NANOPARTICLE TRACKING TO PROBE TRANSPORT IN POROUS MEDIA

by

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Abstract

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Porous materials are ubiquitous in nature and industrial applications, ranging from porous subsurface environment to functional polymer membranes used in separation processes, such as water treatment and pharmaceutical sterilization. However, there are currently no universal models capable of predicting mass transport of nanoparticles or macromolecules in porous media based on a simple description of the porous materials because of the complexity of heterogeneity of real-world porous materials, and because many coupled dynamic mechanisms (e.g. adsorption, steric effects, hydrodynamic effects, electrostatic interactions, etc.) give rise to the observed macroscopic transport phenomena.

Conventional experimental techniques, like dynamic light scattering and nuclear magnetic resonance, measured mass transport in porous media at the ensemble level, where the microscopic mechanisms that give rise to macroscopic transport behaviors are obscured, making it difficult to determine the origin of complex phenomena such as anomalous diffusion, hindered pore-space accessibility, and unexpected retention under flow. To address this issue, we have developed refractive index matching imaging systems, combined with single-particle tracking methods, allowing the direct visualization of single particle motion within a variety of porous materials from single-pore to macroscopic length scales.

In this thesis, we investigated transport mechanisms (i.e., diffusion, advection and self-propulsion) of nanoparticles in porous media using single-particle tracking methods in both fundamental and applied scenarios. From the fundamental research perspective, we used a highly ordered porous medium as a model system to directly explore the effects of geometric parameters (i.e., pore size, pore throat size, and tracer particle size) and microscopic interactions (i.e., electrostatic and hydrodynamic interactions) on

nanoparticle transport in porous environments. We found a linear scaling relation between the macroscopic diffusion coefficient and microscopic diffusion behavior involving a combination of parameters associated with pore-scale features and phenomena, including both geometric effects and particle-wall interactions. In addition, we found that self-propulsion effects could significantly facilitate the nanoparticle transport in complex porous media due to nuanced effects associated with self-propulsion, including enhanced mobility in confinement, efficient surface-mediated searching for holes, and the abolition of barriers at cavity exits. From the applied research perspective, we used commercially available polymer microfiltration membranes as a model porous medium to investigate the mechanisms of particle fouling and remobilization in filtration processes. We elucidated the mechanisms of particle remobilization during flow interruption, and membrane fouling mechanisms of bidisperse particle solutions condition by directly visualizing the entire process. These findings presented here provided important insights into understanding particle transport in complex and confined environments, which can be used to advance and optimize filtration processes.

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Chapter 1 Introduction



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1.1 Background

Porous media are a class of materials containing pores (voids). The skeleton of the material is a solid, while the pores are usually filled with a liquid or gas. The characteristics of a porous material depend on the size and shape of the pores, porosity (the volume fraction of void space) and the composition of the material.¹ Porous media are used for a wide range of applications, from filtration membranes²⁻³ to bioreactors⁴⁻⁵ to heterogeneous catalysis.⁶ Moreover, many geological and biological environments are porous. Different applications require specific transport properties; for example, porous media used for filtration membranes require a narrow pore size distribution to achieve selective filtration,³ while porous media designed for bioreactors require bimodal pore size distribution since enzymes are often immobilized in small pores and large pores are used for mass transport of reactants and products.⁵

Although mass transport within porous media is critical to many applications, we have an incomplete understanding of the microscopic processes that give rise to the macroscopically observed transport. Most experimental studies involve macroscopic functional measurements made the length scales that are orders of magnitude larger than the pore size, making it difficult to obtain mechanistic information

about the microscopic interactions (e.g., electrostatics, hydrodynamics, transient binding, etc.) and complex geometries that combine to produce the macroscopic transport phenomena.⁷⁻¹¹ However, theoretical models developed to explain the transport in porous media usually begin with a microscopic description of particle dynamics at single pore length scales, which is then averaged over the porous matrix to predict macroscopic transport properties.¹²⁻¹⁶ Therefore, the gap between experimental approaches and theoretical frameworks has made it difficult to determine the underlying origins of the complex transport phenomena that are frequently observed such as anomalous diffusion¹⁷ and unexpected retention under flow.¹⁸

Tracking the motion of individual particles from sub-pore to macroscopic length scale is a promising way to bridge this gap and elucidate the microscopic transport mechanisms in porous media. While there is a long history of tracking flow in two-dimensional model systems, it has traditionally been difficulty to track particles deep within a fully three-dimensional material. One challenge is due to the mismatch of refractive indices of the solid matrix and voids, which causes significant light scattering at solid-void boundaries. Although the use of materials with characteristic length scales much smaller than the wavelength of visible light (such as gel networks¹⁹ or nanoporous glass²⁰) can reduce scattering, this represents only a subset of porous materials and excludes many technologically important materials, like sterilization membranes. Another approach to reduce scattering is to permeate the voids with a liquid that has the same refractive index as the solid matrix.²¹⁻²² A carefully designed refractive-index matching system can enable high quality single-particle tracking in various industrially relevant porous materials, like nitrocellulose and poly(ethylene glycol). By combining highly multiplexed single-particle tracking methods with refractive index matched systems, we have directly visualized the motion of individual particles in various porous media, providing new insights into the microscopic mechanisms underlying anomalous mass transport phenomena.

1.2 Single-Particle Tracking in Porous Media

1.2.1 Single-Particle Tracking

Single-particle tracking has played an important role in physical, chemical and biological areas science since Robert Brown's observation of the motion of pollen grains suspended in water using an optical microscope.²³ In modern single-particle tracking experiments, particles of interest are imaged using an optical microscope with a photon detector, particle motion is continuously recorded in a video fashion, and sophisticated tracking algorithms are employed to localize particles positions and generate trajectories. Nanoparticles, which are smaller than the intrinsic resolution of the microscope, are localized by identifying the centroid locations of their point spread functions (PSF); successive localizations associated with the same particle are connected chronologically frame-by-frame to create a single-particle trajectory (Figures 1-1a and b).²⁴ In practice, trajectories are created for many particles in parallel. These localization and trajectory linking processes are performed using automated computational algorithms that have been optimized to yield as many as tens of thousands of spatiotemporal trajectories for a given experiment, enabling detailed statistical analysis, and the observation of relatively rare-but-important events. Given rare image data with high signal relative to background levels, the location of a particle can be determined with a spatial resolution that is much better than the apparent diffraction limited size of the point spread function. In practice, a localization precision of ± 10 nm is readily achieved. The temporal resolution of trajectories is determined by the acquisition time of each movie frame, which may be on the order of 10 ms. These specific values of spatial and temporal resolution depend on the equipment employed and the conditions of the experiments.

In addition to conventional 2D single-particle tracking approaches, several 3D tracking methods have been developed recently through point spread function engineering (encoding axial position in the shape of the PSF)²⁵⁻²⁷ and interference-, interface-, or intensity-sensing methods (which measure the position of the particle relative to features in the imaging geometry).²⁸⁻²⁹ The ability to track particles in 3D provides unique opportunities to study fundamental transport problems that are intrinsically three-

dimensional, such as cavity translocation problems in porous environments. In particular, we utilized double-helix point spread function (DH-PSF) imaging to achieve three-dimensional single-particle trajectories, Figure 1-1c shows a representative schematic diagram of imaging equipped with DH-PSF module. In this method, the engineered PSF is converted from an Airy disk shape to a double-helix shape with two lobes in the image plane whose angular orientation rotates with the axial (*z*) position of the emitting particle (Figure 1-1d). Therefore, the transverse (x-y) position of an emitting particle is estimated from the midpoint of the two lobes, and the axial (*z*) position is estimated from the angle of the line connecting the two lobes by using a calibration plot that correlates axial position to azimuthal angle.



Figure 1-1. Schematic diagrams of single-particle tracking methods. (a) Particles are localized by finding the central locations of point spread functions. (b) Single-particle trajectories are generated by connecting successive localizations of the same particle based in a time series. (c) Schematic diagram of imaging system equipped with double-helix point spread function (DH-PSF) module, which enabled 3D single-particle tracking. (d) Images of a fluorescent particle at different axial positions, with 0 being centered in the focal plane. Panel (a) and (b) were adopted from reference 24, panel (c) was adopted from reference 36, and panel (d) was adopted from reference 25.

1.2.2 Refractive-Index Matching Liquid

One challenge associated with imaging particle motion deep in a porous medium is the significant light scattering at the ubiquitous solid-void boundaries. To overcome this challenge, we have formulated various refractive-index matching liquid mixtures for different porous media. An effective refractive-index matching liquid should have the following properties: (1) transparency, (2) refractive index tunability near the index of the porous matrix, (3) chemical compatibility with the porous matrix and tracer particles, and (4) wettability of the porous medium.²¹ We have made good use of the obscure solvent thiodiethanol, which has a relatively high refractive index (RI) of n=1.52, good wetting properties, and broad chemical compatibility. Since it is miscible with surfactants and many solvents with lower RI, one can easily formulate index-matching mixtures to enable optical imaging within various technologically relevant porous media, including biomaterial scaffolds, like poly(L-lactic acid) ($n \sim 1.46$) and poly(ethylene glycol) $(n \sim 1.46)$, and separation membranes, like nitrocellulose $(n \sim 1.50)$ and poly(vinylidene fluoride) $(n \sim 1.42)$. In some applications, aqueous systems are desirable (e.g., for their ability to dissolve salts and stabilize biomolecules, and we have achieved effective refractive index matching for various porous materials using glycerol/water mixtures. Generally speaking, the RI of mixtures will span the RI range of the components, and it is straightforward to empirically test a series of mixtures in parallel by simply immersing a piece of the porous material in each mixture. The material will effectively vanish to the naked eye when immersed in an index-matched liquid.

1.2.3 Fluidic Platform for Direct Observation of Filtration Processes

Advection is an important particle transport mode in porous media, especially in filtration processes, where a pressure gradient is generally employed as the main driving force for particle transport. As opposed to the simpler diffusion experiments, a flow cell is necessary to study convective flow. We have developed flow cells based on PDMS fluidic devices, which are easy to prepare in conventional laboratories, and are readily customized. Figure 1-2 shows a schematic diagram of a setup

that combines fluorescent microscopy imaging system with a fluidic platform to directly observe of filtration processes. Small rectangular sections of the membranes were cut and inserted in a custommade PDMS fluidic device, and the transmembrane particle flow was imaged using a fluorescence microscope employing alternating-laser excitation. Tracer particle solutions were introduced into the inlet and flowed through the membrane being imaged, employing a dead-end filtration process geometry where the flow direction is normal to the membrane surface. Fluorescent latex nanobeads were used as tracer particles, and flow conditions were controlled by a syringe pump. In addition, by incorporating alternating-laser excitation, which excited the two types of fluorescent particles in an alternating fashion, we able to image two nanoparticle populations (e.g. of different sizes) simultaneously. With proper design and modification, this platform can be used to investigate various important filtration problems at a single-particle level, including particle remobilization mechanisms during flow interruption and fouling mechanisms under various experimental conditions.



Figure 1-2. Schematic diagram of a fluidic imaging platform equipped with alternating laser excitation system.

1.3 Statistical Analyses of Single-Particle Trajectories

1.3.1 Analysis of Diffusive Trajectories

Diffusion is a basic but important mode of transport of molecules and particles. It is obviously necessary to understand diffusion as a fundamental baseline. Diffusion experiments are easy to perform, and the analysis of diffusive trajectories provides a great deal of useful information, including diffusive modes (Fickian vs anomalous diffusion), ergodic properties through mean square displacement analysis, and the hydrodynamic radius from the Stokes-Einstein relation. Here, we introduced some fundamental analysis methods that are broadly applicable to diffusion experiment.

(a) Mean squared displacement

Mean squared displacement (MSD) analysis is a classic approach to extract the diffusion coefficient D, and to classify the type of diffusion associated with particle trajectories. A generic formula to calculate MSD is $MSD(\tau) = \langle \Delta r(\tau)^2 \rangle = \langle (r(t + \tau) - r(t))^2 \rangle$, where r(t) denotes the position of the particle at time t, and τ is the lag time. The average $\langle \cdot \rangle$ represents an ensemble-average over several trajectories and/or a time-average over t. In principle, these averages provide similar information for classical diffusive trajectories; however, they are not equivalent for non-ergodic processes, and it is therefore important to compare them explicitly²³. For example, the ensemble-average MSD and time-average MSD of polymer diffusion in polyelectrolyte multiplayers are significantly different because the diffusing molecule can bind to the surrounding molecules or regions in the film and trapped for waiting times that become longer as the trajectory evolves, which lead to the "aging" effect³⁰.

The diffusion process can be classified based on an MSD analysis, by fitting to a power law function $\langle \Delta r(\tau)^2 \rangle \propto \tau^{\alpha}$. The case $\alpha = 1$ is referred to as Fickian (also called normal diffusion), whereas $\alpha \neq 1$, it is an anomalous diffusion (Figure 1-3a). In particular, if $\alpha > 1$, it is a super-diffusive process; if $\alpha < 1$, sub-diffusive.²³ Notably, when $\alpha = 1$, the diffusion coefficient *D* can be extracted using the equation $MSD(\tau) = 2dD\tau$, where *d* denotes the dimension. For example, for two-dimensional trajectories, $MSD(\tau) = 4D\tau$. From

the diffusion coefficient, the hydrodynamic radius of tracer particles can be obtained through the Stokes-Einstein relation. Notably, while MSD analysis is widely used to classify a diffusive process, it does not in itself enable an understanding of underlying mechanisms. For example, there are various situations that may lead to sub-diffusive motion (i.e., a continuous time random walk vs. fractional Brownian motion), and more detailed analyses are required to distinguish between them.

(b) Step-size distributions

In addition to conventional MSD analysis, trajectories are commonly analyzed through the statistics of individual displacements, which can be quantified using step-size distribution such as the van Hove correlation function, $G_s(\Delta x, \Delta t) = \frac{1}{N} \langle \sum_{i=1}^N \delta(x + x_i(t) - x_i(t + \Delta t)) \rangle$, where $\langle \cdot \rangle$ indicates ensemble averaging. This distribution represents the probability that a particle has moved a distance Δx along the x, y or z coordinate during time Δt . A Gaussian distribution is consistent with simple Brownian motion, and the diffusion coefficient can be extracted through equation of $P(x, t) = \frac{1}{\sqrt{4\pi Dt}} \exp(-\frac{x^2}{4Dt})$.³¹ Of course, the distribution need not be Gaussian, and the shape may indicate the presence of multiple superimposed diffusive modes, or anomalous diffusion.

In general, diffusion can be extremely complex, and no single statistical metric is sufficient to make definitive mechanistic conclusions. In addition to the fundamental analysis methods illustrated here, there are various analysis methods for different scenarios, like velocity autocorrelation functions to study caging effects in confined motion and waiting time distributions to analyze continuous-time random walks (CTRW), etc. Moreover, it is helpful to apply various statistical methods and to compare the results for self-consistency. For example, Granick and co-workers observed a linear dependence of MSD on lag time for the motion of fluorescent beads in entangled F-actin networks, suggesting Fickian diffusion. However, the displacement distribution exhibited exponential tails for large displacements, demonstrating that the diffusion was not Brownian. ³² The detailed analysis of diffusive trajectories from single-nanoparticle and

single-molecule tracking experiments in recent years suggest that exotic diffusive mechanisms are actually quite common in complex environments, and this had led to a re-birth of interest in the topic.



Figure 1-3. Analysis methods of single particle trajectories. (a) Schematic mean square displacement curves for different types of diffusion. (b) Schematic diagram illustrating a tortuosity calculation in a porous medium.

1.3.2 Analysis of Advective Trajectories

Advection is another important transport mode of particles, especially in industrial processes, where pressure gradient is the main driving force. Imaging of advection is generally more difficult to perform compared to diffusion, since a well-designed flow cell and well-controlled flow conditions are necessary for these experiments. However, the rewards are significant, advective trajectories can provide microscopic transport insights into important industrial processes, like membrane filtration, which is impossible for conventional ensemble methods. Here, we introduce some fundamental analysis methods that are broadly applicable to advection experiments in porous media.

(a) Local geometry is characterized using tortuosity

The classical concept of tortuosity (τ) is frequently used to characterize the complexity of pathways in porous media, and may be a useful parameter to understand hindered mass transport processes in complex

environments. Tortuosity has been defined in various ways in the literature to relate to the diffusive, hydraulic, or electrical properties of porous media.³³ A conventional way to measure tortuosity from ensemble properties involves the equation $\tau = \sqrt{D_p/D_0}$, where D_p is the apparent diffusion coefficient within a porous medium under stagnant conditions, and D_0 is the diffusion coefficient in unconfined liquid.³⁴ While this type of calculation is simple to perform, it provides only an average value under static conditions, and implicitly treats the porous environment as a static effective medium.

Microscopically, it is more proper to define the geometric tortuosity of a given trajectory, instead of the material as a whole. In particular, the tortuosity of a trajectory may be defined mathematically as the ratio of contour length to the end-to-end Euclidean distance of trajectory (Figure 1-3b).³⁵ Given large-scale single-particle tracking data, the geometric tortuosity can be explicitly measured for each trajectory (or even for sub-trajectories), and these data sets can be viewed as probability distributions, or treated statistically (e.g., calculating the mean, median, variance, etc.).

In our experiments, we generally calculate tortuosity values of equal length sub-trajectories, which we call local tortuosity (τ_L). This accounts for the fact that trajectories can vary significantly in length and takes advantage of the greater information content of long trajectories. In particular, each trajectory is divided into sub-trajectories with equal numbers of time steps. For each sub-trajectory, the equation $\tau_s=L_s/C_s$ is employed to calculate the local tortuosity, where L_s is the contour length and C_s is the end-to-end distance of the sub-trajectory. The distribution of local tortuosity values can then be further analyzed, e.g., by calculating the mean local tortuosity ($\langle \tau_L \rangle$) in a given location, field of view, time interval, of experiment. Depending on the choice of sub-trajectory duration, the local tortuosity values can provide spatial information about porous media on length scales of approximately 1-100 times the characteristic pore size.

Interestingly, at a local level, the net direction of an individual trajectory can diverge significantly from the macroscopic flow direction. Therefore, it can be interesting to measure the projection of tortuosity on the macroscopic flow axis, which we label the global tortuosity, which provides additional information regarding the overall flow directions of trajectories (e.g., side flow and backflow). The global tortuosity τ_{G}

is calculated as the ratio of the sum of all contour lengths to the sum of all displacements projected onto the global flow direction, $\tau_G = \frac{\sum L_n}{\sum C_{n,p}}$, where L_n and $C_{n,p}$ are the contour length and the projection of the displacement of the trajectory in the global flow direction, respectively.

Since trajectories are explicitly connected to specific spatial regions, information about spatial heterogeneity is intrinsically encoded in the large data sets. For example, a trajectory map can be created by overlaying all acquired trajectories in a given field of view. These visualizations can provide interesting qualitative information about mass transport, as well as about the porous structure of materials. For example, in a heterogeneous material, more tortuous intervals of trajectories often correspond to spatial regions with smaller pores, while less tortuous trajectories correspond to regions with larger pore diameters. Most importantly, direct observations of trajectory maps provide a unique and highly intuitive perspective on complex transport processes like membrane fouling and particle remobilization during flow interruption.

(b) Characterizing local dynamics

Tortuosity analysis provides quantitative structural and geometric information about trajectories but ignores the dynamic information intrinsic to spatiotemporal trajectories (i.e., the local instantaneous velocity of particles). Residence time analysis is one way to extract this dynamic information, which is also embedded in single-particle trajectories. Specifically, we divide the field of view into small spatial bins, identify all trajectories passing through a given bin, and then calculate the mean residence time for all sub-trajectories passing through a given bin. Therefore, spatial regions where particles move slowly will be associated with large residence time values, and vice versa. Based on these residence times data, spatial residence time heatmaps and residence time distribution can be created to investigate the heterogeneity of the porous medium.³⁵

In addition, for porous media that have well-defined cavities or galleries, it is interesting to extract the time required for a particle to escape from a cavity and translocate to a neighboring cavity, often through a narrow constriction. This first passage time represents a special type of residence time, which describes the time interval required for a stochastic process, starting at given initial position, to arrive a certain position for the first time. In the scenario of porous media, it is the residence time for a confined particle's first escape from a given pore space.³⁶⁻³⁷

1.3.3 Analysis of Self-Propelled Trajectories

In addition to diffusion and advection, self-propulsion is currently being explored as a novel transport mechanism that is underutilized industrially, but widely seen in nature. Self-propulsion describes the autonomous motion of macro-, micro- and nanoscropic natural and artificial objects, and is readily implemented in the laboratory using artificial active colloids. Self-propulsion has significant potential in applications, like drug delivery, medical diagnosis and environmental remediation. The general methods used to analyze diffusive trajectories can also be employed for self-propelled trajectories.

The fundamental difference between diffusive trajectories and self-propelled trajectories is the propulsion speed v due to self-propulsion effects; as v increases, self-propelled trajectories are more likely to exhibit trajectories with persistent directed motion. However, over long time scales the directional motion of the particle is randomized by its rotational diffusion. Therefore, in addition to the translational diffusion coefficient D_R and propulsion speed v are the other two parameters unique to self-propelled trajectories which are extracted from trajectory analysis. In particular, the translational diffusion coefficient can be obtained using $D_T = \frac{k_B T}{6\pi\eta R}$, and the rotational diffusion coefficient can be obtained using $D_T = \frac{k_B T}{6\pi\eta R}$, and the rotational diffusion coefficient can be obtained using $D_T = \frac{k_B T}{6\pi\eta R}$, and the rotational diffusion coefficient can be obtained using $D_T = \frac{k_B T}{6\pi\eta R}$, and the rotational diffusion coefficient can be obtained using $D_T = \frac{k_B T}{6\pi\eta R}$, and the rotational diffusion coefficient can be obtained using $D_R = \frac{k_B T}{8\pi\eta R^3}$, where k_B is the Boltzmann constant, T is the temperature, η is the viscosity, and R is the particle radius. The propulsion speed v can be extracted from the equation $D_{T_active} = D_{T_passive} + v^2/6D_R$ in three-dimensional space, where D_{T_active} represents the apparent translational diffusion coefficient of active particle with self-propulsion effects, and $D_{T_passive}$ represents the translational diffusion coefficient of the corresponding passive particle with Brownian motion only.³⁸ Both D_{T_active} and $D_{T_passive}$ can be extracted from mean squared displacement of trajectories.

1.4 Objectives

The objective of this thesis is to understand nanoparticle transport mechanisms in complex porous environments. To accomplish this objective, single-particle tracking methods were utilized to visualize nanoparticle diffusion, advection and self-propelled motion in various kinds of porous media from both fundamental and applied research perspectives. From the fundamental research perspective, we used a highly ordered porous medium as a model system to directly explore the effects of geometric parameters (i.e., pore size, pore throat size, and tracer particle size), microscopic interactions (i.e., electrostatic and hydrodynamic interactions) and self-propulsion effects on nanoparticle transport in porous environments. From the applied research perspective, we used commercially available polymer microfiltration membranes as the model porous medium to investigate the mechanisms of particle fouling and remobilization in filtration processes. We elucidated the particle remobilization mechanisms during flow interruption, and the membrane fouling mechanisms of bidisperse particle solutions by directly visualizing the entire process. In particular, the work was broken down into two categories and six main aims:

Fundamental research:

- 1. Understanding cavity escape and pore accessibility in nonaqueous solution. (Chapter 2)
- 2. Understanding cavity escape and pore accessibility in aqueous solution. (Chapter 3)
- 3. Connecting nanoparticle diffusion in porous media from single-pore to macroscopic. (Chapter 4)
- 4. Understanding self-propulsion effects in cavity escape and pore accessibility. (Chapter 5)

Applied research:

- 1. Understanding the particle remobilization process during flow interruption. (Chapter 6)
- 2. Understanding membrane fouling mechanisms of bidisperse particle solutions. (Chapter 7)

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Chapter 2 Diffusive Escape of a Nanoparticle from a Porous Cavity

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2.1 Abstract

Narrow escape from confinement through a nano-channel is the critical step of complex transport processes including size exclusion-based separations, oil and gas extraction from the microporous subsurface environment, and RNA translocation through nuclear pore complex channels. While narrow escape has been studied using theoretical and computational methods, experimental quantification is rare because of the difficulty in confining a particle into a microscopic space through a nanoscale hole. Here, we studied narrow escape in the context of continuous nanoparticle diffusion within the liquid-filled void space of an ordered porous material. Specifically, we quantified the spatial dependence of nanoparticle motion and the sojourn times of individual particles in the interconnected confined cavities of a liquid-filled inverse opal film. We found that nanoparticle motion was inhibited near cavity walls and cavity escape was slower than predicted by existing theories and random-walk simulations. A combined computationalexperimental analysis indicated that translocation through a nano-channel is barrier-controlled rather than diffusion-controlled.

2.2 Introduction

In the absence of confining interfaces, the mean-square displacement (MSD) of Brownian motion grows linearly over time and the corresponding displacement is Gaussian distributed ¹⁻². This simple picture breaks down within tortuous interconnected porous environments, where the internal geometry of void spaces can confine diffusion ³⁻⁵. Observing spatially confined diffusional trajectories can potentially provide

information about the local microstructure ⁶⁻¹⁴. Moreover, confinement can fundamentally change the statistics of Brownian motion via e.g., transient adsorption/desorption ¹⁵⁻¹⁶, particle-wall interactions ¹⁷⁻¹⁸, entropic ¹⁹⁻²⁰ or hydrodynamic ²¹⁻²² effects, and other noncovalent interactions. These effects cause deviations from Brownian motion, manifesting as a range of anomalous behavior, such as obstructed slow transport, nonlinear MSD and/or non-Gaussian-distributed displacements. Notably, recent observations suggest that the accessible volume explored by a particle in heterogeneous porous materials was smaller than expected ²³⁻²⁵; the underlying mechanisms are not understood yet.

The rate-determining factor of limited accessibility is the narrow escape through bottlenecks within porous void spaces. To study the origin of the transport mechanism, a straightforward approach is to quantify the sojourn time in a well-defined confined cavity prior to escape. A successful escape event consists of two naturally decoupled steps ²⁶: (1) the search for an opening, and (2) the subsequent translocation of a particle through the opening. The former is connected to the mathematical "narrow escape problem" that calculates the mean first passage time (MFPT) of a Brownian particle to an opening on a reflecting boundary of a confined domain. The current understanding of the latter is that the passage is controlled by a diffusion-limited barrier ²³. A variety of theoretical frameworks have been proposed that allow asymptotic evaluation of MFPT in various physical scenarios ²⁷⁻³⁸; however, it remains challenging to test theoretical predictions experimentally. A pioneering "pore-cavity-pore" device involving the active entrapping of particles via an applied electric field, represented an important first step to characterize the narrow escape problem ³⁹.

2.3 Results and Discussion

Here, we experimentally study the escape of individual fluorescent polystyrene nanoparticles from a confined silica cavity in an indexed-matched solution consisting of thiodiethanol and the surfactant Triton X-100. The confined space comprises a silica inverse opal film consisting of a hexagonally close-packed interconnected network of spherical cavities, where each cavity has twelve circular holes that connect to adjacent cavities ⁴⁰. The holes allow the escape of a Brownian particle from one cavity to another, exploiting passive diffusion. We employed a multiplexed 3D single-particle imaging approach to record the sojourn time in a given cavity by identifying consecutive escape events of many particles simultaneously. This fully 3D tracking capability allowed us to address the "many-window escape problem" ^{32, 34}.

The silica inverse opals were prepared by evaporative deposition of monodisperse polystyrene spheres ⁴⁰⁻⁴¹. The resulting structure contained a high-quality silica inverse opal layer with extremely uniform cavity size over macroscopic length scales (Figures. 2-1a, and 2-1b). The resulting structure had a cavity radius R = 0.25 um and an adjustable hole diameter *L* that was controlled by varying the concentration of the silicate precursor solution (Figure A-2). The particles (hydrodynamic radius of 24 nm) were dispersed in a 1:5 mixture of thiodiethanol and Triton X-100 at a concentration of approximately 10^{-13} – 10^{-14} M. This particular formulation was chosen because it simultaneously matches the refractive index of silica, minimizes particle adhesion to the interior silica surfaces, and is sufficiently viscous to permit highly-resolvable observations of particle motion. As such, the solution (while not commonly found in natural porous environments) serves as an appropriate model system for observing confined nanoparticle mobility.



Figure 2-1. Schematic diagram of 3D single-particle tracking of nanoparticle in inverse opals. (a) Representative scanning electron microscope image of an inverse opal film. The scale bar represents 1 μ m. (b) Confocal laser scanning microscopy image of an inverse opal film filled with Rhodamine 6G solution. The scale bar represents 5 μ m. (c) A variable angle wide-field microscope body was equipped with a phase

mask that enabled DH–PSF imaging. (d) A representative 3D trajectory of a particle confined in an inverse opal structure.

Some particles diffused passively and spontaneously into the interior void space. However, even under optimized conditions, a small fraction of particles was immobilized and accumulated over the course of the experiment, due to the presence of various attractive interactions (see Table A-1) and fact that nanoparticle samples often exhibit surface heterogeneity ⁴²⁻⁴³. While particle immobilization and retention is important in its own right, here we focused on the mobile nanoparticle population. We employed a 3D single-molecule imaging approach, which combined variable-angle illumination epifluorescence microscopy and double-helix point spread function (DH–PSF) optics (SPINDLETM module, Double Helix LLC, Boulder, Figure 2-1c) to accumulate 3D spatiotemporal positions of mobile particles ⁴⁴⁻⁴⁵. As shown in Figure 2-1d, 3D trajectories reflected the internal structural geometry of the inverse opal and provided definitive evidence of escape events not generally possible from 2D projections (Figure A-10).



Figure 2-2. Hindered cavity escape of nanoparticle in inverse opals under nonaqueous condition. (a) Plots of MSD versus lag time τ for free diffusion (black square) and confined diffusion at different *L* (hole diameter). (b) Representative displacement probability distributions for free and confined diffusion at $\tau =$

0.05 s. (c) Representative VACF for free and confined diffusion. (d) Spatially dependent diffusion coefficient as a function of the distance to the wall. (e) Representative trajectory passing through three cavities. (f) Cartesian coordinates of the trajectory from panel (e) as a function of time. The vertical blue lines represent escape events.

