Amyloid fibrils in disease and structure-based discovery of small molecules that disaggregate fibrils

David Eisenberg Lab 5/20/22, GoldLab Symposium

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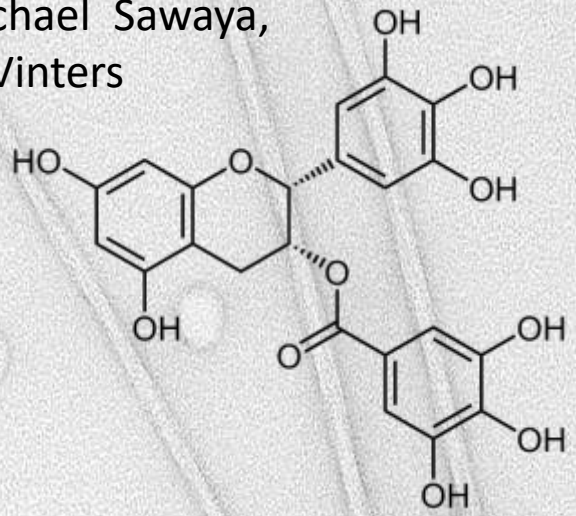

Stability and common features of pathogenic amyloid fibrils

How do small molecules (e.g. EGCG) disassemble ultra-stable tau amyloid fibrils of Alzheimer's disease?

How can cryo-EM structures of brain-extracted tau fibrils complexed with EGCG identify small molecules that disaggregate tau fibrils

Disclosure: DE is SAB chair and equity holder of ADRx

Amyloid Related Conditions (>50)

Disease	Protein fibrils	Disease	Protein fibrils	Prion (infectious) Disease	Protein
Alzheimer's	Amyloid β Tau	ALS (Lou Gehrig's]	SOD1, TDP-43…	CJD, GSS, Kuru, FFI	PrP
Parkinson's	α-synuclein	CTE	Tau	BSE, vCJD (mad cow)	PrP
Diabetes type 2	Amylin aka IAPP	Pick's	Tau	CWD (Elk)	PrP
Light chain amyloidosis	IgG light chains	Huntington's	Huntingtin	[Psi+]	Sup35
Senile amyloidosis	Trans-thyretin	Some cancers	p53	[Ure2]	Ure3
Insulin amyloidosis	Insulin	Kidney dialysis amyloidosis	β2-microglobulin		

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Parkinson's	α-synuclein	CTE	Tau	BSE, vCJD (mad cow)	PrP
Diabetes type 2					
Light chain amyloidosis					35
Senile amyloidosis					3
Insulin amyloidosis					

Association or Causation?

Mutations in disease-associated proteins often cause early disease onset or greater severity

Doubling of chromosome 17 which encodes α-synuclein causes early onset Parkinson's

Transfection of mutant genes into experimental animals mimics aspects of human diseases

0 nm

Amyloid Related Conditions (>50)

Disease	Protein	Disease	Protein	Prion (infectious)
Alzh				
Par				
Dial type				
Ligh amy				
Sen amy				
Insu amy				

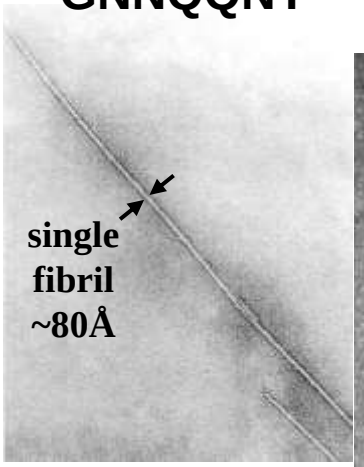
Hypotheses

- Fibrils, or smaller aggregates, are agents of disease
- If we can inhibit protein aggregation, we can halt disease progression
- A sound route to effective inhibitors is structure-based design

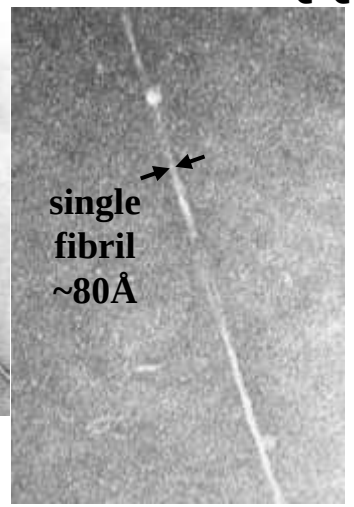
Short segments of fiber-forming proteins are the adhesive units and form both amyloid fibers and microcrystals that contain fibrils

Balbirnie et al. PNAS 2001

GNNQQNY



NNQQ

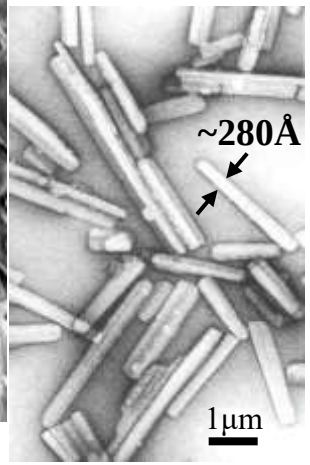


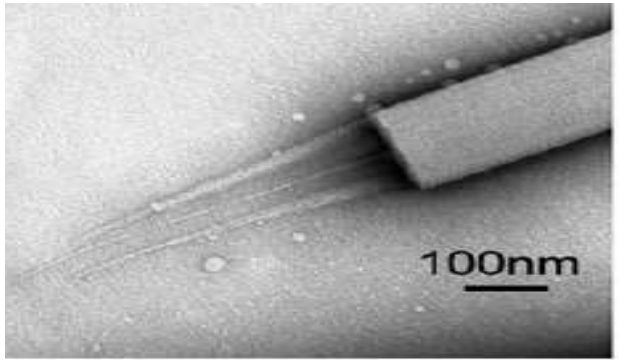
NNQQ

In both micro-crystals and fibrils, β-strands are normal to the long axis.

NNQQNY

GNNQQNY

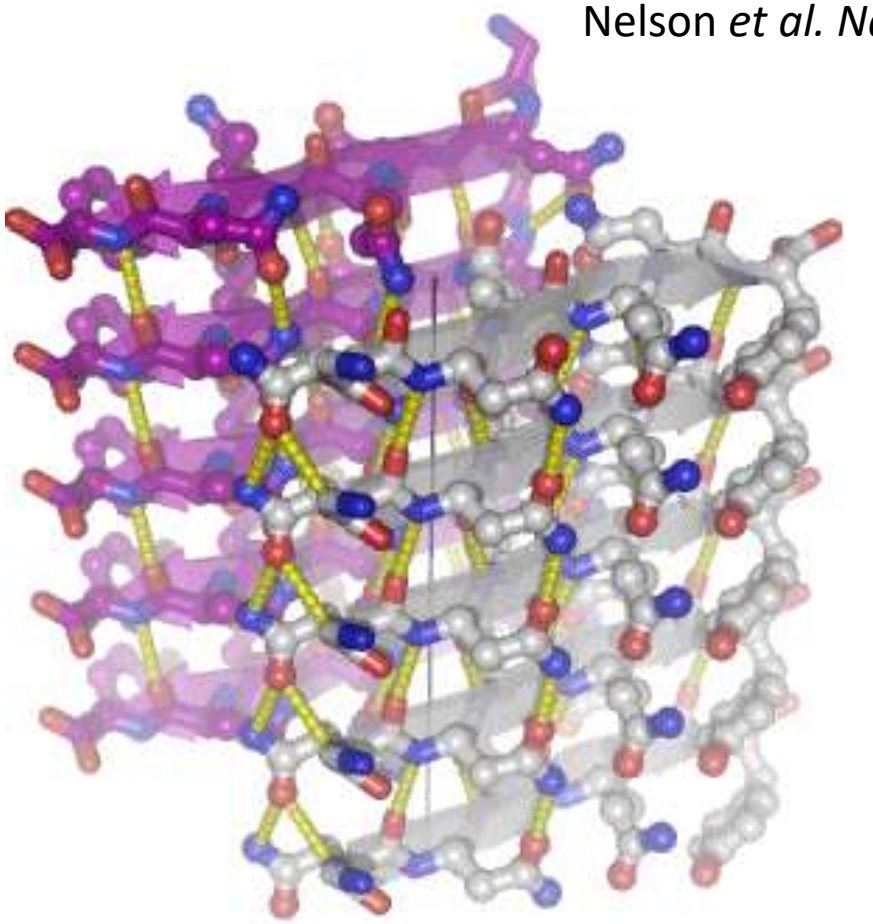




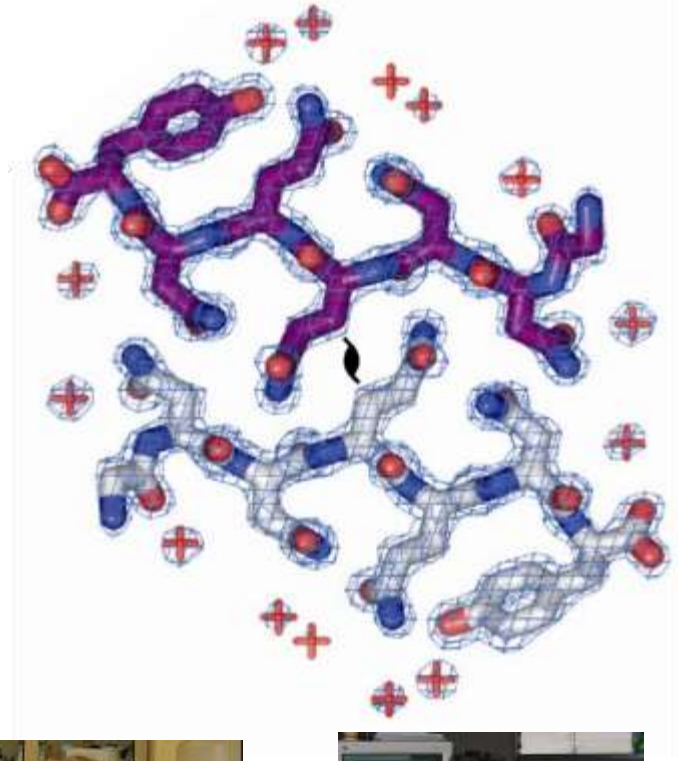
Fibers seem to grow from tips of crystals

The adhesive segments of amyloid forming proteins are extended chains of amino acids. The chains stack into sheets. Two sheets form a stable "steric zipper".

Nelson *et al. Nature,* 2005



View down the fibril axis of ~ 100,000 layers



Stabilizing features:

- Polarized H-bonds
- Tightly mating sheets
- Dry interface
- Residue ladders on surface

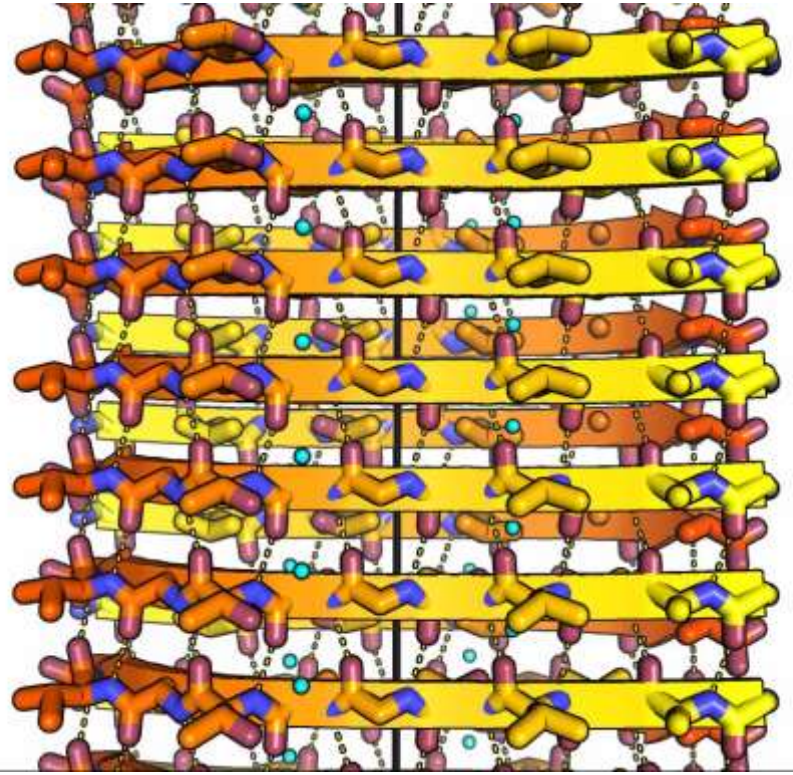
Rebecca Nelson

Mike Sawaya

Pathogenic amyloid fibrils are stabilized by adhesive segments that form mated β-sheets
Example of NACore of α-synuclein GAVVTGVTAVA forms a steric zipper

