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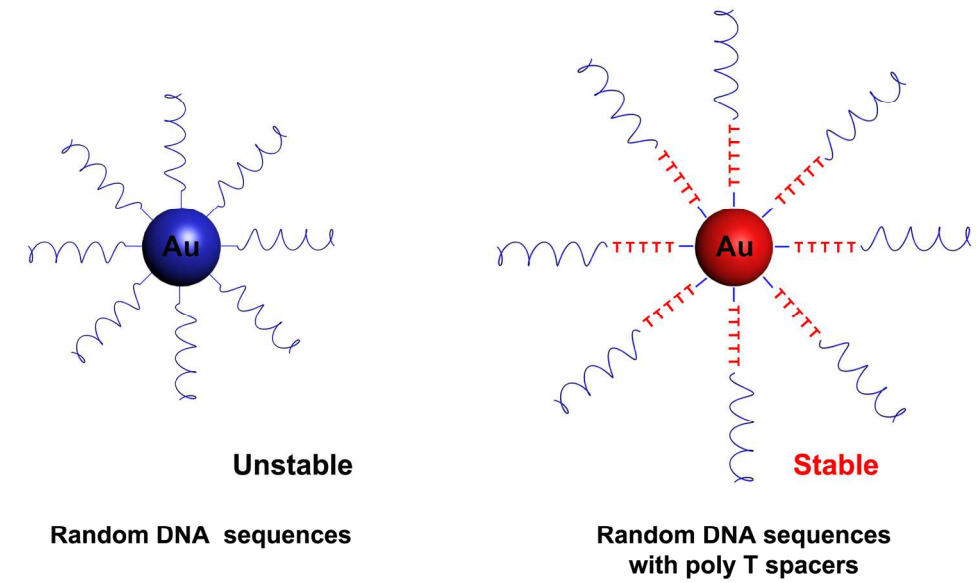

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Surfactant-free nanoparticle-DNA complexes with ultrahigh stability against salt for environmental and biological sensing

Jun Hyuk Heo[1], Hui Hun Cho[2], Jung Heon Lee*[1,2]

We report the development of surfactant free-gold nanoparticle (AuNP)-DNA complexes that remained stable in solutions with extremely high ionic strength, using seawater as a model solution. Although the stability of AuNPs can be increased to a certain degree by functionalizing negatively charged DNA strands on their surface, they still have limited stability in highly concentrated salt solutions. However, we found that AuNPs functionalized with poly T bases have exceptional stability in high ionic strength solutions. For example, AuNPs functionalized with a 5T spacer remained highly stable in seawater, with no color change and no red-shift in absorbance spectra for up to 9 days. Using this surprising property of poly T spacers, we prepared highly stable AuNP-DNA complexes containing random sequences by introducing 5T spacers on the random sequenced DNA strand. The random sequenced AuNP-DNA complexes remained stable in seawater, several molar concentrations of monovalent metal ion solutions (6.1 M Na^+ or 4.8 M K^+), and millimolar concentrations of diverse divalent metal ions. In addition, the highly stable AuNP-DNA complex maintained biological activity in seawater, which was demonstrated by complementary reaction and aptamer based biosensing. These results provide important insight into NP use for various applications under harsh biological and environmental conditions.

Introduction

Nanoparticles (NPs) have many interesting chemical and physical properties that can be used for wide-ranging applications, including electronics[1-4], photonics,[5-7] energy,[8-11] catalysis,[8, 12-17] biological imaging[18-21], drug delivery,[22-24] and environment monitoring.[25-28] Although nanoscale materials have unique optical and electrical properties that are not observed in materials with larger dimensions, most of their physical properties depend closely on the stability of the NPs. For example, the color of gold nanoparticles (AuNPs) generally changes from red to blue when they become close to each other due to coupling of the surface plasmon resonance of the particles.[29, 30] Similarly, CLIO (cross-linked dextran iron oxide) NPs, which are frequently used MRI contrast agents, tend to decrease the spin-spin relaxation time (T2) of surrounding water protons much more efficiently

as they become aggregated.[31-33] As NPs are exposed to diverse environments, their stability can be an important issue. In particular, as NPs have an extremely high surface area to volume ratio,[12] they can be easily aggregated in the presence of metal ions due to the screening of the repulsive charges on the NP surfaces. Although various molecules such as polyethylene glycol (PEG), sodium dodecyl sulfate (SDS), polystyrene sulfonate (PSS), and phosphine have been used to increase AuNP stability, it is still very difficult to prevent aggregation of NPs.[34-39] Because of this stability issue, applications of NPs in severe conditions, such as in seawater, have only been rarely reported.[34]

DNA is a versatile biomolecule that can provide transcendent functions to NPs. Because diverse functional groups can be placed on any desired position of DNA,[40, 41] DNA can be easily conjugated on the surface of a NP.[42, 43] The strong negative charge of the DNA backbone helps to increase NP stability. Taking advantage of the target recognition capability of DNA functionalized on the NP surface, based on the Watson–Crick base pairing model, NPs can be self-assembled in a programmed manner. More importantly, DNA not only interacts with a complementary DNA strand but also binds to a wide types of molecules, including metal ions[44-51], peptides[52-55], proteins[56, 57], small molecules[58-60], organic dyes[61-63], bacteria[64], and cells[65, 66] with specificity. Therefore, DNA provides the multifunctional capability NPs need to broaden their use in biosensing[46-51, 67, 68], bioimaging[69], and environmental monitoring.[70, 71]

Although DNA conjugated NPs are more stable than bare NPs,[72] they have limited stability in a high concentrated salt solution. This restricted stability of NP-DNA conjugates is a huge barrier for their practical applications. NPs are generally exposed to severe conditions such as high salt concentration for most biological and environmental applications. Although various polymers can be used to protect NPs[34-36], they have a critical drawback because their presence can hinder attachment and activity of DNAs functionalized on the NP surface.

In this study, we report on a DNA conjugated gold NP (AuNP-DNA) complex that remained stable in an extremely concentrated salt solution, using seawater as the model solution. Seawater is one of the harshest environmental systems, containing diverse and concentrated divalent and monovalent metal ions (Table S1). We show that a short thymine (poly T) based spacer maintained the stability of the AuNP-DNA complexes in solutions with high metal ion concentrations, including seawater. Furthermore, we demonstrated that the AuNP-DNA complexes maintained their target specificity and functionality in highly concentrated metal ion solutions and can be used for colorimetric detection of biomolecules in seawater. To our knowledge, this is the first study to demonstrate a stable NP-DNA complex system for practical applications in seawater. This study is significant as the results can be used for potential applications of NPs in diverse harsh environments.

