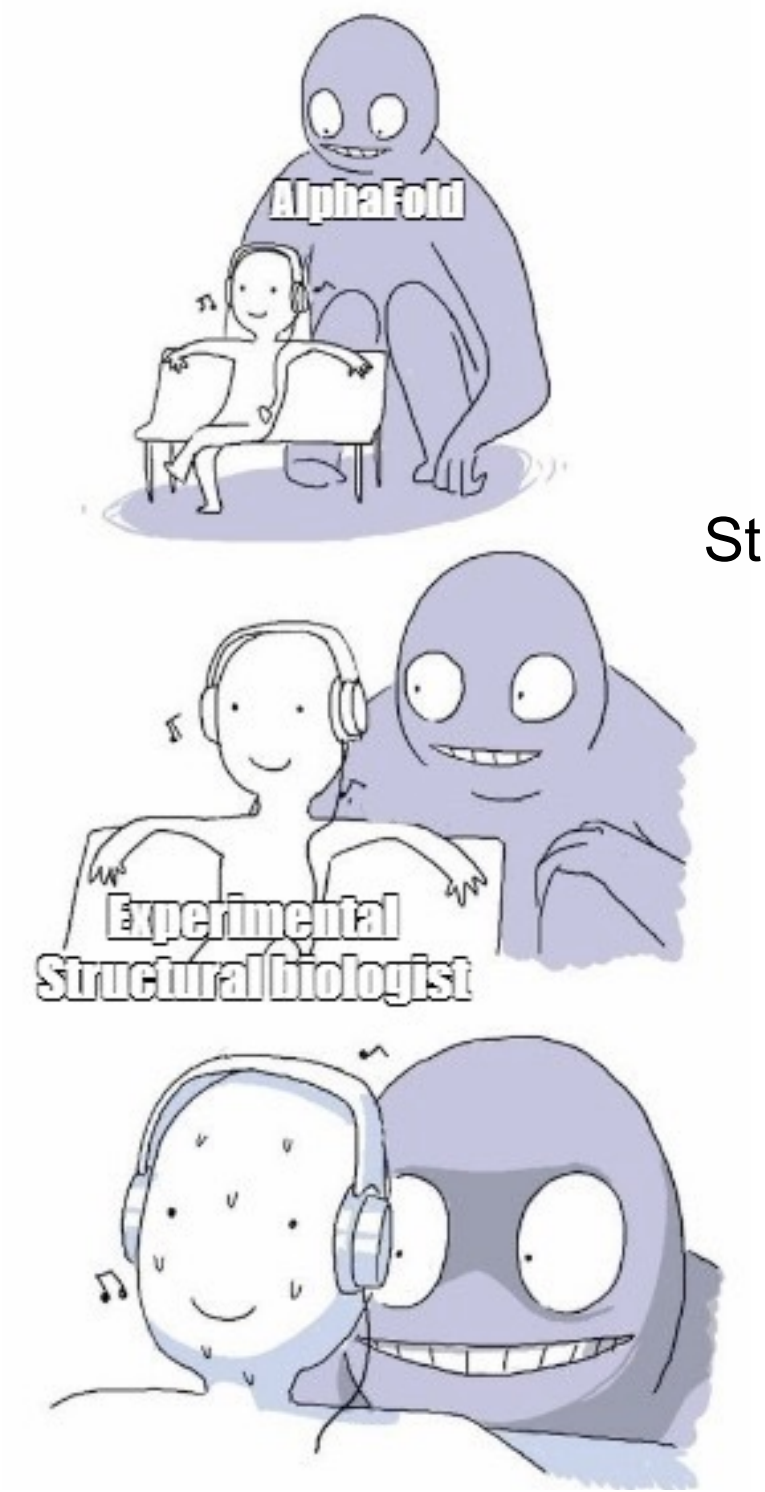
Centre de Biologie Structurale
Montpellier

Combined Use of NMR and SAS for Flexible Systems

Pau Bernadó

pau.bernado@cbs.cnrs.fr

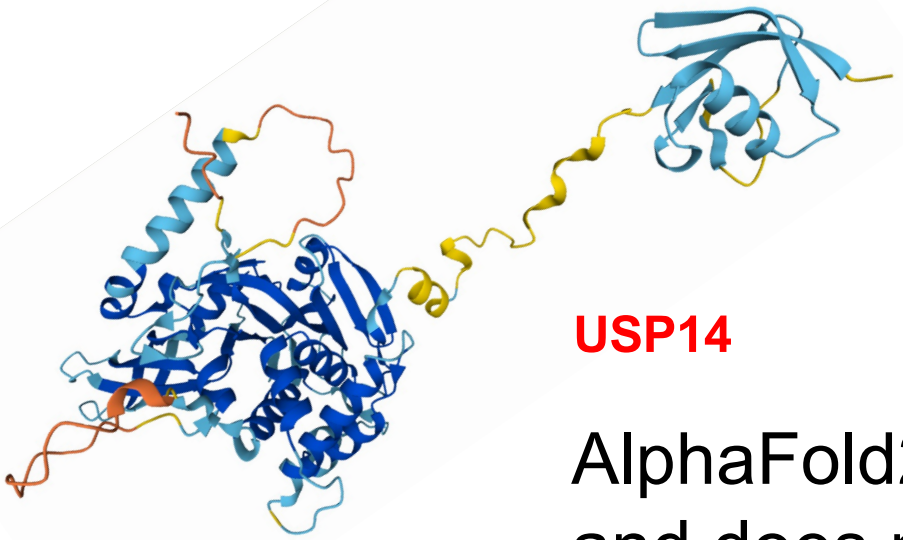
Grenoble 9/2024



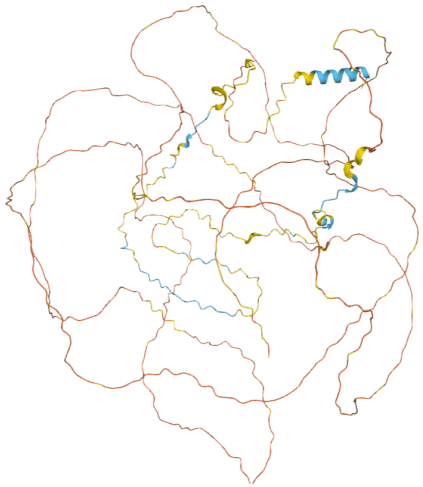
The AlphaFold Era

Structural models for all proteins on earth

Functionally tailored protein design

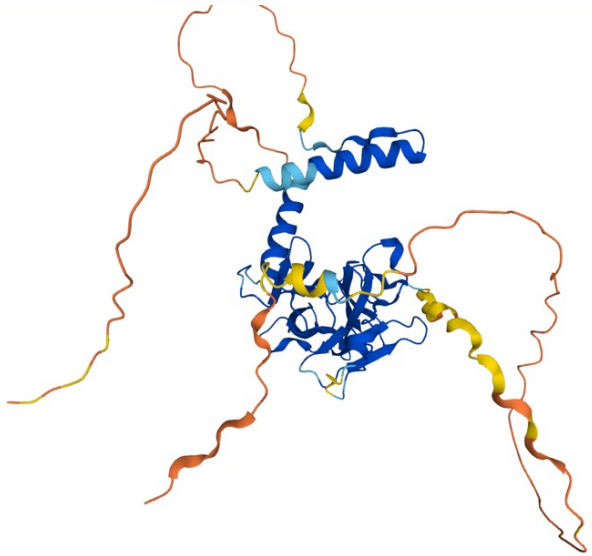


USP14

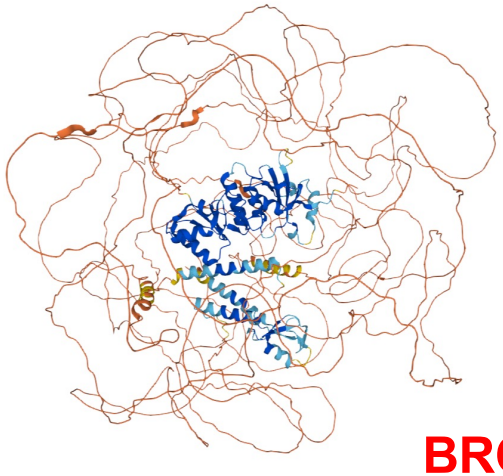


Tau

AlphaFold2 produces static structures and does not “understand” disordered segments



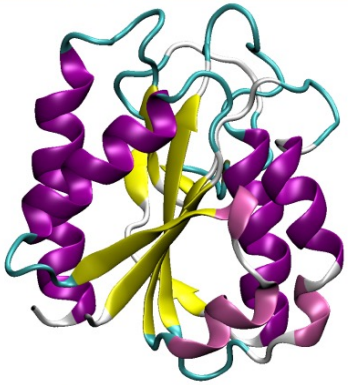
p53



BRCA1

Proteins are Dynamic Molecules

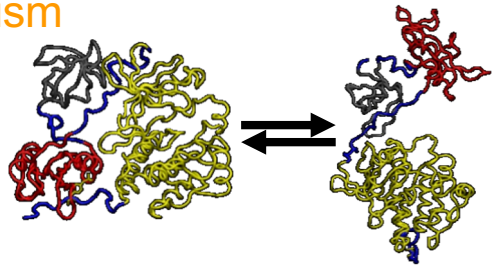
ORDER



Concerted Motions

On/off mechanisms

Allosterism



IDPs

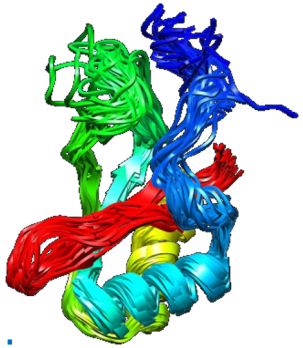
Signalling and control

PTM

Recognition

LLPS

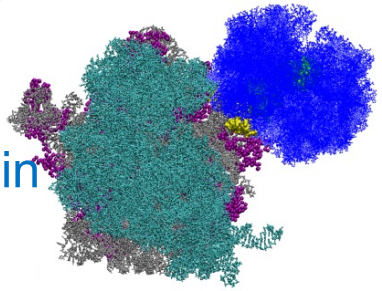
DISORDER



Protein vibrations

Catalysis

Partner Recognition



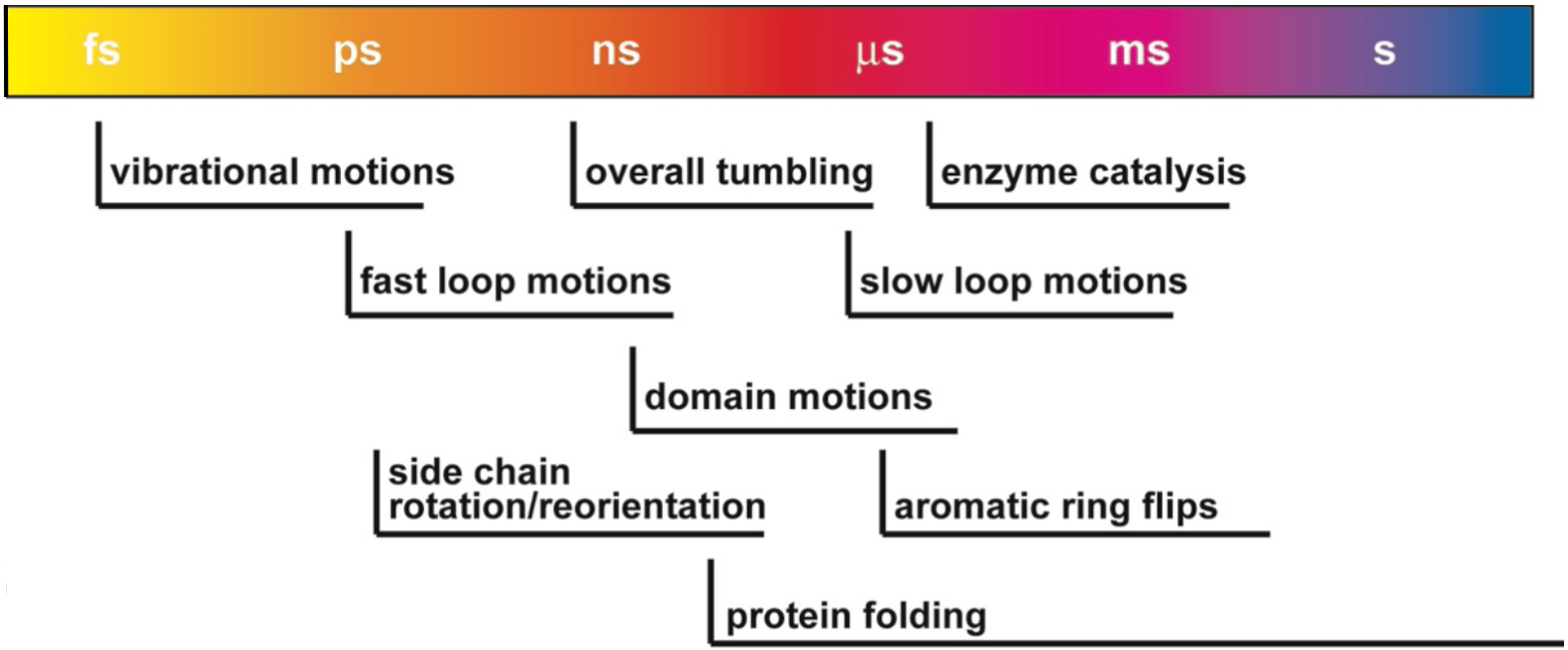
Flexible Multidomain Proteins

Springs

Entropic Linkers

Protein Motions: Conformational Disorder and Time-dependence

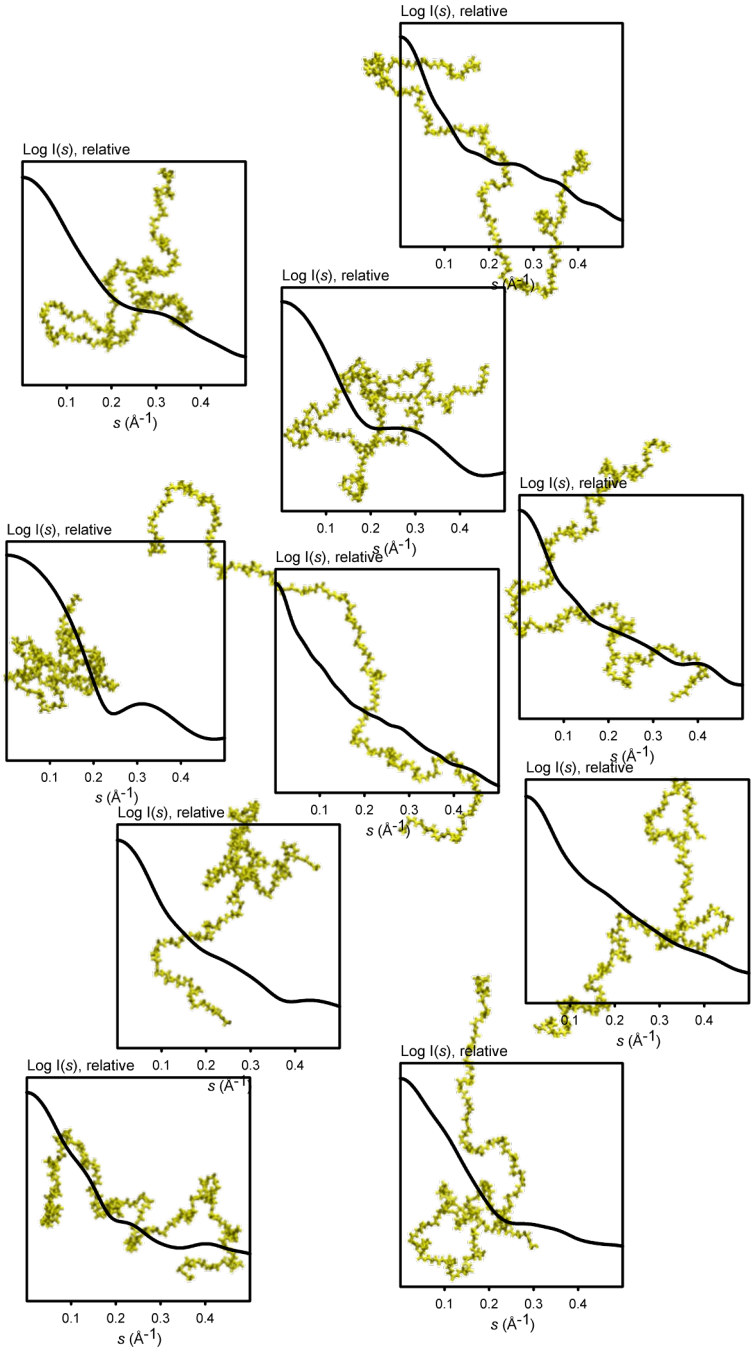
Time-Dependence ← ***Dynamics*** → Conformational plasticity

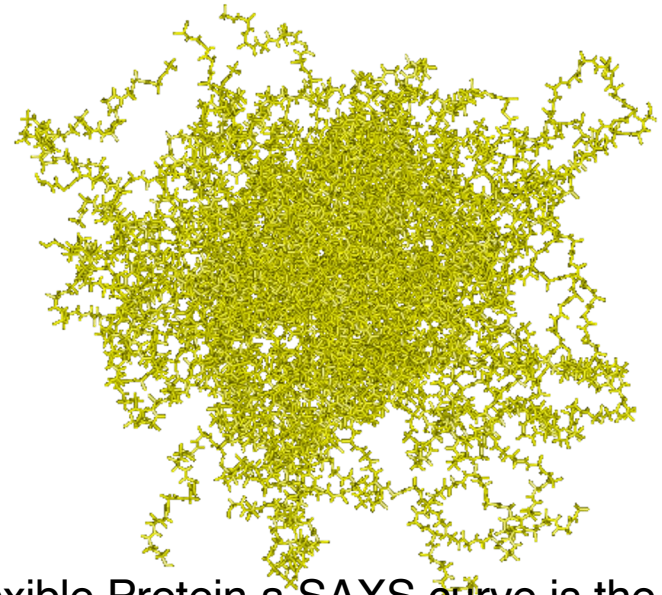


SAS is sensitive to motions notably changing the size and shape of biomolecules

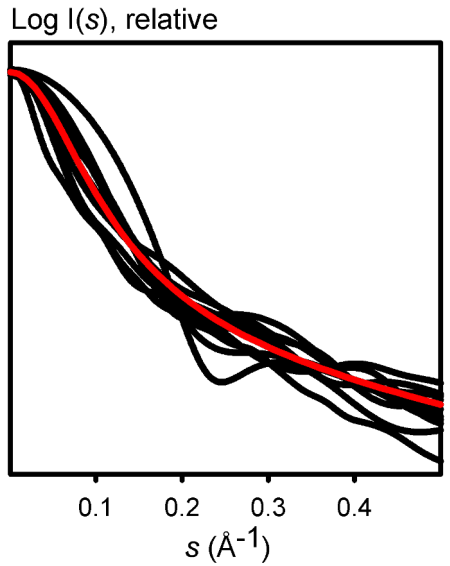
Time-Dependence can be measured through special setups

SAS in Flexible Proteins





In a flexible Protein a SAXS curve is the average of all conformations coexisting in solution



Two Crucial Concepts

Assumption of a dynamic system implies the use of the concept of ‘ensemble of conformations’ to properly describe the data (SAS and others).

No *ab initio* or Rigid-body models of flexible proteins

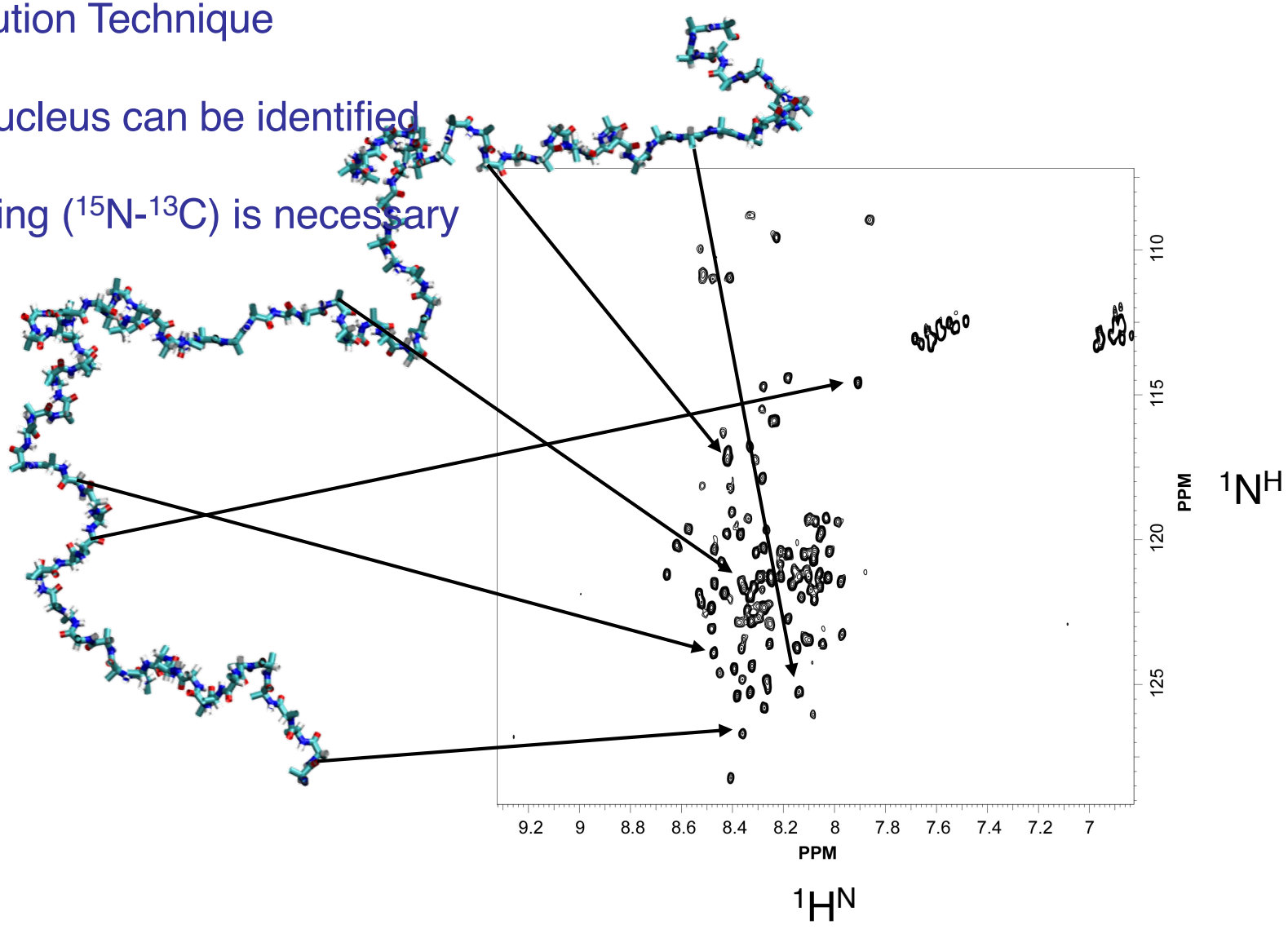
Reliable and structurally meaningful dynamic information of biomolecules can only be derived from SAXS if the system can be modelled and their form factors calculated

Nuclear Magnetic Resonance (NMR)

Atomic Resolution Technique

Each active nucleus can be identified

Isotopic labelling (^{15}N-^{13}C) is necessary



NMR, a Versatile Source of Structural Information

Local Conformations
Chemical Shifts, J-Couplings

Interatomic Distances
^{1}H-^{1}H NOEs, PREs, Pseudocontact Shifts

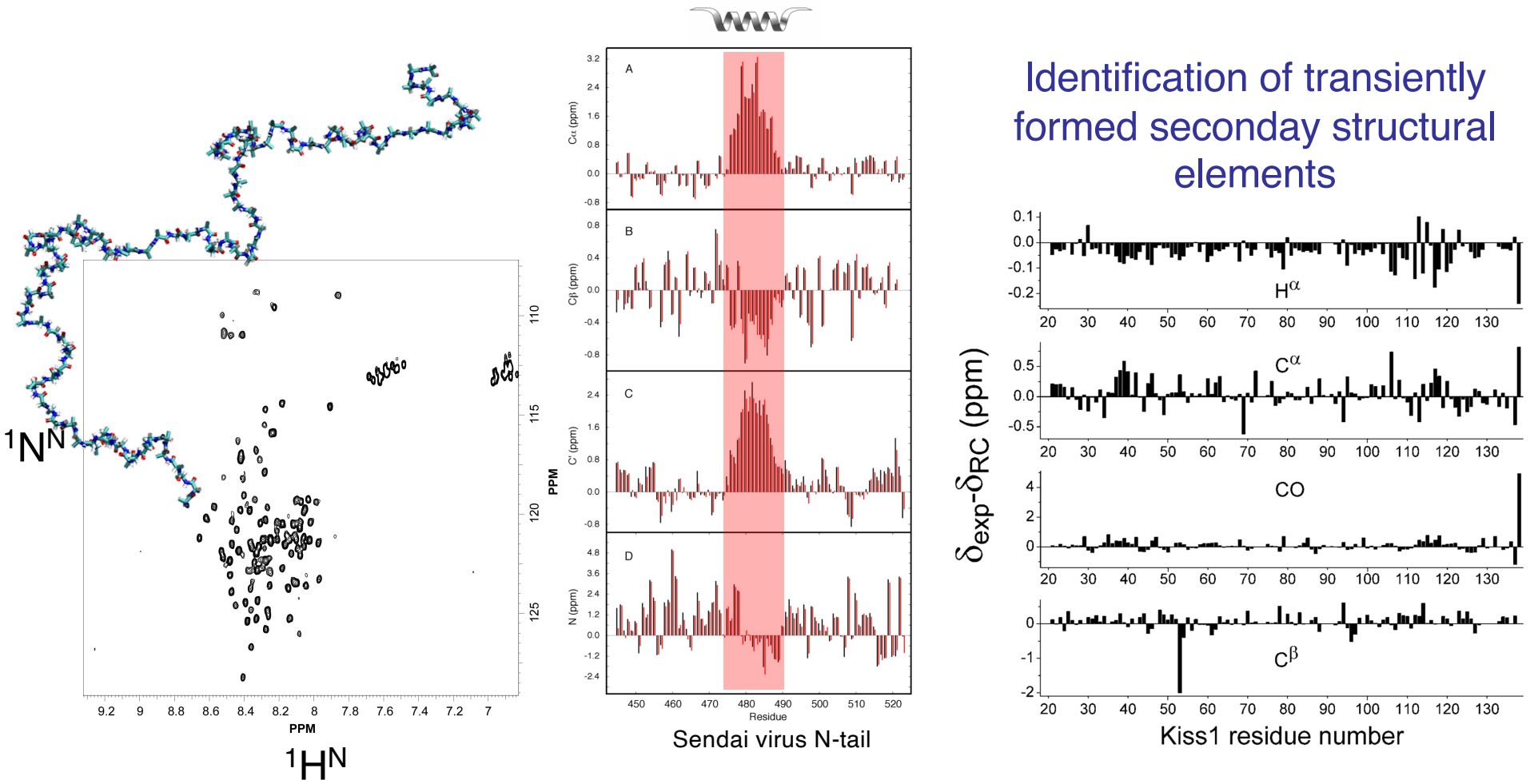
Orientational Restrictions
RDCs, Relaxation (R_2/R_1), Pseudocontact Shifts

