Multiple Structural States of Proteins Revealed by NMR

Appetizer: The enzyme cyclophilin in action

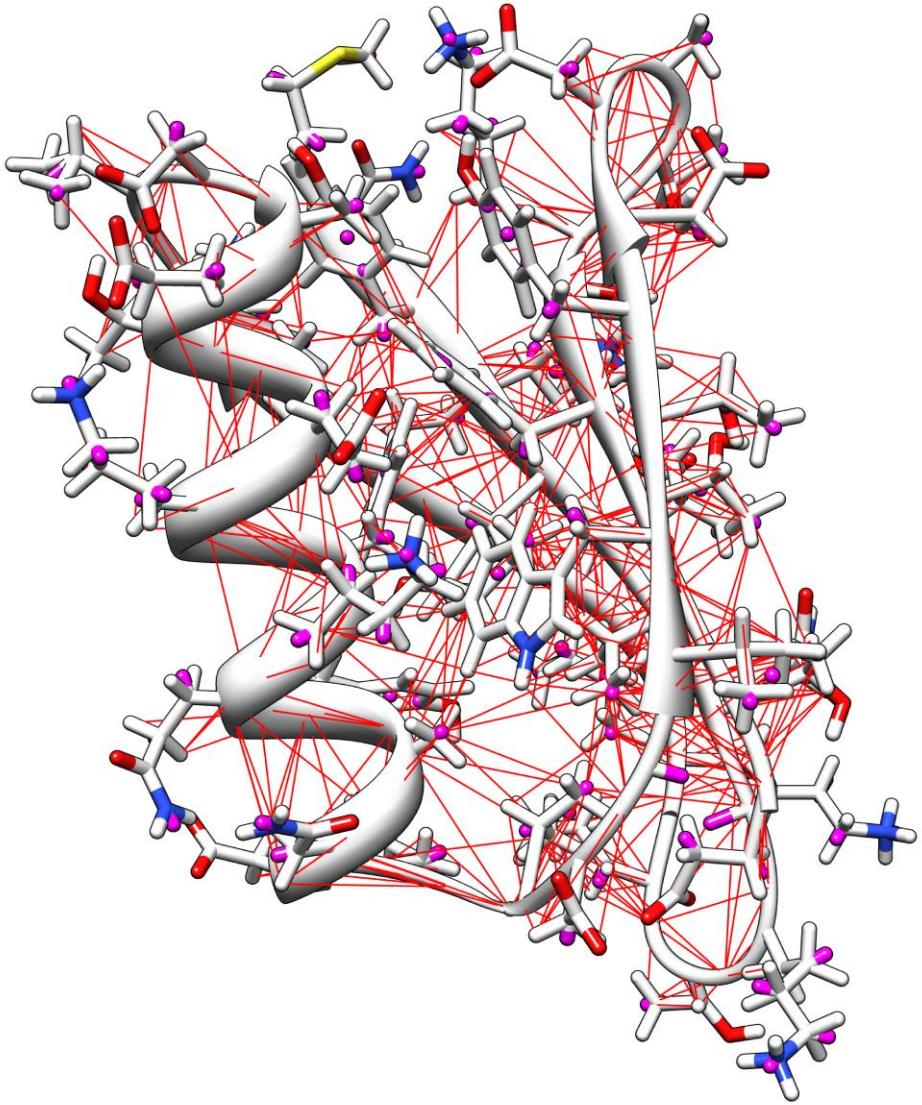
First dish: Protein allostery

Second dish: Dynamical interplay between membrane lipids and membrane protein

Third dish: Multiple states in vivo revealed by in cell NMR

Main Dish: The multiple states of a-synuclein associated with Parkinson's disease

The Nuclear Overhauser Effect (NOE)

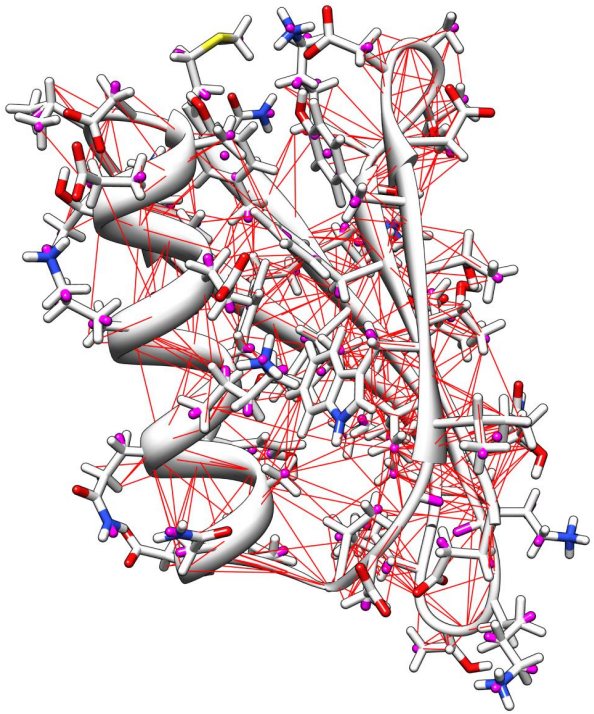


The Nuclear Overhauser Effect (NOE)

$$\sigma_{KL} = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma^4 h^2}{40\pi^2} \frac{1}{(r_{KL}^{\text{rigid}})^6} \left[J(0) - 6J(2\omega)\right]$$

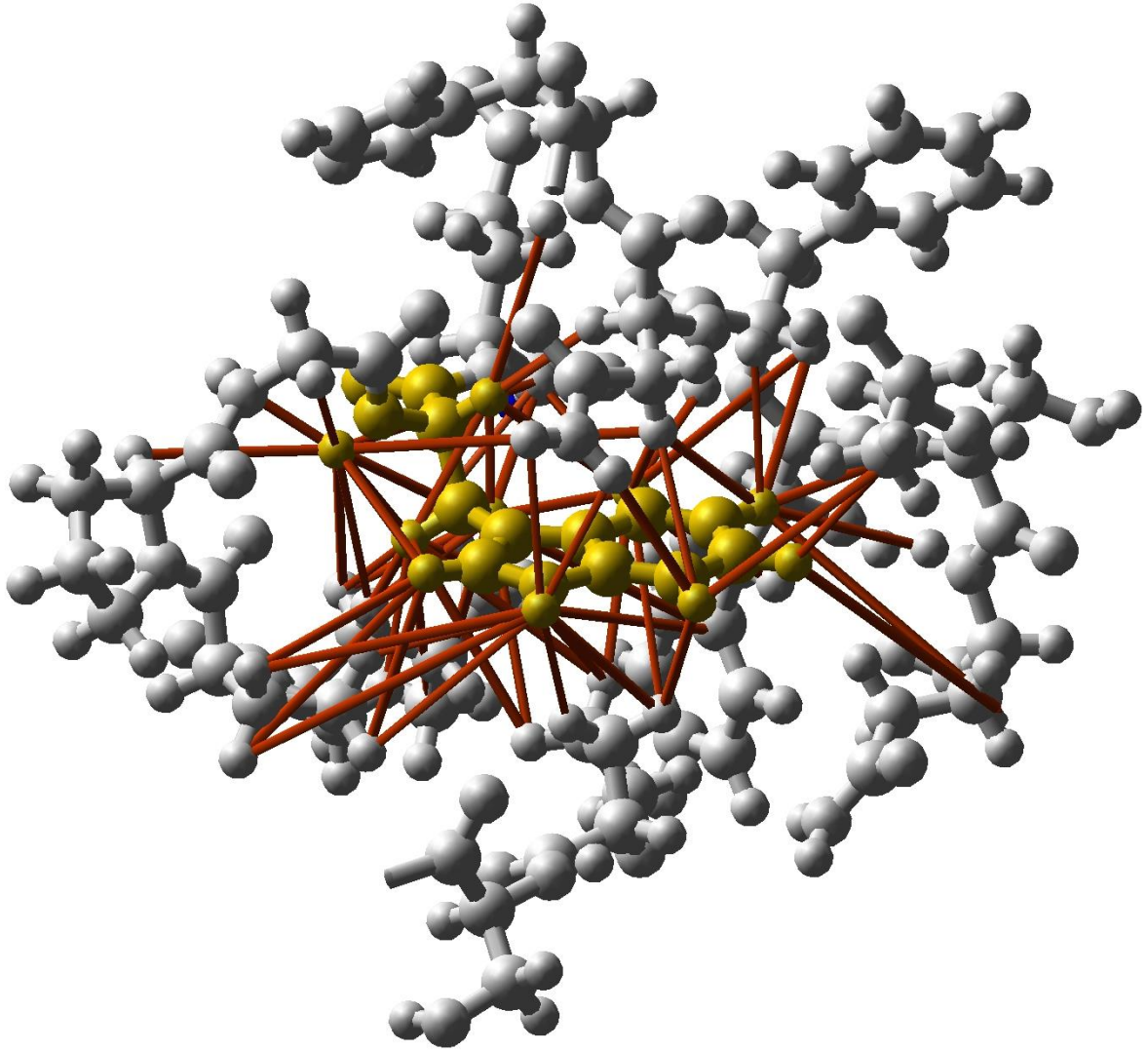
$$J(\omega) = S_{KL}^{\text{fast}\,2} \frac{\tau_c}{1+(\tau_c\omega)^2} + \left((r_{KL}^{\text{rigid}})^6 \left\langle \frac{1}{r_{KL}^6} \right\rangle - S_{KL}^{\text{fast}\,2} \right) \frac{\tau_{\text{tot}}}{1+(\tau_{\text{tot}}\omega)^2}$$

$$\frac{1}{\tau_{\text{tot}}} = \frac{1}{\tau_c} + \frac{1}{\tau_{\text{int}}}$$



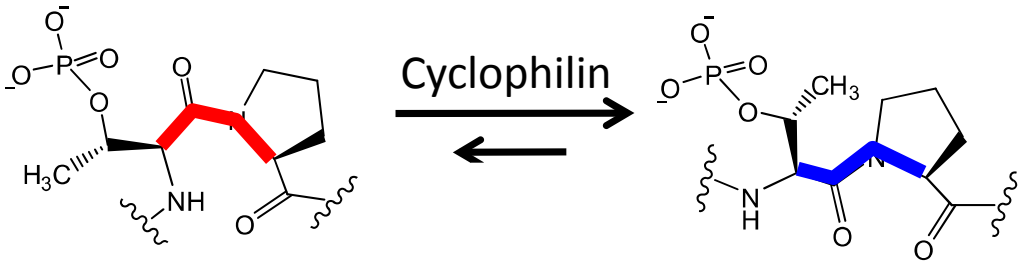
$$\sigma = (\mu_0/4)^2 \frac{\gamma^4 h^2}{40\pi^2} \tau_c < \frac{1}{r^6} >$$

Example of collected distances in WW domain



- 66 eNOE-derived Distance restraints
- 30 with a precision of 0.1 Å
- 4 degrees of freedom
- Fulfilled in the 2 state structure calculation with an average error of 0.01 Å per distance

Cyclophilin, which is a Proline Cis/Trans Isomerase

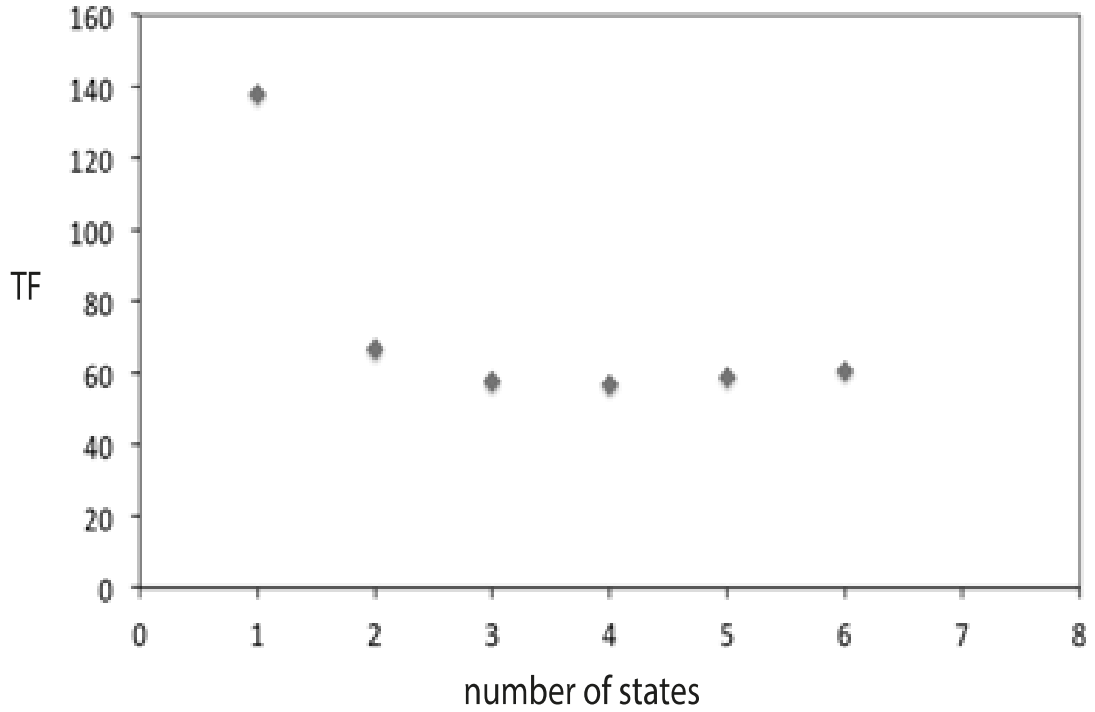


(Wuthrich, Kern, Eisenmesser, Kay, Vendruscolo et al.)

Picture from Peng (2015)

Two State Structural Ensemble of the Enzyme Cyclophilin (Chi, Vögeli, Riek et al., Angewandte Chemie 2015)

(3640 eNOEs, 396 RDCs, 96 *J* couplings)

