Supporting Information

Rational Design of a (S)-Selective-Transaminase for Asymmetric Synthesis of (1S)-1-(1,1'-biphenyl-2-yl)ethanamine

Daniel F.A.R. Dourado [1,2‡], Stefan Pohle [2‡], Alexandra T.P. Carvalho [1,2‡], Dharmendra S. Dheeman [2], Jill M. Caswell [2], Timofey Skvortsov[1,2], Iain Miskelly[2], Rodney T. Brown [2], Derek J. Quinn [2], Christopher C.R. Allen[3], Leonid Kulakov [3], Meilan Huang [1,], & Thomas S. Moody [2]*

[1] School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG, Northern Ireland, UK

[2] Almac Sciences, Department of Biocatalysis and Isotope Chemistry, 20 Seagoe Industrial Estate, Craigavon BT63 5QD, Northern Ireland, UK

[3] School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

‡ These authors contributed equally to this work

* Correspondence and requests for materials should be addressed to M.H.

(email: m.huang@qub.ac.uk**)**

Table S1 Rationally designed *V. fluvialis* TAm mutants. In the first *in vitro* screening round 72 mutants were tested while 41 mutants constituted the second round. The study is focused on residues: G55, L56, W57, H83, B: R88, V153, K163, A228, E257, V258, I259, V422, R415. Additionally, we also mutated residues that interact with PMP: Y150, S118, S284, and L287 (interacts with S284), and residues that interact with L56 and with B: R88: N58, F19 and F85.

First screening round	Second screening round
F19K/W57G	W57F/I259A/V422A
F19R/W57G	W57F/I259M/V422A
G55A/W57G	W57F/R415A
G55S/W57G	W57F/R88H
L56A/W57G	W57F/R88H/V153S/K163F/I259A/R415A/V422A
L56F/W57G	W57F/R88H/V153S/K163F/I259M/R415A/V422A
L56I/W57G	W57G/ K163F/I259M/V422A
L56V/W57G	W57G/I259A/R415A
W57A	W57G/I259A/R415A/V422A
W57A/N58V	W57G/I259A/R415G/V422A
W57A/R415L	W57G/I259A/R415P/V422A
W57F	W57G/I259M/ R415G/V422A
W57G/A228S	W57G/I259M/ R415P/V422A
W57G/A228S/V422A	W57G/I259M/R415A
W57G/A228T	W57G/I259M/R415A/V422A
W57G/A228V	W57G/K163F/I259A/V422A
W57G/E257A	W57G/K163F/R415A
W57G/E257A/V258A	W57G/R415A/V422A
W57G/E257G	W57G/R88H /I259A/R415A/V422A
W57G/E257G/V258A	W57G/R88H/I259A
W57G/E257N	W57G/R88H/I259A/V422A
W57G/F85A	W57G/R88H/I259M
W57G/F85G	W57G/R88H/I259M/R415A/V422A
W57G/F85V	W57G/R88H/I259M/V422A
W57G/G55A/S284G	W57G/R88H/K163F
W57G/H83I	W57G/R88H/R415A
W57G/H83L	W57G/R88H/R415G
W57G/H83M	W57G/R88H/R415P

W57G/H83N	W57G/R88H/V153S
W57G/H83P	W57G/R88H/V153S/I259A/R415A/V422A
W57G/I259A	W57G/R88H/V153S/I259M/R415A/V422A
W57G/I259A/V422A	W57G/R88H/V153S/K163F/I259A/R415A/V422A
W57G/I259A/V422Y	W57G/R88H/V153S/K163F/I259A/R415G/V422A
W57G/I259M	W57G/R88H/V153S/K163F/I259A/R415P/V422A
W57G/I259M/V422A	W57G/R88H/V153S/K163F/I259M/R415A/V422A
W57G/I259Q	W57G/R88H/V153S/K163F/I259M/R415G/V422A
W57G/I259V	W57G/R88H/V153S/K163F/I259M/R415P/V422A
W57G/K163F	W57G/R88H/V422A
W57G/K163L	W57G/V153S/I259A/V422A
W57G/L287I	W57G/V153S/I259M/V422A
W57G/L287V	W57G/V153S/R415A
W57G/L56A/I259M	
W57G/L56A/I259V	
W57G/N58A	
W57G/N58I	
W57G/N58P	
W57G/N58T	
W57G/N58V	
W57G/R415A	
W57G/R415F	
W57G/R415L	
W57G/R415P	
W57G/R415V	
W57G/R415W	
W57G/R88H	
W57G/R88K	
W57G/R88Q	
W57G/S118A	
W57G/S284A	
W57G/S284G	
W57G/V153A	
W57G/V153K	
W57G/V153R	

W57G/V153S	
W57G/V258A	
W57G/V258I	
W57G/V258T	
W57G/V422A	
W57G/V422I	
W57G/V422L	
W57G/V422Y	
W57G/Y150F	

Table S2 *V. fluvialis* TAm WT and engineered variants reaction rates and kinetic parameters towards acetophenone.

Enzyme	**Wild type**	**W57G/ R415A**	**W57G/ K163F/ R415A**	**W57G/ I259M/ R415A**	**W57F/R88 H/V153S/ K163F/I25 9M/R415A /V422A**
K_M **[mM]**	14.7	4.53	12.7	5.80	6.70
K_{cat} **[s^{-1}]**	$1.83x10^{-2}$	$2.28x10^{-2}$	$5.80x10^{-2}$	$1.73x10^{-2}$	$1.16\ x10^{-1}$
K_{cat}/K_M **[$M^{-1}\cdot s^{-1}$]**	1.24	5.03	4.55	2.98	16.6
Fold increase in K_{cat}/K_M [a]	1	4	4	2	13
Reaction rate [mM/h][b]	0.442	0.706	1.24	0.469	2.93
Reaction rate fold increase	1	2	3	1	7

[a] Fold change relative to WT values.

