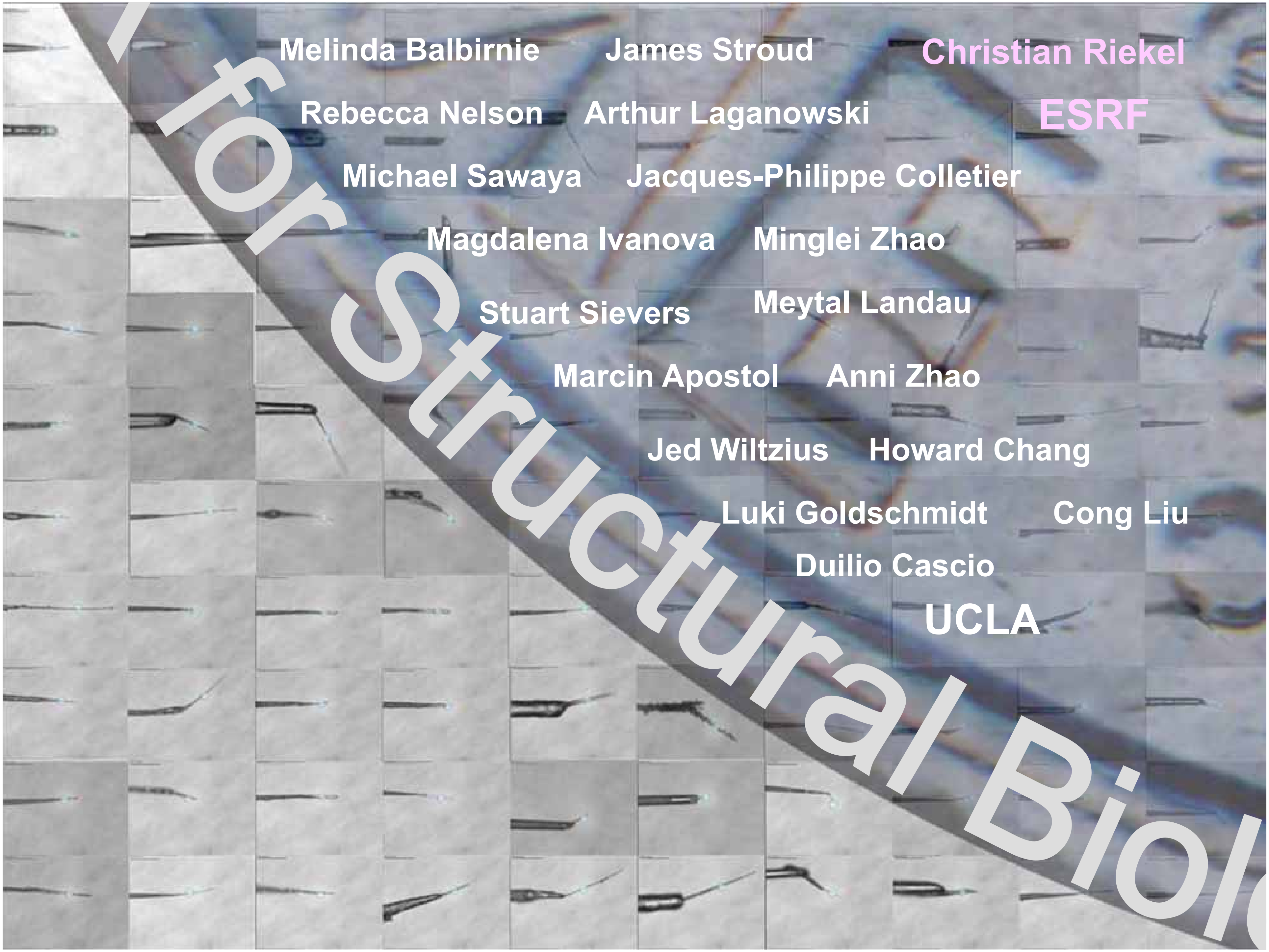
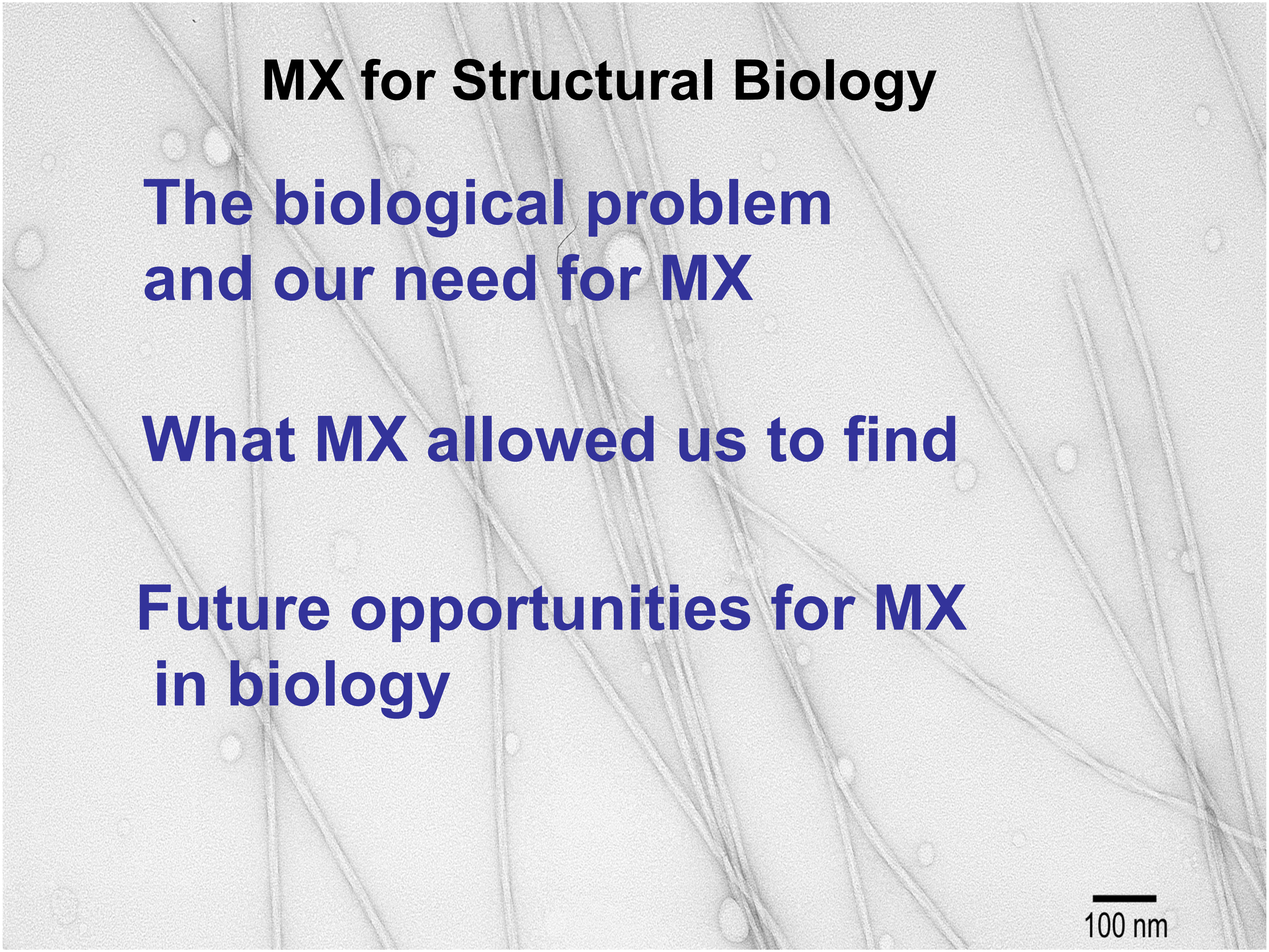

for Structural Biol
Melinda Balbirnie
James Stroud
Christian Riekel
Rebecca Nelson
Arthur Laganowski
ESRF
Michael Sawaya
Jacques-Philippe Colletier
Magdalena Ivanova
Minglei Zhao
Stuart Sievers
Meytal Landau
Marcin Apostol
Anni Zhao
Jed Wiltzius
Howard Chang
Luki Goldschmidt
Cong Liu
Duilio Cascio
UCLA

MX for Structural Biology

The biological problem and our need for MX

What MX allowed us to find

Future opportunities for MX in biology

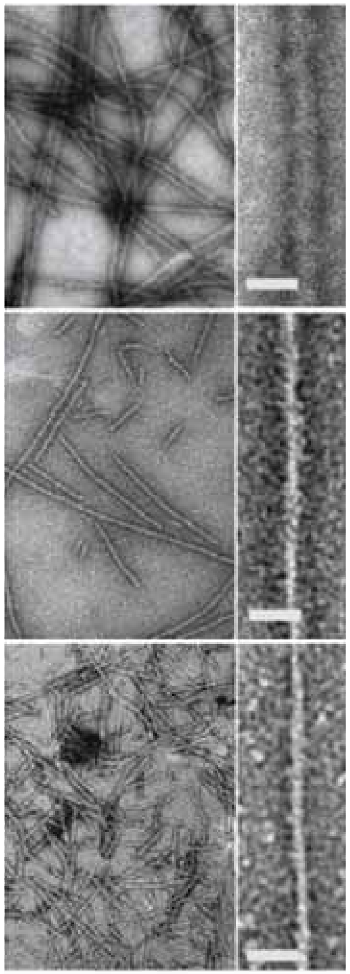


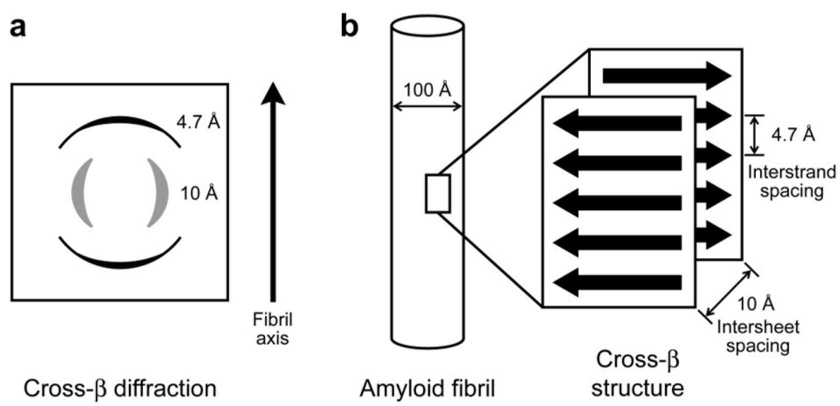
Amyloid

- Unbranched, elongated protein fibrils
- Associated with varied diseases (e.g. CJD Alzheimer's, Dialysis-related amyloidosis)
- Bind Congo Red with green birefringence
- Functional and denatured amyloids
- Cross-β diffraction pattern shows β strands perpendicular to fiber axis: common spine

Pathol-ogists

Bio-physicists





Kishimoto, Namba et al (2004)

Amyloid Fibril-Related Conditions

Amyloid (24)		Prion (transmissible)		Amyloid-like	
Alzheimer's	Aβ	[Psi+]	Sup35		
Alzheimer's	Tau	[Ure2]	Ure3	Parkinson's	α-synuclein
Diabetes II	Amylin aka IAPP			LouGehrig's (ALS)	Superoxide Dismutase TDP-43
Injection amyloidosis	Insulin	CJD, GSS Kuru	PrP	HIV Sexual trans-mission	SEVI
Dialysis amyloidosis	β2-micro-globulin	BSE, vCJD (mad cow)	PrP	Cancer	p53
Senile amyloidosis	Trans-thyritin				

100 nm

My Scientific Dilemma in 2001

Important biological problem—structure of amyloid fibers

5.4 M Alzheimer’s patients in US in 2010

~19 M patients expected by 2050

Economic burden:

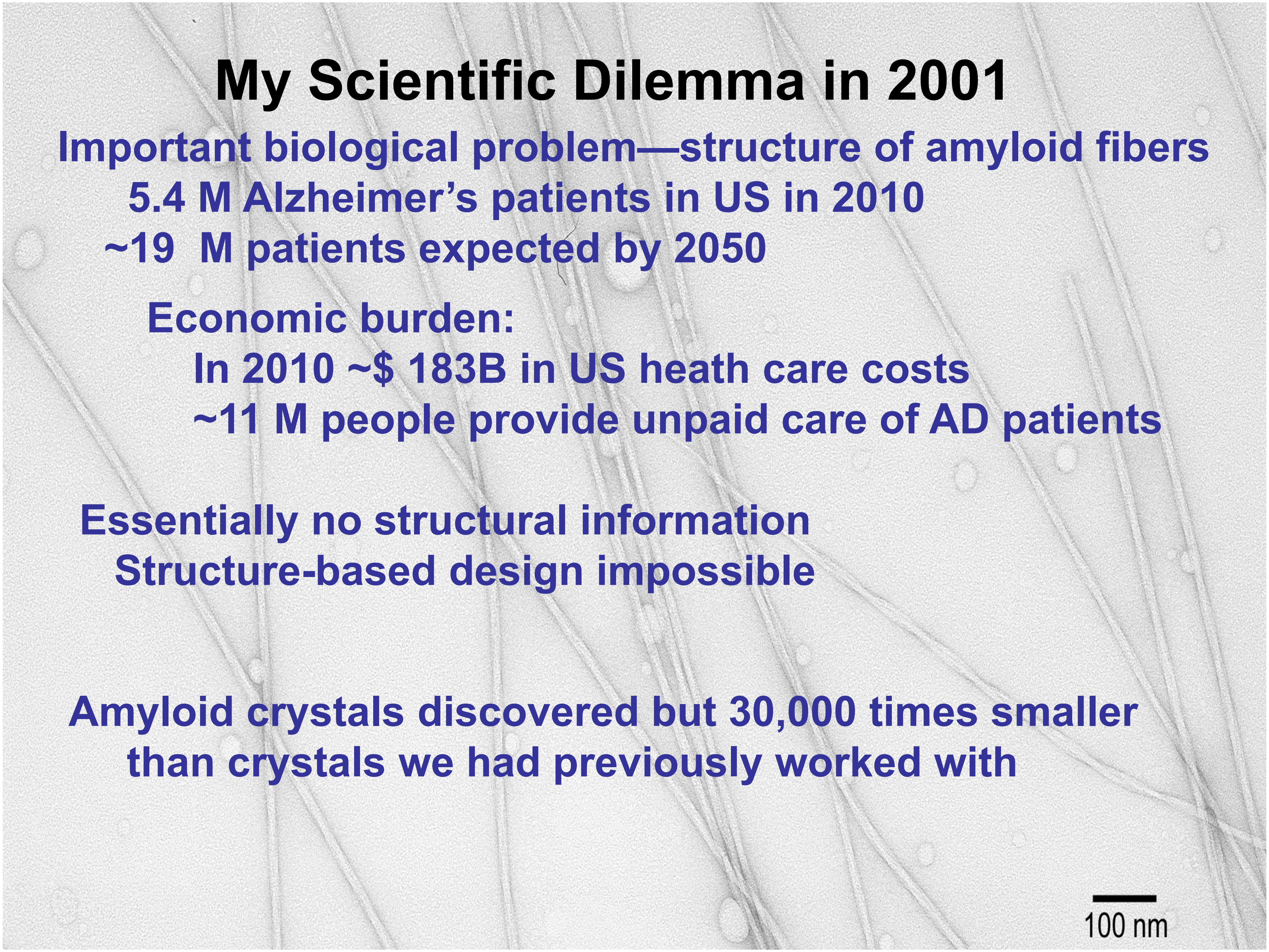
In 2010 ~$ 183B in US heath care costs

~11 M people provide unpaid care of AD patients

Essentially no structural information

Structure-based design impossible

Amyloid crystals discovered but 30,000 times smaller than crystals we had previously worked with

