Structural Folds of Amyloid Fibrils

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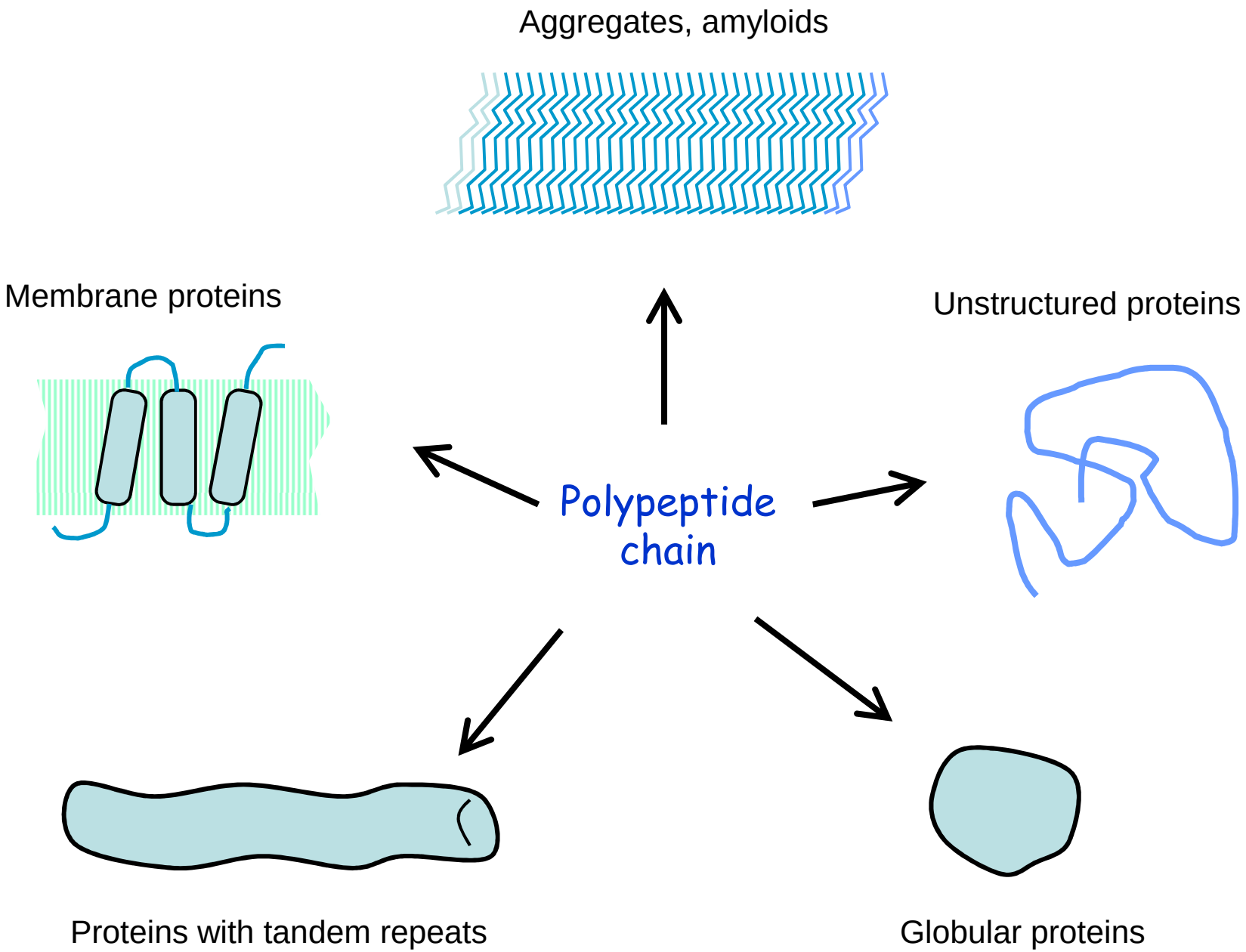
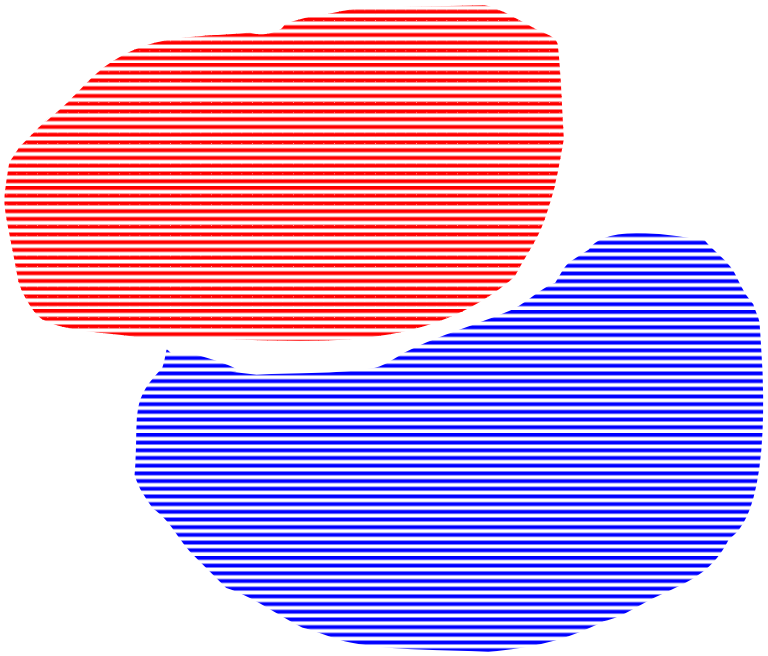
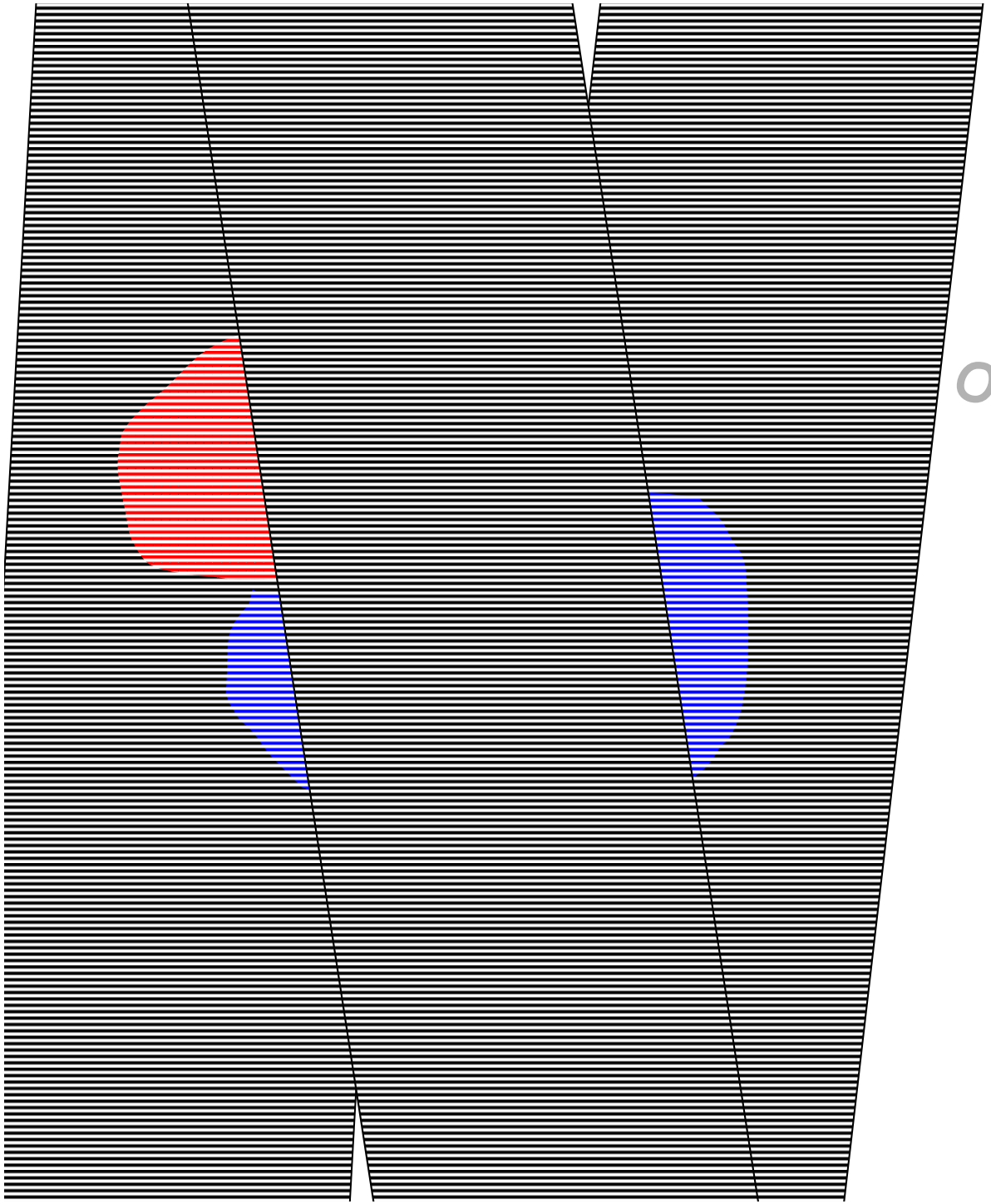
Montpellier, France


Aggregates, amyloids
Membrane proteins
Unstructured proteins
Polypeptide chain
Proteins with tandem repeats
Globular proteins

Structure of Amyloid Fibrils



Limited size
and
Optimal stability
of
Protein
Structures



Limited size
and
Optimal stability
of
Proteins
Structures

Stable structures
of
Unlimited size

Presence of amyloid fibrils is connected with serious neurodegenerative diseases, including Alzheimer's disease, Parkinson's desease, Huntington's disease, and also the transmissible prion diseases.

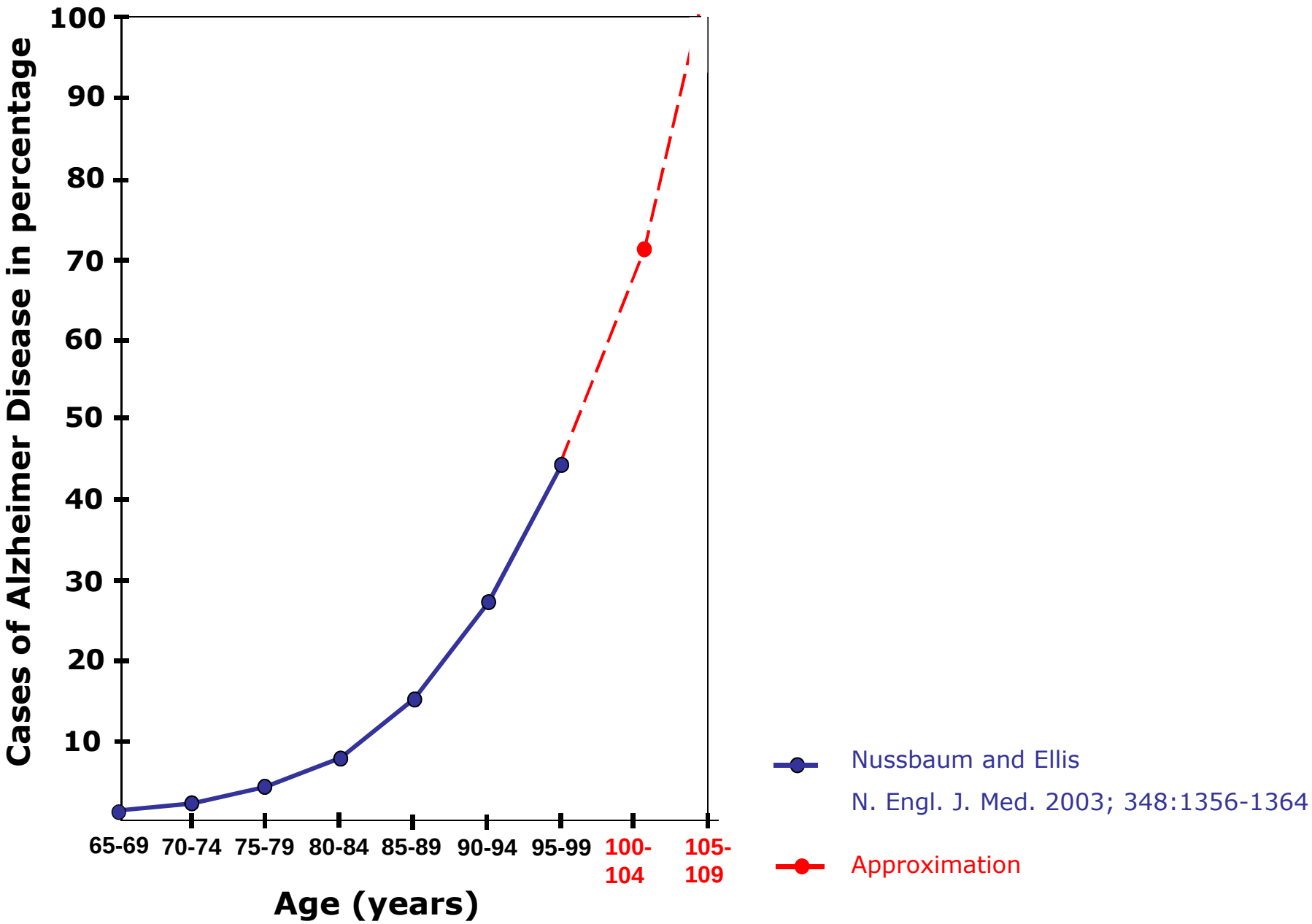
Destroy neuronal tissues
in the human **brain**

Span over **many years**

Intense care

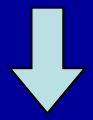
In France
Patients ~ 2% of population

Cost for each citizen ~**350 euros** annually in 2005
Represents **0.6%** of GDP in 2005; **0.8%** in 2020; **1.8%** in 2040


Cases of Alzheimer Disease in percentage
100
90
80
70
60
50
40
30
20
10
65-69
70-74
75-79
80-84
85-89
90-94
95-99
100-104
105-109
Age (years)
Nussbaum and Ellis
N. Engl. J. Med. 2003; 348:1356-1364
Approximation

The 3D structure of amyloid fibrils?

