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DNA Aptamers as Analyte-Responsive Cation Transporters in Fluorogenic Vesicles: Signal Amplification by Supramolecular Polymerization

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In the presence of amphiphilic counterions, polyions such as DNA[1,2] or cell-penetrating peptides (CPPs)[3,4] can dissolve in nonpolar media, phase transfer from water into organic solvents, and carry hydrophilic counterions into and across bulk and lipid bilayer membranes. The usefulness of this counterion-mediated function to enable cellular uptake of DNA[2] and CPPs[4] and for sensing applications[1,3] has been demonstrated. To further explore the usefulness of DNA chemistry in the general context of sensing systems[1,3,5,6] made from synthetic ion carriers, channels, and pores,[1,3,7] DNA aptamers[6] appeared ideal. Comparable to antibodies but much easier to obtain, DNA aptamers can be selected for any analyte. Moreover, selection methods imply that DNA aptamers act as ss-DNA and that ds-helices formed with their antiaptamer dissociate in response to analyte binding (Figure 1).

Aptamer **1** recognizes adenosine (A) and adenosine triphosphate (ATP, Table 1, entry 1).[6] The ability of dodecylguanidinium (DG) as a representative countercation to activate aptamer **1** as a cation transporter was determined in EYPC-LUVs⊃DPX/HPTS following previously established procedures (i.e., egg yolk phosphatidylcholine large unilamellar vesicles loaded with the anionic fluorophore 8-hydroxy-1,3,6-pyrenetrisulfonate and the cationic quencher *p*-xylene-bis-pyridinium bromide).[1] This assay reports transport activity as fluorescence recovery. At saturation with DG, the maximal fluorescence recovery Y_{MAX} accessible with aptamer **1** was 40% of that of calf-thymus DNA (Table 1, entry 1, Figure S1). This activity of aptamer **1** did not change in the presence of ATP (Figure S2).

The activity of polyion transporters increases with the number of charges in an overadditive manner because of multivalency effects.[3b] As expected for charge duplication upon dimerization, the activity of aptamer **1** increased in the presence of antiaptamer **2** at constant oligonucleotide concentration (Figure S3). Their Job plot showed with $Y_{MAX} = 75\%$ highest activity at 1:1 stoichiometry (Table 1, entry 2; Figure S3). DG-activated ds-DNA **1•2** responded to ATP (Table 1, entry 2; Figure 2A, ○). This finding validated the envisioned sensing concept to generate a signal by duplex disassembly in response to analyte binding.

Inactivation of **1•2** by ATP occurred with an $IC_{50} = 2.1$ mM and did not reach completion ($\Delta Y < Y_{MAX}$, Table 1, entry 2; Figure 2A, ○). Reduced competition by duplex destabilization with shortened antiaptamer strands **3**−**5** was considered to improve sensitivity for ATP (Table 1, entries 3−5). However, reduction of duplex charge and stability at the same time reduced the multivalency effects that determine polyion−counterion activity Y_{MAX} and response ΔY. As a result, the total efficiency $\eta = IC_{50}/\Delta Y$ was not improved and decreased rapidly with antiaptamer shortening (Table 1, entries 3−5).

To increase ATP sensitivity without losses in transport activity, duplex destabilization with mismatched rather than shortened antiaptamers seemed more promising. ATP recognition indeed improved with increasing mismatch in antiaptamers **6**−**8**, whereas

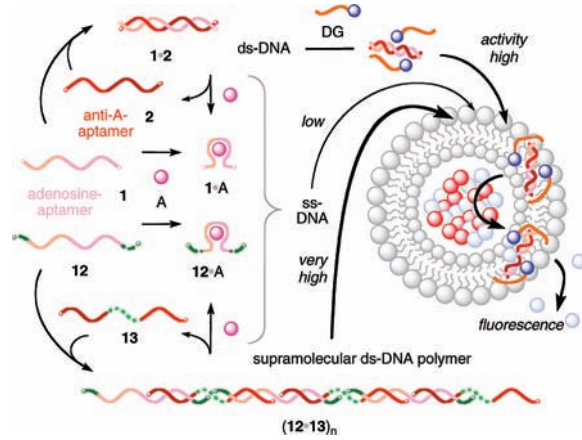

Figure 1. Activated by countercations (here DG), DNA can act as cation transporter in fluorogenic vesicles. For sensing with DNA aptamers, the differences in activity of ss-DNA (low), ds-DNA (high), and supramolecular ds-DNA polymers (very high) are used. The disassembly of aptamer/antiaptamer duplexes without (e.g., **1•2**) or with (e.g., $(\mathbf{12•13})_n$) sticky ends in response to the binding of analyte A is thus reported as a decrease in activity, i.e., fluorescence emission.

activity and response decreased, presumably due to incomplete duplex formation in the absence of ATP (Table 1, entries 6−8). Contrary to antiaptamer shortening, the total efficiency improved with mismatch from $\eta = 42$ for **1•2** to $\eta = 28$ for **1•8** (Table 1, entry 2 vs 8).