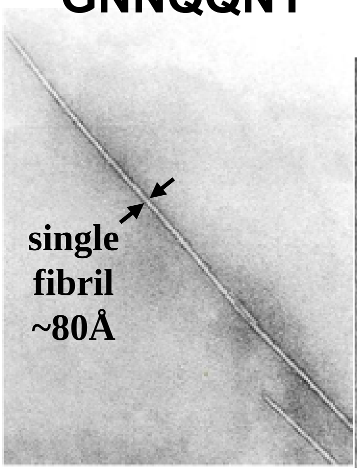


Short segments of fiber-forming proteins form both amyloid fibers and microcrystals

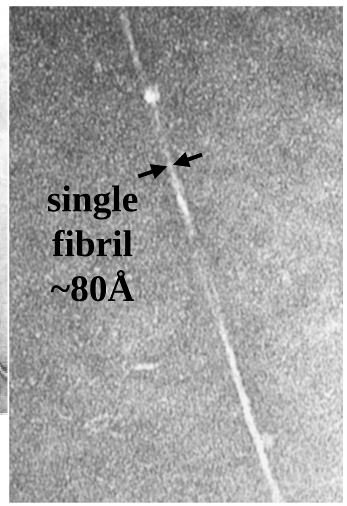
Balbirnie et al. PNAS 2001

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

GNNQQNY



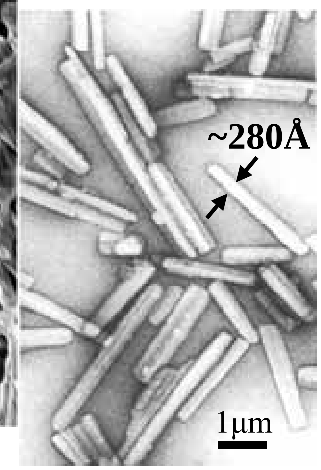
NNQQ



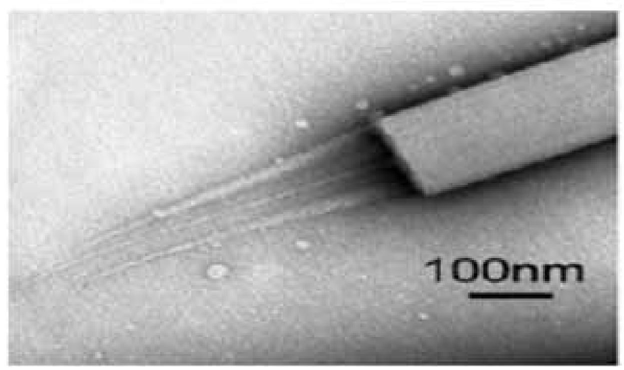
NNQQ

NNQQNY

GNNQQNY



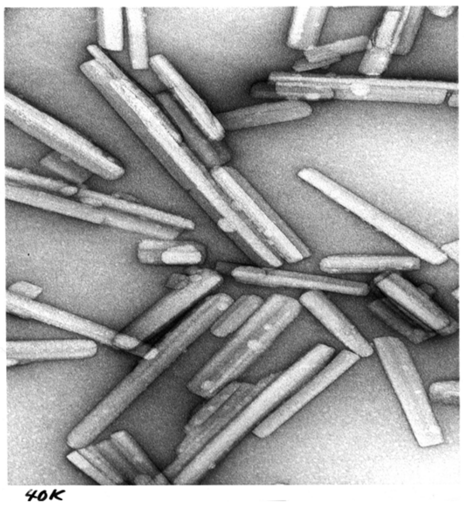
GNNQQNY fibrils exhibit all properties of amyloid fibrils: dye binding, cooperative aggregation kinetics, stability, cross-β diffraction



Fibers seem to grow from tips of crystals

GNNQQNY microcrystals

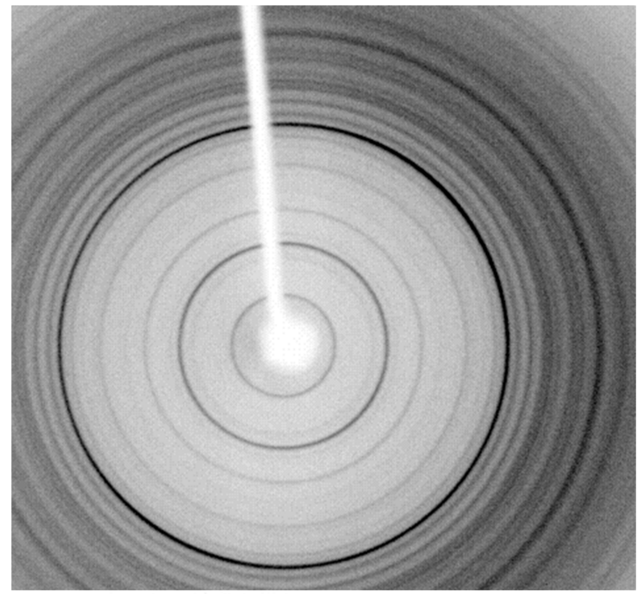
10-100 mM < 24 hr



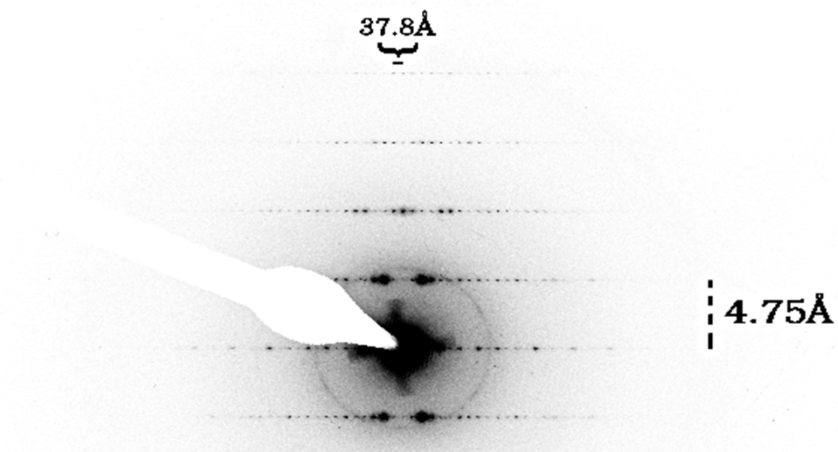
Crystal width = ~280 Å

Balbirnie et al. PNAS 2001

X-rays

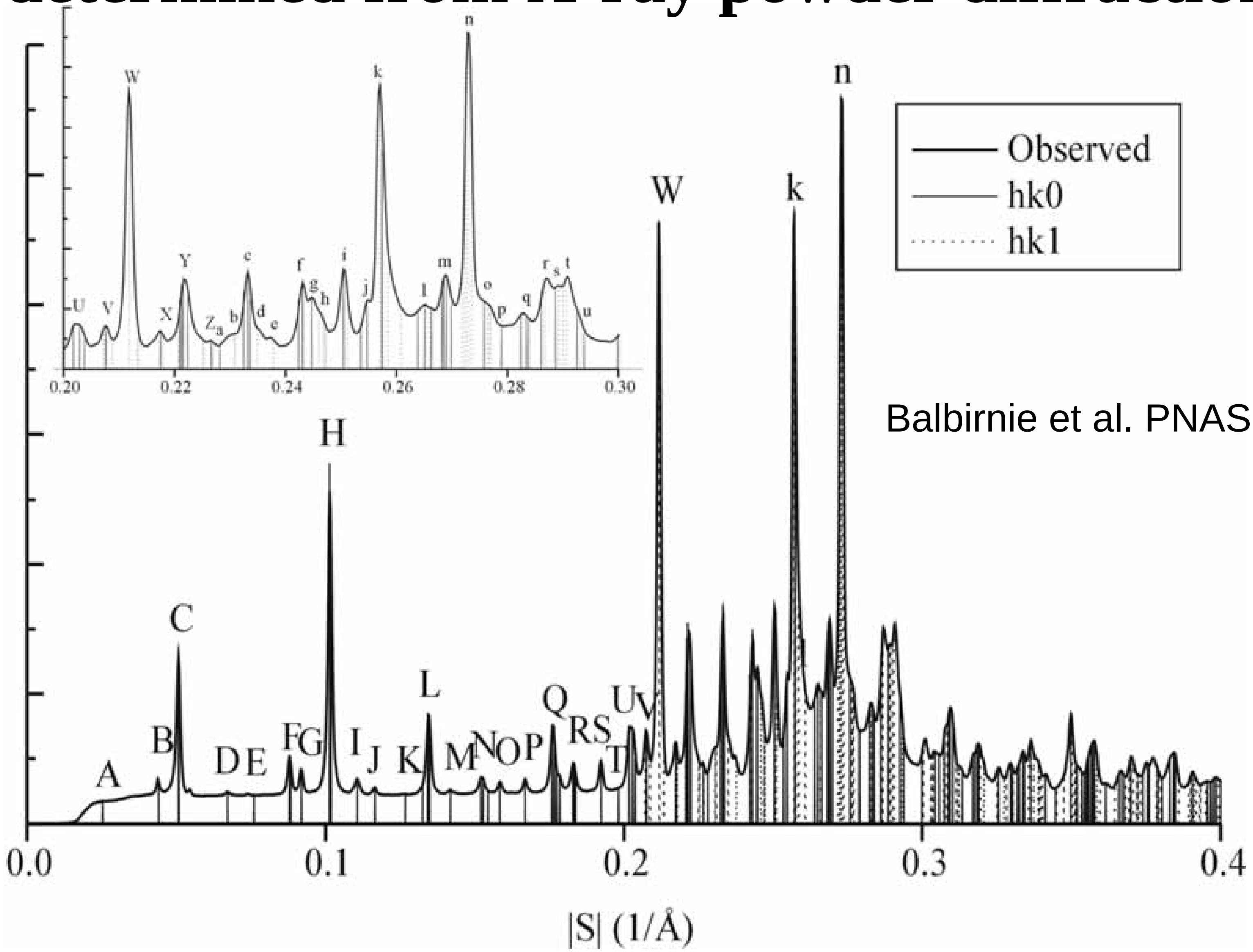


Electrons



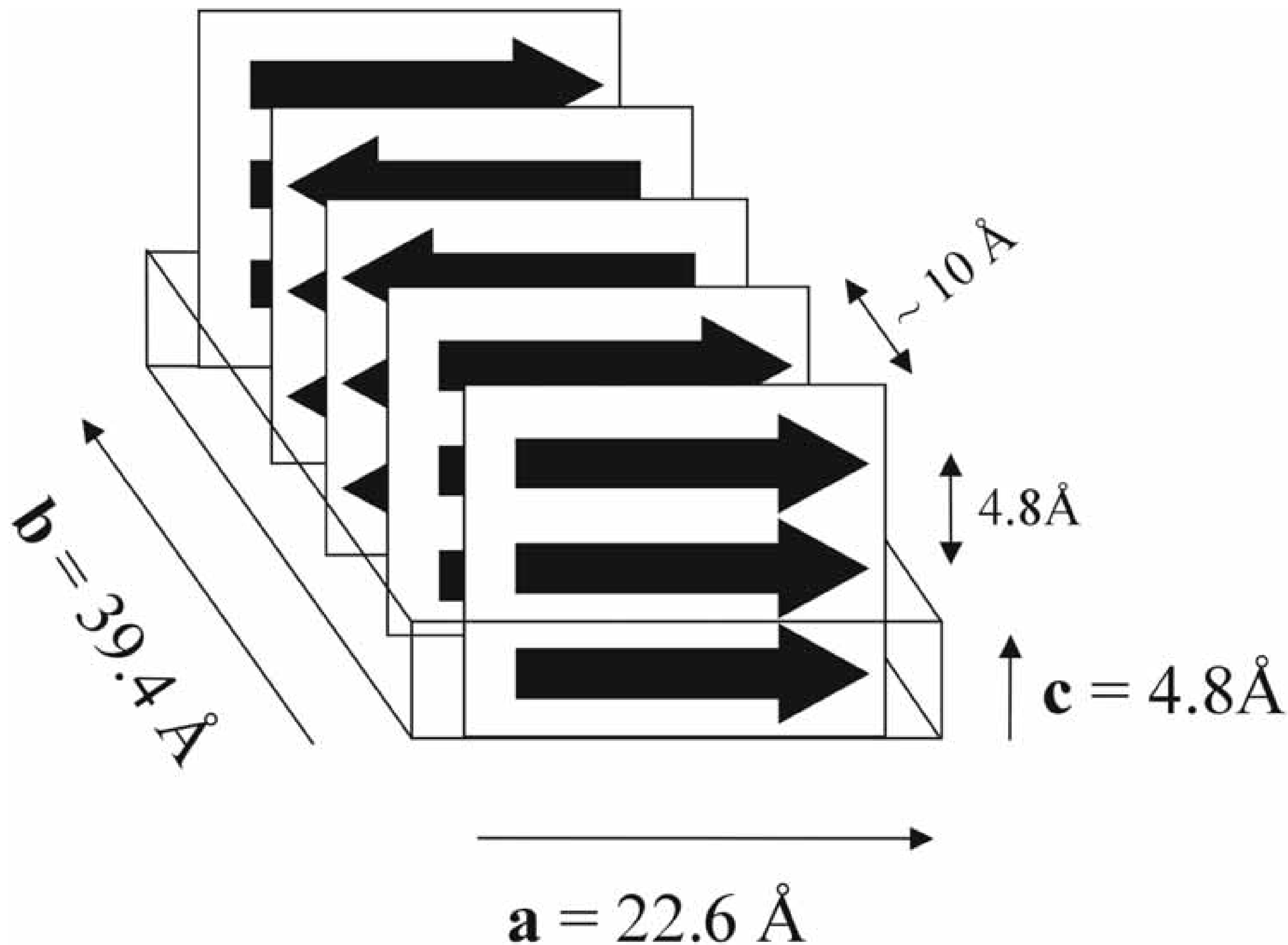
Research of Ruben Diaz & Donald Caspar

Crystal unit cell dimensions and space group determined from X-ray powder diffraction



