

Supporting Information

Exploring the thermal stability of DNA-linked gold nanoparticles in ionic liquids and molecular solvents

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1. Materials and Methods.

Chemicals. All the IL samples were purchased from Ionic Liquids Technologies (Tuscaloosa, AL). All the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA). Two different thiolated DNA were used to respectively functionalize AuNPs as shown in Figure S1A. The linker is a 24-mer DNA. There is a nine-adenine spacer to separate the hybridization sequence from each AuNP surface. Two different fluorescent probes were used; both contained the same DNA sequences but one with a pH sensitive FAM label (Figure S1B) and the other with a pH insensitive Alexa Fluor 488 label (Figure S1C). HAuCl_4 was purchased from Sigma-Aldrich. Hydrochloric acid was purchased from VWR (Mississauga, ON). Sodium citrate, sodium chloride and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonate (HEPES) were purchased from Mandel Scientific (Guelph, ON). Milli-Q water was used for all experiments.

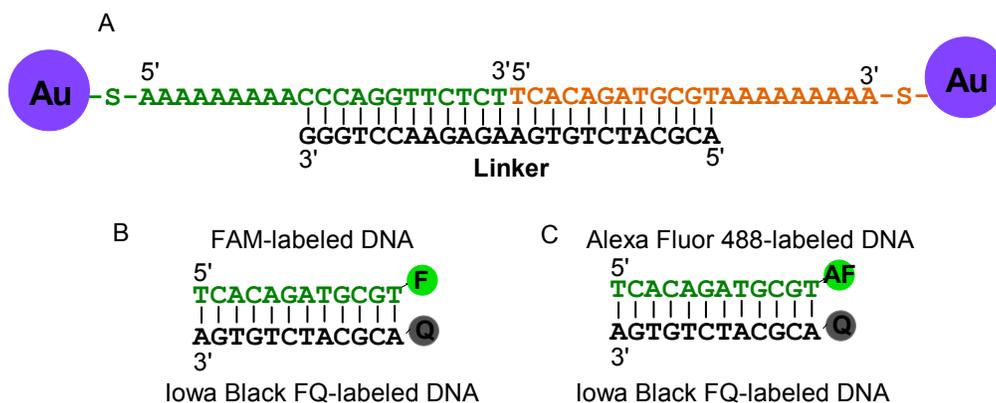


Figure S1. DNA sequences, modifications and their linkages to AuNPs. In the Figure, “F” denotes for FAM, “Q” denotes for Iowa Black FQ, and “AF” denotes for Alexa Fluor 488.

AuNP preparation. Citrate capped 13 nm AuNPs were prepared using the standard citrate reduction reaction using 1 mM HAuCl_4 and a final of 3.88 mM trisodium citrate.¹ The detailed procedures will not be repeated here. The average hydrodynamic diameter was determined to be 14.5 nm using dynamic light scattering acquired with Malvern Zetasizer Nano ZS90 (Figure S2).

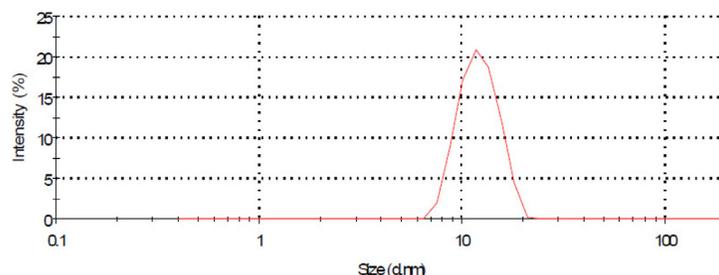


Figure S2. AuNP size characterization using dynamic light scattering.

