Electronic Supplementary Material (ESI) for Molecular BioSystems

Differential binding of 2'-biotinylated analogs of c-di-GMP with c-di-GMP riboswitches and binding proteins

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Table S1: Different ligands (c-di-GMP, endo-S-c-di-GMP, compound **5** and compound **6**) docking into Vc2 RNA (PDB: 3IRW). The different conformers of each ligand were optimized using Guassian with fixed C5'-C5' distance of two guanines. Dockings were done in triplicate for each c-di-GMP and analog conformers.

Ligand	Distance (Å) between C5 of two guanines in Gaussian optimized structure	Distance (Å) between C5 of two guanines after docking			Docked binding affinity (kcal/mol)		
c-di-GMP	5.5	5.81	5.82	5.82	-12.2	-12.2	-12.2
	6.5	6.12	6.10	6.10	-11.8	-11.8	-11.8
	6.6	6.46	6.46	6.46	-14.6	-14.6	-14.7
	6.7	6.51	6.51	6.51	-15.1	-15.1	-15.1
	6.8	6.46	6.46	6.45	-13.6	-13.6	-13.5
	6.9	6.93	6.65	6.65	-11.3	-16.2	-16.2
	7.0	6.71	7.00	6.72	-16.7	-11.3	-16.7
	7.1	6.79	6.79	6.79	-17	-17.1	-17
	7.2	6.85	6.86	6.86	-17.1	-17.3	-16.9
	7.3	6.93	6.92	6.93	-17.4	-17.4	-17.4
	7.4	6.99	6.99	6.99	-17.5	-17.5	-17.5
	7.5	6.92	6.93	6.92	-16.9	-16.9	-16.9
	7.6	7.13	7.13	7.13	-17.5	-17.5	-17.5
	7.7	7.20	7.20	7.20	-17.3	-17.4	-17.4
	7.8	7.28	7.28	7.28	-17.1	-17	-17.1
	7.9	7.35	7.35	7.35	-16.7	-16.7	-16.7
	8.0	7.42	7.43	7.42	-16.2	-16.1	-16.2
	8.5	7.48	7.46	7.45	-12.5	-12.5	-12.5
	9.5	7.75	8.37	8.37	-10.4	-11.4	-11.3
endo-S-c-di-GMP	5.5	5.75	5.75	5.75	-11.1	-11.1	-11.2
	6.0	6.12	6.11	6.12	-11.5	-11.5	-11.5
	6.5	6.87	6.88	6.88	-13.1	-13	-13
	6.6	6.95	6.95	6.95	-13.3	-13.3	-13.2
	6.7	7.17	7.17	7.17	-13.1	-13.2	-13.1
	6.8	7.12	7.12	7.12	-12.6	-12.6	-12.6

