The Abdus Salam
International Centre for Theoretical Physics

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Educational, Scientific and
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IAEA
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SMR/1845-15

Conference on Structure and Dynamics in Soft Matter and Biomolecules: From Single Molecules to Ensembles

4 - 8 June 2007

Congregatio NEC Ordinatio
Proline and glycine control protein self-aggregation into amyloid or elastomer fibrils

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Molecular Structure and Function
Hospital for Sick Children
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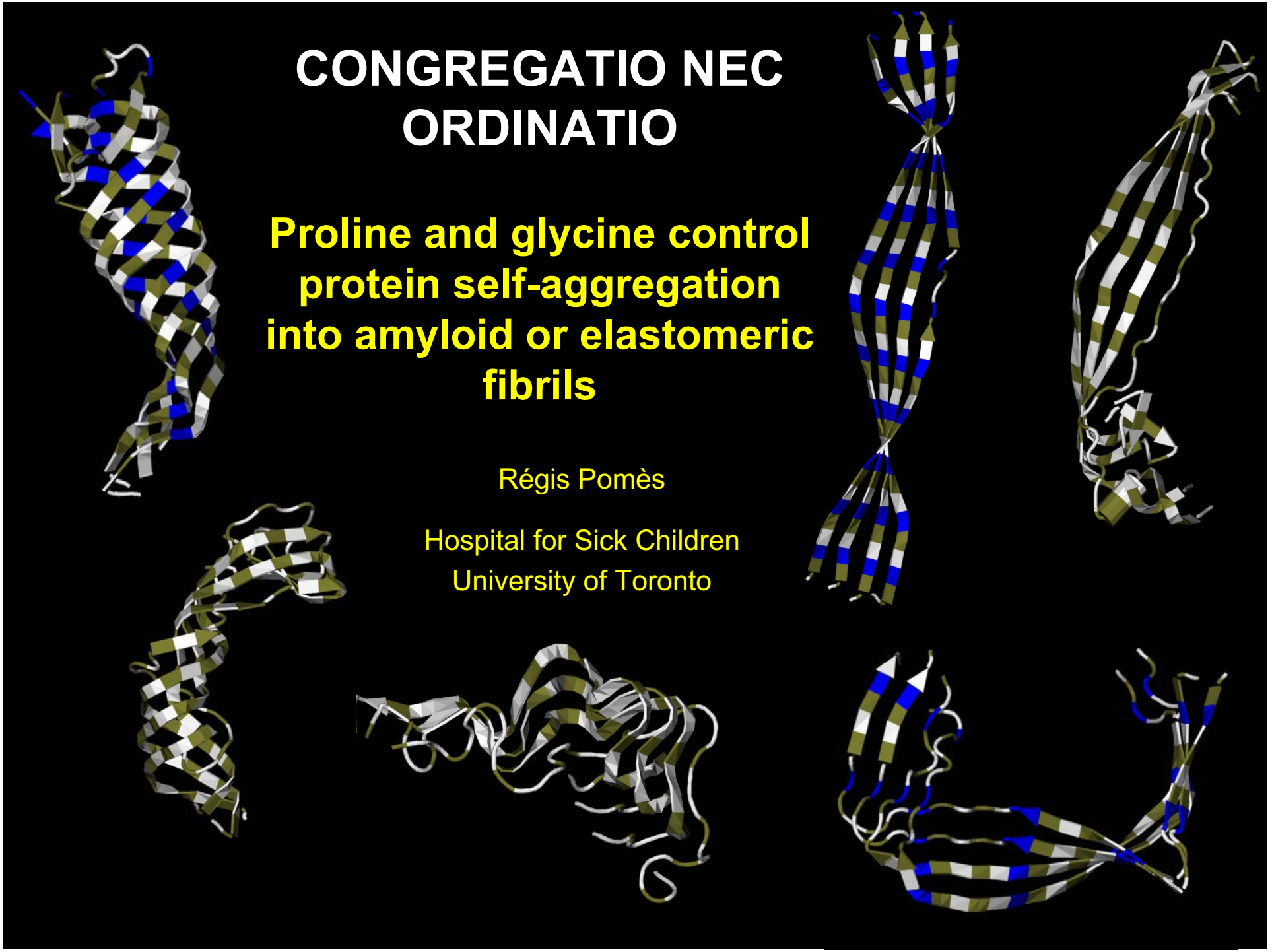
CONGREGATIO NEC ORDINATIO

Proline and glycine control protein self-aggregation into amyloid or elastomeric fibrils

Régis Pomès

Hospital for Sick Children
University of Toronto




Nilu Chakrabarti
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Rowan Henry
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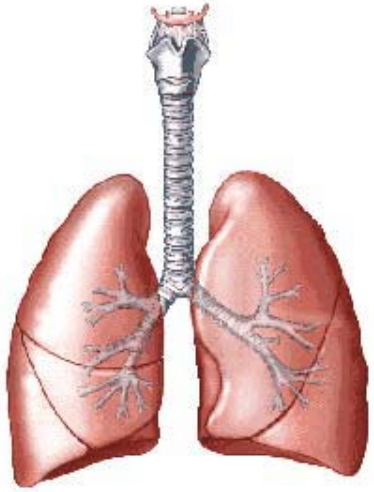
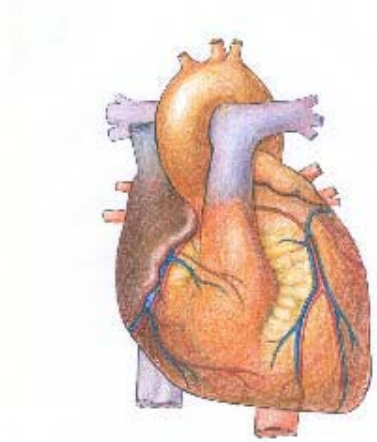
Sarah Rauscher
Grace Li
Elisa Fadda
Régis Pomès
Collaborators: Fred Keeley, Ming Miao. $$: NSERC, HSFO, SickKids

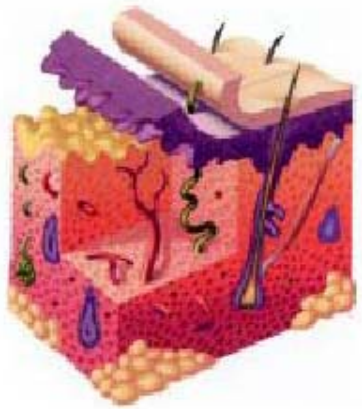
Biological Role of Elastin



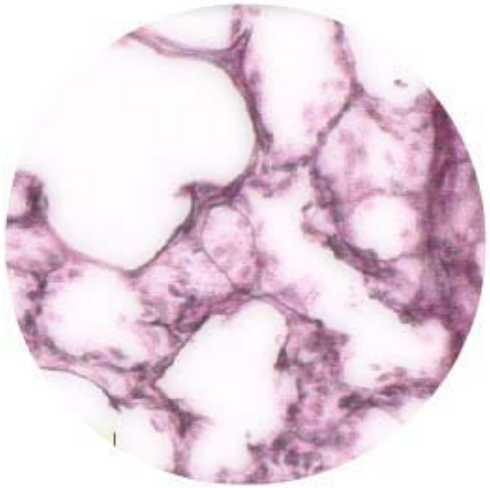
Lungs

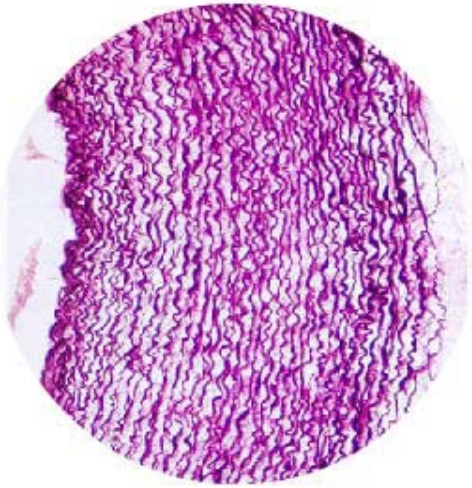


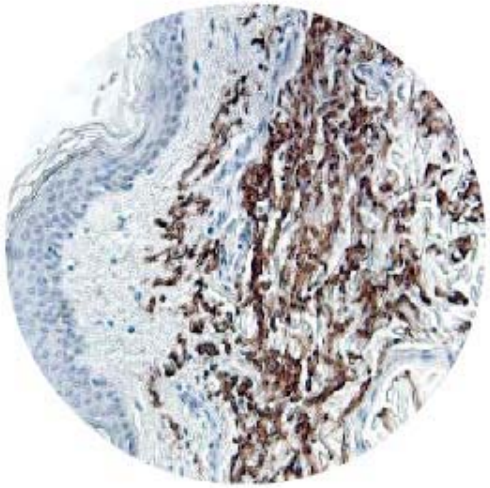
Arteries



Skin

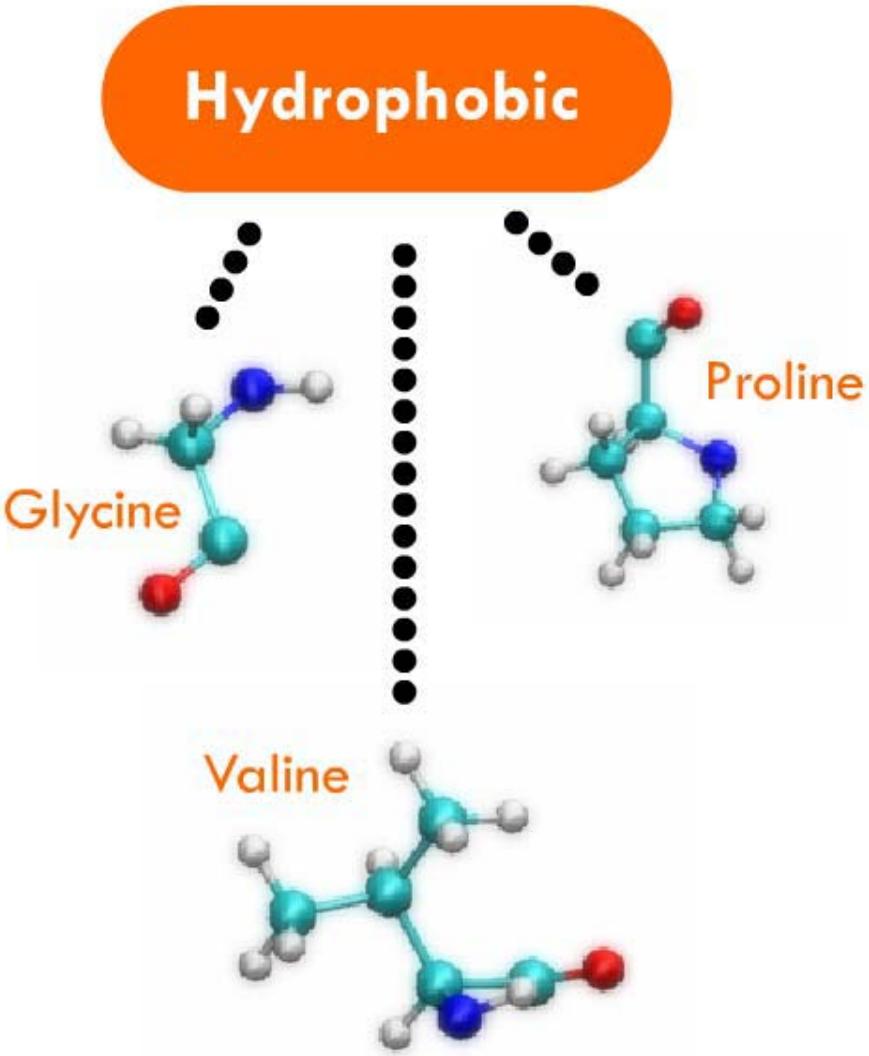


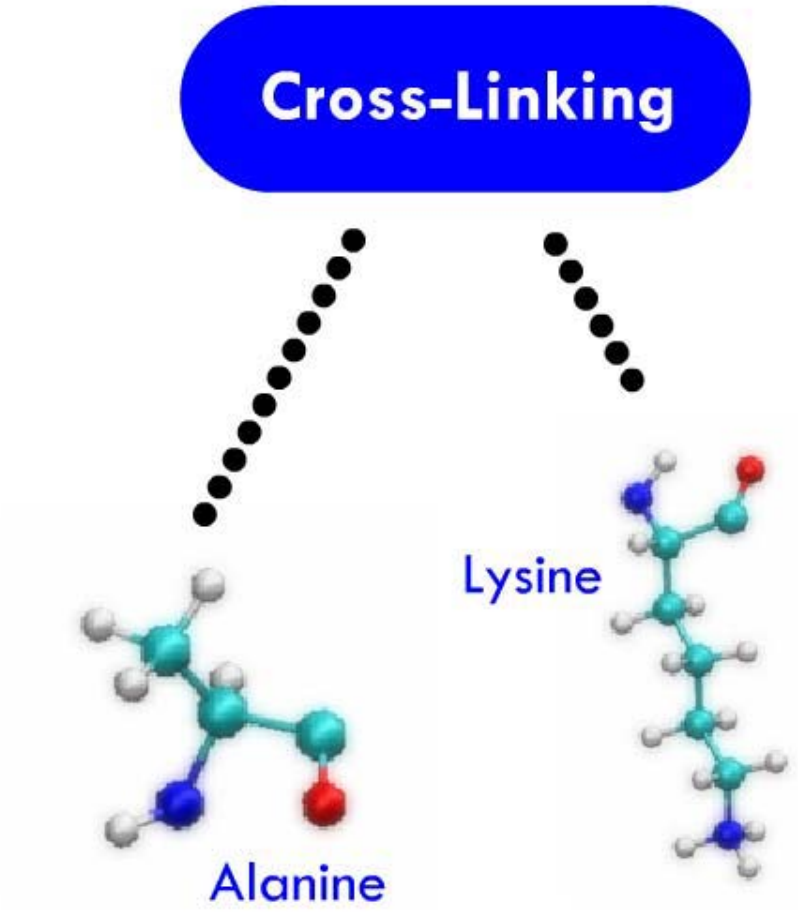




Sequence of Elastin

Elastin has two types of domains:
1. cross-linking
2. hydrophobic

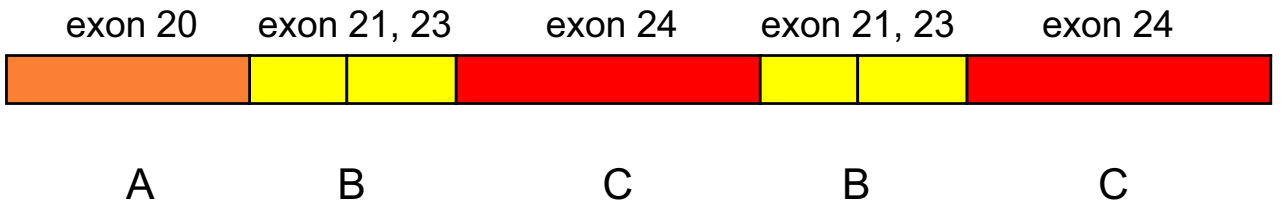




Synthesizing biomimetic materials uising recombinant methods

Reference Elastin-Like Polypeptide (EP20-24-24)

