Supporting Information

Formyl-coenzyme A (CoA):oxalate CoA-transferase from the acidophile *Acetobacter aceti* has a distinctive electrostatic surface and inherent acid stability

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Running title: Structure of *A. aceti* UctB

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Table I: Comparison of selected FCOCT · CoA crystal structures.

	H6UctB 3ubm	YfdW 1q6y	FRC 1p5r
Accessible surface area (Å^2)[a]	31 746	29 968	31 039
Non-polar (%)	48	52	48
Polar (%)	36	36	36
Charged (%)	15	12	15
Positive N (%)	8.2	5.3	6.8
Negative O (%)	7.2	7.0	8.6
Packing[b]			
Expected/observed volume (%)	91	97	98
No. packing defects	304	152	145
No. buried charges	32	32	36
Hydrogen bonds[c]			
Total no. (excluding solvent)	460	449	442
No. inter-subunit[d]			
All contacts	51	56	55
Contacts involving side chains	41	44	45
Buried residue pairs	10	16	12
No. intra-subunit[e]			
All contacts	409	393	387
Contacts involving side chains	163	153	141
Buried residue pairs	41	41	44
Salt bridges[f]			
No. inter-subunit			
All residues	1	4	5
Buried residue pairs	0	0	0
No. intra-subunit			
All residues	33	38	36
Buried residue pairs	7	6	2

[a] Values computed for the AB dimer using PyMOL.
[b] Values computed for the AB dimer using VADAR.
[c] Values computed for subunit A.
[d] Values computed using PDBSUM.
[e] Values computed using HBPLUS.
[f] Values determined for the AB dimer by manual inspection using a 3.5 Å cutoff.

Table II: Differential analysis of selected surface residues.[a]

	Residue			Category[b]			RSCASA[c]	Contacts[d]		
Entry	3ubm	1q6y	1p5r	3ubm	1q6y	1p5r	(%)	3ubm	1q6y	1p5r
1	A087	E083	E081	S	–	–	75.5	sol	sol	sol
2	W095	E091	K089	H	–	+	17.2	sol	sol	SM
3	Q120	E116	E114	P	–	–	84.2	sol	sol	sol
4	S143	K139	K137	P	+	+	58.8	sol	SS[e]	SL
5	n176	d159	d157	P	–	–	24.7	SS[e]	sol	sol
6	d283	p266	a276	–	S	S	36.5	SS[e]	sol	sol
7	K313	A296	A306	+	S	S	65.3	sol	sol	sol
8	T339	D322	D332	P	–	–	57.8	sol	SS	SM+SS
9	H346	Y329	W339	+	H	H	28.9	SM	SM	SS[f]
10	K349	Q332	Q342	+	P	P	83.6	sol	sol	sol
11	R351	F333	Y343	+	H	H	88.2	sol	sol	sol
12	A366	D349	D359	S	–	–	26.1	sol	SS	SM
13	D368	S351	S361	–	P	P	19.7	sol	SS	SM
14	k382	l365	i375	+	H	H	65.7	sol	sol	sol
15	Q409	E393	E403	P	–	–	56.6	SM	SM	SS
16	a422	d406	d416	S	–	–	70.9	sol	sol	SS

[a] The residues listed are (1) surface accessible, (2) members of the same residue category in YfdW (1q6y) and FRC (1p5r) but are in a different residue category in H6UctB (3ubm), and (3) predicted to alter the surface charge (i.e., at least one of the categories is charged). Residues located at structurally conserved positions are shown in capital letters.

[b] Categories: + = positive (HRK); – = negative (DE); H = large/hydrophobic (FILMVW); P = polar (CNQSTY); S = small (AGP). Entry 2 was obtained when charged residues were considered as a single category. Entries 9 and 11 were obtained when Tyr was grouped in the H set. Other changes to the groupings did not yield new entries.

[c] Relative side-chain accessible surface area in H6UctB, computed using NACCESS using default parameters derived from sidechain areas in Ala-X-Ala tripeptides.

[d] Type of side chain interaction(s): sol = solvent-exposed; SL = side chain/ligand; SM = side chain/main chain; SS = side chain/side chain. All three roles are given where there is any difference.

[e] Contacts a residue in the partner subunit.

[f] W339 forms a π-cation interaction with K331 N^{ϵ}.

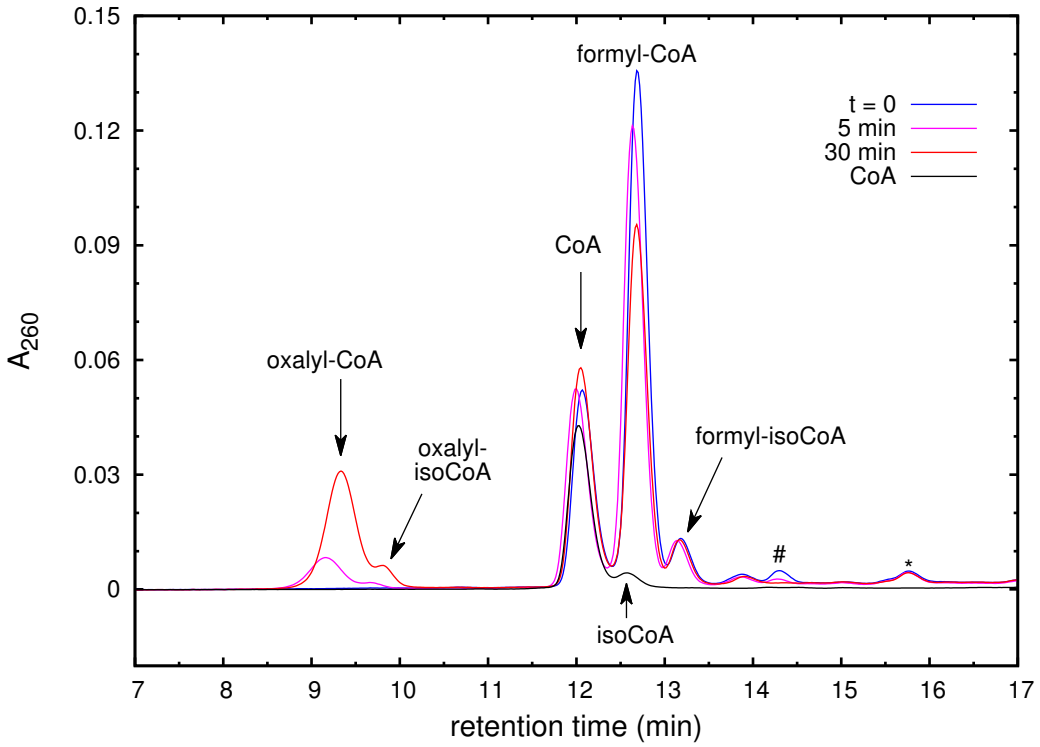

Figure 1: A representative HPLC time series used for FCOCT activity measurements. Indicated (acyl-)CoA peaks were identified by reference to authentic standards, except for oxalyl-(iso)CoA. The peak labeled with an asterisk has the same retention time as acetyl-CoA, which is a potential contaminant. However, acetyl-CoA was not detected in mass spectrometric analysis of the synthetic formyl-CoA, and the unassigned peak remains a constant size throughout the assay. The peak labeled with a pound sign is unassigned and was observed to decrease over time in the absence of enzyme.