Overall Size
Diffusion Coefficients, Relaxation (Rotational Tensor), RDCs (Alignment Tensor)

Time-Scale of Motions
Relaxation (R_1,R_2, Het-NOE), Relaxation Dispersion, $R_1\rho$, CEST

The Majority of these Observables are also Ensemble Averaged

Chemical Shifts



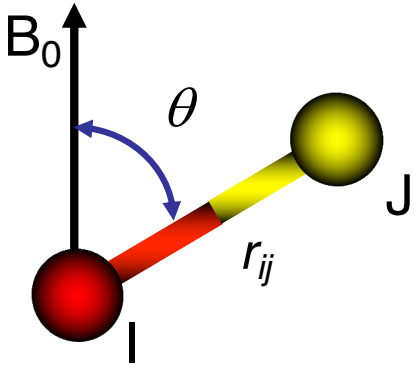
Sendai virus N-tail

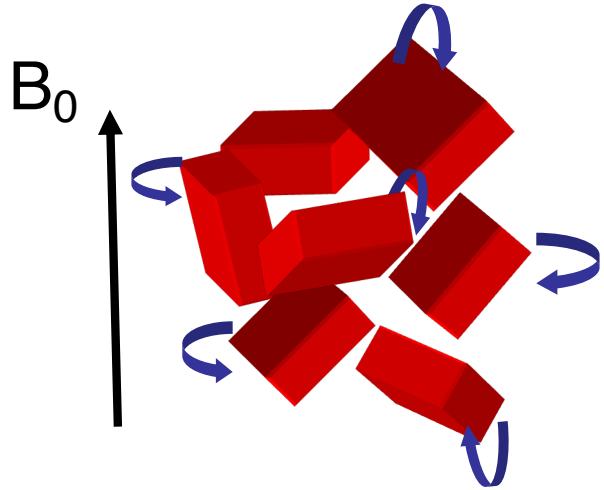
Identification of transiently formed seconday structural elements

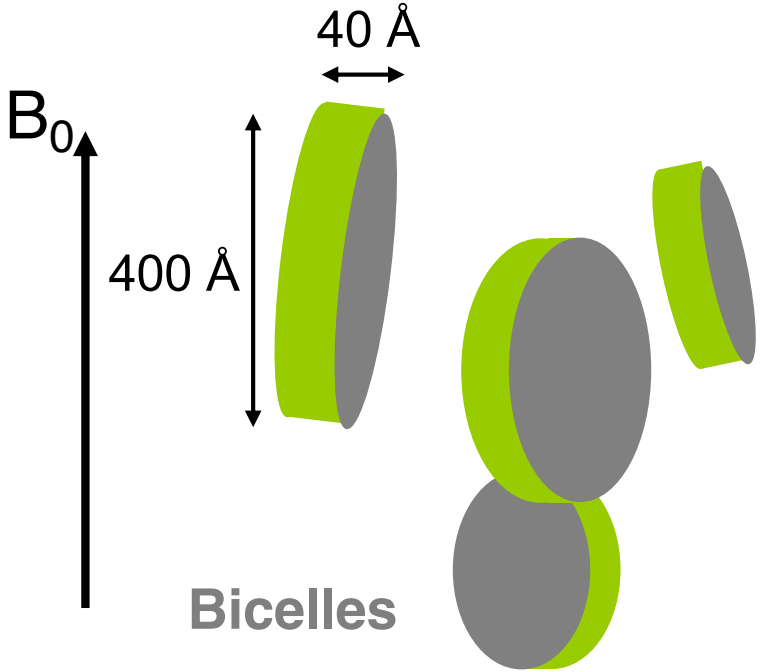
Kiss1 residue number

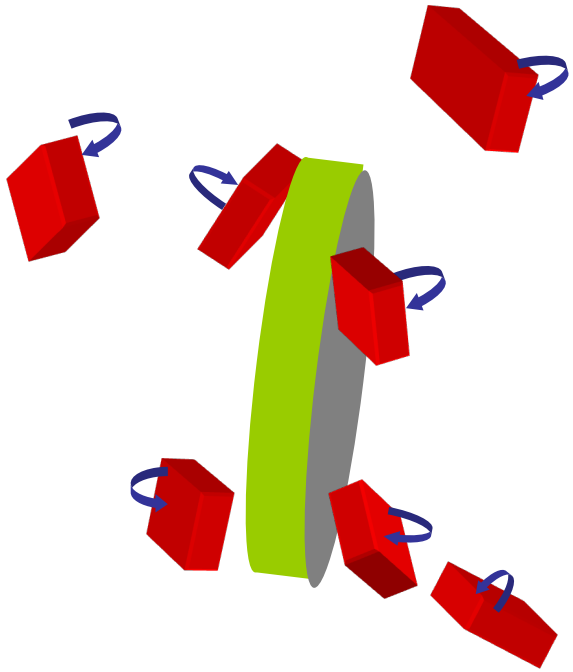
Residual Dipolar Couplings (RDCs)

$$D_{ij} = -\frac{\gamma_i \gamma_j \mu_0 h}{8\pi^3}\left\langle \frac{P_2(\cos\theta(t))}{r_{ij}^3}\right\rangle$$



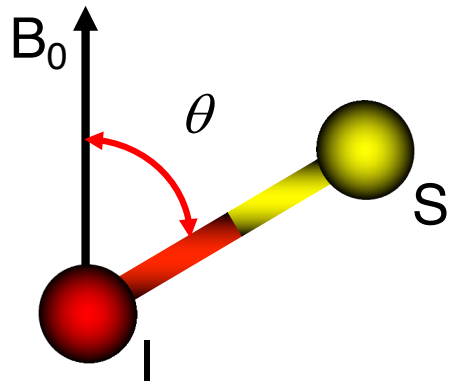






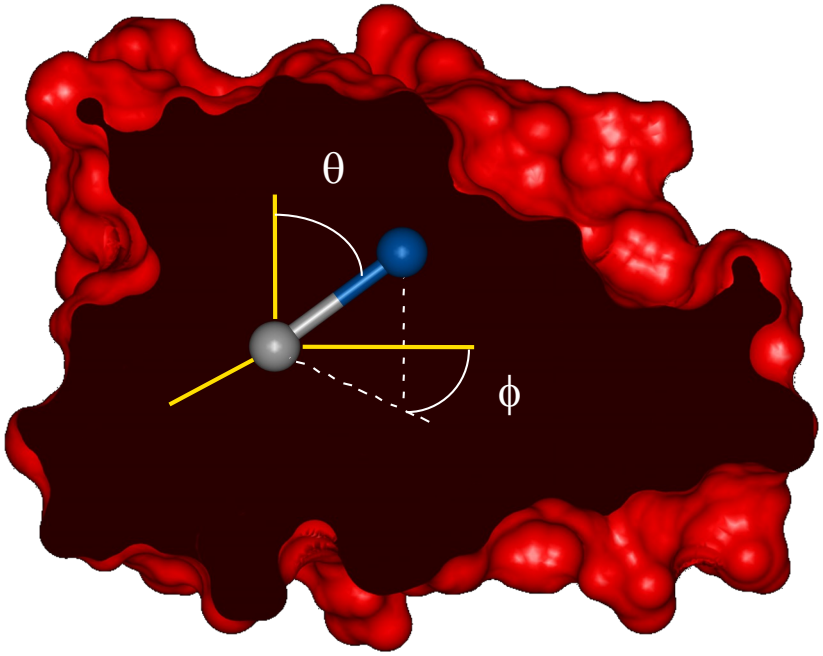
Tjandra, N.; Bax, A. *Science*, **1997**, *278*, 1111-1114.

Residual Dipolar Couplings (RDCs)



$$D_{ij} = -\frac{\gamma_i \gamma_j \mu_0 h}{8\pi^3}\left\langle \frac{P_2(\cos\theta(t))}{r_{ij}^3} \right\rangle$$

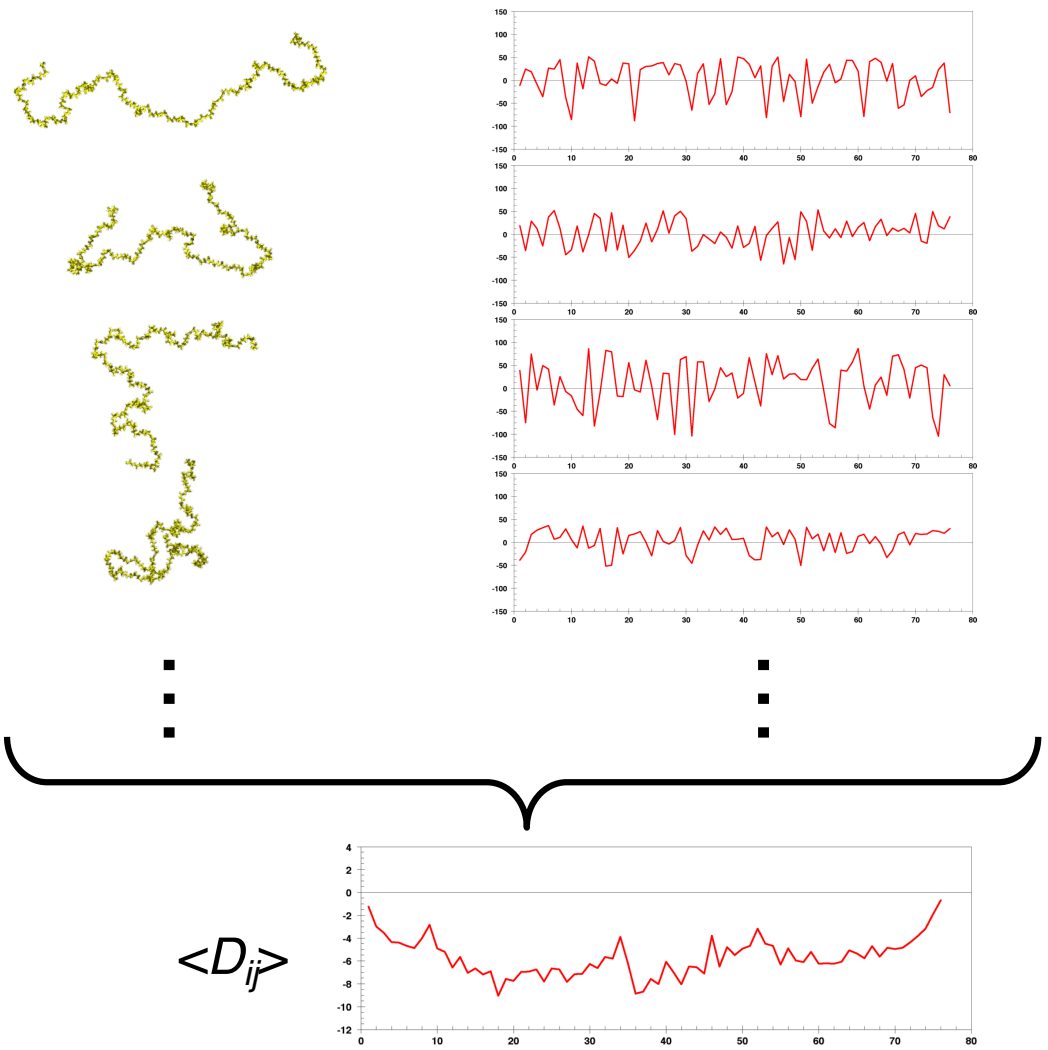
$$D_{ij} = -S\frac{\gamma_i \gamma_j \mu_0 h}{16\pi^3 r_{ij}^3}\left(A_a\left(3\cos^2\theta - 1\right) + \frac{3}{2}A_r \sin^2\theta\cos 2\varphi \right)$$

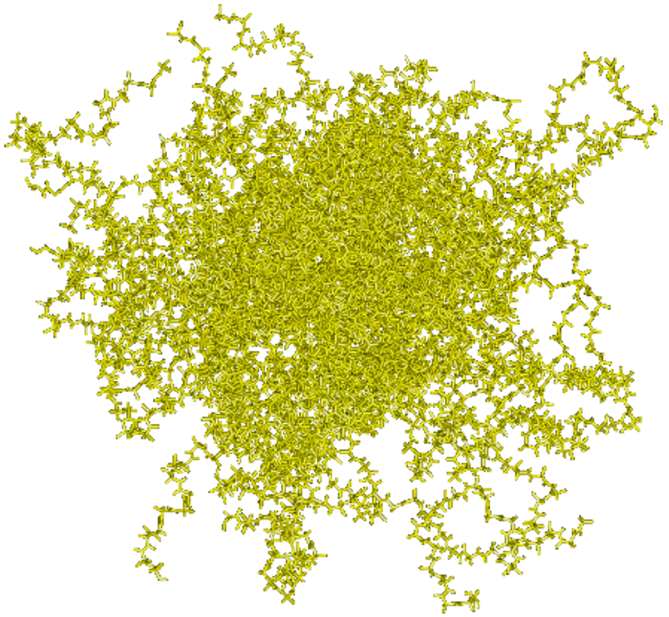


Residual dipolar couplings depend on the orientation of internuclear vectors relative to the alignment frame

RDCs are a valuable source of information to study structure and dynamics of biomolecules

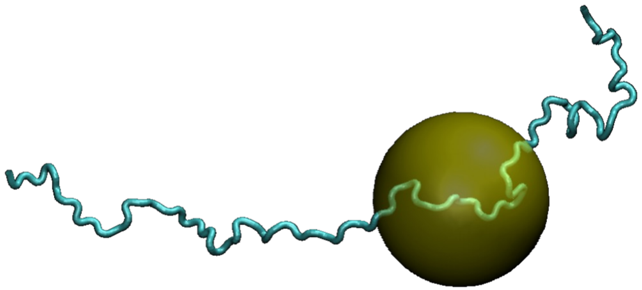
RDCs in Flexible Proteins

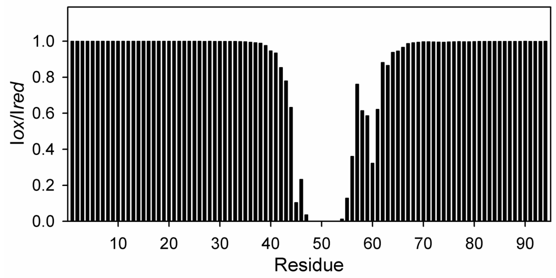




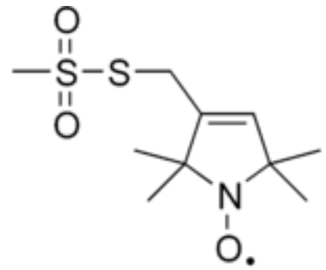
RDCs are the averaged value for all conformations in the ensemble

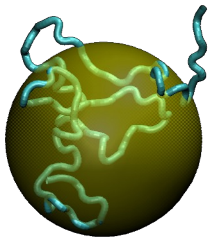
Paramagnetic Relaxation Enhancement (PREs)

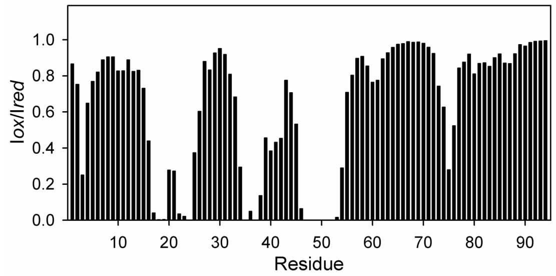




Free electron Radical attached to a Cys mutant



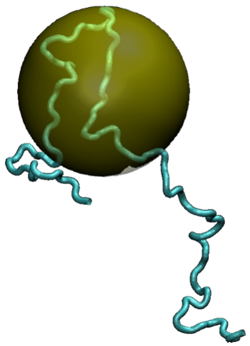


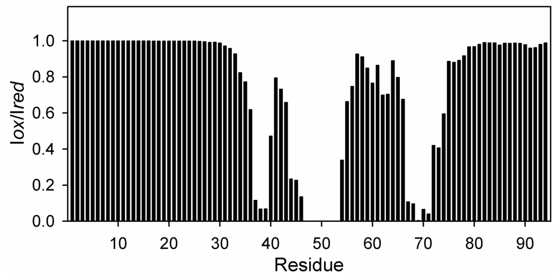


Residues in the proximity experience an enhancement of their R_2 relaxation

Effect up to r < 25 Å

r^6 dependence

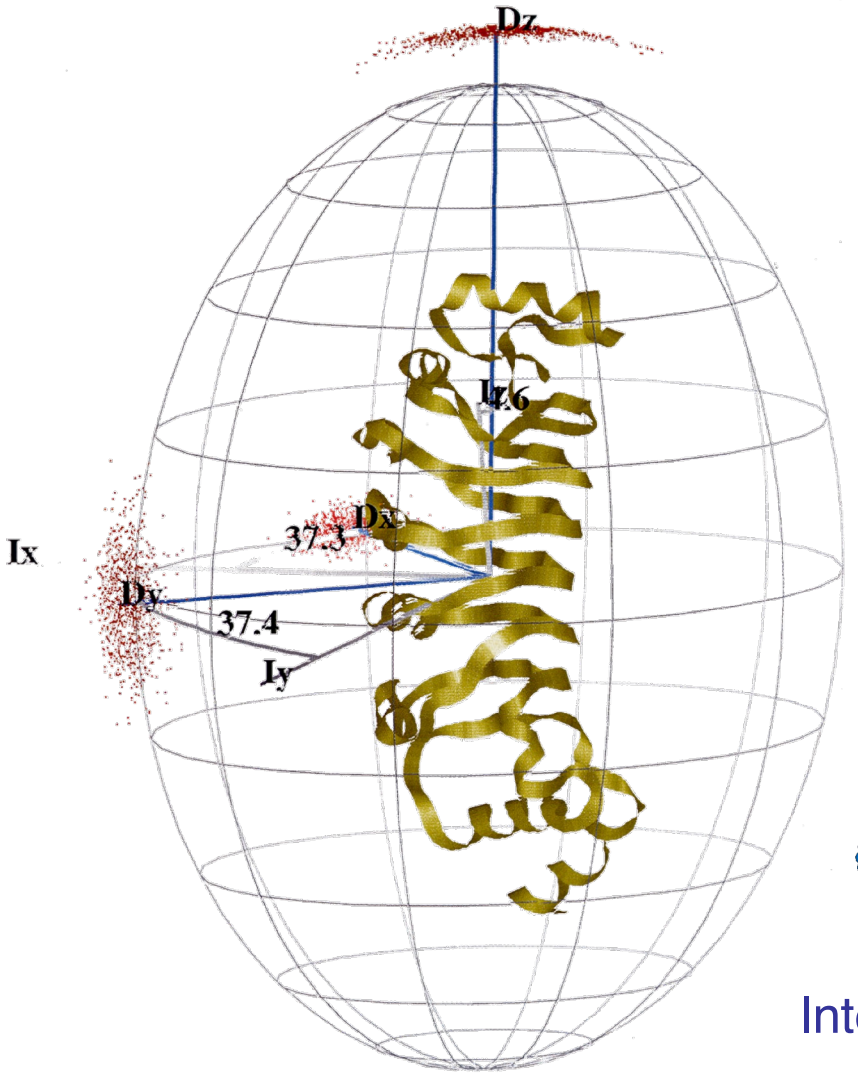


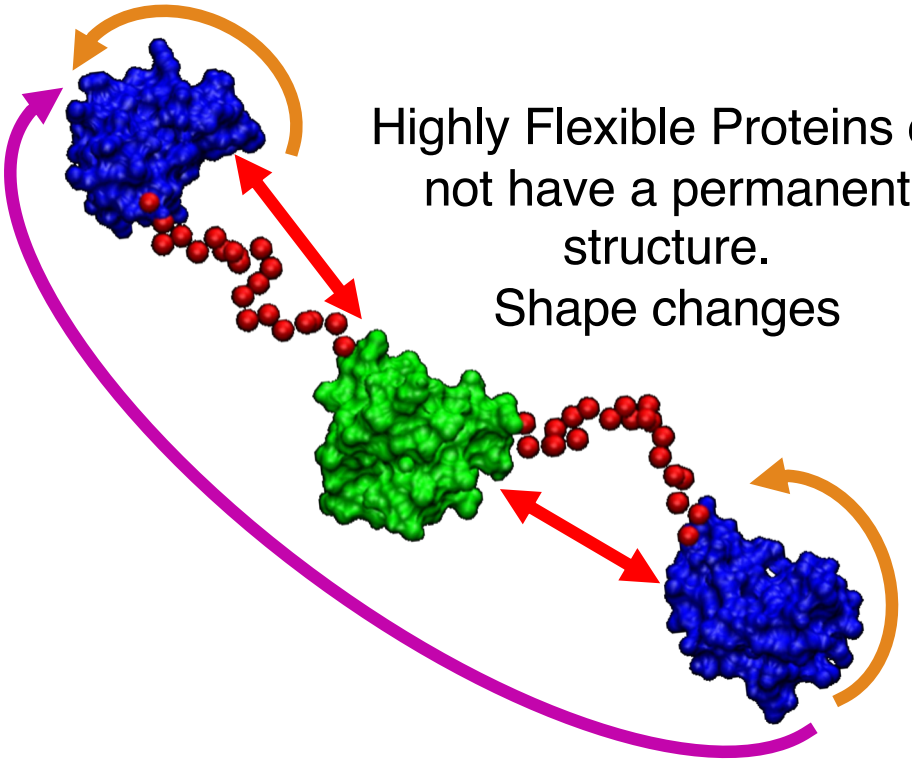


Although it is a relaxation phenomena, in ensembles properties are normally averaged

Protein Dynamics from Relaxation Rates

Hydrodynamic Properties
Rotational Diffusion Tensor (ns)

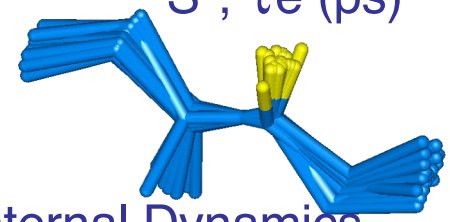




Highly Flexible Proteins do not have a permanent structure.
Shape changes

Different interdomain motions are probably active at the same time-scale

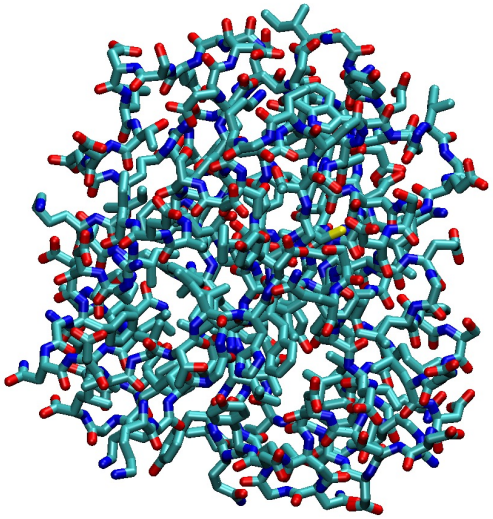
S^2, τe (ps)



Internal Dynamics

NMR		SAXS
Close Contacts: ^{1}H-^{1}H nOes LR relationship: RDCs, R_2/R_1	**Globular Proteins**	Overall Shape Validation
Interfaces: ^{1}H-^{1}H nOes, CS, PRE Orientation: RDCs, R_2/R_1	**Complexes and Rigid Multi-Domain Proteins**	Translational Information Overall Shape Validation
Conform. Sampling: CS, RDCs LR Contacts: PREs	**Unstructured Proteins**	Dimensions of the Ensemble
Time-Scale Information: Spin Relaxation	**Flexible Multi-Domain Proteins**	Volume Sampled by the Domains

