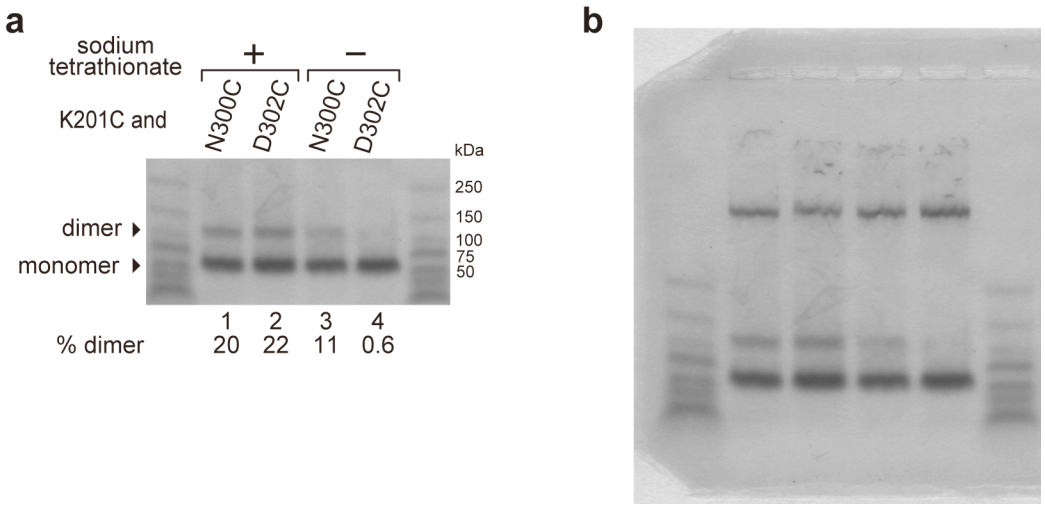
Supplementary Information

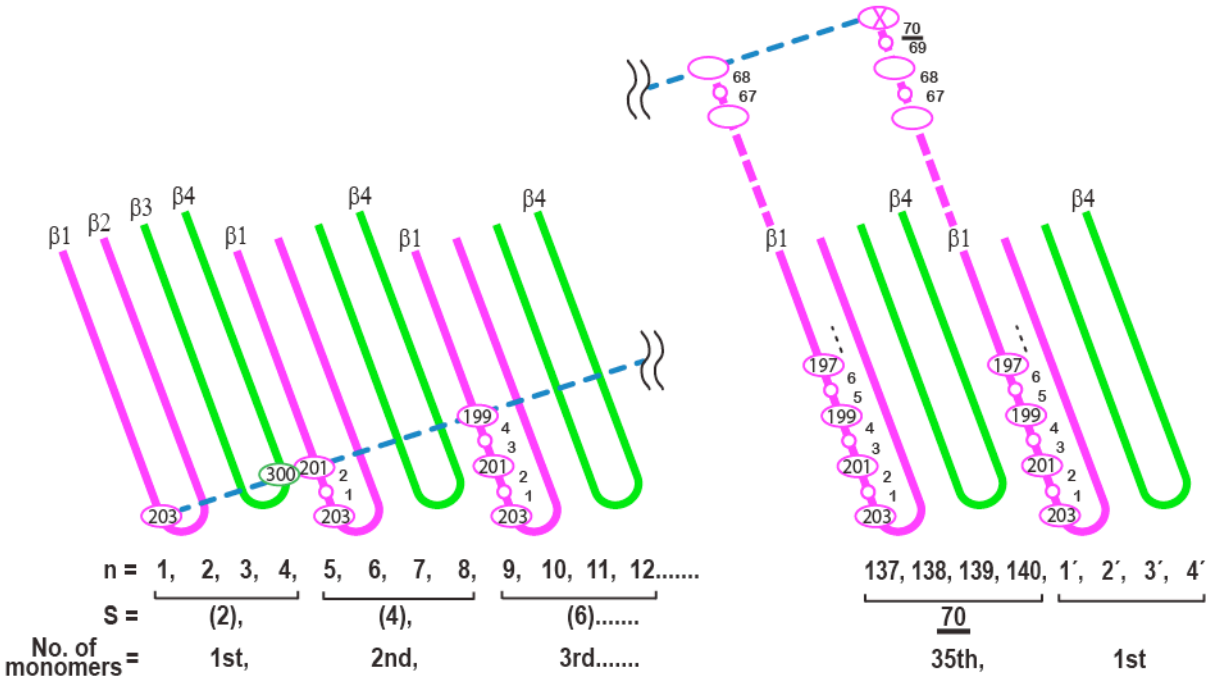
Disulfide-Bond Scanning Reveals Assembly State and β-Strand Tilt Angle of PFO β-Barrel

