Simulations of amyloid aggregation:
From coarse-grained models of fibril formation to atomistic models of potential inhibitors

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"Ich habe mich verloren"

(Auguste Deter, 1901)
Alzheimer’s disease

PREVALENCE OF ALZHEIMER’S DISEASE
(BY DECADES IN U.S.A. FROM 1900-2050)

YEARS


NUMBER OF VICTIMS

0 2,000,000 4,000,000 6,000,000 8,000,000 10,000,000 12,000,000 14,000,000

AGE 65-74 YEARS  AGE 75-84 YEARS  AGE 85+ YEARS
Alzheimer’s disease

- Misfolding and aggregation

Microscopy

Morphology

Diffraction Pattern

Cross-β structure
Prevent misfolding and aggregation
Models and Simulation Methods

I. Monomeric Aβ (10⁰ peptide): Atomistic model, Monte Carlo sampling (A. Vitalis)

II. Oligomerization inhibitors (10¹ peptides): Atomistic model, MD (M. Convertino)

III. Fibrillation kinetics (10² − 10³ "peptides"): Simple (coarse-grained phenomenological) model, MD (R. Pellarin)
Methods

- Explicit Aβ (OPLS partial charges) and ions (130 mM), implicit water (ABSINTH model, Vitalis and Pappu, JCC 2009)
- Monte Carlo simulations in torsional space (75% backbone and 15% side chain moves) and rigid-body motion (10% moves)
- 12 replica-exchange (REMC) runs for each of the two alloforms
- In each REMC run, four replicas at temperatures of 280, 295, 310, and 325 K
- For each run and replica, $10^8$ Monte Carlo steps in the NVT ensemble
- Standard errors are standard deviations of trajectory average values from 12 independent runs (all interblock correlations eliminated)
$$A\beta_{40} \circ$$
$$A\beta_{42} \triangle$$

ubiquitin
tendamistat
calbindin
average value
I. Monomeric Aβ ensemble

Central structures (large ribbon) of most populated clusters at 280 K and backbone of nine other structures from the same cluster (thin ribbons)
I. Monomeric Aβ ensemble

Residue-Residue Contact Probabilities

Equilibrium data at 310 K (upper-left) and standard errors (lower-right). Contact definition: any atom-atom distance between a specific pair of residues < 5.5 Å.
I. Monomeric $\beta$ ensemble

Monte Carlo simulations

Experimentally determined structures (NMR)

$\beta$-propensity at 310 K

Fibril model of $\text{A}_1^\text{42}$
Riek, PNAS 2005 (PDB 2BEG)

$\text{A}_1^\text{40}$ bound to an affibody protein identified by phage display (Hoyer et al., PNAS 2008)
I. Monomeric Aβ ensemble

Results

Collapsed structure for both Aβ_{40} and Aβ_{42} with fluid hydrophobic core

The 11 N-terminal segment is completely unstructured

All charged side chains, particularly Glu22 and Asp23 exposed to solvent

Low $\beta$-sheet propensity but sequence specific and consistent with solid-state NMR

The sequence partially encodes fibril structure, but fibril elongation must be thought of as a templated assembly step

Vitalis and A.C., J. Mol. Biol. 403, 148, 2010
Models and Simulation Methods

I. Monomeric Aβ ($10^0$ peptide): Atomistic model, Monte Carlo sampling (A. Vitalis)

II. Oligomerization inhibitors ($10^1$ peptides): Atomistic model, MD (M. Convertino)

III. Fibrillation kinetics ($10^2$ – $10^3$ "peptides"): Simple (coarse-grained phenomenological) model, MD (R. Pellarin)
II. Aβ oligomerization inhibitors
II. Aβ oligomerization inhibitors

Methods

• Explicit Aβ heptapeptides and inhibitor (CHARMM), implicit solvent (SASA, FACTS)
• Aβ-heptapeptide concentration of 5 mg/ml, Aβ:inhibitor ratio of 3:1
• Periodic boundary conditions (NVT ensemble)
• Langevin dynamics simulations (low friction) at 330 K
• 10 independent 2.5 µs runs for each Aβ heptapeptide segment
• Divide and conquer using three overlapping heptapeptides covering the H\textsubscript{14}QKLVFFAEDV\textsubscript{24} segment: Aβ\textsubscript{14−20}, Aβ\textsubscript{16−22}, and Aβ\textsubscript{18−24}
• Control simulations with explicit solvent, started from most populated "binding mode(s)"
II. Aβ oligomerization inhibitors

