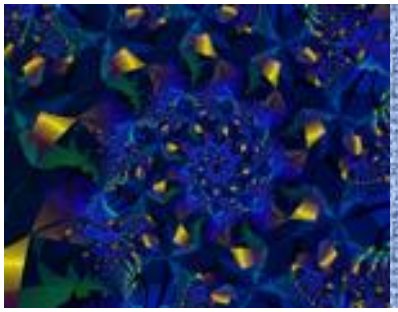


Theoretical Chemistry at UNICAMP
Molecular Dynamics / Skaf Group

Biophysical Molecular Dynamics Simulations

Munir S. Skaf
skaf@iqm.unicamp.br

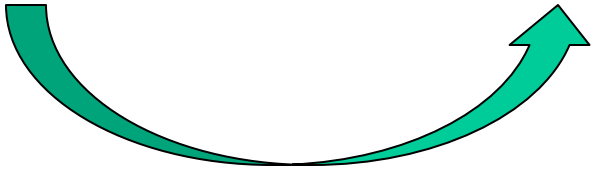
Institute of Chemistry / State University of Campinas

BIOEN Workshop on Molecular Mechanisms of Photosynthesis

São Paulo, October 25, 2010

The Holy Grail of Structural Molecular Biology

Protein structure  **Biological function**

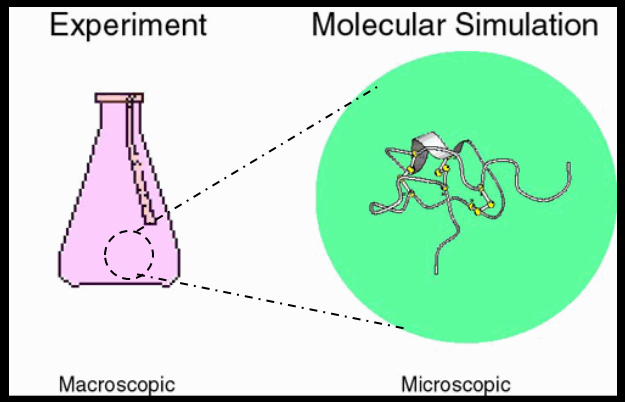


Dynamics

Understanding how molecules move requires knowledge of interactions at the atomic level

MD simulations are a powerful tool for studying atomic motions

MD Simulations in a Nutshell



Initial Configuration: $\mathbf{R}^N$, $\mathbf{V}^N$ ← PDB

↓

Save atomic positons

↓

Potential $V(\mathbf{r}_i)$ at i

↓

Newton's Second Law

$$\mathbf{a}_i(t) = -\frac{1}{m_i}\frac{\partial V}{\partial \mathbf{r}_i}$$

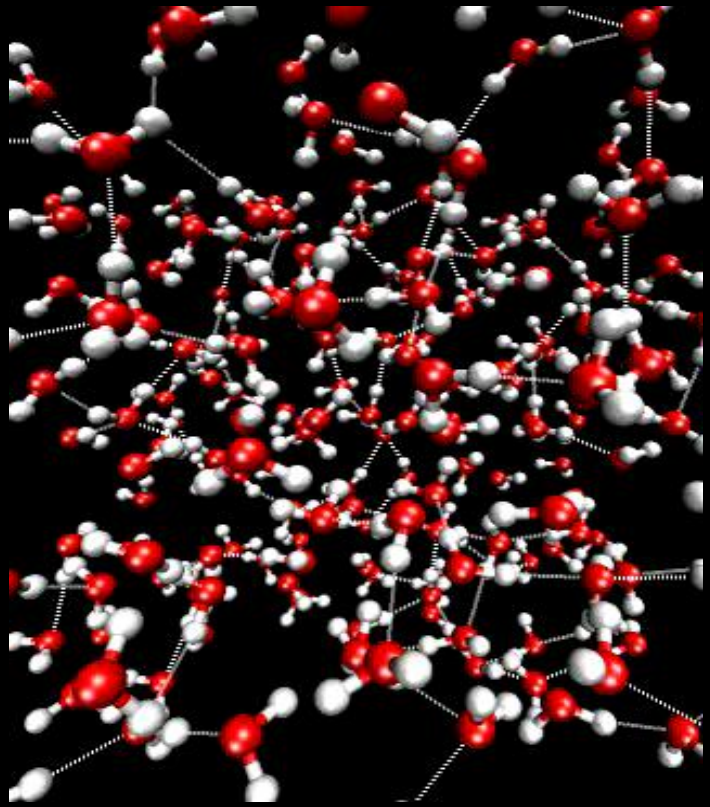
↓

Integrations of the Equations of Motion

$$\mathbf{r}_i(t+\Delta t) = \mathbf{r}_i(t) + \mathbf{v}_i(t)\Delta t + \frac{\mathbf{a}_i(t)\Delta t^2}{2}$$

$$\mathbf{v}_i(t+\Delta t) = \mathbf{v}_i(t) + \mathbf{a}_i(t)\Delta t$$

iterate

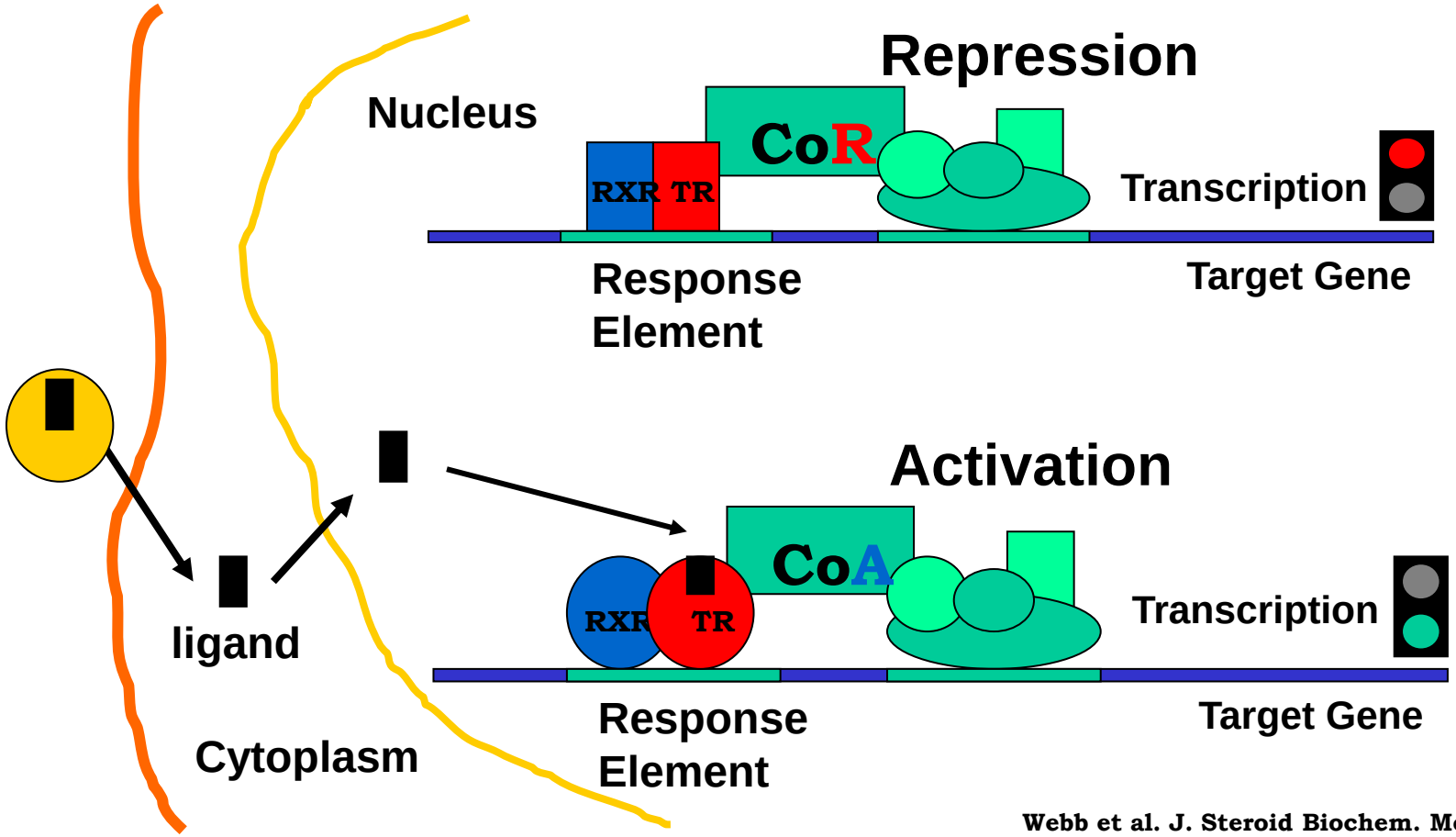


Nuclear Receptors (transcription proteins)

Role: Modulate gene expression by means of hormone binding

Importance: Cell differentiation, metabolism, sexual diff. & function, diabetes, cancer, inflammatory processes, etc

Vitamins A&D, steroids, estrogen, progesterone, corticoids, fatty acids, etc



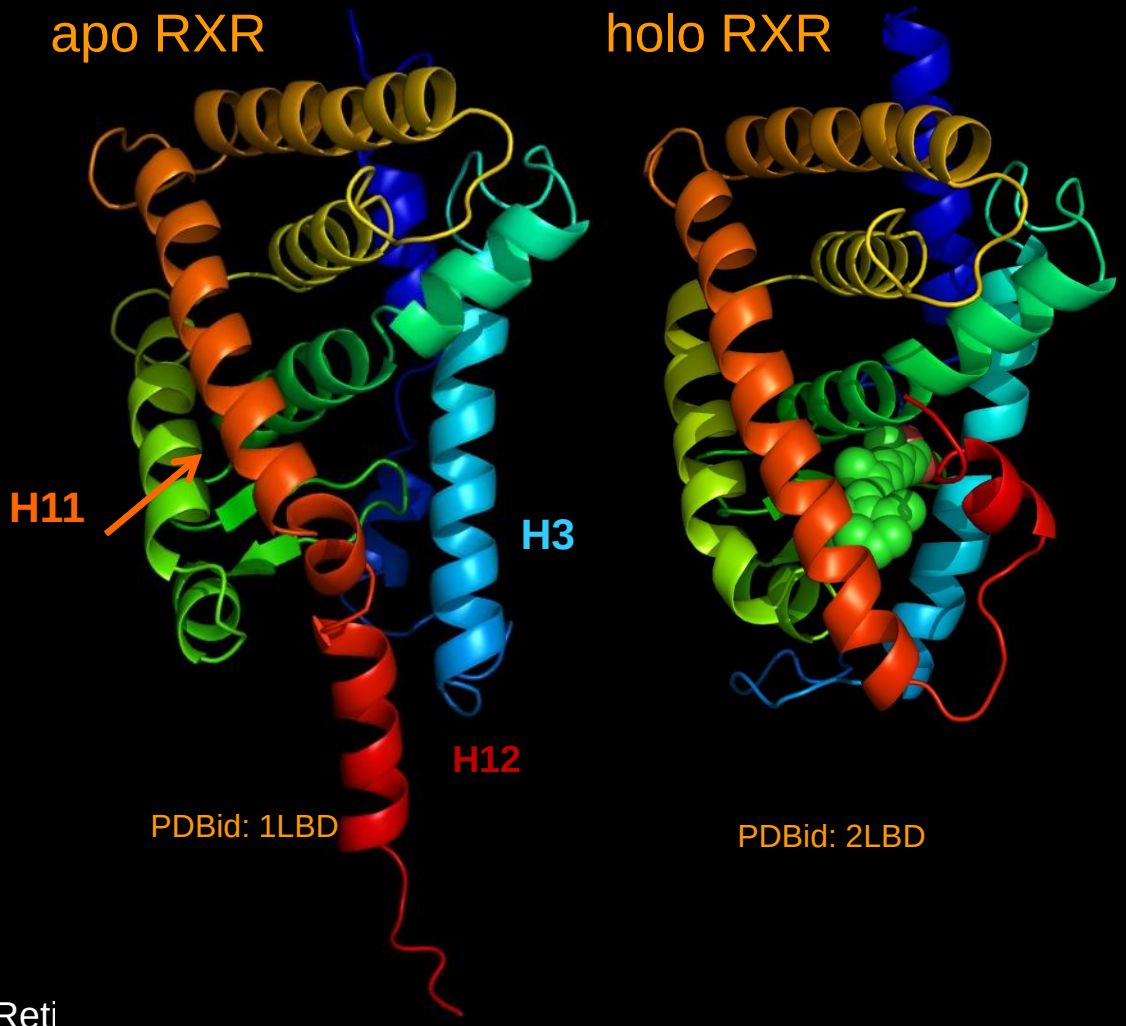
Webb et al. J. Steroid Biochem. Mol. Biol. 83, 59 (2003)

HOW DO LIGANDS ENTER & EXIT NR´s HYDROPHOBIC BINDING POCKET ?

