Artificial Metalloenzymes: Challenges and Opportunities (An Introduction)

Theracat Workshop

Sept 25, 2019

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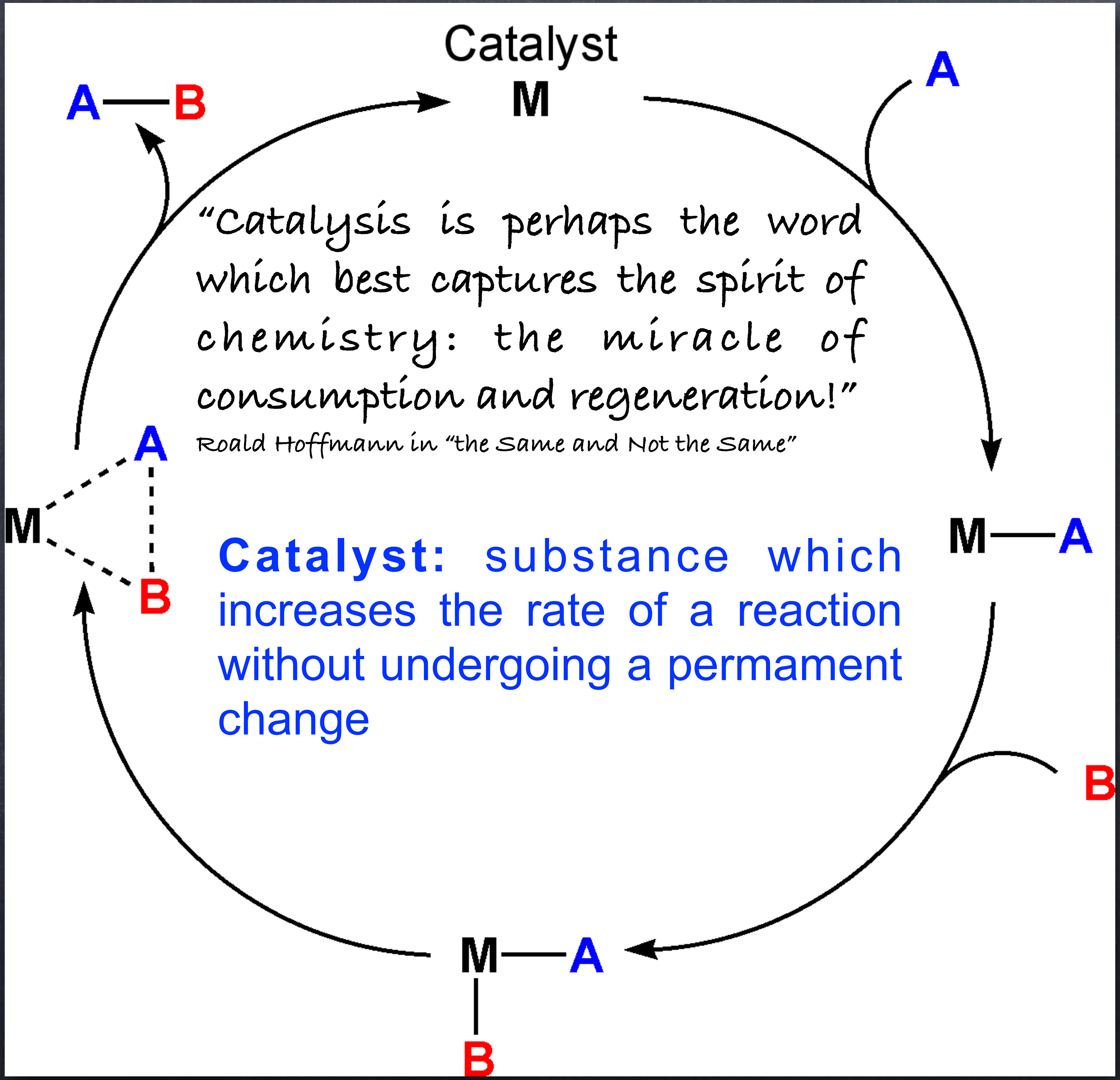
University of Basel

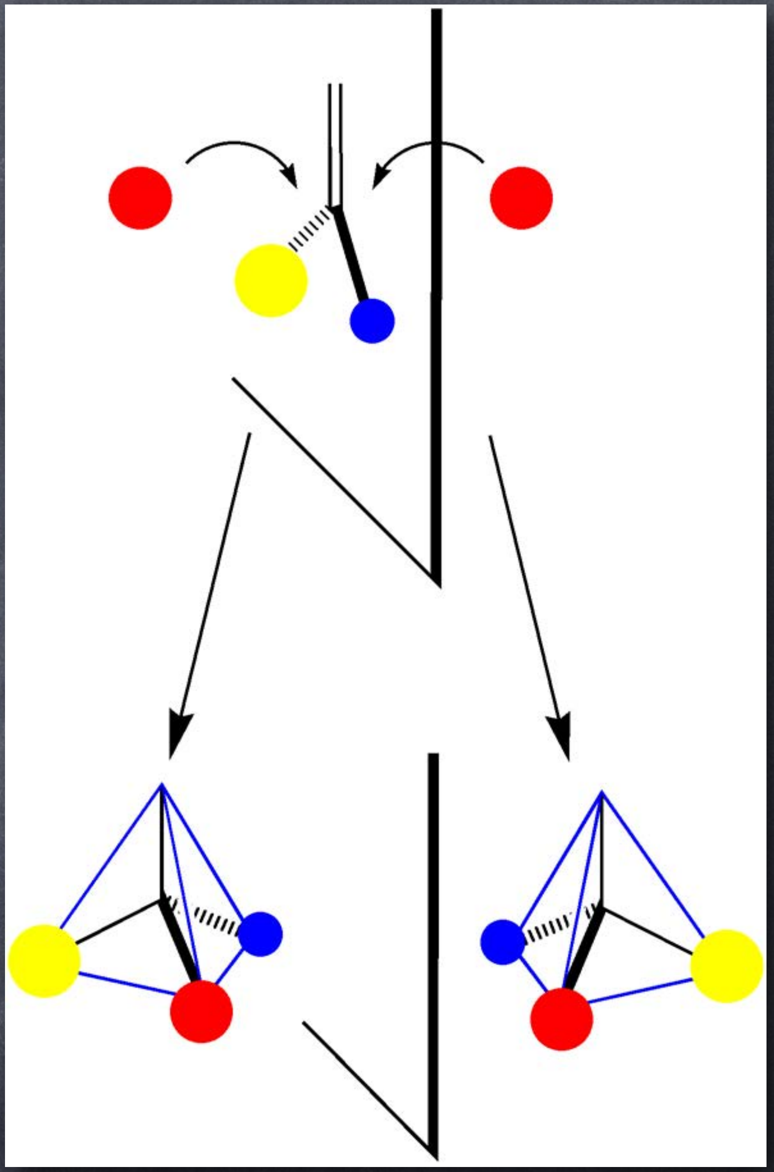
Catalysis: Definition



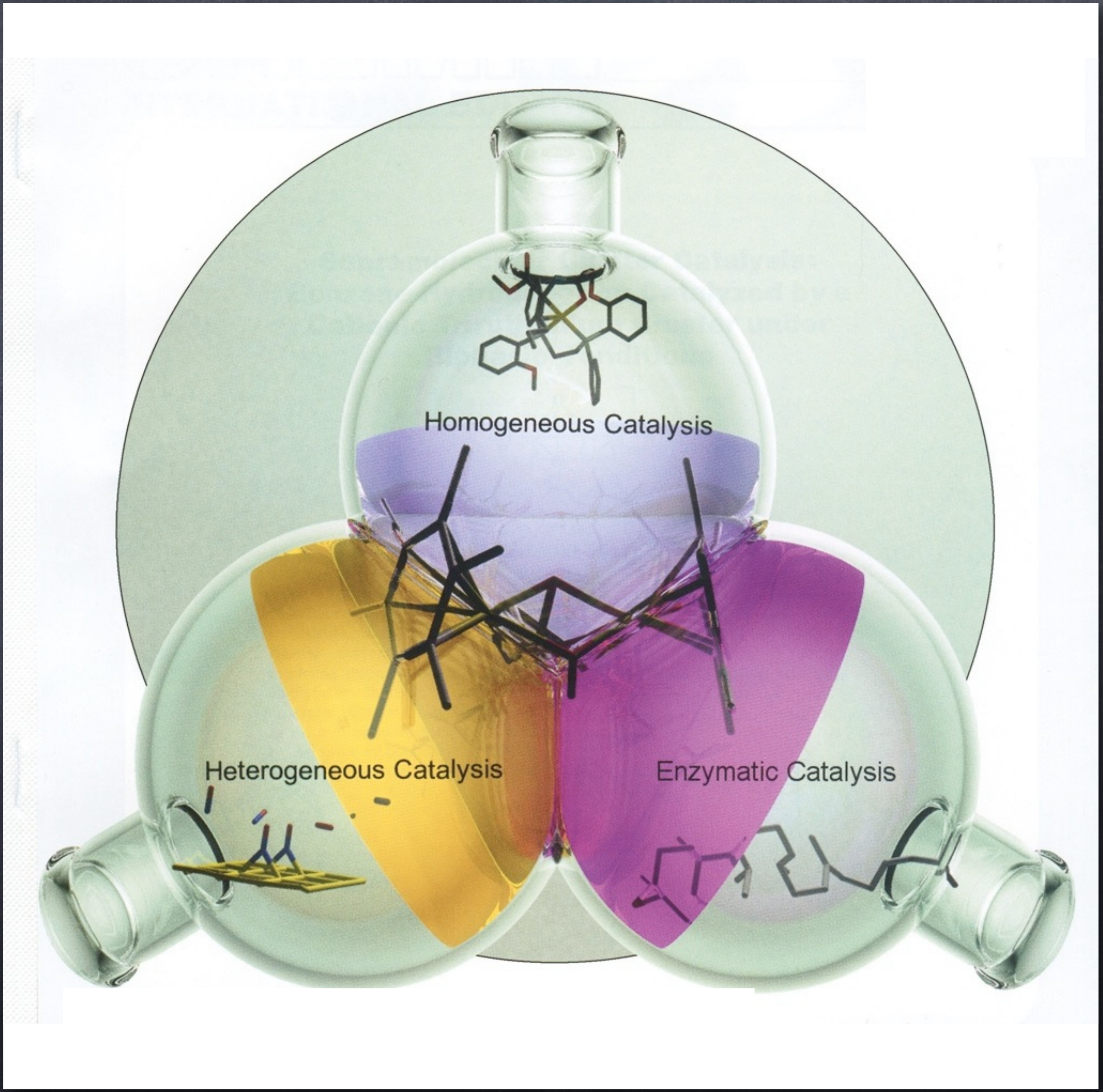
"Catalysis is perhaps the word which best captures the spirit of chemistry: the miracle of consumption and regeneration!"

Roald Hoffmann in "the Same and Not the Same"

Catalyst: substance which increases the rate of a reaction without undergoing a permament change



Heterogeneous-, Homogeneous- and Enzymatic Catalysis: Three Kingdoms



The Most Important Discovery of the 20th Century: Haber-Bosch Nitrogen Fixation

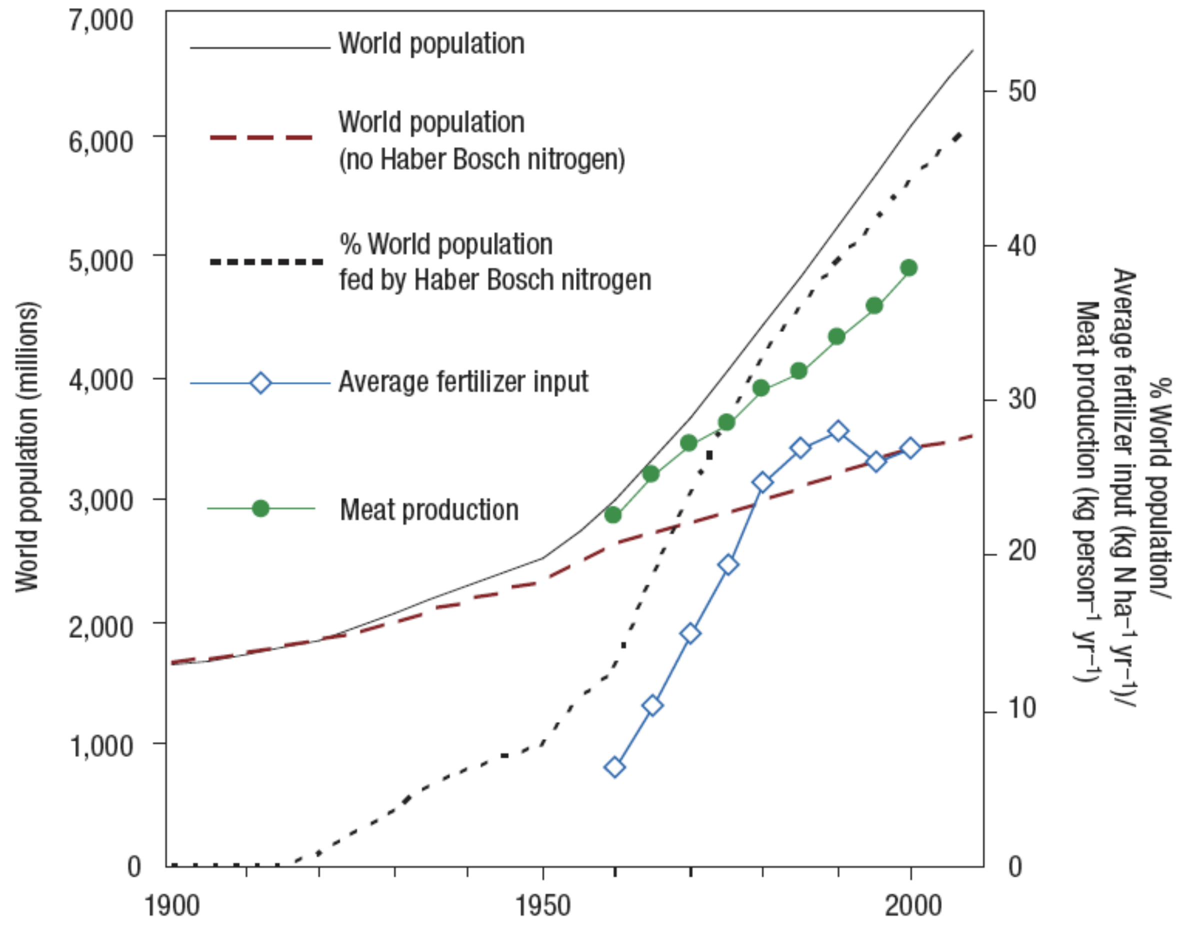
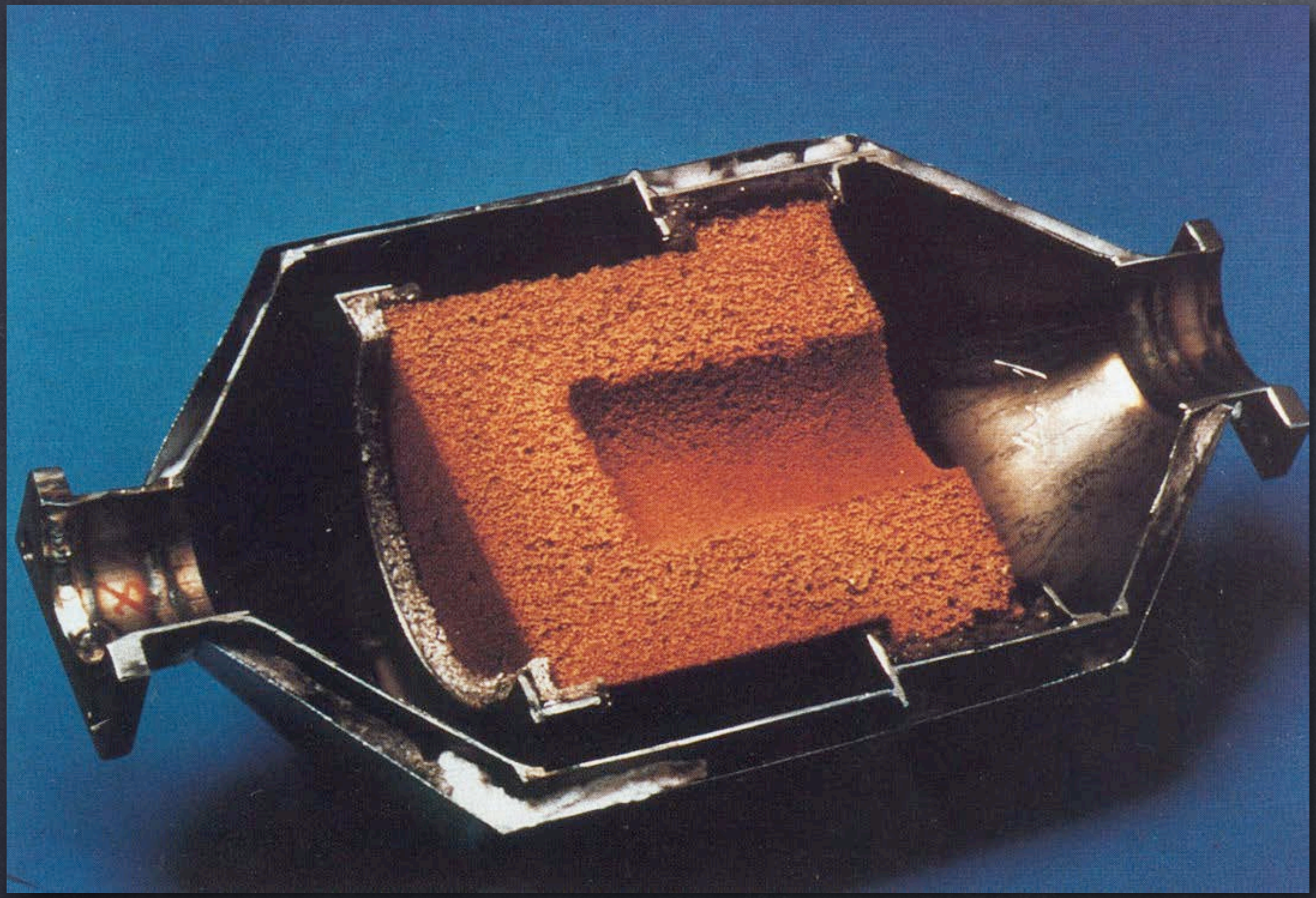
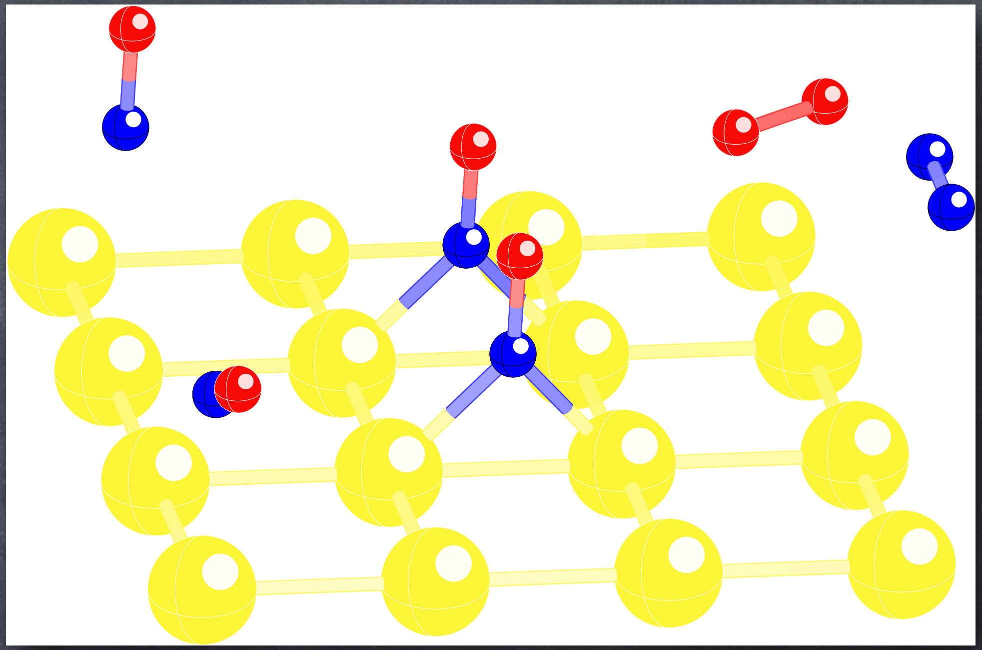


Figure 1 Trends in human population and nitrogen use throughout the twentieth century. Of the total world population (solid line), an estimate is made of the number of people that could be sustained without reactive nitrogen from the Haber–Bosch process (long dashed line), also expressed as a percentage of the global population (short dashed line). The recorded increase in average fertilizer use per hectare of agricultural land (blue symbols) and the increase in per capita meat production (green symbols) is also shown.

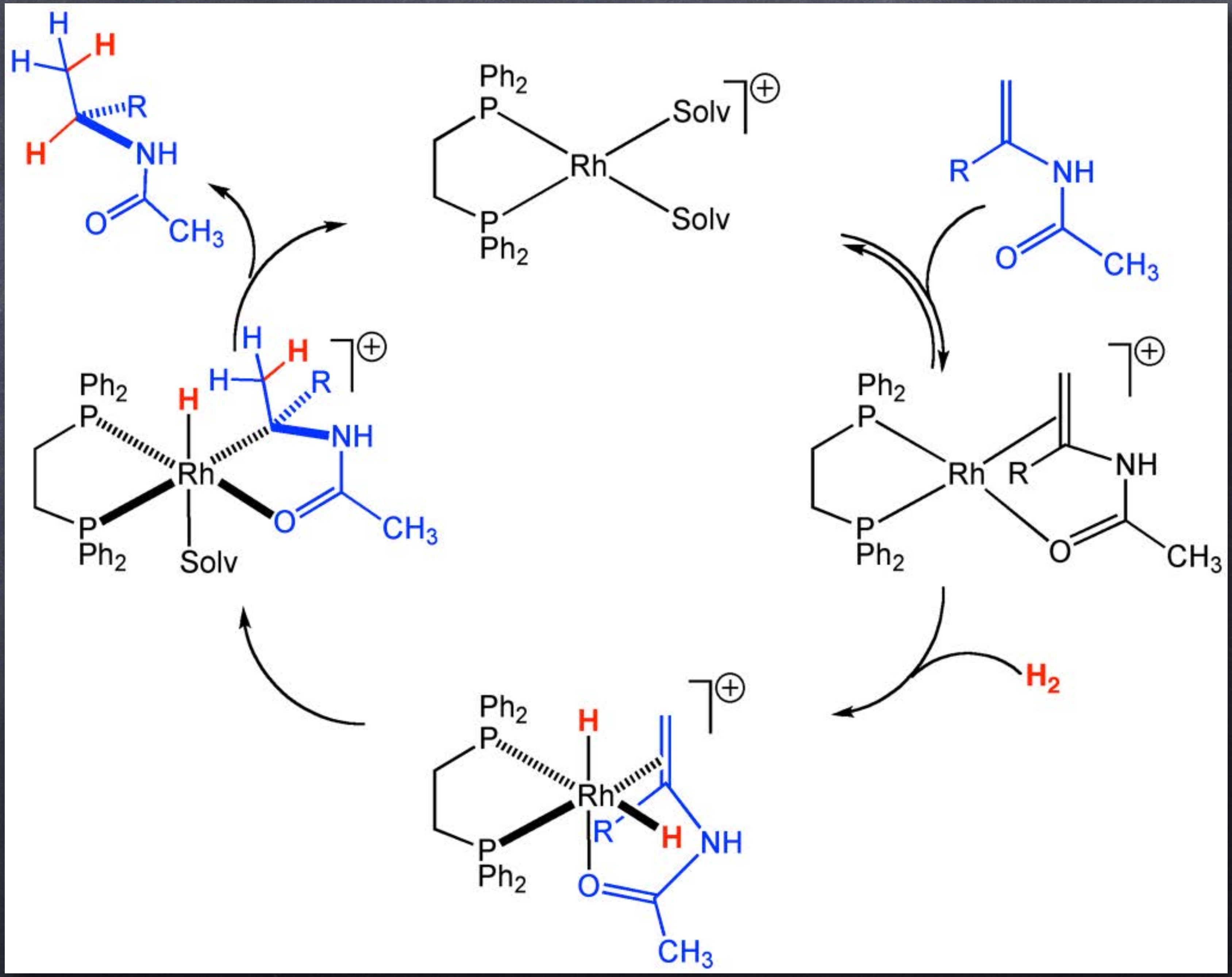
$$N_2 + 3H_2 \rightleftharpoons 2NH_3$$

A Tiger in your Engine and...
A Catalyst in your Exhaust Pipe
Ertl Nobel Prize 2007 for Heterogeneous Catalysis

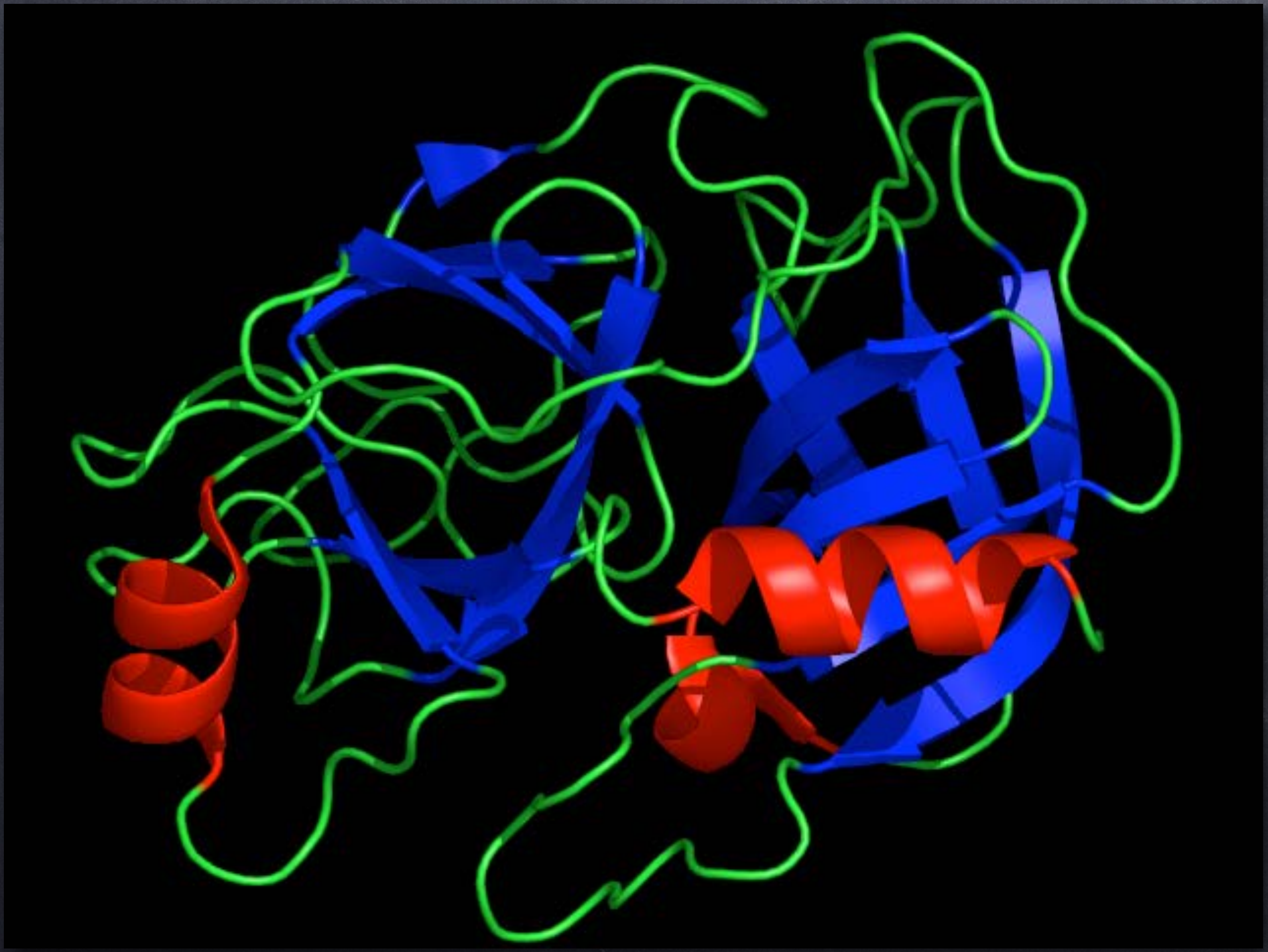


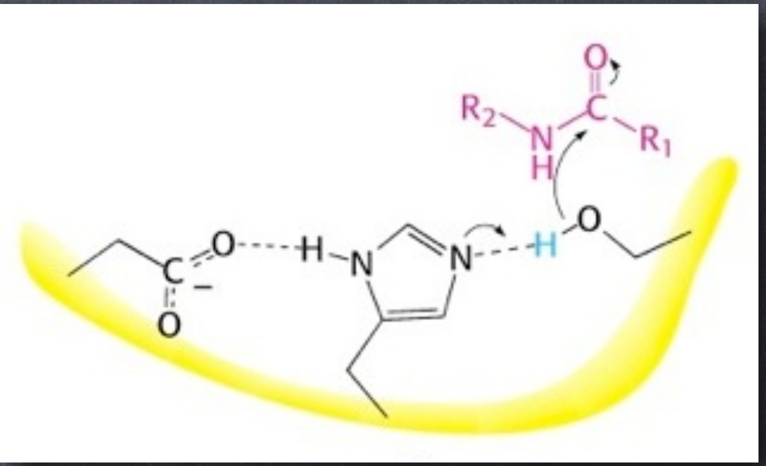


Homogeneous Catalysis:
The Elegance of a Man-Made Catalyst
Three Nobel Prizes since 2001



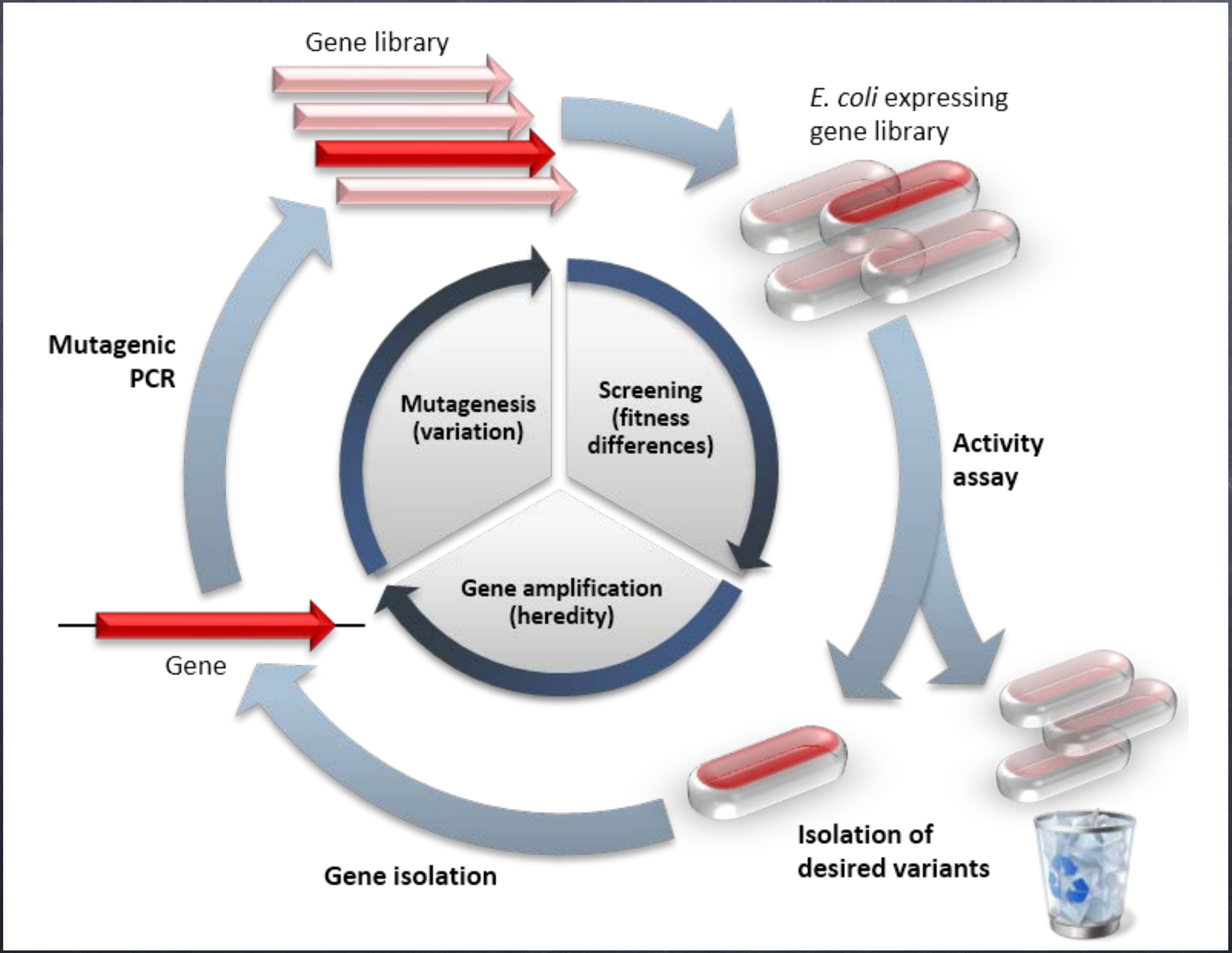
Subtilisin: A Serine Protease Used in your Laundry- and Dishwashing Powder



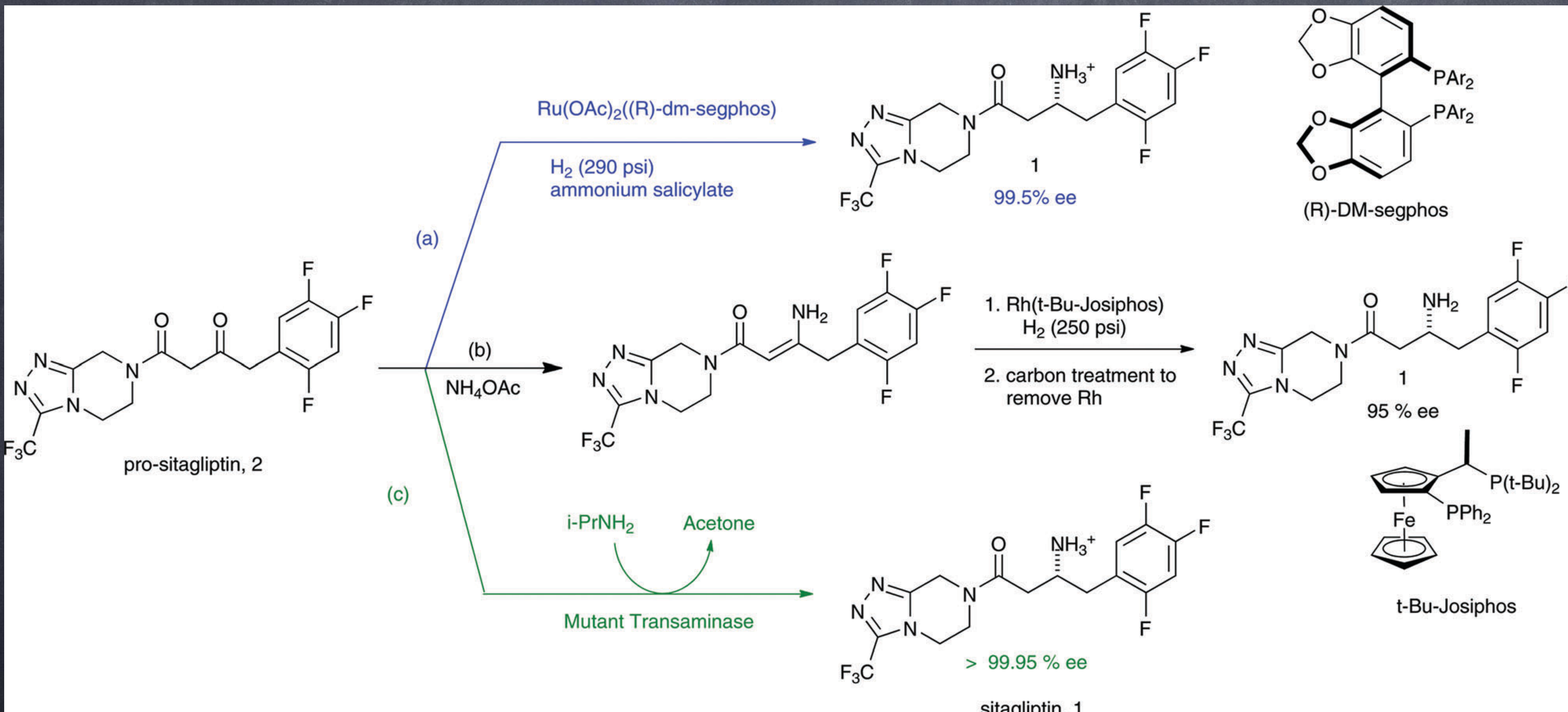


900 Tons/Year in EU

2018 Nobel Prize: Frances Arnold (1/2) for Directed Evolution



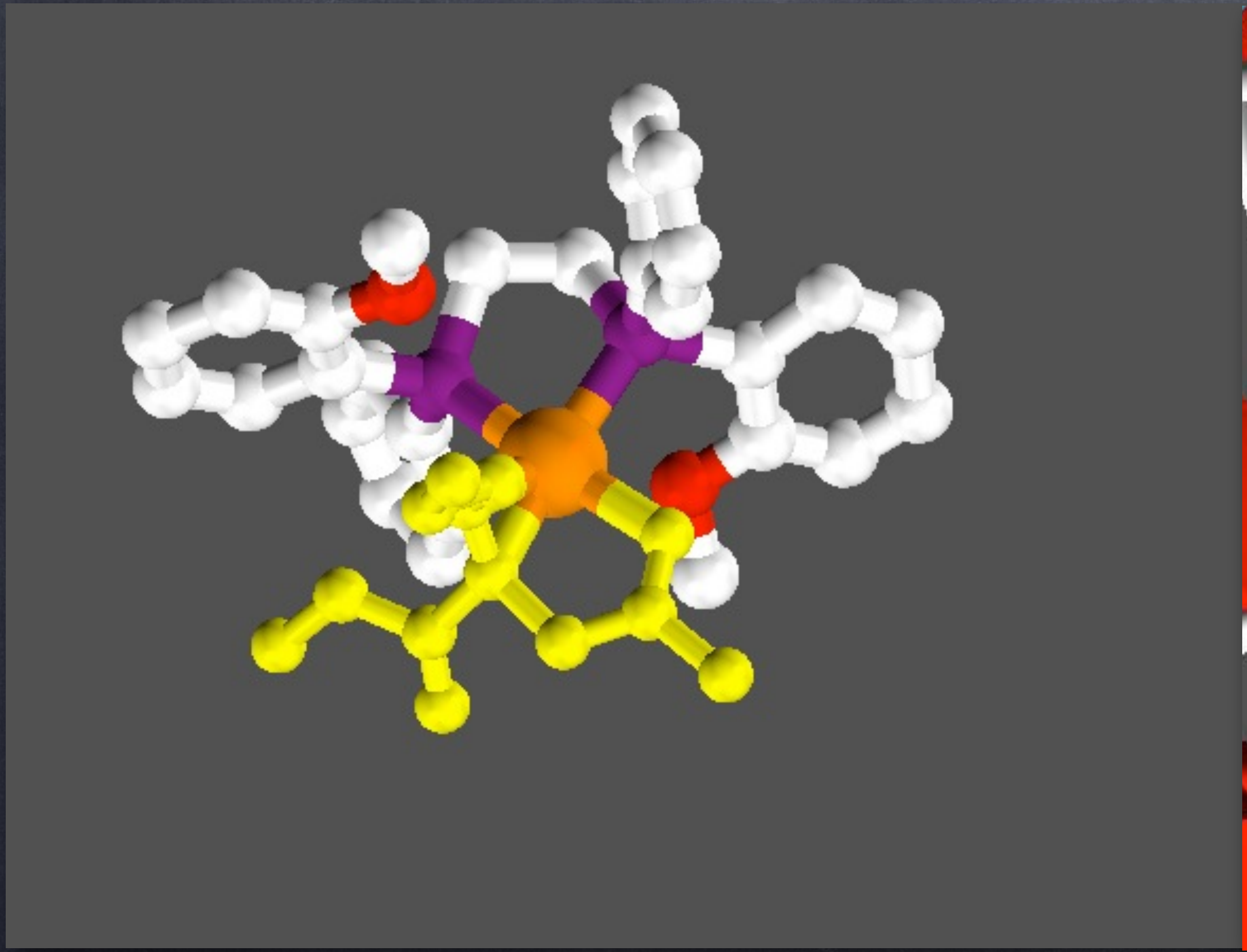
Sitagliptin (Merck): Two Green Chemistry Awards