[b] Reaction rates are initial reaction rates. Reaction conditions: 20 mM substrate, 1M IPA, 0.01 mM enzyme, 0.1 M K·phosphate buffer (pH 8.0), 10% DMSO (v/v), 40 °C. Reactions were performed in duplicates.

Table S3 Residual activities of the WT enzyme and best mutants. Residual activity is defined as the [amount of product formed in a reaction with preincubated enzyme]/[amount of product formed in control reaction]*100%.

Variant	**40°C - 18 h**	**50°C - 18 h**
W57G/R415A	91.1%	28.2%
W57G/K163F/R415A	109%	34.1%
W57G/I259M/R415A	38.8%	16.4%
W57F/R88H/V153S/K163F/ I259M/R415A/V422A	97.9%	89.6%

Table S4 Amount of amine product formed (mM) in the reactions with the preincubated enzymes.

Enzyme	40°C - 0 h	40°C - 18 h	50°C - 0 h	50°C - 18 h
Wild type	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000
W57G/R415A	0.0425	0.0306	0.0364	0.0093
	0.0299	0.0320	0.0322	0.0096
	0.0315	0.0321	0.0334	0.0099
W57G/K163F/R415A	0.108	0.114	0.149	0.0506
	0.110	0.116	0.150	0.0488
	0.102	0.120	0.149	0.0535
W57G/I259M/R415A	0.0220	0.0074	0.0880	0.0150
	0.0182	0.0082	0.0838	0.0132
	0.0197	0.0076	0.0840	0.0137
W57F/R88H/V153S/K163F/ I259M/R415A/V422A	0.744	0.705	1.14	0.990
	0.730	0.717	1.12	1.01
	0.736	0.742	1.12	1.03

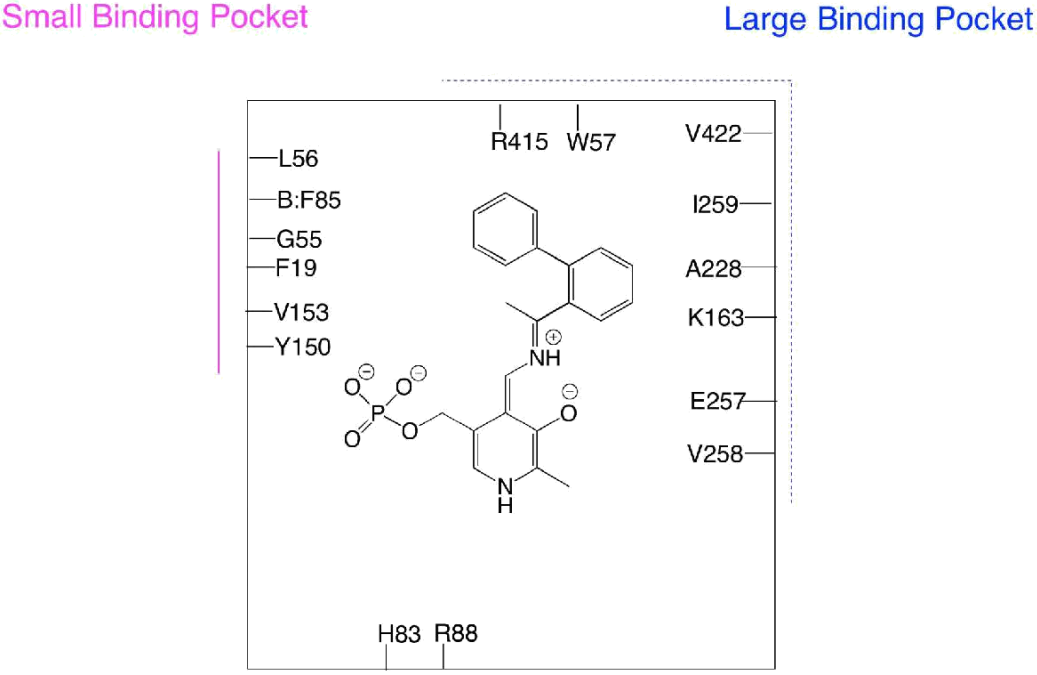
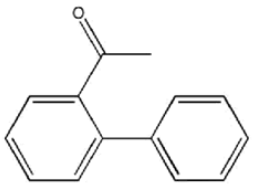


Figure S1. The large and small pockets of *V. fluvialis* amine transaminase.

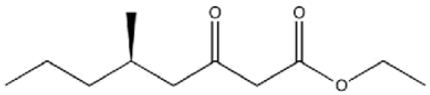
a



2-acetylbiphenyl

Chemical Formula: $C_{14}H_{12}O$
Exact Mass: 196.09
Molecular Weight: 196.25

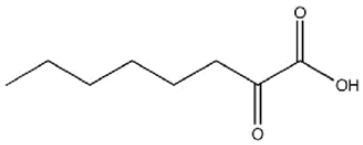
b



(R)-ethyl 5-methyl 3-oxooctanoate

Chemical Formula: $C_{11}H_{20}O_3$
Exact Mass: 200.14
Molecular Weight: 200.28

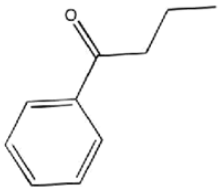
c



2-oxo-octanoate

Chemical Formula: $C_8H_{14}O_3$
Exact Mass: 158.09
Molecular Weight: 158.20

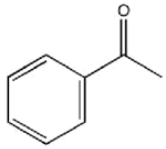
d



butyrophenone

Chemical Formula: $C_{10}H_{12}O$
Exact Mass: 148.09
Molecular Weight: 148.21

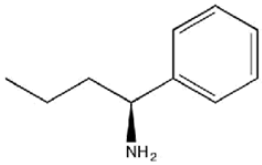
e



acetophenone

Chemical Formula: C_8H_8O
Exact Mass: 120.06
Molecular Weight: 120.15

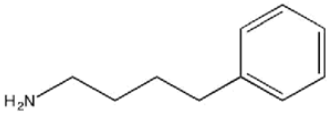
f



(S)-1-phenylbutylamine

Chemical Formula: $C_{10}H_{15}N$
Exact Mass: 149.12
Molecular Weight: 149.24

g



4-phenylbutylamine

Chemical Formula: $C_{10}H_{15}N$
Exact Mass: 149.12
Molecular Weight: 149.24

Figure S2. Substrates studied in the engineering of (S)-selective-TAms. **a)** Substrate studied in the present research (ketone **1**); **b**)-**f**) Substrates studied in previous literature. **b**) Substrate studied in Ref. 6e; **c**) Substrate studied in Ref. 6f; **d-e**) Substrates studied in Ref. 6g; **f**) Substrate studied in Refs. 10&11; **g)** Substrate studied in Ref. 8.

