FG-Nup Aggregation with Specific Crowding Agents

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Abstract
A eukaryotic cell's genetic material is stored in the nucleus, surrounded and protected by the nuclear envelope. Spanning the nuclear envelope are nuclear pore complexes (NPC). The NPC's mechanism to regulate which macromolecules may pass between the nucleus and cytoplasm and which macromolecules are blocked remains undetermined. It is known that the NPC is filled with nucleoporins that have repeating phenylalanine and glycine residues (FG-nups). These FG-nups are intrinsically disordered proteins that act as the filter to determine what moves between the nucleus and cytoplasm. These proteins readily aggregate within living cells and their cellular state is unclear. Moreover, FG Nup aggregation resembles that of many other, primarily pathological, protein aggregates, and can be used as a model system to determine what features promote the aggregation state of this broad class of proteins. It is important to research the NPC and its selective filter because it is essential to understanding how the body maintains normal physiology by microcellular control. Alzheimer’s disease and other amyloid pathologies are associated with the NPC’s and its role in protein production. A fibrillation assay has been established to investigate amyloid formation of a specific protein domain taken from the NPC, FG124. In vitro molecular crowding is often used to simulate cellular conditions. Here we show that crowding agents differently affect amyloid formation. A 96-well plate and plate reader was used to record thioflavin fluorescence, which is an established readout of amyloid formation, to investigate how specific conditions affect FG124 over time. The basic goal is to observe how fast it aggregates, how much it aggregates and any other notable differences between specific agents that modulate aggregation. These properties were quantitated by using MATLAB's curve fitting algorithms. Based on our results, we hypothesized that each specific crowder will have an individualized interaction with proteins due to the unique molecular structure. However, to prove
this speculation, future experiments are required to test FG124 interactions with different concentration of crowders and other aromatic polymers.
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**Introduction:**

The nucleus is where the genetic code is stored for eukaryotic living cells. As such, effective communication between the nucleus and the cytoplasm is essential for any eukaryotic organism. One critical mediator of a cell’s communication between the nucleus and the cytoplasm is the Nuclear Pore Complex (NPC). The NPC is a massive structure that expands across the nuclear envelope and regulates the flow of macromolecules between the cytoplasm and nucleus. The nuclear pore is both fast and specific. However, its ability to be a selective barrier and let some cargo molecules pass through while blocking other macromolecules remains poorly characterized. It is important to research the NPC and its selective filter because it is essential to understanding how the body maintains cellular homeostasis and responds to external stimuli. This evidence may provide researchers with possible therapeutic approaches to halting or avoiding protein aggregation in human diseases.

A standard biochemical paradigm relates protein structure and function. It is believed that function of a protein in the cell is dependent on the structure it attains. Ordered proteins are a series of amino acids linked to together that fold into a defined structure. Intrinsically disordered proteins go directly from amino acid sequence to function, bypassing folding into a specific and consistent structure. Importantly, these are the types of proteins that are overrepresented in disease and could be the future targets for therapeutic drugs.

Dysfunctional proteins include, but are not limited to, intrinsically disordered proteins that are aggregated. This aggregation can originate from the NPC function malfunctioning and allowing for problematic protein expression. Recent studies have begun to explore these how alternations in the nuclear envelope could explain disease pathogenesis (Sheffield, 2006). One
type of protein aggregation that can occur is the formation of amyloid fibrils. Amyloid fibrils are insoluble fibers that are resistant to degradation. Although essential to persevering homeostasis, they are destructive and accumulate in diseased tissues. They come from normally soluble proteins and their specific role in human pathology is undetermined. It is undetermined because their stability, insolubility and heterogeneous composition makes it so commonly used methods for structure determination are difficult. Therefore, it is vital for the scientist design a method to better understand the assembly of amyloid fibrils and the NPC role in inhibiting aggregation to better prevent the causation of amyloid diseases (Rambaran, 2008).

