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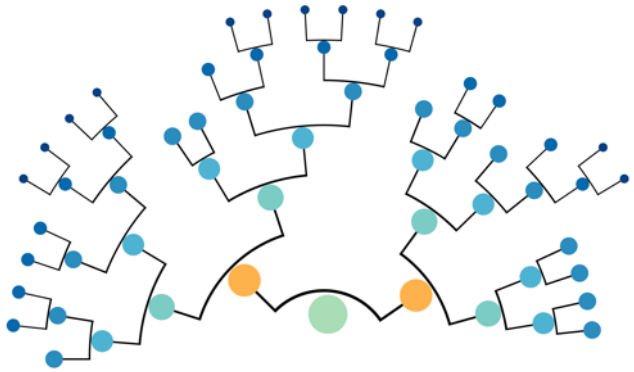
Virtual CECAM-ITCP School 2020

# Using Molecular Simulation to Trace the Role of Conformational Dynamics in Enzyme Evolution

Caroline Lynn Kamerlin

Department of Chemistry - BMC

Uppsala University



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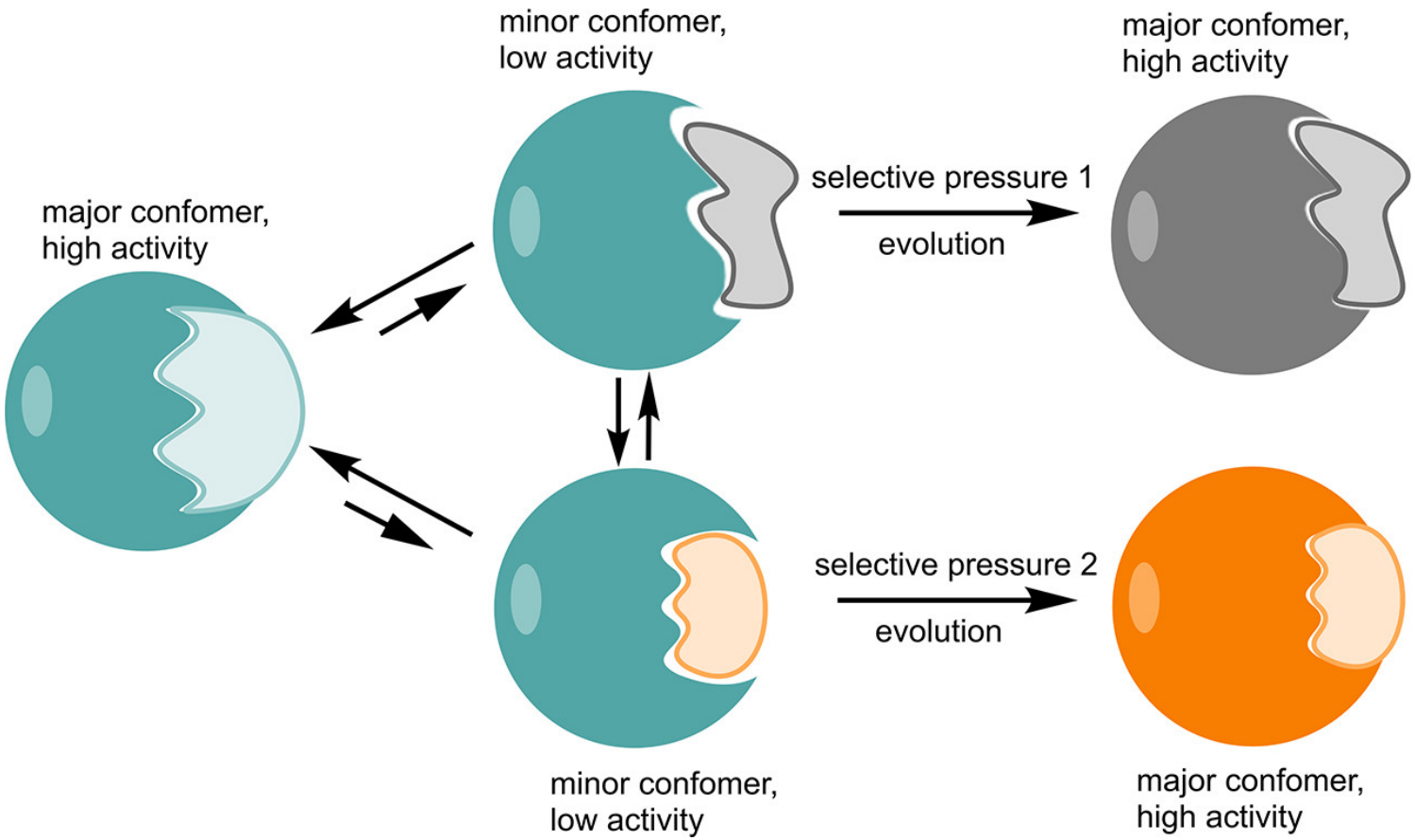
# The Role of Conformational Diversity




Tawfik's "New View": James & Tawfik, *TIBS* 28 (2003), 361

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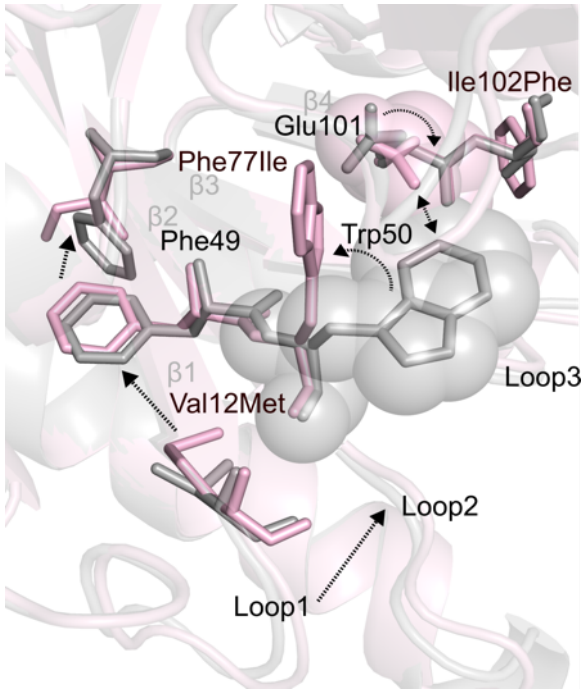
# (Just Some!) Examples of Systems

- Electrostatic cooperativity in alkaline phosphatases.
- Loop dynamics and scaffold flexibility controlling the selectivity of organophosphate hydrolases.
- Active site shuffling in a designed Kemp eliminase.
- Substrate and side chain dynamics during the emergence of new functions on non-enzymatic scaffolds, and in *de novo* active sites.




Regulating conformational dynamics appears to be critical for evolvability!

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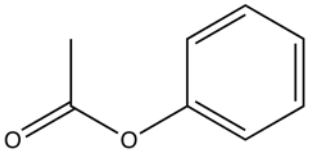
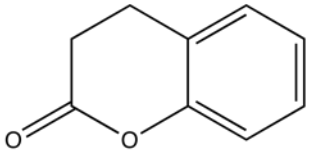
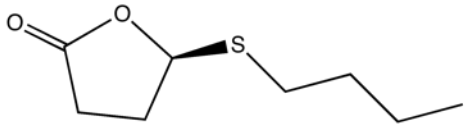


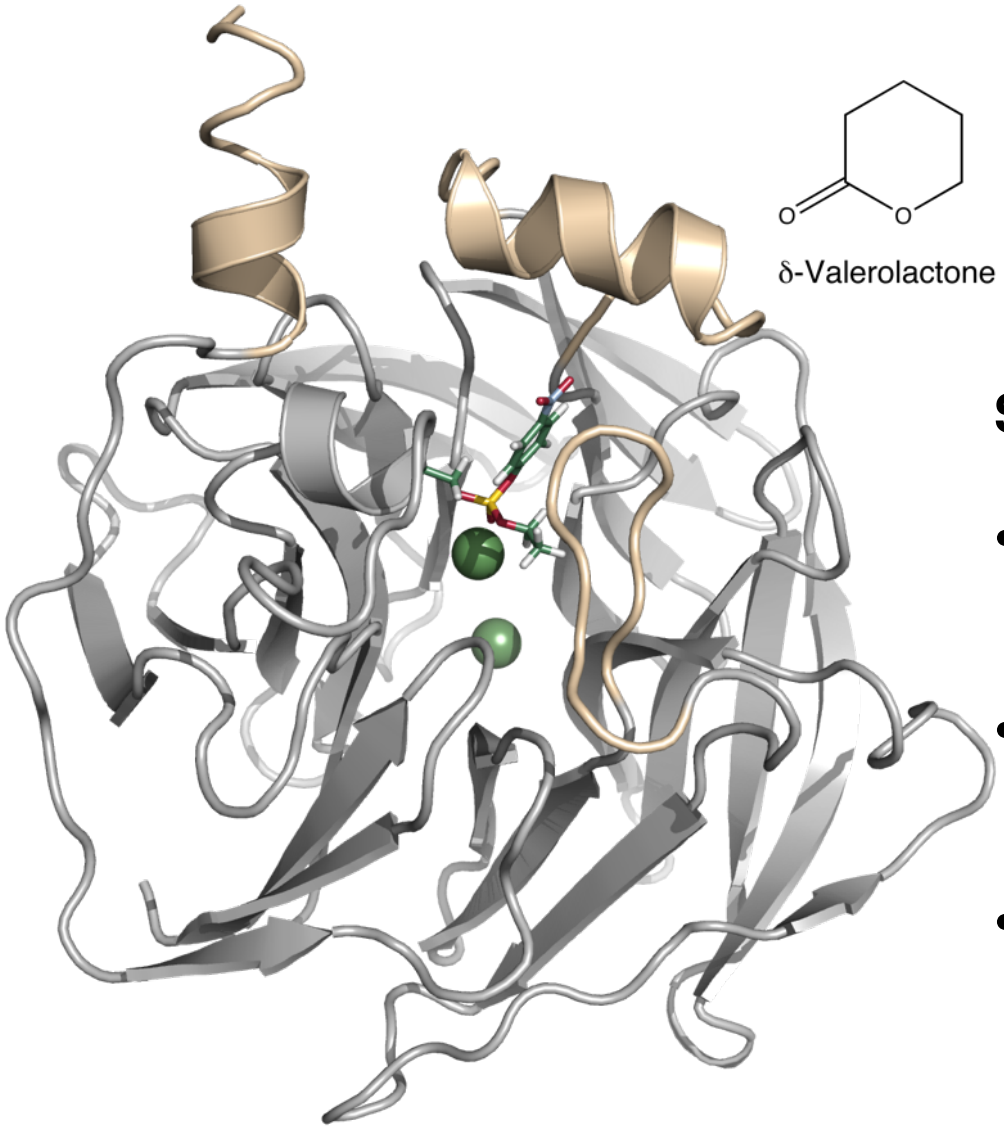
Phenyl Acetate



Dihydrocoumarin

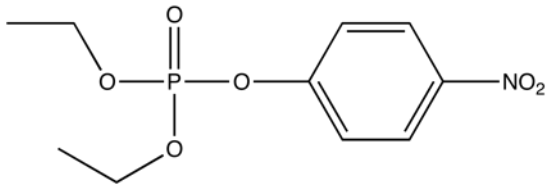


L-5-(thiobutyl)-butyrolactone





δ-Valerolactone




Paraoxon

**Serum paraoxonase 1 (PON1):**

- Anti-atherosclerotic component of high density lipoprotein.
- **<u>Extremely promiscuous</u>** and highly evolvable enzyme.
- Very attractive as a therapeutic agent for treatment of acute organophosphate poisoning.