DNA attachment and AuNP assembly. Thiolated DNAs were attached to AuNPs following literature reported procedures with slight modification.^{1,2} Glass scintillation vials (20 mL volume) were soaked in 12 M NaOH for ~ 1 hr followed by rinsing with an ample amount of water. In a typical preparation, 3

mL AuNPs were transferred to each vial and 3 μM thiolated DNA was added. The DNAs were activated by 4:1 TCEP in pH 5 acetate buffer for 1 hr before mixing with AuNPs. After 2 hr incubation, the solution was brought to 50 mM NaCl by drop-wise adding 1 M NaCl. 4 hr later, an additional 50 mM NaCl was added. After overnight incubation, another dose of 50 mM NaCl was added to reach a final of 150 mM NaCl. The AuNPs were ready for use after incubating for eight more hours. This salt aging procedure is intended to reach a high DNA density and increase AuNP stability.³ The 3'-thiol DNA capped AuNPs precipitated out of solution after 2 days because of the non-specific aggregation, showing the presence of a high DNA density.⁴

To prepare DNA-linked AuNP aggregates, 600 μL of each DNA-functionalized AuNPs was centrifuged at 15000 rpm for 20 min. After removing the supernatant, the AuNPs were dispersed in 530 μL water, allowing aggregated AuNPs to disassemble. Then 10 μL of 500 mM HEPES (pH 7.6) and 60 μL of 3 M NaCl were added to reach a final NaCl concentration of 300 mM. To form aggregates, 300 nM linker DNA was added and the color of the AuNPs changed to purple in ~ 20 min. The aggregates were allowed to incubate at room temperature overnight before use.

Colorimetric tests. For the picture in Figure 2A, 40 μL citrate capped AuNPs were mixed with 10 μL of ILs (100% for PN and EN, 50% (w/w) for the rest three). The picture was taken by a digital camera (Canon PowerShot IS1200) right after the mixing. For the picture in Figure 2B, 49 μL ILs (100% for PN and EN, 50% for the rest three) were mixed with 1 μL concentrated AuNPs. The concentrated AuNPs were prepared by centrifuging 500 μL of each of the two types of DNA-functionalized AuNPs at 15000 rpm for 20 min followed by removing most of the supernatant. The AuNP pellets were combined and the total volume was ~ 20 μL (concentrated by $\sim 50\times$). To each IL sample, 1 μL of such AuNPs were added and mixed. The picture was taken ~ 20 min after mixing.

For the picture in Figure 2C-G, the AuNP aggregates (prepared from 600 μL of each AuNP) were centrifuged and re-dispersed in 150 μL of buffer (500 mM NaCl, 200 mM HEPES, pH 7.6). The 45 μL of IL solutions were prepared by mixing water with IL stock solutions (100% for PN and EN; 75% for DN, MN and CP) such that after adding the 5 μL AuNP aggregates, the designated IL concentration can be reached. For example, to prepare the 50% CP sample, 33.3 μL of 75% CP, 11.7 μL water and 5 μL AuNP aggregates were mixed. The final NaCl concentration was 50 mM and HEPES was 20 mM. A photo was taken at room temperature and the samples were then incubated using a dry bath at 50 $^{\circ}\text{C}$. After 2 min, the tubes were taken out and a photo was taken. Following this, the tubes were incubated in a water bath at ~ 95 $^{\circ}\text{C}$ for ~ 1 min before the final picture was taken. For the picture in Figure 4, the procedures were about the same but one more temperature was tested.

Melting curves using UV-vis. For each melting sample, two solutions (each 100 μL) were mixed. One contained AuNP aggregates dispersed in 40 mM NaCl and 40 mM HEPES, pH 7.6 and the other solution was a 2 \times IL solution. This gives a final NaCl of 20 mM and HEPES of 20 mM with a total volume of 200 μL . The final AuNP concentration is ~ 5 nM for all the samples. This preparation method works for samples containing up to 50% IL. For even higher IL contents, the samples were prepared by mixing a smaller volume of AuNPs into concentrated ILs while still maintaining the same final NaCl and HEPES concentrations. The samples were then transferred into quartz microcuvettes with a pathlength of 1 cm. After sealing the cuvettes to minimize evaporation, the extinction spectra were monitored as a function of temperature using a UV-vis spectrophotometer (Agilent 8453A) equipped with an 8-cell sample holder. The temperature was controlled using a circulating waterbath and the actual temperature for the samples was measured using a digital thermometer. Samples with T_m