To provide an overview of the diffusive behavior, we calculated the equivalent one–dimensional ensemble-average mean squared displacement (MSD). For nanoparticles dispersed in a bulk liquid, the MSD grew linearly with τ , as expected for Brownian motion, yielding a diffusion coefficient $D = 0.12\pm0.01$ μ m²/s over a wide range of time-scales. In contrast, the confined diffusion exhibited a classic MSD three-regime behavior associated with cage dynamics (Figure 2-2a)⁴⁶.

For short lag times, the MSD grew linearly with τ , with an effective confined diffusion coefficient, $D_{\text{confined}} = \text{MSD}/2\tau \approx 0.04 \ \mu\text{m}^2/\text{s}$, that was significantly slower than that in bulk liquid. The ensembleaveraged displacement probability distribution in this time regime deviated only slightly from Gaussian behavior (Figure 2-2b). We calculated the velocity autocorrelation function (VACF) and found negative values of VACF for lag times in the range 0.1 s < τ < 0.3 s (Figure 2-2c). Interestingly, the anticorrelation vanished at $\tau \approx 0.3$ s, consistent phenomenologically with the spatially dependent "diffusing diffusivity" model ⁴⁷⁻⁴⁹, arising from particle-wall interactions ⁵⁰. This caused a gradual decrease in mobility as a function of proximity to the wall (Figure 2-2d).

At intermediate lag times, the MSD plot curved downward, sometimes exhibiting an actual plateau due to transient trapping within a cavity for a probabilistic sojourn time. In the long-time limit, a particle escaped a given cavity and hopped among different cages, giving rise to an approximately diffusive behavior, consistent with the cage dynamics in a transient network ⁵¹⁻⁵². The ratio of the hole diameter to the tracer particle diameter was expected to have a major impact on the frequency of escape associated with the onset of the long-time regime (Figure 2-2a). The effective tracer particle diameter was 48 nm. The hole diameter *L* for a given inverse opal sample, was determined by analyzing scanning electron microscope images.

The key quantity required to characterize the narrow escape problem is the sojourn time. For example, the mean sojourn time, T_{soj} , can be theoretically related to the long-time diffusion coefficient $D_{long} = R^2/6T_{soj}$. To determine these time intervals, we identified the escape from one cavity to another by monitoring the 3D position fluctuation using a maximum allowed displacement approach (Figures 2-2e, 2-2f). Figure 2-3a shows complementary cumulative distributions of sojourn time in inverse opals at varying L. Due to technical limitations, we obtained reliable and statistically meaningful measurements of T_{soj} , only for L in the range 113–187 nm; T_{soj} was inaccessibly long for L<113 nm (i.e. particles rarely escaped a cavity) despite the fact that the hole diameter was more than twice that of the particle. The distribution of sojourn times was adequately described by the single exponential decay $P(t) = \text{Exp}(-t/T_{soj})$, suggesting that the escape was governed by a rate process with a barrier ⁵³. We found that T_{soj} , decreased rapidly with increasing L. For example, as L was increased by a factor of 1.6, T_{soj} decreased more than 4x. This scenario indicates that tightening the escape pathway can effectively decreased the probability of escape (Figure A-3b).




Figure 2-3. Comparison of experimental results and simulations of nanoparticle cavity escape from inverse opals in nonaqueous solvent. (a) Cumulative distribution of sojourn times at different L. (b) sojourn time as a function of L. The circle symbols represent the experimental results. The black and red lines denote simulated results from a RW simulation and a RW simulation involving a repulsive particle-wall potential while the blue and green lines represent theoretical results calculated using the Cheviakov and Lagache models, respectively.

To better understand the mechanisms controlling the cavity escape process, we performed several calculations to model and interpret the sojourn time distribution. First, from the perspective of short-time diffusion, the time t_{α} required for a particle to reach the boundary of a cavity of size *R* can be estimated as $t_{\alpha} = R^2/6D_{\text{confined}} \approx 0.23$ s, which is generally consistent with the transition from the initial linear to plateau region in the plot of MSD vs. τ (Figure 2-2a). However, the calculated t_{α} is nearly two orders of magnitude shorter than the minimum measured value of T_{soj} , suggesting that the cavity escape time is much longer than the cavity exploration time. To evaluate this diffusion-limited process quantitatively, we applied two different asymptotic formulas to calculate the MFPT of pointlike particles to reach multiple openings on a cavity, as shown in Figures 2-3b and A-8^{32, 34}. All parameters used in these calculations were experimentally measured. These two calculations agreed reasonably well with each other and showed a similar trend as experimental measurements, but underestimated the absolute measured sojourn times by a factor of ~20.

In principle, slow particle translocation can arise from factors such as size-exclusion, hydrodynamic effects, or long-range interactions between walls and particles. As detailed in the

supplementary material, computer simulations were performed to determine which factors contributed to the measured phenomena. To isolate size-exclusion effects, we performed a kinetic random walk simulation (RW) simulation to mimic the escape of a finite-size spherical particle from an inverse opal cavity. The RW simulation yielded improved agreement, but still significantly underestimated the measured sojourn times (Figure 2-3b). The RW simulation agreed with a standard Brownian Dynamics (BD) simulation for the calculation of the sojourn time prior to escape, suggesting that size-exclusion (entropic) effects cannot solely explain the slow escape. Since the RW simulation does not consider localized hydrodynamic effects ²²⁻²³, we performed simulations using modified multiparticle collision dynamics (MPCD) to include momentum exchange between the particle and cavity walls. However, the MPCD results predicted only modestly longer sojourn times, ~20% longer than the predictions of BD simulations under the same conditions (Figure A-11).

Additional control experiments were performed to directly control and probe the relevance of repulsive particle-wall interactions, by tracking the escape of tracer nanoparticles in an inverse opal film (L=150 nm) that was immersed in a water/glycerol mixture (1:9 by weight). In this aqueous system, the electrostatic interaction was well-defined and could be controlled by changing the salt (NaCl) concentration. When the salt concentration was decreased from 0.1M to 0.001 M, corresponding to an increase in the Debye length from 0.96 nm to 9.6 nm, the mean sojourn time increased from 2.6 to 9.4 s (Figure A-12) for the same tracer particles in the same porous structure. Importantly, the sojourn time for the higher-salt system was consistent with the RW simulation result, indicating that the escape is adequately described by the size-exclusion (entropic) effects alone when the electrostatic interaction is screened ¹⁹. Under lower-salt conditions, however, escape was significantly slower, consistent with the observations in Figure 2-3b, suggesting that the slow particle translocation indeed originated from the presence of repulsive particle-wall interactions.

These interactions were further evaluated by considering the radial probability distribution of confined particles (Figure A-13), which exhibited apparent repulsion from cavity walls compared to RW simulations. By considering the types of interactions that may be relevant for nanoparticles in porous media

(Table A-1), we find that the repulsion is phenomenologically consistent with Coulombic effects in nonaqueous solutions ⁵⁴⁻⁵⁵ or with dipole interactions for objects having large dipole moments in a medium with small dielectric constant ⁵⁶. Such a force can provide an additional barrier to escape, that would increase T_{soj} as predicted recently by the Grebenkov-Oshanin model ²⁶.

Since the effects of screening in non-aqueous solution are unclear, the RW simulation was simply modified to include a hypothetical repulsive potential, A_{rep} , to represent the dipole-dipole interaction between particle and wall, which biases the probabilities of steps towards or away from the cavity walls. In particular, the probability of moving towards the boundary was given as $P=0.5-A_{rep}/(r+r_0)^6$ where *r* denotes the distance to a wall, and a small intercept r_0 was defined to avoid divergence. The only adjustable parameter was A_{rep} which controlled the strength of the repulsion, and reduced to Brownian motion in the limit of $A_{rep} = 0$ m. By setting $A_{rep} = 3 \times 10^{-6}$ m, excellent agreement was achieved with experimental observations, further supporting a model (Figure 2-3b) involving the presence of a barrier when approaching the boundary. This can also be expressed as an effective narrowing of the escape pathway, providing a mechanistic interpretation for previous reports that have hypothesized the presence of a decrease in the "effective" diffusion coefficient ^{57,59} or an increase in the "effective diffusion length ⁶⁰. In the limiting case, where the particle diameter approaches that of the hole, escape pathways become vanishingly small. Therefore, if the pore size is heterogeneously distributed as expected for most real-world porous materials, the decreased escape pathway makes some openings impermeable to tracer particles, leading to reduced accessibility of a porous network.

2.4 Conclusions

In summary, we used 3D tracking to study the escape of Brownian particles in a uniform liquidfilled inverse opal film. The escape from a given cavity was explicitly quantified by measuring the distribution of sojourn times for particles within cavities by analyzing 3D trajectories in inverse opals. The comparison between experiments and theoretical calculations indicated that escape through the bottleneck was barrier-controlled rather than diffusion-controlled, leading to a decrease in probability of cavity escape and a stronger size-exclusion effect than expected from nominal pore-size alone. These results also highlight the importance of using the sojourn time or MFPT to analyze stochastic processes in confinement ⁶¹⁻⁶²

2.5 References

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Chapter 3 Electrostatic Barriers to Nanoparticle Accessibility of a Porous Matrix



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3.1 Abstract

Translocation from one cavity to another through a narrow constriction (i.e. a "hole") represents the fundamental elementary process underlying hindered mass transport of nanoparticles and macromolecules within many natural and synthetic porous materials, including intracellular environments. This process is complex, and may be influenced by long-range (e.g. electrostatic) particle-wall interactions, transient adsorption/desorption, surface diffusion, and hydrodynamic effects. Here, we used a threedimensional (3D) tracking method to explicitly visualize the process of nanoparticle diffusion within periodic porous nanostructures, where electrostatic interactions were mediated via ionic strength. The effects of electrostatic interactions on nanoparticle transport were surprisingly large. For example, an increase in the Debye length of only a few nm (in a material with a hole diameter of ~100 nm) increased the mean cavity escape time three-fold. A combination of computational and experimental analyses indicated that this hindered cavity escape was due to an electrostatic energy barrier in the region of the hole, which was quantitatively explained using DLVO theory. These findings explicitly demonstrate that the cavity escape process was barrier-limited, and dominated by electrostatic effects.

3.2 Introduction

Confined diffusion has broad implications in diverse physical, chemical, and biological processes, including hindered mass transport within separation membranes¹⁻², crude oil extraction through porous rock³, diffusion of calcium ions in dendritic spines⁴⁻⁵, and RNA translocation between the nucleus and the cytoplasm⁶. The rate-determining factor in these processes is often the narrow escape through bottlenecks within porous void spaces. Extensive work has indicated that nanoparticles, macromolecules and bacteria exhibit unexpected obstructed and/or anomalous diffusion under confinement ⁷⁻¹⁶, and some previous reports have emphasized the importance of cavity escape ¹⁷⁻¹⁹. However, in general, the physical mechanisms underlying the cavity escape process remain incompletely understood. Moreover, despite the fact that particle-wall interactions²⁰, transient adsorption/desorption²¹⁻²², hydrodynamic effects²³⁻²⁴, and electrostatic interactions ²⁵⁻²⁶ are expected to influence confined diffusion, their effects on cavity escape and translocation have not been systematically explored.

To acquire large data-sets on cavity escape, enabling statistical modeling, an effective approach involves the creation of a periodically structured material, which can be used to quantify successive sojourn times within well-defined and identical confined spaces prior to escape. A successful escape event consists of two decoupled processes: (1) the diffusive search for an opening in the cavity, and (2) subsequent translocation through the opening ²⁷. The former process is related to the classical "narrow escape problem", which calculates the first passage time of Brownian particles that escape from a bounded domain through small windows on an otherwise reflecting boundary. A variety of theoretical frameworks have been developed to evaluate the mean first passage time in various physical scenarios ²⁷⁻³⁹. As for the translocation process, the traditional understanding is that the passage is controlled by a diffusion-limited barrier. However, recent experimental and theoretical work^{19, 27} suggest that this process may change from diffusion-limited into barrier-limited if there is strong energy barrier at the exit.

Previous studies of confined diffusion in porous nanostructures have provided important insights toward understanding the escape process ¹⁵⁻¹⁸. For example, by studying the motion of DNA molecules

within an interconnected two-dimensional array of cavities, Nykypanchuk et al. found that DNA molecules localized strongly in the cavities and only sporadically jumped through holes¹⁷. In order to effectively identify cavity translocation events in three dimensions (3D), as a more realistic model for transport in porous media of practical interest, it is necessary to perform fully 3D single-particle tracking. In particular, the analysis of 2D projections of 3D trajectories inevitably omits some translocation events that involve transitions between cavities whose 2D projections significantly overlap. Therefore, to enable robust statistical modeling of the fundamental escape process, we employed wide-field 3D single-particle tracking which permitted highly multiplexed acquisition of nanoparticle trajectories.

Previously, our group studied nanoparticle cavity escape in silica inverse opal films in non-aqueous solution using a 3D single particle tracking technique, and found that the escape process was dramatically slower than predicted by existing theories and random-walk simulations¹⁹. This was ascribed to a translocation energy barrier of unknown origin, which we hypothesized to be caused by Coulombic effects⁴⁰⁻⁴² or dipole interactions⁴³, both of which may theoretically occur in non-aqueous high-dielectric media. However, because it was difficult to quantify, or systematically vary, the magnitude and/or extent of electrostatic interactions in non-aqueous solvents, a detailed understanding of the nature of the barrier to escape remained elusive. Here, we explicitly investigate the effects of electrostatic interactions on the dynamics and accessibility of nanoparticles in a porous matrix. To address this, we experimentally studied the influence of ionic strength on the process of nanoparticle escape from a well-defined cavity where the cavity walls and nanoparticle had like surface charges, and we quantitatively connected the cavity sojourn time (i.e. the time required for escape) to the effective narrowing of the holes between cavities induced by electrostatic repulsion using random walk simulations and electrostatic calculations. The confined spaces comprised the cavities within a silica inverse opal film consisting of a hexagonally close-packed interconnected network of spherical cavities, where each cavity had twelve circular holes connecting to adjacent cavities. The connecting holes permitted the escape of a nanoparticle from one cavity to another, exploiting passive diffusion. We employed double-helix point spread function imaging to record the 3D

trajectories of nanoparticles in the silica inverse opal film, and thereby extracted sojourn times using a maximum allowed displacement algorithm. We found that electrostatic repulsion greatly increased the mean sojourn times, resulting in significant decreases in the long-time diffusion coefficient in the porous material. By combining the experimental data with computational simulations and theoretical electrostatic calculations, we explicitly demonstrated that the escape process was barrier-limited and dominated by electrostatic interactions.

3.3 Methods

3.3.1 Preparation of Silica Inverse Opals

Silica inverse opals were prepared via evaporative deposition of monodisperse polystyrene spheres suspended within a hydrolyzed silicate sol-gel precursor solution. Monodispersed colloidal polystyrene beads (mean diameter 502 nm, standard deviation 13 nm, measured by photo correlation spectroscopy) were purchased from Sigma-Aldrich, USA. 0.25 mL of 5 vol% colloidal polystyrene latex suspension was added to 9.75 mL Milli-Q water (18.2MΩ.cm at 25°C), and 90 or 110 µL of hydrolyzed TEOS solution. Different concentrations of TEOS solution resulted in different hole sizes in the inverse opal structure. The standard TEOS solution consisted of TEOS (98% Aldrich), 0.10 M HCl (Fisher Chemical), and anhydrous EtOH (Decon Labs), respectively, with a volume ratio of 1:1:1.5. The standard TEOS solution was stirred for 1 h at room temperature prior to use. A 25 mm diameter microscope glass slide (Electron Microscopy Sciences, US), after cleaning in piranha solution, was vertically suspended in a beaker containing the polystyrene colloid/TEOS suspension. The solution was evaporated in a 65 °C oven for 2 days on a vibration-free table, to deposit a thin film onto the surface of a glass slide. The resulting films were then pyrolyzed in air at 500°C for 2 h, to remove the polystyrene spheres and transform the silicates into a high-quality, crack-free silica inverse opal layer, with uniform cavity size and periodic holes over macroscopic

length scales. A field emission scanning electron microscope (FE-SEM) (JEOL JSM-7401F) was used to characterize the structure and size of the inverse opal film.

3.3.2 Particle Suspensions

Fluorescently labeled nanoparticles were purchased from Life Technologies (dark red, F-8789). The particles were dispersed in a mixture of glycerol and sodium chloride solution, with different salt concentrations (0.001 M, 0.01 M and 0.1 M), where the weight fraction of glycerol was 90%. The hydrodynamic radius of the nanoparticle was 23 nm, determined by single-particle tracking observations of thousands of fluorescently labeled nanoparticle Brownian motion in solution. Fluorescent particles were introduced into the inverse opal structure via passive diffusion. The refractive index of 90 wt% glycerol/sodium chloride solution was 1.46, very close to refractive index of silica (n=1.45), this index matching solution eliminated light scattering from the internal and external liquid-solid interfaces.

3.3.3 Imaging Nanoparticle Diffusion

A Nikon Ti-Eclipse microscope with 100 X oil immersion objective (NA = 1.45) was used for single-particle tracking experiments. A 647 nm diode laser was used as an excitation source, incident at angles of 60—65° to decrease out-of-focus light. Emission was collected by the objective and passed through a sequence of a dichroic mirror, a bandpass filter, a long pass filter, and a SPINDLETM module including a phase mask (Double Helix LLC, Boulder) to transform the standard point spread function to double helix point spread function.

Approximately 50 movies per sample (3 minutes each) were captured with 50 ms acquisition time, at different regions within the inverse opal samples. DH-TRAXTM software was used to identify the positions of nanoparticles, with approximate precisions of 10 nm laterally and 20 nm axially. The x and y

positions of the tracer particles were determined from the midpoint of the positions of the two lobes, and the z position was determined from the angle of the two lobes by using a calibration plot that mapped angles to z positions. The calibration of the z positions was described in the supporting information.

3.4 Results and Discussion

3.4.1 Tracking Particles in the Porous Matrix

The silica inverse opal films were prepared via evaporative deposition of monodisperse polystyrene spheres suspended in a hydrolyzed silicate sol-gel precursor solution ⁴⁴. The resulting silica inverse opal structure exhibited a periodic arrangement of cavities and holes over macroscopic length scales (Figures 3-1a and 1b), with a cavity radius $R = 0.25 \mu m$ and an adjustable hole diameter L that was controlled by the concentration of the silicate precursor solution. The hole diameter L for a given inverse opal sample, was determined by analyzing scanning electron microscope images. The measurement details are described in the supporting information. The tracer particles used in this study were carboxylate-modified polystyrene nanoparticles (hydrodynamic radius of 23 nm), they were dispersed into a water/glycerol mixture (1:9 by weight) at concentrations in the range of $10^{-13} - 10^{-12} M$. Nanoparticles diffused passively and spontaneously into the interior void space. To study the effects of electrostatic interactions, we changed the concentration of NaCl in the solution; 0.001M, 0.01M and 0.1M concentrations were employed.

To directly and quantitatively characterize the three-dimensional escape process, we employed a 3D single-molecule imaging approach that combined variable-angle illumination epifluorescence microscopy with double-helix point spread function (DH-PSF) optics (SPINDLETM module, Double Helix LLC, Boulder) with a high-efficiency phase mask (Figure 3-1c) to record 3D spatiotemporal sequences of single-particle positions ⁴⁵⁻⁴⁷. The fully 3D tracking capability allowed us to correctly identify all the escape events, where 2D projections of 3D trajectories generally fail to identify some cavity translocation events.



Figure 3-1. Schematic diagram of experimental setup of 3D particle tracking of nanoparticles in inverse opals. (a) Representative scanning electron microscopy image of an inverse opal film, the scale bar represents 500 nm. (b) Representative scanning electron microscopy image of an inverse opal film, the scale bar represents 1 μ m. (c) A variable angle wide-field microscope body was equipped with a phase mask that enabled DH-PSF imaging.

We quantified the confined diffusion by calculating the ensemble-average mean square displacement (MSD) via $\Delta r(\tau)^2 = \langle |r(t + \tau) - r(t)|^2 \rangle$, where t denotes elapsed time within a trajectory, τ denotes the lag time, and r is the 3D position. Figure 2a shows plots of MSD vs τ for confined nanoparticle diffusion at different salt concentrations, as well as for nanoparticle Brownian motion in free solution. Compared to the Brownian motion in solution, in which the MSD grew linearly with τ , the confined diffusion in the inverse opal structure was slower and exhibited a more complicated three-regime MSD behavior associated with transient cage dynamics ⁴⁸⁻⁴⁹. The caging effects became more apparent under lower salt conditions, where the effects of electrostatic repulsion were amplified. For example, at a salt concentration of 0.001 M, the MSD plot exhibited a clear plateau, indicative of strong confinement prior to escape.



Figure 3-2. Hindered cavity escape of nanoparticles from inverse opals under aqueous condition. (a) Mean square displacement vs. lag time for free diffusion and confined diffusion at different salt concentrations. The dashed line in the figure indicates the length scale associated with confinement caused by the cavity wall. (b) Representative velocity autocorrelation function for free and confined diffusion. (c) Representative 3D trajectory for confined nanoparticle diffusion in an inverse opal film, where the color indicates the progression of time within the trajectory. (d) Cartesian coordinates of the trajectory from figure (c) as a function of time. The vertical lines indicate escape events.

For short lag times, the MSD grew linearly with τ , with an effective confined diffusion coefficient $D_{confined} = MSD/6\tau = 0.04 \ \mu m^2/s$, which was slightly smaller than that in free solution, which was 0.05 $\ \mu m^2/s$. In this regime, nanoparticles were still exploring the cavity, and had not yet felt the confinement induced by the cavity wall. Note that this diffusion coefficient calculation for the confined MSD data slightly underestimated the value of $D_{confined}$ due to the gradual leveling-off of the MSD that occurred as τ increased. In this short time-lag regime, most steps corresponded to free diffusion within the interior of a cavity, and

the effects of confinement by cavity walls were not apparent. Consistent with this interpretation, the apparent diffusion coefficients in this regime were nearly independent of salt concentration.

At intermediate lag times, the MSD plot curved downward, even exhibiting a distinct plateau in the lowest salt condition due to transient trapping within cavities for stochastic sojourn times. We calculated the velocity autocorrelation function and found evidence for temporal anticorrelation for lag times in the range 0.1 - 0.3s (Figure 3-2b), consistent with cavity confinement due to particle-wall interactions⁵⁰. In this confined regime, MSD curves were salt concentration dependent, suggesting that electrostatic interactions played a role in controlling nanoparticle escape from the cavity. At long lag times, nanoparticles escaped a given cavity and hopped amongst different cavities, giving rise to an approximately diffusive behavior, consistent with the cage dynamics in a transient network⁵¹⁻⁵². Therefore, the long-diffusion coefficient can hypothetically be related to the dimensions of the confined geometry and the characteristic escape time.

Qualitatively similar caging and jump behaviors (Figure 3-2c) are observed in other confined environments, including mesh-like materials, such as those formed by an entangled F-actin network⁵³⁻⁵⁵. Interestingly, although these two diverse systems exhibit similar caging and escape behaviors in their spatiotemporal trajectories, the mean squared displacement plots are substantially different. In contrast with the three-regime MSD shown in Figure 3-2a, the MSD in an entangled F-actin network exhibits power law dependent anomalous subdiffusion, presumably due to the convolution of the particle's Brownian motion and fluctuations of individual actin filaments. In contrast, the confined space within the inverse opal is static.

3.4.2 Electrostatic Interactions and Sojourn Times

The key measurement required to characterize the narrow escape problem is the sojourn time, i.e. the first passage time through a given cavity. To determine these time intervals, we applied a criterion to identify the escape from one cavity to another by monitoring the positional fluctuations in all three dimensions using a maximum allowed displacement algorithm (Figure 3-2d). Specifically, when a

nanoparticle was confined in a given cavity, the x, y and z positions of the particle fluctuated around a central point with a particular characteristic distance (i.e. the position fluctuated about the center of a cavity with the cavity radius as the relevant distance). Therefore, when the radial position exceeded that threshold, it was identified as a successful escape to an adjacent cavity. The details regard to identify escape events are described in the supporting information. Figure 3-2d shows the results of this method to identify escape events and extract corresponding sojourn times. By extracting hundreds of sojourn times for each experimental condition, we accumulated complementary cumulative distributions of sojourn times in inverse opals at varying salt concentrations, as shown in Figure 3a for holes 149 nm in diameter. The distribution of sojourn times was satisfactorily described by a double exponential decay function $P(t)=A*exp(-t/T_1)+(1-A)*exp(-t/T_2)$, permitting the calculation of the mean sojourn time, $T_{soj} = A*T_1+(1-t)T_2$ A)*T₂. Interestingly, we found that mean sojourn time, decreased dramatically with increasing salt concentration. For example, for holes 149 nm in diameter, cavity 500 nm in diameter, as the salt concentration was increased from 0.001 M to 0.1 M, the mean sojourn time decreased more than three-fold, from 9.49 s to 2.60s, while the Debye length changed by only 9 nm. This scenario suggested that electrostatic repulsion between the diffusing nanoparticle and confined wall surface dramatically tightened the effective escape pathway, decreasing the probability of escape and extending the mean sojourn time. Experiments using cavities with different hole sizes resulted in similar findings, confirming this conclusion (Figure B-8).



Figure 3-3. Electrostatic interactions on sojourn time distribution and diffusion coefficients of nanoparticle in porous media. (a) Complementary cumulative distributions of sojourn times at different salt concentrations, for holes 149 nm in diameter connecting cavities 500 nm in diameter. (b) Normalized long-time diffusion coefficient of nanoparticle in inverse opal films with different hole sizes versus the salt concentration. Error bars are smaller than the symbols.

The long-time diffusion coefficient of tracer particle (normalized by the diffusion coefficient in solution) is shown in Figure 3b as a function of salt concentration and hole size. Clearly, the long-time diffusion coefficient was much slower than the bulk diffusion coefficient D_0 , and exhibited a strong dependence on the salt concentration. For example, for 149 nm holes, the normalized long-time diffusion coefficient increased from 0.04 to 0.17 when increasing the salt concentration from 0.001 M to 0.1 M. This change was directly related to the sojourn time of confined tracer particles, suggesting that electrostatic interactions directly influenced the narrow escape process.

Einstein first derived a predicated the relation between the long-time diffusion coefficient and successive random displacements of magnitude⁵⁶; extrapolated into three dimensions, his theory predicts $D = \lambda^2/6T$. Equating λ to the cavity radius and substituting T by T_{soj} , the predicted diffusion coefficients were in good agreement with the experimentally measured diffusion coefficients. In the plot of measured long-time diffusion coefficient calculated from MSD data versus $R^2/6T_{soj}$ in different hole sizes and salt concentrations (Figure B-9), all of the data, collapsed on a linear trend-line, indicating that the phenomena associated with confinement and escape could be directly related to the macroscopic random motion in the long-time regime.

From the perspective of short time diffusion, the time t_{α} required for a confined particle to explore the boundary of a well-defined cavity can be estimated as $t_{\alpha} = R^2/6D_{confined}=0.26$ s, this is consistent with the time required for the MSD plot to transition from the initial linear region to the "plateau" region, where the effects of confinement are manifested. Importantly, t_{α} was generally an order of magnitude smaller than T_{soj} , again suggesting that the escape from the bounded spherical domain as barrier-limited instead of diffusion-limited. The barrier to escape comprises a combination of the entropic barrier at the hole exit and the energy barrier induced by electrostatic repulsion^{27, 57}. In particular, we hypothesize that the electrostatic repulsion between the nanoparticle and the cavity wall, which not only repelled the nanoparticle from the edge of the cavity, but also potentially narrowed the effective hole and inhibiting translocation. The distribution of radial nanoparticle positions within a cavity, as a function of salt concentration, is consistent with this hypothesis. For example, a nanoparticle was significantly more likely to approach the cavity wall closely at higher salt concentrations (0.1 M compared to 0.001 M) potentially increasing the opportunity for escape (Figure B-10).

3.4.3 Energy Profile at the Hole Exit

To quantitatively understand the effects of electrostatic repulsion, we calculated the potential energy barrier in the vicinity of the hole using a model for the interaction between a charged particle and a

hole in a like-charged membrane surface. Specifically, the potential energy experienced by the nanoparticle near a hole was evaluated using an approach described by Bowen et al ⁵⁸⁻⁵⁹. This model divides the hole area into three regions; in region I, the particle predominantly interacts with a single flat plate adjacent to the hole; in region II, the particle interacts with the entrance of the hole, and in region III, the particle primarily interacts with the cylindrical inner surface of the hole (Figure B-4a). Here, we focus on the area of the hole entrance, which is the connecting zone between region II and region III. The interaction energy in this connecting zone is assumed to be proportional to the weighted average of the interaction energy due to the fraction of particle surface in region II and in region III.



Figure 3-4. Theoretical calculations of electrostatic interactions in hole exits. (a) Schematic of a model of the interaction of a charged particle and a pore in a like-charged membrane surface. (b) Dimensionless potential energy when the nanoparticle is located in the entrance plane of a hole, for holes 149 nm in diameter connecting cavities 500 nm in diameter. The horizontal axis represents the lateral position of the center of the nanoparticle. The black dashed line indicates the value (potential energy) / $k_BT=1$.

According to DLVO theory, there are two distinct types of interactions between a charged particle and a charged surface: (1) Electrostatic repulsion caused by the overlapping of electrical double layers of the particle surface and hole surface; (2) attractive van der Waals interactions. Therefore, the explicit formula for the interaction energy of two curved charged surfaces in terms of the closest distance, h_0 , between them, is

$$\begin{split} U(h_0) &= U_e(h_0) + U_m(h_0) \\ &= \frac{\pi \epsilon_0 \epsilon_r}{g} \Big(\big(\psi_p + \psi_m \big)^2 \ln(1 + \exp(-\kappa h_0)) + \big(\psi_p - \psi_m \big)^2 \ln(1 - \exp(-\kappa h_0)) \Big) - \frac{A}{6gh_0} \end{split}$$

Where κ is the inverse Debye length, ϵ_0 is the permittivity of the vacuum, ϵ_r is the relative dielectric constant of the medium, ψ_p is nanoparticle surface potential, ψ_m is the inverse opal surface potential, A is the Hamaker constant, h_0 is the closest distance between wall and nanoparticle, and g is a steric factor determined by the geometrical characteristics of the system.

