SUPPORTING INFORMATION

FOR

Unexpected complex formation between coralyne and c–di-AMP provides a simple fluorescent turn-on assay to detect this bacterial second messenger.

Jie Zhou[a], David A. Sayre[a], Yue Zheng[a] and Henryk Szmacinski[b]
*Herman O. Sintim[a]**

[a]Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742.

[b]Department of Biochemistry and Molecular Biology, Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, 725 West Lombard St, Baltimore, MD 21201.

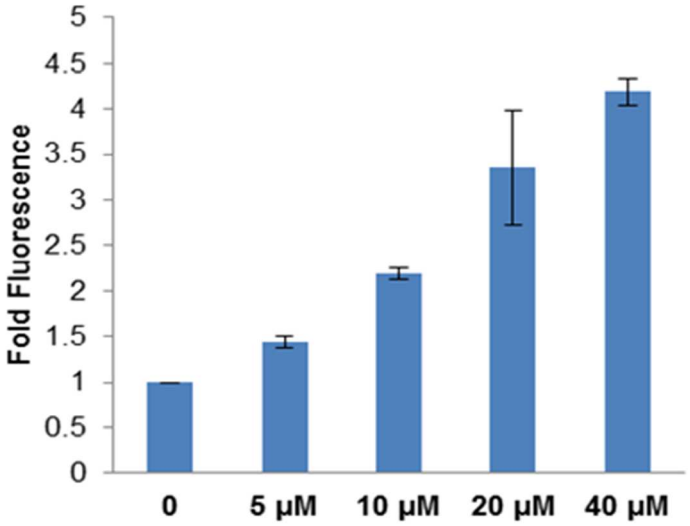

Figure S1. The fold fluorescence of coralyne in the presence of c-di-AMP. In the absence of a halogen quencher the unbound coralyne is also fluorescent and therefore changes in fluorescence intensity in the presence of different concentrations of c-di-AMP are not large. For example, the fluorescence fold changes for 20 µM and 40 µM c-di-AMP are almost similar. The addition of bromide or iodide improves the discrimination between different c-di-AMP concentrations (see Figure 4 of main text). Condition: Coralyne = 10 µM, Buffer Tris-H_3PO_4 (pH 7.5), [c-di-AMP] = 0, 5, 10, 20 or 40 µM. Temperature = 10 ^{o}C. ex. 420 nm, em. 475 nm.

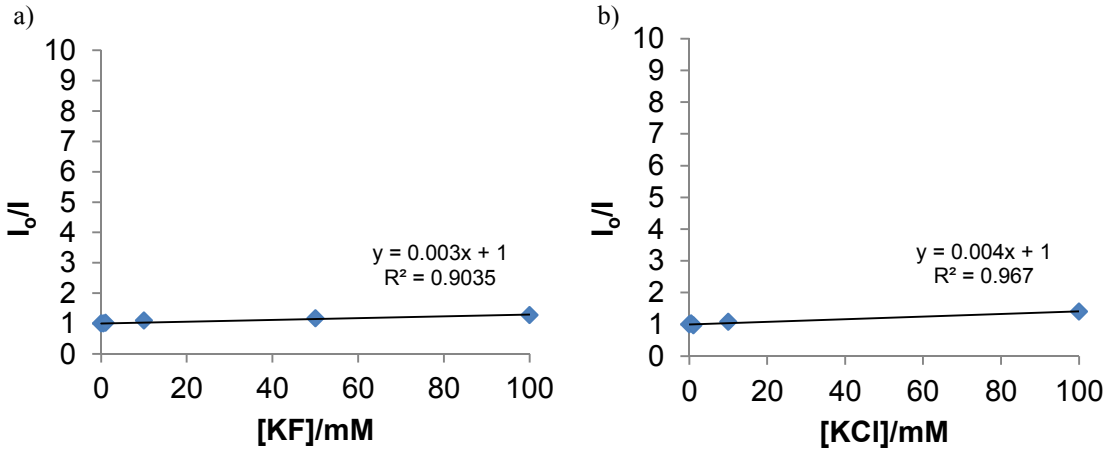

Figure S2. Stern-Volmer plots for KF and KCl quenching in the absence of c-di-AMP indicate these are not good fluorescence quenchers, in contrast to KBr or KI. Condition: [coralyne] = 10 µM, Buffer: 50 mM Tris-H_3PO_4 (pH 7.5) containing various concentrations of KF (a) (0, 0.5, 1, 10, 25, 50, 75, 100, 250 or 500 mM) and KCl (b) (0, 0.5, 1, 10, 25, 50, 75, 100, 250 or 500 mM)

