Structure determination of macromolecular assemblies by solid-state NMR

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IECB - Institut Européen de Chimie et Biologie, Bordeaux

Structure determination of **macromolecular assemblies** by solid-state NMR

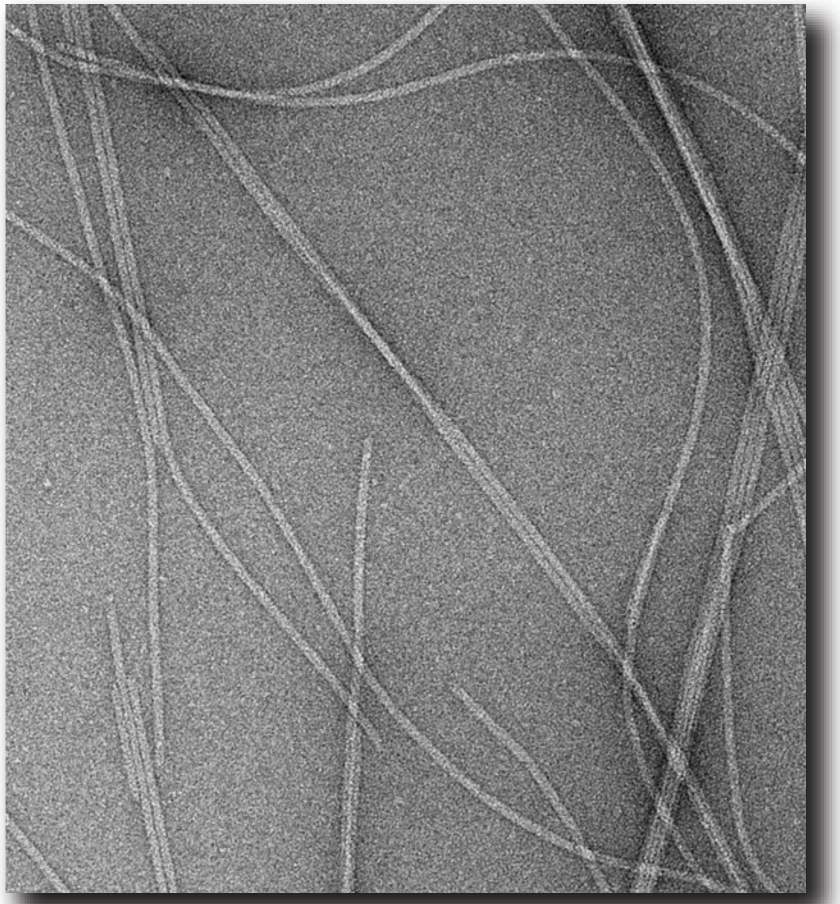
→ **Non covalent assembly of multiple copies of subunits into a large suprastructure**

Self-organizations, supramolecular assemblies

Macromolecular assemblies, macromolecular complexes

Mesoscopic scale:

. **Fibrils**
. **Filaments, protofilaments**
. **Nanotubes**
. **Oligomers**
. **Aggregates, etc.**



. **Crucial in many fundamental cellular processes:**

Propagation of information, protein transport, bacterial and viral infection, cell motility, etc.

. **Assembly driven by weak interactions, but they are usually well defined in morphology**

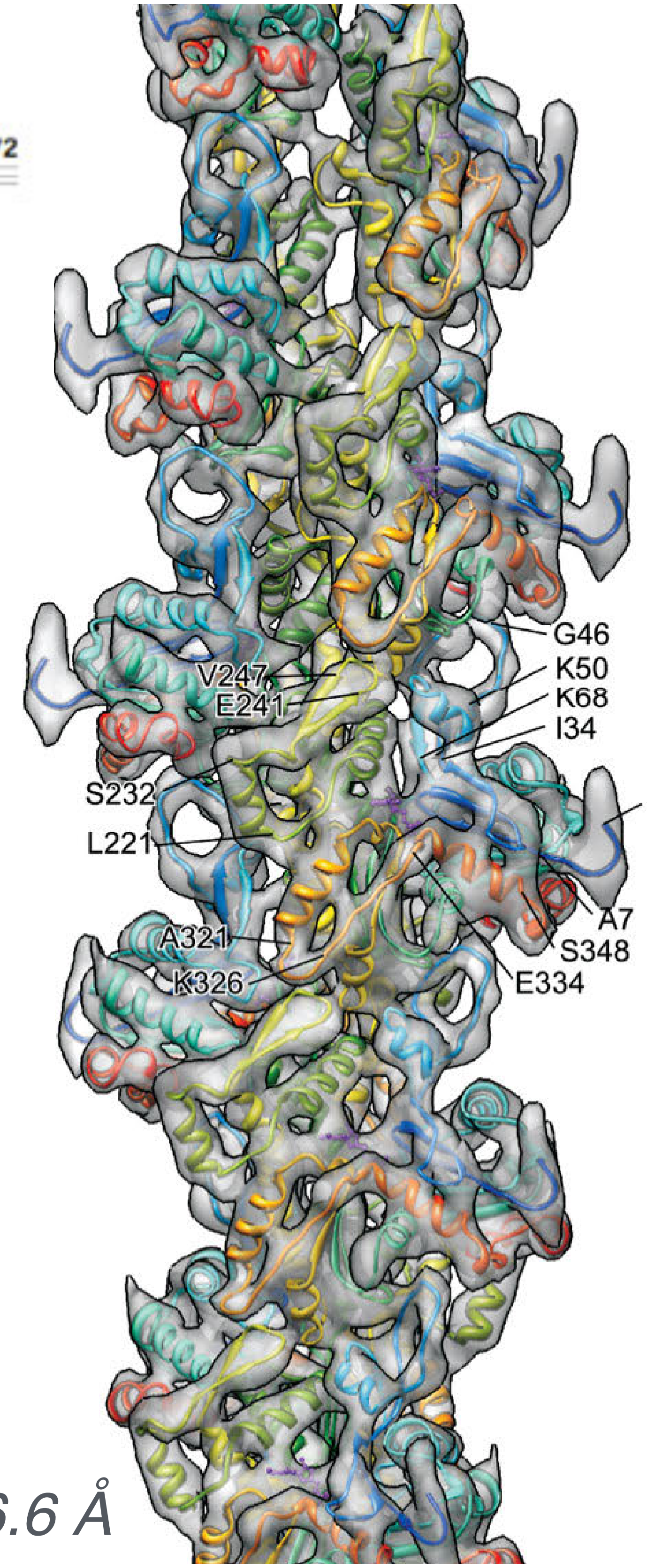
THE STATE-OF-THE-ART APPROACH

LETTER

doi:10.1038/nature09372

Direct visualization of secondary structures of F-actin by electron cryomicroscopy

K. Namba *Nature* **2010**



F-actin: 6.6 Å

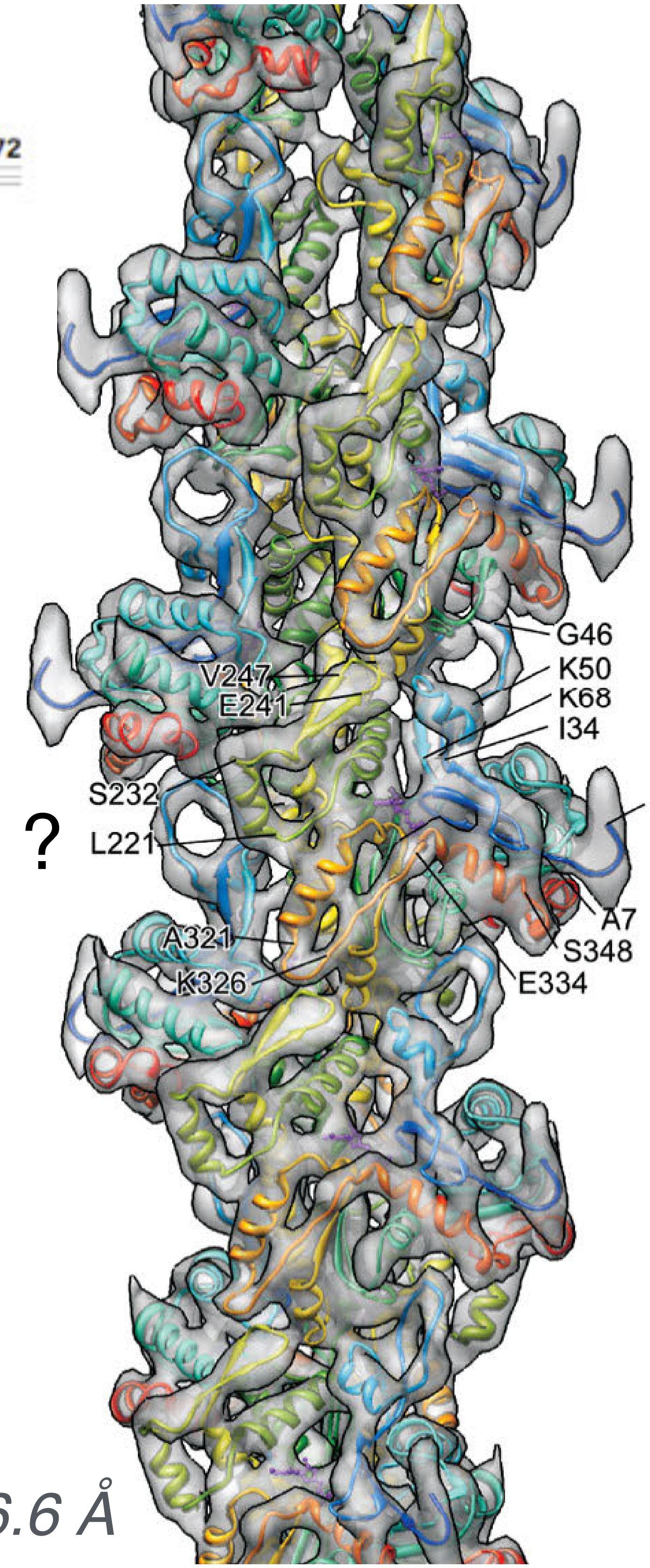
THE STATE-OF-THE-ART APPROACH

LETTER

doi:10.1038/nature09372

Direct visualization of secondary structures of F-actin by electron cryomicroscopy

K. Namba *Nature* **2010**



. **Native conformation** / **refolding** upon the assembly ?

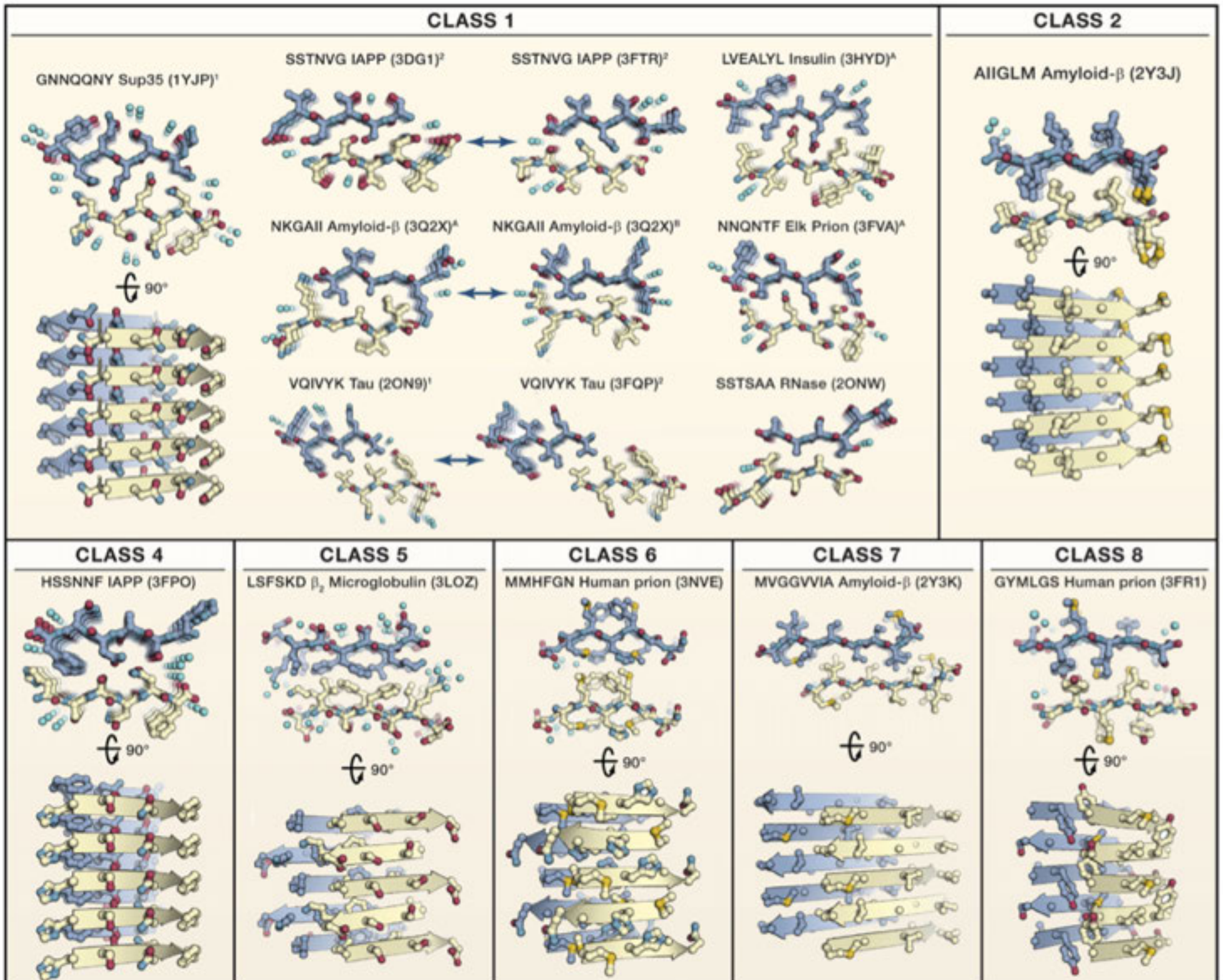
. Subunit-subunit **interfaces** ?

→ **Assumptions** are required to generate 3D models

F-actin: 6.6 Å

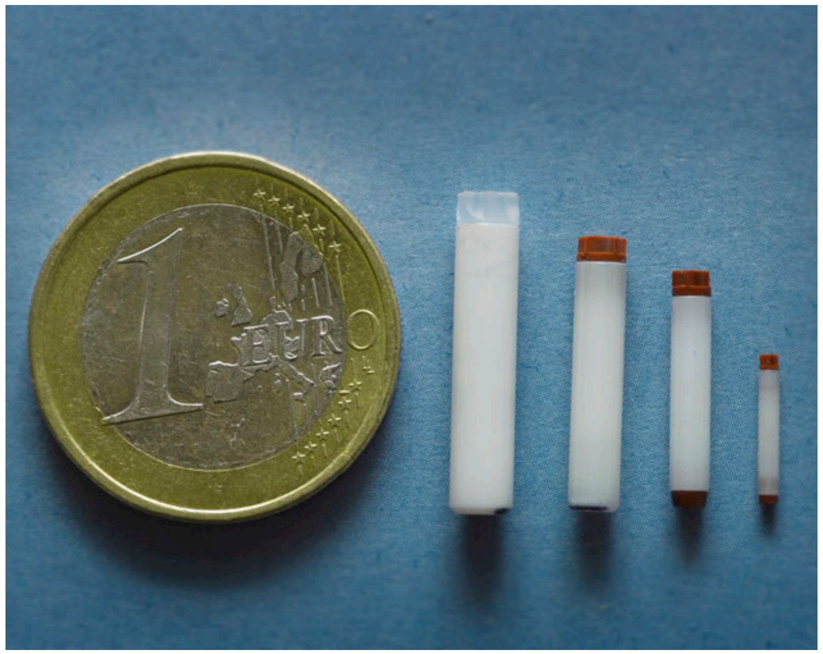
3D MODELS FROM CRYSTAL STRUCTURE

see Eisenberg and co.

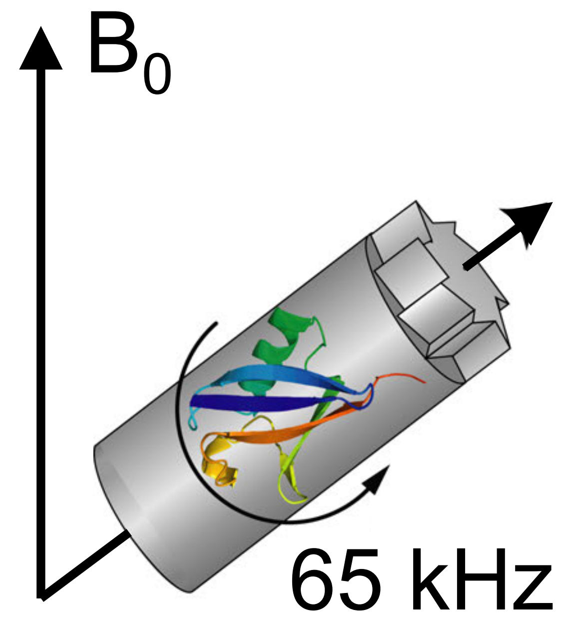


Urgent need for new approaches to investigate self-assemblies in the **intact assembled state** at **atomic resolution**

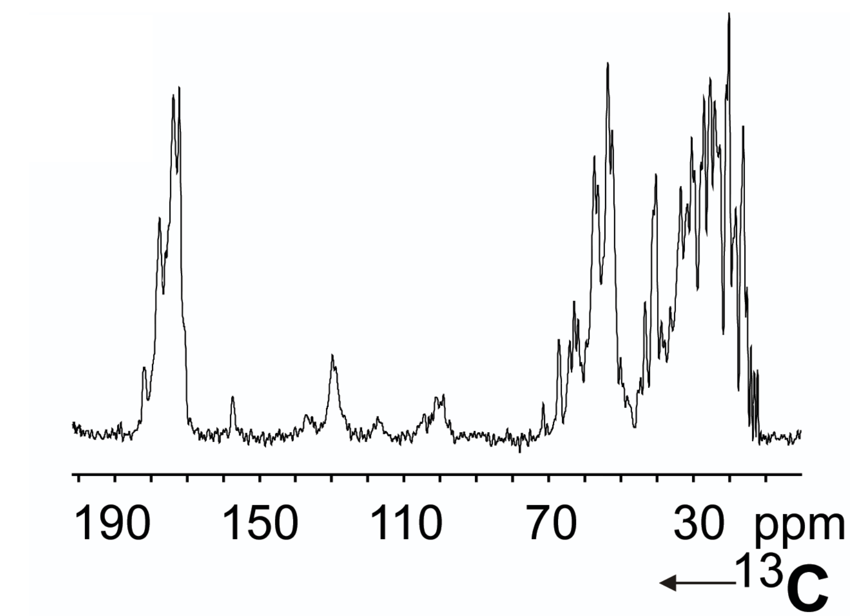
SOLID-STATE NMR (SSNMR)



SSNMR rotors

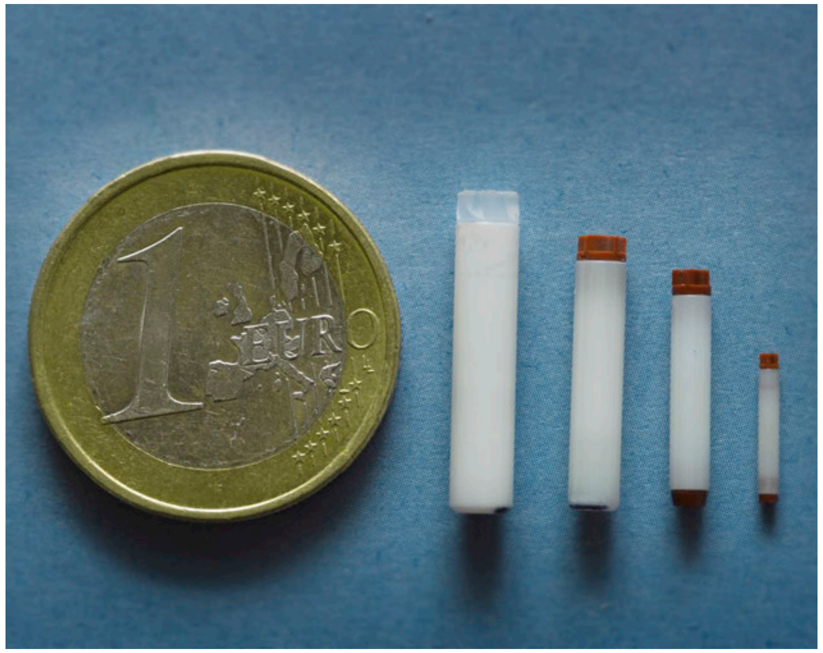


Magic-angle spinning

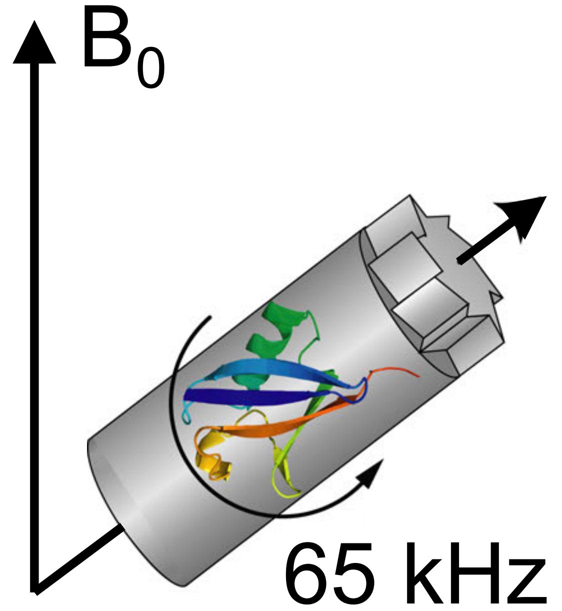


High resolution

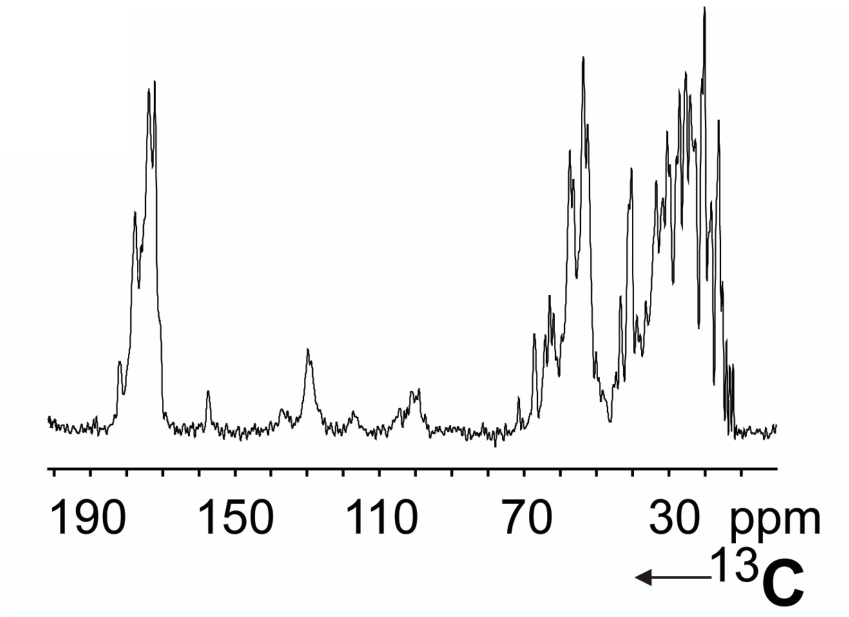
SOLID-STATE NMR (SSNMR)



SSNMR rotors



Magic-angle spinning



High resolution

. Neither **longe-range order** nor **solubility** required

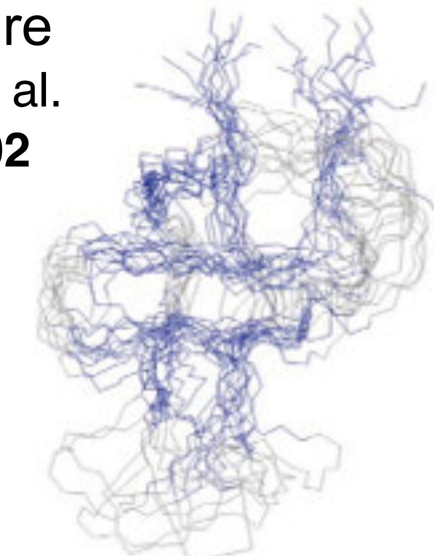
. **Solid** to **semi-solid** samples

. Proteoliposomes, oligomers, fibrils, filaments, precipitates, gels, frozen solutions, nanoparticles, etc...