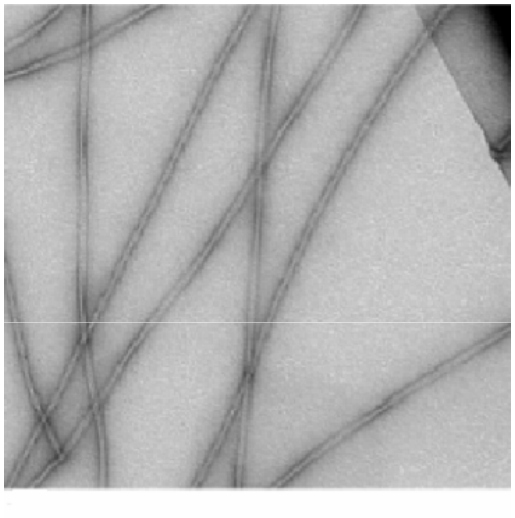
Incomplete structural information from electron-microscopy, X-ray fiber diffraction, solid-state NMR etc



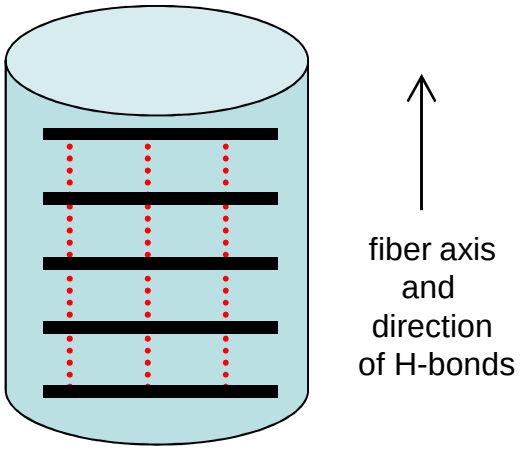
Structural model

COMMON FEATURES OF AMYLOID FIBRILS

From EM
straight, unbranched fibrils
4 to 15 nm in diameter



From X-ray diffraction
« cross-beta »structures



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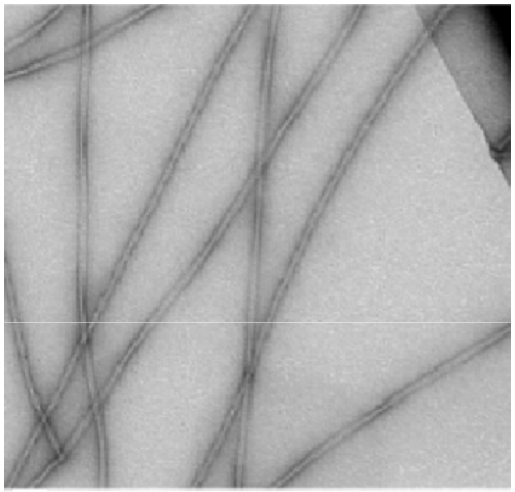

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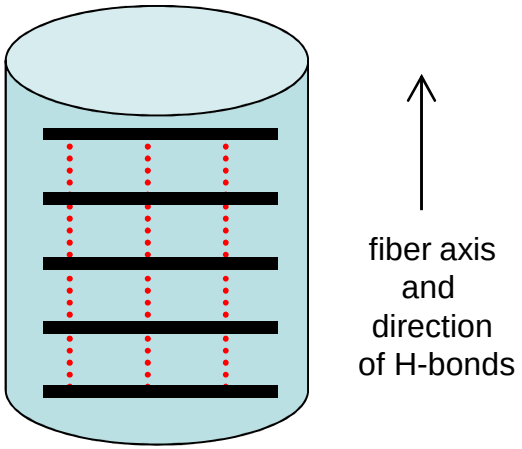
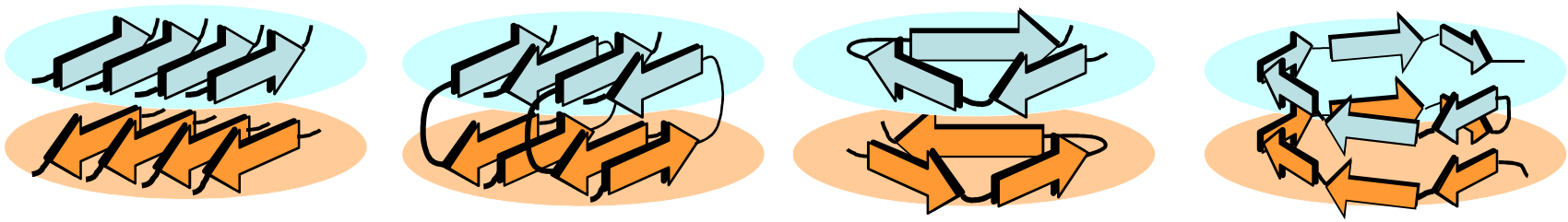
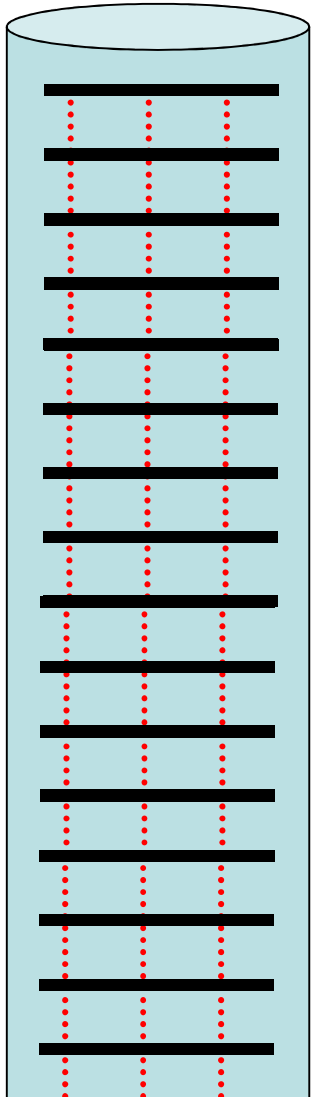


?

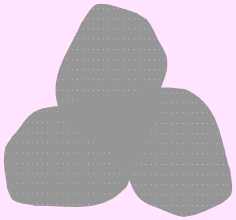


EM and
X-ray fiber diffraction
(diameter, twist, coiling, cross-beta structures)



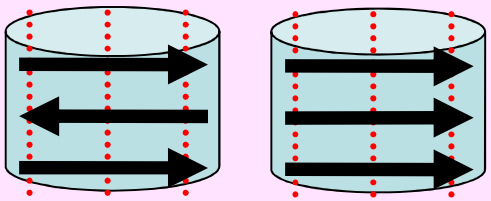
NEW METHODS

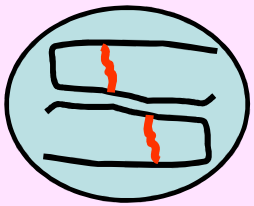
✓ Cryo-EM



✓ STEM (number of peptides in fibril cross-section)

✓ ssNMR,
EPR spectroscopy





EM and
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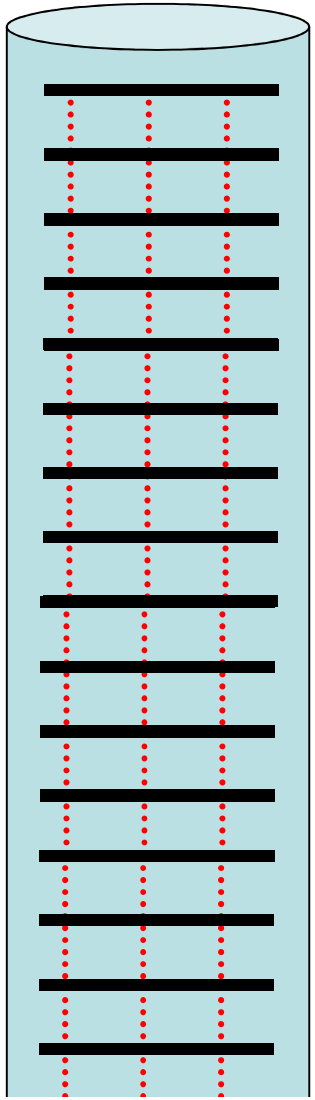

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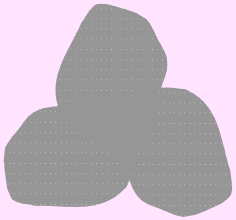

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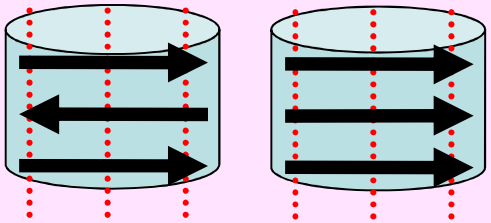
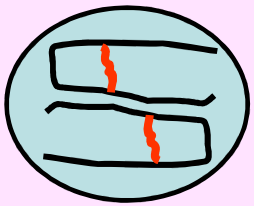
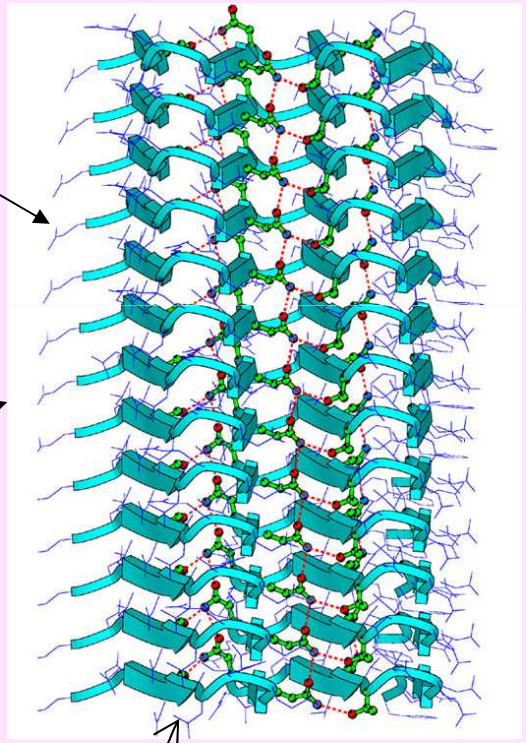


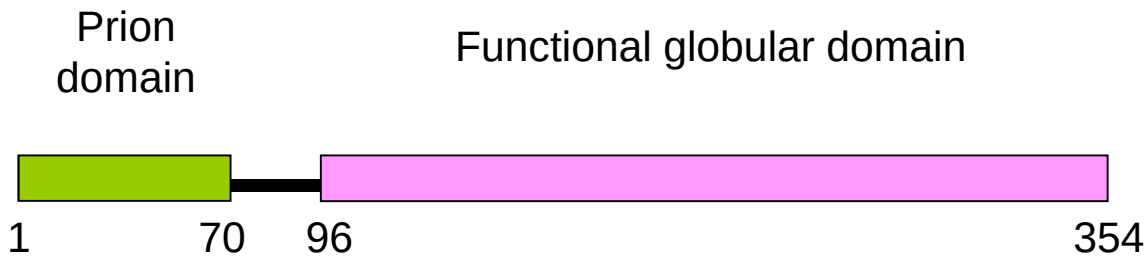


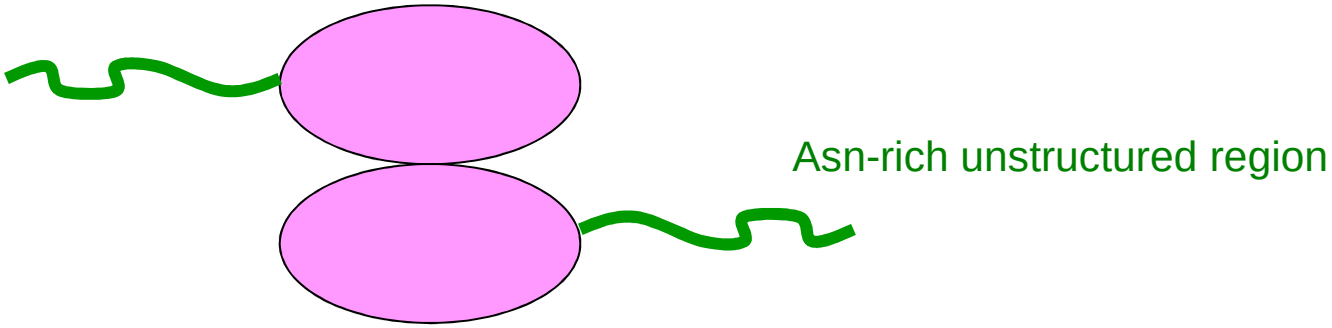


Yeast prion filaments

formed by Ure2p

Yeast prion filaments formed by Ure2p





Homodimer, interacts with GATA transcription factor Gln3p

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 44, Issue of October 31, pp. 43717–43727, 2003
Printed in U.S.A.

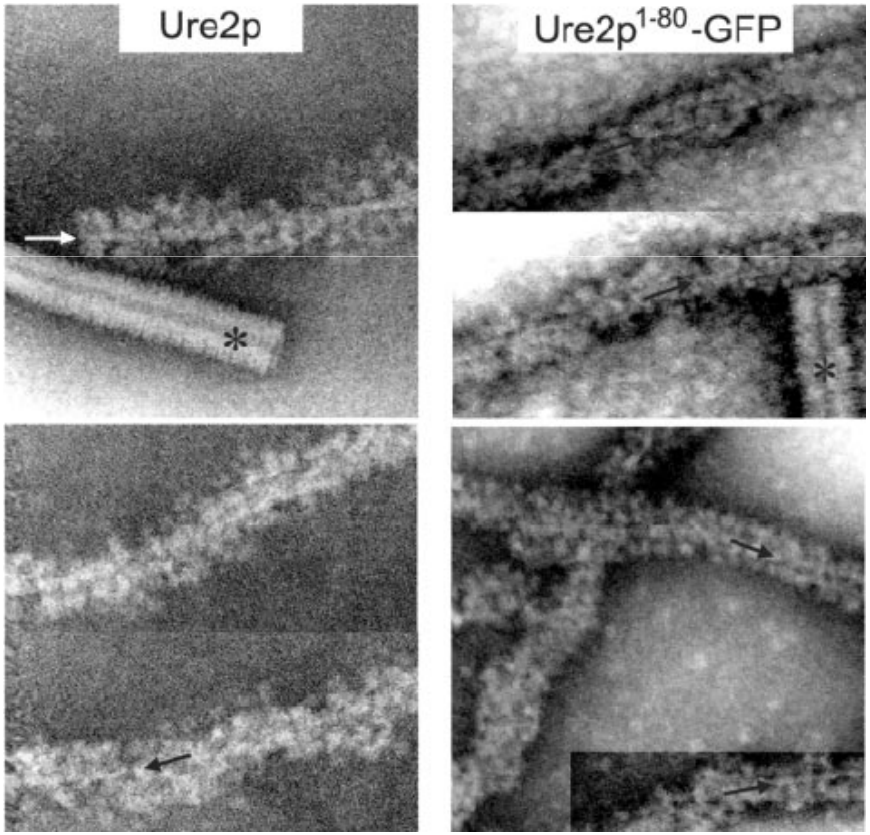
Architecture of Ure2p Prion Filaments

THE N-TERMINAL DOMAINS FORM A CENTRAL CORE FIBER*[S]

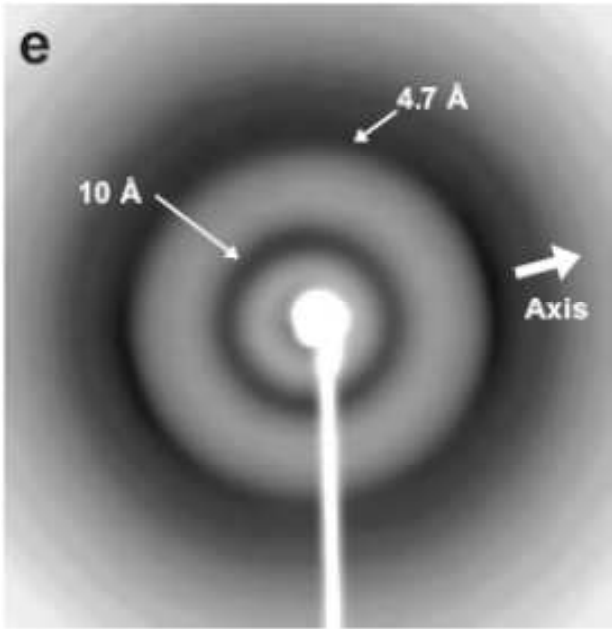
Received for publication, June 6, 2003, and in revised form, August 6, 2003
Published, JBC Papers in Press, August 12, 2003, DOI 10.1074/jbc.M306004200

Ulrich Baxa‡§, Kimberly L. Taylor§¶, Joseph S. Wall‖, Martha N. Simon‖, Naiqian Cheng‡, Reed B. Wickner§, and Alasdair C. Steven‡****