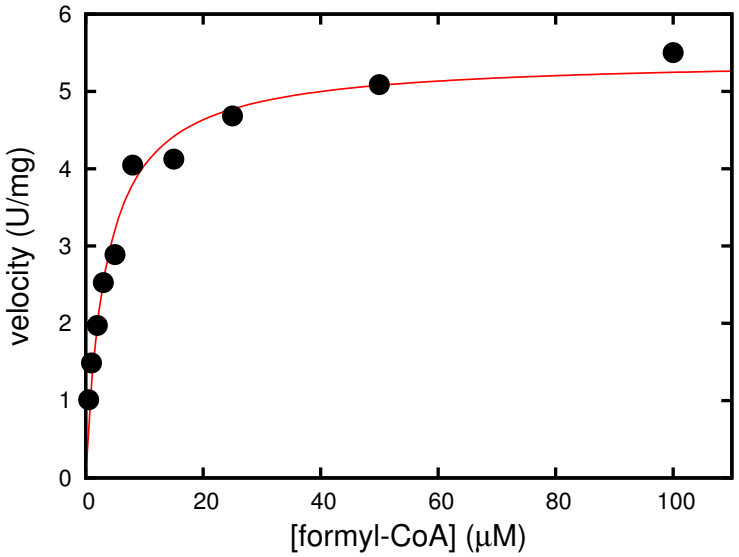

Figure 2: Saturation curve for formyl-CoA determined at 50 mM oxalate with 20 – 100 ng H6UctB.

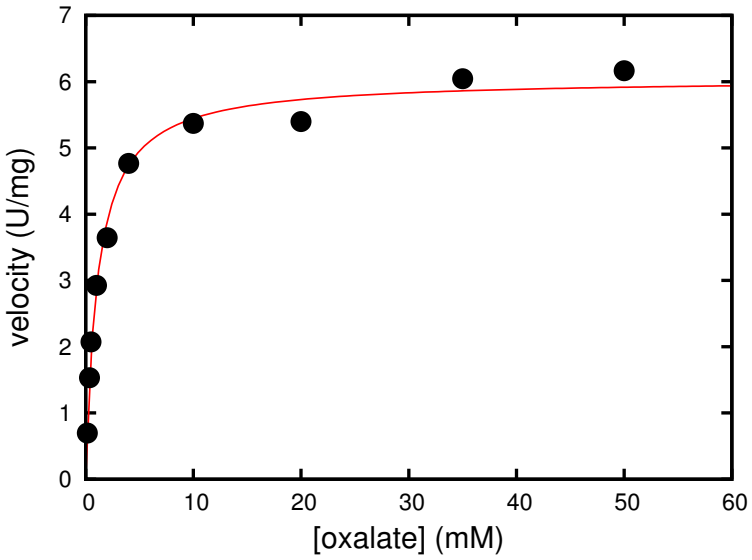

Figure 3: Saturation curve for oxalate determined at 0.1 mM formyl-CoA with 100 ng H6UctB.

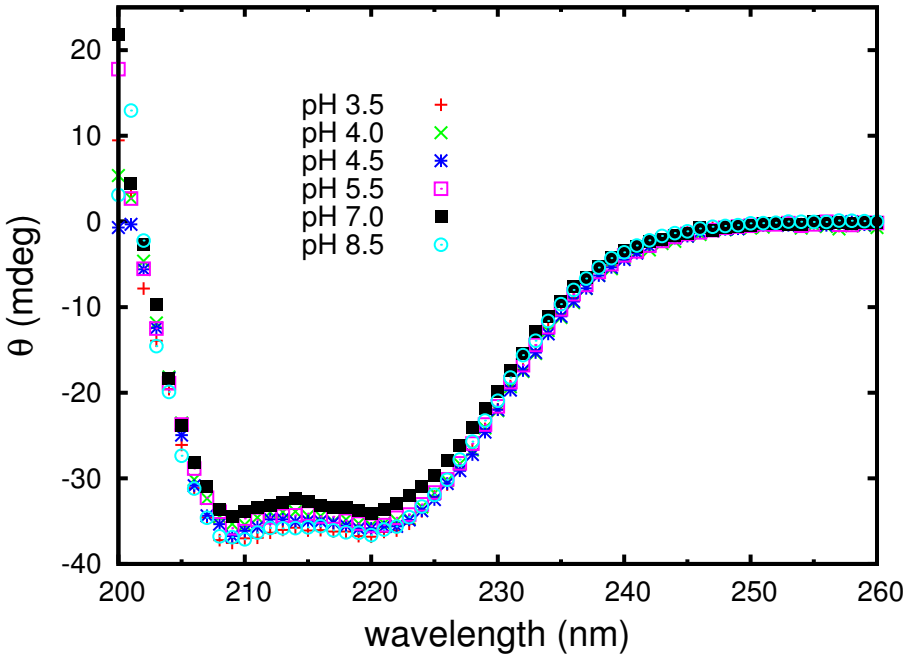

Figure 4: Ultraviolet CD spectra for H6UctB acquired at 20 °C at the indicated pH values.

