

Stimuli-Responsive Releasing of Gold Nanoparticles and Liposomes from Aptamer-Functionalized Hydrogels

Firas El-Hamed, Neeshma Dave, and Juewen Liu*

**Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, 200
University Avenue West, Waterloo, Ontario, N2L 3G1, Canada**

Email: liujw@uwaterloo.ca

Abstract

Controlled release of therapeutic agents is important for improving drug efficacy and reducing toxicity. Recently, hydrogels have been used for controlled release applications. While majority of the previous work focused on releasing the cargo in response to physical stimuli such as temperature, light, electric field, and pH, we aim to trigger cargo release in the presence of small metabolites. In our system a DNA aptamer that can bind to adenosine, AMP and ATP was used as a linker to attach either DNA-functionalized gold nanoparticles and or liposomes to DNA-functionalized hydrogels. In the presence of the metabolite, both the nanoparticle and liposome cargos were released. The effect of salt, temperature, target concentration, and drying has been systematically studied. Interestingly, we found that the gel can be completely dried while retaining the DNA linkages, where upon rehydration; the adenosine-induced releasing was still achieved. Our work demonstrates that aptamers can be used to control release of drugs and other materials attached to hydrogels.

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1. Introduction

Controlled release of therapeutic agents is important for improving drug efficacy and reducing toxicity [1, 2]. Hydrogels are ideal for such applications because of their good biocompatibility, high loading efficiency, and more importantly, their response can be triggered by a number of environmental conditions [2-10]. For example, hydrogels that respond to temperature [11, 12], pH [13], electric or magnetic field [14, 15], and light [16] have all been reported. For biomedical applications, however, it is often more desirable to achieve controlled drug release in response to specific chemical or biological stimuli [4, 17], such as the release of insulin in the presence of high glucose concentration [18]. To achieve this, molecular recognition elements need to be incorporated into the hydrogel formulation.

Previously, chemically responsive hydrogels controlled release applications have been achieved by employing antibodies or protein enzymes as the molecular recognition elements [4, 17, 19]. However, in the case of proteins, they are susceptible to irreversible denaturation and therefore, more stable alternatives may provide improved performance [20-28]. Aptamers are DNA or RNA-based ligands that can bind to a diverse range of molecules including metal ions, small molecules, to proteins and even whole cells [29-31]. Compared to antibodies, aptamers are smaller in size, highly stable, easier to modify, and have less batch-to-batch variations. In many cases, aptamers have been shown to rival antibodies in terms of binding affinity and specificity. As a result, many aptamer-based sensors and smart materials have been prepared to change their optical properties or assembly states in response to the presence of specific target molecules [32-36].

Incorporation of aptamers, especially DNA aptamers into hydrogels appears to be a viable option to construct chemically responsive drug release systems [37-41]. For example, the Tan group reported the use of DNA aptamer as a crosslinker to assemble DNA-functionalized hydrogels [37, 41], where

addition of the target molecule resulted in gel dissolution and release of entrapped nanoparticles or enzymes. In such studies, a high concentration (e.g. ~1 mM) of DNA was required to crosslink the gel. In another approach, aptamers were attached to the hydrogel side chain [38-40, 42]. In all these cases, the aptamers were able to bind to their respective targets and target release can be achieved by adding a complementary DNA to the aptamer to disrupt aptamer binding [43]. Instead of using complementary DNA, we aim to employ small molecule metabolites to trigger cargo release, which is more practical for cell-based and *in vivo* applications. This can be made possible by taking advantage of the two properties of an aptamer DNA. First, an aptamer DNA can still bind to its complementary strand. Therefore, aptamers can be used to link DNA functionalized cargos to DNA functionalized hydrogels. Second, an aptamer can bind to its target molecule and this binding can be engineered to displace DNA base pairing interactions and release the cargo. In our system, this whole process does not lead to the dissolution of the hydrogel since it is not crosslinked by DNA. As a result, a low DNA concentration is required. In this work, we employed monolithic hydrogels as a matrix to test the idea presented above and the model cargos were gold nanoparticles (AuNPs) and liposomes.

