

Summary and Closing Remarks

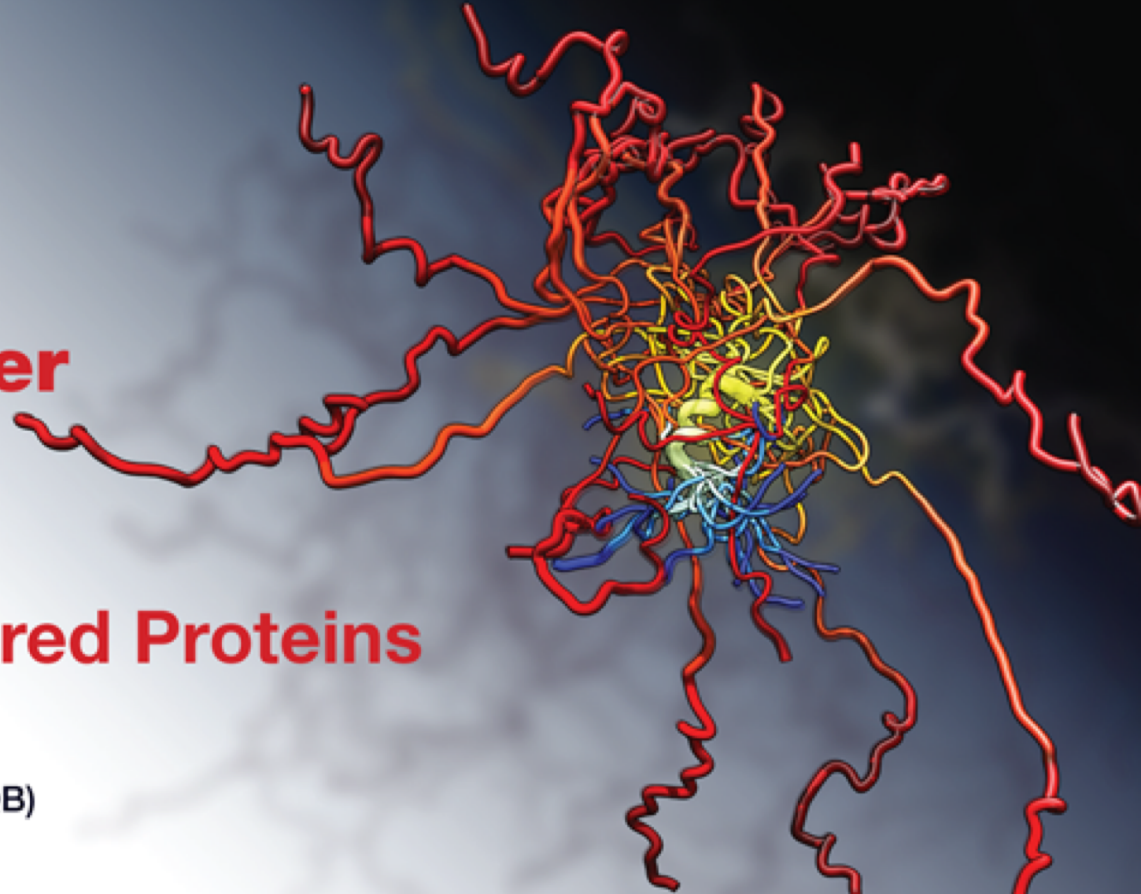
RUTGERS

CRASH COURSE:

Order and Disorder in Biology and Human Disease: Intrinsically Disordered Proteins

Organized jointly by:

I Institute for Quantitative Biomedicine (IQB)
Chemistry & Chemical Biology (C&CB)

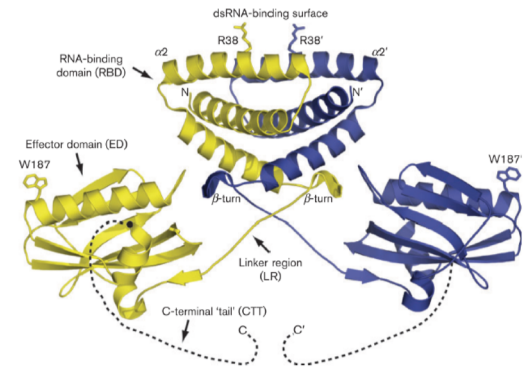
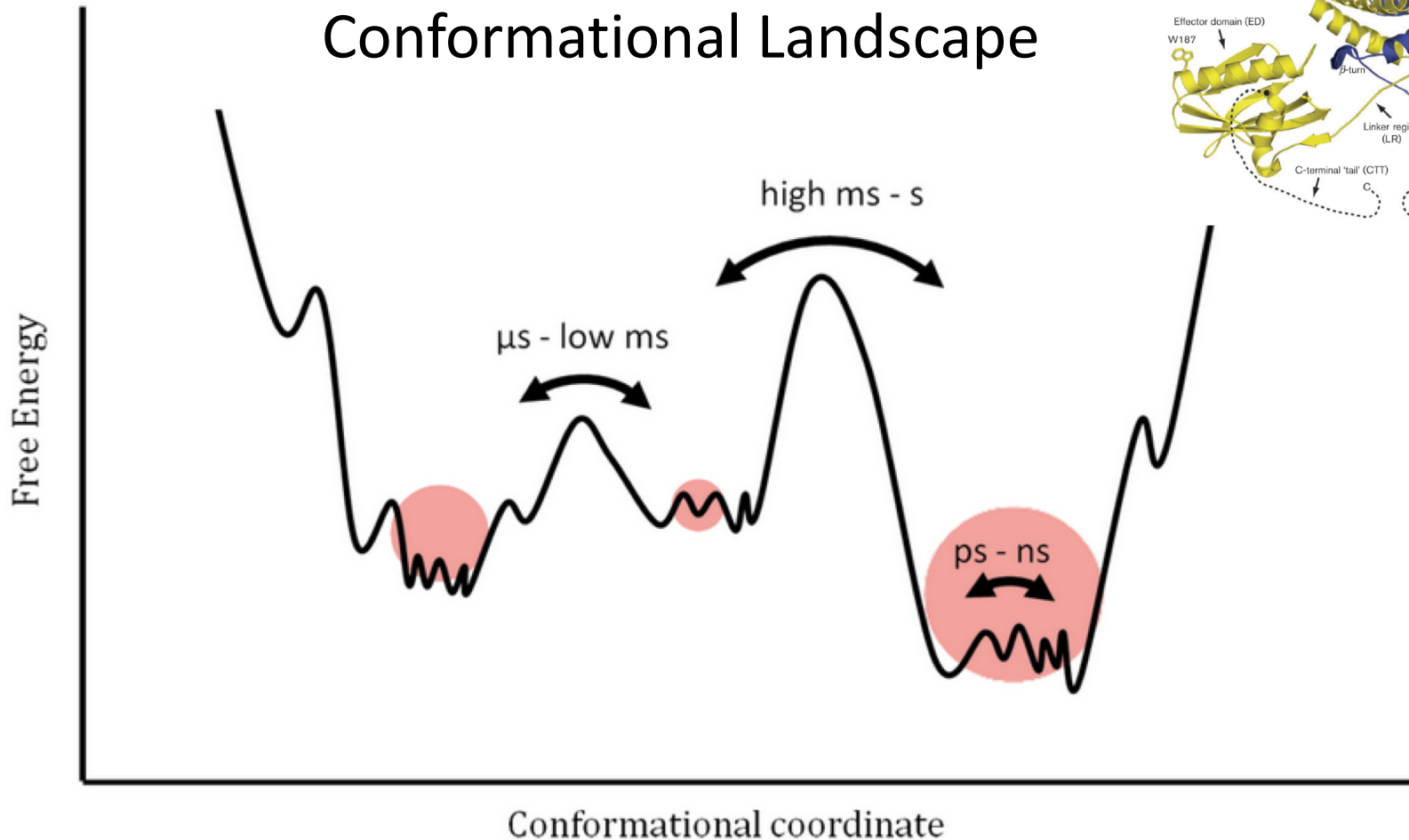


Gaetano T. Montelione
February 26, 2019

IDPs in Disease

Protein Dynamics

Protein Structures Sample a Conformational Landscape





IDPs are an Essential Component of the Structure -> Function Paradigm

Keith Dunker, Indiana University

• Sequence → Structure → Function

- Catalysis,
- Membrane transport,
- Binding with DNA, RNA, Proteins, **IDPs** & molecules

• Sequence → IDP Ensemble → Function

- Signaling, Dunker AK, et al., *Biochemistry* 41: 6573-6582 (2002)
- Regulation, Dunker AK, et al., *Adv. Prot. Chem.* 62: 25-49 (2002)
- Recognition, Xie H, et al., *Proteome Res.* 6: 1882-1898 (2007)
- Control. Vucetic, S. et al., *Proteome Res* 6: 1899-1916 (2007)
Xie H, et al., *Proteome Res* 6: 1917-1932 (2007)