Table S1. KI quenching of coralyne's fluorescence in the absence of c-di-AMP

Quencher KI [mM]	τ_I (νσ)	f_i	$\tau_{\alpha\varpi\gamma}$ (νσ)	χ^2	τ_o / τ (I_0/I)	I
0	14.22 9.82 <32>	1 0.317 0.683	14.22 24.97	1.92 1.16	1 (1)	228
0.125	8.54	1	8.54	1.08	1.67 (4.36)	42.5
0.25	8.65	1	8.65	1.11	1.64 (9.38)	
0.5	8.84	1	8.84	1.11	1.61 (16.4)	13.9
1	7.3	1	7.3	0.92	1.95 (36.8)	6.2
5	4.85	1	4.85	1.38	2.93 (101.3)	5

[a] Condition: [coralyne] = 10 μM, Buffer: 50 mM Tris-H_3PO_4 (pH 7.5) at 25 °C with KI concentration indiciated in the first column; Average lifetime $\tau_{avg} = \Sigma f_i \tau_i$. [b] For relative lifetime single lifetime fit was used for KI and average lifetimes from double-exponential fit in the presence of c-di-AMP.

Table S2. KI quenching of coralyne's fluorescence in the presence of 40μM c-di-AMP

Quencher KI [mM]	τ_I (νσ)	f_i	$\tau_{\alpha\varpi\gamma}$ (νσ)	χ^2	τ_o / τ (I_0/I)	I
0.5	12 2.38 19.02 1.23 5.35 26.75	1 0.14 0.86 0.074 0.128 0.798	12 16.69 22.13	52 2.07 1.24	1 (1)	410
1	11.52 2.04 18.19 0.67 3.33 21.22	1 0.149 0.851 0.122 0.115 0.763	11.52 15.78 16.65	86 3.17 1.57	1.06 (1.12)	365
5	11.07 2.24 17.66 0.58 3.37 20.45	1 0.159 0.841 0.149 0.129 0.723	11.07 15.21 15.3	40 1.89 1.07	1.1 (1.92)	213
10	10.25 2.21 16.09 0.9 3.99	1 0.181 0.819 0.106 0.157	10.25 13.58	70 2.71	1.23 (1.39)	294

	19.92	0.737	15.4	1.42		
25	9.68 2.53 15.95 1.25 4.44 20.14	1 0.214 0.786 0.099 0.188 0.712	9.68 13.08 15.31	76 2.17 1.3	1.28 -1.73	237
50	9.11 2.4 14.43 0.69 3.16 15.83	1 0.228 0.772 0.115 0.199 0.686	9.11 11.69 11.57	69 1.94 1.26	1.43 -2.53	162
75	9.01 2.24 12.79 1.63 6.13 17.2	1 0.229 0.771 0.157 0.239 0.604	9.01 10.38 12.11	25 1.18 1	1.61 -3.13	131
100	8.86 2.11 12.27 1.69 6.71 16.24	1 0.22 0.78 0.166 0.249 0.585	8.86 10.03 11.45	41 1.35 1.16	1.66 -3.5	117

[a] Condition: [coralyne] = 10 µM, [c-di-AMP] = 40 µM, Buffer: 50 mM Tris-H_3PO_4 (pH 7.5) at 25 °C with KI concentration indicated in the first column; Average lifetime $\tau_{avg} = \Sigma f_i \tau_i$. [b] For relative lifetime single lifetime fit was used for KI and average lifetimes from double-exponential fit in the presence of c-di-AMP.

