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DNA Nanogels To Snare Carcinogens: A Bioinspired Generic Approach with High Efficiency

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Abstract: *Polycyclic aromatic hydrocarbons (PAHs) are combustion-related pollutants and are ubiquitous in the environment, including in sources of drinking water. Upon contact with DNA, stable PAH–DNA adducts form rapidly as the first step towards their toxic effects. In this work, we prepared hydrophilic DNA nanogels to exploit this generic complexation process as a biomimetic scavenging method. This approach relies on interaction between PAHs and the complete network that constitutes the water-swollen nanogels, and is not restricted to interfacial adsorption. Up to 720 μg of PAH per gram of DNA nanogel are taken up, meaning that 1 mg of DNA nanogel is sufficient to purify a liter of water containing the critical PAH concentration for cancer risk (600 ng L^{-1}). As a result of short diffusion pathways, PAH uptake is rapid, reaching 50 % loading after 15 minutes. Beyond PAHs, DNA nanogels may be useful for the generic detoxification of water containing genotoxins, since most known molecules that strongly associate with DNA are mutagenic.*

Polycyclic aromatic hydrocarbons (PAHs) are combustion-related pollutants. Despite being hydrophobic, PAHs are to a certain extent water soluble and have been found in sources of drinking water such as surface water in concentrations up to 830 ng L^{-1}.[1] PAHs are metabolized to bay-region diepoxides,[2] which intercalate between the nucleotide bases, followed by covalent binding. This causes genetic miscoding,[3,4] and a number of cancers have been directly linked to PAH–DNA adducts.[5–7] Accordingly, long-term PAH exposure in humans increases cancer risk,[8] with a critical value for drinking water at 600 ng L^{-1}.[9] To date, several material concepts have been applied for PAH scavenging (Table S1 in the Supporting Information), including a semi-rigid cyclophane (ExBox),[10] cyclodextrins,[11] butyl rubber,[12] and recently, photoswitchable water-soluble nanoparticles that aggregate upon irradiation.[13] These approaches usually exploit interfacial adsorption of PAH on hydrophobic materials.

Initial PAH intercalation with DNA occurs through physical interaction of the planar PAH molecules with the hydrophobic spaces between adjacent base pairs of DNA strands.[14–16] This binding is generic, and broader and better PAH binding has been shown for DNA compared to cyclodextrins.[17] In addition, a recent investigation revealed that beyond PAHs, DNA binds a broad range of hydrophobic aromatic molecules.[18]

Chemically cross-linked DNA hydrogels present an interesting new class of materials,[19–21] and they have, for example, recently been explored as drug-delivery systems.[22,23] In contrast to the interfacial adsorption exploited so far for PAH sequestration, these highly swollen DNA networks would enable a volume-based scavenging mechanism for PAHs. In particular, colloidal systems seem highly attractive since the diffusion pathways are short and thus high uptake capacity might be combined with rapid sequestration. The intrinsic hydrophilicity of such systems would in addition ensure complete wetting and swelling with aqueous solutions, and furthermore, DNA-based materials are biocompatible and biodegradable.

Herein, we pursued this bio-inspired approach for PAH scavenging. DNA nanogels were fabricated from double- (ds) and single-stranded (ss) DNA molecules in inverse miniemulsion. Chemical cross-linking was achieved using the diepoxide cross-linker poly(ethylene oxide) digylcidyl ether (PEG-DGE) in the presence of tetramethylethylenediamine (TEMED) as a catalyst (Figure 1) at various concentrations and compositions (Table S2). After preparation and washing, the nanogels were further purified by dialysis against 4 mM NaBr solution to protect the conformation of the DNA strands in the nanogels. Transmission electron microscopy (TEM) and cryo-field emission scanning electron microscopy (cryo-FESEM) investigations (Figure 1 b,c) showed that the nanogels exhibit a spherical shape without any indication of aggregation. UV/Vis measurements of nanogel dispersions in comparison to DNA solutions at 260 nm (DNA absorption band) showed that the average yields for the nanogel preparation were in the range of 50–70 %. The measurements also revealed that the concentration of the DNA solution used for nanogel preparation and the resulting DNA nanogel concentration show a linear correlation (Figure 1 d, inset).