Multi-Pronged and Multi-Targeted Interactions are Key for Efficient Inhibition of IDP α S Aggregation

Jean Baum – Rutgers University

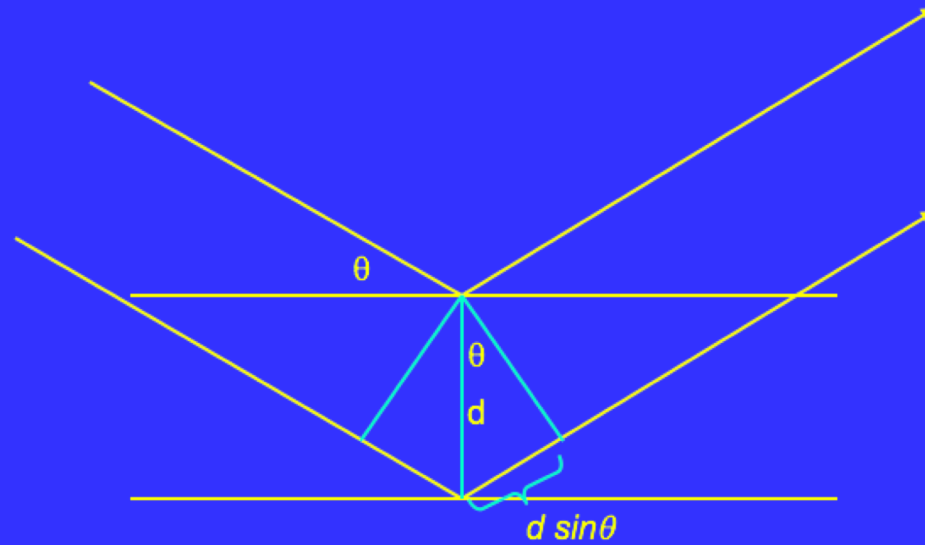
- **The IDP β -synuclein interacts with the IDP α -synuclein at multiple points along its disease associated aggregation pathway.**
- Simultaneous interactions of multiple β -synuclein domains with α -synuclein are beneficial to slow down aggregation.
- β -synuclein interferes with the seeding ability of α -synuclein fibrils to template the aggregation of endogenous α -synuclein monomers.
- **The multi-pronged and multi-targeting ability of an IDP to delay/inhibit amyloid formation at the earliest and latest stages of aggregation represent a powerful platform for future therapeutic design.**

Characterizing IDPs

How do you know your protein is an IDP or has IDRs?

X-ray Crystallography

Bragg Diffraction



For constructive interference $2d \sin \theta = n \lambda$

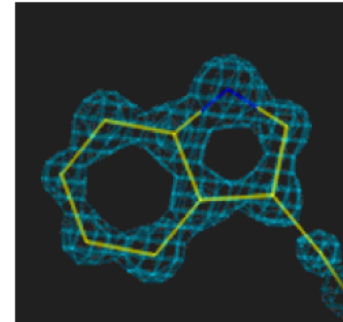
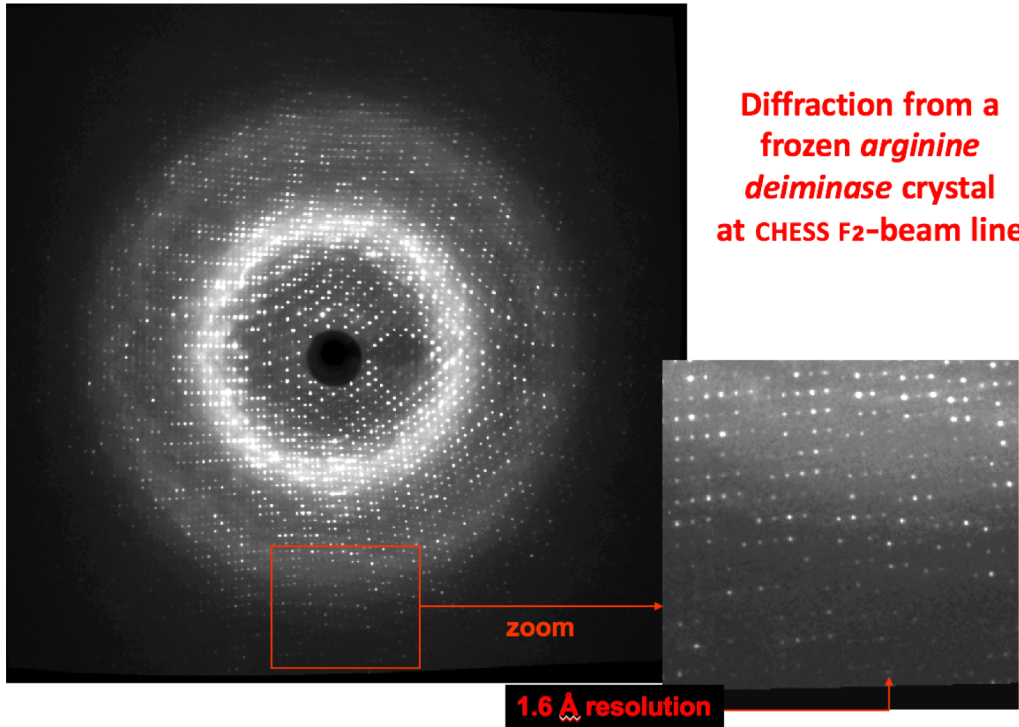
d - Spacing between two atoms

θ - Angle of incidence of X-ray

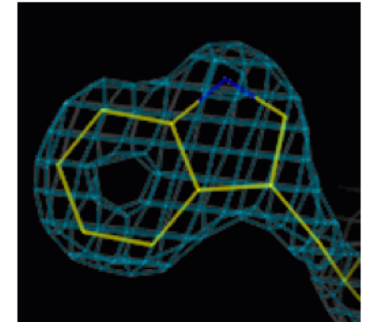
λ - Wavelength of X-ray
(1.5418 Å for $\text{CuK}\alpha$)

