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Three-Dimensional DNA Crystals as Molecular Sieves

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DNA has proved to be a successful nanoscale building block because of its inherent programmability and its predictable structural features.[1] A variety of two-dimensional (2D) arrays have been assembled from DNA,[2] and these have been used to template the assembly of protein and nanoparticle arrays.[3] One long-standing goal of DNA nanotechnology has been the rational design and assembly of three-dimensional (3D) DNA crystals.[1] Three-dimensional DNA crystals have been proposed to be used as molecular scaffolds for structure determination of guest molecules,[4] as molecular sieves,[2a] and in the assembly of high-density molecular electronics.[5] We recently described the crystal structure of a continuously base paired 3D DNA lattice and demonstrated that the structural motifs observed in this lattice could be used to rationally design crystals with expanded lattice dimensions.[6] Here we demonstrate that these expanded DNA crystals can function as molecular sieves by selectively adsorbing proteins based on size.

Porous crystalline solids, such as zeolites, are used in catalysis, ion exchange, adsorption, and separation. The ability of these materials to selectively adsorb molecules based on size and shape has prompted their designation as molecular sieves.[7] Considerable effort has been devoted to developing meso- and macroporous materials with spatially ordered features for their improved zeolitic properties, their potential use as photonic crystals, optical information storage devices, and in microelectronics assembly.[8]

We have rationally designed and crystallized a 3D DNA array with mesoporous features. These crystals are composed of a 24 nucleotide assembly strand and an 11 nucleotide spacer strand that is complementary to a contiguous portion of the assembly strand. In the presence of Mg^{2+}, interactions between assembly strands generate an antiparallel Watson−Crick duplex and a parallel-stranded homopurine base pairing region. When separated at an appropriate distance by the spacer strand, these DNA oligos self-assemble into a 3D array (Figure 1a). This periodic assembly is predicted to form solvent channels running the length of the crystals both parallel and perpendicular to the 6-fold symmetry axis (Figure 1b and 1c), generating a pore network with axially distinct aperture sizes. These crystals have diffracted to only ~5 Å resolution, likely due in part to their high solvent content, but indexing of the low-resolution diffraction confirmed the predicted hexagonal space group with unit cell dimensions within 3 Å of those predicted from modeling.[6]

Adsorption of molecules into porous materials is dependent upon the size and shape of the molecules and pore structure of the material. In the expanded DNA crystals, the predicted ~9 nm solvent channel diameter parallel to the 6-fold symmetry axis could adsorb a perfectly spherical protein of ~300 kDa based on an average specific volume of 0.73 cm^3/g.[9] To test if proteins could be adsorbed into the solvent channels, DNA crystals were soaked with proteins of various molecular weights. To minimize nonspecific binding to the negatively charged DNA, we selected primarily proteins with net negative charges at near neutral pH. The exceptions were lysozyme (positive) and myoglobin (neutral).

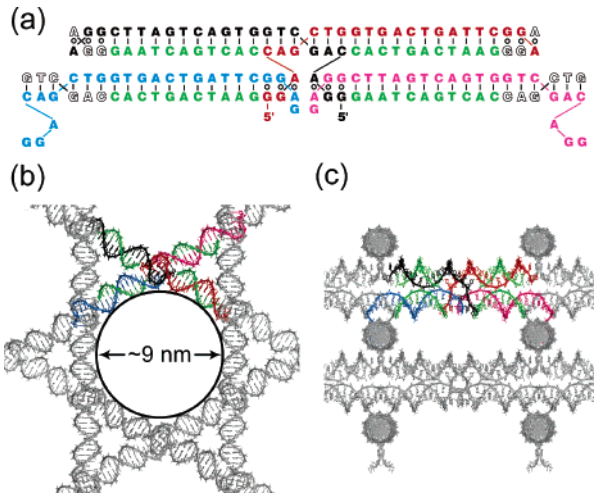

Figure 1. A 3D DNA lattice. The secondary structure of the expanded DNA lattice is shown in (a). Four assembly strands are shown in different colors, and the spacer strands in green. The resulting model of the lattice structure as seen down the 6-fold axis is shown in (b) and orthogonal to the 6-fold in (c). Assembly and spacer strands are colored as in (a).

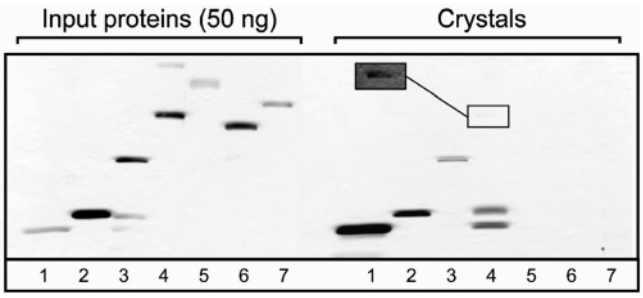

Figure 2. Adsorption of proteins into the DNA crystals examined by SDS−PAGE. On the left are the control lanes with 50 ng of the input proteins. The lanes on the right show equal volumes of crystals (2−4 crystals) after being soaked in 50 mg/mL of the input proteins, washed, and dissolved. The lane numbers correspond to the following: (1) lysozyme (14 kDa); (2) myoglobin (18 kDa); (3) carbonic anhydrase (28 kDa); (4) ovalbumin (45 kDa); (5) BSA (68 kDa); (6) alcohol dehydrogenase (tetramer, 140 kDa); (7) β-amylase (tetramer, 206 kDa). The boxed region in the ovalbumin-soaked crystal lane is shown with altered contrast in the inset image. No protein bands could be detected in soak lanes 5−7 with modified contrast.