In region II, we assume a hole with rounded edges to avoid mathematical discontinuities at the hole entrance. Therefore, the energy in region II was evaluated as the sum of the energy between the particle and the two rounded surfaces that form the hole entrance, with the steric factor $g = \sqrt{\frac{1}{a}(\frac{1}{a} + \frac{1}{b})}$, where a is the hydrodynamic radius of the tracer particle, and b is the radius of the rounded edge at the hole entrance. To estimate the b value, we used the standard deviation of the hole size measurements from scanning electron microscopy, which was about 5% of the measured hole radius. The final results of the calculation were relatively insensitive to the choice of b value. For example, in the case of a 149 nm hole and 0.001 M salt solution, changing the b value from 2% to 8% of the measured hole radius, changed the effective hole size from 100 nm to 99 nm (Figure S6b). In these calculations, we assumed that the nanoparticle and inverse opal surfaces had equal values of surface potential. A sensitivity analysis was performed to understand the influence of this assumption. In particular, varying the surface potential of the inverse opal surfaces in range 0.8 $\psi_p - 1.2 \psi_p$ resulted in a minimal change of the effective pore size (only a few nm), which had a

negligible influence on the trends observed or conclusions drawn (Figure B-11). The calculation details are described in the supporting information.

The dimensionless potential energy (normalized by the thermal energy k_BT) for a 46 nm nanoparticle at the entrance of 149 nm hole is shown in Figures 3-4b and B-4. Notably, the potential energy near the walls increases dramatically when ionic strength is decreased. At high salt concentration, there is a deep potential valley across nearly the entire the hole area. However, at low salt concentration, the potential valley narrows significantly (Figure B-4), essentially narrowing the effective size of the hole available for nanoparticle escape. Specifically, if one considers the diameter of the effective hole to be the region where the potential energy is less than $k_B T$ (as a rough criterion for particle escape over an energy barrier via Brownian motion), decrease in salt concentration from 0.1 M to 0.001 M narrows the effective hole size by 47 nm. Varying the criterion from $0.8k_BT$ to $1.2k_BT$ changed the effective pore size only a few nm, which had a negligible influence on the trends observed and main conclusions of this work (Figure B-12). Note that the abscissa of Figure B-4b represents the center-of-mass position of the nanoparticle, thus the effective hole size equals the size associated with the potential well plus the hydrodynamic diameter of the nanoparticle. This dramatic decrease of the effective hole size explains why a small change in salt concentration had such a significant effect on the mean sojourn time. A back-of-the envelope calculation might lead to an intuitive guess that the effective hole diameter could decrease by roughly four Debye lengths, since both the nanoparticle and the hole have double layers on all sides. However, this estimate is overly simplistic, and the actual narrowing will generally depend on the zeta potential as well as the Debye length. For example, given the parameters of our experiments, decrease in salt concentration from 0.1 M to 0.001 M, which increased the Debye length by ~ 9 nm, resulted in an apparent reduction in the effective hole size by more than five Debye lengths.

3.4.4 Comparison between Experiments and Simulations

To better understand the mechanism behind the nanoparticle translocation process, we modeled the narrow escape process using kinetic random walk simulations. To mimic the process of a finite-size nanoparticle escape from an inverse opal cavity, we created a spherical cavity structure with twelve holes on the surface in a three-dimensional Cartesian coordinate system, the walker was initially placed at the origin at the start time t = 0. The simulation time step was dt = 0.05 s, each step was drawn from a Gaussian distribution. In addition, we used the short-time diffusion coefficient obtained empirically from experiments to include hydrodynamic effects in the confined environment. The simulation details are described in the supporting information. As expected, the simulated mean sojourn times increased significantly as the hole size decreased. Interestingly, the mean sojourn time for the higher-salt condition was in good agreement with the simulation results, suggesting that the escape process was adequately described by size-exclusion effects alone when electrostatic repulsion was effectively screened (Figure B-5a). However, under lowersalt condition, the escape process was significantly slower (by as much as a factor of three) than expected for diffusion-limited narrow escape. Specifically, for the 149 nm hole size inverse opal films, the mean sojourn time for [NaCl]=0.001 M was 9.4 s, compared to a simulated prediction of < 3 s. According to the Brownian random walk simulation, without electrostatic repulsion, a 9.4 s mean sojourn time would correspond to an effective hole size of only 101 nm. Interestingly, when including the electrostatic energy barrier effect described above, the calculated effective size for these conditions was 99 nm, consistent with the random walk simulation. Figure B-5b re-plots the mean sojourn time data using effective hole sizes calculated using the narrowing calculated from electrostatic repulsion calculations, and these data fall very close to the predictions from Brownian random walk simulations. The calculation details are shown in Table B-1 in the supporting information. This suggests that the effects of surface charge on cavity escape can be largely accounted for simply by calculating the effective hole size associated with electrostatic energy barriers.



Figure 3-5. Comparison of experimental results and simulations of nanoparticles cavity escape from inverse opals in aqueous solvent. (a) Mean sojourn time vs. actual hole size determined from SEM measurements. (b) Mean sojourn time vs. effective hole size calculated based on an electrostatic energy barrier model at the hole entrance.

Combined, these findings demonstrate that electrostatic repulsion between a nanoparticle and the surface of a bounded domain extended the sojourn time significantly by creating an energy barrier at the exit hole area, which can be quantitatively predicted by DLVO theory.

3.5 Conclusions

Previous work has suggested that nanoparticle diffusion under confinement may be influenced by multiple effects, e.g., hydrodynamics, adsorbate-surface interaction, entropic barriers and electrostatics. The results presented here provide a comprehensive picture of how and why electrostatic interactions influence nanoparticle confined nanoparticle diffusion and the related escape problem. In particular, we found a clear and dramatic trend in the mean sojourn time, where particles escaped slowly in the presence of electrostatic repulsion. A detailed analysis of the potential energy near the hole showed that this trend was due to electrostatic repulsion created a high energy barrier at the entrance of the hole, which narrowed the effective hole size significantly and caused slow escape under low salt conditions. Interestingly, under low salt conditions, this system represents a physical realization of the scenario where a mean first passage time (MFPT) calculation is not sufficient to fully explain the escape problem, since the mean sojourn time may be orders of magnitude larger than the MFPT calculated solely based on diffusion-limited transport¹⁹. To theoretically study such a barrier-limited escape problem in a porous medium, or similarly, a first-reaction time problem in a setting involving very low concentrations of reactive species, the mean first passage time is insufficient, it is necessary to obtain the full time distribution of passage times due to the strong defocusing of the relevant time scales^{57, 60-61}.

Confined diffusion of nanoparticles or macromolecules in the presence of electrostatic interactions has important implications in separations and biological processes. This work shows that pore accessibility can be easily and quantitatively controlled by tuning electrostatic interactions. These effects are likely to be quite general; indeed, a recent experimental study in our group¹⁹ found strong barriers to cavity escape even in a non-aqueous system. Together with the empirically observed (but poorly understood) electrostatic interactions in nonpolar solvent with surfactant⁴⁰⁻⁴², the results presented here provide a potential explanation for these observations, and suggest that electrostatic barriers to cavity escape, and the associate reduction in mobility and pore-space accessibility, may extend to a wide range of liquid-filled porous materials.

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Chapter 4 Connecting Hindered Transport in Porous Media Across Length Scales: From Single-Pore to Macroscopic



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4.1 Abstract

Hindered mass transport is widely observed in various porous media, however, there is no universal model capable of predicting transport in porous media due to the heterogeneity of porous structures and the complexity of the underlying microscopic mechanisms. Here, we used a highly ordered porous medium as a model system to directly explore the effects of geometric parameters (i.e. pore size, pore throat size and tracer particle size) and microscopic interaction parameters (e.g. controlled by ionic strength) on nanoparticle transport in porous environments using single-particle tracking. We found a linear scaling relation between the macroscopic diffusion coefficient and microscopic diffusion behavior involving a combination of parameters associated with microscopic features and phenomena, including both geometric effects and particle-level interactions. The proportionality coefficient relating micro and macro behaviors was complex and related to the connectivity of the matrix and pore-size variation, which could lead to tortuous diffusion pathways, hindering macroscopic transport.

4.2 Introduction

Porous media are used extensively in various industrial situations, like filtration membranes used in water treatment^{1, 2}, food/beverage applications,^{3, 4} pharmaceutical purification,^{5, 6} and controlled release for drug delivery.^{7, 8} Moreover, many geological and biological process involve mass in porous networks.^{9, ¹⁰ Therefore, significant efforts have been devoted to study the transport of nanoparticles, macromolecules and bacteria in porous media, leading to observations of various complex and obscure phenomena, including anomalous diffusion, hindered transport, and limited pore accessibility.¹¹⁻¹⁹ However, there is no universal model capable of predicting transport in porous media due to the heterogeneity of porous structures and the fact that the observed macroscopic behaviors are influenced by various microscopic effects, like particle-wall interactions,²⁰ hydrodynamic effects^{21, 22} and electrostatic interactions.²³⁻²⁵}

In an effort to understand connections between microscopic mechanisms and macroscopic behavior, we have employed highly-ordered silica inverse opal films as a model system, and directly visualized nanoparticle transport within porous materials using three-dimensional (3D) single-particle tracking methods by matching the refractive index of the solvent with that of the porous matrix. 3D tracking methods were enabled by a SPINDLETM module (Double Helix LLC, Boulder), which transformed the standard Airy-disk point spread function into a double helix point spread function.²⁶⁻²⁸ The results provided a comprehensive picture of hindered nanoparticle transport in this class of porous material from single-pore to macroscopic scales. In particular, the highly-ordered pore structure significantly reduced the complexity of studying and interpreting the transport and made it possible to quantify the effects of geometric parameters and microscopic interactions on nanoparticle motion. Importantly, 3D tracking was necessary to develop a quantitative understanding of this problem since 2D projections of 3D trajectories failed to comprehensively identify translocation events that involve transitions between cavities whose 2D projections significantly overlap (Figure 4-1a).

4.3 Methods

4.3.1 Preparation of Silica Inverse Opals

Silica inverse opals were prepared via evaporative deposition of monodispersed polystyrene beads suspended within a hydrolyzed silicate sol-gel precursor solution. In particular, 0.25 mL of 5 vol% monodispersed polystyrene suspension (Sigma-Aldrich) was added to 9.75 mL Milli-Q water, and 20 - 150 µL of hydrolyzed TEOS solution. The cavity size was defined by the size of colloidal polystyrene beads, while the hole size was determined by the concentration of the TEOS solution. A standard TEOS solution consisted of tetraethoxysilane (98% Aldrich), 0.10 M hydrogen chloride (Fisher Chemical), and anhydrous ethanol (Decon Labs), with a volume ratio of 1:1:1.5. The standard TEOS solution was stirred for 1h at room temperature prior to use. A 25 mm diameter microscope glass slide was used as a substrate. This glass slide was cleaned in piranha solution before use, then vertically suspended in a beaker containing the polystyrene beads and standard TEOS solution. The solution was evaporated in a 65°C oven for 2 days, to deposit a thin film of opal structure onto the glass slide. The resulting opal film was then pyrolyzed in a muffle furnace at 500 °C for 2 hours, to remove the template polystyrene beads and form a high-quality silica inverse opal film.

4.3.2 Imaging Nanoparticle Diffusion in Inverse Opals

A Nikon total internal reflection fluorescence microscope was used for single-particle tracking experiments. Three-dimensional tracking was enabled by a SPINDLETM module including a phase mask and a filter (Double Helix LLC, Boulder), which transformed the standard point spread function to a double helix point spread function, where the position in the axial (z) direction was encoded into the shape of double helix point spread function. The experimental setup is shown in Figure S8. Approximately 50 movies per sample were captured with 50 ms acquisition time at different spatial locations within the silica

inverse opal samples. DH-TRAXTM tracking software was used to identify the particle position, with precisions of 10 nm laterally and 20 nm axially.

4.4 Results and Discussion

The parameters used to characterize the diffusive transport of nanoparticles or macromolecules in porous materials can be summarized into two categories; (i) geometric parameters (pore size, pore throat size and tracer particle size), and (ii) microscopic interaction parameters (solvent quality, ionic strength). Here, we systematically varied these parameters, and investigated their effects on microscopic and macroscopic transport. In particular, silica inverse opal films were prepared by evaporative deposition of a crystalline layer monodisperse polystyrene beads, which served as the removable template for a silica solgel reaction.²⁹ The resulting structure contained a hexagonally close-packed interconnected network of cavities, where each cavity had 12 holes connecting to adjacent cavities (Figure 4-1b). In this system, cavities served as pores and holes served as pore throats, cavity size was controlled by the diameter of the template polystyrene beads, and hole size was controlled by varying the concentration of the silicate precursor solution. We used 0.02 µm and 0.04 µm diameter carboxylate-modified polystyrene beads as tracer particles; they were dispersed into solvent at concentrations in the range of $10^{-13} - 10^{-12}$ M, which permitted single particle localization. The solvent systems were designed to achieve refractive-index matching with the silica matrix;^{18, 25} both nonaqueous and aqueous solvent systems were investigated. In particular, a mixture of thiodiethanol and Triton X-100 (volume ratio 5:1) was used as a nonaqueous solvent, and a mixture of water and glycerol (mass ratio 1:9) was used as an aqueous solvent. 0.001 M, 0.01 M and 0.1 M concentrations of NaCl were added into the aqueous solvent to study the effects of electrostatic interactions on particle transport.

We quantified the macroscopic particle transport process in a traditional way by calculating the ensemble-average mean squared displacement (MSD), which reflects a classic three-regime cage dynamics

(Figures 4-1b, c).^{30, 31} In the short lag time regime, particles were still exploring the cavity and had not been influenced by the geometric confinement induced by the cavity wall, so the MSD grew linearly with lag time, with a reduced diffusion coefficient compared to the particle in unconfined liquid. This reduction of the short-time diffusion coefficient was due to hydrodynamic effects in confined environments, which is more apparent in less viscous liquid. For example, the reduction in nonaqueous solvent (mixture of thiodiethanol and Triton X-100) is much more significant than that in aqueous solvent (glycerol/water mixture), since the viscosity of nonaqueous solvent is about four-fold smaller than the aqueous solvent (Figures 4-2a, b). In the intermediate lag time regime, as particles began to feel the confinement caused by the cavity wall, the MSD plot curved downward, even exhibiting a plateau in some cases. In the long lag time regime, particles escaped the confined cavity and hopped between different cavities, giving rise to an effective diffusive behavior for long times and large length scales. As another visualization of these three transport regimes, we calculated the lag time-dependent diffusion coefficient $\tilde{D}(\tau)$ based on the relation $\langle r^2 \rangle = 6D\tau^{\alpha} = 6(D\tau^{\alpha-1})\tau = 6\widetilde{D}(\tau)\tau$, where τ is the lag time, and α is the anomalous diffusion exponent.³² For Fickian diffusion, $\alpha = 1$ and $\widetilde{D}(\tau)$ is constant for all lag times. Figure 1c shows representative changes in $\widetilde{D}(\tau)$ as a function of lag time. $\widetilde{D}(\tau)$ was almost constant for short lag times, reflecting random Brownian motion in the cavity, consistent with the linear increase of the MSD. $\tilde{D}(\tau)$ decreased gradually at intermediate lag times, indicating that the confined particles bounced back and forth, reflected by cavity walls. These bouncing events decrease the magnitude of displacements, leading to a reduced diffusion coefficient, consistent with the curved downward part in the MSD plot. The transition of $\widetilde{D}(\tau)$ from the initial time-independent to the intermediate time-dependent regime corresponds conceptually to the transition from the initial Fickian diffusion in the cavity interior to the subdiffusion associated with confinement by cavity walls. After a gradual decrease at intermediate times, $\widetilde{D}(\tau)$ saturated at a constant value for long lag times, reflecting a well-defined long-time diffusion coefficient. In this long-time regime, the particle motion is dominated by hops between adjacent cavities through connecting holes, and the particle motion approximates macroscopic random diffusion with a constant value of D_{long}.
The transition of $\tilde{D}(\tau)$ from the intermediate time-dependent to the final time-independent regime corresponds conceptually to a mean escape time for a particle from the initial confined cavity to an adjacent cavity, also known as the mean sojourn time. A variety of theoretical frameworks have been developed to evaluate the mean sojourn time in various physical scenarios.³³⁻³⁹ With the use of 3D single-particle tracking, we were able to accurately identify escape events and quantitatively extract each sojourn time. In particular, we identified an escape event by monitoring the positional fluctuations in all three dimensions using a maximum allowed displacement algorithm (Figure 4-1d). The mean sojourn times were then extracted from the distributions of sojourn times (Figure C-9).



Figure 4-1. Representative diffusive particle trajectory in inverse oapls. (a) Representative diffusive particle trajectory in an inverse opal film. (b) Representative mean squared displacement plot in unconfined liquid or inverse opal films, under nonaqueous solvent conditions. Inset: representative SEM image of an inverse opal structure. The scale bar represents 1 μ m. (c) Representative plot of mean squared displacement and time-dependent diffusion coefficient versus lag time. (d) Cartesian coordinates of the trajectory from (a) as a function of time. The vertical dashed black lines represent escape events.

Geometric conditions modified the three-regime cage dynamics by controlling the time scales associated with the intermediate lag time regime. In particular, increasing the cavity size increased the time required to reach the intermediate lag time regime. Also, the MSD plot exhibits stronger confinement effects in the intermediate regime with decreasing hole size or increasing tracer particle size (Figure 4-2a). Moreover, solution conditions influenced the cage dynamics significantly as well. In the aqueous solvent, both the tracer particle and cavity wall were negatively charged; therefore, electrostatic repulsion increased with decreasing salt concentration. In fact, at low salt concentration of 0.001 M, the MSD plot even

exhibited an apparent "plateau" in the intermediate regime (Figure 4-2b), indicating strong confinement. Moreover, as reported previously, electrostatic interactions played an important in controlling the nanoparticle cavity escape process. In particular, an increase in the Debye length of only a few nm (compared to a hole diameter of ~100 nm) increased the mean sojourn time three-fold, and this hindered cavity escape was due to an electrostatic energy barrier in the region of the hole.²⁵



Figure 4-2. Mean squared displacement as a function of lag times of diffusive nanoparticle in inverse opals. (a) Mean squared displacement as a function of lag time for various geometric conditions for a nonaqueous solvent system. (b) Mean squared displacement as a function of lag time at different salt concentrations for an aqueous solvent system.

Interestingly, we found that all these effects associated with the variation of geometric and solution conditions could be embedded into a single parameter, R^2/T_{soj} , where R is the cavity radius, and T_{soj} is the

mean sojourn time for given experimental conditions. Figure 4-3a shows a plot of the long-time diffusion coefficient (D_{long}) versus R²/T_{soj} for various geometric and solution conditions, exhibiting a universal linear relationship. The details of the experimental parameters for each condition are shown in Table C-1 in the Supporting Information. Here, Dlong indicates the macroscopic diffusion coefficient at long lag times, and R^2/T_{soj} is related to parameters associated with diffusion at the single-pore scale. Therefore, the linear scaling relation connects macroscopic to microscopic diffusion behavior. In particular, this relationship indicates that the macroscopic diffusion coefficient is determined by the characteristic dimensions of the porous medium (i.e., cavity size, hole size and tracer particle size) and the characteristic sojourn time. For a porous medium with a well-defined mean cavity size (or even a Gaussian distribution of sizes), the longtime diffusion process can be simplistically considered as hopping in the porous network with characteristic hopping distance and waiting time. Specifically, the hopping distance is defined by the geometric cavity size, and the waiting times can be related to experimentally measured sojourn time distributions, where cavity, hole and particle sizes, as well as microscopic interactions (e.g., hydrodynamic and electrostatic interactions) are embedded in these distributions. In previous work, we found that mean sojourn times can be predicted by a combination of kinetic random walk simulations and theoretical calculations for various solvent conditions.²⁵ Electrostatic interactions influence sojourn times by creating an energy barrier at cavity exits, which can be modeled using an effective hole size approximation and predicted by DLVO theory. Therefore, the linear scaling relationship presented here has several practical implications. For example, the relationship could be used to design materials with particular transport properties. Viewed in another way, if macroscopic transport is measured, the relationship could be used as an analytical tool to infer microscopic properties, and vice versa. For example, Bhattacharjee and Datta used the same underlying relationship to predict the long-time diffusion coefficient of bacteria diffusion in porous media based on microscopic parameters (i.e. hopping distance and waiting times), and demonstrated that bacteria exhibit hopping and trapping, instead of run-and-tumble transport behaviors in porous media.¹⁷



Figure 4-3. Scale law between long-time diffusion coefficient and short-time cavity escape processes. (a) Long-time diffusion coefficient versus R^2/T_{soj} for various geometric and solution conditions. (b) Simulations and experimental observations of the long-time diffusion coefficient versus R^2/T_{soj} for various geometric and solution conditions.

To better understand the mechanism behind nanoparticle transport in theses porous media, we simulated the macroscopic transport process using kinetic random walk simulations. We computationally created a network mimicking the inverse opal connectivity with a given pore size. The random walker began at the origin, and moved to one of 12 adjacent pores (with equal probability) after a time drawn from a distribution obtained empirically from experiments. Further simulation details are described in the supporting information. Interestingly, we found that, like the experimental diffusion coefficients, the simulated long-time diffusion coefficients also scaled linearly with R^2/T_{soj} , but the proportionality constant

was significantly larger, resulting in faster simulated diffusion compared to the experimental observations (Figure 4-3b). Notably, while involving realistic parameters, such as a distribution of sojourn times, the results from our simulations agree with theoretical expectations for a simple three-dimensional (3D) random walk. In particular, taking the diameter of the cavity (2R) as the hopping length and the mean sojourn time (T_{soj}) as the waiting time, the expected theoretical proportionality constant between R^2/T_{soj} and D_{long} is 2/3, which is consistent with the proportionality constant generated by the simulated long-time diffusion coefficients in Figure 4-3b. To understand the discrepancy between experimental observations and simulations, we noted anecdotal observations suggesting that confined particles were more often more likely to translocate through certain connecting holes than others (Figure C-10). Based on this, we hypothesized that the discrepancy was due to reduced pore connectivity. In particular, in real experimental scenarios, a given cavity may have a distribution of hole sizes, and so a particle confined within that cavity may be more likely to translocate to certain neighboring cavities and unlikely to translocate to other cavities. This variability was not incorporated in the simulations, which would potentially lead to larger long-time diffusion coefficients than the experiments. In addition, the existence of fully blocked holes due to nanofabrication defects would further increase this effect (Figure C-1b).

To test this hypothesis, we performed two types of Monte Carlo simulations to investigate the (1) the effects of hole sizes on the nanoparticle's translocation route; (2) the effects of hole sizes and blocked holes on the long-time diffusion coefficient. In particular, we performed simulations of nanoparticle escape from an inverse opal cavity with holes of two different sizes. We found that the probability of translocating through large holes could be 2-3 fold greater than translocating through small holes when the size difference was 20 nm (Figure C-5b), which is the magnitude of hole size variation observed in our materials (Table C-1). Therefore, we concluded that hole size variation and blocked holes could significantly influence nanoparticle transport, creating inhibited pathways, resulting in tortuous pathways and decreasing overall cavity accessibility. To study the influence of porous network structure ("blocked" holes) on the long-time diffusion coefficient, we performed kinetic random walk simulations in a 2D lattice bond percolation model.

In this model, nodes represent the cavities, bonds represent the holes connecting cavities, and a point tracer executes a random walk on a 2D square lattice with fractional bond connectivity given by the parameter b. For example, b = 1 indicates a fully connected porous network (four connections per node) and b = 0.75indicates a partial blocked porous network (average three connections per node). For long simulations (2000 steps), we found that the diffusion coefficient by 10% when the fraction of bonds b was reduced from 1 to 0.9 and decreased by 50% when the fraction of bonds b was reduced from 1 to 0.7 (Figure C-7). The simulation details are described in the supporting information. Although this is a simplified simulation model compared to the actual experimental geometry, it suggests that inhibited pathways can significantly decrease the long-time diffusion coefficient. In addition, more sophisticated bond percolation simulations with various connection geometries also demonstrated that blocked pore connections and inhibited pathways led to significantly reduced long-time diffusion coefficients.⁴⁰⁻⁴³ However, to fully explain the reduced diffusion that was observed experimentally on the basis of blocked holes alone would require blockage of approximately 35% of the connecting holes in a cubic lattice model,⁴¹ which is not consistent with the very small fraction of completely blocked holes observed in SEM images. Considering all of these factors, we conclude that the combination of hole size variation and sporadically blocked holes caused by fabrication defects significantly influenced nanoparticle transport behavior inside the inverse opals. They were more likely to translocate through larger holes and occupy cavities with larger holes, leading to the reduced long-time diffusion coefficient.

4.5 Conclusions

The transport of particles and macromolecules in a porous medium can be influenced by three distinct factors: porosity, constrictivity, and tortuosity.⁴³ Porosity is defined as the volume fraction of void space in a porous material, and is a well-known parameter often used to characterize porous media. Constrictivity is a refinement of the porosity concept; because porous media usually have both large pores and much smaller pore constrictions, so translocation through pore constrictions (throats) is the rate-limiting

process. Constrictivity has been recognized in various scenarios, but rarely investigated quantitatively.⁴⁴⁻⁴⁷ The tortuosity of a pathway is generally defined as the ratio of contour length to the Euclidean end-to-end distance. In practice, when applied to actual nanoparticle trajectories, tortuosity is not a material property, but depends on operating parameters such as particle size and flow conditions.^{48, 49} In addition to porosity, constricitivity and tortuosity play important roles in determining particle transport in porous media; thus, knowing porosity alone is insufficient to predict the transport behavior. For example, one study showed that nanoparticles exhibited significantly different diffusion behaviors in two porous materials with same porosity and nominal pore sizes, but different topologies.⁴⁴

In this work, we employed 3D single-particle tracking to visualize particle transport in a highly ordered porous medium and quantified the constrictivity by the mean sojourn time. We found a linear scaling relation between macroscopic diffusion coefficient and microscopic diffusion behaviors (captured by R^2/T_{soi}), which included the overall effects of geometric confinement and microscopic interactions. This linear scaling relation connected the studies from single-pore to macroscopic scales, providing insights into interpreting macroscopic behaviors from microscopic perspective (and vice versa). Interestingly, we observed a discrepancy between experimentally observed long-time diffusion coefficients and Monte Carlo simulated diffusion coefficients under the assumption of fully connected uniform inverse opal structures. We found that this was due to the tortuous pathways traveled by particles in the real inverse opal structure, which was caused by the variation of hole size and sporadic blocked holes, which is inevitable in real-world materials. This emphasizes the important effects of tortuosity on transport in real-world porous media, where tortuous pathways can limit the macroscopic diffusion coefficient. These combined findings suggest that hole-size variability and blocked holes can have both local and global effects. Locally, the escape from a given cavity is inhibited, which is captured by the parameter T_{soi}. Globally, hole-size variability influences the network connectivity, which may in turn affect the proportionality coefficient between microscopic and macroscopic behaviors.

These observations were uniquely enabled by single-particle tracking methods, which directly visualized spatio-temporal particle trajectories in complex environments, providing microscopic insights into transport process in porous materials that can be challenging to understand from macroscopic measurements alone. In addition to the diffusion experiments presented here, particle tracking methods can be applied to study advection in porous media, and understand the microscopic mechanisms of membrane fouling⁵⁰ and particle remobilization under intermittent flow conditions.⁵¹

4.6 References

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Chapter 5 Mechanisms of Transport Enhancement for Self-Propelled Nanoswimmers in a Porous Matrix

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5.1 Abstract

Micro/nanoswimmers convert diverse energy sources into directional movement, demonstrating tremendous promise for biomedical and environmental applications, many of which involve complex tortuous or crowded environments. Here, we investigated the transport behavior of self-propelled catalytic Janus particles in a complex interconnected porous void-space, where the rate-determining step involves the escape from a cavity and translocation through holes to adjacent cavities. Surprisingly, self-propelled nanoswimmers escaped from cavities more than 20x faster than passive (Brownian) particles, despite the fact that the mobility of nanoswimmers was less than 2x greater than that of passive particles in unconfined bulk liquid. Combining experimental measurements, Monte Carlo simulations and theoretical calculations, we found that the escape of nanoswimmers was enhanced by nuanced secondary effects of self-propulsion which were amplified in confined environments. In particular, active escape was facilitated by anomalously rapid confined short-time mobility, highly efficient surface-mediated searching for holes, and the effective abolition of entropic and/or electrostatic barriers at the exit hole regions by propulsion forces. The latter mechanism converted the escape process from barrier-limited to search-limited. These findings provide general and important insights into micro/nanoswimmer mobility in complex environments.

5.2 Introduction

Micro- and nanoscale robots are capable of taking energy from their environment and converting it to directional motion. A variety of micro/nanoswimmers based on different actuation principles have been developed recently, including chemically powered colloidal particles ¹⁻², magnetic swimmers ³⁻⁴,

photocatalytic nanomotors ⁵⁻⁶ and acoustic microrods ⁷⁻⁸. They have attracted considerable interest because of their potential biomedical and environmental applications, such as biomedical diagnosis ⁹⁻¹⁰, drug delivery ¹¹⁻¹² and environmental remediation ¹³⁻¹⁴. However, these applied scenarios often require efficient motion of micro/nanoswimmers within complex tortuous, crowded and interconnected environments, like microfluidic devices and biological tissues, and the current understanding of nanoswimmer transport remains incomplete in this regard.

The transport of micro/nanoswimmers in confined environments is intrinsically complex due to the strong coupling of translational and orientational motion with interfaces, among other effects. Moreover, their motion is influenced by both thermal fluctuations and self-propulsion, and while the motion can be simulated computationally using ad hoc models, there is no comprehensive theoretical paradigm that captures the full range of out-of-equilibrium effects. A variety of experimental and theoretical frameworks have been employed to study micro/nanoswimmers motion in various scenarios ¹⁵⁻²³, leading to interesting discoveries about transport behaviors near interfaces. For example, Das et al. found that boundaries could be used to steer self-propelled Janus particles since they tend to move along solid/liquid interfaces ²¹. Similarly, Brown et al. found that self-propelled Janus particles orbit individual colloids and stochastically hop between neighboring colloids with a hopping rate inversely correlated with fuel concentration when swimming in a two-dimensional colloidal crystal ²².

While previous studies have provided important insights about the effects of interfaces on micro/nanoswimmer transport. The dynamics in complex interface-rich environments remain to be clarified. Since potential applications often involve transport in porous media where objects must search for and then translocate through narrow pores/openings, technological use of micro- and nanoscale robots will rely on an understanding of how self-propulsion influences transport in a three-dimensional porous medium, which in turn relies on elementary process involving narrow hole searching and translocation.