FIGURE 1: Amyloid fibers on a marco and micros-scale. The photograph on the left was taken using electron microscopy.

Amyloids are currently associated with more than 40 human pathologies known as protein deposition diseases (Giehm, 2010). The growing number of human diseases linked to formation of amyloid fibrils has sparked great interest in characterizing the aggregating process in vitro. Some amyloid diseases include Alzheimer’s disease, Type 2 Diabetes, and various forms of Amyloidosis. Biopsies of diseases individuals will show extracellular deposits of amyloid fibrils in organs and tissues. For example, Alzheimer’s disease is characterized by Beta-Plaque amyloids and Type 2 diabetes is characterized by islet amyloids in the pancreas (Marzban, 2003). The mechanisms responsible for islet amyloid formation in Type 2 Diabetes are still unclear. However, amyloid formation of islets are found in up to 90% patients with Type
In Alzheimer’s disease, a pathogenic characteristic are the neurofibrillary tangles and presence of beta-plaque amyloids. In fact, a recent study has prompted further investigation of the NPC and nucleocytoplasmic transport to help prevent the progression of Alzheimer’s disease (Sheffield, 2006). The ability to determine what species are responsible for the toxicity of amyloids, what the exact mechanism for toxicity and how it can be reversed, prevented and cured remains unsolved. However, since protein aggregation and protein control is regulated by NPC, a link between studying the NPC and amyloid formation has ignited a large interest.

**NPC Regulation**

The nuclear pore complex regulates the flow of macromolecules between the cytoplasm and nucleus. These macromolecules (including RNA, transcription factors, and many others) help the body maintain homeostasis. Problems with the structure or function of the NPC could affect nearly all cellular functions, including transcription, translation and protein synthesis (Sheffield, 2006). When molecules leak out or into the nucleus it can cause a series of complications that further lead to the development of diseases, such as Alzheimer’s disease (Sheffield, 2006).

**NPC Structure**

To accommodate the selective transport of cargo across the nuclear envelop, proteins, referred to as nucleoporins, are attached to the membrane embedded scaffold. These nucleoporins comprise 30 different types, with repeating phenylalanine (F) and glycine (G) residues (Frey, 2007). The phenylalanine and glycine residues are a defining feature, which is why these nucleoporins are called FG-nups. Although these FG-nups account for 50% of the mass in the NPC (Wente, 2010), these proteins are not well understood by the scientific community.
community. In particular, little is known about their native state and what determines their aggregation state. However, it is clear that they are intrinsically disordered proteins.

FG repeats interact with nuclear transport receptors (NTRs) and restrict the diffusion molecules. They are crucial to the NPC’s ability selectivity and rapidly transport molecules (Frey, 2007). The FG repeats have sections of hydrophobic regions that NTRs are believed to attach to, facilitating the NPC’s ability to receive cargo (D’angelo, 2009). This ability to receive cargo is vital for the nucleus to maintain genomic control over living organisms. The purpose of my project is study the aggregation of an FG-nup to gain insight on it’s natural state in the body. By developing a systematic model to study FG124, we can better understand dysfunctional proteins, amyloid formation, the proteins of the Nuclear Pore Complex and how they attribute to human pathology and physiology.

Fibrillation Assay

In the Hough lab, we developed a fibrillation assays with different crowding agents to analyze how a specific FG nup, FG124, aggregates in certain conditions that imitate the nuclear pore. FG124 is a fragment of the nucleoporin, Nsp1. Part of the Nsp1 nup is ordered and adheres
to the inside of the wall. However, Nsp1 also contains a disordered region of approximately 500 amino acids. FG124 is a 124 amino acid long section of the disordered region that is known to aggregate in solution. The reason we studied this particular nup is that by understanding the aggregation mechanism of FG124, one can better understand the aggregation of Nsp1 and nucleoporins in general. More specifically, if one can understand how the protein interacts with different crowding agents, one can also understand the protein’s state in the nuclear pore complex. In addition, we established a reproducible way to investigate amyloid formation. It is believed that understanding how the NPC and amyloid’s function, it will contribute to scientist’s ability to diagnosis, treat, and even prevent the development of some human diseases.