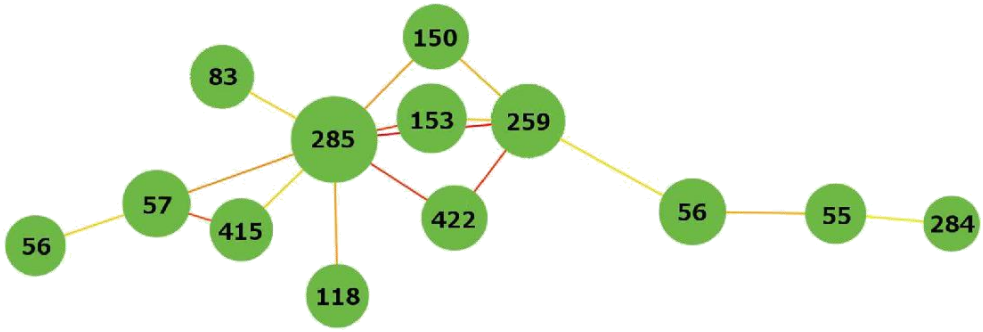
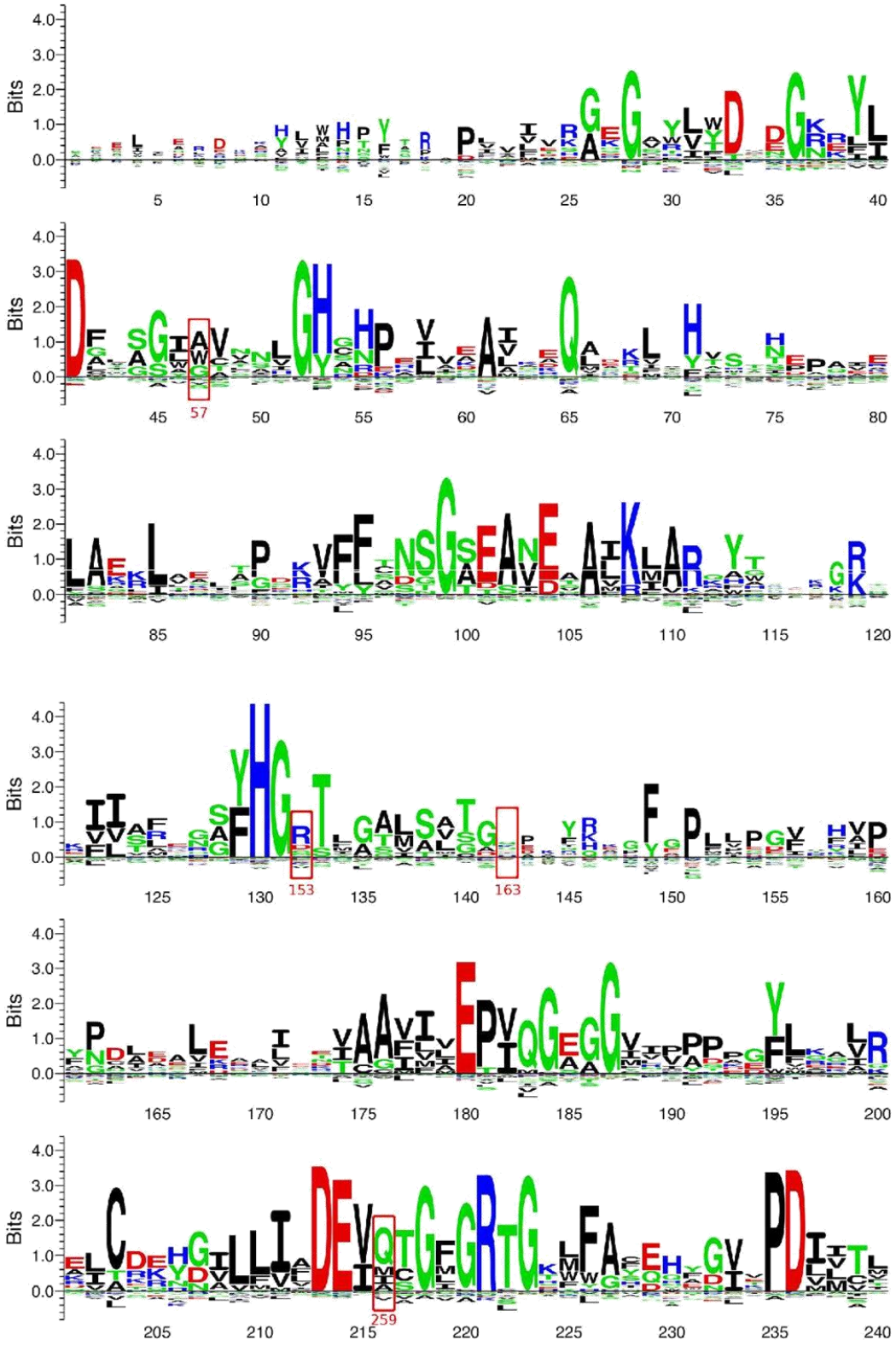


Figure S3. Co-evolution network of pyridoxal-phosphate-dependent aminotransferase super family (30,000 sequences) with a coefficient higher than 0.8.


