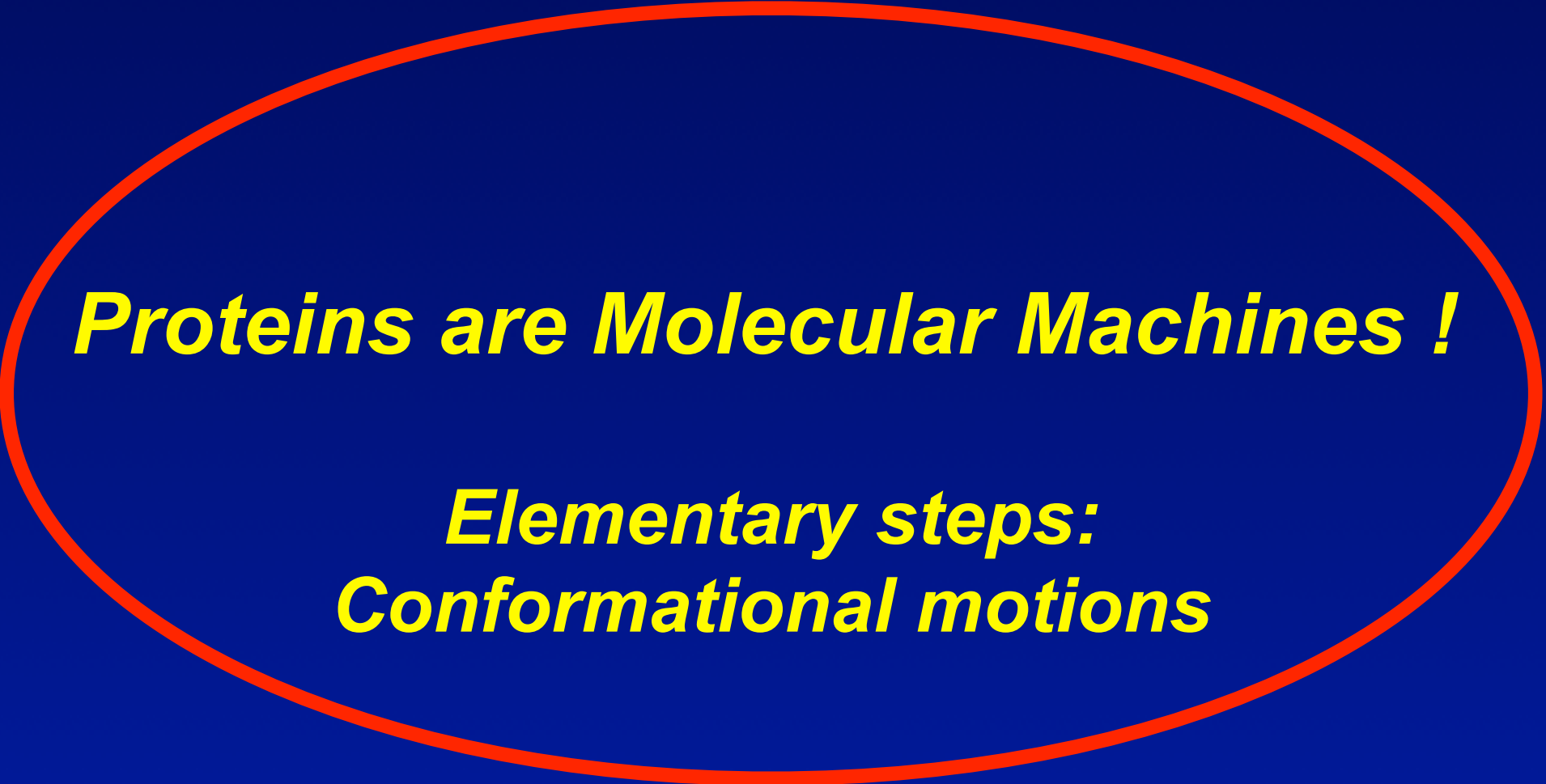




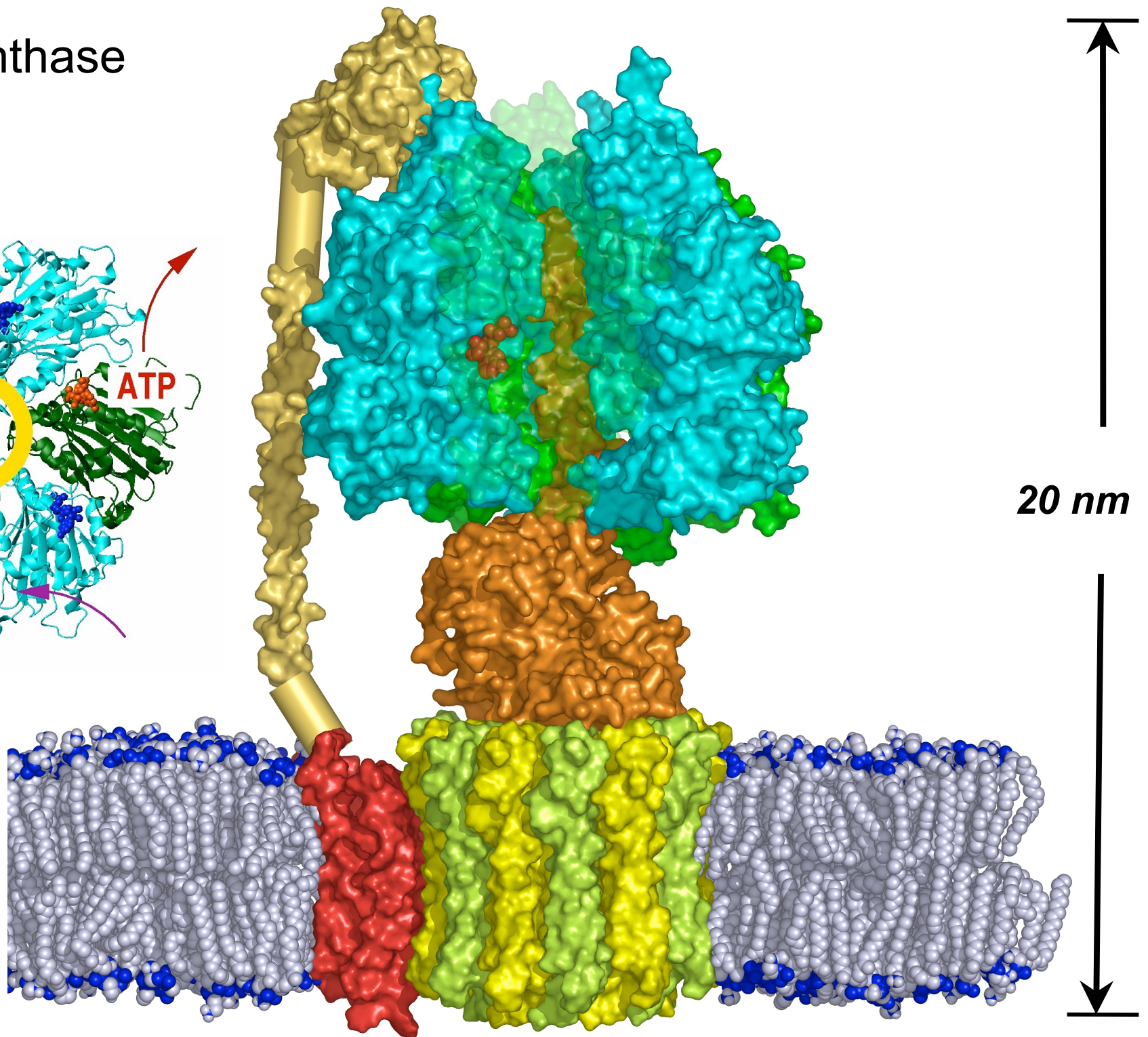
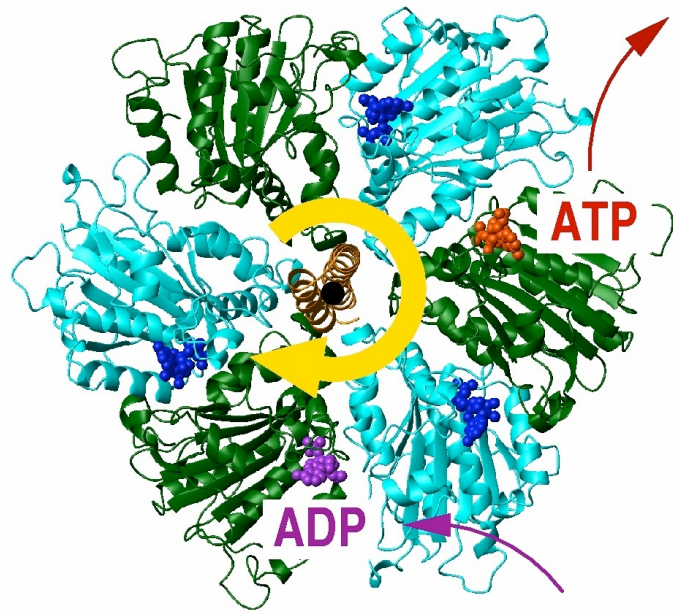
Positions available



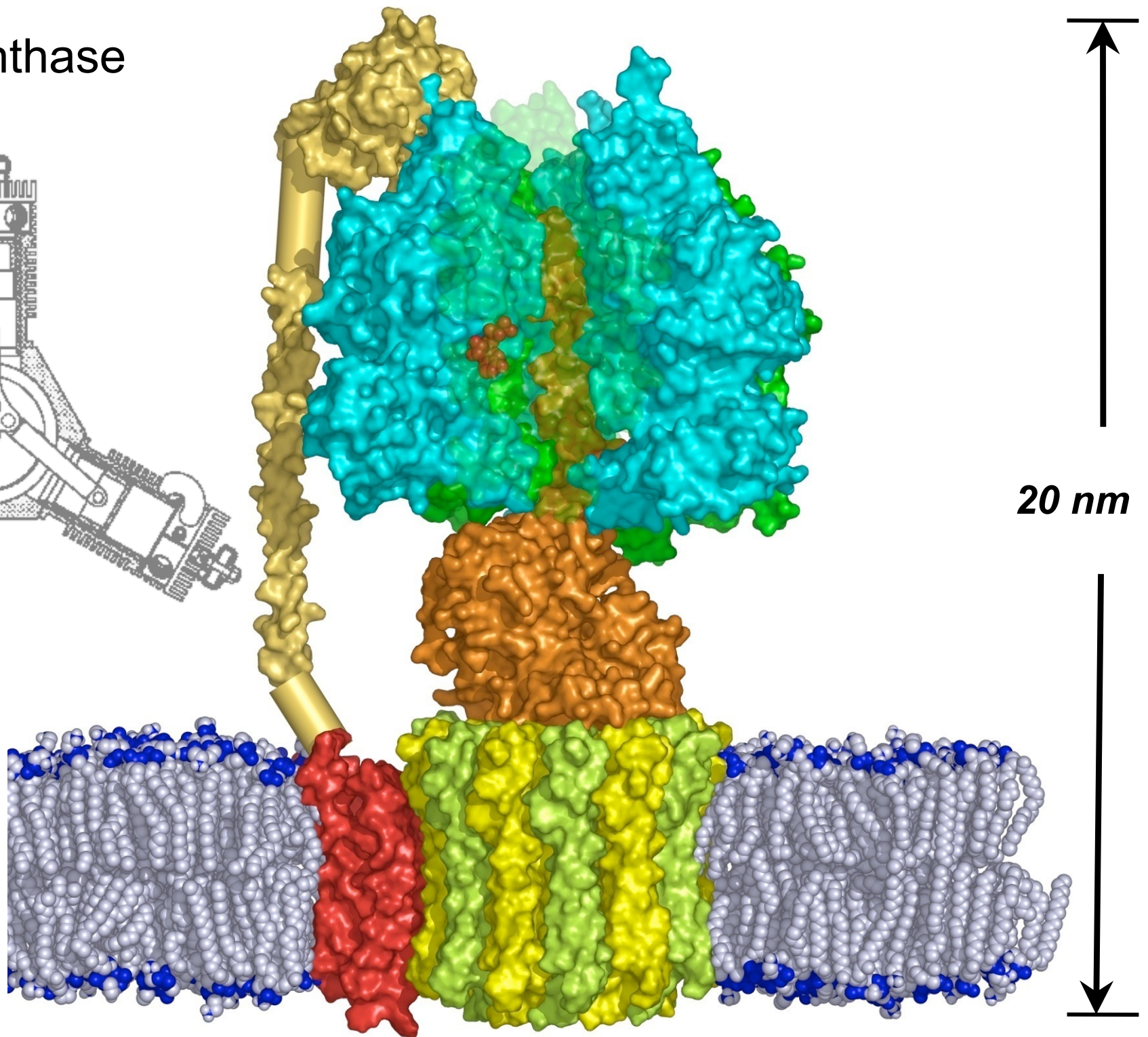
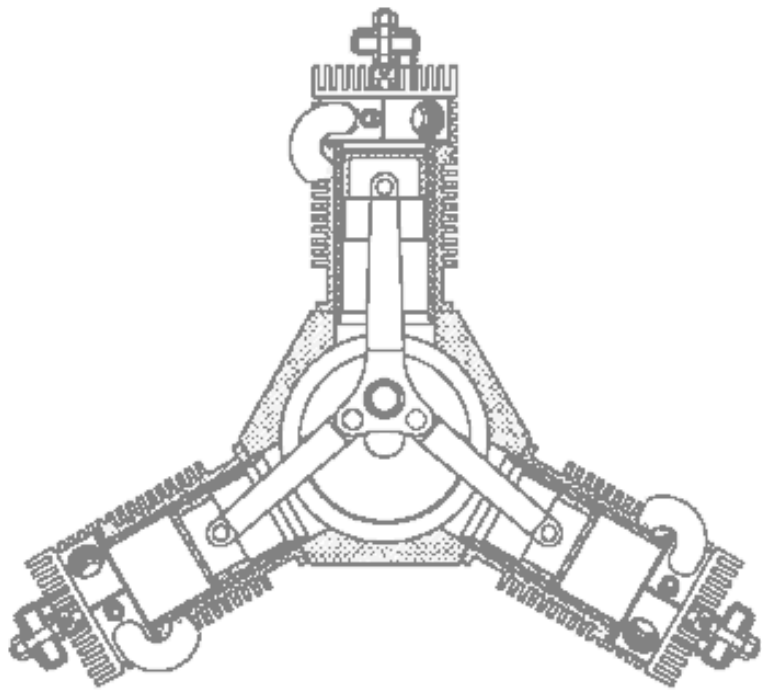
Proteins are Molecular Machines !

***Elementary steps:
Conformational motions***

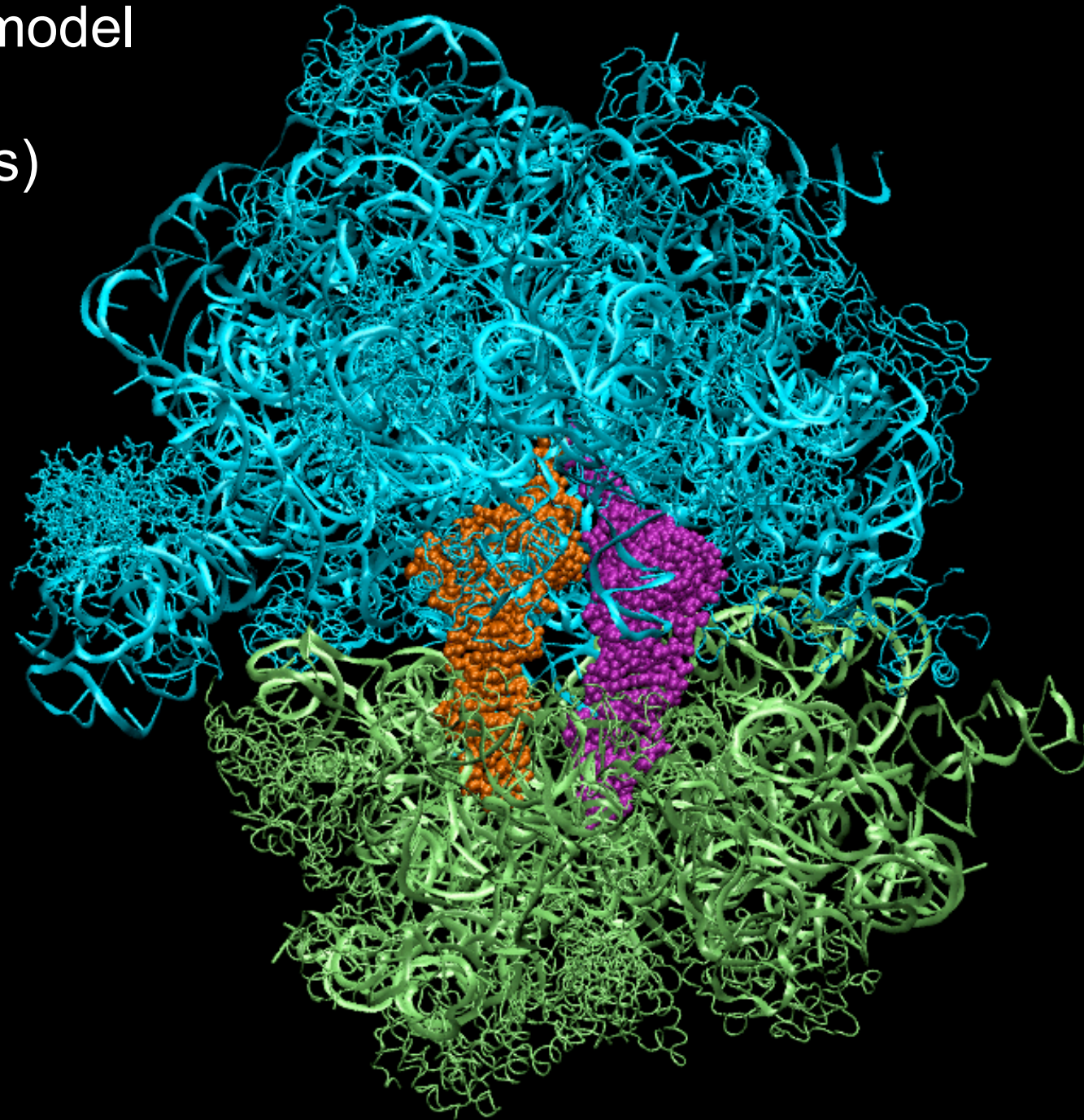
F-ATP Synthase



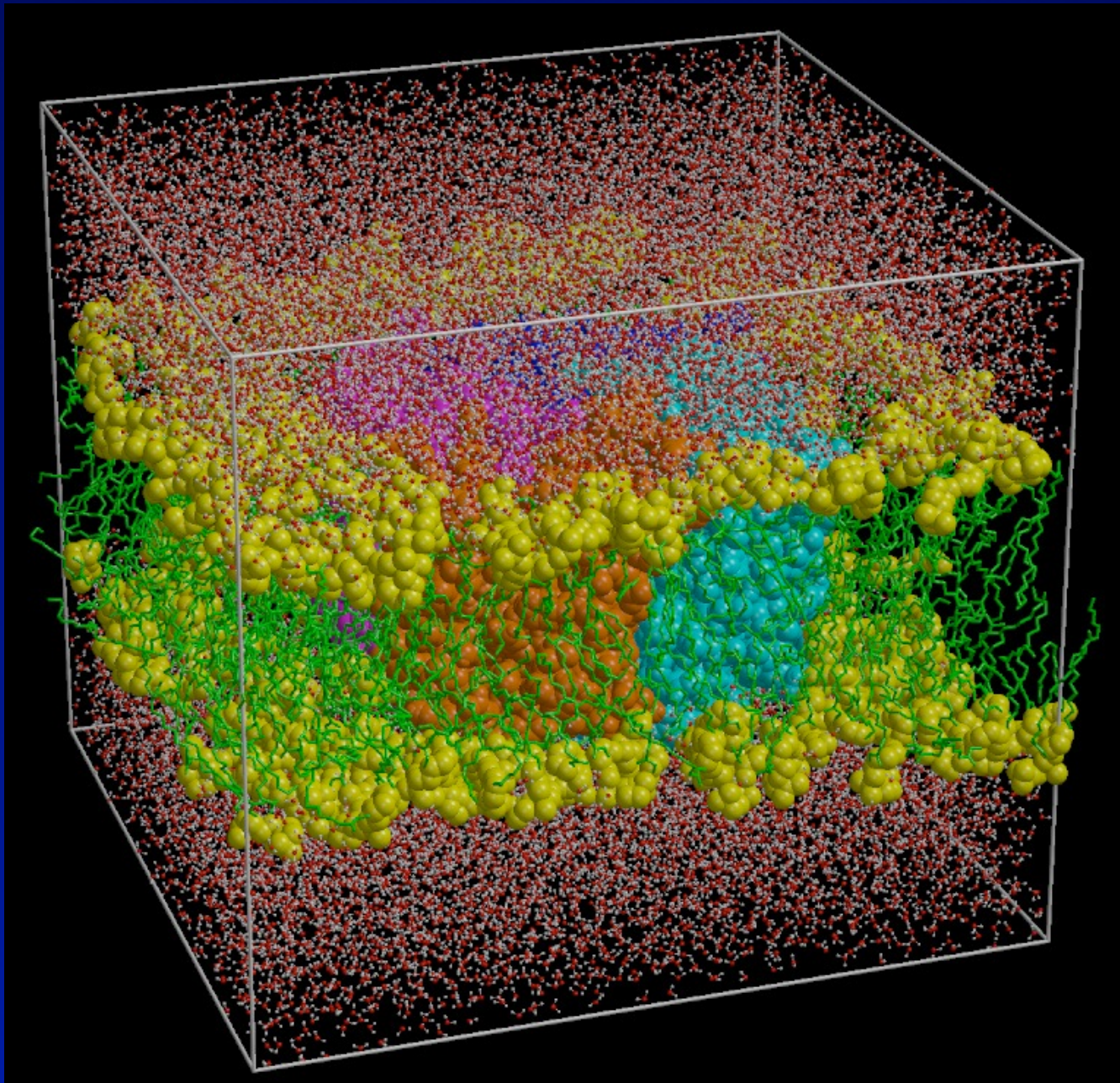
F-ATP Synthase



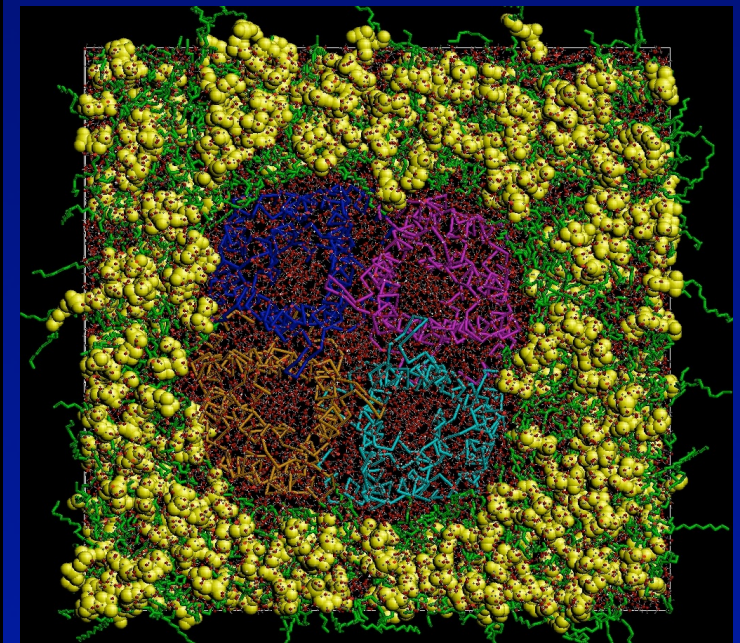
MD-Fit of
atomic xray model
to EM maps
(23 structures)



MD simulations of water transport

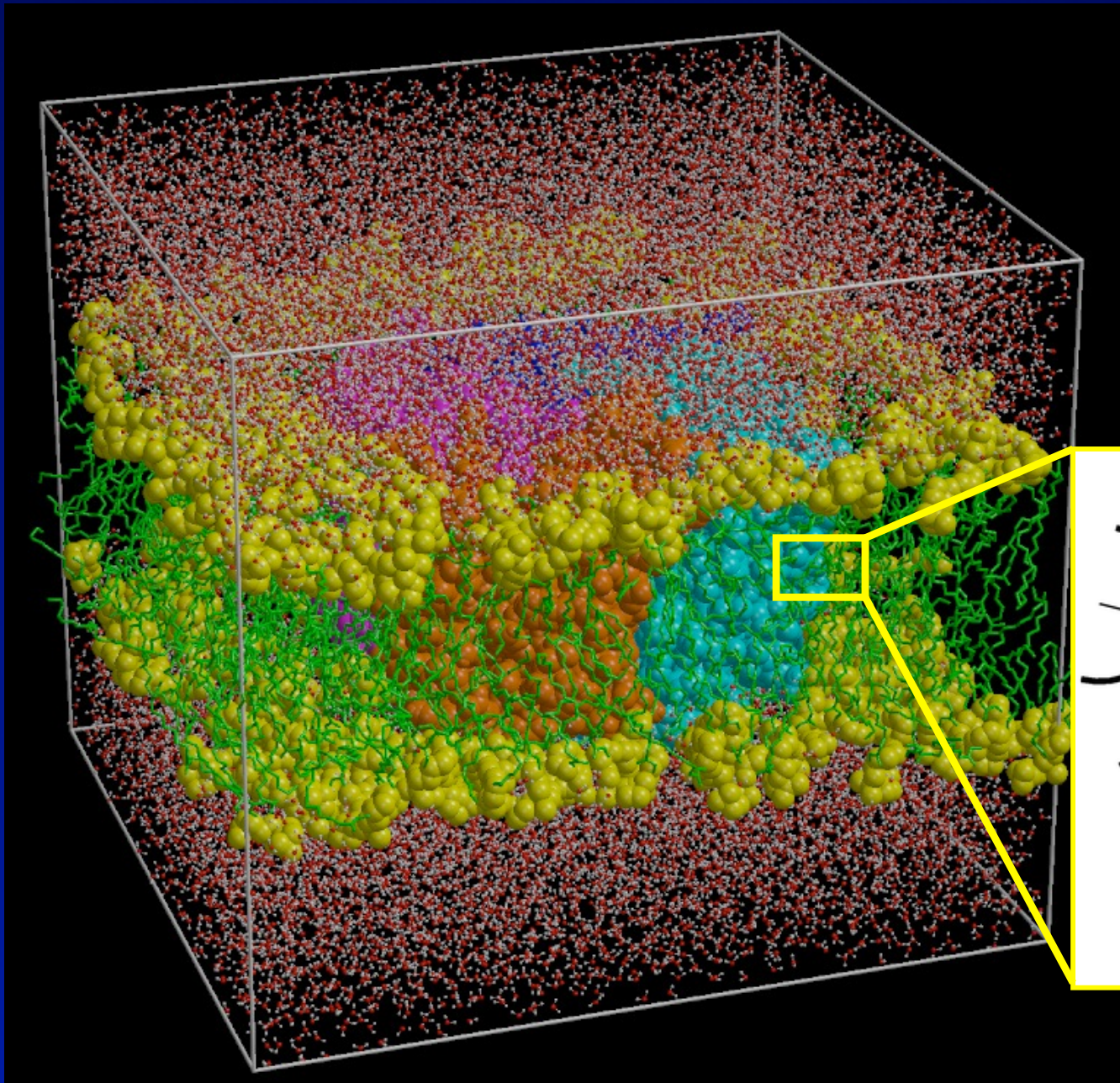


- *ca. 100 000 atoms*
- *full electrostatics, periodic boundary*
- *10 ns simulation time*

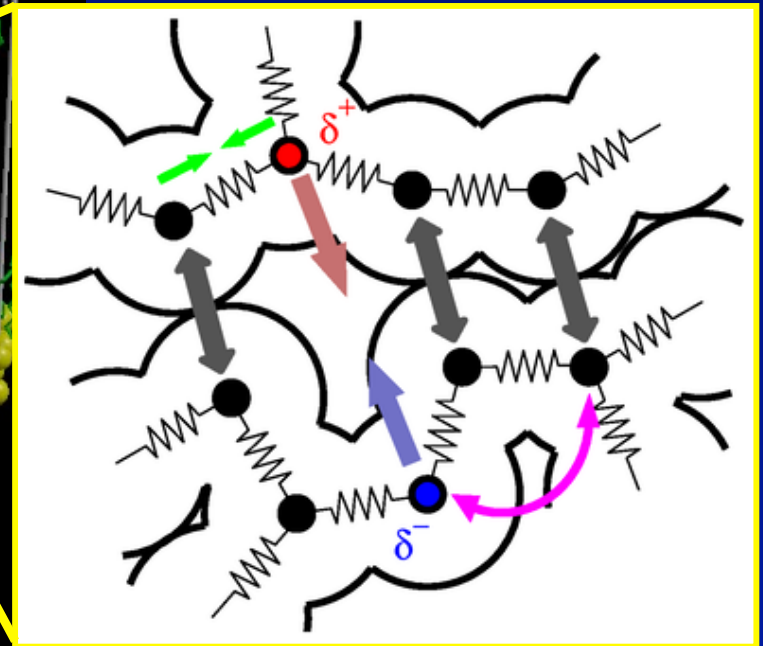


top view

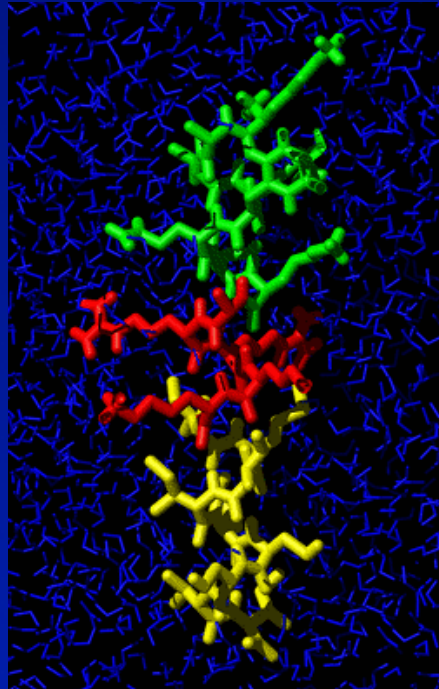
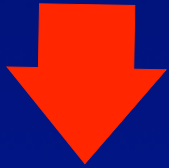
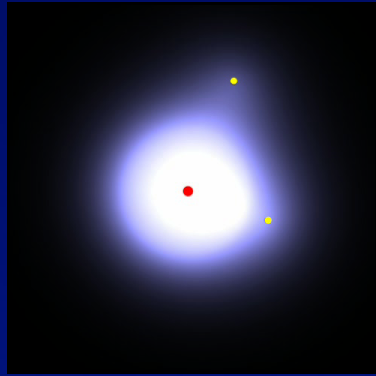
MD simulations of water transport