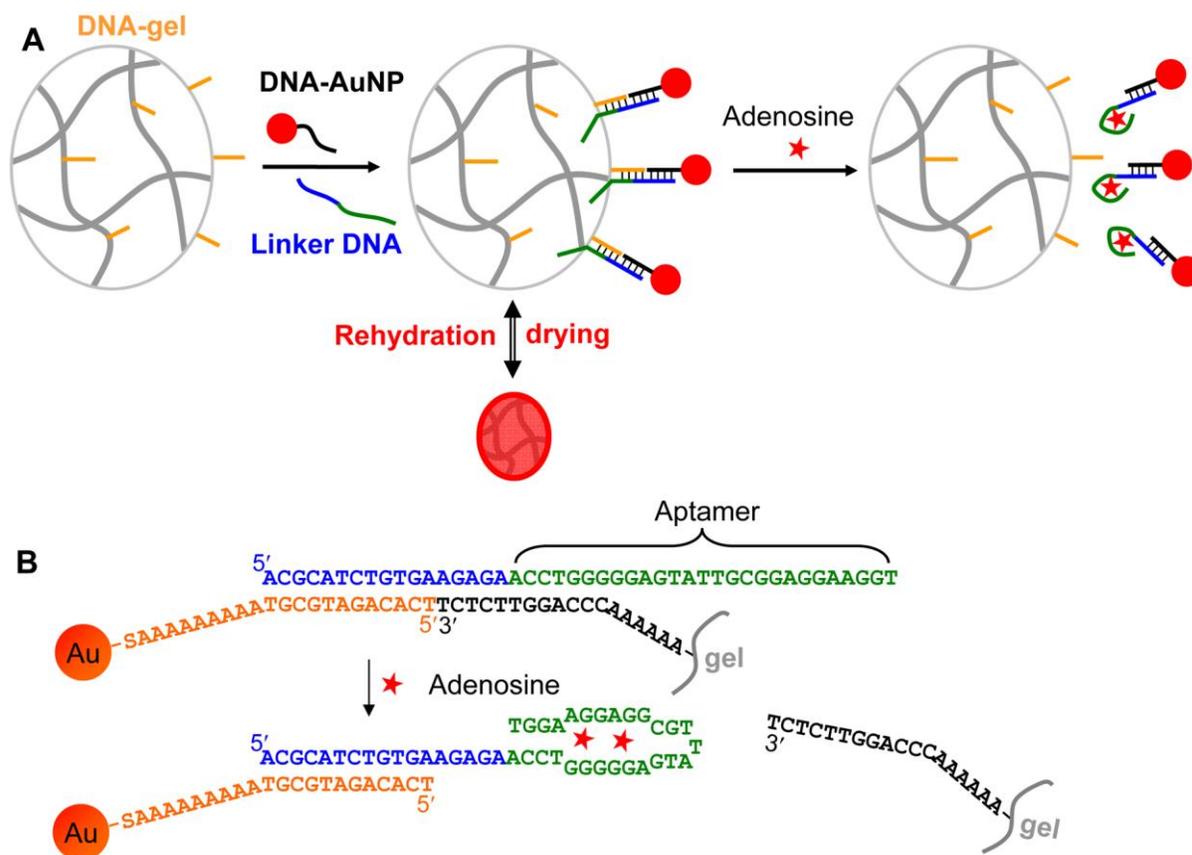


Figure 1. (A) Acrydite-modified DNA was covalently linked to a polyacrylamide hydrogel. In the presence of an aptamer-based linker DNA, DNA-functionalized AuNPs were attached to the gel surface, resulting in a red colored gel. This gel can be dried and rehydrated. Addition of the target molecule, adenosine, resulted in AuNP release. (B) The DNA sequences used in this triggered release reaction.

2. Experimental section

2.1. Chemicals

All of the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA) and purified by standard desalting. Acrylamide/bis-acrylamide 29:1 40% gel stock solution, ammonium persulphate (APS), N,N,N',N'-tetramethylethylenediamine (TEMED), and modified Eagle's medium

(EMEM), with Earle's balanced salt solution, without L-glutamine and phenol red were purchased from VWR. Sodium nitrate, sodium citrate, adenosine and other ribonucleosides, and tris(hydroxymethyl)aminomethane (Tris) were purchased from Mandel Scientific (Guelph, Ontario, Canada). Hydrogen tetrachloroaurate(III) hydrate was purchased from Sigma-Aldrich. Phospholipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rh-PE) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(*p*-maleimidophenyl)butyramide], sodium salt (MPB-PE) were purchased from Avanti Polar Lipids (Alabaster, AL).

2.2. Hydrogel preparation

To prepare hydrogels the following solutions were mixed: 100 μ L 40% gel solution (29:1), 40 μ L 2 M NaNO₃, 80 μ L 0.5 M Tris nitrate, pH 8.0, 12 μ L 0.5 mM acrydite-modified DNA, and 528 μ L water. This mixture contained a final gel percentage of 5% and 100 mM NaNO₃, 50 mM Tris nitrate, and 10 μ M DNA. To initiate polymerization, a fresh initiator solution was made by dissolving 50 mg APS in 500 μ L water to which 25 μ L TEMED was finally added. 40 μ L of the initiator solution was added to the above mixture to initiate the polymerization reaction. A 96-well plate was used for gel preparation. To each well 70 μ L of the gel solution was added. The gels were polymerized for 1 hr at room temperature and then soaked in 40 mL water overnight to remove free monomers, initiator, and unincorporated DNA. Subsequently the gels were soaked in a buffer containing 100 mM NaCl, 25 mM HEPES, pH 7.6 overnight. From our previous work, it has been calculated that ~60% of the DNA was polymerized inside the gel and the remaining gets washed away [28, 39].