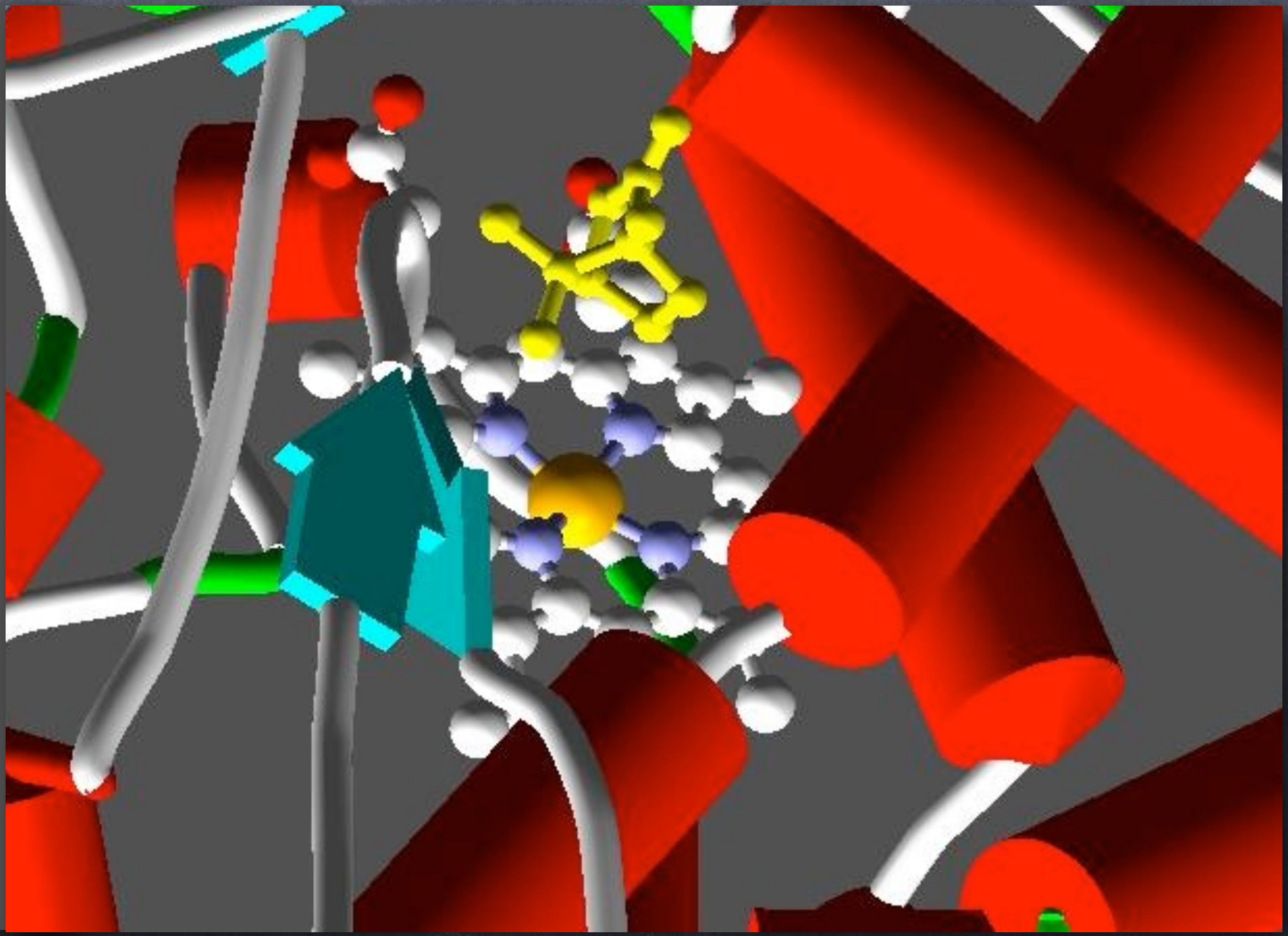


Homogeneous- vs. Enzymatic Catalysis

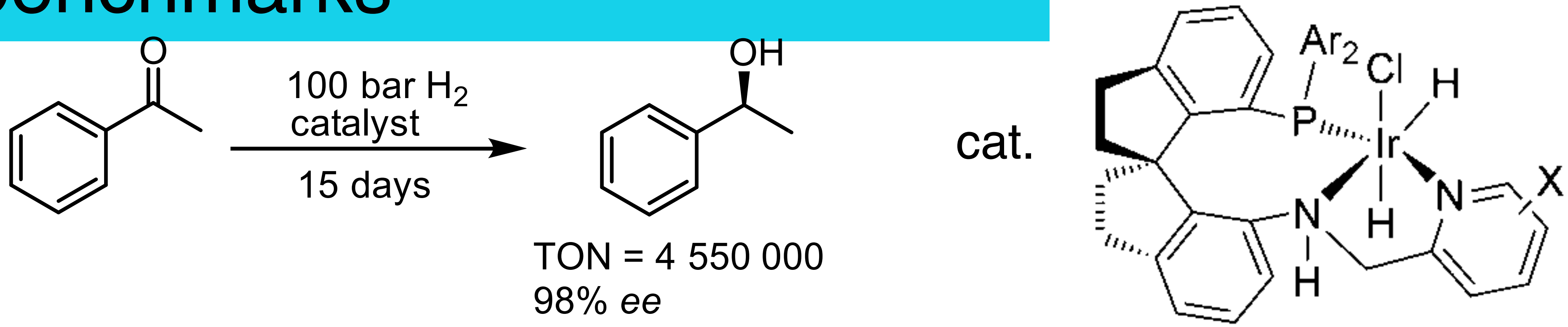
Homogeneous		Enzymatic
	Enantiomers	
Both		Single
	Solvent Tolerance	
Organic		Aqueous
	Substrate Specificity	
Broad		Narrow
	Optimization	
Chemical		Genetic
	Catalyst Lifetime	
Limited		Extended
	In vivo Compatibility	
Limited		Excellent

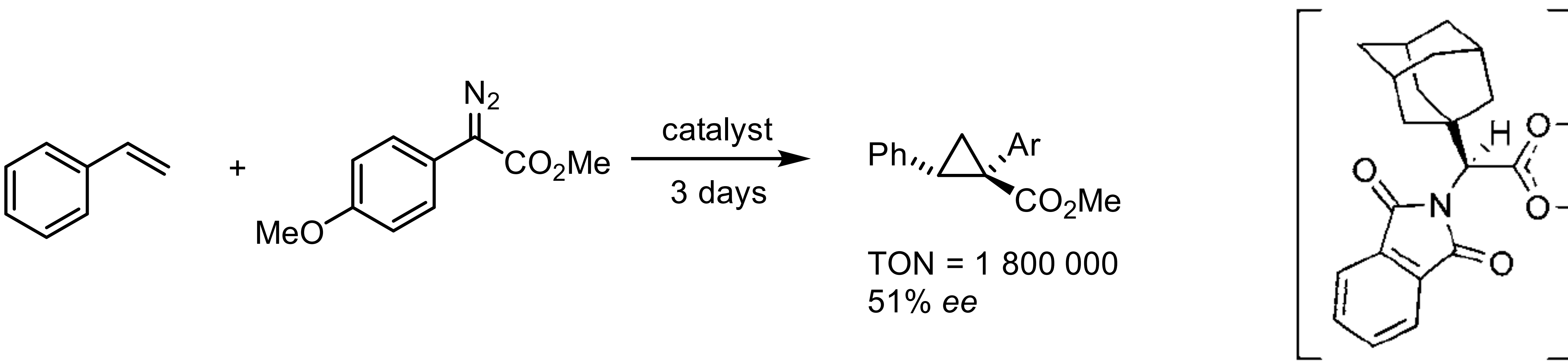
Second Coordination Sphere Environments





what is a good catalyst? a few benchmarks





$Rh_2(S\text{-}PTAD)_4$

$CO_2 + H_2O \xrightarrow{\text{carbonic anhydrase}} HCO_3^- + H^+$ $k_{cat} = 8.2$ ´ $10^5\ s^{-1}$ $k_{cat}/k_{uncat} = 7.7 \times 10^6$

Q. L. Zhou, Angew. Chem. Int. Ed. 2011, 50, 7329.
H. M. L. Davies, Chem. Sci. 2010, 1, 254.
A. Fersht >Structure and Mechanism in Protein Science<.

what is the motivation?

ii) make better catalysts (more efficiently)

rate

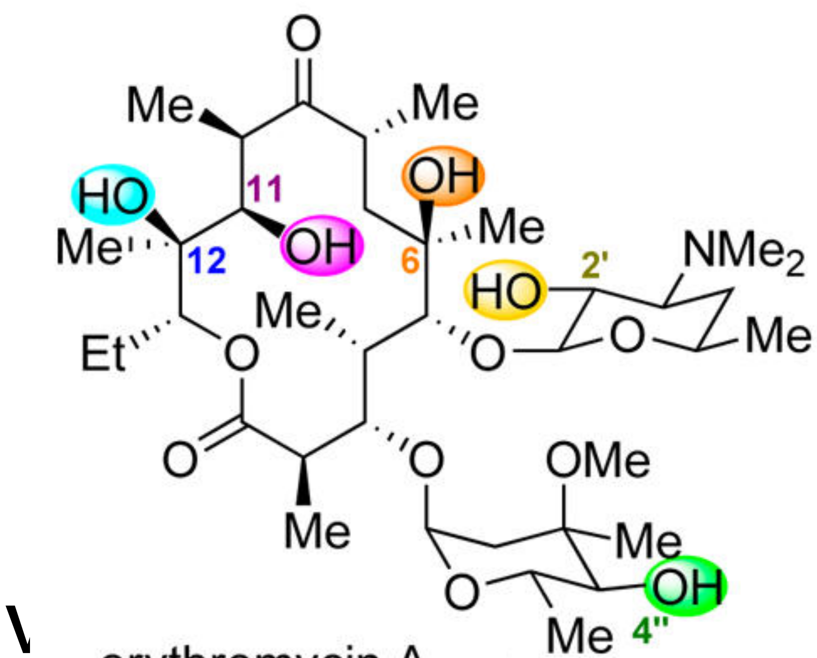
robustness

selectivity (chemo-, regio-, and stereoselectiv

compatibility (with other catalysts, biological environment)

accessibility (e.g. hydrogenases)

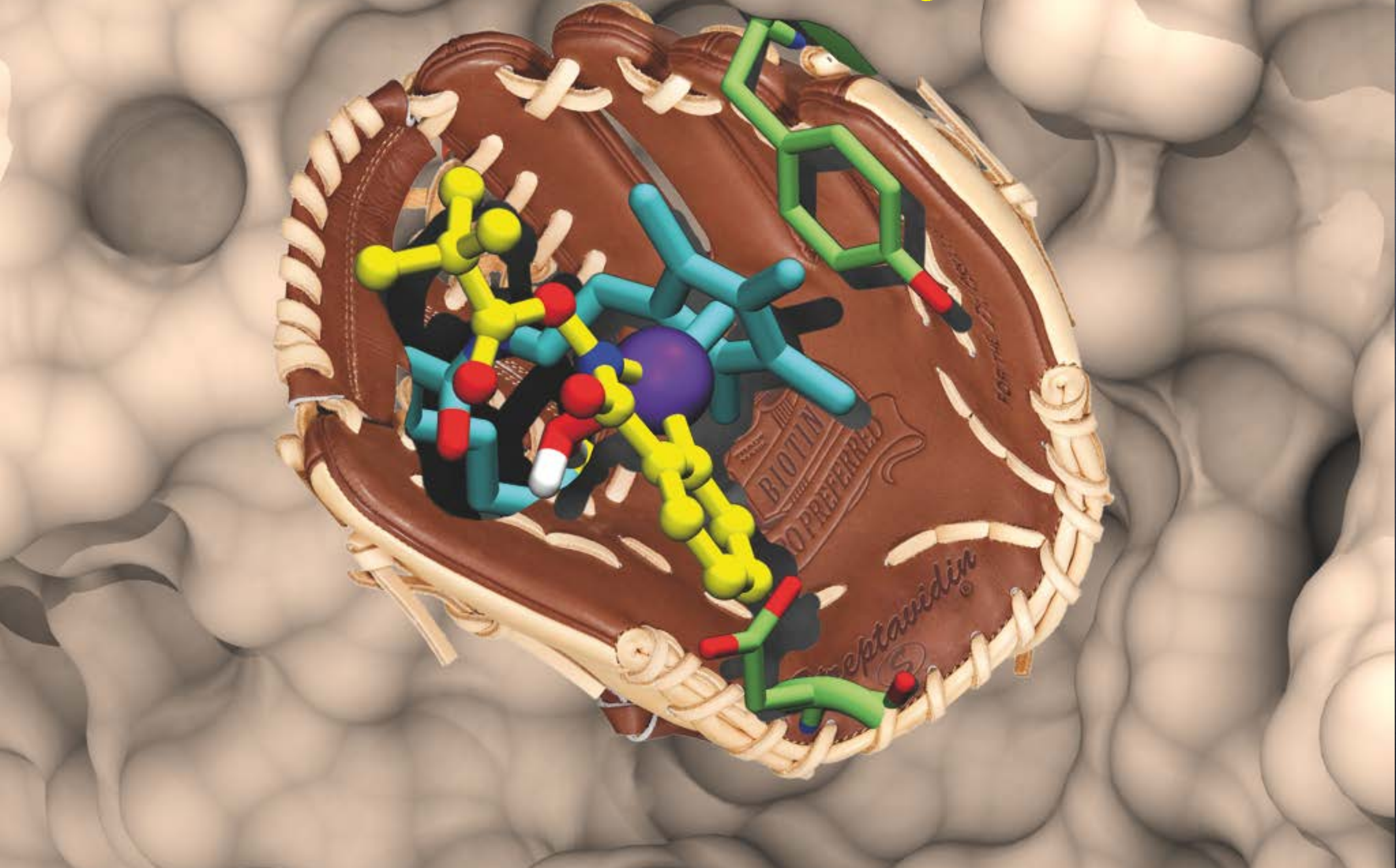
controllability (allostery)

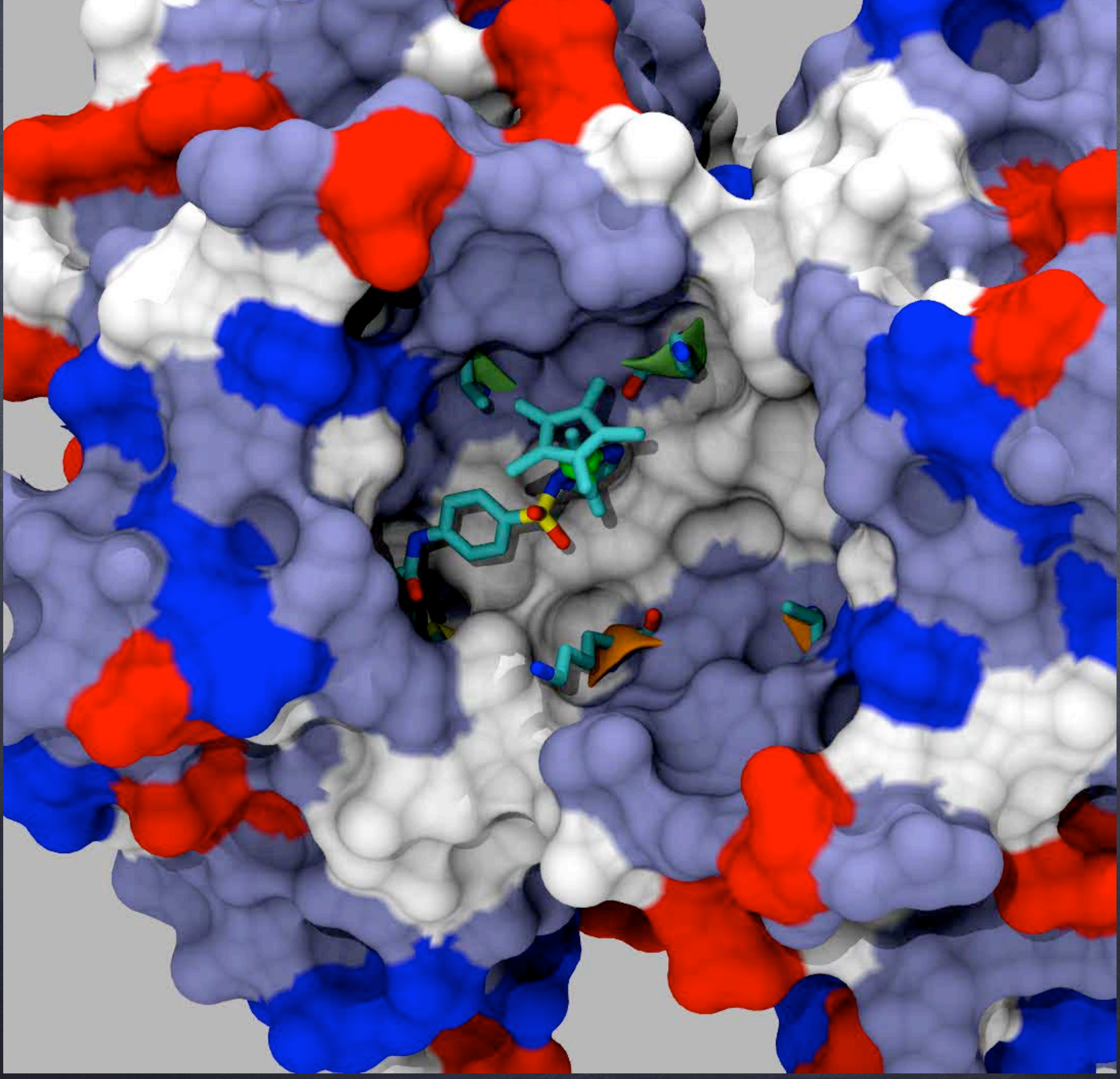


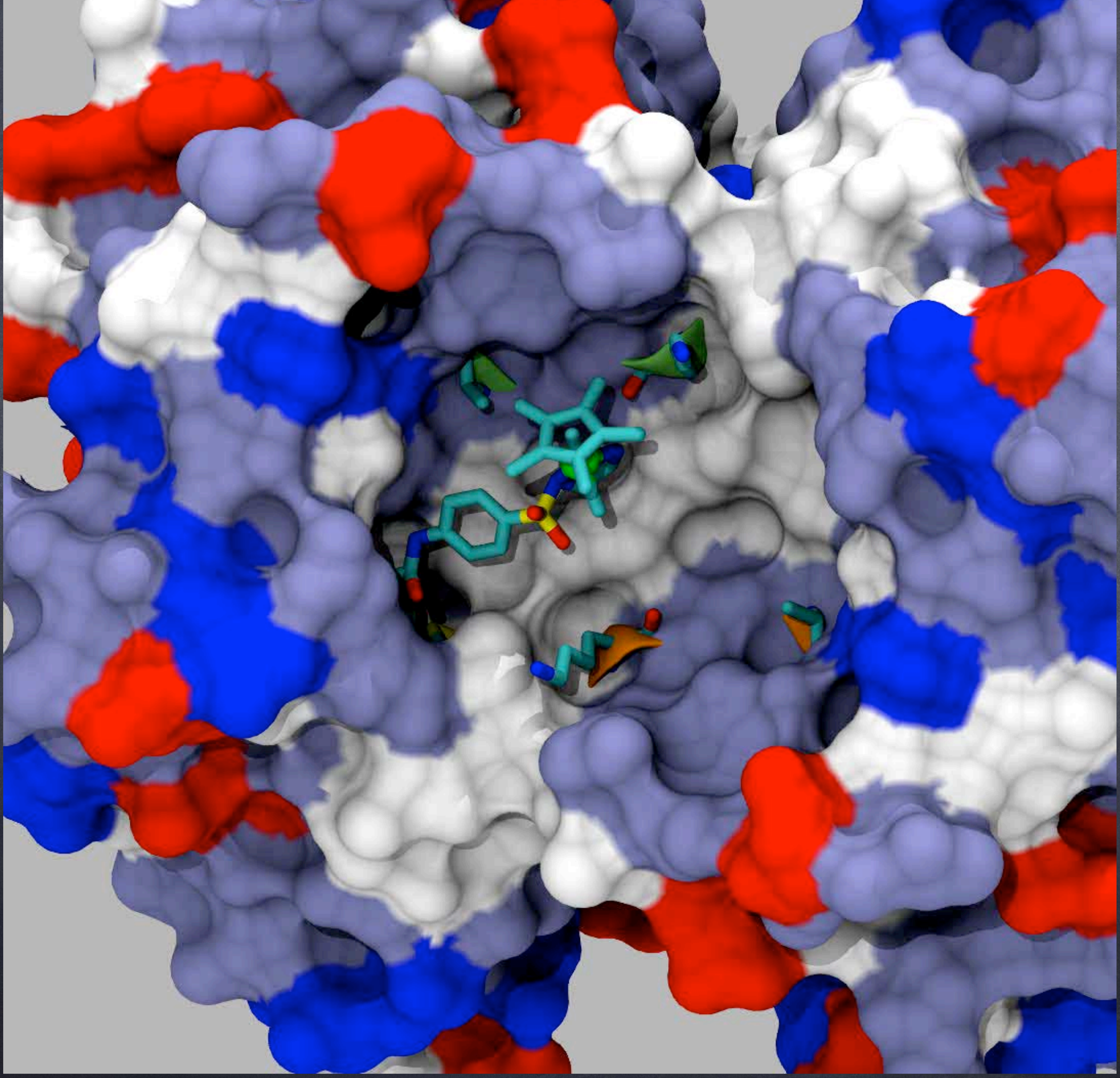
erythromycin A

review on late stage functionalization: S. J. Miller >Applications of Nonenzymatic Catalysts to the Alteration of Natural Products< Chem. Rev. 2017, 117, 11894.

Proteins as Host for Abiotic Cofactors: Artificial Metalloenzymes?





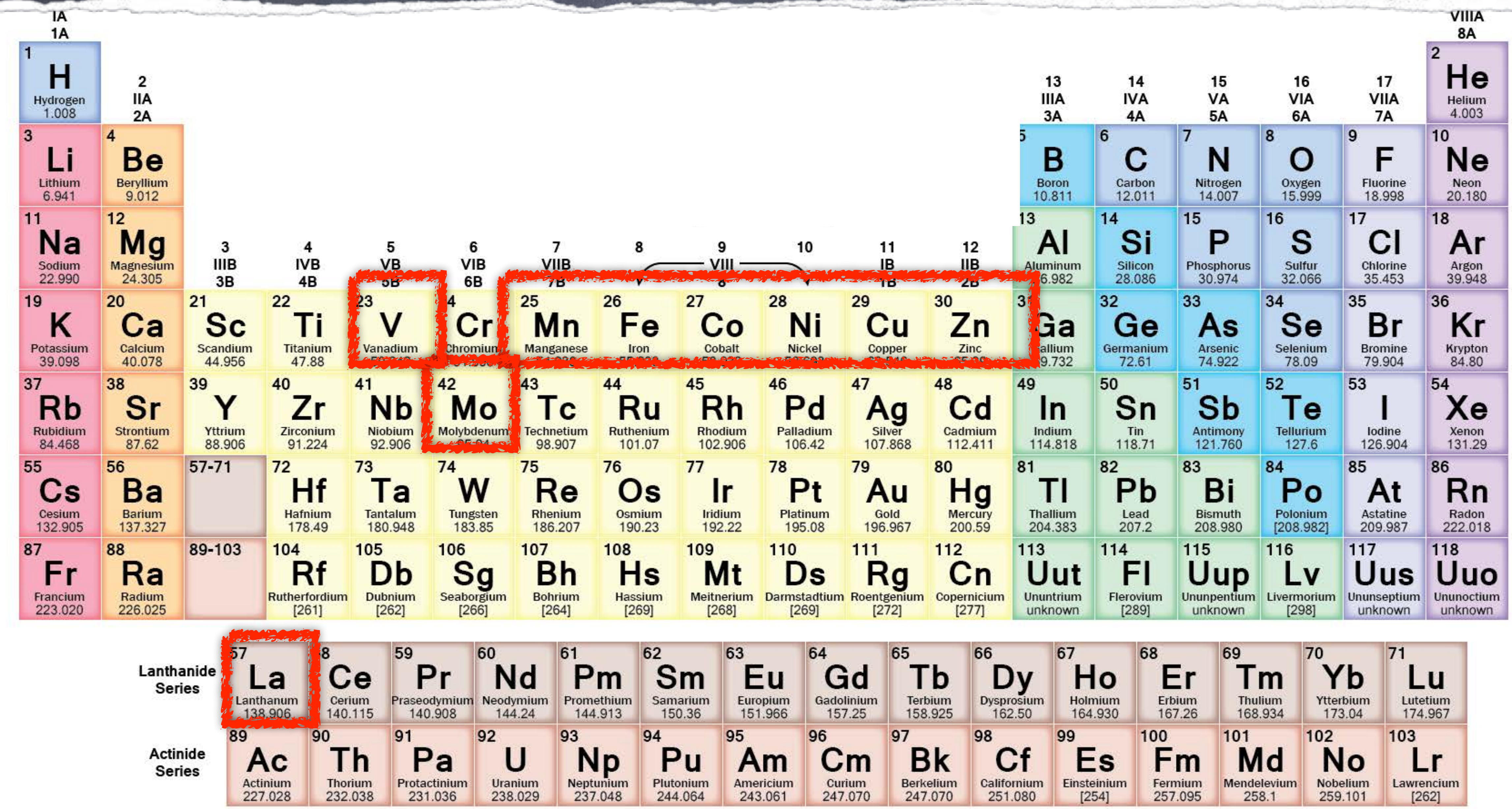


The Periodic Table of The (Metalloenzymes) Elements

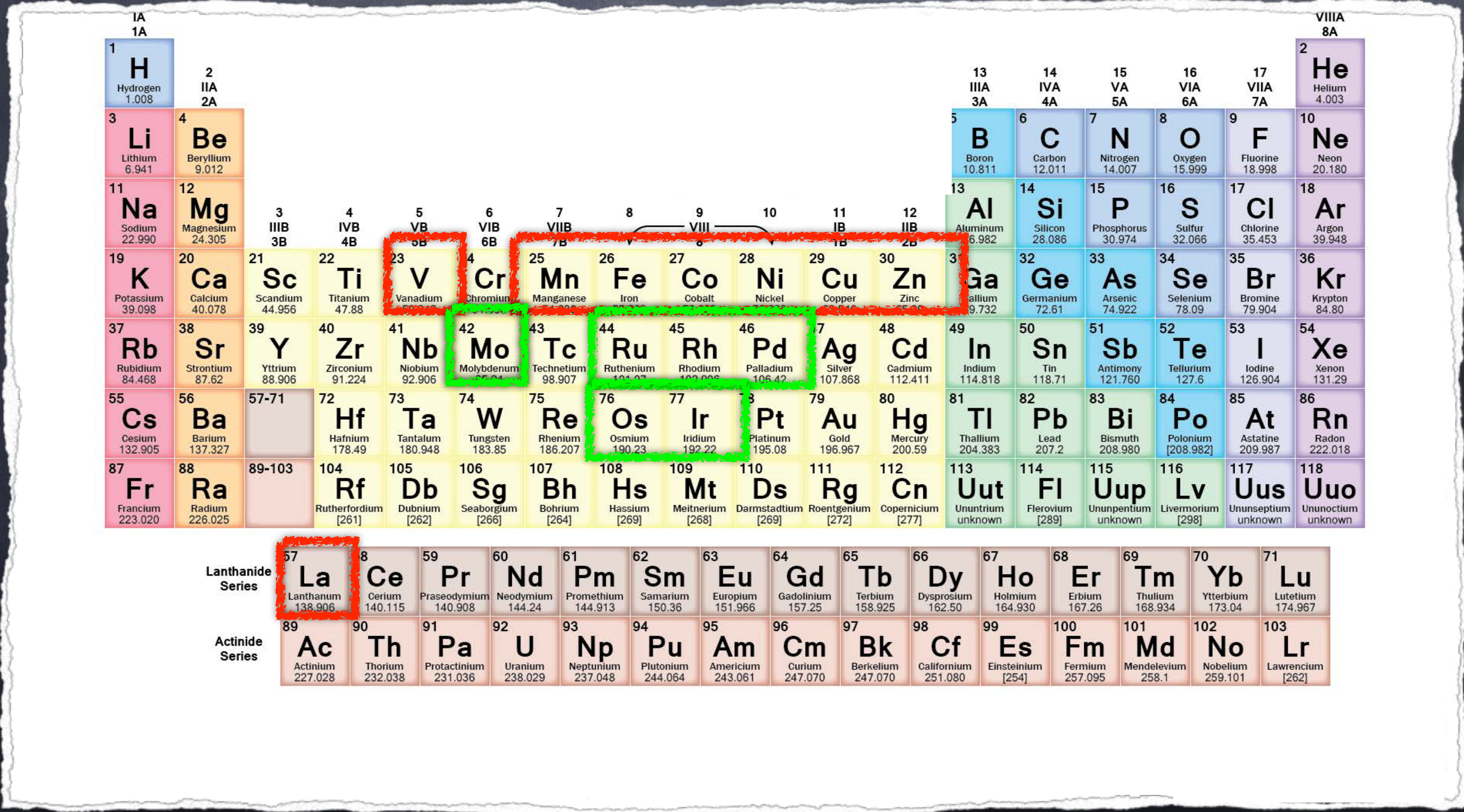
IA 1A	2 IIA 2A	3 IIIB 3B	4 IVB 4B	5 VB 5B	6 VIB 6B	7 VIIB 7B	8	9 VIII 8	10	11 IB 1B	12 IIB 2B	13 IIIA 3A	14 IVA 4A	15 VA 5A	16 VIA 6A	17 VIIA 7A	VIIIA 8A
1 H Hydrogen 1.008																	2 He Helium 4.003
3 Li Lithium 6.941	4 Be Beryllium 9.012											5 B Boron 10.811	6 C Carbon 12.011	7 N Nitrogen 14.007	8 O Oxygen 15.999	9 F Fluorine 18.998	10 Ne Neon 20.180
11 Na Sodium 22.990	12 Mg Magnesium 24.305											13 Al Aluminum 26.982	14 Si Silicon 28.086	15 P Phosphorus 30.974	16 S Sulfur 32.066	17 Cl Chlorine 35.453	18 Ar Argon 39.948
19 K Potassium 39.098	20 Ca Calcium 40.078	21 Sc Scandium 44.956	22 Ti Titanium 47.88	23 V Vanadium 50.942	24 Cr Chromium 51.996	25 Mn Manganese 54.938	26 Fe Iron 55.933	27 Co Cobalt 58.933	28 Ni Nickel 58.693	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 69.732	32 Ge Germanium 72.61	33 As Arsenic 74.922	34 Se Selenium 78.09	35 Br Bromine 79.904	36 Kr Krypton 84.80
37 Rb Rubidium 84.468	38 Sr Strontium 87.62	39 Y Yttrium 88.906	40 Zr Zirconium 91.224	41 Nb Niobium 92.906	42 Mo Molybdenum 95.94	43 Tc Technetium 98.907	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.906	46 Pd Palladium 106.42	47 Ag Silver 107.868	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.71	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.904	54 Xe Xenon 131.29
55 Cs Cesium 132.905	56 Ba Barium 137.327	57-71	72 Hf Hafnium 178.49	73 Ta Tantalum 180.948	74 W Tungsten 183.85	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.22	78 Pt Platinum 195.08	79 Au Gold 196.967	80 Hg Mercury 200.59	81 Tl Thallium 204.383	82 Pb Lead 207.2	83 Bi Bismuth 208.980	84 Po Polonium [208.982]	85 At Astatine 209.987	86 Rn Radon 222.018
87 Fr Francium 223.020	88 Ra Radium 226.025	89-103	104 Rf Rutherfordium [261]	105 Db Dubnium [262]	106 Sg Seaborgium [266]	107 Bh Bohrium [264]	108 Hs Hassium [269]	109 Mt Meitnerium [268]	110 Ds Darmstadtium [269]	111 Rg Roentgenium [272]	112 Cn Copernicium [277]	113 Uut Ununtrium unknown	114 Fl Flerovium [289]	115 Uup Ununpentium unknown	116 Lv Livermorium [298]	117 Uus Ununseptium unknown	118 Uuo Ununoctium unknown

Lanthanide Series	57 La Lanthanum 138.906	58 Ce Cerium 140.115	59 Pr Praseodymium 140.908	60 Nd Neodymium 144.24	61 Pm Promethium 144.913	62 Sm Samarium 150.36	63 Eu Europium 151.966	64 Gd Gadolinium 157.25	65 Tb Terbium 158.925	66 Dy Dysprosium 162.50	67 Ho Holmium 164.930	68 Er Erbium 167.26	69 Tm Thulium 168.934	70 Yb Ytterbium 173.04	71 Lu Lutetium 174.967
Actinide Series	89 Ac Actinium 227.028	90 Th Thorium 232.038	91 Pa Protactinium 231.036	92 U Uranium 238.029	93 Np Neptunium 237.048	94 Pu Plutonium 244.064	95 Am Americium 243.061	96 Cm Curium 247.070	97 Bk Berkelium 247.070	98 Cf Californium 251.080	99 Es Einsteinium [254]	100 Fm Fermium 257.095	101 Md Mendelevium 258.1	102 No Nobelium 259.101	103 Lr Lawrencium [262]

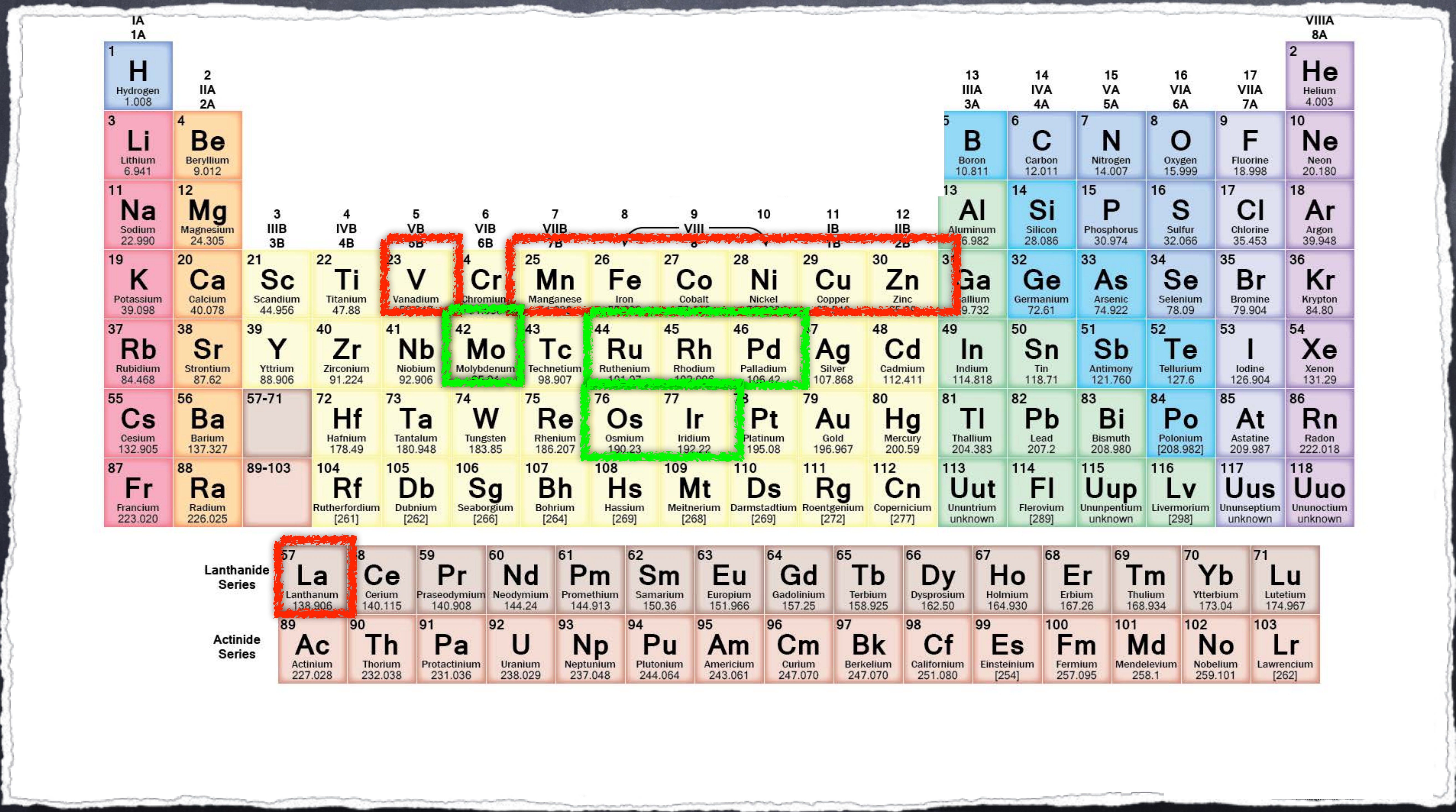
The Periodic Table of The (Metalloenzymes) Elements



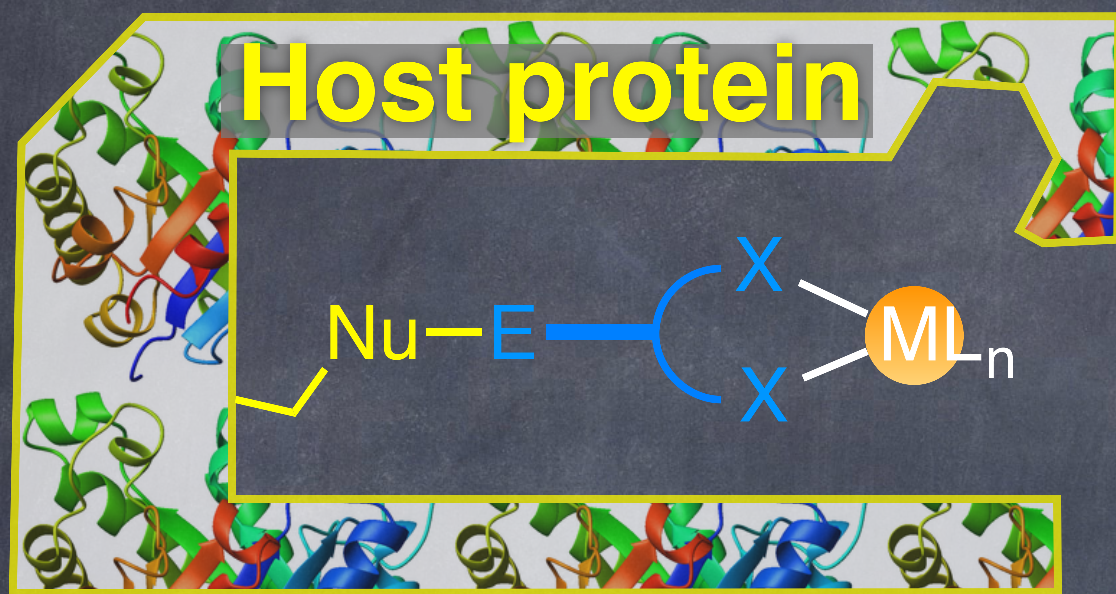
The Periodic Table of The (Metalloenzymes) Elements



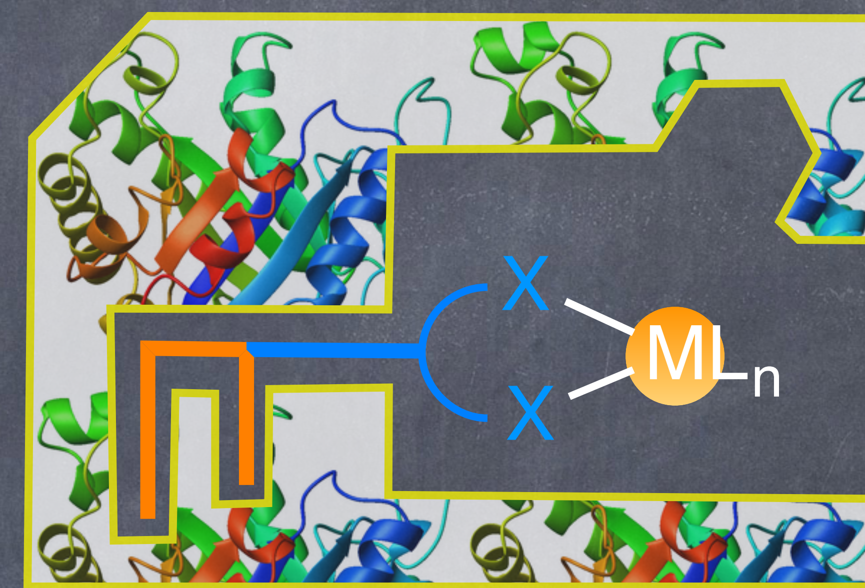
The Periodic Table of The (Metalloenzymes) Elements