	6.9	7.18	7.19	7.19	-13.9	-13.9	-13.9
	7.0	7.27	7.27	7.27	-13.9	-13.9	-13.9
	7.1	7.35	7.35	7.35	-14	-14	-14
	7.2	7.43	7.43	7.43	-14	-14	-13.9
	7.3	7.50	7.50	7.50	-13.9	-13.9	-13.9
	7.4	7.57	7.57	7.57	-13.9	-13.9	-13.9
	7.5	7.66	7.74	7.66	-12.6	-12.6	-12.6
	7.6	7.88	8.36	7.88	-12.3	-11.7	-12.3
	7.7	7.96	7.96	7.96	-12	-12	-12
	7.8	7.20	7.21	7.20	-13.5	-13.5	-13.5
	7.9	7.95	7.95	7.95	-12.9	-12.9	-12.9
	8.0	8.03	8.03	8.03	-12.5	-12.5	-12.5
	8.5	8.62	7.65	8.61	-11.3	-12.8	-11.2
	9.5	8.71	8.71	8.72	-12.1	-12.1	-12.1
Compound 5 (model for 2'-[Biotin]-endo-S-c-di-GMP)	5.5	7.50	7.19	7.18	-11.2	-11.3	-11.2
	6.5	6.73	6.73	6.73	-11.2	-11.2	-11.2
	6.8	7.00	6.99	7.00	-11.4	-11.3	-11.4
	6.9	7.09	7.09	7.09	-11.4	-11.4	-11.4
	7.0	7.20	7.20	7.20	-11.4	-11.4	-11.4
	7.1	7.20	7.20	7.20	-11.4	-11.4	-11.4
	7.2	7.41	7.41	7.41	-11.2	-11.2	-11.2
	7.3	7.53	7.53	7.53	-10.6	-10.8	-10.6
	7.4	7.48	7.62	7.48	-11.1	-10.5	-11.1
	7.5	7.68	7.68	7.68	-11.7	-11.7	-11.7
	7.6	7.84	7.84	7.84	-11.6	-11.6	-11.5
	7.7	7.98	7.98	7.98	-11.6	-11.6	-11.6
	7.8	9.26	9.26	9.26	-11.5	-11.5	-11.5
	8.0	9.48	9.48	9.48	-11.7	-11.6	-11.7
	8.5	9.47	9.47	9.69	-10.8	-10.8	-11.6
	9.5	10.50	10.07	10.49	-10.2	-10.9	-11.3
Compound 6 (model for 2'-[Biotin] -AHC-c-di-GMP	5.5	6.52	6.52	6.52	-9.7	-9.7	-9.7
	6.5	6.08	6.09	6.08	-10.2	-10.1	-10.1
	6.8	6.35	6.35	6.35	-10.1	-10.1	-10.1
	6.9	7.56	7.59	7.55	-10.4	-10.3	-10.3
	7.0	7.67	7.73	7.67	-10.3	-10.3	-10.4
	7.1	7.78	7.80	7.85	-10.2	-10.2	-10.1
	7.2	7.87	7.88	8.05	-10.2	-10.2	-10.2
	7.5	7.98	8.00	8.05	-10.6	-10.6	-10.5
	8.0	8.43	8.55	8.56	-10.4	-10.5	-10.6
	8.5	9.24	9.23	8.40	-11.7	-11.7	-9.8
	9.5	9.16	9.96	9.92	-10.1	-12.1	-11.4

Table S2: Different ligands (c-di-GMP, endo-S-c-di-GMP, compound **5** and compound **6**) docking into CdA RNA (PDB: 3Q3Z). The different conformers of each ligand were optimized using Guassian with fixed C5'-C5' distance of two guanines. Dockings were done in triplicate for each c-di-GMP and analog conformers.

Ligand	**Distance (Å) between C5 of two guanines in Gaussian optimized structure**	**Distance (Å) between C5 of two guanines after docking**			**Docked binding affinity (kcal/mol)**		
c-di-GMP	5.5	5.97	5.97	5.97	-10.1	-10.1	-10.1
	6.5	6.5	6.5	6.5	-10.1	-10.1	-10.8
	6.6	6.6	6.6	6.6	-12.5	-12.5	-12.5
	6.7	6.7	6.7	6.7	-13.1	-13.1	-13
	6.8	6.7	6.8	6.7	-12.9	-12.9	-12.9
	6.9	6.8	6.8	6.8	-14	-14	-14
	7.0	6.9	6.9	6.9	-14.3	-14.3	-14.3
	7.1	7.0	7.0	7.0	-14.6	-14.6	-14.6
	7.2	7.0	7.0	7.0	-14.7	-14.7	-14.7
	7.3	7.1	7.1	7.1	-14.8	-14.8	-14.8
	7.4	7.2	7.2	7.2	-14.7	-14.7	-14.7
	7.5	7.2	7.2	7.2	-15.1	-15.1	-15.1
	7.6	7.3	7.3	7.3	-14.4	-14.4	-14.4
	7.7	7.4	7.4	7.4	-14.2	-14.2	-14.2
	7.8	7.4	7.4	7.4	-13.9	-13.9	-13.9
	7.9	7.5	7.5	7.5	-13.5	-13.5	-13.5
	8.0	7.6	7.6	7.6	-13.1	-13.1	-13.1
	8.5	7.6	7.6	7.6	-13.4	-13.4	-13.4
	9.5	8.1	8.1	8.1	-13.1	-13.1	-13.2
endoscdGMP	5.5	5.5	5.5	5.5	-9.5	-9.5	-9.5
	6	5.8	5.8	5.8	-9.7	-9.6	-9.7
	6.5	6.4	6.4	6.4	-9.8	-9.8	-9.8
	6.6	6.4	6.5	6.4	-9.8	-9.8	-9.8
	6.7	7.4	7.4	7.4	-9.8	-9.8	-9.8
	6.8	6.7	6.7	6.7	-10.7	-10.7	-10.6
	6.9	7.5	7.5	7.5	-9.9	-9.8	-9.9
	7	7.5	7.5	7.5	-9.9	-9.9	-9.9
	7.1	7.6	7.6	7.6	-9.9	-9.9	-9.9
	7.2	7.7	7.7	7.7	-9.9	-9.9	-9.9
	7.3	7.1	7.1	7.1	-10	-10	-10
	7.4	7.2	7.2	7.2	-10.1	-10	-10.1
	7.5	7.7	7.7	7.7	-10.9	-10.9	-10.9
	7.6	8.1	8.1	8.1	-10.1	-10.1	-10.1
	7.7	8.2	8.2	8.2	-10.1	-10.1	-10.1
	7.8	8.2	8.2	8.3	-10.1	-10.1	-10.1
	7.9	7.3	7.3	7.3	-11.1	-11.1	-11.1