- ca. 100 000 atoms
- full electrostatics, periodic boundary
- 10 ns simulation time



Molecular Dynamics Simulations



Schrödinger equation

$$i\hbar\partial_t\Psi(r, R) = H\Psi(r, R)$$

Born-Oppenheimer approximation

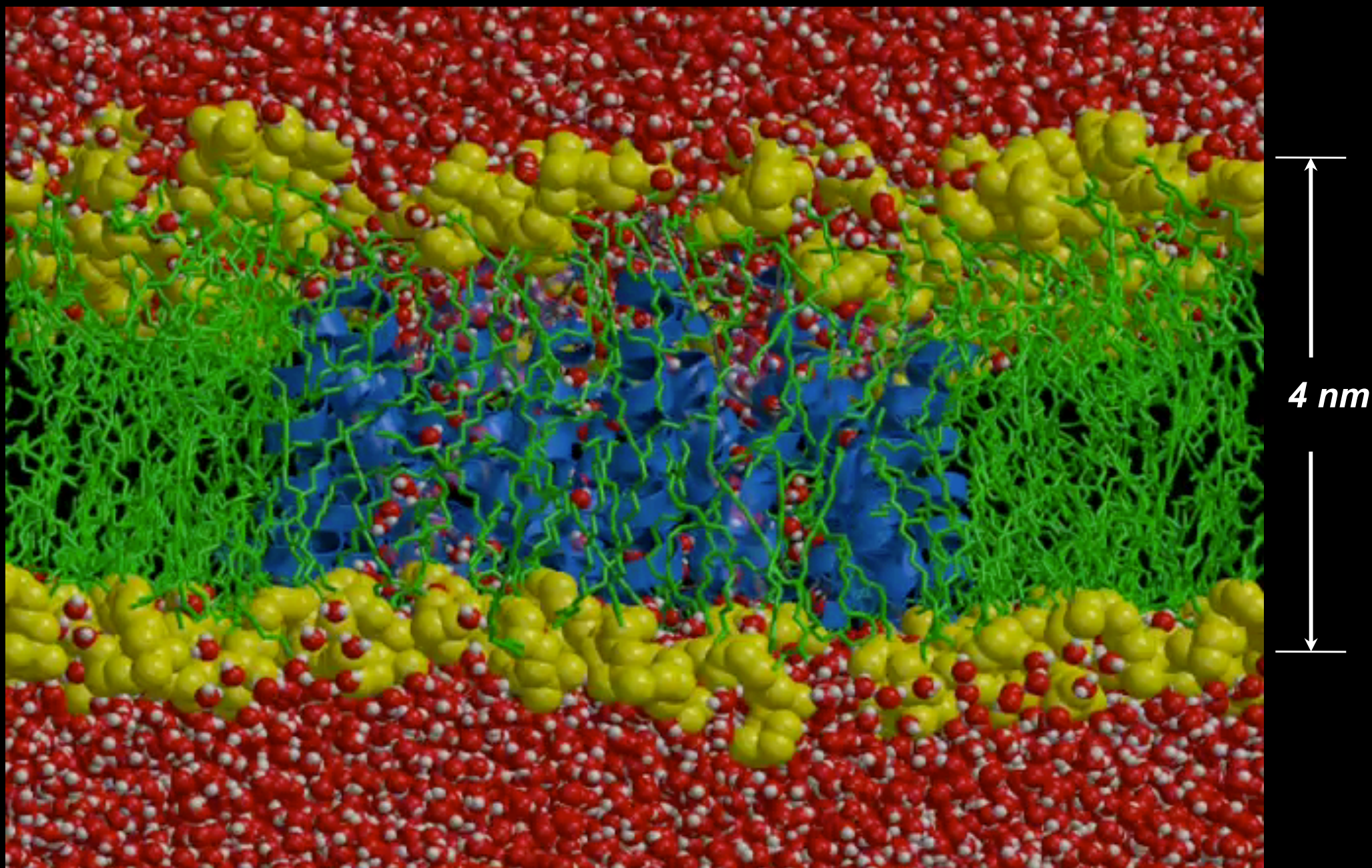
$$H_e\Psi_e(r; R) = E_e(R)\Psi_e(r; R)$$

Nucleic motion described classically

$$m_i\frac{d^2}{dt^2}\vec{R}_i = -\vec{\nabla}_i E(\vec{R})$$

Empirical Force field

$$E(\vec{R}) = \sum_{\text{bonded}} E_i(\vec{R}) + \sum_{\text{non-bonded}} E_i(\vec{R})$$



Molecular dynamics simulation, $1\text{s} \hat{=} 2 \cdot 10^{-11}\text{s}$

Water Permeation proceeds in steps

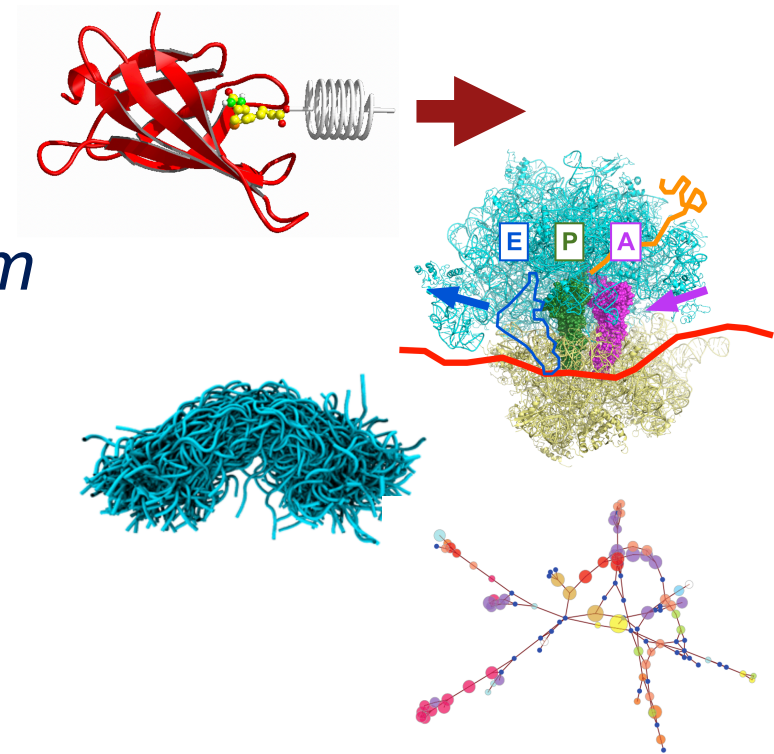
***one out of 16 full
spontaneous
permeation events
(2 ns)***

***(outside the channel, only
few water molecules are
shown)***



Towards a mechanistic understanding of protein function

- (1) Ligand unbinding revisited*
- (2) Ribosomal antibiotics mechanism*
- (3) Ribosomal Frameshifting*
- (4) Intrinsically Disordered Proteins*
- (5) The Dynasome*



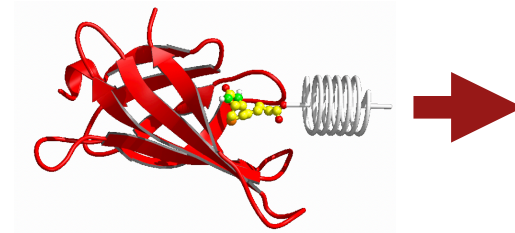
AFM+ X-ray + cryo EM + MD

***Lars Bock, Christian Blau, Michal Kolar, Andrea Vaiana, Andreas Russek,
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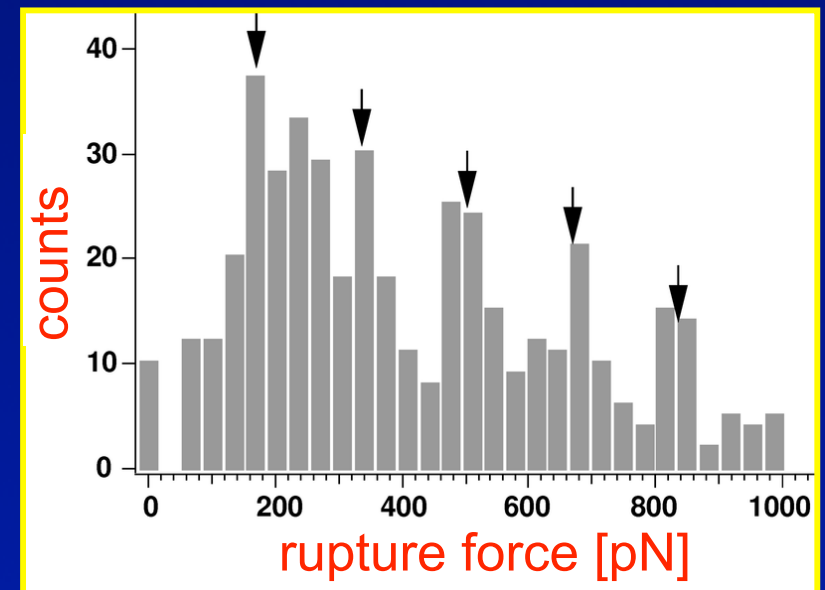
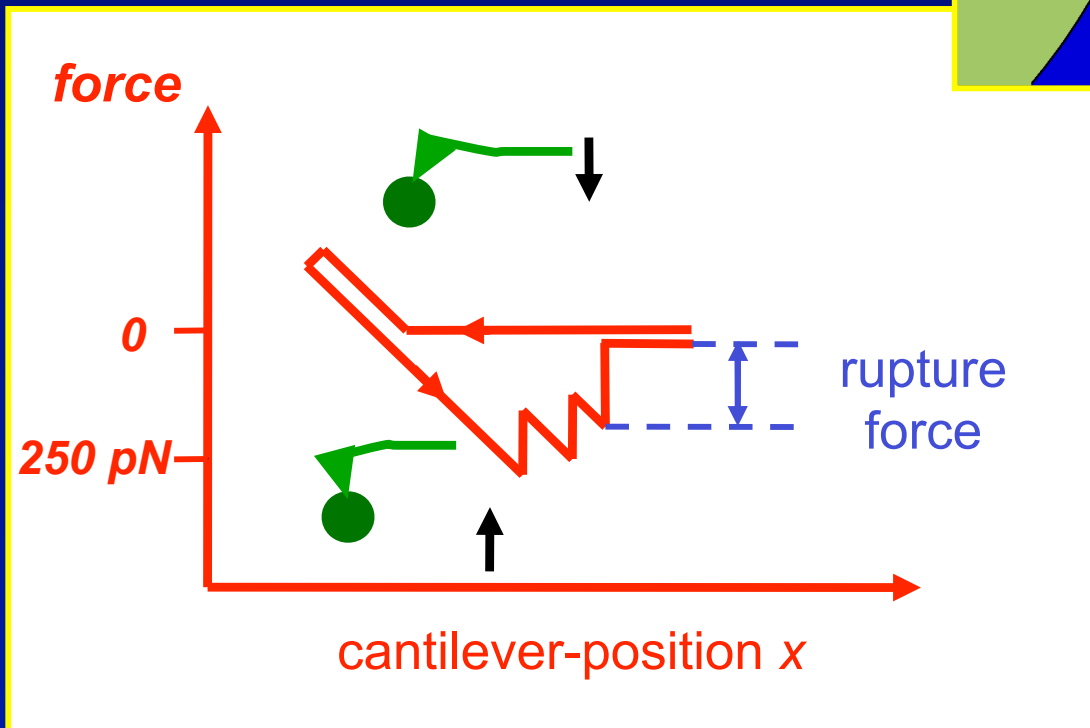
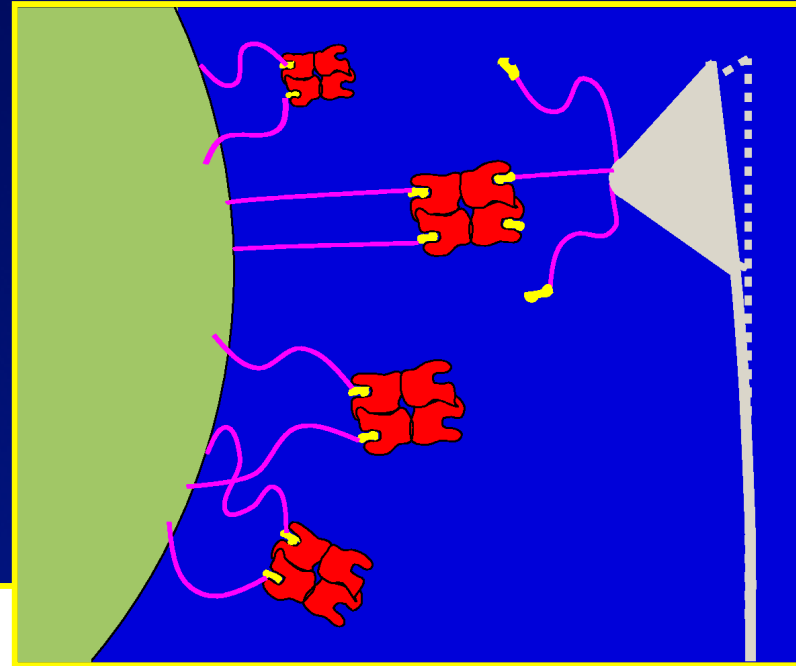
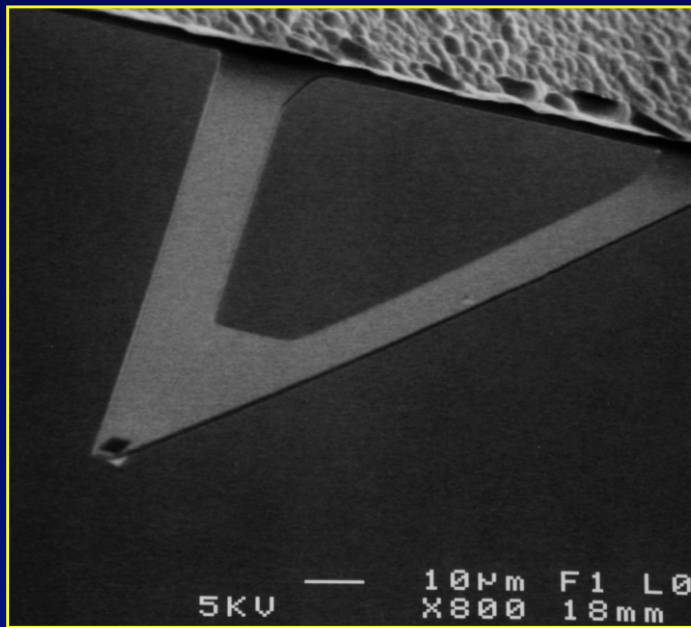
(5) The Dynasome

AFM+ X-ray + cryo EM + MD

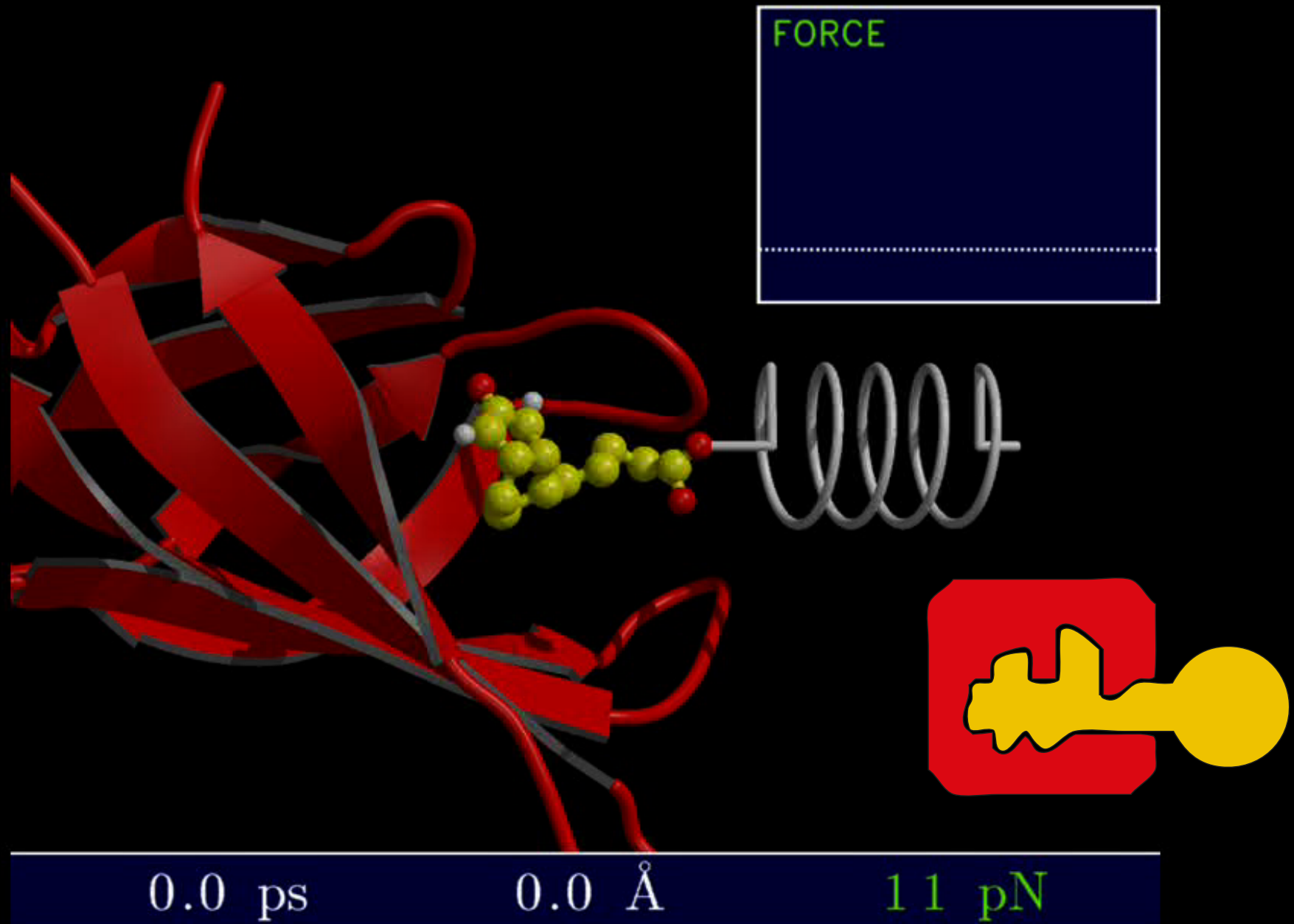
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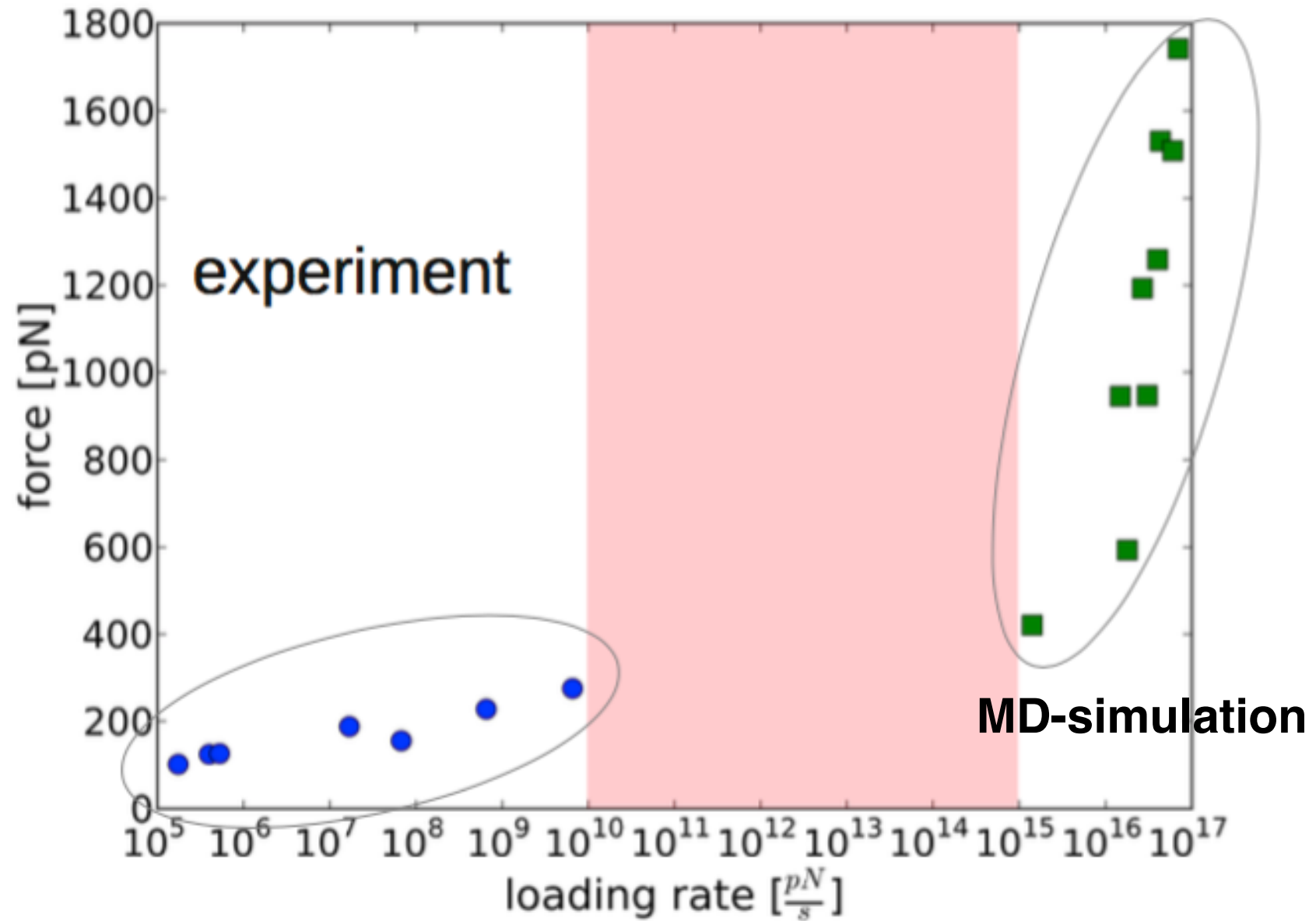
Single Molecule Force Spectroscopy



Force probe simulations

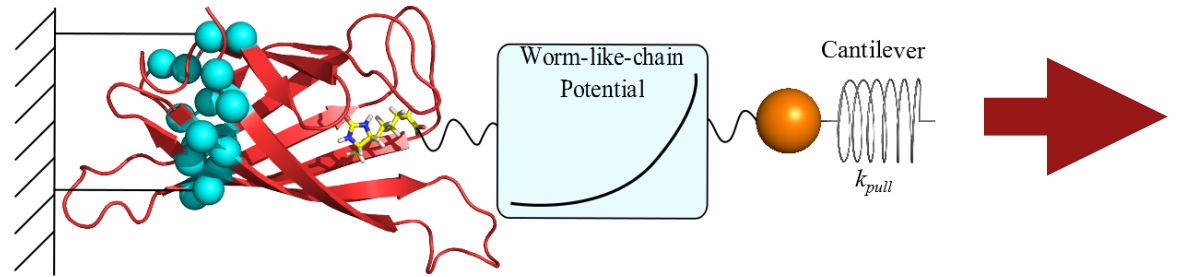


AFM unbinding: Simulation vs Experiment (1996)

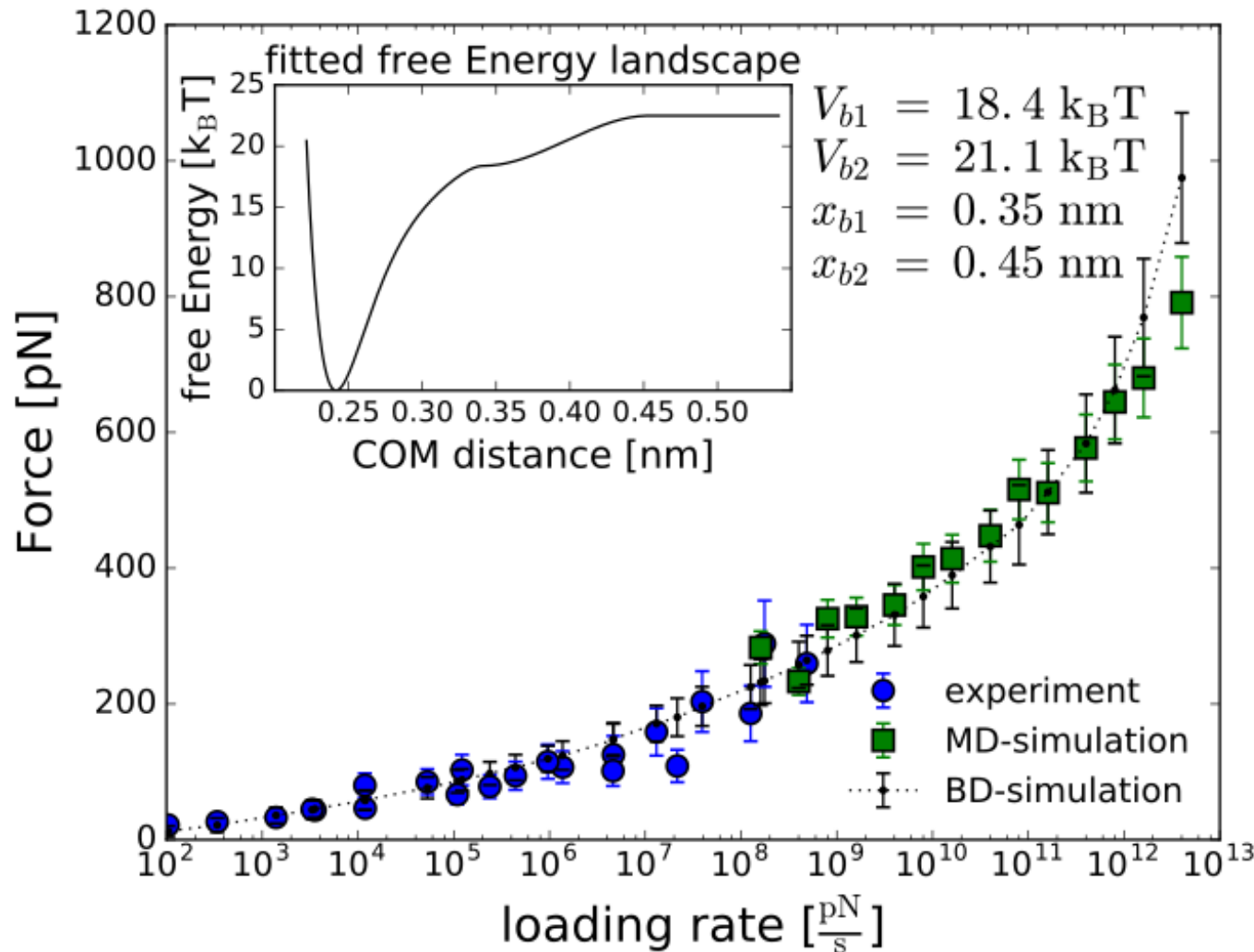


MD Simulation Methods

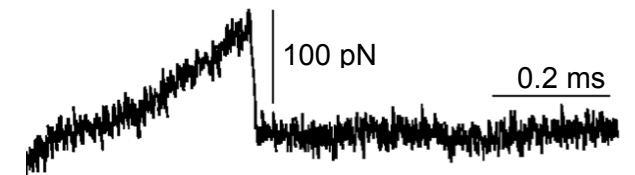
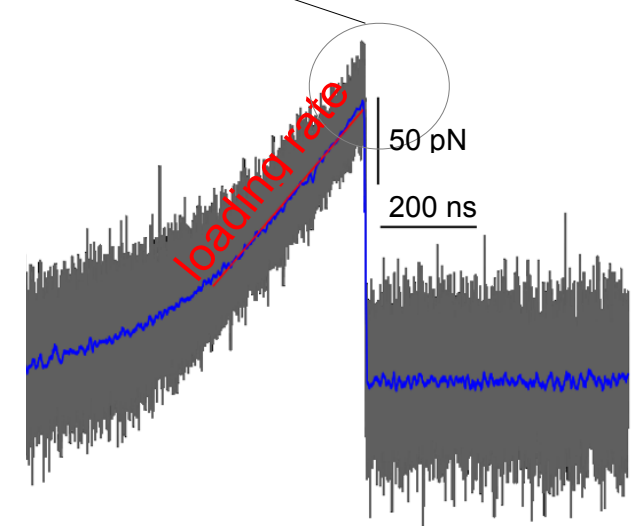
- Tetramer and monomer
- Pulling spring of cantilever
- Loading rates: $10^7 \dots 10^{13}$ pN/s
- Simulation lengths up to 8 μ s
- 10...20 simulations per velocity
- WLC-Potential to mimic PEG



