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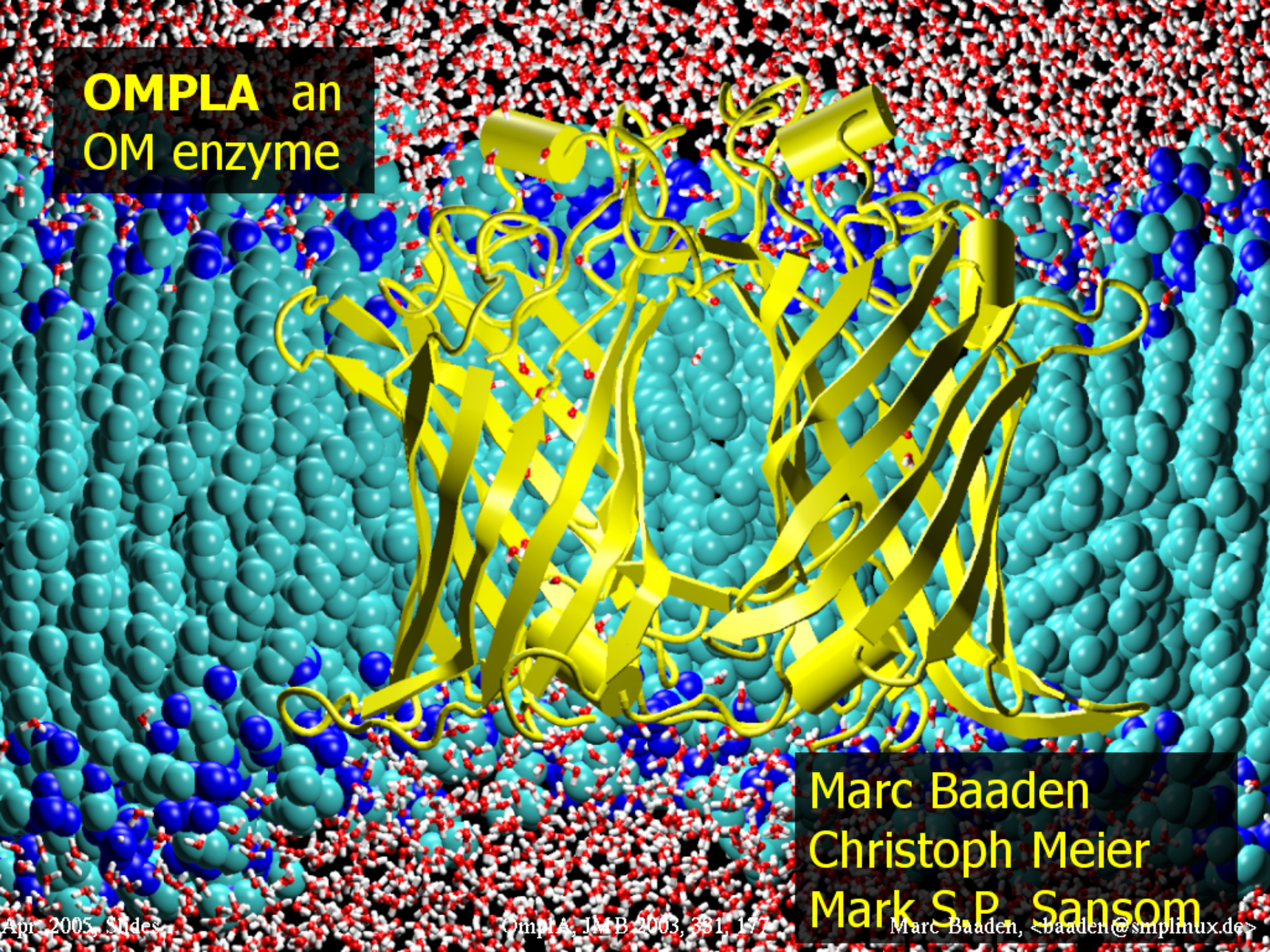
OMPLA an OM enzyme



Marc Baaden
Christoph Meier
Mark S.P. Sansom

OmpLA, JMB 2003, 331, 177

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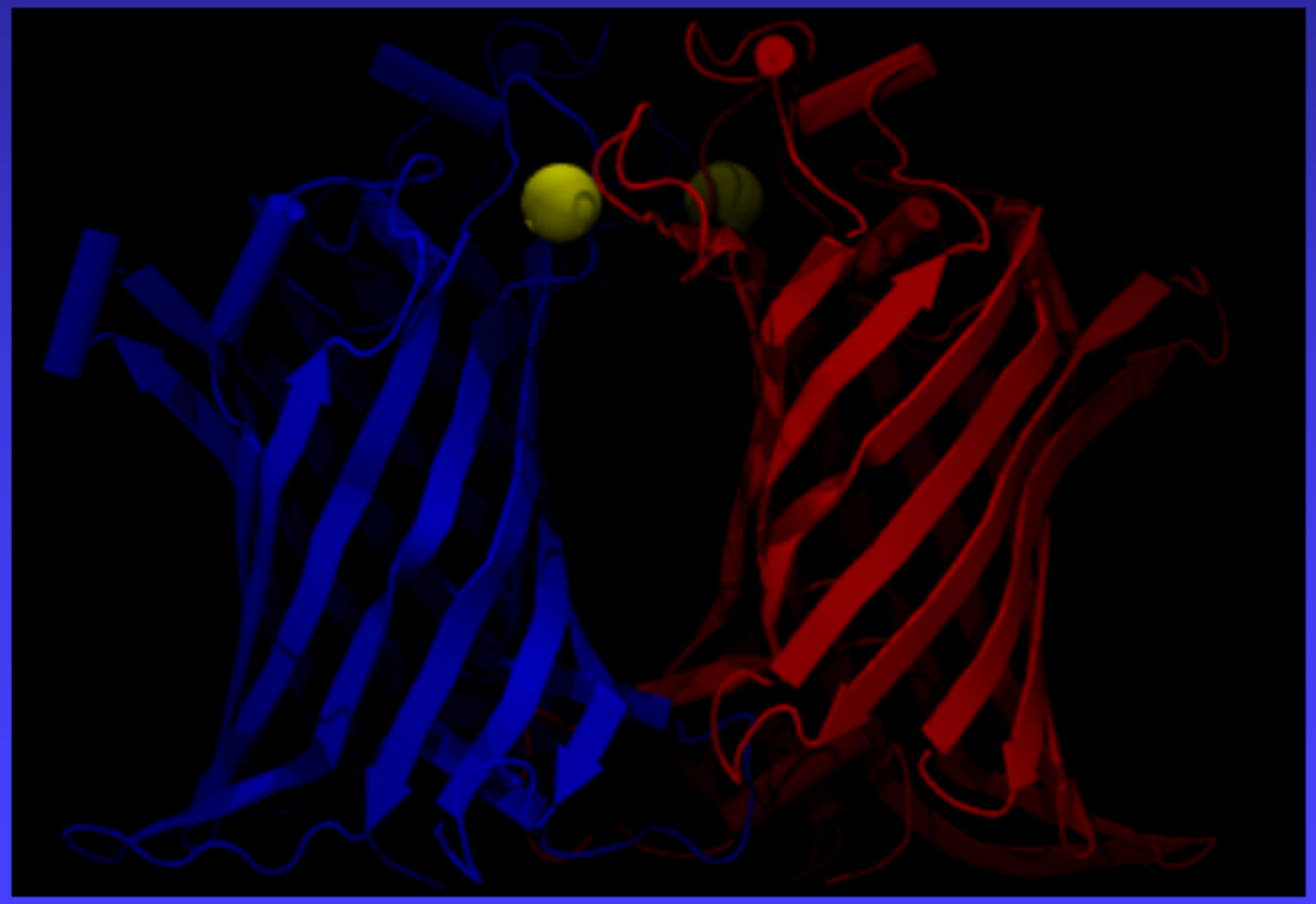
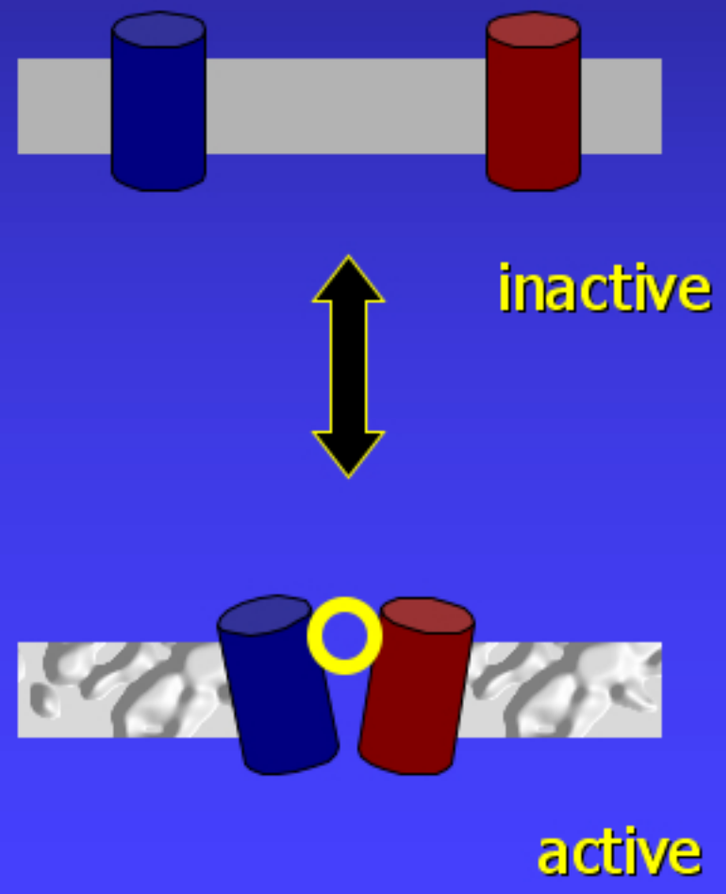
OMPLA: an outer membrane enzyme

- OMPLA
  - outer membrane lipase
  - dimerises to form active site
  - perturbed cell envelope required
- Ca^{2+} ions stabilize dimer and active site
- Catalytic triad related to Ser proteases





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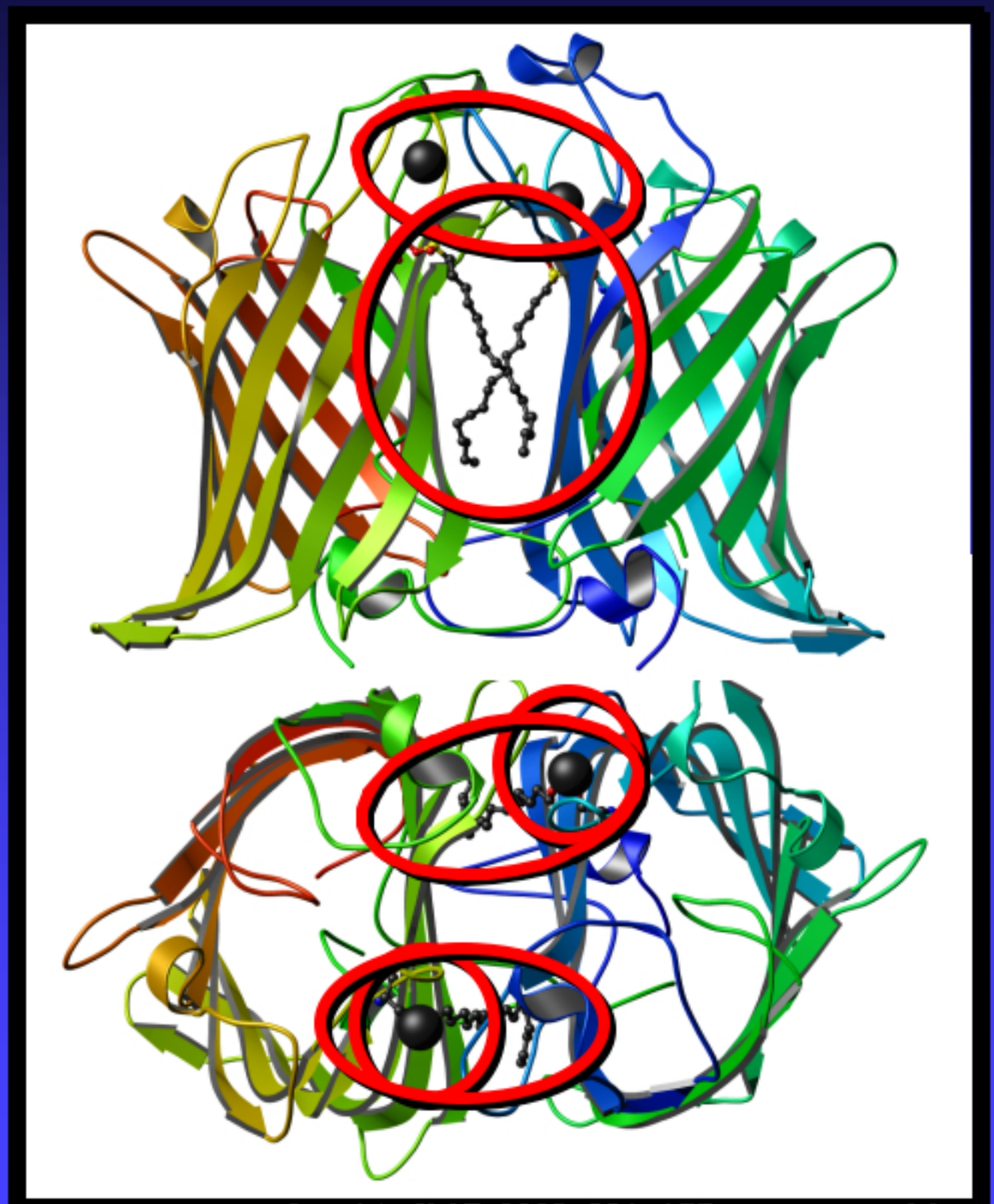
Ompla dimer with calcium and bound inhibitor



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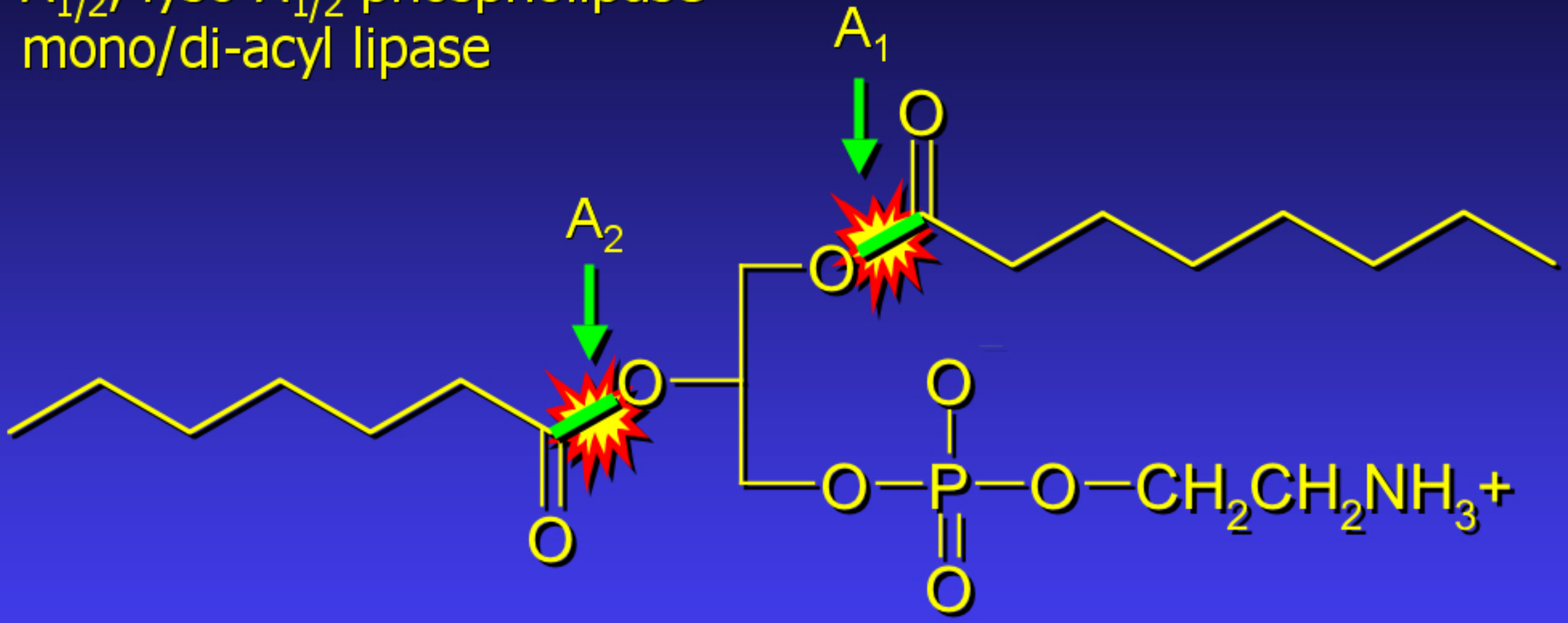
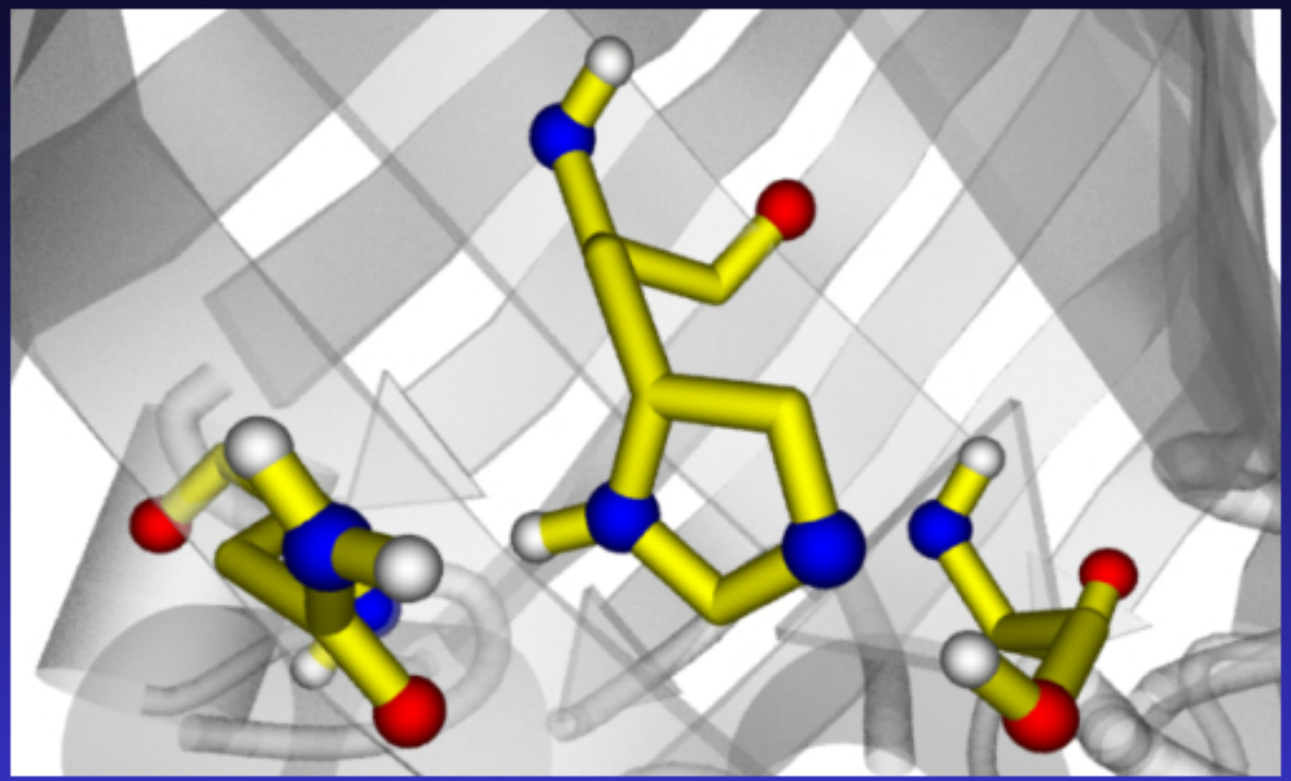
OMPLA phospholytic activity