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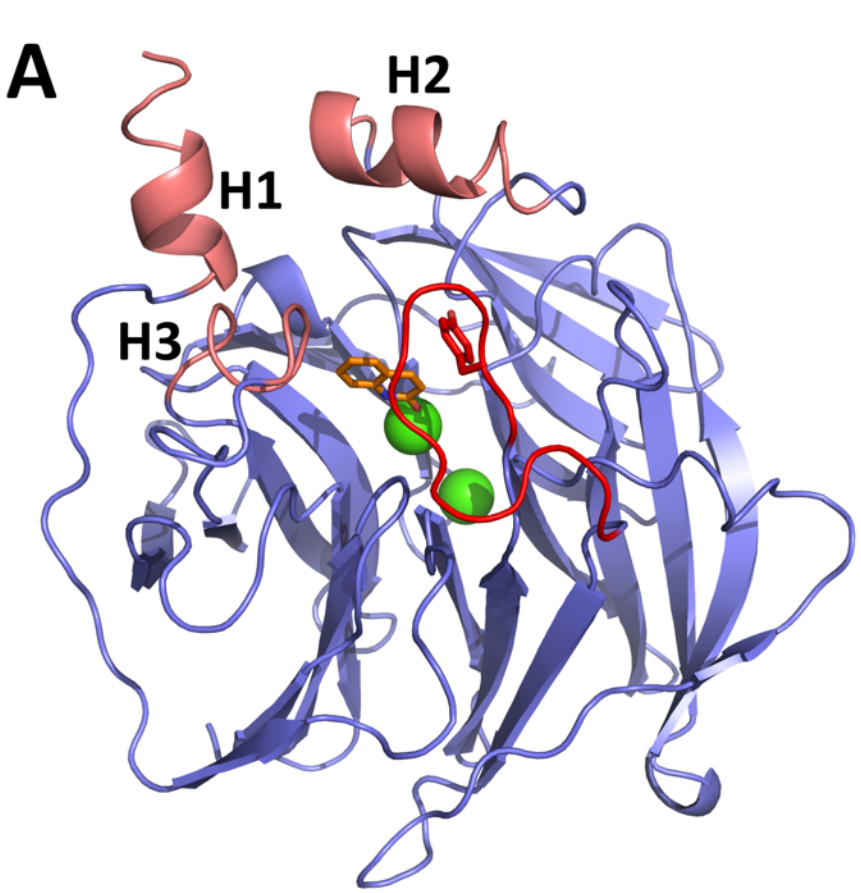
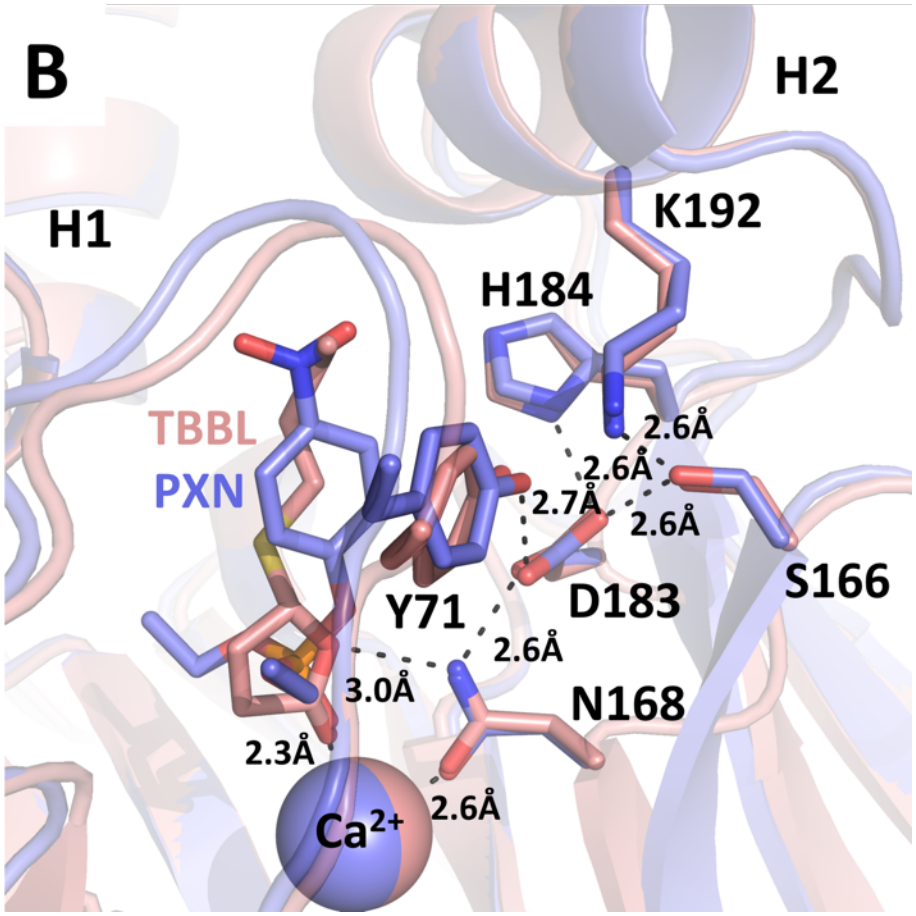
# PON1 Active Site Architecture




Ben-David *et al.*, *J. Mol. Biol.* **427** (2015), 1359

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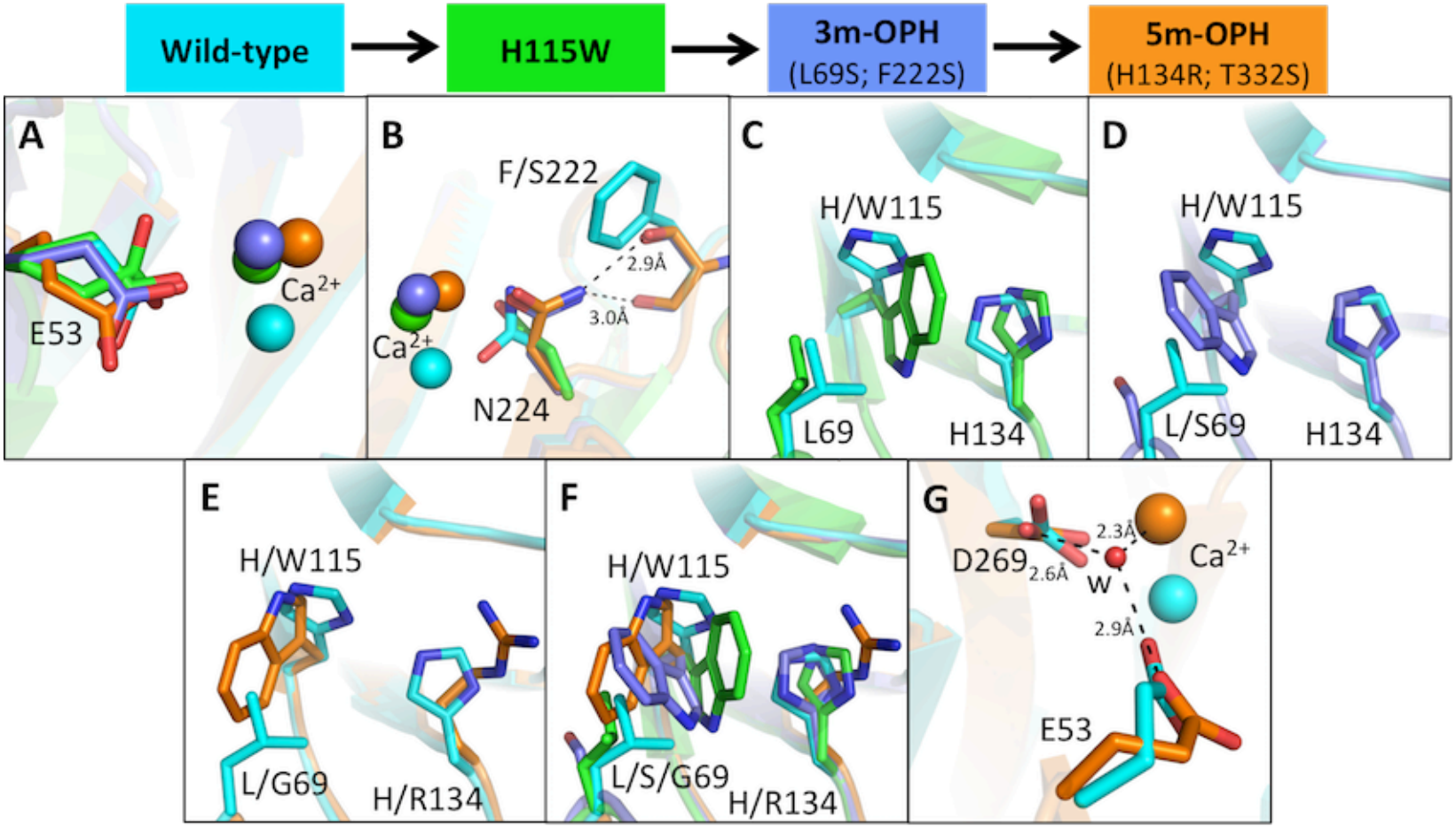
# PON1 Neo- *vs.* Re-Functionalization




Ben-David *et al.*, *Mol. Biol. Evol.* **37** (2020), 1133.

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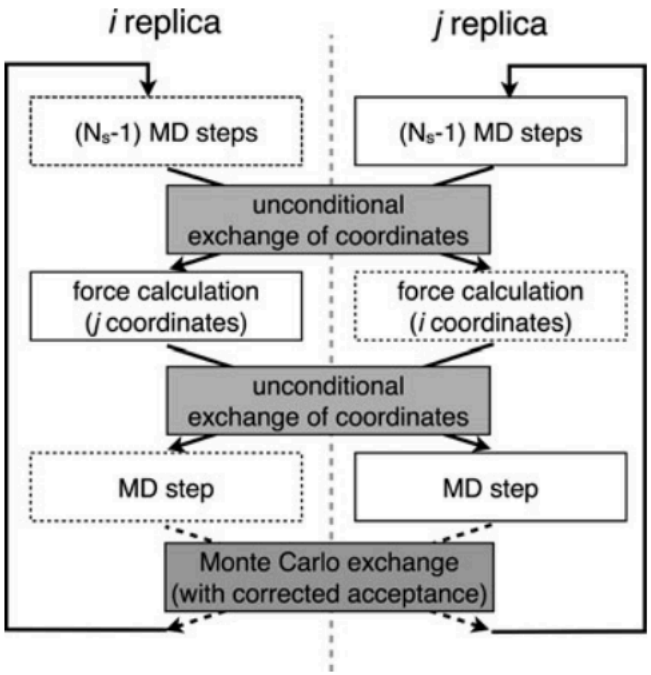
# Hamiltonian Replica Exchange




- System has coordinates *r* + potential U(*r*).

- Couple to thermal bath, so that probability of exploring a configuration is: $P(r) \propto e^{-\frac{U(r)}{k_B T}}$

- REX samples “cold” replica from which unbiased statistics can be extracted + “hot” replicas used to accelerate sampling.