Diameter of core fiber is about 4 nm

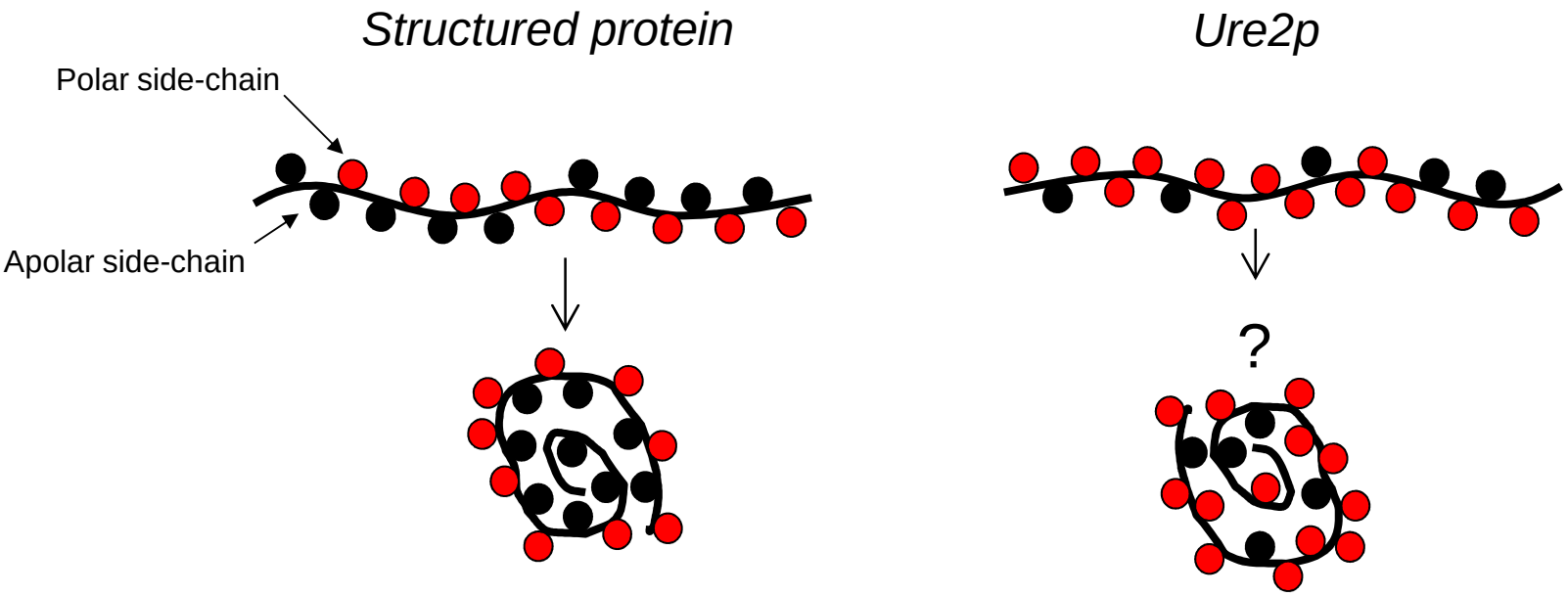


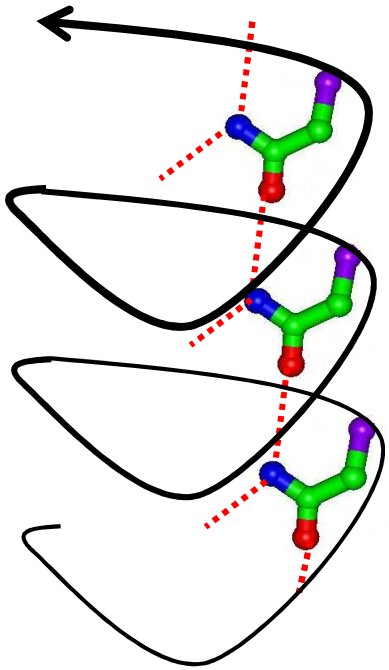
Ure2p core fibril has cross-beta structure

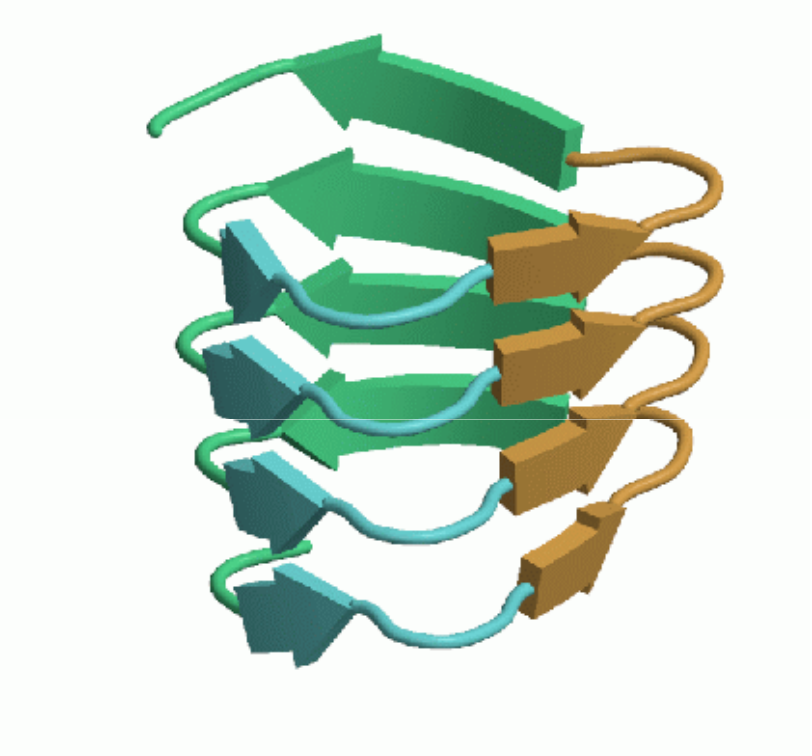


X-ray and electron fiber diffraction
(Baxa et al., J. Struct. Biol. 2005)

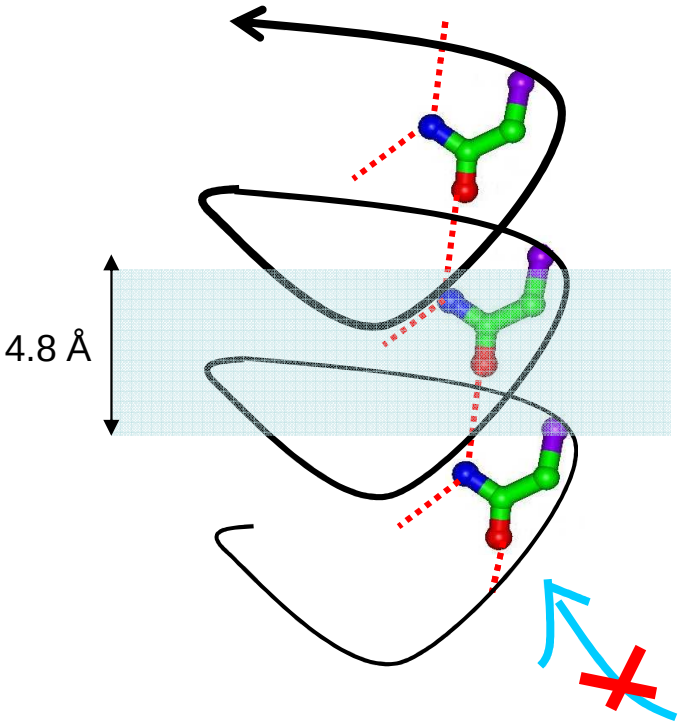
Ure2p prion domain has Asn-rich amino acid sequence







Structural fold for Ure2p prion domain



- One peptide per one beta-layer, according to scanning transmission EM (*Baxa et al., (2003) JBC, 278, 43717)*
- Parallel and in-register beta-structure according to solid state NMR *(Chan and Tycko, (2005) Biochemistry 44, 10669)*

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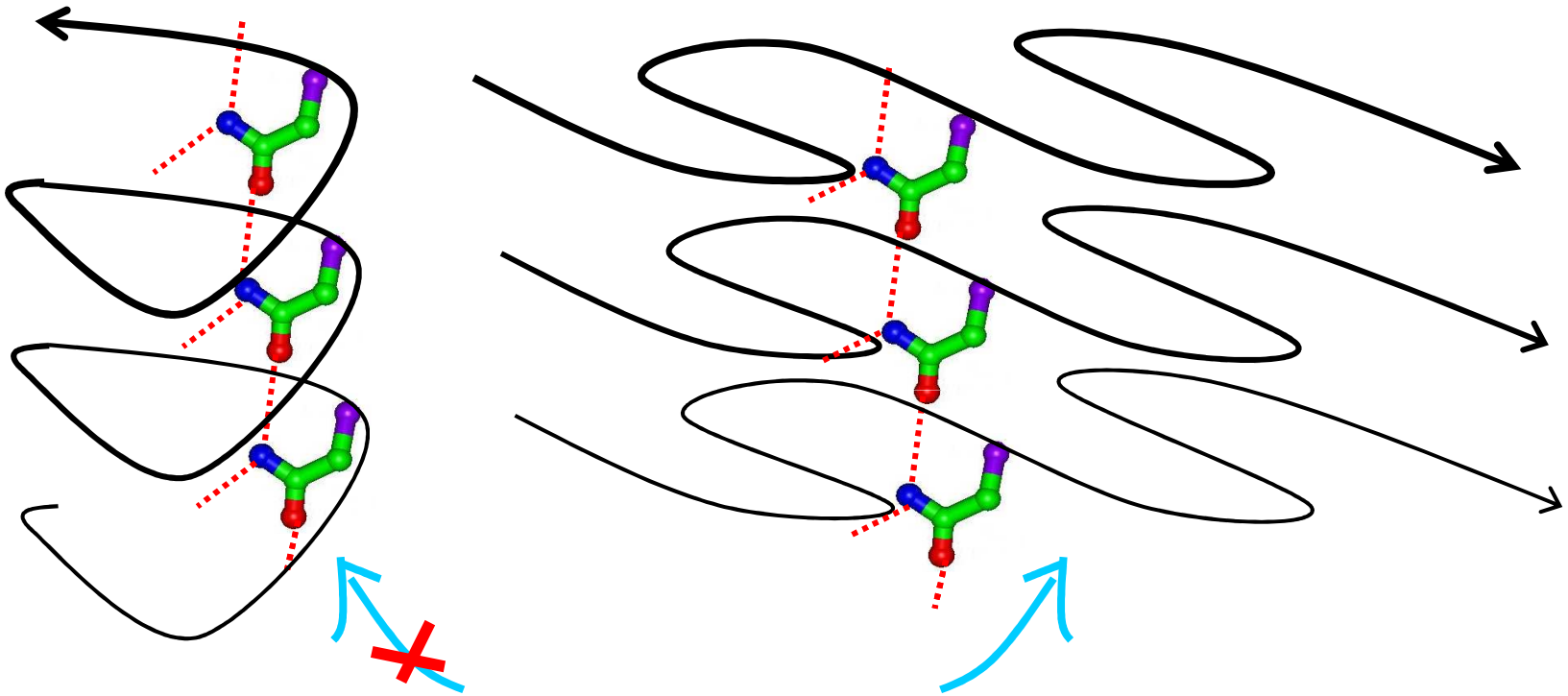

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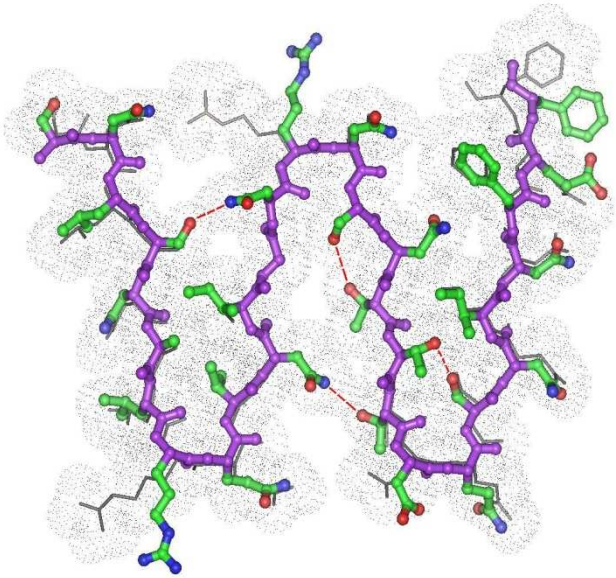
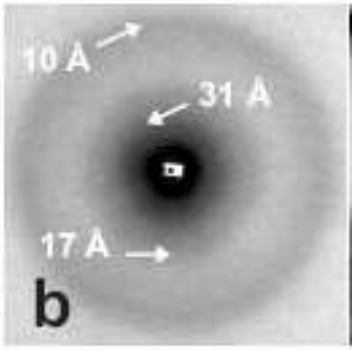
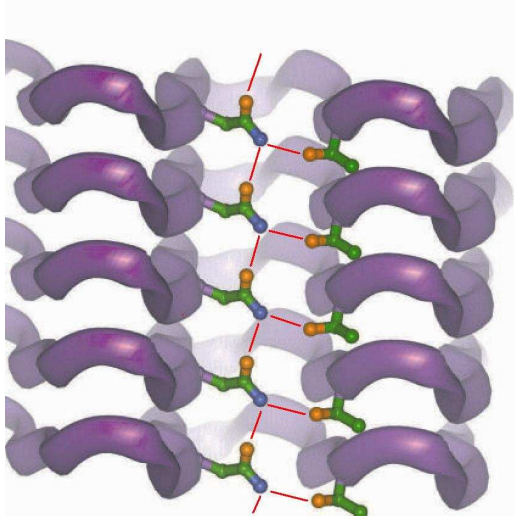


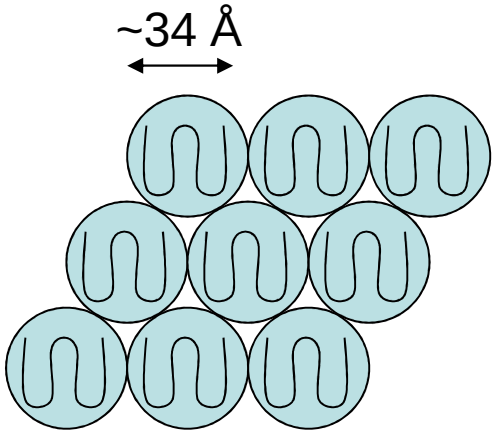
Axial projection
Ure2p (10-39)



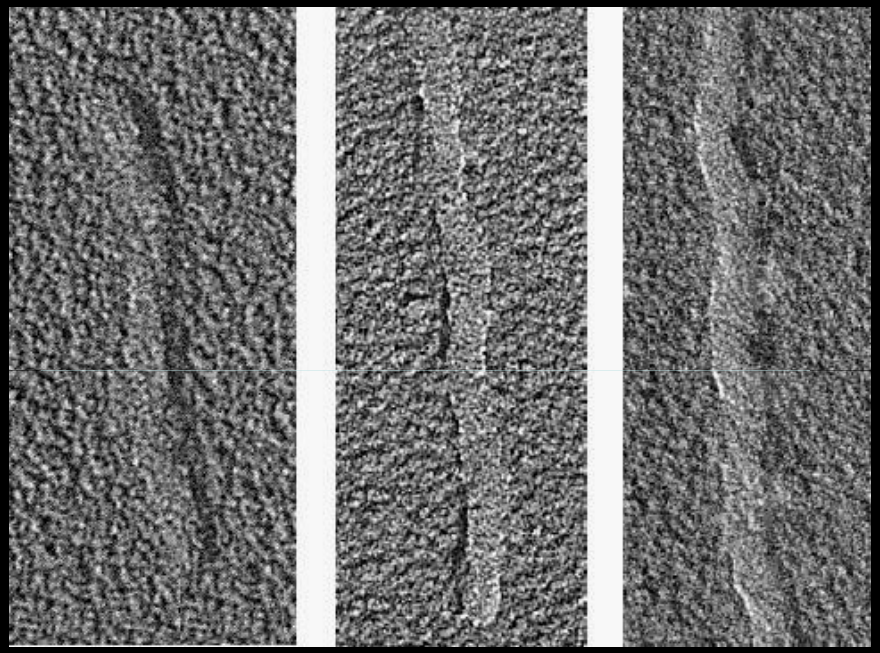
(Adapted from Baxa et al., J. Struct. Biol. 2005)



