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Substrate promiscuity of a *de novo* designed peroxidase

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Supplementary information

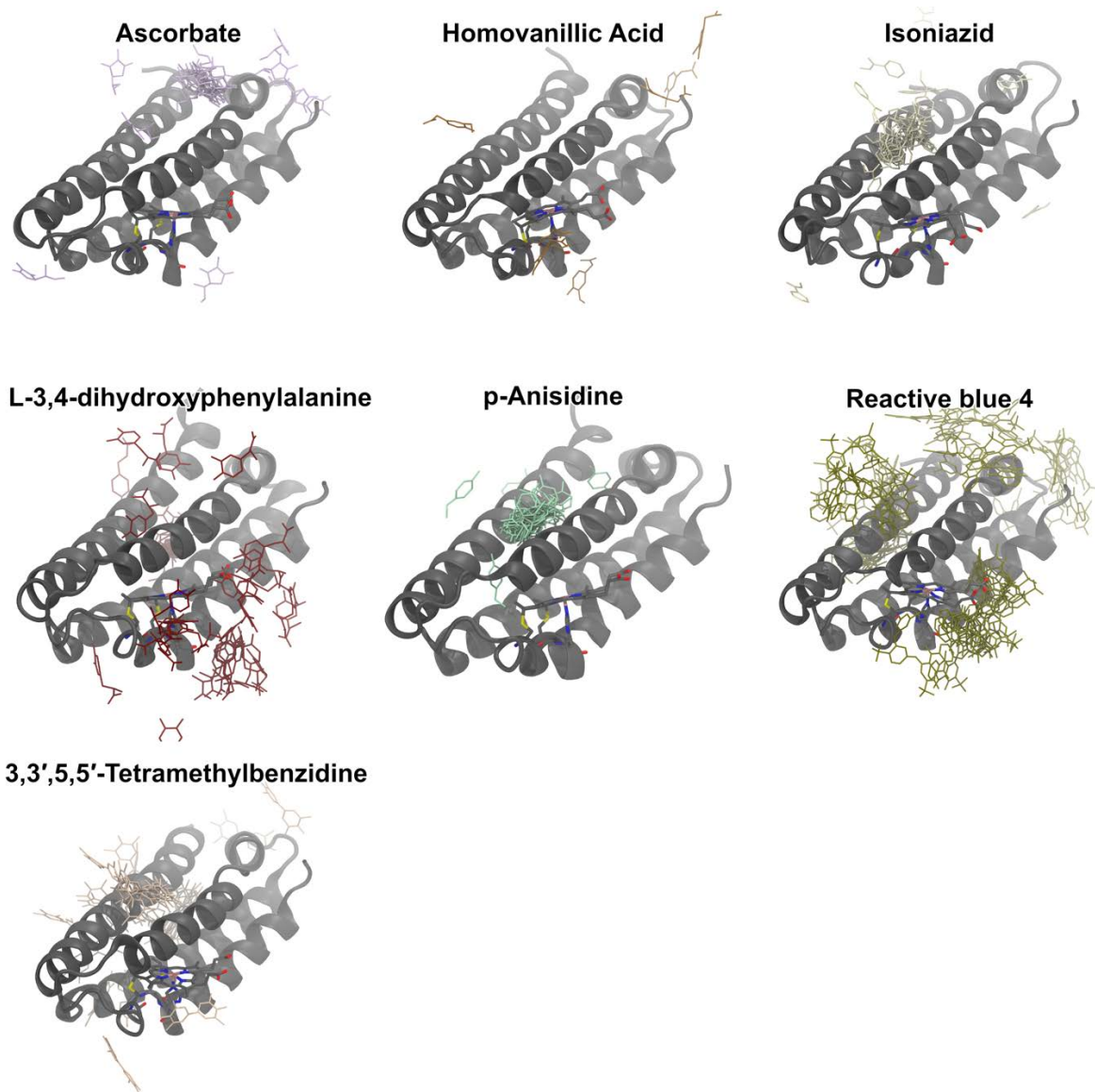
Includes:

SI Figures 1-3

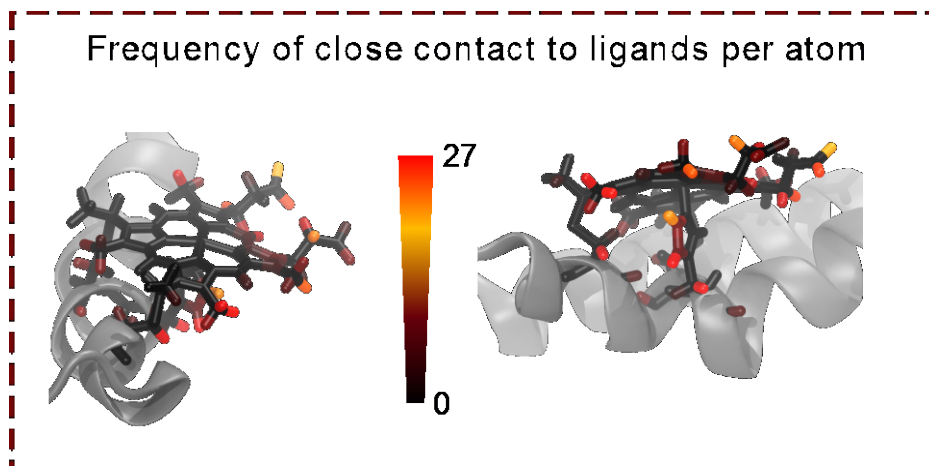
SI Table 1

SI References

SI Figure 1. Computational binding screen results for individual substrates (part 1). An overlay of poses associated with protein after 5 ns is shown for each substrate.



SI Figure 2. Computational binding screen results for individual substrates (part 2). An overlay of poses associated with protein after 5 ns is shown for each substrate.



SI Figure 3. Frequency of close contacts between screened substrates and the C45 heme. Heat map of C45 displaying the frequency of close contacts between substrates and the *c*-type heme of C45.

	E^0 oxidation potential (mV vs <u>N</u> SHE)	Conditions	REF
Cytochrome <i>c</i> Fe ³⁺ /Fe ²⁺	261	pH 8.3	[1]
<i>o</i> -Phenylenediamine	400	pH 7.0	[2]
Homovanillic Acid	430	pH 7.0	[3]
Amplex Red	600	pH 7.4	[4]
Guaiacol	680	pH 7	[5]
Luminol	750	pH 9	[6]
ABTS	760	pH 4	[7]
<i>p</i> -Anisidine	766	pH 5.1	[8]
<i>o</i> -Dianisidine	809	In water	[9]
Reactive Orange 2	868	pH 8	[10]
TCP	930	pH 5.1	[11]
HRP CPDI/CPDII	949	pH 7.0	[12]
TBP	984	pH 6 90% Ethanol	[13]
HRP CPDII/Fe ³⁺	991	pH 7.0	[12]
C45 CPDI/CPDII	~1000	Indirect pH 8.6 ^a	This work
4-Aminobenzoic Acid	1080	pH 5.1	[8]
CcP CPDI/CPDII	1087	pH5.3	[14]
HRP CPDI/CPDII	1120	indirect low pH	[15]
APX CPDI/CPDII	1156	pH 7	[16]
1,4-Dimethoxybenzene	1300	pH 4.5	[17]
MnP CPDI/CPDII	1300	pH <4	[18]
Veratrole Alcohol	1360	pH 3.6	[19]
LiP CPDI/CPDII	~1450	Indirect ^b	[15]

SI Table 1. Redox potentials of peroxidase substrates and key redox couples of peroxidases. Reduction potentials of tested peroxidase substrates and reactive peroxidase species quoted vs the Nernst Hydrogen Electrode (NHE). Blue shading denotes peroxidase substrates, and yellow shading indicates key oxidising species of horseradish peroxidase (HRP), cytochrome *c* peroxidase (CcP), ascorbate peroxidase (APX), manganese peroxidase (MnP) and lignin peroxidase (LiP). CPDI and CPDII refer to peroxidase compounds I and II respectively. ^a C45 CPDI/CPDII is an estimate based on the substrates it will oxidise. ^b Inferred though reactivity with a series of substrates. The last column contains the literature references (REF).

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