NACore is necessary for fibril formation and toxicity of alpha-synuclein

Crystals are 10,000,000,000 times smaller than the hemoglobin crystals of Perutz



First electron diffraction structure of a previously unknown protein

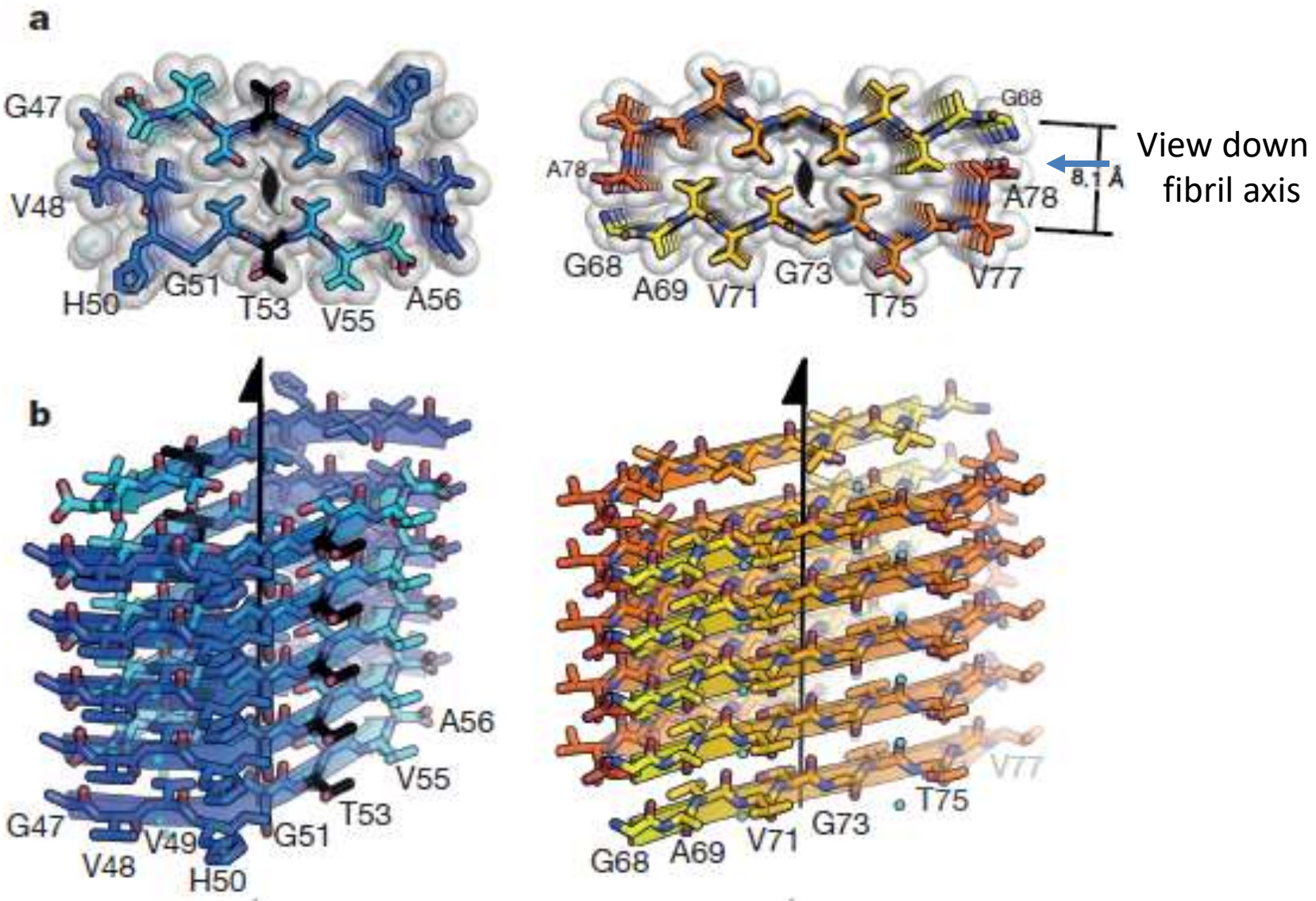
Resolution 1.5 Å
H atoms visible

Rodriguez et al.
Nature 2015

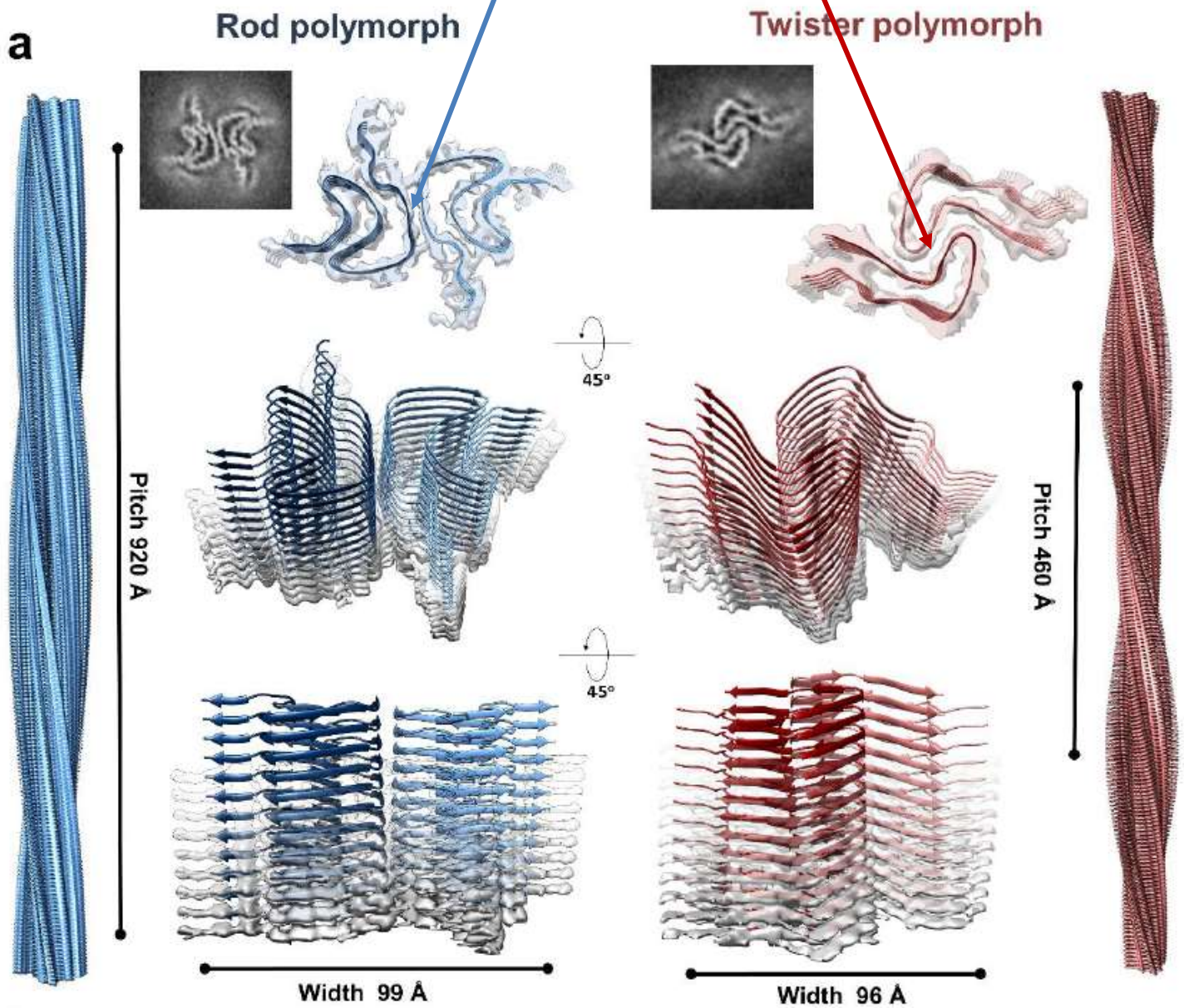
Stabilization from:
- Tightly mated β-sheets
- Polarized H-bonds
- Stacked Tyr, Phe, Gln, Asn

PreNAC and NACore

form Steric zippers

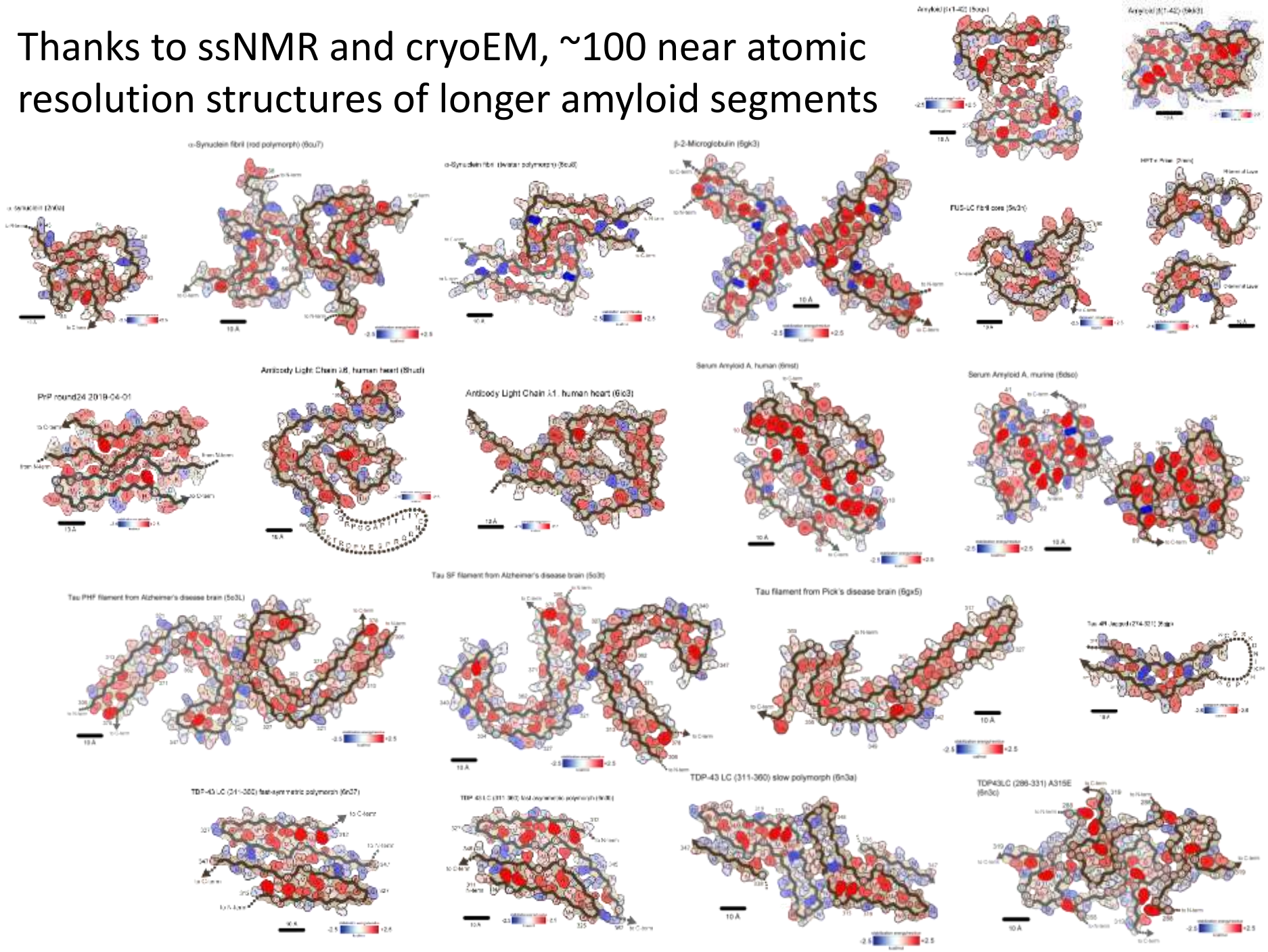


CryoEM structures of α-synuclein full fibril polymorphs show both PreNAC and NACore steric zippers