2.3. AuNP preparation

AuNPs (13 nm diameter) were synthesized following literature reported procedures by reducing 1 mM HAuCl₄ using sodium citrate [44]. The resulting AuNPs were ~13 nm in diameter with a concentration of ~10 nM AuNPs. The 3'-thiol modified DNA was first activated by treating 1.5×TCEP solution at pH 5 and incubating for 1 hr [45]. The resultant activated DNA was incubated overnight with AuNPs with a final DNA concentration of 3 μM. After overnight incubation, the pH of the solution was brought to 7.6 by drop wise addition a final of 5 mM HEPES buffer, pH 7.6 and 100 mM NaCl. The AuNPs were further incubated for 24 hrs before use. To remove free DNA, the AuNPs were centrifuged at 15000 rpm for 15 min and the supernatant solution was discarded.

2.4. Liposome preparation

We prepared and functionalized liposomes using the same procedure we previously published [46]. In brief, 2.5 mg DOPC was mixed with 5% MPB-PE and 1% Rh-PE in chloroform. Chloroform was removed under a gentle N₂ flow in the fume hood and trace amounts of residual chloroform was removed by storing the samples under vacuum overnight. The dried lipids were kept under a N₂ environment and then stored at -20 °C prior to use. To prepare liposomes, the lipid was rehydrated with 0.5 mL of buffer A (150 mM NaCl, 25 mM HEPES, pH 7.6). After ~ 1 hr, this cloudy lipid suspension was extruded through a polycarbonate membrane (pore size = 100 nm) 21 times. To attach DNA, 50 μL of 5 mg/mL liposomes were reacted with 60 μM TCEP activated thiol-modified DNA at room temperature overnight. After incubation, the salt was adjusted to 500 mM NaCl to induce aggregation and the sample was stored at 4 °C for 12 hrs. These liposomes were harvested after centrifugation (13000 rpm) at 4 °C to remove unreacted DNA in the supernatant. Finally, the liposome was dispersed in buffer A at a concentration of 2.5 mg/mL.

2.5. Linking AuNPs and liposomes to hydrogel

Each gel was placed in 1 mL buffer B (300 mM NaCl, 20 mM HEPES, pH 7.6) containing 1 nM 13 nm AuNPs and 10 nM linker DNA. The gel was warmed up to 60 °C for 2 min in a water bath and was cooled to room temperature overnight. The supernatant was then removed and the gels were re-suspended in the same buffer. To attach the liposome, 10 µL of the above prepared liposome was added to each gel in the presence of 50 nM linker DNA to react overnight in 1 mL of buffer B.

2.6. Melting Curves

To measure melting curves, a gel with immobilized AuNPs was soaked in a quartz micro-cuvette containing 500 µL of buffer. The gel size was large enough to sit on top of the optical window for detection while still small enough to move freely in the buffer. The cuvette was sealed on the top by parafilm to prevent evaporation. Extinction at 520 nm was then monitored as a function of temperature using an Agilent 8453 spectrophotometer. The temperature was increased at a rate of 1 °C/min. The sample was equilibrated for 2 min at each temperature before taking the measurement.

2.7. Adenosine induced release

Adenosine induced AuNP release was carried out in a quartz micro-cuvette. A gel was placed in the cuvette containing 500 µL buffer at a designated temperature (typically 35 °C). The extinction spectrum of the cuvette was measured every minute. After 6 min, a small volume of 50 mM adenosine was added and the extinction spectrum was collected every 1 or 2 min. To study the release of liposomes, each gel was placed in a 1cm×1cm fluorescence quartz cuvette filled with 2 mL buffer A. The temperature was adjusted to 35 °C. Fluorescence intensity at 590 nm was monitored every 30 sec with 570 nm excitation. 4 mM adenosine was added after 5 min.

2.8. Hydrogel drying and rehydration

Hydrogel drying was carried out by placing each gel in a plastic weighing boat with the round side facing down. The remaining buffer around the gel was removed using a pipette. After incubating for 3 days at room temperature in dark, the gels were completely dried. To rehydrate the gel, each dried gel was transferred to a 1.7 mL microcentrifuge tube and 1 mL of buffer A was added. After ~1 hr at room temperature, gels were fully rehydrated to their original volume and the AuNPs remained on the surface.

3. Results and Discussion

3.1. Adenosine-induced AuNP release. We chose to use AuNPs as a model cargo since they have a high extinction coefficient allowing them to be easily monitored using the naked eye and by UV-vis spectroscopy. In addition, DNA can be attached to the AuNP surface using well-established Au-thiol chemistry. The design of our stimuli-responsive system is shown in Figure 1A. A 5'-acrydite-modified DNA was co-polymerized with the polyacrylamide hydrogel using bis-acrylamide as a crosslinker. The AuNPs were functionalized with a DNA containing a 3'-thiol modification. Upon mixing the gel with AuNPs in the presence of a linker DNA, the AuNPs attached to the gel surface. As can be observed from Figure 2A, the gel was transparent in the absence of the linker DNA, suggesting that no non-specific binding occurred. Addition of 10 nM linker DNA resulted in a red colored gel, confirming DNA-mediated AuNP attachment.