Takehiro K. Sato, Rodney K. Tweten, and Arthur E. Johnson

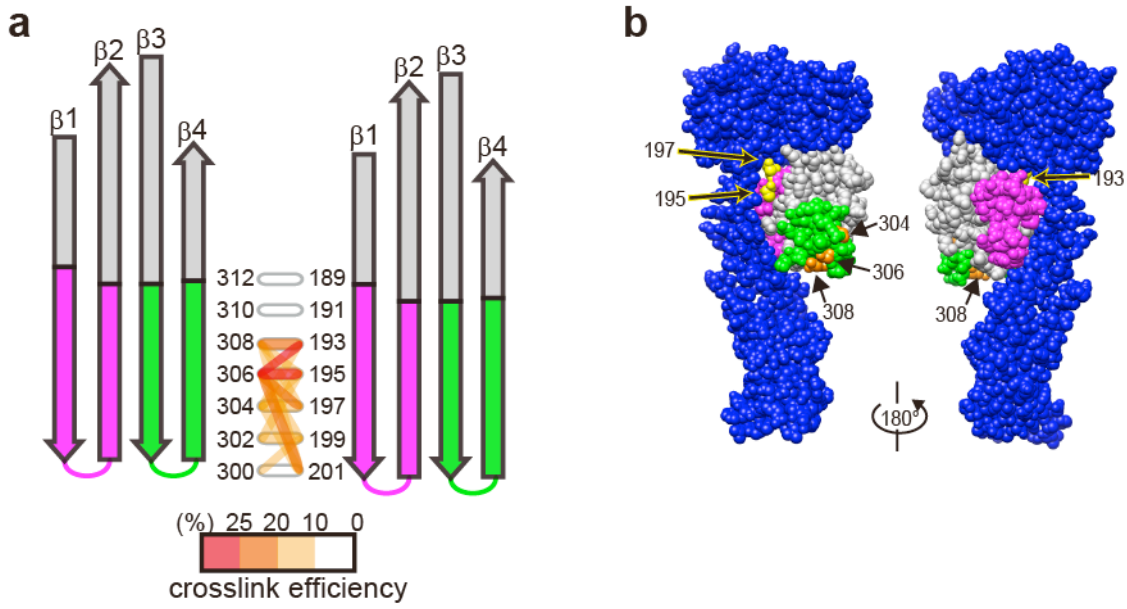
Supplementary Results



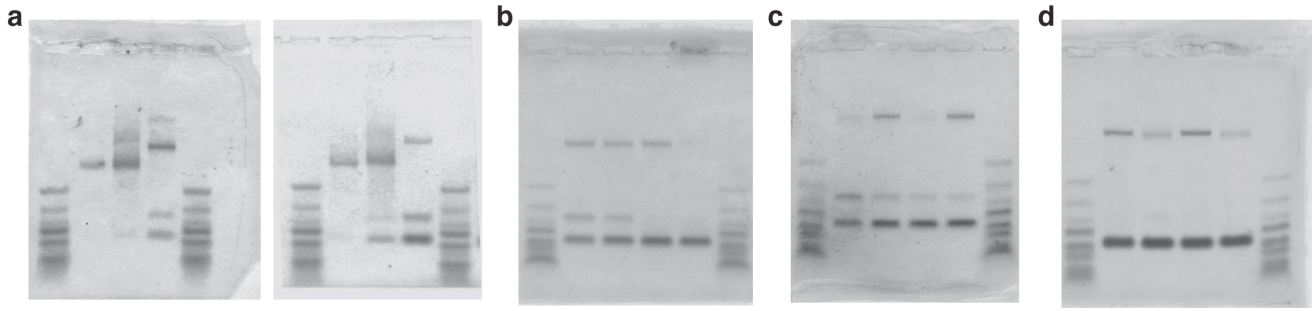
Supplementary Figure 1. Residues 201 and 300 are juxtaposed in the PFO β-barrel, while 201 and 302 are only transiently proximal. (a) An equimolar mixture of K201C and either N300C or D302C PFO derivatives were incubated for 40 min at 37°C to form pore complexes. Pore complexes were then purified and incubated either in the presence or absence of tetrathionate. Disulfide-linked dimers were analyzed by SDS-AGE, and the % yield of dimer is indicated below the lanes. **(b)** Entire gel from which panel **a** was excised.



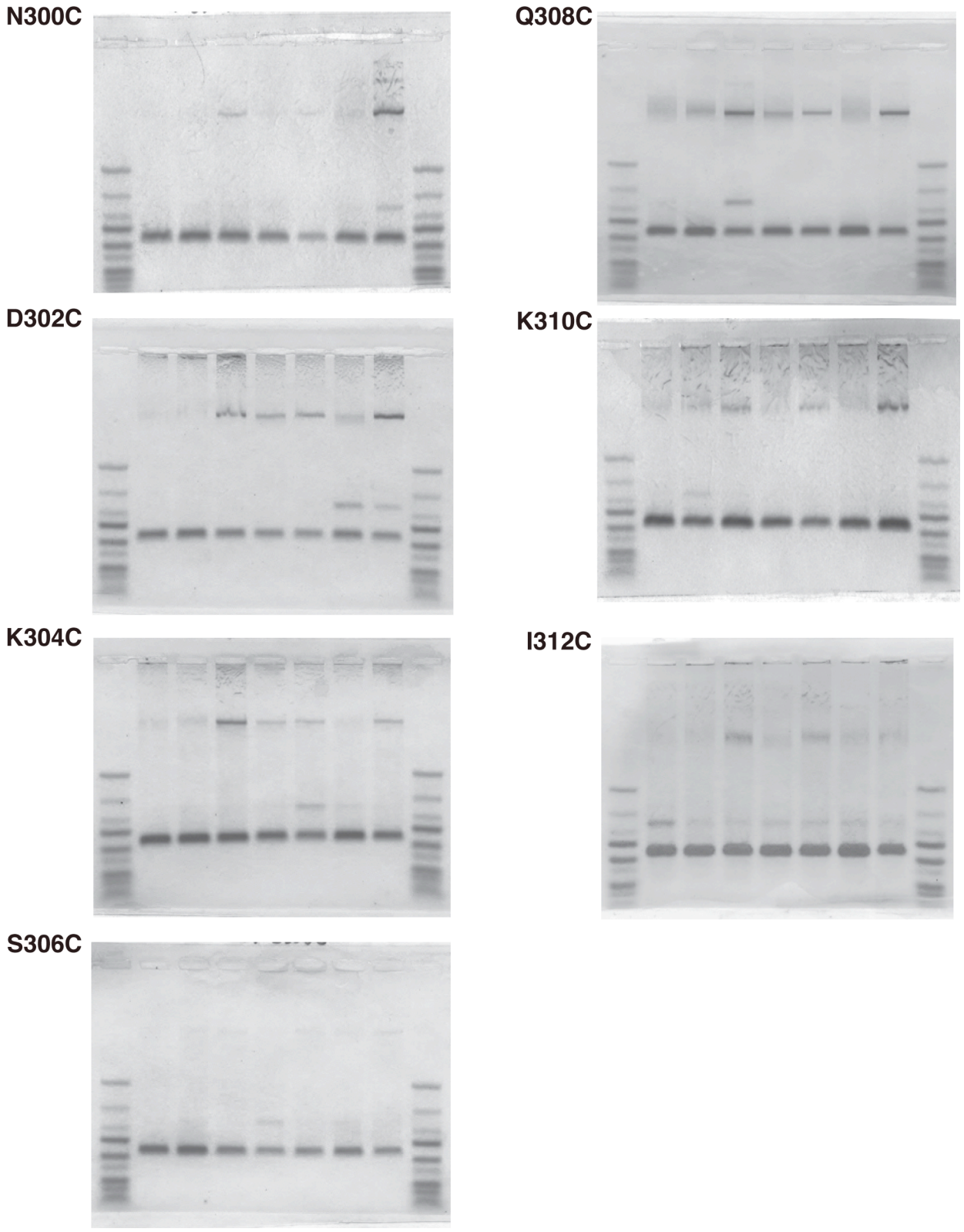
Supplementary Figure 2. Parameters of PFO β-barrel structure. A PFO β-barrel consists of ~35 PFO monomers, each of which inserts two TMHs (one shown in magenta, the other in green) into the bilayer and hence contributes four β-strands to the β-barrel. The total number of β-strands (n) in the PFO β-barrel is therefore 140. These strands are depicted above as an unfolded β-sheet in the plane of the paper. The β1 strand of the leftmost hairpin in the figure is defined to be n = 1; β1 in the next monomer to the right is therefore n = 5, and so on. The shear number (S) is defined as the total number of residues that are offset in circling the β-barrel once and returning to the starting strand[10]. The number of offset residues can be determined by extending a line (blue dashes) perpendicularly from residue 203 in the first strand (n =1) until it returns to the first strand (n = 1'). S is the stagger (number of offset residues) from residue 203 in n = 1 to the termination point, X, at the completion of one turn around the β-barrel. For PFO, S = 70 (underlined) when n = 140. The S/n ratio is therefore 1/2 since the offset is two residues per PFO monomer (4 β-strands) in the large PFO β-barrel.



