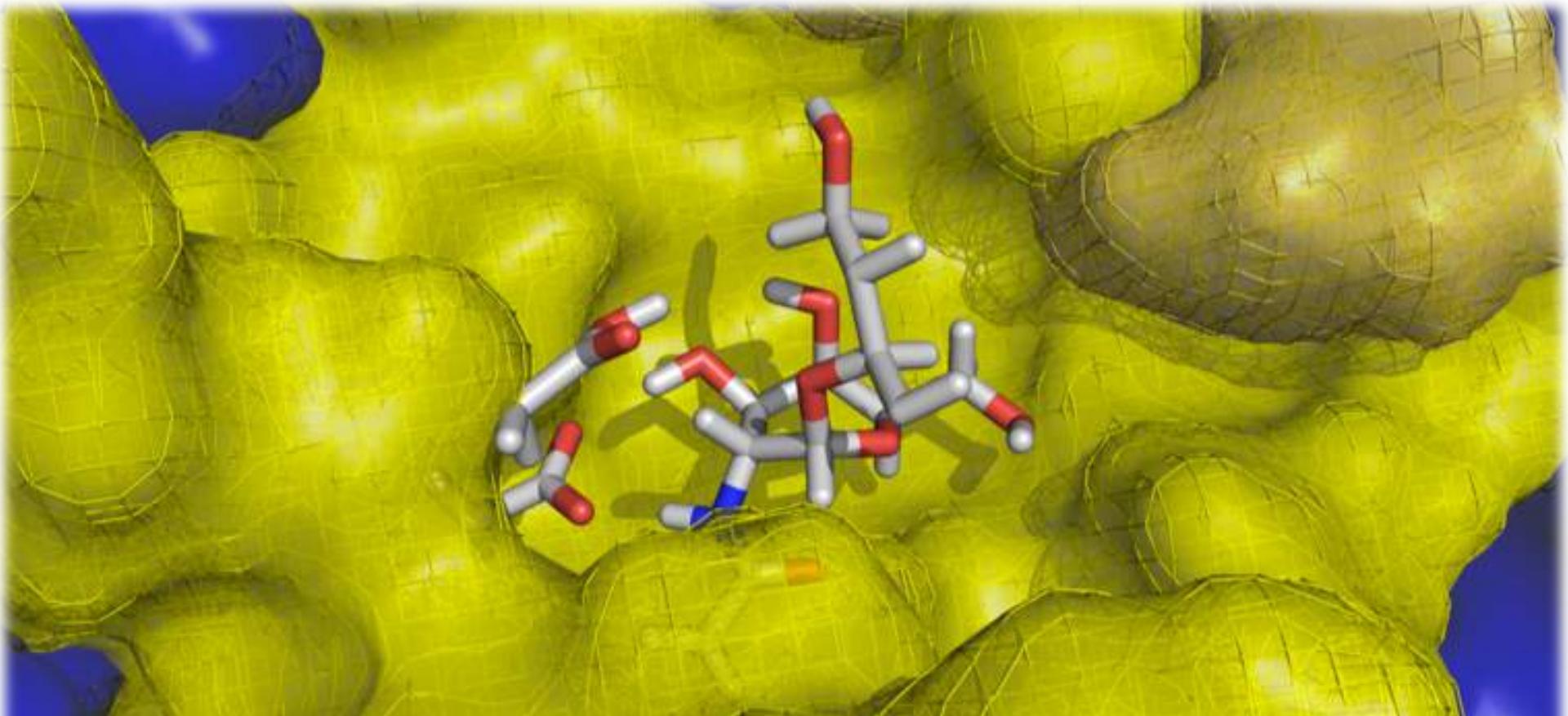


Protein:Protein Association Analyzed by Alanine Scanning Mutagenesis

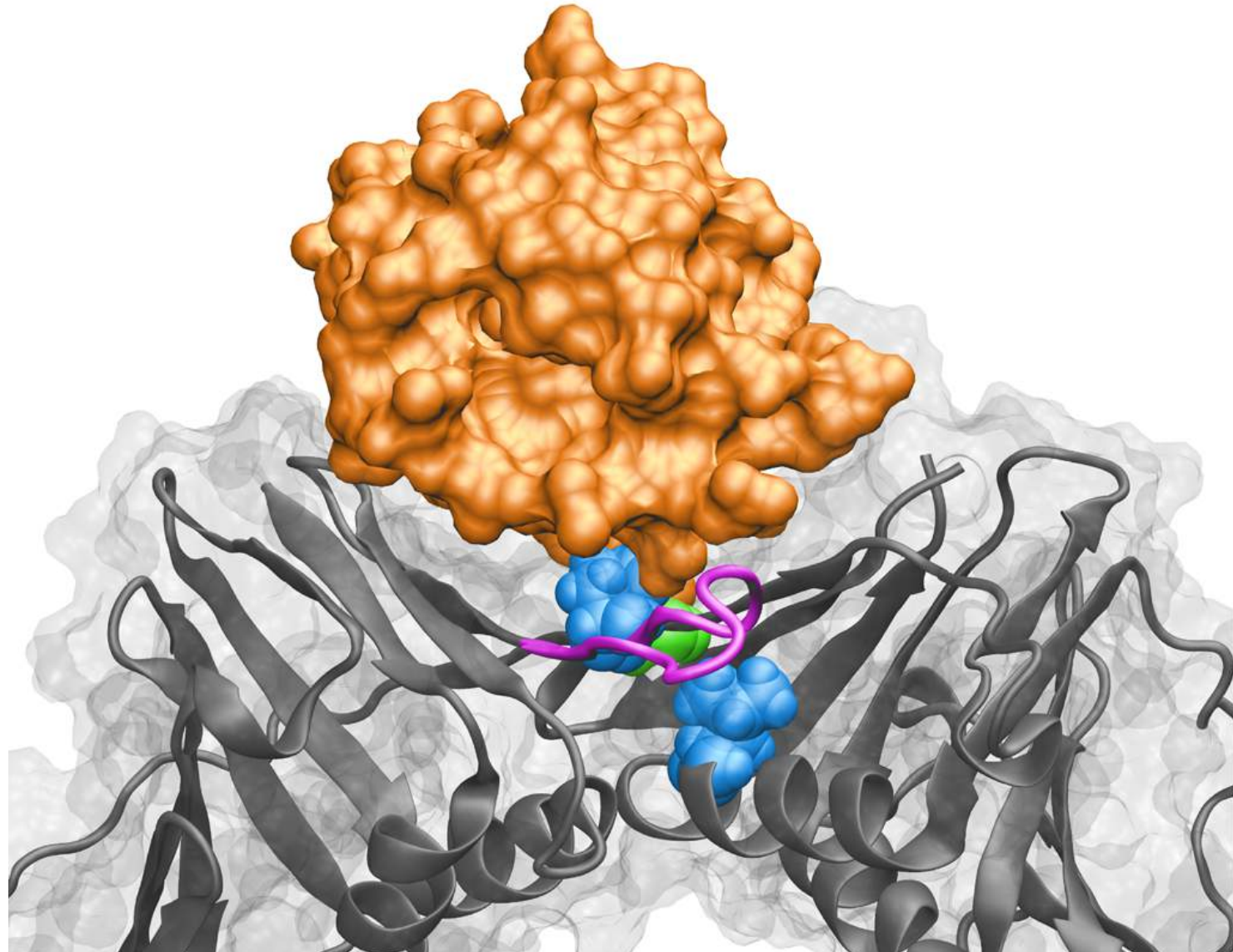
Pedro Alexandrino Fernandes,

Dep. Chemistry & Biochemistry, University of Porto, Portugal

pedro.fernandes@fc.up.pt



Protein:Protein Interfaces



Protein:Protein Interfaces



Mostly non-covalent

Protein:Protein Interfaces



Mostly non-covalent

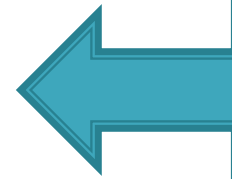
Ubiquitous in nature

Protein:Protein Interfaces



Mostly non-covalent

Ubiquitous in nature



- Quaternary Structure
- Signal transduction
- Intercell Communication
- Post-translational modifications

Protein:Protein Interfaces



Mostly non-covalent

Ubiquitous in nature

Very peculiar structure

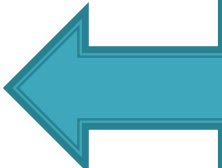
Protein:Protein Interfaces



Mostly non-covalent

Ubiquitous in nature

Very peculiar structure

- 
- Large (mostly 1000 – 2000 Å²)
 - Flat
 - Hydrophobic, with hydrophilic spots

Protein:Protein Interfaces



Mostly non-covalent

Ubiquitous in nature

Very peculiar structure

Very peculiar energetics

Protein:Protein Interfaces

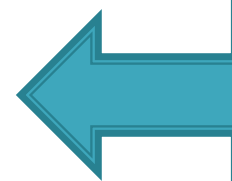


Mostly non-covalent

Ubiquitous in nature

Very peculiar structure

Very peculiar energetics



- Most residues do not contribute for binding
- A few residues contribute the most
- These are spatially complementary

Protein:Protein Interfaces




Mostly non-covalent

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Why we Study the Energetics?



To interfere with protein:protein association

Why we Study the Energetics?



To interfere with protein:protein association



To regulate cellular events

Why we Study the Energetics?



To interfere with protein:protein association



To regulate cellular events



To produce therapeutic effects

Why we Study the Energetics?



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Agonists/Antagonists



Interface Active sites

What is ASM?



ASM measures the free energy contribution of **each sidechain** for protein binding by the following procedure:

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1. Measure ΔG_{bind} for the wild type complex

What is ASM?

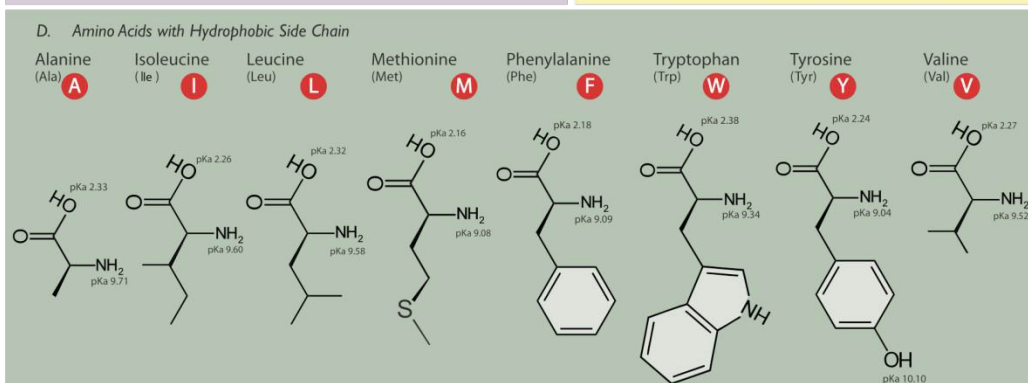
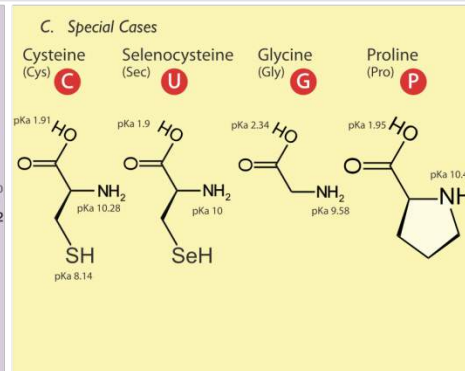
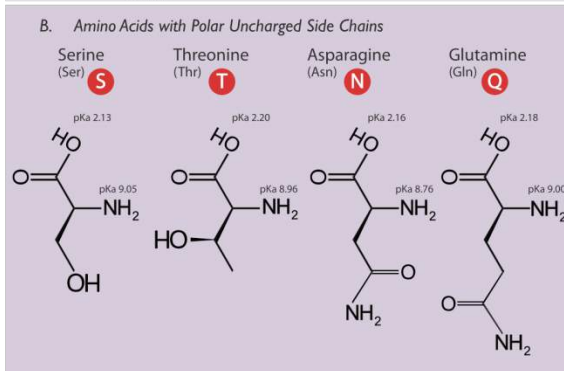
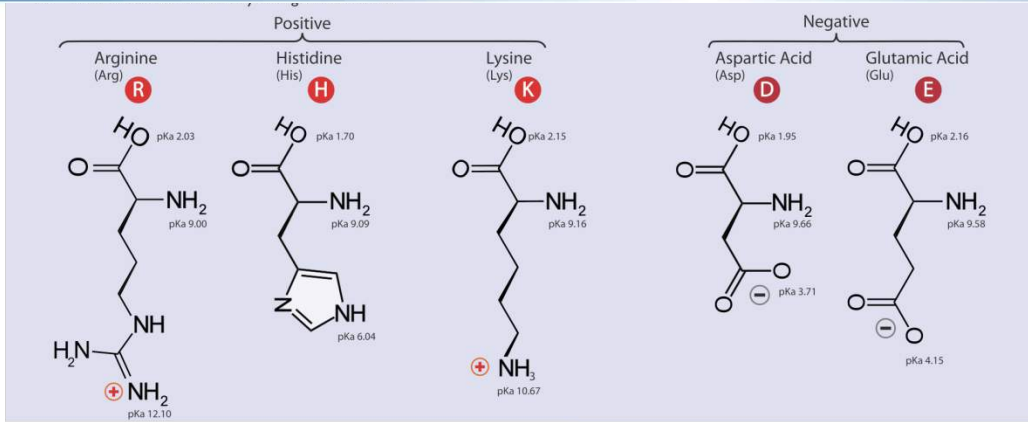


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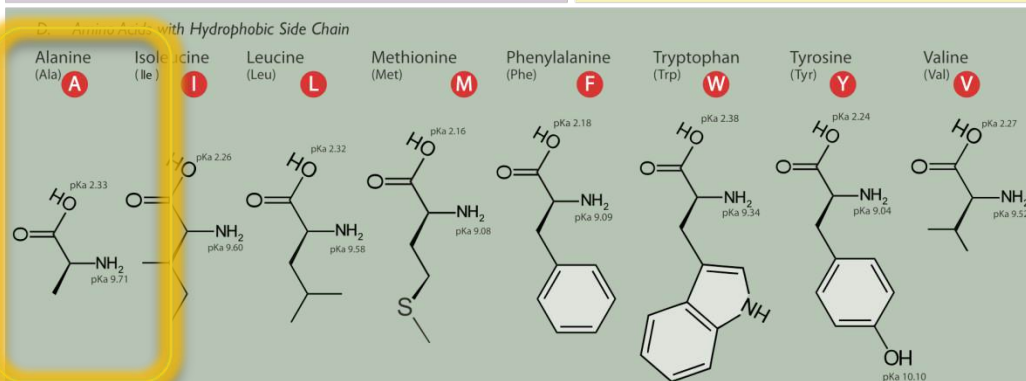
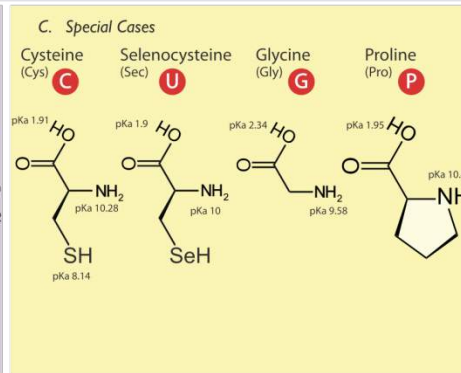
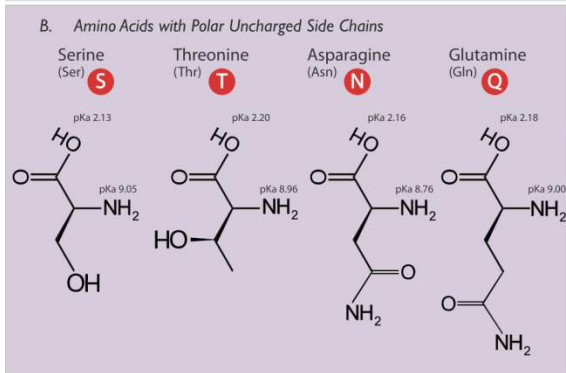
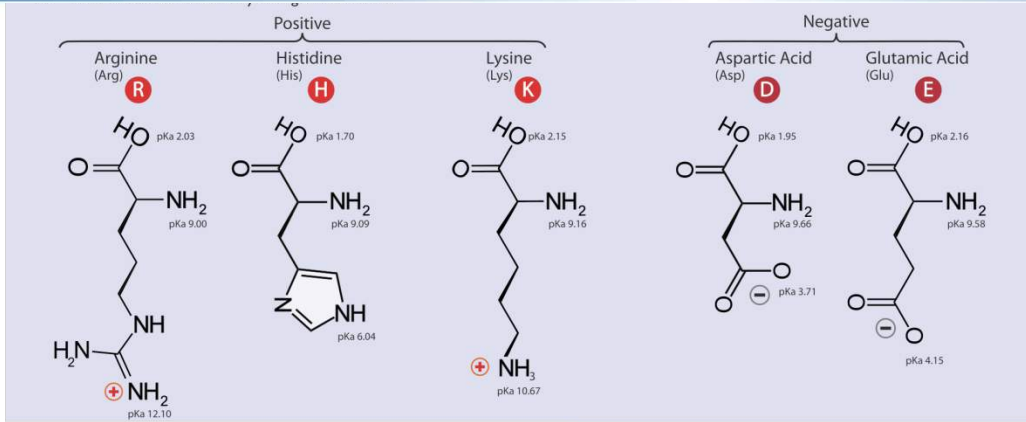
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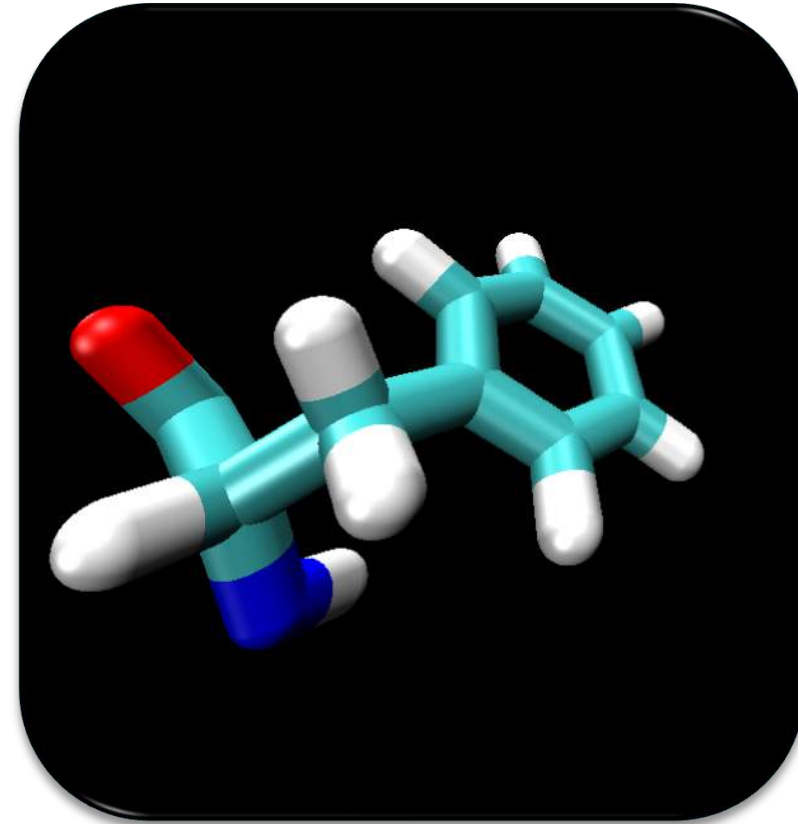
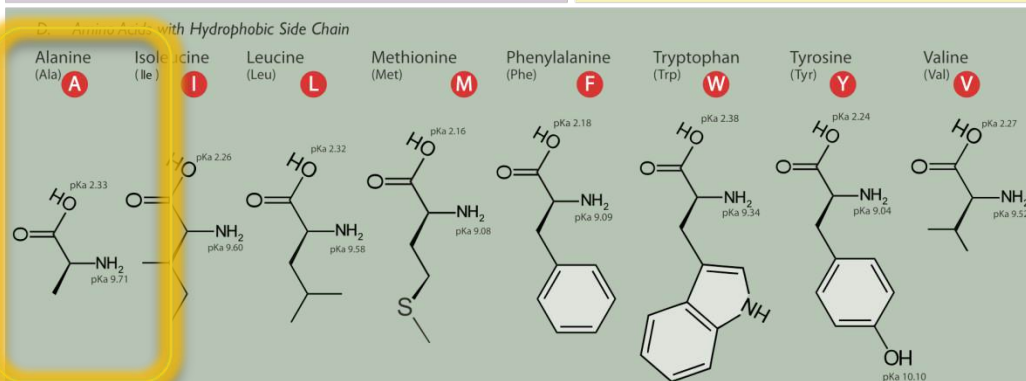
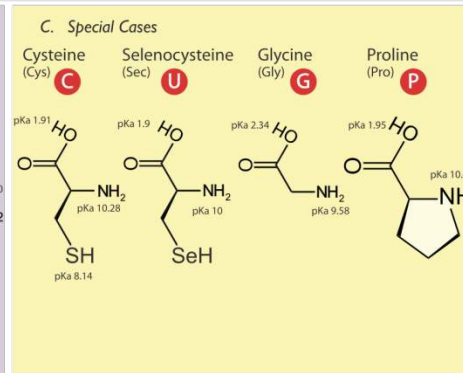
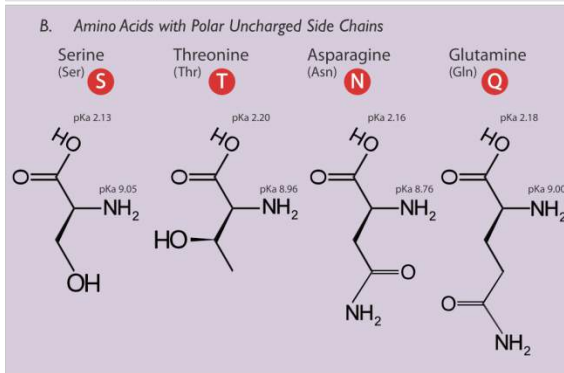
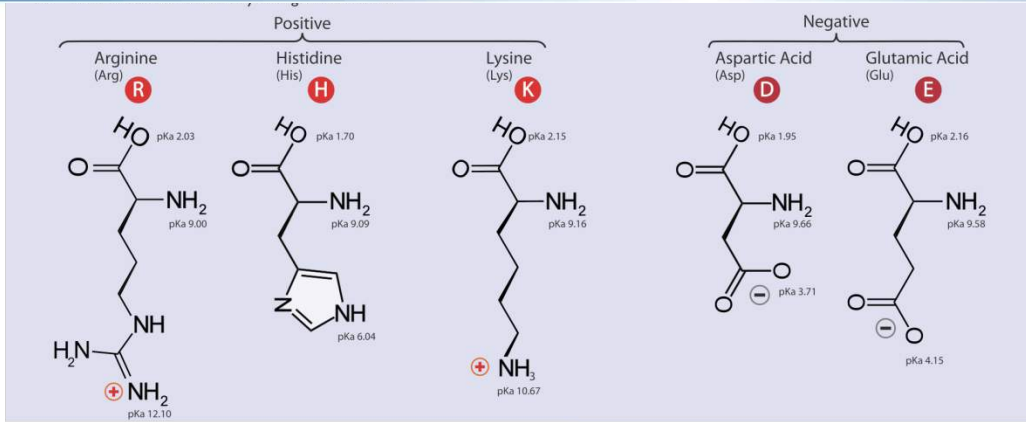
What is ASM?