Results and discussion

The effect of sequence on AuNP-DNA complex stability in seawater

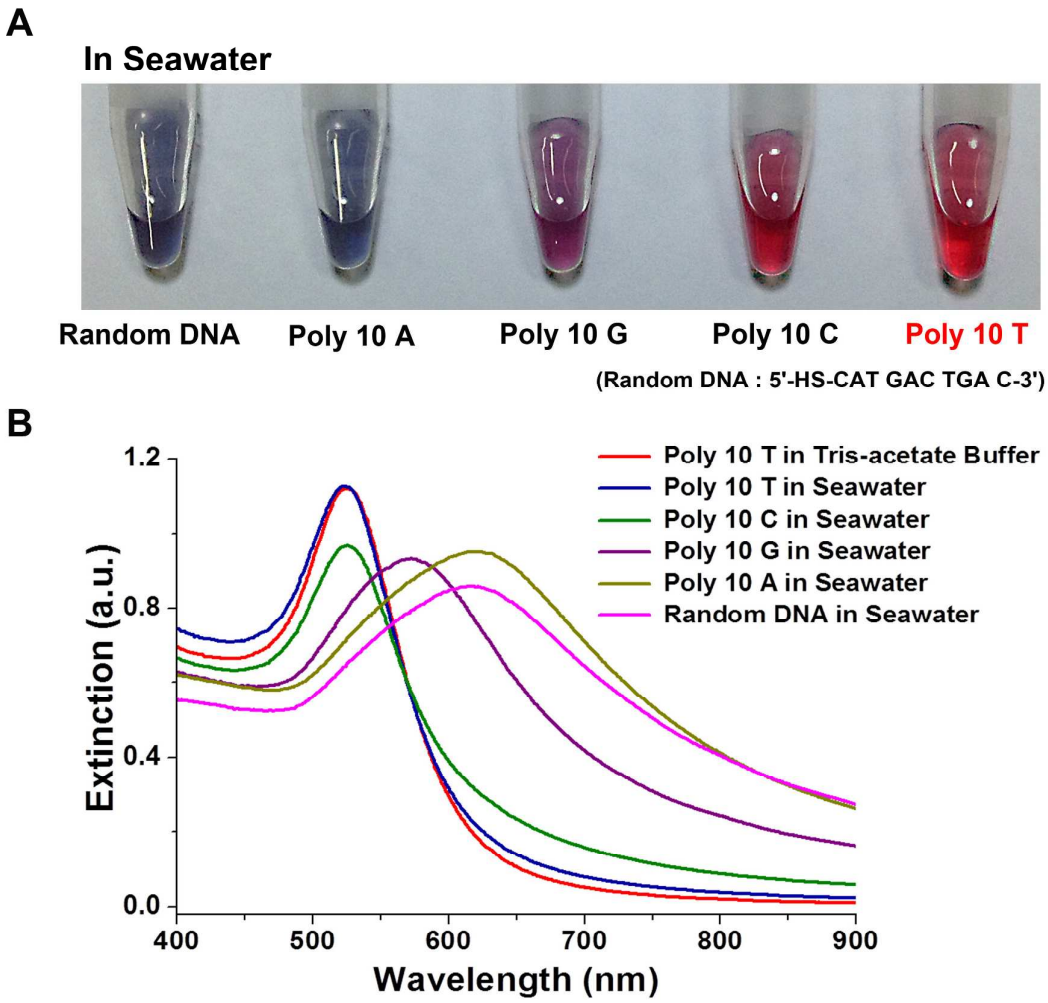

Fig 1. Stability of AuNP-DNA complexes in seawater. (A) The color of AuNPs functionalized with different sequences of DNA. AuNPs functionalized with 10 mer poly A, poly G, and random sequences aggregated in seawater. However, AuNPs functionalized with 10 mer poly C and poly T bases remained stable. AuNPs functionalized with 10T DNA (AuNP-[10T]) remained stable in seawater for at least 7 days. (B) Extinction spectra of AuNPs functionalized with different sequences of DNA. The spectrum of AuNP-[10T] in seawater is similar to that of AuNP-[10T] in buffer solution.

AuNP-DNA is expected to be aggregated due to the presence of highly concentrated metal ions in seawater (see Table S1 for the list and concentration of metal ions existing in the seawater used in this work). When AuNPs functionalized with random sequenced 10 mer DNA (AuNP-[Random]) were dissolved in seawater, we observed that the color of the AuNP-[Random] changed immediately from red to dark blue (Fig. 1A). When 13

nm AuNPs were dispersed, they were generally red, as AuNPs have a plasmon resonance peak at approximately 522 nm. However, as the AuNPs moved closer to each other, plasmon resonance coupling occurred between AuNPs. This coupling generated a red shift of their absorbance peak and made the AuNPs blue. This color change indicates that the AuNP-[Random] was aggregated by the metal ions in seawater. Because all four bases have different chemical and physical properties[73, 74], we were interested in whether each base may have a different influence on stability of the AuNP-DNA complex. Thus, to investigate the effect of each base on AuNP-DNA stability, we prepared four sets of AuNP-DNA complexes, including AuNPs functionalized with 10 mer poly A, G, T, and C, respectively. When all four AuNP-DNA complexes were dissolved in seawater, we observed a contrasting trend in the color change of the complexes (Fig. 1A). AuNPs functionalized with 10 mer poly A bases (AuNP-[10A]) showed an immediate color change from red to dark blue. This color change was similar to what was observed with AuNP-[Random]. AuNPs functionalized with 10 mer poly G bases (AuNP-[10G]) turned purple when dissolved in seawater. In contrast, AuNPs functionalized with 10 mer poly C (AuNP-[10C]) or poly T (AuNP-[10T]) remained red. We used a UV-vis spectrophotometer to quantitatively obtain the change in the surface plasmon resonance peak of each AuNP-DNA complex in seawater (Fig. 1B). As expected from the color changes of the complexes, the absorbance peak shift of AuNP-[10A] was 94 nm (= 616 – 522 nm), which was the largest among the four AuNP-DNA complexes we tested and was comparable to AuNP-[Random]. The absorbance peak shift of AuNP-[10G] was still significant by 50 nm but was much less than that observed from both AuNP-[10A] and AuNP-[Random]. However, the maximum surface plasmon resonance peak of both the AuNP-[10T] and AuNP-[10C] complexes remained at 522 nm and showed minimal change. Decreased absorbance at 522 nm and increased scattering at longer wavelengths were still observed from the extinction spectra of AuNP-[10C], indicating that a certain amount of aggregation occurred from the complex. In contrast, the extinction spectra of AuNP-[10T] taken in seawater was very similar to the spectra of AuNP-[10T] taken in Tris-acetate buffer. These results suggest that the DNA bases functionalized on the AuNP surface had a contrasting influence on stability of the AuNP-DNA complexes, and that poly T DNA has the potential to make AuNP-DNA complexes stable in solutions with ultrahigh ionic strengths such as seawater.

We attribute this dissimilarity in the stability of AuNP-DNA complexes with different bases to the interaction between the bases and the Au surface.[75, 76] Purines bind more strongly to Au surfaces than do pyrimidines.[73, 74] Purines tend to directly lie on the Au surface, whereas pyrimidines generally stand up perpendicularly. This difference in packing orientation can result in a significant difference in the loading density of DNA on the AuNP surface.[72, 77] As all DNA strands are strongly negative due to the phosphate backbone, which all

nucleotides have in common, higher loading of DNA strands should produce higher stability of AuNP-DNA complexes under high ionic strength conditions. This was observed in our data, as the AuNP-[10T] and AuNP-[10C] had much higher stability compared to that of the AuNP-[10G] and AuNP-[10A]. Between the two pyrimidine bases, it is reported that C binds to Au more strongly than T. Therefore, once DNA is functionalized on AuNP surface through Au-thiol chemistry, poly T based DNA should be packed on the NP surface with the highest density and help AuNP remain most stable in high ionic strength solutions.[77-79] In addition, as thymine has the highest electronegativity compared to other bases,[73, 74] poly T spacers can help AuNP-DNA complexes to have strong negative charge on the surface and remain stable in high ionic strength media.

The effect of poly T DNA length on AuNP-DNA complex stability in seawater

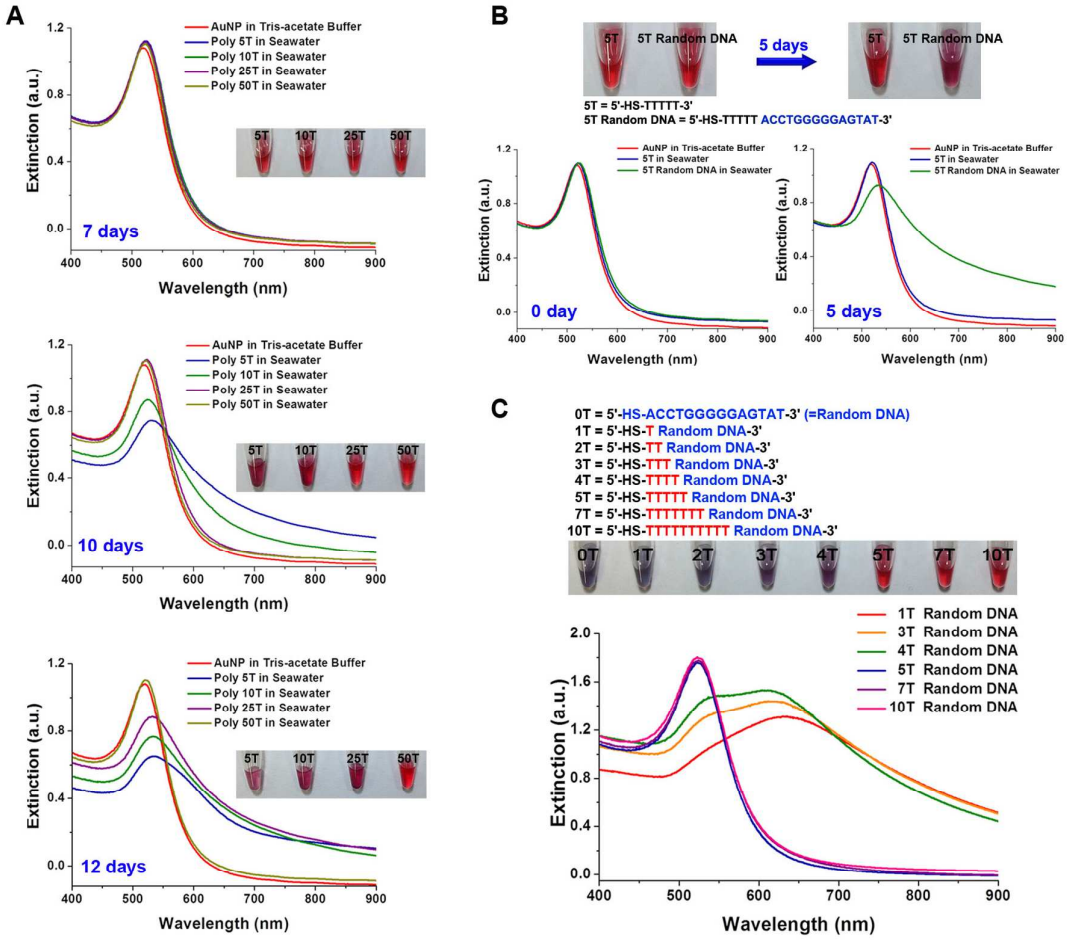

Fig 2. (A) Effects of the number of poly T bases on the stability of AuNPs in seawater. Although poly T spacers longer than 5T were all effective to keep AuNP-DNA stable, longer strands are better for long term stabilization. AuNPs functionalized with 50T were stable even after 12 days (B) The stability

of AuNPs functionalized with 5T DNA and AuNPs functionalized with a random DNA with 5T spacer in seawater. AuNP functionalized with a random DNA with 5T spacer can be stable in seawater for up to 3~4 days. (C) The number of T bases needed to keep AuNPs functionalized with a random DNA stable in seawater. With 5T based spacers, AuNP-DNA stays stable.

