

## A DNA nanoswitch incorporating the fluorescent base analogue 2-amino purine detects single nucleotide mismatches in unlabelled targets

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Supplementary Material

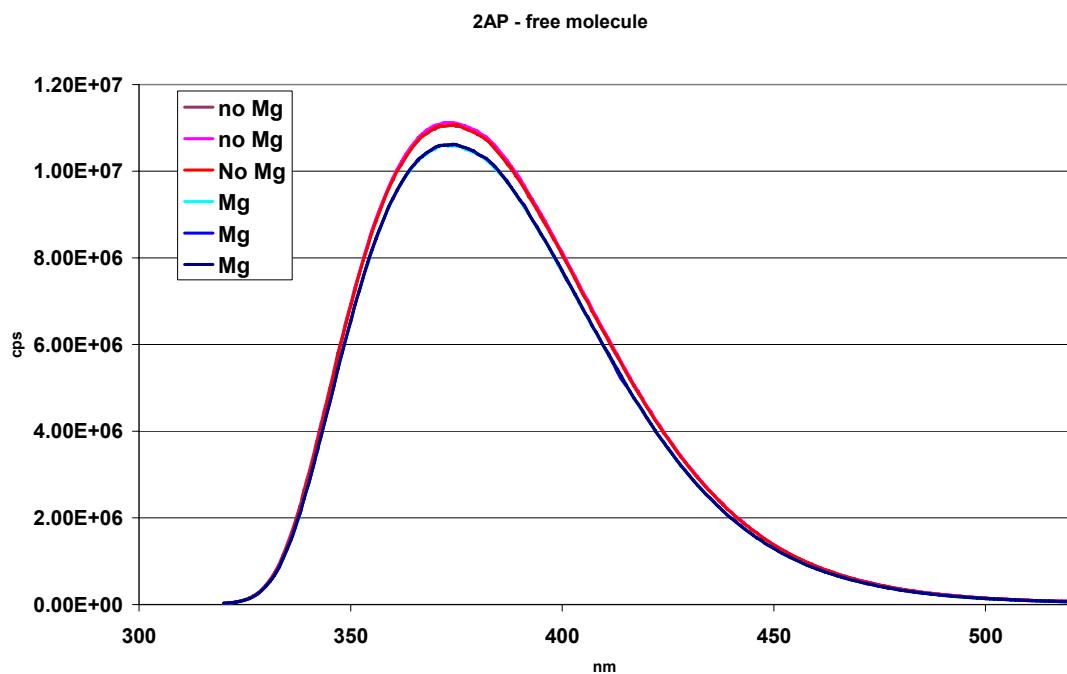


Figure S1  
Emission spectra of AP (1 μM) with and without MgCl<sub>2</sub> (5 mM)

