Role of conformational dynamics in the evolution of novel enzymatic activities

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RES Meeting 2017, Santiago de Compostela

Universitat de Girona

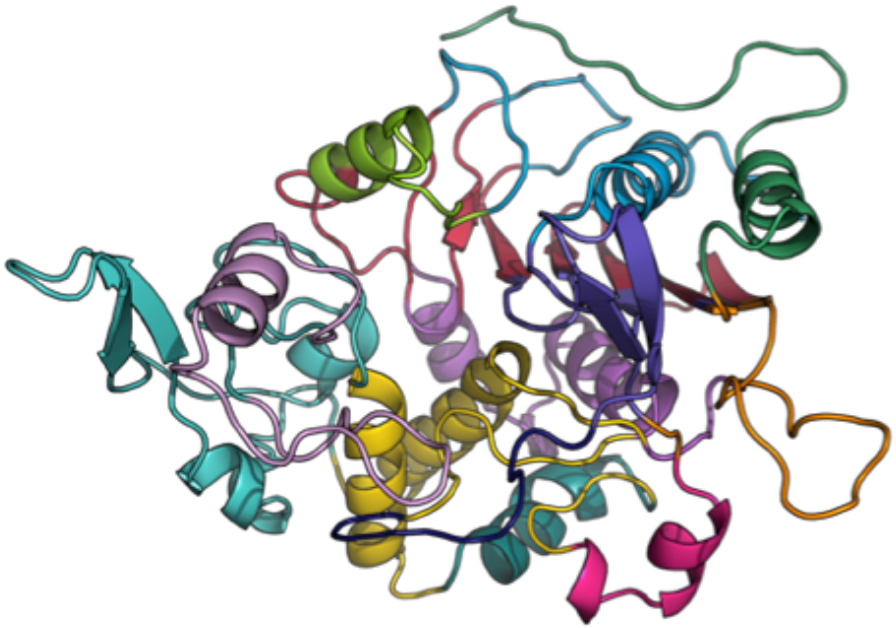
iQCC

CompBioLab

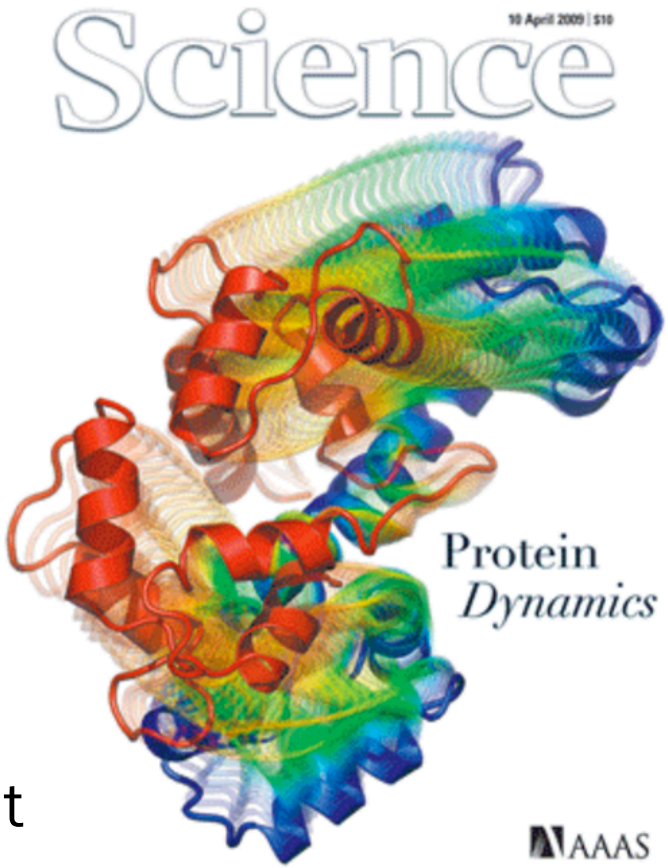
Enzyme Conformational Dynamics

Protein structure–function relationship: RCSB PDB PROTEIN DATA BANK An Information Portal to 107620 Biological Macromolecular Structures

Static view

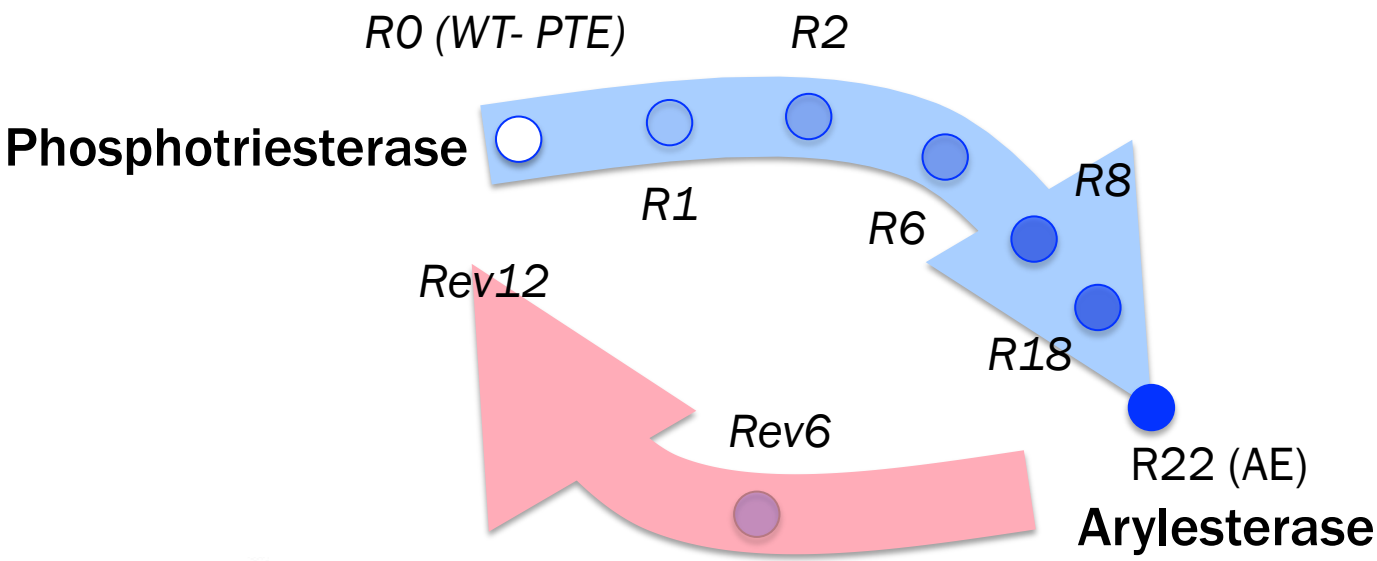


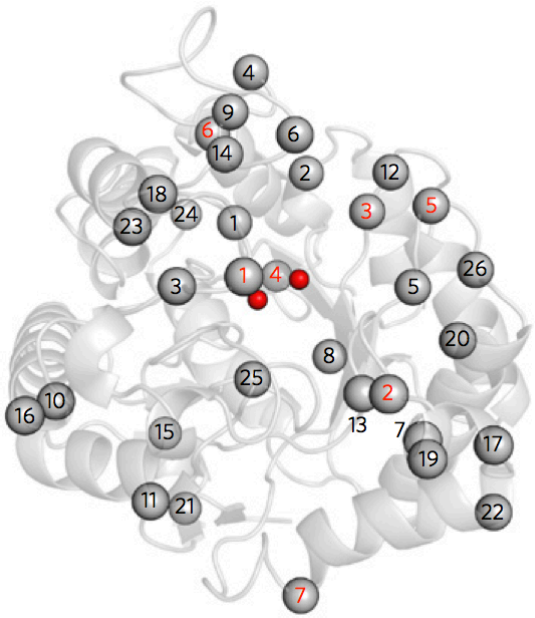
Dynamic view

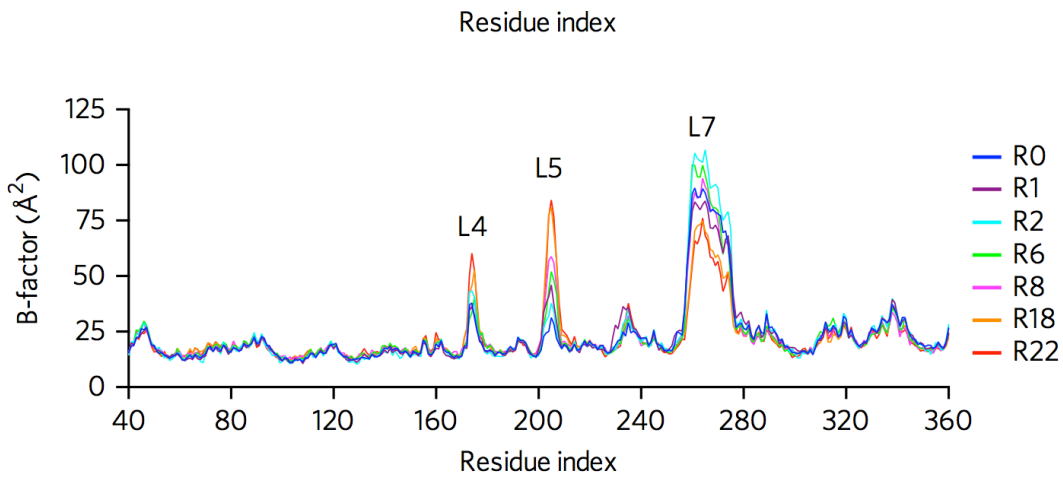


Enzyme **function** originates from the **different structures they adopt**

The importance of enzyme Conformational Dynamics





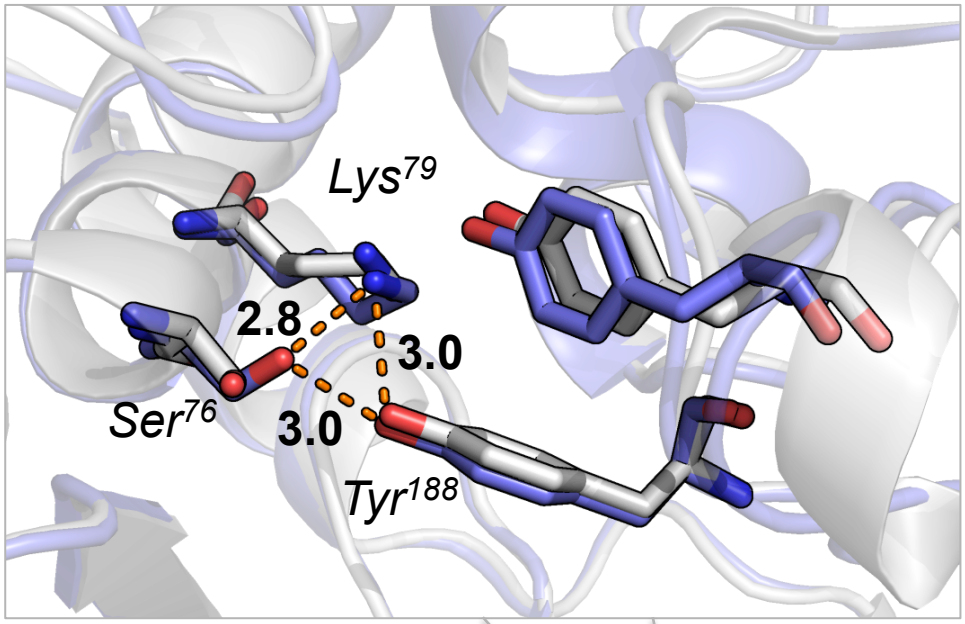


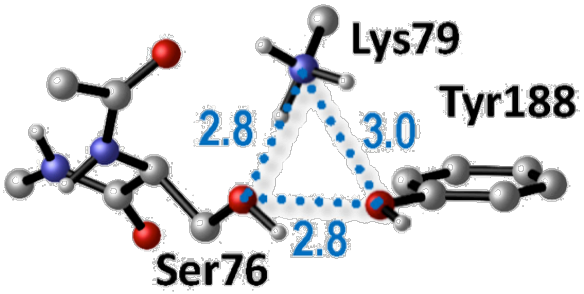
Tokuriki and Jackson *Nat. Chem. Biol.* **12**, 944-950 (**2016**)

The importance of enzyme Conformational Dynamics

Static view

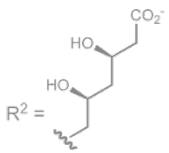
Nature Chemical Biology **2014**, *10*, 431-436

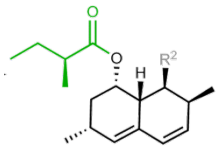




LovD enzyme:

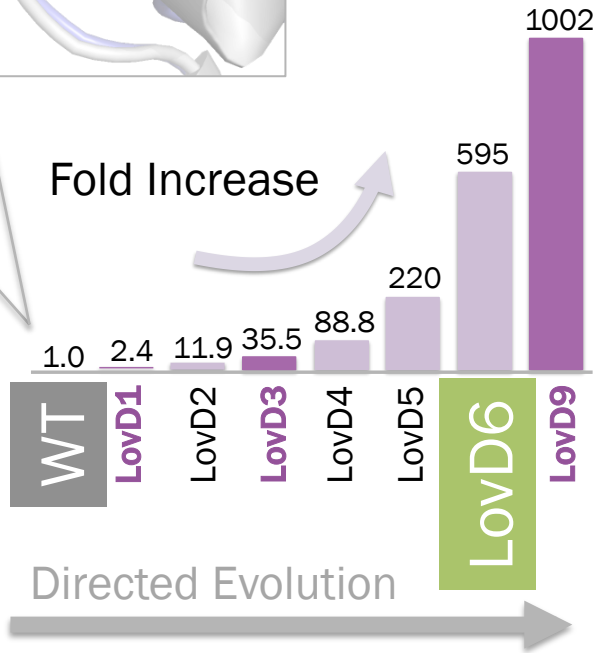
Acyltransferase used for the synthesis of

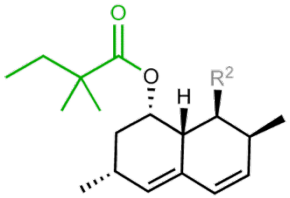




Lovastatin acid (LVA)

Mevacor



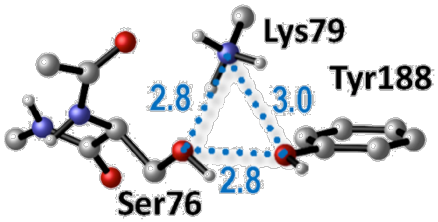


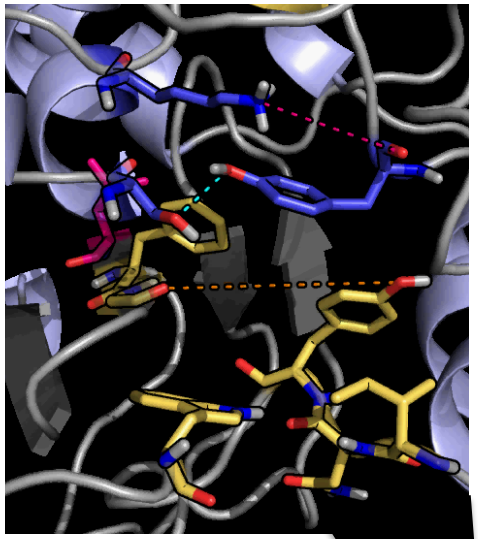
Simvastatin acid (SVA)

