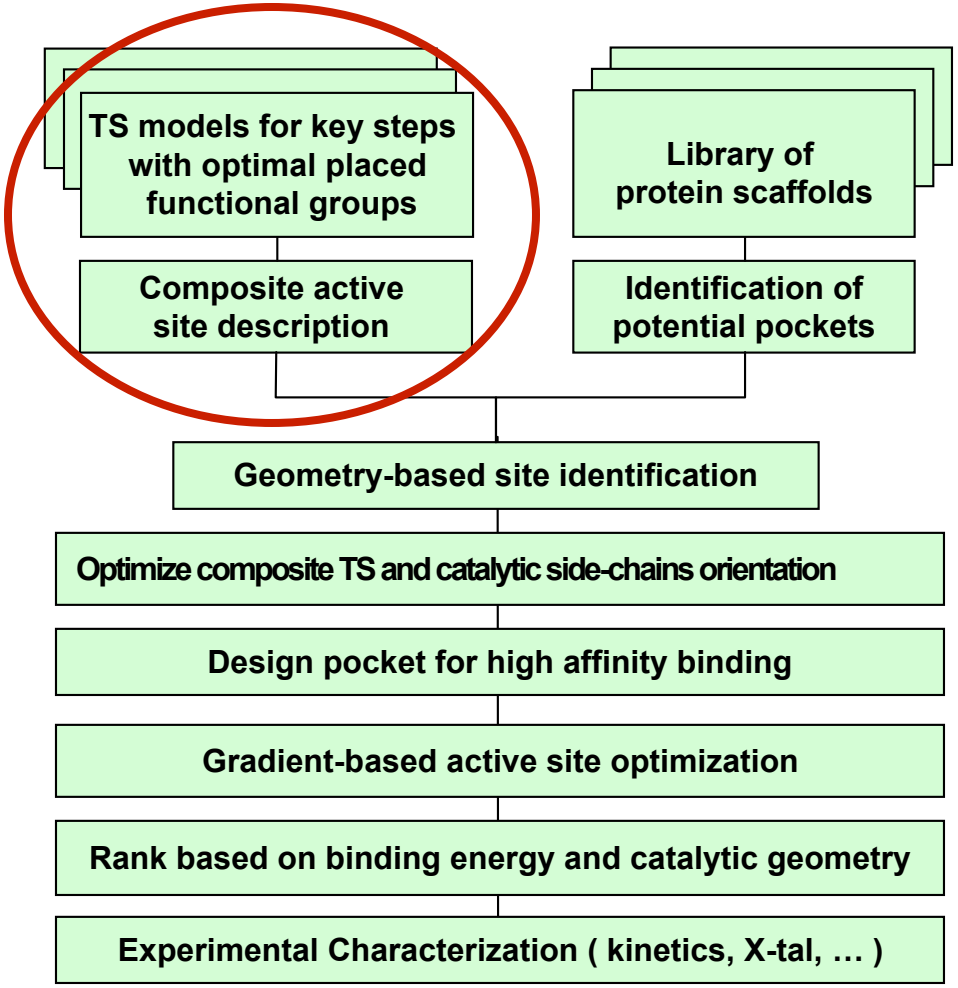
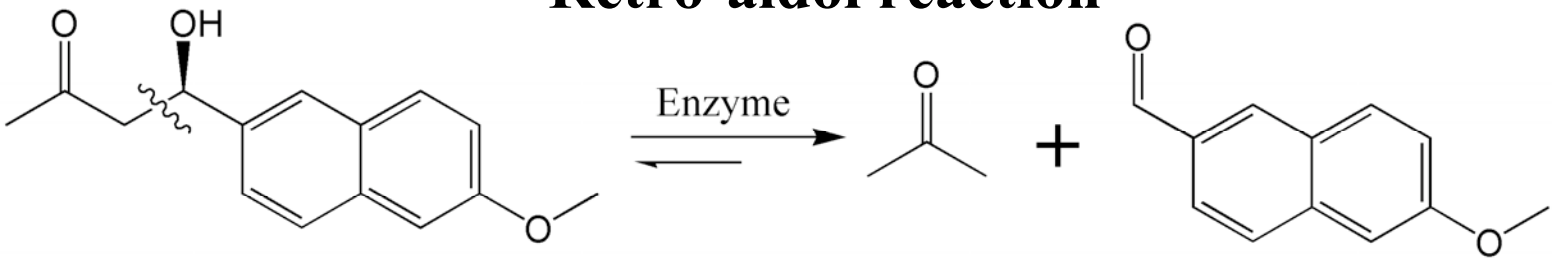
Improved Enzyme Designs -since last RosettaCon

Eric Althoff (and Lin Jiang)
University of Washington

Flow chart of computational enzyme design for multi-step reaction



Retro-aldol reaction



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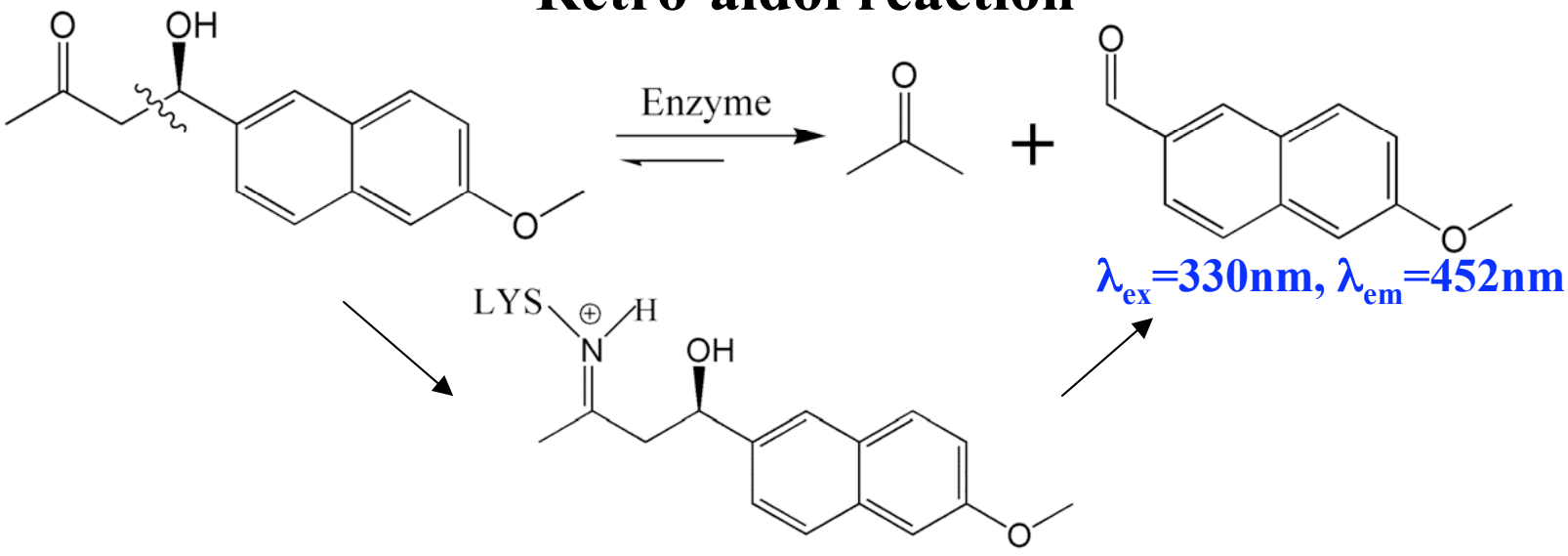
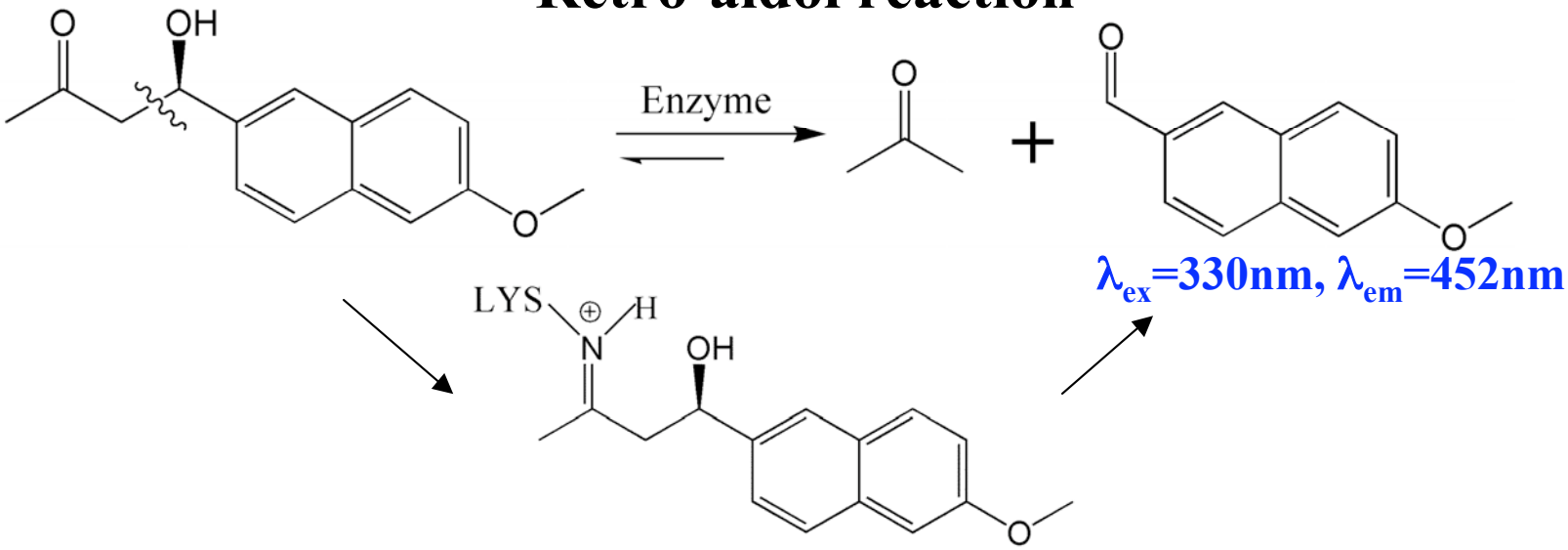


