Aptamer Based Molecular Recognition and Extraction by Reversible DNA Induced Hydrogel System

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Supporting Information

All eight DNA strands (G1, G2, A1, A2, A', D1, D2 and D'; sequences designed using Uniquimer 3D,[1] are shown in Table S1), purchased from Integrated DNA Technologies Inc., were stored in stock solutions in 1×TAE/Mg^{2+} buffer (40 mM tris(hydroxymethyl)aminomethane (Tris), 20 mM acetic acid, 2 mM EDTA, 12.5 mM magnesium acetate; pH 8.0) at a concentration of 3 mM. The G1/G2 solution was prepared in a 1.8 mM buffer (4% acrylamide, 1×TBE). Then, a catalyst solution (ammonium persulphate 0.05 g and N,N,N',N'-tetramethylethylenediamine 25 μL in 0.5mL deionized water) was added to the G1/G2 solution for polymerization at 1.4% volumetric ratio with nitrogen bubbling through the solution for 5 minutes. 1 mM γ-[6-aminohexyl]-ATP-5FAM and γ-[6-aminohexyl]-GTP-Texas Red solution in deionized water were purchased from Jena Bioscience GmbH. A solution of 67 μM γ-[6-aminohexyl]-ATP-5FAM, 67 μM γ-[6-aminohexyl]-GTP-Texas Red and 2 mM strand A1 was prepared in the binding buffer (1×TAE/Mg^{2+} buffer, 30 μL) at room temperature for 1 hour. In order to form the hydrogel, the crosslinker DNA strand A1 and strands G1 and G2 were mixed in stoichiometric concentrations. After incubation at room temperature for 10 minutes, the mixture became hydrogel. The total volume of hydrogel is 90 μL. The concentrations of γ-[6-aminohexyl]-ATP-5FAM, γ-[6-aminohexyl]-GTP-Texas Red and strand A1 are 22 μM, 22 μM and 667 μM respectively in the hydrogel before washing. The control hydrogel was prepared similarly and the only difference was that the crosslinker strand A' did not have the aptamer segment. And the one-species system was prepared in similar way without adding γ-[6-aminohexyl]-GTP-Texas Red.

Table S1. DNA sequences for the hydrogel system

Name	Sequence 5' to 3'
G1	/5'-Acrydite/ACCGCAACGCAGGCATTCGG
G2	/5'-Acrydite/ATGAACGGCAGGAACAAGTA
A1	CCGAATGCCTGCGTTGCGGTACCTGGGGGAGTATTGCGGAGGAAGGTTTTACTTGTTCCTGCCGTTCATTCTCACTGGC
A2	CCGAATGCCTGCGTTGCGGTATCAGCGTTGGTTGGTGTGGTTGGTTCGCTGATTTTACTTGTTCCTGCCGTTCATTCTCACTGGC
A'	CCGAATGCCTGCGTTGCGGTATCAGCGTTTTCGCTGATTTTACTTGTTCCTGCCGTTCATTCTCACTGGC
D1	GCCAGTGAGAATGAACGGCAGGAACAAGTAAAACCTTCCTCCGCAATACTCCCCCAGGTACCGCAACGCAGGCATTCGG
D2	GCCAGTGAGAATGAACGGCAGGAACAAGTAAAATCAGCGAACCAACCACACCAACCAACGCTGATACCGCAACGCAGGCATTCGG
D'	GCCAGTGAGAATGAACGGCAGGAACAAGTAAAATCAGCGAAAACGCTGATACCGCAACGCAGGCATTCGG