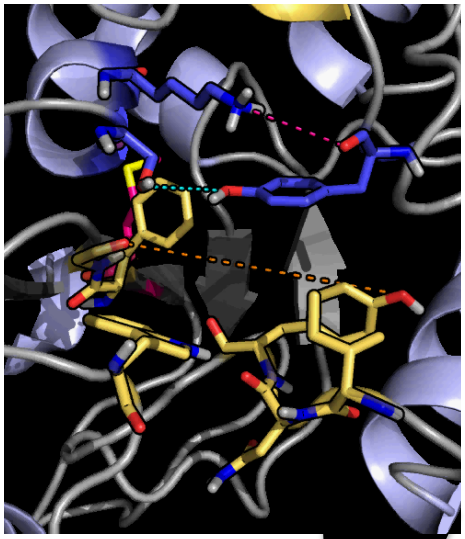
Zocor

The importance of enzyme Conformational Dynamics

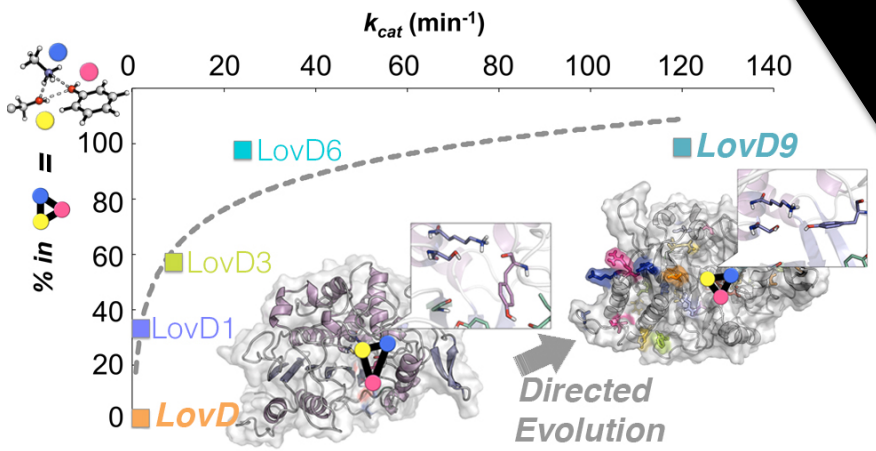
Dynamic view

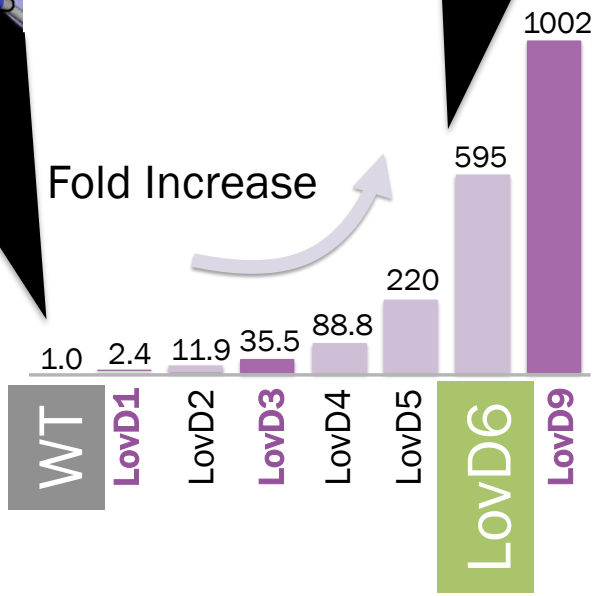






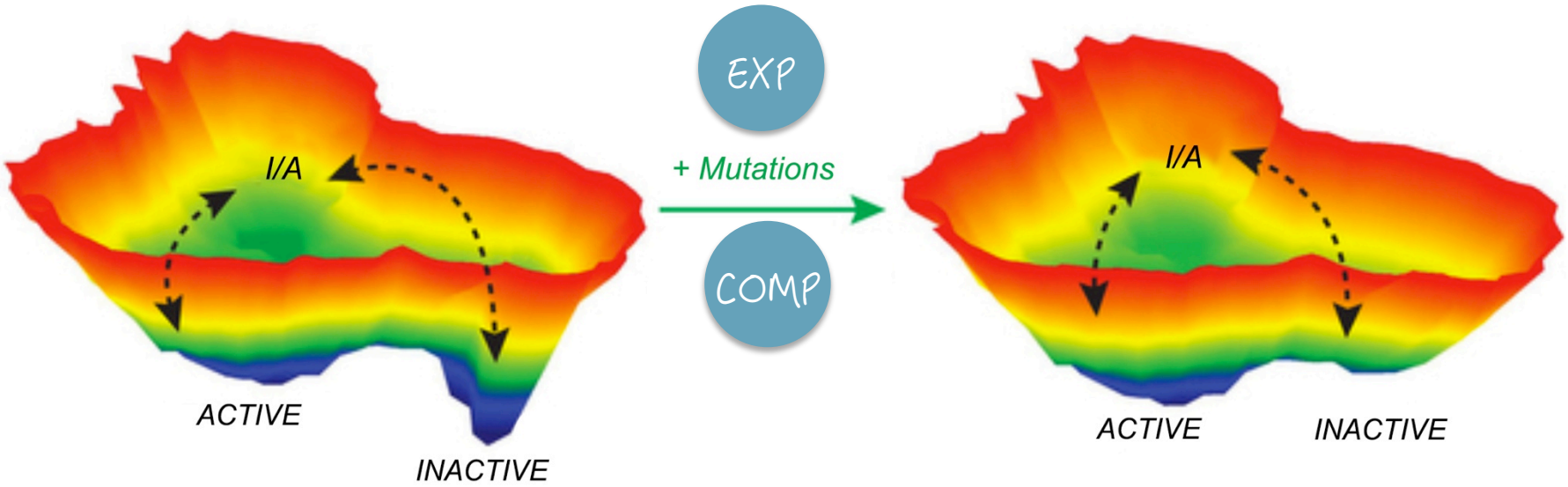
ANTON
1-1.4 μs





Enzyme Evolution towards new function

Population shift towards the Active state:



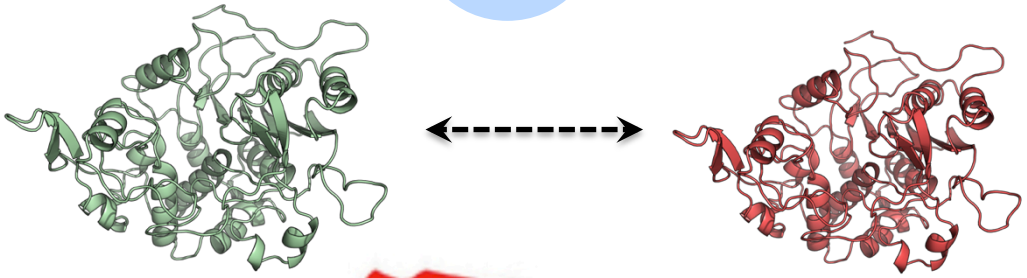
Change the conformational landscape of enzymes to **progressively stabilize pre-existing catalytically active** conformational sub-states

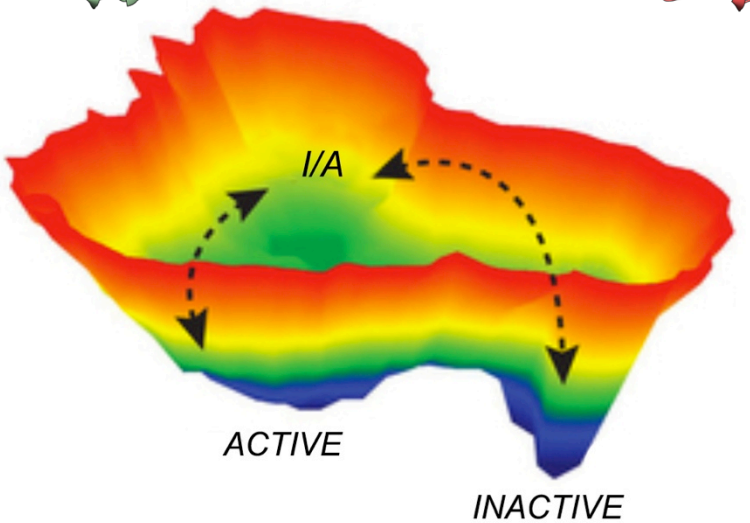
Tokuriki and Jackson *Nat. Chem. Biol.* 12, 944-950 (**2016**)

This Talk

Evaluation of evolved variants

1. Can we explore the enzyme major and minor conformational states?





A. Currin et al., *Chem Soc. Rev.* **2015**, *44*, 1172

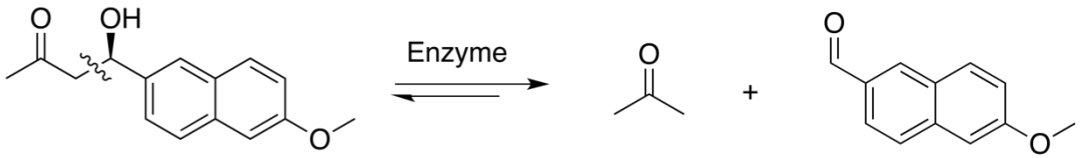
Active site
Distal positions

Correlation-based tools

2. Can we identify which residues are involved in the conformational state conversions?

1. Evaluation of evolved variants: Retro-Aldolase Story

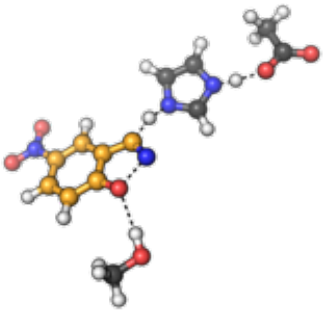
Retro-Aldol:

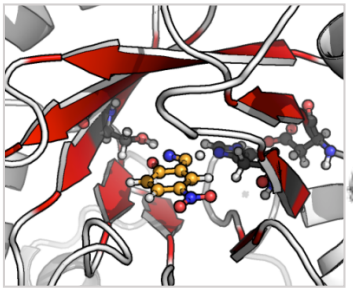


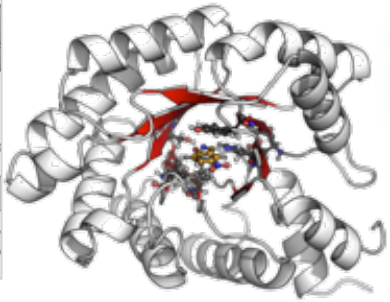
1.

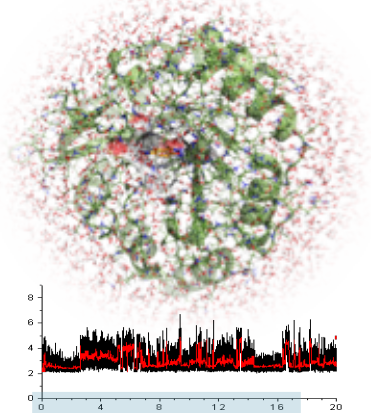
De Novo Computational Design:
Baker & Houk *Science* **2008**, *319*, 1387
Baker, *Prot. Sci.* **2012**, *21*, 717

Houk & Baker
Inside-out protocol









Transition structure GAUSSIAN-QM theozyme

-Ideal geometry
-Rate acceleration

Match theozyme to protein backbone RosettaMatch-EDGE – rank matches

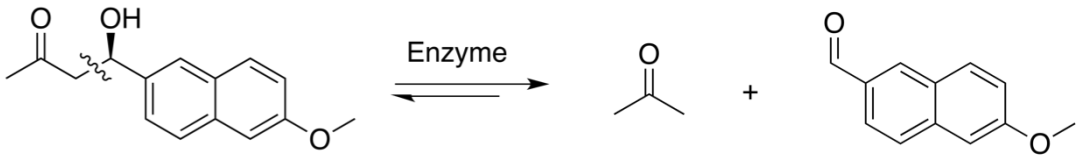
Design/ Repack RosettaDesign Rank and Filter

MD simulations Protein structure, stability

RA95.0

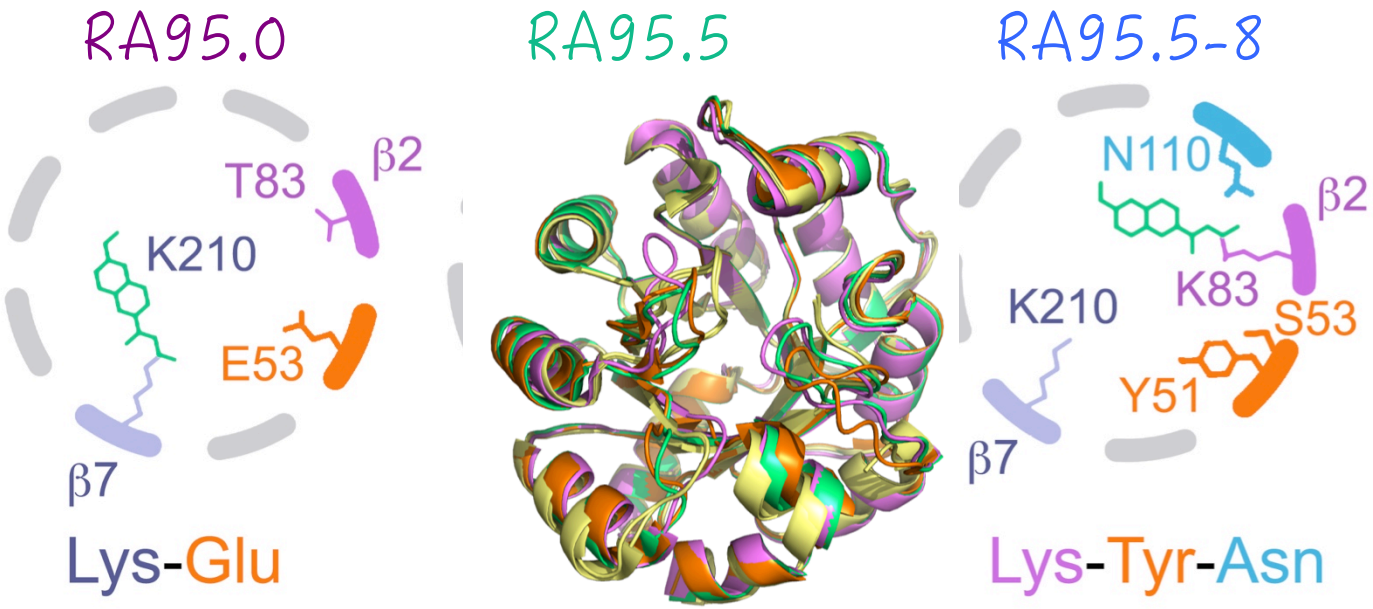
1. Evaluation of evolved variants: Retro-Aldolase Story

Retro-Aldol:



2.

Directed Evolution:
Complete active site remodeling
Ban & Hilvert *Nat. Chem. Biol.* 2013, 9, 494



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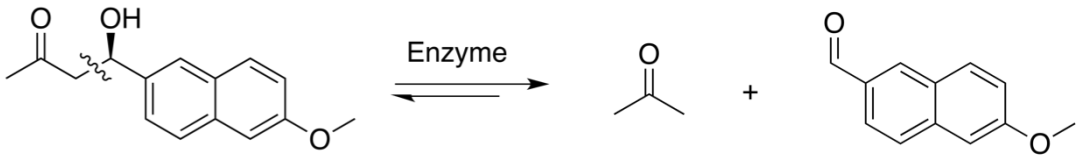
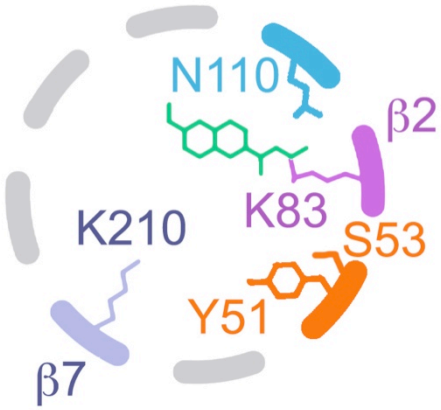
Retro-Aldol:



3.