The Mouse Trap Model

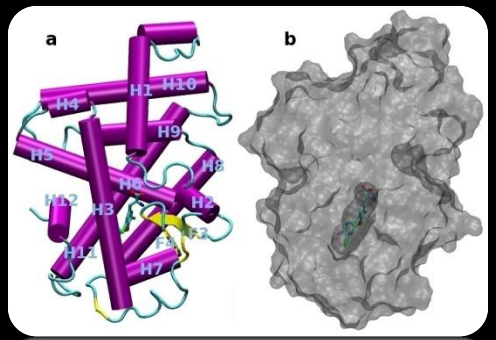
apo RXR

holo RXR



PDBid: 1LBD

PDBid: 2LBD

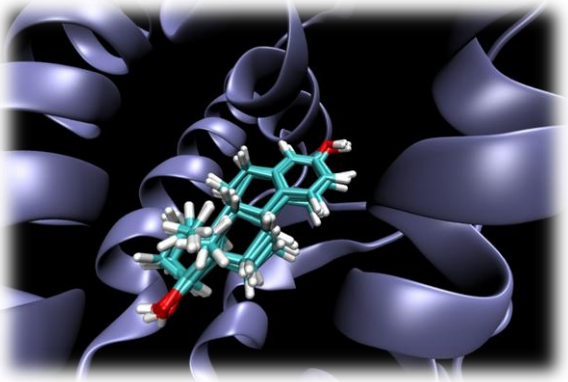


RXR: 9-*cis*-Reti

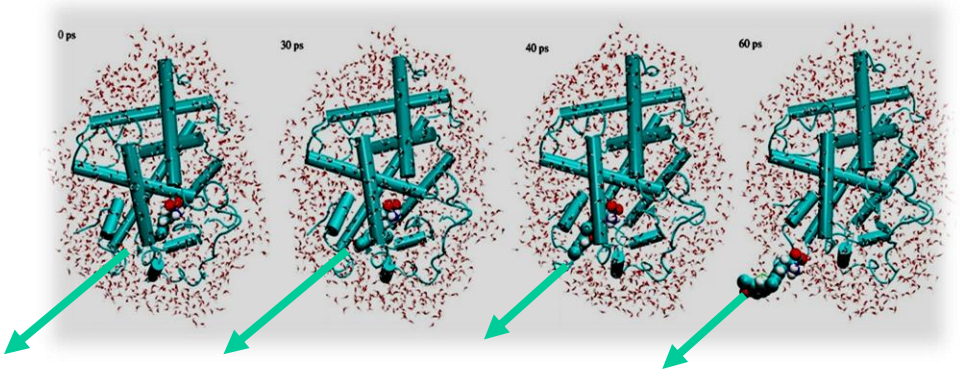
Moras & Gronemeyer; *Curr. Opin. Cell Biol.* (1998) **10** 384

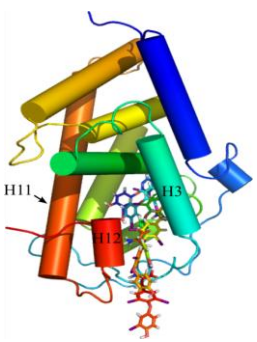
Mechanisms of Ligand Dissociation

Locally Enhanced Sampling:
Search for dissociation pathways

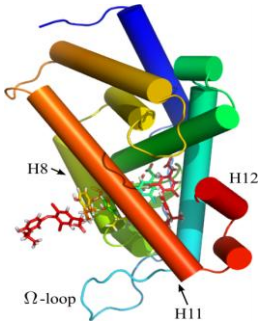


Steered MD:
Relative importance of pathways

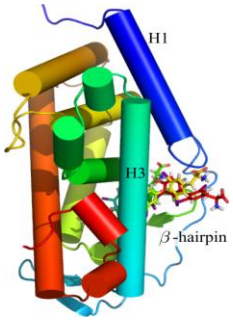




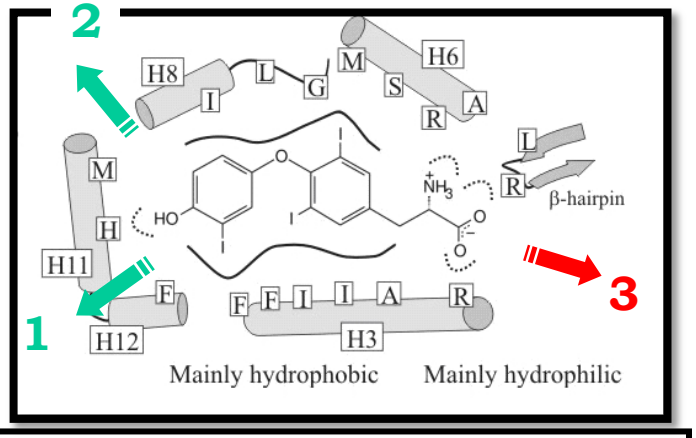
Path I



Path II



Path III

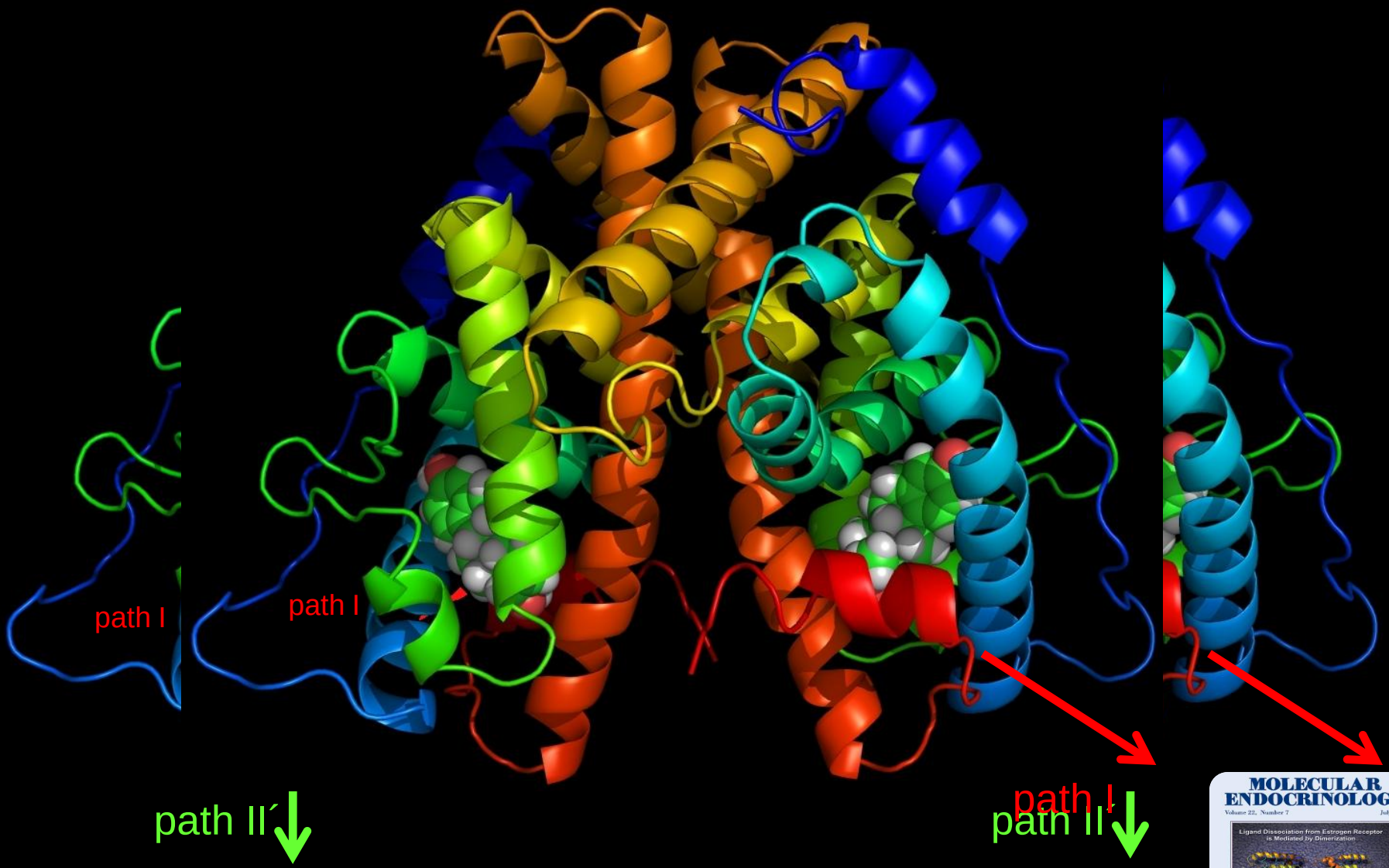


Path 3: Ligand-protein hydrophilic contacts are readily replaced by ligand-solvent interactions

NR can harbor multiple ligand escape pathways

Martínez et al. Biophys. J. 2005 ; J. Med. Chem. 2006

Dissociation Pathways From Estrogen Receptor Dimers



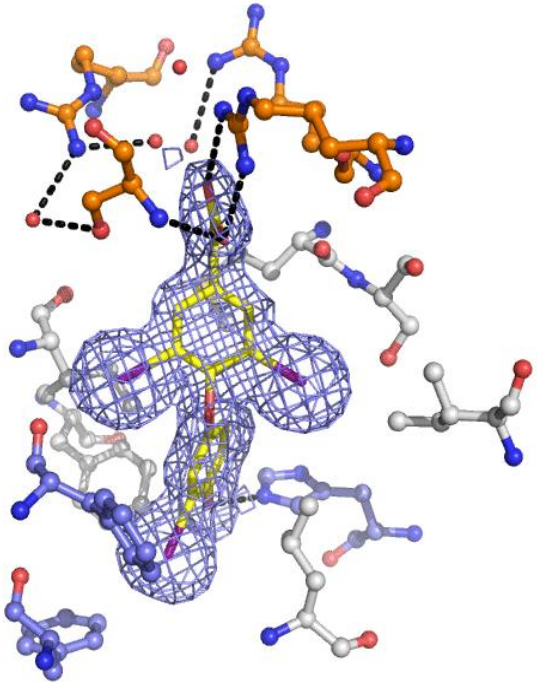
Dimerization strongly suppresses some dissociation paths. MD suggests explanation for ligand dissociation kinetics in ER.