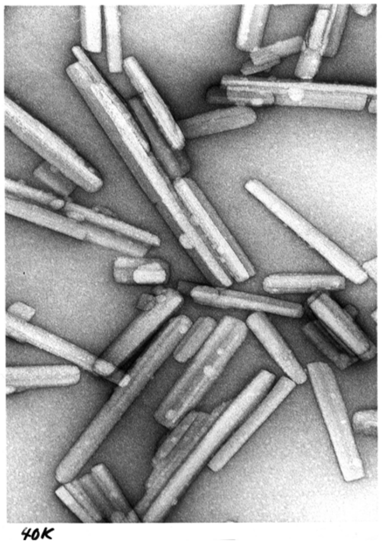
Balbirnie et al. PNAS 2001

Packing of GNNQQNY peptides in microcrystals



Balbirnie, Grothe, & Eisenberg PNAS 2001

Microcrystals of Sup35 Peptide: GNNQQNY



- Density $\rho = 1.39 \pm .01$ g×cm^{-3}
- Unit cell volume = 4.23×10^3 Å^3
- $V_M = 1.26$ Å^3/Da
  (Densest protein crystal so far observed)

From $nM = N\rho V$:

- 4 peptides/cell
- 11 ± 4 water molecules/cell

Conclusions:

- Numerous side chain H-bonds/peptide, other than to water
- Highly H-bonded
- The GNNQQNY amyloid is nearly anhydrous
- Dense network of hydrogen-bonded sidechains

Approaches to the Structure 2001-2005

X-ray powder diffraction -- ongoing

Textured X-ray powder diffraction -- ongoing

Electron diffraction-- ongoing

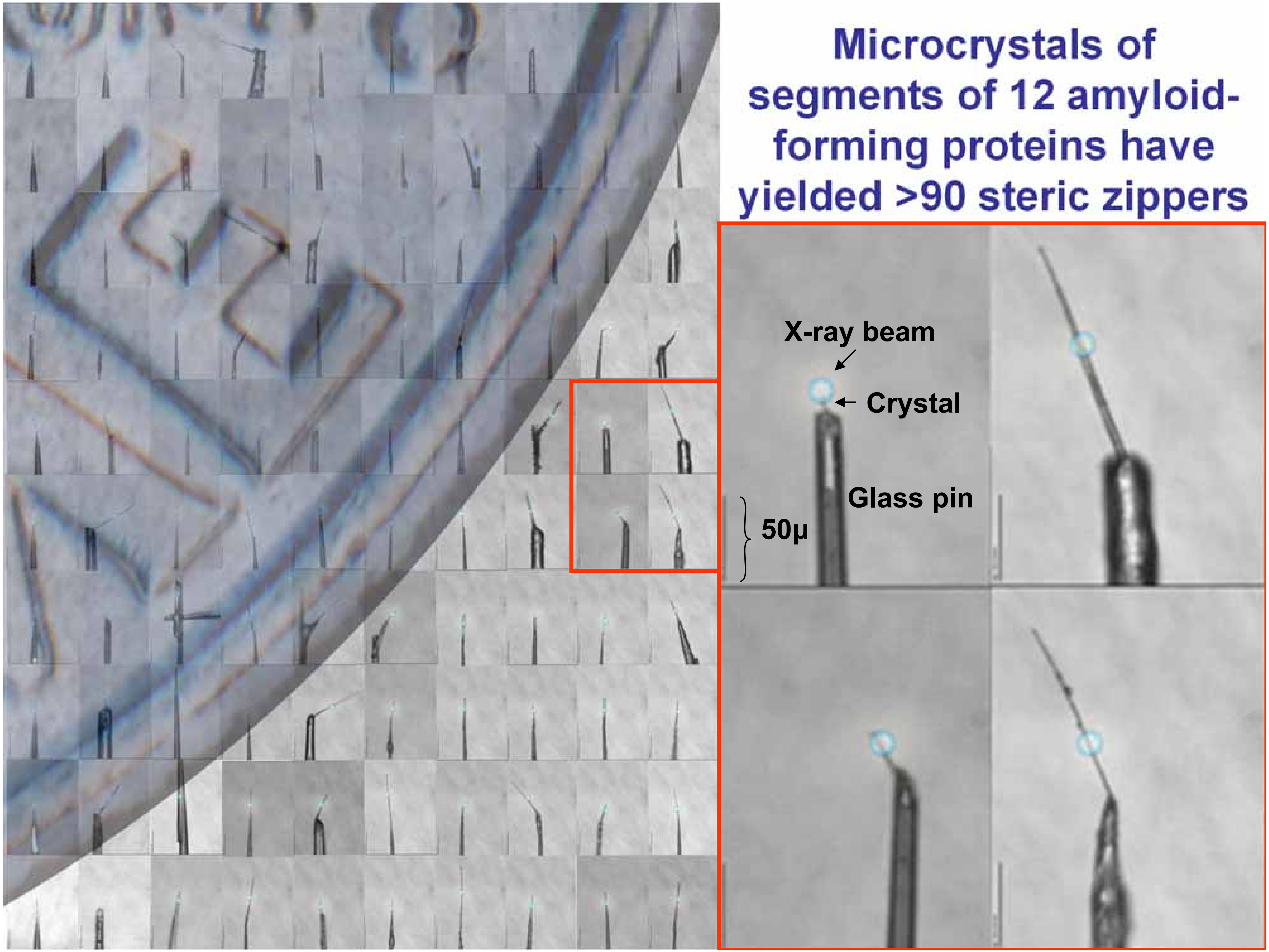
Solid-state NMR -- ongoing

Microfocus is required to reduce background noise

100 μm beam diameter
Standard at home or synchrotron
Only a fraction of incoming X-rays impinge crystal.
High background obscures reflections

1 μm beam diameter
ESRF ID13
All X-rays impinge crystal
Low background, good I/σ.

Microcrystals of segments of 12 amyloid-forming proteins have yielded >90 steric zippers



GNNQQNY, a dry steric zipper

Fibers and microcrystal have 50,000 layers

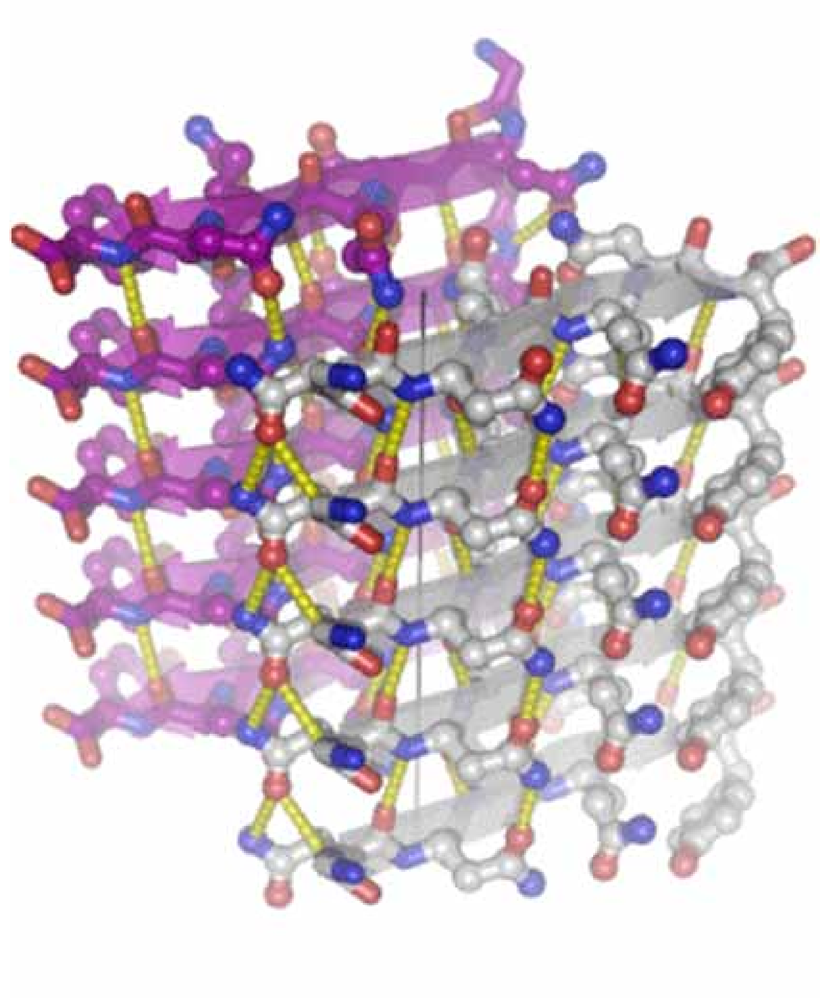
Extended strands, H-bonded 4.8Å
apart into in register β-sheets

Gln, Asn, Tyr sidechains also H-bonded

Two sheets, interdigitated, with tightly
complementary sidechains, bonded
by van der Waals forces

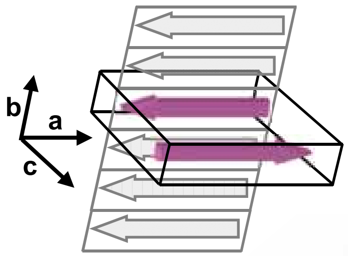
More tightly complementary than
any previous structure in PDB

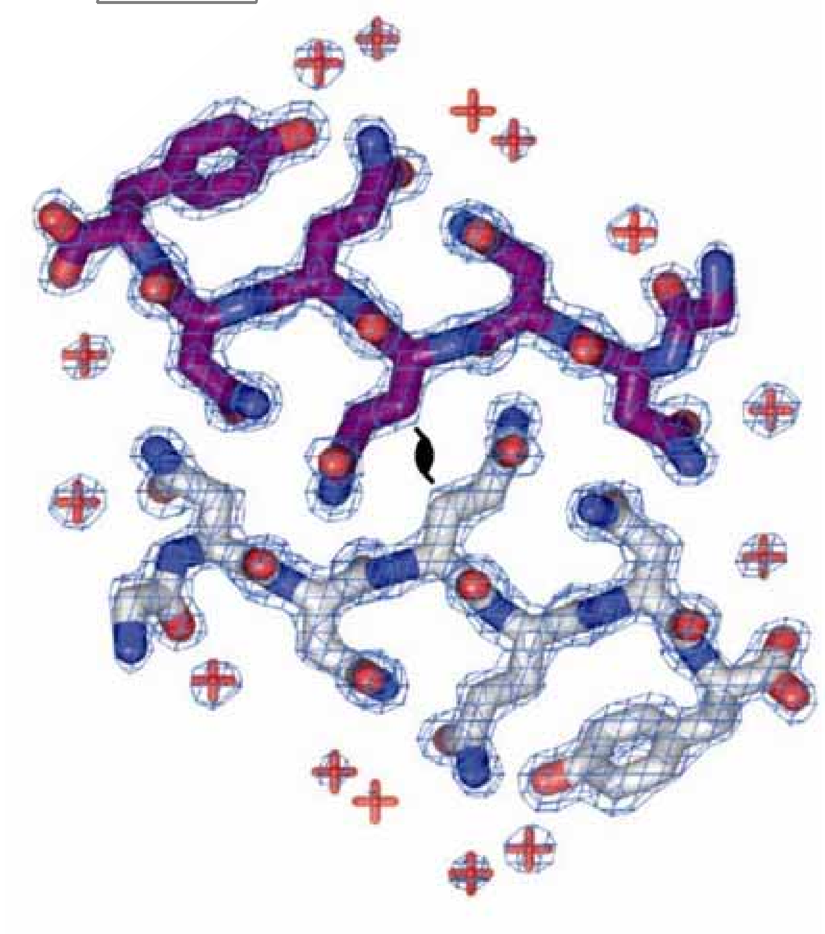
Dry between the β-sheets



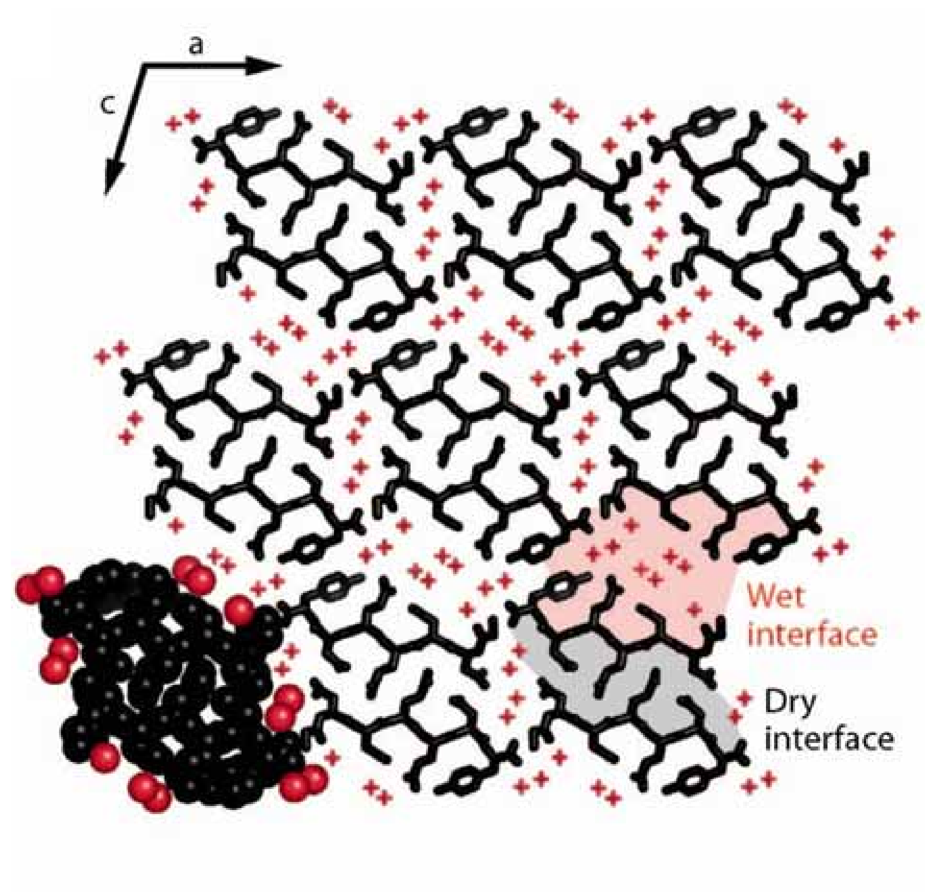
Nelson et al. Nature 2005

View down the fibril axis shows self-complementary interactions between paired beta sheets of the steric zipper and weak interactions between pairs