X-ray Crystallography

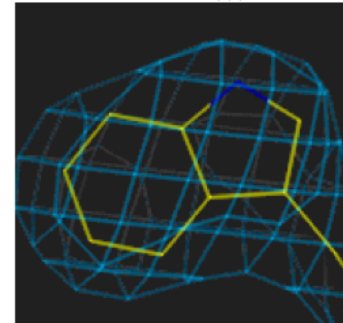
Atomic Resolution



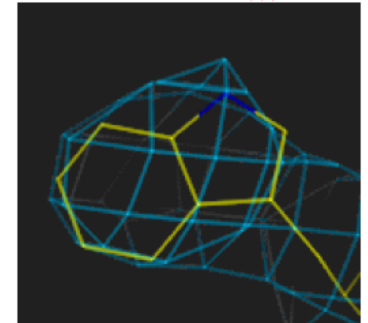
1.0 Å



2.5 Å



3.5 Å



4 Å

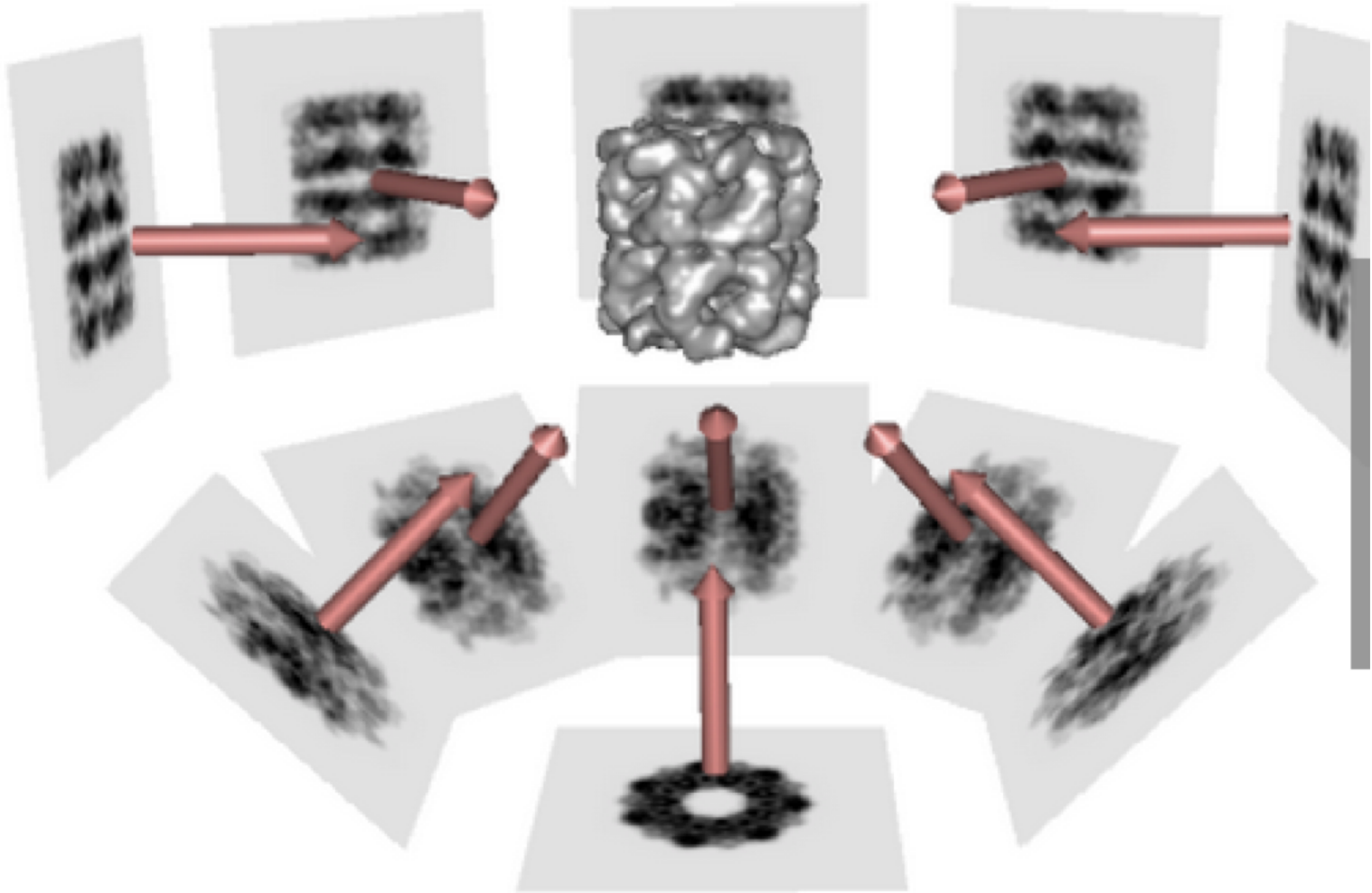
Crystallography Provides Extensive Information about IDRs

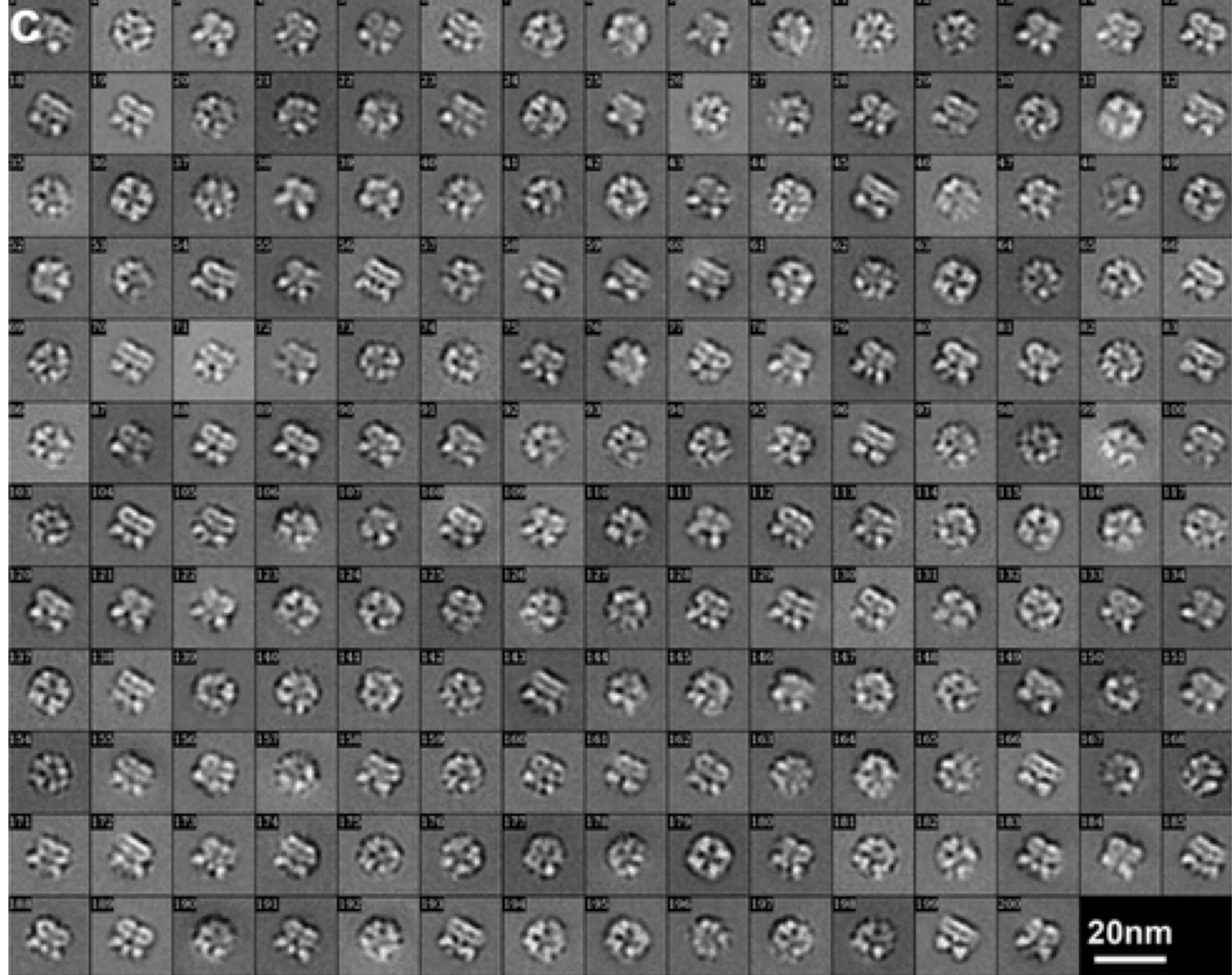
Stephen Burley - Rutgers University

Learning about IDPs from PDB Data (MX)

- Structures of IDPs post Disorder → Order Transition
 - 4E-BP1 } Marcotrigiano *et al.* (1997) *Cell* 89, 951-961.
 - eIF4G } Marcotrigiano *et al.* (1999) *Molecular Cell* 3, 707-716.
- Many proteins really do look like “beads on a string”
 - Eukaryotic even more so than Bacterial
- Many protein structures have disordered segments that cannot be visualized with crystallography
- Many protein structures have poorly-ordered segments that are hard to see with crystallography

Single Particle Image Reconstruction from cryoEM Data





Structural basis for the gating mechanism of the type 2 ryanodine receptor RyR2

Wei Peng,^{1,2*} Hualong Shen,^{1,2,3*} Jianping Wu,^{1,2,3*} Wenting Guo,⁴ Xiaojing Pan,^{1,2} Ruiwu Wang,⁴
S. R. Wayne Chen,⁴ Nieng Yan^{1,2,3*}

