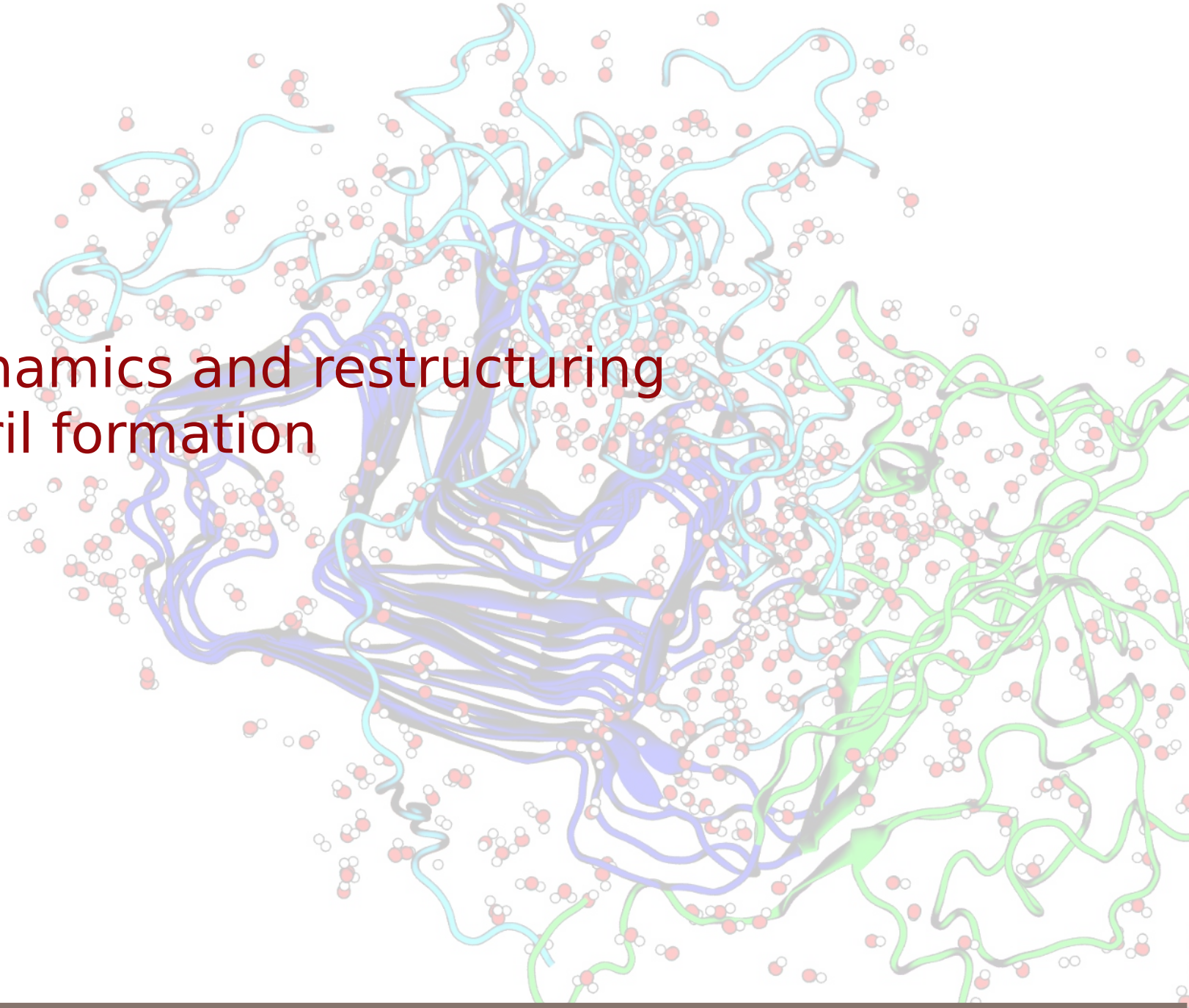
Unraveling water dynamics and restructuring upon α-synuclein fibril formation

Kevin Pounot



NEUTRONS FOR SOCIETY

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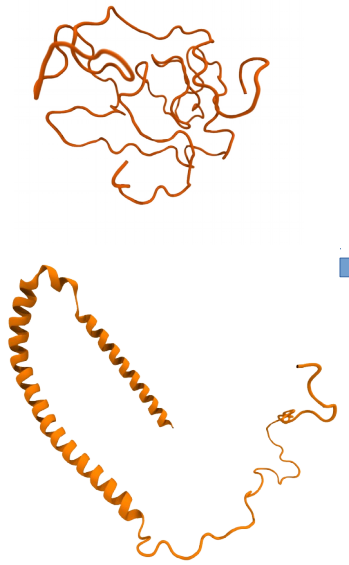
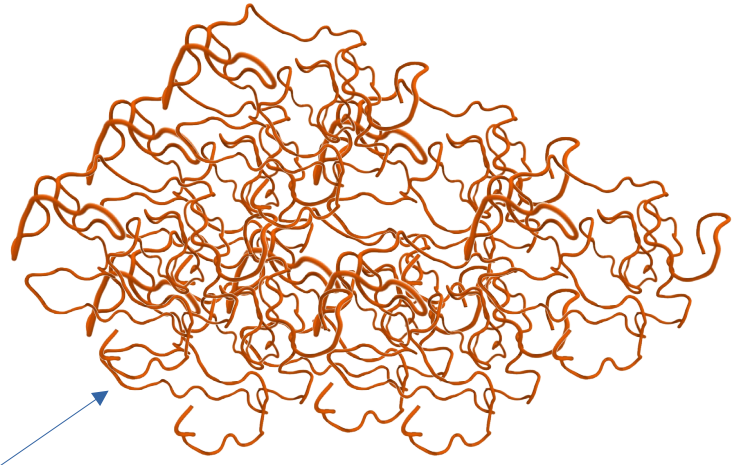
UCIRVINE

Introduction – protein conformation and stability

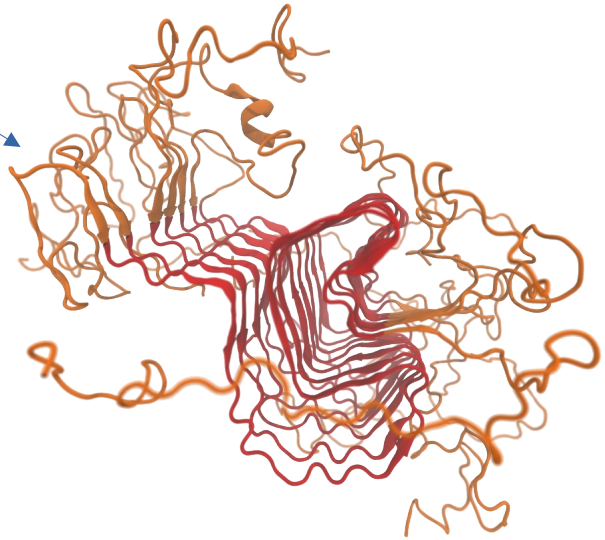
native conformations



oligomers

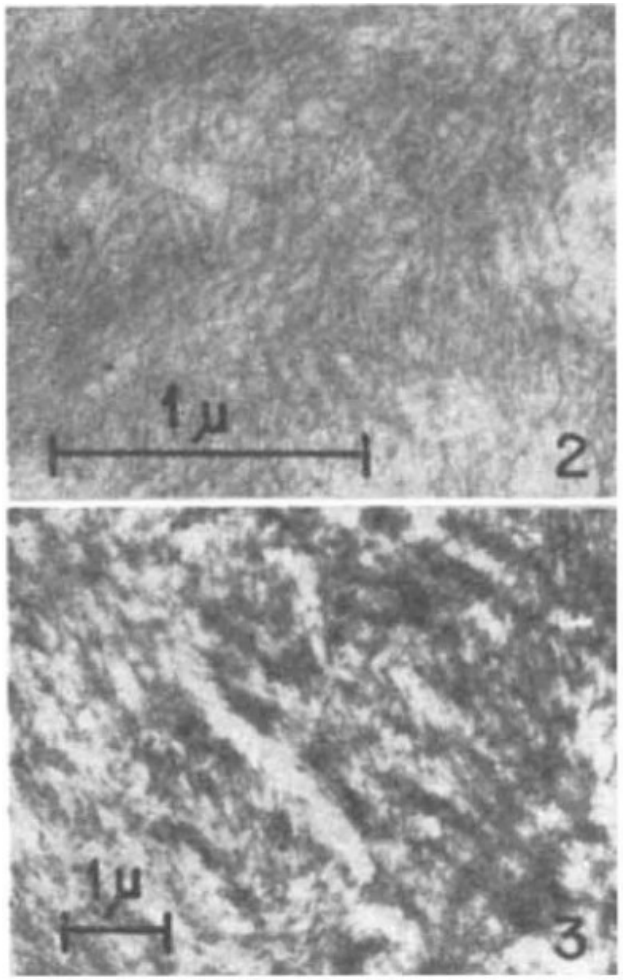


disordered aggregates

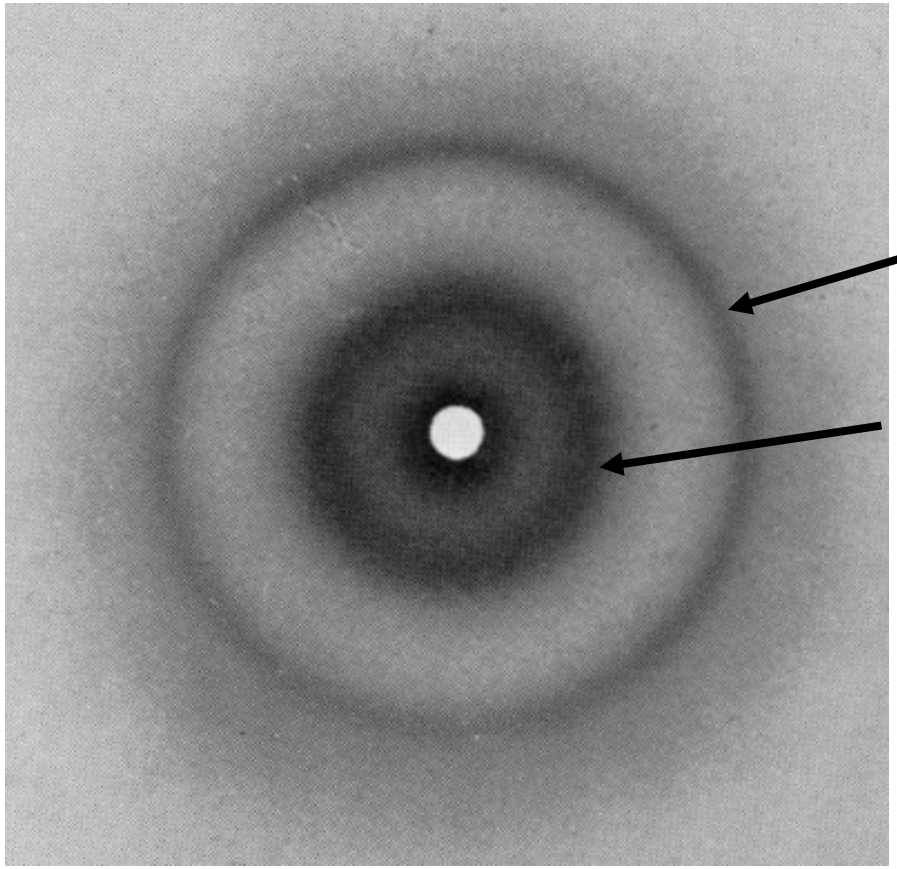


ordered (amyloid) aggregates

Introduction – the amyloid fibril

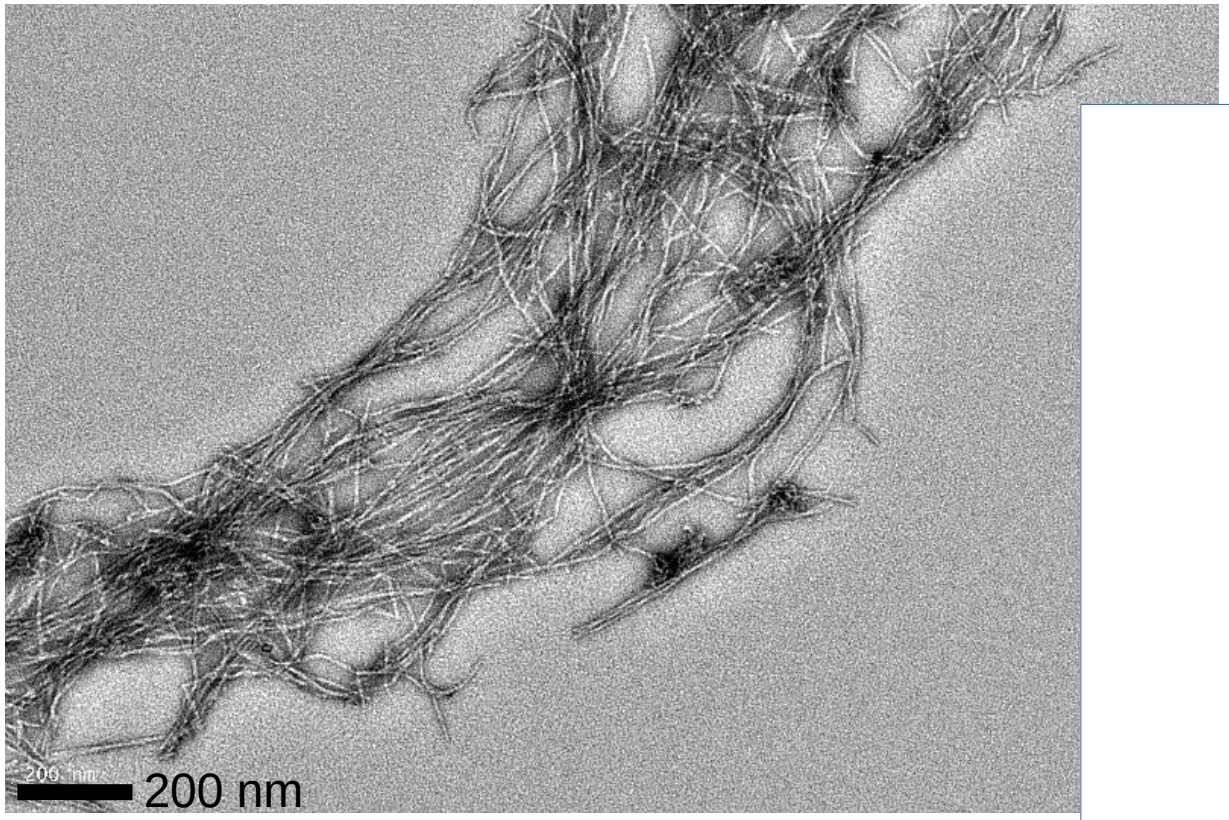


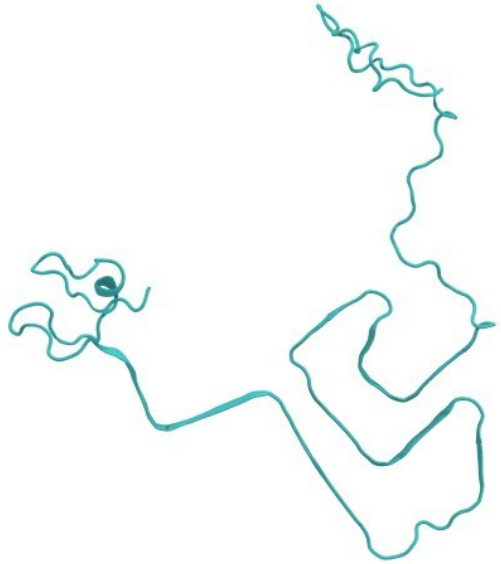
Cohen and Calkins, Nature (1959)



Astbury et al., The Biochemical Journal (1935)

