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DNA Core@Inorganic Shell

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Abstract: A chemically well-defined Bio Core@Inorganic Shell nanohybrid, which consists of rationally designed DNA molecule core with a size of ~100 nm and spherical inorganic nanoshell with an overall thickness of ~10 nm reassembled with exfoliated layered metal hydroxide (MH nanosheets), is prepared. The DNA encapsulation and its release, due to the pH-dependent solubility of the MH nanoshell, plays a crucial role in maximizing the stability of base sequence-manipulated and probe-functionalized DNA molecules with designed information. The present DNA Core@MH Shell nanohybrid can provide wide bioinspired applications converged with nanotechnology, such as an advanced gene delivery system and a biomedical diagnostics, tracing/collection/sensing system for DNA-based information.

Bioconjugated inorganic nanohybrids have been extensively explored in biorelated applications involving drug delivery systems, biomedical imaging, molecular diagnostics, biochemical sensing, and biocatalysts.[1,2] In these exploitations, many attempts were made to enhance the chemical and biological stability of fragile bioactive molecules by hybridizing them with functional inorganic materials; they were simply prepared by intercalating biomolecules into layered or porous inorganic frameworks or by immobilizing them onto a surface of inorganic nanoparticles. It has been, however, well-known that such conventional routes could not result in a high level of encapsulation and the uniformly controlled nanosize of bio-inorganic hybrids. In addition, thus prepared hybrids might not be fully protected from harsh chemical and biological conditions due to the unencapsulated or surface-bound disclosed parts of biomolecules. In this communication, we describe a chemically well-defined "Bio Core@Inorganic Shell" nanohybrid, with rationally designed DNA in the core encapsulated by an inorganic lattice-engineered nanoshell. Furthermore, the thus prepared DNA@Inorganic nanohybrid is applied to the "Nanoforensics" system as a newly adopted integrative concept combining nanochemistry and DNA-based molecular sensing.

To prepare this Core@Shell nanohybrid, we utilized base sequence-manipulated and fluoroprobe-functionalized DNA molecules, which can offer great potential for therapeutic and diagnostic applications in molecular nanotechnology,[3] and exfoliated layered metal hydroxide (MH nanosheets), which serve as secondary hosts or building blocks to prepare desired heterostructures in the presence of charge-balancing guest species.[4,5] Since the layered MH is well-known to undergo spontaneous exfoliation under appropriate conditions,[6] the negativley charged DNA molecules can be encapsulated into a positively charged inorganic nanocavity of self-assembled MH nanosheets, as illustrated in Figure 1a. The designed DNA solution (150 μM) dissolved in a mixed solvent of decarbonated water/formamide was dropped into a diluted colloidal suspension of MH nanosheets (125 μM) at ambient temperature with gentle shaking under a N_2 atmosphere. The thus prepared nanohybrid was then immediately freeze-dried.

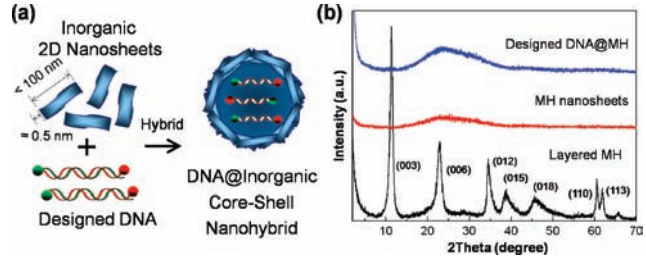

Figure 1. (a) Scheme for the designed DNA@Inorganic Core−Shell nanohybrid and (b) XRD patterns of the layered MH, MH nanosheets and DNA@MH nanohybrid.