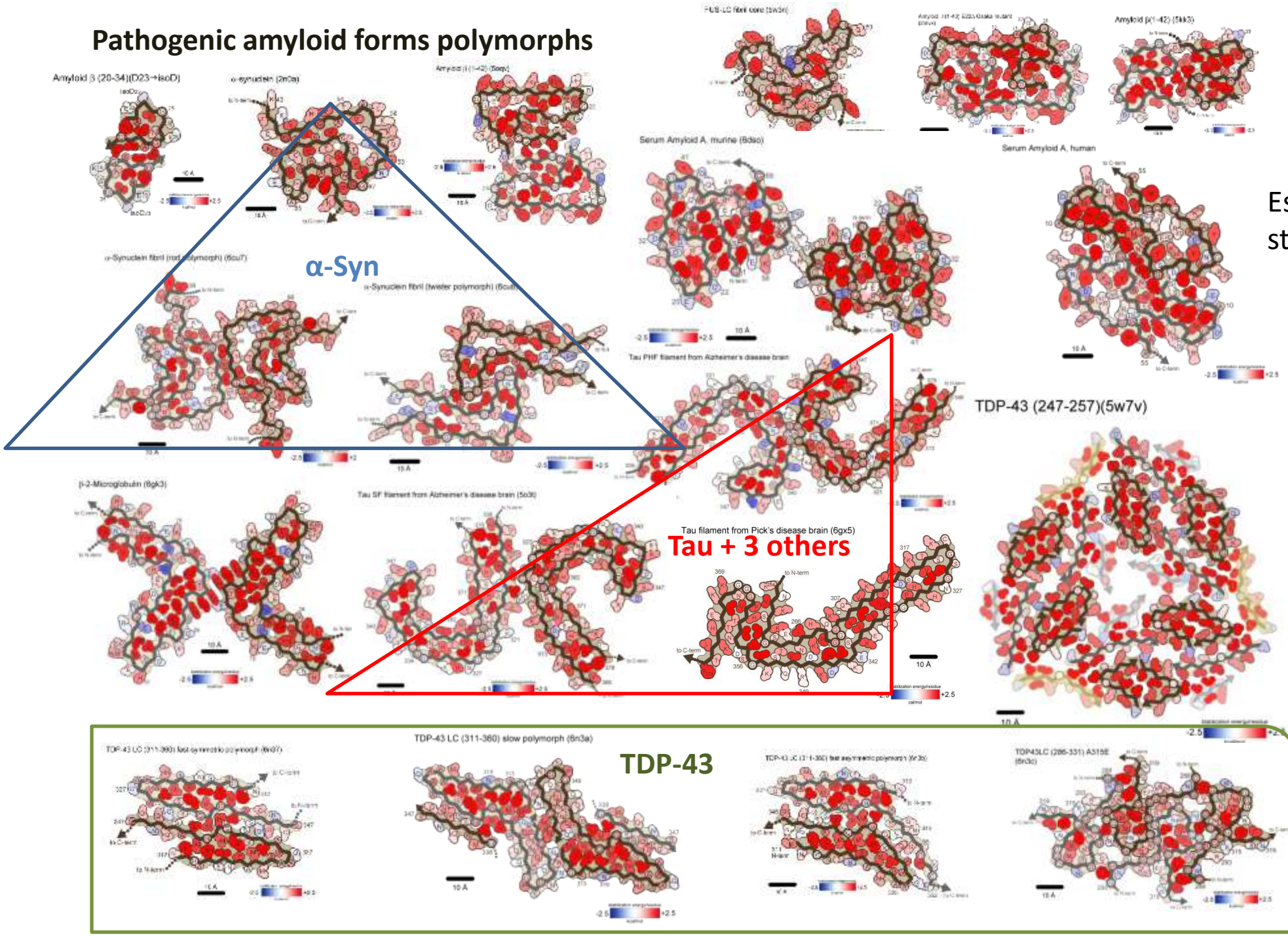


Lin Jiang, Binsen Li, Kevin Murray, et al. Nature Communications (2018)

Thanks to ssNMR and cryoEM, ~100 near atomic resolution structures of longer amyloid segments



Pathogenic amyloid forms polymorphs



Essentially 2D structures

9 polymorphs of tau: Why so many?

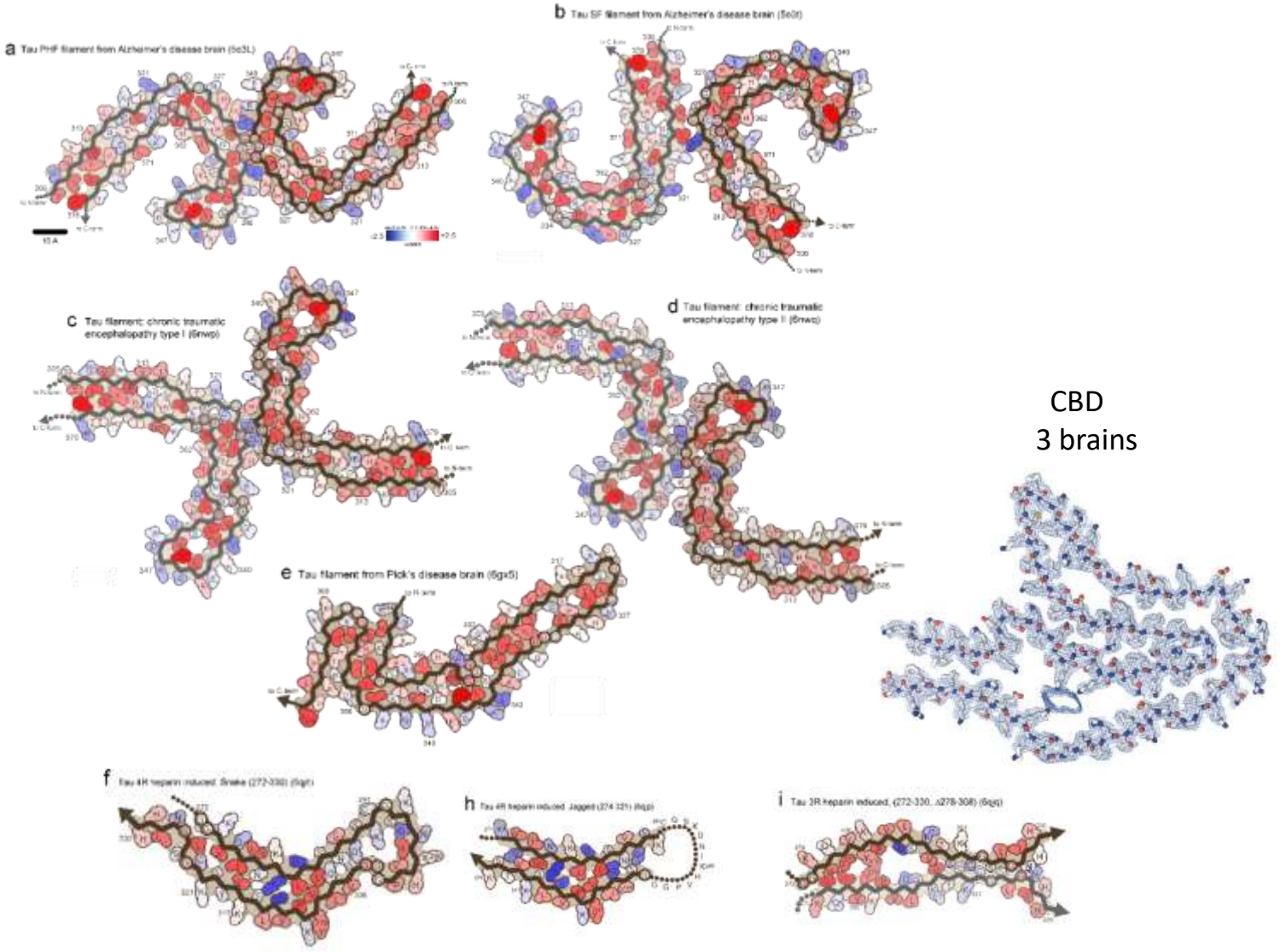
Alzheimer's
17 brains
(4 cryoEM, + 13
antibody maps)

CTE
3 brains

Pick's
3 brains

Heparin-induced
(4 structures)

CBD
3 brains



Structures from Scheres, Goedert, Falcon, Zhang et al. (2017-2019)

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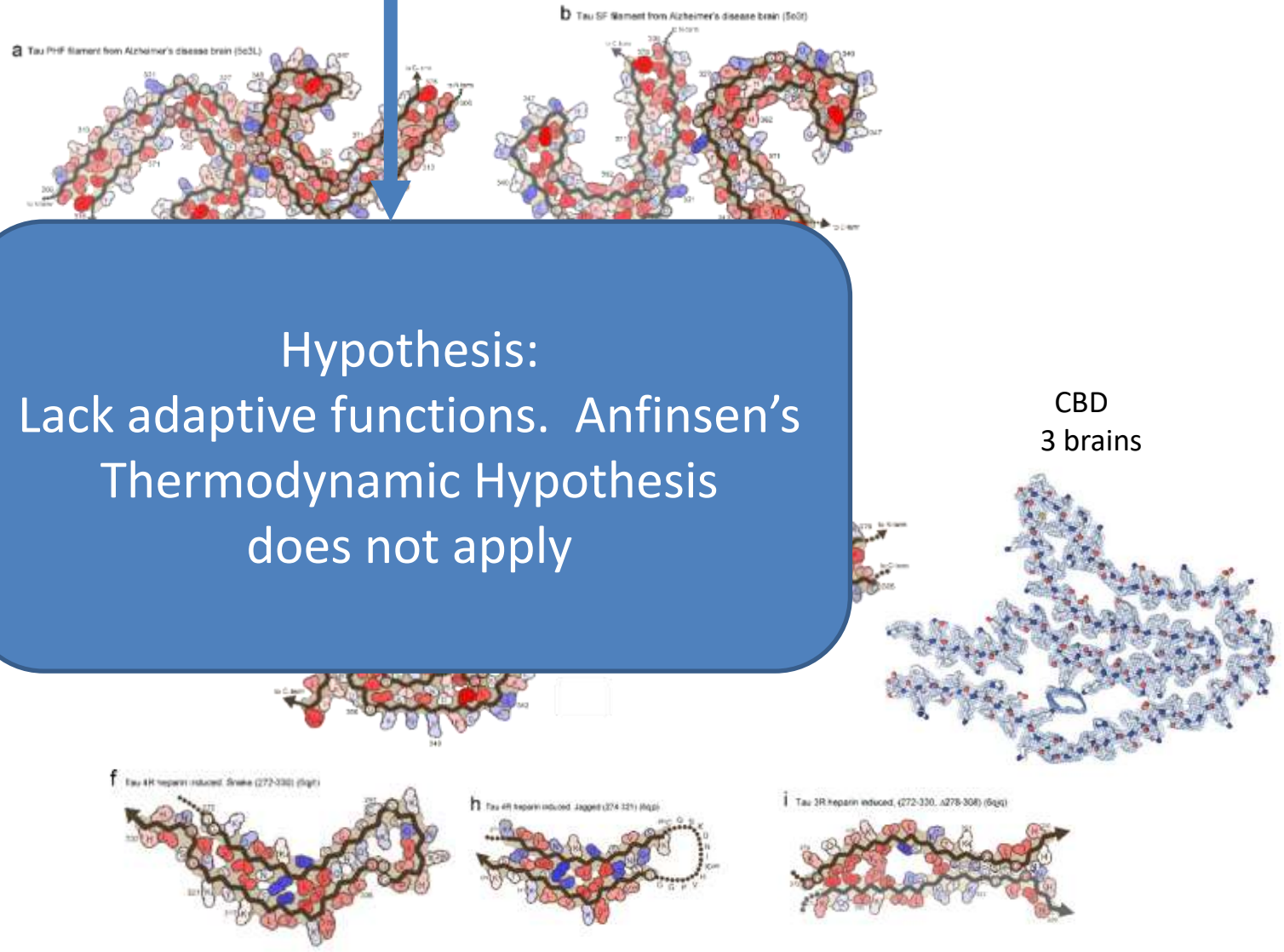
Hypothesis:
Lack adaptive functions. Anfinsen's
Thermodynamic Hypothesis
does not apply



Structures from Scheres, Goedert, Falcon, Zhang et al. (2017-2019)

9 polymorphs of tau: Why so many? Why all the same in AD brains?