EP 20-24-24



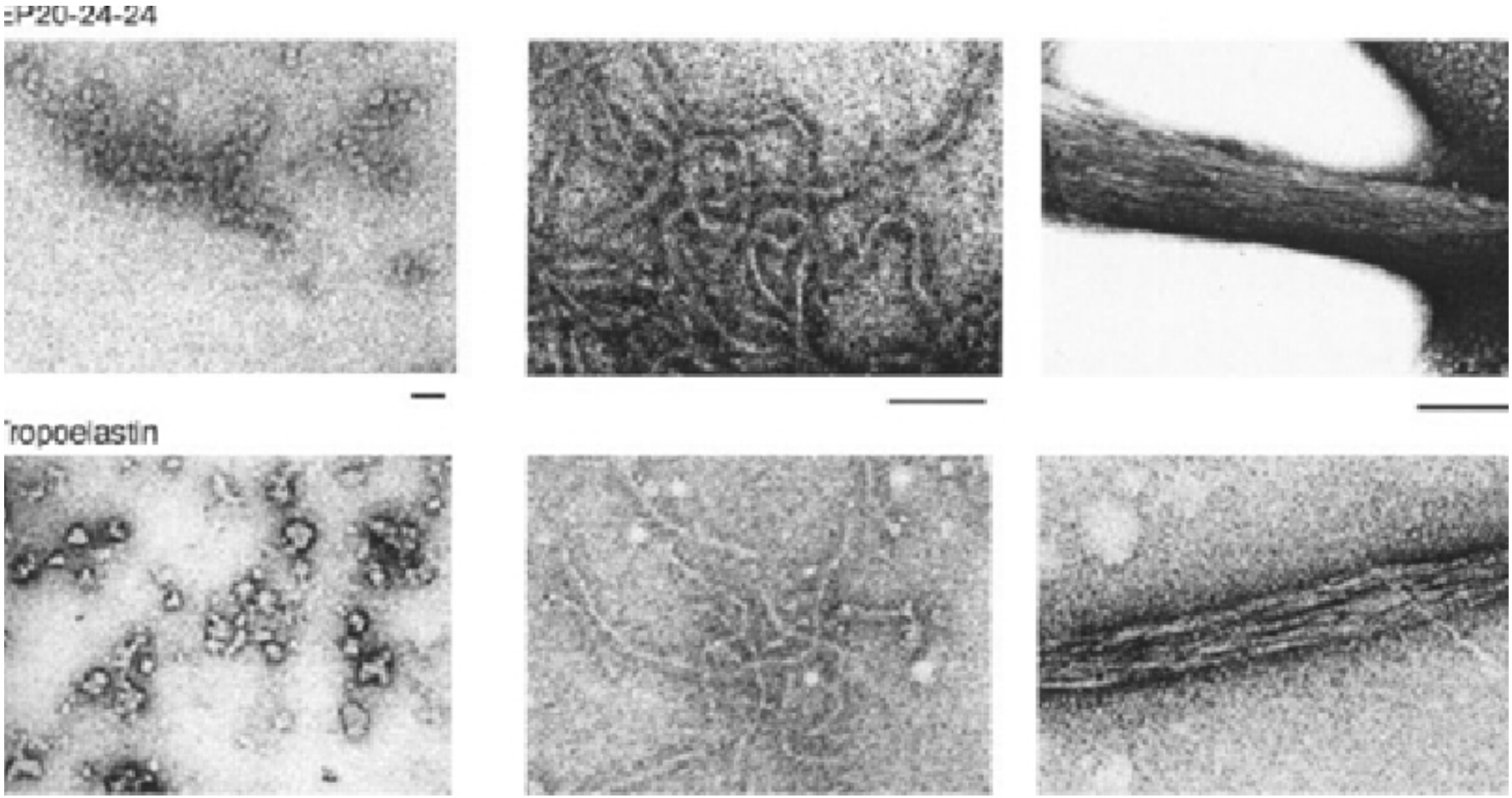
MW: 16,992 Da

Exon 20: FPGFGVGVGGIPGVAGVPGVGGVPGVGGVPGVGI

Exon 21, 23: PEAQAAAAAKAAKYGVGTPAAAAAKAAAKAAQF

Exon 24: GLVPGVGVAPGVGVAPGVGVAPGVGLAPGVGVAPGVGVAPGVGVAPAIGP

Elastin spontaneously self-aggregates into biomimetic materials



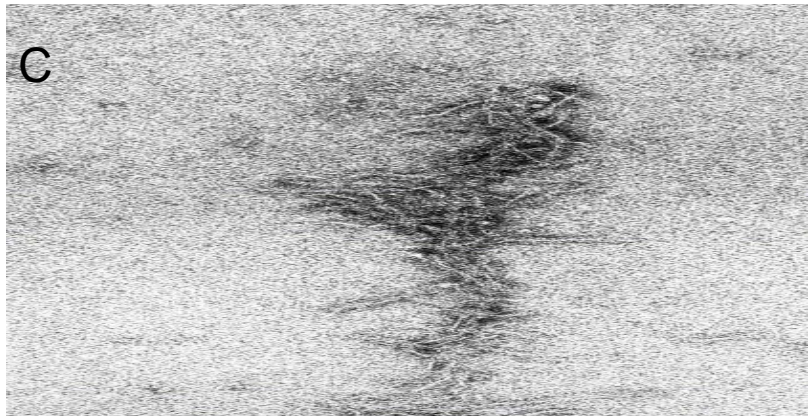
Bellingham, C. et al (2003) Biopolymers. 70. 445-455.

A

coacervation

Tc

B

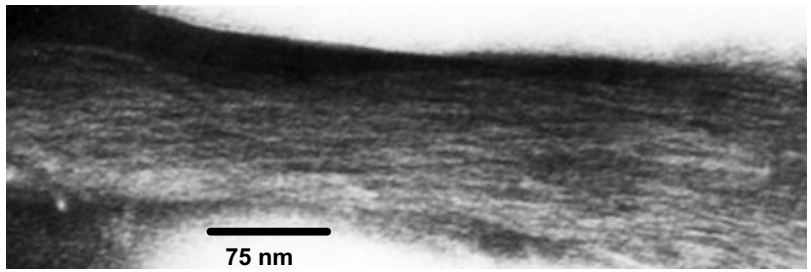


C

10 min. post-coacervation

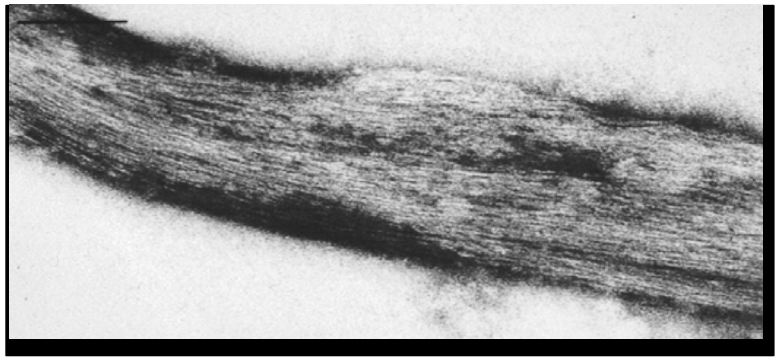
spontaneous maturation

D



EP20-24-24

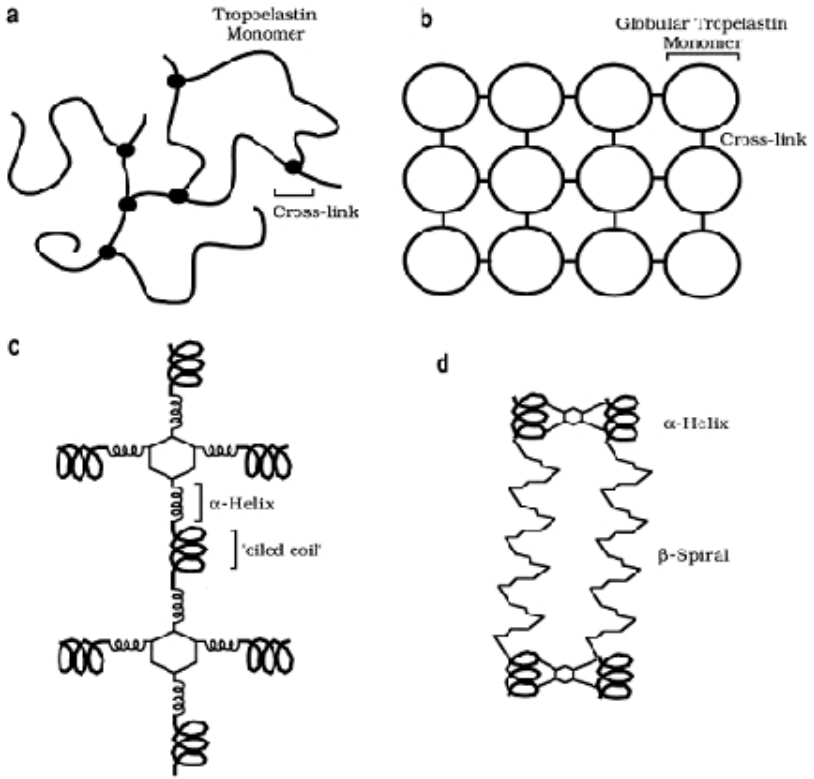
E



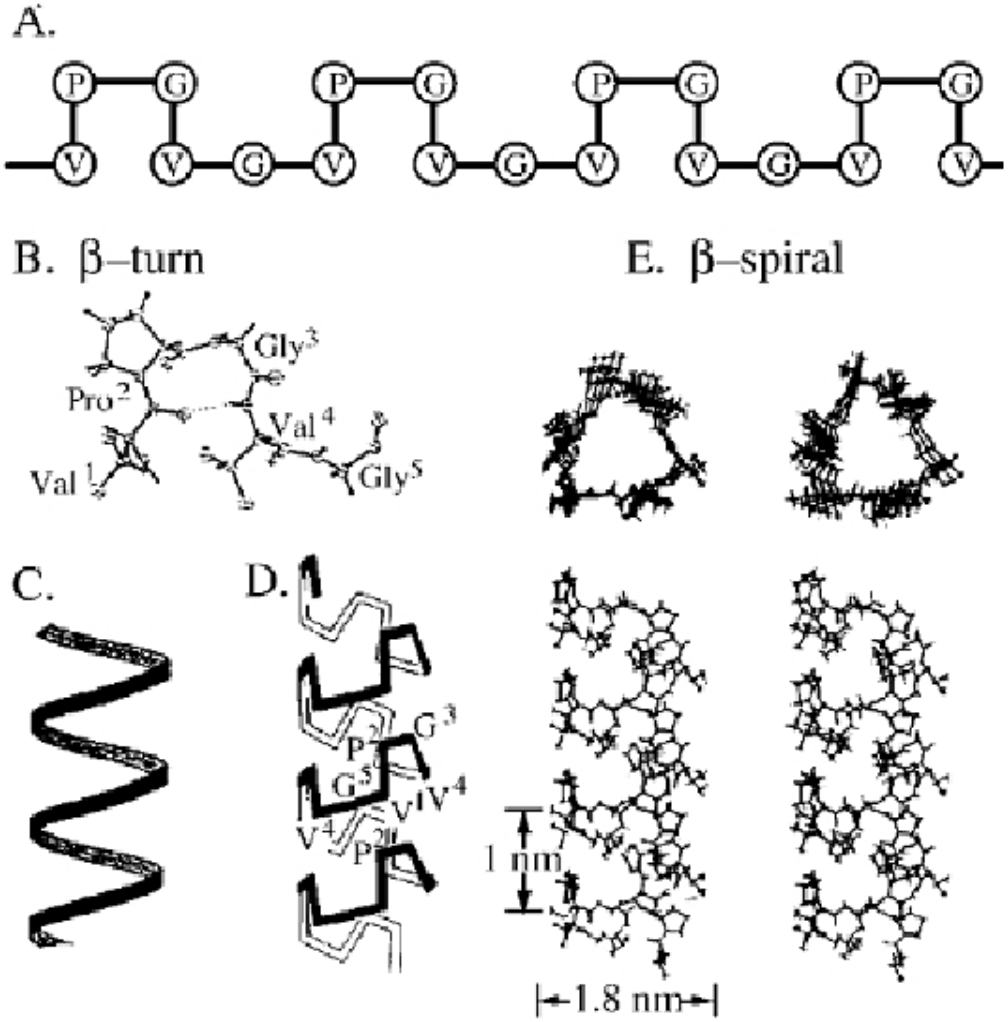
full-length tropoelastin

Recombinant polypeptides based on sequences of human and chicken elastin self-assemble and exhibit elastin-like extension and elastic recoil

Structural Models of Elastin



Vrhovski, B. and A. Weiss. 1998. Eur. J. Biochem. 258. 1-18.



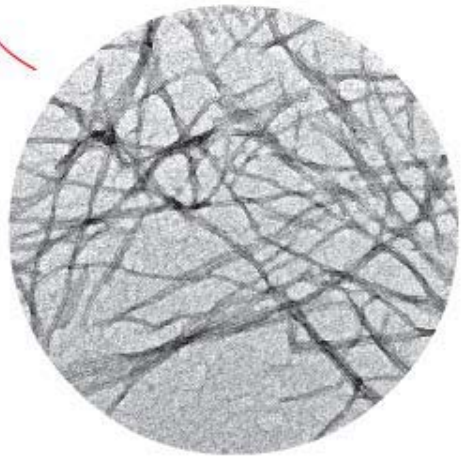
Urry, D. and Parker, T. 2002. Journal of Muscle Research and Cell Motility. 23. 543-559.

