Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Repurposing designed mutants: a valuable strategy for computer-aided laccases engineering. The case of POXA1b.

Valerio Guido Giacobelli[a #], Emanuele Monza[b #], M. Fatima Lucas[b] , Cinzia Pezzella[a] , Alessandra Piscitelli[a], Victor Guallar[b,c *], Giovanni Sannia[a *]

[a]Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4, 80126 Naples, Italy
[b]Joint BSC-CRG-IRB Research Program in Computational Biology, Barcelona Supercomputing Center, c/Jordi Girona 29, 08034 Barcelona, Spain
[c]Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain

#Equally contributed to this work
*Corresponding authors: victor.guallar@bsc.es, sannia@unina.it.

Information presented here includes:

Examples of PELE input:

- Sampling
- Design

Table S1. Variants from round 1 showing improved descriptor values.

Protein variant	Binding energy (kcal/mol)	SASA
Wild type	-8	0.19
A336R	-22	0.10
A336Q	-13	0.17
A336K	-15	0.14
G161R	-16	0.12
G161Q	-20	0.11
G161K	-16	0.11
G161M	-15	0.09
G161S	-19	0.13
G161Y	-14	0.10
G392R	-18	0.10
G392F	-14	0.04
G392W	-15	0.02
G392Y	-13	0.04
L511R	-18	0.13
L511N	-18	0.18
L511Q	-17	0.17
L511H	-14	0.16
L511K	-14	0.15
L511T	-17	0.17
F331R	-16	0.12
F331Q	-15	0.17
F331K	-15	0.17
F331Y	-19	0.15
P163R	-17	0.18
P163N	-20	0.19
P163Q	-16	0.14
P163K	-14	0.13
V162N	-20	0.10
V162C	-18	0.09

V162H	**-20**	**0.09**
V162S	**-16**	**0.17**
V162T	-18	0.21

Table S2. Variants from round 2 of the V162H branch showing improved descriptor values.

Protein variant	Binding energy (kcal/mol)	SASA
V162H_A336N	-28	0.09
V162H_A336K	-26	0.10
V162H_L511R	-28	0.08
V162H_L511Q	-27	0.10
V162H_F331Y	**-29**	**0.09**

Table S3. Variants from round 2 of the V162S branch showing improved descriptor values.

Protein variant	Binding energy (kcal/mol)	SASA
V162S_A336R	-22	0.16
V162S_A336Q	-21	0.16
V162S_A336K	-22	0.17
V162S_G161R	-22	0.10
V162S_G392R	-22	0.05
V162S_G392Q	-22	0.12
V162S_G392F	-23	0.03
V162S_G392W	-23	0.03
V162S_G392Y	-23	0.03
V162S_L511R	-25	0.14
V162S_L511N	-25	0.20
V162S_L511Q	-24	0.17
V162S_L511T	-24	0.18
V162S_F331R	-23	0.13
V162S_F331K	-23	0.16
V162S_F331Y	**-26**	**0.14**
V162S_P163R	-24	0.14
V162S_P163N	-28	0.18
V162S_P163K	-23	0.14
V162S_P163M	-21	0.17

Table S4. Variants from round 3 of the V162H branch showing improved descriptor values.

Protein variant	Binding energy (kcal/mol)	SASA
V162H_F331Y_A336R	-36	0.08
V162H_F331Y_A336N	**-35**	**0.07**
V162H_F331Y_G392R	-35	0.10
V162H_F331Y_L511R	-35	0.08
V162H_F331Y_L511N	-36	0.10
V162H_F331Y_P163K	-35	0.03
V162H_F331Y_P510R	-35	0.08

Table S5. Variants from round 3 of the V162S branch showing improved descriptor values.

Protein variant	Binding energy (kcal/mol)	SASA
V162S_F331Y_A336N	**-31**	**0.15**
V162S_F331Y_G161K	-34	0.15
V162S_F331Y_P163R	-33	0.10
V162S_F331Y_P163N	-35	0.13
V162S_F331Y_P163Q	-32	0.13
V162S_F331Y_P163H	-32	0.10
V162S_F331Y_P163K	-33	0.09

Table S6. Computational ionization energy of 2,4- and 2,5- dabsa.