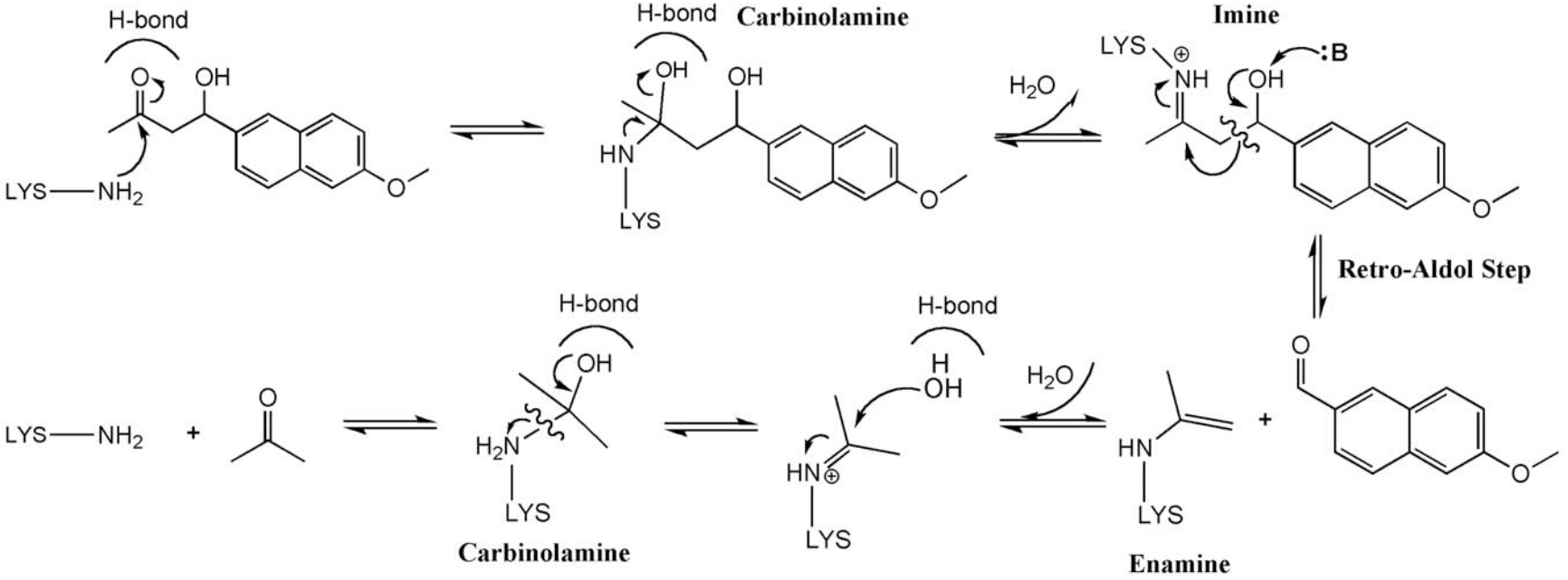
λ_{ex}=330nm, λ_{em}=452nm

Retro-aldol reaction

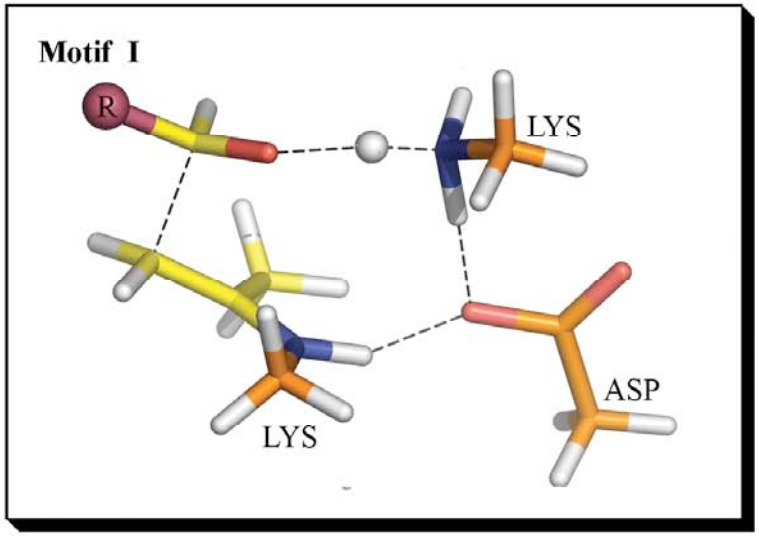


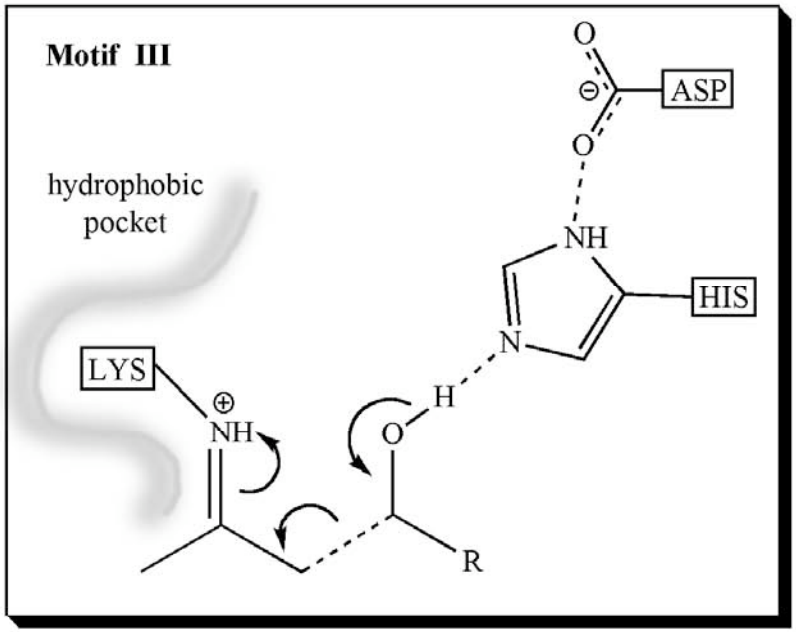
λ_{ex}=330nm, λ_{em}=452nm

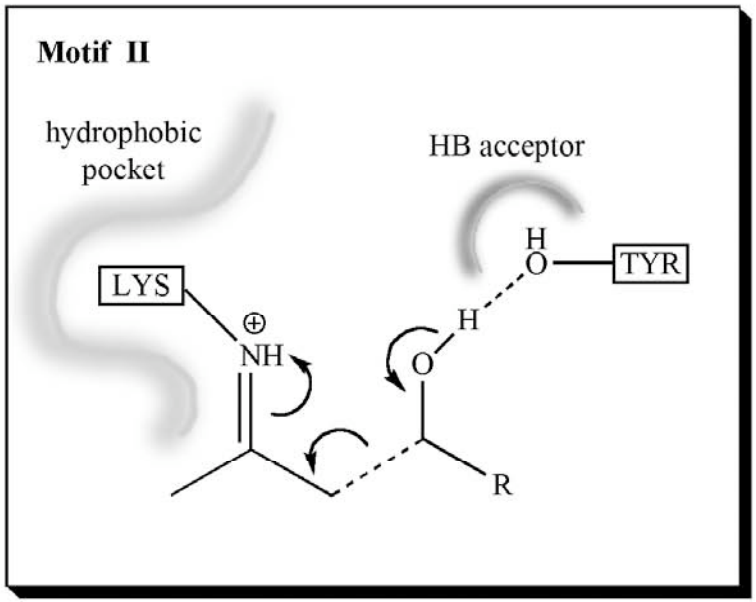
Aldol reaction pathway through enamine formation

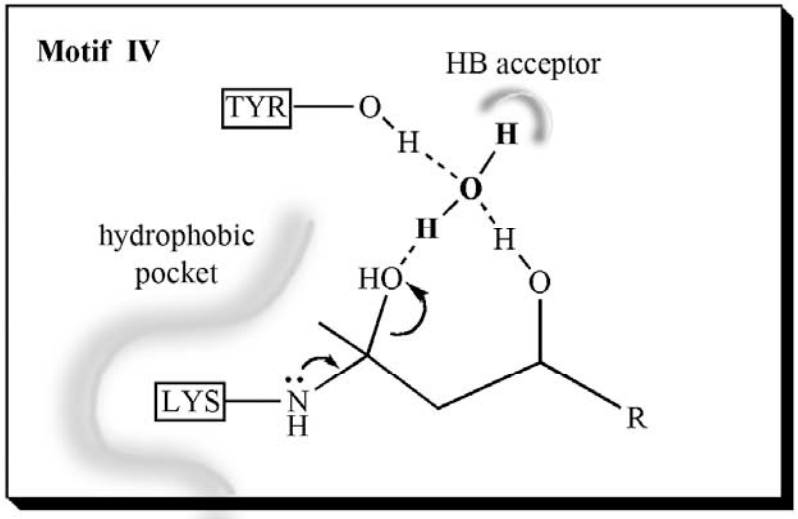


Catalytic motifs used in designs

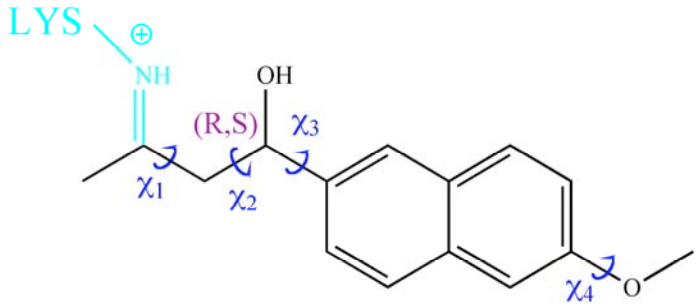








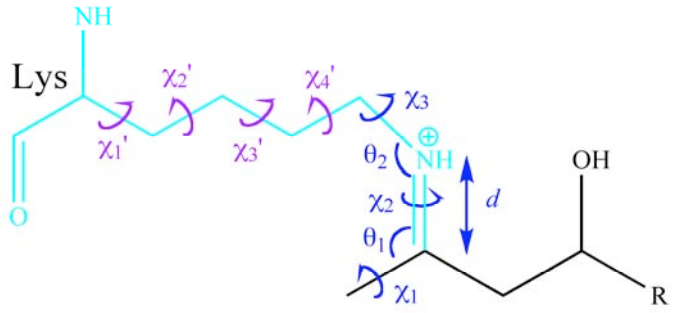
Diversification of transition state



	optimal value[a]	deviation	# of conformations
χ_1	91°,-89°	+/-10°	6
χ_2	-177°	+/-10°	3
χ_3	109°,-71°	+/-10°	6
χ_4	0,180°	+/-20°	6
(R,S)-enantiomers			2
total			1,296

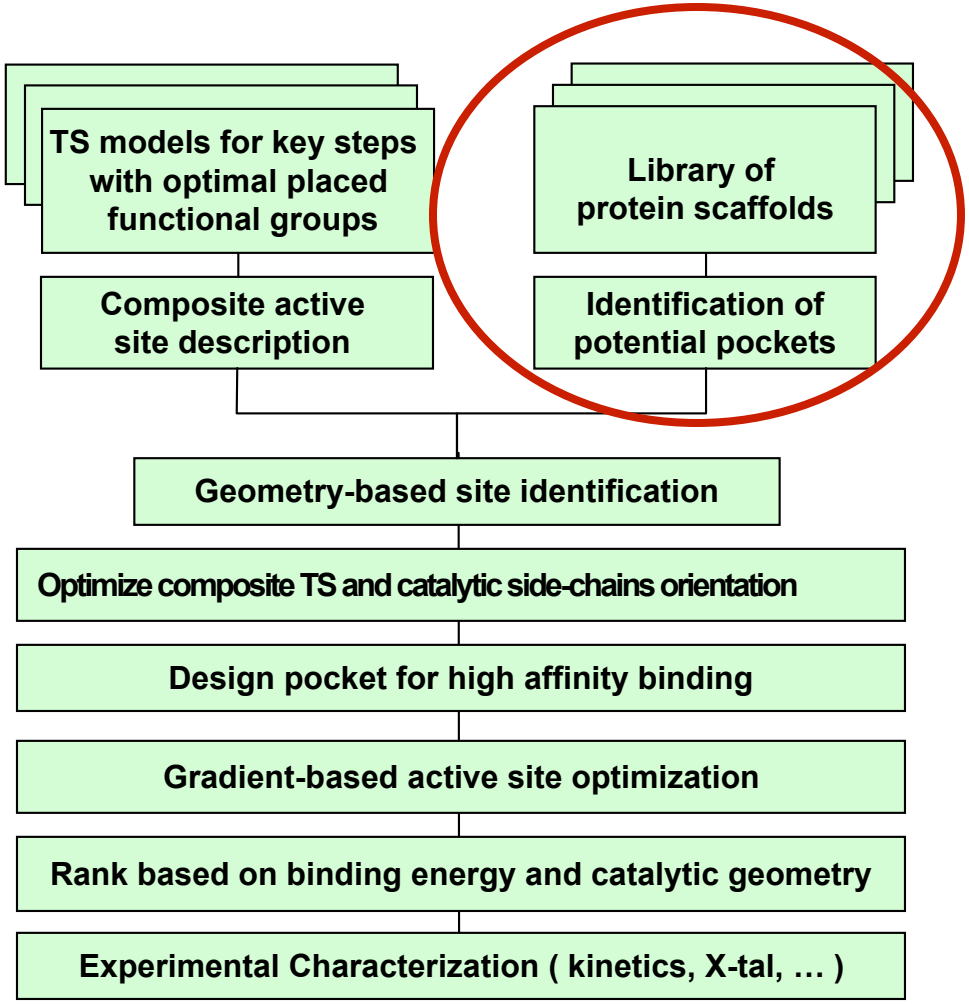
[a]. all torsion angles take their starting values from the QM lowest energy TS model of the R-enantiomer based on the QM caculation for C-C bond-breaking step

Diversification of side-chains

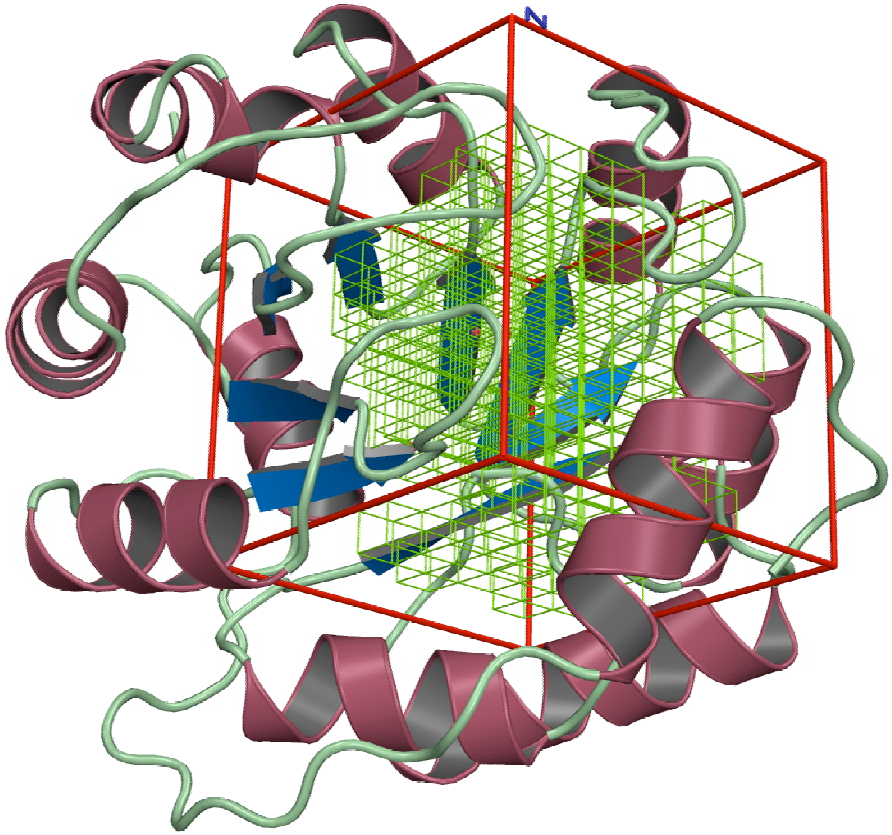


	ideal value	deviation	# of conformations
d	1.3Å	+/-0.2Å [a]	1
θ_1	125°	+/-5° [a]	1
θ_2	120°	+/-5° [a]	1
χ_1	180° [b]	+/-10°	3
χ_2	0°,180° [b]	+/-10°	6
χ_3	0° [c]	every 60°	6
χ_1'	64.3° [d]	+/-7.6°	
χ_2'	178.9° [d]	+/-8.1°	
χ_3'	177.5° [d]	+/-10.0°	
χ_4'	-179.2° [d]	+/-9.6°	
$\chi_1' - \chi_4'$			28x81[e]
all			244,944

