Supplemental Material

Rosetta command lines

Design runs used Rosetta3 version 47627M with the following command lines:

Command line 1:

```
fixbb.macosgccrelease  \
-database rosetta_database \
-multi_cool_annealer 10 \
-resfile resfile \
-correct \
-linmem_ig 10 \
-nstruct 20 \
-s 3GBS_strip.pdb
```

Command line 2:

```
fixbb.macosgccrelease  \
-database rosetta_database \
-multi_cool_annealer 10 \
-resfile resfile \
-linmem_ig 10 \
-nstruct 10 \
-score:weights design_hpatch.wts \
-s 3GBS_strip.pdb
```

Command line 3:

```
fixbb.macosgccrelease  \
-database rosetta_database \
-multi_cool_annealer 10 \
-resfile resfile \
-linmem_ig 10 \
-nstruct 20 \
-score:weights design_hpatch.wts \
-soft_rep_design \
-s 3GBS_strip.pdb
```

All three runs used the following resfile:

```
PIKAA GPAVLIMFYWKRQNEDST
EX 1 LEVEL 4 EX 2
USE_INPUT_SC
start
#disulfide
37 A NATRO
113 A NATRO
#disulfide
```

63 A NATRO
76 A NATRO
#disulfide
177 A NATRO
184 A NATRO
#triad
181 A NATRO
194 A NATRO
126 A NATRO
#other active site res
90 A NATRO
#args
162 A NATAA
82 A NATAA
65 A NATAA
#pro
36 A NATRO
39 A NATRO
51 A NATRO
60 A NATRO
81 A NATRO
88 A NATRO
93 A NATRO
116 A NATRO
169 A NATRO
192 A NATRO
193 A NATRO

The job with 3rd command line listed above was aborted after 2 structures were generated, giving 32 total predictions. Command lines 2 and 3 made use of the 'hpatch' mode to avoid large hydrophobic surface patches (R. Jacak, A. Leaver-Fay, and B. Kuhlman (2012) Proteins 280 825–838).

Mutations for variant 11 were generated with the following command line and resfiles:

```
fixbb.macosgccrelease  \
-database rosetta_database/ \
-multi_cool_annealer 10 \
-resfile resfile \
-correct \
-linmem_ig 10 \
-nstruct 20 \
-s 3GBS_strip.pdb
```

resfile:

```
NATAA
EX 1 LEVEL 4 EX 2 EX 3
USE_INPUT_SC
start
```

#disulfide
37 A NATRO
113 A NATRO
#disulfide
63 A NATRO
76 A NATRO
#disulfide
177 A NATRO
184 A NATRO
#triad
181 A NATRO
194 A NATRO
126 A NATRO
#other active site
90 A NATRO
#pro
36 A NATRO
39 A NATRO
51 A NATRO
60 A NATRO
81 A NATRO
88 A NATRO
93 A NATRO
116 A NATRO
169 A NATRO
192 A NATRO
193 A NATRO
design
102 A PIKAA D
105 A PIKAA R
106 A PIKAA E
new
97 A PIKAA GPAVLIMFYWKRQNEDST
98 A PIKAA GPAVLIMFYWKRQNEDST
99 A PIKAA GPAVLIMFYWKRQNEDST
101 A PIKAA GPAVLIMFYWKRQNEDST
107 A PIKAA GPAVLIMFYWKRQNEDST
109 A PIKAA GPAVLIMFYWKRQNEDST
110 A PIKAA GPAVLIMFYWKRQNEDST
135 A PIKAA GPAVLIMFYWKRQNEDST
139 A PIKAA GPAVLIMFYWKRQNEDST