Chemical species	Ionization energy (eV)
2,4-dabsa	4.44008397
2,5-dabsa	4.26511468

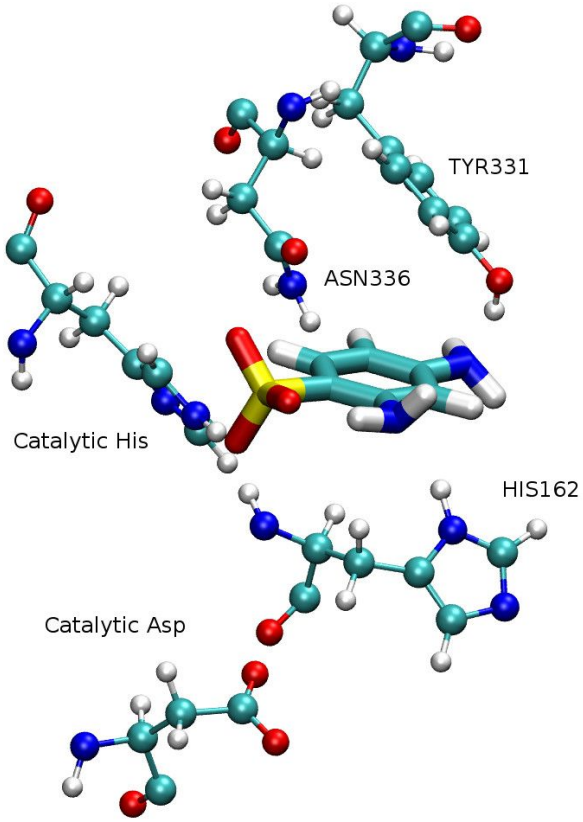

Figure S1. PELE sampling result for the V162H branch triple mutant.

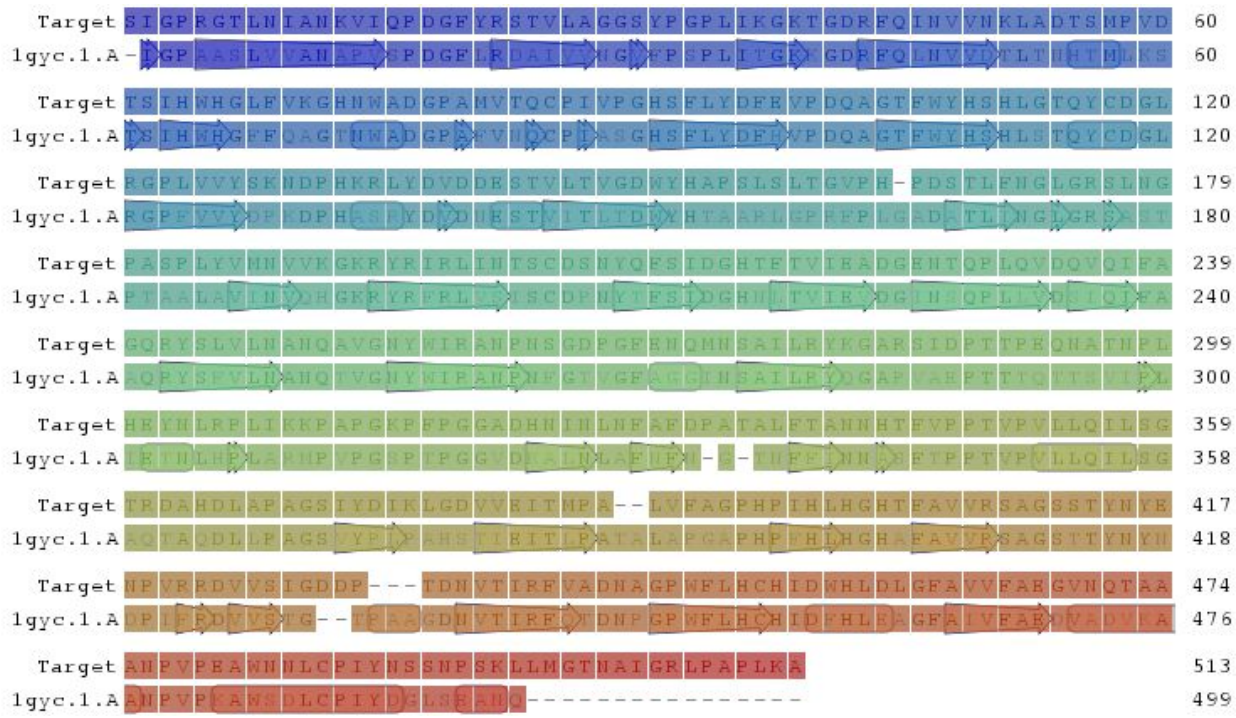

Figure S2. Sequence alignment of POXA1b with its homology model template.