Biological Role of Amyloid Fibrils

Amyloid Diseases:

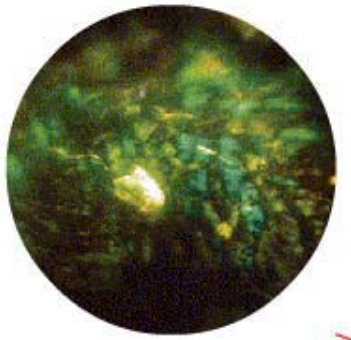
Parkinson's Disease
BSE (Mad Cow)
Type II Diabetes

Alzheimer's Disease



A Protective Amyloid:

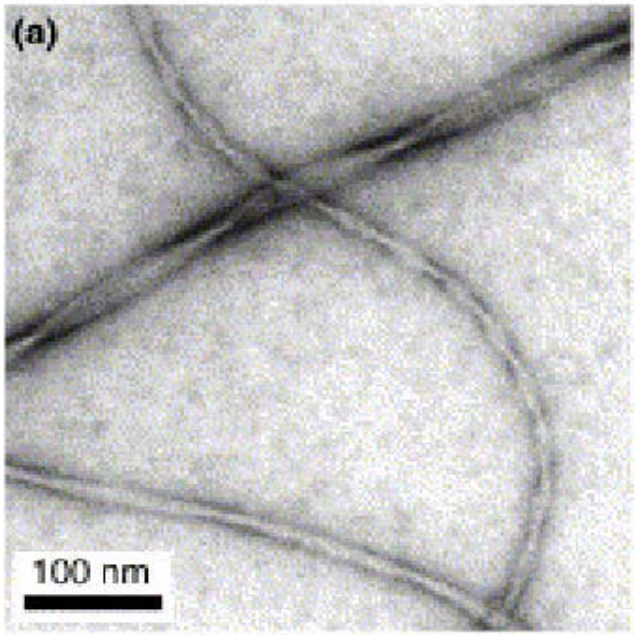
There is also a positive role for amyloids in nature

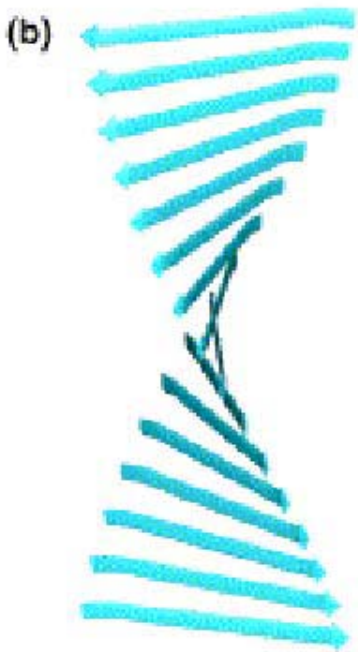


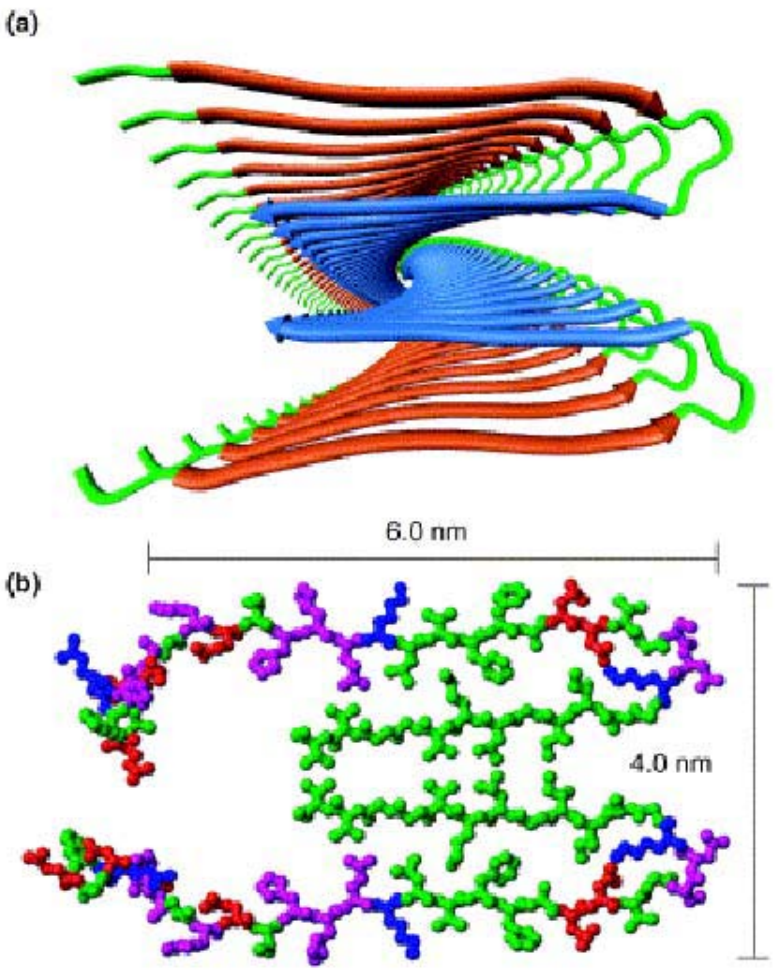
Silk Moth
Chorion Protein

Hamodrakas,S. 2003. Journal of Structural Biology. 145. 226-235.

Structural Models of Amyloids: Cross-β Sheets







-beta strands running nearly perpendicular to the long axis of the fibre

-backbone H-bonding parallel to the long axis

Current Opinion in Structural Biology
14 , 2004 , 96-103

The hydrophobic domains of elastin are pseudo-periodic

Human Exon 20:

GARPGVGVGGIPTYGVGAGGFPGFGVGVGGIPGVAGVPSVGGVPGVG
GVPGVGIS

Human Exon 24:

GLVPGVGVAPGVGVAPGVGVAPGVGLAPGVGVAPGVGVAPGVGVAPG
IGPGGVA

Chicken Exon 20:

GAGVPGVGVPGVGIGGVPGVPGVPGVPGVPGVPGVPGVPGVPGVPG
VPGVPGVVPGVGV

Chicken Exon 24:

GAGRVPGVGVPGAVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPG
VGVPGVGVPGVGVPGVGVPGVGVPGVGVPG

Sequence Motifs:

PGVPGV

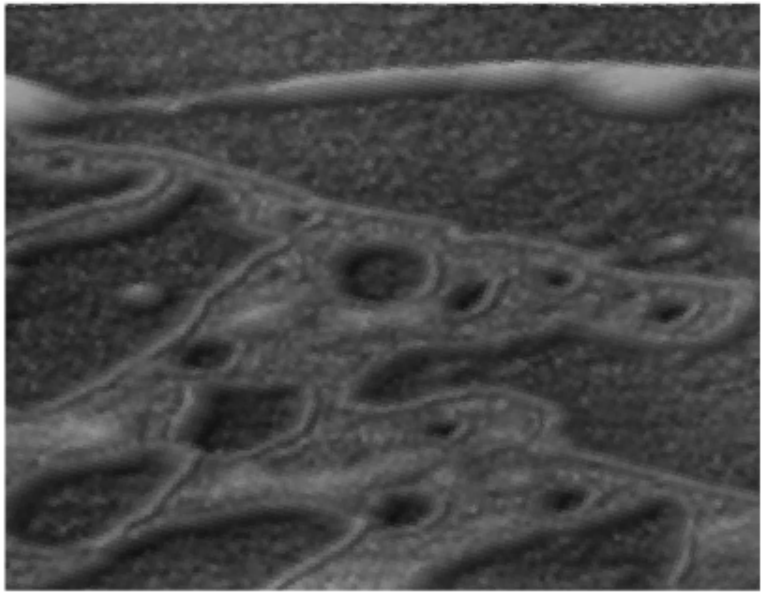
PGVGGV

PGV GV

PGV GVA

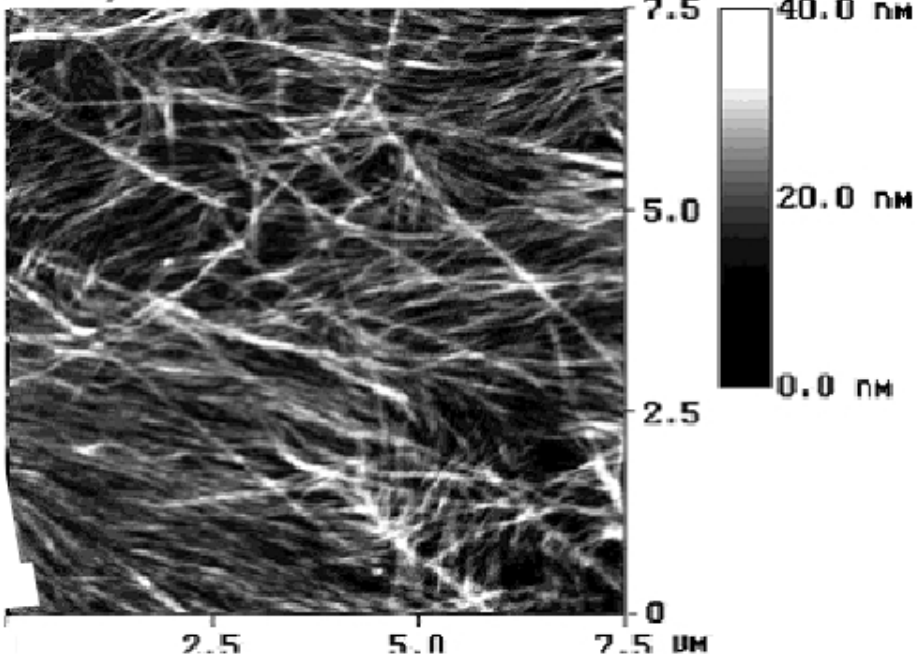
GGVGV can make either elastin or amyloid

Elastin

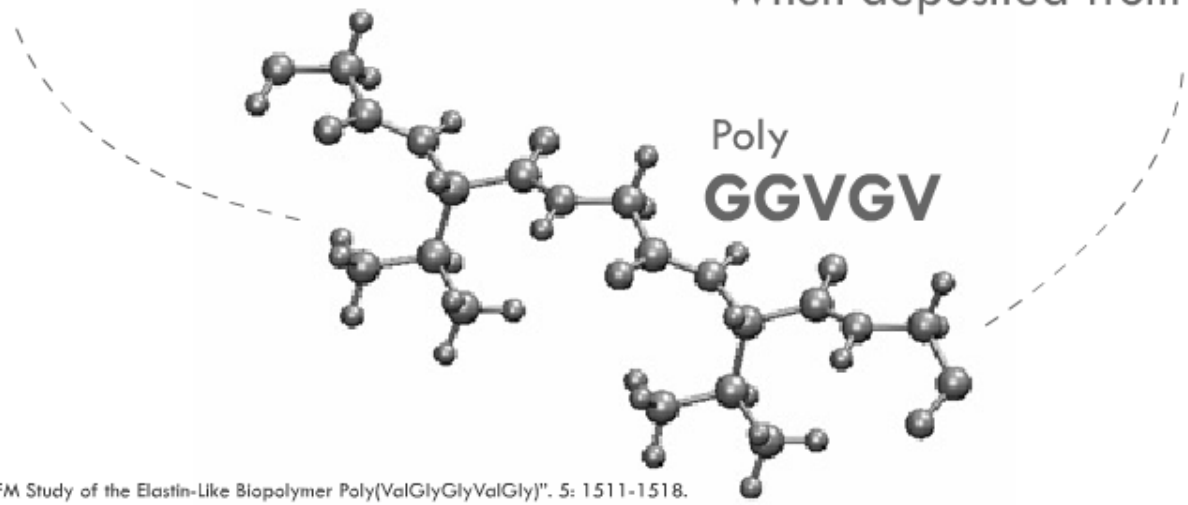


When deposited from methanol

Amyloid



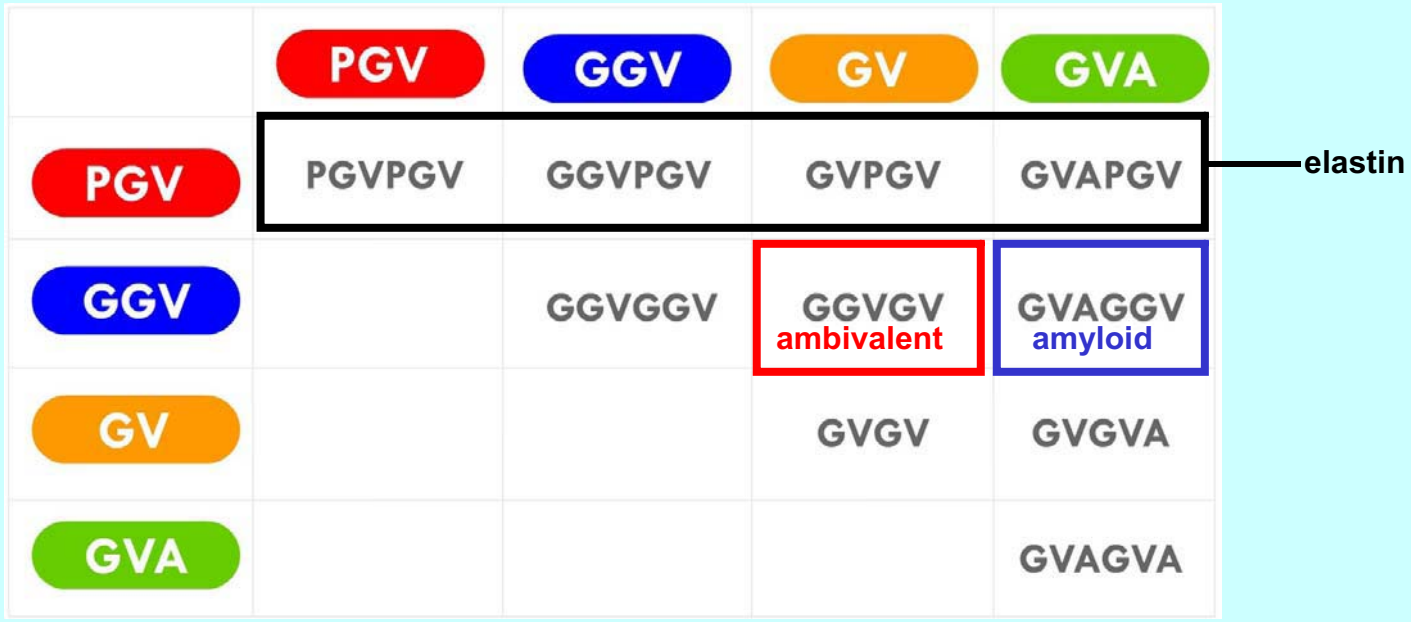
When deposited from aqueous solution



Poly
GGVGV

Flamia, R. et al. (2004) " AFM Study of the Elastin-Like Biopolymer Poly(ValGlyGlyValGly)". 5: 1511-1518.