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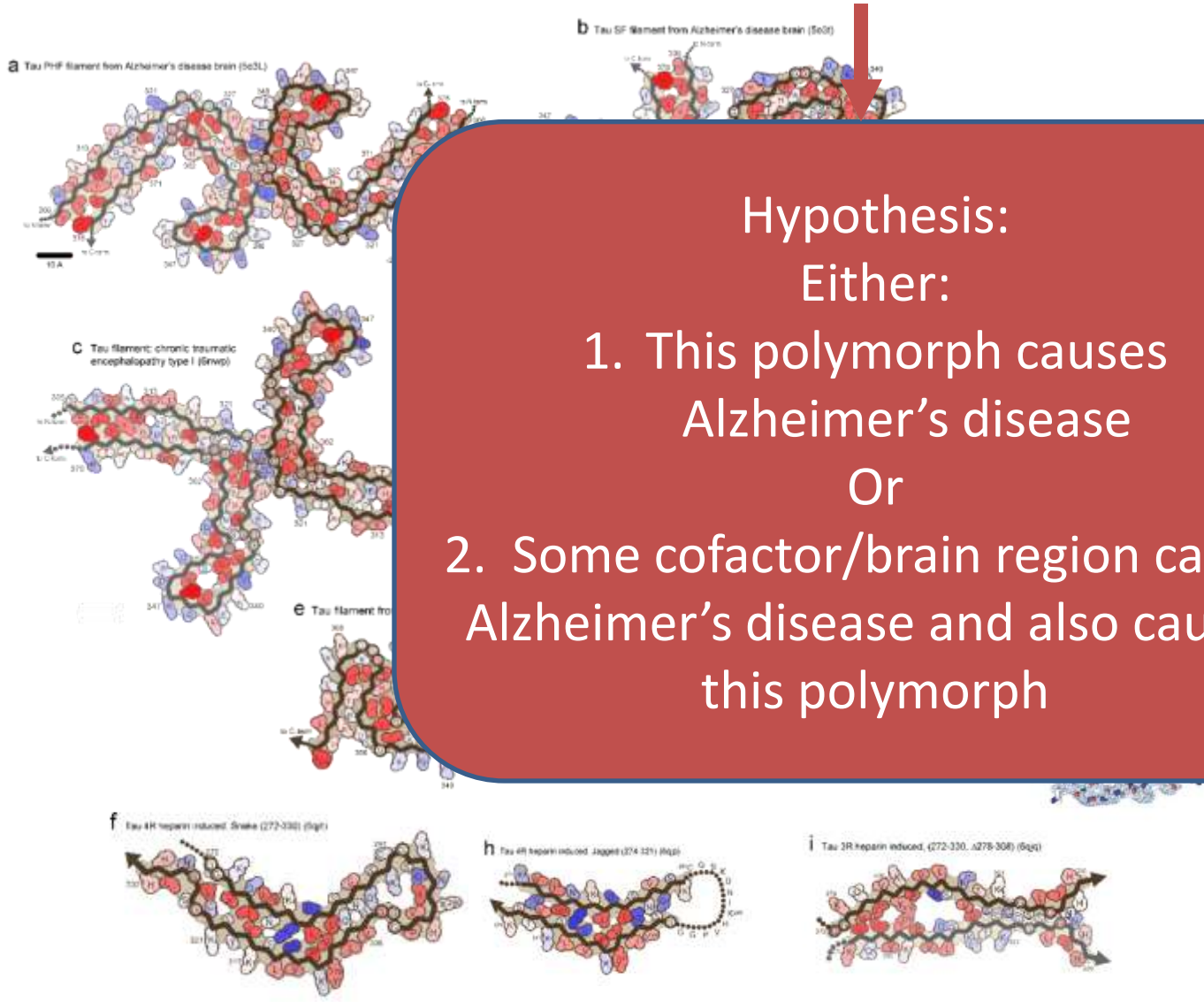


Hypothesis:
Either:
1. This polymorph causes Alzheimer's disease
Or
2. Some cofactor/brain region causes Alzheimer's disease and also causes this polymorph

Structures from Scheres, Goedert, Falcon, Zhang et al. (2017-2019)