To address this question, we designed and employed an experimental system to quantitatively investigate nanoswimmers transport in porous media, and performed comprehensive computational simulations to isolate the effects of individual transport mechanisms. In particular, we used self-propelled Janus particles as model nanoswimmers and highly-ordered silica inverse opals as a model porous medium. We quantitatively investigated the cavity escape process (i.e., the translocation through bottleneck areas between cavities, which is the rate-determining process for transport in many porous environments ²⁴⁻³⁰) using 3D single-particle tracking. A successful cavity escape consists of two processes: (1) the search for an opening on the cavity and (2) the subsequent translocation through the opening. By directly comparing the motion of nanoswimmers and equivalent passive Brownian particles ³¹⁻³⁹, we found that nanoswimmers exhibited significantly (greater than 20x) accelerated cavity escape compared to passive Brownian particles, which was due to a combination of enhanced mobility, efficient searching for holes due to preferential motion along internal cavity surfaces, and self-propelled thrust-enhanced translocation across repulsive barriers to hole escape. In particular, enhanced mobility and surface-mediated searching facilitated the search step, while the additional force due to self-propulsion facilitated the translocation step, converting the cavity escape process from barrier-limited to search-limited.

5.3 Methods

5.3.1 Preparation of Silica Inverse Opals

Silica inverse opals were prepared by evaporative deposition of monodispersed polystyrene beads from a hydrolyzed silicate sol-gel precursor solution. Specifically, 0.25 mL of 5 vol% monodispersed colloidal polystyrene suspension (2 μ m in diameter) was added to 9.75 mL Milli-Q water, and 30 – 100 μ L of hydrolyzed TEOS solution. The cavity size was determined by the size of colloidal polystyrene beads, while the hole size was determined by the concentration of TEOS solution. A standard TEOS solution consisted of anhydrous ethanol, tetraethoxysilane, and 0.10 M hydrogen chloride, at a volume ratio of 1:5:1:1. The standard TEOS solution was stirred for 1h at room temperature prior to use. A 25 mm diameter microscope glass slide was used as a substrate, it was vertically suspended in a beaker containing the colloidal polystyrene beads and standard TEOS solution. The solution was evaporated in a 70°C oven for 2 days, to form a thin film of opal structure onto the glass slide. The resulting opal film was then pyrolyzed in a furnace at 500 °C for 2 hours, to remove the templating polystyrene particles and form a silica inverse opal film.

5.3.2 Imaging Nanoparticle Diffusion in Inverse Opals.

A Nikon N-STORM super resolution microscope equipped with a phase mask was used for singleparticle tracking experiments. Three-dimensional tracking was enabled by a SPINDLETM module including a phase mask and a filter (Double Helix Optics, Inc.), which transformed the standard point spread function to a double helix point spread function, where the position in the Z direction was encoded into the shape of double helix point spread function. Approximately 50 movies per sample were captured at different spatial locations within the silica inverse opal samples. DH-TRAXTM tracking software was used to identify the particle position, with precisions of 10 nm laterally and 20 nm axially.

5.4 Results and Discussion

5.4.1 Tracking Particles in the Porous Medium

As a porous medium, we employed a silica inverse opal film, prepared by evaporative deposition of monodispersed polystyrene colloidal particles from a hydrolyzed silicate sol-gel precursor solution 40 . The resulting structure exhibited a hexagonally close-packed interconnected network of spherical cavities, where each cavity had 12 holes connecting to neighboring cavities. The connecting holes allow the confined particle to hop between adjacent cavities and explore the porous structure. As tracer particles, we employed fluorescently labeled platinum-polystyrene Janus particles (hydrodynamic radius of 129 nm), which were dispersed into a glycerol/water mixture (1:1 by weight) at concentrations in the range $10^{-16} - 10^{-15}$ M. Tracer particles were prepared by dispersing a dilute suspension of carboxylate-modified fluorescent polystyrene

beads (Thermo Fisher Scientific) on a glass slide, sputter coating a thin layer of platinum, and subsequently liberating the particles from the glass slide by sonication into pure water. Hydrogen peroxide (3wt%) was used as chemical fuels to convert the passive Brownian particles into nanoswimmers. When a Janus platinum-polystyrene particle is immersed in an aqueous solution containing hydrogen peroxide, the platinum-coated hemisphere catalyzes the hydrogen peroxide decomposition reaction, while the polystyrene hemisphere remains inert. This asymmetric reaction surrounding the Janus particles generates a concentration gradient, leading to self-propulsion behaviors governed by self-diffusiophoresis¹.

We performed three-dimensional (3D) single-particle tracking to directly visualize tracer particle transport within the inverse opal void space ⁴¹. The refractive index of the liquid filling the medium was matched to that of the silica framework, and 3D single-particle tracking was enabled by double helix point spread function imaging using a SPINDLETM module (Double Helix Optics, Inc.), where the position in the axial direction was encoded into the shape of the engineered point spread function ⁴²⁻⁴³. Conventional 2D single-particle tracking approaches have been widely used to study the motion of micro/nanoswimmers^{19-22, 44-47}; however, 3D tracking is necessary to quantitatively study the cavity escape process. In particular, 3D tracking uniquely enables unambiguous identification of all escape events, since 2D projections of 3D trajectories often fail to identify events that involve transitions between cavities whose 2D projections have significant overlap (Figure 5-1). Figure 5-1C and 1E show representative particle trajectories of a passive Brownian particle and a nanoswimmer within inverse opals, respectively. Figure 5-1D and 1F show the corresponding particle coordinates as a function of time for these two types of particles. Qualitatively, passive particles were more likely to explore the full volume of a given cavity, while nanoswimmers tended to move along the internal cavity surface. This behavior is consistent with previous observations of micro/nanoswimmers in the vicinity of planar solid-liquid interfaces ^{16, 19, 21}.



Figure 5-1. Experimental schematic and representative trajectories. (A) A microscope was equipped with a phase mask and relay optics that enabled double-helix point spread function imaging. (B) Representative scanning electron microscope image of an inverse opal film structure, the scale bar represents 1 μ m. (C) A representative 3D trajectory of a passive Brownian particle in an inverse opal. (D) Corresponding particle coordinates of a passive Brownian particle in an inverse opal. The vertical dashed lines represent escape events. (E) A representative 3D trajectory of an active nanoswimmer in an inverse opal. (F) Corresponding particle coordinates of an active nanoswimmer in an inverse opal. (G) A representative 3D trajectory of a passive Brownian particle in an inverse opal. (G) A representative 3D trajectory of a passive Brownian particle in an inverse opal. (H) Corresponding particle coordinates of a passive Brownian particle in an inverse opal. (H) Corresponding particle coordinates of a passive Brownian particle in an inverse opal. (H) Corresponding particle coordinates of a passive Brownian particle in an inverse opal with added salt. (H) Corresponding particle coordinates of a passive Brownian particle in an inverse opal with added salt. Inverse opals used in these three representative trajectories have 2 μ m cavities with 498 nm connecting holes.

5.4.2 Nanoswimmers have Enhanced Mobility in Confinement

The mechanisms of transport at various time-scales can be visualized using the ensemble-averaged mean squared displacement (MSD) $\Delta r(\tau)^2 = \langle |r(t + \tau) - r(t)|^2 \rangle$, where *t* represents elapsed time within a trajectory, τ represents the lag time, and *r* denotes the 3D position. Figure 5-2A shows plots of MSD versus τ for platinum-polystyrene Janus particles in various solvents. In unconfined liquid, the passive particles exhibited an MSD that was linear in lag time (Fickian diffusion) with a diffusion coefficient of 0.28+/-0.01 μ m²/s, and the active nanoswimmers were only slightly superdiffusive, with an apparent diffusion coefficient of 0.47+/-0.03 μ m²/s, an approximate 70% enhancement. The uncertainty of the diffusion coefficient was estimated as the standard error from multiple independent experiments. While nanoswimmers hypothetically exhibit ballistic motion for short time scales, at times longer than the characteristic rotational diffusion time $\tau_R = \frac{k_B T}{8\pi \eta R^3} = 0.08 s$ (which is smaller than the shortest lag time measured), the propulsion direction was randomized, and an active nanoswimmer exhibited apparent Brownian motion with an enhanced apparent translational diffusion coefficient, D_{active} = D_{passive} + V² $\tau_R/6$ in three-dimensional space, where V is the propulsion velocity ¹⁶.

Compared to motion in the unconfined liquid, both passive Brownian particles and nanoswimmers exhibited slower movement in the porous medium, consistent with the various confinement effects caused by the porous environment. At short times, both passive and active particles in confinement exhibited approximately Fickian behavior, since particles were still exploring the cavity and had not yet experienced the confinement induced by the cavity wall. In this regime, passive particles exhibited an apparent diffusion coefficient $D_{passive} = MSD/(6 \tau) = 0.1 +/- 0.01 \mu m^2/s$, and nanoswimmers showed a faster apparent short-time diffusion coefficient, with $D_{active} = 0.3 +/- 0.02 \mu m^2/s$. In both cases, this short-time diffusion was inhibited compared to diffusion in unconfined liquid, presumably due to enhanced drag from hydrodynamic effects within cavities. Unexpectedly, this slowing was significantly greater for passive particles (whose diffusion coefficient was 3x smaller in confinement compared to unconfined) than for active nanoswimmers (whose apparent short-time diffusion was reduced by only 35% under confinement). As a result, the short-time diffusion was reduced by only 35% under confinement).

time diffusion for nanoswimmers, which was only \sim 70% greater than that of passive particles in unconfined liquid, was 3x greater under confinement. This anomalously high mobility within the geometrically confined void space may be due to enhancement of concentration gradients, which has the potential to intensify self-propulsion effects associated with self-diffusiophoresis. In particular, while conventional hydrodynamic effects associated with geometric confinement (e.g. enhanced drag near walls and recirculation of displaced fluid) are of course expected to inhibit mobility of all particles, the unique selfpropulsion mechanisms of nanoswimmers, which depend on concentration gradients, may be enhanced, counteracting the increased drag ¹⁵. This anomalously high confined mobility represents one contribution towards the enhanced cavity escape of nanoswimmers relative to Brownian particles.

Over longer time scales in inverse opals, passive Brownian particles exhibited the classic threeregime MSD behavior for "caged" motion. As described above, for lag times shorter than the time required to explore the cavity, the MSD grew linearly with lag time. At intermediate lag times, the MSD plot curved downward, even exhibiting a distinct plateau due to transient trapping within cavities. At long lag times, the MSD gradually increased again as passive Brownian particles escaped out of the confined cavity and hopped among neighboring cavities. This three-regime MSD behavior of passive Brownian particles was qualitatively similar to the caging and jump behavior observed for a particle confined in a porous medium ⁴⁸⁻⁴⁹. The MSD of nanoswimmers, on the other hand, gradually evolved from Fickian to modestly subdiffusive motion, but did not exhibit distinct caging behavior, qualitatively suggesting efficient searching for holes and rapid translocation.



Figure 5-2. Statistical analyses of experimental results of Janus platinum-polystyrene particle in various solvent conditions. (A) Mean square displacement of catalytical Janus particle under various conditions. A 50wt% glycerol/water mixture was used as a solvent for passive particles; passive experiments with salt employed 50wt% glycerol/water with 0.001M NaCl added. Nanoswimmer experiments employed a 50wt% glycerol/water mixture with 3wt% hydrogen peroxide. The porous media used in the experiments were inverse opals with 2 µm diameter cavities and 498 nm connecting holes. The tracer particle radius was 129 nm. (B) Complementary cumulative probability distribution of sojourn time under various solution conditions.

5.4.3 Accelerated Cavity Escape of Nanoswimmers

We recently demonstrated an explicit connection between macroscopic long-time diffusion in a porous medium and microscopic pore-level processes ³⁷. In particular, we found that the macroscopic

diffusion coefficient can be predicted with a knowledge of cavity escape kinetics and geometrical properties of the medium. The key parameter to characterize cavity escape is the sojourn time, also known as the first passage time through a given cavity. To determine these time intervals, we identified the escape from one cavity to another by monitoring the positional fluctuations in all three dimensions using a maximum allowed displacement algorithm ³⁶. Figure 5-1D and 1F show representative results employing this method. By extracting hundreds of sojourn times for each experimental condition, we calculated complementary cumulative distributions of sojourn times in inverse opals for both passive Brownian particles and active nanoswimmers (Figure 5-2B). We found that the mean sojourn time of a nanoswimmer was more than 20x shorter than that of corresponding passive Brownian particles (Figure 5-3A). For example, in an inverse opal film with 2 μ m in diameter cavities and 498 nm holes, the mean sojourn time decreased from 68.2 s to 2.8 s after adding chemical fuel to convert passive Brownian particles into nanoswimmers. This suggested that the active motion of nanoswimmers dramatically accelerated the cavity escape process, enhancing transport process in porous media. Moreover, at intermediate lag times, the MSD for nanoswimmers curved slightly downward but did not exhibit a distinct plateau like a passive Brownian particle. This suggested that nanoswimmers were weakly confined (i.e., their transport was only slightly influenced by the cavity escape process), which is consistent with their significantly reduced mean sojourn time.

While the three-fold enhancement in the short-time mobility contributes to faster searching for holes, it clearly cannot explain the >20x reduction of the mean sojourn time. Moreover, the short-time mobility is not always simply related to the mean sojourn time. Since the cavity escape process involves both search and escape steps, faster diffusion alone does not guarantee a decrease of the sojourn time if it is an escape-limited process. On the other hand, as illustrated by the trajectories in Figure 5-1C and 1E, passive Brownian particles and nanoswimmers exhibited qualitatively different search modes. In particular, passive Brownian particles executed 3D searches, typically avoiding the cavity walls, while nanoswimmers tended to move along the walls. The radial position probability distribution provided quantitative confirmation of this observation (Figure 5-3B). While the detailed mechanisms are not entirely understood,

for certain regimes of self-propelled speed and rotational diffusion coefficient, self-propelled particles have been observed to move along solid-liquid interfaces ^{16, 18-19, 21}. In the current experiments, this leads to a distinctive search process for holes, where nanoswimmers preferentially move along the internal cavity surface. Interestingly, it has been predicted theoretically that related surface-mediated motion can accelerate the search process in the so-called "narrow escape" problem ⁵⁰⁻⁵¹. For example, a surface-mediated diffusive search can accelerate the search process more than three-fold for a small hole in a circular 2D domain. Therefore, in addition to enhanced mobility, we hypothesized that efficient surface-mediated searching, enabled by a nanoswimmer's tendency to move along boundaries, could accelerate the cavity escape process.

5.4.4 Comparison between Experiments and Simulations

To fully understand this significant enhanced escape process of nanoswimmers, we performed kinetic Monte Carlo simulations for both passive Brownian particles and nanoswimmers. In these simulations, we created a spherical cavity structure with 12 holes on the surface in a three-dimensional Cartesian coordinate system; a particle was initially placed at the cavity center. In the passive Brownian particle scenario, the particle motion was modeled as Brownian motion with the short-time diffusion coefficient obtained from experiments (thereby incorporating hydrodynamic effects empirically). In the nanoswimmer scenario, the nanoswimmer motion was modeled as the combined action of random translational and rotational diffusion processes and directed self-propulsion ⁵². In particular, the self-propulsion effects were simulated as a motion with constant directional speed, where the direction depends on the particle orientation which undergoes rotational diffusion. The simulation details are described in the Supporting Information.

Nanoswimmer simulations show that self-propelled motion resulted in an enhanced probability of the particle being near the internal wall of the spherical cavity, consistent to the experimental observations. In contrast, both experimental and simulations of passive Brownian particles exhibited depletion near the cavity wall. For simulated particles, this depletion was presumably due to steric effects, with the length scale dominated by the particle size. However, the depletion region obtained from experimental observations of passive Brownian particles was significantly more extended, suggesting long-range repulsion from cavity walls (Figure 5-3B), consistent with previous findings ^{35-36, 41}. In particular, repulsion between cavity walls and passive Brownian particles can inhibit the cavity escape process and decrease the effective hole size, leading to a barrier-limited escape process. Importantly, we found that nanoswimmers simulations were in good quantitative agreement with experimental mean sojourn times, but that Brownian simulations underestimated the mean sojourn times (Figure 5-3A) by approximately 5x. This comparison suggested that nanoswimmer escape was search-limited but Brownian passive escape was barrier-limited.

Based on these observations, we hypothesized that the significantly enhanced cavity escape process of nanoswimmers compared to corresponding passive Brownian particles was due to the combination of three effects: (1) enhanced short-time mobility, (2) an efficient surface-mediated search mechanism, (3) and effective abolition of barriers at hole exits, which converted the cavity escape process of nanoswimmers to search-limited from barrier-limited. As described below, a combination of control experiments and kinetic Monte Carlo simulations allowed us to isolate and quantify the effects of these three mechanisms.



Figure 5-3. Comparison of experiments and kinetic Monte Carlo simulations. (A) Mean sojourn time versus hole size. The uncertainty of the hole size represents the standard deviation of the hole size measurements of from SEM images. The uncertainty of the mean sojourn time represents the standard error of the sojourn time measurements. (B) Radial position probability distribution of confined particle in inverse opal cavity.

5.4.5 Efficient Searching for Holes Along Cavity Surfaces

The effects of enhanced mobility and surface-mediated searching were decoupled by modeling the cavity escape process of a passive Brownian particle under different translational diffusion coefficients and boundary conditions (Figure D-6). In particular, reflective boundary conditions at the cavity walls were used to mimic the 3D searching behavior of passive particles, while an adsorptive boundary condition was used to mimic the 2D searching behavior of nanoswimmers, i.e., after a pseudo-nanoswimmer first encountered a wall, it subsequently diffused along the interior cavity surfaces. Experimental measured

short-time diffusion coefficients of passive particle and nanoswimmers were used to represent the respective short-time mobilities.

These individual effects are visualized in Figure 5-4A, which uses passive particles with reflective boundary conditions (3D searching) as a baseline, and graphs the enhancement in search time associated with (1) faster effective short-time mobility due to self-propulsion, (2) surface-mediated (2D) searching without enhanced mobility, and (3) both effects combined. Interestingly, surface-mediated searching significantly accelerated the escape process in the small hole size regime, while the impact of faster short-time mobility increased with increasing hole size. For the specific hole size (498 nm) employed in the experiments, each effect contributed approximately equally to the reduction in search time, for a total enhancement of approximately 5x, compared to the experimentally measured nearly 25x enhancement of mean sojourn time.

Interestingly, the simulated sojourn times that were based on simplified Monte Carlo simulations of passive Brownian motion with qualitative nanoswimmer transport features (i.e., enhanced short-time mobility and surface-mediated search) were in reasonably good agreement with the experimental measured values for the nanoswimmer (Figure 5-4B). This agreement further confirms the importance of the combined effects of enhanced mobility and surface-mediated searching in facilitating the cavity escape process, in that simply incorporating qualitative transport features of nanoswimmers into passive Brownian particle simulations provided a reasonably accurate approximation of mean sojourn times. The full-featured nanoswimmer simulations predicted the experimental measured value with somewhat better accuracy over the full range of hole sizes. A significant difference between these two types of simulations involved the assumption of purely 2D surface diffusion in the simplified passive Brownian particle simulation, which overestimated the escape rate in the small hole size regime while underestimating the escape rate in the large hole size regime.



Figure 5-4. Effects of enhanced mobility, surface-mediated searching and barrier elimination on mean sojourn times. (A) Mean sojourn time ratio as a function of hole size, showing the decoupled effects of enhanced mobility and surface-mediated searching. (B) Comparison of experimental observation of nanoswimmers with two types of Monte Carlo simulations, one is nanoswimmer simulations, and the other is passive Brownian simulations with nanoswimmer features. (C) Comparison of passive Brownian particle simulations with experimental observations of passive Brownian particles with added salt condition. The green inverted triangles represent the same added -salt experimental data but with effective hole sizes calculated based Bowen's model.

5.4.6 Self-Propulsion Overcomes Repulsive Barriers to Cavity Escape

We hypothesized that the discrepancy between the experimentally observed mean sojourn time of passive particles (68.2 s) and the predicted value from simulations (14s) was due to a reduction in the effective hole size caused by electrostatic repulsion at walls and hole exits (as suggested in Figure 5-3B), since the carboxylate-modified particles and silica framework are both negatively charged. Previous studies showed that like-charge electrostatic interactions can inhibit cavity escape, by forming energy barriers at hole exits. This can be modeled as a reduction in the apparent hole size. Added salt can screen this repulsion, increasing the effective hole size and accelerating cavity escape ³⁶. Therefore, we performed additional experiments using the same passive particles and medium with 0.001 M sodium chloride added, and as expected, the mean sojourn time was reduced from 68.2 s to 32.5 s (Figure 5-2B). Figure 5-1G shows a representative trajectory obtained under these conditions, it is qualitatively similar to a Brownian trajectory in the absence of salt in that the confined particle explored the 3D volume of the cavity. However, the MSD plot (Figure 5-2A) in the presence of salt exhibits less confinement at intermediate times, consistent with facilitated hole translocation that led to faster escape to adjacent cavities. However, as illustrated in Figure 5-4C, even in the presence of salt, the cavity escape was still slower than predicted by 3D simulations, suggesting the remaining presence of a small barrier to escape.

To understand this effect more quantitatively, the effective hole size was calculated using a potential energy profile model developed by Bowen et al. $^{53-54}$, which was based on DLVO theory and included both electrostatic and van der Waals interactions. The effective hole was taken to be the region where the potential energy was less than k_BT and the calculation details are described in the supporting information. The effective hole sizes for a physical hole of 498 nm were calculated to be 368 nm and 416 nm, in the absence of salt and the presence of 0.001 M salt, respectively. Consistent with our previous findings, the discrepancies between experimental and simulated sojourn times were successfully explained by calculating effective hole sizes associated with electrostatic energy barriers (Figure 5-4C).

Interestingly, the simulated mean sojourn time of a surface-searching nanoswimmer for an equivalent effective hole size (368 nm) was calculated to be ~8s, which was more than 2x the measured sojourn time of 2.8s. Additional experiments performed with different hole sizes showed similar discrepancies (Figure D-7). These findings, combined with the agreement between simulated and experimental nanoswimmer sojourn times in the absence of salt, suggested that the active nanoswimmers were not significantly affected by electrostatic barriers at hole exits despite the like charge repulsion that clearly limits the escape of the same particles in the absence of fuel. We hypothesized that self-propulsion provides forces that can overcome repulsion, leading to an escape process for nanoswimmers that is entirely search limited, with no additional effect due to barriers to translocation. To test the plausibility of this hypothesis, we compared the self-propulsion force to the barrier force due to electrostatic repulsion. The self-propulsion force was estimated using the Stokes equation $F_p = 6\pi\mu RV = 55$ fN, and the barrier force was calculated based on Bowen's model ⁵³, where the largest barrier force under 0.001M salt condition was approximately 35 fN (Figure D-3C). This comparison provides a consistency check, suggesting that selfpropulsion can provide additional forces of the same order of magnitude as typical electrostatic repulsion, thereby allowing confined nanoswimmers to overcome the barrier at hole exits, converting the cavity escape process from a barrier-limited to a search-limited process.

5.4.7 Mechanisms of Accelerated Nanoswimmer Cavity Escape

Overall, the accelerated cavity escape of nanoswimmers, compared to Brownian particles in the absence of chemical fuel, was due to the combined effects of an enhanced short-time mobility, surfacemediated searching, and the effective abolition of repulsive barriers at hole exits. In particular, the negation of repulsive barriers due to self-propulsion force facilitated the translocation step, converting the barrierlimited process to search-limited; enhanced short-time mobility and efficient surface-mediated searching facilitated the search step. Taking the hole size of 498 nm as a representative example, the mean sojourn time of nanoswimmer was nearly 25x shorter than that of a passive Brownian particle in salt-free glycerol/water solvent (Figure 5-5). Breaking down this enhancement factor, the negation of repulsive barriers reduced the mean sojourn time five-fold, and the combined effects of enhanced diffusion and surface mediated searching reduced the mean sojourn time by five-fold.



Figure 5-5. Mechanisms of accelerated nanoswimmer cavity escape.

5.5 Conclusions

The rate determining process for the transport of particles or macromolecules in porous media involves translocation through pore constrictions. Here we quantitatively studied the cavity escape of passive Brownian particles and nanoswimmers using a well-defined system enabled by 3D single-particle tracking. We found that nanoswimmers escaped from cavities more than an order of magnitude faster than expected relative to passive Brownian particles, despite of the fact that their mobility in unconfined liquid was less than two-fold faster. This anomalously rapid rate of escape suggests that micro/nanoswimmers have tremendous transport efficiency in complex and tortuous environments, which are broadly relevant to biomedical and environmental applications. The fundamental difference between a micro/nanoswimmer and its corresponding passive Brownian particle is self-propulsion, which is naively characterized by speed. However, we found that selfpropulsion induces additional nuanced effects whose impacts are dramatically amplified in complex environments, leading to extremely rapid cavity escape. In particular, the slowing effects of hydrodynamic drag in a confined environment were mitigated compared to a Brownian passive particle, resulting in anomalously fast short-time mobility. Moreover, self-propulsion resulted in qualitatively different phenomena, such as surface-mediated searching and the abolition of energy barriers at hole exits. The results reported here provide a comprehensive picture of the mechanisms that enhance micro/nanoswimmer transport in confined and porous media, and also hint at the immense potential of micro/nanoswimmers in biomedical and environmental applications. For example, low ionic strength conditions are often necessary in various technological applications to prevent aggregation and surface adsorption via electrostatic repulsion ⁵⁵⁻⁵⁷. However, these conditions have undesirable side effects, such as anomalously slow transport in porous materials. Our findings suggest that micro/nanoswimmers can effectively address these undesirable side effects through self-propulsion.

It is interesting to consider how the phenomena observed here might apply to biological microswimmers. Such a comparison must distinguish between "pusher" and "puller" type microbes. Since "pusher" type microbes hydrodynamically attracted to solid surfaces, the searching process for exit holes may be enhanced because of efficient surface-mediated searching, while "puller" type microbes may exhibit longer search times since they are hydrodynamically repelled from solid surfaces ⁵⁸⁻⁶⁰. Therefore, compared to "pusher" type microbes, the transport of "puller" type microbes in porous media is expected to be inhibited. While it may be challenging to create "puller" type colloidal nanoswimmers in a purely viscous environment, such motion may be possible in a structured or viscoelastic medium.

5.6 References

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Chapter 6 Particle Remobilization in Filtration Membranes during Flow Interruption

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6.1 Abstract

Anomalously high levels of particles are often observed to pass through filtration membranes upon resumption of flow following interruption. However, it has been challenging to obtain direct information about the underlying mechanisms. Here, we employed a highly multiplexed single-particle tracking approach, which enabled the visualization of particle transport in microfiltration membranes under flow conditions that included stoppage intervals and therefore provided direct evidence for particle remobilization during flow stoppage. Our results suggest that during flow interruption, a sub-population of trapped/retained particles detach from their retention sites and may escape to less trapped areas through Brownian motion within the pore space. Particles that do not diffuse far enough during flow stoppage generally become trapped again upon the resumption of flow. As a result, the fraction of particles that are remobilized increases with stoppage time, since longer periods of Brownian motion permit diffusing particles to enter less confined regions of the membrane. This mechanistic information about particle transport remobilization will permit the design of filtration flow profiles that meet customized requirements for particle retention during complex filtration cycles.

6.2 Introduction

Microfiltration as a separation process to remove suspended particles and microorganisms from process liquids, has been widely used in water treatment ¹⁻³, pharmaceutical sterilization ⁴⁻⁶ and dairy and

beverage processing ⁷⁻⁸. Process interruption and pressure release are common in these separation processes, due to the use of multiple feed tanks, switching between feed tanks, or the use of a buffer flush to recover residual product. However, studies have found that disruption of a filtration process can cause significant bioparticle remobilization and decrease membrane retention ⁹⁻¹². For example, the Viresolve® NFP and Ultipor® DV20 membranes showed 10-100 fold increase of phage transmission after a short pressure release ¹².

Particle transport in porous media is conventionally studied using so-called breakthrough curve experiments, based on the analysis of particle concentration at the inlet and outlet of porous media, showing the elution concentration as a function of volumetric throughput ¹¹⁻¹⁴. A detailed analysis of such data allows interpretations about particle retention during the filtration process. However, these conclusions are based on indirect model-dependent analysis of ensemble-averaged information about particle transport. As a result, direct measurements and detailed understanding of the complex mechanisms underlying particle retention and remobilization are limited.

Woods and Zydney's pioneering work first visualized the spatial distribution of fluorescently labeled phage retained within a filtration membrane using confocal microscopy, providing important insights into the effect of process disruption on filtration process¹¹. However, we report experiments that directly visualized the complex individual motion of large numbers of particles within filtration membranes during processes designed to mimic flow interruption, in order to better understand particle remobilization in filtration processes.

Single-particle tracking is a powerful approach to study heterogeneous dynamic processes in confined and complex environments, including porous materials ¹⁵⁻²¹ and cell membranes ²²⁻²³. In these experiments, we used fluorescent nanoparticles as tracer particles and tracked particle flow, retention, remobilization, and resumed flow processes during intermittent flow conditions. These flow conditions were controlled by a syringe pump, and the trajectories of fluorescent tracer particles were directly imaged using epifluorescence microscopy. Compared to conventional methods that provide ensemble-averaged
information about particle transport in porous media, particle-tracking methods enable the direct observation of individual spatio-temporal trajectories, which provide explicit identification of retention and remobilization events. The detailed information provided by this approach provides new insights into particle remobilization mechanisms as well as valuable reference data for the rational design of filtration processes to control particle remobilization.

6.3 Methods

6.3.1 Materials and sample preparation

Hydrophilic Durapore (PVDF) membrane filters were purchased from MilliporeSigma with a nominal pore size of 0.65 μ m, a thickness of 125 μ m and a porosity of 70% (Figure 6-1a and b). Small rectangular sections of the membranes were cut and inserted in a custom-made PDMS microfluidic device, and the transmembrane particle flow was imaged using a fluorescence microscope (further details are described in the Supporting information). A schematic diagram of the experimental setup is shown in Figure 6-1c. Tracer particle solutions were introduced into the inlet and flowed through the membrane being imaged, employing a dead-end filtration process geometry where the flow direction is normal to the membrane surface. FluoSpheres carboxylate-modified microspheres (Invitrogen) with a nominal size of 0.04 μ m (dark red fluorescent, 660/680) were used as tracer particles. The tracer particle concentration in solution was 10¹¹ particles/mL for single particle tracking experiments. This concentration was low enough to minimize particle-particle interactions and enable localization of individual particles in membrane. The flow was controlled by a syringe pump (Chemyx Inc.). The initial flux values were calculated using the particle velocity and membrane porosity, which was ~15 L/(h m²). Due to imaging limitations, these flow rates were substantially lower than those employed in actual industrial filtration processes.