between 20 and 53 °C were measured using this method. For samples that melted at lower or higher temperatures, visual observation of AuNP color change was used. This was carried out to avoid water condensation at low temperature and water leakage at too high temperatures. The temperature was increased every ~ 1 °C with a holding time of 2 min. Each condition was run at least in duplicates. For very low T_m samples, an ice/water mixture was prepared in a 500 mL beaker and the samples were placed in a microcentrifuge tube and incubated in the beaker. A magnetic stir bar was placed in the beaker to achieve even temperature distribution. Overtime the temperature in the beaker gradually increased and the temperature where a red color was observed was recorded as the melting temperature. For high T_m samples, a water bath was heated with a temperature increase rate of ~ 1 °C/min and the same visual observation method was used.

To allow an effective comparison of T_m , the melting curves have been normalized by treating every extinction value (A) by a function of $A'=aA+b$ so that the lowest extinction is 0.6 and the highest is 1.2. This linear normalization treatment does not change the T_m value.

Melting curves using fluorescence. A fluorescently labelled and a quencher labelled DNA were first hybridized in buffer (5 μ M fluorophore-labeled DNA, 6 μ M quencher labeled DNA, 200 mM NaCl, 200 mM HEPES, pH 7.6). 5 μ L of this DNA solution was added to 45 μ L solvent solution to achieve a final fluorescent DNA concentration of 500 nM, NaCl = 20 mM and HEPES = 20 mM. 15 μ L of the sample was transferred into a PCR plate and the plate was sealed. The melting curves were collected using a real time PCR thermocycler (CFX96, Bio-Rad) using heating lid at 103 °C. The temperature was increased from 4 to 95 °C with 1 °C increment. The fluorescence in the FAM channel was read at each temperature after a holding time of 20 sec. This measurement was performed in triplicates.

2. The pH artifacts of CP.

With a choline cation and a dihydrogen phosphate anion, CP is considered to be highly biocompatible and is often used to dissolve proteins and nucleic acids. Unlike the other ILs used in this work, the H_2PO_4^- group could still deprotonate and thus change the solution pH. This effect has not been addressed in the literature. Since DNA stability could also be affected by changes in pH, we performed careful studies and have identified several potential artifacts.

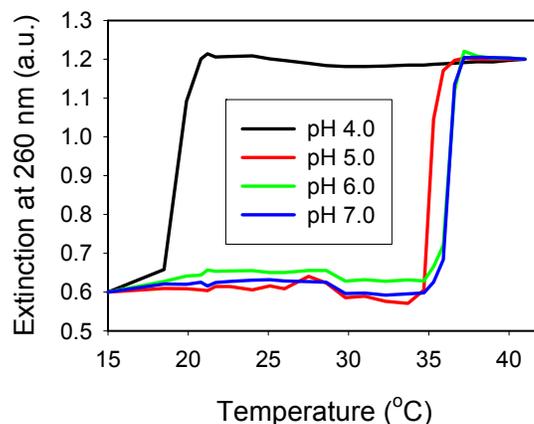


Figure S3. DNA melting curves measured with DNA-linked AuNPs as a function of pH. The buffer contained 10 mM sodium citrate at various pH values adjusted with HCl and 10 mM NaCl.

T_m as a function of pH. We measured the T_m of DNA-linked AuNP aggregates in sodium citrate buffers with pH values ranging from 4.0 to 7.0. The buffers contained 10 mM citrate and 10 mM NaCl and the melting curves are shown in Figure S3. While the melting of DNA occurred at the same temperature for pH 6 and pH 7, a slight drop (~ 1 °C) was observed at pH 5 and a significant drop (~ 16 °C) occurred at pH 4. Therefore, if pH can be controlled to be higher than 5.0, its effect on DNA melting should be minimal. However, below pH 5, care needs to be taken for quantitative comparison of T_m values. This could be related to the protonation of DNA bases. For example, the cytidine base has a pK_a value of 4.2.