- Hottest replica samples system fast enough to cross barriers for the process of interest, intermediate replicas smoothing.

- Normally replicas biased by temperature, HREX biased by temperature + potential.

Bussi, *Mol. Phys.* **112** (2020), 1133.

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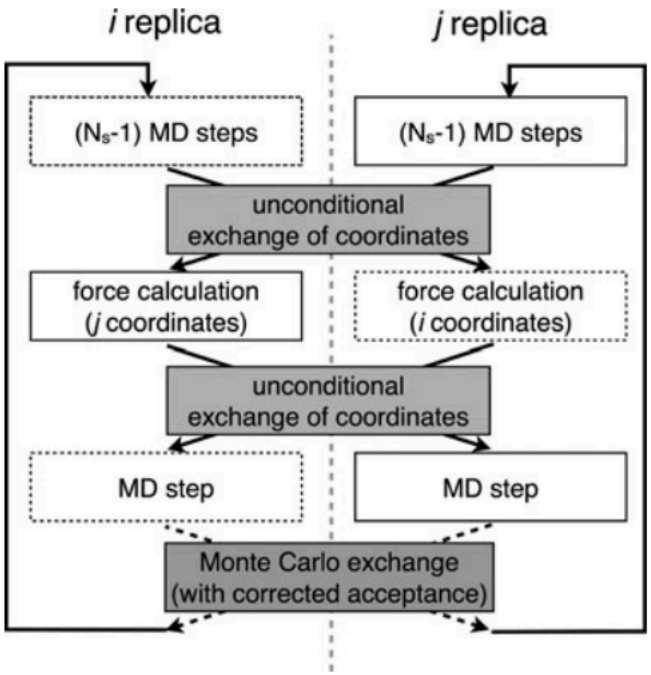


- Simulate each replica at a different temperature with different potential.

- Energy is an extensive property (temperature is intensive) so in HREX can choose specific part of the system to sample (separate "hot", *H*, and "cold", *C*, regions).

- Charges, Lennard Jones and dihedral parameters of hot region scaled by $\sqrt{\lambda}$, $\lambda$, and $\lambda$ ($1^{st}$ and $4^{th}$) or $\sqrt{\lambda}$ ($1^{st}$ or $4^{th}$).

- Interactions in hot region kept at $T_{eff}$ $1/\lambda$, *H* and *C* at $T_{eff}$ $1/\sqrt{\lambda}$ and in *C* at $\lambda$.

- $\lambda$ is chosen to be a real number $0 < \lambda < 1$.

Bussi, *Mol. Phys.* **112** (2020), 1133.

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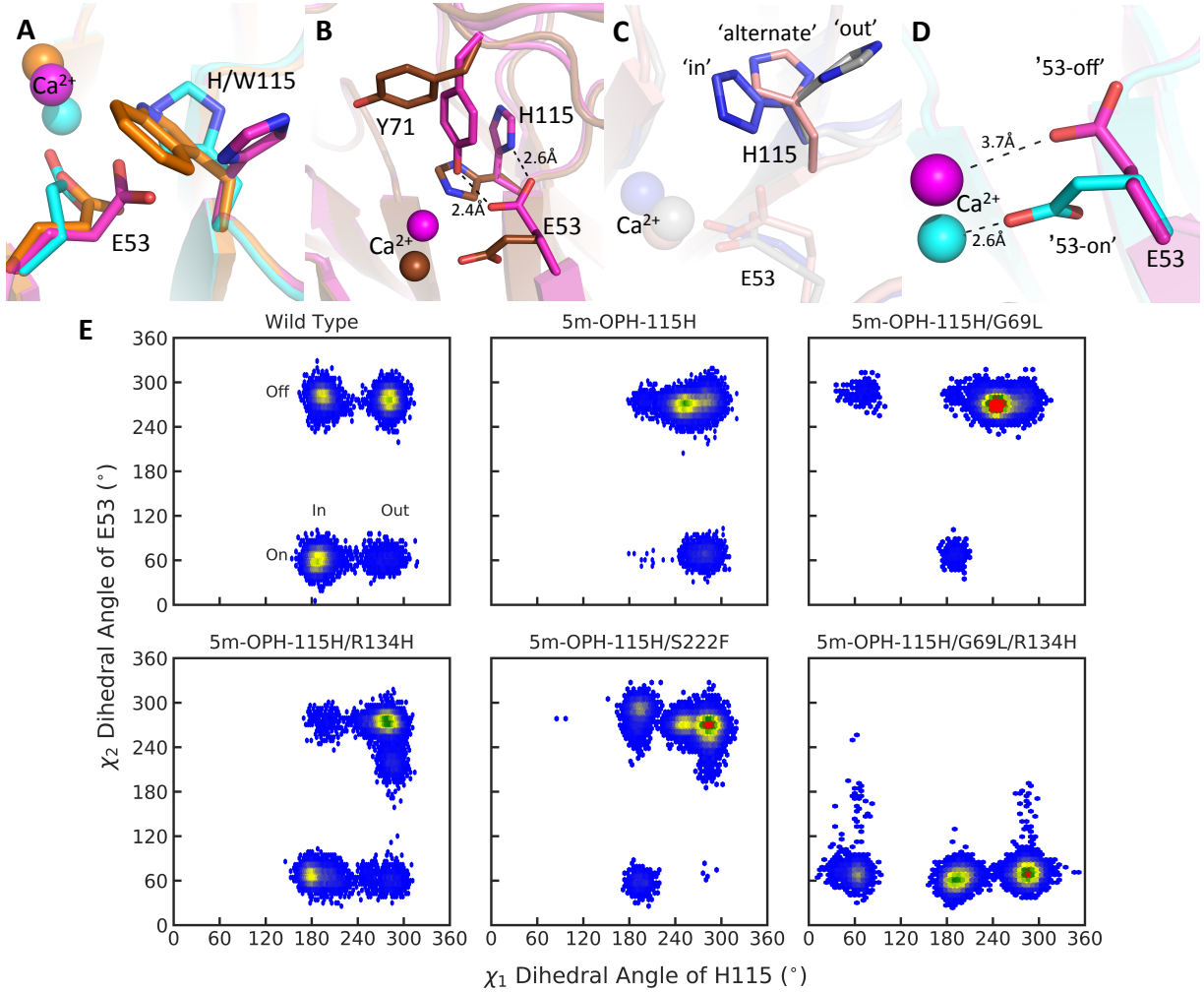


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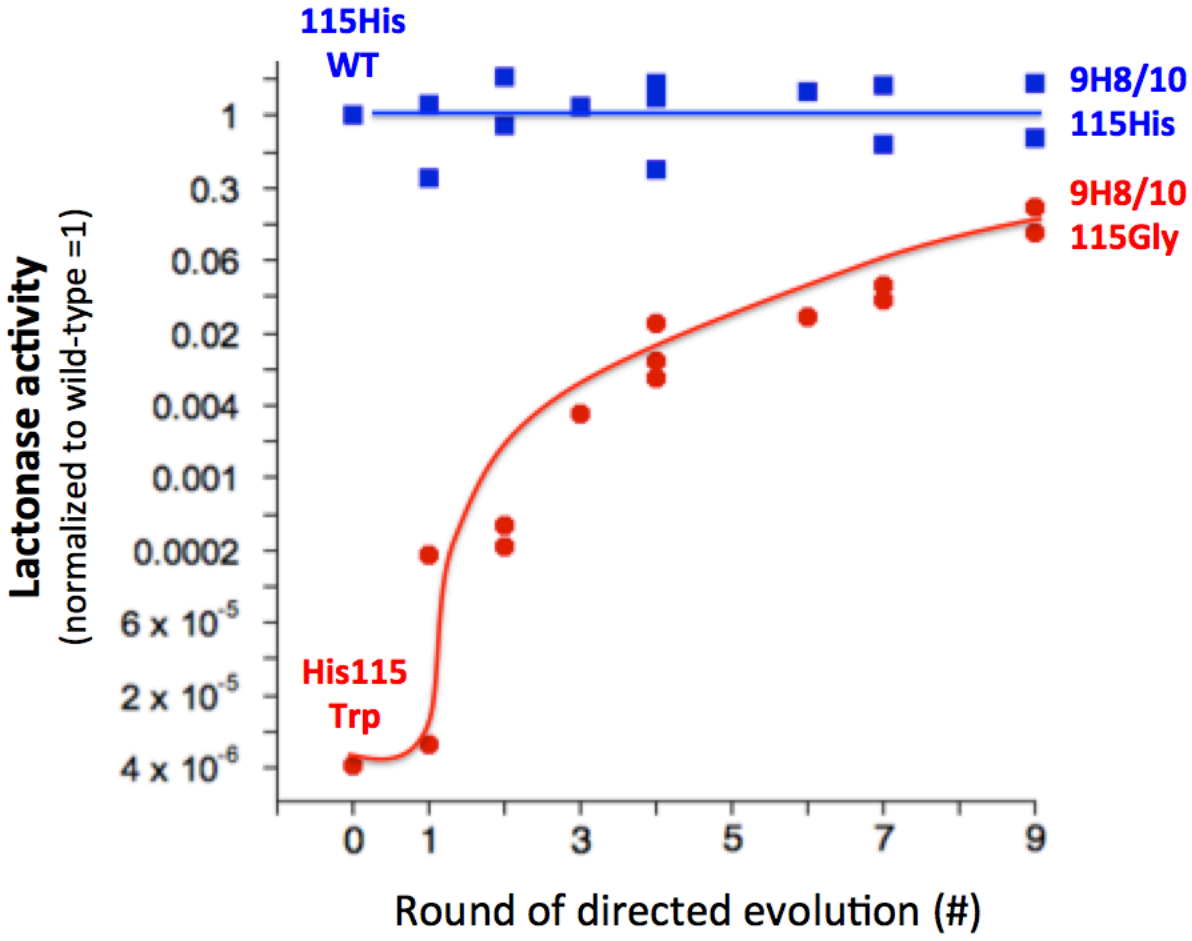


Ben-David *et al.*, *Mol. Biol. Evol.* **37** (2020), 1133.