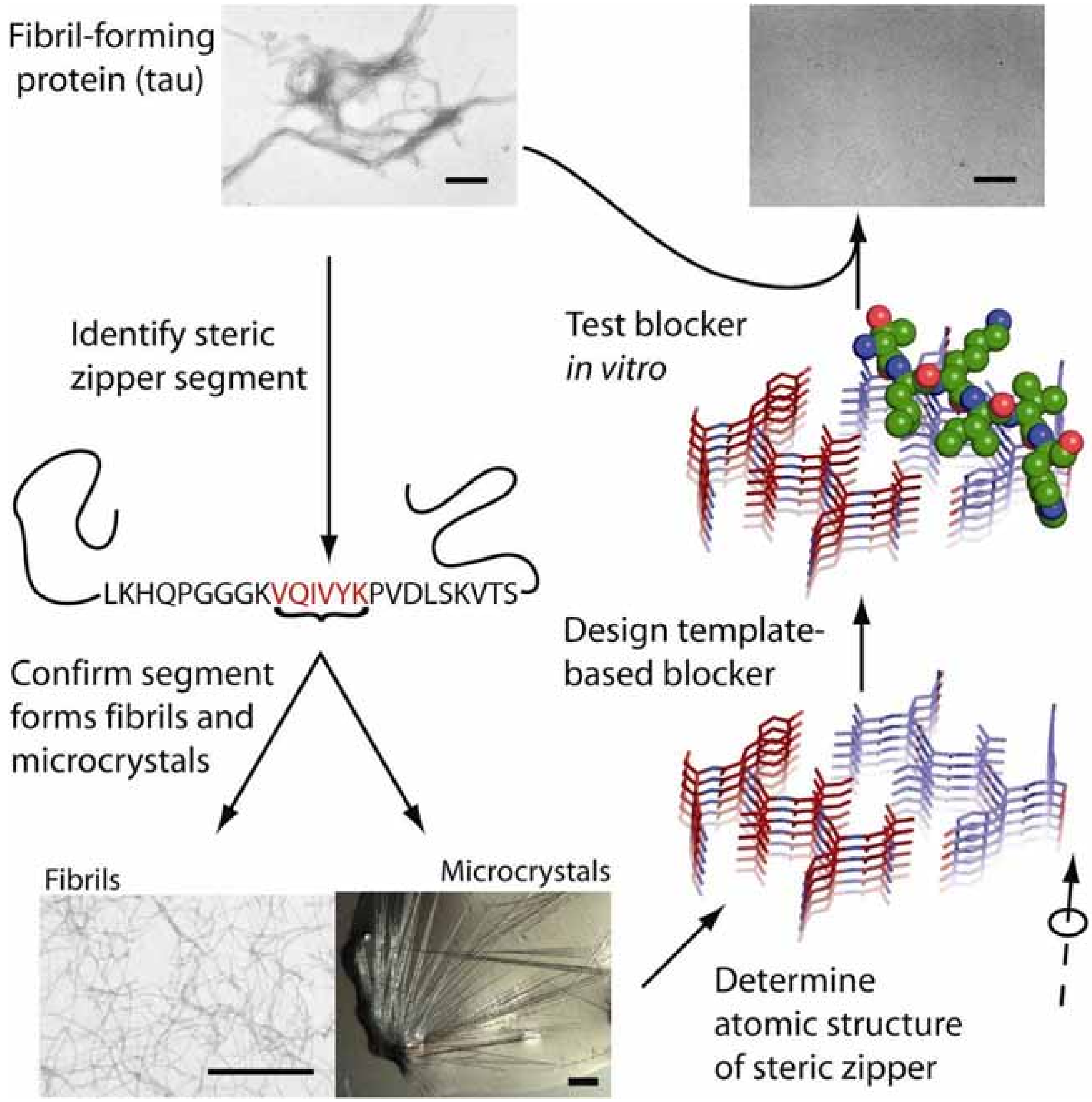
One unit cell



Nine unit cells

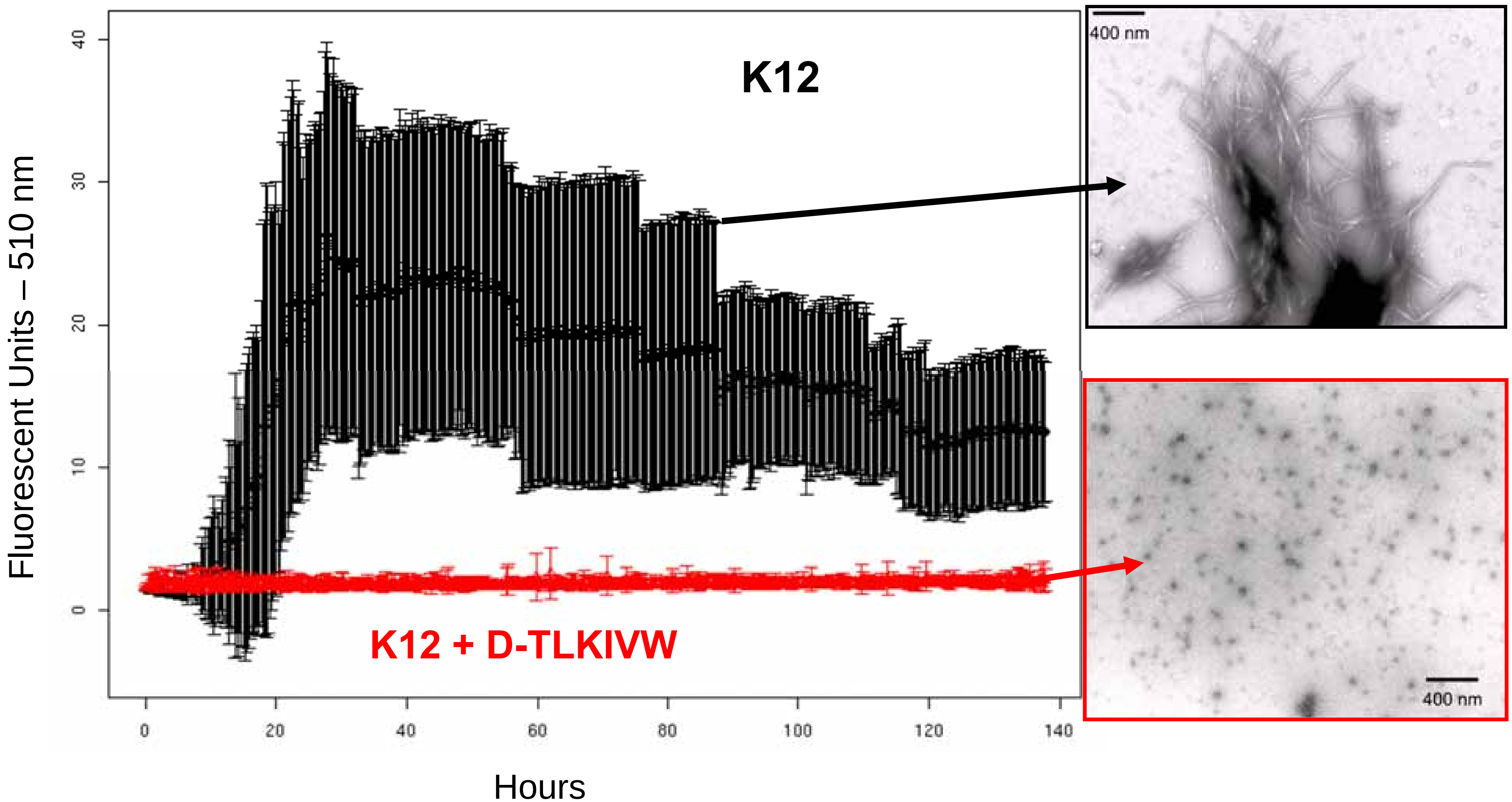
Nelson *et al. Nature,* 2005

Structure-based design of a blocker of fibril formation for Tau



Sievers et al.
Nature 2011

D-TLKIVW prevents fibril formation by Tau K12

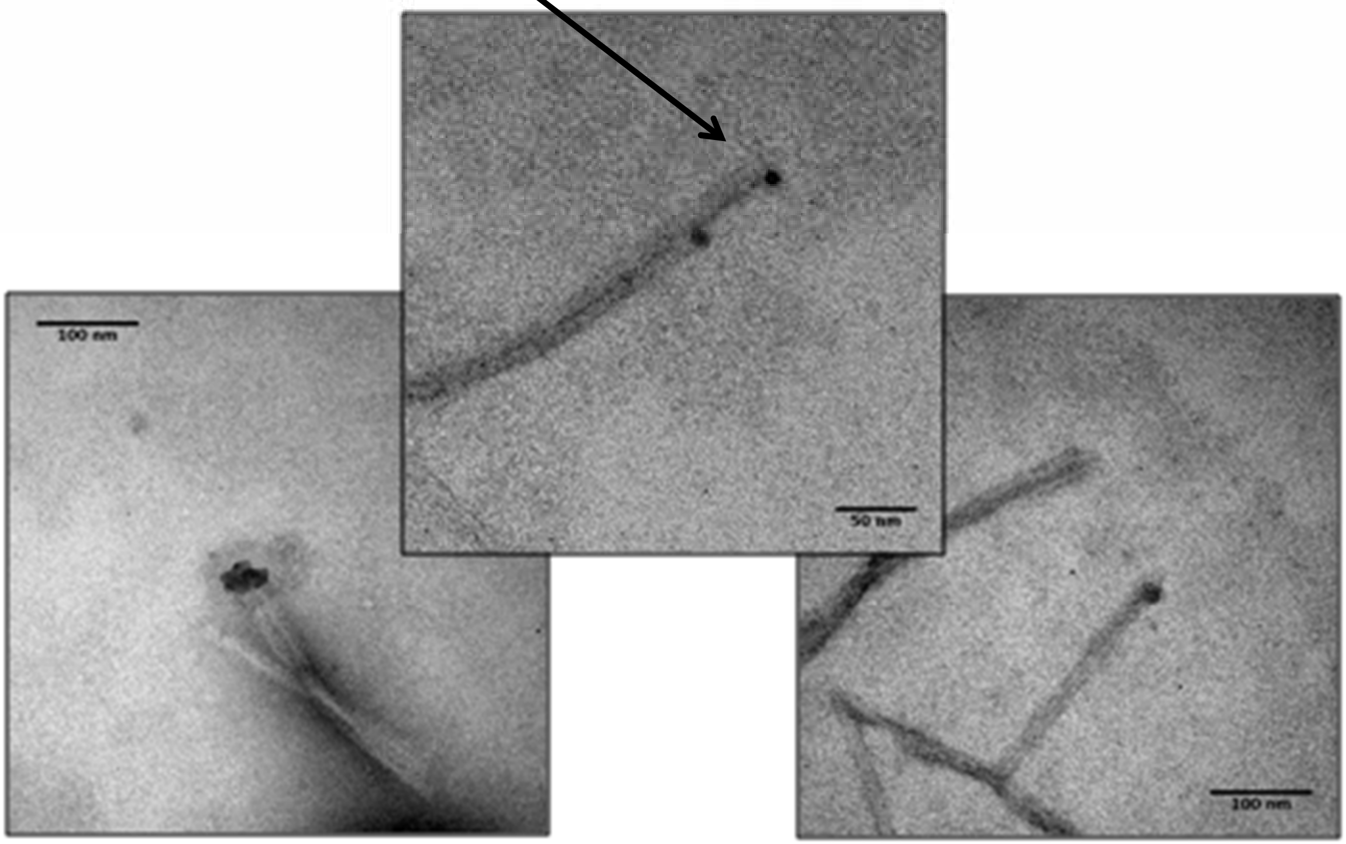


Research of Stuart Sievers & Howard Chang

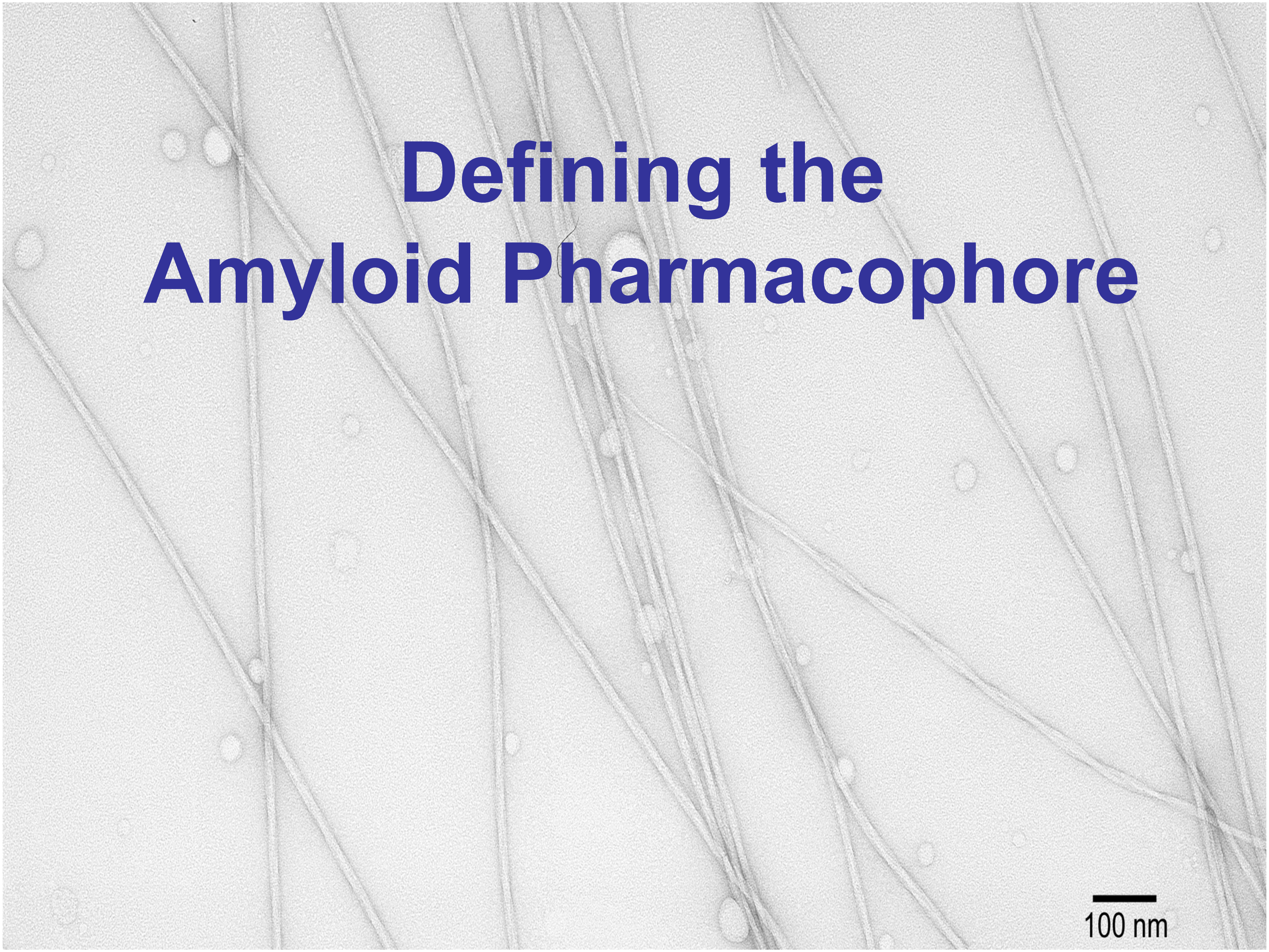
Nano-gold coupled to D-blocker binds to fibril ends