To increase the responsiveness of aptamer sensors, the activity of the initial ds-DNA had to be increased without an increase in activity of the final ss-DNA. Domain rearrangement in antiaptamer **9** compared to antiaptamer **2** was expected to produce a supramolecular ds-DNA polymer $(\mathbf{1•9})_n$. However, decreasing rather than increasing Y_{MAX} found for aptamer **1** with antiaptamer **9** was characteristic for the presence of ss-DNA (Table 1, entry 9). This suggested that polymer $(\mathbf{1•9})_n$ did not form, even when assisted by 1 mM Mg^{2+} (Figure S4). To polymerize the DNA double helices, sticky ends[8] were added in aptamer **10** and complemented in the middle of antiaptamer **11**. The increase in activity from $Y_{MAX} = 75\%$ with duplex **1•2** to $Y_{MAX} = 92\%$ was consistent with supramolecular polymerization into $(\mathbf{10•11})_n$ (Table 1, entry 2 vs 11). Stickier ends in aptamer **12** gave the higher $Y_{MAX} = 100\%$ expected for stabilized supramolecular polymers $(\mathbf{12•13})_n$ (Table 1, entry 13). Aptamer dimerization rather than polymerization with **14•15•13** was correctly reflected in a reduction to $Y_{MAX} = 87\%$ (Table 1, entry 15).

Because access to the individual binding sites is less hindered than in duplex **1•2**, the increase in activity with supramolecular polymerization coincided with increasing sensitivity and selectivity (Figure 2). For example, the total efficiency of ATP recognition improved from $\eta = 42$ for **1•2** more than four times to $\eta = 10$ for $(\mathbf{12•13})_n$ (Table 1, entry 2 vs 13). The selectivity sequence A >

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Table 1. Aptamer Transporters, Their Activation with DG, Inactivation with ATP, and Signal Amplification with Antiaptamers[a]

	Aptamer	Anti-aptamer	Sequence[b]	T_M (°C)[c]	Activation with DG		Inactivation with ATP		
					EC_{50} (μM)[d]	Y_{MAX} (%)[e]	IC_{50} (μM)[f]	ΔY (%)[g]	$IC_{50}/\Delta Y$[h]
1	**1**		5′-ACCTGGGGGAGTATTGCGGAGGAAGGT-3′	-	41.5 ± 4.7	40.0 ± 3.1	-	0	-
2		**2**	3′-TGGACCCCCTCATAACGCCTCCTTCCA-5′	57.7	24.7 ± 0.8	75.3 ± 1.8	2100 ± 180	50	42
3		**3**	3′CCCCTCATAACGCCTCCTTCCA-5′	51.6	24.2 ± 3.4	68.6 ± 4.5	1710 ± 60	39	43
4		**4**	3′-CATAACGCCTCCTTCCA-5′	41.8	23.9 ± 0.9	58.6 ± 1.4	1490 ± 30	28	53
5		**5**	3′-CGCCTCCTTCCA-5′	34.2	24.5 ± 1.3	50.0 ± 1.6	1360 ± 80	15	94
6		**6**	3′-TGGACTCCCTTATAACGCCTCCTTCCA-5′	49.5	30.9 ± 2.4	71.8 ± 4.1	1580 ± 110	50	32
7		**7**	3′-TGGACTCCCTTATAATGCCTCCTTCCA-5′	44.0	27.6 ± 1.6	70.0 ± 2.4	1230 ± 60	44	28
8		**8**	3′-TGGACTCCCTTATAATGCCTTCTTCCA-5′	37.8	32.0 ± 1.8	68.0 ± 3.5	970 ± 80	35	28
9[i]		**9**	3′-CGCCTCCTTCCATAATGGACCCCCTCA-5′	43.0	28.7 ± 1.0	67.3 ± 1.6	1890 ± 130	35	54
10[i]	**10**		5′-GCACCTGGGGGAGTATTGCGGAGGAAGGTGC-3′	-	-	-	-	-	-
11[i]		**11**	3′-ACGCCTCCTTCCACGACGTGGACCCCCTCAT-5′	52.4	26.2 ± 1.5	91.8 ± 3.5	990 ± 80	76	13
12[i]	**12**		5′-CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG-3′	-	-	-	-	-	-
13[i]		**13**	3′-ACGCCTCCTTCCACGGCAGCCGTGGACCCCCTCAT-5′	58.9	28.1 ± 1.0	100.4 ± 2.7	870 ± 50	87	10
14[i]	**14**		5′-ACCTGGGGGAGTATTGCGGAGGAAGGTGCCG-3′	-	-	-	-	-	-
15[i]	**15**		5′-CGGCACCTGGGGGAGTATTGCGGAGGAAGGT-3′	58.9	24.4 ± 0.7	87.1 ± 1.6	920 ± 80	75	12

