

Inter-Species Cross-Seeding: Stability and Assembly of Rat - Human Amylin Aggregates

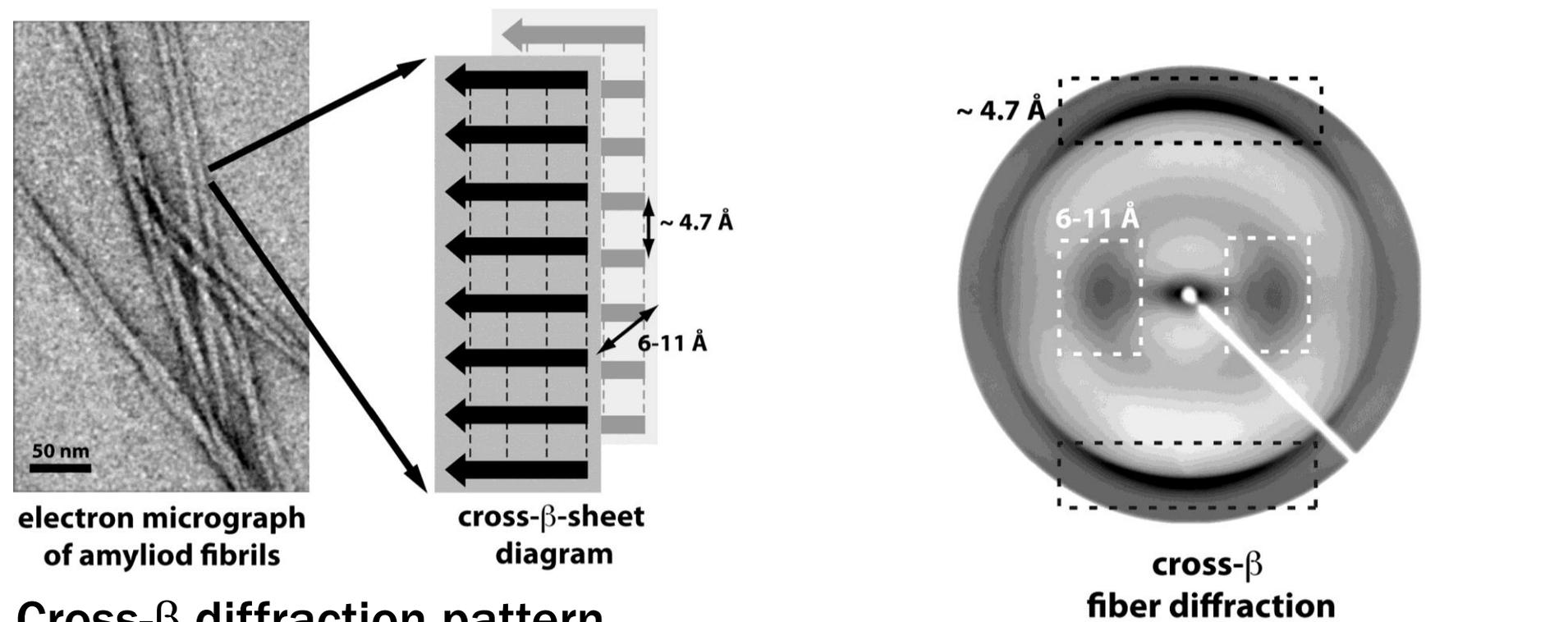
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STRUCTURE OF AMYLOIDS & ROLE IN AMYLOID DISEASE

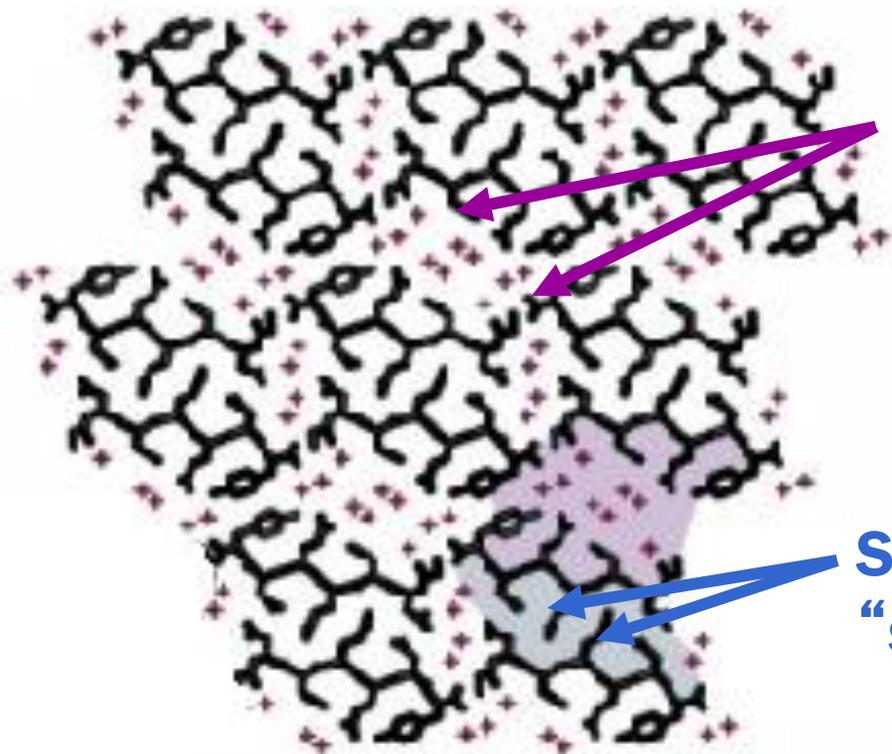
Greenwald J. Structure, 18 (2010), pp. 1244–1260



Cross- β diffraction pattern

- 4.7 Å spacing between β -sheets strands, β -strands oriented perpendicular to the fibril axis
- ~6-10 Å spacing between two mating β -sheets
- **mature fibrils are considered harmless**