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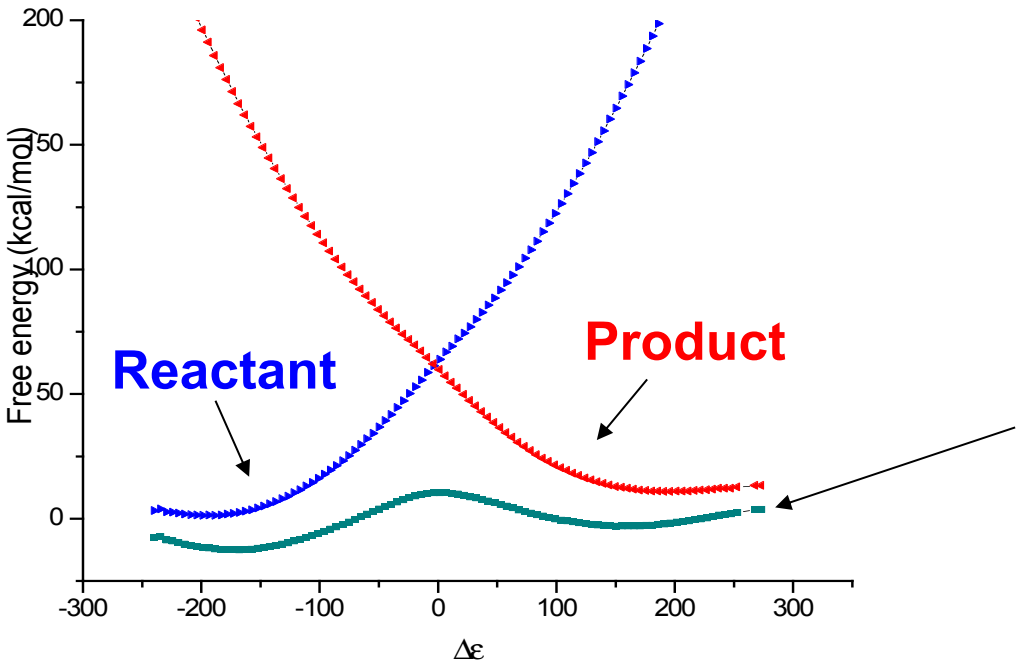
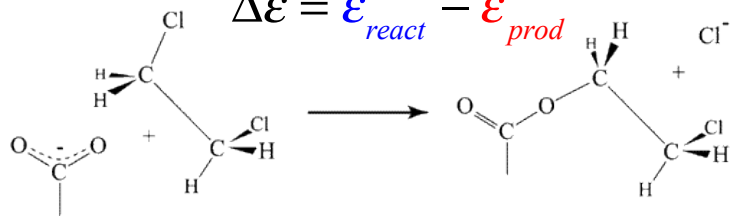
# Empirical Valence Bond Approach




$$\Delta\varepsilon = \varepsilon_{react} - \varepsilon_{prod}$$



**Reactant**:
Force field-like functions describing the reactants' bonding pattern

**Product**:
Force field-like functions describing the products' bonding pattern

**Ground State**:
Eigenvalue of 2x2 Hamiltonian built from Reactant and Product energies and off-diagonal function ($H_{12}$).

$$H = \begin{pmatrix} \varepsilon_{react} & H_{12} \\ H_{12} & \varepsilon_{prod} \end{pmatrix}$$

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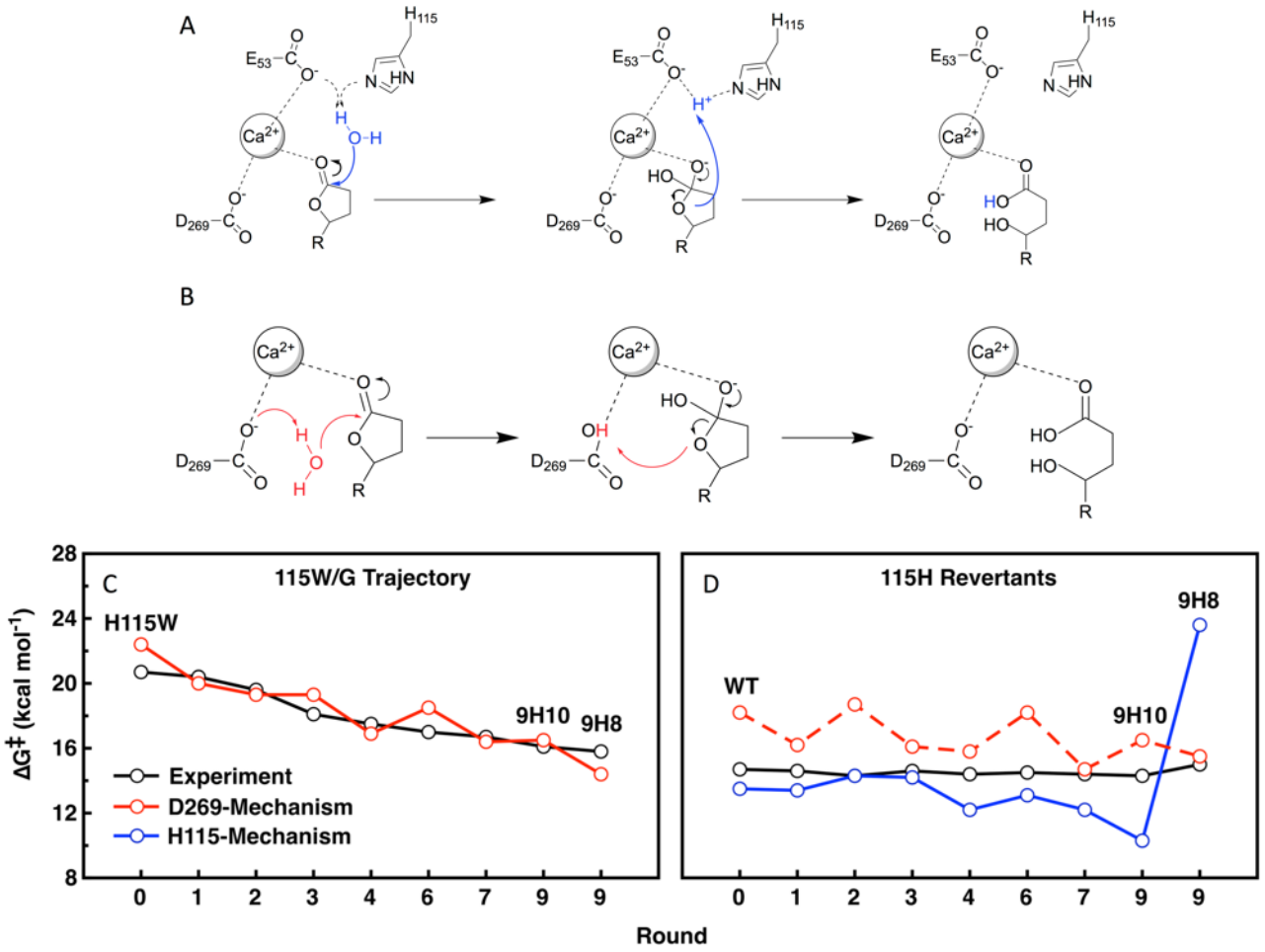
# PON1 Neo- *vs.* Re-Functionalization




Ben-David *et al.*, *Mol. Biol. Evol.* **37** (2020), 1133.

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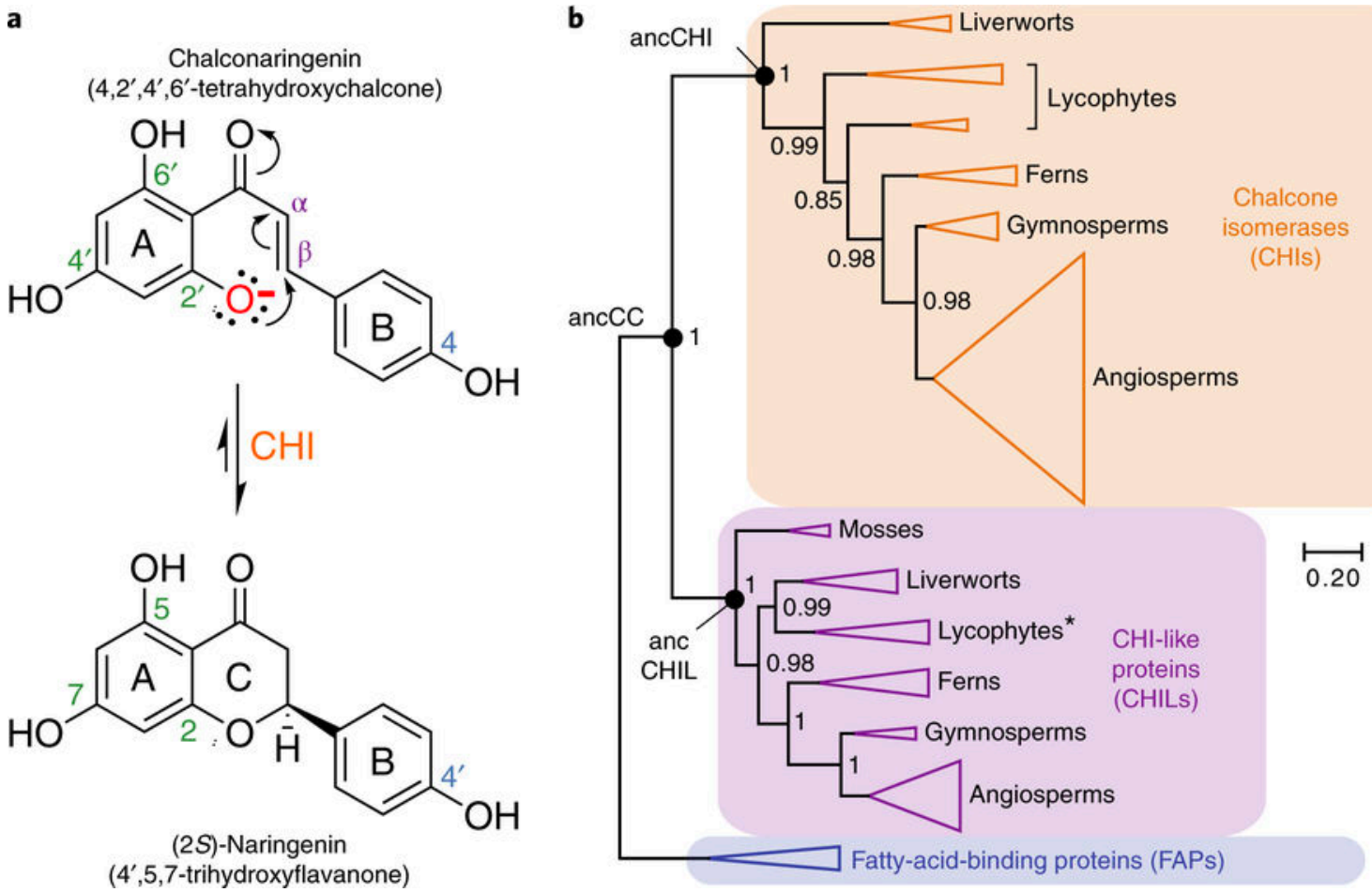
# How Do New Enzymes Emerge?