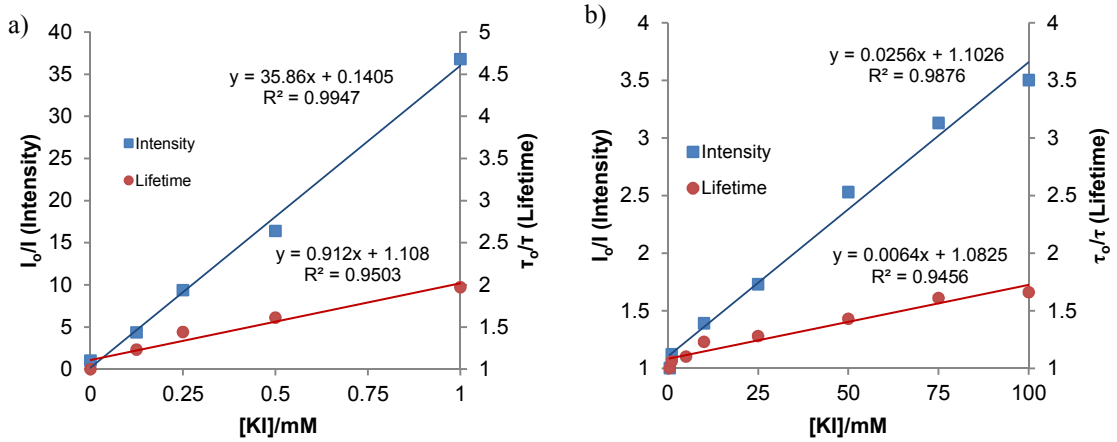

Figure S3. KI quenching of coralyne's fluorescence in the (a) absence and (b) presence of 40 µM c-d-AMP at 25 °C. Buffer: 50 mM Tris-H_3PO_4 (pH 7.5)

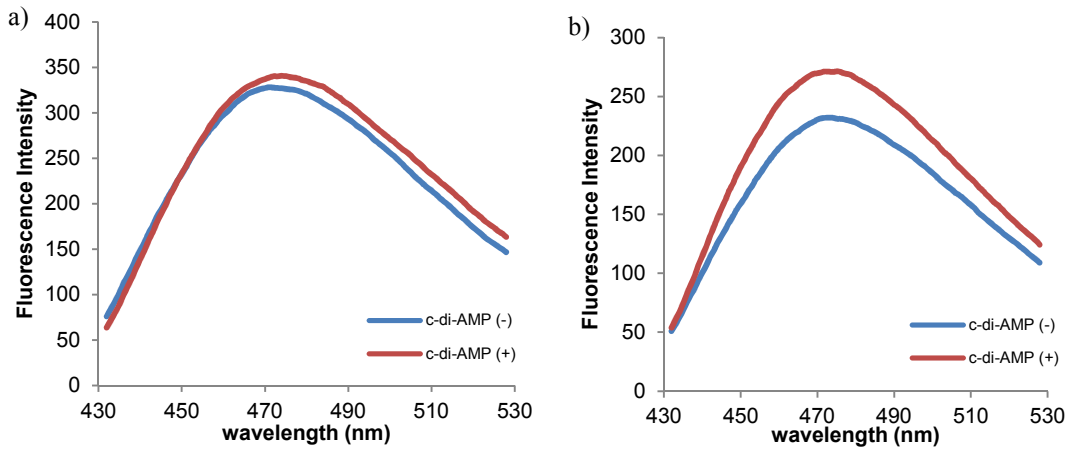

Figure S4. KBr quenching of coralyne's fluorescence in the absence of c-di-AMP or in the presence of c-di-AMP at 60 ^{o}C. Condition: [Coralyne] = 10 µM, [c-di-AMP] = 0 or 40 µM, Buffer: 50 mM Tris-H_3PO_4 (pH 7.5) containing (a) KBr = 1 mM or (b) KBr = 100 mM. ex. 420 nm, em. 475 nm.

In contrast to the data at 10 ^{o}C (Figure S5), where c-di-AMP "protected" coralyne from quenching, at 60 ^{o}C, KBr can quench the fluorescence of coralyne even in the presence of c-di-AMP. This is consistent with a model whereby coralyne forms a complex with c-di-AMP to become "protected" from quenching but at higher temperature, the complex would collapse and hence the protection from quenching will be minimal.