As we found that poly T bases help keep AuNPs stable in seawater, we next were interested in the number of poly T bases necessary for efficient protection of AuNPs. We first compared AuNPs functionalized with 5T, 10T, 25T, and 50T DNA to investigate the effect of the length of poly T bases (Fig. 2A). Because poly T bases can protect AuNPs in seawater, we initially predicted that AuNPs functionalized with longer poly T DNA might have higher stability. When all four AuNPs functionalized with different lengths of poly T DNA (AuNP-[5T], [10T], [25T], and [50T]) were dissolved in seawater, they all remained red for up to 7 days (Fig. 2A, 7 days). A negligible difference in the absorbance peak of each complex was observed from UV-spectrophotometry as well. This observation confirmed that poly T DNA strands with diverse lengths were helpful to protect AuNPs in seawater for at least 7 days. However, 10 days after incubating the AuNP-DNA complexes in seawater, the color of AuNP-[5T] and AuNP-[10T] started to change slightly, whereas the color of AuNP-[25T] and AuNP-[50T] remained red (Fig. 2A, 10 days). This color change trend was also observed from a red-shift in the absorbance peak of AuNP-[5T] and AuNP-[10T] obtained from the UV-vis spectrophotometer. When we monitored the color of the AuNP-DNA complexes further, we determined that AuNP-[50T] remained stable in seawater for at least 12 days, whereas AuNP-[25T] started to aggregate (Fig. 2A, 12 days). These results indicate that a longer poly T DNA is much more effective in protecting AuNPs from aggregation under high salt conditions. Nevertheless, short poly 5T sequences were still sufficient to keep AuNPs stable in seawater for up to 9 days. Additional information on the stability of AuNP-[poly T] complexes at different exposure time can be found in Fig. S1.

Longer T spacers have much lower loading density than those with shorter poly T spacers on the surface of AuNPs. The longer T spacers form mostly disordered layers on the AuNP to similar random coiled shape, while the shorter poly T spacers have a regular monolayer. As a result, longer poly T spacers functionalized with AuNP prevent further interactions between AuNPs and other T spacers.[75, 80, 81]

We have also observed this phenomenon. When we synthesized AuNP-DNA complexes without sonication, AuNPs functionalized with a longer T were much less stable than those functionalized with shorter strands (data not shown). However, after sonicating (10 sec) the samples three times during AuNP-DNA complex synthesis, stability improved in the AuNPs functionalized with longer DNA. From this result, we determined that the

sonication process not only helped to detach DNA bases physically bound on the AuNP surface but also to unfold the long T strands densely packed on the AuNP surface. By releasing the long DNA strands through sonication, the number of DNA strands functionalized on the AuNPs can be increased, resulting in the increased stability of AuNP-DNA complexes.

After we determined that short 5T sequences were sufficient to make stable AuNPs in seawater, we investigated the possibility of extending T based DNA with a random sequenced DNA to make a random sequenced AuNP-DNA complex stable in seawater. First, we extended 5T DNA with 14 bases including 3A, 6G, 2C, and 3T ([5T-random]) and compared the stability of the AuNP-[5T-random] complex with that of the AuNP-[5T] complex in seawater. Surprisingly, we did not observe any instantaneous difference between the two complexes in terms of their color or extinction spectra (Fig. 2B). This finding verifies that the existence of random sequenced bases with a short T based spacer does not make a significant difference in terms of AuNP-DNA complex stability. This finding also indicates that stable random sequenced AuNP-DNA complexes can be made by simply introducing a few T bases on functionalized DNA. The AuNP-DNA complexes with a random sequence maintained their stability for up to 3 days, although they started to change color after 4 days in seawater. Additional data demonstrating the stability of AuNP-[5T-random] dissolved in seawater for different times can be found in Fig. S2.

As we observed that a random DNA with 5T spacer can make AuNPs stable in seawater, we were interested in the influence of the length of poly T spacers on the stability of the complexes. We functionalized AuNPs with DNA strands containing an identical random sequence with 1–10T long spacers to define the optimal length of T bases for high stability. As presented in Fig. 2C, the AuNP-DNA complexes with 5, 7, and 10T bases remained reddish in seawater and showed negligible changes in absorbance spectra. This result indicates that 5, 7, and 10T spacers can keep AuNPs functionalized with random sequenced DNA strands stable in seawater. In contrast, when random DNA strands with 1–4T long spacers were functionalized on the AuNP surface, the AuNP-DNA complexes showed an abrupt color change from red to dark blue. This color change was similar to what occurred with AuNP-[Random] (Fig. 1A). In addition, when extinction spectra were taken from the AuNPs functionalized with random DNA strands containing different lengths of T spacers, we only observed a significant red-shift in the absorbance peak from AuNPs functionalized with 1–4T spacers. This finding indicates that 1–4T based spacers are insufficient to protect AuNPs in seawater. However, spacers longer than 5T bases were sufficient to keep AuNPs stable over the long term in seawater.

Stability of AuNP-DNA complexes against various metal ions

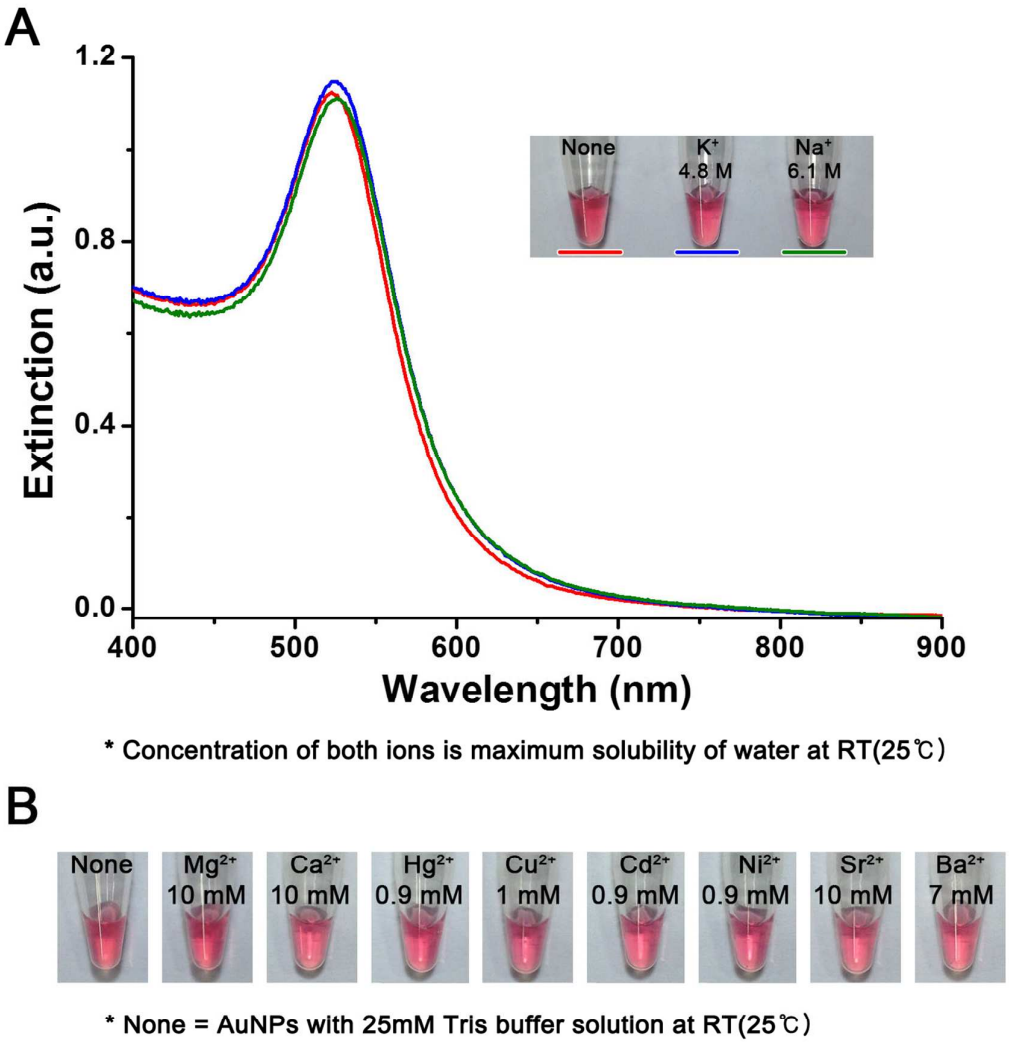

Fig 3. Effect of AuNPs functionalized with random DNA with 5T spacer [5T-random = 5'-HS-TTTTT-ACCTGGGGGAGTAT-3'] in the presence of various metal ions. (A) Negligible change was observed when AuNP-[5T-random] were transferred into a solution with extremely high concentration of monovalent metal ions (6.1 M Na^+, 4.8 M K^+). (B) The AuNP-[5T-random] also remained stable in the presence of diverse divalent metal ions.