Energy maps

α-synuclein

Heterotypic zippers

Homotypic zippers

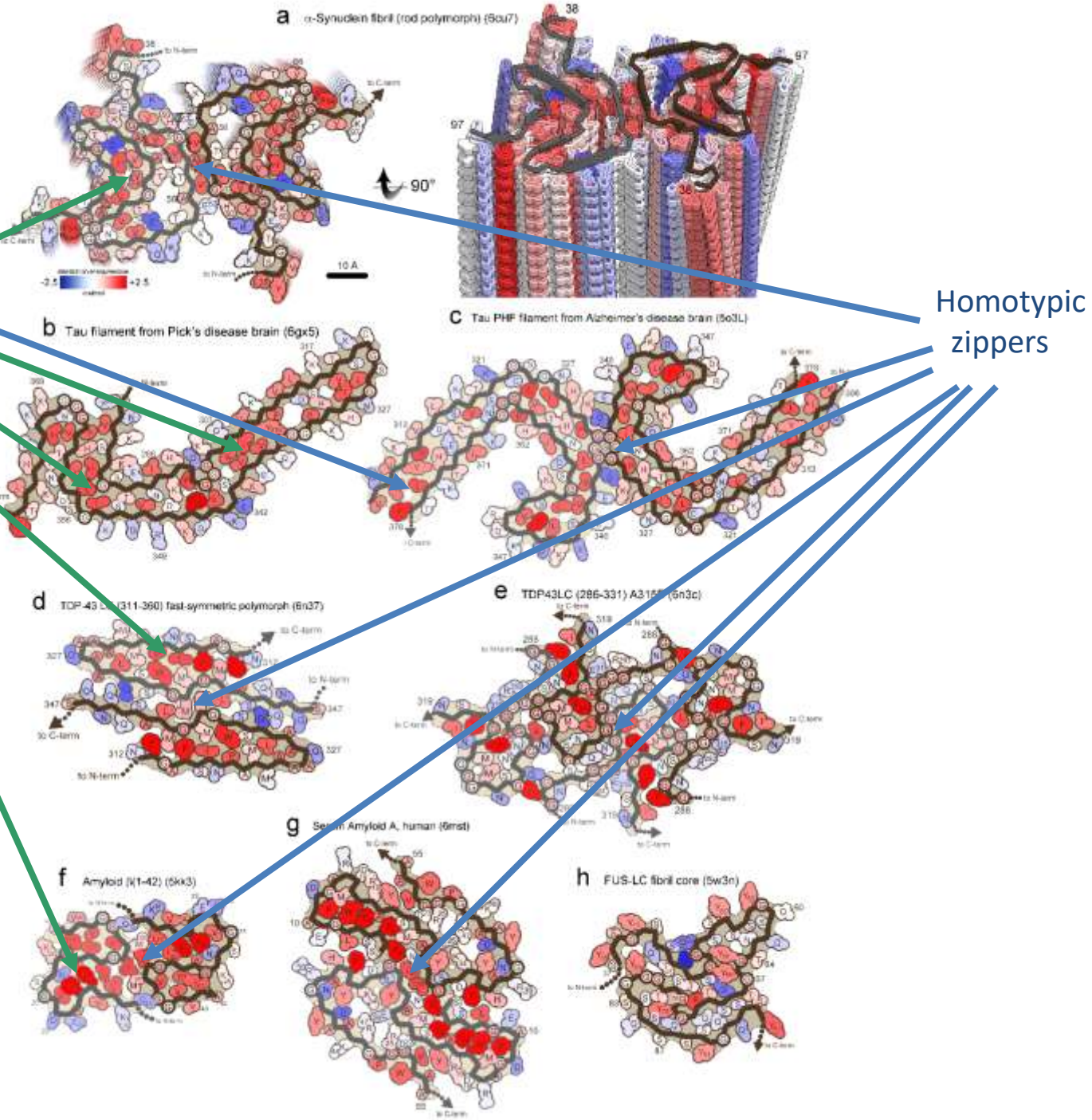
Tau

TDP-43

Amyloid-β

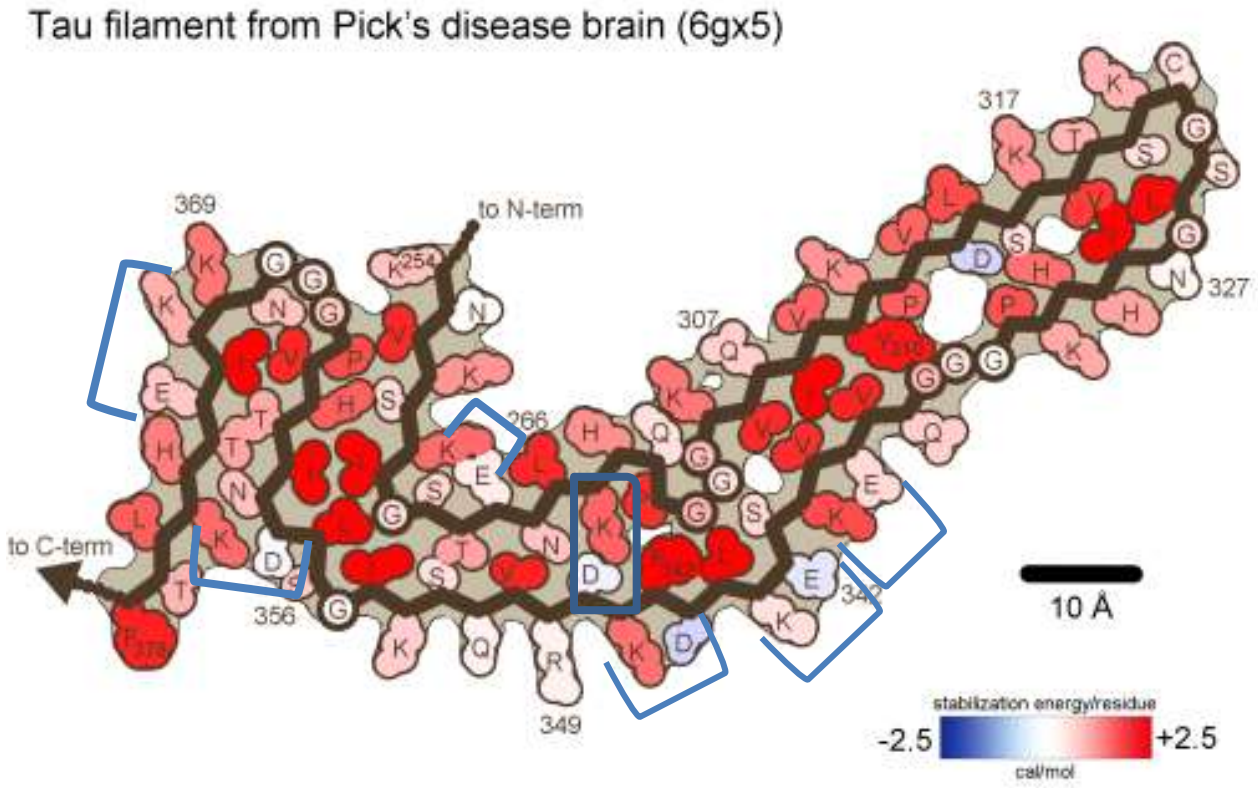
Serum amyloid A

FUS



Neighboring columns of oppositely charged sidechains

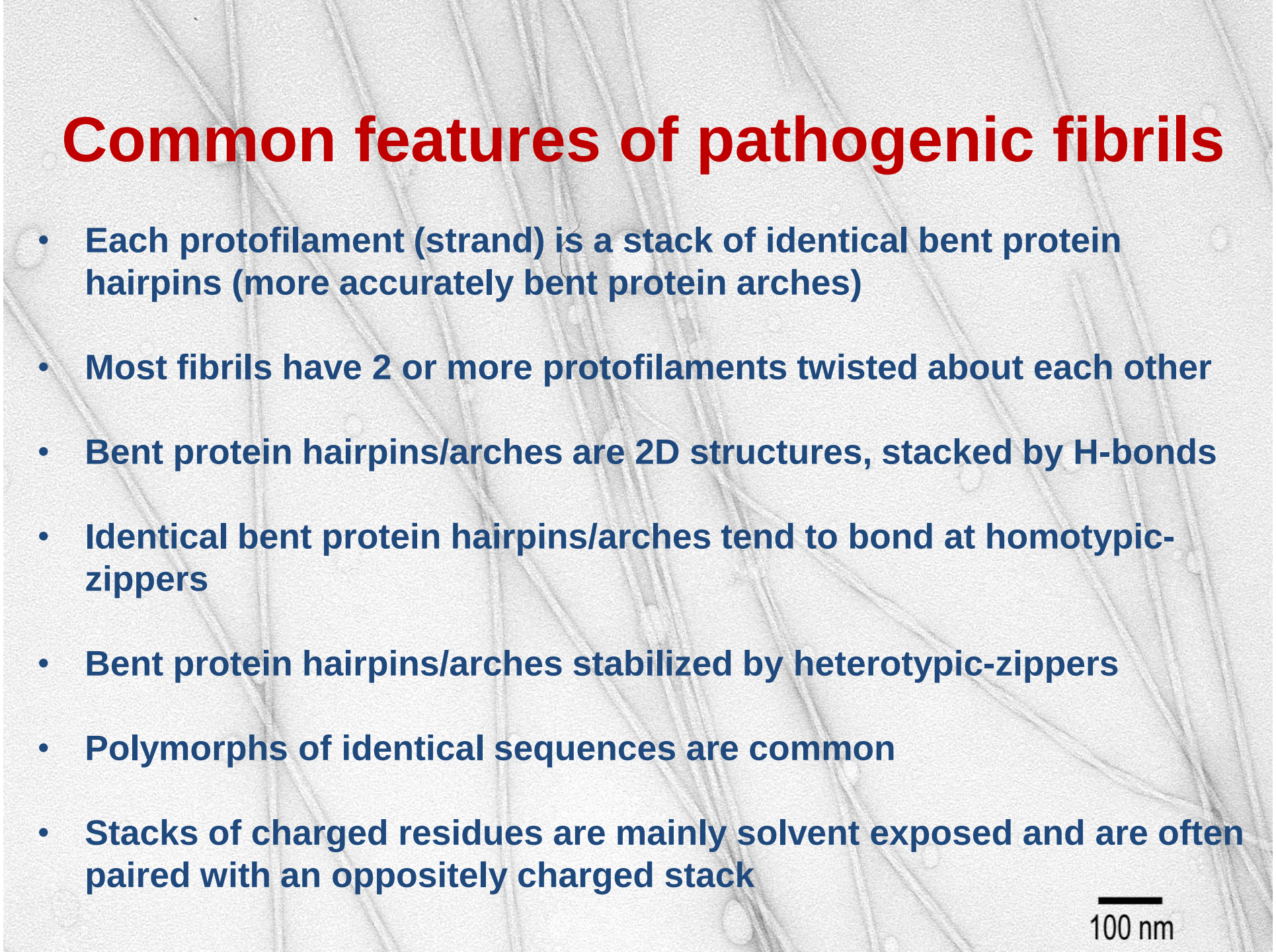
Are these 1-dimensional ionic crystals?



Falcon…Scheres, Goedert, Nature, 2018

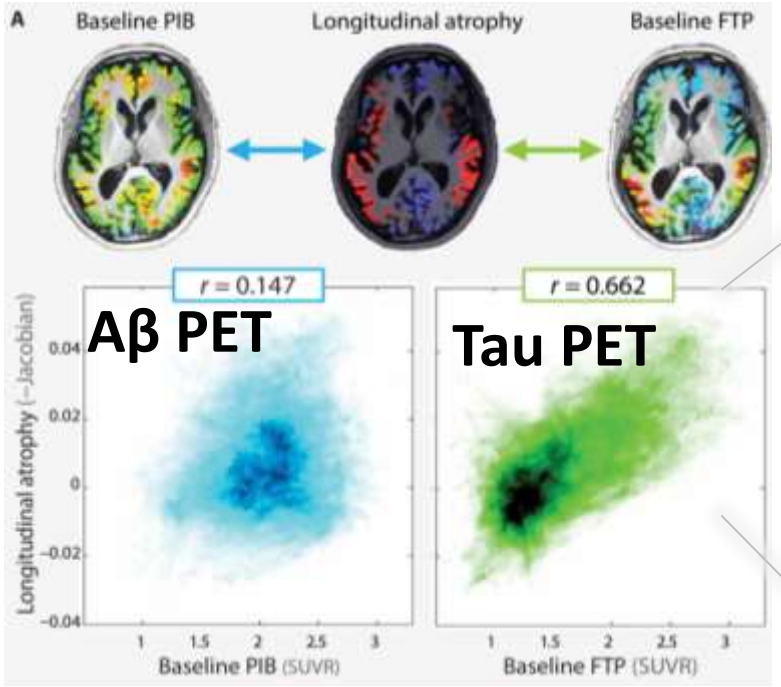
Common features of pathogenic fibrils

- Each protofilament (strand) is a stack of identical bent protein hairpins (more accurately bent protein arches)
- Most fibrils have 2 or more protofilaments twisted about each other
- Bent protein hairpins/arches are 2D structures, stacked by H-bonds
- Identical bent protein hairpins/arches tend to bond at homotypic-zippers
- Bent protein hairpins/arches stabilized by heterotypic-zippers
- Polymorphs of identical sequences are common
- Stacks of charged residues are mainly solvent exposed and are often paired with an oppositely charged stack

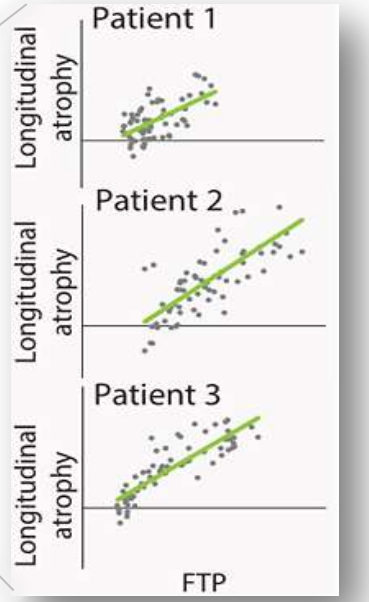


Tau amyloid fibrils as the target for potential Alzheimer's drugs

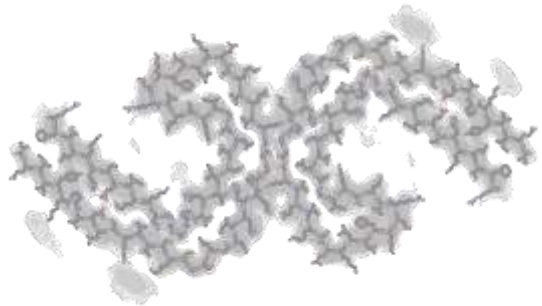
Tau aggregation tracks with brain atrophy, Aβ does not



La Joie et al.
Sci Transl Med. 2020

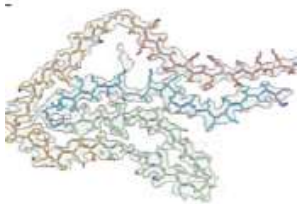


Tau amyloid fibrils extracted from dozens of Alzheimer's brains have the same polymorph structure (paired helical filaments, **PHF**)



Fitzpatrick…Goedert 2017

Recombinant tau fibrils and tau fibrils from other tauopathies have other structures



EGCG disaggregates fibrils of tau PHF from AD brain

Paul Seidler

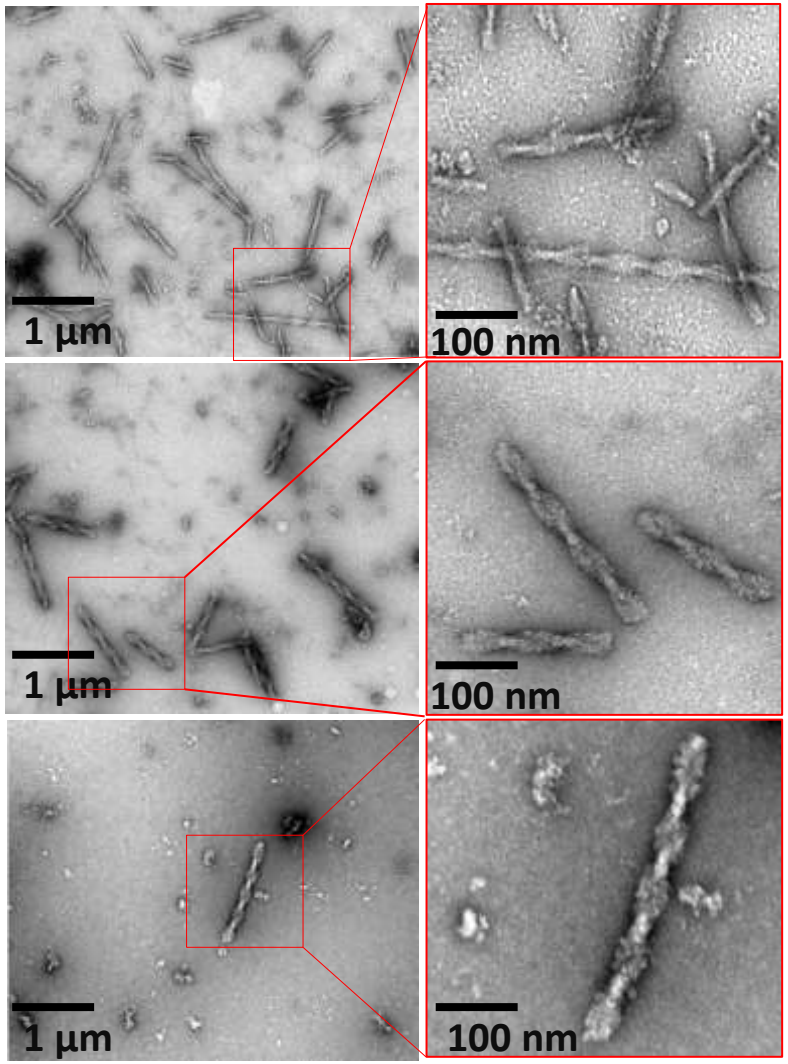
AD tau-PHF

whole field of view | zoomed

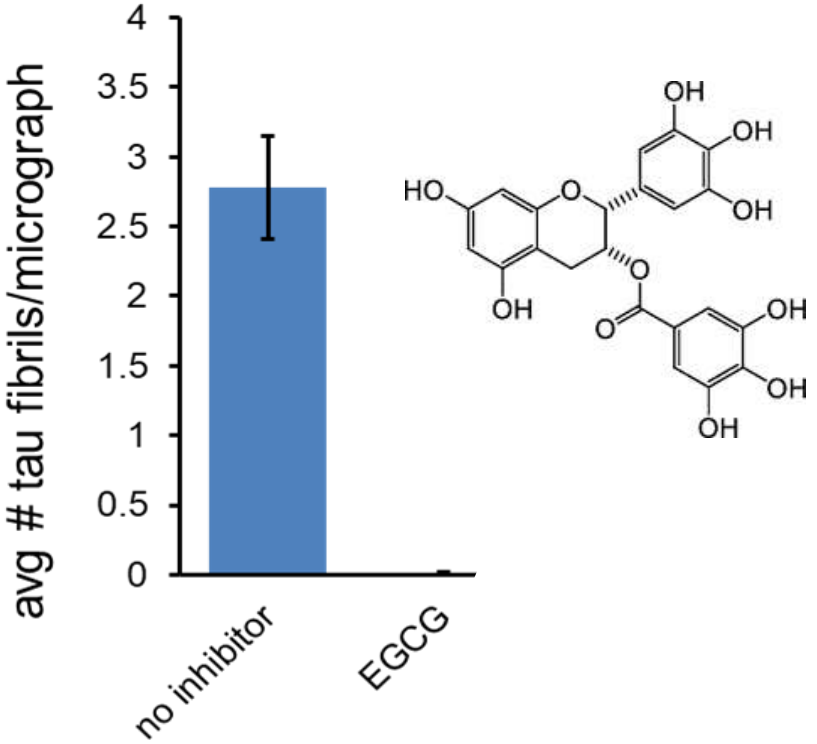
No EGCG

3 hr EGCG

overnight EGCG



Fibril density determined from 100 micrographs

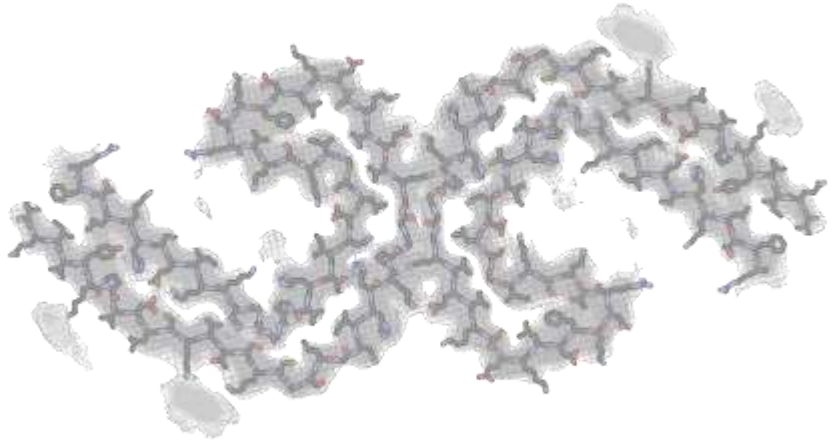


EGCG is not a drug: poor brain penetration; modified in body fluids; reacts with many proteins.