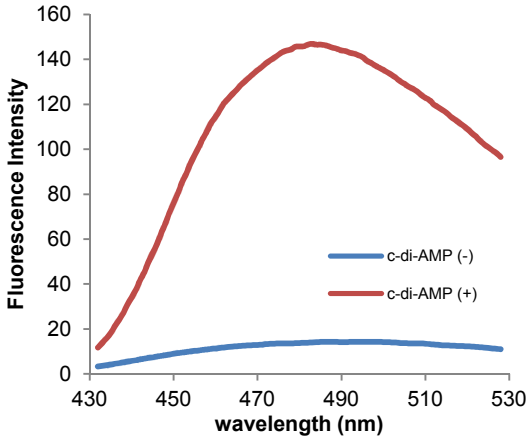

Figure S5. At 10 ^{o}C and in the presence of c-di-AMP, KBr is not effective at quenching the fluorescence of coralyne. Condition: [Coralyne] = 10 µM, [c-di-AMP] = 0 or 40 µM, Buffer: 50 mM Tris-H_3PO_4 (pH 7.5) containing KBr = 100 mM. ex. 420 nm, em. 475 nm.

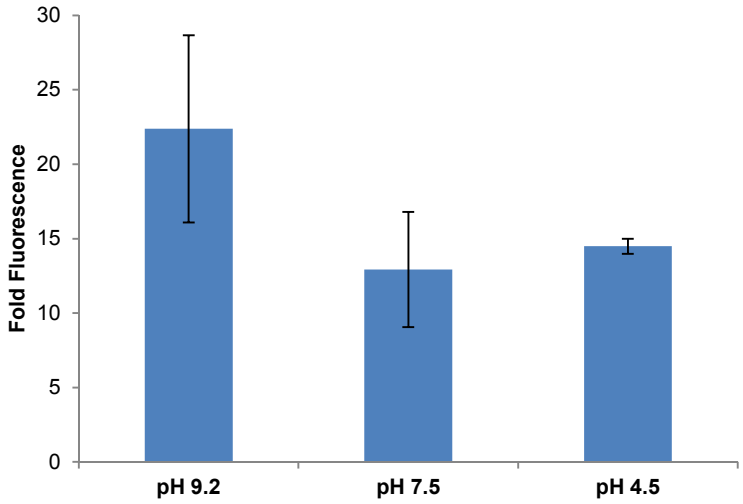

Figure S6. Fold fluorescence profiles of coralyne in the presence of c-di-AMP at different pHs. Condition: [coralyne] = 10 μM, [c-di-AMP] = 40 μM, Buffer: 50 mM Tris-H_3PO_4 (pH 4.5, 7.5 or 9.2), containing 250 mM KBr. Temperature = 10 ^oC. ex. 420 nm, em. 475 nm.