After we determined that AuNPs functionalized with random sequenced DNA with a 5T spacer (AuNP-[5T-random]) remained stable in seawater, we investigated how well AuNP-[5T-random] remained stable in the presence of diverse metal ions. Although seawater contains a large amount of metal ions, it is a solution containing diverse metal ions with dominant Na^+, K^+, Ca^{2+}, and Mg^{2+}.[67] Thus, seawater is not a perfect system to

study the stability of AuNP-DNA complexes in the presence of individual metal ions. Thus, we first transferred AuNP-[5T-random] to Tris-acetate buffer containing highly concentrated monovalent metal salt to carry out this experiment and monitored the color change of the AuNPs. Surprisingly, when we placed AuNP-[5T-random] in both 6.1 M NaCl and 4.8 M KCl solutions, which are close to the maximum solubility of NaCl and KCl at room temperature, they remained red with negligible changes in the absorbance peaks (Fig. 3A). This result indicates that AuNP-[5T-random] was remarkably stable in highly concentrated monovalent metal ion solutions. We inspected the stability of AuNP-[5T-random] in divalent metal ion solutions. We prepared highly concentrated metal salt stock solutions, added a small amount of stock solution into AuNP-[5T-random], and monitored the extinction spectra of the AuNPs 3h after adding the concentrated salt solutions to determine the concentration of divalent metal ions at which AuNP-[5T-random] remained stable (Fig. S3). As a result, AuNP-[5T-random] complexes remained stable in the presence of 0.9–10 mM of various divalent metal ions. In Fig. 3B, we specifically show AuNP-[5T-random] samples incubated in 10 mM Mg^{2+}, 10 mM Ca^{2+}, 0.9 mM Hg^{2+}, 1 mM Cu^{2+}, 0.9 mM Cd^{2+}, 0.9 mM Ni^{2+}, 10 mM Sr^{2+}, and 7 mM Ba^{2+} solutions. It is evident that the AuNP-[5T-random] samples remained red, indicating that the complexes remained stable in the presence of millimolar concentrations of divalent metal ion solutions.

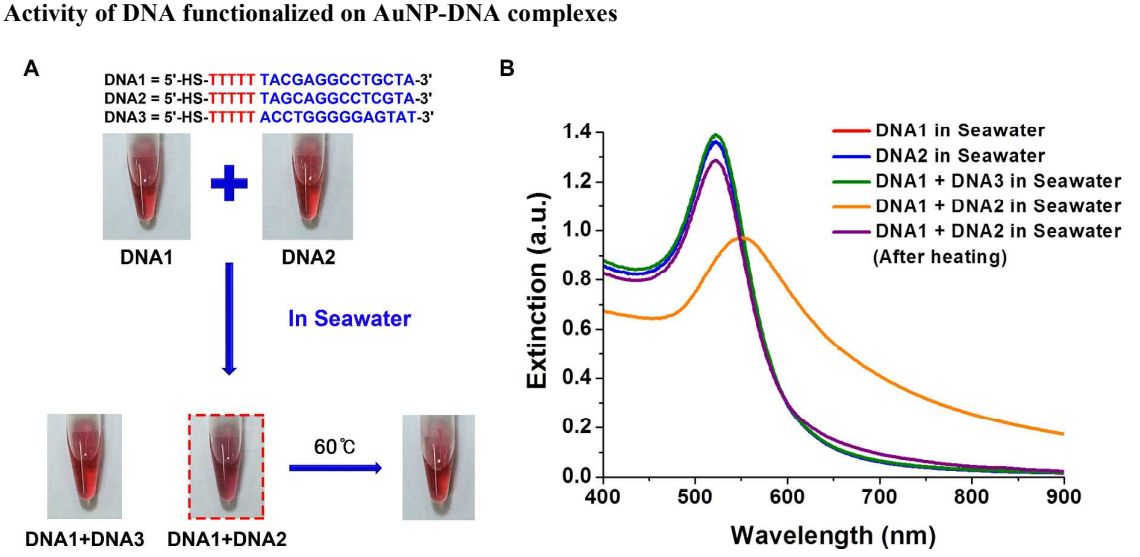

Fig 4. Sequence specific activity of AuNP-DNA complexes in seawater. (A) When two complementary AuNP-DNA complexes were mixed together (DNA1+DNA2), color change of AuNP was observed. On the other hand, when two noncomplementary AuNP-DNA complexes were mixed together (DNA1+DNA3), no color change was observed. (B) Extinction spectra of the AuNP-DNA complexes in seawater. Red shift of the resonance peak was observed only when two complementary AuNP-DNA complexes were mixed together.

As we determined that AuNP-DNA remains stable in high ionic strength solutions by introducing a few T spacers into the DNA, we were interested in whether the DNA functionalized on the AuNP surface still possessed sequence-specific activity and could induce target-directed biological functions, which are critical for biosensing applications. We prepared a pair of AuNP-DNA complexes functionalized with two complementary DNA strands with 5T spacers and compared the color of the AuNPs before and after mixing the pair with seawater to check the sequence-specific activity of DNA functionalized on the AuNP surface (Fig. 4A). Before mixing the AuNP-DNA pair (DNA 1 and 2), the two samples remained red. This result indicates that both AuNP-DNA complexes remained stable in seawater. However, as the two AuNP-DNA complexes were mixed together, they showed an immediate color change from red to blue. This color change was also observed by the red-shift in the absorbance peak (Fig. 4B). When we heated the mixed sample up to 60 °C, above the melting temperature of the two complementary DNA strands, the color of the sample immediately changed from blue to red with a blue-shift in the absorbance peak. This observation indicates that the mixture of the two AuNP-DNA complexes induced DNA-directed aggregation of AuNPs. In addition, when we carried out a control experiment by mixing two non-complementary AuNP-DNA complexes, we did not observe color change after mixing the

two solutions (DNA 1 and 3, Fig. 4A and B). This result suggests that the aggregation of the two complementary AuNP-DNA complexes is sequence specific and that the random sequenced DNAs functionalized on the surface of AuNPs are biologically active in seawater.

Selectivity and sensitivity of an AuNP-based adenosine sensor in seawater

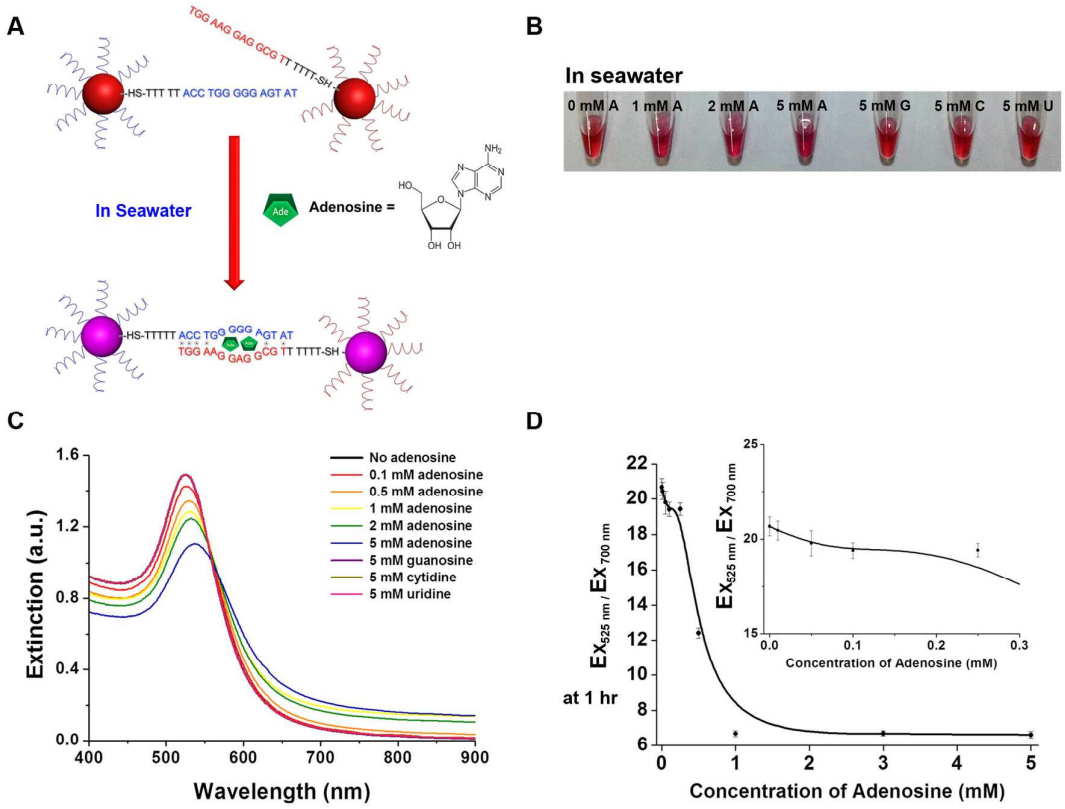

Fig 5. Colorimetric detection of adenosine using aptamer-functionalized AuNP sensor in seawater at room temperature. (A) The AuNPs are functionalized with two different DNA stands. The two AuNP-DNA complexes can be linked by adenosine to form aggregates in seawater. In the presence of adenosine, AuNPs aggregate and the color of AuNP will change. (B) Color change of adenosine aptamer-functionalized AuNP in seawater. The color change only happens in the presence of adenosine (A= adenosine). No color change was observed when the sensor was exposed to same amount of G, C, and U (G= guanosine, C= cytidine, U= uridine) (C) UV-vis spectra of adenosine aptamer sensor with varying adenosine concentrations. (D) Calibration curve of adenosine sensor. The sensor has a detection limit of 0.5 mM in seawater.