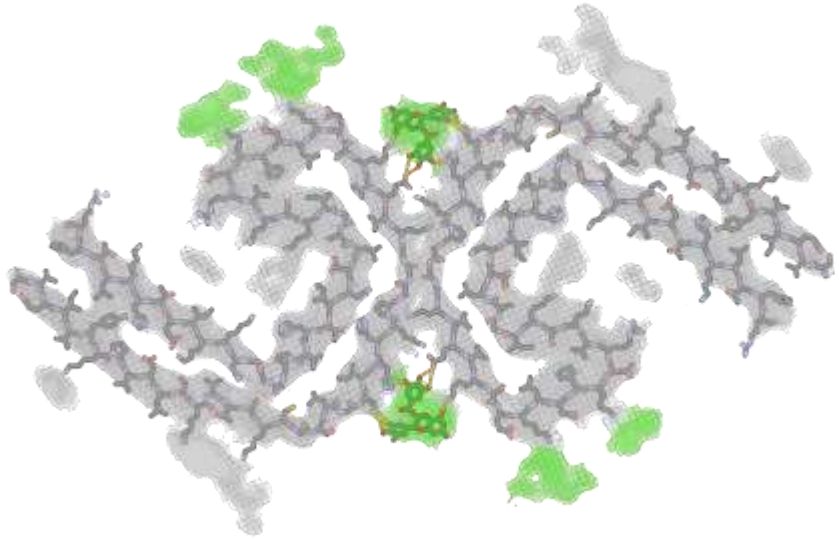
Cryo-EM structure of cryogenically-trapped 3-hour EGCG-treated tau fibrils from AD brains

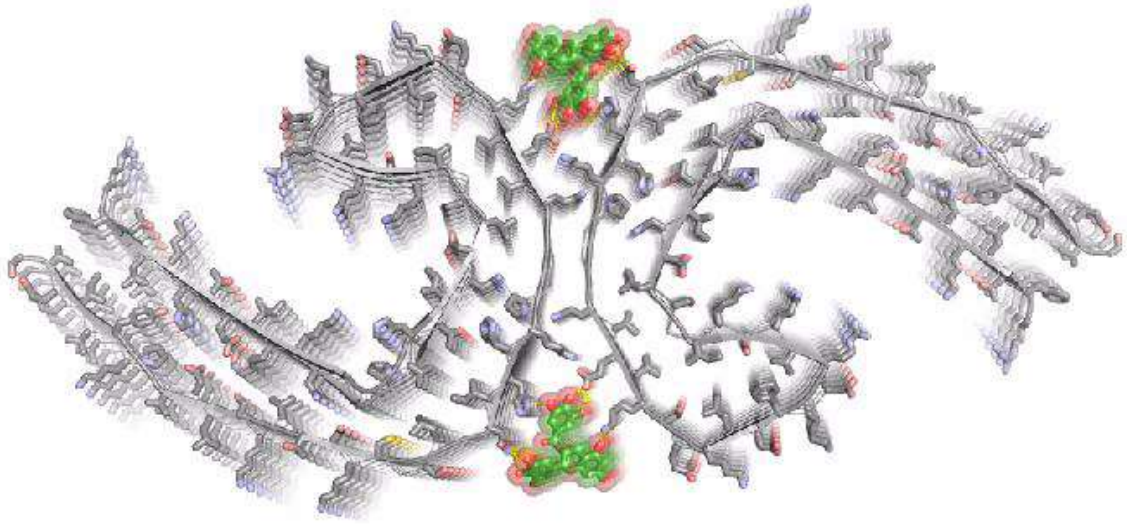
Paul Seidler
David Boyer
Poster #5

No EGCG



3 hr EGCG





New density appearing after 3hr incubation with EGCG

Cryo-EM structure of cryogenically-trapped 3-hour EGCG-treated tau fibrils from AD brains

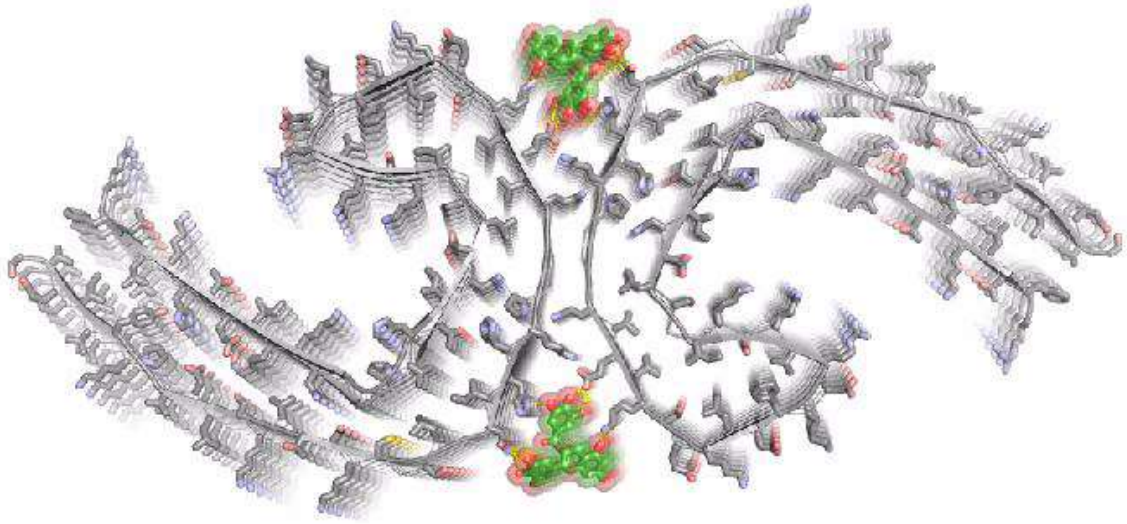
Paul Seidler
David Boyer
Poster #5

The 3-hour cryogenically trapped structure is a transient intermediate on the pathway to tau fibril disassembly

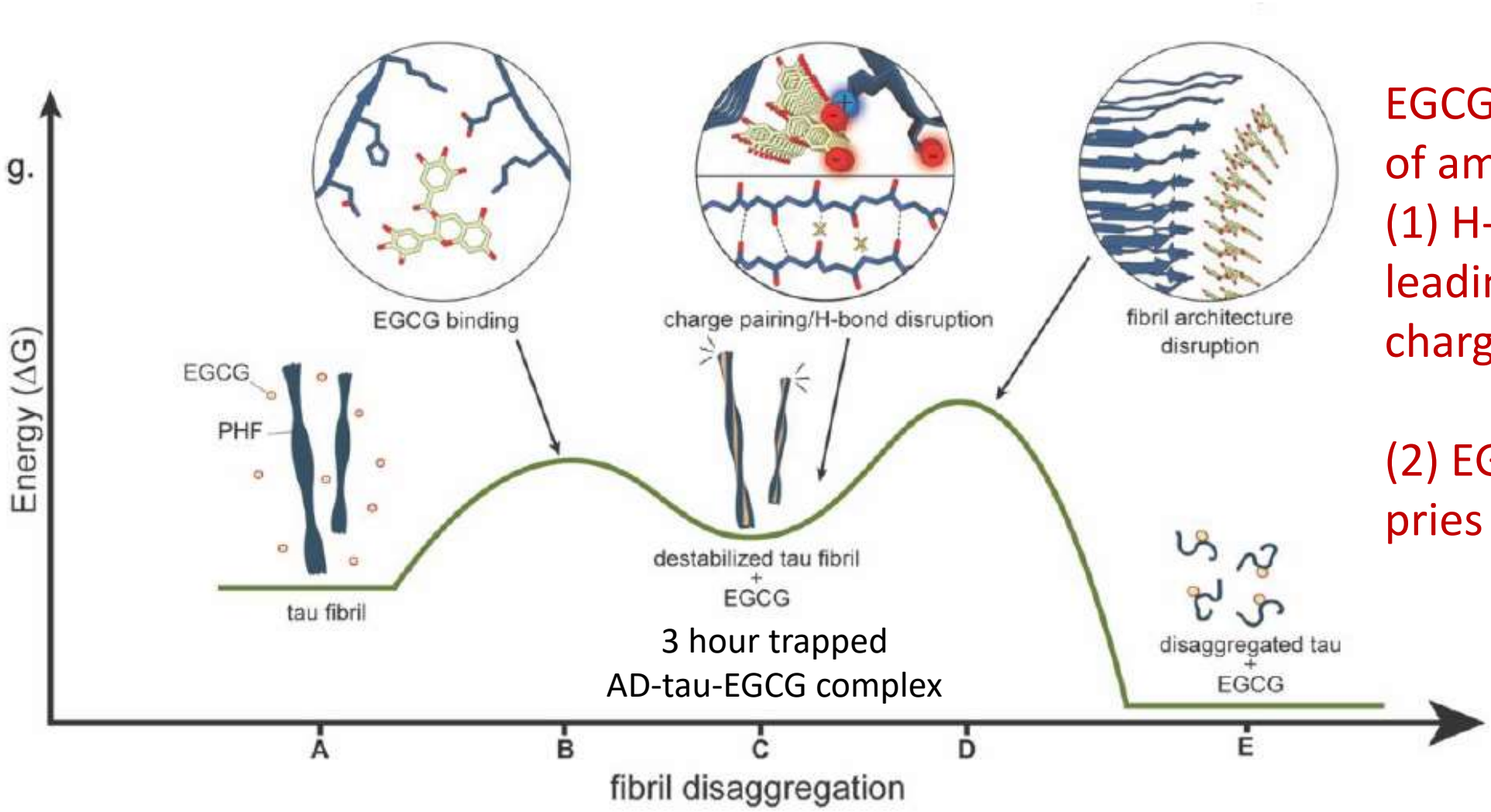
EGCG molecules are stacked in two polar clefts at the junctions of the two protofilaments of brain tau PHFs

Tau molecules and EGCG molecules are both spaced 4.8 Å apart along the fibril axis. EGCG O-H groups apparently hydrogen bond to charged tau residues

The 4.8 Å spacing of EGCG molecules is greater than their minimum energy separation of ~3.9 Å



Proposed reaction coordinate for disaggregation of Alzheimer's disease tau fibrils by EGCG



EGCG's disaggregation of amyloid is enabled by (1) H-bond competition leading to tau interlayer charge repulsion.

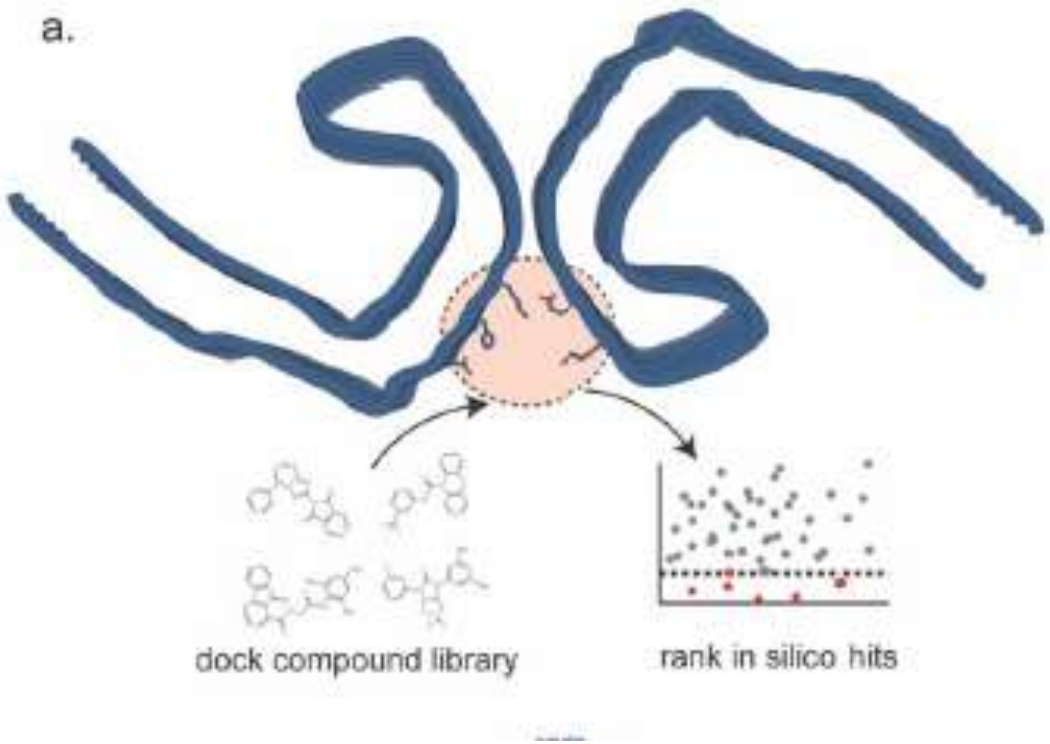
(2) EGCG-EGCG stacking pries tau layers apart.