The DNA sequences and AuNP arrangement on the hydrogel surface are shown in Figure 1B. The adenosine aptamer sequence is in green and the blue portion of the aptamer binds to the DNA on the hydrogel surface. Addition of adenosine resulted in aptamer folding, decreasing the number of base

pairs for binding to the gel and detaching the AuNP from the gel surface. Indeed as shown in Figure 2B, most of the AuNPs were dissociated upon 4 mM adenosine and the gel appeared to be almost colorless. Aptamer-functionalized AuNPs have been previously attached to gold surfaces [47, 48], however this is the first systematic study using a hydrogel surface.

A tool that is often used to study DNA-linked nanomaterials is DNA melting experiments, in which the thermal stability of the linkage can be deduced. Using a previously reported method [28], the gel was loaded into a quartz micro-cuvette and 500 μ L buffer was added. The gel was large enough to sit on the top of the optical window. Initially the buffer was clear since all of the AuNPs were associated with the gel. If AuNPs dissociation occurred, increased AuNP extinction at 520 nm can be observed. As shown in Figure 2C, the 520 nm surface plasmon peak increased gradually with increasing temperature and a large transition occurred at \sim 55 $^{\circ}$ C in the presence of 300 mM NaCl. If the extinction value was plotted as a function of temperature, a melting transition at 55 $^{\circ}$ C can be observed (Figure 2D, dots). With 100 mM NaCl, the melting temperature was at 45 $^{\circ}$ C (squares). The fact that a higher salt resulted in a higher melting temperature supported that the AuNPs were linked by DNA to the gel surface.

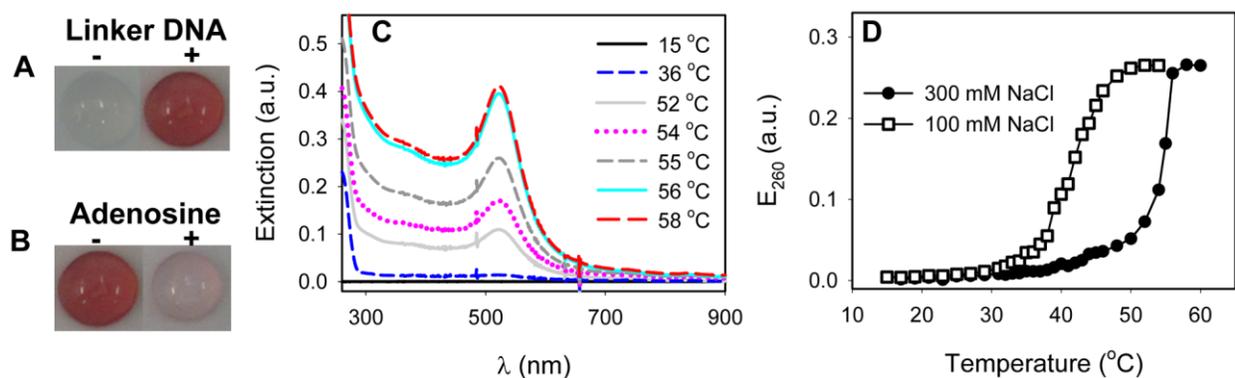


Figure 2. (A) Photograph of the gels mixed with DNA-functionalized AuNPs in the presence and absence of the linker DNA. (B) Addition of adenosine induced AuNPs release from the hydrogel surface leading to a close to transparent gel. The incubation time was overnight for this sample in the presence of 4 mM adenosine. (C) Selected UV-vis spectra during a melting study (300 mM NaCl, 20 mM HEPES, pH 7.6). Most AuNPs were released at close to 55 °C. (D) Melting curves in two buffers containing different salt concentrations.