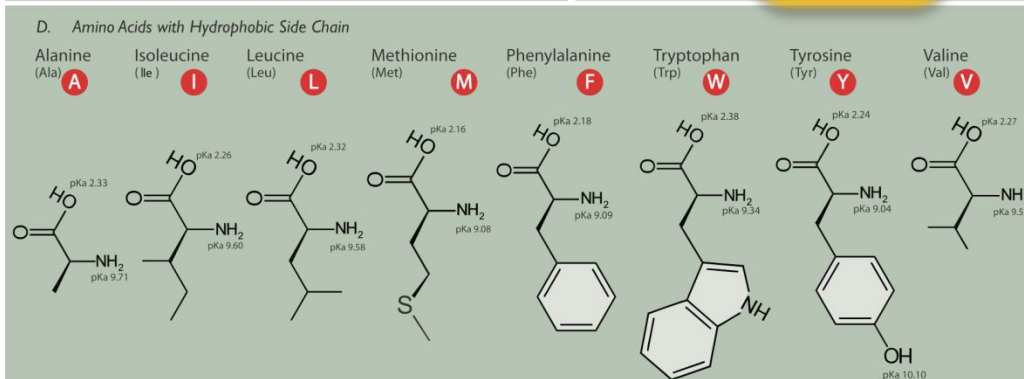
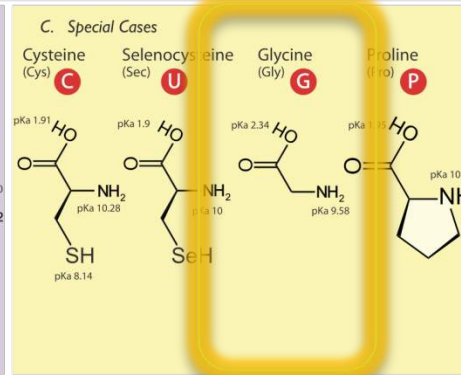
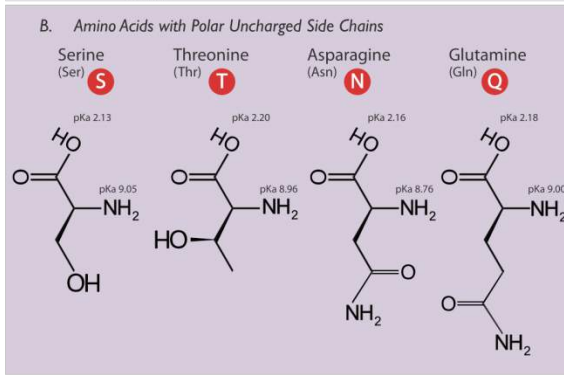
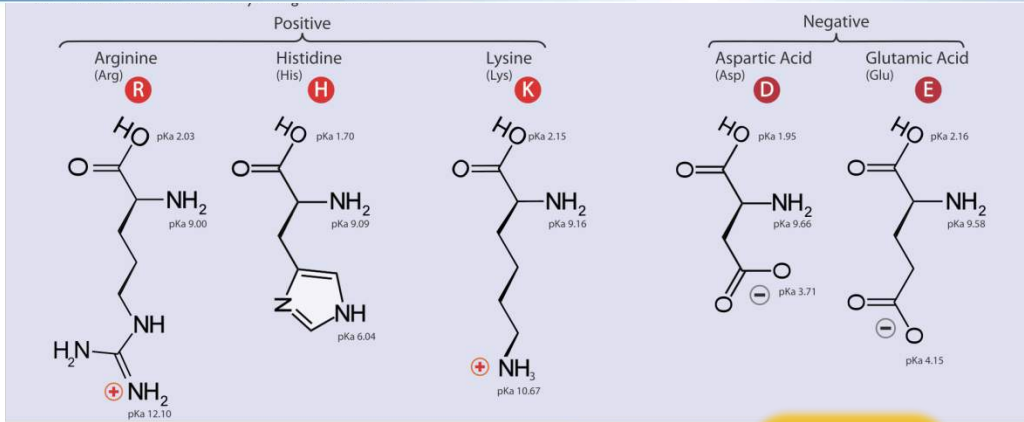
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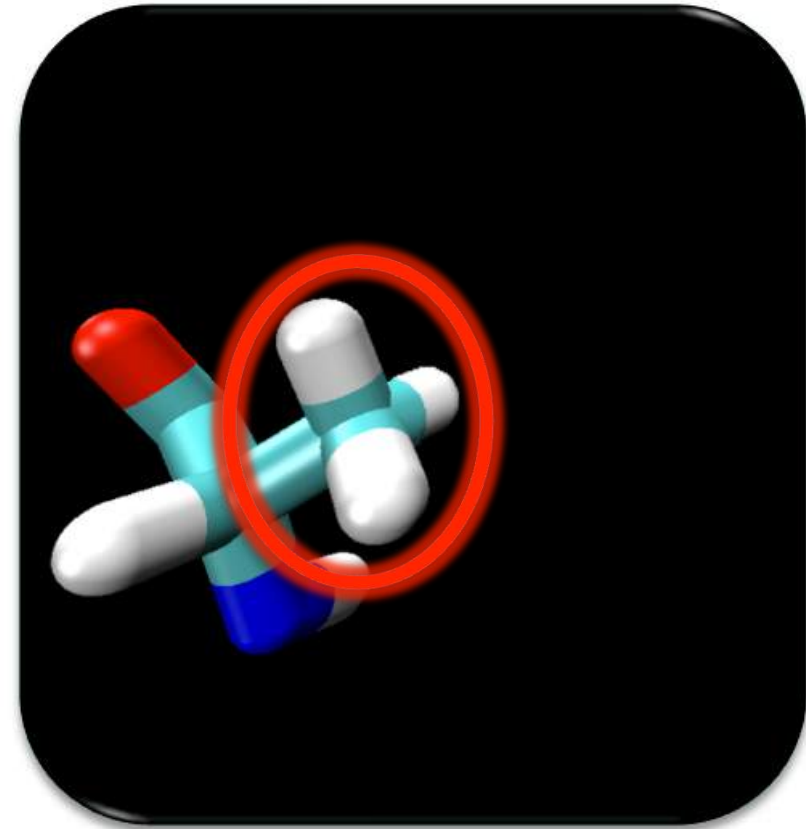
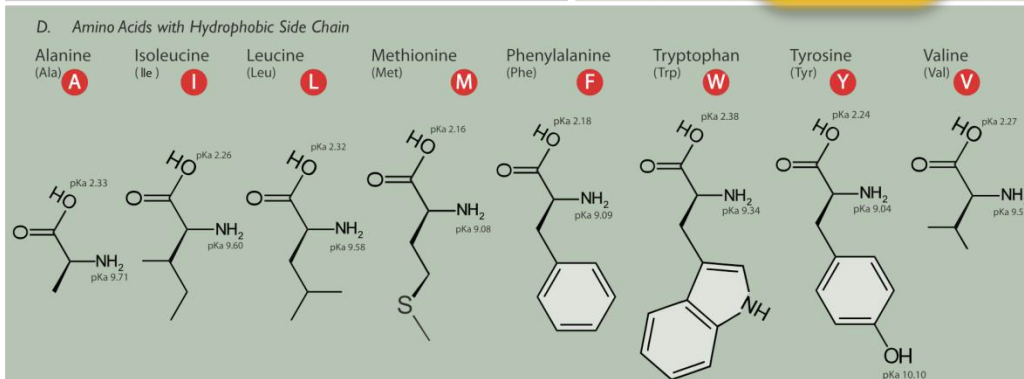
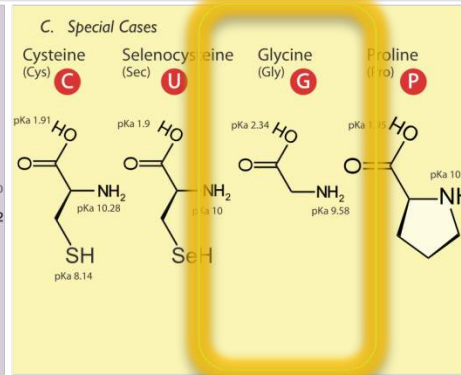
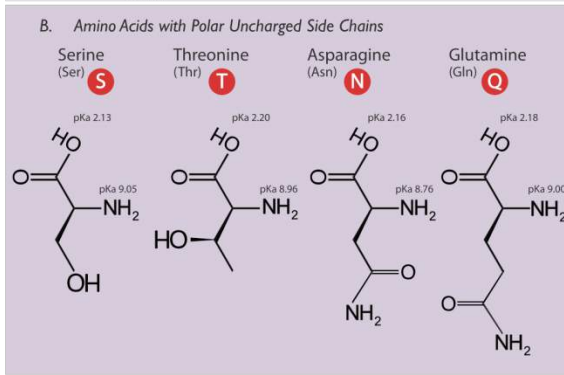
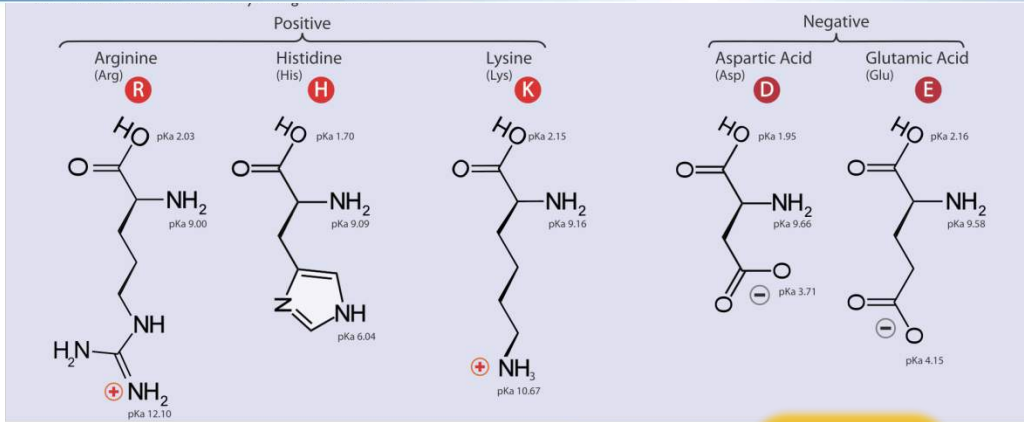
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4. Calculate $\Delta\Delta G_{\text{bind}} = \Delta G'_{\text{bind}} - \Delta G_{\text{bind}}$

How to measure ΔG_{bind} ?



How to measure ΔG_{bind} ?



MM-GBSA

How to measure ΔG_{bind} ?



MM-GBSA

MM-PBSA

How to measure ΔG_{bind} ?



MM-GBSA

MM-PBSA

FEP

How to measure ΔG_{bind} ?



MM-GBSA

MM-PBSA

FEP

TI

How to measure ΔG_{bind} ?



MM-GBSA

MM-PBSA

FEP

TI

Etc...

How to measure ΔG_{bind} ?



MM-GBSA

MM-PBSA

FEP

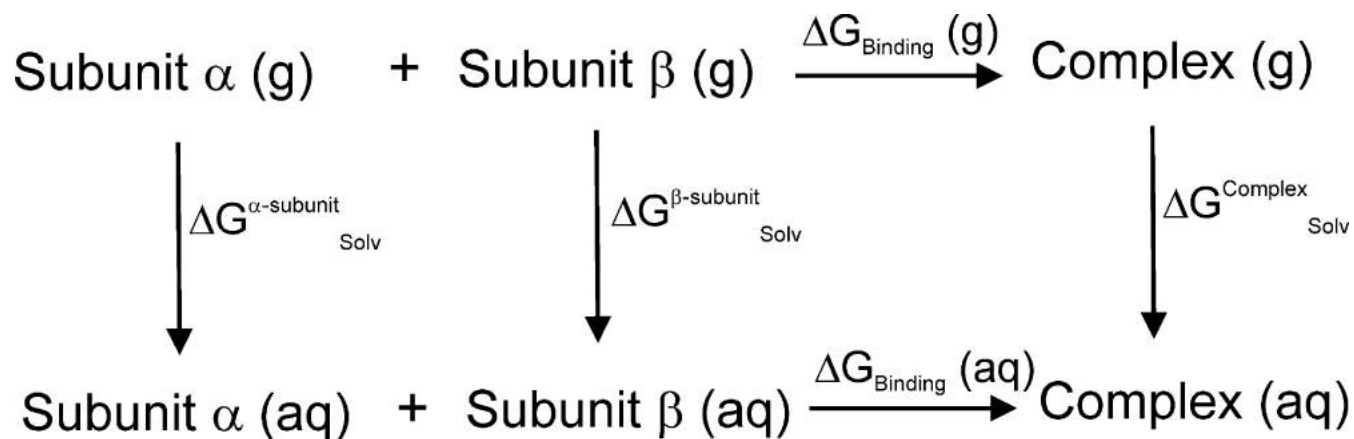
TI

Etc...

How to measure ΔG_{bind} ?



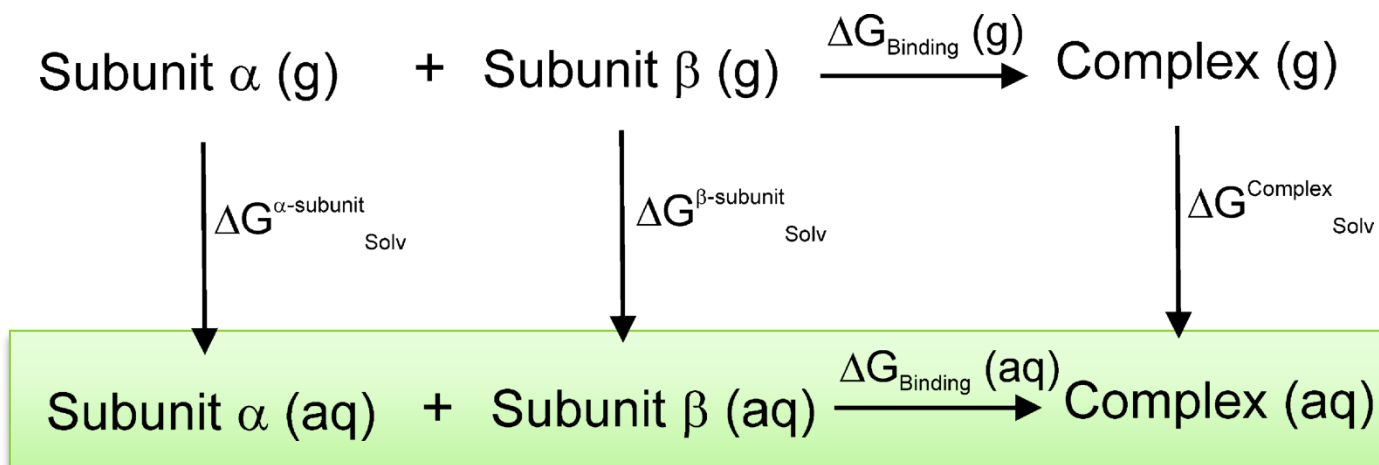
MM-PBSA



How to measure ΔG_{bind} ?



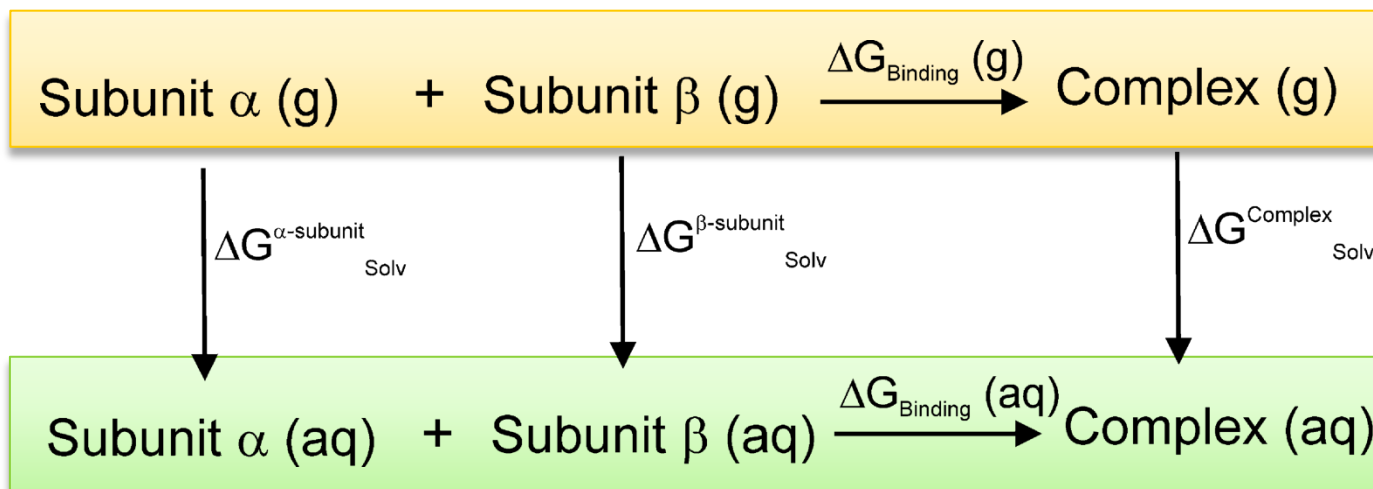
MM-PBSA



How to measure ΔG_{bind} ?