Thyroid Hormone (TR) Isoform Selectivity

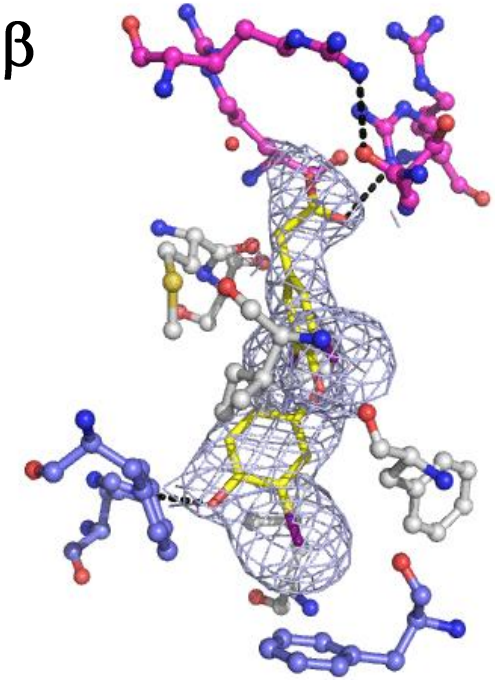
TRα : Abundant in the heart / TRβ : Abundant in the liver

TRIAC is a natural ligand that binds TRβ with higher affinity.

TRα



TRβ



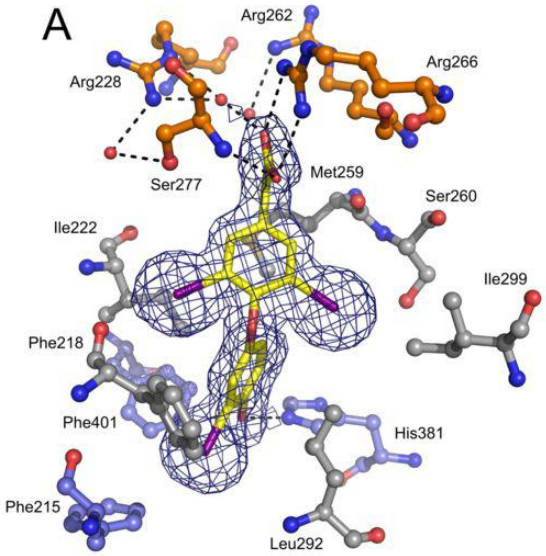
Crystal structures

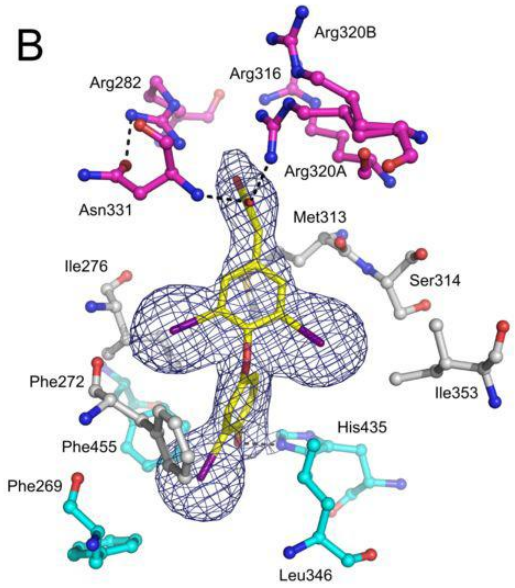
Thyroid Hormone (TR) Isoform Selectivity

TRα : Abundant in the heart / TRβ : Abundant in the liver

TRIAC is a natural ligand that binds TRβ with higher affinity.

Apparent paradox: X-ray structures clearly indicate stronger Triac binding to TRα, not to TRβ!





Crystal structures

MD

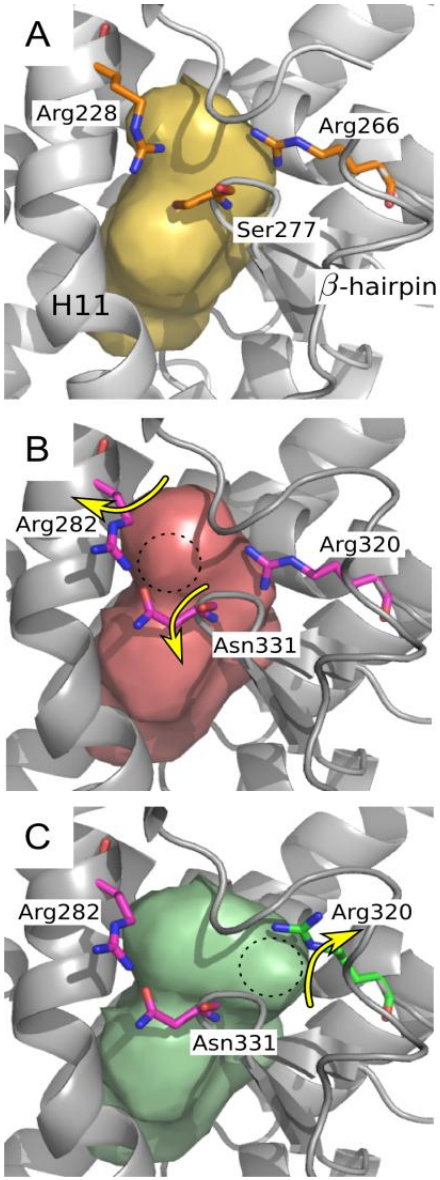
Ligand interactions and the role of water

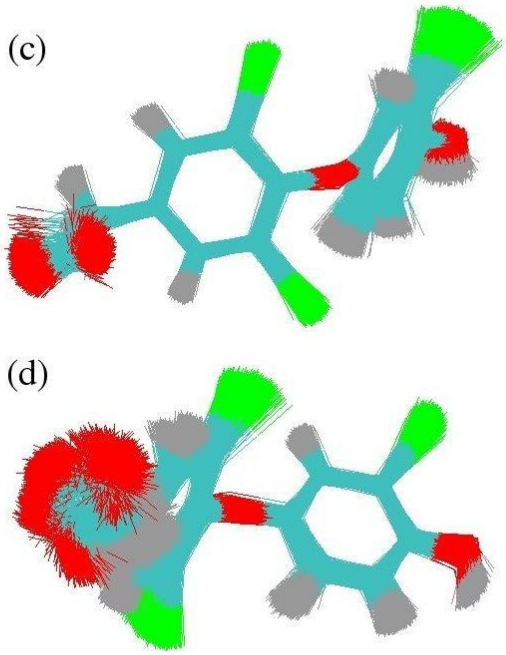
TRIAC Interaction with:	TRα / kcal mol^{-1}	TRβ / kcal mol^{-1}
LBD residues	-66.63	-46.96
Whole environment	-192.74	-192.52
Water molecules	-18.37	-48.47

- Ligand-LBD interactions with TRα are, indeed, stronger!
- Interactions with the environment are similar...
- Water substitutes Ligand-LBD interactions!

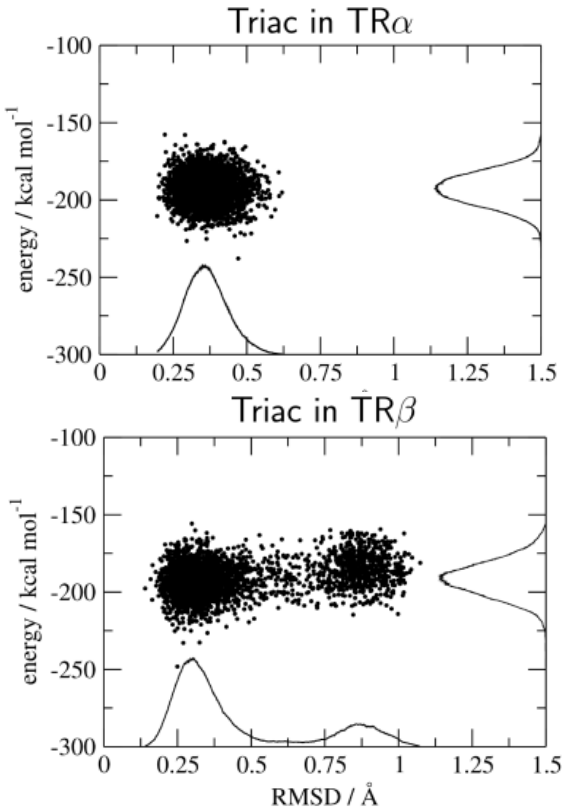
Triac/TRα: 1 water at a time
Triac/TRβ: up to 4 waters in cavity

TRIAC is substantially more mobile in TR-β cavity





Computing entropy difference



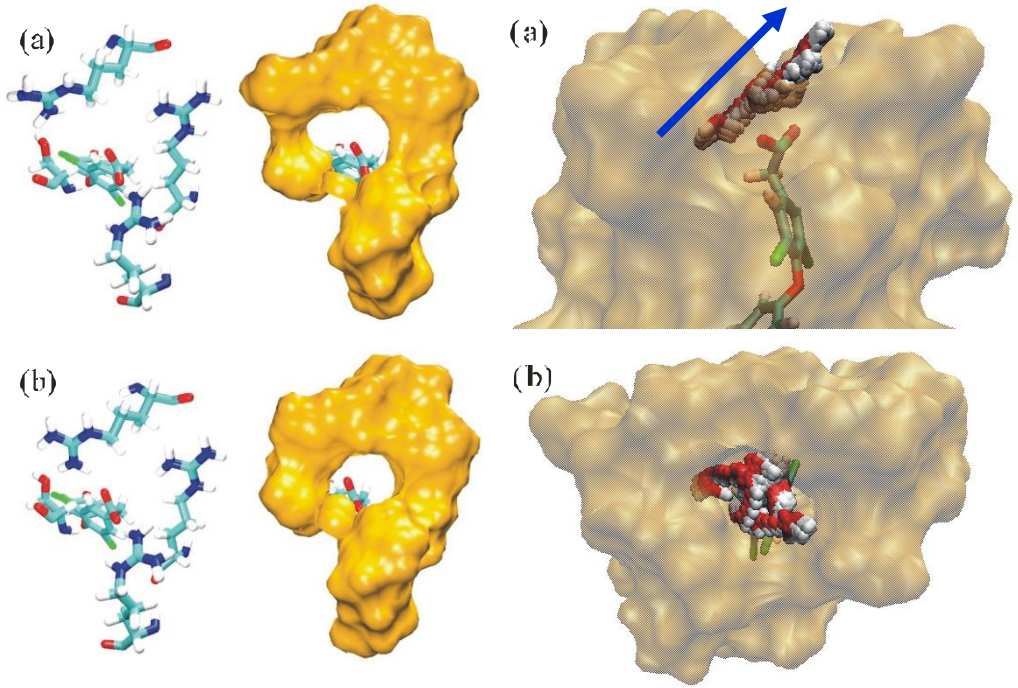
$\Delta S = R \ln (\Omega_B / \Omega_A)$

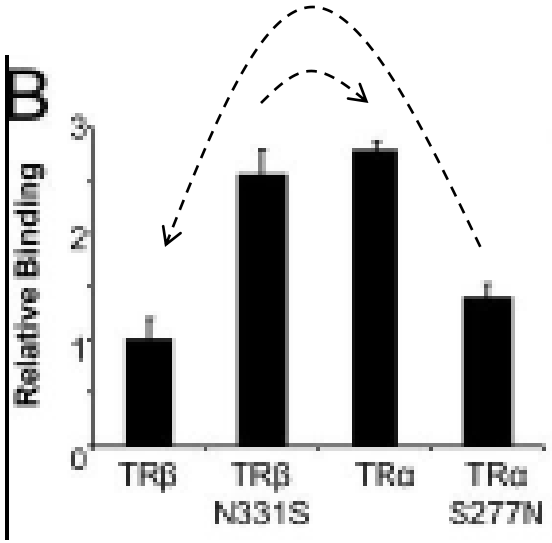
MD: Entropy accounts for Triac selectivity

MD: ~ 0.4 kcal/mol

Exp: ~ 0.4-0.6 kcal/mol

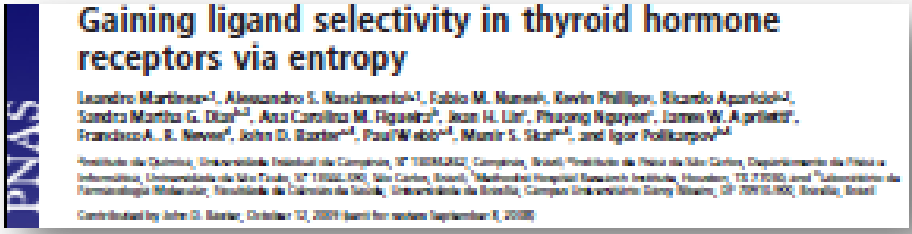
Further analyses of binding pocket suggest that specific S277/N331 mutation should lead to near reversal of TRα/TRβ selectivity.