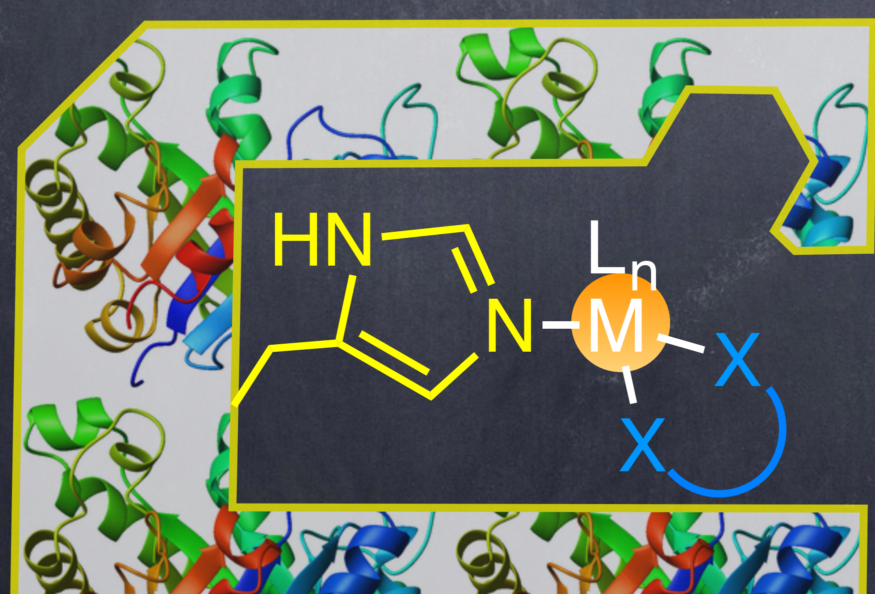
Anchoring of the Catalyst:
Four Alternatives to Ensure Localization



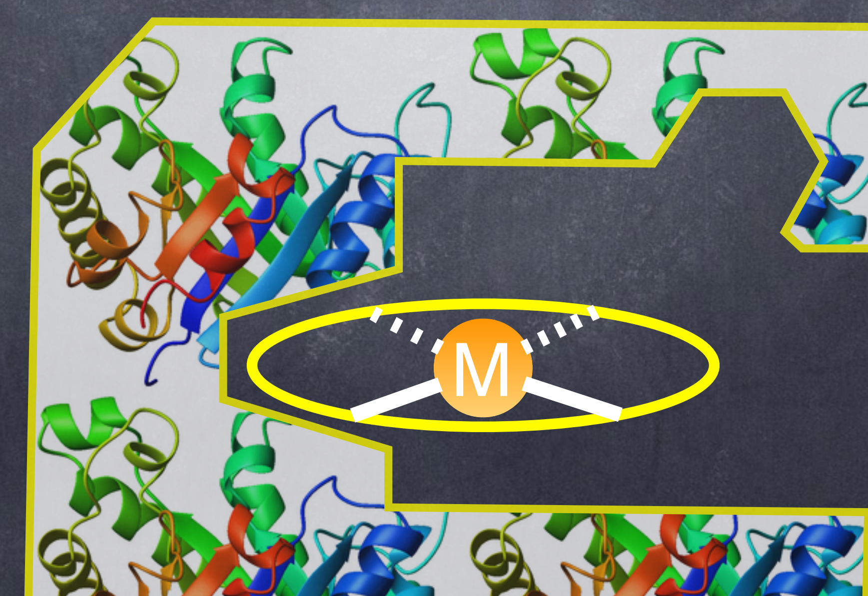
Covalent anchoring



Supramolecular anchoring



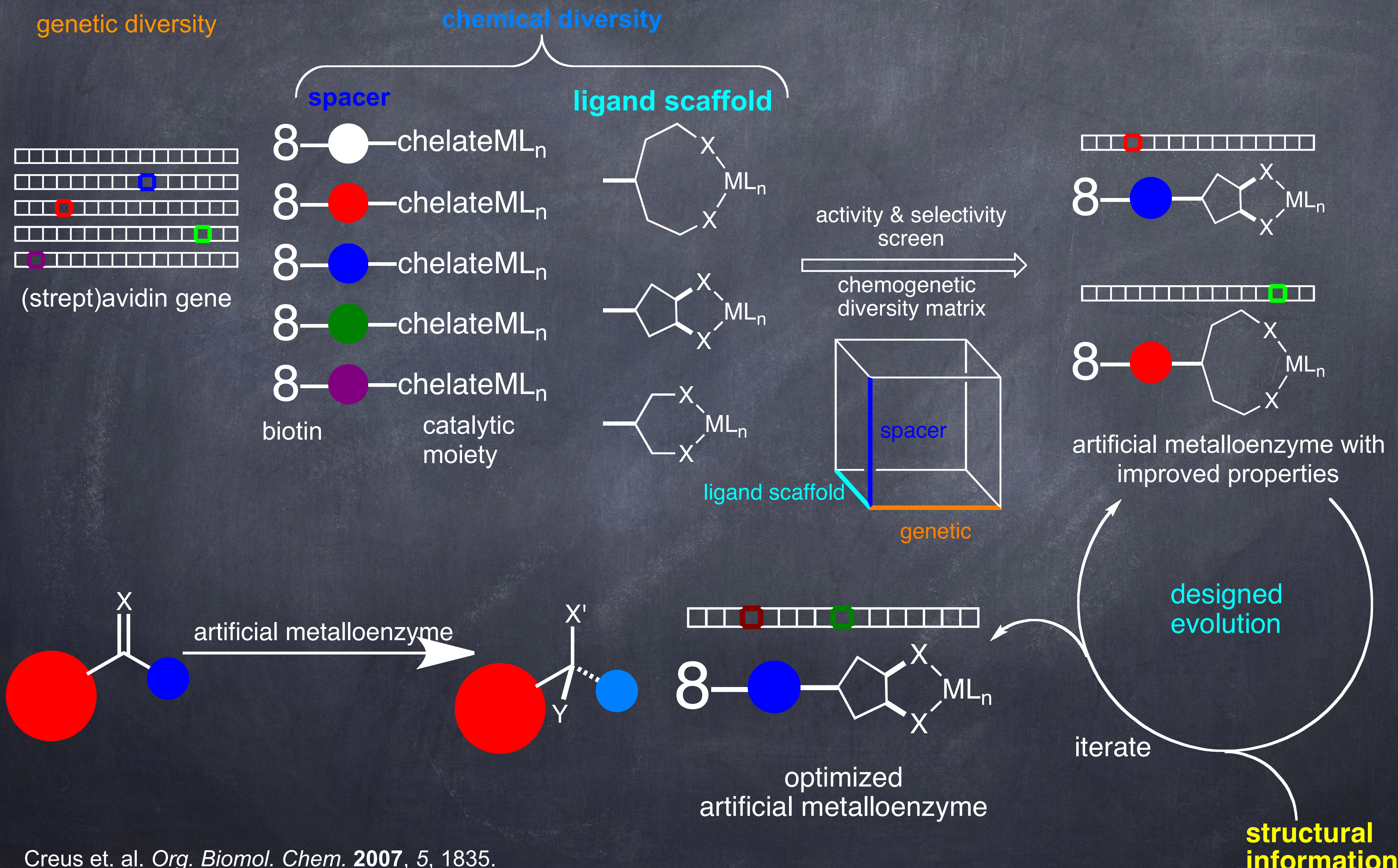
Dative anchoring



Metal substitution

Lewis, Ward *et al.*, *Chem. Rev.* **2018,** *118*, 142.

ArMs: Chemo-Genetic Optimization Strategy

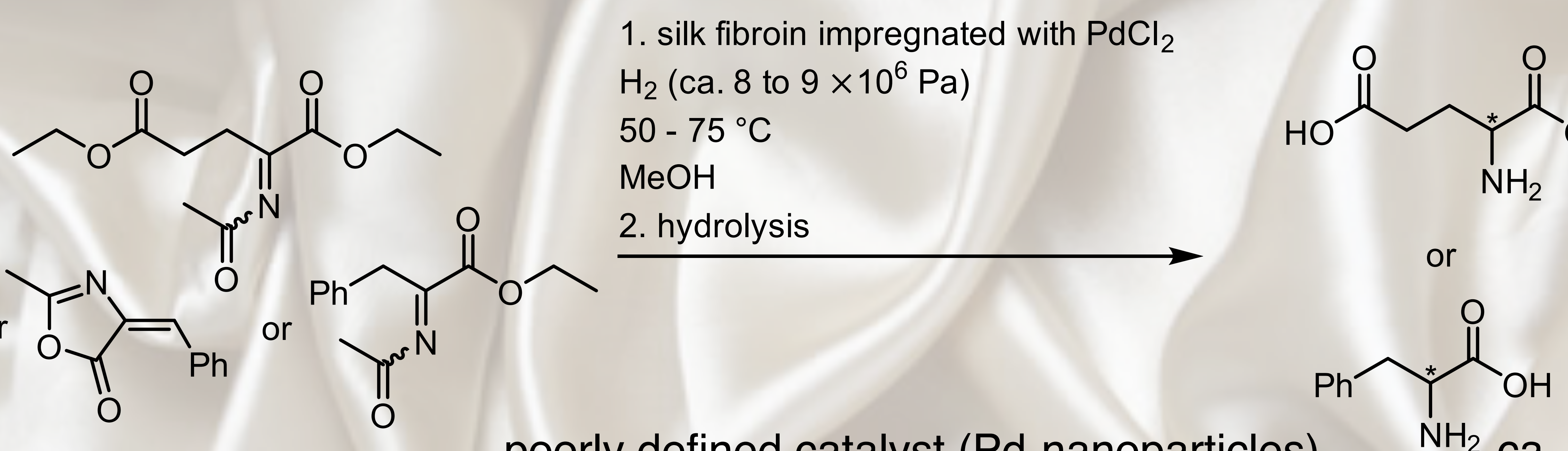


Creus et. al. *Org. Biomol. Chem.* **2007**, *5*, 1835.

Standing on the Shoulders of Giants:
A Historical Perspective

historical perspective – a few milestones

artificial metalloenzymes were at the beginning of asymmetric transition metal catalysis by chemical means! Akabori et al. 1956

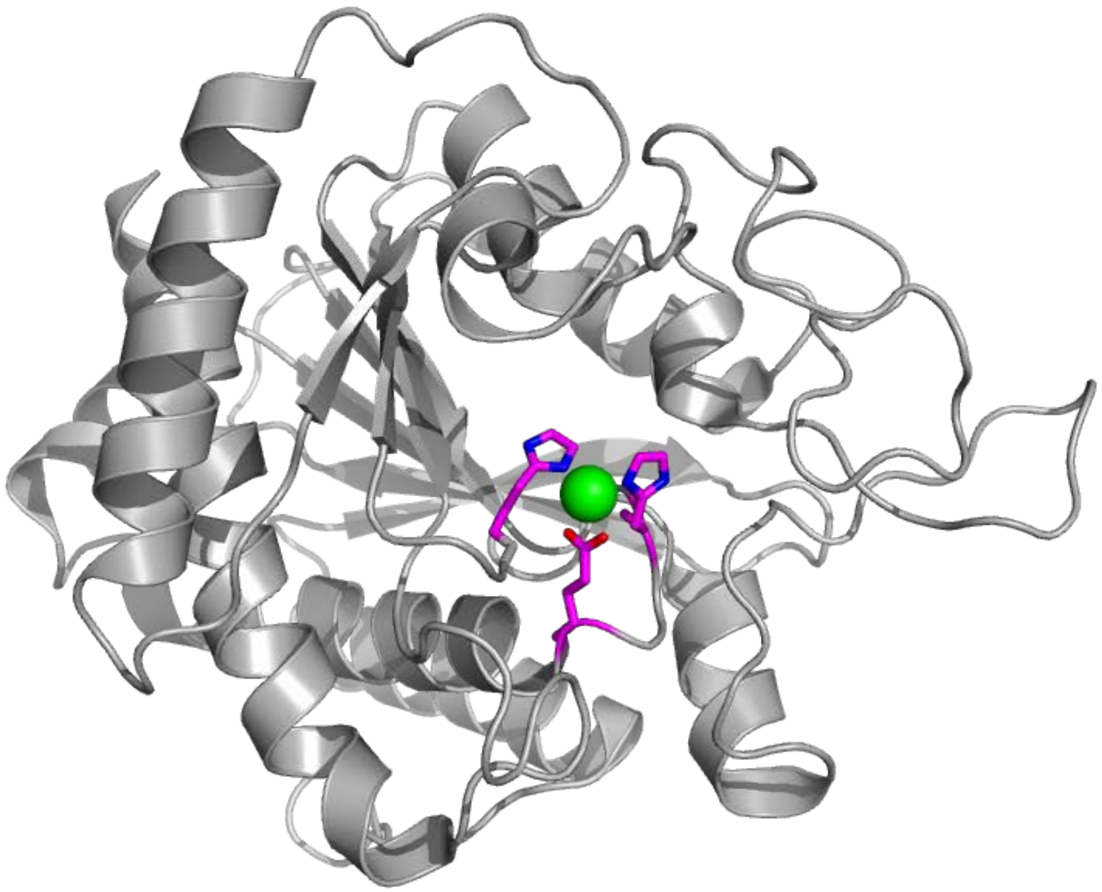


ca. 25% ee

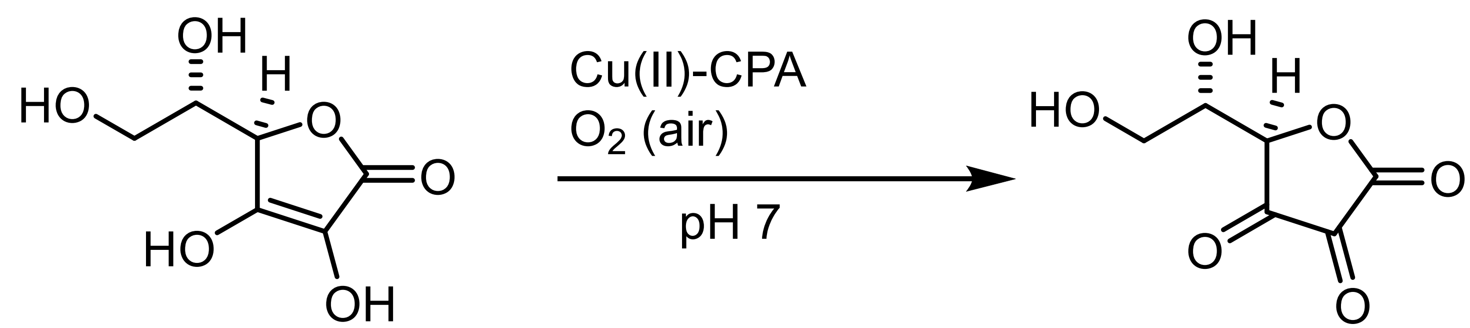
- poorly defined catalyst (Pd-nanoparticles)
- reproducibility issues

S. Akabori et al. >An Asymmetric Catalyst< Nature 1956, 178, 323-324.
H. B. Kagan in >Compr. Asym. Catal.< 2000, Eds. A. Pfaltz/E.N. Jacobsen, Springer Ber

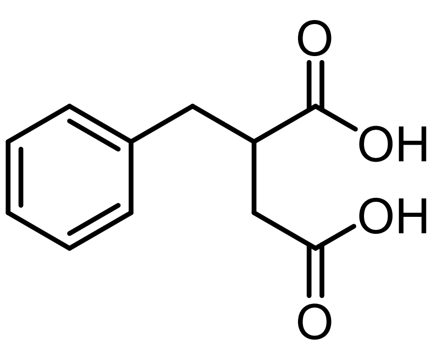
non-native reaction after metal exchange: E. T. Kaiser (1976)



carboxypeptidase A
PDB 1M4L



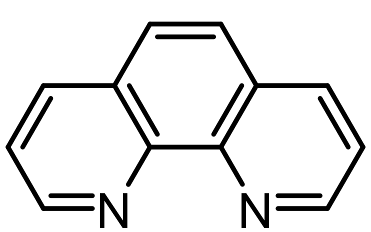
effective inhibitor



k_{cat} = 6 min^{-1} and K_m = 0.24 mM

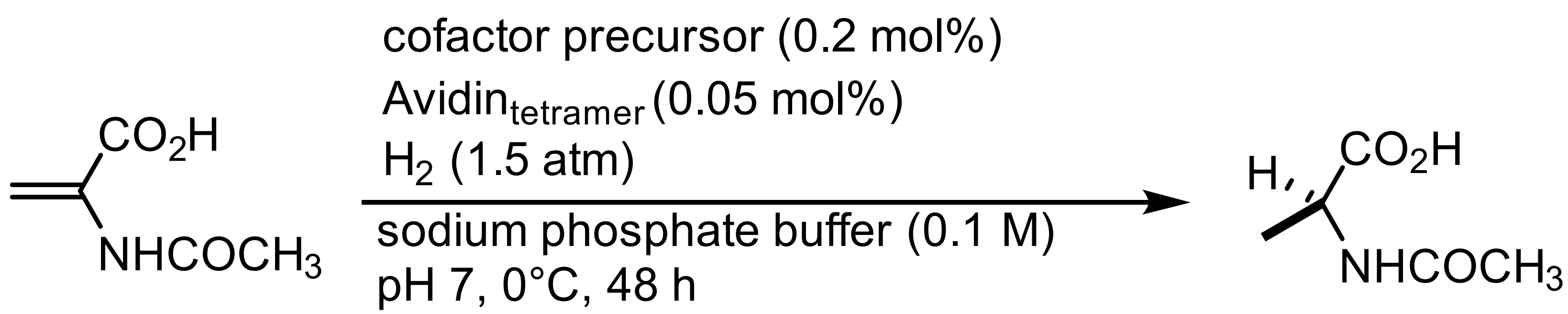
metal exchange:

dialysis against:



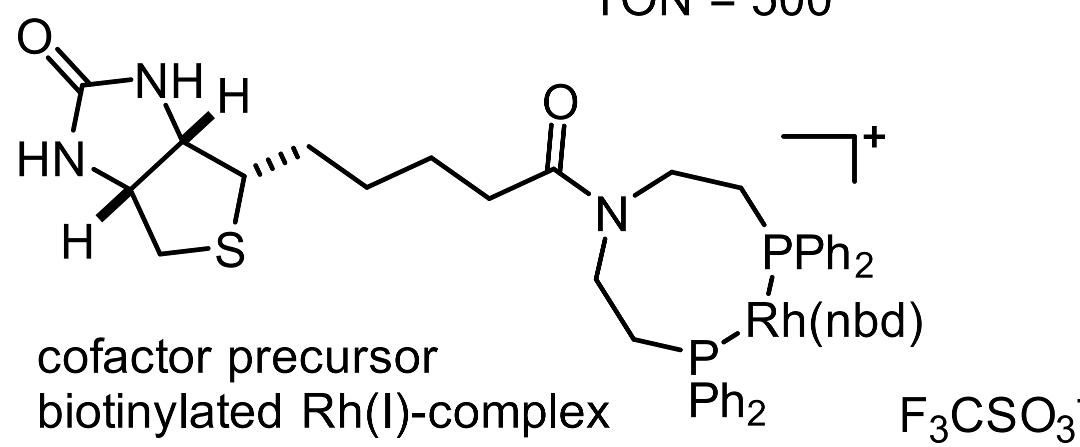
E. T. Kaiser, J. Chem. Soc. Chem. Commun. 1976, 830-83

hydrogenation - Whitesides (1978)



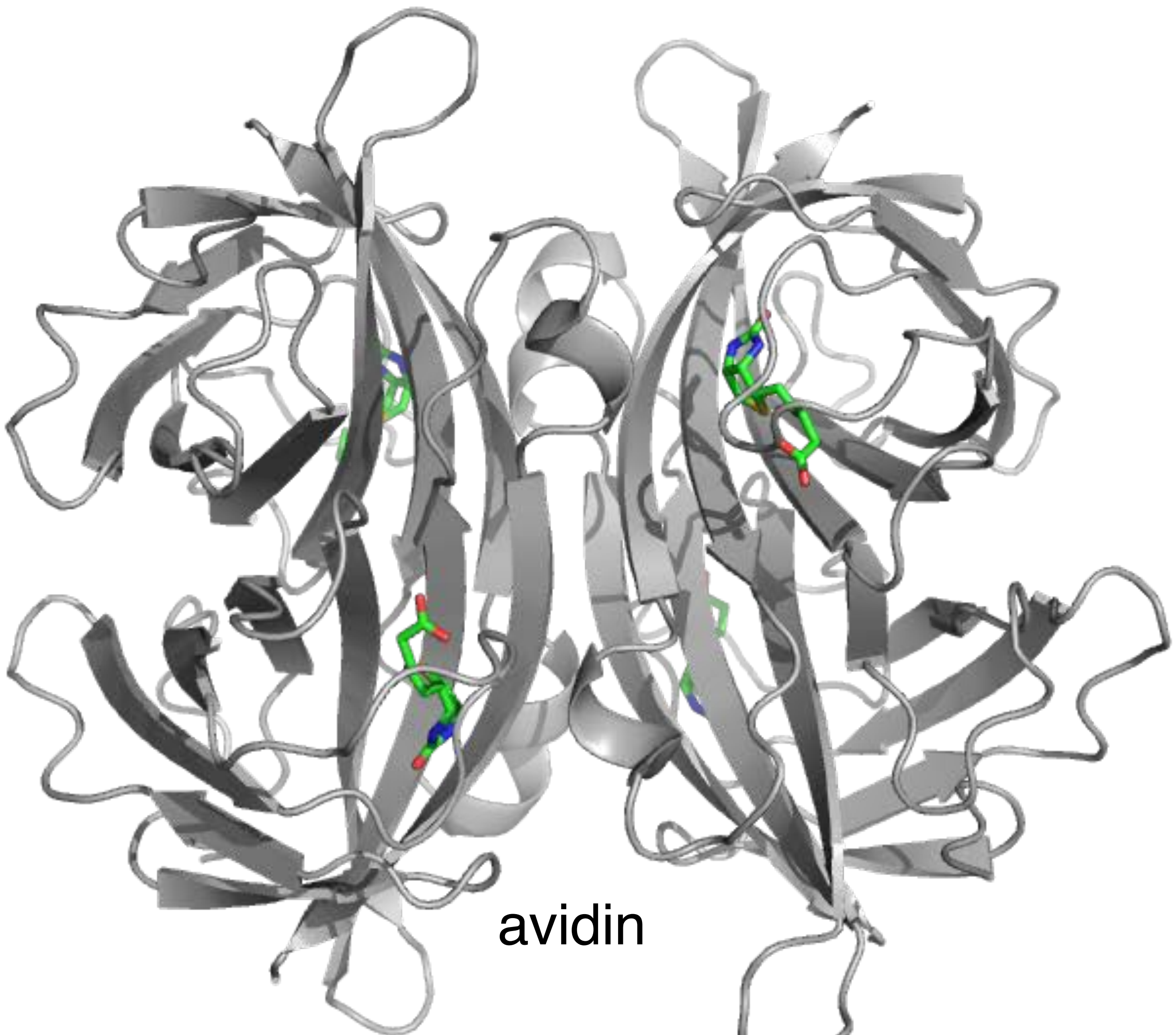
0.04 M

41% ee
TON = 500



cofactor precursor
biotinylated Rh(I)-complex

nbd = 2,5-norbornadiene



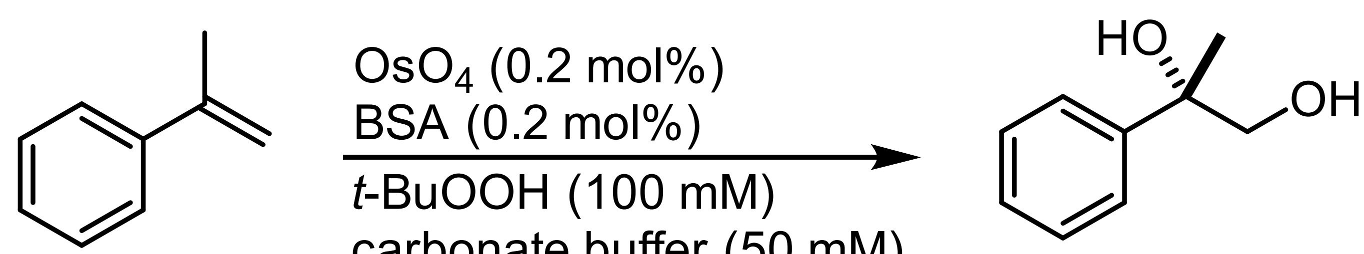
PDB 2AVI

K_D(biotin-avidin) ≈ 10^{-15} M

of the catalyst (CA by approximately a factor of 10). The presence of 1 equiv of avidin in solution (assuming each avidin subunit to be associated with 1 equiv of **1**) resulted in a definite *increase* in activity, and in the production of **3** with ~40% *S* enantiomeric excess. When the ability of avidin to bind **1** was blocked by prior exposure to ether a 10% excess or a tenfold

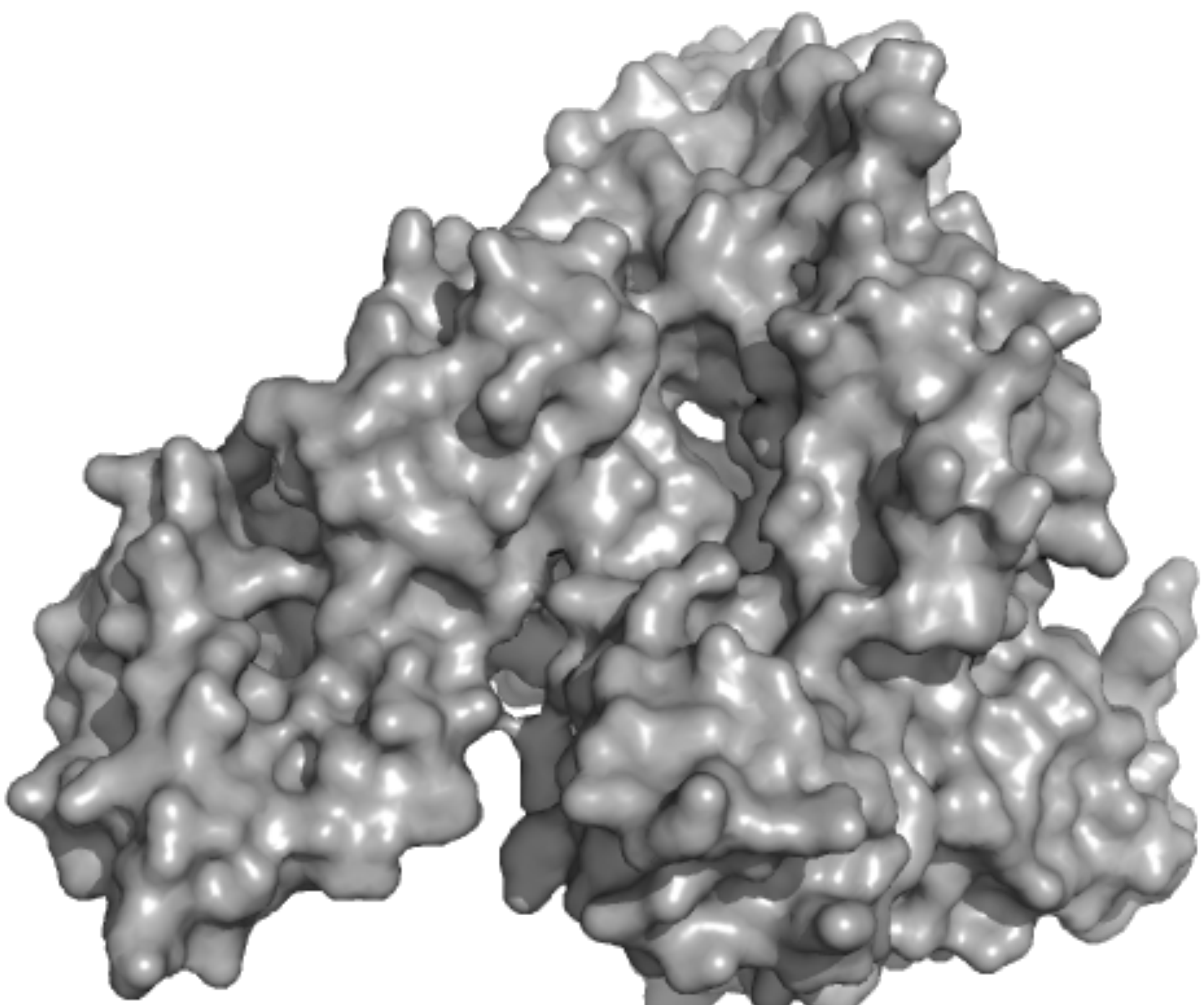
G. M. Whitesides, J. Am. Chem. Soc. 1978, 100, 306

alkene dihydroxylation. Kokubo et al. (1983)



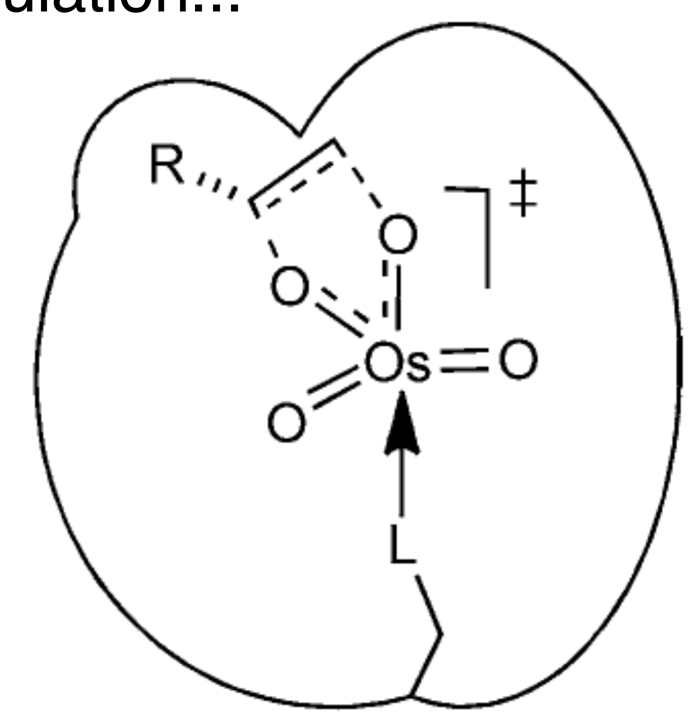
100 mM

68 % *ee*
TON = 40



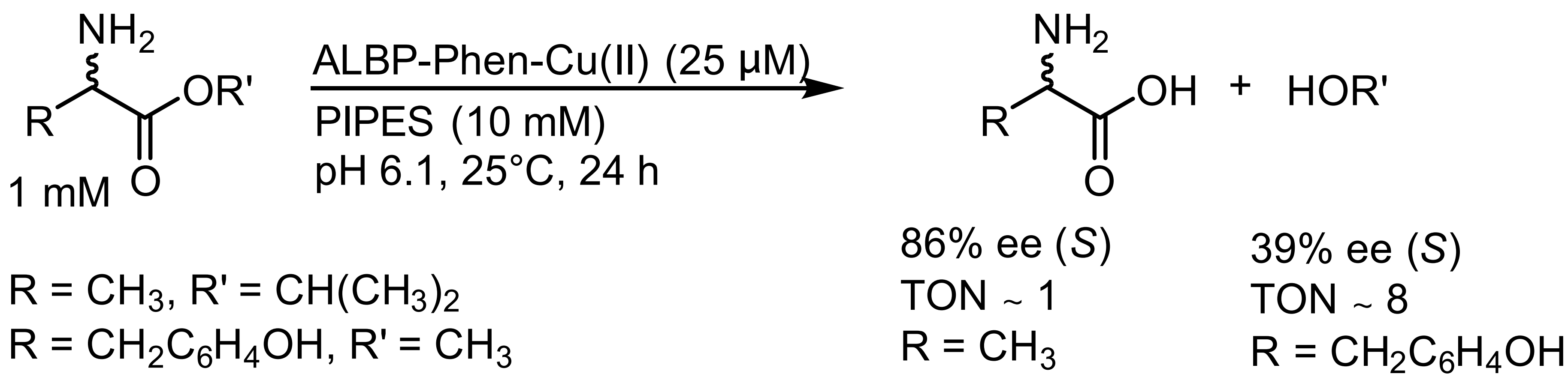
bovine serum albumin (BSA)
PDB ID 4FS5

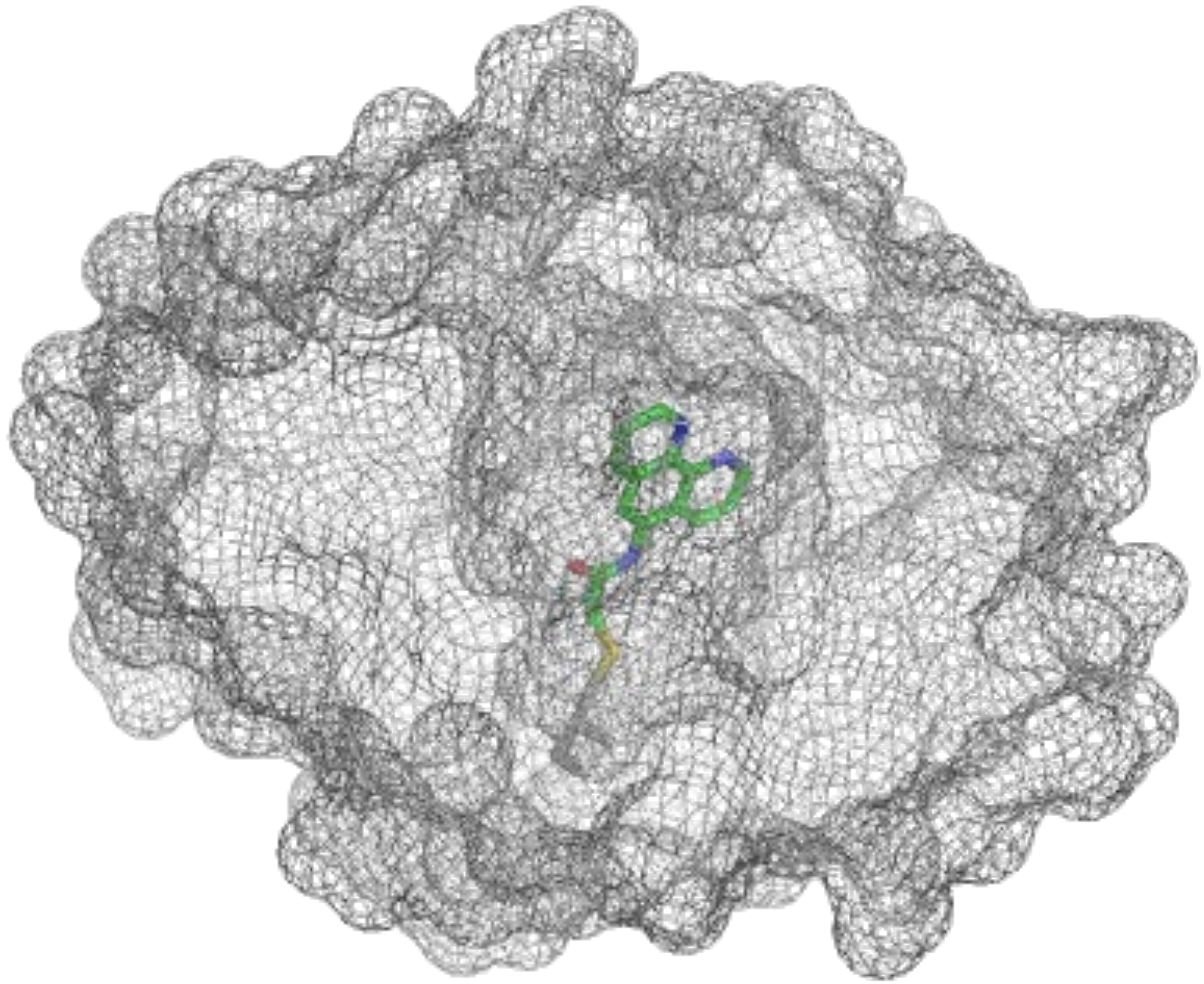
speculation...