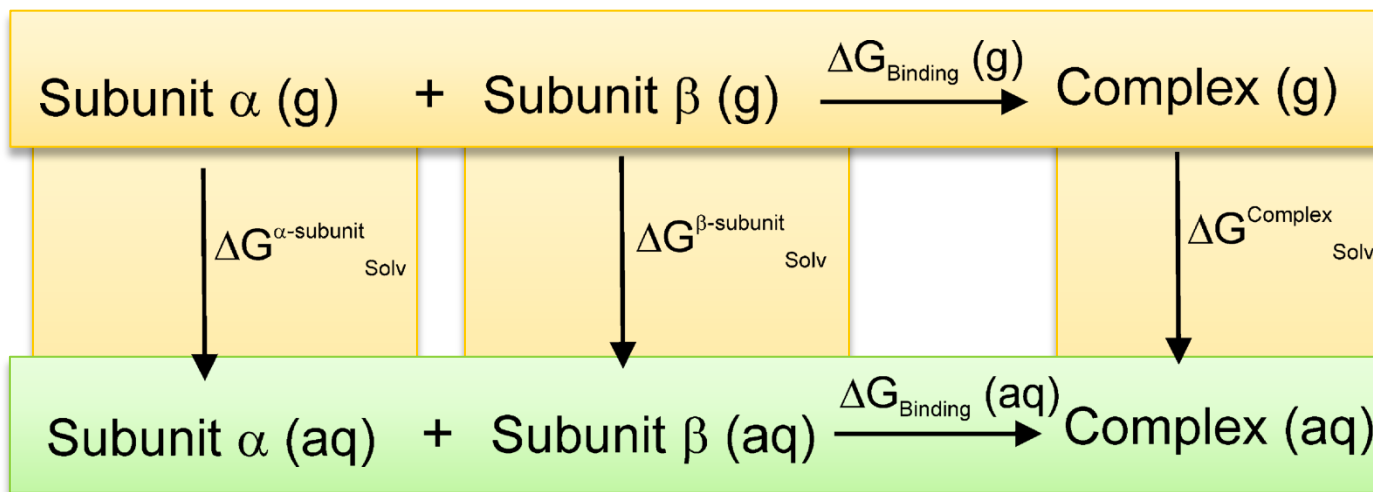
MM-PBSA



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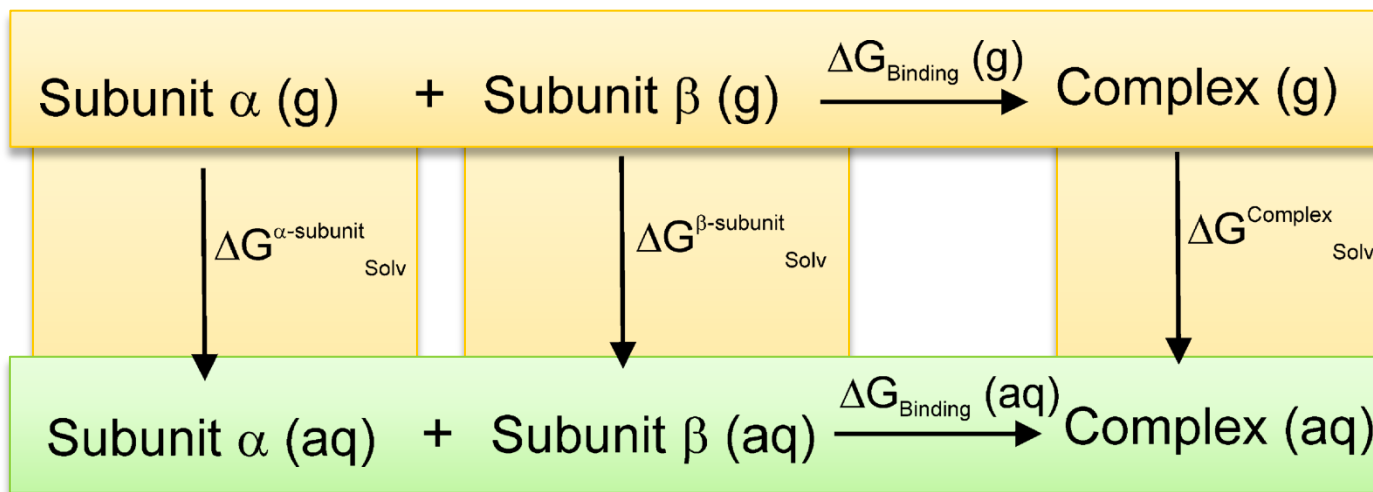
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How to measure ΔG_{bind} ?

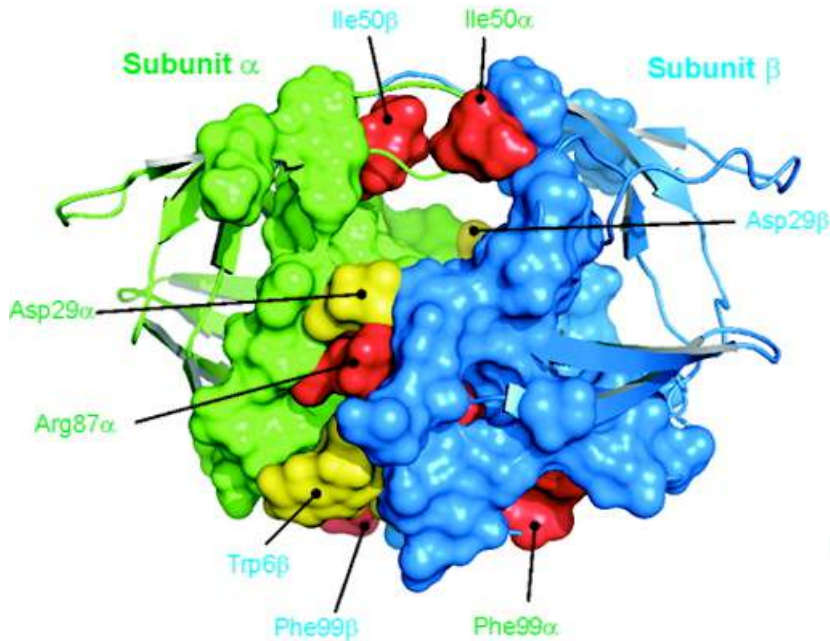


MM-PBSA

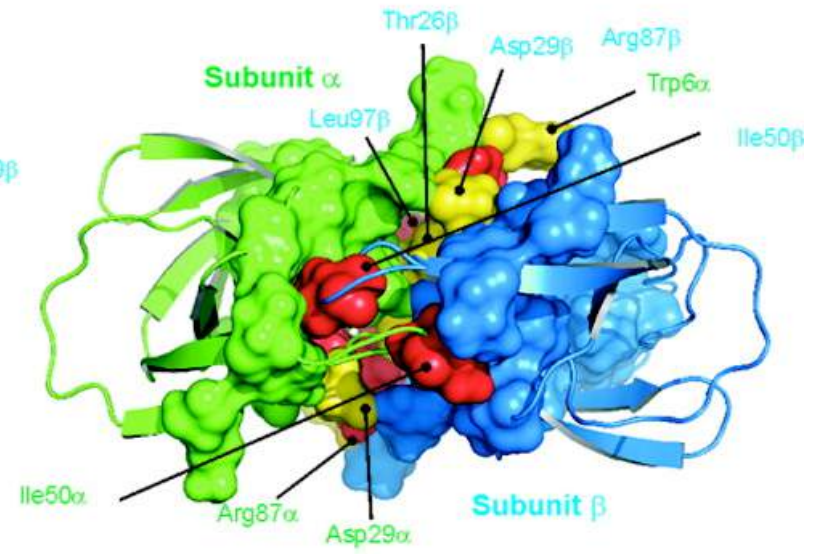


$$\Delta\Delta G_{\text{bind}} = \Delta G'_{\text{bind}} - \Delta G_{\text{bind}}$$

Alanine Scanning Mutagenesis



Hot Spot Warm Spot Subunit α Subunit β



Effect of mutations
for Ala

Hot Spot $\Delta\Delta G_{\text{bind}} > 4$ Kcal/mol

Warm Spot $\Delta\Delta G_{\text{bind}} = 2-4$ Kcal/mol

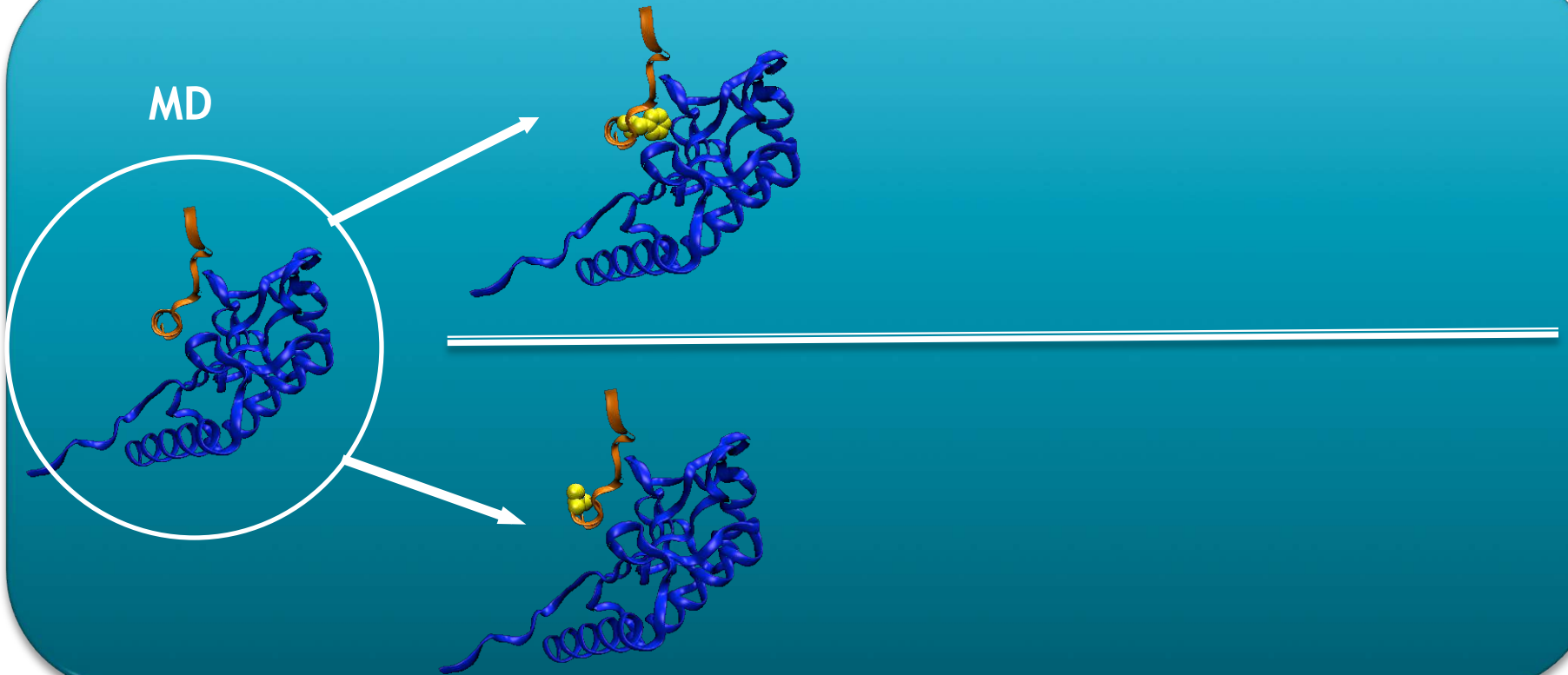
Null Spot $\Delta\Delta G_{\text{bind}} = 0-2$ Kcal/mol

Cold Spot $\Delta\Delta G_{\text{bind}} < 0$ Kcal/mol

Computational Alanine Scanning Mutagenesis

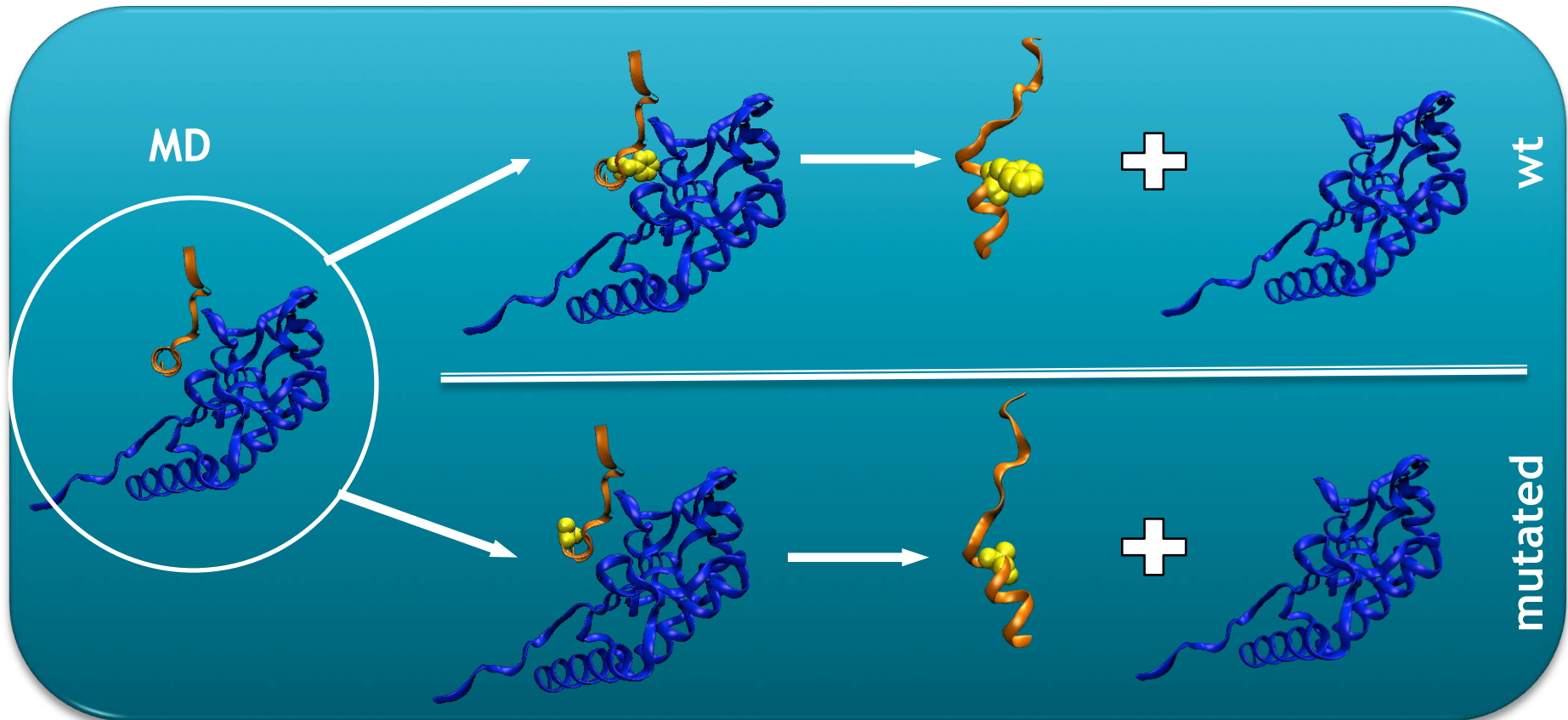
Single MD Protocol

MD



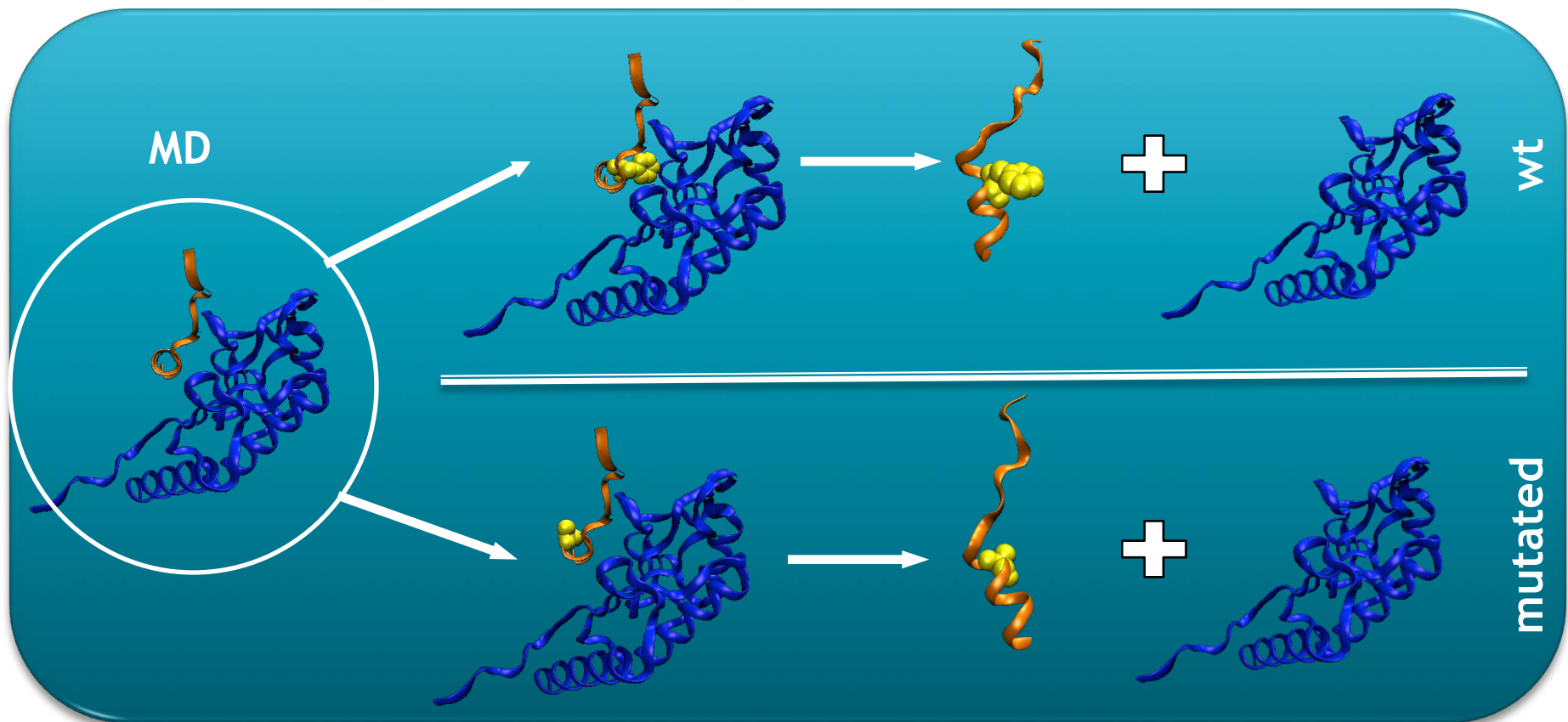
Computational Alanine Scanning Mutagenesis

Single MD Protocol



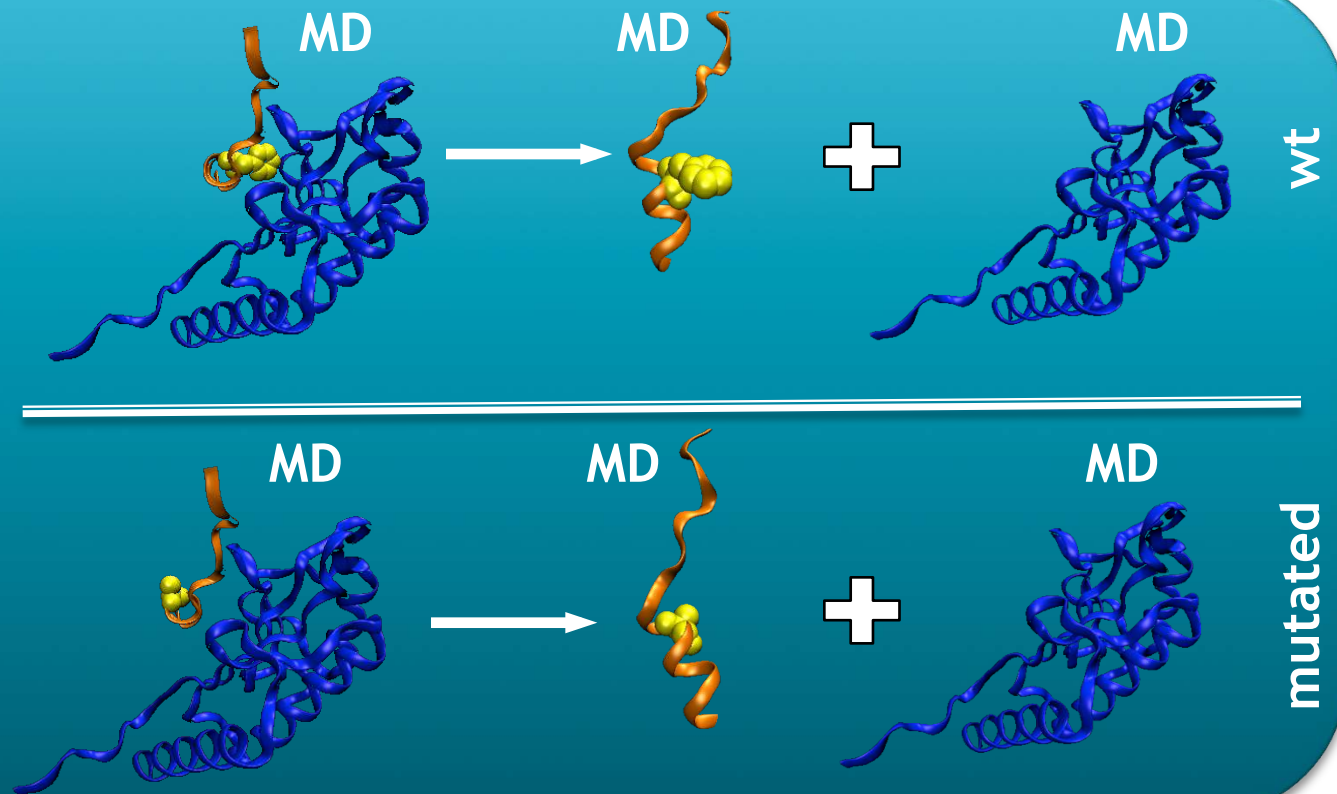
Computational Alanine Scanning Mutagenesis

Single vs Multiple MD Protocols...