Bits
4.0
3.0
2.0
1.0
0.0
5 10 15 20 25 30 35 40
45 57 50 55 60 65 70 75 80
85 90 95 100 105 110 115 120
125 130 153 135 140 163 145 150 155 160
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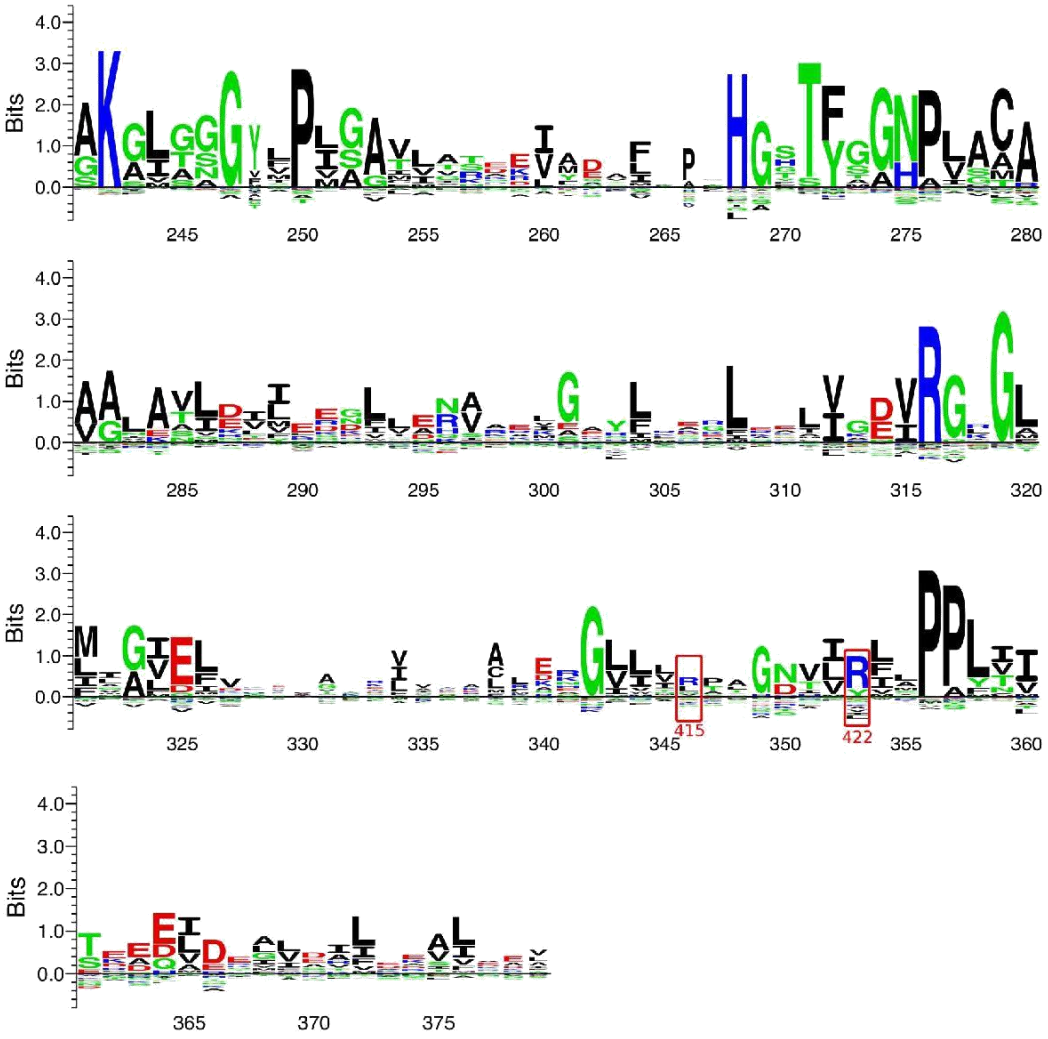

Figure S4. Amino acid distribution of the Pyridoxal-phosphate-dependent aminotransferase super family. Total number of sequences: 10623; the sequences coverage is at least 60 % of the *V. fluvialis* Tam sequence; sequence identity >25% and < 95%; Residue positions mutated in the best variants are highlighted with redboxes.

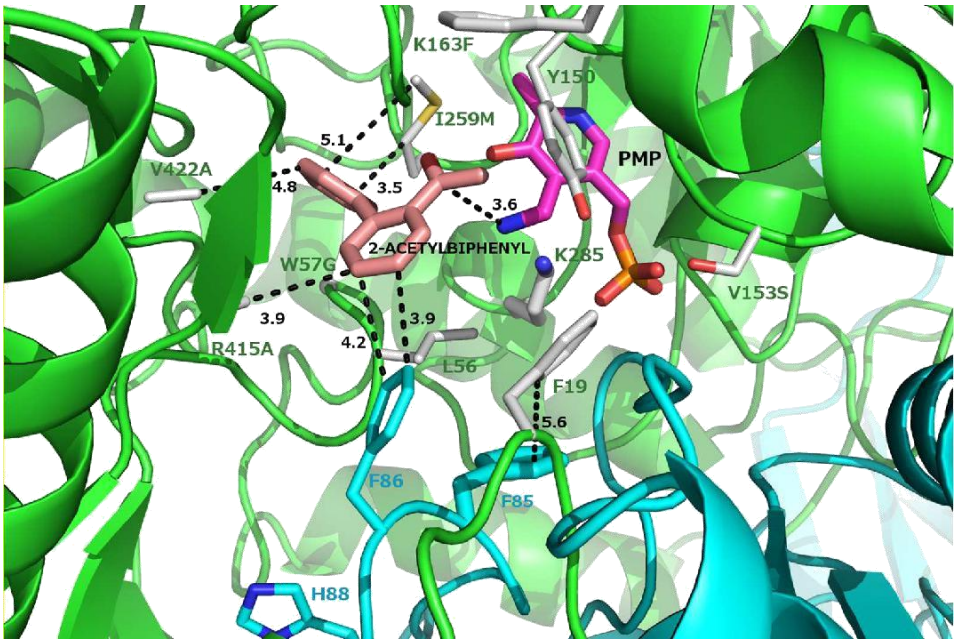

Figure S5. MD reference structure of ketone **1** docked into *V. fluvialis* TAm W57G/R88H/V153S/K163F/I259M/V422A/R415A in presence of PMP. The active centre residues are represented by sticks with the carbons of Chain A (green ribbon) coloured in grey and the carbons of Chain B (cyan ribbon) coloured in cyan. Relevant distances are shown (Å).