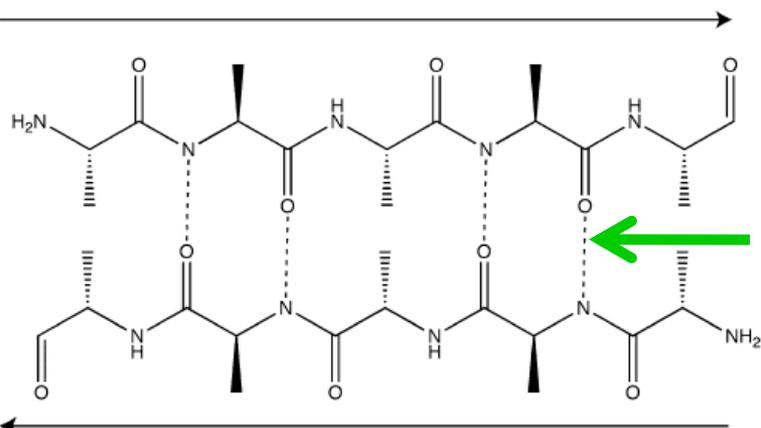
ATOMISTIC DETAILS REVEAL THREE TYPES OF INTERACTIONS IMPORTANT FOR AGGREGATION



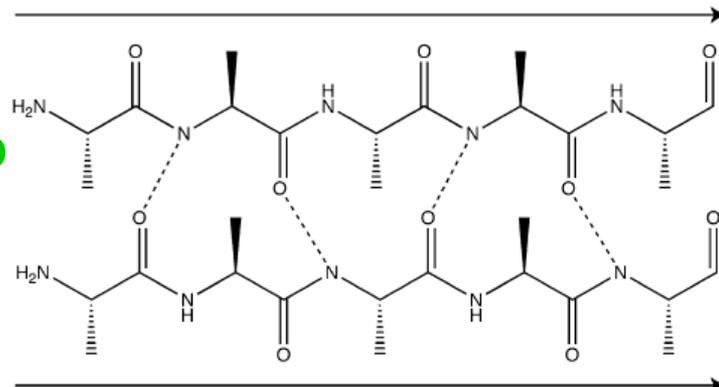
Side chain-side chain (hydrophilic)

Nelson et al. (2005) Nature, 435,773–778

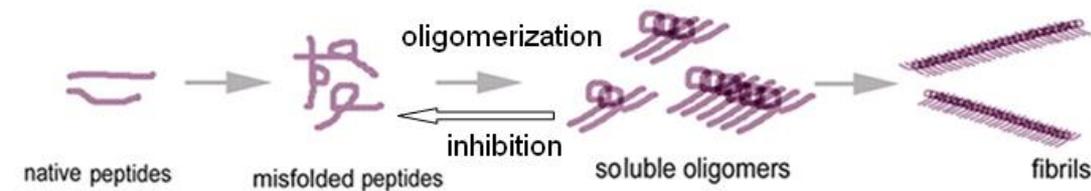
Side chain-side chain (hydrophobic
"steric zipper")



Main chain to
main chain
H-bond

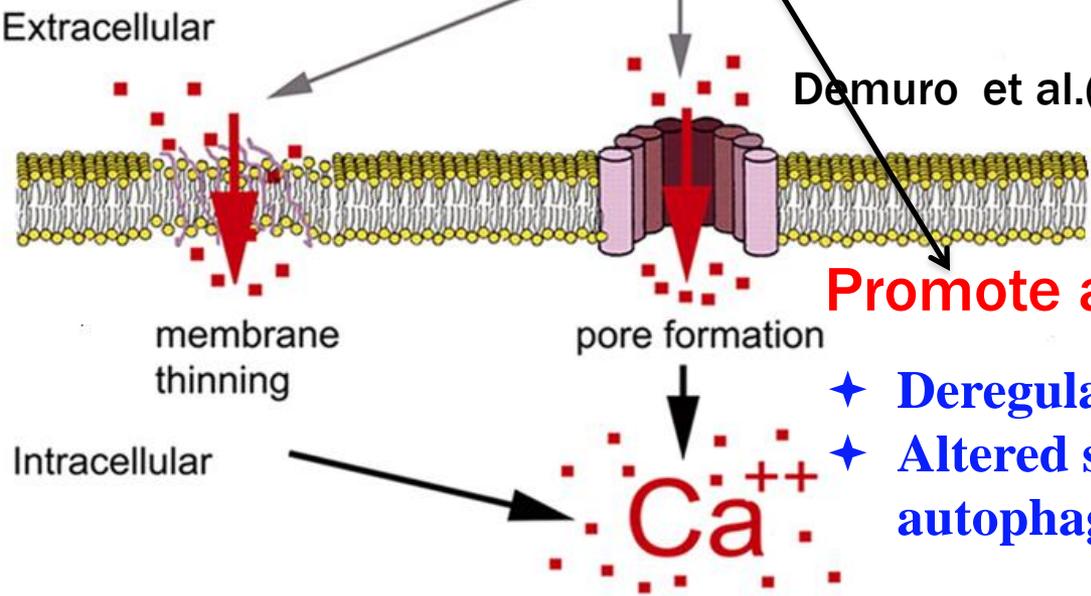


AMYLOID TOXICITY AND PROTEIN AGGREGATION DISEASES



Over 30 human diseases:

No cure, symptomatic treatments



Demuro et al.(2010) *J. Biol. Chem.* 285, 12463-12468

Promote abnormal protein interactions

- ◆ Deregulate essential cellular functions
- ◆ Altered signaling, mitochondria dysfunction, autophagy

Neurodegenerative diseases

Alzheimer's disease → A β peptides (aa1-40/42)

