Improvisations in phototrophy. Protein engineering and functional investigation of rhodopsin proton-pumps

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Appendix

A.1 Sources of special chemicals

Company	Special chemicals/Materials
Addgene	MSPDE31-plasmid
Anatrace	1-n-dodecyl-β-D maltopyranoside (DDM), octyl-β-D-glucopyranoside neopentyl glycol (OGNG)
Avanti polar lipids	*E. coli* polar lipid extract
Buchem	Retinals MOA2, DMAR, MMAR, [8-15, 19, 20]-$^{13}C_{10}$-labelled A1
Cray valley	SMA2000
Gifts	Retinals A2, ALL-E, PHE, MOA2, nonyl β-D-glucopyranoside, asolectin, dodecyl phosphocholine (DPC)
Novagen	1,4-Dithiothreitol (DTT), benzonase nuclease
Promega	isopropyl β-D-1-thiogalactopyranoside (IPTG)
Roche	EDTA-free protease inhibitor tablets, anti-His_6 mouse monoclonal antibody,
Thermo-Scientific Fischer	Ni^{2+}-NTA resin and columns, Coomassie brilliant blue G-250, restriction enzymes, protein ladder, *Pfu* DNA polymerase
Sigma-Aldrich	Retinal A1, ampicillin, valinomycin, lysozyme, carbonyl cyanide m-chlorophenylhydrazone (CCCP), imidazole, bis-tris propane, hydroxylamine, L-Serine, HEPES, β-cyclodextrin, benzoase nuclease, octyl-β-D-glucopyranoside (OG), TritonX-100, Tergitol NP-40, Blue-dextran

A.2 Absorbance spectra, λ_{max} and ε of free retinals and proteorhodopsins

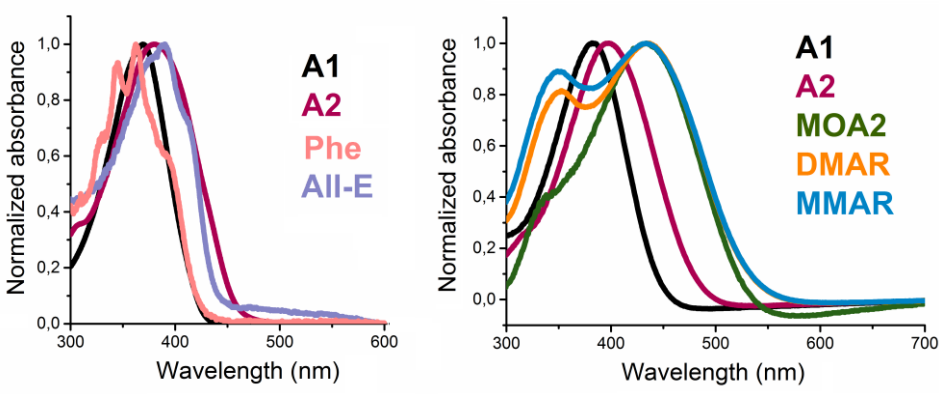

Figure A.2: Absorbance spectra of retinal analogs in organic solvents. [a] A1, A2, ALL-E (hexane); PHE (methanol). [b] A1, A2, MOA2, DMAR, MMAR (DMF).

Retinal	λmax (nm)			
	[a]DMF	[b] 1% DDM	[c] Oxime DMF	[d] Oxime 1% DDM
A1	382	388	365	368
A2	400	404	375	378
PHE	360	nd	nd	nd
ALL-E	395	nd	nd	nd
MOA2	430	441	415	402
DMAR	430	433	358	370
MMAR	423	438	358	350

Table A.2.1: λ_{max} of [a] free retinal in DMF, with the exception of PHE (in methanol) and ALL-E (in hexane). [b] free retinal in 1% DDM solution (pH 7); [c] retinal oxime in DMF solution; [d] retinal oxime in 1% DDM solution (pH 7). Data are the average of at least two measurements, with standard deviation ≤2 nm. nd: not determined.