The cross-linked hydrogel was washed with 40 μL binding buffer for 3 times to leach out the unbound ATP and GTP, followed by collecting the washing buffer. Then, the hydrogel was transferred to a centrifuge device and dissolved when immersed in a solution of strand D with 20% excess amount relative to strand A1 (for the control, strand D', which is fully complementary to strand A', was added to displace strand A'). In the end, dissolved hydrogel solution was centrifuged though the filter column and collected. The G1/G2 solution, the hydrogel, and the dissolved hydrogel were applied for flow behavior observation. The amount of γ-[6-aminohexyl]-ATP-5FAM, γ-[6-aminohexyl]-GTP-Texas Red release, after dissolution of the hydrogel, and in the washing buffer were calculated from the concentrations measured by fluorescence spectrophotometry. The standard curves of them were constructed to determine the concentrations (Figure S1). The fluorescence intensities were recorded on a LS 55 Luminescence Spectrometer (PerkinElmer Inc.) with excitation at 492 nm and emission at 523 nm for γ-[6-aminohexyl]-ATP-5FAM, and excitation at 583 nm and emission at 612 nm for γ-[6-aminohexyl]-GTP-Texas Red.

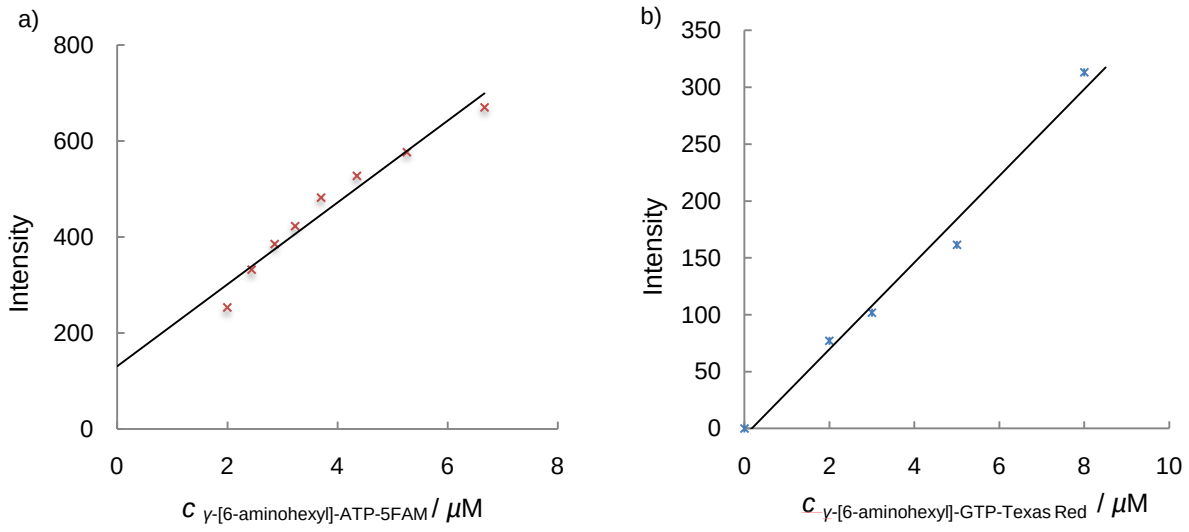

Figure S1. a) Standard curve of *γ*-[6-aminohexyl]-ATP-5FAM, the equation is fluorescence intensity=100.02*c* + 65.947, correlation coefficient = 0.955; b) standard curve of *γ*-[6-aminohexyl]-GTP-Texas, the equation is fluorescence intensity = 38.145*c* – 10.274, correlation coefficient = 0.985.

Table S2. ATP and GTP concentrations of the released solution and the washed-out solution from the hydrogel

	Crosslinker DNA with the aptamer segment		Crosslinker DNA without the aptamer segment	
Concentrations in dissolved hydrogel* (*μ*M)	Ratio	66:34	Ratio	50:50
	ATP	18.9	ATP	11.0
	GTP	9.9	GTP	10.9
Concentrations in washing buffer** (*μ*M)	Ratio	16:84	Ratio	56: 44
	ATP	1.8	ATP	11.8
	GTP	9.4	GTP	9.3

*The volume of dissolved hydrogel solution is 90 *μ*L.
** The volume of washing buffer is 90 *μ*L.