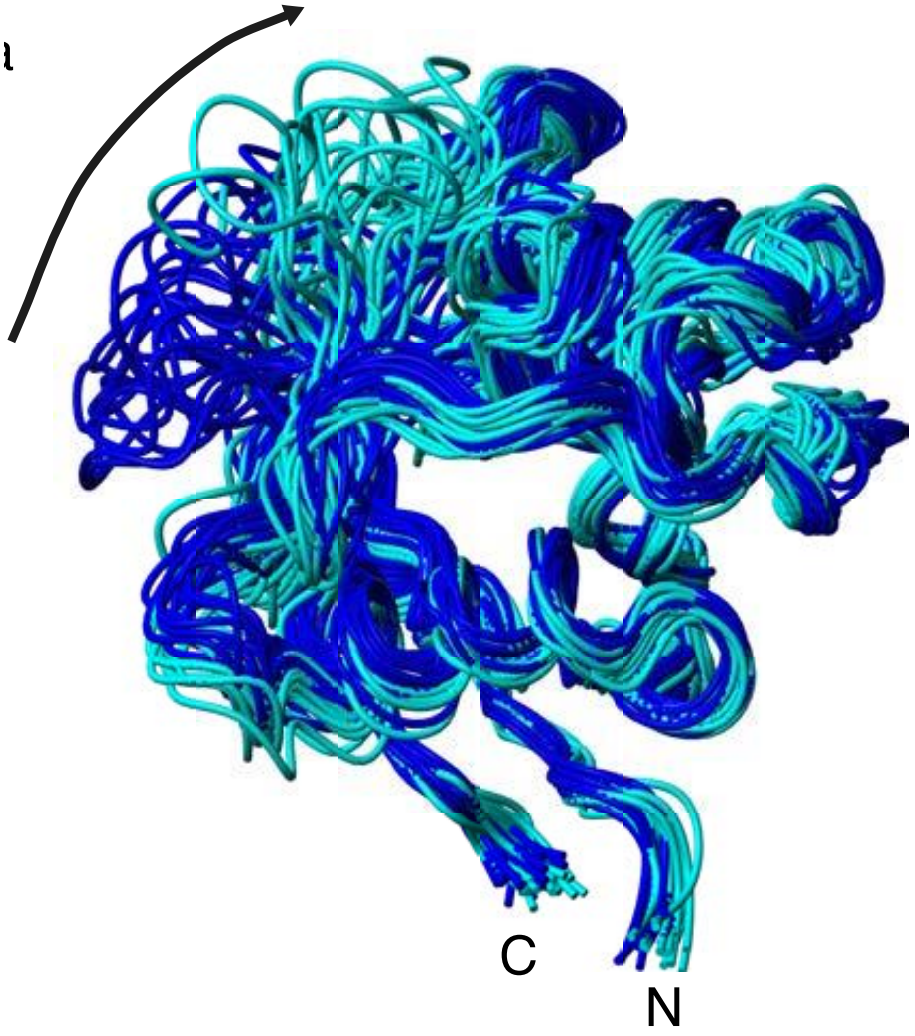


$$\sigma = (\mu_0/4)^2 \frac{\gamma^4 h^2}{40\pi^2} \tau_c < \frac{1}{r^6} >$$

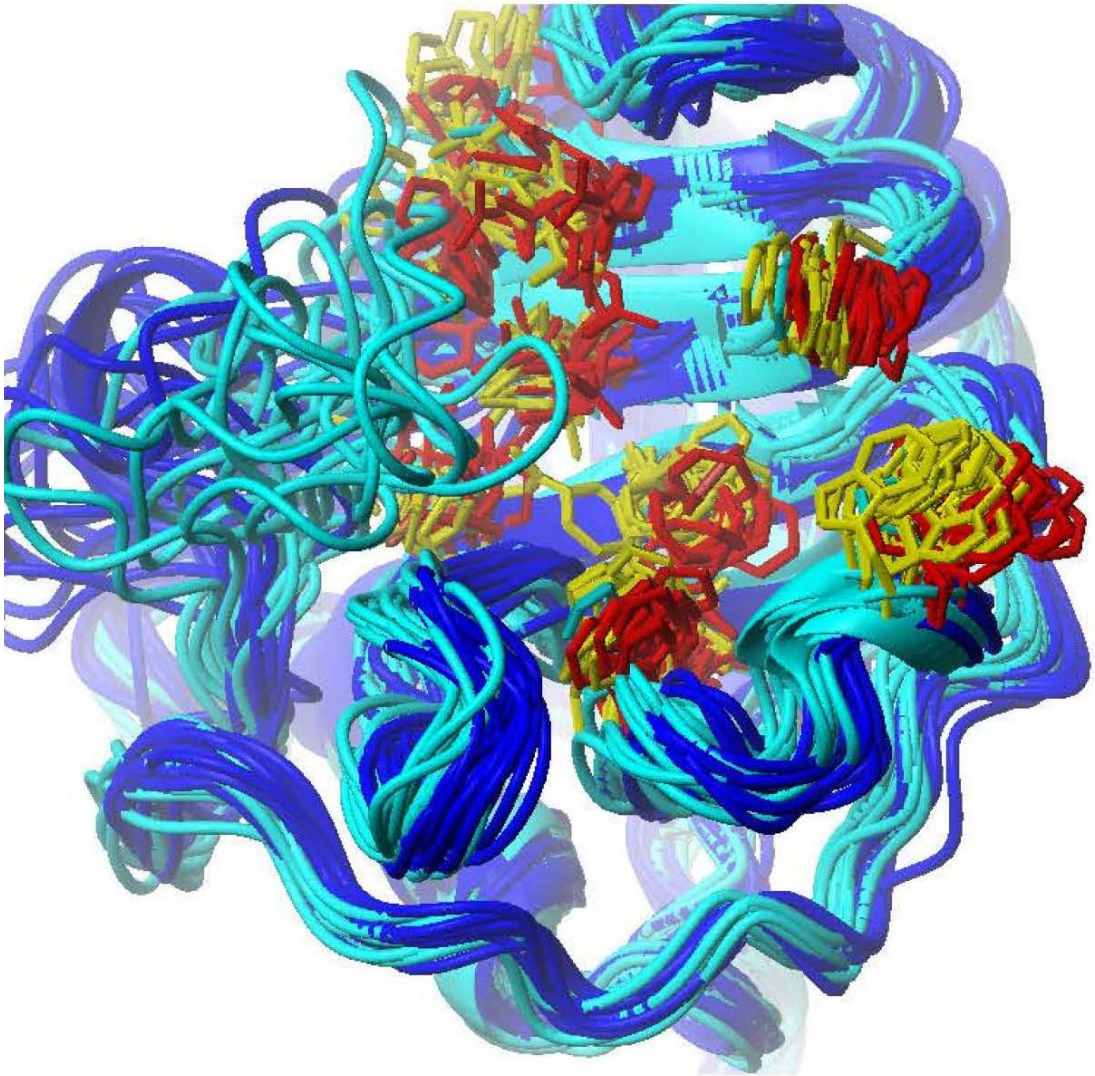
Enzyme Action - Capture structural intermediates:

Two States structural ensemble of the enzyme cyclophilin (Chi, Vögeli, Riek et al., Angewandte Chemie 2015)

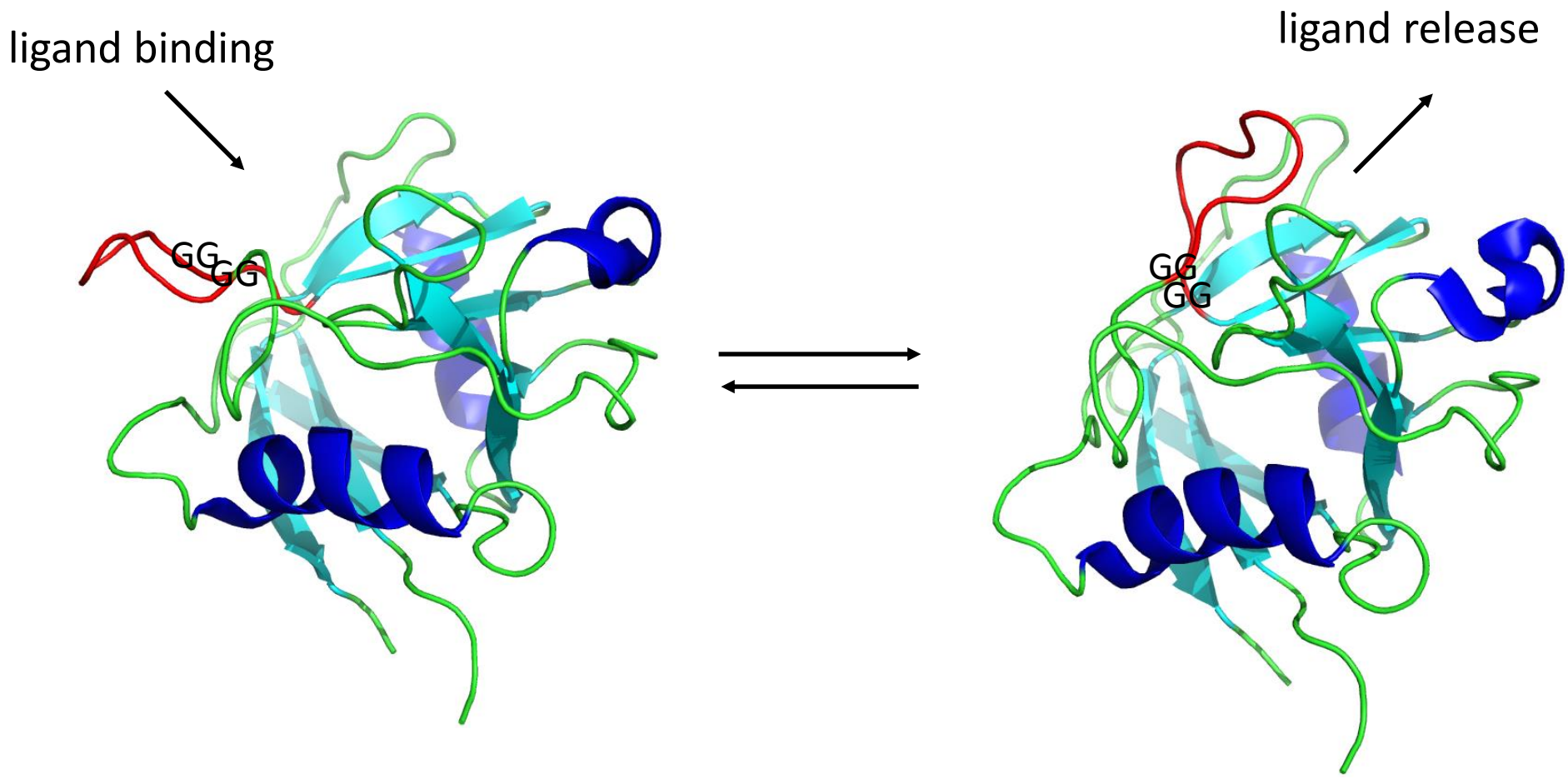
(3640 eNOEs 396 RDCs, 96 *J* couplings)



Two states ensemble of Cyclophilin in its apostate



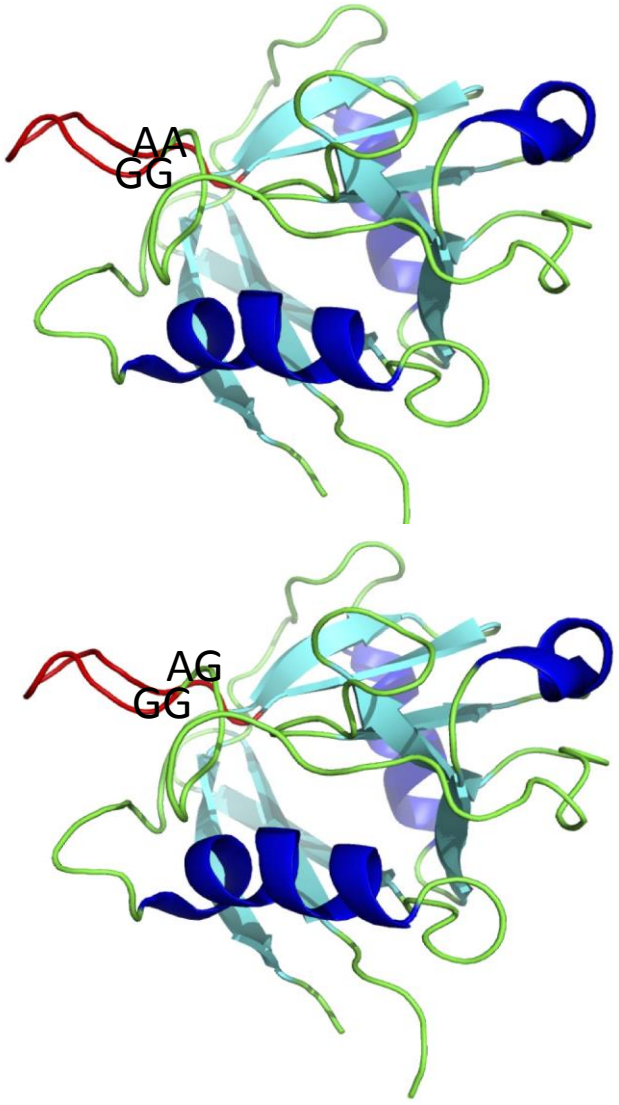
Proposed Mechanism of the Action of Cyclophilin



Extending the eNOE data set of large proteins by evaluation of NOEs with unresolved diagonals**. Chi et al J.Bio NMR 2015**

A Structural Ensemble for the Enzyme Cyclophilin Reveals an Orchestrated Mode of Action at Atomic Resolution**. Chi et al Angewandte Chemie Int Ed. 2015**

A 10 times more active mutant



Pro Cis/trans turn over rate k_{conf}

Protein	k_{conf} (s^{-1})	Errors
Wild type	2.4	±0.04
G74AG75A	0.5	±0.01
G75A	32.1	±0.69

(Vögeli, Chi et al. Angewandte Chemie 2016; Chi, Vögeli, Riek et al., Angewandte Chemie 2015)

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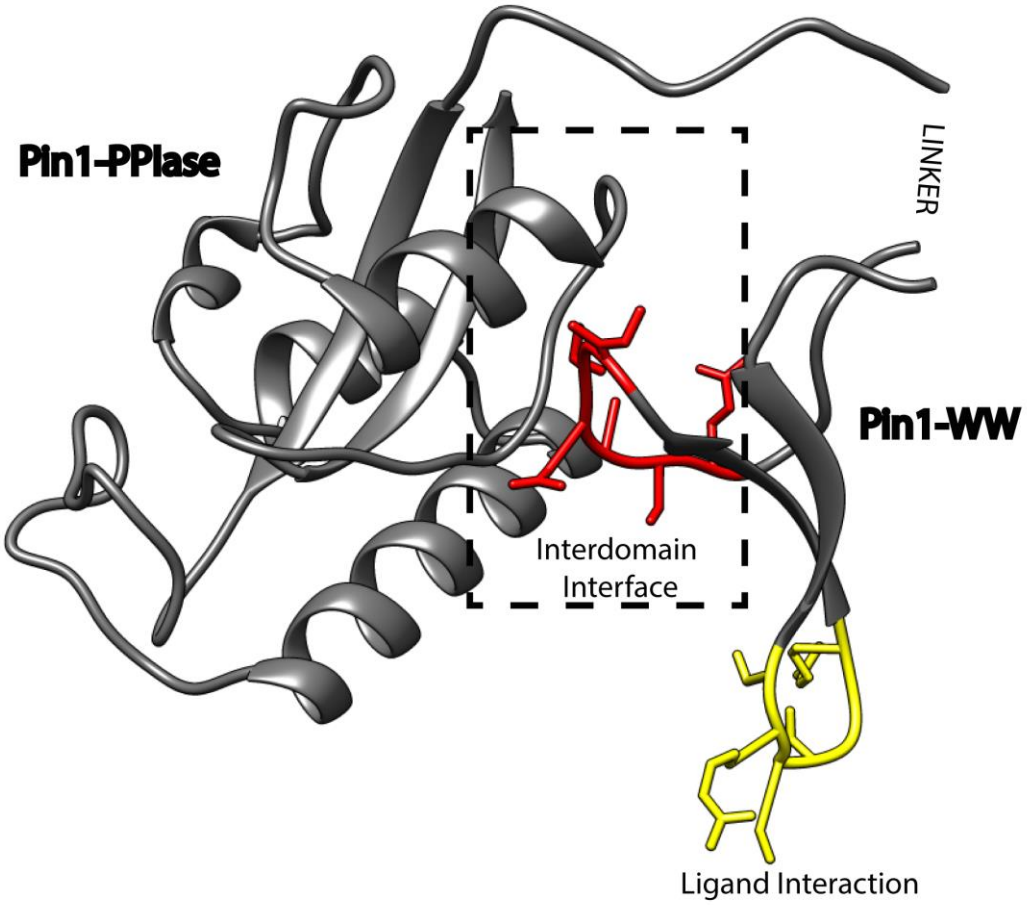
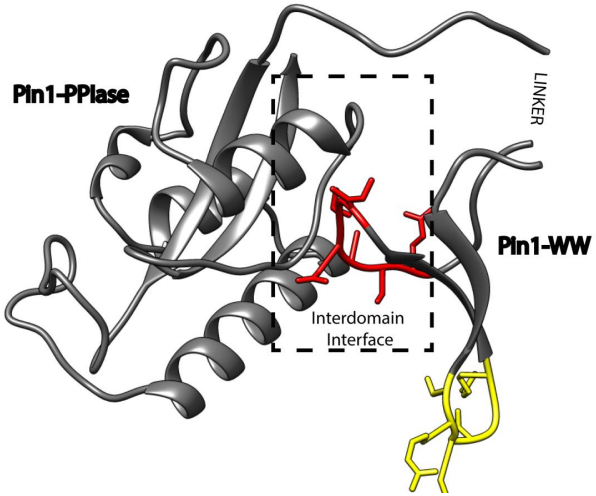
Desert: On the nature of time

Allostery of a WW domain of Pin1



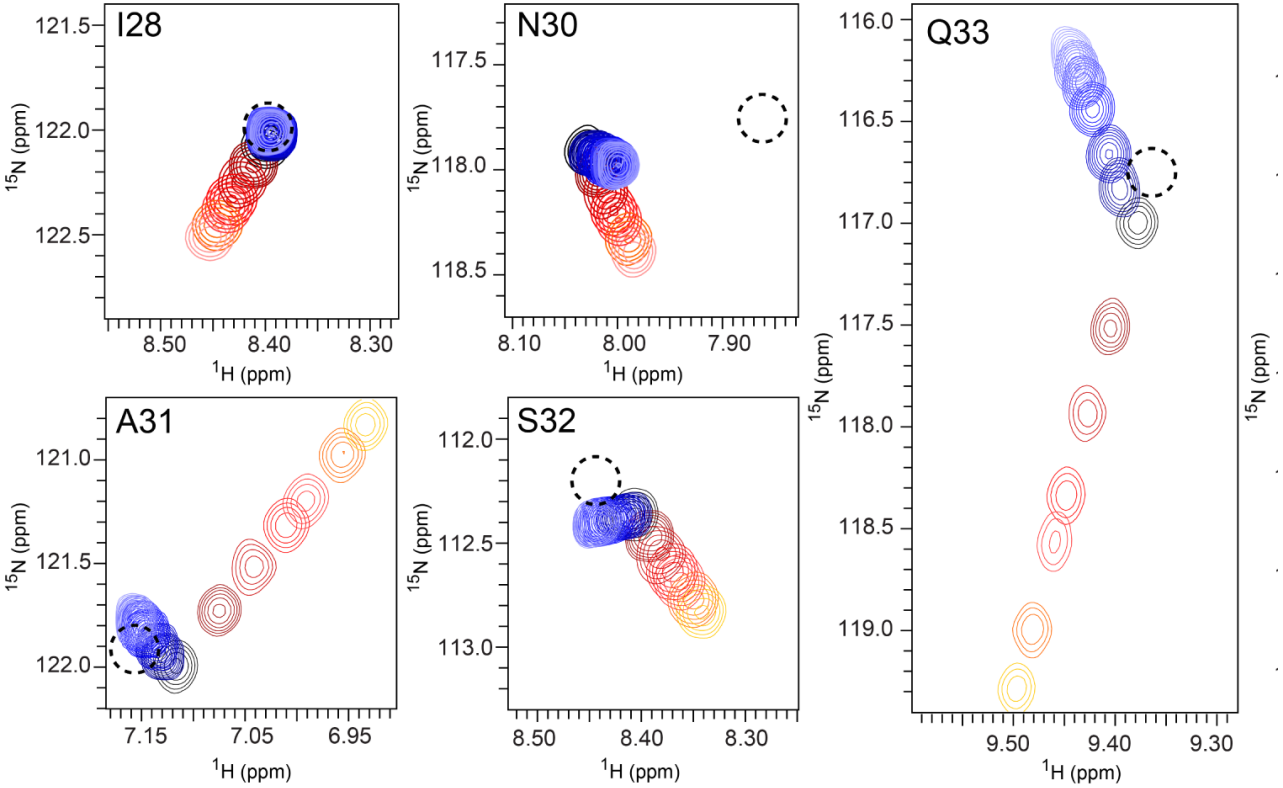
(Ramanganathan, Noel, Kelly, Peng et al.)