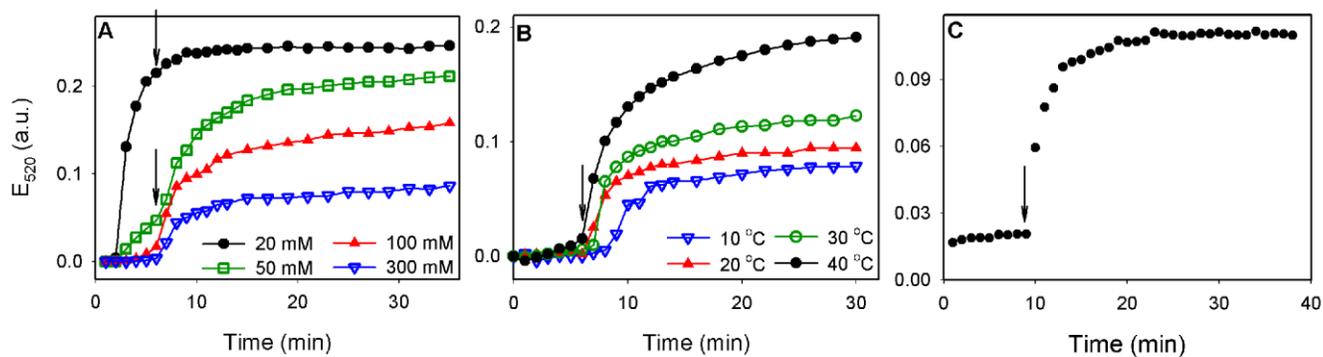


Figure 3. The kinetics of adenosine induced AuNP release as a function of NaCl concentration (A) and temperature (B). The arrows indicate the time when 4 mM adenosine was added. The experiments in (A) were performed at 35 °C adding 4 mM adenosine. The experiments in (B) were performed in 100 mM NaCl buffer, 20 mM HEPES, pH 7.6, also adding 4 mM adenosine. (C) Kinetics of AuNP release in modified Eagle's medium (EMEM) with Earle's balanced salt solution at 35 °C.

3.2. Effect of salt and temperature. The melting study showed that both temperature and salt were important for the dissociation of AuNPs. Therefore, the effect of these two parameters on adenosine-induced release was studied next. First, the temperature was fixed at 35 °C and the concentration of NaCl was varied. After incubating the gel for 6 min, 4 mM adenosine was added and the buffer extinction was monitored as a function of time. As can be observed in Figure 3A, with 300 mM NaCl, the background color was very stable and AuNP release occurred immediately after the addition of adenosine. With 100 mM NaCl, the release in the absence of adenosine was also very slow and a faster release was observed upon adenosine addition. At even lower salt concentrations, AuNPs already started to dissociate even in the absence of adenosine, suggesting that the reaction temperature (i.e. 25 °C) exceeded the melting temperature of the linker DNA. This experiment suggests that the rate of release can be tuned by varying the ionic strength of the solution. The reaction can occur in a wide range of salt concentrations and the optimal concentration at 35 °C should be between 100 and 300 mM NaCl, which is close to the physiological condition.

The effect of temperature was tested by fixing the NaCl concentration to 100 mM. As can be observed from Figure 3B, the adenosine induced release was progressively faster at higher temperature. At high temperature, the binding between DNA became less stable, making the structure switching of aptamer easier [49, 50]. To test the releasing kinetics under physiological conditions, the gel was soaked in modified Eagle's medium (EMEM) with Earle's balanced salt solution at 35 °C. Addition of adenosine also resulted in a release of AuNPs (Figure 3C), suggesting that this reaction can also be carried out in a cellular environment.

3.3. Effect of target concentration. Under optimized salt and temperature conditions, we next tested the release of AuNPs as a function of adenosine concentration. We chose to use 100 mM NaCl and 35

°C for this set of experiment. As can be observed from Figure 4A, the AuNPs were released in a concentration-dependent manner with higher adenosine triggering a faster release. In the presence of 4 mM adenosine, a releasing rate constant of 0.25 min^{-1} was obtained using a first-order reaction model. In addition to adenosine, this aptamer is also known to bind to other molecules including ATP and AMP. Therefore, the responses to these molecules were also tested (Figure 4B). The dissociation in the presence of adenosine was the fastest followed by AMP and ATP. This is consistent with previous reports confirming that the aptamer has a higher affinity for adenosine [51, 50]. Finally the responses of the gel to other ribonucleosides were tested and little AuNP release was observed with 4 mM of guanosine, uridine, or cytidine. These experiments confirmed that the AuNPs were released only in the presence of molecules that can trigger aptamer binding and therefore this process was highly specific.

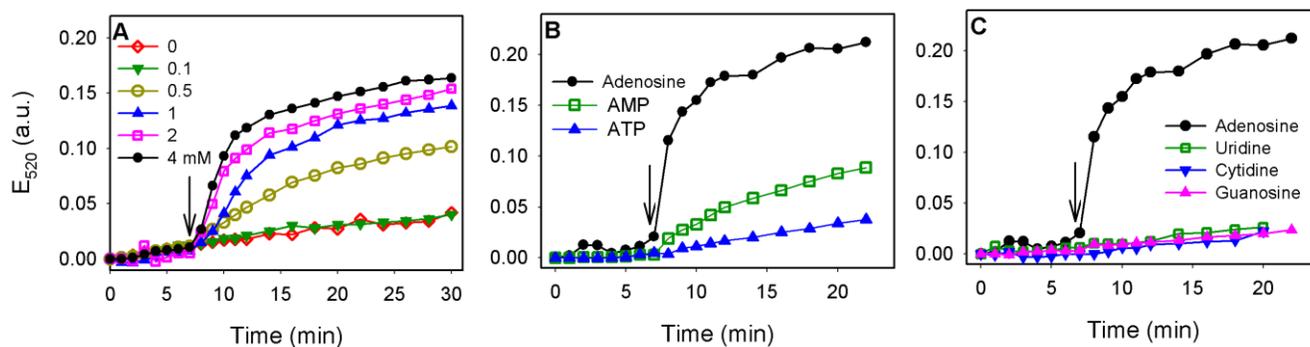


Figure 4. (A) Kinetics of AuNP release as a function of adenosine concentration. (B) Kinetics of AuNP release in the presence of 4 mM adenosine, AMP, and ATP. (C) Kinetics of AuNP release in the presence of other ribonucleosides. All the experiments were performed in 100 mM NaCl, 20 mM HEPES, pH 7.6 at 35 °C. The arrows indicate the time point when the molecules were added.