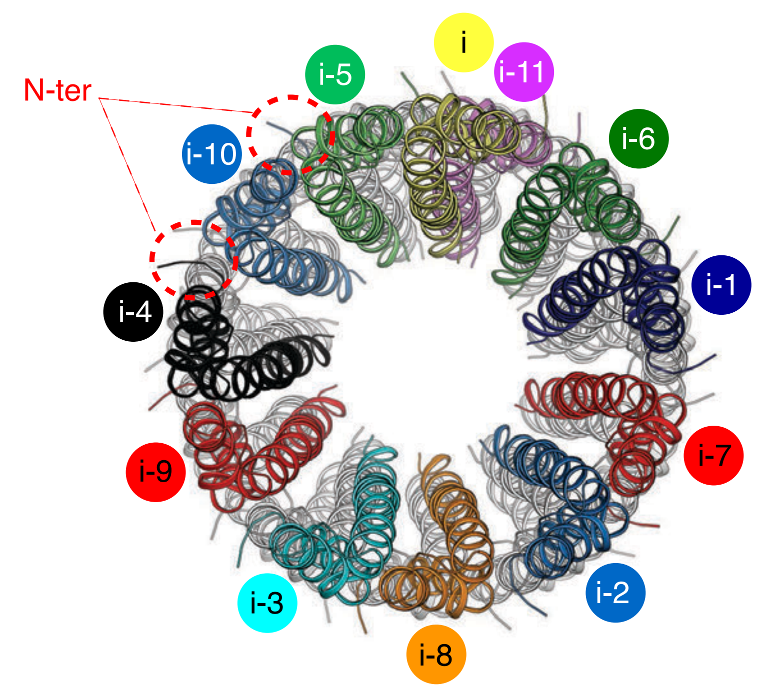
Solid-state NMR
and
structural biology

SSNMR & STRUCTURAL BIOLOGY

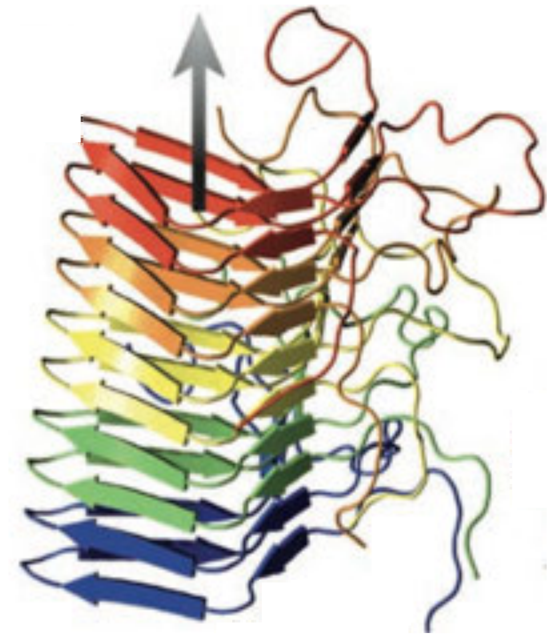
1st structure
Castellani et al.
Nature **2002**



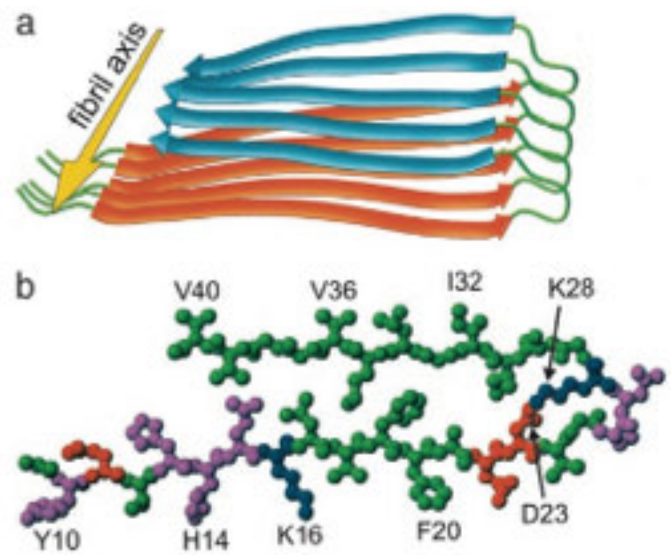
T3SS needle structure
Loquet et al. *Nature* **2012**



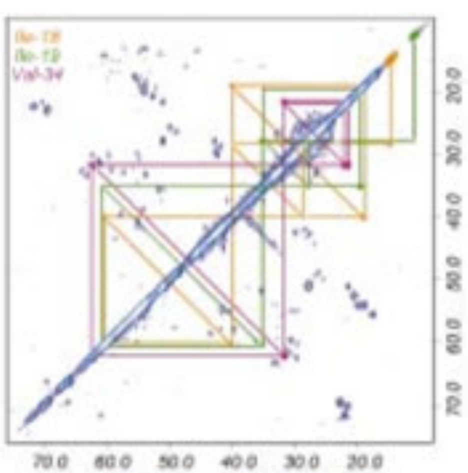
HET-s structure
Wasmer et al. *Science* **2008**



Aβ 3D model
Petkova et al. *PNAS* **2002**



1st assignment
McDermott et al.
JBNMR **2000**



2000 **2002** **2004** **2006** **2008** **2010** **2012**

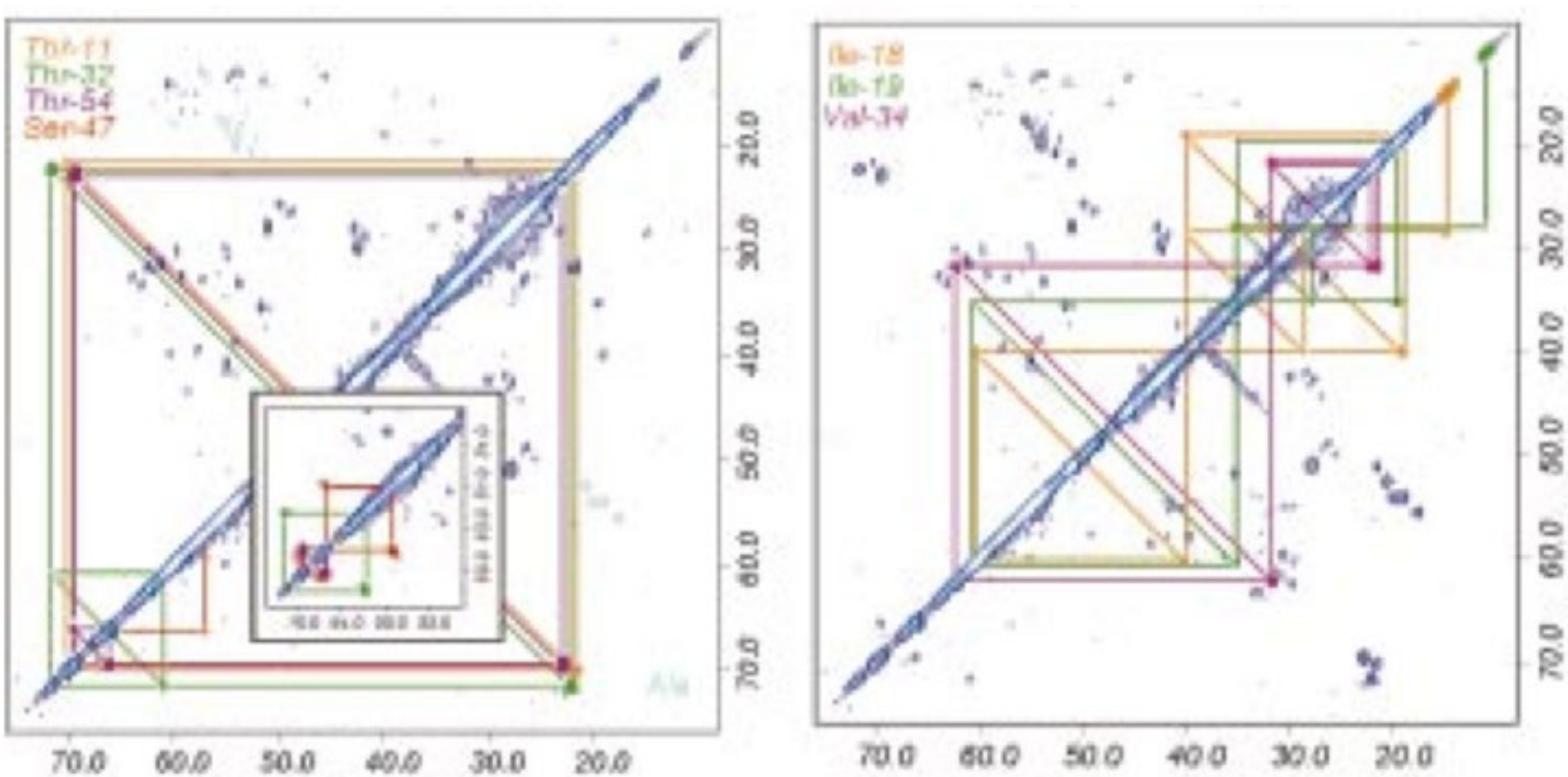
Cristalline samples

2000 1st SSNMR assignment

Journal of Biomolecular NMR, **16:** 209–219, 2000.
KLUWER/ESCOM

Partial NMR assignments for uniformly (^{13}C, ^{15}N)-enriched BPTI in the solid state

Ann McDermott[a,*], Tatyana Polenova[a,**], Anja Bockmann[a,***], Kurt. W. Zilm[b,*], Eric K. Paulsen[b], Rachel W. Martin[b] & Gaetano T. Montelione[c]



2003 SSNMR on protein nanocrystals

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Magnetic Resonance 165 (2003) 162–174

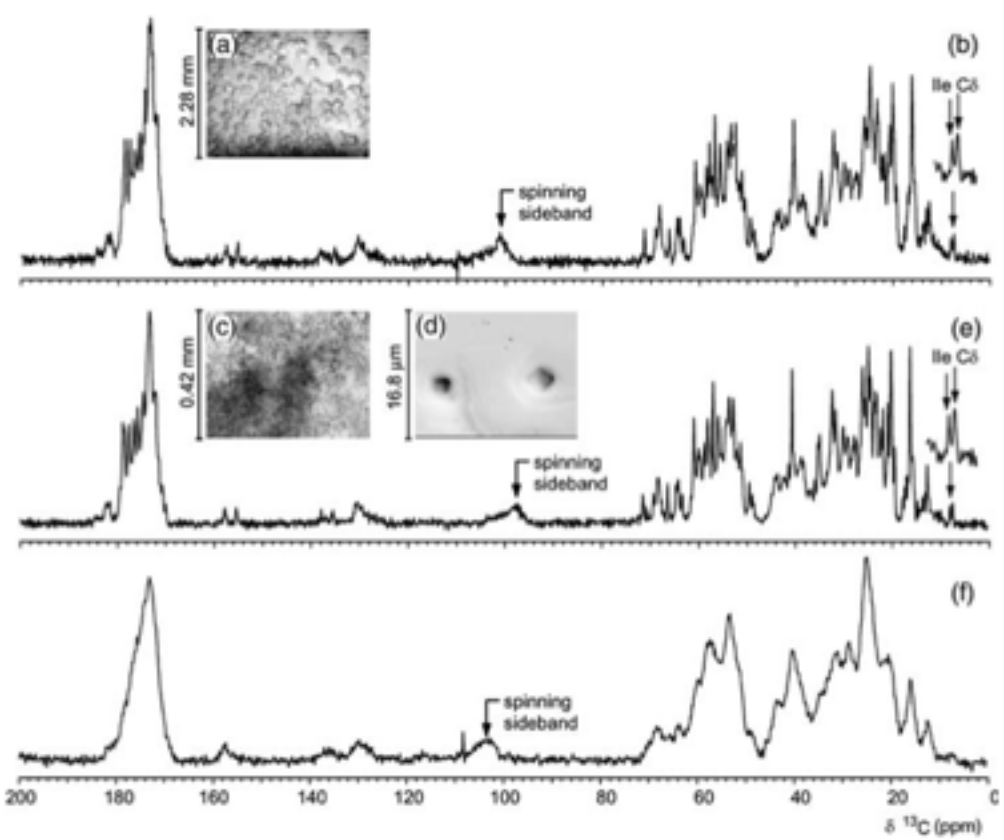
JMR Journal of Magnetic Resonance

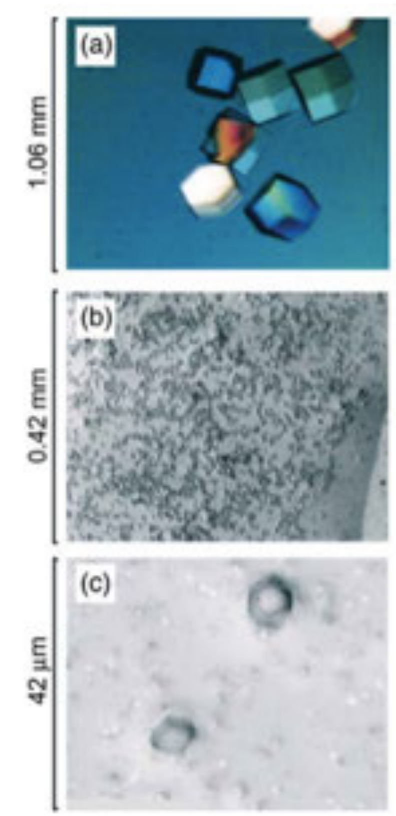
www.elsevier.com/locate/jmr

ELSEVIER

Preparation of protein nanocrystals and their characterization by solid state NMR

Rachel W. Martin and Kurt W. Zilm*



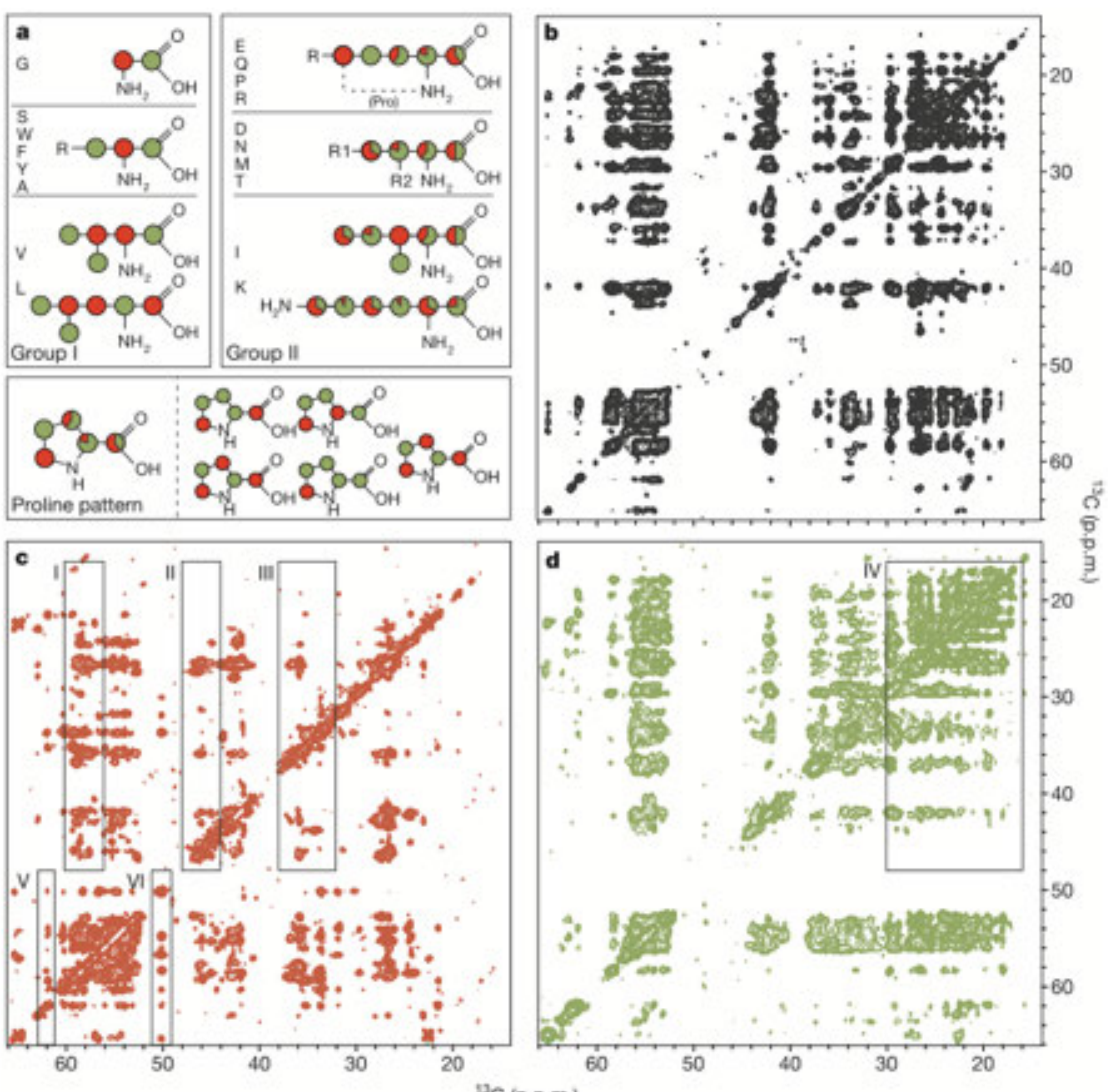


2002 1st protein structure (microcrystals)

letters to nature

Structure of a protein determined by solid-state magic-angle-spinning NMR spectroscopy

Federica Castellani, Barth van Rossum, Annette Diehl, Mario Schubert, Kristina Rehbein & Hartmut Oschkinat



2008 Uniformly $^{13}C/^{15}N$ labeled samples

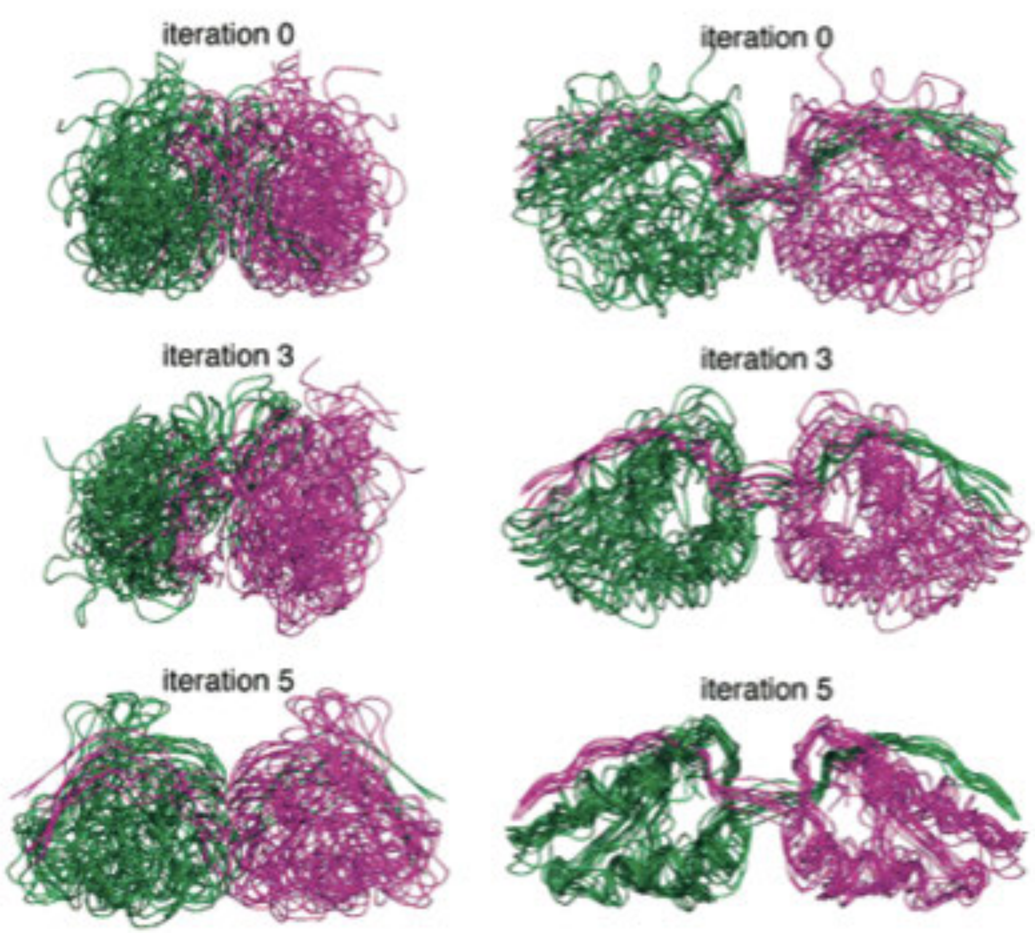
3D Structure Determination of the Crh Protein from Highly Ambiguous Solid-State NMR Restraints

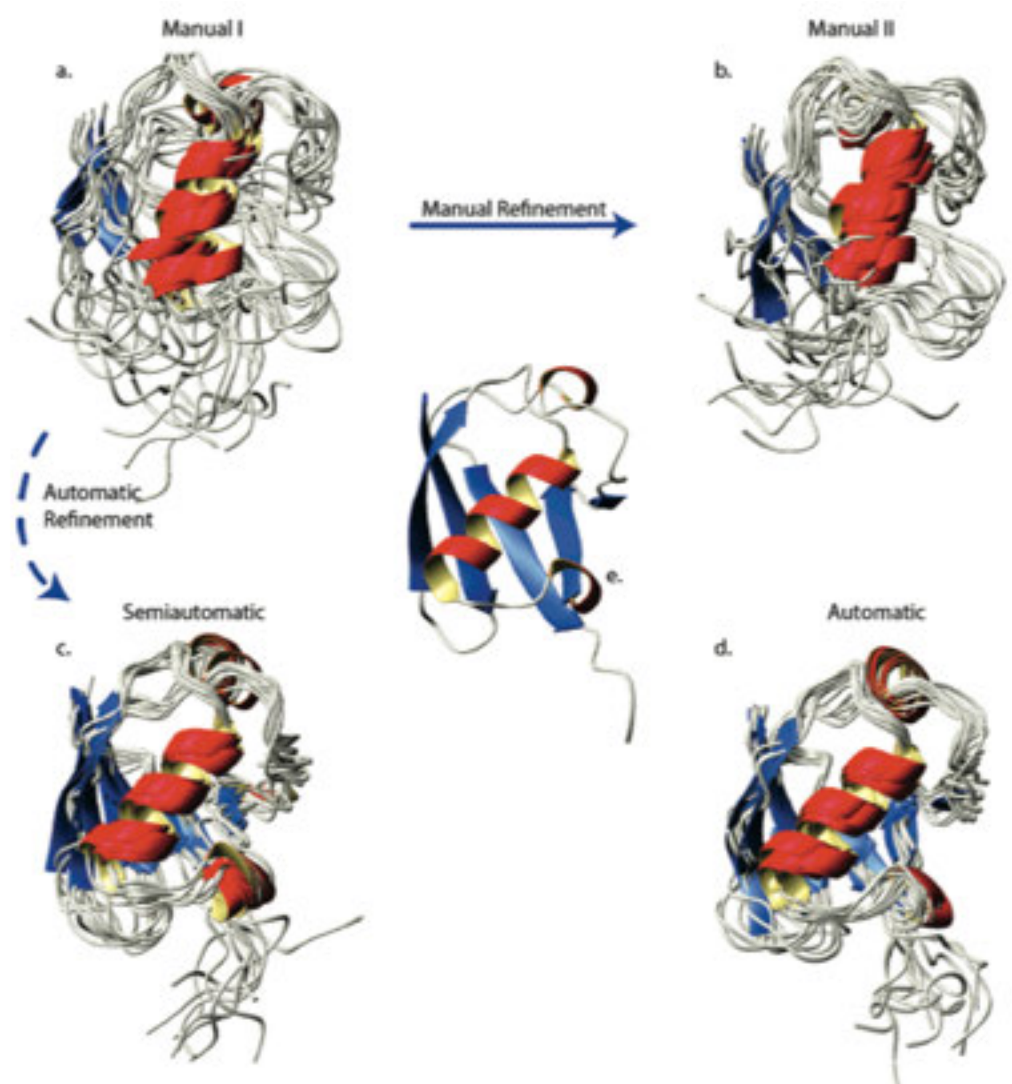
Antoine Loquet,[‡] Benjamin Bardiaux,[#] Carole Gardiennet,[‡] Christophe Blanchet,[‡] Marc Baldus,[§] Michael Nilges,[#] Thérèse Malliavin,[#] and Anja Böckmann[*,‡]

J|A|C|S
ARTICLES
Published on Web 02/20/2008

Protein Structure Determination from ^{13}C Spin-Diffusion Solid-State NMR Spectroscopy

Theofanis Manolikas,[†] Torsten Herrmann,[‡] and Beat H. Meier[*,†]

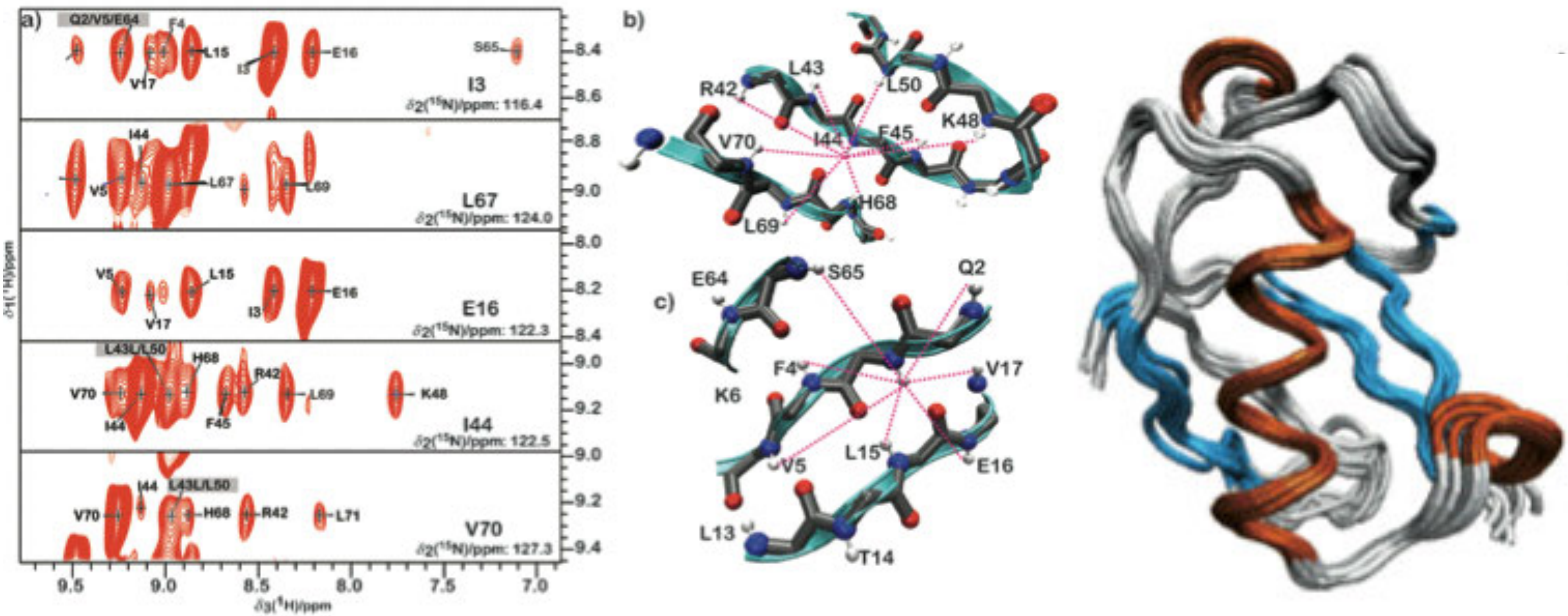




2015 Sub-mg microcrystalline protein

De Novo 3D Structure Determination from Sub-milligram Protein Samples by Solid-State 100 kHz MAS NMR Spectroscopy**