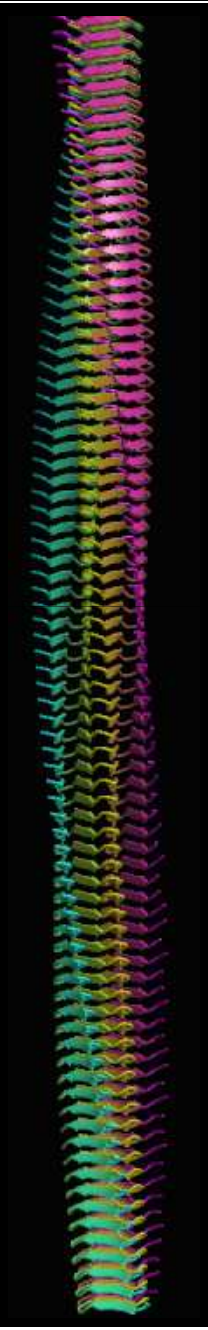
Radial projection

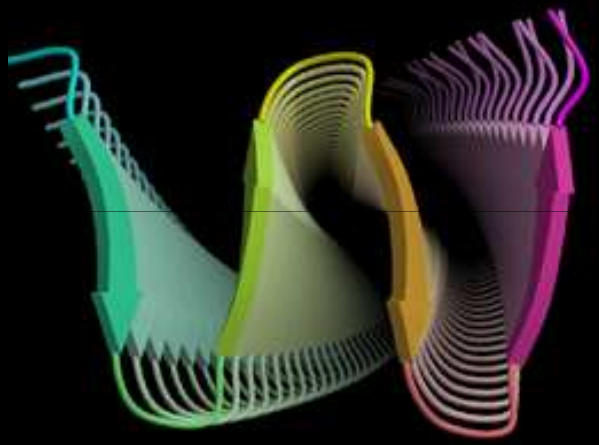


Left-handed twist of fibrils



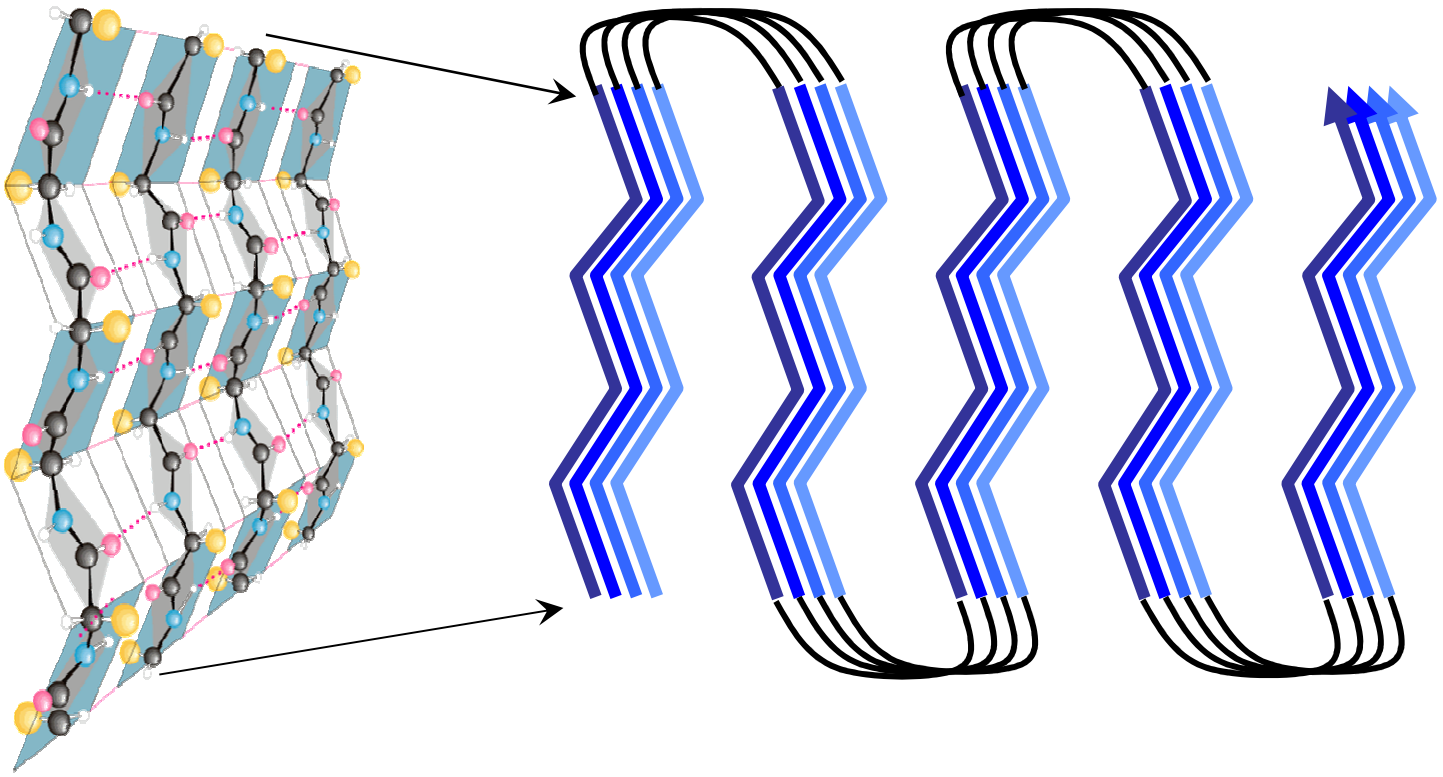
Unidirectional shadowing
of Ure2p(10-80)-GFP





Ure2p(10-39)

Kajava, Baxa, Wickner and Steven
PNAS (2004) 101, 7885.



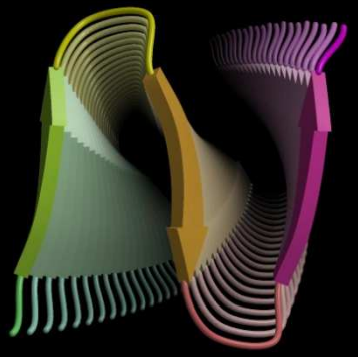
Canonical pleated β-structure

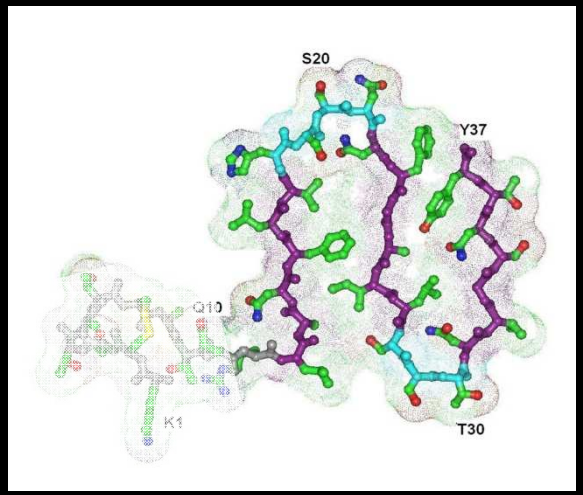
Superpleated β-structure

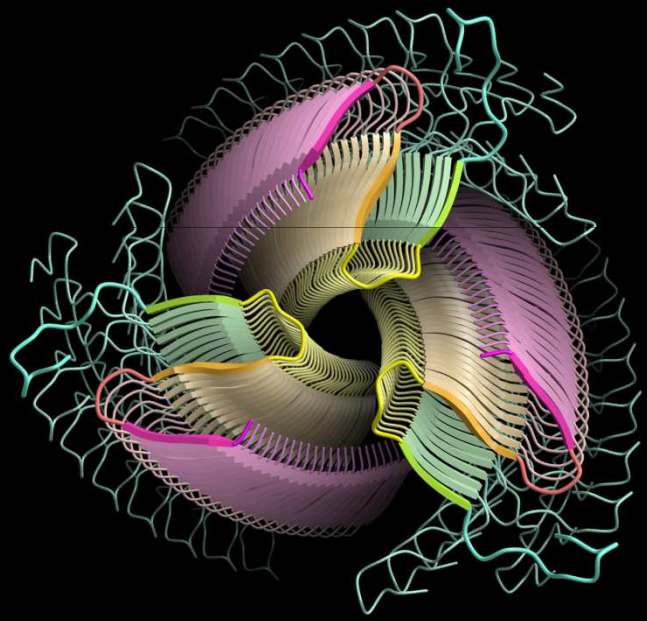
Amyloid Fibrils of Human Amylin

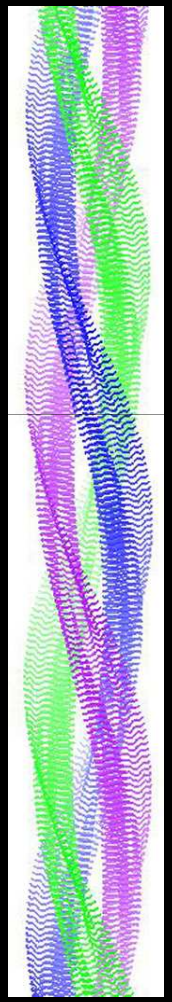
Human amylin is the major component of pancreatic amyloid deposits found in ~ 90% of persons with non-insulin-dependent (type 2) diabetes mellitus.

STEM + EM + X-ray fiber diffr + EPR



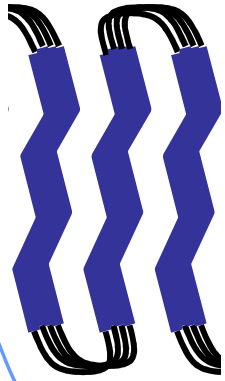






Kajava, Aebi and Steven (2005) J. Mol. Biol 348, 247

Applicability of the superpleated β-structure to other amyloids



Poly(Q) tracts (Huntingtin disease)

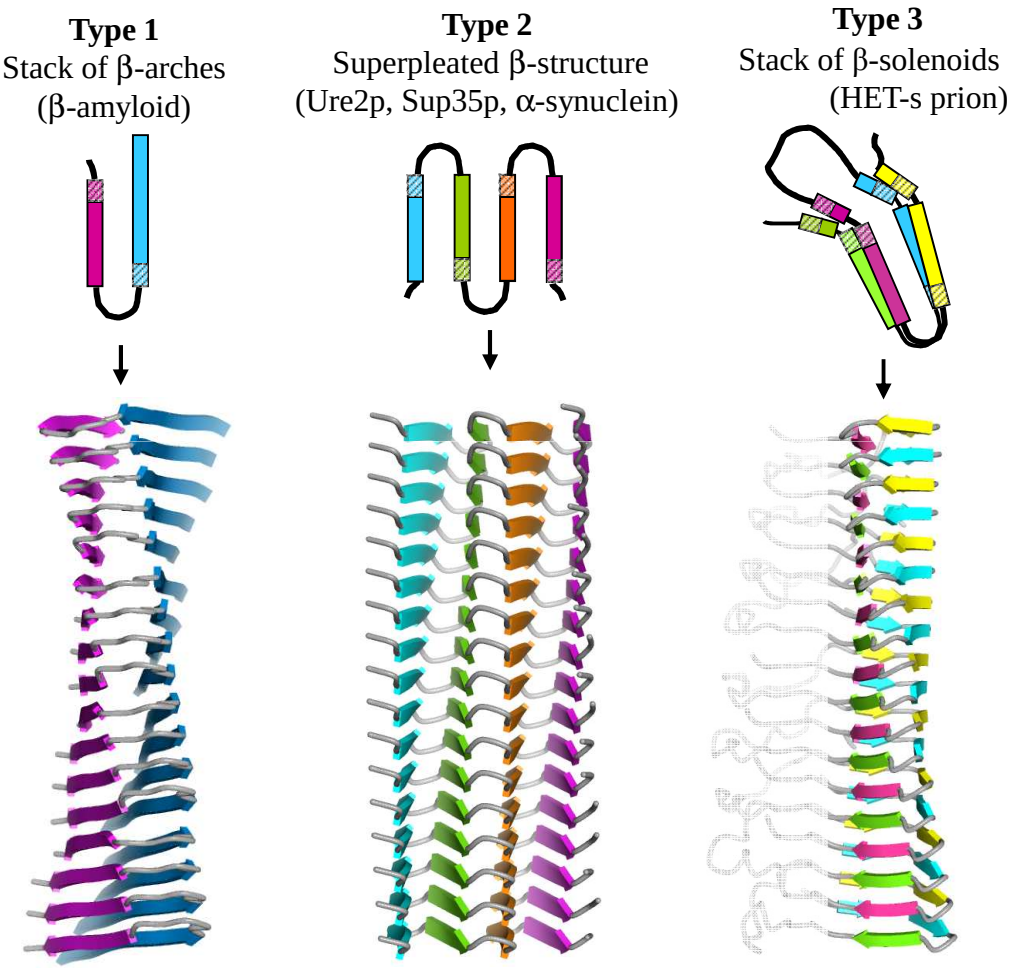
α-synuclein (Parkinson's disease)
(Der-Sarkissian et al., 2003, JBC, 278, 37530)

Tau protein (Alzheimer's disease)
(Margittai and Langen, 2004, PNAS, 101, 10278)

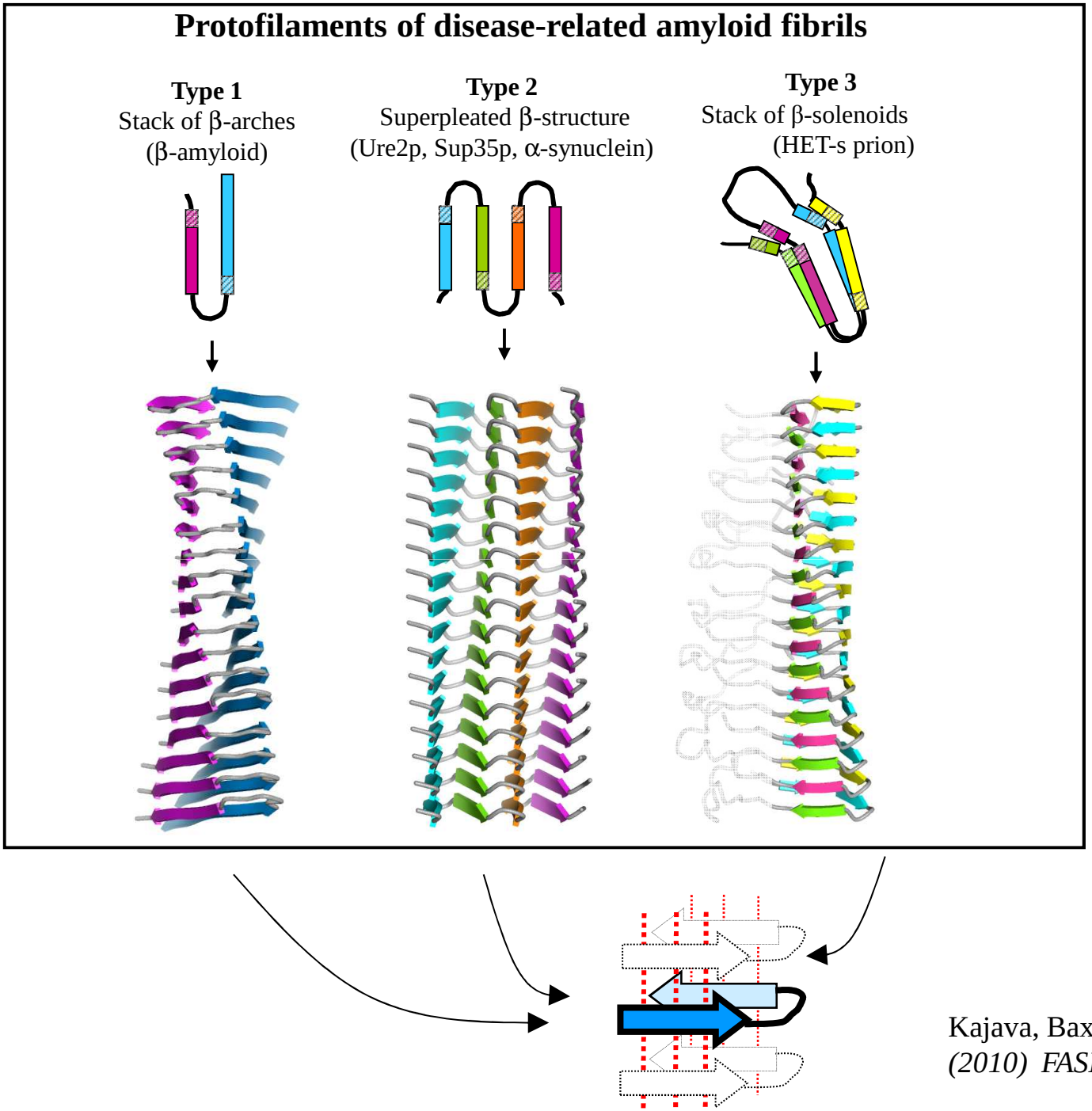
Prion domains of yeast proteins Sup35
(Shewmaker et al., PNAS. 2006103(52):19754)

Kajava, Baxa, Wickner and Steven PNAS (2004) 101, 7885.