According to the *α*-thrombin and BSA system, the *α*-thrombin, purchased from Sigma–Aldrich, was from human plasma lyophilized in saline sodium citrate buffer (pH 6.5). All components for hydrogel were added together (0.6 mM strand A2, 0.6 mM acrydite modified strand G1 and G2, 480 *μ*M *α*-thrombin and bovine serum albumin (BSA), 4% acrylamide 100 *μ*L with catalyst solution 2 *μ*L) in the binding buffer (50 mM Tris-HCl, 100 mM NaCl, 3 mM KCl, 1 mM EDTA; pH 7.5) with an incubation time of 2 hours at room temperature. Control hydrogel was prepared similarly but with the crosslinker strand A' excluding the aptamer segment. When the hydrogel is successfully formed, the following process is similar to the steps described above. The amount of *α*-thrombin and BSA release, after dissolution of the hydrogel in the strand D2 solution, and in the washing buffer were calculated from the protein concentration measured by the Bradford method, which employed a Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA).

Table S3. *α*-Thrombin and BSA concentrations of the released solution and the washed-out solution from the hydrogel.

	Crosslinker DNA with the aptamer segment		Crosslinker DNA without the aptamer segment	
protein concentrations in dissolved hydrogel* (*μ*g/*μ*L)	total protein	2.32	total protein	1.40
	ratio	60:40	ratio	36:64
	α-thrombin	1.41 (38.5 *μ*M)	*α*-thrombin	0.51 (13.8 *μ*M)
	BSA	0.91 (16.0 *μ*M)	BSA	0.89 (13.5 *μ*M)
protein concentrations in washing buffer** (*μ*g/*μ*L)	total protein	1.05	total protein	1.80
	ratio	24:76	ratio	37:63
	α-thrombin	0.25 (6.9 *μ*M)	*α*-thrombin	0.67 (18.2 *μ*M)
	BSA	0.80 (12.0 *μ*M)	BSA	1.13 (17.0 *μ*M)

*The volume of dissolved hydrogel solution is 102 *μ*L.

** The volume of washing buffer is 102 μL.

The capture and release of ATP was examined by native PAGE. ATP and strand A1 were mixed at a ratio of 1:1 in 1×TAE/Mg^{2+} buffer. After incubation at room temperature for 1 hour, the solution was then stcoichiometrically mixed with strand D1 at room temperature. (As a positive control, strand A1 and strand D1 were also mixed in the absence of ATP). An aliquot of sample for each step was taken for PAGE. In order to observe the fluorescence of ATP in PAGE, a mixture of ATP and strand A1 with large quantity (1 nmol) was also prepared for PAGE. A 12% native PAGE assay was run in 1×TBE buffer at room temperature for 2 hours at a constant voltage of 80 V. The gel was stained with GelRed (Biotium, Inc.).