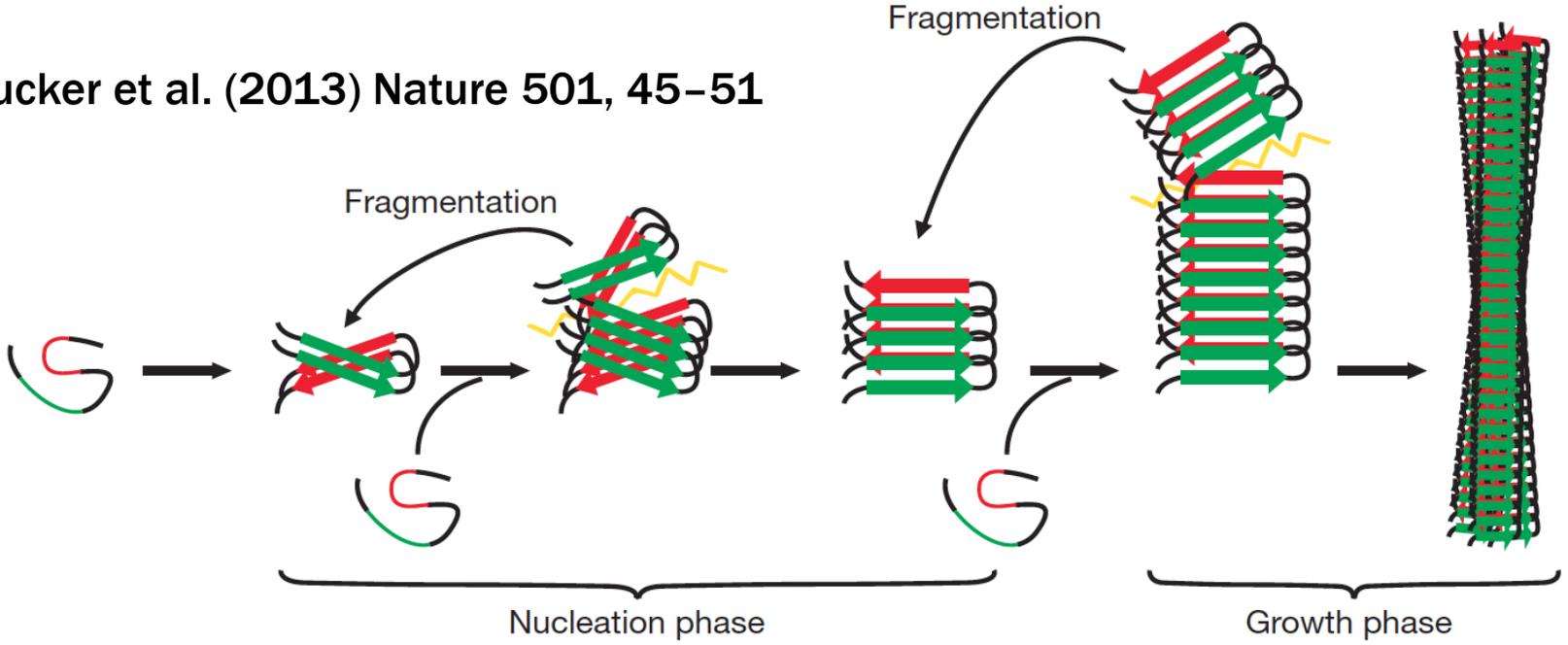
Non-neuropathic

Type II diabetes → Amylin (aa 1-37)

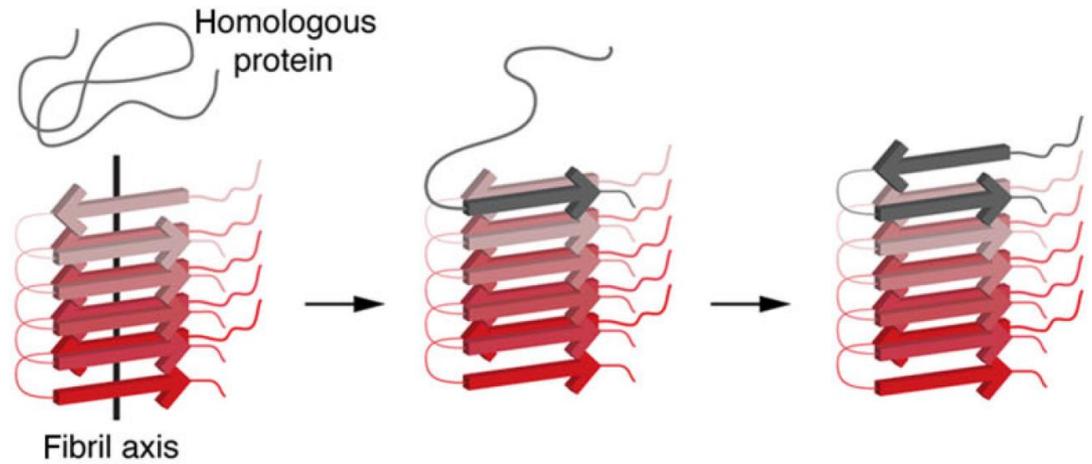
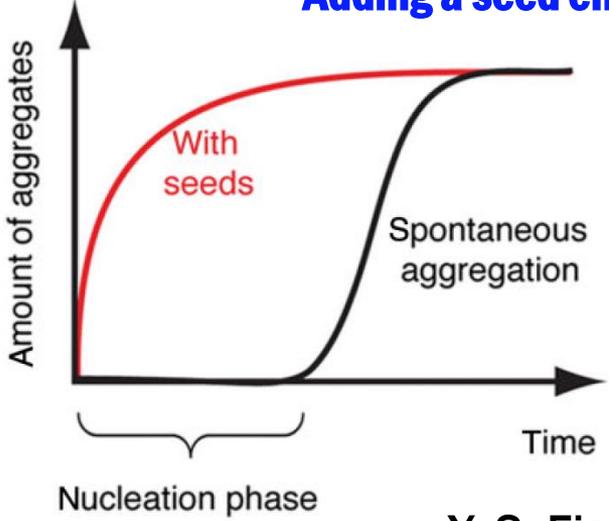
Buxbaum, J. N. et al. (2012) *J. Mol. Biol.* 421, 142–159

