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

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Protein engineering and functional
investigation of rhodopsin proton-pumps

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Dr. R. van der Steen

Dr. A. Pandit

For all my inner and outer guides...

*“Willingness to be puzzled by what seem to be obvious truths is the first step towards gaining understanding of how the world works” -
Noam Chomsky*

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Abbreviations

A1	all- <i>trans</i> -retinal
A2	all- <i>trans</i> -3,4-dehydroretinal
ACRs	anion channelrhodopsins
ALL-E	all- <i>trans</i> -all-E-locked-retinal
AR3	Archaerhodopsin3
ATP	adenosine triphosphate
bR	Bacteriorhodopsin
CCCP	carbonyl cyanide m-chlorophenyl hydrazine
CCRs	cation channel rhodopsins
cfu	colony forming units
ChR2	Channelrhodopsin2
DDM	aqueous solution of 1-n-dodecyl- β -D-maltopyranoside
DPC	dodecyl phosphocholine
DMAR	all- <i>trans</i> -3-dimethylamino-16-nor-1,2,3,4-didehydroretinal
DMF	dimethylformamide
DTT	1,4-dithiothreitol
GR	<i>Gloeobacter violaceus</i> rhodopsin
GR _{CTRL}	<i>E. coli</i> MG1655 Δ <i>iscS</i> MUT pJBS1257- IPTG
GR _{MUT}	<i>E. coli</i> MG1655 Δ <i>iscS</i> -MUT pJBS1257-IPTG, retinal, arabinose
GR _{WT}	<i>E. coli</i> MG1655 Δ <i>iscS</i> -MUT pJBS1257-IPTG, retinal
GR:A1	pigment of GR with A1 as the chromophore
GR-FS	GR single mutant F260S
IPTG	Isopropyl β -D-1-thiogalactopyranoside
HR	Halorhodopsin
MMAR	all- <i>trans</i> -3-methylamino-16-nor-1,2,3,4-didehydroretinal

MOA2	all- <i>trans</i> -3-methoxy-3,4-dehydroretinal
MSP	membrane scaffold protein
MUT	mutagenesis plasmid
OG	octyl- β -D-glucopyranoside
OGNG	octyl glucose neopentyl glycol
PAR	photosynthetically active radiation
PHE	all- <i>trans</i> -1,2,3,4-didehydro-1,5-desmethyl-retinal
pmf	proton motive force
PR	Proteorhodopsin from Monterey Bay SAR86 γ proteobacterium
PR _{CTRL}	<i>E. coli</i> MG1655 Δ <i>iscS</i> MUT pJBS1255 IPTG
PR _{MUT}	<i>E. coli</i> MG1655 Δ <i>iscS</i> -MUT pJBS1255 IPTG, retinal, arabinose
PR _{WT}	<i>E. coli</i> MG1655 Δ <i>iscS</i> -MUT pJBS1255 IPTG, retinal
PR:A1	pigment of PR with A1 as the chromophore
PR-DNFS	PR double mutant D212N, F234S
PR-TA	PR single mutant T101A
NIR	near-infrared
Rh	bovine visual rhodopsin
RT	room temperature
SB	starvation buffer
SBAz	starvation buffer supplemented with 30 mM sodium azide
SMA	styrene maleic acid copolymer
ssNMR	solid-state nuclear magnetic resonance spectroscopy
SRI	Sensory rhodopsin I
SRII	Sensory rhodopsin II
TD-DFT	time dependent density functional theory
WT	wild type