$A_{1/2}$, lyso-$A_{1/2}$ phospholipase
mono/di-acyl lipase

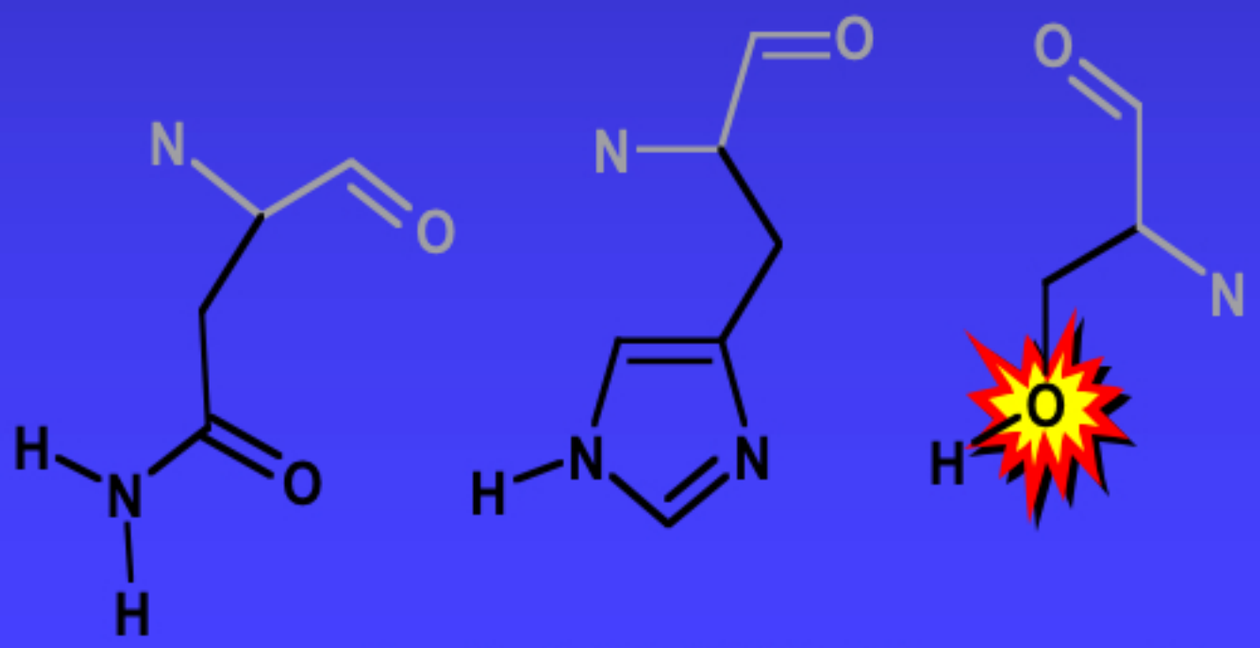


- *Escherichia coli*
- 269 amino acids
- 20 a.a. signal seq.
- Cofactor calcium
- Broad specificity
- Widespread in Gram- bacteria

Active site catalytic triad



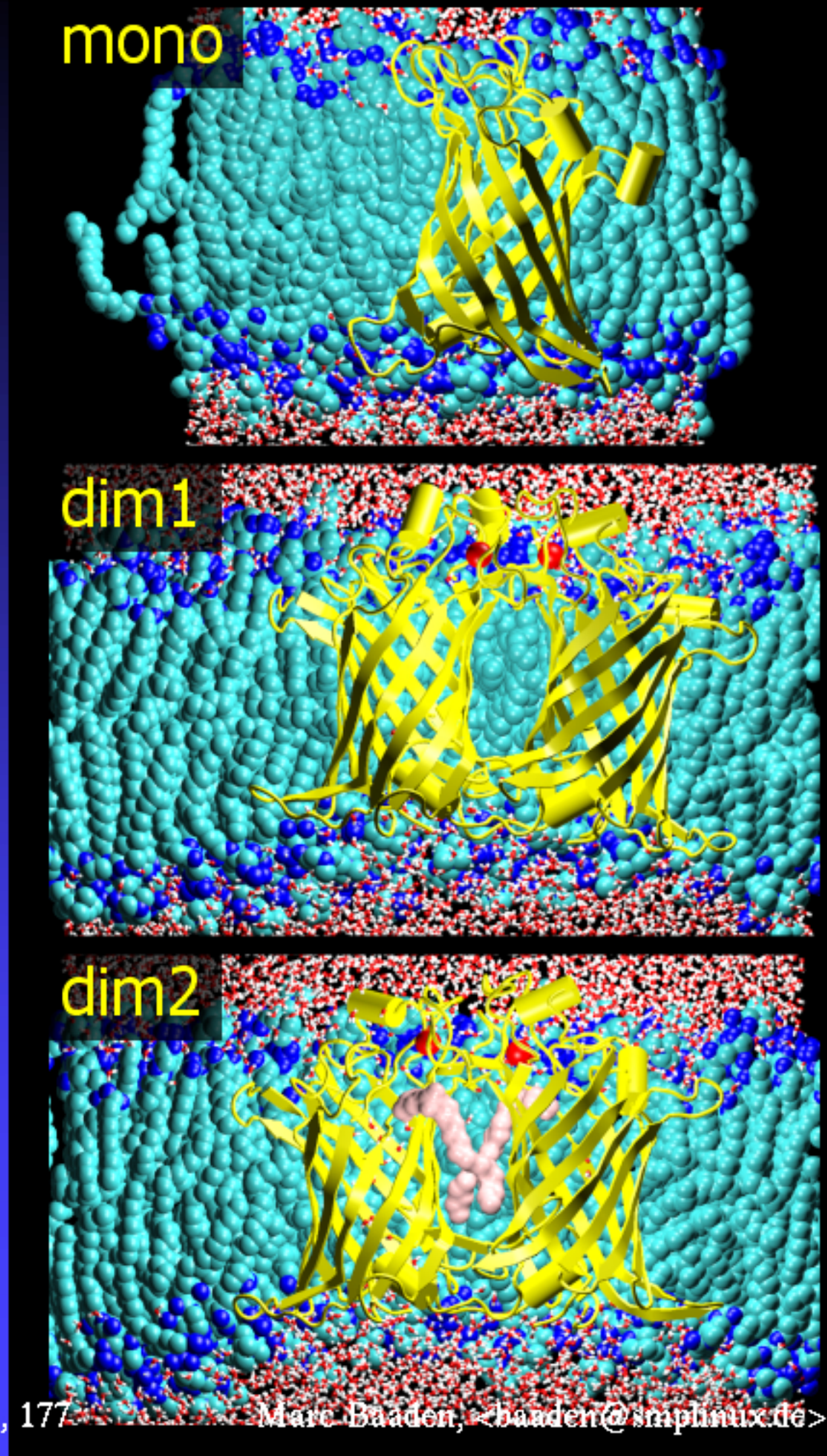
Asn_{156} His_{142} Ser_{144}



OMPLA simulation systems

(***Baaden, Meier*** & ***Sansom, JMB*****, 2003**)

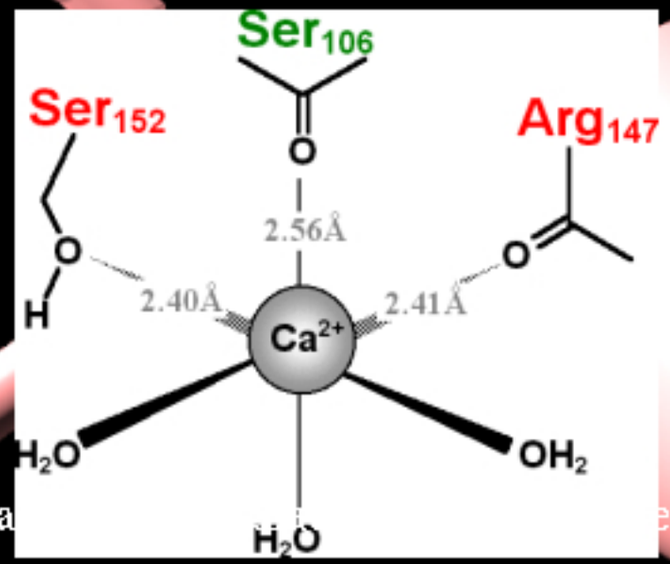
- XR structures by Snijder *et al.* (2.1 - 2.8 Å)
- Molecular Dynamics simulations
- In POPC bilayer
- 5 ns each (Cutoff) … now 10 ns PME
- Monomer
  vs. dimer, Ca^{2+}
  vs. dimer-inhibitor complex, Ca^{2+}



Arg147

Ser106

Ser152



OmplA, JMB 2003, 331, 177

Challenges in modeling OMPLA

Calcium

- parameters adapted from Shiratori & Nakagawa and Meulenhoff
- high affinity Ca^{2+} site (K_d=36μM)
- coordination controlled by distance restraints

- substrate parameterization based on Gromacs-87 forcefield
- membrane insertion
- symmetry

Calcium implementation

Interaction		Standard forcefield[27]			Modified forcefield[33,34]		
		Charge	r_{12}	r_6	Charge	r_{12}	r_6
Ca^{2+}	Ser-152 O^{γ}	-0.548	$0.15062 \cdot 10^{-9}$	$0.22617 \cdot 10^{-2}$	-0.598	$0.20104 \cdot 10^{-9}$	$0.30321 \cdot 10^{-2}$
Ca^{2+}	Ser-152 C^{β}	+0.15	0.3533310^{-4}	$0.90975 \cdot 10^{-2}$	+0.20	0.3533310^{-4}	$0.90975 \cdot 10^{-2}$
Ca^{2+}	Arg-147 O	-0.38	$0.74158 \cdot 10^{-5}$	$0.22617 \cdot 10^{-2}$	-0.764	$0.74158 \cdot 10^{-5}$	$0.22617 \cdot 10^{-2}$
Ca^{2+}	Arg-147 C	+0.38	$0.33740 \cdot 10^{-5}$	$0.23402 \cdot 10^{-2}$	+0.764	$0.33740 \cdot 10^{-5}$	$0.23402 \cdot 10^{-2}$
Ca^{2+}	Ser-106 O	-0.38	$0.74158 \cdot 10^{-5}$	$0.22617 \cdot 10^{-2}$	-0.764	$0.74158 \cdot 10^{-5}$	$0.22617 \cdot 10^{-2}$
Ca^{2+}	Ser-106 C	+0.38	$0.33740 \cdot 10^{-5}$	$0.23402 \cdot 10^{-2}$	+0.764	$0.33740 \cdot 10^{-5}$	$0.23402 \cdot 10^{-2}$
Ca^{2+}	Water O^{W}	-0.82	$0.26331 \cdot 10^{-5}$	$0.26171 \cdot 10^{-2}$	-0.82	$0.26331 \cdot 10^{-5}$	$0.26171 \cdot 10^{-2}$

***Table 2.3:* The forcefield modification of the calcium ligands in dimeric OMPLA.**
Modifications were made to the atomic charge and Lennard-Jones parameters (cf. appendix A) of the ligand atoms of calcium.
In the ab initio calculation the energy profile phospholipase A2 was generated and the nonbonded interaction energy parameters (for the Lennard-Jones and Coulomb interaction) were subsequently fitted to the calculated profile. The detailed derivation of these figures is discussed in appendix C. After a method by Shiratori et al.[33] and Meulenhoff[34].