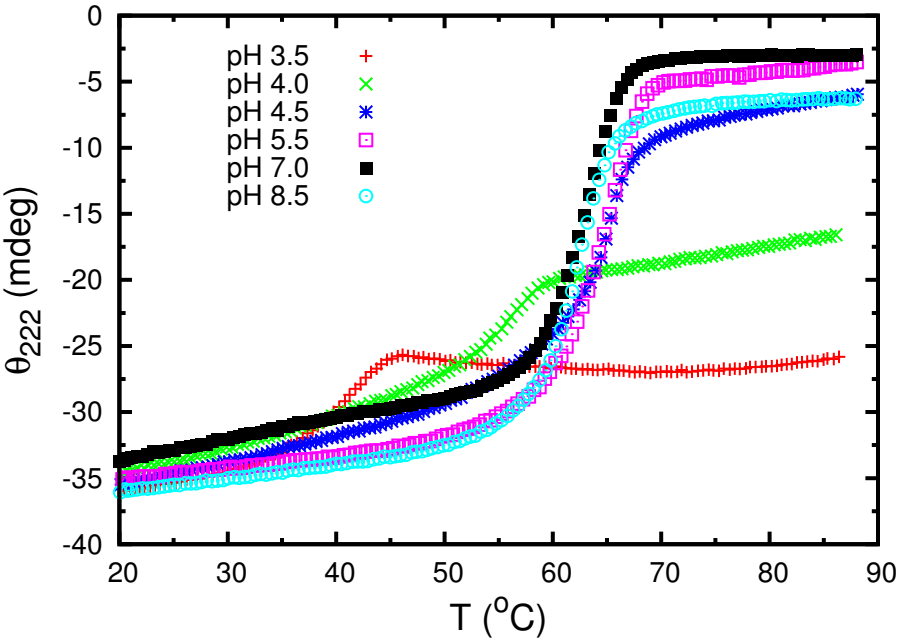

Figure 5: Thermal denaturation profiles used to measure T_m values for H6UctB.

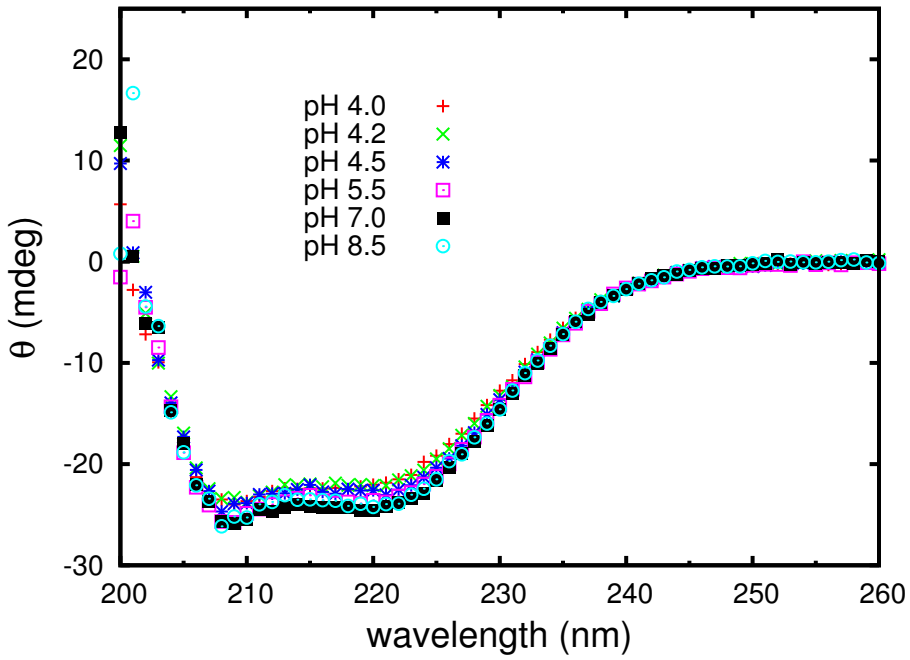

Figure 6: Ultraviolet CD spectra for H6YfdW acquired at 20 °C at the indicated pH values.

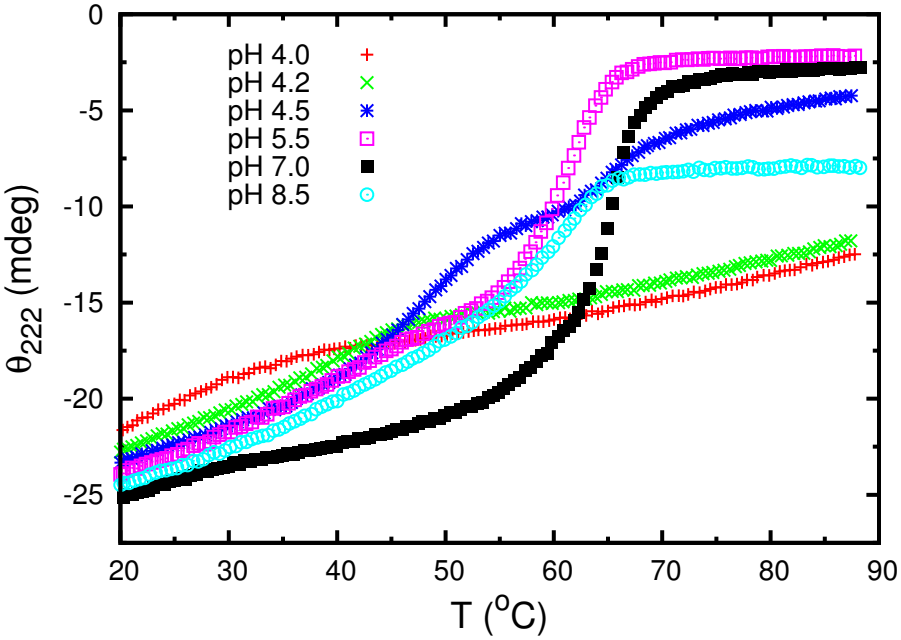

Figure 7: Thermal denaturation profiles used to measure T_{m} values for H6YfdW.