Minimalist approach: a ten-suspect line up



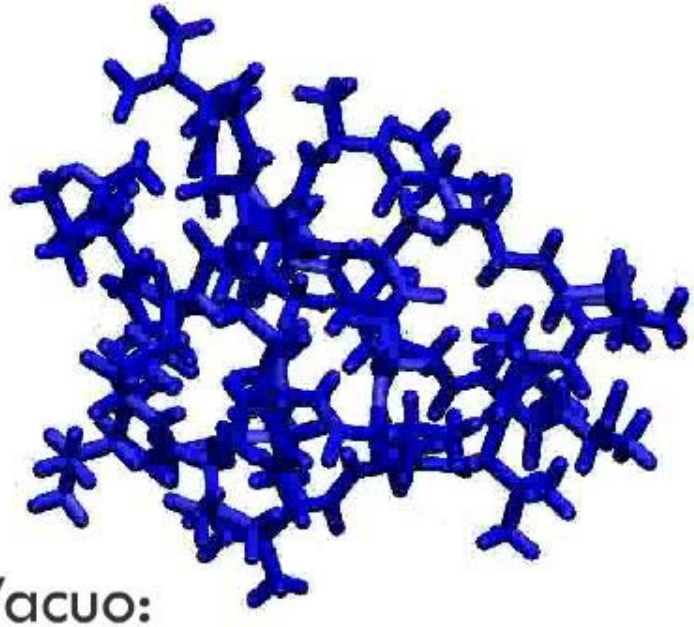
Study periodic repeats of these motifs:

$(GVAGGV)_6$, $(GVPGV)_7$…

→ simplify sequence to isolate properties

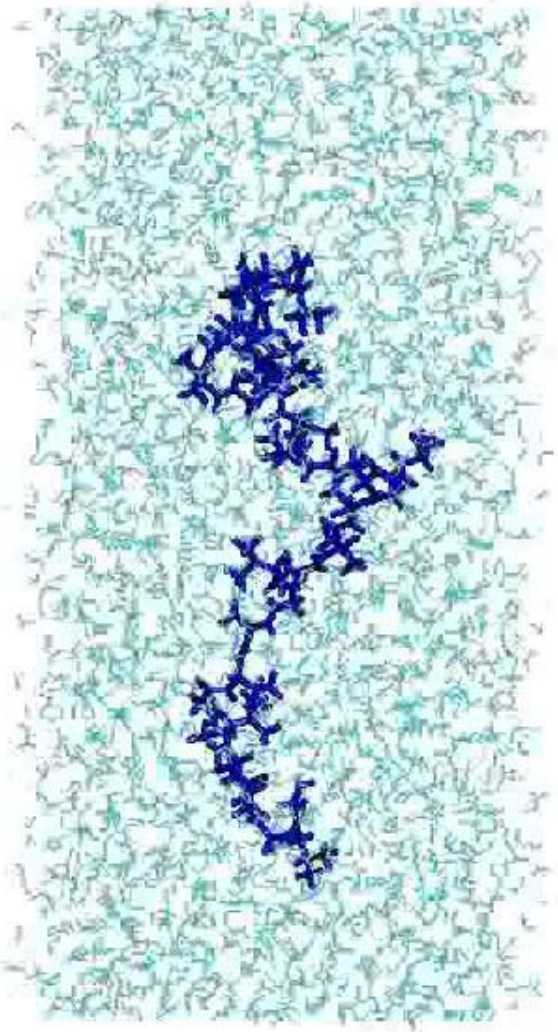
→ amplify SNR

Molecular Dynamics



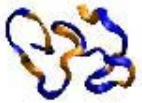
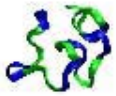
In Vacuo:
~400 atoms

N,V,T
Empirical force field (OPLS-AA)
GROMACS MD Simulation Package



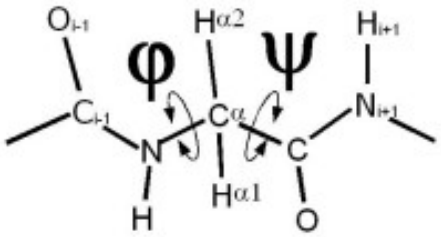
In Water
~9000 atoms

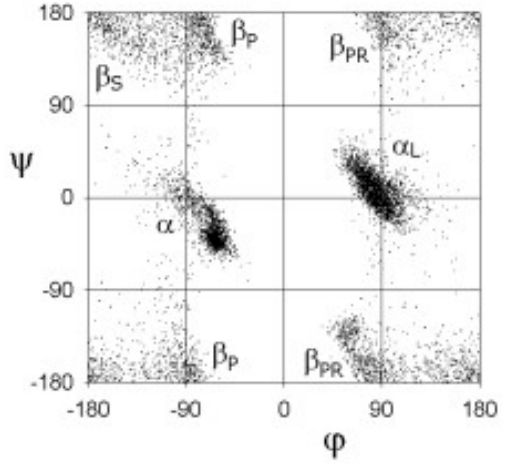
Monomers in Water

	PGV	GGV	GV	GVA
PGV	PGVPGV 	GGVPGV 	GVPGV 	GVAPGV 
GGV		GGVGGV 	GGVGV 	GVAGGV 
GV			GVGV 	GVGVA 
GVA				GVAGVA 

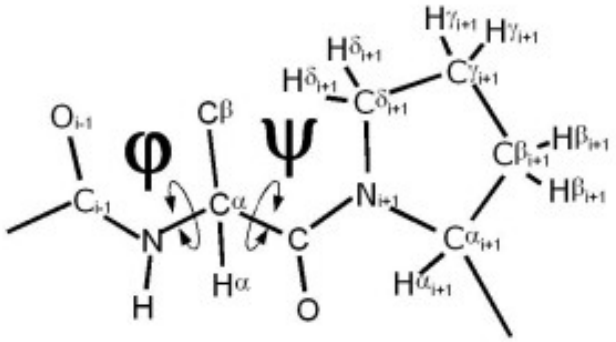
Inherent Plasticity of the Backbone

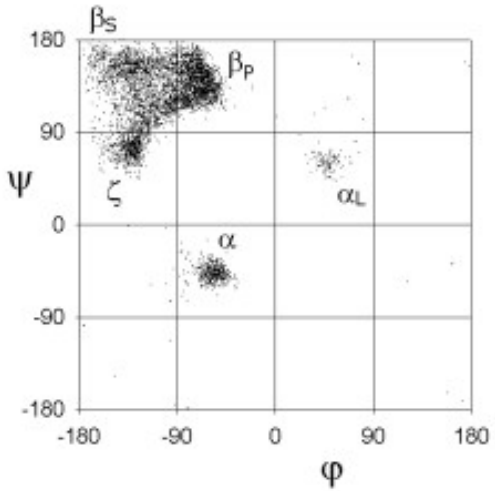
A



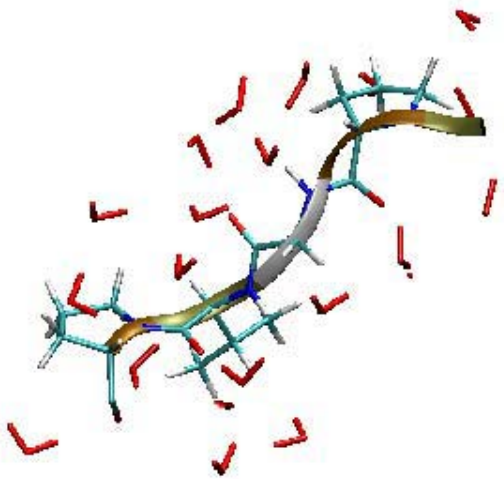


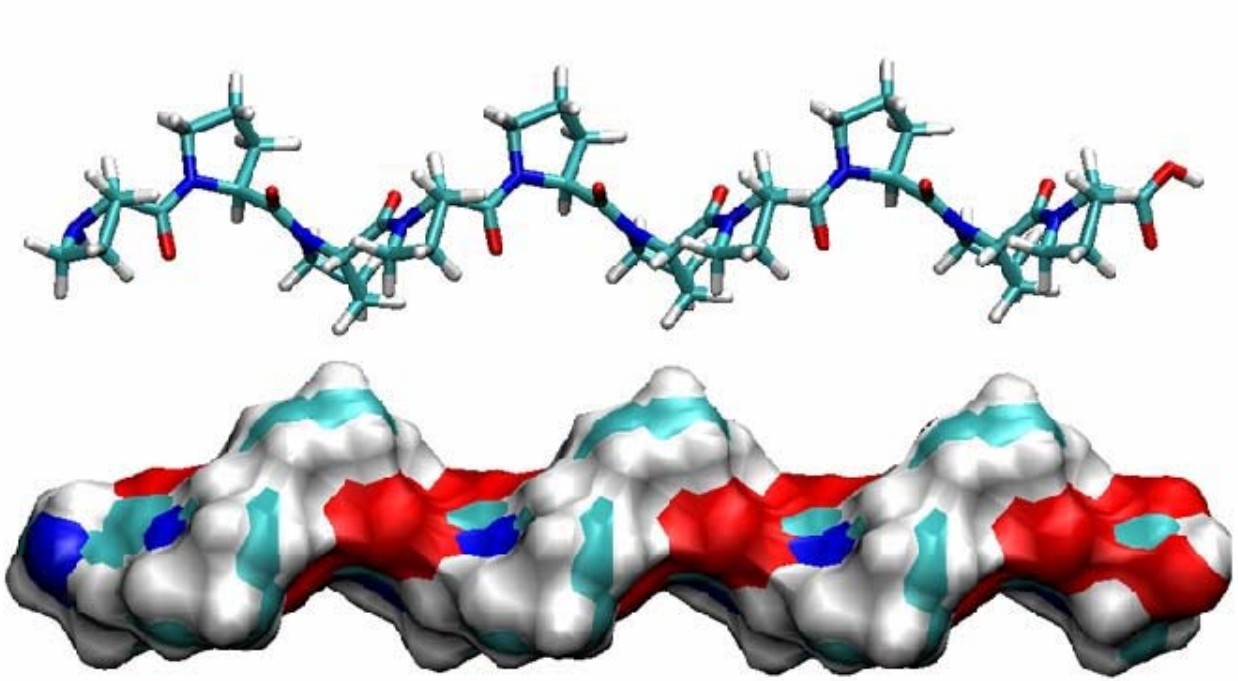
B

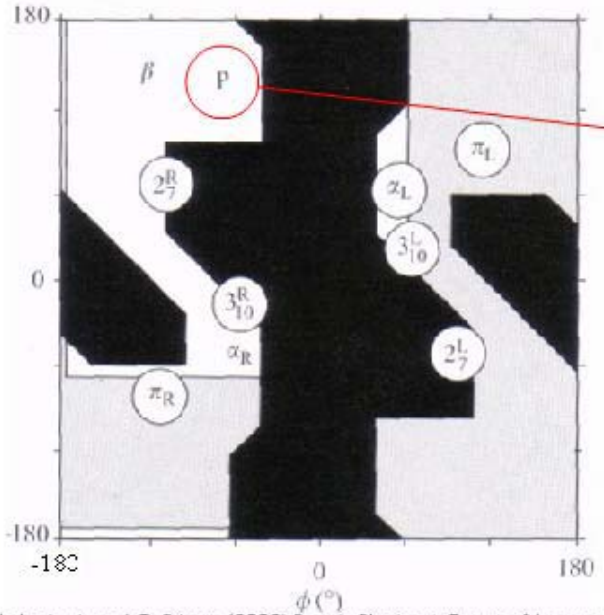




Polyproline II





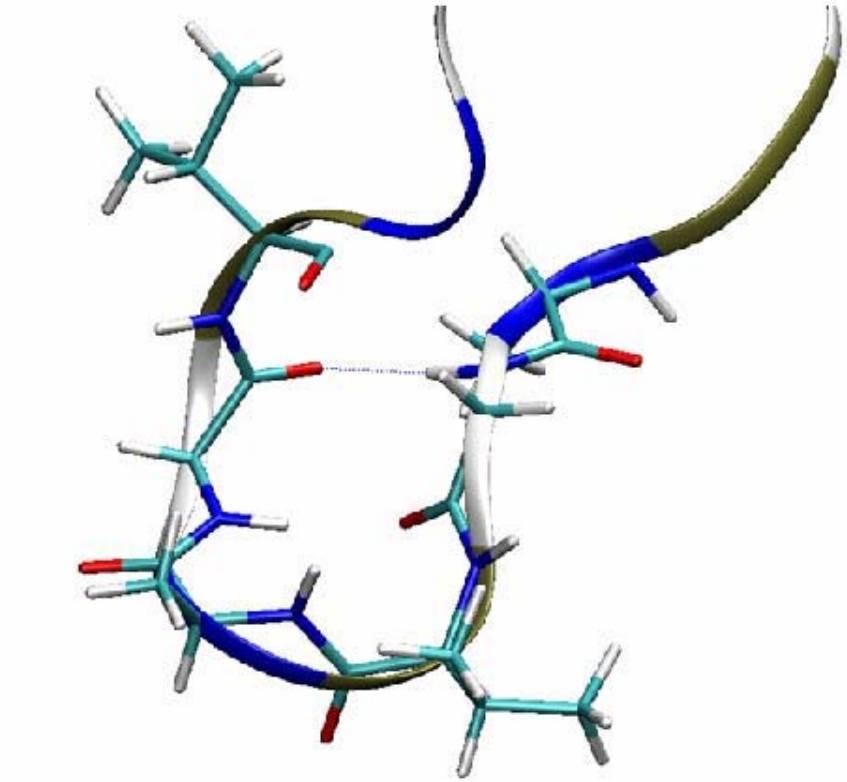


Finkelstein, A. and O. Ptitsyn. (2002) Protein Physics: A Course of Lectures. Academic Press.

Polyproline II is classified as having $\varphi = -75°$ and $\psi = 145°$.

The backbone of PPII structures is accessible to solvent.

Hydrogen Bonded Turns

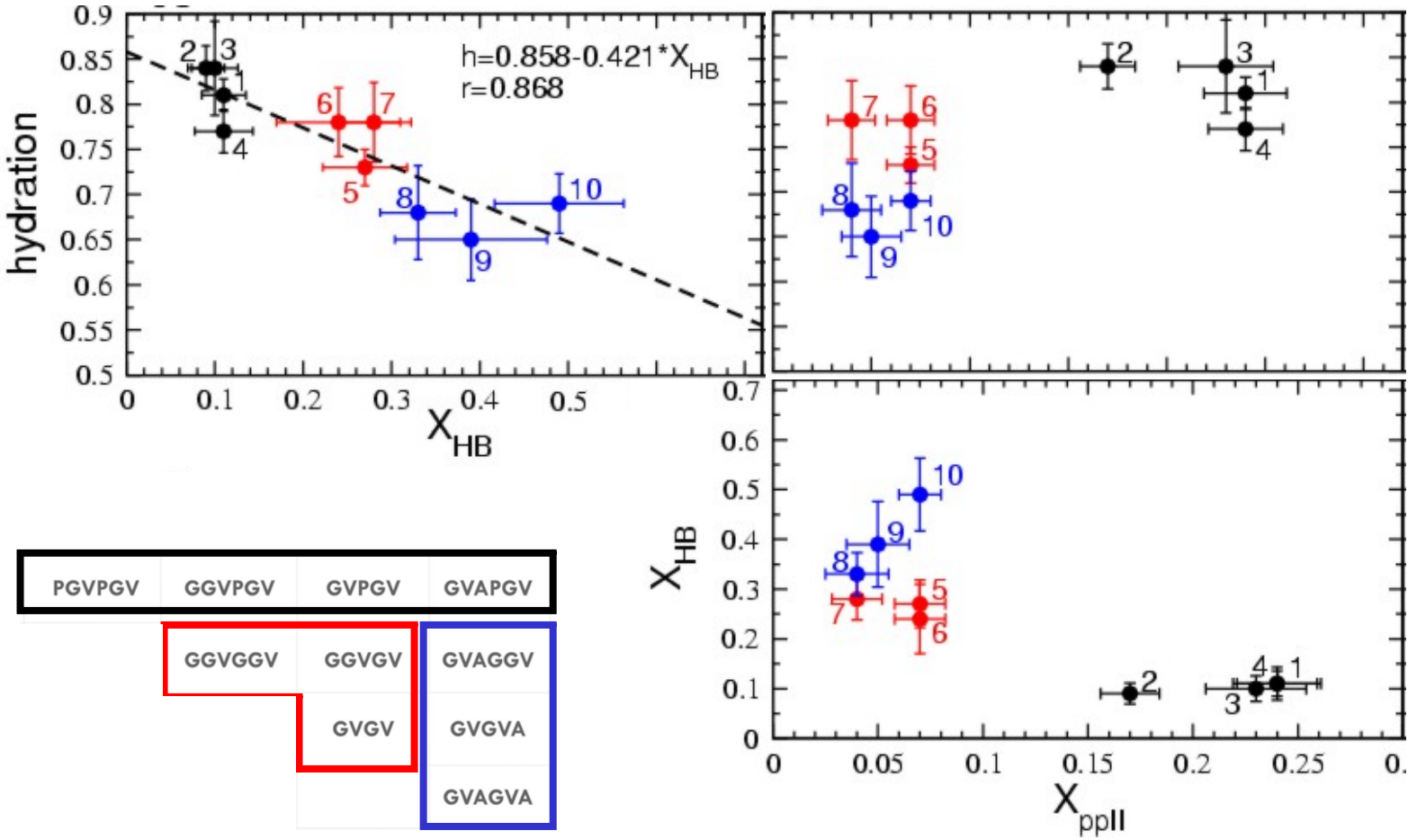


* There are no alpha helices or beta sheet structures observed during the simulations. All structure is local.