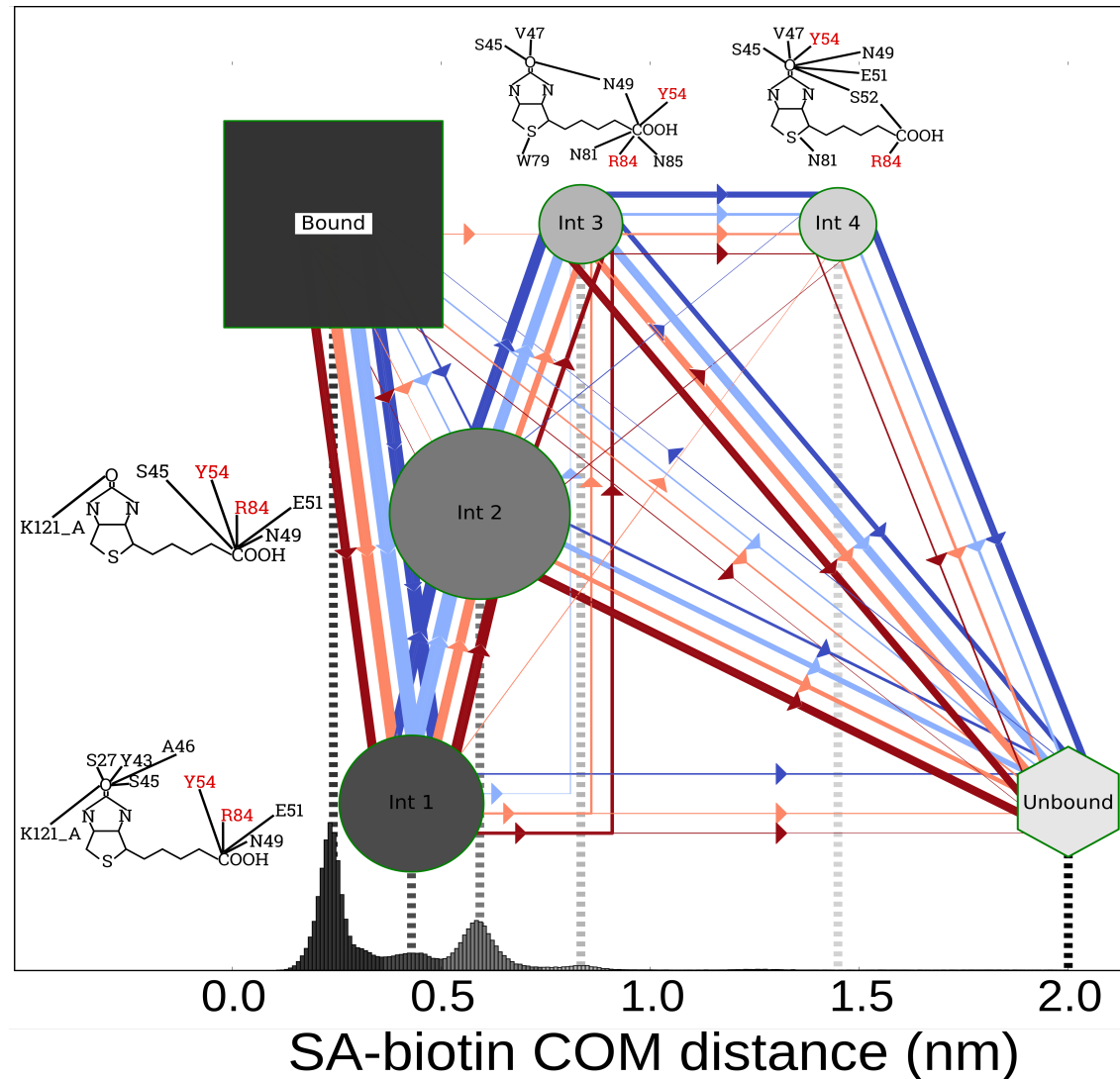
Exp + Sim -> Free energy landscape



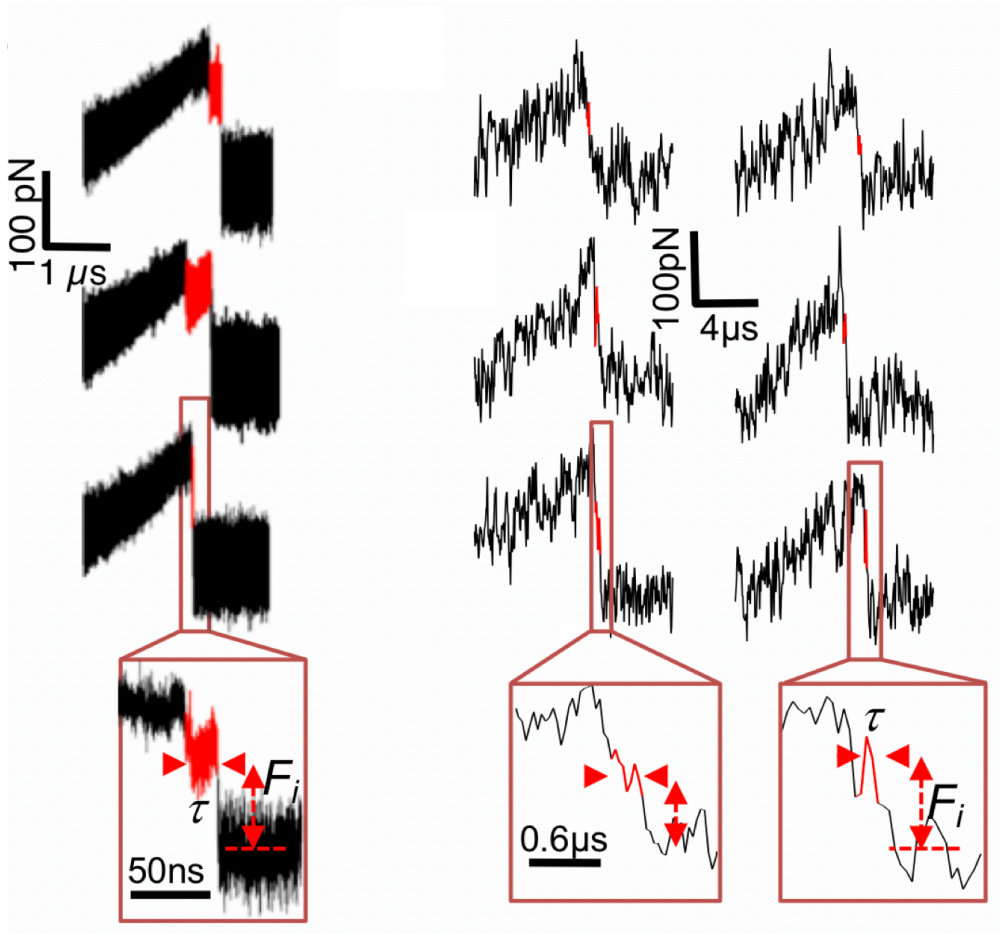
unbinding force



Unbinding pathways change with loading rate

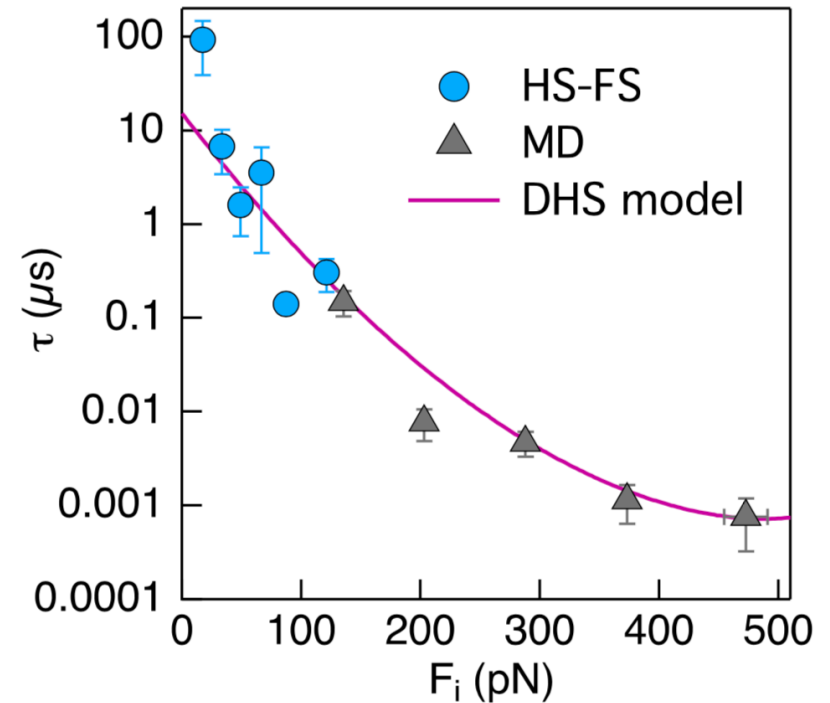


Outer Intermediate



Simulation

Experiment



$$\tau(F) = \tau_0 \left(1 - \frac{\nu F x^\ddagger}{\Delta G^\ddagger}\right)^{1-1/\nu} \exp\left(-\beta \Delta G^\ddagger \left[1 - \left(1 - \frac{\nu F x^\ddagger}{\Delta G^\ddagger}\right)^{1/\nu}\right]\right)$$

$$x^\ddagger = 0.23 \text{ nm}$$

$$\Delta G^\ddagger = 22.5 k_B T$$

[1] Dudko, O. K., Hummer, G. & Szabo, A. *Phys. Rev. Lett.* **96**, 108101 (2006).

Towards a mechanistic understanding of protein function

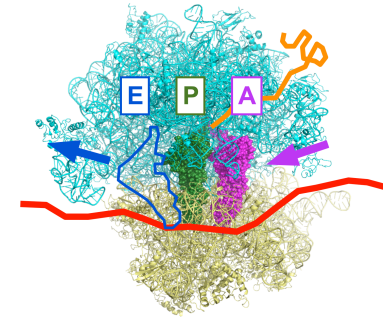
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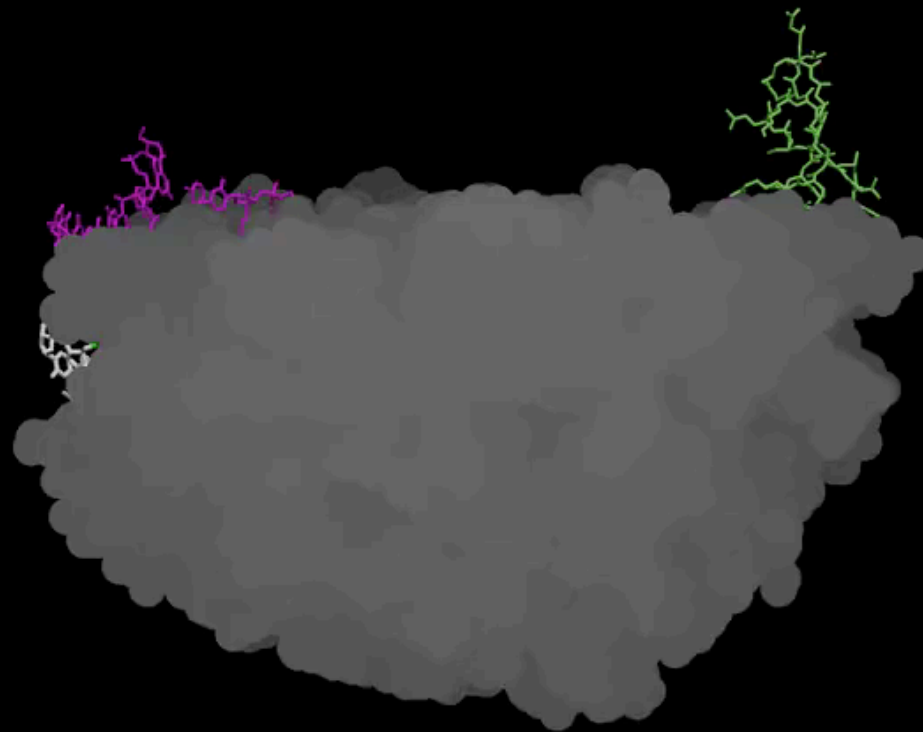


AFM+ X-ray + cryo EM + MD

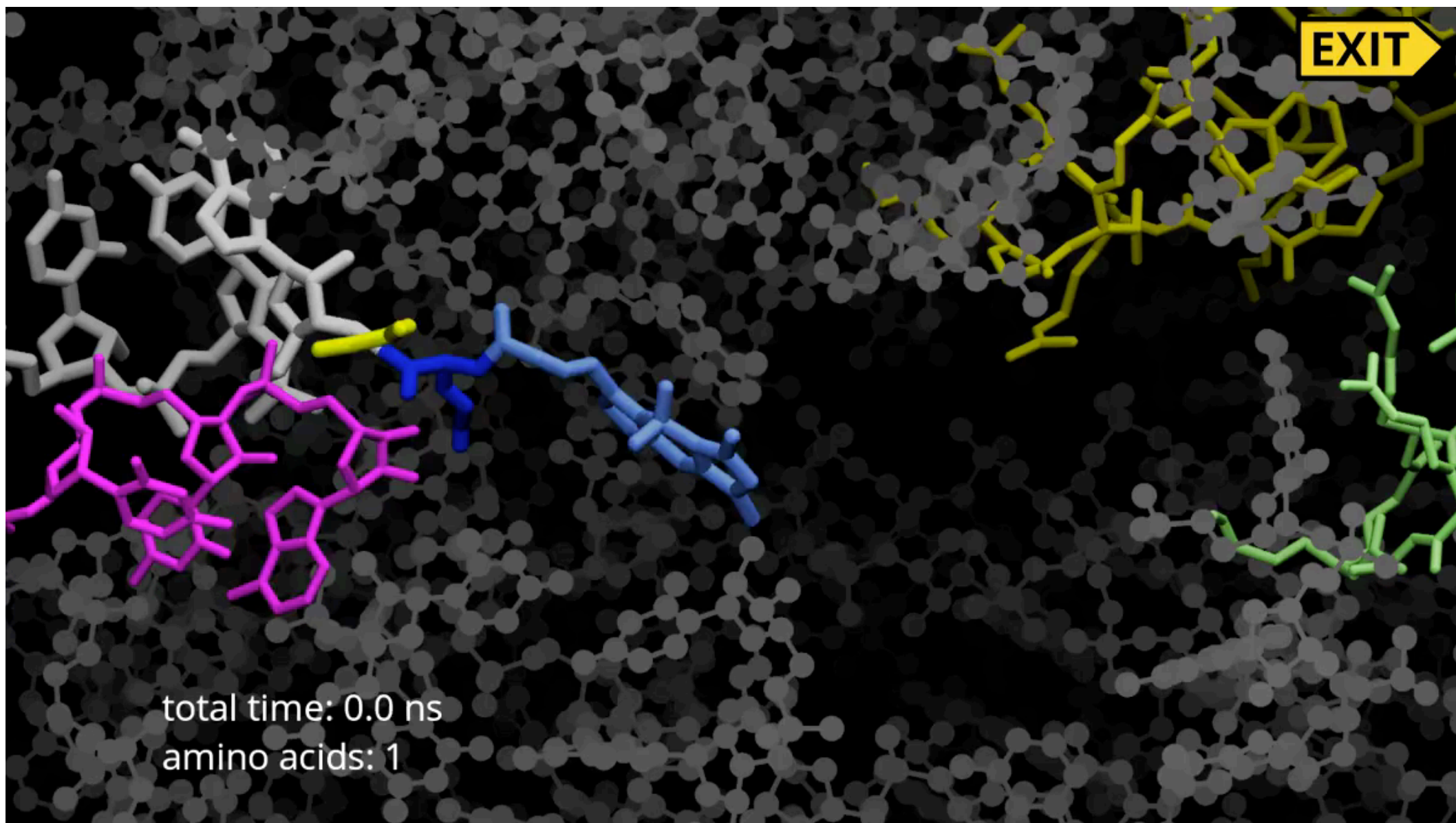
***Lars Bock, Christian Blau, Michal Kolar, Andrea Vaiana, Andreas Russek,
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Elongation dynamics of the nascent peptide in the exit tunnel

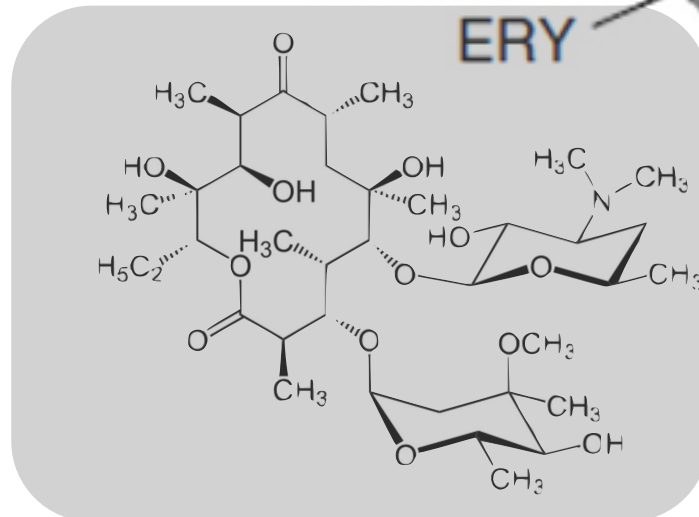
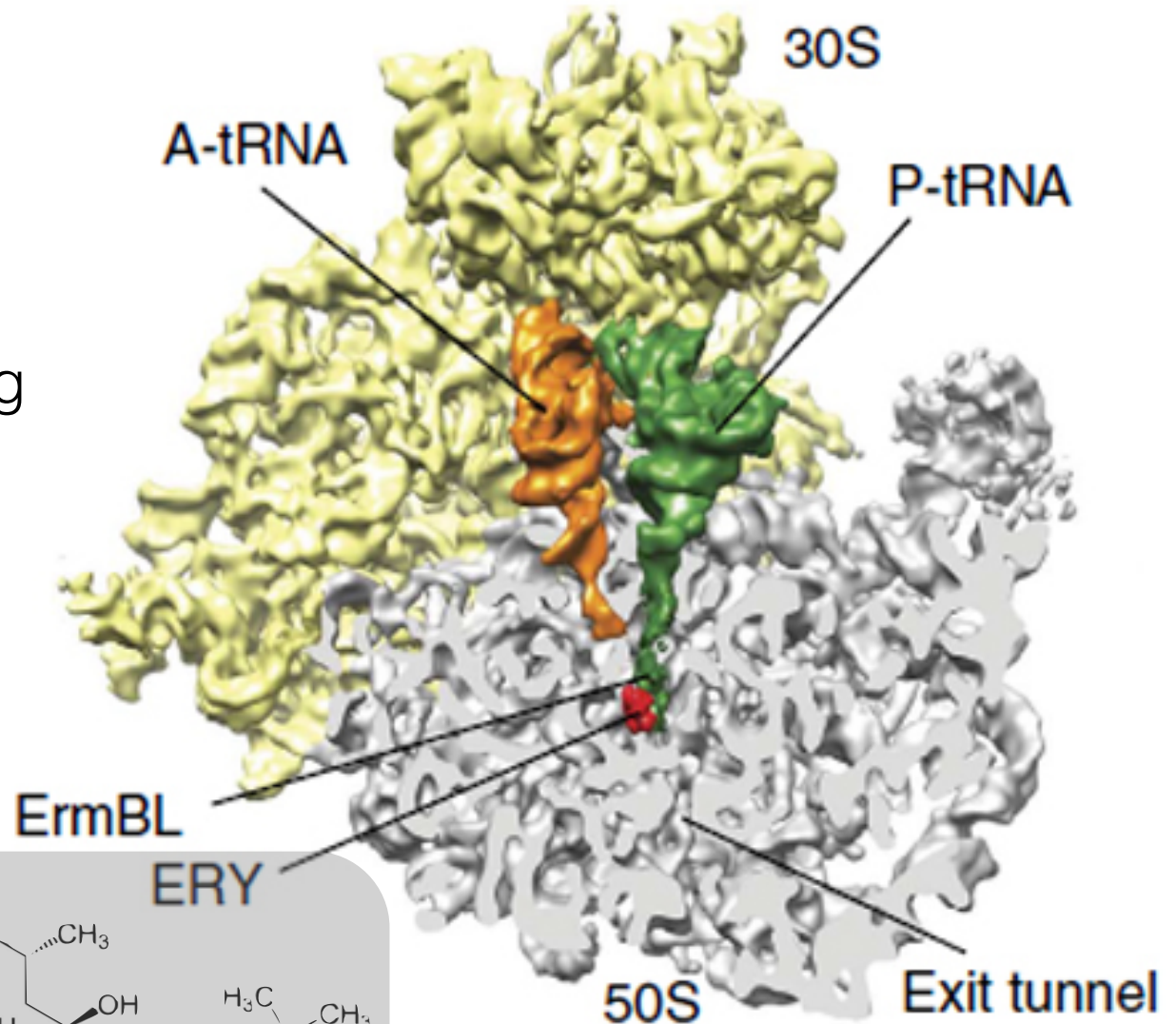


Elongation dynamics of the nascent peptide in the exit tunnel



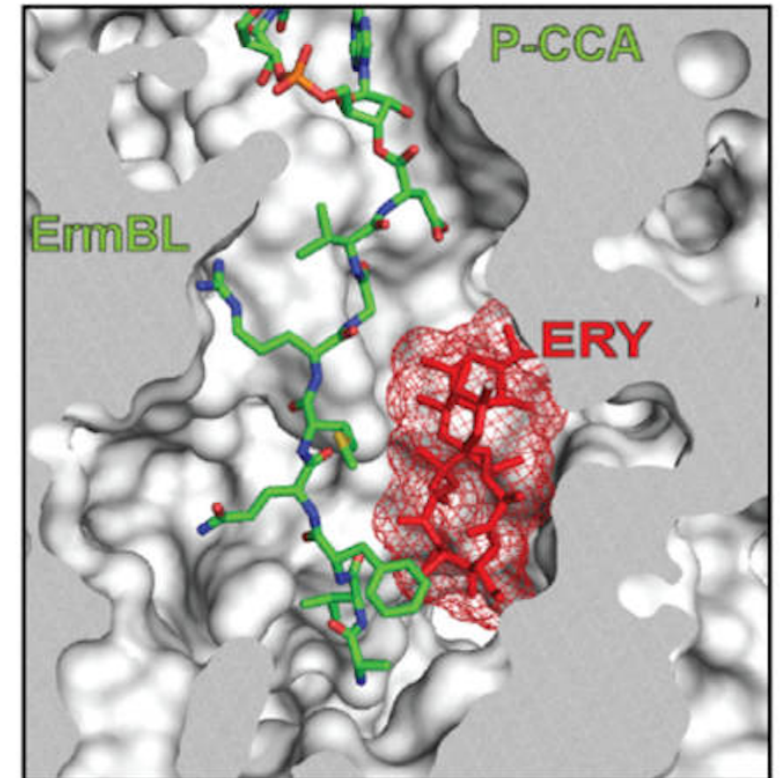
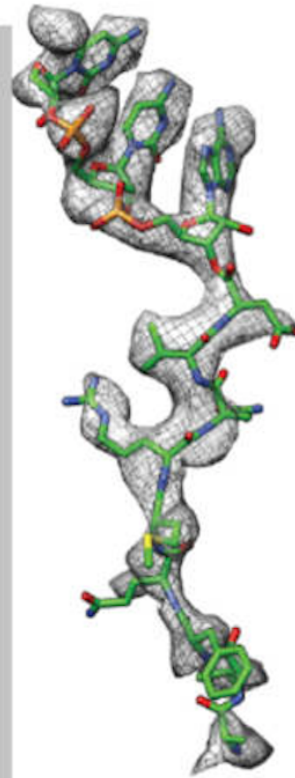
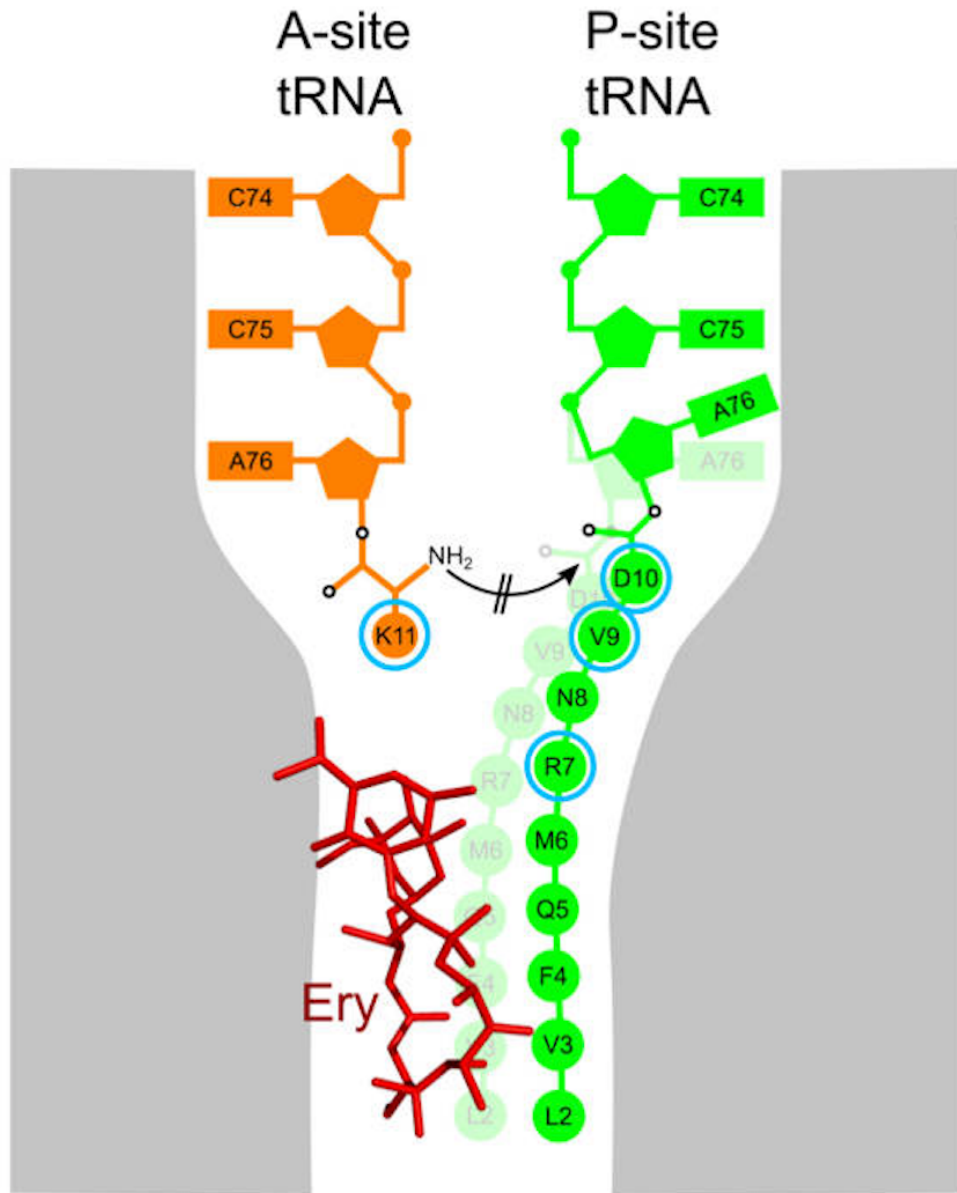
Erythromycin (Ery) binds in the exit tunnel

- Most peptides translated
- Stalls the ribosome during ErmBL translation (and others)



Arenz *et al* Nat. Commun. (2014)