Retinal	ε ($M^{-1}cm^{-1}$)				
	[a] DMF	[b] 1% DDM	[c] Oxime 1% DDM	[d] PR:retinal 1% DDM	[e] GR:retinal 1% DDM
A1	45600	39500	52500	54200	55500
A2	44500	38000	44000	46800	49200
MOA2	30400	31500	30400	nd	nd
DMAR	31800	29000	42000	40500	nd
MMAR	28000	31000	36000	35200	34200

Table A.2.2: Molar absorbance (ε) of retinals and corresponding proteorhodopsins. [a] free retinal in DMF solution; [b] free retinal in 1% DDM solution; [c] retinal oxime in 1% DDM solution; [d] PR containing the various retinal analogs, after solubilization in 1% DDM solution; [e] GR containing the various retinal analogs, after solubilization in 1% DDM solution. The DDM solution is at pH 7. Data are averages of duplicate assays, with standard deviation ≤ 8%. nd: not determined.

A.3 Fluorinated retinal analogs

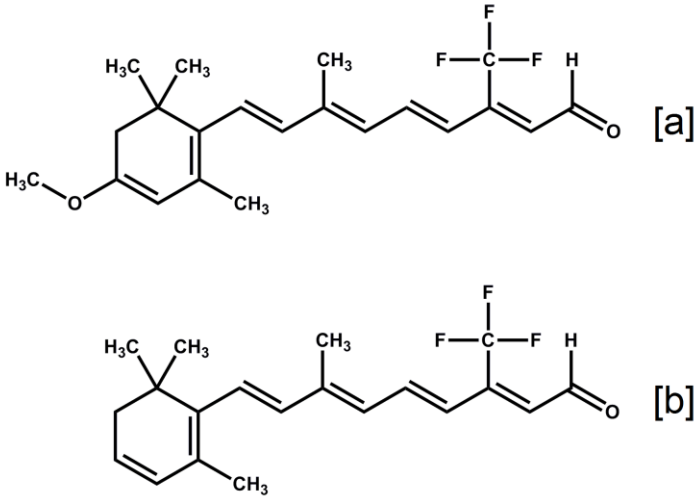

Figure A.3: Chemical structures of retinal analogs that do not yield stable pigments with PR or GR, upon addition to culture or regeneration in membrane vesicles. [a] 3-methoxy-13-trifluoromethyl retinal A2 (λmax = 453 nm in hexane); [b] 13- trifluoromethyl retinal A2 (λmax = 446 nm in hexane). Both are strongly red-shifted relative to retinal A2 (λmax = 381 nm in hexane).

A.4 Primer sequences used in this study for site-directed mutagenesis

Primer name	Sequence
1267_vector_for	GTGAGCGGATAACAATTTCACACAGG
2893_PR_D212N_for	CACAGGTTACCTGATGGGTAACGGTGGATCAGCTCT
2894_PR_D212N_rev	AGAGCTGATCCACCGTTACCCATCAGGTAACCTGTG
2895_PR_F234S_for	GACTTTGTTAACAAGATTCTATCCGGATTAATTATATG
2896_PR_F234S_rev	CATATAATTAATCCGGATAGAATCTTGTTAACAAAGTC
2897_vector_rev	GTATCCGCTCATGAGACAATAACCC
2922_PR_T101A_for	AGATACATTGATTGGTTGCTAGCAGTTCCTCTATTAATATGTG AATTCTAC
2923_PR_T101A_rev	GTAGAATTCACATATTAATAGAGGAACTGCTAGCAACCAATCA ATGTATCT
2924_PR_L105K_for	CATTGATTGGTTACTAACAGTTCCTCTTAAGATATGTGAATTC TACTTAA
2925_PR_L105K_rev	TTAAGTAGAATTCACATATCTTAAGAGGAACTGTTAGTAACCA ATCAATG
2926_PR_A178R_for	CTGCATGCAATACTCGAAGTCCTGCTGTGCAATCAGCT
2927_PR_A178R_rev	AGCTGATTGCACAGCAGGACTTCGAGTATTGCATGCAG
2970_GR_F260S_for	GCCTGTATCAGGTCTACTAGTCTTCGCGA
2971_GR_F260S_rev	GAAGACTAGTAGACCTGATACAGGCTTCGC

