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Positive Staining for Improved Contrast Of Macromolecules In Transmission Electron Microscopy

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The study of materials is governed by the relationship between structure and function. A quintessential example of this phenomenon is deoxyribonucleic acid (DNA), in which molecules assemble in a particular pattern at the nanometer scale, organize into functional configurations at the micrometer scale, and consequently constitute the basis of life at the macroscale [1]. The visualization of nucleic acids and proteins is essential to our understanding of the human body; however, DNA in particular has additionally proven useful in applications beyond biology, such as the functionalization of particles for disease therapy or the patterning of functional substrates [2,3]. In order to characterize and fully control how DNA and other macromolecules may assist in these applications, we must visualize the distribution of DNA strands and observe the mechanisms by which they adhere to surfaces.

The primary challenge in electron microscopy of soft materials is achieving reasonable contrast at electron doses low enough to avoid sample damage. Traditional staining methods to overcome signal-to-noise issues require heavy metal contrast agents, which are effective for visualizing the cell membrane due to strong lipid adherence but do not provide adequate staining of nuclear material. A DNA staining method recently developed by Ou et al. and leveraging a technique produced by Maranto (1982) [4,5], chromatin electron microscopy tomography (ChromEMT), allows for positive staining of DNA and other macromolecules in the nucleus of human cells. This method first labels cells with a fluorescent molecule that selectively binds to DNA, and then bathes the cells in diaminobenzidine (DAB). Upon photobleaching, excited fluorescent molecules generate a reactive oxygen species that catalyzes DAB polymerization localized to DNA. Then, cells are stained with osmium tetroxide (OsO_4), which binds to DAB with high affinity. The ChromEMT staining technique is tunable to different macromolecules by using appropriate selective fluorescent tags, and is not limited to material in cells [4].

We extended ChromEMT to directly image DNA on the surface of polymer-core spherical nucleic acids (SNAs) [2], demonstrating the applicability of the method to isolated nucleic acid strands rather than macromolecules in cell nuclei. SNAs were deposited directly onto grids for transmission electron microscopy (TEM), and the ChromEMT staining procedure was performed by transfer of the grids into droplets of solution for the appropriate step. Four conditions were imaged using a Hitachi HD-2300 STEM in high angle annular dark field (HAADF) mode in order to determine the effectiveness of the staining procedure: 1) SNAs without a staining agent; 2) SNAs stained with OsO_4; 3) SNAs labeled with the fluorescent tag cyanine 5 (Cy5), washed in DAB and stained with OsO_4; and 4) SNAs labeled with Cy5, washed in DAB, photobleached, and stained with OsO_4 (the entirety of the ChromEMT method). All preparations and microscopy were performed at room temperature. Figures 1a-d show each of the

four conditions. While the SNAs are visible at all conditions, a ring structure around the core appears with the DAB wash (Fig. 1c-d), both with and without photobleaching, which is not present in the SNA-only and SNA with OsO_4 conditions (Fig. 1a-b). The staining of the shell suggests that Cy5 successfully polymerizes DAB localized to the fluorescent dye. However, this fluorophore only labels the end of the chain; the method will be attempted using deep-red anthraquinone Nr. 5 (DRAQ5), a DNA-specific tag that intercalates along the length of the chain [4], to assess it as a candidate dye to stain the entire chain and potentially visualize individual strands.

DNA strands could be resolved by repeating ChromEMT at cryogenic temperatures, which will reduce surface contamination and more effectively preserve the structure of DNA and DAB by diminishing DAB drift after polymerization. Correlation with spectroscopic mapping of the phosphate backbone in DNA will provide confirmation of the specificity of DNA labeling. We plan to use this method to visualize other types of SNAs, such as protein-core SNAs with improved control over the core structure and the distribution of DNA on the surface, as well the strands in DNA-assisted assembly systems such as nanoparticle superlattices [3]. We will additionally explore other biomimetic nanoparticle constructs and use 3-dimensional tomography for improved visualization of the 3D distribution of the stain. [6]

References:

[1] CL Woodcock, RP Ghosh, Cold Spring Harbor Perspectives in Biology **2** (2010), p. a000596.
[2] Z Li, Y Zhang, P Fullhart, CA Mirkin, Nano Letters **4** (2004), p. 1055-1058.
[3] Q Lin, JA Mason, Z Li, W Zhou et al, Science (2018), p. eeaq0591.
[4] HD Ou, S Phan, TJ Deerinck, A Thor et al, Science **357** (2017), p. eaag0025.
[5] AR Maranto, Science **217** (1982), p. 953-5.
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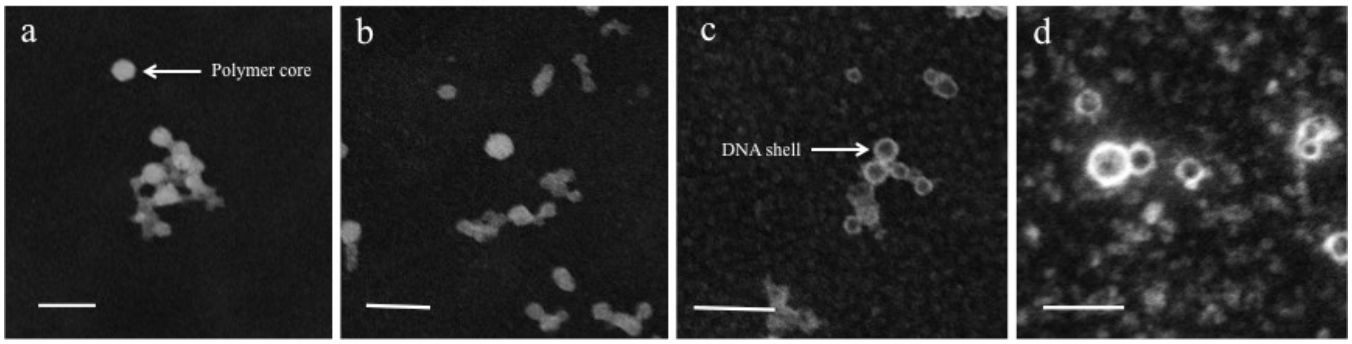

Figure 1. HAADF images of protein-core SNAs with different staining conditions. a) SNAs without staining agent. b) SNAs stained with OsO_4 only. c) SNAs tagged with cyanine 5 fluorescent label, washed in DAB, and stained with OsO_4. A ring structure begins to appear, with higher contrast around the shell of the particle than in the core. d) SNAs with cyanine 5 fluorescent tag, DAB wash, photobleaching, and OsO_4 staining, also with the presence of a ring around the core. Scale bars 200 nm.

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