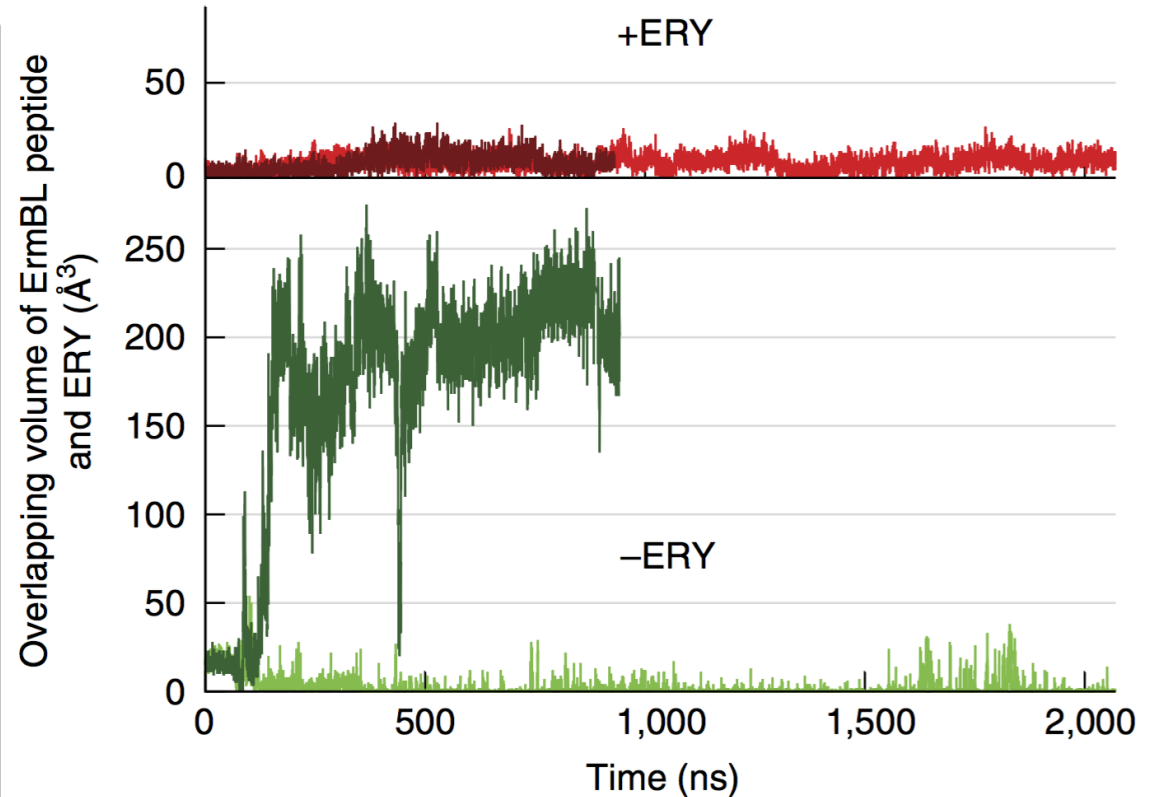
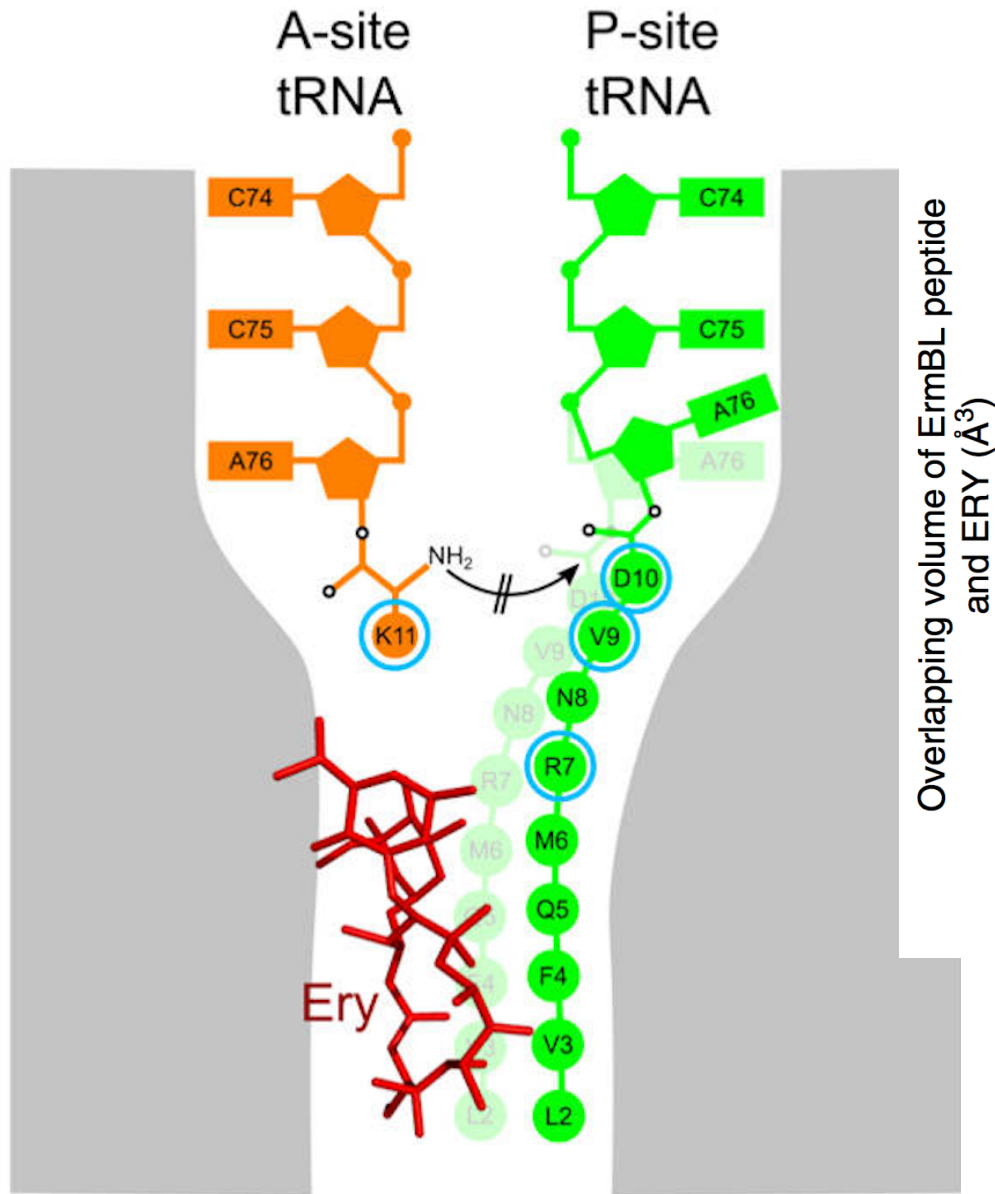
Erythromycin stalls the ribosome: Codon 10 of ErmBL



3.6 Å cryo-EM structure of ErmBL stalled complex

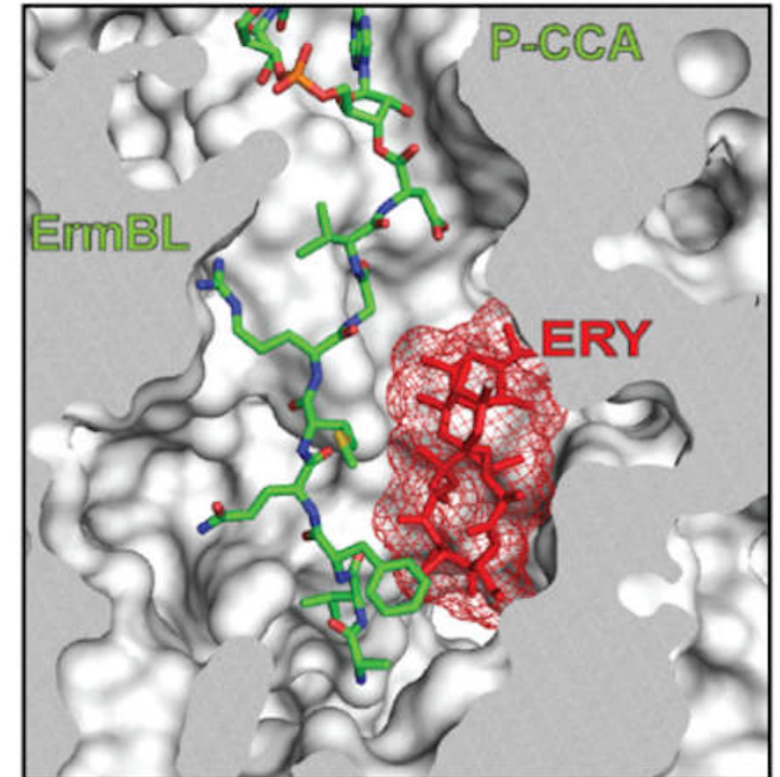
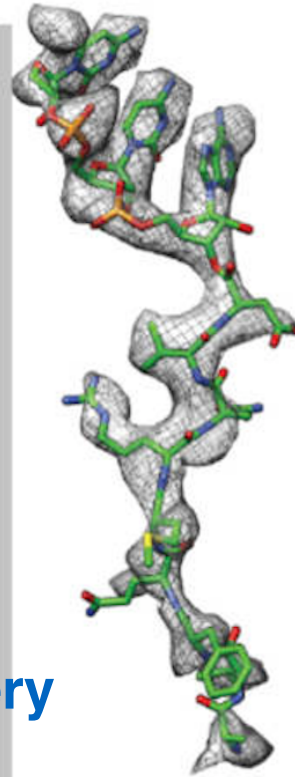
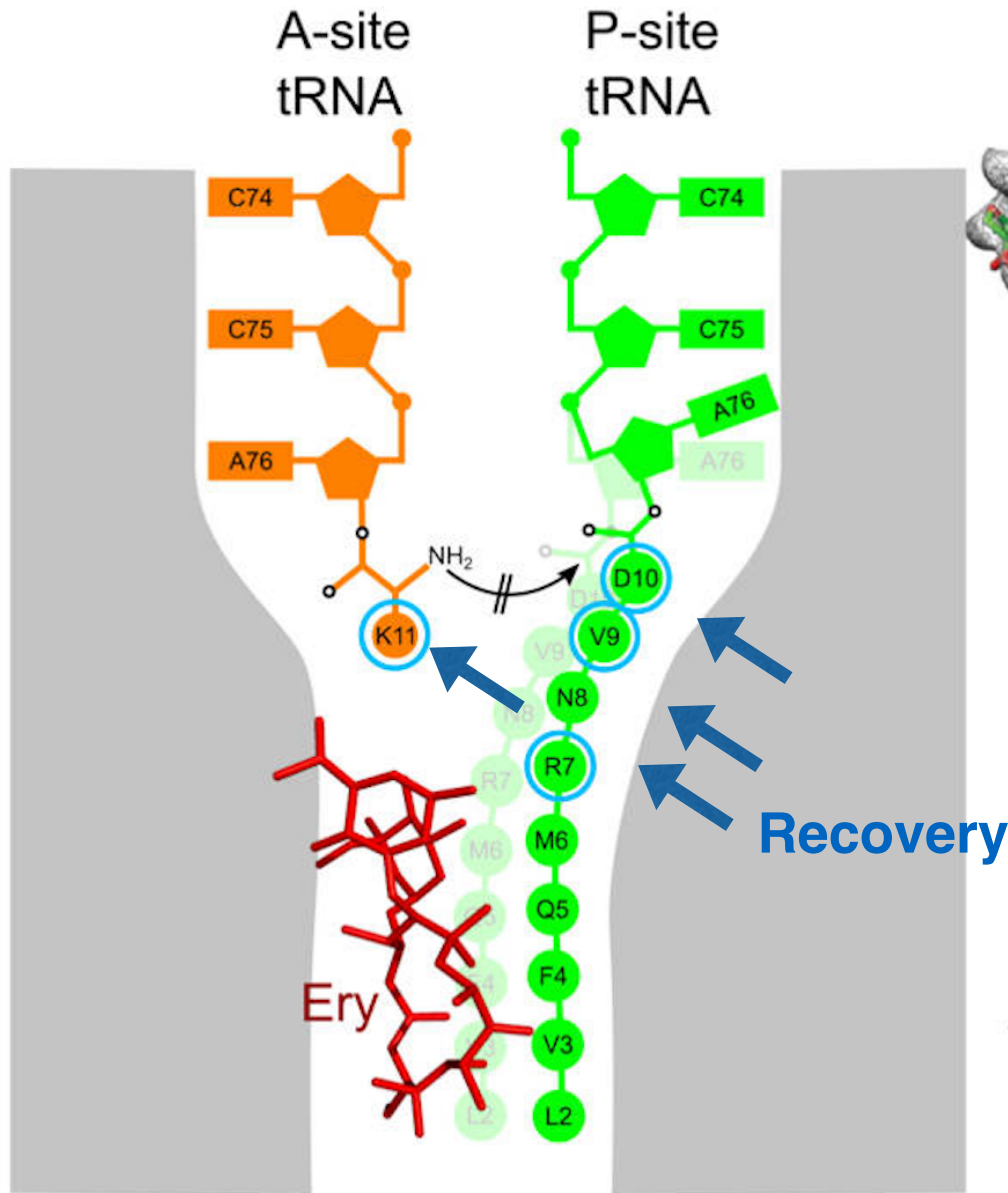
ErmBL with erythromycin

Erythromycin stalls the ribosome: Codon 10 of ErmBL



ErmBL with erythromycin

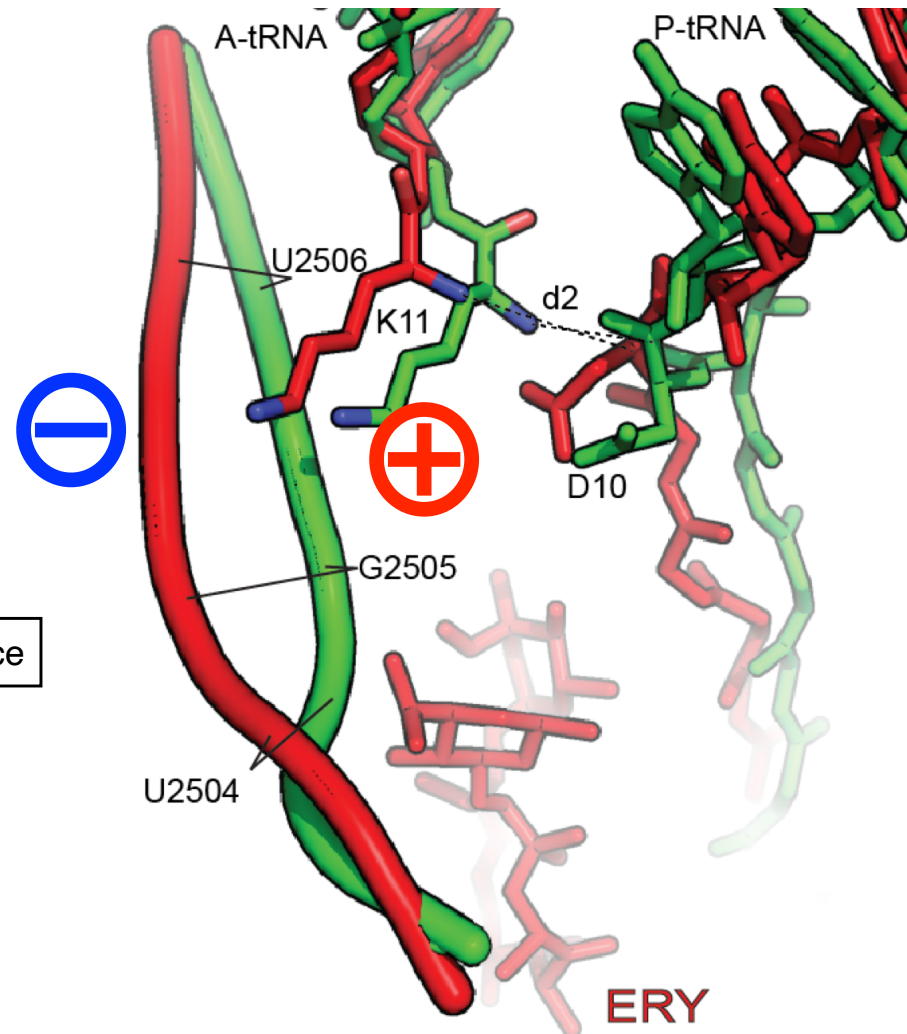
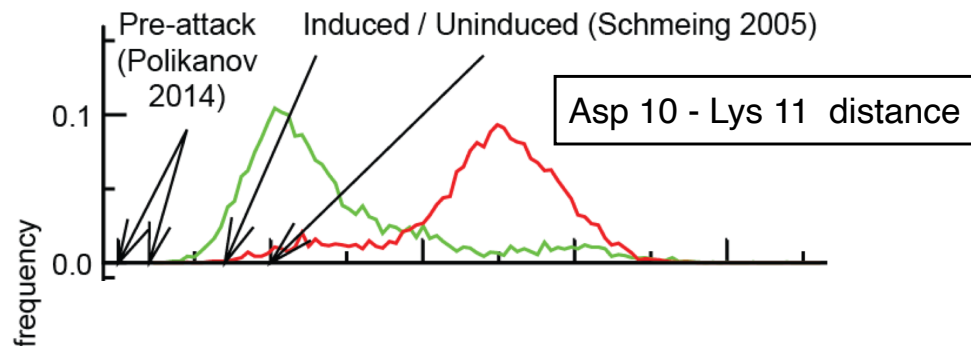
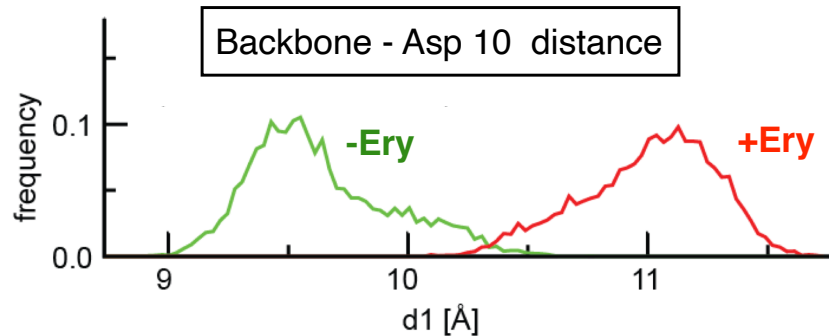
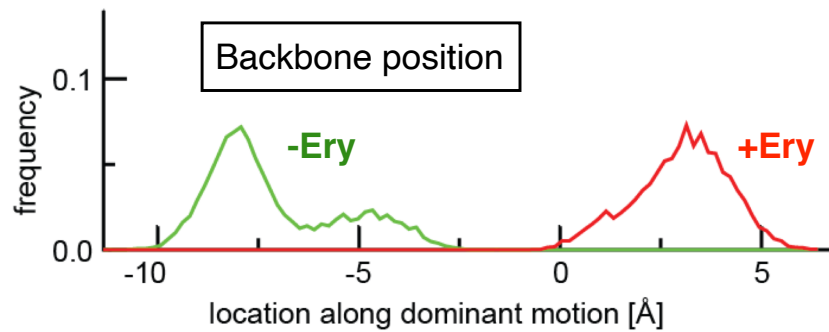
Erythromycin stalls the ribosome: Codon 10 of ErmBL



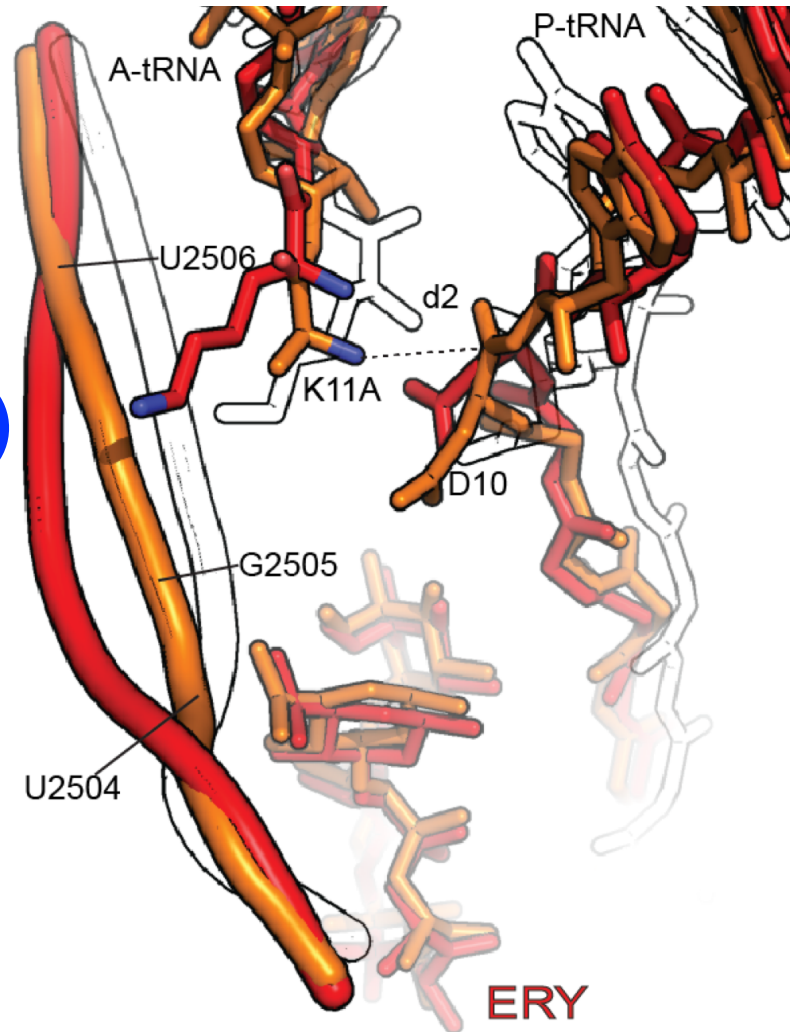
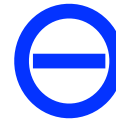
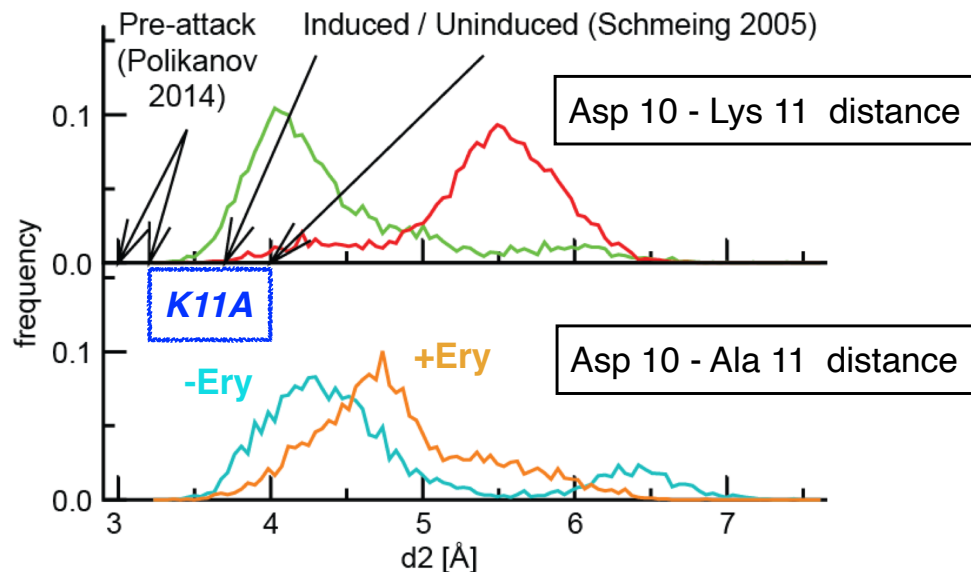
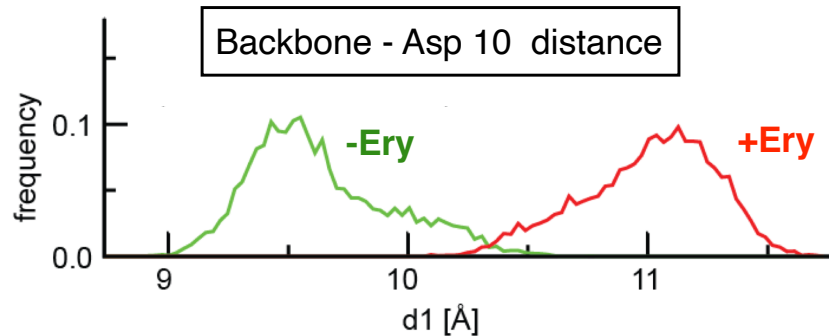
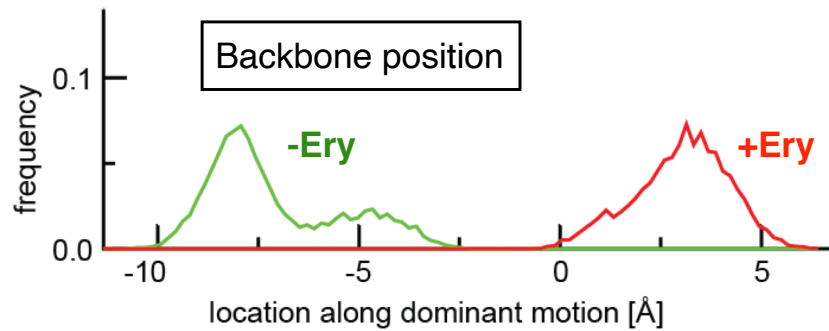
3.6 Å cryo-EM structure of ErmBL stalled complex

ErmBL with erythromycin

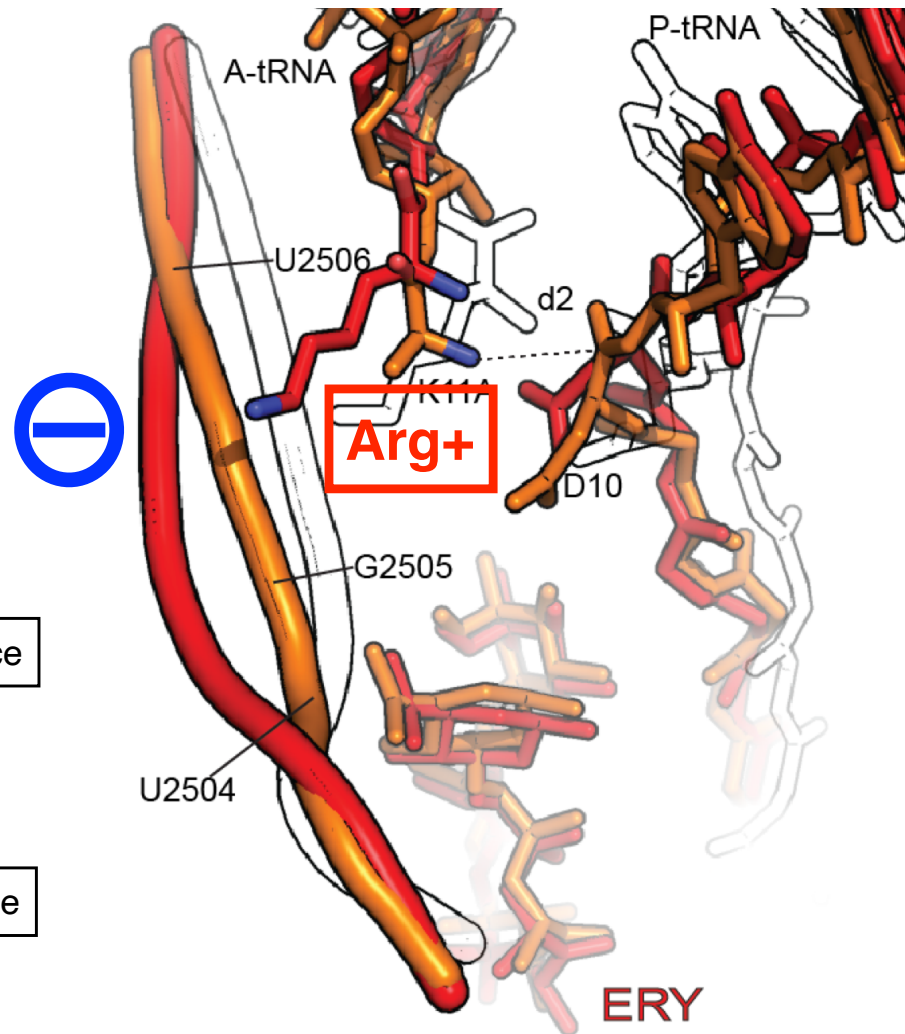
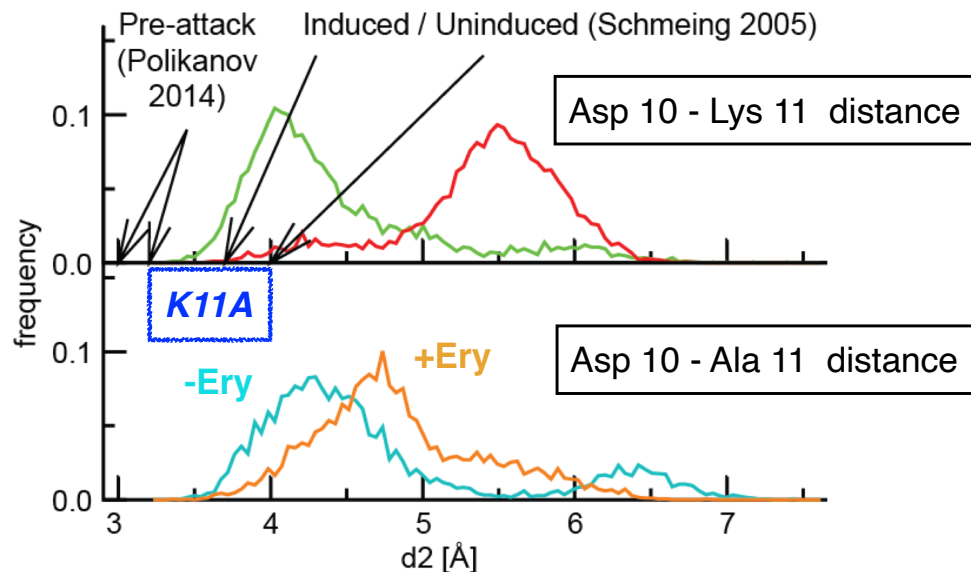
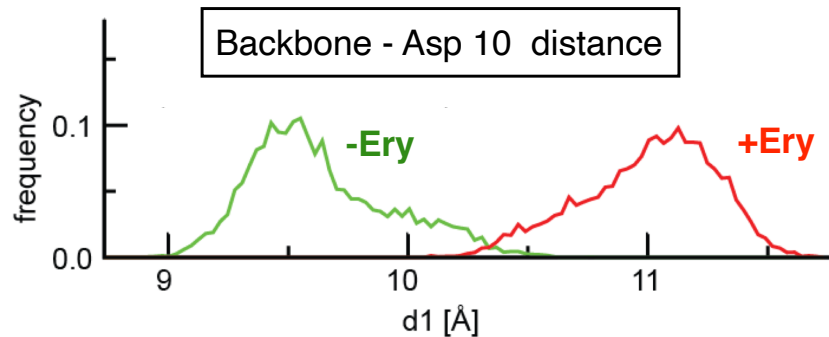
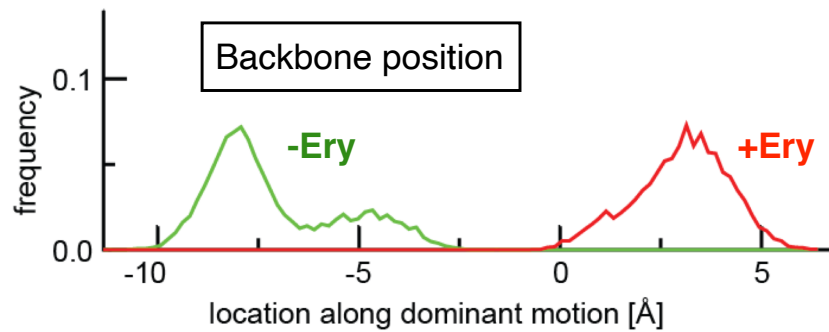
MD: Backbone shift increases $\text{NH}_2\text{-C}$ distance



