

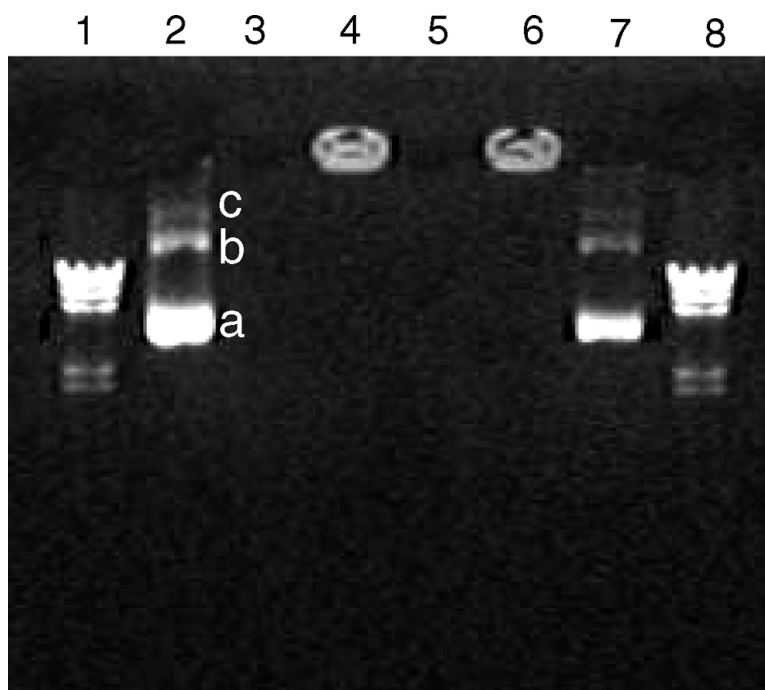
Communication

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Bioconjugated Nanoparticles for DNA Protection from Cleavage

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We have developed a novel method to protect DNA from cleavage based on bioconjugated nanoparticles. Genetic engineering has brought new challenges and opportunities for medicine and biomedical research and development.¹ However, genetic engineering technology is limited in DNA manipulations because DNA strands would be cleaved in cellular environments.^{2–4} Although a few measures have been taken to protect the DNA from cleavage, such as adding inhibitors in DNA solutions and entrapping DNA in liposome,^{5,6} these methods may hinder further manipulation of DNA. Nanomaterials with unique properties such as large surface area, pore structure, embedded effect, and size effect have been used effectively in bioanalysis and drug delivery.^{7–9} It is thus expected that they might shield the embedded DNA sequences due to their large surface and pore structures. In this Communication, we report the application of amino-modified silica nanoparticles to protect DNA from cleavage.

Amino-modified silica nanoparticles (NPs) (45 ± 4 nm, shown in Figure 1) are directly synthesized by using the synchronous hydrolysis of tetraethoxysilane and *N*-(β -aminoethyl)- γ -aminopropyltriethoxysilane in water-in-oil microemulsion. Briefly, a well-distributed mixture of the two silanes (with volume ratio 1:1) is added to the microemulsion containing cyclohexane, Triton X-100, and *n*-hexanol (with volume ratio 4:1:1) and synchronously hydrolyzed. Zeta potential measurements have shown that the separated amino-modified silica NPs possess a positive charge of nearly +30 mV at neutral pH, due to the protonation of the amino group on the NPs. This can serve as the foundation for an effective enrichment of negatively charged DNA strands onto the positively charged amino-modified silica NP surfaces. In this work, green fluorescence protein (GFP) plasmid DNA was selected as a model DNA. Agarose gel electrophoresis also demonstrated that the amino-modified silica NPs could bind with DNA to form DNA–NP complexes. As shown in Figure 2, lanes 2 and 4, plasmid DNA moved in the electric field, and amino-modified silica nanoparticles–plasmid DNA complexes were retained around the sample well. The reason the DNA–NP complexes did not move toward the positive electrode lies in the charge and the large size of the complexes. In a control experiment, pure silica NPs with negative zeta potential at neutral pH could not enrich plasmid DNA. As shown in lane 5, there was no DNA in the lane after the incubation of the pure silica NPs with plasmid DNA. This clearly shows that it is necessary to have amino-modified NPs for DNA binding.

To investigate whether the NPs are sufficient and efficient in providing protection against enzymatic cleavage of DNA, 2 U of DNaseI was added respectively to the plasmid DNA and the plasmid DNA–NP complexes, containing 3.6 μ g of DNA, and incubated for 1 h at 37 °C. As shown in Figure 2, lanes 3 and 6, free plasmid DNA was degraded completely, while plasmid DNA–NP complexes were seen around the sample pore after the same digestion process, and the intensity is quite similar to that of the plasmid

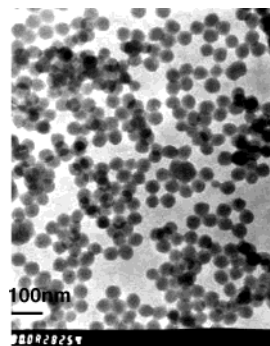


Figure 1. TEM image of the amino-modified silica nanoparticles (45 ± 4 nm).