DNA nanogels were obtained with hydrodynamic diameters ranging from 150 nm to 650 nm and polydispersity index (PDI) values of 0.2–0.5, which varied depending on the DNA and cross-linker concentrations (Figure S4). Increasing the PEG-DGE/DNA ratio leads to a decrease in size due to

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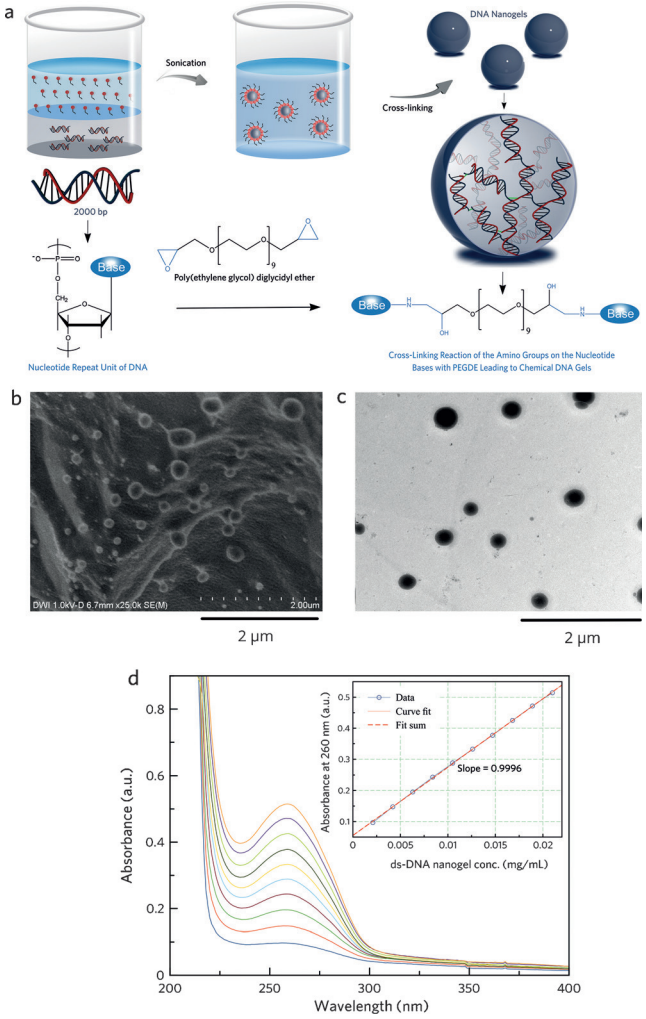

Figure 1. Synthesis pathway and characterization of DNA nanogels. a) Scheme of DNA nanogel preparation in inverse miniemulsion. b) Cryo-SEM and c) TEM images of DNA nanogels prepared from ds-DNA molecules. d) UV/Vis spectra of ds-DNA nanogel dispersions at elevated nanogel concentration with corresponding absorbance change at 260 nm (inset).

denser cross-linking until a critical concentration of PEG-DGE is reached, above which the nanogel size remained constant. This behavior can be attributed to an antagonistic effect of the PEG-DGE–TEMED pair (for a detailed discussion see the Supporting Information). Zeta potential measurements (Figure S3) and circular dichroism (CD) values (Figure S4d) support preservation of the native right-hand B-form of the DNA double-helix in the ds-DNA nanogels. However, a high amount of cross-linking induces an α-helix-to-coil transition (Figure S4d, inset).