T. Kokubo et al., J. Chem. Soc. Chem. Commun. 1983, 769

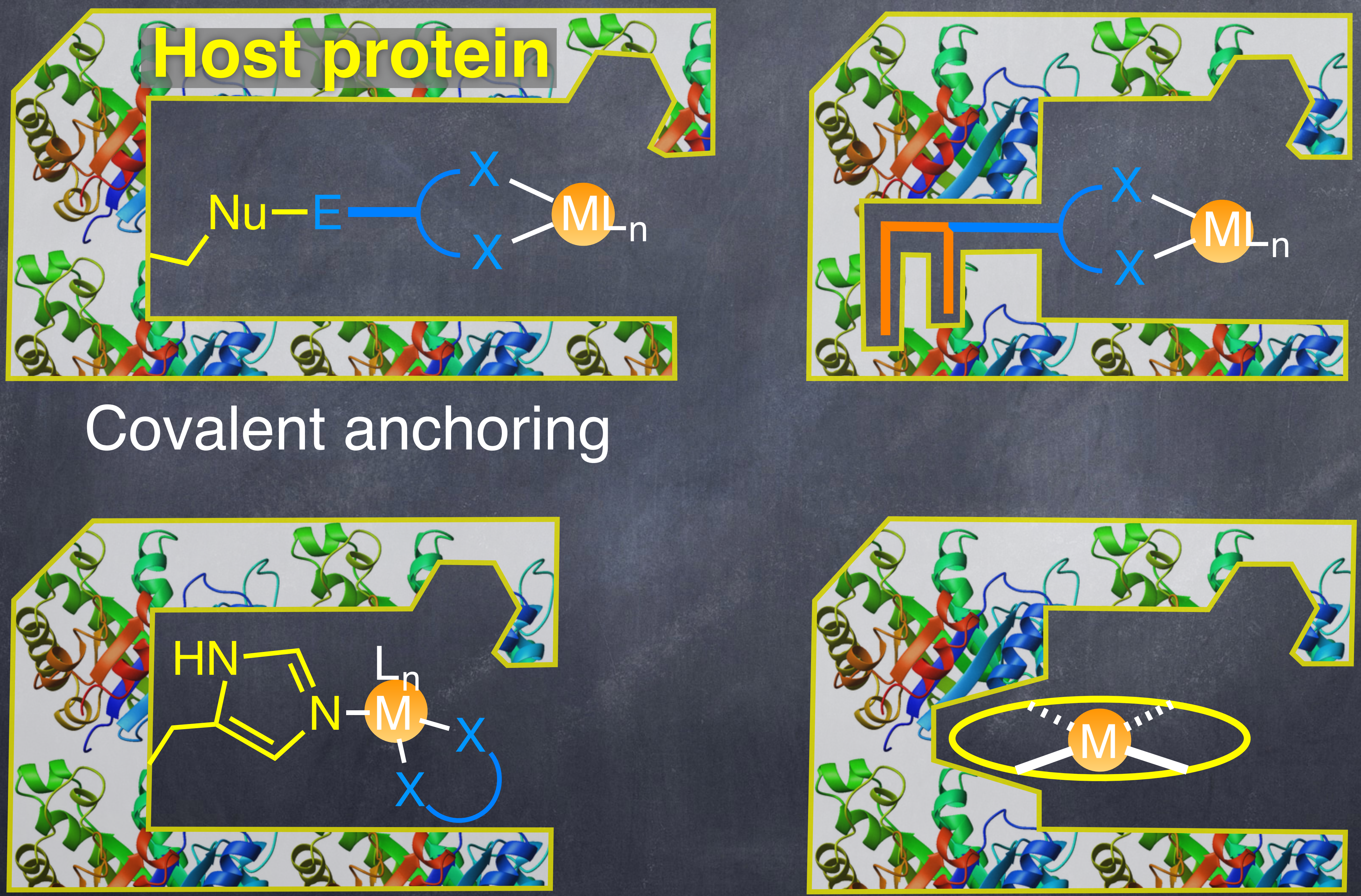
ester hydrolysis. Distefano (1997)





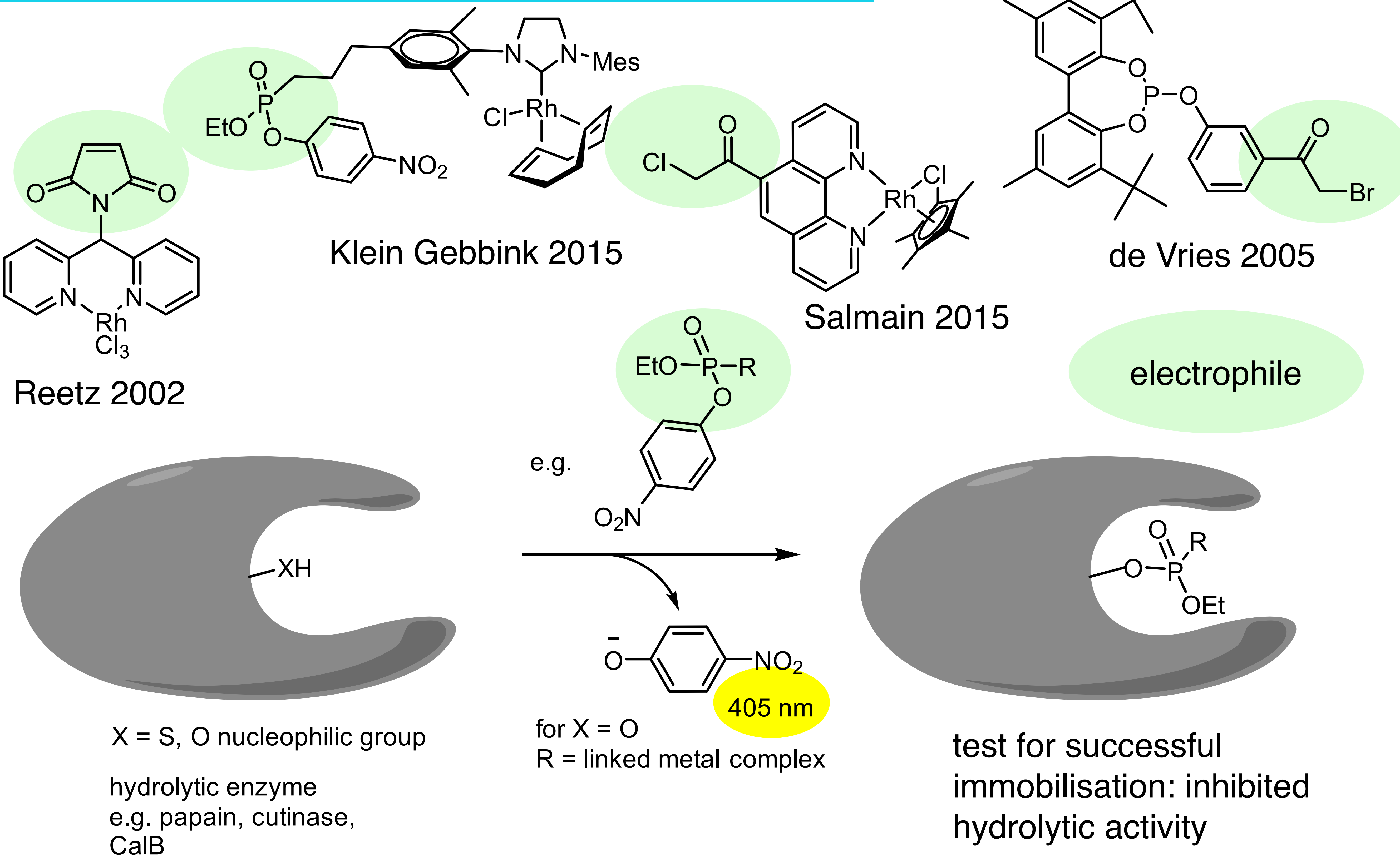
M. D. Distefano et al., J. Am. Chem. Soc. 1997, 119, 11643

Anchoring of the Catalyst:
Four Alternatives to Ensure Localization



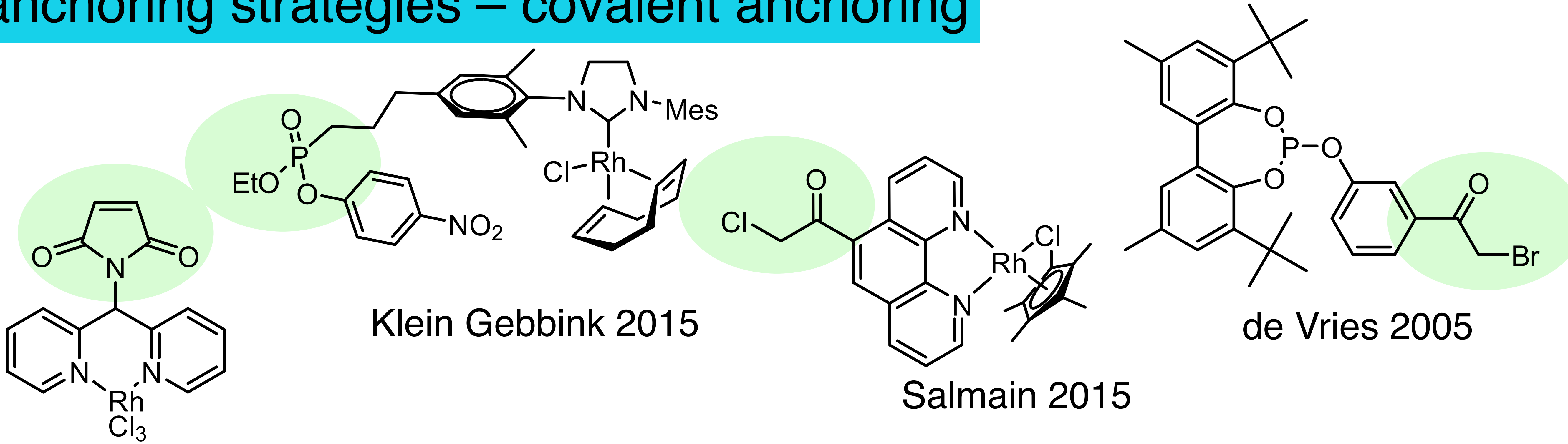
Lewis, Ward *et al.*, *Chem. Rev.* **2018,** *118*, 142.

anchoring strategies – covalent anchoring



E. T. Kaiser, Biochem. Biophys. Res. Commun. 1977, 76, 64; M. T. Reetz, Chimia, 2002, 56, 721;
R. J. M. Klein Gebbink, Chem. Commun. 2015, 51, 6792; M. Salmain, J. Mol. Catal. B, 2015,

anchoring strategies – covalent anchoring



Reetz 2002

Klein Gebbink 2015

Salmain 2015

de Vries 2005

only modest enantioselectivities observed in hydrogenation and transfer hydrogenation reactions –

poorly defined catalyst environment? Too much flexibility?

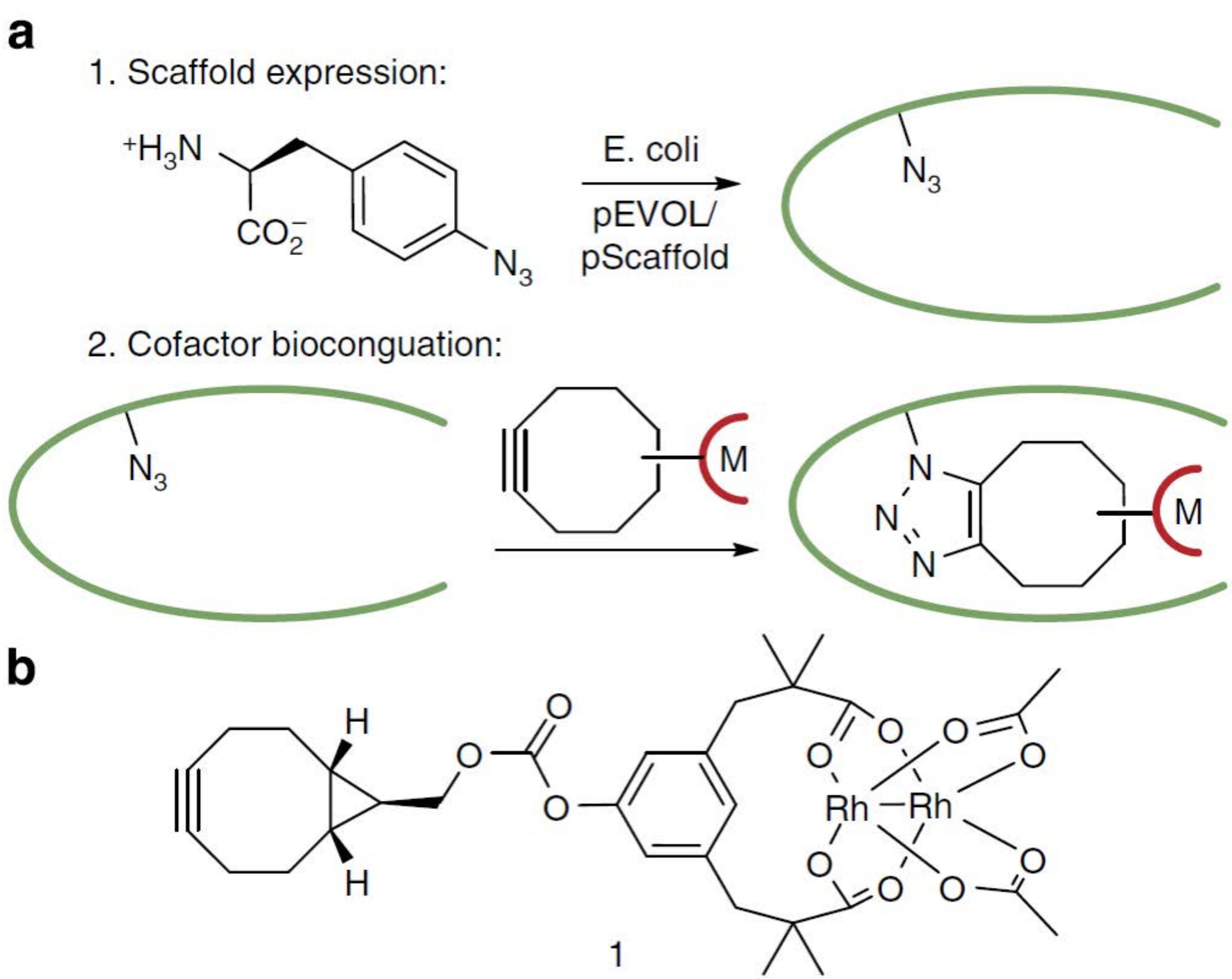
E. T. Kaiser, Biochem. Biophys. Res. Commun. 1977, 76, 64; M. T. Reetz, Chimia, 2002, 56, 721;

R. J. M. Klein Gebbink, Chem. Commun. 2015, 51, 6792; M. Salmain, J. Mol. Catal. B, 2015,

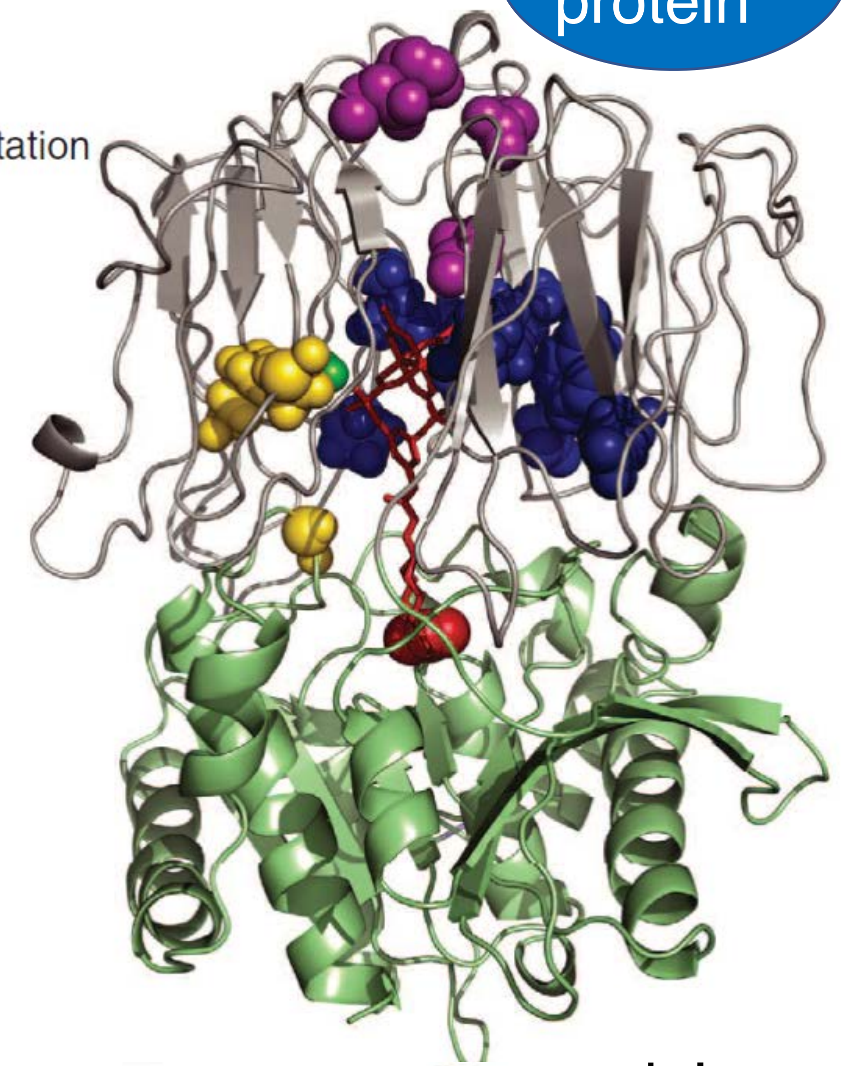
‘biocompatible cofactors’ – more options

purified protein

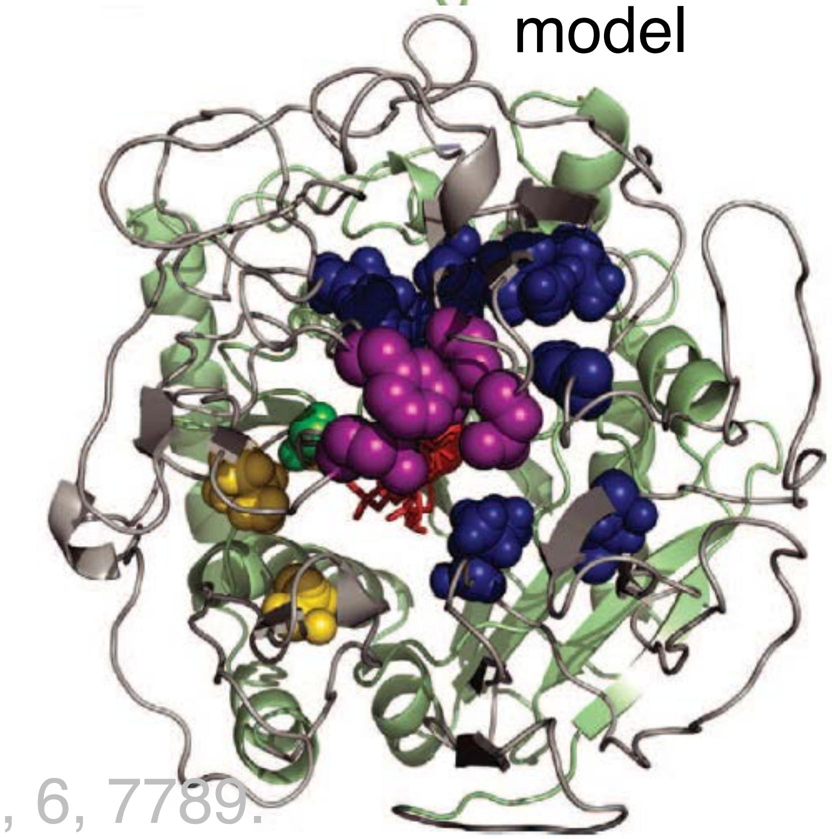
1. installing the cofactor



Alanine mutations (A_4)
Histidine mutations
Histidine/phenylalanine mutation
Phenylalanine mutations



model



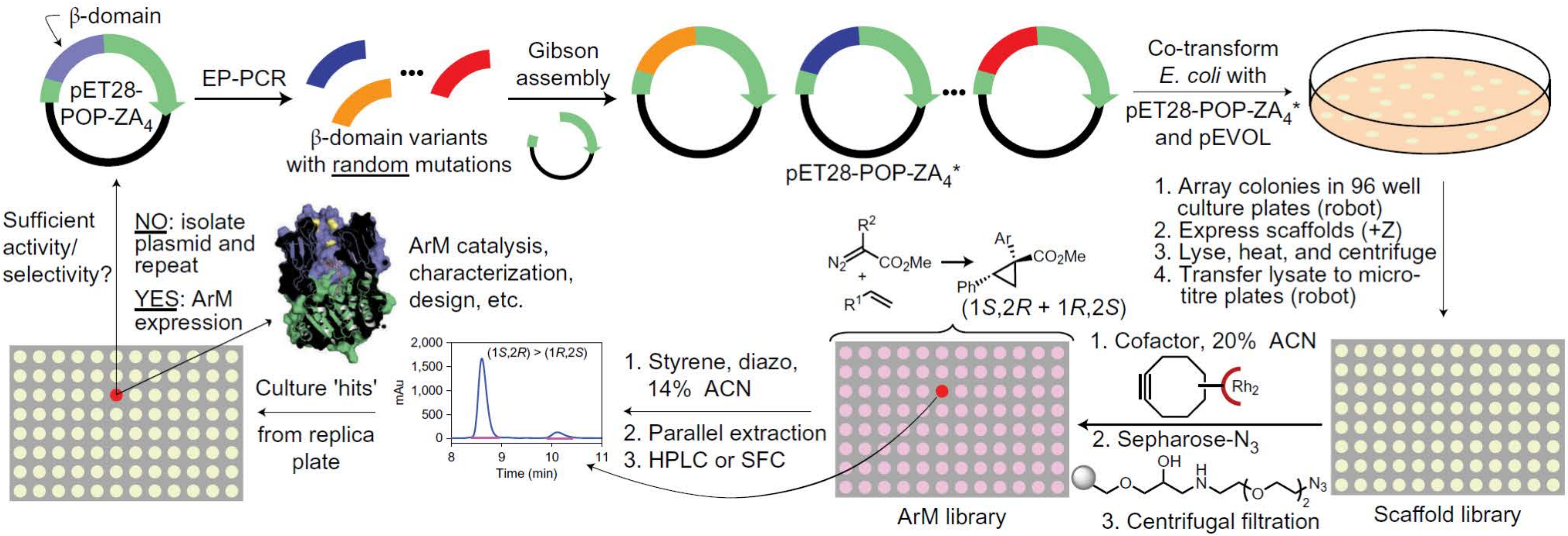
mutant identified which can bind the cofactor (4 Alanine mutations) and is active and selective (1 His mutation) in cyclopropanation $0\text{-}ZA_4$

J. C. Lewis, Nat. Commun. 2015, 6, 7789.

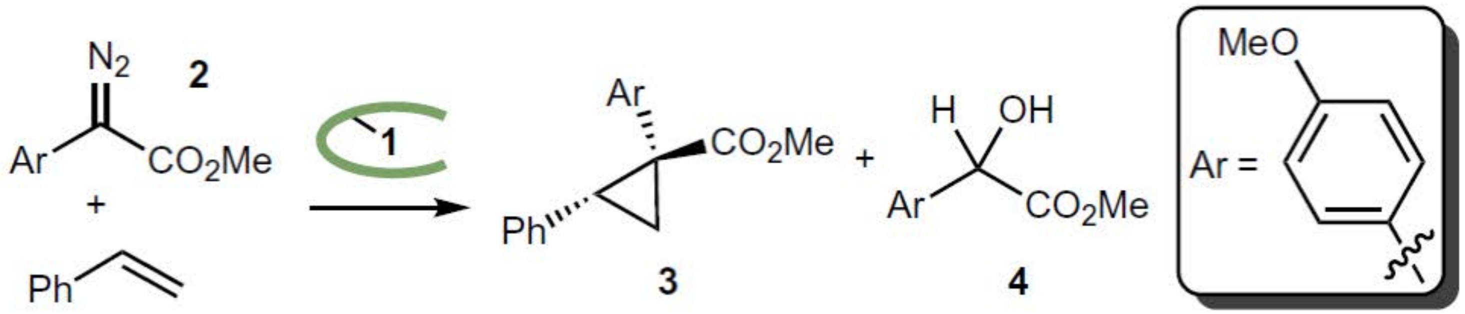
'biocompatible cofactor' – random mutagenesis

cfe heat treated

2. library generation and screening



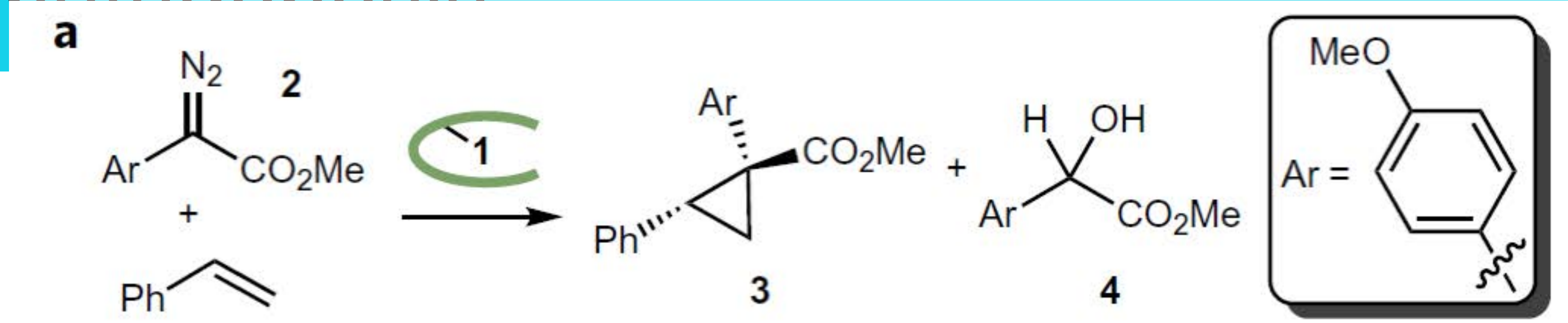
a



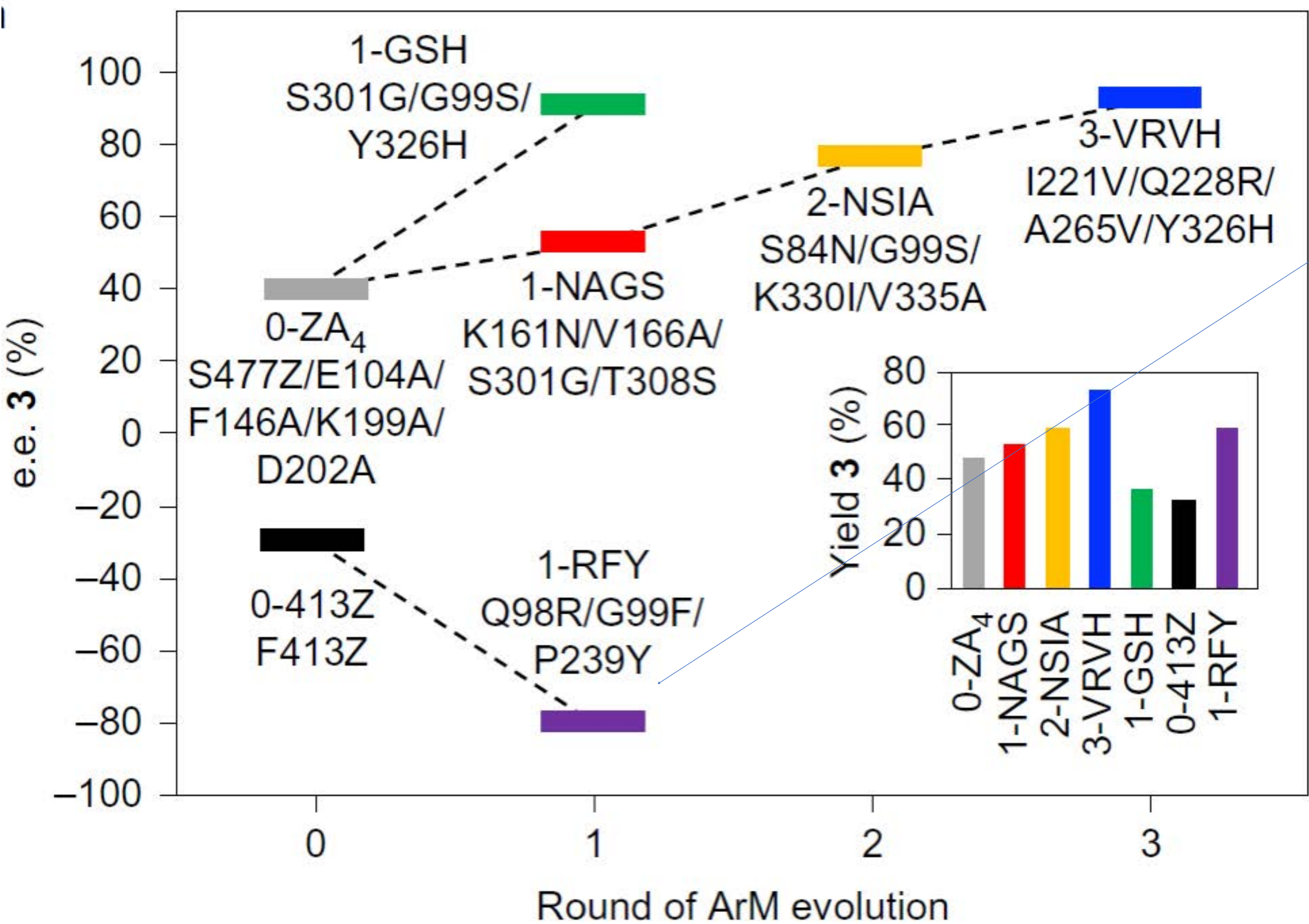
scavenge excess cofactor

J. C. Lewis, Nat. Chem. 2018, 10, 318

‘biocompatible cofactor’ – random mutagenesis



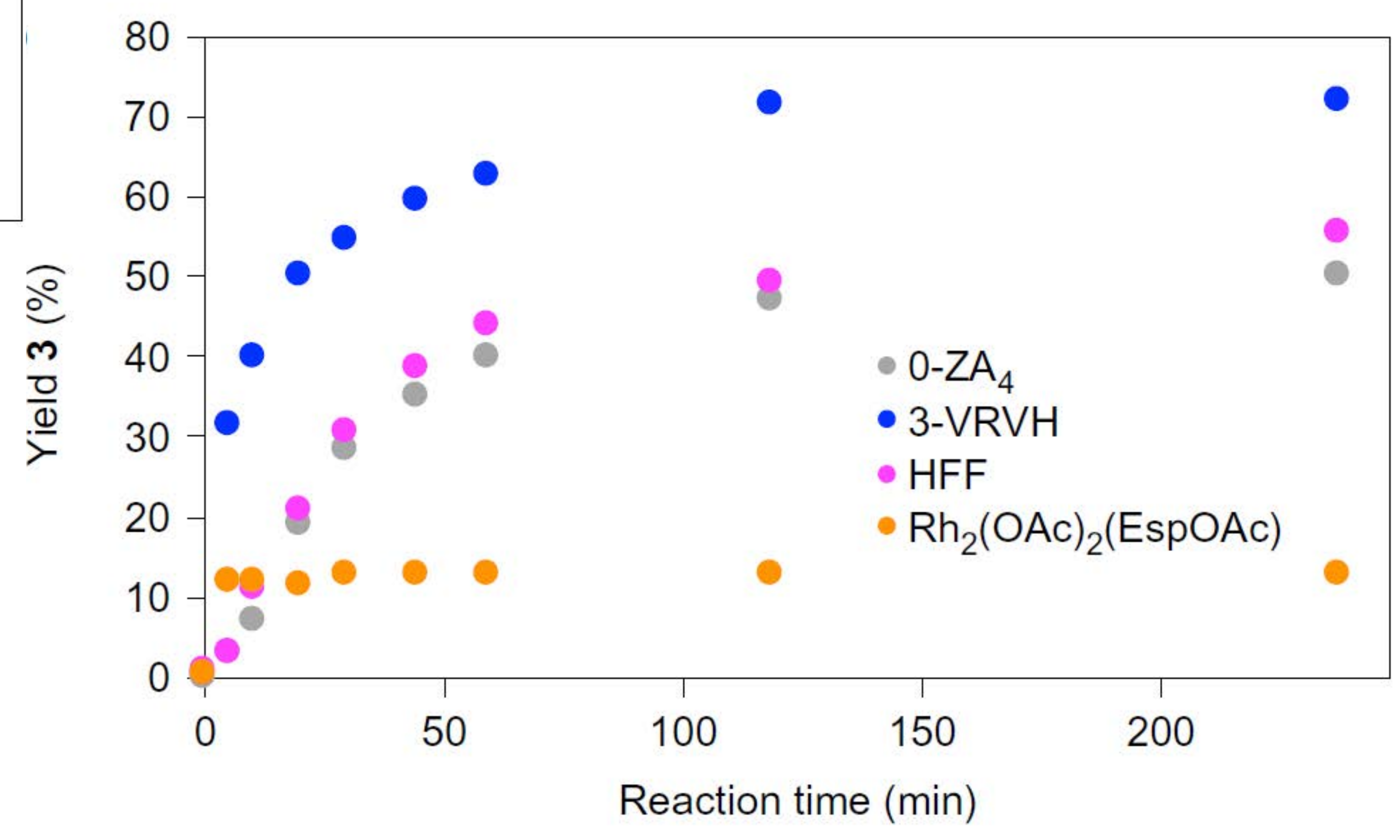
TON up to 66



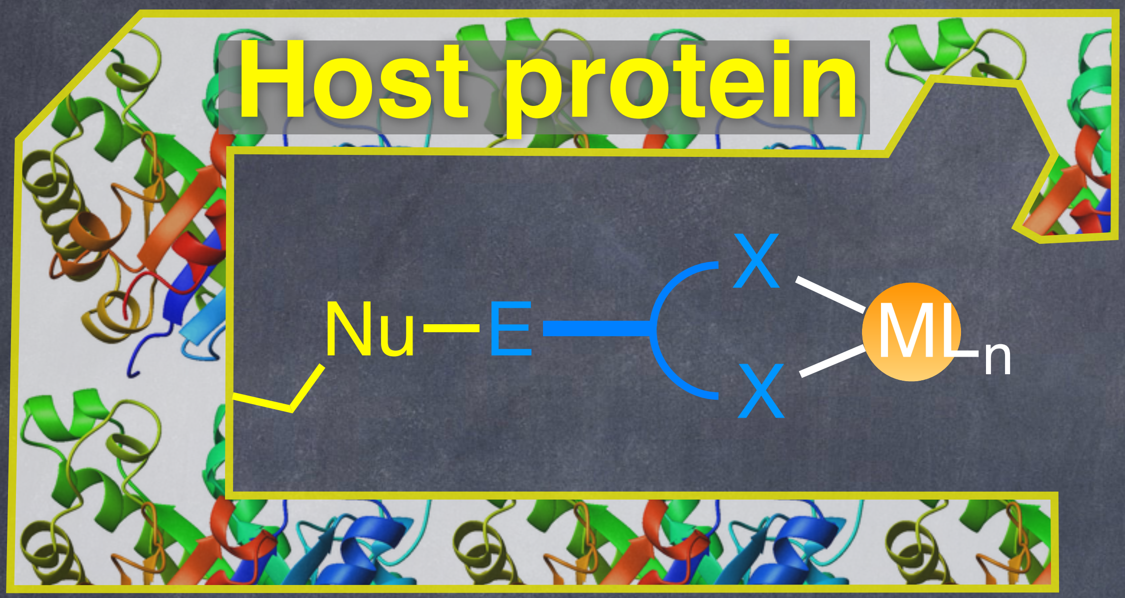
random mutagenesis of selected sites based on crystal structure inspection with degenerate codons

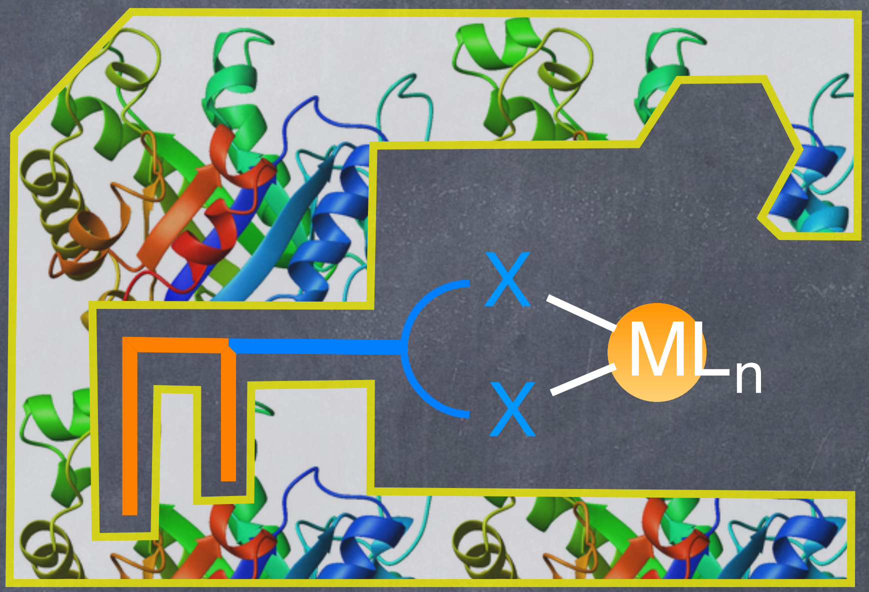
variants/ round 96 48 576

high mutation frequency

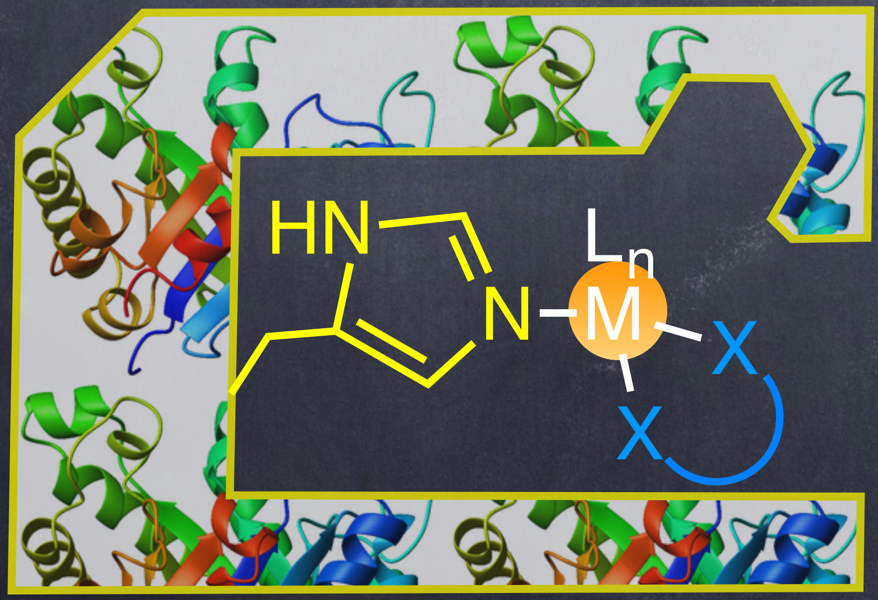


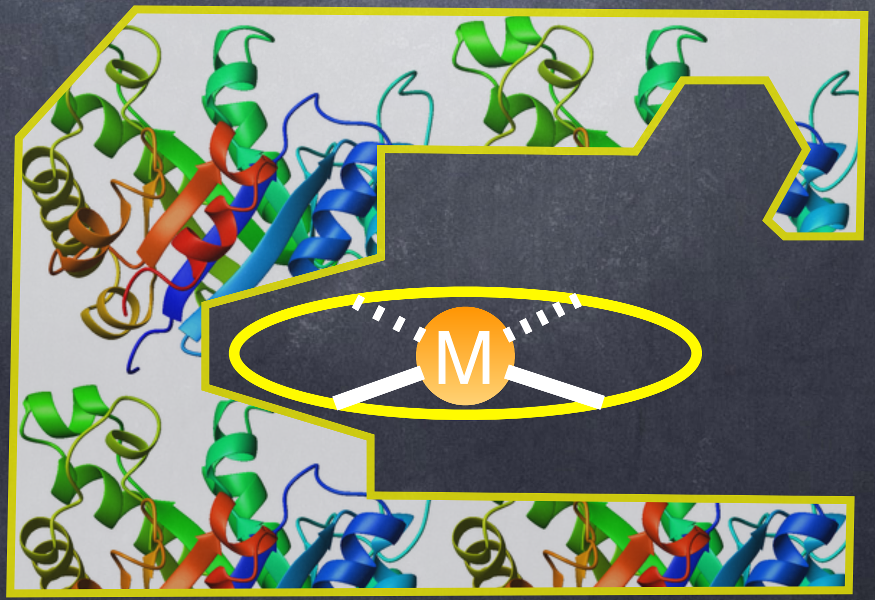
Anchoring of the Catalyst:
Four Alternatives to Ensure Localization





Supramolecular anchoring

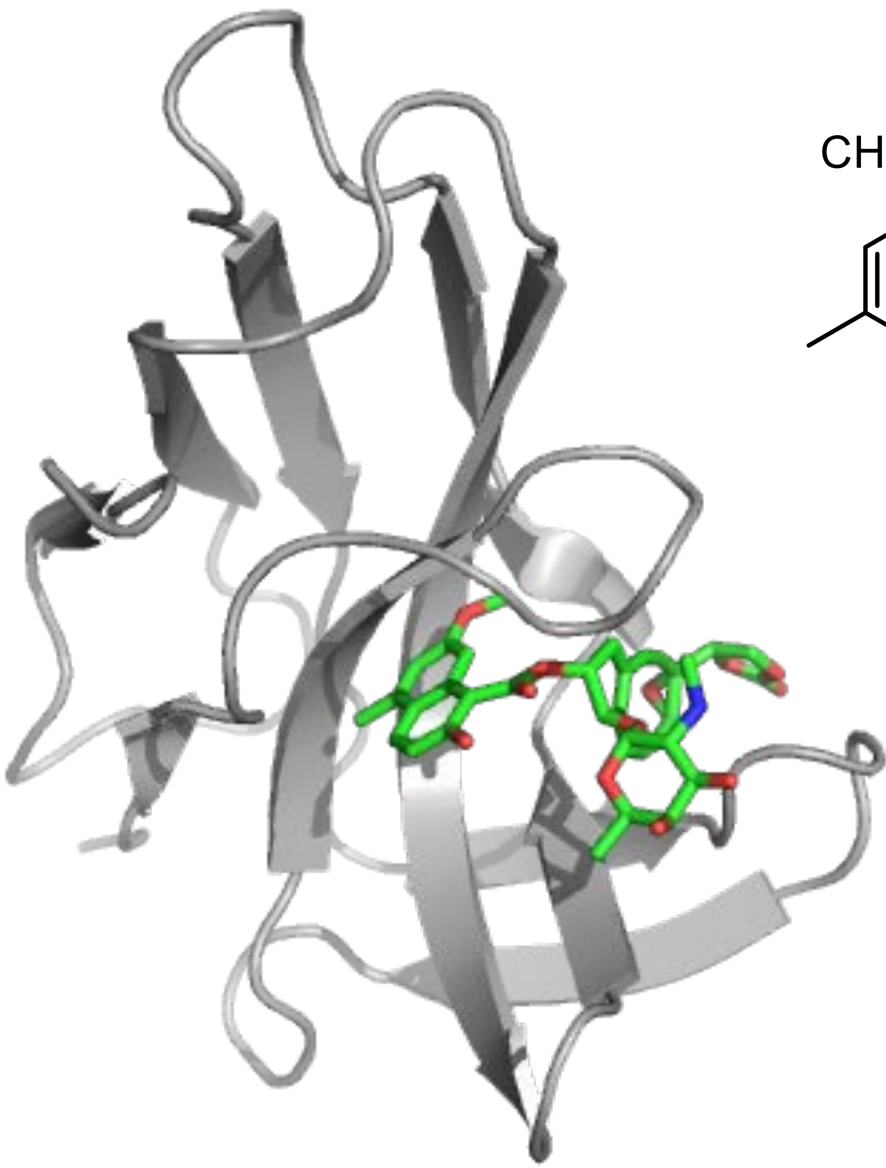




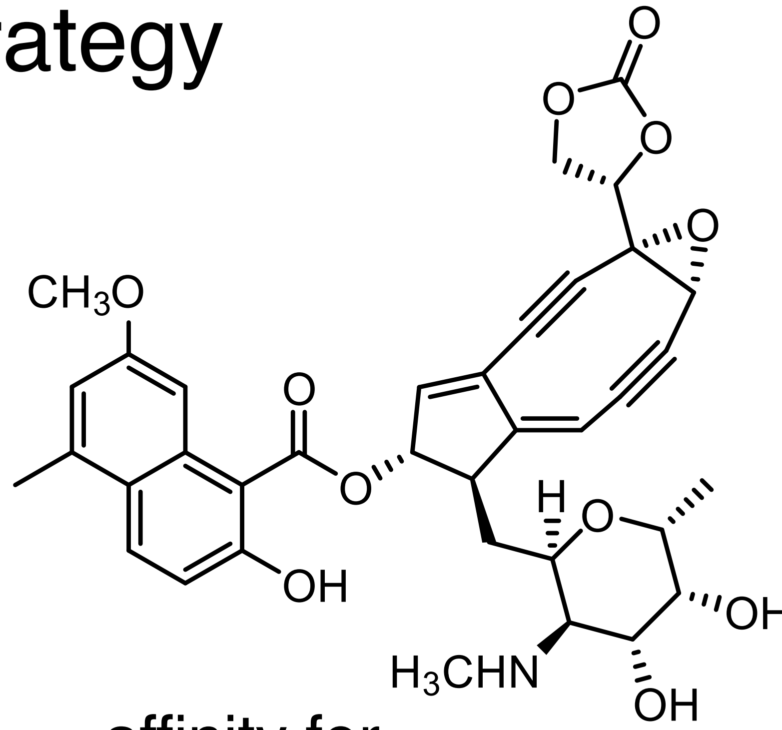
Lewis, Ward *et al.*, *Chem. Rev.* **2018,** *118*, 142.