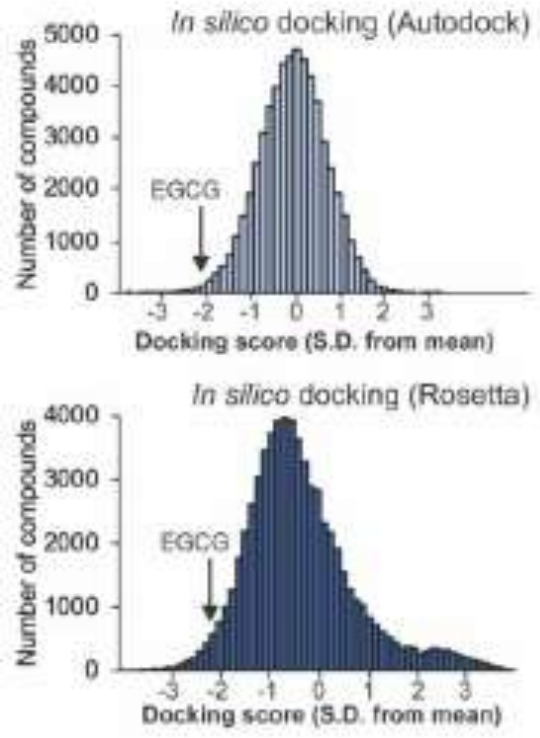
Structure-based discovery of disaggregants of tau fibrils extracted from autopsied AD brains (AD-tau)

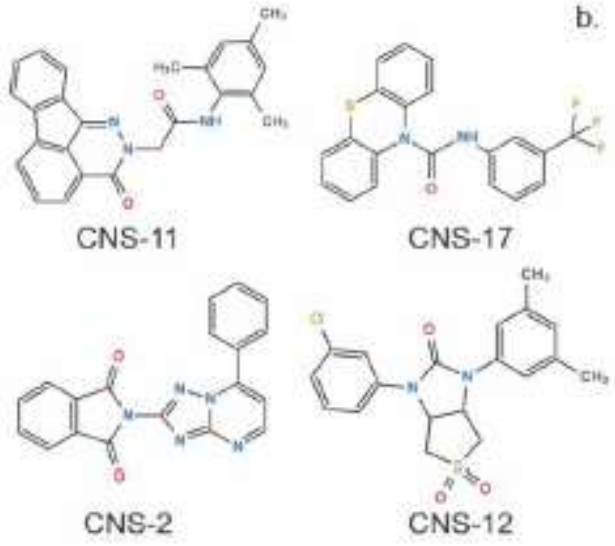
Use EGCG binding site as pharmacophore for disaggregants

Consider the EGCG site on AD-Tau fibrils as a **pharmacophore** for disaggragants
Screen *in silico* for CNS-friendly compounds binding the pharmacophore

Kevin Murray







~60,000 ChemBridge CNS-compatible compounds
~ 1700 FDA approved small-molecule drugs

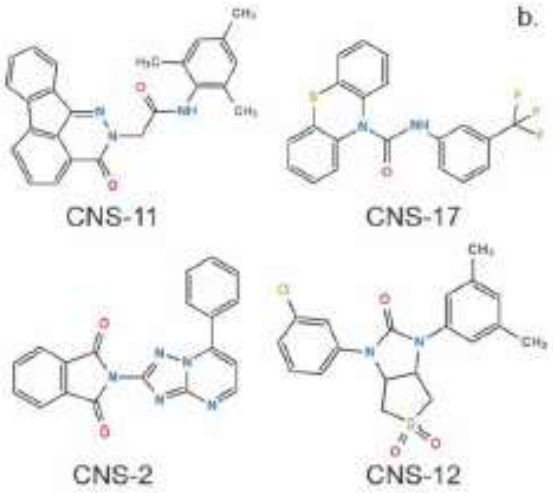
100 conformations docked for each compound

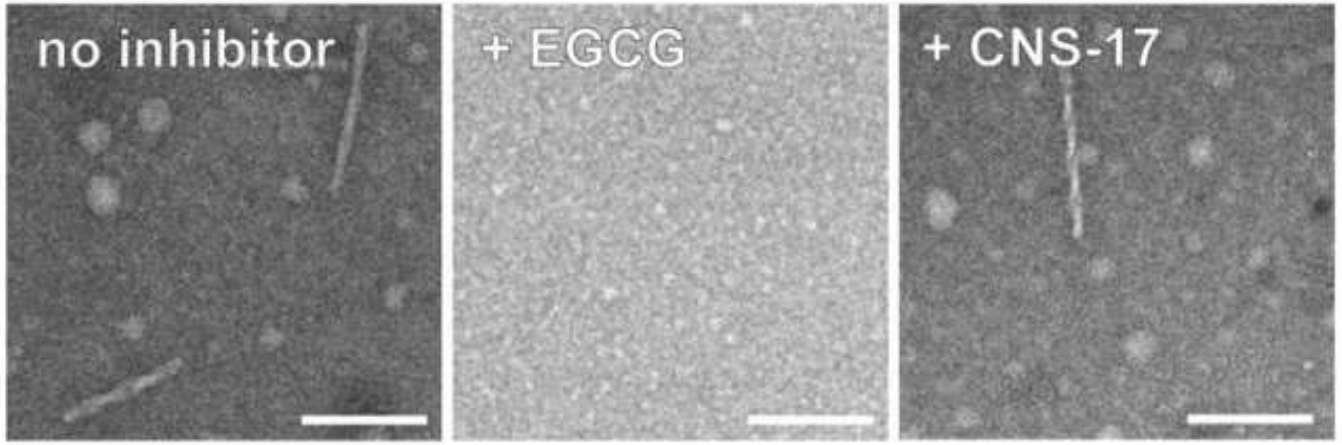
Positive control EGCG binds with predicted energy >2 SD below mean.

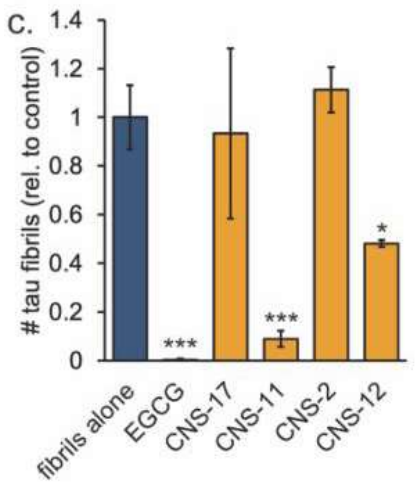
46 compounds obeying Lipinski rule of 5 (i.e. drug-like) selected for experimental study

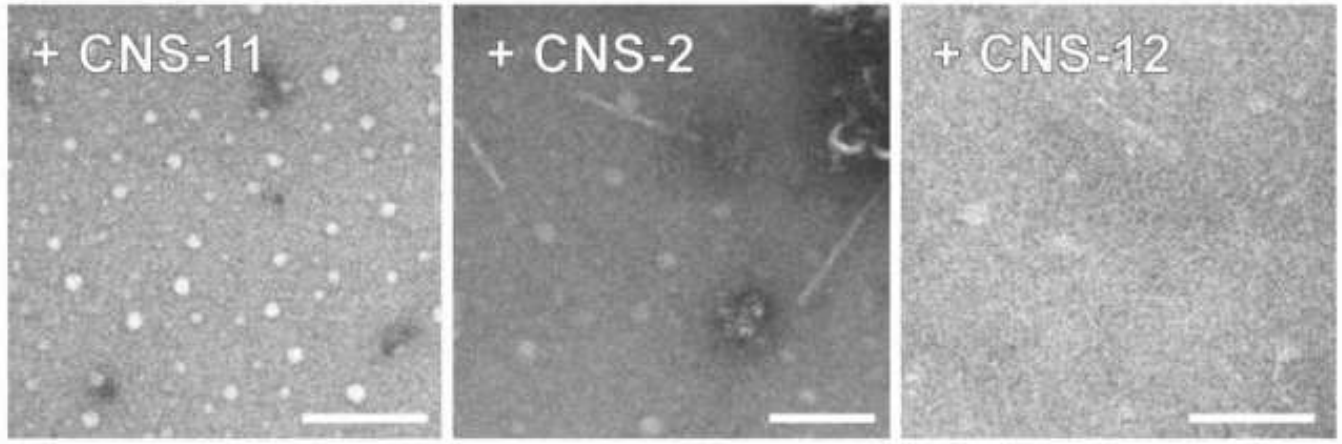
Experimental assessment of potential disaggregating compounds

Direct measures of fibril disassembly of selected compounds





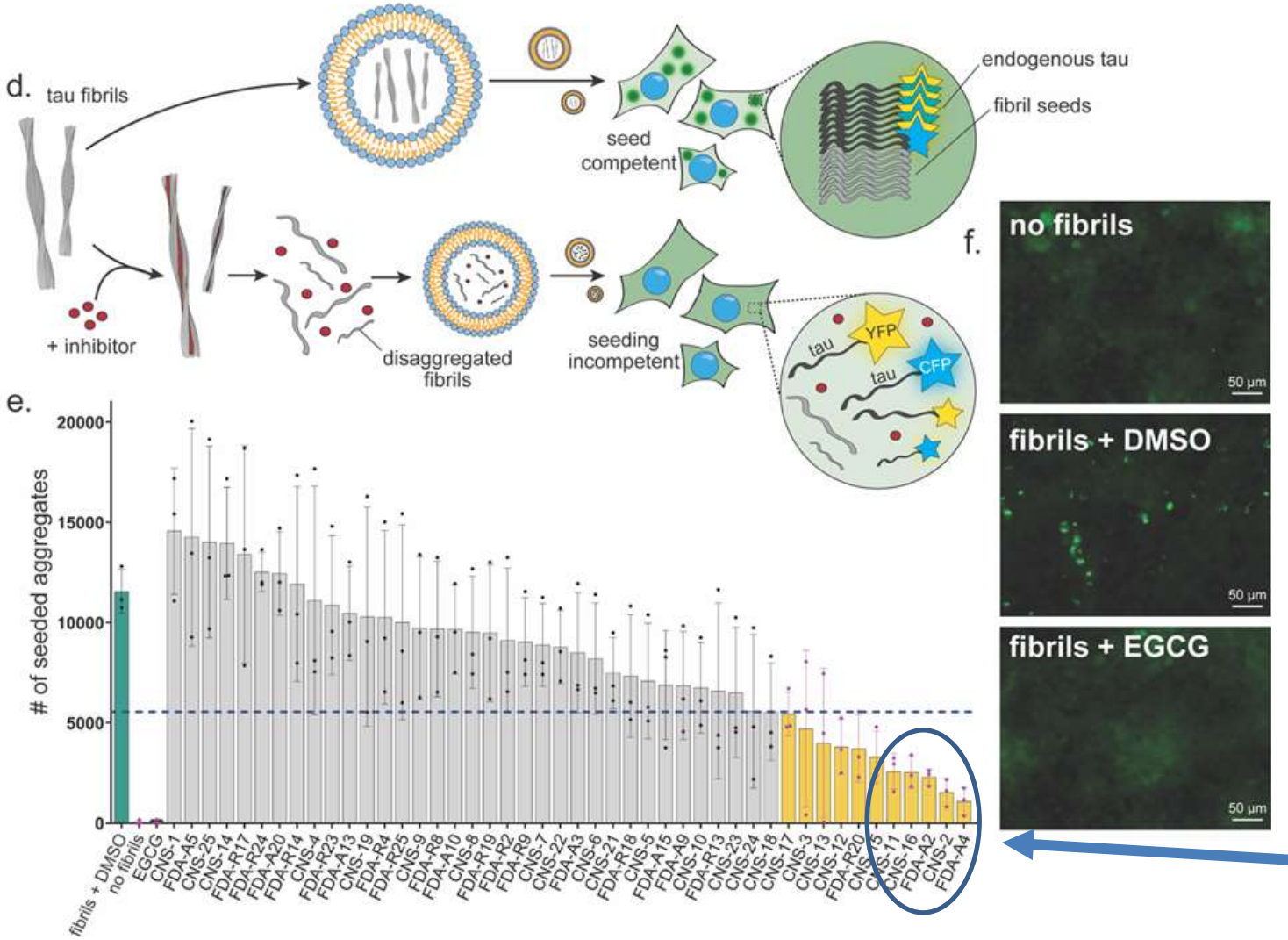




Several pharmacophore-selected compounds disaggregate AD-tau fibrils. But none (so far) as effectively as EGCG

Effective disaggregants must not produce disaggregated products that seed growth of tau amyloid fibrils

Transduce disaggregated products into biosensor cells to test seeding capacity



These compounds block seeding most successfully.