Three $\text{H}_{14}\text{QKLVFF}_{20}$ heptapeptides with/without inhibitor

A

$P_\gamma$

$t=865.46$ ns

$t=865.56$ ns

$t=865.62$ ns

$t=865.72$ ns

$t=865.96$ ns

$t=866.02$ ns
II. Aβ oligomerization inhibitors

Convertino et al., Protein Sci. 18, 792, 2009
II. Aβ oligomerization inhibitors

Quinone-Trp "hybrid": 1,4-naphthoquinon-2-yl-L-tryptophan (NQTrp)
II. A\(\beta\) oligomerization inhibitors

![Figure 1](image1)

![Figure 2](image2)
II. Aβ oligomerization inhibitors

Figure 7
II. Aβ oligomerization inhibitors
Results

II. Aβ oligomerization inhibitors

The linking of naphtoquinone and Trp yields a potent inhibitor of Aβ oligomerization and fibrillation

Destabilization of inter-peptide β-sheet hydrogen bonds, increase of structural disorder within the oligomer

Atomistic details of interactions: van der Waals and aromatic interactions with Phe19 and Phe20, hydrogen bonds with backbone polar groups of residues 20-22 (in agreement with NMR data)

Scherzer-Attali, Pellarin, Convertino et al., PLoS ONE 5, e11101, 2010
Endomorphins: endogenous designed

Tyr-Pro-Trp-Phe  Tyr-Pro-Phe-Phe

Figure 1

Figure 2
**Figure 1.**

(a) Western blot analysis showing the expression of proteins in the indicated samples. The proteins are labeled with their respective molecular weights (kDa). The samples are labeled as follows: lane 1, a; lane 2, b; lane 3, c; lane 4, d; lane 5, e.

(b) Bar graph showing the fluorescence intensity (arbitrary units) of the analyzed samples. The samples are labeled as follows: Aβ₁₋₁₂, 100 μM; End-1, 100 μM; End-1, 50 μM; End-1, 5 μM; Aib-1, 100 μM; Aib-1, 50 μM; Aib-1, 5 μM; Positive control.
Models and Simulation Methods

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II. Oligomerization inhibitors (10¹ peptides): Atomistic model, MD (M. Convertino)

III. Fibrillation kinetics (10² – 10³ "peptides"): Simple (coarse-grained phenomenological) model, MD (R. Pellarin)
III. Coarse-grained simulations

Outstanding questions in protein aggregation:

1. Why are fibrillation kinetics complex and heterogeneous?

2. Are there multiple pathways?

3. Is there a kinetic control?

4. How do oligomers and (proto)fibrils interact with the membrane?
Methods

Phenomenological coarse-grained model of an amphipathic "peptide" (10 beads per peptide)

Peptide concentration of 8 mM

Periodic boundary conditions (NVT ensemble)

Langevin dynamics (low friction) at 310 K

For each amyloidogenity value, 100 runs of length up to 20 $\mu$s each (40 CPU-days/run)

Initial frame: 125 (or 1000) monodispersed "peptides"
IIIa. Peptide aggregation kinetics

Only 1 degree of freedom ($\phi$) and 10 interaction centers
Two states, amphipathic, 125 monomers in simulation box
IIIa. Peptide aggregation kinetics

Only 1 degree of freedom ($\phi$) and 10 interaction centers
Two states, amphipathic, 125 monomers in simulation box
Dependence on amyloidogenicity (dE)

High amyloidogen. (dE $\geq 0$ kcal/mol): Nucleation faster than micelle.
Low amyloidogen. (dE $\leq -2$ kcal/mol): Nucleation slower than micelle.
Dependence on amyloidogenicity ($dE$)

High amyloidogen. ($dE \geq 0$ kcal/mol): Nucleation faster than micelle.
Low amyloidogen. ($dE \leq -2$ kcal/mol): Nucleation slower than micelle.