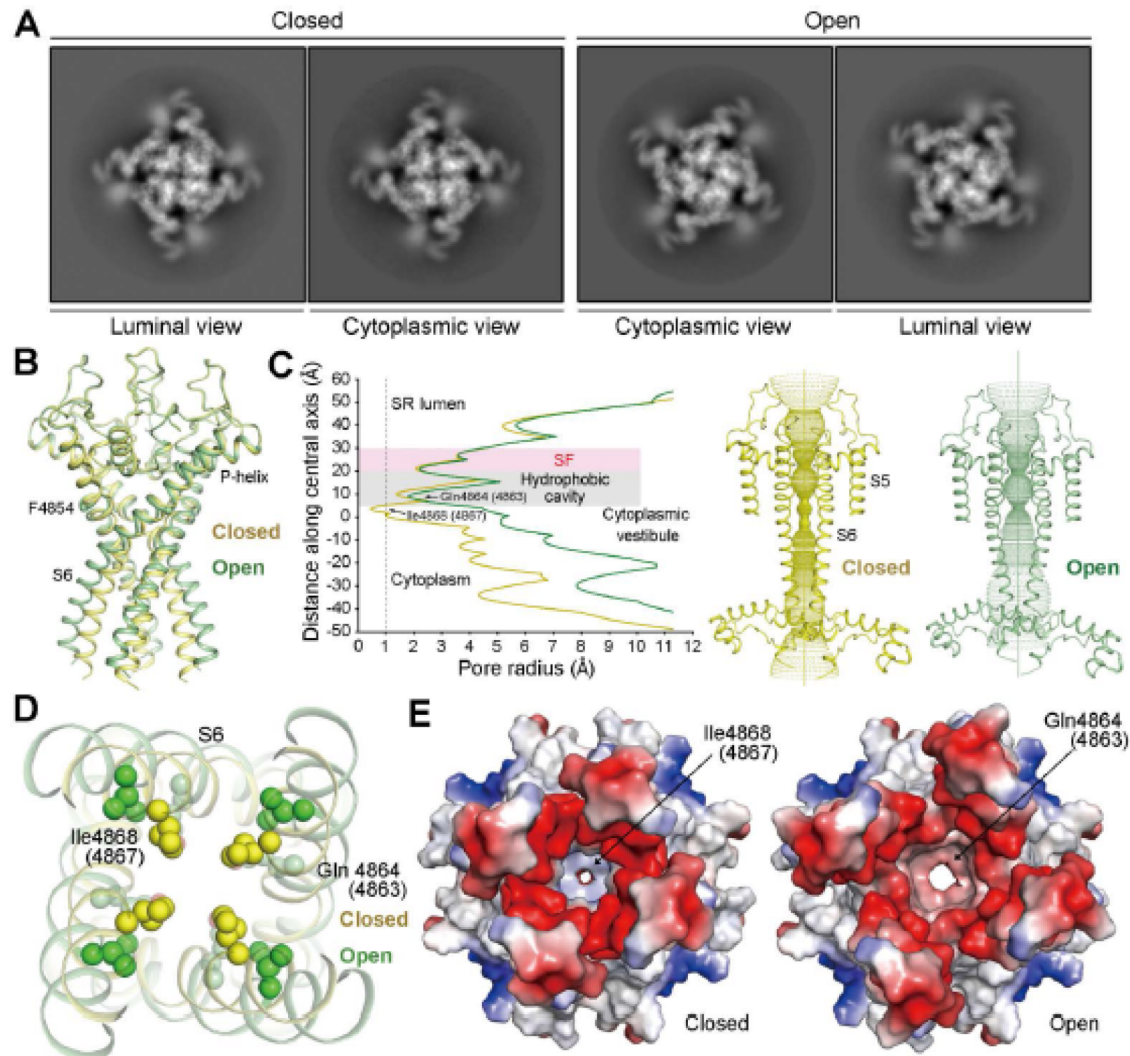
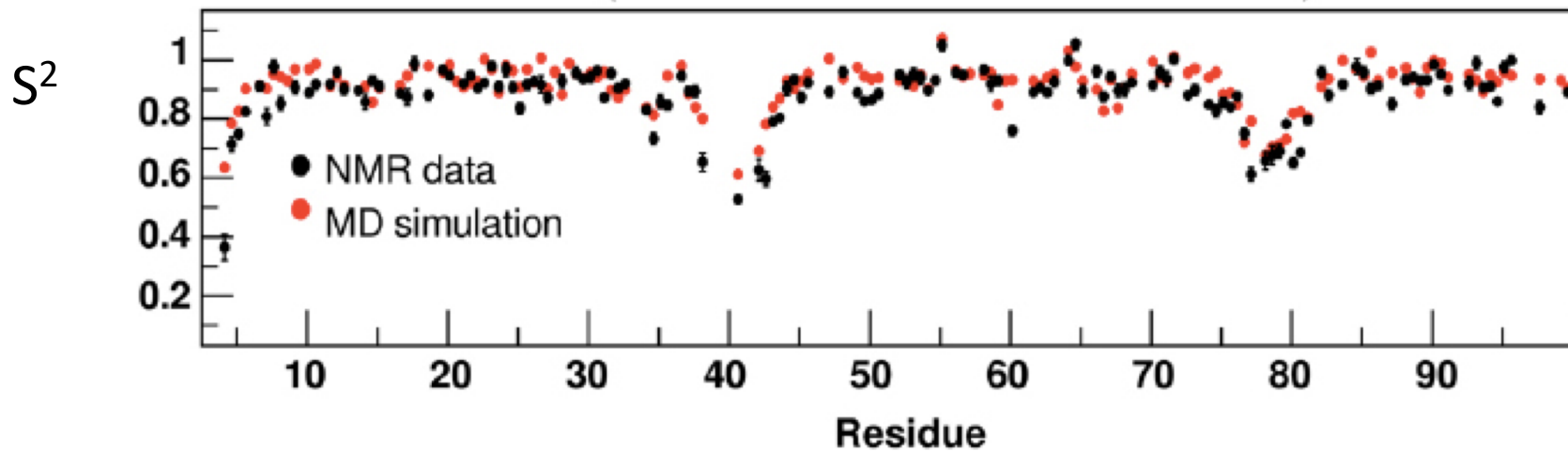
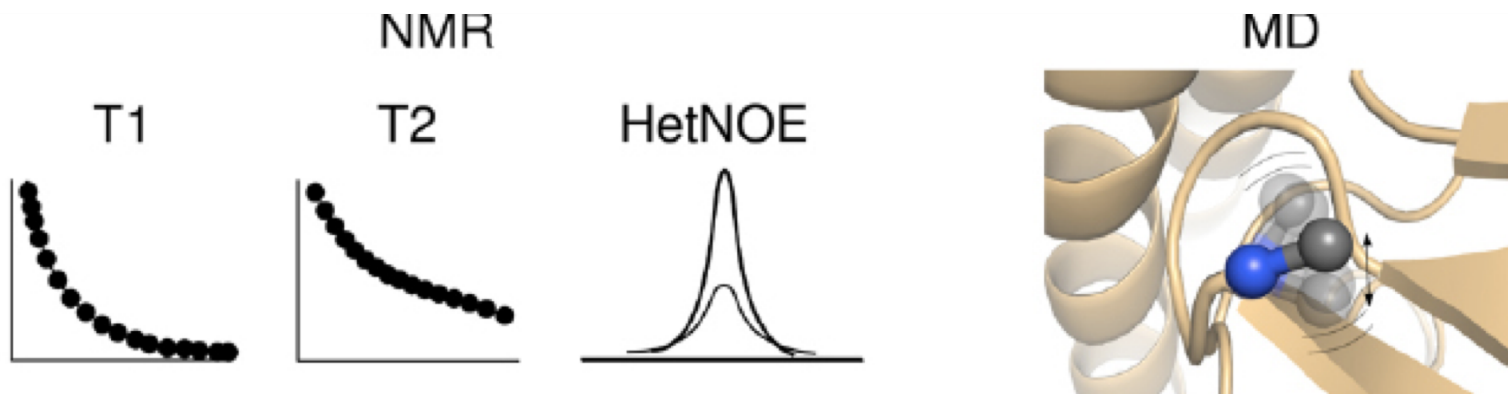
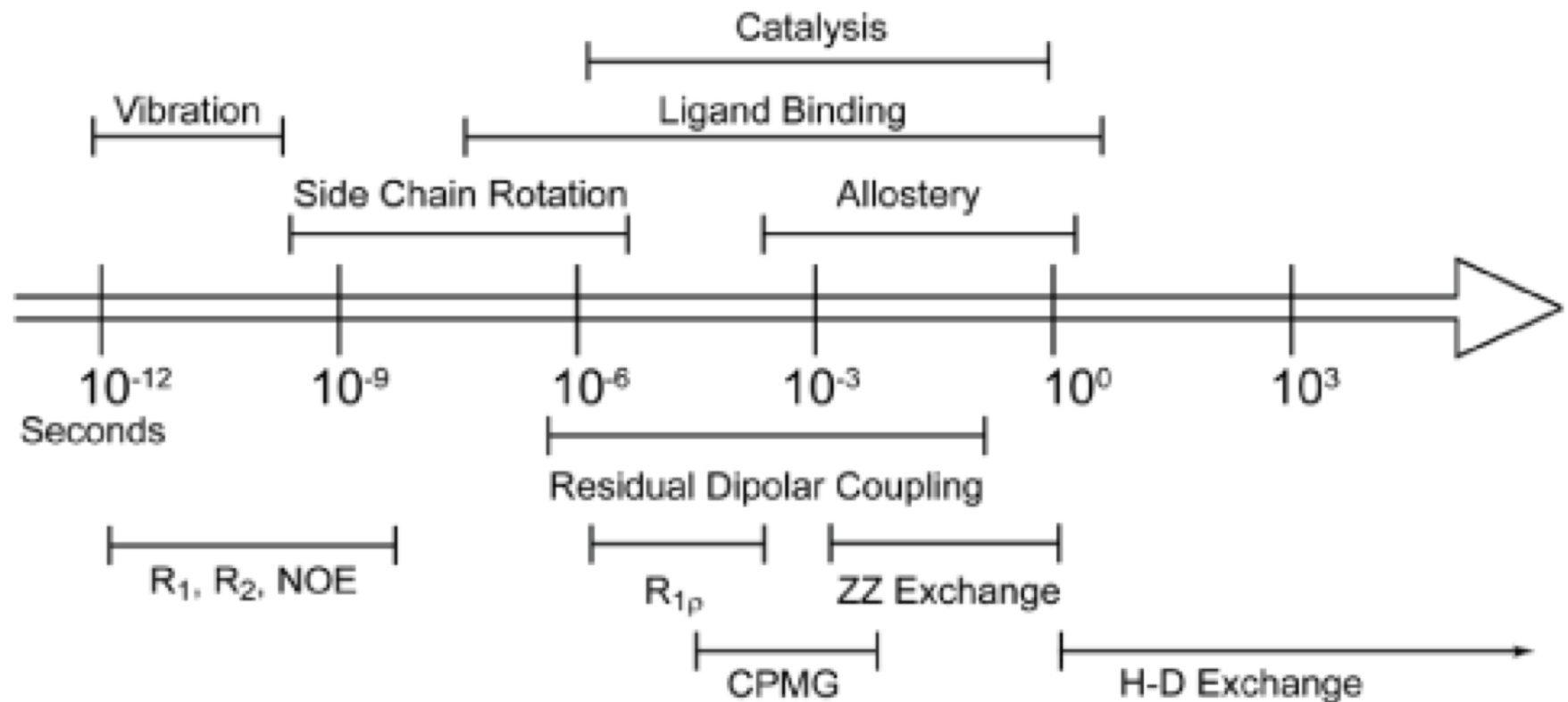