After 1 week in the protein solutions, the crystals were washed, dissolved in SDS loading buffer, and their protein content was examined by silver stained SDS−PAGE (Figure 2). The proteins with molecular weights below ~45 kDa were visible, with the apparent amount of protein retained inversely proportional to its size. Ovalbumin was the largest protein that could be detected, while BSA, alcohol dehydrogenase, and β-amylase could not be detected. In the case of ovalbumin, several lower molecular weight impurities (likely ovomucoid and lysozyme) were preferentially adsorbed,

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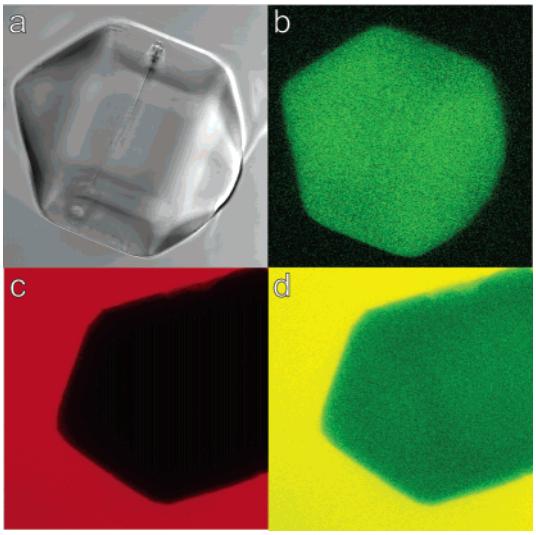

Figure 3. Direct observation of proteins adsorbed into and excluded from the crystals. (a) A light microscope image of an expanded lattice crystal soaked in GFP. (b) A fluorescent confocal image through the center of the crystal shows that GFP is distributed throughout the interior of the crystal. The depth of field corresponds to ~50 unit cells. Confocal images of a crystal soaked in GFP and MBP−RFP show that MBP−RFP is too large to enter the solvent channels and is excluded from crystal interior (c), while GFP is present inside and outside the crystal in the merged image (d).

though these were in low enough amounts that they could not be detected in the control lane.

Molecular modeling experiments were consistent with the ~45 kDa adsorption cutoff observed in the SDS−PAGE experiments (Supporting Information). These data reflect the theoretical ability of the proteins to fit within the solvent channel, but do not reflect their ability to enter into a pore from outside the crystal. The inverse relationship between the apparent amount of each protein adsorbed and their molecular weights is consistent with known sieving effects and can be explained at least in part by a decreased ability to enter into the pores and an increase in frictional resistance that restricts travel within a pore.[10]

The SDS−PAGE detection of proteins provides only indirect observation of adsorbed proteins. To directly observe proteins adsorbed into or excluded from the crystal interior, crystals were soaked with fluorescent proteins of different sizes and were observed by laser-scanning confocal microscopy. Crystals were soaked in green fluorescent protein (GFP, 28 kDa) and a maltose-binding protein/tetrameric red fluorescent fusion protein (MBP−RFP, 280 kDa). Confocal images using a 0.46 μm depth of field through the approximate center of the crystals showed that GFP was adsorbed into the crystals and remained inside the crystal after washing (Figure 3a and 3b). Importantly, GFP was distributed throughout the interior of the crystal, indicating that the protein was adsorbed and not simply associated with the crystal surface. Crystals were also incubated with GFP and MBP−RFP simultaneously for 1 week and imaged in the solution containing both proteins. MBP−RFP was excluded from the crystal with a sharp boundary between the crystal and the MBP−RFP in solution (Figure 3c). GFP was observed both inside and outside the crystals in the merged image (Figure 3d).

Creation of 3D DNA crystals has been recognized as an important step toward a number of DNA nanotechnology applications. The results described here (i) demonstrate that DNA self-assembly can be used to create mesoporous 3D objects; (ii) confirm the approximate size of the solvent channels in the expanded DNA crystals in absence of high-resolution structural data; (iii) show that guest proteins can be incorporated into the solvent channels of these crystals. The crystals described here constitute self-assembled, periodic, mesoporous arrays that may have applications such as those proposed for similar inorganic materials. They could also be used for chromatographic separations as has been described for cross-linked protein crystals.[11]

Fundamentally, most proposed applications of DNA nanotechnology (in 2D or 3D) will require positioning guest molecules relative to the DNA array. The precision required in positioning will be dependent upon the application. For 3D DNA crystals to be useful as molecular scaffolds in structure determination, guest molecules will likely need to be statically positioned with angstrom-level precision to provide coherent diffraction of X-rays. For molecular sieving applications or to template the assembly of other nanoscale objects, guest molecules need only be constrained dynamically. This demonstration shows that guest molecules can be selectively incorporated into 3D DNA crystals after crystallization and provides a framework for developing crystals in which guest molecules can be positioned with higher precision.

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Supporting Information Available: Methods and molecular modeling of protein guests in a solvent channel. This material is available free of charge via the Internet at http://pubs.acs.org.

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