MD Predicts: **K11R** should enhance stalling

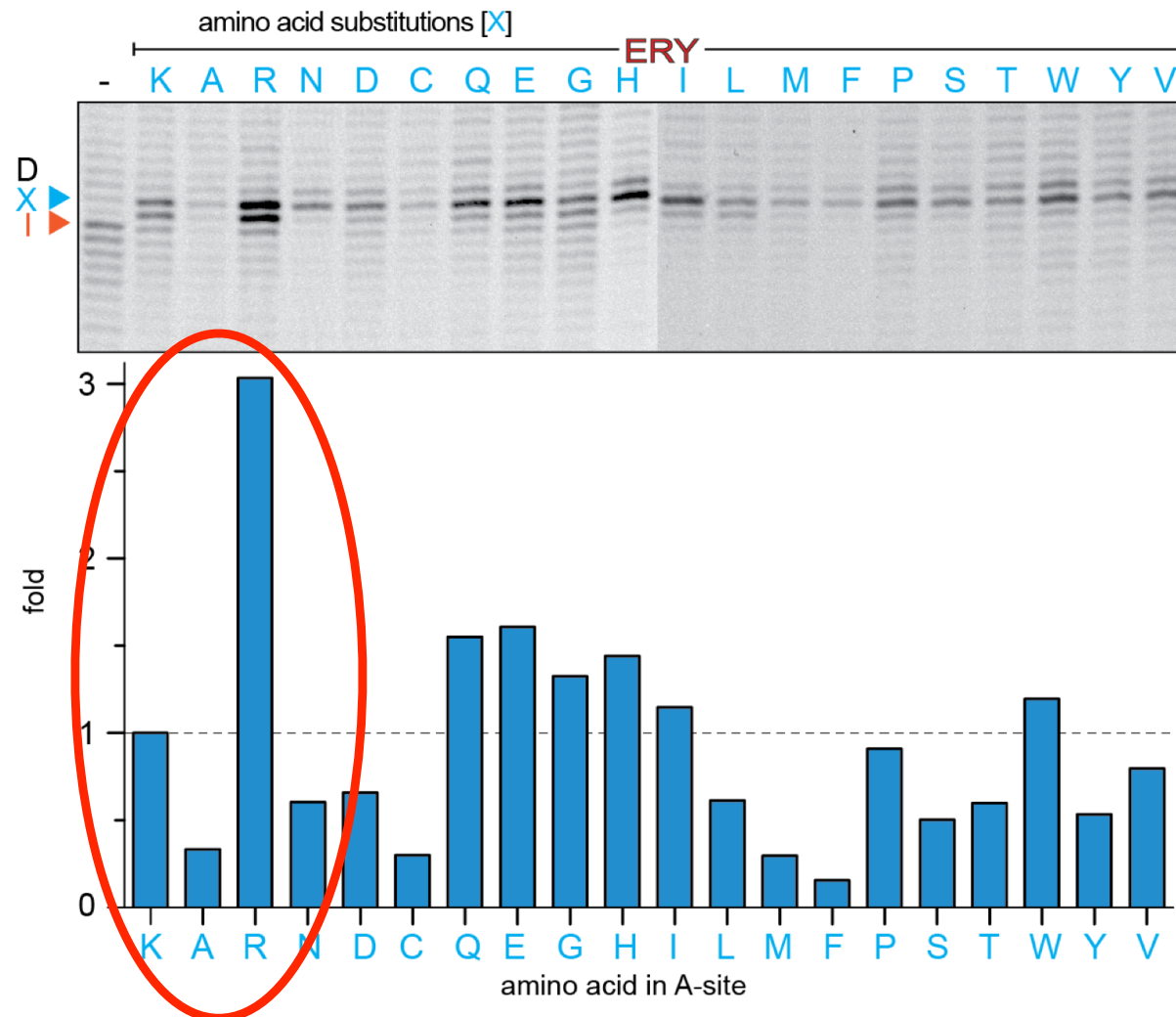


MD Predicts: K11R should enhance stalling



Prediction confirmed

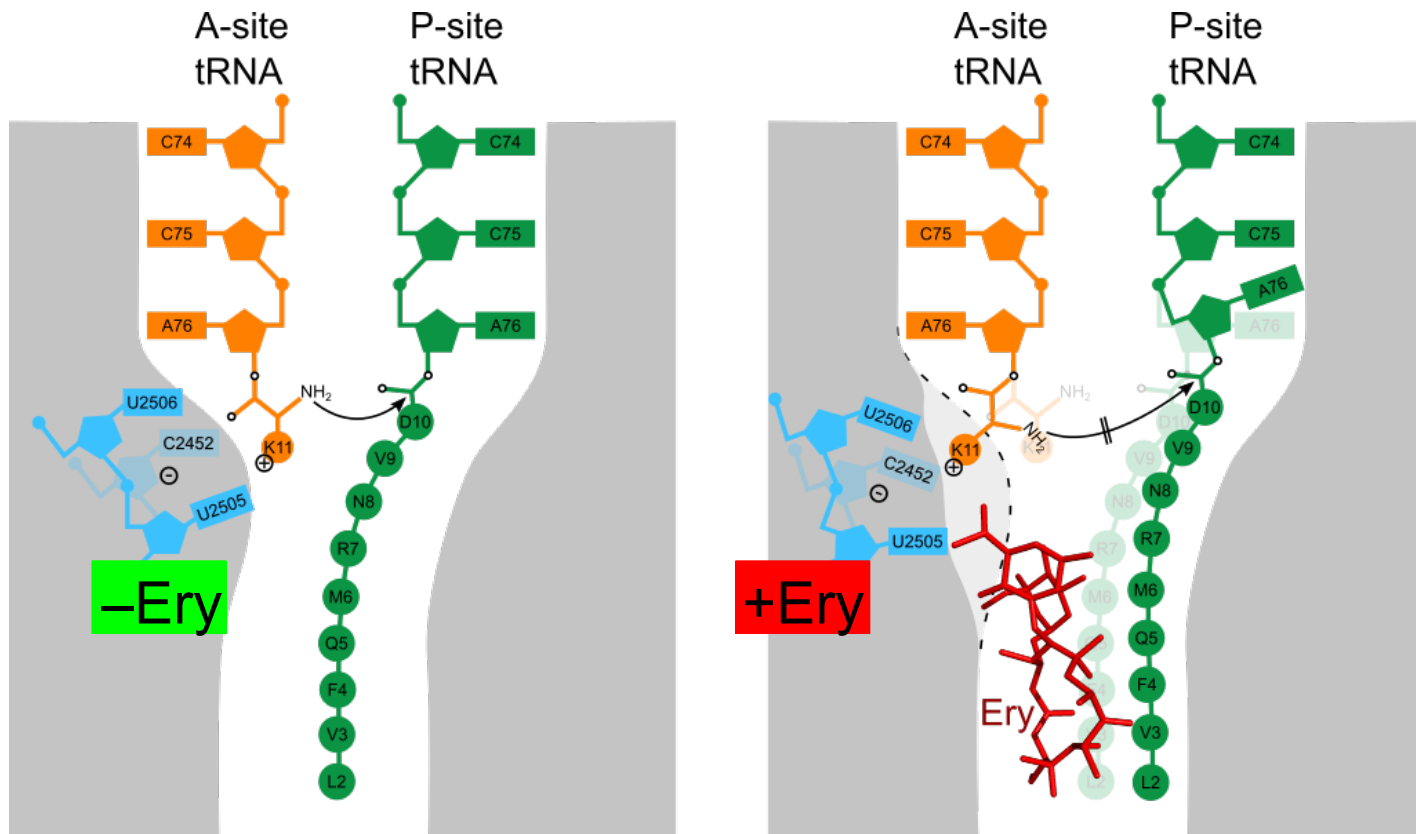
An Arg at codon 11 also stalls the ribosome



(Daniel Wilson, unpublished data)

Mechanism of stalling by erythromycin

Collaboration: Daniel Wilson (Univ. Munich)



+Erythromycin:

1) peptide conformation changed
→ P-site tRNA

2) A-site crevice perturbed → A-site amino acid K11 shifts

Distance between attacking NH_2 and carbonyl C increased

→ Inhibition of peptide bond formation

Towards a mechanistic understanding of protein function

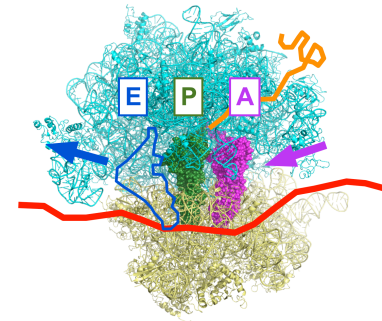
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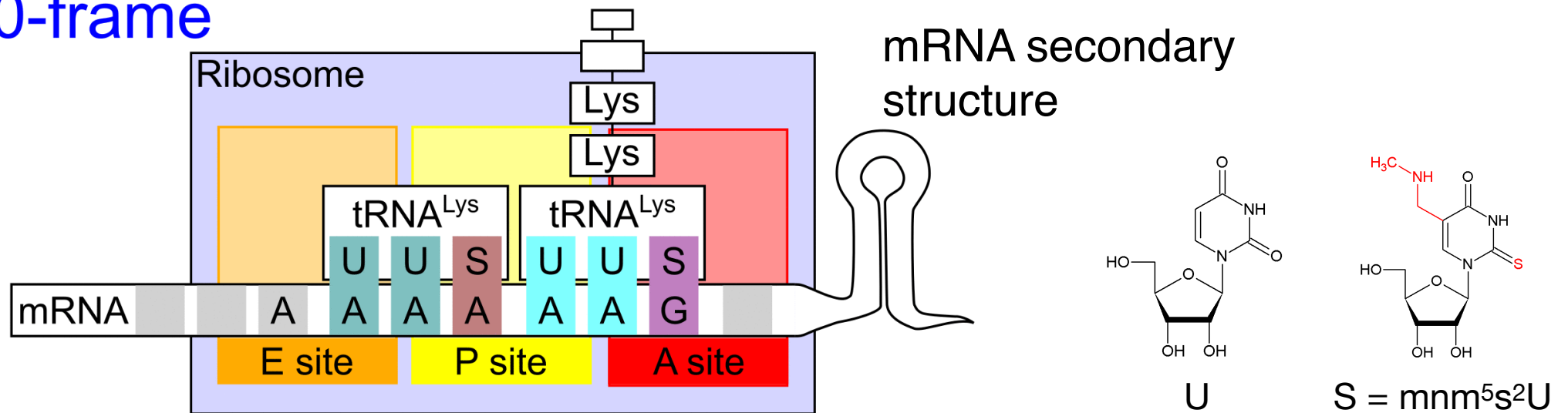
*Holger Stark, Marina Rodnina (MPI Göttingen)
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Simon Scheuring (Cornell Univ.)*

Thermodynamic Control of Ribosomal Frameshifting

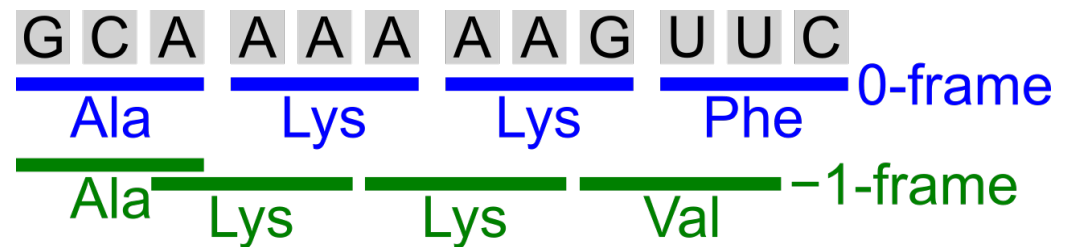
Programmed ribosomal frameshifting is a controlled reading-frame shift

- increase information content of genome (1 mRNA → 2 proteins)
- regulation of expression

0-frame



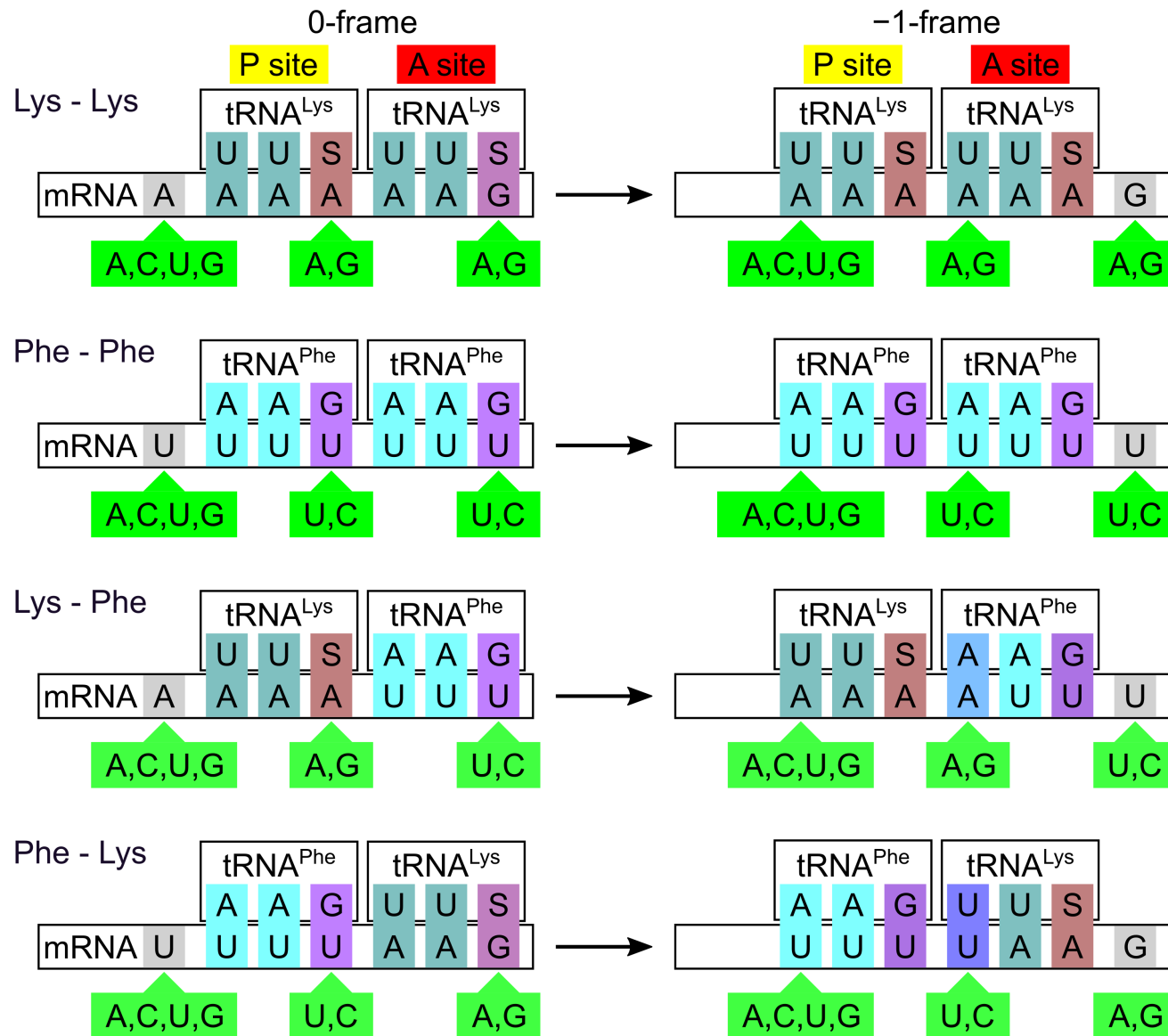
Example of –1 frame shifting:
E. coli gene *dnaX* encodes
 two polymerase subunits



Frameshift efficiency (FS): probability of ending up in the –1 frame

Thermodynamic Control of Ribosomal Frameshifting

Measured FS for 64 mRNA sequences with *dnaX* secondary structure

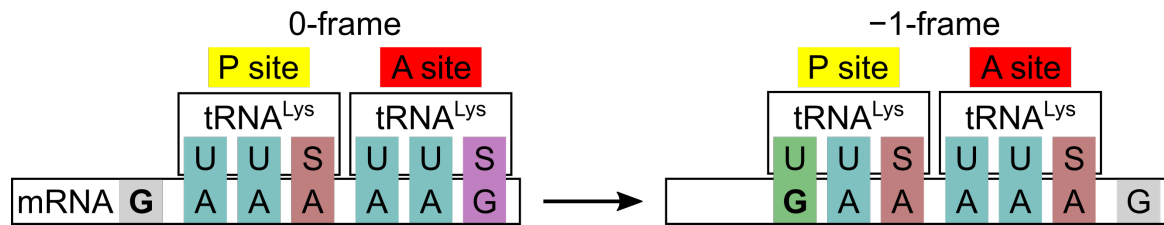


Thermodynamic Control of Ribosomal Frameshifting

Bayesian Statistics to Obtain Free Energies

Assumptions:

Frameshifting in equilibrium



$$FS = \frac{\exp\left(-\frac{\Delta G}{k_B T}\right)}{1 + \exp\left(-\frac{\Delta G}{k_B T}\right)}$$

base-pair
free-energy
differences

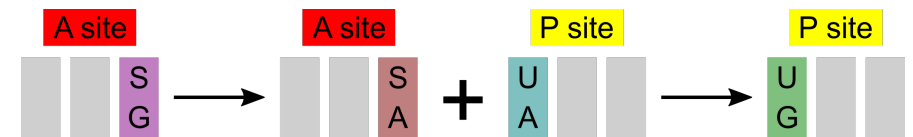
efficiencies from
experiment

$$P(\Delta G_{bp} | \mathbf{FS}_{\text{experiment}}) \propto P(\mathbf{FS}_{\text{experiment}} | \Delta G_{bp}) \cdot P(\Delta G_{bp})$$

probability of obtaining
experimental efficiencies

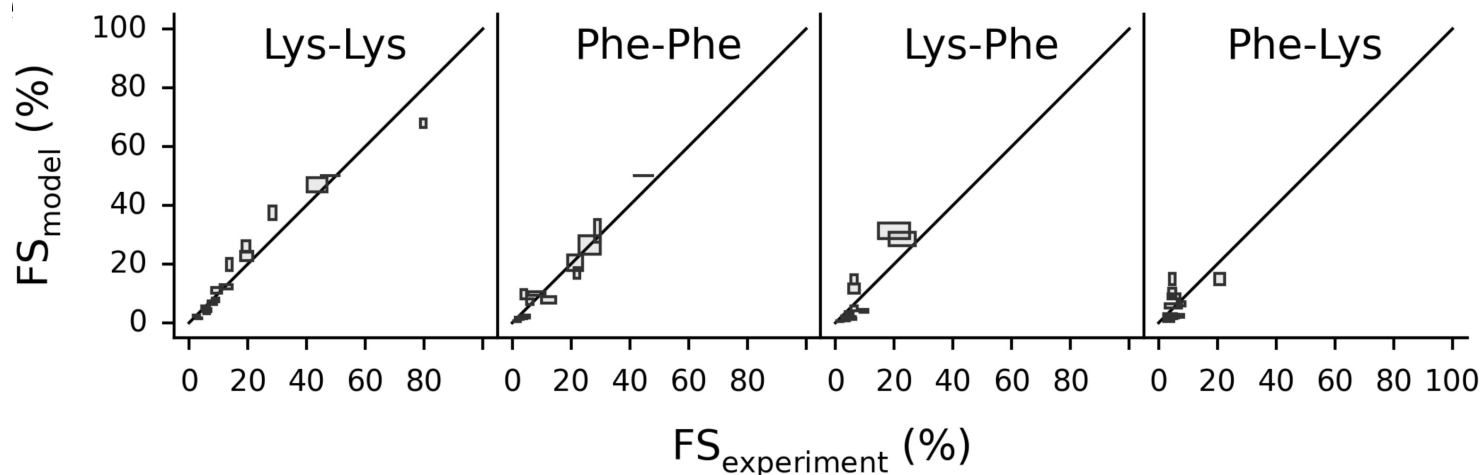
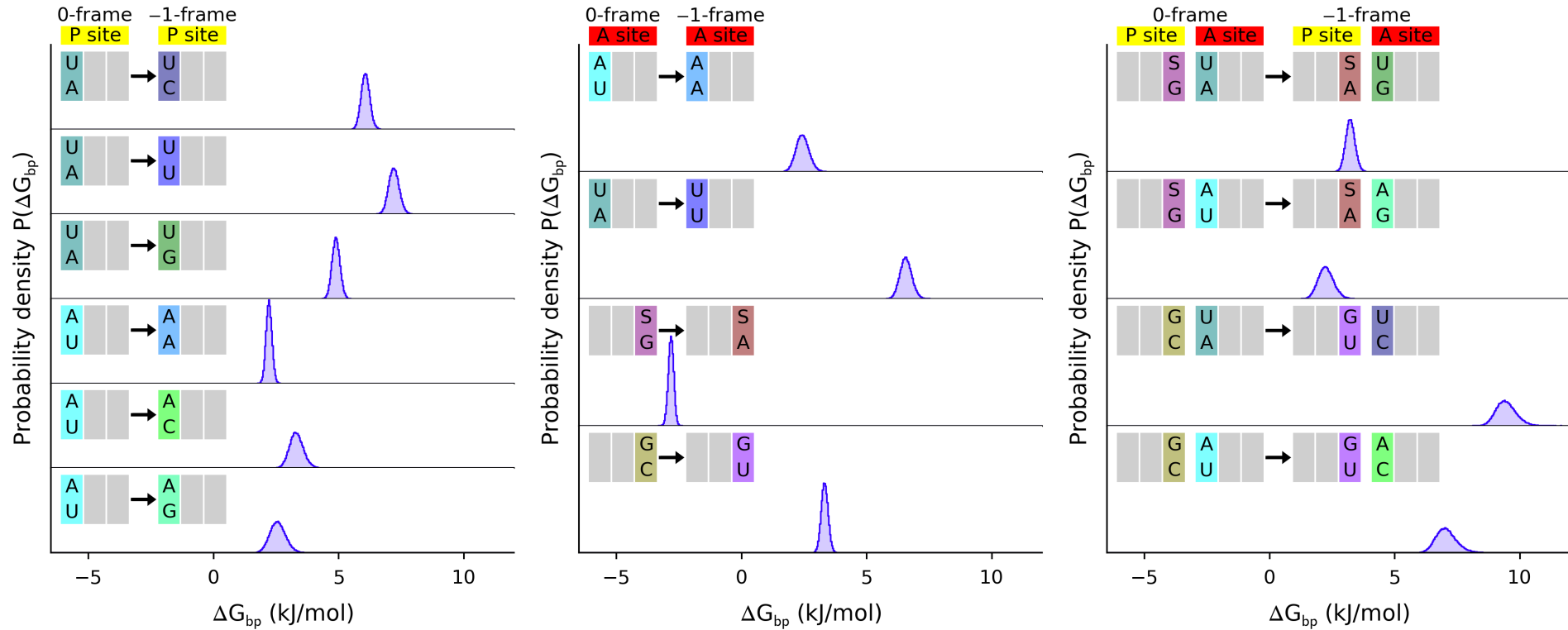
prior probability of base-pair
free-energy differences

base-pair free-energy differences
additive



$$\Delta G = \sum_{\text{bp changes}} \Delta G_{bp}$$

Thermodynamic Control of Ribosomal Frameshifting



no kinetic contribution

$$k_t \ll k_f, k_b$$

Bock, Caliskan, Korniy, Peske, Rodnina, Grubmüller *Nat. Comm.* 2019

Peng, Bock, Belardinelli, Peske, Grubmüller, Rodnina *Sci. Adv.* 2019

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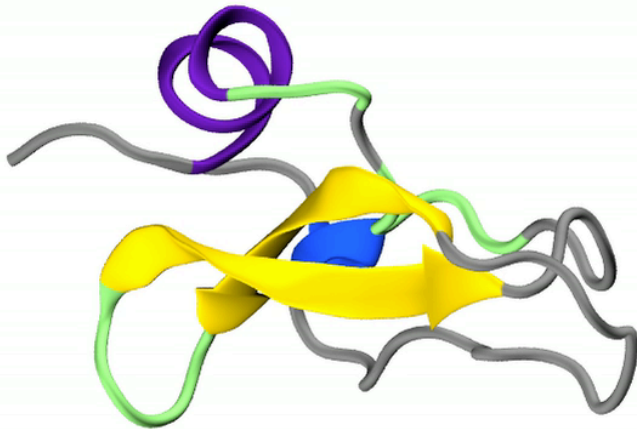
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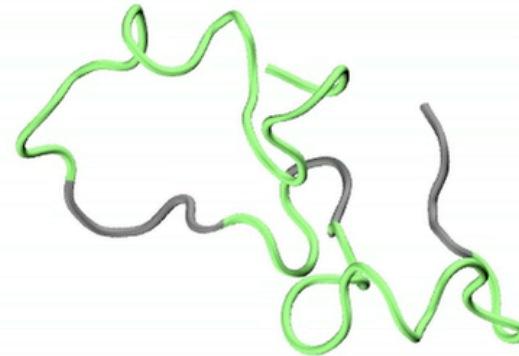
***Holger Stark, Marina Rodnina (MPI Göttingen)
Roland Beckmann, Daniel Wilson (Univ. Munich)
Simon Scheuring (Cornell Univ.)***