We introduced a pair of partially complementary adenosine-specific aptamers on the AuNPs to investigate the possibility of using these AuNP-DNA complexes to detect biological molecules in seawater. Aptamers are nucleic acid-based molecules with extremely high sensitivity and selectivity to specific chemical or biological molecules. As the general properties of aptamers are similar to antibodies, they are called chemical antibodies and are often combined with diverse nanoscale materials or devices to construct highly sensitive and specific biosensors.[82-86] We used a pair of adenosine aptamers, which were modified from the sequences reported from

other groups, to demonstrate that a biosensor could be operated in seawater (Fig. 5A).[84, 85] We inserted 5T bases on the end of each DNA strand to make them stable in seawater. When the AuNP-aptamer complexes were separately transferred to seawater, we did not observe a color change. This finding indicates that both AuNP-aptamer complexes were stable in seawater. When both AuNP-aptamer complexes were mixed together, we did not observe a color change occurring from the AuNPs as well (Fig. 5B). However, when adenosine was added to the mixture of the AuNP-aptamer complexes, we saw the color of the AuNPs change from red to light purple within 1 h at room temperature. This color change could also be monitored from the red-shift of the AuNP absorbance peak in the presence of adenosine (Fig. 5C). This finding suggests that AuNP aggregation was triggered by introducing adenosine. We added G, C, U into the mixed AuNP-aptamer complexes under identical conditions and monitored the color change to check selectivity of the reaction (Fig. 5B and C). As predicted, we did not see any color change or red-shift in the absorbance peak of the AuNPs, suggesting that this AuNP color change occurred specifically in the presence of adenosine and that no other molecules responded. Although we observed adenosine-induced assembly of AuNP-aptamer complexes within 1 h at room temperature, we could not observe it when we lowered the temperature to 4 °C (see Fig. S4). This suggests that temperature is an important factor that can determine kinetics of this reaction and it should be carefully considered for practical application. The detection limit of adenosine in our AuNP-aptamer system with 5T spacers was about 0.5 mM in seawater (Fig. 5D). This might not be the sensor reporting the best performance for detection of adenosine. To find whether the 5T spacer is the reason for such moderate performance of the sensor, we prepared two sets of AuNP-aptamer sensors with and without 5T spacer and conducted aptamer sensing in 1/10 times diluted seawater, where both sensors remained stable. The AuNP-aptamer sensor without 5T spacer showed color change from red to purple in relatively low adenosine concentration ranges and the detection limit of sensor turned out to be 0.059 mM (see Fig. S5). On the other hand, AuNP-aptamer sensor with 5T spacer had lower detection limit of 0.2 mM in 1/10 diluted seawater. This indicates that the existence of 5T spacer negatively affects the performance of the sensor. We attribute this result to the length of the DNA. As the length of the DNA functionalized on the AuNP becomes longer, the gap between the aggregated AuNPs will become larger, resulting in less surface plasmon resonance shift and less color change of AuNPs. Consequently, as we introduce 5T spacer into our AuNP-aptamer sensor, the color change can be less significant and the sensitivity of the sensor can become low. On the other hand, we observed that the sensitivity of the AuNP-aptamer sensor with 5T spacer has increased when sensing was carried out in 1/10 times diluted seawater. We think this influence of the ionic strength on the sensor is related to property of the DNA aptamer we used. The binding

affinities of aptamers are highly dependent on the biochemical conditions of the media including ionic strength and pH. Therefore, to make a sensor that works most efficiently in high ionic strength media, like seawater, it would be necessary to use new aptamers that are selected or modified for optimal use in those media. However, since most existing aptamers, including the one used in our work, are designed for use in moderate ionic strength buffer conditions,[84, 85] they might not be desirable for high efficiency sensing in extremely harsh media like seawater. By further optimizing the sensing conditions and aptamer strands, we believe we can develop high performance sensors for application in harsh environmental and biological conditions.

Conclusions

In summary, we successfully synthesized surfactant-free AuNP-DNA complexes with ultrahigh stability in solutions with highly concentrated ions, using seawater as a model solution. The AuNP-DNA complexes functionalized with random sequenced DNA cannot be stable in seawater. However, the AuNP-DNA complexes became stable in seawater by simply inserting a few T based spacers into the random sequenced DNA. In addition, these AuNP-DNA complexes maintained their stability in solution with several molar monovalent metal ions (6.1 M of Na^+, 4.8 M of K^+) and millimolar concentrations of diverse divalent metal ions. Furthermore, we demonstrated that these highly stable AuNP-DNA complexes can carry out biological activities in seawater through the complementary reaction and adenosine aptamer based biosensor. These results provide important insight into NP use for diverse applications under harsh biological and environmental conditions.

Experimental

Preparation of materials

All oligonucleotides purified by high performance liquid chromatography were purchased from Integrated DNA Technologies Inc. (Coralville, IA, USA) and Bioneer Corp. (Daejeon, Korea). Gold(III) chloride hydrate ($HAuCl_4 \cdot 3H_2O$, CAS: 27988-77-8), sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, CAS: 6132-04-3), adenosine, guanosine, uridine, cytidine, and all other materials were purchased from Sigma-Aldrich Corp (St. Louis, MO, USA). Deionized water (18.2 MΩ·cm) was prepared with a Sartorius Arium® pro Ultrapure water system.

Preparation of seawater

The seawater used in this study was obtained from Daeboo Island beach, Korea. The seawater was filtered with a 0.2 μm filter to remove sediments before use. The concentration of major and minor metal ions in the seawater was analyzed with inductively coupled plasma mass spectrometry at the National Instrumentation Center for Environmental Management, Seoul National University and appended to supporting information (Table S1)

Synthesis of AuNPs

Thirteen nanometer AuNPs were synthesized by a previously reported citrate reduction method.[84, 87] Specifically, 100 mL of 1 mM gold(III) chloride hydrate solution was mixed with 10 mL of 38.8 mM sodium citrate dihydrate solution in a two-neck flask, and the reaction was carried out through reflux. Then, the synthesized 13 nM AuNPs solution was stored in a dark room before use.

DNA functionalization on AuNPs

First, 3 mL of AuNP solution was placed in a vial, pre-treated with 10 M sodium hydroxide solution, and rinsed several times in deionized water. A 3 μL aliquot of 10 mM TCEP was added to a mixture of 9 μL 1 mM stock solution of DNA and 1 μL 500 mM acetate buffer solution (pH = 5.2) and incubated for 1 h to active the thiolate DNA. The DNA solution was transferred to the AuNP solution and kept in the dark overnight. We added 100 μL of 1 M NaCl solution and sonicated for 10 sec after gentle shaking to minimize physical adsorption and increase chemical conjugation of DNA on the AuNP surface. This whole process was repeated three times, so that the final concentration of NaCl of the solution was 100 mM. This solution was stored in a dark room for 1 day before use.[87]

Detection of adenosine in seawater

The AuNP-DNA complexes were centrifuged at 17000 rcf for 10 min, and the supernatant was replaced with 25 mM Tris buffer solution (pH = 8.20) to remove free DNA from the solution. After two rounds of additional purification, the supernatant was replaced with seawater. A particular amount of adenosine stock solution was added to a mixture of AuNP-aptamer complexes and the AuNP color change was monitored using the naked eye or UV-vis spectrophotometer for the biosensing experiment.

Stability measurement of the AuNP-[5T-random] complexes

To measure the stability of AuNP-DNA complexes in a diverse solution of metal ions, AuNPs-[5T-random = 5'-HS-TTTTT-ACCTGGGGGAGTAT-3'] were incubated in concentrated NaCl, KCl, $Hg(ClO_4)_2$, $CaCl_2$, $BaCl_2$, $MgCl_2$, $SrCl_2$, $CuSO_4$, $NiSO_4$, and $CdCl_2$ solutions, and the color change of the AuNPs was monitored with the naked eye and a UV-vis spectrophotometer, respectively. The concentration of AuNP-[5T-random] solution used in this experiment was half of the concentration of the AuNP-[5T-random] solution used for the detection of adenosine.

