Supplemental Figures

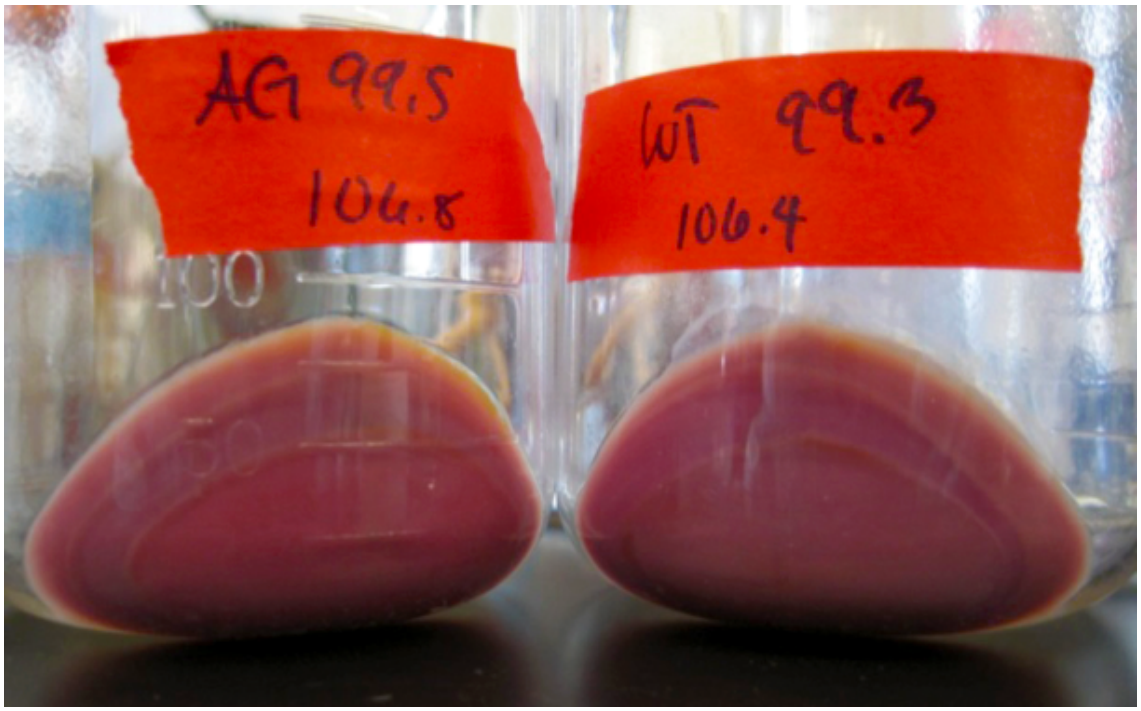

Figure S1: Colored bacterial pellets. *E. coli* cell pellets expressing GBCDEFA permutation mutant (left) and wild-type *H. turkmenica* bacteriorhodopsin (right). Correct targeting to the membrane and folding of a mutant is indicated by a WT-like pink coloration.