Kinetic traces analogous to V18I (A), V18Q (B), and V18P (C) mutants of A$\beta$40 (Fändrich, 2005).
Illa. Peptide aggregation kinetics

Protofibrillar intermediates and templated protofilament assembly

\[ dE = -1.5 \text{ kcal/mol} \]

\[ dE = -2.5 \text{ kcal/mol} \]

Pellarin et al., J. Mol. Biol. 374, 917, 2007
IIIA. Peptide aggregation kinetics

Dependence on concentration

Lag phase \([dE = -2.5 \text{ kcal/mol}]\)

The micelle aggregation number reaches a plateau \((N=20-23)\): Agreement with A\(_{42}\) surfactant behavior.

Critical concentration for micelle formation = 4.4 mM.

High amyloidogenicity: first order elongation.
Low amyloidogenicity: competitive polymerization, monomer/micelle equilibrium maintains a nearly constant concentration of isolated monomers.
IIIa. Peptide aggregation kinetics

Dependence on concentration

The micelle aggregation number reaches a plateau (N=20-23): Agreement with Aβ42 surfactant behavior.

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Influence of concentration on insulin fibril formation (Fink, Biochemistry 2001).
Ill a. Peptide aggregation kinetics

β-Stable scenario

small nucleus
fast
downhill
no intermediates
main pathway
$k_{elongation}$ depends on conc.

Phe-Phe, GNNQQNY, transthyretin, $\beta_2$-microglobulin Pmel17, other $\beta$-proteins?

β-Unstable scenario

large nucleus
slow
micellar intermediates
protofibrillar intermediates
multiple pathways
$k_{elongation}$ conc. independent

Aβ peptide, Sup35, prion protein, myoglobin, (other $\alpha$-proteins?)
Outstanding questions in protein aggregation:

1. Why are fibrillation kinetics complex and heterogeneous?

2. Are there multiple pathways?

3. Is there a kinetic control?

4. How do oligomers and (proto)fibrils interact with the membrane?
Prefibrillar Intermediates

- **M**
  - Micellar oligomers
  - **N**=15-20
  - **N**^p^f?=0.1
  - **N**^n?=15-18

- **2PP**
  - 2-protofilament protofibril
  - **N**=55-75
  - **N**^p^f?=2
  - **N**^n?=25-35

- **3PP**
  - 3-protofilament protofibril
  - **N**=60-85
  - **N**^p^f?=3
  - **N**^n?=20-30
  - P1=0.3

- **3PF**
  - 3-protofilament fibril (rare)
  - **N**>90
  - **N**^p^f?=3
  - **N**^n?=5-10
  - P1=0.1

Mature Fibrils

- **4PF**
  - 4-protofilament fibril
  - **N**>90
  - **N**^p^f?=4
  - **N**^n?=5-10
  - P1=variable

Mature Fibrils Morphologies

- **4PF^1^/(+,−)**
  - P1=0.1
  - l_{min}=0.13-0.15

- **4PF^2^−**
  - P1=0.37
  - l_{min}=0.11

- **4PF^2^+**
  - P1=0.35
  - l_{min}=0.12

- **4PF^3^**
  - P1=0.0
  - l_{min}=0.12
IIIa. Kinetic control

![Graphs showing ΔF (kcal/mol) vs. Zₐ/Z and dE (kcal/mol) vs. nucleation frequency for different species: 4PF₁, 4PF₂⁺, and 4PF₂⁻.](image)
IIIa. Kinetic control

A. $\beta$-stable

B. $\beta$-unstable

C. Fibril maturation

Lag phase

Micellar oligomers

Monomers

4PF1, 4PF3, 4PF2+, 4PF2-

3PP1, 3PP2+, 3PP2, 3PP2-

2RP

Nucleus
IIIa. Kinetic control

Pellarin et al., JACS 132, 14960, 2010
IIIb. Interactions with membrane

Large hydrophilic head: Formation of micelles
Three-bead lipid model

Small hydrophilic head: Formation of liposome

Begin

End
Fibril growth on vesicle
Membrane leakage

Protofibril growth at vesicle surface promotes leakage

Time = 20 ns

Time = 750 ns
Membrane leakage

Branching at vesicle surface promotes leakage

- time = 89 ns
- time = 90 ns
Influence of amyloidogenicity

Average number of polar contacts vs. Time (ns)

- high, without vesicles
- high, with preformed fibril
- intermediate, without vesicles
- intermediate
- low
- very low