Colorimetric assay in CP without buffer. In the absence of a buffer, a low concentration of CP (e.g. 2-5%) produced a red color but high concentration of CP stabilized (Figure S4). This is a pH artefact since with 20 mM HEPES, all the samples showed a purple color at room temperature (Figure 2G of the main paper).

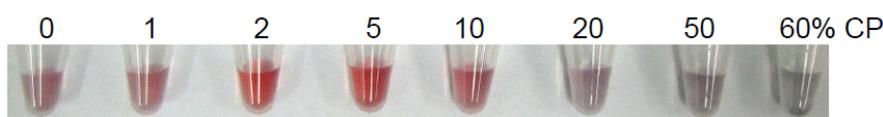


Figure S4. The color of DNA-linked AuNP aggregates in various concentrations of CP without a buffer. Red color indicates melted DNA.

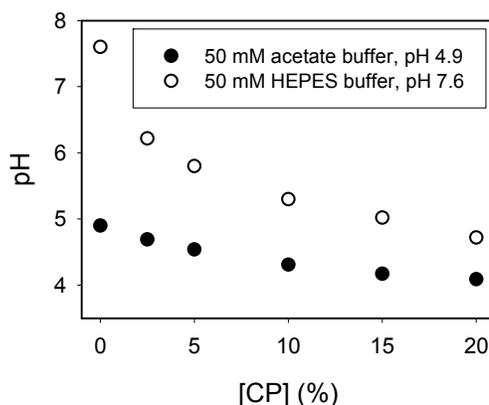


Figure S5. Solution pH as a function of CP concentration.

pH measurement with CP. Using a pH meter, we have measured the solution pH as a function of CP concentration. If water is used, 2.5% CP dropped the pH to 3.7. Interestingly with 50% CP, pH =4.5. Therefore, CP suppressed its own ionization. This also explained the data in Figure S4 that melting was observed only up to 20% CP. If 50 mM HEPES, pH 7.6 was used as a buffer, a large pH drop was still observed (Figure S5, open circles). At 20% CP, the pH dropped to below 5, or ~ 3 pH units drop from the starting pH. If a lower pH buffer (e.g. pH 4.9 acetate buffer) was used, the drop was only ~ 1 unit at 20% CP, but the absolute value was close to 4.0. As shown above, DNA T_m is affected strongly by pH when pH is below 5. Therefore, artifacts associated with pH change for CP needs to be carefully considered.

Fluorescence-based melting curves. Using the FAM-labeled fluorescent probe (Figure S1B), we measured the melting curves in the presence of CP. As shown in Figure S6A, in the absence of CP, the background fluorescence was at ~ 40 , which increased to ~ 100 upon melting. Note that the background of the instrument was at ~ 20 (e.g. reading of an empty plate). Therefore, the fluorescence increased ~ 4 -fold upon melting for this probe. Even though 20 mM HEPES buffer was added, addition of just 1% CP has significantly dropped the fluorescence. This fluorescence drop is an indication of pH change. Note that 1% CP has a concentration of ~ 50 mM, higher than the 20 mM buffer concentration. At high CP concentration of 50% and 60%, the background increased again, consistent with our previous observation that CP was able to suppress its own ionization at high concentration. Nevertheless, T_m values can still be extracted from each melting curves as plotted in Figure S6B, which clearly showed a trend of T_m increase followed by decrease.