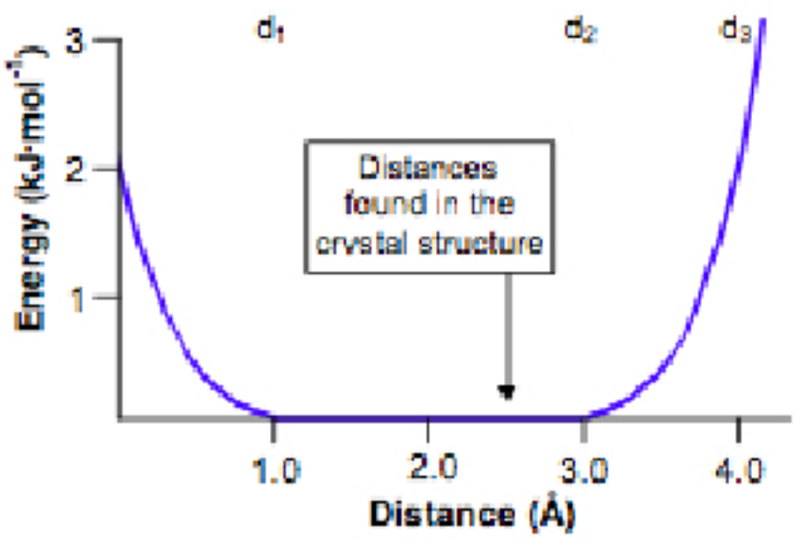
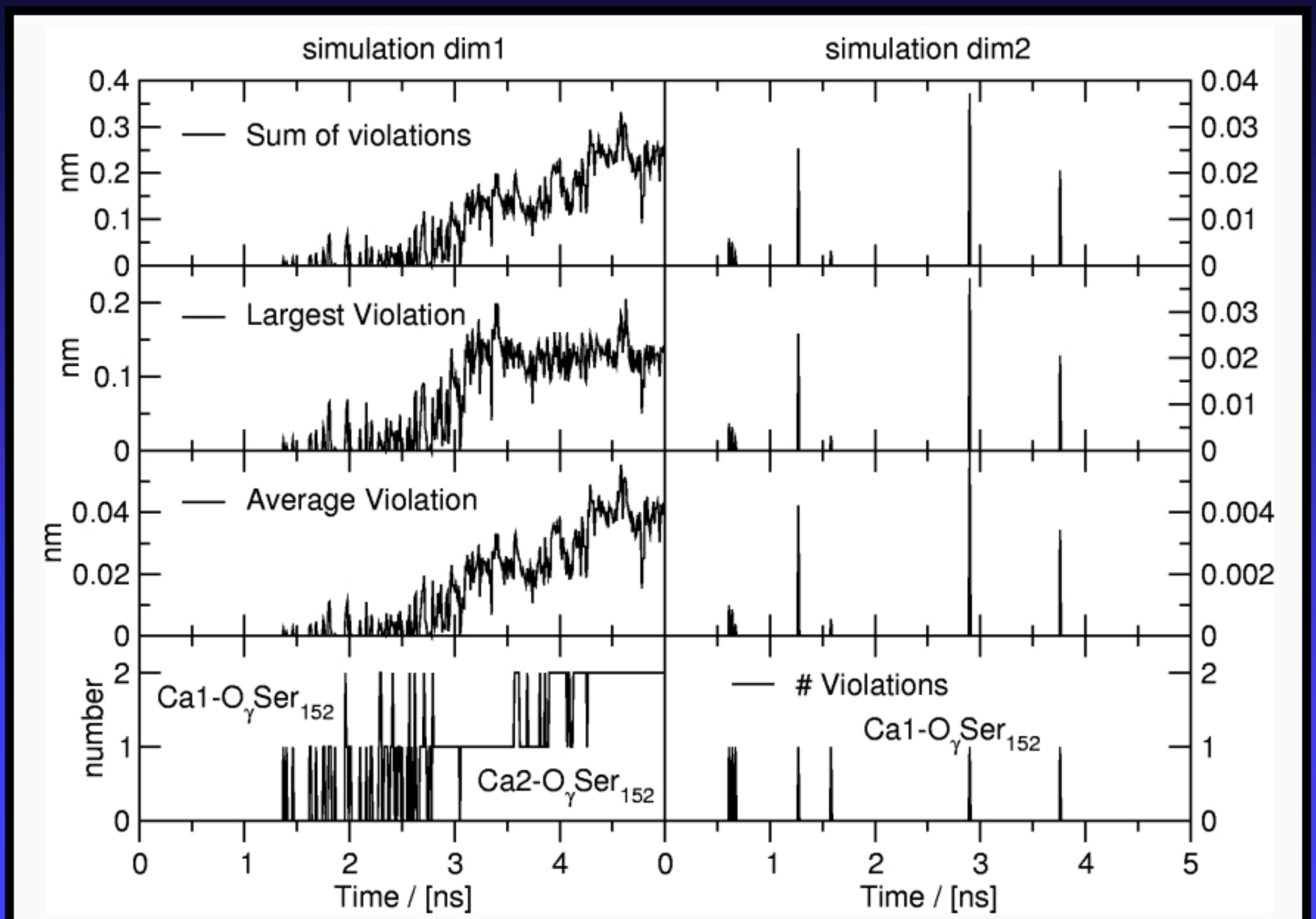
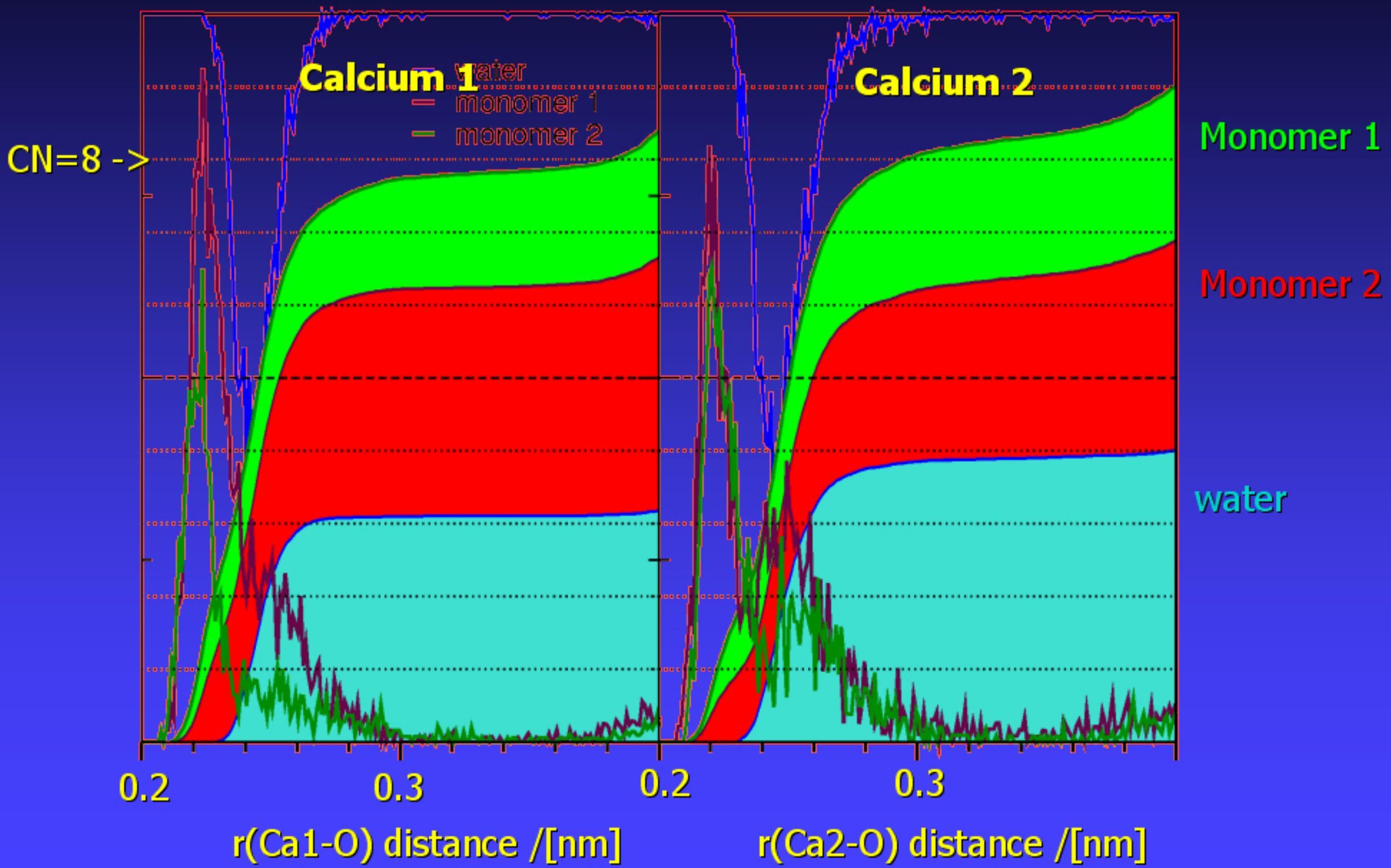


***Figure 2.7:* Distance restraints in dimeric, calcium-containing OMPLA.** *An energetic penalty is added to the potential when the distance between specified pairs of atoms exceeds a threshold value. The potential form is quadratic below 1Å, and between 3Å and 4Å, and linear beyond 4Å. The cut-off distances are set such that the restraints do not act on distances close to those observed in the crystal structure of OMPLA. They are equivalent in strength to less than the weakest covalent bonds found in the protein (Force constant 200 kJ·mol^{-1}·nm^{-2}).*

Violation of Ca^{2+} distance restraints



Calcium coordination - g(r)



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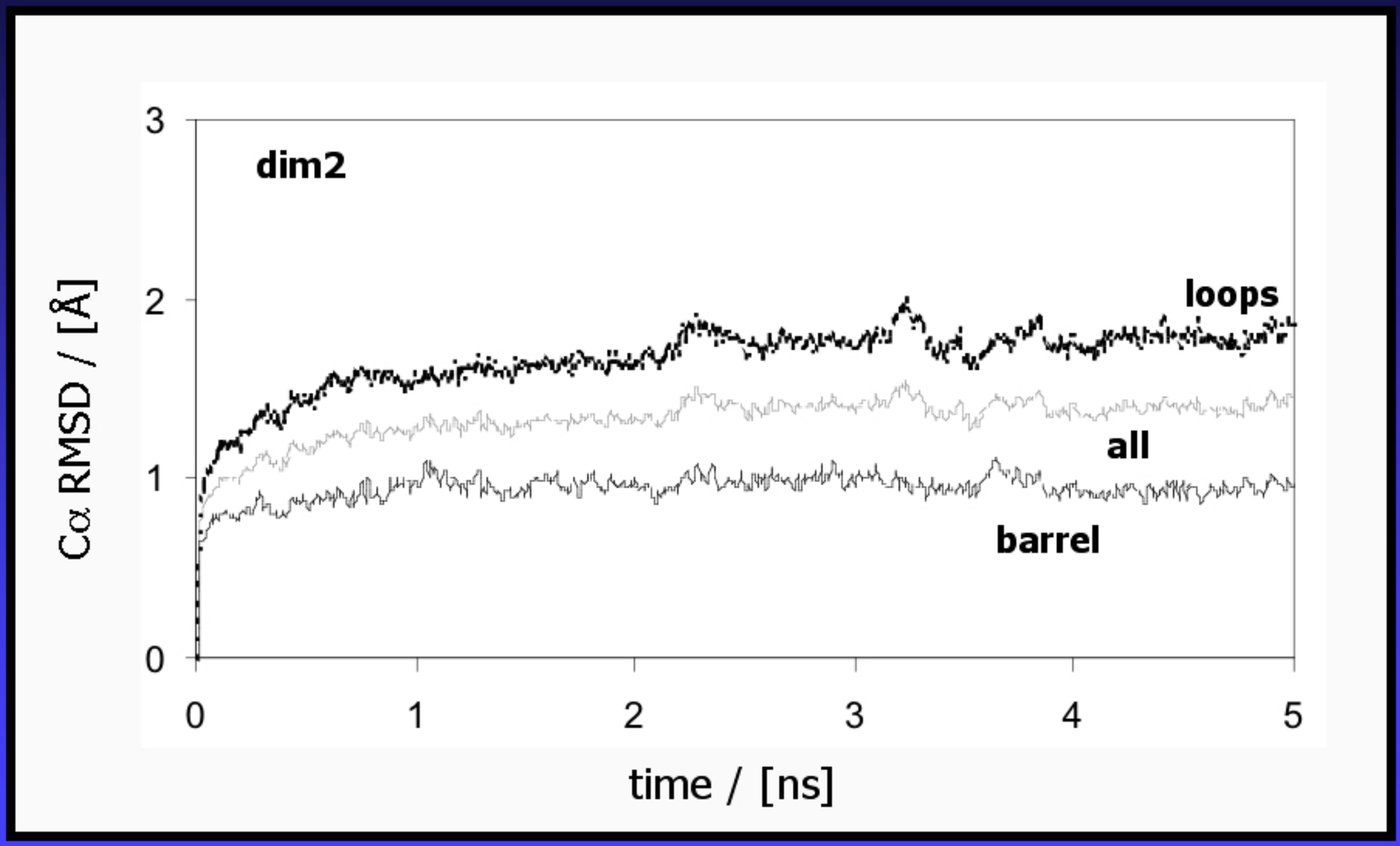
Structural drift (RMSD) from XR



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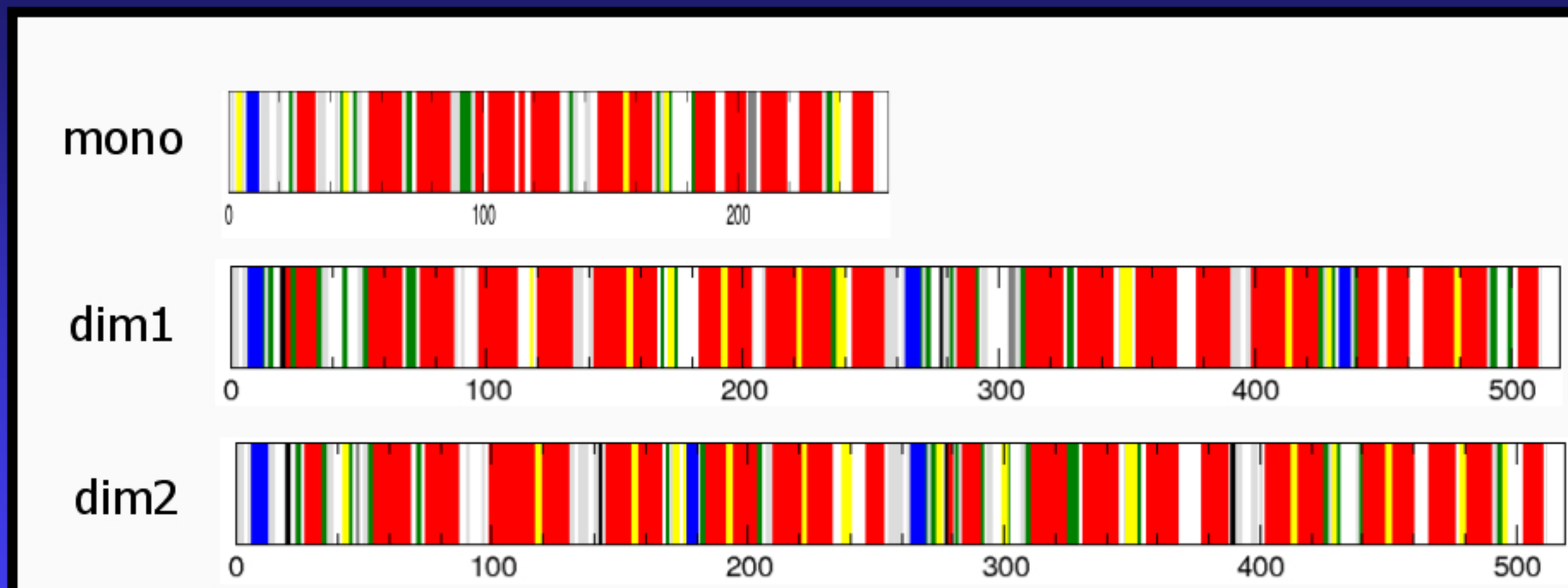
Dominant secondary structure