Kaltenbach *et al*., *Nat. Chem. Biol.* **14** (2018), 548

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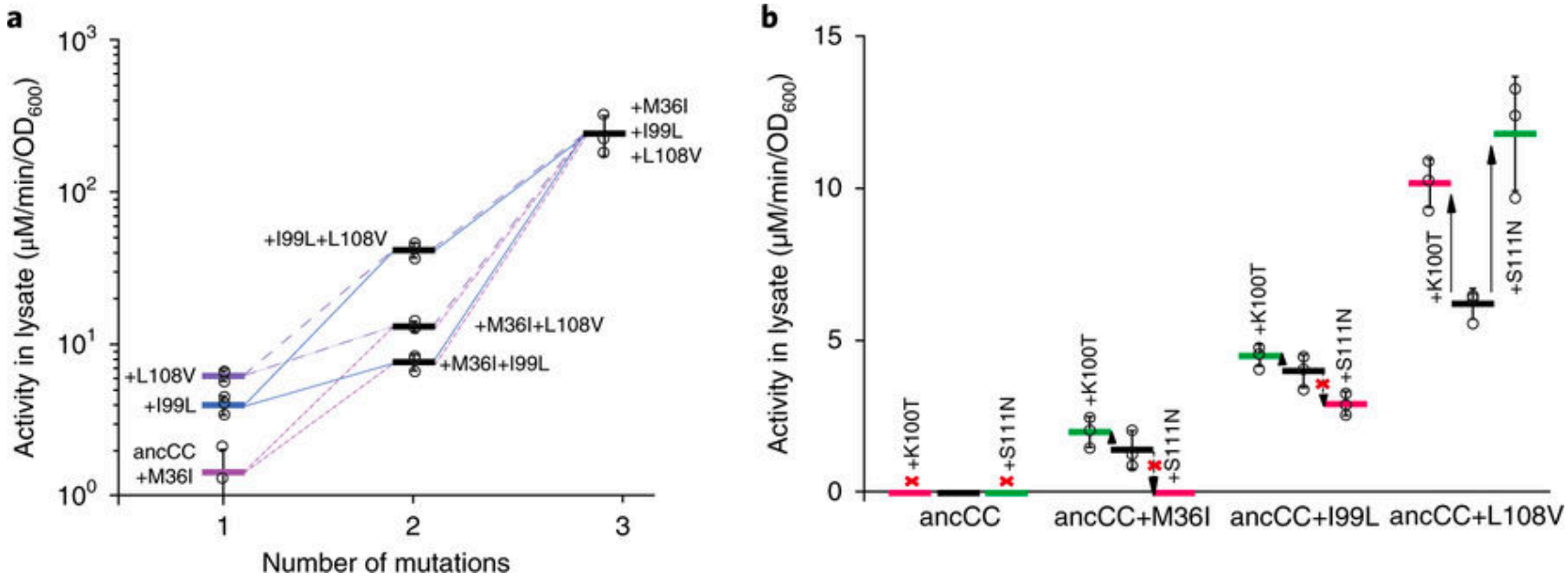
# Additivity *vs.* Epistasis in CHI Evolution




Kaltenbach *et al*., *Nat. Chem. Biol.* **14** (2018), 548

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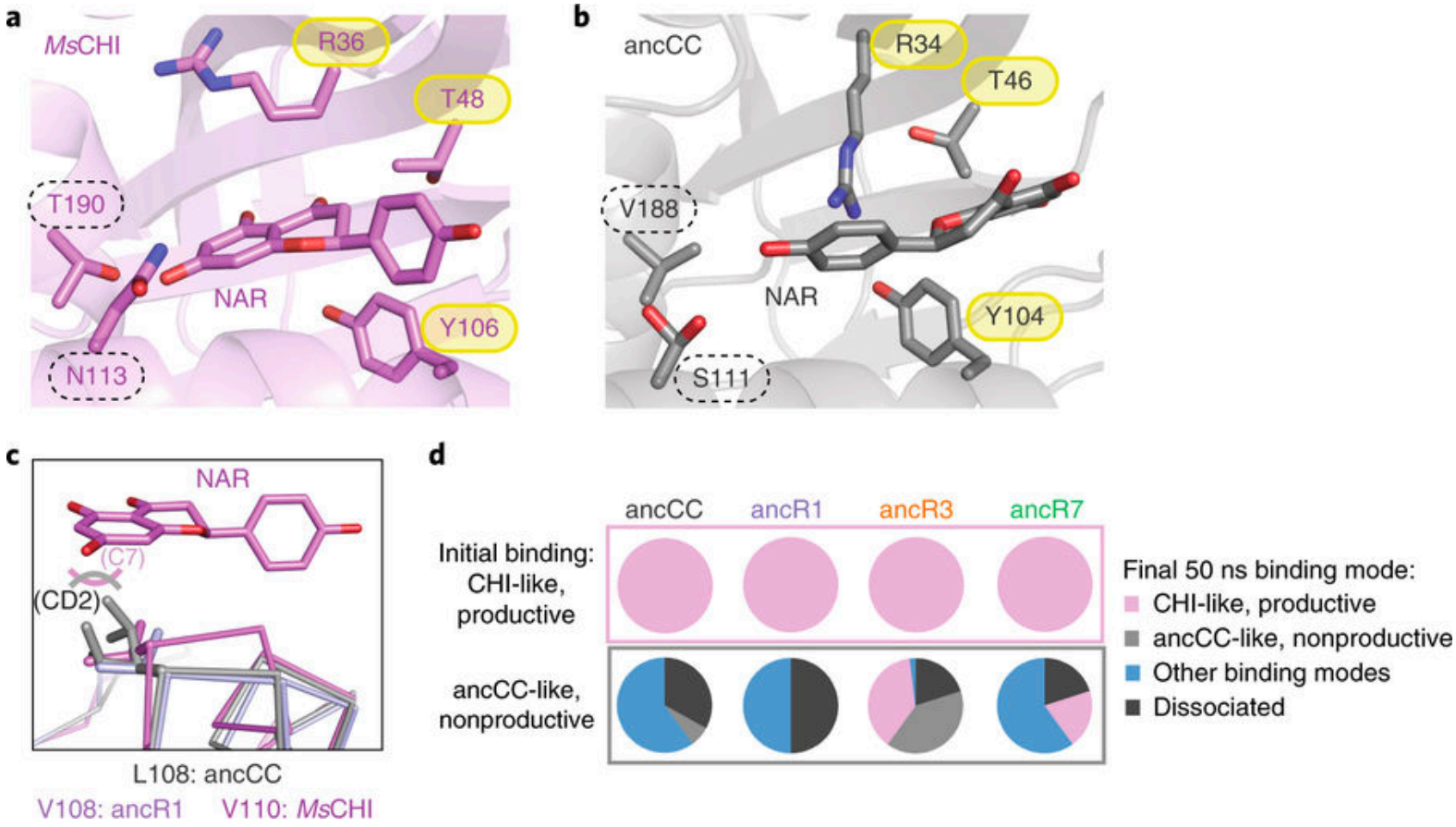
# Structural Changes During Evolution




Kaltenbach *et al*., *Nat. Chem. Biol.* **14** (2018), 548

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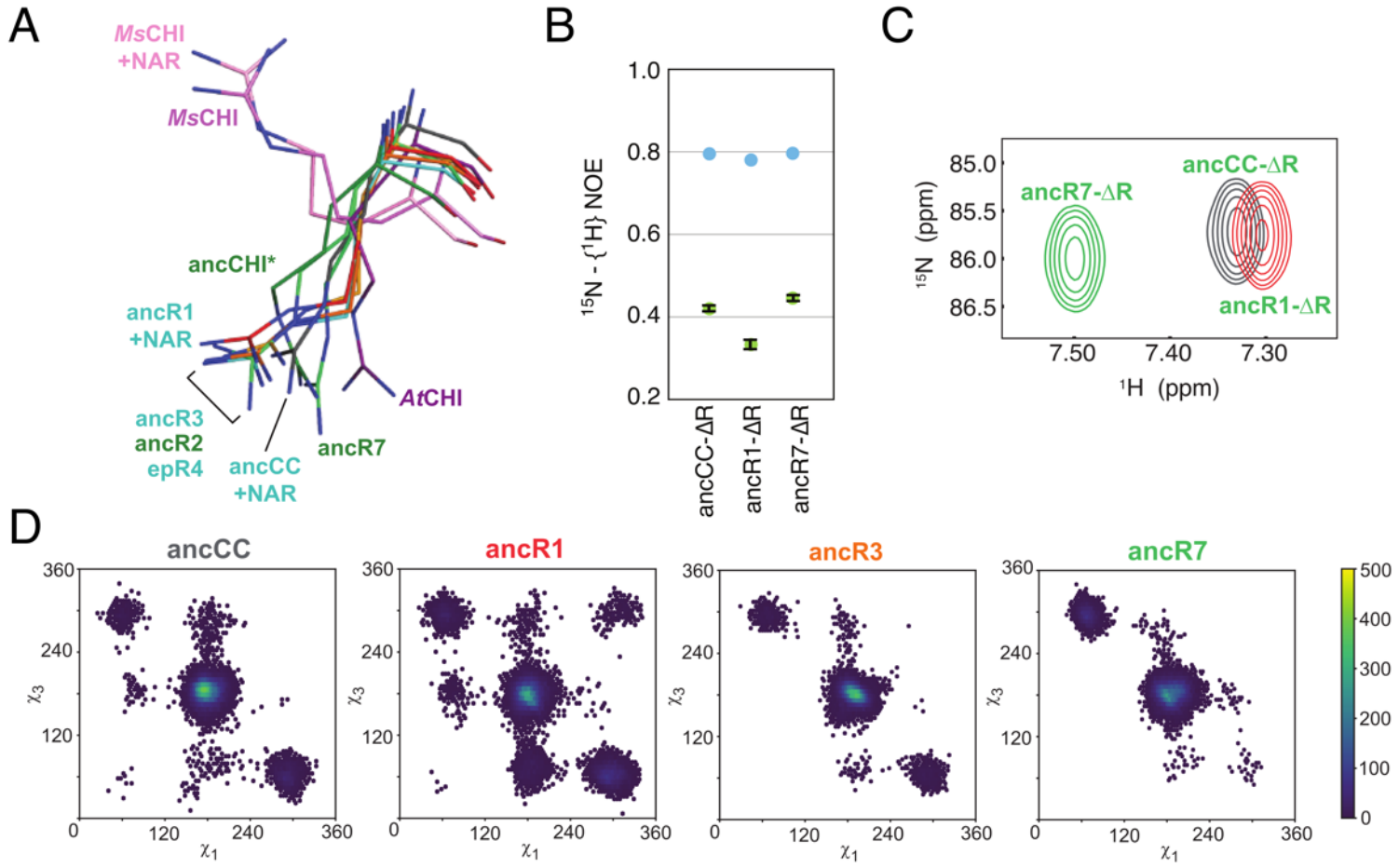
# Evolution Rigidifies a Key Residue