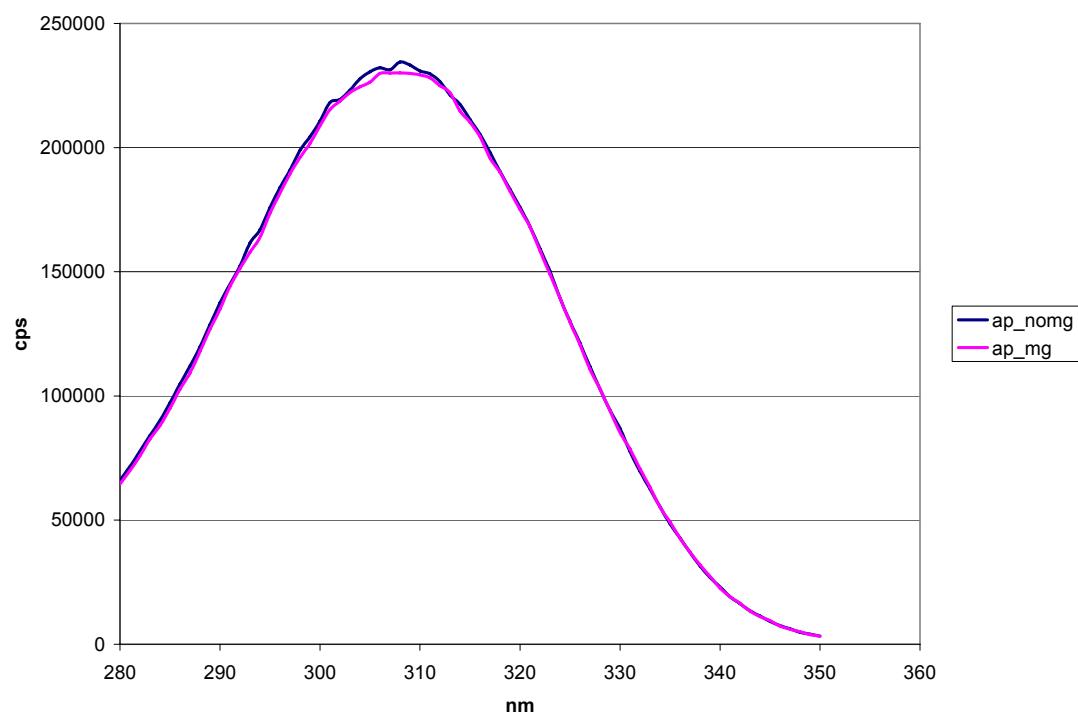


Figure S2  
Excitation spectra of free AP, with and without MgCl<sub>2</sub> (5 mM)

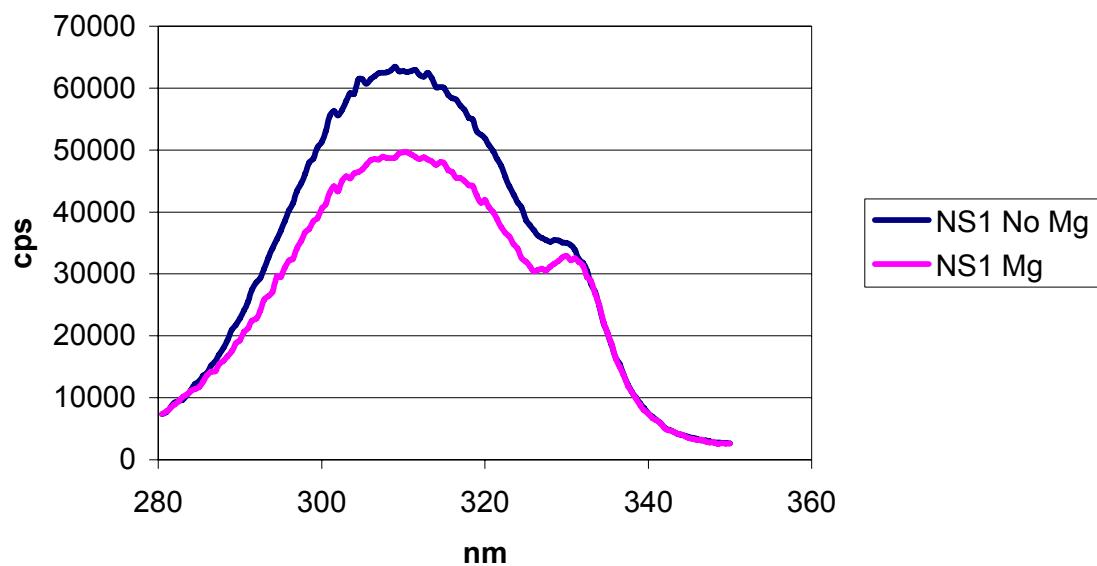


Figure S3  
Excitation spectra of NS1 with and without  $\text{MgCl}_2$  (5mM)

