Atomistic dye description

(a) Alexa488 C5 maleimide / Alexa647 C2 maleimide

(b)

(c) Molecular dynamics simulations

(d) **Representations of coarse-grained dyes**

Credible Volume (NPS) — Localized antenna

Accessible Volume — Free diffusion

Accessible Contact Volume — Free diffusion + contact

Weighted Accessible Volume — Gaussian chain diffusion

(e) **Orientational & spatial density along R**

Isotropic orientation model / Single cone model / Mean position

Förster Resonance Energy Transfer (FRET) combined with single-molecule spectroscopy probes macromolecular structure and dynamics and identifies coexisting conformational states. We review recent methodological developments in integrative structural modeling by satisfying spatial restraints on networks of FRET pairs (hybrid-FRET). We discuss procedures to incorporate prior structural knowledge and to obtain optimal distance networks. Finally, a workflow for hybrid-FRET is presented that automates integrative structural modeling and experiment planning to put hybrid-FRET on rails. To test this workflow, we simulate realistic single-molecule experiments and resolve three protein conformers, exchanging at 30 µs and 10 ms, with accuracies of 1–3 Å RMSD versus the target structure. Incorporation of data from other spectroscopies and imaging is also discussed.