Fig. 2. Structural comparison of the open and closed RyR2. (A) Representative 2D class averages of cryo-EM images of the closed and open RyR2. Note the marked difference of the central pore. (B) Conformational changes of the S6 bundle of the channel domain

NMR is Particularly Powerful for Studying Protein Dynamics



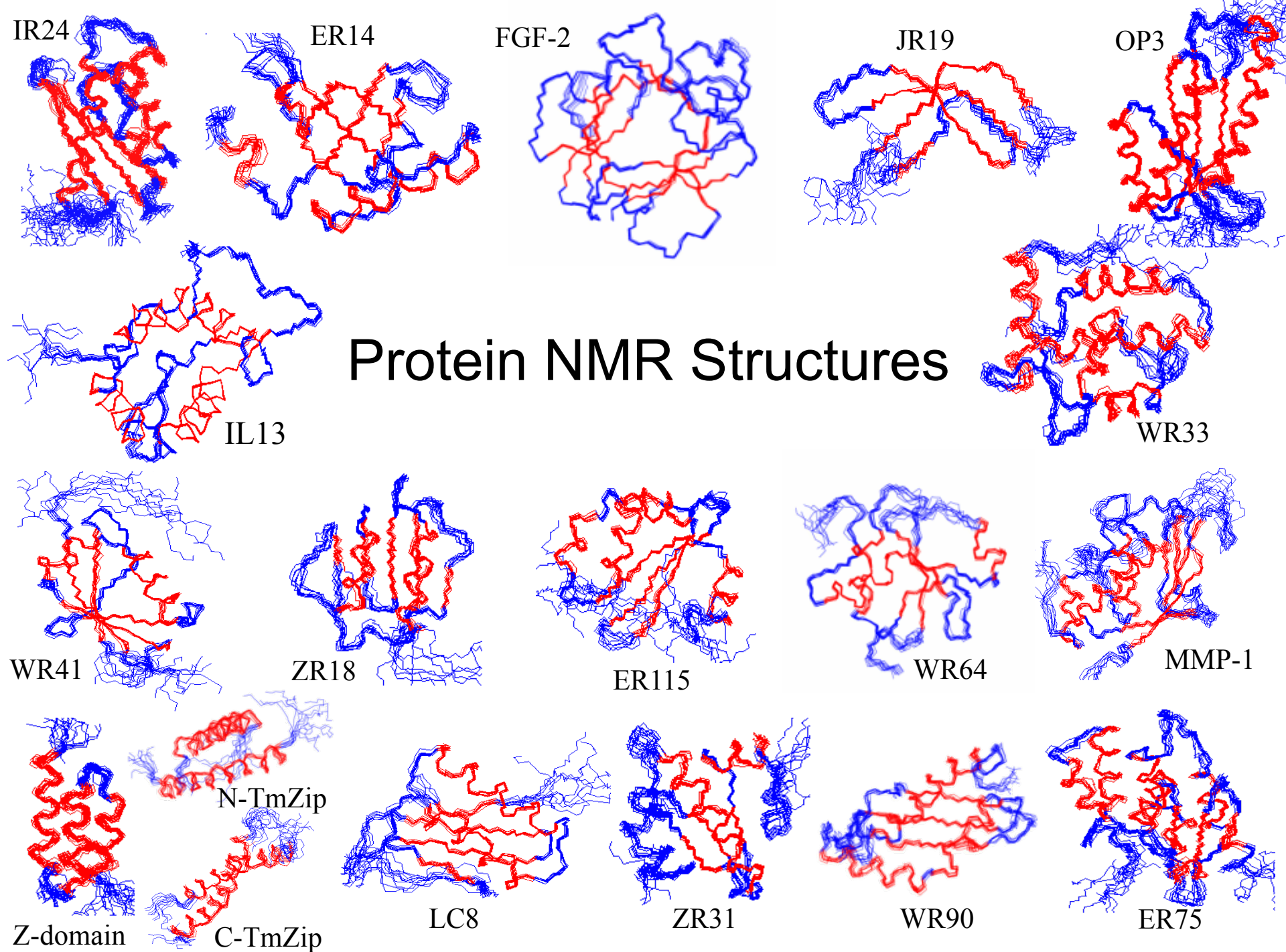
NMR Dynamic Time Scales



IDP's: The BMRB Perspective

Pedro Romero – Univ of Wisconsin

- BMRB is the primary database for experimental NMR data.
- BMRB carries most kinds of NMR data, and supports many NMR experiments.
- NMR is an important experimental technique for the study of the many aspects of IDPs.
- Most IDP NMR experiments are not deposited to BMRB.
- BMRB works with IDP researchers to ensure IDP NMR studies are archived, and asks for researchers' collaboration.



Ordered / Disordered vs. Well-Defined / Not-Well-Defined

Ordered: Those parts of the biomolecular structure which, in nature are sampling a narrow distribution of conformations

Disordered: Those parts of the biomolecular structure which, in nature, are sampling a wide distribution of conformations

Well-defined: Those parts of the biomolecular structure for which the ensemble of coordinates represent a well-converged structure. High precision.

Not-well-defined: Those parts of the biomolecular structure for which the ensemble of coordinates **do not** represent a well-converged structure. Low precision.

Ordered / Disordered vs. Well-Defined / Not-Well-Defined

Ordered / Disordered

- nuclear relaxation measurements
- RDC measurements
- chemical shift data

Non-structural Data

Well-Defined / Not-Well-Defined

- rmsd (local vs global)
- Dihedral Circular Variance
(Dihedral Angle Order Parameters)
- Distance Variance Matrix

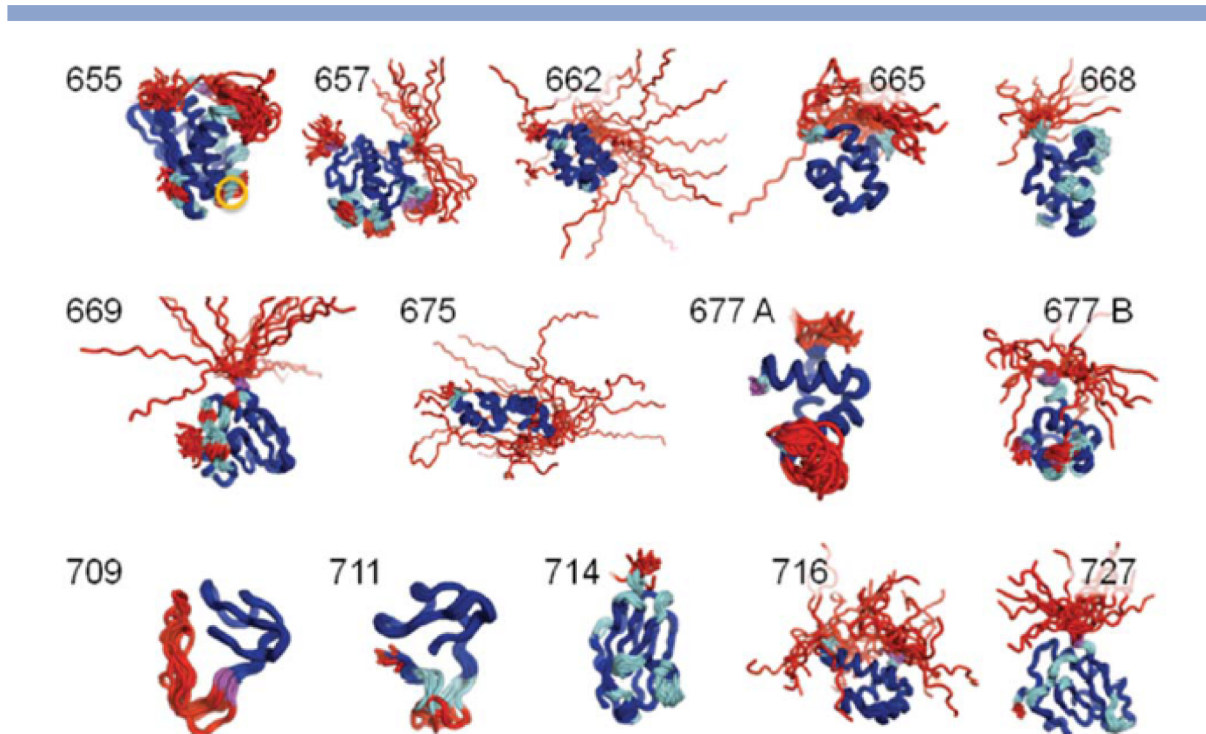
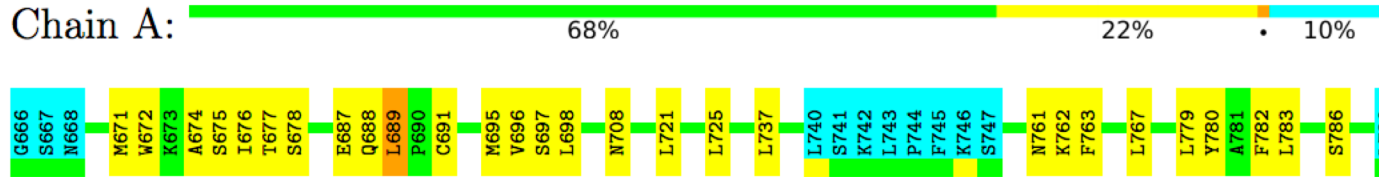
Based on Atomic Coordinates