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† Electronic Supplementary Information (ESI) available: Photographs, and UV-vis spectra of AuNP-DNA complex in the presence of poly T spacers, and the concentration of ionic components in seawater are included. See DOI : 10.1039/b000000x/

1. S. Jeong, K. Woo, D. Kim, S. Lim, J. S. Kim, H. Shin, Y. N. Xia and J. Moon, *Adv. Funct. Mater.*, 2008, 18, 679-686.
2. P. C. Sherrell, J. Chen, J. M. Razal, I. P. Nevirkovets, C. Crean, G. G. Wallace and A. I. Minett, *Energy Environ. Sci.*, 2010, 3, 1979-1984.
3. G. Maruccio, *Nat Nano*, 2012, 7, 147-148.
4. J. Lawrence, J. T. Pham, D. Y. Lee, Y. Liu, A. J. Crosby and T. Emrick, *ACS Nano*, 2014, 8, 1173-1179.
5. A. G. Brolo, *Nat Photon*, 2012, 6, 709-713.
6. S. H. Ko, K. Du and J. A. Liddle, *Angew. Chem. Int. Ed.*, 2013, 52, 1193-1197.
7. A. Sharma, S. Dhar and B. P. Singh, *Appl. Surf. Sci.*, 2013, 273, 144-149.

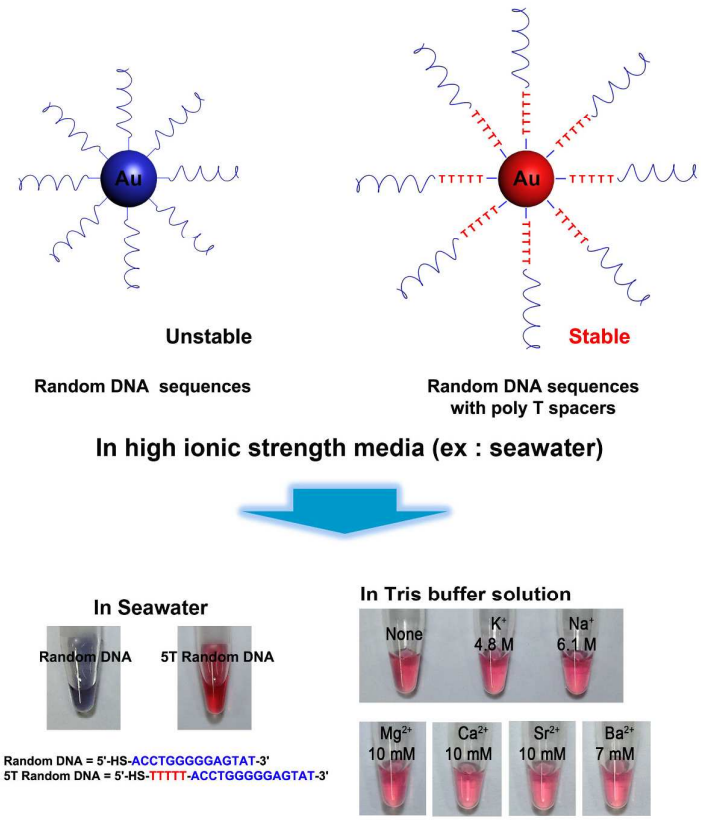
8. K.-J. Jeon, H. R. Moon, A. M. Ruminski, B. Jiang, C. Kisielowski, R. Bardhan and J. J. Urban, *Nat Mater*, 2011, 10, 286-290.
9. O. Neumann, A. S. Urban, J. Day, S. Lal, P. Nordlander and N. J. Halas, *ACS Nano*, 2012, 7, 42-49.
10. Z. Wei Seh, W. Li, J. J. Cha, G. Zheng, Y. Yang, M. T. McDowell, P.-C. Hsu and Y. Cui, *Nat Commun*, 2013, 4, 1331.
11. J. L. Yang, J. J. Wang, Y. J. Tang, D. N. Wang, X. F. Li, Y. H. Hu, R. Y. Li, G. X. Liang, T. K. Sham and X. L. Sun, *Energy Environ. Sci.*, 2013, 6, 1521-1528.
12. R. Narayanan and M. A. El-Sayed, *J. Phys. Chem. B*, 2005, 109, 12663-12676.
13. N. Wu, J. Wang, D. N. Tafen, H. Wang, J.-G. Zheng, J. P. Lewis, X. Liu, S. S. Leonard and A. Manivannan, *J. Am. Chem. Soc.*, 2010, 132, 6679-6685.
14. S. Wu, J. Dzubiella, J. Kaiser, M. Drechsler, X. Guo, M. Ballauff and Y. Lu, *Angew. Chem. Int. Ed.*, 2012, 51, 2229-2233.
15. L. R. Baker, G. Kennedy, J. Krier, M. Spronsen, R. Onorato and G. Somorjai, *Catal. Lett.*, 2012, 142, 1286-1294.
16. X.-H. Li, X. Wang and M. Antonietti, *Chem. Sci.*, 2012, 3, 2170-2174.
17. T. K. Townsend, N. D. Browning and F. E. Osterloh, *Energy Environ. Sci.*, 2012, 5, 9543-9550.
18. X. Gao, Y. Cui, R. M. Levenson, L. W. K. Chung and S. Nie, *Nat Biotech*, 2004, 22, 969-976.
19. F. Wang, D. Banerjee, Y. Liu, X. Chen and X. Liu, *Analyst*, 2010, 135, 1839-1854.
20. Z. Tao, G. Hong, C. Shinji, C. Chen, S. Diao, A. L. Antaris, B. Zhang, Y. Zou and H. Dai, *Angew. Chem. Int. Ed.*, 2013, 52, 13002-13006.
21. J. Liu, J. Bu, W. Bu, S. Zhang, L. Pan, W. Fan, F. Chen, L. Zhou, W. Peng, K. Zhao, J. Du and J. Shi, *Angew. Chem. Int. Ed.*, 2014, 53, 4551-4555.
22. K. S. Soppimath, T. M. Aminabhavi, A. R. Kulkarni and W. E. Rudzinski, *J. Controlled Release*, 2001, 70, 1-20.
23. S. Mornet, S. Vasseur, F. Grasset and E. Duguet, *J. Mater. Chem.*, 2004, 14, 2161-2175.
24. D. H. M. Dam, J. H. Lee, P. N. Sisco, D. T. Co, M. Zhang, M. R. Wasielewski and T. W. Odom, *ACS Nano*, 2012, 6, 3318-3326.
25. G. K. Darbha, A. K. Singh, U. S. Rai, E. Yu, H. Yu and P. Chandra Ray, *J. Am. Chem. Soc.*, 2008, 130, 8038-8043.
26. R. A. Alvarez-Puebla and L. M. Liz-Marzan, *Energy Environ. Sci.*, 2010, 3, 1011-1017.
27. K. M. Mullaugh and I. I. I. G. W. Luther, *J. Environ. Monit.*, 2010, 12, 890-897.
28. R. I. MacCuspie, K. Rogers, M. Patra, Z. Suo, A. J. Allen, M. N. Martin and V. A. Hackley, *J. Environ. Monit.*, 2011, 13, 1212-1226.
29. M. Das, K. H. Shim, S. S. A. An and D. K. Yi, *J. Toxicol. Environ. Health*, 2011, 3, 193-205.
30. E. C. Dreaden, A. M. Alkilany, X. Huang, C. J. Murphy and M. A. El-Sayed, *Chem. Soc. Rev.*, 2012, 41, 2740-2779.
31. D. E. Sosnovik, M. Nahrendorf and R. Weissleder, *Basic Res Cardiol.*, 2008, 103, 122-130.
32. J. R. McCarthy and R. Weissleder, *Adv. Drug Del. Rev.*, 2008, 60, 1241-1251.
33. C. Tassa, S. Y. Shaw and R. Weissleder, *Acc. Chem. Res.*, 2011, 44, 842-852.
34. J. H. Heo, K.-I. Kim, M. H. Lee and J. H. Lee, *J. Nanosci. Nanotechnol.*, 2013, 13, 7254-7258.