Kabsch, W. and C. Sander (1983). "Dictionary of Protein Secondary Structure: Pattern Recognition of Hydrogen-Bonded and Geometrical Features". Biopolymers. 22. 2577-2637.

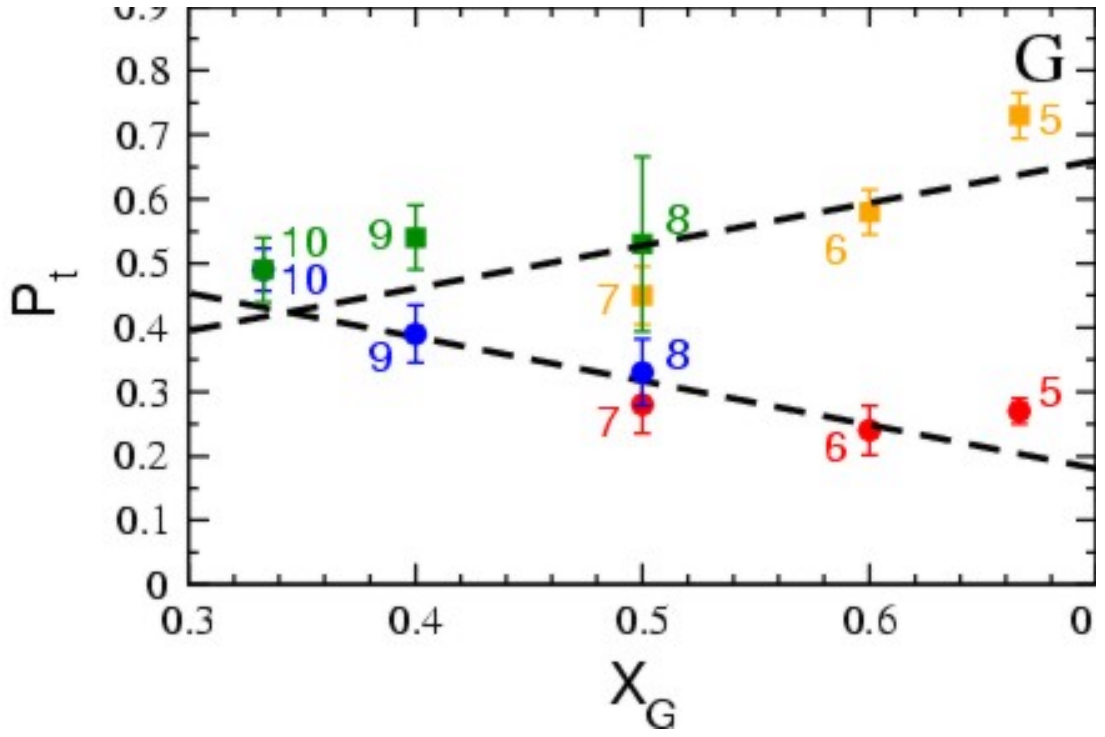
TURNS	Hydrogen Bond Between Residues:
γ-turn (2-turn)	i and i+2
β-turn (3-turn)	i and i+3
α-turn (4-turn)	i and i+4
π-turn (5-turn)	i and i+5
6-turn	i and i+6
7-turn	i and i+7

Hydration and Conformational Propensity of the Backbone



Those ambivalent glycines

Modulation of backbone self-interactions

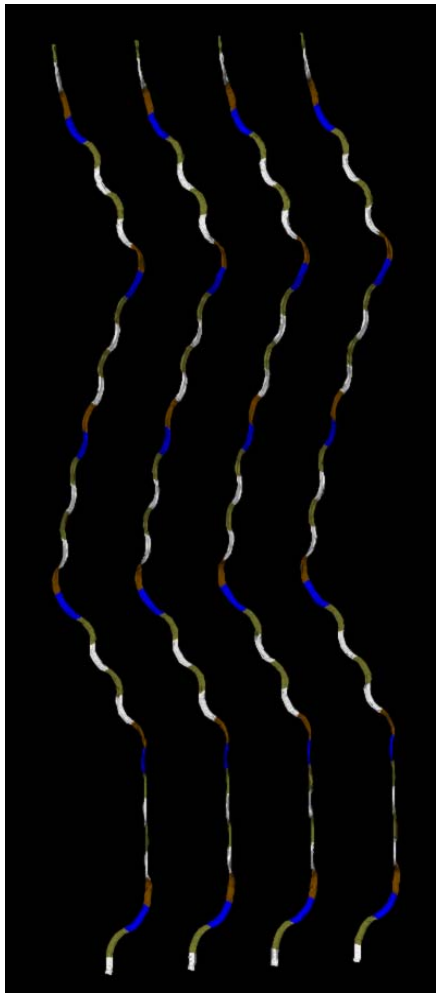


in vacuo

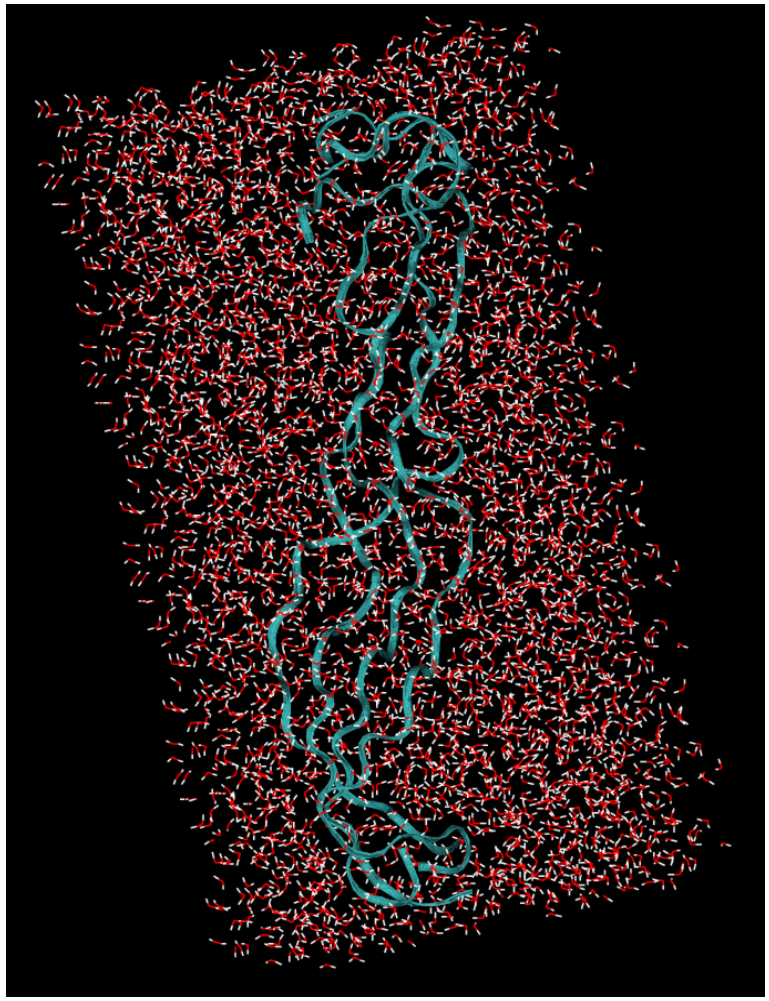
in water

Can aggregates make β sheets?

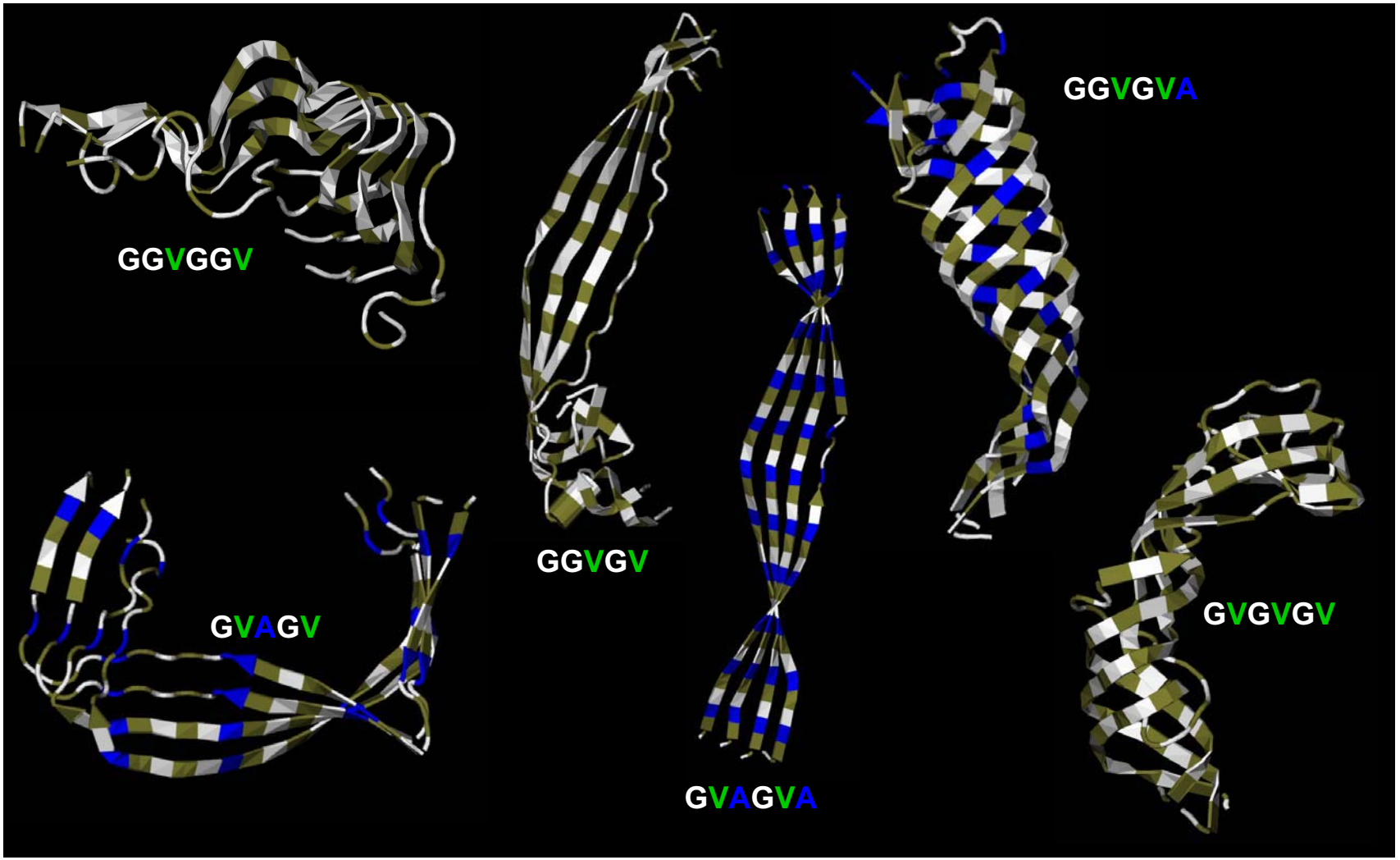
in vacuo



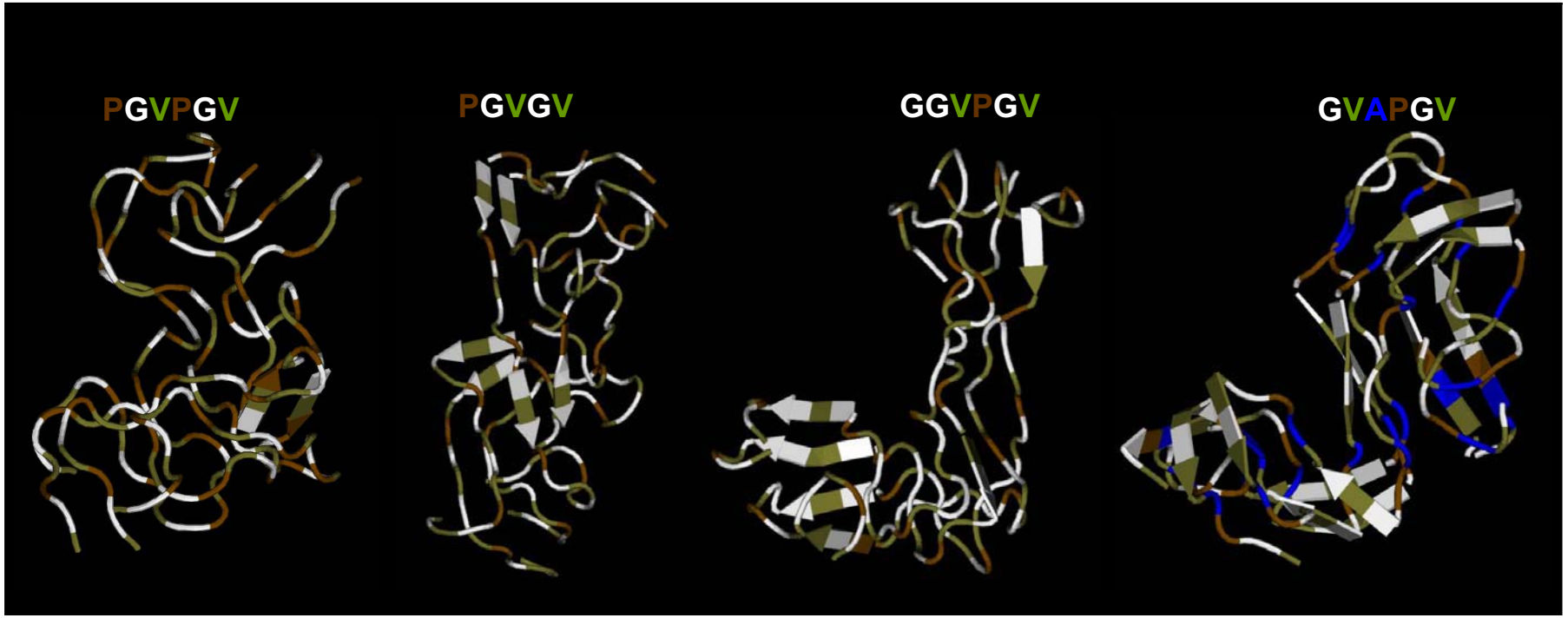
in water



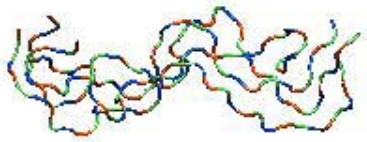
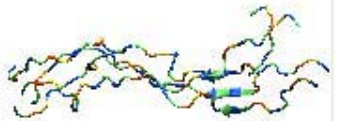
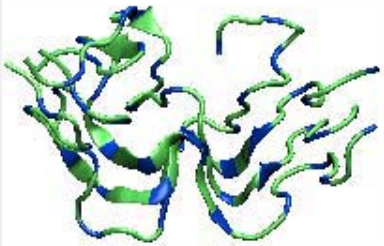
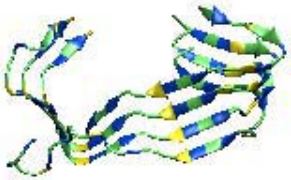
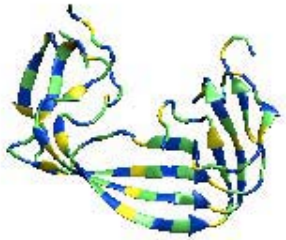
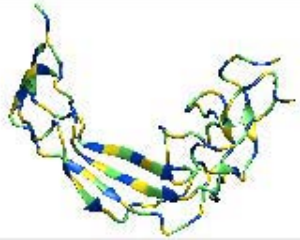
Structural Features of Aggregates in Vacuo