Flow chart of computational enzyme design for multi-step reaction



Precalculate Active Site Grid in Scaffold Proteins



-high resolution structures
-binding pocket

Andrew Wollacott

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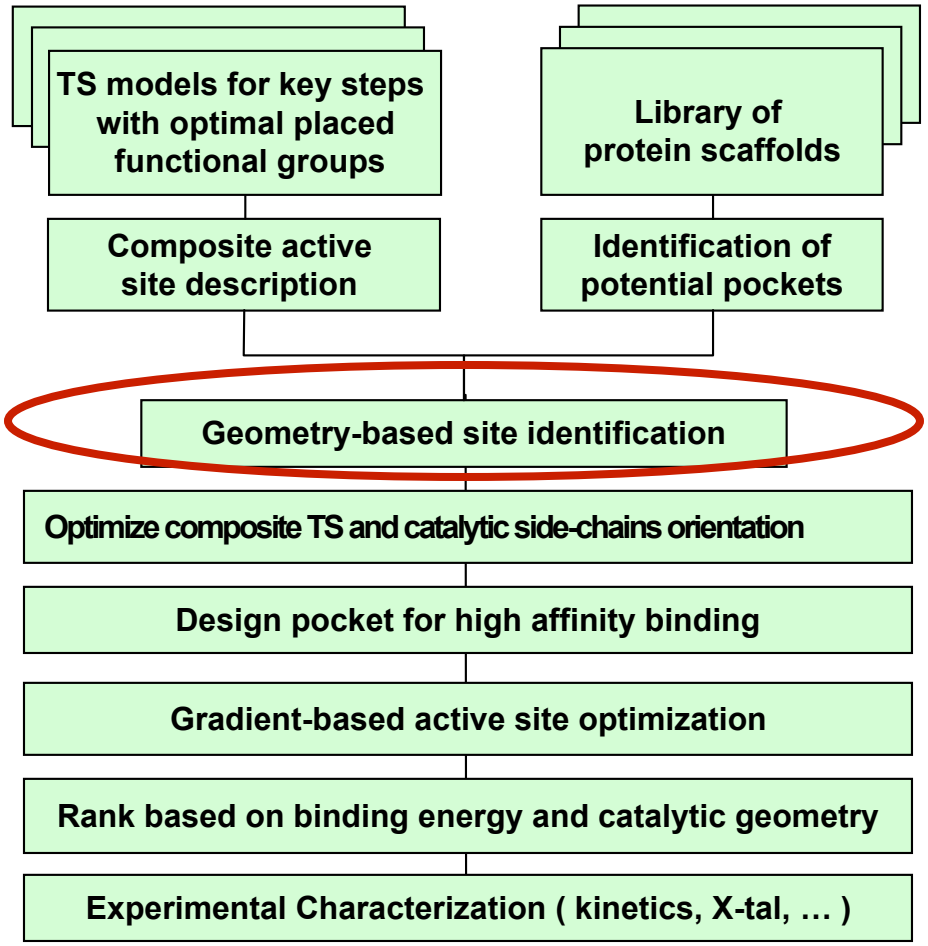
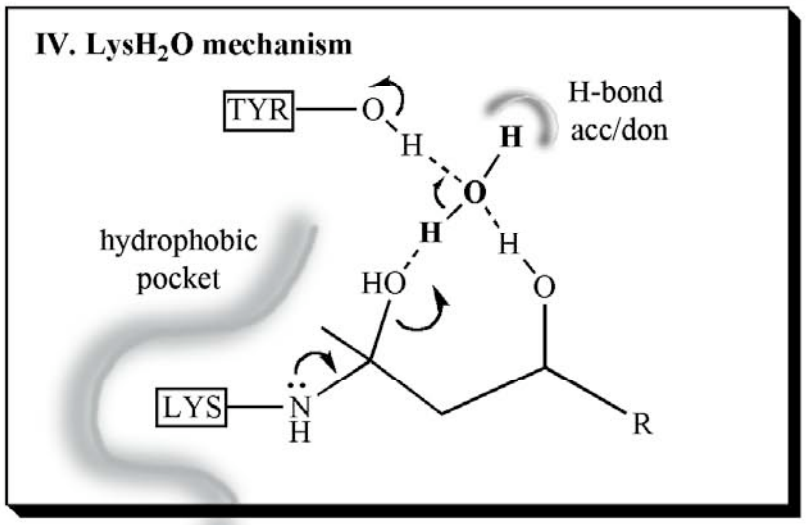


Generation of active site description: the transition state model and functional group positioning