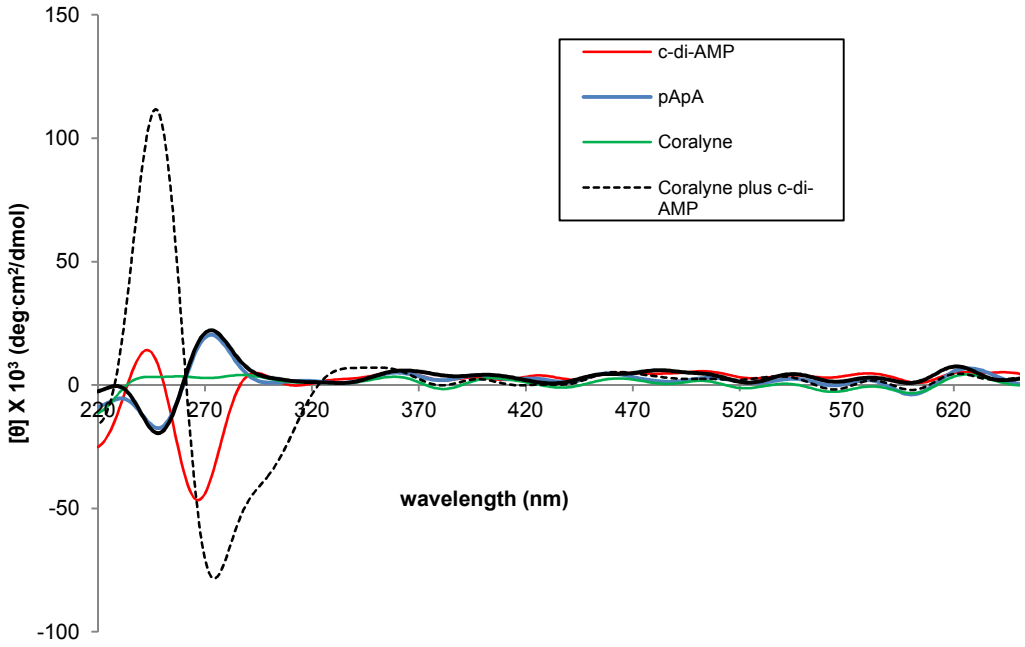

Figure S7. CD of coralyne-c-di-AMP or pApA complex (220-700 nm). Condition: [coralyne] = 10 μM, [c-di-AMP] = 40 μM, Buffer: 50 mM Tris-H_3PO_4 (pH 7.5) containing 250 mM KBr. Temperature = 10 ^oC.