Vipin Agarwal, Susanne Penzel, Kathrin Szekely, Riccardo Cadalbert, Emilie Testori, Andres Oss, Jaan Past, Ago Samoson, Matthias Ernst,* Anja Böckmann,* and Beat H. Meier**



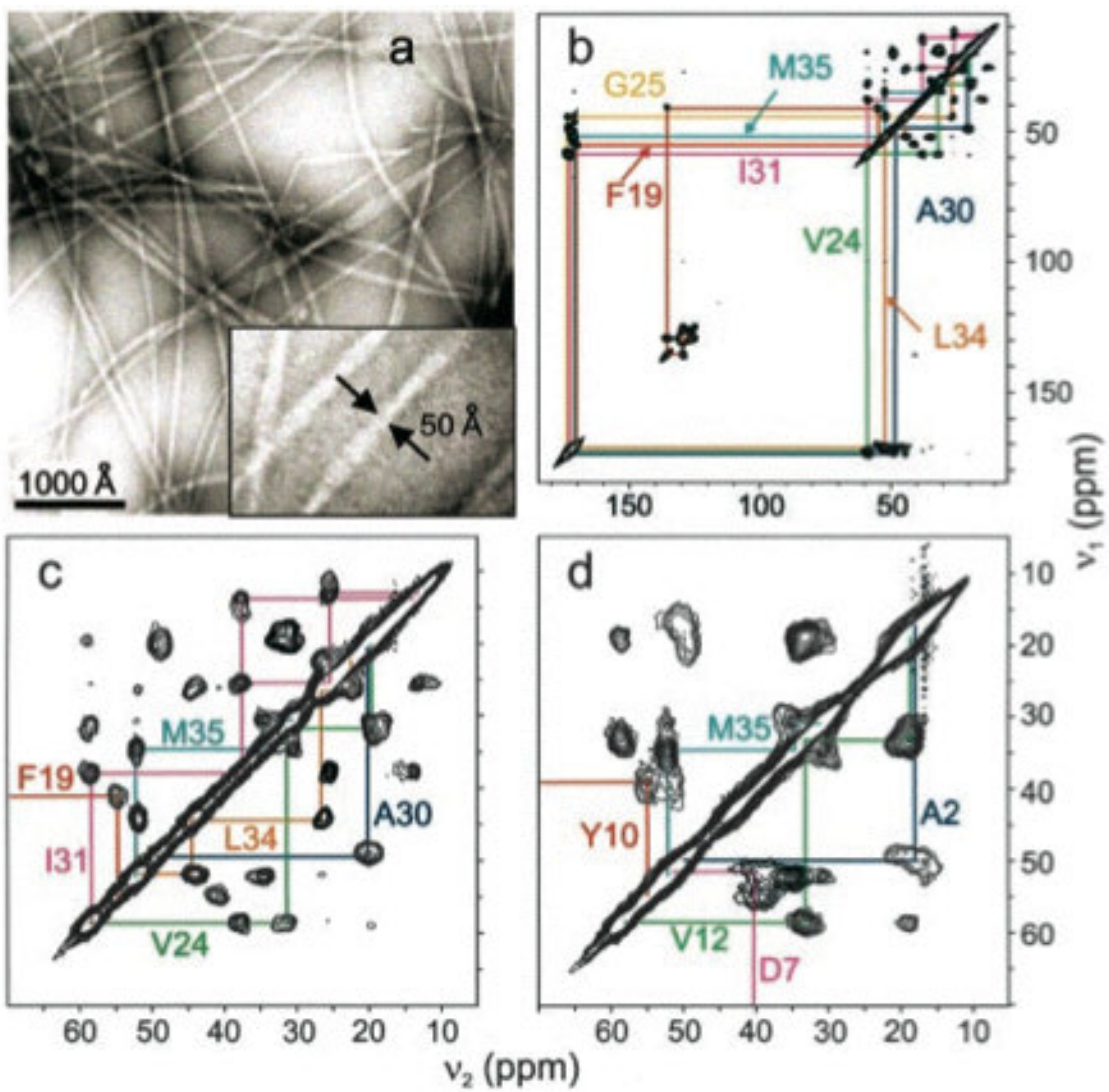
Amyloid fibrils

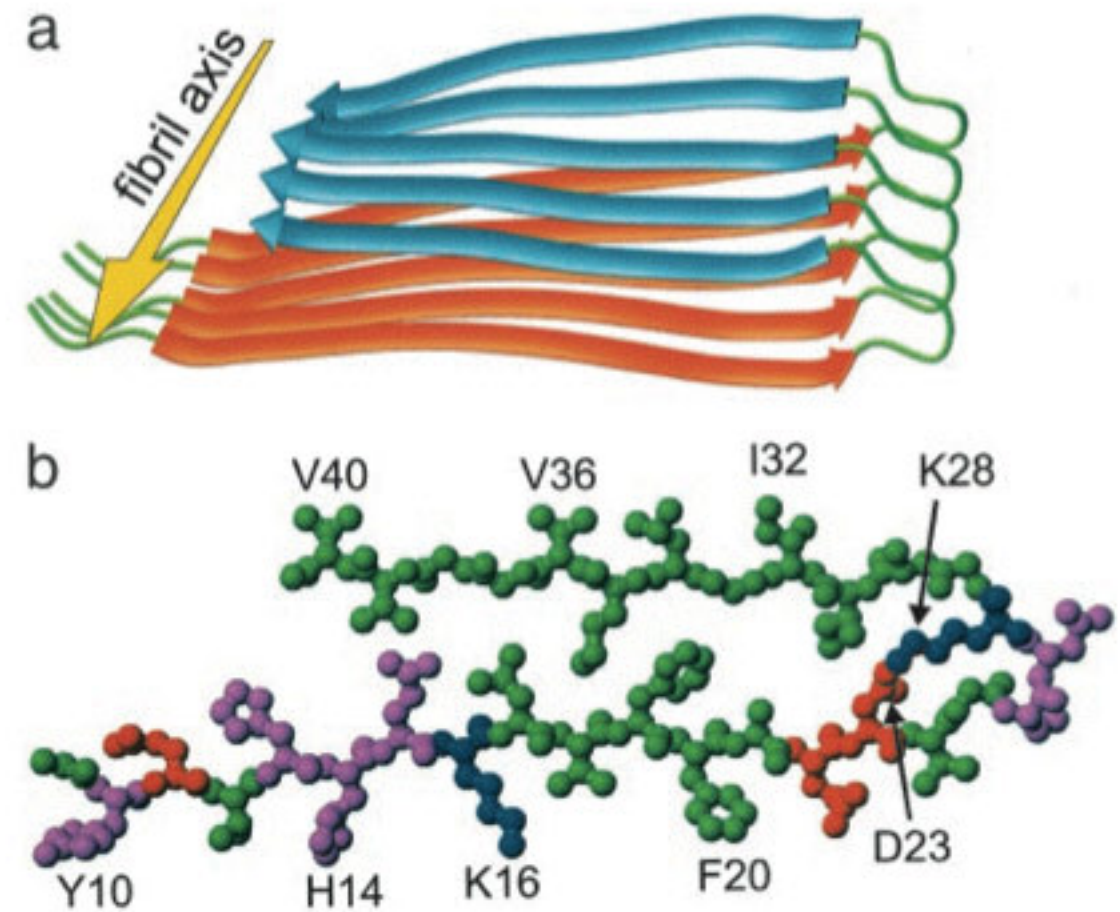
2002

Amyloid fibril: 3D model

A structural model for Alzheimer's β-amyloid fibrils based on experimental constraints from solid state NMR

Aneta T. Petkova*, Yoshitaka Ishii*†, John J. Balbach*, Oleg N. Antzutkin‡, Richard D. Leapman§, Frank Delaglio*, and Robert Tycko*¶



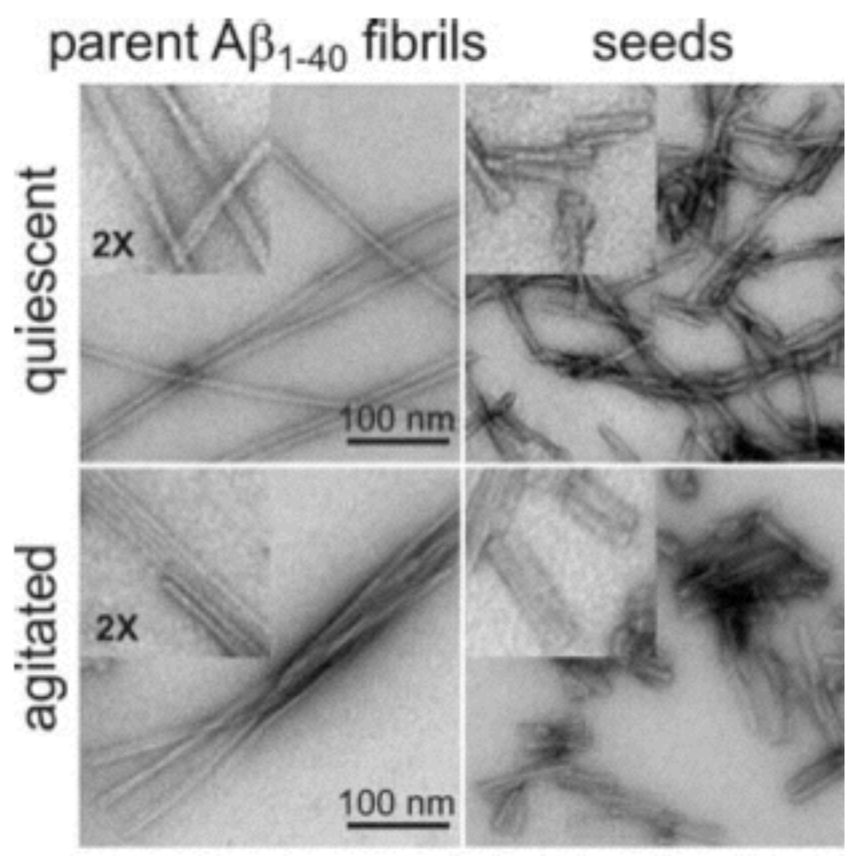


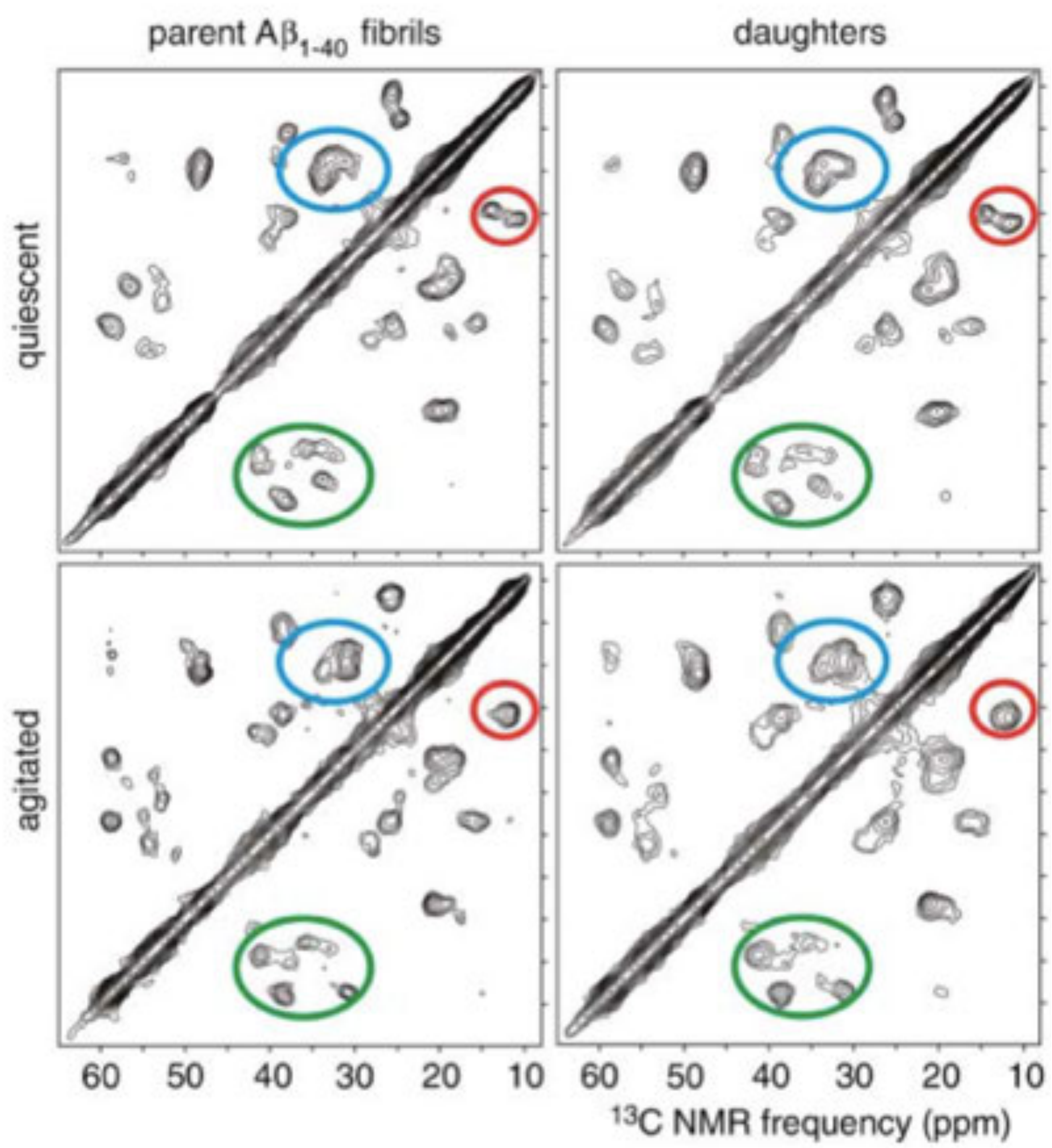
2005 Structure - polymorphism - toxicity

Science AAAS

Self-Propagating, Molecular-Level Polymorphism in Alzheimer's β-Amyloid Fibrils

Aneta T. Petkova,[1] Richard D. Leapman,[2] Zhihong Guo,[3] Wai-Ming Yau,[1] Mark P. Mattson,[3] Robert Tycko[1]*

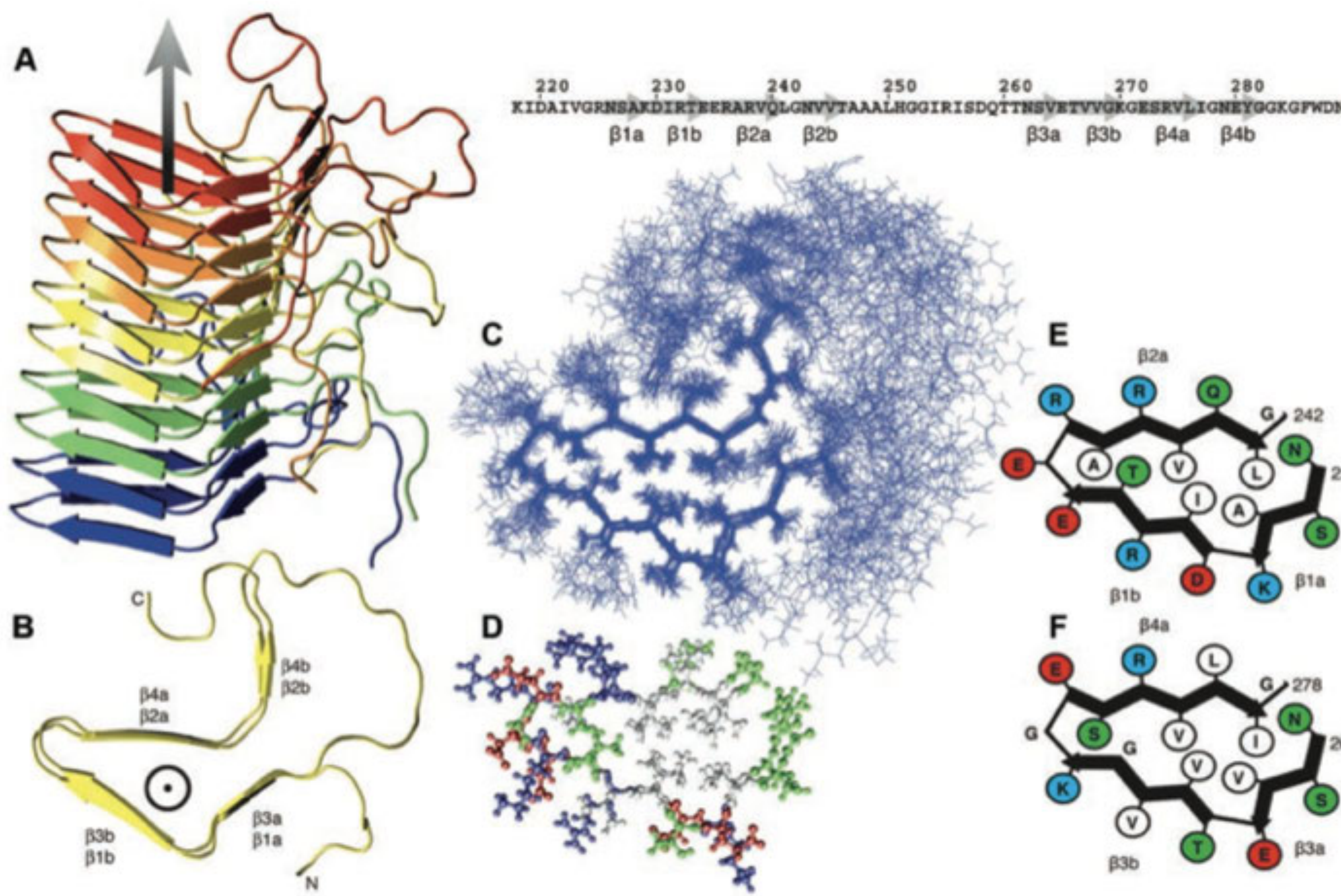




2008 Amyloid fibril: atomic structure

Amyloid Fibrils of the HET-s(218–289) Prion Form a β Solenoid with a Triangular Hydrophobic Core

Christian Wasmer,* Adam Lange,* Hélène Van Melckebeke,*
Ansgar B. Siemer,† Roland Riek, Beat H. Meier‡

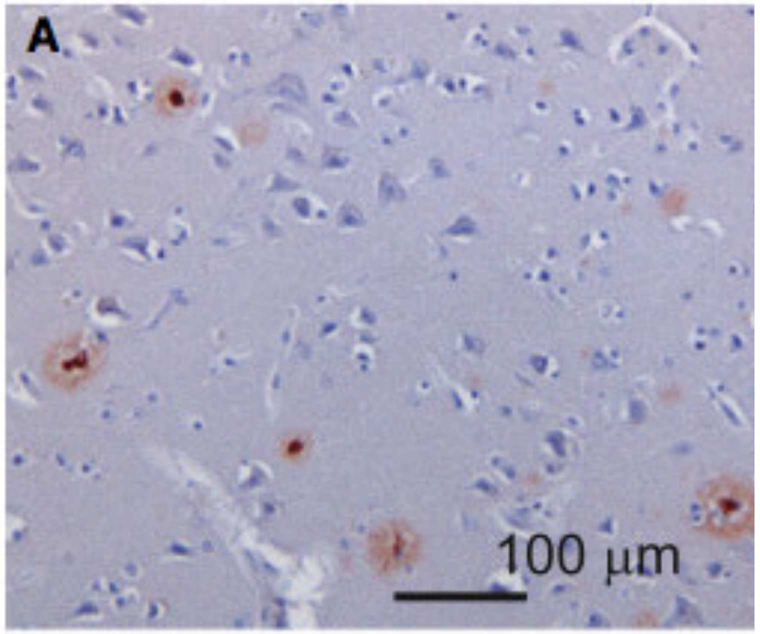


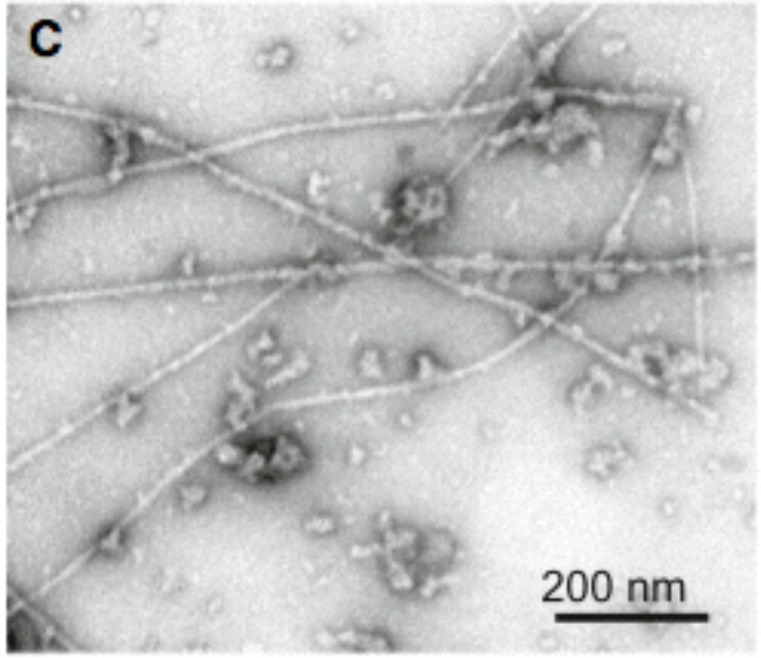
2013 Amyloid beta in brain tissue

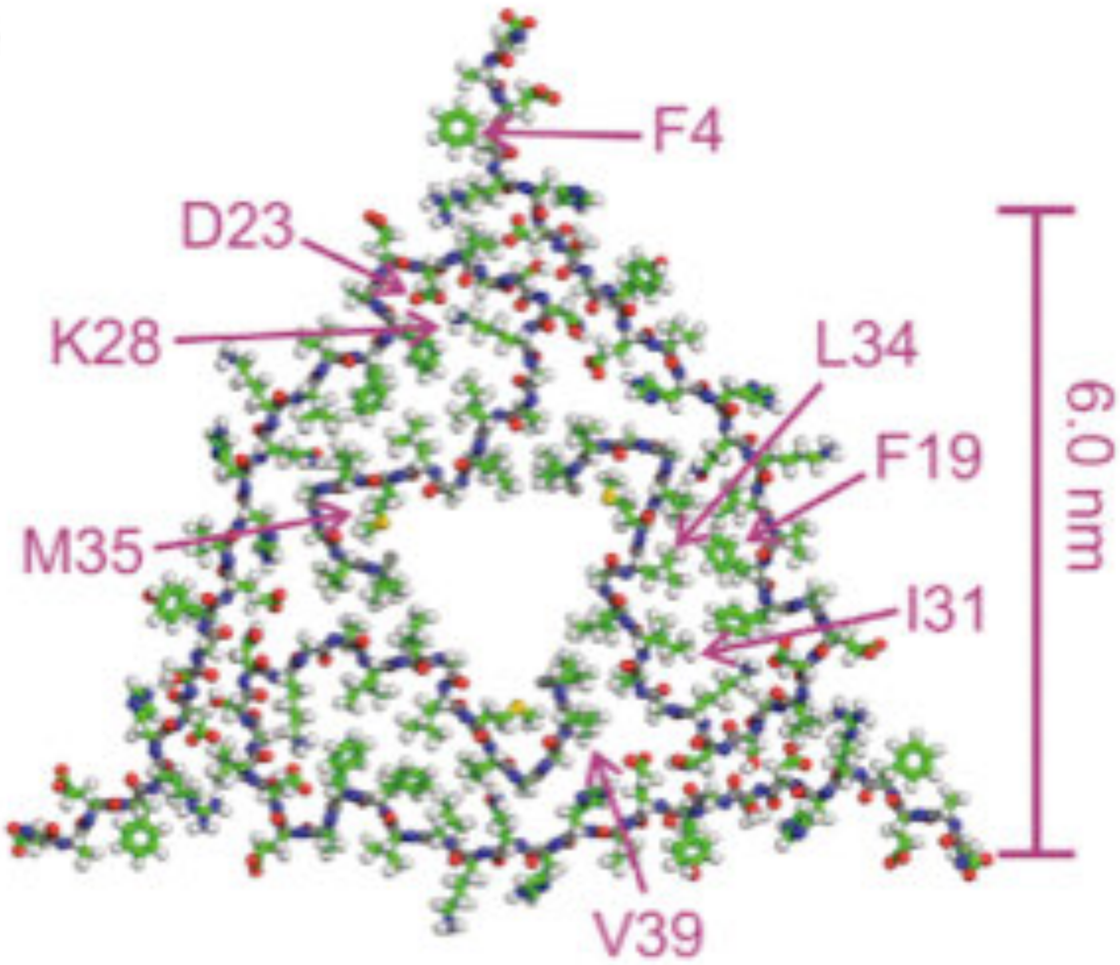
Molecular Structure of β-Amyloid Fibrils in Alzheimer's Disease Brain Tissue

Cell

Jun-Xia Lu,[1] Wei Qiang,[1] Wai-Ming Yau,[1] Charles D. Schwieters,[2] Stephen C. Meredith,[3] and Robert Tycko[1,*]