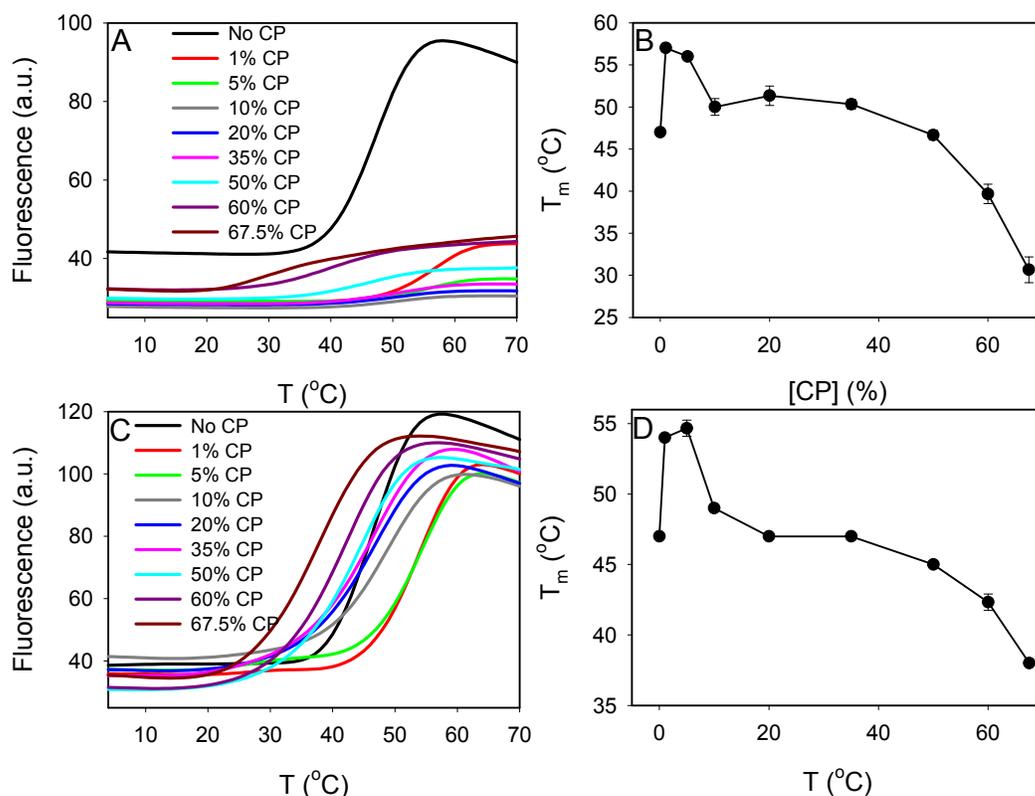


Figure S6. Fluorescence based melting curves (A) and T_m as a function of CP concentration (B) for the DNA probe in Figure S1B. Fluorescence based melting curves (C) and T_m as a function of CP concentration (D) for the DNA probe in Figure S1C.

To confirm our observation, we next employed a pH insensitive Alexa Fluor 488 fluorophore and repeated the experiment. As shown in Figure S6C, the heights of all the melting curves are comparable, regardless of CP concentration, which indicates the large fluorescence intensity variation in Figure S6A was indeed due to pH affecting FAM intensity. The change of T_m as a function of CP concentration was not affected by the use of different probes by comparing Figure S6B with Figure S6D. In this fluorescence-based assay, concentrated CP destabilized the DNA, but in Figure 2G of the paper, 60%

CP appeared to caused AuNP stabilization. To further understand this, we collected AuNP aggregates disperse in 60% CP after heating to 95 °C, removed the solvent, and re-dispersed the AuNP aggregates in water. Upon heating, no color change was observed, suggesting that the AuNPs were irreversibly aggregated. In other words, the low pH of concentrated CP might have damaged the DNA-AuNP conjugate.

Colorimetric assay using choline chloride. Although choline chloride has a melting temperature of 302 °C and thus does not qualify under the definition of IL, it was also tested for comparison. Since chloride cannot release protons, there should be no pH artifact. In this case, the 67.5% sample at 50 °C showed red color (Figure S7), indicating the presence of destabilizing effect that was not observed with 67.5% CP for the colorimetric assay (Figure 2G of the main paper). This led us to believe that the trend in Figure 2G to be a pH related artifact, where no destabilization effect was observed.