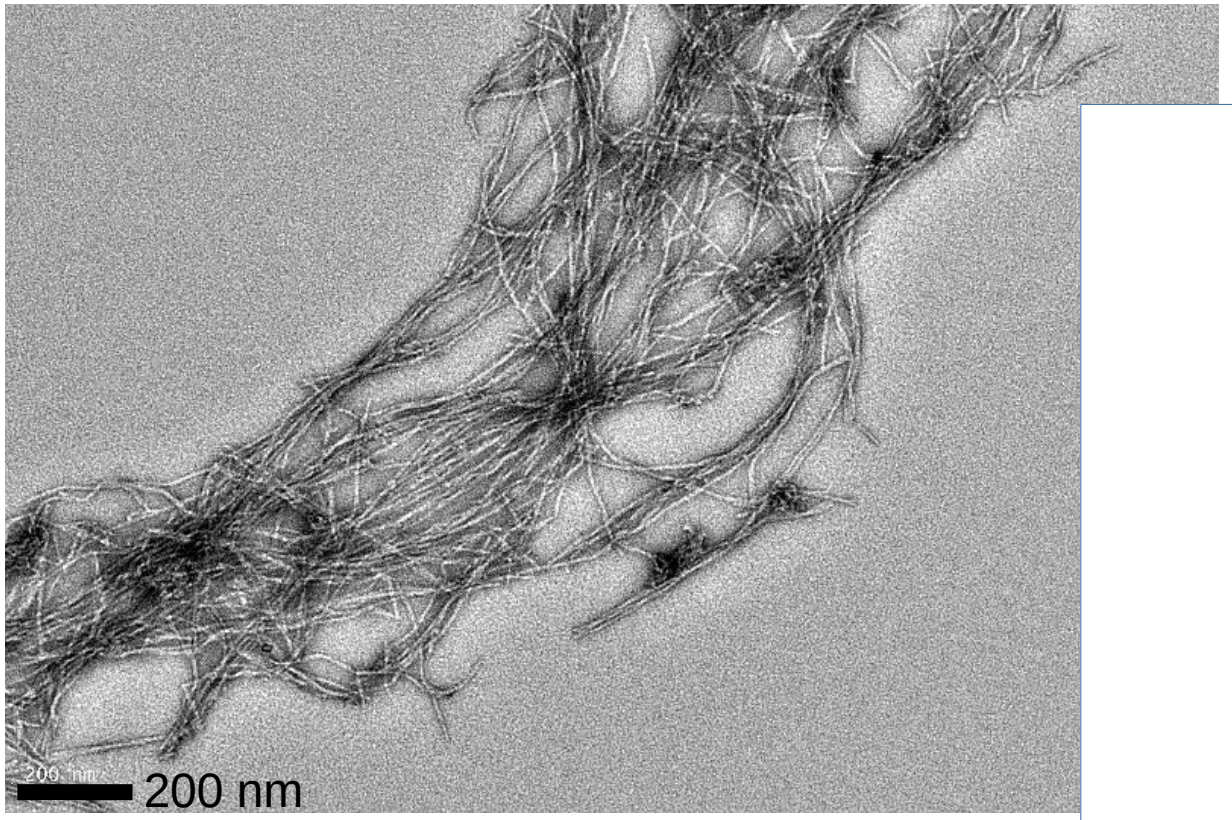
Introduction – the amyloid fibril





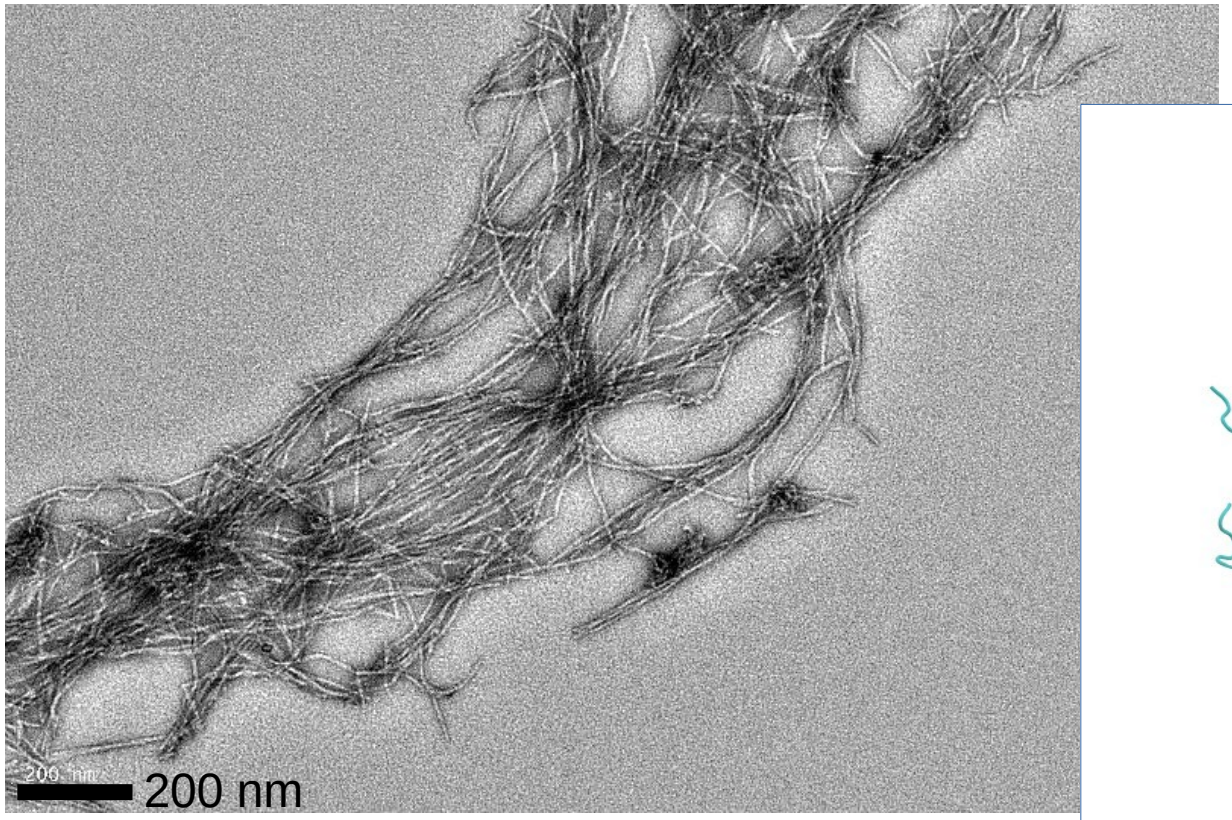
Tuttle et al., Nature Structural & Molecular Biology (2016)

Introduction – the amyloid fibril



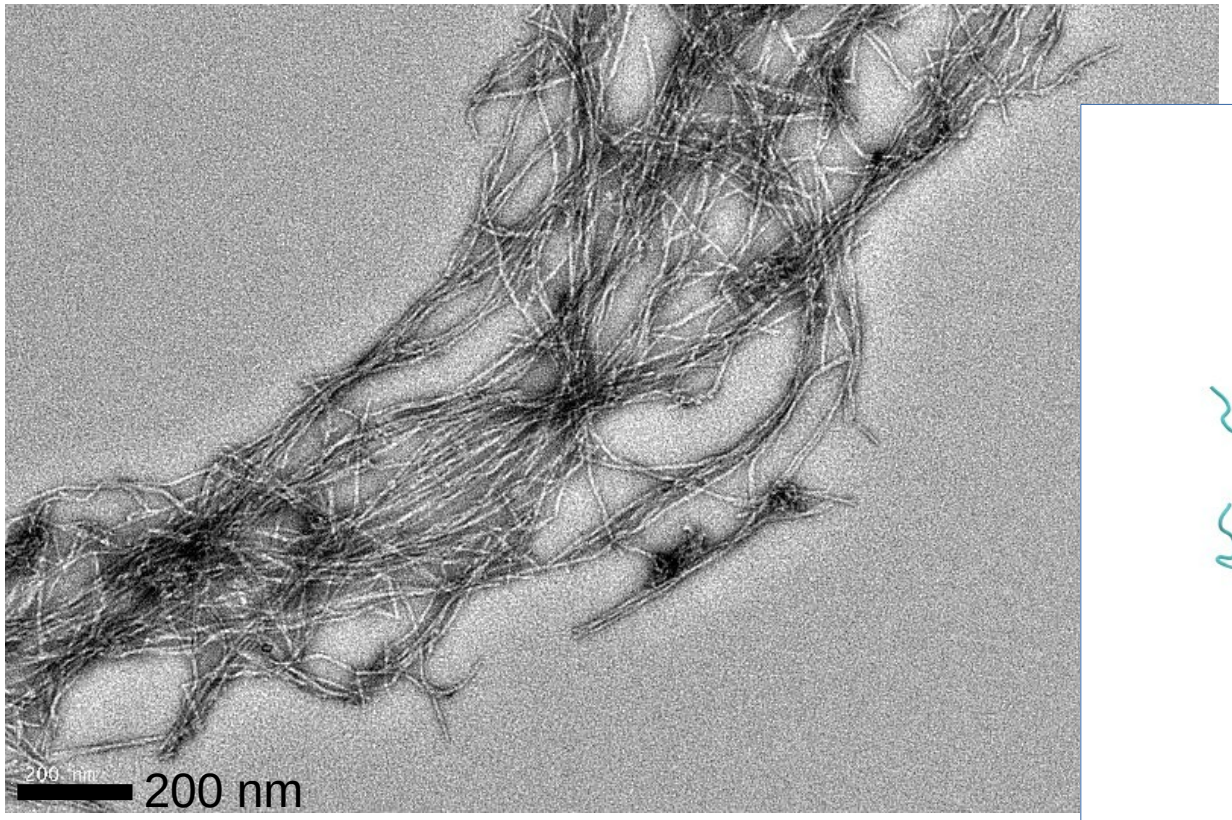
Tuttle et al., Nature Structural & Molecular Biology (2016)

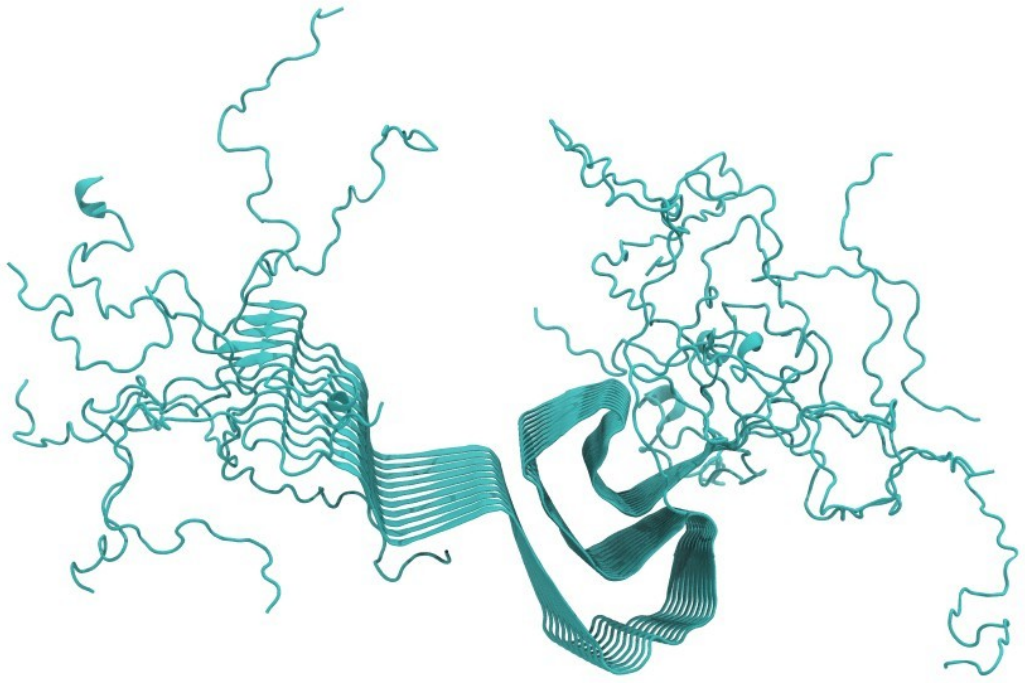
Introduction – the amyloid fibril



Tuttle et al., Nature Structural & Molecular Biology (2016)

Introduction – the amyloid fibril



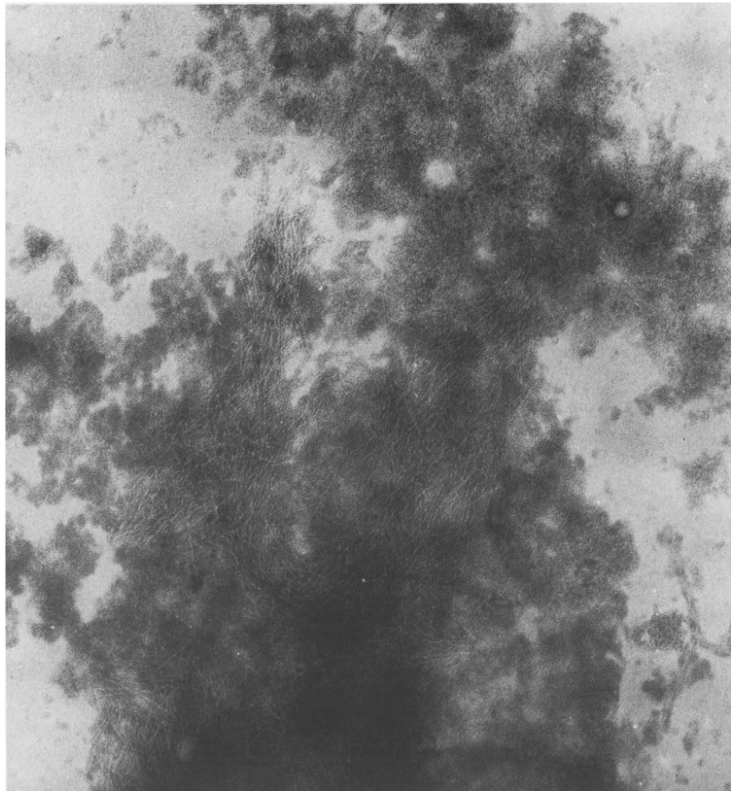


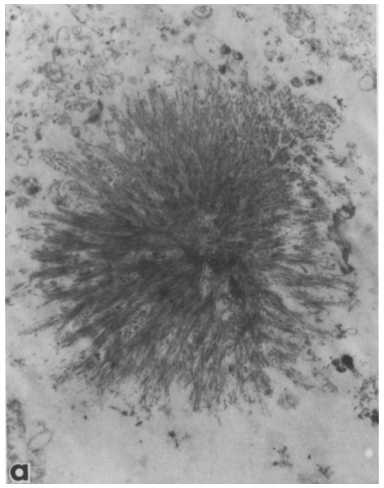
Tuttle et al., Nature Structural & Molecular Biology (2016)

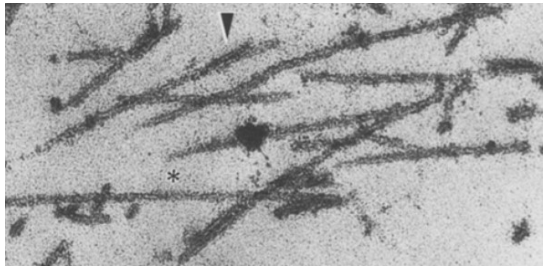
Introduction – why studying the amyloid aggregation process?

Dementia
Alzheimer's, Parkinson's

amyloid plaques





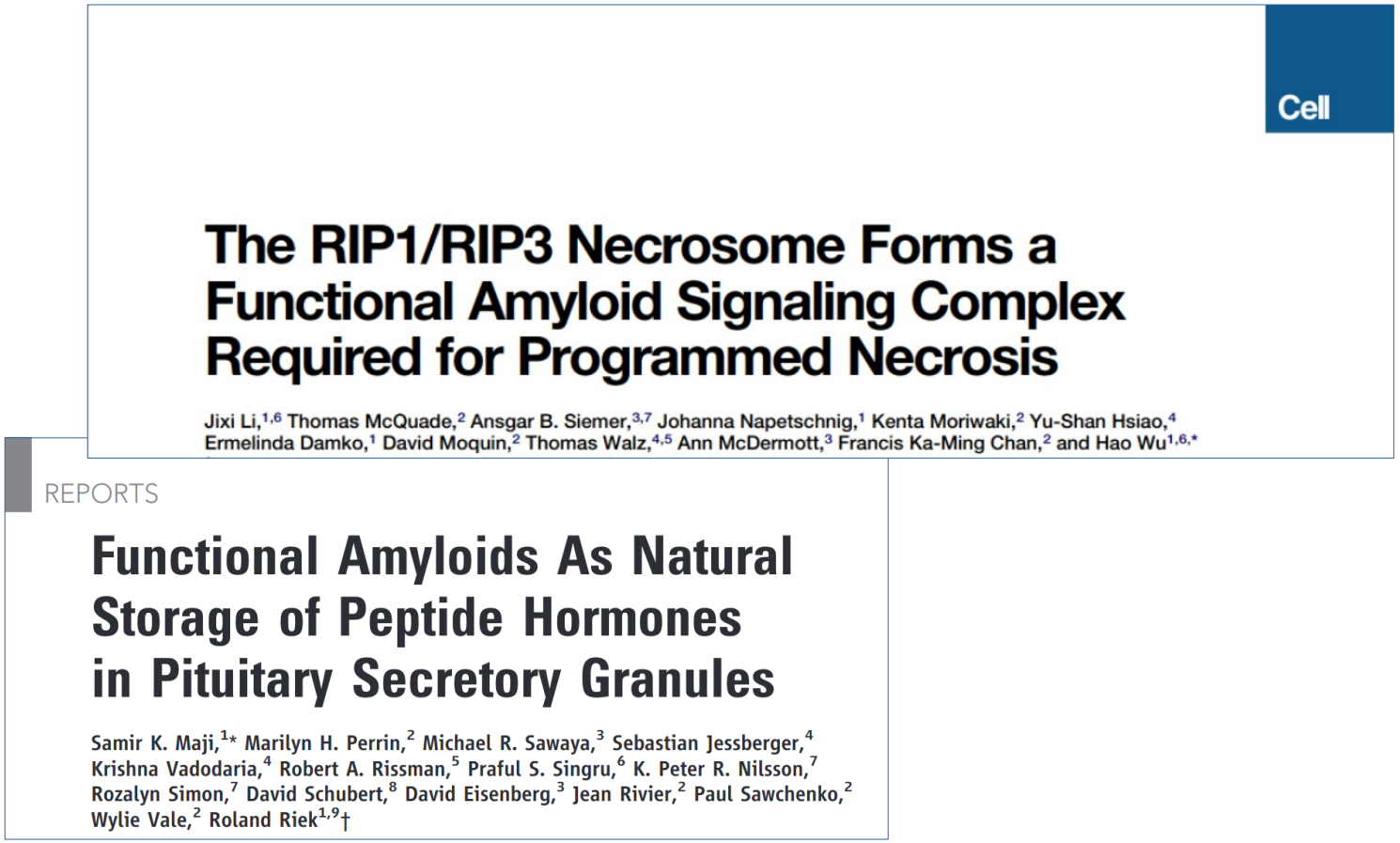


Merz et al., Acta Neuropathologica (1983)

Introduction – why studying the amyloid aggregation process?

Dementia
Alzheimer's, Parkinson's

Functional aggregation



Cell

The RIP1/RIP3 Necrosome Forms a Functional Amyloid Signaling Complex Required for Programmed Necrosis

Jixi Li,[1,6] Thomas McQuade,[2] Ansgar B. Siemer,[3,7] Johanna Napetschnig,[1] Kenta Moriwaki,[2] Yu-Shan Hsiao,[4] Ermelinda Damko,[1] David Moquin,[2] Thomas Walz,[4,5] Ann McDermott,[3] Francis Ka-Ming Chan,[2] and Hao Wu[1,6,*]

REPORTS

Functional Amyloids As Natural Storage of Peptide Hormones in Pituitary Secretory Granules

Samir K. Maji,[1*] Marilyn H. Perrin,[2] Michael R. Sawaya,[3] Sebastian Jessberger,[4] Krishna Vadodaria,[4] Robert A. Rissman,[5] Praful S. Singru,[6] K. Peter R. Nilsson,[7] Rozalyn Simon,[7] David Schubert,[8] David Eisenberg,[3] Jean Rivier,[2] Paul Sawchenko,[2] Wylie Vale,[2] Roland Riek[1,9†]

Introduction – why studying the amyloid aggregation process?