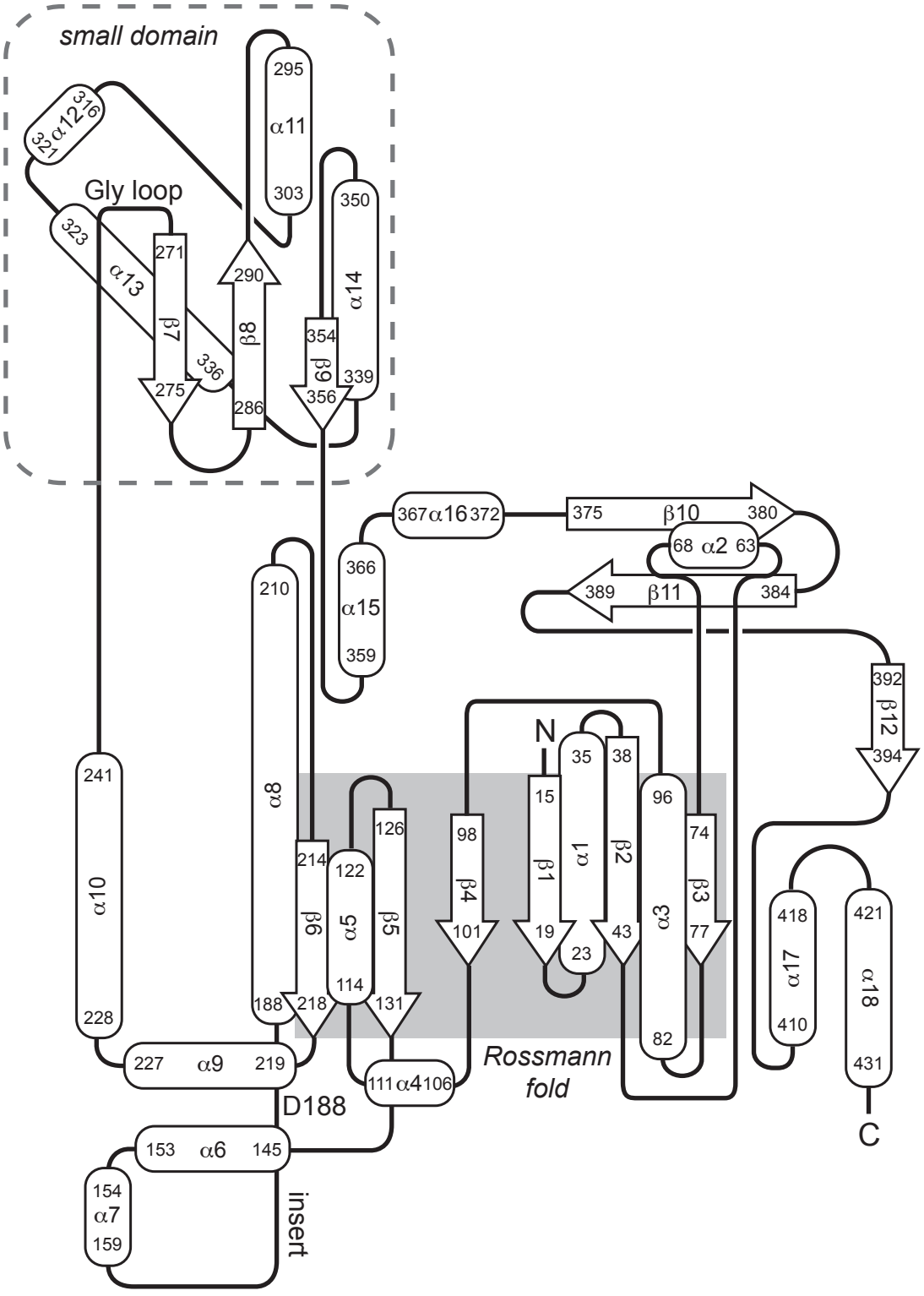

Figure 8: Topology diagram for H6UctB. Strand β12 interacts with strand β6 of the partner subunit.

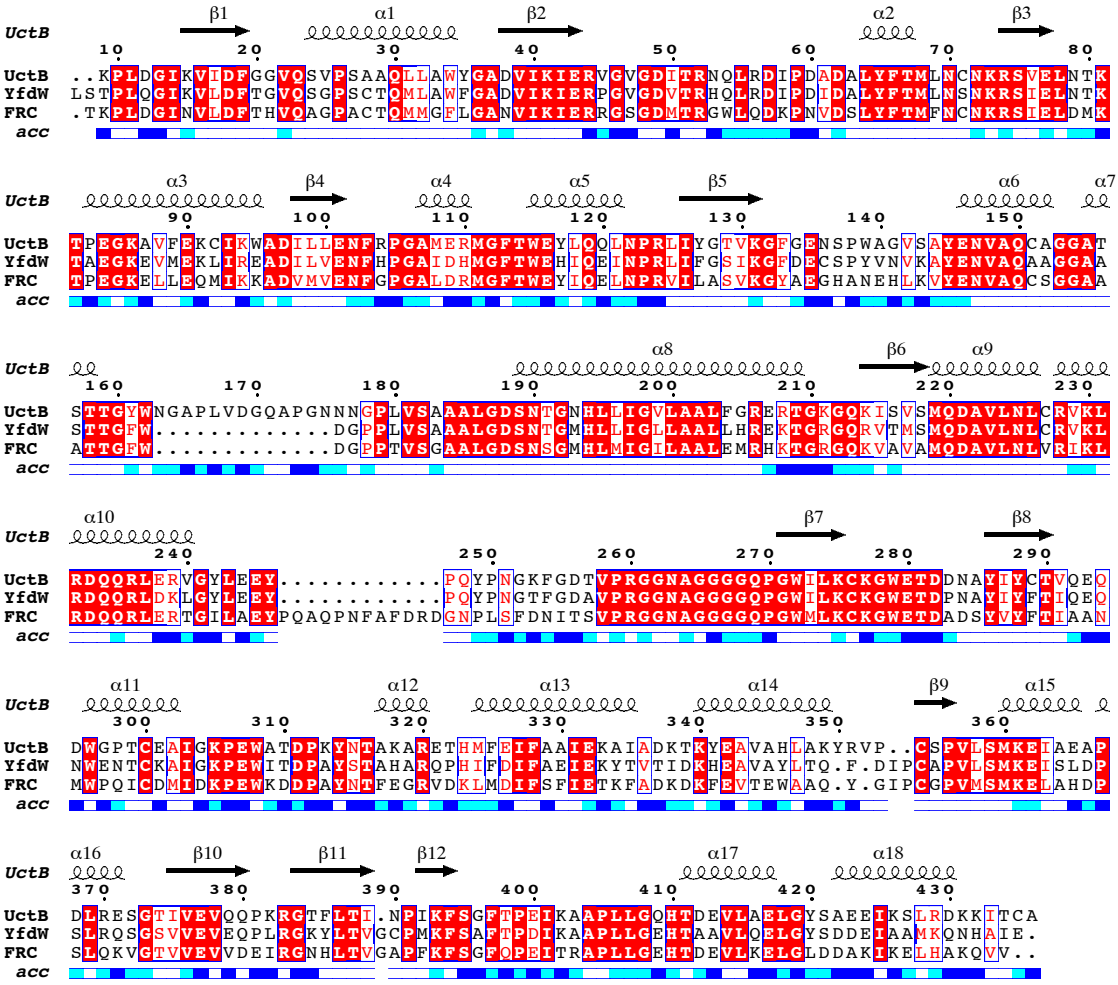

Figure 9: Structure-based sequence alignment of FCOCT enzymes. UctB, *A. aceti* H6UctB (PDB id 3ubm); YfdW, *E. coli* YfdW (PDB id 1q6y); FRC, *O. formigenes* FRC (PDB id 1p5r). The alignment was computed using SSM using subunit A of each structure, all of which are bound to CoA. H6UctB secondary structure assignments (omitting three 3_{10} helices) and relative solvent accessibilities (*acc*) were computed using DSSP implemented within ESPript. Accessible positions ($acc > 0.4$) are dark blue, intermediate positions ($0.4 > acc > 0.1$) are cyan, and buried positions ($acc < 0.1$) are white.