Globular Protein



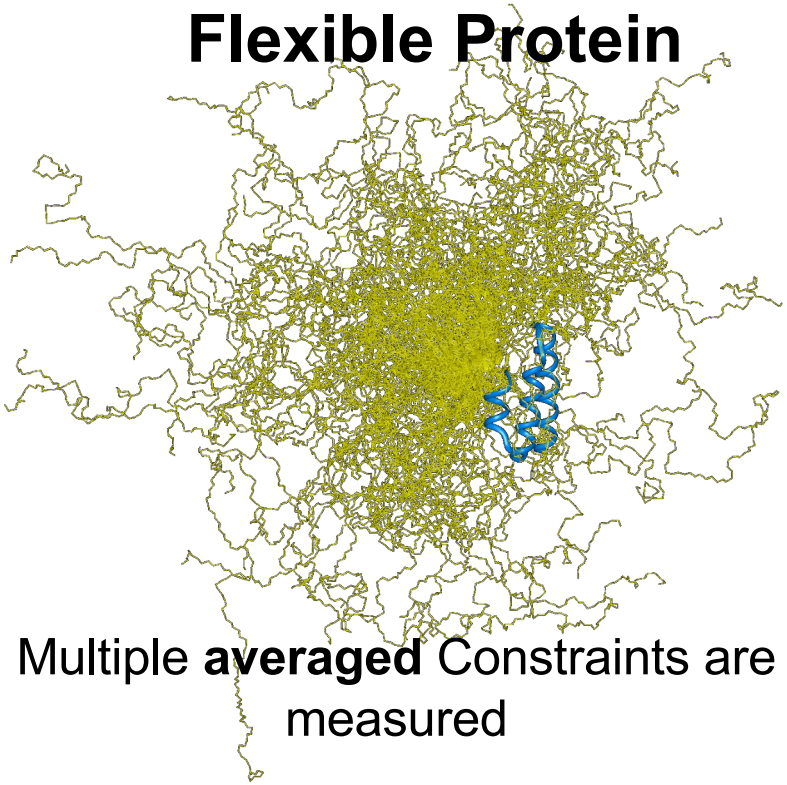
Multiple Constraints are measured (Short and Long Range)

Optimization of a single set of coordinates to simultaneously describe all data

Single Structure

Limited number of degrees of freedom (previous knowledge)

Flexible Protein



Multiple **averaged** Constraints are measured

Optimization of an **ensemble of coordinates** to simultaneously describe all data available

(non unique) Ensemble Model

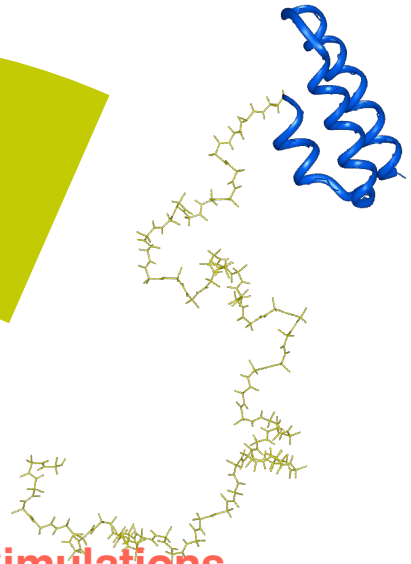
Ill-defined problem…

Data - Information - Model validation and cross-validation

Structural and Forward Models

SAXS:
CRYSOL, AXES, FOXS, AquaSAXS, pepsiSAXS, WAXSIS…

NMR:
CS: Sparta, Sparta+, ShiftX, CamShift…
RDCs: PALES, Flexible-Meccano
PREs: Flexible-Meccano
Hydrodynamics: HydroPro, SOMO



MD-Simulations
Ensemble
Flexible Meccano
MoMA
IDPConformerGenerator
CG-Simulations
Ranch…

Conformation-dependent Property

Structural and Forward Models

SAXS:

CRYSOL, AXES, FOXS, AquaSAXS, pepsiSAXS, WAXSIS…

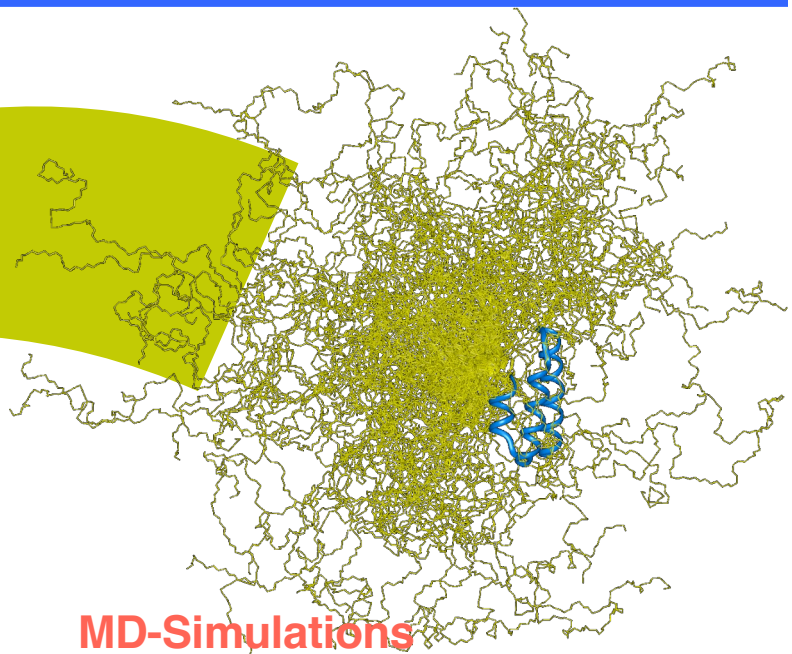
NMR:

CS: Sparta, Sparta+, ShiftX, CamShift

RDCs: PALES, Flexible-Meccano

PREs: Flexible-Meccano

Hydrodynamics: HydroPro, SOMO



MD-Simulations
Ensemble
Flexible Meccano
MoMA
IDPConformerGenerator
CG-Simulations
Ranch…

Warning!!!

Not all properties can be averaged

Not all properties require the same number of conformations

Ensemble-Averaged Property

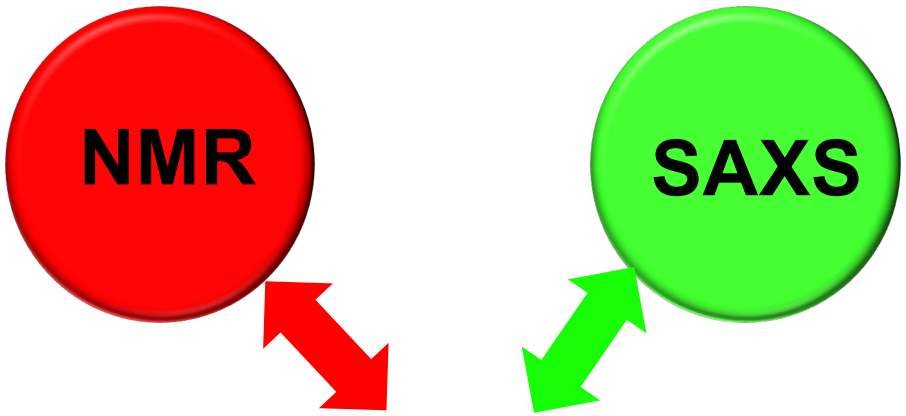
Combining NMR and SAXS

Capacity to address complex biological systems

Structural models with better resolution

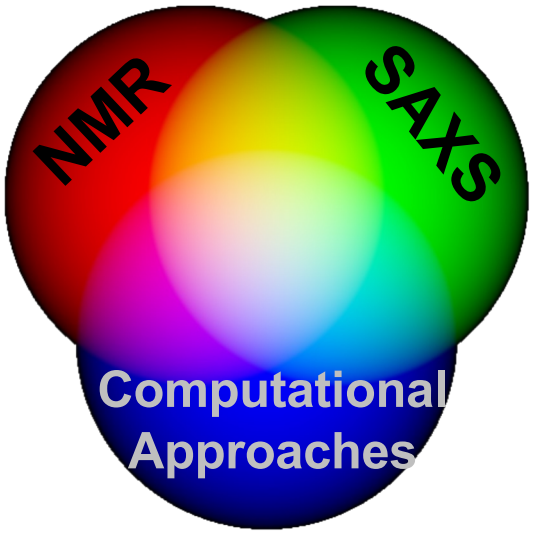
More complete models embedding structure and dynamics

Model Validation



Structural Model

Data-Driven Modelling



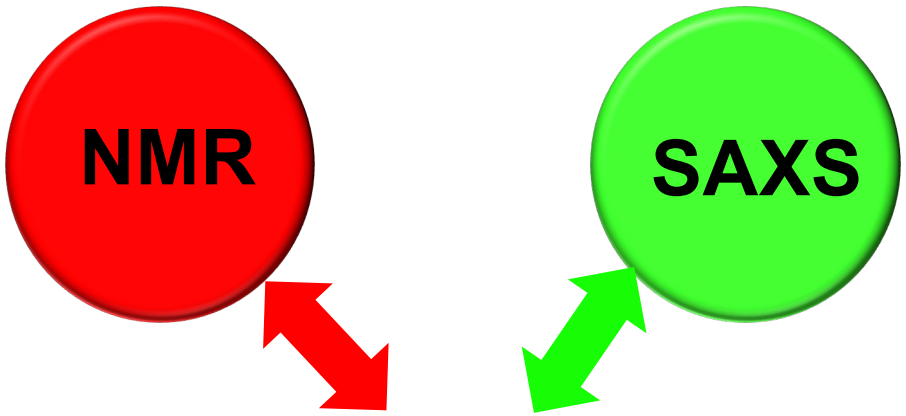
Combining NMR and SAXS

Capacity to address complex biological systems

Structural models with better resolution

More complete models embedding structure and dynamics

Model Validation



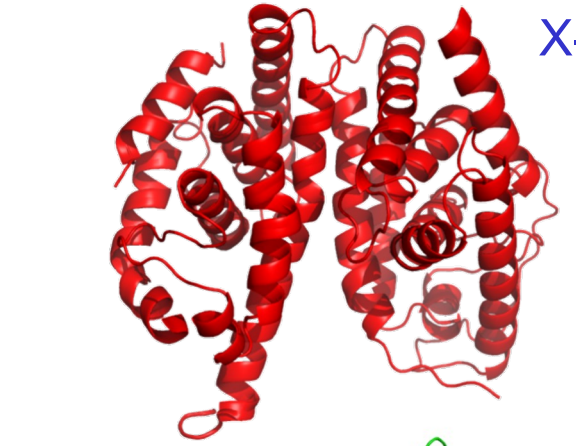
Structural Model

Data-Driven Modelling

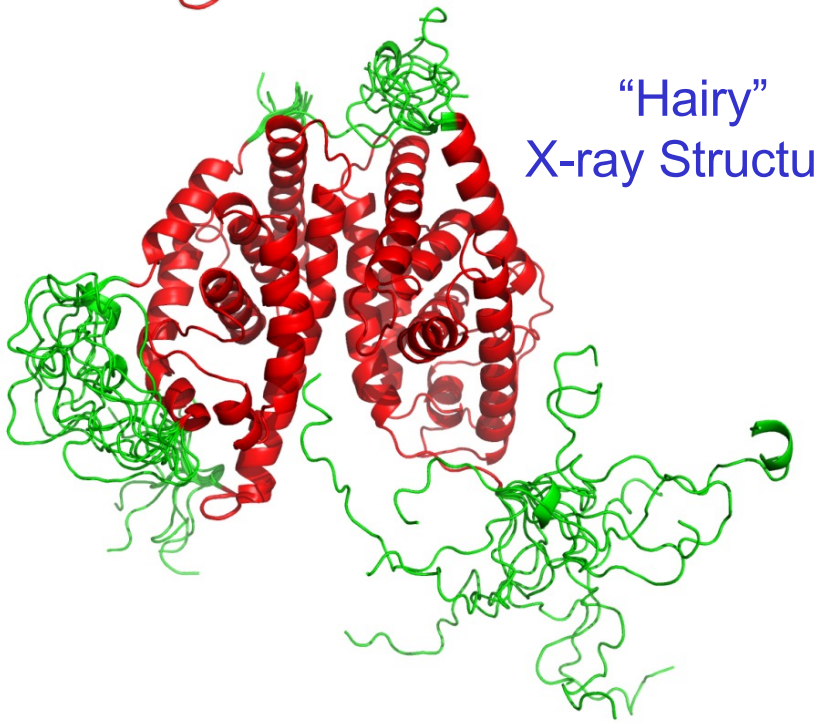
SAXS/NMR as Validation Tools for Ensembles

- SAXS/NMR are used in combination with molecular dynamics simulations or other computational approaches
- Snapshots are collected, their individual SAXS/NMR properties are computed with adapted **forward models**, averaged and compared with the experimental one… **Not fitted**
- Therefore is a *validation method* for a computational model that either works or does not work.
- SAS and NMR data can be used to to parametrize force-fields
- In SAS for relatively rigid systems, the effects of moderate dynamics can be ‘compensated’ with small structural perturbation or changes in the hydration

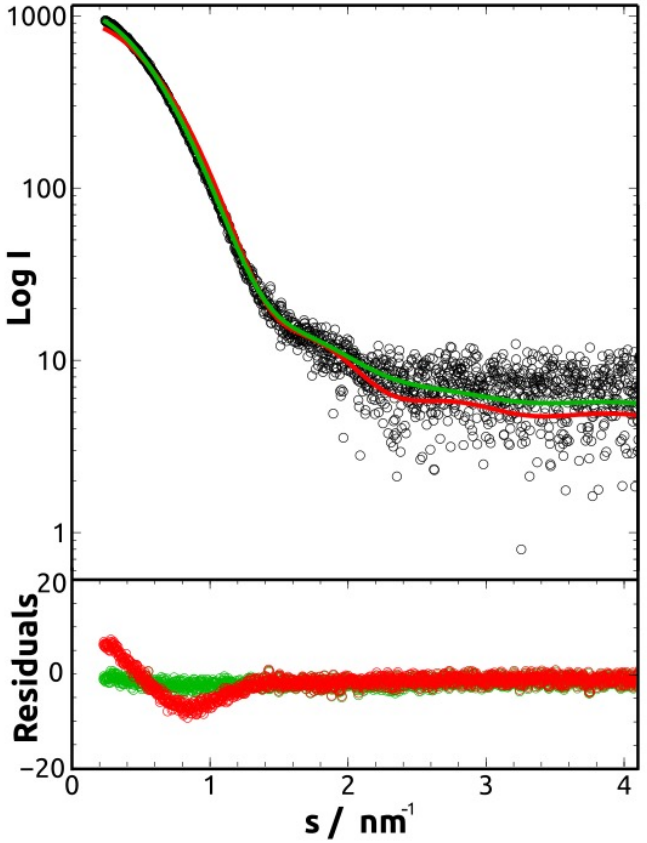
SAXS as a Validation Tool of Ensemble Models



X-ray Structure

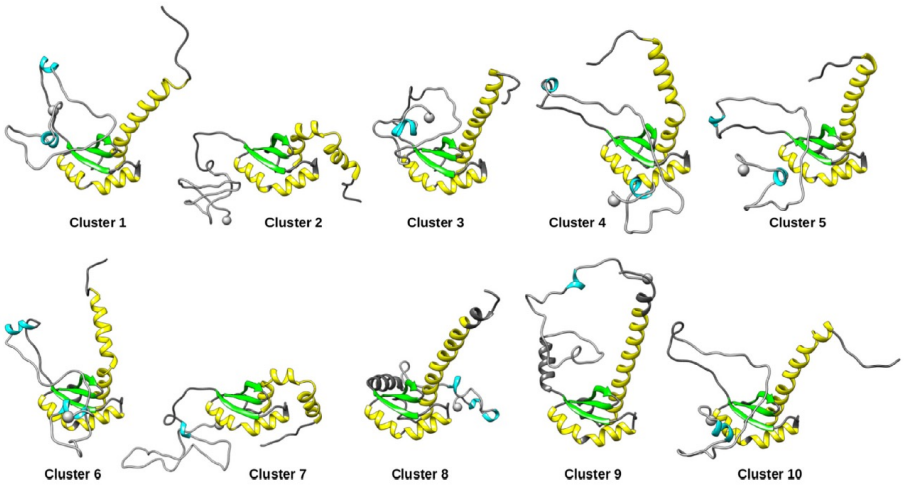


"Hairy"
X-ray Structure



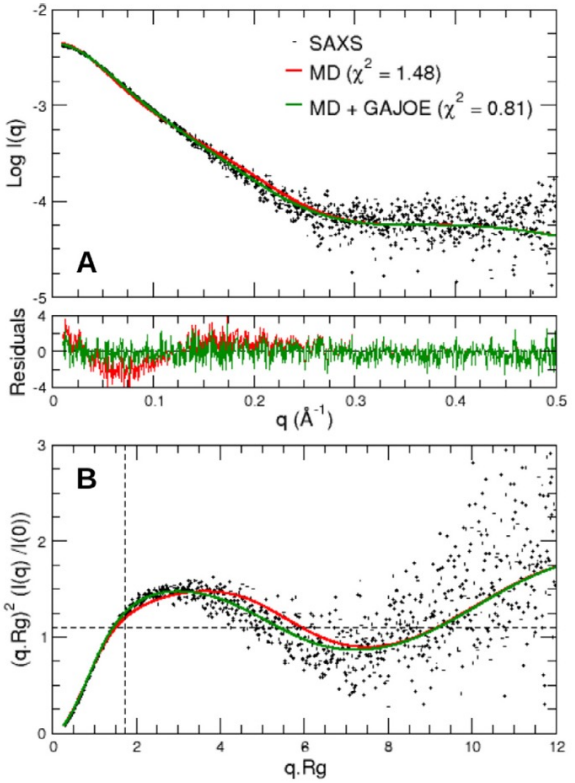
Cordeiro et al. Structure 2019,27, 1270.

SAXS as a Validation Tool for MD trajectories

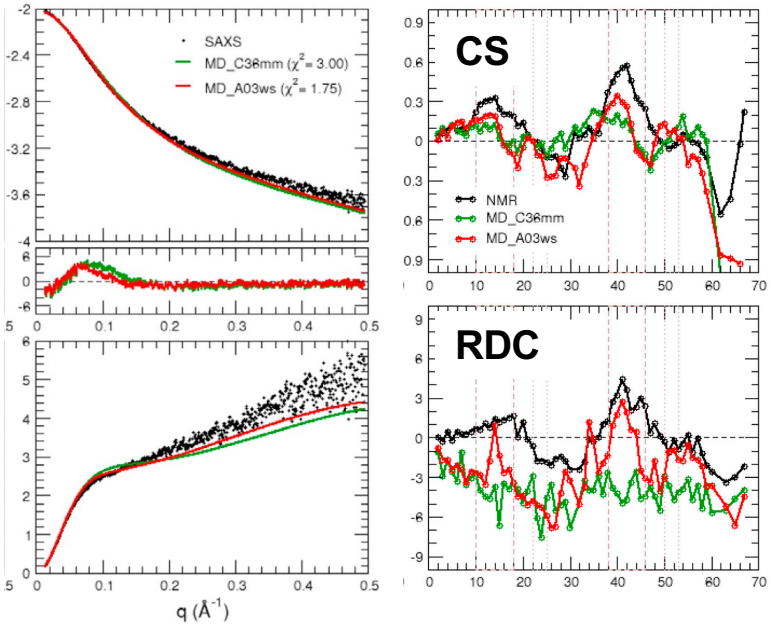


Chan-Yao-Chong et al. J. Struct. Biol. 2020, 21, 107573.

DciA



N-WASP (70-residue long IDP)

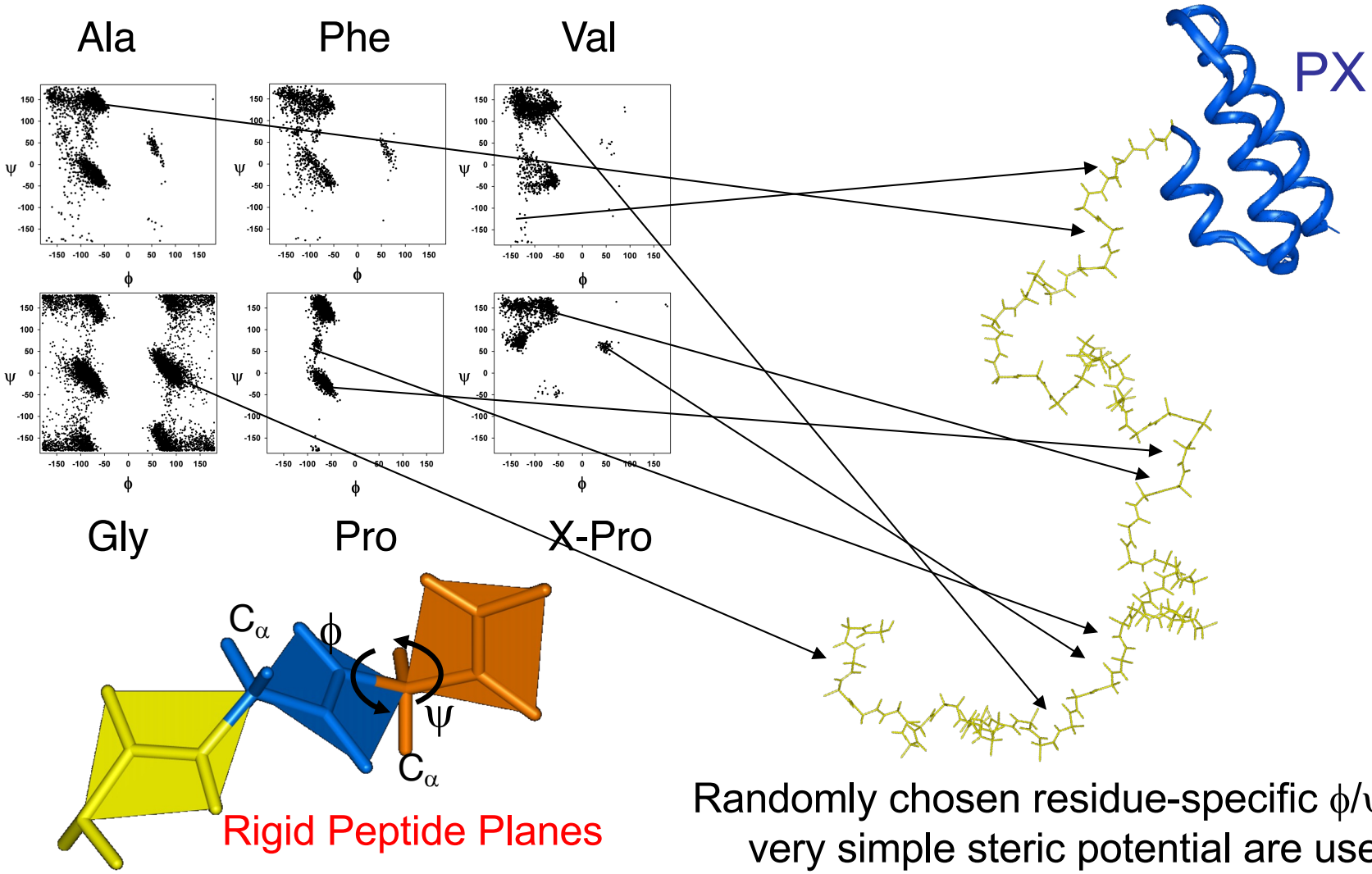


Ensembles are very sensitive to the Selection of Force-Field and water model. **SAXS/NMR to validate (or improve) physical models**

Care must be take to conformational sampling problems

Chan-Yao-Chong et al. BJ 2019, 116, 1216.

Random Chain Generators

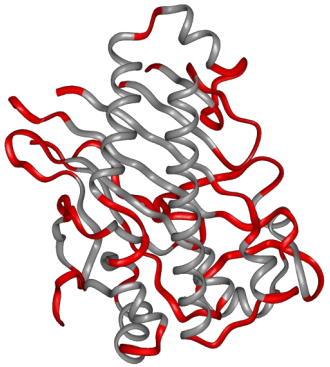


Randomly chosen residue-specific ϕ/ψ and a very simple steric potential are used to define a single conformation

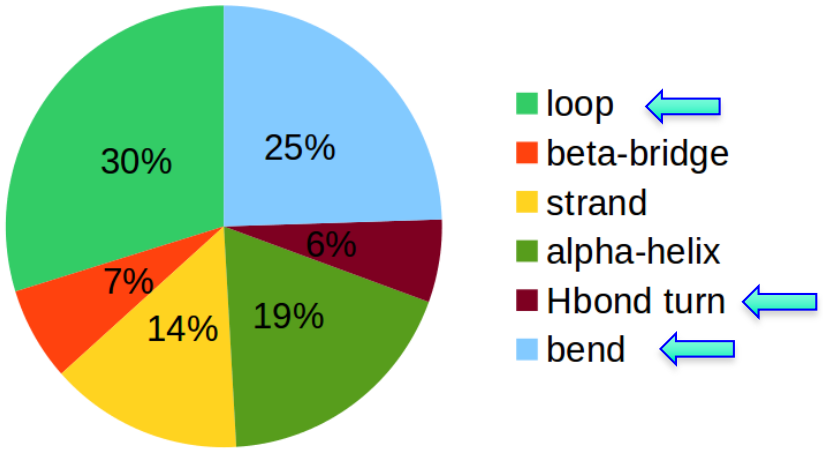
FM

Bernadó et al. *PNAS* 2005, 102, 17002
Ozenne et al. *Bioinformatics* 2012, 28, 1463

Realistic Models of IDPs – Tri-peptide Coil Model



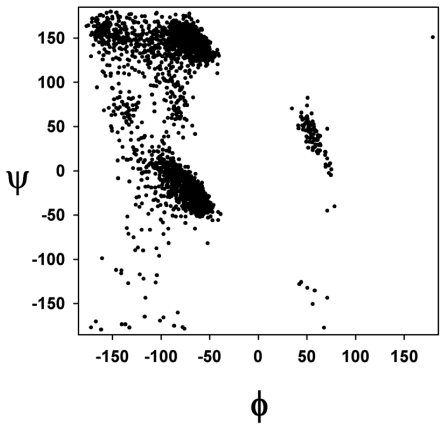
81.749 Proteins from SCOPe < 95% identity

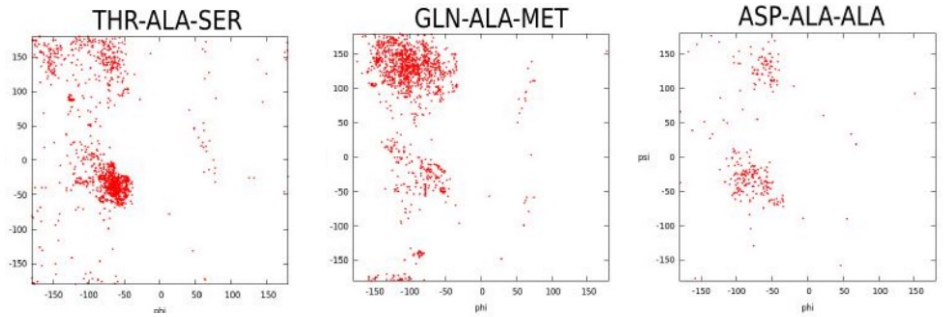


COIL Database (LTS)

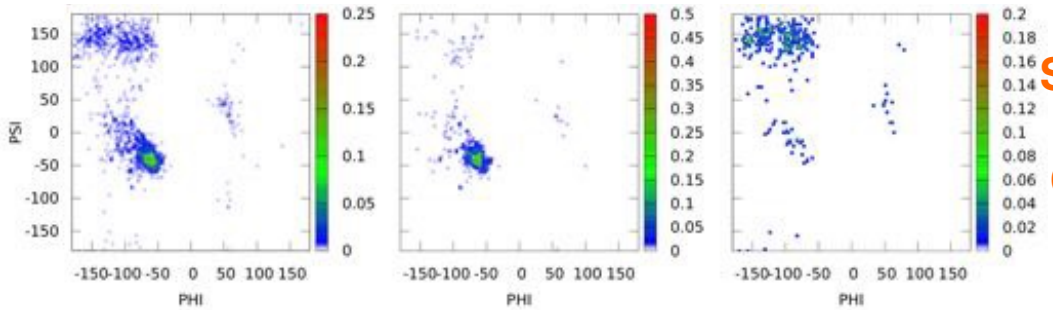
Tri-Peptides = 3.645.381

Alanine





SEQUENCE- Dependent Conformation



SER-ARG-ALA SER(α)-ARG-ALA(α) SER(β)-ARG-ALA(β)

STRUCTURE- Dependent Conformation

Estaña et al. *Structure*, 2019, 27, 381

Application to NMR Residual Dipolar Couplings

Single-Residue Sampling (SRS)

M V K P G T F D P E M K...