wwPDB NMR Structure Validation Report

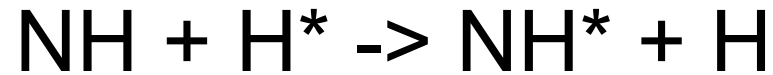
4 Residue-property plots

● Molecule 1: Sorting nexin-25

Blue – Not well defined



Amide Proton Exchange



Hydrogen Bonds

Ligand-binding Sites

Ligand-induced Conformational Changes

- Allosteric Changes

Conformational Breathing

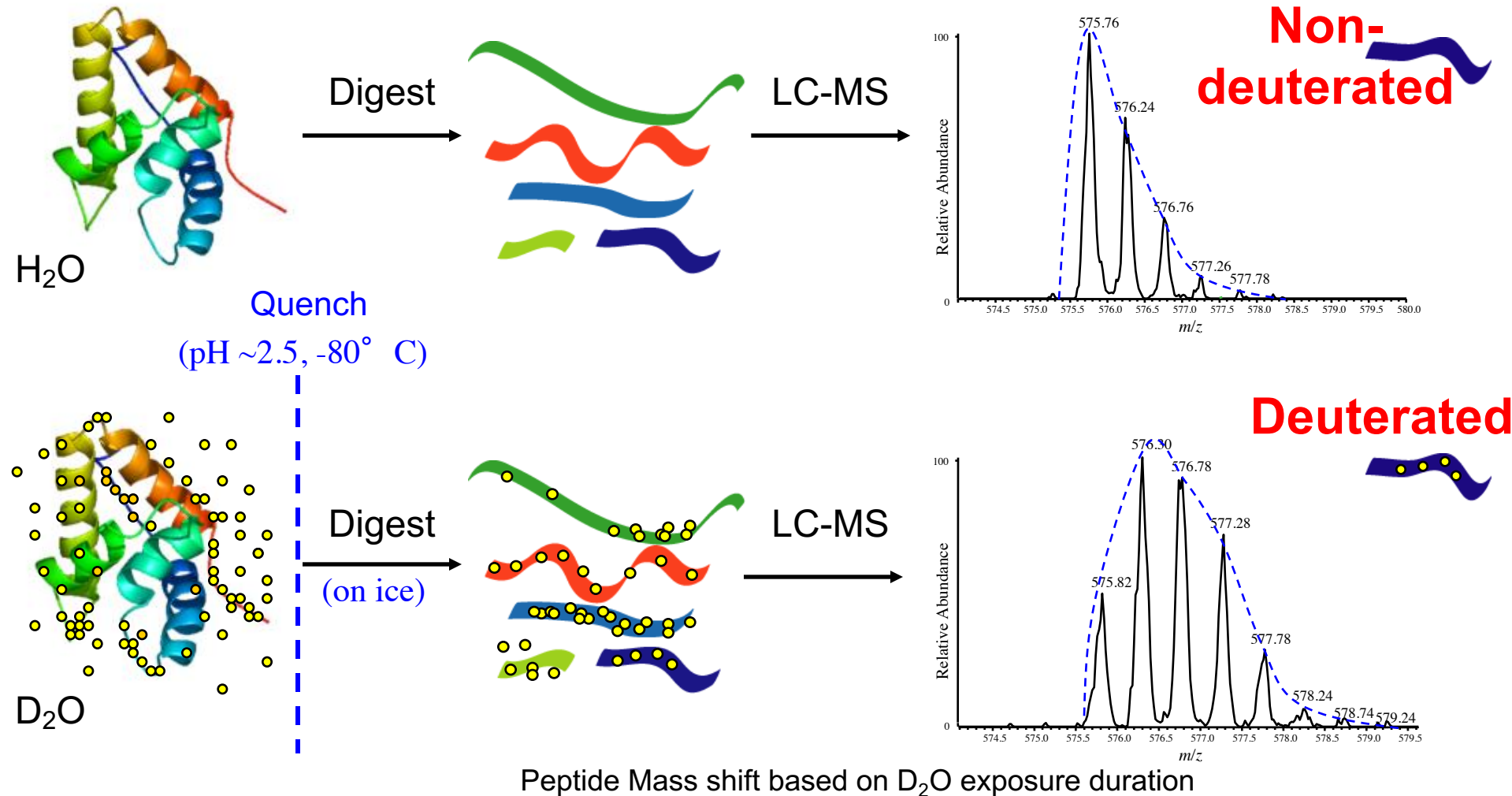
Energy Landscape

Identify Flexible Regions of Proteins

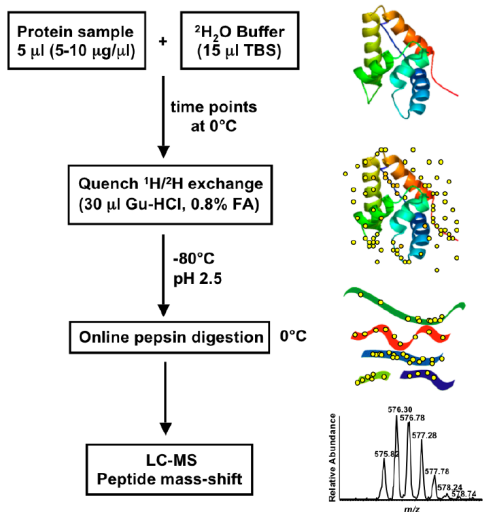
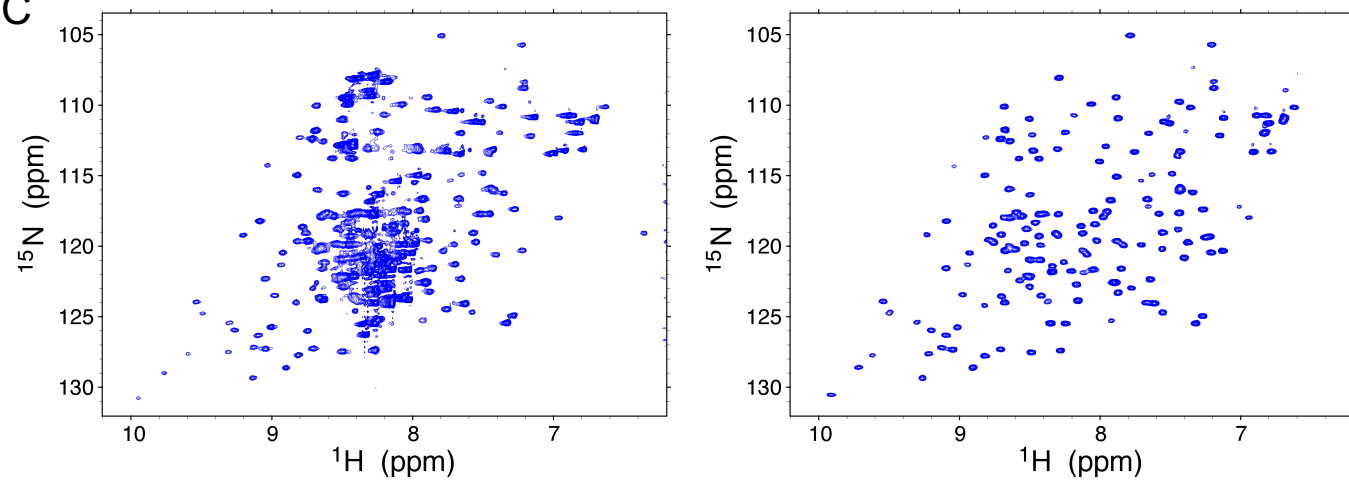
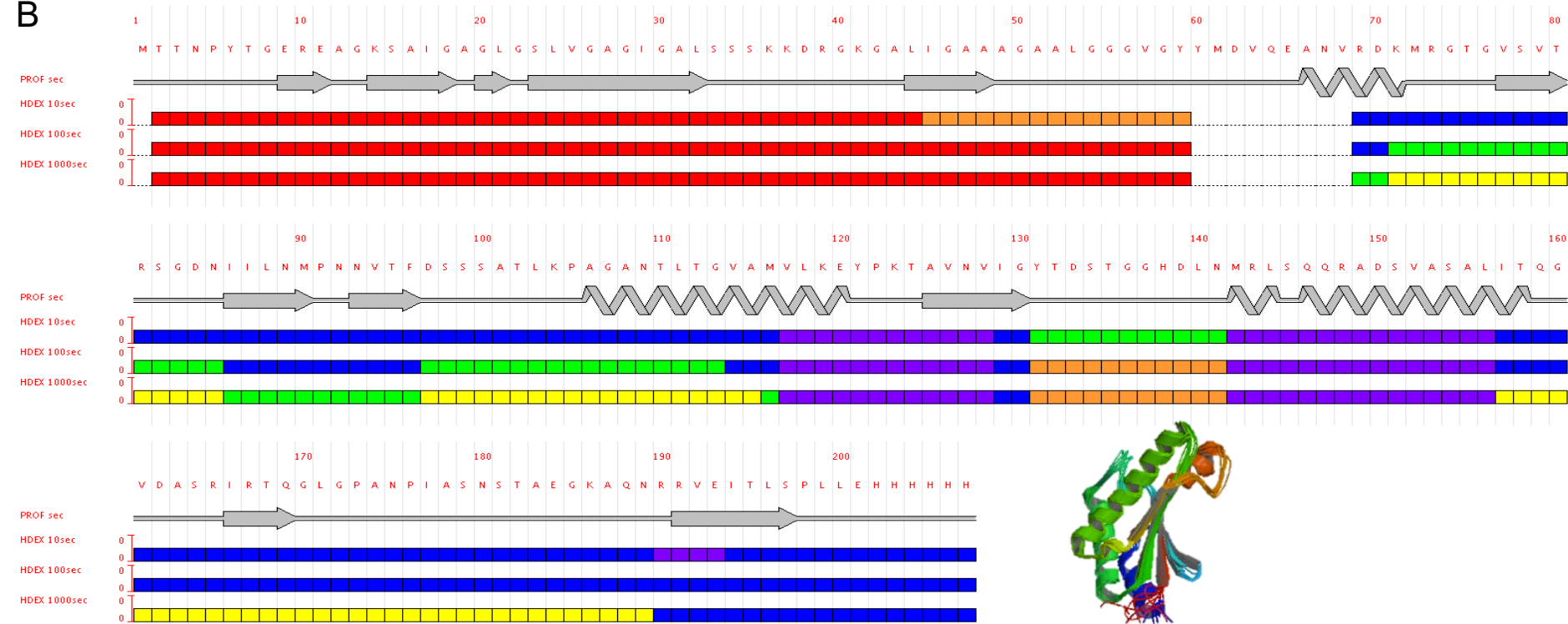
Protein Folding Mechanisms / Intermediates

Amide H/D Exchange MS (HDX-MS)

H. Zheng



1. ZL Zhang, DL Smith, Determination of amide hydrogen exchange by mass spectrometry: A new tool for protein structure elucidation, *Protein Sci.* 1993, 2, 522 – 531.
2. D Pantazatos. et al., Rapid refinement of crystallographic protein construct definition employing enhanced H/D exchange MS, *PNAS*, 2004, 101, 3, 751 – 756.