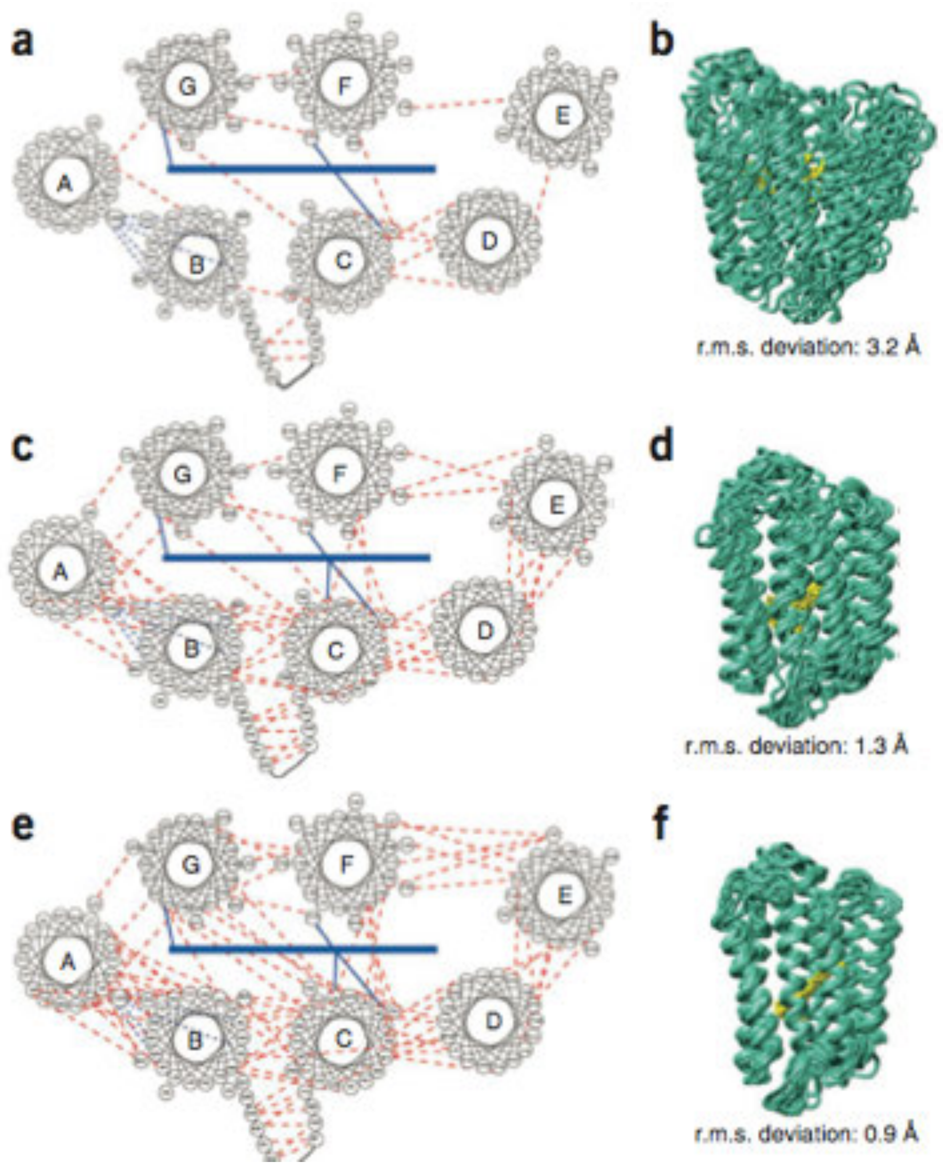


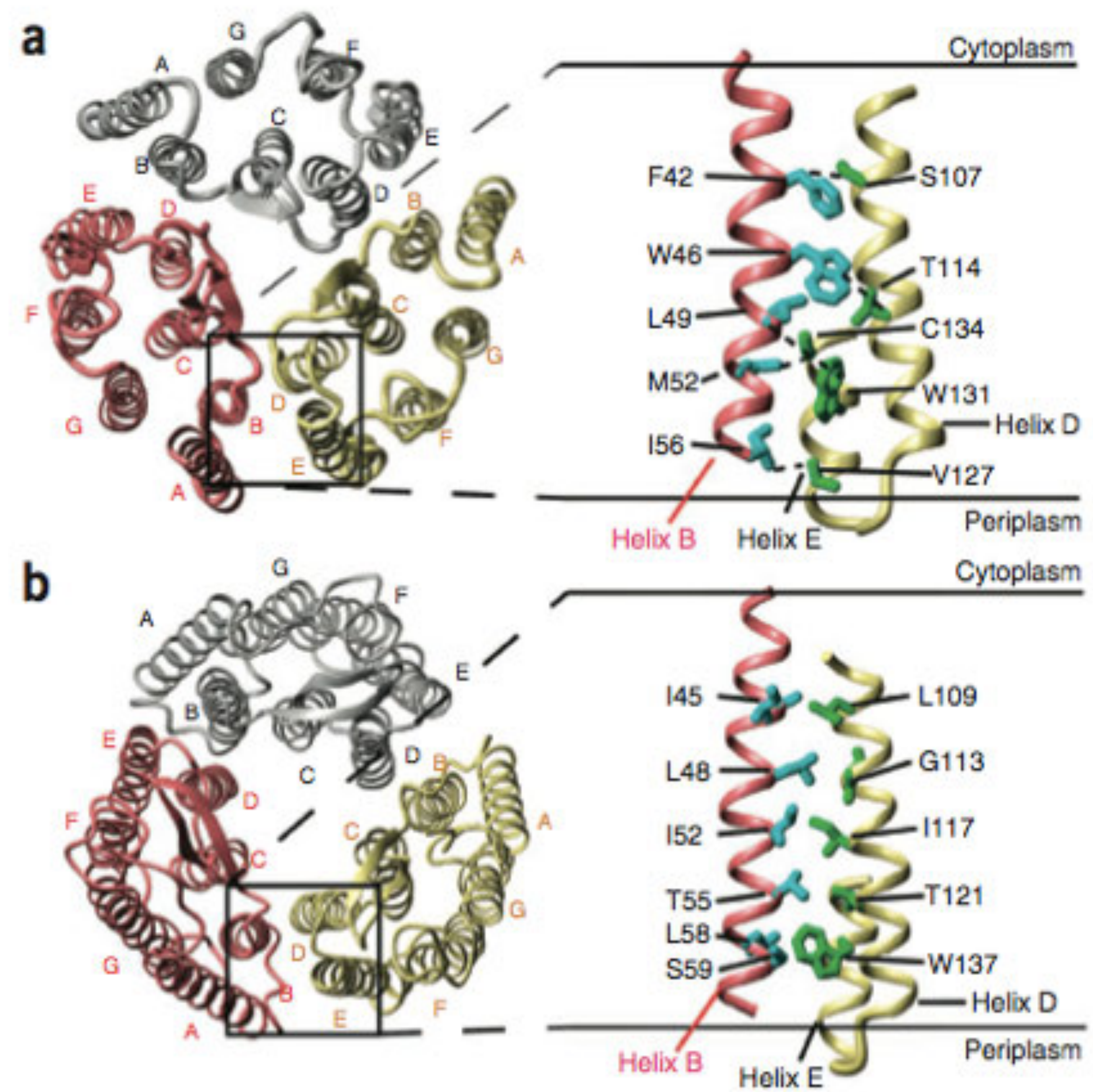
Membrane proteins

2013 Membrane protein structure

Solid-state NMR spectroscopy structure determination of a lipid-embedded heptahelical membrane protein

Shenlin Wang[1,2], Rachel A Munro[1,2], Lichi Shi[1,2,6], Izuru Kawamura[1,6], Takashi Okitsu[3], Akimori Wada[3], So-Young Kim[4,5], Kwang-Hwan Jung[4,5], Leonid S Brown[1,2] & Vladimir Ladizhansky[1,2]

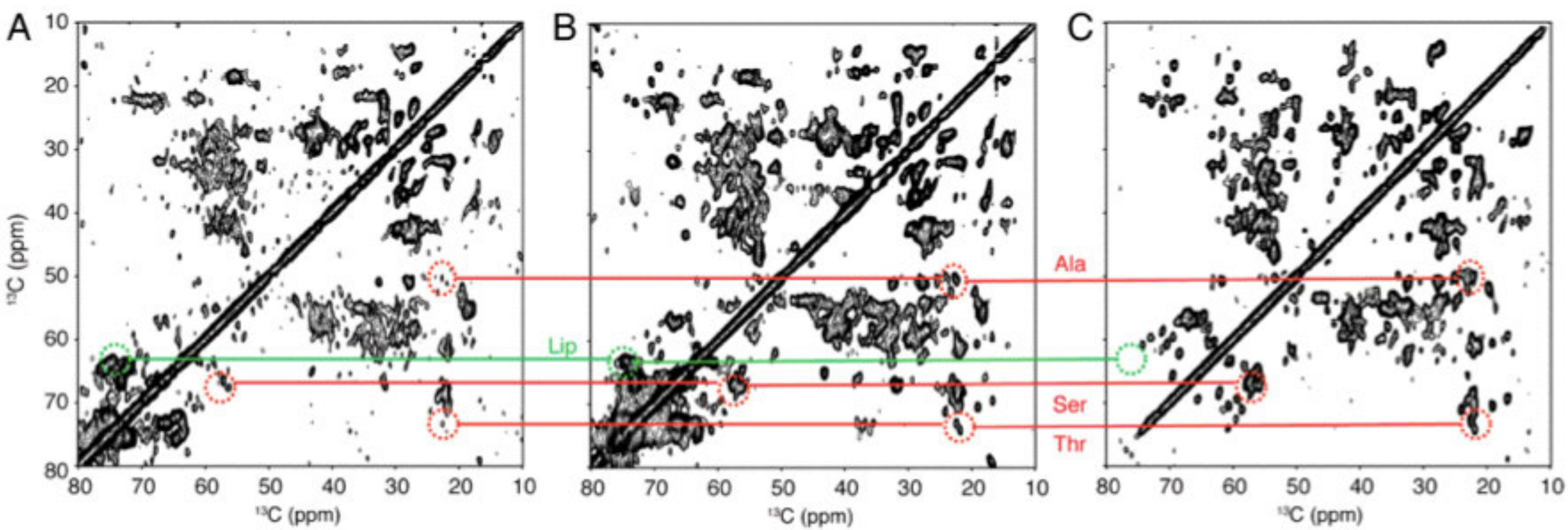




2012 In-cell SSNMR of membrane proteins

Cellular solid-state nuclear magnetic resonance spectroscopy

Marie Renault[a], Ria Tommassen-van Boxtel[b], Martine P. Bos[b], Jan Andries Post[c], Jan Tommassen[b,1], and Marc Baldus[a,1]



New SSNMR approaches to tackle macromolecular protein assemblies

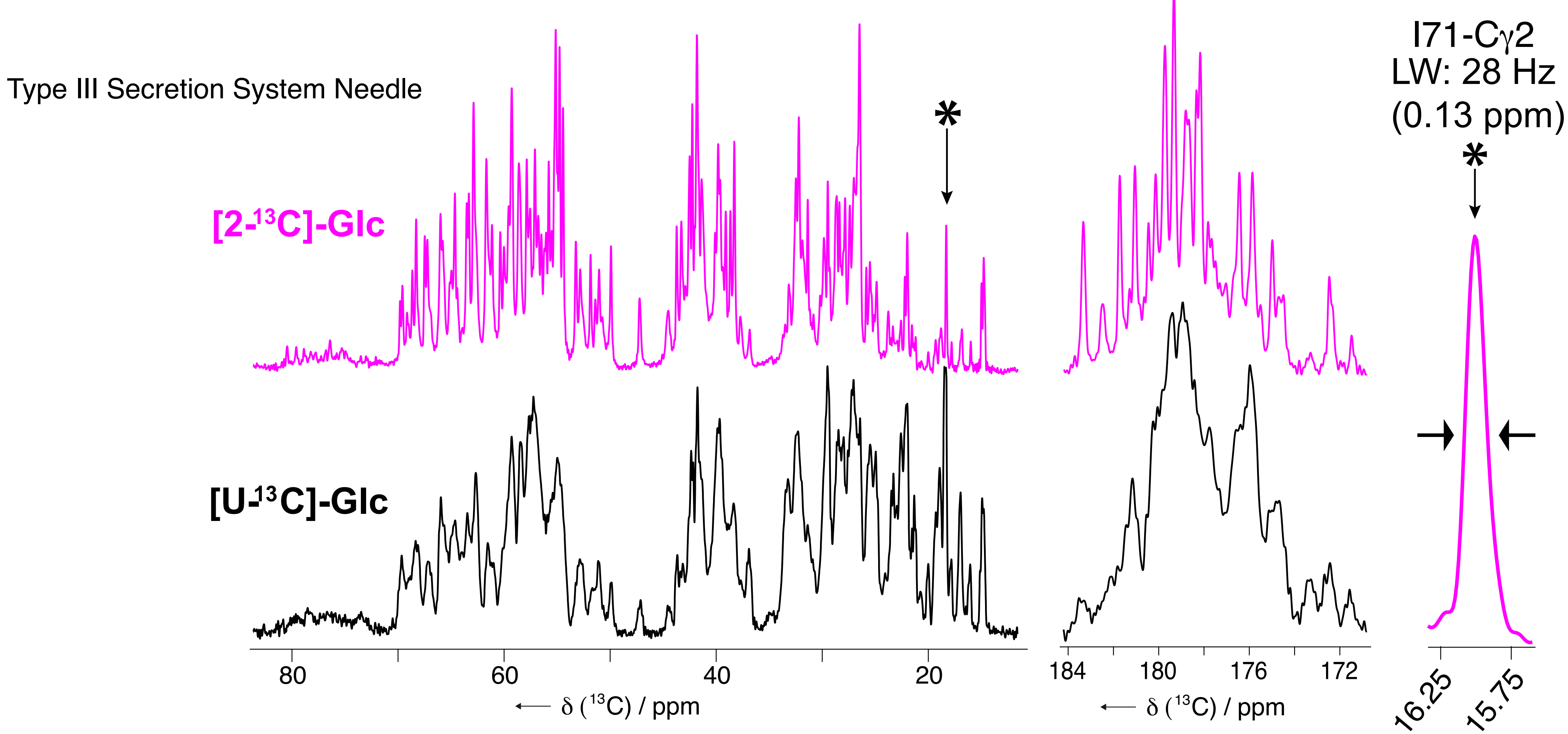
^{13}C SPIN DILUTION

Dilution of the ^{13}C spin network to reach ultra-sparsely labeled materials

[U-^{13}C]-Glc
CH(O)
CH(OH)
CH(OH)
CH(OH)
CH(OH)
CH2(OH)

[1-^{13}C]-Glc
CH(O)
CH(OH)
CH(OH)
CH(OH)
CH(OH)
CH2(OH)

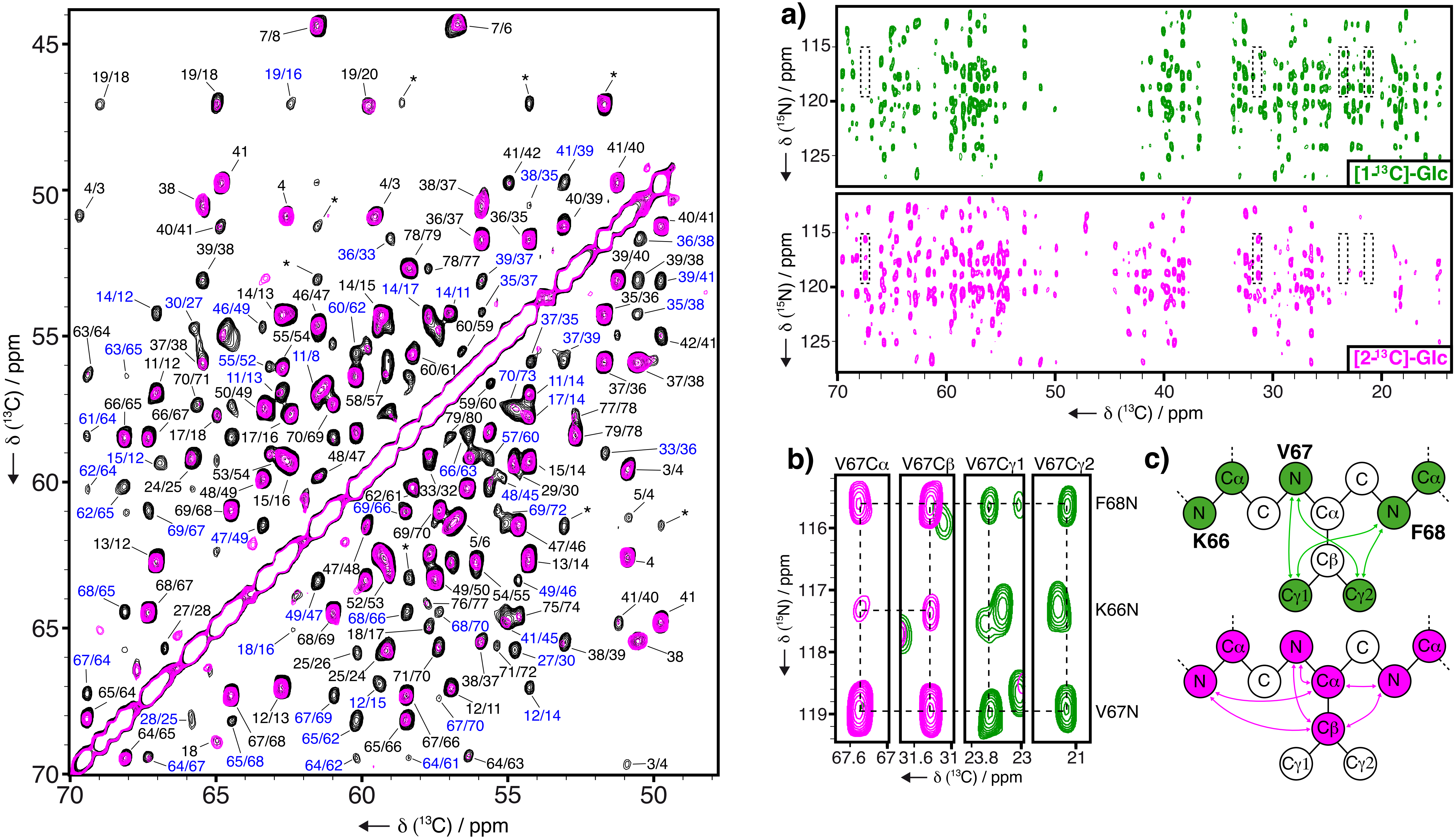
[2-^{13}C]-Glc
CH(O)
CH(OH)
CH(OH)
CH(OH)
CH(OH)
CH2(OH)