Allostery of a WW domain of Pin1

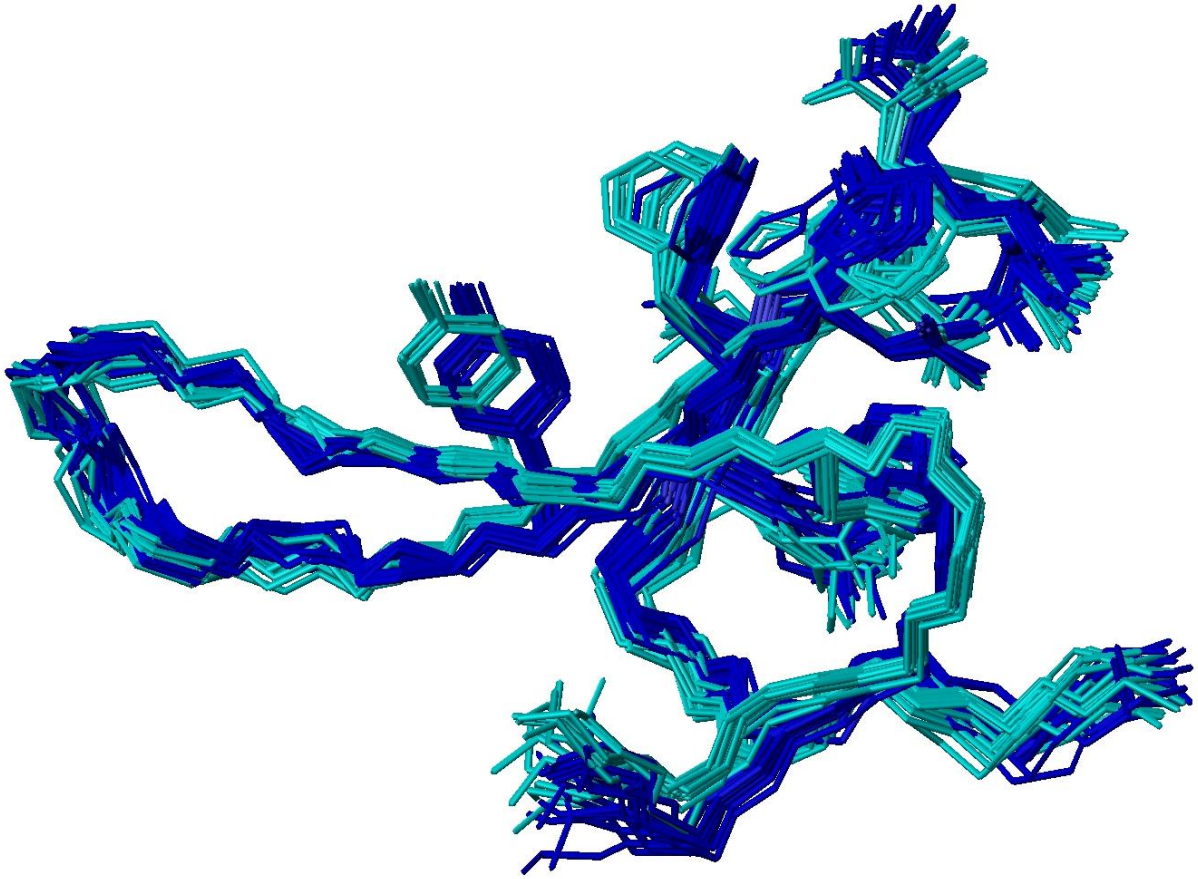


peptide pCdc25C is a negative allostery ligand

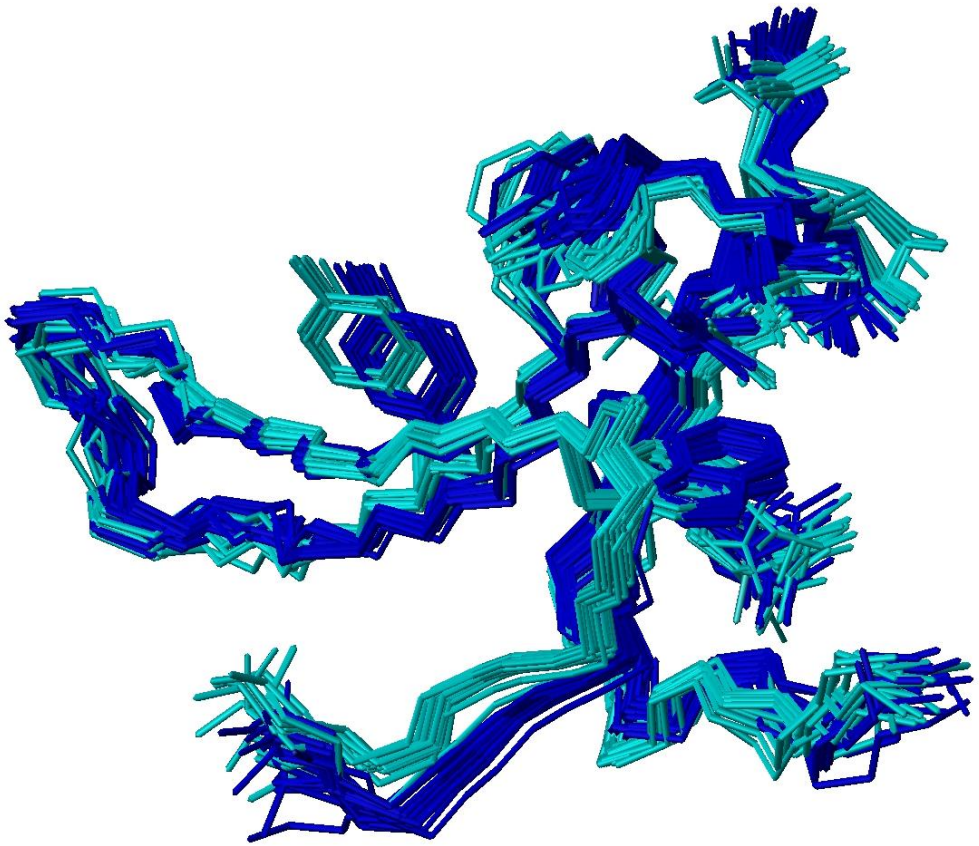
peptide FFpSPR is a positive allostery ligand



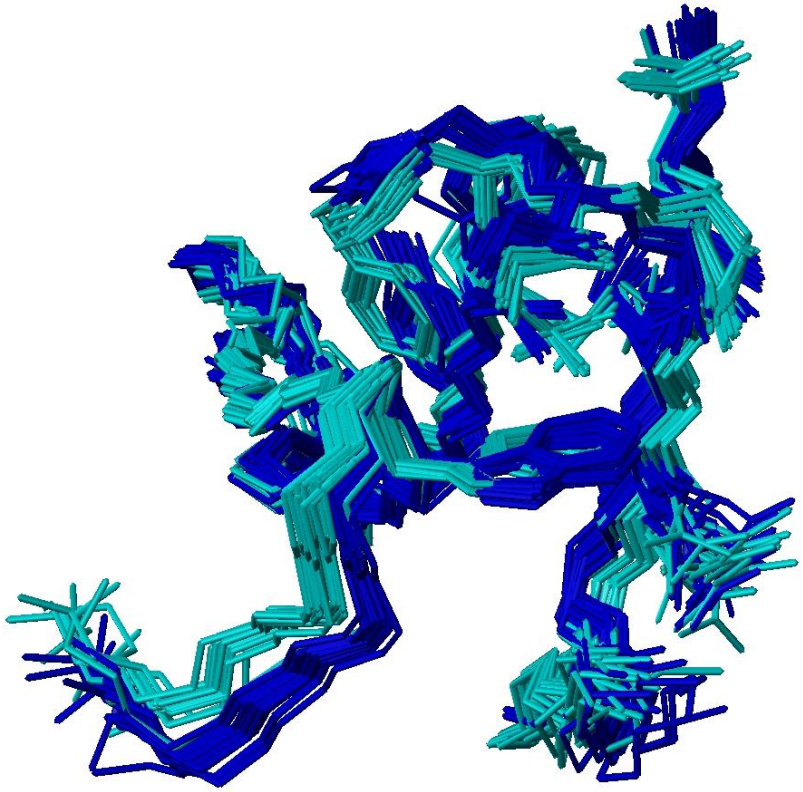
2 State eNOE Structure of ApoWW



2 State eNOE Structure of ApoWW



2 State eNOE Structure of ApoWW



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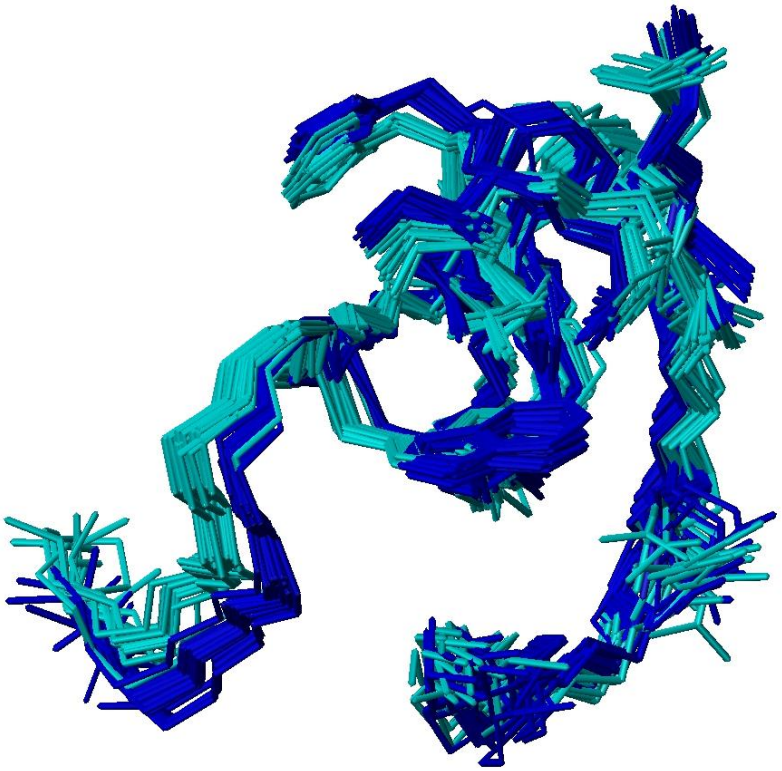
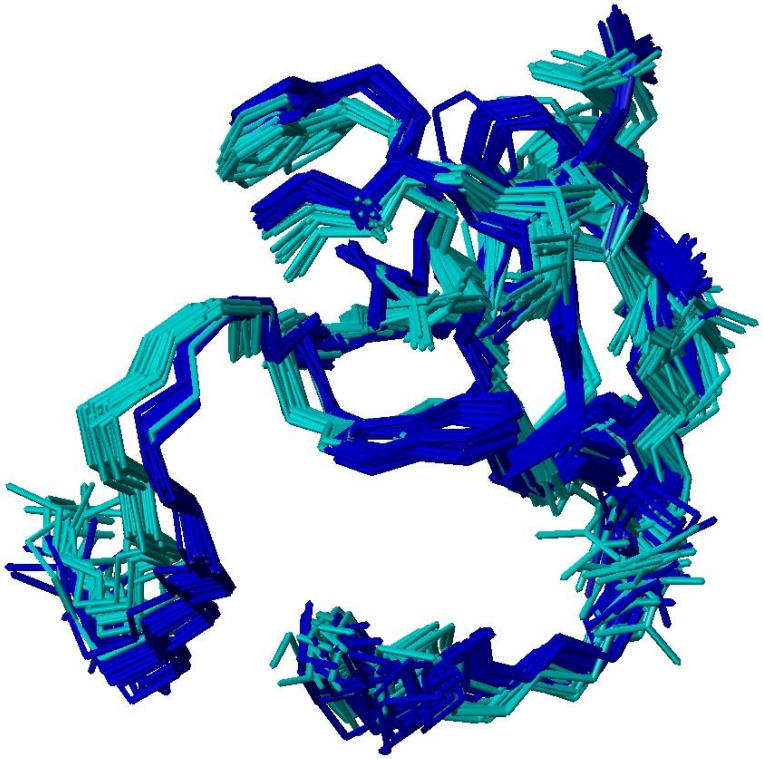
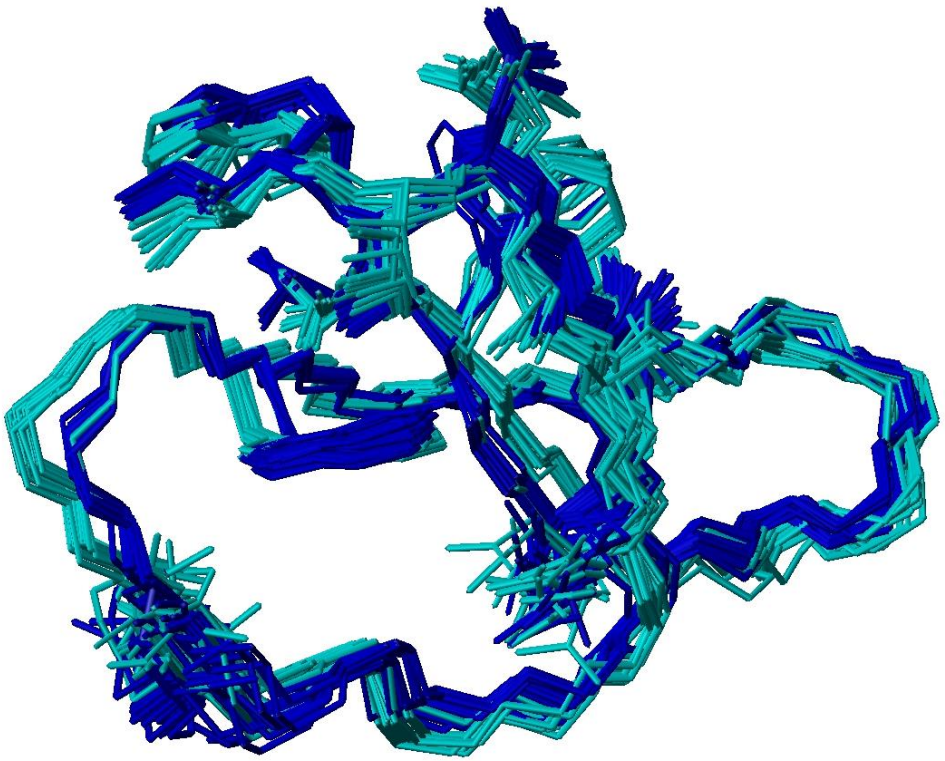