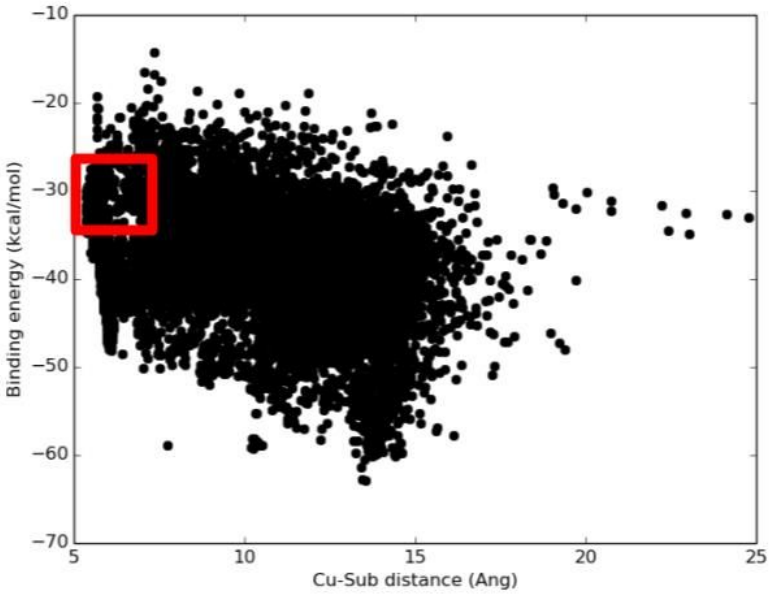

Figure S3. PELE binding energy vs. 2,4-dabsa-Cu distance. Reactive enzyme-substrate conformations are localized in the red rectangle.

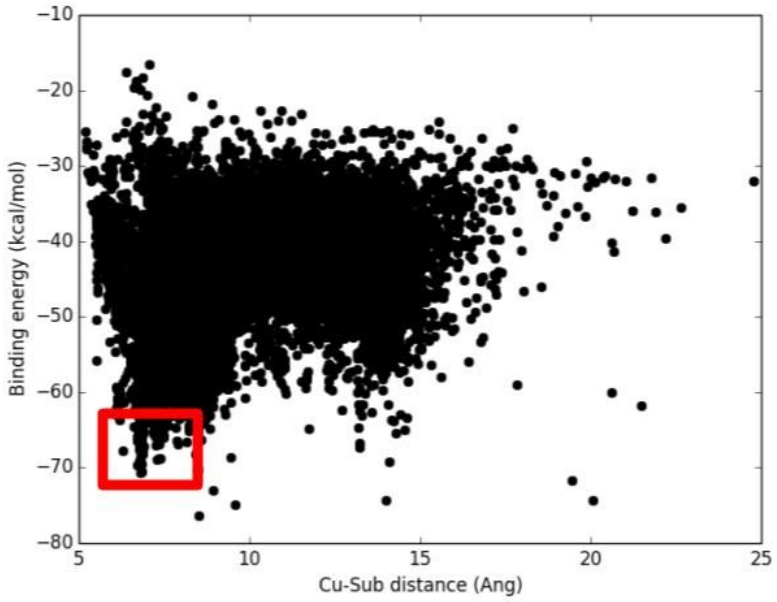

Figure S4. PELE binding energy vs. 2,5-dabsa-Cu distance. Reactive enzyme-substrate conformations are localized in the red rectangle.

PELE sampling input file

POXA1b-2,4-dabsa sampling

file datadir /home/bsc72/bsc72328/plop/data
file log dyn_24dabsa.log
energy params solvent vdgbnp
energy params ionic 0.15
load pdb poxa1b-24dabsa_fixed.pdb waters no ions no het yes