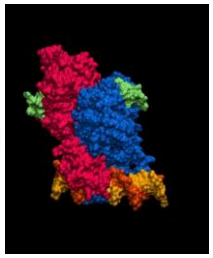
Functional studies confirms MD hypothesis

Martínez et al. PNAS 106, 20717 (2009)



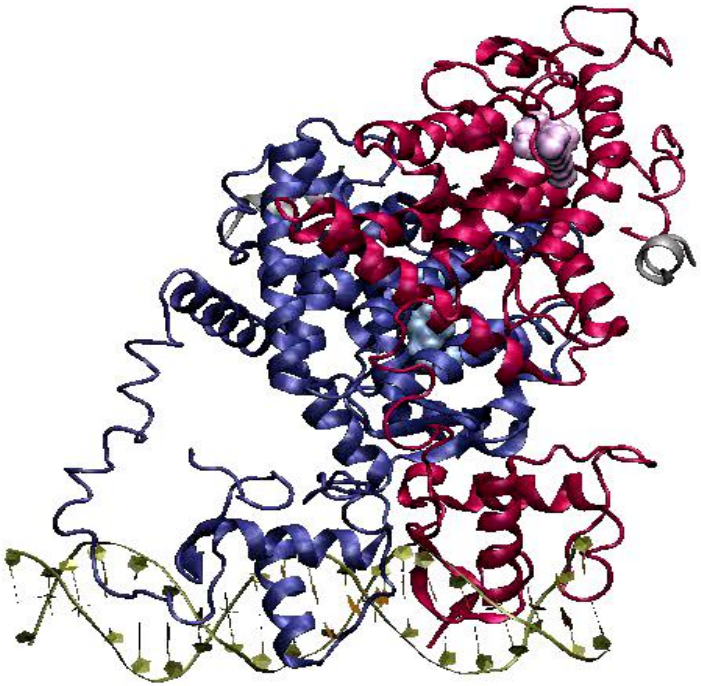
Full-length RXR/PPARg Complex: Dynamical Cross-Correlations

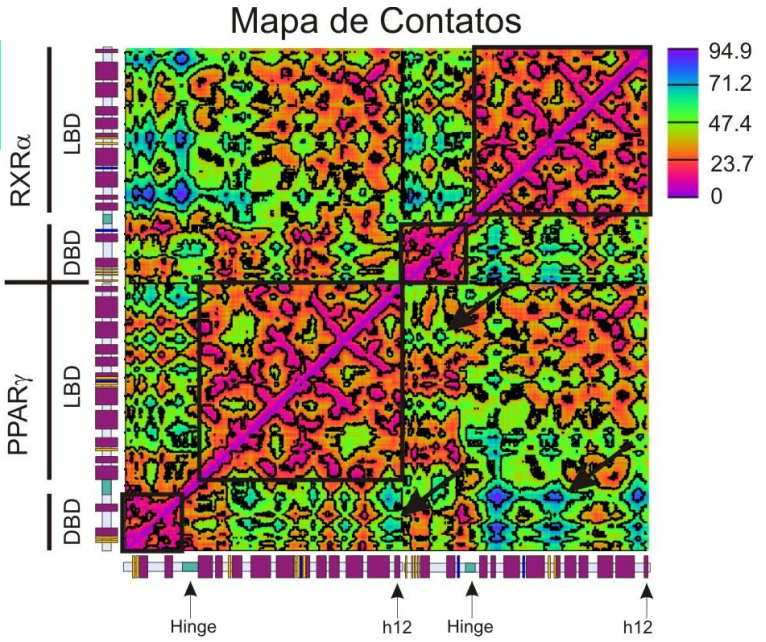
(Rastinejad, *Nature* 2008)

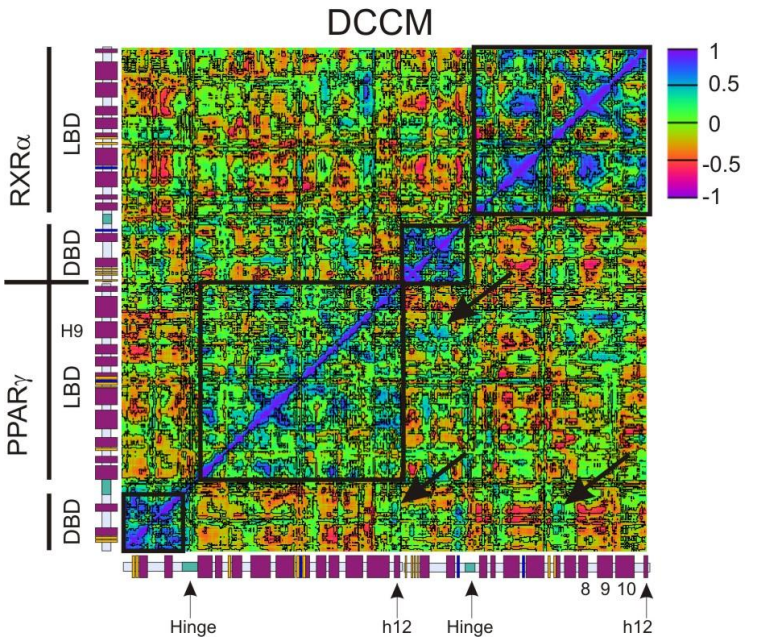


MD reveals correlated motions between DBD & H12 (~80 A apart !)

K. Yamamoto et al, Science 2009:
DNA sequence affects NR activity --
"We therefore propose that DNA is a sequence-specific allosteric ligand…"



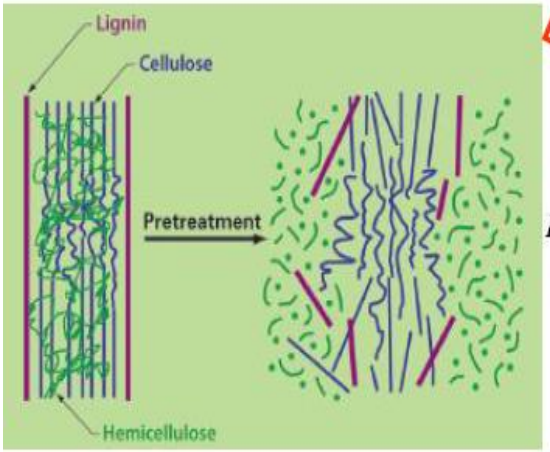




Glycosyl Hydrolases:Ethanol from Cellulose

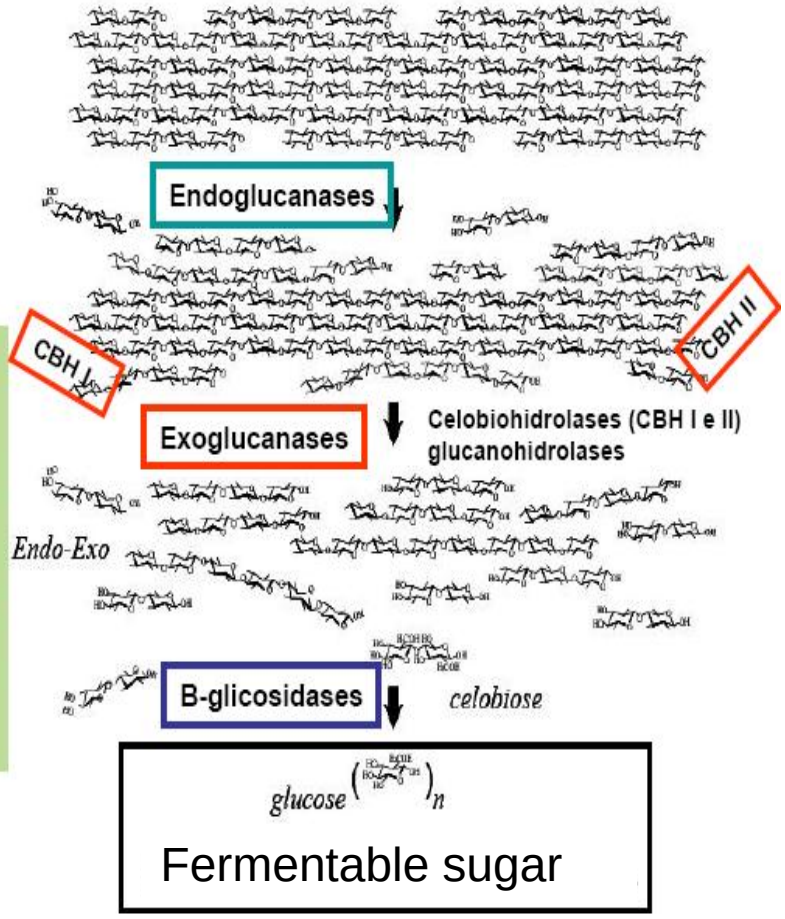
Sugarcane Bagasse

Parede celular



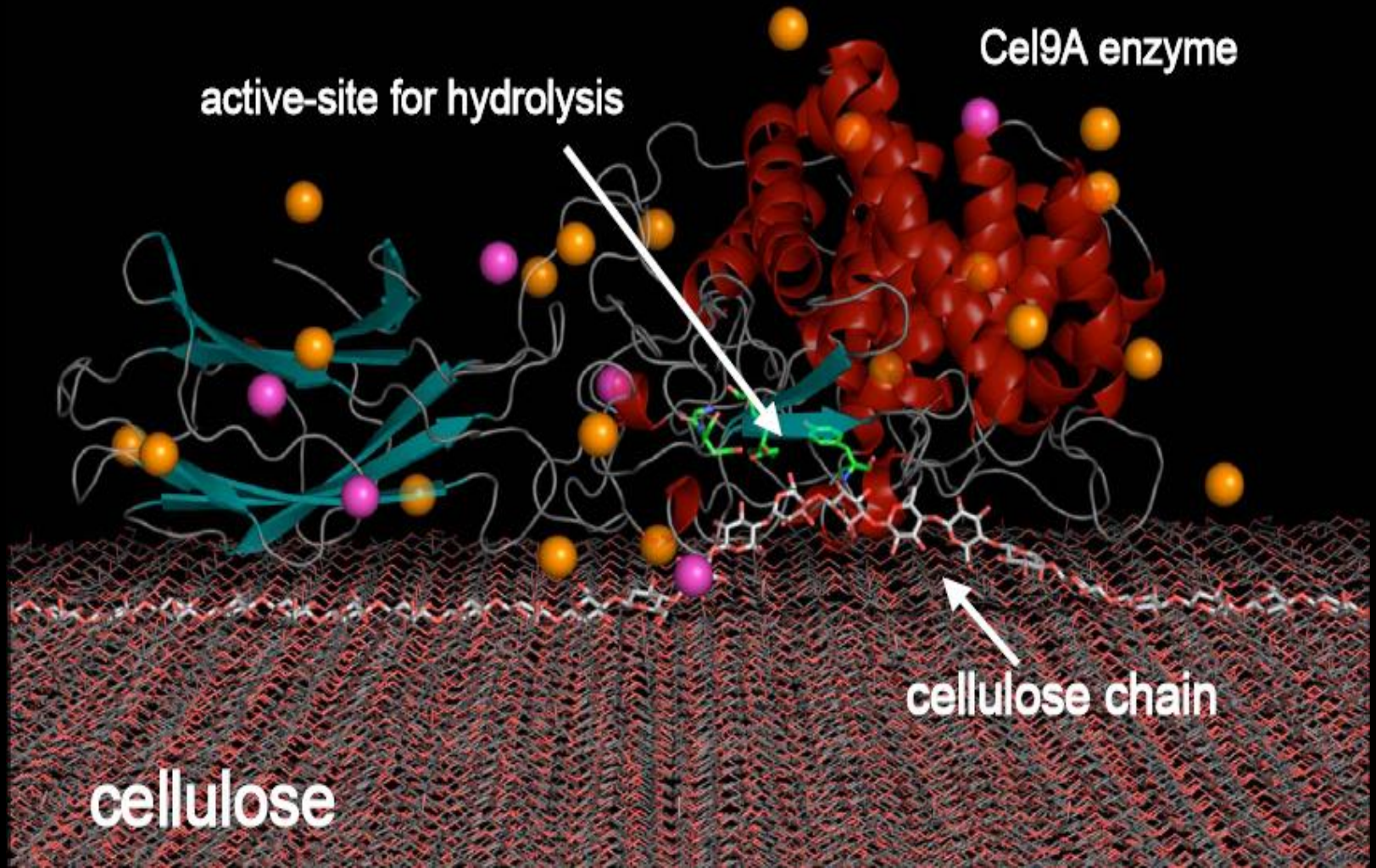
Lignina: 20%
Hemicelulose: 28%
Celulose: 36%