Dementia
Alzheimer's, Parkinson's

Functional aggregation

Biomaterials
drug delivery
electronics
scaffold, adhesive

ADVANCED MATERIALS
www.advmat.de
Materials Views
www.MaterialsViews.com

Amyloid Fibrils as Building Blocks for Natural and Artificial Functional Materials

Tuomas P. J. Knowles and Raffaele Mezzenga**

Protein Spherulites for Sustained Release of Interferon: Preparation, Characterization and *in vivo* Evaluation

YANBO JIANG,[1] KAI SHI,[1] DENGNING XIA,[1] SHUO WANG,[1] TAO SONG,[2] FUDE CUI[1]

[1]Department of Pharmaceutics, School of Pharmaceutical Science, Shenyang Pharmaceutical University, Shenyang 110016, China

[2]Liaoning Satellite Biotechnology Products Research Institute (Limited Company), Shenyang 110044, China

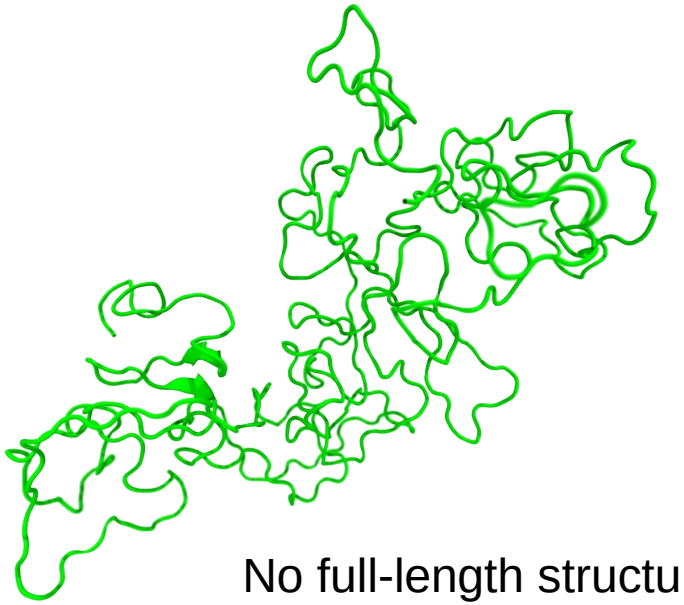
Objectives

- Fundamental understanding of protein amyloid aggregation process in the light of dynamics

Objectives

- Fundamental understanding of protein amyloid aggregation process in the light of dynamics

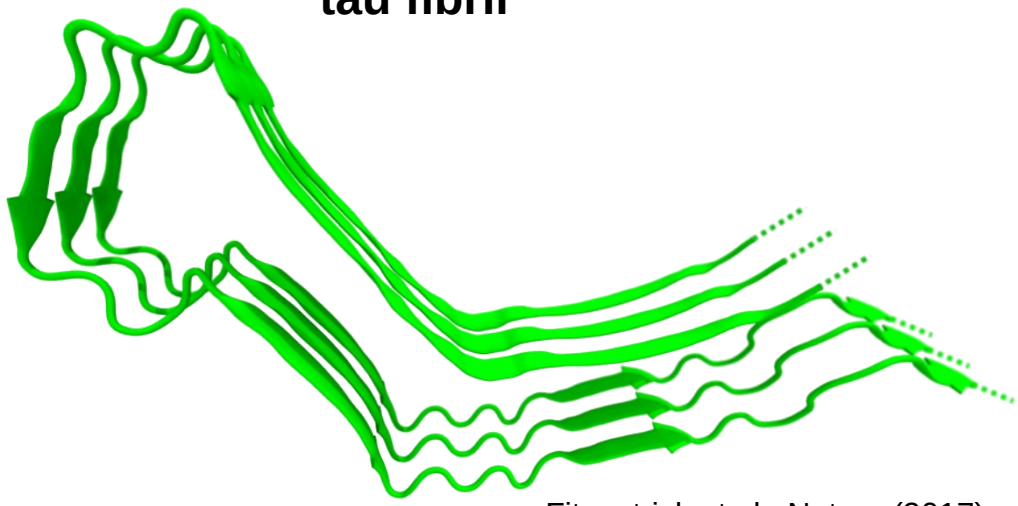
tau monomer



water dynamics increased

protein dynamics unaffected

tau fibril



Fitzpatrick et al., Nature (2017)

No full-length structure available for fibril ➔ no simulations for atomic detail

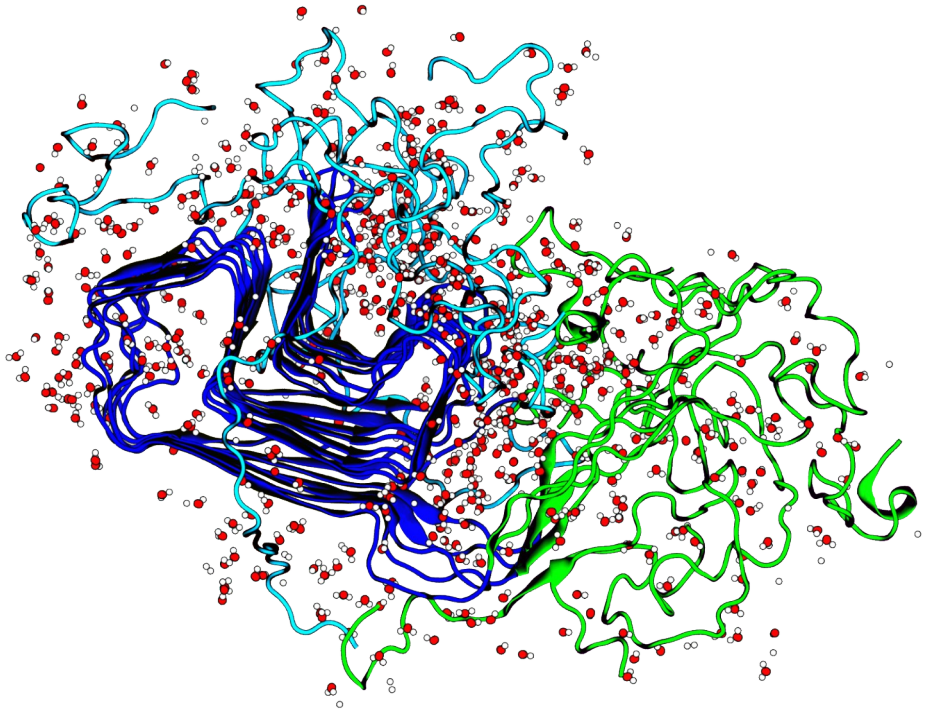
Fichou, et al. PNAS (2015)

Objectives

- Fundamental understanding of protein amyloid aggregation process in the light of dynamics

- Include other proteins for comparative study and obtain atomistic details

Proteins studied during this work



α-synuclein – Parkinson’s disease

140 aa – intrinsically disordered protein

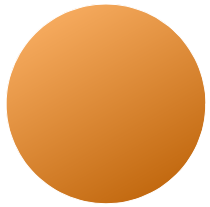
Protein and hydration water dynamics upon fibrillation

Methods – incoherent neutron scattering

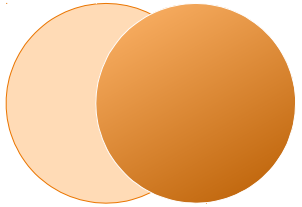
Methods – incoherent neutron scattering

$$\left(\frac{d^2\sigma}{d\Omega dE'}\right) \propto \sum_j \left\langle e^{-iq\cdot r_j(0)} e^{iq\cdot r_j(t)} \right\rangle$$

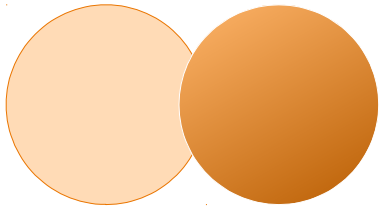
Time t=0

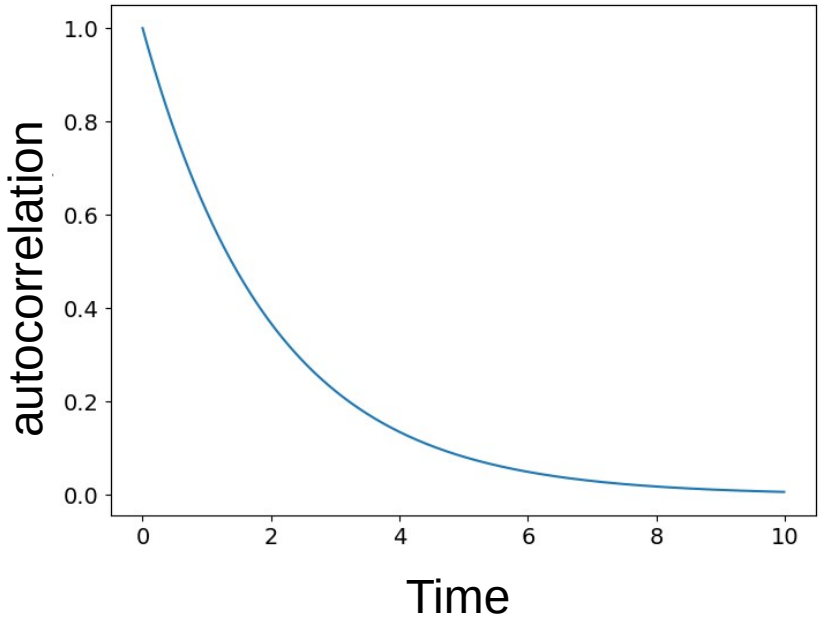


Time t=δt



Time t=2δt

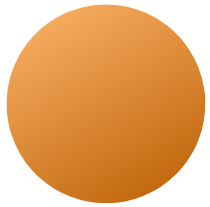




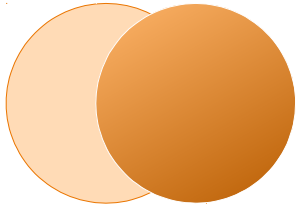
Methods – incoherent neutron scattering

$$\left(\frac{d^2\sigma}{d\Omega dE'}\right) \propto \sum_j \int \left\langle e^{-iq\cdot r_j(0)} e^{iq\cdot r_j(t)} \right\rangle e^{i\omega t} dt$$

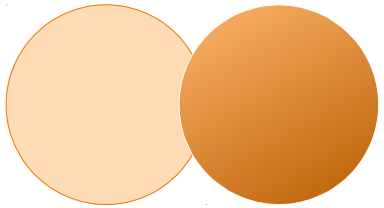
Time t=0

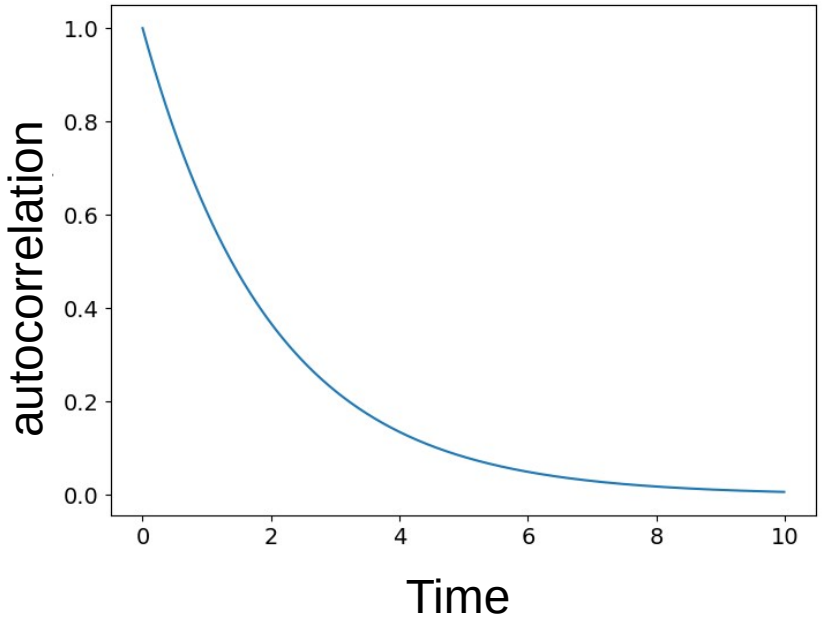


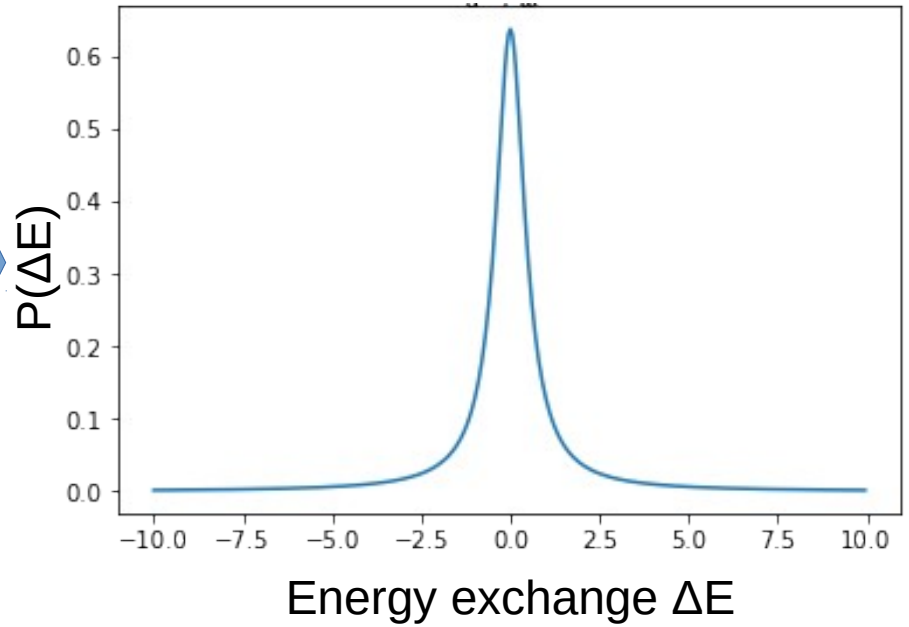
Time t=δt



Time t=2δt







Methods – incoherent neutron scattering

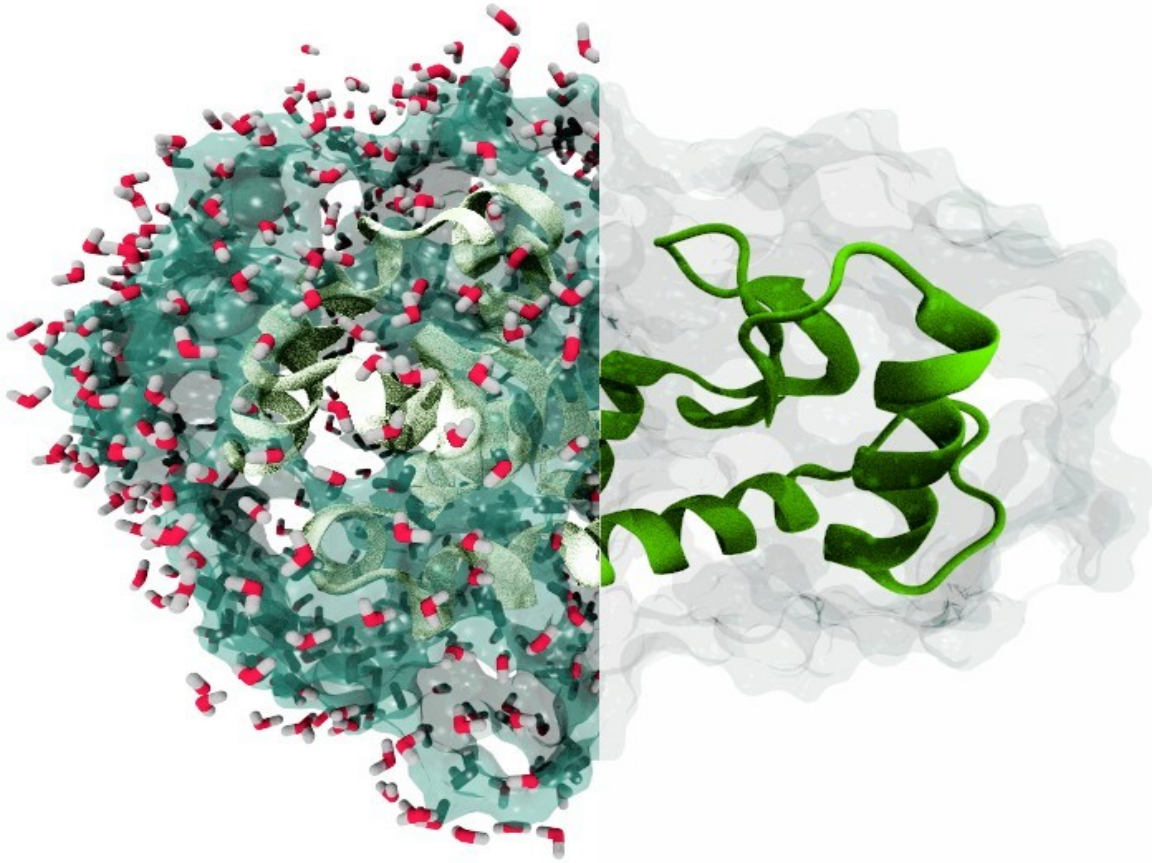
$$\left(\frac{d^2\sigma}{d\Omega dE'}\right) \propto \sigma_{inc} \sum_j \int \left\langle e^{-iq\cdot r_j(0)} e^{iq\cdot r_j(t)} \right\rangle e^{i\omega t} dt$$

~ 80 barns for hydrogen
~ 2 barns for deuterium

Methods – incoherent neutron scattering

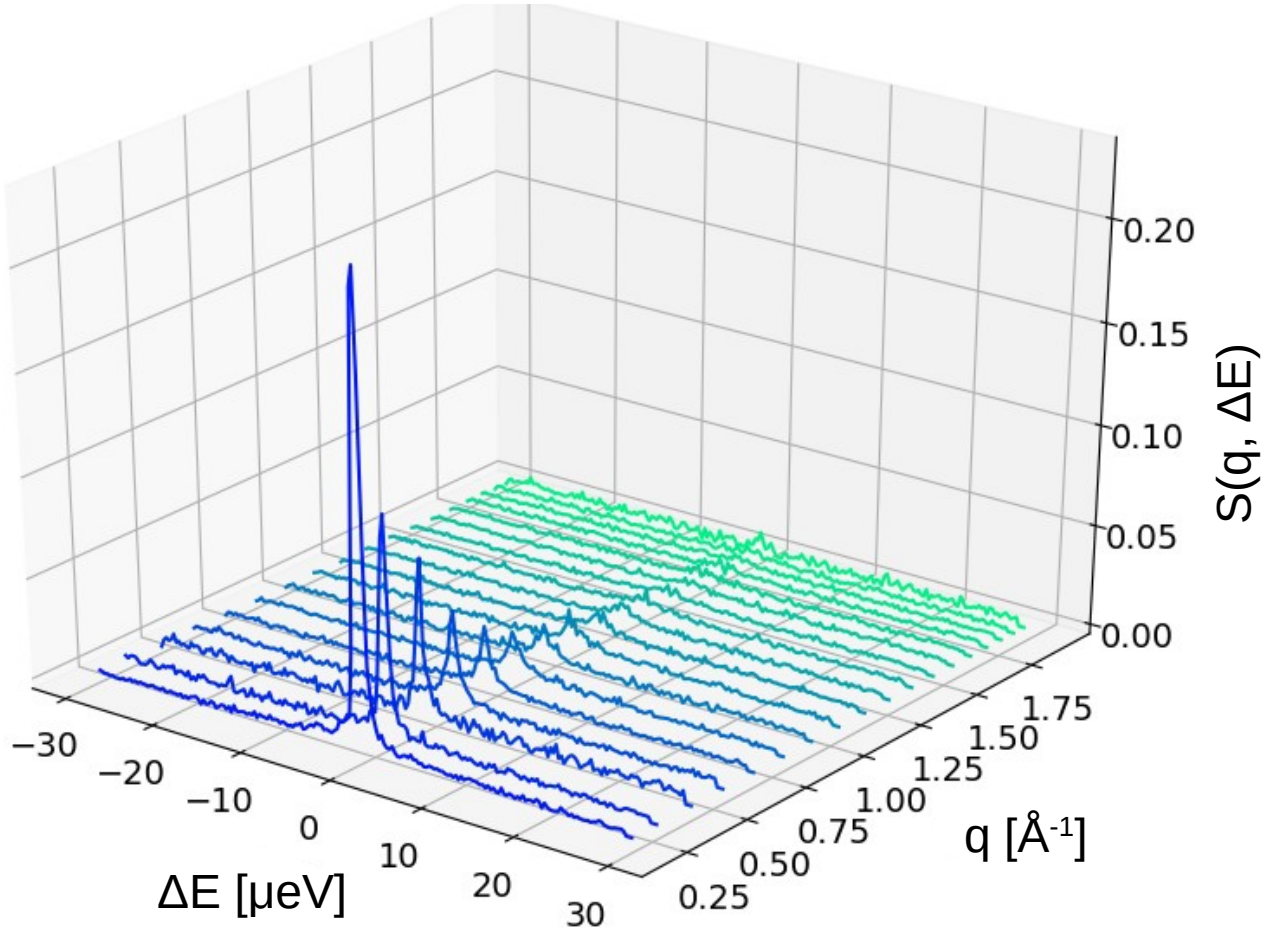
$$\left(\frac{d^2\sigma}{d\Omega dE'}\right) \propto \sigma_{inc} \sum_j \int \left\langle e^{-iq\cdot r_j(0)} e^{iq\cdot r_j(t)} \right\rangle e^{i\omega t} dt$$