Folded vs. disordered proteins

> 30% of eukaryotic proteins
> 75% cancer linked proteins



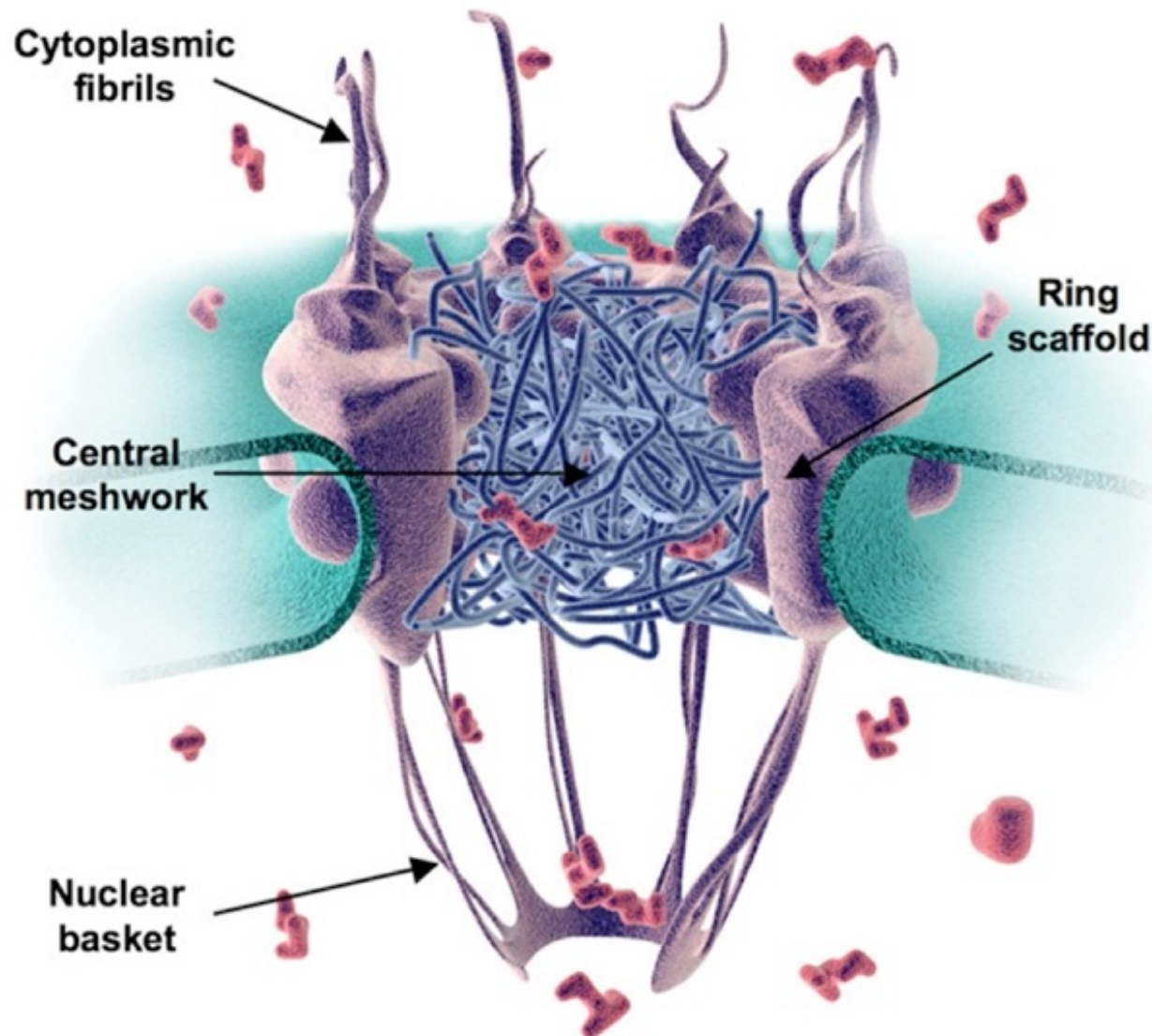
Folded



Disordered

Nucleoporins are prototypic disordered proteins

CYTOPLASM

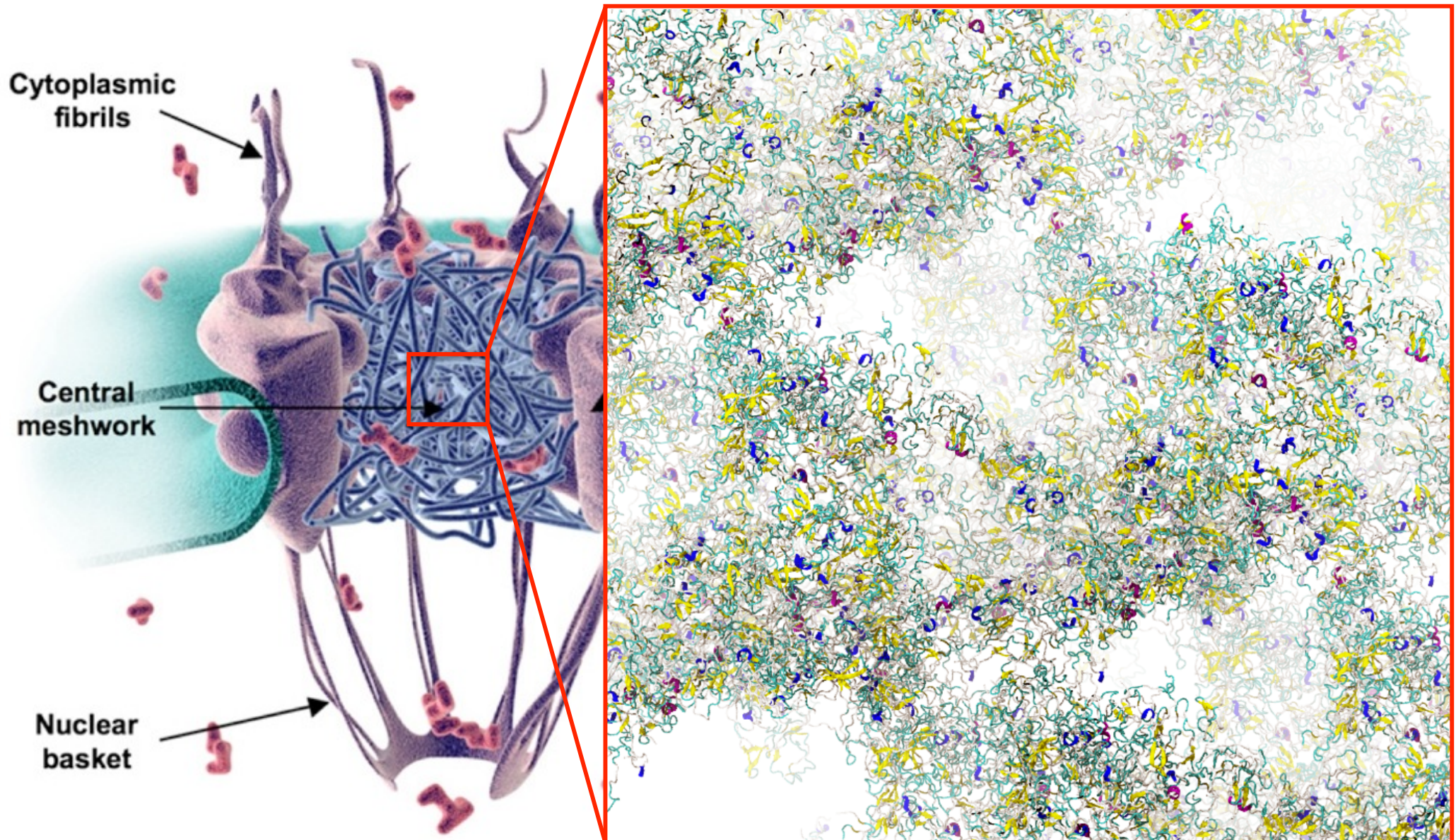


NUCLEUS

The nuclear pore complex is responsible for selective transport of macromolecules larger than 40 kDa into and out of the nucleus.

Nucleoporins are prototypic disordered proteins

CYTOPLASM

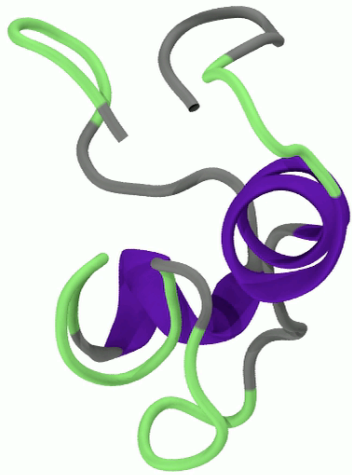


(unpublished data)

NUCLEUS

MD Simulations of Disordered Proteins

ff99sb*-ildn



CHARMM 22*



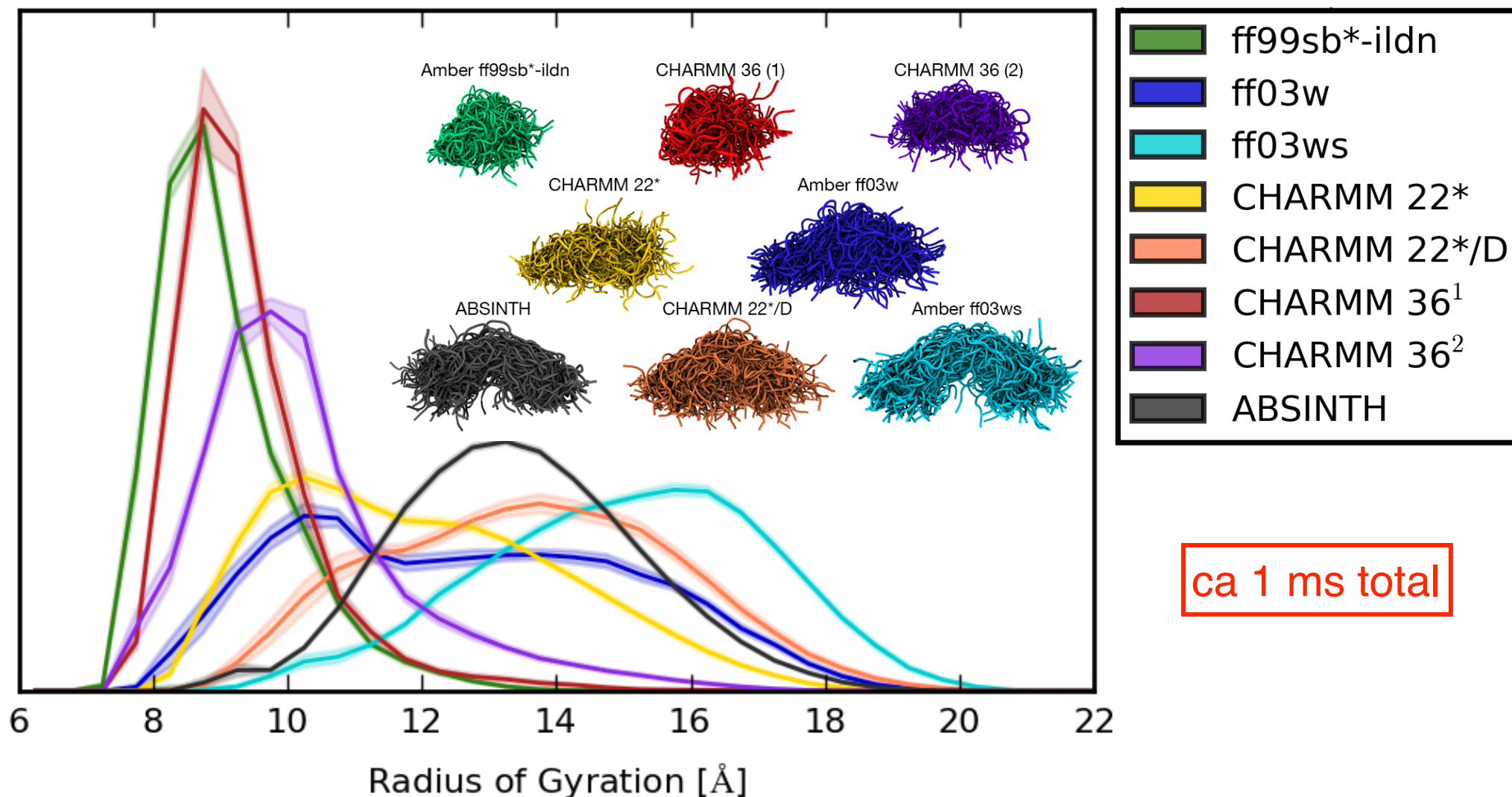
Force Fields Compared for Disordered Proteins

Force Field	Peptide Force Field	Water Model	References
ff99sb*-ildn	amber ff99sb*-ildn	TIP3P	Piana, S. <i>et al</i> (2011) <i>Biophys. J.</i> , 100 : L47-L49. Jorgensen, W. L. <i>et al</i> (1983) <i>J. Chem. Phys.</i> 79 : 926–935.
ff03w	amber ff03w	TIP4P/ 2005	Best, R. B. and Mittal, J. (2010) <i>J. Phys. Chem. B</i> , 114 : 14916–14923. Abascal J. L. and Vega C. (2005) <i>J. Chem. Phys.</i> 123 : 234505.
ff03ws	amber ff03ws	TIP4P/ 2005	Best, R. B., Zheng, W. and Mittal, J. (2014) <i>J. Chem. Theor. Comput.</i> , 10 : 5113–5124. Abascal J. L. and Vega C. (2005) <i>J. Chem. Phys.</i> 123 : 234505.
CHARMM 22*	CHARMM 22*	CHARMM-modified TIP3P	Piana, S. <i>et al</i> (2011) <i>Biophys. J.</i> , 100 : L47-L49. MacKerell, A. D. <i>et al</i> (1998) <i>J. Phys. Chem. B</i> 102 : 3586–3616.
CHARMM 22*/D	CHARMM 22*	TIP4P-D	Piana, S. <i>et al</i> (2011) <i>Biophys. J.</i> , 100 : L47-L49. Piana, S. <i>et al</i> (2015) <i>J. Phys. Chem. B</i> . (online)
CHARMM 36¹	CHARMM 36	CHARMM-modified TIP3P	Best, R. B. (2012) <i>J. Chem. Theor. Comput.</i> 8 : 3257-3273. MacKerell, A. D. <i>et al</i> (1998) <i>J. Phys. Chem. B</i> 102 : 3586–3616.
CHARMM 36²	CHARMM 36	TIP3P	Best, R. B. (2012) <i>J. Chem. Theor. Comput.</i> 8 : 3257-3273. MacKerell, A. D. <i>et al</i> (1998) <i>J. Phys. Chem. B</i> 102 : 3586–3616.
ABSINTH	OPLS-AA/L	ABSINTH (implicit water)	Vitalis A. and Pappu R. V. (2009) <i>J Comput Chem</i> 30 : 673–699.

Force Fields Differ Dramatically in Compactness

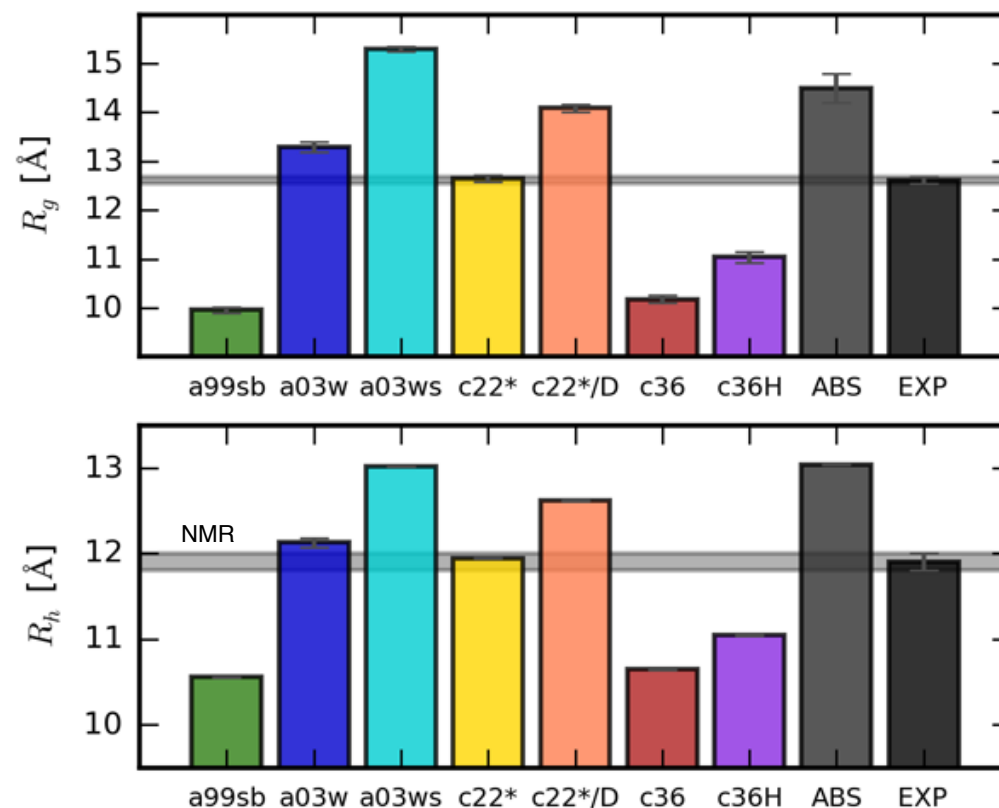
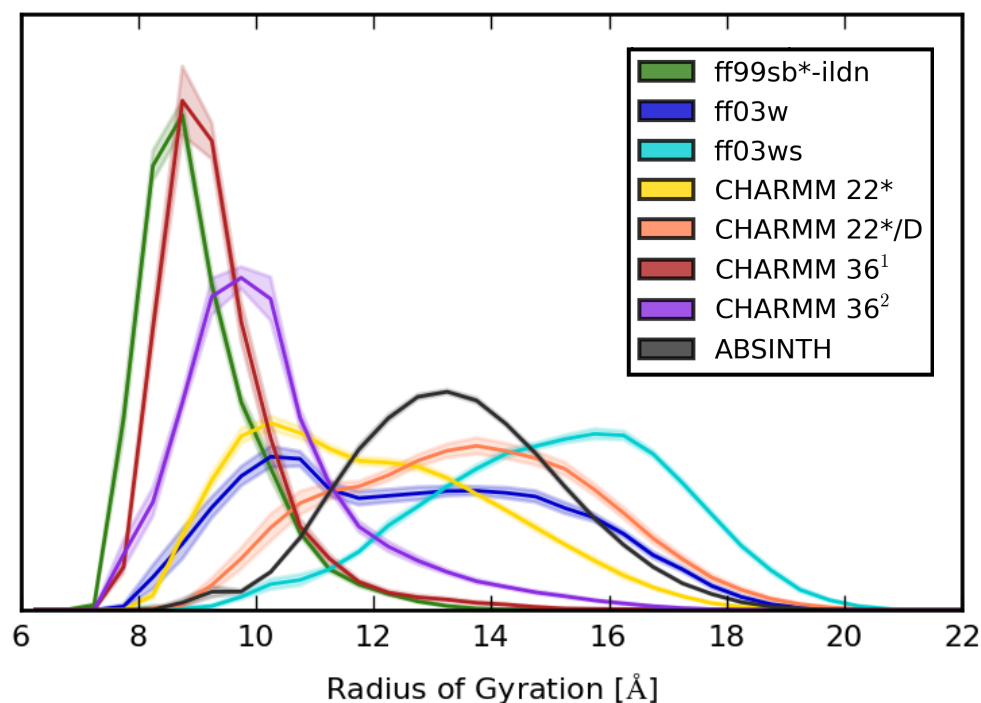
'RS-Peptide' **GAMGPSYGRSRSRSRSRSRSRSRSRS**

Xiang, S. Q. et al Structure , 2162–2174 (2013).