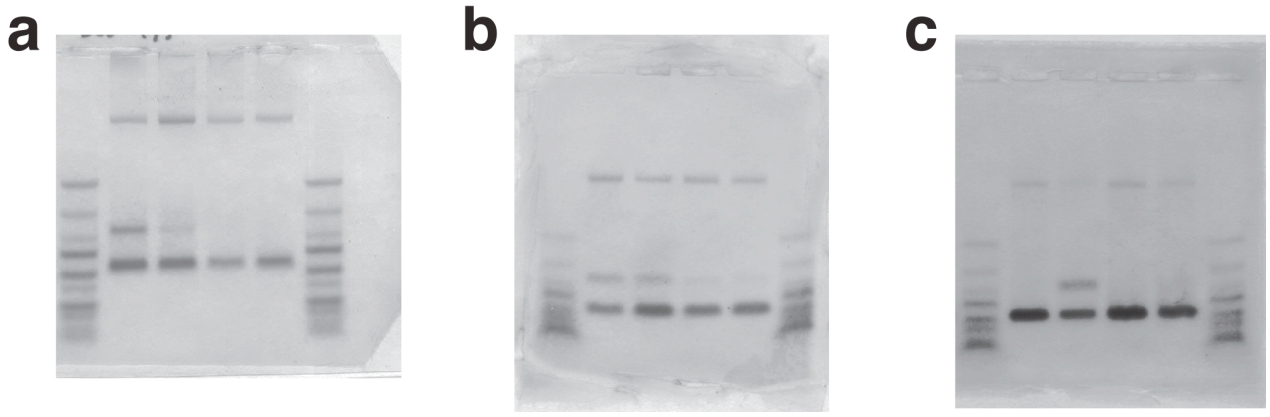
Supplementary Figure 3. β4-β1 cross-linking in PPCs. (a) Crosslink efficiencies for each pair of β1 and β4 residues in PPCs are indicated by the color of the lines linking the two residues. **(b)** The locations of the TMH residues that are frequently disulfide-linked in the PPC are shown in space-filling representations of monomeric PFO rotated 180° relative to each other. Images were generated using Chimera.



