New and Notable

Single-Molecule Vibrational Spectroscopy Adds Structural Resolution to the Optical Trap

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ABSTRACT The ability to apply forces on single molecules with an optical trap is combined with the endogenous structural resolution of Raman spectroscopy in an article in this issue, and applied to measure the Raman spectrum of ds-DNA during force-extension.

The resounding success of single-molecule biophysical techniques has encouraged the development of additional tools for more detailed exploration. The unique ability of single-molecule methods to apply force and torque, to disentangle heterogeneity, and to watch equilibrium kinetics would pair beautifully with the ultrafast time resolution and atomistic structural sensitivity of vibrational spectroscopy. However, the weak signal levels endemic to vibrations have left them mostly in the domain of bulk spectroscopy; cross-sections for Raman scattering are typically 10^{14} times smaller than for fluorescence emission. In this issue, Rao et al. (1) overcome this gap using surface-enhanced Raman spectroscopy (SERS) (2,3) to add vibrational spectroscopic resolution to their optical trap. In this experiment, a single DNA strand is brought into the near-field vicinity of a silver nanoparticle-coated silica bead that enhances its Raman scattering, and the spectrum is recorded as the DNA is extended in the optical trap. The authors find that applying force shifts the phosphate-stretching vibrational frequency. Molecular dynamics and density functional theory calculations were used to explain these results by showing that external load applied to the DNA backbone induces Ångstrom-level displacements in the P-O bonds.

This work is immediately relevant to the communities interested in DNA mechanics and single-molecule Raman spectroscopy. While the authors' results may refine our structural models for DNA in the low-force regime (1–9 pN), the ongoing debate about the molecular nature of the transition into overstretched DNA ($\geq$65 pN) (4) would be well served by additional structural resolution. For the SERS community, the optical trap provides a fantastic control as it allows one to unambiguously verify that a single-molecule is probed and systematically control its distance and orientation to the metal surface, which may finally resolve long-standing mysteries about the mechanism of SERS. Ideally, both methods will be advanced in concert at the expense of coercing as much information as possible out of a single molecule.

While this work is groundbreaking, the real excitement is in its potential. One limitation in most implementations of both single-molecule force and fluorescence spectroscopy is acute sensitivity to distance changes >5 nm, which diminishes upon approaching the subnanometer scale. Raman scattering and infrared absorption vibrational spectroscopies offer a complementary distance sensitivity as molecular oscillators sense their local environment and couple to one another on scales of ~0.1 nm; see Fig. 1 for a comparison. The optical trap can now be used to initiate specific structural changes to be probed by SERS. In such mechanistic studies, one benefits from the fact that the vibrational spectrum is an endogenous probe, arising from oscillations in all the different bonds present (enzyme as well as substrate), that directly encodes the kinetics and dynamics of structural changes. Such a detailed view of hydrogen-bond rearrangements, covalent-bond formation/breaking, and symmetry changes can offer subtle details that are impossible to tag with fluorophores or directly monitor via a force measurement. As vibrational spectroscopy is rapidly approaching the molecular fingerprinting level with DNA base resolution (5) and protein identification (6), there is an optimistic future for this apparently new multiplexed technique across the various divisions of biophysics.

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Submitted August 16, 2012, and accepted for publication September 20, 2012.

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Editor: Matthias Rief

0006-3495/13/01/0004/2 $2.00

http://dx.doi.org/10.1016/j.bpj.2012.09.044

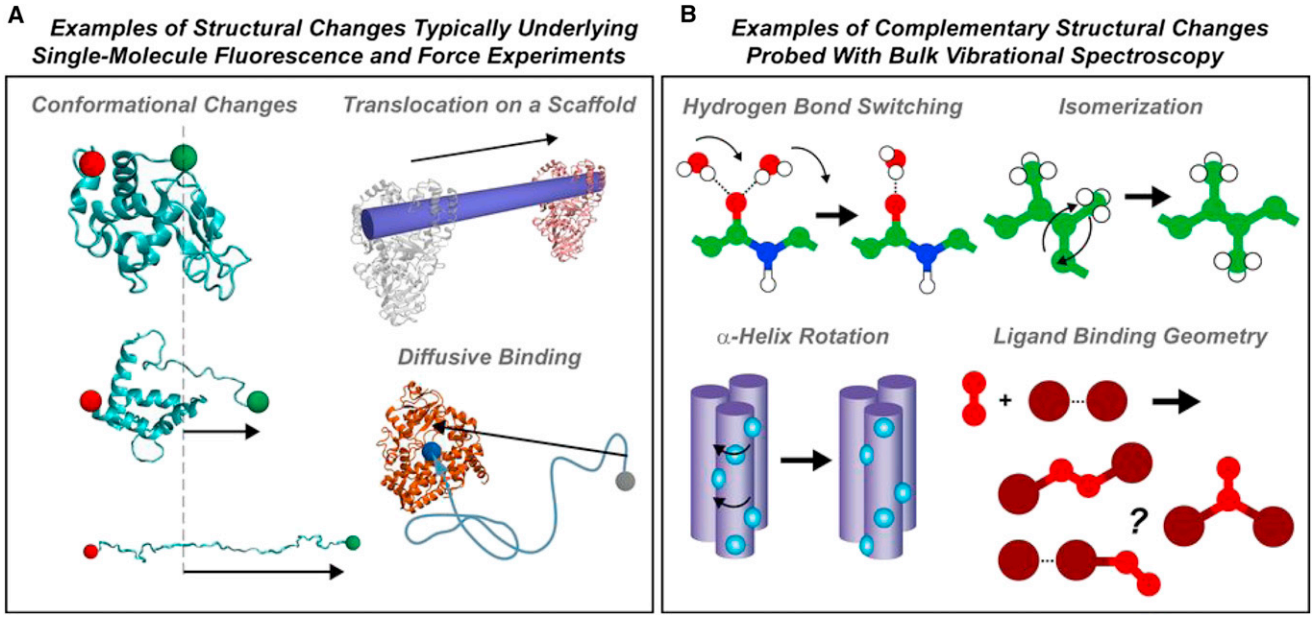

FIGURE 1 (*A*) Examples of mesoscopic structural changes typically underlying single-molecule experiments, such as unfolding of DNA and proteins; translocation of enzymes on a scaffold such as the motor proteins, dynein and kinesin, and replication proteins; and binding of substrates such as ATP and FAD (7,8). (*B*) Examples of microscopic structural changes probed by bulk vibrational spectroscopy, which may complement single-molecule studies such as hydrogen bonding, isomerization, subtle secondary structural changes such as α-helix rotation and β-sheet reordering, and ligand-binding geometry and kinetics (9–12).

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