for = forward primer

rev = reverse primer

A.5 Site-directed mutants, their λ_{max} and relative proton-pump activity

Site-directed mutants generated in this study	[a]λmax (nm)	[b]H+
GR WT	540	+++
GR F260S	549	+++
PR WT	520	+++
PR D212N	521	+++
PR F234S	540	+
PR L105K	538	+
PR T101A	539	++
PR A178R	540	-
PR D212N, F234S	540	++
PR D212N, F234S, L105K	545	+
PR D212N, F234S, T101A	555	+
PR D212N, F234S, A178R	548	-

Table A.5: WT pigments and their corresponding single, double and triple mutants generated in this study. [a] λ_{max} of the purified pigments in 0.1% DDM solution, pH 8. [b] proton pumping scores relative to the corresponding WT pigment. +++ 70-100% WT, ++ 50-70% WT, + 30-50% WT, - 0-30% WT.

A.6 pH effect with PR-DNFS:MMAR

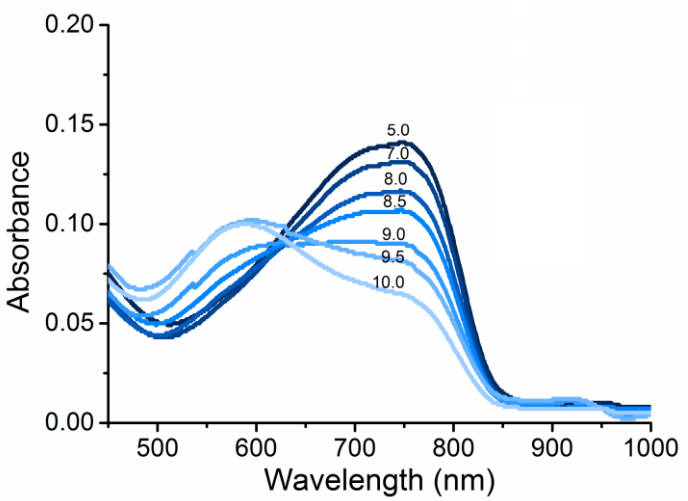

Figure A.6: Absorbance spectra of His-tag purified PR-DNFS:MMAR in DDM solution at pH 5, 7, 8, 8.5, 9, 9.5, 10. pH values are indicated next to the corresponding spectrum in the graph. Spectra represent separate samples, not a titration of a single sample.

A.7 Fluorescence of PROPS with MMAR

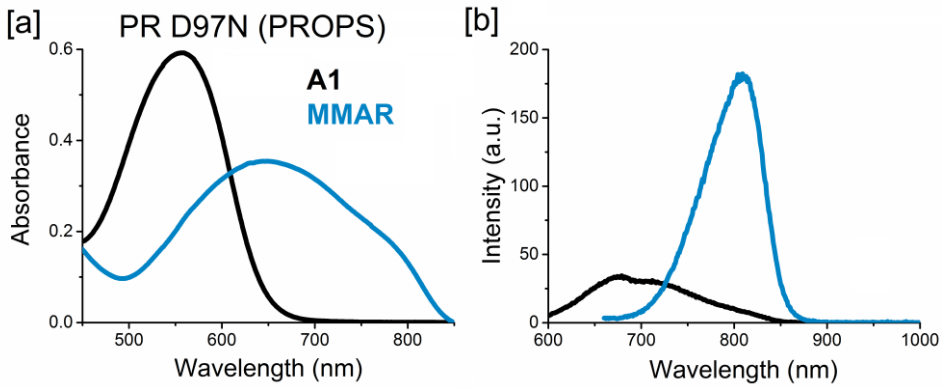

Figure A.7: Absorbance spectra of His-tag purified PROPS: A1 and PROPS:MMAR in DDM solution, pH 8 [a] and their corresponding emission spectra upon excitation at 540 nm for PROPS:A1 and 650 nm for PROPS:MMAR[b].