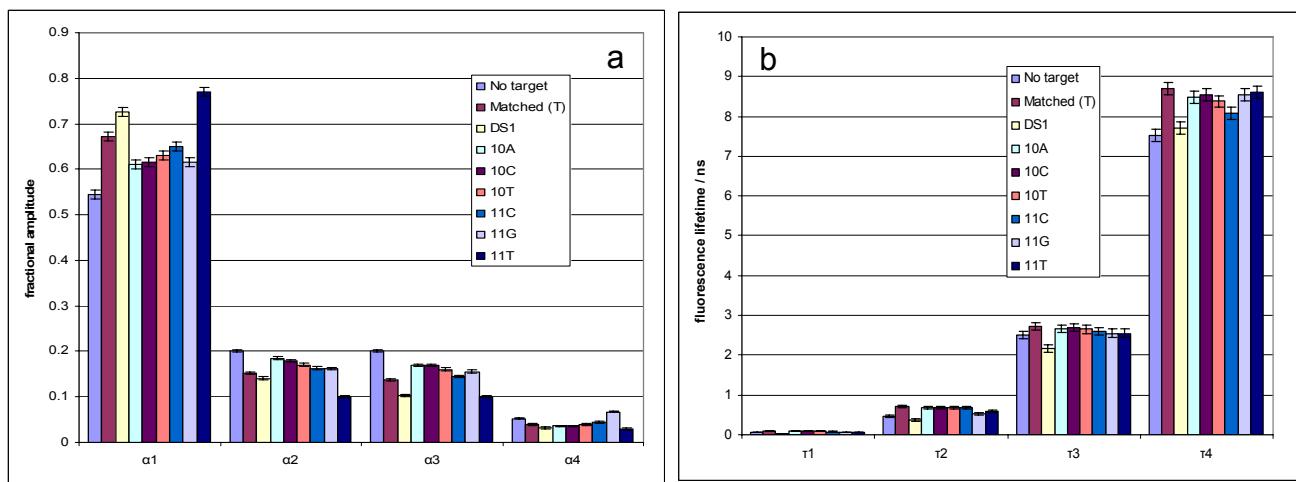


Figure S4: a – population,  $\alpha_i$  and b – fluorescence lifetime,  $\tau_i$ , data for nanoswitches in the absence of  $Mg^{2+}$  (the open configuration). Error bars represent standard deviations calculated from individual fits.

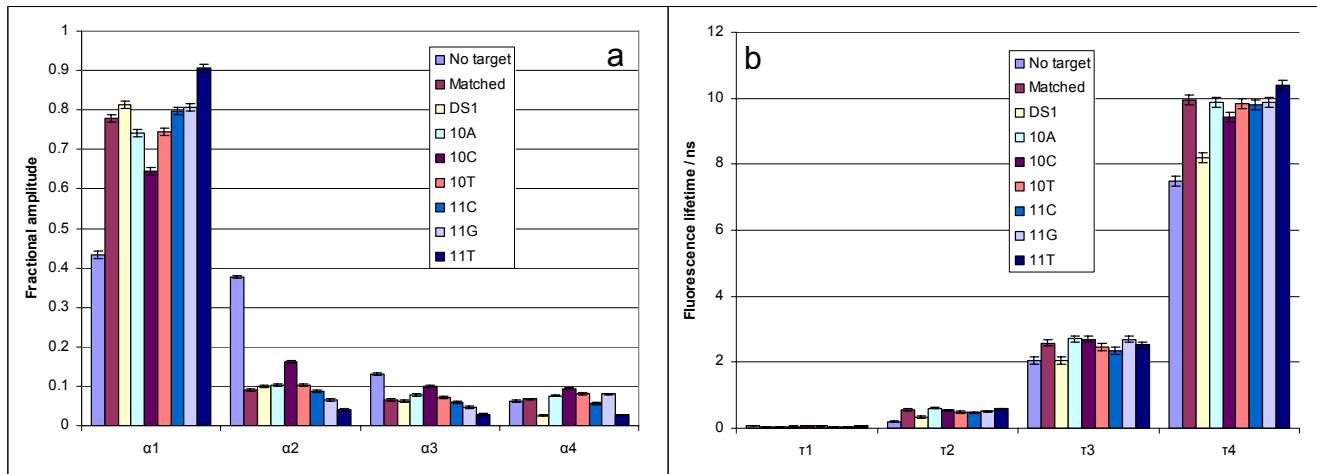


Figure S5: a – population,  $\alpha_i$  and b – fluorescence lifetime,  $\tau_i$ , data for nanoswitches in the presence of 5 mM  $Mg^{2+}$  (the closed configuration). Error bars represent standard deviations calculated from individual fits.