The PAH scavenging capacities of the DNA nanogels were explored by incubation with solutions of phenanthrene, anthracene, coronene, and perylene, followed by the examination of aliquots by fluorescence spectroscopy after filtration. Throughout the study, the nanogel samples dsDNA1-1 and ssDNA1-1 were used in the experiments, except where otherwise noted (see Table S2). For phenanthrene, the initial concentration (0.871 µg/mL) was decreased to 0.304 µg/mL by the ds-DNA nanogels and to 0.451 µg/mL by the ss-DNA nanogels after 170 min exposure (Figure 2a). The binding of phenanthrene to ssDNA strands can be attributed to aromatic stacking on nucleobases, as well as intercalation to some degree with reformed ds regions in the gels (for a detailed discussion, see the Supporting Information). Time-dependent studies (Figure 2b) showed that complete loading takes hours. However, 50% loading capacity was reached for the ds-DNA nanogels in only 15 minutes. These findings were confirmed by UV/Vis spectroscopy (320 to 380 nm) in distilled water at 25°C (Figure 2c). Furthermore, our data reveals that 1 mg of DNA nanogel are sufficient to completely purify a liter of water containing the critical concentration of PAH for cancer risk (600 ng L^{-1}).[9]

The effectiveness of PAH scavenging was also tested with anthracene, coronene, and perylene by measuring the fluorescence at the respective excitation and emission wavelengths. Figure 3 shows the fluorescence spectra of PAH solutions before and 10 min after exposure to the DNA

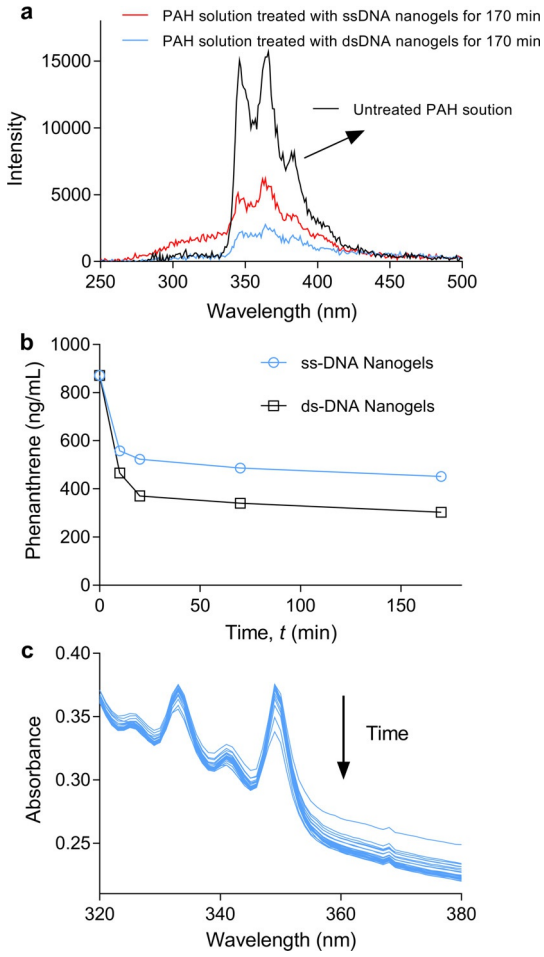

Figure 2. PAH scavenging properties of the DNA nanogels. a) Fluorescence spectra of a phenanthrene solution before and after treatments with dsDNA (dsDNA1-1) and ssDNA (ssDNA1-1) nanogels for 170 min. b) Phenanthrene concentration after treated with dsDNA and ssDNA nanogels as a function of time. (c) Time-dependent UV/Vis spectra of ds-DNA nanogels exposed to phenanthrene. A full-wavelength spectrum scan was performed every 2 min.