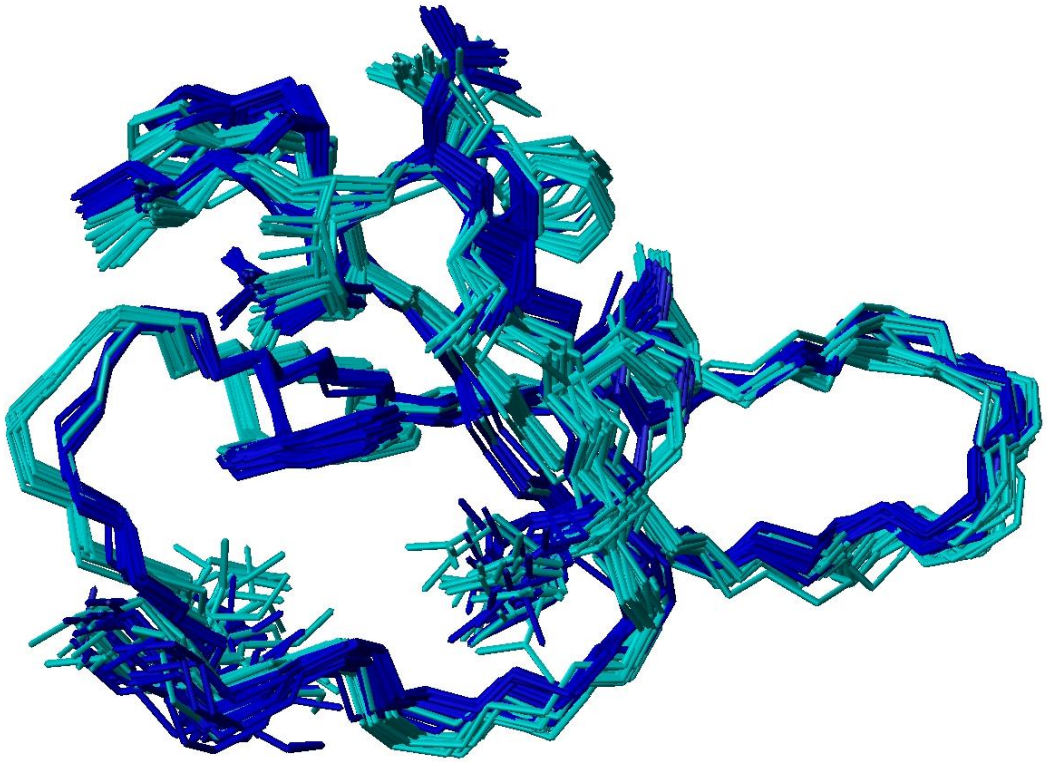
2 State eNOE Structure of ApoWW



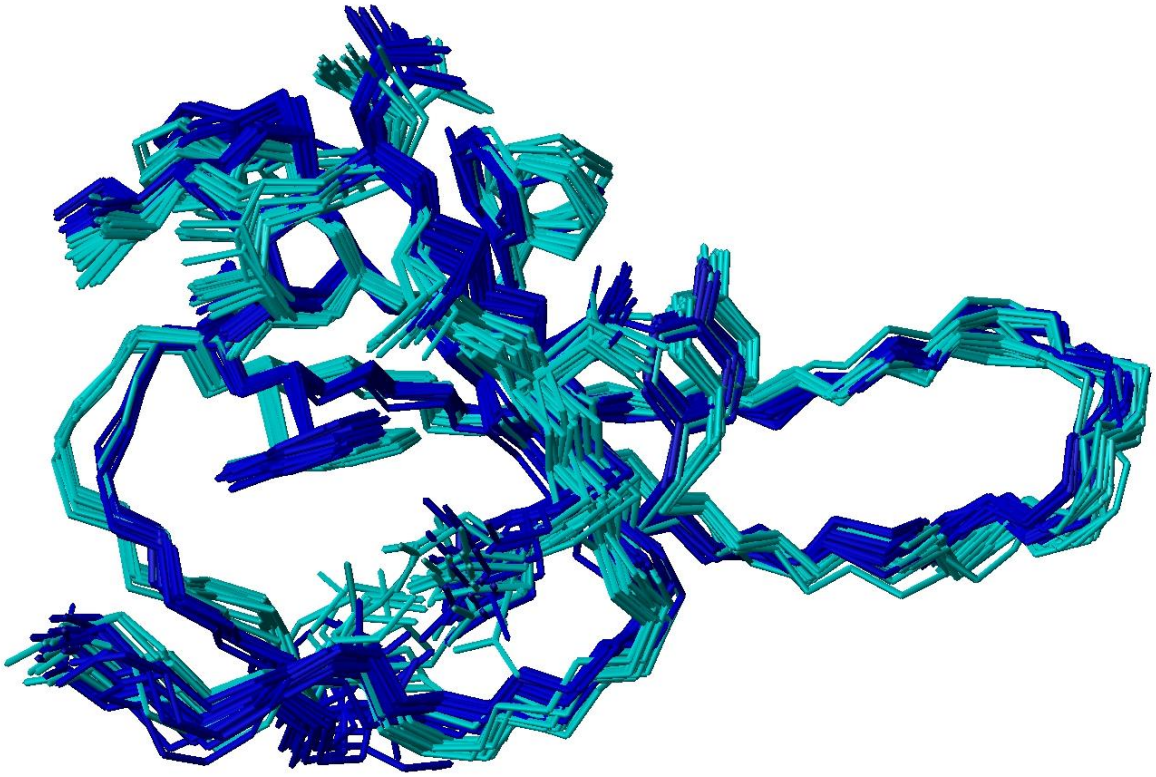
2 State eNOE Structure of ApoWW



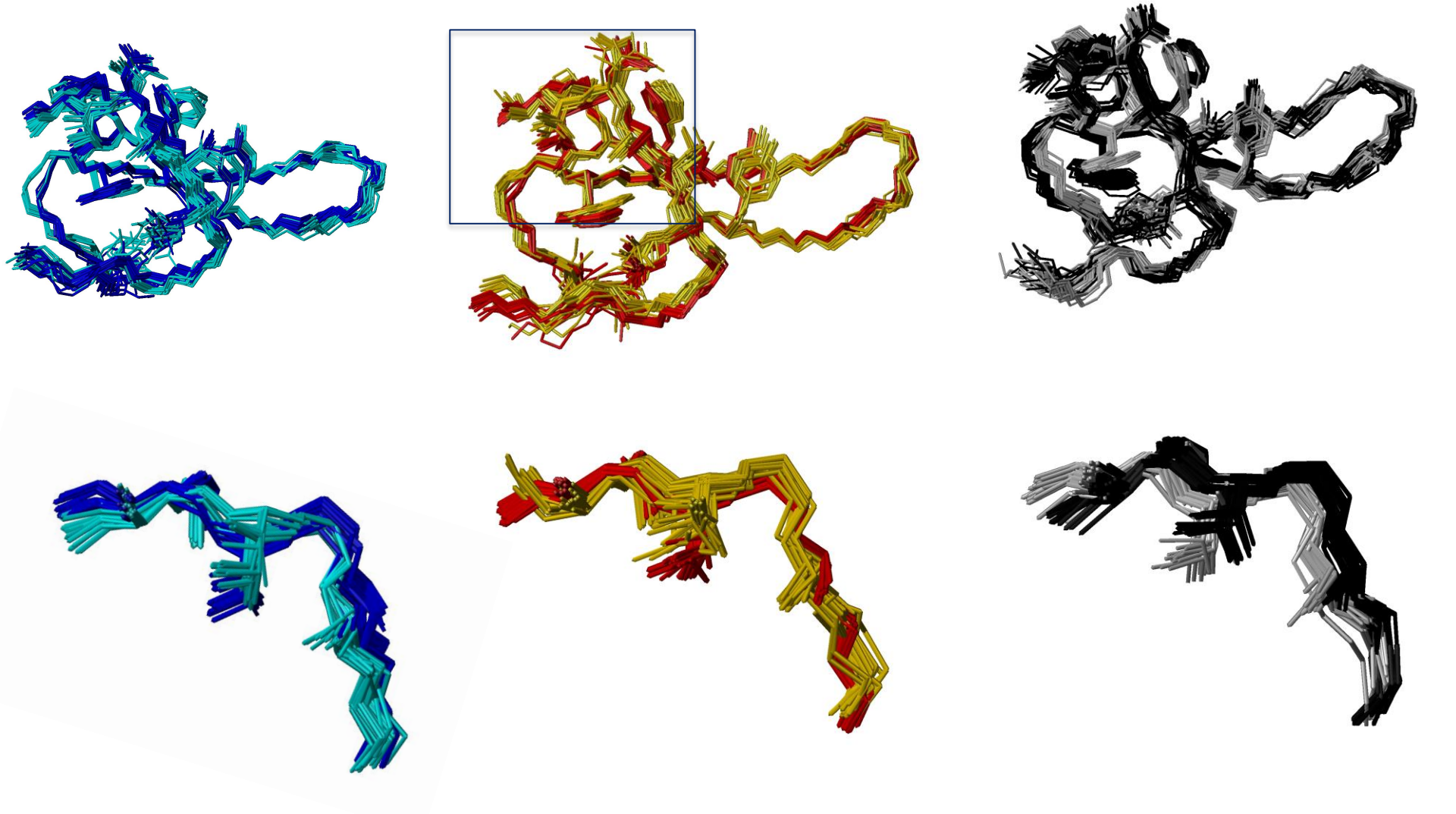
2 State eNOE Structure of ApoWW



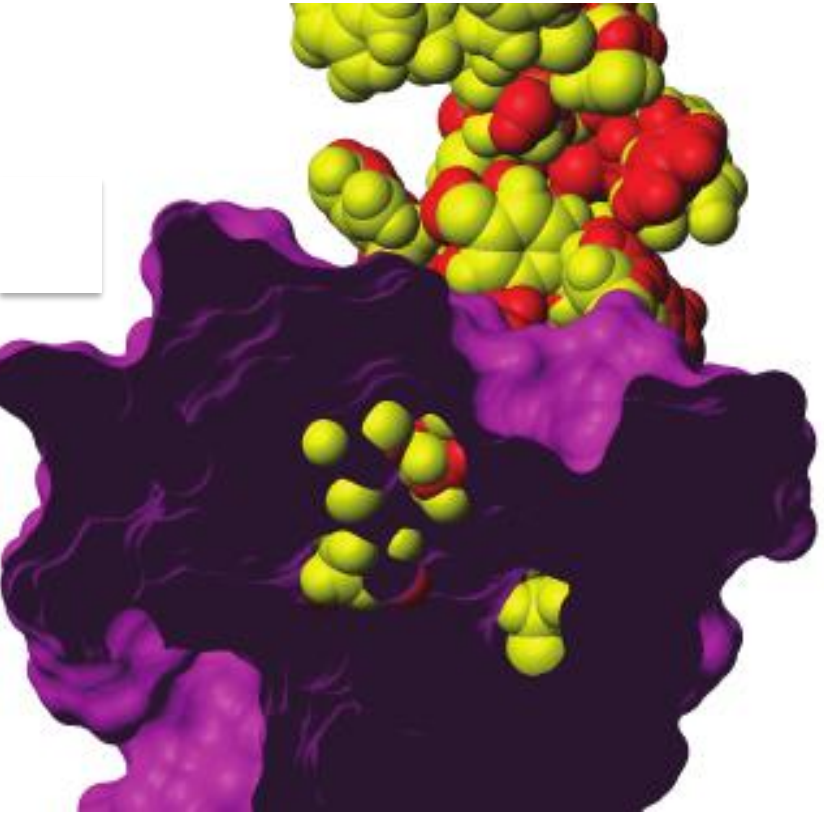
2 State (1:1) eNOE Structure of ApoWW

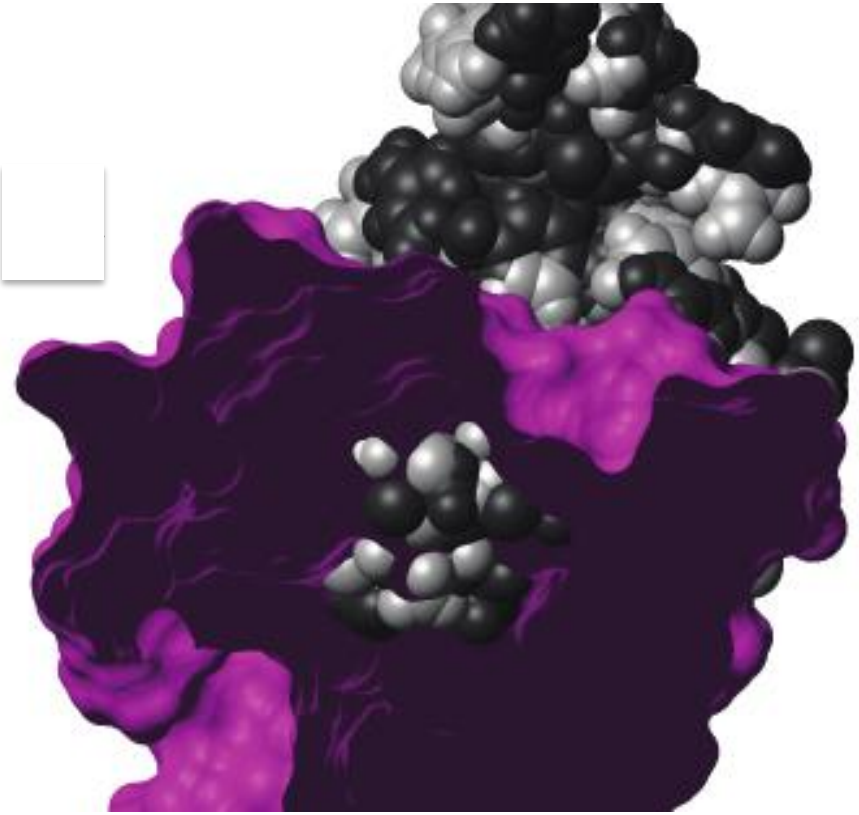


2 State eNOE Structure of pCdc25C and FFpSPR

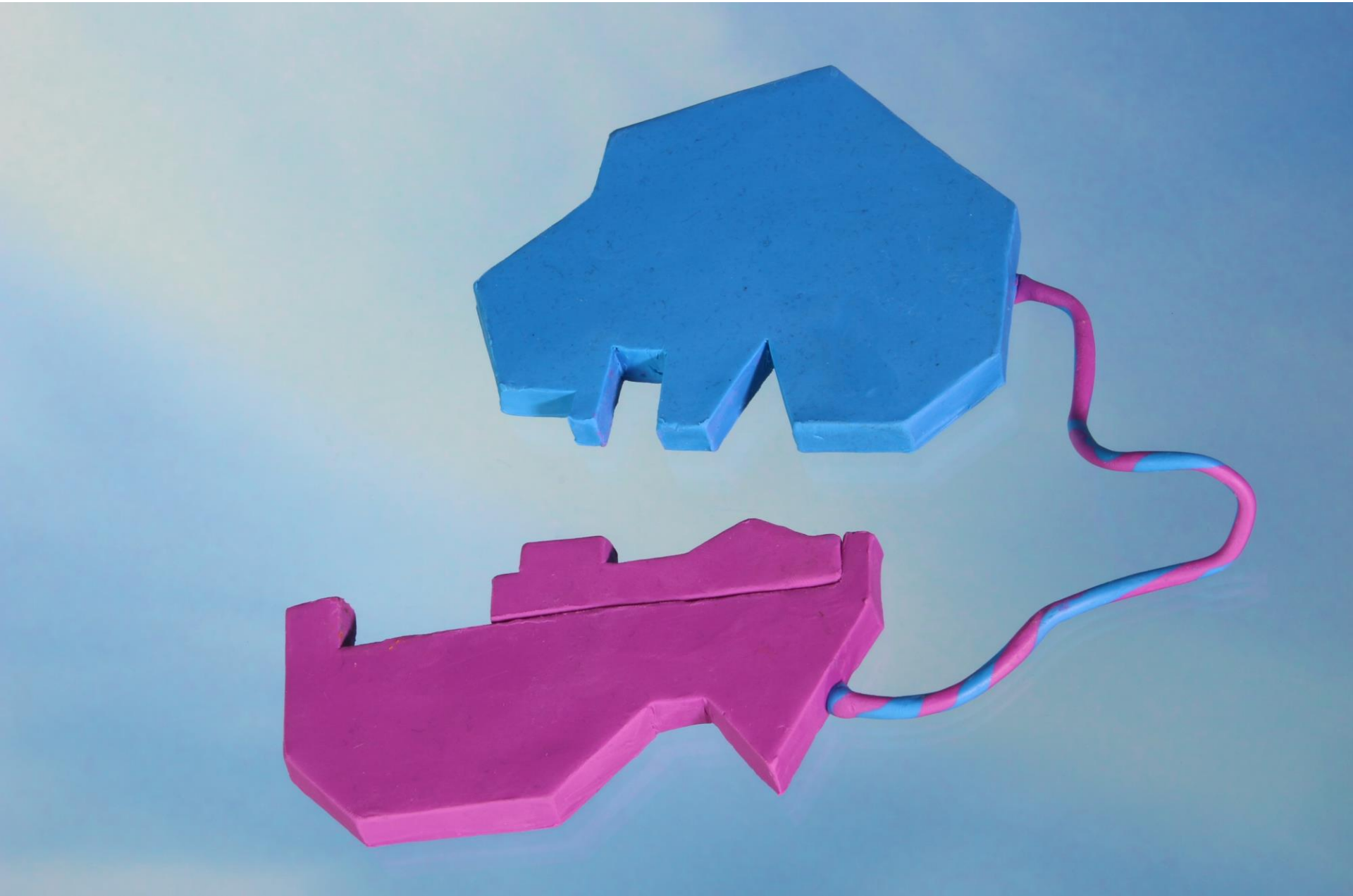


The two states in the context of the full-length Pin 1

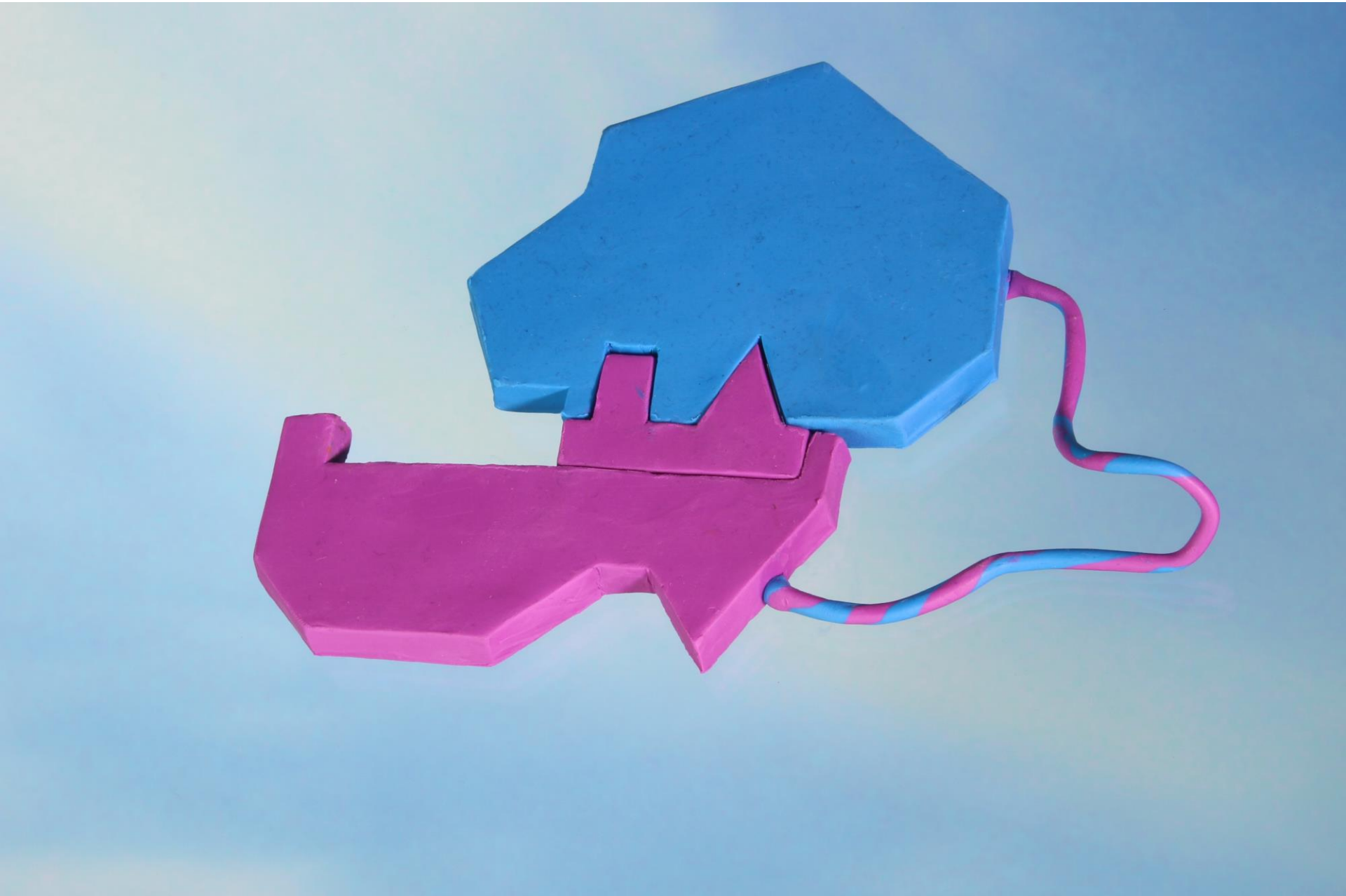




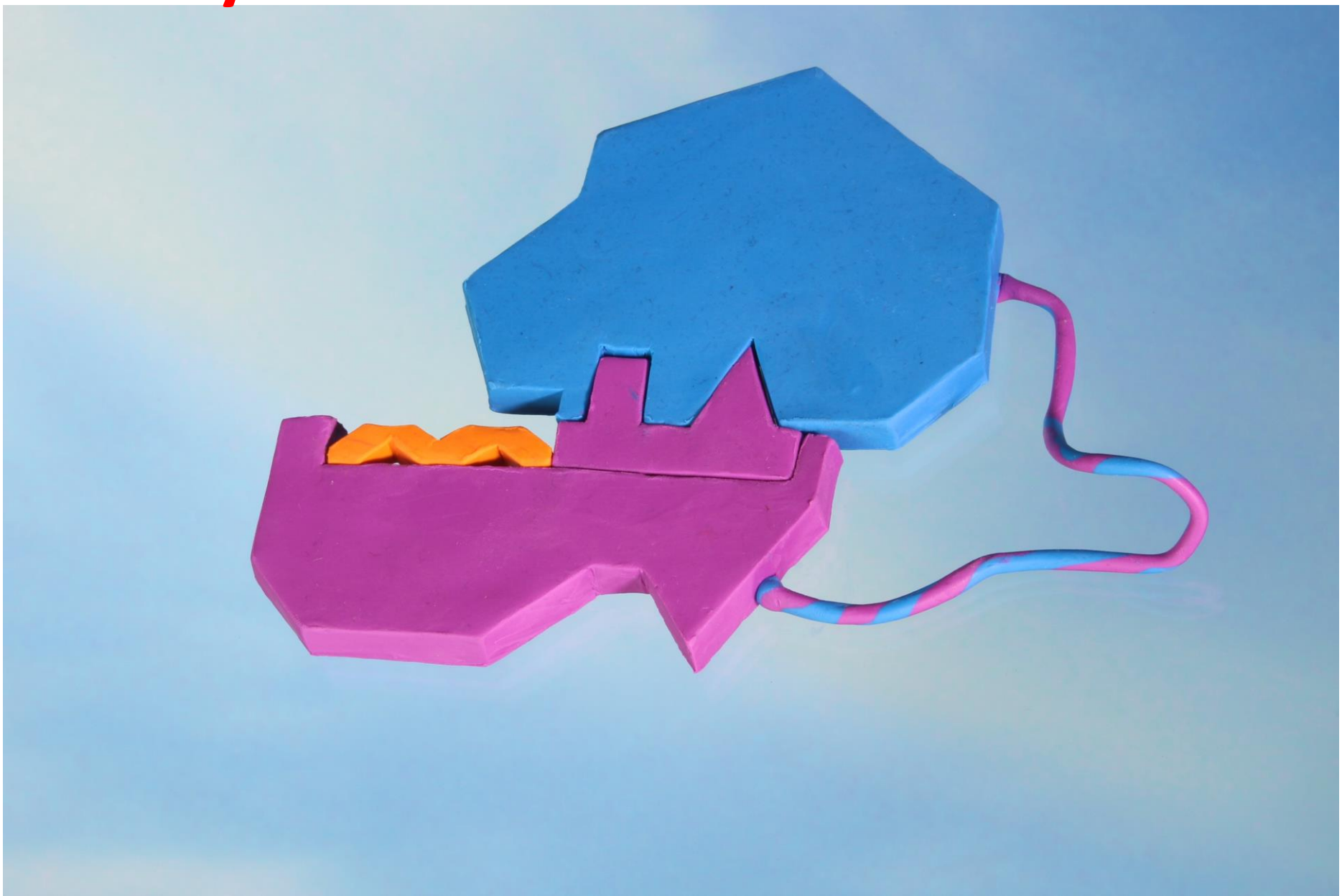
2 state of the apo form



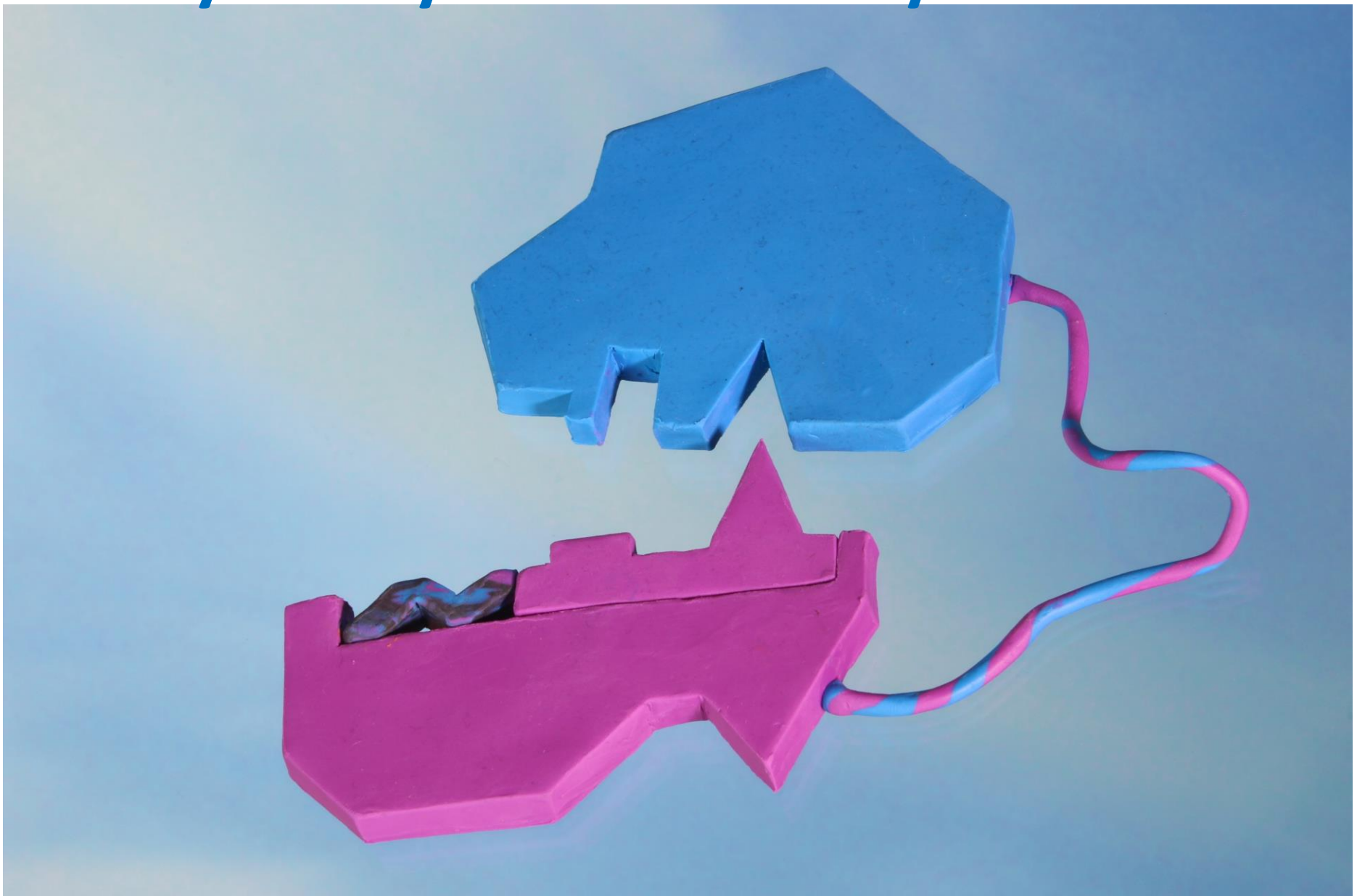
2 state of the apo form



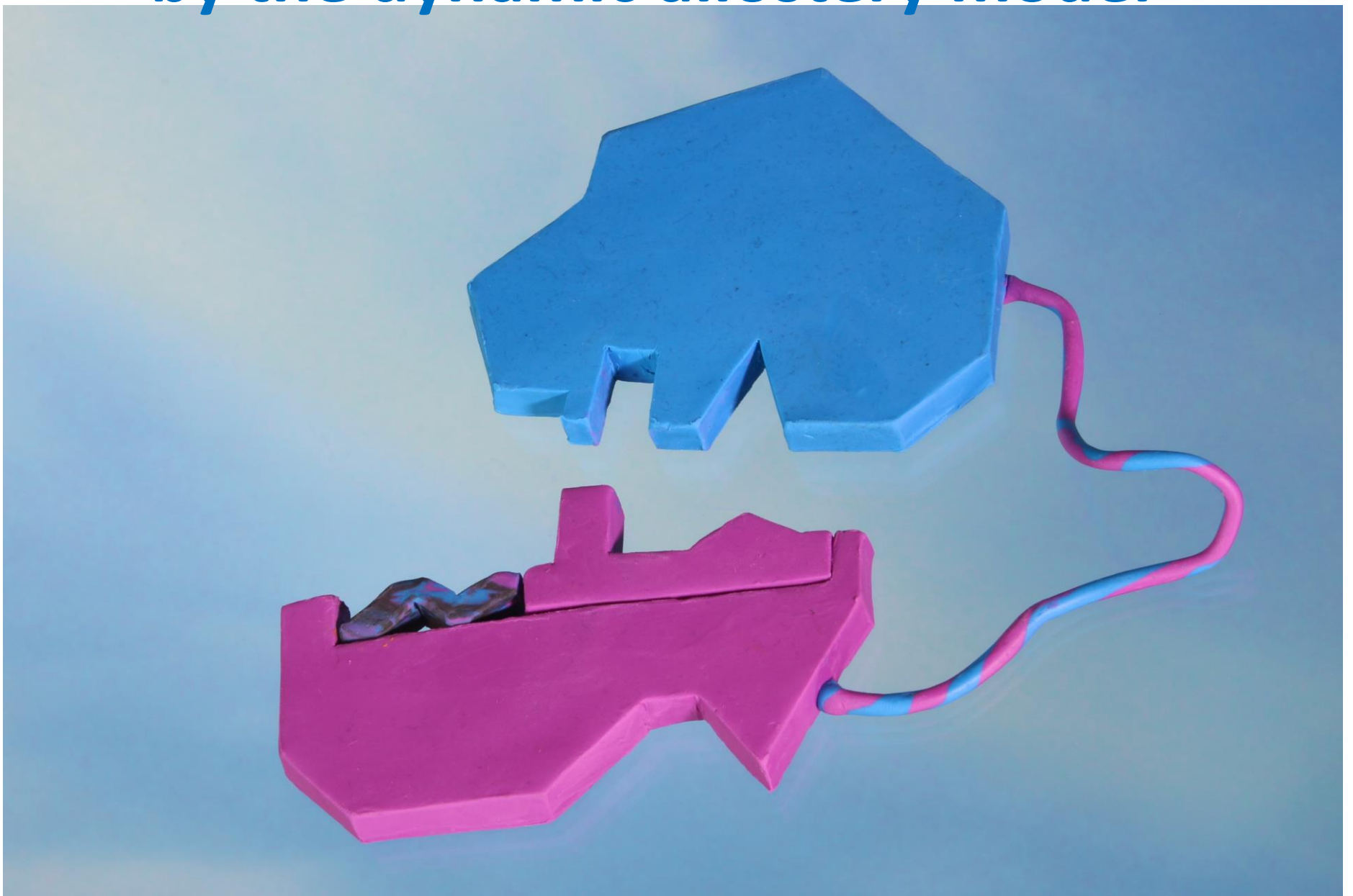
positive allostery ligand FFpSPR acts by Conformational Selection