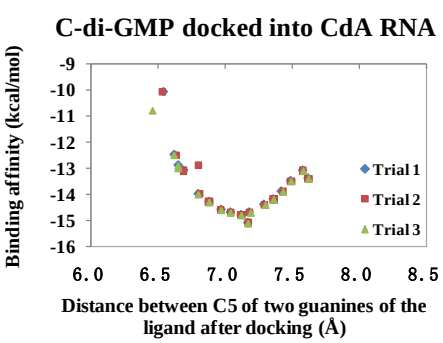
	8	7.4	7.4	7.4	-11.1	-11.1	-11
	8.5	7.9	7.9	8.0	-11.3	-11.3	-11.3
	9.5	9.2	9.2	9.2	-10.7	-10.7	-10.7
Compound 5 (model for 2'-[Biotin]-endo-S-c-di-GMP)	5.5	6.6	6.8	6.6	-9.8	-10	-9.8
	6.5	7.5	7.5	7.5	-10	-10	-10
	6.6	7.4	7.6	7.6	-10.4	-10	-10
	6.7	7.4	7.4	7.4	-10.5	-10.8	-10.5
	6.8	7.5	7.5	7.5	-10.9	-11	-10.9
	6.9	7.6	7.6	7.6	-10.8	-10.9	-10.9
	7	7.7	7.7	7.7	-11.1	-11	-10.9
	7.1	7.7	7.7	7.7	-11	-11	-10.9
	7.2	8.1	8.1	8.1	-10.3	-10.3	-10.3
	7.3	8.2	8.2	8.2	-10.2	-10.2	-10.2
	7.4	8.3	8.3	8.3	-10.3	-10.3	-10.3
	7.5	8.8	8.8	8.8	-11.4	-11.4	-11.4
	7.6	9.0	9.0	9.0	-10.5	-10.5	-10.5
	7.7	9.1	9.1	9.1	-10.4	-10.4	-10.4
	7.8	9.2	9.2	9.2	-10.3	-10.3	-10.3
	8	9.4	9.4	9.4	-10	-10	-10
	8.5	9.4	9.4	9.4	-11.1	-11.1	-11.1
	9.5	10.4	10.4	10.4	-11	-11	-11
Compound 6 (model for 2'-[Biotin]-AHC-c-di-GMP	5.5	6.4	6.4	6.5	-8.8	-8.8	-8.9
	6.5	7.4	7.4	7.4	-10	-10	-10
	6.8	7.0	7.0	7.0	-8.6	-8.7	-8.6
	6.9	8.0	8.0	8.0	-9.1	-9.1	-9.1
	7	8.1	8.1	8.1	-9.2	-9.2	-9.2
	7.1	8.2	8.2	8.2	-9.3	-9.3	-9.3
	7.2	8.3	8.3	8.3	-9.4	-9.4	-9.4
	7.5	8.3	8.3	8.3	-9.2	-9.2	-9.2
	8	8.9	8.9	8.9	-9.4	-9.4	-9.4
	8.5	9.4	9.4	9.4	-10.5	-10.5	-10.5
	9.5	10.4	10.4	10.4	-10.5	-10.6	-10.6