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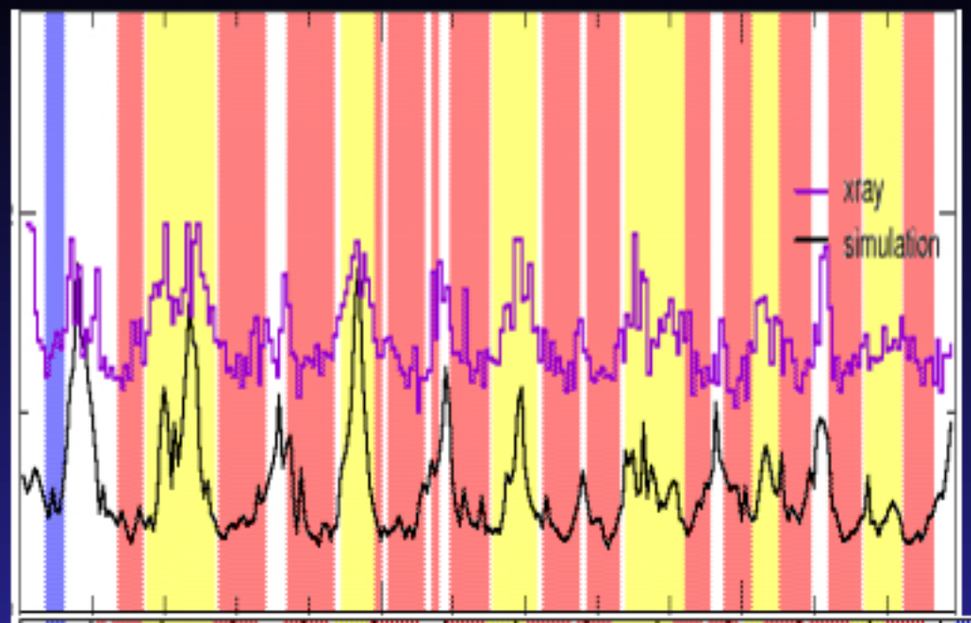
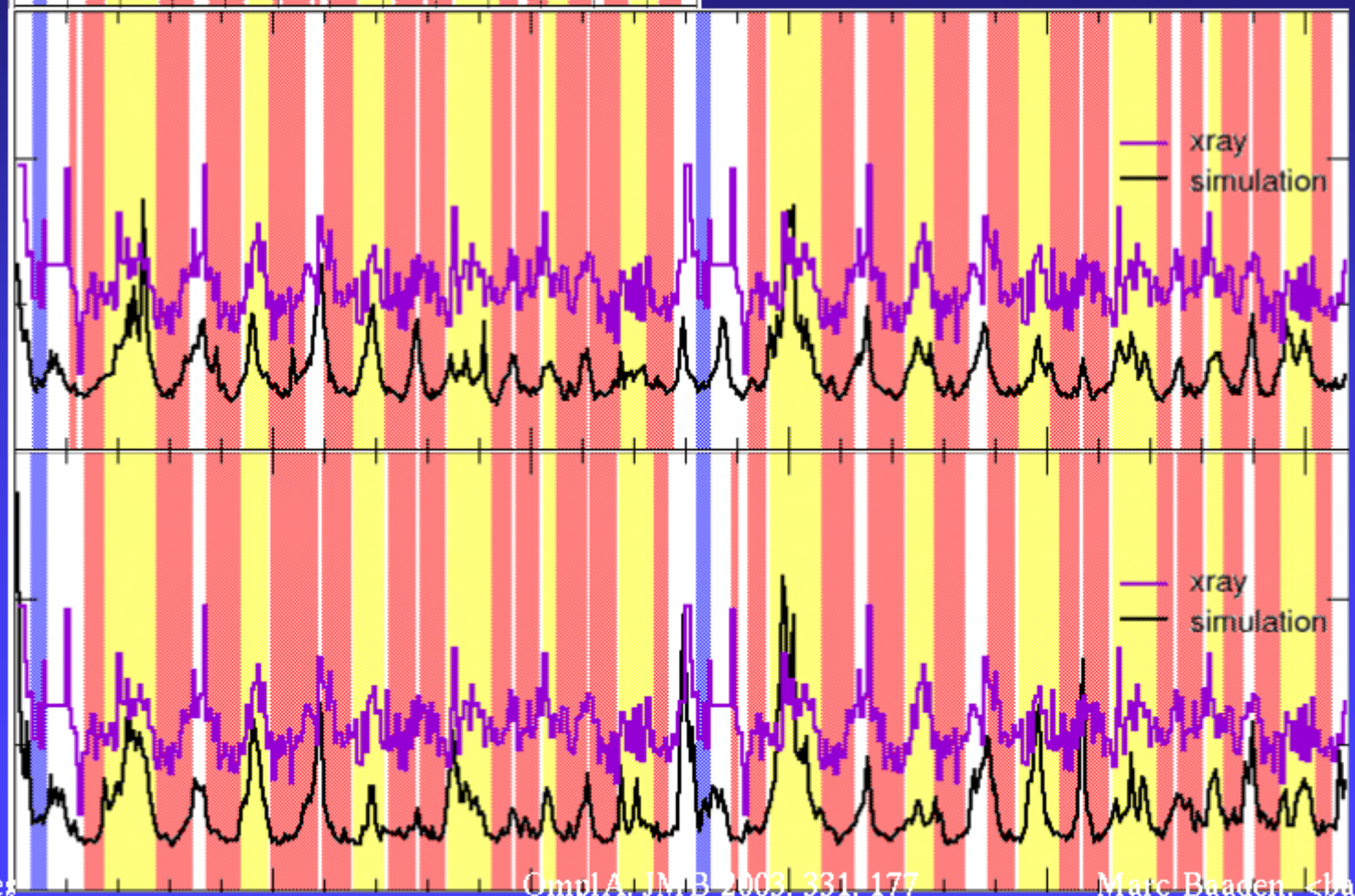
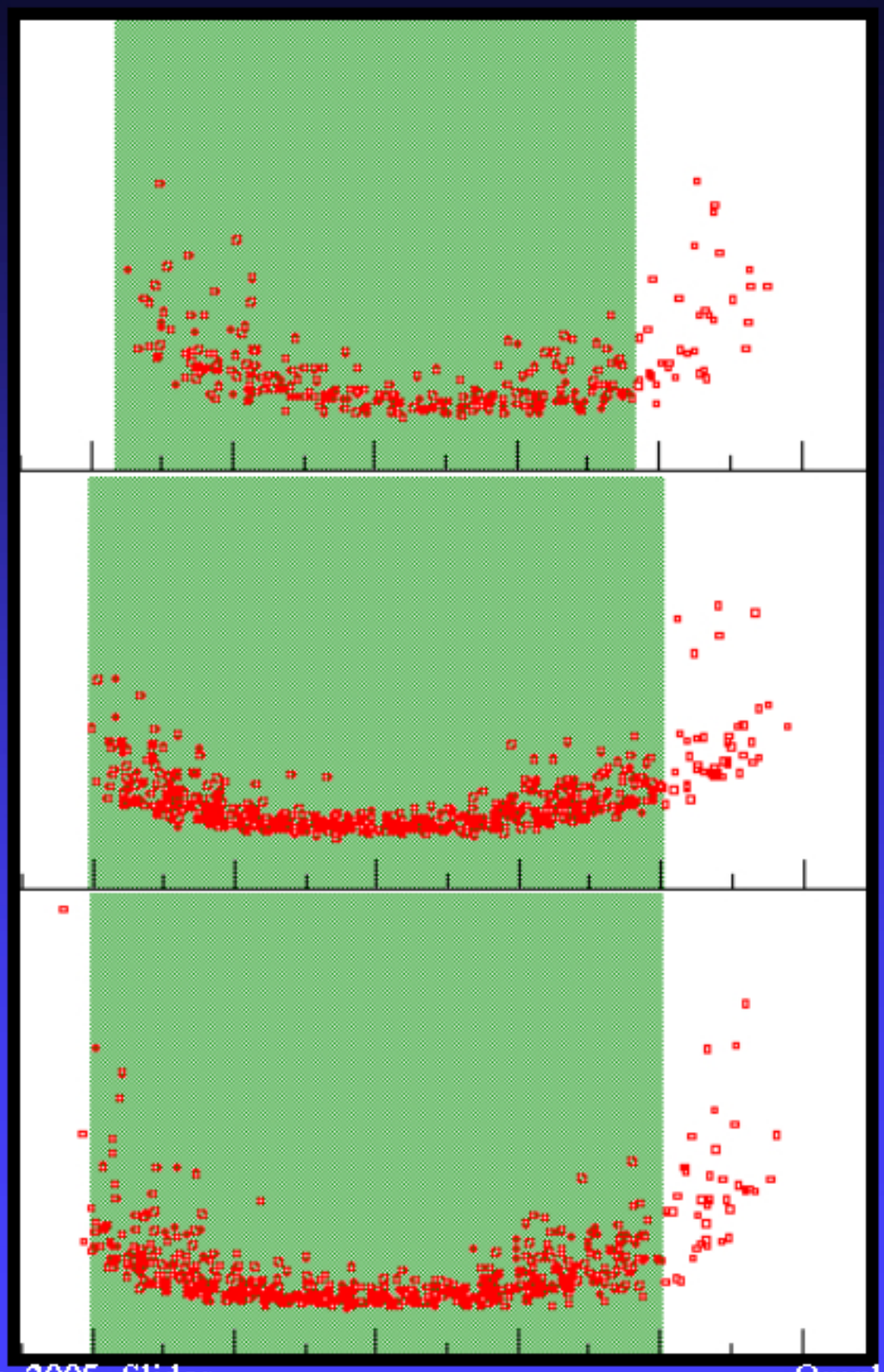
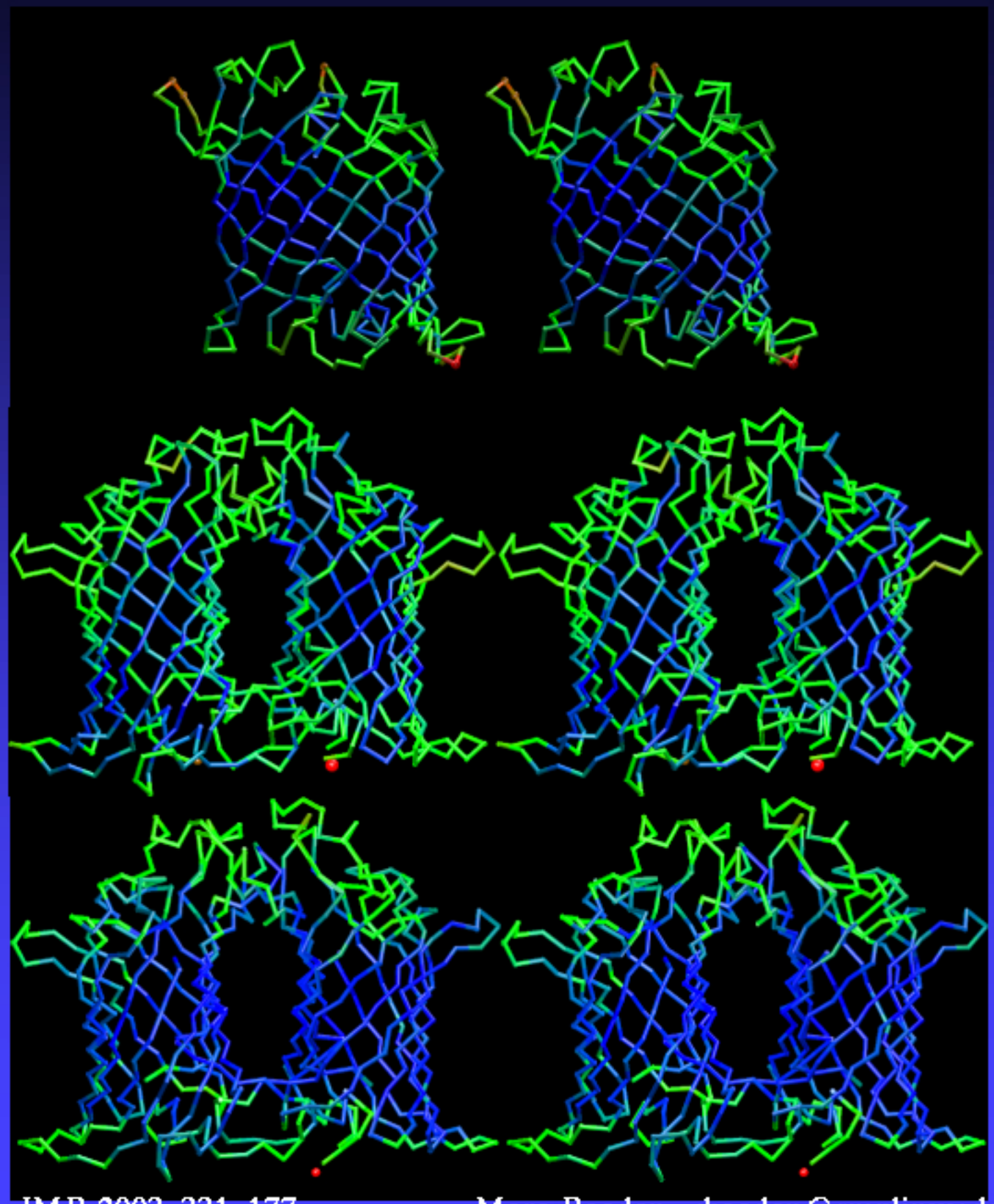
OMPLA RMSF vs B-factors





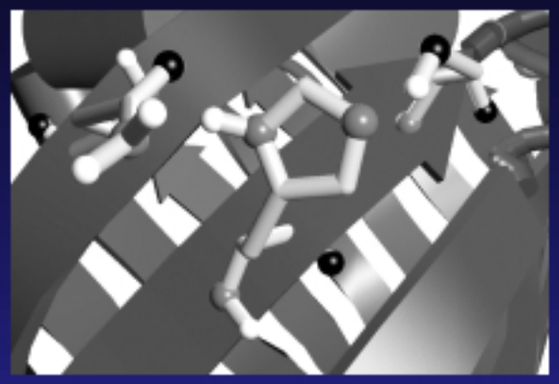
RMSF vs membrane position



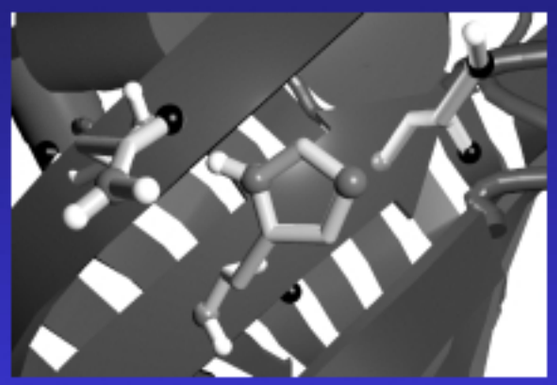


Active site dynamics

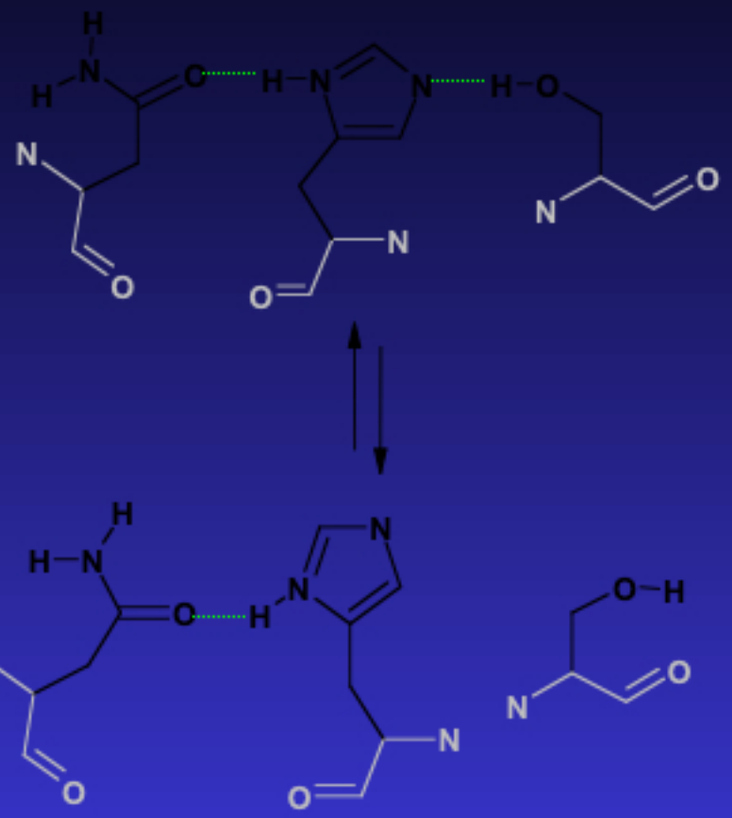
A



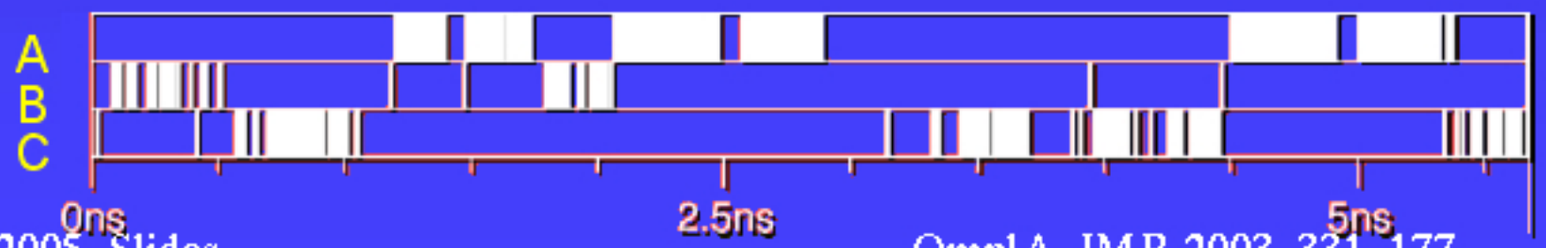
B



C



N156 **H142** **S144**



- catalytic triad fluctuates
- Ca^{2+} stabilizes
- Substrate induces changes in monomer:monomer interactions

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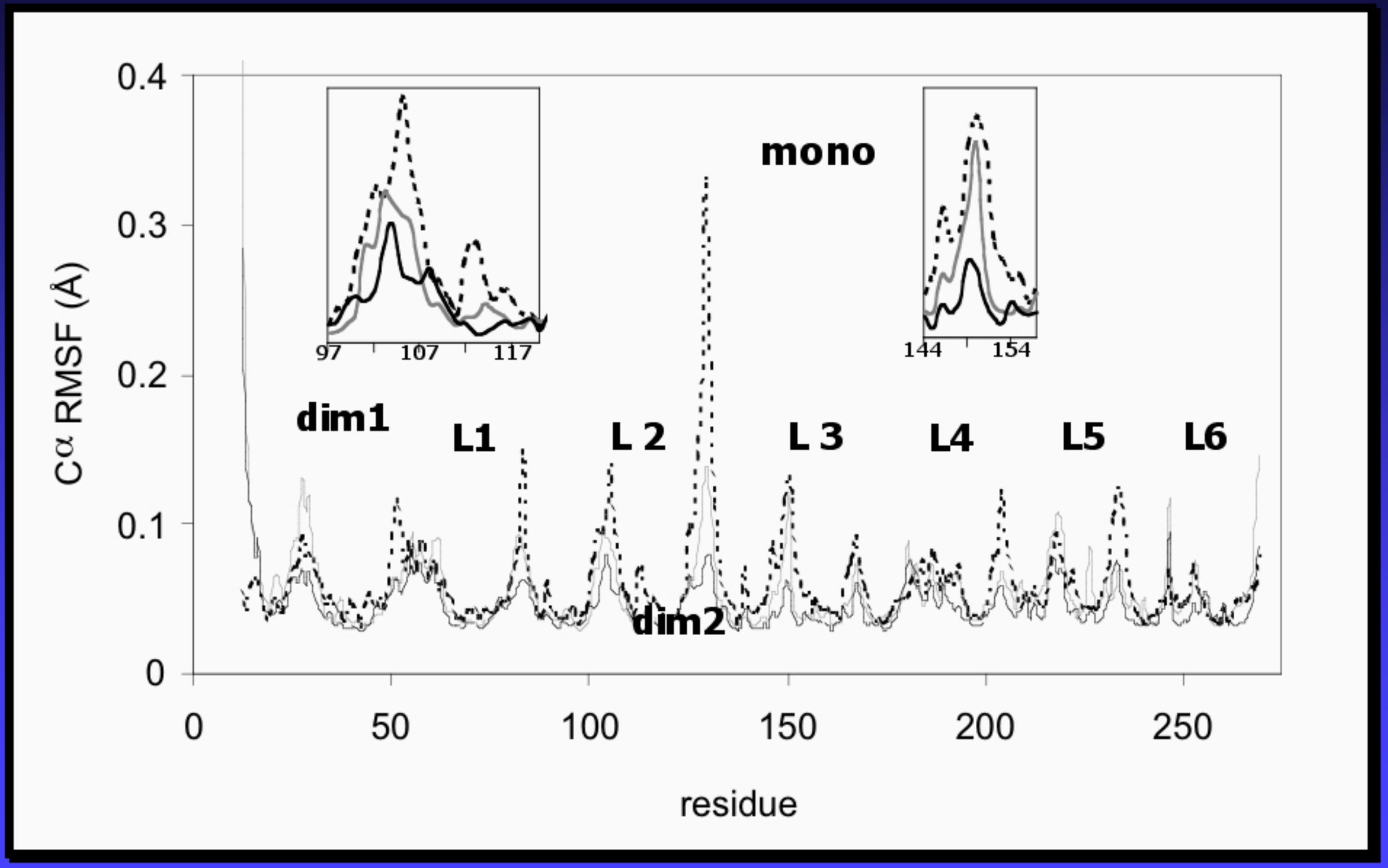
Stabilising role of calcium