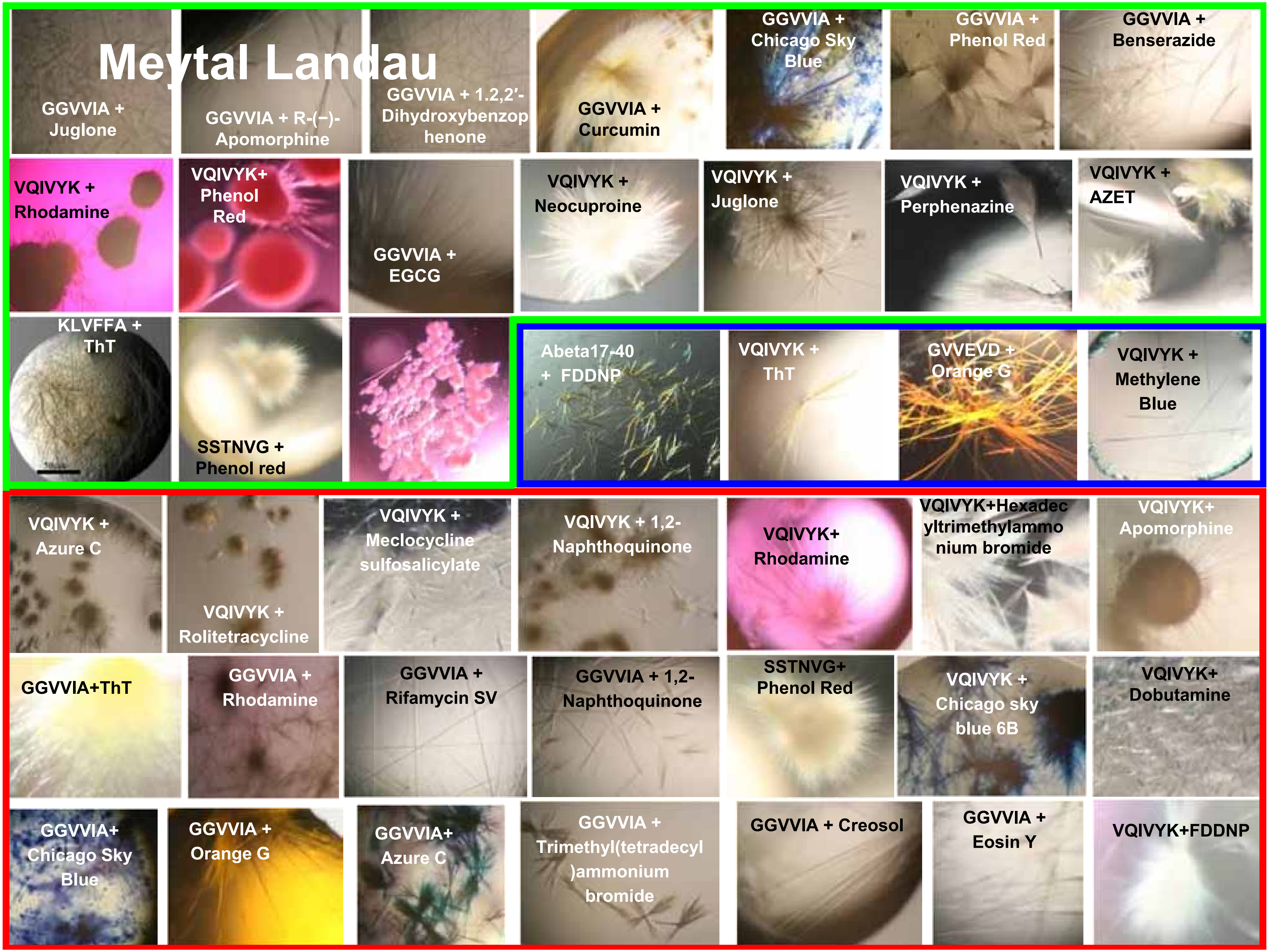
(Howard Chang)

NanoAu-CGG-TLKIVW



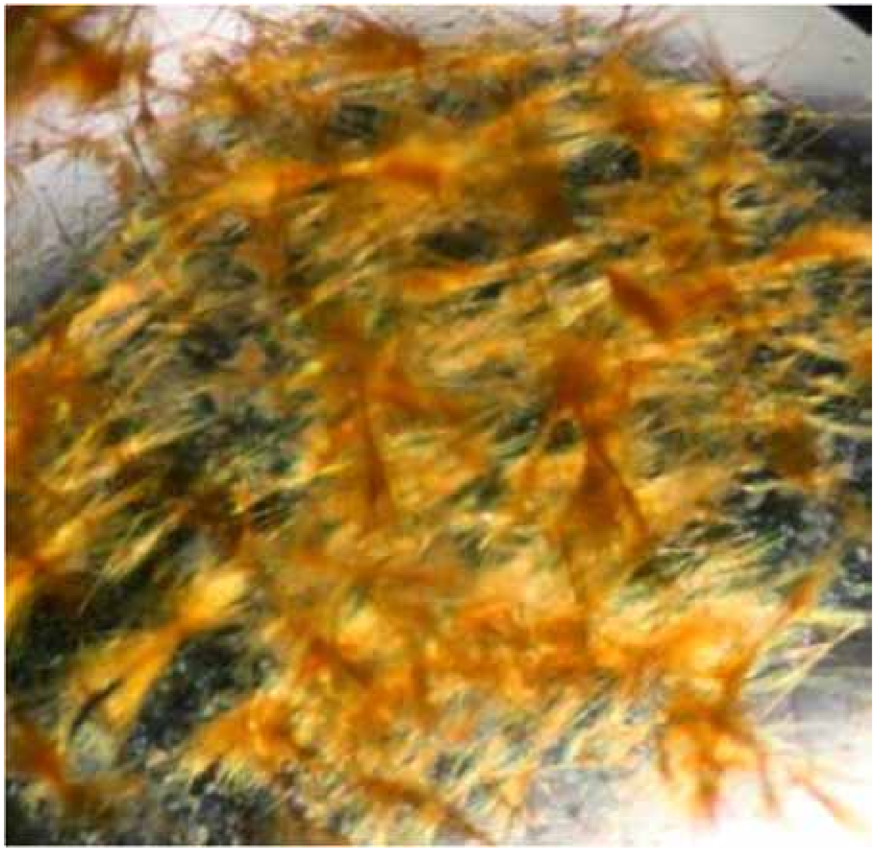
Defining the Amyloid Pharmacophore





Meytal Landau
GGVVIA + Juglone
GGVVIA + R-(−)-Apomorphine
GGVVIA + 1.2,2′-Dihydroxybenzophenone
GGVVIA + Curcumin
GGVVIA + Chicago Sky Blue
GGVVIA + Phenol Red
GGVVIA + Benserazide
VQIVYK + Rhodamine
VQIVYK+ Phenol Red
GGVVIA + EGCG
VQIVYK + Neocuproine
VQIVYK + Juglone
VQIVYK + Perphenazine
VQIVYK + AZET
KLVFFA + ThT
SSTNVG + Phenol red
Abeta17-40 + FDDNP
VQIVYK + ThT
GVVEVD + Orange G
VQIVYK + Methylene Blue
VQIVYK + Azure C
VQIVYK + Rolitetracycline
VQIVYK + Meclocycline sulfosalicylate
VQIVYK + 1,2-Naphthoquinone
VQIVYK+ Rhodamine
VQIVYK+Hexadecyltrimethylammonium bromide
VQIVYK+ Apomorphine
GGVVIA+ThT
GGVVIA + Rhodamine
GGVVIA + Rifamycin SV
GGVVIA + 1,2-Naphthoquinone
SSTNVG+ Phenol Red
VQIVYK + Chicago sky blue 6B
VQIVYK+ Dobutamine
GGVVIA+ Chicago Sky Blue
GGVVIA + Orange G
GGVVIA+ Azure C
GGVVIA + Trimethyl(tetradecyl)ammonium bromide
GGVVIA + Creosol
GGVVIA + Eosin Y
VQIVYK+FDDNP

KLVFFA (from Amyloid Beta) + Orange G

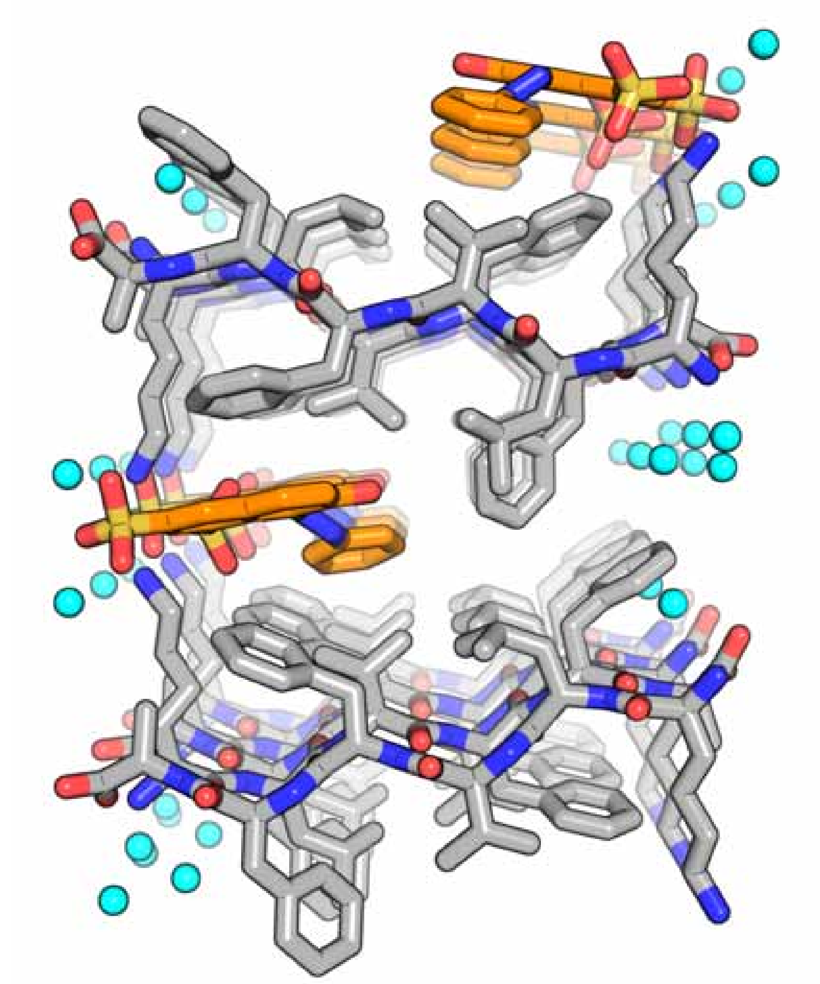


Structure of KLVFFA with Orange G

KLVFFA + Orange G 5-10:1mM
10-30% w/v Polyethylene glycol 1,500,
20-30% v/v Glycerol

