Electronic Supplementary Material (ESI) for Molecular BioSystems.

Table S1. Structural features of AOX and AOXfad and AAO (3fim)

Structural Features	**AOX**	**AOXfad**	**AAO(3fim)**
Sheets	7	8	7
β-α-β unit	1	1	2
β Hairpins	6	6	6
β bulges	3	6	7
Strands	21	24	25
Helices	25	23	26
Helix-Helix Interactions	23	24	18
β turns	104	106	42
γ turns	14	18	6
Disulphide Bridge			1

Legends for Supplementary Figures

Fig. S1. Ramachandran Plot to visualize energetically allowed regions for backbone dihedral angles ψ against φ of amino acid residues in alcohol oxidase model; Residues in most favored regions are 84.8%, and residues in generously allowed regions are 15.2%. There were no residues in generously allowed regions and disallowed regions. The red, dark yellow and light yellow regions represent the favored, allowed, and "generously allowed" regions respectively.

Fig. S2. VERIFY 3D-1D plot (a) AOX model without FAD cofactor (AOX). The plot shows more than 95% amino acids are in good profile region. In AOX residue number 185-198, 370 and 552-555 are present in negative 3D-1D average score. (b) AOX model with FAD cofactor (AOX_{fad}). Residues number 185-199, 470-476 and 650-663 in AOX_{fad} are found to be in the negative regions. Most of these residues fall in the loop region of the modeled structure of AOX and AOX_{fad}.

Fig. S3. Structural Pocket in AOX model as predicted by CASTp program (a) Pocket shown in green spherical atoms and rest of protein shown in wireframe, (b) Structure is shown in hydrophobic surface view using UCSF Chimera program: pocket is sown in green color, FAD in red color and protein in gray color.

Fig. S4. Structure based multiple sequence alignment of AOX model (*Candida boidinii,* AOX*), Alcohol oxidase (*Pichia pastoris,* 5HSA), aryl alcohol oxidase (*Pleurotus eryngii,* 3FIM), formate oxidase (*Aspergillus oryzae*, 3Q9T), glucose oxidase (*Aspergillus niger,* 1GAL) and choline oxidase (*Arthrobacter globiformis*, 2JBV). MultiAlignViewer feature of Chimera program was used for alignment. Highly conserved residues are highlighted in red color and partially conserved in yellow color. Red star on the residue indicating FAD binding sites in AOX* (*Candida boidinii*). Common FAD binding sites in the GMC family enzymes are highlighted in green boxes. Gapped

alignment has showed more number of residues (blue boxes) in AOX* compared to the other family member enzymes. Most of these residues constitute the loop regions in AOX*. Figure was generated by using ESPript utilizing the ENDscript server[1].

Fig. S5. Topological diagram for AOX and its complexes with cofactor and ionophores; (a) AOX, (b) AOX+FAD, (c) AOX+CCCP , (d) AOX+DNP. Topology has shown that two helixes (residues 546 to 550 and 177 to 181) in AOX converted into loop after binding with ligands in all complexes. Residue 99 to 101, 130-131 and 148-149 showing additional β-sheets in AOX ligand complexes that was absent in AOX.

Fig. S6. Secondary strucutre wire diagrams for (a) AOX (b) AOX + FAD (c) AOX + CCCP (d) AOX + DNP. Static view of secondary structure elements (α-helices and β-sheets) together with various structural motifs such as β- and γ-turns, and β-hairpins. Residues no. 99-100 and 148-149 has converted to β-strand from γ-turns (a) against (b), (c) & (d). Length of β-hairpin was reduced from 72-81 amino acids to 75-79 amino acids that contributed to the β-strand length increment in all complexes. FAD and inhibitor binding sites are shown by the red dots above the single letter amino acids that are further enclosed in red boxes.

Fig. S7. Graphical representation of solvent accessibility of amino acid residues in proteins; (a) ASAview graphical representation of AOX Model without FAD (AOX), (b) ASAview graphical representation of AOX Model with FAD (AOX_{fad}), SASA positions of tryptophan (W) residues are shown by the red arrow and particular number is highlighted in the red box outside the spiral graph.

