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Imaging Individual Molecules Using Liquid-phase TEM - Surprises and Research Opportunities

Huan Wang[1], Hima Manasa Kandula,[2] Ye-Jin Kim,[3,4] Oh-Hoon Kwon,[3,4] and Steve Granick[3,4]

[1] College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, P. R. China.
[2] Department of Physics, University of Massachusetts, Amherst, MA 01003, USA.
[3] Department of Chemistry, UNIST, Ulsan 44919, South Korea.
[4] Center for Soft and Living Matter, Institute for Basic Science (IBS), Ulsan 44919, South Korea.

Exploring the potential of liquid-phase TEM to image individual molecules and their mutual interactions, this laboratory has explored multiple systems. Regarding individual polymer molecules, polystyrene sulfonate and poly(ethylene oxide) are visualized with and without added salt, trapped in liquid pockets between creased graphene sheets. The projected sizes and conformational fluctuations of adsorbed molecules and adsorption–desorption events are analyzed. Confirming the identification of the observed objects, statistical analysis is made of datasets of hundreds of images for times up to 100 s, with variation of the chemical makeup of the polymer, the molecular weight of the polymer, and the salt concentration, as described in detail elsewhere [1].

Likewise using graphene liquid-cell electron microscopy, with electrons of low energy at low dose we resolve the time dependence of conformational adaptations of macromolecules for times up to minutes, the resolution determined by motion blurring, with DNA as the test case. Single-stranded DNA molecules are observed in real time as they hybridize near the solid surface to form double-stranded helices; we contrast molecules the same length but differing in base-pair microstructure (random, blocky, and palindromic hairpin) whose key difference is that random sequences possess only one stable final state, but the others offer metastable intermediate structures. Hybridization is observed to couple with enhanced translational mobility and torsion-induced rotation of the molecule. Prevalent transient loops are observed in error-correction processes. Transient melting and other failed encounters are observed in the competitive binding of multiple single-stranded molecules. Among the intermediate states reported here, some were predicted but not observed previously, and the high incidence of looping and enhanced mobility come as surprises. As described in detail elsewhere [2], these error-producing mechanisms, failed encounters, and transient intermediate states would not be easily resolved by traditional single-molecule methods.

A simple strategy enables us to be less limited than traditionally by the fact that aqueous samples tend to suffer from water radiolysis and other chemical degradation caused by the high energy of incident electrons. As reported in detail elsewhere [3], aqueous liquid pockets in graphene liquid cells at room temperature display significantly improved stability when using deuterated water, D_2O. Use of D_2O outperforms adding radical scavengers to H_2O regardless of imaging details; it increases the lifetime of dissolved organic macromolecules by a factor of 2–5, and it delays by even longer the appearance of radiolysis-induced bubbles, by a factor of time up to 10 [3].

In fact, in recent research we find that single protein catalytic events can also be resolved using this method; the proteins of interest to us currently are DNA binding proteins. A picture emerges in which simple experiments, performed at single-particle and single-molecule resolution, can dissect microscopic phenomena in ways that surprise.

References:

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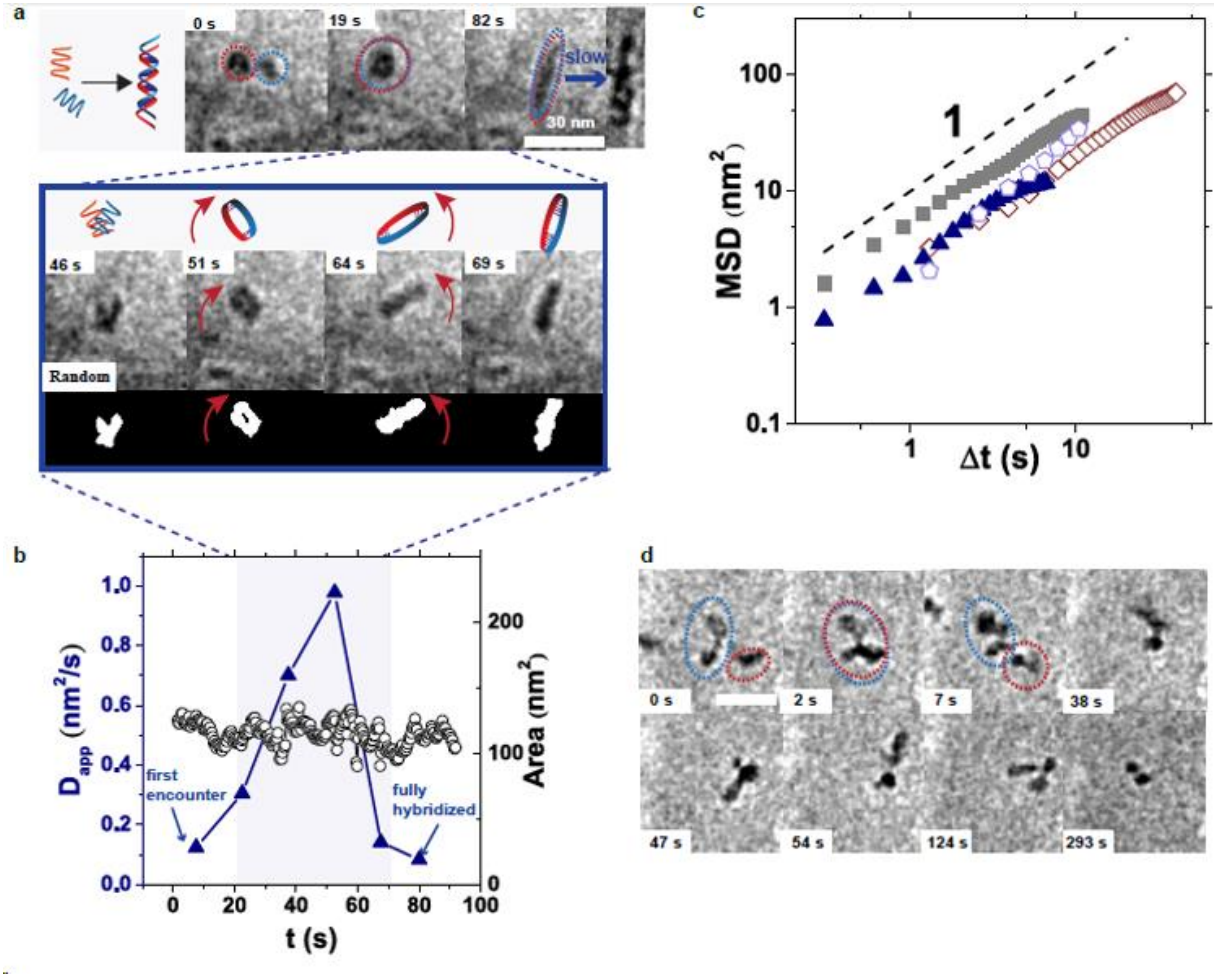

Figure 1. DNA self-assembly visualized in aqueous buffer by electron microscopy. (a) Example of single stranded molecules with random base pair sequence. Two complementary strands are imaged for 82 s while they hybridize while the expanded view includes, for better visual contrast, accompanying binarized images obtained from threshold intensity. Dotted circles in the top row identify single molecules. Red arrows highlight rotations in the indicated directions at the indicated times. (b) Translational mobility, D_{app}, plotted against time from considering 4 s intervals at times indicated, for experiment in Fig. 1a. (c) On log-log scales, mean square displacement of the duplex center-of-mass is plotted against time with reference slope unity with different symbols signifying different detection conditions. (d) Example of complementary pentablock base pair sequences reverse assembly in d and the relative angle θ to the horizontal (triangles). Dashed lines are guides to the eye. Adapted with permission from reference 2.

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