Structural Features of Aggregates in Vacuo



Aggregates in Water

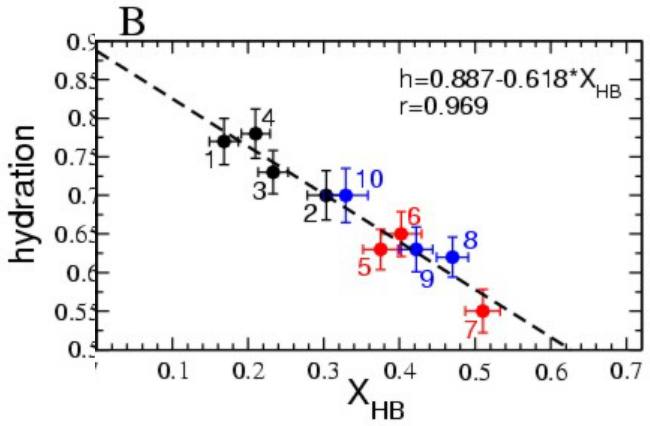
	PGV	GGV	GV	GVA
PGV				
GGV				
GV				
GVA				

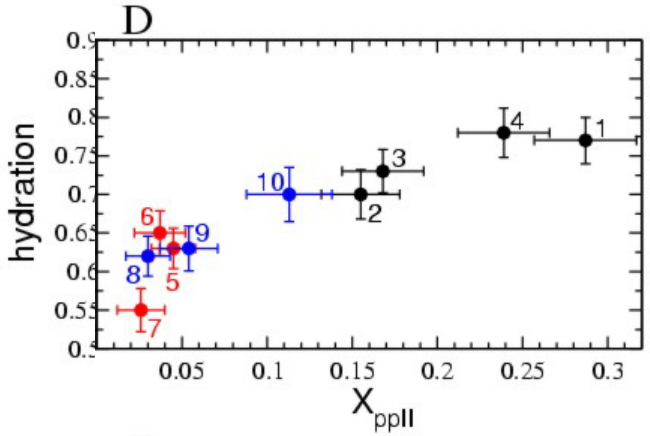
Structurales Properties of Aggregates

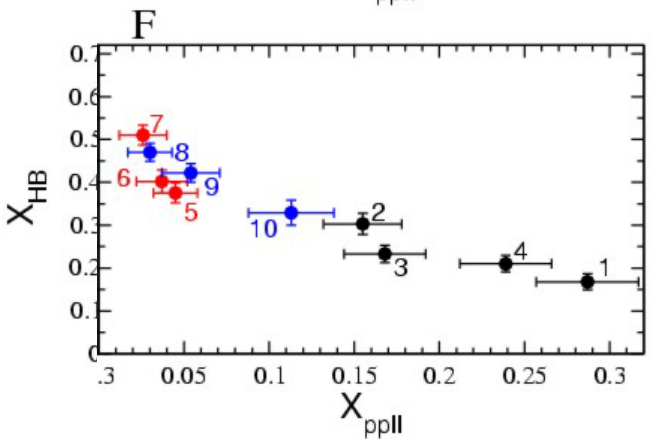
Peptide-peptide HB now mostly β sheet

P-containing peptides still separate from the other 2 groups, both of which can form extended β sheets

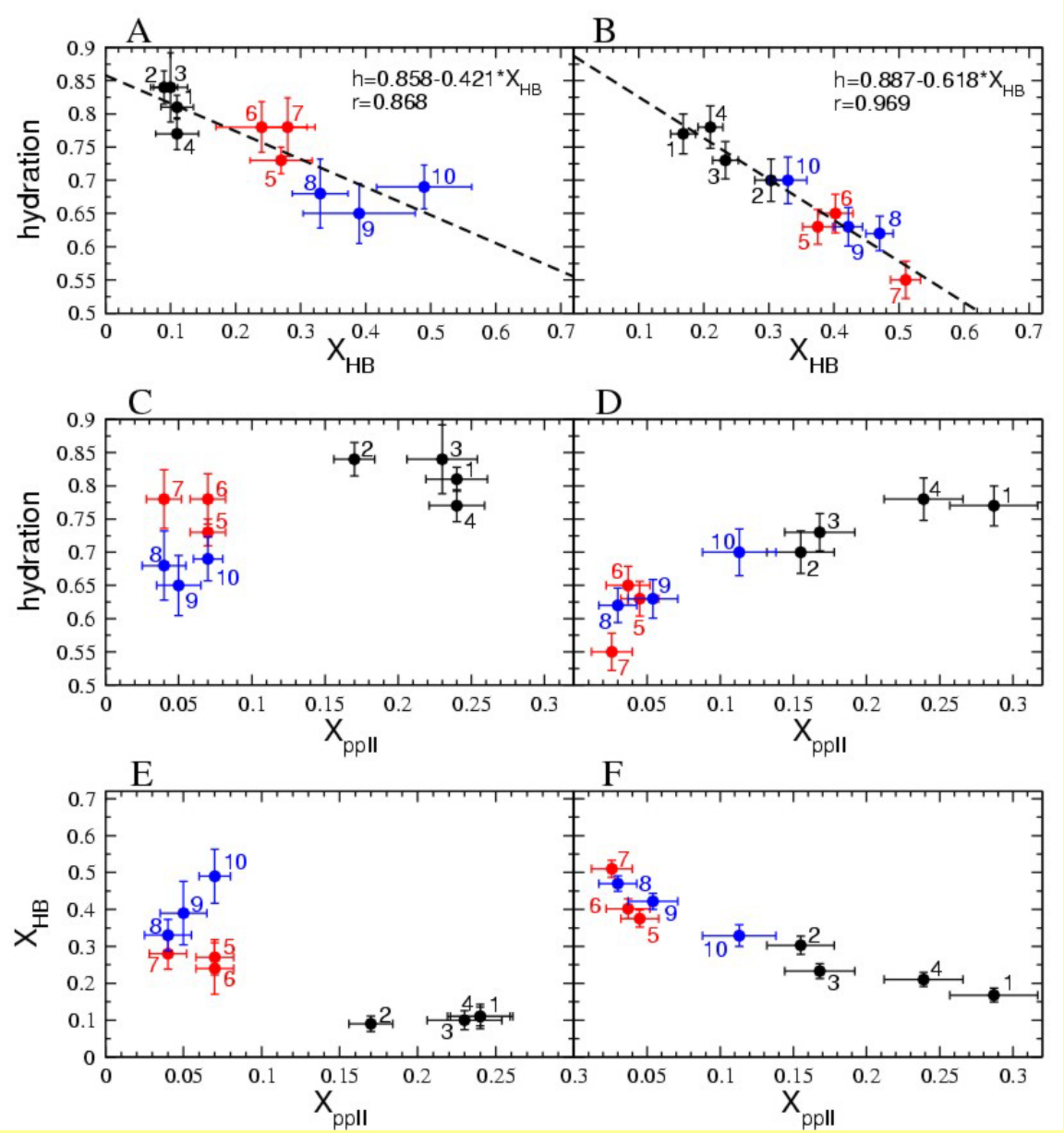
PGVPGV	GGVPGV	GVPGV	GVAPGV
	GGVGGV	GGVGV	GVAGGV
		GVGV	GVGVA
			GVAGVA







monomers self-assemblies



Elastin-like and amyloid-like peptides consistently separate on the basis of conformational backbone propensities

Primary determinant = Proline: prevent secondary structure formation (peptide-peptide HB)

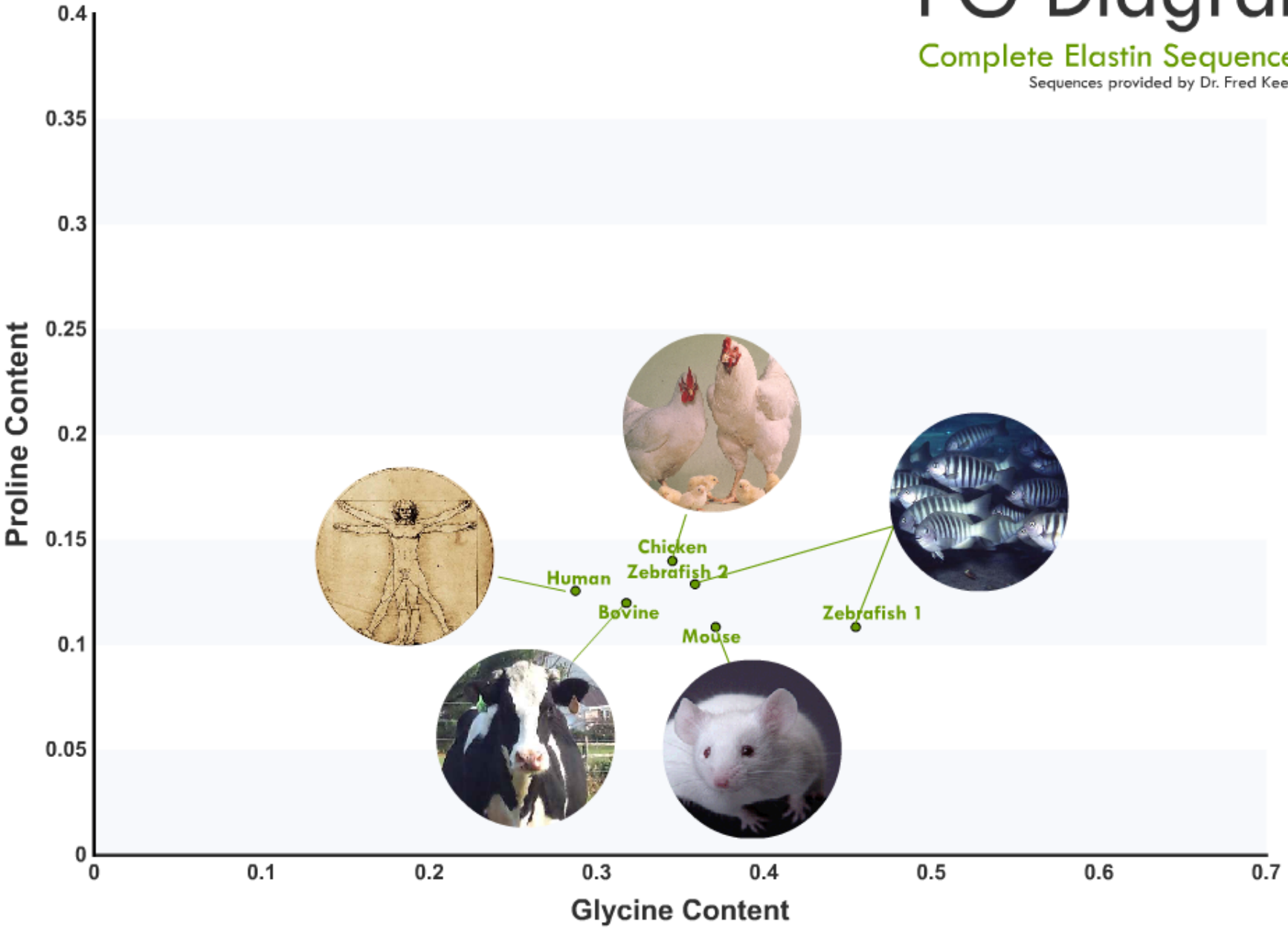
Secondary determinant = Glycine content.

In the absence of Prolines, it is the fraction of G content that modulates HB turn propensity of single chains and separates presumed-schizoids from presumed-amyloids.

PG Diagram

Complete Elastin Sequences

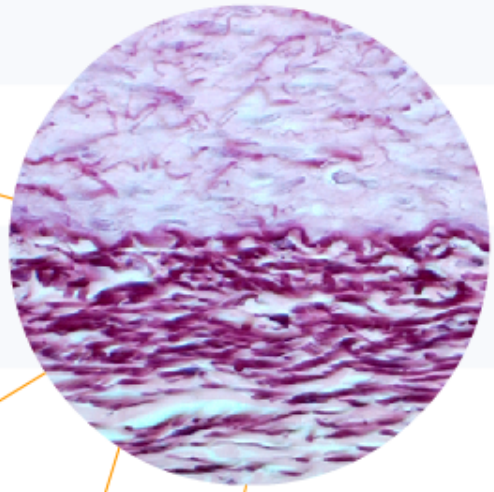
Sequences provided by Dr. Fred Keeley



PG Diagram

Exons of Human Elastin

Tamburro, A. et al. (2003) "Dissection of Human Tropoelastin:
Exon-By-Exon Chemical Synthesis and Related Conformational Studies"