Amyloidogenicity
Leakage depends on amyloidogenicity

The graph shows the average number of probe molecules over time (in ns) for different levels of amyloidogenicity:
- **No peptides**
- **With preformed fibril**
- **Very low**
- **Low**
- **Intermediate**
- **High**

As the amyloidogenicity increases, the leakage also increases, indicating a direct correlation between the two.
Influence of amyloidogenicity

![Graph showing the influence of amyloidogenicity on the average number of polar contacts and probe molecules over time. The x-axis represents time in nanoseconds (ns), and the y-axes represent the average number of polar contacts and probe molecules. The graphs illustrate the comparison between high, intermediate, and low amyloidogenicity scenarios, with and without vesicles, highlighting the impact on molecular interactions.]
Interactions with membrane

Intermediate amyloidogenicity

Low amyloidogenicity

Number of peptides

Number of probe molecules

Time (ns)

fibril growth on vesicle

fibril growth in the bulk
Interactions with membrane

Legend:
- Red: Peptide in β-conformation
- Blue: Peptide in non-β-conformation
- Orange: Peptide in either conformation

1. Low amyloidogenicity
2. High amyloidogenicity
3L. Slow leakage
3H. Fast leakage
4L. Slow
4H. Fast
5.

– p. 50
### Interactions with membrane

<table>
<thead>
<tr>
<th>scaling of pept-lip. int.</th>
<th>amyloidogenicity</th>
<th>Number of runs with np&gt;100</th>
<th>Lag time $t_{50}$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>with membr.</td>
<td>without membr.</td>
</tr>
<tr>
<td>0.87</td>
<td>high</td>
<td>10/10</td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td>interim.</td>
<td>29/29</td>
<td>89 ± 29</td>
</tr>
<tr>
<td></td>
<td>low</td>
<td>17/20</td>
<td>958 ± 503</td>
</tr>
<tr>
<td></td>
<td>very low</td>
<td>0/10</td>
<td>&gt; 2000</td>
</tr>
<tr>
<td>0.90</td>
<td>high</td>
<td>10/10</td>
<td>10 ± 1</td>
</tr>
<tr>
<td></td>
<td>interim.</td>
<td>30/30</td>
<td>69 ± 23</td>
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</tr>
<tr>
<td></td>
<td>very low</td>
<td>0/20</td>
<td>&gt; 2000</td>
</tr>
</tbody>
</table>

Friedman et al., JMB 387, 407, 2009
Conclusions

- Protofibril growth but not mature fibril promotes cell leakage.
- Membrane accelerates (slows down) fibril formation of peptides with high (low) \( \beta \)-aggregation propensity.

Friedman et al., JMB 387, 407, 2009
Acknowledgments

A. Vitalis
R. Pellarin
M. Convertino
R. Friedman

Tel Aviv: R. Scherzer-Attali, A. Frydman-Marom, Prof. E. Gazit
Coarse-grained simulations

Seeding eliminates lag phase
Effect of seeding

Number of polar contacts

- Seeded
- Unseeded

Time (μs)
Coarse-grained simulations

Seeding eliminates lag phase

Effect of seeding

Fink, Biochemistry 2001
Coarse-grained simulations

Fibrils are dynamic assemblies despite their ordered arrangement.

Models which assume irreversible aggregation are not realistic!

Number of unrecycled monomers as a function of time for nine runs started from a preformed fibril of the β-unstable model. The decay time (inset) does not depend on concentration.

C=16.0 mM dE=-2.5 kcal/mol
Convergence of dispersed monomer concentration in aggregation and disaggregation runs.

Critical concentration of fibril formation (2.5 mM) is lower than critical concentration of micelle formation (4.4 mM). Hence, micelles disappear at monomer-fibril equilibrium.
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Critical concentration of fibril formation (2.5 mM) is lower than critical concentration of micelle formation (4.4 mM). Hence, micelles disappear at monomer-fibril equilibrium.

S26P mutant of Aβ40
(Wetzel, Biochemistry 2005)
Conclusions II

• Seeding eliminates lag phase.
• Molecular recycling: Fibrils are dynamic assemblies.
• Coarse-grained model is useful to explore large range of aggregation kinetics from very slow (pathological aggregation) to very fast ("Functional" amyloids).