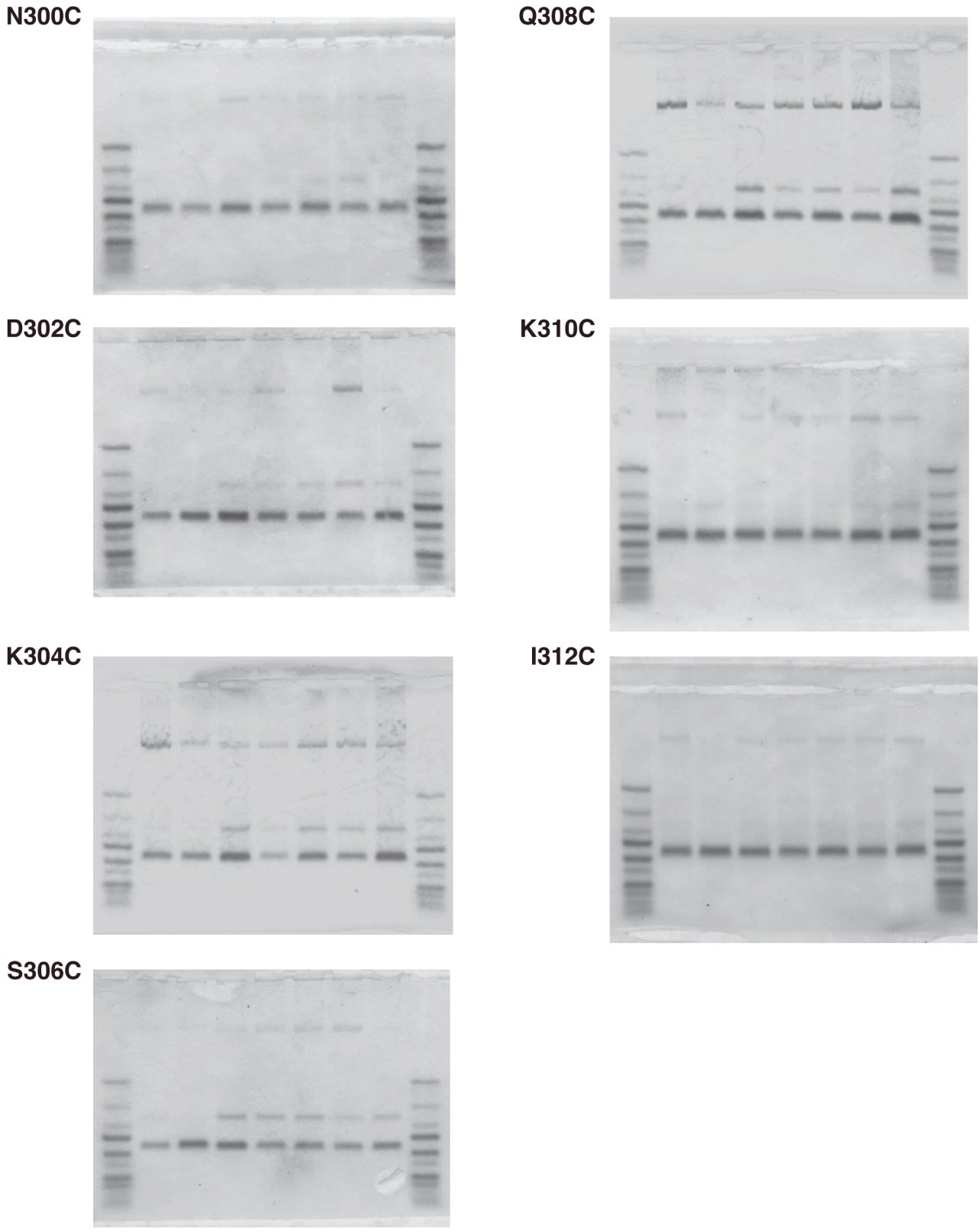
Supplementary Figure 4. Detection of disulfide-bonded PFO dimers in PPC and pore complexes. The whole gels from which the corresponding panels of Figure 2 were derived.