Mechanism overview

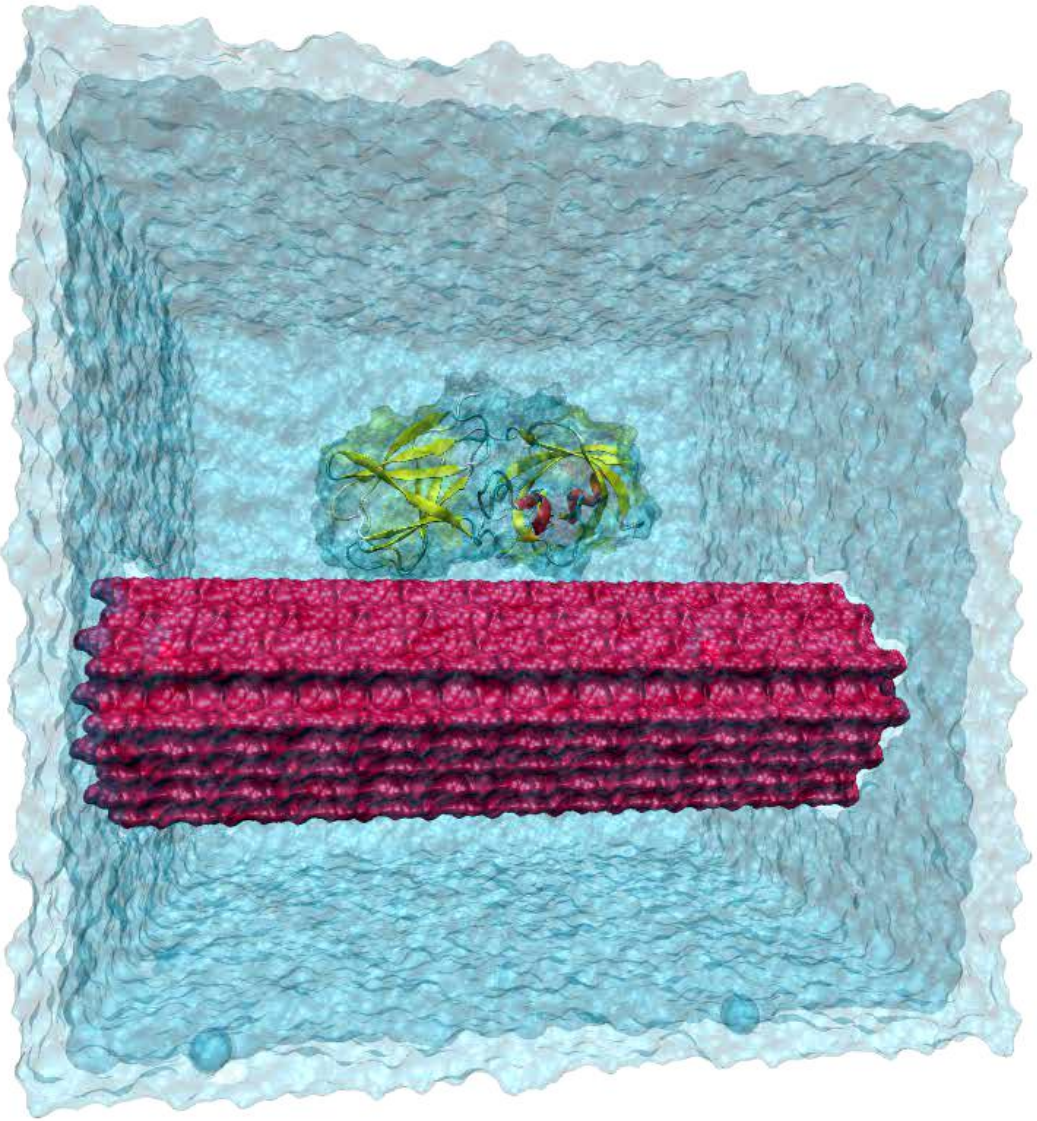


Goal: to develop efficient enzyme cocktails to render hydrolysis economically viable

Atomistic MD studies of GH

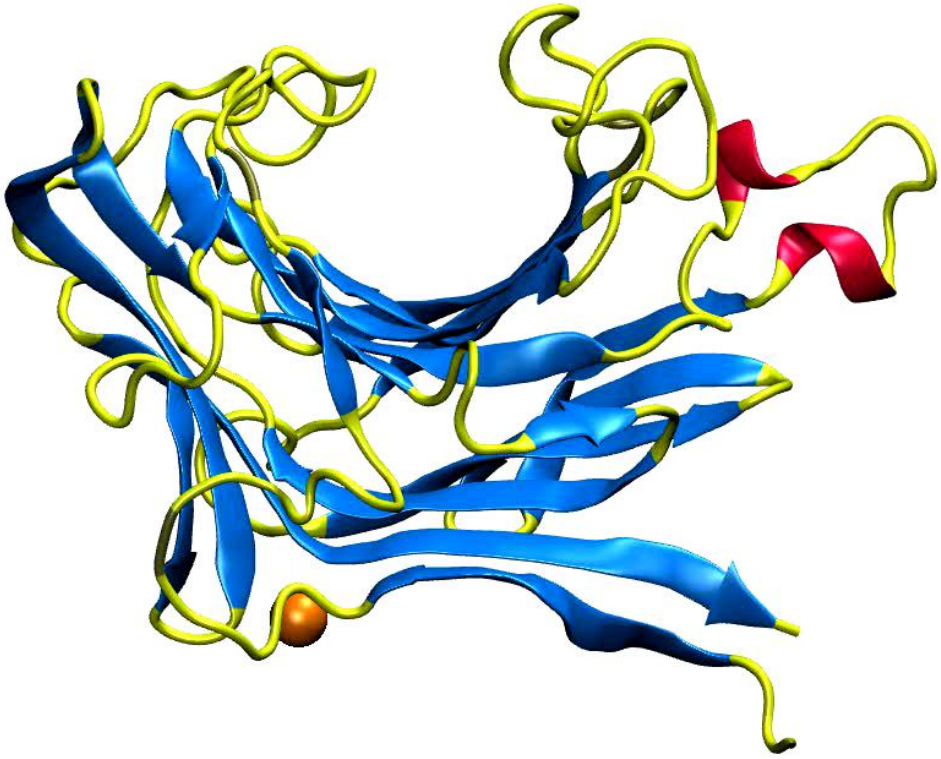


Binding to cellulose microfribils (200,000 atoms)



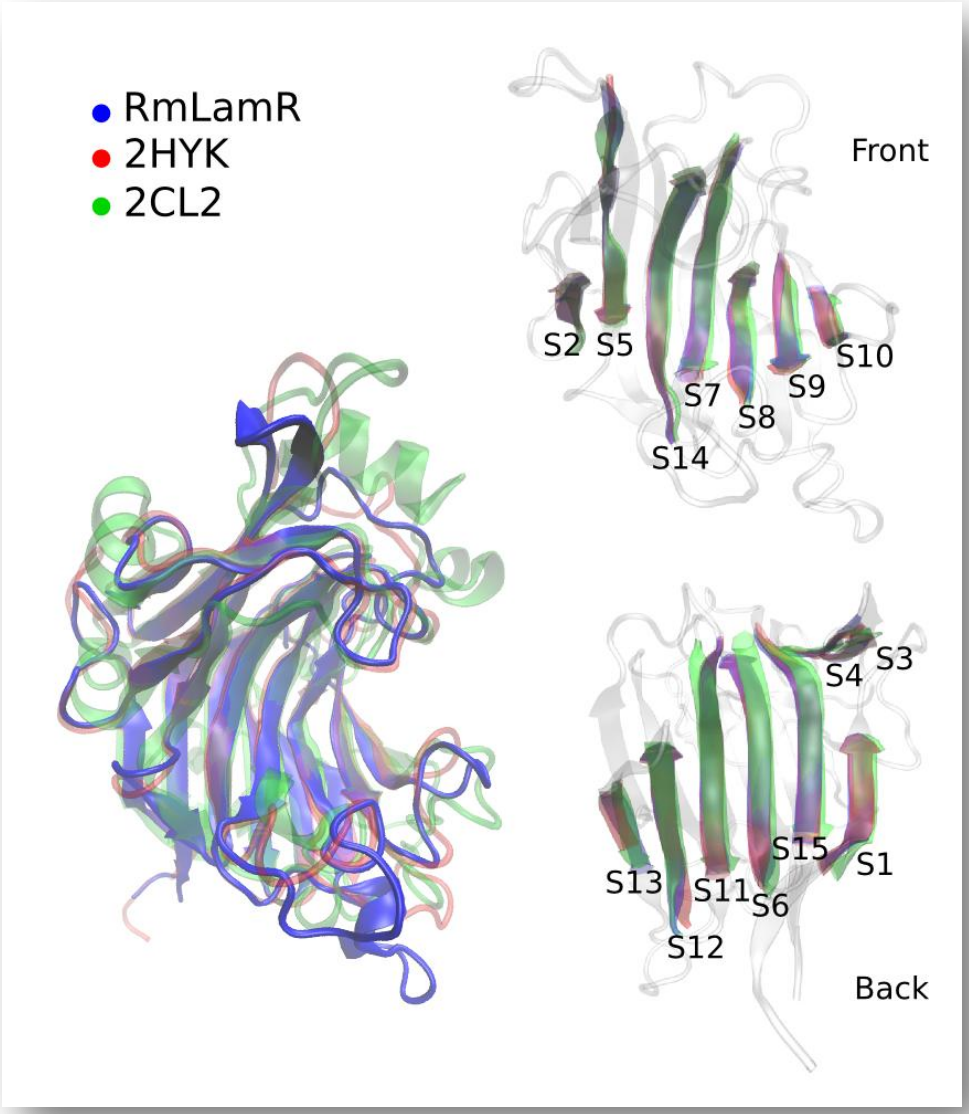
Hyperthermophilic Laminarinase from R. Marinus (Family 16 GH; endoglucanase)

X-ray: 1.95 A Resolution (I. Polikarpov)



Structural Homologs

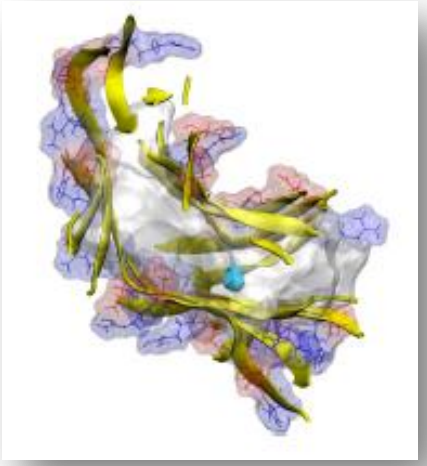
2HYK (*Nocardiopsis sp.*) : Thermophile
2CL2 (*P. chrysosporium*): Mesophile