Thioflavin (ThT) is a fluorescing molecule used to measure protein aggregation. ThT undergoes a significant increase in fluorescence when bound to amyloid fibrils (Giehm, 2010). Samples of protein, ThT and a crowding reagent are loaded into a 96-well plate and then the plate reader measures fluorescence over time. The plate reader records an intensity value approximately every 10 minutes. The data generates a protein’s aggregation over time graph, which is what we refer to as our fibrillation assay. This fibrillation assay is used to screen the effects of external factors on the FG124 nup. These external factors include; serine, lystate solution, polyethylene glycol, and polyvinylpyrrolidone.

The crowding reagents:

Macromolecular crowding can alter the properties of molecules in a solution. Most importantly, these conditions simulate the environment of living cells. Crowding can affect several properties of proteins, including their dynamics and shape. This leads to reduced volume of solvent which in some cases increases macromolecules chemical activity. In a cell the concentration of macromolecules ranges from 80-400mg/ml, which corresponds to a volume
occupancy of 5-40% (Homouz, 2008). In some in vitro systems, the crowding agents accelerate the folding process because a protein in a secondary, tertiary or quaternary state will occupy less volume than staying in its primary state.

Serine is an amino acid derived from glycine or threonine. It is considered a non-essential amino acid which means it can be synthesized in human bodies. This distinction is used because some amino acids cannot be made by our cells and need to be consumed from diet. Serine is vital in biosynthesis of purines, pyrimidines and other amino acids. It is found in high concentration in cellular membranes. Serine worked as a positive control in the experiment due the previous literature stating that FG-nups aggregation is promoted when crowded in a high concentration of serine (Milles, 2013).

Lysate is the mix of cellular components that are released when cells are broken. A lysate mixture contains various cellular components, though not necessarily in their physiological state. As FG-nups aggregation state in the cell remains unknown, using a crowding agent to mimic the numerous cellular components is beneficial because it can potentially simulate the state of FG124 within its own similar environment.

Polyethylene glycol (PEG) was also chosen for this experiment because it is commonly used as an inert crowder. It is considered biologically inert, safe and found as a liquid or a low temperature melting solid. It is flexible and water soluble polymer that can be used to create high osmotic pressure. These properties make PEG very useful in biochemistry and biomembrane experiments, when using the osmotic stress technique.

Polyvinylpyrrolidone (PVP) is also typically considered an inert molecule which is very polar and soluble in water. However, it also binds to other macromolecules due to its polarity. PVP was chosen for a crowding agent in the experiment because of the previous conception that
inert molecules promote aggregation. PVP contains an aromatic ring. The aromatic ring has been studied to influence protein aggregation (Cukaleyski, 2012). Aromatic rings can align in an energetically favorable manner which impacts protein aggregation.

**Aromatic Aggregation**

Phenylalanine and other aromatic amino acid are found in many proteins that self-assemble and aggregate to cause human diseases. When two aromatic groups interact, molecular structures become more ordered and organized (Cukalevski, 2012). Aromatic molecules have a series of pi orbitals surrounding the points of the ring. These orbitals create electron dense areas above and below the ring, making it sit flat. Due to electrons constantly moving, it creates a partial negative and positive charges. Therefore, it is energetically favorable for the rings the align so that the opposite charges are closest to each other. The interaction of the pi orbitals drives a favorable conformation in which stacks of aromatic molecules in a particular pattern. Consequently, this leads a possible aligning of the amino acids so that it instigates protein aggregation.