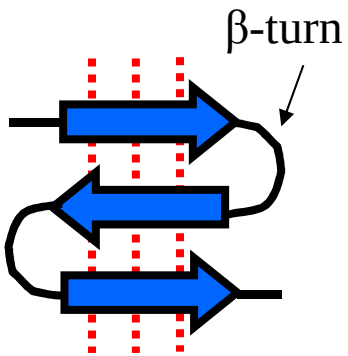
Protofilaments of disease-related amyloid fibrils



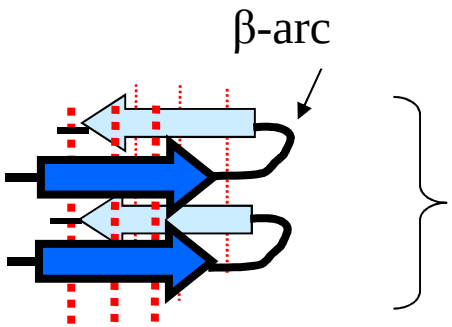
Kajava, Baxa and Steven *(2010) FASEB J. 24:1311*



Kajava, Baxa and Steven
(2010) FASEB J. 24:1311

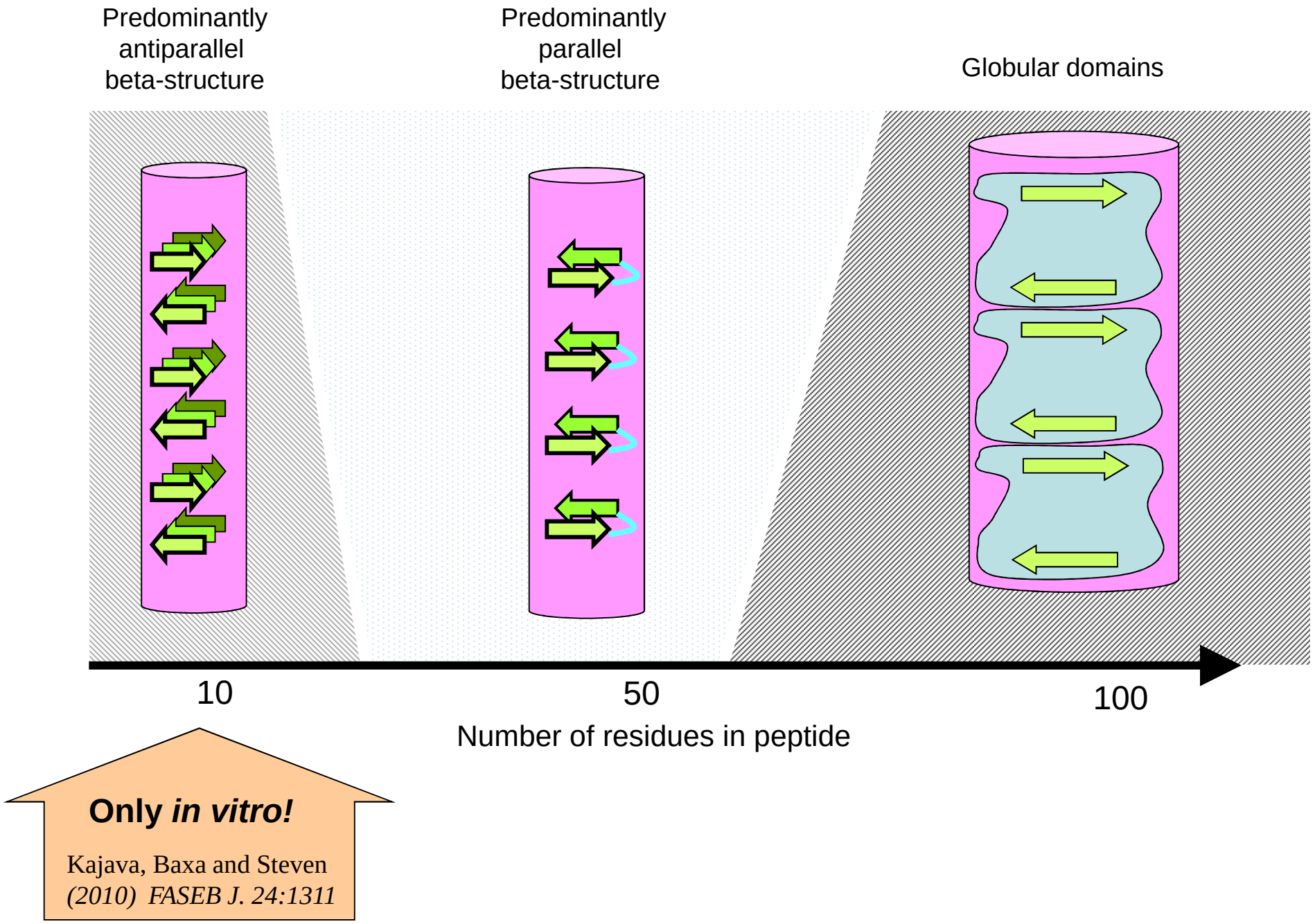


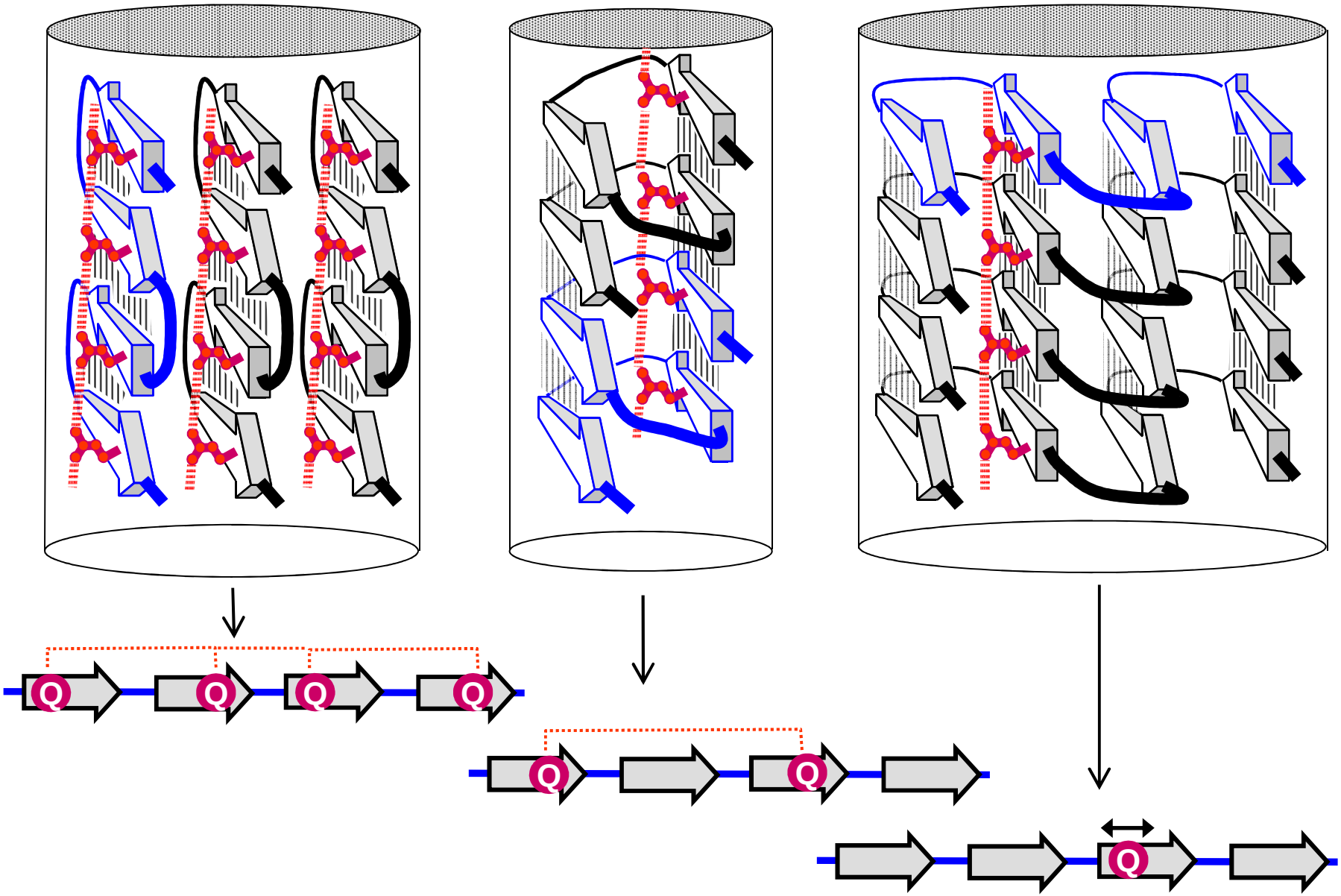
β-hairpins



β-arcade

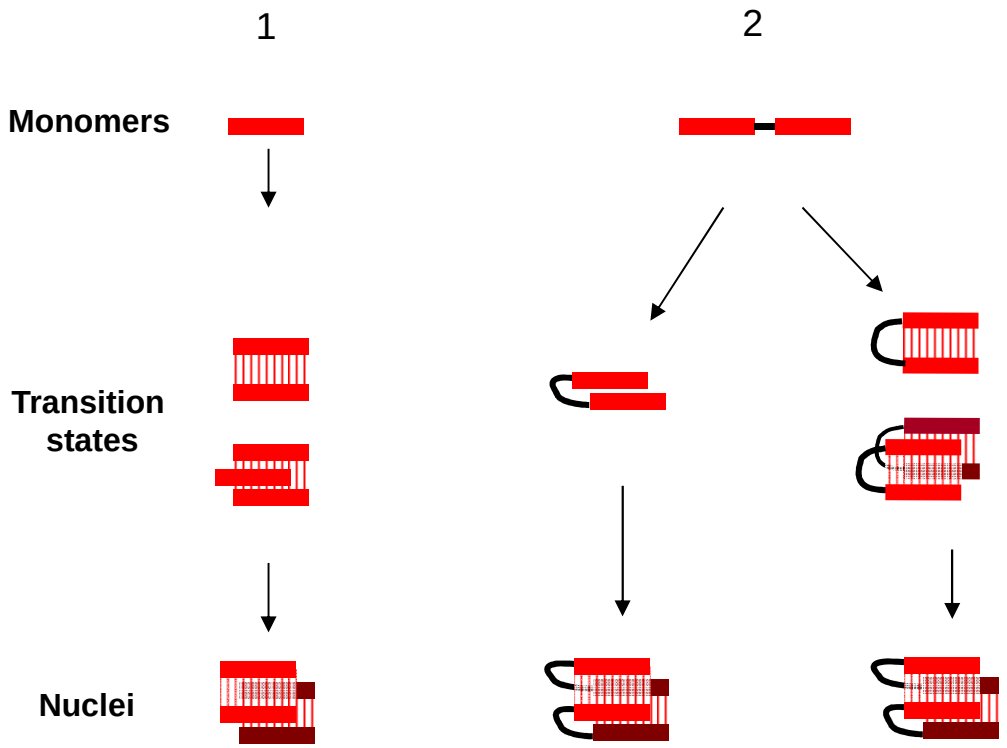
β-arches


Predominantly antiparallel beta-structure
Predominantly parallel beta-structure
Globular domains
10
50
100
Number of residues in peptide
Only *in vitro!*
Kajava, Baxa and Steven *(2010) FASEB J. 24:1311*

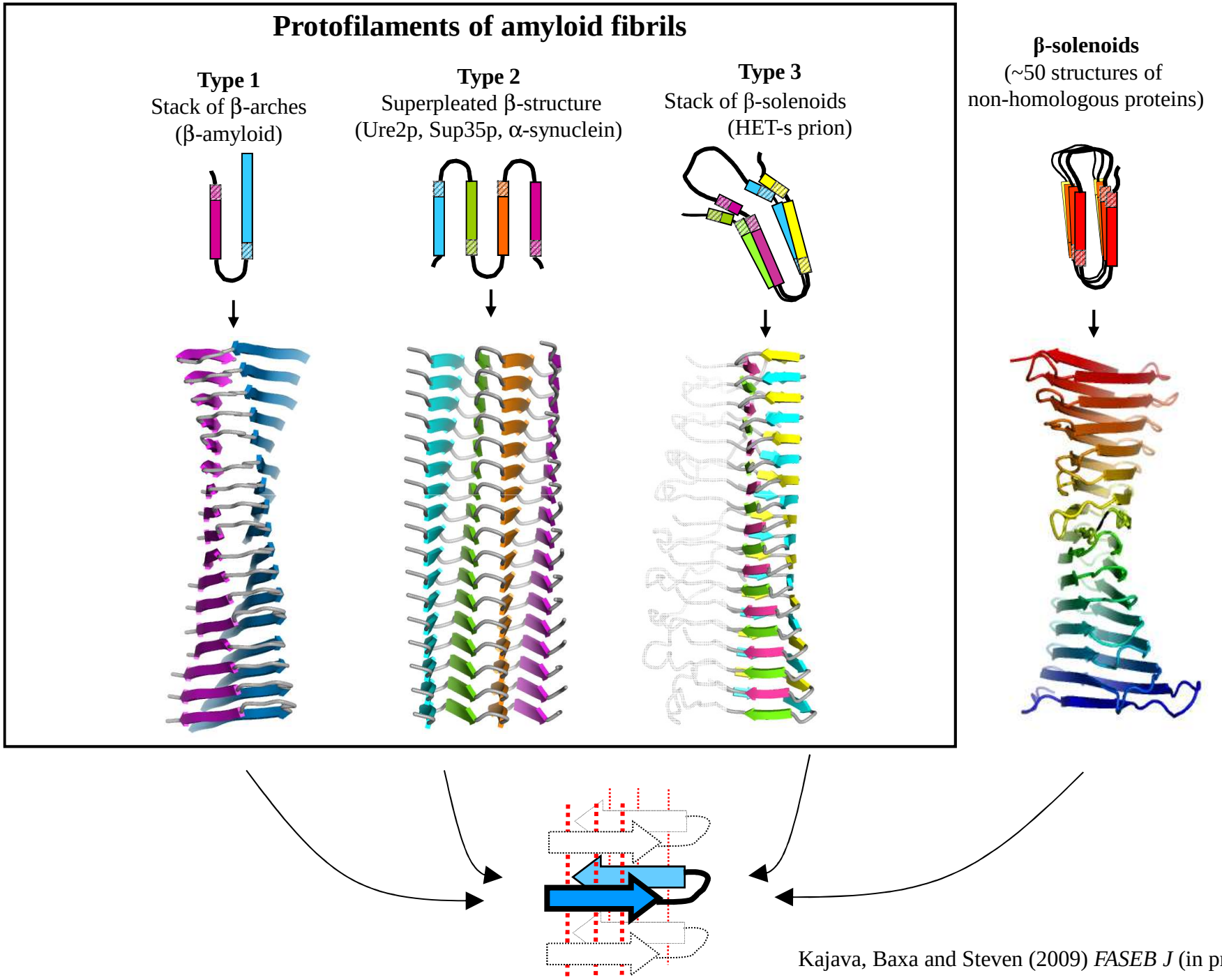


Kajava, Baxa and Steven *(2010) FASEB J. 24:1311*

Beta-arches may provide the best nuclei for fibrillogenesis

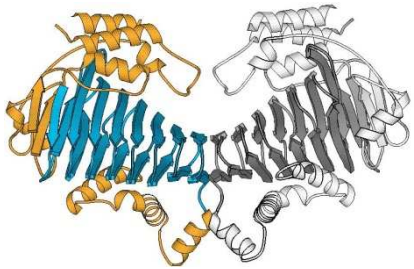


Kajava, Baxa and Steven *(2010) FASEB J. 24:1311*

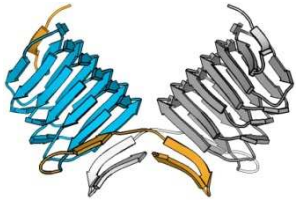


Kajava, Baxa and Steven (2009) *FASEB J* (in press)