Figure 6-1. SEM images of filtration membranes and schematic diagram of single-particle tracking experimental setup. (a) and (b) Scanning electron microscope (SEM) images of Durapore (PVDF) filtration membranes with a nominal pore size of 0.65 μ m. (c) Schematic diagram showing the flow cell and imaging system.

To track particle motion deep in the membranes, we formulated a refractive-index matching liquid to minimize light scattering from the interior surfaces of membranes. In particular, the refractive-index matching liquids satisfy the following requirements: (1) the refractive index matches that of the membrane ($n \sim 1.42$); (2) transparency; (3) does not swell or dissolve the solid matrix or tracer particles; (4) wets the membrane materials ²⁰⁻²¹. Here, refractive index-matching liquids (n = 1.42) were formulated with isopropanol and Triton X-100 (volume ratio 64:36) for the PVDF membranes. In addition, Triton X-100 also functioned as a nonionic surfactant to reduce nonspecific adsorption of nanoparticle to the interior surface of membranes.

6.3.2 Single-particle tracking experiments

All experiments were performed at room temperature on a Nikon Ti-Eclipse microscope with a through-objective TIRF unit and a 100X Nikon APO oil immersion objective. Fluorescent tracer particles were excited by a 640 nm OBIS continuous wave laser (Coherent). Emission was collected by the objective and passed through a sequence of a dichroic mirror, a bandpass filter, and a long pass filter. A cooled EMCCD camera (iXon DU897) was used to capture sequences of fluorescence images with a pixel size of 0.16 µm and a frame rate of 20 Hz. Multiple movies were collected for each time period in every observation. Custom algorithms implemented in MATLAB were used to determine trajectories of individual particles as described previously ²⁴.

6.4 Results and Discussion

6.4.1 Visualizing particle trajectories during filtration processes

We used single-particle tracking methods to investigate the impact of flow profiles (i.e. interrupted flow during filtration) on particle transport. To study this, we observed particle trajectories during continuous steady flow, then paused the flow for a pre-determined time interval (the fluid within the membranes was stagnant during this time), and finally resumed the flow after pausing. The flow was controlled by a syringe pump. Different pause times were investigated systematically to understand the influence on particle remobilization.

As expected, tracer particles showed significantly different transport phenomena in the presence or absence of convective flow. As one visualization of particle transport under different flow conditions, trajectory maps (Figure 6-2) were constructed by overlaying spatial trajectories obtained in representative experiments for 40 nm tracer particles in PVDF membrane. In Stage 1, with steady particle flow through the membrane, the trajectories were long and straight, and aligned with the flow direction on average. In Stage 2, when the flow was interrupted, the trajectories were generally compact and tortuous, consistent

with apparent random motion as expected in the absence of convective flow forces. In Stage 3, when the flow resumed, the trajectories became long and straight again, deterministically with the flow direction. Interestingly, we initially observed more trajectories passing through a given field of view after resumption of the flow. For example, during a three minutes tracking period after resuming the flow, we observed an average of 1078 particle trajectories passing through the field of view, compared with an average of 780 trajectories during the three minutes tracking period before pausing the flow, an increase of more than 38%. Since the experiments were performed under same conditions in Stages 1 and 3, we hypothesized that retained particles became remobilized during the interruption of flow, and that some of these subsequently passed through the field of view after flow resumption. In particular, we hypothesized that, in the absence of convective flow during flow interruption, previously trapped particles had the opportunity to diffusively explore alternative pathways to escape from a previously trapped region ²⁵⁻²⁶. This hypothesis was tested by direct analysis of the remobilized trajectories as described below.



Figure 6-2. Representative trajectory maps of 40 nm tracer particles in a PVDF membrane under different flow conditions. (a) Stage 1, steady flow, (b) Stage 2, interrupted flow, (c) Stage 3, resumed the flow. Inset of (b) represents a magnified trajectory, exhibiting more random motion compared to the directionally biased trajectories in (a) and (c). The scale bars are $5 \,\mu$ m.

6.4.2 Visualizing particle remobilization

As shown in Fig. 2, single-particle trajectories provided direct evidence for the remobilization of trapped particles during flow interruption. Consistent with the trajectories maps shown above, the portions

of trajectories associated with Stage 1 (flow) were directionally biased in the flow direction (magenta line in Figure 6-3). In some cases, particles became immobilized, presumably by adsorption or trapping, resulting in a subsequent series of extremely short displacements. Interestingly, after flow had been paused for some time, a significant number of these immobilized particles became mobile again, moving in apparently random directions in the stagnant liquid within the filter pore space (blue line in Figure 6-3). As a result, some of these particles were successfully remobilized, and moved away from the trapped region when flow resumed. Figure 6-3 shows four representative remobilized trajectories, which represent two types of remobilization phenomena observed in the experiments. In particular, Figure 6-3a, c and e represent completely remobilized trajectories, where the particles moved away from the previously trapped location upon the resumption of flow. Figure 6-3g, on the other hand, represents a temporarily remobilized trajectory, where an immobilized particle remobilized during flow stoppage, but was quickly immobilized again in the original trapped location upon flow resumption. We hypothesized that the temporary remobilization was due to immobilized particles that did not escape completely from the confined region, and therefore, tended to revert to the previously trapped pore constriction upon flow resumption. For the same reason, we hypothesized that longer stoppage times may facilitate the remobilization process, since a remobilized particle would have a higher possibility of complete escape from a given confined region, permitting the particle to access an entirely new pathway, instead of moving back to the previous trap upon the resumption of flow. A detailed analyses of the influence of stoppage time on the remobilization processes is discussed below.

Importantly, while the trajectories after remobilization were generally random, the direction of each particle's first movement in the remobilized regime was strongly biased to be against the original flow direction (indicated by the black arrows in Figure 6-3). These remobilization directions were determined by direct analysis of particle position versus time, by identifying the first displacement larger than a threshold associated with small fluctuations. A sensitivity test was performed to test the influence of the choice of the threshold. The results (Figure E-6) indicated the choice of the threshold value in relatively

large ranges had a negligible influence on the trends observed or conclusions drawn. A detailed description of the methods used to calculate remobilization angle and of the sensitivity test of the threshold used to calculate the remobilization angle are included in the Supporting information.



Figure 6-3. Representative remobilized trajectories, and Cartesian particle positions versus time. (a), (c), (e) and (g) are representative two-dimensional trajectories. Black circles denote the beginning of the trajectories, green circles denote the end of the trajectories, red circles denote immobilized locations, and black arrows denote remobilized directions. Magenta lines represent the trajectory in Stage 1, blue lines represent the trajectory in Stage 2, and yellow lines represent the trajectory in Stage 3. The scale bar in (a), (c), (e) represents 5 μ m, while the scale bar in (g) represents 1 μ m. The flow direction is from right to left. (b), (d), (f) and (h) are the corresponding particle positions over time. The blue dashed lines indicate the moment of flow interruption, and the yellow dashed line indicates the resumption of flow. In panels (b), (d) and (f) the particles left the field of view before resumption of flow.

While visualization of trajectories provided qualitative information about remobilization mechanisms, we performed more robust statistical analyses to understand the details of the particle remobilization process. Specifically, we extracted the remobilization directions from over 50 distinct remobilized trajectories, creating a distribution of remobilization directions as shown in Figure 6-4. According to this probability distribution, over 95% of the remobilized trajectories began in a direction against the original flow direction. This suggested that in order to escape from a trapped position, a particle must generally move against the previous convective flow direction to escape a pore constriction. Following this initial escape, remobilized particles moved randomly throughout the period of interrupted flow, potentially enabling the particle to move completely away from the previously trapped region.



Figure 6-4. Distribution of remobilization direction. The blue arrow indicates the original average flow direction during Stage 1, the orange circular sectors indicate the remobilization direction during flow stoppage. The error bars indicate the standard deviation of the measured values based on Poisson statistics.

6.4.3 Remobilized fraction

To quantify the extent of remobilization, the remobilization fraction was calculated under different experimental conditions based on the particle trajectory maps. Specifically, we identified the locations of immobilized particles immediately preceding flow interruption and then again after flow resumption (Figure 6-5). If a particle did not appear in a previously immobilized position after flow resumption, it was assumed to be remobilized. The criterion used to identify immobilization was that the end-to-end displacement was less than a Euclidean distance threshold. The threshold was chosen to be slightly larger than the localization error of an immobilized particle, and substantially smaller than the step size of a diffusively moving particle. The final results were found to be insensitive to the specific choice of threshold size between these limits. A location was labeled as a remobilization event if it corresponded to the position of an immobilized particle only before the pause of flow and not after, whereas a location was labeled as immobilized if a particle was identified in that location both before and after the pause session. The remobilization fraction was calculated as the ratio of the number of remobilization locations to the number of immobilized locations before pausing the flow. For example, in Figure 6-5, the remobilized fraction is 0.31. A sensitivity test was performed to understand the influence of the choice of thresholds. In particular, varying the thresholds in a relatively large range resulted in a minimal change in the remobilized fraction (+/-0.01), which had a negligible influence on the trends observed or conclusion drawn (Figure E-3 and Figure E-4). A detailed description of the methods used to create remobilization maps and of the sensitivity test of the thresholds used to calculate the remobilized fraction are shown in the Supporting information.



Figure 6-5. Representative remobilization map. Red circles indicate the locations of immobilized particle at the end of Stage 1 (flow), green circles indicate the locations of immobilized particle at the beginning of Stage 3 (resumed flow following interruption). Therefore, the locations with only red circles, indicate previously immobilized particles that were remobilized after flow interruption. The locations with both red and green circles, indicate previously immobilized particles that remained trapped, or returned to the initial trapped position after flow interruption. The scale bar indicates 5 μ m.

6.4.4 Remobilized fraction as a function of stoppage time

To investigate the influence of pause time on particle remobilization, various pause times were tested with the same initial flow rate and duration; experiments were performed in different membrane locations to account for membrane heterogeneity. Notably, the experiments were performed over a short time window, about 1.5 - 2 hours, where irreversible immobilization was not very significant. Figure 6-6 shows the remobilization fraction versus pause time. Interestingly, the remobilization fraction increased systematically with pause time up to about 6 minutes, and then appeared to saturate for longer pause times. We hypothesized that this behavior could be related to the fact that the area explored by Brownian motion of particles during flow interruption increased monotonically with the duration of the pause session. In particular, the longer the pause time, the higher the probability that a remobilized particle could diffuse into a less trapped region, permitting untrapped transport after the resumption of flow. If a particle diffused only a small distance during flow interruption, it was presumably more likely to return to its initial trapped position upon flow resumption. Thus, it is useful to consider the approximate distance explored by particles

during flow stoppage, as indicated by the upper horizontal axis of Figure 6-6. In particular, we estimated the escape distance based on the particle diffusion coefficient during flow stoppage (Figure E-2). Interestingly, the approximate escape distance associated with a stoppage time of 6 minutes was about 10 μ m, which is approximately an order of magnitude larger than the nominal pore size of the membranes used in these experiments. Considering that the actual physical pore size is usually an order larger than the nominal pore size ²⁷, it is consistent with our hypothesis that a successful remobilization event occurred only when a particle fully escaped from the previously trapped pore space.

Generally speaking, to be fully remobilized, trapped particles must diffuse over a length scale on the order of the actual physical pore size, which is potentially related to the first passage time problem in a confining domain. A variety of theoretical frameworks ²⁸⁻³⁵ and experimental approaches ^{16-17, 36-38} have been developed to evaluate the first passage time in various physical scenarios. The results presented here represent a physical realization of the scenario where a nanoparticle escapes from a highly heterogeneous pore. For example, the remobilized fraction can be approximated as the fraction of particles, initially randomly distributed in a confined space, successfully escaped out of the confinement via Brownian motion for a given stoppage time. Therefore, the curve in Figure 6-6 also can be interpreted as a cumulative distribution of first passage time (after rescaling the y axis), that is, the probability that a diffusive particle has escaped out of the confining domain for given stoppage time. Moreover, given a short stoppage time window, the remobilized fraction is directly related to how fast the confined particle travels (i.e., diffusion coefficient). Therefore, for a given stoppage time, the remobilized fraction is expected to increase with decreasing particle size and fluid viscosity according to Stokes-Einstein equation.



Figure 6-6. Average remobilization fraction versus flow stoppage time. The error bars represent the standard error of replicate experiments under the same pause time conditions. The upper horizontal displacement axis indicates the corresponding average root-mean-squared displacement for given pause time, which was calculated using the diffusion coefficient within the membrane during the flow stoppage period.

These observations may provide some interesting insights into particle retention in these filtration membranes. The mechanisms of particle captured by filtration can be summarized into four categories: (1) simple sieving at pore constrictions; (2) inertial impaction (relatively large particles cannot follow the fluid flow lines through the tortuous pores and are eventually captured as they impact the pore wall); (3) Brownian diffusion and wall-adsorption (small particles are subject to random motion that occasionally brings them into contact with the pore walls, with which they are captured) (4) Adsorption based on electrostatic attraction ^{27, 39-40}. In our experiments, remobilized particle represent a sub-set of a population that spontaneously begins to move during flow stoppage, suggesting that they are trapped by a pore constriction and are not irreversibly adsorbed (in which case they would not likely detach simply because of flow stoppage). The maximum value of the remobilized fraction (~0.33) versus pause time suggests that approximately one-third of the particles retained in our experiments were captured by sieving at pore constrictions, because only particles trapped in a narrowed pore throat are likely to escape from the trapped

region during flow stoppage. The remaining two-thirds of particles may be strongly surface adsorbed, and would presumably require high or reversed flow for removal ⁴¹⁻⁴².

As a comparison, we also determined the remobilized fraction under continuing convective flow condition. In particular, instead of analyzing the trajectories in Stage 1 and Stage 3 to identify the remobilized events, we analyzed trajectories at two time points in Stage 1 to investigate the potential for remobilization in the presence of hydrodynamic forces from flow. We found that the remobilized fraction was 0.10 under convective flow (Figure E-5), which we speculated mainly due to desorption of adsorbed particles, potentially enhanced by hydrodynamic shear forces. Since the remobilization fraction was as high as 0.33 during flow stoppage, this suggests that flow interruption may significantly facilitate particle remobilization.

6.4.5 Remobilization mechanisms

By visualizing particle trajectories that include both immobilization and remobilization, and by analyzing the influence of pause time on remobilization fraction, we obtain insights into the remobilization process permitting a proposed particle remobilization mechanism during flow stoppage. As summarized in Fig. 7, during the filtration process, particles may become trapped in confined regions, such as pore throats or other constrictions, due to the directional convective flow. The most likely way to escape such a trapped region involves motion against the convective flow direction, which is unlikely in the presence of convective flow, but possible via Brownian motion. Consistent with this, as shown in Figure 6-4, the remobilization directions in over 95% of remobilized trajectories were observed to be against the original convective flow direction, suggesting that the initial Brownian motion was highly biased by the geometry of the trapping site. Therefore, the particle remobilization observed during flow stoppage (Figure 6-3) may be directly related to the spikes observed in elution profiles (e.g. of virus particles) following pressure release and flow resumption in ensemble measurements ^{11-12, 26}. Moreover, we found that the duration of the pause time affected the remobilization fraction by influencing the potential for a particle to escape to a

less trapped region. As shown in Figure 6-3 (g) and (h), although some particles may escape from their original trapped region, if they do not travel far enough away to access an entirely new pathway, they may move back to the previous trap upon the resumption of flow. For longer pause times, untrapped particles have a higher likelihood of escaping to different pathways, and are thus less likely to return to the previously trapped region upon the resumption of flow.

The remobilization mechanisms proposed here may provide guidance for membrane filtration process design. For example, the results described above provide guidance regarding the effect of the duration of flow interruption on particle remobilization, which can be directly tested for a given filtration process. Moreover, since Brownian motion enhances the remobilization of particles that are trapped during convective flow, it may be advisable to employ continued flow rates during feed exchange, to inhibit "backward" Brownian motion and loss of retention. On the other hand, remobilization could be helpful for membrane cleaning processes. Membrane cleaning is often carried out using flow reversal, which mainly removes immobilized particle that are too large to enter deep into the filtration membrane, and may not be sufficient to remove smaller particles that are retained in pore constrictions deep in the membrane. Periodic pauses during membrane cleaning process may be helpful to remove this population of residual bioparticles by allowing them to diffuse out of pore constrictions.

The nanoparticle convective entrapment described here is similar to "straining" phenomena described in studies of water flow through natural porous media, in which colloidal particles are immobilized by straining of individual particles of flocs ⁴³. Thus, the mechanisms proposed here may provide insights into the colloidal-facilitated transport of metals and radionuclides in subsurface environments ⁴⁴ and may aid the design of efficient environmental remediation processes that involve nanoparticles ⁴⁵⁻⁴⁶.



Figure 6-7. Schematic diagram of remobilization mechanisms.

6.5 Conclusions

Bioparticle remobilization in porous media during pressure release is an important concern in virus purification and downstream processing of therapeutic proteins, as filtration membranes are widely used to remove viruses and other contaminating particles in these processes. A single-particle tracking approach was employed to study the effects of flow conditions on particle remobilization during flow stoppage. Using direct observations of particle trajectories in filtration membranes under different flow conditions, we visualized the process of particle immobilization and remobilization, confirming the general observation that flow stoppage can cause particle remobilization at a single particle level.

Moreover, single-particle remobilization trajectories provided insights into underlying particle remobilization mechanisms during flow stoppage. In particular, Brownian motion during flow stoppage provided the opportunity for a trapped particle to escape from a pore constriction; the longer the pause time, the higher the possibility for the trapped particle to escape to a less confined region enabling remobilization upon the resumption of flow. The mechanisms proposed here provide a general understanding of nanoparticle transport in porous media under interrupted flow, which can be used to design better membrane filtration processes and potentially enhance the environmental remediation processes involving nanoparticles. These advances were uniquely enabled by a single-particle tracking approach, which provided direct visualization of spatio-temporal particle trajectories in a confined environment, where particles exhibit complex, heterogeneous, and intermittent motion that can be challenging to understand from ensemble-averaged measurements alone.

6.6 References

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Chapter 7 Fouling of Microfiltration Membranes by Bidisperse Particle Solutions

7.1 Abstract

Fouling of filtration membranes is a key process that degrades performance and reduces membrane lifetime. However, it remains challenging to obtain direct information about fouling mechanisms, especially fouling that occurs within the internal pore spaces. Here, we employed highly multiplexed single-particle tracking with alternating laser excitation, which enabled the direct visualization of nanoparticle transport within microfiltration membranes, where the advective motion and retention of 40 nm and 200 nm diameter particles was simultaneously imaged within filtration membranes with 650 nm nominal pore size, as membranes became increasingly fouled as a function of total particle throughput. We found that internal membrane fouling consisted of three stages, fouling site formation, fouling site growth, and fouling site coalescence. Larger particles were more likely to be retained initially, nucleating the fouling sites. These sites, tended to capture other particles passing through the membrane, causing the fouling sites to grow in size. Eventually, isolated fouling sites grew large enough to coalescence with neighboring sites, blocking a large area of membrane pores, leading to significantly fouling. Importantly, the presence of small numbers of large particles significantly increased the retention of small particles and also accelerated the rate of particle retention. This mechanistic information about internal membrane fouling will assist in the design and optimization of filtration process to reduce membrane fouling as well as advance the understanding of complex mass transport of polydisperse particle distributions.

7.2 Introduction

Microfiltration is a pressure-driven separation process used to separate micron-sized particles and microorganisms from process liquids. Microfiltration has been widely used in industrial applications,

including pharmaceutical sterilization ¹⁻³, water treatment ⁴⁻⁶, and beverage and dairy processing ⁷⁻⁸. Fouling is a crucial problem during separations using microfiltration membranes, which can be categorized as external fouling and internal fouling. External fouling is caused by cake formation, which occurs on the upstream surface of the membrane due to the accumulations of large particles that cannot enter the membrane pores, while internal fouling is caused by pore plugging and/or pore narrowing, which occurs within the porous structure of the membrane due to physical deposition and/or adsorption of small particles and macromolecules that are able to enter the membrane pores.

Microfiltration membrane fouling is conventionally studied by measuring permeate concentration and flux rate as a function of time ⁹⁻¹³. Interpretations drawn from such experiments are based on indirect model-dependent analysis of ensemble-averaged information about particle transport and membrane fouling. While such interpretations can have practical utility, they provide limited insights into the complex transport behavior of particles within the highly heterogeneous and interconnected membrane environment. Supplementing these measurements, scanning electron microscope (SEM) images taken before and after fouling can serve to verify proposed mechanisms ¹⁴⁻¹⁵. For example, Tracey and Davis ⁹ proposed protein fouling mechanisms in microfiltration membranes by studying resistance to flow versus time curve, and these experiments were complemented by cross-sectional SEM images of the fouled membranes ¹⁴, which showed that fouling mechanisms changed from internal fouling dominated to external fouling with increasing foulants size. This type of endpoint characterization provides important qualitative insights into fouling mechanisms but does not allow visualization of the entire fouling process, which is required for a comprehensive understanding of fouling and fouling reduction.

Foulants in real-world scenarios have a wide range of particle size, and particle size can affect the performance of the microfiltration significantly ¹⁶⁻¹⁹. For example, Chang et al. ¹⁶ found that permeate quality was better in the presence of multiple sized particles because a cake layer deposited on the membrane surface consisting of spheres of varying sizes will normally have a smaller porosity than one consisting of spheres of a single size. Although size effects in the microfiltration process have been

extensively studied, the effects of polydisperse particle distributions, especially when the particles are smaller than the pore size are not well understood.

Here, we combined highly multiplexed single-particle tracking methods with alternating-laser excitation (ALEX) imaging to simultaneously visualize nanoparticle motion deep in the porous microfiltration membrane and elucidate fouling processes associated with the presence of both large and small particles. Single-particle tracking is a powerful approach to study heterogeneous dynamic processes in complex environments, including porous polymer membranes and biological materials ²⁰⁻²⁷. In these single-particle tracking experiments, particle motion was continuously imaged using fluorescence microscopy, particle positions were localized and linked into trajectories using a custom tracking algorithm. To track two particle populations, we employed alternating-laser excitation, which enables imaging of multiple fluorescent signals simultaneously by rapid switching between excitation wavelengths ²⁸⁻³⁰. By using nanoparticles of two different sizes with contrasting excitation spectra, we were able to visualize bidisperse particle trajectories provided explicit information about the formation and development of fouling sites in the membranes. The findings presented here provide new insights into internal fouling mechanisms as well as valuable reference data for the rational design of microfiltration processes to reduce membrane fouling.

7.3 Methods

7.3.1 Materials and sample preparation

Hydrophilic Durapore (PVDF) membrane filters were purchased from MilliporeSigma with a nominal pore size of 0.65 μ m, a thickness of 125 μ m and a porosity of 70% (Figure 7-1a, 1b). Small rectangular sections of the membranes were cut and inserted in a custom-made PDMS microfluidic device, and the transmembrane particle flow was imaged using a fluorescence microscope equipped with

alternating-laser excitation. A schematic diagram of the experimental setup is shown in Figure 7-1c. Tracer particle solutions were introduced into the inlet and flowed through the membrane being imaged, employing a dead-end filtration process geometry where the flow direction is normal to the membrane surface. FluoSpheres carboxylate-modified microspheres (Invitrogen) with a nominal size of 0.04 μ m (dark red fluorescent, 660/680) and 0.20 μ m (orange fluorescent, 540/560) were used as tracer particles. In the particle tracking experiments, the particle solution contained 200 nm tracer particle at a concentration of 10⁹ particles/mL and 40 nm tracer particles at a concentration of 10¹⁰ particles/mL. The flow condition was controlled by a syringe pump (Chemyx Inc.). The flux rates employed in the experiments were approximately 5 - 25 L/(h m²), which were substantially lower than those employed in actual industrial filtration processes.





Figure 7-1. SEM images of filtration membranes and schematic diagram of single-particle tracking experimental setup. (a) and (b) Scanning electron microscope (SEM) image of a PVDF membrane with nominal pore size of 0.65 μ m. (c) Schematic diagram showing the flow cell and imaging system.

To image particle motion deep in the microfiltration membranes, we formulated a refractive index matching liquid to minimize light scattering at the interface between membrane surface and immersing liquid. In particular, the refractive index matching liquids need to satisfy the following requirements: (1) same refractive index as the membranes; (2) does not swell or dissolve the membrane of tracer particles; (3) transparency; (4) wets the membrane materials. A mixture of isopropanol and Triton X-100 (volume ratio 64:36) was used as refractive index-matching liquid (n ~ 1.42) for the PVDF membranes used in the experiments ³¹⁻³². Notably, Triton X-100 as a nonionic surfactant also function to reduce nonspecific adsorption of nanoparticle to the interior surface of membranes.

7.3.2 Single-particle tracking experiments

All experiments were performed at room temperature using a Nikon N-STORM microscope with a through-objective TIRF unit and a 100X Nikon Apo oil immersion objective. To image two populations of nanoparticles simultaneously, we employed alternating-laser exciting method, which excited the two types of fluorescent particles in an alternating fashion. Specifically, 200 nm fluorescent tracer particles were excited by a 560 nm wavelength laser, and 40 nm fluorescent tracer particles were excited by a 640 nm wavelength laser. Emission was collected by the objective and passed through a sequence of a dichroic mirror, a bandpass filter, and a long pass filter. A cooled sCMOS camera (Hamamatsu ORCA-Flash4.0 V2) was used to capture sequences of fluorescence images with a pixel size of 0.13 µm and a frame rate of 20 Hz. Multiple movies were collected at different membrane locations to account for spatial heterogeneity of the membrane. Custom developed algorithms implemented in MATLAB were used to identify fluorescent particles and determine trajectories of individual particles as described previously ³³⁻³⁴.

7.4 **Results and Discussion**

7.4.1 Simultaneous visualization of large and small nanoparticle trajectories

To visualize particle motion in the membrane structure, trajectory maps were constructed by overlaying trajectories obtained in the experiments for both 40 nm and 200 nm tracer particles at various flow rates in PVDF membrane samples. We were able to distinguish and visualize 40 nm and 200 nm particles in the same membrane region under the same flow conditions and to analyze their motion separately. For example, Figure 7-2 (a), (b), (d) and (e) show 40 nm and 200 nm tracer particle motion individually, and Figure 7-2 (c) and (f) show both 40 nm and 200 nm tracer particle motion in the same field of view. As noted previously, we found that the apparent tortuosity of the trajectories was a functional property that depended on the flow conditions. For example, with increasing fluid velocity, trajectories of both particle sizes became less tortuous (Figure 7-2).



Figure 7-2. Trajectory maps obtained with 40 nm and 200 nm, at low and high flow rates. The trajectory colors in panel a, b, d, and e are arbitrary for the sake of visibility. In panels c and f, the red color indicates the 40 nm tracer particle trajectories, and the blue color indicates the 200 nm tracer particle trajectories. Scale bar represents 5 μ m.

Different sized particles experience different hydrodynamic drag and therefore execute different Brownian motion. As a result, at the same fluid velocity, 40 nm and 200 nm particles actually experience different flow conditions. To account for these effects, we characterized trajectory tortuosity as a function of Peclet number (*Pe*). The Peclet number is a dimensionless group that represents the ratio of the advective transport rate to the diffusive transport rate, which can be calculated using *Pe*=Lv/D, where L is the characteristic length of the porous medium (the nominal membrane pore size of 0.65 µm was used), v is the local velocity of tracer particles, and D is the corresponding diffusion coefficient of tracer particles in unconfined liquid. The diffusion coefficient (D) for each type of particle was calculated from the mean squared displacement (MSD) of particles in unconfined liquid using the equation MSD=4Dt. In the refractive index matching liquid used in these experiments, the diffusion coefficient was $0.18+/-0.01 \ \mu m^2/s$ for the 200 nm particles and $0.83+/-0.01 \ \mu m^2/s$ for the 40 nm particles. The trajectory tortuosity (τ) was calculated using the equation of τ =L/C, where L is the contour length and C is the end-to-end displacement of a trajectory. For each experimental condition, the mean tortuosity was calculated as the mean of all trajectory tortuosities in the field of view.

The mean tortuosity of the two sized tracer particles as a function of Pe is shown in Figure 7-3. These results explicitly confirmed that the tortuosity relevant to particle transport is not simply a structural material property but is instead a functional property that depends on operating conditions. In particular, tortuosity decreases with increasing Pe or decreasing particle size. As noted previously, the decrease of tortuosity with increasing Pe was due to the fact that particles flowed more deterministically in the flow direction at higher Pe because of reduced diffusive exploration of alternative pathways ³⁵⁻³⁶. The increase in apparent tortuosity for larger tracer particles was due to the fact that larger particles were more likely to be forced into alternative pathways because of their inability to pass through small pore constrictions ³⁷⁻³⁸. These findings were consistent with previous studies that investigated two tracer particle sizes separately ²⁶. This consistency suggests that motion of nanoparticles was insensitive to the presence of other sized particles.



Figure 7-3. Tortuosity for 40 nm and 200 nm tracer particles as a function of *Pe*. Error bars were calculated as the standard error of measurements from multiple experiments.

7.4.2 Visualizing internal fouling processes

During the microfiltration process, foulants can deposit on the membrane surface or in the pores, depending on the size of the particles. Particles smaller than the nominal membrane pore size can deposit internally, which leads to pore narrowing or blocking, causing membrane fouling. In our experiments, we directly visualized the entire sequence of the internal fouling process using the fluorescent tracer particles themselves as model foulants. We observed a three-stage internal fouling process: (1) initial fouling site formation, (2) fouling site growth, and (3) fouling site coalescence. A representative example (Figure F-5) was shown in the Supporting Information.

Quantitatively, this three-stage internal fouling process was analyzed by calculating the number and sizes of the fouling sites in the membrane as a function of particle flux through the membrane. In these experiments, fouling sites were identified as the locations where particles were immobilized using criteria described in the Supporting Information. Figure 7-4a shows a representative fouling site map generated by enumerating and labeling the fouling sites in a given field of view.