J. Am. Chem. Soc. **2011**

ULTRA HIGH RESOLUTION SSNMR

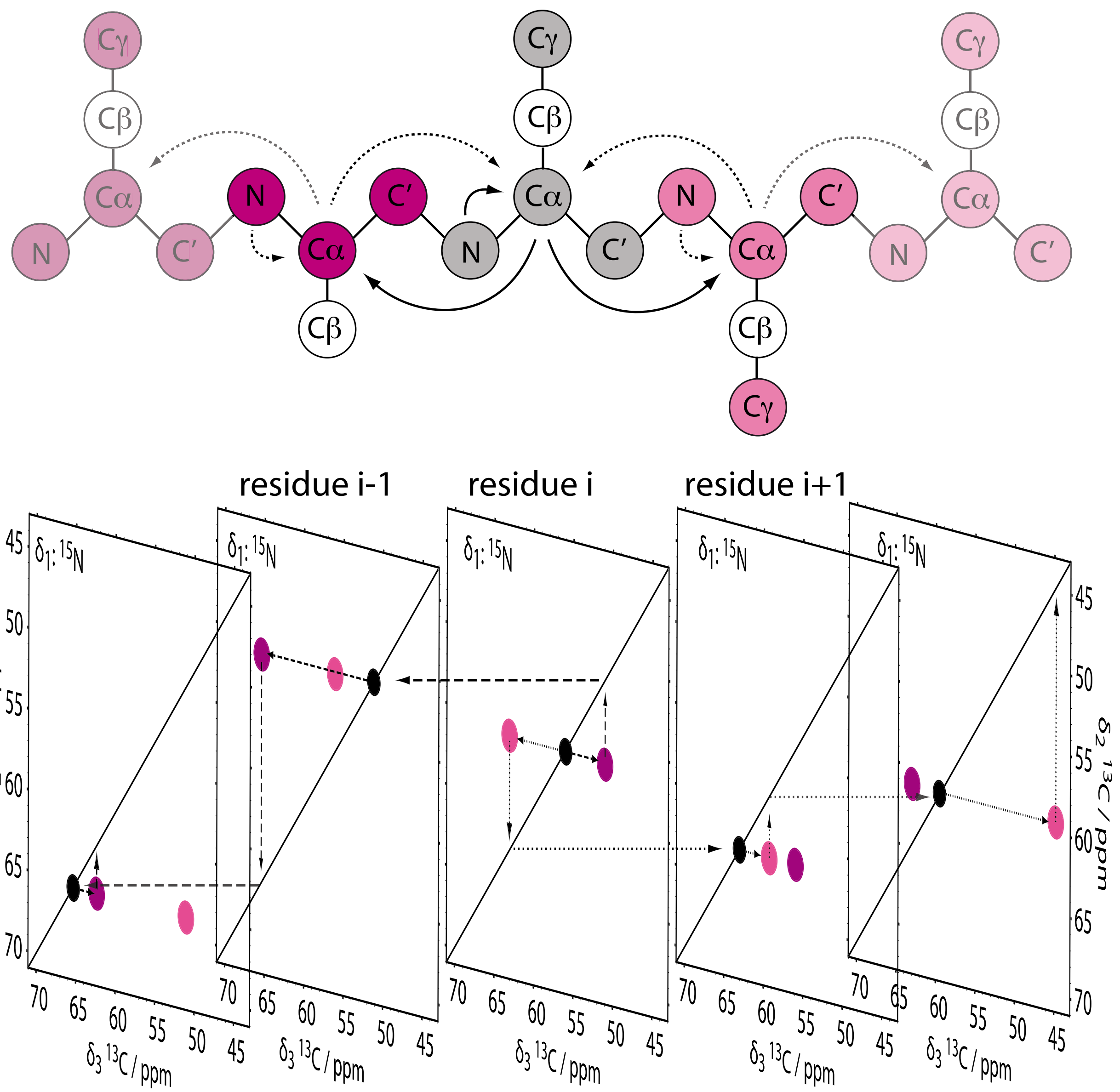
Type III Secretion System Needle

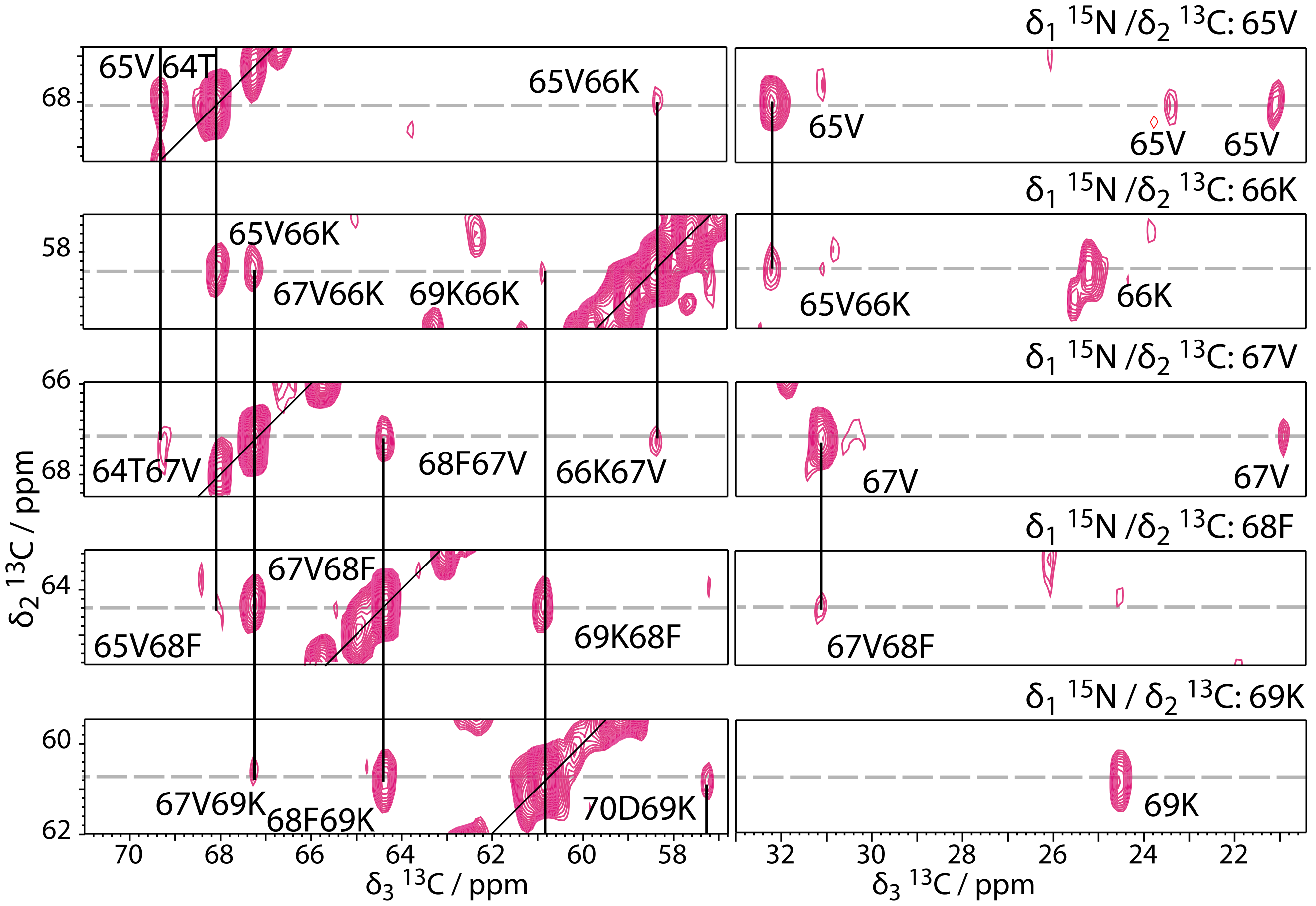


J. Am. Chem. Soc. **2011**

SPIN DILUTION + 3D SPECTROSCOPY

NMR assignment & secondary structure from a single 3D

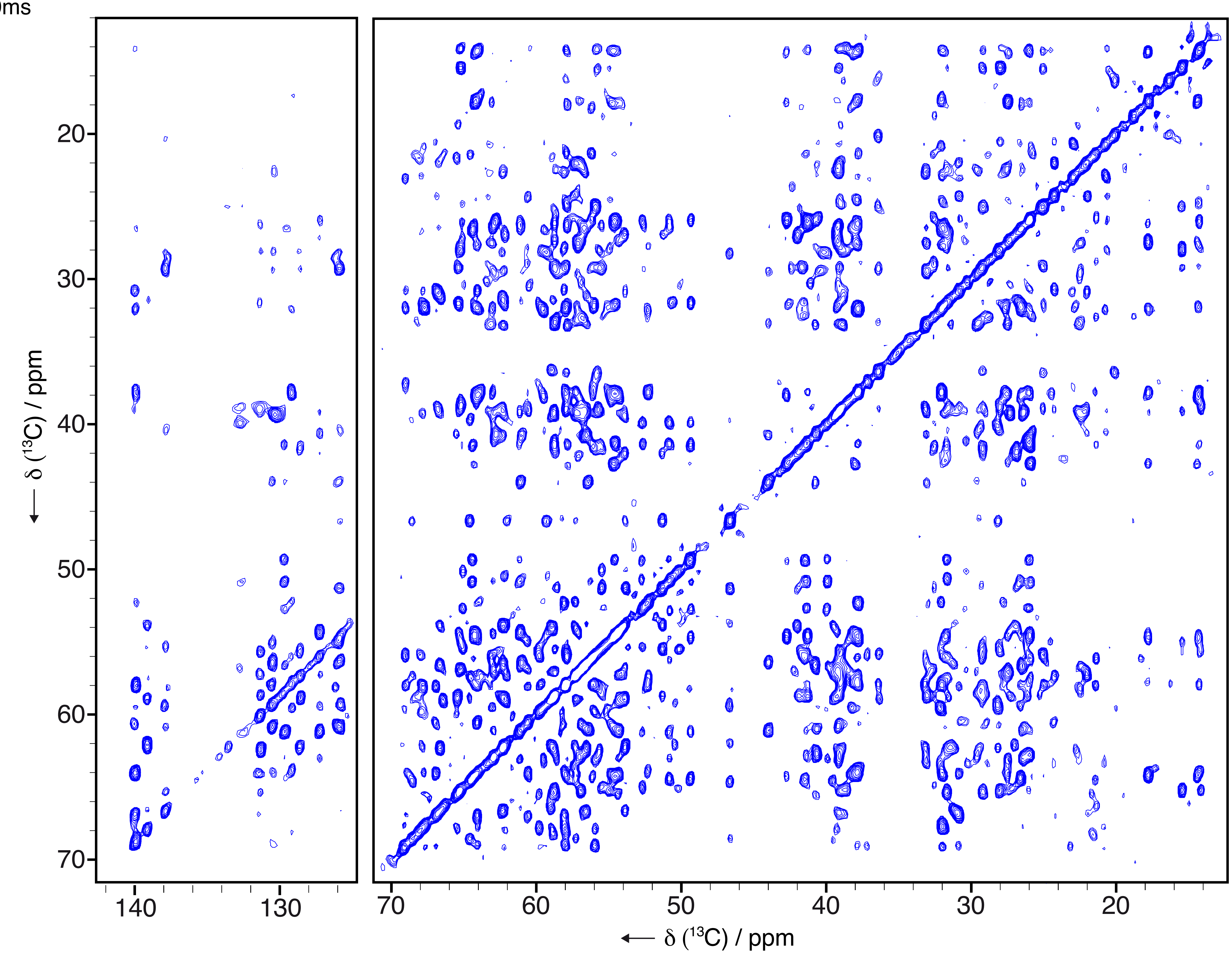




J. Biomol. NMR **2013**

DETECTION OF DISTANCE RESTRAINTS

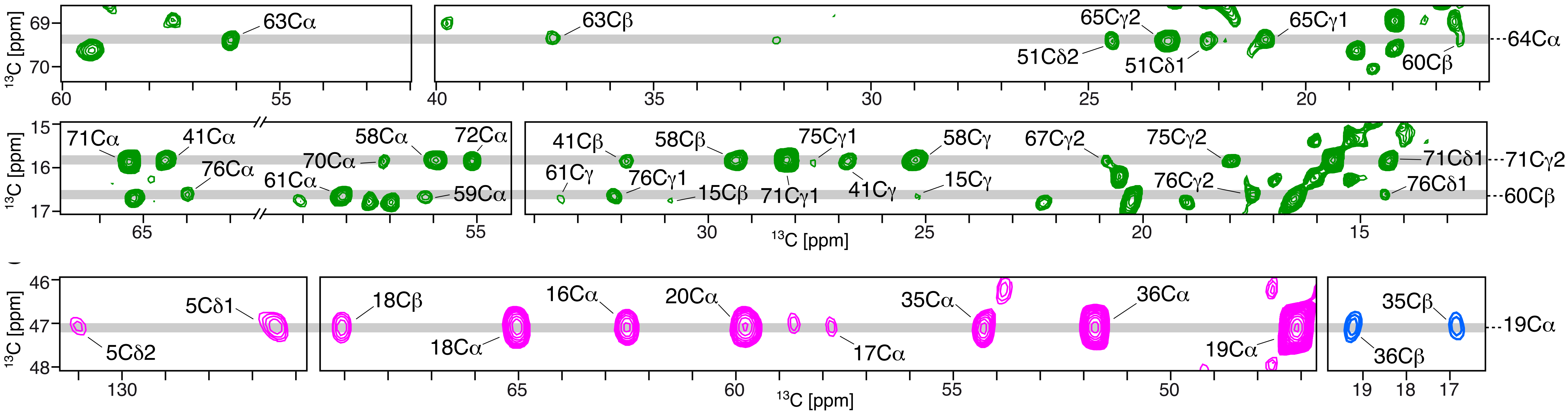
Type III Secretion System Needle
PDSD 850ms



DETECTION OF DISTANCE RESTRAINTS

Unambiguous assignment of ^{13}C-^{13}C and ^{15}N-^{13}C distances

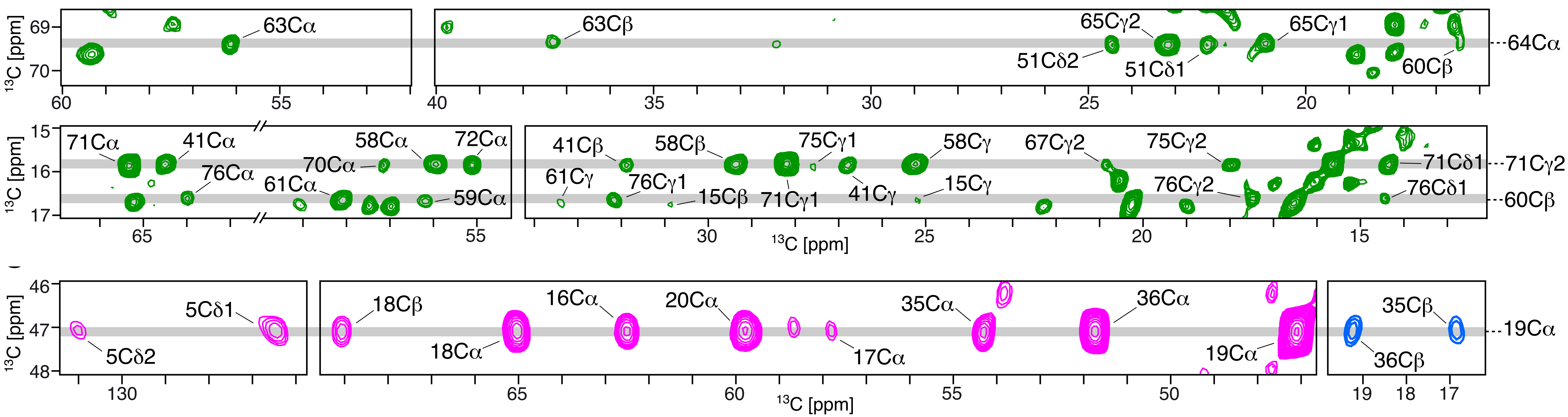
Type III Secretion System Needle



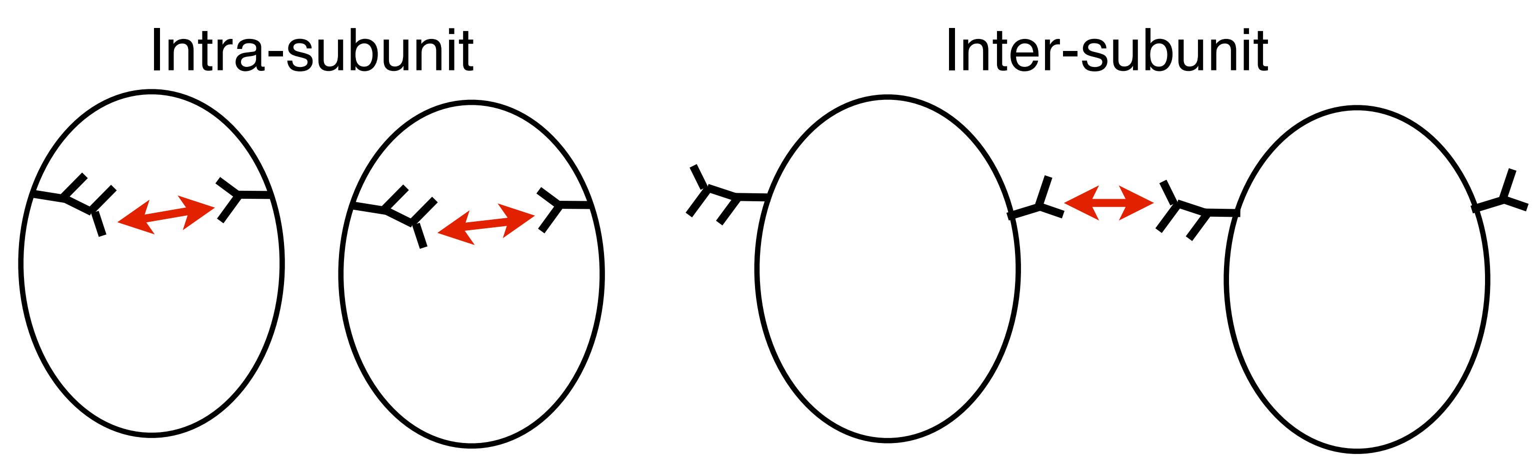
DETECTION OF DISTANCE RESTRAINTS

Unambiguous assignment of ^{13}C-^{13}C and ^{15}N-^{13}C distances

Type III Secretion System Needle

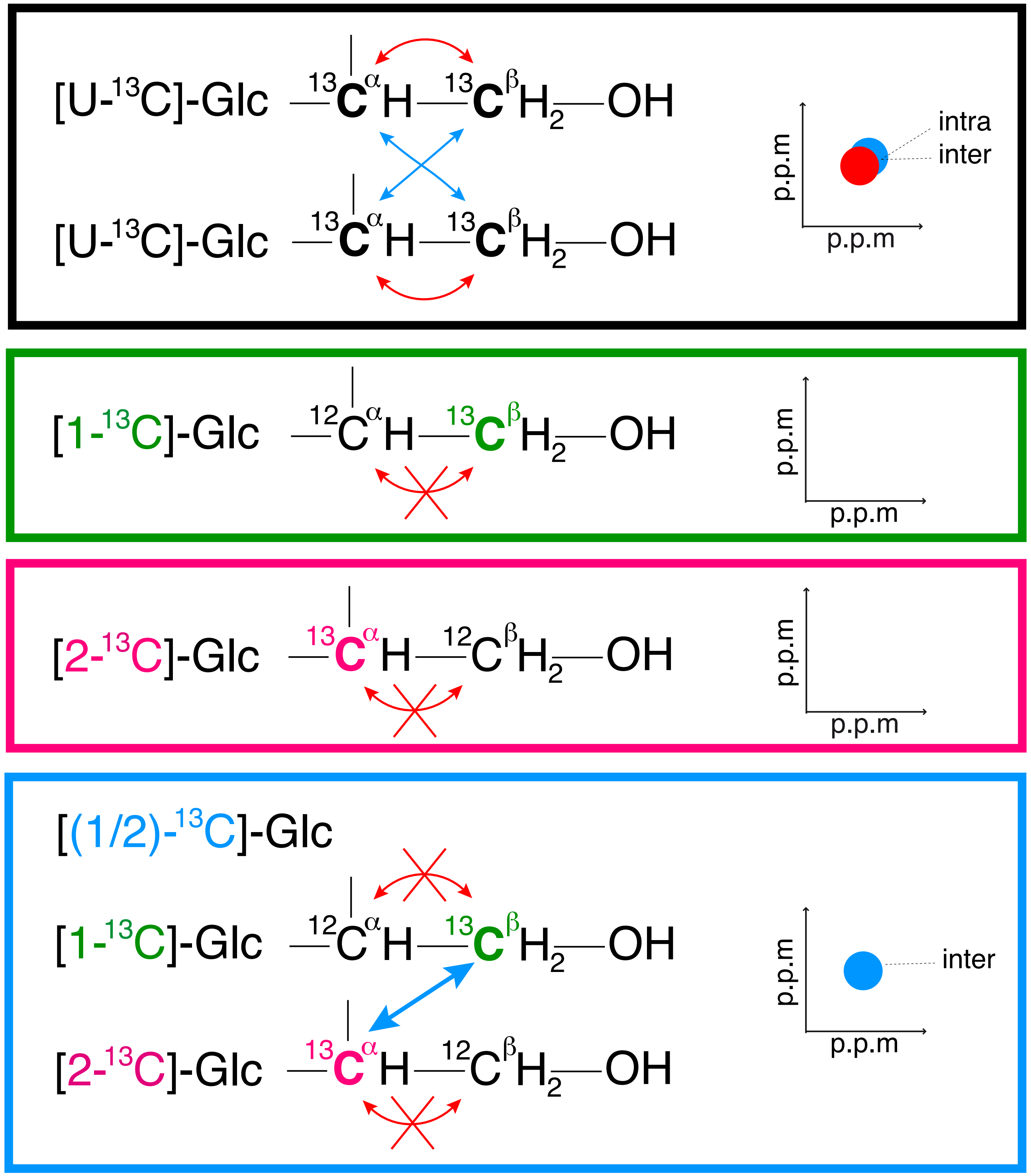


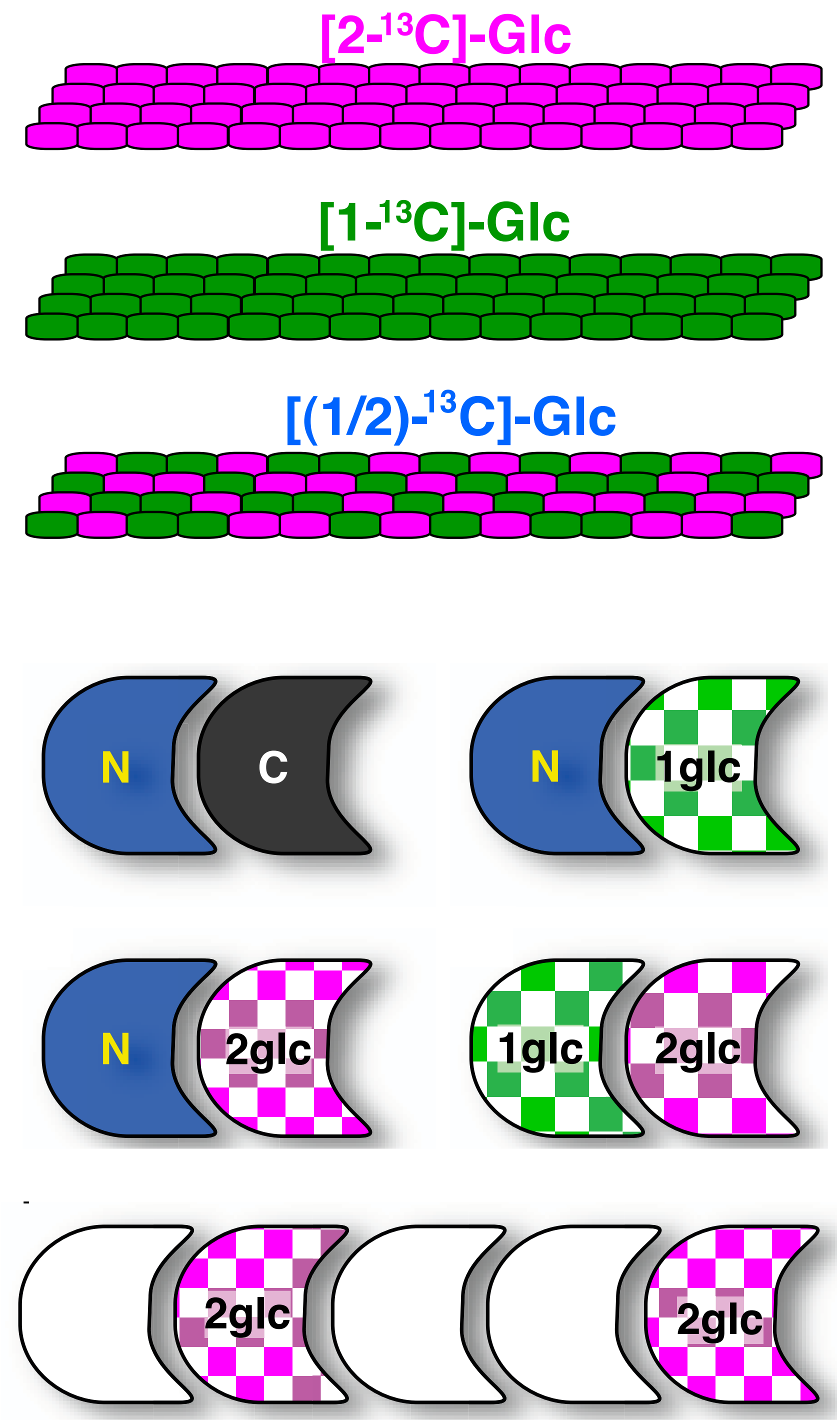
Distinction between **intra-subunit** and **inter-subunit** restraints