D-protein
H_2O

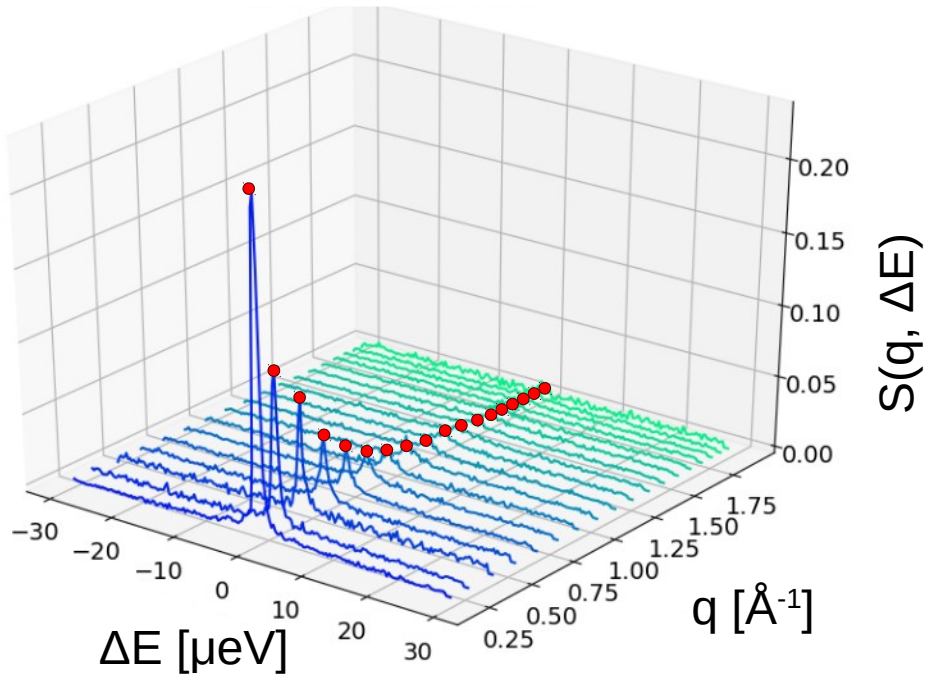


H-protein
D_2O

Methods – incoherent neutron scattering



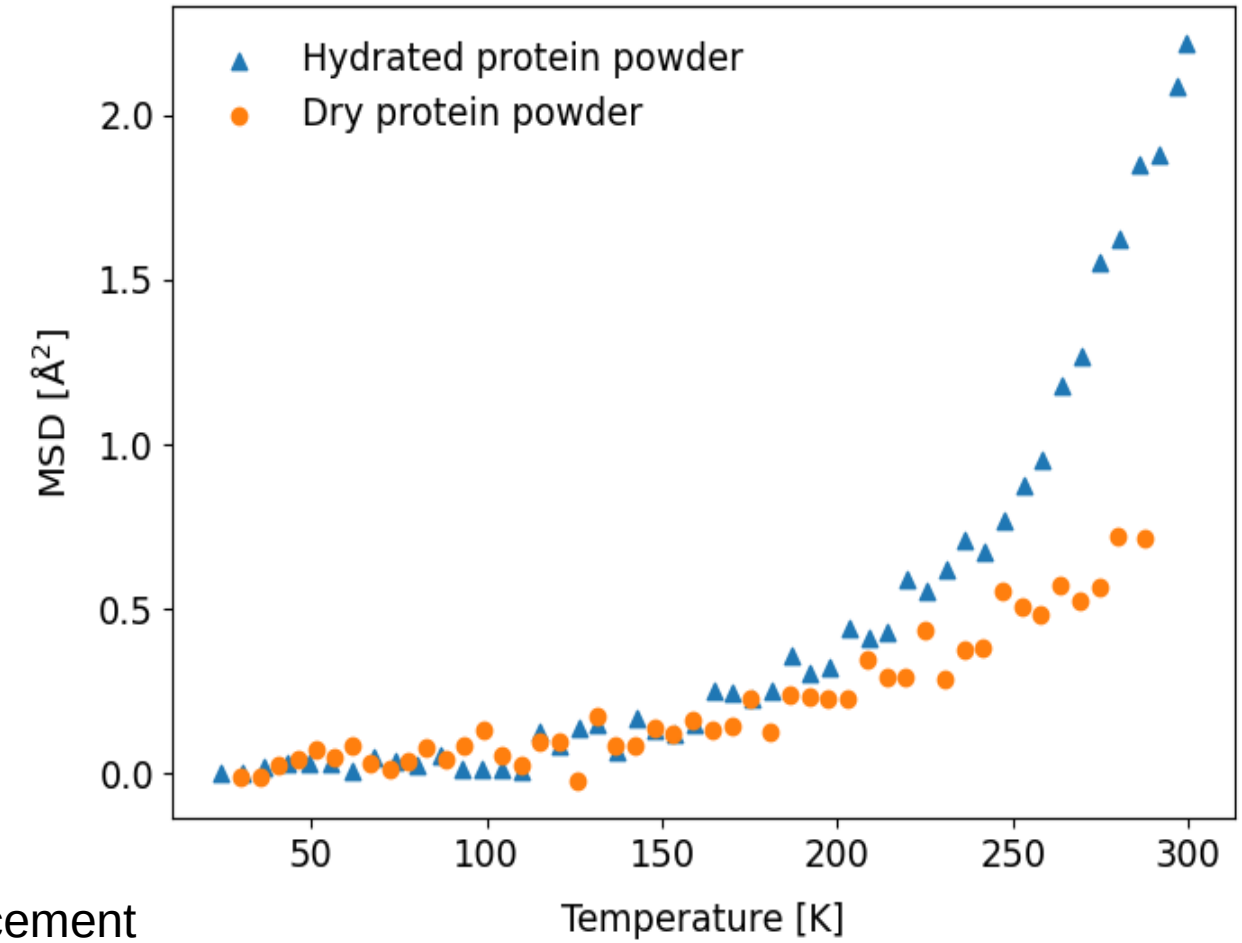
Methods – incoherent neutron scattering



$$\mathrm{EISF(q)} = e^{\frac{1}{3}q^2\langle r^2\rangle_{\Delta E=0}}$$

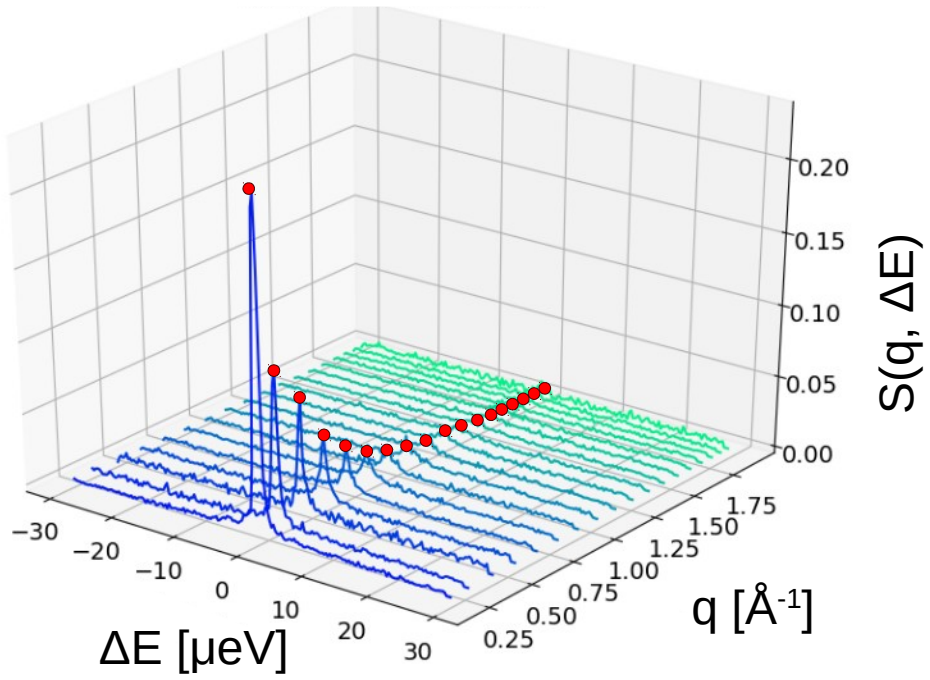
Mean square displacement

Mean square displacement (MSD)

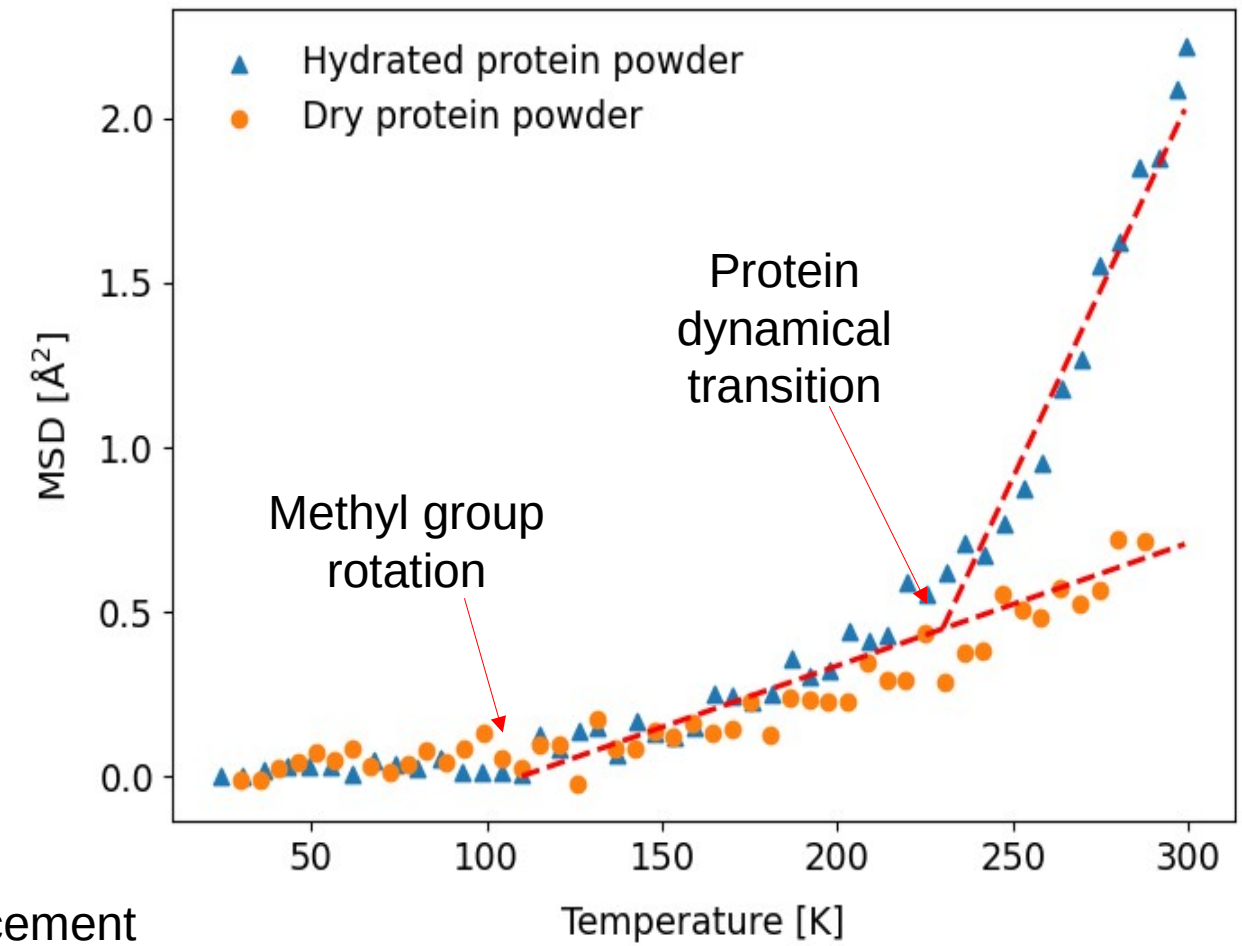


Yi et al., Journal of Physical Chemistry B (2012)
Peters and Kneller. The Journal of Chemical Physics (2013)

Methods – incoherent neutron scattering



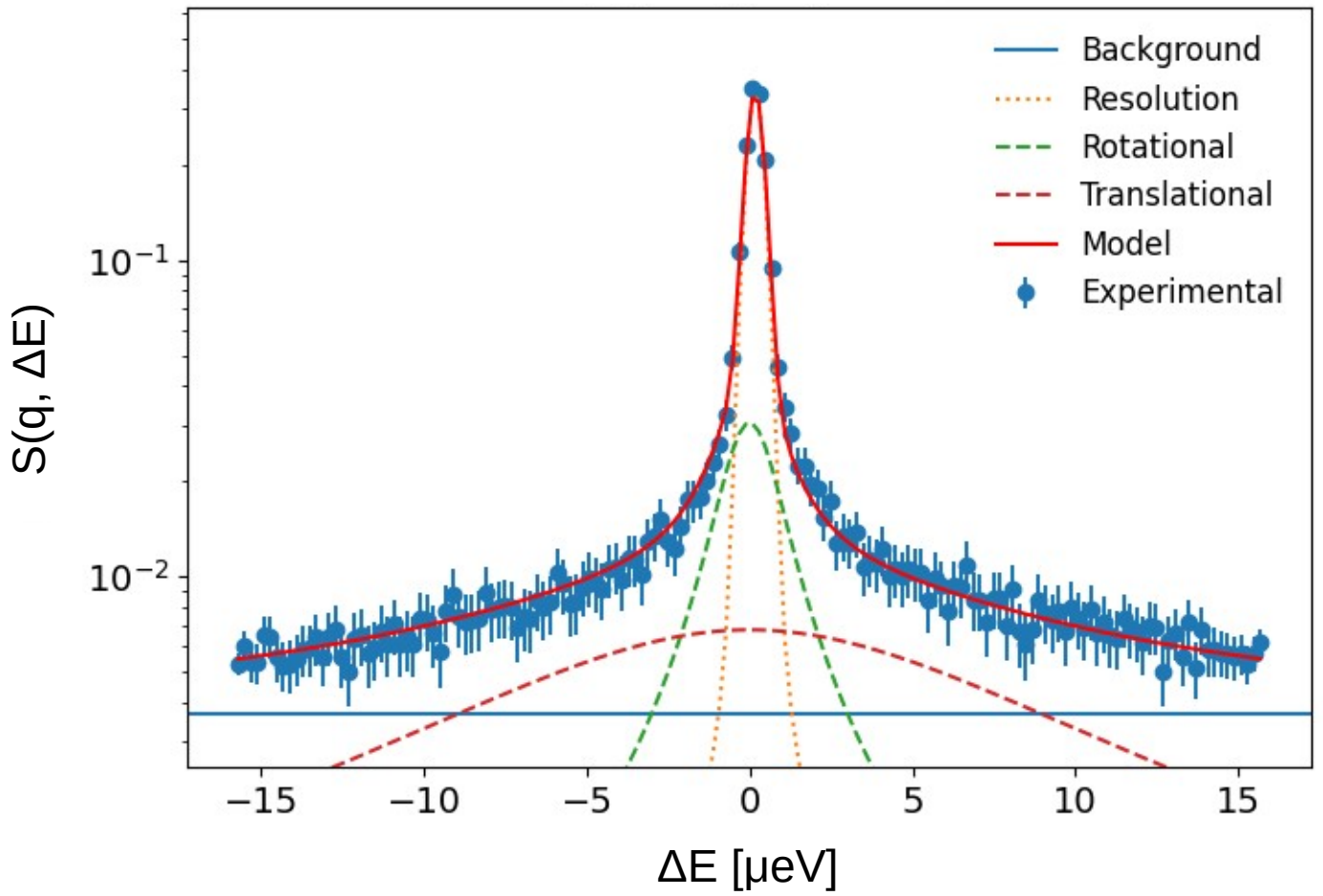
Mean square displacement (MSD)



$$\mathrm{EISF(q)} = \mathrm{e}^{\frac{1}{3}\mathrm{q}^2\langle \mathrm{r}^2\rangle_{\Delta \mathrm{E}=0}}$$