GROWTH CURVE AND EFFECT OF SEEDS ON LAG PHASE

M Jucker et al. (2013) Nature 501, 45-51



Adding a seed eliminates the fibril formation lag time

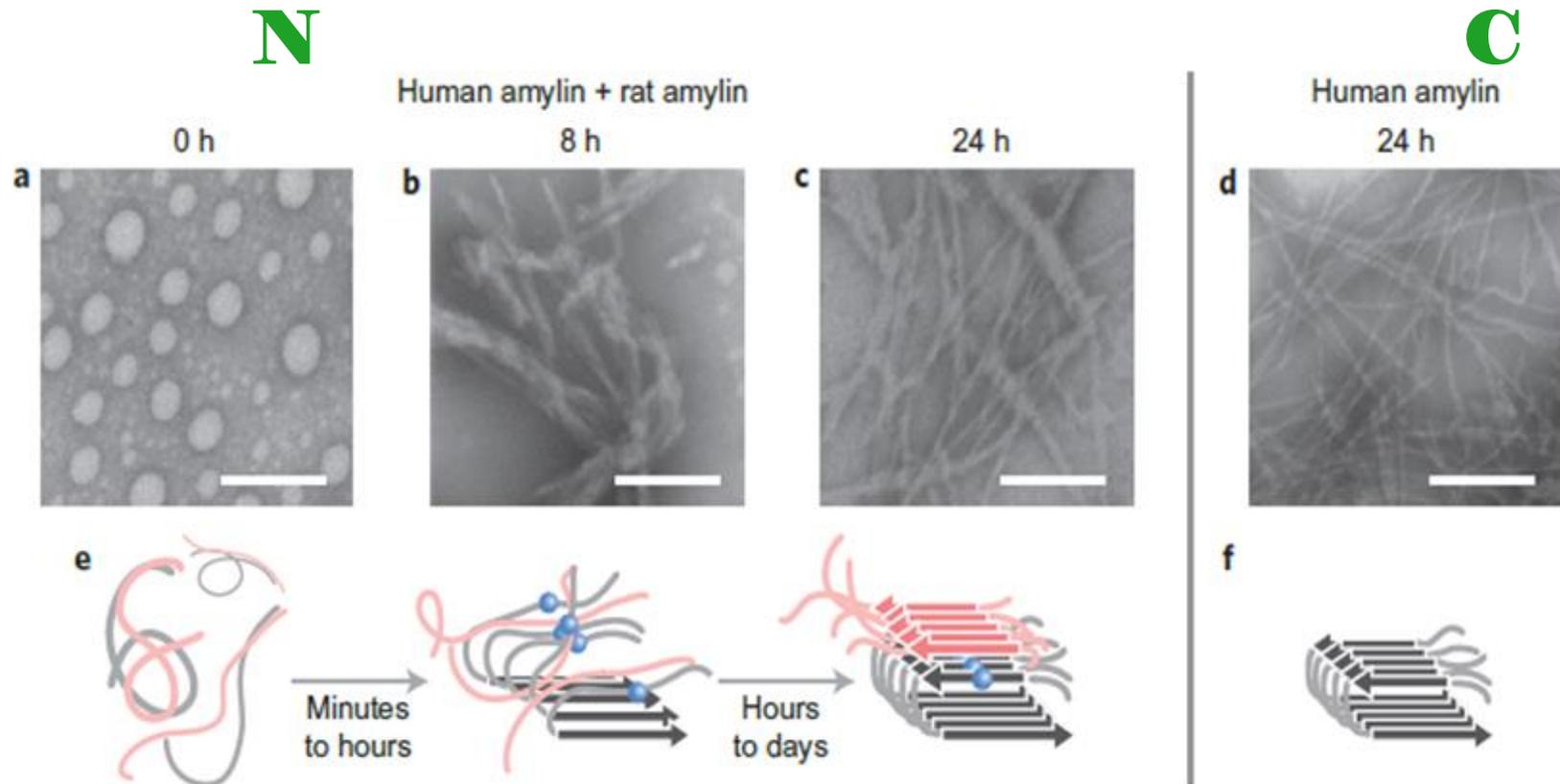


Y. S. Eisele (2013) Brain Pathology 23, 333-341

All-atom molecular modeling of weak amyloid-inhibiting properties of rat amylin

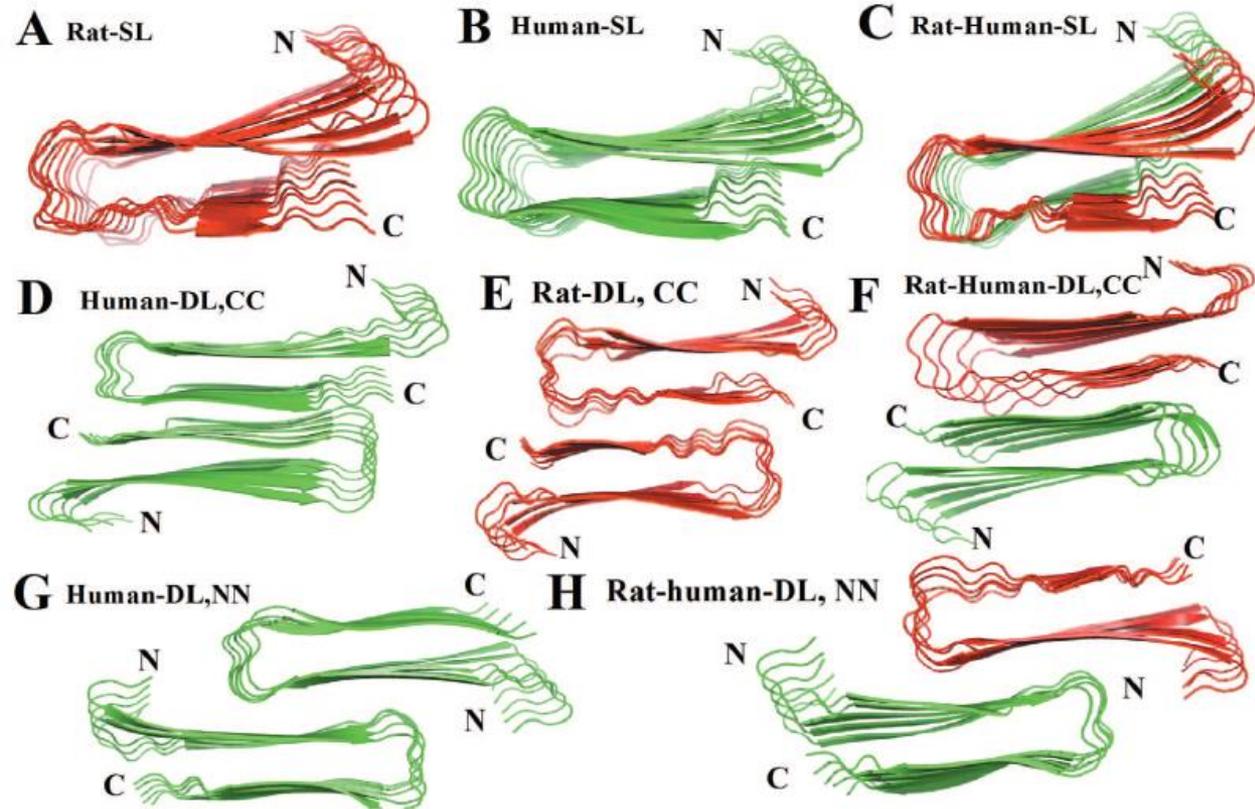
hIAPP $\left[\begin{array}{c} \text{S-S} \\ \text{KCNTATCATQ}^{10}\text{RLANFLVHSS}^{20}\text{NNFGAILSS}^{30}\text{NVGSNTY-NH}_2 \end{array} \right]$

rIAPP $\left[\begin{array}{c} \text{S-S} \\ \text{KCNTATCATQ}^{10}\text{RLANFLVRRSS}^{20}\text{NNLGPVLPPT}^{30}\text{NVGSNTY-NH}_2 \end{array} \right]$



Nature Chemistry (2012), 4, 355–360

MOLECULAR DYNAMICS SIMULATION PROTOCOL



The mixed oligomers can be

- longer proto-filament single layer (elongation)
- merged via either N or C-terminal contacts double layer (thickening),

Simulation parameters:

Software: GROMACS/4.5.5

Simulation: 3 runs, 300ns

Target pressure: 1bar (NPT)

Force field: ff99SB

Temperature: 310K

Explicit solvent: 3TIP3P

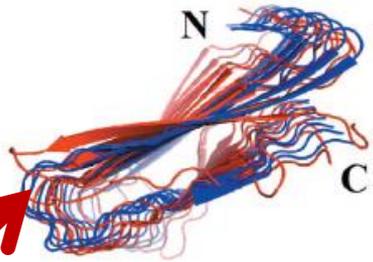
Analysis of the simulations: tools available in Gromac

