Inhibiting Protein Amyloid Aggregation with Nanoparticles

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Protein misfolding diseases

- An increasing list of protein misfolding diseases
  - Alzheimer’s disease – Aβ
  - Parkinson’s disease – α-synuclein
  - Huntington’s disease – huntingtin
  - Type-2 Diabetes – Islet Amyloid Polypeptide
  - Amyotrophic Lateral Sclerosis – SOD1

- Common hallmarks
  - Fibrillar aggregates – regular structures from different precursors
  - Long process; rare nucleating events
  - Symptoms typically appear in mid to later life (50-70 years)

Amyloid fibril – the common cross-beta structure

Nucleation Process – Sigmoidal Kinetics

Inhibitor design – targeting each of the step
Nanoparticles as catalysts for protein fibrillation

Linse S. et al, PNAS 104:8691-6, 2007
Colvin VL and Kulinowski KM, PNAS 104:8679-8, 2007
Aggregation promoting or inhibiting – the contrasting effects of NPs?

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Proteins</th>
<th>Effects on Amyloid Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-walled CNT, QDs, Copolymer NP, CeO$_2$ NP$^{23}$</td>
<td>β-2 microglobulin</td>
<td>Promotion</td>
</tr>
<tr>
<td>TiO$_2$ NP$^{43}$</td>
<td>Aβ</td>
<td></td>
</tr>
<tr>
<td>AuNP$^{75}$</td>
<td>lysozyme</td>
<td></td>
</tr>
<tr>
<td>Graphene oxide$^{67}$</td>
<td>Aβ</td>
<td></td>
</tr>
<tr>
<td>AuNP$^{45}$</td>
<td>Aβ</td>
<td>Inhibition</td>
</tr>
<tr>
<td>CNT$^{42}$</td>
<td>Aβ$_{16-22}$</td>
<td></td>
</tr>
<tr>
<td>Carbon Dots$^{76}$</td>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>Polymeric NP$^{68}$</td>
<td>Aβ</td>
<td>Either promotion or inhibition</td>
</tr>
<tr>
<td>Polystyrene NP$^{37}$</td>
<td>Aβ</td>
<td></td>
</tr>
</tbody>
</table>

Q: What are the **determinants** of NPs and/or proteins for the complex and seemingly contrasting behaviors?

Objective – Amyloid-inhibiting nanomedicine
Outline

- Multiscale modeling approach
  - DMD simulations
  - Multiscale models
- Uncovering the effects of NPs on protein aggregation
  - Varying NP-Protein attractions
  - Competing aggregation in solution and on NP surface
  - A complete picture of protein aggregation influenced by NPs
- Applications of anti-amyloid Nanomedicine
  - Graphene oxide
  - Dendrimer
Challenges in computational modeling: multiscale modeling

Large gaps of time and length scales between experimental observation and the underlying molecule system

Approaches:
Enhanced sampling methods
Simplified protein models

Ding F. and Dokholyan N.V., Trends in Biotechnology, (2005)
Enhanced MD method: DMD

\[ m \ddot{\alpha}_i = \sum_j \vec{F}_{ij} \]

Dynamics become event-driven:
- collision prediction,
- sorting for next collisions,
- updating the colliding atoms

Alder and Wainwright, J. Chem. Phys. 27:1208 (1957);
Zhou Y and Karplus M, PNAS, 94, 14429 (1997);
Multi-scale protein models

**Two-Bead**

Time scale: \(~\text{seconds-hours}\)
Applications: Protein folding/misfolding, Protein aggregation,


**Four-Bead**

Time scale: \(~\text{seconds}\)
Applications: 2\textsuperscript{nd} structure transition, Protein folding/misfolding, Protein aggregation


**Pseudo all-atom**

Time scale: \(~\mu\text{s-ms}\)
Applications: Protein folding/misfolding, aggregation of short peptides


**All-atom**

Time scale: \(~\mu\text{s}\)
Applications: Folding of small proteins; near-native dynamics; and protein unfolding

Multiscale DMD simulations

*Ab initio* protein folding

Coarse-grained simulation of protein aggregation

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Coarse-grained modeling of NPs

Effects: NP-protein attractions (affinities), relative concentrations, competition between bulk and surface, etc.

Complex effects of NP-Protein attractions on protein aggregation
The dependence of protein surface concentration on NP-Protein attractions

Increasing NP-protein attractions leads to more proteins on NP surface
The dependence of diffusion on NP-protein attractions

Increasing NP-protein attractions leads to **decreased protein diffusion** on NP surface.
Dependence of protein concentrations (fixed attraction)

Aggregation on NP surface is concentration dependent
Effect of relative protein/NP concentration
A multi-factorial effects of NPs on aggregation

Conditional promotion (concentrations)

inhibition

Depends on NPs and Proteins

Radic, S., Ke PC, Davis, TP, Ding F., RSC Adv., 2016
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Graphene oxide inhibits IAPP aggregation and cytotoxicity
Graphene oxide sequesters IAPP

Nedumpully-Govindan et al, PCCP, 18:94-100 (2016)
Biophysical characterization of GO-IAPP interaction

Nedumpally-Govindan et al, PCCP, 18:94-100 (2016)
GO reduces cytotoxicity of IAPP

Control

GO

hIAPP

hIAPP + GO

Cell death (%)
n=4, One–way ANOVA (24 h treatment)

Nedumpully-Govindan et al, PCCP, 18:94-100 (2016)
PAMAM dendrimer inhibits IAPP aggregation and cytotoxicity
PAMAM dendrimer binds the amyloidogenic region of Amyin monomer
PAMAM dendrimer inhibits dimerization

E.N. Gurzov et al, Small, in press (2016)
Biophysical characterization of the anti-aggregation effects – DLS, ThT, TEM

E.N. Gurzov et al, Small, in press (2016)
Inhibition of IAPP cytotoxicity *in vitro* and *ex vivo*

E.N. Gurzov et al, Small, in press (2016)
Summary

- A multiscale approach for modeling protein aggregation at the Nano-Bio interface with long time scales and large system sizes
- A mechanistic insight about the complex and seemingly contrasting effects of NPs on amyloid aggregation
- Utilizing the anti-aggregation effects of NPs for anti-amyloid nanomedicine design.
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