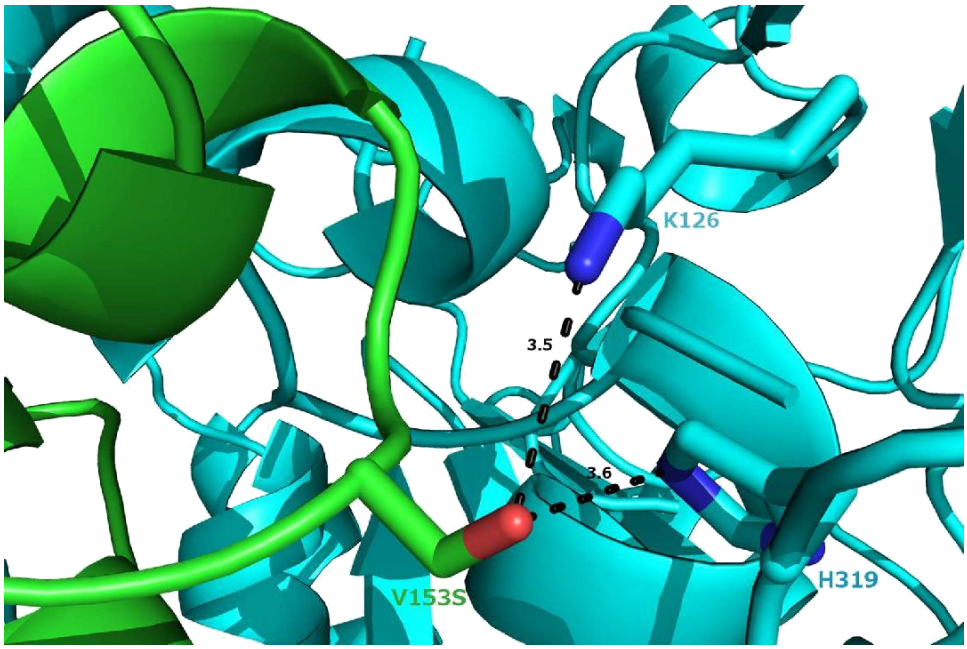

Figure S6. The predicted interactions along the dimeric interface of the *V. fluvialis* TAm V153S mutant from ZEMu calculation. The mutant structure was built based on the crystal structure of the WT *V. fluvialis* TAm (pdb: 4E3Q). Chain A is shown by green ribbon and Chain B is shown by cyan ribbon. Relevant distances are shown (Å).

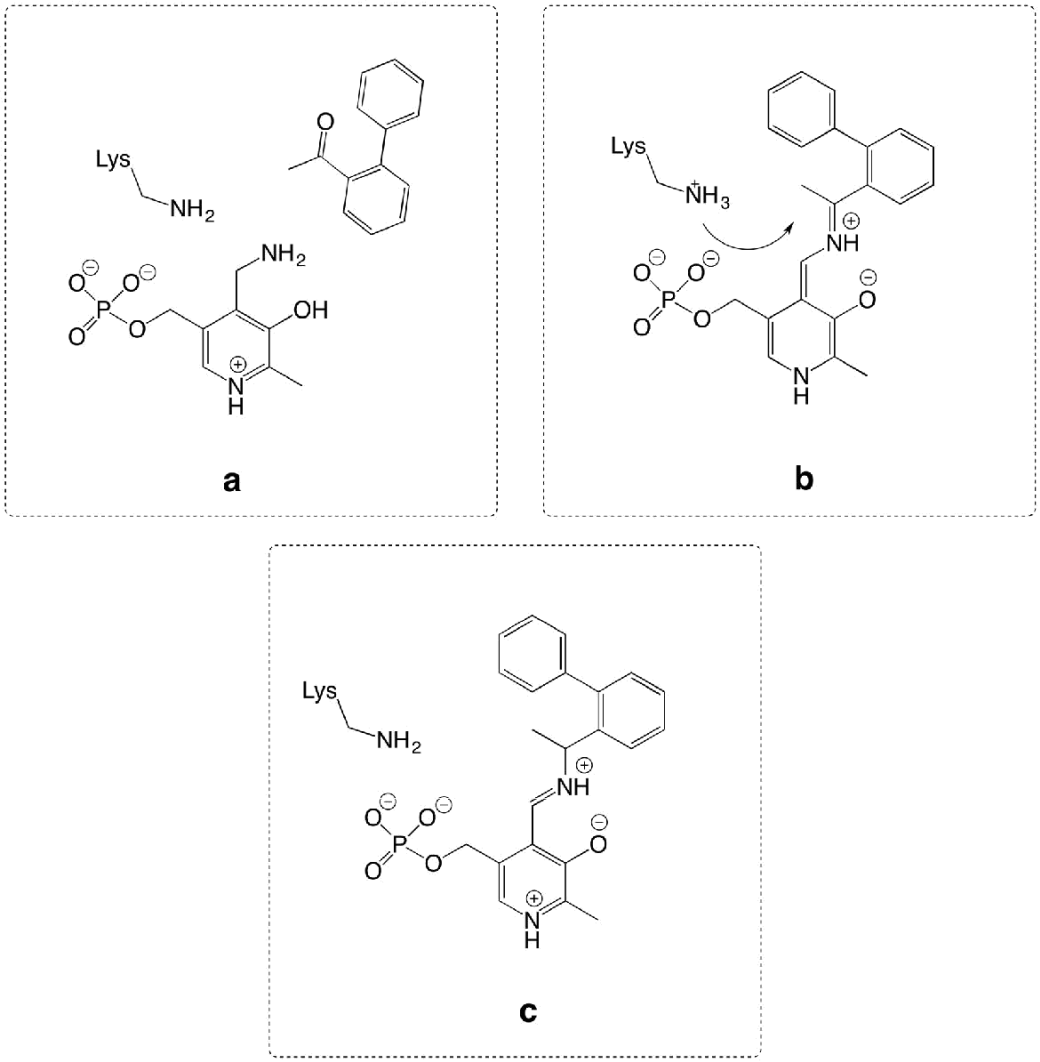

Figure S7. Intermediates in the transamination reaction pathway modelled in this study: (**a**) E: PMP; (**b**) Planar quinonoid; (**c**) External aldimine.