The layered MH with a chemical composition of $[Mg_{2.1}Al(OH)_6][NO_3]\cdot 1.6H_2O$ exhibited sharp (*00l*) X-ray reflections attributable to the corresponding layered structure, as expected. In contrast, the DNA@MH nanohybrid with a spherical structure, $[Mg_{1.9}Al(OH)_6][\text{Designed DNA}]_{2.4}\cdot 2.6H_2O$, showed the disappearance of (*00l*) peaks in the low region ($2\theta < 15°$) of Bragg's angle as shown in Figure 1b. Its broad XRD feature indicates that the present encapsulation route results in the amorphous core−shell nanohybrid, where the DNA molecules are stabilized in the nanocavity of the spherically reassembled MH nanosheets.

According to the scanning electron microscopy (SEM) and tapping-mode atomic force microscopy (AFM) images shown in Figure 2a and 2b, the morphology and topography of the DNA@MH nanohybrid are fairly uniform with a nanosized spherical structure. The particle size of the present nanohybrid is determined to be around 120 ± 20 nm in diameter, which is consistent with the size distribution data based on a dynamic light scattering (DLS) analysis. Though the present nanohybrid showed a spherical morphology, its cell dimension of a hexagonal unit ($a = 0.30$ nm) calculated by a spotty-ring selected area electron diffraction (SAED) pattern is in excellent agreement with the in-plane lattice parameter of the layered MH (Supporting Information). Referring to the cross section transmission electron microscopy (TEM) image in Figure 2c, we can see the nanoscale DNA Core@MH Shell heterostructure, namely, the designed DNA core with a size of 100 ± 20 nm in the inorganic nanoshell with a wall thickness of 10 nm. The spherical nature of the present DNA Core@MH Shell structure can be rationalized due to the fact that the lateral edges of the MH nanosheets curl up gradually around the spherical DNA molecules, similar to the transformation of 2D nanosheets into 1D nanotubes,[8] and in turn, the MH nanosheets would be scrolled into concentric multilayers due to the turbostratic reassembling of 10−20 MH nanosheets with water molecules. The formation of such a DNA@MH nanohybrid is not surprising since the negatively

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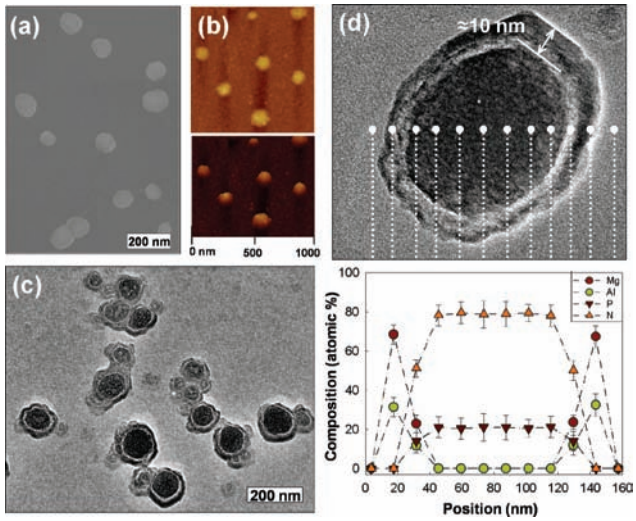

Figure 2. (a) SEM image, (b) AFM image, and (c) cross section TEM image of the DNA Core@MH Shell nanohybrid and (d) EDS linescan profile of the cross-sectional DNA Core@MH Shell nanohybrid at discrete steps.

charged DNA molecules ($\xi = -55.2$ mV) are likely to be encapsulated in the positively charged MH nanosheets ($\xi = +23.7$ mV) to form a charge-neutralized nanohybrid ($\xi = +11.1$ mV) *via* electrostatic assembly (Supporting Information).

As shown in the energy dispersive X-ray spectroscopy (EDS) linescan profile across the cross-sectional nanohybrid (Figure 2d), the formation of the DNA core and MH nanoshell is clearly demonstrated. High Mg and Al contents in the shell region are surely due to the reassembled MH nanosheets, whereas the P and N contents with a high steady plateau are from the DNA molecules within the core region. The measured compositional ratios of Mg/Al = 2.0 and N/P = 3.7 are in good agreement with those from elemental analyses, indicating that the DNA molecules are indeed encapsulated within the MH nanoshell to form a chemically well-defined DNA Core@MH Shell heterostructure.