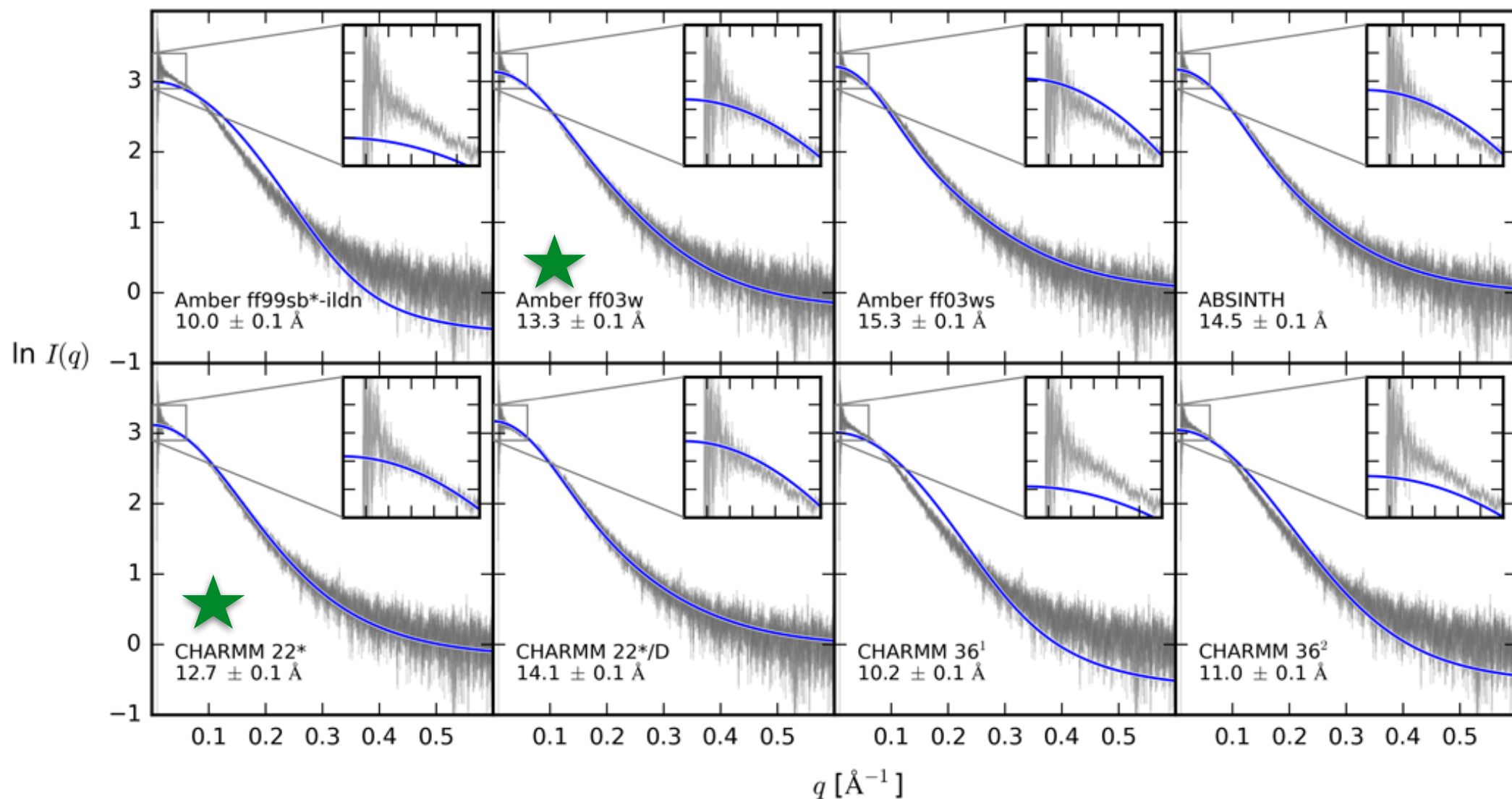
Comparison to Experiment

I. Small Angle X-Ray Scattering (Compactness)



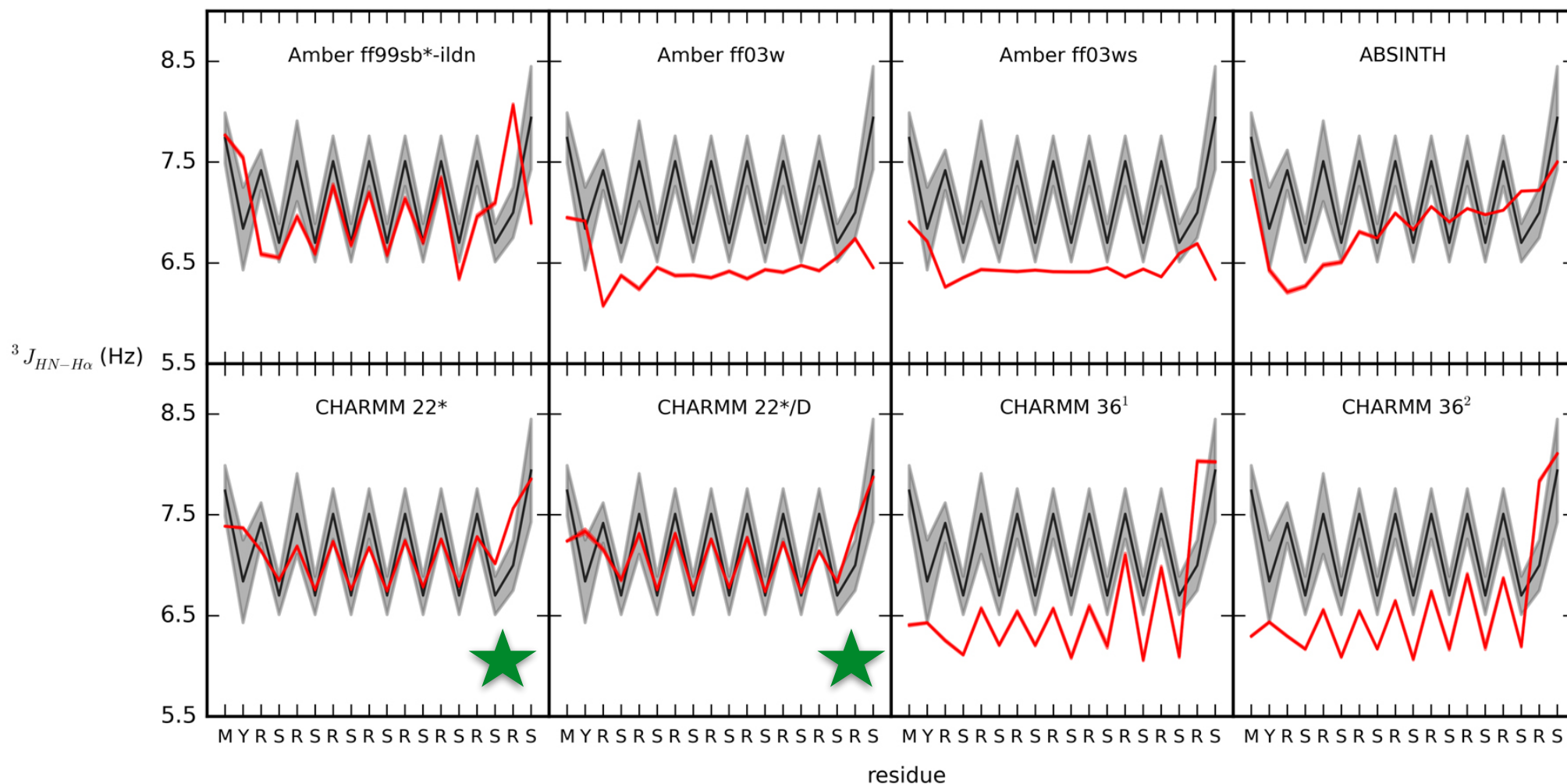
Comparison to Experiment

I. Small Angle X-Ray Scattering (Compactness)



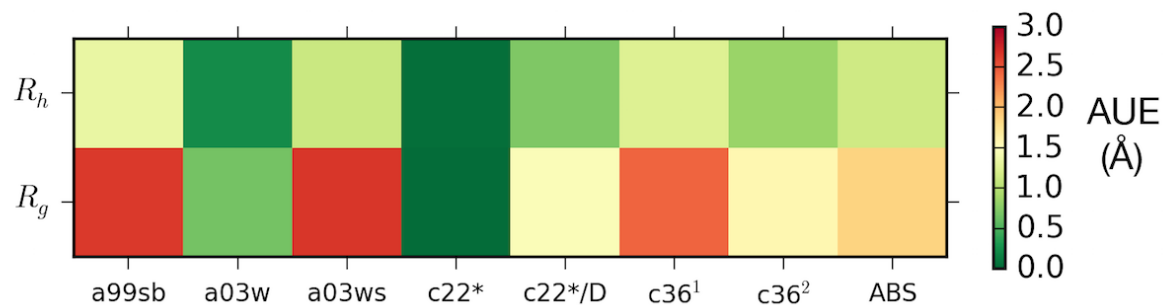
Comparison to Experiment

II. NMR 3J(HNHA)-Coupling (Secondary Structure)



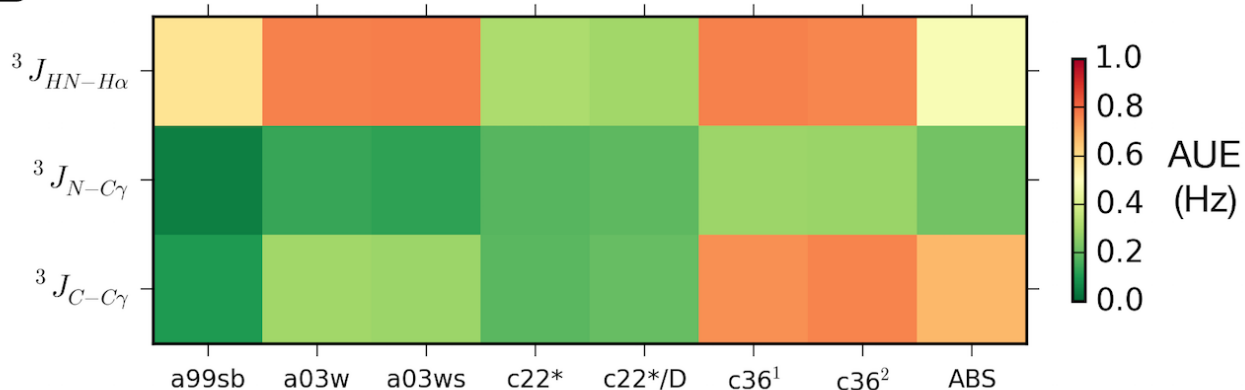
Summary: Comparison to Experiment

A

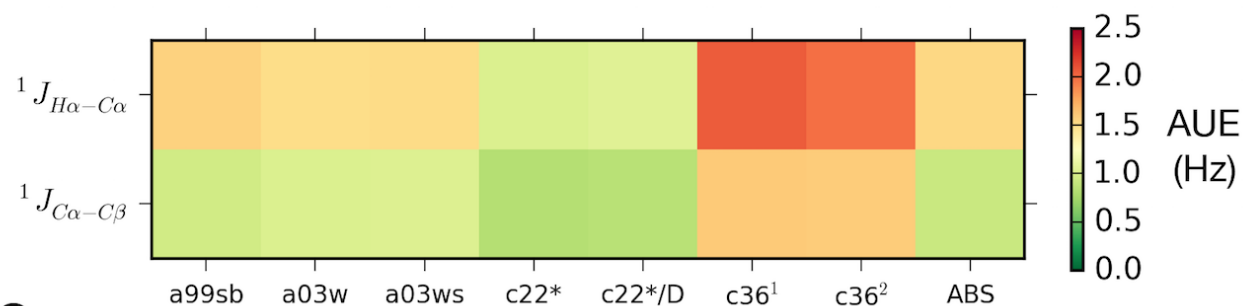


Chain Dimensions

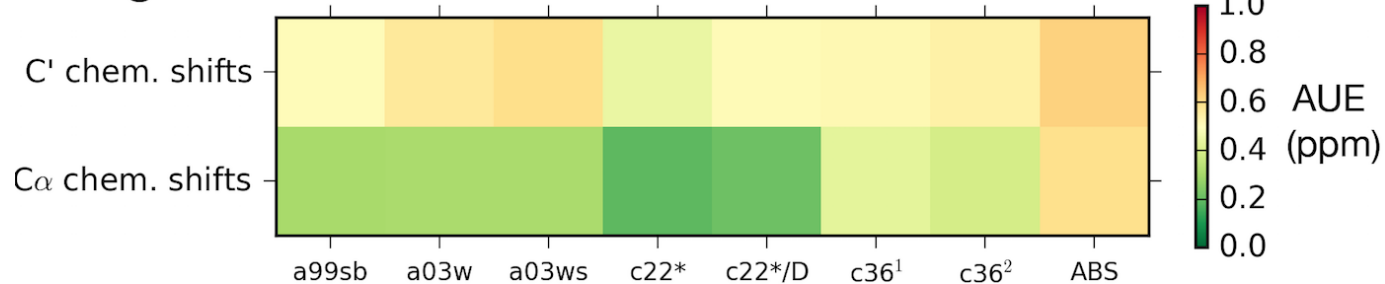
B



Scalar Couplings

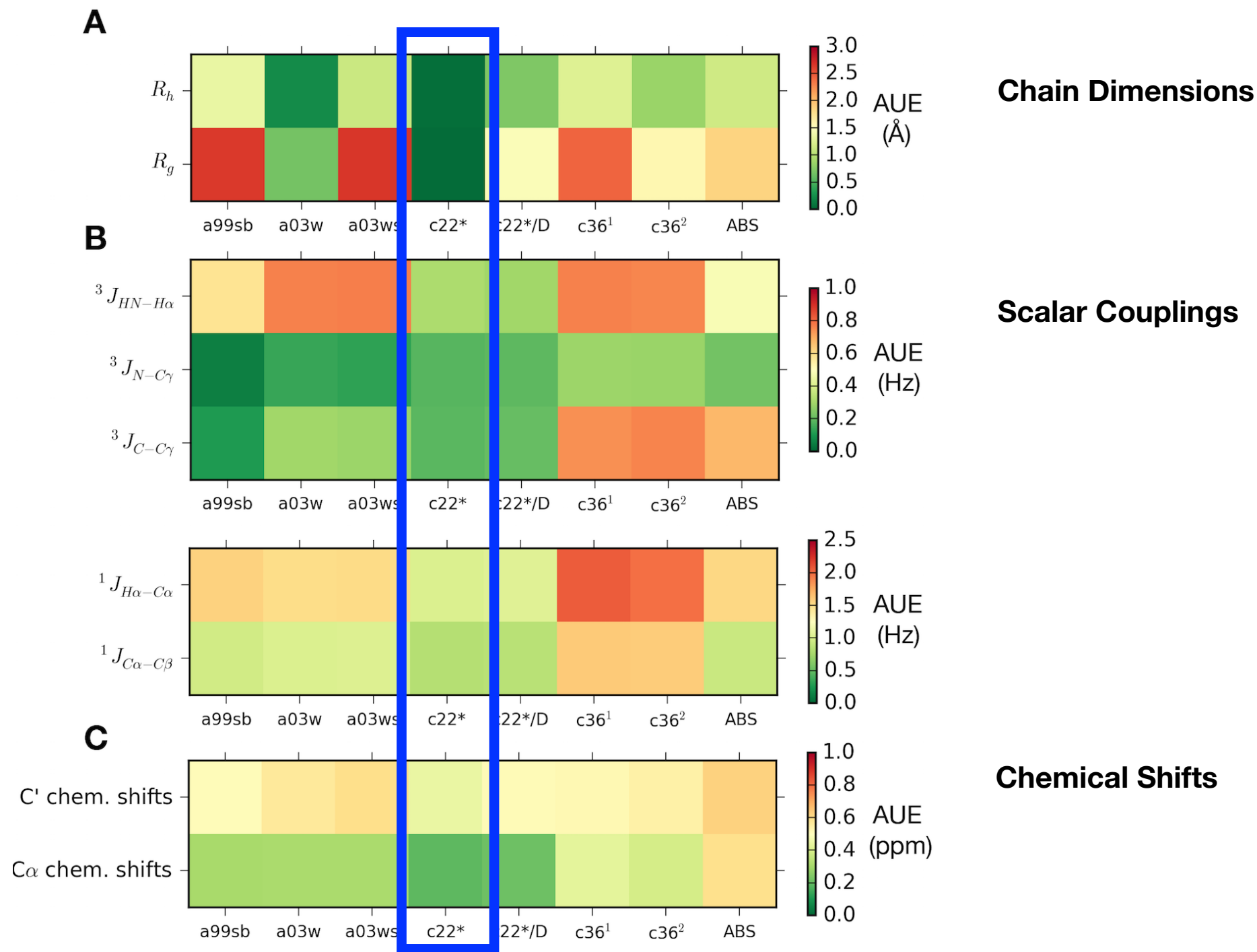


C



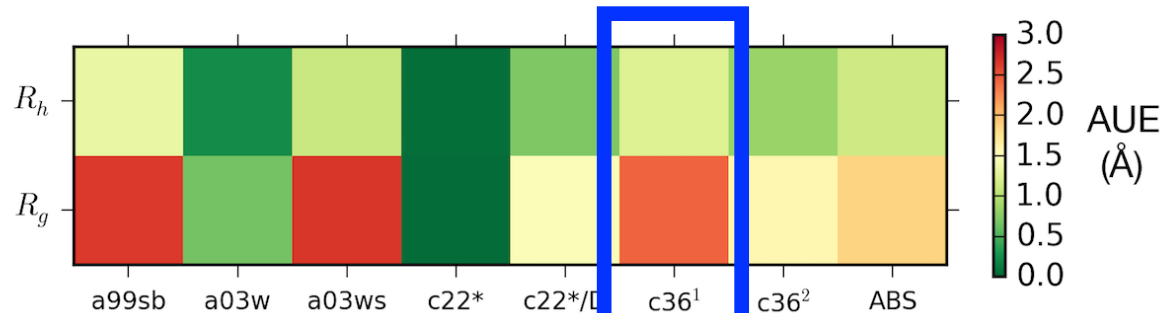
Chemical Shifts

Summary: Comparison to Experiment



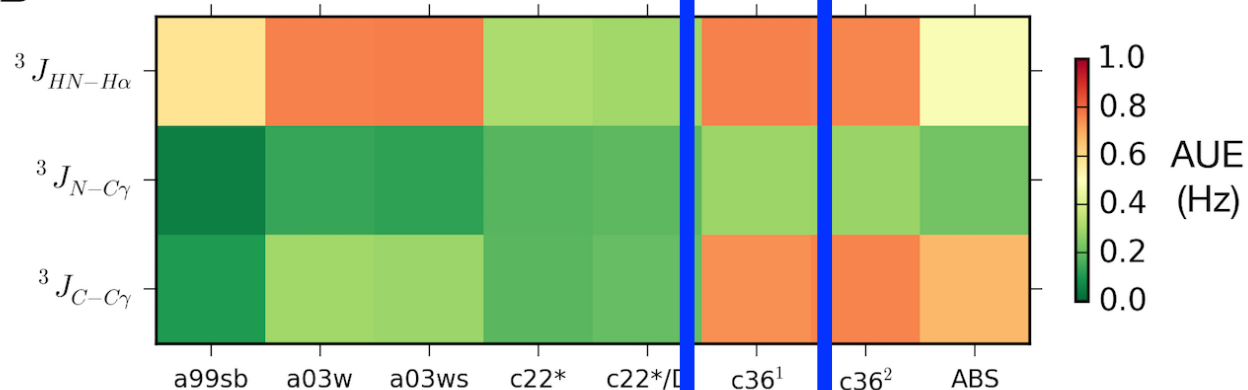
Summary: Comparison to Experiment

A



Chain Dimensions

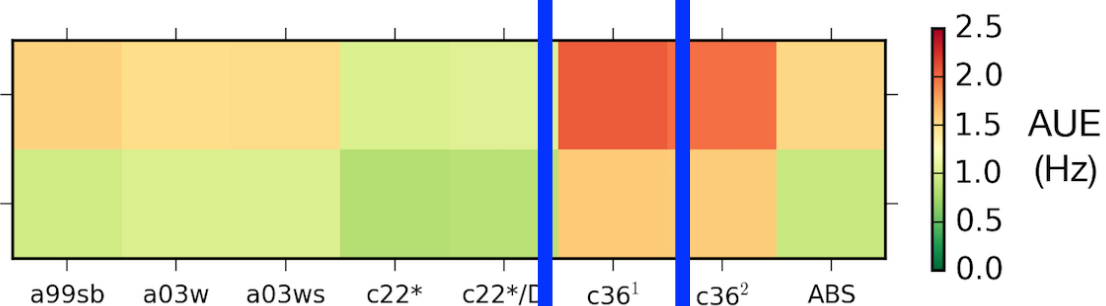
B



Scalar Couplings

$^1J_{H\alpha-C\alpha}$

$^1J_{C\alpha-C\beta}$



C

C' chem. shifts

C α chem. shifts

Chemical Shifts

New Force Field Version:

Validated against 14 peptides and 15 proteins,
cumulative simulation time of more than 500 μ s

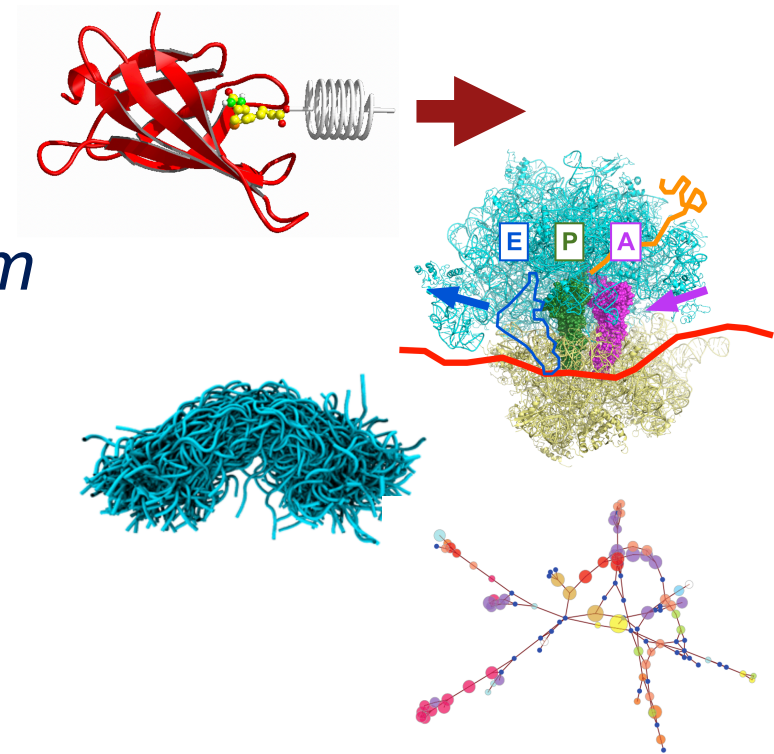
CHARMM36m: Improved conformational sampling in simulations of intrinsically disordered peptides

Jing Huang,¹ Sarah Rauscher,² Ting Ran,¹ Michael Feig,³ Bert de Groot,² Helmut

Grubmueller,² Alexander D. MacKerell Jr.^{1*}

Towards a mechanistic understanding of protein function

- (1) Ligand unbinding revisited*
- (2) Ribosomal antibiotics mechanism*
- (3) Ribosomal Frameshifting*
- (4) Intrinsically Disordered Proteins*
- (5) The Dynasome*



AFM+ X-ray + cryo EM + MD

*Lars Bock, Christian Blau, Michal Kolar, Andrea Vaiana, Andreas Russek,
Sarah Rauscher, Ulf Hensen*

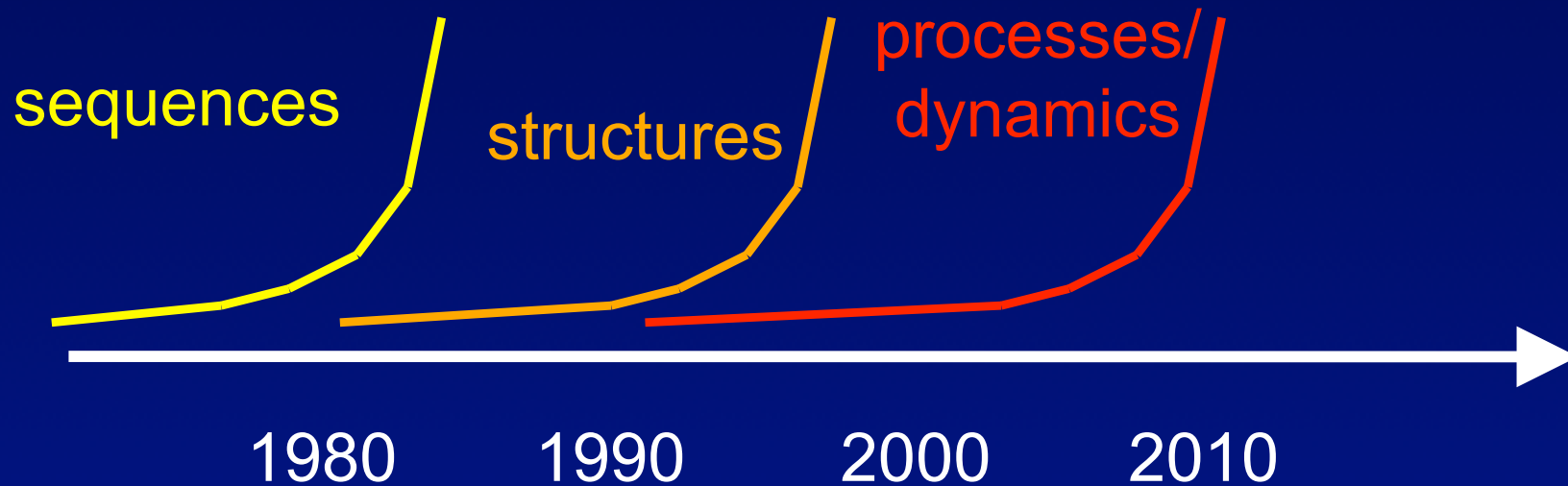
*Holger Stark, Marina Rodnina (MPI Göttingen)
Roland Beckmann, Daniel Wilson (Univ. Munich)
Simon Scheuring (Cornell Univ.)*

Charting the Protein Dynamics Landscape: The Dynasome

Ulf Hensen, Tim Meyer, Jürgen Haas, Rene Rex

Collab.: Gert Vriend (Nijmegen, NL)

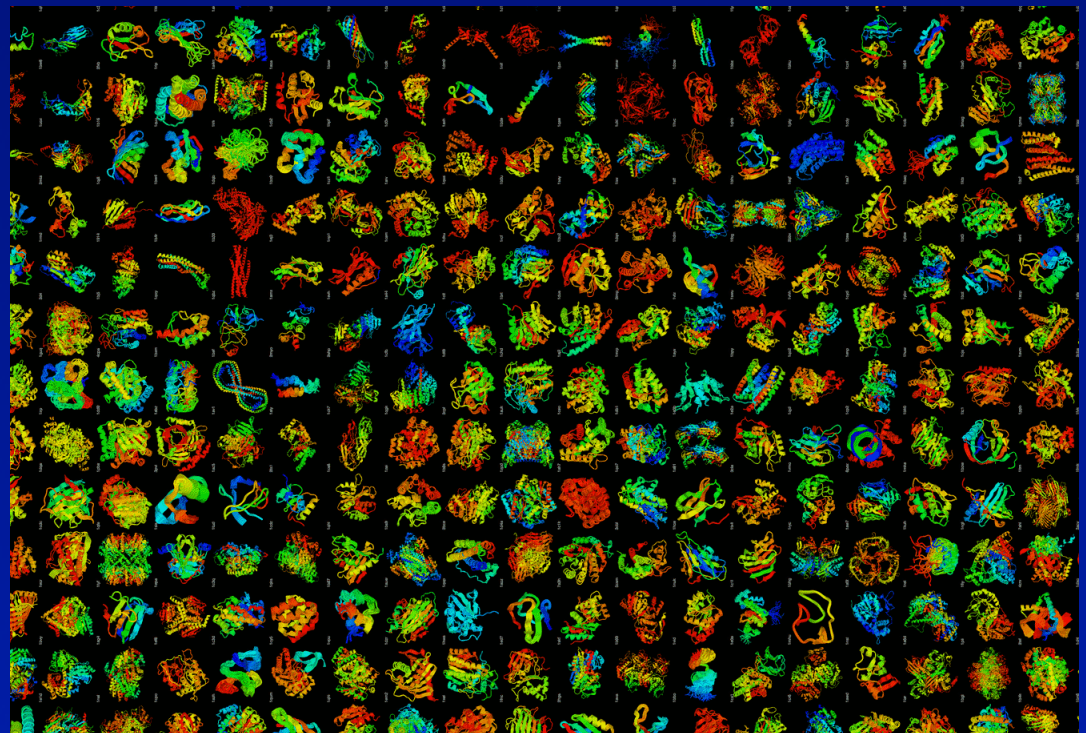
Perspective: The 'Dynasome'



Phylogenetic trees

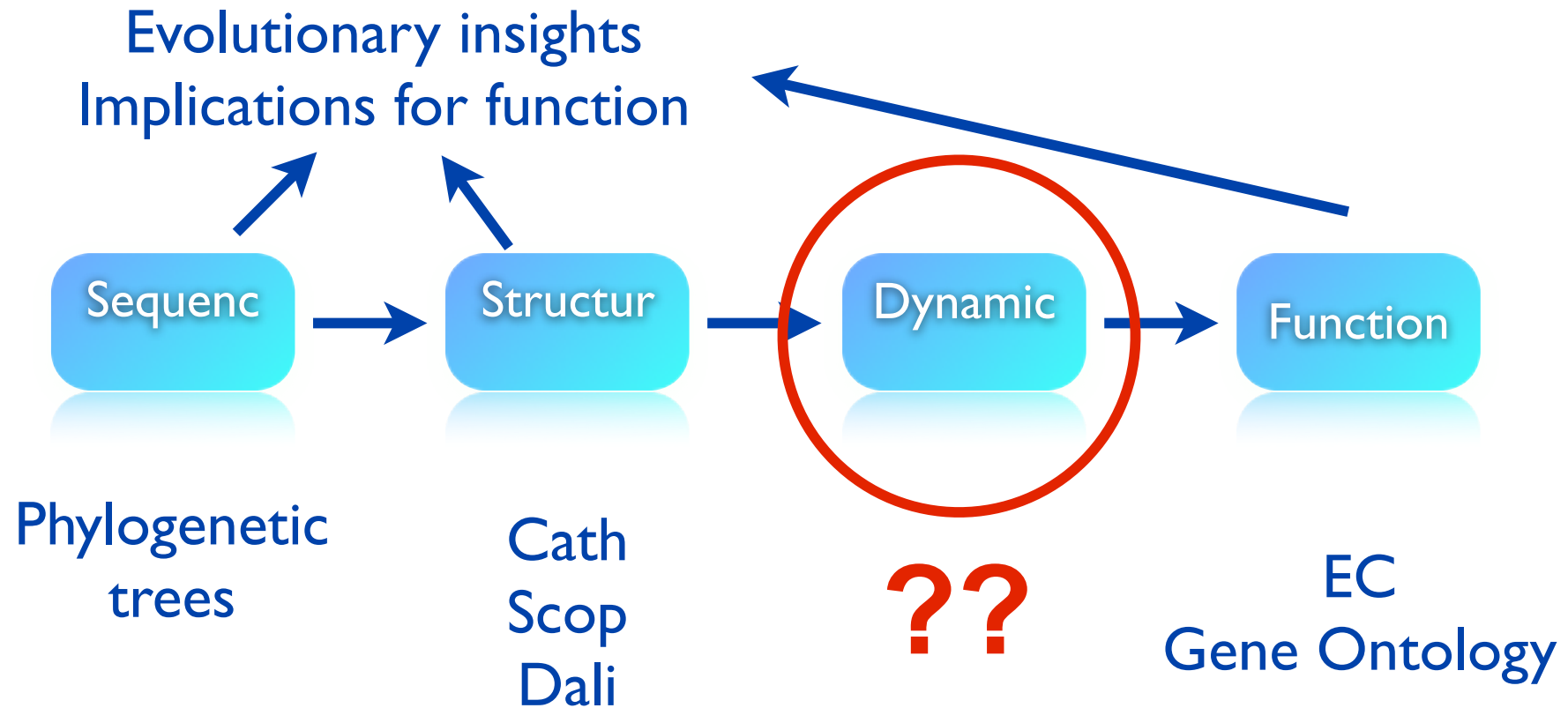
Protein structures fall
into families

-> Can we also identify
and classify
dynamics motifs?



Exploring the protein dynamics space:

Structure / dynamics / function relationship



Ulf Hensen, Tim Meyer,
Jürgen Haas, René Rex,
Collaboration: Gert Vriend

Pang A, Arinaminpathy Y, Sansom M, Biggin P (2005) Proteins 61: 809–822.
Meyer T, de la Cruz X, Orozco M (2009) Structure 17: 88–95.
Jonsson AL, Scott KA, Daggett V (2009) Biophysical Journal 97: 2958–2966.

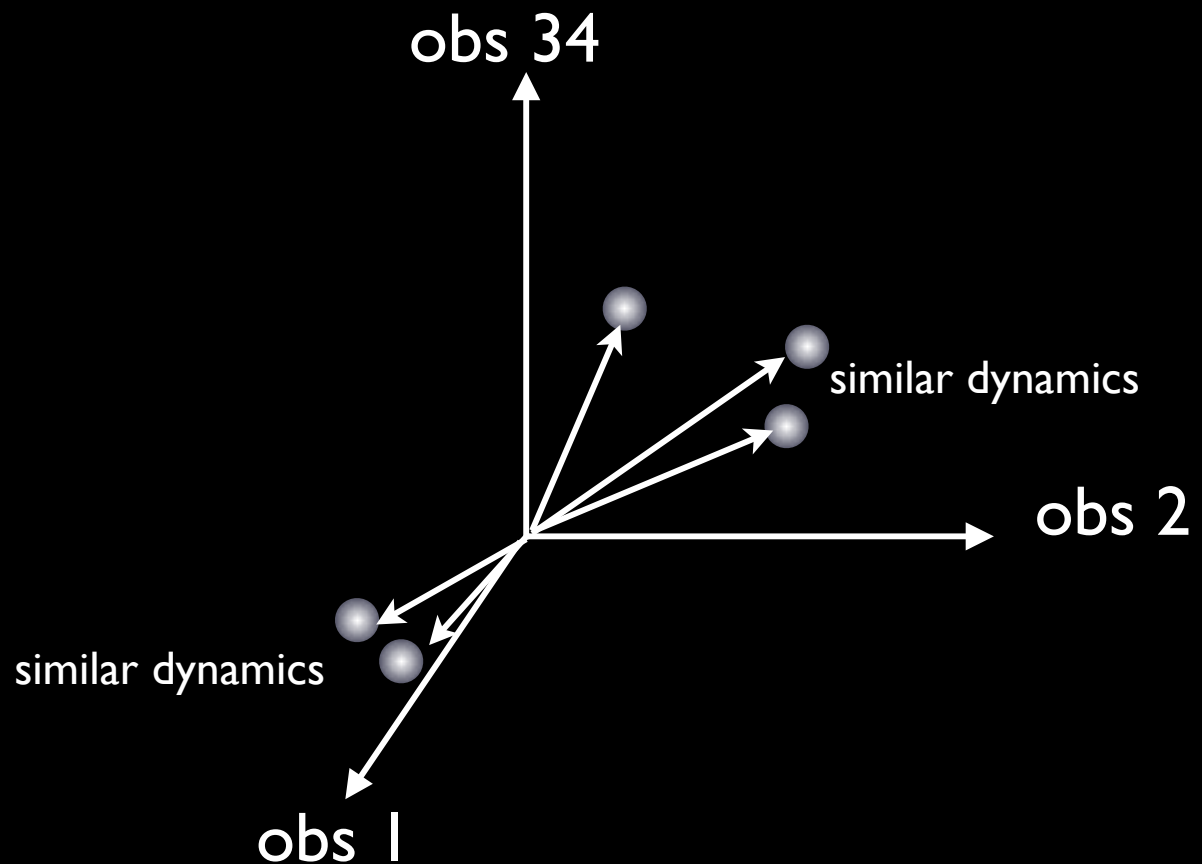


34 Dynamics observables

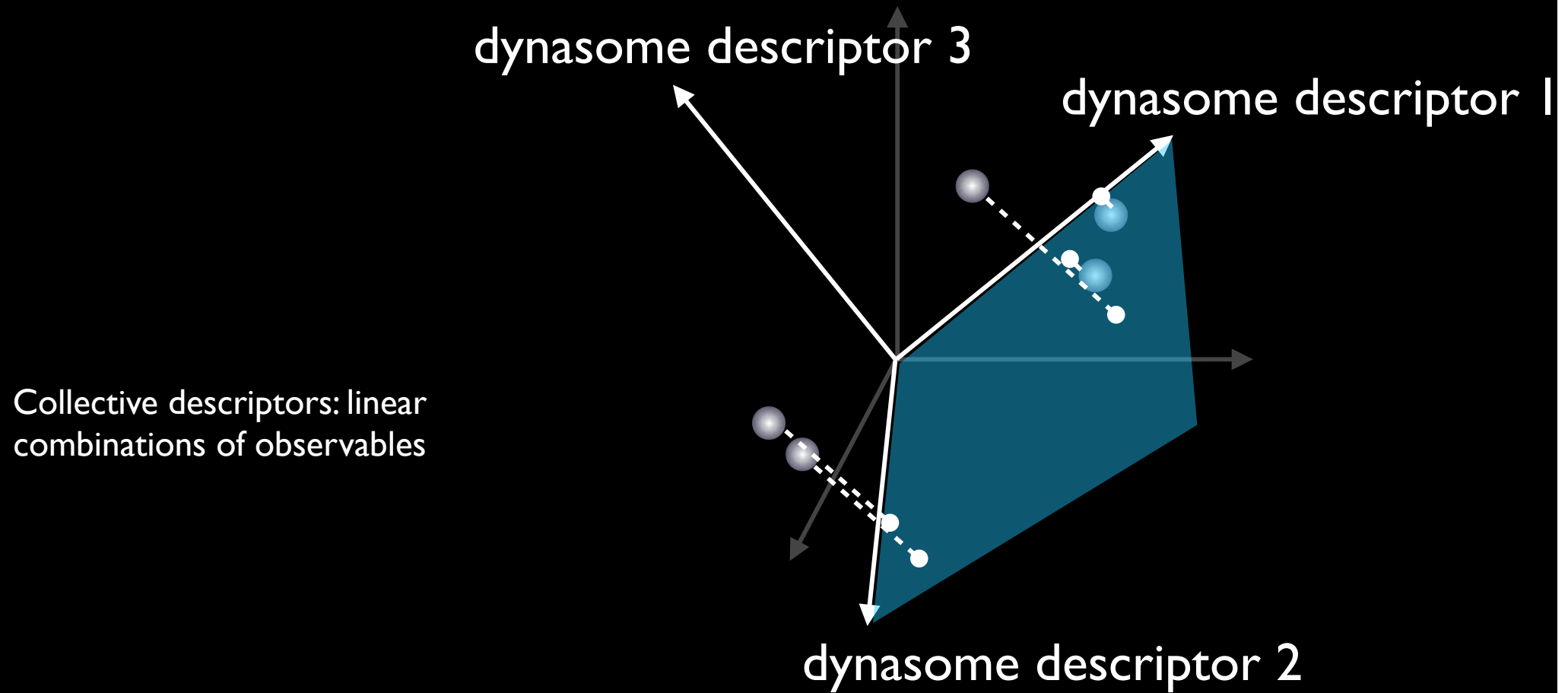
from 100 ns Simulations of 112 representative proteins