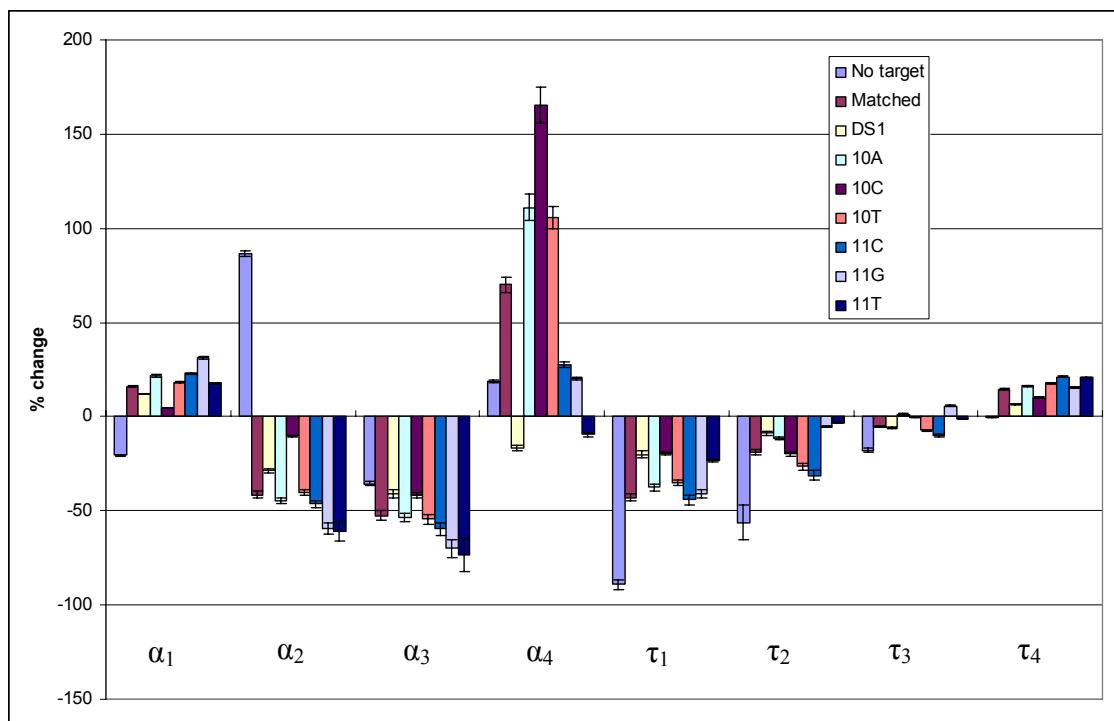


Figure S6. Change in proportion,  $\alpha_i$ , and lifetime,  $\tau_i$ , of each component, i on the addition of  $Mg^{2+}$ . Error bars represent standard deviations calculated from individual fits.

	[Mg <sup>2+</sup> ]	$\alpha_1$	$\alpha_2$	$\alpha_3$	$\alpha_4$		$\tau_1$ (ns)	$\tau_2$ (ns)	$\tau_3$ (ns)	$\tau_4$ (ns)	$\chi^2$
No target	0 mM	0.54	0.20	0.20	0.05		0.07	0.46	2.50	7.52	1.09
No target	10 mM	0.43	0.38	0.13	0.06		0.08	0.20	2.05	7.50	1.05
Matched (T1)	0 mM	0.67	0.15	0.14	0.04		0.08	0.70	2.73	8.70	1.18
Matched (T1)	10 mM	0.78	0.09	0.07	0.07		0.05	0.57	2.59	9.96	1.03
DS1	0 mM	0.73	0.14	0.10	0.03		0.04	0.36	2.17	7.70	1.05
DS1	10 mM	0.81	0.10	0.06	0.03		0.03	0.33	2.04	8.21	1.05
T10A	0 mM	0.61	0.18	0.17	0.04		0.09	0.69	2.66	8.47	1.078
T10A	10 mM	0.74	0.10	0.08	0.08		0.05	0.61	2.70	9.87	1.028
T10C	0 mM	0.62	0.18	0.17	0.04		0.08	0.68	2.70	8.55	1.063
T10C	10 mM	0.64	0.16	0.10	0.10		0.07	0.54	2.69	9.41	1.051
T10T	0 mM	0.63	0.17	0.16	0.04		0.09	0.67	2.65	8.38	1.127
T10T	10 mM	0.74	0.11	0.07	0.08		0.06	0.49	2.46	9.86	1.024
T11C	0 mM	0.65	0.16	0.14	0.04		0.07	0.68	2.61	8.09	1.103
T11C	10 mM	0.80	0.09	0.06	0.05		0.04	0.47	2.34	9.81	1.073
T11T	0 mM	0.77	0.10	0.10	0.03		0.07	0.60	2.55	8.60	1.175
T11T	10 mM	0.90	0.04	0.03	0.03		0.06	0.58	2.52	10.40	1.067
T11G	0 mM	0.62	0.15	0.16	0.07		0.07	0.54	2.55	8.54	1.024
T11G	10 mM	0.80	0.07	0.05	0.08		0.04	0.51	2.69	9.86	1.12

Table S1. Fluorescence lifetimes and amplitudes for all nanoswitches and model molecules studied. Although global fitting software reports  $\chi^2$  as a measure of fit quality and not individual error measurements, we can estimate, using individual decays, the following standard deviations in the measured parameters:  $\alpha_1 \pm 0.01$ ,  $\alpha_2 \pm 0.003$ ,  $\alpha_3 \pm 0.003$ ,  $\alpha_4 \pm 0.002$ ,  $\tau_1 \pm 0.002$ ,  $\tau_2 \pm 0.03$ ,  $\tau_3 \pm 0.1$ ,  $\tau_4 \pm 0.15$ .