Mean square displacement

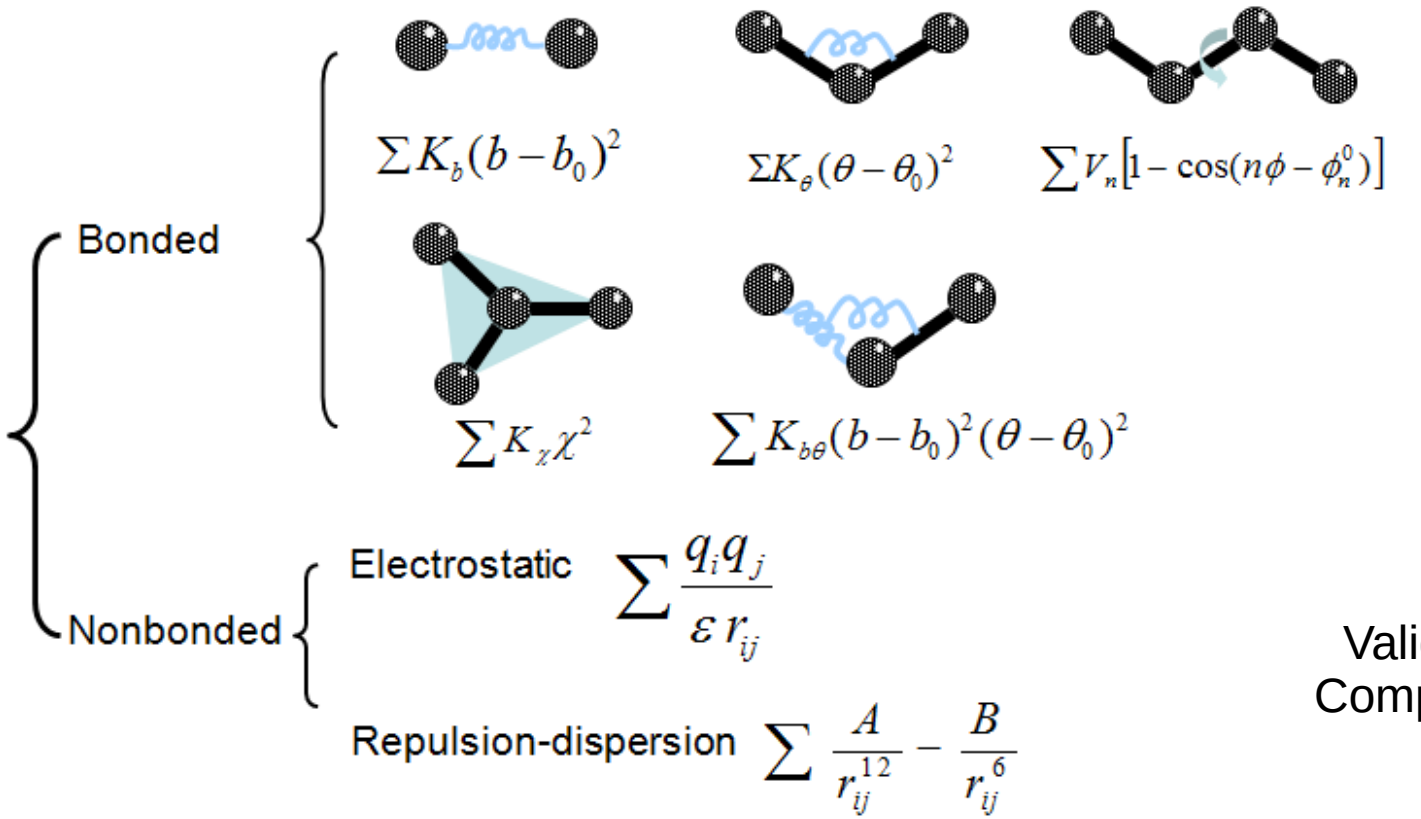
Methods – incoherent neutron scattering



$$S(q,\Delta E) = e^{-q^2 MSD/6} R(q,\Delta E) \otimes [a_0 \delta(\Delta E) + a_t \mathcal{L}_{trans} + a_r \mathcal{L}_{rot}] + bkgd$$

Methods – all-atoms MD simulations

Methods – all-atoms MD simulations



NAMD 2.13

CHARMM36 force field

Validation against experimental data
Computed MSD and scattering spectra

Methods – all-atoms MD simulations

Access to the position of every atom in the system:

- number density $$\rho_i(r) = \left\langle \frac{1}{N} \frac{1}{4\pi r^2 dr} [n_i(r+dr) - n_i(r)] \right\rangle$$

- hydrogen bond (HB) autocorrelation

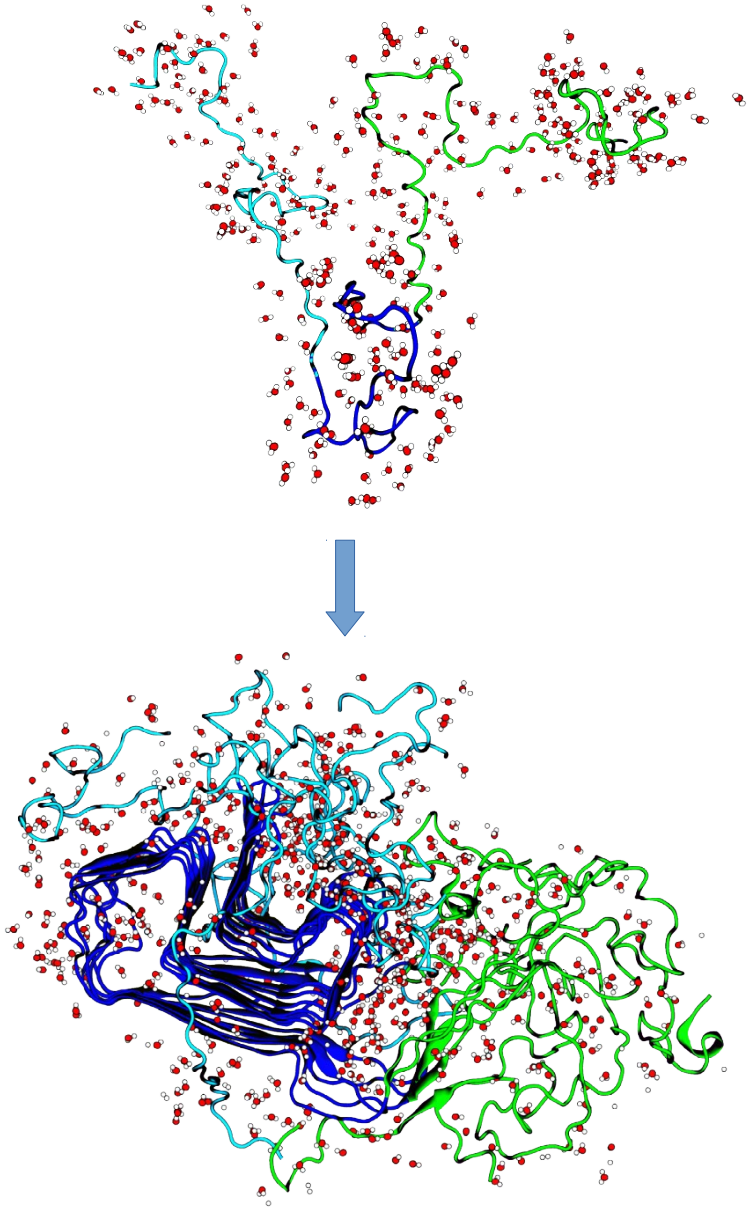
$$C_{ij}(t) = \left\langle \frac{\sum_{i \neq j} h_{ij}(t) h_{ij}(0)}{\sum_{i \neq j} h_{ij}^2(0)} \right\rangle$$

$$h_{ij} = \begin{cases} 1 \text{ if HB } (||X - H|| < 2.5\text{Å and } \widehat{XHX} > 130°) \\ 0 \; otherwise \end{cases}$$

- average distance between protein residues $$\overline{d_{ij}} = \frac{1}{\text{numFrames}} \sum_{t_0}^{\text{numFrames}} \|r_i(t_0) - r_j(t_0)\|$$

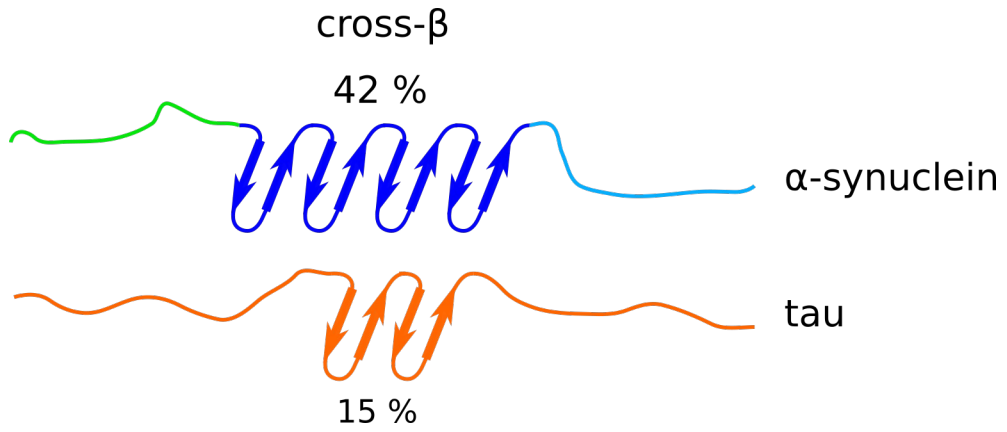
Results

Results - α-synuclein



Hydrated powders of α-synuclein

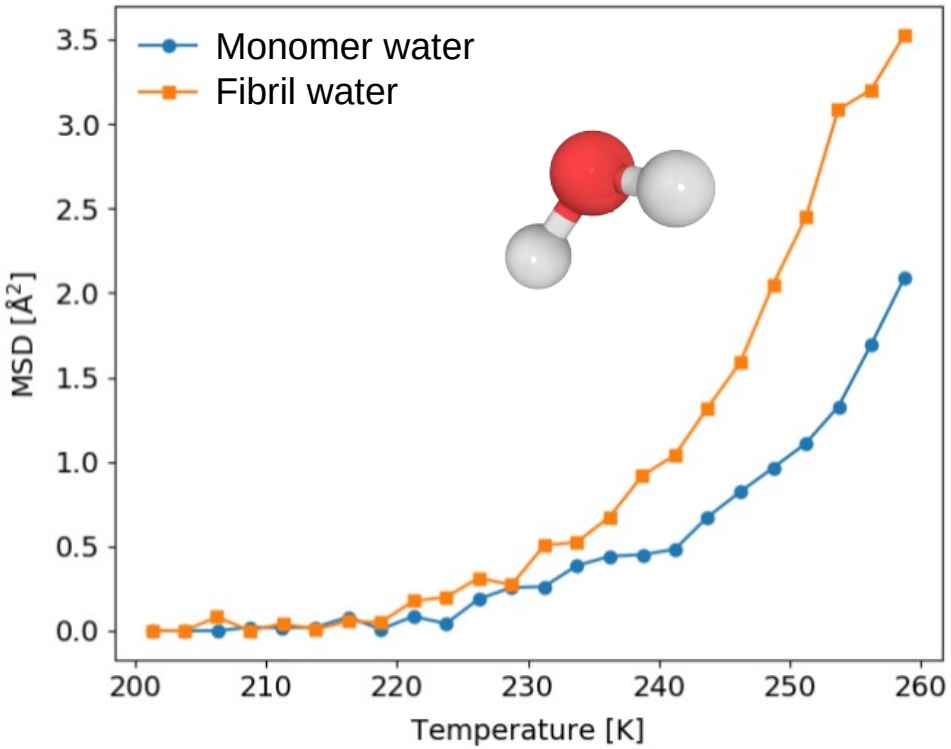
- 3 main regions:
  - N-ter – lipid binding
  - cross-β core – amyloid aggregation
  - C-ter – calcium binding
- Initial structures available for monomers and fibrils
- Higher proportion of cross-β than tau



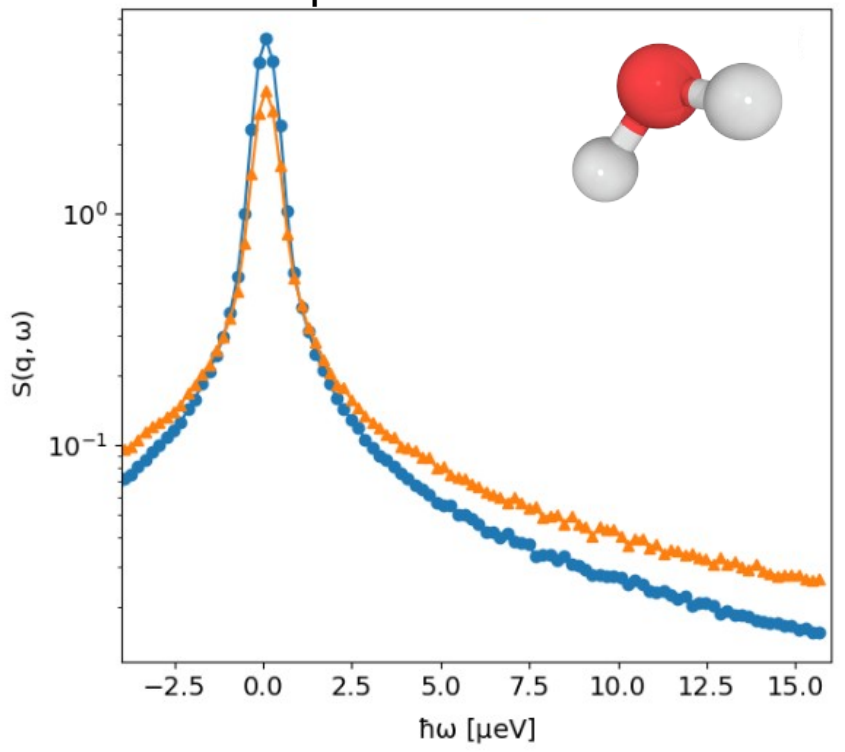
Lautenschläger et al., Nature Communications (2018)

Results - α-synuclein

Mean-square displacement of water



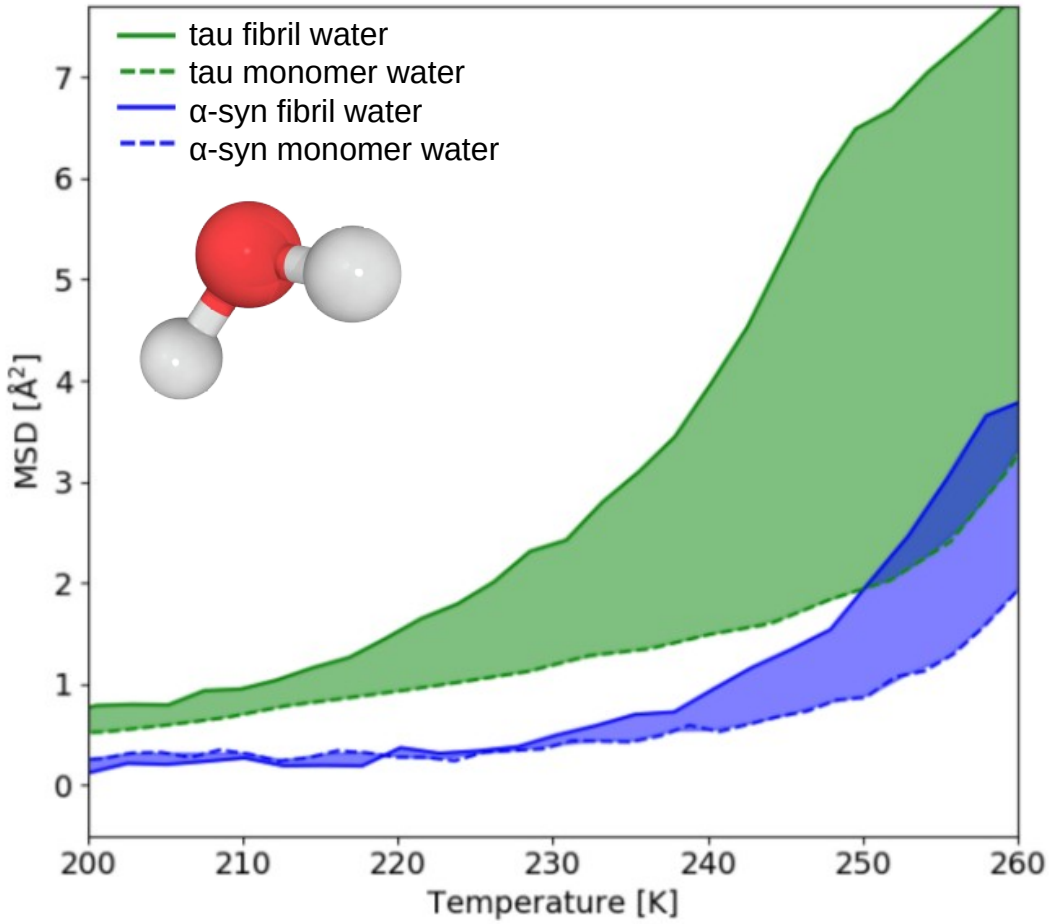
Quasi-elastic neutron scattering spectrum of water



Protein dynamics unaffected

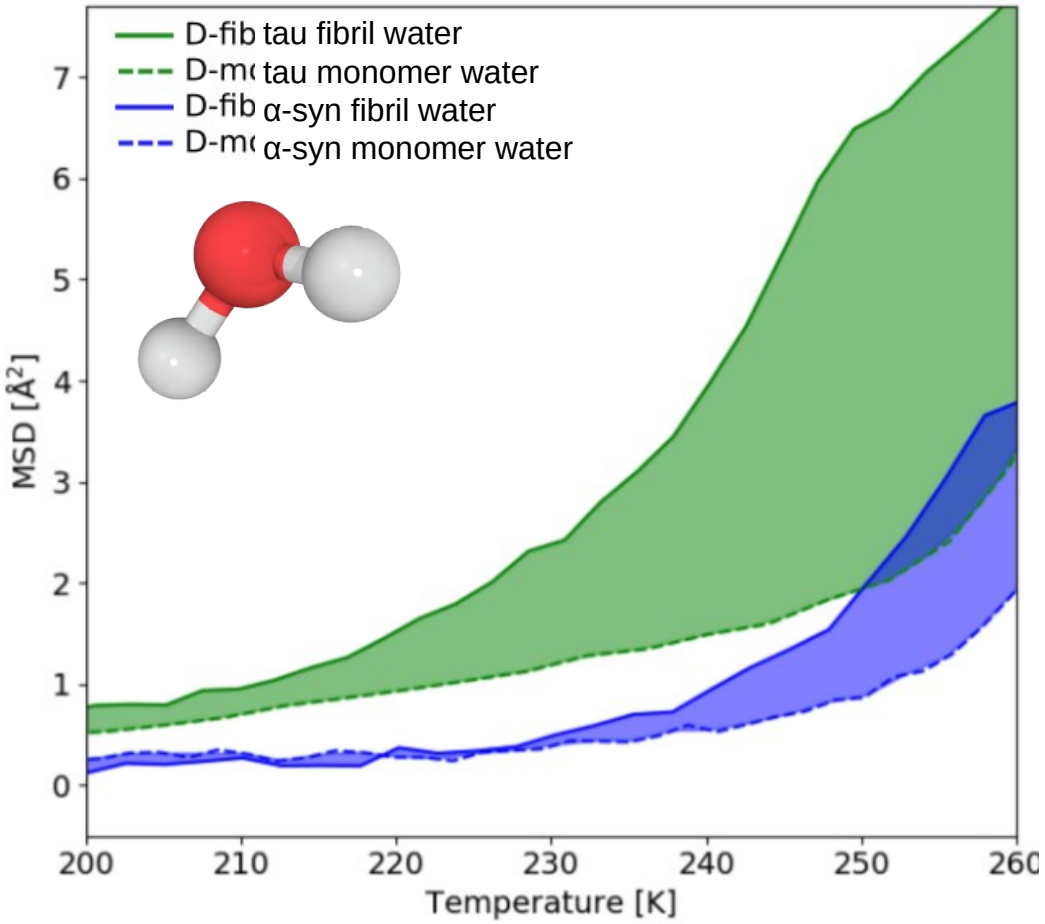
Results - α-synuclein

Mean-square displacement of water for α-syn (blue) and tau (green)