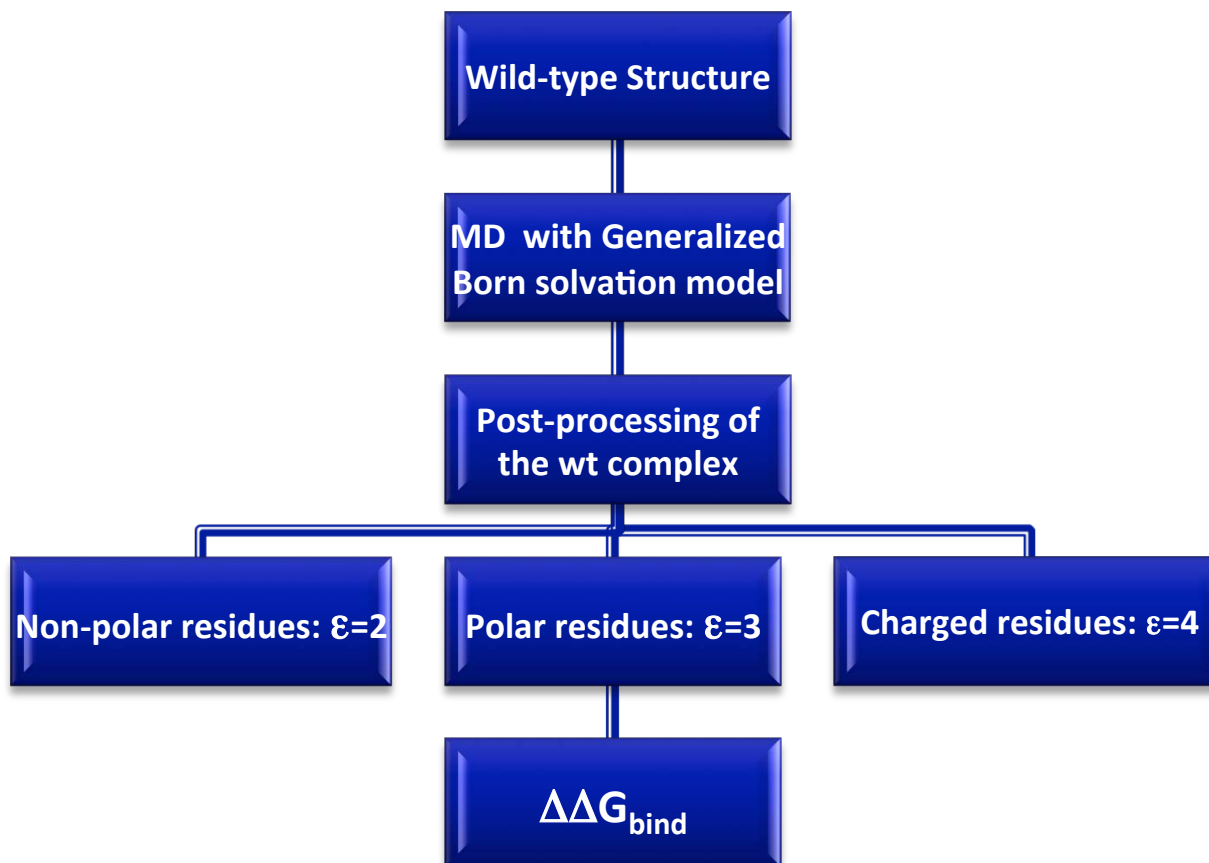


Computational Alanine Scanning Mutagenesis

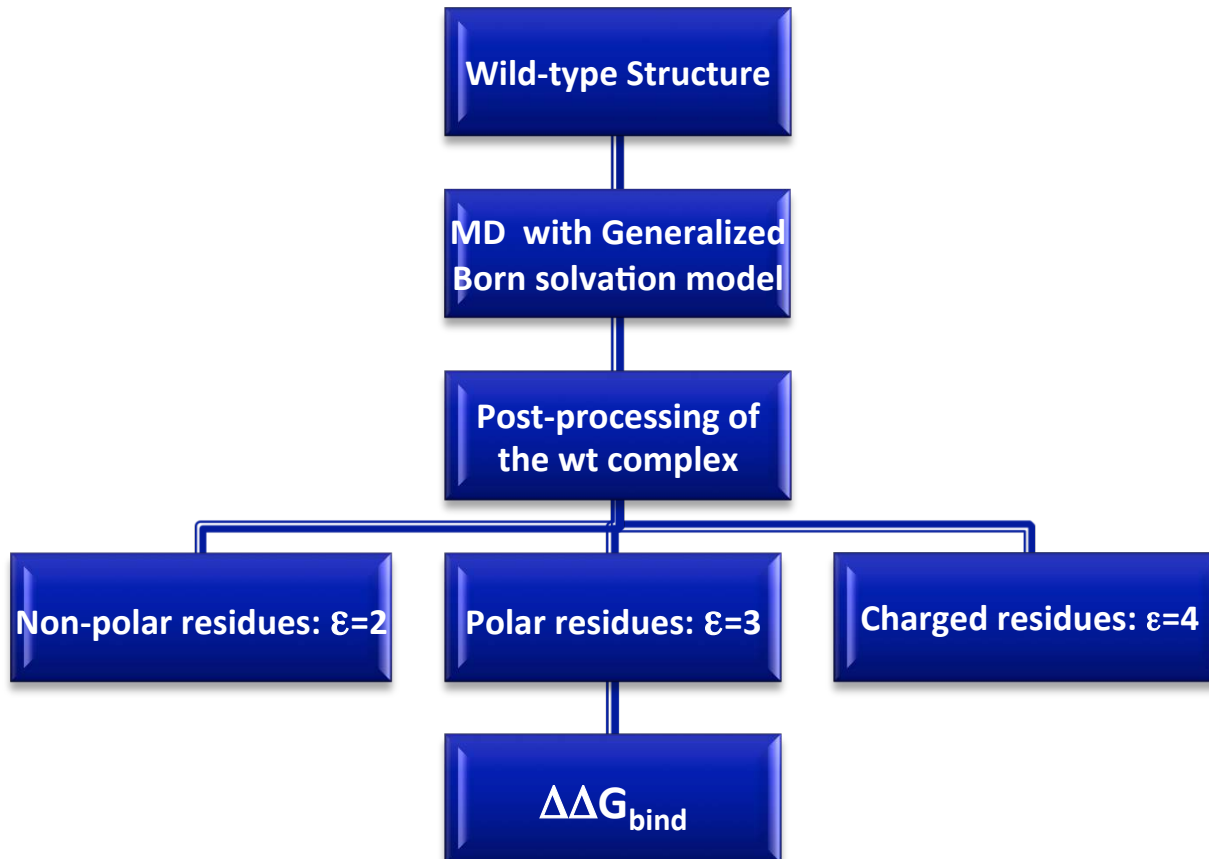
Single vs Multiple MD Protocols...



How to account for the interface reorganization?

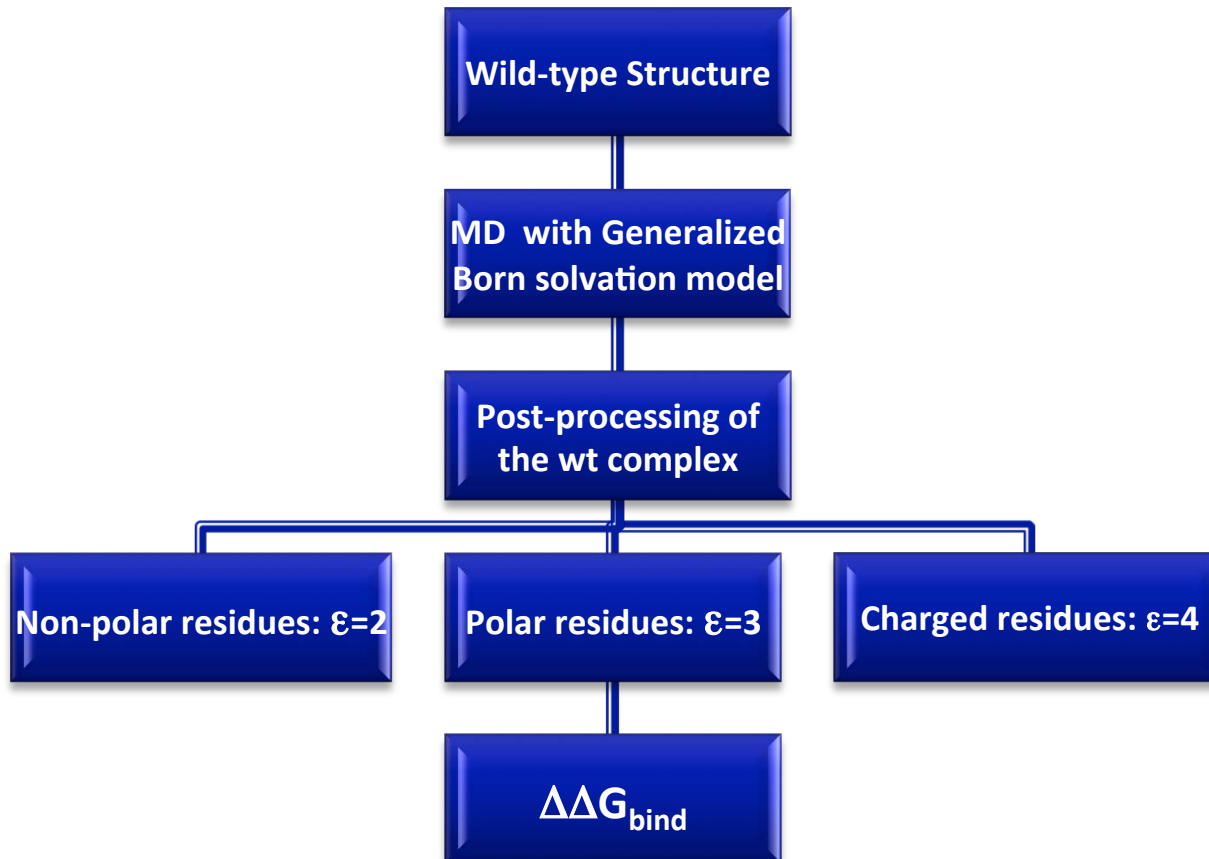


How to account for the interface reorganization?



- the different ϵ account for the different degree of relaxation of the interface when different types of amino acids are mutated for Ala

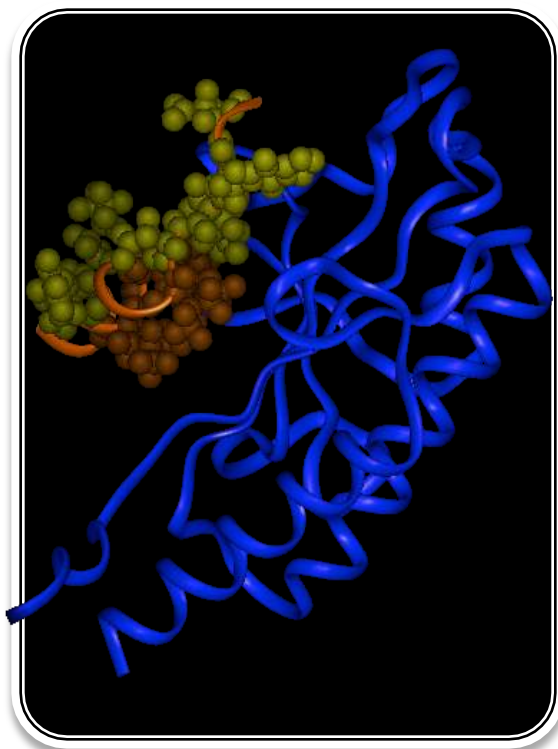
How to account for the interface reorganization?



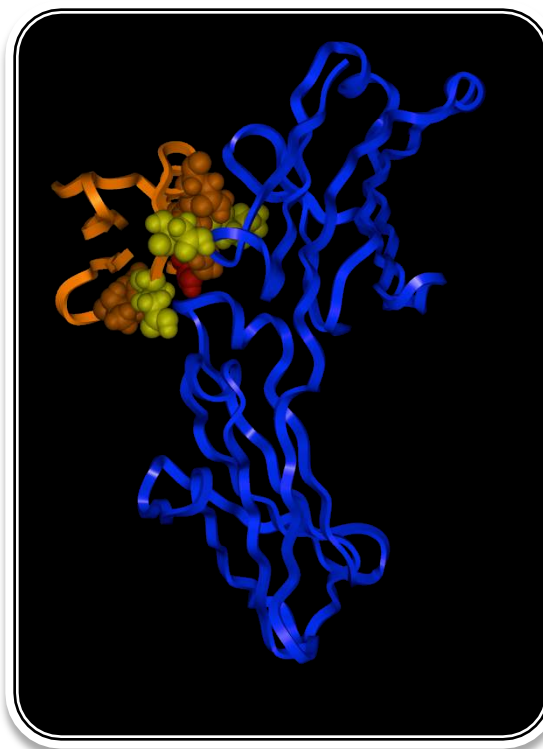
- the stronger the interactions these amino acids establish, the more extensive the relaxation should be, and the greater ϵ must be to mimic these effects.

Calibration – Results of ≈ 50 mutants

ZipA:FtsZ



IgG:C2 frag. of protein G



immunoglobulin:lysozyme



MUE= 0.8 Kcal/mol

MaxUE= 2.4 Kcal/mol

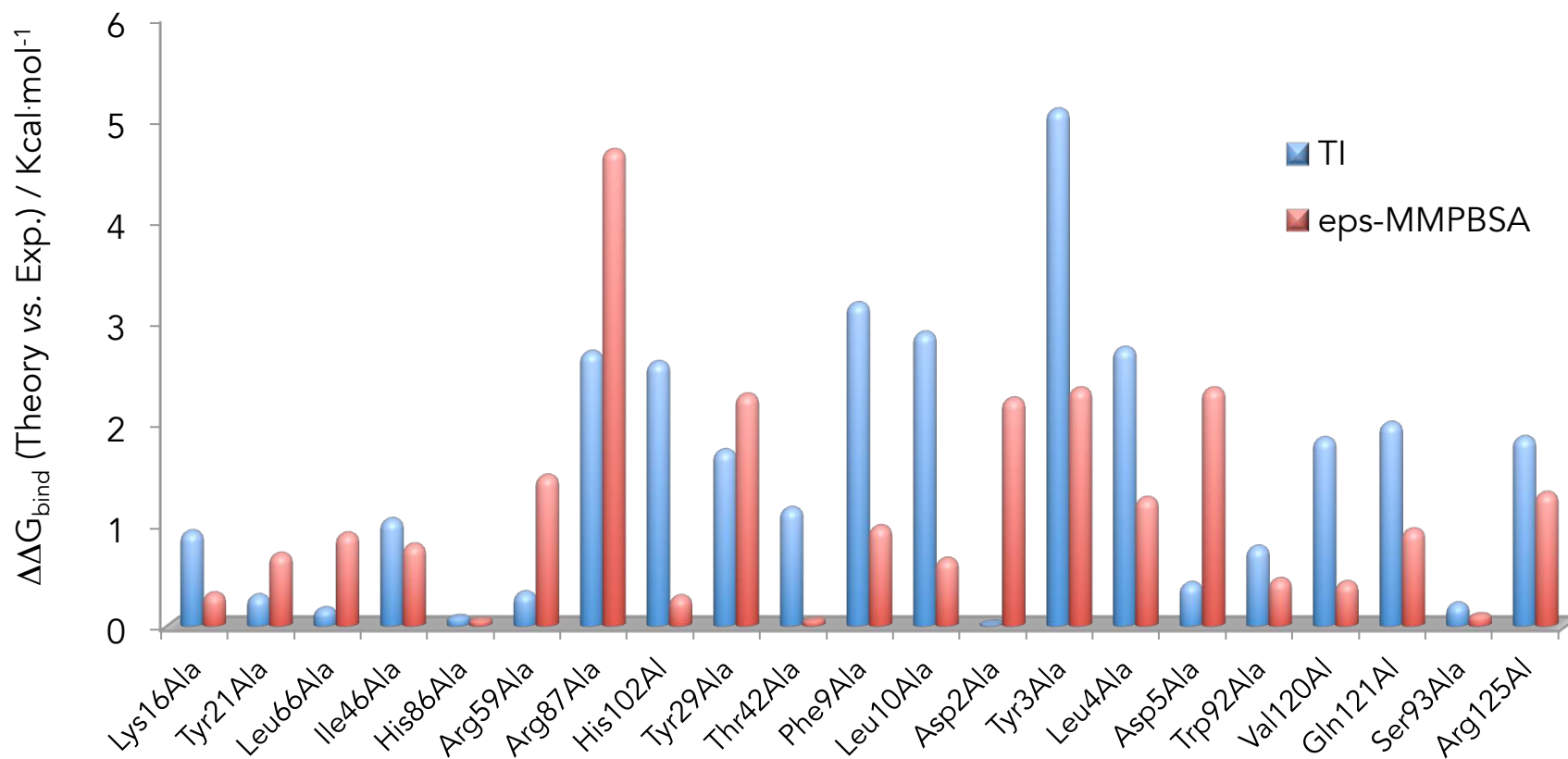
Overall success close to 100% in the detection of hot-spots

Calibrated ϵ -MMPBSA as accurate as TI

3 Protein:Protein complexes, 25 mutations

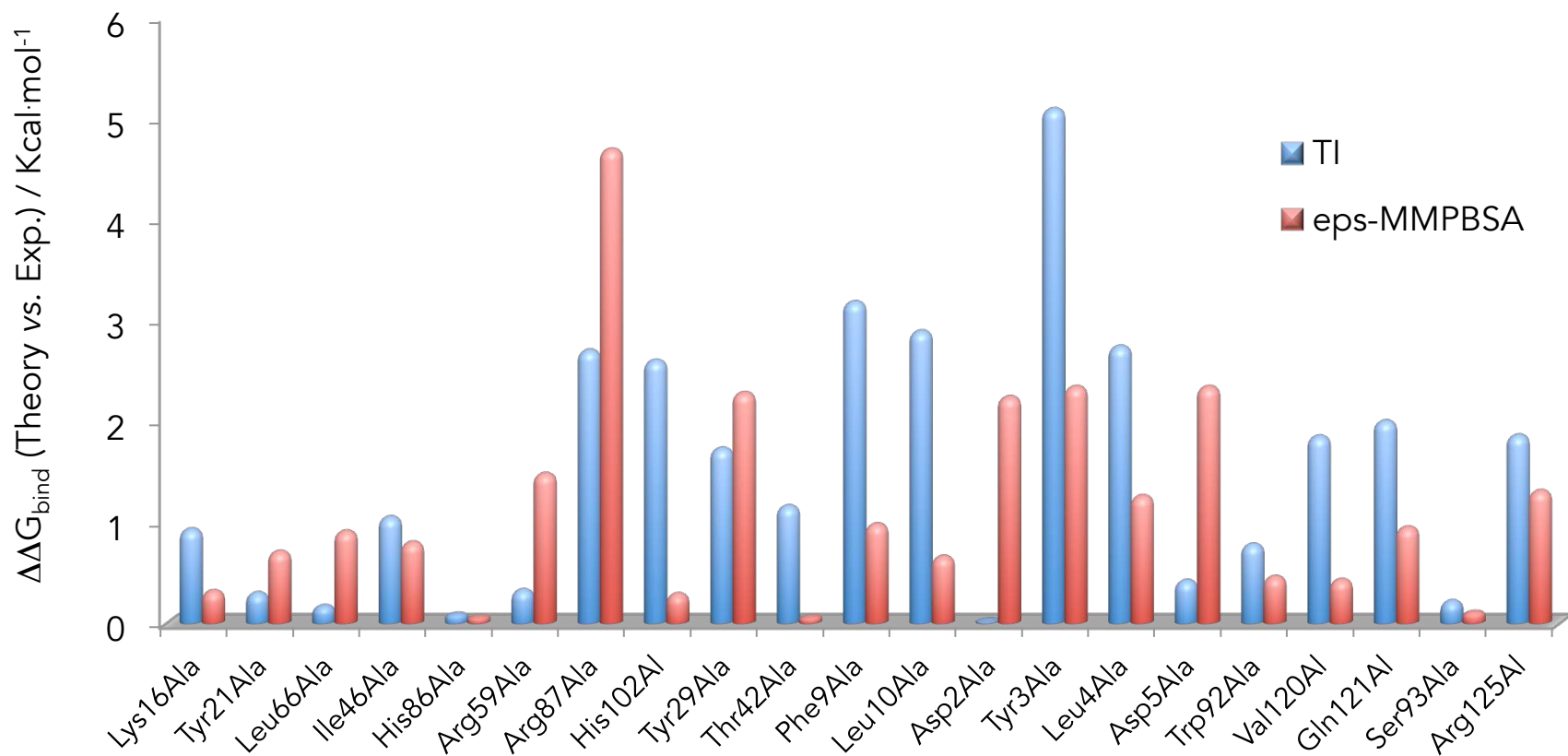
$\Delta\Delta G$ (theory vs. exp.) with TI: 1.5 kcal·mol⁻¹ (max: 5.1 kcal·mol⁻¹)

$\Delta\Delta G$ (theory vs. exp.) with ϵ -MMPBSA: 1.2 kcal·mol⁻¹ (max: 4.7 kcal·mol⁻¹)