anchoring strategies - supramolecular

the Trojan horse strategy



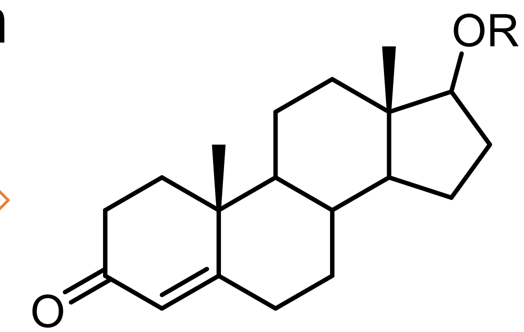
neocarzinostatin with natural ligand bound
PDB 1NCO



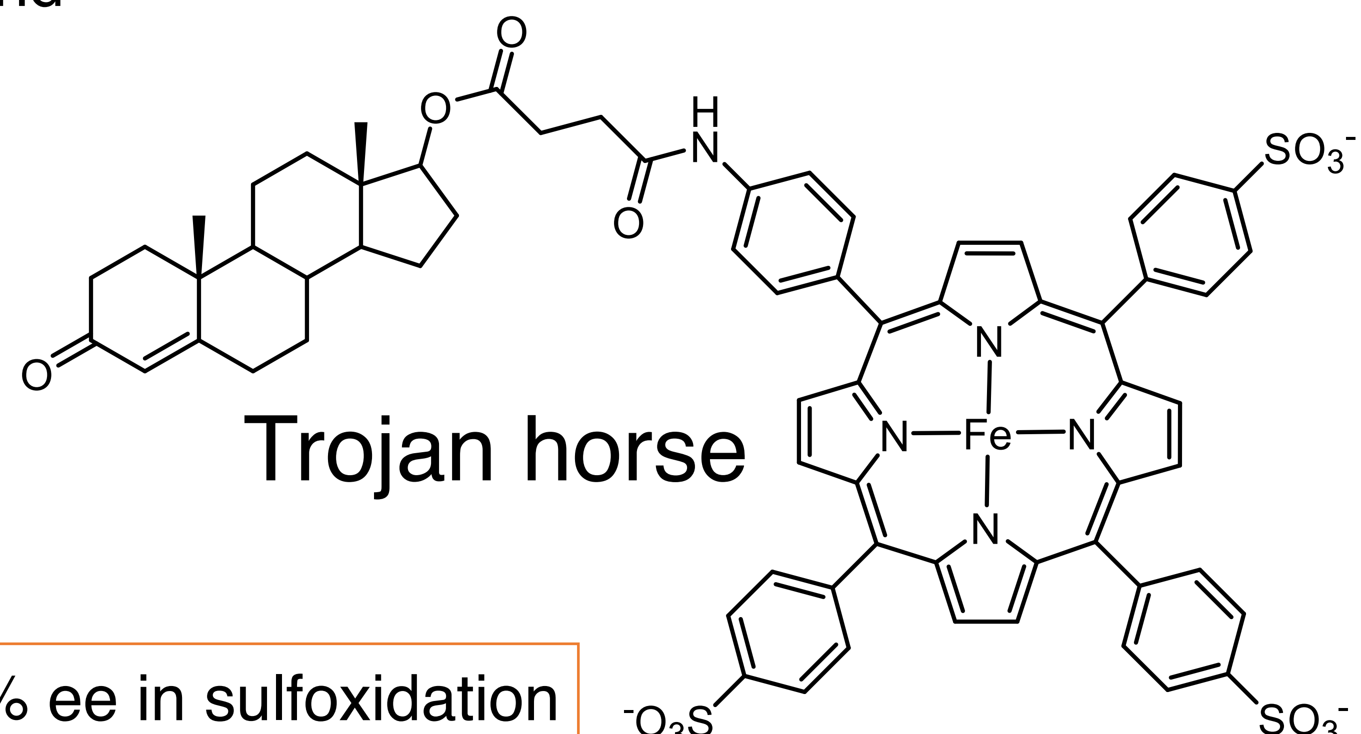
affinity for
natural ligand

analysis
directed evolution
(phage display)

7 mutations



affinity for new ligand
$K_d = 4 \times 10^{-5}$ M



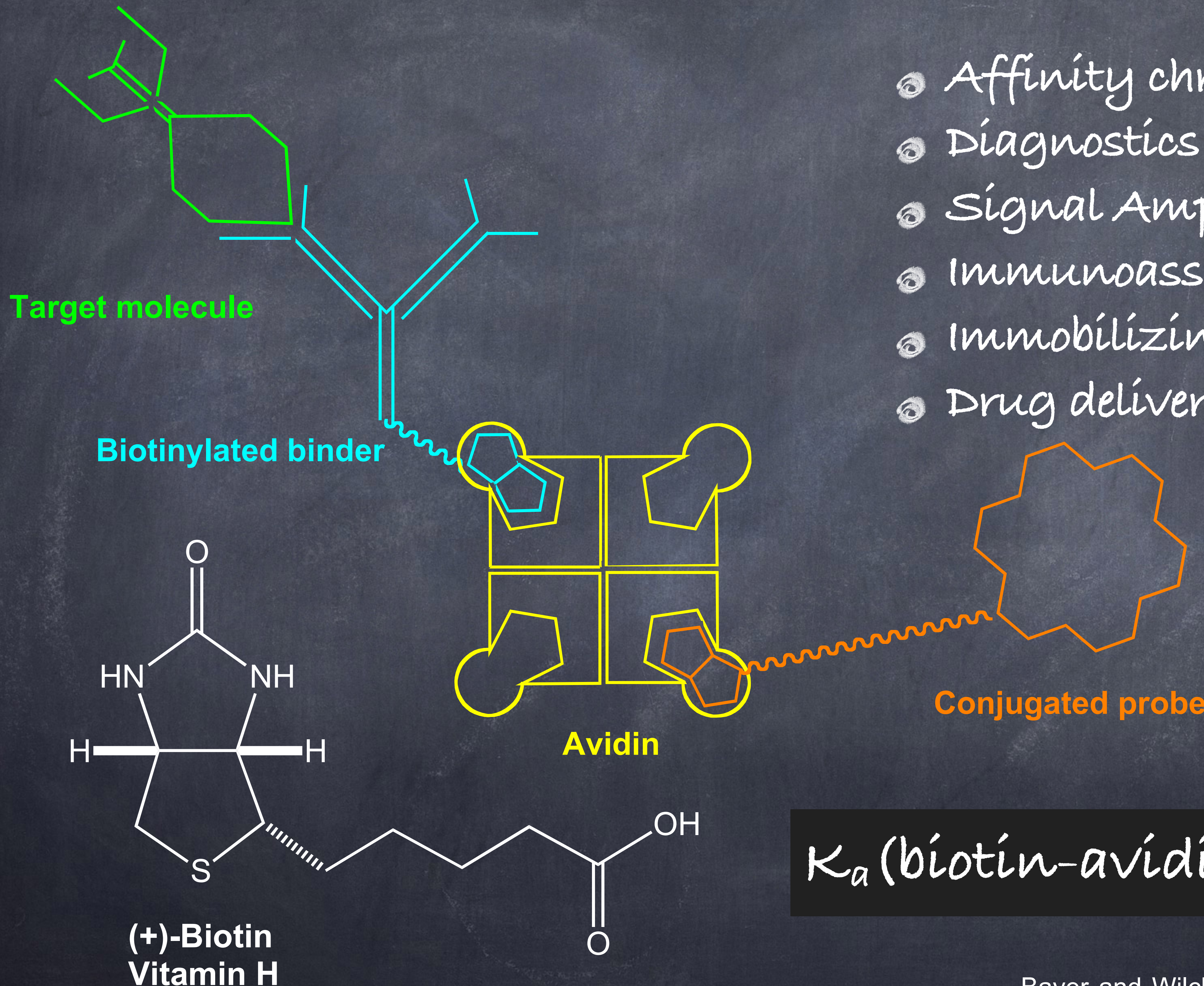
Trojan horse

13% ee in sulfoxidation

catalysis: J.-P. Mahy, R. Ricoux, ChemBioChem 2016, 17, 433; review catalysis: J.-P. Mahy Chem. Commun. 2015, 2476, protein engineering: P. Minard, Biochemistry 2003, 42, 5674.

Biotin-Avidin Technology: Molecular Velcro

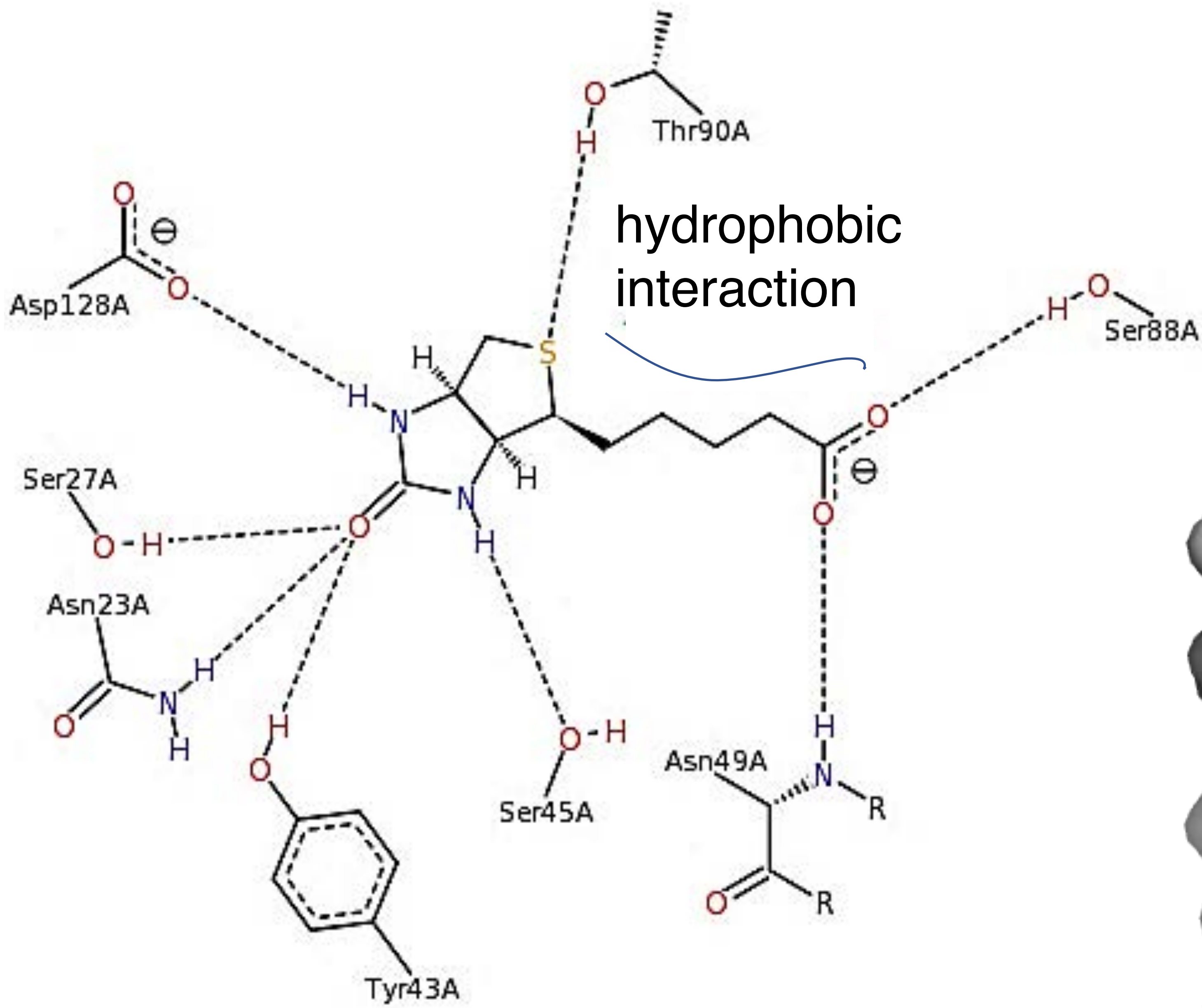
- Affinity chromatography
- Diagnostics
- Signal Amplification
- Immunoassay
- Immobilizing agent
- Drug delivery

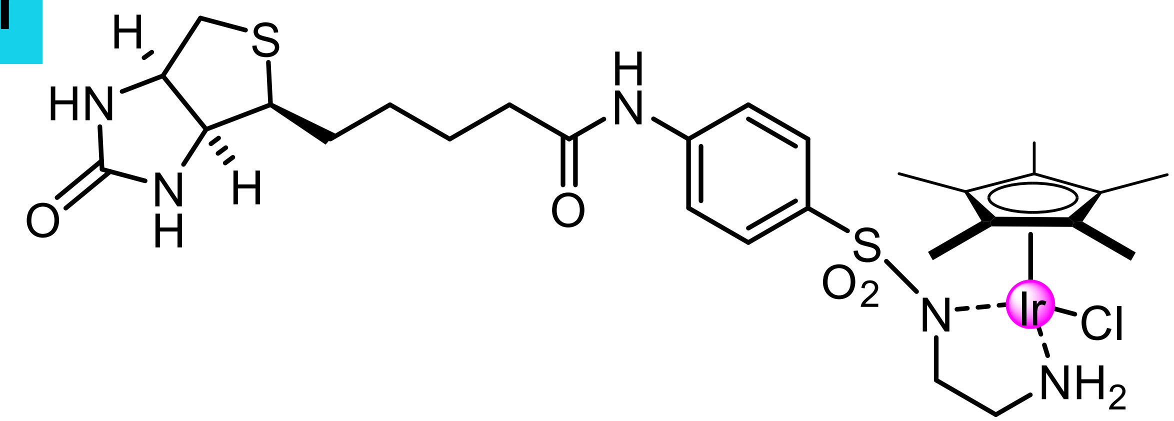


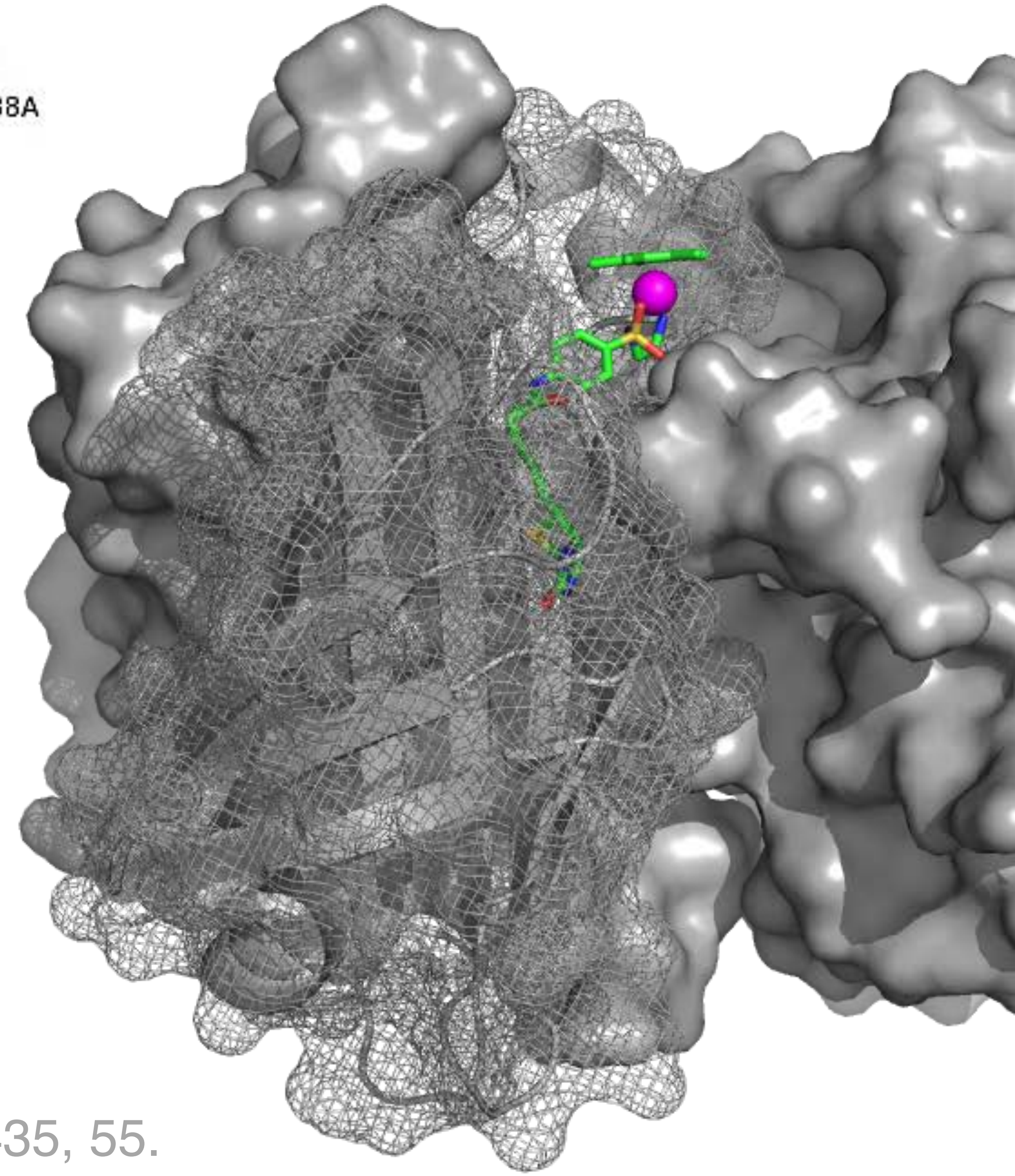
K_a (biotin-avidin) $10^{14} M^{-1}$

Bayer and Wilchek. *Methods in Enzymology*, Vol. 184, Academic Press, **1990.**

anchoring strategies - supramolecular



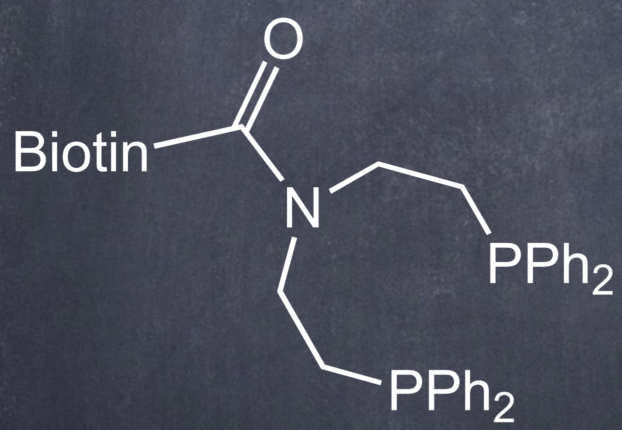




biotin-streptavidin interactions; $K_D \approx 10^{-14}$ M

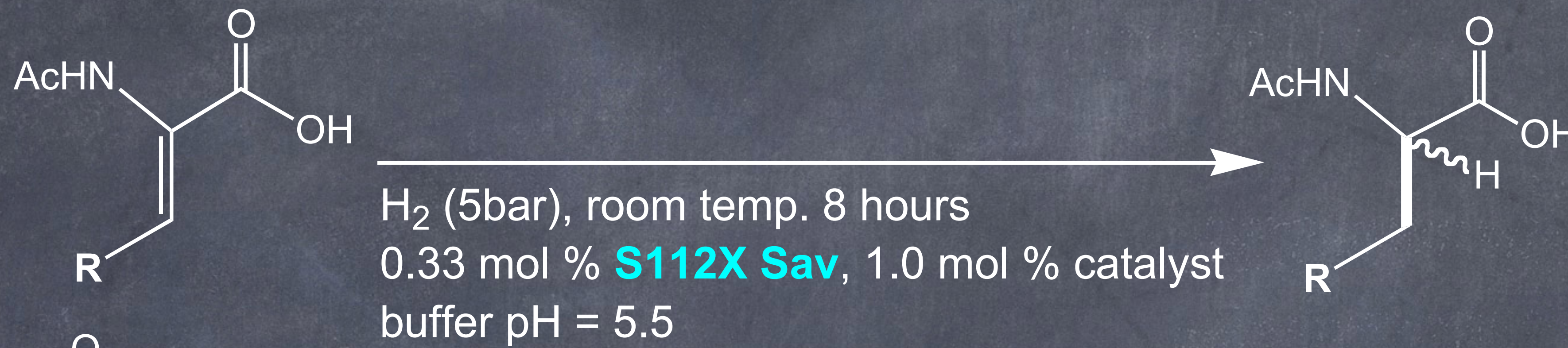
affinity engineering: M. Howarth, Biochem J. 2011, 435, 55.
Ircp* catalysis: T. R. Ward, Angew. Chem. Int. Ed. 2011, 50, 3026.

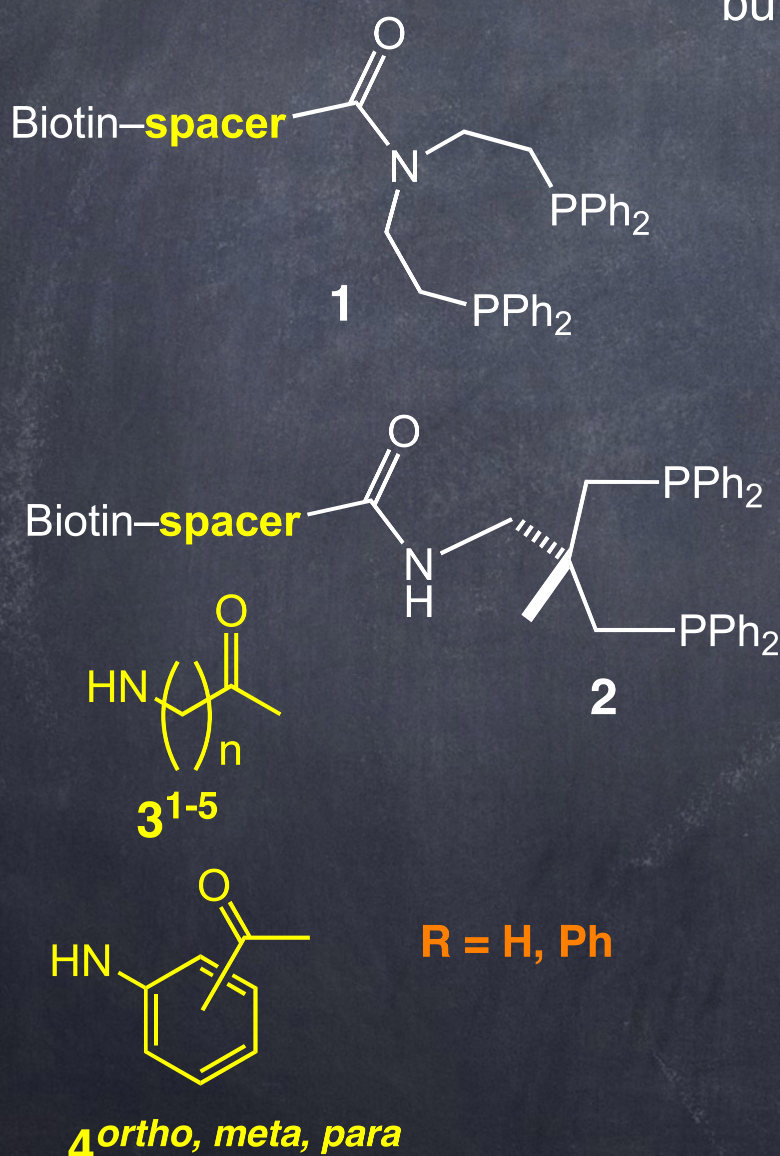
Substrate Specificity: Enzyme-Like or Homogeneous Catalyst-Like ?



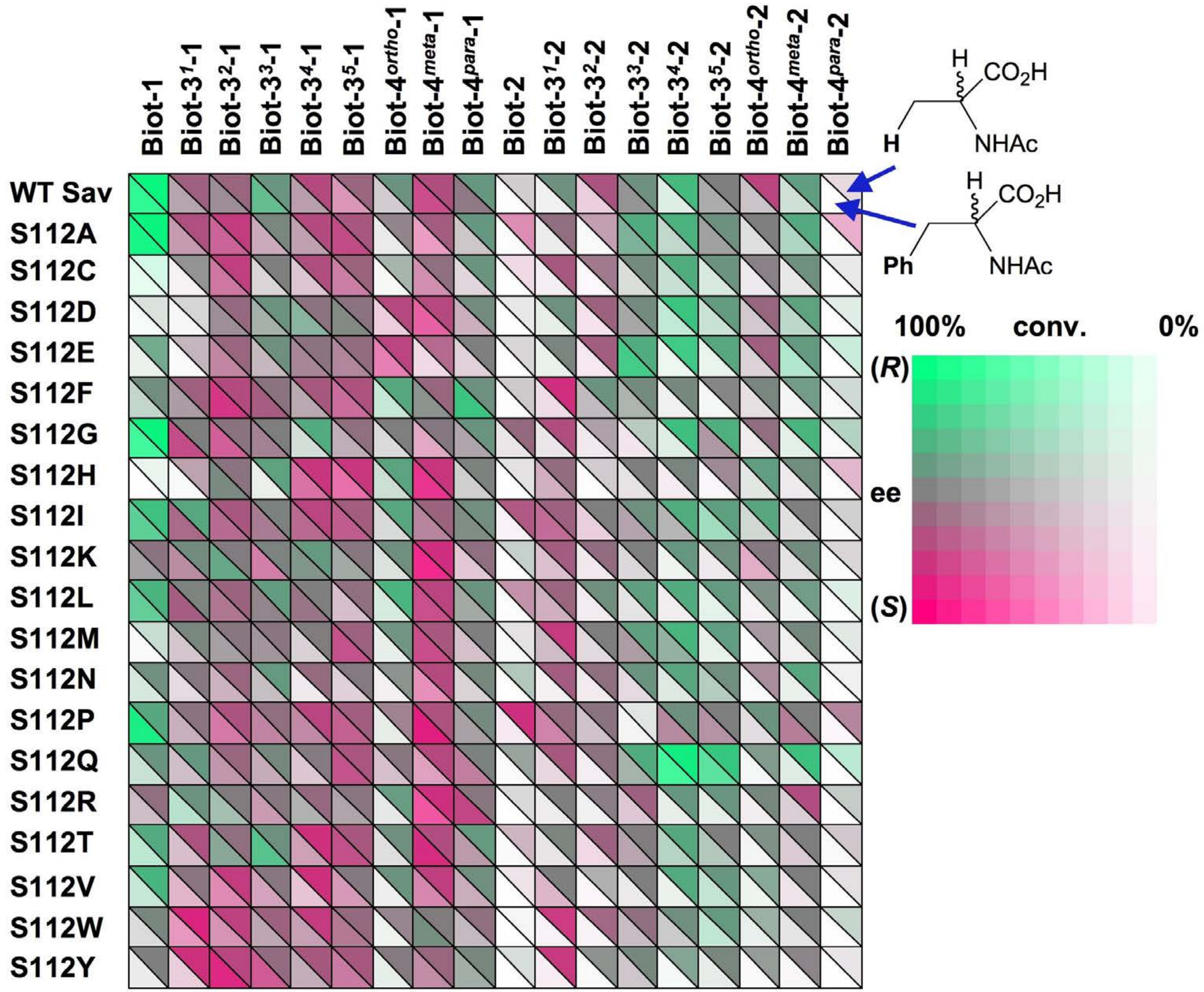
R = H	R = Ph
94 % ee (R)	93 % ee (R)
quant.	quant.

Chemo-Genetic Optimization:
18 Ligands Combined with 20 Proteins



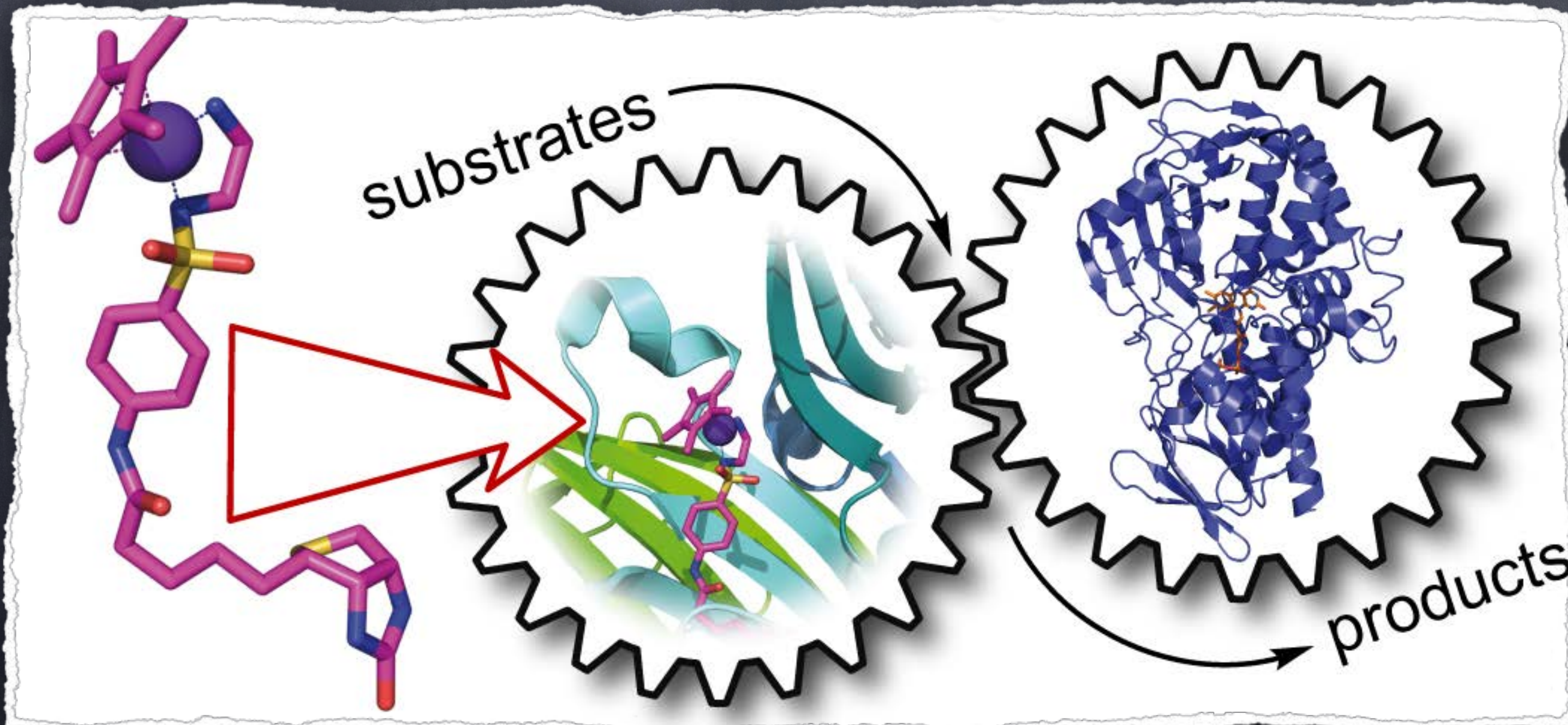


Chemo-Genetic Optimization: Fingerprints

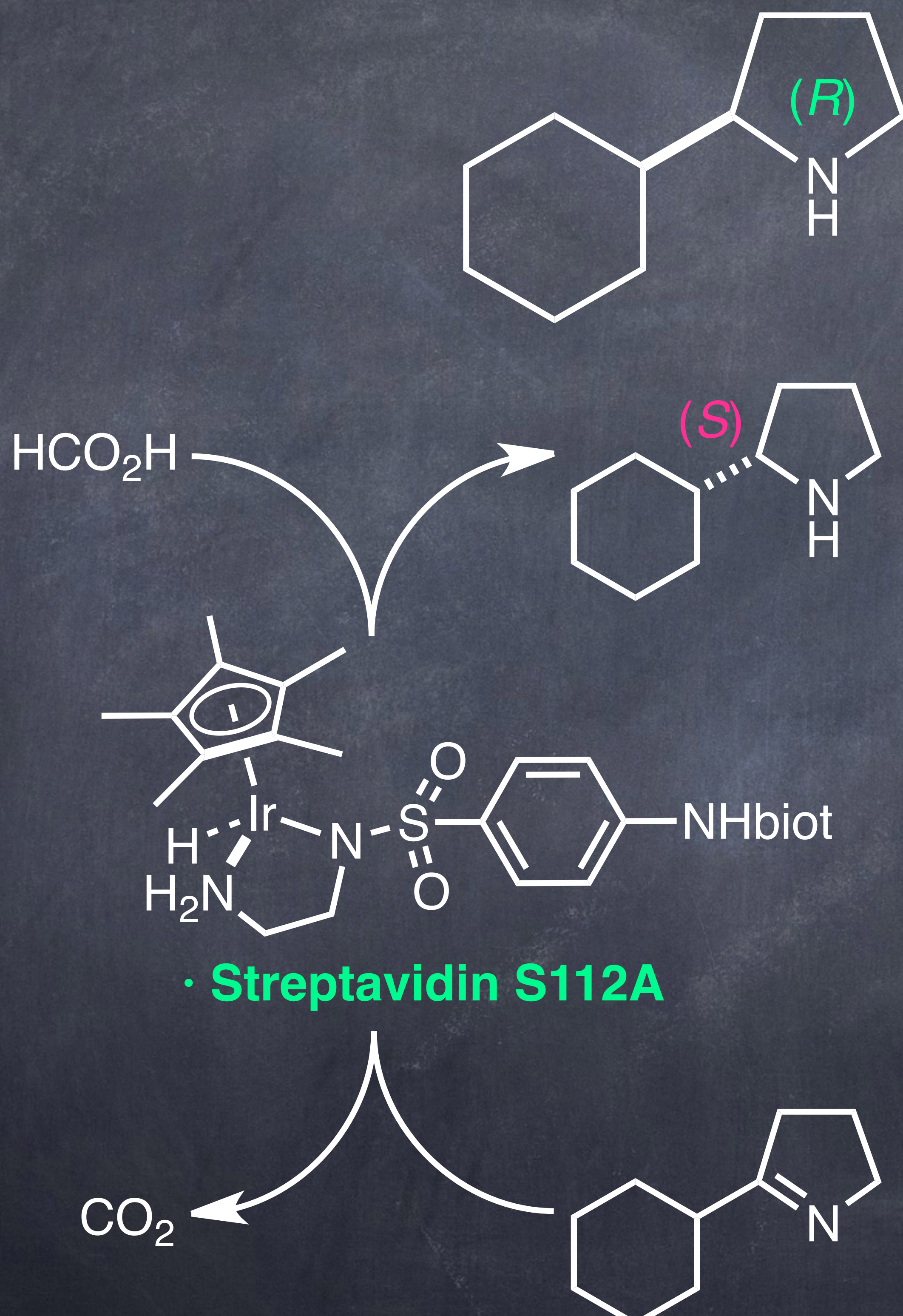


Klein et. al. *Angew. Chem. Int. Ed.* **2005**, *44*, 7764.

Enzyme Cascades...