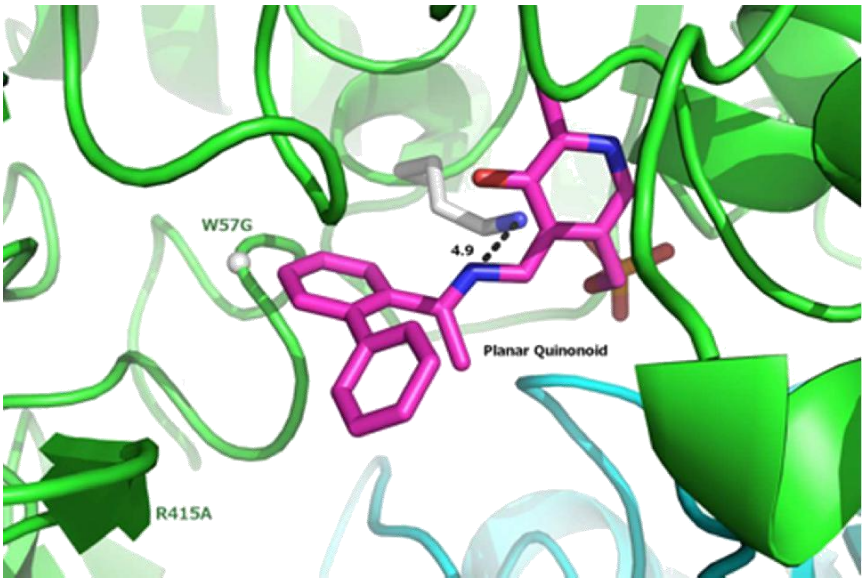

Figure S8. *V. fluvialis* TAm W57G/R415A docked into the planar quinonoid. The active centre residues are represented by sticks with the carbons of Chain A (green ribbon) coloured in grey and the carbons of Chain B (cyan ribbon) coloured in cyan. Relevant distances are shown (Å).

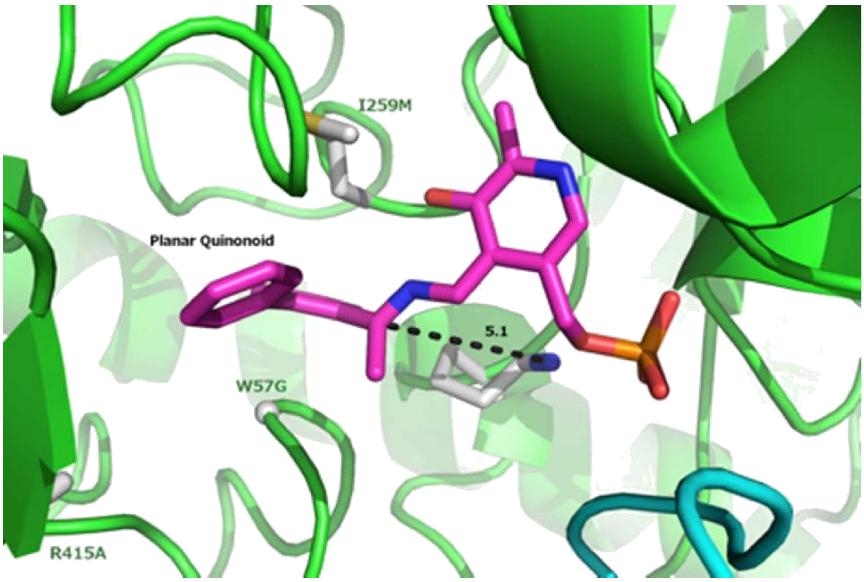

Figure S9. *V. fluvialis* TAm W57G/R415A/I259M docked into the planar quinonoid. The active centre residues are represented by sticks with the carbons Chain A (green ribbon) coloured in grey and the carbons of Chain B (cyan ribbon) coloured in cyan. Relevant distances are shown (Å).

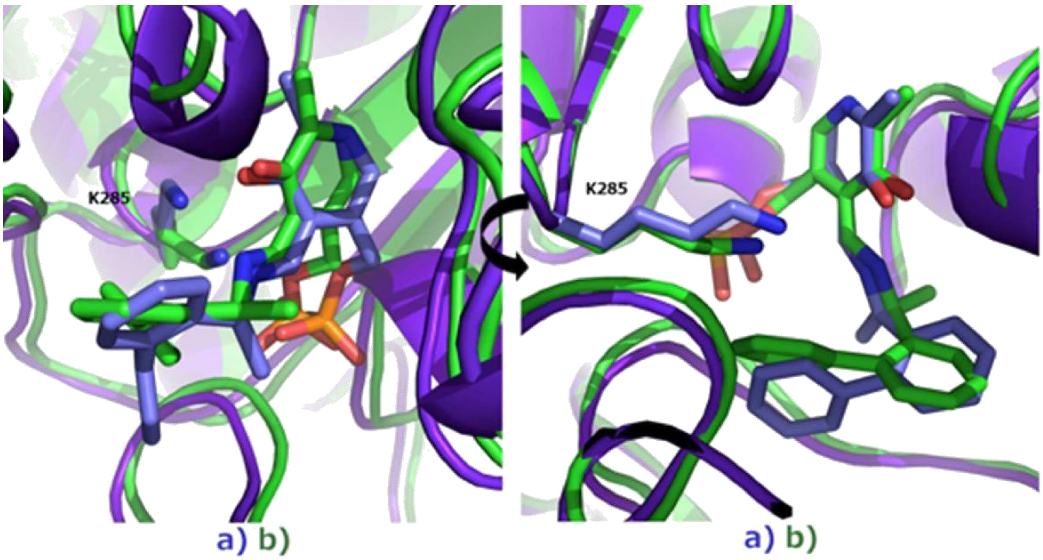

Figure S10. Superimposition of the MD reference structures of *V. fluvialis* TAm W57F/R88H/V153S/K163F/I259M/R415A/V422A with **(a)** planar quinonoid and **(b)** pro – (S) external aldimine (main chain rms = 1.2 Å). Two different orientations are shown.