Supplementary Figure 5. β4-β1 crosslinking in pore complex The whole gels from which the corresponding panels of Figure 3a were derived.



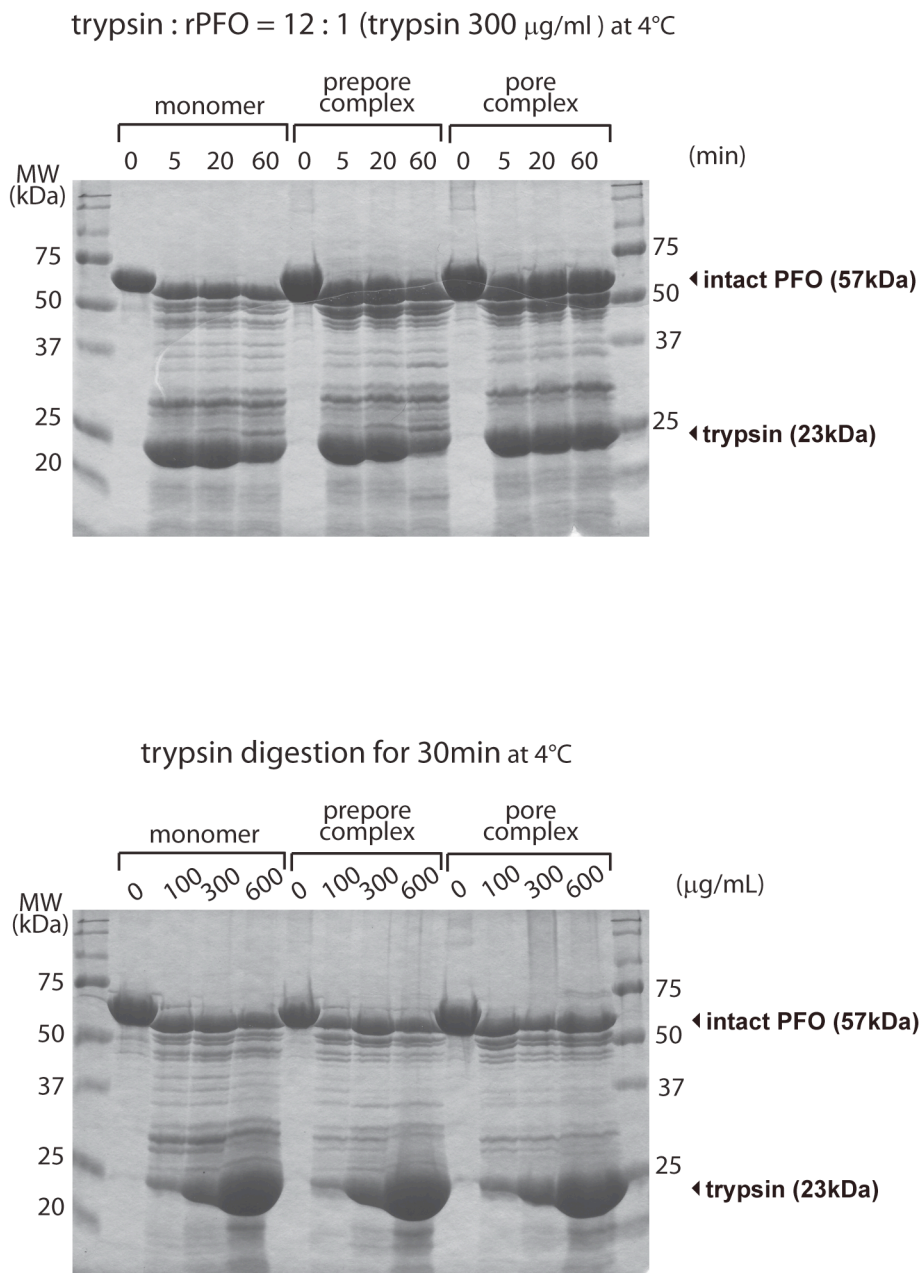
Supplementary Figure 6. Dimer formation in PPCs. The whole gels from which the panels of Figures 4a, 4b, and 4c were derived.