Unambiguous detection of supramolecular interactions

CONCEPT OF THE METHOD





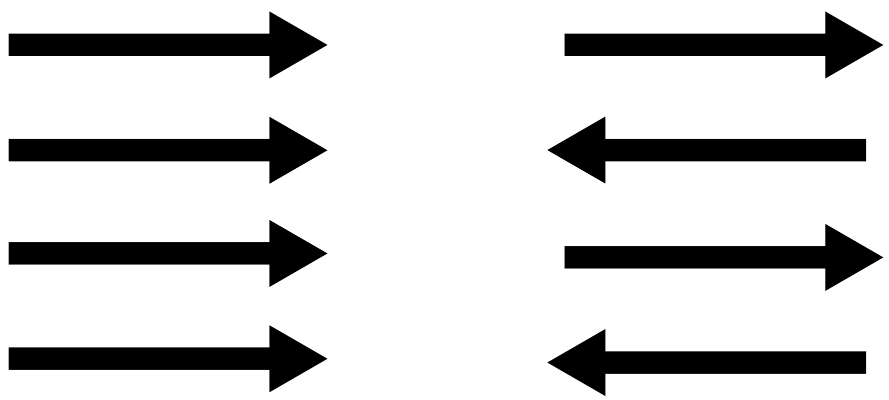
J. Am. Chem. Soc. **2010**

Nature **2012**

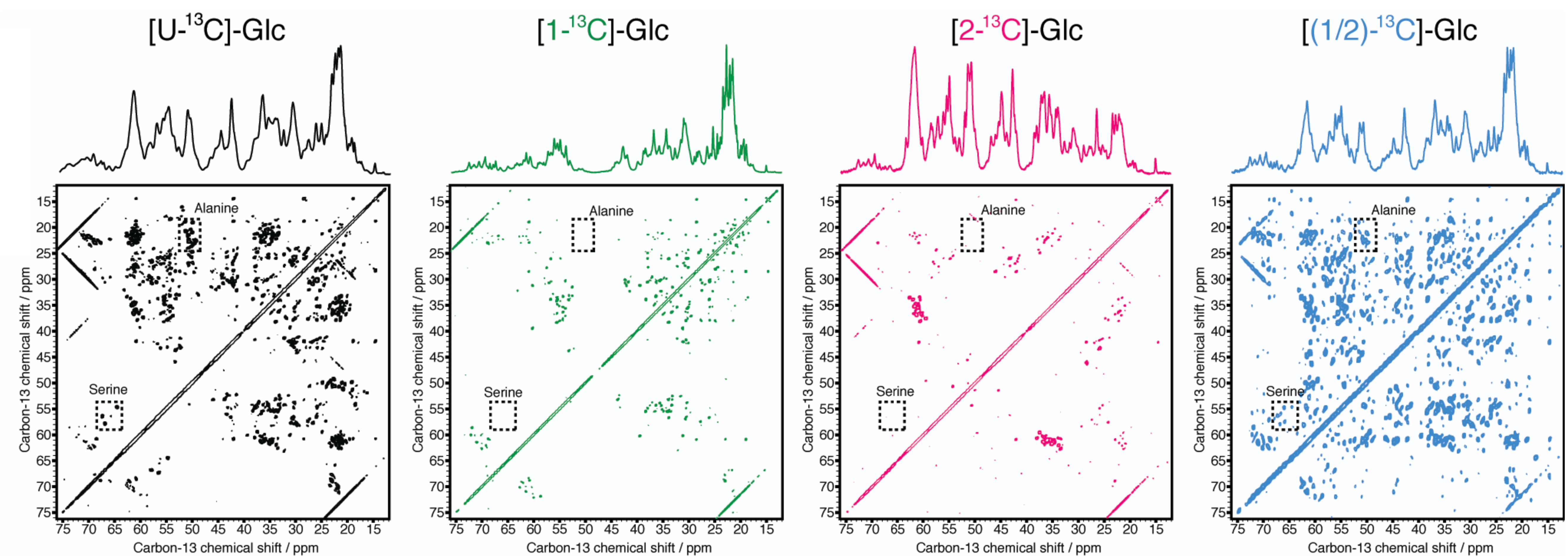
Acc. Chem. Res. **2013**

AMYLOID FIBRILS

Parallel Anti-parallel

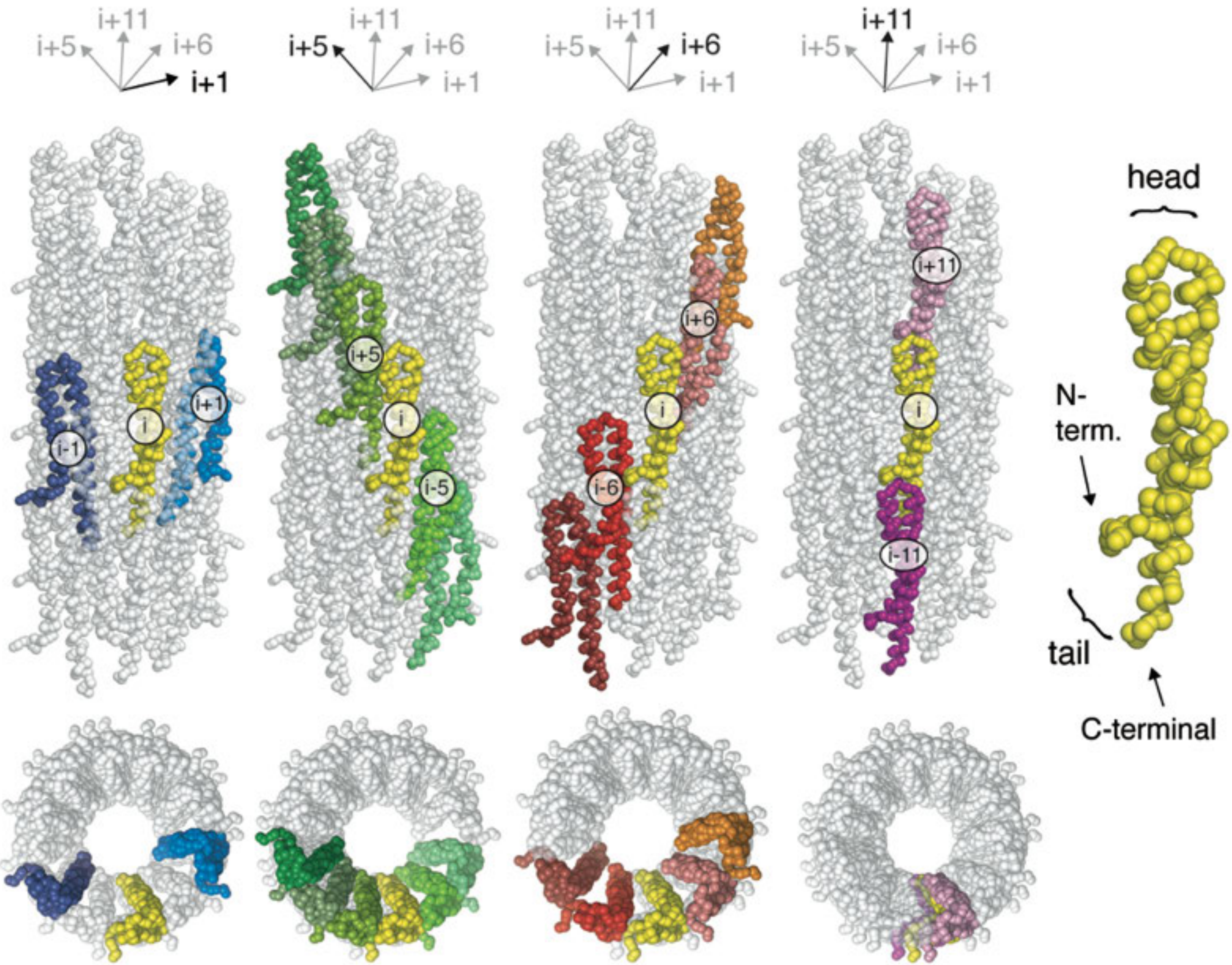


Alpha-synuclein amyloid fibrils



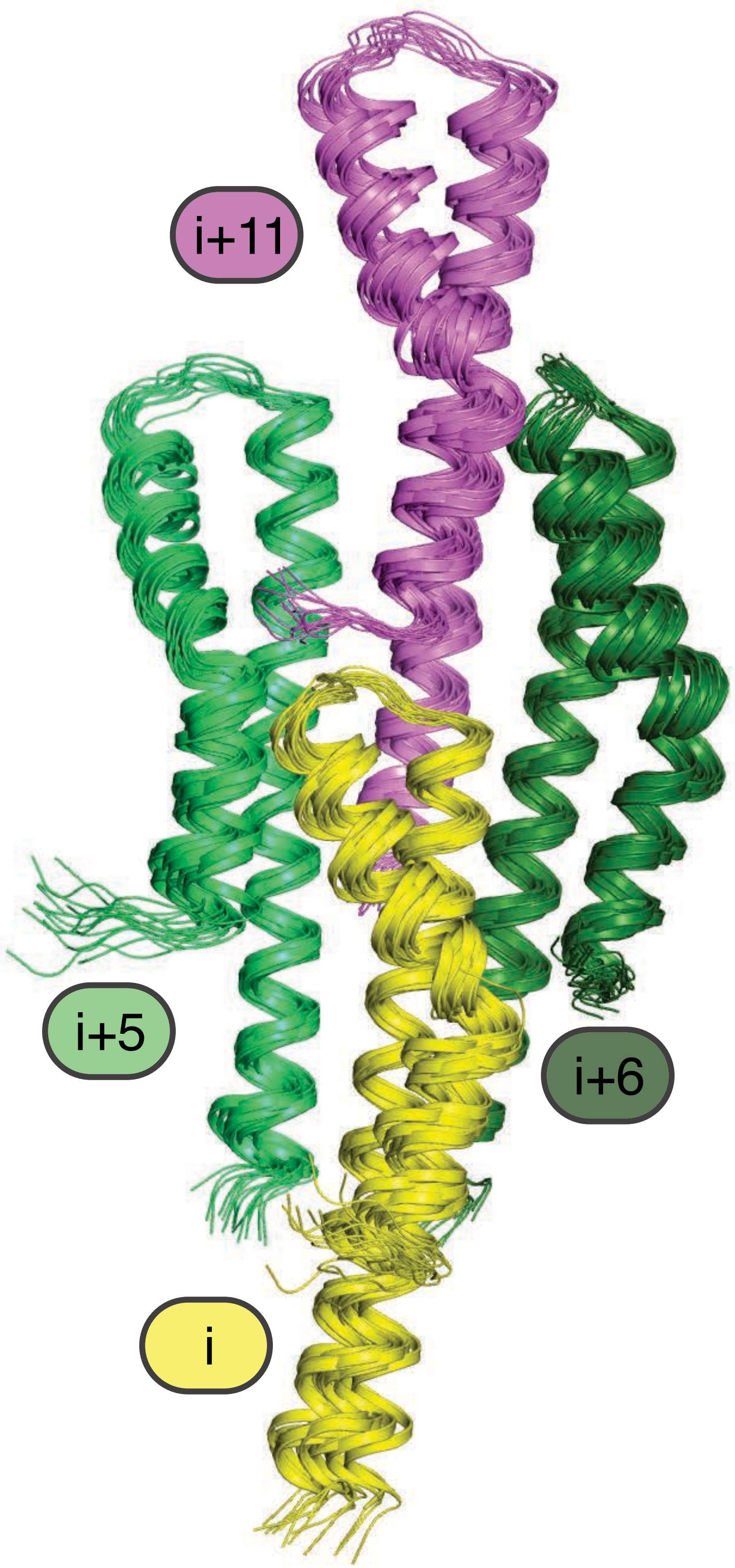
J. Am. Chem. Soc. **2010**

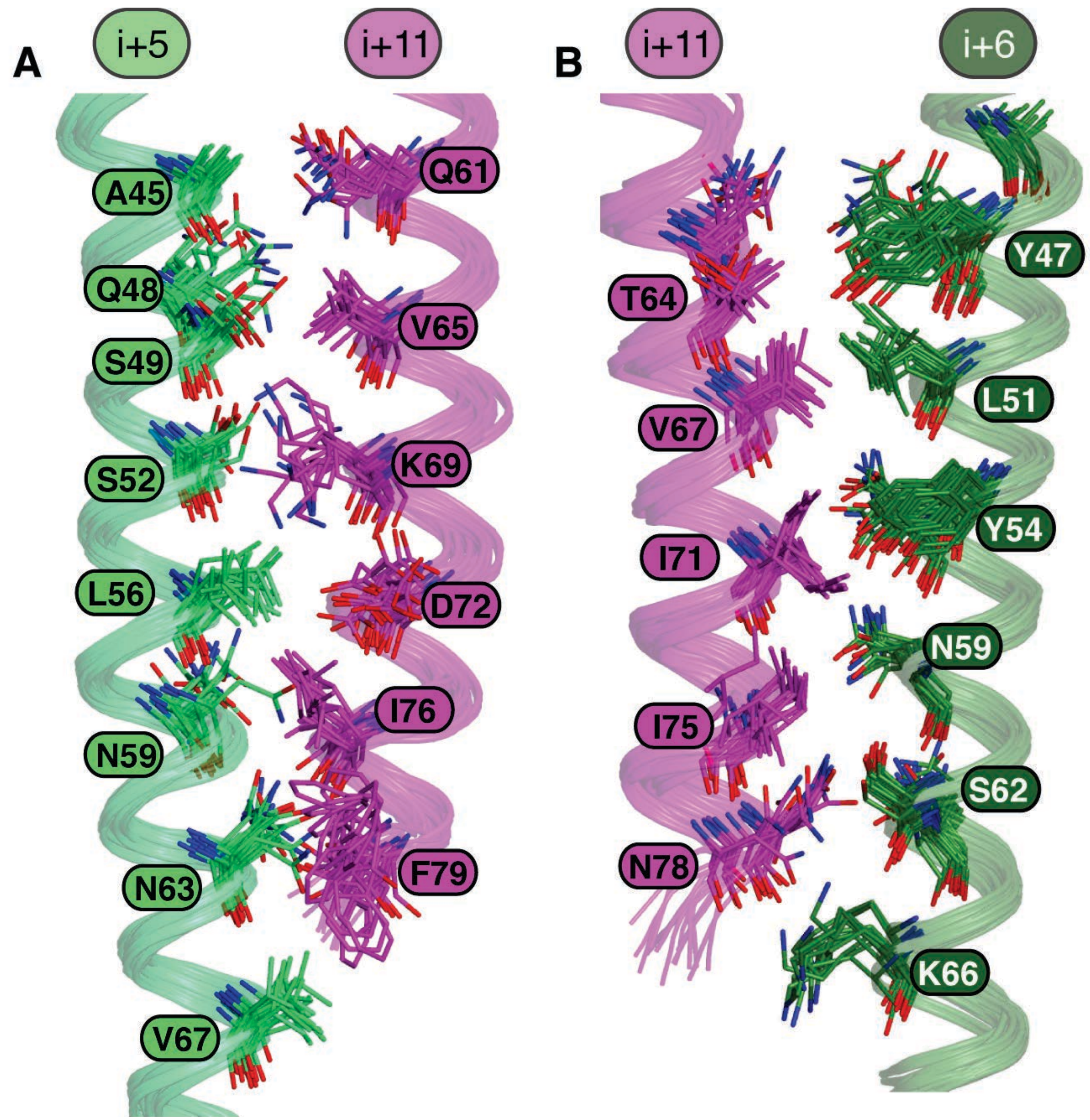
HELICAL FILAMENTS



Acc. Chem. Res. **2013**

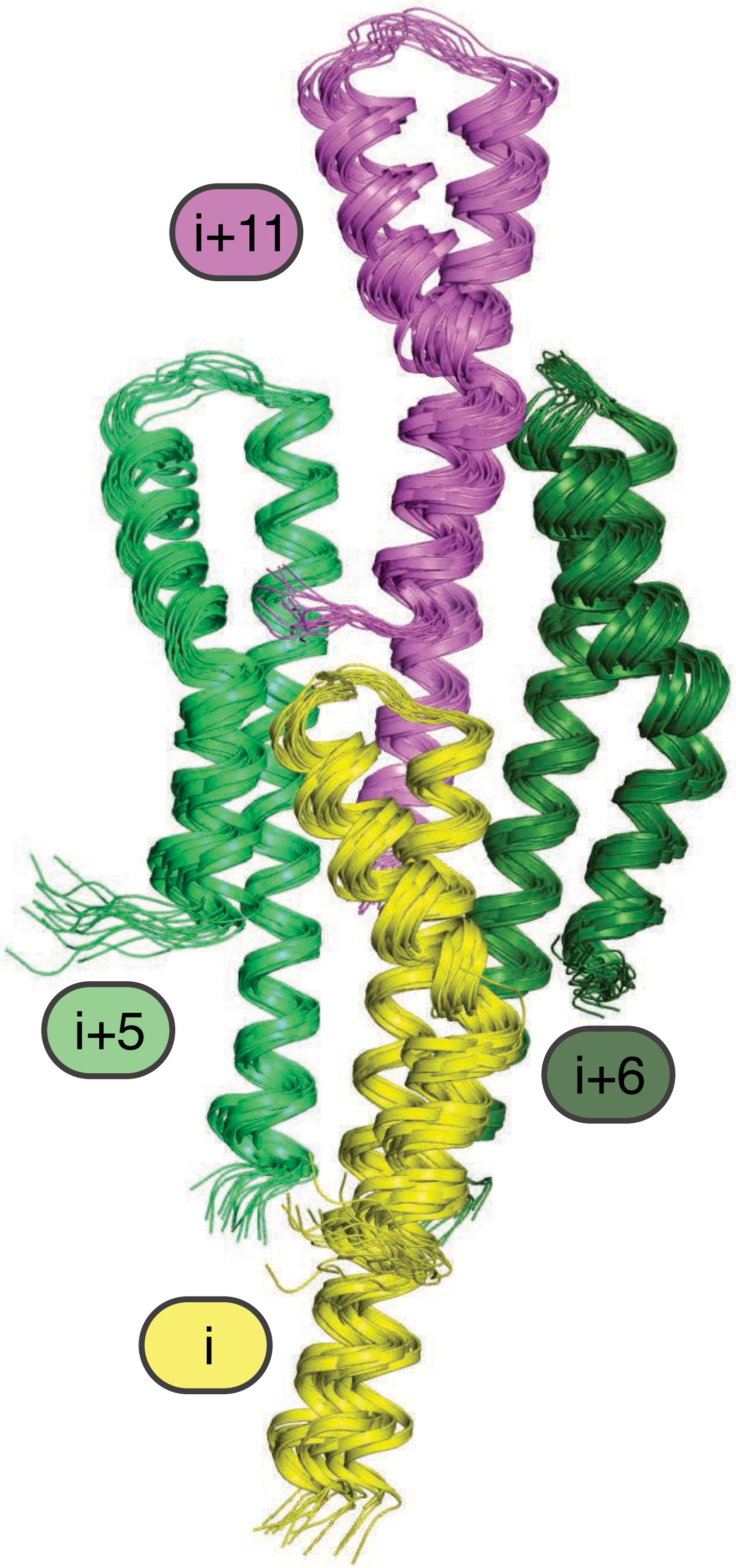
BUILDING-BLOCK STRUCTURE



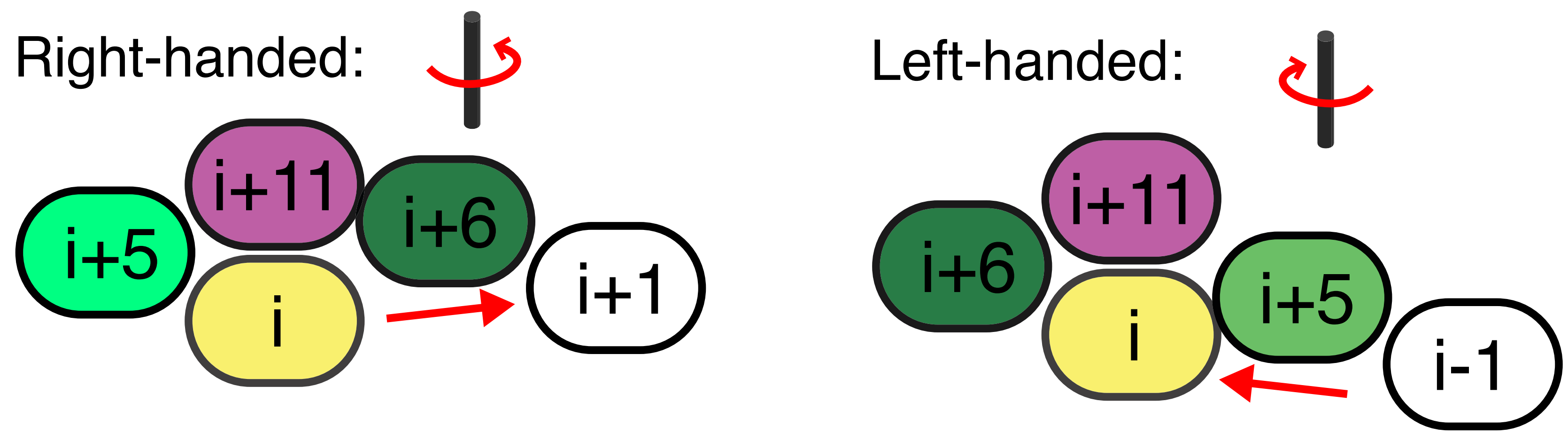


J. Am. Chem. Soc. **2013**

HELICAL HANDEDNESS



Handedness of the filament

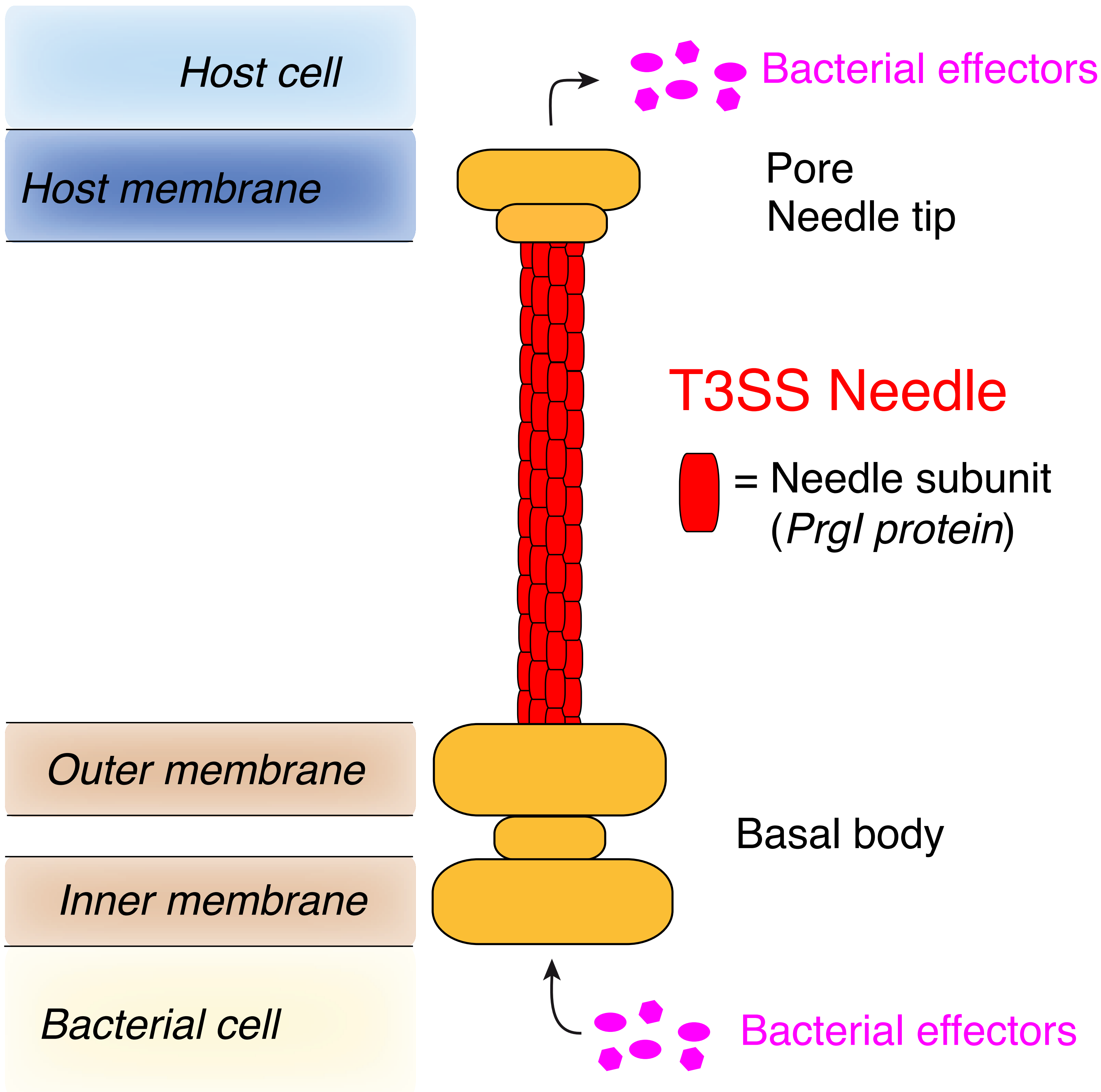


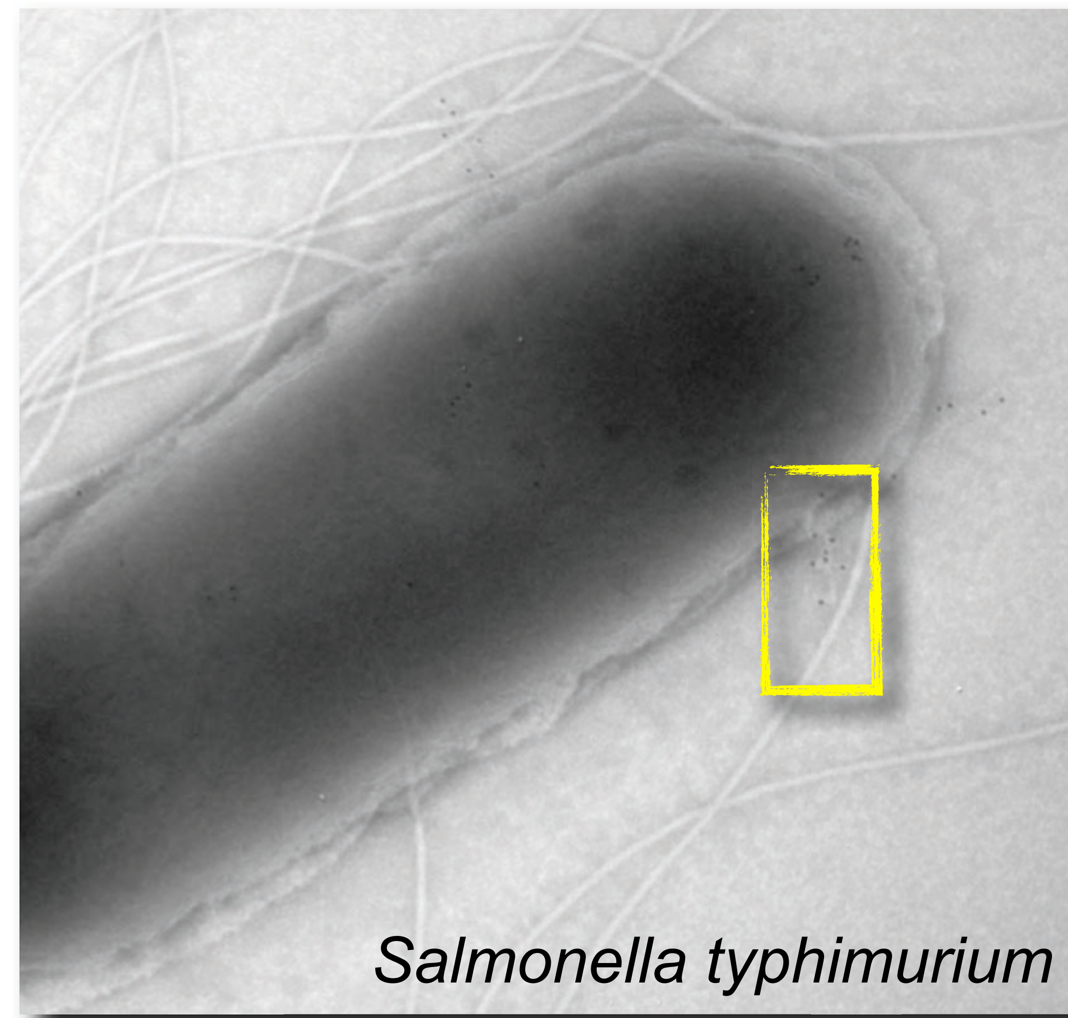
- Right-handed filaments
- Axial rise per unit of 4.16 ± 2.1 Å (SSNMR)
- Axial rise per unit of 4.2 Å (STEM)

J. Am. Chem. Soc. **2013**

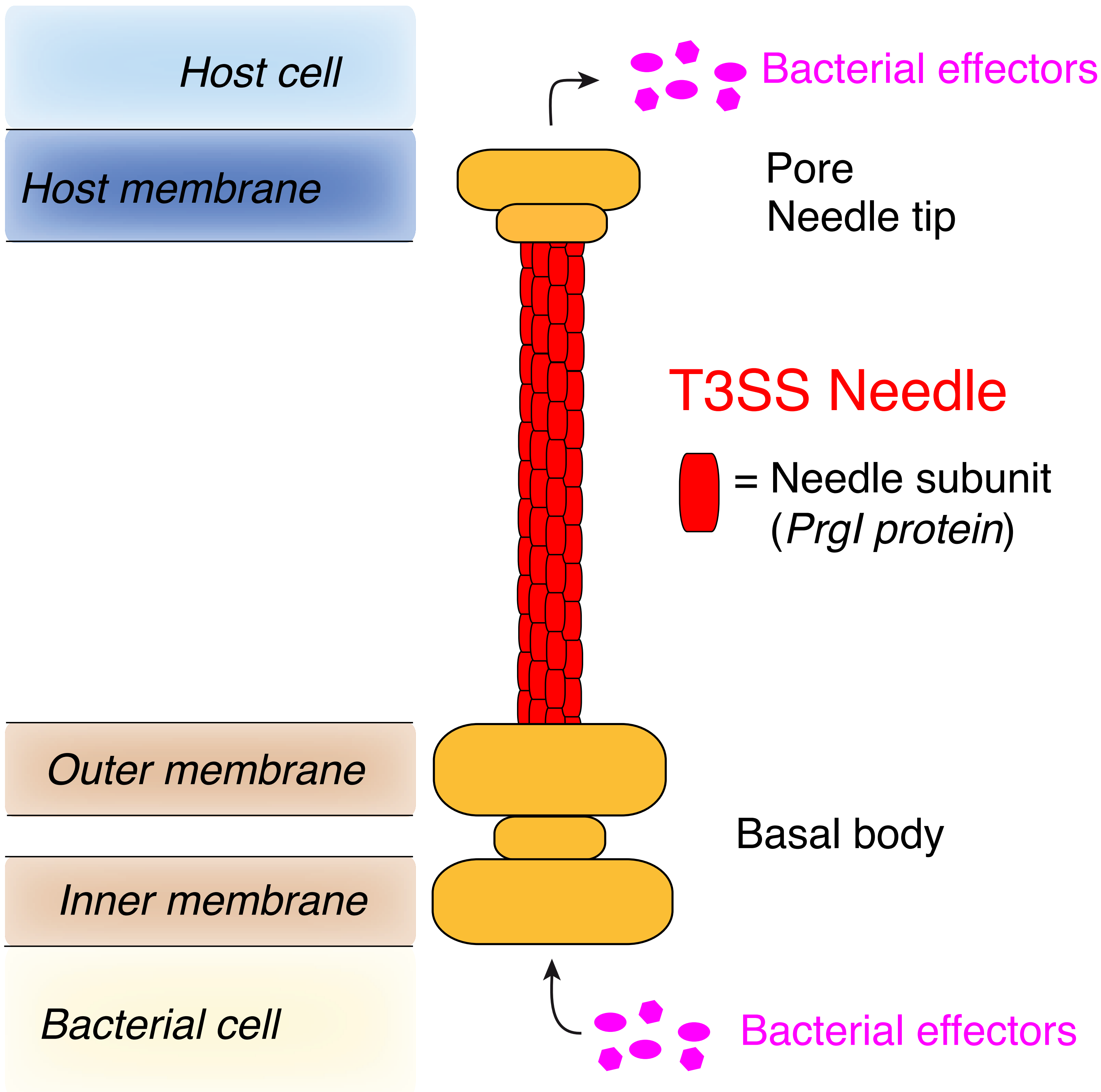
Towards atomic structures of complex biological supramolecular assemblies