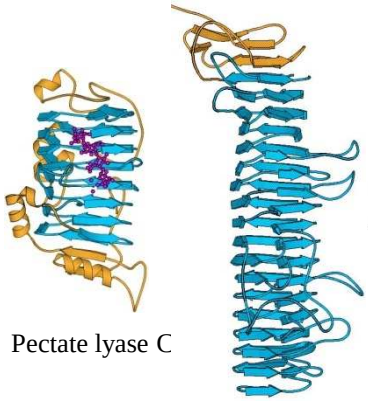
Known β-solenoids



Stabilizer of iron transporter SufD

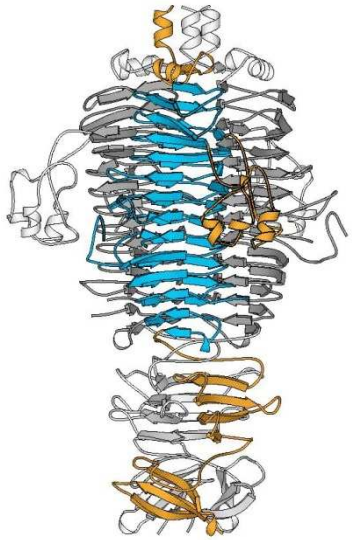


Cyclase-associated protein



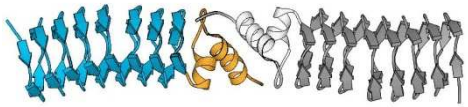
Pectate lyase C

P.69 pertactin

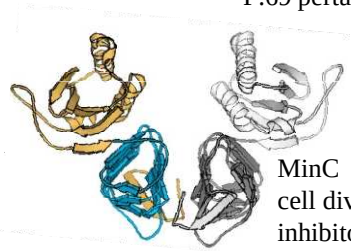


Tailspike endorhamnosidase

Antifreeze protein



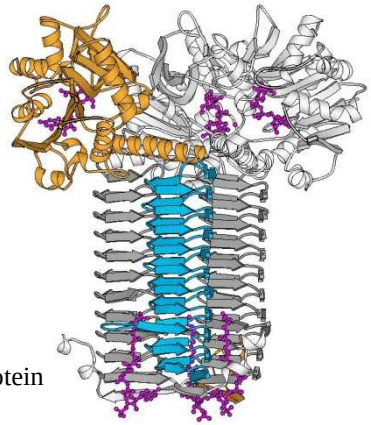
MfpA inhibitor of DNA gyrase



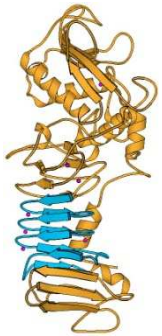
MinC cell division inhibitor

YadA adhesin

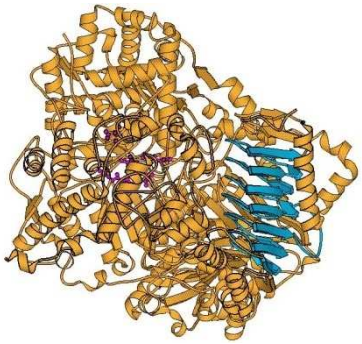
Antifreeze protein



N-acetyl-glucosamine 1-phosphate uridyltransferase



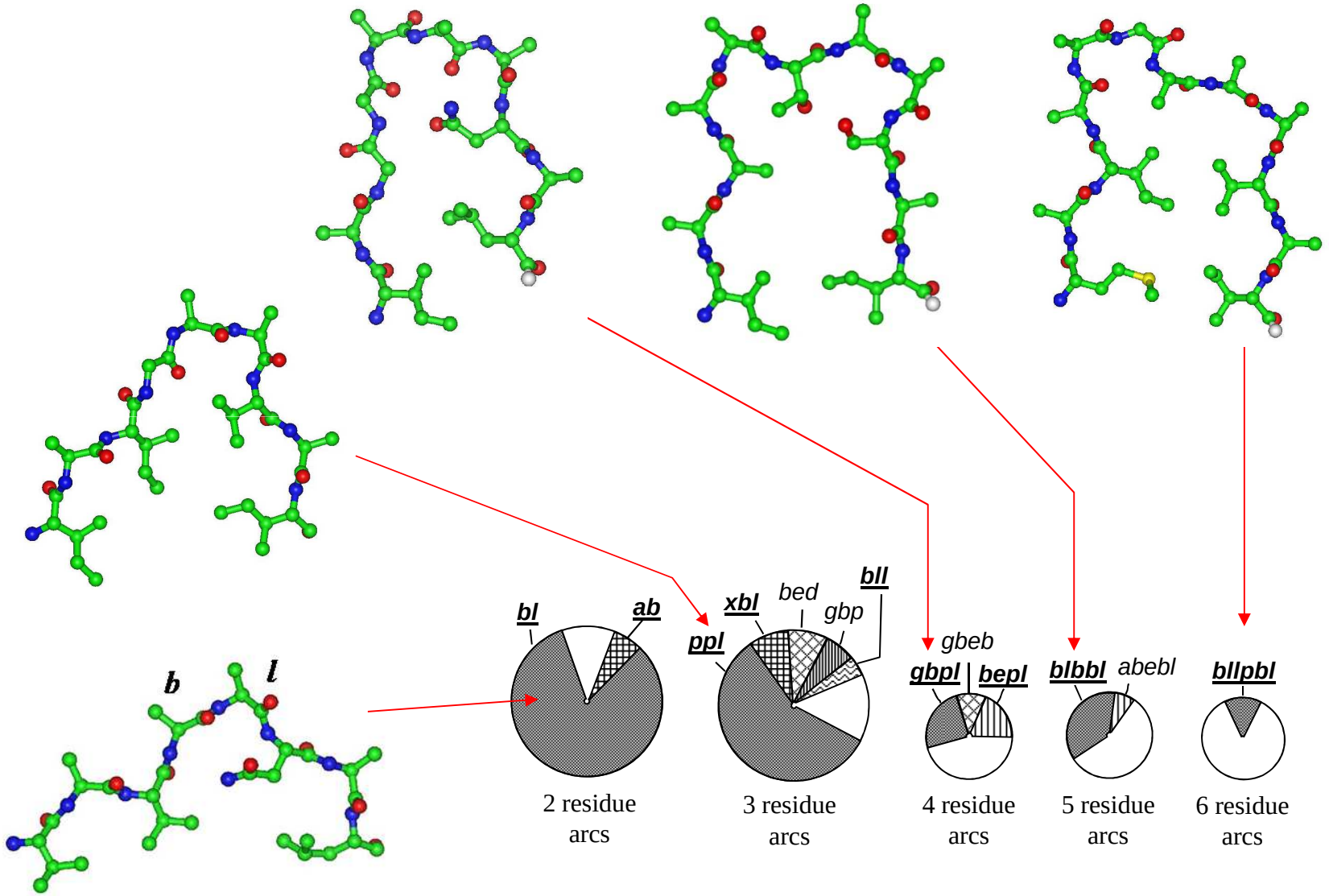
PrtC protease C



Glutamate synthase

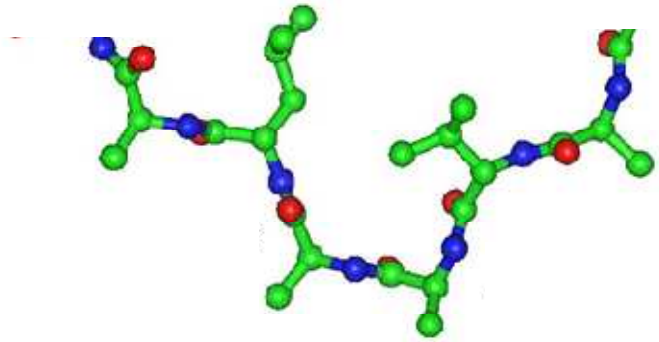
A.V. Kajava and A.C. Steven –(2006) *Advances in Protein Chemistry"* 73:55-96.

Standard conformations of β-arches

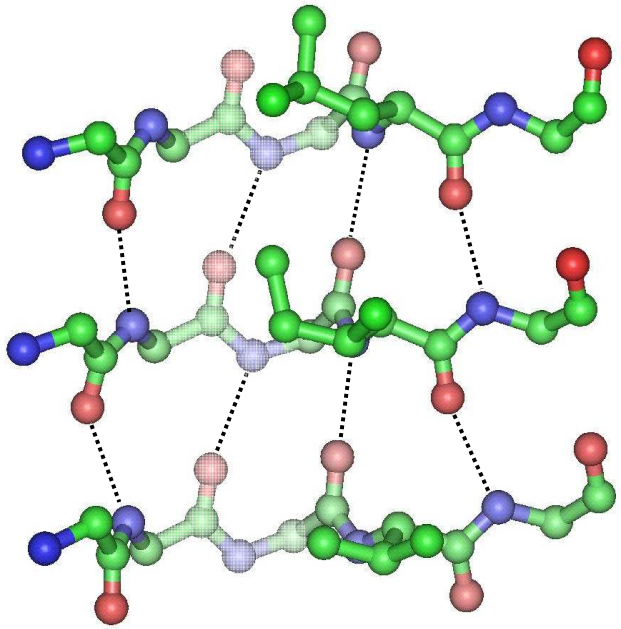


Standard conformations of beta-arches in beta-solenoid proteins
Hennetin, Julien, Stevene and Kajava (2006) J.Mol.Biol., 358, 1094

Standard conformations of β-arches

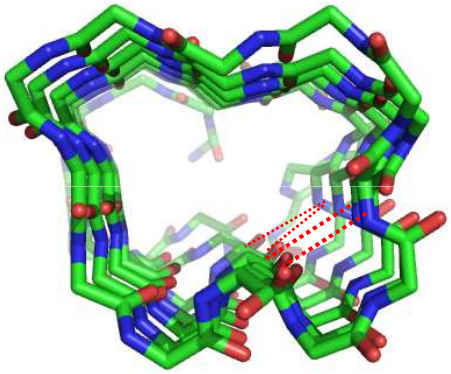


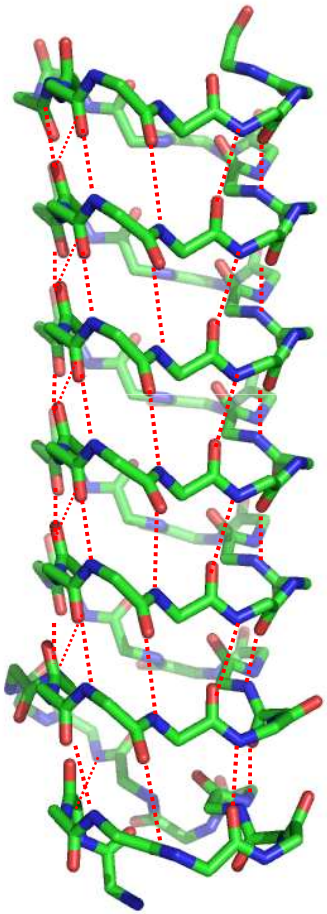
Axial projection
of beta-arch



Lateral projection of
beta-arch stack

CRYSTAL STRUCTURE OF ANTIFREEZE PROTEIN
FROM THE BEETLE, *TENEBRIO MOLITOR*



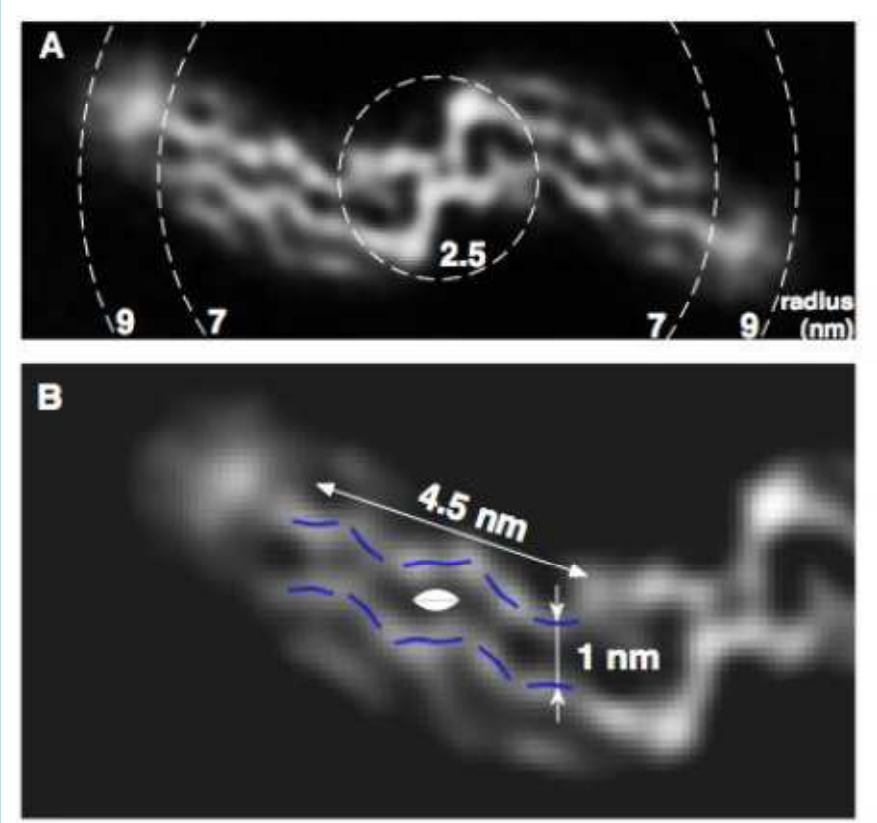


Liou, Y.C., Tocilj, A., Davies, P.L., Jia, Z. (2000) Nature **406:** 322-324

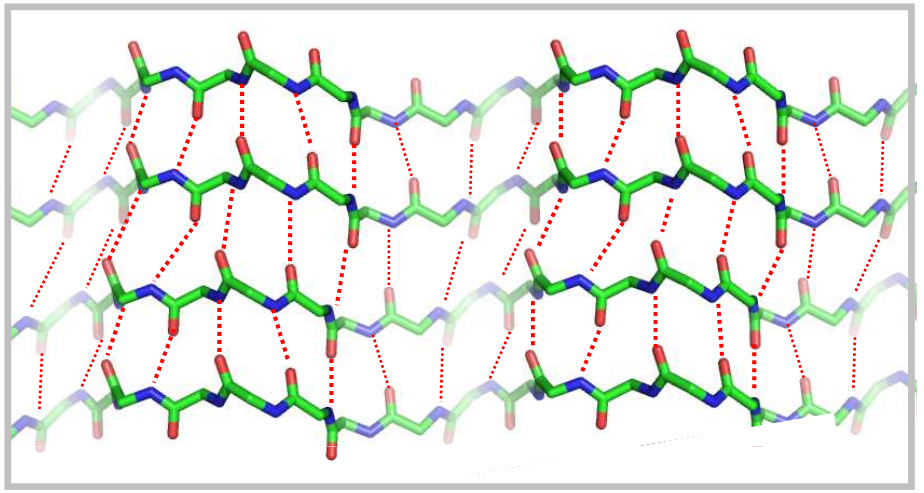
Corrugated paired beta-sheet

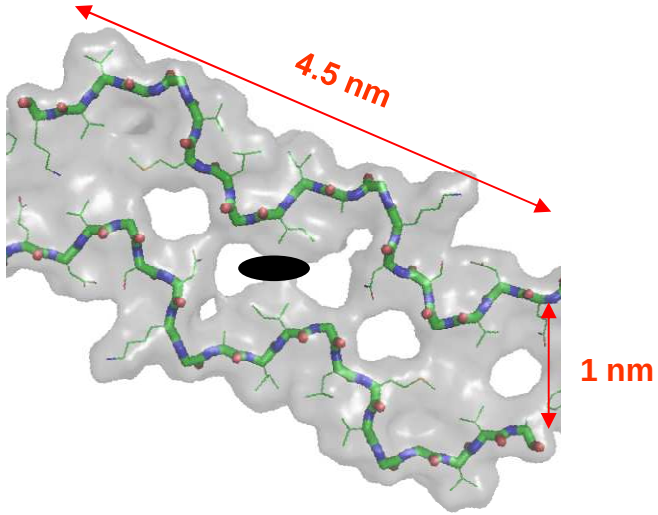
Paired beta-sheet structure of an Abeta(1-40) amyloid fibril revealed by electron microscopy.