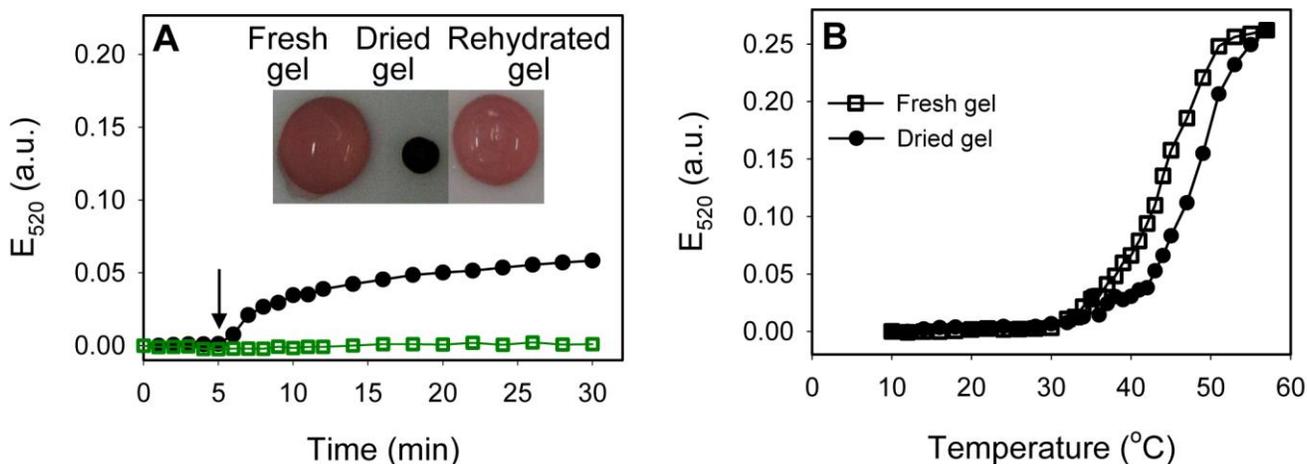


Figure 5. (A) Kinetics of AuNP release for the rehydrated gels in the presence of 4 mM adenosine (dots) or uridine (squares). The black arrow indicates the time for target addition. The reactions were performed at 35 $^{\circ}$ C in the presence of 100 mM NaCl, 20 mM HEPES, pH 7.6. Inset: photographs of fresh gel, dried gel and rehydrated gel. (B) Melting curves of freshly prepared gel and rehydrated gel collected in 100 mM NaCl, 20 mM HEPES, pH 7.6. The rehydrated gel melted at a higher temperature.

3.4. Hydrogel drying and rehydration. We next tested whether it was possible to dry and rehydrate the AuNPs functionalized hydrogel, since drying is important for long-term storage of the material. Usually, a protecting agent (e.g. sucrose or trehalose) is added to protect biomolecules during the drying process [52]. We found that it is possible to achieve direct drying in our hydrogel system. The AuNP attached hydrogels were taken out from the buffer (100 mM NaCl, 25 mM HEPES, pH 7.6) and dried on a plastic weighing boat under ambient conditions. After three days, the gels were completely dry. A picture of fresh gel and the dried gel are shown in the inset of Figure 5A. The dried gel showed a much darker color since all of the AuNPs were concentrated on the gel surface with the reduction in gel volume. The dried gel was then placed in 100 mM buffer for rehydration. Interestingly, the gel achieved the original volume in \sim 1 hr and there was no free AuNP released from the gel, suggesting that the binding between AuNP and gel was very strong.

Addition of adenosine still resulted in AuNP release while uridine did not show any effect (Figure 5A). The rate of AuNP release, however, was slower for the rehydrated gel compared to the freshly prepared ones. We hypothesize that during drying, more DNA linkages were formed, possibly due to shrinkage of the gel network establishing more contact areas between gel and AuNPs. To test this hypothesis, the melting curve of this rehydrated gel was measured and indeed it showed a higher melting transition temperature (Figure 5B). This can only be explained by the increased DNA linkages between AuNP and the gel surface. It is well known that the melting of such multivalent systems is related to the number of DNA linkages [53].