Berhanu WM, Hansmann UHE (2014) PLoS ONE 9(5): e97051

Proline in C terminus makes N-terminal interface more stable

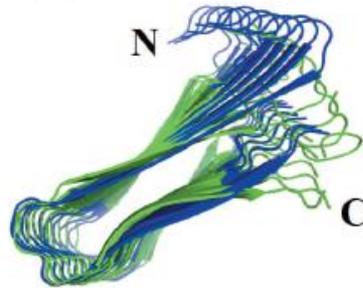
Single layer modes

(A) Rat-SL



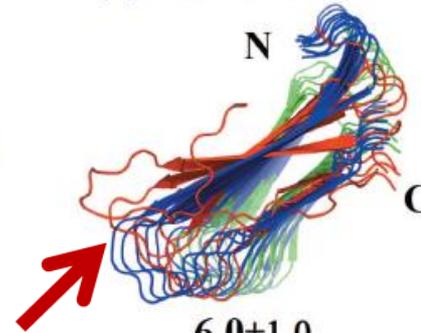
4.5 ± 0.6

(B) Human-SL



5.0 ± 0.4

(C) Rat-human-SL

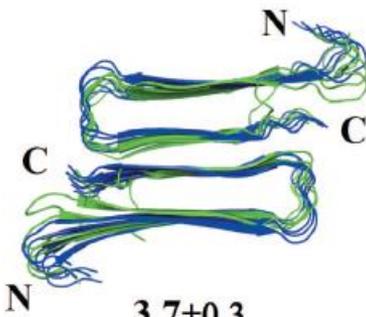


6.0 ± 1.0

Disturbance of U-shaped fibril topology in C-terminal of rat system due to pro

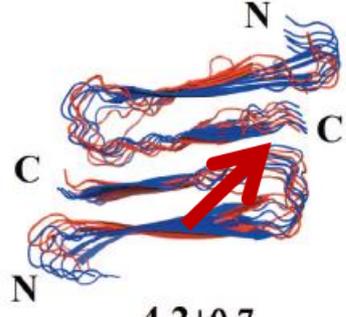
Double layer CC interface modes

(D) Human-DL, CC



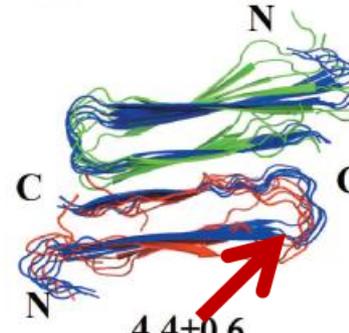
3.7 ± 0.3

(E) Rat-DL, CC



4.2 ± 0.7

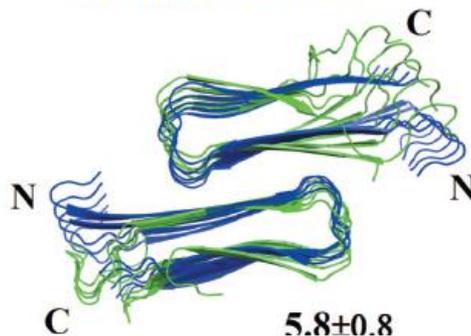
(F) Rat-human-DL, CC



4.4 ± 0.6

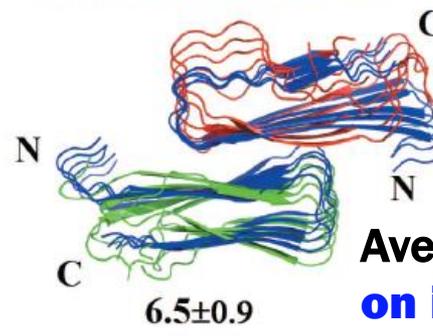
Double layer NN interface modes

(G) Human-DL, NN



5.8 ± 0.8

(H) Rat-human-DL, NN

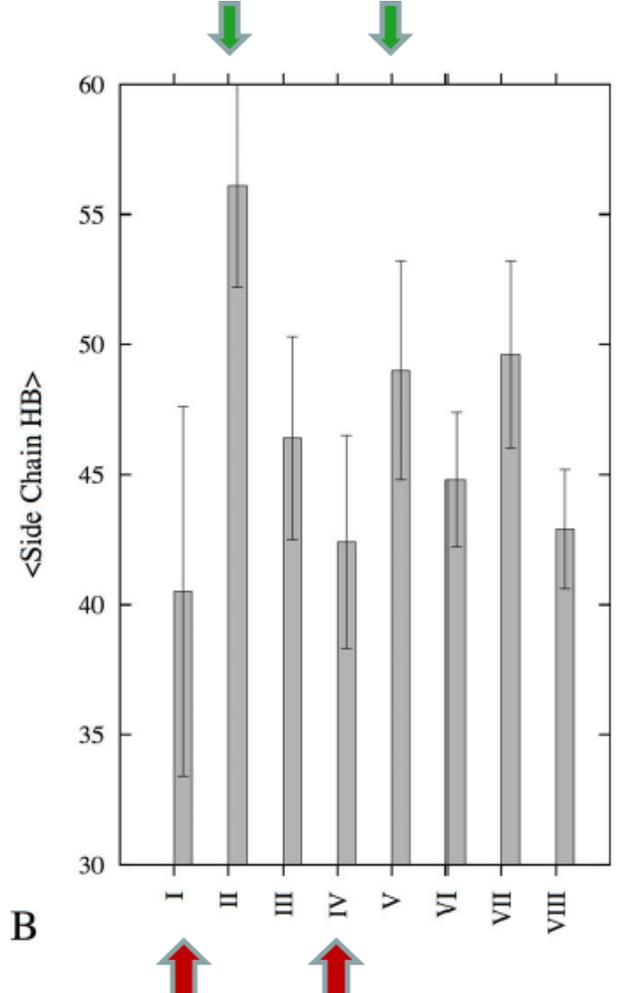
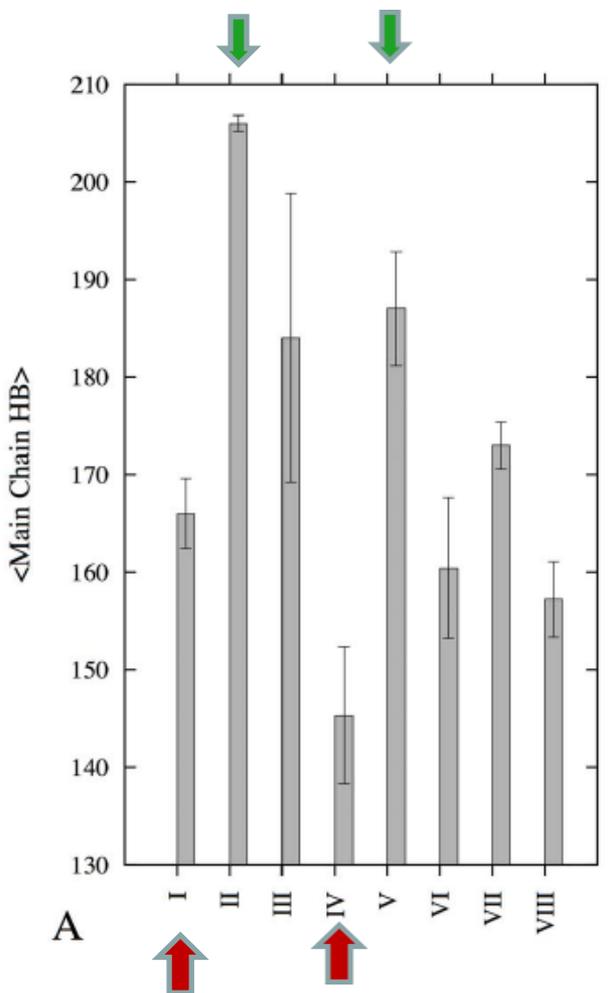