EX12

EX20

EX5

EX30

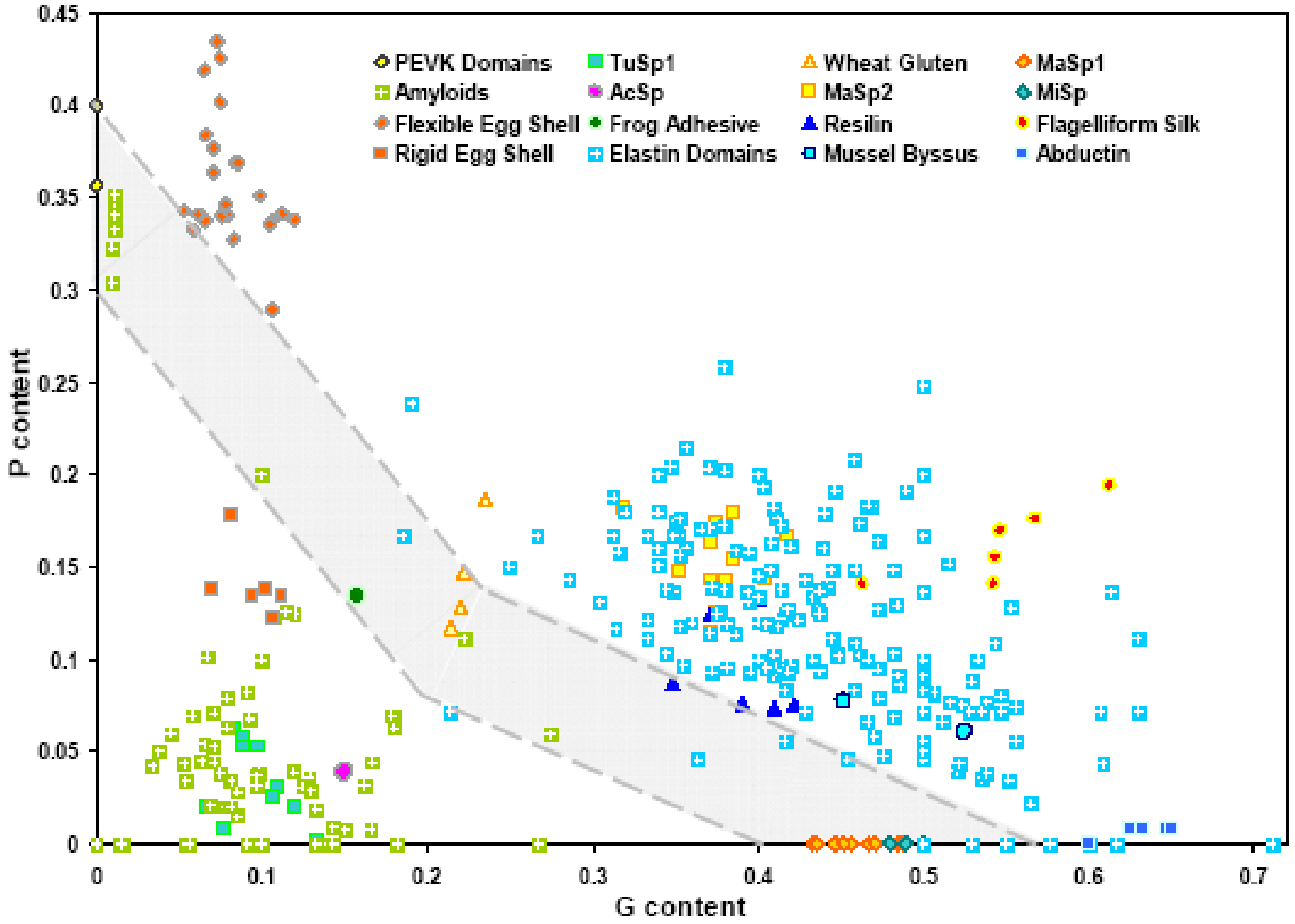
Proline Content

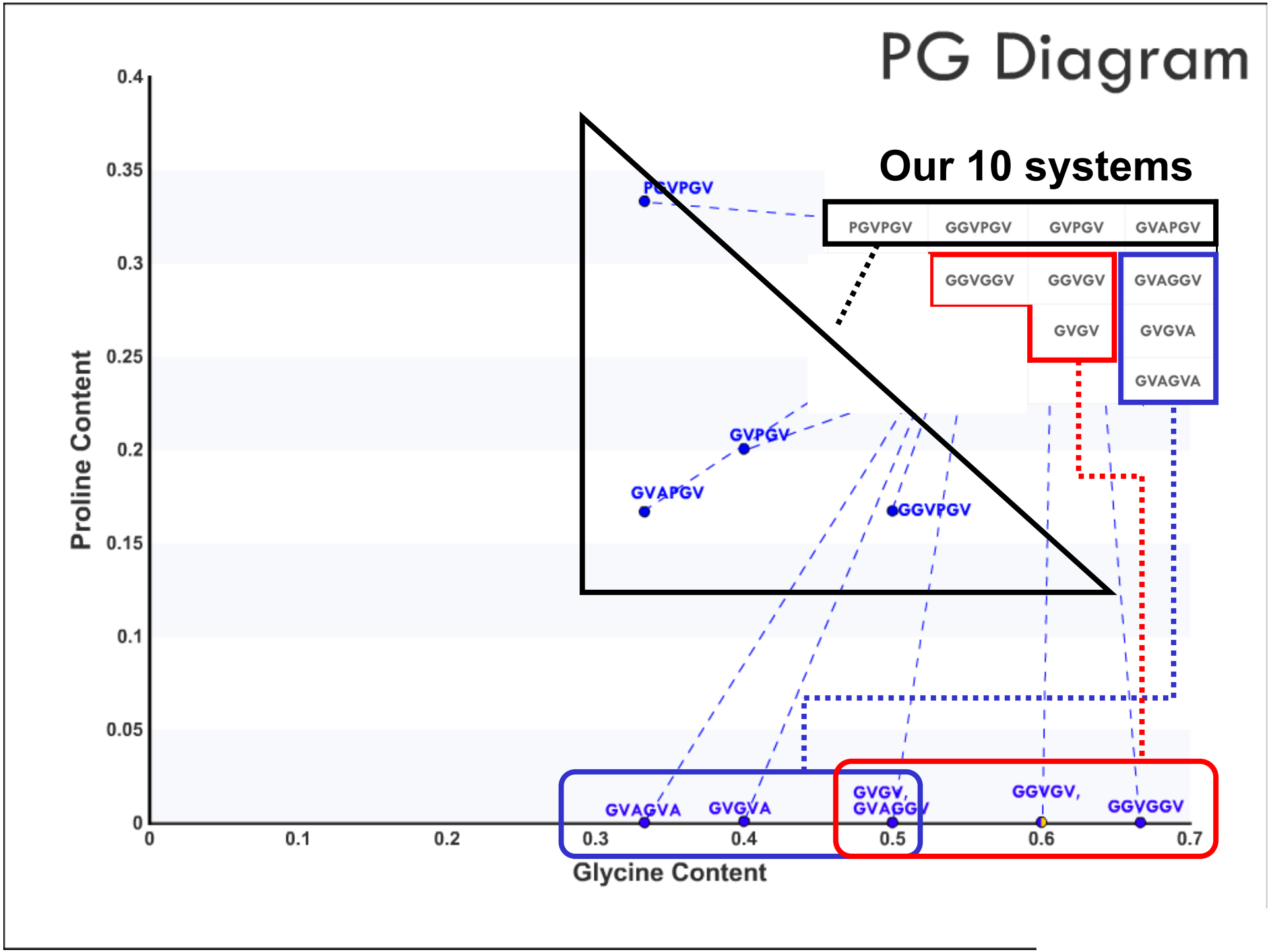
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0

0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Glycine Content

P and G control self-organization into elastomeric or amyloid fibrils




PG Diagram
Our 10 systems
PGVPGV
GGVPGV
GVPGV
GVAPGV
GGVGGV
GGVGV
GVAGGV
GVGV
GVGVA
GVAGVA
PGVPGV
GVPGV
GVAPGV
GGVPGV
GVAGVA
GVGVA
GVGV,
GVAGGV
GGVGV,
GGVGGV
Proline Content
Glycine Content
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Why combine P and G?

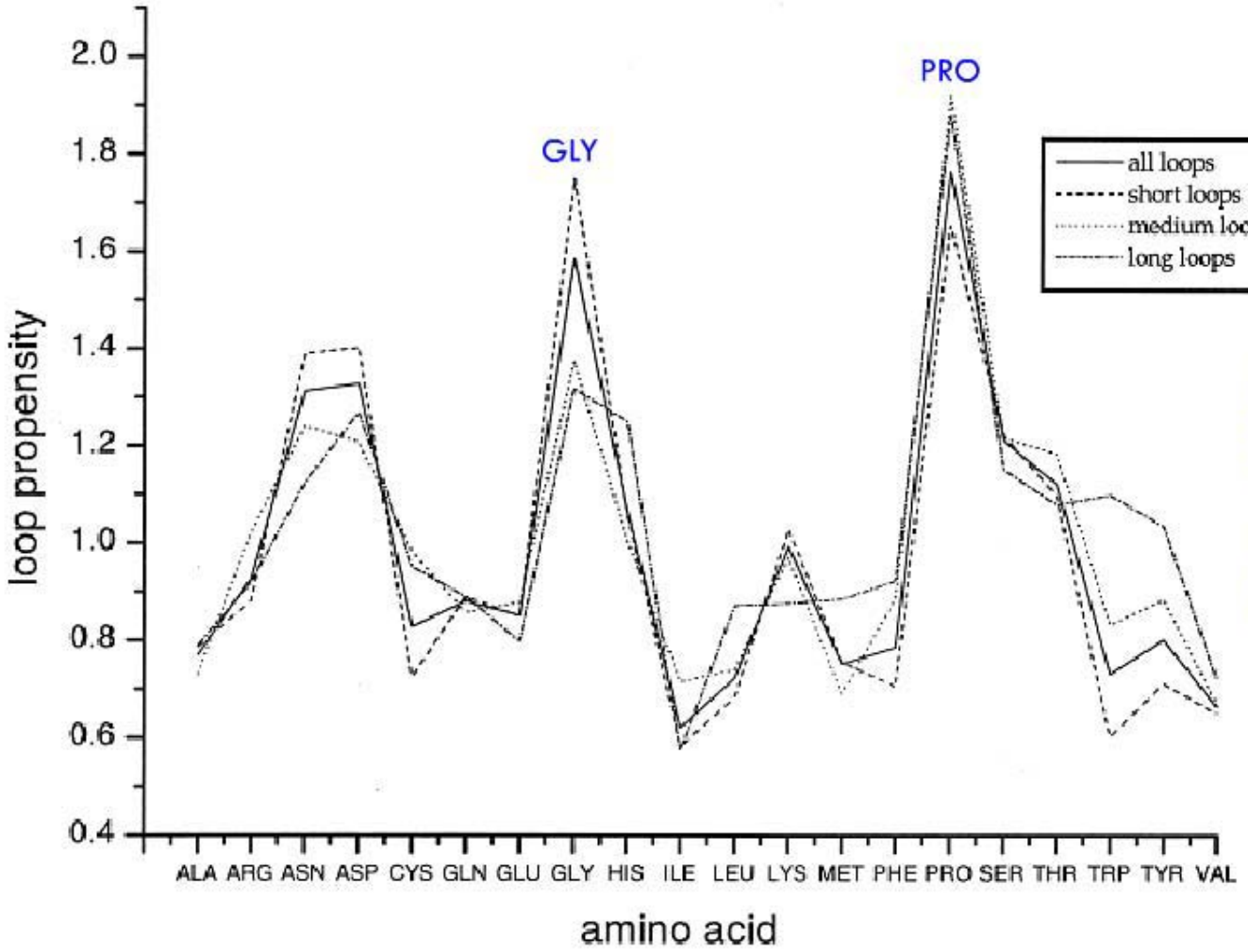
= the two outliers of the Ramachandran plot!

Both conspire to keep backbone disordered and hydrated, though for opposite reasons:

P ← rigid, can't comply

G ← extremely compliant in absence of water
but won't stay put in water:
too flexible, chain entropy

The Importance of Proline & Glycine



-Proline and glycine are the amino acids most commonly observed in disordered loops (from the PDB)

-Both proline and glycine are known to be "amyloid breakers"

Crasto, C. and J. Feng. (2001) Proteins: Structure, Function and Genetics. 42:399-413.
Uversky, V. (2005) "A GLYmmer of Insight into Fibril Formation". Structure. 13: 1090-1091.
Williams, A. et al (2004) "Mapping A-beta Fibril Secondary Structure Using Scanning Proline Mutagenesis". J. Mol. Bio. 335: 833-842.

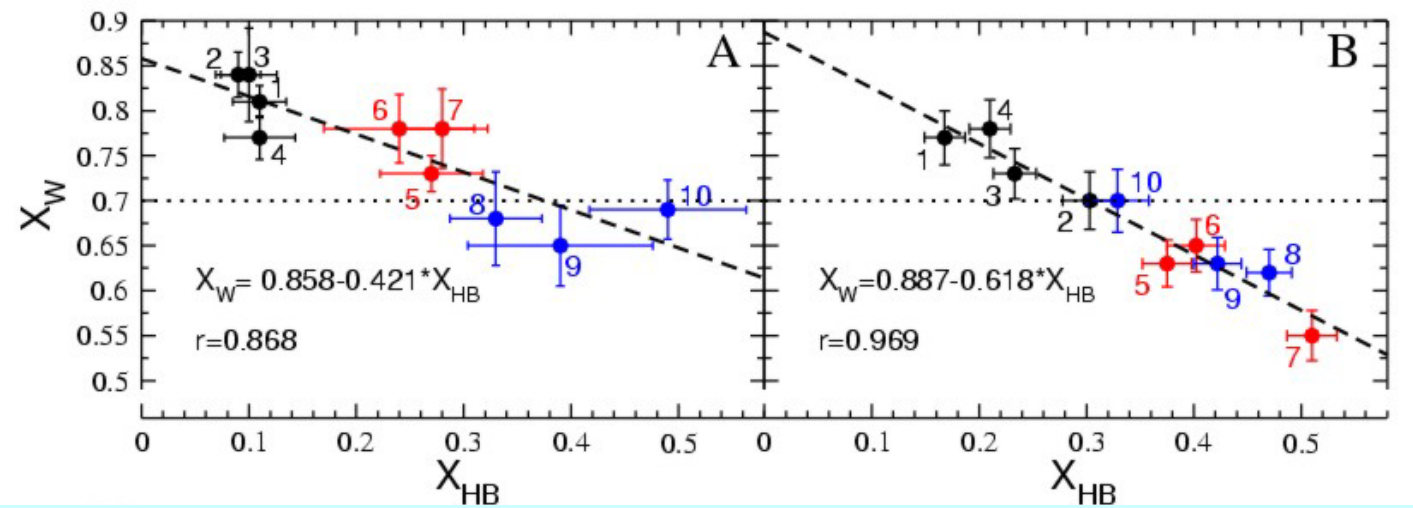
Why keep backbone hydrated?