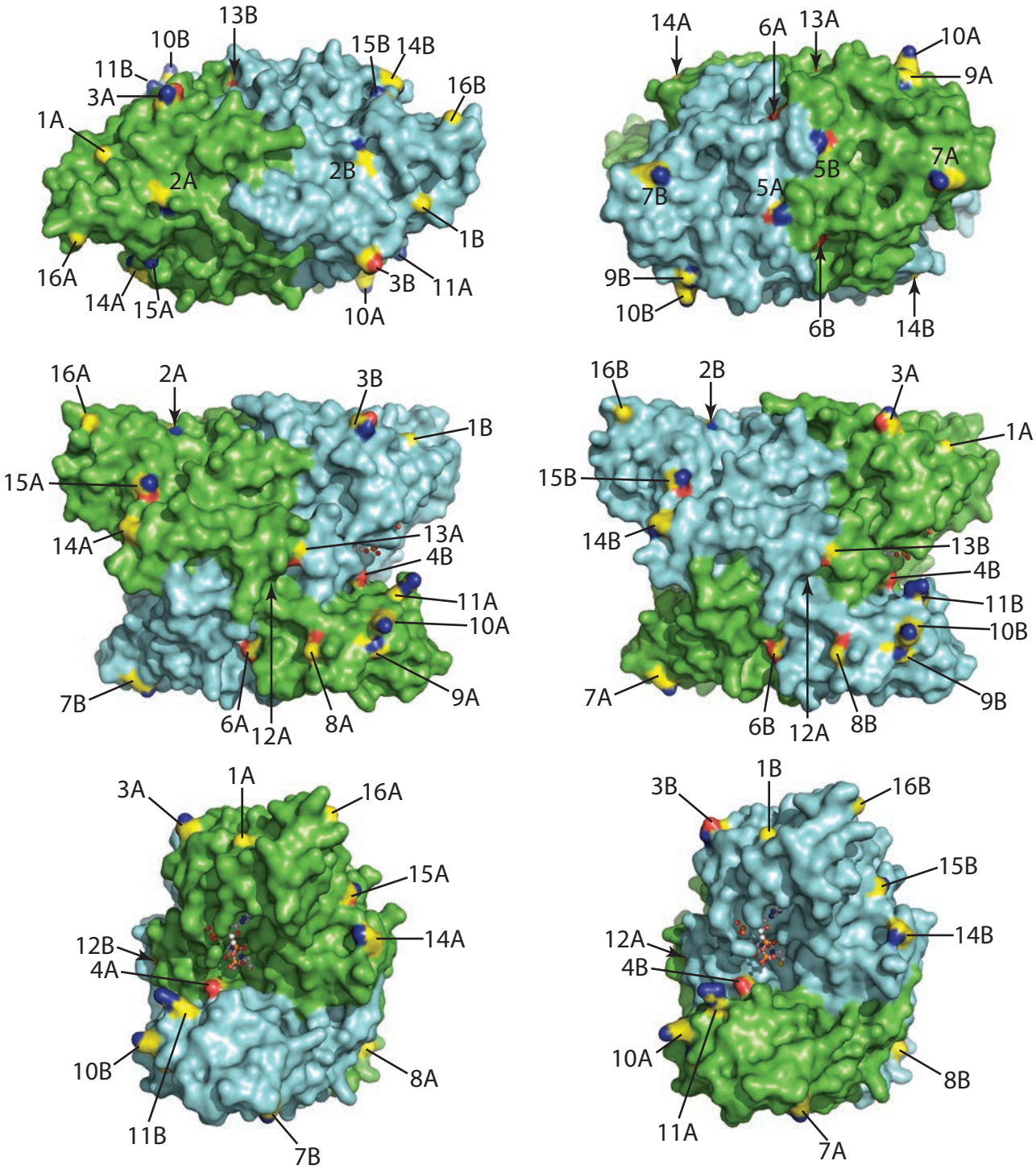

Figure 10: The molecular surface of H6UctB (PDB id 3ubm) colored by chain (chain A, green; chain B, light blue) or by atom for the side chains of residues that alter the surface charge of H6UctB relative to YfdW/FRC. The labels denote entry numbers in Table II and chain (A or B). CoA is rendered as balls and sticks. Rotations on the indicated axis are given relative to the view illustrated in Figure 3B or the left center image. *Top row*, "top" (rotated 90° on x) and "bottom" (rotated 270° on x). *Middle row*, "front" (no rotation) and "back" (rotated 180° on y). *Bottom*, "left side" (rotated 90° on y) and "right side" (rotated 270° on y).

