Plastic Particles and Molecular Crowding Agents Strongly Influence the Fibrillation of Two Proteins Involved in Human Amyloid Disease

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Background

Amyloid Fibrils: insoluble protein aggregates dominated by beta sheet structures

- Accumulation of fibrils in tissues causes amyloid diseases including Parkinson's, Alzheimer's, type 2 diabetes, etc.[1]

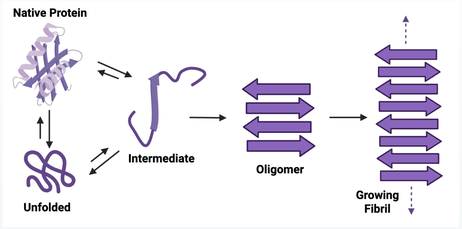
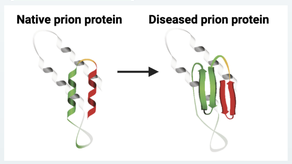


Figure 1. Diagram of protein fibril formation

Prion Protein (PrP) Common, flexible protein on the surface of nerve cells. Misfolding causes prion diseases, like mad cow disease



- Diseased prion protein acts as a template to convert other native prion proteins to diseased form.
- Associates with other misfolded prions causing neuronal damage

Human Lysozyme Anti-bacterial enzyme whose fibrillation is associated with a form of human systemic amyloidosis[2]

Nano plastics

- Extremely small pieces of plastic (<1 µm) produced from the breakdown or manufacturing of plastics.
- Able to enter the bloodstream and tissues and can interact with proteins

Crowding Agents

- Used to mimic crowded cellular environment in in-vitro fibrillation experiments.
- Typical examples include small carbohydrates and synthetic polymers

How do small molecules such as crowding agents and nano plastics impact the different stages and rate of protein fibrillation?

Methodology

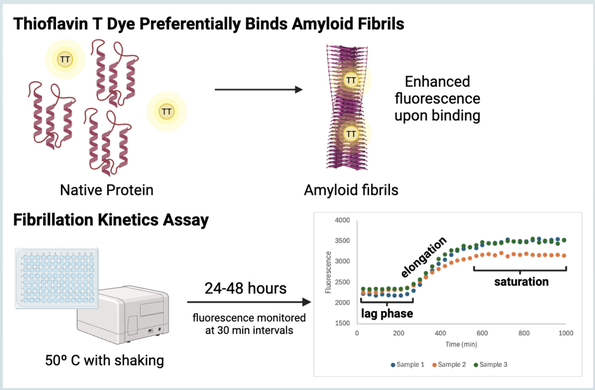


Figure 2. Overview of fibrillation assay methods utilizing the amyloid fibril binding properties of Thioflavin T fluorescent dye

Custom fitting functions used to obtain kinetic data from AmyloFit[1]

No Slope in Post-Growth Phase

$$\frac{m_1 + m_2(e^{m_3*(m_4-t)})}{(1+(e^{m_3*(m_4-t)})}$$

With Slope in Post-Growth Phase

$$\frac{(m_1+m_5t) + m_2(e^{m_3*(m_4-t)})}{(1+(e^{m_3*(m_4-t)})}$$

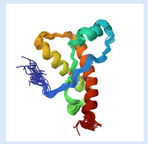
Important Parameters: m_3 = rate of fibrillation, m_4 = half-time of fibrillation

Results: Prion Protein with Nano plastics

Prion Protein stock: non-infectious bank vole prion protein fibrillating conditions: pH 7.4 & 0.02% SDS

Testing effects of polystyrene plastic nanospheres:

1. **Small nanospheres** (0.05 µm diameter)
2. **Large nanospheres** (0.3 µm diameter)



x represents 0.01% mg/mL solution of nanospheres

Effects of different concentrations of nanospheres

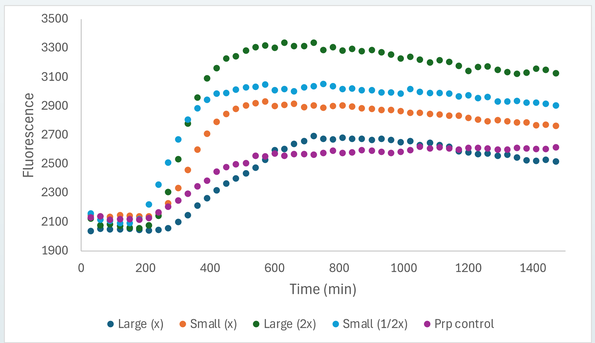


Figure 3. Average Fibrillation Kinetics of Prp for Large and Small Nanospheres at different concentrations. Legend labels correspond to the concentration and size of nanospheres added to each condition. Samples were run in triplicate and averaged to obtain each curve

Condition	Average Rate (min^{-1})	Average Half-time (min)
Buffer	0.01627 (+/- 0.00414)	424.3 (+/- 22.2)
Small Nanospheres (1/20x)	0.01329 (+/- 0.0042)	441.0 (+/- 155)
Small Nanospheres (1/2x)	0.0291 (+/- 0.00328)	280.7 (+/- 10.1)
Small Nanospheres (x)	0.03037 (+/- 0.0029)	339.3 (+/- 16.7)
Small Nanospheres (2x)	0.01643 (+/- 0.0014)	488.0 (+/- 63.0)
Small Nanospheres (20x)	0.02880 (+/- 0.0072)	219.3 (+/- 9.87)
Large Nanospheres (1/2x)	0.01241 (+/- 0.0041)	543.7 (+/- 66.2)
Large Nanospheres (x)	0.02487 (+/- 0.0050)	447.3 (+/- 131)
Large Nanospheres (2x)	0.03320 (+/- 0.0024)	315 (+/- 31.2)
Large Nanospheres (5x)	0.01817 (+/- 0.0028)	365.7 (+/- 7.37)
Large Nanospheres (10x)	0.03457 (+/- 0.032)	449.7 (+/- 145)

Table 1. Average rate of fibrillation (min^{-1}) and half-time (min) from fibrillation kinetic assays of different concentrations of large and small nanospheres

Additional Experiments:

- Binding absorbance assay to assess Prp-nanosphere interactions
  - Non-significant absorbance change = minimal binding
- Circular Dichroism (CD) to monitor secondary structure changes of Prp after 24-hour exposure to large nanospheres
  - Altered Prp structure but not confident in specific trend

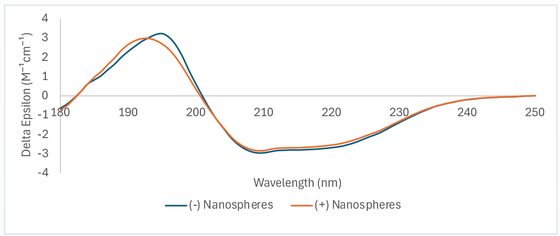


Figure 4. CD spectra comparison of Prp incubated with and without Large nanospheres for 24 hours.

	Helix	Strand	Turn	Other
(-) Large Nanospheres	29.6	11.9	16.6	41.9
(+) Large Nanospheres	21.7	23.8	15.2	39.4
Bank Vole Prp Crystal Structure (2K56)	53.1	3.5	2.7	40.7

Table 2. Bestsel[3] secondary structure estimations from CD spectra analysis for samples incubated with and without nanospheres for 24 hours compared to a crystal structure segment of Bank vole Prp

Results: Human Lysozyme under Crowding Conditions

Effects of titrations of crowding agents on human lysozyme fibrillation

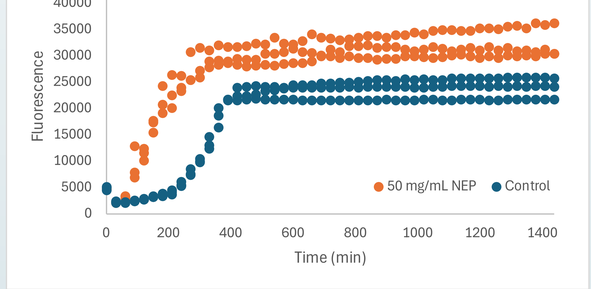


Figure 5. NEP molecules induces human lysozyme to fibrillate faster at a lower concentration.

