

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

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Information presented here includes:

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

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

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

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

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

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

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.

Figure S4. PELE binding energy vs. 2,5-dabsa-Cu distance.

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

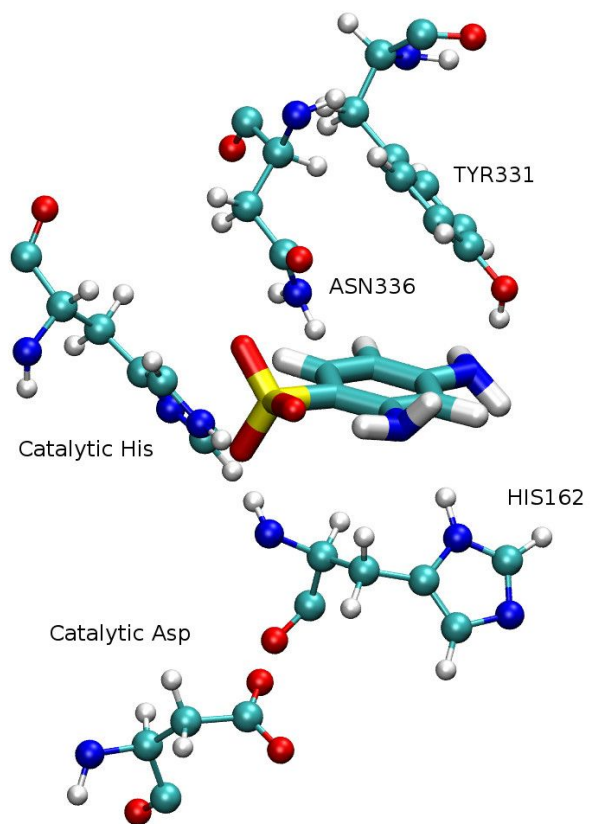


Figure S1. PELE sampling result for the V162H branch triple mutant.

Target	SIGPRGTLNLANKVIQPDGFRSTVLAGGSYPGPLIKGKTGDRFQINVVYKLA DTSMPVD	60
1gyc.1.A	DGPASLVYAHAPSPDGFIRDAIYHGGYFPPLITGKYGDRFQLNHYDYLTLHTMLKS	60
Target	FSIHVHGLFVKGHNWADGPA MVTCQPIVPGHSFLYDFEVPDQAGTEFWYHSHLGTOYCDGL	120
1gyc.1.A	FSIHVHGGFFQAGTNWADGPAFVHDCPIASGHSFLYDFEVPDQAGTEFWYHSHLS TOYCDGL	120
Target	RGPLVYVYSKNDPHKRLYDVDDESTVLTVGDNYHAPSLSLTGVPH-PDSITLFLNGLGRSLIG	179
1gyc.1.A	RGPEVYVYDFDPHRSYDIDDERSTVITLTDYHTAAPLSPAPPAGADITLNGLGRSAIT	180
Target	PASPLYVMNVYKGRYRIRLINTSCDSNYQFSIDGHTFTVIRADGENTOPLOYDOVQIFA	239
1gyc.1.A	PTAALAVMLNDRGKRYRIRLINTSCDPHYIYFSIDGHNITVIRADGENTOPLOYDQLQIFA	240
Target	GQRYSLVLYNANQAVGNYVIRANPNSGDYGFENQMHSAIIRYKGRARSIDPTTPRQATHPL	299
1gyc.1.A	GQRYSEVLYNANQAVGNYVIRANPNSGFAGSNHSAIIRYDGAFAARPTTICVTVVPL	300
Target	HEYNLRPLIKKPAAGKPPSPGGADHNINLNEAFDPATALEPTANNHTEVPPPTVPVLLQILSG	359
1gyc.1.A	RTNLRPLIKRMPVPGPIPGGDAINLNEAFDPATALEPTANNHTEVPPPTVPVLLQILSG	358
Target	TPDADDLAPAGSIYDIKLGDVVEITMPA--LVFAGPHPIHLHGHTFAVYRSAGSSTYNVE	417
1gyc.1.A	QTAADDLAPAGSIYEPYHSLRITIPATALEPAPHPPIHLHGHTFAVYRSAGSSTYNVH	418
Target	NPVREDVYSIGDDP---TDMVTIREVADNAGPWFLHCHIDVHLDLGFVVVFARGVNQTAA	474
1gyc.1.A	DPVREDVYSEIG---TDFSGDMVTIREVDNMPGWFLHCHIDVHLBAGFAVVFARVADVLA	476
Target	ANPVPEAVNHLCPYNSNPSKLLMGTNAIGRLPAPLK	513
1gyc.1.A	ANPVPEAVNHLCPYNSNPSKLLMGTNAIGRLPAPLK-----	499