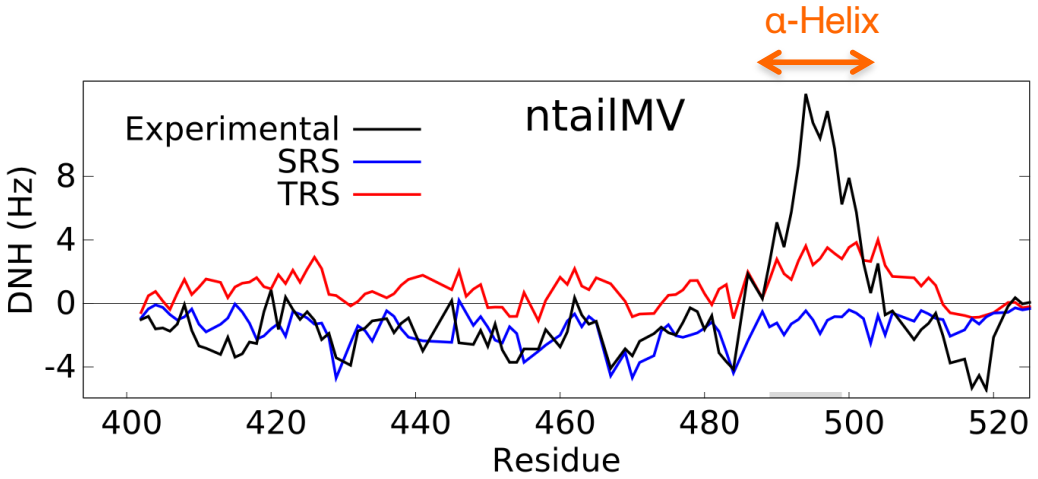
Tripeptide-Residue Sampling (TRS)

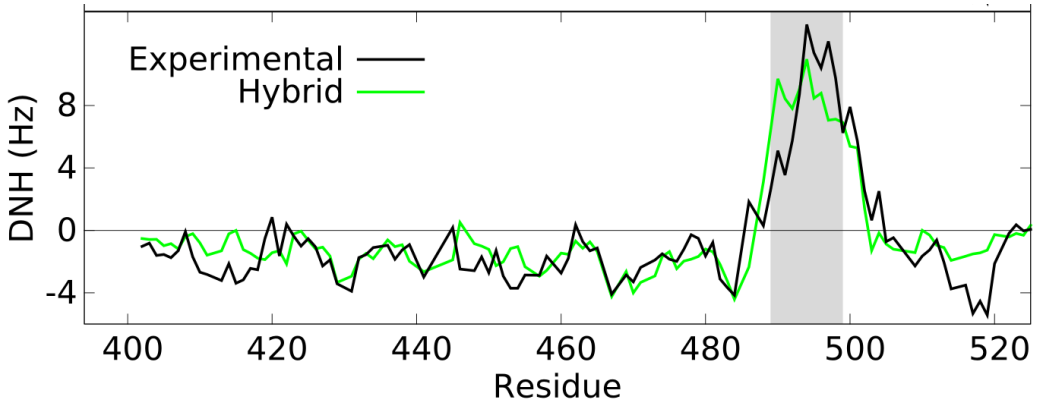
M V K P G T F D P E M K...

M V K P G T F D P E M K...

M V K P G T F D P E M K...

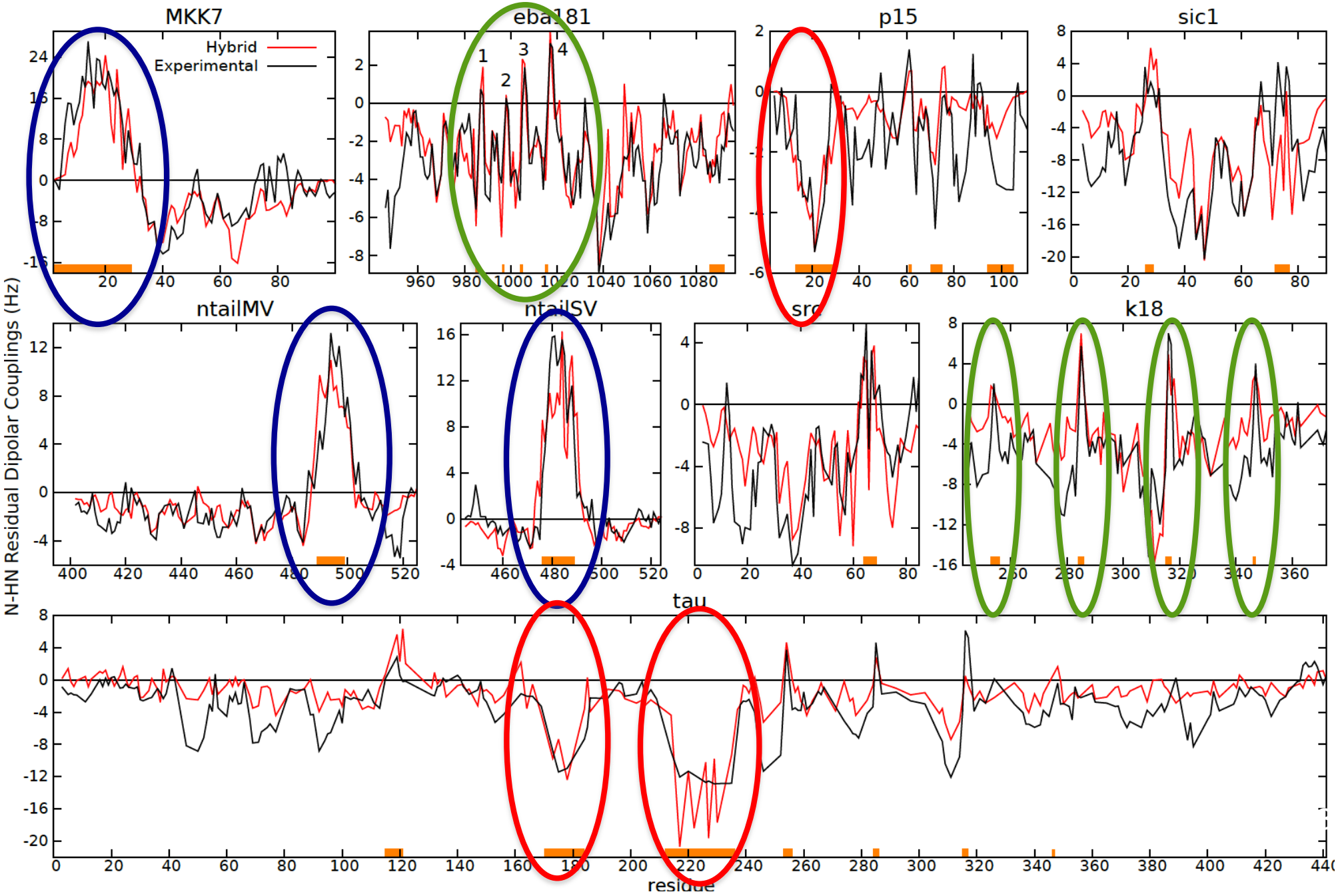
M V K P G T F D P E M K...



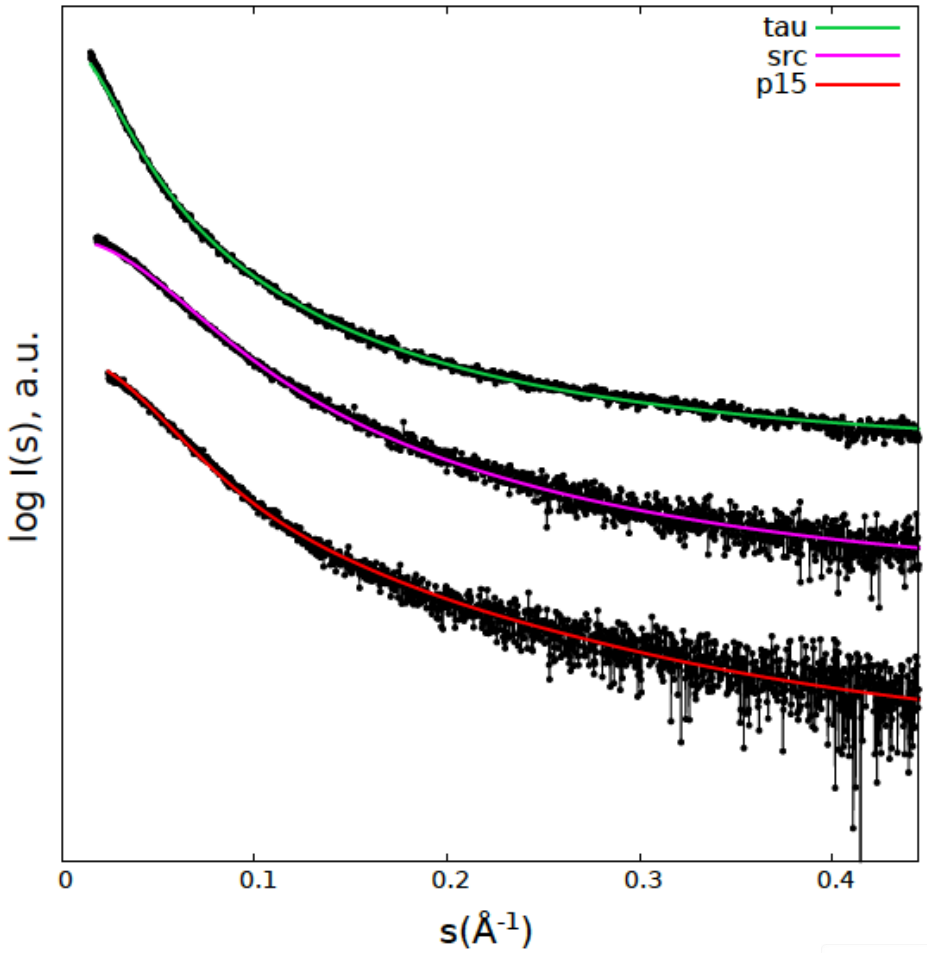


The appropriate combination of the SRS and TRS strategies provides an excellent description of the RDCs measured for ntail MV

Application to NMR Residual Dipolar Couplings



Application to SAXS curves

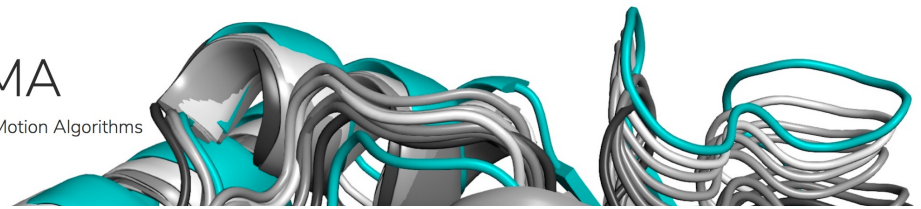


The ensembles generated are also in agreement with the SAXS curves

The building strategy based on the tri-peptide database produces realistic ensembles of IDPs that are compatible with NMR and SAXS data

However, long-range contacts are not accounted for…

MoMA
Molecular Motion Algorithms



Combining NMR and SAXS

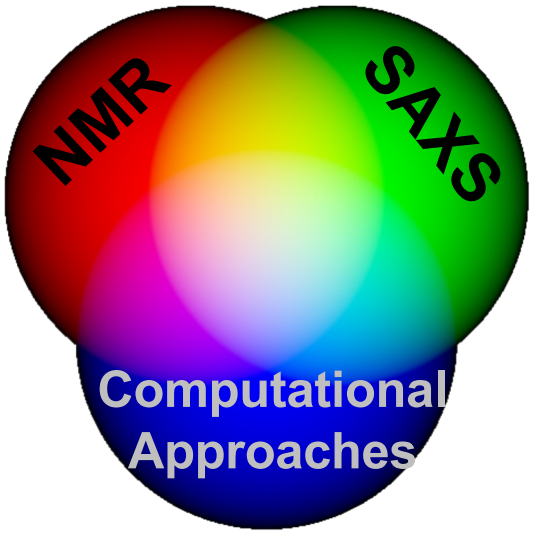
Capacity to address complex biological systems

Structural models with better resolution

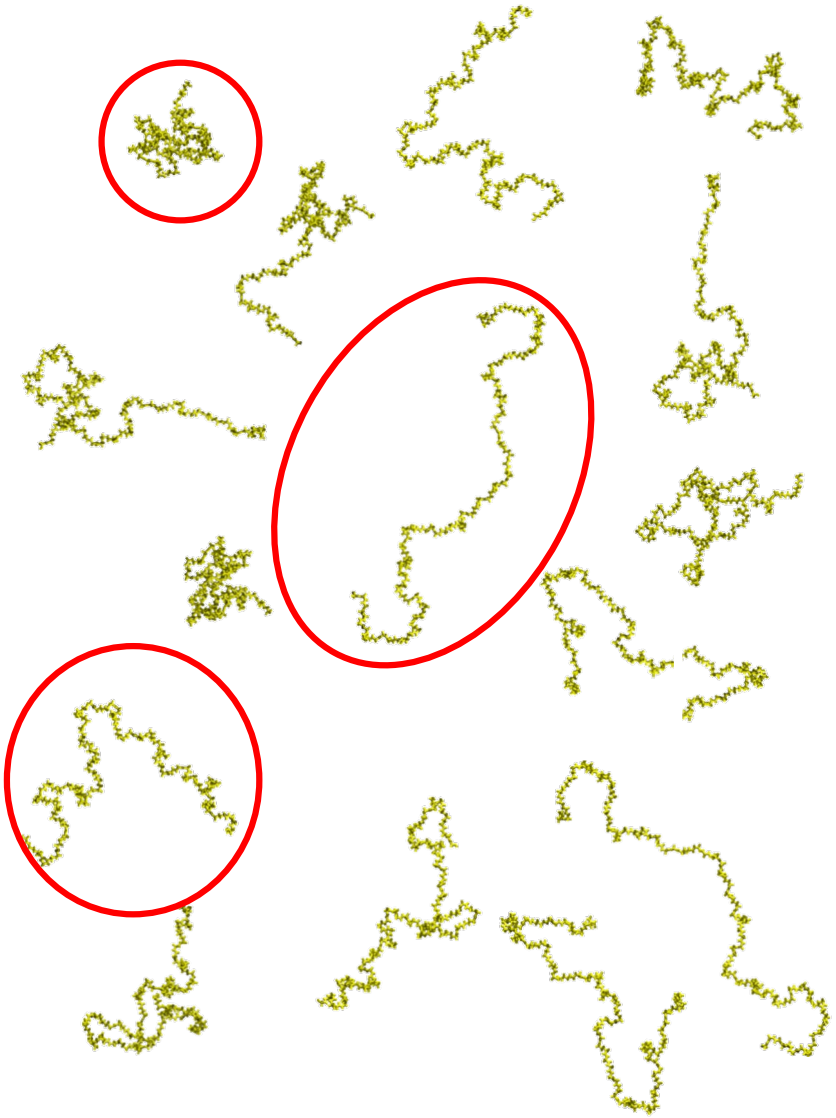
More complete models embedding structure and dynamics

Model Validation

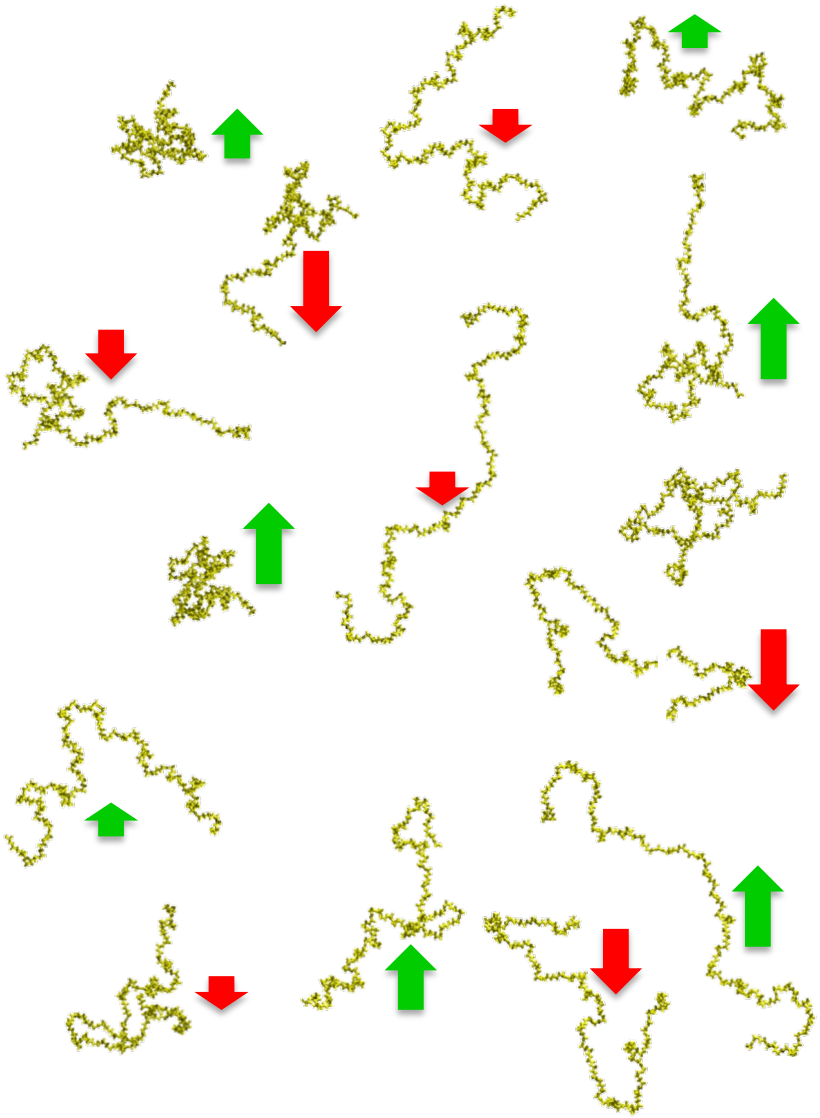
Data-Driven Modelling



Ensemble Methods: Two Philosophies



Maximum Parsimony



Maximum Entropy

The Ensemble Optimization Method (EOM) MP

Bernadó, Mylonas, Petoukhov, Blackledge, and Svergun. Structural characterization of flexible proteins using small-angle X-ray scattering. *J Am Chem Soc* 2007, **129**:5656-64.

Minimal Ensemble Search (MES) MP

Pelikan, Hura, and Hammel. Structure and flexibility within proteins as identified through small angle X-ray scattering. *Gen Physiol Biophys* 2009, **28**:174–189.

Ensemble Refinement of SAXS (EROS) ME

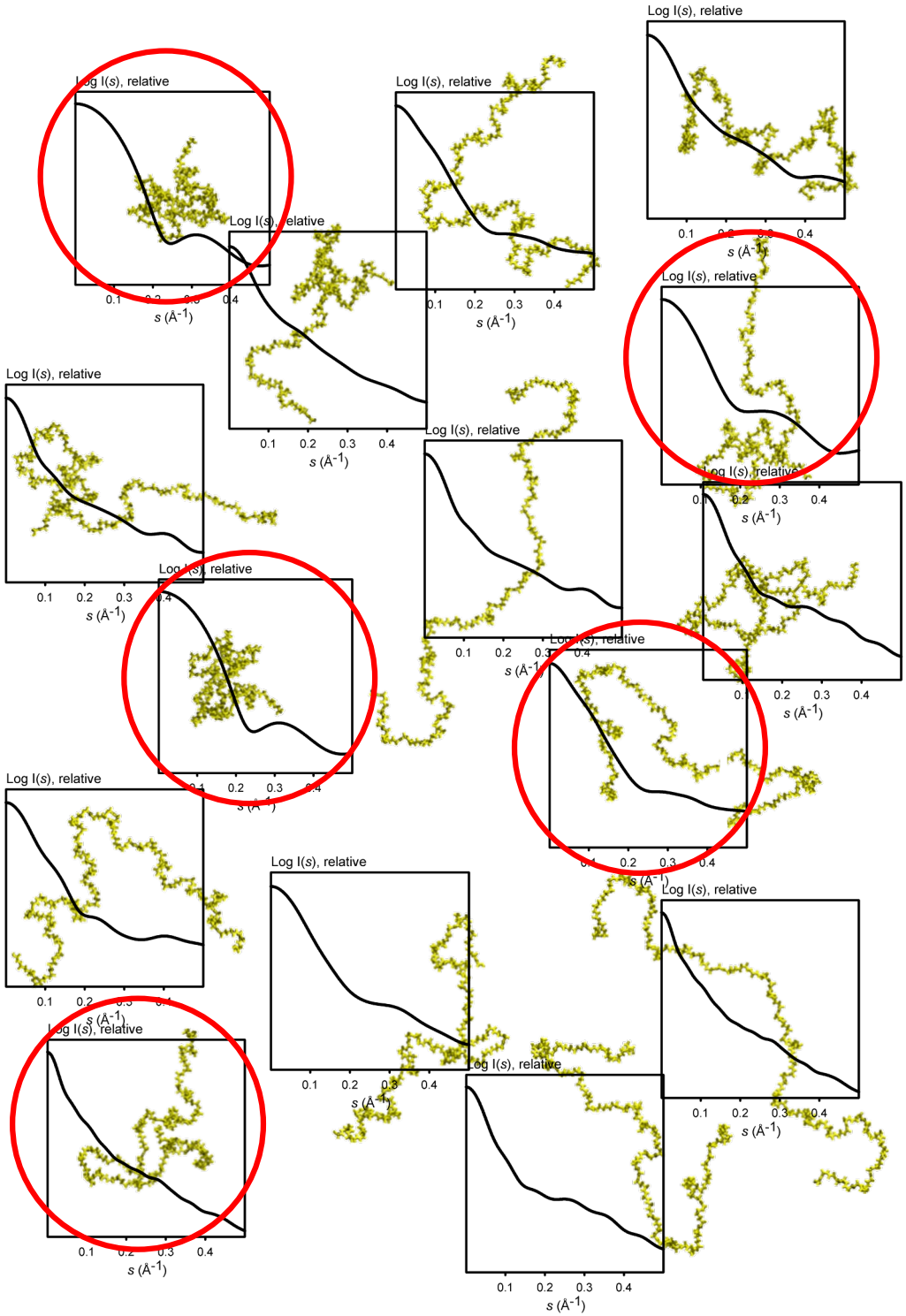
Rozycki, B., Kim, Y.C., Hummer, G. SAXS ensemble refinement of ESCRT-III CHMP3 conformational transitions. Structure 2011, **19**:109-116.

Bayesian Maximum Entropy (BME) ME

Bottaro S, Bengtsen T, Lindorff-Larsen K. Integrating Molecular Simulation and Experimental Data: A Bayesian/Maximum Entropy Reweighting Approach. *Methods Mol. Biol.* 2020, 2112:219-240.
Pesce F, Lindorff-Larsen K. Refining conformational ensembles of flexible proteins against small-angle x-ray scattering data. *Biophys J.* 2021, 120(22):5124-5135.

Basis-Set Supported SAXS (BSS-SAXS) Bayesian

Yang, Blachowicz, Makowski and Roux. Multidomain assembled states of Hck tyrosine kinase. *PNAS* 2010, **36**:15757-15762.



1. Calculation of a conformational ensemble
2. Computation of the theoretical SAXS profiles
3. Selection of a subensemble-Reweighting
4. Structural interpretation of the subensemble

The Ensemble Optimization Method (EOM) MP

Bernadó, Mylonas, Petoukhov, Blackledge, and Svergun. Structural characterization of flexible proteins using small-angle X-ray scattering. *J Am Chem Soc* 2007, **129**:5656-64.

Minimal Ensemble Search (MES) MP

Pelikan, Hura, and Hammel. Structure and flexibility within proteins as identified through small angle X-ray scattering. *Gen Physiol Biophys* 2009, **28**:174–189.