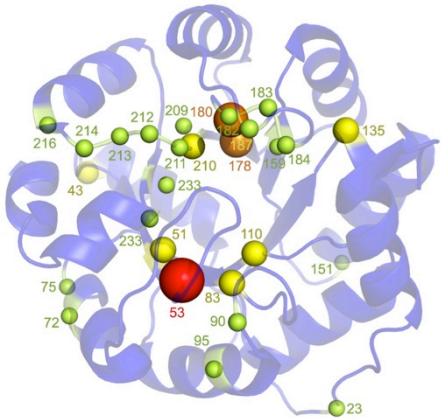
Ultrahigh-throughput screening:
Emergence of a catalytic tetrad
Griffiths & Hilvert *Nat. Chem.* **2017**, 9, 50

RA95.5-8

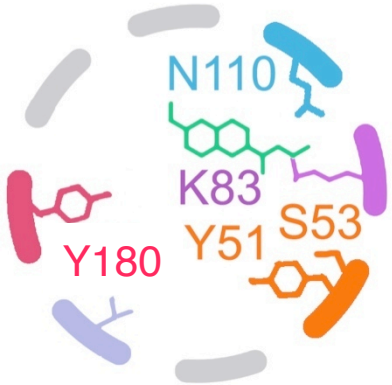


Lys-Tyr-Asn

Rounds of DE



RA95.8F



Lys-Tyr-Asn-Tyr

1. Evaluation of evolved variants: Retro-Aldolase Story

1. De Novo Computational Design:
Baker & Houk *Science* **2008**, *319*, 1387
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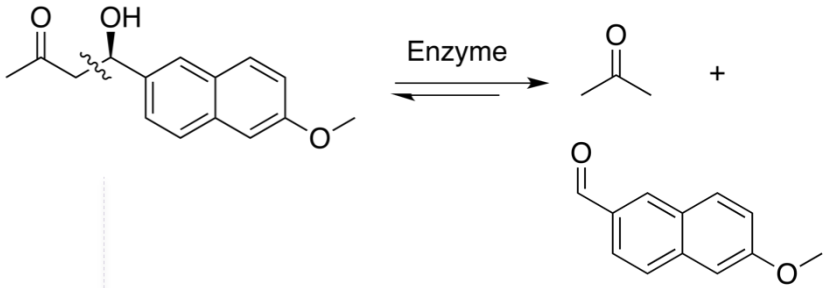
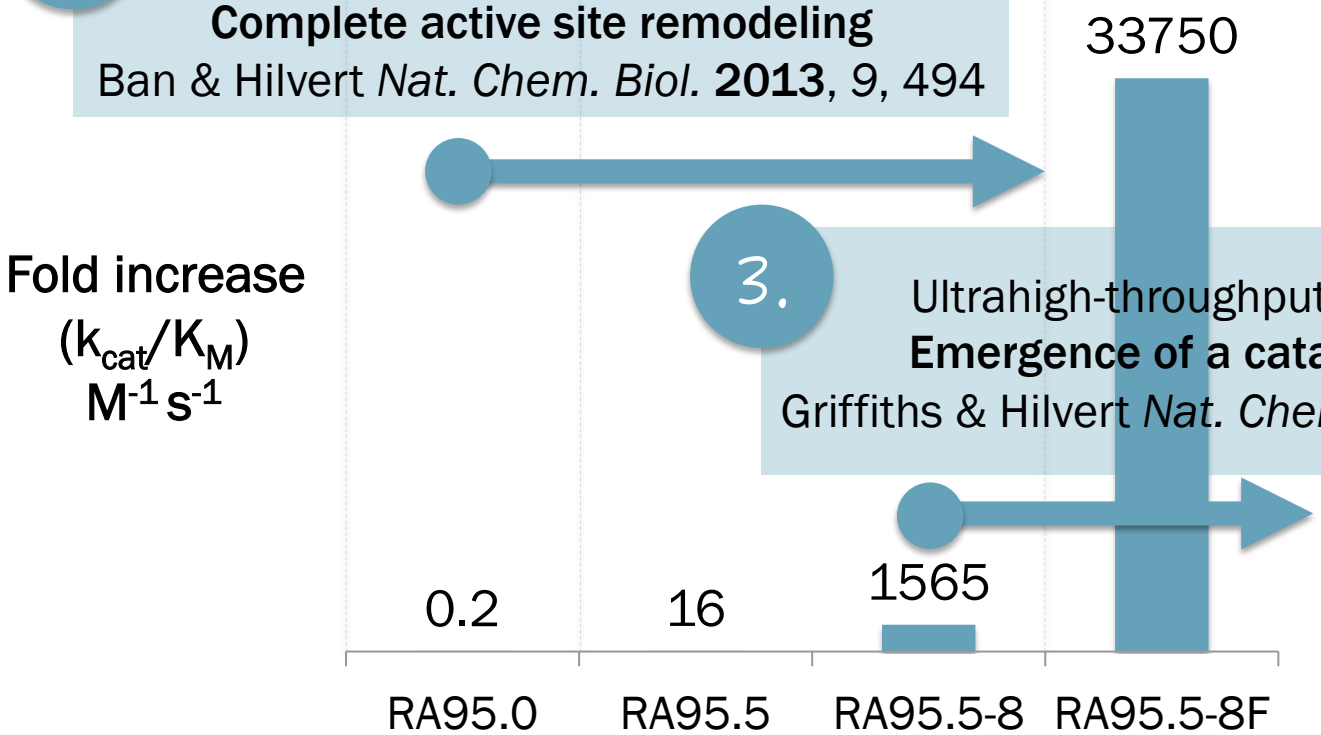
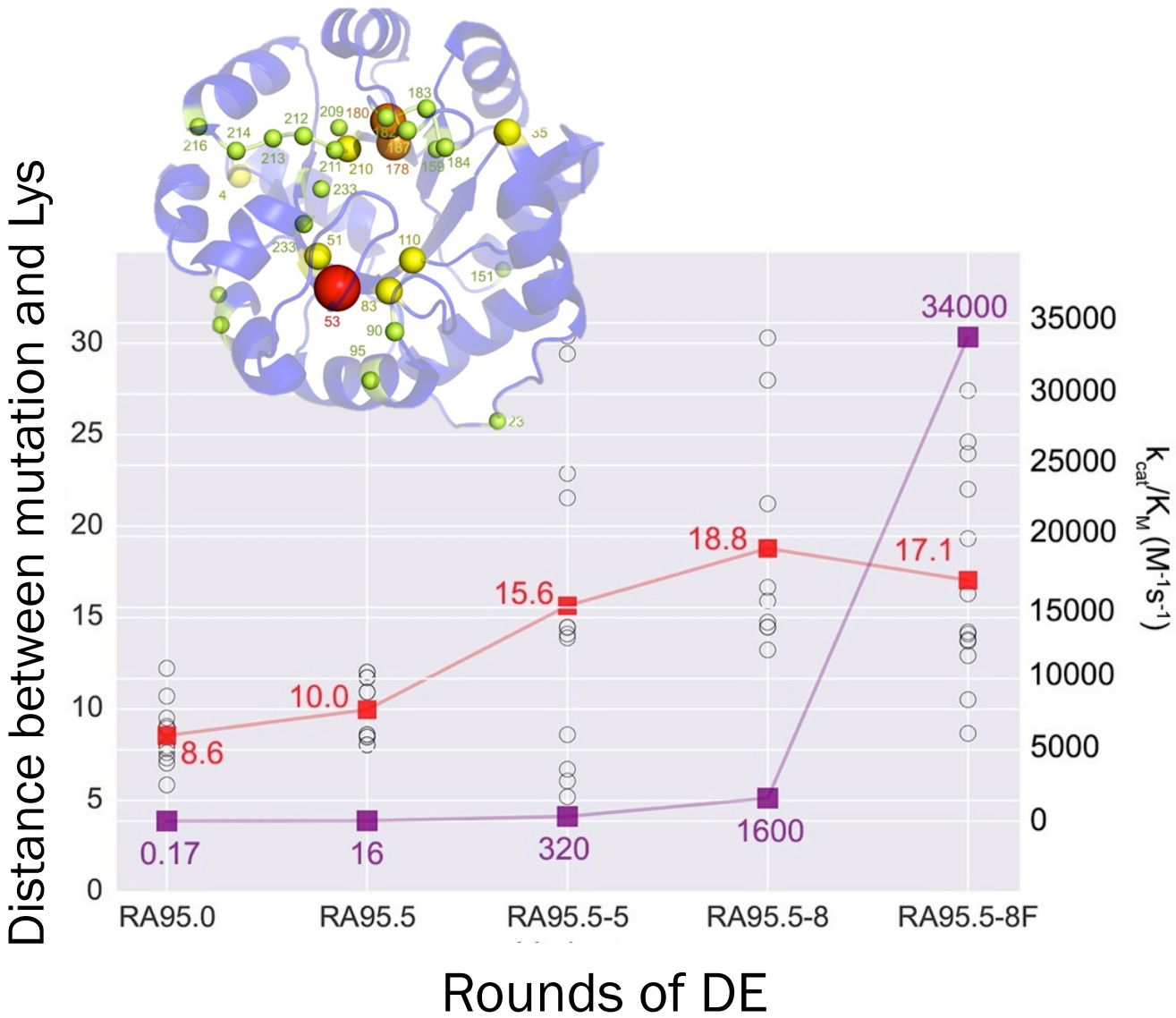
Retro-Aldol:



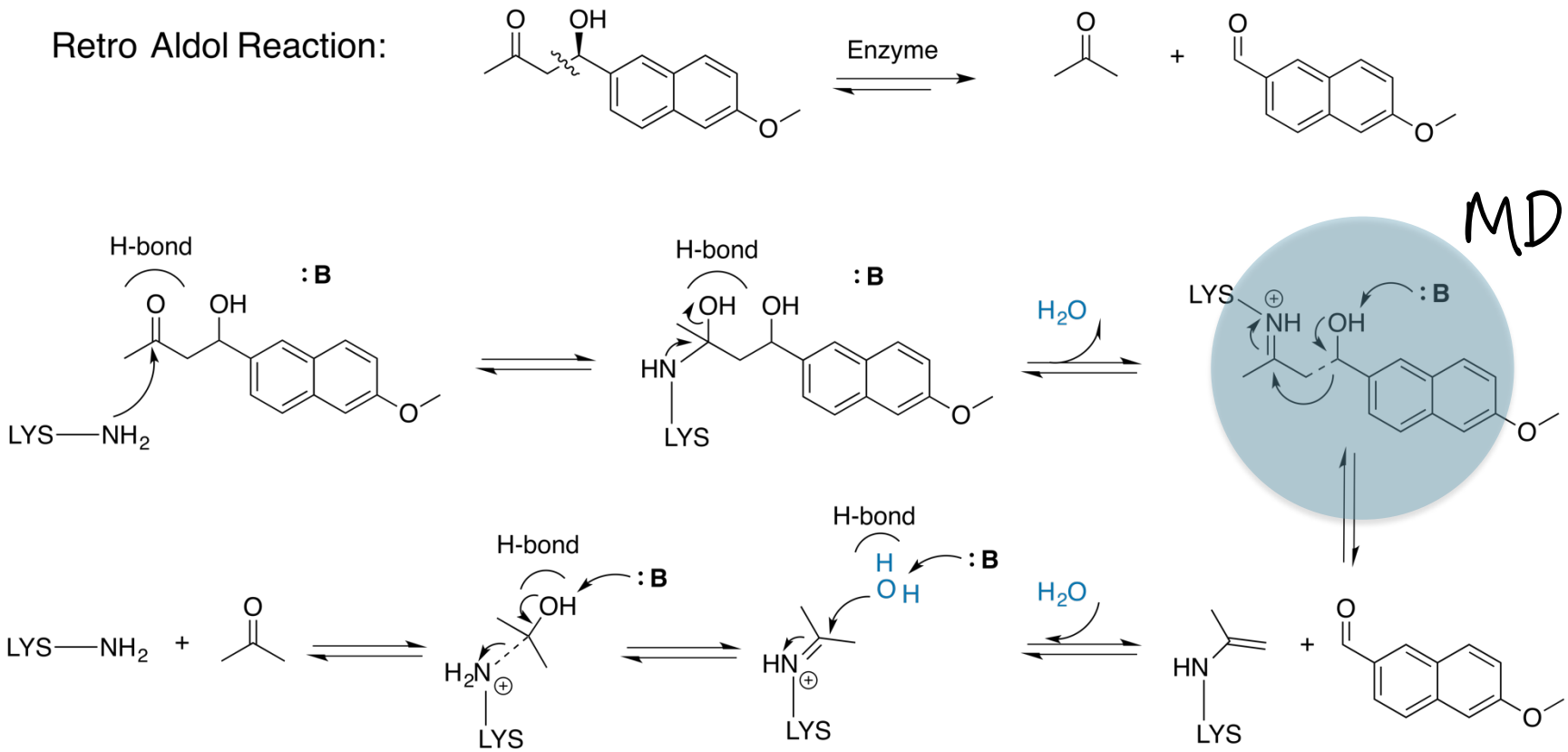


1. Evaluation of evolved variants: Distal mutations



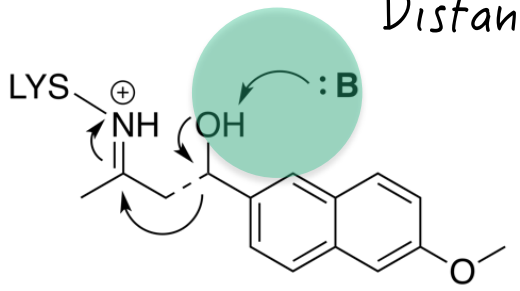
1. Evaluation of evolved variants: Retro-Aldolase mechanism

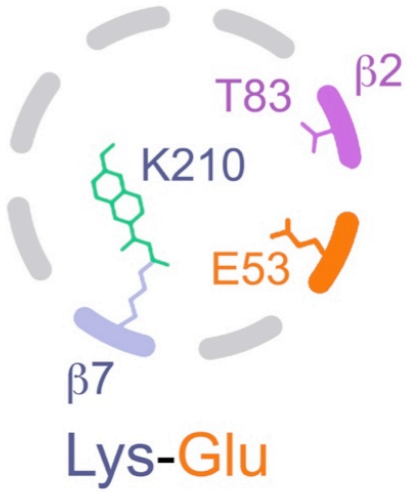
Retro Aldol Reaction:

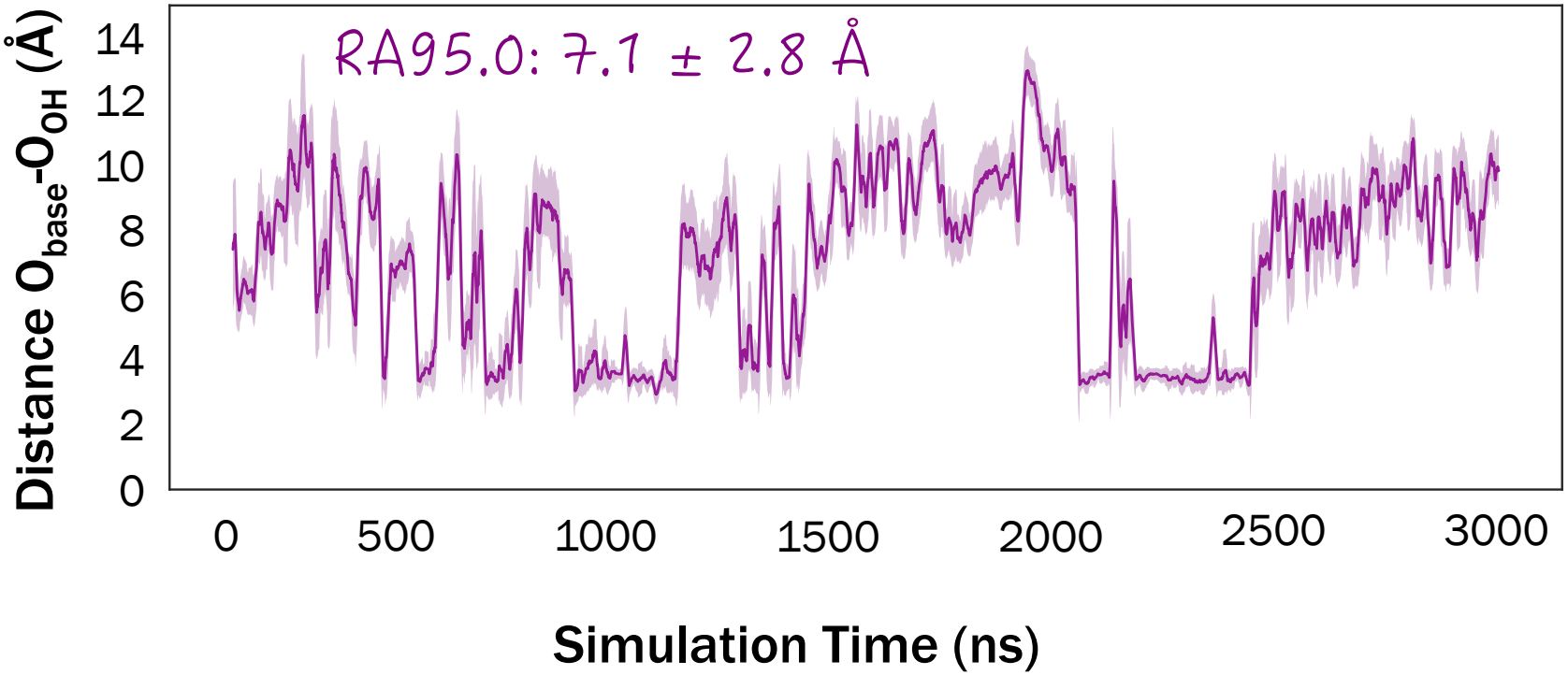


1. Evaluation of evolved variants: RA95.0

Distance base and OH

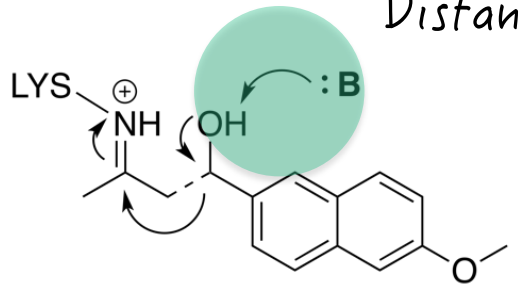


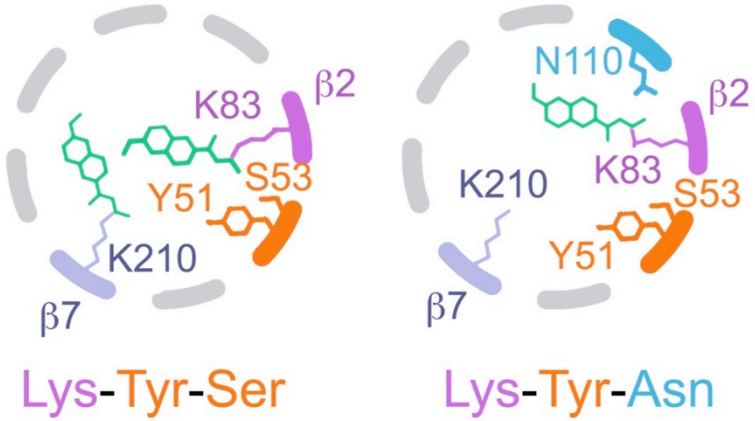


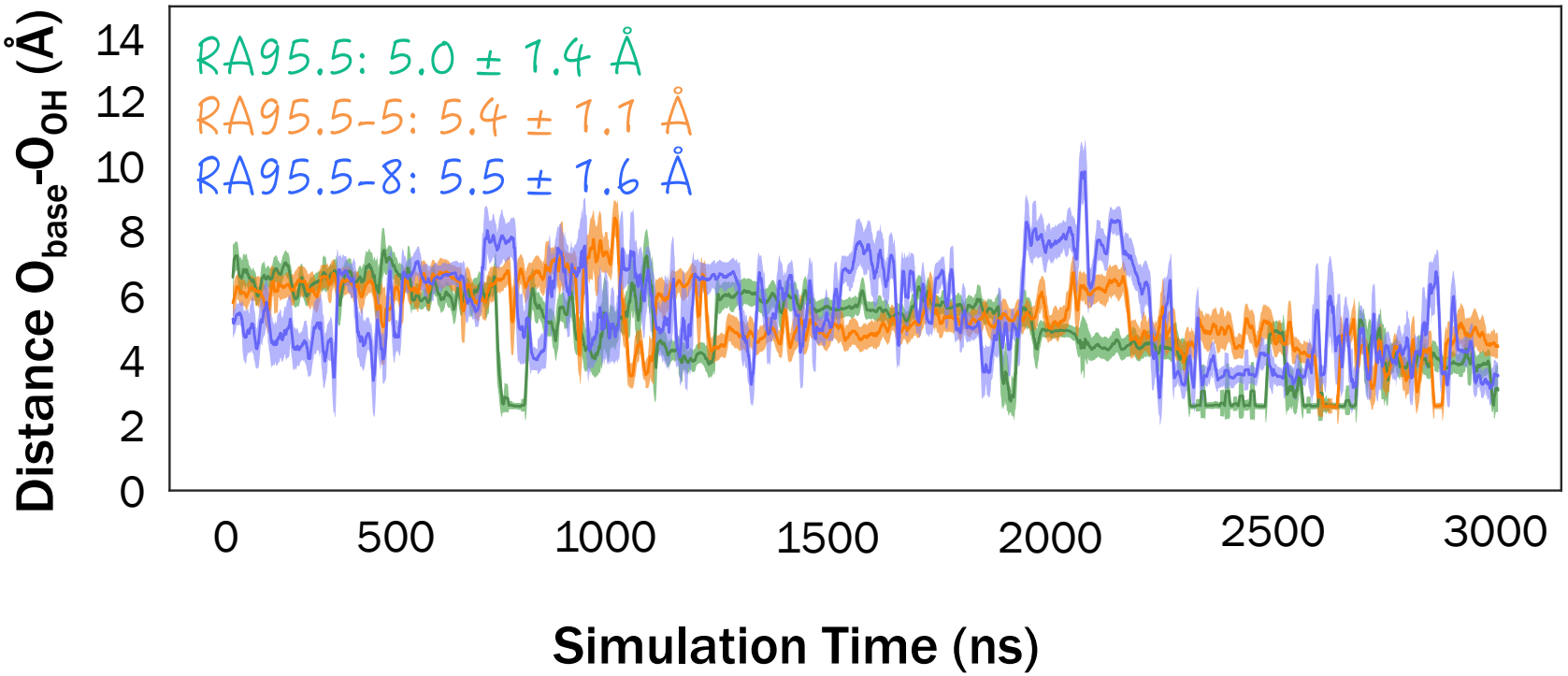


1. Evaluation of evolved variants: RA95.5 ⟶ RA95.5-8

Distance base and OH

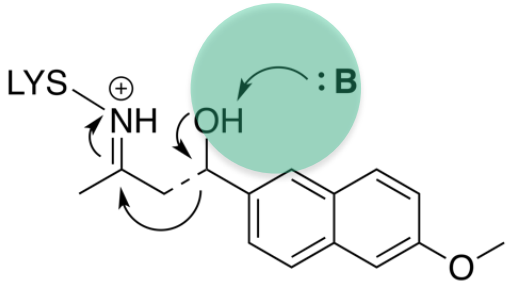


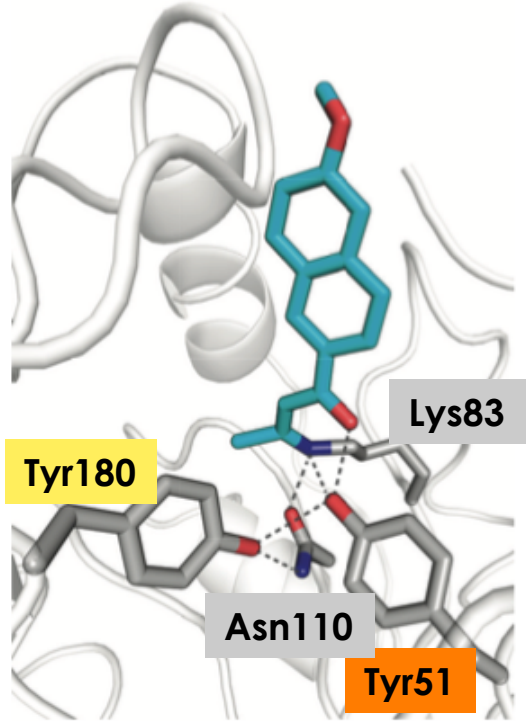


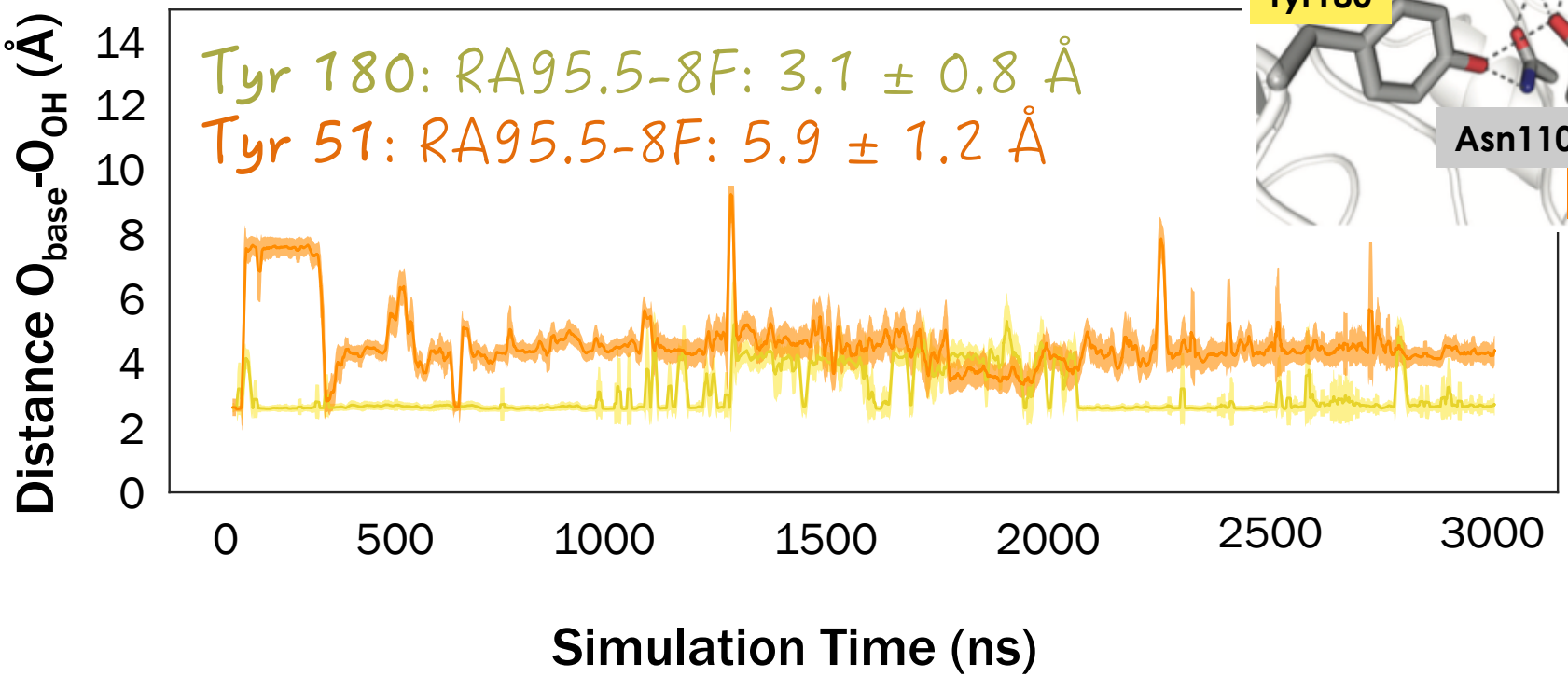


1. Evaluation of evolved variants: RA95.5-8 ⟶ RA95.5-8F

Distance base and OH

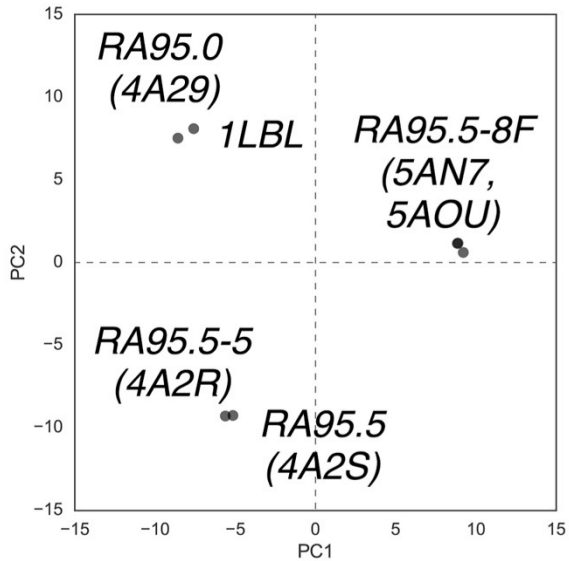


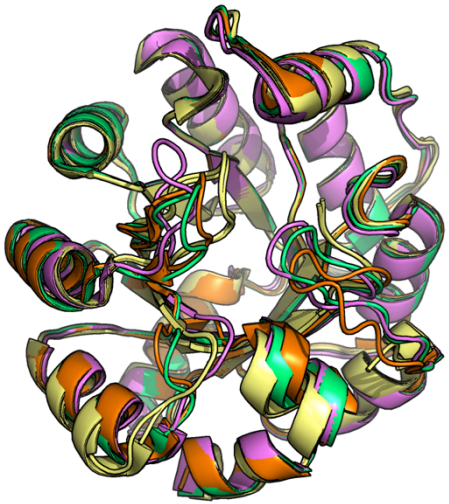


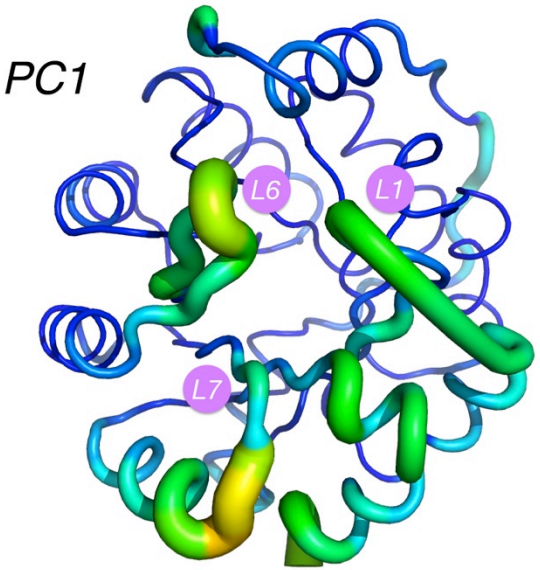


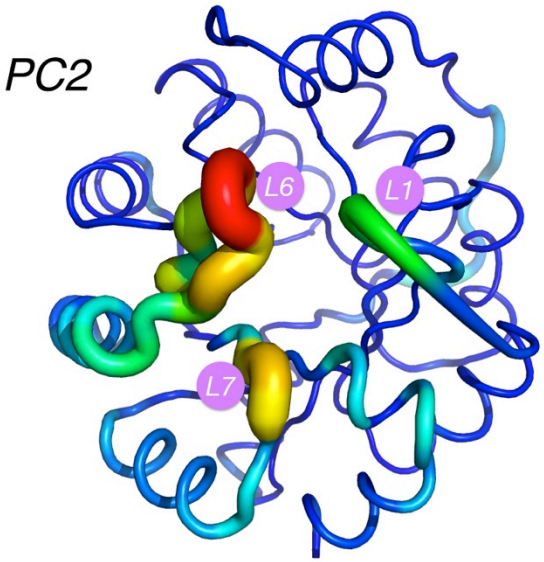
1. Evaluation of evolved variants: PCA analysis on X-ray

Principal Component Analysis on available X-ray structures:



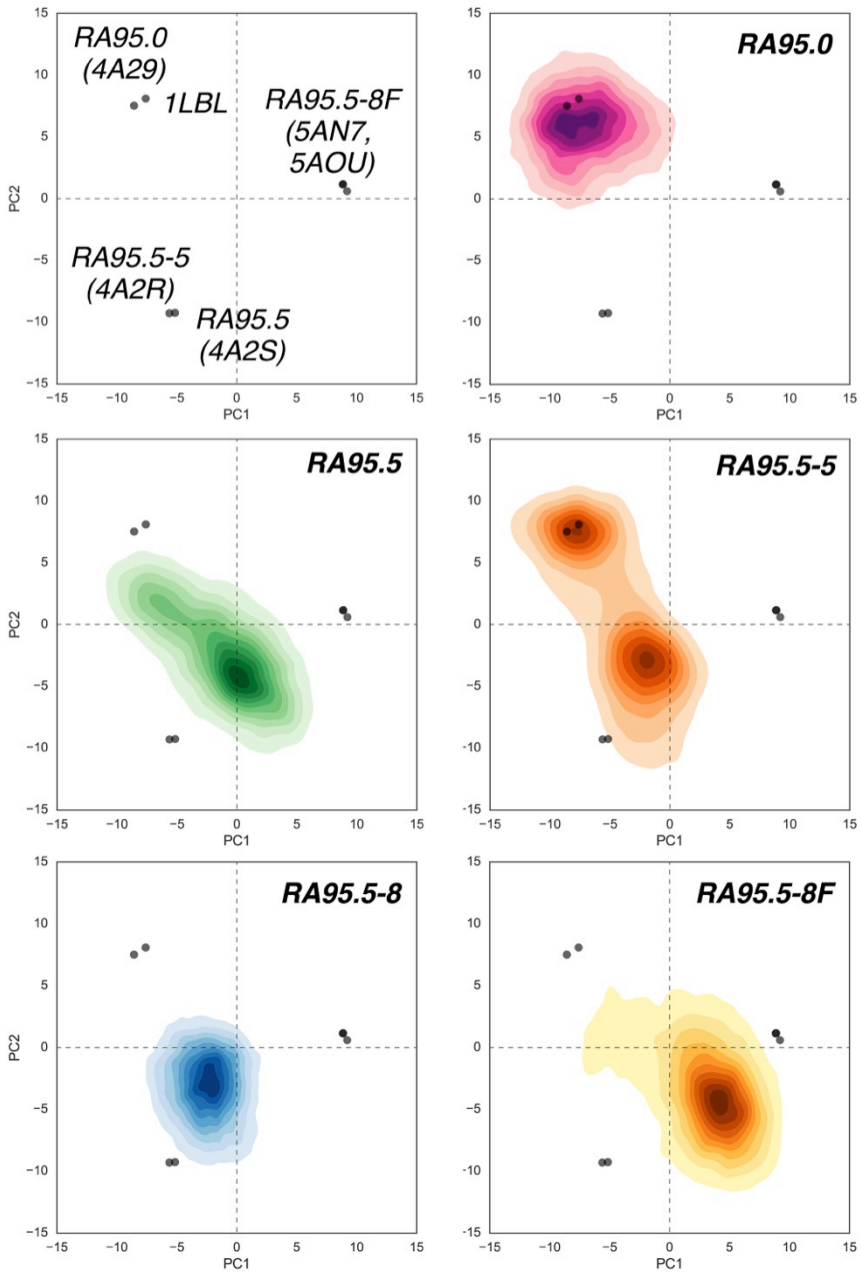




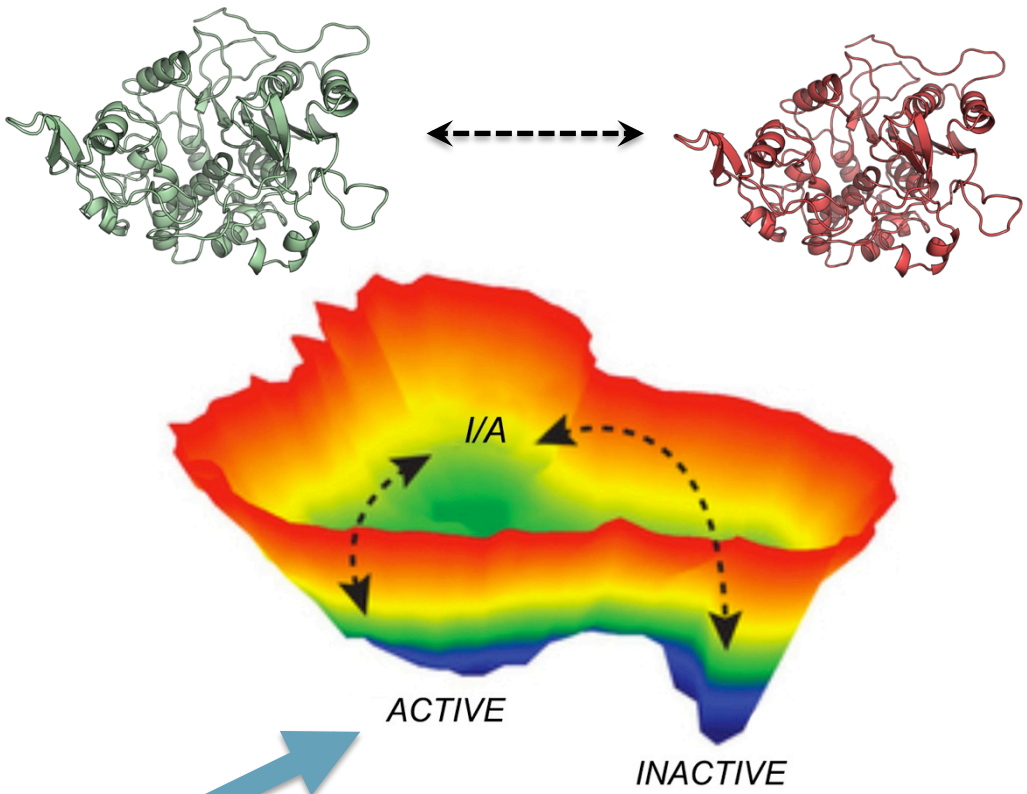


1. Evaluation of evolved variants: PCA analysis on X-ray

MD trajectories projected on Principal Component Analysis on available X-ray structures:



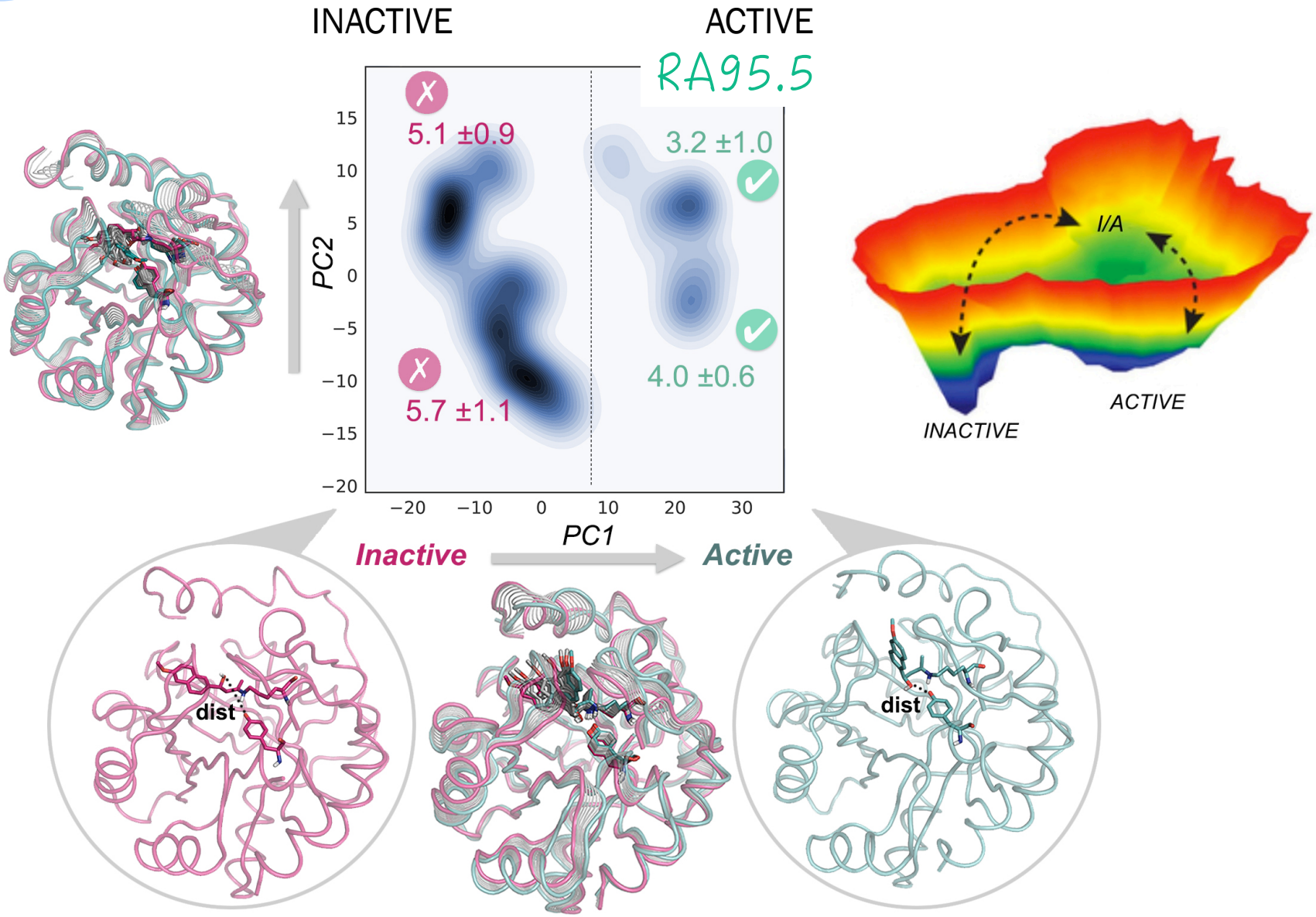
1. Evaluation of evolved variants: PCA analysis on MD data



Can we identify active and inactive states?

Principal Component Analysis on MD trajectories

1. Evaluation of evolved variants: Active and Inactive states



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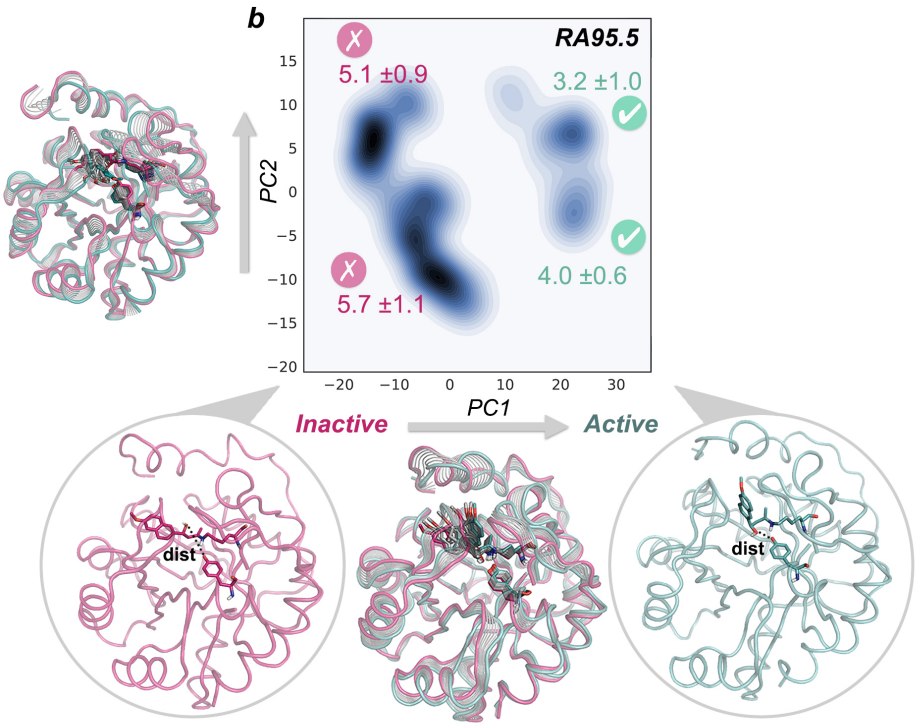

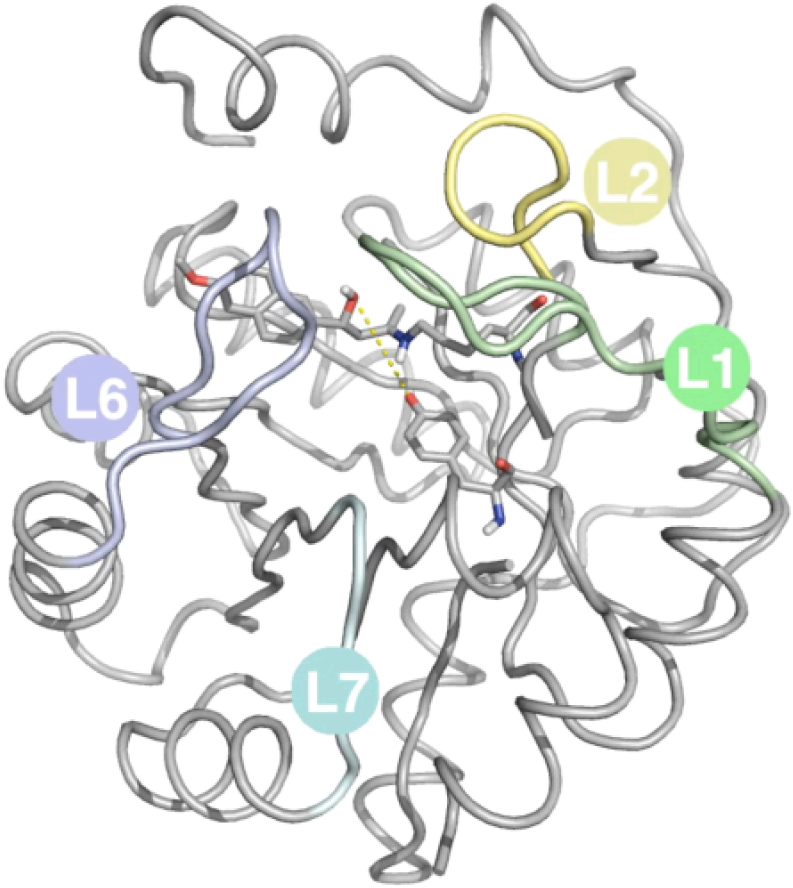

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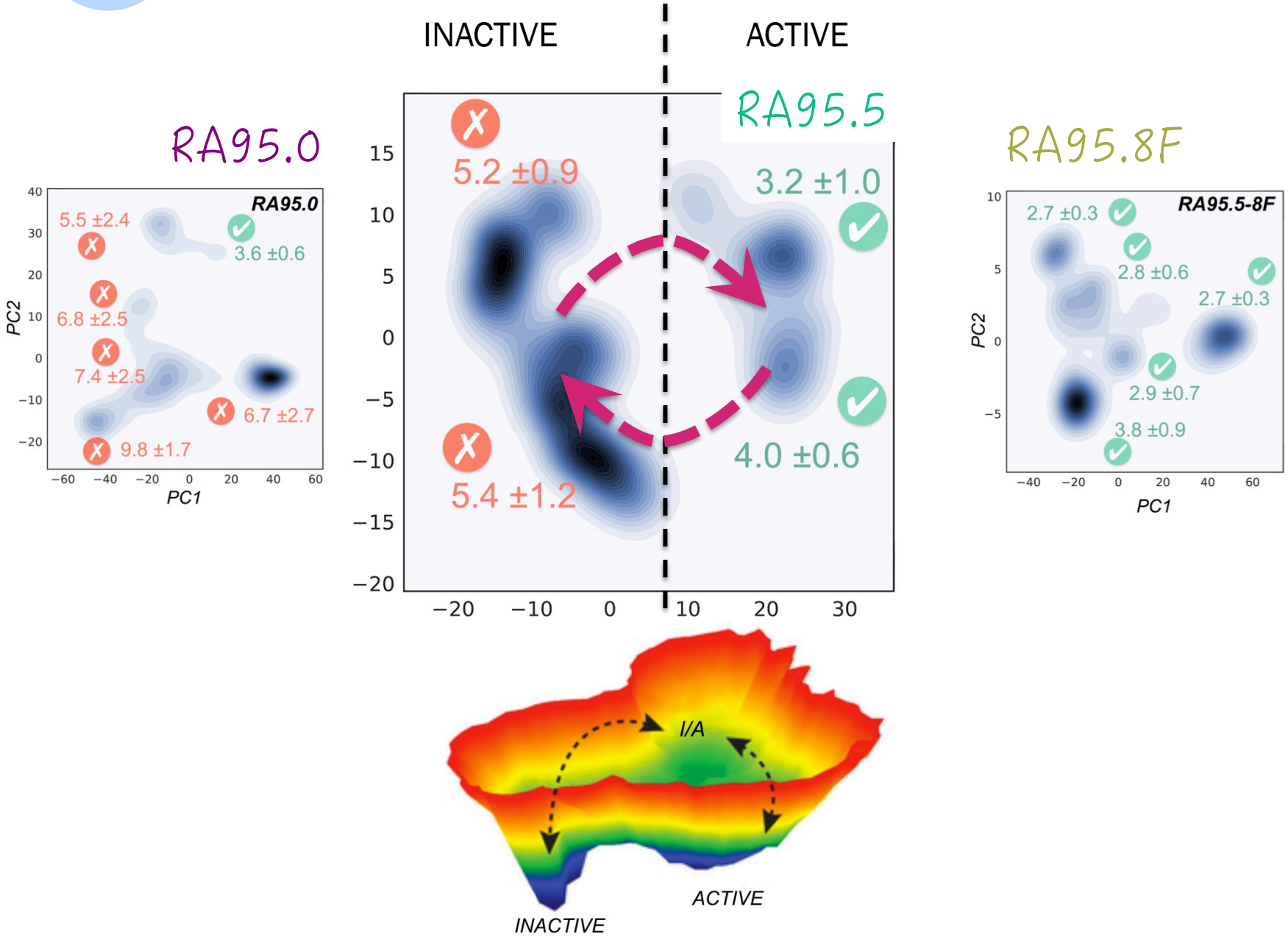