When CP is used as a solvent, its concentration is so high that no buffer can be effective in controlling the pH. For this reason and supported by the examples in this work, care needs to be taken when performing quantitative analysis in the presence of CP.

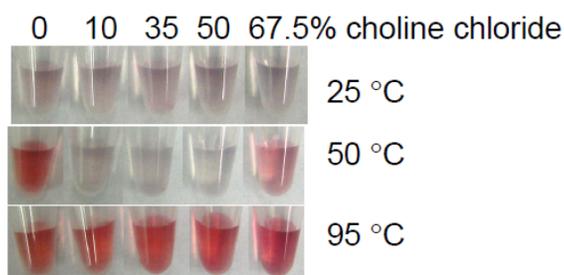


Figure S7. Colorimetric assay in the presence of choline chloride. The conditions are the same as listed in Figure 2 of the paper.

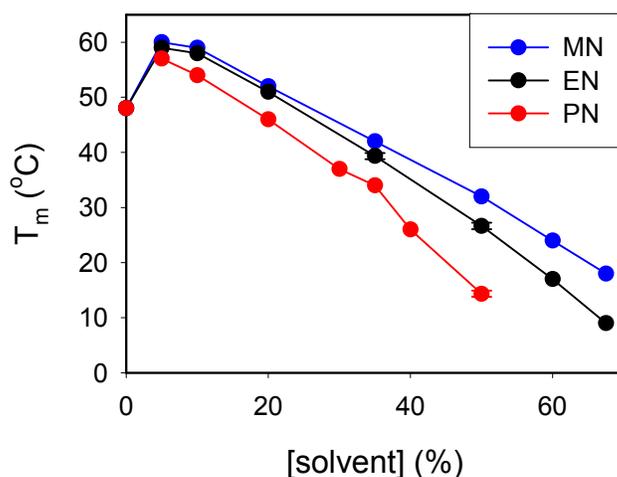


Figure S8. DNA T_m as a function of IL concentration measured using the DNA probe in Figure S1B.

3. T_m of other ILs

In the paper we presented the T_m values of PN. In Figure S8, the DNA T_m values of two other ILs as a function of their concentrations are presented. MN is the least hydrophobic among the three and shows the highest T_m while the most hydrophobic PN has the lowest T_m 's. This indicates that PN has the largest effect in destabilizing DNA duplex.

4. pH change caused by EPB and MIN.

To study the effect of pH change caused by EPB and MIN, these two ILs were added into 2 mL of water samples containing 20 mM HEPES, pH 7.6. The pH was measured using a pH meter. The change of pH is plotted in Figure S9, where EPB does not cause pH change but MIN induced significant pH drop.

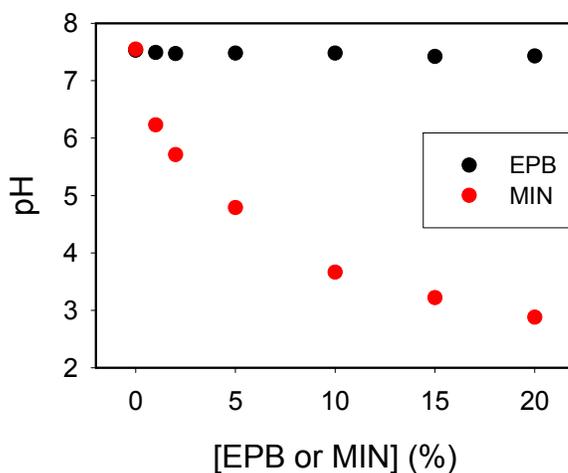


Figure S9. pH change caused by EPB and MIN ILs. The buffer contained 20 mM HEPES, pH 7.6.