6.5 ± 0.9

Average structures superimposed on initial structure

Berhanu WM, Hansmann UHE (2014) PLoS ONE 9(5): e97051

HYDROGEN BONDING NETWORK STABILIZES THE AGGREGATES

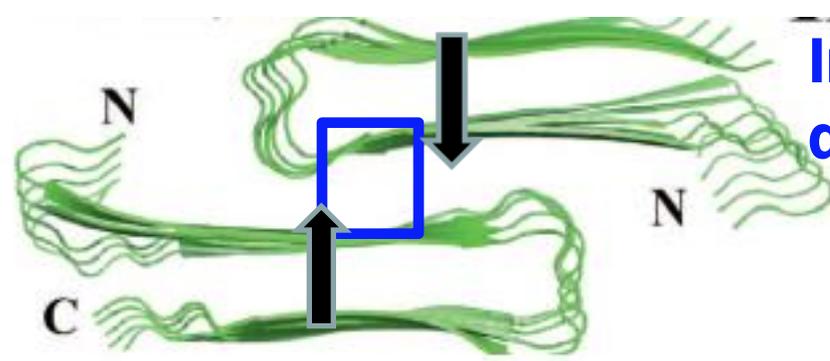


- I) SL Rat
- II) SL Hum
- III) SL mix
- IV) DL rat
- V) DL Hum-CC
- VI) DL Mix CC
- VII) DL Hum NN
- VIII) DL Mix NN

• Both inter- and intra-molecular hydrogen bond are less in rat and mixed aggregate models

Inter-sheet distance in the NN-interface double layer models are comparable

Similar hydrophobic contacts and shape complementarity



$\langle F_{15}/V_{17} \rangle$	Human amylin oligomer
	Sh ₁ -St ₂ /Sh ₂ -St ₂ *
Run1	8.5 (0.4)
Run2	8.5 (0.4)
Run3	8.4 (0.5)
Mean±SD	8.5±0.1

	Human-rat amylin complex
	Sh ₁ -St ₂ /Sh ₂ -St ₂
	10.0 (0.3)
	9.2 (0.4)
	8.8 (0.4)
	9.3±0.6

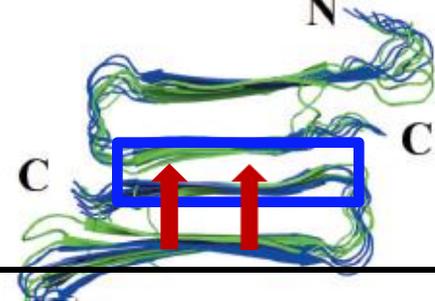
$\langle V_{17}/F_{15} \rangle$	Sh ₁ -St ₂ /Sh ₂ -St ₂ *
Run1	8.7 (0.5)
Run2	10.0 (0.5)
Run3	9.2 (0.8)
Mean±SD	9.3±0.6

	Sh ₁ -St ₂ /Sh ₂ -St ₂
	10.3 (0.3)
	10.5 (0.6)
	11.6 (0.4)
	10.8±0.7

C to C interface distances

Short distance = strong & favorable interactions

larger distance = signal for unfavorable contacts



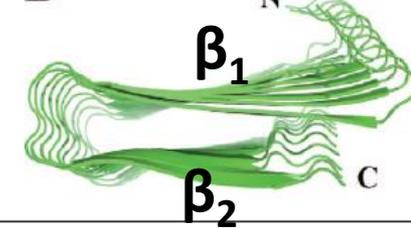
$\langle L_{27}/G_{33} \rangle$	Human amylin oligomer			$\langle N_{31}/S_{29} \rangle$	Sh ₁ -St ₂ /Sh ₂ -St ₂	Sh ₁ -St ₃ /Sh ₂ -St ₃	Sh ₁ -St ₄ /Sh ₂ -St ₄
	Sh ₁ -St ₂ /Sh ₂ -St ₂ *	Sh ₁ -St ₃ /Sh ₂ -St ₃	Sh ₁ -St ₄ /Sh ₂ -St ₄				
Run1	7.3 (0.3)	7.3 (0.2)	7.4 (0.3)	Run1	5.8 (0.2)	5.7 (0.2)	5.9 (0.3)
Run2	7.2 (0.2)	7.4 (0.2)	7.3 (0.2)	Run2	5.7 (0.2)	6.1 (3.1)	6.9 (0.4)
Run3	7.1 (0.4)	7.2 (0.3)	7.3 (0.3)	Run3	5.6 (0.2)	5.7 (0.3)	6.0 (0.3)
Mean±SD	7.2±0.1	7.3±0.1	7.3±0.1	Mean±SD	5.7±0.1	5.8±0.6	6.3±0.2

L_{27}/G_{33}	Rat amylin oligomer			Sh ₁ -St ₂ /Sh ₂ -St ₂	Sh ₁ -St ₃ /Sh ₂ -St ₃	Sh ₁ -St ₄ /Sh ₂ -St ₄	
	Sh ₁ -St ₂ /Sh ₂ -St ₂	Sh ₁ -St ₃ /Sh ₂ -St ₃	Sh ₁ -St ₄ /Sh ₂ -St ₄				
Run1	9.7 (0.6)	10.0 (0.7)	10.9 (1.1)	Run1	7.0 (0.4)	7.1 (0.4)	7.4 (0.3)
Run2	9.6 (0.4)	9.4 (0.5)	10.3 (0.8)	Run2	7.2 (0.4)	7.0 (0.3)	7.0 (0.4)
Run3	9.8 (1.5)	9.8 (1.7)	10.5 (2.1)	Run3	7.6 (0.7)	9.0 (0.8)	9.3 (0.9)
	9.7±0.1	9.7±0.3	10.6±0.3		7.3±0.5	7.7±1.1	7.9±1.2

L_{27}/G_{33}	Mixed human-rat amylin oligomer			Sh ₁ -St ₂ /Sh ₂ -St ₂	Sh ₁ -St ₃ /Sh ₂ -St ₃	Sh ₁ -St ₄ /Sh ₂ -St ₄	
	Sh ₁ -St ₂ /Sh ₂ -St ₂	Sh ₁ -St ₃ /Sh ₂ -St ₃	Sh ₁ -St ₄ /Sh ₂ -St ₄				
Run1	8.7 (1.6)	8.2 (0.8)	7.6 (0.5)	Run1	6.9 (0.4)	6.7 (0.4)	6.7 (0.4)
Run2	10.2 (0.9)	10.1 (0.7)	9.7 (0.7)	Run2	6.6 (0.5)	6.1 (0.5)	6.2 (0.5)
Run3	10.3 (0.6)	8.7 (0.5)	9.7 (0.4)	Run3	6.9 (0.4)	7.0 (0.3)	7.1 (0.3)
	9.8±0.9	9.0±1.0	9.0±1.2		6.8±0.5	6.6±0.5	6.7±0.3

fewer residue contacts , result in poor packing at the interfaces

Rat and mixed SL and DL-CC models have less perfect U-shape and less populated β -structure than Mixed DL-NN