negative allostery ligand pCdc25C acts by the dynamic allostery model



negative allostery ligand pCdc25C acts by the dynamic allostery model



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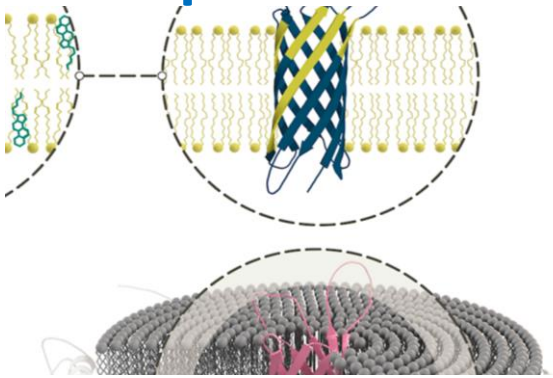
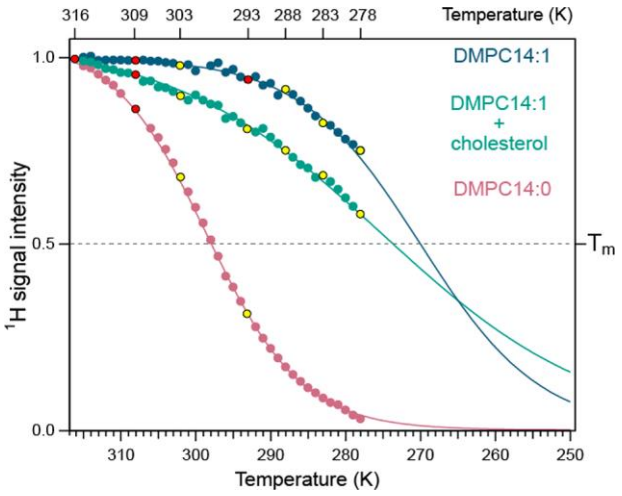
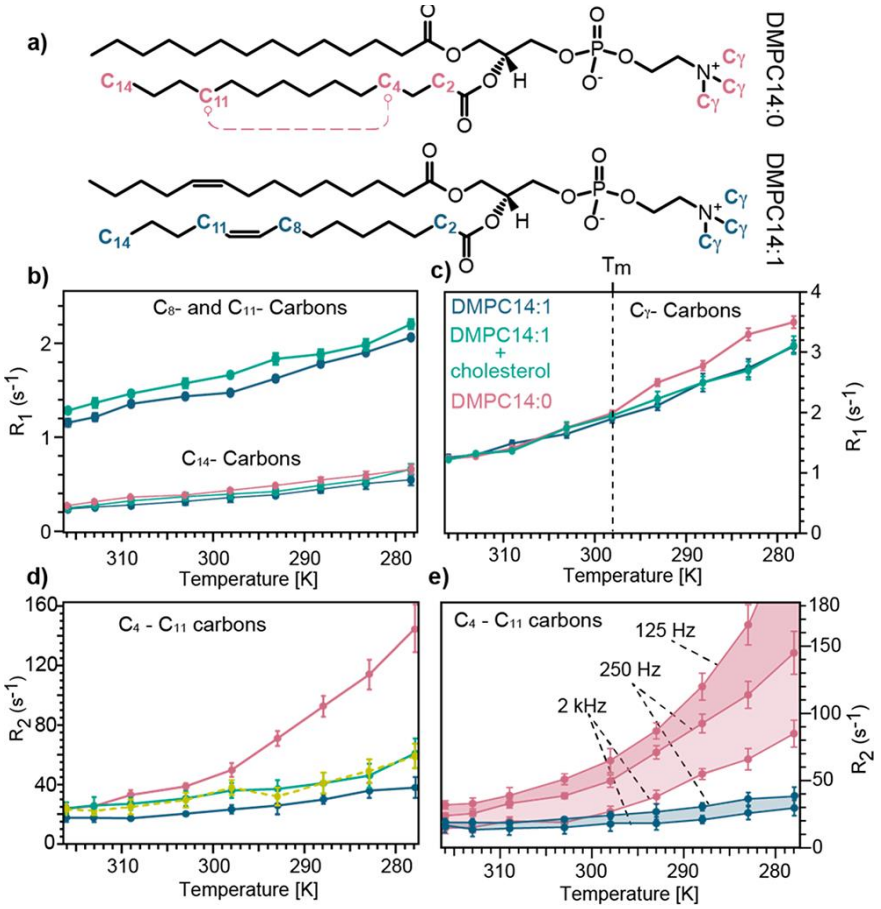
Dynamical interplay between membrane lipids and membrane protein

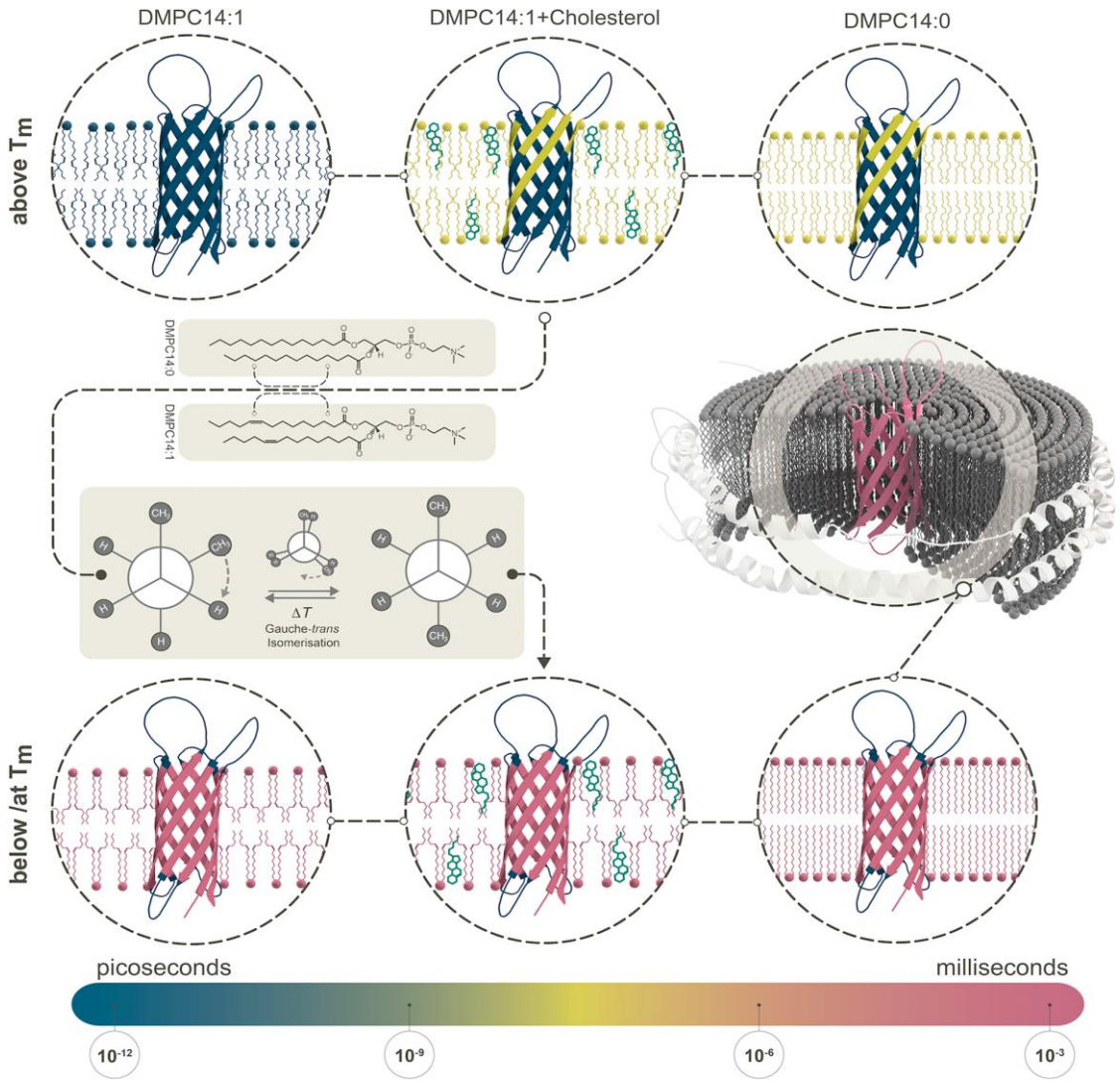






(Frey, Riek et al. JACS, 2018)

Dynamical interplay between membrane lipids and membrane protein



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Aim and Strategy of Our In-Cell NMR Research

- Protein delivery to cells by **electroporation** **for both folded and intrinsically disordered proteins** in **mammalian cells** and characterization by **in-cell NMR**.