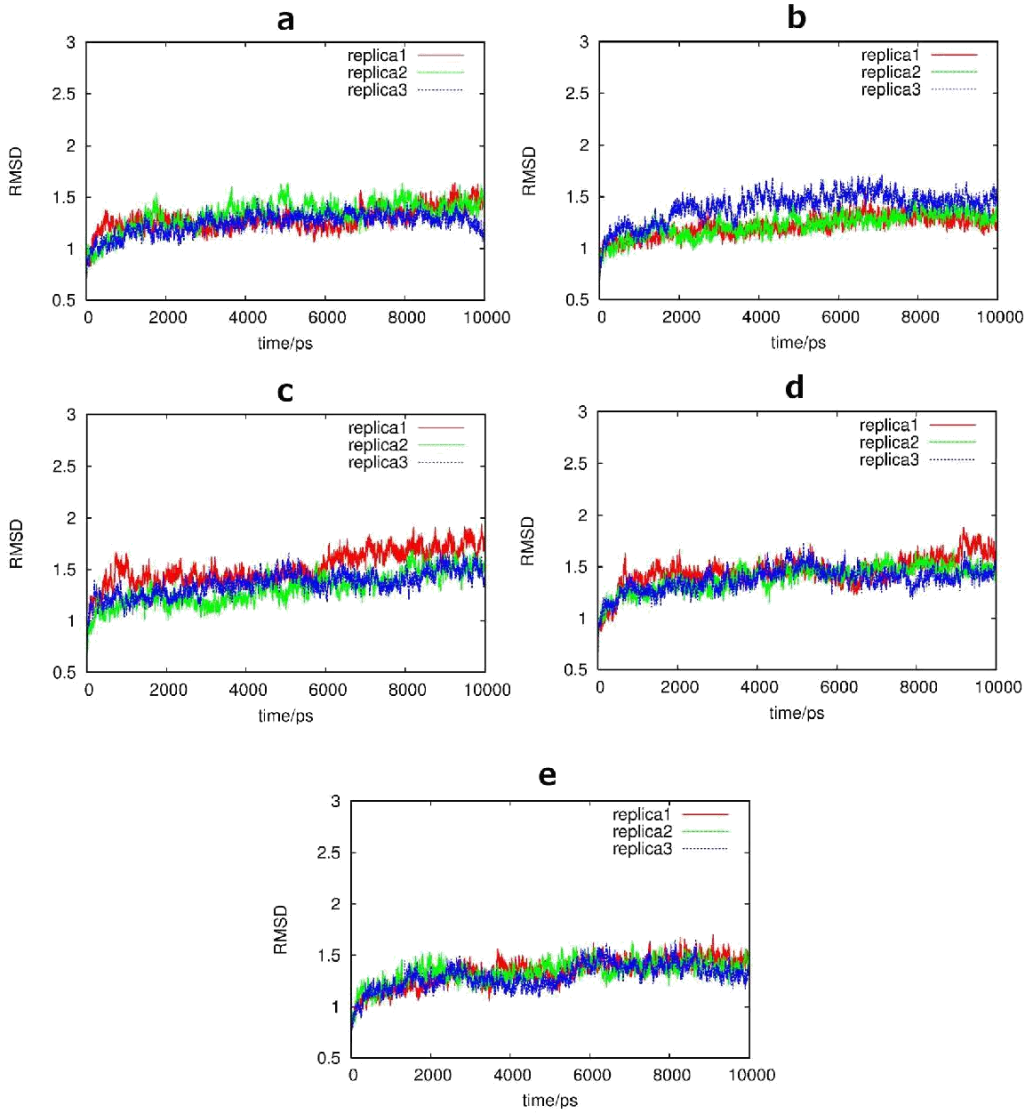

Figure S11. RMSD of the alpha-carbon atoms for (**a**) *V. fluvialis* TAm WT; (**b**) *V. fluvialis* TAm W57G/R415A; (**c**) *V. fluvialis* TAm W57G/ K163F/R415A; (**d**) *V. fluvialis* TAm W57G/ I269M/R415A; (**e**) *V. fluvialis* TAm W57F/ R88H/V153S/K163F/I259M/R415A/V422A. All the complexes contain ketone **1** and the PMP cofactor. No significant difference was found in the respective MD replicas.

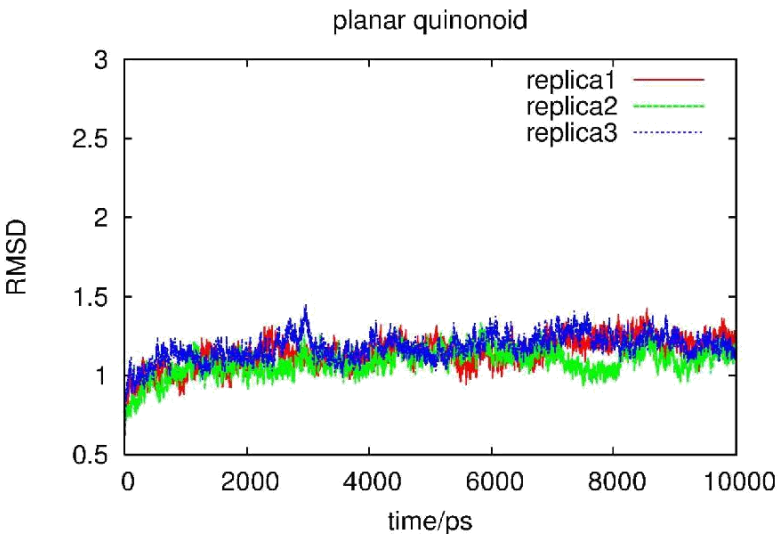

Figure S12. RMSD of the alpha-carbon atoms for the *V. fluvialis* TAm W57F/ R88H/V153S/K163F/I259M/R415A/V422A bound with the planar quinonoid. No significant difference was found in the respective MD replicas.