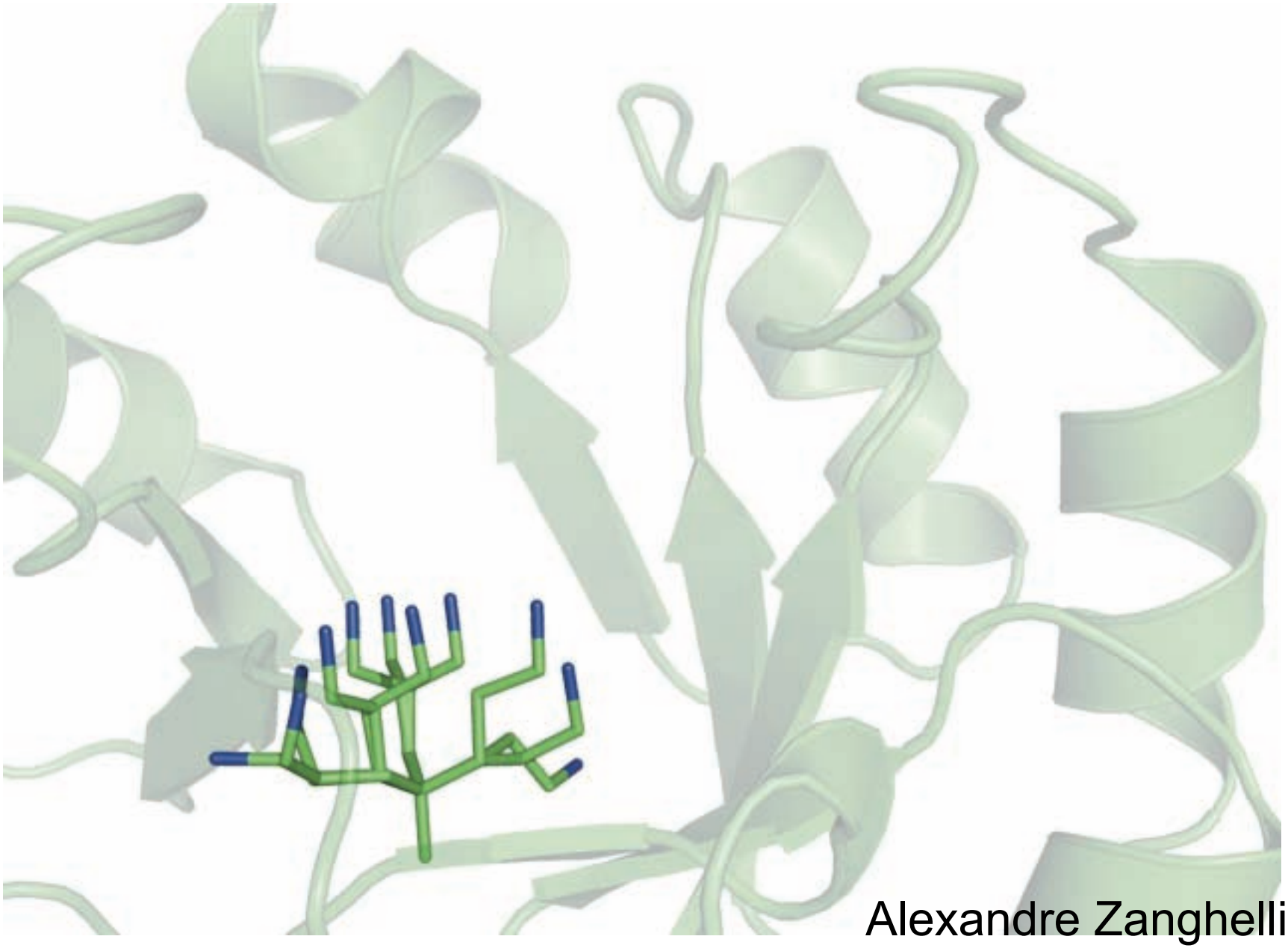
TS

TYR

SER

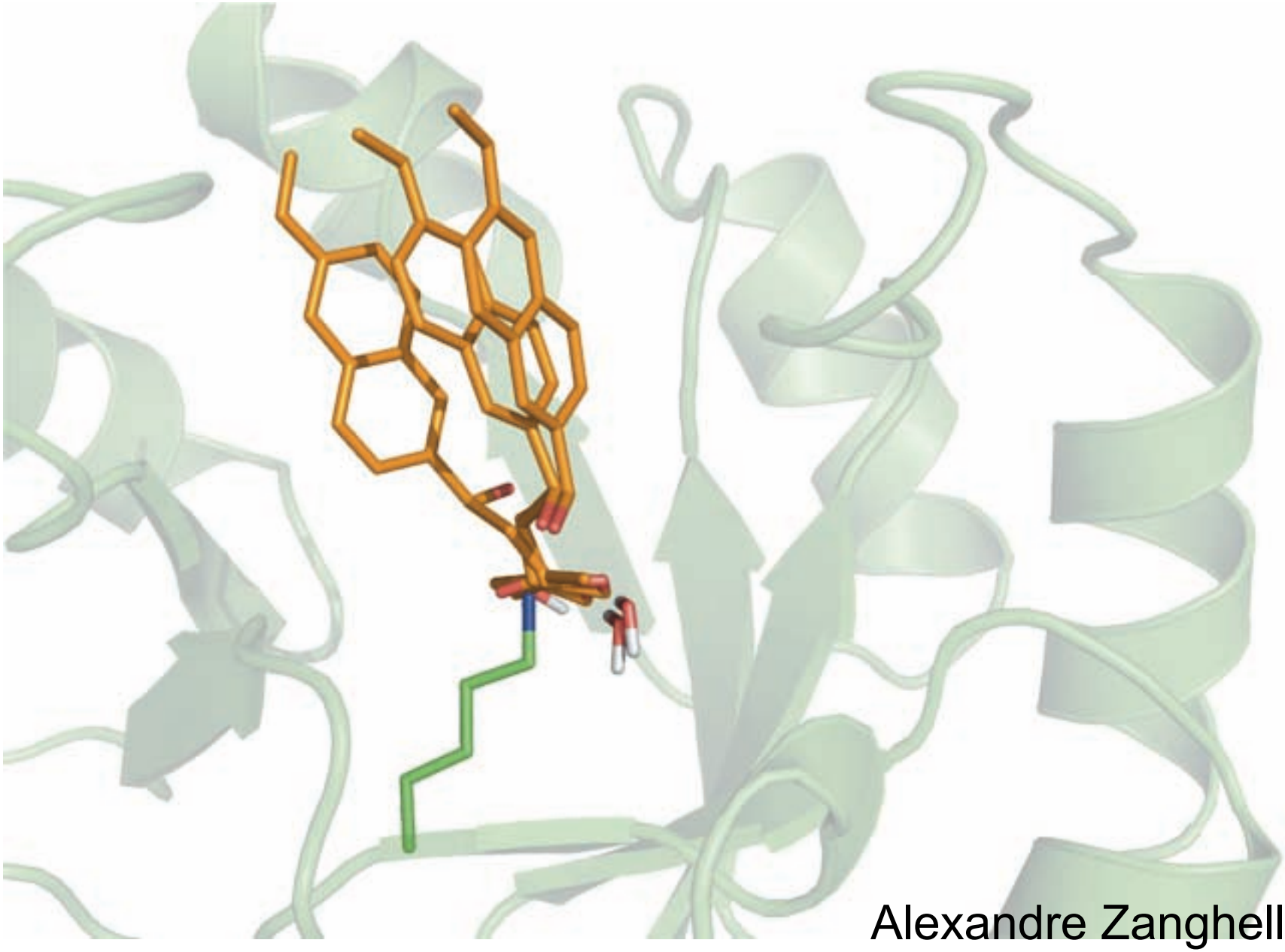
LYS

Placement of first catalytic sidechain rotamers



Alexandre Zanghellini

Placement of an ensemble of TS models



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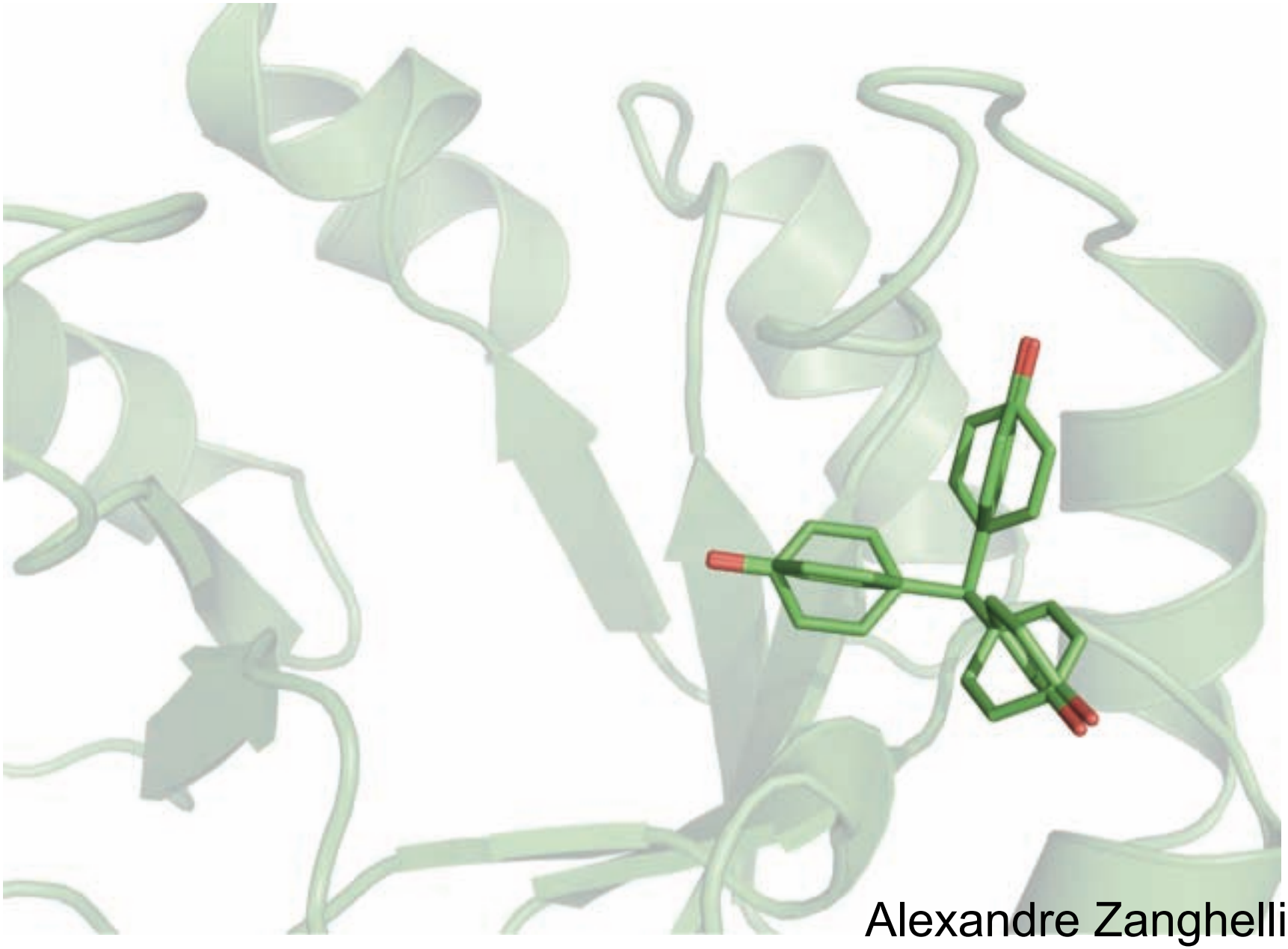
Placement of second catalytic sidechain rotamers



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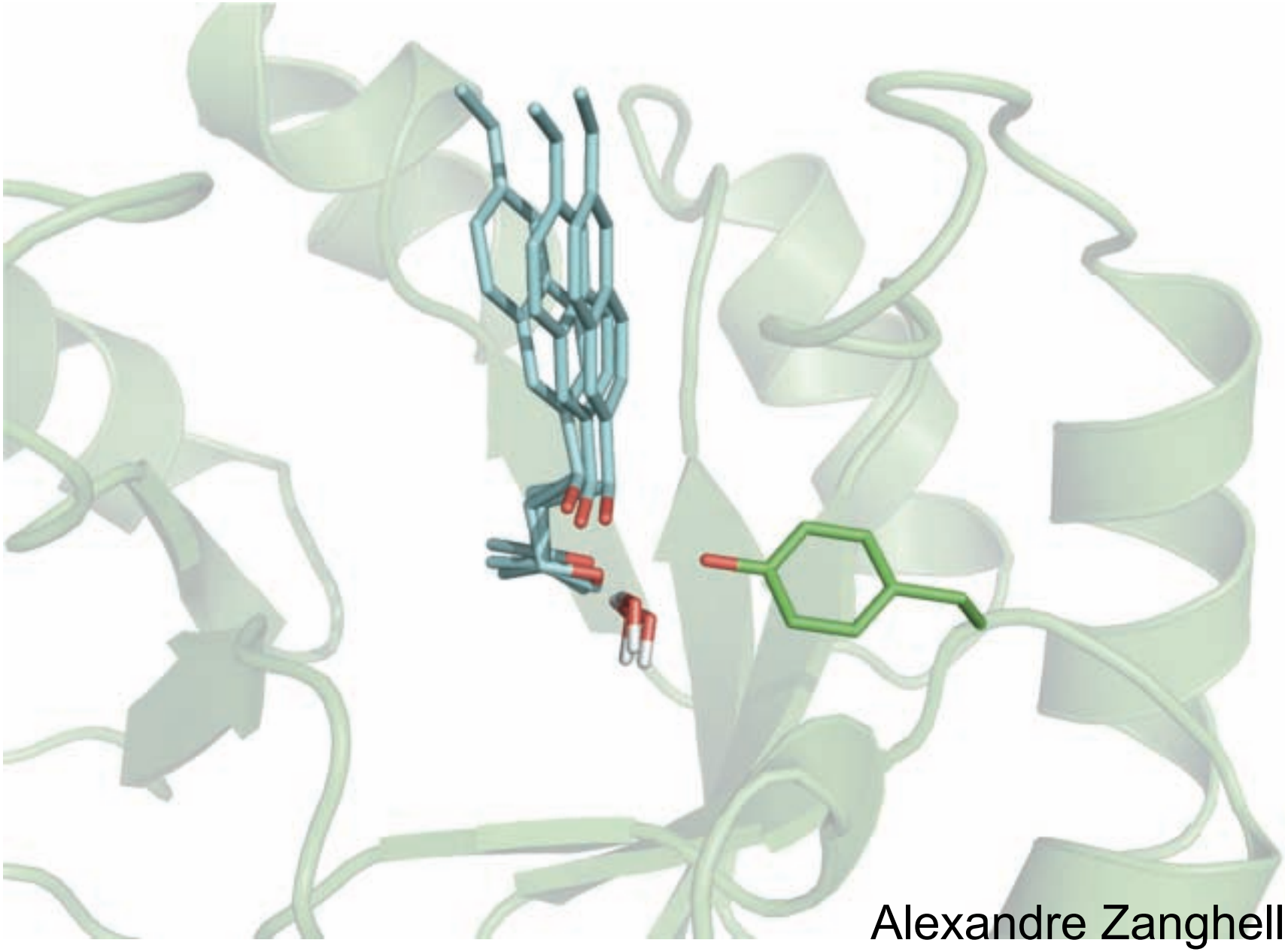

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Placement of third catalytic sidechain rotamers

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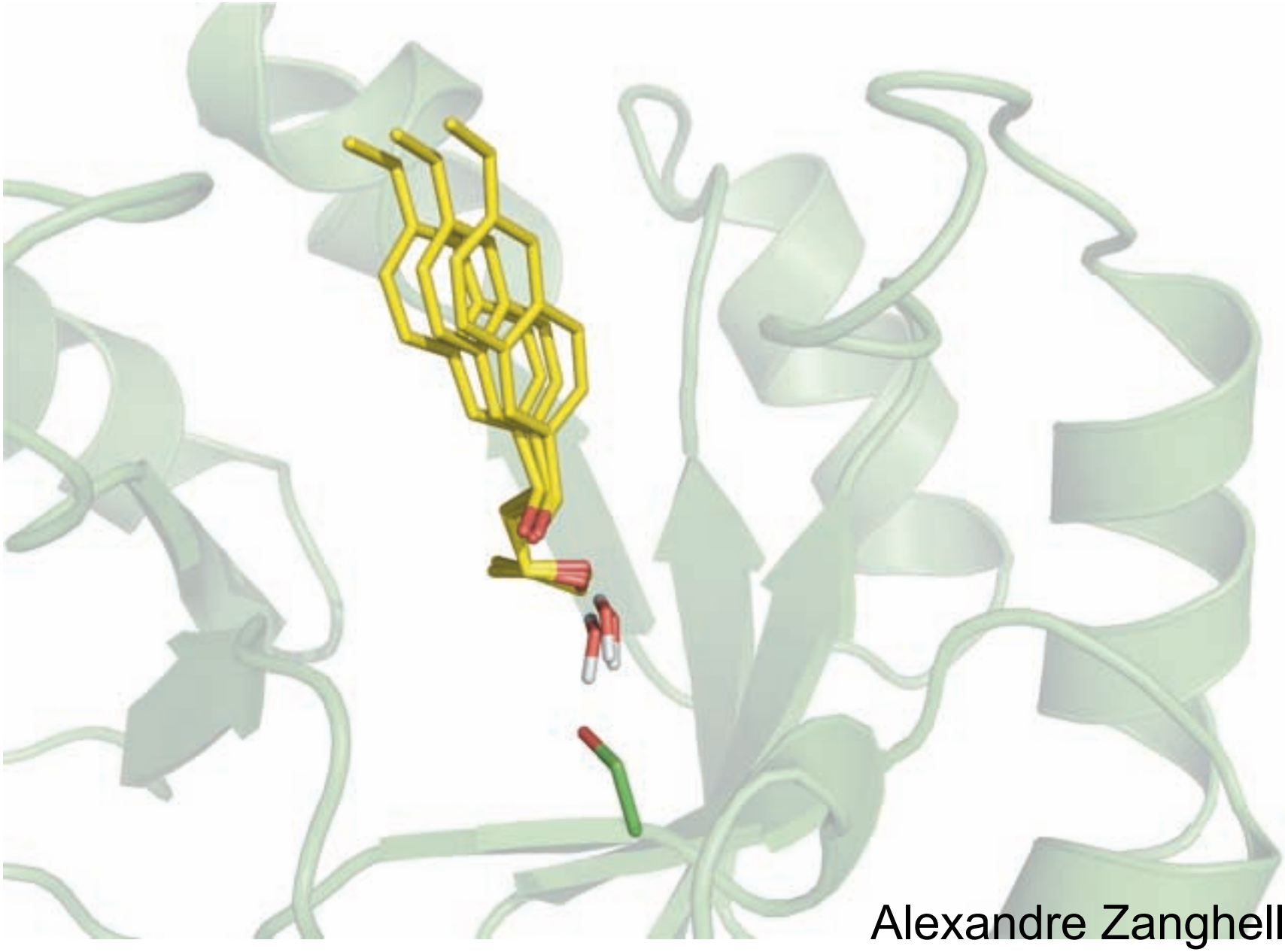
Placement of an ensemble of TS models



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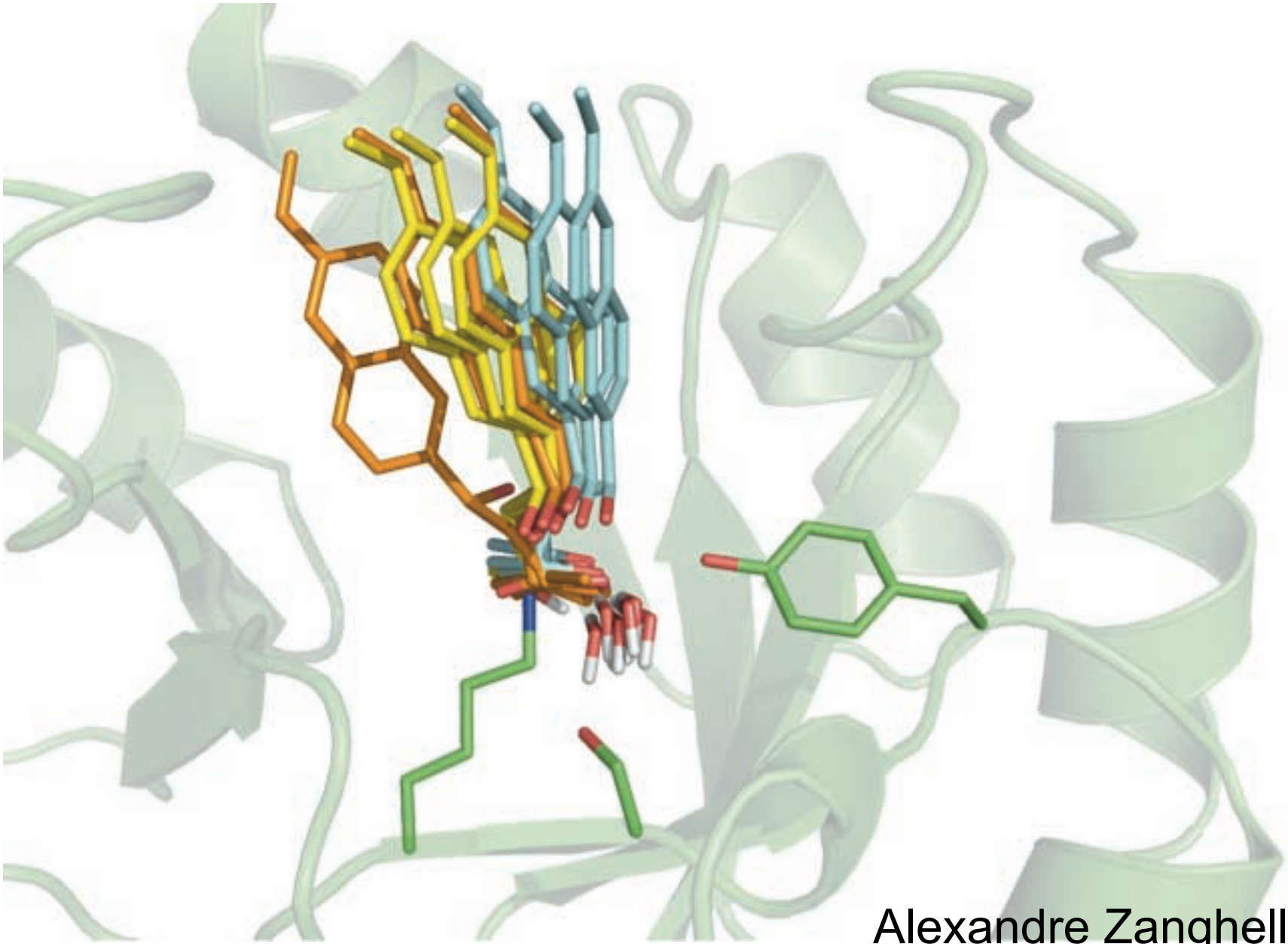
Identification of overlap among TS ensembles by hashing



