Supplement to:

Control of phosphodiesterase activity in the regulator of biofilm dispersal RbdA from *Pseudomonas aeruginosa*

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This supplement contains:

Supplementary Table S1

Supplementary figures S1+S2

Colour	MS volume (Å ³)	SA volume (Å ³)	Pocket MS area (Å ³)	Pocket SA area (Å ³)	Openings #	lining amino acids
green	430.8	56.2	403.6	144.1	0	Ser5, Tyr6, Gly9, Glu10, Trp13, Asp49, Met52, Ile70, Asn75, Ile83, Phe96 , Leu102, Trp103 , Gly106, Val148, Ala151, Ala152, Phe155
green	13.2	0.0	27.1	0.0	0	Phe96, Ile100, Trp103, Phe155
blue	35.3	0.2	59.1	3.2	0	Trp13, Pro46, Arg50, Asp107, Val110
grey	22.5	0.1	39.4	1.1	0	Gly9, Leu12, Asp49 , Gly73, Asn75
purple	51.2	1.2	72.0	8.4	0	Gln17, Tyr109, Val110, Leu113, Ile144, Val148

Table S1. Analysis of cavities in the RbdA periplasmatic domain $RbdA_{38-201}$ (computed model, AF2). Color code relates to amino acids lining the cavity as seen in Figure S2. The largest pocket volume is explored in Figure 4.



Figure S1. Experimental size determination of RbdA constructs. The PDE domain (RbdA₅₄₉₋₇₉₇) mainly eluted as a dimer at approximately 59.5 mL, with a small peak present for the monomer at approximately 71 mL. The linked DGC-EAL construct (RbdA₃₇₆₋₇₉₇) eluted as a dimer at approximately 53.3 mL. The autonomous DGC domain was (mainly) monomeric (RbdA₃₇₆₋₅₃₆) and eluted at approximately 78.4 mL. Determined using a HiLoad 16/600 Superdex 75 prep grade column.



Figure S2. Computed model of RbdA₃₈₋₂₀₁ periplasmatic domain. The colors indicate amino acids lining different pockets as given in Table S1. Selected amino acid side chains lining the pockets are shown as sticks.



Figure S3. Superposition of of RbdA (green, translucent) and RmcA (orange, solid; 5M3C⁵⁴). A, the dimerisation interface, cartoon representation; compare Figure 1. B, helices α 5 and α 6 from the interface in RmcA (orange and light orange) with a small β -sheet, as opposed to RbdA (dark and light green).