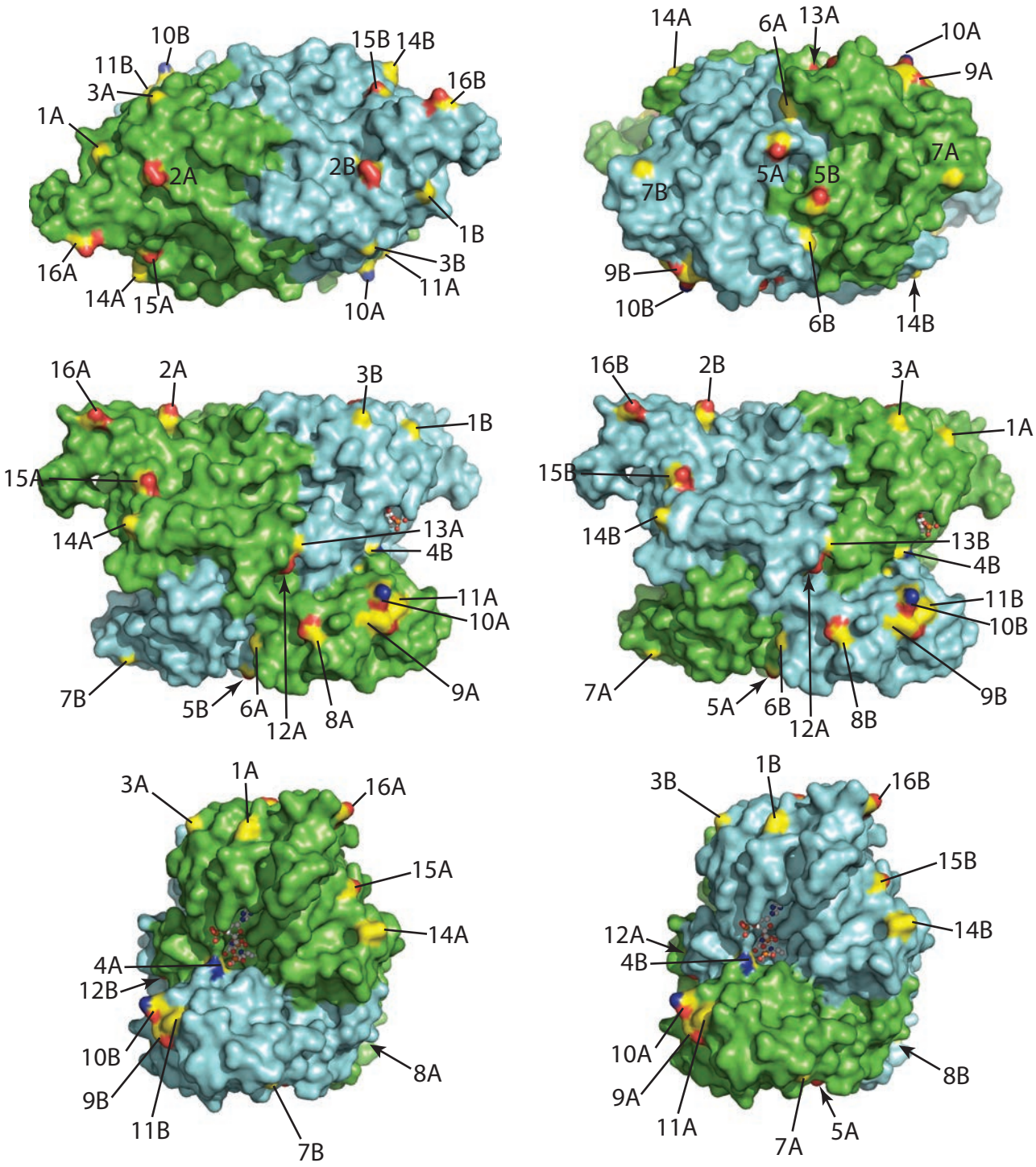

Figure 11: The molecular surface of YfdW (PDB id 1q6y) oriented and rendered as in Supplementary Figure 10. Crystallographic symmetry operators were used to generate the dimeric biological unit from the monomeric asymmetric unit.

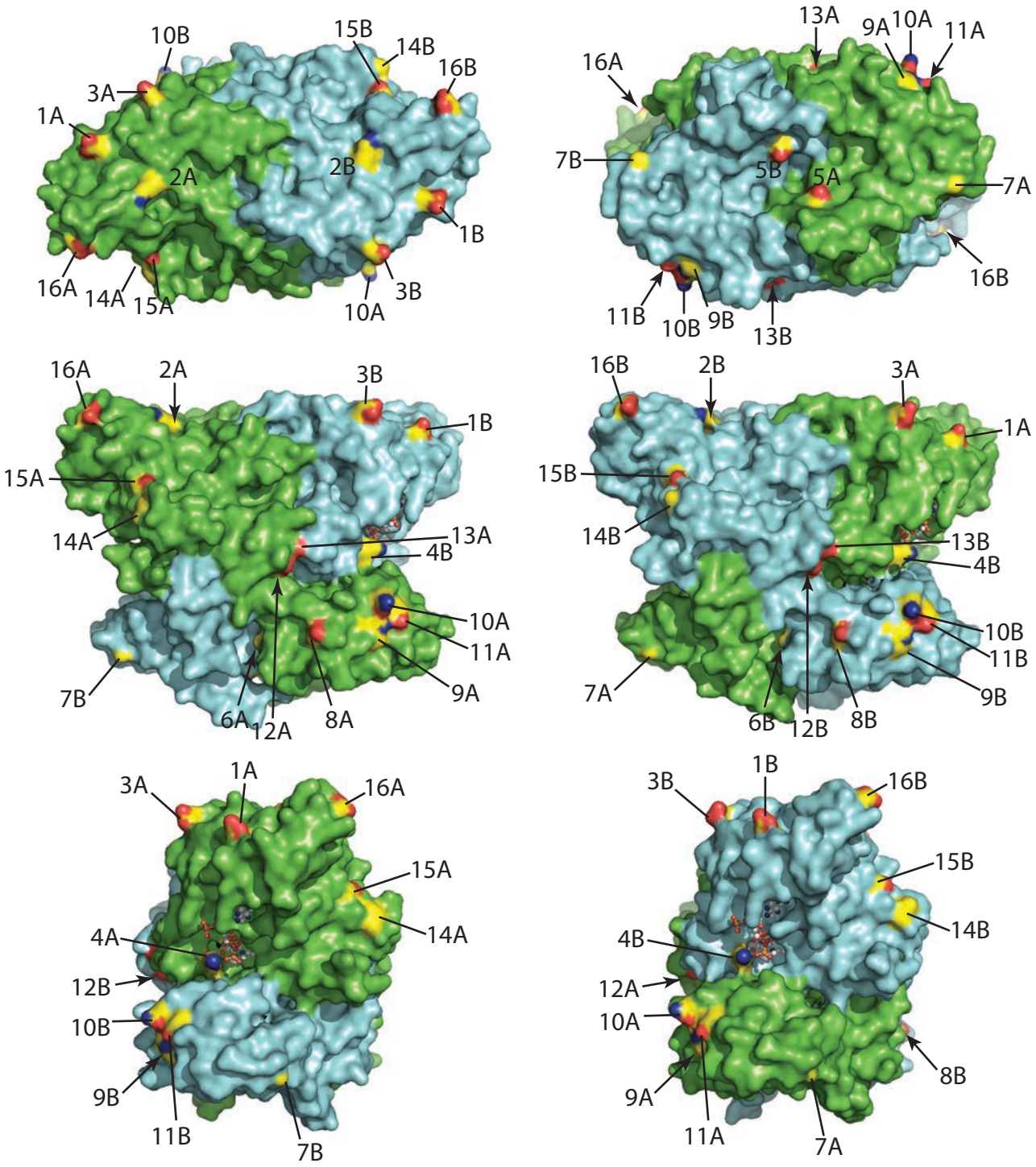

Figure 12: The molecular surface of FRC (PDB id 1p5r) oriented and rendered as in Supplementary Figure 10.