Index	Symbol	Description
1, ..., 5	$\lambda_1, \dots, \lambda_5$	Eigenvalues 1, ... 5
6	m^λ	Slope of the middle third of the eigenvalue spectrum
7	χ_λ^2	R^2 value of that fit
8, ..., 12	$\cos_1, \dots, \cos_5$	Cosine content of the principal components 1-5
13, 14, 15	$\chi_{\mathcal{N},1}^2, \chi_{\mathcal{N},2}^2, \chi_{\mathcal{N},3}^2$	Goodness of fit of a Gaussian fit to principal components 1-3
16, ..., 20	$f_1^{\text{acf}}, \dots, f_5^{\text{acf}}$	Friction constant derived from a fit to the autocorrelation function of principal components 1-5
21	$\mu(\gamma)$	Measure of the average ruggedness of the energy landscape: Average slope of a linear fit to the time dependent eigenvalue spectrum λ .
22	$\text{skew}(\gamma)$	Skewness of the distribution of these ruggedness values (6) of each collective degree of freedom.
23	$\text{kurt}(\gamma)$	Kurtosis thereof.
24	μ^{RMSD}	Average root-mean square deviation from the X-ray structure
25	c_v^{RMSD}	Coefficient of variation thereof
26	μ^{RMSF}	Average residual fluctuations with respect to the average ensemble structure
27	c^{r_g}	Coefficient of variation of the radius of gyration
28, ..., 31	$c^{\text{struct}}, c^\alpha, c^\beta, c^{\text{turn}}$	Coefficient of variation of secondary structure content: total, α -helix, β -sheet, turn
32	μ^{SAS}	Average solvent accessible surface
33	c^{SAS}	Coefficient of variation thereof
34	S_{RMSF}	RMSF entropy

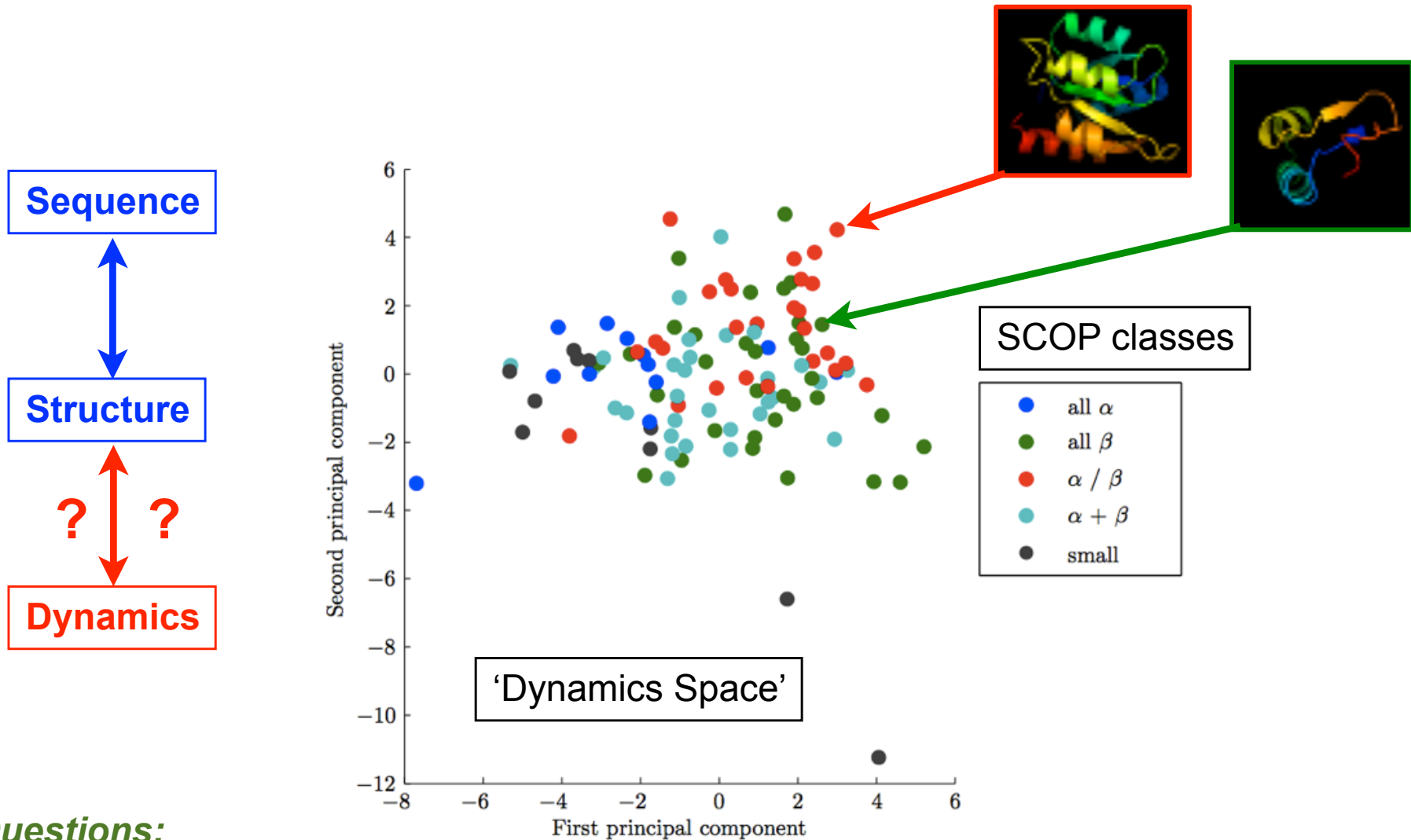
Each protein $\rightarrow$ vector in dynamics space



Projection into essential subspace: PCA



Structure / dynamics relationship



Questions:

- What is the meaning of the principal components?
- Does similar structure imply similar dynamics?
- Does similar dynamics require similar structure?
- How does function correlate with dynamics?

Collective dynamics descriptors

Descriptor 2

$f_1^{\text{acf}}(-)$	(12 %)
$f_2^{\text{acf}}(-)$	(8 %)
$f_4^{\text{acf}}(-)$	(7 %)
$\chi_{N,1}^2(-)$	(7 %)
$\text{kurt}(\gamma)(+)$	(7 %)

Kinetics

slow

....

fast

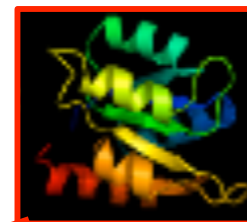
'Dynamics Space'

flexible

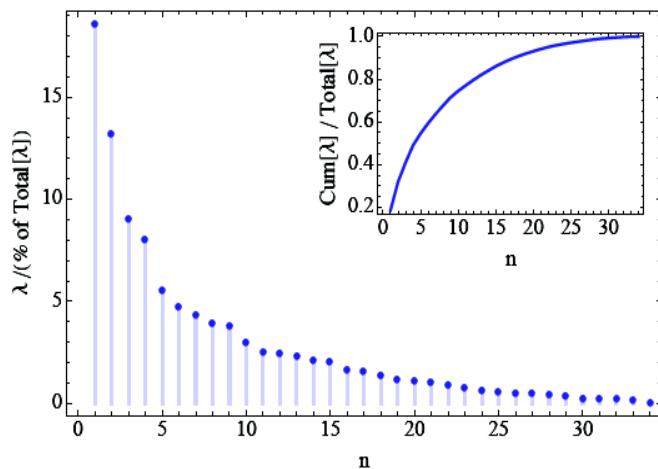
....

rigid

Thermodynamics



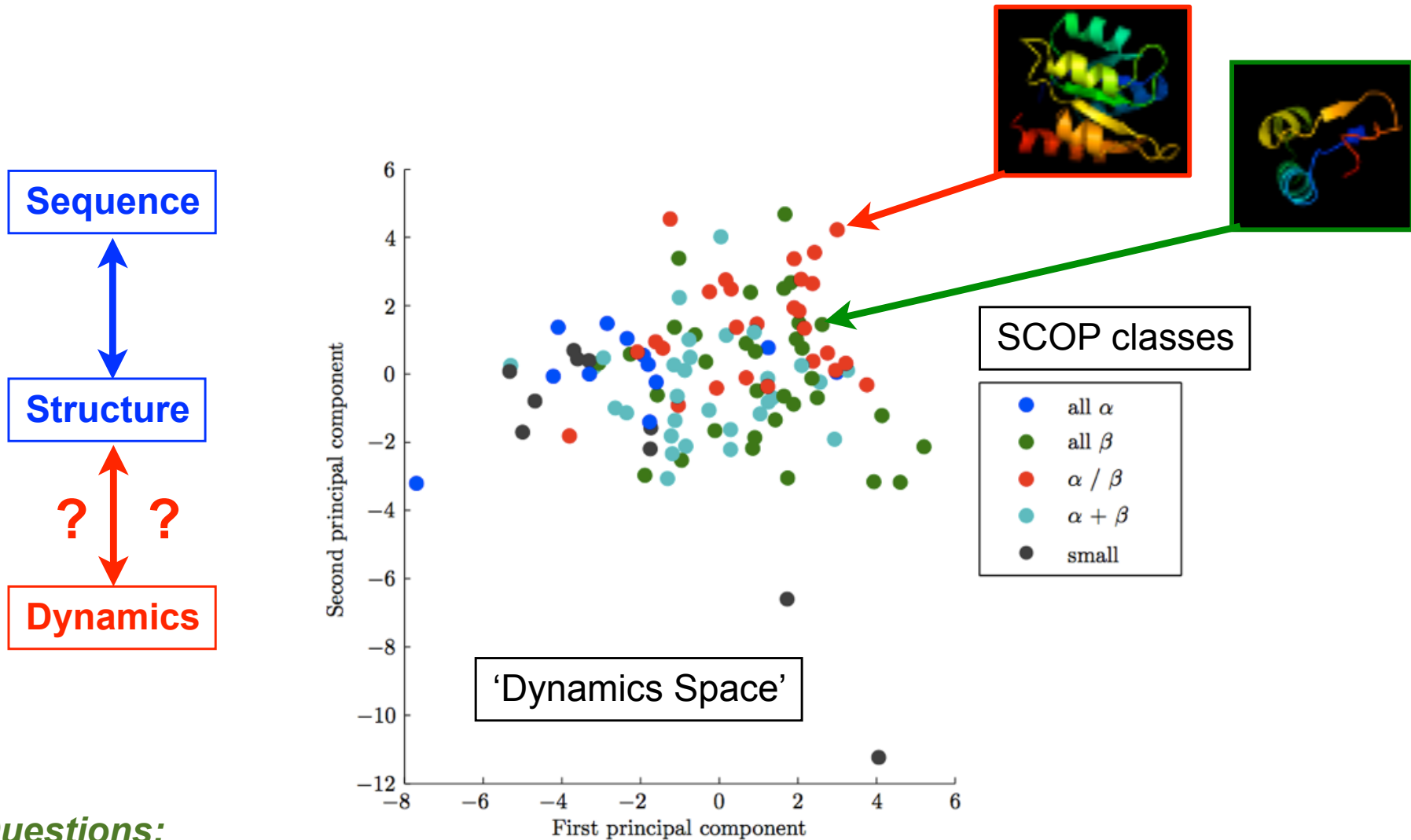
- all α
- all β
- α / β
- $\alpha + \beta$
- small



Descriptor 1

$\mu^{\text{RMSF}}(-)$	(10 %)
$\mu^{\text{RMSD}}(-)$	(9 %)
$c^{rg}(-)$	(9 %)
$\text{skew}(\gamma)(+)$	(7 %)
$\mu(\gamma)(+)$	(7 %)

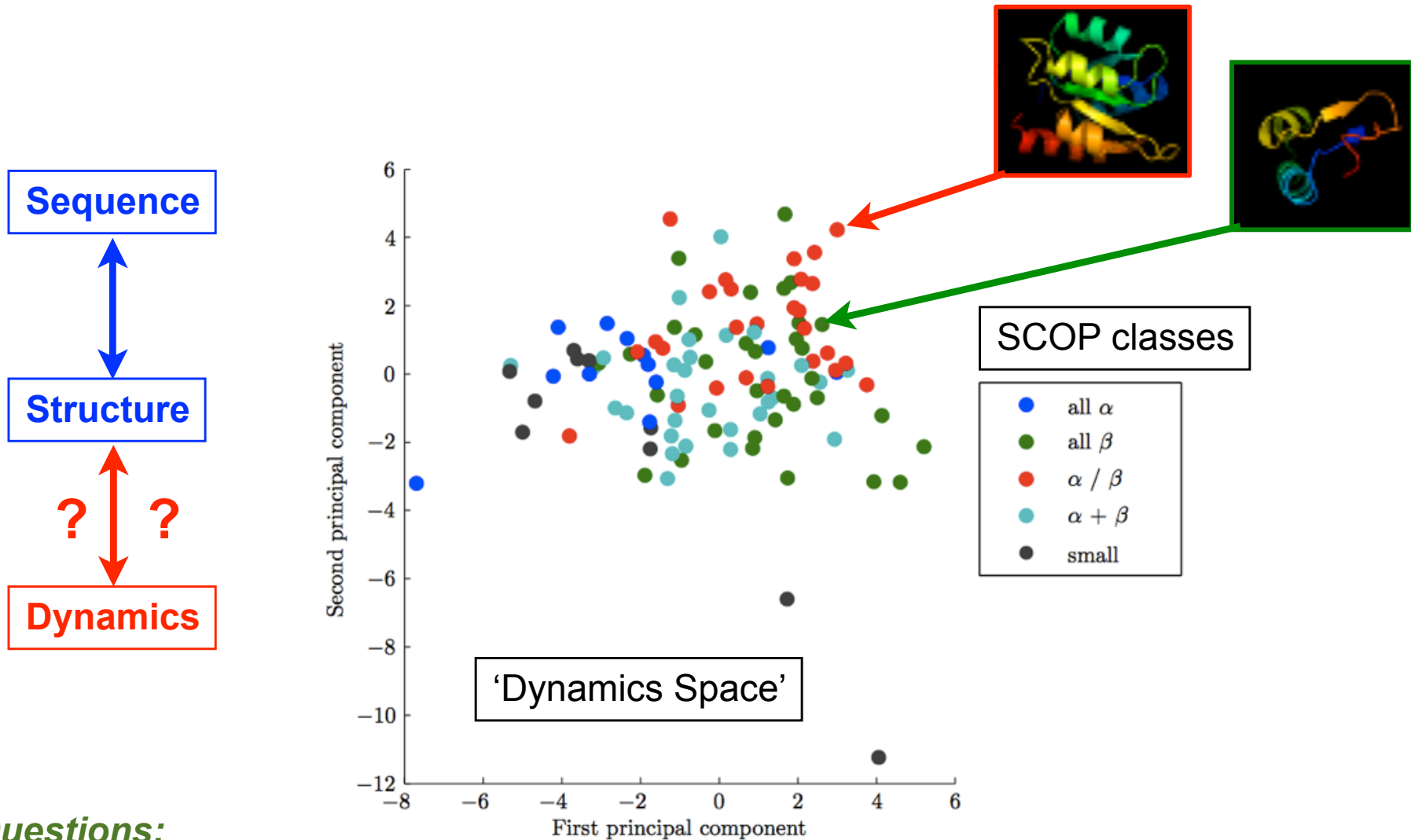
Structure / dynamics relationship



Questions:

- What is the meaning of the principal components?
- Does similar structure imply similar dynamics?
- Does similar dynamics require similar structure?
- How does function correlate with dynamics?

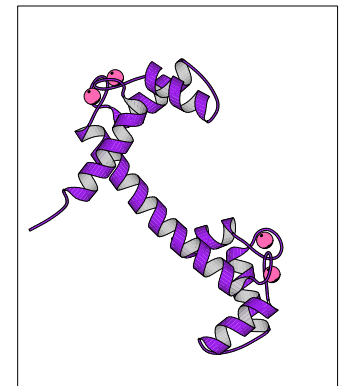
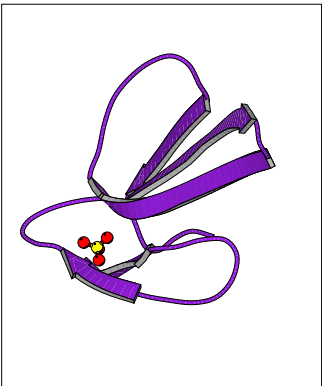
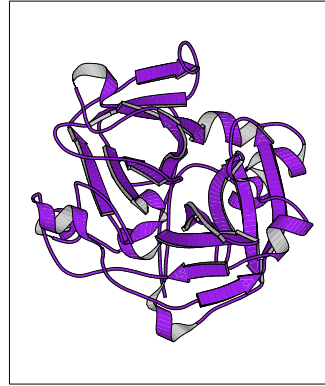
Structure / dynamics relationship



Questions:

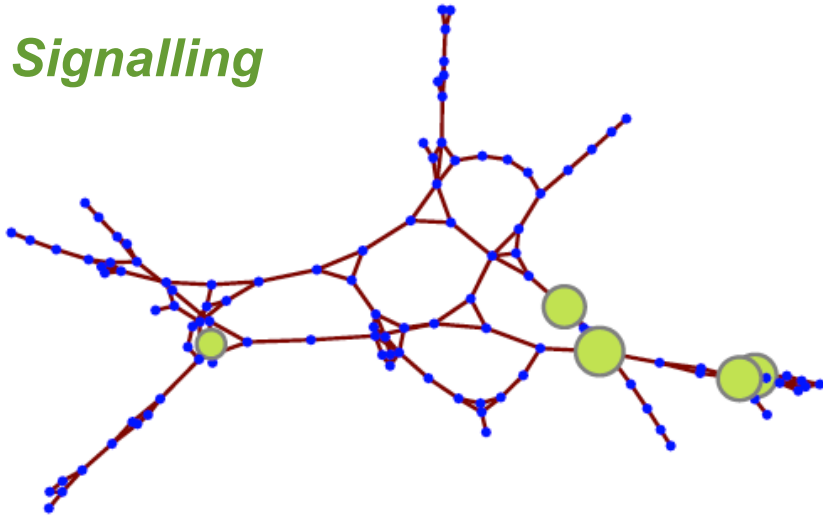
- What is the meaning of the principal components?
- Does similar structure imply similar dynamics?
- Does similar dynamics require similar structure?
- How does function correlate with dynamics?

The fine structure of the dynasome

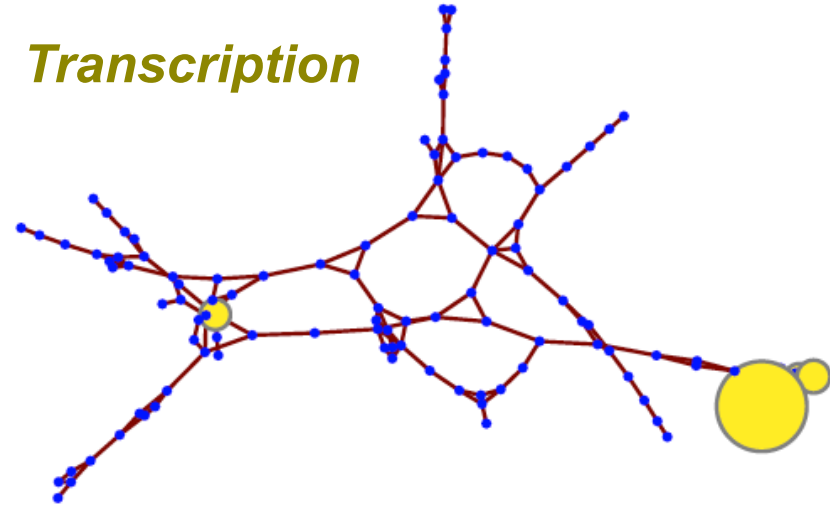


Dynamics correlates with function!

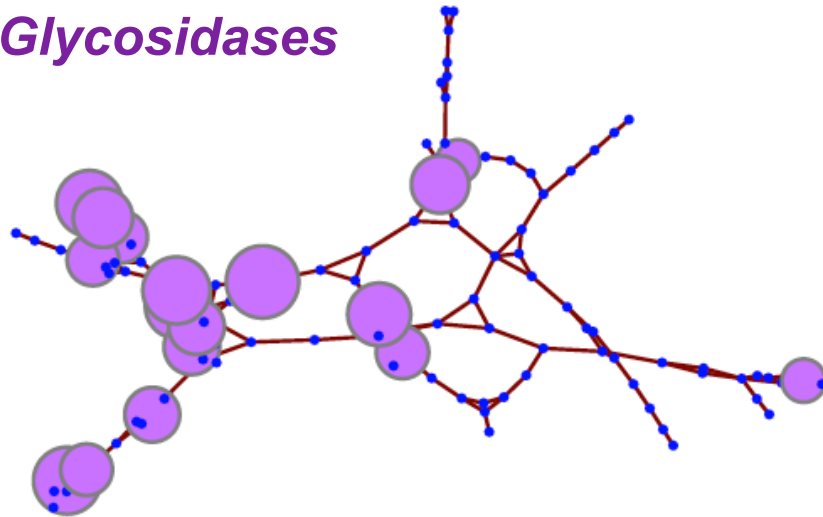
Signalling



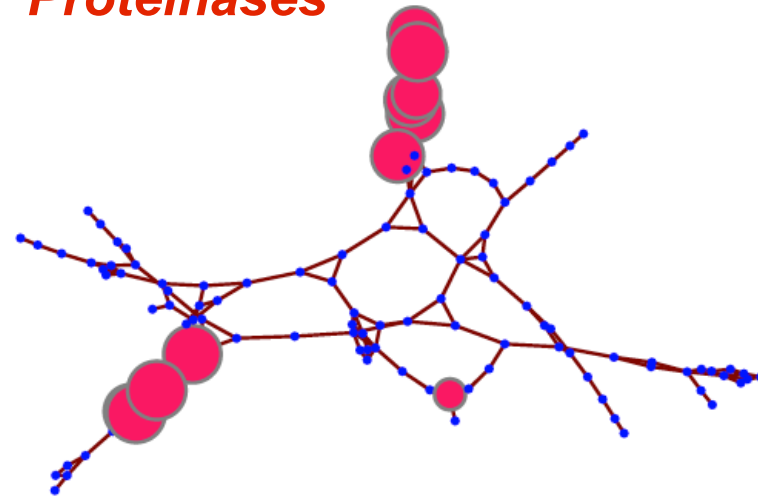
Transcription



Glycosidases

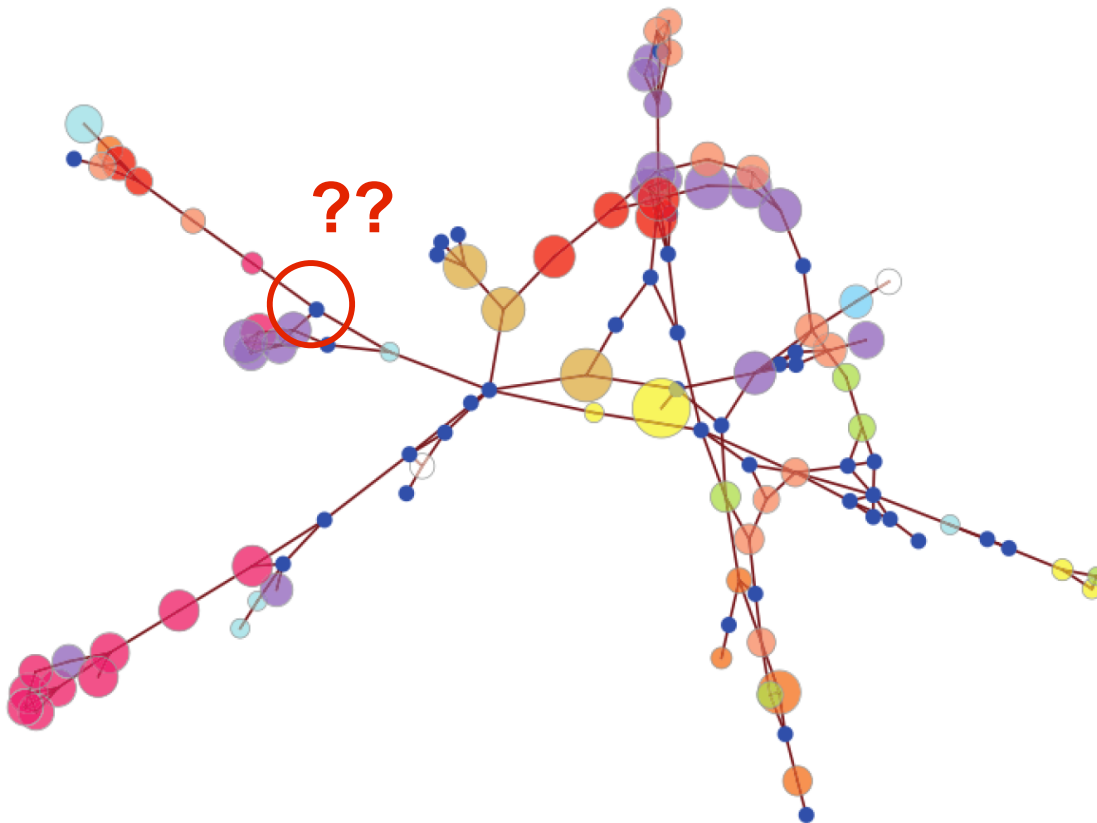


Proteinases



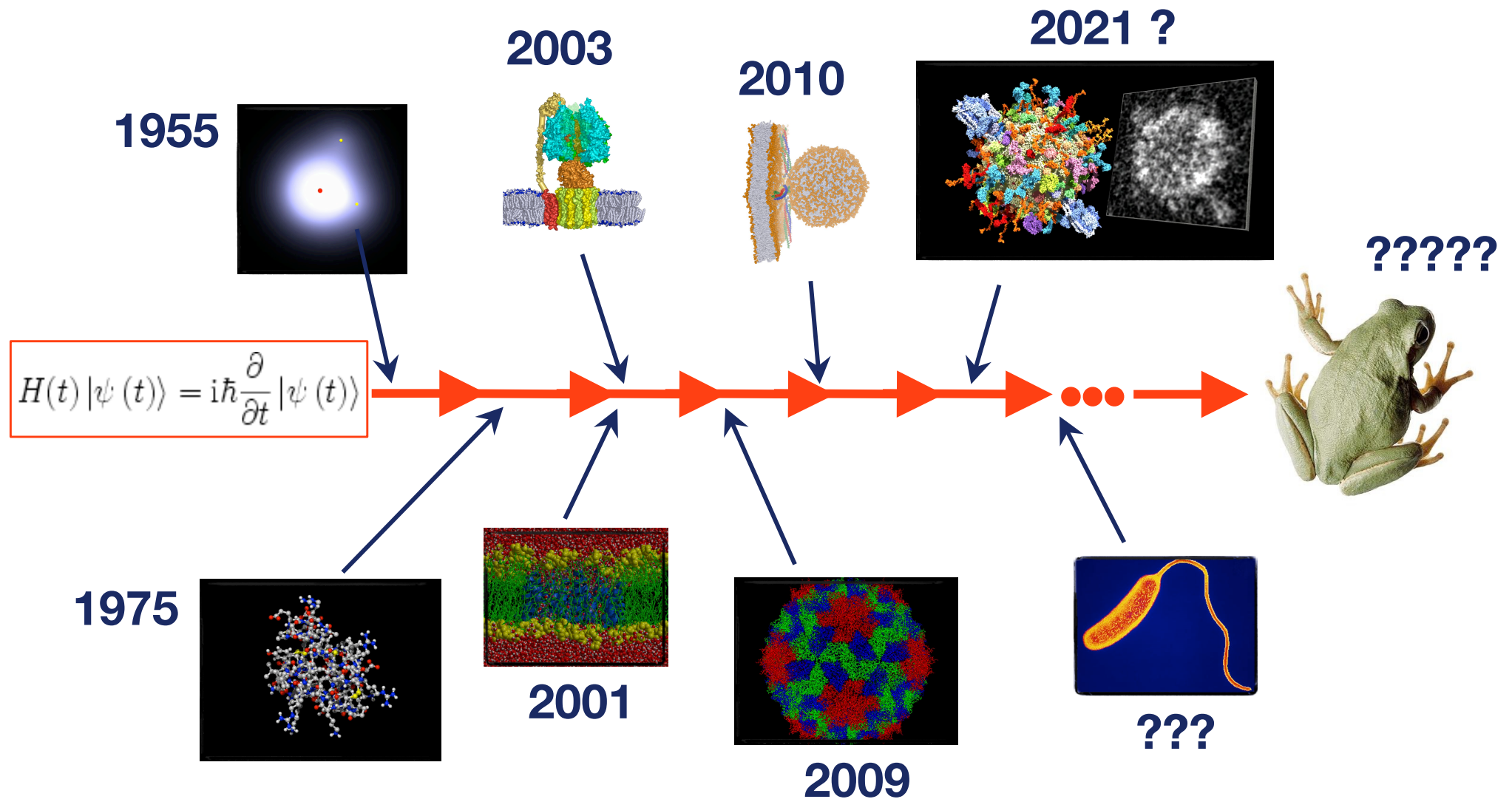
Protein Function Prediction

1. New protein of unknown function:
2. Based on its dynamics, determine its position in the graph.
3. function from closest cluster.



<i>dyn</i>	<i>struct</i>	<i>random</i>
46 %	32 %	11 %

Towards a *fundamental understanding of life* processes from *first principles*



What have we learned ?

(1) AFM + force probe MD -> Overlap, 11 orders total

- Solved puzzle of too low AFM forces
- Unbinding free energy landscape, 3rd barrier
- Loading rate dependent unbinding paths

(2) Ribosomal antibiotics mechanism:

- new allosteric stalling mechanism

(3) Ribosomal Frameshifting:

- Thermodynamic control (+ kinetics)

(4) Intrinsically disordered proteins:

- new force field version: CHARMM36m

(5) The Dynasome:

- Structure -> Dynamics -> Function



Positions available