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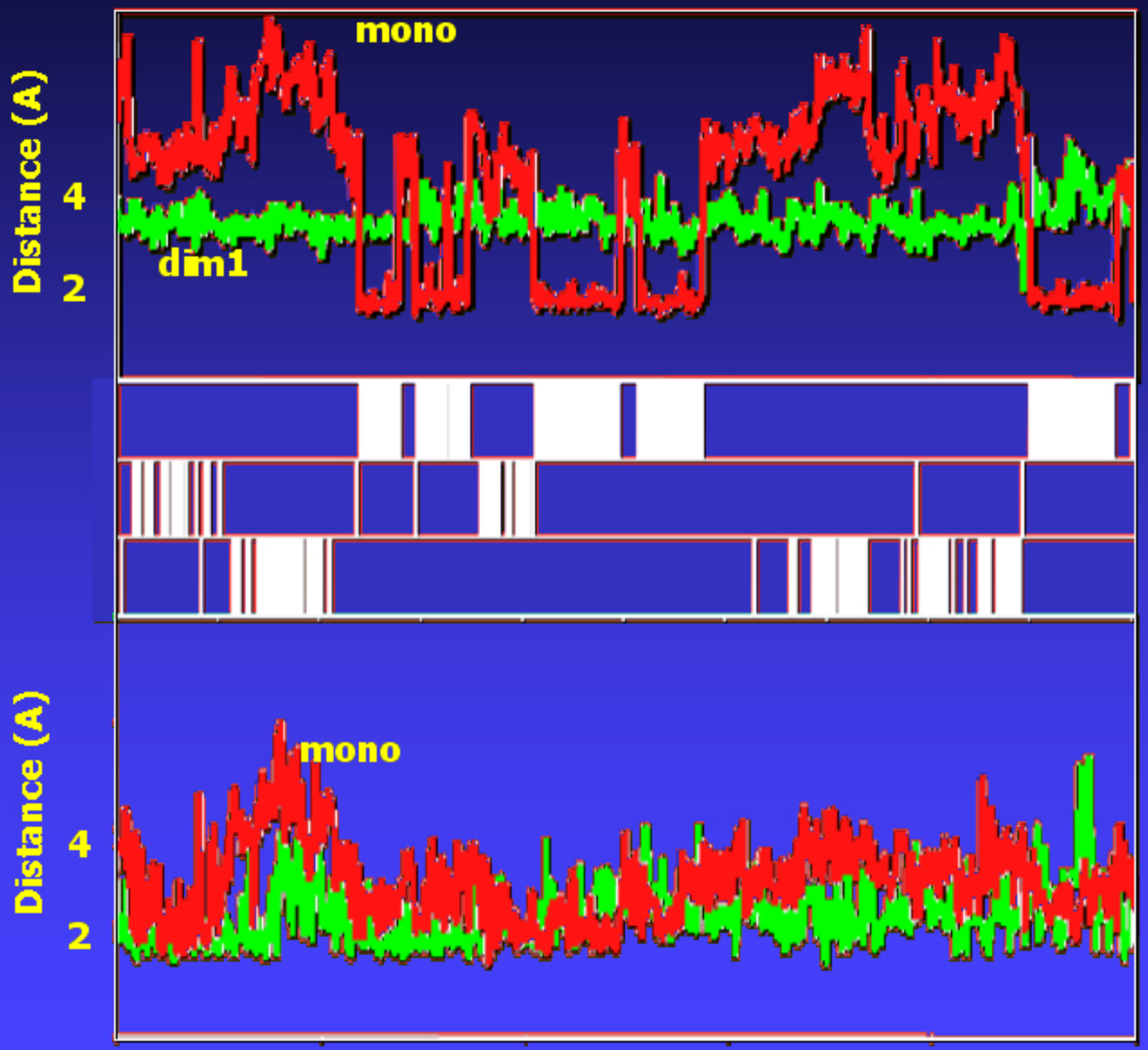
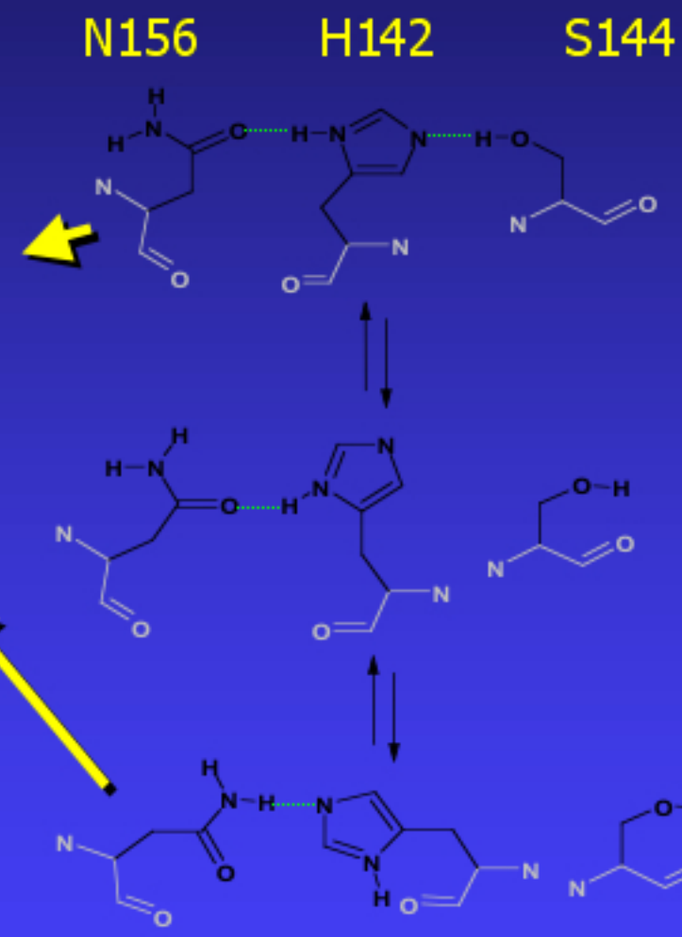
Visual analysis *vs.* H-bond distances



N(H142) – HO(S144)



O(N156) – HN(H142)

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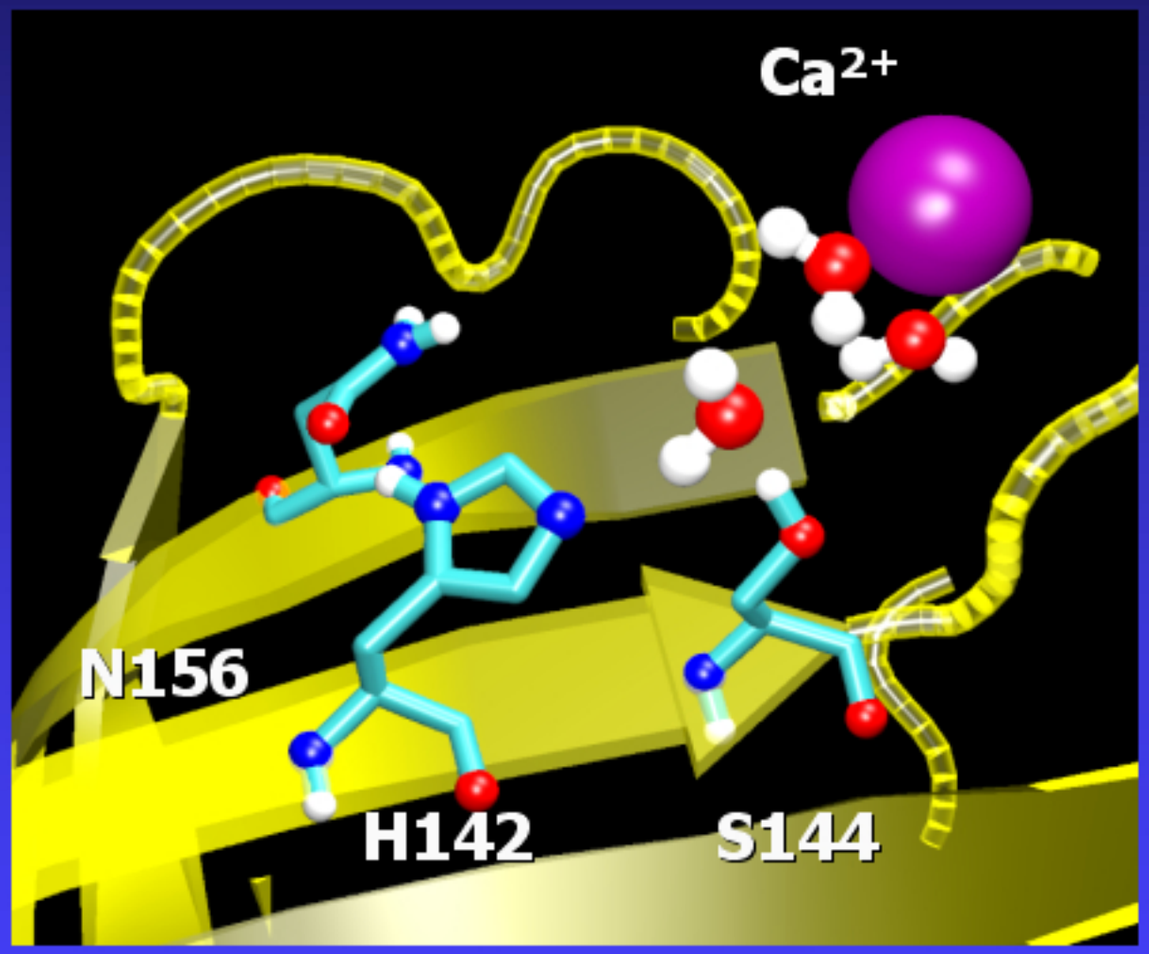
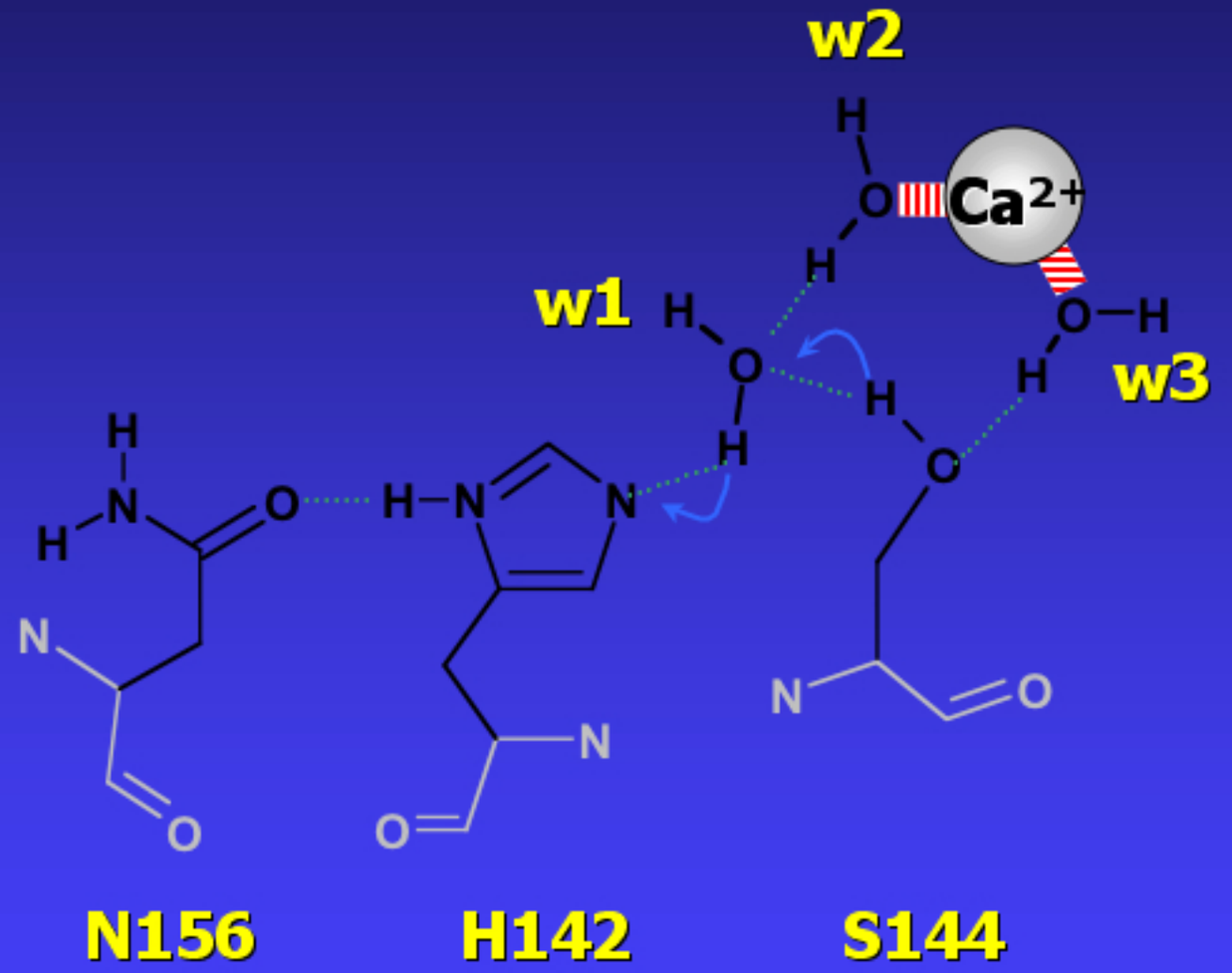
Is there a fixed hydrogen bond network near Ca^{2+} ?





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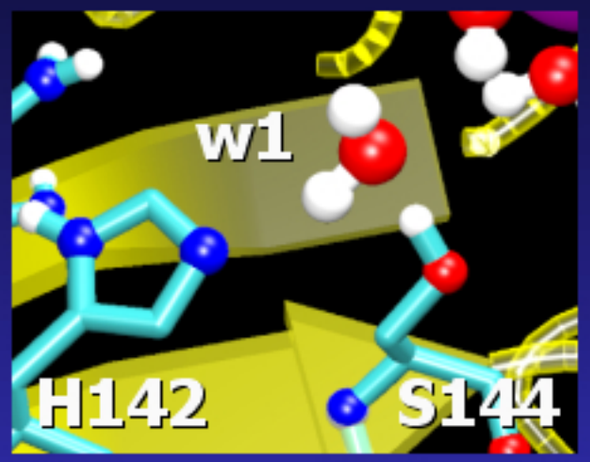
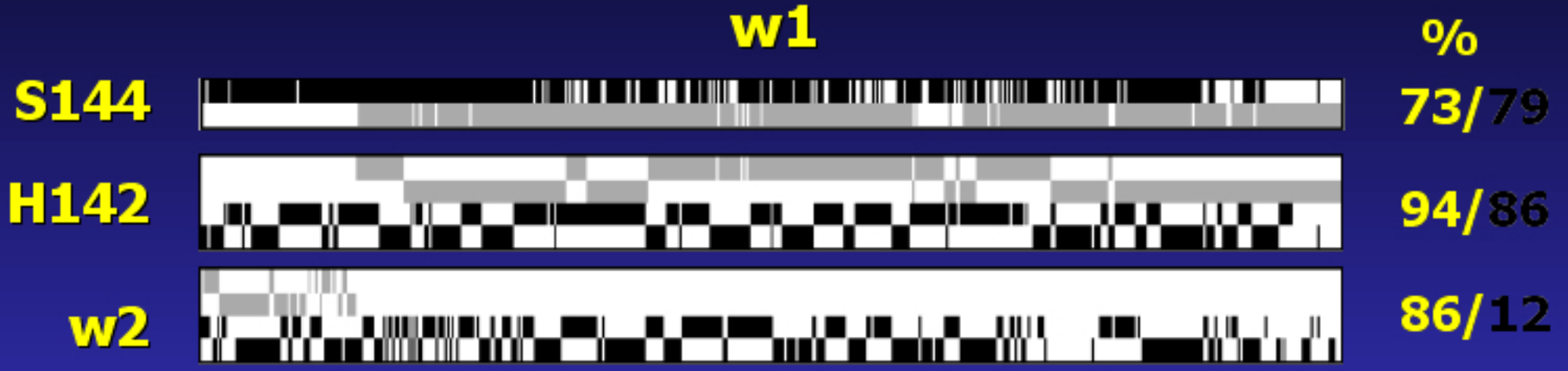
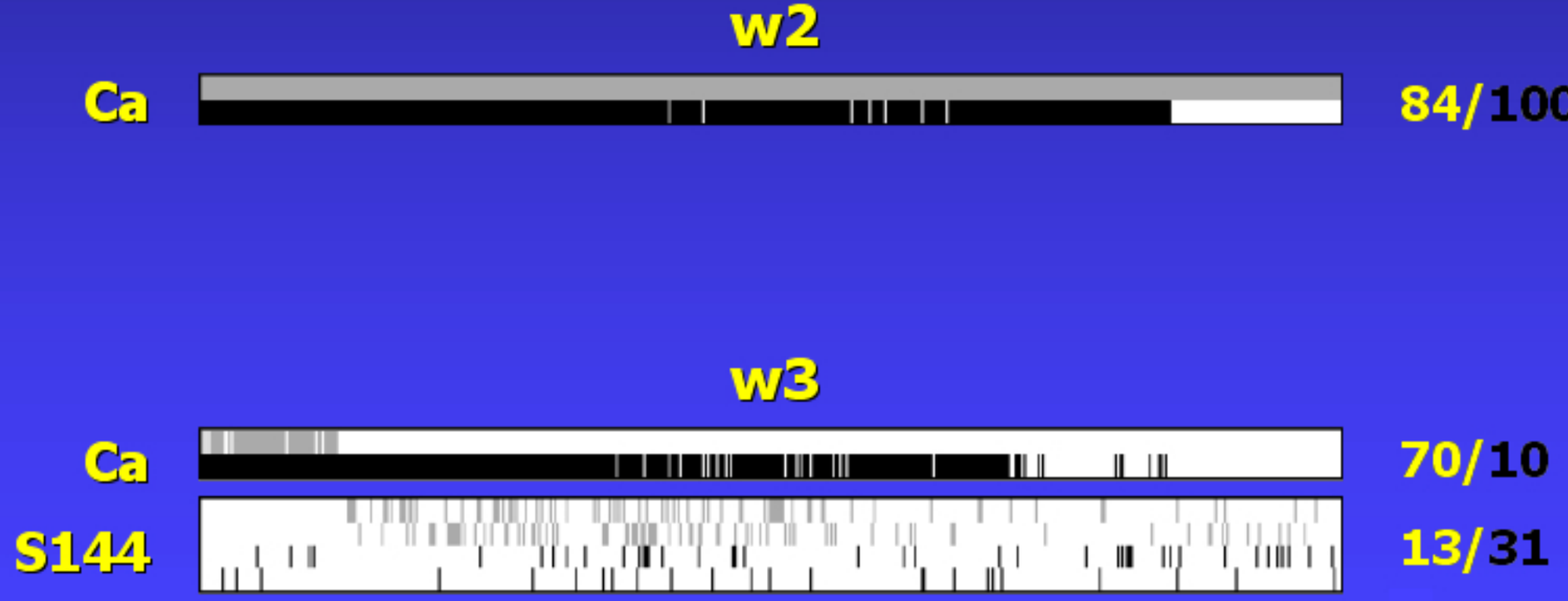
Dominant water interactions