Kaltenbach *et al*., *Nat. Chem. Biol.* **14** (2018), 548

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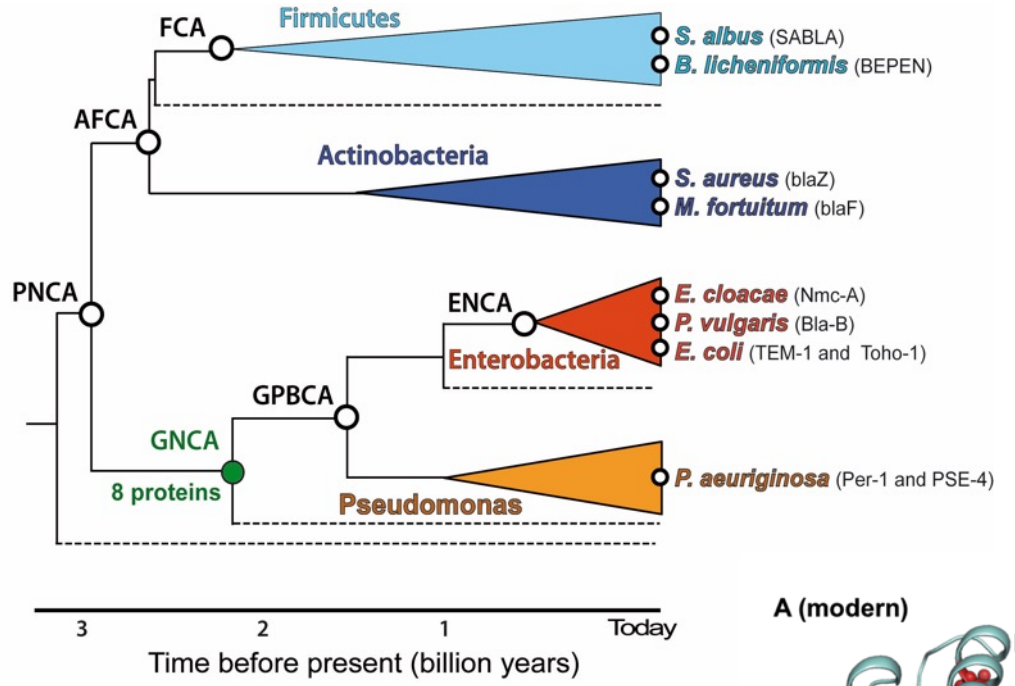
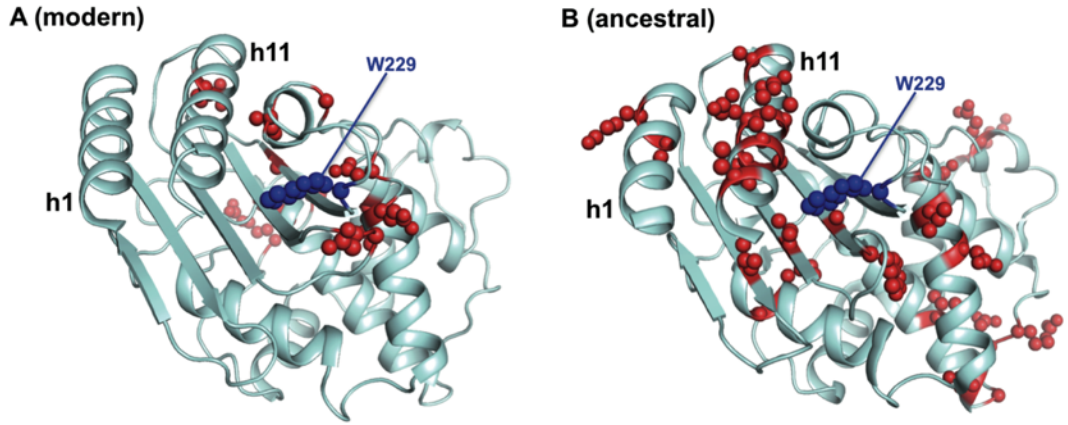
# *De Novo* Active Sites in β-Lactamases




Generating *de novo* active sites, put into resurrected Precambrian β-lactamases, identified through ancestral sequence reconstruction.




Risso *et al.*, *Nat. Commun.* **8** (2017), 16113

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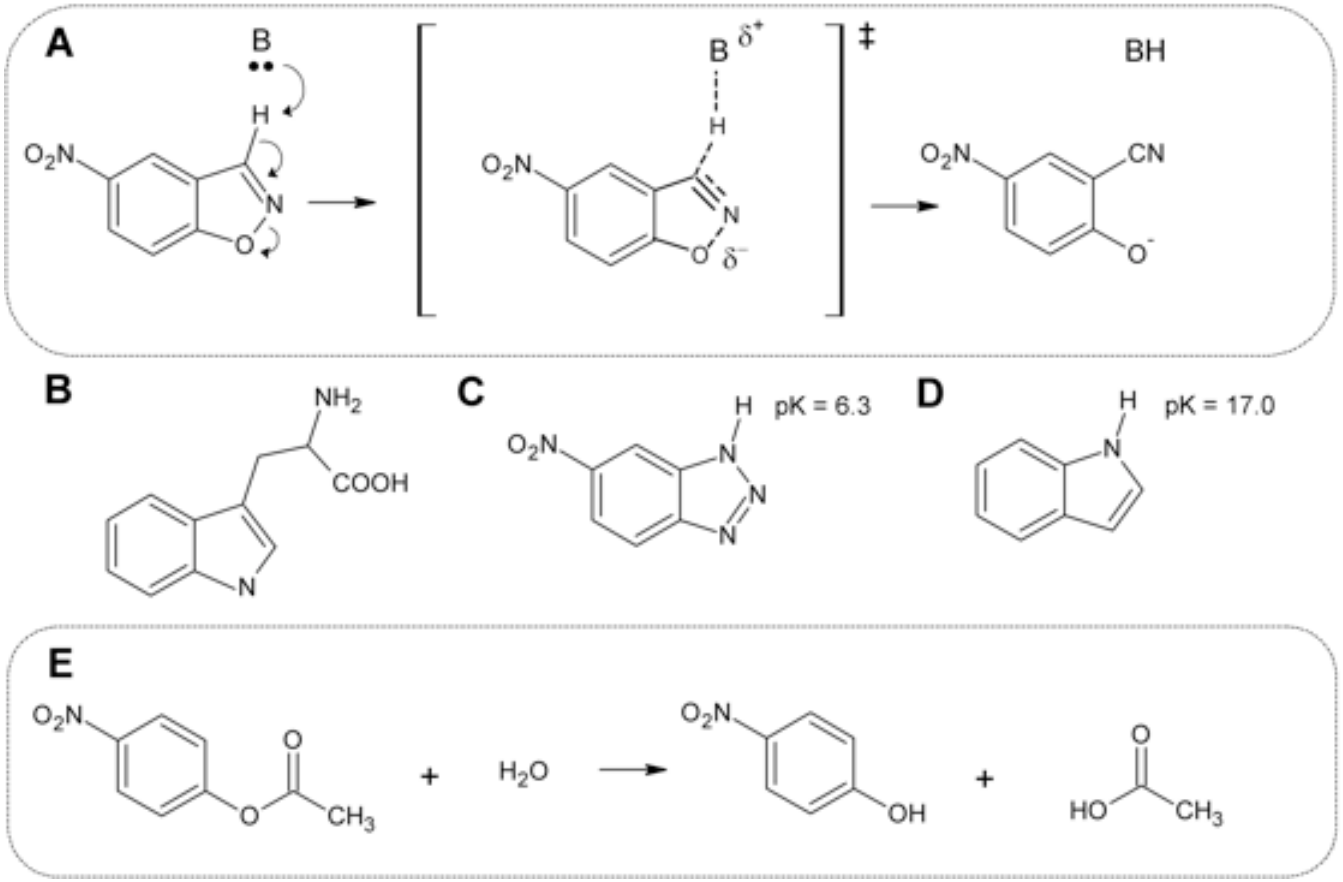


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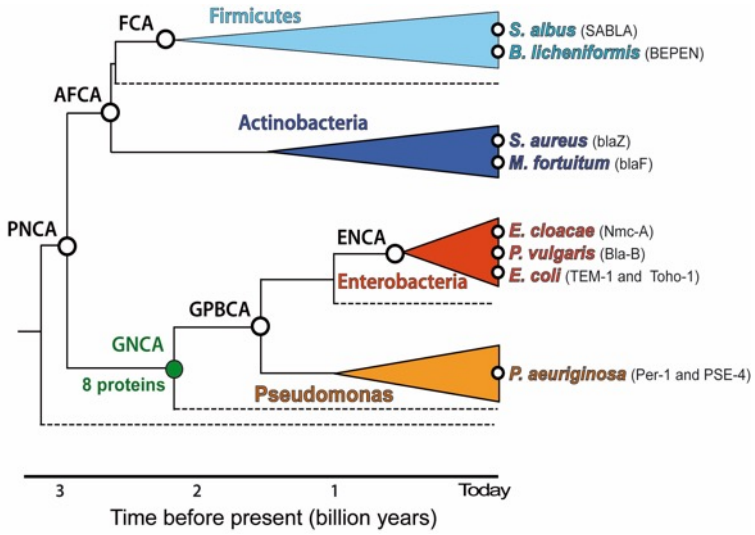