Ensemble Refinement of SAXS (EROS) ME

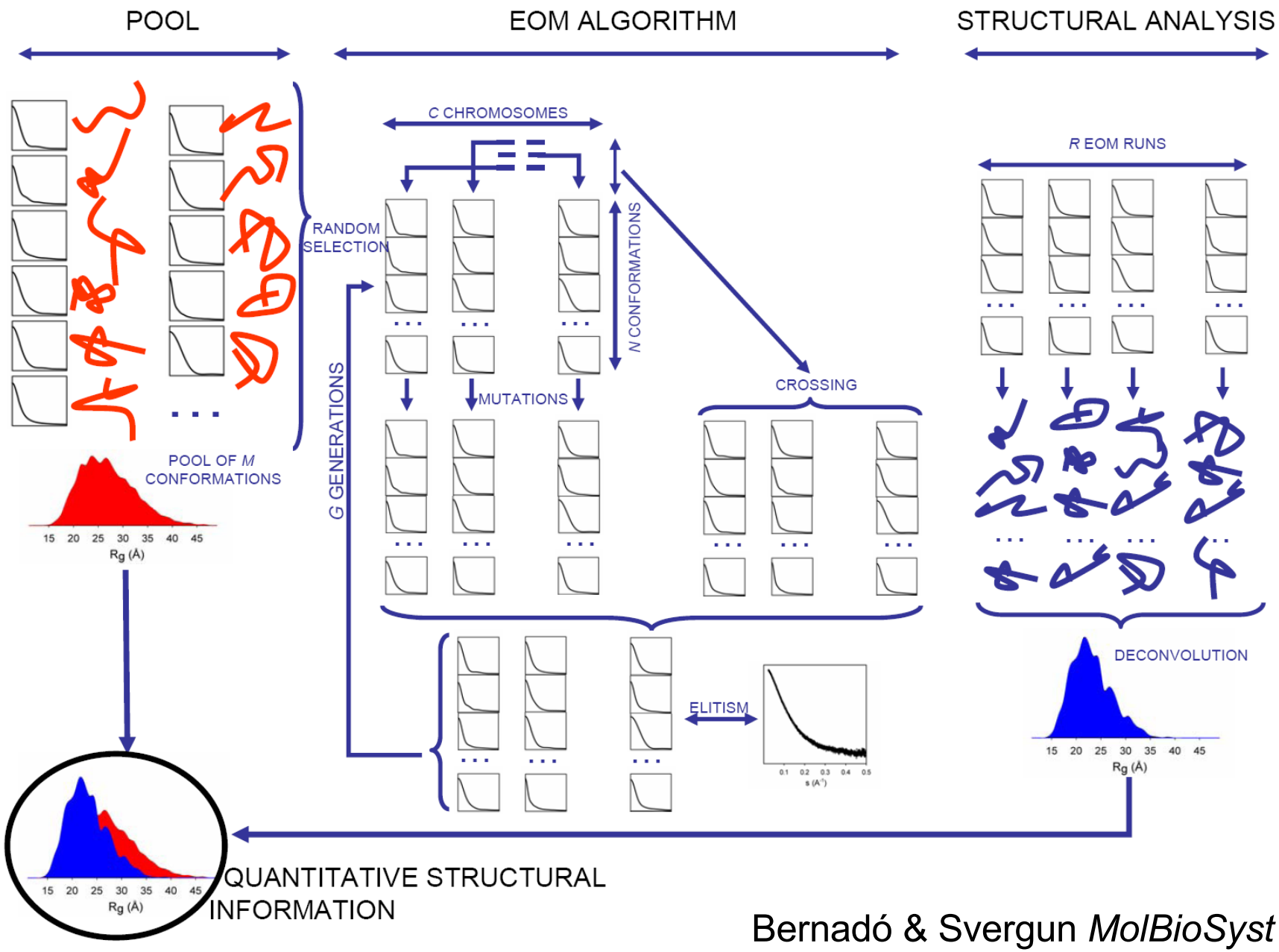
Rozycki, B., Kim, Y.C., Hummer, G. SAXS ensemble refinement of ESCRT-III CHMP3 conformational transitions. Structure 2011, **19**:109-116.

Bayesian Maximum Entropy (BME) ME

Bottaro S, Bengtsen T, Lindorff-Larsen K. Integrating Molecular Simulation and Experimental Data: A Bayesian/Maximum Entropy Reweighting Approach . Methods Mol. Biol. 2020, 2112:219-240.
Pesce F, Lindorff-Larsen K. Refining conformational ensembles of flexible proteins against small-angle x-ray scattering data. Biophys J. 2021, 120(22):5124-5135.

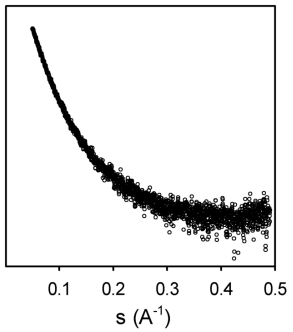
Basis-Set Supported SAXS (BSS-SAXS) Bayesian

Yang, Blachowicz, Makowski and Roux. Multidomain assembled states of Hck tyrosine kinase. *PNAS* 2010, **36**:15757-15762.



Bernadó & Svergun *MolBioSyst* 2011

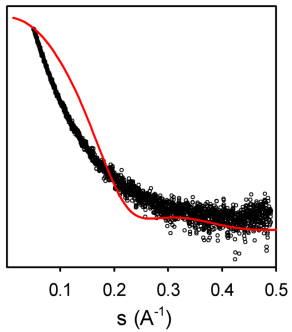
Denatured Lysozyme as Example

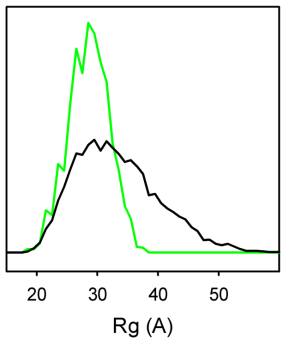


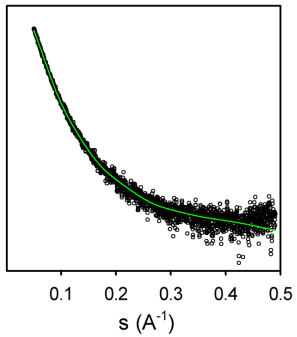
8M Urea, 10 mM DTT

R_g = 26.3 Å

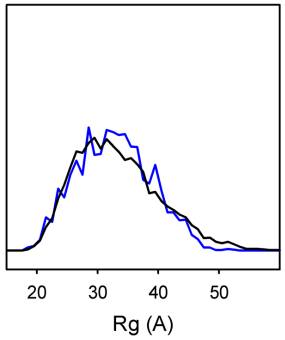
R_g (native) = 15.1 Å

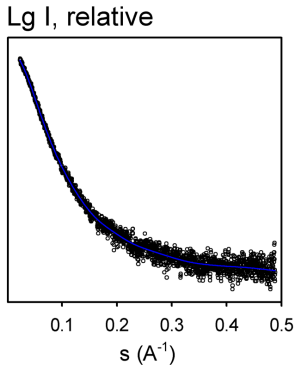






8M Urea, 10 mM DTT



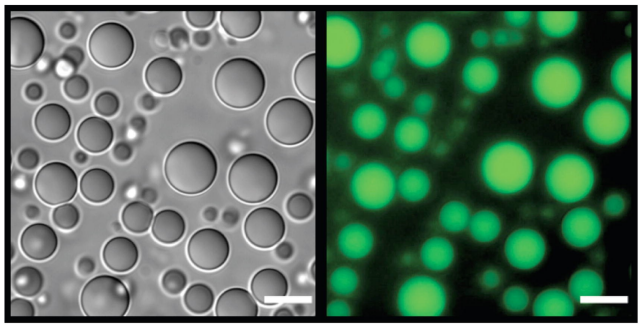


8M Urea, 100 mM DTT

R_g = 30.0 Å

Bernadó et al. JACS 2007, 129, 5656

Chain Compaction as a Proxy for LLPS

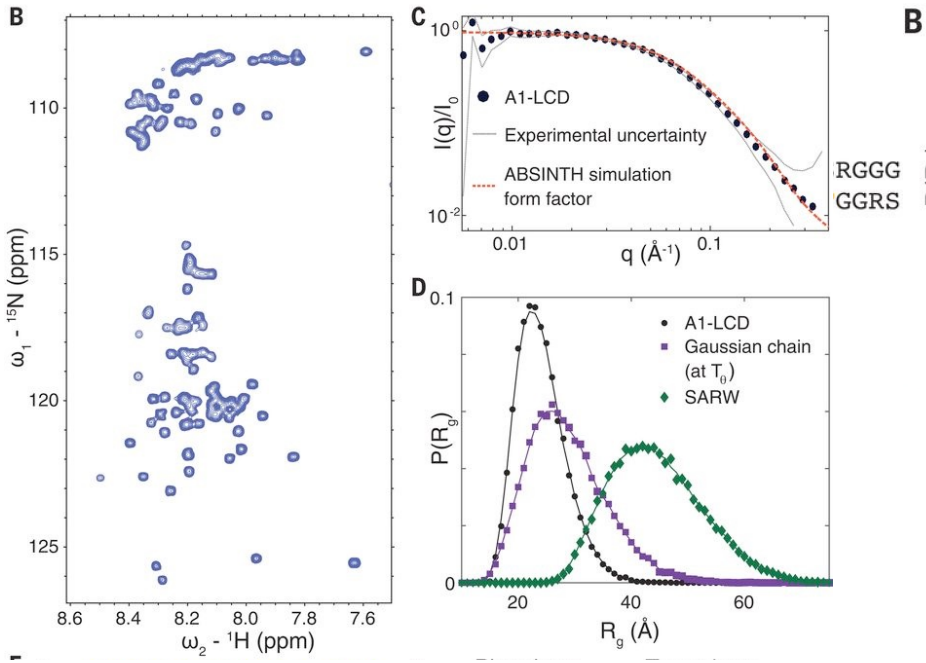


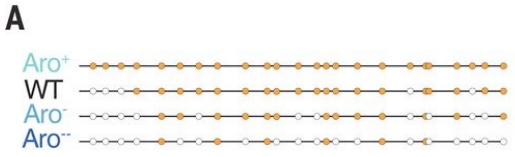
Liquid-Liquid Phase Separation is an emerging phenomenon linked to multiple biological functions

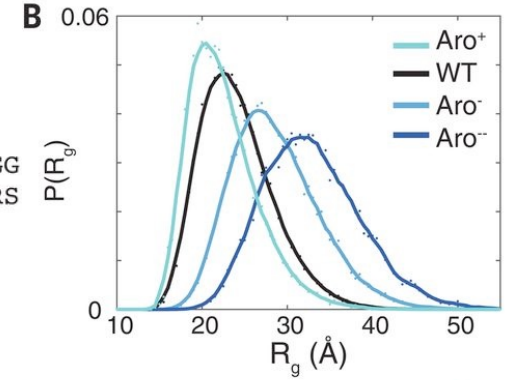
LCD of hnRNPA1

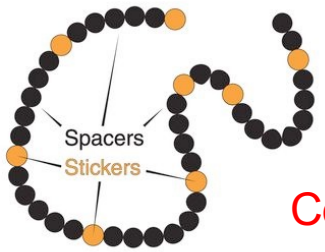
MASASSSQRG RSGSGNFGGG RGGGFGGNDN FGRGGNFSGR GGFGGSRGGG
GYGGSGDGYN GFGNDGSNFG GGGSYNDFGN YNNQSSNFGP MKGGNFGGRS
SGPYGGGGQY FAKPRNQGGY GGSSSSSSYG SGRRF

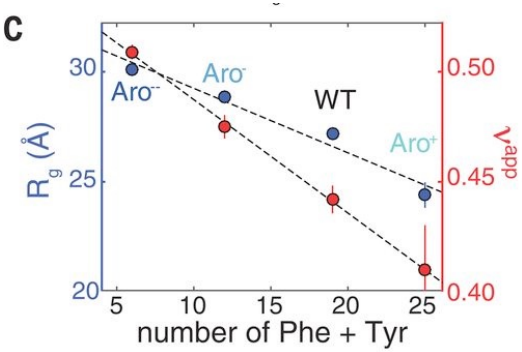
This protein is disordered and more compact than a random coil

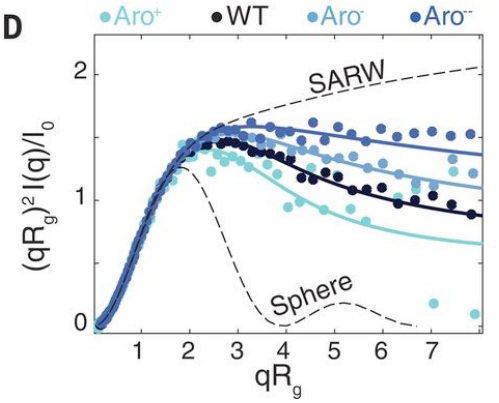








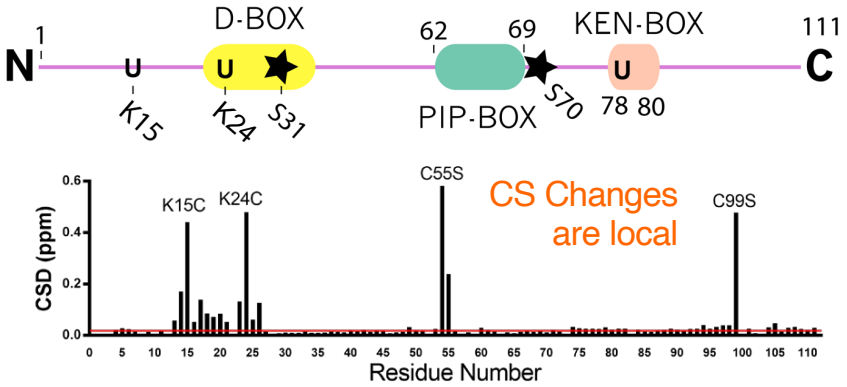


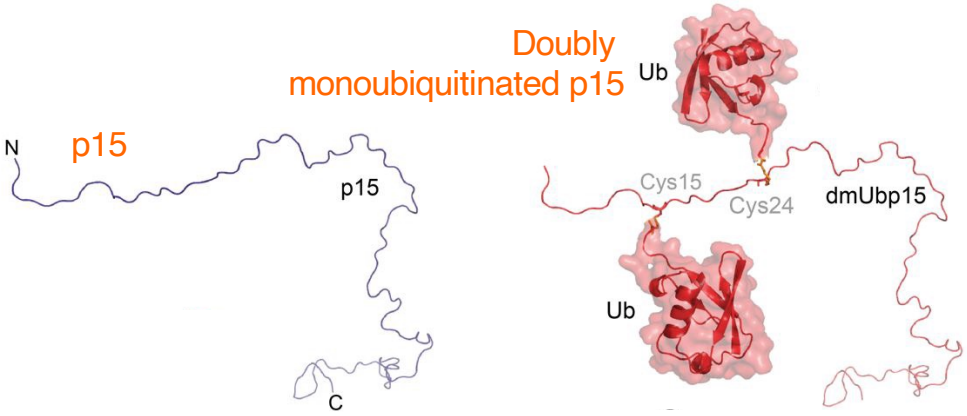


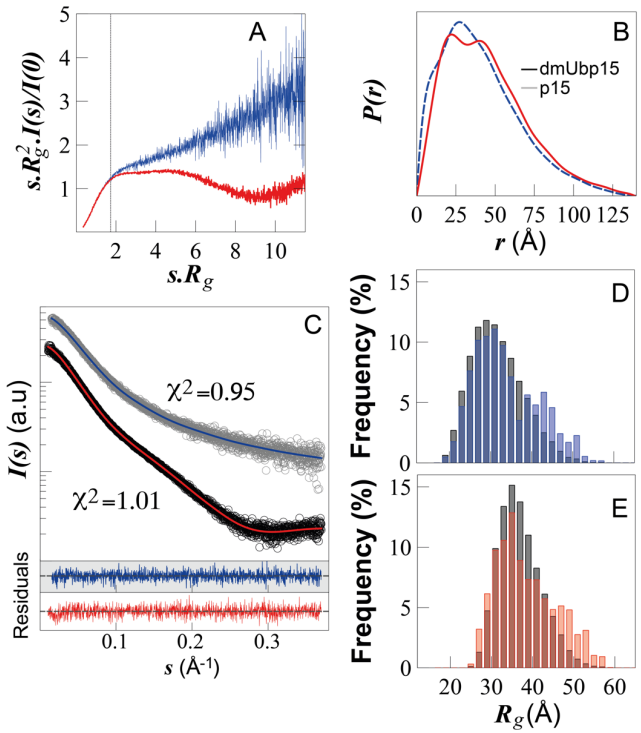
Compacness is governed by the patterning of aromatic residues

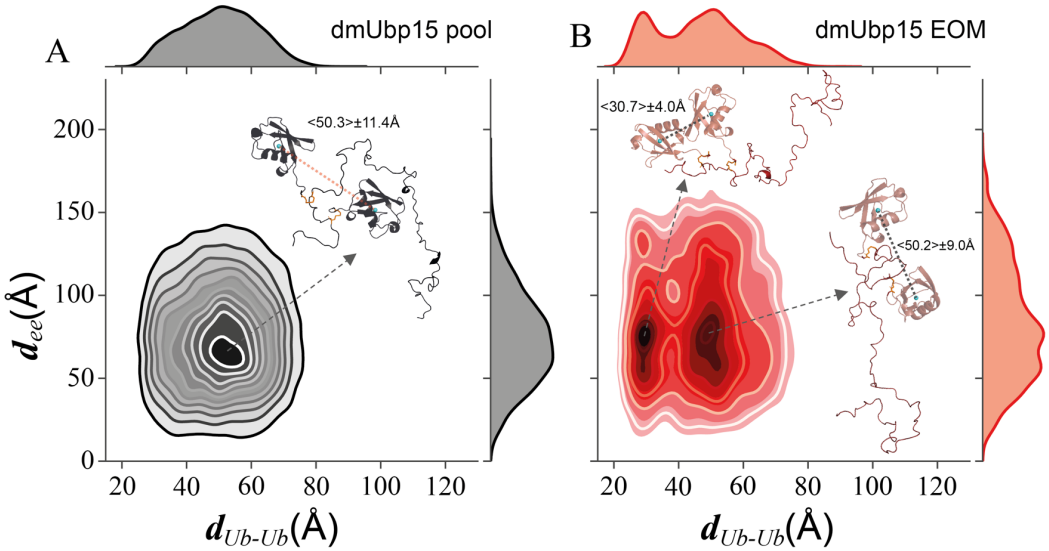
Martin et al. *Science* 2020, 367, 694-699

Doubly monoubiquitinated p15









The EOM analysis indicates that both ubiquitins form transient dimers when grafted to p15
The exact dimerization surface is undefined

Coll. Francisco Blanco (CICBiogune-Bilbao) and Tiago Cordeiro (ITQB-Lisbon)

González-Magaña et al. ACS Chem Biol, 2019

Structural Meaning of the Selected Conformations

Disordered proteins sample an astronomical number of conformations…
But SAXS data can be fitted with a small ensemble (20-50 conformations).
SAXS is a low resolution technique!!!!

You can get more or less the same results with smaller subensembles…
These ensembles are just low-resolution representations of reality

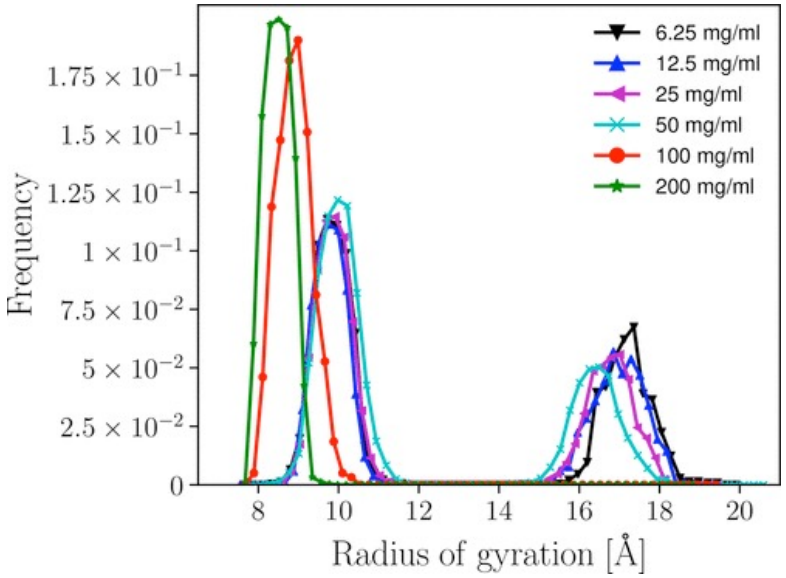
Structures collected are simply a TOOL to describe the size and shape distributions

It is tempting to look at the structures at atomic/residue level… Don't do this because (Remember that) SAXS is a low resolution technique and the information content is limited

If certain structure is collected at each run… It does not necessarily mean that it is prevalent in solution

Results are biased by the original structural model used

EOM… Not Always Easy to Interpret



Histatin (24 AA-long peptide)

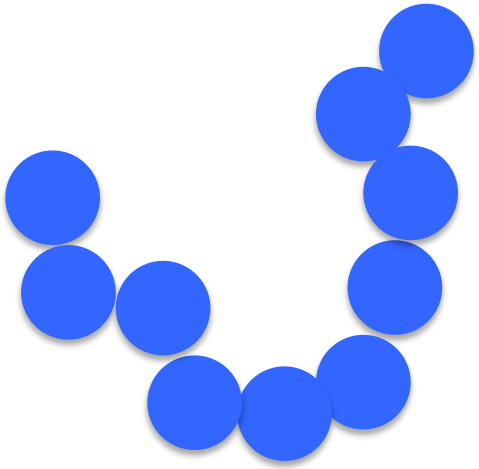
The unphysical bimodal R_g distributions suggest that EOM is not useful in this case

Fagerberg E. et al. *J Chem Theory Comput.* 2019, 15:6968-6983.

Histatin is a peptide

Use of MP approach (minimal ensemble size)

Use of a Dummy Residue (DR) Approach



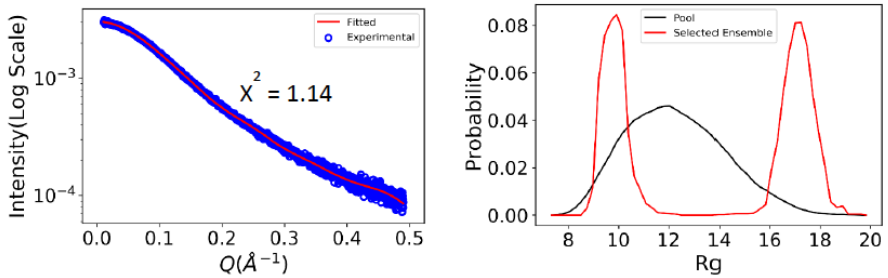
All amino acids are considered equivalent in terms of scattering

EOM… Not Always Easy to Interpret

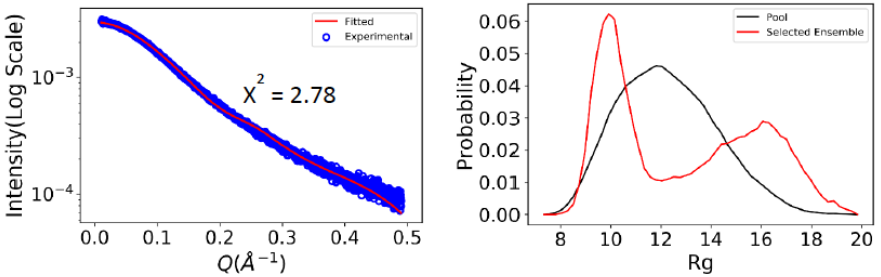
Coll. C. Jeffries, M. Petoukhov and D.Svergun (EMBL-HH)

RanCh (Dummy Residues)

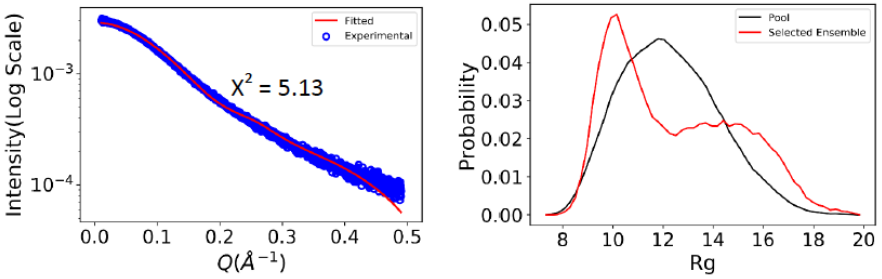
Default



20 Structures

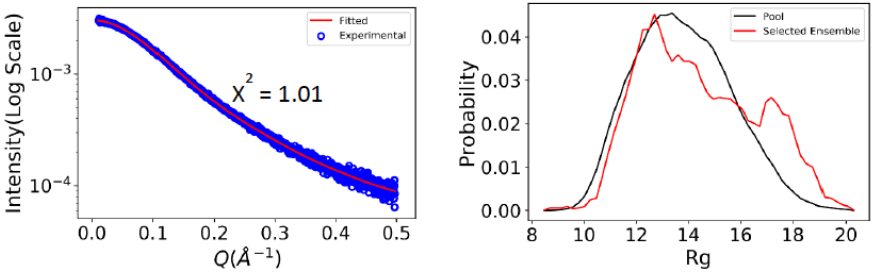


50 Structures

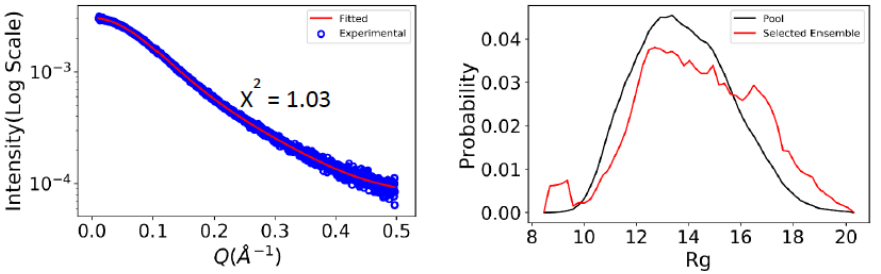


Flexible-Meccano (Atomistic)

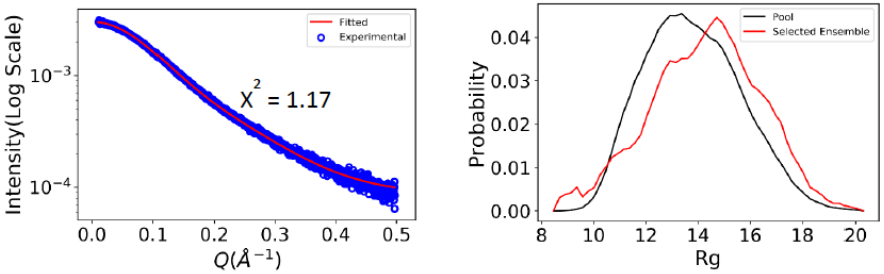
Default



20 Structures



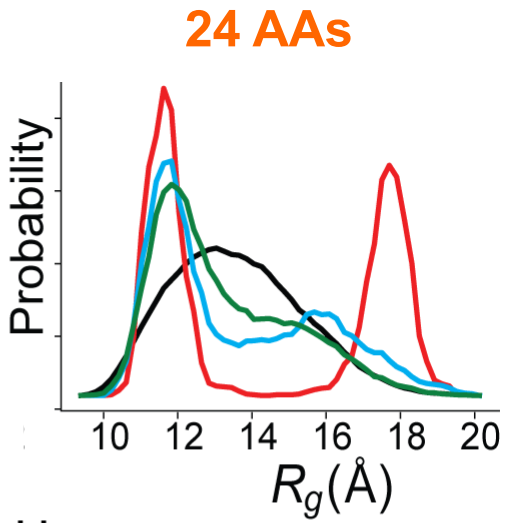
50 Structures



Atomistic Description is not bimodal… even for the MP scenario

Sagar A et al. *J Chem Theory Comput.* 2021, 17(4):2014-2021.