Number of Salt Bridges:

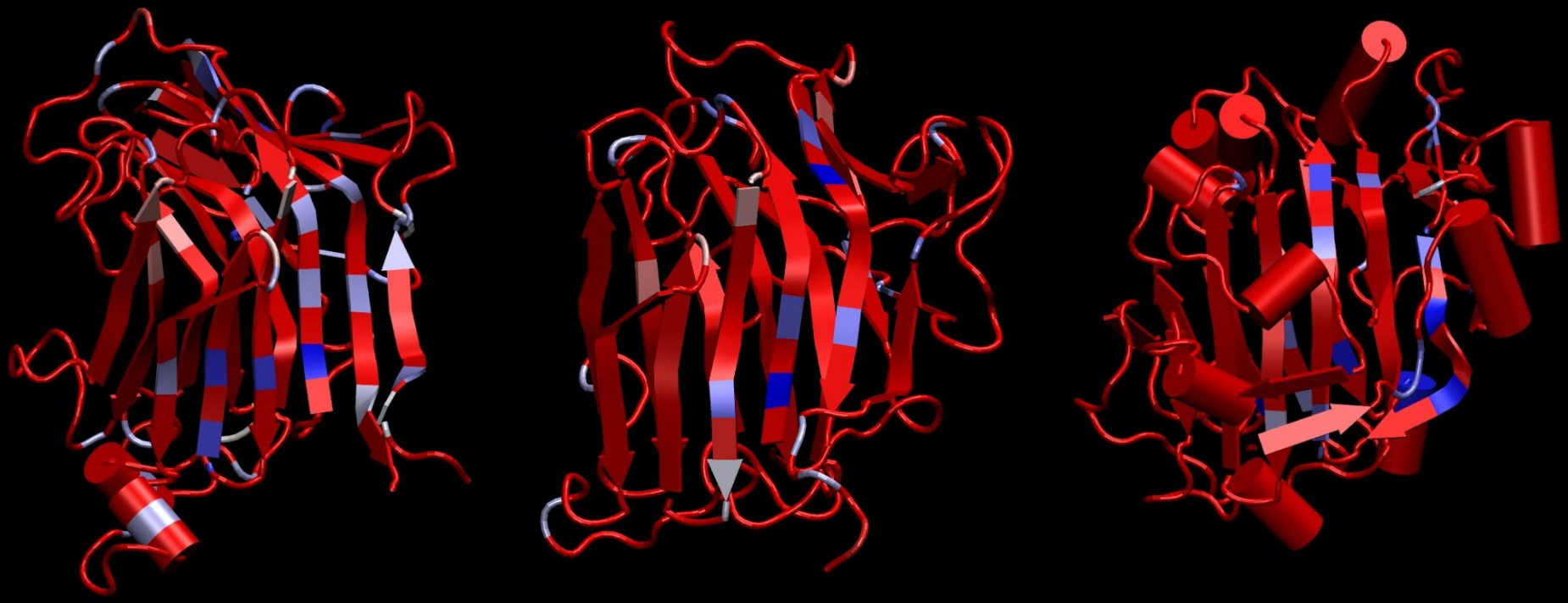
RmLamR	**17 (22)**
2HYK	**7**
2CL2	**11**

Charged residues interact equally well with water, so what is the enthalpic gain upon folding ?

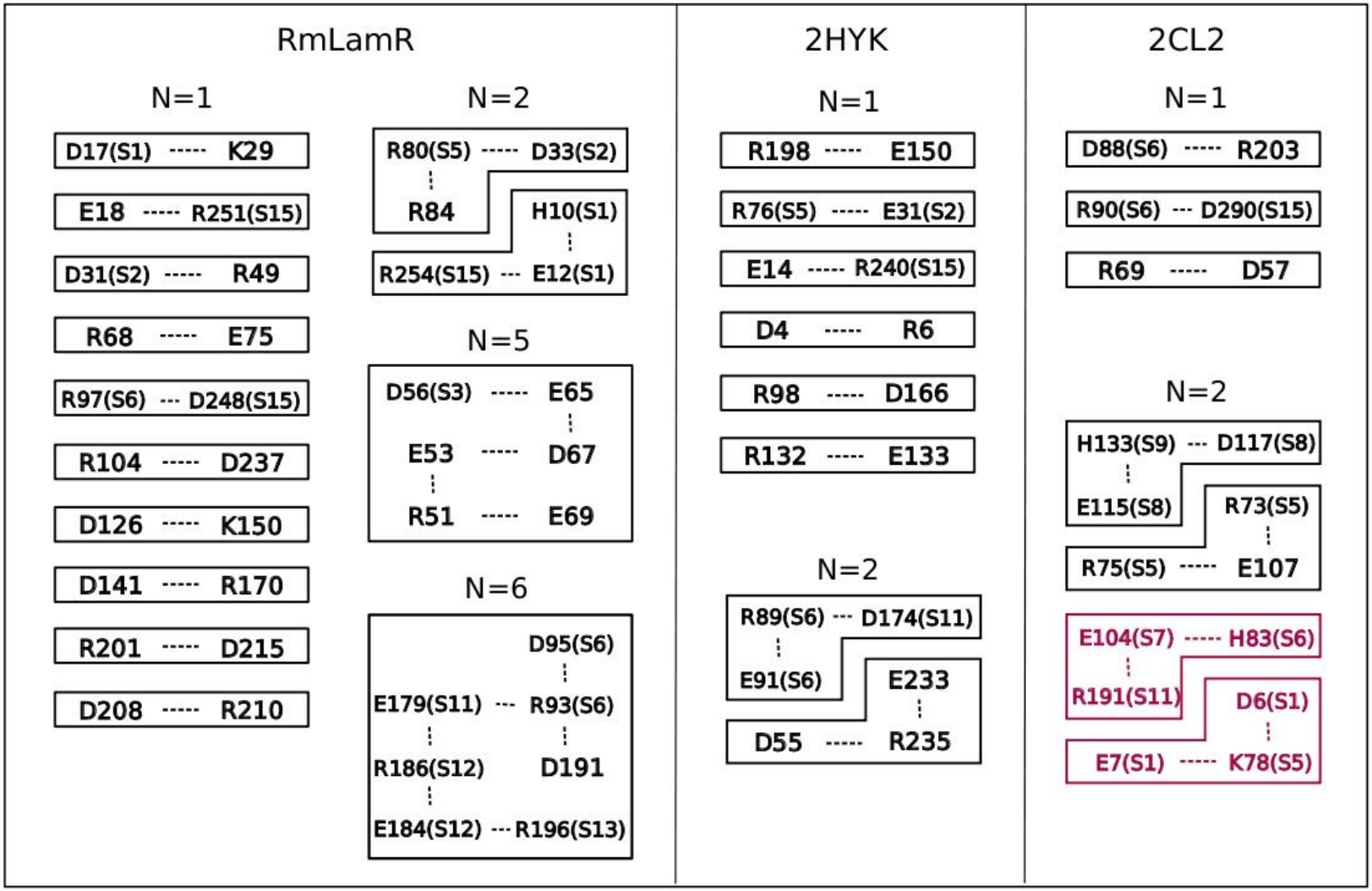


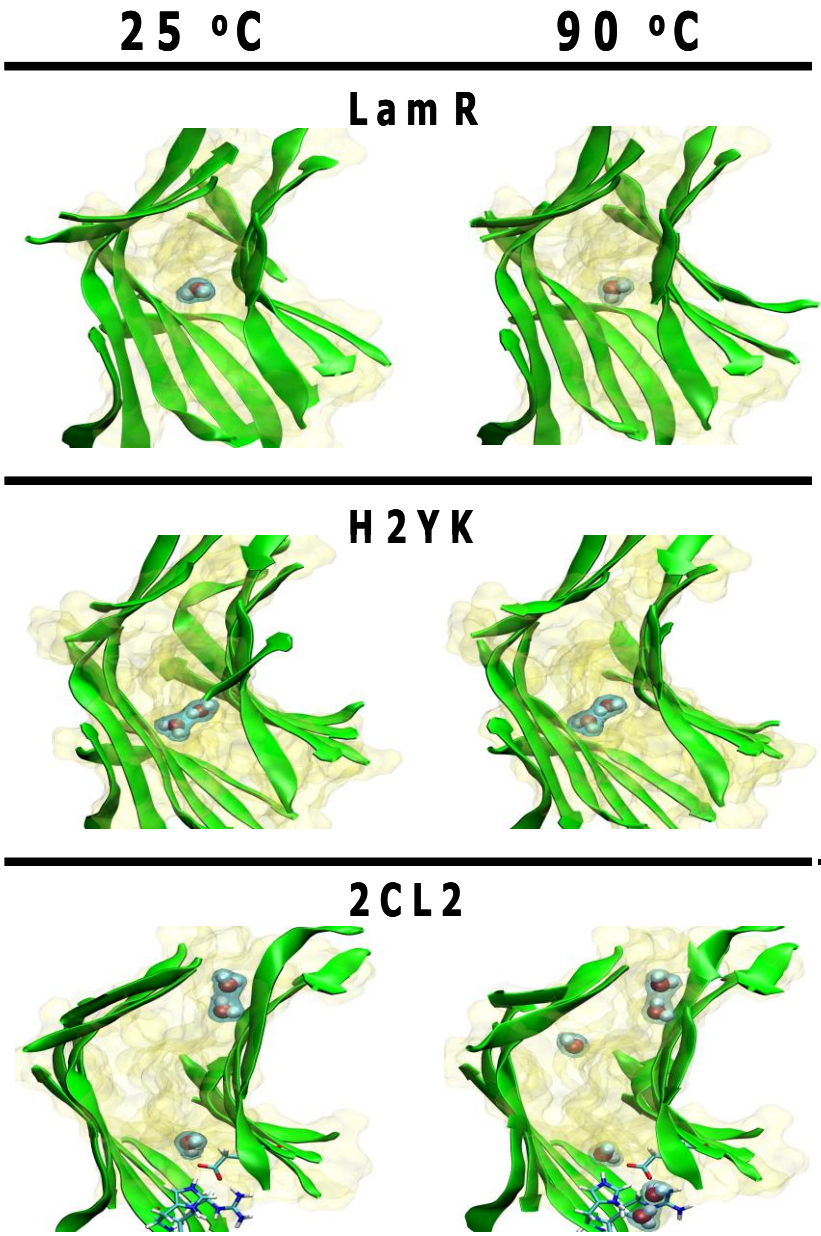
HYDROPHOBIC CONTACTS AND SALT BRIDGES

Enzyme	RmLamR		2HYK		2CL2	
Temp.	25 °C	90 °C	25 °C	90 °C	25 °C	90 °C
HC	166 ± 2	165 ± 3	150 ± 2	151 ± 3	153 ± 3	152 ± 4
SB	24 ± 3	24 ± 2	9 ± 1	10 ± 2	11 ± 1	11 ± 1



Salt Bridges Spatial Distribution (Topology)





Molecular basis for thermostability

2CL2

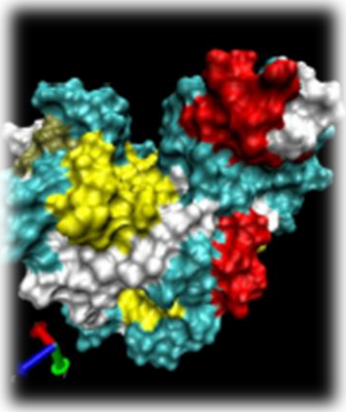
Charged residues in opposite leaflets interact via salt bridges.

More water molecules can diffuse into the hydrophobic core.