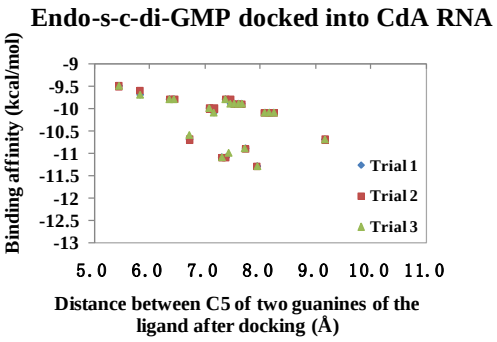
Table S3: Different ligands (c-di-GMP, endo-S-c-di-GMP, compound **5** and compound **6**) docking into WspR protein (PDB: 3I5A). The different conformers of each ligand were optimized using Guassian with fixed C5'-C5' distance of two guanines. Dockings were done in triplicate for each c-di-GMP and analog conformers.

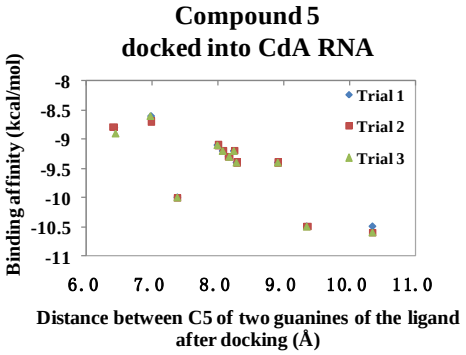
Ligand	**Distance (Å) between C5 of two guanines in Gaussian optimized structure**	**Distance (Å) between C5 of two guanines after docking**			**Docked binding affinity (kcal/mol)**		
c-di-GMP	5.5	6.1	6.1	6.1	-8.6	-8.6	-8.6
	6.5	6.5	6.5	6.5	-10.2	-10.1	-10.2
	6.6	6.6	6.6	6.6	-11.3	-11.3	-11.3
	6.7	6.7	6.7	6.7	-11.4	-11.4	-11.4
	6.8	6.7	6.7	6.7	-10.7	-10.7	-10.7
	6.9	6.8	6.8	6.8	-11.6	-11.6	-11.6
	7	6.9	6.9	6.9	-11.7	-11.7	-11.7
	7.1	6.9	6.9	6.9	-11.7	-11.7	-11.7
	7.2	7.0	7.0	7.0	-11.7	-11.7	-11.7
	7.3	7.0	7.0	7.0	-11.7	-11.7	-11.7
	7.4	7.1	7.1	7.1	-11.6	-11.6	-11.6
	7.5	7.2	7.2	7.2	-11.2	-11.3	-11.3
	7.6	7.2	7.2	7.2	-11.5	-11.4	-11.5
	7.7	7.3	7.3	7.3	-11.3	-11.3	-11.3
	7.8	7.3	7.3	7.2	-11	-11	-11
	7.9	7.2	7.2	7.2	-11	-11	-11
	8	7.3	7.3	7.3	-11	-11	-11
	8.5	7.4	7.4	7.4	-11.8	-11.8	-11.8
	9.5	8.2	8.2	8.2	-12.4	-12.4	-12.4
	10.5	9.1	9.1	9.1	-12.5	-12.5	-12.5
endo-S-c-di-GMP	6	6.4	6.5	5.2	-8.1	-8.1	-8
	6.5	6.6	6.6	6.6	-8.5	-8.5	-8.5
	6.6	6.7	6.7	6.7	-8.6	-8.6	-8.6
	6.7	7.6	7.6	7.6	-9	-9	-9
	6.8	7.5	7.5	7.5	-8.8	-8.8	-8.8
	6.9	7.0	7.1	7.0	-9.1	-9.2	-9.2
	7	7.8	7.8	7.8	-10	-10	-10
	7.1	7.5	7.5	7.5	-10.3	-10.3	-10.3
	7.2	7.6	7.6	7.6	-10.2	-10.2	-10.2
	7.3	7.8	7.7	8.0	-10.3	-10.3	-10.3
	7.4	7.8	7.8	7.8	-10.3	-10.2	-10.2
	7.5	7.4	7.4	7.4	-9.3	-9.3	-9.3
	7.6	7.7	7.7	7.7	-9.1	-9.1	-9.1
	7.7	7.8	7.8	7.8	-9.1	-9.1	-9.1
	7.8	7.8	7.8	7.8	-9.2	-9.2	-9.2