constraint atom A:597:CU__ current 200 0.0 &
atom A:451:_SG_ current 200 0.0 &
atom A:451:_CB_ current 200 0.0 &
atom A:456:_ND1 current 200 0.0 &
atom A:456:_CB_ current 200 0.0 &
atom A:456:_CG_ current 200 0.0 &
atom A:394:_ND1 current 200 0.0 &
atom A:394:_CB_ current 200 0.0 &
atom A:394:_CG_ current 200 0.0 &
atom A:453:_CD1 current 200 0.0 &
atom A:453:_CG1 current 200 0.0 &
atom A:461:_CE1 current 200 0.0 &
atom A:461:_CD1 current 200 0.0 &
atom A:461:_CG_ current 200 0.0 &
atom A:598:CU__ current 200 0.0 &
atom A:599:CU__ current 200 0.0 &
atom A:600:CU__ current 200 0.0 &
atom A:399:_NE2 current 200 0.0 &
atom A:111:_NE2 current 200 0.0 &
atom A:450:_NE2 current 200 0.0 &
atom A:452:_NE2 current 200 0.0 &
atom A:109:_NE2 current 200 0.0 &
atom A:66:_ND1 current 200 0.0 &
atom A:397:_NE2 current 200 0.0 &
atom A:64:_NE2 current 200 0.0 &

```
pele &
        het L:1 &
        pdbmodel yes &
        top_side 10 &
        init_min no &
        task &
                show bind_ene 1 &
                show SASA 1 &
                if random 1 gt 0.5 then rot_r 0.02 else rot_r 0.25 endif &
                if random 2 gt 0.5 then tra_r 2.0 else tra_r 0.75 endif &
                spawn atom 1 A:597:CU__ within 15.0 &
                exit steps gt 2000 &
        end_task &
        temp 1000 &
        tries 1 &
        anmfreq 1 &
        spfreq 1 &
        mifreq 1 &
        wrfreq 1 &
        omit_sp A:394 A:394 &
        omit_sp A:451 A:451 &
        omit_sp A:456 A:456 &
         omit_sp A:453 A:453 &
        omit_sp A:461 A:461 &
        side &
                randomize no &
                verbose no &
                failsafe no &
        sideend &
        path traj_24dabsa_ &
        mirad 20  &
        sprad 10  &
        min &
                rmsg 0.02 &
                nbup yes &
```

```
        gbup yes &
        alphaup yes &
minimend &
caconst 0.05 &
lcom_con 0.1 &
rem_bulk_mov 3 &
anm_eig_freq 100000 &
anm_altm_freq 1 &
anm_altm_type 3 &
lanmanm neig 6 &
lanmanm ualig YES &
lanmanm move_ca 0.2 &
lanmanm mix_modes 0.75 &
lanmmin &
        mxitn 100 &
        iter 1 &
        rmsg 0.02 &
        nbup yes &
        alphaup no &
minimend
```

PELE design input file

POXA1b-2,4-dabsa design

file datadir /home/bsc72/bsc72328/plop/data
file log dyn.log
energy params solvent vdgbnp
energy params ionic 0.15
load pdb candidate_refined.pdb waters no ions no het yes

constraint atom A:597:CU__ current 200 0.0 &
atom A:451:_SG_ current 200 0.0 &
atom A:451:_CB_ current 100 0.0 &
atom A:456:_ND1 current 200 0.0 &
atom A:456:_CB_ current 100 0.0 &
atom A:456:_CG_ current 100 0.0 &
atom A:394:_ND1 current 200 0.0 &
atom A:394:_CB_ current 100 0.0 &
atom A:394:_CG_ current 100 0.0 &
atom A:453:_CD1 current 100 0.0 &
atom A:453:_CG1 current 100 0.0 &
atom A:461:_CE1 current 100 0.0 &
atom A:461:_CD1 current 100 0.0 &
atom A:461:_CG_ current 100 0.0 &
atom A:598:CU__ current 200 0.0 &
atom A:599:CU__ current 200 0.0 &
atom A:600:CU__ current 200 0.0 &
atom A:399:_NE2 current 100 0.0 &
atom A:111:_NE2 current 100 0.0 &
atom A:450:_NE2 current 100 0.0 &
atom A:452:_NE2 current 100 0.0 &
atom A:109:_NE2 current 100 0.0 &
atom A:66:_ND1 current 100 0.0 &
atom A:397:_NE2 current 100 0.0 &

```
atom A:64:_NE2 current 100 0.0 &

pele &
        het L:1 &
        pdbmodel yes &
        top_side 10 &
        init_min no &
        task &
                show bind_ene 1 &
                show SASA 1 &
                spawn atom 1 A:597:CU__ within 15.0 &
                exit steps gt 2000 &
        end_task &
        rot_r 0.02 &
        tra_r 0.25 &
        temp 1000 &
        tries 1 &
        anmfreq 1 &
        spfreq 1 &
        mifreq 1 &
        wrfreq 1 &
        omit_sp A:394 A:394 &
        omit_sp A:451 A:451 &
        omit_sp A:456 A:456 &
        omit_sp A:453 A:453 &
        omit_sp A:461 A:461 &
        side &
                randomize no &
                verbose no &
                failsafe no &
        sideend &
        path traj_24dabsa_ &
        mirad 20  &
        sprad 10  &
        min &
                rmsg 0.02 &
```

```
        nbup yes &
        gbup yes &
        alphaup yes &
minimend &
caconst 0.05 &
lcom_con 0.1 &
rem_bulk_mov 3 &
anm_eig_freq 100000 &
anm_altm_freq 1 &
anm_altm_type 3 &
lanmanm neig 6 &
lanmanm ualig YES &
lanmanm move_ca 0.5 &
lanmanm mix_modes 0.75 &
lanmanm omit_sp A:597 A:600 &
lanmmin &
        mxitn 100 &
        iter 1 &
        rmsg 0.02 &
        nbup yes &
        alphaup no &
minimend
```