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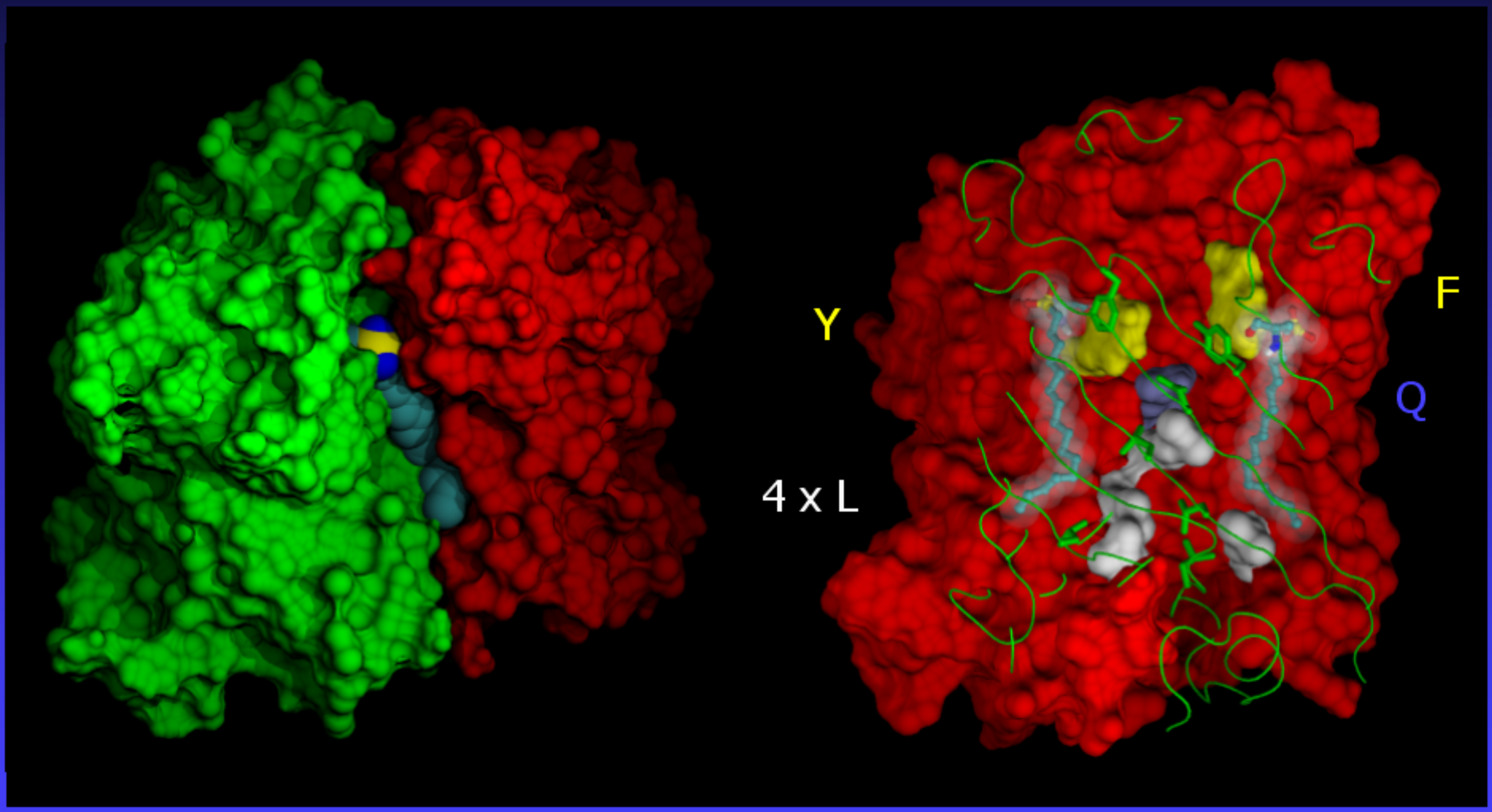
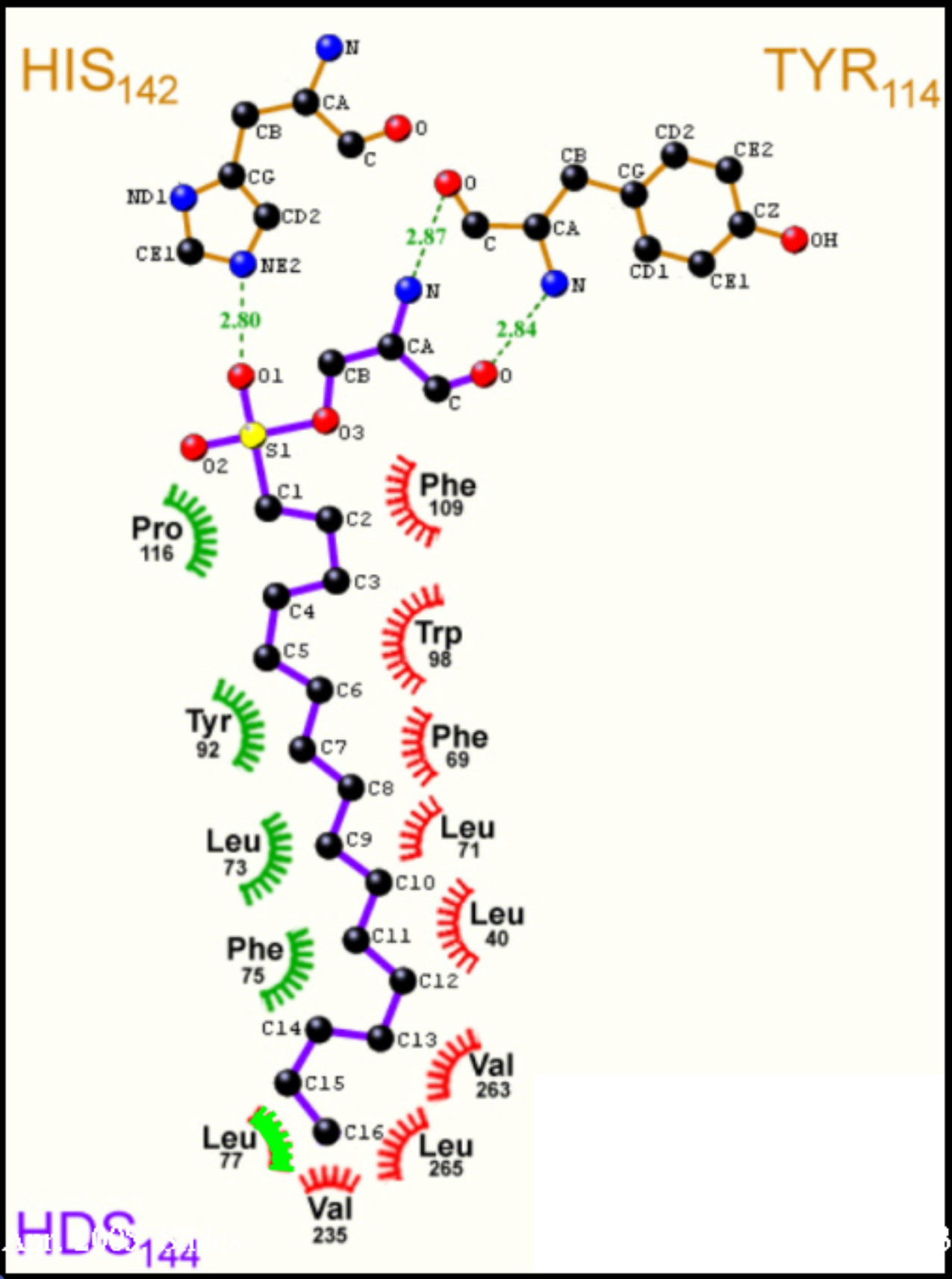
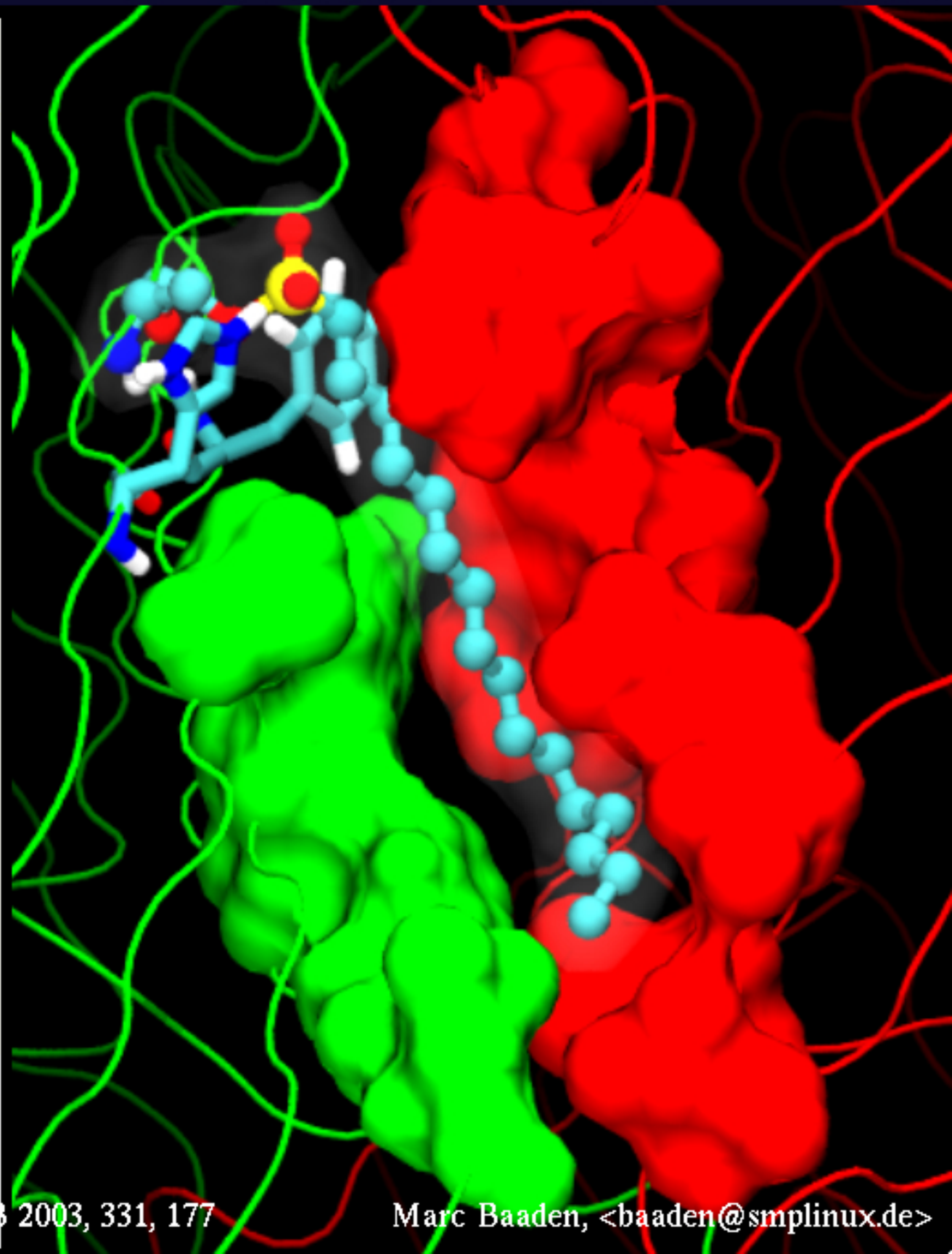
OMPLA dimer interface



- stacked aromatic rings
- complementary Leu bulges
- central polar Gln
- hydrogen bonds

HDS inhibitor binding pocket





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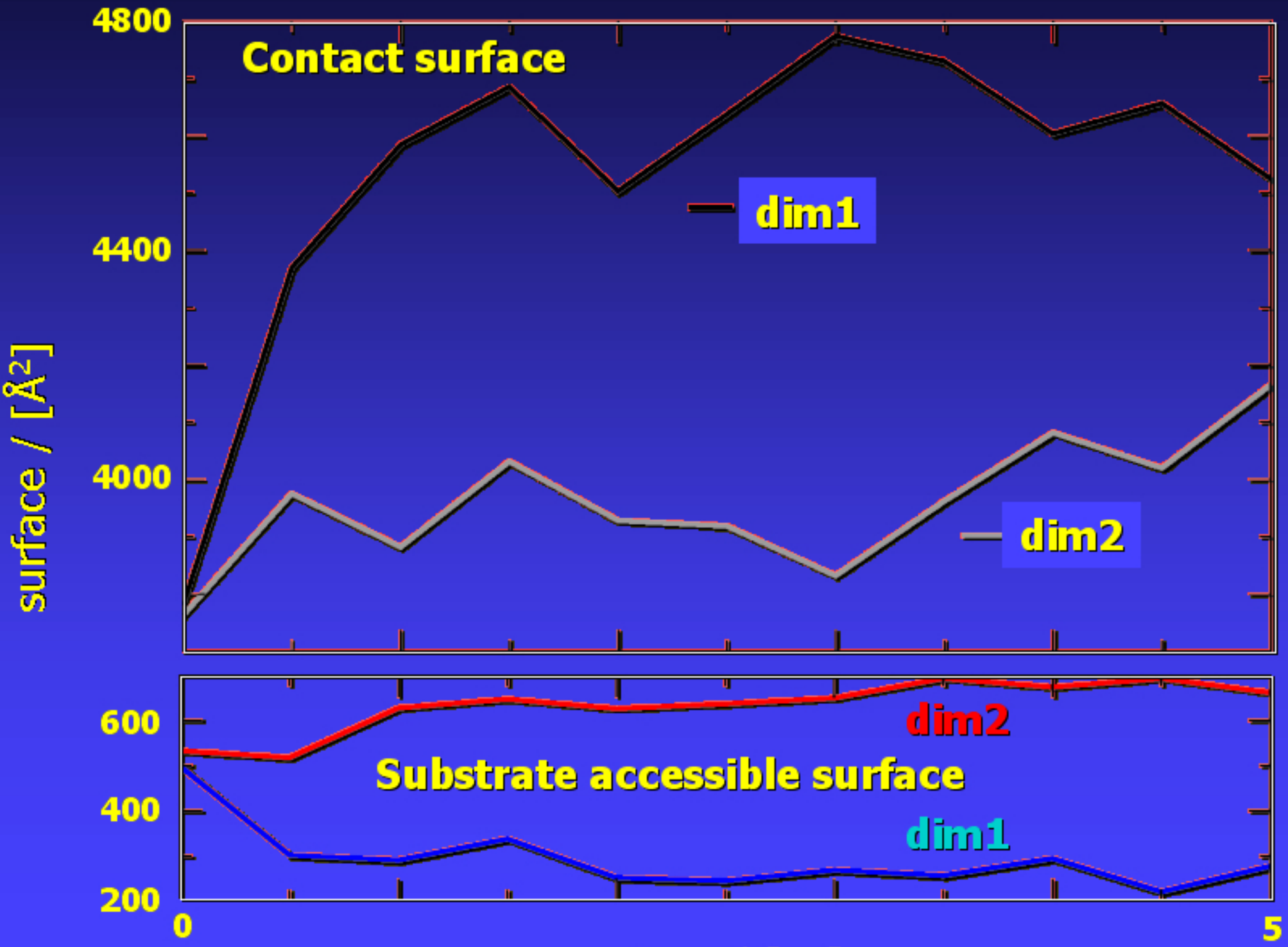
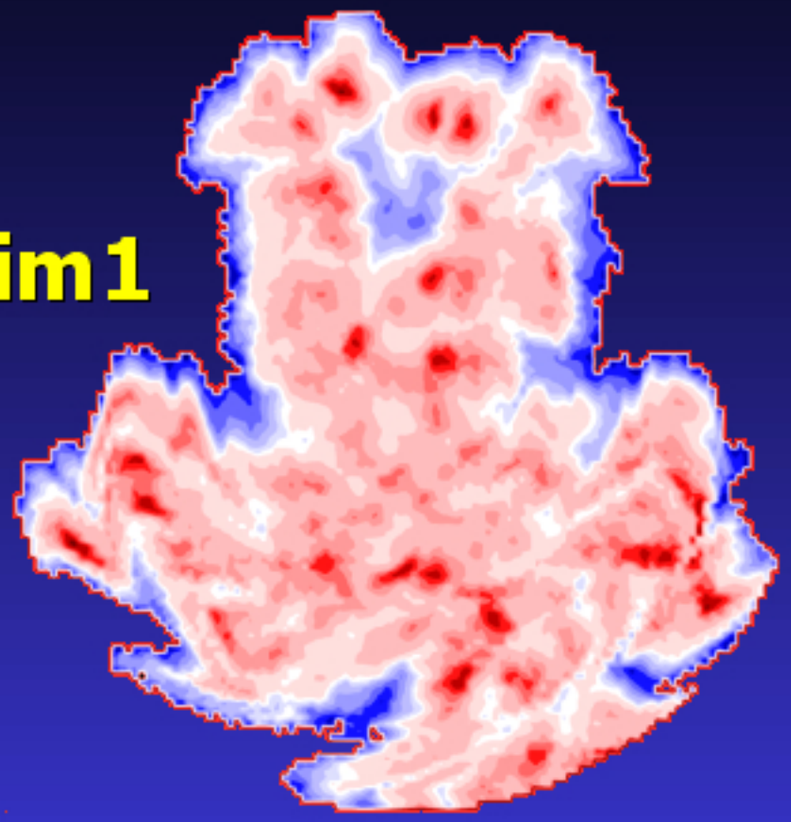
Collapse of the substrate binding pocket