Sachse, Fändrich, Grigorieff, *PNAS*, 2008, 105:7462

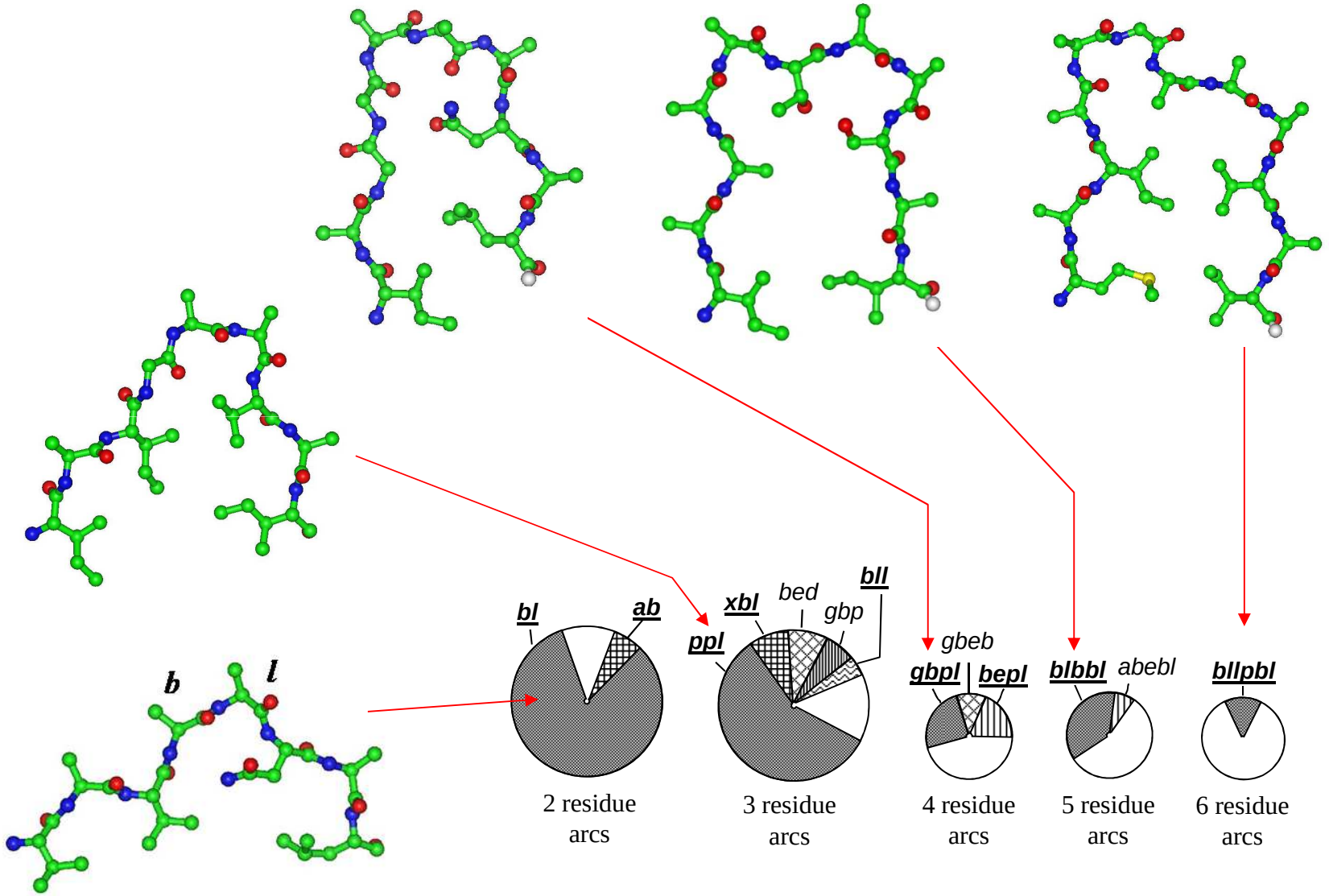


Molecular model



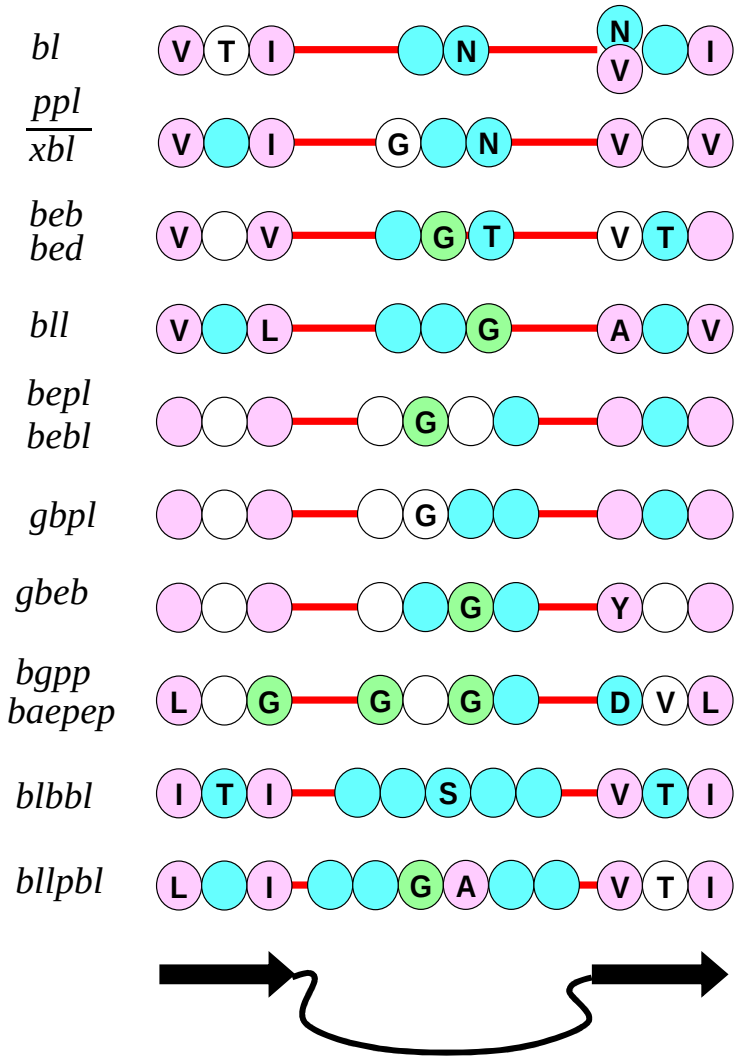


Standard conformations of β-arches



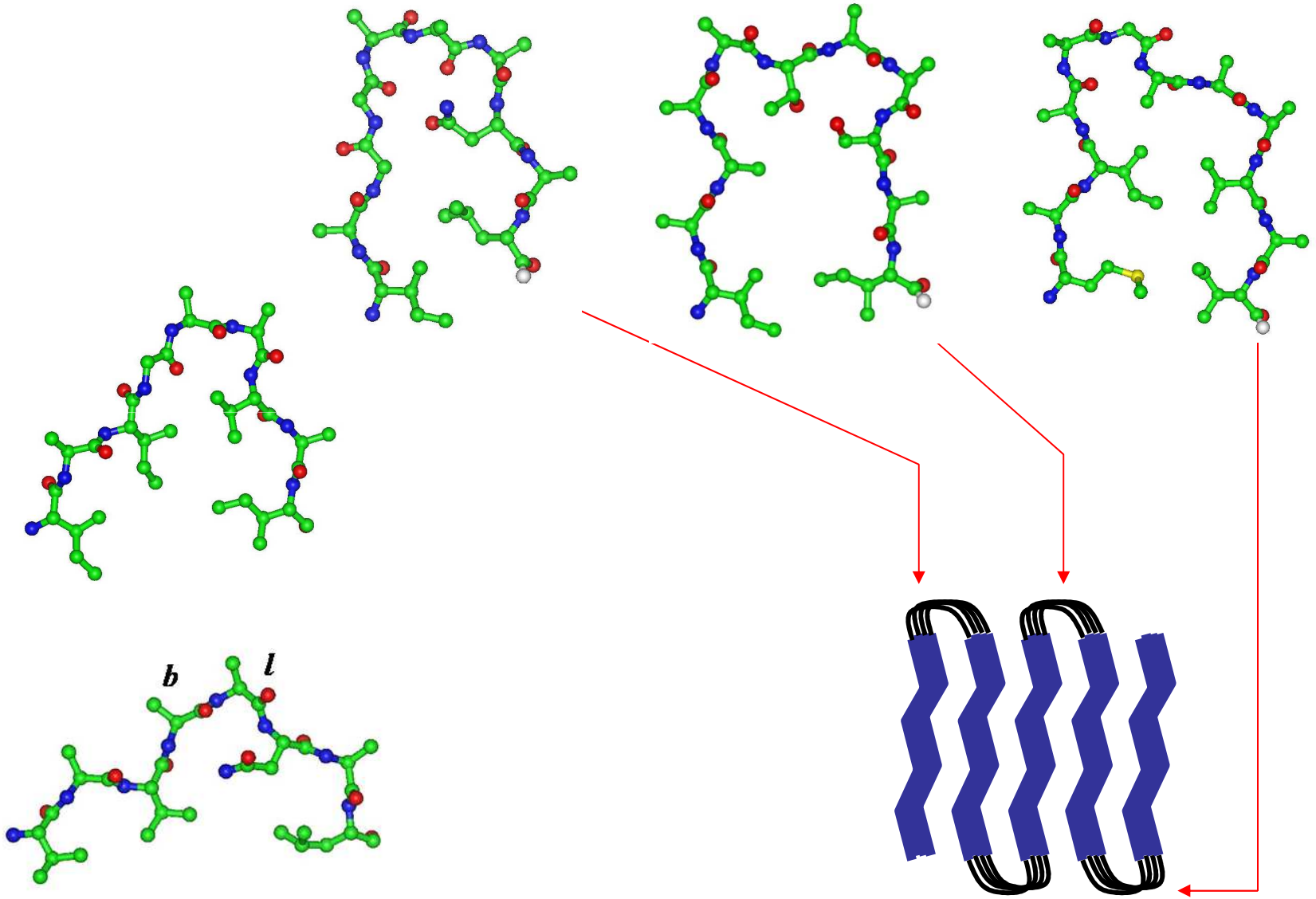
Standard conformations of beta-arches in beta-solenoid proteins
Hennetin, Julien, Stevene and Kajava (2006) J.Mol.Biol., 358, 1094

Standard conformations of β-arches



Hennetin et al., (2006) J.Mol.Biol., 358, 1094

Prediction of amyloidogenicity of proteins

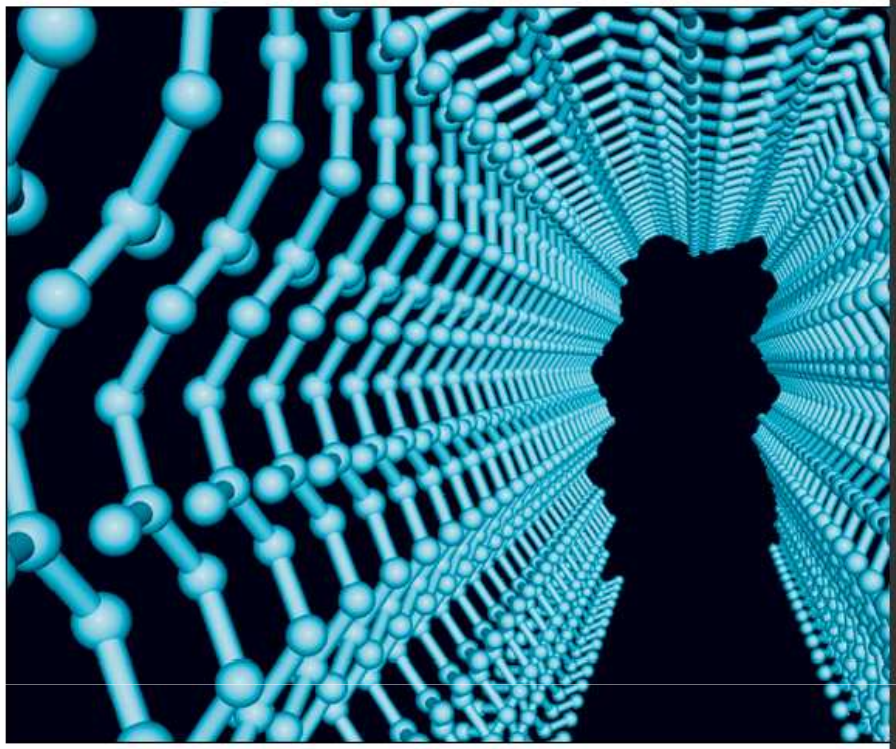


CONCLUSIONS

Stack of β-arches is a common arrangement of disease-related amyloid fibrils.

This can be explained by capacity of β-arch stacks
(1) to be stabilized not only by apolar residues but also by polar residues,
(2) to be the most efficient nuclei for amyloidogenesis

Known β-arcs have preferred conformations and sequence motifs. We identified them. This information can be used for prediction and modeling of amyloid fibrils.



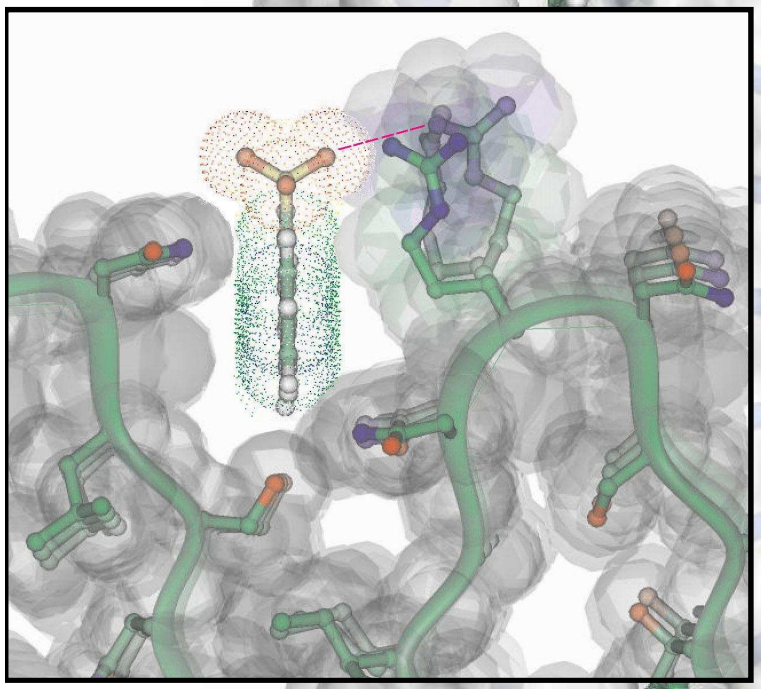
ФИНИШ ПОД БЕЛКОВОЙ АРКАДОЙ?

Человечество находится в постоянном поиске лекарств, медицинских технологий и рецептов образа жизни, которые бы позволили ему жить дольше. И успехи бесспорны: в течение последнего полувека средняя продолжительность жизни в экономически развитых странах возрастала каждые пять лет на один год. Сегодня люди, живущие 80 лет и более, уже не редкость. Однако, чем дольше мы живём, тем отчётливей вырисовывается очередное серьёзное препятствие на нашем пути к дальнейшему увеличению продолжительности жизни. Это нейродегенеративные заболевания: болезни Альцгеймера, Паркинсона, синдром Хантингтона и другие, ведущие к старческому слабоумию. На сегодняшний день эти болезни неизлечимы и заканчиваются постепенным угасанием психических функций и неминуемой смертью. Рост случаев нейродегенеративных заболеваний по мере старения впечатляет. Среди доживших до семидесяти такими болезнями страдает не более чем каждый тридцатый, а среди девяностолетних это уже чуть ли не каждый третий.

Андрей КАЯВА, научный директор
Исследовательского центра макромолекулярной биохимии, CNRS, Франция.