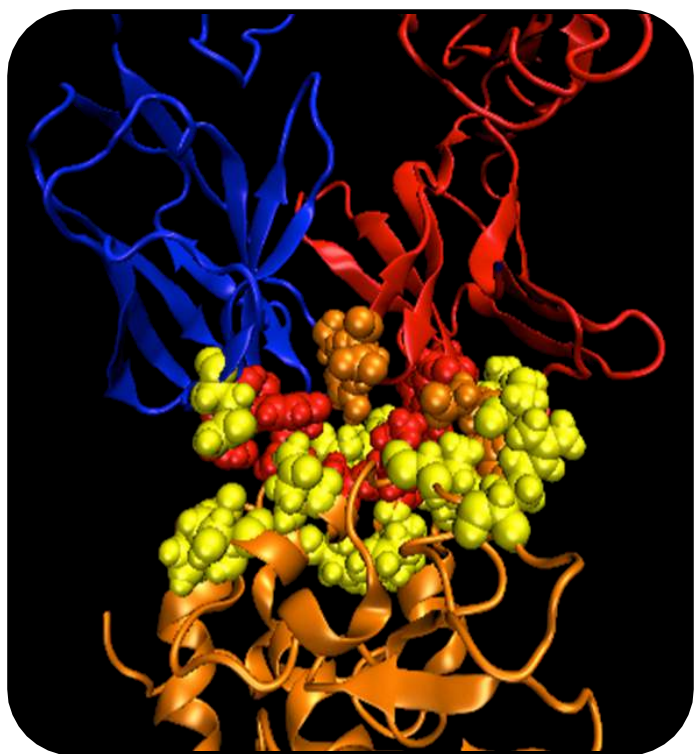
Calibrated ϵ -MMPBSA as accurate as TI

ϵ -MMPBSA CPU Time: $\approx 90\%$ MD + $\approx 10\%$ MM-PBSA (the last grows linearly with n° mutations)
TI CPU Time: longer MDs, growing linearly with n° mutations





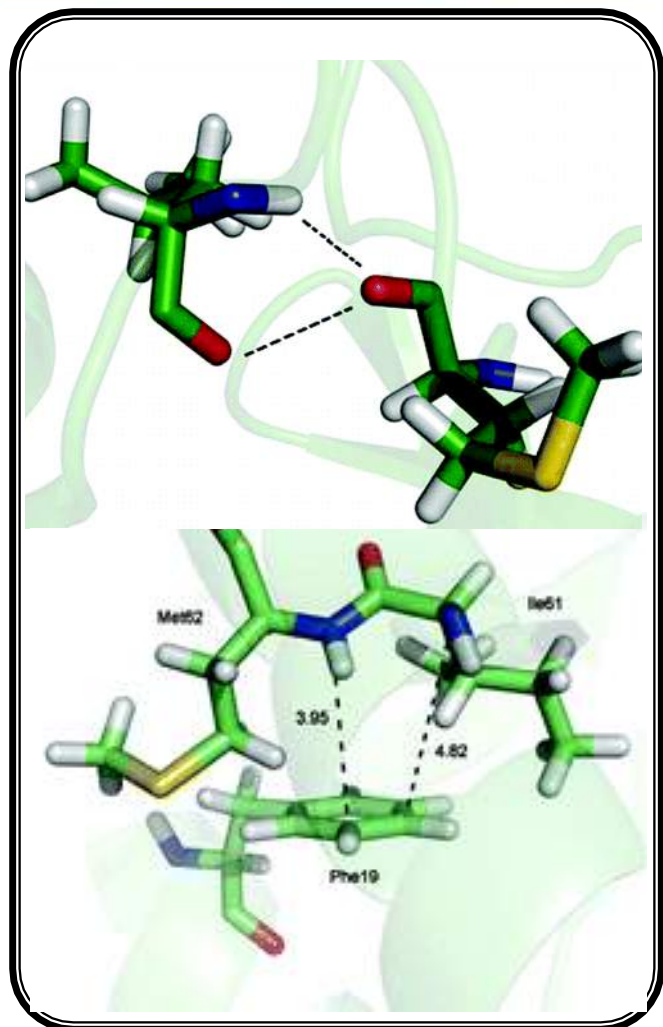
Map whole protein interfaces with a single MD



J PHYS CHEM B, 2525 2010

Proteins 68, 803-812, 2007
J Chem Theory & Comput 3, 885, 2007
J Phys Chem B 111, 2697, 2007

Insights



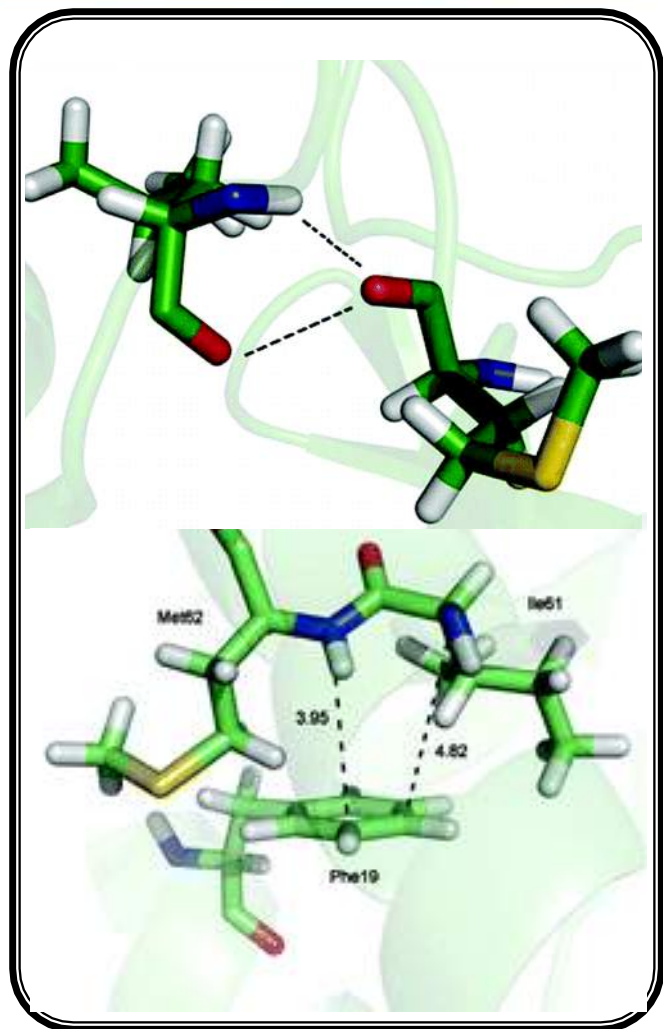
Map whole protein interfaces with a single MD

Quantify the backbone contribution for ΔG_{bind}

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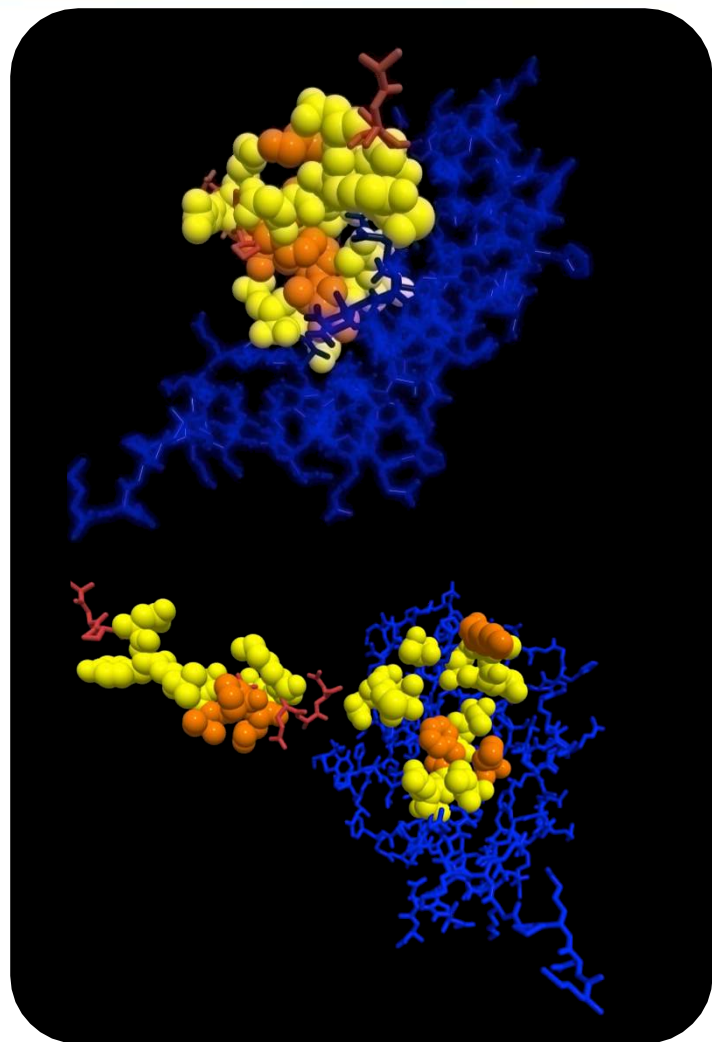
Quantify the backbone contribution for ΔG_{bind}

Decompose ΔG_{bind} in electrostatic, dispersive and solvation contributions

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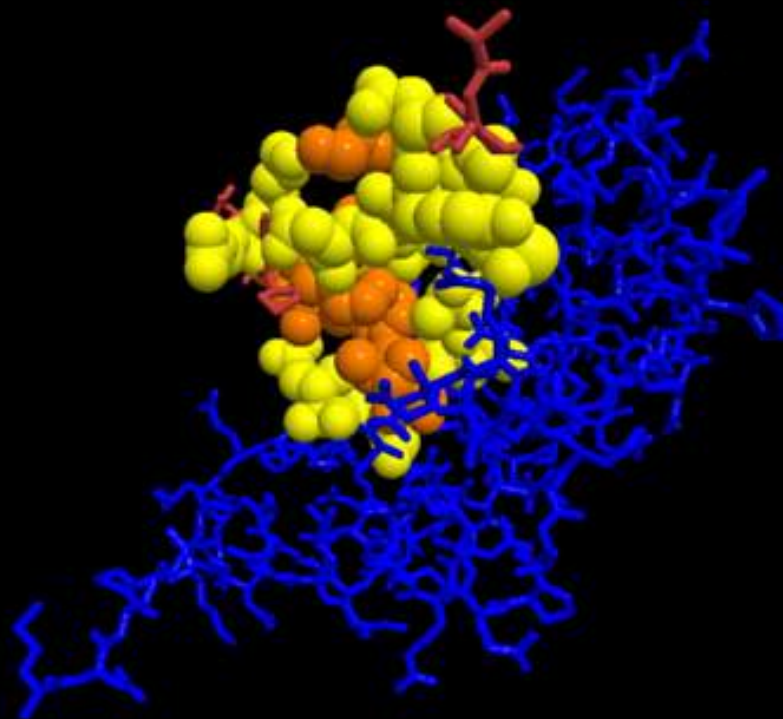
Analyse hot spot complementarity and detect druggable sites

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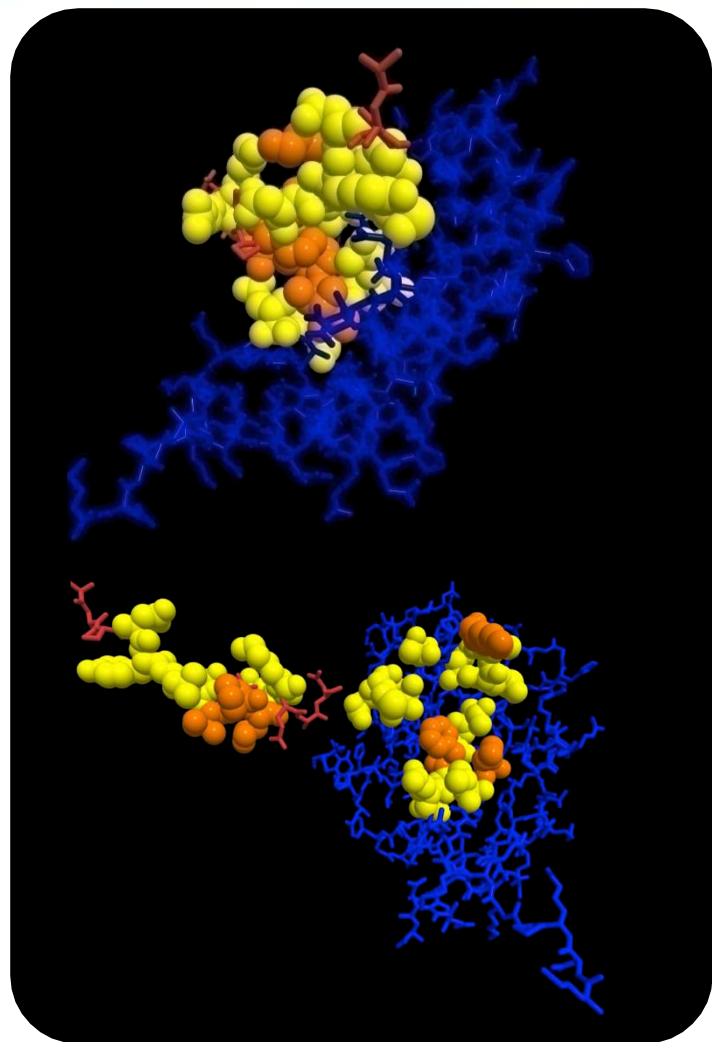
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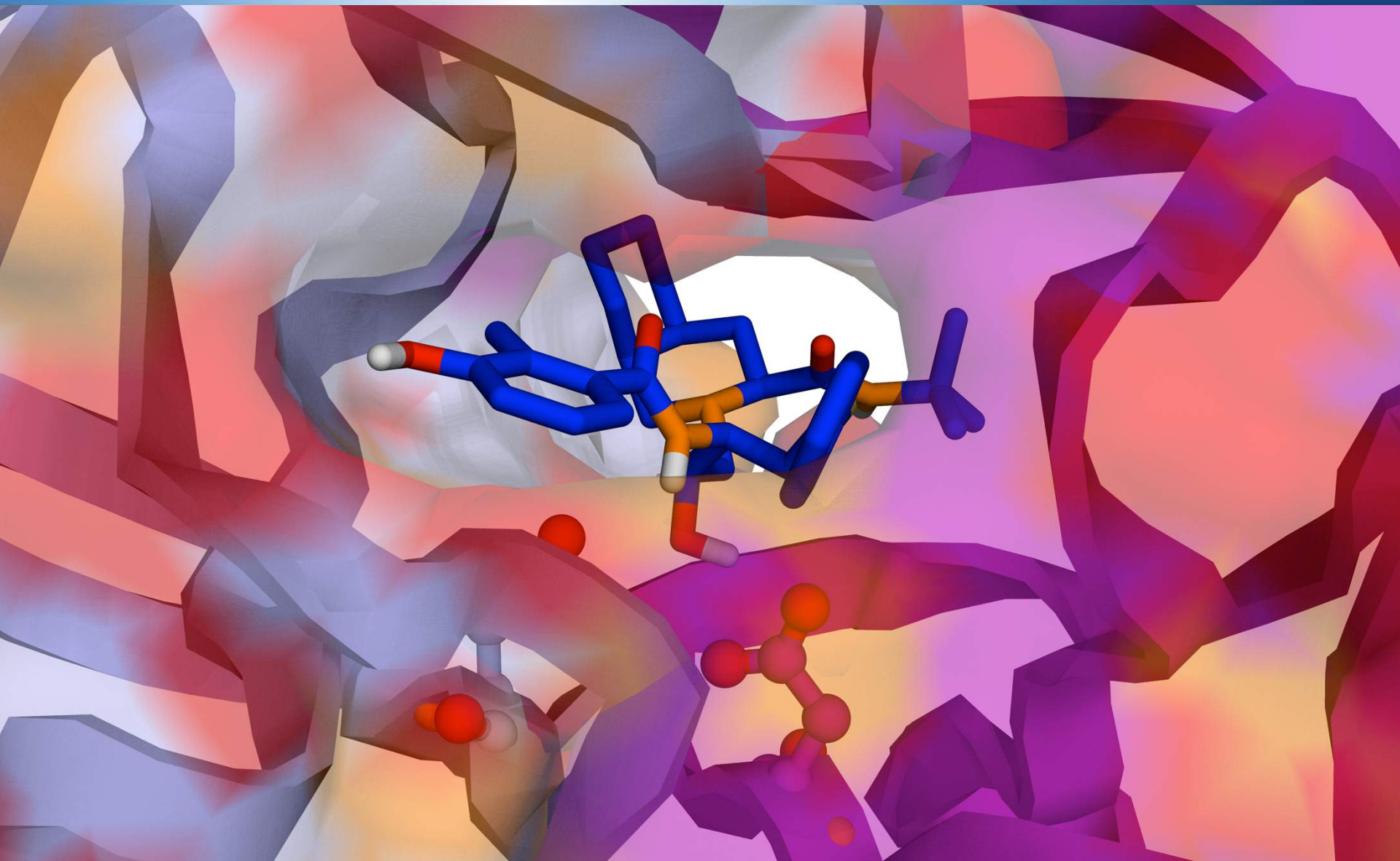
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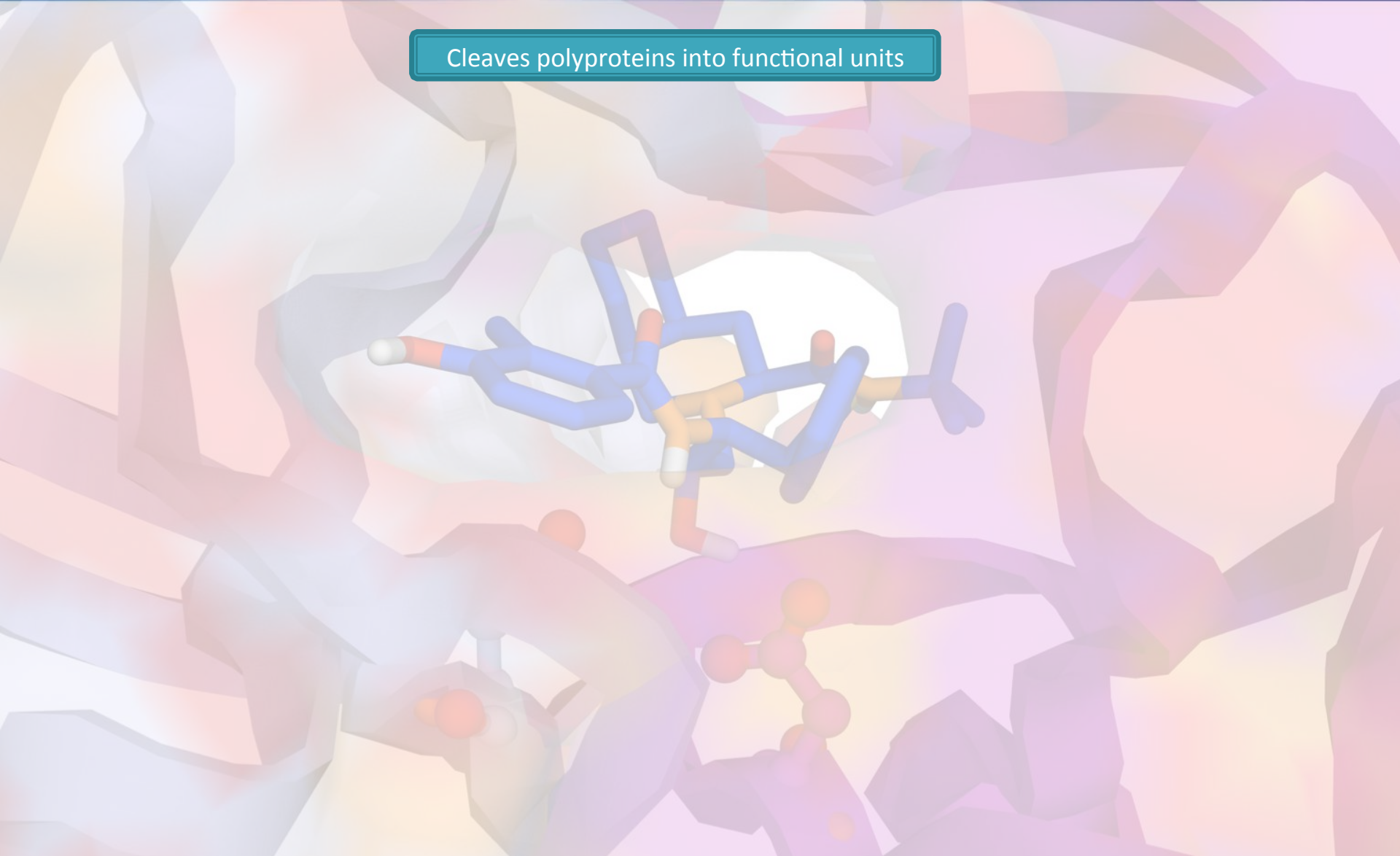
Example: HIV-1 Protease