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[1] X. Robert and P. Gouet, Nucleic Acids Res., 2014,42 (Web Server Issue) W320-324. DOI:10.1093/nar/gku316

Fig. S8. Principal component PC1 vs PC2 plot: (a) AOX (b) AOX_{FAD} (c) AOX_{CCP} (d) AOX_{DNP}. Trajectories of AOX and each complex were projected onto the plane formed by the first two PCs (PC1 & PC2). AOX showed wider distribution of conformations along the PC1 vs PC2 plane compared to other complexes of all the ligands studied in this work. Projection of trajectory among all the systems displays unidirectional conformation changes.

Fig. S9. The large concerted motions of AOX and its complexes described by the first principal component. Ensemble of structures represents PDB format trajectory of PC1 for AOX and its complexes. The color of the structure ensemble is according to the B-factor value, red color shows high mobility, while the blue color shows low mobility of structure. The scale bar is shown at the center of the image. Positions of ligands interacting with AOX are shown in green color in respective complex. Each panel of the ensemble is represented as (a) AOX, (b) AOX+FAD, (c) AOX+CCCP , (d) AOX+DNP.

Supplementary Figures

Figure S1

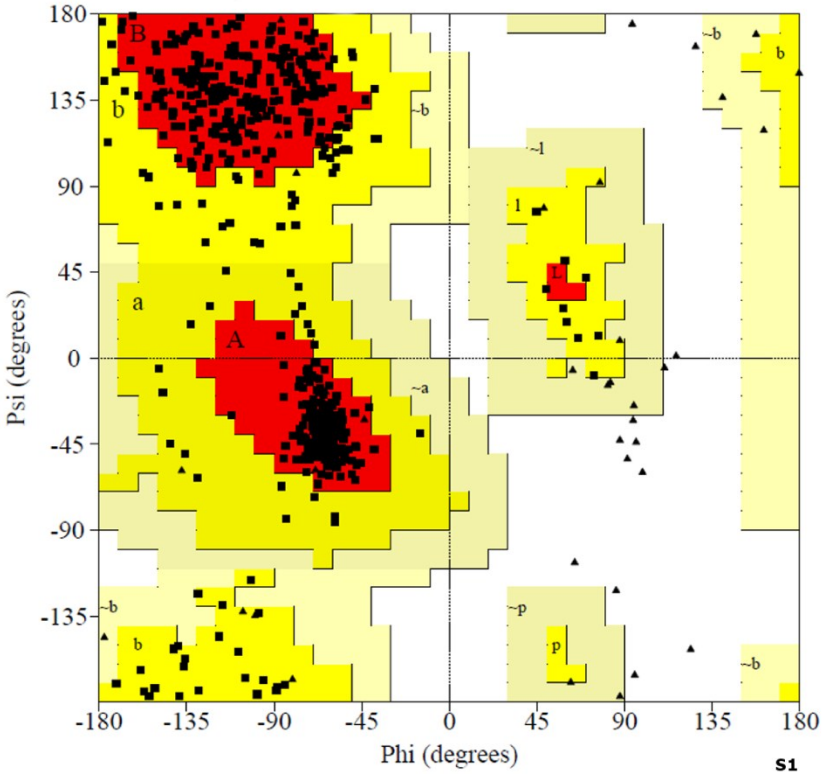
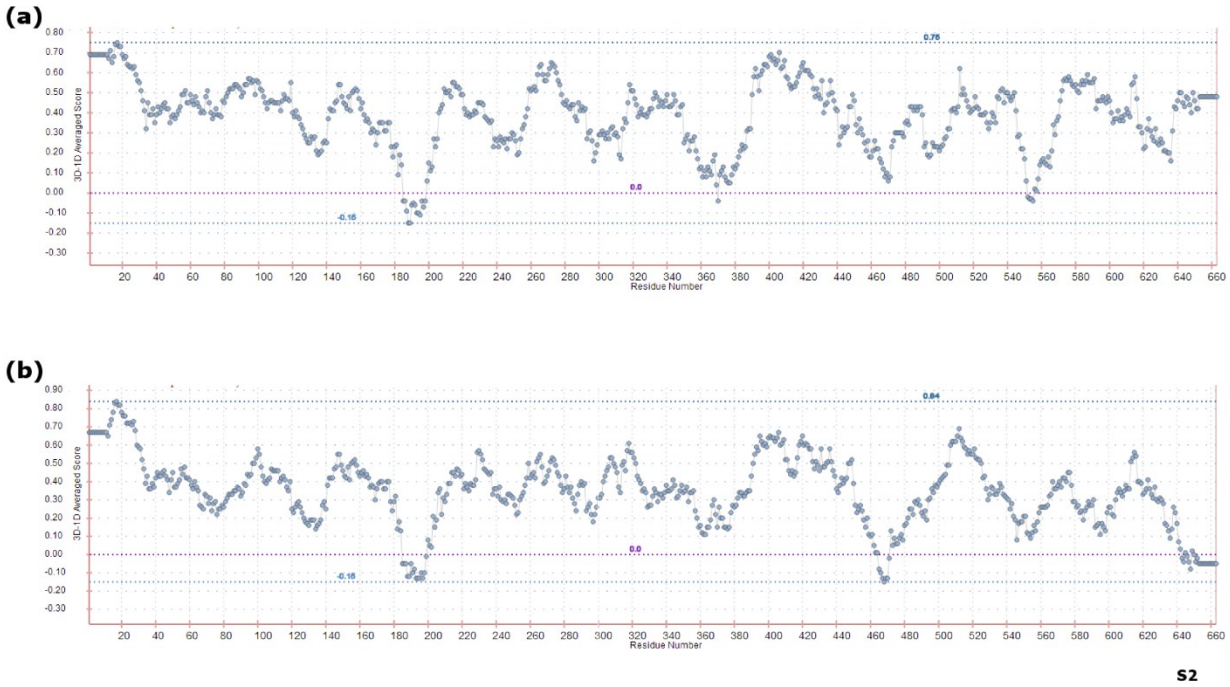