Rmerge = 18.4%; Resolution=1.8A;
Completeness=96.4%

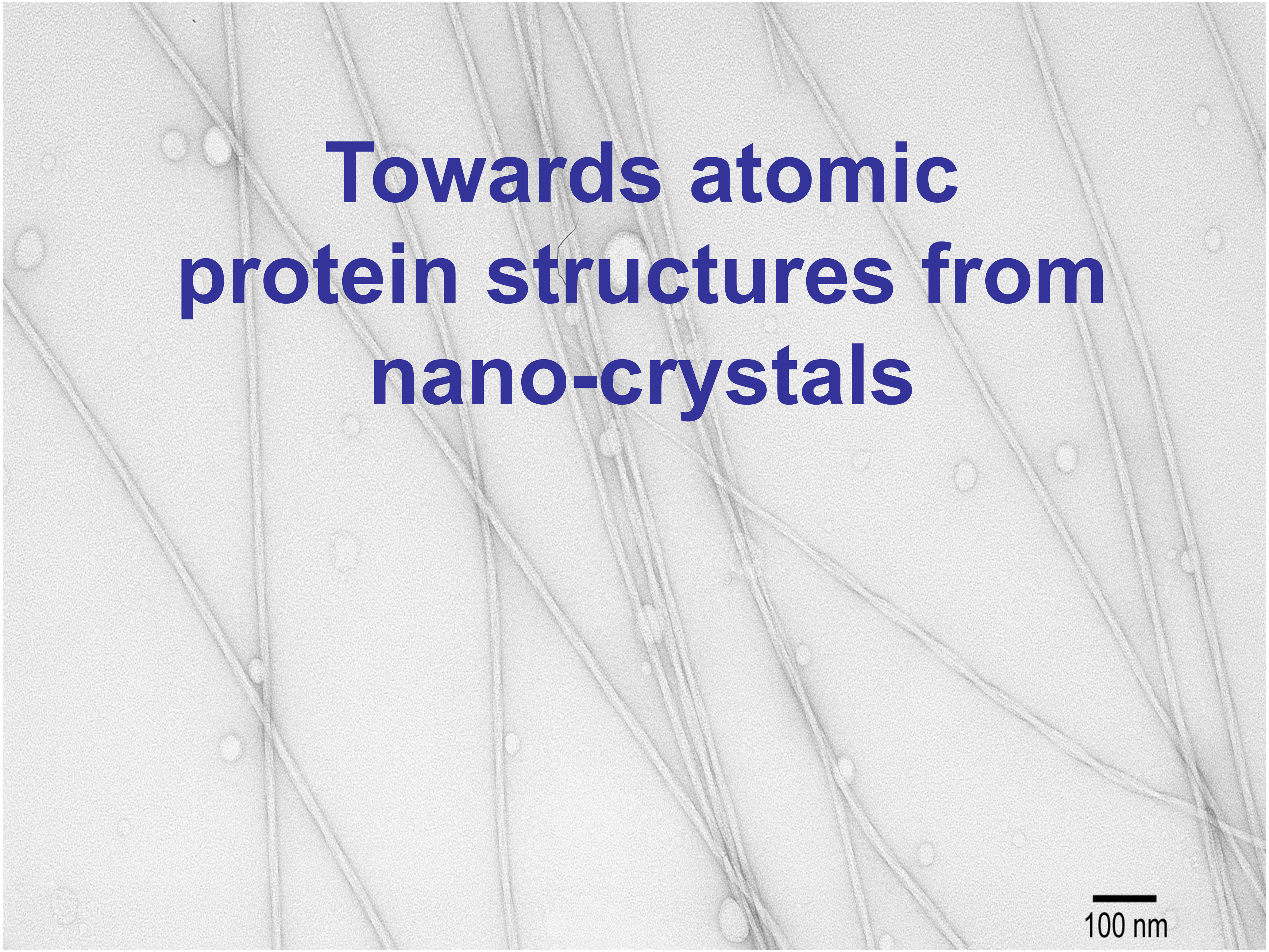
C2;
a, b, c 43.64 26.85 9.55Å;
β 91.55 °
Rwork/Rfree(%)= 22.6/27.6

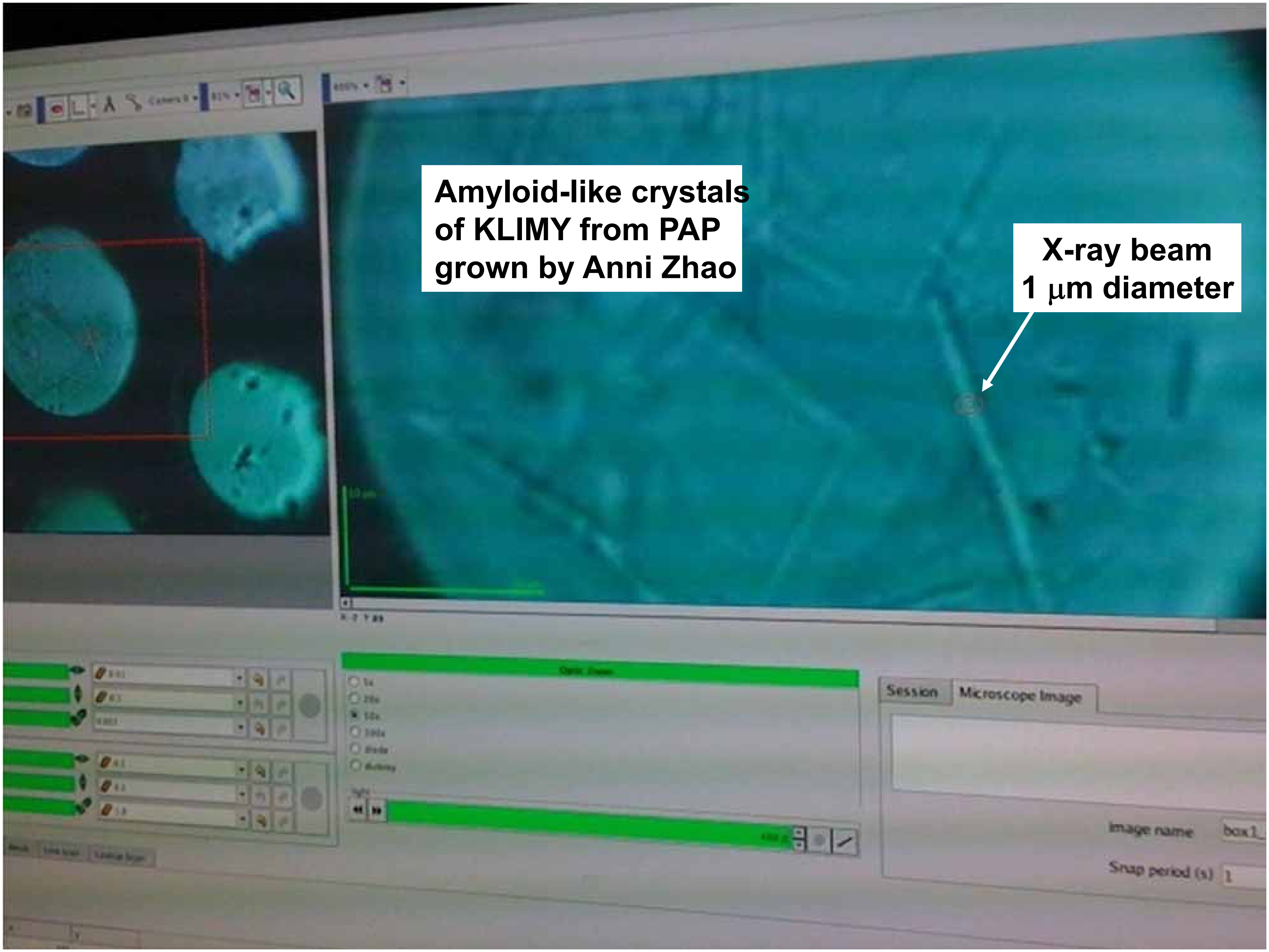


The asymmetric unit contains:
- Two peptide segments
- One Orange G
- Six water molecules

Landau et al. PLoS Biology
In press

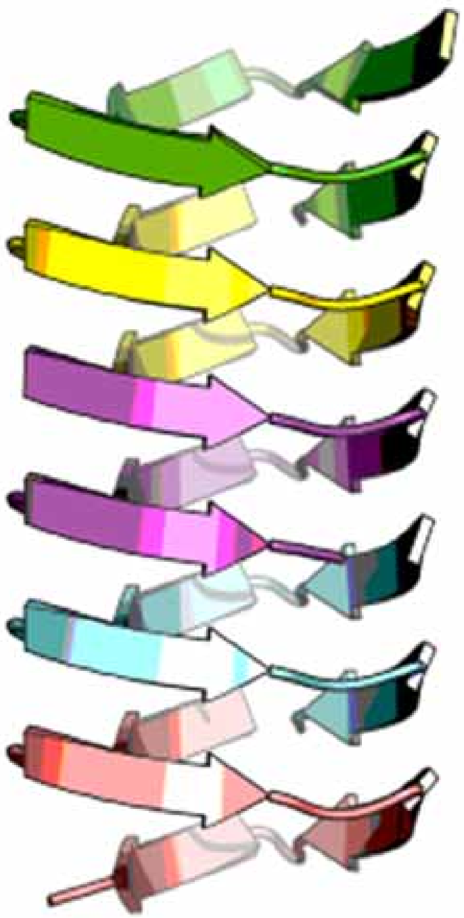
Towards atomic protein structures from nano-crystals

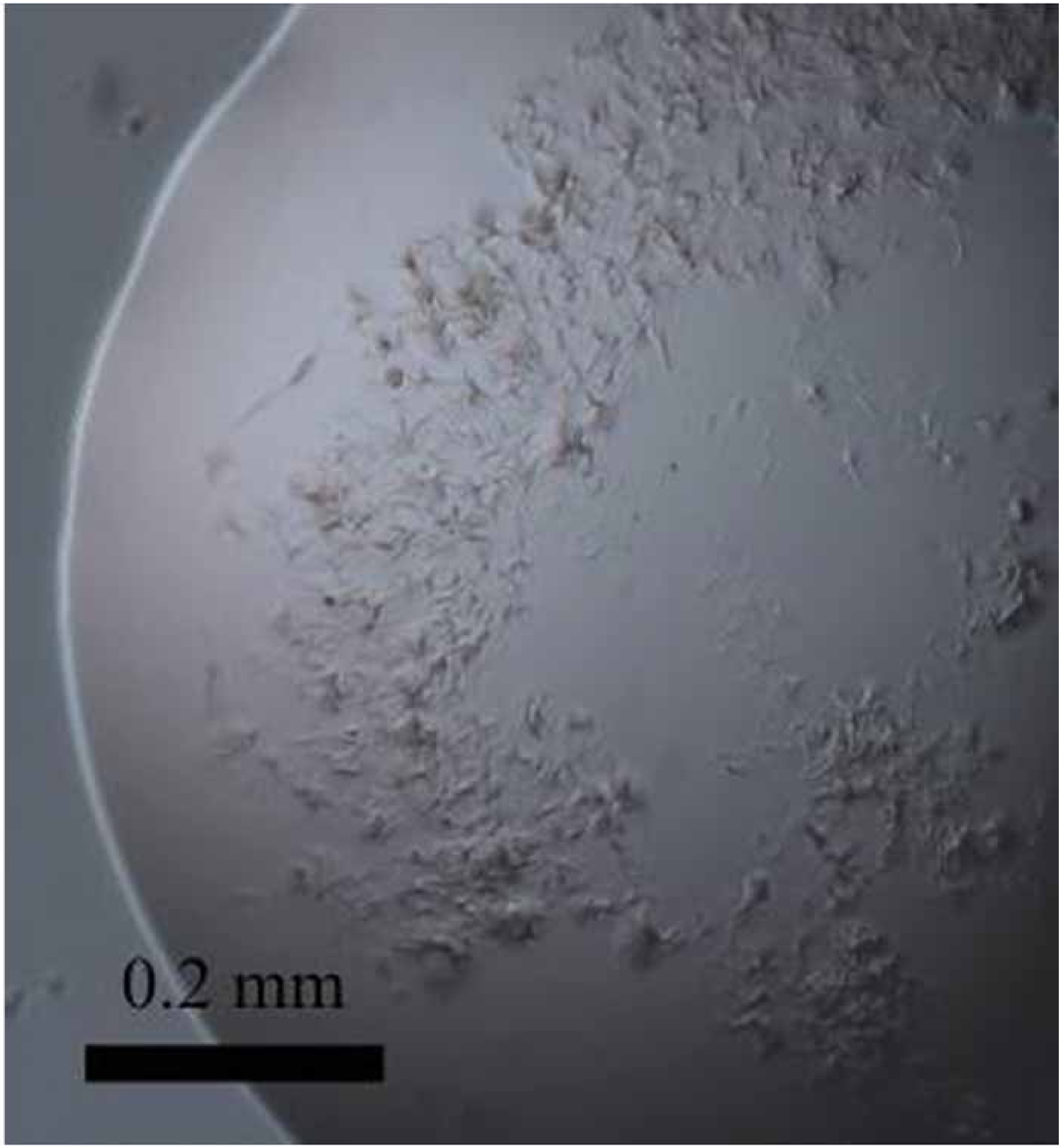


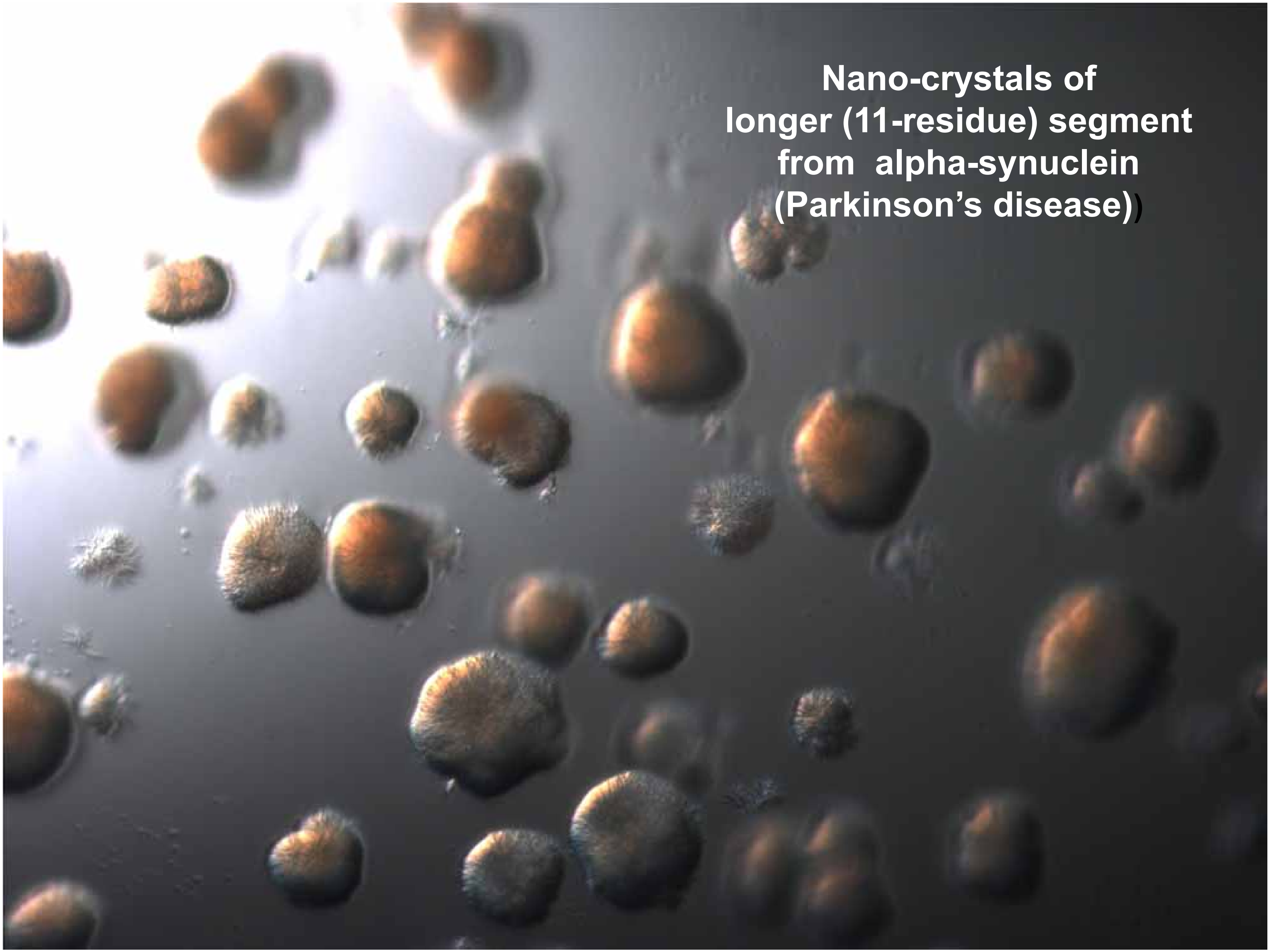

Amyloid-like crystals of KLIMY from PAP grown by Anni Zhao
X-ray beam 1 μm diameter
Session
Microscope Image
image name
Snap period (s)

A 3-sheet prion structure?

