Key residues for the light-regulation of the blue light activated adenylyl cyclase (bPAC) from *Beggiatoa sp.*

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Supporting Information

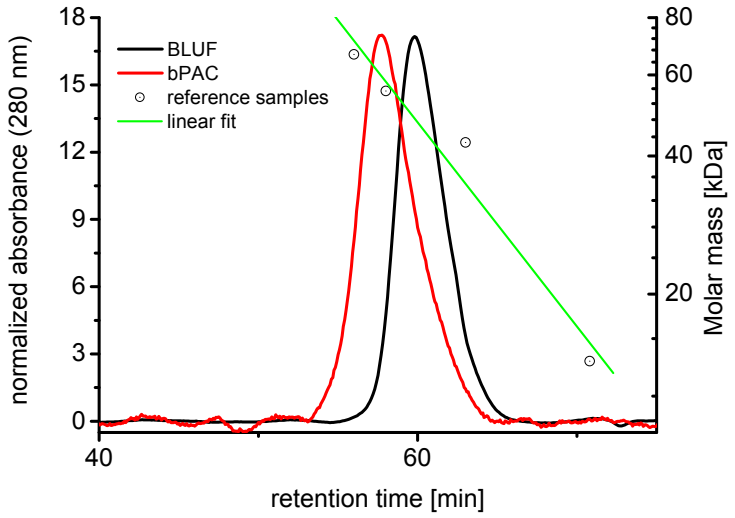

Figure S1. Size exclusion chromatography of bPAC and bPAC-BLUF. Protein samples were applied to a Superose 6 10/300 GL column (GE healthcare) at 0.3 ml/min and detected by

absorption at 280 nm using an Äkta prime setup (GE healthcare). Calibration was performed using bovine serum albumin, ovalbumin, alpha-amylase and lysozyme (Sigma-Aldrich). Full-length bPAC accordingly appears at an apparent molecular weight of ~60 kDa, the bPAC BLUF domain at ~45 kDa. The expected molecular weight of full-length protein and BLUF domain in dimeric form are 80 kDa and 35 kDa, respectively. Given the apparent size being strongly dependent on the hydrodynamic radius we consider both proteins to be in dimeric form.

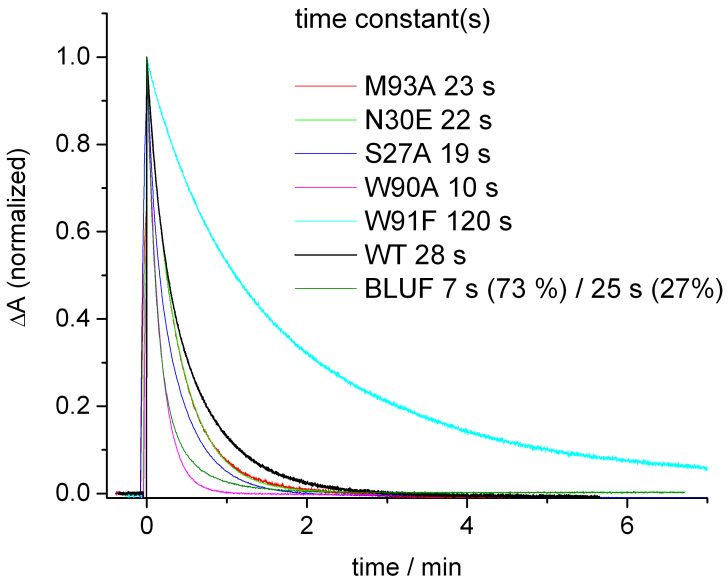

Figure S2. Absorbance change at 490 nm after blue light illumination. The time dependent decay of the absorption is fitted with a mono- or bi-exponential decay function with the indicated lifetime(s).

Table S1. Enzymatic activity of bPAC and mutants in light and dark (nmol cAMP min^{-1} mg $protein^{-1}$). Additionally the dark activities of bPAC and S27A are given for 5 times higher protein concentration*.

Protein	Dark	Light	St. deviation Dark	St. deviation Light
cyc	18	n. d.	5	n. d.
bPAC	121	2008	30	286
W91A	370	993	181	417
W91F	1157	1842	142	142
M93A	1589	2821	122	140
Q49A	1010	1781	404	192
Y7F	1817	1237	405	338
N30E	319	2842	119	97
S27A	134	2087	14	11
***bPAC**	99	n.d.	10	n.d.
***S27A**	38	n.d.	1	n.d.