5. DNA melting in molecular solvents.

We noticed that the color of DNA-functionalized AuNPs (without linker DNA) immediately turned blue in molecular solvents (Figure S10A). Such AuNP aggregation was reversible since if the solvents were removed after centrifugation, the color went back to red in aqueous buffer. The aggregation of AuNPs reflected the non-specific interaction of DNA. Due to the presence of such non-specific interactions, a question arises as to whether the DNA linkages in DNA-linked AuNPs were still maintained in this case. To test this, we mixed AuNP aggregates with 80% of the various molecular solvents and then the samples were spun down. After removing the solvent, buffer was added and a blue color was observed for all the samples except for the one incubated with DMSO (Figure S10B). Heating the samples to 70 °C produced red color for all the samples, indicating that DNA-linkages were maintained. Therefore, high concentration of DMSO appeared to wash away the linker DNA but the other three solvents maintained the specific DNA linkages.

Since high concentration of DMSO disrupted the DNA linkages, we collected the AuNP aggregates in 80% from the other three solvents and washed with buffer. Finally the melting curves were measured in the normal aqueous buffer (20 mM NaCl, 20 mM HEPES, pH 7.6) and the results are shown in Figure S10. Sharp melting transitions were observed for all the samples at the temperature similar to the

sample never exposed to organic solvents. Therefore, we conclude that when dispersed in 80% ethanol, ACN, or DMF, the DNA linkages have been maintained. Although non-specific interactions (e.g. the interactions that make AuNPs aggregate in Figure S9A) are likely to occur, after re-dispersed in aqueous buffer, these non-specific interactions should disappear.

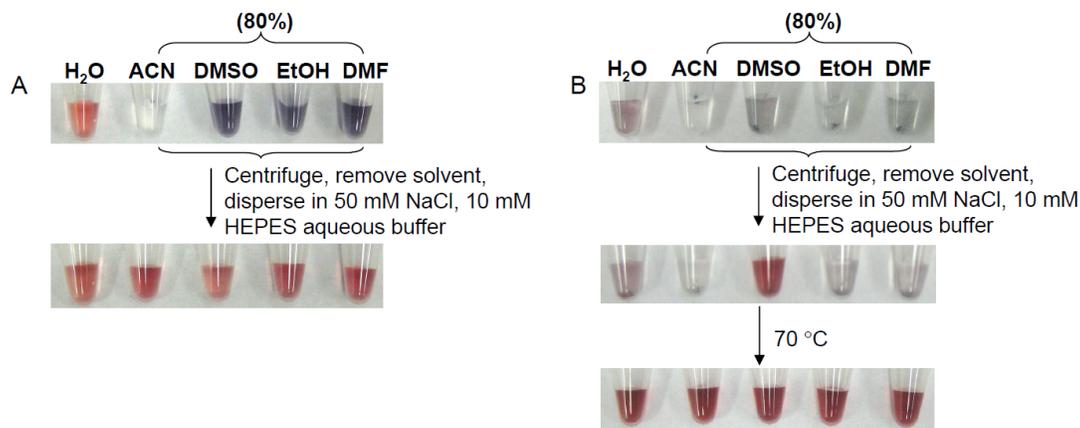


Figure S10. (A) Studying non-specific DNA-functionalized AuNPs aggregation (no linker DNA) in various molecular solvents. 80% ACN produced a close to clear color due to formation of very large aggregates. The other three solvents all induced non-specific AuNP aggregation. After centrifugation, removing solvents and adding aqueous buffer, red color was achieved for all the samples, indicating that solvent induced aggregation was reversible. (B) Effect of solvents on DNA-linked AuNP aggregates. After removing the solvents, only the DMSO soaked sample showed red color, indicating that the linker DNA might have been dissociated from the aggregates. The other samples remained aggregated after removing the solvents and re-dispersing in aqueous buffer. Heating to 70 °C produced red color for all the samples, indicating that the aggregates were assembled by the linker DNA.