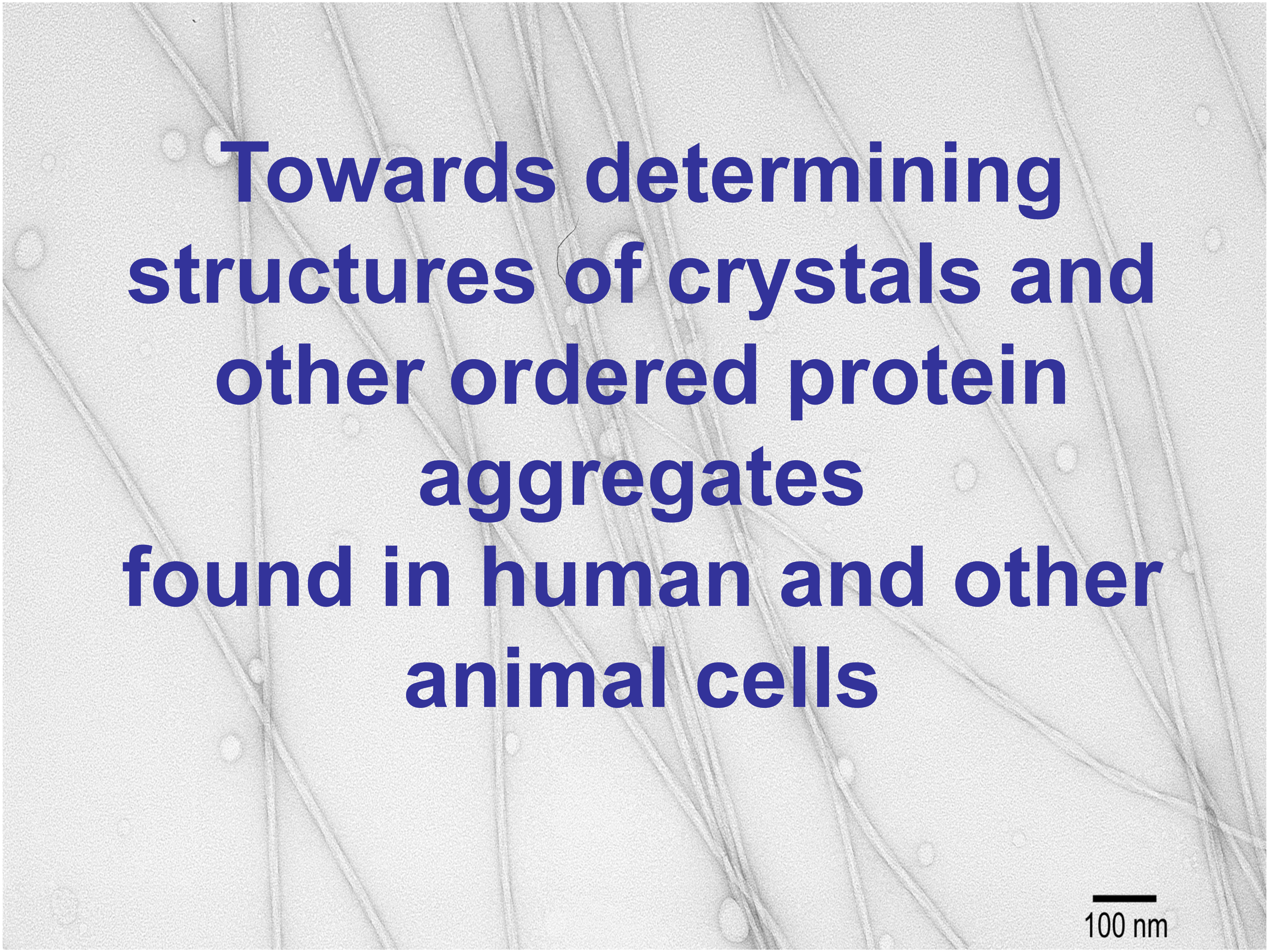
Only nanocrystals available



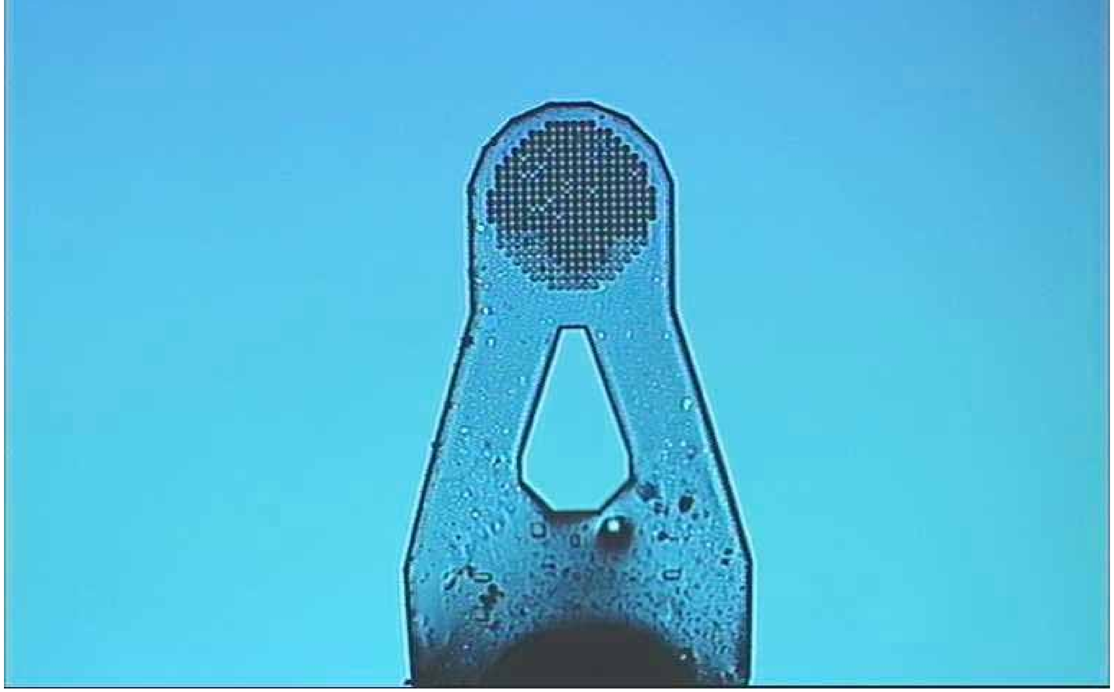


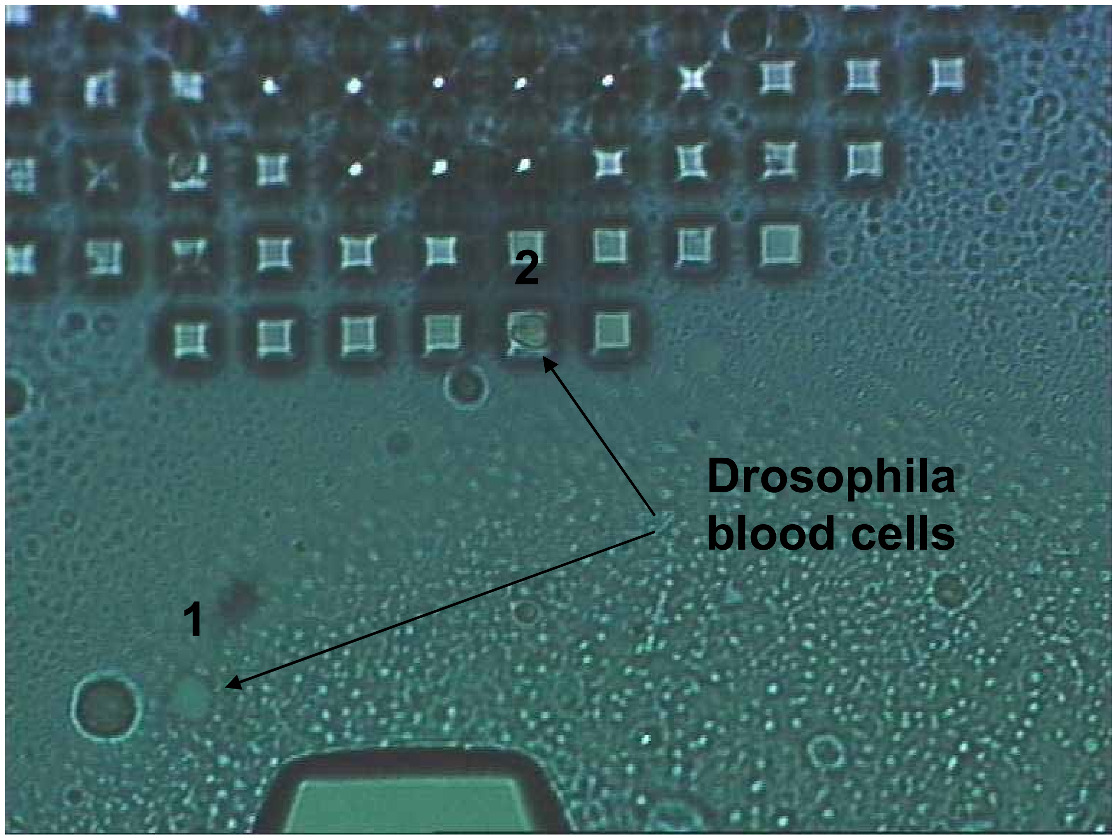

Nano-crystals of
longer (11-residue) segment
from alpha-synuclein
(Parkinson's disease))

Towards determining structures of crystals and other ordered protein aggregates found in human and other animal cells



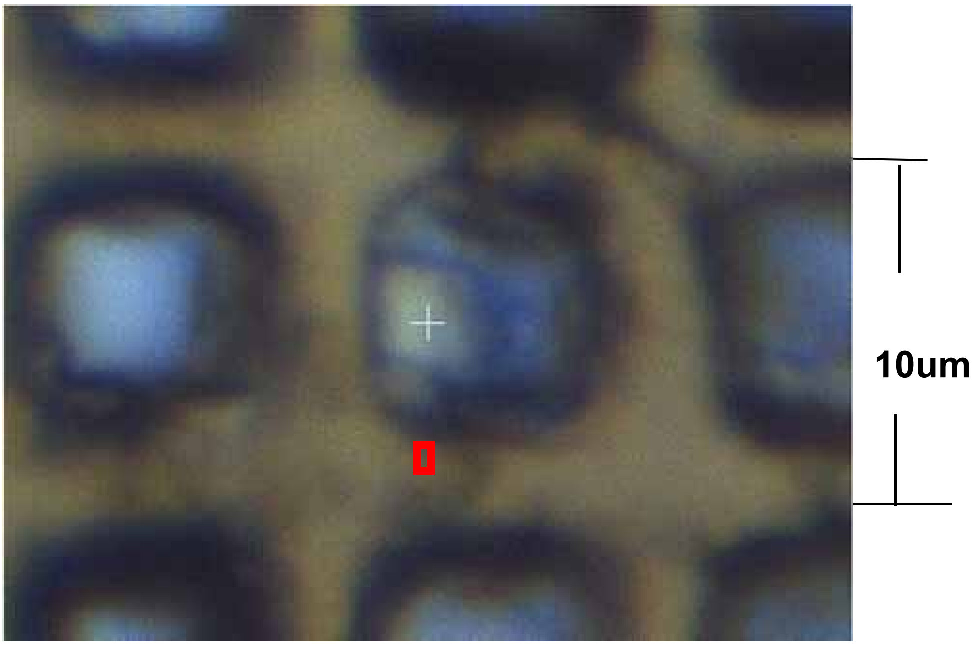
10 micron Mictigen micromesh in cryo-stream