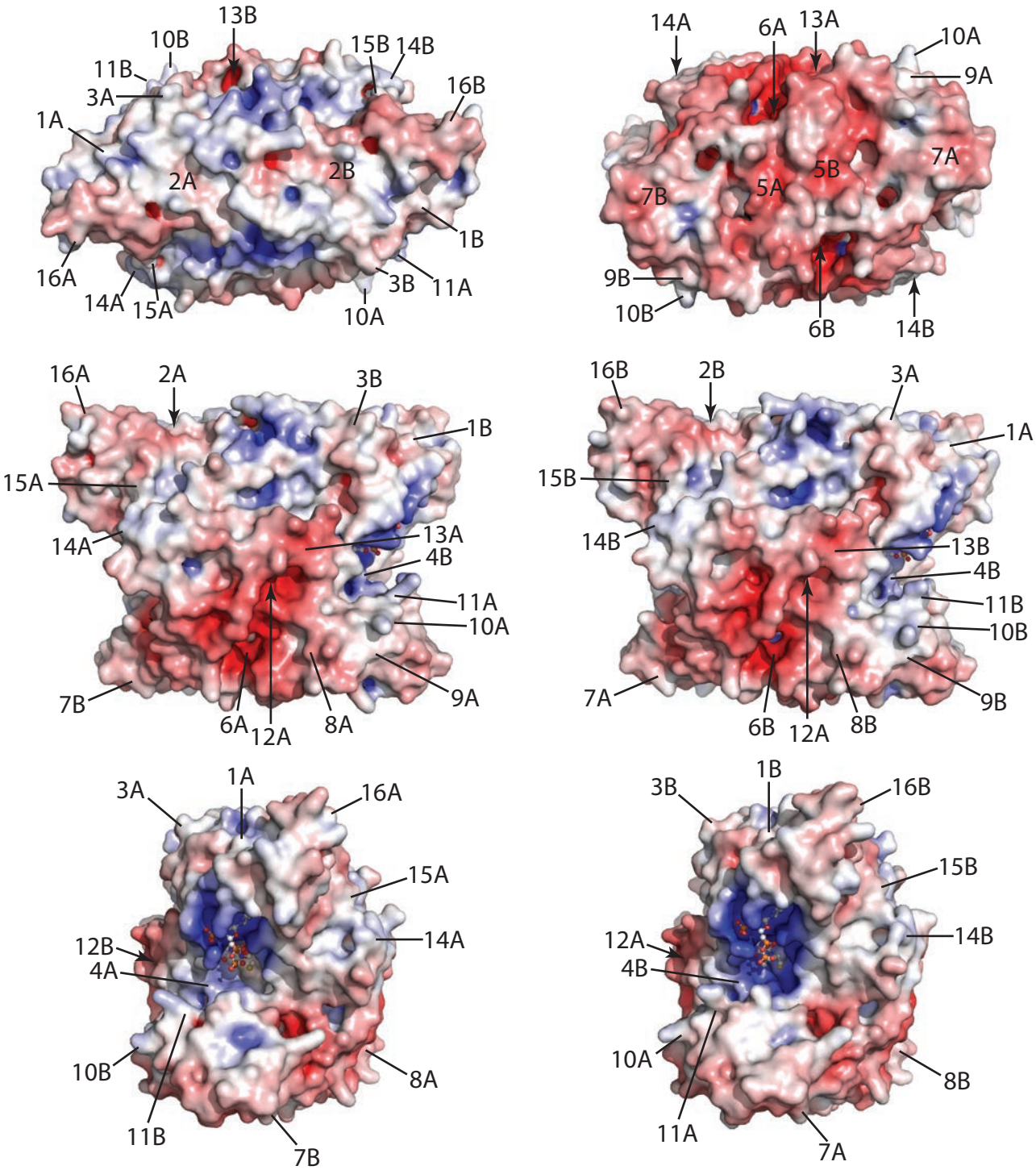

Figure 13: The molecular surface of H6UctB (PDB id 3ubm) colored by electrostatic potential. Negatively and positively charged regions are colored red and blue, respectively. The saturation of the color is proportional to the degree of charge, ranging from -8 to $+8$ kT/e. The surface is otherwise identical to that in Supplementary Figure 10. The left middle depiction is the same as Figure 3B.