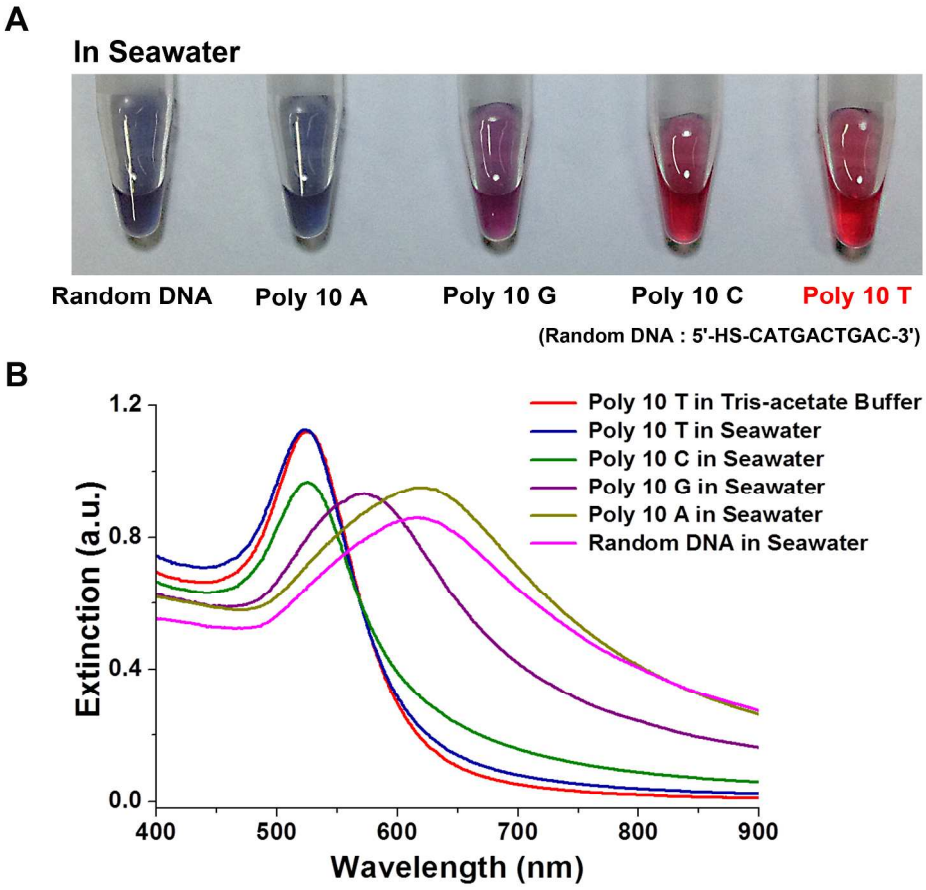
35. X. Zhang, M. R. Servos and J. Liu, *J. Am. Chem. Soc.*, 2012, 134, 9910-9913.
36. J. Shin, X. Zhang and J. Liu, *J. Phys. Chem. B*, 2012, 116, 13396-13402.
37. B. Ding, Z. Deng, H. Yan, S. Cabrini, R. N. Zuckermann and J. Bokor, *J. Am. Chem. Soc.*, 2010, 132, 3248-3249.
38. J. Zhou, J. Ralston, R. Sedev and D. A. Beattie, *J. Colloid Interface Sci.*, 2009, 331, 251-262.
39. S. S. Kumar, C. S. Kumar, J. Mathiyarasu and K. L. Phani, *Langmuir*, 2007, 23, 3401-3408.
40. J. H. Lee, D. P. Wernette, M. V. Yigit, J. Liu, Z. Wang and Y. Lu, *Angew. Chem. Int. Ed. Engl.*, 2007, 46, 9006-9010.
41. J. H. Lee, N. Y. Wong, L. H. Tan, Z. D. Wang and Y. Lu, *J. Am. Chem. Soc.*, 2010, 132, 8906-8908.
42. A. P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez Jr and P. G. Schultz, *Nature*, 1996, 382, 609-611.
43. C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, 382, 607-609.
44. H. Li and D. Kong, *Prog Chem*, 2013, 25, 2119-2130.
45. P. J. J. Huang, J. Lin, J. Cao, M. Vazin and J. Liu, *Anal. Chem.*, 2014, 86, 1816-1821.
46. J. Li and Y. Lu, *J. Am. Chem. Soc.*, 2000, 122, 10466-10467.
47. J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2003, 125, 6642-6643.
48. J.-S. Lee, M. S. Han and C. A. Mirkin, *Angew. Chem. Int. Ed.*, 2007, 46, 4093-4096.
49. J. Liu and Y. Lu, *Chem. Commun.*, 2007, 46, 4872-4874.
50. T. S. Dalavoy, D. P. Wernette, M. Gong, J. V. Sweedler, Y. Lu, B. R. Flachsbart, M. A. Shannon, P. W. Bohn and D. M. Cropek, *Lab on a Chip*, 2008, 8, 786-793.
51. A. K. Brown, J. Liu, Y. He and Y. Lu, *ChemBioChem*, 2009, 10, 486-492.
52. W. Bal, M. Dyba, Z. Szewczuk, M. Jezowska-Bojczuk, J. Lukszo, G. Ramakrishna and K. S. Kasprzak, *Mol. Cell. Biochem.*, 2001, 222, 97-106.
53. N. Sewald, S. D. Wilking, R. Eckel, S. Albu, K. Wollschläger, K. Gaus, A. Becker, F. W. Bartels, R. Ros and D. Anselmetti, *J. Pept. Sci.*, 2006, 12, 836-842.
54. X. Lv, J. Wang, Z. Dong, F. Lv and Y. Qin, *Peptides*, 2009, 30, 681-688.
55. K. Fujimoto, M. Kajino, I. Sakaguchi and M. Inouye, *Chem. Eur. J.*, 2012, 18, 9834-9840.
56. H. J. Sung, B. Kayhan, T. Ben-Yedidia and R. Arnon, *J. Biol. Chem.*, 2004, 279, 48410-48419.
57. T. H. Koch, D. Smith, E. Tabacman and D. A. Zichi, *J. Mol. Biol.*, 2004, 336, 1159-1173.
58. M. N. Stojanovic, P. De Prada and D. W. Landry, *J. Am. Chem. Soc.*, 2000, 122, 11547-11548.
59. H. Saitoh, A. Nakamura, M. Kuwahara, H. Ozaki and H. Sawai, *Nucleic Acids Res Suppl*, 2002, 215-216.
60. S. H. Han and J. S. Lee, *Langmuir* 2012, 28, 828-832.
61. H. Ihmels and D. Otto, in *Supermolecular Dye Chemistry*, (Eds.: F. Würthner), Springer Berlin Heidelberg, 2005, vol. 258, pp. 161-204.
62. B. Armitage, in *Heterocyclic Polymethine Dyes*, ed. L. Strekowski, Springer Berlin Heidelberg, 2008, vol. 14, ch. 109, pp. 11-29.
63. Y. Liu, Y. Wang, J. Jin, H. Wang, R. Yang and W. Tan, *Chem. Commun.*, 2009, 665-667.
64. S. D. Aguirre, M. M. Ali, P. Kanda and Y. Li, *J. Vis. Exp.*, 2012, 63, e3961.
65. J. G. Bruno and J. L. Kiel, *Biosens. Bioelectron.*, 1999, 14, 457-464.

66. C. Wang, M. Zhang, G. Yang, D. Zhang, H. Ding, H. Wang, M. Fan, B. Shen and N. Shao, *J. Biotechnol.*, 2003, 102, 15-22.
67. M. W. J. H. U. Sverdrup, R. H. Fleming, *in the oceans : their Physics, Chemistry, and General Biology*, Prentice Hall Inc., 1942, vol. 1, pp. 229-247.
68. J. M. Kim, S. M. Chang, H. Muramatsu and K. Isao, *Korean J. Chem. Eng.*, 2011, 28, 987-1008.
69. H. Xu, Q. Li, L. Wang, Y. He, J. Shi, B. Tang and C. Fan, *Chem. Soc. Rev.*, 2014, 43, 2650-2661.
70. C. Sicard and J. D. Brennan, *MRS Bull.*, 2013, 38, 331-334.
71. C. Wang and C. Yu, *Rev. Anal. Chem*, 2013, 32, 1-14.
72. S. J. Hurst, A. K. R. Lytton-Jean and C. A. Mirkin, *Anal. Chem.*, 2006, 78, 8313-8318.
73. Q. Zhang and E. C. M. Chen, *Biochem. Biophys. Res. Commun.*, 1995, 217, 755-760.
74. L. Song, J. Gao, K. Han, X. Wang and J. He, *Toxicol. Environ. Chem.*, 2013, 95, 970-983.
75. H. Kimura-Suda, D. Y. Petrovykh, M. J. Tarlov and L. J. Whitman, *J. Am. Chem. Soc.*, 2003, 125, 9014-9015.
76. J. J. Storhoff, R. Elghanian, C. A. Mirkin and R. L. Letsinger, *Langmuir*, 2002, 18, 6666-6670.
77. L. M. Demers, M. Östblom, H. Zhang, N. H. Jang, B. Liedberg and C. A. Mirkin, *J. Am. Chem. Soc.*, 2002, 124, 11248-11249.
78. M. Östblom, B. Liedberg, L. M. Demers and C. A. Mirkin, *J. Phys. Chem. B*, 2005, 109, 15150-15160.
79. Z. Wang, L. Tang, L. H. Tan, J. Li and Y. Lu, *Angew. Chem. Int. Ed.*, 2012, 51, 9078-9082.
80. D. Y. Petrovykh, V. Perez-Dieste, A. Opdahl, H. Kimura-Suda, J. M. Sullivan, M. J. Tarlov, F. J. Himpsel and L. J. Whitman, *J. Am. Chem. Soc.*, 2006, 128, 2-3.
81. D. Y. Petrovykh, H. Kimura-Suda, L. J. Whitman and M. J. Tarlov, *J. Am. Chem. Soc.*, 2003, 125, 5219-5226.
82. L. Yang, X. Zhang, M. Ye, J. Jiang, R. Yang, T. Fu, Y. Chen, K. Wang, C. Liu and W. Tan, *Adv. Drug Del. Rev.*, 2011, 63, 1361-1370.
83. Y. Nakamura, *Adv. Polym. Sci.,* 2012, vol. 249, pp. 135-152.
84. J. Liu and Y. Lu, *Nat. Protoc.*, 2006, 1, 246-252.
85. F. Li, J. Zhang, X. Cao, L. Wang, D. Li, S. Song, B. Ye and C. Fan, *Analyst*, 2009, 134, 1355-1360.
86. N. K. Navani, W. K. Mok and L. Yingfu, *Methods Mol. Biol.*, 2009, 504, 399-415.
87. H. L. Jung, Z. Wang, J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2008, 130, 14217-14226.