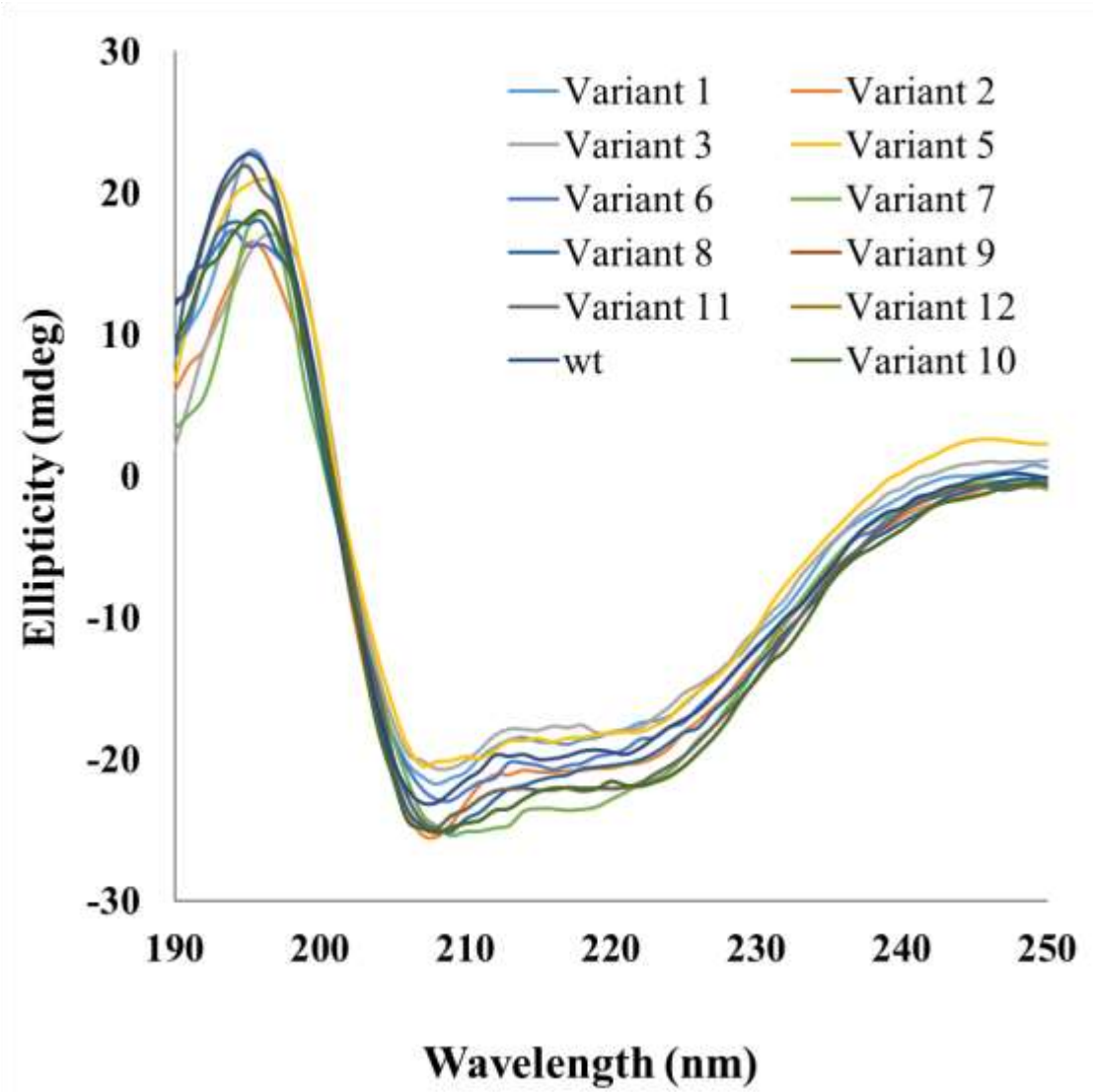

Figure S1: CD wavelength scan overlays to compare the secondary structures of variants in comparison with Wt-AoC. Analysis was performed on 10µM protein solutions in 20mM pH 8 Phosphate buffer at room temperature.

