

1 **Supplementary material**

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3 **Table S1.** Number of MS hits detected for 777 proteins, divided between four protein fractions: AP-
4 soluble, AP-membrane, HI-soluble and HI-membrane.

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6 **Table S2.** Known or predicted (KOP), and putative novel c-di-GMP binding proteins in *Bdellovibrio*
7 *bacteriovorus* HD100 as they were detected by CCMS. Data is provided as “the number of MS hits
8 detected in a binding experiment / the number of hits detected in a competition control”. For details see
9 materials and methods. A. KOP c-di-GMP binding proteins include all previously identified c-di-GMP
10 network proteins in *B. bacteriovorus* and c-di-GMP orthologous binders in other bacteria. B. Putative
11 novel binders are proteins hitherto not known to bind c-di-GMP.

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13 **Table S3.** Functional classification of the 84 CCMS candidate binders.

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15 **Figure S1.** Pairwise sequence alignment of *Xanthomonas campestris* Clp (Clp_XCC0472) and *B.*
16 *bacteriovorus* HD00 CRP (Bd2590). D70, R154, R166 and D170 are involved in c-di-GMP binding by Clp
17 (11). Bd2590 retains 3 of the four positions. E99 is responsible for Clp DNA-binding. The HTH motif is
18 colored in blue.

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20 **Figure S2.** Protein affinity purification. Bd2717 (A), Bd2402 (B) and Bd2924 (C) were His-tagged, produced
21 and purified with Ni-NTA resin. Tot. – total protein lysate, F.T. – flow through unbound fraction, W. -
22 wash, M. size marker, Elution – Elution fraction, Dial. – post dialysis.

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24 **Figure S3.** Bd2717 binds two cyclic di-GMP molecules. The arginine residue, positioned adjacent to the
25 RxxxR motif (R¹⁵⁵RXXXR) in the PilZ protein Bd2717, is predicted to promote binding of an intercalated c-
26 di-GMP dimer rather than a c-di-GMP monomer (32). Microscale thermophoresis measurements carried

27 with saturating concentrations of c-di-GMP, resulting in saturation curve that shows a characteristic kink,
 28 when saturation is reached. Bd2717 bound 1.7 cyclic di-GMP molecules, *i.e.* two c-di-GMP molecules.

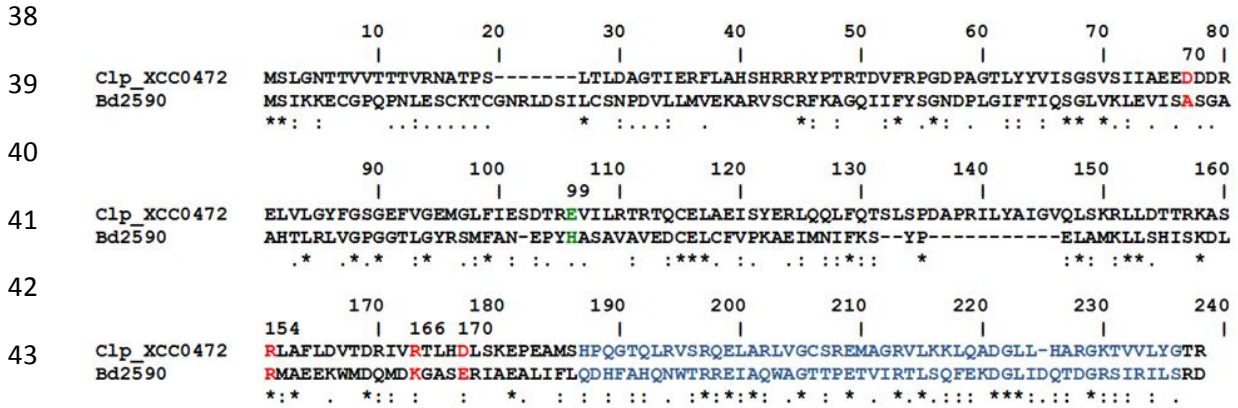
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30 **Figure S4.** Bd2402 and Bd2924 do not interact with cyclic di-AMP. Fluorescently labeled (A) Bd2402, a
 31 two-component response regulator, and (B) Bd2924, an acyl-coA dehydrogenase, were subjected to MST
 32 analysis with increasing concentrations of cyclic di-AMP, which had no apparent effect on the
 33 thermophoretic movement of the two proteins. Average values are of three triplicates. Error bars indicate
 34 standard deviation.

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37 **Figure S1**



53 **Figure S2**

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67 **Figure S3**

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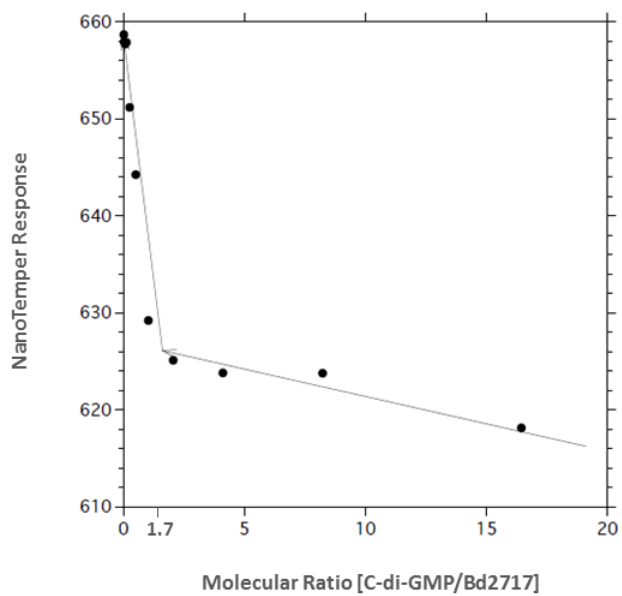
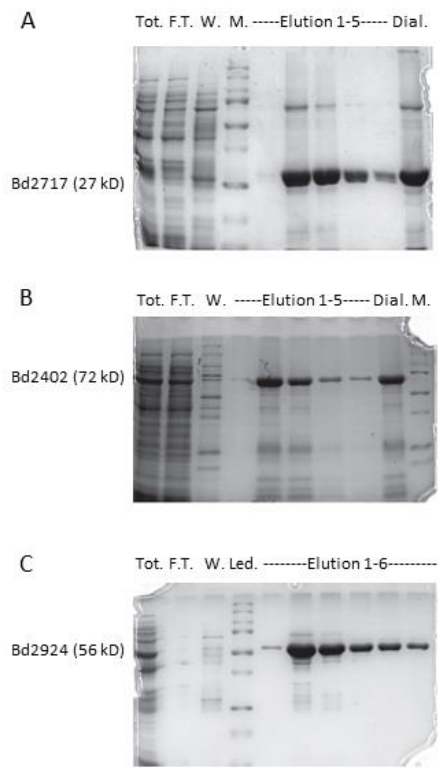
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78 **Figure S4**

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