	7.9	7.3	7.3	7.3	-10.3	-10.3	-10.2
	8	7.3	7.3	7.3	-10.2	-10.2	-10.2
	8.5	7.7	7.7	7.7	-10	-10	-10
	9.5	8.6	8.6	8.6	-9.9	-9.9	-9.9
Compound 5 (model for 2'-[Biotin]-endo-S-c-di-GMP)	6.5	7.3	7.3	7.3	-9.4	-9.4	-9.4
	6.6	7.3	7.4	7.3	-9.3	-9.4	-9.3
	6.7	7.4	7.4	7.4	-9.4	-9.4	-9.4
	6.8	7.5	7.5	7.5	-9.4	-9.4	-9.4
	6.9	7.6	7.6	7.6	-9.3	-9.4	-9.4
	7	7.7	7.7	7.7	-9.2	-9.2	-9.2
	7.1	7.7	7.7	7.7	-9.3	-9.3	-9.2
	7.2	7.9	8.2	8.2	-8.9	-8.8	-8.8
	7.3	7.2	7.2	7.2	-10	-10	-10
	7.4	7.3	7.3	7.3	-9.9	-9.9	-9.9
	7.5	8.9	8.9	8.9	-8.4	-8.4	-8.4
	7.6	9.0	9.0	9.0	-9.1	-9.1	-9.1
	7.7	9.1	9.1	9.1	-9	-9.1	-9.1
	7.8	9.2	9.2	9.2	-9	-9	-9
	8	8.3	9.4	8.3	-9.1	-8.9	-9
	8.5	9.9	9.6	9.5	-7.8	-7.9	-7.8
	9.5	9.7	9.7	9.7	-7.6	-7.6	-7.6
Compound 6 (model for 2'-[Biotin] -AHC-c-di-GMP	5.5	6.2	6.5	6.5	-7.4	-7.4	-7.4
	6.5	6.6	6.6	6.6	-8.5	-8.6	-8.7
	6.8	6.8	6.8	6.8	-8.6	-8.6	-8.6
	6.9	7.4	7.4	7.4	-8.6	-8.6	-8.6
	7	7.4	7.4	7.5	-7.9	-8.7	-8.5
	7.1	7.5	7.5	7.5	-7.8	-7.9	-7.9
	7.2	7.9	7.9	7.6	-8	-8	-7.9
	7.5	7.4	7.4	7.4	-8.3	-8.4	-8.4
	8	8.8	8.8	8.8	-8.4	-8.4	-8.5
	8.5	9.3	9.3	9.5	-8.3	-8.4	-8.3
	9.5	10.4	10.4	10.4	-8.4	-8.4	-8.4

Figure S1: Plots of binding energies versus distance between the two guanine nucleobases (after docking) of c-di-GMP, endo-S-c-di-GMP, compound **5** or compound **6** when docked into CdA RNA (PDB: 3Q3Z). Dockings were done in triplicate for each c-di-GMP or analog conformer.







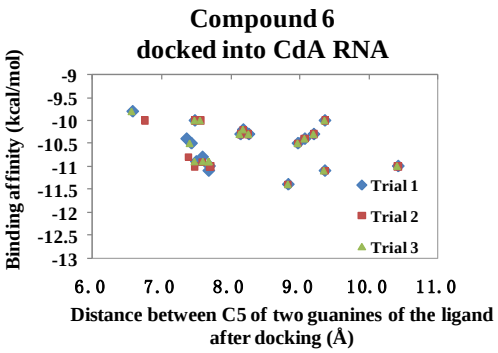


Figure S2: Plots of binding energies versus distance between the two guanine nucleobases (after docking) of c-di-GMP, endo-S-c-di-GMP, compound **5** or compound **6** when docked into WspR protein (PDB: 3I5A). Dockings were done in triplicate for each c-di-GMP or analog conformer.