Supplementary Information for

Structure and mechanism of a pentameric formate channel

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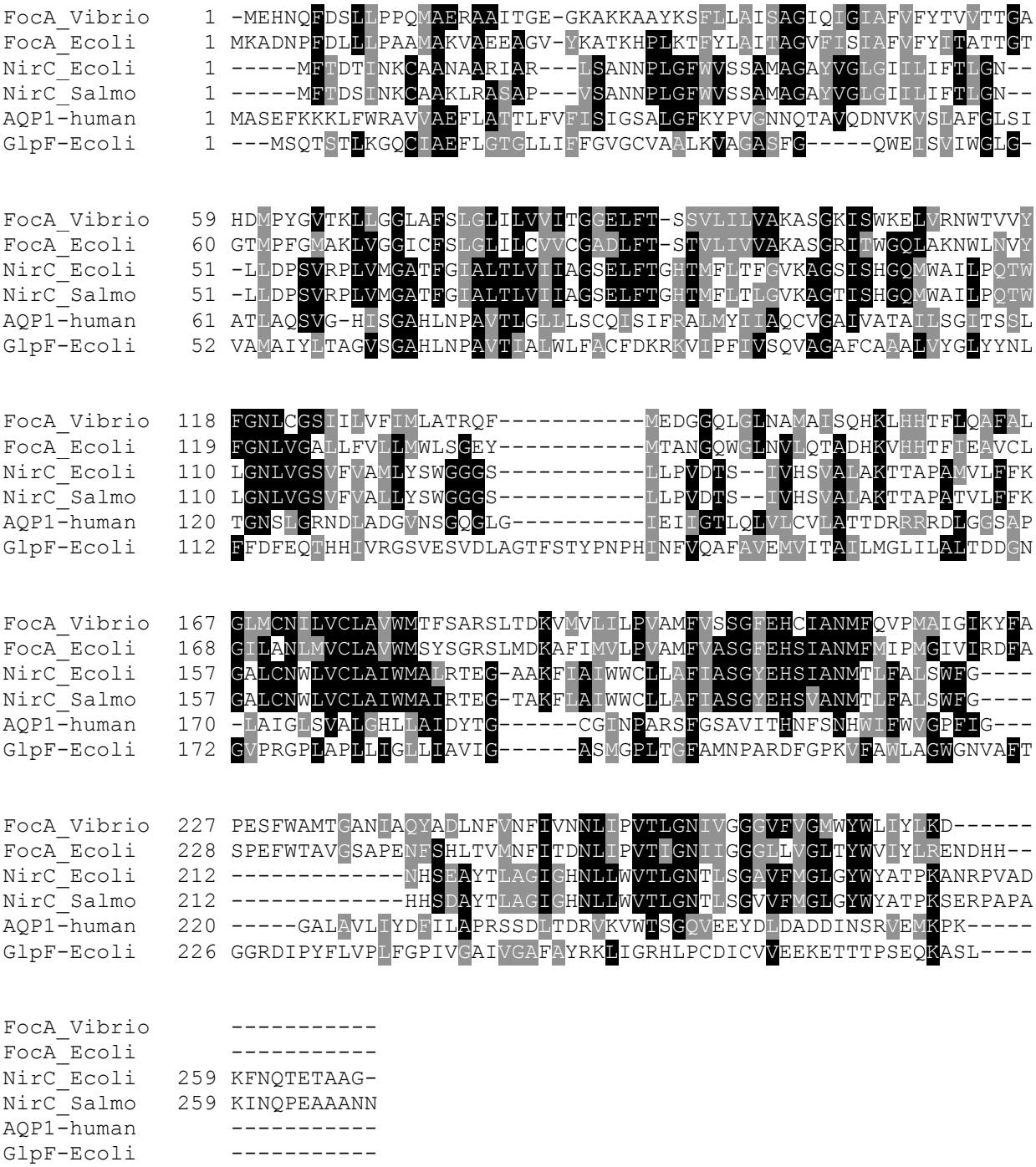

Fig. 1. Sequence alignment between the formate nitrite transport (FNT) protein family and the water/glycerol channel (AQP/GlpF) family.

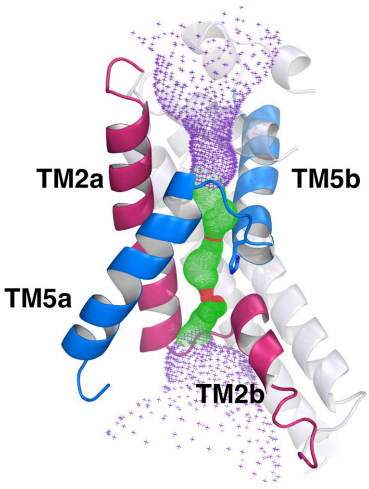

Fig. 2. Two broken transmembrane helices (TM2 and TM5) in Monomer A.