Figure S2


(a)
3D-1D Averaged Score
Residue Number
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0.0
-0.16
(b)
3D-1D Averaged Score
Residue Number
0.84
0.0
-0.16

S2

Figure S3

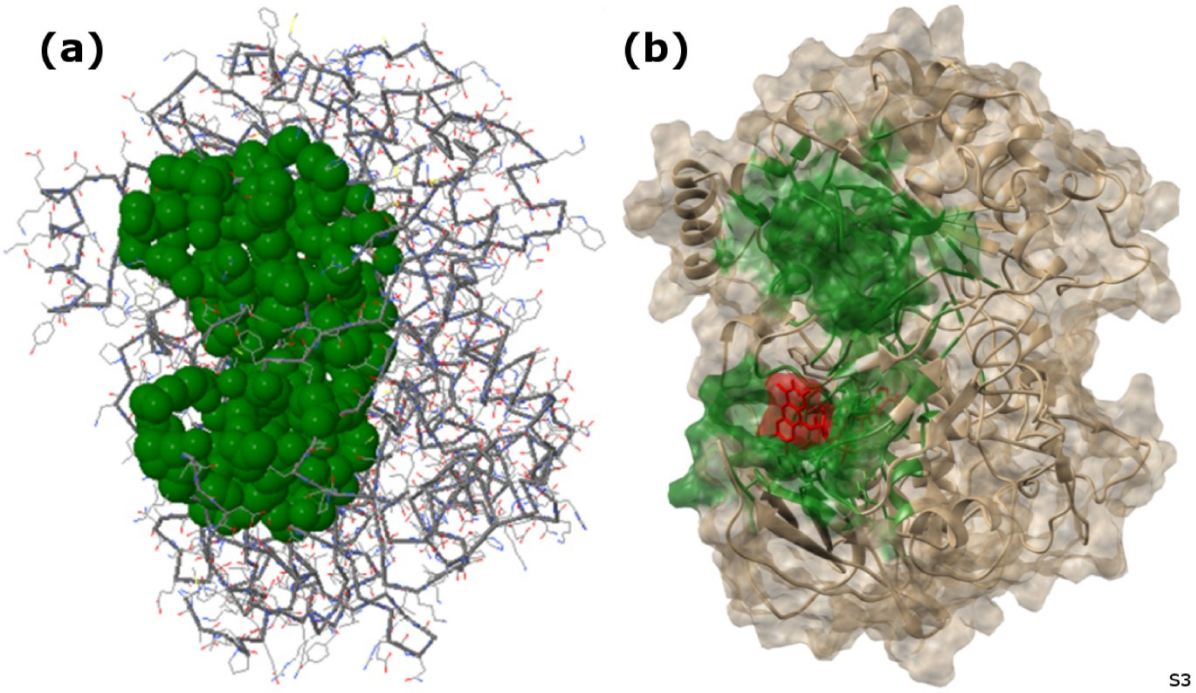

Figure S4