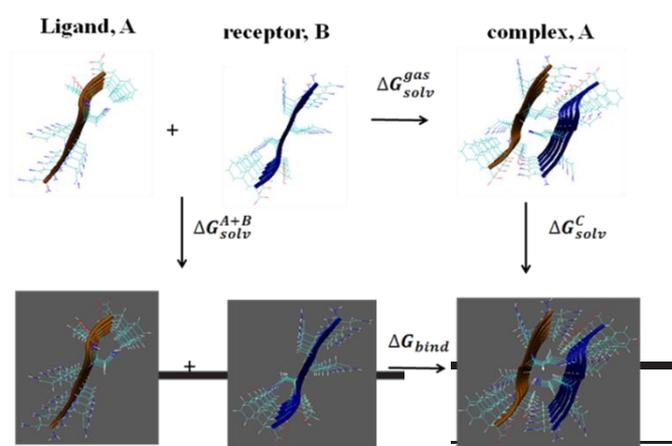


β_1 segment, N terminal (residue 8-17)	Secondary structure, first 100 ns		Secondary structure, last 100 ns	
	β -sheet*	Turn*	β -sheet*	Turn*
Rat-SL	81.7 (6.0)	18.3 (6.0)	81.9 (4.0)	18.1 (3.0)
Human-SL	81.4 (5.0)	18.6 (5.0)	79.4 (3.0)	21.6 (3.0)
Rat-human-SL	84.6 (5.0)	15.4 (5.0)	83.6 (5.0)	16.4 (5.0)
Rat-DL, CC →	78.2 (2.0)	21.8 (2.0)	77.1 (1.0)	22.9 (1.0)
Human-DL, CC	87.8 (2.0)	12.2 (2.0)	87.7 (1.0)	12.3 (1.0)
Rat-Human-DL, CC →	77.0 (6.0)	23.0 (6.0)	77.6 (3.0)	22.4 (3.0)
Human-DL, NN	85.0 (3.0)	15.0 (3.0)	82.7 (1.0)	17.3 (1.0)
Rat-Human-DL, NN →	87.7 (3.0)	12.3 (3.0)	86.0 (8.0)	14.00 (8.0)

NN hybride higher β -structure

β_2 segment, C terminal (residue 28-37)	β -sheet	Turn	β -sheet	Turn
	Rat-SL	45.3 (2.0)	54.7 (2.0)	42.6 (1.0)
Human-SL	66.7 (4.0)	32.7 (3.0)	65.2 (5.0)	34.8 (5.0)
Rat-human-SL	56.9 (4.0)	43.1 (4.0)	54.7 (4.0)	45.3 (3.0)
Rat-DL, CC →	46.0 (8.0)	54.0 (9.0)	43.9 (1.1)	56.1 (10)
Human-DL, CC	69.2 (3.0)	29.8 (3.0)	66.9 (8.0)	33.1 (8.0)
Rat-Human-DL, CC →	55.9 (2.0)	44.1 (1.0)	54.4 (2.0)	46.6 (11.0)
Human-DL, NN	61.0 (6.0)	39.0 (7.0)	49.4 (7.0)	50.6 (7.0)
Rat-Human-DL, NN →	52.7 (1.0)	47.3 (2.0)	46.0 (10.0)	54.0 (10.0)

BINDING FREE ENERGY OF SINGLE LAYER AND DOUBLE LAYER :THE MIXED STRUCTURE WITH NN INTERFACE MORE STABLE



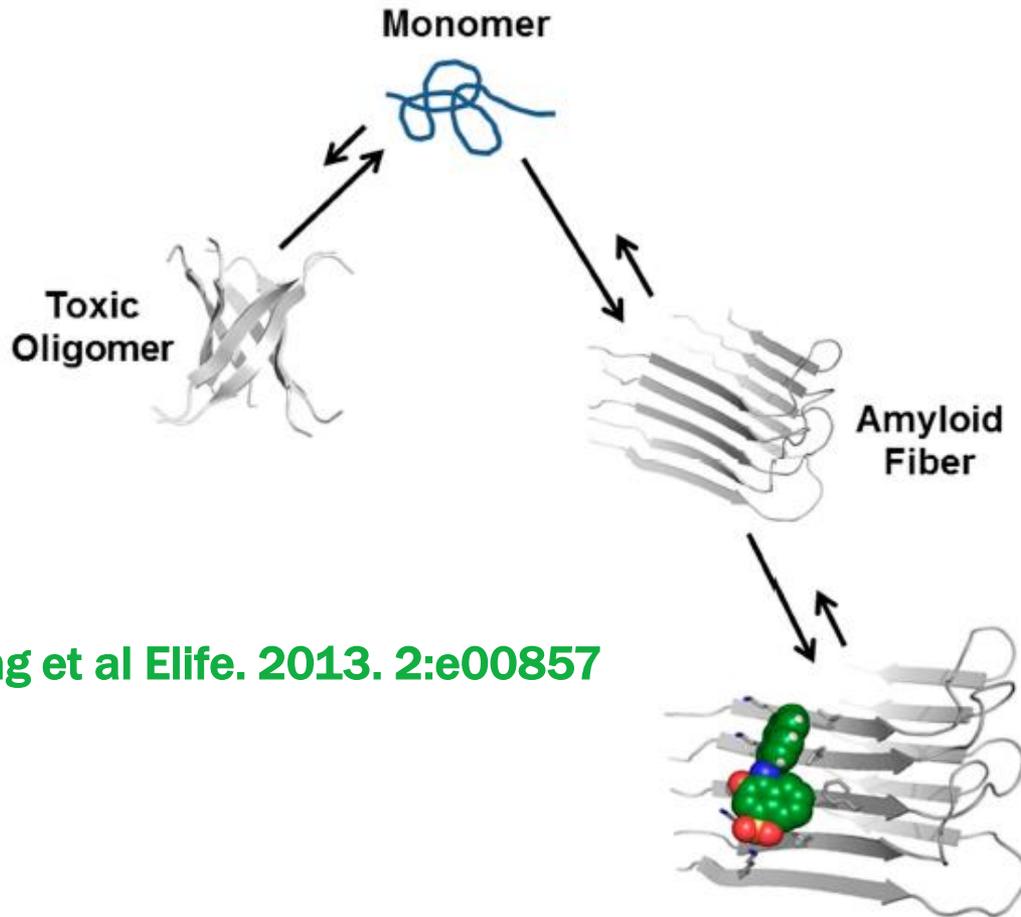
$$\Delta G_{\text{binding}} = \Delta E_{\text{vdw}} + \Delta E_{\text{elec}} + \Delta E_{\text{PB}} + \Delta E_{\text{SA}}$$