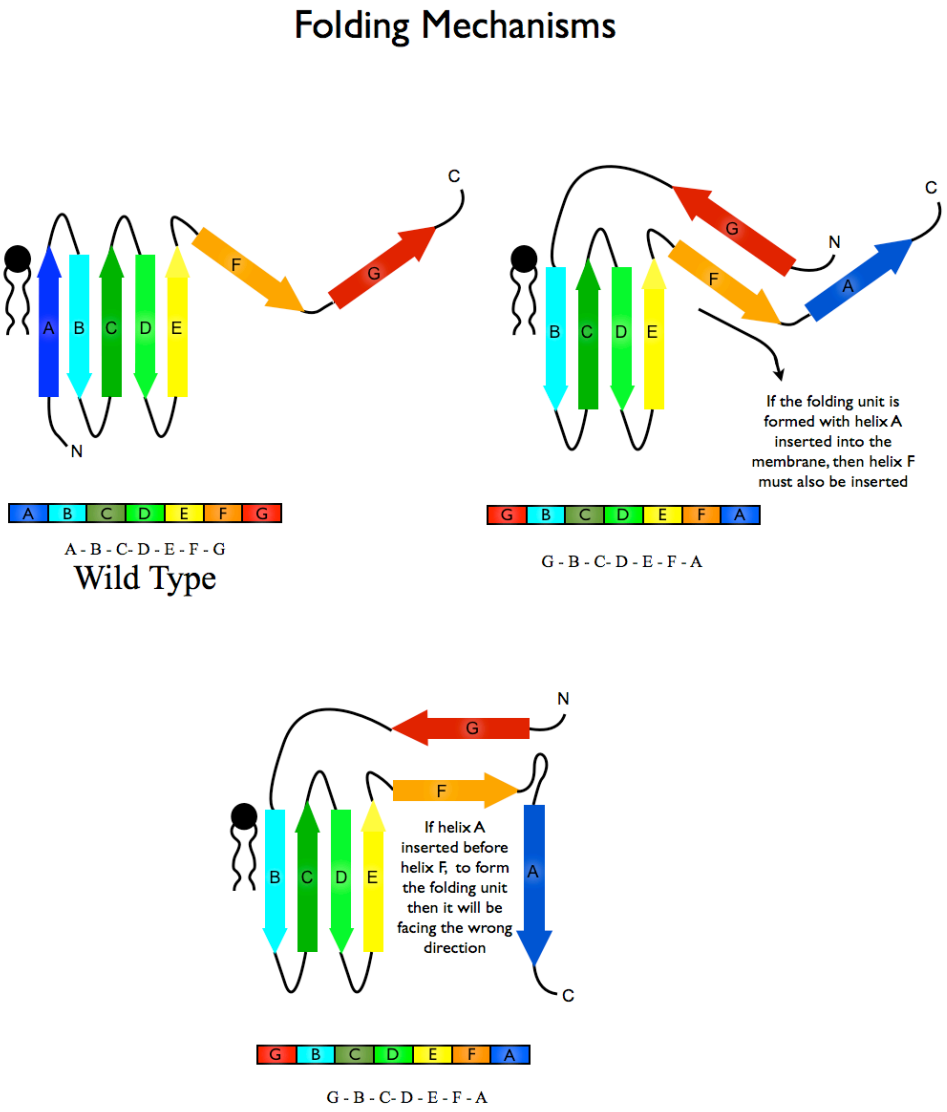

Figure S2: Proposed folding mechanisms for bR. Upper left: Conventional folding mechanism for wild-type bR, in which helices F and G cannot insert into the membrane until helices A-E have already inserted and associated. Upper right: Our GBCDEFA mutant cannot follow the proposed wild-type folding mechanism, since it is physically impossible for helices A-E to insert and associate before helix F is inserted. Lower middle: If helix A in fact inserts and associates with helices B-E before F is inserted, then helix A will have the wrong polarity in the membrane, and helix F will be left in solution.