FIGURE 3: This figure shows the generically favorable interaction of pi orbital stacking in aromatic molecules

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http://pubs.rsc.org/en/content/articlehtml/2012/sc/c2sc20045g
**Materials and Methods**

In the Hough lab, I have worked to improve and make more reproducible methods to monitor protein fibrillation and aggregation. I optimized a wide range of steps as described below. The overall strategy I used was to use bacteria to produce large quantities of FG124, and then to monitor its aggregation by thioflavin (ThT) intensity in different conditions. The procedure involved a protein transformation, followed by growing the protein, and the purifying it and determining aggregation with a microplate reader. The individual steps were as follows.

**Transformation**

The purpose of a transformation is to introduce a foreign plasmid into bacteria and then the bacteria will make large quantities to the desired protein. The plasmid contains both the DNA sequence encoding for the protein of interest, as well as the gene to make the cells resistant to an antibiotic. The transformation is completed using competent BL21 DE3 Gold cells from E. coli that are kept frozen. Cells were thawed over ice. First, the gold cells were mixed with the plasmid, and the mixture was incubated on ice for 30 minutes. Then, the mixture was placed on a heat block to heat shock the cells for 1 minute at 42 degrees. Next, the mixture was incubated again on ice for 5 minutes. Using sterile technique, SOC was added as a food source for the cells. The SOC is specifically rich in nutrients to help the cells grow to the best of their ability after being damaged by heat shocking. The last incubation occurred for 1 hour, then the mixture was spread on a kanamycin plate under sterile conditions and left to incubate over-night. Kanamycin is an antibiotic added to the plate that keeps it from becoming contaminated. Only those cells containing the plasmid, and so resistance to Kanamycin, grow.

**Growing FG124**

The FG124 growing processes took place over two days. The first day a preculture was prepared using cells from the transformation plate, LB, and antibiotic under sterile conditions.
The plasmid has a gene that encodes for kanamycin resistance, so that is why the antibiotic kanamycin is specifically used. The preculture was left to incubate over night at 37 degrees. The next morning the when the samples appears to have grown, the growing of a one liter culture was performed.

The growing of a one liter culture began with adding the preculture and a mL of Kanamycin into one liter of autoclaved LB. LB was the source of food for the bacteria grow in the culture. Feeding the bacteria and shaking the culture contributed to a greater concentration of the desired protein produced. Everything was mixed together under sterile conditions to keep the culture from being contaminated. The culture was placed in a shaking incubator at 37 degrees and spun 180 rpm. The culture remained shaking until it had an optical density of 0.6. Once the optical density was achieved, the induction began by adding IPTG. A pre and post induction sample was collected to be run on a gel after the purification processes is completed. Lastly, after the induction with IPTG, to induce the T7 promoter, the desired gene began being expressed. The culture was spun down and the cell pellets were kept frozen until the purification started. The frozen pellets can remain in the -80 freezer until the purification process can begin. This is extremely beneficial because the purification of a specific is a long and extensive process.

Purification Process

To purify the protein, it began with lysing the cell pellet and then running it through a cobalt affinity column. The column allowed for the ability to retrieve the purified protein desired for our experiments. A protease inhibitor cocktail was added to inhibit general degradation of proteins. The desired protein bound to the column due the His-tag allowing for it be separated and collected. The protein was purified in to a buffer that contains 7 M Guanidine Hydrochloride (GuHcl). The GuHcl was used because it keeps the protein from aggregating. The protein
remained in this solution until it was run through a desalted column and used in a fibrillation assay. A final gel was performed at the end of the process that included samples from the flow through, supernatant, washes and elutions. The final gel determined if the desired protein was expressed so that the fibrillation assay can follow. If for any reason the protein does not appear in the final elutions, one can uses the gel to determine where exactly in the process the protein disappeared.

**Concentrating FG124**

Before the fibrillation assay can begin, the protein required concentrating. The elutions were pooled together and then run through centrifuge filter units. Due to the protein’s size, it did not run through the filter and the liquid without protein will flew through and was discarded. 1mL of concentrated protein was the desired volume after centrifuging multiple times.