A**C****B**

Disorder Prediction



Dismeta

Disorder Prediction
Meta-Server



Main

The Dismeta server shows consensus results of these protein disorder predictors:

DISEMBL	DISOPRED2	DISpro	DRIPPRED	FoldIndex
FoldUnfold	GlobPlot2	IUPred	RONN	VSL2

It also reports predictions from these sequence analysis tools:

coils	ANCHOR	SignalP	TMHMM	SEG	PROFphd	PSIPred
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All tools use default setup. Tools in **green** run on DisMeta server site.

Email address:

Protein name or NESG targetID (no longer than 10 characters):

Sequence for non-NESG target(letters only):

SignalP option - Organism:

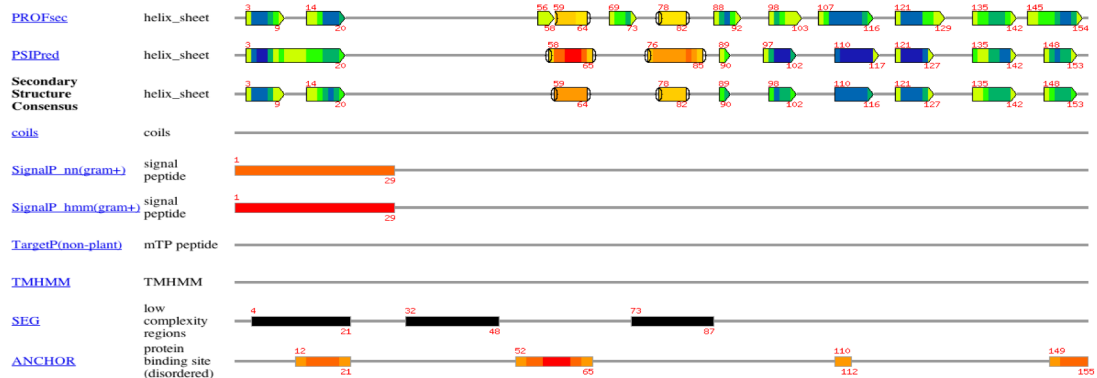
gram+ gram- euk

www-nmr.cabm.rutgers.edu/bioinformatics/disorder

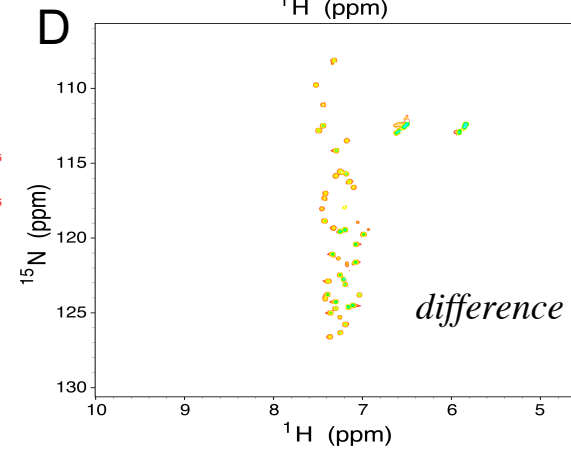
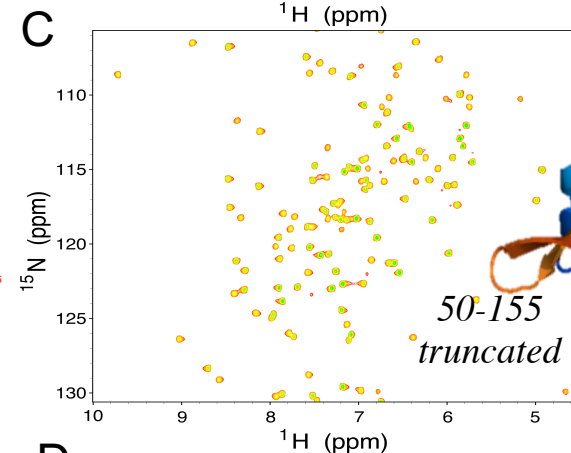
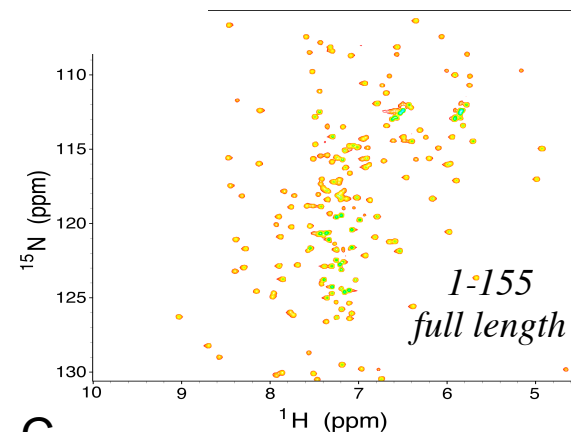
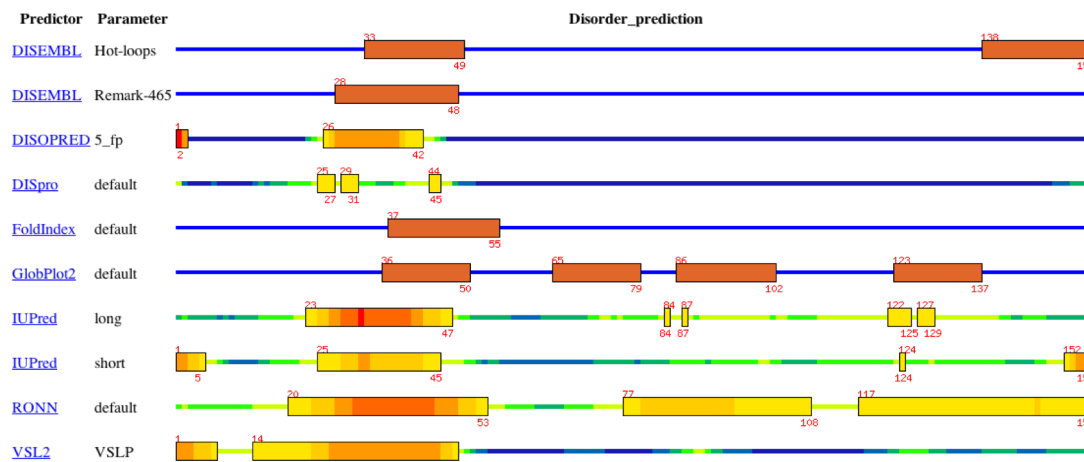
submit