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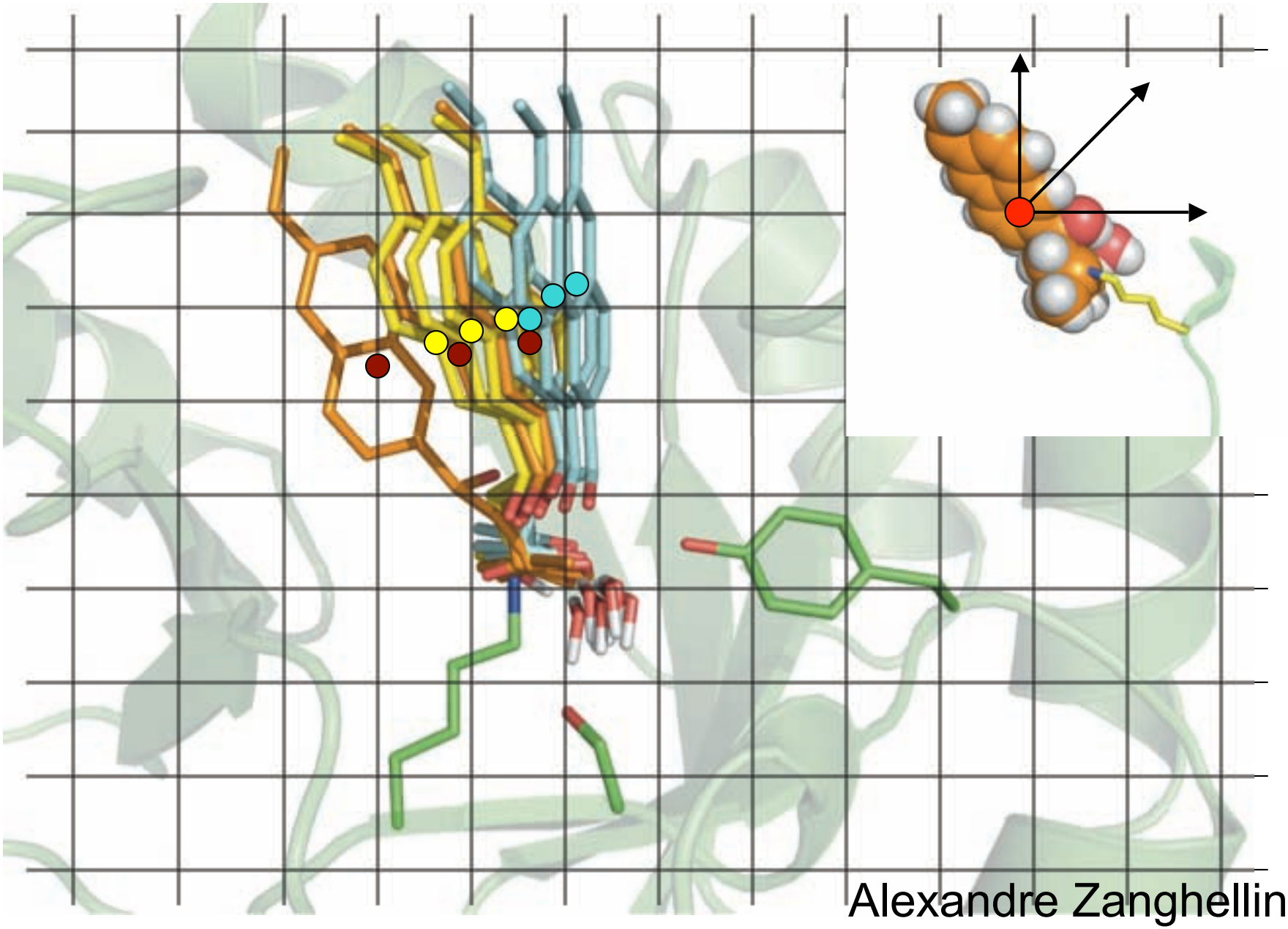

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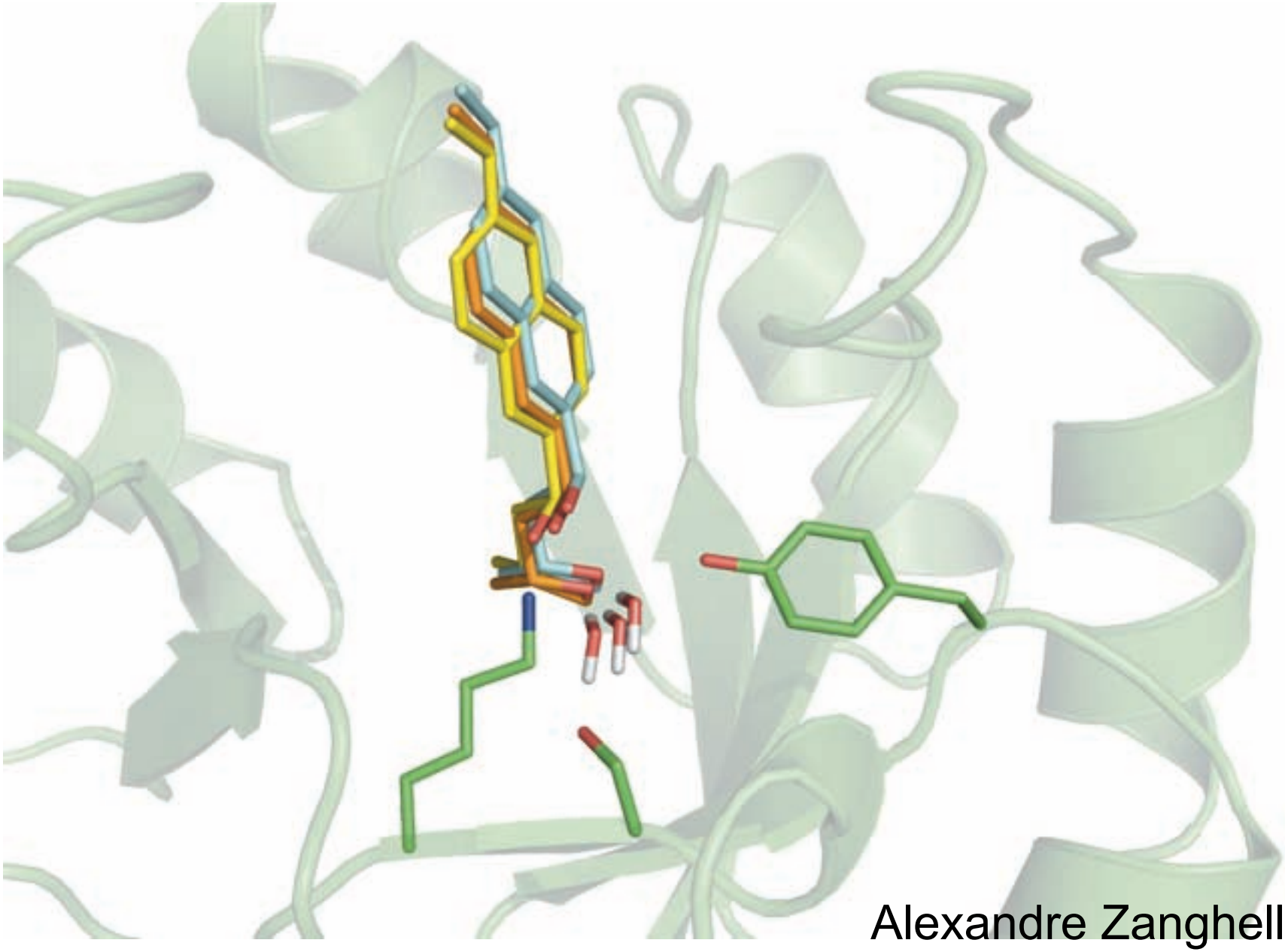

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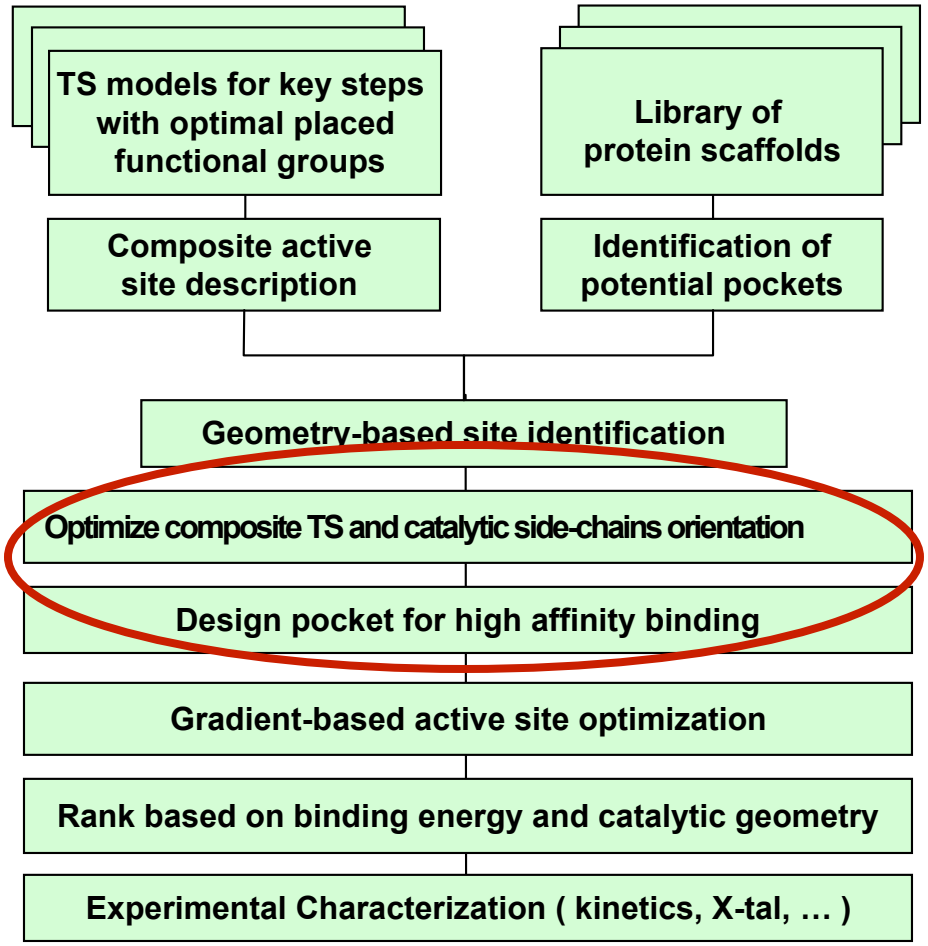
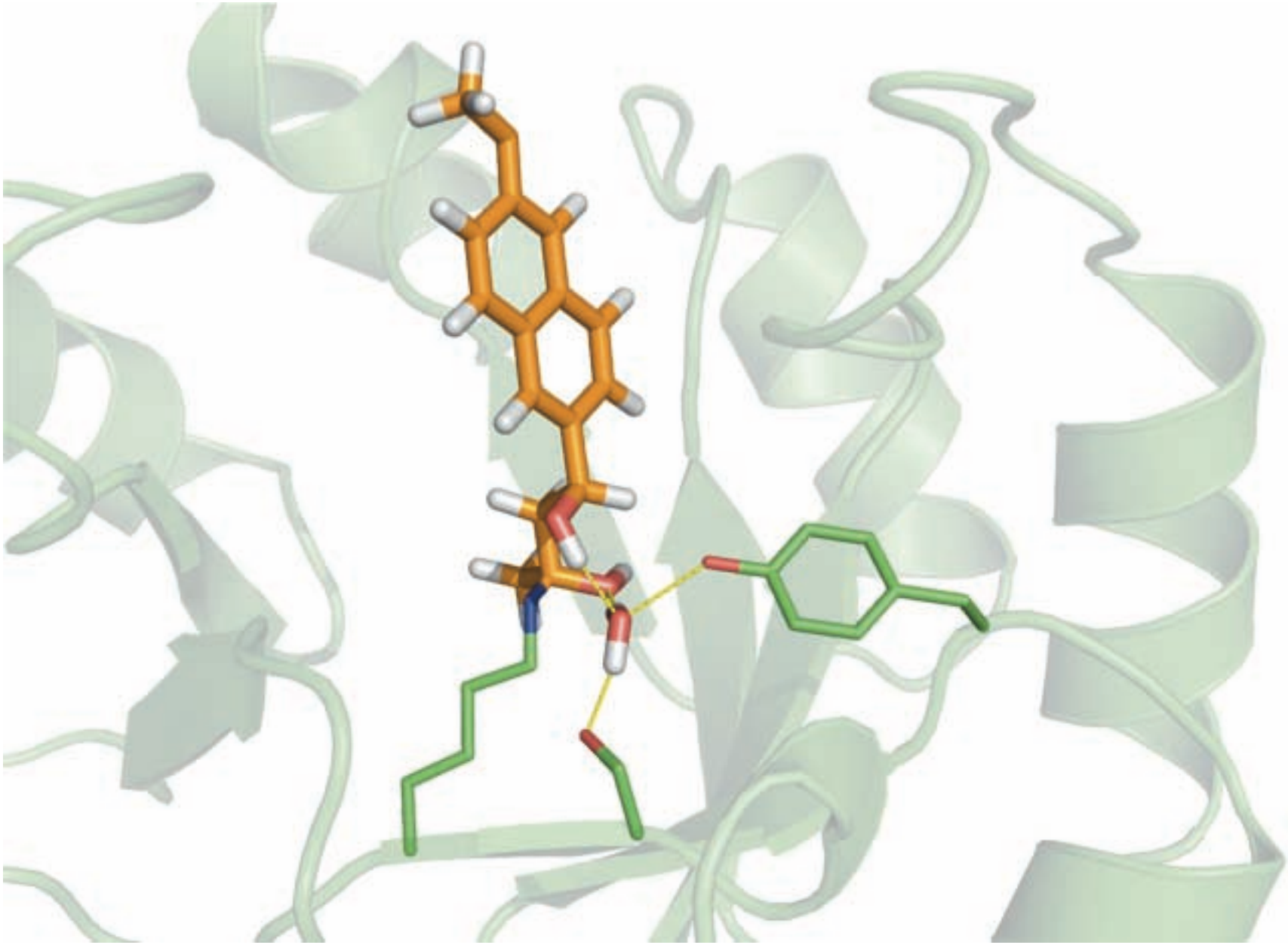
Flow chart of computational enzyme design for multi-step reaction