**The Fibrillation Asaay**

Next, the thioflavin assay was used in a fibrillation assay to analyze the FG124 aggregation. A 96-well plate and plate reader was required to record the aggregation state over 10 hours, with 100 different time points and monitor the protein to investigate how specific conditions affect FG124 over time. The plate reader measures fluorescents from the bottom of the plate. The excitation wavelength was set at 450nm and the emission was measured at wavelength of 482nm. The plate reader also shook in an orbital manner during the assay. The temperature inside the plate reader set to be at 29 degrees. The basic goal of the assay was to obverse how fast FG124 aggregates, how much it aggregates and any other notable differences between specific agents that modulate aggregation.
FIGURE 4: The figure above shows the mechanism that is occurring within the plate reader. On the left it shows what is exactly added into each well. On the right, it shows that over time, as the protein aggregates, the thioflavin fluoresces brighter and plate reader records the intensity of the Tht fluorescence.

The experiment began with a desalting column. To mimic physiologically relevant conditions, we used a buffer which we called PTB. PTB stands for potassium transport buffer. It contains 150mM KCL, 20mM Hepes, and 2mM MgCl\(_2\). It is closely related to the buffer in which nuclear transport has been recapitulated using isolated nuclei. Also, PTB was used throughout the experiment keeping the solutions as homologous as possible. The desalting column was stored at room temperature and then equilibrated first using 1x PTB. The protein was added to the column and then fractions were collected. A small sample from each fraction was mixed with the Bradford reagent. The Bradford assay provided a mechanism which a change in color shows which collected fractions had the greatest concentration of protein. The fractions with the most protein present were combined and used exclusively in the fibrillation assay. A Bicinchoninic Acid assay (BCA) was completed once the plate reader was set up so the exact concentration of protein can be recorded. The protein used in the experiments averaged to about 1 mg/mL. This is not important for one specific experiment but it allows the ability to compare all experiments, especially if they have different concentrations of our protein.
The collected fractions were dispersed into Eppendorfs which included the specific crowding reagent and thioflavin. The preparation for the crowding conditions is completed the previous day and left to nutate overnight to ensure that the mixture is fully dissolved and homologous. Six replicates of 150microL of each condition were placed in the wells. A glass bead was also added to each well to help make the measurements more consistent. I found in the literature that glass beads help to create reproducible data and that is why it was included in these experiments (Gihem, 2010).

**The Fibrillation Assay Prep**

To compare our protein conditions, controls and blanks were included in each trial to more accurately understand the recorded aggregation. The positive controls in our study included serine and buffer. Serine was a positive control because previous literature claims it promotes aggregation (Milles, 2013). Buffer was used because it allows for the comparison of crowding conditions versus non-crowding conditions. GuHcl was the negative control because it keeps FG-nups from aggregating. The blanks in the experiment were an important tool used for the analysis. The blank included everything that would normally be in an experimental well besides the protein. The information gained from using the blanks allows for normalization. The blank information provided what the crowding agents do alone in the plate reader. This helped determine what are the exact effects of the protein in the crowding agent. The plate was loaded and then the BCA was completed. This was to ensure that the plate reader captured protein aggregation as soon as the protein had been desalted and was able to begin aggregating. The stocks were all prepared beforehand to expedite the process of getting the samples into the plate reader. The more time the protein sits desalted, the more time it has to aggregate without being
documented by the plate reader. Lastly, the next morning the data was collected and analyzed using Microsoft Excel.

In this experiment, a 30% solution of serine was made and then added to each well to a final concentration of 19%. A solution of 20% PVP was created and added to each well to a final concentration of 13%. The same procedure for PVP is used for preparing PEG.

Lysate was prepared by lysing BL21 DE3 Gold cells using either sonication or a homogenizer. The samples were then lyophilized in an ammonium bicarbonate buffer, which decomposed during lyophilization. A lyophilizer created a vacuum to slowly remove all liquid from the sample. The reason to lyophilize the lysate is to make replicable aliquots of lysate in a solid state. A limitation of lyophilizing proteins was that structure damage to the proteins may occur simultaneously which destroyed some cellular components.