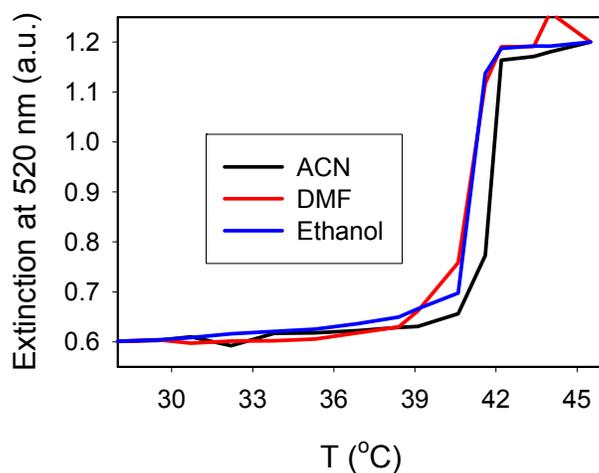


Figure S11. Melting curves of the samples from Figure S10B. A further wash was performed and the aggregates were dispersed in 20 mM NaCl and 20 mM HEPES, pH 7.6 for melting curve measurement. The extinction values at 520 nm were normalized for comparison of the melting transitions.

6. Melting curves of the fluorescent probe in DMSO.

Some of the melting curves for the DMSO data shown in Figure 3F of the paper are given in Figure S12. Normal melting curves were obtained with up to 50% DMSO. Further increase of DMSO resulted in no DNA dissociation, consistent with the colorimetric assay results. The initial fluorescence values for the high DMSO samples were low, indicating that the fluorophore and quencher were close to each other, although the duplex structure might be disrupted (e.g. DNA formed non-specific complexes/aggregates).

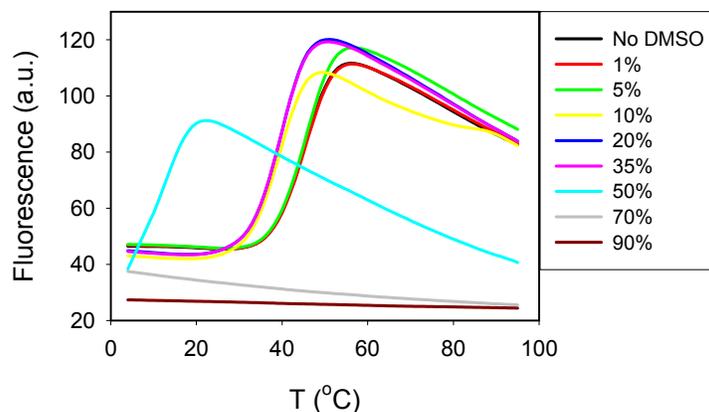


Figure S12. Melting curves of DNA in Figure S1B in various concentrations of DMSO.

7. Absorption spectra of sodium nitrate solution.

The absorption peak at 300 nm for the alkylammonium nitrate ILs was from the nitrate. For example, the spectrum in Figure S12 is from 200 mM NaNO₃ (water as blank, no buffer). With a > 10% such ILs, the nitrate can make the 260 nm absorption greater than 4, making it difficult to accurately measure the DNA melting based on 260 nm extinction.

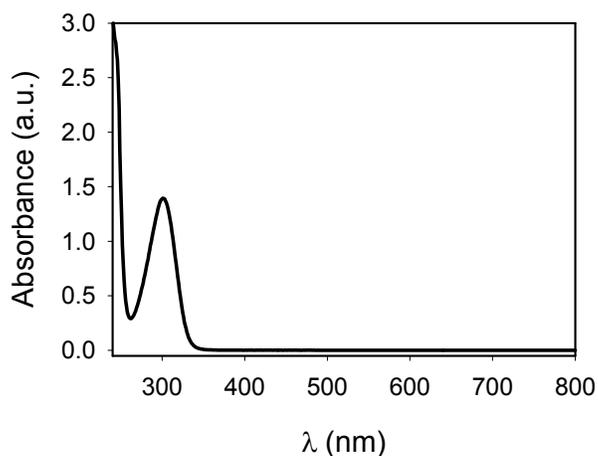


Figure S13. Absorption spectrum of 200 mM NaNO₃ (blanked with water).

Additional references.

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