Optimization of TS and catalytic side-chains orientation



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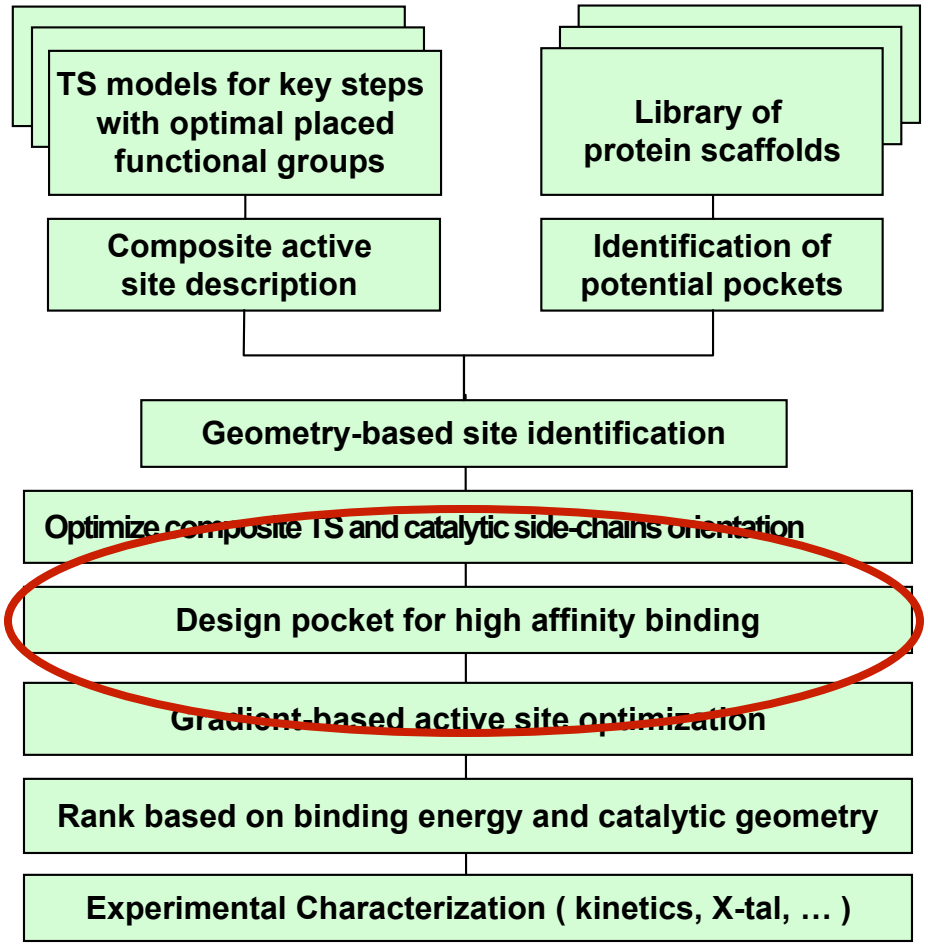
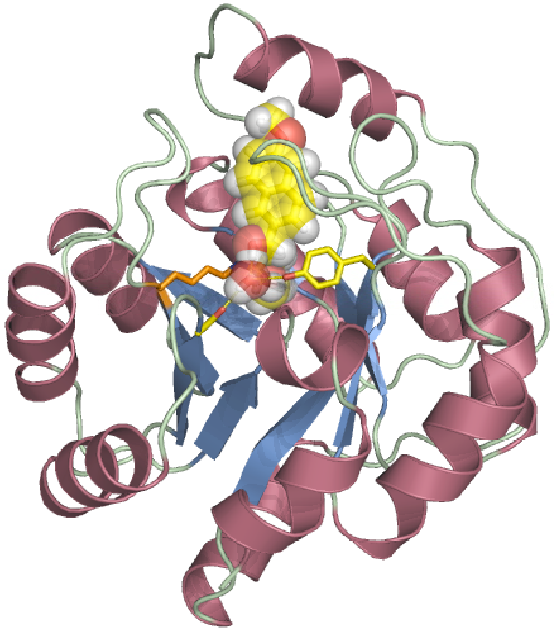
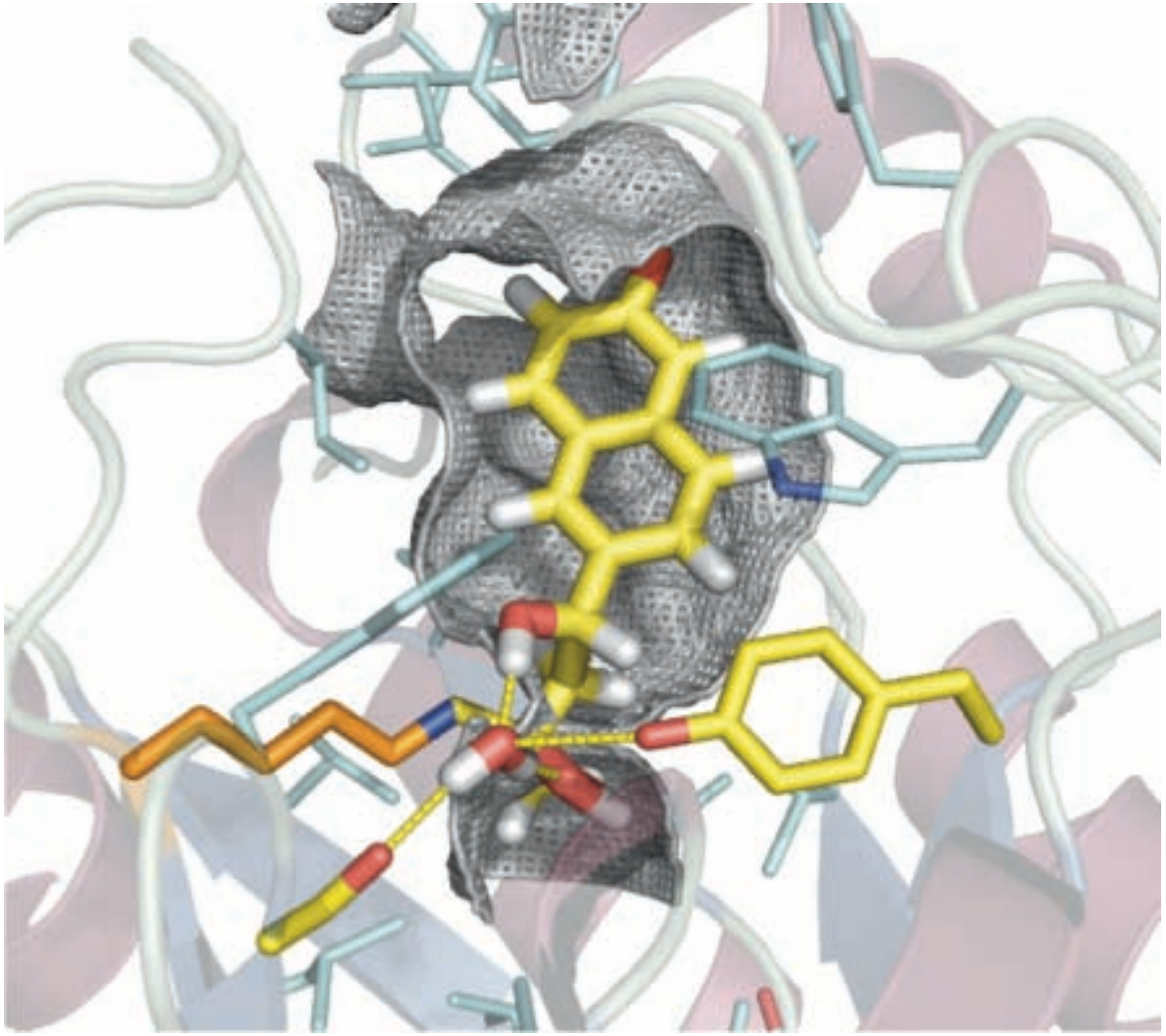