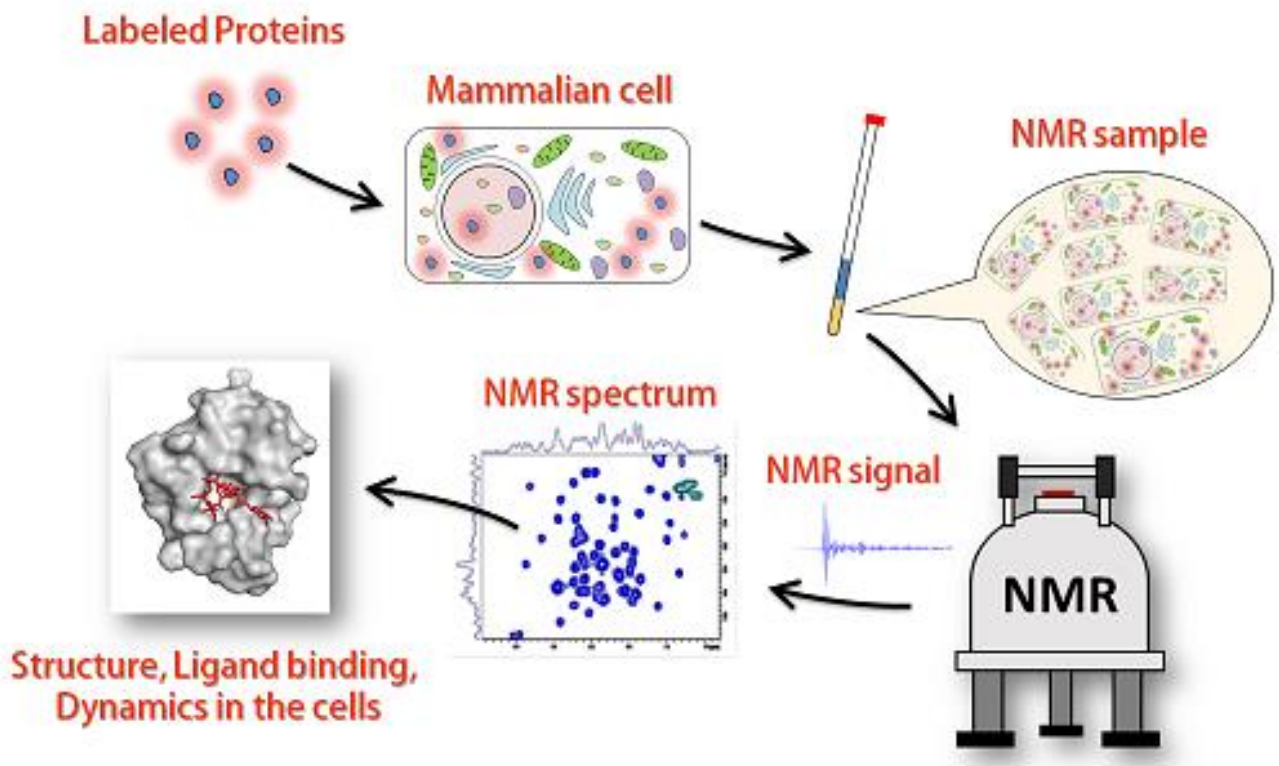
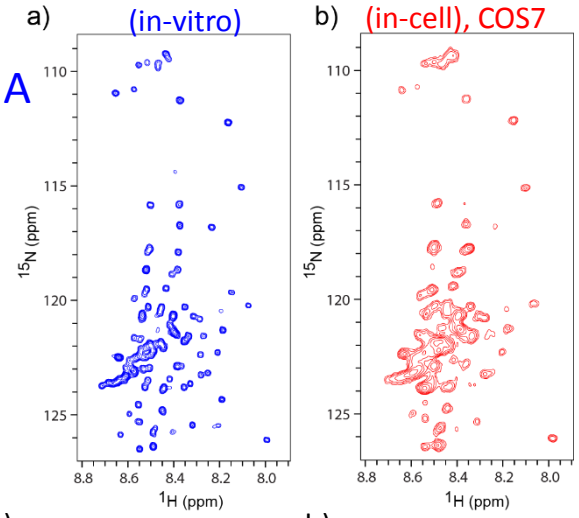


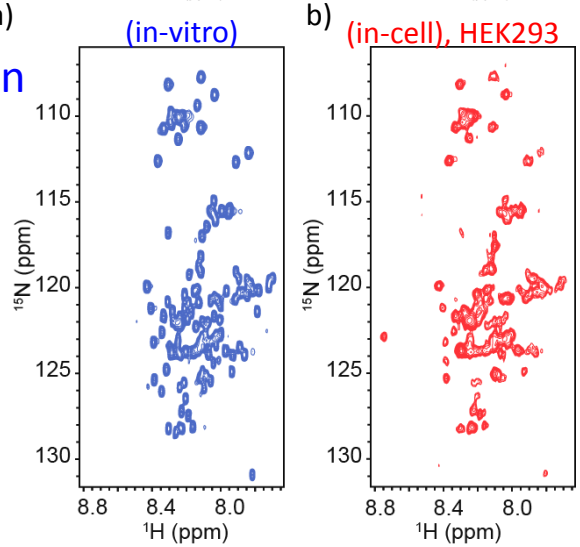
Image courtesy to http://mb.biophys.kyoto-u.ac.jp/research.html

In-Cell NMR Structure of GB1

PTMA



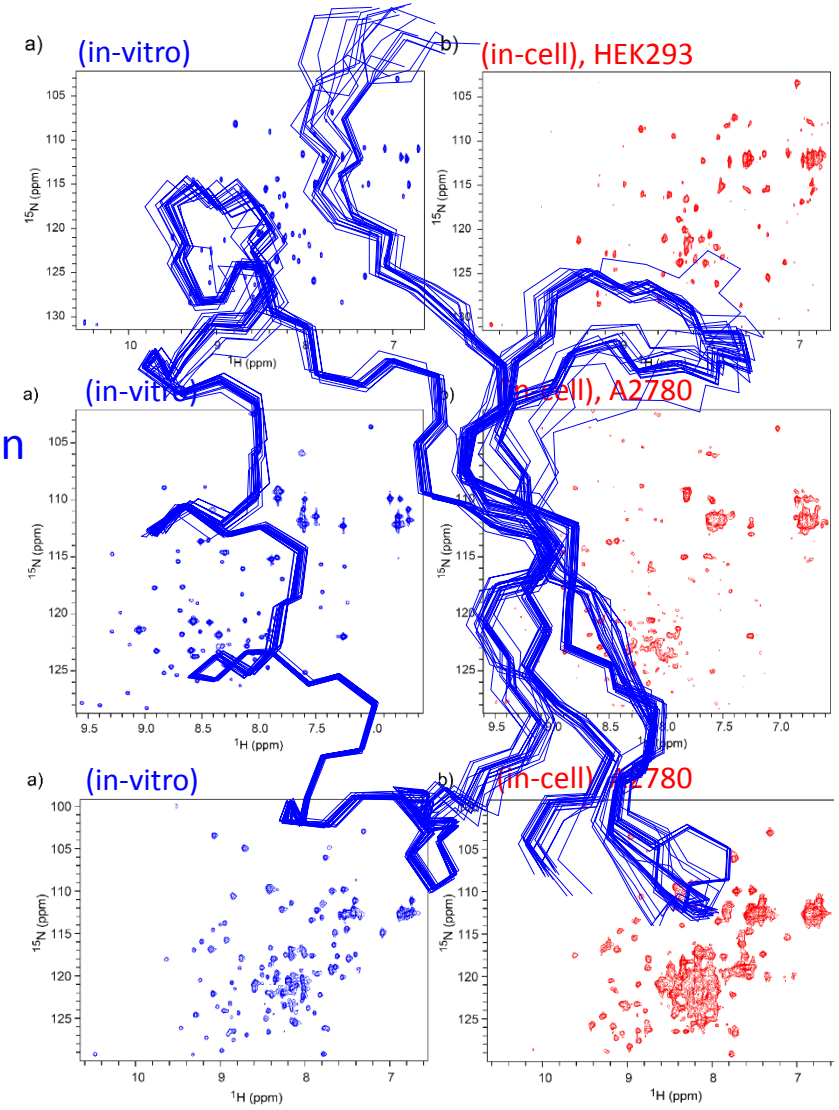
αSyn



GB1

Ubiquitin

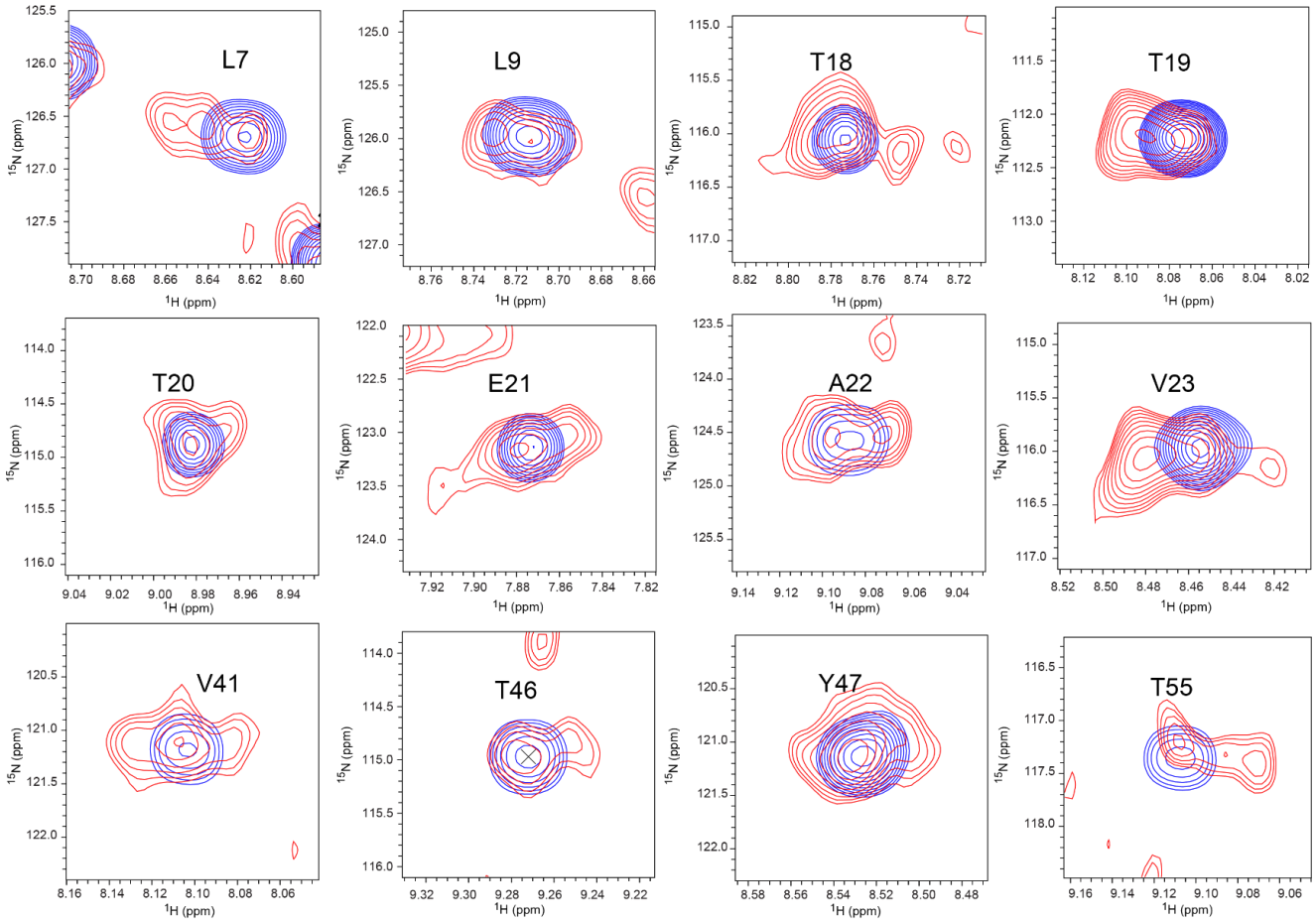
PDZ2



Multiplicity of GB1 Resonances in Living Cells

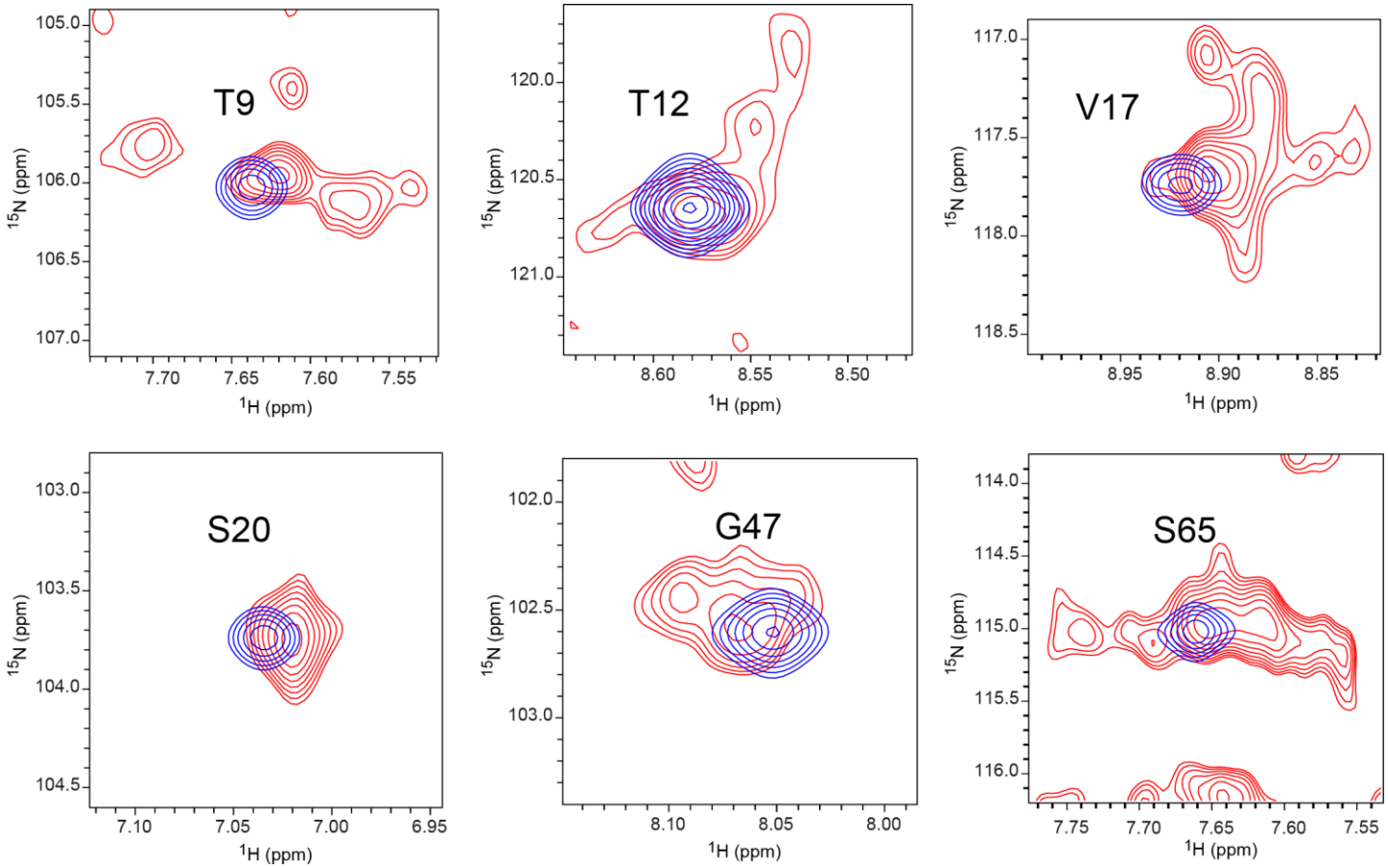
^{1}MGTYK**L**I**L**N GKTLKGE**TTT EAV**DAATAEKVFKQYANDNG**V**DGEW**TY**DDATKTF**T**VT^{57}