Structures	ΔE_{vdw}	ΔE_{elec}	ΔE_{PB}	ΔE_{SA}	$\Delta G_{\text{binding}}$
Rat-SL	-184.1 ± 1.7	2117.8 ± 45.4	-2043.0 ± 33.8	-106.7 ± 1.7	-2.6 ± 2.8
Human-SL	-188.9 ± 3.0	1042 ± 93.5	-1007.0 ± 91.6	-108.0 ± 0.4	-45.7 ± 0.9
Rat-Human-SL	-185.6 ± 3.6	1527.9 ± 35.3	-1478.9 ± 44.9	-109.8 ± 0.5	-26.8 ± 8.7
Rat-DL, CC	-326.5 ± 64.5	1774.9 ± 39.3	-1665.0 ± 54.6	-202.9 ± 33.3	-13.7 ± 5.9
Human-DL, CC	-233.6 ± 24.7	432.6 ± 34.4	-393.6 ± 33.8	-124.3 ± 1.3	-70.2 ± 15.9
Human-DL, NN	-330.2 ± 4.8	395.1 ± 26.5	-318.7 ± 30.9	-196.2 ± 2.7	-57.7 ± 2.3
Rat-Human-DL, NN	-420.6 ± 16.2	1259.0 ± 49.3	-1143.0 ± 51.5	-255.3 ± 5.5	-49.2 ± 8.6

Cross-seeding is possible & likely to through NN interface

HYDROPHOBIC (β_1 and β_2) CORE ARE IMPORTANT

- β -region are crucial for stability and growth of aggregate
- should be considered as a potential target for design



Jiang et al Elife. 2013. 2:e00857

Amylin

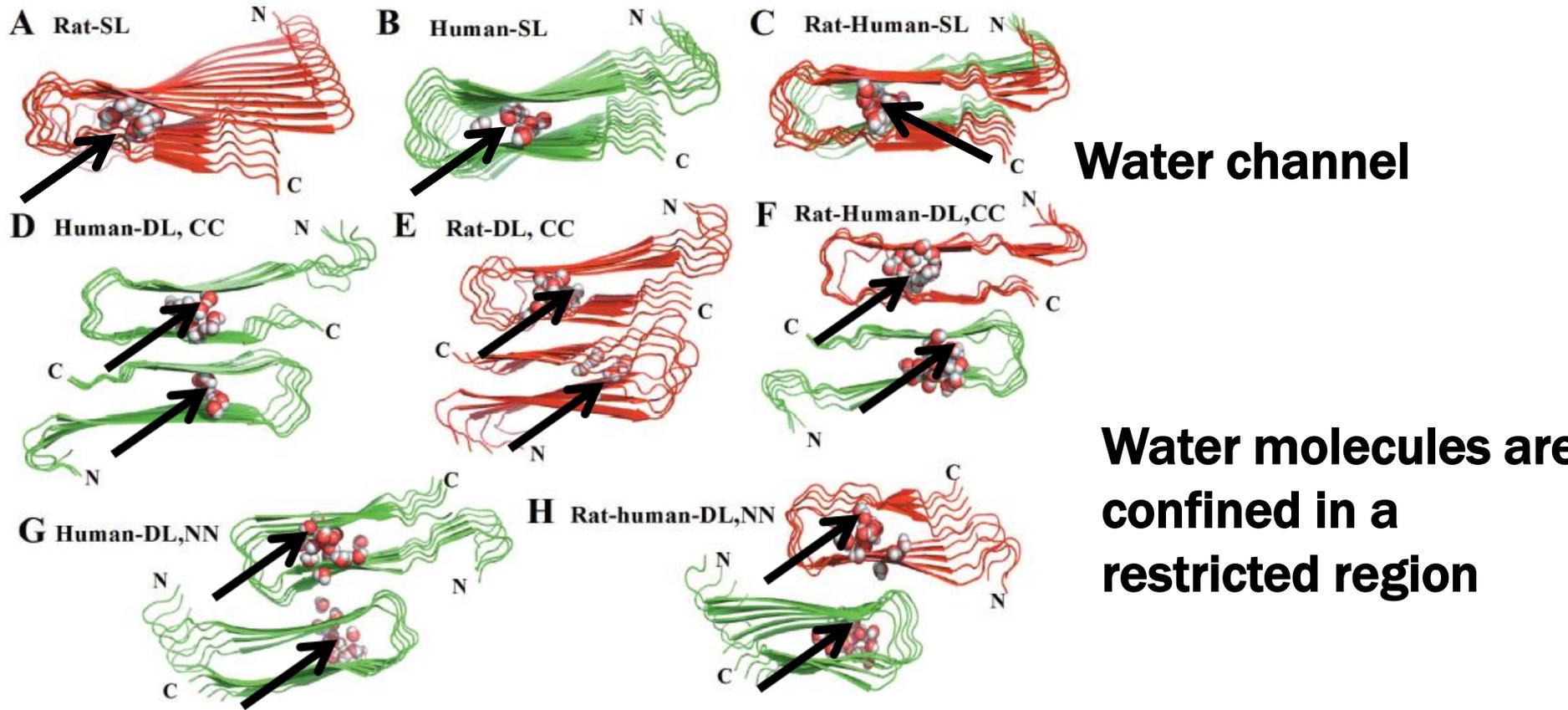
L₁₃ANFL₁₇

A₂₅ILSS₂₉

hetero-assembly

all of the above

WATER PERMEATION THROUGH FIBRIL-LIKE OLIGOMER COULD ACCOUNT FOR CYTOTOXICITY



- Experimentally determined fibril does not contain water molecules
- Simulation reveals hydrophilic water channel at different location
- Water flow was observed even in the cross-seeded oligomer

CONCLUSIONS

- **The β -hairpin motif is partially lost in presence of proline in rat with CC interface, \longrightarrow making NN interface in mixed complex**
- **Binding free energy show aggregate growth is favorable for human amylin and rat-human NN interface cross-seeded oligomers**
- **Hydrophobic cores in β -sheet regions are crucial for stability and elongation**
 - **Could be target for SBDD of aggregation inhibitors**
- **Water molecules flowing internally in homo-polymers and hetero-polymers explains the toxicity of human and rat amylin**

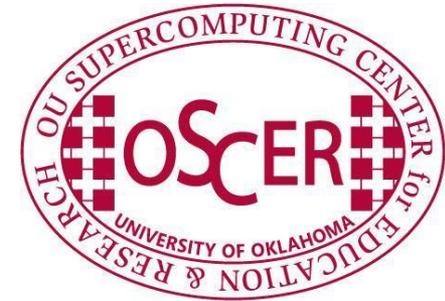
ACKNOWLEDGEMENT

Prof Ulrich H. E. Hansmann

All group members

We thank OSCER and NERSC
for providing supercomputer
time

NIH (grant GM62838)



Thank You !