Summary

Structure-based discovery of disaggregants of tau fibrils extracted from autopsied AD brains (AD-tau)

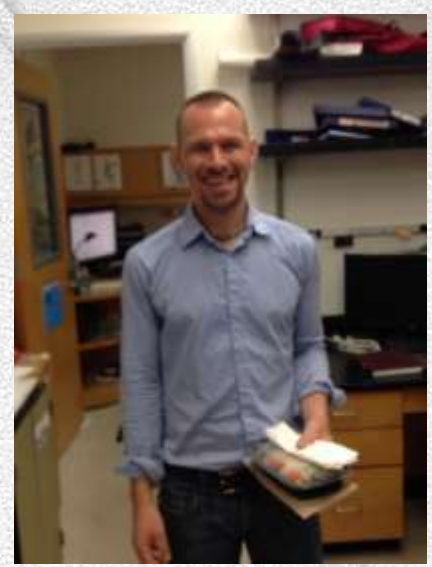
The cryogenically-trapped EGCG-AD-tau complex suggests how small molecules disassemble tau fibrils

The EGCG-AD-tau complex offers a pharamacophore for discovery of disaggregants of AD-tau fibrils

Structure-based drug design, so effective for cancer and metabolic diseases, may be possible for amyloid conditions

UCLA Amyloiders: Duilio Cascio, Michael Sawaya, Daniel Anderson, Sarah Griner, Qin Cao, Paul Seidler, Jeanette Bowler, David Boyer, Melinda Balbirnie, Romany Abskhron, Jaihui Lu, Gregory Rosenberg, Einav Tayeb-Fligelman, Cindy Cheng, Sean Jiang, Ke Hou, Hope Pan

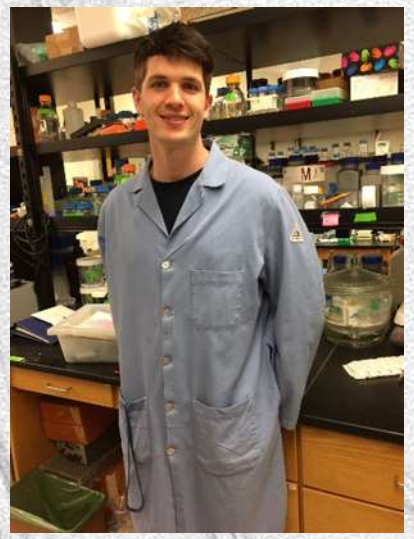
Collaborators: Patrick Harran, Ben Novitch, Lin Jiang, Joe Loo, Jose Rodriguez, Christina Sigurdson, Marc Diamond, Steve McKnight, Greg Cole, Sally Frautschy



Paul Seidler

Jiahui Lu

David Boyer
Poster #5



Kevin Murray

Michael Sawaya

Support: HHMI NIA, DOE

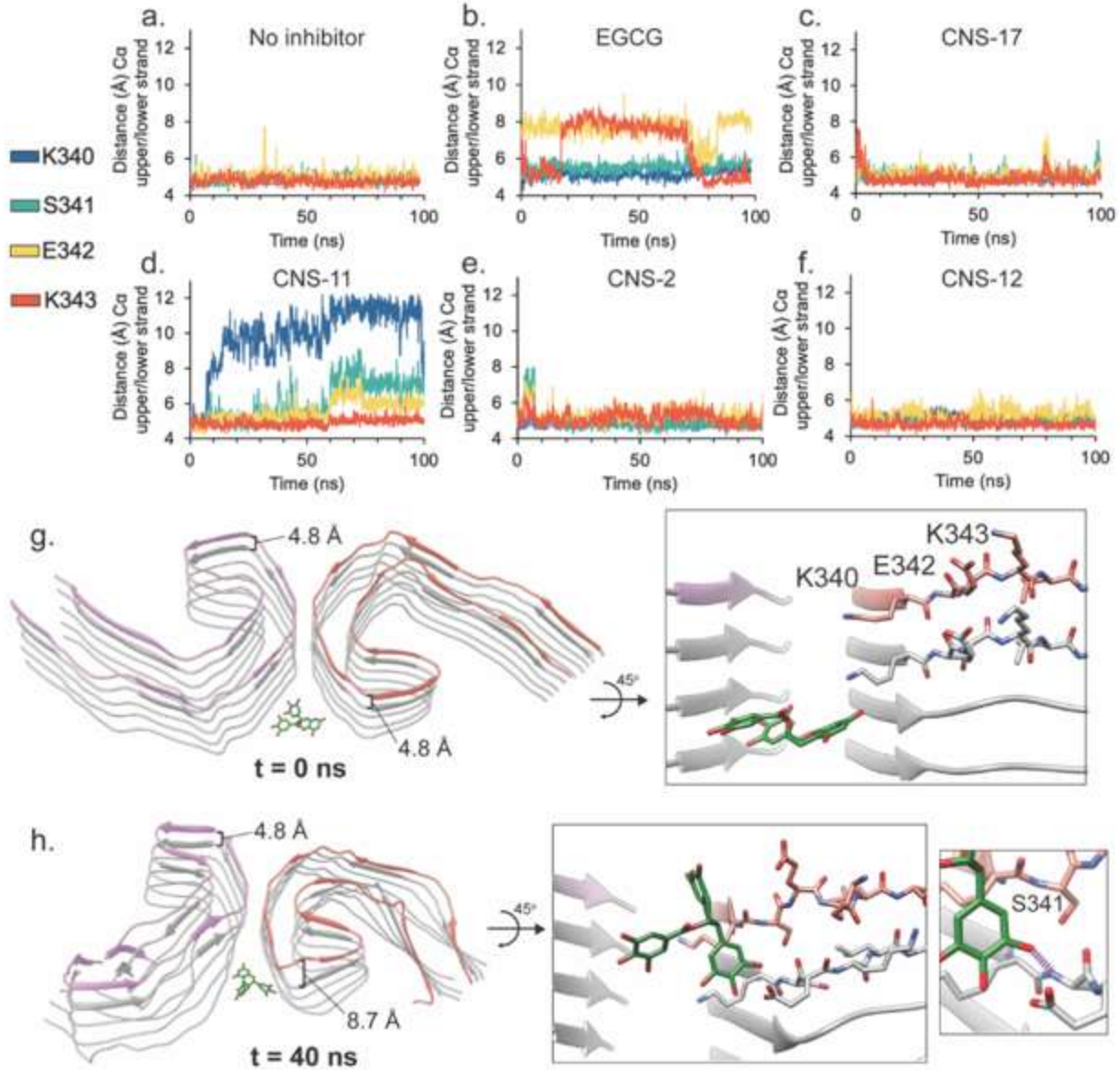
Experiments in progress

Brain penetration: measure of concentration of CNS-11 and CNS-11G in mouse brain after tail vein injection

Behavioral efficacy: Assessment of behavioral changes in P302L mice treated with compounds

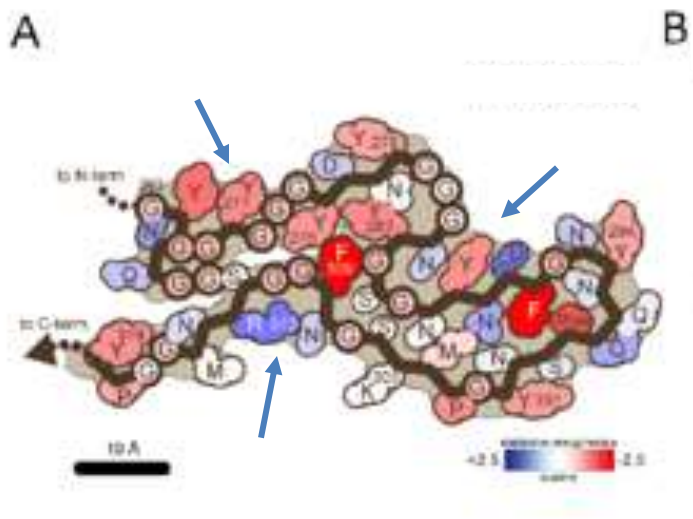
Target efficacy: measure of diminished tau fibril load in brains of P301L tau mice

Extension to Parkinson's disease: parallel experiments on alpha-synuclein fibrils and treatment of PD mice


a.
No inhibitor
b.
EGCG
c.
CNS-17
d.
CNS-11
e.
CNS-2
f.
CNS-12
K340
S341
E342
K343
Distance (Å) Cα upper/lower strand
Time (ns)
g.
4.8 Å
4.8 Å
t = 0 ns
45°
K343
E342
K340
h.
4.8 Å
8.7 Å
t = 40 ns
45°
S341

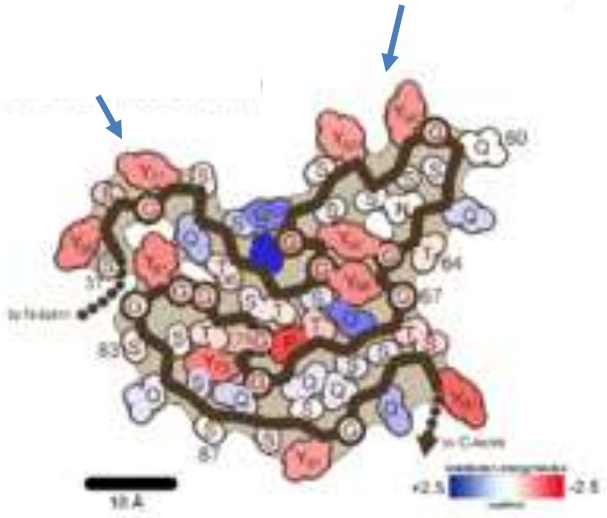
Functional vs Pathogenic Amyloid Fibrils

hnRNPA2 LCD



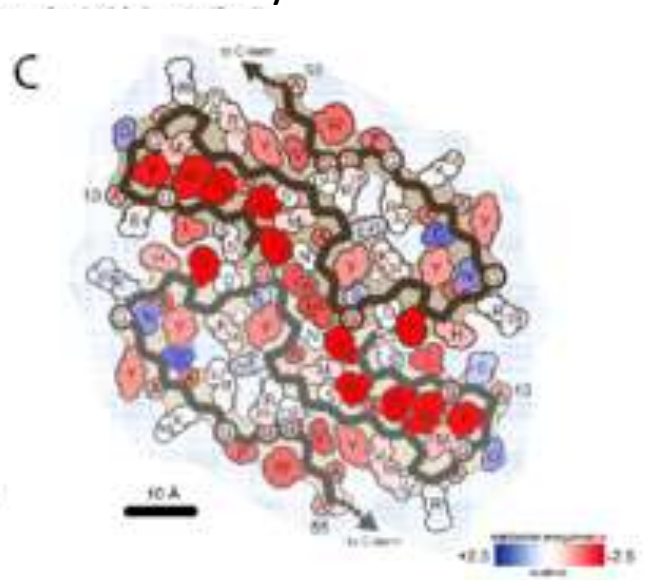
Lu et al. UCLA 2019

FUS LCD



Murray, Tycko, McKnight 2017

Serum amyloid A



Li, Fandrich et al. 2019

Functional fibrils are less stable than pathogenic fibrils

Functional fibrils seem monomorphic

Functional fibrils contain putative LARKS

Pathogenic amyloid forms polymorphs