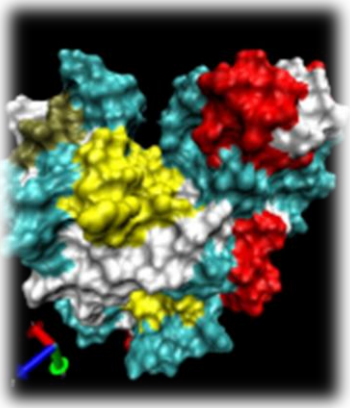
Molecular basis for thermophilicity

RmLamR: substrate binding channel is preserved

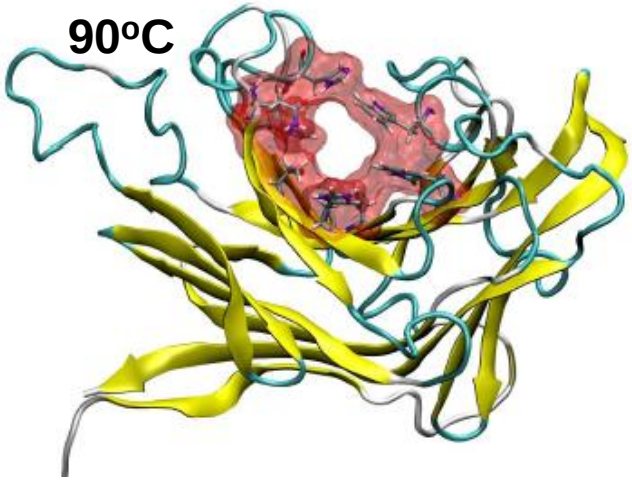
25°C



90°C

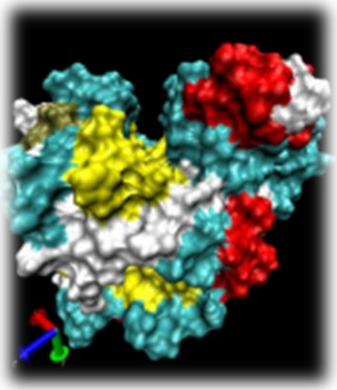


90°C

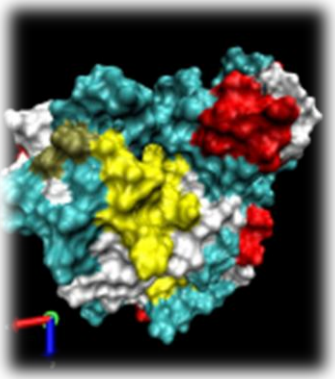


2CL2: substrate binding channel is obstructed at 90°C

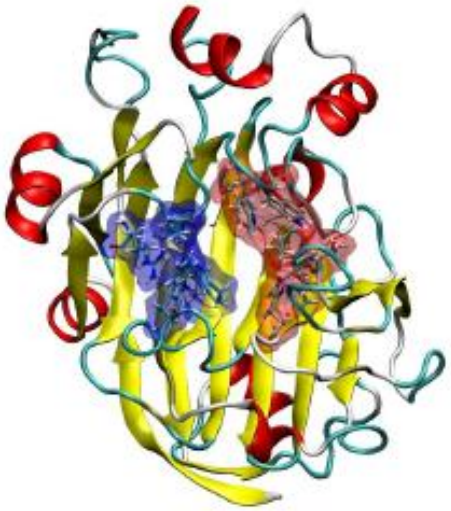
25°C



90°C



90°C



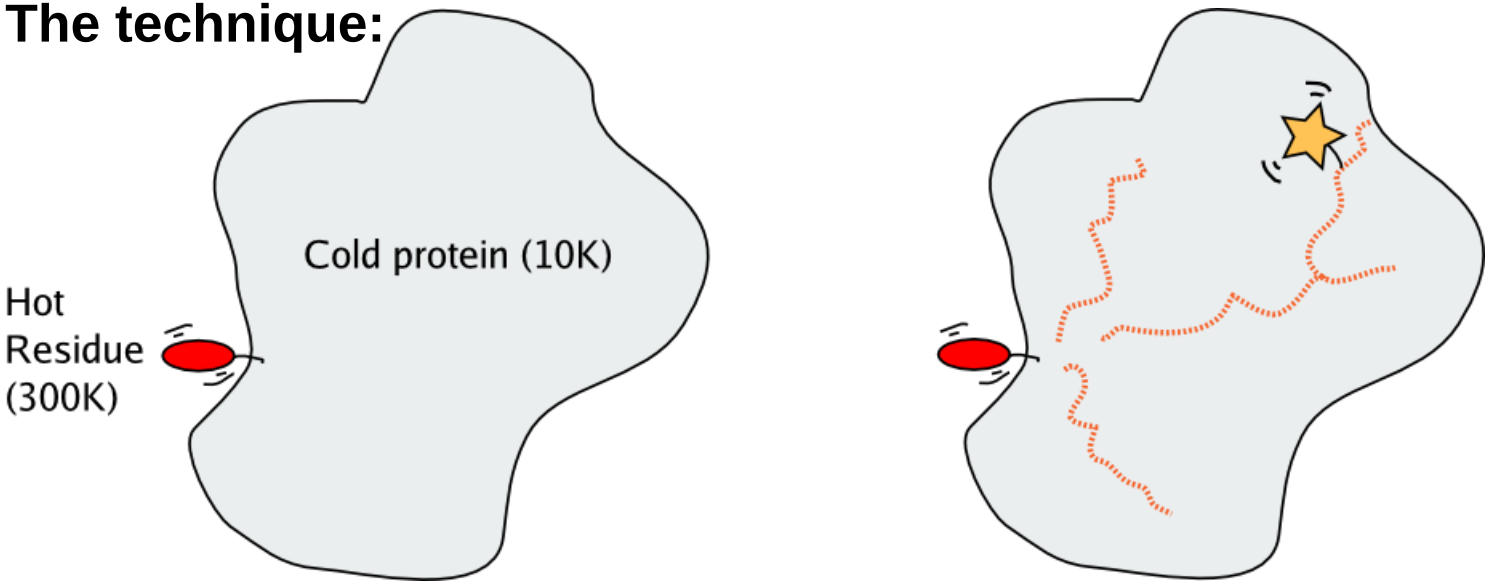
Intramolecular Vibrational Energy Transfer in Proteins (anisotropic thermal diffusion)

1. The method

1.1. Ota and Agard (2005):

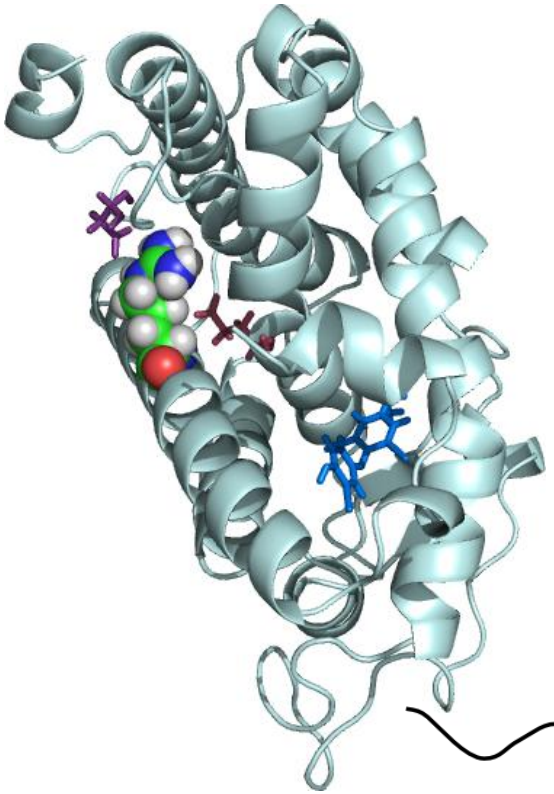
Thermal diffusion seems to explain networks of evolutionary connectivity (Ranganathan, Science 1999) - does not seem to be confirmed.

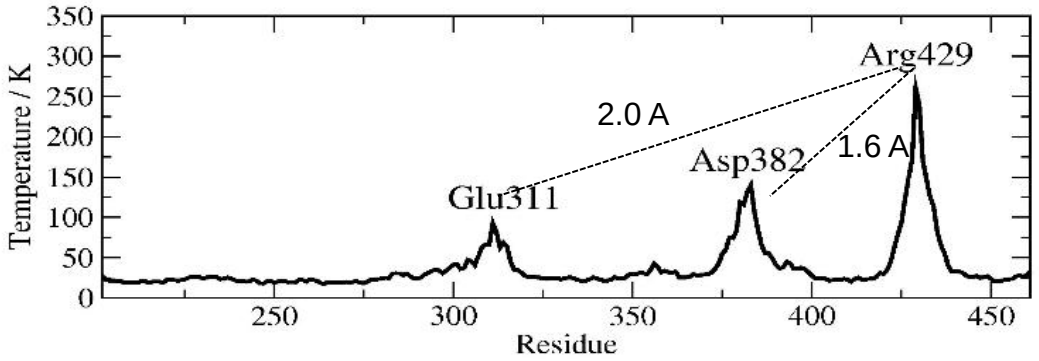
1.2. The technique:



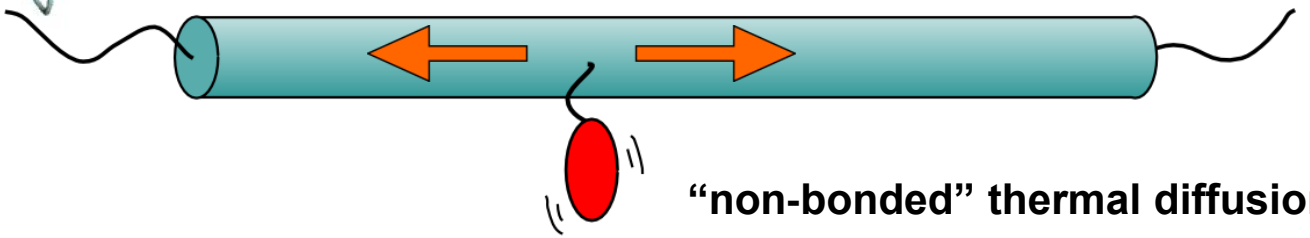
2. Mechanisms of thermal diffusion

Typical heating profile: Arg429

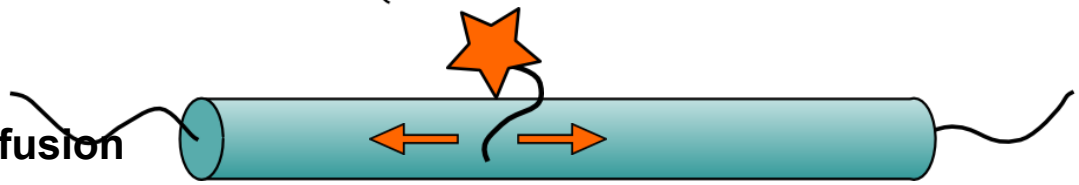




“bonded” thermal diffusion



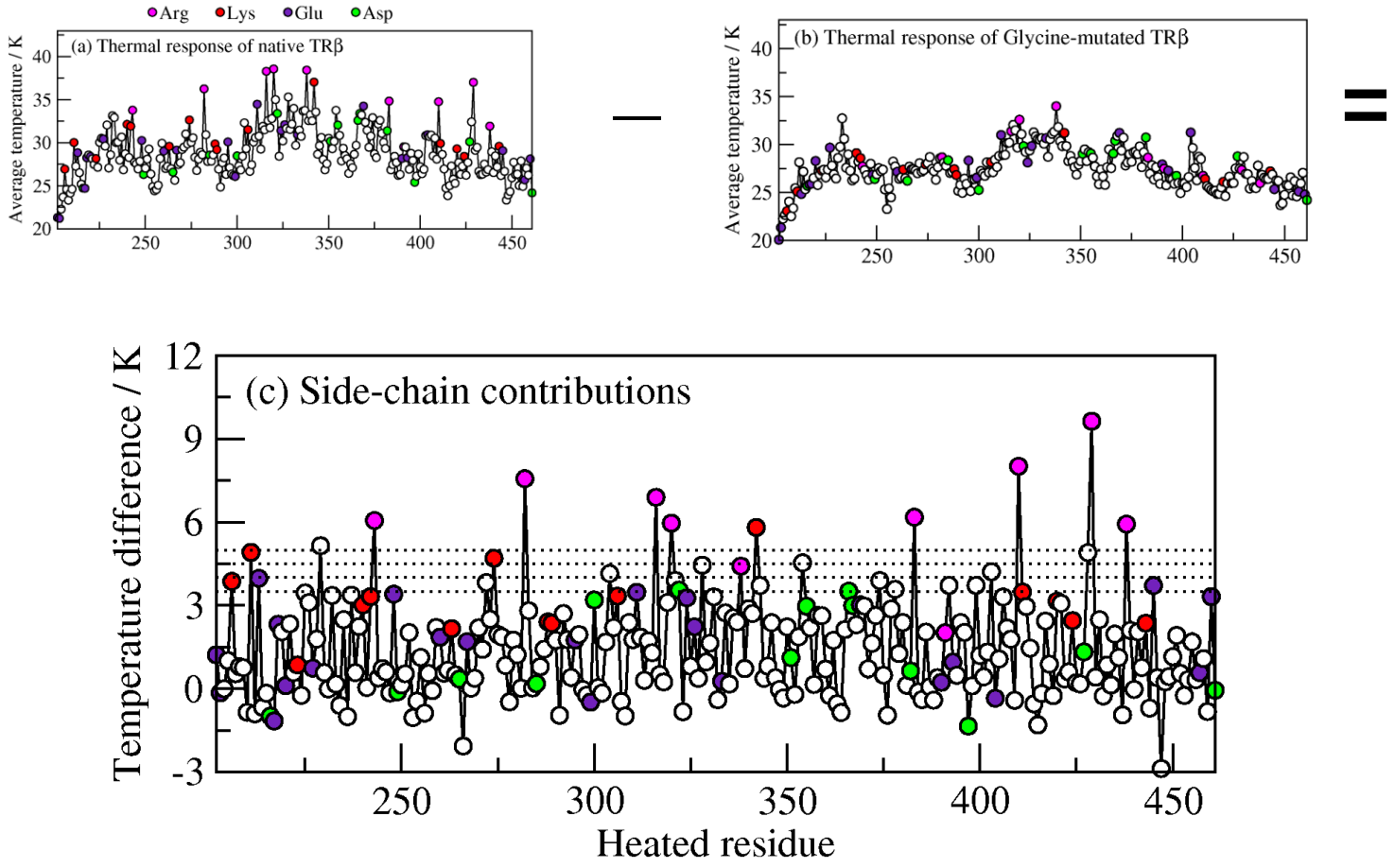
“non-bonded” thermal diffusion

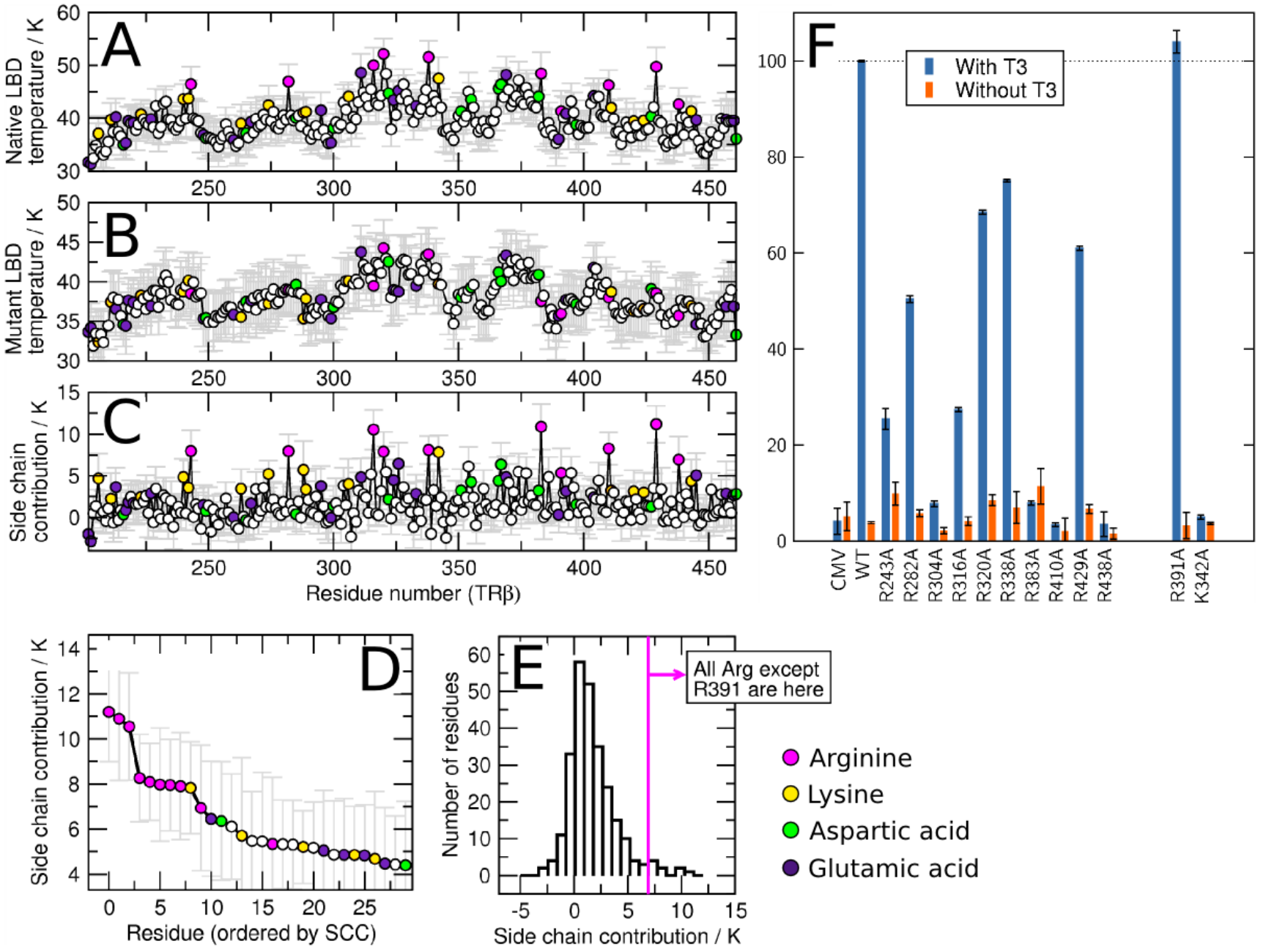


2^{ary} “bonded” thermal diffusion

2. Mechanisms of thermal diffusion

Side-chain contributions




A
Native LBD temperature / K
B
Mutant LBD temperature / K
C
Side chain contribution / K
Residue number (TRβ)
F
With T3
Without T3
CMV
WT
R243A
R282A
R304A
R316A
R320A
R338A
R383A
R410A
R429A
R438A
R391A
K342A
D
Side chain contribution / K
Residue (ordered by SCC)
E
Number of residues
All Arg except R391 are here
Side chain contribution / K
Arginine
Lysine
Aspartic acid
Glutamic acid

Take home message

- MD simulations provide a powerful means of bridging the gap between protein structure and function
- MD may reveal unsuspected roles played by protein dynamics and water molecules (non-crystallographic) in protein function
- Experimentalists understand the results !

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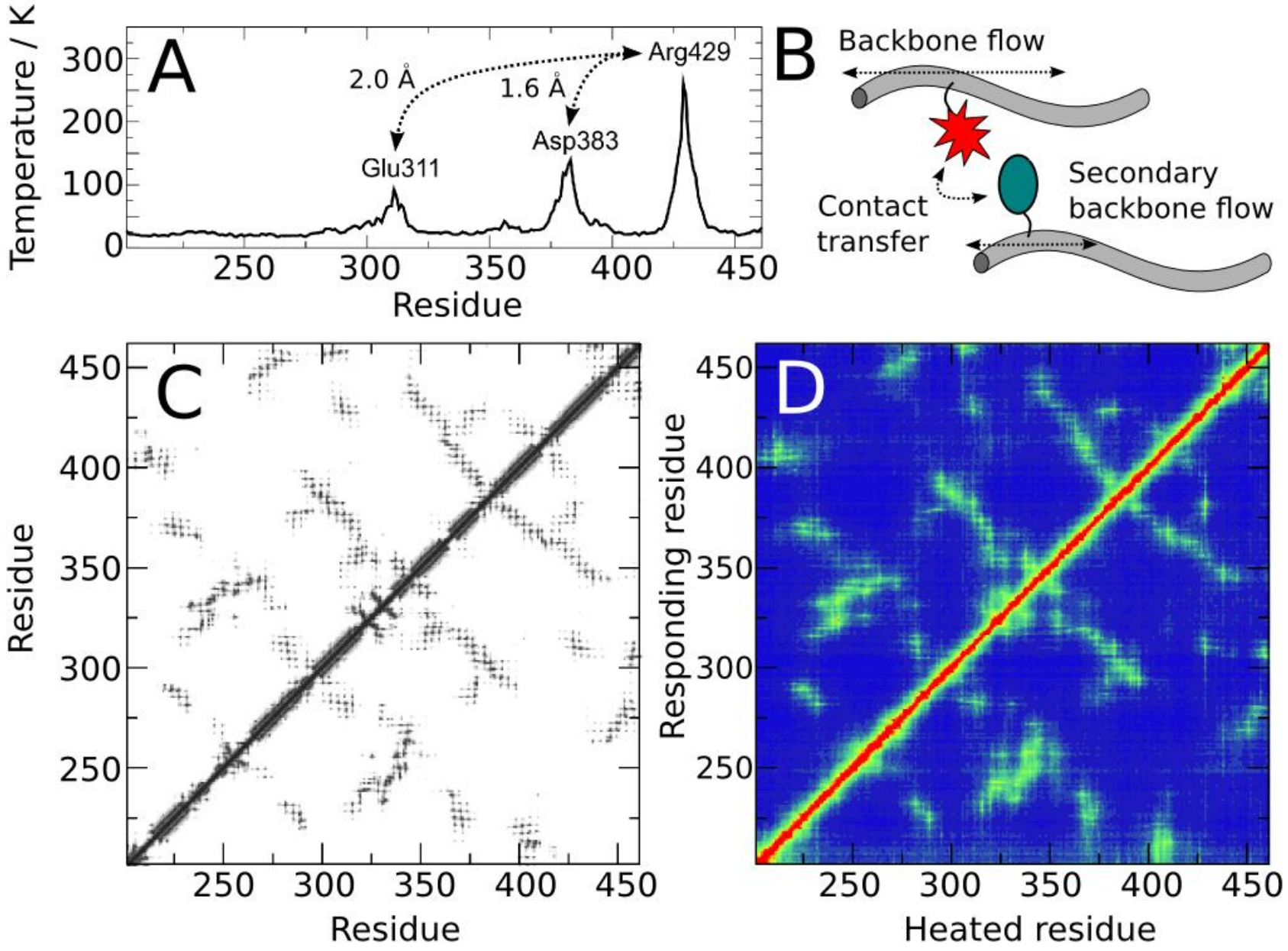
A
Temperature / K
300
200
100
0
250
300
350
400
450
Residue
2.0 Å
1.6 Å
Arg429
Asp383
Glu311
B
Backbone flow
Contact
transfer
Secondary
backbone flow
C
Residue
450
400
350
300
250
250
300
350
400
450
Residue
D
Responding residue
450
400
350
300
250
250
300
350
400
450
Heated residue