3.5. Release of liposomes. After using AuNP releasing as a model system, we next prepared rhodamine-labeled DOPC liposomes that were functionalized with the same DNA. Liposomes have been studied as drug delivery vehicles for a wide range of drugs and therefore are more practically related to drug delivery. The liposomes were assembled on the hydrogel surface using the same linker DNA. As shown in Figure 6A, the gel became highly fluorescent after the liposome attachment. After adding adenosine, the solution became fluorescent, indicating released liposomes in solution. A releasing rate constant of 0.22 min^{-1} was obtained, which was very similar to the 0.25 min^{-1} obtained using AuNPs, suggesting that the use of different types of nanoparticles had relatively little effect on the releasing kinetics. Removal of the solution and adding new buffer to the remaining gel showed an almost non-fluorescent gel. This process was also monitored using a spectrofluorometer. As shown in Figure 6B, addition of adenosine resulted in an increase in the solution fluorescence.

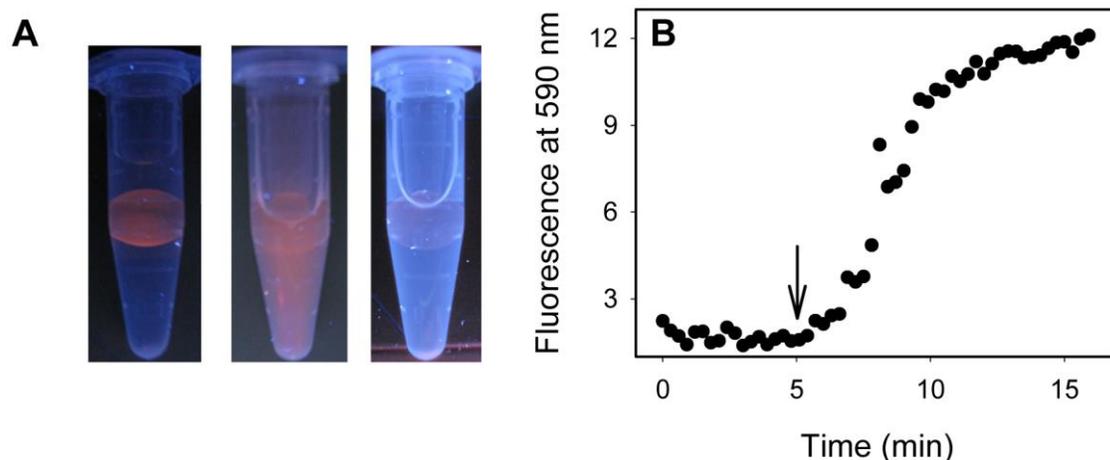


Figure 6. (A) A Photograph of rhodamine-labeled liposome coated hydrogel (left), after adding adenosine (middle) and after changing buffer (right). (B) Kinetics of fluorescence increase after adding 4 mM adenosine to hydrogel soaked in a quartz fluorescence cuvette. The arrow indicated the time when adenosine was added. This experiment was performed in 100 mM NaCl, 20 mM HEPES, pH 7.6 at 35 °C.

Conclusions. In summary we have demonstrated the use of a DNA aptamer to assemble DNA-functionalized AuNPs and liposomes to a monolithic polyacrylamide hydrogel surface. In the presence of adenosine, ATP, or AMP, the nanoparticles were released. The release was very specific and occurred only in the presence of molecules that the aptamer can bind. At the same time, the nanoparticle release kinetics was dependent on salt and temperature. The hydrogel can be easily dried for storage and rehydrated. The rehydrated gel showed an even tighter AuNP binding and adenosine induced release can still be achieved. After this initial proof-of-concept experiment using monolithic gels, future work will be focused on the use of hydrogel microparticles to increase the specific surface area and to facilitate delivery.

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References:

- [1] Peer D, Karp J M, Hong S, Farokhizad O C, Margalit R and Langer R 2007 *Nat. Nanotechnol.* **2** 751-60
- [2] Langer R and Tirrell D A 2004 *Nature* **428** 487-92
- [3] Gil E S and Hudson S M 2004 *Prog. Polym. Sci.* **29** 1173-222
- [4] Qiu Y and Park K 2001 *Adv. Drug Deliver. Rev.* **53** 321-39
- [5] Lee K Y and Mooney D J 2001 *Chem. Rev.* **101** 1869-79
- [6] Jeong B, Kim S W and Bae Y H 2002 *Adv. Drug Deliver. Rev.* **54** 37-51
- [7] Hoffman A S 2002 *Adv. Drug Deliver. Rev.* **54** 3-12
- [8] Hennink W E and van Nostrum C F 2002 *Adv. Drug Deliver. Rev.* **54** 13-36
- [9] Tsitsilianis C 2010 *Soft Matter* **6** 2372-88
- [10] Banta S, Wheeldon I R and Blenner M 2010 *Annu. Rev. Biomed. Eng.* **12** 167-86
- [11] Tanaka T 1978 *Phys. Rev. Lett.* **40** 820-3
- [12] Chen G H and Hoffman A S 1995 *Nature* **373** 49-52
- [13] Cheng E J, Xing Y Z, Chen P, Yang Y, Sun Y W, Zhou D J, Xu L J, Fan Q H and Liu D S 2009 *Angew. Chem. Int. Ed.* **48** 7660-3
- [14] Osada Y, Okuzaki H and Hori H 1992 *Nature* **355** 242-4
- [15] Tanaka T, Nishio I, Sun S T and Uenonishio S 1982 *Science* **218** 467-9
- [16] Kang H, Liu H, Zhang X, Yan J, Zhu Z, Peng L, Yang H, Kim Y and Tan W 2011 *Langmuir* **27** 399-408
- [17] Miyata T, Urugami T and Nakamae K 2002 *Adv. Drug Deliver. Rev.* **54** 79-98