[a] Data for antiaptamers refer to 1:1 mixtures with the aptamer listed above; entry 15, data refer to a 1:1:1 mixture of **13**, **14**, and **15**; total oligonucleotide concentrations were 100 nM in all cases, 40 μM DG for inactivation. Data were obtained from dose−response curves for HPTS/DPX export from EYPC-LUVs; see Figure 2 and Supporting Information. [b] Mismatches (black) and sticky ends (green) are underlined; color codes refer to Figure 1. [c] Calculated melting temperatures of aptamer/antiaptamer duplexes. [d] Effective DG concentration needed to reach 50% activity. [e] Maximal fluorescence emission (= activity) obtained at saturation with DG activators, calibrated relative to maximal activity of DG-activated calf-thymus DNA under identical conditions.[1] [f] Inactivator concentration needed to reduce activity to 50%. [g] Response $\Delta Y = Y_{MAX,i} - Y_{MIN,i}$, $Y_{MAX,i}$: maximal fluorescence emission (= activity) without inactivators; DG concentrations were reduced to $Y_{MAX,i}$ to ensure maximal, quasi-linear initial response ($Y_{MAX,i} \sim 0.85\ Y_{MAX}$). Maximal possible ΔY is thus $\Delta Y \approx 85\%$; $Y_{MIN,i}$: minimal fluorescence emission obtained at saturation with inactivators. [h] Efficiency $\eta = IC_{50}/\Delta Y$ (sensitivity/response). [i] 1 mM Mg^{2+} in the extravesicular buffer.

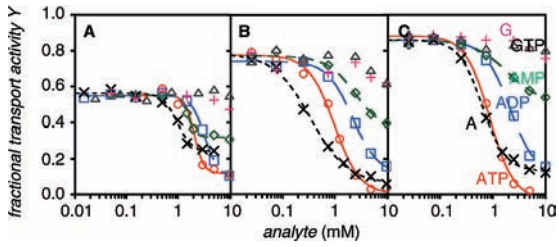

Figure 2. Response ΔY of DG-activated aptamer/antiaptamer mixtures **1**+**2** (A, 30 μM DG), **10**+**11** (B, 40 μM DG), and **12**+**13** (C, 40 μM DG, 100 nM oligonucleotides) to adenosine (×), ATP (○), ADP (□), AMP (◇), guanosine (+), and GTP (Δ). ΔY is the difference in emission (= activity) Y without and with analyte. Y was calibrated against maximal activity with calf-thymus DNA ($Y = 1.0$); to ensure quasi-linear initial response, DG concentrations were slightly reduced to give $Y_{MAX,i}$ ($Y_{MAX,i} \approx 0.85\ Y_{MAX}$, Table 1).

ATP ≫ ADP > AMP was even more pronounced with $(\mathbf{12{\bullet}13})_n$ than with **1**•**2**, the insensitivity toward G and GTP was maintained (Figure 2A vs 2C). Controls confirmed the DG-activated calf-thymus DNA is not inactivated by 100 mM ATP (not shown).

In previous membrane-based sensing systems, enzymes were used as analyte-specific signal generators, pores or transporters as signal transducers, and bifunctional in-/activators as signal amplifiers.[1,3,5a] In this report, aptamers are introduced to unify signal generation and signal transduction with the disassembly of supramolecular dimers and polymers as a new concept for signal amplification. The objective of this study was to introduce DNA chemistry to membrane-based sensing systems[1,3,5,7] and not to improve anything related to aptamer chemistry.[6] The sensitivity of adenosine aptamers was indeed as poor as in other systems.[6b,c] Also as expected from the literature,[6] aptamer selectivity was outstanding with regard to nucleobase recognition and significant but naturally condition dependent with regard to nucleotide charge. Our findings thus demonstrate that the intrinsic characteristics of aptamers are preserved in membrane-based sensing systems. Moreover, we show that other grand principles of DNA nanotechnology such as hybridization, mismatch, or sticky-end polymerization are applicable to membrane transport with high precision and fidelity. This suggests that the unique properties of membrane-based systems can be generally used to build DNA-based sensors.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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