By tracking fouling sites over time during a flow experiment, we were able to analyze the number of fouling sites as a function of particles passing through membrane, which exhibited three-regime behavior (Figure 7-4b). We hypothesized that this behavior corresponded to the three-stage of fouling: fouling sites formation, growth and coalescence. In this picture, the first regime involves the initial formation/nucleation of fouling sites via particle capture by pore constrictions and/or adsorption to highly retaining regions of the pore walls. In the second regime, the most likely locations for fouling nuclei already filled, the existing fouling sites grow by capturing other particles from the flow. In the third regime, with the growth of fouling sites, neighboring fouling sites coalescence into enormous fouling sites (that potentially percolate across large regions of the membrane); the coalescence of existing sites results in a decrease in the number of sites in this regime. To verify this hypothesized mechanism, we calculated the average 2D- projected area of fouling sites as a function of the number of particles passing through the membrane (Figure 7-4c). In particular, the average size of fouling sites grew slowly in the second regime (reflecting the gradual capture of particles by individual sites), but increased dramatically in the final regime, suggesting that the large fouling sites resulting from coalescence were particularly effective at capturing additional particles. Figure F-5 qualitatively shows this three-stage fouling mechanisms as indicated by the quantity and size of the fouling sites as a function of the number of particles passing through the membrane.



Figure 7-4. Representative fouling site map and three-stage fouling process. (a) Representative fouling site map. Red circles indicate fouling sites containing immobilized 40 nm particles, and green circles indicate fouling sites containing immobilized 200 nm particles. Scale bar represents 5 μ m. (b) Number of fouling sites versus cumulative particles passing through membrane. (c) Average area of fouling sites versus cumulative particles passing through membrane. Error bars were calculated as the standard error of measurements from multiple experiments.

7.4.3 Influence of co-existence nanoparticle sizes

By employing alternating-laser excitation, we were able to distinguish the particle motion of 40 nm and 200 nm tracer particles and to investigate how the co-existence of multiple particle sizes influenced the fouling mechanisms. To isolate the effects of different particle sizes, we identified and counted the fouling sites based on their composition. In particular, as illustrated in Figure 7-4a, we identified fouling sites composed of 40 nm particles alone, 200 nm particles alone, or both 40 nm and 200 nm particles. We found that the colocalization fraction (Figure 7-5a and 5b) of fouling sites was high, i.e., the probability was greater than 90% that a fouling site containing immobilized 200 nm particles also contained immobilized 40 nm particles. More importantly, by investigating the time sequence of formation for more than 100 fouling sites, we found that more than 92% of the fouling sites were initially formed by immobilized 200 nm particles, suggesting that the retention of small particles (40 nm) was mediated by the retention of large particles (200 nm).

To quantify the effects of co-existence nanoparticle sizes on the fouling process, we compare the retention fraction of small particles under conditions with or without co-existence of large particles (Figure 7-5c). The retention fraction was defined as the fraction of the total particles seen to enter the membrane that was observed to be retained by the porous membrane structure. The retention fraction of small particles was approximated as the ratio of the number of immobilized small particles trajectories to the total number of immobilized and mobile small particle trajectories. We found that presence of large particles influences the retention of small particles by increasing the retention fraction and also by accelerating the retention of small particles size (40 nm) was significantly smaller than the nominal pore size (0.65 μ m) of the membrane. However, the initial retention fraction increased to 5% in the presence of a small particles increased in the presence of large particles increased in the presence of large particles increased in the membrane, the retention fraction of small particles increased in the presence of large particles increased in the development of fouling sites. However, the addition

of large particles dramatically increased the acceleration of the retention fraction as indicated by the considerably increased slope of the retention fraction curve (Figure 7-5c).

Generally speaking, the mechanisms of internal particle capture within a porous structure can be summarized into four categories: (1) sieving at pore constrictions; (2) adsorption due to electrostatic attraction or other noncovalent interactions; (3) inertial impaction (relatively large particles are captured as they impact a pore wall by inertia since they cannot follow the fluid flow lines through the tortuous pores); (4) Brownian motion (small particles are captured as they occasionally contact pore walls because of random motion) ³⁹⁻⁴⁰. In our experimental conditions, the nonaqueous immersion liquid system and the addition of nonionic surfactant Triton X-100 significantly reduced the adsorption probability. Therefore, sieving and inertial impaction presumably became the main mechanisms to capture floating particles, consistent with the observation that large particles (200 nm) formed the initial fouling sites since they were more likely to be trapped in anomalously small pore constrictions. As a result, these fouling sites increased the retention fraction, accelerated the increase of retention of small particles, and ultimately accelerated the membrane fouling process overall.



Figure 7-5. Fouling processes of co-existence of nanoparticle sizes. (a) Number of fouling sites versus cumulative particles passing through membrane. (b) Colocalization fraction as versus cumulative particles passing through membrane. (c) Retention fraction of small particles (40nm) versus cumulative small particles passing through membrane under conditions with or without the coexistence of large particles (200 nm). For the experiments including large particles, the concentration ratio of small particles to large particles was 10:1.

7.4.4 Fouling mechanisms of bidisperse particle solutions

By visualizing the trajectories of two different particles sizes simultaneously, and by analyzing the number, size, and composition of fouling sites as a function of time, we obtained insights into the membrane fouling process permitting a proposed internal microfiltration mechanism under conditions where multiple particles sizes are dispersed in the filtration medium. The fouling mechanisms can be summarized into three stages, as shown in Figure 7-6. During the initial stage of fouling site formation/nucleation, as polydisperse particles passing through the membrane, larger particles are more likely to be trapped in confined regions, such as anomalously small pore constrictions. These trapped particles become the initial fouling sites, capturing other floating particles (by narrowing pore sizes and/or serving as attachment sites) to growth during the subsequent fouling site growth stage. As the sites continue to grow in size, the fouling reaches a third stage, which neighboring fouling sites coalescence into enormous sites, completely blocking a large area of porous structure and capturing small particles at a greatly enhanced rate.

Kelly et al. ¹¹⁻¹² proposed a two-stage mechanism for protein fouling during microfiltration by studying ensemble flux decline over time, in which the initial flux decline was due to the deposition of large protein aggregates, with these aggregates then serving as attachment sites for the continued deposition of other proteins. The proposed three-stage fouling mechanisms here provided microscopic verification of a similar fouling mechanism by directly visualizing the entire internal fouling process.



Figure 7-6. Three-stage of membrane fouling mechanisms. (a) Fouling site formation: nanoparticles (especially larger sized) become immobilized in pore constrictions, forming the initial fouling sites. (b)

Fouling site growth: fouling sites size grow by capturing floating nanoparticles. (c) Fouling site coalescence: fouling sites continue to grow and neighboring sites coalescence into huge fouling sites that block a large area of the membrane.

7.5 Conclusions

Compared to external membrane fouling, internal fouling is more difficult to characterize and extremely challenging to remove because of the complexity and heterogeneity of the porous structure. A single-particle tracking approach was employed to study the internal membrane fouling mechanisms. By direct observations of particle trajectories, we visualized the entire process of membrane fouling and proposed a three-stage fouling mechanism: (1) fouling site formation; (2) fouling site growth; and (3) fouling site coalescence. Moreover, the combination of single-particle tracking with alternating-laser excitation method enabled us to observe trajectories of two different particle sizes simultaneously and to analyze the composition of fouling sites. Importantly, we found that the retention of small particles was mediated by the previous retention of large particles, elucidating the fouling mechanisms of polydisperse particle suspensions.

The mechanisms proposed here provide a comprehensive understanding of internal membrane fouling mechanisms, which can potentially be used to design better membrane filtration processes with reduced fouling effects. For example, fouling sites in the first stage are more likely to be removed by backflow or flow stoppage since most fouling sites formed in this stage are due to larger particles trapped in the pore constrictions. This is consistent with the observation that backpulsing and backwashing can significantly reduce membrane fouling in the early stage ⁴¹⁻⁴². These findings were uniquely enabled by single-particle tracking approaches, which provided direct visualization of spatio-temporal particle trajectories in complex and confined environments, where particles exhibit complex and heterogeneous motion that can be challenging to understand from ensemble-averaged methods alone.

7.6 References

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Chapter 8 Concluding Remarks

In this thesis, single-particle tracking methods were utilized to probe nanoparticle transport mechanisms in complex porous environments by visualizing nanoparticle diffusion, advection and self-propulsion in both highly-ordered and extremely heterogeneous porous materials. Along with Monte Carlo kinetic simulations, statistical modeling and theoretical calculations, we elucidated the fundamental mechanisms of hindered accessibility of nanoparticle transport in porous media, and particle remobilization membrane fouling mechanisms during microfiltration processes.¹

By employing highly-ordered porous materials, we investigated the effects of geometric parameters (i.e., pore size, pore throat size, and tracer particle size) and microscopic interactions (i.e., electrostatic and hydrodynamic interactions) in nanoparticle transport. We found a linear relation to connect nanoparticle transport from a single-pore to macroscopic scale. The rate determining process at the single-pore scale is nanoparticle cavity escape through the bottlenecks of the porous voids, which can be search-limited or barrier-limited. In a search-limited cavity escape process, a nanoparticle escapes immediately upon encountering an exit hole, so this process can be accurately predicted by theoretical first-passage-time model and Brownian simulations. In a barrier-limited cavity escape process, free energy barriers (i.e. entropic and/or electrostatic) at the exit hole can significantly delay the cavity escape process. However, by incorporating energy barriers as effective hole size, the cavity escape process be can accurately predicted based on Brownian simulations with and effective hole size.²⁻³ The macroscopic diffusion coefficient is directly related to the single-pore scale process with characteristic length (i.e. well-defined cavity diameter) and time (i.e. mean sojourn time) parameters, because the macroscopic level transport can be simplistically considered as hopping in the porous network with a characteristic hopping distance and waiting time.⁴

We also employed the same high-ordered porous medium as a model system to investigate selfpropulsion effects in confinement. By comparing the cavity escape process of both passive Brownian particles and active self-propelled nanoswimmers, we found that self-propulsion effects can induce nuanced secondary effects, such as anomalous short-time high mobility, surface-mediated searching and abolition of energy barriers, which significantly enhanced self-propelled nanoswimmer transport in complex confined environments. These findings justified the great potential of utilizing active self-propelled nanoswimmers in various applications, including biomedical diagnosis, drug delivery and environmental remediation.⁵

By employing commercial filtration membranes as the model porous materials, we answered questions about fundamental transport mechanisms during filtration processes, which are difficult to resolve using conventional ensemble methods. We found that the decreasing of particle retention after process disruption during filtration processes is due to particle remobilization when pressure is released. In particular, process disruption provides a unique opportunity for confined particles to retract from a confined region via random Brownian motion.⁶

In addition, we also visualized the entire membrane fouling process associated with the coexistence of two nanoparticle populations with distinctly different sizes. We found that membrane fouling can be summarized as three regimes: (1) fouling site formation (larger particles tend to be captured by pore constrictions and form the initial fouling sites); (2) fouling site growth (the initial fouling sites serve as nucleation sites and capture other floating particles in the flow); and (3) fouling site coalescence (neighboring fouling sites coalescence and form gigantic fouling sites, completely blocking a large area of membrane). Therefore, the retention of small particles is mediated by transport behavior of large particles.

The findings described here provide fundamental understanding about nanoparticle transport in porous media, and can be used to optimize membrane filtration processes and advance the industrial applications of nanoparticles and nanoswimmers in complex environments. Most importantly, the methodologies developed in this thesis are readily employed in laboratories with access to conventional fluorescence imaging and are broadly applicable to a wide range of confined environment scenarios. Compared to conventional ensemble methods, the ability to track the motion of individual particles deep within a porous material opens new opportunities to quantitatively study microscopic interactions, leading

to a microscopic understanding of macroscopic transport phenomena, like hindered diffusion, inhibited accessibility, enhanced transport of active matters, particle remobilization and membrane fouling in filtration processes.

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Appendix A: Supporting Information for Chapter 2

A.1 Materials and Sample Preparation

Fabrication of silica inverse opals Monodisperse colloidal polystyrene particles (522 nm diameter, approximately 2.5% standard deviation) were purchased from microParticles GmbH, Germany and used as the template particle. The standard deviation of the size is <2%, consistent with the polydispersity index of 1.03 provided by the manufacturer and used as received. The silica precursor solution is a mixture of 98% tetraethyl orthosilicate (Sigma-Aldrich), 0.10 M HCl solution, and 100% ethanol (Decon Labs), respectively, with a volume ratio of 1:1:1.5. The silica precursor solution was hydrolyzed by stirring using a magnetic rotor at 200 rpm for 1 h at room temperature prior to use. In order to fabricate the silica inverse opals on a solid substrate, 0.5 mL of a 5 vol% "stock" colloidal suspension was added to 9.5 mL of Milli-Q water (18.2M Ω .cm at 25°C), and x mL of hydrolyzed TEOS solution, where x was varied from 0.024 to 0.150 mL. A round microscope glass slide (Electron Microscopy Sciences, US) with a diameter of 25 mm and thickness of 150 μ m, cleaned in piranha solution, was vertically suspended in a beaker containing the mixture of particles and the silica precursor solution. The aqueous solution was evaporated in a 65 °C oven over a 2-day period on a vibration-free table, to deposit a thin film onto the surface of a glass slide. The resulting structure contained sol-gel silicates, distributed uniformly in the interstitial spaces of the polystyrene opal film with a thickness of about 8–9 µm. Upon pyrolysis in air at 500°C for 2 h, the polystyrene spheres were removed and silicates transformed to a high-quality silica inverse opal layer with uniform cavity size and almost crack-free, periodic structure of holes over macroscopic length scales (Figure A-1). The hole size was systematically varied by changing the amount of silicate precursor solution (Figure A-2).



Figure A-1. SEM image of inverse opals at low magnification. Scanning electron microscopy (SEM) image of a silica inverse opal film at low magnification showing a highly uniform structure with rare cracks. The image looks blurred due to electrostatic charging effects associated with SEM images of nonconductive samples.



Figure A-2. SEM images of inverse opals at high magnification. Representative scanning electron microscopy images of inverse opal films with varying hole sizes.

<u>*Particle suspensions*</u> Fluorescent polystyrene particles were purchased from Life Technologies (dark red, F-8789). The particles were dispersed in a mixture of thiodiethanol (TDE, Sigma-Aldrich) and Triton X-100 (Sigma-Aldrich) where the volume fraction of Triton X-100 was 16.7%. The particle concentration is approximately 10^{-13} – 10^{-14} M. The viscosity of this mixture is 73 cP determined by a TA Instruments ARES

Rheometer. The hydrodynamic radius of the particle was determined to be 24 nm by tracking the diffusion of more than three thousand fluorescent particles in dilute solution.

A.2 Sample preparation

The particle suspension was placed on top of an inverse opal film (Figure S3) gently. The fluorescent particles were introduced into the inverse opal structure via passive diffusion. The refractive index, n, of the TDE/Triton X-100 mixture was 1.48, very close to that of silica (n = 1.45), permitting high-resolution optical imaging by eliminating scattering from liquid-solid interfaces. Moreover, the surfactant Triton X-100 dramatically reduced the sticking of particles to the inner walls of the inverse opals.

A.3 Microscope

A Nikon Ti-Eclipse microscope comprising a through-objective TIRF unit and a 100× Nikon APO oil immersion objective (NA = 1.45) mounted on a perfect focus module (Nikon) was used for particle tracking. The fluorescent particles were excited using a 647 nm diode laser (Coherent) at a laser power of 1.18 mW. The incident angle of the excitation laser was adjusted at angles of 60—65° to decrease out-of-focus light. Emission was collected by the objective, passed through a sequence of a dichroic mirror (Chroma, 86100bs), a bandpass filter (Chroma, ZET405/488/561/640X), a long pass filter (Semrock, 647 nm RazorEdge® ultrasteep long-pass edge filter), and a SPINDLETM module including a phase mask (Double Helix LLC, Boulder) to transform the standard point spread function to double helix point spread function (DH-PSF) and finally directed to a cooled Electron-multiplying charge-coupled device (EMCCD) camera (iXon DU897). The EMCCD camera was operated at -70 °C to capture sequences of fluorescence images by a frame rate of 20 Hz at a high signal-to-noise ratio. In DH-PSF imaging, a standard 4f imaging system, with a phase mask in the Fourier plane of the image train, converts the conventional Airy disc PSF into two lobes of intensity whose relative azimuthal angle encoded information about the axial position of the emitter.



Figure A-3. Photograph of a silica inverse opal film. The green arrow indicates the direction of evaporation during the coating process. The field of view (observation area) in single-particle tracking measurements was approximately 100-300 μ m below the upper line of the inverse opal layer. The diameter of microscope glass slide is 25 mm.

The frame rate, 20 Hz, was an optimal setting that was sufficiently fast to track the confined diffusion while retaining a high signal-to-noise ratio. The 3D position of emitters was determined using DH-TRAXTM software and the positions of adjacent objects in consecutive images were strung together to generate molecular trajectories using a custom-developed algorithm. The localization uncertainty of DH-PSF depended on the number of photons detected. For the system studied here (involving fluorescent particles in inverse opals excited by a 647 nm diode laser at a laser intensity of about 1.18 mW), the number of photons detected fluorescent colloidal particles was in the range of 1000—3000, corresponding to x, y, z localization precision of approximately 5, 5, and 10 nm, respectively. We placed the field of view approximately 100-300 μ m below the upper surface of the inverse opal layers (Figure A-3) and moved the field of view parallel to the upper line in order to search for diffusing fluorescent particles that were confined in the inverse opal film. Scanning electron microscope measurements were performed at similar regions to determine the sizes of interconnecting circular holes.

Overall, diffusion and escape of fluorescent particles in fourteen inverse opal samples of varying interconnecting circular hole sizes were measured. For each sample, a series of approximately 100 independent movies (3 min each) were recorded at different regions within the inverse opals (the fields of view usually moved parallel to the upper line of the inverse opal layer). Despite the presence of surfactant, some immobile particles were observed to adsorb on the inner wall of the inverse opal, perhaps because of the well-recognized heterogeneity of nanoparticle surface properties, probably due to variation of functionalization and/or charge. The increased number of immobile particles over the course of the experiment caused overlapping of DH-PSF in the field of view and ultimately limited the time window for a given experiment to be 4-5 h, which was sufficient to accumulate data sets of diffusion and escape in inverse opals at a particle concentration of 10⁻¹⁴ M. DH-TRAXTM software was used to identify the positions of only the "mobile" particles. Typically, about 100 mobile trajectories, exploring at least three cavities, were successfully collected over the time window of a single tracking experiment.



3 µm

4 μm

Figure A-4. DH-PSF Images of a fluorescent bead at different axial positions (from left to right) 1, 2, 3, and 4 µm.

A.4 Calibration of the axial position

1 um

To calibrate the axial position using the relative azimuthal angle between the lobes, we imaged ~ 4 um diameter red fluorescent beads embedded within a polymer matrix (Thermo Fisher Scientific TetraSpeck kit T14792, NO. 4). The piezo stage was used to scan the objective lens axially through the sample in 0.2 μ m steps over a range of 4 μ m, recording 5 images at each step with an acquisition time of 0.05 s. A single bead in the slide was imaged and analyzed. As shown in Figure S4, the two lobes rotated by approximately 150 degrees over a 4 µm depth. The lateral (x, y) particle position was determined by

finding the midpoint of the two lobes' centers, and the *z* position was extracted by interpolating the relative azimuthal angle between two lobes from a cubic polynomial fit $\theta = a_1 * h^3 + a_2 * h^2 + a_3 * h + a_4$ where θ is the relative angle, *h* is the axial position, and a_1, a_2, a_3 and a_4 , are fitting parameters. A representative calibration curve is shown in Figure A-5.



Figure A-5. Calibration curve of relative azimuthal angles between two lobes as a function of the axial position.

A.5 Scanning electron microscope

The structures of inverse opal films on a microscope glass slide (Figure A-3) were imaged using a JEOL JSM-7401F scanning electron microscope operating at 2 kV and a 1 mA emission current. Because the microscope glass slide was nonconductive, electrostatic charging effects caused whitening during imaging, especially for large fields of view (Figure A-1). Large-scale images were still of sufficient quality to show that the inverse opal structure was extremely uniform over a large distances in the region approximately 100 - 300 um below the upper line of the inverse opal. In order to determine the size of the interconnecting circular holes, we performed systematic scanning electron microscope measurements at a low emission current, to reduce electrostatic charging effects.

The SEM characterization was carried out in the CU-Boulder nanomaterial characterization facility, which regularly performs SEM magnification calibration to decrease and eliminate SEM magnification

error. Moreover, we checked for any potential systematic error of the SEM magnification by imaging the template particles and cavity diameters. According to the manufacturer's data, the hydrodynamic diameter of the template particle is 522 nm, as determined by photon correlation spectroscopy. SEM measurements showed that the diameters of the template particle and cavity were 511 ± 10 and 515 ± 14 nm, respectively (Figure A-6). The standard deviation was <2%, consistent with the polydispersity index of 1.03 provided by the manufacturer. It is well known that the hydrodynamic radius is often greater than the true size. Therefore, this consistency reflected the accuracy of our SEM characterization.



Figure A-6. Size distribution of template particles. (a) Representative SEM image of the template particle. Size distribution of (b) = the template particle and (c) the silica inverse opal cavity. The solid lines in panels b and c represent a Gaussian fit.

A.6 Confocal microscope

Laser scanning confocal microscopy was used to image the three-dimensional pore network of the inverse opals. First, the index-matching liquid mixture described above (TDE/Triton X-100) was dyed with 10^{-8} M of Rodamine 6G. This dye was chosen because of its strong adsorption to the silica surface. The inverse opal was prepared on a microscope glass slide and permeated with the dye doping solution, which was then imaged using a Nikon A1R laser scanning confocal microscope. The acquired volumes were 256 × 256 pixels with a pixel size of 0.25 µm. A three-dimensional reconstruction was made using ImageJ software.

A.7 Criteria to measure the size of the interconnecting circular holes

Due to the angle of the SEM image relative to the interconnected cavity network, the planar projection of the circular holes connecting the spherical cavities appear as elliptical areas whose major axis is the actual diameter of the hole. Therefore, the black areas were modeled as ellipses to determine the major axis length. SEM images were first converted into a 8-bit gray image, and a threshold was defined to distinguish the black area from the background. The black areas thus identified were fit to elliptical shapes to determine the outer contour lines. The major axis was then obtained by measuring the fitted ellipse. The distribution of the identified major axis length in a SEM image is shown in Figure S6. For each inverse opal sample, at least three SEM images were used to do the fitting and the obtained values were averaged. Finally, we used the maximum likelihood estimation to obtain the mean value, which denoted as the diameter of the interconnecting circular hole for this inverse opal sample.



Figure A-7. Size distribution of inverse opal holes. (a) A representative SEM image for identification of the hole size. The black scale bar denotes 500 nm. (b) A representative hole size distribution.

A.8 Criteria to identify the escape events

As shown in Figure 1d of the main text, nanoparticle trajectories reflected the inner shape of inverse opals void space. Therefore, we extracted the sojourn time in each cavity according to spatial position. In doing so, we used a maximum allowed displacement method to calculate the sojourn time in a given cavity. In particular, the amplitude of the time-dependent position fluctuation for a tracer particle confined in a given cavity was assumed to be related to the cavity diameter. The center of the first cavity was defined by averaging the particle position in x, y, and z dimensions. An escape event was identified when the position of the particle relative to the center of the cavity exceeded a maximum allowed displacement, i.e., a threshold that was closely associated with the cavity diameter. For an inverse opal with a cavity diameter of 522 nm, a threshold of 500 nm was found to be optimal. After an escape event, a new center is set by averaging the following particle positions in three dimensions, and the process was repeated until the end of the trajectory was reached. This method was robust with respect to variations in the data analysis parameters. For example, changing the distance threshold used to define immobilization by 40 nm still resulted in the correct identification of escape events.

A.9 Theoretical calculations of the escape time

We employed two recent theoretical models to calculate the mean first passage time (MFPT) of a Brownian particle in a spherical cavity that contains multiple holes on the boundary. Conceptually, both methods calculated how long a Brownian particle takes to find a target by exploiting stochastic processes. There is a subtle but important difference between the definition of MFPT and sojourn time. The MFPT is defined as the time a particle takes to search and reach any one of the holes on the boundary. The sojourn time is defined as the time taken required to leave the cavity. Thus, compared to the MFPT, the sojourn time may include an additional term that takes into account the time a particle takes to pass through the hole (translocation). For a zero-dimensional pointlike particle that has no volume, the MFPT is theoretically equivalent to the sojourn time. However, in realistic systems, all particles have non-zero size; the effect of steric confinement should be taken into account, resulting in an increase of the overall time required for escape.

First, we followed a method derived by Cheviakov et al. that calculated the mean first passage time from a unit sphere having multiple holes on the boundary as follows,

$$MFPT = \frac{V}{4LDN} \left[1 + \frac{L}{\pi} log(\frac{2}{L}) + \frac{L}{\pi} \left(-\frac{9N}{5} + 2(N-2)\log 2 + \frac{3}{2} + \frac{4}{N}H(x_1, \dots, x_N) + O(L^2\log L)\right]\right]$$

where *V* is the volume of the cavity, *L* and *N* are the radius and number of the holes, *D* denotes the diffusion coefficient of the particle, the third therm includes the function $H(x_1,...x_N)$:

$$H(\mathbf{x}_{1},...\mathbf{x}_{N}) = \sum_{i=1}^{N} \sum_{j=i+1}^{N} \left(\frac{1}{|\mathbf{x}_{i} - \mathbf{x}_{j}|} - \frac{1}{2} \log |\mathbf{x}_{i} - \mathbf{x}_{j}| - \frac{1}{2} \log(2 + |\mathbf{x}_{i} - \mathbf{x}_{j}|) \right)$$

where x indicates the position of the *n*th hole. Using the experimental parameters as inputs, including $V = 4\pi R^3/3 = 0.065$ where R is the radius of the cavity, $D_{confined} = 0.04 \,\mu\text{m}^2/\text{s}$ in confinement, N = 12, and the assumption of a hexagonally arranged hole distribution on the boundary. This expression is expected to be valid when LN/R <<1 where R is the radius of the cavity. We calculated the asymptotic result using both the two-term and three-term predictions as a function of L in the range 2.4 < LN/R < 4.8, as shown in Figure A-7. The result with LN/R=1 is shown in Figure A-7. These two calculations exhibit small difference. However, the calculation result is approximately two orders of magnitude smaller than the experimental ones. Although LN/R in our case is not in the valid range as described by the authors, the large inconsistency with experimental results suggests that this theoretical calculation of MFPT does not provide even a rough estimate for the experimental escape process.



Figure A-8. Mean first passage time as a function of ε using two different methods. The parameter used is described in the text of the supporting information.

A theory derived by Lagache et al. was also used to estimate the MFPT. In this case, a stochastic process described by the Survival Fokker–Planck equation was developed to study the reverse problem, i.e., a Brownian particle moves from an outer space into a smaller, confined space having many entry holes on the boundary. The method is based on the Neumann–Green's function, for the case in which there are many small holes (N > 1000) on the smaller confined space. If we set the size of two confined spaces to be equal, this method can calculate the MFPT of a Brownian particle in a confined cavity to any one of the holes on the boundary. The formula is given as follows,

$$MFPT = \frac{V}{D} \left(\frac{1}{4\pi R} + \frac{f(\sigma)}{4\pi L} \right)$$

and

$$f(\sigma) = 1 - 8\frac{\sigma}{\pi} + \frac{L}{a\pi}(1 - 4\sigma)\log(\frac{L}{R}) + o(\frac{L}{R})$$

is the correction term, where σ is the overall surface coverage of the hole. Using Eq. S3 with experimental parameters as inputs, we obtained the asymptotic result of the MFPT as a function of *L* for escape from a cavity having randomly or uniformly distributed holes. The results as shown in Figure S7 are systematically smaller than those of the Cheviakov method, likely due to the difference in the treatment of the boundary conditions. Most importantly, both methods fail to reproduce the experimental findings; both theoretical treatments underestimate the MFPT by approximately two orders of magnitude. These large differences indicated that Brownian motion with a reduced diffusion coefficient was not the reason for the long sojourn times.

A.10 Random walk simulation procedure

Here we describe the random walk simulation in a spherical cavity having twelve holes on the surface. The generalized random walk simulations were performed on a three-dimensional Cartesian coordinate system. The walker was initially placed at the origin at the initial time t = 0. The simulation time step was dt = 0.05 s. The total simulation time of each walk was 180 s. Each step was drawn from

a Gaussian distribution
$$P = (4\pi D\Delta t)^{-1/2} \exp\left(-\frac{r^2}{4D\Delta t}\right)$$
 where P is the probability, r represents

displacement in a time interval Δt , and D is the diffusion coefficient of a tracer particles proteins in inverse opals as determined by experimental results at short lag time regimes, shown in Figure 2a in the main text. The center of the spherical cavity locates at the origin with a radius of R = 250 nm.

The origin of the coordinate system was defined as the center of the cavity. In order to create twelve uniformly distributed holes on this cavity, we considered a cube centered at the origin with an edge length of $\sqrt{2R}$. The twelve edge centers of this cube were used to define the centers of twelve holes with varying diameter *L*. The walkers were sterically confined by the boundary of the cavity. The walker could escape from the cavity only through a hole. The sojourn time was then defined as the average time that walkers spent in a cavity prior to escape. More than ten thousand trajectories were simulated in a cavity with varying *L* to yield statistically meaningful results of the cumulative sojourn time distribution.

In order to consider a presumably dipolar repulsion between particle and wall, the random walk simulation was modified to include a sextic-polynomial-like repulsive potential, A_{rep} , between particle and wall; probabilities of steps in a direction to the surface or to the center were biased by the presence of repulsions. In particular, the probability of moving towards the boundary was given as $P=0.5-A_{rep}/(r+r_0)^6$ where *r* denotes the distance to the boundary, and a small intercept $r_0 = 5$ nm was defined to infinite repulsion at the boundary. The only adjustable parameter was A_{rep} . The model reduced to Brownian motion in confinement in the limit of $A_{rep} = 0$ while increasing A_{rep} represents an increase in the strength of particle-wall repulsion. By setting $A_{rep} = 3 \times 10^{-6}$, excellent agreement was achieved with experimental observations.