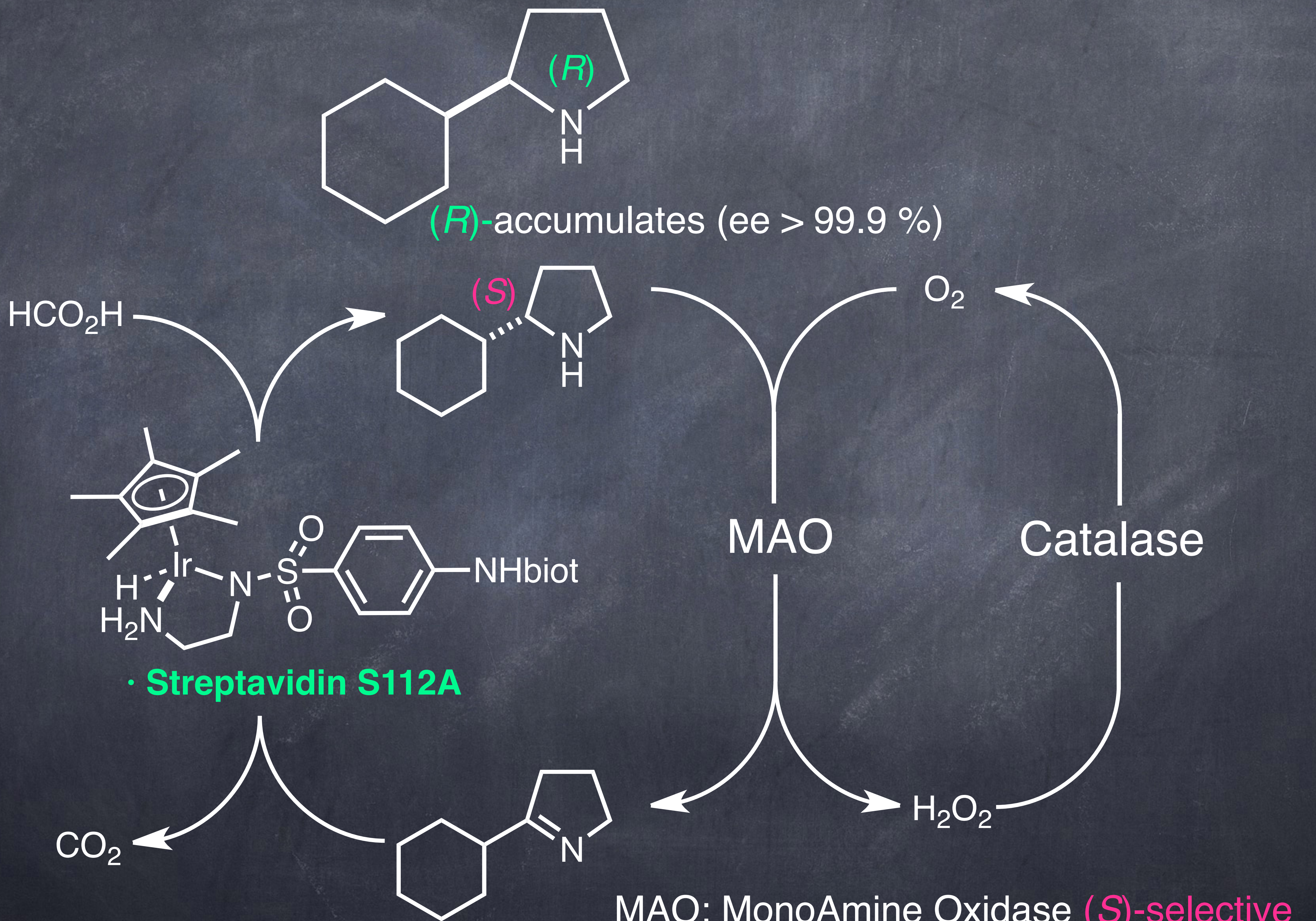


Orthogonal Redox Cascades



M. Dürrenberger, V. Köhler
with N. Turner (U. Manchester)

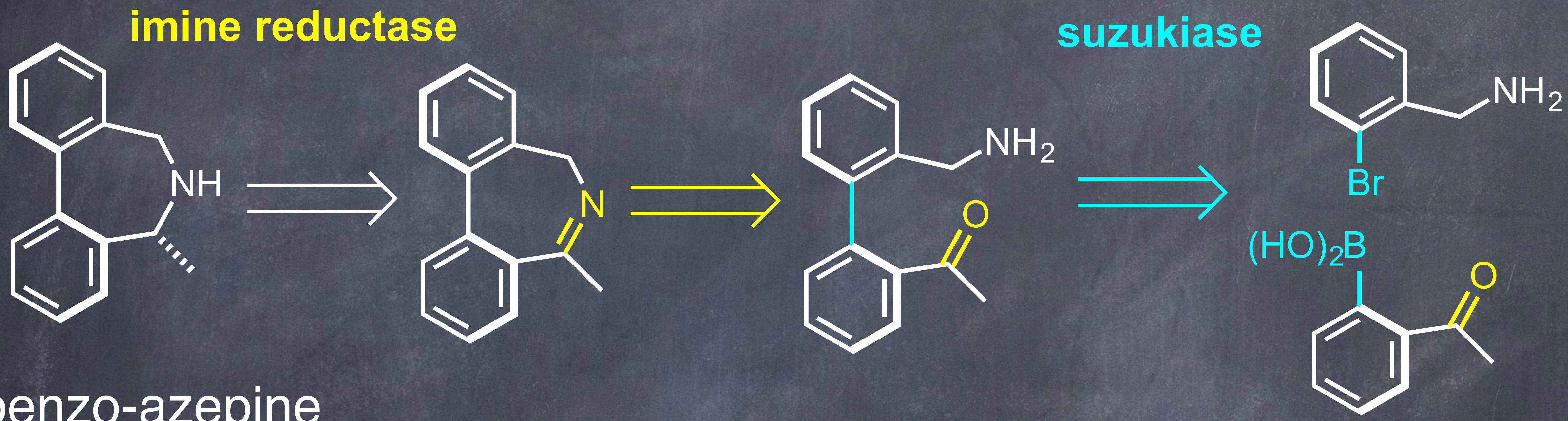
Orthogonal Redox Cascades



MAO: MonoAmine Oxidase (S)-selective

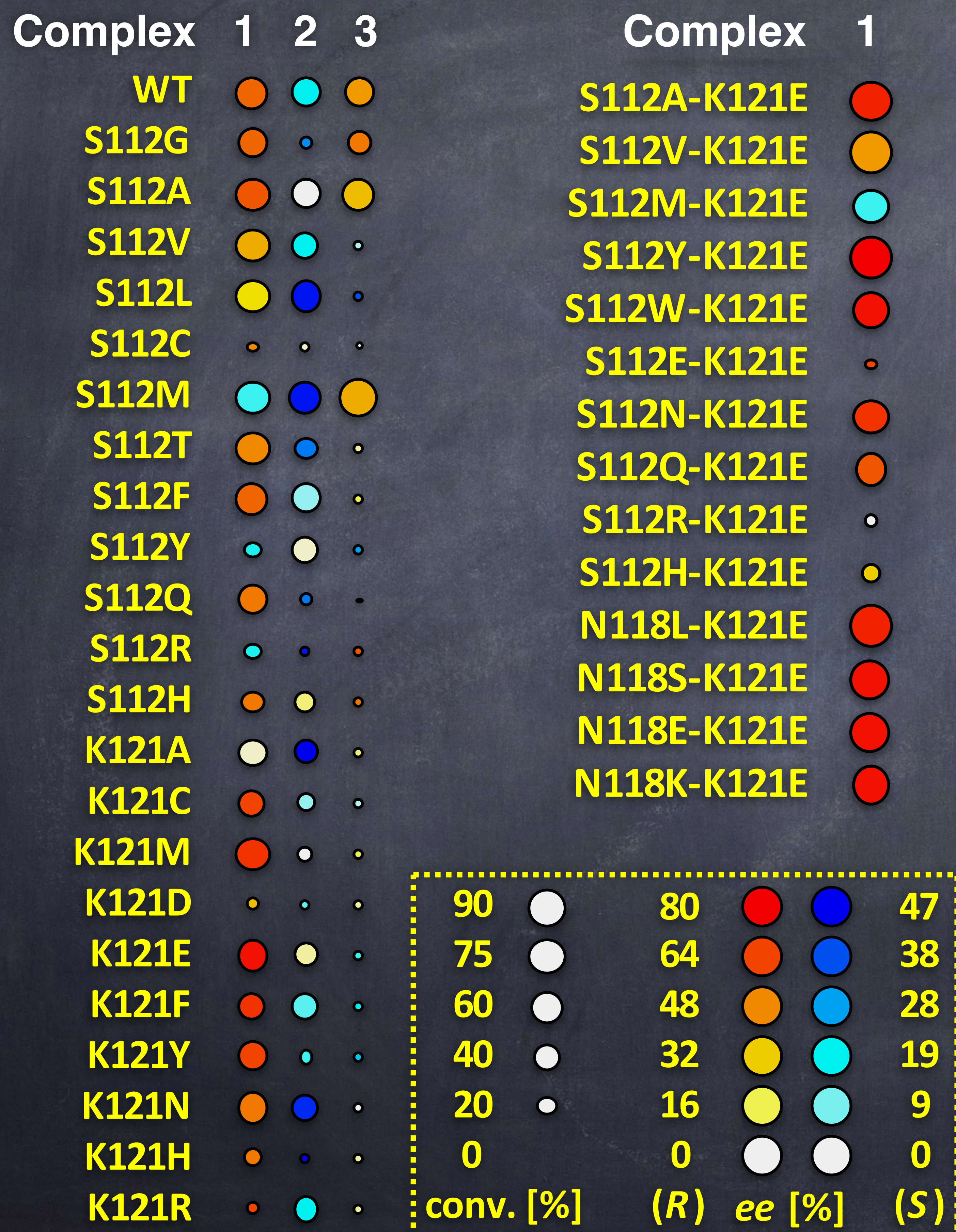
M. Dürrenberger, V. Köhler
with N. Turner (U. Manchester)

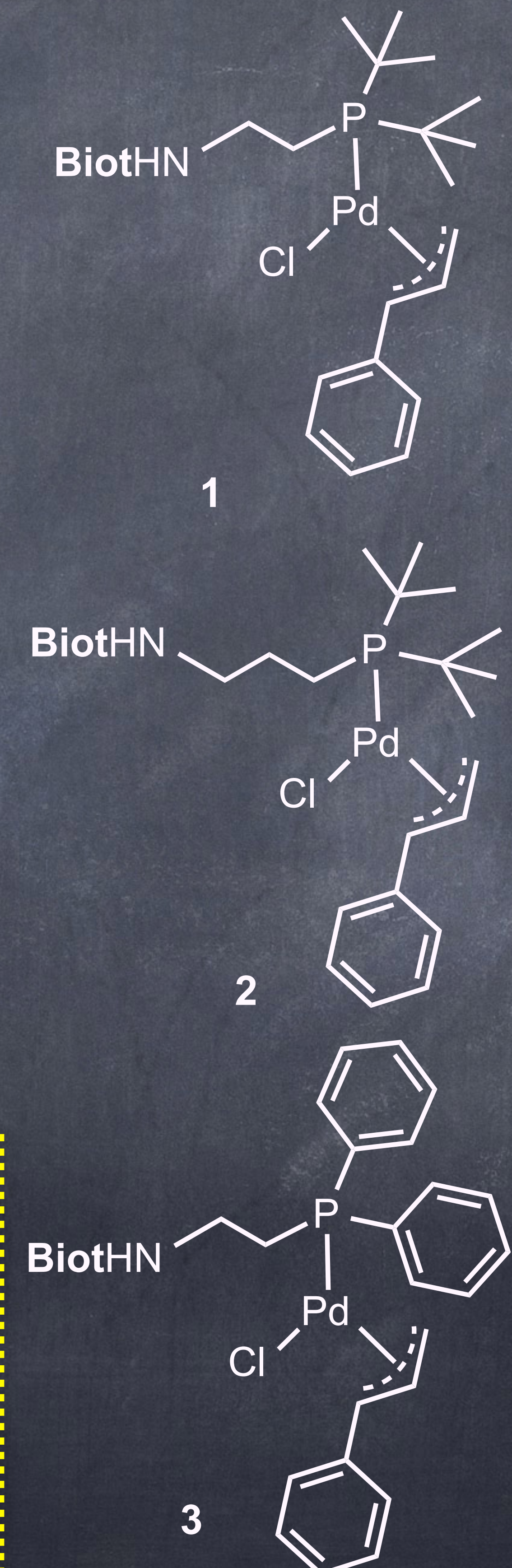
Can Artificial Metalloenzymes Complement Natural Enzymes?



Dibenzo-azepine

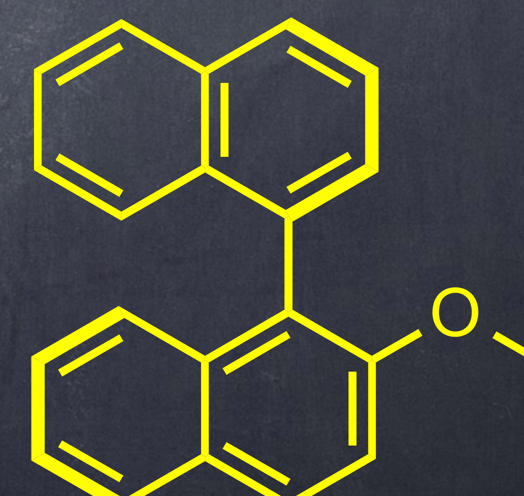
Artificial Suzukiase: Chemo-genetic Optimization





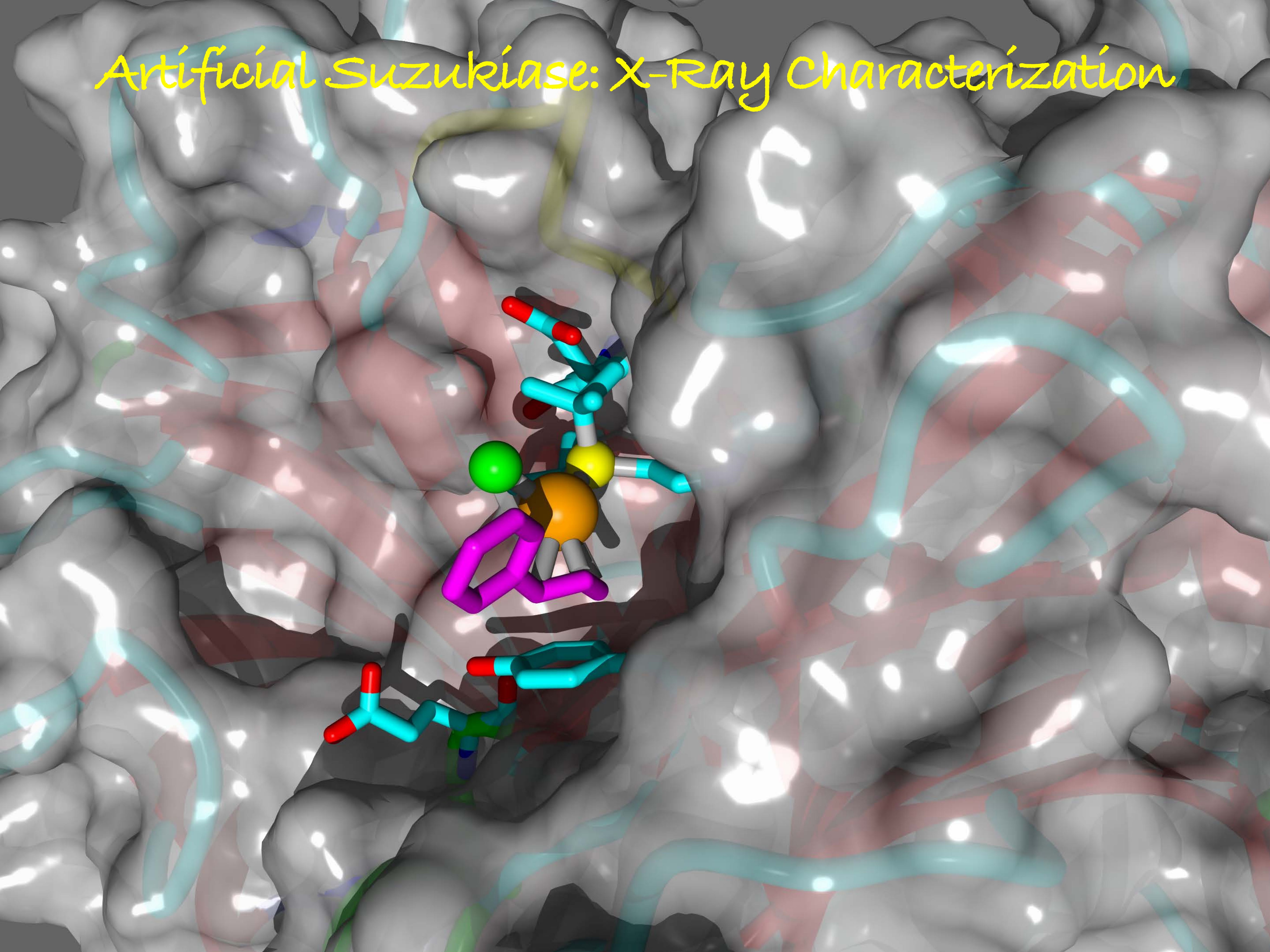
complex 1
no Sav *rac.*, 10 TON
WT 58 (*R*), 78
K121M 67, 59
K121E 76, 50
S112A 60, 58
S112Y 16 (*S*), 14
S112A-K121E 70, 80
N118L-K121E 72, 86
S112Y-K121E 90, 50

complex 2
WT 10 (*S*), 73
S112L 51, 45
S112M 44, 53
K121A 47, 32

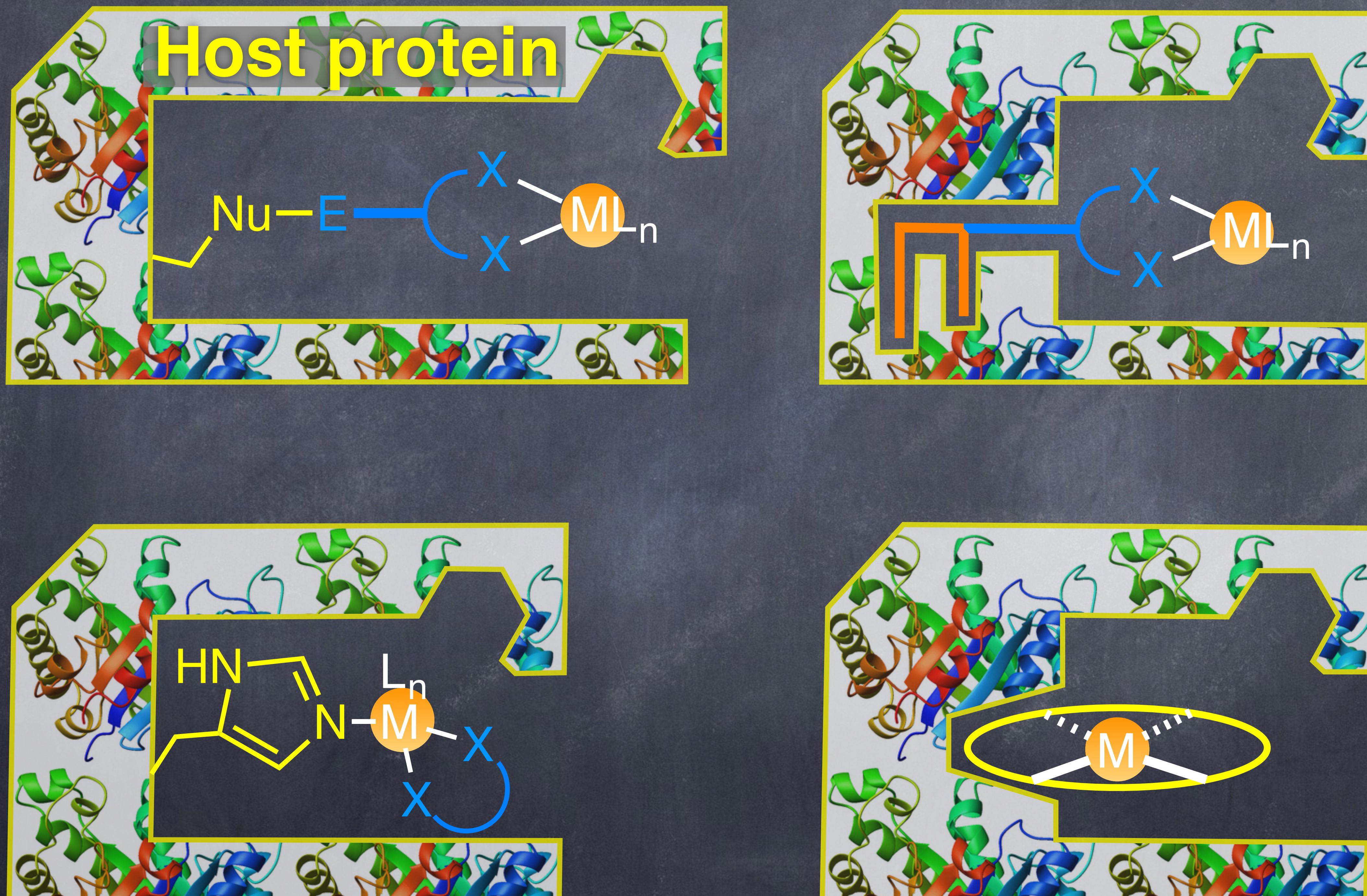


A. Chatterjee et al., *Chem. Sci.*, **2016**, *7*, 673.

Artificial Suzukiase: X-Ray Characterization



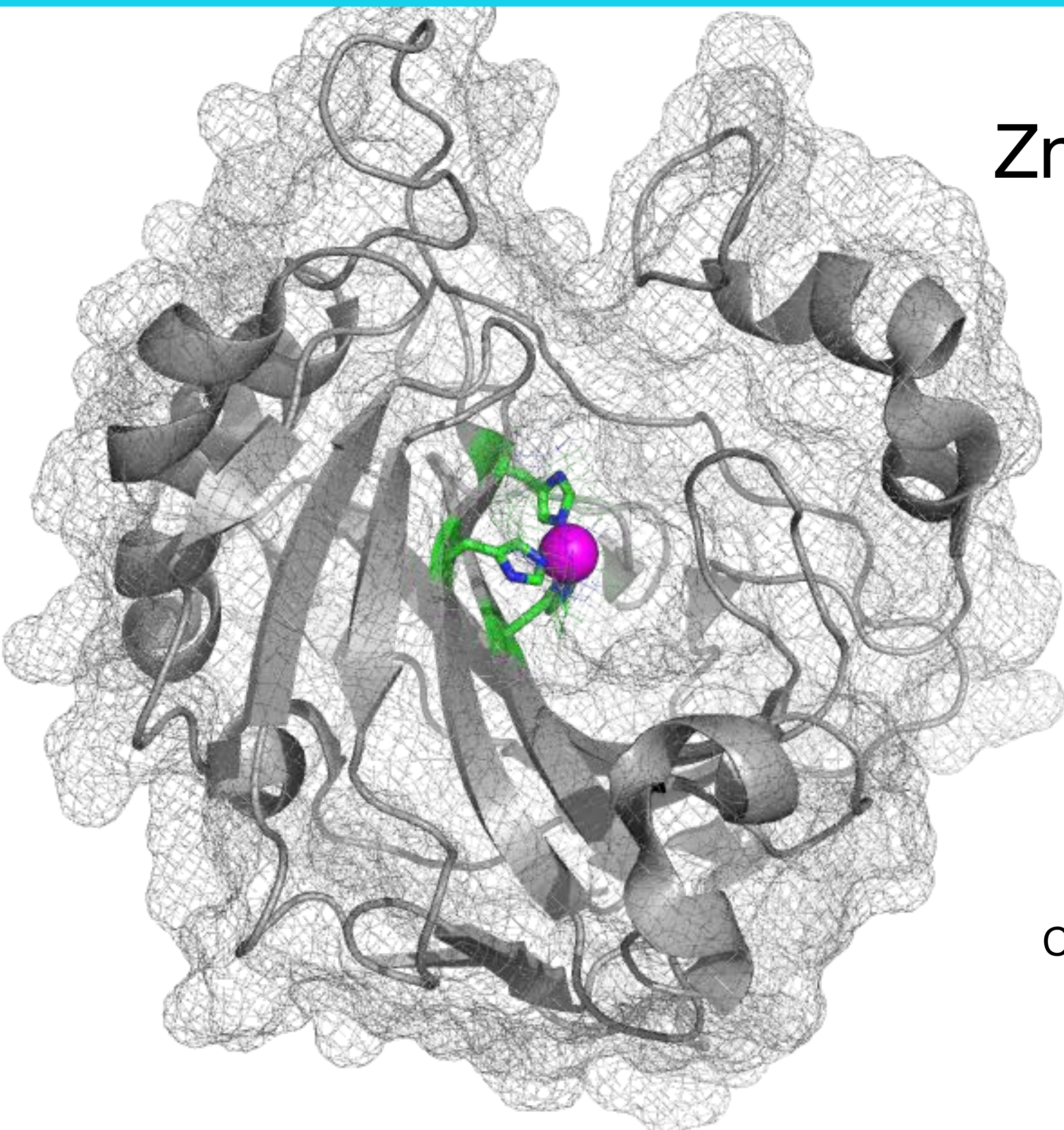
Anchoring of the Catalyst:
Four Alternatives to Ensure Localization



Dative anchoring

Lewis, Ward *et al.*, *Chem. Rev.* **2018,** *118*, 142.

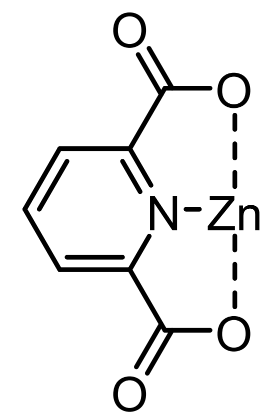
anchoring strategies – dative – metal coordination by aa-side chains

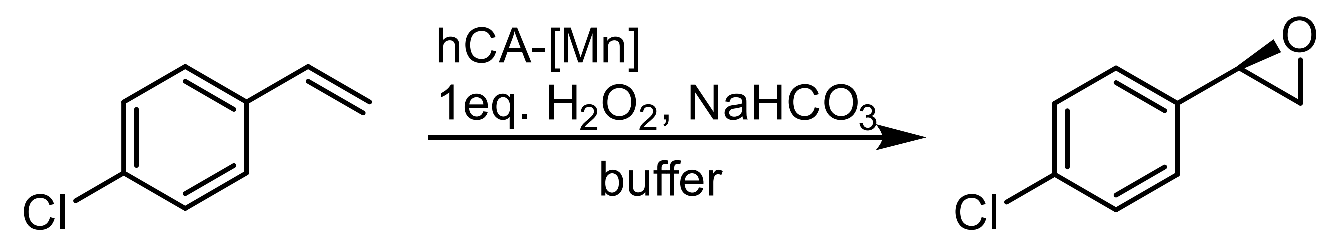


human carbonic anhydrase
PDB 1G1D

1. dialysis against chelating ligand
2. incubation with Mn^{2+}

Zn^{2+} ⇨ Mn^{2+}



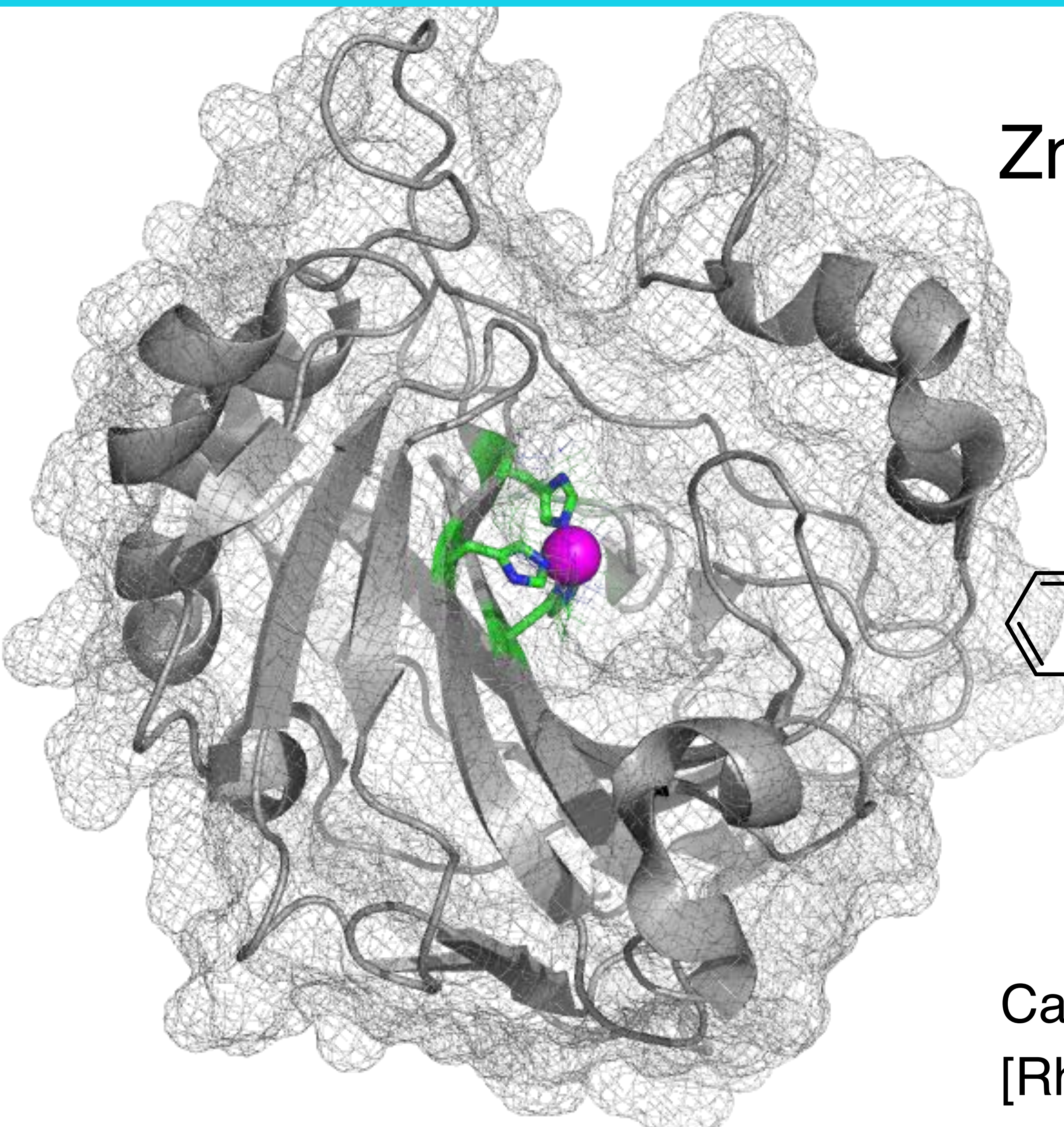


67% *ee*
TTON = 22

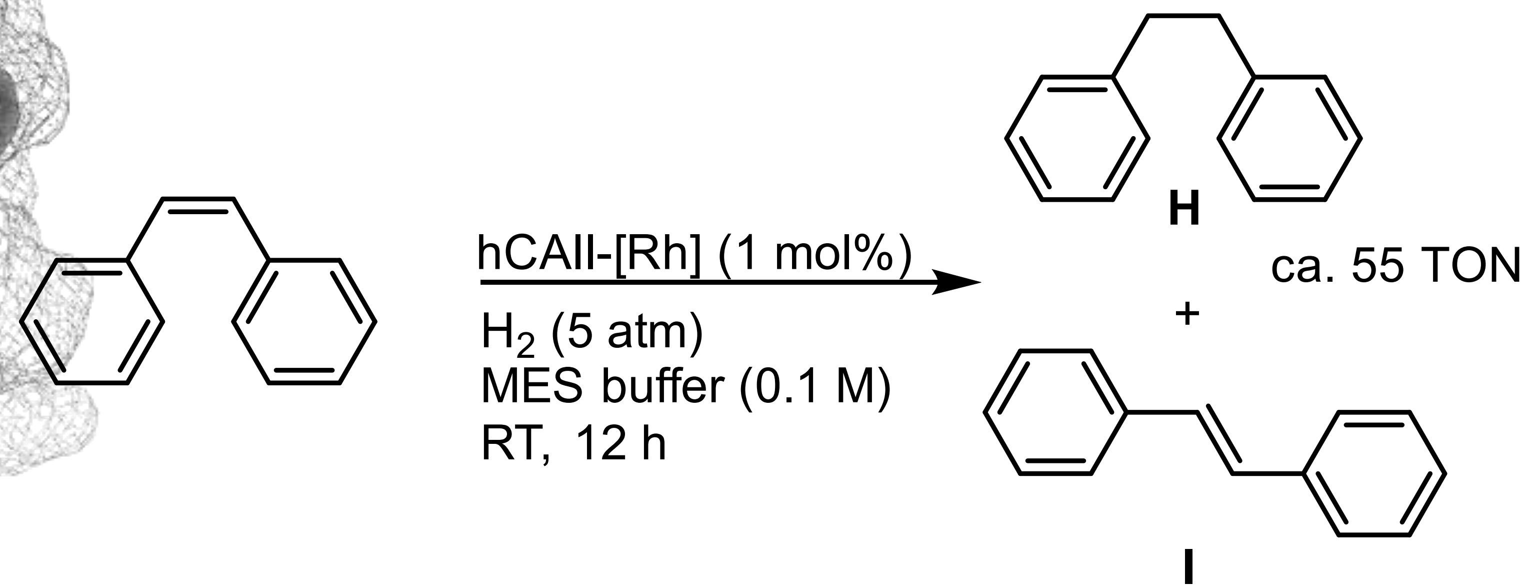
anchoring strategies – dative – metal coordination by aa-side chains

1. dialysis against chelating ligand
2. incubation with Rh+

Zn^{2+} ⇒ Rh^{+}



human carbonic anhydrase
PDB 1G1D

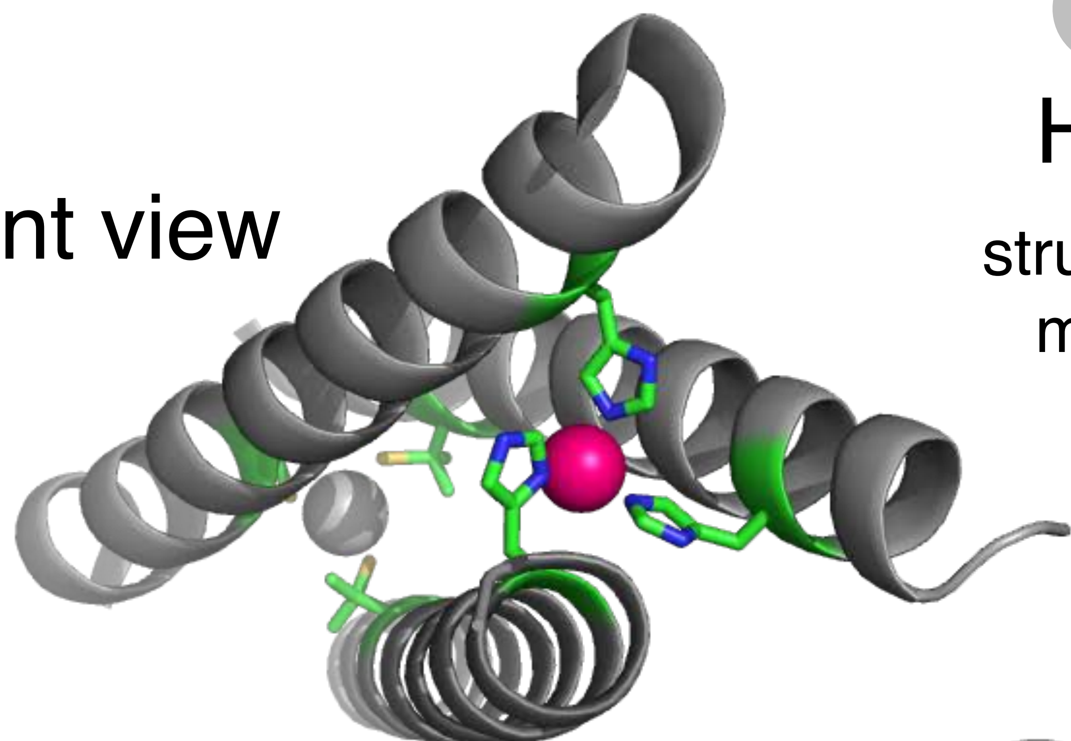


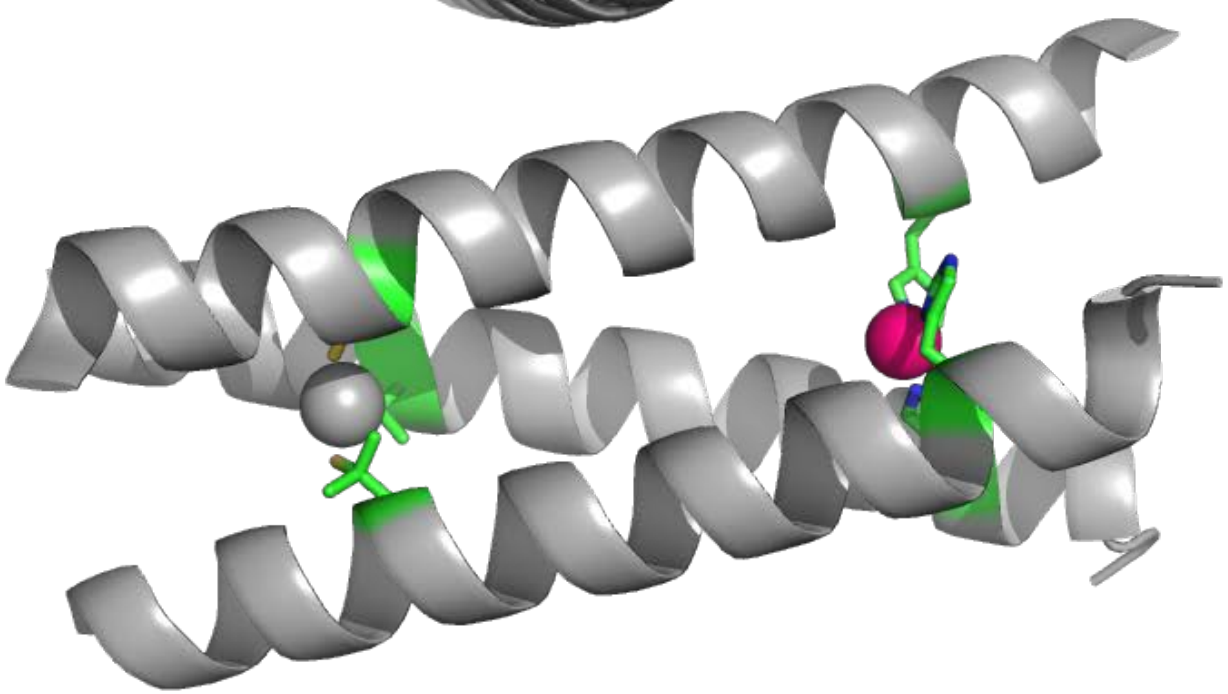
Catalyst	Rh / hCAII	H / I
$[Rh(cod)_2]BF_4$	-	6.3
hCAII-[Rh]	6.5	3.5
9*His-hCAII-[Rh]	1.8	20.6

R. Kazlauskas Chem. Eur. J. 2009, 15,

anchoring strategies – dative – in de novo design

front view





side view

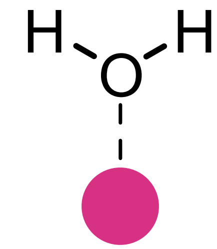
crystal structure of analogue
PDB 3PBJ

Hg^{2+}

structural
metal

Zn^{2+}

catalytic
center



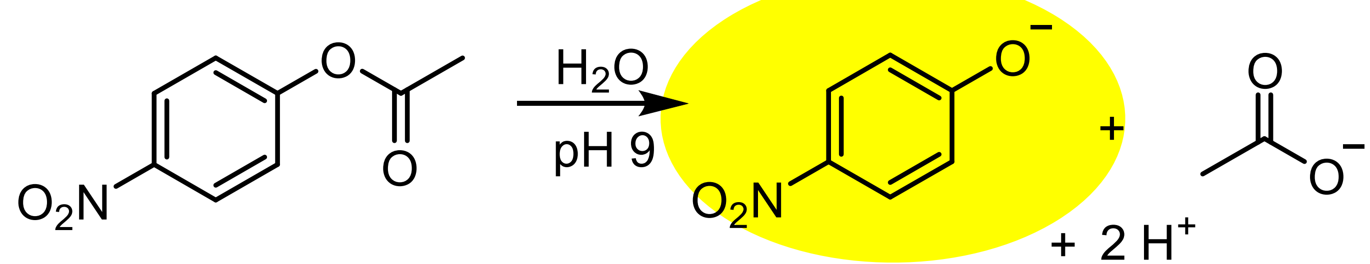
pKa 8.5 (mimic)
6.5 (CA)

a carbonic anhydrase (CA) mimic

$$CO_2 + H_2O \xrightarrow{\text{catalyst}} HCO_3^- + H^+$$

	CA (phys. pH)	mimic (pH 9.5)
k_{cat} (s^{-1})	8.2×10^5	$1.8 (\pm 0.4) \times 10^3$
K_M (mM)	89	10.0 ± 2.4

promiscuous activity:

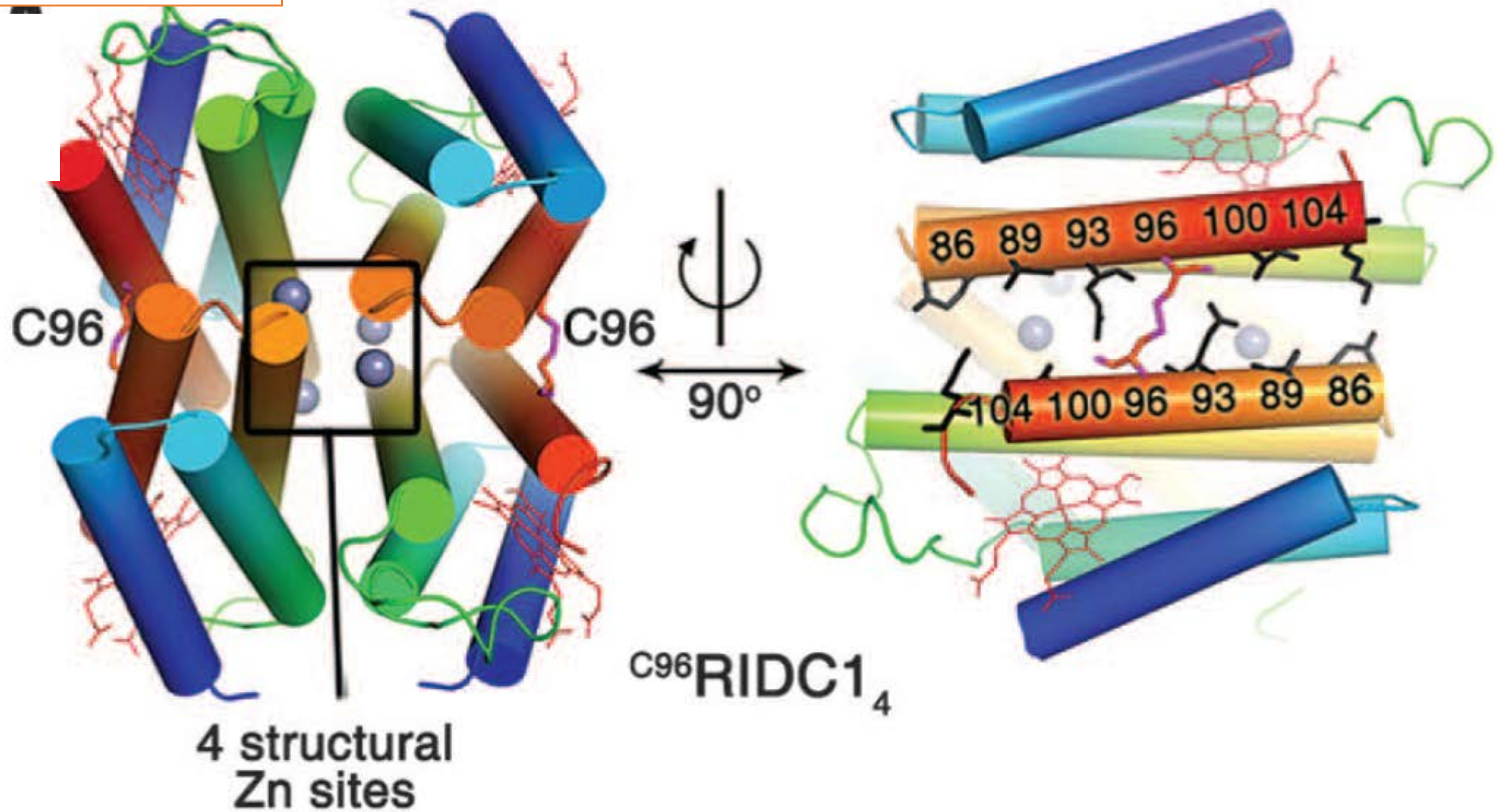


	CA	mimic
k_{cat} (s^{-1})	56 ± 10	$38 (\pm 10) \times 10^{-3}$
K_M (mM)	23.9	2.1 ± 0.6