HIV-1 Protease



Cleaves polyproteins into functional units

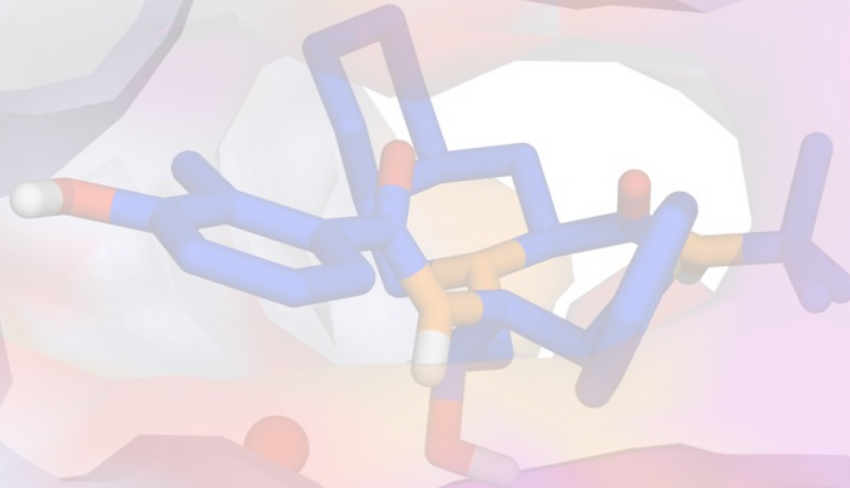


HIV-1 Protease



Cleaves polyproteins into functional units

Inhibition controls HIV-1 proliferation



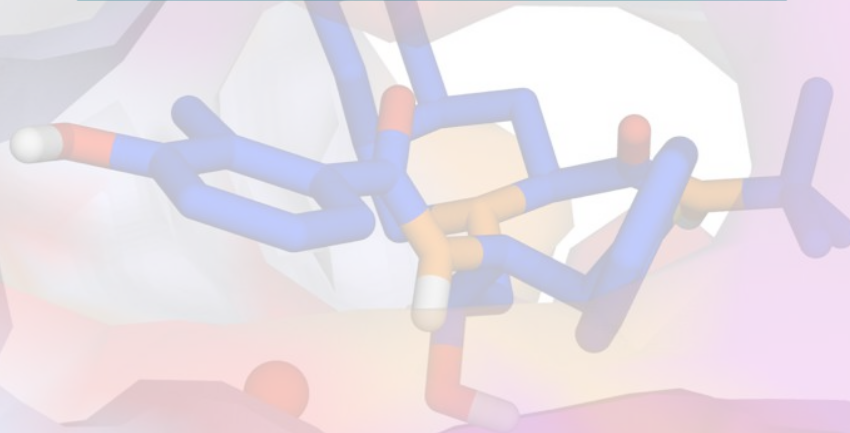
HIV-1 Protease



Cleaves polyproteins into functional units

Inhibition controls HIV-1 proliferation

Present in all HAART therapies



HIV-1 Protease



Cleaves polyproteins into functional units

Inhibition controls HIV-1 proliferation

Present in all HAART therapies

Develops resistance to antiretrovirals



HIV-1 Protease



Cleaves polyproteins into functional units

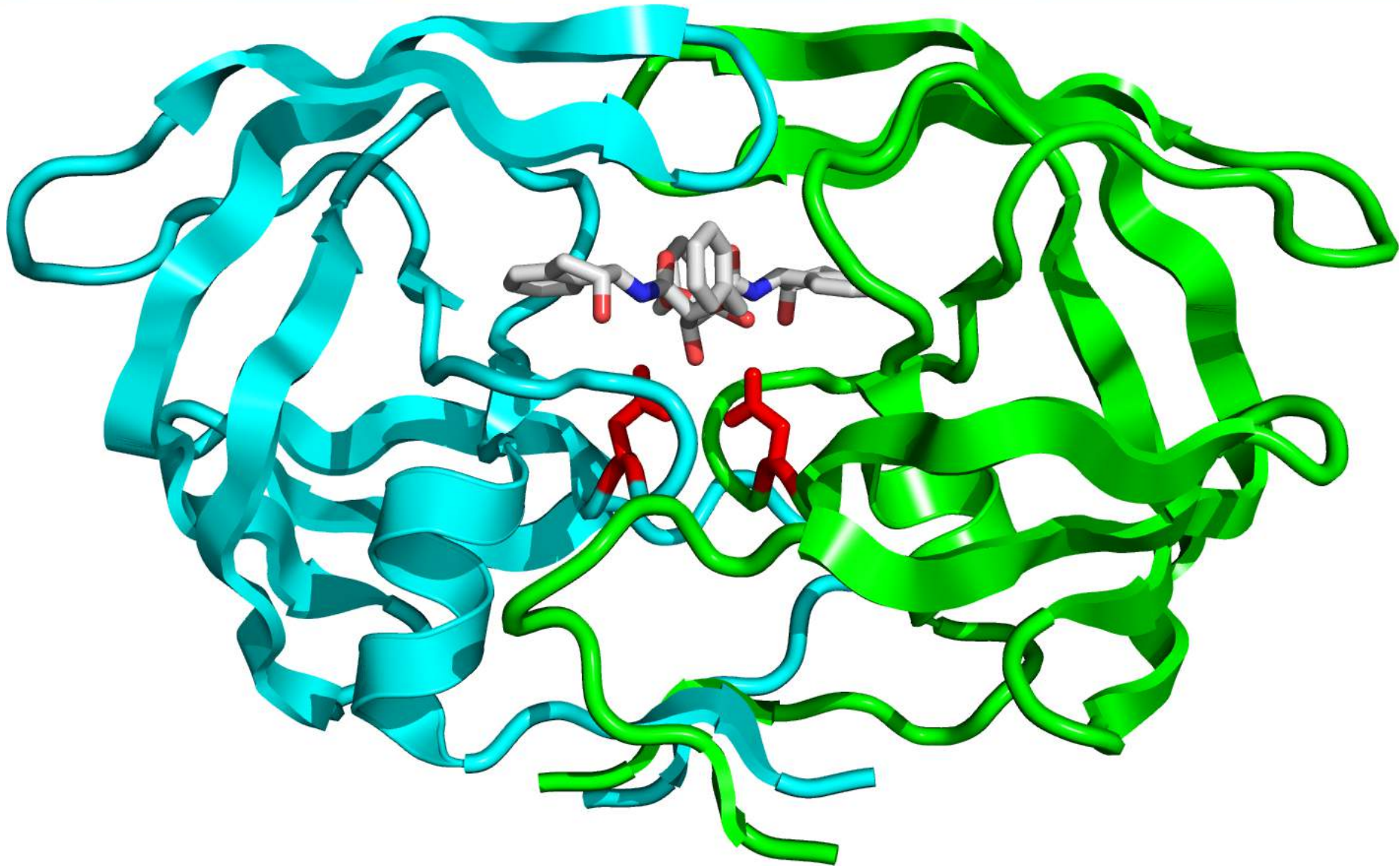
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Dimmerization Inhibitors?

Active Site at the Dimer Interface



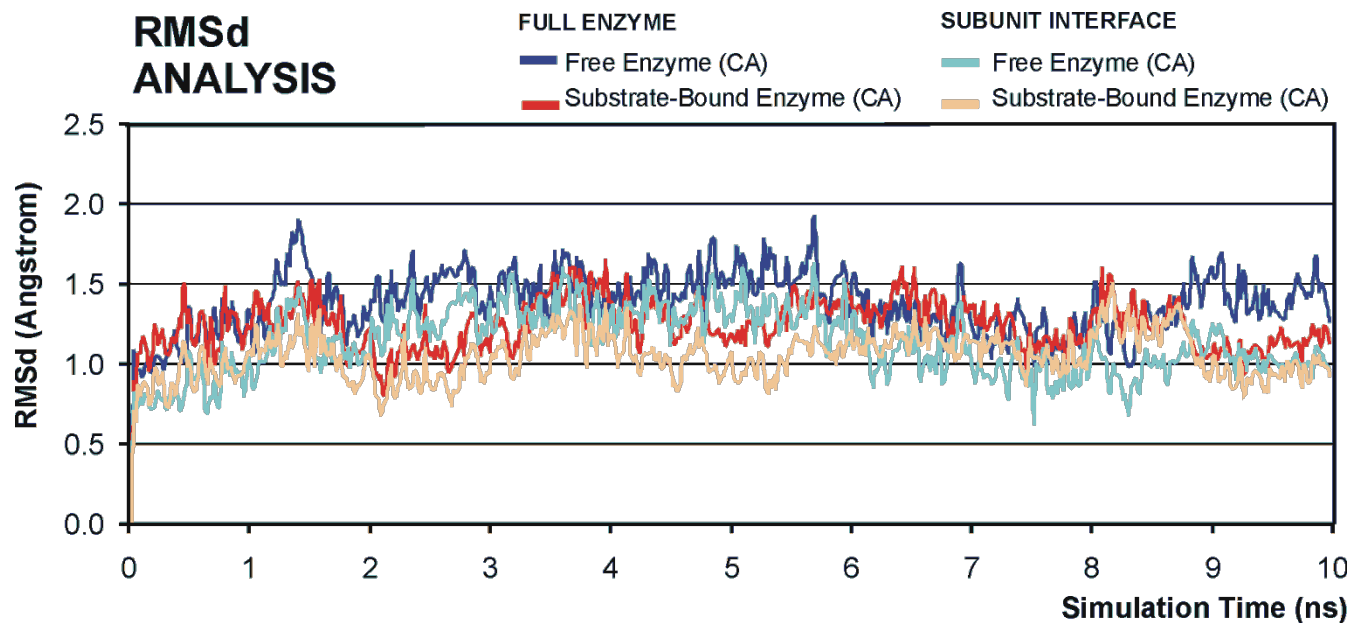
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Present in all HAART therapies

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Mutation	Subunit α $\Delta\Delta G_{\text{Binding}}$	Subunit β $\Delta\Delta G_{\text{Binding}}$	Average $\Delta\Delta G_{\text{Binding}}$	Difference between chains	SASA lost in Dimmer	SASA lost in Dimmer (\AA^2)
Gln2Ala	1.51±0.53	2.21±0.53	1.86	0.70	17.7%	50.4
Ile3Ala	1.73±0.55	1.86±0.56	1.80	0.13	19.1%	52.6
Thr4Ala	0.35±0.54	0.38±0.53	0.37	0.03	7.7%	18.5
Leu5Ala	4.98±0.55	5.02±0.55	5.00	0.04	45.6%	129.4
Trp6Ala	2.49±0.54	3.76±0.54	3.13	1.27	13.7%	49.5
Gln7Ala	0.24±0.53	0.24±0.53	0.24	0.00	2.6%	7.5
Arg8Ala	2.28±0.53	1.85±0.54	2.07	0.43	17.9%	62.2
Val11Ala	0.37±0.56	0.44±0.55	0.41	0.07	2.7%	7.0
Leu23Ala	0.88±0.55	1.03±0.55	0.96	0.15	7.2%	20.3
Leu24Ala	1.08±0.55	1.36±0.55	1.22	0.28	11.4%	32.3
Asp25Ala	0.94±0.53	4.44±0.53	2.69	3.50	14.3%	35.0

Statistics



	Hot Spots	Warm Spots	Null Spots	Total
Overall results	5	4	26	35
$\Delta\Delta G_{\text{Binding}}$	6,0	2,7	0,8	1,8
$\Delta\Delta E_{\text{electrostatic}}$	-0,8	6,8	1,1	1,5
$\Delta\Delta E_{\text{vdW}}$	7,2	1,8	0,9	1,9
$\Delta\Delta G_{\text{PolarSolv}}$	-0,8	-6,1	-1,2	-1,7
$\Delta\Delta G_{\text{NonPolarSolv}}$	0,3	0,1	0,0	0,1
Hydrophilic Contribution	-1,5	0,8	-0,1	-0,2
Hydrophobic Contribution	7,5	1,9	0,9	2,0
SASA lost upon Dimerization (%)	45%	25%	8%	15%
SASA lost upon Dimerization (\AA^2)	134	66	22	43

Statistics



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$\Delta\Delta G_{\text{PolarSolv}}$	-0,8	-6,1	-1,2	-1,7
$\Delta\Delta G_{\text{NonPolarSolv}}$	0,3	0,1	0,0	0,1
Hydrophilic Contribution	-1,5	0,8	-0,1	-0,2
Hydrophobic Contribution	7,5	1,9	0,9	2,0
SASA lost upon Dimerization (%)	45%	25%	8%	15%
SASA lost upon Dimerization (\AA^2)	134	66	22	43

Statistics



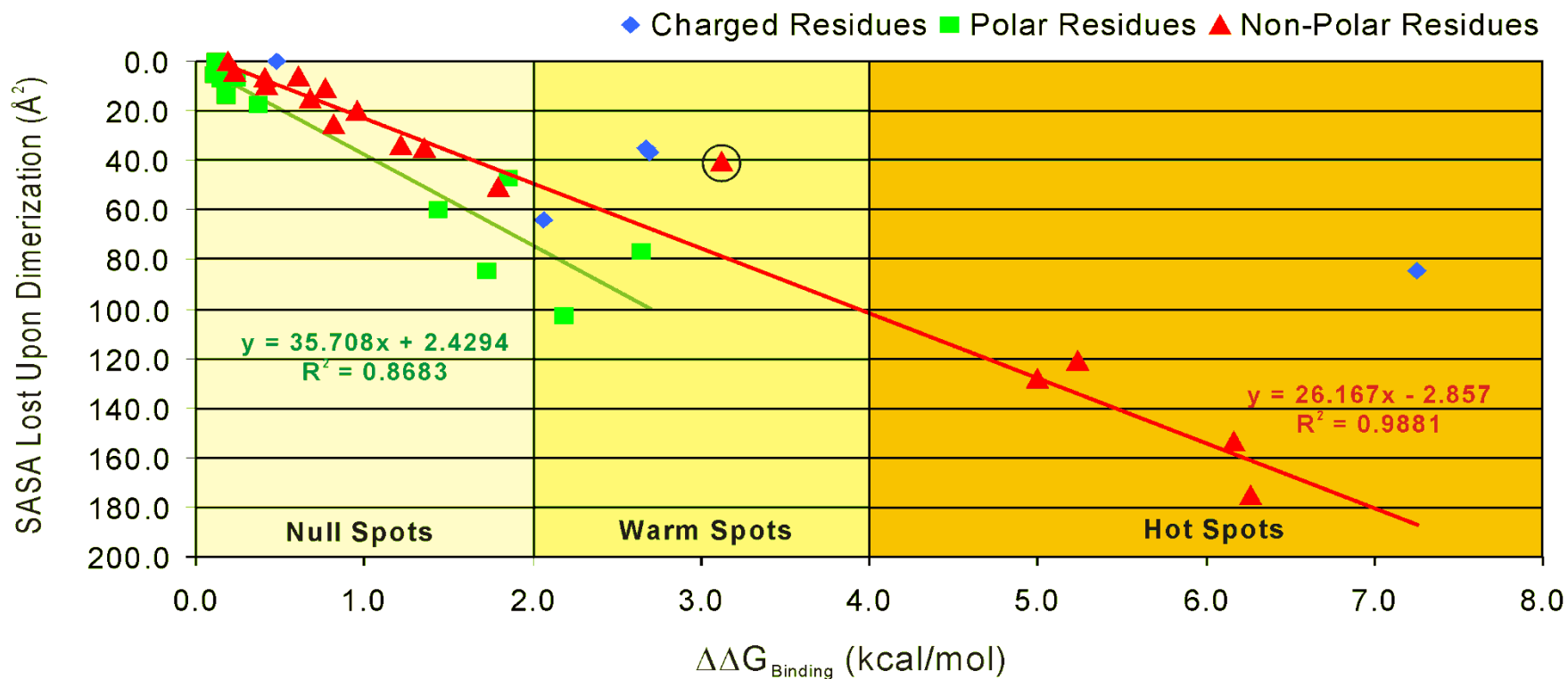
	Hot Spots	Warm Spots	Null Spots	Total
Overall results	5	4	26	35
$\Delta\Delta G_{\text{Binding}}$	6,0	2,7	0,8	1,8
$\Delta\Delta E_{\text{electrostatic}}$	-0,8	6,8	1,1	1,5
$\Delta\Delta E_{\text{vdW}}$	7,2	1,8	0,9	1,9
$\Delta\Delta G_{\text{PolarSolv}}$	-0,8	-6,1	-1,2	-1,7
$\Delta\Delta G_{\text{NonPolarSolv}}$	0,3	0,1	0,0	0,1
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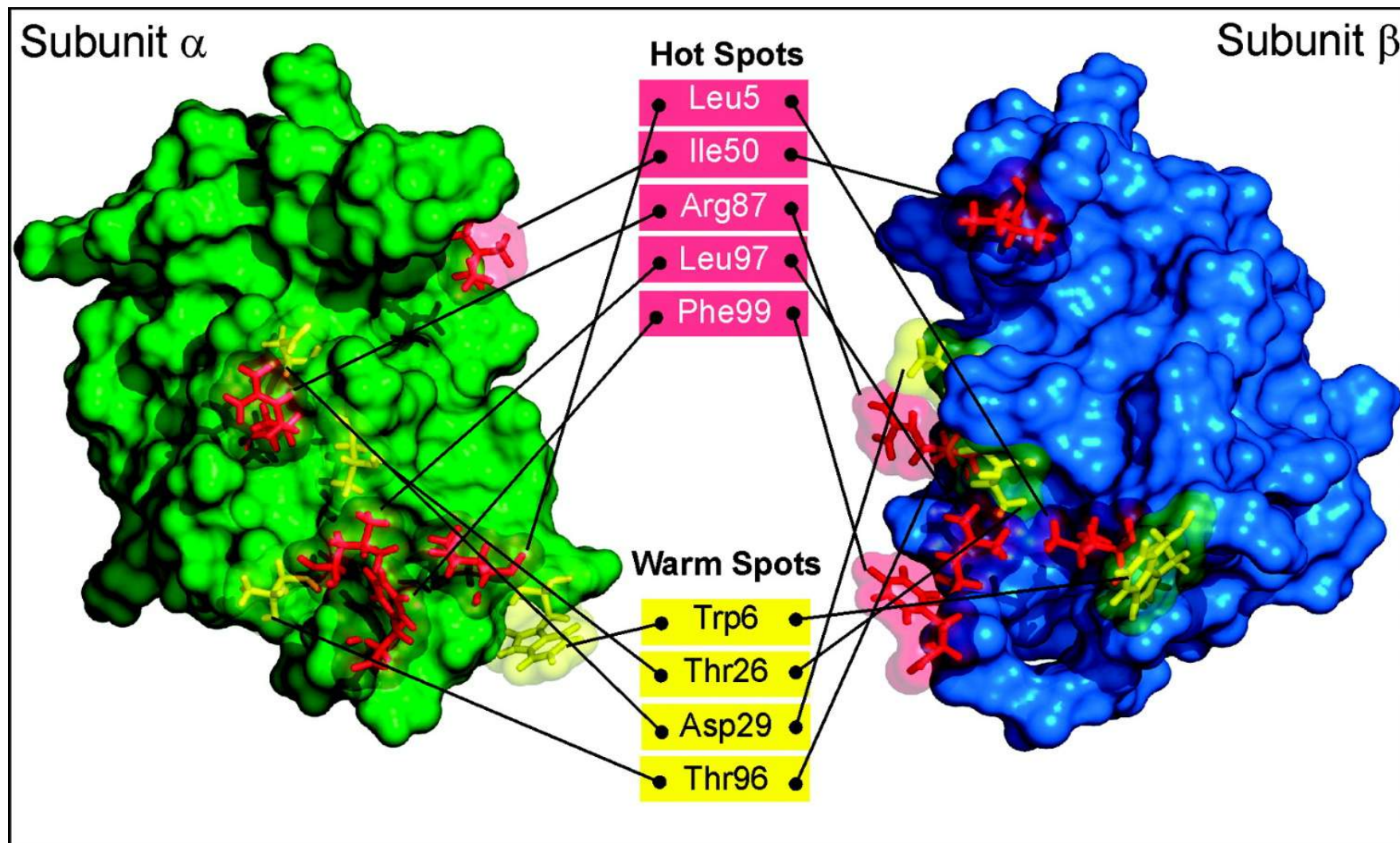


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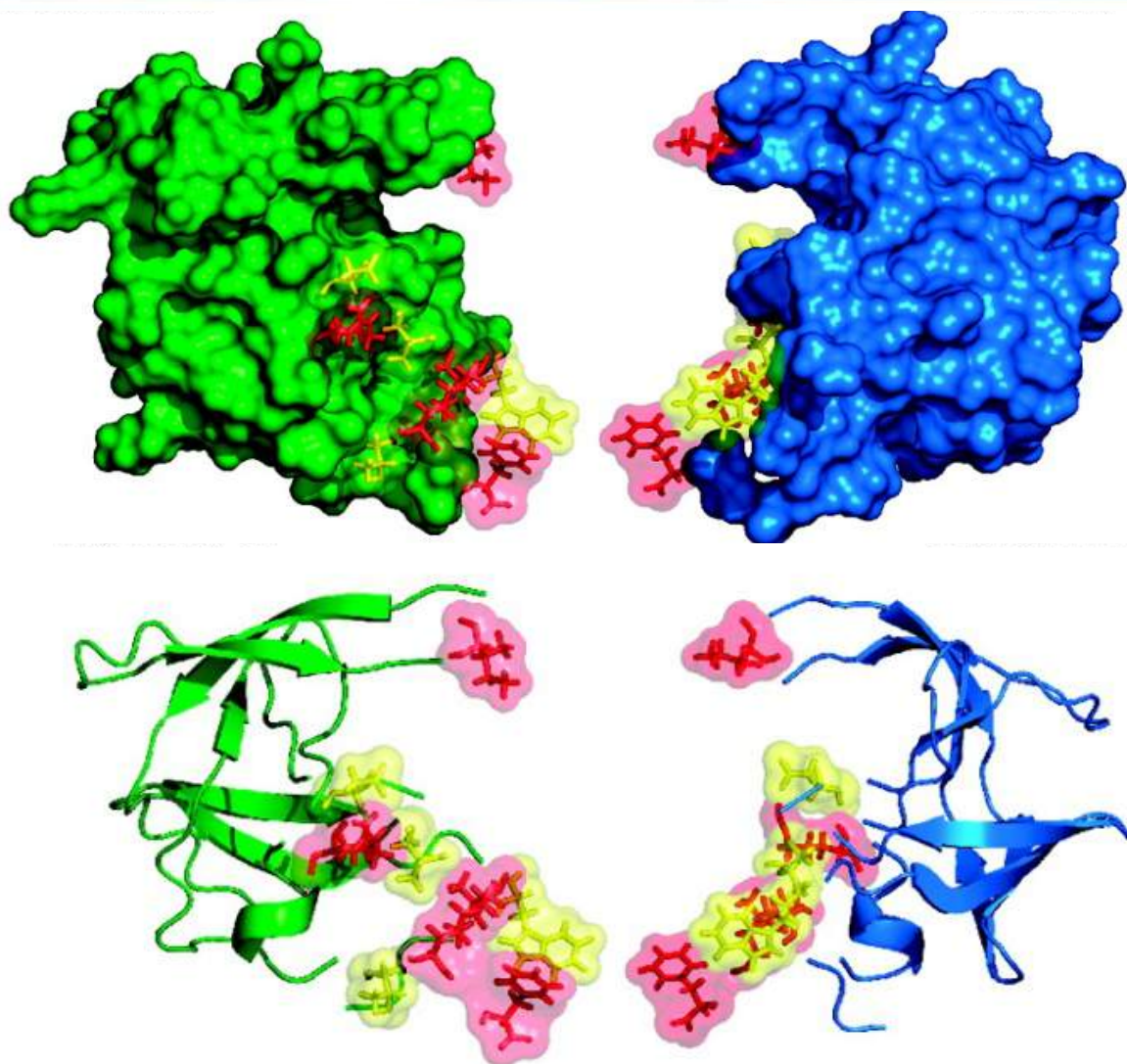
Statistics