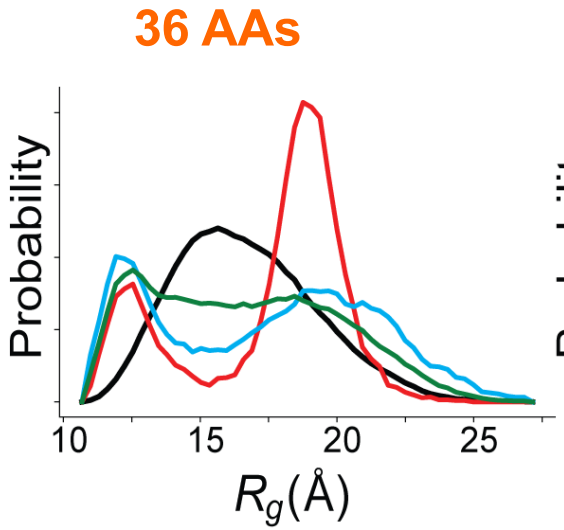
Protein Length is important (synthetic data)

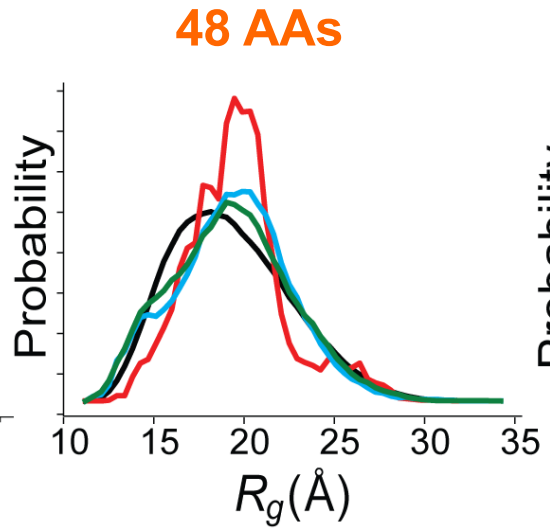


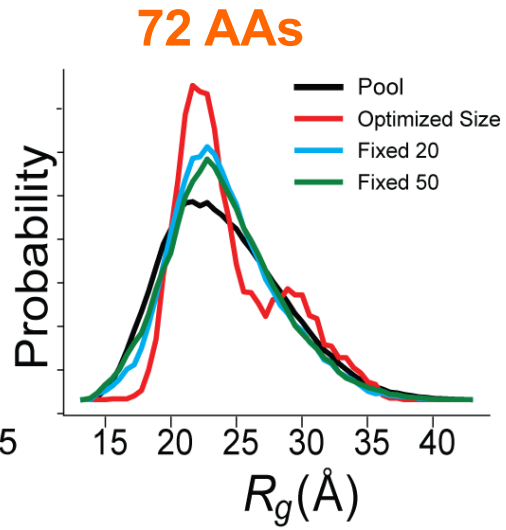
Pool
MP
Fixed 20
Fixed 50

When using DR fitting, MP approach gives bimodal distributions

For larger peptides fixed-size ensembles are unimodal

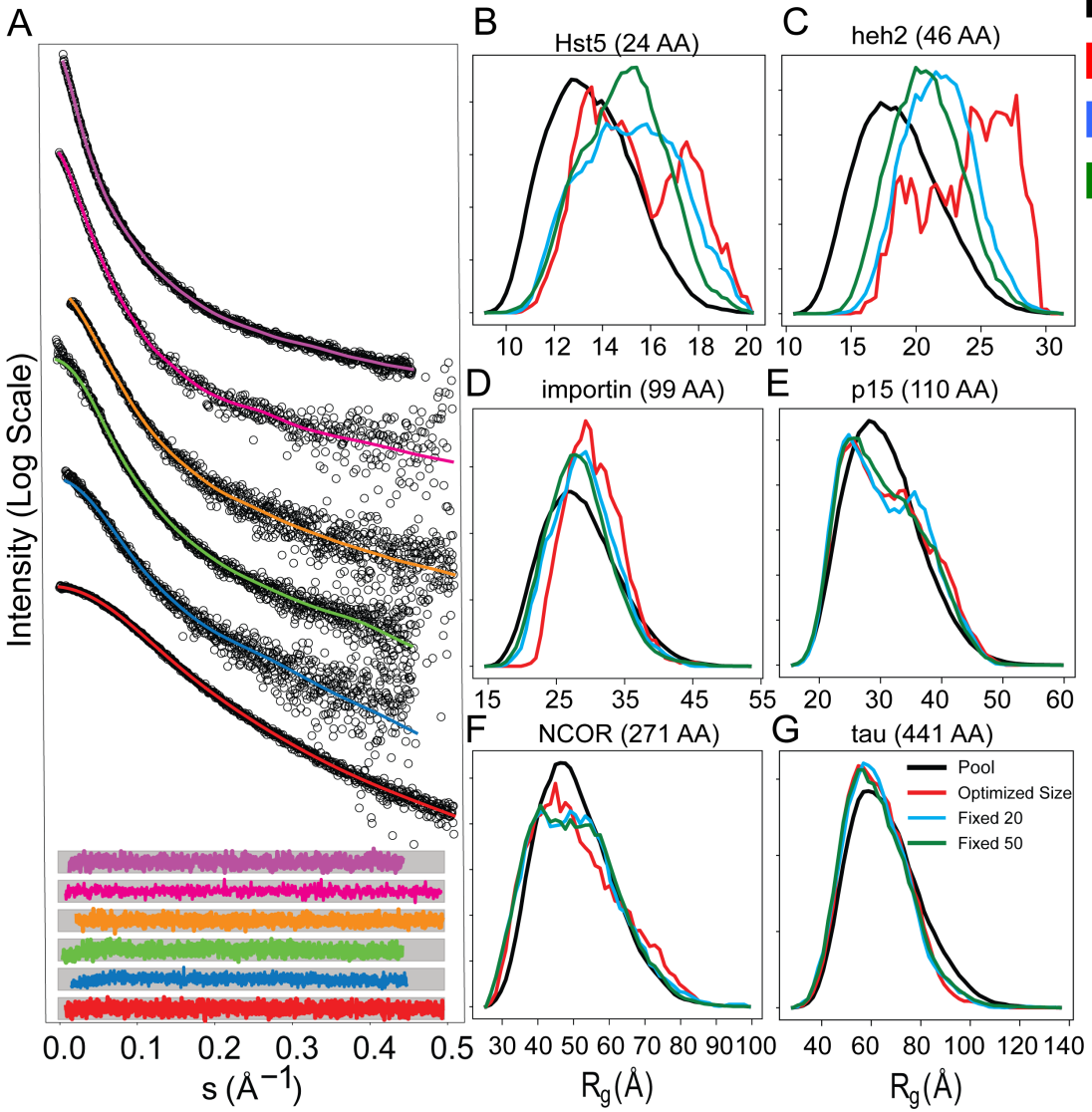




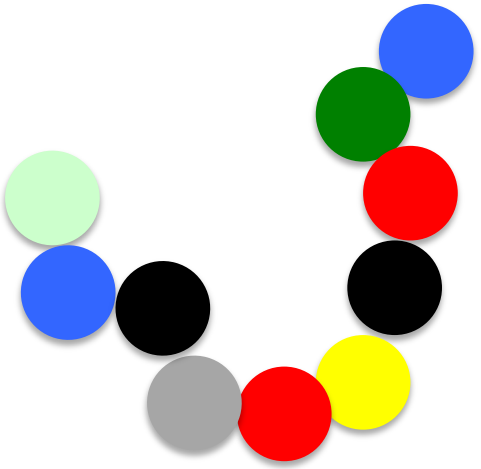


Sagar A et al. *J Chem Theory Comput.* 2021, 17(4):2014-2021.

Use of Amino-Acid specific Form Factors



Pool
MP
Fixed 20
Fixed 50



Use atomistic or Residue-specific form factors (RanCh)

Do not use the minimum ensemble size unless you have good reasons

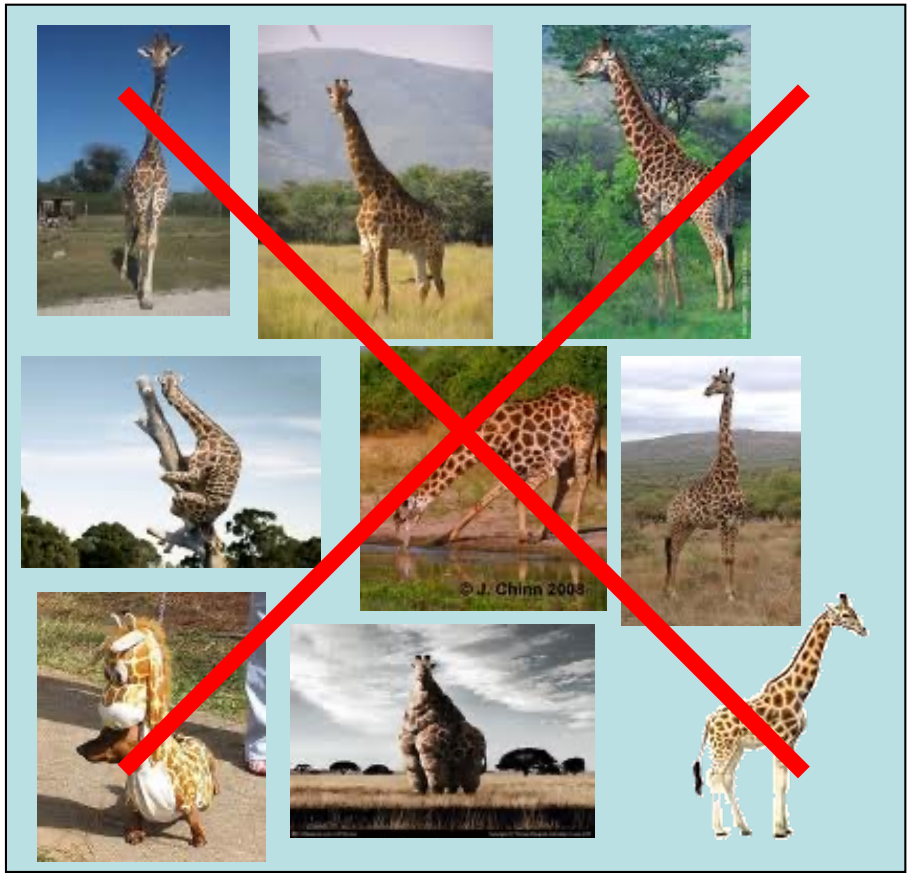
Sagar A et al. *J Chem Theory Comput.* 2021, 17(4):2014-2021.

A Frequent Wrong Intuition about EOM

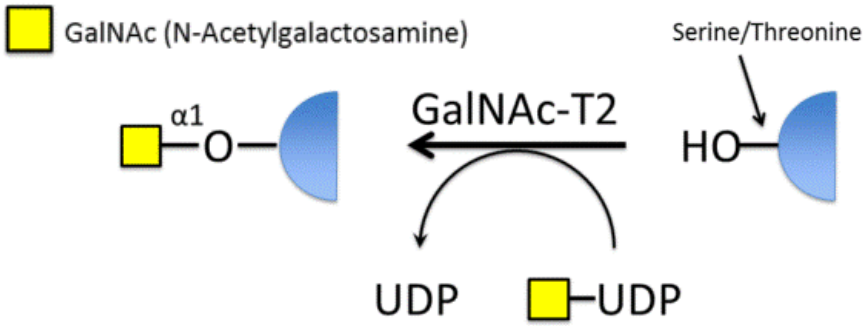
"With EOM you can fit an elephant if you want…"

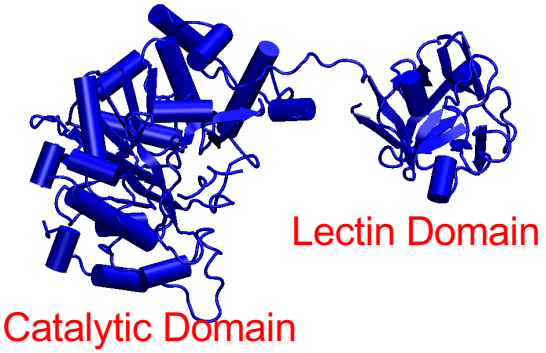
Maria Garcia-Parajo

THIS IS NOT TRUE

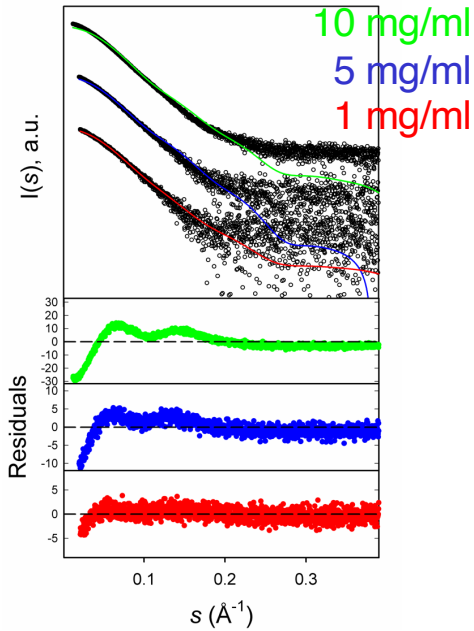


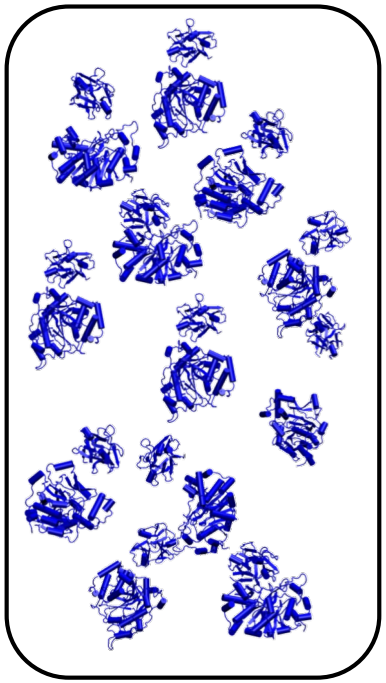
GalNAc-T2





EOM (single Chain)

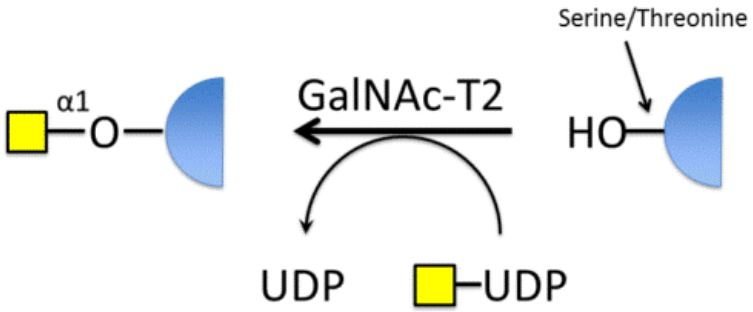


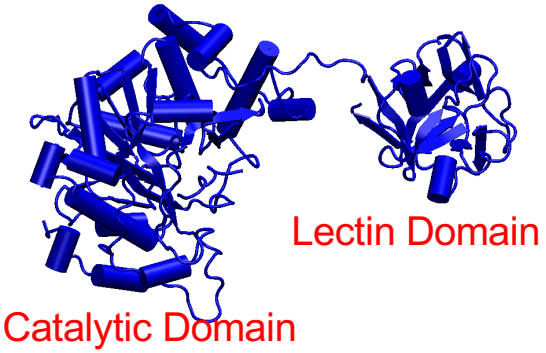


Col. Ramón Hurtado (Zaragoza-Spain)

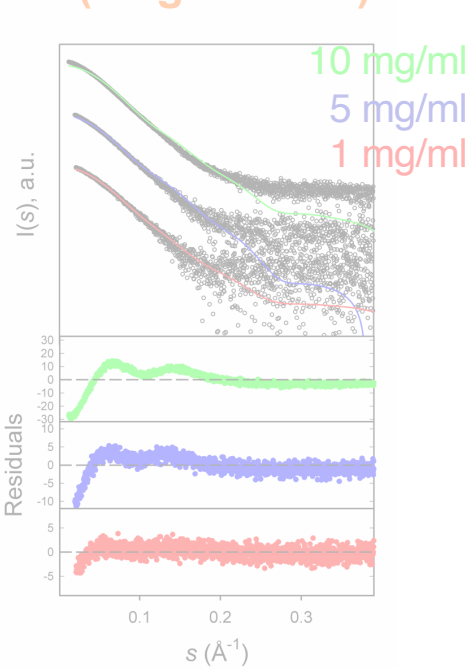
Lira-Navarrete et al. Nat. Comm 2015

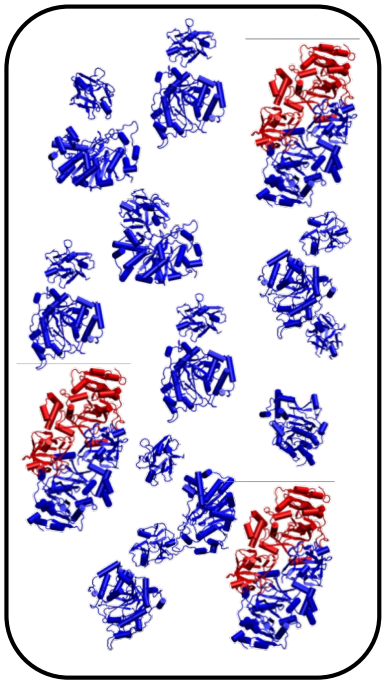
GalNAc-T2



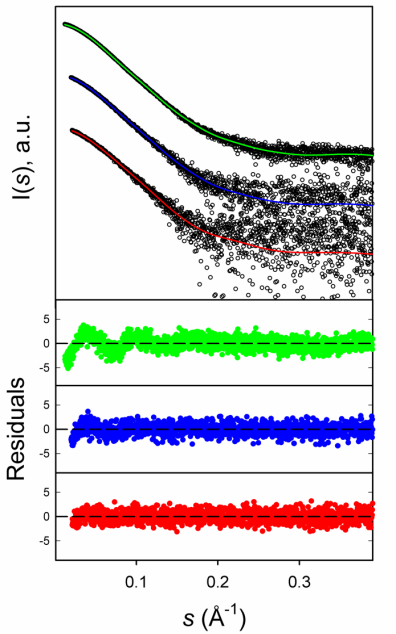


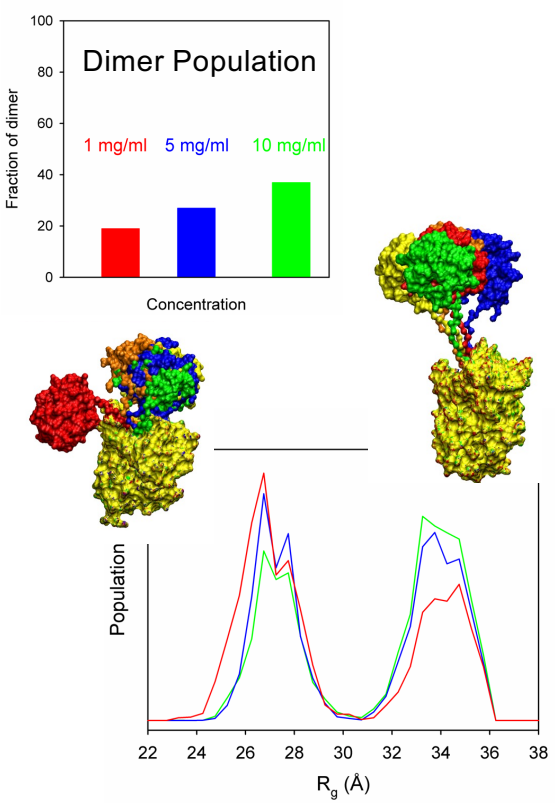
EOM (single Chain)





EOM (with dimers)





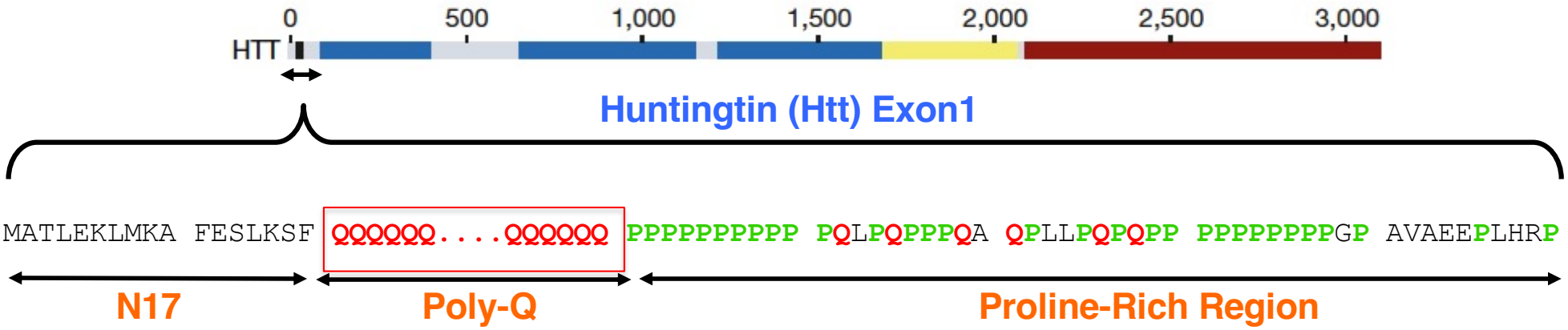
Col. Ramón Hurtado (Zaragoza-Spain)

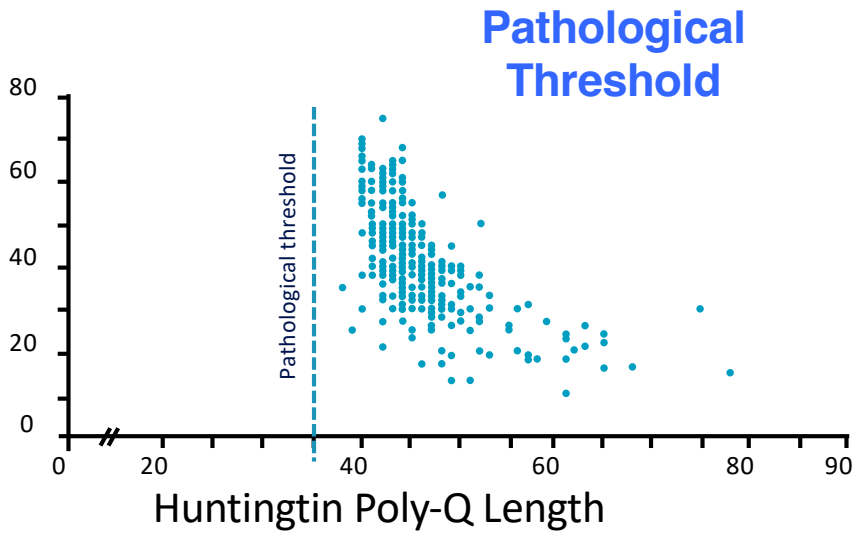
Lira-Navarrete et al. Nat. Comm 2015

Is SANS useful for IDPs?