Design model RA46 on indole-3-glycerol phosphate synthase scaffold





1. Hydrogen Bonding Satisfied 2. SASA optimized

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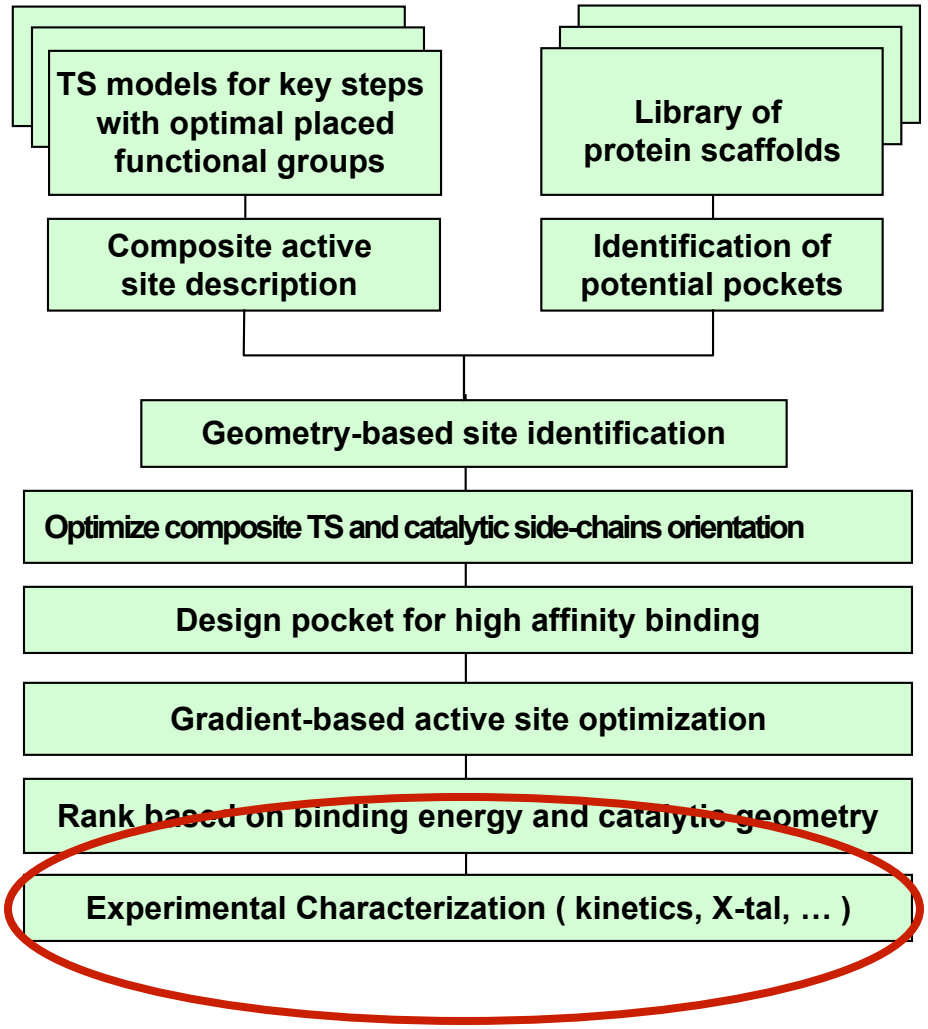
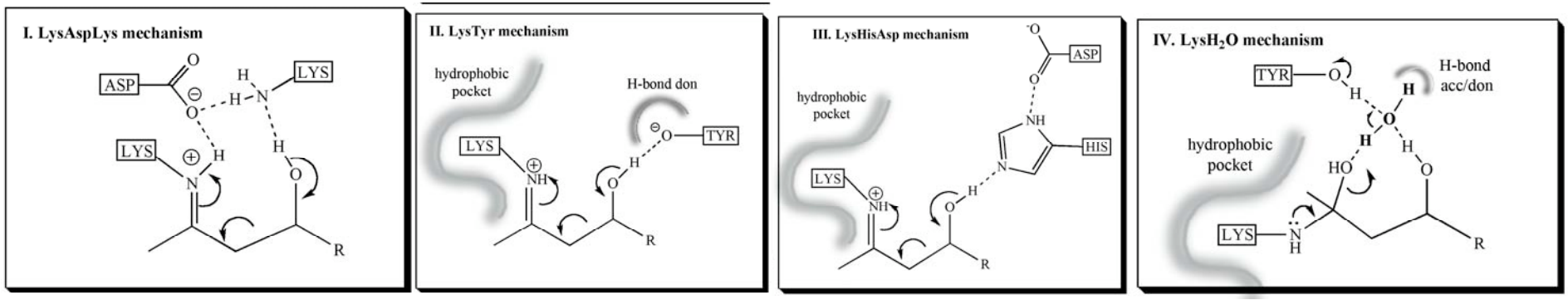
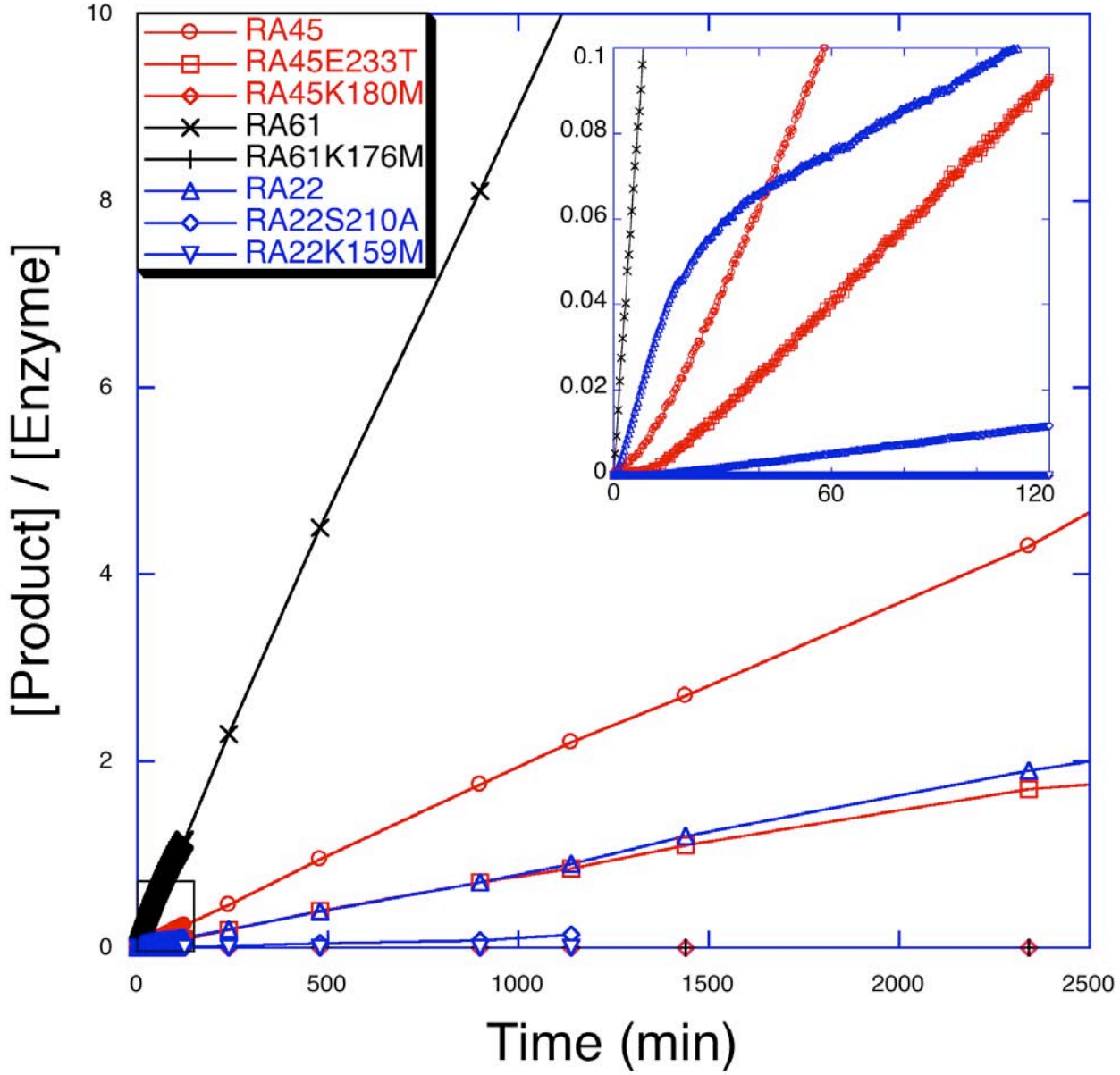


Schiff-base formation and enzyme activity for different catalytic sites

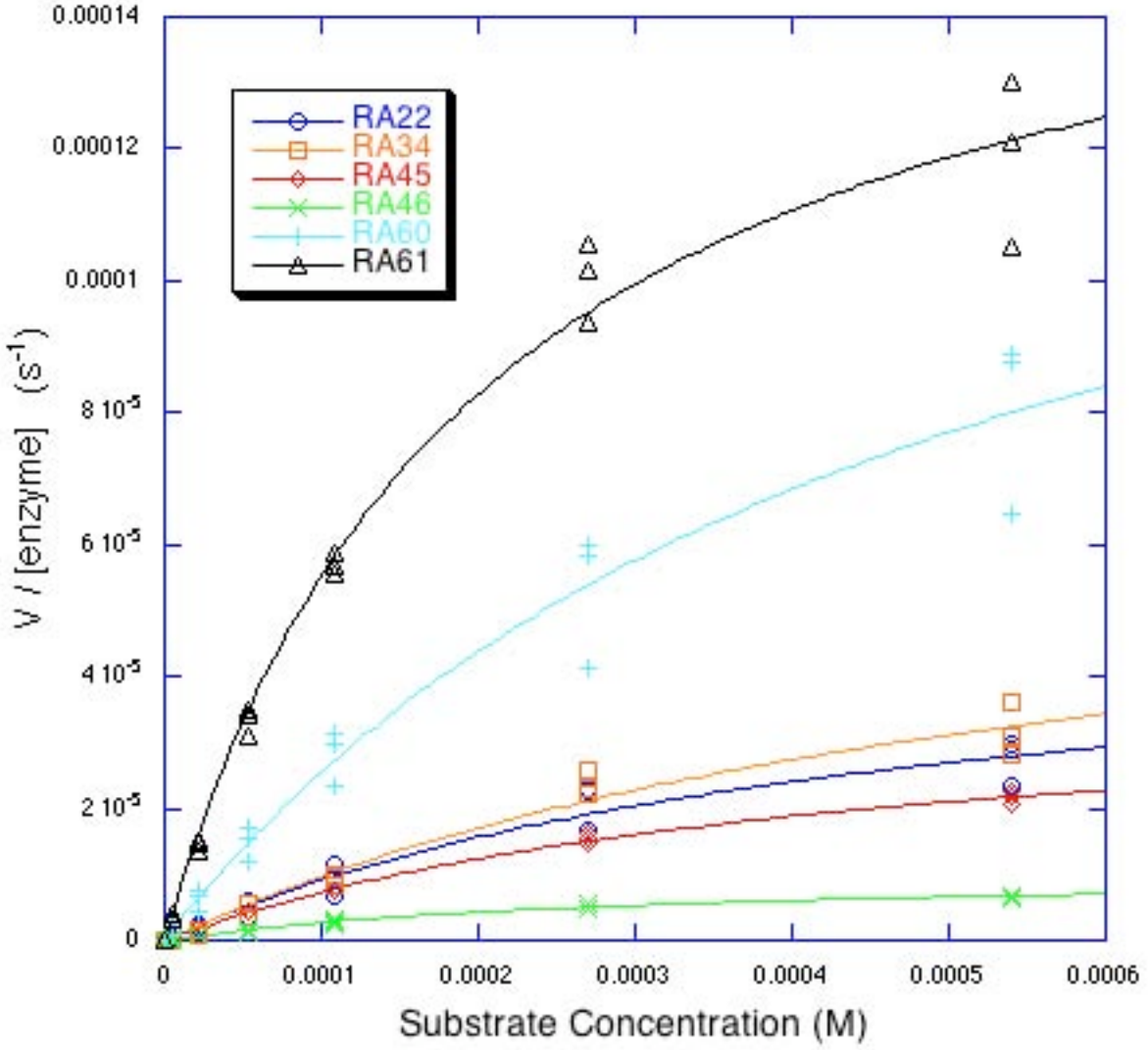


Motif	Catalytic lysine environment	Carbinolamine stabilization	Proton abstraction	# tested	# forming enaminone	# active designs	Rate enhancement
I	Polar	-	Lys/Asp dyad	12	2	0	
II	Hydrophobic	-	Tyr	9	1	0	
III	Hydrophobic	H-bond acceptor/donor	His/Asp dyad	13	10	10	10^2~10^3
IV	Hydrophobic	Water, H-bond acceptor	Water	38	20	22	10^3~10^4

Progress Curves Aldolase Design



Determination of Kinetic Parameters

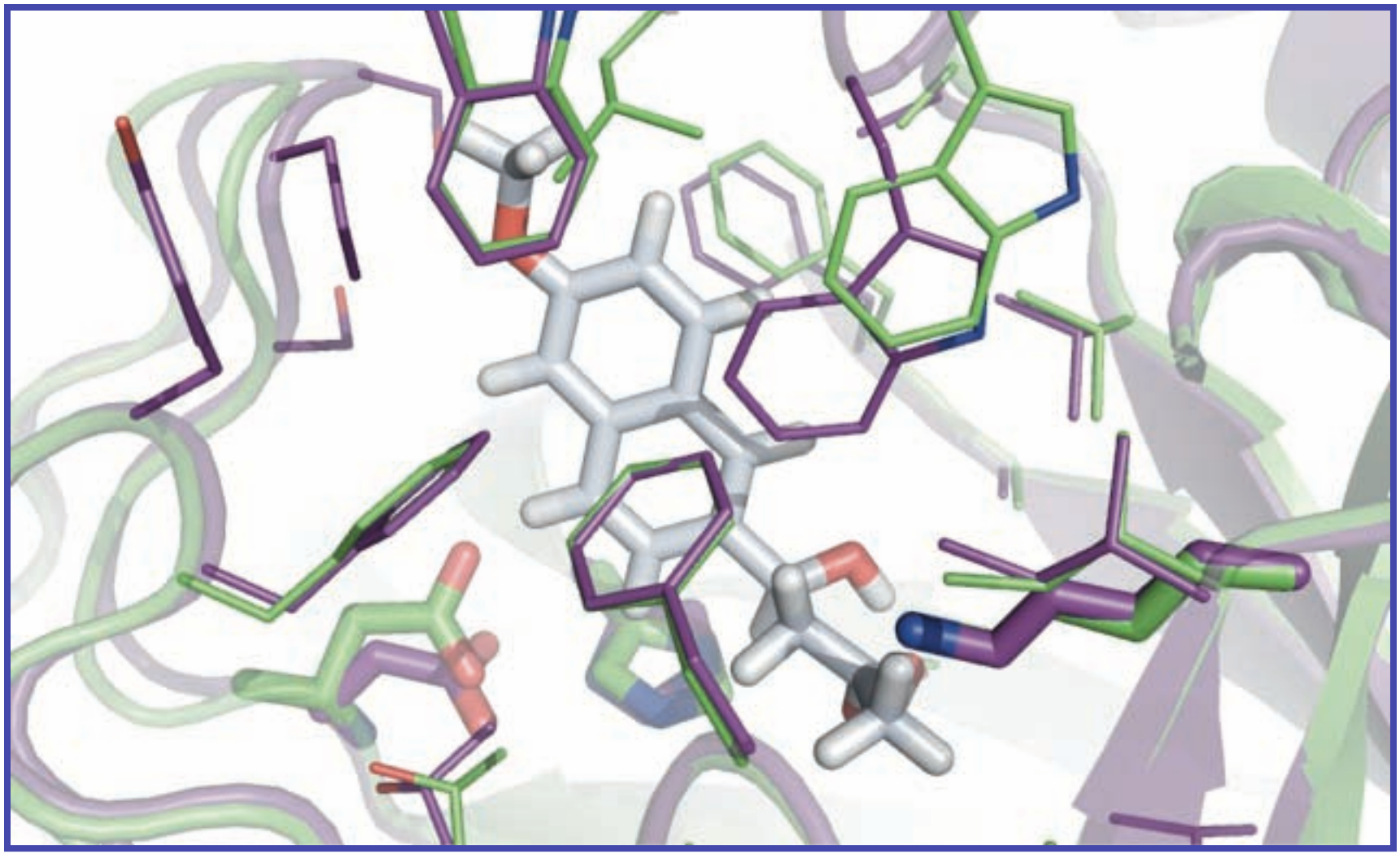


Determination of Kinetic Parameters

Design	k_{cat} ($x10^{-3}$ min^{-1})	K_M (μ M)	k_{cat}/K_M ($M^{-1}s^{-1}$)	k_{cat}/k_{uncat}*
RA22	$_b$3.1 ± 0.3 $_s$0.5 ± 0. 1	$_b$480 ± 130 $_s$450 ± 210	$_b$0.11 ± 0.03 $_s$0.018 ± 0.006	$_b$8.1 x 10^3 $_s$1.2 x 10^3
RA34	$_b$4.2 ± 1.1 $_s$0.6 ± 0. 1	$_b$620 ± 180 $_s$600 ± 140	$_b$0.11 ± 0.01 $_s$0.016 ± 0.004	$_b$1.1 x 10^4 $_s$1.5 x 10^3
RA45	2.3 ± 0.2	430 ± 48	0.091 ± 0.004	6.0 x 10^3
RA46	0.62 ± 0 . 5	290 ± 60	0.037 ± 0.006	1.6 x 10^3
RA60	9.3 ± 0 . 9	510 ± 33	0.30 ± 0.06	2.4 x 10^4
RA61	9.0 ± 1 . 0	210 ± 50	0.74 ± 0.11	2.3 x 10^4