Condition	Average Rate	Average Half-time
Control	0.02643 (+/- 0.006637)	321.3 (+/- 12.42)
50 mg/mL Glucose	0.01627 (+/- 0.003093)	355.3 (+/- 40.41)
50 mg/mL Sucrose	0.01407 (+/- 0.002201)	373.0 (+/- 8.0)
50 mg/mL Ficoll	0.01227 (+/- 0.001419)	418.0 (+/- 71.58)
50 mg/mL Dextran	0.01733 (+/- 0.006294)	386.0 (+/- 54.29)
50 mg/mL PVP10	0.01036 (+/- 0.003765)	284.0 (+/- 51.39)
50 mg/mL PVP40	0.01146 (+/- 0.002706)	335.0 (+/- 28.51)
50 mg/mL NEP	0.01860 (+/- 0.002706)	160.7 (+/- 6.429)
100 mg/mL Glucose	0.02010 (+/- 0.004139)	416.3 (+/- 61.70)
100 mg/mL Sucrose	0.02283 (+/- 0.007275)	378.3 (+/- 51.03)
100 mg/mL Ficoll	0.02450 (+/- 0.003923)	286.7 (+/- 26.27)
100 mg/mL Dextran	0.01553 (+/- 0.003722)	313.0 (+/- 26.00)
100 mg/mL PVP10	0.01447 (+/- 0.002040)	336.0 (+/- 11.53)
100 mg/mL PVP40	0.01139 (+/- 0.002504)	386.3 (+/- 22.12)
100 mg/mL NEP	0.05173 (+/- 0.01472)	145.33 (+/- 9.073)

Table 3. Average rates of reaction and half-lives of human lysozyme fibrillation with titrations of different crowding agents

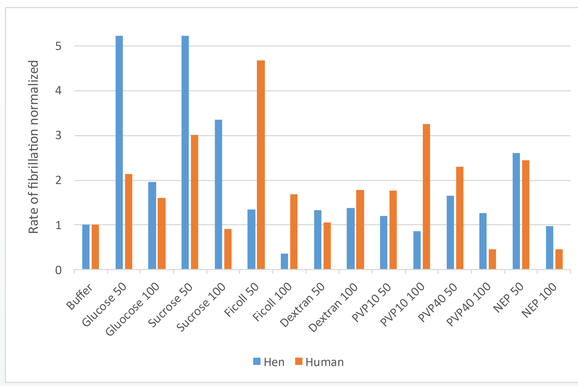


Figure 6. Synthetic polymers are more effective at promoting the fibrillation of human lysozyme in comparison to hen lysozyme[4]

Conclusions

Nanosphere Effects on Prion Protein

- **Nanospheres increased overall rate of Prp fibrillation**
  - Effects of nanosphere concentration differed between different sizes
- **Specific Nanosphere-Prp interactions still unknown**
  - Nanospheres don't significantly bind to Prp
  - Incubation with nanospheres affects Prp secondary structure but specific trend is not known

Future directions:

- More in depth analysis methods needed to determine mechanism of nano plastics interaction with Prp
  - Monitor change in secondary structure over a time interval with CD
  - Use IR spectroscopy to determine further structural changes

Crowding Effects on Human Lysozyme

- Crowding agents tend to have promoting effect on human lysozyme fibrillation overall
- Human lysozymes receive stronger promotive effect from polymers in higher concentration, while hen lysozymes are more sensitive to monomers/small molecules in lower concentration

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