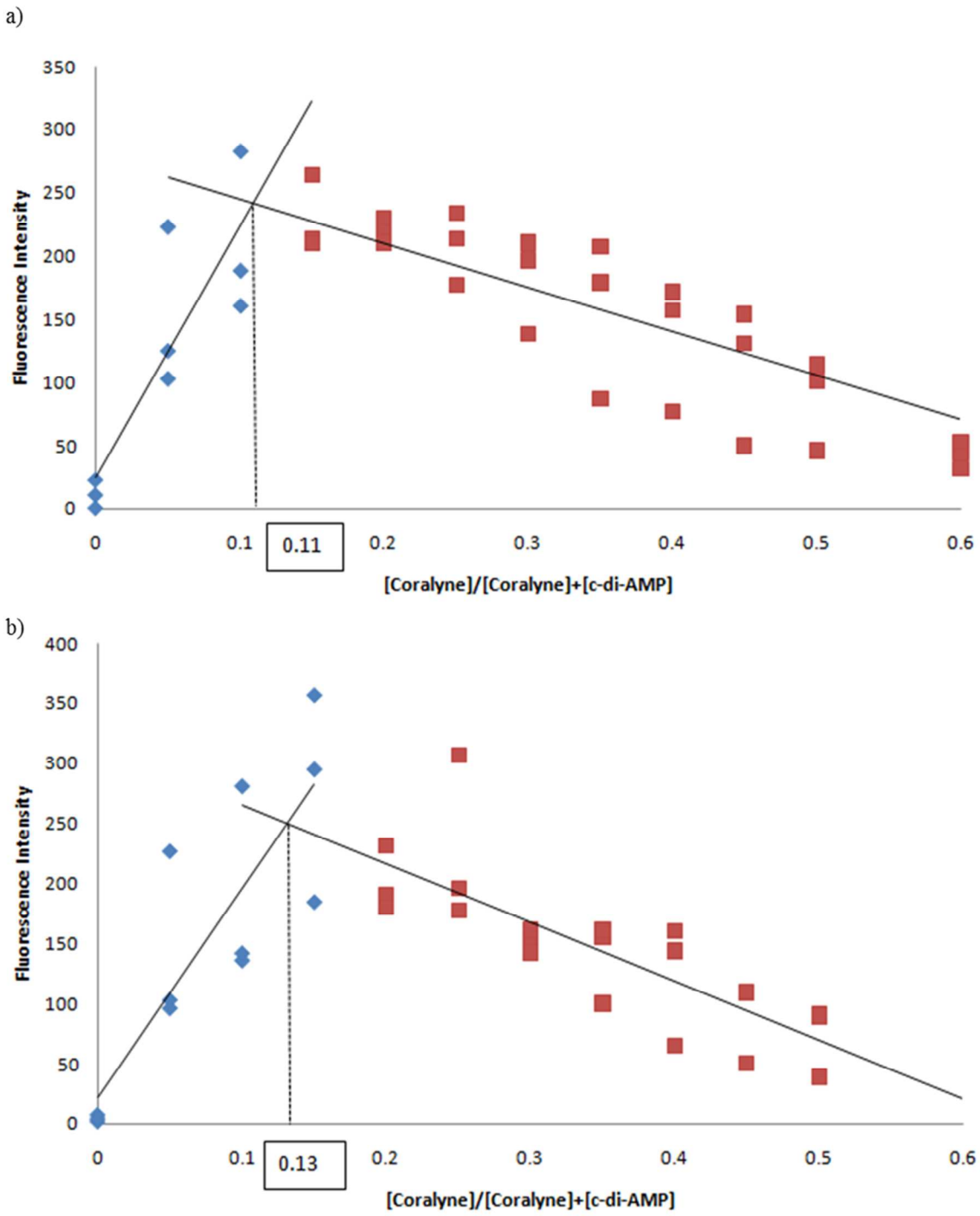

Figure S8. Job plot. [Coralyne] + [c-di-AMP] was fixed at 50 µM. The experiment was done in triplicate and plotted together on the graphs. Buffer: 50 mM Tris-H_3PO_4 (pH 4.5, Figure S6a or pH 9.2, Figure S6b) containing 250 mM KBr. Temperature = 10 ^{o}C. ex. 420 nm, em. 475 nm.

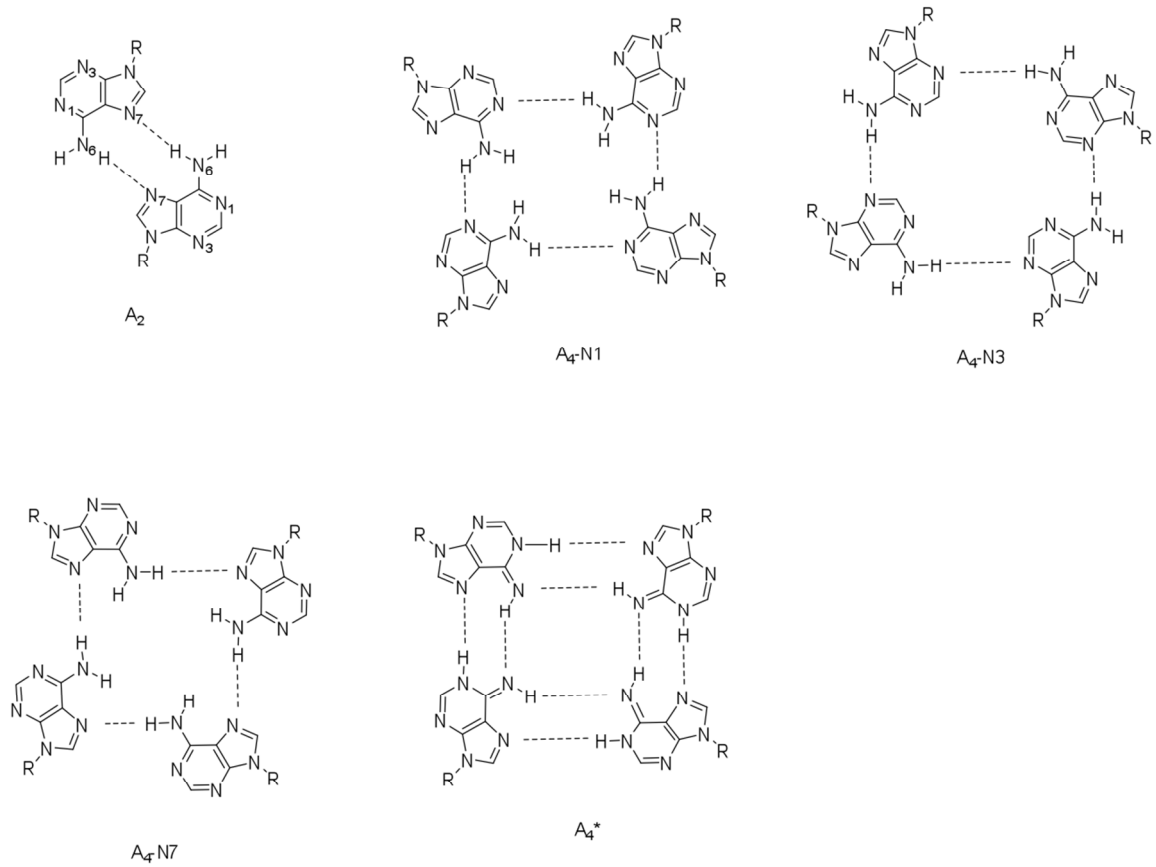

Figure S9. Adenine-adenine hydrogen bonding in duplex and quartets.