Furthermore, we applied this DNA@MH nanohybrid to the DNA-based document identification system. To validate the feasibility, we integrated the present nanohybrid into conventional ink and then printed the security elements of the document with the nanohybrid-formulated ink, where the secured [designated as "S"] signature was made as an exclusive covert feature: although both documents bearing [S] look the same to the naked eyes as shown in Figure 3a, one can differentiate each document by fluorescence analysis, since the DNA molecules encapsulated in the MH nanoshell are concealed in [S] and its flouroprobes can respond to the unique optical signals. As shown in Figure 3b, the nanohybrid-suspended [S] shows the merged yellow color [S] resulting from the mixture of green and red flouroprobes, demonstrating the high dispersibility of nanoscale hybrids as well as the secured molecular barcode. For further identification of base sequence information, the DNA molecules were intentionally recovered out of the DNA@MH nanohybrid by resolving the MH shell with an weak acidic solution (pH = 4) in the presence of EDTA, chelating agent, to trap Mg^{2+} and Al^{3+} ions. Then released DNA molecules were denatured into single strands at 95 °C for 2 min, which were immediately detected by a biosensor assay as illustrated in Figure 3c. Hybridizations of prepatterned microarray can be depicted as the image of the "S" shape within the 12 × 12 array (144 spots) as shown in Figure 3d. The DNA-chip composed of two different capture DNAs that recognize the unique complementary base sequence of the designed DNA can demonstrate their potential cross-reactivity and nonspecific binding. The DNA information hidden on the authentic document was then identified as a two-color "S" graphic of representative spots as shown in Figure 3e. These results imply that the present nanohybrid-driven rational identification system can be used for molecular barcoding, item level tagging, security label sensing, and a traceability/authentication system.[8]

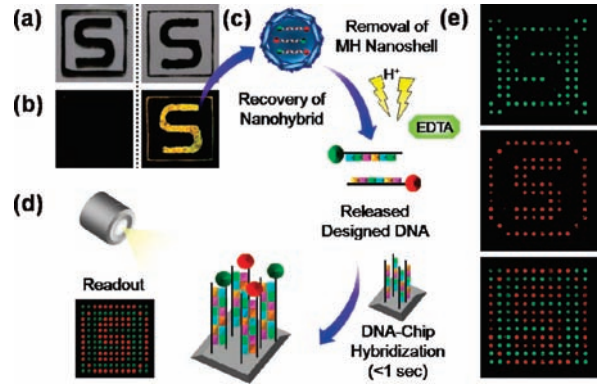

Figure 3. (a) Photograph images of the [S] shaped signatures, pure black ink (left) and DNA@MH nanohybrid-suspended black ink (right); (b) fluorescence microscopy images; (c) schematic procedure of identifying for the designed DNA information encapsulated in MH nanoshell by DNA-chip hybridization (<1 s, 100 pmol); (d) Designed DNA is determined On/Off by a specific "S" graphic image under illumination; and (e) readout images of "S" graphic.

In conclusion, we were able to demonstrate a nanoscale Bio Core@Inorganic Shell hybrid, with a rationally designed DNA core with a size of 100 ± 20 nm in the spherical inorganic nanoshell of reassembled MH nanosheets with a wall thickness of 10 nm, *via* a soft-chemical encapsulation route. The DNA Core@Inorganic Shell nanohybrid can provide wide bioinspired applications converged with nanotechnology, such as an advanced gene delivery system and a biomedical diagnostics, tracing/collection/sensing system for DNA-based information.

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Supporting Information Available: Experimental, further characterization data, and discussions. This material is available free of charge via the Internet at http://pubs.acs.org.

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