This procedure has taken very long to perfect. Small changes have been applied to ensure the best procedure. For example, we tested the affects of shaking and not shaking, the presence of beads, and the variability with changing pH. We additionally used a microscope during the experiment to directly observe the aggregates. Running the fibrillation assay all weekend has also been tried. Also, many different conditions have been tried to get the most replicable and interesting results. For example, experiments have been run with other amino acids, BSA, NTF2, FSFG. NTF2 is a nuclear transporter also studied in the lab. FSFG is part of the same disordered region of nucleoporins that FG124 comes from. However, FG124 is known to aggregate whereas FSFG does not. The use of data to make decisions and support change has been valued in our lab. These techniques are used to replicate the FG124 environment in a cell to better understand its naturally occurring aggregation state.
Analysis

Results from the plate reader were transferred into Microsoft Excel. Excel was used to graph each individual replicate and then normalize the replicate to its blank. Each sample’s intensity was divided by the blank’s intensity, so that if there was no aggregation the value would be one. Each experiment produced 30-50 individual graphs and the best way to analyze them is using MATLAB to fit and summarize the graphs. MATLAB will fit the individual graphs to a sigmoidal curve. A sigmoidal curve was used to present aggregation results because it includes a lag phase, an exponential growth phase and a stationary phase (Giehm, 2010). We used the sigmoidal curves from MATLAB to analyze the fits and averages and then derive conclusions.
FIGURE 5: This figure shows a flow chart of the many steps of preparation and analysis in a fibrillation assay. steps.

Results

During the past two years, many fibrillation assays were performed. The results that are presented in this paper are derived from the most recent experiments. The previous experiments have varied on many factors including what conditions crowd FG124, shaking vs. non-shaking, running overnight vs. running over the weekend, different concentrations of GuHCl, beads vs. no beads and many others. The results in this paper are from a series of fibrillation assay’s run most recently, with beads, stored in 7M GuHCl, orbital shaking, and over the time frame of 10 hours that collect 100 different time points.

The final analysis included approximately 30 replicates of each crowding condition, excluding lysate. Each crowding agent has unique characteristics that set it apart from the others. Previous literature assumed that all inert molecules have the same affect. It did not consider the potential interactions that could occur due to the crowders molecule structure. The data that has been collected shows otherwise. It is hypothesized that each specific crowder will have an individualized interaction with proteins based on their molecular structure.

Fits

The equation we have fit our individual plots is:

\[ y = \frac{A}{1+e^{-K(t-T)}}. \]

MATLAB will generate a value for \( A \), \( K \) and \( T \) for each replicate. An average for individual fibrillation assays or an average over the entire research experience can be easily obtained by writing a script for MATLAB.

\( A \) is the value assigned for amplitude, which measure how high the measurements go. For this experiment, \( A \) is not a great way to compare to conditions due the variety of results. The amplitude of an aggregation curve does not give researchers a good idea as to what underlying mechanism may be. Aggregates can easily move out of the light that measures the fluorescence...
due to being consistently shaken. Although this is rare, it can affect the data collected. Which directly affects the reported average of \( A \). Also, the thioflavin can directly influence the measured value for \( A \). \( A \) is not valuable because this research is not interested in the thioflavin fluorescence, it is interested in protein aggregation.

Previous literature uses a combination of \( K \) and \( T \) to determine lag time. Lag time is described as the time it takes for the protein to begin to aggregate. This is also referred to as the time it takes to plant the seed and begin nucleation. \( K \) more specifically measures the exponential rate of the growth phase. \( T \) records more specifically when the graph reaches a threshold. The combination of the two give a more accurate description of the protein and its aggregation mechanism within a specific crowder.