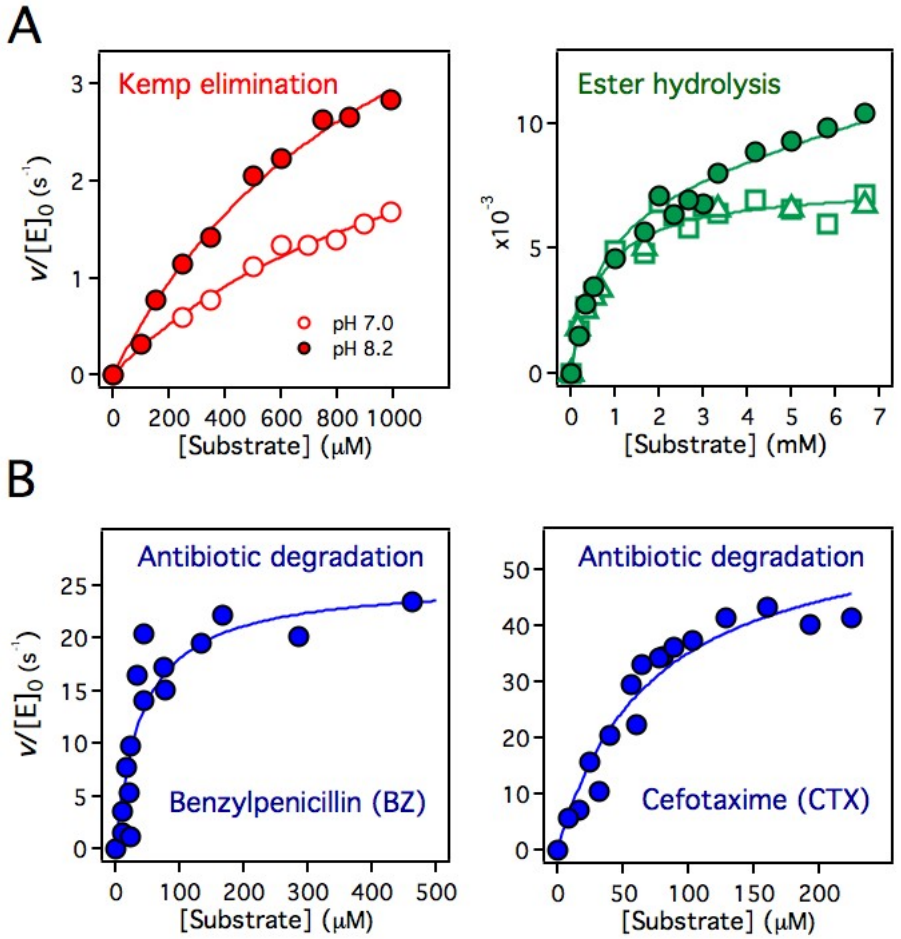


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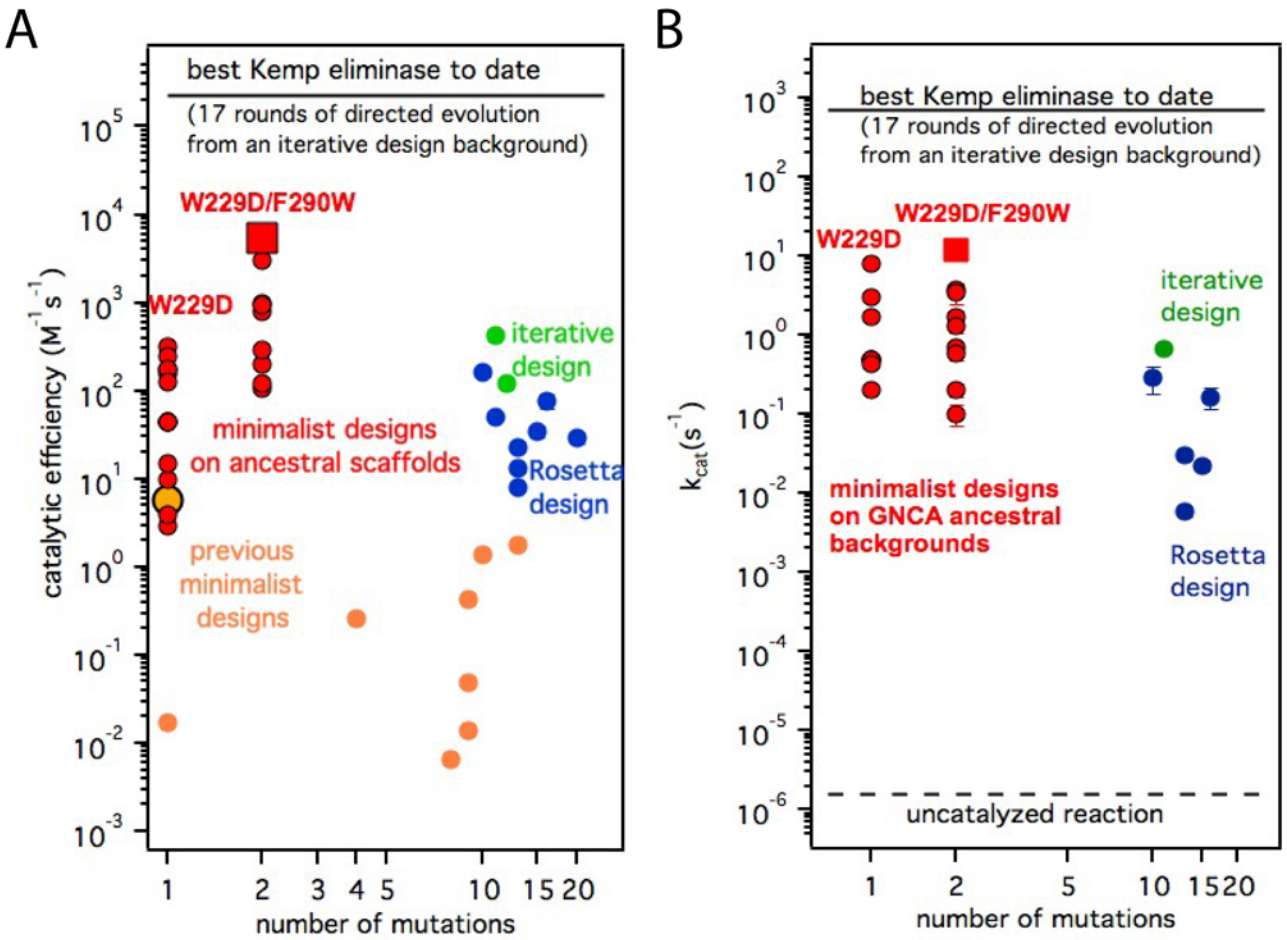


Risso *et al.*, *Nat. Commun.* **8** (2017), 16113

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# Random Library Screening

| Clone | $k_{cat}$ / $K_M$ ($M^{-1}$ $s^{-1}$) | $T_M$ (°C) |
|---|---|---|
| GNCA4-WT | **3047±282** | 80 |
| 3C11 | 608±68 | 77 |
| 4B4 | 1770±126 | 81 |
| 8F11 | **5980±117** | 80 |
| 6D5 | 2476±420 | 81 |
| 7C1 | 600±56 | 72 |
| 8E12 | 2222±167 | 70 |
| 6A12 | 1036±159 | 79 |
| 7D1 | 1880±155 | 67 |
| 2H4 | 2280±146 | ND |
| 5H8 | 2066±67 | 64 |

Library of variants with random mutations / average mutational load of 3-5 mutations:

- 522 tested, 300 with greatly diminished activity
- Best variant carried 6 mutations, only 2-fold more active than wild-type

Risso *et al.*, *Chem. Sci.* 2020, DOI: 10.1039/D0SC01935F

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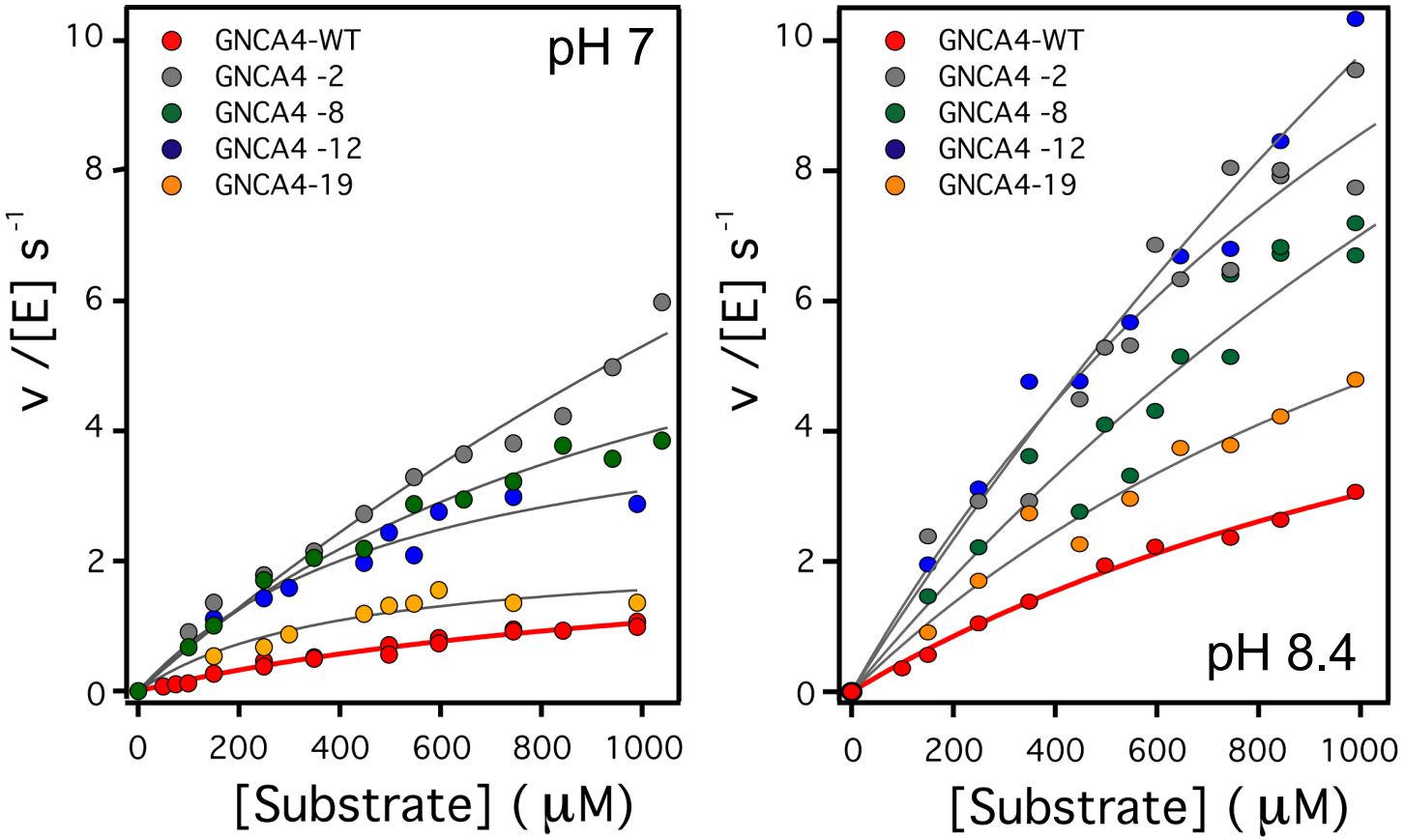
# Activity Enhancement with FuncLib

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http://funclib.weizmann.ac.il

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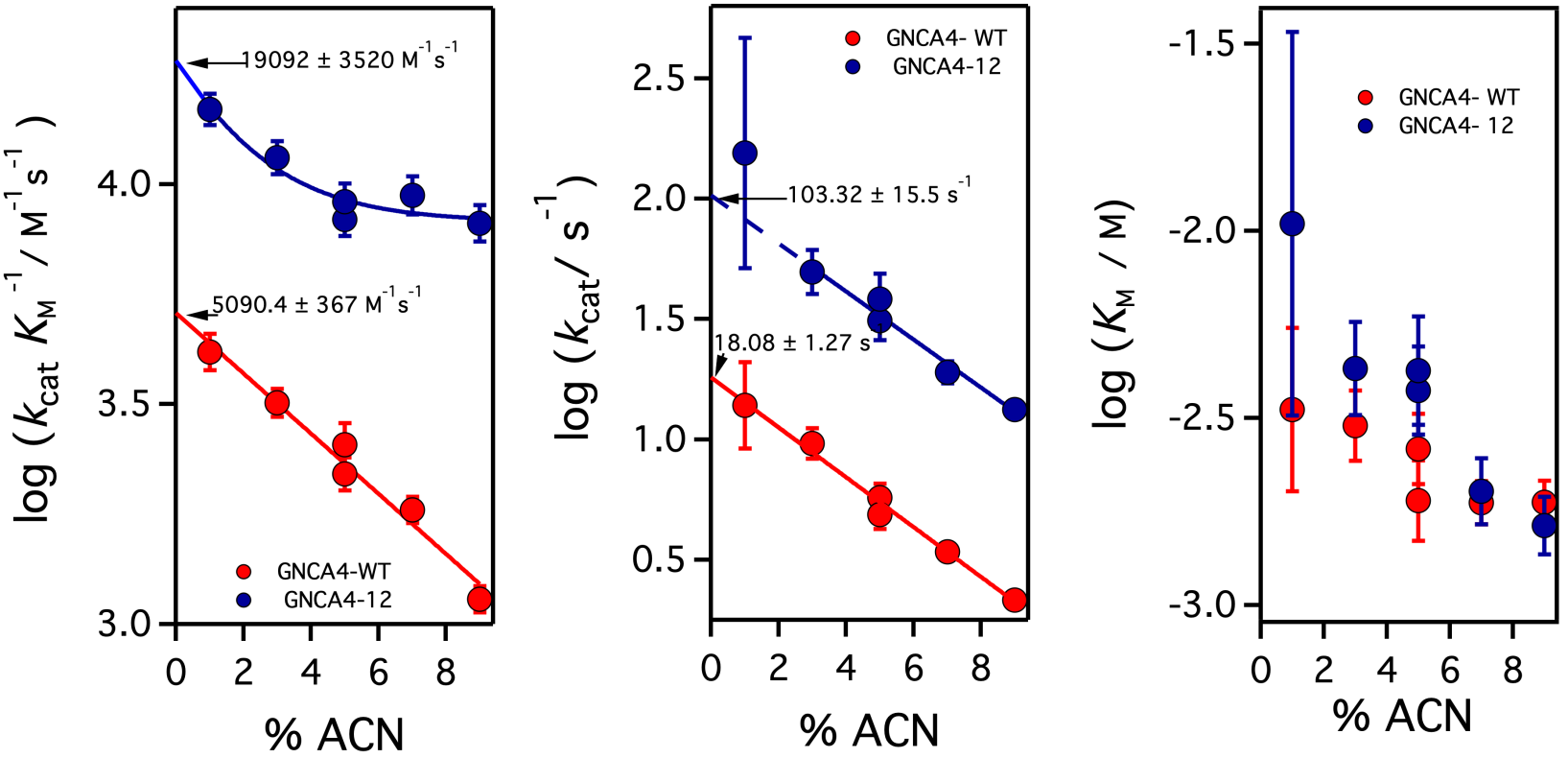