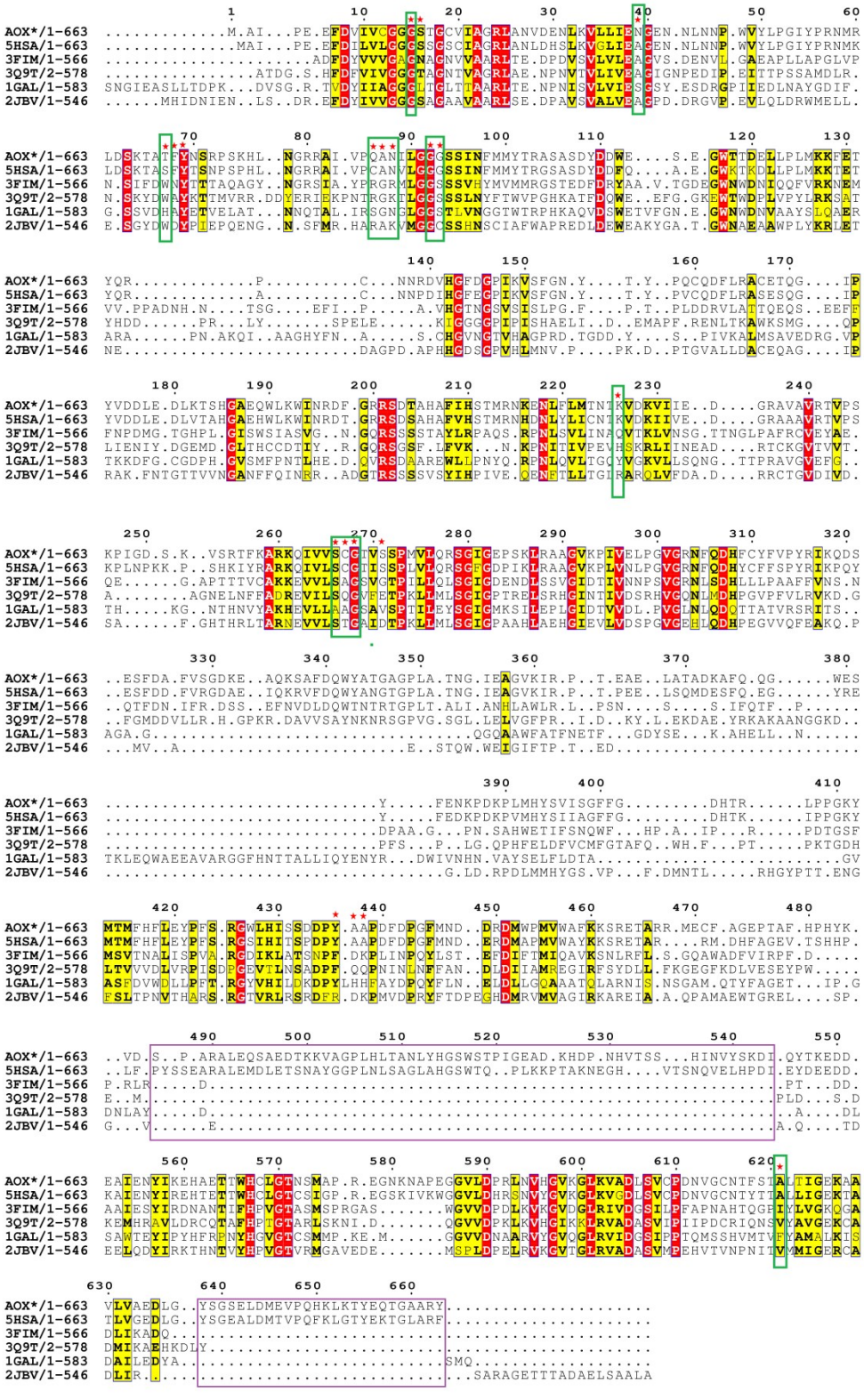

Figure S5

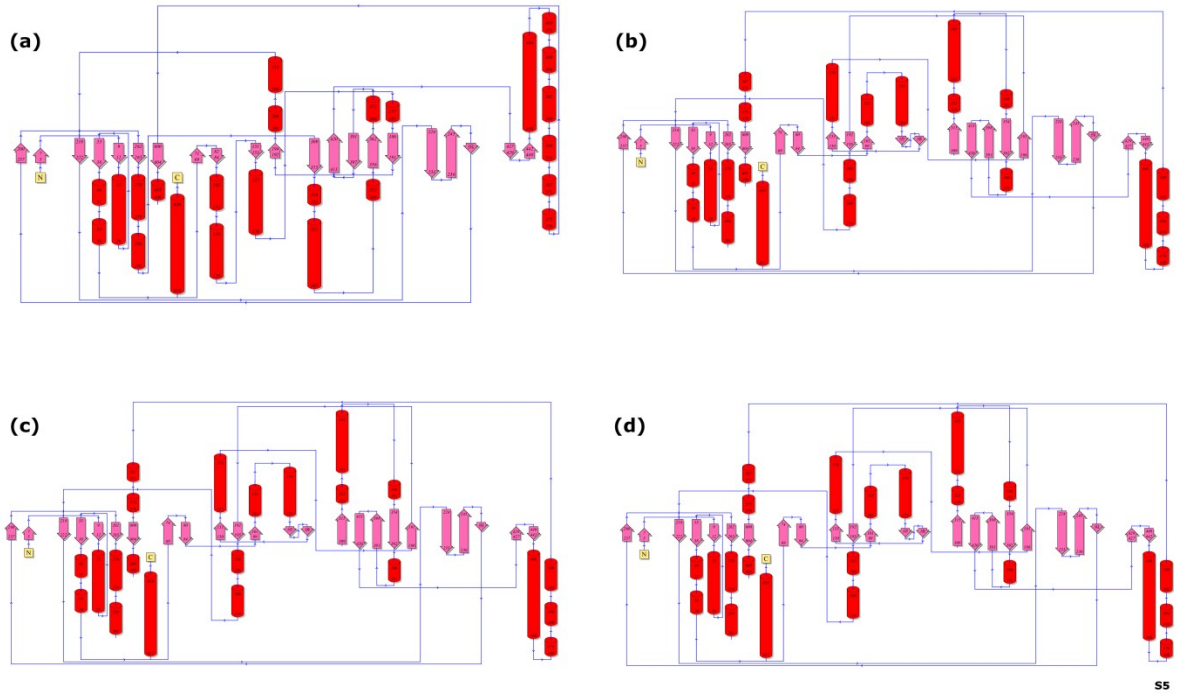

Figure S6

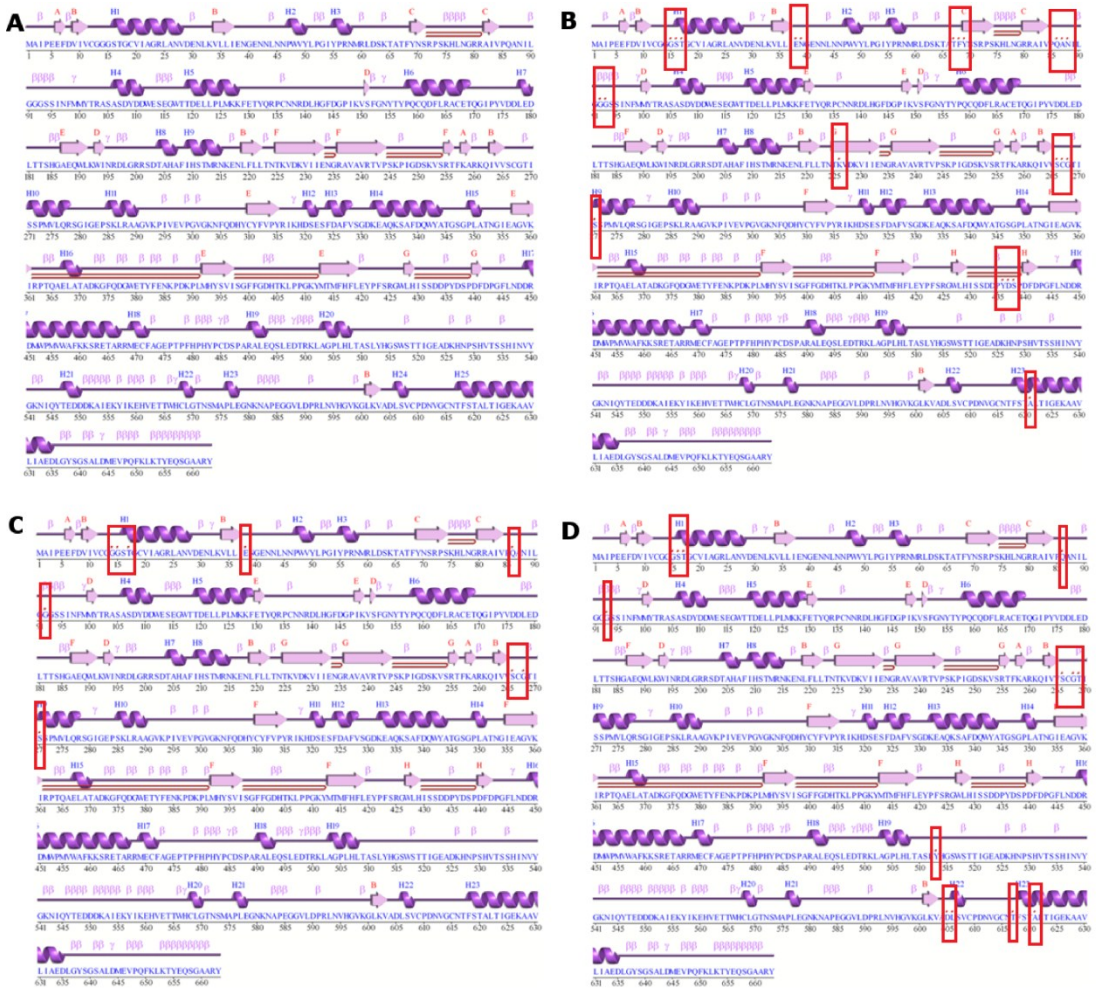