Table of content

AuNP-DNA complex highly stable in extremely high ionic strength media, such as seawater, was obtained by inserting a few thymine bases into the DNA strands.





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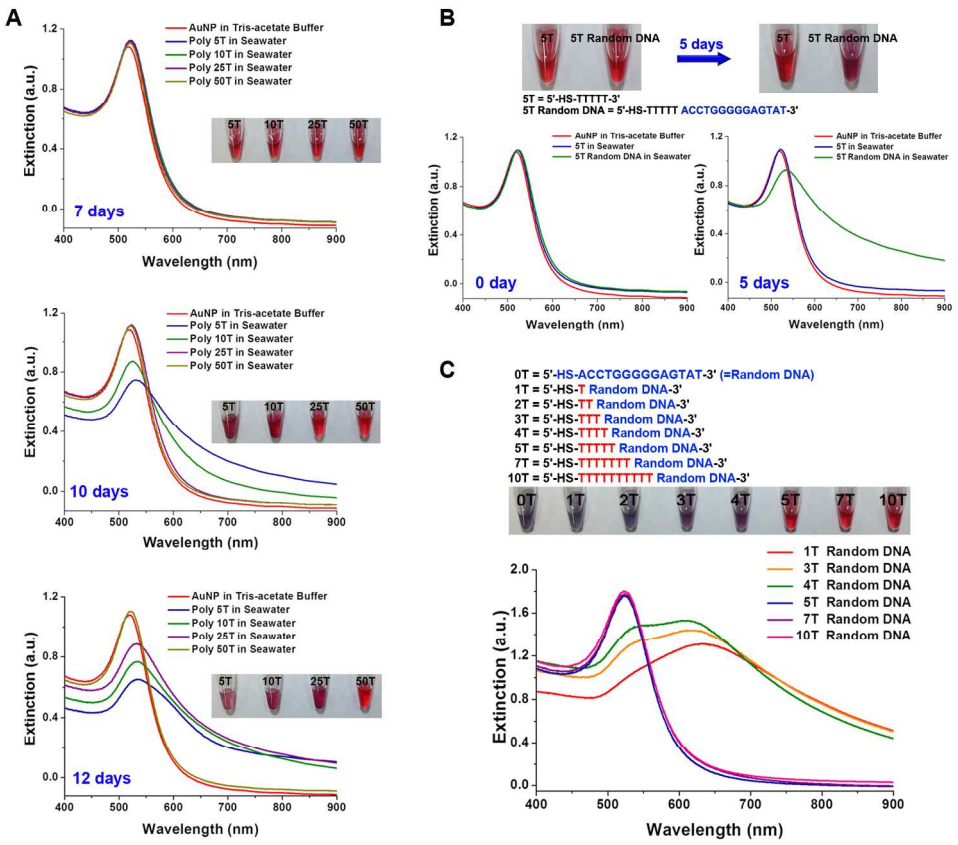

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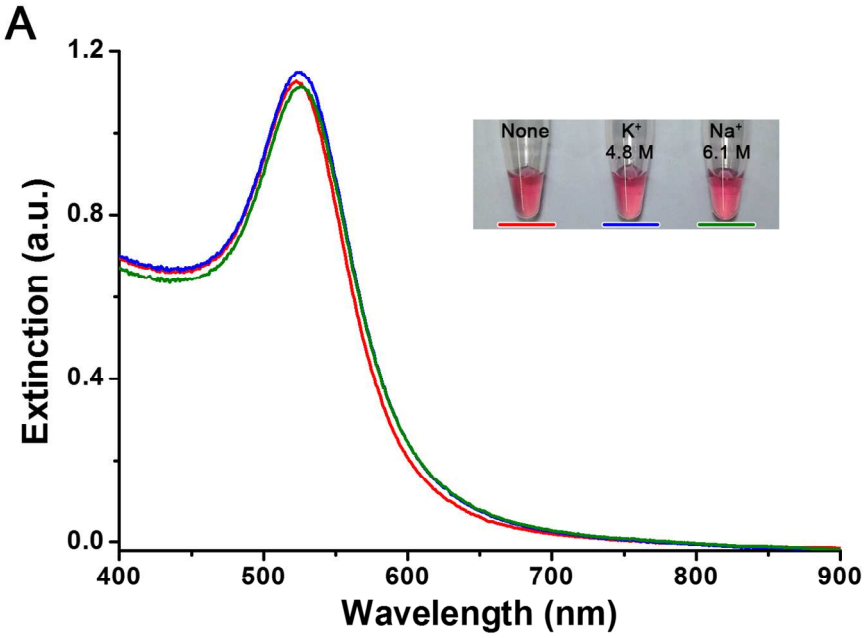
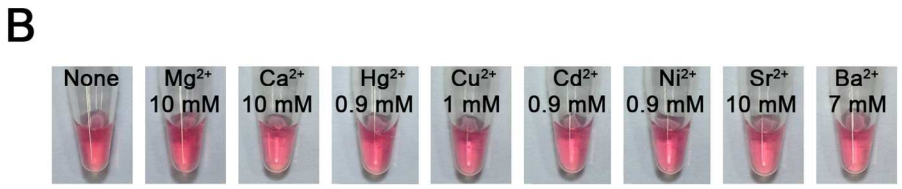


* Concentration of both ions is maximum solubility of water at RT(25℃)



* None = AuNPs with 25mM Tris buffer solution at RT(25℃)

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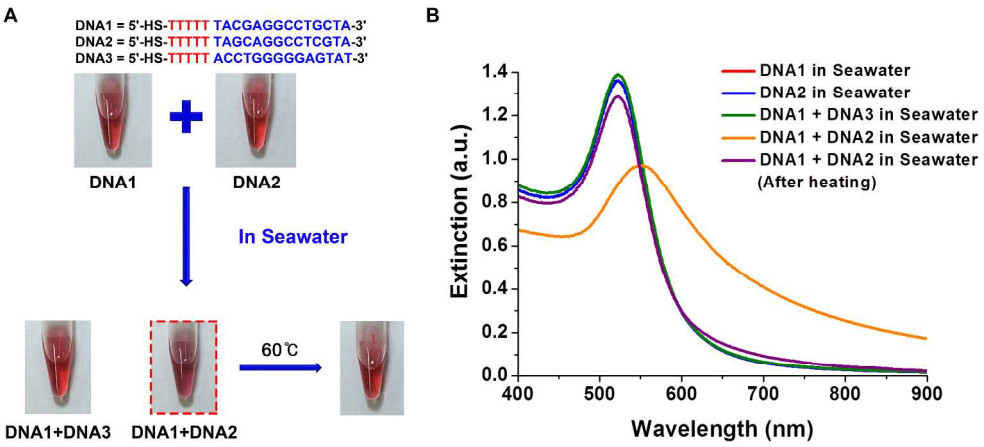

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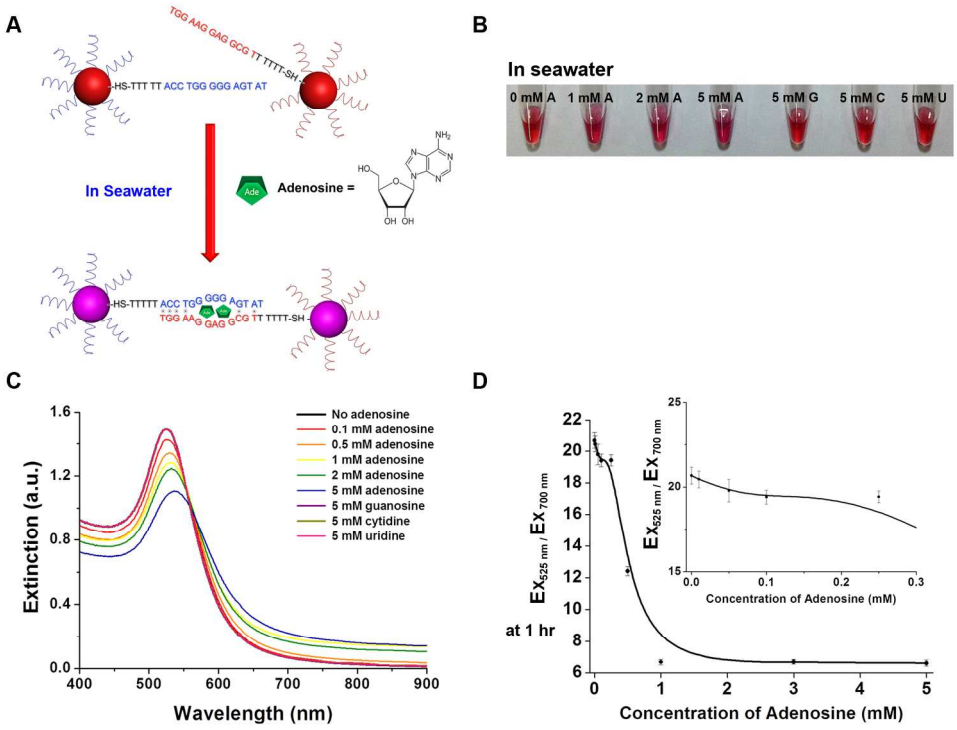

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