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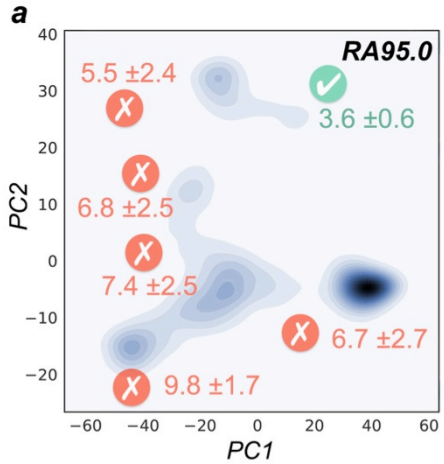
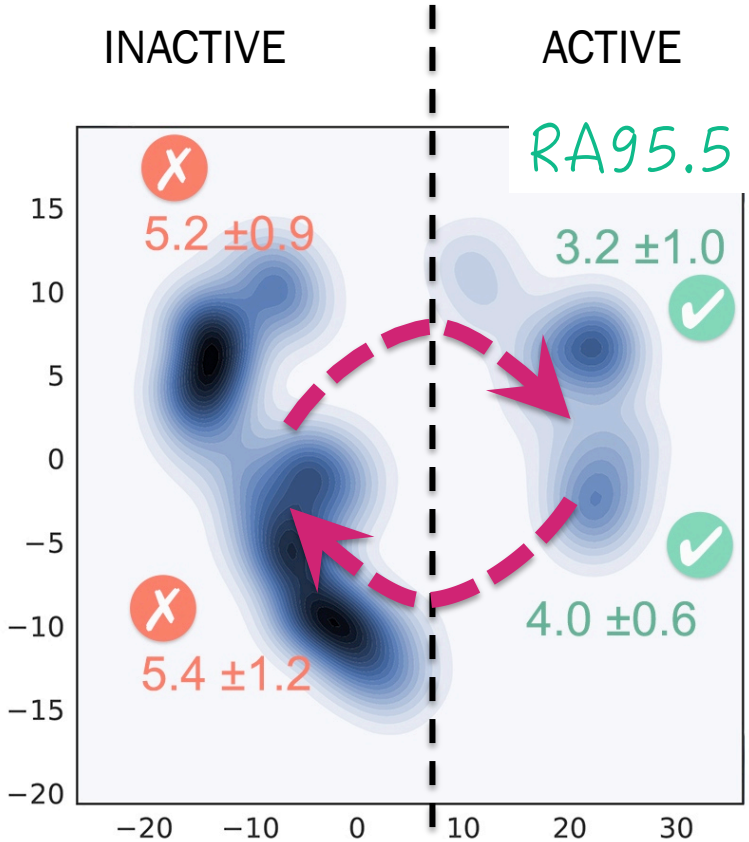
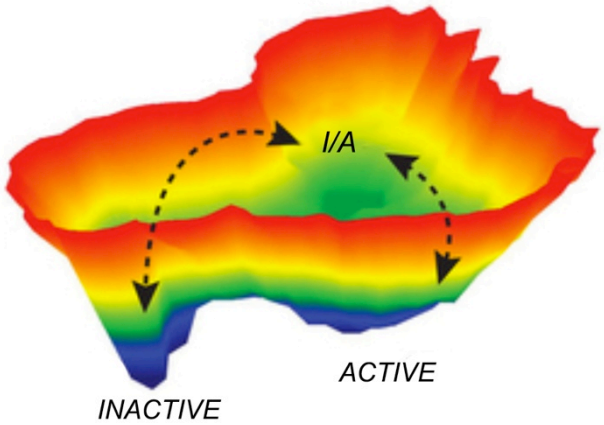


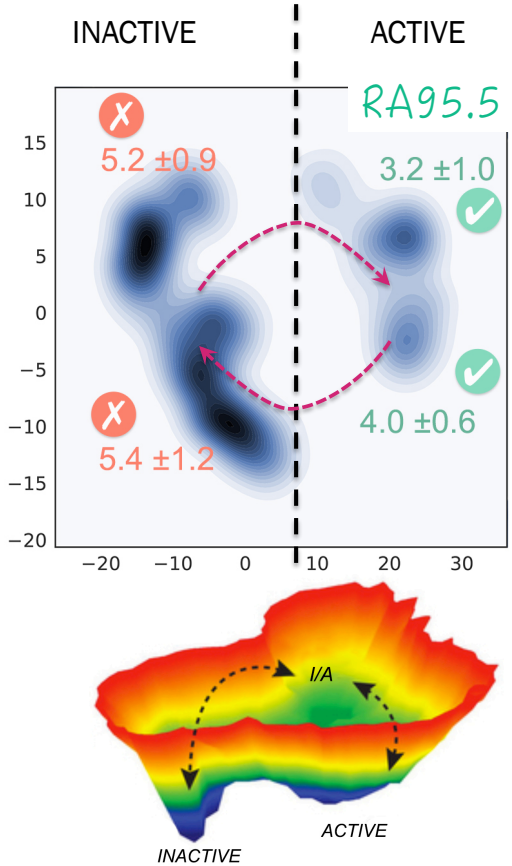


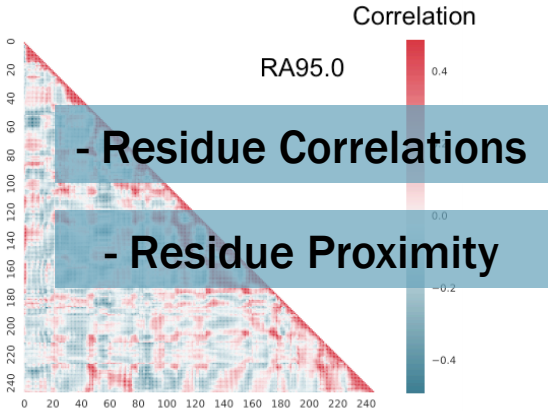


2. Development of new tools

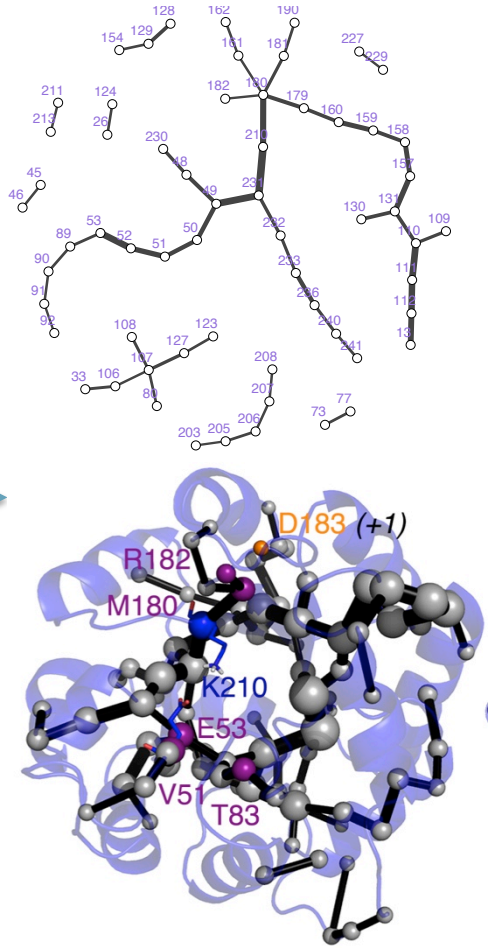
Can we identify which residues are involved in the conformational state conversions?

2. Development of new tools Computational Design of Enzymes





- Residue Correlations
- Residue Proximity

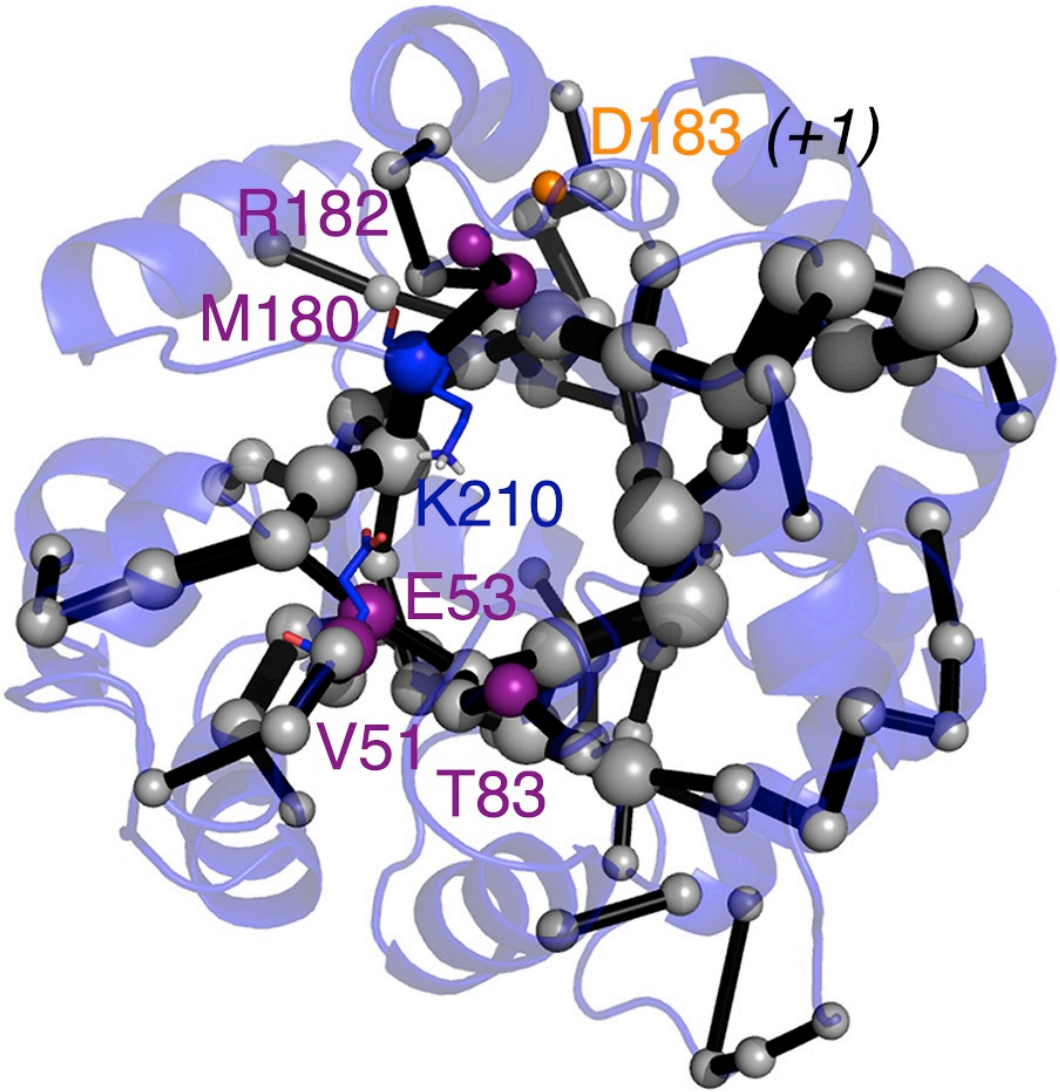


Shortest Path MAP

Identifying residues involved:

INACTIVE → ACTIVE transitions

RA95.0 → RA95.5



6 DE mutations:

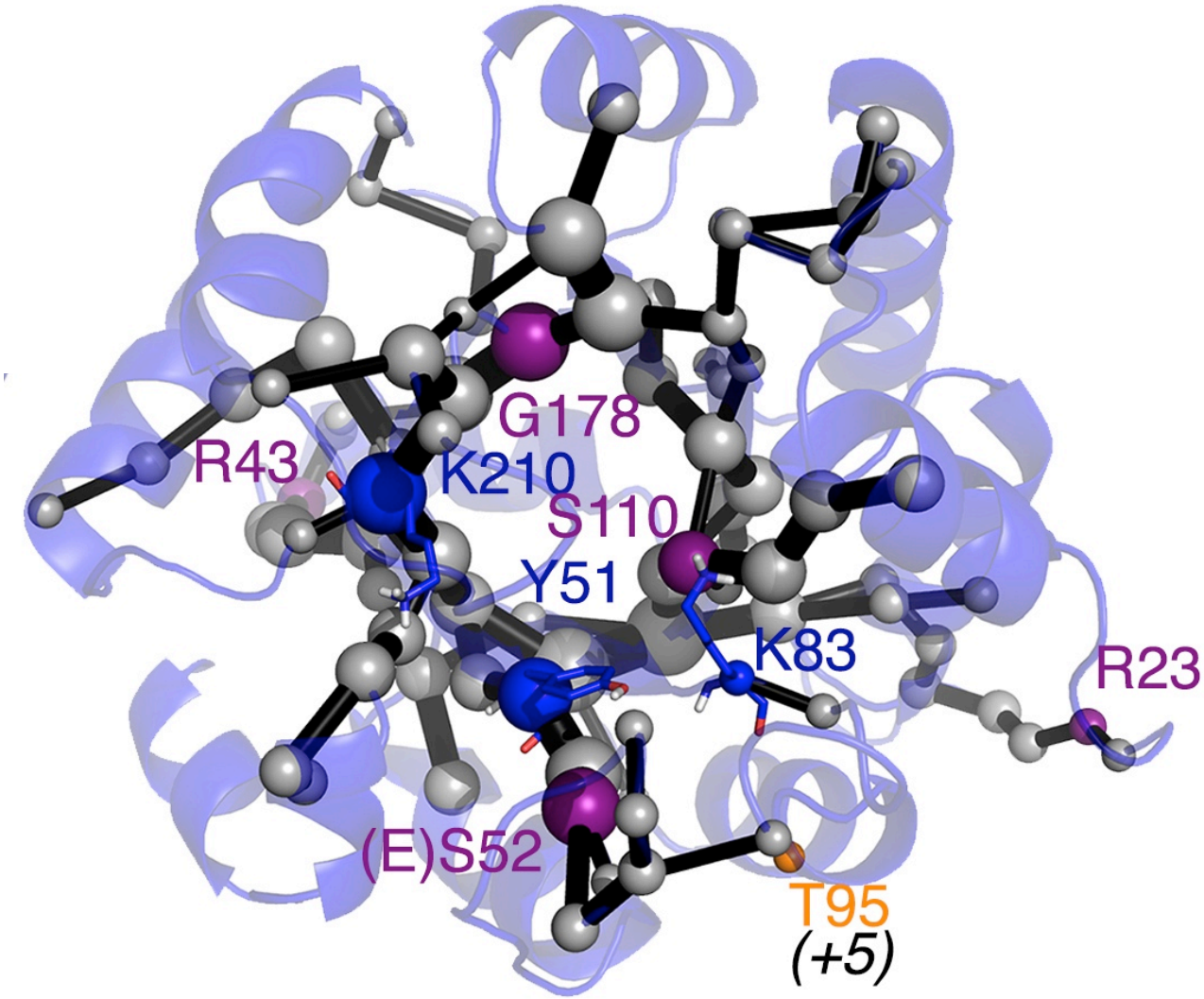
5 identified with Shortest Path Analysis