~ “lubrication”

Hydration threshold below which elastin is brittle

= 20-30% by weight

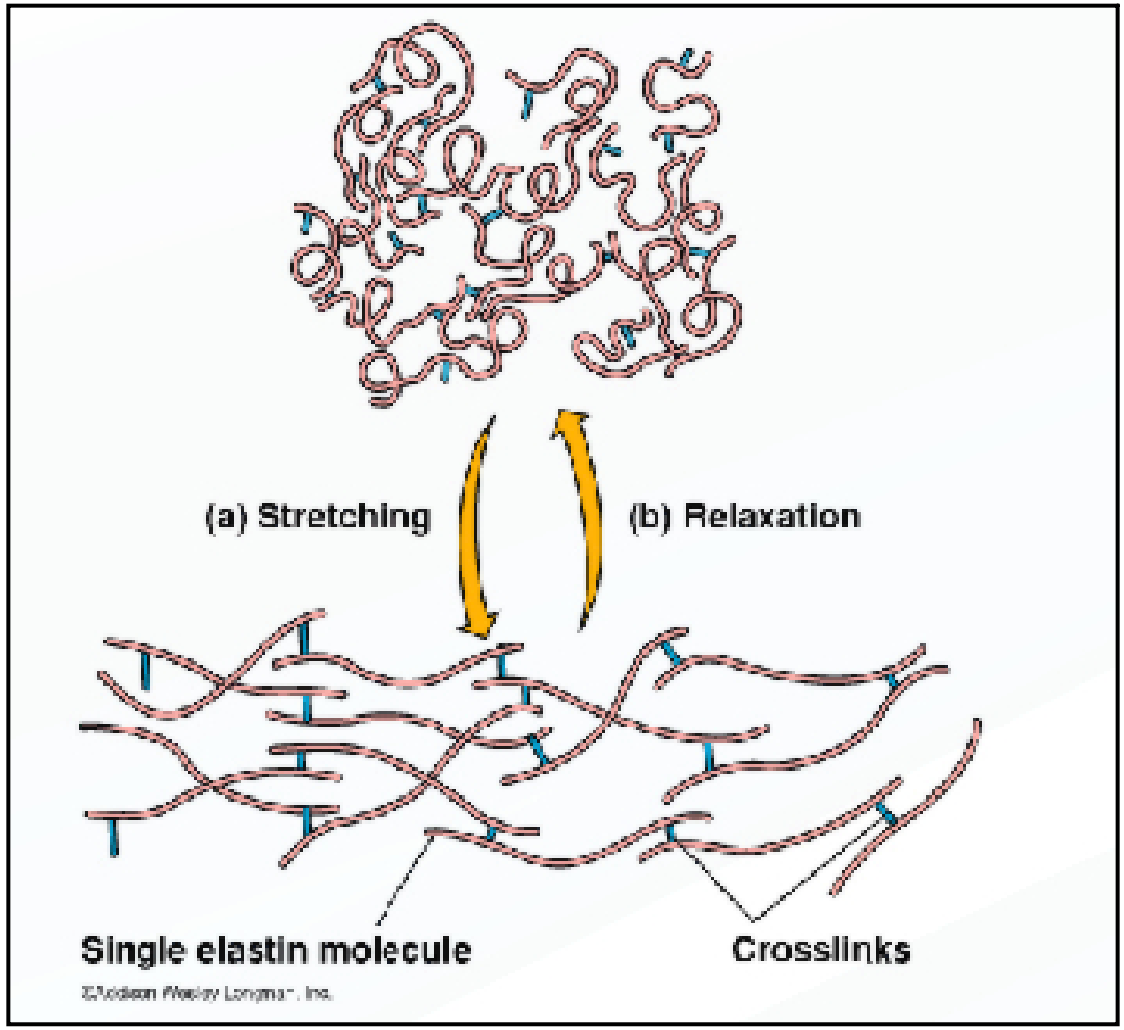
Consistent with the fraction of water required to solve the backbone as obtained from our calculations



… and disordered?

Conformational chain entropy as a factor driving elastic recoil

Entropic model of rubber-like elasticity



Chain Entropy

$S_C = R \ln \Omega$

Disordered chain ("random")

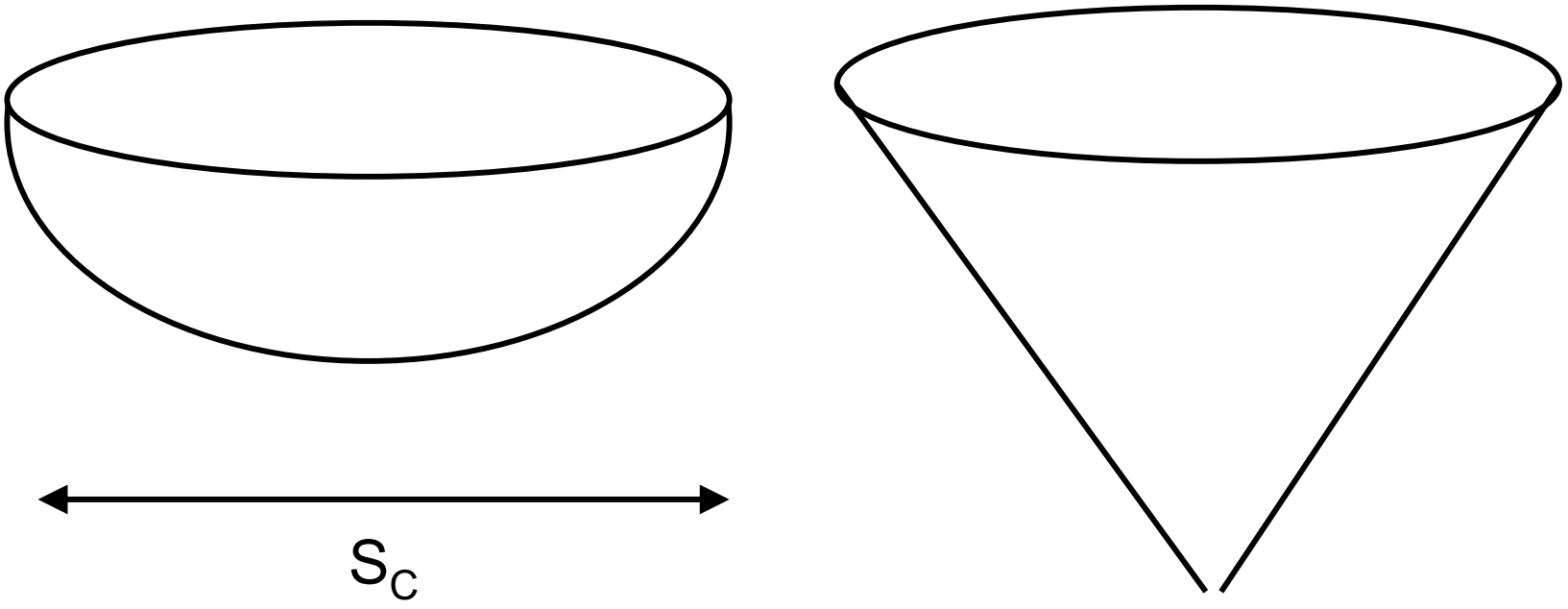
Ω large

Ordered (stretched) Chain

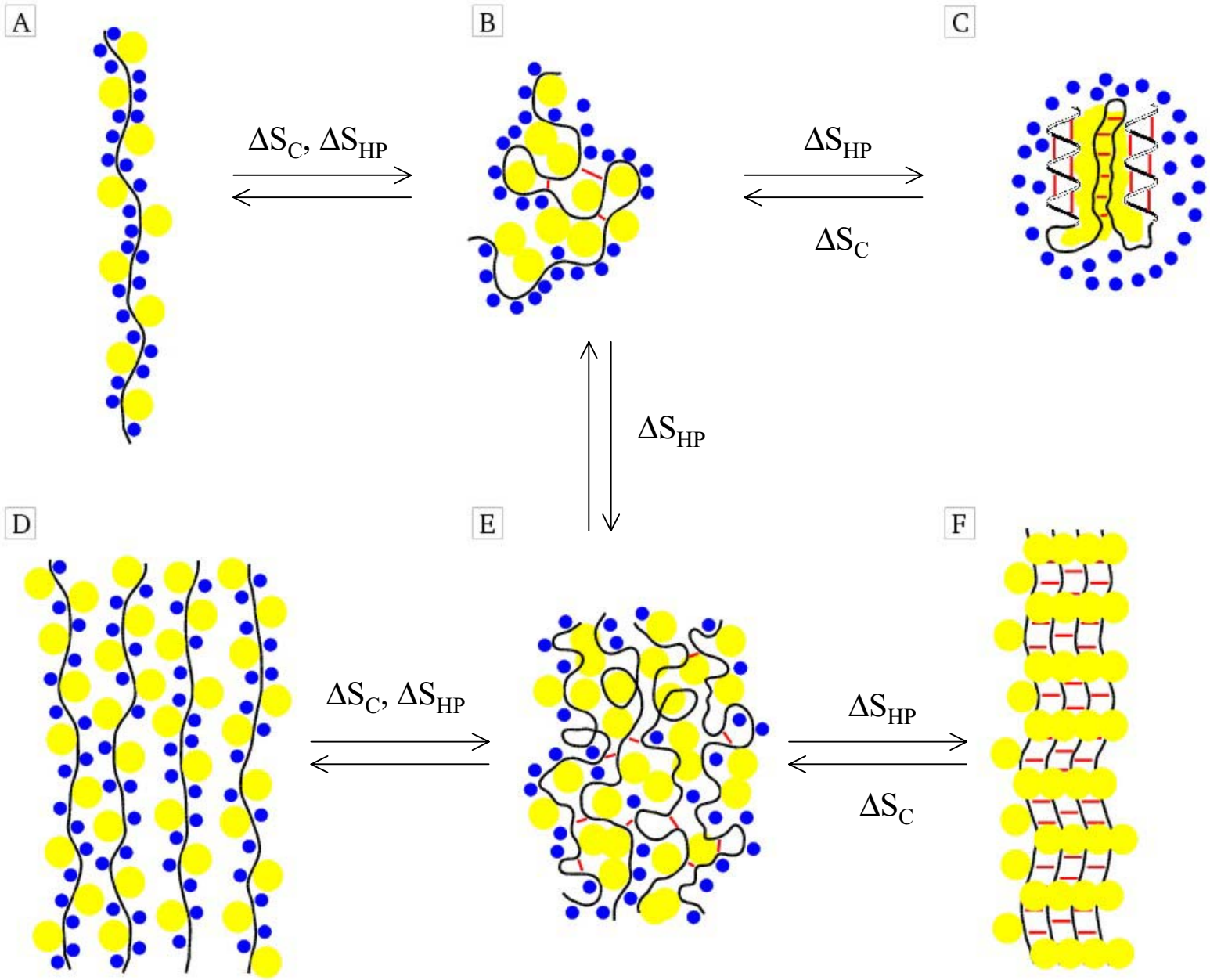
Ω small

Underlying free energy landscape

More Wok than Funnel



Hydration and entropy in folding, aggregation, and recoil



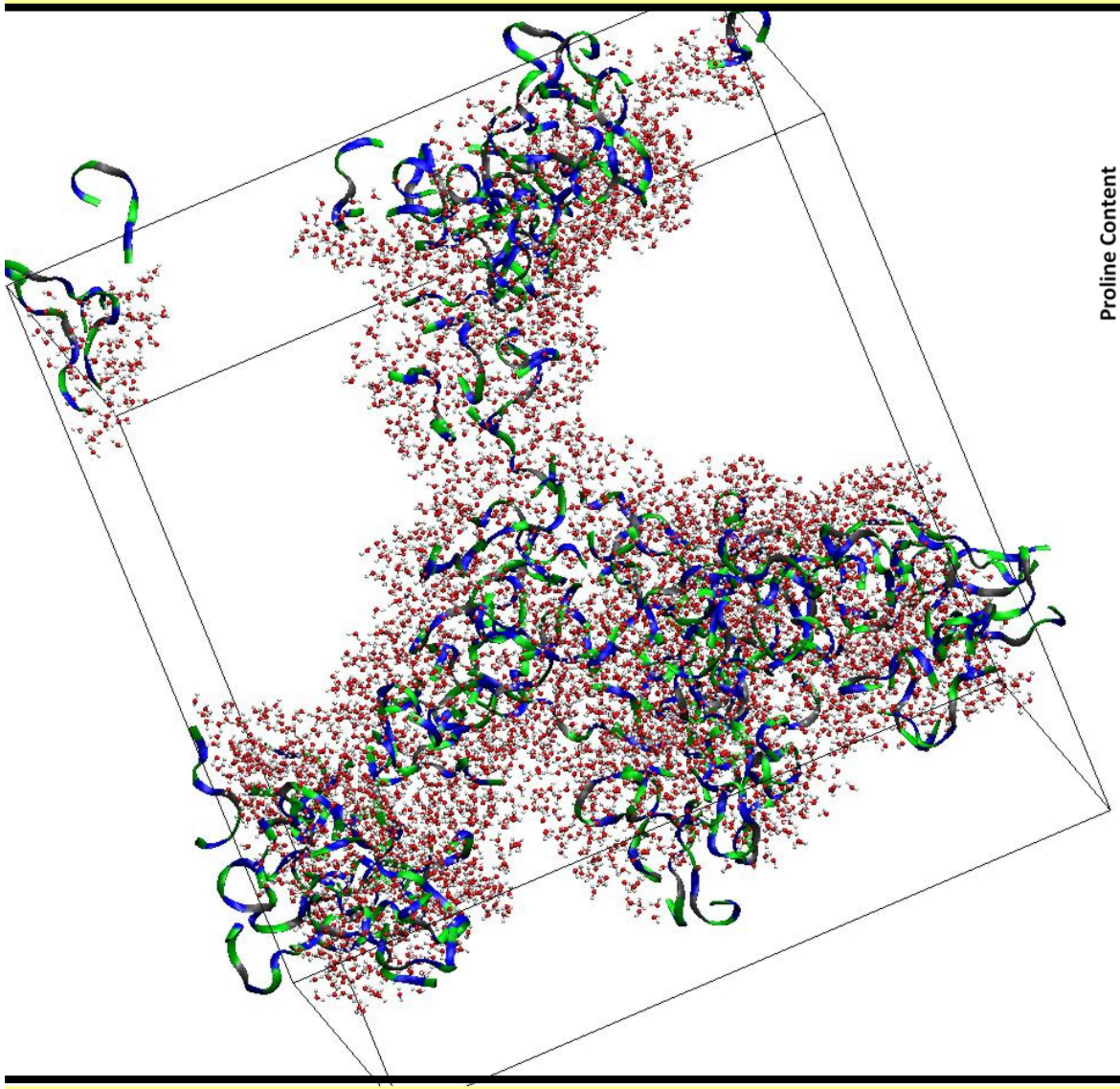
Elastin,the amyloid that wouldn’t

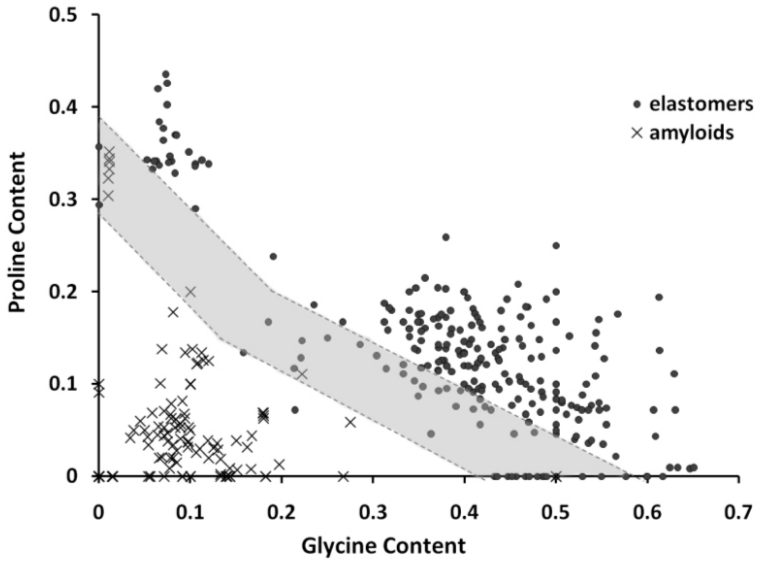
Molecular basis of elasticity

Backbone hydration and conformational disorder
are required for elastomeric function

Satisfying a combined threshold in proline and glycine
is a necessary condition

These properties are incompatible with amyloid fibre formation





Perspectives

Sequence determinants?

Structure of aggregates?

Modulation of elasticity?