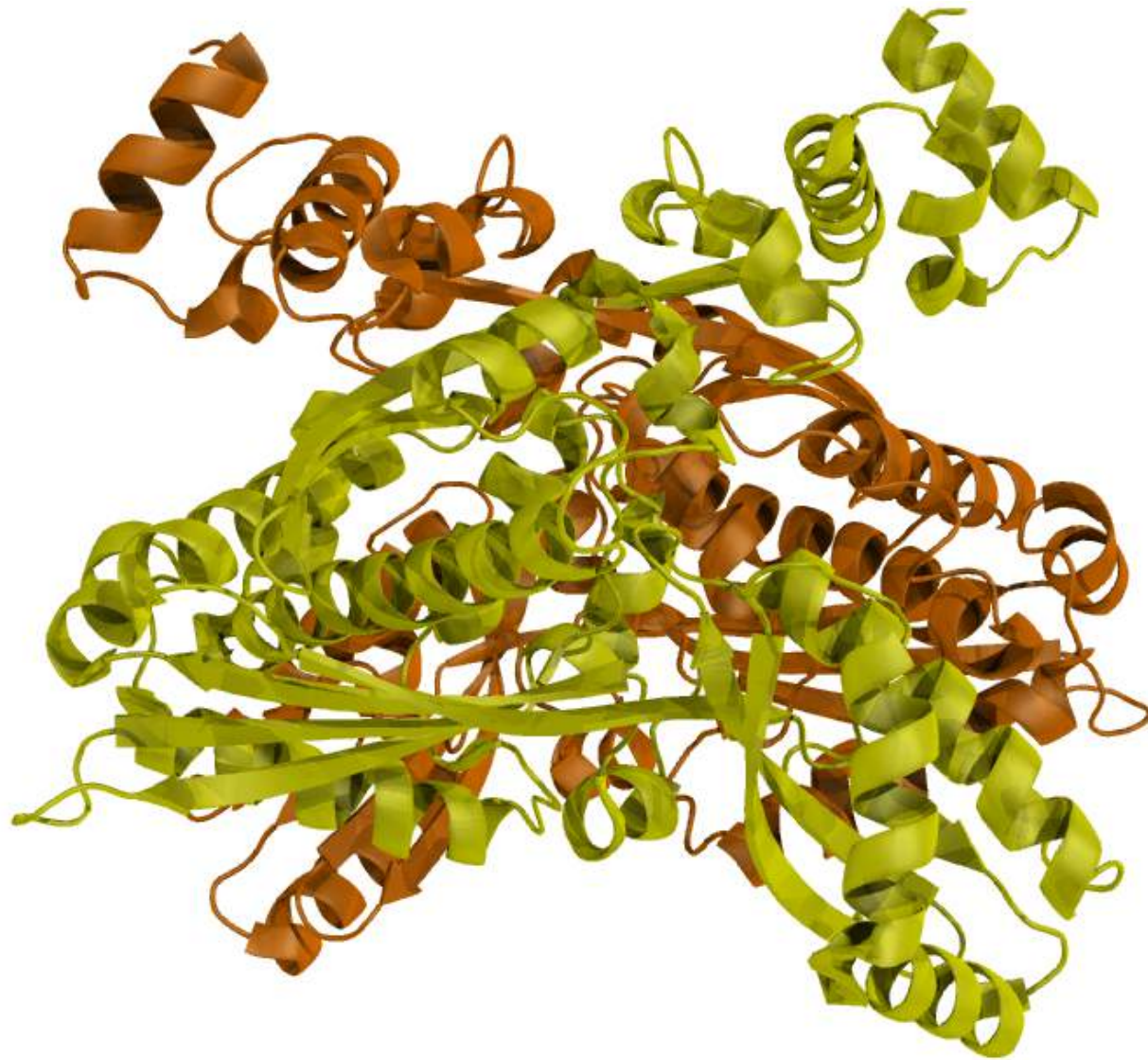
Interface Map



Interface Pockets ...

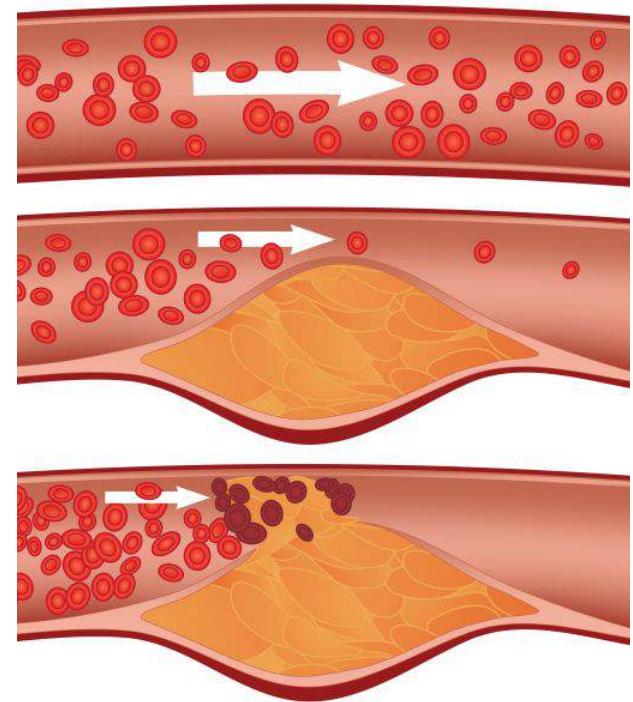


HMG-CoA-Reductase

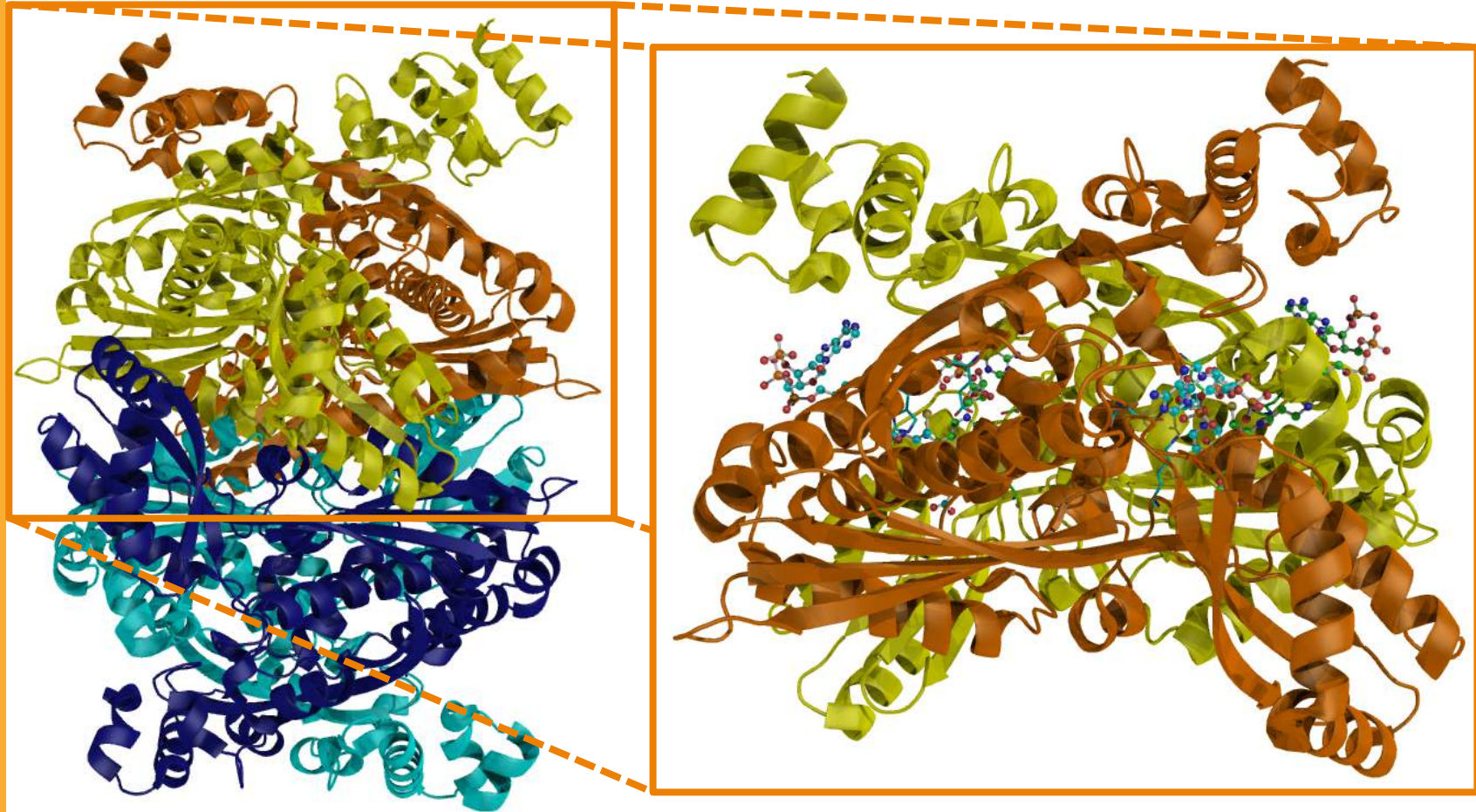


Cholesterol

- High blood cholesterol causes atherosclerosis, which is related to other diseases:
 - Ischemic heart disease (IHD).
 - Stroke.
- In 2008, IHD was the **number 1** leading cause of death (12.8%).
- Stroke and other cardiovascular diseases was **number 2** (10.8%).
- Hypercholesterolemia affects most people after a certain age.
 - The number of young people who suffer from high blood cholesterol is rising.

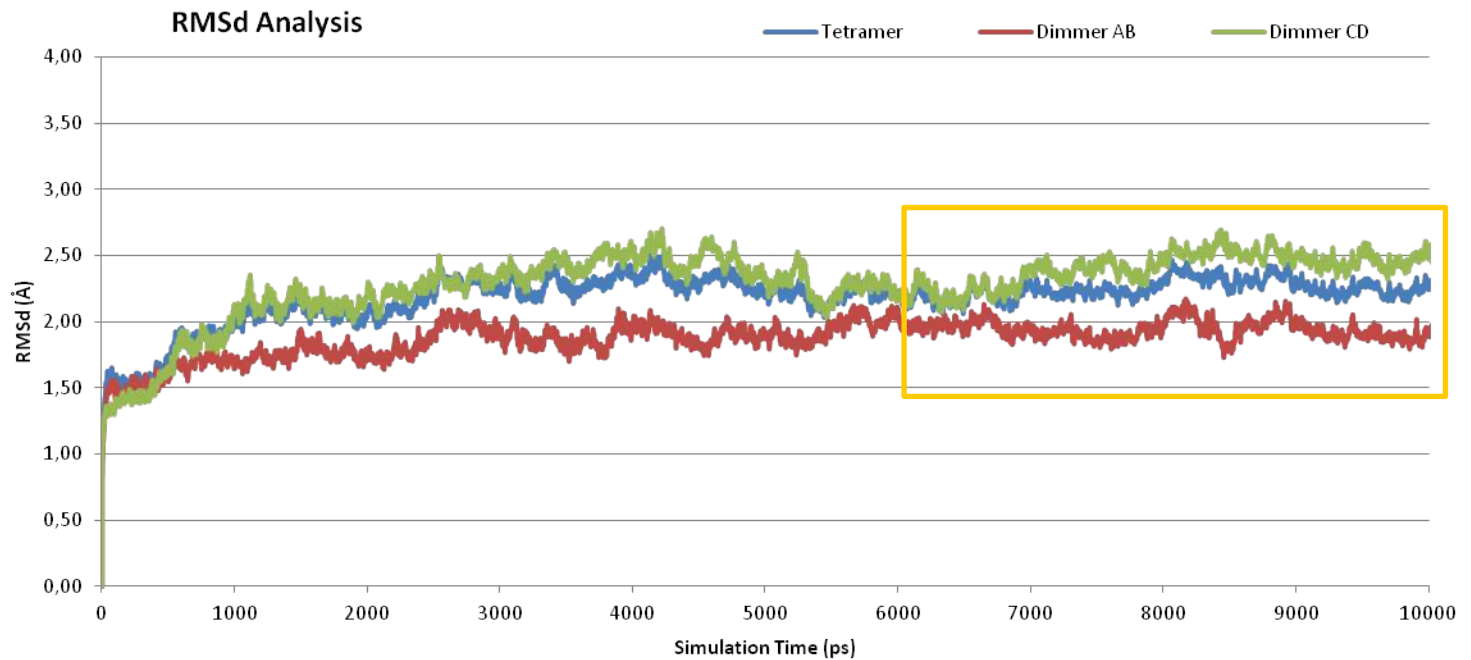


Structure of HMG-CoA Reductase

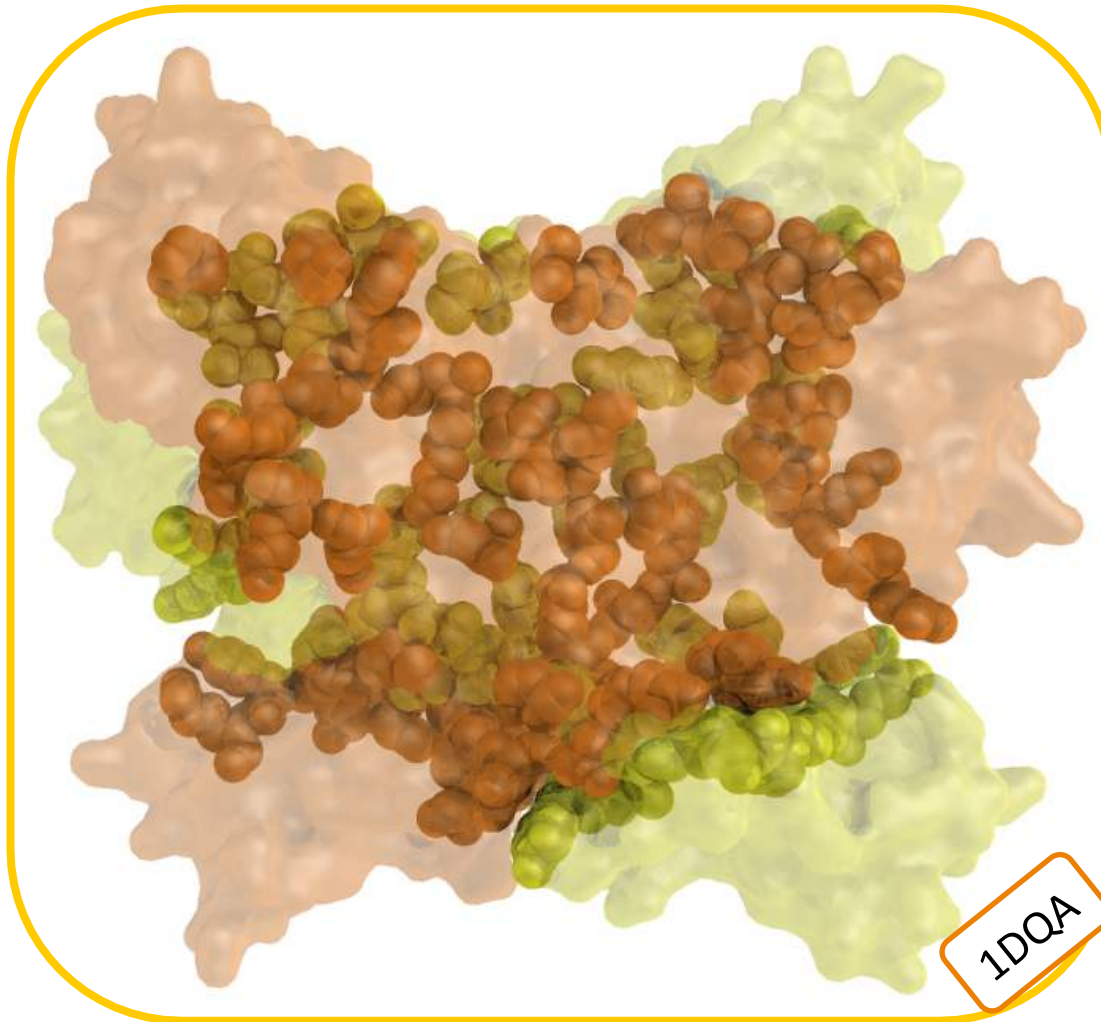


Molecular Dynamics

10 ns Molecular Dynamics



Interface Studied



Entire interface is too large...

Over 120 mutations per subunit.

So:

Buried surface > 40 Å².

Total mutations:

232 residues

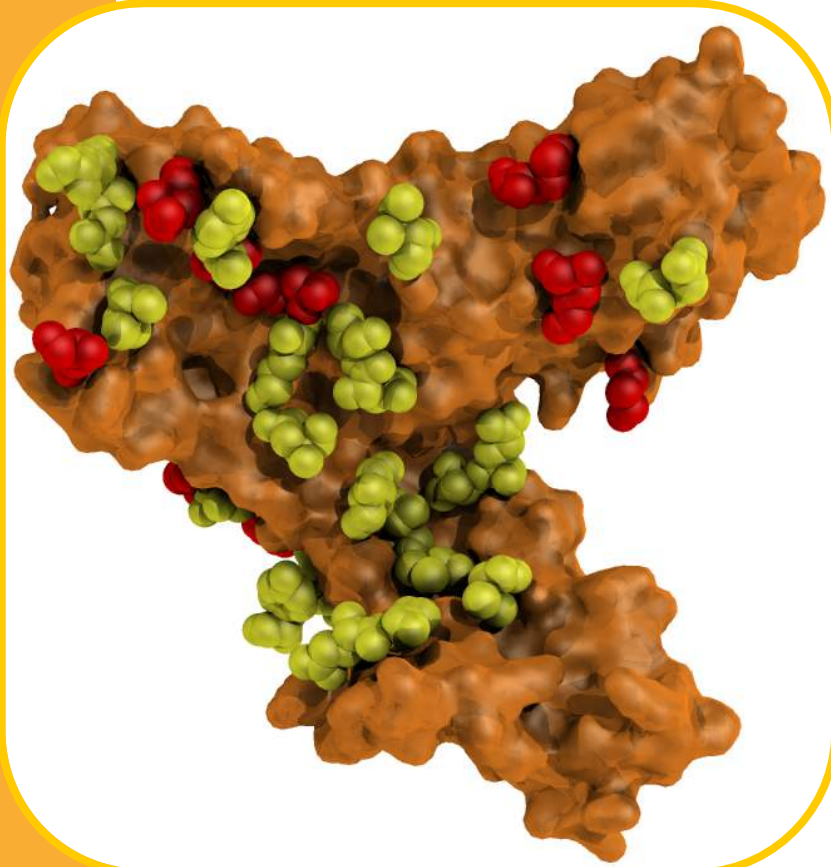
(58 for each subunit)

ASM and SASA Results

- Hot spots are usually residues which have very little surface exposed to the solvent upon dimerization.
- SASA allows us to evaluate the surface of a residue that is exposed.