V. L. Pecoraro, Nat. Chem. 2012, 4, 118.

'bio-available cofactor' – applying selection

1. starting point

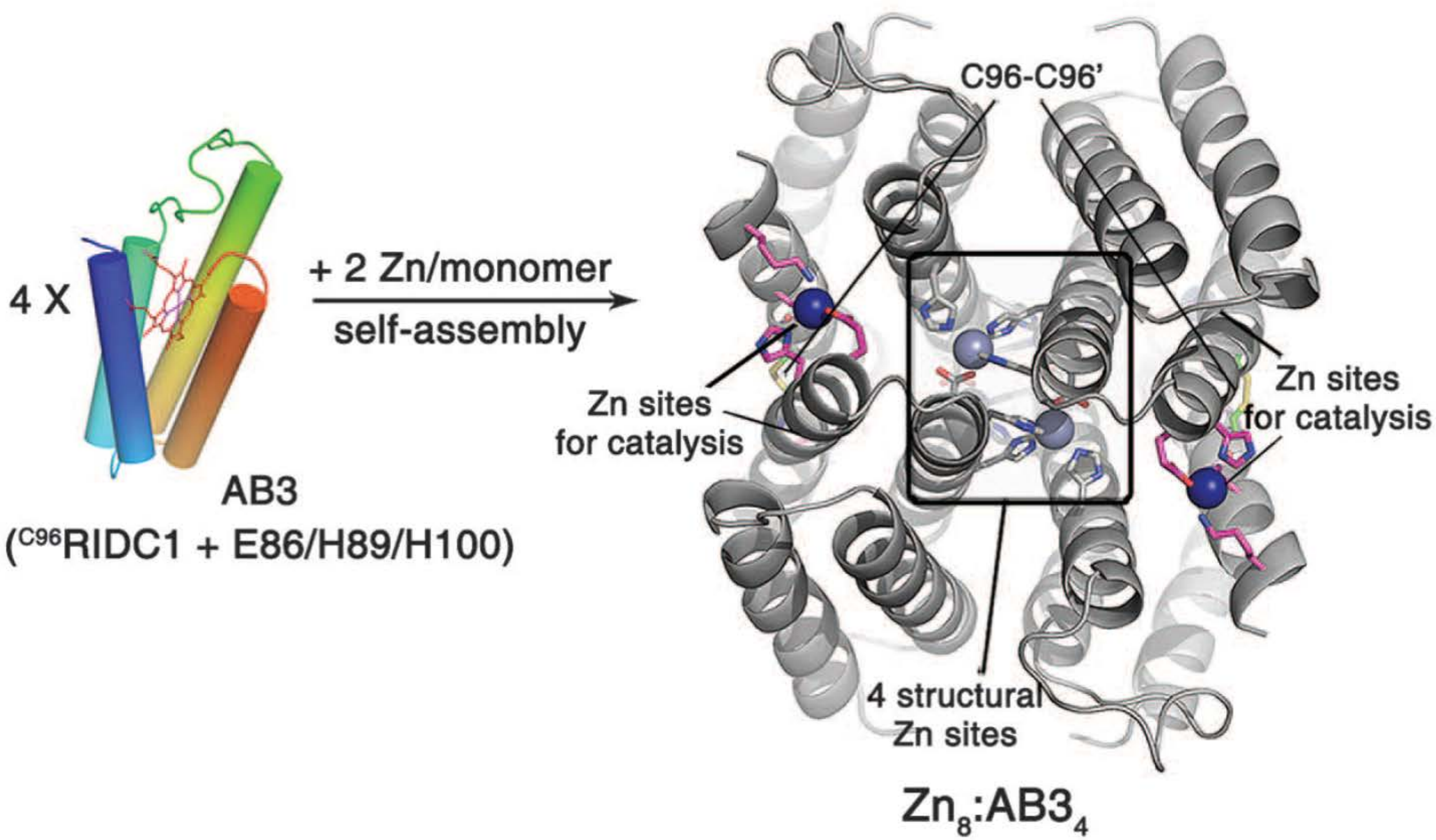


- cytochrome cb562 matures in the periplasm
- an engineered mutant of cytochrome cb_{562} self-assembles into a tetramer upon Zn^{2+} coordination
- the assembly is stabilized by S-S bridges

F. A. Tezcan, J. Am. Chem. Soc. 2007, 129, 1337

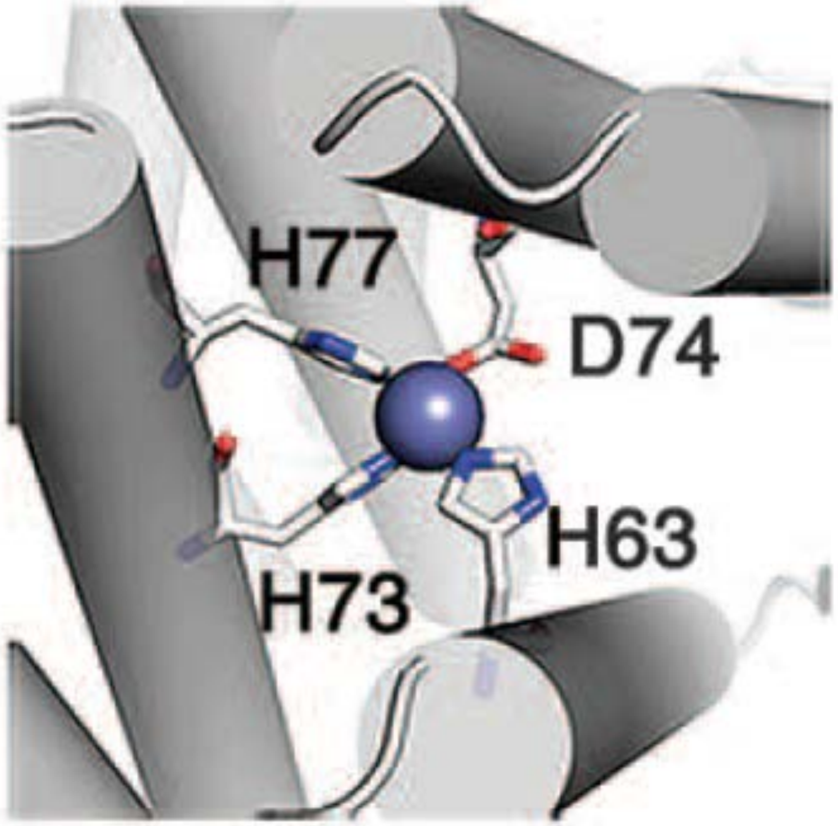
‘bio-available cofactor’ – applying selection

2. installing additional Zn-binding sites



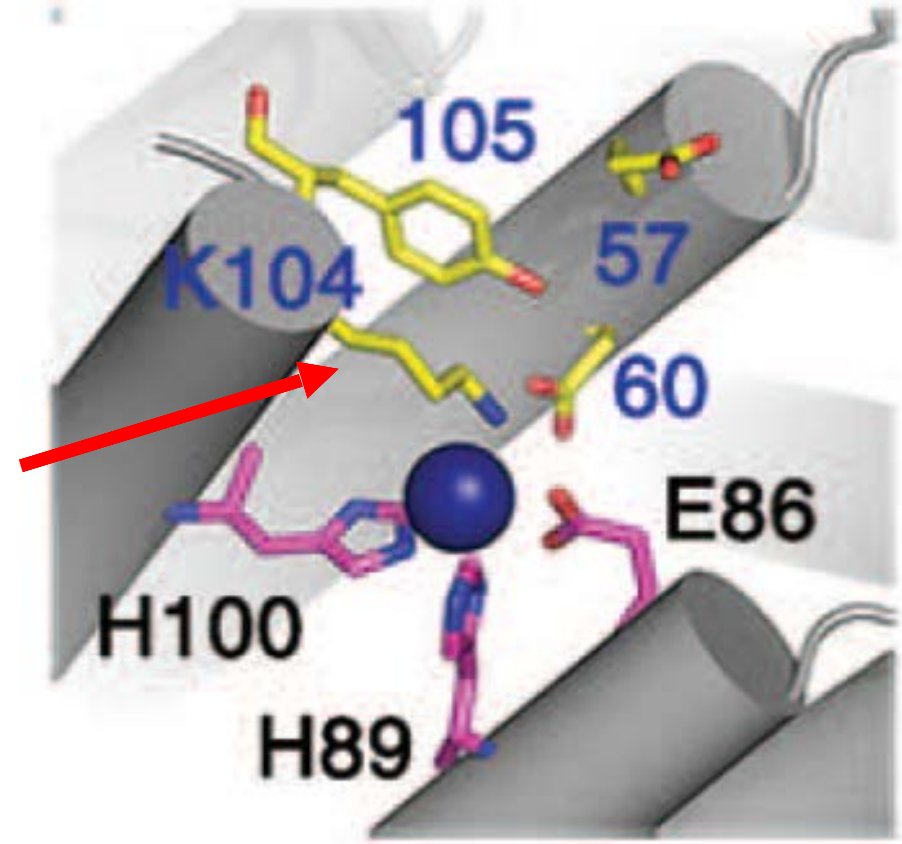
F. A. Tezcan, Science 2014, 346, 1525

‘bio-available cofactor’ – applying selection



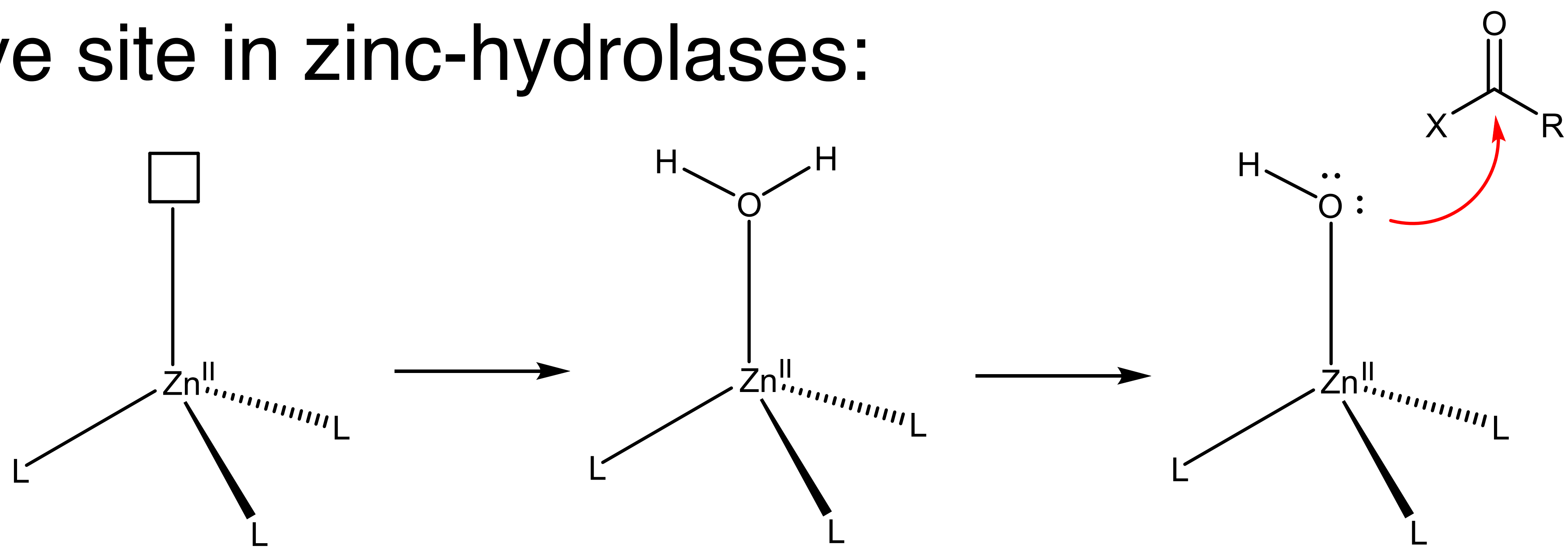
structural Zn site

unexpected coordination of K104 ɹ mutate to A104



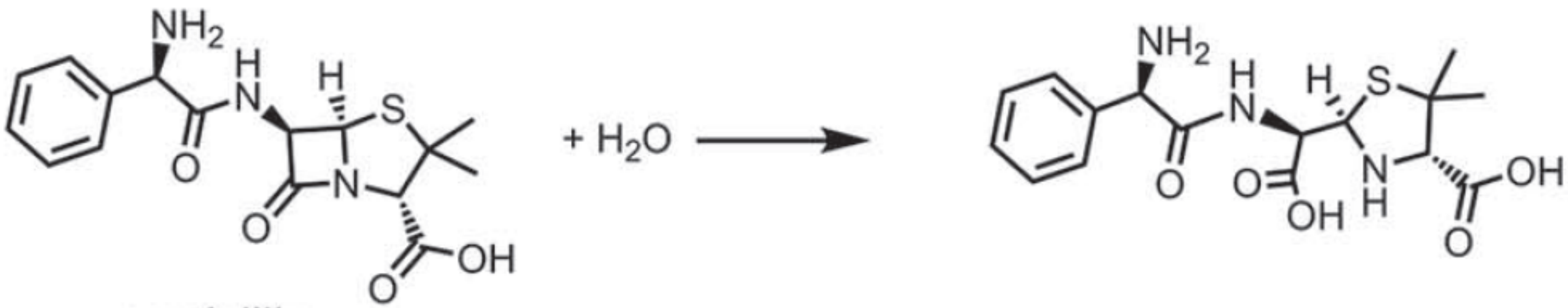
Zn site for catalysis

active site in zinc-hydrolases:

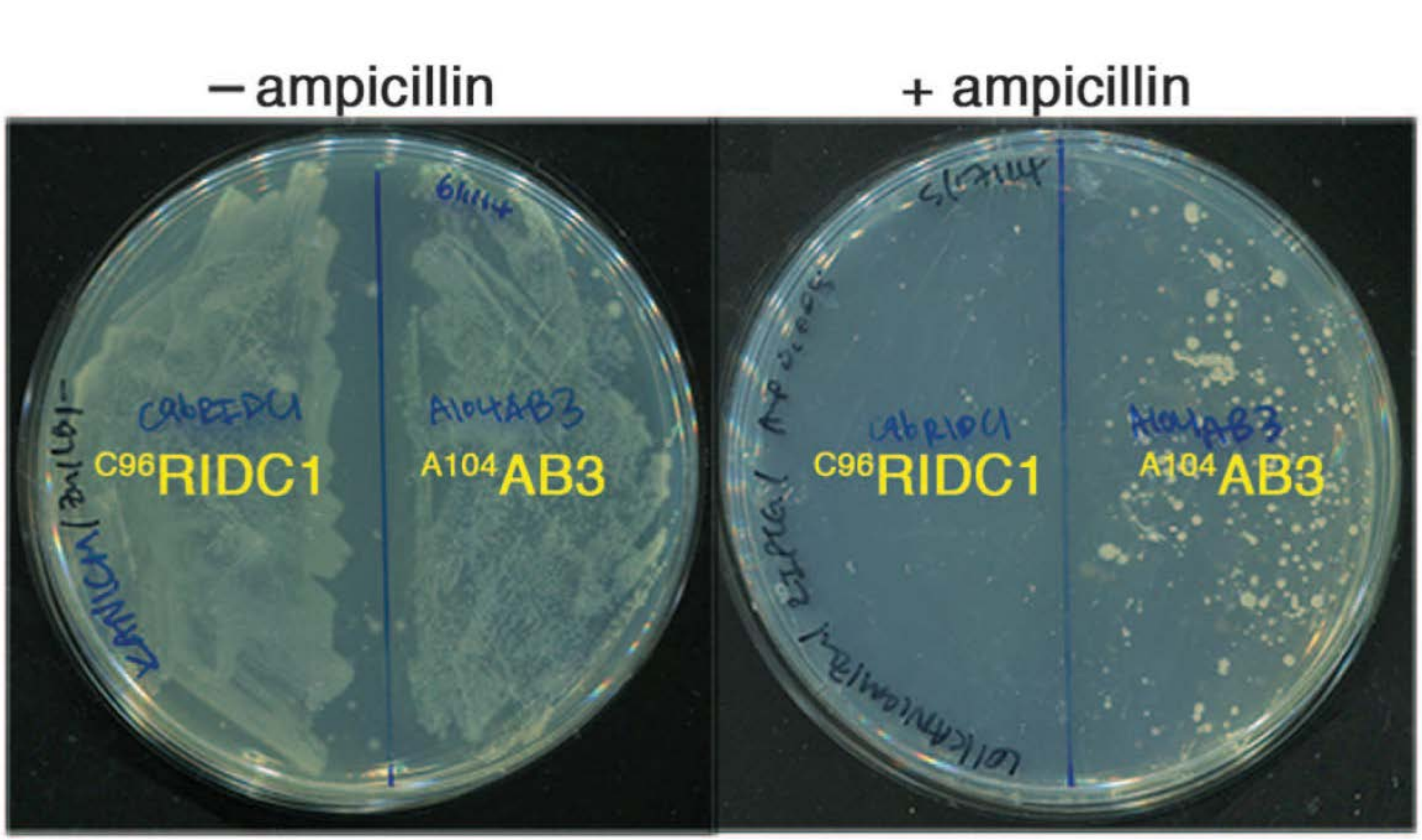


‘bio-available cofactor’ – applying selection

in vivo

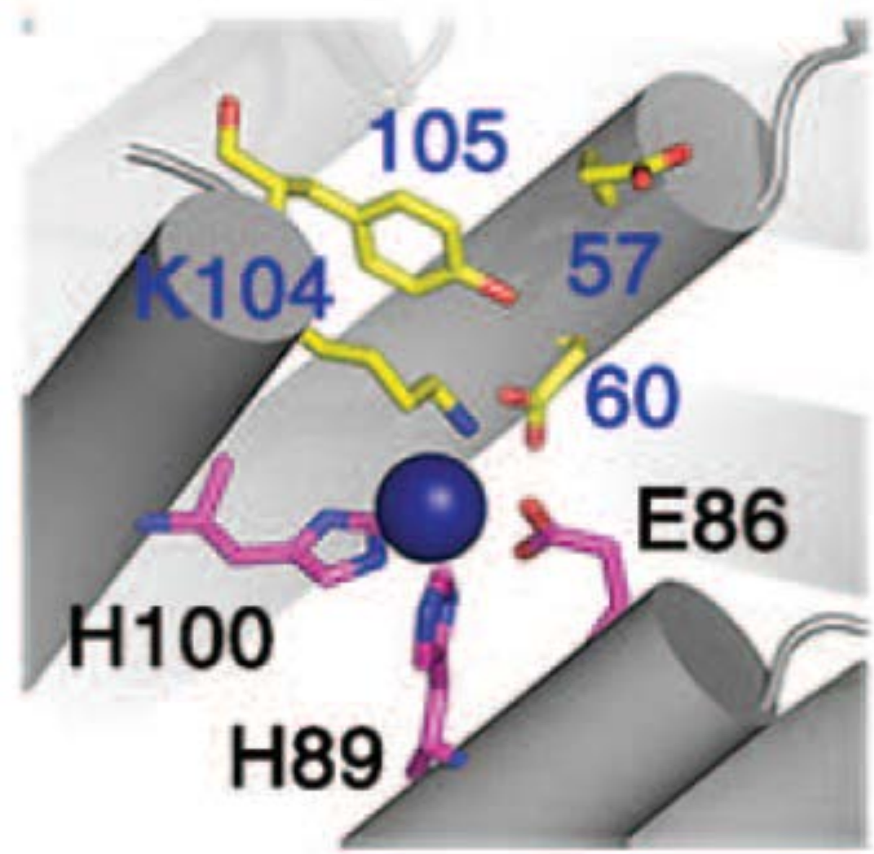


ampicillin



‘bio-available cofactor’ – applying selection

in vivo



Zn site for catalysis

randomisation of 4 active site residues Glu57, Asp60, Lys104, Tyr105

weak correlation with survival frequency, but the two mutants with highest survival frequency were also the most active in vitro A104-G57 and A104-T105

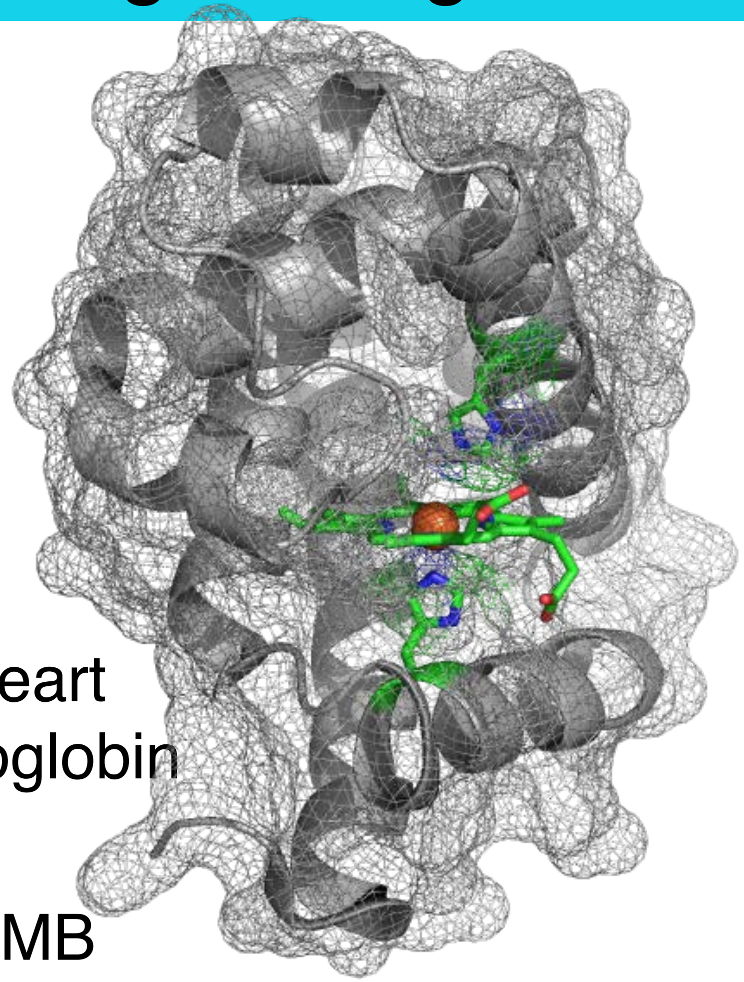
mutant A104-G57 displays Michaelis-Menten kinetics

Anchoring of the Catalyst:
Four Alternatives to Ensure Localization

Metal/cofactor substitution

Lewis, Ward *et al.*, *Chem. Rev.* **2018,** *118*, 142.

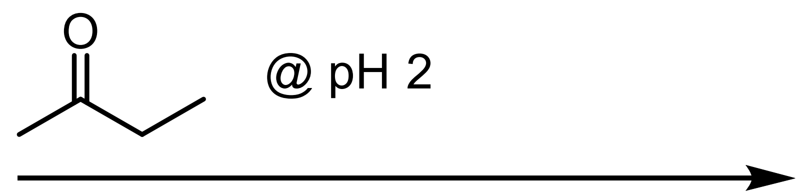
anchoring strategies – metal/cofactor substitution



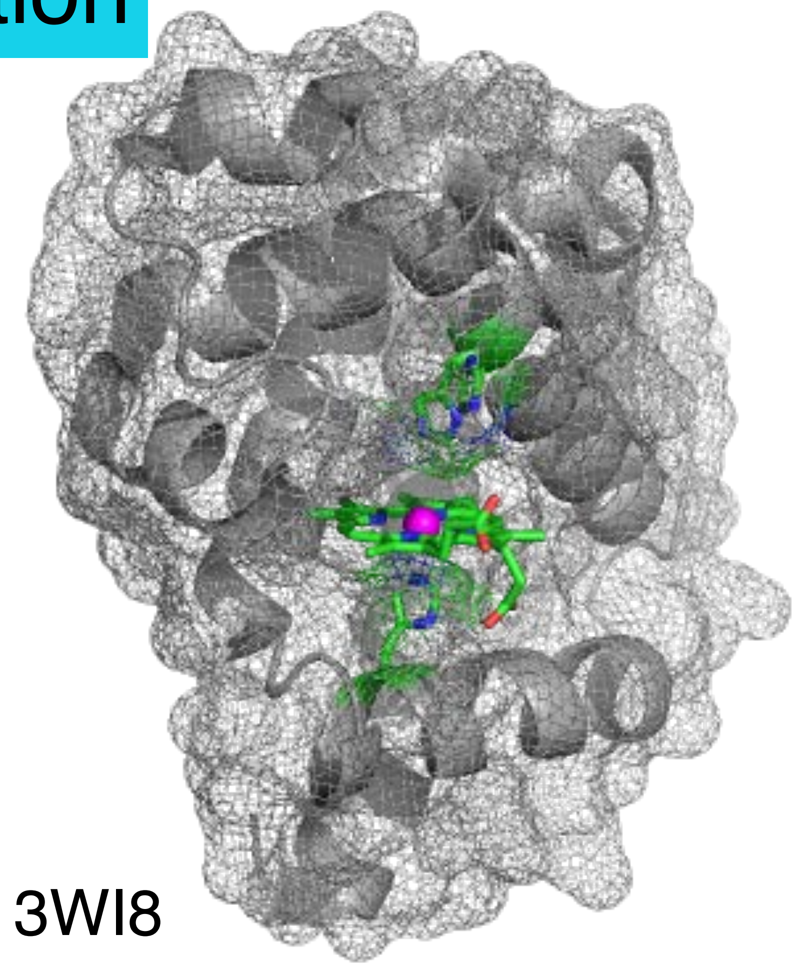
horse heart metmyoglobin

PDB 1YMB

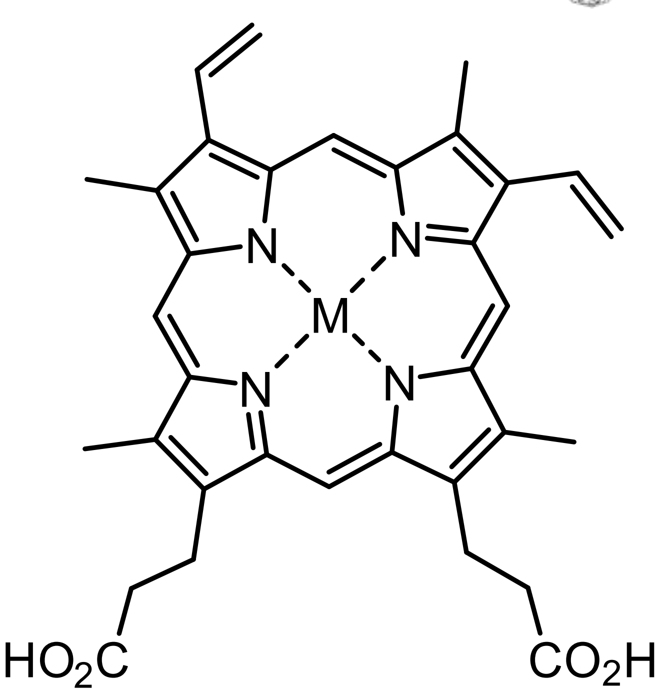
1. removal of heme B
extraction with



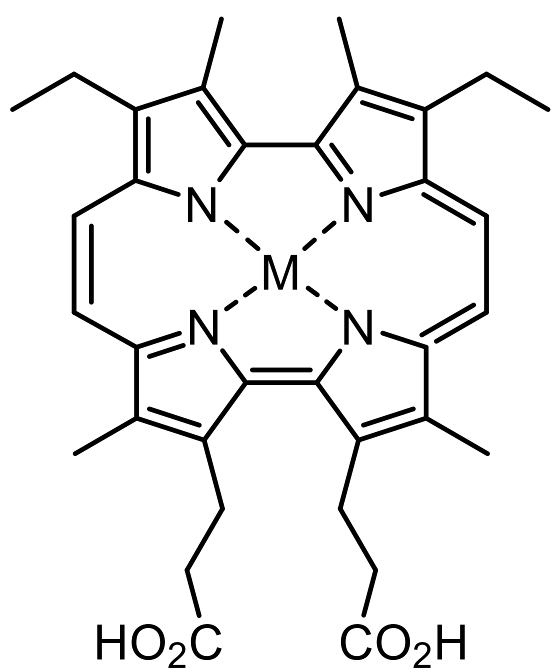
2. reconstitute
with non-natural cofactor
in buffer + some organic solvent



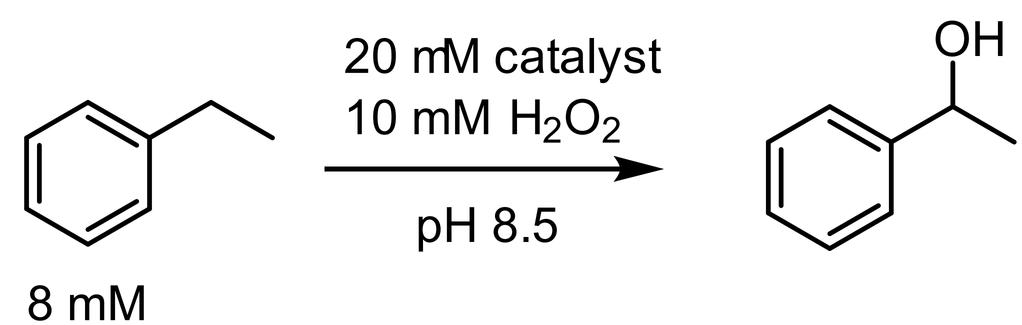
PDB 3WI8



heme B (M = Fe)
Mn-porphyrine (M = Mn)



Mn-porphycene (M = Mn)
Fe-porphycene (M = Fe)



cofactor of protein	TTON	TOF (h^{-1})
heme B	0	0
Mn-porphyrine	0	0
Fe-porphycene	0	0
Mn-porphycene	13	33

T. Hayashi, J. Am. Chem. Soc. 2013, 135, 17282.

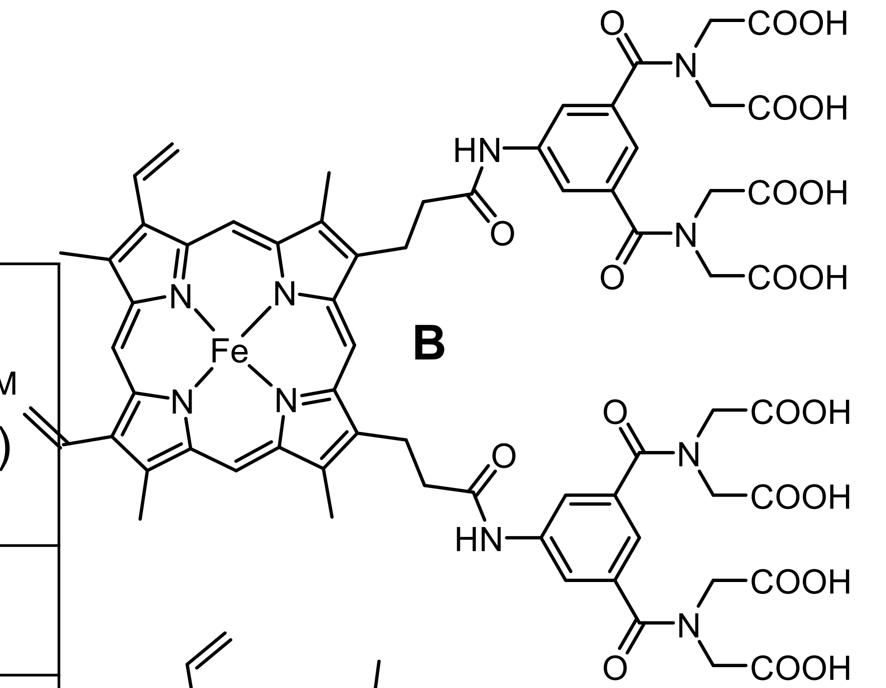
improving catalytic efficiency through effective molarity?

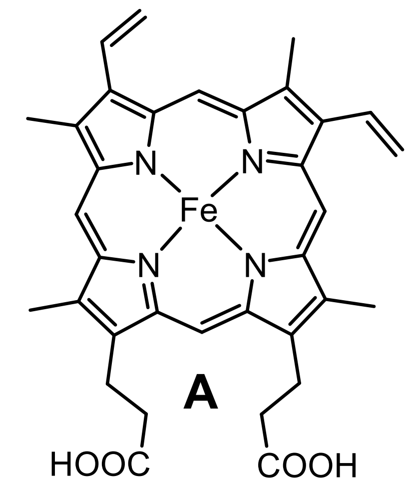
purified protein

0.025-20 mM

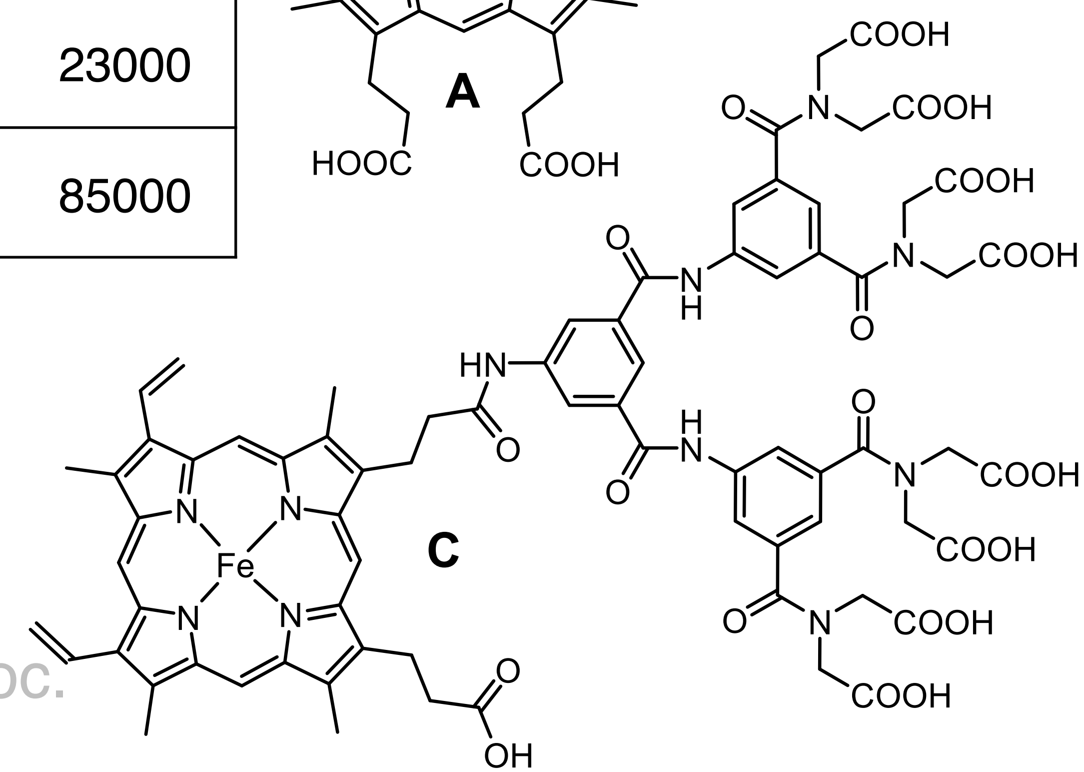
H_2O_2 (4-100 mM)
Buffer (20-100 mM)
pH 6, 7
20-25 °C

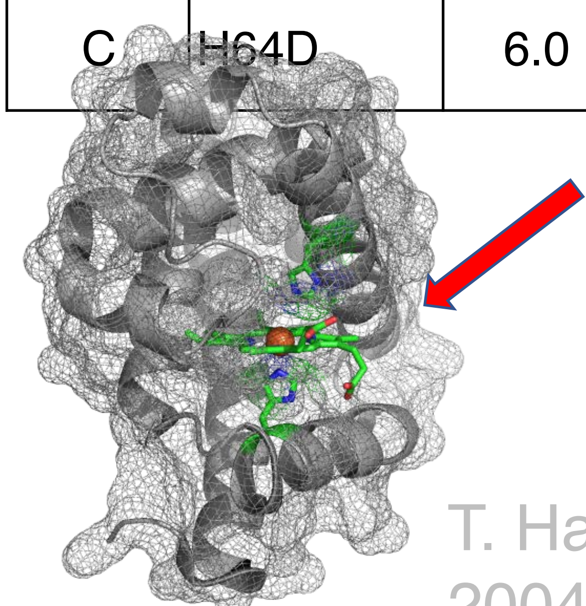
Cof.	Protein	pH	T (°C)	[Cof] (μM)	[H_2O_2] (mM)	K_M (mM $^{-1}$)	k_{cat} (s $^{-1}$)	k_{cat}/K_M ($s^{-1}M^{-1}$)
A	WT	7.0	20	1	9.7	32	0.36	11
B	WT	7.0	20	1	9.7	7.4	1.1	149
A	WT	6.0	25	2	100	54	2.8	53
B	WT	6.0	25	2	100	3.4	6.2	1800
A	H64D	6.0	25	2	15	1.8	9.0	5100
B	H64D	6.0	25	2	15	0.052	1.2	23000
C	H64D	6.0	25	4	100	0.29	24	85000





A



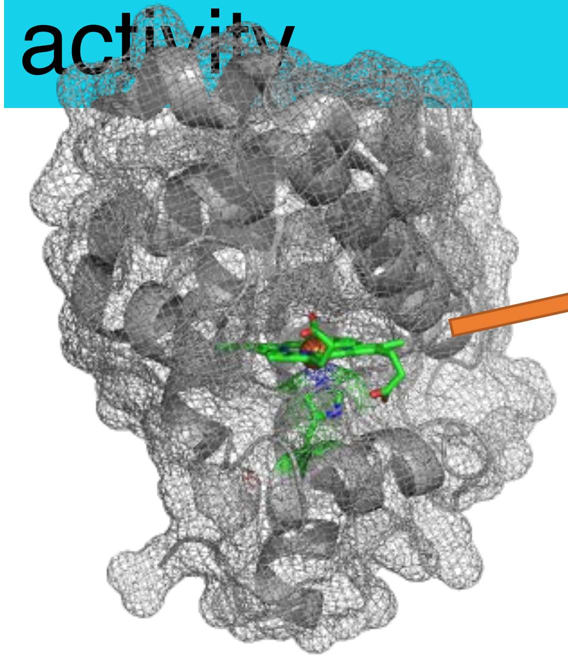


A ↓ B/C

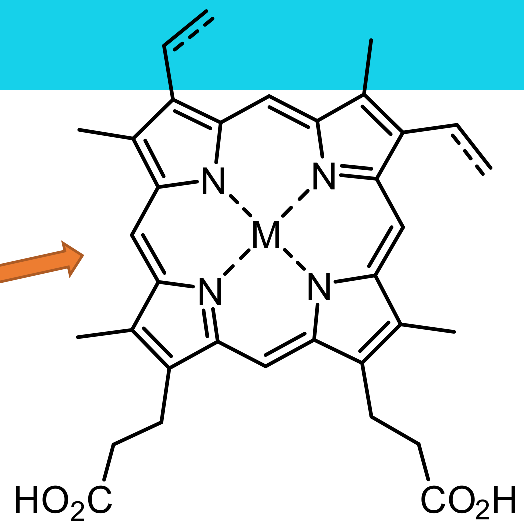
T. Hayashi, Y. Watanabe, J. Am. Chem. Soc. 2004, 126, 436.