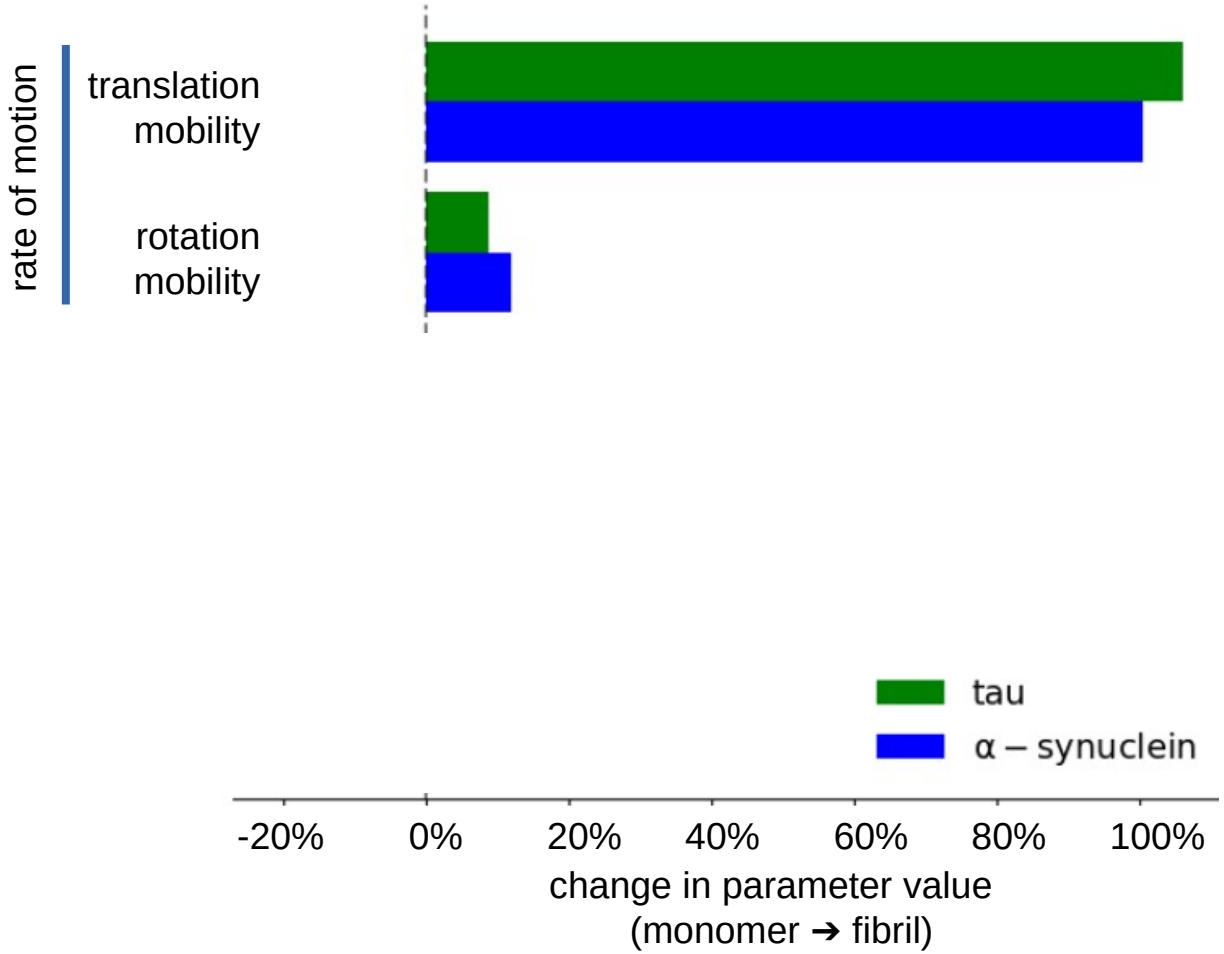


Results - α-synuclein

Mean-square displacement of water for α-syn (blue) and tau (green)

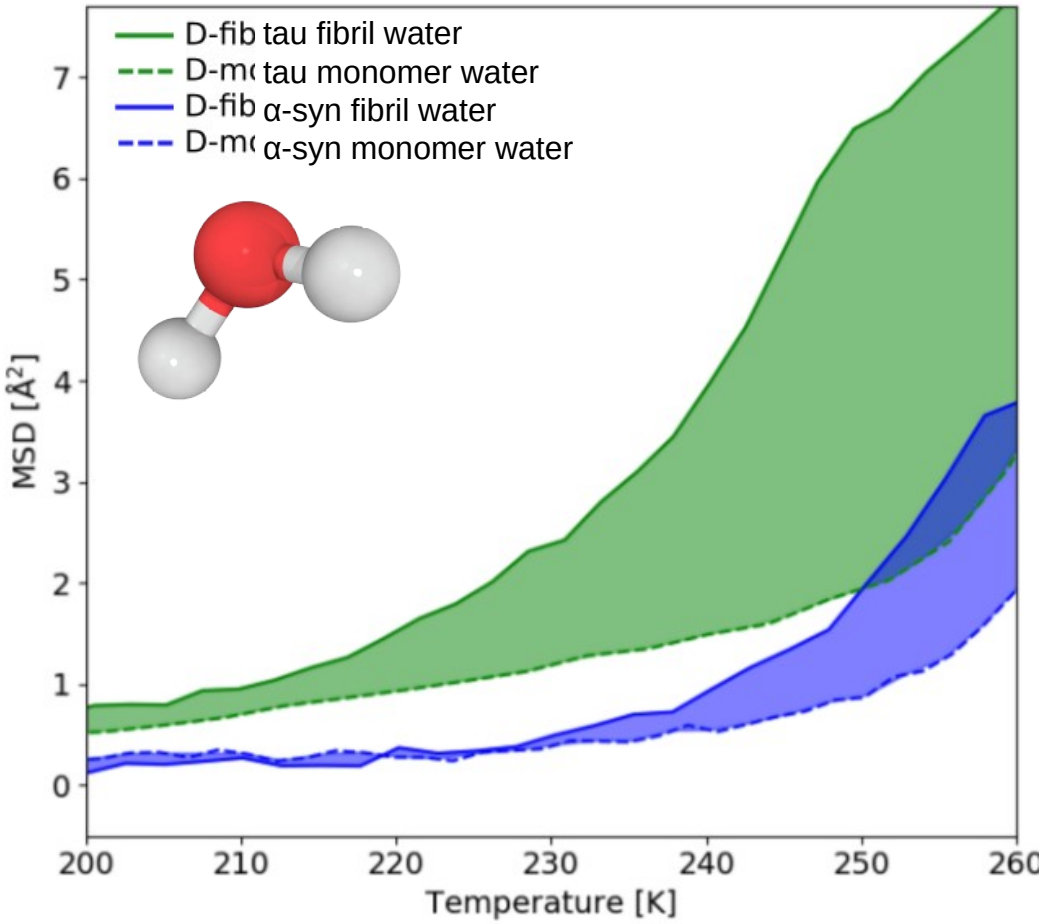


% change in parameter values upon fibril formation

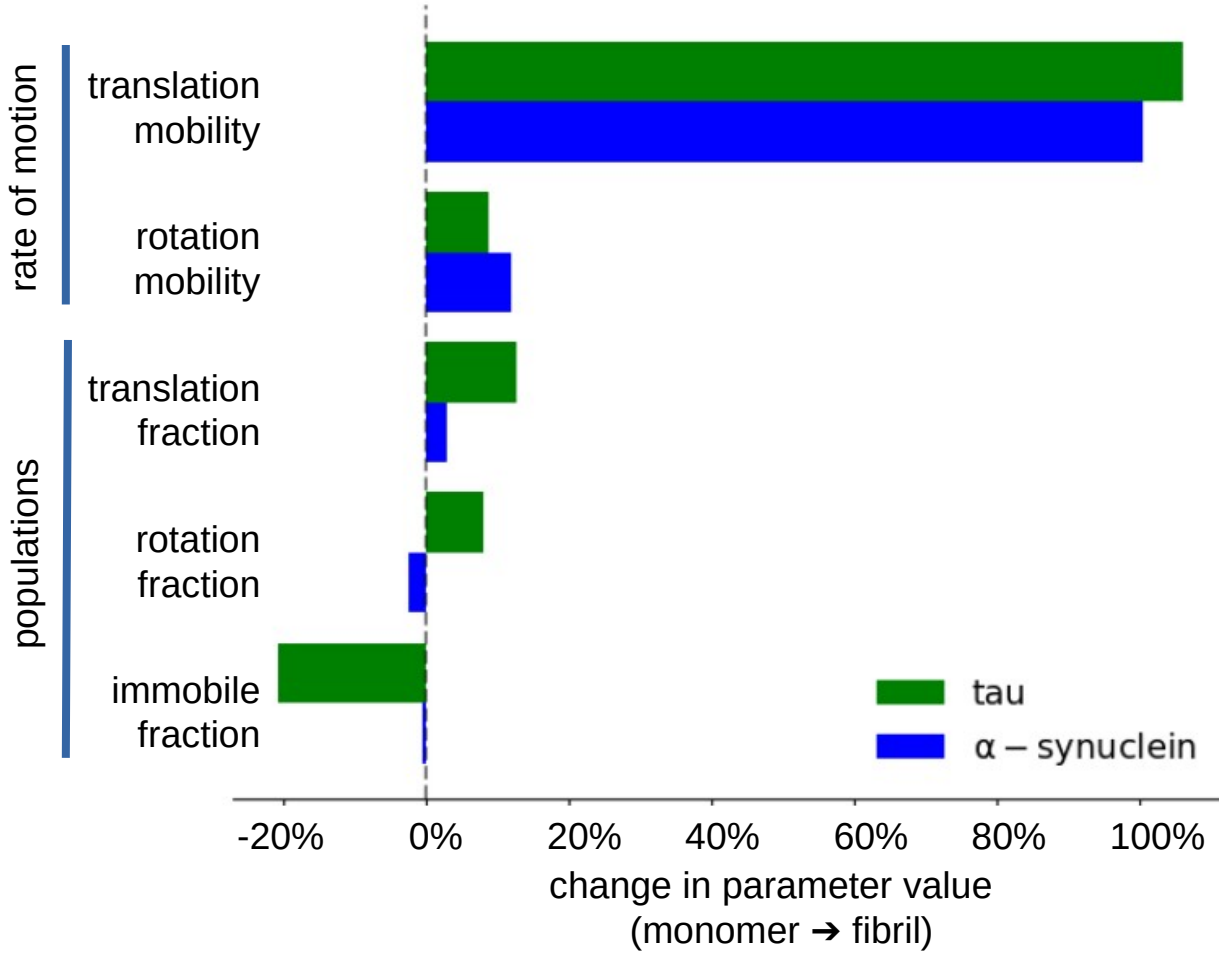


Results - α-synuclein

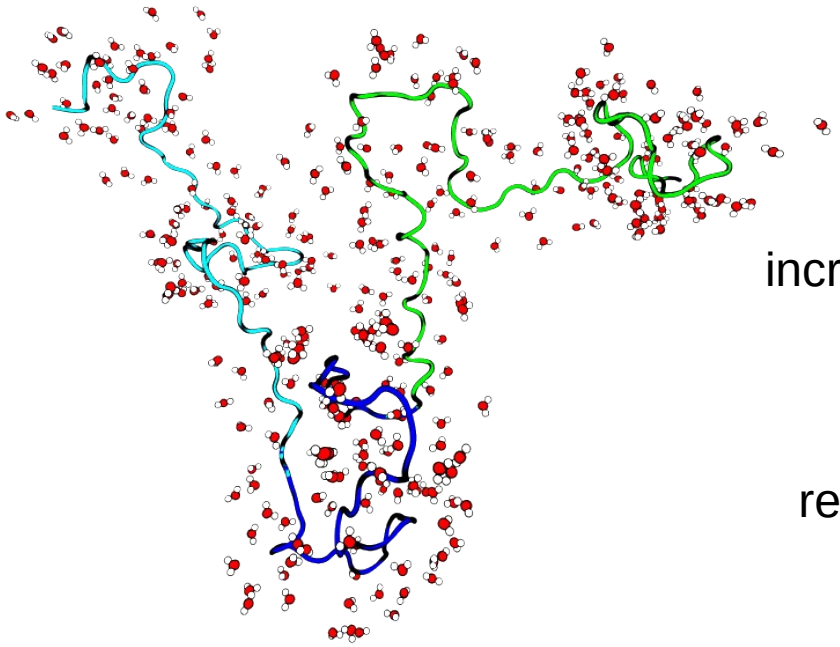
Mean-square displacement of water for α-syn (blue) and tau (green)



% change in parameter values upon fibril formation

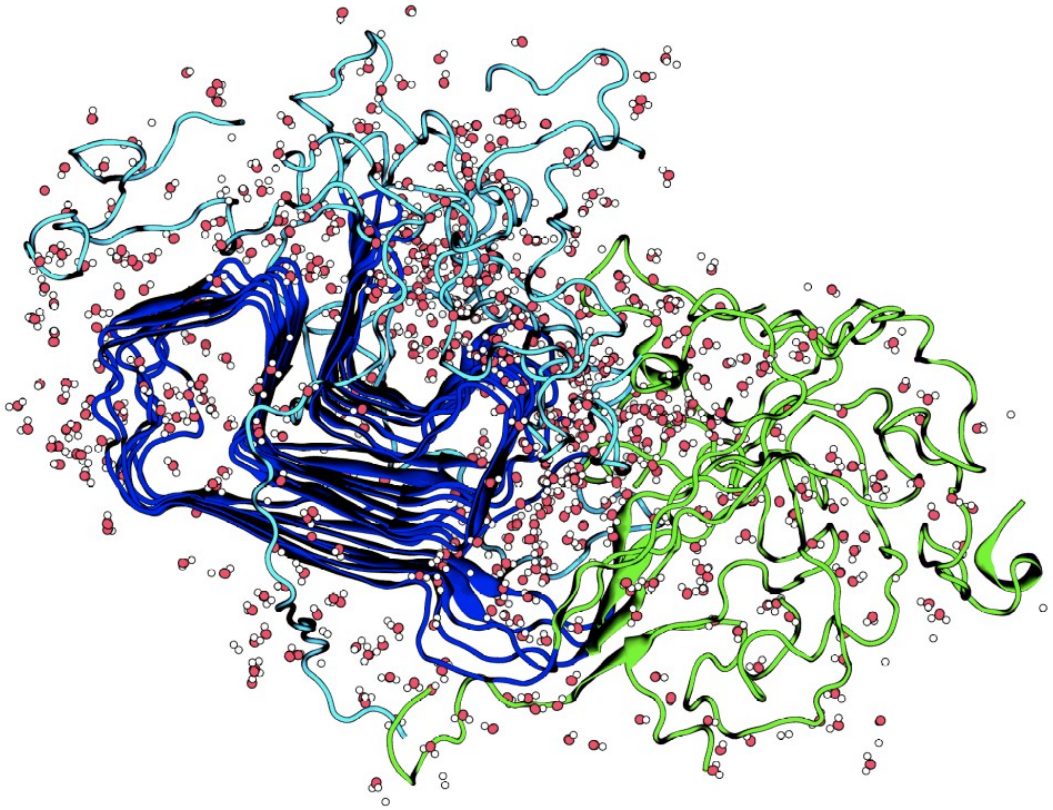


Results - α-synuclein



increased water dynamics
and entropy

reorganization of water
populations

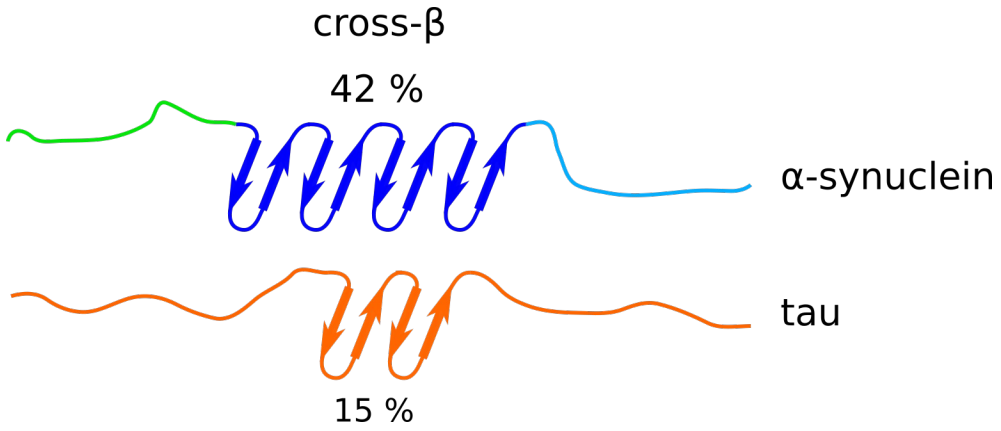


Results - α-synuclein

Neutron scattering ➔ ensemble-averaged dynamics

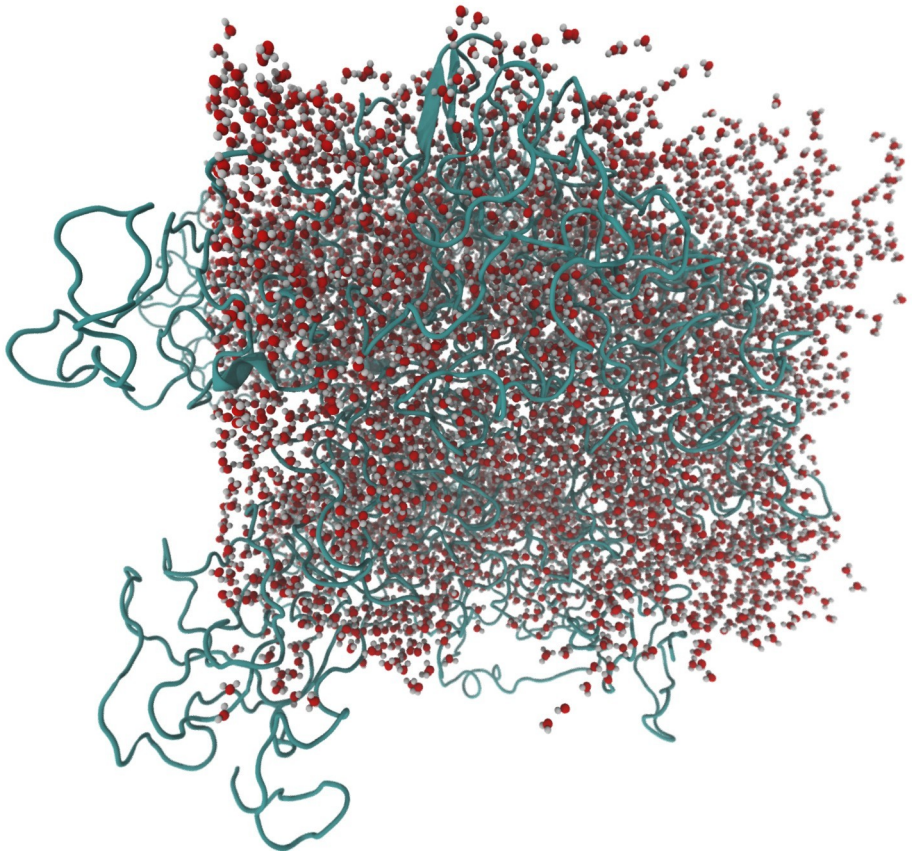
local dynamics ➔ simulations

Supervised by **Prof. Douglas Tobias** during 3 months stay at University of California, Irvine