Молекулярные механизмы нейродегенеративных заболеваний до конца не выяснены и остаются объектом жарких дискуссий. Очевидно одно — мозговые ткани всех пациентов, страдающих такого рода болезнями, содержат нерастворимые отложения (белковые «бляшки»). Белки — основные молекулы живых организмов, представляющие собой цепочки из соединённых пептидными связями аминокислот. Существует 20

Potential impact of the results



More precise models

⇩

Structure-based design
of inhibitors of fibrillogenesis

Better prediction
of amyloidogenicity
of proteins

⇩

Patient-oriented risk prediction
to develop age-related,
neurodegenerative
and other diseases

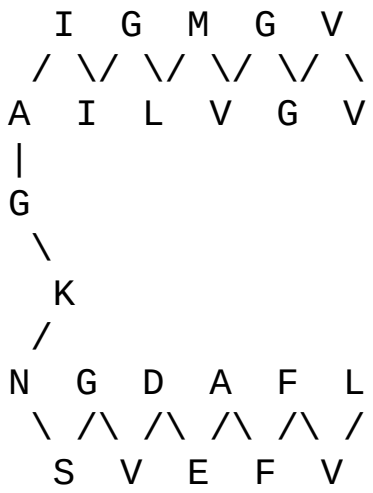
Computer program for identification of regions that can form beta-arcades and prediction of their 3D structure

arch candy

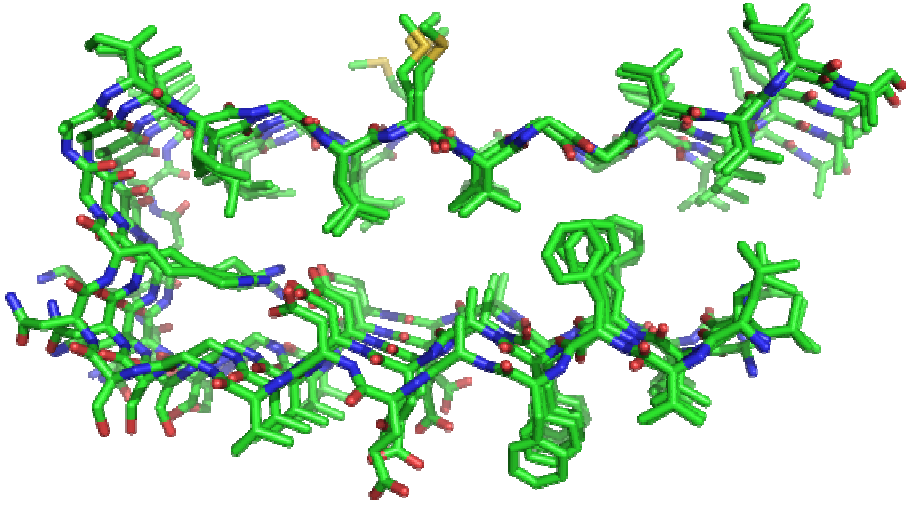
It correctly predicts 3D structures available in PDB : 2LMN, 2BEG, 2LQN - different forms of Abeta, 2E8D - beta2-microglobulin, 2NNT Human CA150

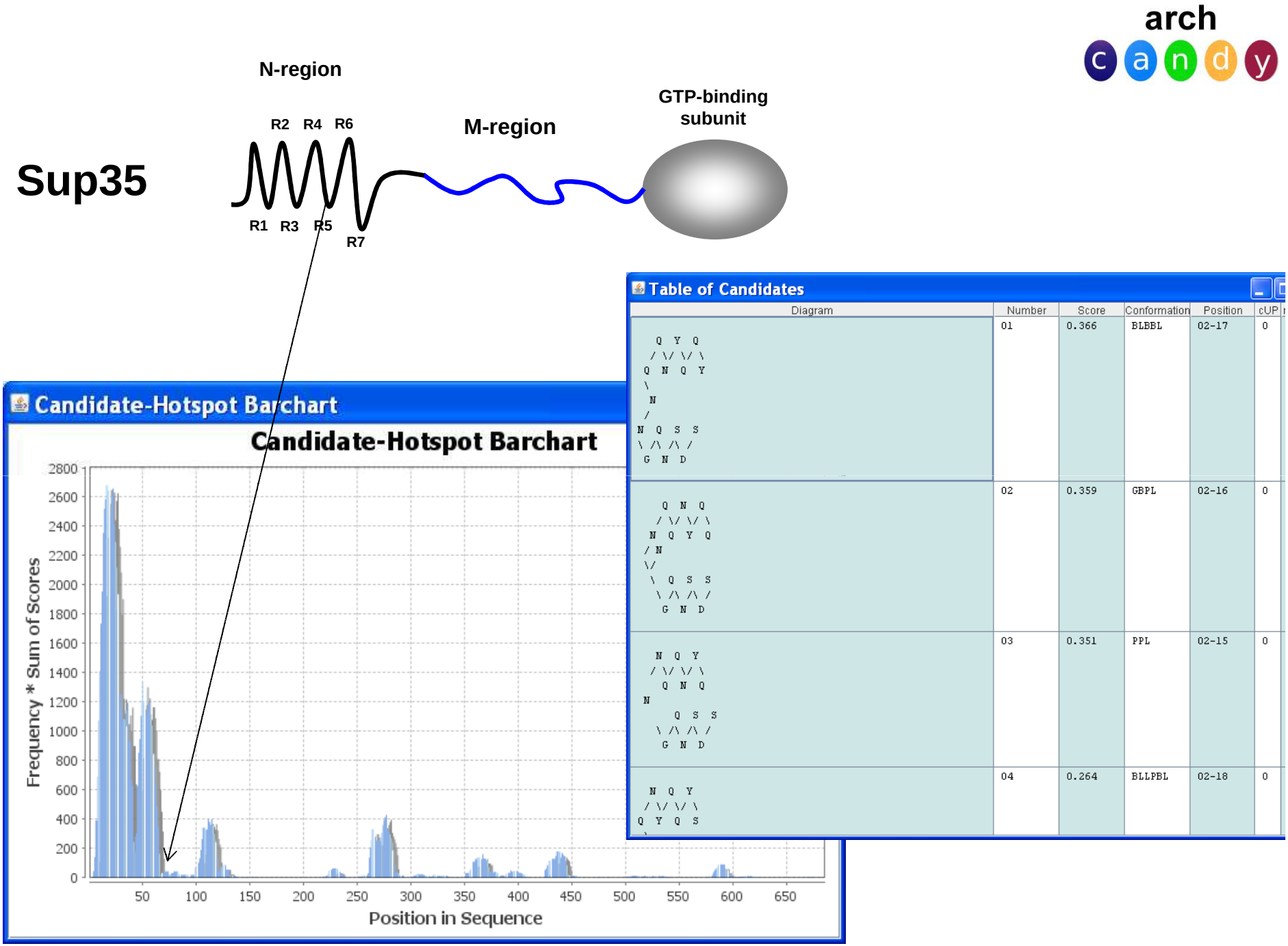
and explains the increase of amyloidogenicity in Abeta mutants linked to FAD.

Prediction of the 3D Structure of Alzheimer's Abeta(1-42) fibrils



Known structure PDB code 2BEG




arch candy
N-region
GTP-binding subunit
R2 R4 R6
M-region
Sup35
R1 R3 R5
R7
Table of Candidates
Diagram
Number
Score
Conformation
Position
cUP
01
0.366
BLBBL
02-17
0
02
0.359
GBPL
02-16
0
03
0.351
PPL
02-15
0
04
0.264
BLLPBL
02-18
0
Candidate-Hotspot Barchart
Candidate-Hotspot Barchart
Frequency * Sum of Scores
Position in Sequence
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
50
100
150
200
250
300
350
400
450
500
550
600
650

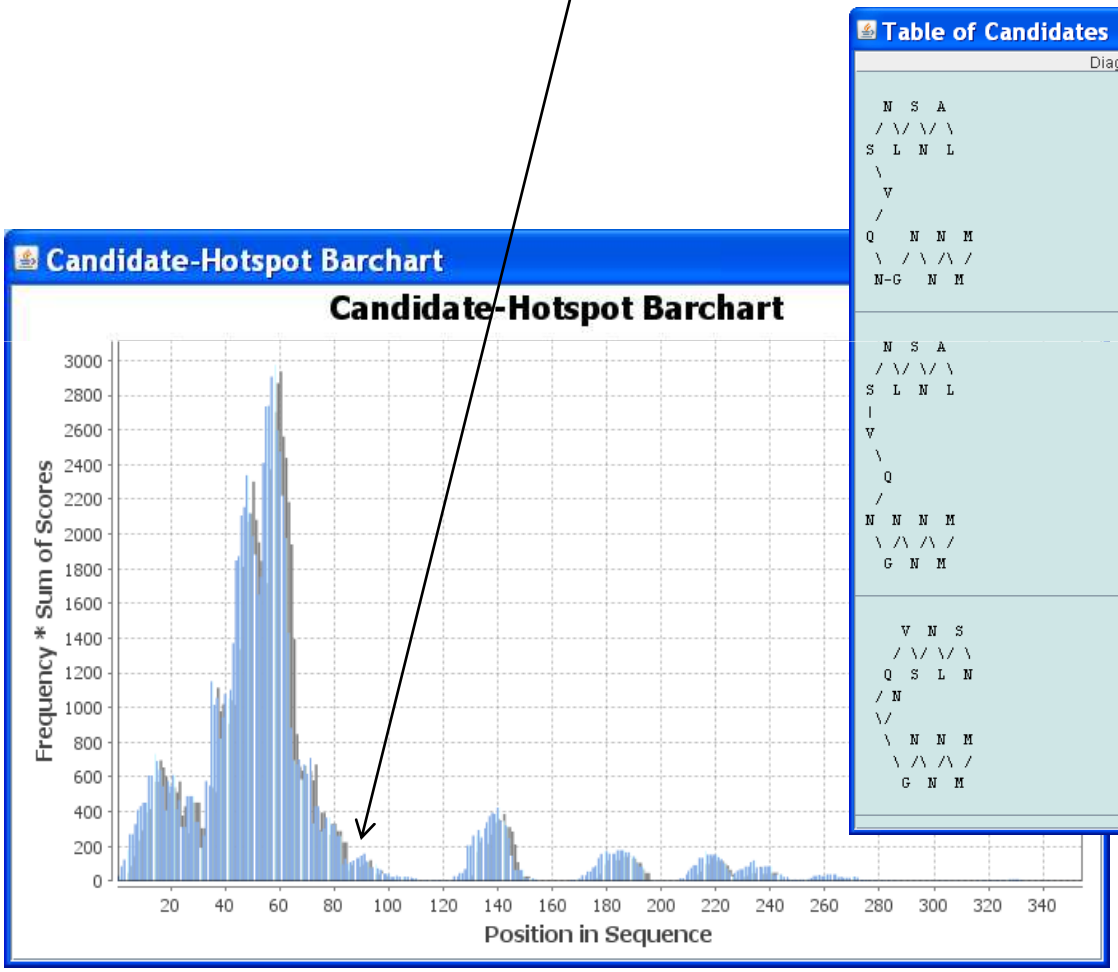
arch candy

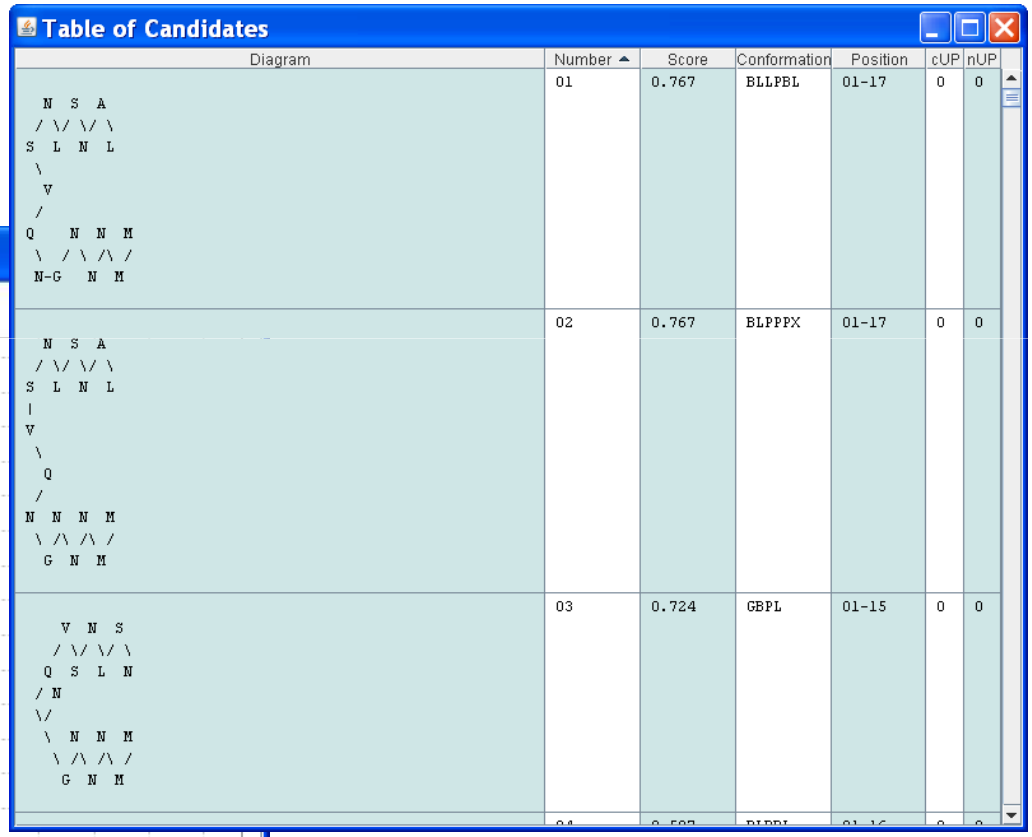
Prion domain

Functional globular domain

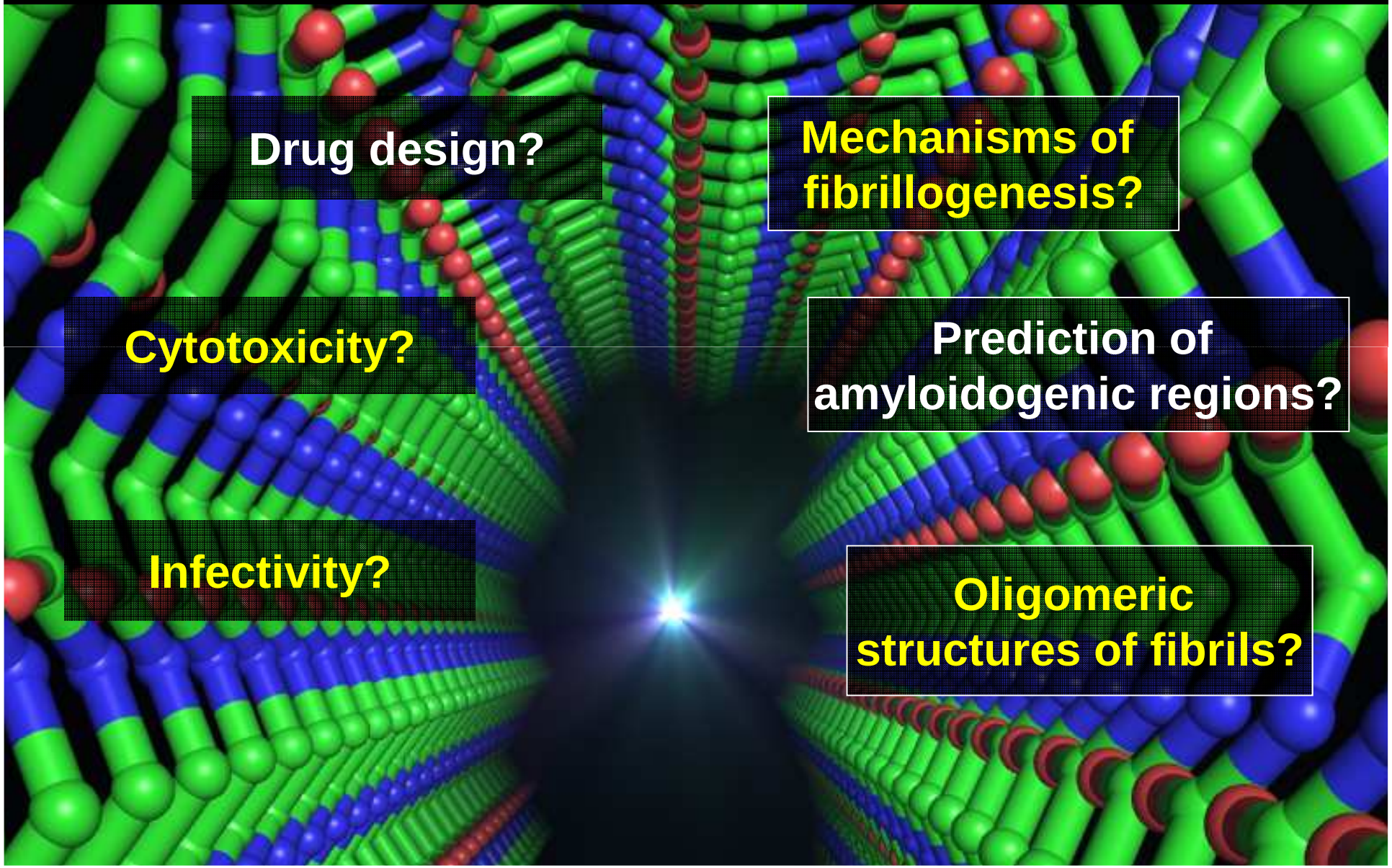
Ure2p

1 70 96 354





Where do beta-arcades lead?



Alasdair Steven NIAMS, NIH, USA

Ulrich Baxa NIAMS, NIH, USA

Reed Wickner NIDDK, NIH, USA (Ure2p)

Ueli Aebi Biozentrum Basel, Switzerland (Amylin)

Galina Zhouravleva S.Petersbourg University, Russia

Jerome Hannetin CRBM, CNRS, France (Beta-arches)
Berangere Jullian , CRBM, CNRS, France (Beta-arches)
Abdullah AHMED , CRBM, CNRS, France (Beta-arches)