Supplementary Figure 7. β4-β1 crosslinking in prepore complexes. The whole gels from which the corresponding panels of Figure 4d were derived.

Domain	Cleavage site	PFO C459A MW = 56.8 kDa C-terminal fragment (kDa)	PFO C459A MW = 56.8 kDa N-terminal fragment (kDa)	TMH locations
D1	34	52.1	4.7	
D1	49	50.5	6.3	
D2	59	49.4	7.4	
D2	66	48.6	8.2	
D2	69	48.3	8.5	
D2	70	48.2	8.6	
D2	74	47.8	9	
D2	80	47.1	9.7	
D2	82	46.8	10	
D1	101	44.8	12	
D1	113	43.6	13.2	
D1	125	42.1	14.7	
D1	126	42	14.8	
D1	139	40.5	16.3	
D1	145	40	16.8	
D1	153	39	17.8	
D1	164	37.9	18.9	
D1	168	37.4	19.4	
D1	178	36.3	20.5	
D3	189	34.9	21.9	TMH1
D3	201	33.7	23.1	TMH1
D3	219	31.8	25	
D3	220	31.7	25.1	
D3	227	30.9	25.9	
D1	239	29.5	27.3	
D1	255	27.7	29.1	
D1	257	27.5	29.3	
D3	275	25.6	31.2	
D3	280	25	31.8	
D3	288	24.1	32.7	TMH2
D3	295	23.4	33.4	TMH2
D3	299	23	33.8	TMH2
D3	304	22.4	34.4	TMH2
D3	300	21.6	35.2	TMH2
D3	332	19.3	37.5	
D3	336	18.6	38.2	
D3	342	18.1	38.7	
D3	343	18	38.8	
D3	346	17.6	39.2	
D1	354	16.7	40.1	
D1	368	15.1	41.7	
D2	378	14.1	42.7	
D4	391	12.6	44.2	
D4	393	12.4	44.4	
D4	417	9.7	47.1	

Supplementary Table 1. Trypsin cleavage sites in PFO. PPC TMHs are unfolded, flexible, and moving, while the TMHs in monomers are tightly folded in helices and the TMHs in pore complexes form the β-barrel that is embedded in the membrane. Limited protease digestion may therefore detect PPC-specific cleavages in the PFO polypeptide. Trypsin was chosen because PFO contains 45 Lys and Arg residues, with 2 in TMH1 and 5 in TMH2. SIngle cleavages in either TMH would yield peptides of 22-24 kDa and 33-35 kDa, but we expexted TMHs in the membrane-embedded pores would not be cleaved.



Supplementary Figure 8. Trypsin digestion of PPCs, monomers, and membrane-embedded pore complexes. PFO monomers, PPCs, and pore complexes were exposed to trypsin under the same conditions while the concentration of trypsin and the time of digestion were varied systematically. A few proteolytic fragments near the size expected for TMH cleavage were present in the monomer and PPC gels, but missing in the pore complex gels, as expected. However, we did not observe any proteolytic fragments that were unique to the

PPC (this was also true when the gels were run for shorter times and we examined peptides <20 kDa). It therefore appears that the partially unfolded and moving TMHs on the inside of the circular PPC oligomer are not sufficiently accessible for trypsin cleavage to yield a trypsin fragment that differs from those observed in monomers. Thus, this approach was not very promising for detecting TMH conformations unique to the PPC.