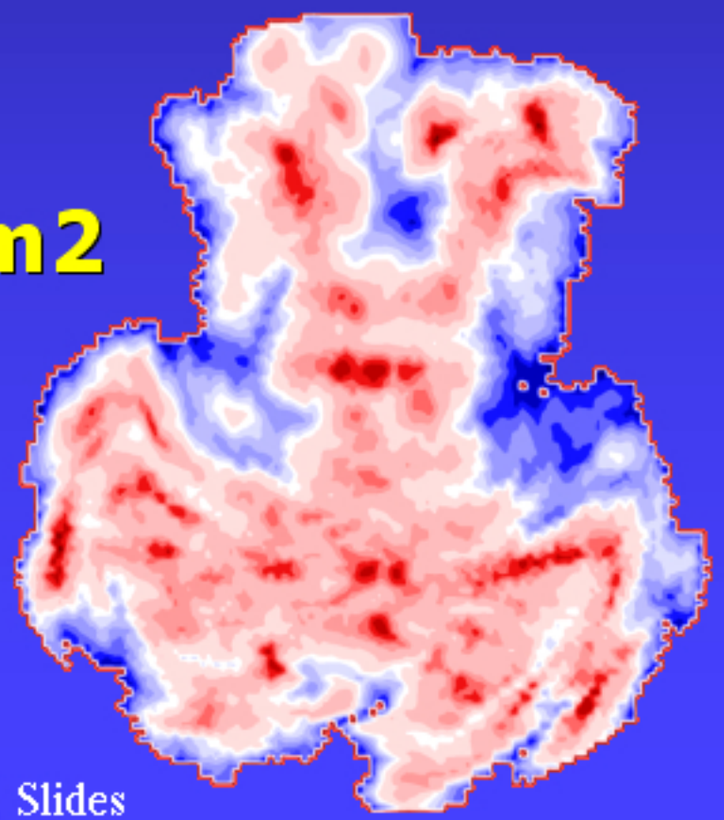


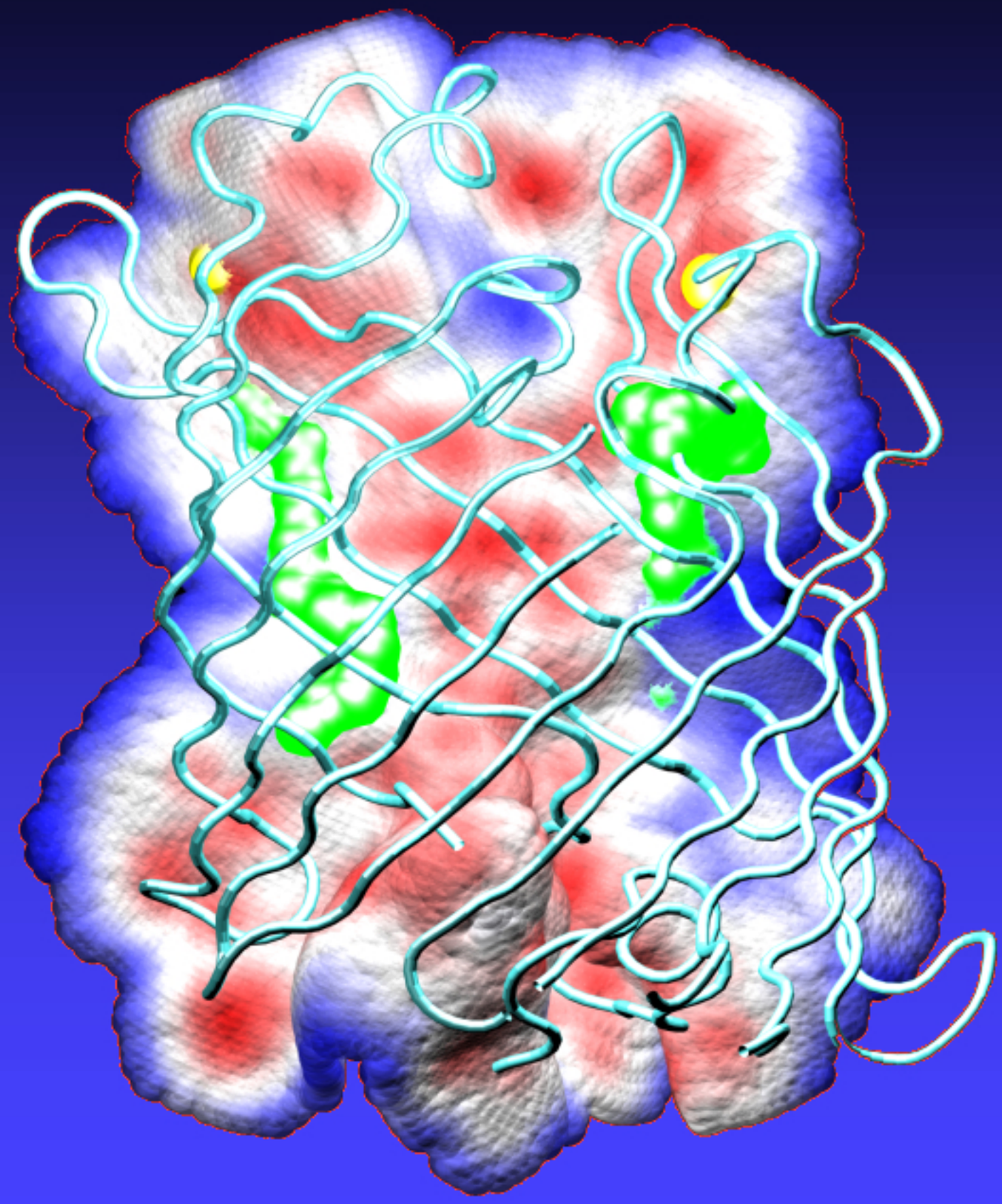
Interactions between monomers

dim1



dim2





d (Å)

10

6

2

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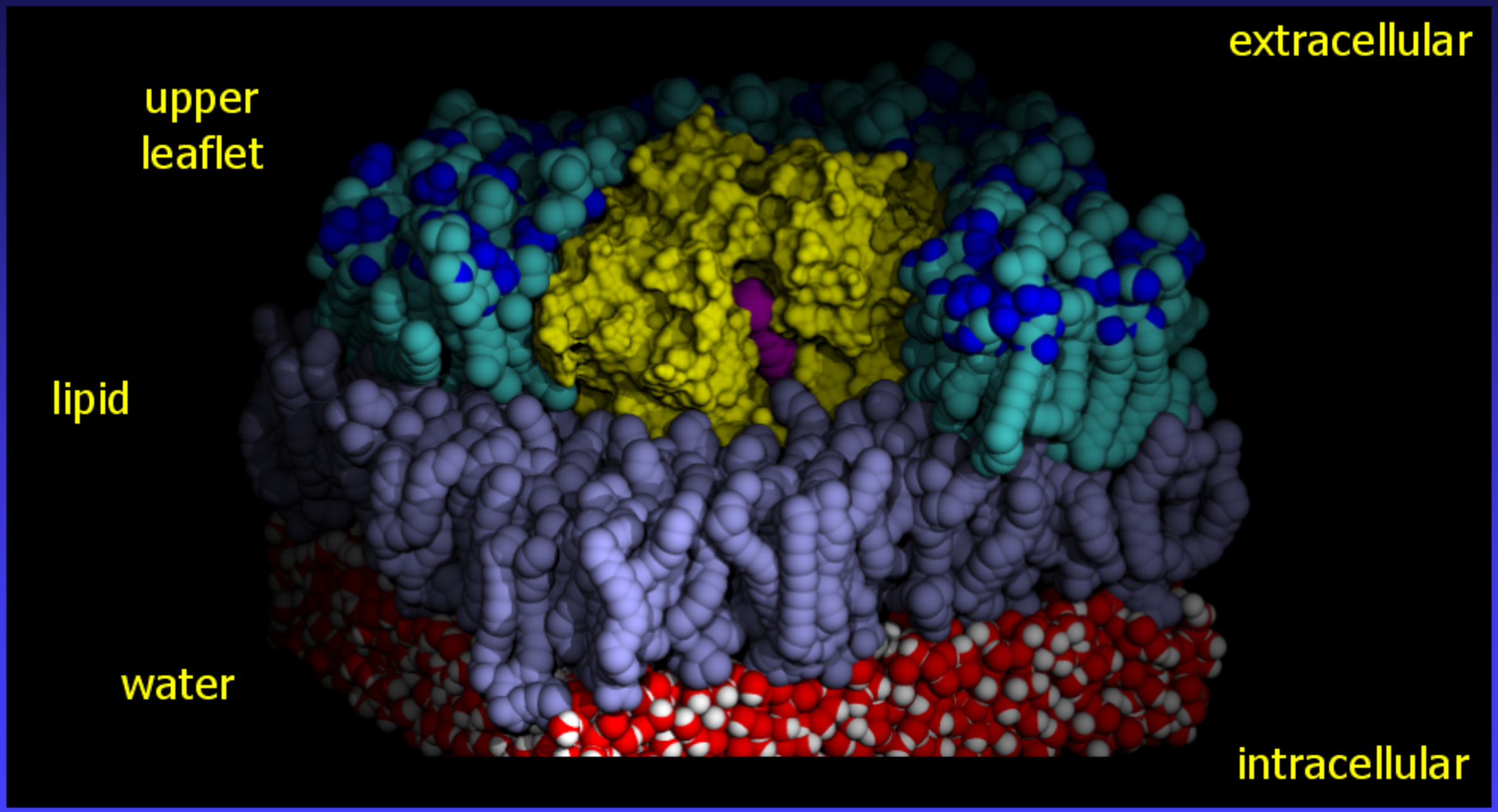
Lipid binding site



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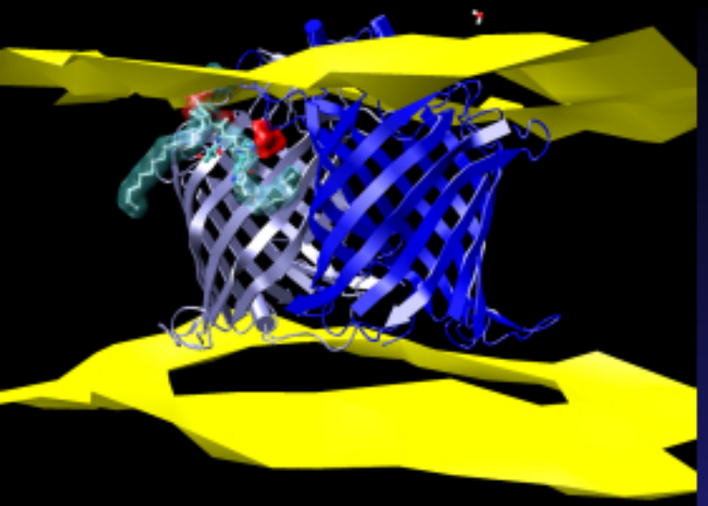
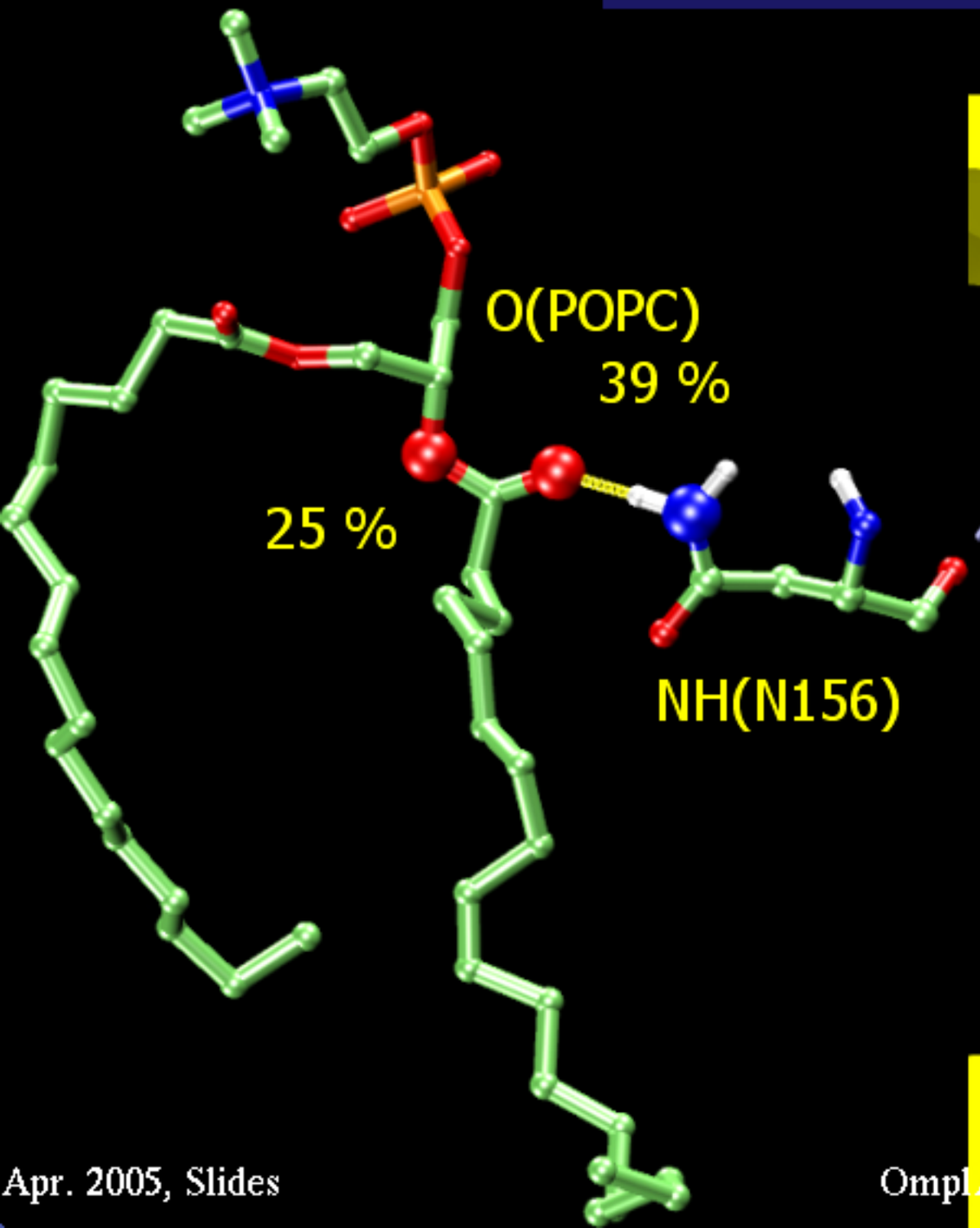
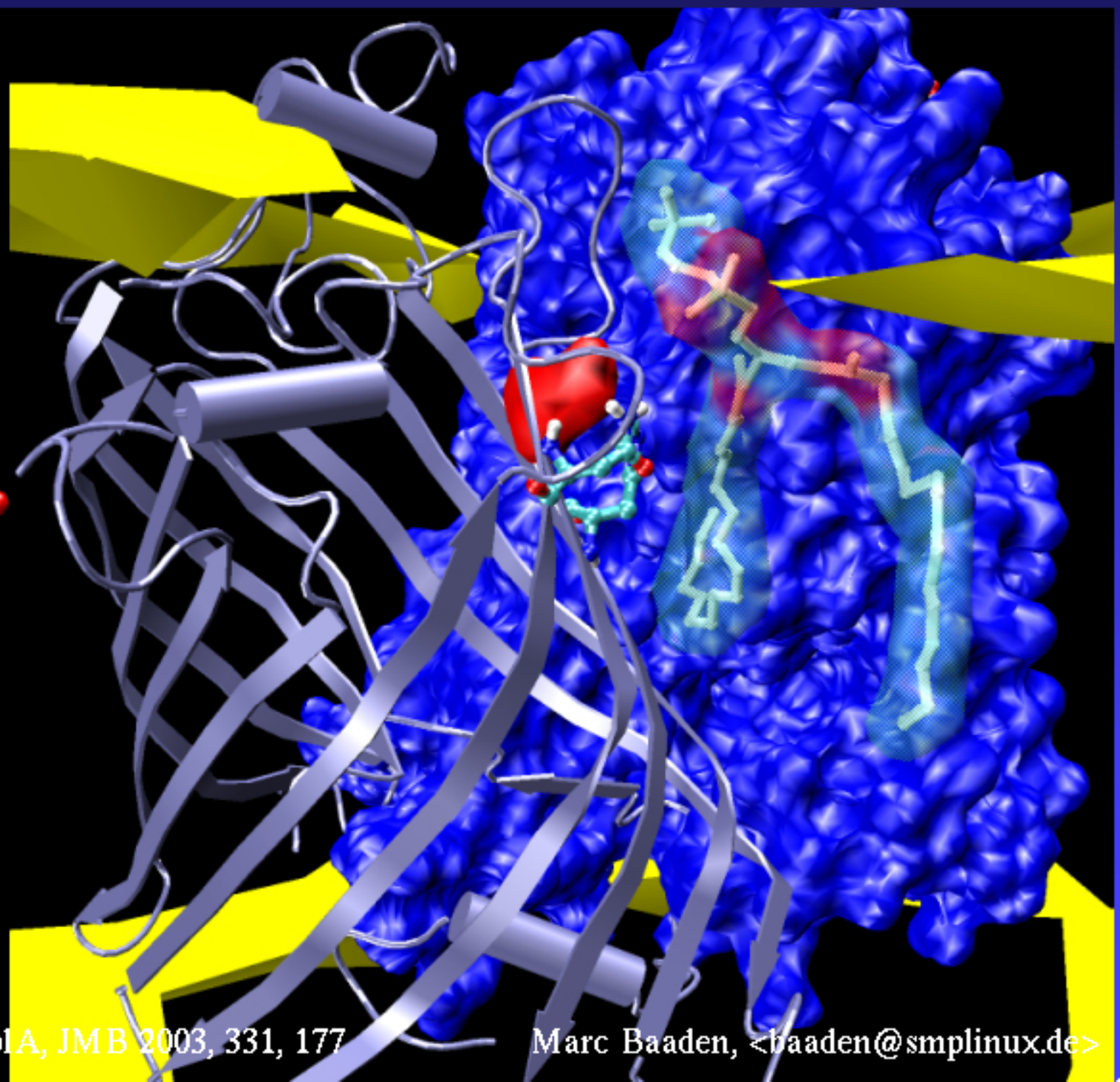
Lipid-protein interaction