Jain “Come” (2016)

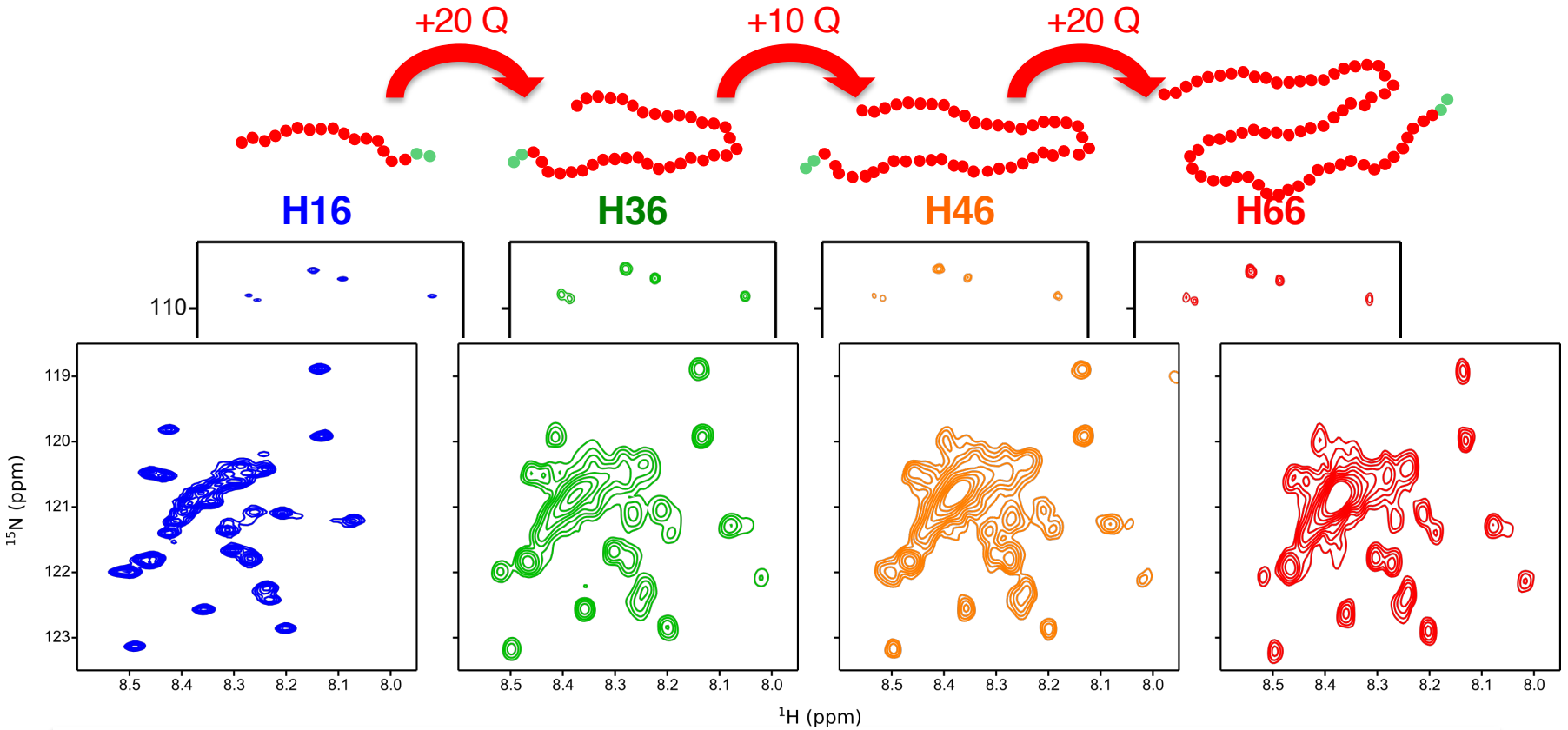
Huntingtin and Huntington's Disease

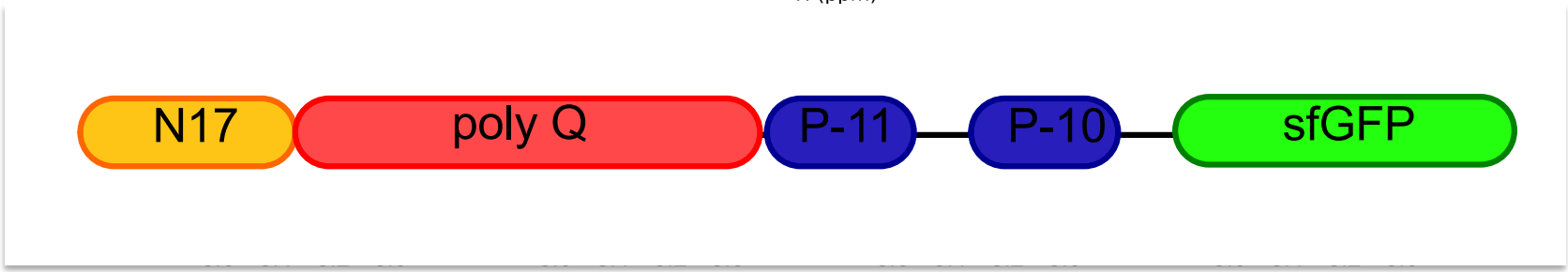




Margolis *et al. Arch. Gen. Psychiatry* 1999, 56, 1019.

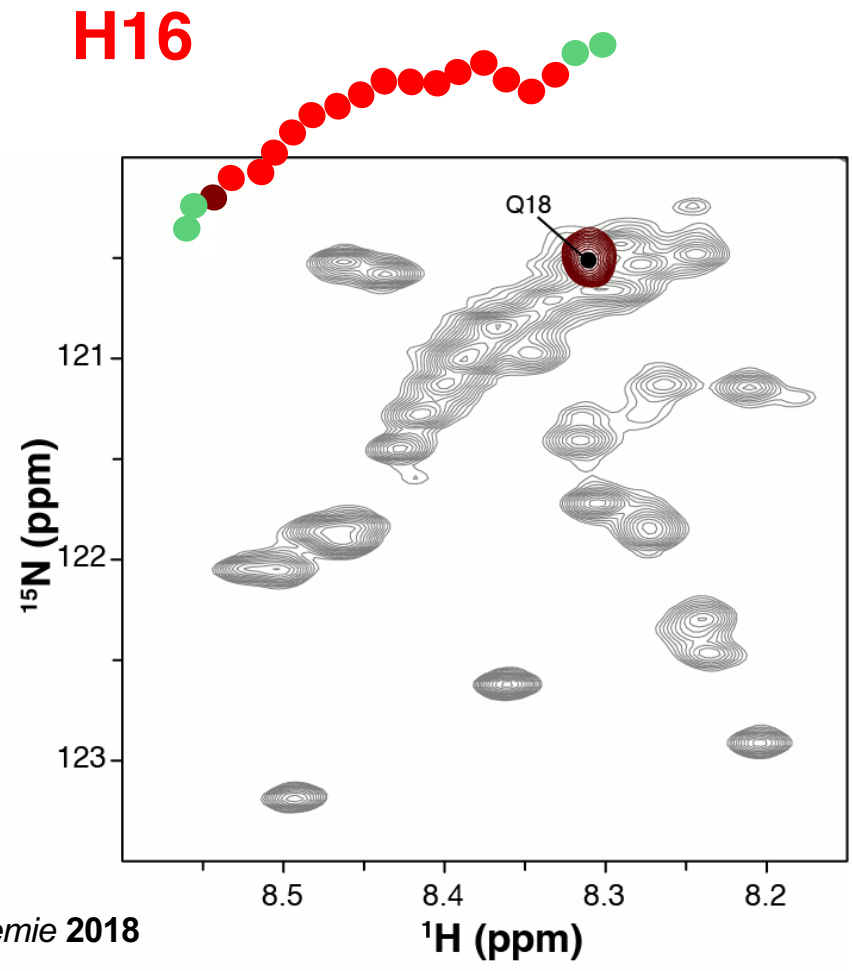
Compositional Bias and NMR





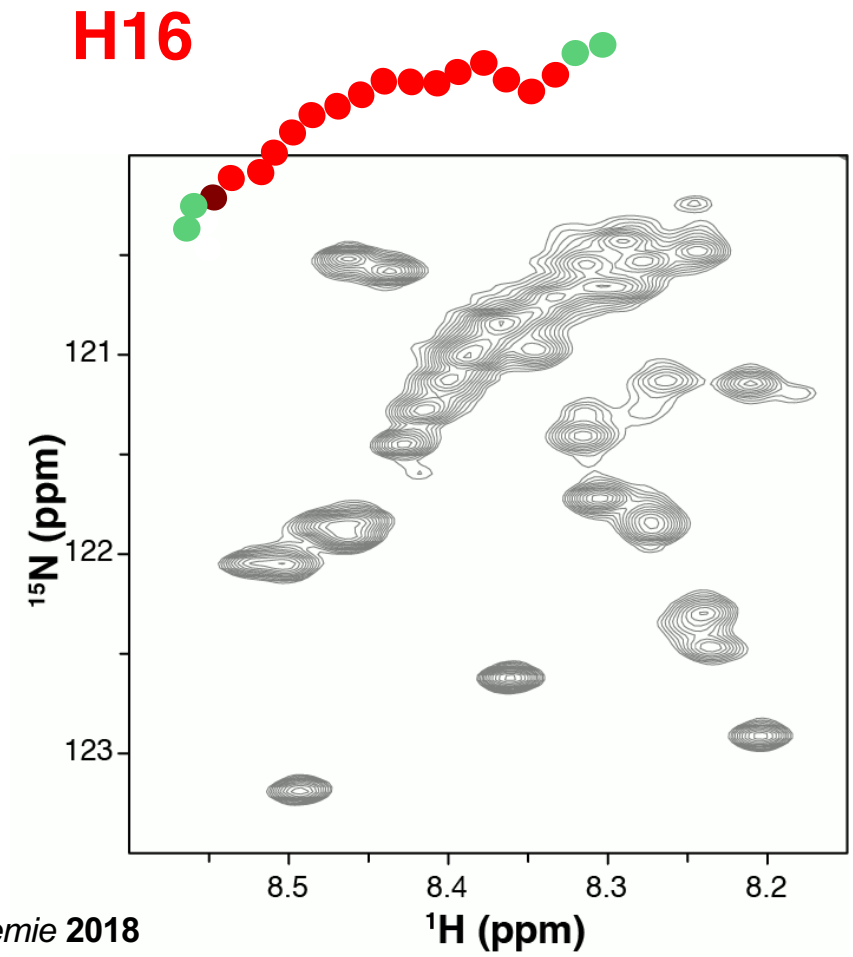
^{1}H (ppm)

Site-Specific Isotopic Labelling



Urbanek et al. *Angewante Chemie* **2018**
Urbanek et al. *Structure* **2020**
Urbanek et al., *ChemBioChem* **2020**
Morató et al., *Biomolecules* **2020**
Urbanek et al., *JACS* **2020**
Elena-Real et al. *Structure* 2023
Elena-Real et al., *NSMB* **2023**

Compositional Bias and NMR



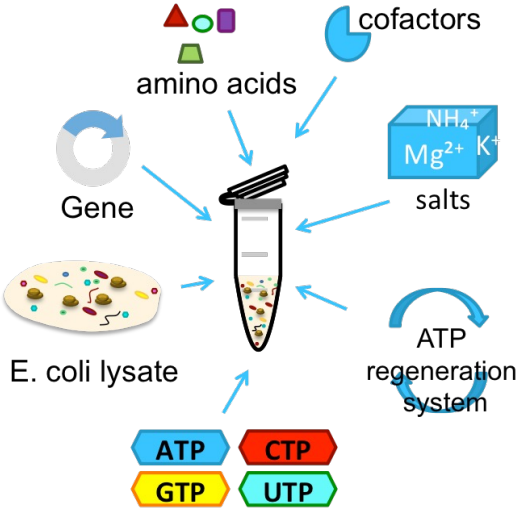
Urbanek et al. *Angewante Chemie* **2018**
Urbanek et al. *Structure* **2020**
Urbanek et al., *ChemBioChem* **2020**
Morató et al., *Biomolecules* **2020**
Urbanek et al., *JACS* **2020**
Elena-Real et al. *Structure* 2023
Elena-Real et al., *NSMB* **2023**

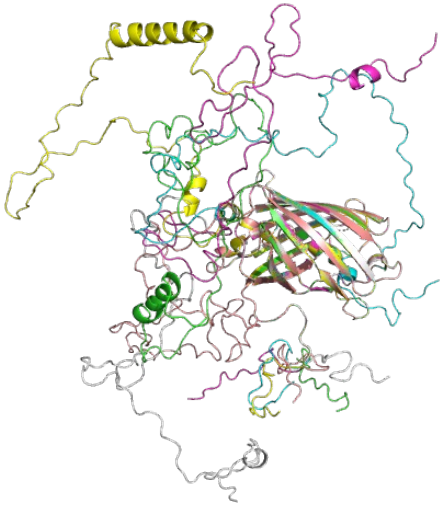
SANS and Segmental Labelling in Huntingtin

Xamuel Loft Lund

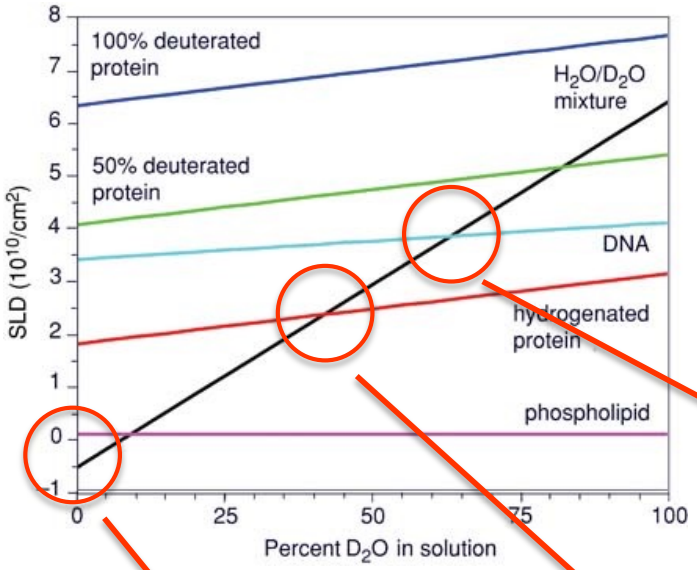
Anne Martel

Frank Gabel



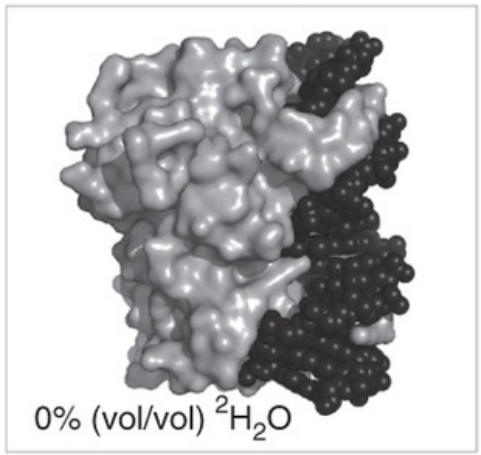


Contrast Matching in SANS

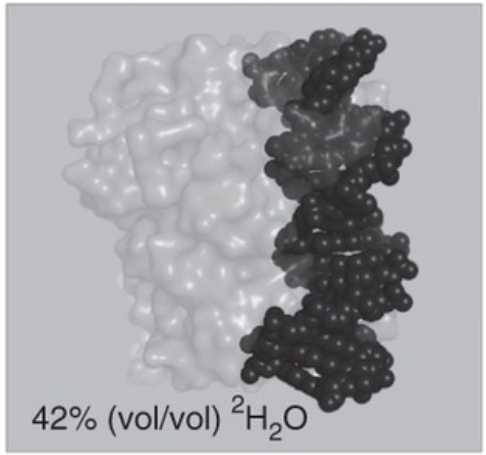


By modulating the deuteration level of the buffer, SANS experiments can highlight individual components of the assembly by 'making invisible' the others if they have a distinct density of exchangeable hydrogens.

Protein/DNA complex



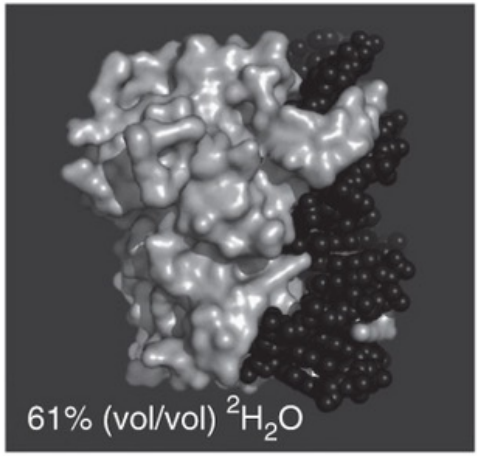
Protein match point



$\Delta\rho_{protein} \approx 0$

$I(q) \approx (\Delta\rho V)^2 P(q)_{DNA}$

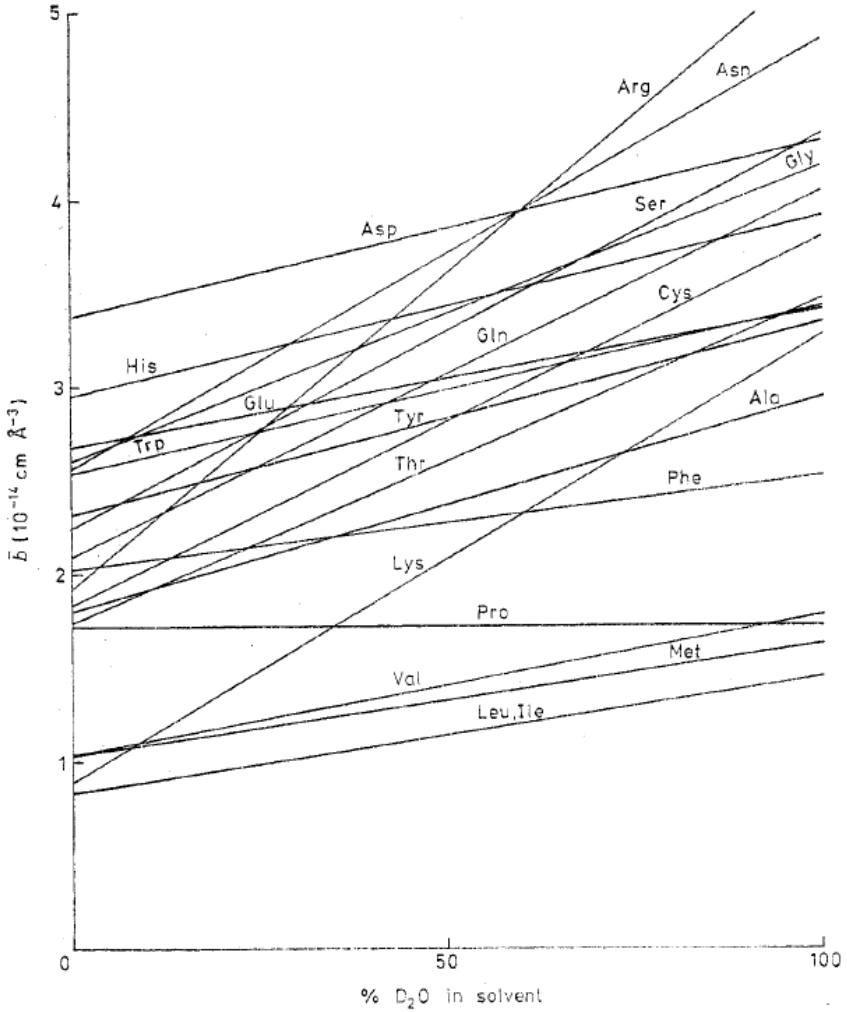
DNA match point



$\Delta\rho_{DNA} \approx 0$

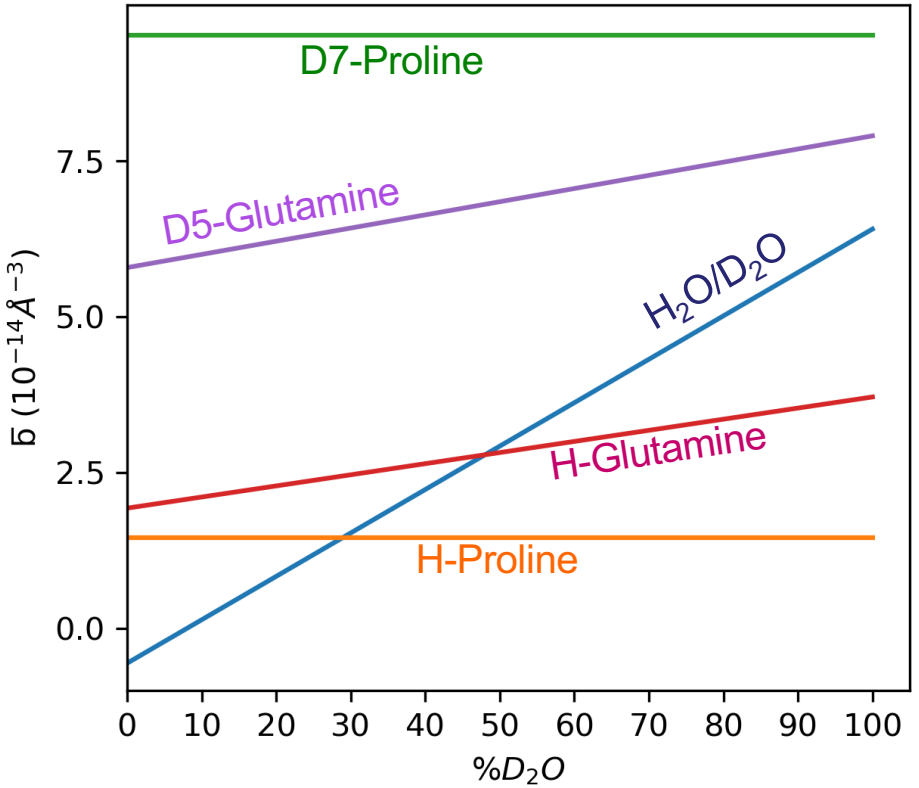
$I(q) \approx (\Delta\rho V)^2 P(q)_{protein}$

Contrast Variation is Amino Acid Dependent



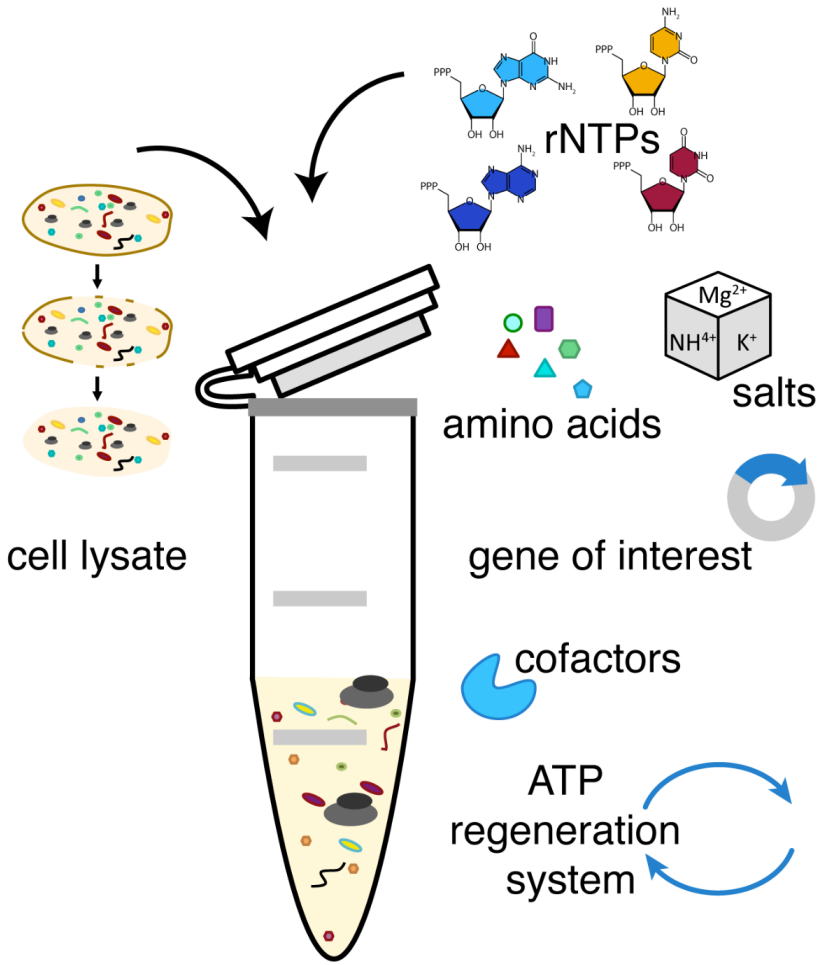
Each amino acid has a different scattering length depending on the H_2O/D_2O, its composition and the number of exchangeable protons

Jacrot *Rep. Prog. Phys.* 1976, 39, 911



…and we can modify it by introducing deuterated amino acids

Cell-Free Protein Synthesis



Cell-free contains the transcriptional and translational machineries from *E.coli* to express protein directly in a tube.