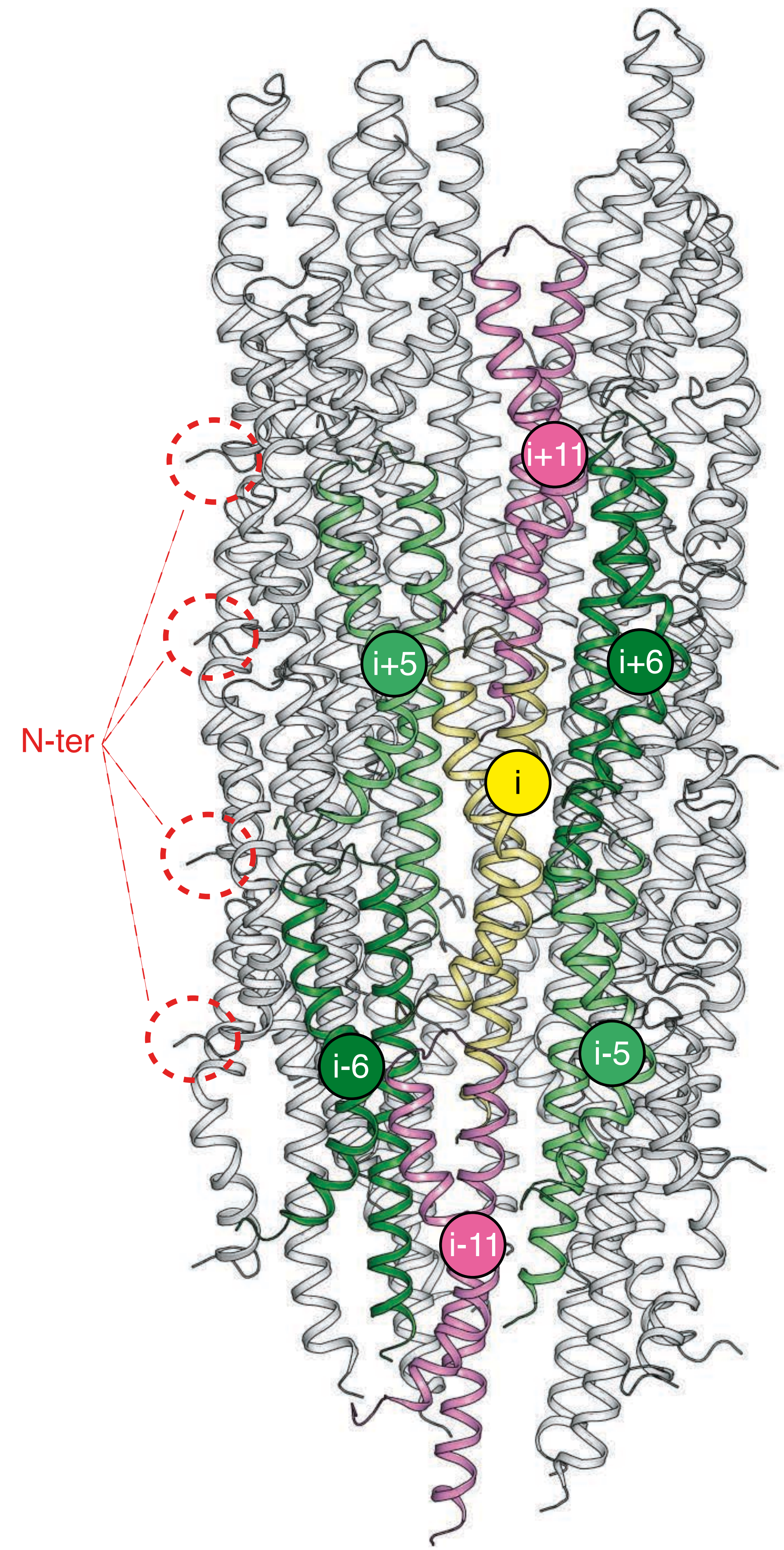
Type-III secretion system needle



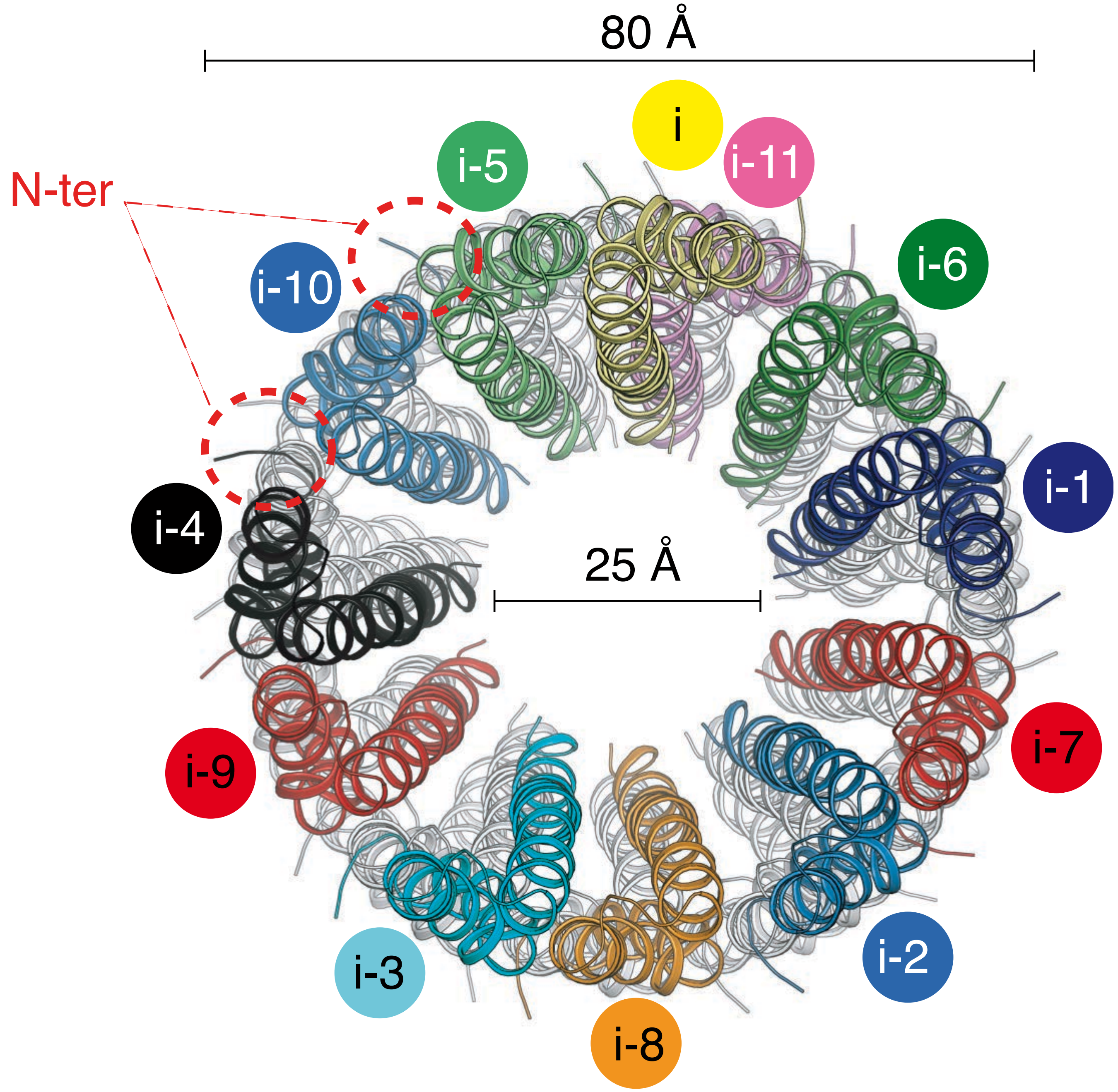


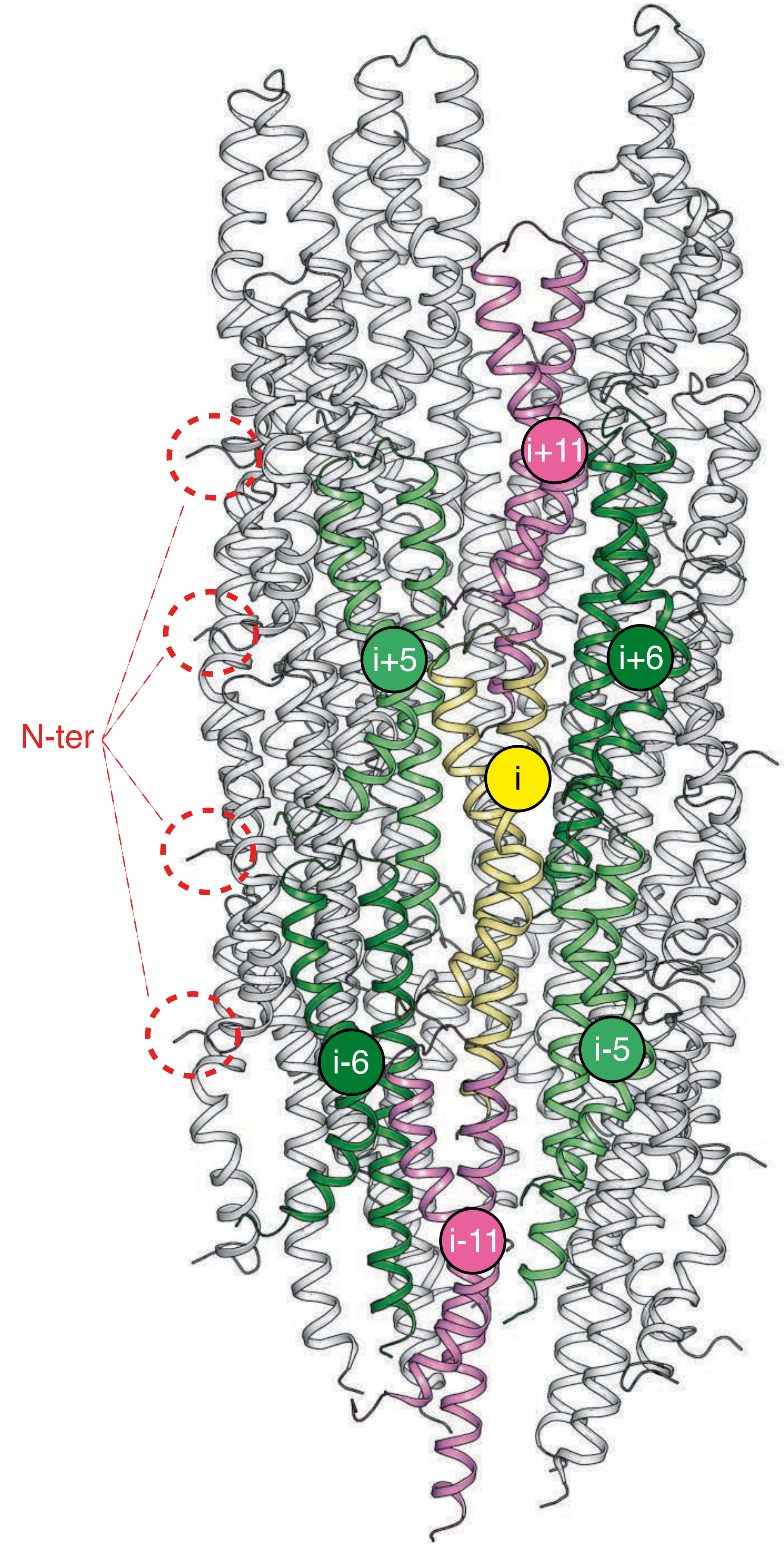
Type-III secretion system needle





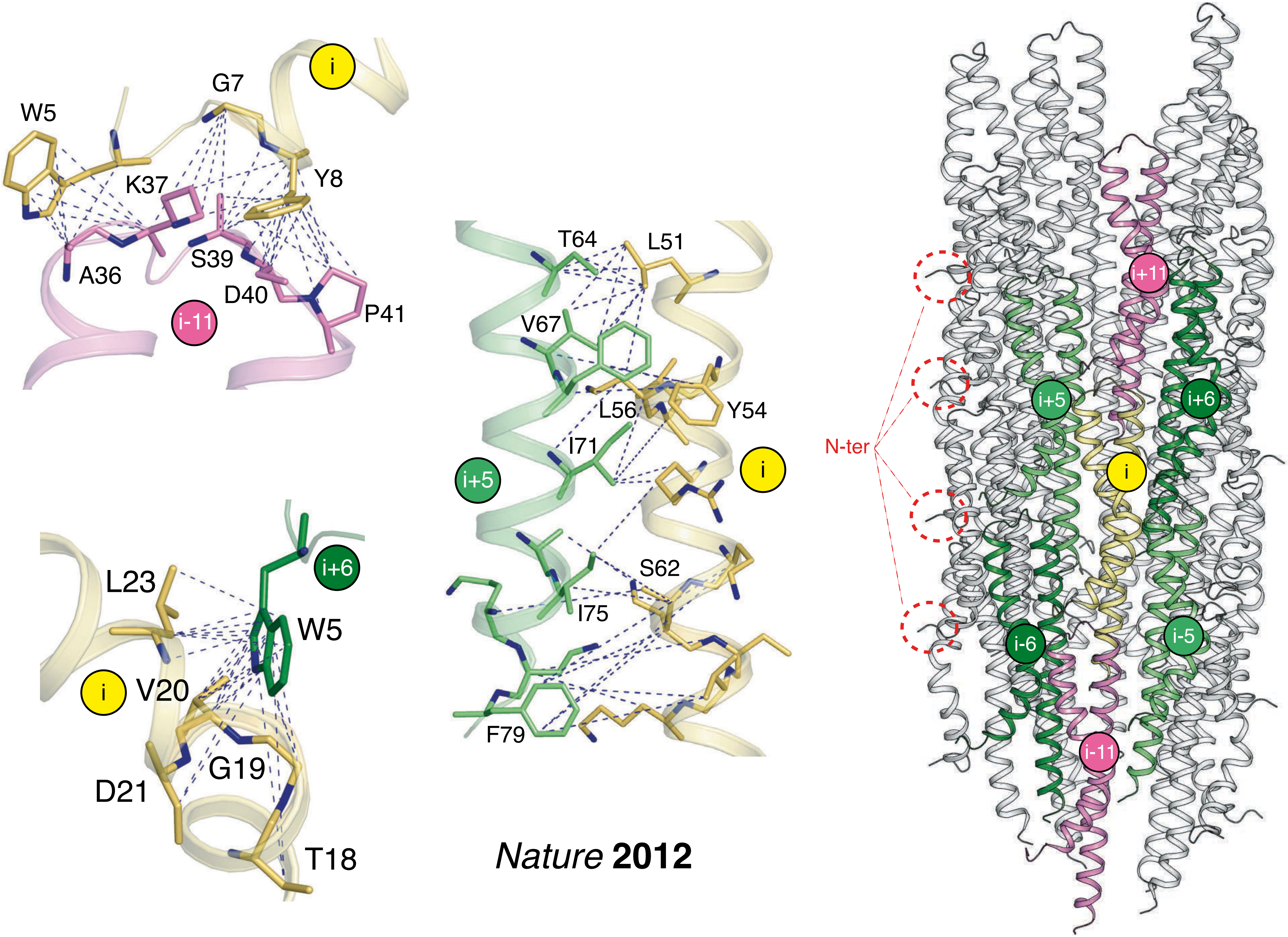
Type-III secretion system needle





Nature **2012**

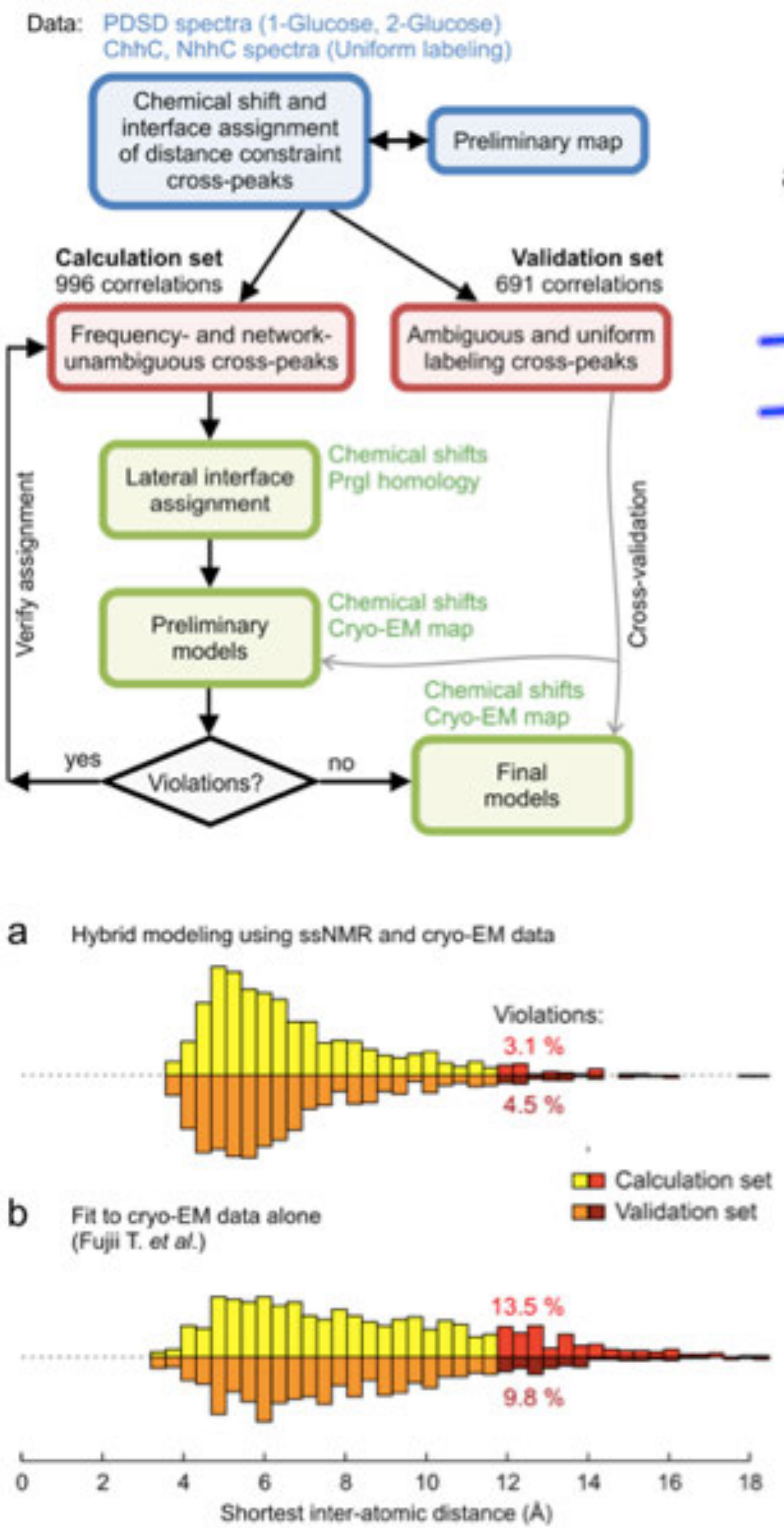
Type-III secretion system needle

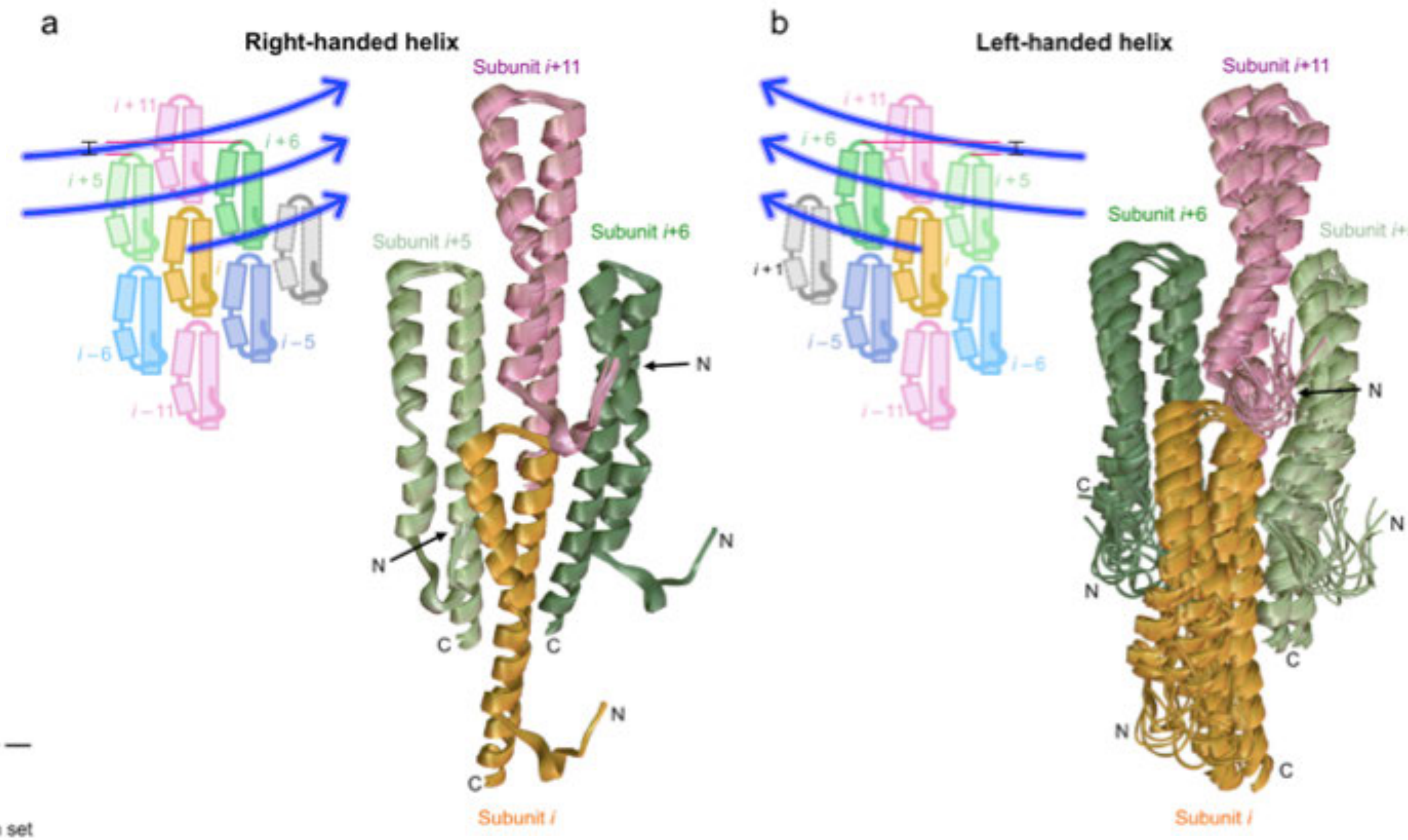


Nature **2012**

TOWARDS HYBRID APPROACHES

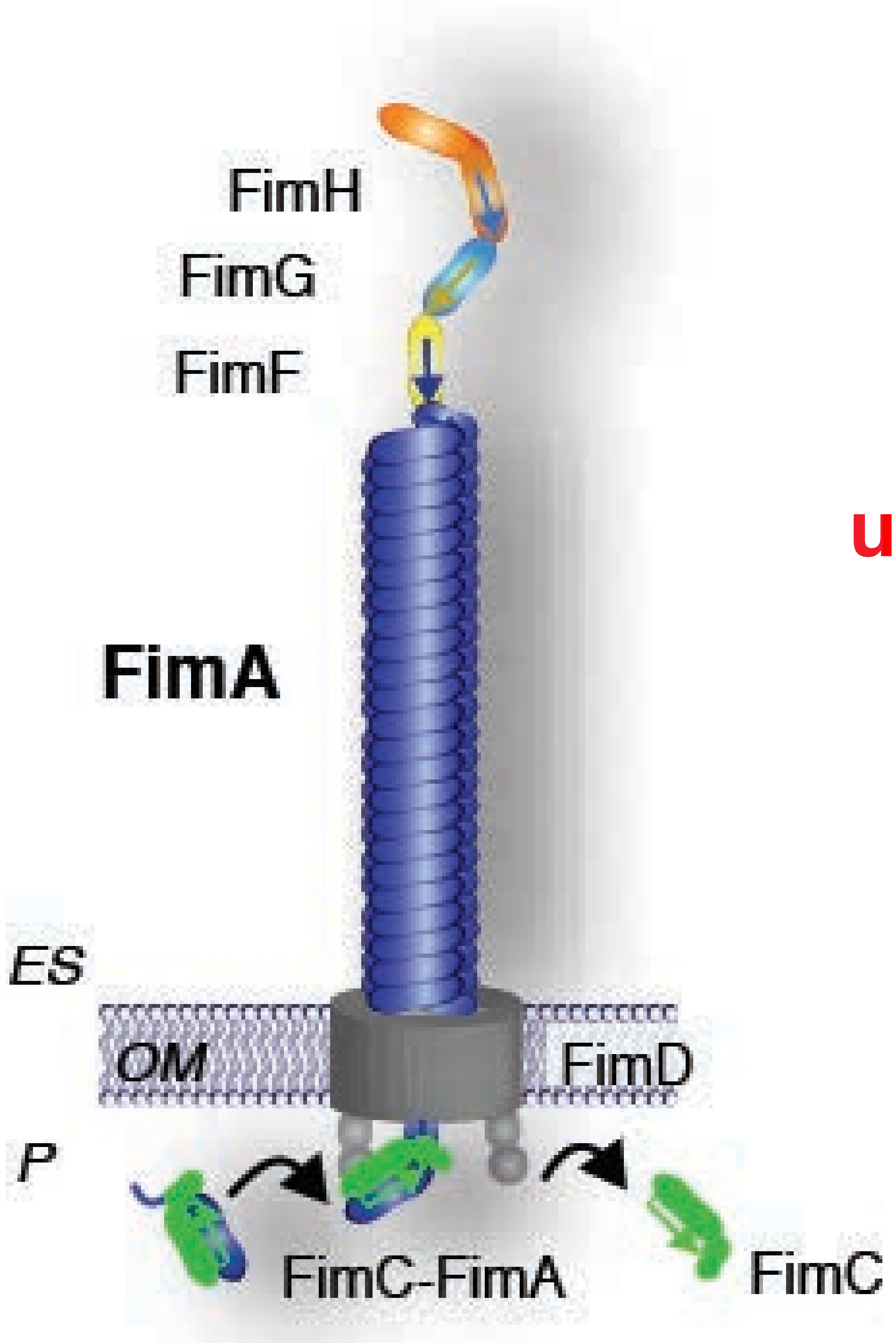
Protocols to combine cryo-EM and SSNMR



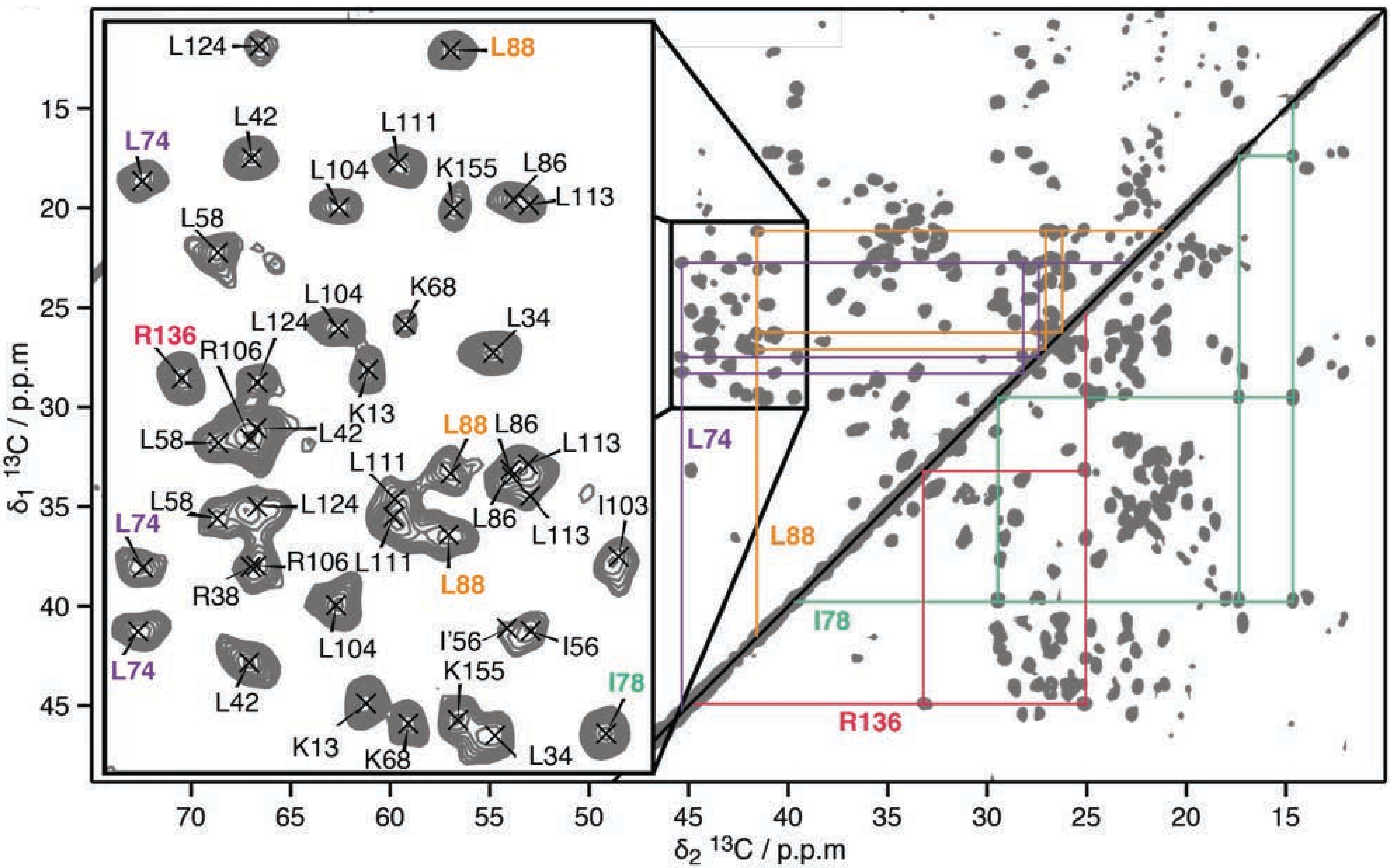


Nature Comm. **2014**

TOWARDS COMPLEX ASSEMBLIES

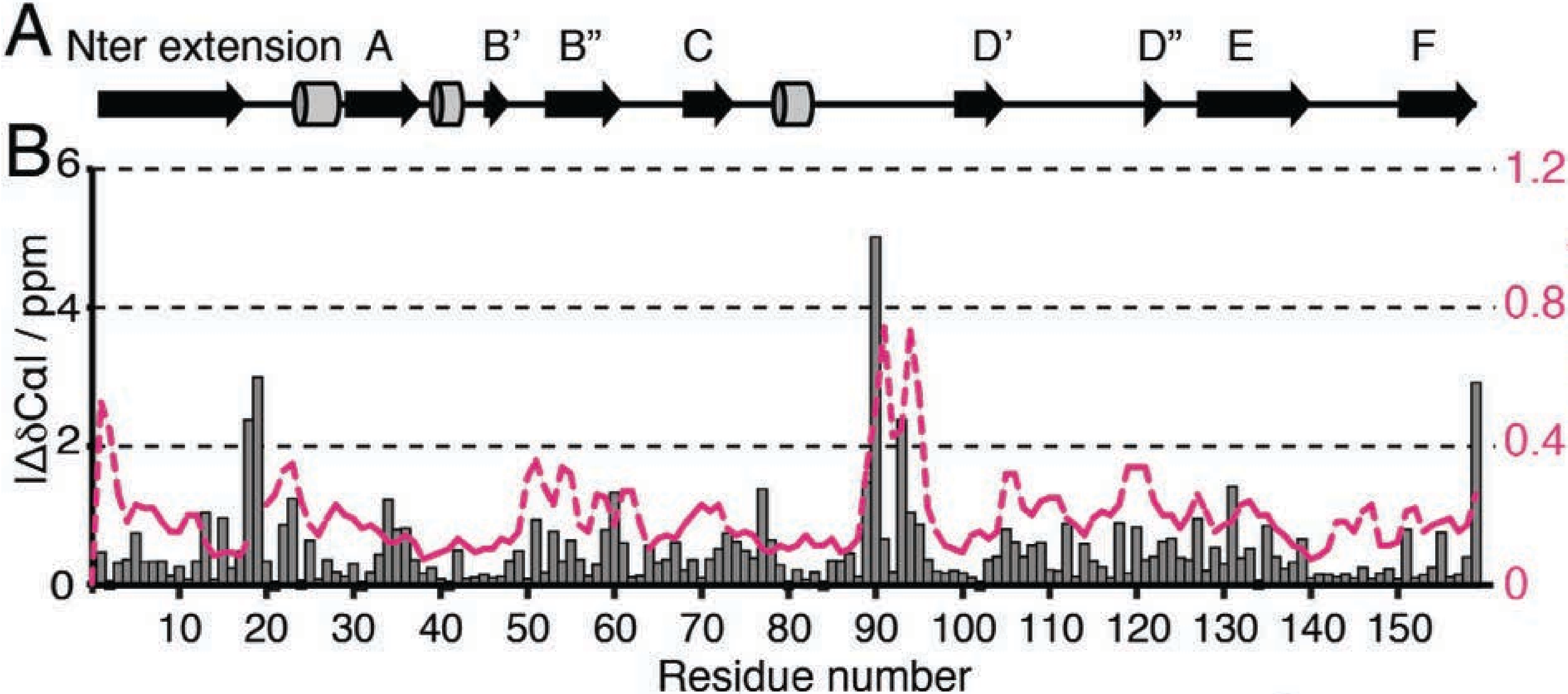


Type I pilus of uropathogenic *E. coli*

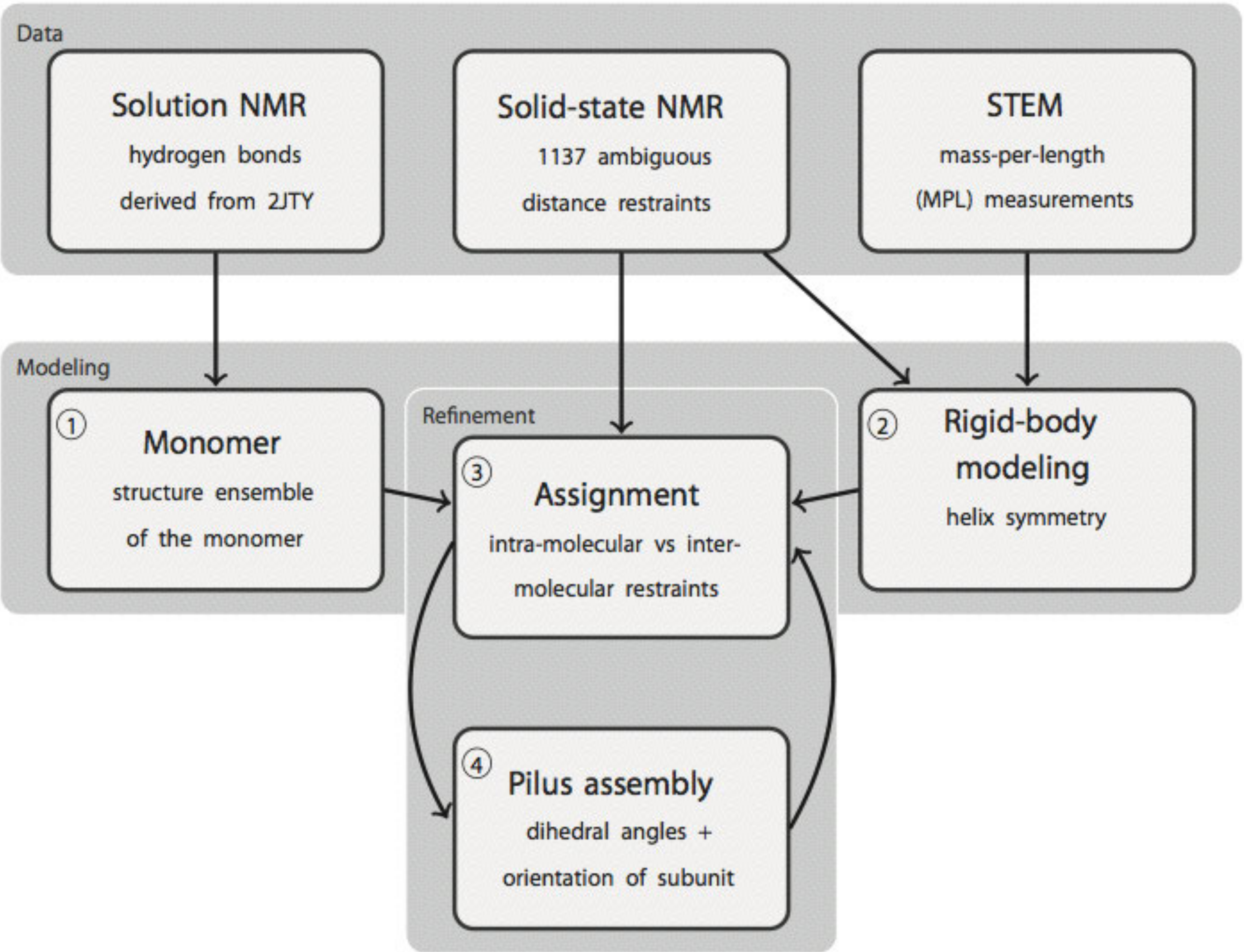


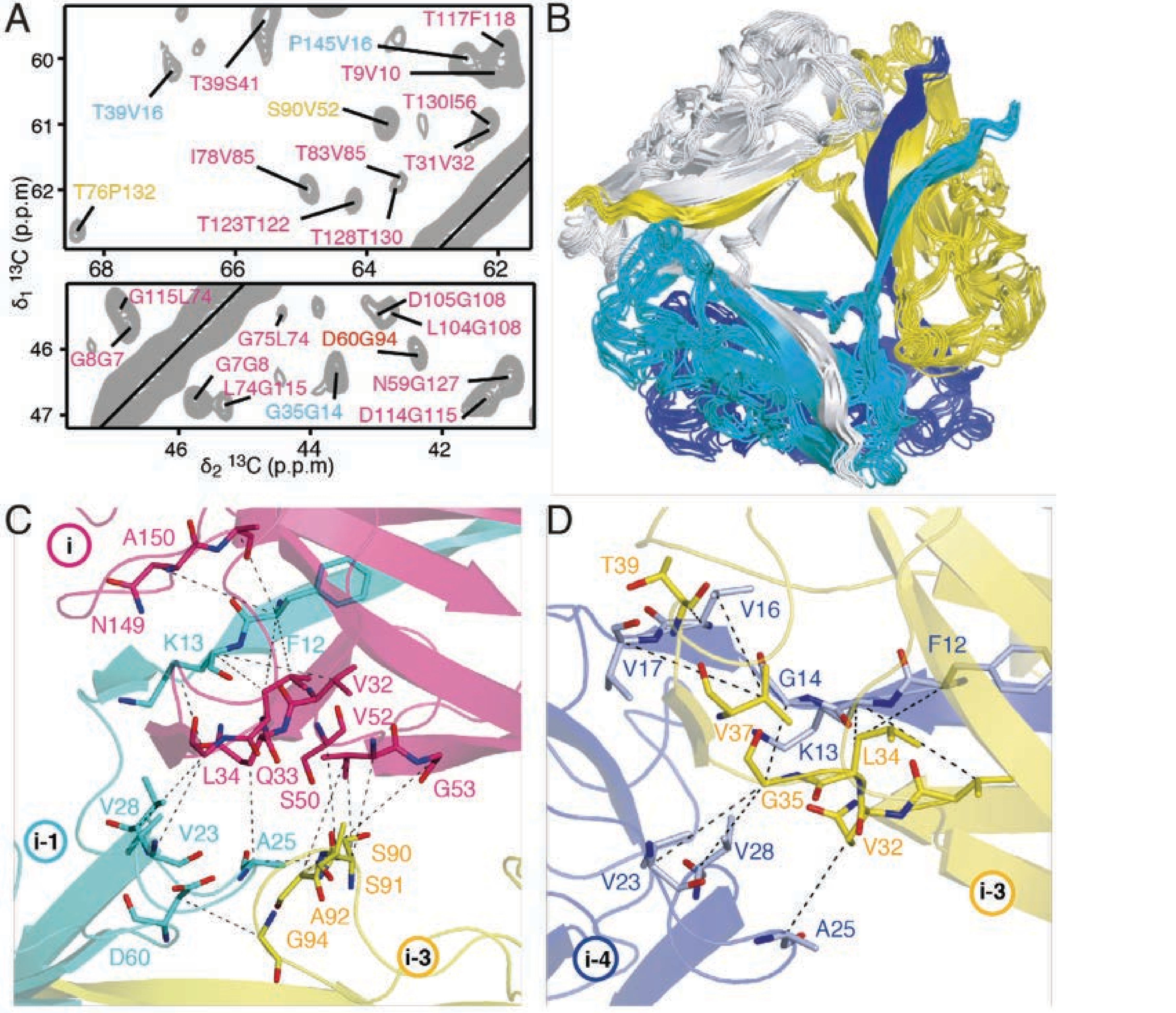
L124
L88
L42
L111
L74
L104
K155
L86
L113
L58
K68
L104
L124
L34
R136
R106
K13
L42
L58
L88
L86
L111
L113
L58
L124
L86
I103
L74
L113
R106
L111
R38
L88
L74
L104
I'56
I56
L74
K155
L42
I78
K13
K68
L34
L88
I78
R136
δ1 13C / p.p.m
δ2 13C / p.p.m

Submitted



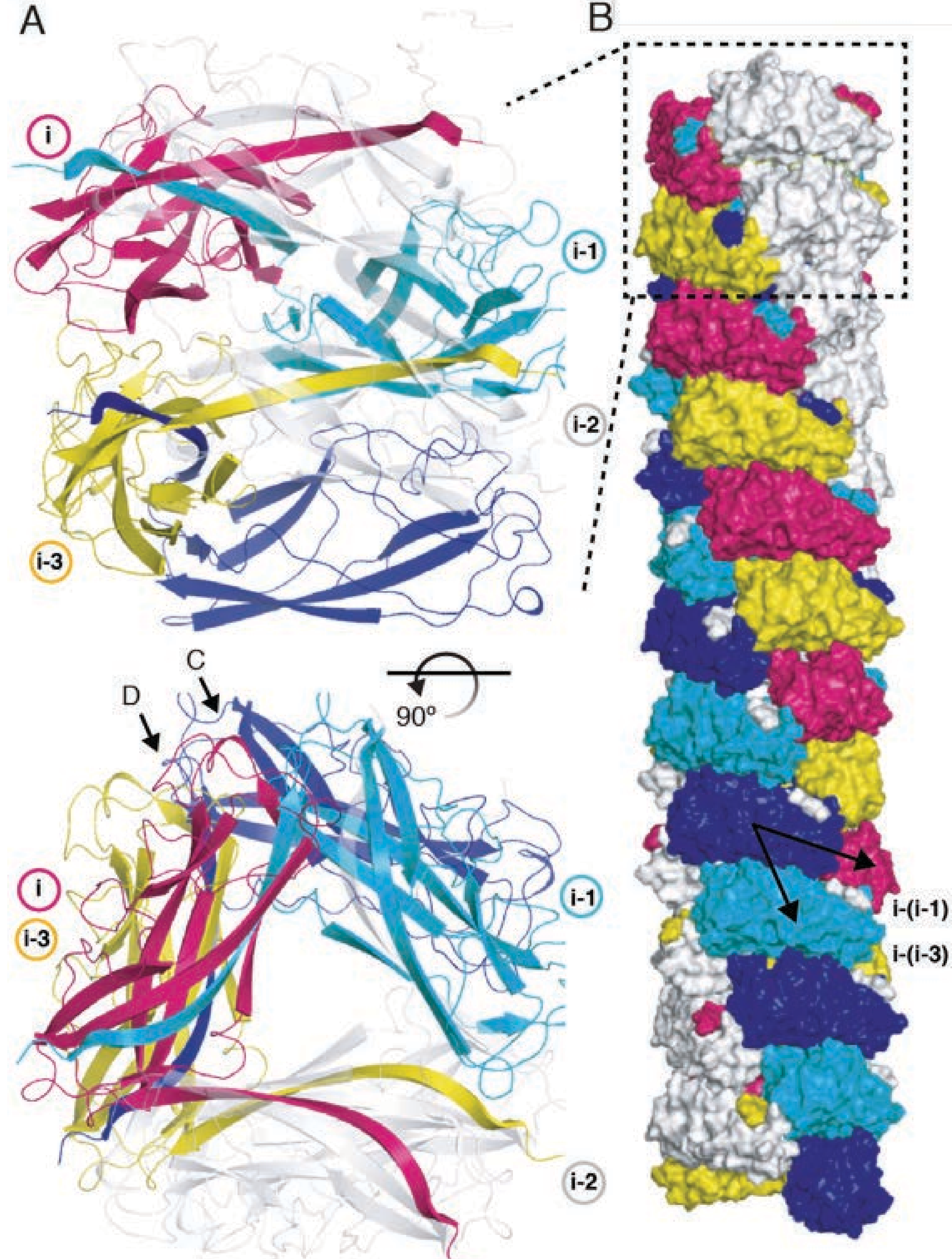
Comparison SSNMR assignments vs. LSNMR data


Data
Solution NMR
hydrogen bonds derived from 2JTY
Solid-state NMR
1137 ambiguous distance restraints
STEM
mass-per-length (MPL) measurements
Modeling
1 Monomer
structure ensemble of the monomer
Refinement
3 Assignment
intra-molecular vs inter-molecular restraints