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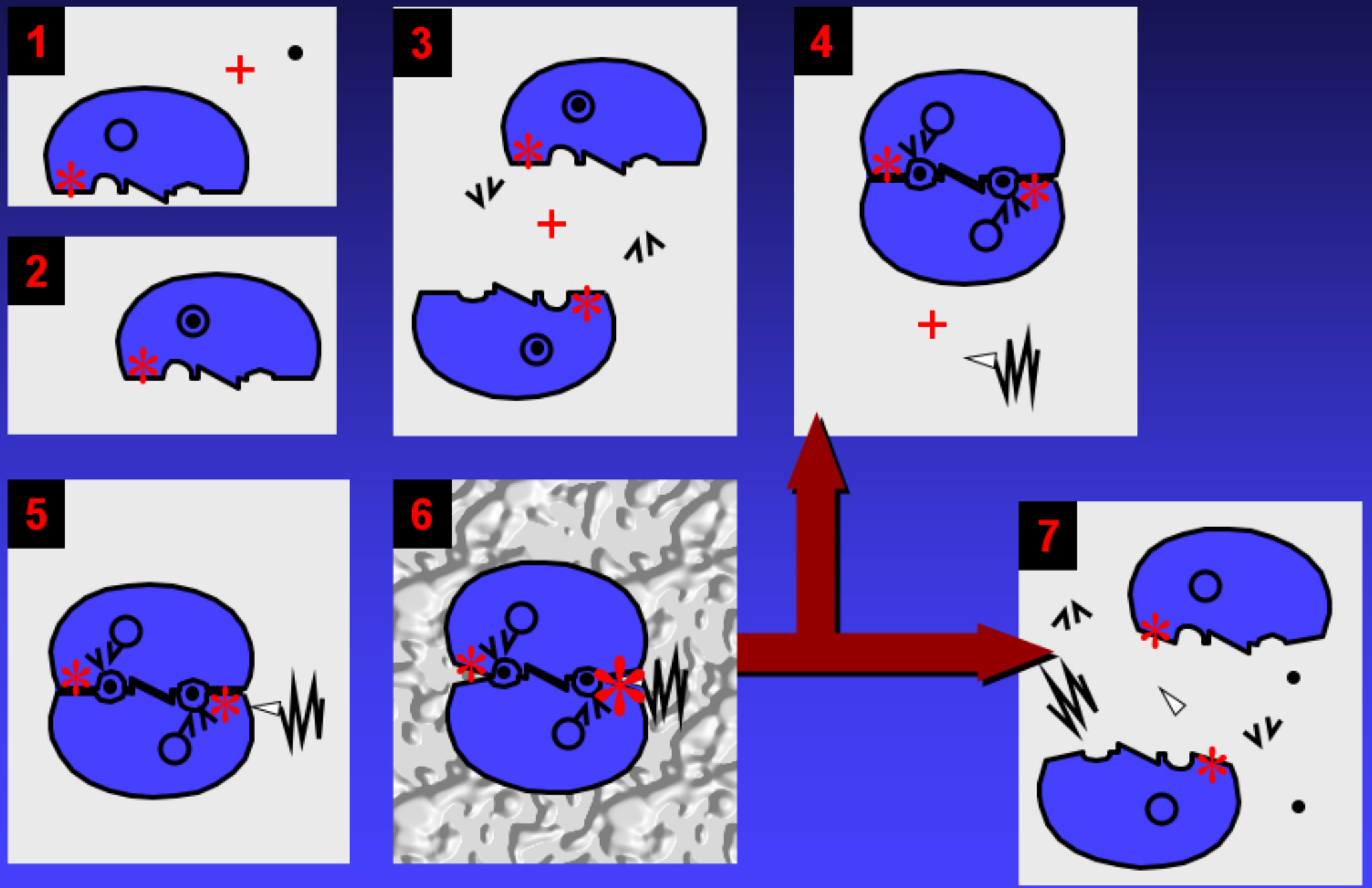
Hypothetical enzymatic cycle



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Which link to BioNanoTech ?

Ompla as potential security valve for membrane based devices …

.. in order to maintain the membrane integrity

Many biological nano-devices are based on membranes (e.g. liposomes and nanosomes). To maintain membrane integrity under various conditions one might add specific proteins, like anti-freeze (glyco)proteins. In this context the Ompla enzyme could help to develop a security valve with respect to mechanical stress.

The Ompla enzyme functions as a kind of security valve in the bacterial outer membrane. It's enzymatic cycle is activated by a mechanical deformation of the membrane, which triggers lysis of phospholipids. The mechanical trigger may be caused by an imbalance in membrane composition or by physical stress. Lysis and hence removal of phospholipids from the membrane helps to restore it's integrity.

If the activity of Ompla could be controlled and fine-tuned, one might be able to develop self-regulating devices, with a capacity of dealing with a certain amount of environmental stress and membrane distorsion.

A first step is to fully uncover the enzymatic mechanism of Ompla, which is related to the presence of calcium ions, a specific hydration shell, dimerization and membrane distorsion.

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Molecular Dynamics Simulations of Outer Membrane Phospholipase A

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INTRODUCTION

OmpLa a phospholipase located in the bacterial outer membrane of *E. coli*, degrades a wide variety of phospholipids in cells with a perturbed envelope. It is a **12-stranded β-barrel** and exists in monomeric and dimeric forms.

Enzymatic activity is regulated by reversible dimerization in conjunction with Ca^{2+}-binding, leading to 2 active sites at the monomer-monomer interface.

Recently, crystal structures of monomeric and dimeric OmpLa with and without Ca^{2+} were solved (Snijder et al., 1999 and 2001) revealing open and inactive conformations.

An improved understanding of the **structure-function relationship** in terms of dimerization, the role of Ca^{2+} and structural impact of the cell envelope can be gained from simulation approaches.

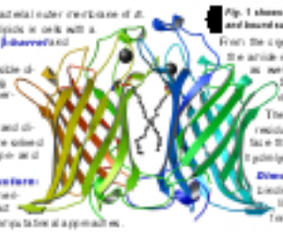


Fig. 1 shows the OmpLa dimer with its two calcium binding sites and bound substrate analogues.

From the crystal structures it appears that **Ca^{2+}-binding** occurs via the amide oxygens of S106, R167 and the 3 oxygen of S107, as well as 2 water molecules with an octahedral geometry. S106 and S107 belong to one monomer and R167 to the other.

The **catalytic active site** consists of two highly conserved residues H73 and S144 and one less conserved N106. They face the outer leaflet of the lipid bilayer. OmpLa is believed to hydrolyze its substrate through a nucleophilic attack by S144.

Dimerization results in formation of two extended substrate binding clefts and is partially substrate dependent. There is little structural difference between monomeric and dimeric forms (RMSD 0.82Å).

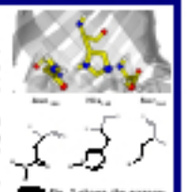
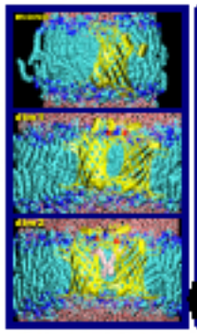


Fig. 2 shows the arrangement of active site residues in OMPLA, which resembles that of serine proteases.

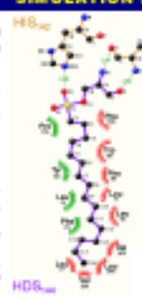


SIMULATION SETUP

Starting structures for the protein in its monomeric and dimeric forms were taken from the **1qd5** and **1qd6** entries of the PDB respectively and missing parts repaired. Residues 1-12 were replaced by an acetyl group.

Protonation states were assigned first by Delphi-difference solutions to the Poisson-Boltzmann equation at pH 7 taking into account the influence of the environment by comparing the bare protein (i.e. water-like environment) and the protein with a low dielectric medium. **Active site** protonation states were assigned manually.

Three systems were considered for molecular dynamics simulations in a membrane-like environment: the Ca²⁺-free monomer, the Ca^{2+}-bound dimer and the dimer with inhibitor bound to S144.



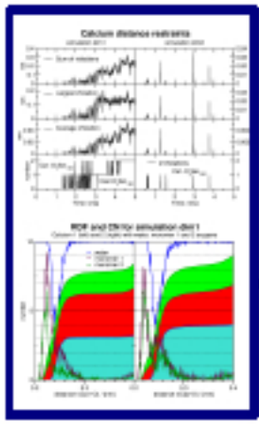
Calcium interactions were implemented using a modified force field for Ca^{2+} ions and the surrounding residues S102, R167 and S106. Partial atomic charges and Lennard-Jones parameters were adapted from Straatsma & Nakagawa, 1991. In addition, distance restraints of 2.0Å, 2.4Å and 2.4Å were applied between calcium and the three residues.

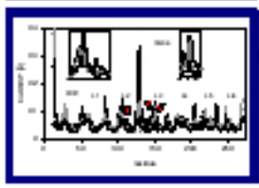
The **hexadecanesulphonyl inhibitor** was modelled by modifying S144, leading to a hexadecanesulphonyl serine (HDS) residue generated via PRODRG (van Aalten, 1996).

The system was then inserted in a **lipid bilayer** consisting of palmitoyloleoylphosphatidylcholine (POPC) and hydrated with an aqueous phase of about 0.6M NaCl.

Fig. 3 highlights the membrane part of the 3 simulation systems. The protein is shown in yellow, calcium in red and the inhibitor in pink. The bilayer consists of POPC.

Fig. 4 shows the modified residue 144 (HDS) and a two-dimensional representation of its interactions with residues forming the surface exposed binding cleft.





MOLECULAR DYNAMICS IN A LIPID BILAYER

Molecular dynamics simulations in full atomic detail have been initiated in order to study the conformational dynamics of mono- and dimeric OmpLa in a membrane-like environment.

- Timescale ~6 ns per system
- System size ~ 81 x 81 x 80 Å³
- Membrane consists of ~ 128 POPC
- Total of ~ 14118 Å

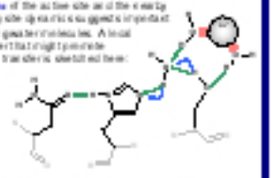
One point of interest and concern is the behavior of calcium in these simulations. As can be seen on the left, the distance restraints that were applied were necessary for system dim1, but not for dim2. The radial distribution functions of Fig. 5 show that calcium is coordinated by 2 to 4 water molecules and 2 to 3 residues. This multi character, leading to **average coordination number** (CN) of 6.

Fig. 5. Top: Violation of imposed distance restraints in the dimer simulations. Bottom: radial distribution functions of calcium in simulation dim1 separated in contributions from monomer1, monomer2 and water.

The comparison of the **root mean square fluctuations** (RMSF) in the mono, dim1 and dim2 systems shows that dimerization leads to reduced fluctuations, in particular near the residues involved in calcium binding. This agrees well with the overall root mean square deviation values which are lower for the dimer simulations. As a general trend beta barrel regions with a RMSD in range from 1.0 to 1.2 Å and some 0.8-1.1 Å lower than the mono reference simulations.

Fig. 6. Root mean square fluctuations for simulations mono, dim1 and dim2 with inlays focusing on the residues near the calcium-binding site. Coordinating residues D106, R167 and D102 are indicated by red arrows.

First analysis of the active site and the nearby calcium binding site dynamics suggests important roles for bridging water molecules. A local order of water that might provide catalytic proton transfer is identified here:



Another important feature seems to be the collapse of the **substrate-binding cleft** in the dim1 simulation as compared to dim2 (with inhibitor bound). This can eg be seen by an increase of the monomer-monomer contact area or the change of accessible surface of the substrate-binding cleft.

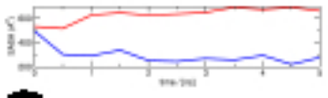


Fig. 7. Solvent accessible surface area of one substrate binding cleft for simulations dim1 and dim2 as a function of time (ignoring the bound inhibitor for simulation dim2).

REFERENCES & ACKNOWLEDGEMENTS

1. Snijder, et al., 1999, Nature 401,717.
2. Snijder et al, 2001, JMB 309,3443.
3. Straatsma & Nakagawa, 1991, J. Comp. Chem. 12,71.
4. Van Aalten et al., 1996, J. Comp. Aid. Mol. Des. 10,255.

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Future directions

Further analysis

Lipid-protein interactions

Membrane

Water

Methodology

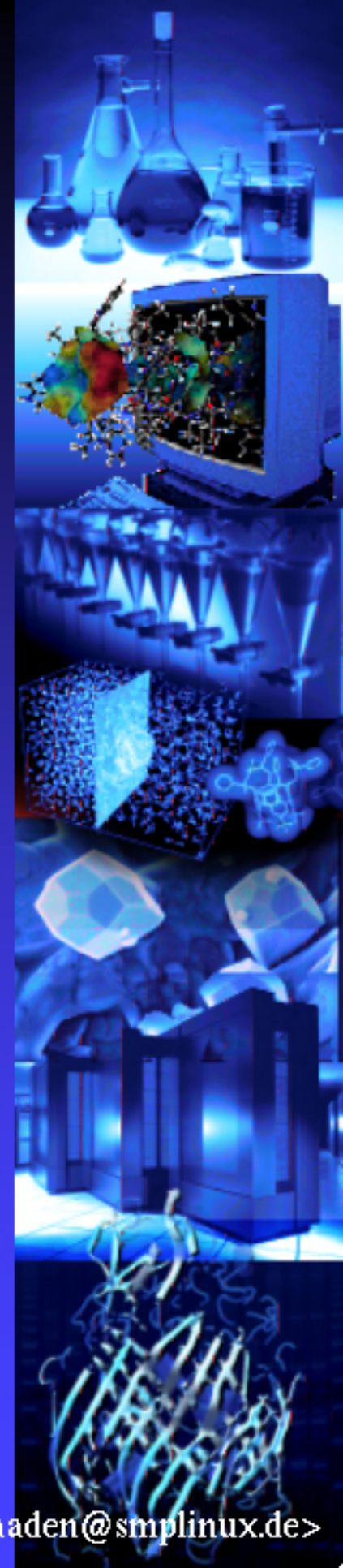
Extended simulations

PME *vs* Cutoff

micellar system

Enzymatic reaction

Mixed approaches (Car-Parrinello, QM/MM)



Additional documents

Movies and animations

http://www.baaden.ibpc.fr/pub/ompla/t_hdshow.avi

http://www.baaden.ibpc.fr/pub/ompla/t_omplarot.avi

http://www.baaden.ibpc.fr/pub/ompla/ompla_substrate_pocket.mov

Poster

http://glabaune.free.fr/omplpost.pdf

Further information at

http://www.baaden.ibpc.fr