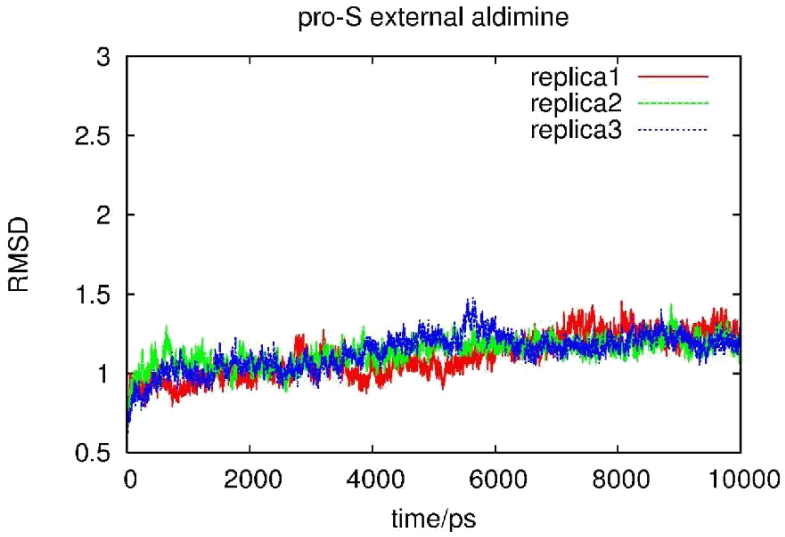

Figure S13. RMSD of the alpha-carbon atoms for the *V. fluvialis* TAm W57F/ R88H/V153S/K163F/I259M/R415A/V422A bound with the pro (S) external aldimine. No significant difference was found in the respective MD replicas.

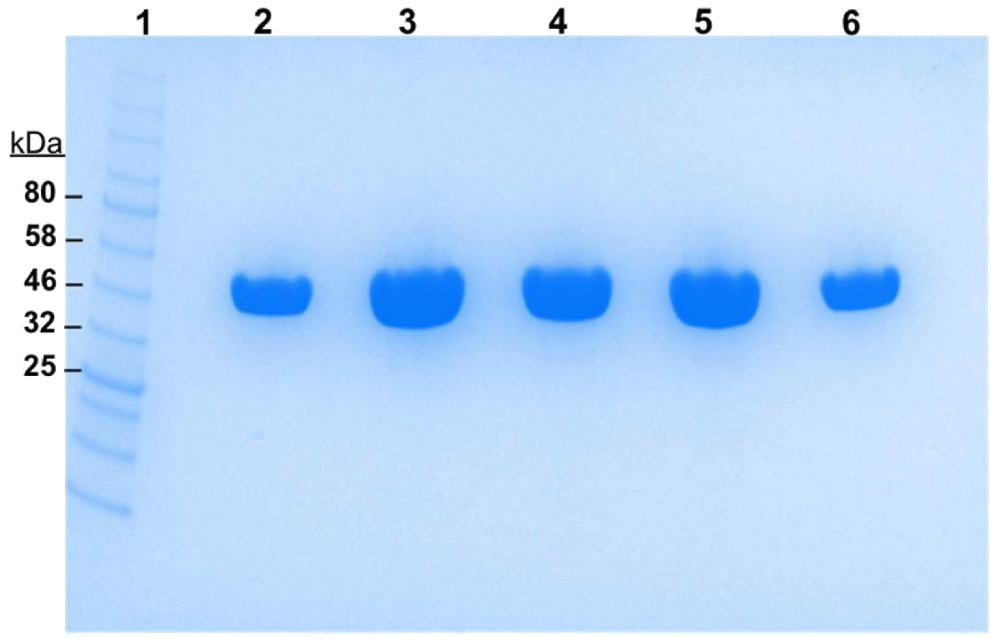

Fig S14. SDS-PAGE analysis of purified WT *V. fluvialis* TAm and its four variants. The enzymes were purified using immobilised metal affinity chromatography (IMAC) as described in material and methods. (1) Molecular weight markers; (2) WT; (3) W57G/R415A; (4) W57G/K163F/R415A; (5) W57G/I259M/R415A; (6) W57F/R88H/V153S/K163F/I259M/R415A/V422A.

$$\Delta G = (V_{bound}^{L-L} - V_{unbound}^{L-L}) + (V_{bound}^{P-P} - V_{unbound}^{P-P}) + \left(V_{bound}^{P-L} - V_{unbound}^{P-L} + \Delta S_{conf}\right)$$

Equation S1. Autodock 4.2 free energy equation. ΔS_{conf} is the entropy lost upon binding; V corresponds to the pairwise energy terms; L refers to the ligand and P the protein Ref. 24.

$$V = W_{vdw} \sum_{i.j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) + W_{hbond} \sum_{i.j} E_{(t)} \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}\right) + W_{elec} \sum_{i.j} \frac{q_i q_j}{e(r_{ij}) r_{ij}} + W_{sol} \sum_{i.j} \left(S_i V_j + S_j V_i\right) e^{\left(-r_{ij}^2/2\sigma^2\right)}$$

Equation S2. Autodock 4.2 pairwise energy terms. *Ws* are weighting constants optimized to calibrate a data set of 188 diverse protein–ligand complexes of known structure and binding energy. The first term is the Lennard-Jones potential with A and B based on the amber force field; the second term is a directional H-bond term; the third term describes electrostatics with the Coulomb potential; the fourth corresponds to the desolvation potential (Ref. 24).