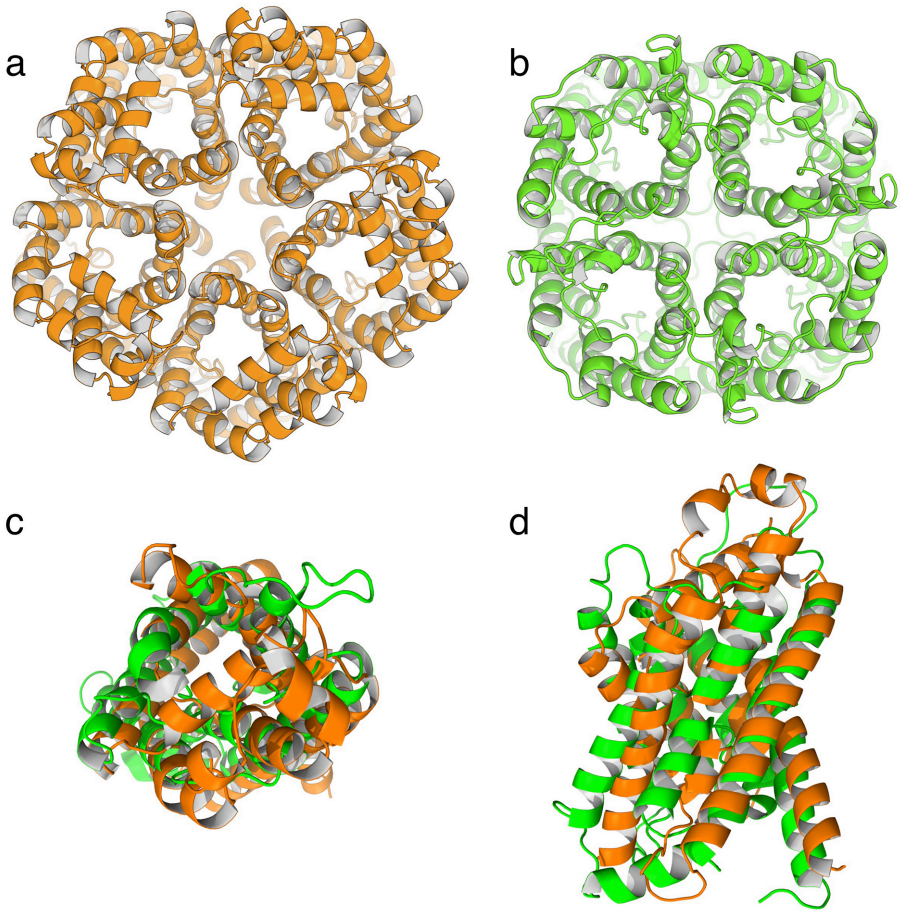

Fig. 3. Structural comparison between FocA and AQP1 water channel (PDB ID: 1J4N). (**a**) FocA pentamer. (**b**) AQP1 tetramer. (**c**) Superposition of FocA and AQP1 monomers, top view from the extracellular side. (**d**) Superposition of FocA and AQP1 monomers, side view from within the membrane. FocA molecules are colored orange, and AQP1 molecules green. The r.m.s.d for α-carbon between FocA and AQP1 monomers is 7 Å for the 212 amino acids in the middle of the sequence.

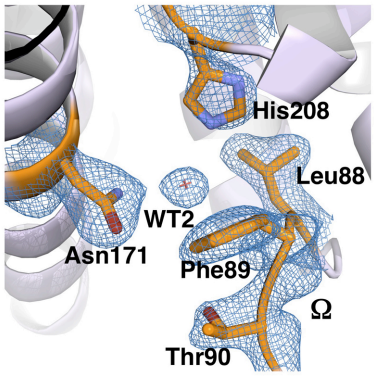

Fig. 4. $2F_o$-F_c map of Monomer E in the low-formate crystal form. The Ω loop moves in the cytoplasmic direction along with the Thr90 residue. A water molecule (WT2) occupies the original position of Thr90 as in Monomer A and forms a hydrogen bond with His208.