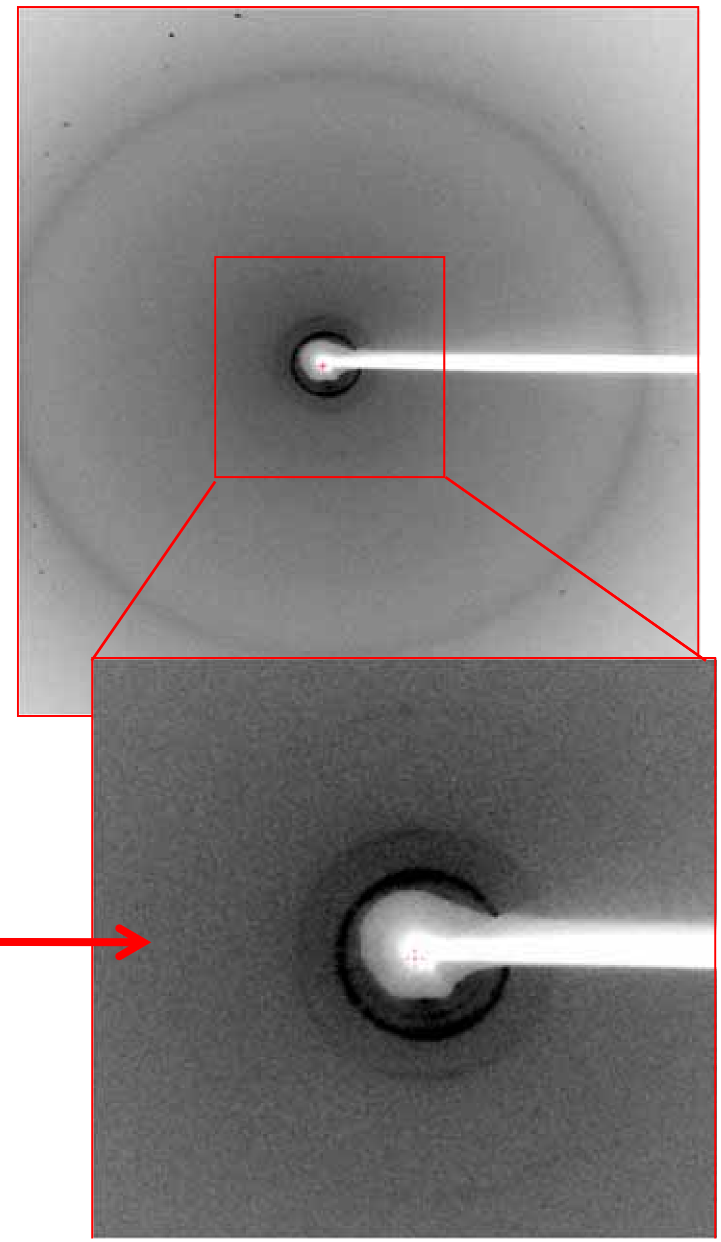




2
1
Drosophila
blood cells

Drosophila crystal cell on 10 μm mesh

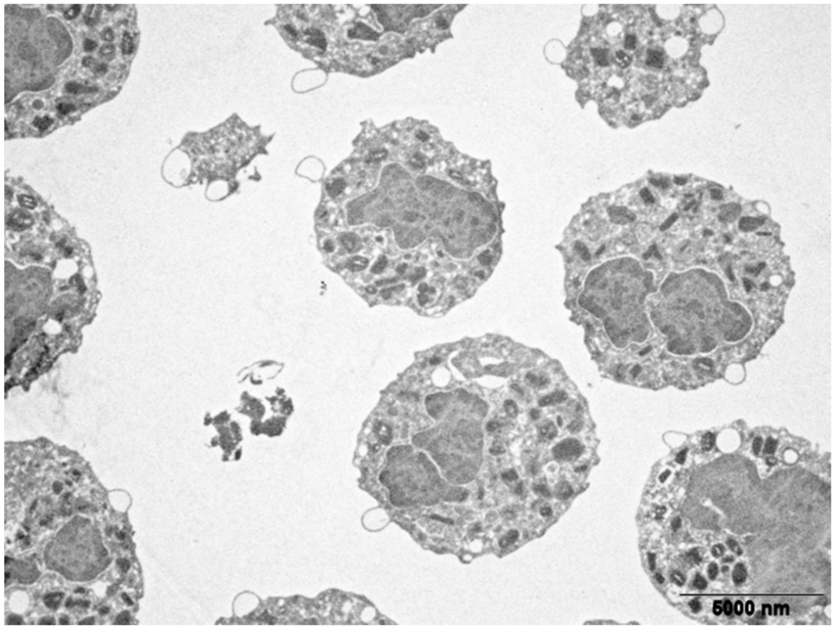


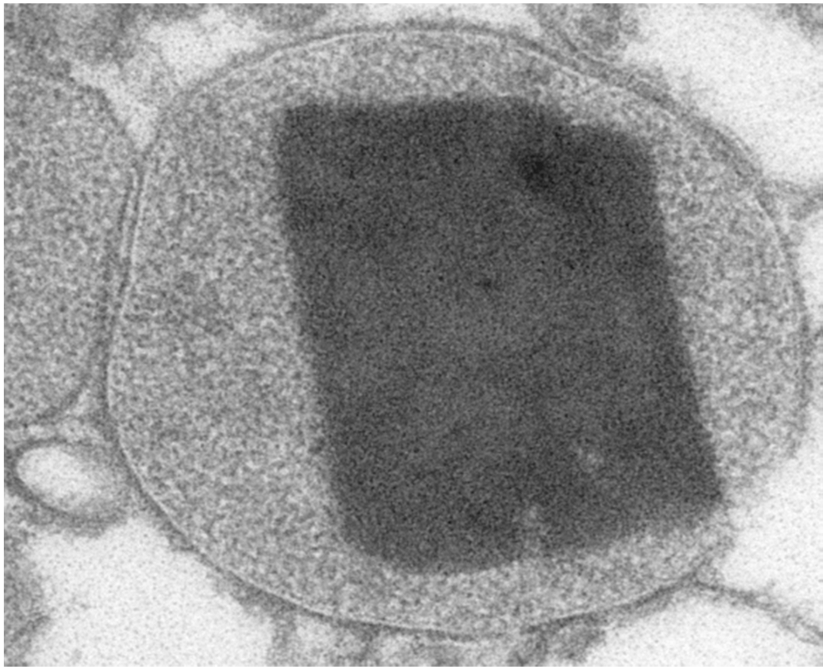


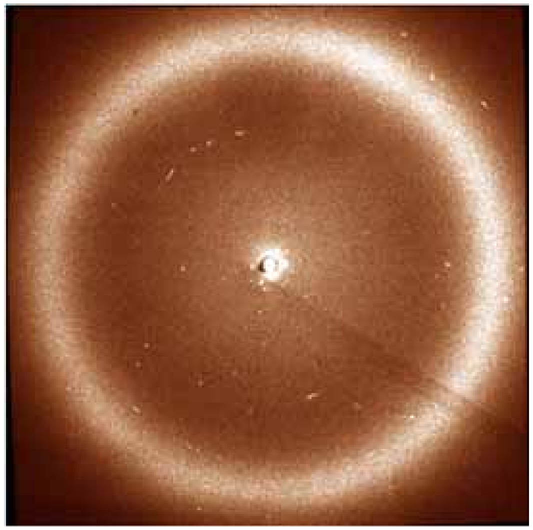
The diffraction pattern reveals powder rings at 50, 38, 26, 13, 4.2, and 3.7 Å spacings

Michael Sawaya, Mari Gingery, Duilio Cascio
Group of Utpal Banerjee, UCLA
Jacques Colletier, Christian Riekel, ESRF-IBS

Eosinophile (type of white blood cell) granules Characterized by George Palade et al. (1965) by EM







Unit cell dimensions tentatively determined by Alice Soragni, Jacques Colletier, Manfred Brunner, Christian Riekel

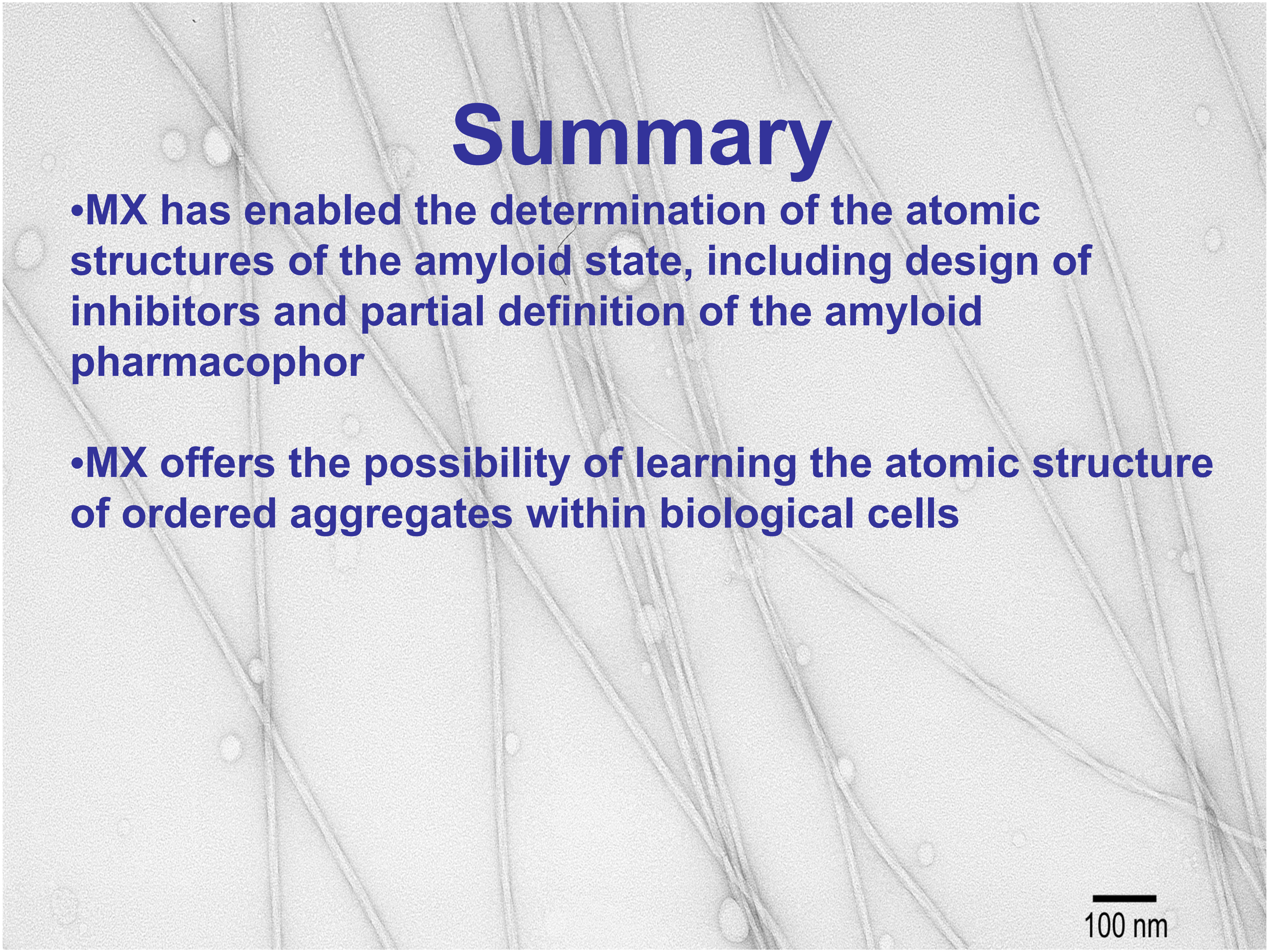
Cell Types Containing Intracellular Crystalline Inclusions

Species	Cell type	Description of crystals	Protein	Reference
Drosophila	Crystal cells	Intracellular inclusions [lamellar]	Prophenol oxidase	(Shrestha & Gateff 1982); T. M. Rizki & R. M. Rizki 1980)
Human Rat Guinea pig Mouse	Eosinophil leukocytes	Membrane-bound granules [0.3-1.2 um] Granule cores [lamellar]	?	(Miller et al. 1966)
Human	B cell lymphomas	ER-bound crystal rods [lamellar]	Ig	(Peters et al. 1984)
Human	Abnormal mitochondria in muscle myopathies	Crystal rods in outer mitochondrial membrane compartment	Mitochondrial creatine kinase	(Stadhouders et al. 1994)
Human	Kidney mitochondria	Helical crystals in outer mitochondrial compartment; linear and flexuous crystals in matrix	?	Jasmin 1978
Human Dog Monkey	Liver mitochondria	Intramitochondrial [lamellar]	?	Wills 1965
Human	Ad5-infected KB cells	Intranuclear adenovirus-induced inclusions	heteromeric capsid protein formed of penton base and fiber subunits	(Franqueville et al. 2008); (Carstens et al. 1975)
Frog	Oocyte mitochondria	Intramatrix & intracristae inclusions [lamellar]	?	Spornitz 1972
Armadillo	Epididymus	Single membrane-bound cytoplasmic crystalline rods [lamellar]	?	(Edmonds et al. 1973)
Earthworm	Spermatazoa	Intranuclear inclusions [lamellar]	?	(Anderson et al. 1968)
Tomato	Young leaf mesophyll	Intracellular inclusions [Cubic]	?	Singh 1976
Helicobacter pylori	Causative agent of gastric diseases	Cytoplasmic paracrystalline inclusions	*Pfr*, bacterial ferritin	(Frazier et al. 1993)
Photorhabdus luminescens	Entomopathogenic bacteria	Intracellular inclusions	*cipA* *cipB*	(Bintrim et al. 1998)
Bacillus thuringiensis	Insecticidal bacteria	parasporal crystals	*Cry*	(Hofte et al. 1989)
Paenibacillus popilliae	Insecticidal bacteria	parasporal crystals	?	Weiner 1978
Brevibacillus laterosporus	Mosquitocidal bacteria	parasporal crystals	?	(Smirnova et al. 1996); (Orlova et al. 1998)

Summary

•MX has enabled the determination of the atomic structures of the amyloid state, including design of inhibitors and partial definition of the amyloid pharmacophor

•MX offers the possibility of learning the atomic structure of ordered aggregates within biological cells



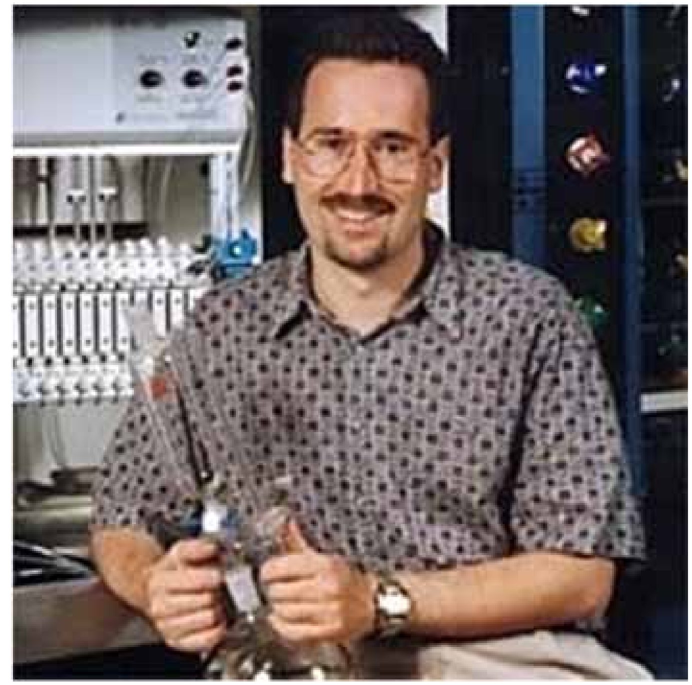
The Amyloid State of Proteins

UCLA: Rebecca Nelson, Michael Sawaya, Marcin Apostol, Melinda Balbirnie
Magdalena Ivanova, Stuart Sievers, Jed Wiltzius, Minglei Zhao, Cong Liu
Luki Goldschmidt, Heather Mcfarlane, Howard Chang, Anni Zhao
Lin Jiang, Jiyong Park, Jacques Colletier, Poh Teng, Boris Bhrumstein
Univ. of Washington: John Karanicolis, David Baker
ESRF: Christain Riekel ETH: Roland Riek, Alice Soragni

Collaborators

Christian Riekel
ESRF

David Baker
University of Washington



James Nowick
UCI

Roland Riek (ETH Zurich), John Karanicolas (U. Kansas), Jan Münch (Ulm)