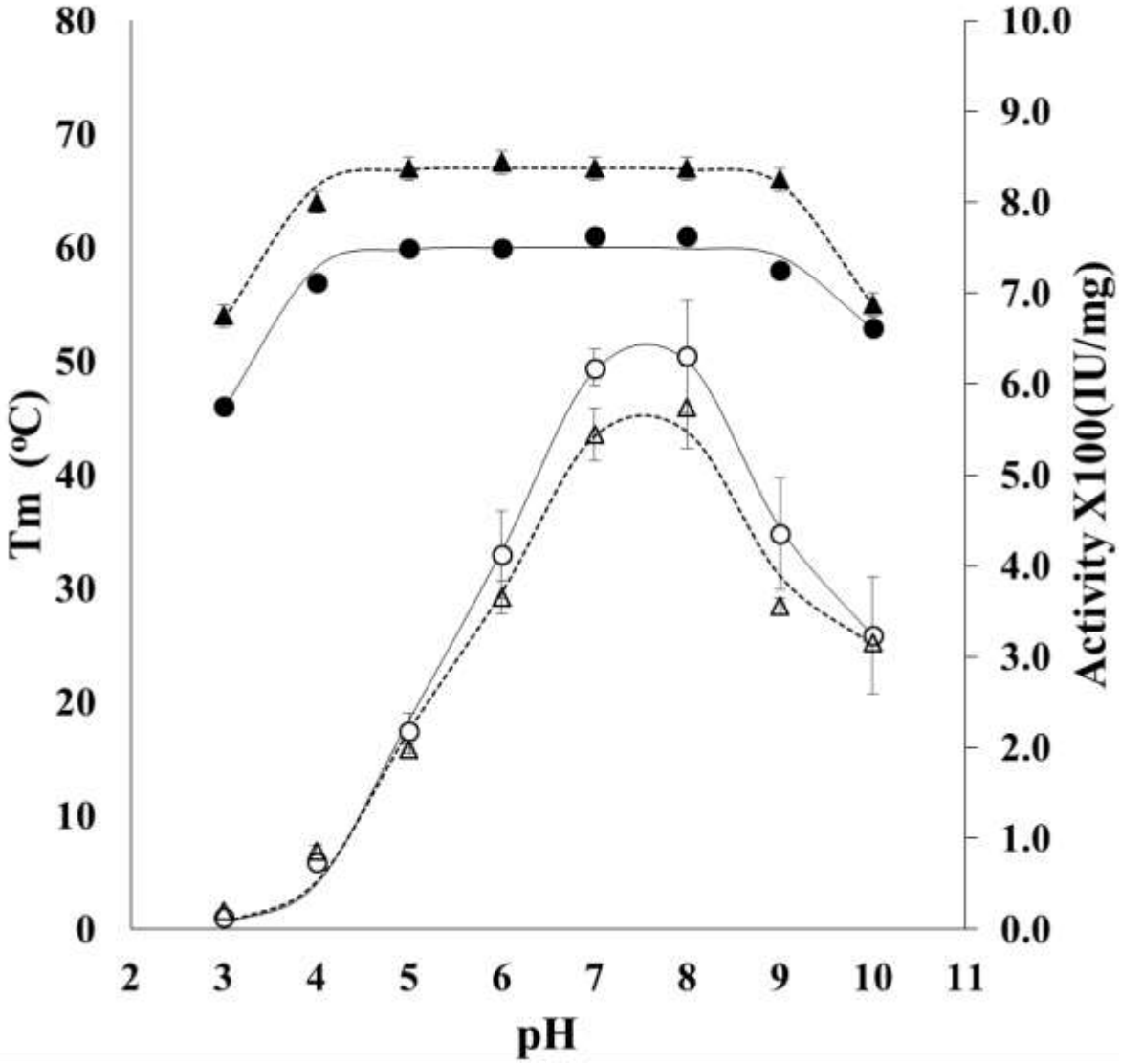

Figure S2 - Comparison of pH optima for the activity and stability of Variant 3 and wt-AoC (circles, wt-AoC; triangles - Variant 3; filled symbols - T_m; open symbols, PCL hydrolysis activity). The lines are fits to the Henderson-Hasselbalch equation for two ionizable groups (T_m data) and at least four ionizable groups (activity data). Variant 4 and wt-AoC have equivalent pKa values.