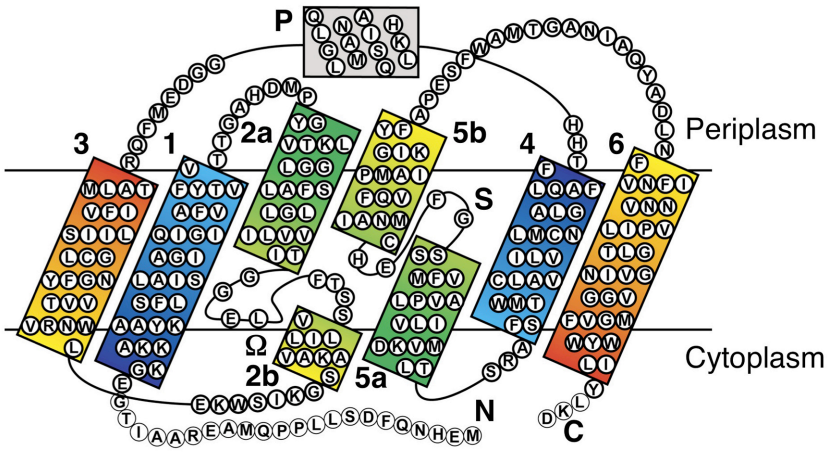

Fig. 5. Topology of Monomer E. Compared with Monomer A, TM2b in Monomer E is longer and a different segment in the sequence forms the helix. The color coding and helix numbering scheme are the same as for Figure 2d.

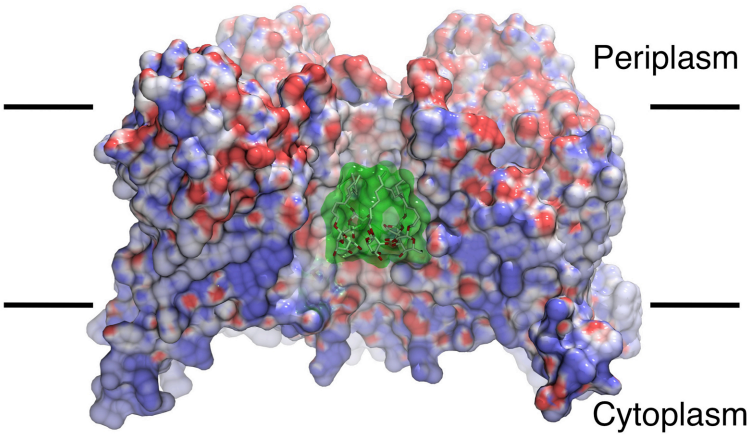

Fig. 6. Surface representation of the central pore around the fivefold axis of the pentamer. Five octylglucoside molecules (green) are found in the middle of the pore.

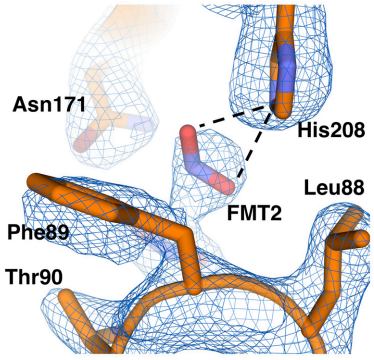

Fig. 7. $2F_o$-F_c map of Monomer E in the high-formate crystal form showing His208 forming a bidentate hydrogen bond with the two oxygen atoms of Formate 2 (FMT2).

Table 1. Structural variations among FocA monomers

Monomer	Ω Loop position	Formate/water in selectivity filter	TM2b position in sequence	Disordered residues (1σ)	β-OG found in cytoplasmic funnel	Involvement in crystal contact
Low-formate crystal structure (2.13 Å)						
Monomer A	UP	None	90-94	97-100	No	Yes
Monomer B	UP	None	90-92	93-107	Yes	No
Monomer C	UP	None	90-93	94-99	No	No
Monomer D	DOWN	1 HOH	N/A	93-104	Yes	No
Monomer E	DOWN	1 HOH	93-101	None	Yes	Yes
High-formate crystal structure (2.5 Å)						
Monomer A	UP	None	90-95	None	No	Yes
Monomer B	UP	None	90-93	94-107	No	No
Monomer C	UP	None	90-93	94-104	No	No
Monomer D	DOWN	1 FMT	N/A	93-100	No	No
Monomer E	DOWN	2 FMT	93-101	None	Yes	Yes

Table 2. Protein-formate contact in Monomer E (high-formate)

Residue	Atom	Formate	Atom	Distance (Å)
Leu88	CG	**FMT2**	O1	3.77
Phe89	CB		C	3.43
	CB		O1	3.43
	CB		O2	3.56
	CD2		C	3.29
	CD2		O2	3.30
	CG		C	3.71
	CG		O2	3.60
Asn171	CG		C	3.70
	CG		O2	3.76
	ND2		C	2.85
	ND2		O1	3.75
	ND2		O2	3.10
His208	CD2		O2	3.44
	CE1		C	3.72
	CE1		O1	3.53
	CE1		O2	3.12
	NE2		C	3.20
	NE2		O1	3.00
	NE2		O2	2.76
Leu88	O	**FMT1**	C	3.53
	O		O2	3.47
Thr90	CB		C	3.78
	CG2		C	3.70
	CG2		O1	3.58
	OG1		C	2.86
	OG1		O1	3.29
	OG1		O2	3.41
Asn171	CG		O1	3.76
	ND2		O1	3.42
	OD1		O1	3.29