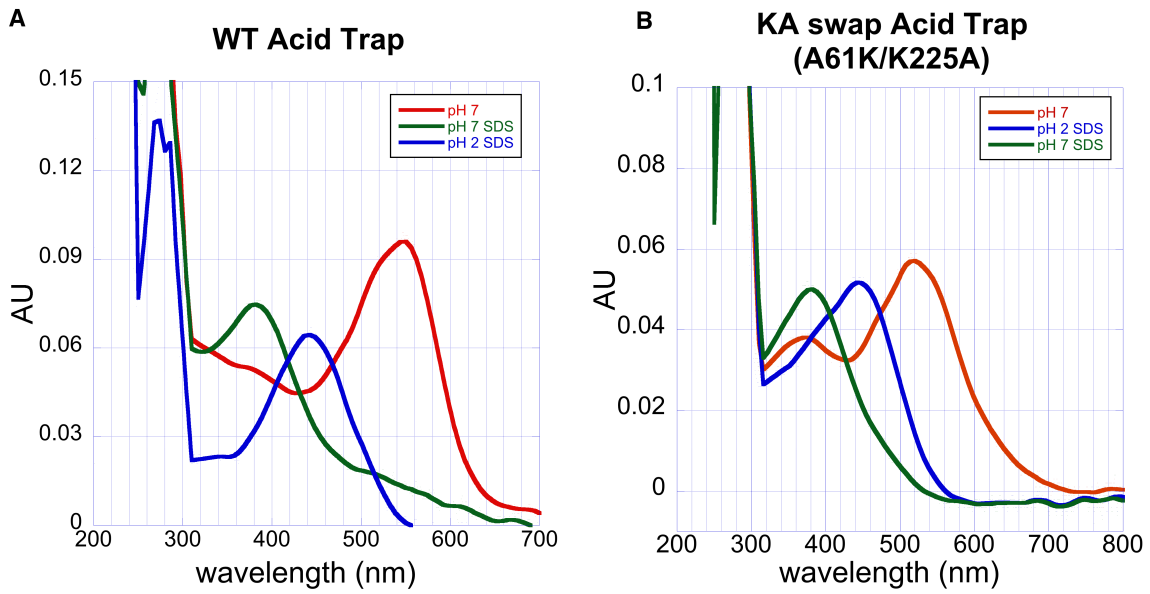

Figure S3: Acid trap experiments for (A) WT bR and (B) the A61K/K225A lysine swap mutant. For each protein, the absorbance spectra for three conditions are overlaid: in red, native protein at pH 7; in green, denatured protein at pH 7 with 2% SDS; in blue, denatured protein at pH 2 with 2%SDS. Native protein at neutral pH displays a distinctive red-shifted absorbance maximum near 550 nm. When the protein is denatured at neutral pH, the lysine-retinal Schiff base linkage hydrolyzes, free retinal is exposed to water, and it displays a 380 nm absorbance maximum. On the other hand, when protein is denatured under acidic conditions, the Schiff base remains protonated and does not hydrolyze. However, since the protein is denatured, the covalently bound retinal is no longer bound in the protein's retinal-binding pocket, the retinal is exposed to aqueous solution, and it displays an absorbance maximum of about 440 nm.

Table S1: Individual helix sequences, as diagrammed in Figure 2. Sequences of each helix as determined using homology to solved bR structures.

Helix Name	Sequence
Helix A	AALAPPMAATVGPESIWLWIGTIGMTLGTLYFVGRGRGVRDR
Helix B	KMQEFYIITIFITTIAAAMYFAMATGFGVTEVM
Helix C	VGDEALTIYWARYADWLFTTPLLLLDLSLLAG
Helix D	ANRNTIATLIGLDVFMIGTGAIAALSST
Helix E	PGTRIAWWAISTGALLALLYVLVGTLSENARNR
Helix F	APEVASLFGRLRNLVIALWFLYPVVWILGTEGTF
Helix G	GILPLYWETAAFMVLDLSAKVGFGVILLQSRSVLERVATPTAAPT

Table S2: Mutant sequences, related to Figure 2. Sequences of wild-type and permuted bacteriorhodopsin constructs. Sequence numbering is based on the wt protein.