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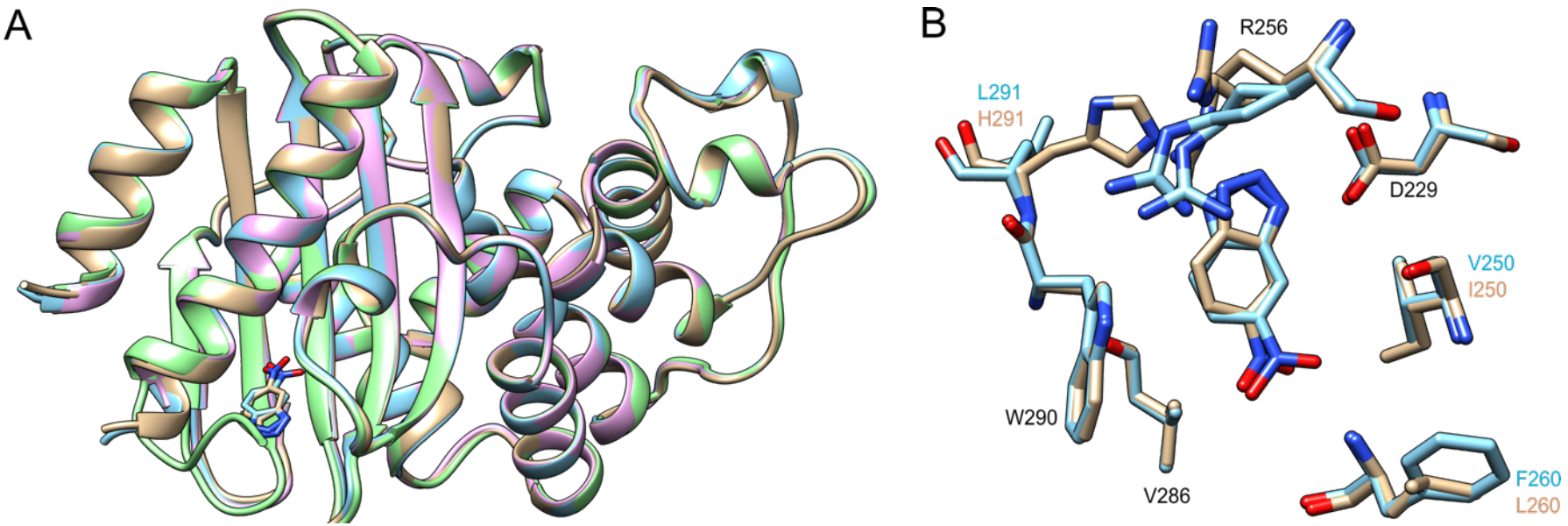
# Minimal Structural Changes




Risso *et al.*, *Chem. Sci.* 2020, DOI: 10.1039/D0SC01935F

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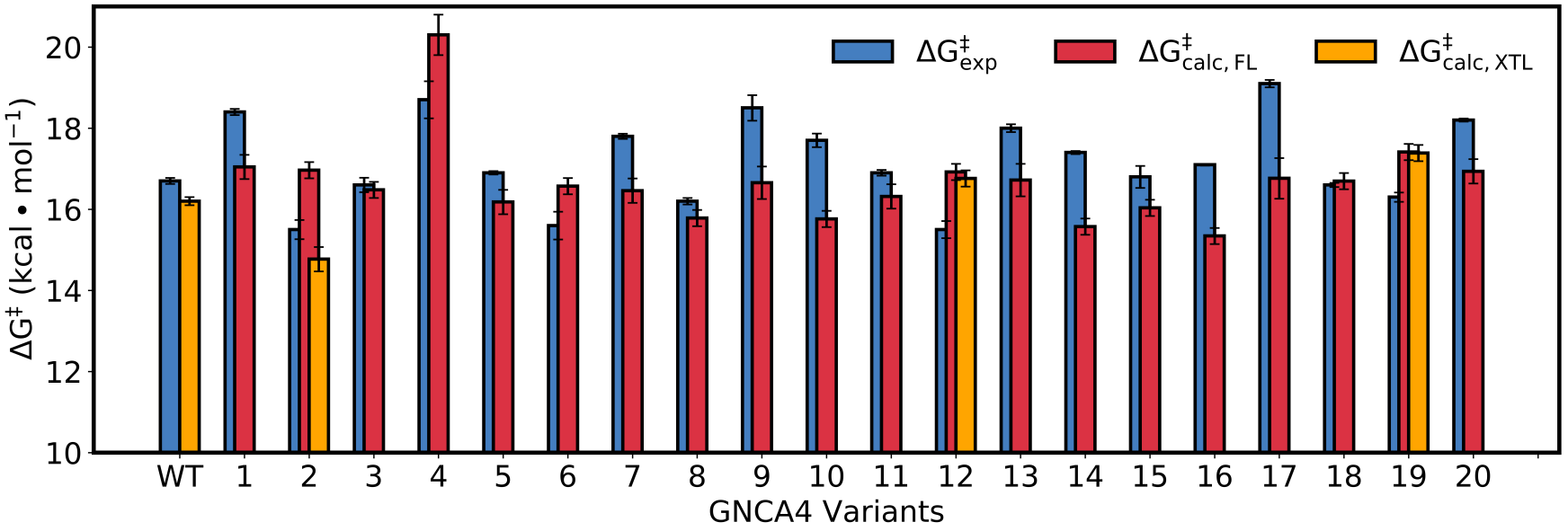
# Can EVB Further Refine the Ranking?




Risso *et al.*, *Chem. Sci.* 2020, DOI: 10.1039/D0SC01935F

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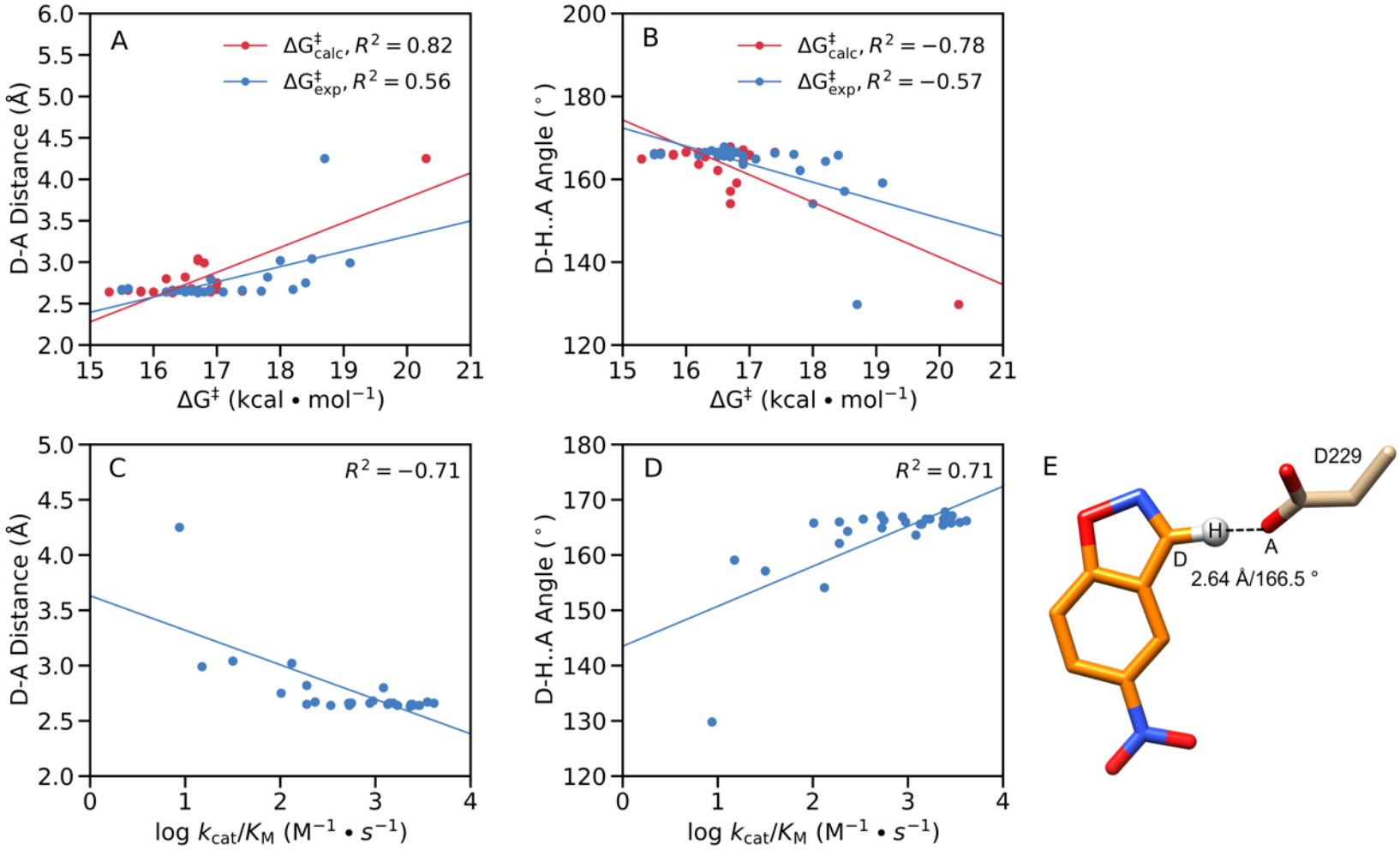
# Geometric Preorganization and Activity




Risso *et al.*, *Chem. Sci.* 2020, DOI: 10.1039/D0SC01935F

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# So What Drives Enzyme Evolution?

- Comparison of several enzymes shows strong correlation between the structural and electrostatic features of their active sites and variations in substrate selectivity.
- These enzymes don't know in advance what substrate will bind, but exploit conformational dynamics to adjust their active site environment to a given substrate after the binding step.
    - *Just having key catalytic residues in place is not enough*!
- Regulating both local and global conformational dynamics appears to be an important factor in allowing for the emergence of new enzyme activities.

Conformational dynamics needs to be accounted for in both experimental and computational protein engineering studies!

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# Further Reading if Interested



ACS Catalysis

pubs.acs.org/acscatalysis Viewpoint

## Manipulating Conformational Dynamics To Repurpose Ancient Proteins for Modern Catalytic Functions

Jasmine M. Gardner, Michal Biler, Valeria A. Risso, Jose M. Sanchez-Ruiz,* and Shina C. L. Kamerlin*

Cite This: *ACS Catal.* 2020, 10, 4863–4870 Read Online



J|A|C|S JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/JACS Perspective

## Harnessing Conformational Plasticity to Generate Designer Enzymes

Rory M. Crean, Jasmine M. Gardner, and Shina C. L. Kamerlin*

Cite This: *J. Am. Chem. Soc.* 2020, 142, 11324–11342 Read Online

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