2 Rigid-body modeling
helix symmetry
4 Pilus assembly
dihedral angles + orientation of subunit



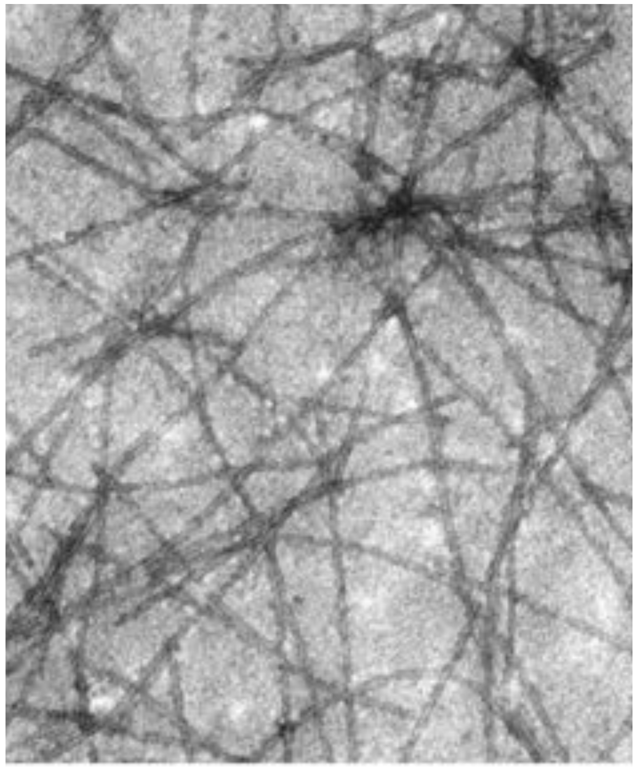
A
T117F118
P145V16
T39S41
T9V10
T39V16
S90V52
T130I56
T31V32
I78V85
T83V85
T76P132
T123T122
T128T130
δ_1 ^{13}C (p.p.m)
δ_2 ^{13}C (p.p.m)
G115L74
D105G108
L104G108
G75L74
D60G94
G8G7
G7G8
L74G115
N59G127
G35G14
D114G115
B
C
i
A150
N149
K13
F12
V32
V52
L34
Q33
S50
G53
V28
V23
A25
S90
S91
A92
G94
D60
i-1
i-3
D
T39
V16
V17
G14
F12
V37
K13
L34
G35
V32
V28
V23
A25
i-3
i-4

Submitted

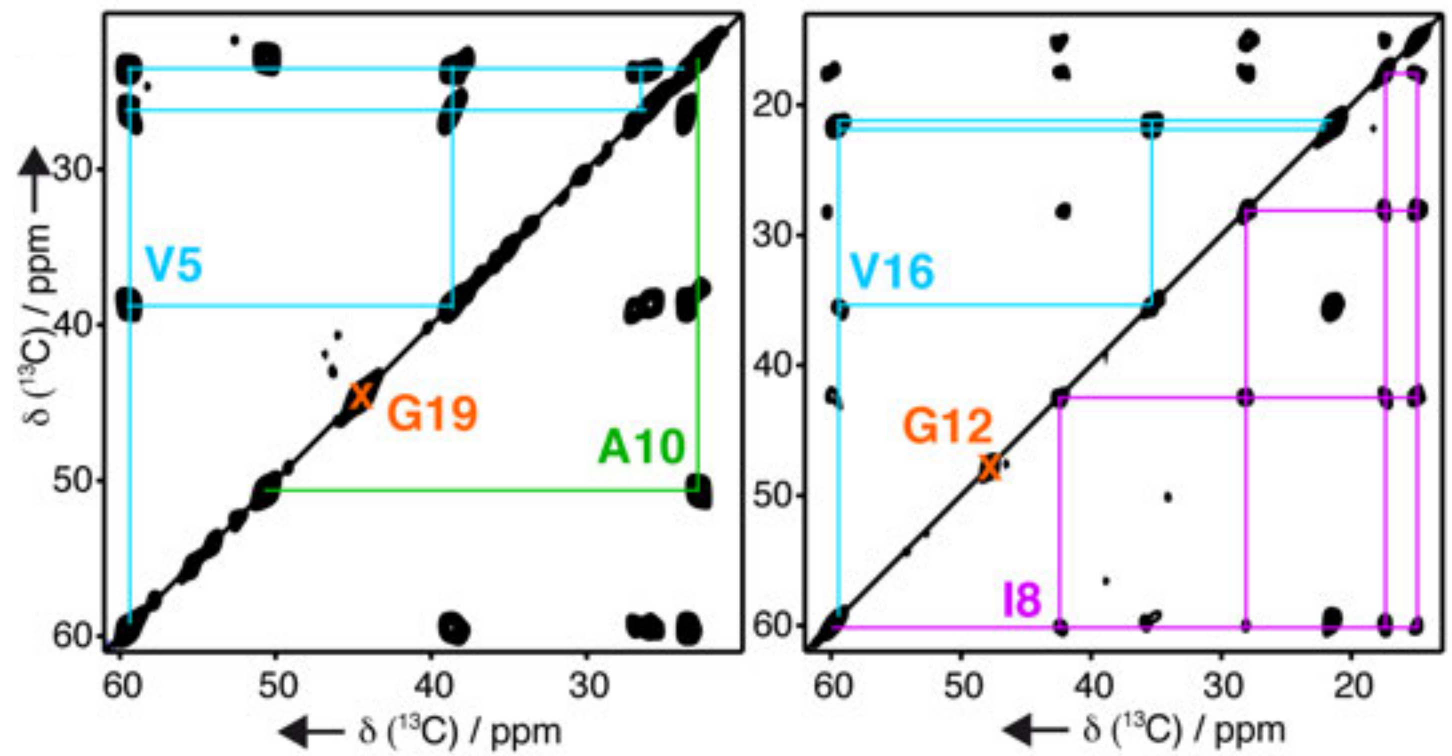


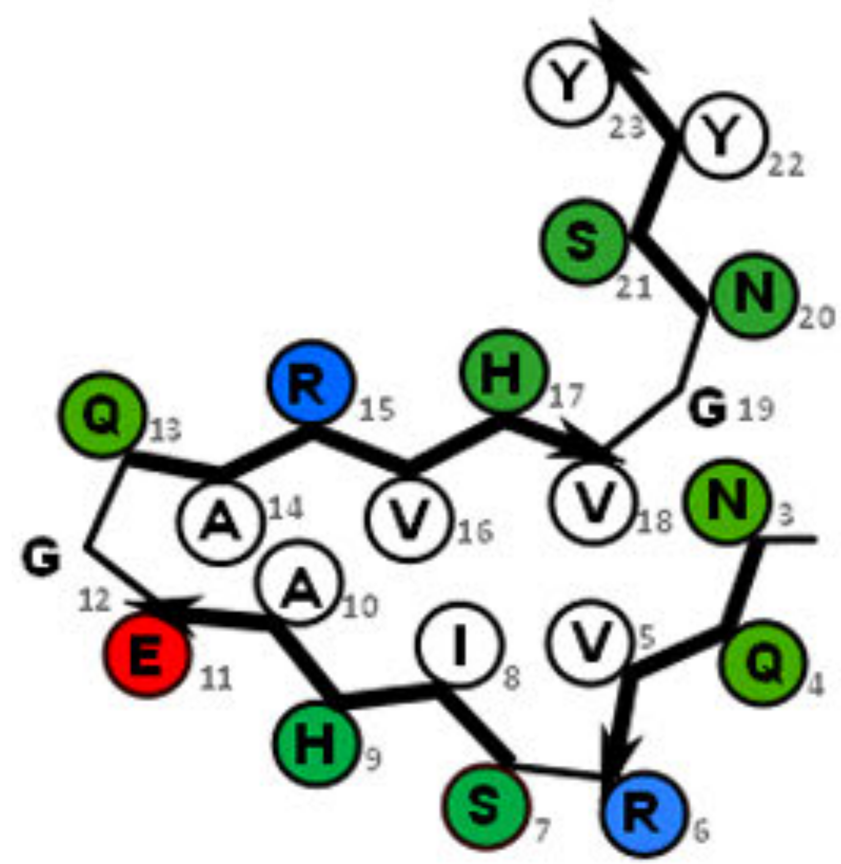
A
B
i
i-1
i-2
i-3
D
C
90º
i
i-3
i-1
i-2
i-(i-1)
i-(i-3)

AMYLOID FIBRILS IN APOPTOSIS



NWD2 amyloid fibrils





Plos Biology, *2015*

PRACTICAL ASPECTS

. **Sample quantity:**

. 1-20mg for ^{13}C / ^{15}N detection

. few mg for ^{1}H detection, but:

. you need to perform deuteration
. use small rotor
. distance restraints are more difficult to measure

. **Sample state:**

. from solid to semi-solid (no liquid !)
. as concentrated as possible

. **Molecular size:**

. site-specific study: up to 350 residues
. structure determination: up to 150-200 residues
. no limitation on the object size

. **Structural order:**

. as high as possible, to limit the number of conformers
. standard MAS SSNMR: rigid part of the protein
. J-based MAS SSNMR: mobile regions