Figure S7

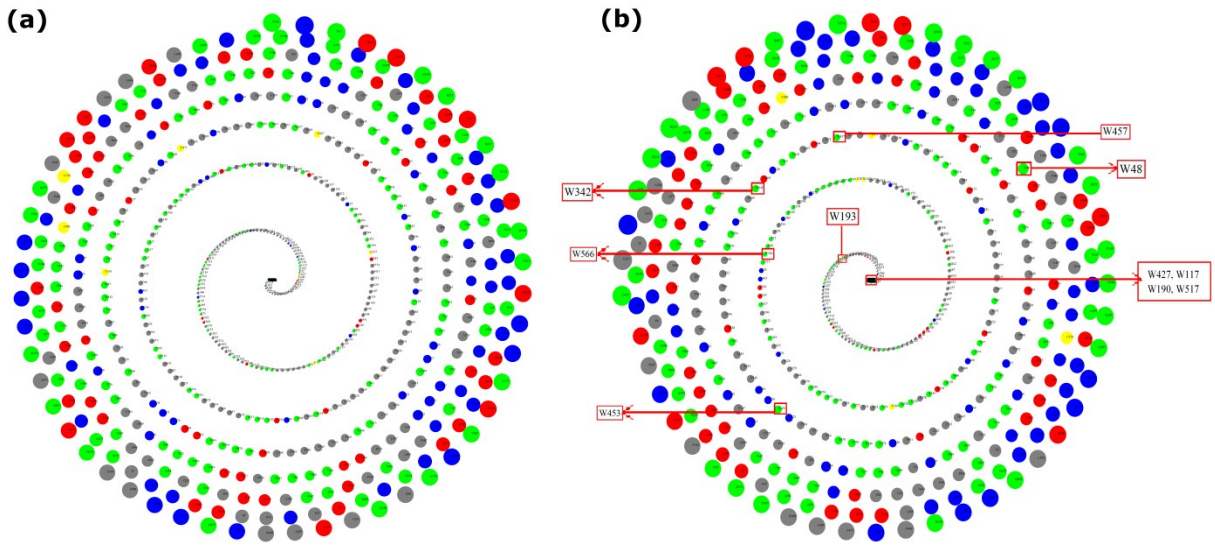

Figure S8

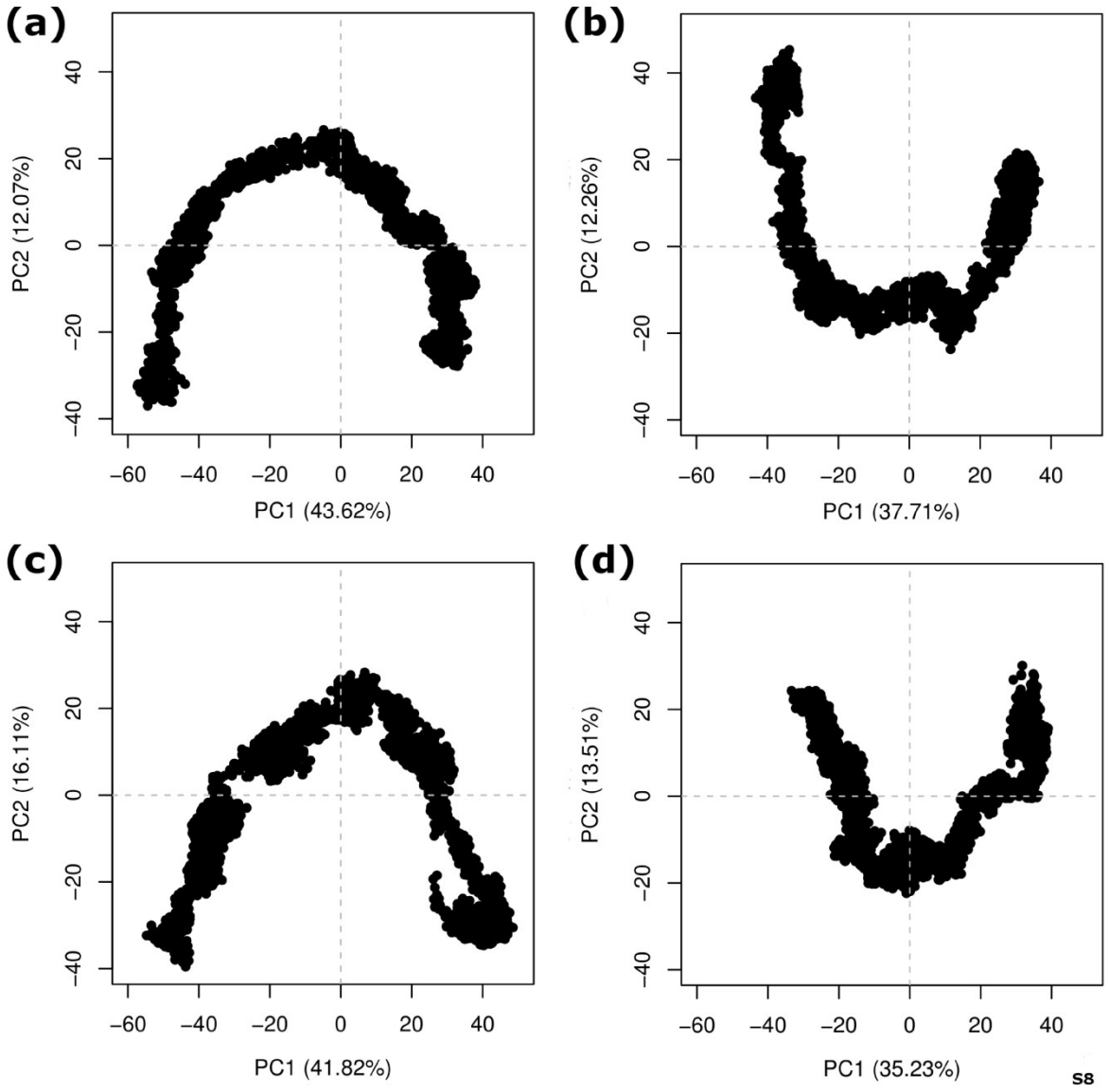
(a)
PC2 (12.07%)
PC1 (43.62%)
(b)
PC2 (12.26%)
PC1 (37.71%)
(c)
PC2 (16.11%)
PC1 (41.82%)
(d)
PC2 (13.51%)
PC1 (35.23%)
S8

Figure S9