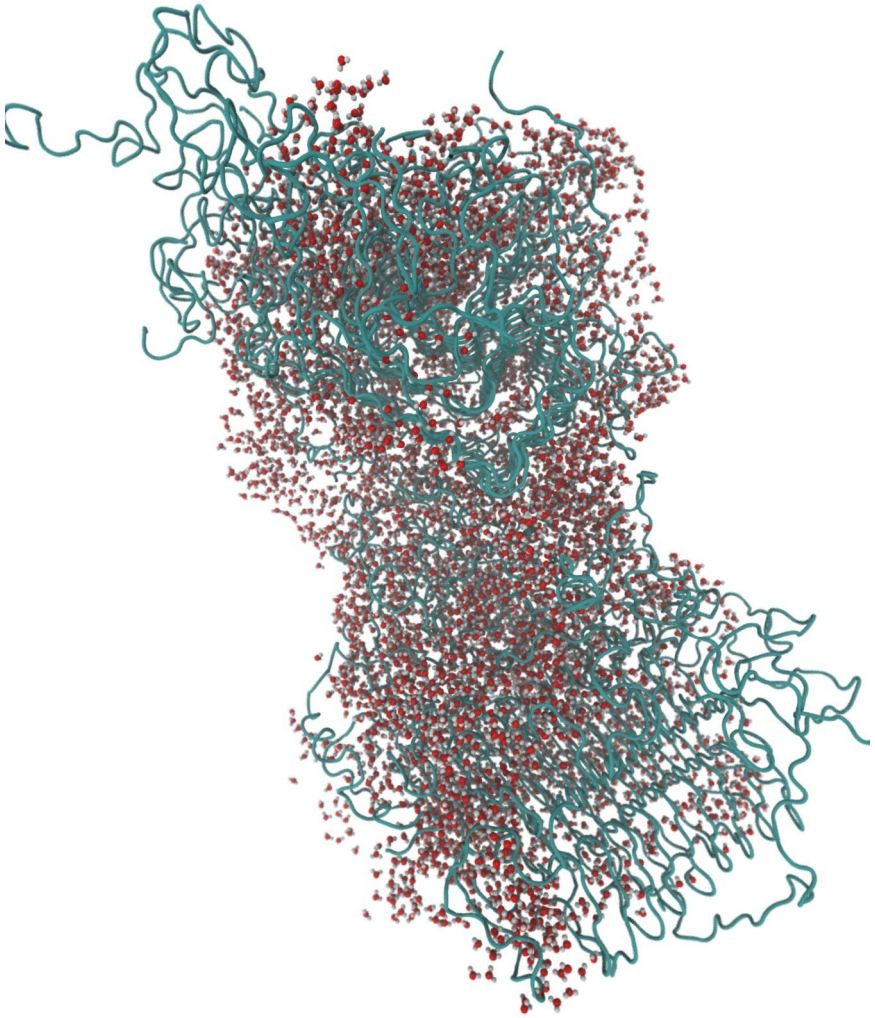
Results - α-synuclein

Hydrated powder of α-synuclein monomers



Min-Kyu et al., Protein Science : A Publication of the Protein Society (2009)

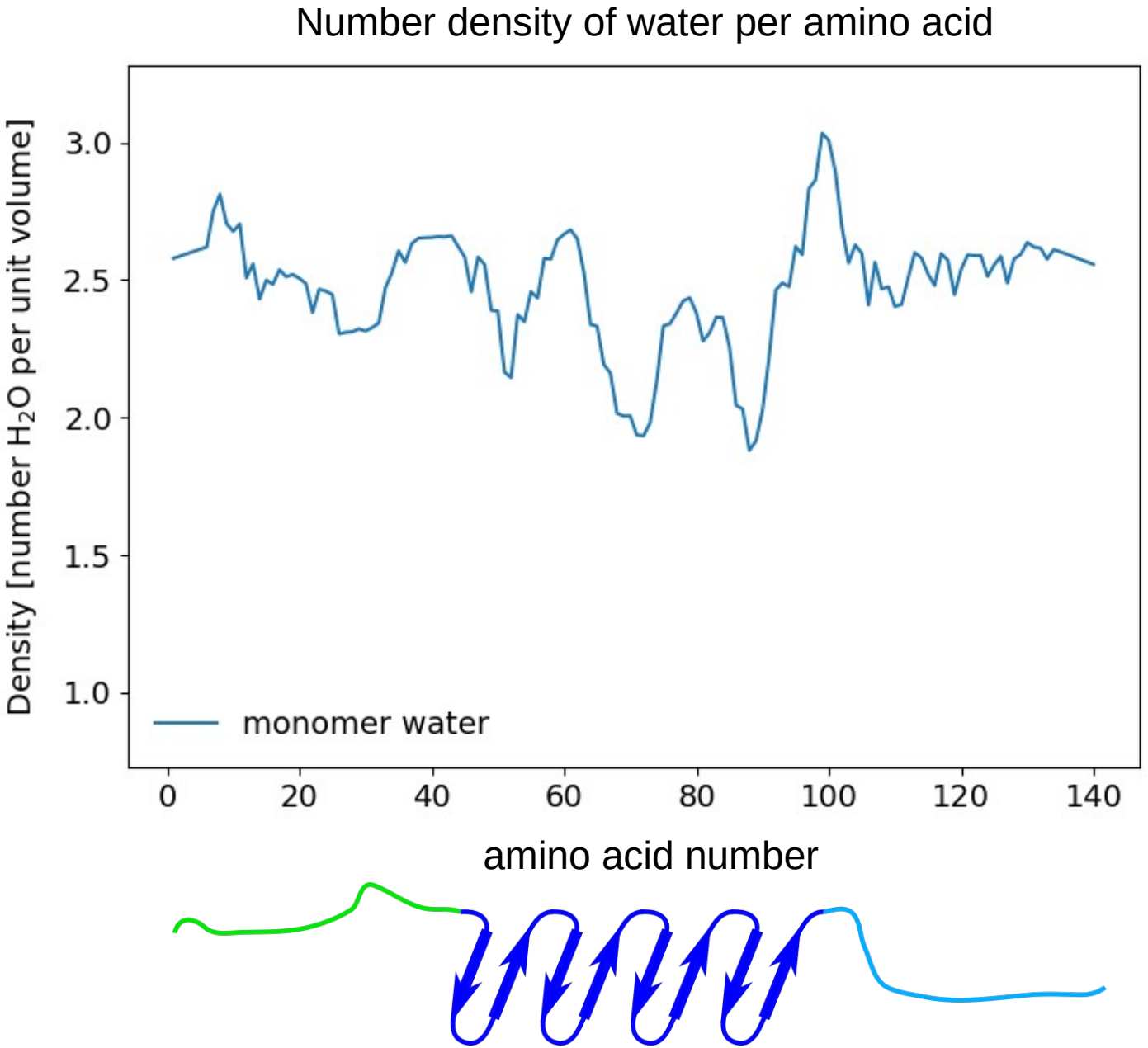
Hydrated powder of α-synuclein fibrils



Tuttle et al., Nature Structural & Molecular Biology (2016)

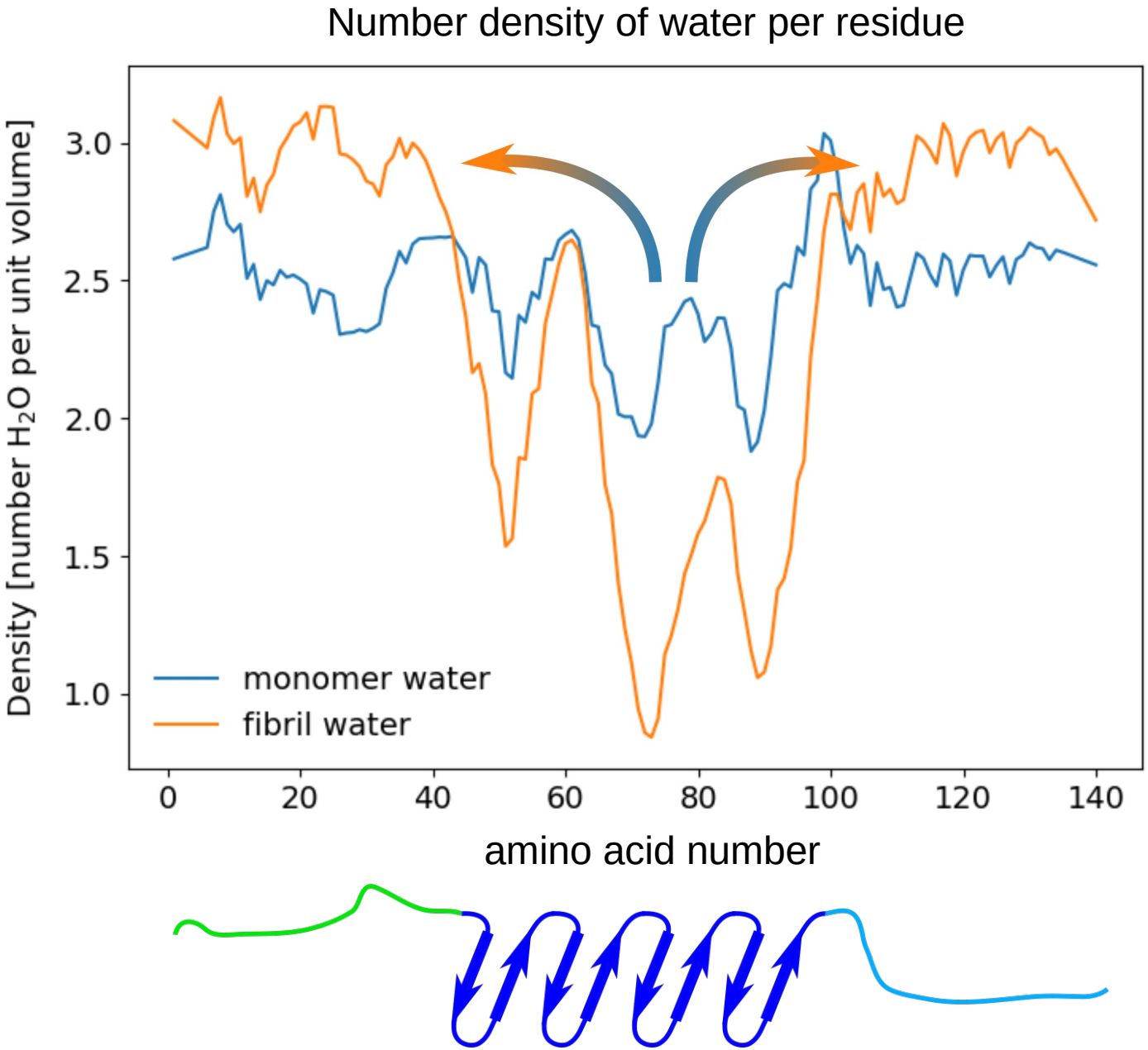
100 ns equilibration, 20 ns run
Simulations validated against experimental data

Results - α-synuclein



Water around monomer
➔ 2.5 water molecules per amino acid

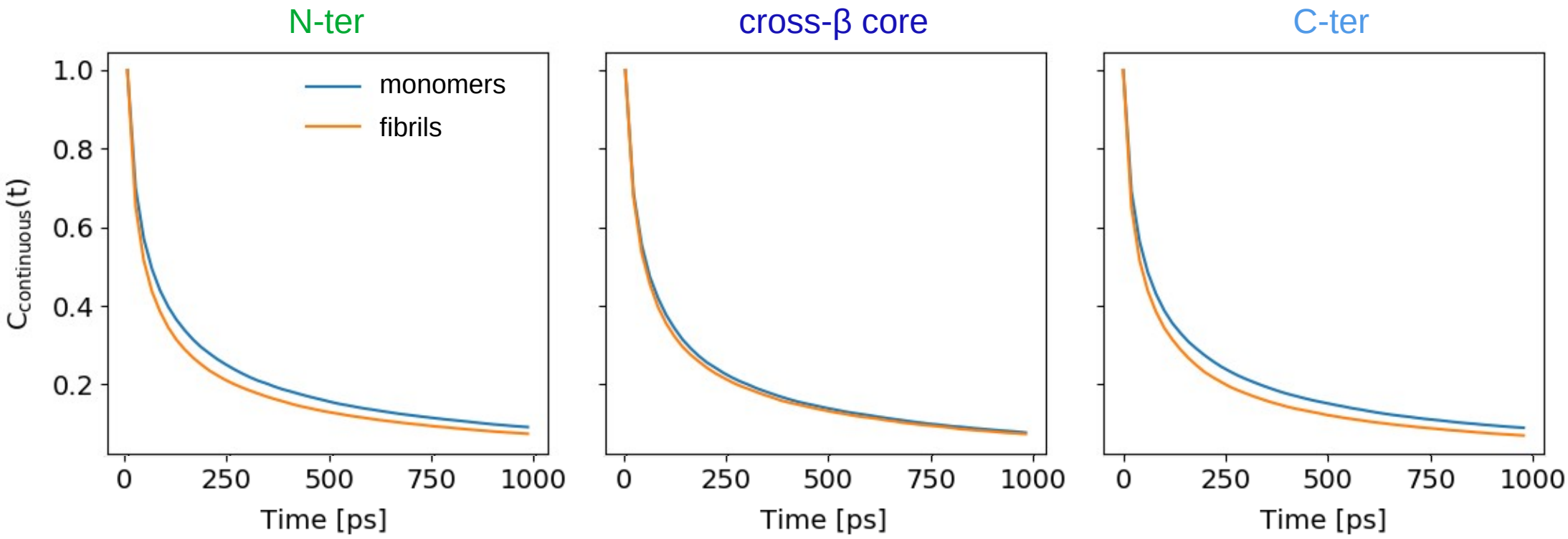
Results - α-synuclein



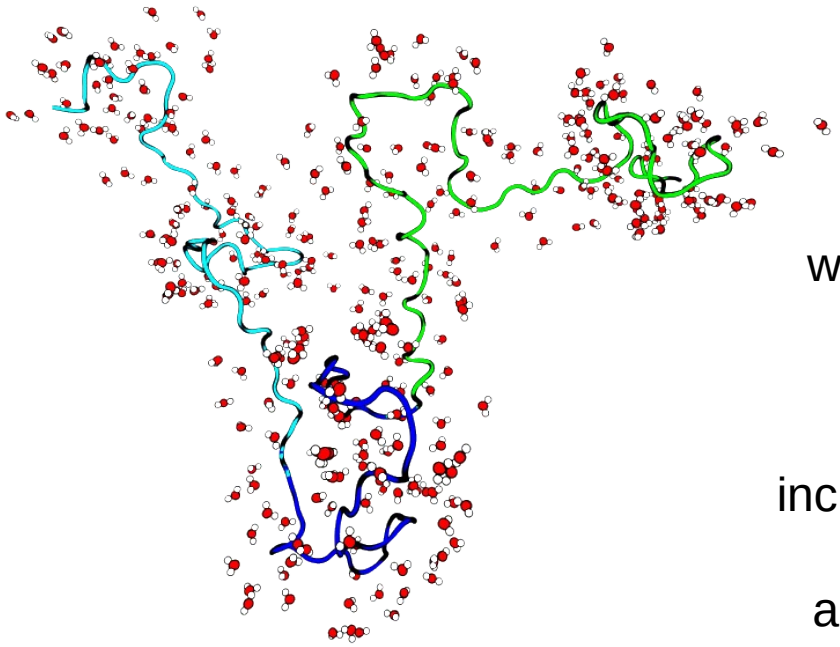
Water around monomer
➔ 2.5 water molecules per amino acid