A.11 Multi-particle collision dynamics simulation procedure

In our model system, solvent molecules were described as point-like particles through the multiparticle collision dynamics (MPCD) algorithm, while the probe nanoparticle was modeled by standard molecular dynamics (MD). As shown previously, the hybrid approach MPCD is suitable to describe systems involving hydrodynamic interactions (HIs). The MPCD algorithm consists of alternating streaming and collision steps. In a streaming step, solvents move ballistically within the time interval h between collisions and the position of solvent i is updated using the expression

$$\mathbf{r}_i(t+h) = \mathbf{r}_i(t) + \mathbf{v}_i(t)h$$

where $r_i(t)$ and $v_i(t)$ denote the position and the velocity of a solvent particle *i* at time *t*, respectively. In a collision step, all particles are sorted into cubic cells with a side length *a*, and their velocities relative to the center-of-mass velocity of the cell are rotated by a fixed angle ϕ around a randomly oriented axis using the expression

$$\mathbf{v}_i(t+h) = \mathbf{v}_i(t) + (\mathbf{R}(\phi) - \mathbf{E})(\mathbf{v}_i(t) - \mathbf{v}_{cm}(t))$$

where v_{cm} denotes the center-of-mass velocity of all particles in the cell, $R(\phi)$ is the rotation matrix, *E* is the unit matrix. During a collision, the mass, local momentum, and kinetic energy are conserved. To guarantee Galilean invariance, a random shift of the grid of collision cells is performed prior to each collision step.

In the simulation, the escape of a single nanoparticle of mass M is considered from a cavity of diameter d_c through one of twelve holes of diameter d_h at the cavity surface. The cavity is fixed in the cubic simulation box with box size L_b . Periodic boundary conditions are applied in all directions. The bounce-back rule is applied between the cavity wall and the solvent, signifying that the velocity of the solvent is inverted from v to -v when approaching the wall. The interactions between the colloid particle with the tube wall is modeled by the Weeks-Chandler-Andersen (WCA) potential:

$$U_{cw}(r) = \begin{cases} 4\varepsilon_{cw} \left[\left(\frac{\sigma_{cw}}{r} \right)^{12} - \left(\frac{\sigma_{cw}}{r} \right)^{6} \right] + \varepsilon_{cw}, & r \le 2^{1/6} \sigma_{cw} \\ 0 & \text{else} \end{cases}$$

where *r* denotes the distance between colloid and the wall, s_{cw} is the colloid-wall collision diameters, and e_{cw} governs the strength of the potential. Similarly, the colloid-solvent interaction potential takes the WCA form

$$U_{cs}(r) = \begin{cases} 4\varepsilon_{cs} \left[\left(\frac{\sigma_{cs}}{r}\right)^{12} - \left(\frac{\sigma_{cs}}{r}\right)^{6} \right] + \varepsilon_{cs}, & r \le 2^{1/6} \sigma_{cs} \\ 0 & \text{else} \end{cases}$$

where s_{cs} is the colloid-solvent collision diameter, e_{cs} governs the strength of potential. The Velocity-Verlet algorithm is used to integrate Newton's equation of motion of colloid particle and solvents with a time step h_{p} . A local Maxwellian thermostat is used to maintain the temperature at the desired value.

The MPCD simulation can be simplified as a standard Brownian dynamics (BD) simulation, the colloid particle is subjected to conservative, frictional, and random forces, with

$$M\ddot{\mathbf{r}} = -\nabla U_{cw} - \zeta v + F^R$$

where ζ is the friction coefficient, v is the colloid's velocity, and $F^{\mathbb{R}}$ is the random force which satisfies the fluctuation-dissipation theorem.

Parameters The simulation parameters are chosen as follows: $f = 130^{\circ}$, the side length of the cell a = 1, the average number of solvent particles in each cell is r = 10, the colloid radius $r_{cc} = 1$ and the colloid diameter $s_{cc} = 2$, the solvent mass m = 1, the colloid mass $M = 4pr_{cc}{}^3r/3 \approx 42$, $s_{cw} = s_{cs} = 2$, $e_{cw} = e_{cs} = 1$; the box size $L_b = 24$, $d_c = 10s_{cc} = 20$, d_h ranges from 2.5s_{cc} to $4s_{cc}$, $k_BT = 1$, $h = 0.1(ma^2/k_BT)^{1/2} = 0.1$, $h_p = 0.025(ma^2/k_BT)^{1/2} = 0.025(ma^2/k_BT)^{1/2}$

twelve holes are
$$(0, -\frac{\sqrt{3}}{3}, -\frac{\sqrt{6}}{3}), (\frac{1}{2}, \frac{\sqrt{3}}{6}, -\frac{\sqrt{6}}{3}), (-\frac{1}{2}, \frac{\sqrt{3}}{6}, -\frac{\sqrt{6}}{3}), (1,0,0), (\frac{1}{2}, \frac{\sqrt{3}}{2}, 0), (-\frac{1}{2}, \frac{\sqrt{3}}{2}, 0)$$

1,0,0),
$$(-\frac{1}{2}, -\frac{\sqrt{3}}{2}, 0)$$
, $(\frac{1}{2}, -\frac{\sqrt{3}}{2}, 0)$, $(0, \frac{\sqrt{3}}{3}, \frac{\sqrt{6}}{3})$, $(-\frac{1}{2}, -\frac{\sqrt{3}}{6}, \frac{\sqrt{6}}{3})$, $(\frac{1}{2}, -\frac{\sqrt{3}}{6}, \frac{\sqrt{6}}{3})$, respectively. The

positions of the holes are selected based on the hexagonal close-packed structure of the spherical cavity in experiments. For each data presented, we averaged on the order of 5000 samples. At the beginning of the simulation, we put the particle at the center of the cavity.



Figure A-9. Schematic diagram of MPCD simulations. (a) Schematic illustration of a colloid particle in a cavity with twelve holes on the surface. Blue and black spheres represent a colloid particle and the solvents, respectively. (b) Representative plots of MSD versus lag time using BD and MPCD simulation at L/R = 3 where L and R denote the diameter of hole and tracer particle, respectively.

A.12 Table and Figures Mentioned in the Main Text



Figure A-10. Representative trajectories of a passive nanoparticle in inverse opal. (a) Two-dimensional projection of a trajectory in an inverse opal film, illustrating the fact that escape events cannot be robustly identified from two-dimensional trajectories. (b) Actual 3D motion for the trajectory shown in panel (a).



Figure A-11. Sojourn time distributions of simulation results of nanoparticle cavity escape in inverse opal. Cumulative distribution of sojourn times at L/R = 3 using BD and MPCD simulations.



Figure_Apx A-12. Sojourn time distributions of nanoparticle cavity escape in inverse opal under various salt conditions. Cumulative distribution of tracer particle sojourn times as a function of NaCl concentration for a cavity with window size L=150 nm.



Figure A-13. The radial probability distribution (P_o) of confined particles as a function of the distance to the center of cavity. Black symbols represent experimental results and red symbols represent results from BD simulations. The vertical line denotes the radial position of the outer cavity wall.

Table A-1. Relevant microscopic interactions for nanoparticle transport in inverse opals under nonaqueous solvent condition.

Interactions	Description
Hydrodynamic interaction	Particles moving in a fluid stimulate long-range flows that
	can influence the motion of other particles, and themselves
	(through back-flow).
Entropic effects	Entropic (excluded volume) effects are ubiquitous in a
	high surface area medium that limits the volume accessible
	to its diffusing components
Coulomb interaction	Attractive or repulsive interactions may be caused by
	opposite or like net charge, respectively. These
	interactions are screened in electrolyte solutions. While
	such interactions are most common in aqueous solutions,
	a growing body of evidence supports the presence of
	electrostatic repulsion in non-aqueous solutions although
	the precise mechanisms are not completely understood.
Dipole interaction	Recent observations demonstrate that dipole-dipole
	interactions may be significant for objects having large

	dipole moments in a medium with a small dielectric
	constant.
van der Waals interaction	This attractive force is generated by induced dipolar interactions between atoms, molecules and/or particles.
	These interactions are ubiquitous, but relatively weak
	except at short range.
Hydrogen bonds	A weak non-covalent interaction between two molecules
	having a proton in one molecule and an electronegative
	atom in the other.
Other non-covalent interaction	These interactions include various short-range
	intermolecular forces including π - π interactions,
	hydrophobic effects, cation- π , anion- π , etc.
Appendix B: Supporting Information for Chapter 3

B.1 Calibration of the axial position

We imaged ~ 4 µm diameter red fluorescent beads embedded within a polymer matrix (Thermo Fisher Scientific TetraSpeck kit T14792, NO.4), to calibrate the axial position using the relative azimuthal angle between the lobes. The piezo stage was used to scan the objective lens axially through the sample in 0.15 µm steps over a range of 3 µm, recording 5 images at each step with an acquisition time of 0.05 s. The z position was extracted by interpolating the relative azimuthal angle between two lobes from a cubic polynomial fit $\theta = a_1h^3 + a_2h^2 + a_3h + a_4$, where θ is the relative angle, h is the axial position, and a_1 , a_2 , a_3 and a_4 , are fitting parameters. A representative calibration curve is shown in Figure S4. For this example, the fitting function was $\theta = -7.8126h^3 - 4.9858h^2 + 77.732h + 97.467$.



Figure B-1. Calibration curve of relative azimuthal angles between two lobes as a function of the axial position

B.2 Criteria to measure the size of interconnecting circular holes

The structures of inverse opal films on a microscope glass slide were imaged using a JEOL JSM-7401F scanning electron microscope.

<u>Cavity size measurement</u>: The size of the template particles was measured before use, these polystyrene particles had an extremely uniform size distribution according to SEM measurement (Figure S2a), with a mean diameter of 495 nm (Figure S2b). This value from SEM measurement was slightly smaller than the value provided by manufacturer, due to the different measurement technique. Specifically, the manufacturer used photon correlation spectroscopy (Coulter Nanosizer N4 Plus) to measure the hydrodynamic radius, which is expected to be slightly larger than geometrical measurements using SEM. The cavity size of the inverse opal structure also measured using SEM, had a mean diameter 494 nm (Figure S2c), very similar to the size of templating particles. These measurements confirm the consistent accuracy of SEM measurement.



Figure B-2. Size distributions of templating nanoparticles. (a) A representative SEM picture of templating nanoparticle. (b)Templating particle size distribution. (c) Silica inverse opal cavity size distribution.

<u>Hole size measurement</u>: Due to the angle of the SEM image relative to the interconnected cavity network, the planar projection of the circular holes connecting the spherical cavities appears as elliptical areas whose major axis is the actual diameter of the hole. Therefore, the black areas (Figure 1b in main content) were modeled as ellipses to determine the major axis length. SEM images were first converted into 8-bit gray images, and a threshold was defined to distinguish the black areas from the background. The black areas

thus identified were fit to elliptical shapes to determine the outer contour lines. The major axis was then obtained by measuring the fitted ellipse. The distribution of the identified major axis length in a SEM image is shown in Figure S3. For each inverse opal sample, at least three SEM images at different location of the sample were used to perform the hole size analysis. Maximum likelihood estimation was used to determine the mean value of the measurement, and this was denoted as the diameter of the interconnecting circular hole for this inverse opal sample.



Figure B-3. Size distributions of inverse opal holes. (a) Hole size measurement with a mean hole size of 127 nm, and a standard deviation of 8 nm. (b) Hole size measurement with a mean hole size of 149 nm, and a standard deviation of 7 nm.

B.3 Criteria to identify escape events

Nanoparticle trajectories reflected the inner shape of the inverse opal structure; therefore by analyzing the spatial positions of nanoparticles, we were able to differentiate confinement in the cavity from hopping between adjacent cavities in order to measure sojourn times. Specifically, we used a maximum allowed displacement method to separate the trajectories into different states, which corresponded to confinement in different cavities. Escape events occurred at transitions between states. Specifically, the amplitude of the time-dependent position fluctuations for a nanoparticle confined in a given cavity was related to the cavity diameter. The center of the first cavity was defined by averaging the particle position in x, y and z dimensions. An escape event was identified when the position of the particle

relative to the center of the cavity exceeded a maximum allowed displacement, i.e., a threshold that was associated with the cavity diameter. For an inverse opal with a cavity diameter of 500 nm, a threshold of 500 nm was used. After an escape event, a new center was set by averaging the following particle positions in three dimensions, and the process was repeated until the end of the trajectory was reached. This method was robust with respect to variations in the data analysis parameters. For example, changing the threshold by \pm 20 nm, still resulted in the correct identification of escape events.

B.4 Theoretical calculation of the energy barrier at the hole entrance

The potential energy between the nanoparticle and the hole of inverse opal was evaluated using the approach described by Bowen et al. based on Derjaguin's approximation, yielding

$$U(h_{0}) = U_{e}(h_{0}) + U_{m}(h_{0})$$

= $\frac{\pi\epsilon_{0}\epsilon_{r}}{g} ((\psi_{p} + \psi_{m})^{2} \ln(1 + \exp(-\kappa h_{0})) + (\psi_{p} - \psi_{m})^{2} \ln(1 - \exp(-\kappa h_{0}))) - \frac{A}{6gh_{0}}$

where κ is the inverse Debye length, ϵ_0 is the permittivity of the vacuum and ϵ_r is the relative dielectric constant of the medium. ψ_p is nanoparticle surface potential, ψ_m is the inverse opal surface potential, A is the Hamaker constant. h_0 is the closest distance between wall and nanoparticle, g is the steric factor determined by the geometrical characteristics of the system.

Here, we set ϵ_r 46.98, which is the permittivity of 90wt% glycerol/water mixture, ψ_p and ψ_m as -25.7 mV. The Hamaker constant A was 2.36*10⁻²¹J and κ^{-1} equals to 0.96 nm for 0.1 M salt concentration solvent, 3.04 nm for 0.01 M salt concentration solvent, and 9.6 nm for 0.001 M salt concentration solvent.

As described in the main text, this model divides the hole area into three regions. In region I, the particle interacts with a flat plate adjacent to the hole; in region II, the particle interacts with the curved entrance of the pore, and in region III, the particle interacts with the cylindrical inner surface of the hole. Here, we focus only on the area of the hole entrance, which is the buffer zone between region II and region

III. The interaction energy in this buffer zone was assumed to be proportional to the fractions of the particle surface projected on the appropriate part of the inverse opal surfaces.

For different region, we used different steric factors, g.

In region I,
$$g_1 = \frac{1}{a}$$
.

In region II,
$$g_2 = \sqrt{\left(\frac{1}{a} + \frac{1}{b}\right)\left(\frac{1}{a} - \frac{1}{r_0 + b}\right)}$$

In region III,
$$g_3 = \sqrt{\frac{1}{a} \left(\frac{1}{a} - \frac{1}{r_0}\right)}$$
.

Here, a is the radius of nanoparticle, equal to 23 nm, r_0 is the hole radius, b is the radius of curvature of the rounded edge of the hole entrance. We used the standard deviation of hole size from SEM measurements as the b value, which was about 5% of the hole size.

The calculations were relatively insensitive to the specific choice of b. For example, when we changed the value from 2% of the hole size to 8% of the hole size, the effective hole size only changed from 100 nm to 99 nm (Figure B-6b).

Using the above parameters and equations, the potential energy near the hole was calculated, then divided k_BT to obtain the dimensionless potential energy. Figure B-4 shows a representative potential energy plot of 46 nm nanoparticle near a 149 nm hole entrance with three different salt concentrations. From this calculation, we can also obtain the potential energy plot for a nanoparticle at the hole entrance (Figure S6a). In this case, the width of the apparent well 53 nm; adding the nanoparticle size of 46 nm, gave an effective hole size for this case of 99 nm.



Figure B-4. Three-dimensional plots of the potential energy in the hole area. (a) 0.1M salt concentration with 149 nm hole size. (b) 0.01M salt concentration with 149 nm hole size. 0.001M salt concentration with 149 nm hole size.



Figure B-5. Potential energy contour plot. Contour plot of dimensionless potential energy of 46 nm nanoparticle near the hole entrance for 149nm hole size. The immersion solution is contained 0.001 M salt.



Figure B-6. Dimensionless potential energy as a functional of tracer particle position. (a) Plot of dimensionless potential energy for a 46 nm nanoparticle at the entrance of a 149 nm hole, the immersion solution is contained 0.001 M salt. (b) Plot shows the lack of sensitivity of the potential energy calculation to the specific choice of the b value.

B.5 Random walk simulation procedure

Here we describe the random walk simulation in a spherical cavity with twelve holes on the surface. The generalized random walk simulations were performed on a three-dimensional Cartesian coordinate system. The walker was initially placed at the origin at the initial time t = 0. The simulation time step was dt = 0.05 s. The total simulation time of each walk was 180 s. Each step was drawn from a Gaussian distribution where P is the probability, r represents displacement in a time interval Δt , and D is the diffusion coefficient of a tracer particles in inverse opals as determined by experimental results at short lag time regimes, shown in Figure 1a in the main text. The center of the spherical cavity is located at the origin with a radius of R = 250 nm.



Figure B-7. Representative example of random walk simulation of confined nanoparticle diffusion in an inverse opal structure

The origin of the coordinate system was defined as the center of the cavity. Twelve holes were created on the cavity surface to mimic the inverse opal structure. The position of the centers of the twelve holes were:

$$\left(0, -\frac{\sqrt{3}}{3}R, -\frac{\sqrt{6}}{3}R\right), \left(\frac{R}{2}, \frac{\sqrt{3}}{6}R, -\frac{\sqrt{6}}{3}R\right), \left(-\frac{R}{2}, \frac{\sqrt{3}}{6}R, -\frac{\sqrt{6}}{3}R\right), (R, 0, 0), \left(\frac{R}{2}, \frac{\sqrt{3}R}{2}, 0\right), \left(-\frac{R}{2}, \frac{\sqrt{3}R}{2}, 0\right), \left(-\frac{R}{2}, -\frac{\sqrt{3}R}{2}, 0\right), \left(-\frac{R}{2}, -\frac{\sqrt{3}R}{2}, 0\right), \left(0, \frac{\sqrt{3}}{3}R, \frac{\sqrt{6}}{3}R\right), \left(-\frac{R}{2}, -\frac{\sqrt{3}}{6}R, \frac{\sqrt{6}}{3}R\right), \left(\frac{R}{2}, -\frac{\sqrt{3}}{6}R, \frac{\sqrt{6}}{3}R\right)$$

respectively. The walkers were sterically confined by the boundary of the cavity. The walker could escape from the cavity only through a hole. The sojourn time was then defined as the average time that walkers spent in a cavity prior to escape. More than ten thousand trajectories were simulated in a cavity with varying L to yield statistically meaningful results of the cumulative sojourn time distribution.

B.6 Table and figures mentioned in the main text

Mean hole	Standard	Salt concentration	Debye	Mean	Effective hole	Hole size	Effective hole size
diameter L	deviation of	(M)	length	sojourn time,	size from	difference,	from calculation
(nm)	hole		(nm)	$T_{soj}(s)$	simulation, L _s	L-L _s (nm)	using DLVO theory,
	diameter				(nm)		L _c (nm)
	(nm)						
127	8	0.001	9.60	19.5 +/-0.47	86	31	76
127	8	0.01	3.04	7.4 +/- 0.92	107	20	112
127	8	0.1	0.96	4.0 +/- 0.20	123	4	123
149	7	0.001	9.60	9.5 +/- 0.33	101	48	99
149	7	0.01	3.04	4.2 +/- 0.09	125	24	135
149	7	0.1	0.96	2.6 +/- 0.15	149	0	146

Table B-1. Data of sojourn time for different hole size and salt concentration.



Figure B-8. Complementary cumulative distribution of sojourn times for different hole sizes and salt concentrations. The lines represent a double exponential fitting function $P(t)=A*exp(-t/T_1)+(1-A)*exp(-t/T_2)$, permitting the calculation of the mean sojourn time, $T_{soj} = A*T_1+(1-A)*T_2$.



Figure B-9. Long-time diffusion coefficient vs. R₂/6T_{soj} for different hole sizes and salt concentrations.



Figure B-10. Distribution of the nanoparticle position when confined in the cavity at different salt concentrations. The standard deviations of are 103 nm, 110 nm, 114 nm, for 0.001 M, 0.01 M, and 0.1 M salt, respectively.



Figure B-11. Dimensionless potential energy when the nanoparticle is located in the entrance plane of a hole. The horizontal axis represents the lateral position of the center of the nanoparticle. The black dashed line indicates the value (potential energy) / $k_BT=1$. Three values of nanoparticle surface potential used to calculate the potential energy for the nanoparticle when located in the entrance plane of the hole, for holes 149 nm in diameter connecting cavities 500 nm in diameter, at 0.001 M, 0.01 M and 0.1 M salt concentrations. This figure indicates that the potential energy calculation is relatively insensitive to the nanoparticle surface potential over a reasonable range.



Figure B-12. Mean sojourn time vs. effective pore size calculated based on an electrostatic energy barrier model at the hole entrance. Three energy values, $0.8k_BT$, $1k_BT$, $1.2k_BT$ were used as criteria to determine the effective pore size here. This figure indicates that chose $1k_BT$ as the criterion is reasonable, and the results are relatively insensitive to the specific choice of the criterion.

Appendix C: Supporting Information for Chapter 4

C.1 Measurement of inverse opal structure

The silica inverse opals prepared by the method described in the main context were high-quality and crack-free over 100 µm scales. **Figure C-1** shows representative SEM images of inverse opals. Notably, some defects like blocked holes in the sample were observed. The inverse opal films were imaged using a JEOL JSM-7401F scanning electron microscope.



Figure C-1. SEM images of inverse opal films. (a) Scanning electron microscopy image of a silica inverse opal film (cavity diameter is 500nm) at 5000X magnification showing a highly uniform structure. (b) Scanning electron microscopy image of a silica inverse opal film. The red box indicates a cavity with a blocked hole.

<u>Cavity size measurement</u>: The size of the template particles was measured before use, and were found to have an extremely uniform size distribution. For example, the nominal 500 nm diameter polystyrene beads exhibited a mean diameter of 495 nm from SEM observations, with a narrow size distribution (**Figure C-**2a, b). The value from SEM measurement was close to the value provided by manufacturer (hydrodynamic mean diameter 502 nm), which was measured by photon correlation spectroscopy (Coulter Nanosizer N4 Plus). The hydrodynamic diameter was expected to be slightly larger than the geometrical measurements using SEM. In addition, the cavity size of the resulting inverse opals, also measured using SEM, had a

mean diameter of 494 nm (Figure C-2c), again very similar to the size of template particles. These measurements confirmed the consistent accuracy of SEM measurement.



Figure C-2. SEM images of templating nanoparticles. (a) A representative SEM picture of template nanoparticles. Scale bar is 1 μ m. (b)Template particle size distribution. (c) Silica inverse opal cavity size distribution.

Hole size measurement: The connecting holes in the inverse opals appear as elliptical areas since the SEM images capture the planar projection of the circular holes. Therefore, the black areas (**Figure C-1b**) were modeled as ellipses to determine the major axis length, which corresponds to the actual diameter of the hole. In particular, SEM images were first converted into 8-bit gray images, and a threshold was defined to distinguish the black areas from the background. Then the identified black regions were fitted to elliptical shapes to determine the outer contour lines. The major axis was then obtained by measuring the fitted ellipse. For each inverse opal sample, at least three SEM images at different locations of the sample were used to perform the hole size analysis. Maximum likelihood estimation was used to determine the mean value of the measurement, which was used as the diameter of the interconnecting hole for given inverse opal sample. Notably, closed or nearly closed holes were excluded from these measurements. **Figure C-3** shows two representative measurement distributions.



Figure C-3. Inverse opal hole size distributions. (a) Hole size measurement with a mean hole size of 127 nm, and a standard deviation of 8 nm. (b) Hole size measurement with a mean hole size of 149 nm, and a standard deviation of 7 nm.

C.2 Criteria to identify escape events

We used the maximum allowed displacement method to identify escape events and extract the corresponding sojourn times. In this method, we distinguished the two states (i.e., confined diffusion in a given cavity and hopping between adjacent cavities) by analyzing the fluctuations of the time-dependent particle positions in three-dimensions. In particular, the amplitude of the time-dependent position fluctuations for a confined nanoparticle in a cavity was directly related to the cavity diameter. Therefore, the center of the first cavity was defined by averaging the particle position in X, Y and Z dimensions. An escape event was identified when the position of the particle relative to the center of the cavity exceeded a maximum allowed displacement (i.e., a threshold that was associated with the cavity diameter). As an example, for the inverse opals with 500 nm diameter cavities, 500 nm was used as the maximum allowed displacement threshold. The choice of threshold was robust; for example, changing the threshold by +/- 20 nm, still enabled the identification of escape events correctly. After an escape event, a new center was set by averaging the following particle positions in three dimensions. This process was repeated until the end of the trajectory was reached. This was an effective, computationally efficient, and robust method to distinguish confined diffusion in given cavity and hopping between adjacent cavities.

C.3 Random walk simulations in inverse opals (macroscopic process)

Here we describe the random walk simulation in an interconnected inverse opal network; the goal of these simulations was to understand the particle transport mechanism in the long lag time regime and to extract the corresponding long-time diffusion coefficient. At long lag times, nanoparticles hopped among different cavities with escape times following a certain distribution controlled by the geometric conditions of the inverse opals and the solution conditions, giving rise to an approximately diffusive behavior. To mimic this behavior, random walk simulations were performed in a three-dimensional Cartesian coordinate system with a well-defined inverse opal network. The random walker was initially placed at the origin at t = 0. The origin was defined at the center of the starting cavity, twelve holes were created on the cavity surface to mimic the connecting holes of an inverse opal structures. The position of the twelve holes centers

were:
$$\left(0, \frac{-2\sqrt{3}}{3}R, \frac{-2\sqrt{6}}{3}R\right), \left(R, \frac{\sqrt{3}}{3}R, \frac{-2\sqrt{6}}{3}R\right), \left(-R, \frac{\sqrt{3}}{3}R, \frac{-2\sqrt{6}}{3}R\right), (2R, 0, 0), \left(R, \sqrt{3}R, 0\right), \left(-R, \sqrt{3}R, 0\right), \left(-2R, 0, 0\right), \left(-R, -\sqrt{3}R, 0\right), \left(0, \frac{2\sqrt{3}}{3}R, \frac{2\sqrt{6}}{3}R\right), \left(-R, \frac{-\sqrt{3}}{3}R, \frac{2\sqrt{6}}{3}R\right), \left(R, \frac{\sqrt{3}}{3}R, \frac{2\sqrt{6}}{3}R\right), \left(R, \frac{\sqrt{3}}{3}R, \frac{\sqrt{6}}{3}R\right), \left(R, \frac{\sqrt{3}}{3}R, \frac{\sqrt{6}}{3}R\right), \left(R, \frac{\sqrt{3}}{3}R, \frac{\sqrt{3}}{3}R\right), \left(R, \frac{\sqrt{3}}{3}R, \frac{\sqrt{$$

respectively. The walker was able to escape from a given cavity to an adjacent cavity only through one of these twelve holes. Therefore, for each simulation step, the algorithm generated a random number between 1 and 12, indicating the escape direction, and a time value indicated the sojourn time in the given cavity. Specifically, the random number generation employed a uniform distribution to represent that equal probabilities of escape through the twelve holes, while the randomly generated sojourn time was drawn from the distribution obtained from experiments, which depended on the geometric conditions, solution conditions and any other potential effects. Based on the randomly generated direction number and time value, the particle was placed in the center of the new cavity, and these steps were repeated until the end of the simulation. Figure C-4 shows one simulated trajectory and its corresponding time-dependent Cartesian particle coordinates. Thousands of trajectories were simulated to generate a long-time diffusion coefficient for each condition (i.e., a defined cavity diameter and sojourn time distribution).



Figure C-4. Representative random walk simulations of nanoparticle diffusion in porous networks. (a) Representative random walk simulation of nanoparticle diffusion in a simplified inverse opal network structure. (b) Time-dependent particle position in X, Y and Z dimensions of the trajectory in (a).

C.4 Random walk simulation in inverse opals (single cavity process)

Here we describe the random walk simulation in a spherical cavity with twelve holes on the surface. The goal was to understand the effects of hole size variation on the escape process. In particular, the 12 holes on the surface were categorized into two groups; 6 holes were chosen as control holes, and held at the same hole size (L) for the entire simulation procedure, while the other 6 holes had a larger hole size (L+ Δ L). The simulations were performed in a 3D Cartesian coordinate system, with the random walker initially placed at the origin. The walker underwent random motion in the cavity, and the simulation ended when the walker escaped the cavity through one of the holes, with the specific hole being identified. After 5000 simulations for each hole size condition, the probability ratio of translocating through large holes to small holes was calculated. **Figure C-5**a shows a representative example of a random walk simulation of confined nanoparticle diffusion in an inverse opal structure. **Figure C-5**b shows the effects of hole size difference on the probability ratio of escaping through large holes. Simulation conditions: cavity diameter 500 nm, baseline hole size L=110 nm, tracer particle diameter 46 nm (hydrodynamic diameter). As a representative example, when the hole size variation was approximately 20 nm, the probability to escape through larger pores was approximately 2-fold larger than through small holes.



Figure C-5. Representative random walk simulations of a confined nanoparticle escape from an inverse opal cavity. (a) Representative example of random walk simulation of a confined nanoparticle escape from an inverse opal cavity. (b) The probability ratio of a confined nanoparticle translocating through large holes to small holes as a function of hole size difference, where the small hole diameter was L=110 nm.

C.5 Random walk simulation in a 2D lattice bond percolation model

Here we describe the random walk simulation in a 2D square lattice bond percolation model. The goal of this simulation was to understand the effects of reduced pore connectivity on the long-time diffusion coefficient. The 2D lattice bond percolation model is a classic model to mimic limited pore connectivity in porous materials and networks. In this model, the nodes represent the pore in a porous network and the bonds connecting the nodes represent the connecting pore constrictions in the porous network.

In these simulations, various porous networks with different pore connection configurations were first created. Specifically, pore connection was characterized by the fraction of bonds (*b*). For example, b = 1 indicates a fully connected porous network (four connections per node) and b = 0.75 indicates a partial blocked porous network (average three connections per node). **Figure C-6** shows three representative 2D lattice networks. The random walker was initially placed at the origin of the given network. As the simulation ran, the walker diffused randomly in the porous network through the connecting bonds until the trajectory ended. Over 10,000 simulated trajectories were used to generate the mean square displacement and extract the long-time diffusion coefficient. **Figure C-7** shows MSD plots as a function of the fraction

of bonds. Apparently, the long-time diffusion coefficient was significantly reduced for porous networks with reduced connectivity. In particular, for long runs (2000 steps), we found that the diffusion coefficient decreased by 10% when the fraction of bonds b was reduced from 1 to 0.9, and decreased by 50% when the fraction of bonds b was reduced from 1 to 0.7.