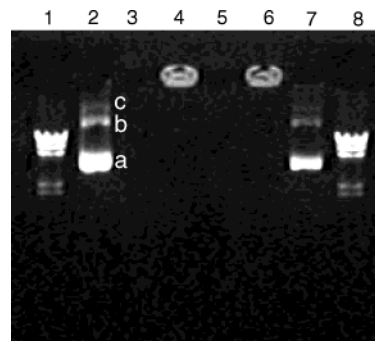


Figure 2. Agarose gel electrophoresis of plasmid DNA and DNA–NP complexes. Lanes 1 and 8 are DNA marker (λ DNA is cleaved by *Hind*III); lane 2 is undigested free plasmid DNA (a, superhelix DNA, b, linear DNA, and c, open circular DNA are the three forms of the plasmid DNA); lane 3 is digested free plasmid DNA; lane 4 is plasmid DNA–NP complexes; lane 5 is pure silica NP after incubation with plasmid DNA; lane 6 is plasmid DNA–NP complexes digested with DNaseI; and lane 7 is the DNA released from the DNA–NP complexes that have been digested with DNaseI.

DNA–NP complexes without digestion of DNaseI (Figure 2, lane 4). To inquire whether the properties of the plasmid DNA in the DNA–NP complexes were changed after digestion with DNaseI, the complexes were treated with 2 mol/L NaCl to release the plasmid DNA from the NPs. It was clear that the DNA released from the DNA–NP complexes gave the same three bands (lanes 2 and 7) as those from the free plasmid DNA. The relative intensities of the bands are different for lanes 2 and 7, and the possible reason is that not all the plasmid DNA is released from the NPs. These results have shown that DNA–NP complex formation was, indeed, efficient to provide protection against enzymatic cleavage.

To further prove the integrity of the GFP plasmid DNA that was protected from the enzymatic digestion, we tested the function of the plasmid DNA in a cellular environment. If GFP plasmid DNA is delivered into cells and is still functional, GFP will be synthesized in the cells through the expression of GFP gene and can then be imaged in real time with fluorescence microscopy.

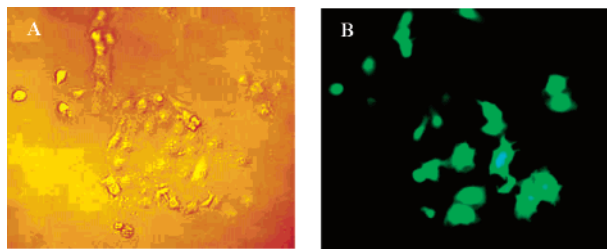


Figure 3. Expression of GFP plasmid DNA in COS-7 cells. (A) Optical image of the cells; (B) fluorescence image.

COS-7 cells were selected as the receptor cells. The plasmid DNA–NP complexes were prepared with a mass ratio of 20:1 (NP vs DNA, containing 1 μg of plasmid DNA) and then incubated with DNaseI for 1 h at 37 °C. The DNA–NP complex was then introduced into COS-7 cells as reported previously.¹⁰ As shown in Figure 3, the green fluorescence was obviously seen for most of the cells in the dish. Control experiments with NP alone or plasmid DNA digested with DNaseI were carried out under the same conditions, and no green fluorescence was observed. From all these experiments, we can conclude, first, that the DNA–NP is protected from DNaseI cleavage and no fragmentation of the plasmid DNA takes place, and second, that the plasmid DNA on the nanoparticle is still functional in a cellular environment, even after being incubated with DNaseI for 1 h.

There might be a few possible reasons for the DNA protection based on amino-modified NP. The first is that the positive charge on the amine group of the nanoparticles keeps Mg^{2+} away from the positively charged NP. This will limit the enzymatic cleavage, as Mg^{2+} is needed for this reaction. The second is that DNA surface binding with the NP results in a variation of the DNA structure due to the size effect and the embedding of DNA onto the NPs. The conformational change will protect DNA from cleavage. To determine the possible reasons for the stability against enzymatic cleavage, a controlled amino-modified glass slide was used to investigate the enzymatic degradation of plasmid DNA. The glass slide was first modified with aminosilanes as previously reported.¹¹ Plasmid DNA was adsorbed onto the amino-modified silica glass. DNaseI was then added to the glass slide for incubation with the DNA for 1 h at 37 °C. The plasmid DNA was degraded completely and could not be seen under fluorescence microscopy when it was dyed with 4',6-diamidino-2-phenylindole (DAPI), while the plasmid DNA without digestion with DNaseI gave bright blue fluorescence on the same type of glass slide (shown in Figure 4). This result illustrates that the positive charge on the amine group of the NPs alone was not the major factor for the protection of DNA from

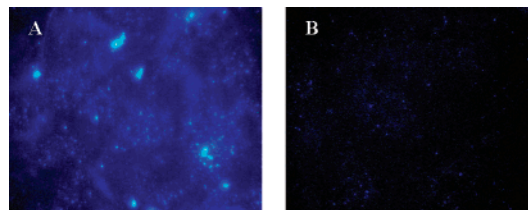


Figure 4. Fluorescence images of the plasmid DNA immobilized on glass slides. (A) DNA immobilized on a glass slide dyed with DAPI. (B) DNA immobilized on a glass slide dyed with DAPI after digestion with DNaseI.

cleavage in the DNA–NP complex. We believe that the smallness of the NPs may force the DNA to become bound in such a way that cleavage is either impossible or at least greatly slowed on the NP surface.

In summary, we have developed a simple method to protect DNA from cleavage by using bioconjugated amino-modified silica nanoparticles. Our results clearly show that DNA could be easily enriched onto the positively charged surface of the nanoparticles for further manipulation. The DNA strands are protected from enzymatic cleavage and have the same properties as free DNA strands when released from the nanoparticles. The released plasmids are biologically active. This bionanotechnology is simple and efficient in protecting DNA strands. It will be highly useful in DNA separation, purification, manipulation, and detection, and possibly in genetic engineering and gene therapy.

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