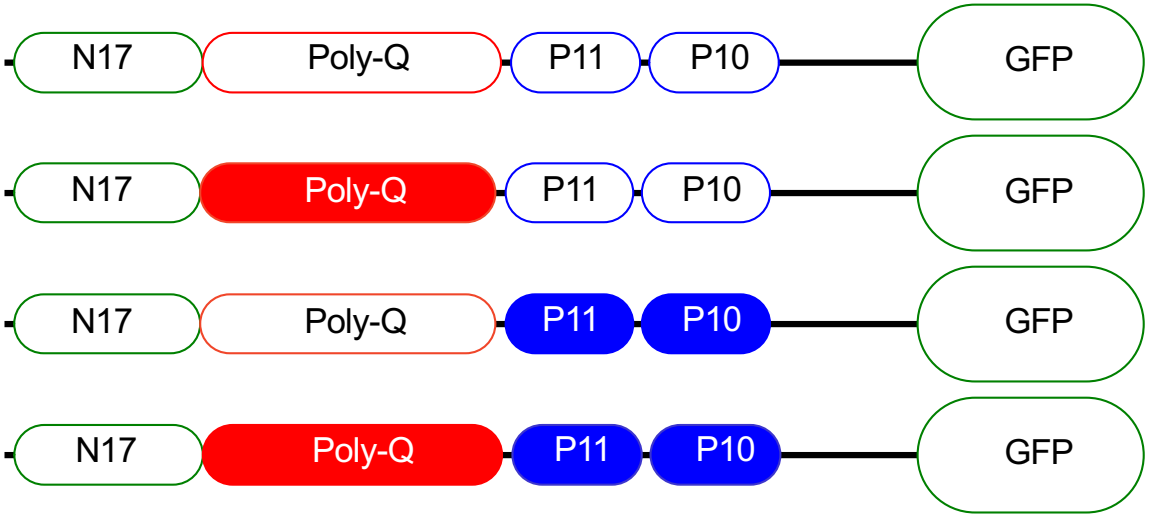
Production of complicated proteins (toxic, membrane proteins, aggregation-prone)

Exquisite control of the amino acid mixture. Deuteration of specific amino acids

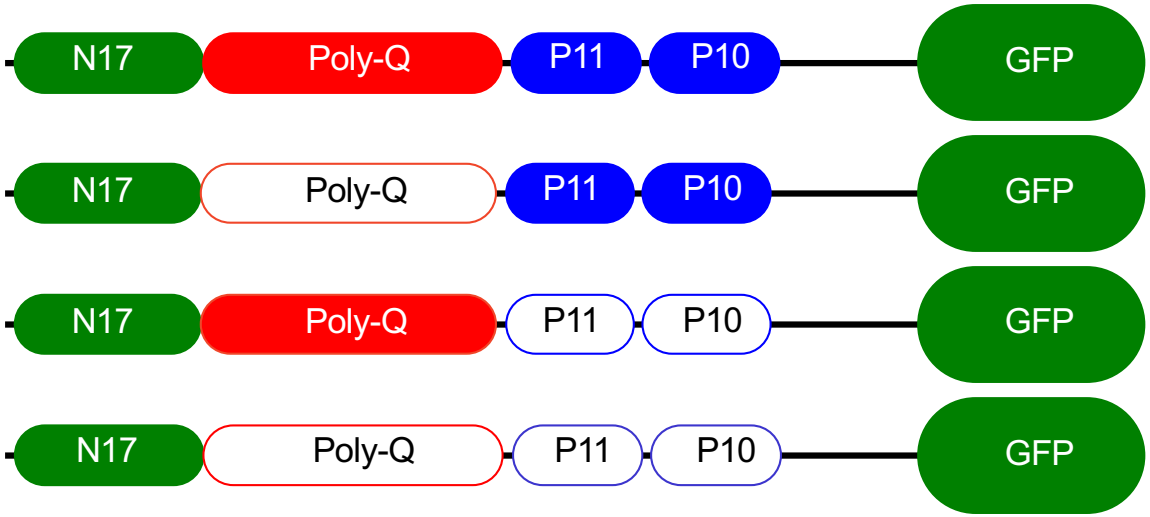
Amino acid scrambling processes are reduced

Reduced yield

Eight Possible Deuteration Patterns

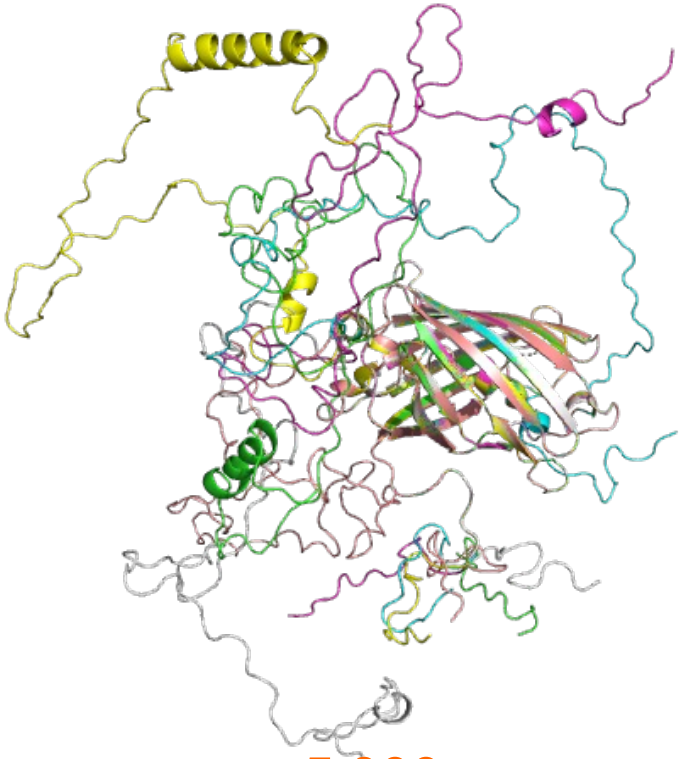


Protonated Patterns

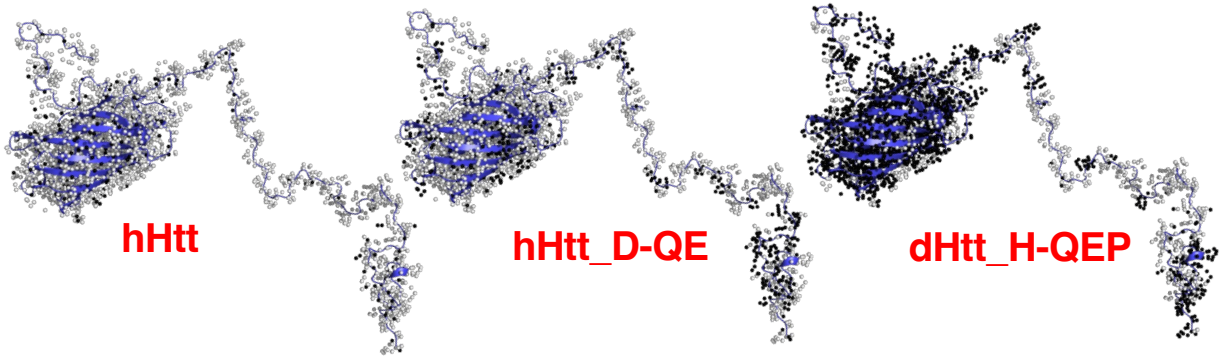


Deuterated Patterns

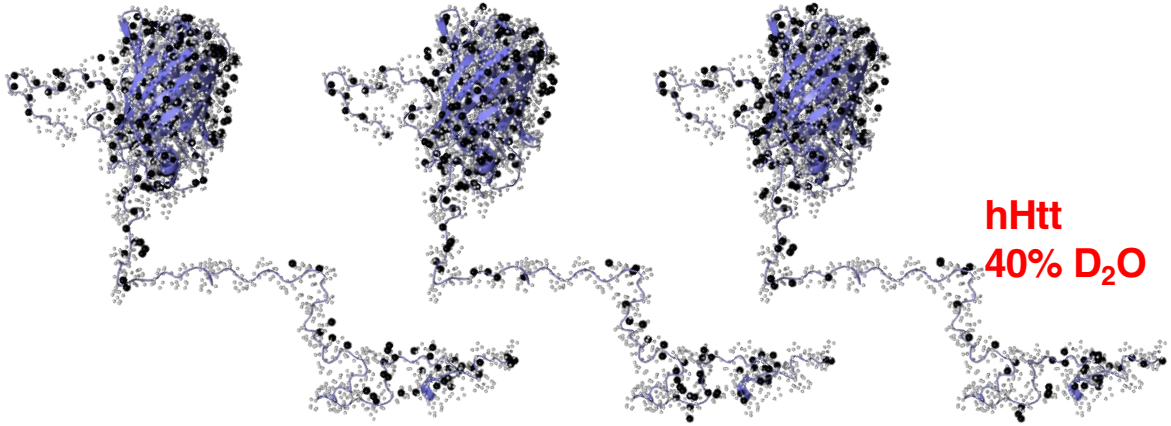
Contrast Variation and Segmental Labelling



5,000 conformations compatible with the NMR data

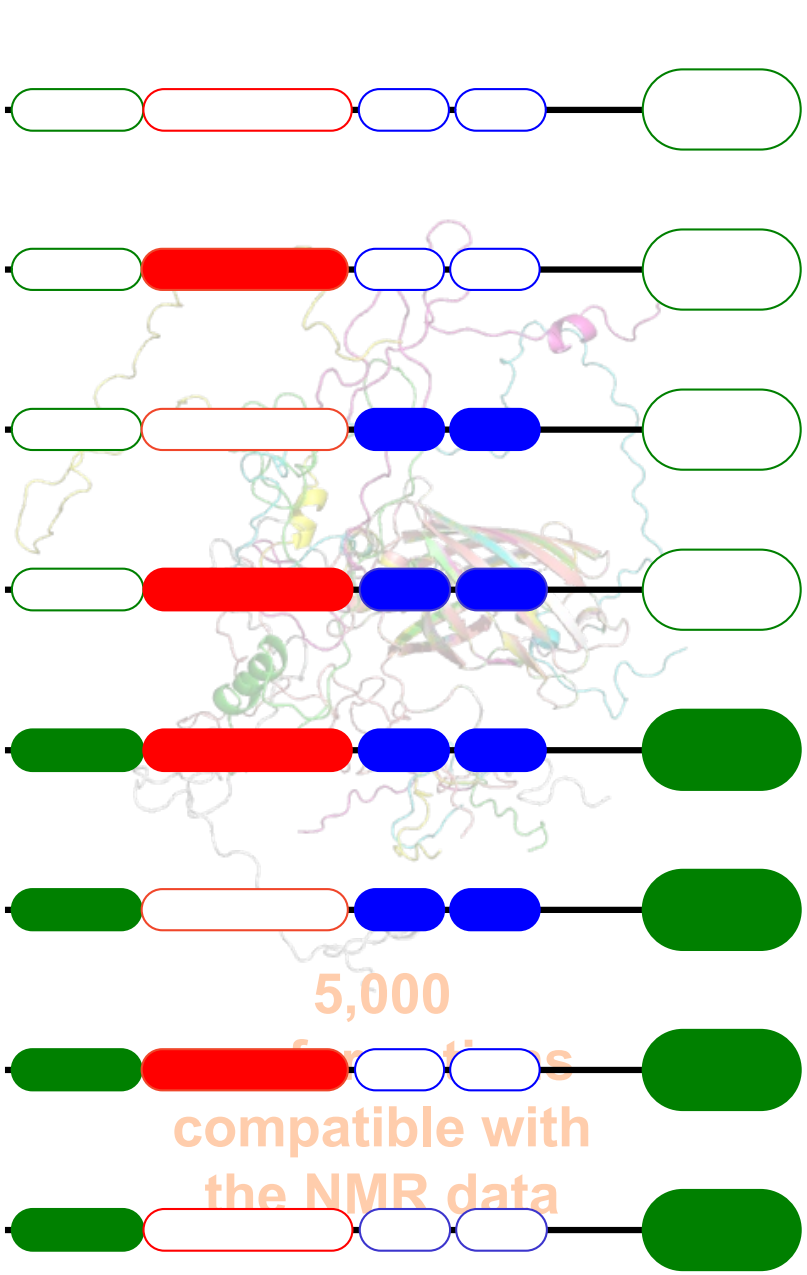


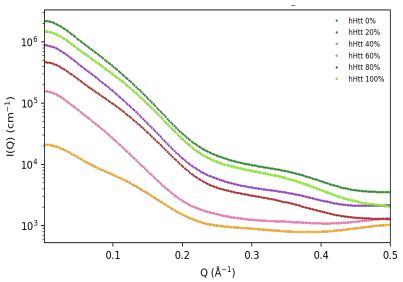
Incorporation of deuterium atoms according to the pattern

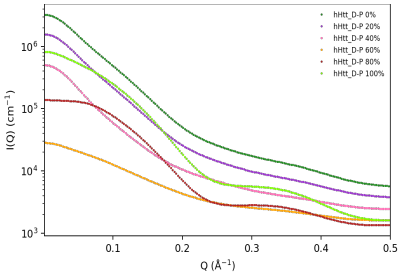


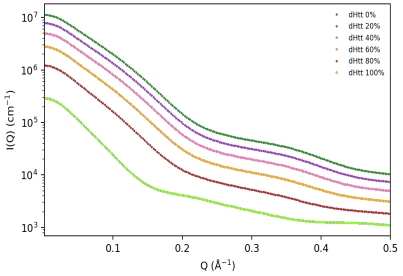
Random incorporation of H/D in labile positions according to 6 D_2O percentages

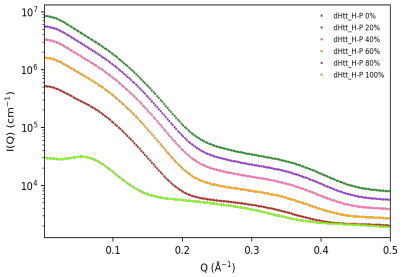
Contrast Variation and Segmental Labelling

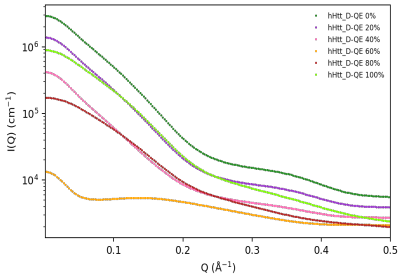


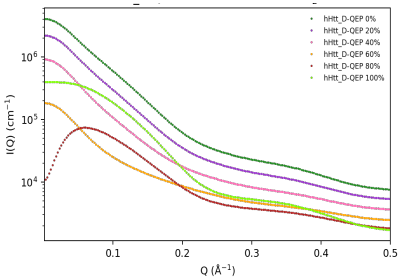


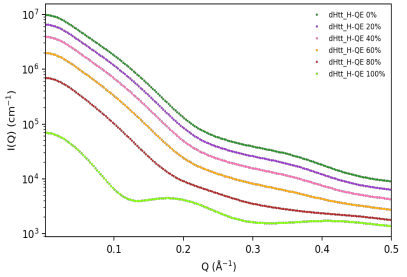


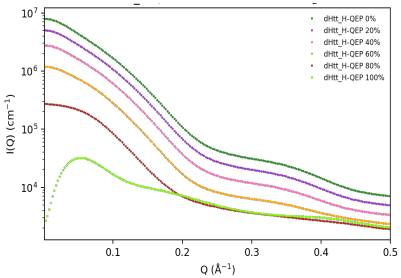




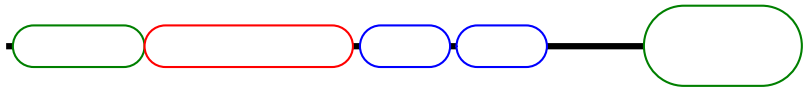


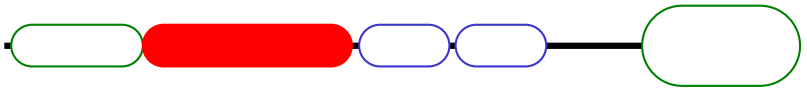


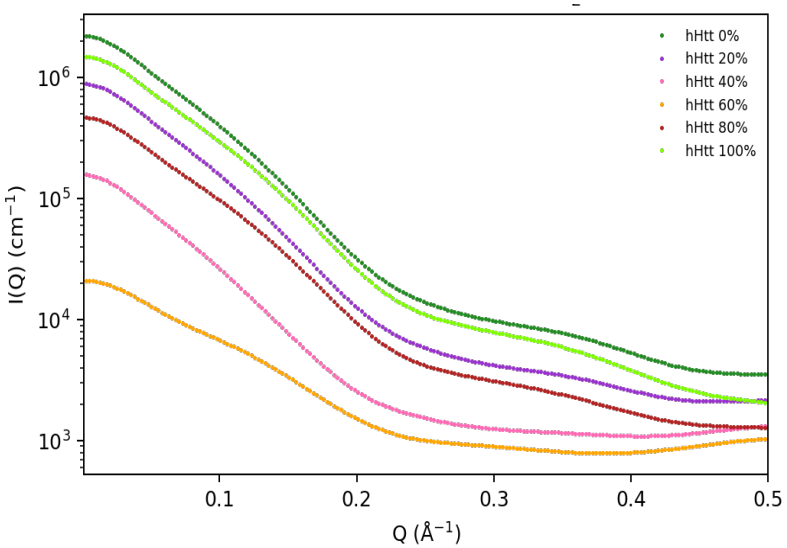


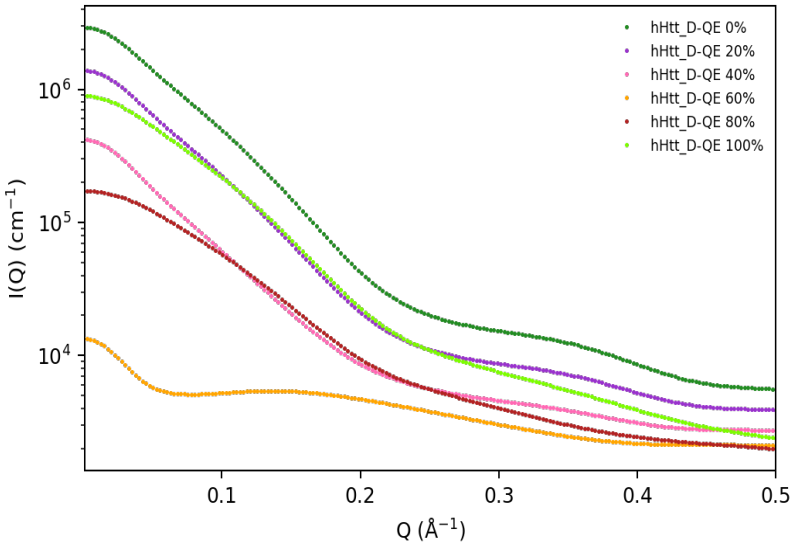


Contrast Variation and Segmental Labelling







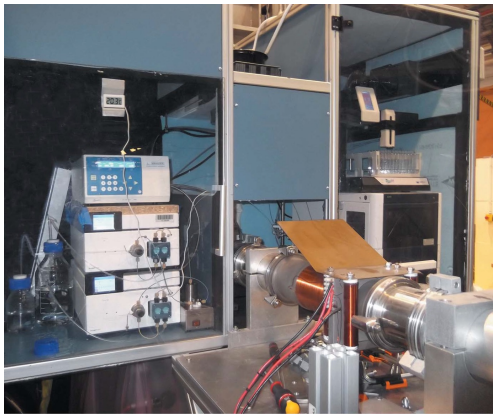


Segmentally labelled samples display a non-homogeneous behaviour along contrast variation experiments

Stronger effects when increasing the number of glutamines in the poly-Q tract

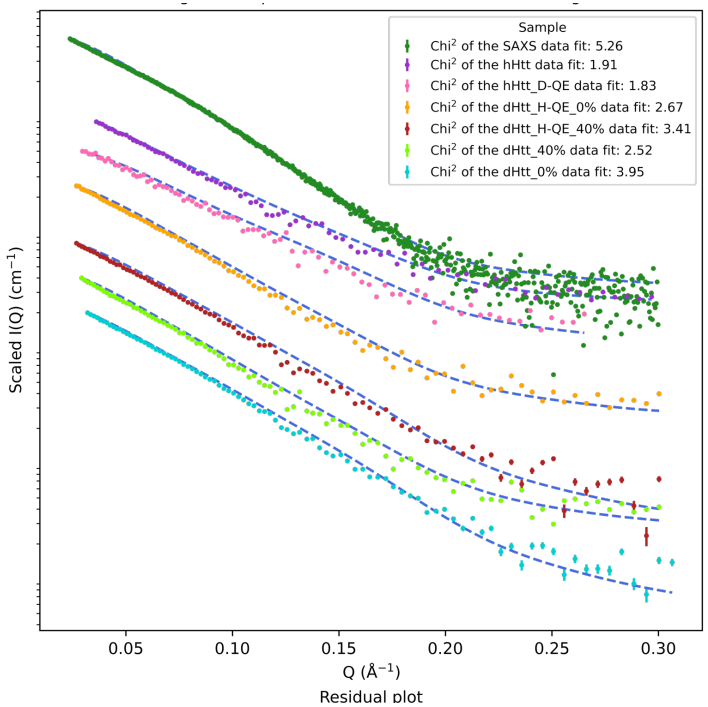
Experimentally Achieved Samples

Sample	% D_2O	R_G	Conc.	Exposure time	Experiment
hHtt (16Q)	20% 100%	41.2 Å 35.1 Å	6.0 mg/mL	2 hours 1 hour 25 minutes	Test_8-03-1020 (04/02-21) (Superdex 75, 10/300)
hHtt_D-P (16Q)	100%	26.1 Å	2.3 mg/mL	6 hours	Test_8-03-1020 (04/02-21) (Superdex 75, 10/300)
hHtt_D-QE (16Q)	0% 100%	39.7 Å 30.8 Å	4.6 mg/mL	2 hours 31 minutes 1 hour 5 minutes	Test_9-13-984 (22/06-21) (Superdex 75, 10/300)
dHtt (16Q)	0% 40%	40.2 Å 39.2 Å	4.8 mg/mL	2 hours 5 minutes 1 hour 8 minutes	Test_8-03-1050 (21/09-21) (Superdex 200, 5/150)
dHtt_H-QE (16Q)	0% 40%	38.0 Å 37.3 Å	4.5 mg/mL	1 hour 7 minutes 1 hour 10 minutes	Test_8-03-1050 (21/09-21) (Superdex 200, 5/150)
hHtt (36Q)	100%	32.6 Å	2.3 mg/mL	1 hour 21 minutes	Test_8-03-1050 (21/09-21) (Superdex 200, 5/150)
hHtt_D-QE (36Q)	100%	27.0 Å	1.7 mg/mL	8 hours 3 minutes	Test_8-03-1050 (21/09-21) (Superdex 200, 5/150)

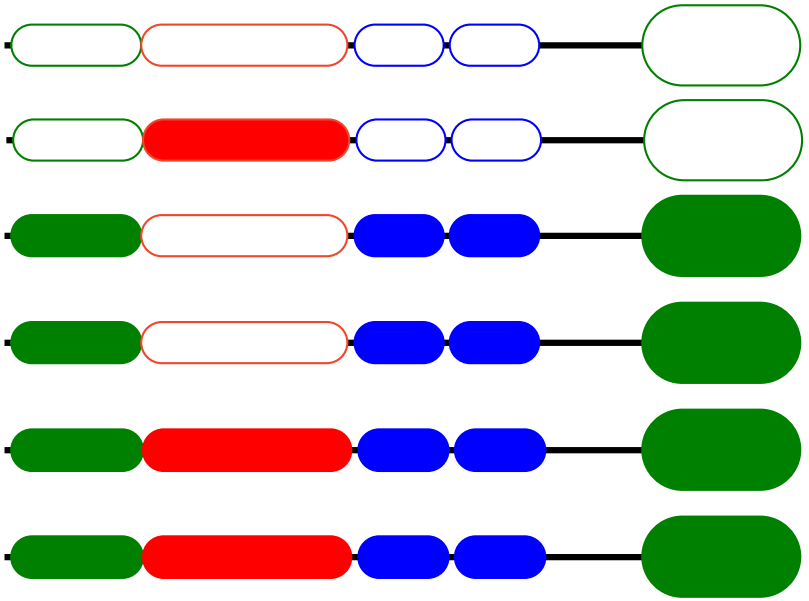


SEC-SANS@ILL

Cross-Validation in SAXS/SANS



SAXS +



100% D_2O

100% D_2O

0% D_2O

40% D_2O

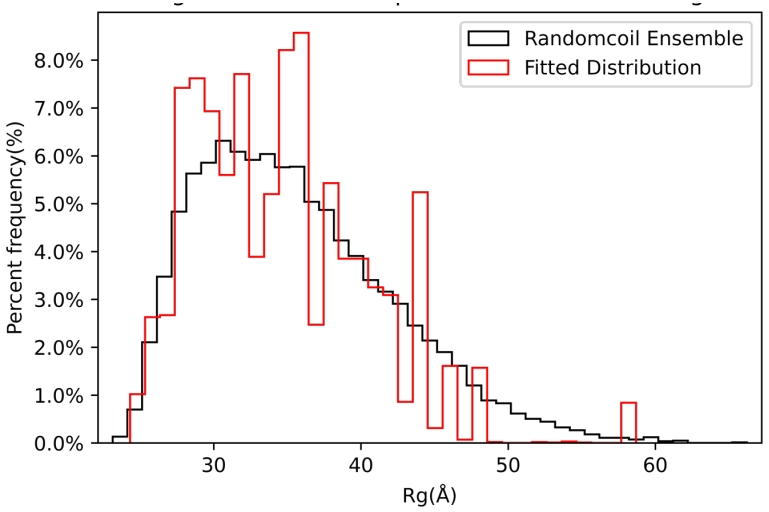
40% D_2O

0% D_2O

Correct simultaneous description of SAXS and 6 SANS datasets

Selection of a subensemble of conformations compatible with all datasets

Equivalent work has to be done with a pathogenic version of Htt with 36 glutamines



Take-Home messages

- In highly flexible proteins, NMR provides the conformational sampling at residue level. SAXS provides the overall size and shape.

- Synergistic application of NMR and SAXS (with the help of computational tools) provides accurate structural/dynamic models for flexible proteins…

- Discerning between flexible and rigid scenarios is fundamental.

- SAXS provides information about large-amplitude motions in biomolecules and reaches novel and biologically relevant information.

- SANS combined with amino acid specific labelling provides insights into the structure of low-complexity regions in proteins.

- Progress in the structural interpretation of SAXS data (in terms of conformational dynamics) will come from the development of theoretical methods to generate and perturb 3D structures…

Even in the AlphaFold era, there is room for experimental structural biology

Structure and Function of Highly Flexible Proteins

Pau Bernadó (DR2)
Nathalie Sibille (CR1)
Frédéric Allemand (IR1)
Aurelie Fournet (AI)

Aurelien Thureau (Soleil)

Xamuel Lund (PhD)
Amin Sagar (Pdoc)
Carlos Elena-Real (Pdoc)
Assia Mouhand (Pdoc)
Alejandro Estaña (PhD)
Annika Urbanek (Pdoc)
Lucile Senicourt (Pdoc)
Matija Popovic (Pdoc)
Anna Morató (IR)
Tiago Cordeiro (Pdoc)

erc
AGENCE NATIONALE DE LA RECHERCHE ANR
Inserm
FONDATION RECHERCHE MÉDICALE
NEUTRONS FOR SCIENCE
MUSE
MONTPELLIER UNIVERSITÉ D'EXCELLENCE
EPIGENMED
REFRACT