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

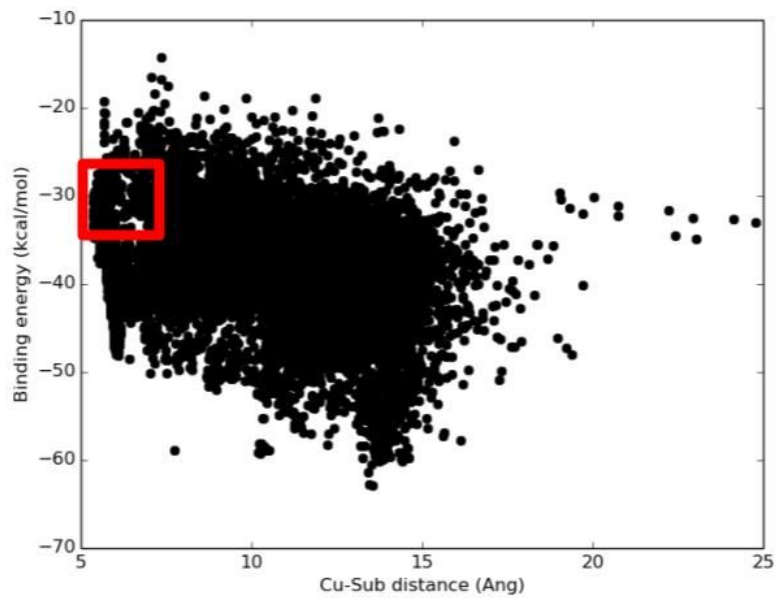


Figure S3. PELE binding energy vs. 2,4-dabsa-Cu distance. Reactive enzyme-substrate conformations are localized in the red rectangle.

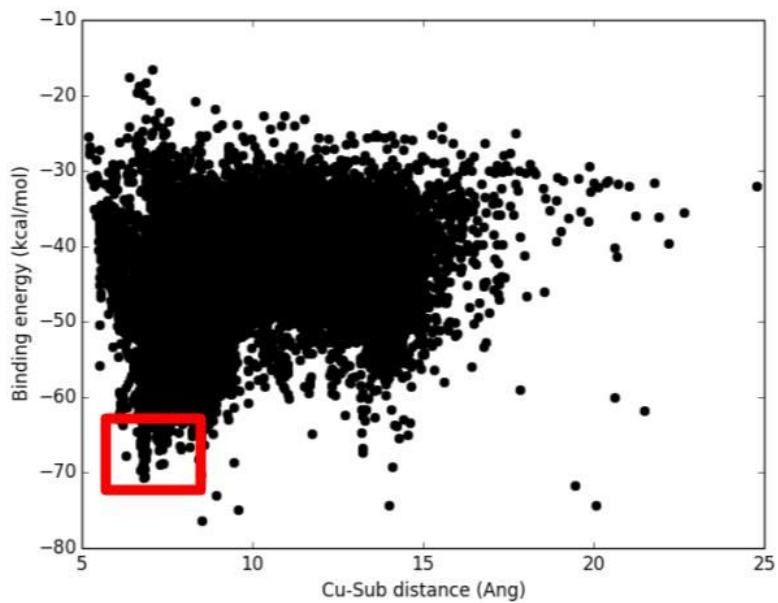


Figure S4. PELE binding energy vs. 2,5-dabsa-Cu distance. Reactive enzyme-substrate conformations are localized in the red rectangle.

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
```