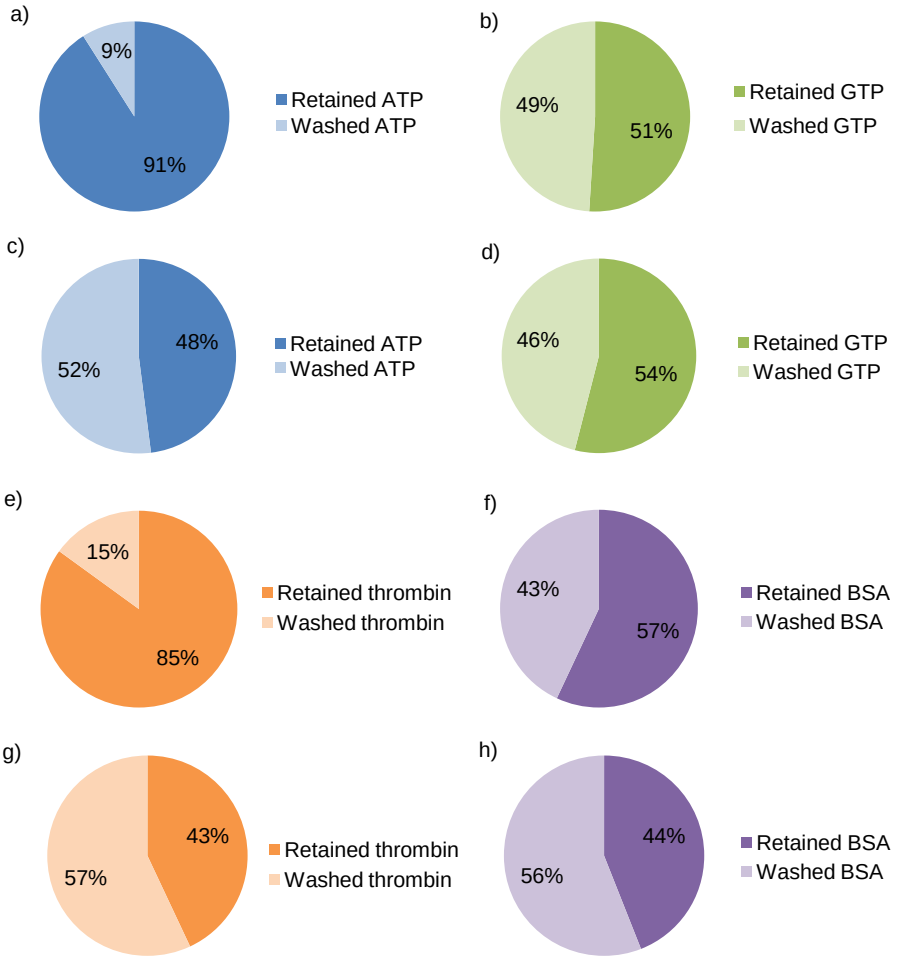

Figure S2. The proportions of the retained and washed targets and nonspecific targets in the hydrogel systems. The proportions of ATP in the dissolved hydrogel solution (the blue bar) and washing buffer (the light blue bar) with the aptamer segment a) and without the aptamer segment c) in the crosslinker. The proportions of GTP in the dissolved hydrogel solution (the green bar) and washing buffer (the light green bar) with the aptamer segment b) and without the aptamer segemt d) in the crosslinker. The proportions of Thrombin in the dissolved hydrogel solution (the orange bar) and washing buffer (the light orange bar) with the aptamer segment e) and without the aptamer segemt g) in the crosslinker. The proportions of BSA in the dissolved hydrogel solution (the purple bar) and washing buffer (the light purple bar) with the aptamer segment b) and without the aptamer segemt d) in the crosslinker.

Since the physical entrapment of the nonspecific molecule species, considerable amount of nonspecific entrapment (as much as 50%) was recorded for our system. We have to admit that it is very important to try realistic sample (such as serum or tissue …) for the efficiency and specificity test of the system. However, it is not practical to apply realistic sample on our hydrogel system with the current rate of nonspecific entrapment. It is not totally impossible to get rid of the residue of nonspecific molecule species. Extremely strong washing buffer or high speed centrifuge is able to get rid of all residues of nonspecific molecule species. Unfortunately, the

binding of target molecules and corresponding aptamers of the hydrogels are not strong enough to withstand the strong washing condition and get washed out together with the nonspecific species. Therefore, we are still try to find a balance between a nice extraction yield of target molecule with specific binding and acceptable selectivity against nonspecific residues. In our opinion, the most important issue of the system is biocompatibility since the most important application of hydrogel is drug delivery. We'll prefer to apply the concept to a biocompatible polymer first and then try to use the biocompatible system to realistic samples for molecular fishing.

Reference

1 J. Zhu, B. Wei, Y. Yuan, Y. Mi, *Nucleic Acids Research* **2009**, *37*, 2164-2175.