- [18] Kost J, Horbett T A, Ratner B D and Singh M 1985 *J. Biomed. Mater. Res.* **19** 1117-33
- [19] Murphy W L, Dillmore W S, Modica J and Mrksich M 2007 *Angew. Chem. Int. Ed.* **46** 3066-9
- [20] Nagahara S and Matsuda T 1996 *Polymer Gels and Networks* **4** 111-27
- [21] Lin D C, Yurke B and Langrana N A 2004 *J. Biomech. Eng.* **126** 104-10
- [22] Yurke B, Lin D C and Langrana N A 2006 *DNA Computing*, ed A Carbone and N A Pierce pp 417-26
- [23] Liedl T, Dietz H, Yurke B and Simmel F 2007 *Small* **3** 1688-93
- [24] Um S H, Lee J B, Park N, Kwon S Y, Umbach C C and Luo D 2006 *Nat. Mater.* **5** 797-801
- [25] Xing Y, Cheng E, Yang Y, Chen P, Zhang T, Sun Y, Yang Z and Liu D 2011 *Adv. Mater.* **23** 1117-21
- [26] Lin D C, Yurke B and Langrana N A 2005 *J. Mater. Res.* **20** 1456-64
- [27] Murakami Y and Maeda M 2005 *Biomacromolecules* **6** 2927-9
- [28] Baeissa A, Dave N, Smith B D and Liu J 2010 *ACS Appl. Mater. Inter.* **2** 3594-600
- [29] Wilson D S and Szostak J W 1999 *Annu. Rev. Biochem.* **68** 611-47
- [30] Jayasena S D 1999 *Clin. Chem* **45** 1628-50
- [31] Famulok M, Hartig J S and Mayer G 2007 *Chem. Rev.* **107** 3715-43
- [32] Katz E and Willner I 2004 *Angew. Chem., Int. Ed.* **43** 6042-108
- [33] Navani N K and Li Y 2006 *Curr. Opin. Chem. Biol.* **10** 272-81
- [34] Liu J, Cao Z and Lu Y 2009 *Chem. Rev.* **109** 1948-98
- [35] Li D, Song S P and Fan C H 2010 *Acc. Chem. Res.* **43** 631-41
- [36] Liu J 2011 *Soft Matter* DOI: 10.1039/C1SM05284E
- [37] Yang H H, Liu H P, Kang H Z and Tan W H 2008 *J. Am. Chem. Soc.* **130** 6320-1
- [38] Soontornworajit B, Zhou J, Shaw M T, Fan T H and Wang Y 2010 *Chem. Comm.* **46** 1857-9
- [39] Dave N, Huang P-J J, Chan M Y, Smith B D and Liu J 2010 *J. Am. Chem. Soc.* **132** 12668-73
- [40] He X, Wei B and Mi Y 2010 *Chem. Comm.* **46** 6308-10

- [41] Zhu Z, Wu C C, Liu H P, Zou Y, Zhang X L, Kang H Z, Yang C J and Tan W H 2010 *Angew. Chem. Int. Ed.* **49** 1052-6
- [42] Joseph K A, Dave N and Liu J 2011 *ACS Appl. Mater. Inter.* **3** 733–9
- [43] Soontornworajit B, Zhou J and Wang Y 2010 *Soft Matter* **6** 4255-61
- [44] Storhoff J J, Elghanian R, Mucic R C, Mirkin C A and Letsinger R L 1998 *J. Am. Chem. Soc.* **120** 1959-64
- [45] Liu J and Lu Y 2006 *Nat. Protoc.* **1** 246-52
- [46] Dave N and Liu J 2011 *Acs Nano* **5** 1304–12
- [47] Li W, Nie Z, Xu X H, Shen Q P, Deng C Y, Chen J H and Yao S Z 2009 *Talanta* **78** 954-8
- [48] Wang J, Munir A and Zhou H S 2009 *Talanta* **79** 72-6
- [49] Nutiu R and Li Y 2004 *Chem. Eur. J* **10** 1868-76
- [50] Nutiu R and Li Y 2003 *J. Am. Chem. Soc.* **125** 4771-8
- [51] Huizenga D E and Szostak J W 1995 *Biochemistry* **34** 656-65
- [52] Kilburn D, Townrow S, Meunier V, Richardson R, Alam A and Ubbink J 2006 *Nat. Mater.* **5** 632-5
- [53] Hurst S J, Hill H D and Mirkin C A 2008 *J. Am. Chem. Soc.* **130** 12192-200