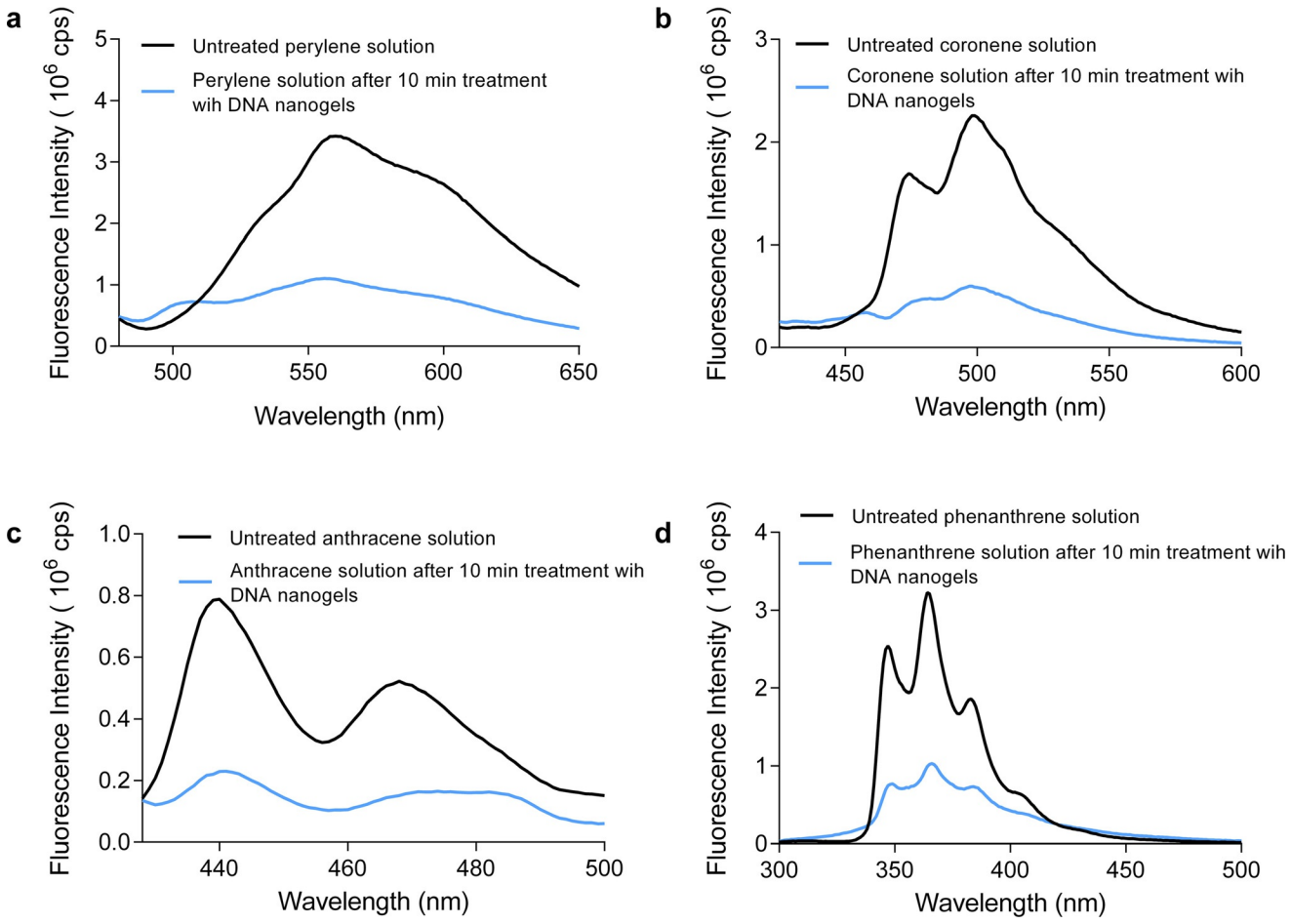

Figure 3. Fluorescence spectra of PAH solutions at $\mu g\,L^{-1}$ concentrations [perylene: 1.0×10^{-2} μM (a), coronene: 4.6×10^{-4} μM (b), anthracene: 2.5×10^{-1} μM (c), phenanthrene: 5.6 μM (d)] before and after DNA nanogel treatment with 10 min exposure time.

nanogels. For all PAH solutions, the nanogels showed good scavenging ability within a short reaction time. The sorption kinetics of phenanthrene into DNA strands fit well to a pseudo-second-order kinetics model (t/q_t versus t), with respective binding constants (k_2) in the range 0.39–0.45 ($g\,mg^{-1}\,min^{-1}$) depending on DNA conformation in the nanogels (Figure S6 and Table S3). Visually, a significant loss of fluorescence was observed for the perylene solutions after 10 min exposure of different concentrations of the DNA nanogels (Figure S5). Complete removal of phenanthrene was observed when the DNA nanogel concentration was increased two-fold at identical phenanthrene concentration (Figure S7).