* k_{uncat}= 3.9 x 10^{-7} min^{-1}(*19*) b = burst phase, s = steady stat e

Comparison between the design model and the X-ray structure (RA22)



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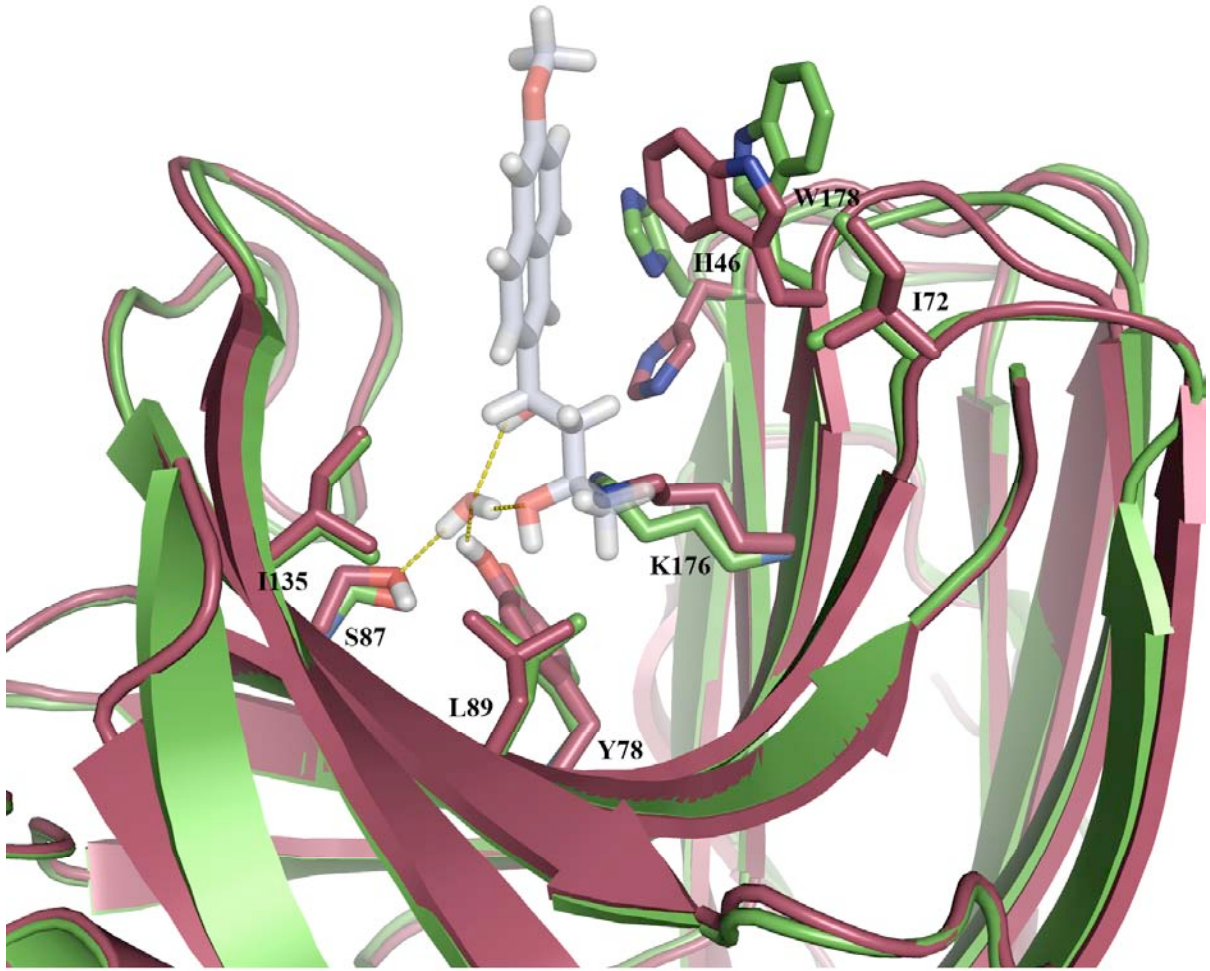
Comparison between the design model and the X-ray structure (RA61)



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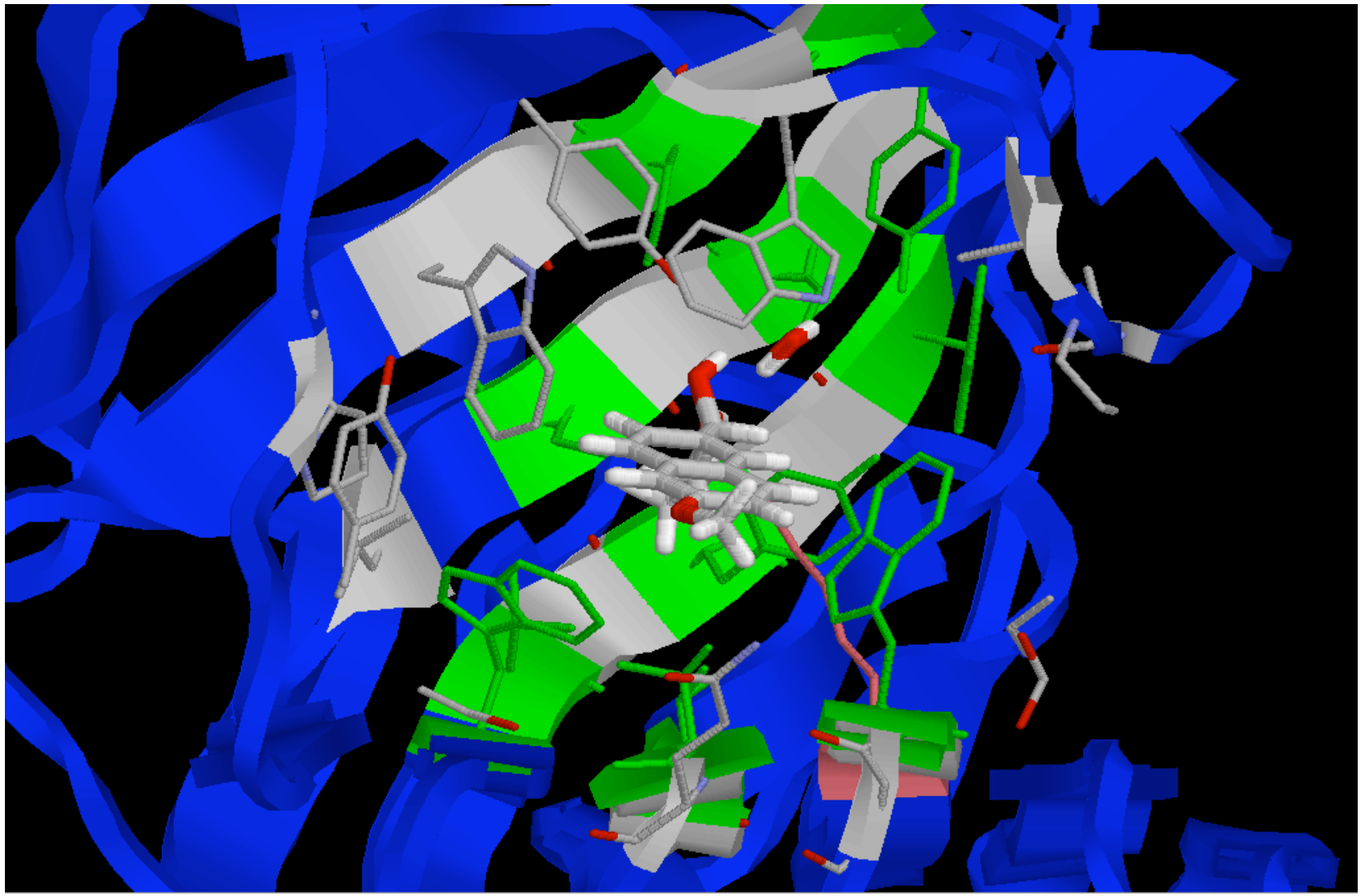
10^4 fold enhancement ceiling

How can we get higher?

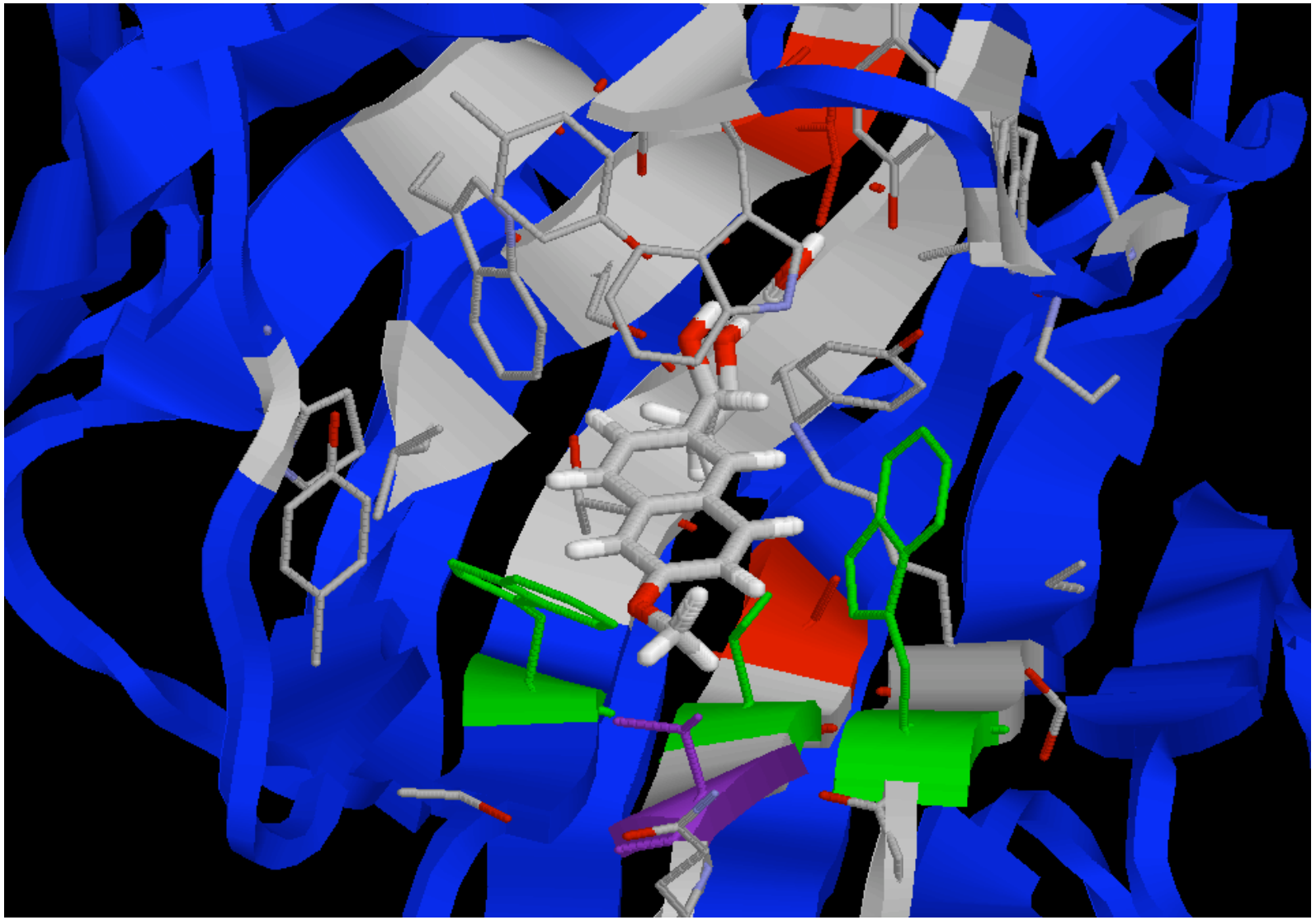
-What are we missing?

RA60 Saturation Mutagenesis



Ling Wang, Zhizhi Wang

S87W



A174M

W74G,S

V176G,I

W46C

V178H

10^5!!!!

Aldolase Experimental Summary

- 70 of 72 designs were soluble
- Retro-Aldol activity detected for 11 lysine positions across 5 scaffolds and 32 design
- Best activity is 2×10^4 fold rate enhancement
- Atomic accuracy of design process shown by crystal structures

Acknowledgments

- Rosetta Community
- David Baker
- Andrew Wollacott
- Lin Jiang
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