GB1 (in-vitro) GB1 (in-cell), HEK 293



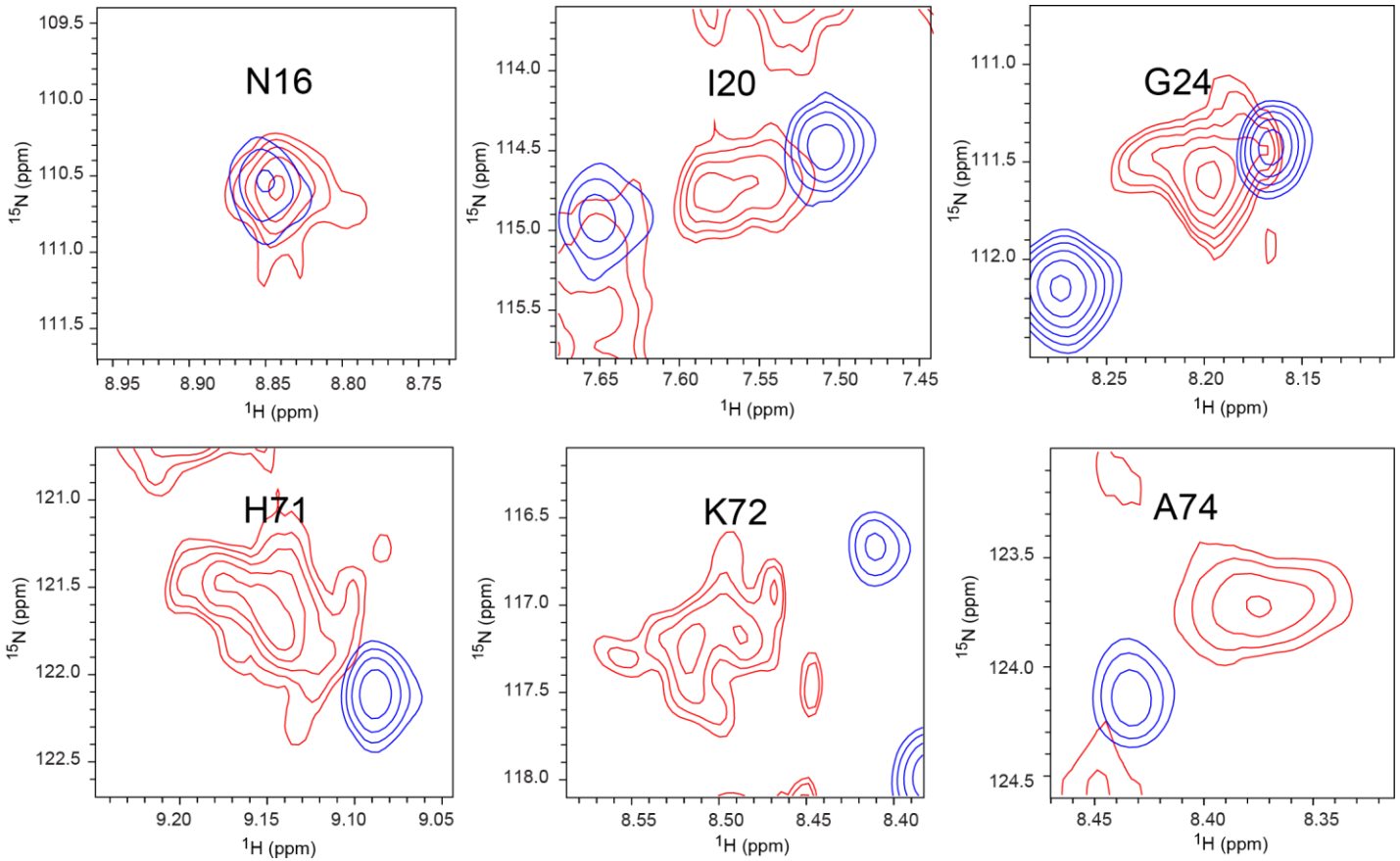
Multiplicity of Ubiquitin Resonances

Ubiquitin (in-vitro) Ubiquitin (in-cell), A2780

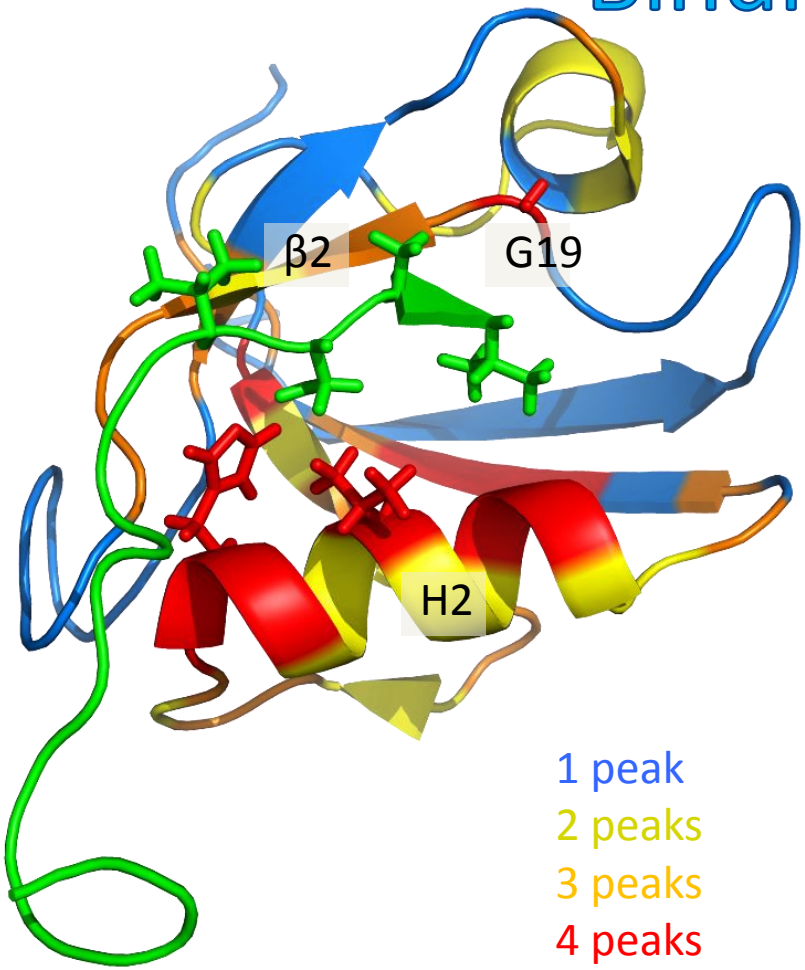


Multiplicity and Chemical Shift Perturbation of PDZ2 Domain Resonances

PDZ2 (in-vitro) PDZ2 (in-cell), A2780

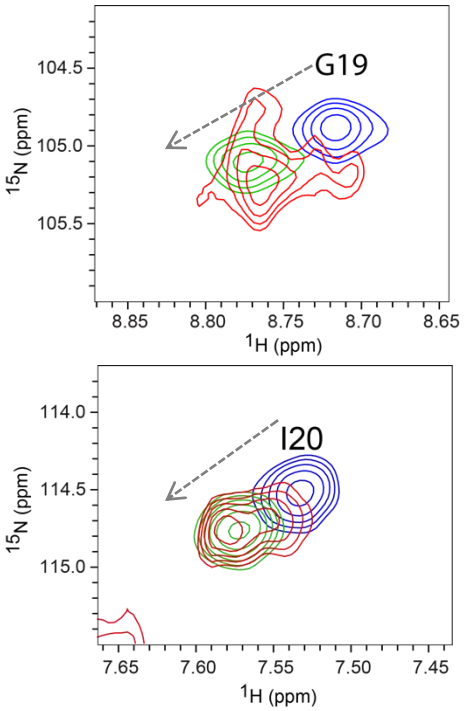


Correlation Between Multiplicity of PDZ2 Domain Residues and Ligand Binding Site



Residues G19, I20, S21 and V22 etc. are shifted in the in-vitro titration of PDZ2 with the C-terminal peptide (**Ac-ENEQVSAV**) of RA-GEF-2.

PDZ2 PDZ2 (in-cell) Complex:PDZ2/Ligand



PDB: 1D5G

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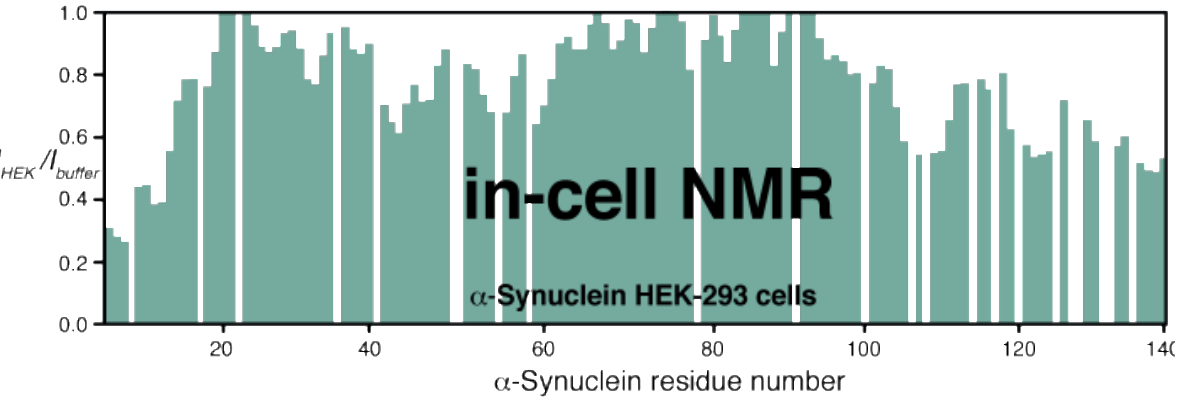
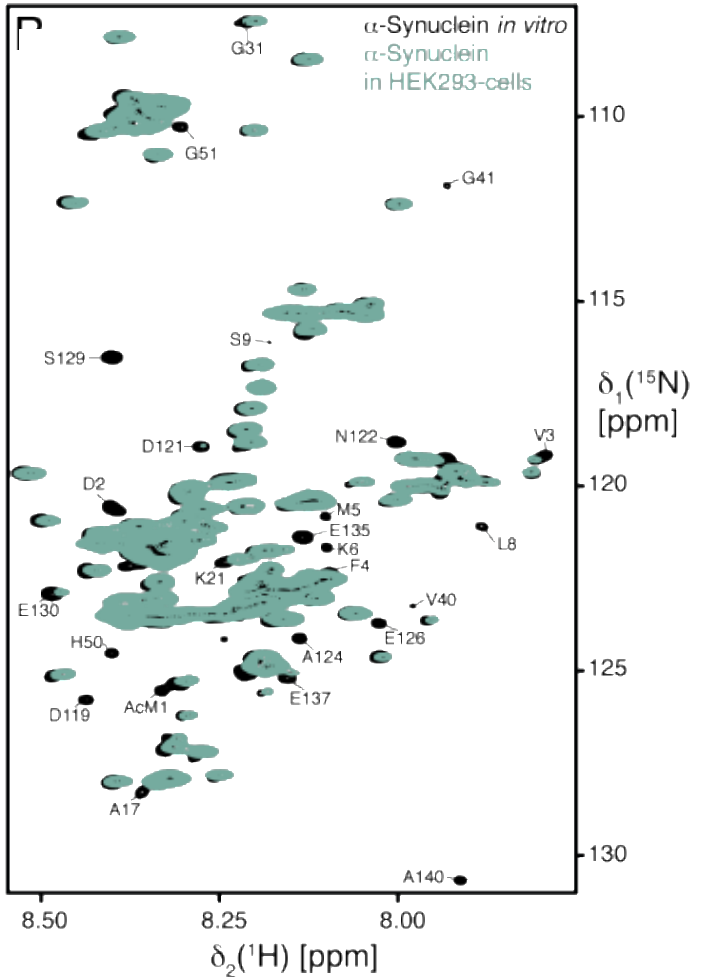
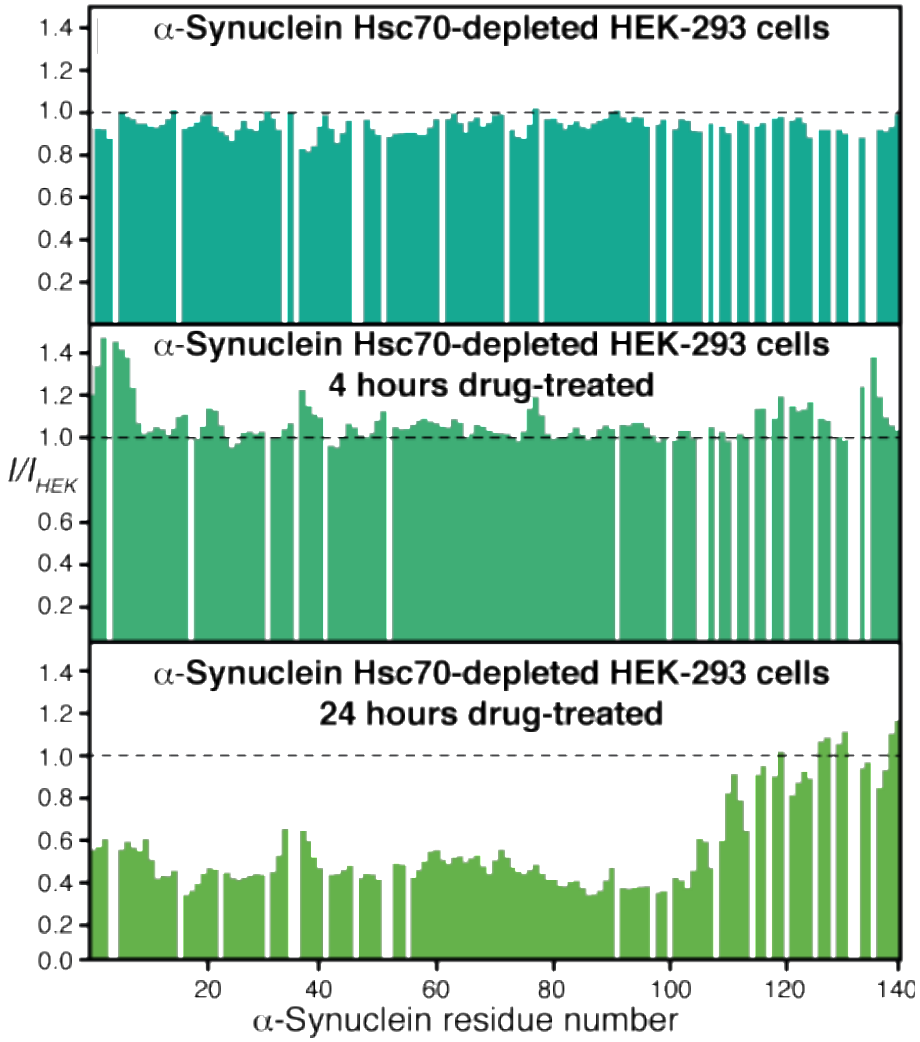
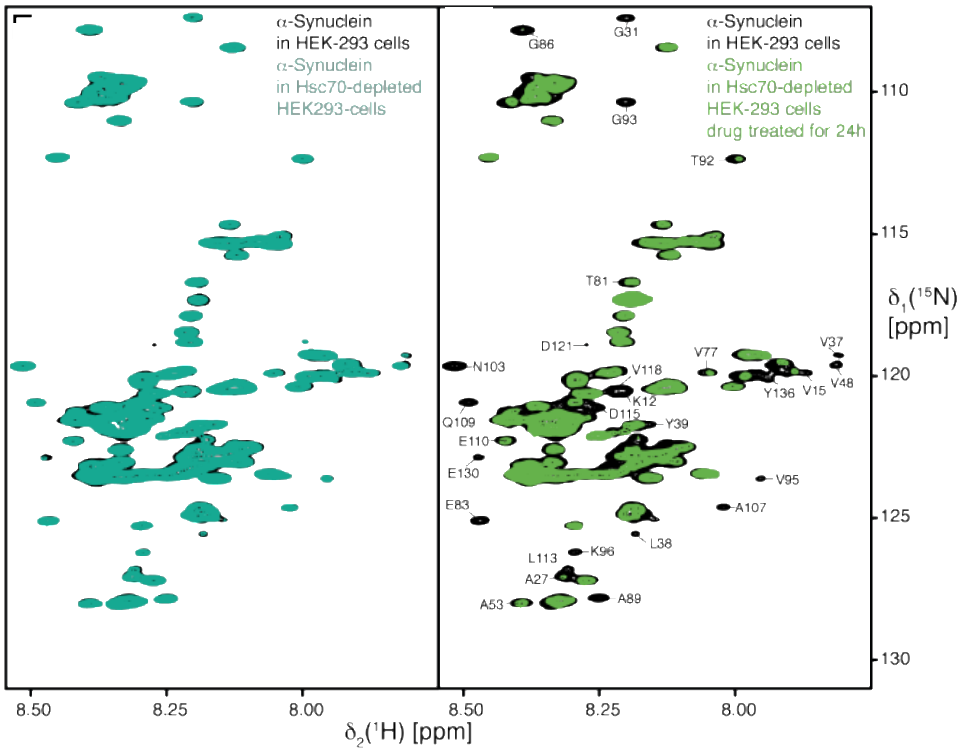
In cell NMR of recombinant ^{15}N-labeled α-Synuclein electroporated into HEK-293 cells (Buhrmann, Gerez, Riek, Hiller et al., Nature, 2020)