Figure C-6. Representative lattice networks. (a) Network with four connections per node, fraction of bonds b = 1. (b) Network with an average of three connections per node, fraction of bonds b = 0.75. (c) Network with an average of two connections per node, fraction of bonds b = 0.5. Note that these are partial fragments of larger networks.



Figure C-7. MSD plots for lattice networks with different connection configurations.

C.6 Table and figures mentioned in the main text

Table C-1. Experiment data of mean sojourn times and long-time diffusion coefficients for various experimental conditions

Solvent system	Cavity diameter	Trace particle	Hole size (nm)	Particle- cavity	Particle- hole size	Mean sojourn	$\frac{R^2/T_{soj}}{(\mu m^2/s)}$	D_{long} ($\mu m^2/s$)
	(nm)	diameter (nm)		size ratio	ratio	time (s)	. ,	
Nonaqueous	500	20	152 +/- 12	0.04	0.13	3.0	0.021	0.005
Nonaqueous	500	20	136 +/- 9	0.04	0.15	4.6	0.014	0.003
Nonaqueous	500	20	124 +/- 8	0.04	0.16	5.8	0.011	0.004
Nonaqueous	500	20	142 +/- 11	0.04	0.14	4.0	0.016	0.004
Nonaqueous	500	20	126 +/- 10	0.04	0.16	5.6	0.011	0.003
Nonaqueous	500	20	134 +/- 10	0.04	0.15	4.9	0.013	0.003
Nonaqueous	500	20	144 +/- 13	0.04	0.14	3.7	0.017	0.004
Nonaqueous	500	40	159 +/- 15	0.08	0.25	6.8	0.009	0.002
Nonaqueous	500	40	130 +/- 11	0.08	0.31	11.9	0.005	0.002
Nonaqueous	500	40	124 +/- 9	0.08	0.32	14.0	0.004	0.001
Nonaqueous	500	40	141 +/- 12	0.08	0.28	9.2	0.007	0.001
Nonaqueous	500	40	126 +/- 11	0.08	0.32	13.7	0.005	0.001
Nonaqueous	500	40	146 +/- 13	0.08	0.27	8.3	0.007	0.002
Nonaqueous	600	40	166 +/- 14	0.07	0.24	7.8	0.012	0.004
Nonaqueous	600	40	189 +/- 15	0.07	0.21	4.8	0.019	0.006
Nonaqueous	800	40	235 +/- 17	0.05	0.17	6.5	0.025	0.007
Nonaqueous	800	40	216 +/- 16	0.05	0.18	8.7	0.018	0.006
Nonaqueous	800	40	198 +/- 15	0.05	0.20	11.6	0.014	0.004
Nonaqueous	800	40	204 +/- 18	0.05	0.20	10.4	0.015	0.005
Nonaqueous	800	40	252 +/- 20	0.05	0.16	5.3	0.030	0.008
Aqueous (0.001 M)	500	40	127 +/- 8	0.08	0.31	19.5	0.003	0.001
Aqueous (0.01 M)	500	40	127 +/- 8	0.08	0.31	7.4	0.008	0.002
Aqueous (0.1 M)	500	40	127 +/- 8	0.08	0.31	4.2	0.015	0.005
Aqueous (0.001 M)	500	40	149 +/- 7	0.08	0.27	9.5	0.007	0.002
Aqueous (0.01 M)	500	40	149 +/- 7	0.08	0.27	4.0	0.016	0.005
Aqueous (0.1 M)	500	40	149 +/- 7	0.08	0.27	2.6	0.024	0.009



Figure C-8. Experimental setup of 3D single-particle tracking in porous media. A variable angle widefield microscope body was equipped with a phase mask that enabled DH-PSF imaging.



Figure C-9. Sojourn time distributions of nanoparticle diffusion in inverse opals. Complementary cumulative distributions of sojourn times of 0.04 μ m tracer particle diffusion in inverse opals (cavity diameter 500nm, hole diameter 149 nm) under different ionic strength conditions. The distribution of sojourn times was described by a double exponential decay function P(t) = A exp(-t/T₁) + (1-A) exp(-t/T₂), permitting the calculation of the mean sojourn times, T_{soj} = AT₁ + (1-A)T₂.



Figure C-10. Representative complex particle trajectory in inverse opals. (a) Representative particle trajectory in an inverse opal film. The numbers indicate the cavity number, the blue arrows indicate the tracer particle translocate direction. Therefore, the trajectory of the confined particle is cavity $1 \rightarrow 2 \rightarrow 1 - 3 \rightarrow 4 \rightarrow 5 \rightarrow 6 \rightarrow 5 \rightarrow 4 \rightarrow 3 \rightarrow 7$. (b) Cartesian coordinates of the trajectory from (a) as a function of time. This representative trajectory indicates that the experimental trajectory is very tortuous, with the tracer particle more likely to translocate through certain holes than others.

Appendix D: Supporting Information for Chapter 5

D.1 Measurement of inverse opal structure

Silica inverse opals were prepared using the methods described in the main text and imaged using a JOEL JSM-7401F scanning electron microscope.

<u>Cavity size measurement:</u> The size of the template particles was measured before use, the polystyrene particles had an extremely uniform size distribution, with a mean diameter of 1927 nm. The cavity size of the resulting inverse opals also measured using SEM, had a mean diameter of 1904 nm, similar to the size of template particles. These measurements confirm the consistency and accuracy of the SEM measurements.



Figure D-1. Size distributions of templating particles. (A) A representative SEM image of template particles. The scale bar represents 1 μ m. (B)Template particle size distribution, with a mean of 1927 nm, and a standard deviation of 38 nm. (C) Silica inverse opal cavity size distribution, with a mean 1904 nm, and a standard deviation of 43 nm.

<u>Hole size measurement</u>: The connecting holes in the inverse opals appear as elliptical areas since the SEM images capture only the planar projection of the circular holes. Therefore, the black areas (Figure D-2A) were fitted to elliptical profiles to determine the major axis length, corresponding to the actual diameter of the circular hole. In particular, SEM images were first converted into 8-bit grayscale images, and a threshold was defined to distinguish the black areas from the background. The identified black regions were then fitted to elliptical shapes to determine the outer contour lines. The major axis was then obtained by

geometrical analysis of the fitted ellipse. For each inverse opal sample, at least three SEM images at different locations within the sample were for the hole size analysis. Gaussian distributions were used to determine the mean value of the measurement, which was used as the diameter of the interconnecting hole for a given inverse opal sample. Note that occasional closed holes and extremely small holes due to defects were excluded from subsequent analysis. Figure D-2B shows a representative hole size distribution.



Figure D-2. Representative SEM image of inverse opals and size distributions of inverse opal holes. (A) A representative SEM image of inverse opal structure. Scale bar is 1 μ m. (B) Representative measured hole size distribution with a mean hole size of 498 nm, and a standard deviation of 26 nm.

D.2 Criteria to identify escape events

We employed a maximum allowed displacement algorithm to identify escape events and to determine the corresponding sojourn times (1). In this approach, we differentiate the two states (i.e., confined diffusion in given cavity vs. hopping between adjacent cavities) by analyzing the fluctuations of the time-dependent position fluctuations for a confined nanoparticle in a given cavity; the magnitude of these fluctuations were directly related to the cavity diameter. Therefore, the center of the first cavity was defined by averaging the particle position in X, Y and Z dimensions. An escape event was identified when the position of the particle relative to the center of the cavity exceeded a maximum allowed displacement (i.e., a threshold that was associated with the cavity diameter). For example, 2000 nm was used as the maximum allowed displacement threshold for 2 μ m diameter cavities. The results were robust with respect to the specific choice of threshold value. For example, a change of the threshold by +/-50 nm did not effect

the algorithms ability identify escape events correctly. After an escape event, a new center was set by averaging the subsequent particle positions in three dimensions. This process was repeated until the end of the trajectory was reached.

D.3 Theoretical calculations of the energy barrier at hole exits

The potential energy between the nanoparticle and the hole of inverse opal was evaluated using an approach described by Bowen et al. based on Derjaguin's approximation, yielding

$$\begin{split} U(h_0) &= U_e(h_0) + U_m(h_0) \\ &= \frac{\pi \epsilon_0 \epsilon_r}{g} \Big(\big(\psi_p + \psi_m \big)^2 \ln(1 + \exp(-\kappa h_0)) + \big(\psi_p - \psi_m \big)^2 \ln(1 - \exp(-\kappa h_0)) \Big) - \frac{A}{6gh_0} \end{split}$$

where κ is the inverse Debye length, ϵ_0 is the permittivity of vacuum and ϵ_r is the relative dielectric constant of the medium. ψ_p is the nanoparticle surface potential, ψ_m is the inverse opal surface potential, A is the Hamaker constant. h_0 is the closest distance between wall and nanoparticle, and g is a steric factor determined by the geometrical characteristics of the system. Here, we set ϵ_r as 65.2, which is the permittivity of the 50wt% glycerol/water mixture. A value of-25.7 mV was used for both ψ_p and ψ_m . The Hamaker constant A was $6.63*10^{-21}$ J. The Debye length κ^{-1} was 0.96 nm for 0.1 M salt solvent, 3.04 nm for 0.01 M salt solvent, and 9.6 nm for 0.001 M salt solvent.

As shown in Figure D-3A, this model divides the hole area into three regions. In region I, the particle interacts with a flat plate adjacent to the hole; in region II, the particle interacts with the curved entrance of the hole; and in region III, the particle interacts with the cylindrical inner surface of the hole. Here, we focus on the area of the hole exits, which is the buffer zone between region II and region III. The interaction energy in this buffer zone was assumed to be proportional to the fractions of the particle surface projected on the appropriate part of the inverse opal surfaces.

For different regions, we used different steric factors, g.

In region I,
$$g_1 = \frac{1}{a}$$
.
In region II, $g_2 = \sqrt{\left(\frac{1}{a} + \frac{1}{b}\right)\left(\frac{1}{a} - \frac{1}{r_0 + b}\right)}$.
In region III, $g_3 = \sqrt{\frac{1}{a}\left(\frac{1}{a} - \frac{1}{r_0}\right)}$.

Here, a is the nanoparticle radius (equal to 129 nm), r_0 is the hole radius, b is the radius of curvature of the rounded edge of the hole entrance. Here we used 1% of hole size to approximate b in the calculations. In previous studies, it was shown that the energy barrier calculations were relatively insensitive to the choice of b.



Figure D-3. Calculations of electrostatic interactions at hole exits. (A) Schematic of the model between the interaction of a charged particle and a hole in a like-charged membrane surface. (B) Dimensionless potential energy when the particle is located in the exit plane of a hole, for an inverse opal with 2 μ m diameter cavities and 498 nm diameter holes, in the solvent of 50% glycerol/water mixture with 0.001 M NaCl. The horizontal axis represents the lateral position of the center of the particle. The blue dashed line indicates the value (potential energy) / (k_BT) = 1. (C) Mean sojourn time versus hole size for passive particles with added salt. Error bars for the hole size represent the standard deviation of the hole size measurements from SEM images. Error bars for the mean sojourn time represent the standard error of the all sojourn time measurements. (D) Comparison of self-propulsion force and barrier force at the hole exits.

Using the above parameters and equations, the potential energy near the hole was calculated, then divided by k_BT to obtain the dimensionless potential energy. Figure S3B shows a representative potential energy plot for a 258 nm particle near a 498 nm hole exit in aqueous solvent with 0.001 M salt. In this case, the width of the apparent well is 158 nm, adding the particle size of 258 nm, gives an effective hole size for this case of 416 nm. With the effective hole size considered (Figure D-3C), the Monte Carlo simulations and experimental results were shown in good agreement.

To test the hypothesis that self-propulsion provides additional force to overcome the energy barrier at the hole exits, we compared the self-propulsion force to the axial component of the barrier force at the hole exits. In particular, we approximated the self-propulsion force using the Stokes equation $F_p = 6\pi\mu RV=55$ fN, and the magnitude of the barrier force as the gradient of the potential energy, where the largest barrier force under 0.001M salt conditions was approximately 35 fN (Figure D-3D). This indicates that self-propulsion can provide additional forces of the same order of magnitude as typical electrostatic repulsion to help confined nanoswimmers overcome the barrier at hole exits, converting the cavity escape process from a barrier-limited to a search-limited process.

Notably, electrostatic interactions could significantly influence the energy profile near the hole area. We calculated the energy profile at three different salt conditions, and found that energy barriers were significantly decreased by increasing the ionic strength (Figure D-4).



Figure D-4. Three-dimensional plots of the potential energy in the hole area. (A) 0.001M salt concentration with 498 nm hole size. (b) 0.01M salt concentration with 498 nm hole size. (c) 0.1M salt concentration with 498 nm hole size.

D.4 Monte Carlo simulation procedure – Passive particle

Here we describe the Monte Carlo simulations of Brownian particle escape from a spherical cavity with twelve holes on the surface. The simulations were performed in a three-dimensional system, where the origin was defined as the center of the cavity, twelve holes were created on the cavity surface to mimic the connecting holes of the inverse opals. The position of the centers of the twelve holes were:

$$\left(0, \frac{-2\sqrt{3}}{3}R, \frac{-2\sqrt{6}}{3}R\right), \left(R, \frac{\sqrt{3}}{3}R, \frac{-2\sqrt{6}}{3}R\right), \left(-R, \frac{\sqrt{3}}{3}R, \frac{-2\sqrt{6}}{3}R\right), (2R, 0, 0), (R, \sqrt{3}R, 0), (-R, \sqrt{3}R, 0), (-R,$$

, respectively. A particle was initially placed at the origin at initial time t = 0. The particles were confined by the boundary of the cavity, they could escape from the cavity only through a hole. For each simulation step, a new particle position was generated using the following equations:

$$x_{i} = x_{i-1} + \sqrt{2D_{T}\Delta t} w_{x,i}$$
$$y_{i} = y_{i-1} + \sqrt{2D_{T}\Delta t} w_{y,i}$$

$$z_i = z_{i-1} + \sqrt{2D_T \Delta t} w_{z,i}$$

Here D_T is translational diffusion coefficient obtained from experimental measurements, Δt is time step, $w_{x,i}$, $w_{y,i}$, and $w_{z,i}$ are uncorrelated sequences of random numbers taken from a Gaussian distribution with zero mean and standard deviation of unity.

Two types of boundary conditions were used for separate sets of simulations. For simulations with reflective boundary conditions, when a simulated particle encountered a cavity wall, a new step was generated based on the reflected trajectory caused by the wall. For simulations with adsorptive boundary conditions, subsequent to the first encounter between particle and cavity wall, the particle continued to move along the wall (diffusing randomly along the inner cavity surface) until it escaped from the cavity. This boundary condition was used to explore the effects of surface-mediated searching on cavity escape. Ten thousand trajectories were simulated with each boundary condition for varying hole sizes to yield statistically meaningful results. The mean sojourn time was defined as the average time that confined particles spent in a given cavity prior to escape.



Figure D-5. Representative Monte Carlo simulations of confined nanoparticle in inverse opals. (A) Passive Brownian particle in inverse opal structure under reflective boundary conditions. (B) Passive Brownian particle in inverse opal structure under adsorptive boundary conditions. (C) Nanoswimmer in inverse opal structure.

D.5 Monte Carlo simulations – Nanoswimmer

Here we describe the Monte Carlo simulations of a nanoswimmer escape from a spherical cavity with twelve holes on the surface. The geometric setup of the simulations was the same as for passive Brownian particles. However, the motion of a nanoswimmer was modeled as the combined action of random diffusion and directional self-propulsion. Therefore, for each simulation step, the new position was simulated using the following equations:

$$\theta_{i} = \theta_{i-1} + \sqrt{2D_{R}\Delta t} \,\omega_{\theta,i}$$

$$\varphi_{i} = \varphi_{i-1} + \sqrt{2D_{R}\Delta t} \,\omega_{\varphi,i}$$

$$x_{i} = x_{i-1} + v\cos\theta_{i-1}\sin\varphi_{i-1}\Delta t + \sqrt{2D_{T}\Delta t} \,\omega_{x,i}$$

$$y_{i} = y_{i-1} + v\sin\theta_{i-1}\sin\varphi_{i-1}\Delta t + \sqrt{2D_{T}\Delta t} \,\omega_{y,i}$$

$$z_i = z_{i-1} + v \cos \varphi_{i-1} \Delta t + \sqrt{2D_T \Delta t} \, \omega_{z,i}$$

Here D_T is the translational diffusion coefficient and D_R is rotational diffusion coefficient, both obtained from experimental measurements. v is the speed resulting by self-propulsion, obtained by subtracting the Brownian motion effects from the overall nanoswimmer motion. Δt is the time step; θ and φ are used to describe particle orientation due to rotational diffusion. $w_{\theta,i}$, $w_{\varphi,i}$, $w_{x,i}$, $w_{y,i}$, and $w_{z,i}$ are uncorrelated sequences of random numbers taken from a Gaussian distribution with zero mean and standard deviation of unity.

D.6 Figures Mentioned in the Main Content



Figure D-6. Monte Carlo simulations of passive Brownian escape from the inverse opal cavity under different short-time diffusion and boundary conditions.



Figure D-7. Comparison of experimental results with simulations of nanoparticle cavity escape processes. Green dashed lines represent effective hole sizes after considering electrostatic repulsion. These results suggested that nanoswimmers are not significantly affected by electrostatic barriers at hole exits despite the like charge repulsion that clearly inhibits the escape of the same particles in the absence of fuel.

Appendix E: Supporting Information for Chapter 6

E. 1 Preparation of polydimethylsiloxane (PDMS) microfluidic device

PDMS mixture was prepared by mixing SYLGARD polymer base with curing agent (SLYGARDTM 184 Silicone Elastomer Kit, Dow Company) in weight ratio 10:1. The mixture was then poured into a master mold to form the desired microchannel. After removing bubbles from the PDMS through evacuation, the PDMS was cured at 60°C for 3h. For flow experiments, two holes were punched in the device for use as inlet and outlet, and a PVDF membrane sample was inserted in the device. The glass coverslip and PDMS surface were activated by UV-ozone treatment for 5 minutes, and the covered PVDF device was sealed by curing the device at 80°C for 30 minutes. The schematic procedure is shown in Figure E-1.



Figure E-1. Schematic description of fabricating PDMS microfluidic device.

E.2 Mean squared displacement of tracer particles during flow stoppage



Figure E-2. Mean squared displacement versus time for 40 nm tracer particles during flow stoppage. The solid line represents a linear fit to the equation MSD = 4Dt. The calculated ensemble diffusion coefficient was $D = 0.09 \ \mu m^2/s$.

E.3 Remobilization fraction calculations

- (1) Analyzing the trajectories generated in the last 30 s of the Stage 1, locations were identified where the end-to-end displacement of the trajectories was less than a certain threshold α, these were marked as immobilized locations associated with Stage 1.
- (2) Analyzing the trajectories generated in the first 15 45 s of the Stage 3, locations were identified where the end-to-end displacement of trajectories was less than a certain threshold α, these were marked as immobilized locations associated with Stage 3. Note that this analysis involved the trajectories generated in the first 15 45 s of the Stage 3 instead of the trajectories in the first 30 s of the Stage 3 to account for the fact that some particles that had become untrapped during flow stoppage required time to return to their previous immobilized position by convective flow.

- (3) The immobilized particle plots generated in Stage 1 and Stage 3 were overlaid. If a given immobilized location in Stage 1, had a corresponding immobilized location in Stage 3 with the center to center distance less than a threshold β , this location was identified as a still immobilized location. However, if a given immobilized location in Stage 1 did not have a corresponding immobilized location in Stage 3 with distance less than the threshold, this location was identified as a remobilized location.
- (4) The remobilized fraction was calculated as the ratio of the number of remobilized locations to the number of immobilized locations in Stage 1.

E.4 Influence of threshold values in remobilization fraction calculations

Two threshold values were employed in the remobilization fraction calculations: (1) the threshold (α) used to identify the immobilized location, and (2) the threshold (β) used to determine a remobilization event. The sensitivity of the calculation to both threshold values were tested, the results indicated the choice of threshold values in these ranges had a negligible influence on the trends observed or conclusions drawn.

(1) Threshold α to identify immobilized locations: locations associated with trajectories where the endto-end Euclidean displacement was less than a threshold were identified as immobilized locations. The threshold used here was 0.8 µm, corresponding to 5 pixels. This threshold was chosen because it was larger than the particle tracking localization error while smaller than the end-to-end displacement generated by mobile trajectories. To determine the sensitivity of the final results to the specific choice of threshold, three threshold α values, 0.32 µm (2 pixels), 0.8 µm (5 pixels), and 1.28 µm (8 pixels) were tested on the same dataset. The resulting remobilized fractions were 0.32, 0.31, 0.32 respectively, which indicated that the choice of the threshold in that range had a negligible influence on the final calculation.



Figure E-3. Remobilization plots for different threshold values. (a) $\alpha = 0.32 \ \mu m$, $\beta = 1.28 \ \mu m$, remobilized fraction = 0.32. (b) $\alpha = 0.8 \ \mu m$, $\beta = 1.28 \ \mu m$, remobilized fraction = 0.31. (c) $\alpha = 1.28 \ \mu m$, $\beta = 1.28 \ \mu m$, remobilized fraction = 0.32. Scale bar represents to 5 μm .

(2) Threshold β to determine remobilized events: immobilized locations in Stage 1 that overlapped with an immobilized location in Stage 3 were identified as still-immobilized locations. However, immobilized locations from Stage 1 with no corresponding immobilized location in Stage 3 were identified as remobilization locations/events. The threshold β was used to determine whether an immobilized location in Stage 1 had a corresponding immobilized location in Stage 3. The threshold used here was 1.28 µm, corresponding to 8 pixels. This threshold was chosen slightly larger than threshold α, considering the potential for image drift during long-time imaging. To determine the sensitivity of the final results to the specific choice of threshold, three threshold values, 0.8 µm (5 pixels), 1.28 µm (8 pixels), and 1.76 µm (11 pixels) were tested on the same dataset. The resulting remobilized fractions were 0.30, 0.31, 0.31 respectively, which indicated that the choice of the threshold in that range had a negligible influence on the conclusions drawn.



Figure E-4. Remobilization plots for different threshold values. (a) $\alpha = 0.8 \ \mu m$, $\beta = 0.8 \ \mu m$, remobilized fraction = 0.30. (b) $\alpha = 0.8 \ \mu m$, $\beta = 1.28 \ \mu m$, remobilized fraction = 0.31. (c) $\alpha = 0.8 \ \mu m$, $\beta = 1.76 \ \mu m$, remobilized fraction = 0.31. Scale bar equals to 5 μm .

E.6 Remobilized fraction under steady convective flow

The remobilized fraction under convective flow was investigated to study the influence of adsorption/desorption on remobilized fraction. In contrast to analyzing the trajectories in Stage 1 and Stage 3 to identify remobilized events, trajectories were analyzed at two time points in Stage 1 to investigate the effects of adsorption/desorption. The remobilized fraction was 0.10 under convective flow, compared to the 0.31 remobilized fraction when flow stoppage was involved in the process, indicating flow stoppage facilitated particle remobilization significantly. In addition, the immobilized location boxed by the blue rectangle is a representative example showing that immobilized particles were remobilized after flow stoppage.



Figure E-5. Remobilization plots under different flow conditions. (a) Remobilization plot under convective flow. Remobilized fraction was 0.10. (b)Remobilization plot involving flow stoppage. Remobilized fraction was 0.31. Blue rectangles indicating two examples of flow stoppage facilitated immobilized particle remobilization. Scale bar represents to 5 µm.

E.7 Remobilization angle calculations

- (1) When an immobilized event was identified as described above, the corresponding immobilized location was identified as (X1, Y1). The Euclidean distance *d* between subsequent positions in the trajectory and the immobilized location (X1, Y1) were then calculated. When *d* was larger than a certain threshold value Δ , the corresponding position (X2, Y2) was identified as the first step of a successfully remobilized trajectory. To make this criterion robust in the presence of noisy positional data, (X2, Y2) was only considered as the initial remobilization displacement when *d* for the subsequent five positions were all greater than the threshold value Δ .
- (2) The remobilization angle θ was calculated based on the angle formed between two points (X1, Y1) and (X2, Y2), with (X1, Y1) as the origin. In particular, the remobilization angle
$$\theta = \arctan(\frac{Y2 - Y1}{X2 - X1}) \qquad if \ X2 - X1 > 0$$

$$\theta = \arctan(\frac{Y2 - Y1}{X2 - X1}) + \pi \quad if \ X2 - X1 < 0, and \ Y2 - Y1 \ge 0$$

$$\arctan\left(\frac{Y2 - Y1}{X2 - X1}\right) + \pi \quad if \ X2 - X1 < 0, and \ Y2 - Y1 \ge 0$$

$$\arctan\left(\frac{Y2 - Y1}{X2 - X1}\right) - \pi \quad if \ X2 - X1 < 0, and \ Y2 - Y1 < 0$$

$$\frac{\pi}{2} \qquad if \ X2 - X1 = 0, and \ Y2 - Y1 < 0$$

$$-\frac{\pi}{2} \qquad if \ X2 - X1 = 0, and \ Y2 - Y1 < 0$$

E.8 Influence of threshold value in remobilization angle calculations

The threshold value $\Delta = 1.28 \ \mu m$ (8 pixels) was used in the remobilization angle calculations, and the sensitivity of the results to variation of this threshold was tested. In particular, three threshold values, 0.8 μm (5 pixels), 1.28 μm (8 pixels), 1.76 μm (11 pixels) were tested on the same dataset. The results (Figure E-6) indicated the choice of the threshold value in these relatively large ranges had a negligible influence on the trends observed or conclusions drawn.



Figure E-6. Distribution of remobilization direction. The orange circular sectors indicate the remobilization direction during flow stoppage. The error bars indicate the standard deviation of the measured values based on Poisson statistics. (a) $\Delta = 0.8 \ \mu m$ (5 pixels). (b) $\Delta = 1.28 \ \mu m$ (8 pixels). (c) $\Delta = 1.76 \ \mu m$ (11 pixels).

Appendix F: Supporting Information for Chapter 7

F.1 Preparation of polydimethylsiloxane (PDMS) microfluidic device

A PDMS mixture was prepared by mixing SYLGARD polymer base with curing agent (SLYGARDTM 184 Silicone Elastomer Kit, Dow Company) in a 10:1 weight ratio. The mixture was then poured into a master mold to form the desired microchannel. After removing bubbles from the PDMS through evacuation, the PDMS was cured at 60°C for 3h. For flow experiments, two holes were punched in the device for use as inlet and outlet, and a PVDF membrane sample was inserted crossing the channel in the device. The glass coverslip and PDMS surface were activated by UV-ozone treatment for 5 minutes, and the covered PVDF device was sealed by curing the device at 80°C for 30 minutes. The schematic procedure is shown in Figure F-1.



Figure F-1. Schematic diagram showing the fabrication of a PDMS microfluidic device.

F.2 Alternating-laser excitation imaging method

Alternating-laser excitation (ALEX) enables the simultaneous imaging of multiple types of fluorescent signals by rapidly switching excitation wavelength. Here, we used 40 nm and 200 nm fluorescent nanoparticles as tracer particles, and 640 nm and 560 nm wavelength lasers as excitation sources. The 40 nm nanoparticles were excited by 640 nm light and the 200 nm nanoparticles were excited by the 560 nm source based on the excitation spectra of the two types of fluorescent particles (Figure F-2). Therefore, each particle tracking video contains three types of images: (1) both 40 nm and 200 nm particles, (2) only 200 nm particles, and (3) only 40 nm particles. As a result, we were able to observe both 40 nm and 200 nm particle motion simultaneously and analyze their transport behavior separately. Fig. S3 shows three representative images with both 200 nm and 40 nm nanoparticles, only 200 nm nanoparticles and only 40 nm nanoparticles in the field of view.



Figure F-2. Fluorescent excitation spectra of 40 nm and 200 nm nanoparticles.



Figure F-3. Representative images of fluorescent 200 nm and 40 nm nanoparticles in filtration membranes. (a) Representative image with both 200 nm and 40 nm fluorescent nanoparticles. (b) Representative image with only 200 nm fluorescent nanoparticles. (c) Representative image with only 40 nm fluorescent nanoparticles. Scale bar represents $5 \mu m$.

F.3 Identification of fouling sites

Fouling sites were identified as the locations where particles were immobilized, which were easily recognized based on the calculation of end-to-end displacements of trajectories in a given region. In particular, the criterion used to identify immobilized particles was that the end-to-end displacement was less than a Euclidean distance threshold. The threshold was chosen to be slightly larger than the combination effects of localization error of an immobilized particles and microscope focus drift, and substantially smaller than the displacement of a convectively moving particle for the given tracking time. For the results shown in the text, a threshold of 5 pixels (0.65 µm) was used for a 10 s tracking time. Notably, the choice of the threshold is very robust because of the dramatic contrast in displacement magnitudes between mobile and immobilized particles. For example, Fig. S4a shows four representative trajectories of mobile 40 nm and 200 nm nanoparticles and of immobilized 40 nm and 200 nm nanoparticles. In this 0.5 s tracking time interval, the end-to-end displacement of trajectories of mobile nanoparticles were clearly dramatically greater than that of immobilized nanoparticles. Based on the identification of fouling sites, the 2D-projected area of fouling sites was approximated using NIS-Elements Advanced Research software (Nikon). We analyzed the immobilized particle trajectories of 200 nm and 40 nm tracer particles separately, then created

a fouling site map by overlaying the fouling sites associated with the 200 nm and 40 nm particles. Therefore, we were able to identify the particle composition of fouling sites. A representative fouling sites map is shown in Figure F-4c.



Figure F-4. Representative example of fouling sites identification. (a) Representative nanoparticle trajectories in a 0.5 s tracking time interval. (1) mobile 40 nm nanoparticle trajectory. (2) mobile 200 nm nanoparticle trajectory. (3) immobilized 40 nm nanoparticle trajectory. (4) immobilized 200 nm nanoparticle trajectory. Scale bar represents 0.1 μ m. (b) Mobile and immobilized nanoparticles in the field of view are distinguished based on the end-to-end displacement of trajectories. Red circles indicate immobilized 40 nm particles, green circles indicate immobilized 200 nm particles, red squares indicate mobile 40 nm particles. Scale bar represents 5 μ m. (c) Fouling sites identified based on the location of immobilized nanoparticles. Scale bar represents 5 μ m.

F.4 Representative example of three-stage internal fouling mechanism



Figure F-5. Representative example of three-stage fouling mechanisms showing fouling site (a) formation, (b) growth, and (c) coalescence. Scale bar represents $2.5 \,\mu m$.