Results - α-synuclein

Water – protein hydrogen bonds autocorrelation

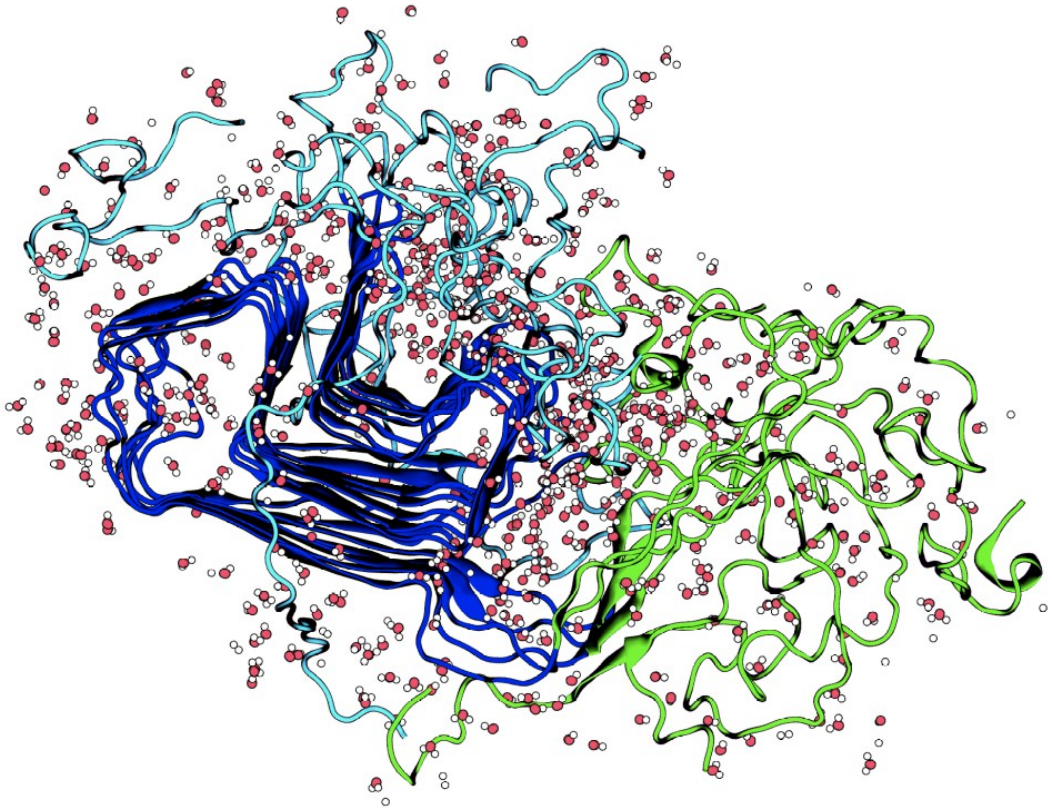


Results - α-synuclein



water pulled away from
cross-β region

increased water dynamics
and entropy
around N-ter and C-ter

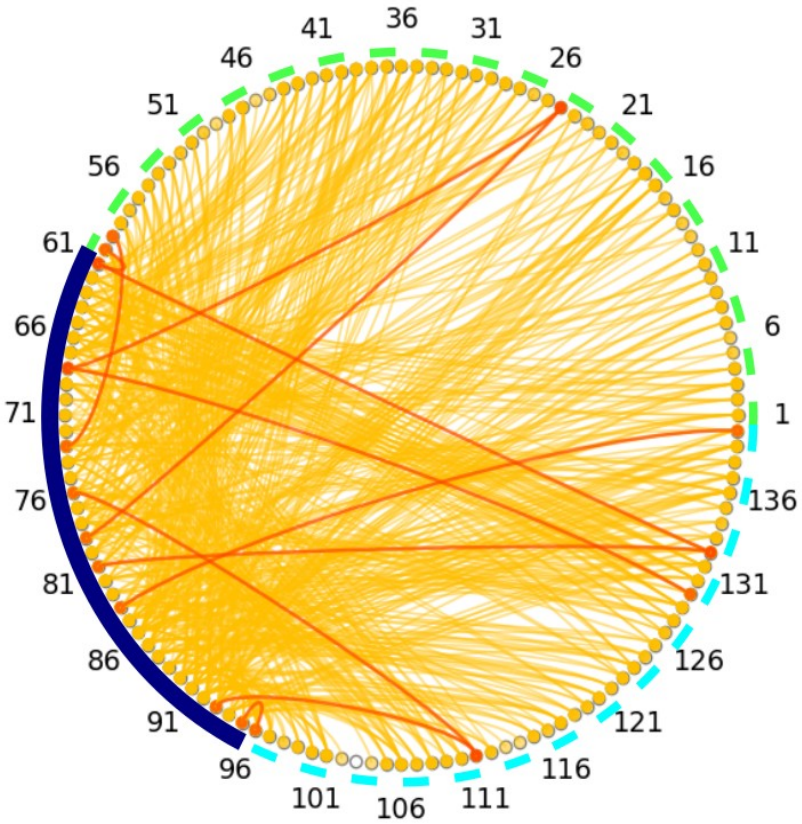


Results - α-synuclein

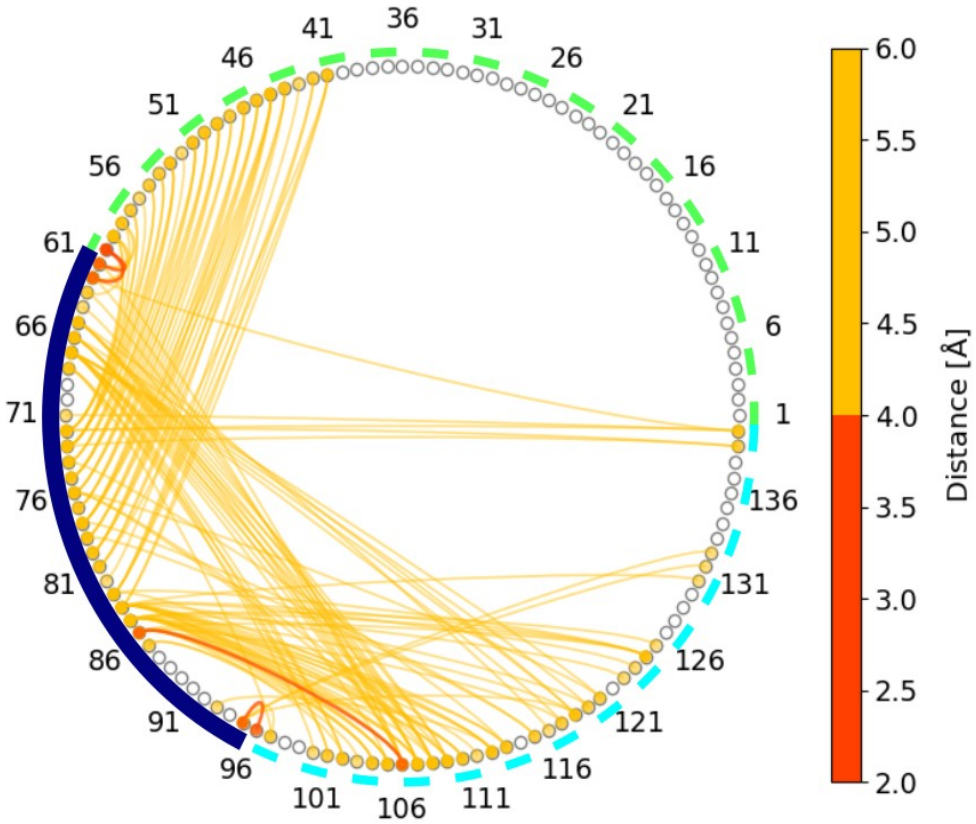
Amino acids distance map in α-synuclein monomers and fibrils

cross-β ➔ N-ter - C-ter

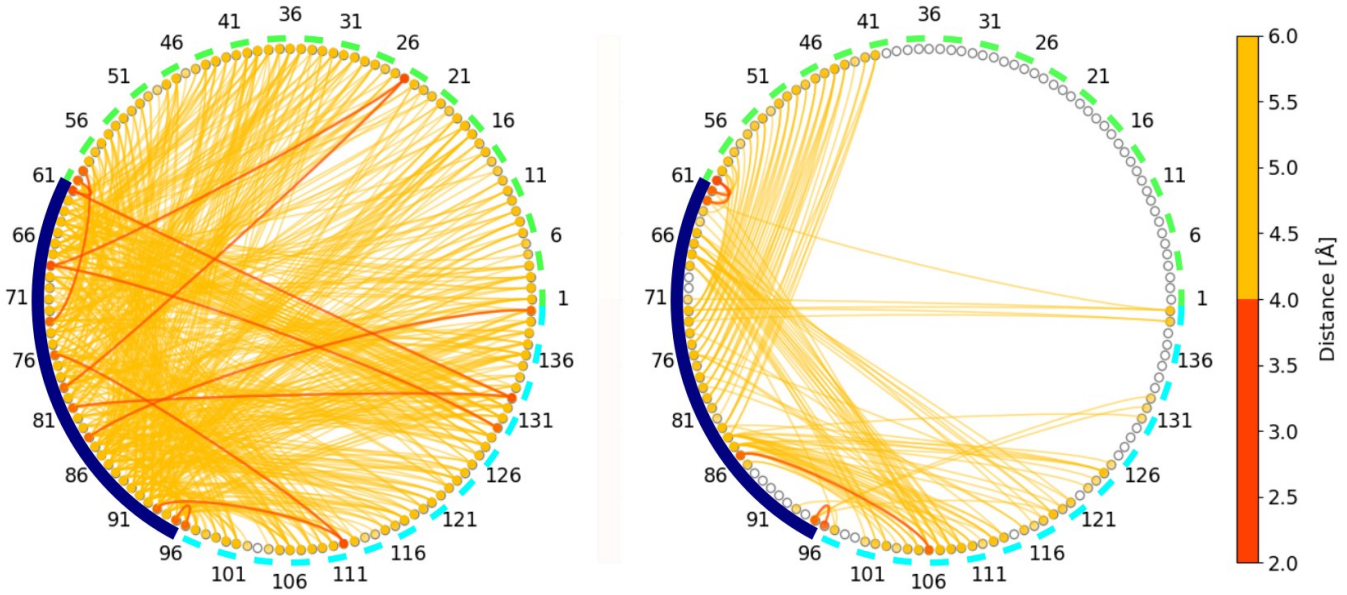
monomers



fibrils



Results - α-synuclein



ARTICLE

doi:10.1038/nature16531

Structural disorder of monomeric α–synuclein persists in mammalian cells

Francois-Xavier Theillet[1]†*, Andres Binolfi[1]†*, Beata Bekei[1]*, Andrea Martorana[2], Honor May Rose[1], Marchel Stuiver[1], Silvia Verzini[1], Dorothea Lorenz[3], Marleen van Rossum[1], Daniella Goldfarb[2] & Philipp Selenko[1]

PLOS COMPUTATIONAL BIOLOGY

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RESEARCH ARTICLE

Conformational ensemble of native α-synuclein in solution as determined by short-distance crosslinking constraint-guided discrete molecular dynamics simulations

Nicholas I. Brodie, Konstantin I. Popov, Evgeniy V. Petrotchenko, Nikolay V. Dokholyan, Christoph H. Borchers

Structure

Volume 27, Issue 11, 5 November 2019, Pages 1710-1715.e4

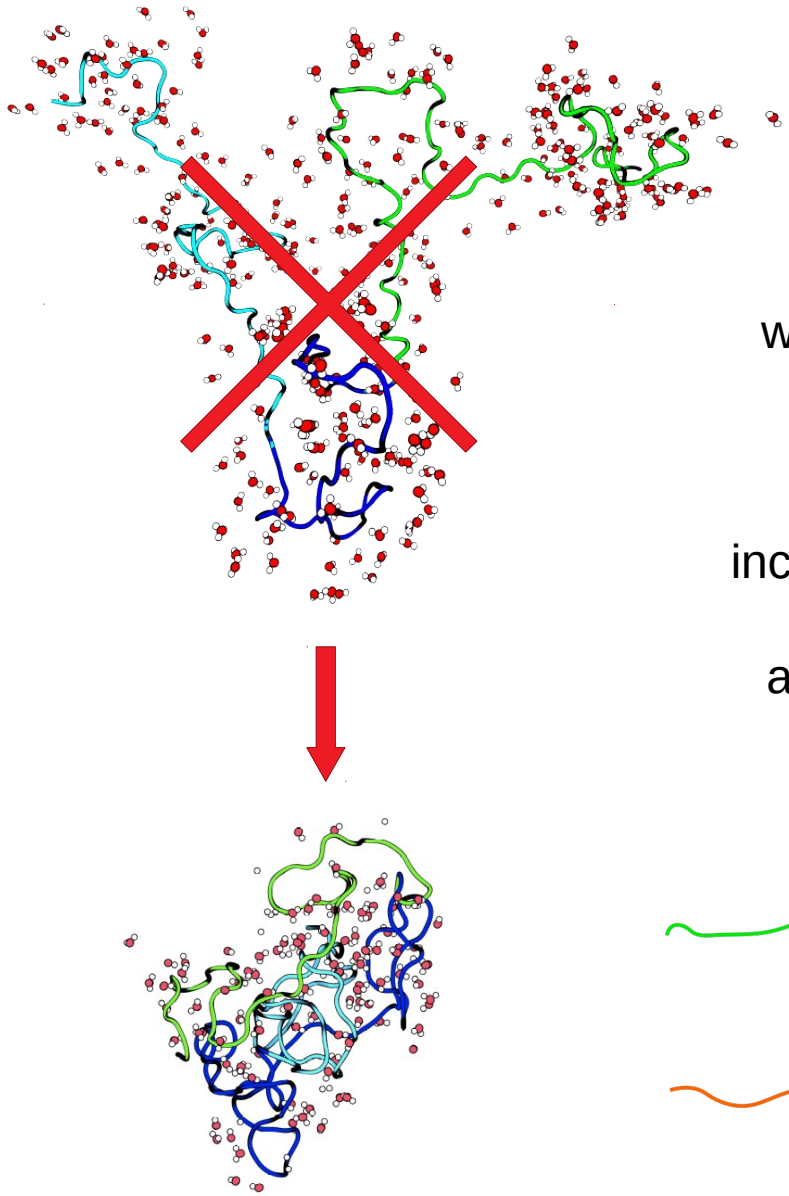
CellPress

Article

Insight into the Structure of the "Unstructured" Tau Protein

Konstantin I. Popov [1, 7], Karl A.T. Makepeace [2, 7], Evgeniy V. Petrotchenko [3], Nikolay V. Dokholyan [4], Christoph H. Borchers [2, 3, 5, 6, 8]

Results - α-synuclein



water pulled away from
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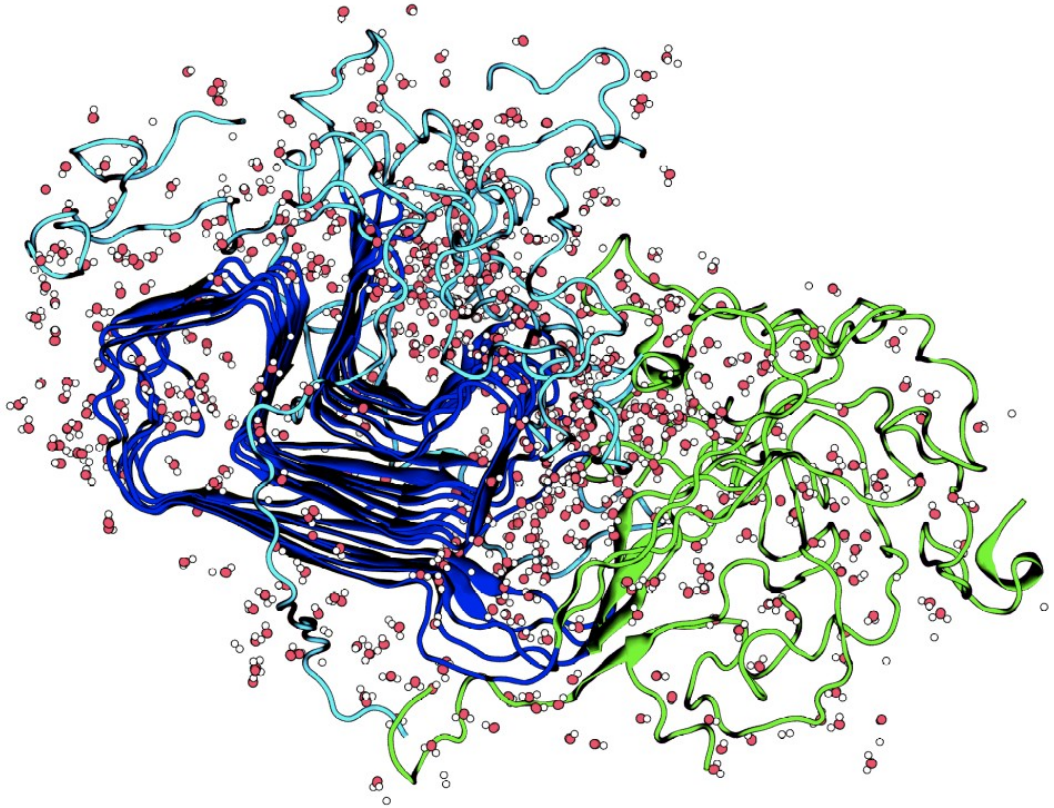
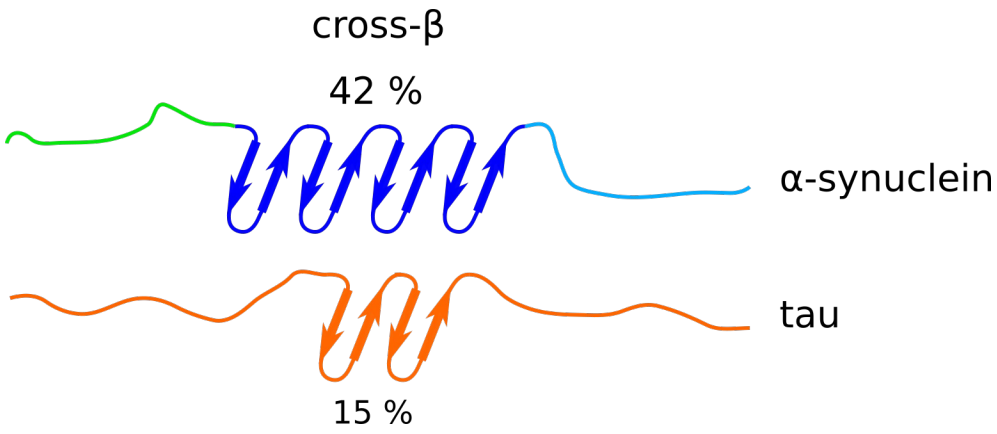
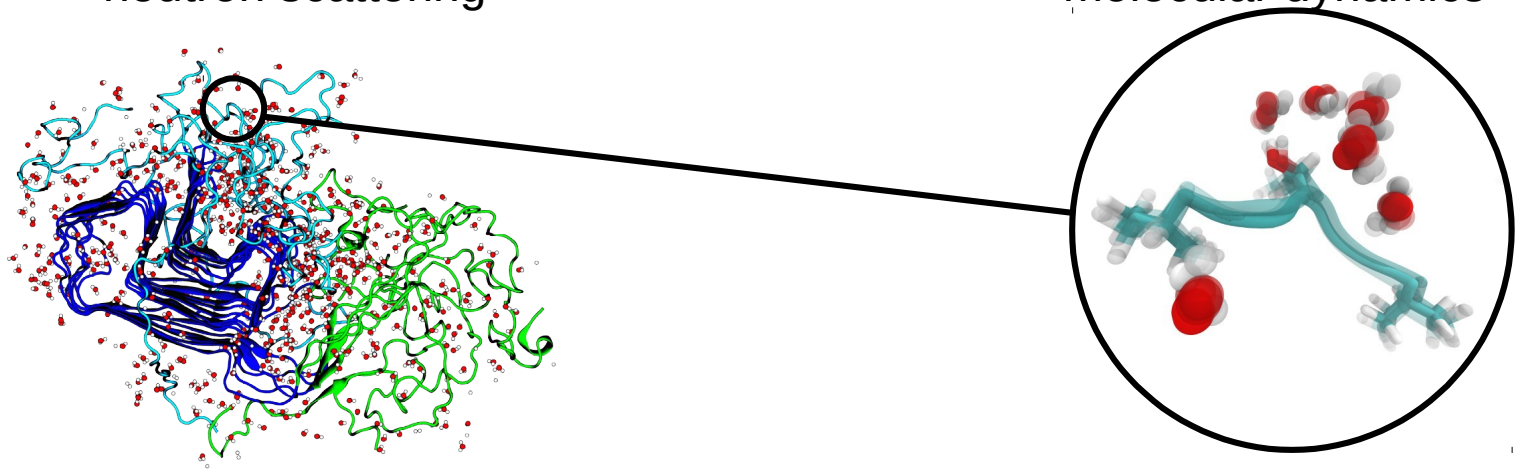




Conclusion and outlook

neutron scattering + molecular dynamics



ensemble-averaged atomistic detail

hydration water
metal ions
time-resolved studies

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