Removal of N-terminal IDR does not affect the structure of the rest of the protein

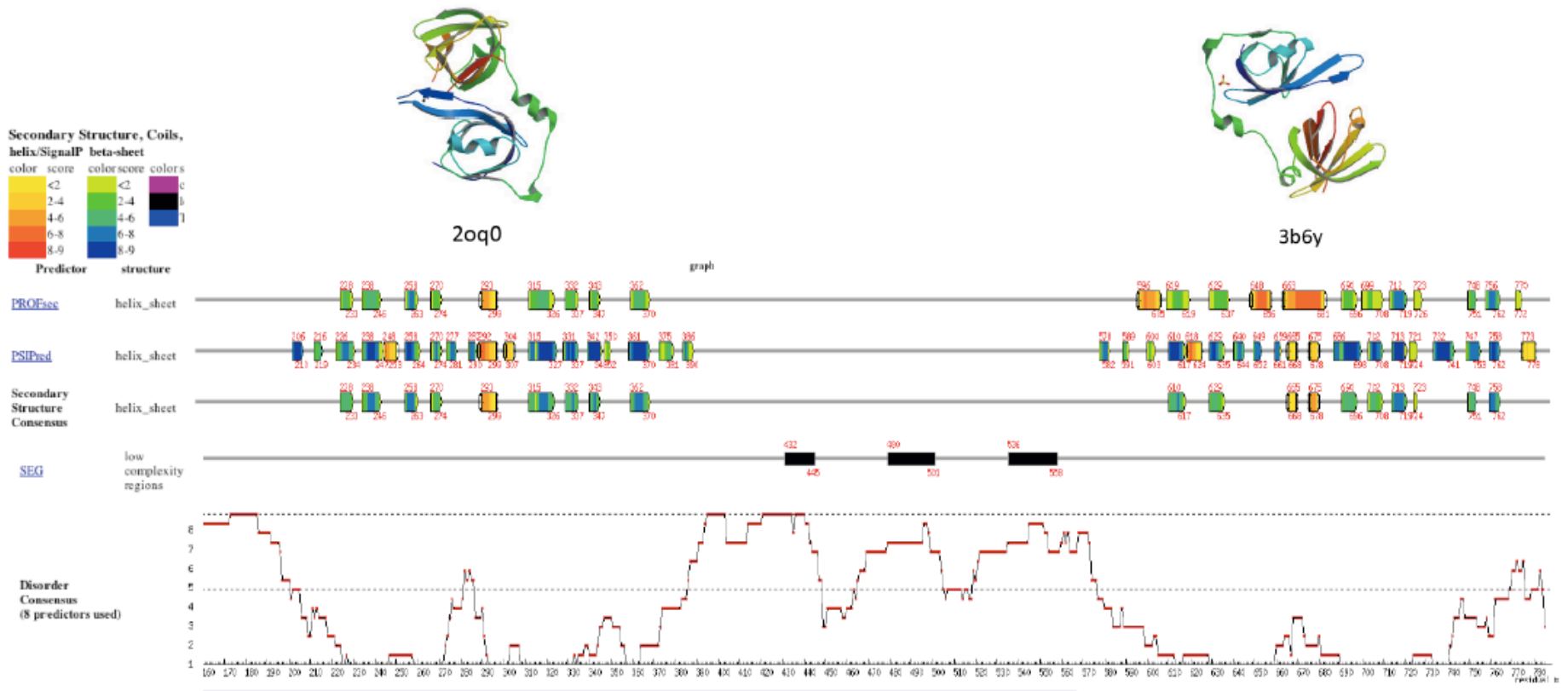
SyR11



Disorder Consensus
(8 predictors used)



DisMeta Disorder Prediction Server For Domain "Parsing"



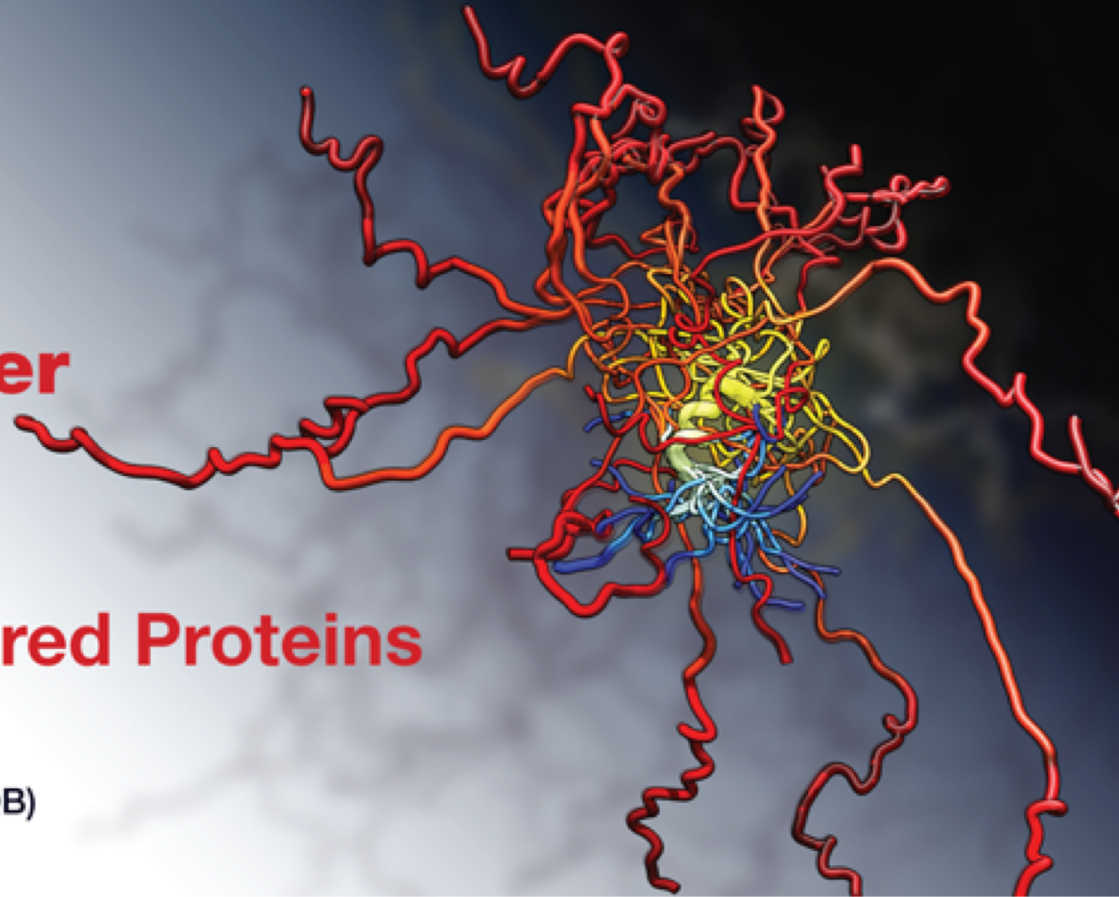
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Jean Baum

Stephen Burley

Biomolecular Structure Visualization Methods