Mutant	Sequence	Sequence numbers (Helix-Name_Residue-numbers_linker)
wt_Ht_bacteriorhodopsin	MCCAALAPPMAATVGPESIWLWIGTIGMTLGTLYFVGRGRGVRDRK MQEFYIITIFITTIAAAMYFAMATGFGVTEVMVGDEALTIYWARYA DWLFTTPLLLLDLSLLAGANRNTIATLIGLDVFMIGTGAIAALSST PGTRIAWWAISTGALLALLYVLVGTLSENARNRAPEVASLFGRLRN LVIALWFLYPVVWILGTEGTFGILPLYWETAAFMVLDLSAKVGFGV ILLQSRSVLERVATPTAAPT	
GBCDEFA	ILPLYWETAAFMVLDLSAKVGFGVILLQSRSVLERGVRDRKMQEFY IITIFITTIAAAMYFAMATGFGVTEVMVGDEALTIYWARYADWLFT TPLLLLDLSLLAGANRNTIATLIGLDVFMIGTGAIAALSSTPGTRI AWWAISTGALLALLYVLVGTLSENARNRAPEVASLFGRLRNLVIAL WFLYPVVWILGTEGTFGSGAALAPPMAATVGPESIWLWIGTIGMTL GTLYFVGRGRGVRDR	G_207-241_gB-F_42-207_sgA_4-45
CDEFGBA	GVTEVMVGDEALTIYWARYADWLFTTPLLLLDLSLLAGANRNTIAT LIGLDVFMIGTGAIAALSSTPGTRIAWWAISTGALLALLYVLVGTL SENARNRAPEVASLFGRLRNLVIALWFLYPVVWILGTEGTFGILPL YWETAAFMVLDLSAKVGFGVILLQSRSVLERVGVRDRKMQEFYIIT IFITTIAAAMYFAMATGFGGSGAALAPPMAATVGPESIWLWIGTIG MTLGTLYFVGRGRGVRDR	C-G_73-241_gB_42-72_ggsgA_4-45
GFABCDE	MILPLYWETAAFMVLDLSAKVGFGVILLQSRSVLERVATPTAAPTA PEVASLFGRLRNLVIALWFLYPVVWILGTEGTFSGAALAPPMAATV GPESIWLWIGTIGMTLGTLYFVGRGRGVRDRKMQEFYIITIFITTI AAAMYFAMATGFGVTEVMVGDEALTIYWARYADWLFTTPLLLLDLS LLAGANRNTIATLIGLDVFMIGTGAIAALSSTPGTRIAWWAISTGA LLALLYVLVGTLSENARNRA	G_207-250F_172-205_sgA-E_4-172
CDEFABG	MGVTEVMVGDEALTIYWARYADWLFTTPLLLLDLSLLAGANRNTIA TLIGLDVFMIGTGAIAALSSTPGTRIAWWAISTGALLALLYVLVGT LSENARNRAPEVASLFGRLRNLVIALWFLYPVVWILGTEGTFSGAA LAPPMAATVGPESIWLWIGTIGMTLGTLYFVGRGRGVRDRKMQEFY IITIFITTIAAAMYFAMATGFGVTEVMVGSGGILPLYWETAAFMVL DLSAKVGFGVILLQSRSVLERVATPTAAPT	C-F_73-206_sgA_4-80_gggsggG_206-250
FG_WALP23_ABCDE	MGSRAPEVASLFGRLRNLVIALWFLYPVVWILGTEGTFGILPLYWE TAAFMVLDLSAKVGFGVILLQSRSVLERGSGWWLALALALALALAL ALALWWASGAALAPPMAATVGPESIWLWIGTIGMTLGTLYFVGRGR GVRDRKMQEFYIITIFITTIAAAMYFAMATGFGVTEVMVGDEALTI YWARYADWLFTTPLLLLDLSLLAGANRNTIATLIGLDVFMIGTGAI AALSSTPGTRIAWWAISTGALLALLYVLVGTLSENARNRGSC	FG_171-241 WABCDE_4-171
DEFG_WALP23_ABC	MGANRNTIATLIGLDVFMIGTGAIAALSSTPGTRIAWWAISTGALL ALLYVLVGTLSENARNRAPEVASLFGRLRNLVIALWFLYPVVWILG TEGTFGILPLYWETAAFMVLDLSAKVGFGVILLQSRSVLERGSGWW LALALALALALALALALWWASGAALAPPMAATVGPESIWLWIGTIG MTLGTLYFVGRGRGVRDRKMQEFYIITIFITTIAAAMYFAMATGFG VTEVMVGDEALTIYWARYADWLFTTPLLLLDLSLLAG	DEFG_110-241W ABC_4-110TEV site
BCDEFG_WALP23_A	MVRDRKMQEFYIITIFITTIAAAMYFAMATGFGVTEVMVGDEALTI YWARYADWLFTTPLLLLDLSLLAGMGANRNTIATLIGLDVFMIGTG AIAALSSTPGTRIAWWAISTGALLALLYVLVGTLSENARNRAPEVA SLFGRLRNLVIALWFLYPVVWILGTEGTFGILPLYWETAAFMVLDL SAKVGFGVILLQSRSVLERGSGWWLALALALALALALALALWWASG AALAPPMAATVGPESIWLWIGTIGMTLGTLYFVGRGRGVRDR	BCDEFG_42-241W A_4-45 TEV