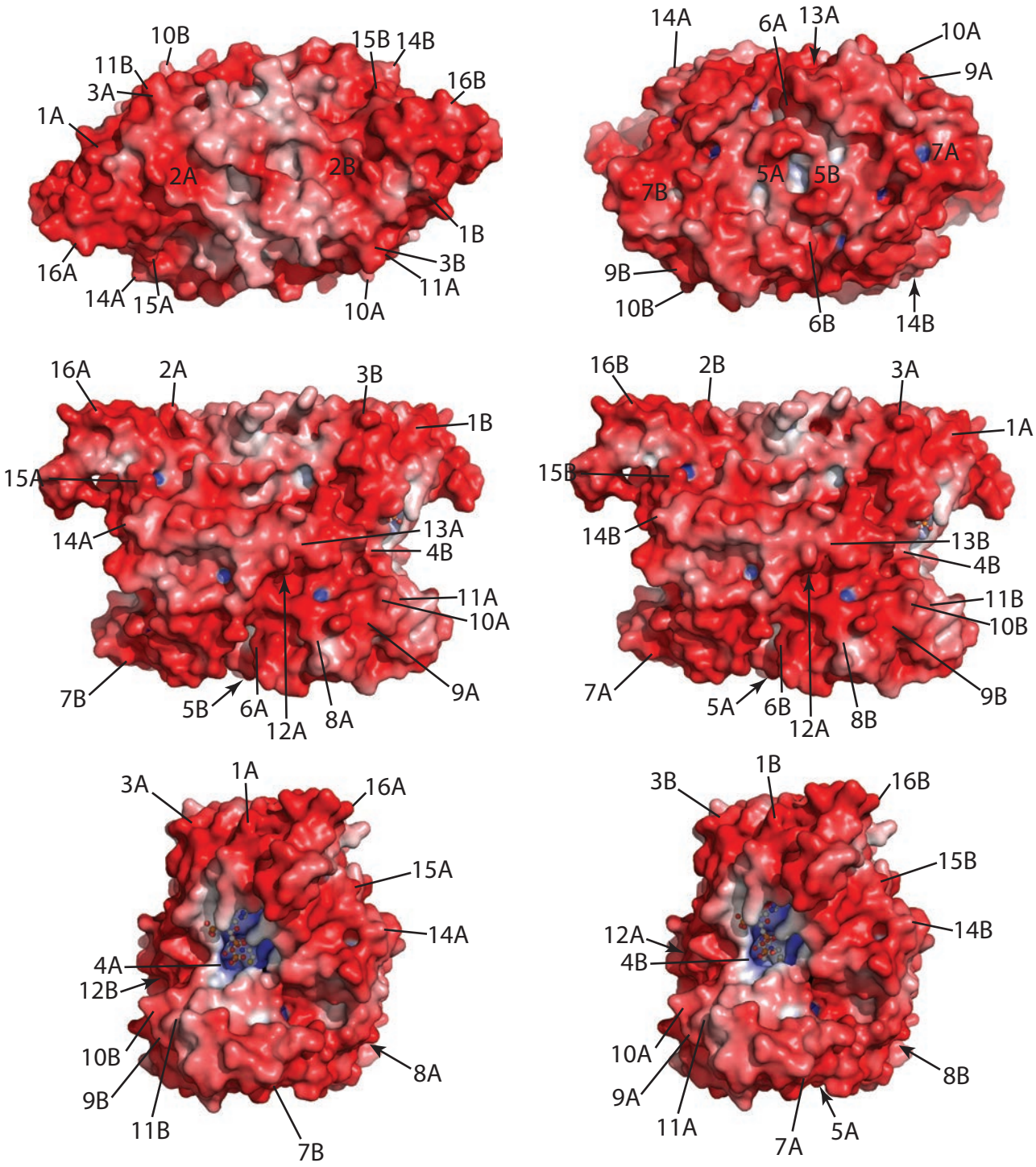

Figure 14: The molecular surface of YfdW (PDB id 1q6y) depicted as in Supplementary Figure 13. Crystallographic symmetry operators were used to generate the dimeric biological unit from the monomeric asymmetric unit.

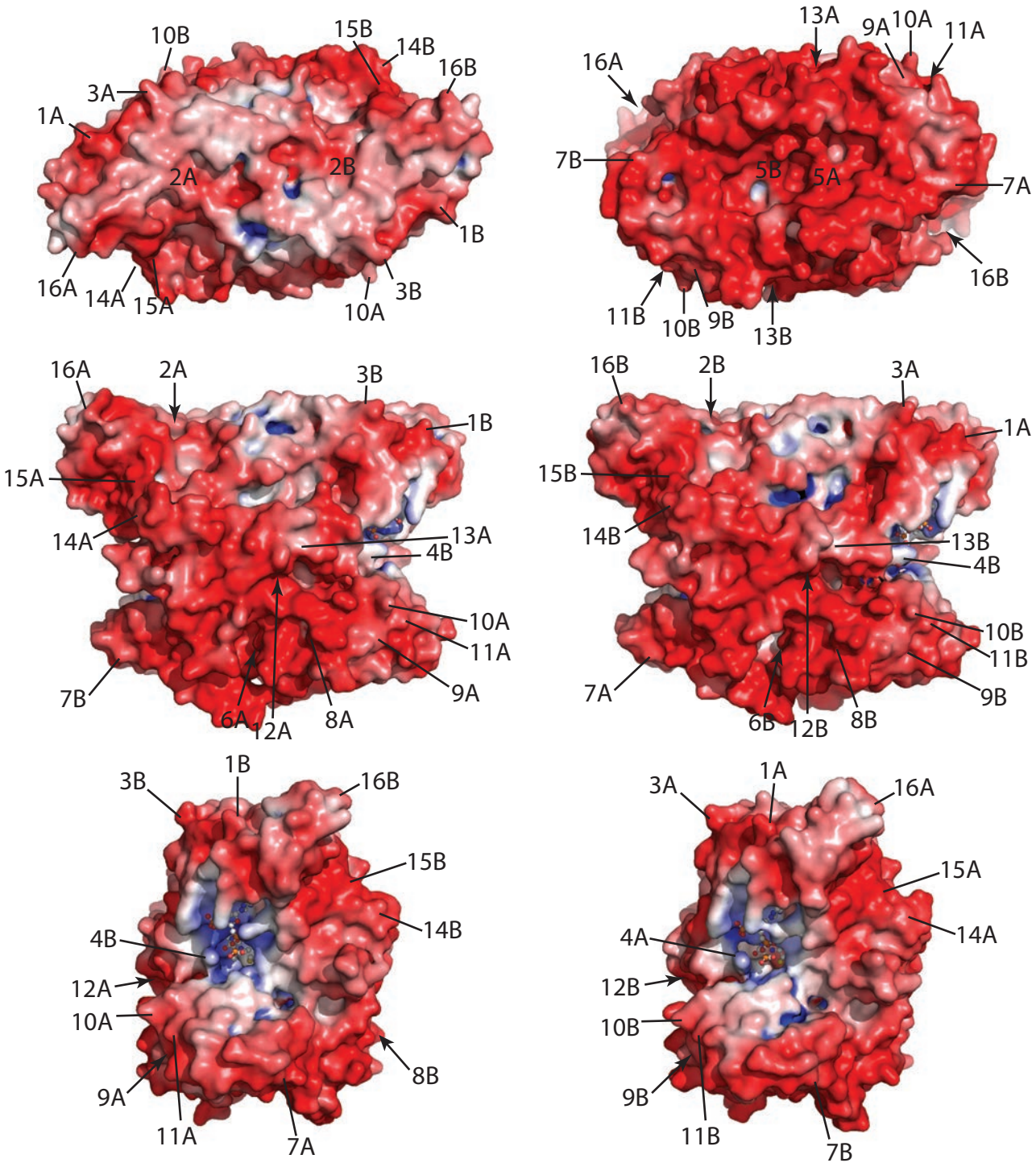

Figure 15: The molecular surface of FRC (PDB id 1p5r) depicted as in Supplementary Figure 13.