![Intensity vs. Time](image)

**FIGURE 6:** The picture shows an example of one of the fits that MATLAB created. This specific plot comes from a buffer replicate in January. The values of \( A \), \( T \), and \( K \) are all generated from the sigmoidal curve

**Averages:**

Overall, the experiments produce similar trend between the averages of \( A \), \( K \), \( T \). This has not always been the case but due to minor changes in the methods, the best reproducible procedure has been achieved.
Although $A$ has defined limitations, it is still important to analyze the results provided. Averages of $A$ show a general trend of buffer reaching the highest amplitude (Figure 7). Therefore, all other aggregation regents inhibit to a certain extent the aggregation of FG124. However, when looking at each individual plot, the crowding agents do not fit the line very well toward the end. It has been hypothesized that the aggregates that form could be breaking down and then rebuilding. This would lead to the scatter toward the end of the plot and affect the trend for $A$.

![FIGURE 7: The figure shows the average $A$ values for the crowding agents generated for 5 different experiments](image)

With everything compared to the positive control buffer, the average for $K$ follows a similar trend throughout experiments. Serine is the crowding regent for the highest value of $K$ in comparison to buffer. This is to be expected because previous literature claims that the crowding of serine promotes aggregation (Milles, 2013). Not only does it support serine’s effect on aggregation, but it provides clarity that a good positive control was selected. PVP and PEG tend to have a similar value of $K$. This can be seen in the overlap of their error bars (Figure 8). The fibrillation assays that include lysate show that lysate promotes slow aggregation and a lower $K$ than buffer.
The values for averaged $T$ also follow a general trend among experiments. This makes it very easy to rate order the crowding agents by their value for $T$. A larger value for $T$ indicates a longer stabilization period of the protein in the crowder. Stabilization means that the protein is unchanging in its disordered state and does not begin aggregating. It was expected that buffer would stabilize it the best, and that serine would promote aggregation. However, it was expected that inert crowders would suppress aggregation which is not supported by the data. The data shows that PVP and serine both influence FG124 by promoting aggregation. Also, PEG is more similar to buffer by showing a long stabilization phase. This is interesting because PEG and PVP were expected to be similar because they are both inert molecules and this was not the case.
FIGURE 9: The figure shows the average T values for the crowding agents generated for 5 different experiments.
**Discussion:**

This is experiment speculates that although PVP and PEG are both inert molecules, they drive different aggregation mechanisms for FG124. PEG has a longer lag time which means its stabilizes the protein relative to buffer. Compared to buffer, on average PEG is equal or higher to its lag time. PEG has a specific interaction with FG124 that stabilizes it in its disordered state. PVP is faster than buffer which means it does not suppress aggregation but rather promotes it. However, it is hypothesized that because of difference of molecular structure is the reason these crowding agents have different results.

We purpose that PVP has a specific interaction that drives aggregation even though it is biologically inert. PVP is aromatic and polar. Due to ring stacking, it can line up the protein in the correct formation so that it can quickly form aggregates with itself. PEG has no ring and therefore can’t assist the Phenylalanine’s stacking up and seeding. Therefore, PEG does a better job at blocking FG124 from coming near other FG124 filaments and aggregating.

However, to prove this speculation, many more experiments are needed in the future. These experiments will not only include repeating higher concentration crowders but also testing FG124 with other aromatic polymers.
Conclusion

Amyloid formation is present in diseases and FG-nups are associated with the nuclear pore allowing proteins to aggregate.

Protein aggregation is determined by the molecular structure of the molecules that crowd it. Even though a molecule is biologically inert, it can drive specific interactions to either promote or stabilization aggregation. Although it was assumed that inert crowders act similarly, we have discovered that this is not always the case.

Lastly, the ability to systematically monitor amyloid formation has received a notable interest in the past decades. Due to all the diseases related to amyloid formation, scientists are looking for a reproducible technique to study amyloid formation. Due to the spontaneity of protein folding, inaccurate ways to measure amyloid formation exist. Therefore, making it difficult to study to amyloid formation. This project has developed a systematic way to study amyloid formation and the nuclear pore complex. In the future, many more experiments can be completed using this method to research more about amyloid formation and the NPC.
Reference List