	Null Spots	Warm Spots	Hot Spots	Total
Residues identified in each monomer	23	25	10	58
Average $\Delta\Delta G_{\text{binding}}$ (kcal/mol)	0.7	2.7	5.6	2.4
Average SASA in Monomer (\AA^2)	82.4	86.6	102.6	90.5
Average SASA in Dimer (\AA^2)	29.6	16.7	8.5	18.3
Average SASA lost upon Dimeration (\AA^2)	52.8	69.9	94.0	72.3
Average SASA lost upon Dimeration (%)	19.5	25.0	33.1	25.9

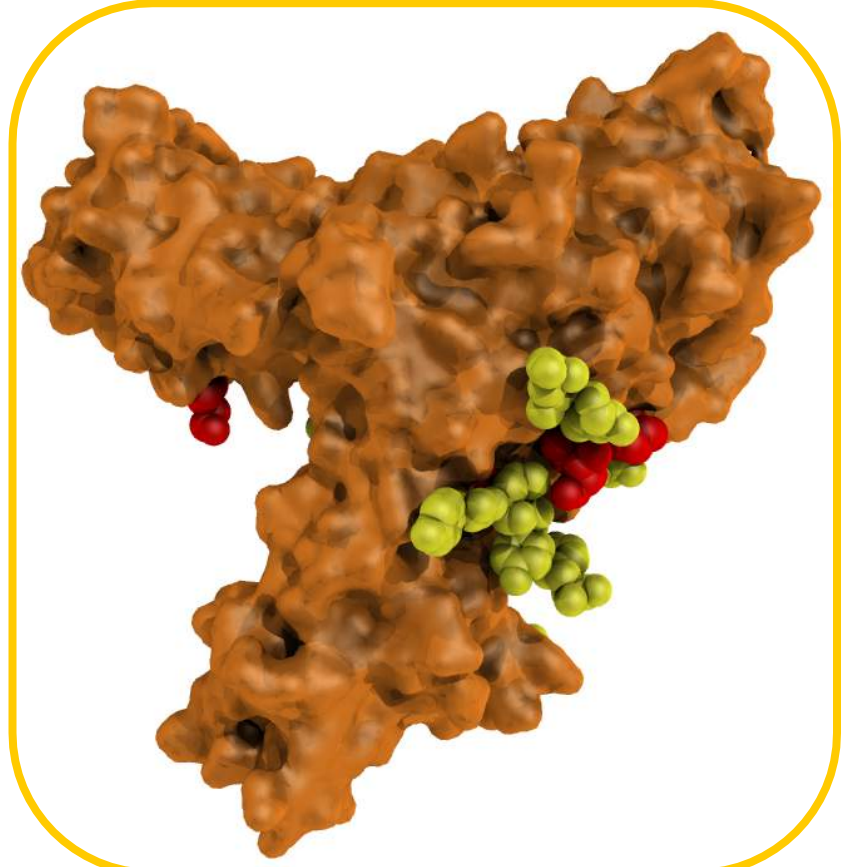
ASM Results



Front View

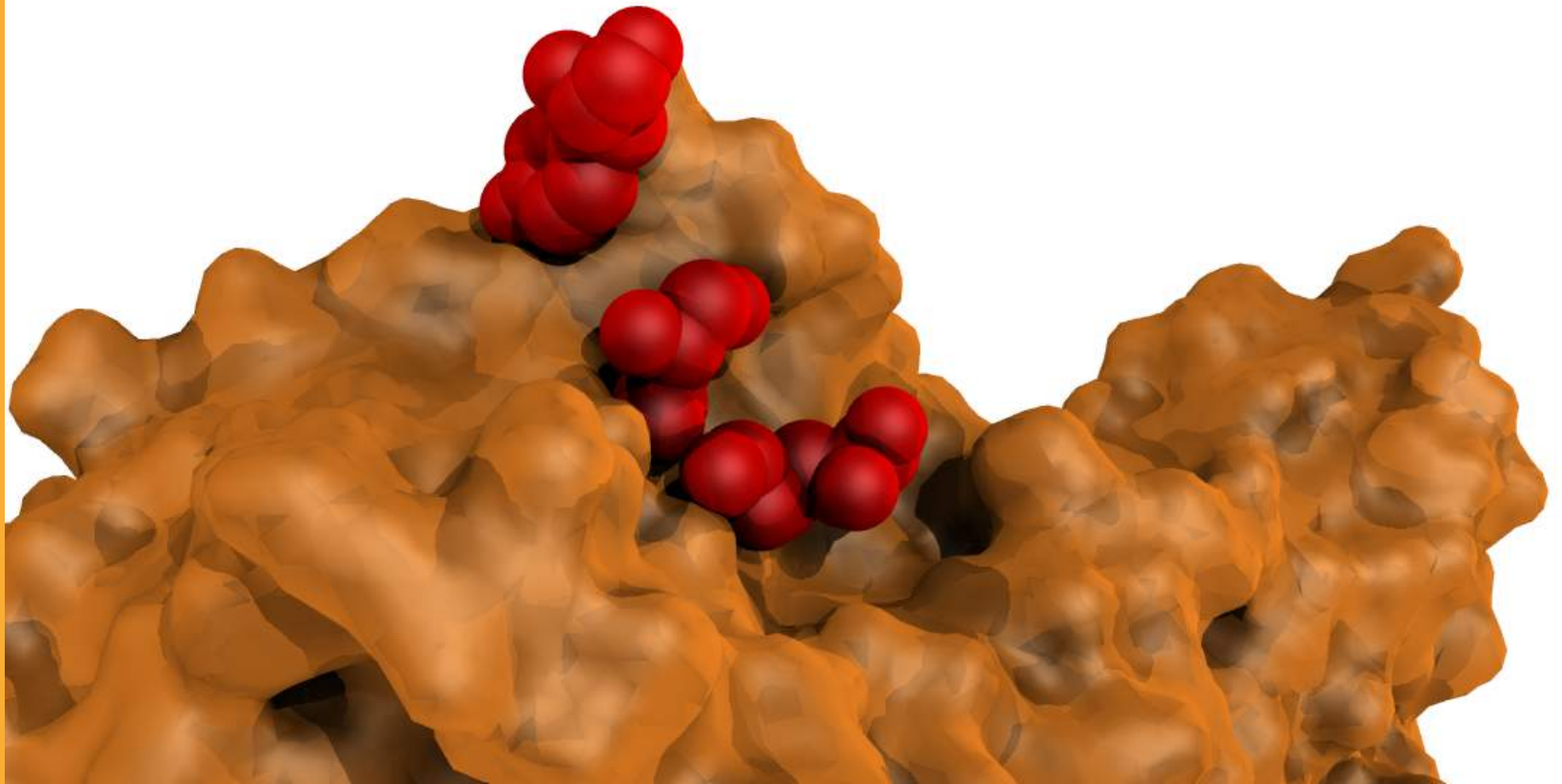
In 58 mutations:

- 10 **Hot spots**
- 25 **Warm spots**
- 23 **Null spots**

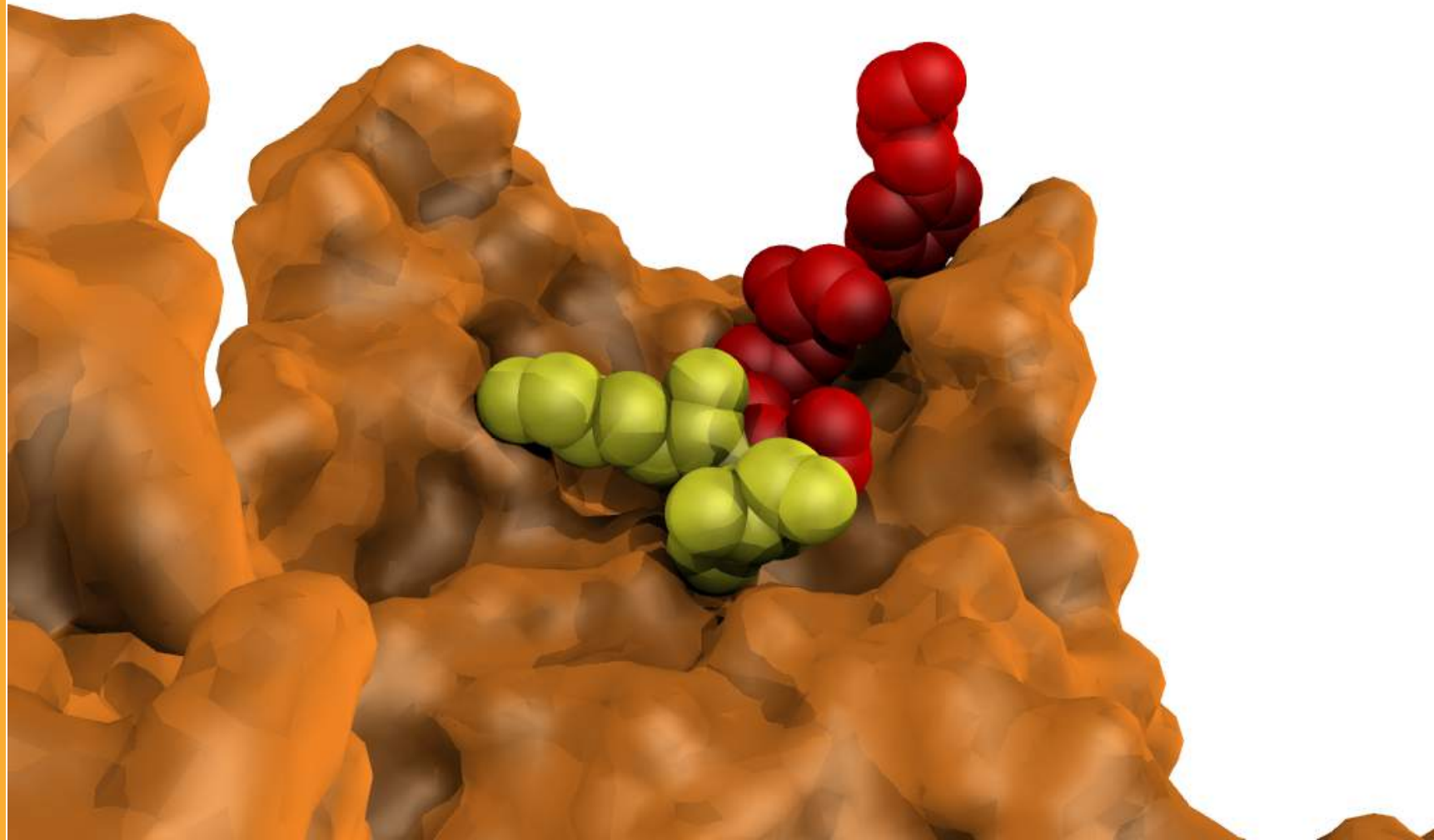


BackView

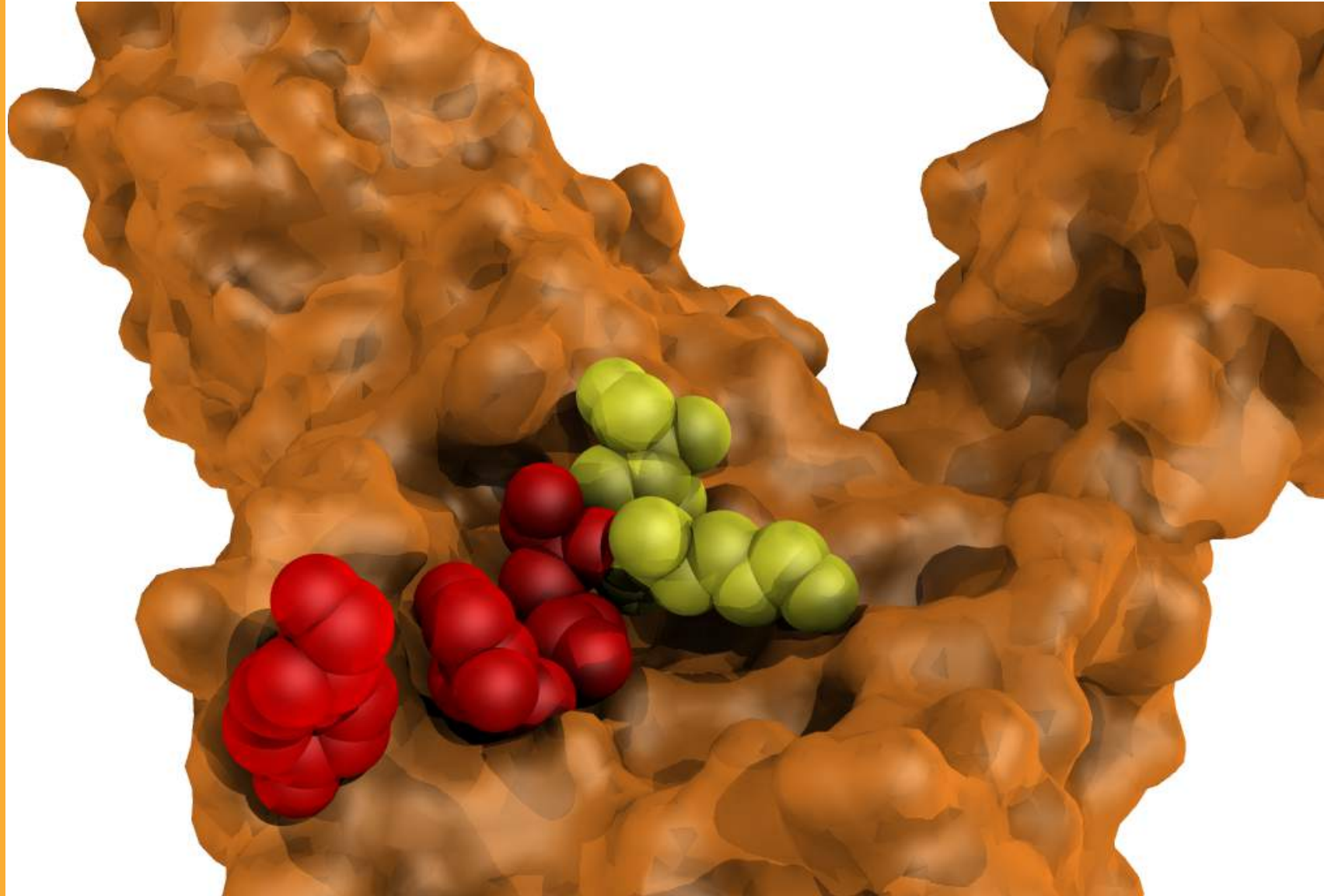
Hot Spot Cavities!!!



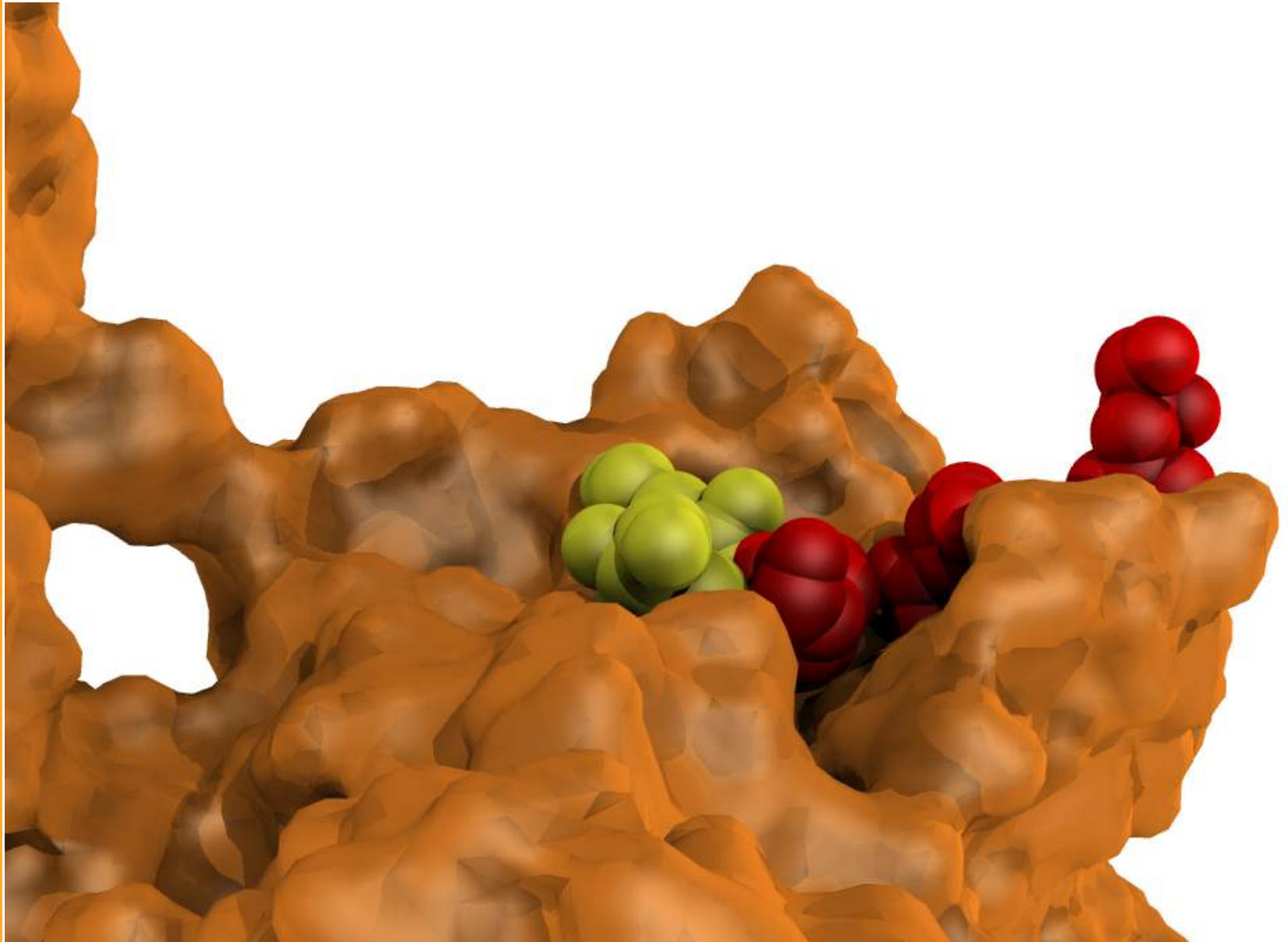
Hot Spot Cavities!!!



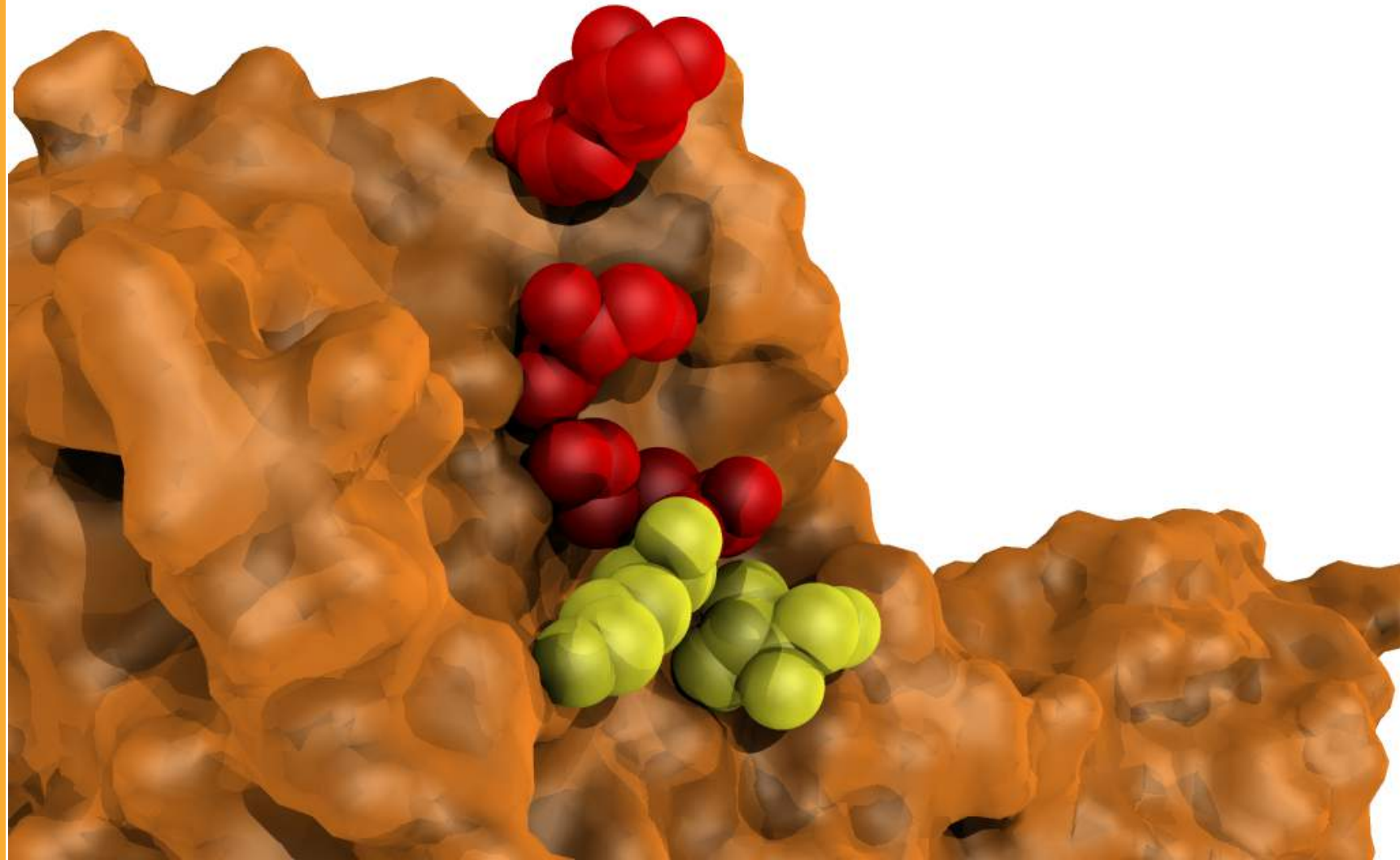
Hot Spot Cavities!!!



Hot Spot Cavities!!!



Hot Spot Cavities!!!



Protein:Protein Association Analyzed by Alanine Scanning Mutagenesis

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