chemogenetic optimization – screening for activity

purified protein

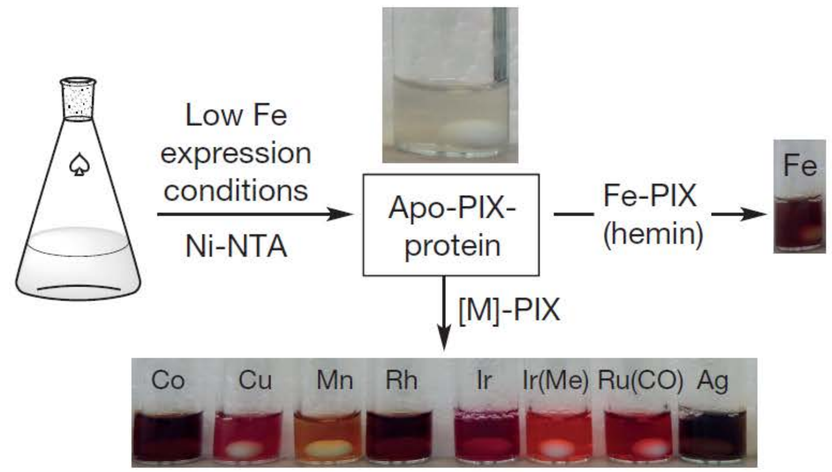


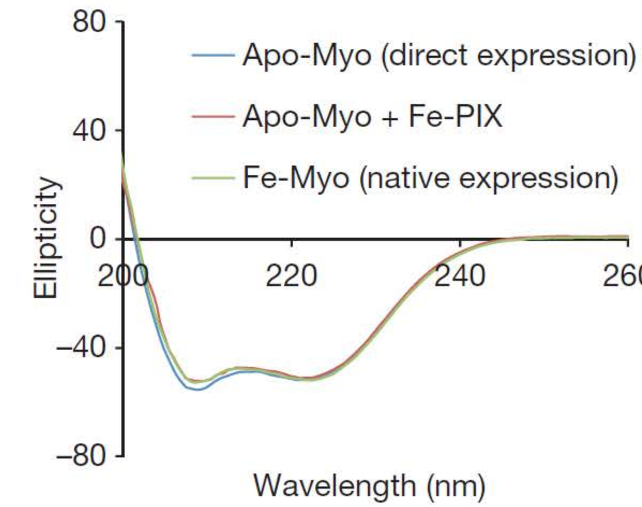
myoglobin

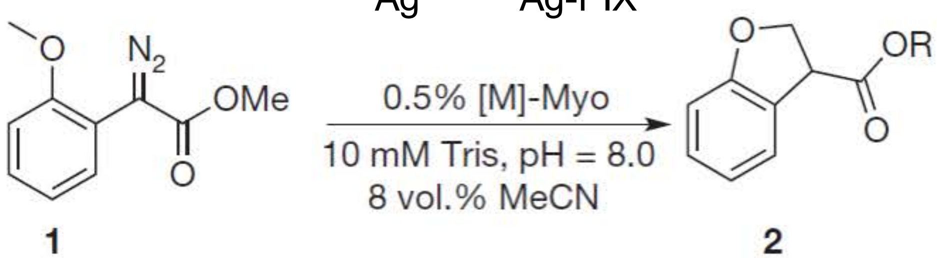


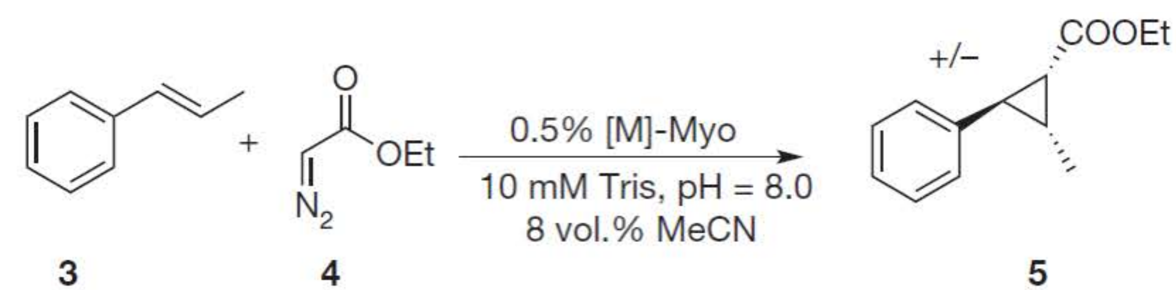
M = Fe^{III}Cl → Fe(Cl)-PIX
Co^{III}Cl Co(Cl)-PIX
Cu^{II} Cu-PIX
Mn^{III}Cl Mn(Cl)-PIX
Rh$^{?}$ Rh-PIX
Ir^{III}Cl Ir(Cl)-PIX
Ir^{III}Me Ir(Me)-PIX
Ru^{II}CO Ru(CO)-PIX
Ag$^{?}$ Ag-PIX

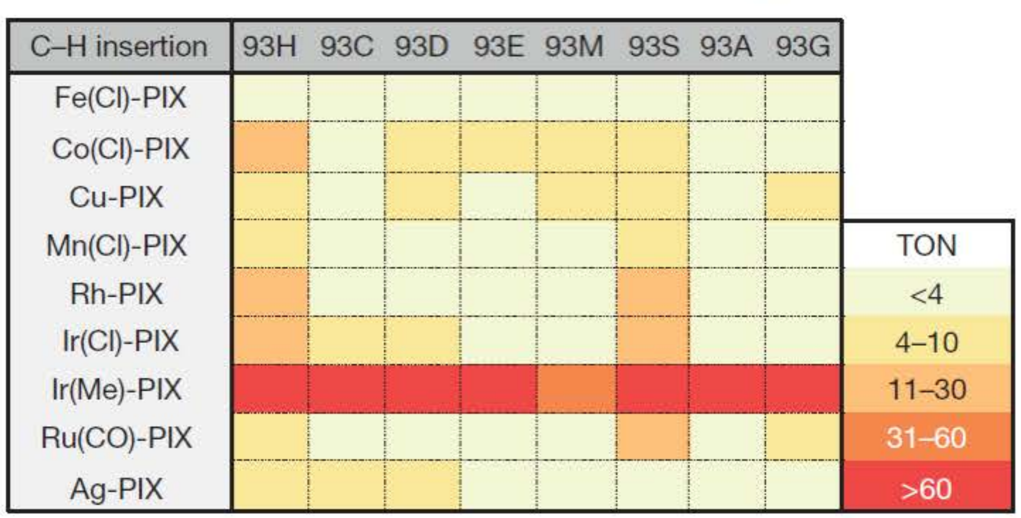
cofactor-free expression

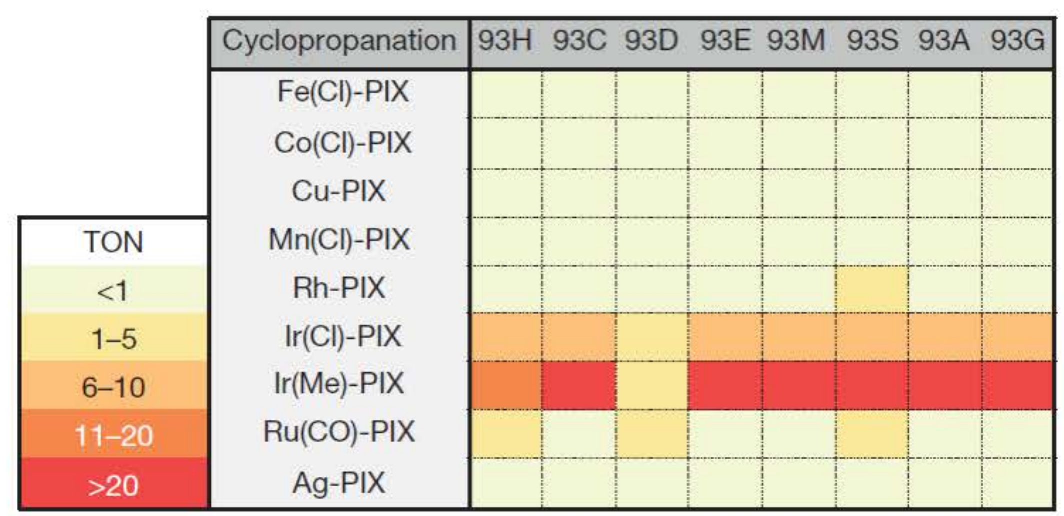








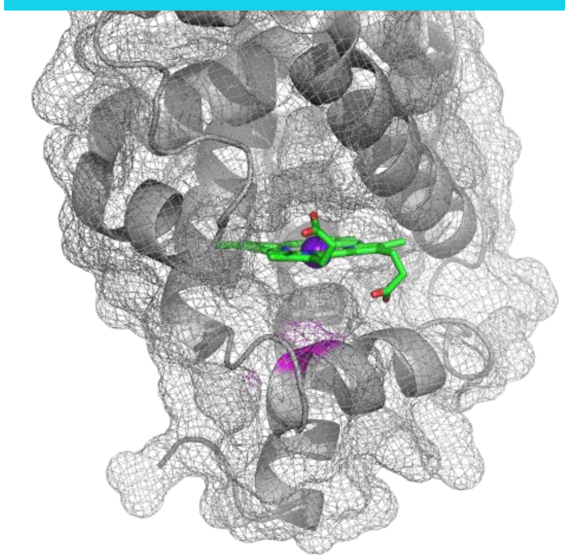


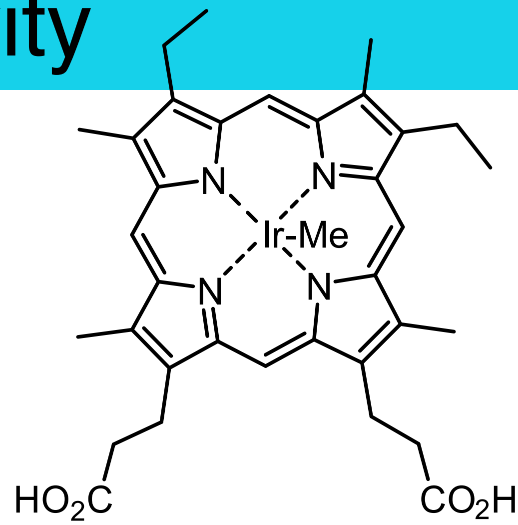


J. F. Hartwig, Nature 2016, 534, 534.

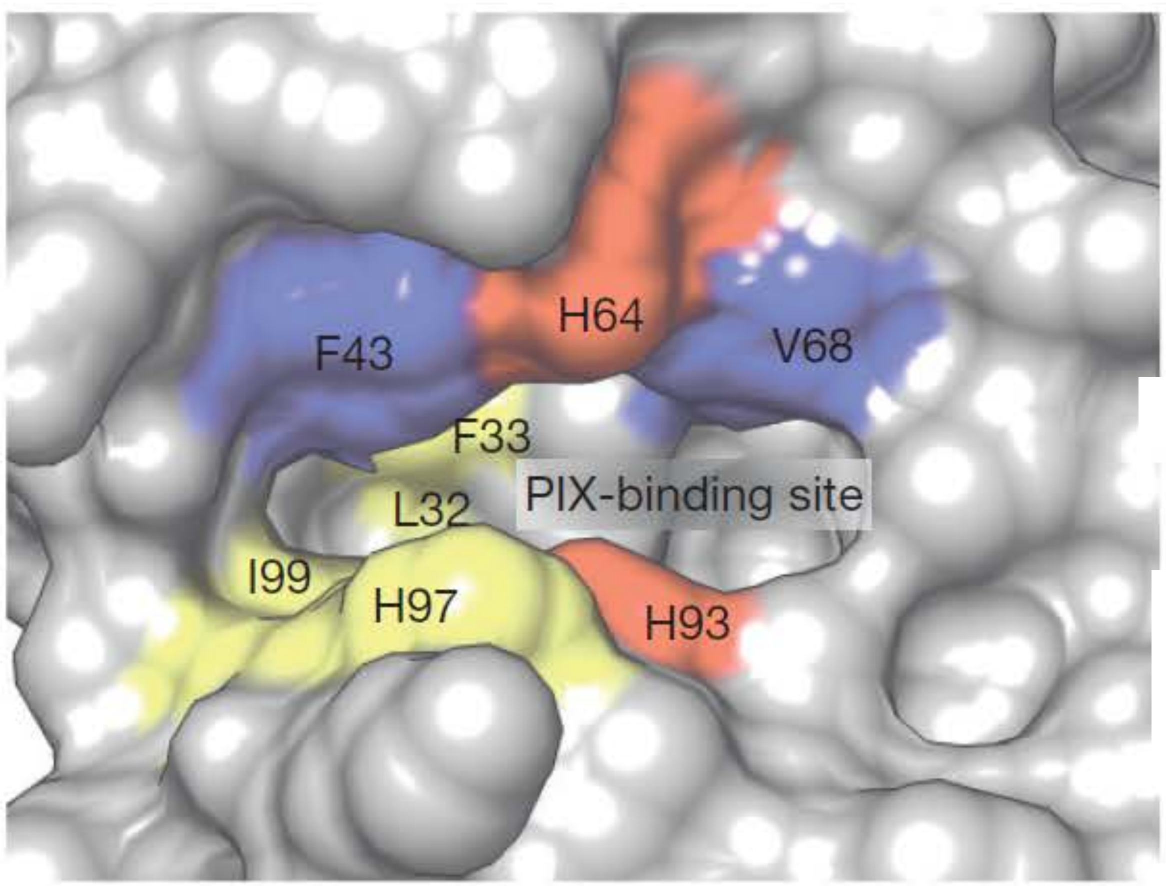
chemogenetic optimization – improving enantioselectivity

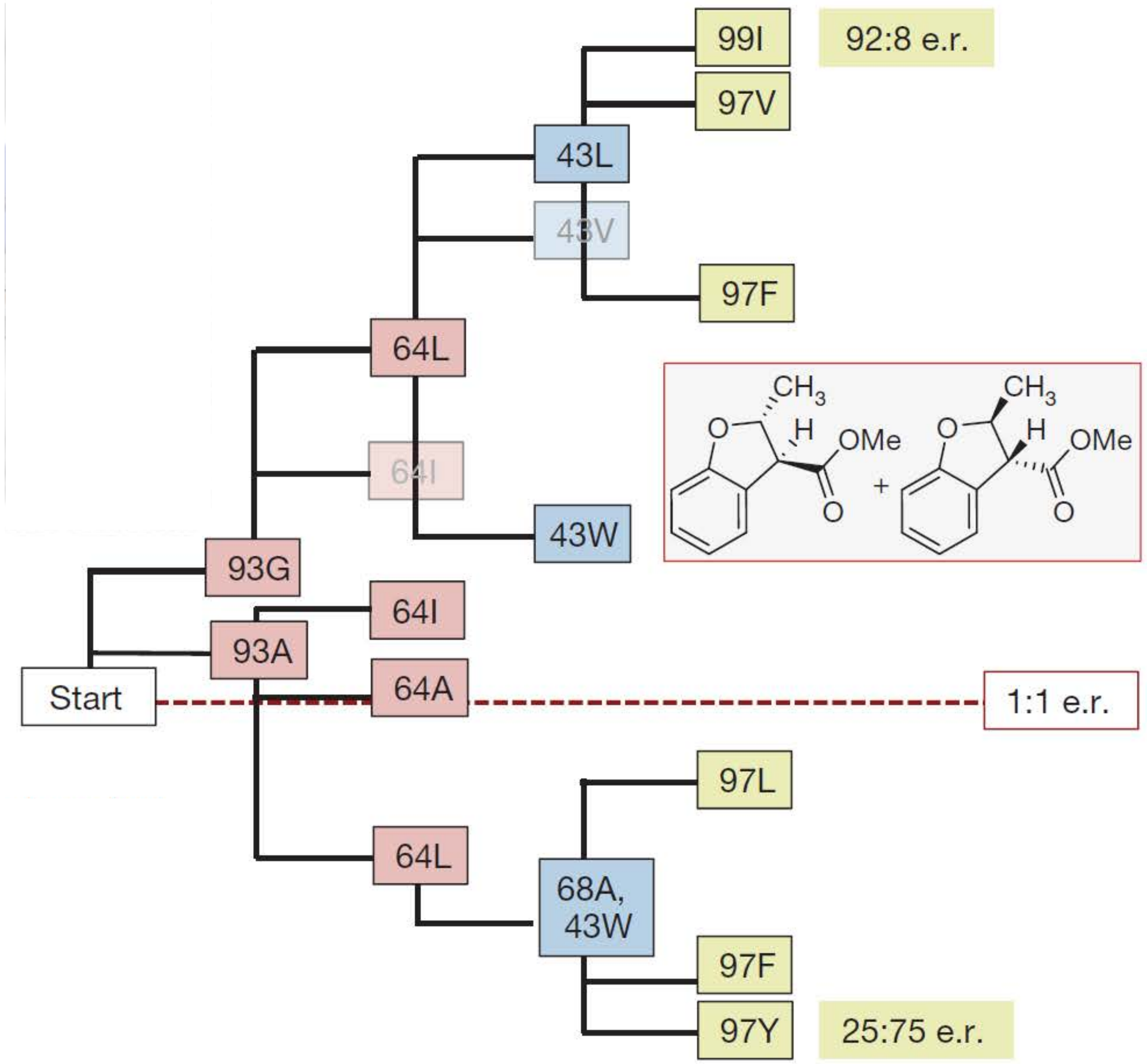
purified protein





ca. 500 mutants screened
degenerate primers

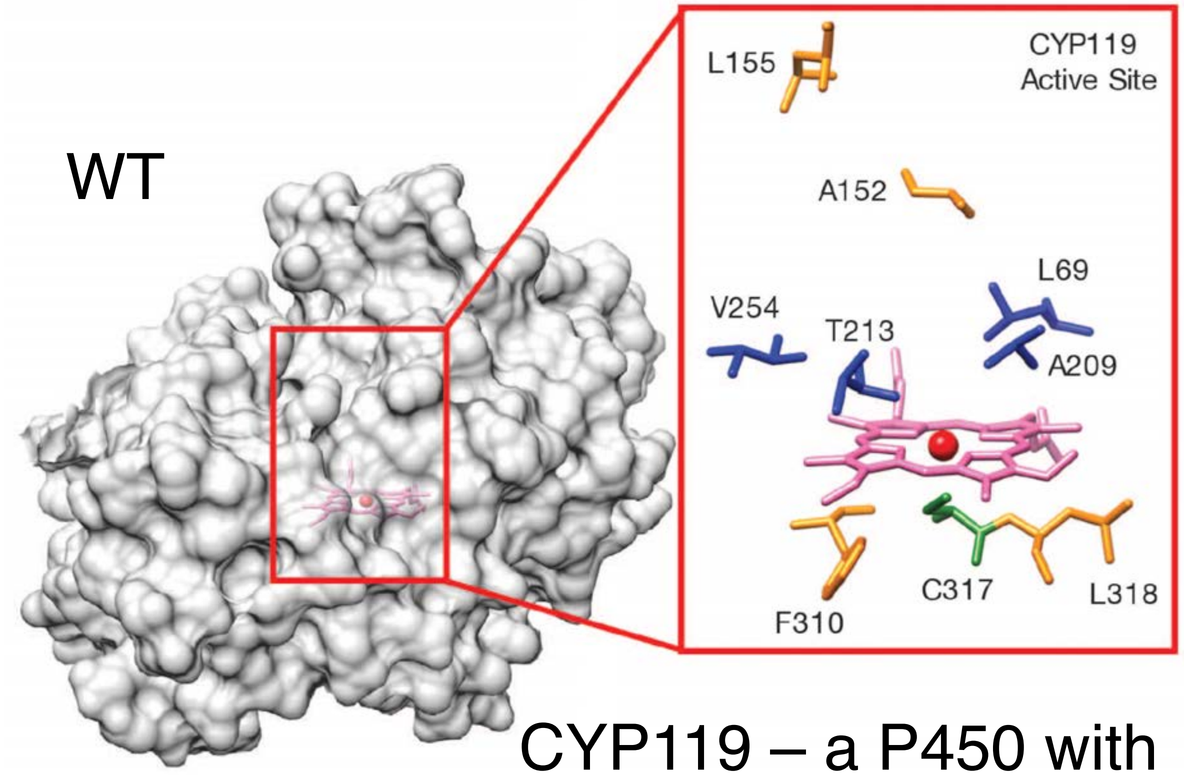




J. F. Hartwig, Nature 2016, 534, 534.

chemogenetic optimization – change scaffold - repeat

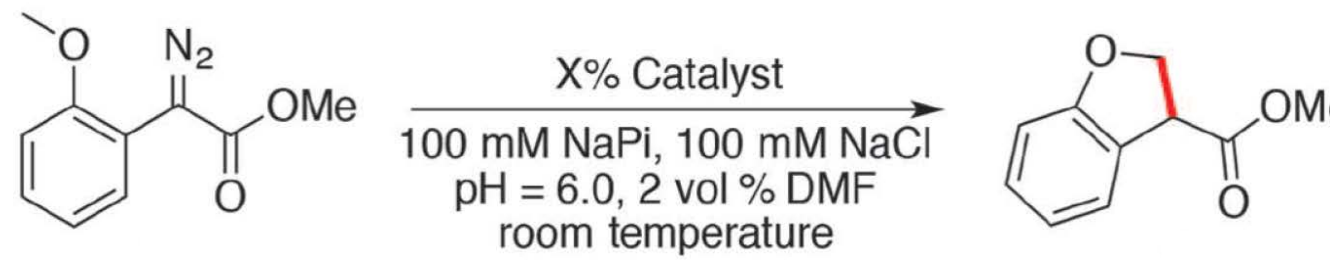
purified protein

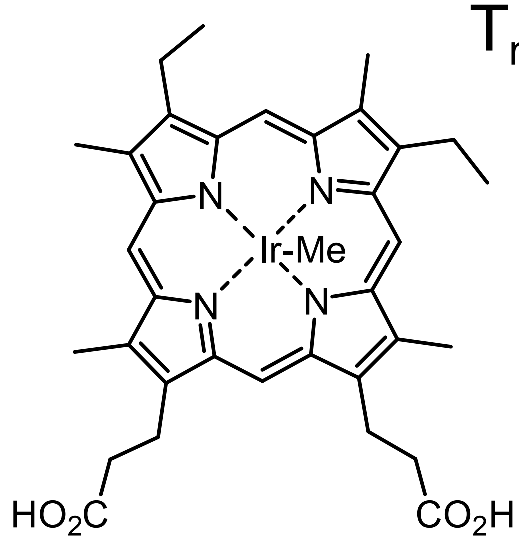


optimization
- exchange C317G to create space for the cofactor
- exchange active site residues for other lipophilic residues

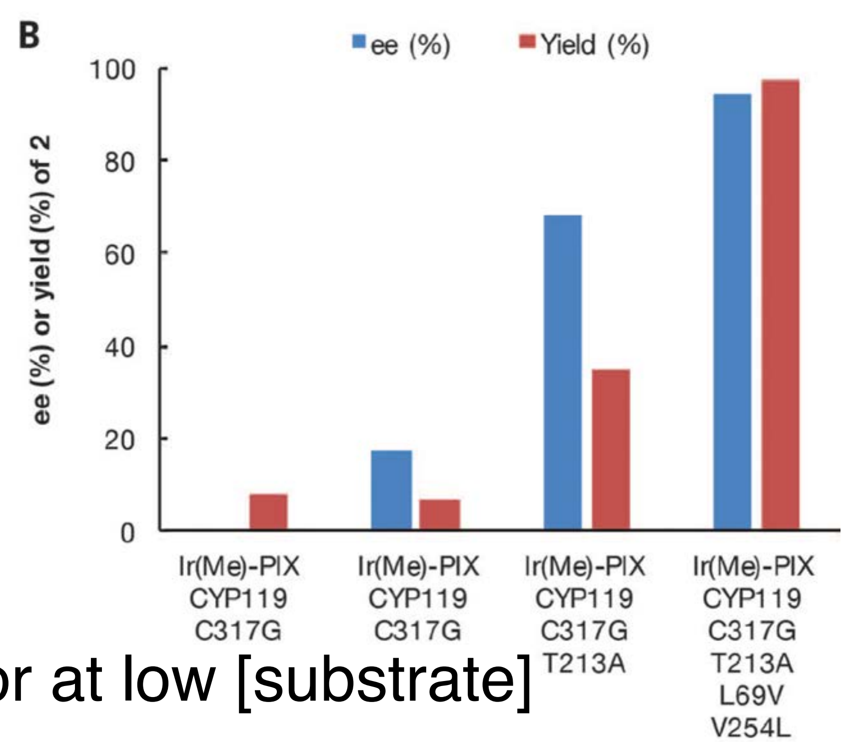
- 24 double mutants tested
- 2 additional mutations introduced

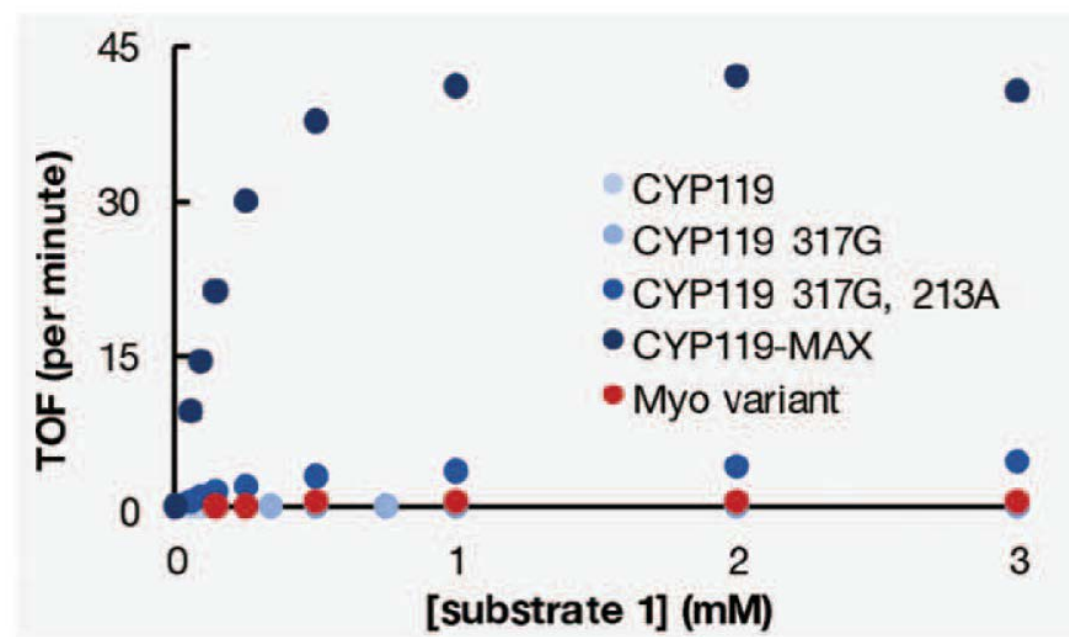
CYP119 – a P450 with high thermostability
T_m = 69°C





TON up to 35 000

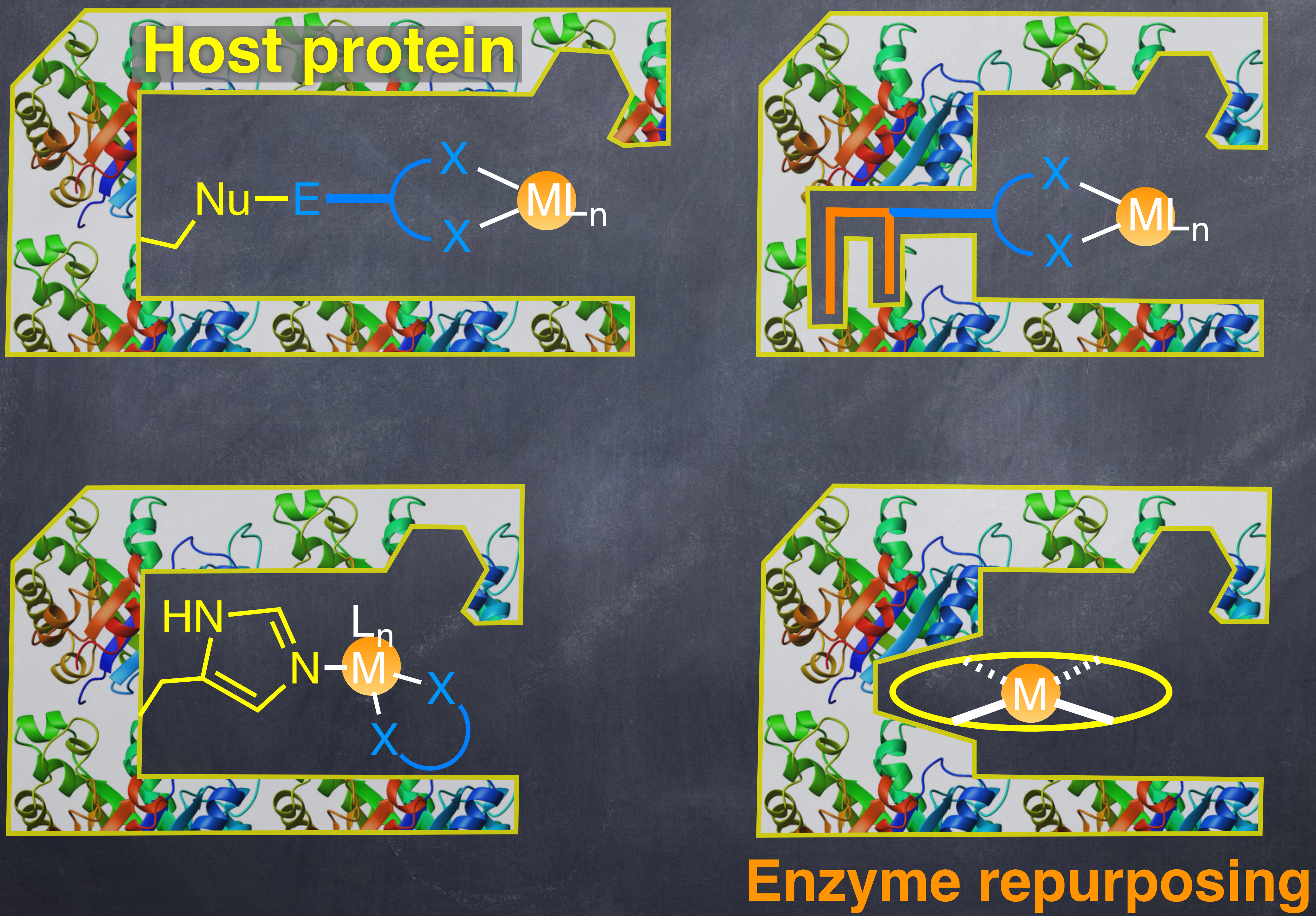




outcompetes free cofactor at low [substrate]

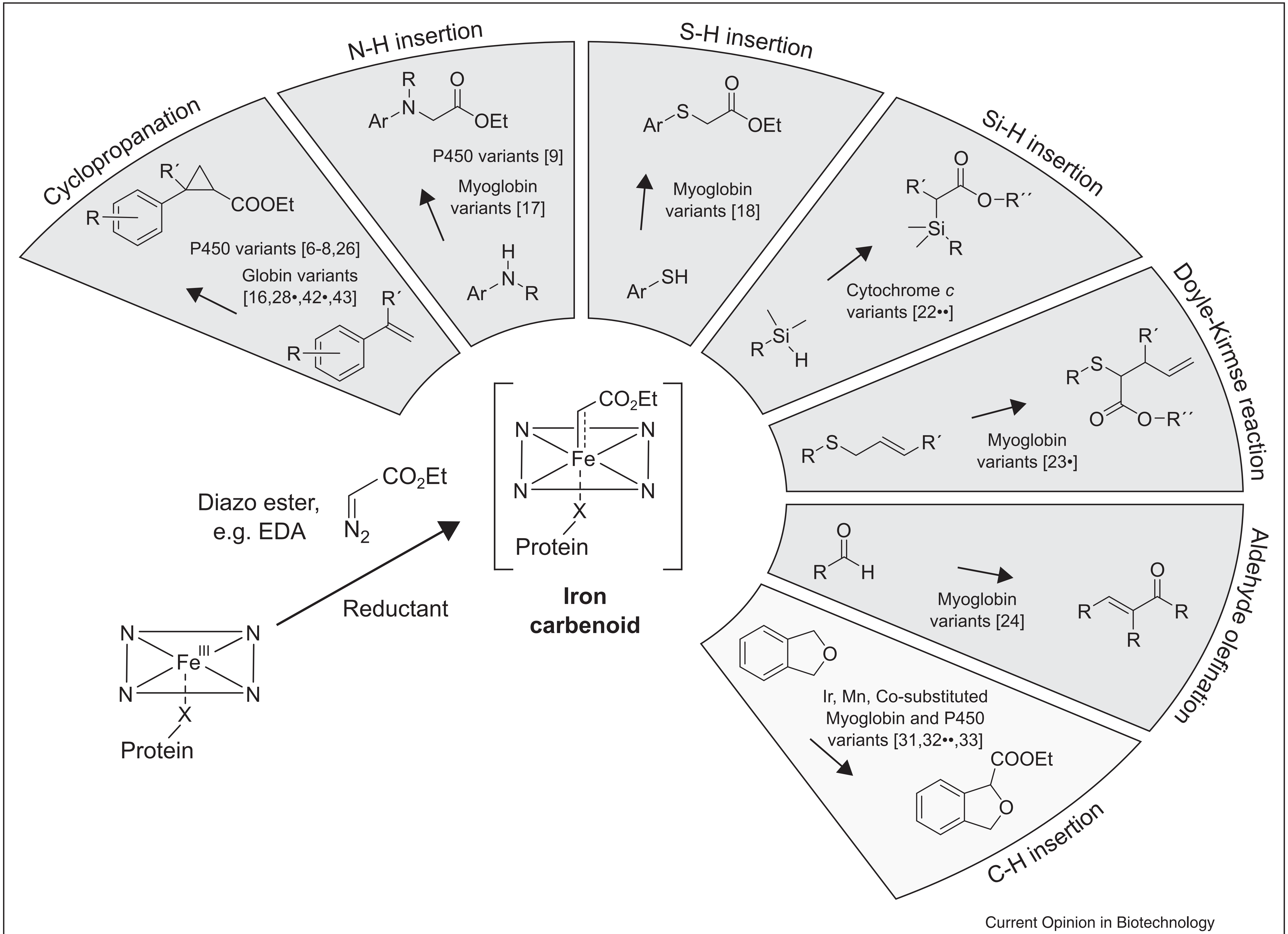
J. F. Hartwig, Science 2016, 354, 6308

Anchoring of the Catalyst:
Four Alternatives to Ensure Localization

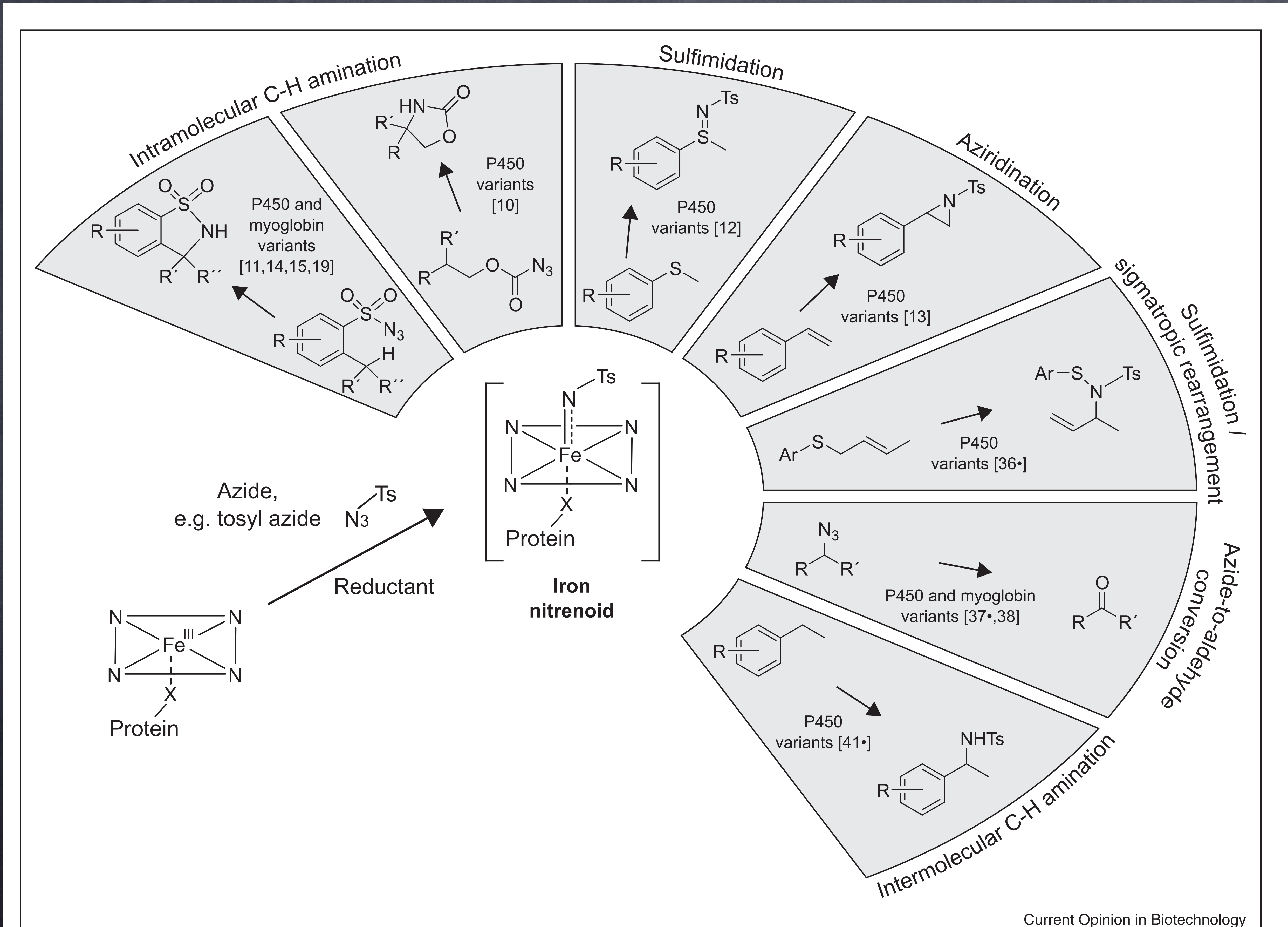


Arnold, Fasan, *et al.*, *Curr. Op. Chem. Biol.* **2017,** *47*, 102.

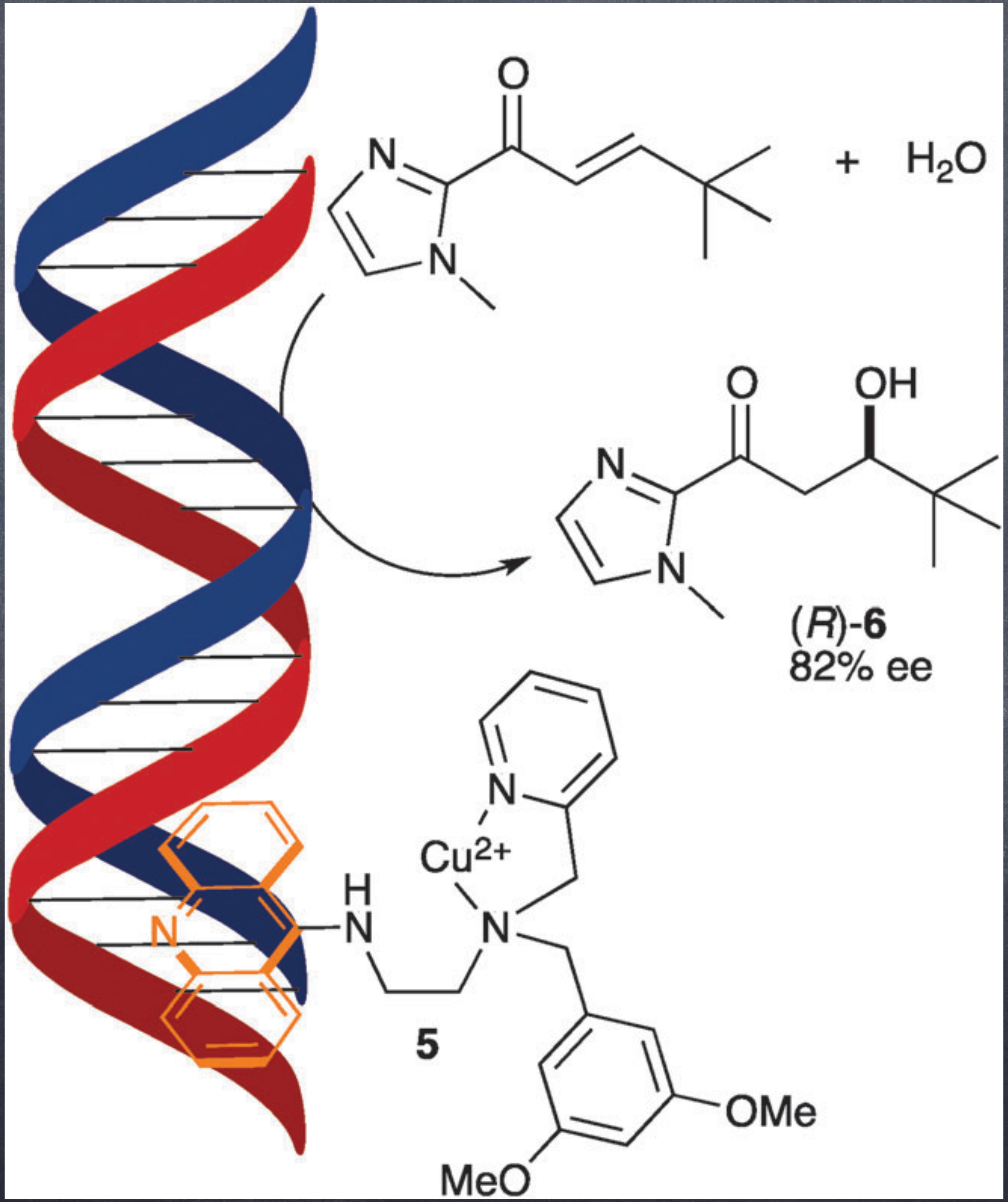
Carbene Transfer Catalyzed by Hemoproteins



Nitrene Transfer Catalyzed by Hemoproteins



DNA as Host for Enantioselective Catalysis



G. Roelfes, Groningen

Artificial Metalloenzymes: Homogeneous- or Enzyme-Like?

Homogeneous		Enzymatic
	Enantiomers	
Both		Single
	Solvent Tolerance	
Organic		Aqueous
	Substrate Specificity	
Broad		Narrow
	Optimization	
Chemical		Genetic
	Catalyst Lifetime	
Limited		Extended
	In vivo Compatibility	
Limited		Excellent