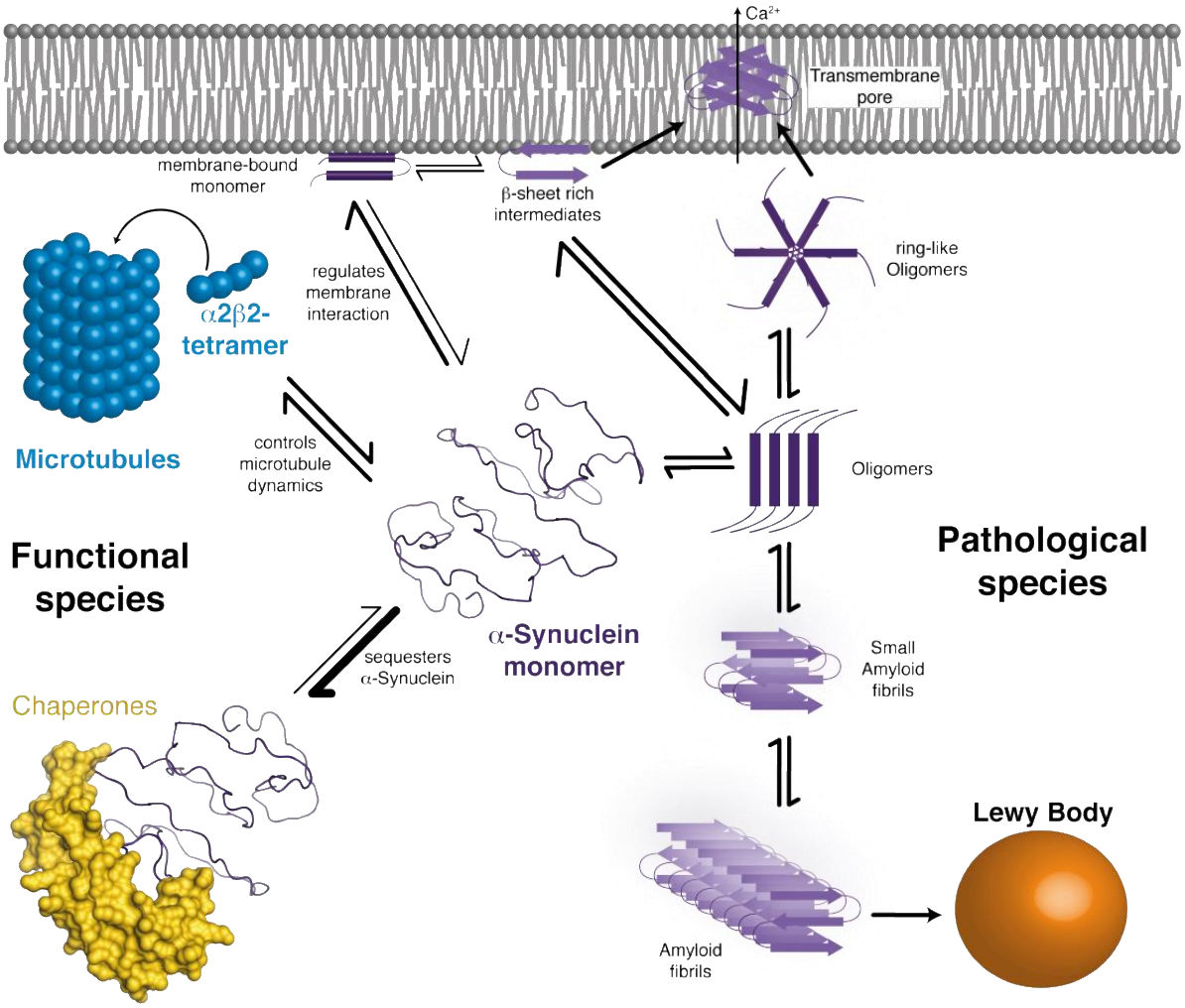
n Cell NMR HEK293 versus Hsc70-depleted and Hsp90 drug-inactivated HEK-293





1 µM Geldanamycin, and 1 µM Radicicol to suppress combined Hsc70 and Hsp90β chaperone expression

The importance of the transient interaction of chaperones with α-Synuclein



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Acknowledgments

Cyclophilin

Chi Chelestine

Beat Vögeli

Julien Orts

Peter Güntert

Membrane protein Dynamics

Lukas Frey

Stefan Bibow

In cell NMR

Bjorn Bormann, Sebastian Hiller

Juan Gerez

Hari Kadavath

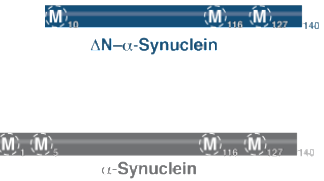
WW

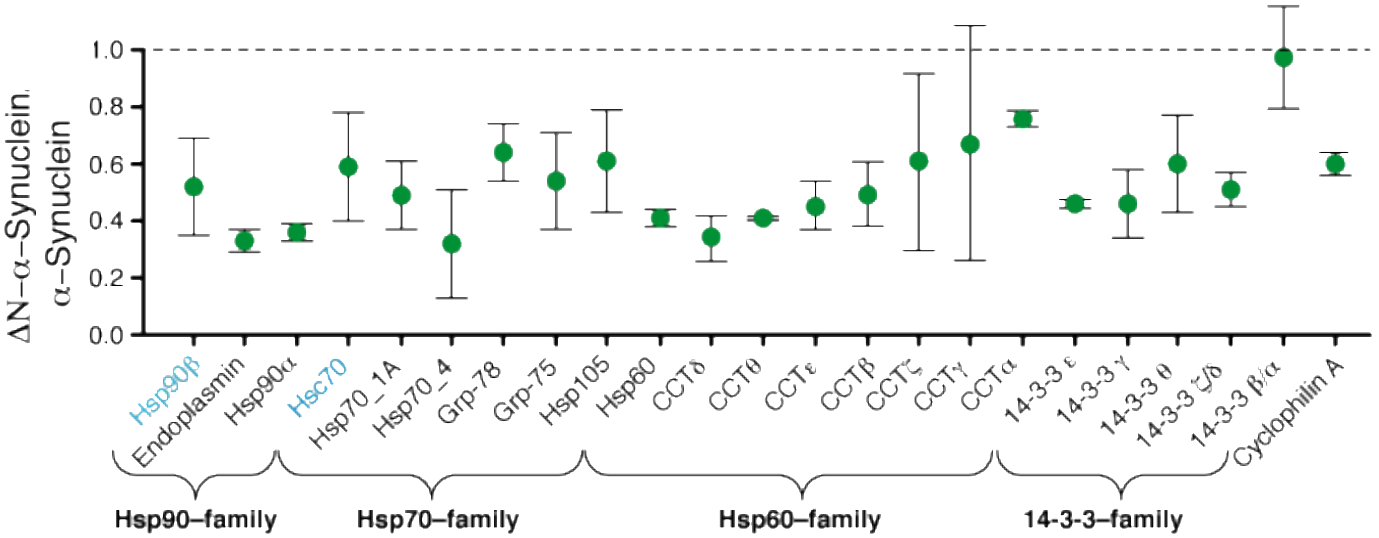
Dean Strotz

Beat Vögeli

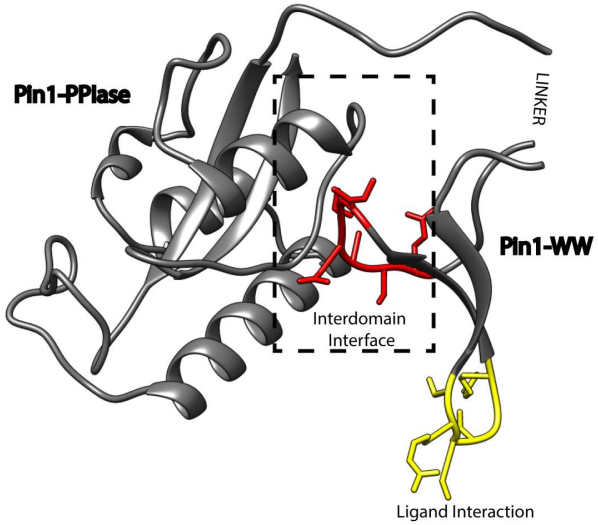
Peter Güntert

Proteomics Approach of HA-tagged α-Synuclein chaperone interaction in HEK-293 cells



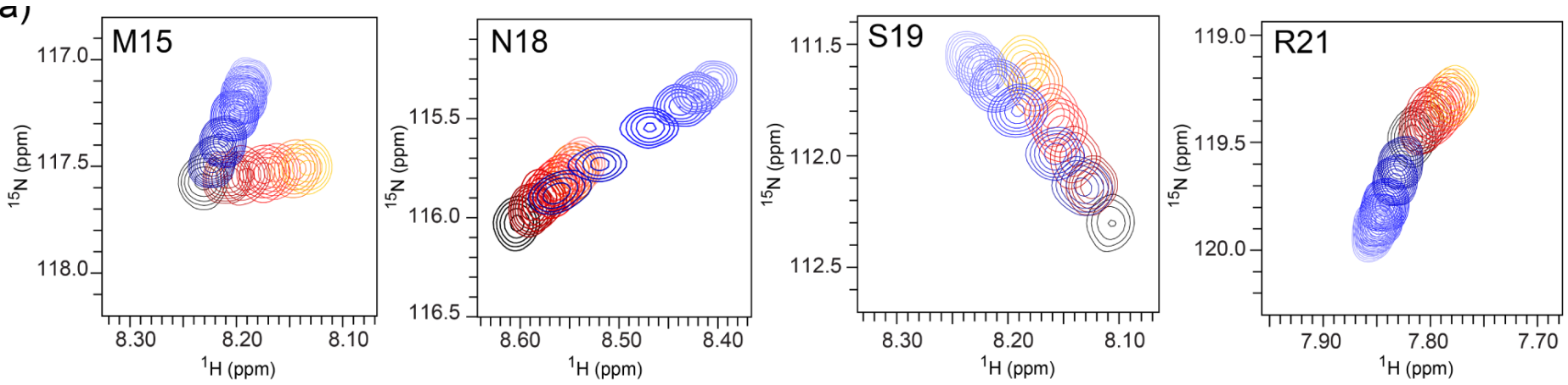


Allostery of a WW domain of Pin1

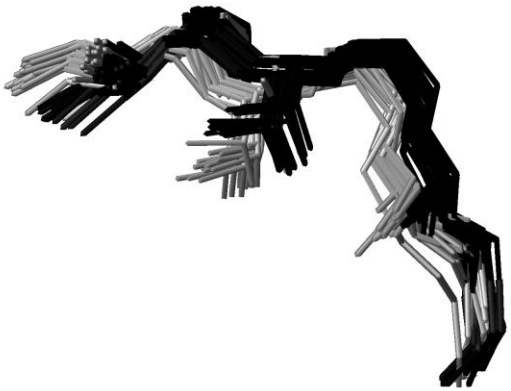


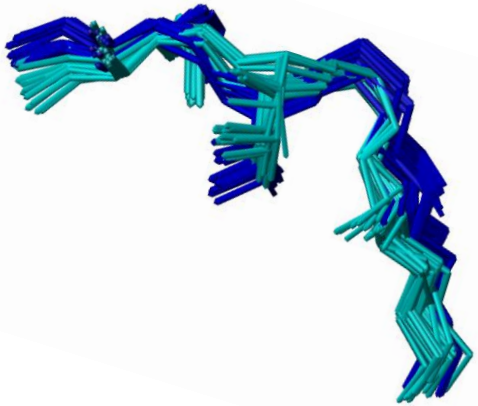
peptide pCdc25C is a negative allostery ligand

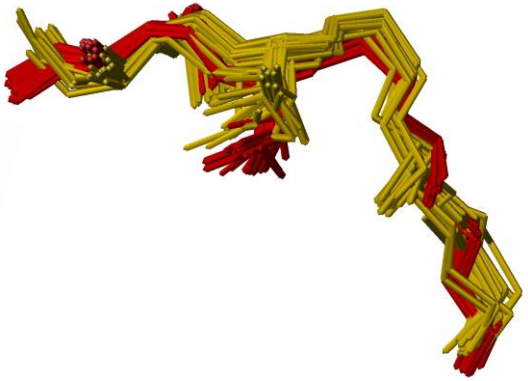
peptide FFpSPR is a positive allostery ligand

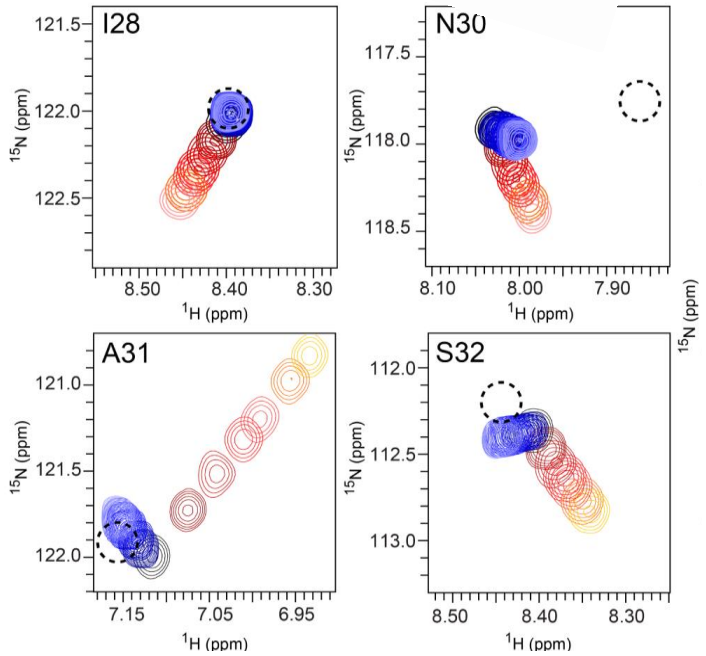


2 State eNOE Structure of pCdc25C (1:1) and FFpSPR (1:4) and Apo (1:1)



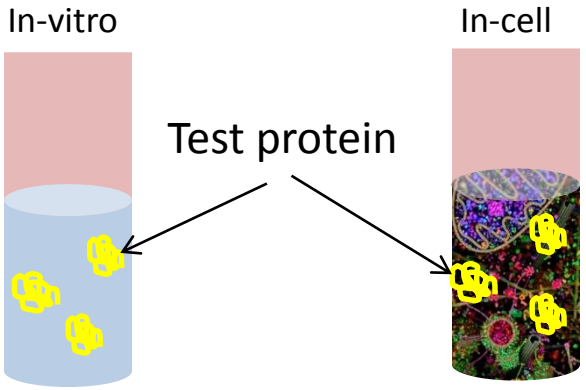


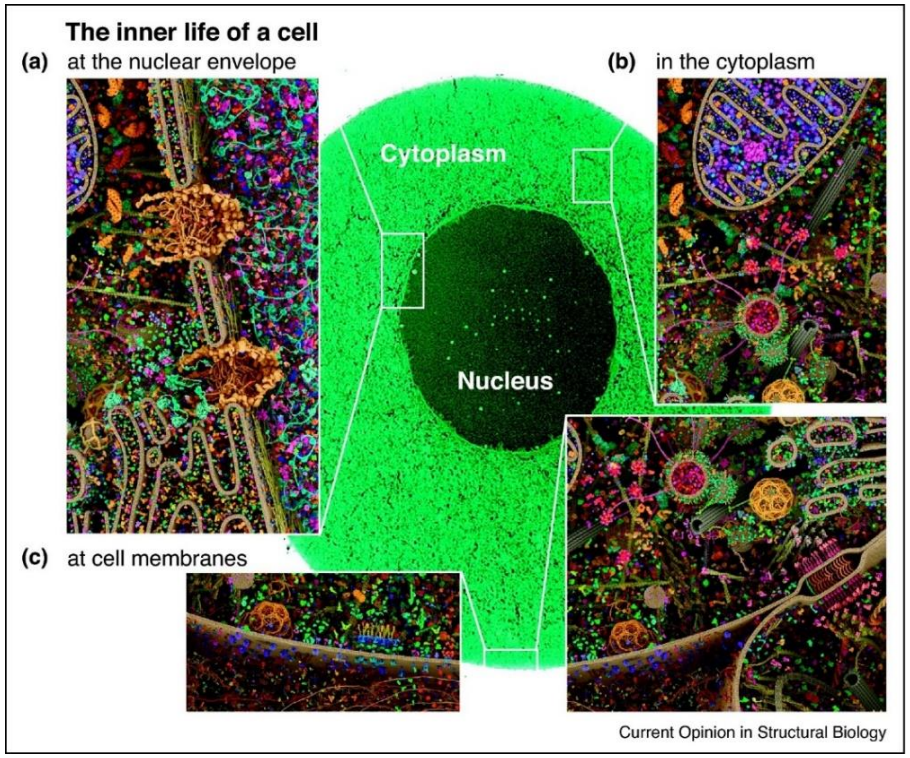




Multiple Structures in vivo?

- In in-vitro studies, molecules may behave differently than in the intracellular millieu.





Plitzko JM et al, *Curr. Opin. Struct. Biol.* (2017)

- The intracellular environment of eukaryotes is of complex heterogeneuos nature containing many cellular machines, protein filaments of cytoskeletal proteins such as microtubules and actin, membranes, etc.

In-cell NMR