1 Adjacent position

2. Development of new tools

RA95.5 ⟶ RA95.5-5

RA95.5 → RA95.5-5



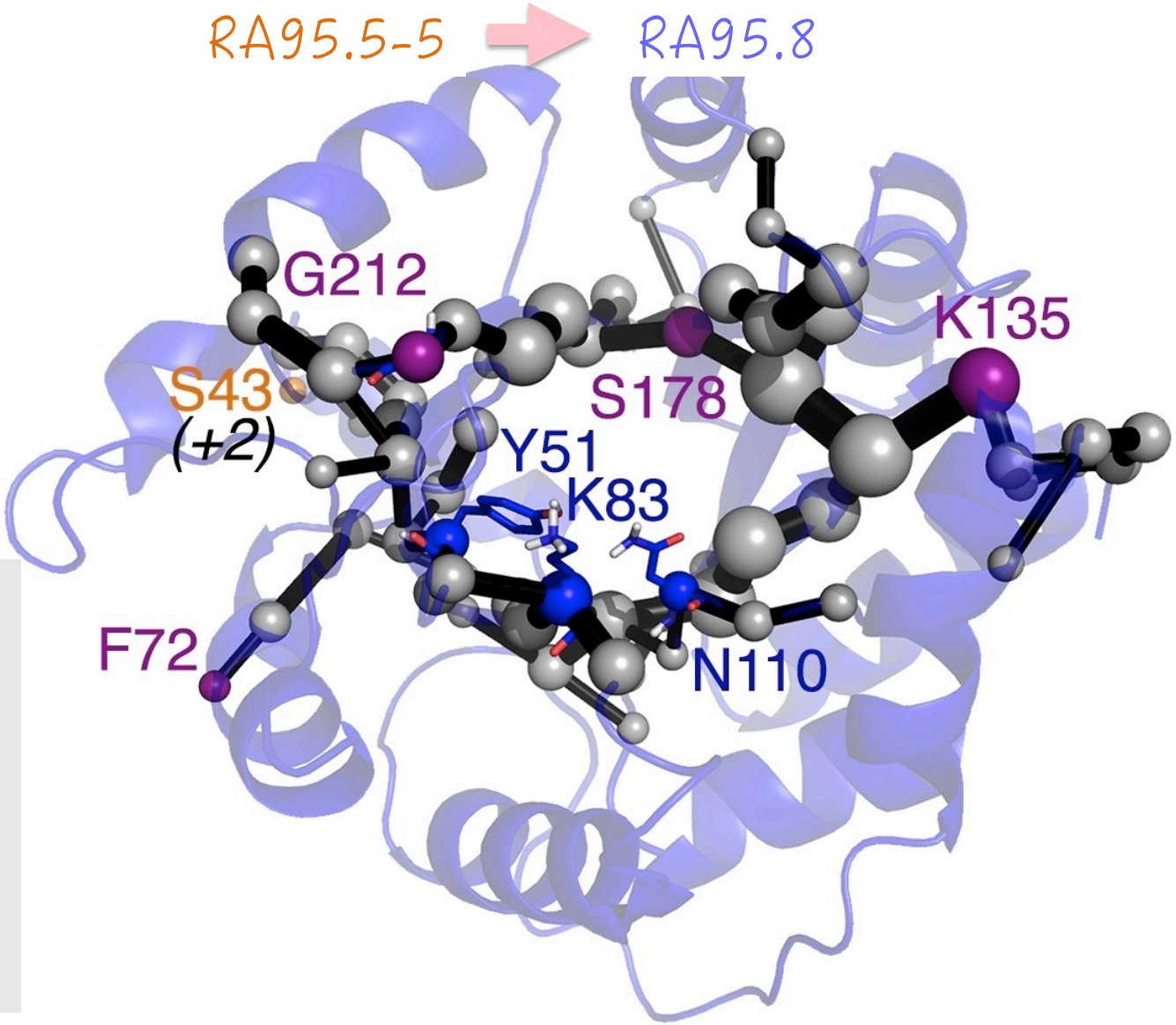
6 DE mutations:

5 identified with Shortest Path Analysis

1 Adjacent position

2. Development of new tools

RA95.5-5 ⟶ RA95.5-8



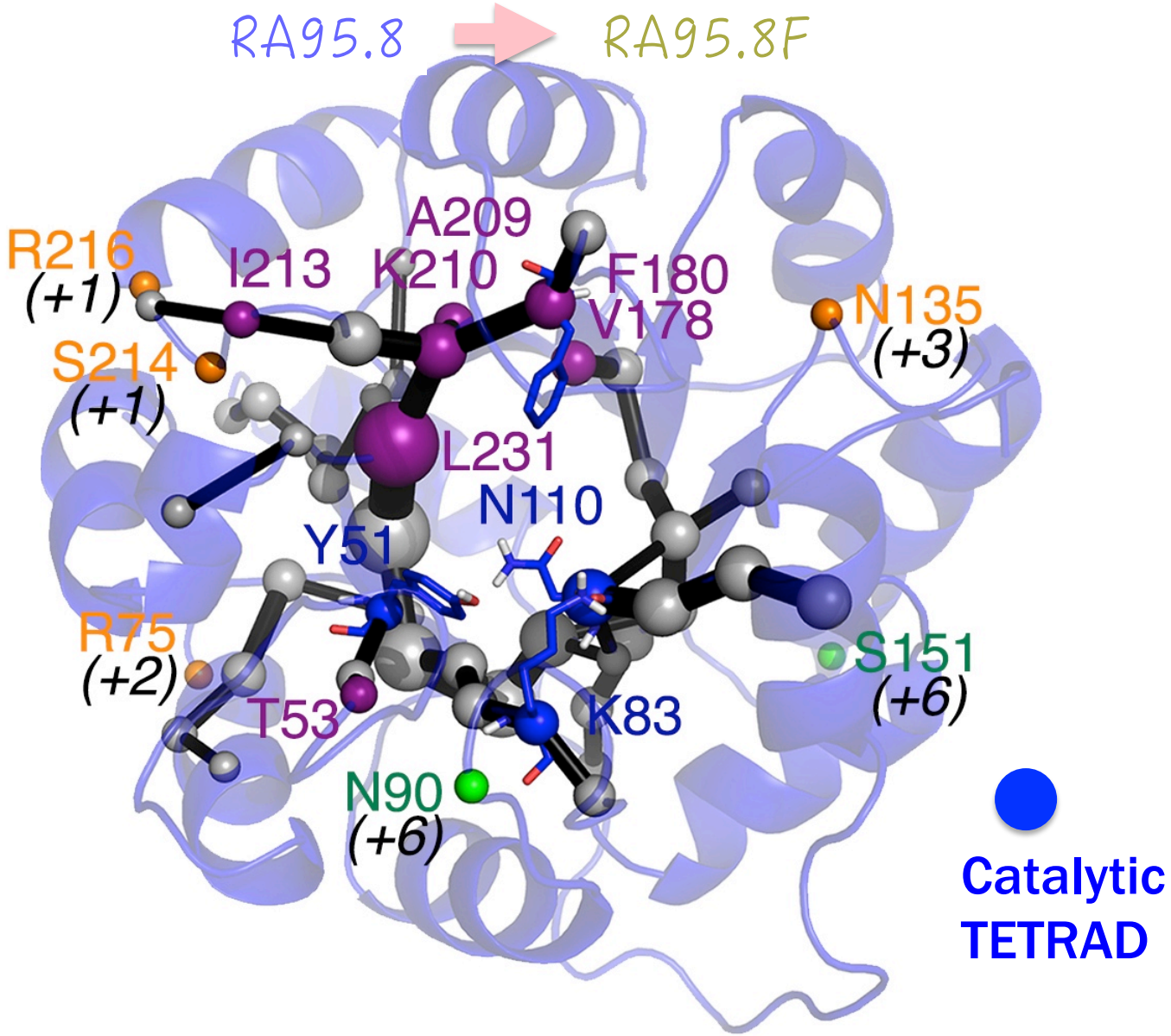
5 DE mutations:

4 identified with Shortest Path Analysis

1 Adjacent position

2. Development of new tools

RA95.5-8 ⟶ RA95.5-8F



13 DE mutations:

7 identified with Shortest Path Analysis

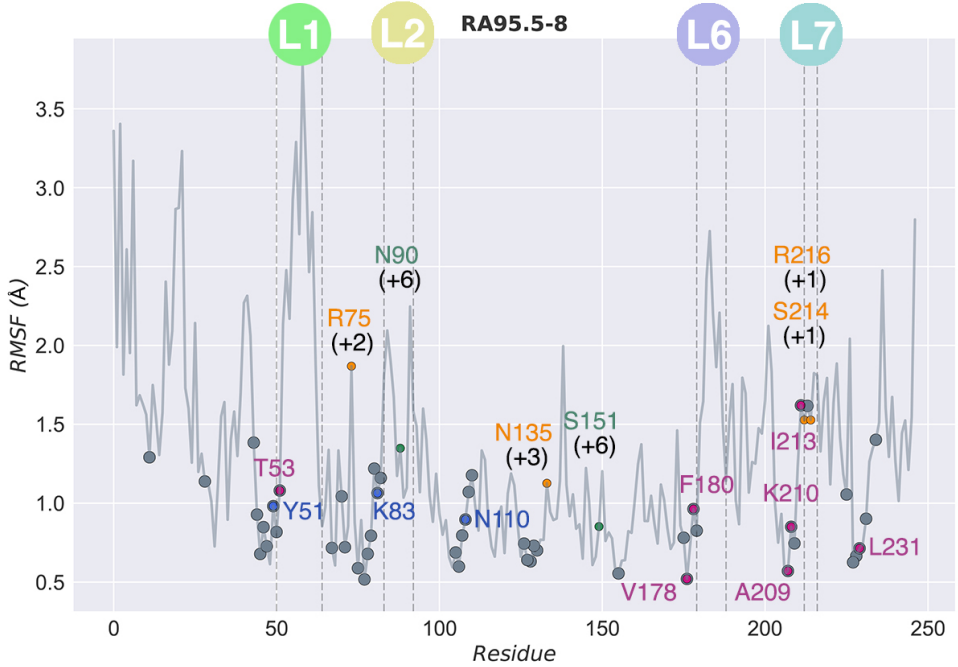
4 Adjacent positions

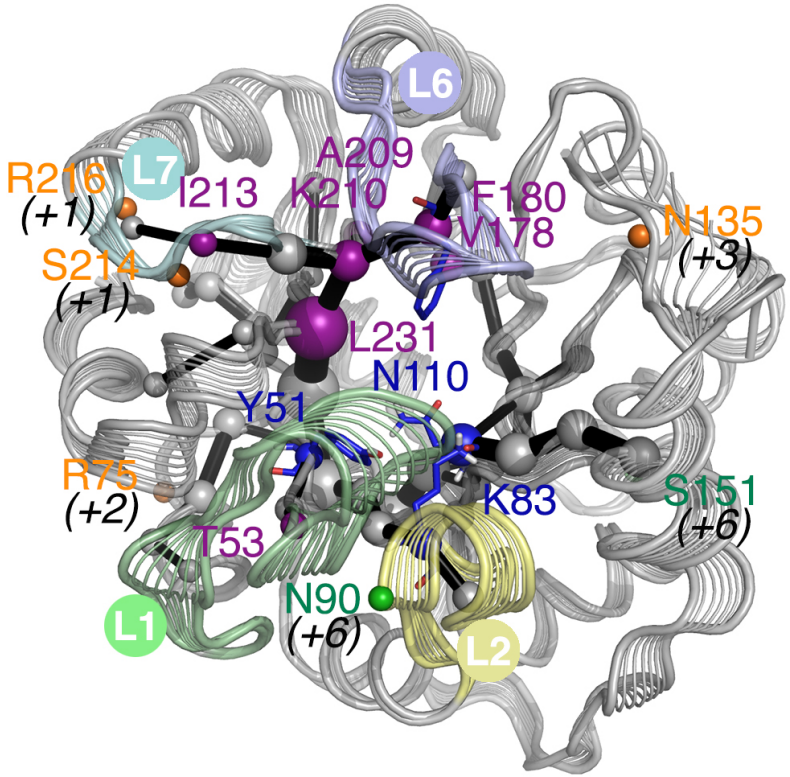
2 +6 position

2. Development of new tools

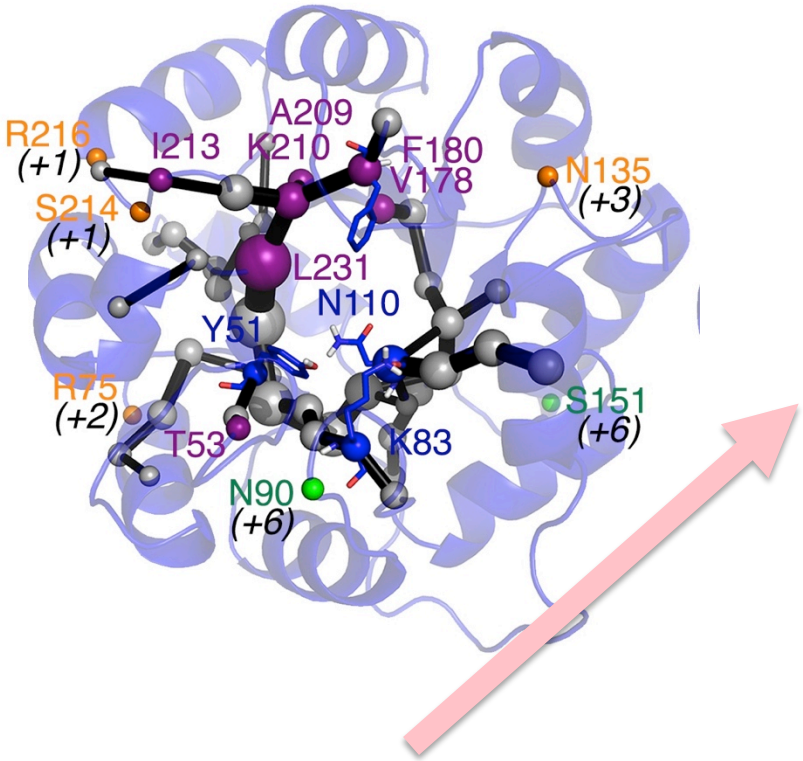
RA95.5-8 ⟶ RA95.5-8F

RA95.8 → RA95.8F

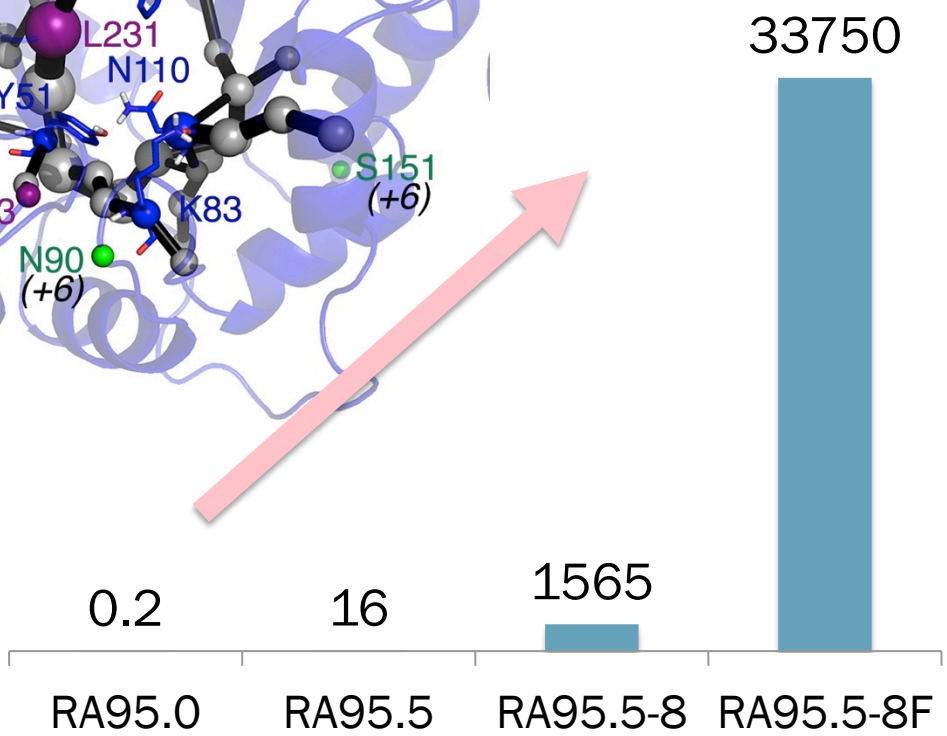




Shortest Path Map



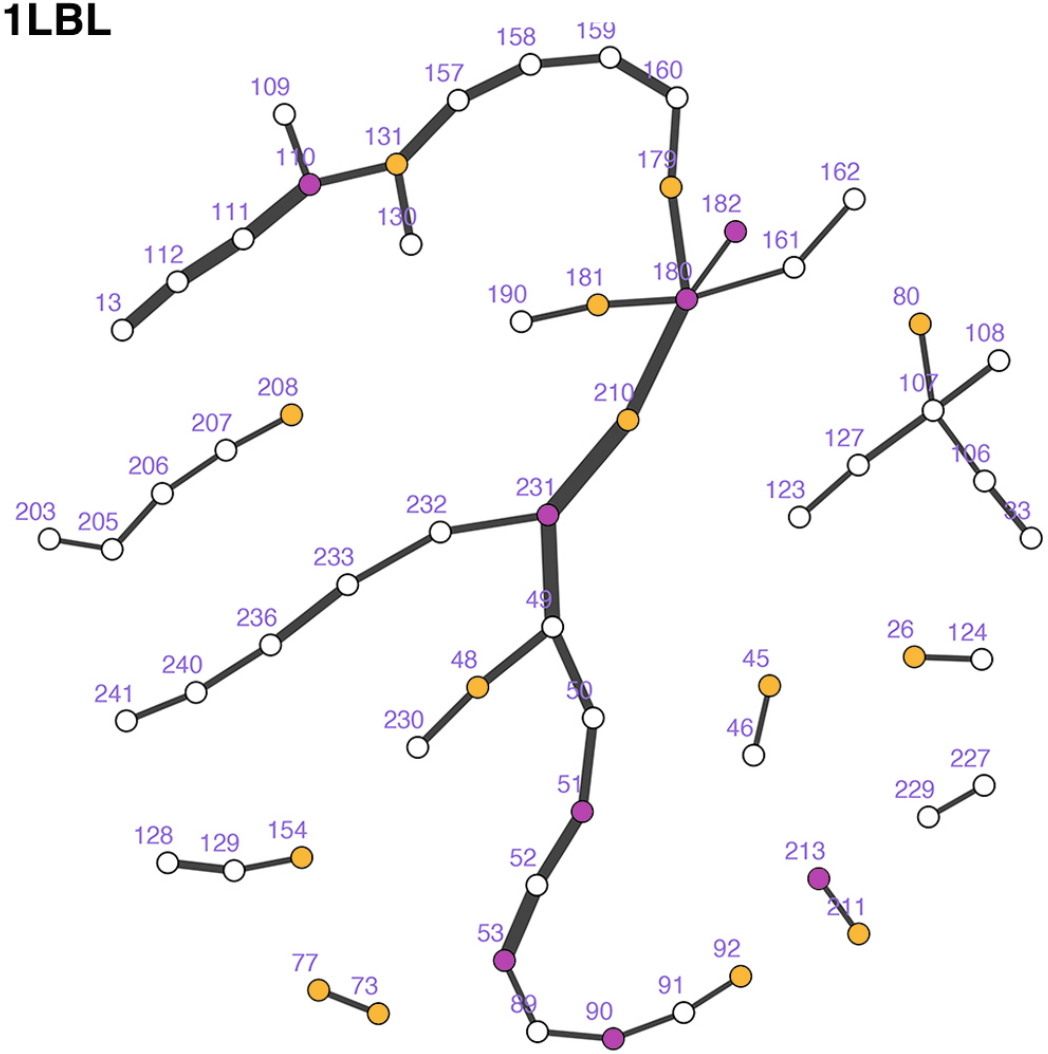
Smarter library of mutation points

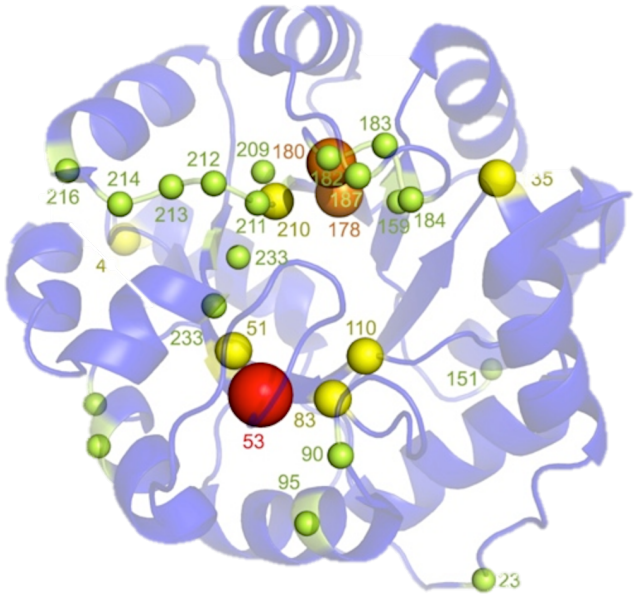


Romero-Rivera, A.; Garcia-Borràs, M.; Osuna, S. The role of conformational dynamics in the evolution of retro-aldolase activity, *ACS Catalysis* **2017**, 10.1021/acscatal.7b02954

Shortest Path Map

1LBL





Positions targeted with Directed Evolution all are predicted by SPM on the original scaffold (1LBL)

Retro-Aldolases are based on 1LBL scaffold:

1. indole-3-glycerol phosphate synthase

30% seq. identity

imidazole-glycerol phosphate synthase

Allosterically regulated enzyme

2. $(\beta\alpha)_8$ barrel enzymes Common enzyme fold in the PDB

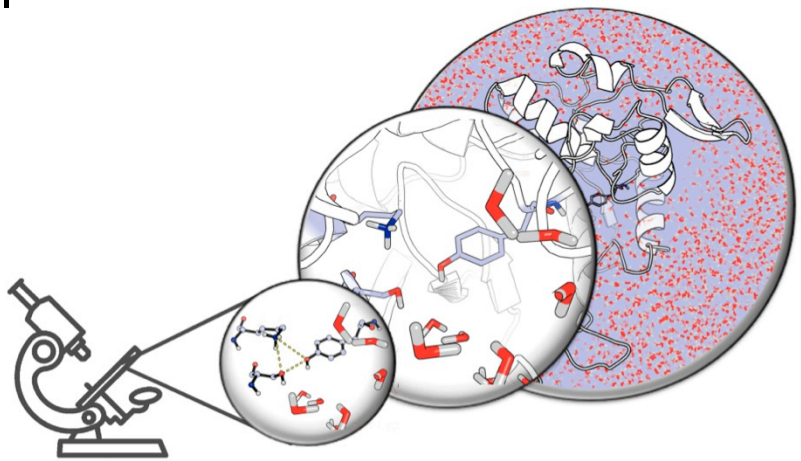
How general is the SPM tool?

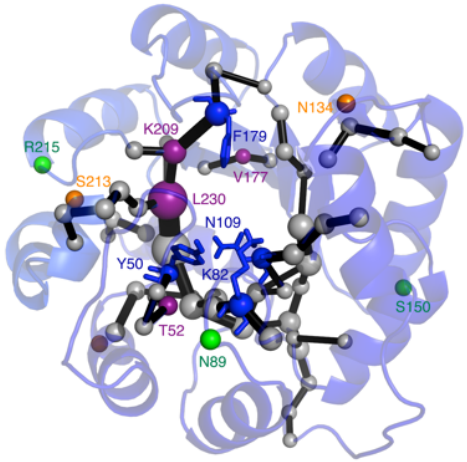
Conclusions

MD simulations are a valuable tool for:

☑ Assessing the catalytic efficiency of enzymes

☑ Predicting mutation points for enhanced activity through SPM tool





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Romero-Rivera, Garcia-Borràs, and Osuna, Computational tools for the evaluation of laboratory engineered biocatalysts, *Chem. Commun.* **2017**, 53, 284.

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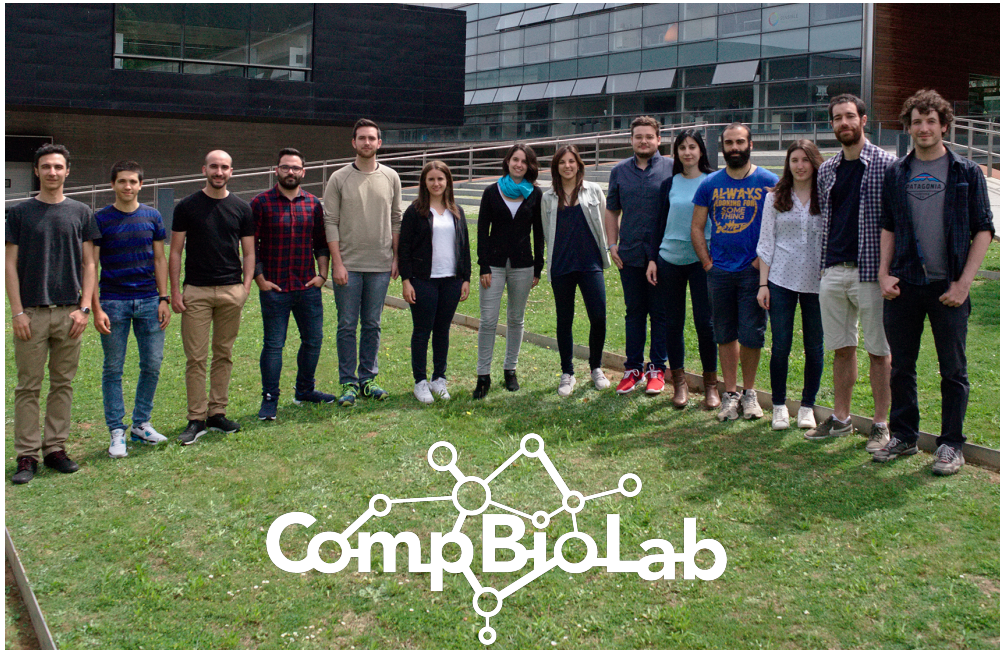

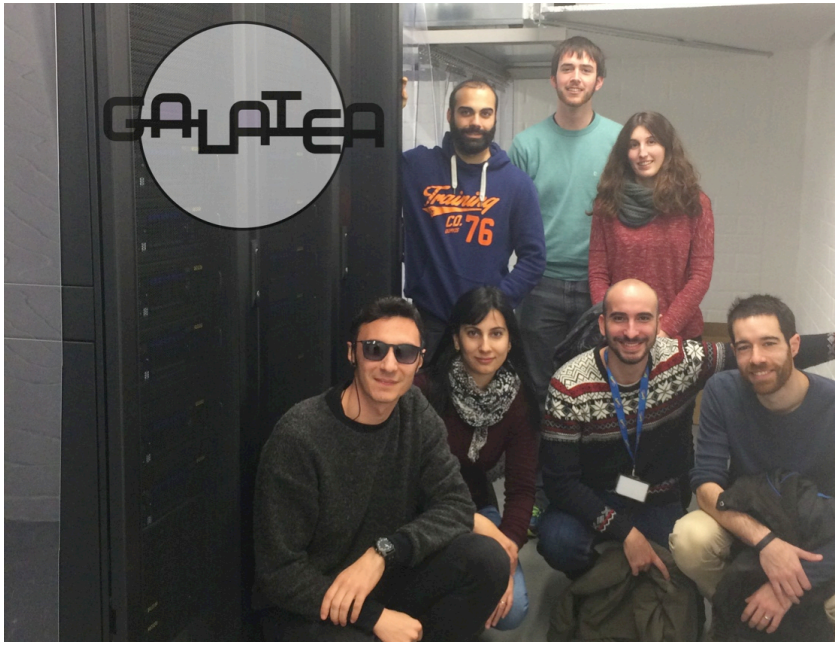

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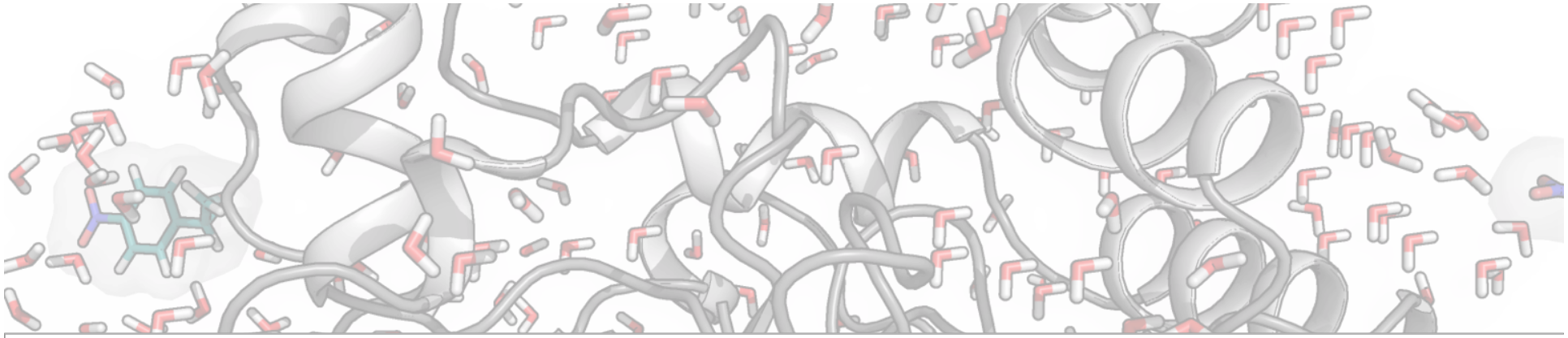
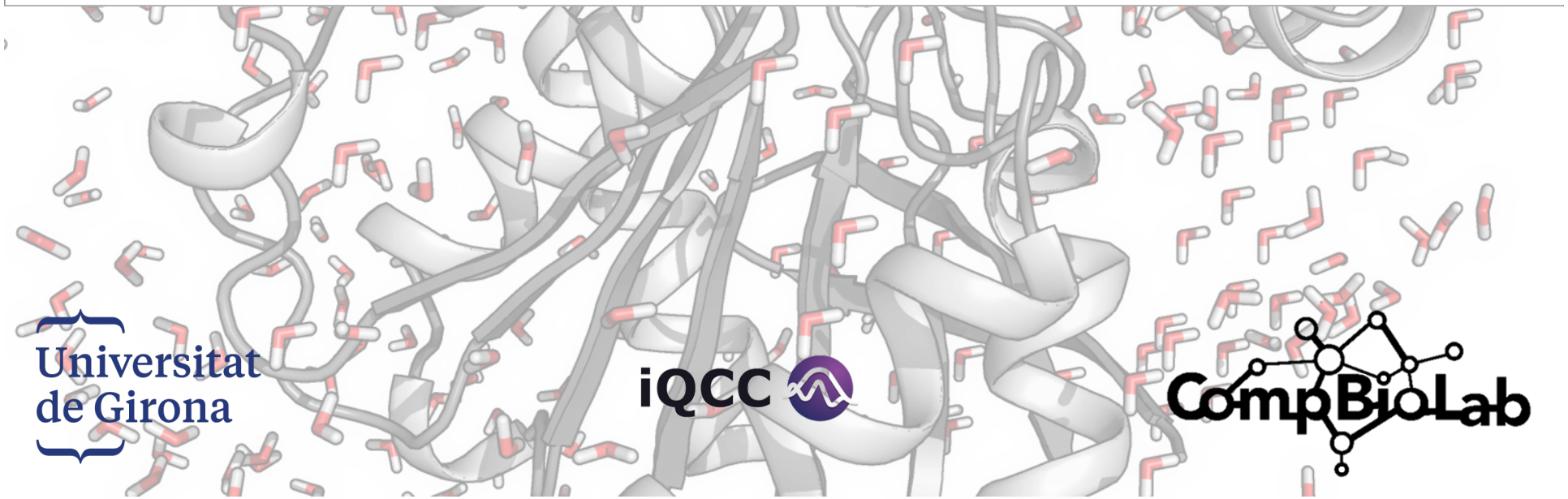
Dr. Marc Garcia-Borràs
Mr. Adrià Romero



Role of conformational dynamics in the evolution of novel retro-aldolase activity

Sílvia Osuna



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