CD spectra of the ds-DNA1-1 and ss-DNA1-1 nanogels (see Table S2) in the presence and absence of phenanthrene were recorded to investigate the scavenging mechanism (Figure 4). The CD spectra of the DNA nanogels show a positive band at 277 nm as a result of base-stacking, which indicates preservation of the native right-hand B-form of DNA. Upon addition of phenanthrene, the intensity of the positive band at 277 nm increases without significant wavelength change, thus indicating an increase in base stacking induced by phenanthrene. In comparison, phenanthrene alone shows a positive peak of minor intensity at around 260 nm due to aromatic stacking, which does not increase in intensity with increasing concentration (Figure S8). These findings support intercalation as the underlying mechanism of PAH sorption by the DNA nanogels.

To conclude, this study introduces a new bioinspired and generic strategy for PAH scavenging through DNA intercalation by hydrophilic DNA nanogels. In contrast to existing strategies, our biomimetic approach relies on a volume-based interaction between PAHs and the network that constitutes the water-swollen hydrogel nanoparticles. This unspecific, but thus also generic, process is efficient and also rapid owing to the nanoscale size of the particles and the short diffusion pathways, with the advantage that the used nanogels are hydrophilic, biodegradable, and even cytocompatible. Beyond PAH binding, recent studies suggest that DNA has a broader capacity for the removal of hydrophobic toxins that act through DNA interaction.[18] Since DNA widely exists in the natural world and can be mass-produced from waste material of the fishing industry such as fish milts, and since we did not find an indication that particle size or dispersity is an important factor for samples with similar DNA content and conformation (% ssDNA portion), the particles can be prepared from cheap raw materials using simple and technologically established processes. DNA nanogels or microgels

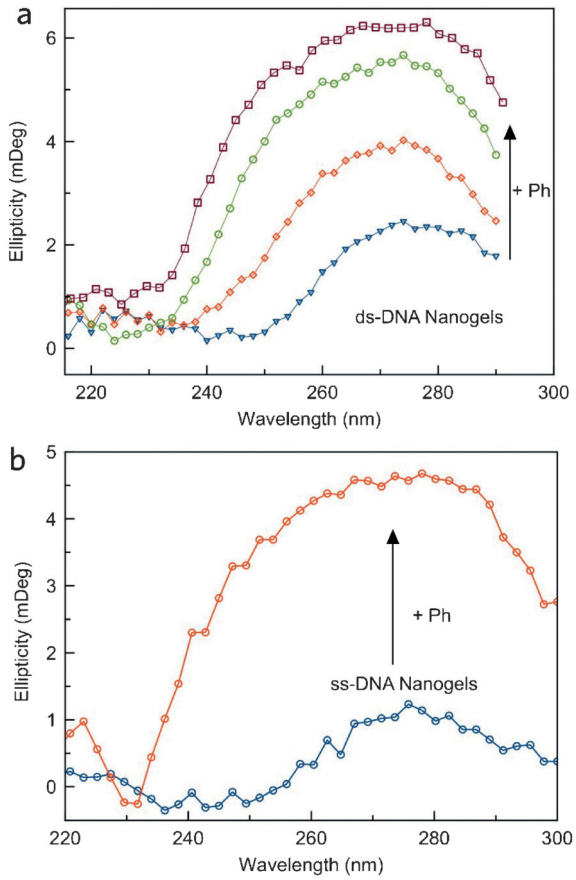

Figure 4. CD spectra of ds-DNA (a) and ss-DNA (b) nanogels before and after exposure to phenanthrene. The concentrations of phenanthrene are 1, 3 and 5 μM for incubation with the dsDNA nanogels (a) and 5 μM for incubation with the ssDNA nanogels (b), with the bottom spectrum representing the plain DNA nanogels before incubation with phenanthrene.

could thus be useful as a scavenging matrix in filters or between membranes, not only for the removal of PAHs from water but for a more generic detoxification of drinking water.

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