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Size dependence of crowding effect and small molecule crowding HSUAN-LEI SUNG, ABHIGYAN SENGUPTA, DAVID NESBITT, Univ of Colorado - Boulder — Cells are exceedingly crowded with 20-40% of the mass taken up by solutes, much different from the dilute buffers where most biological studies are traditionally performed. It's been recognized, for more than a decade, such steric constraint may influence the biomolecule conformations. Previously, macromolecules have been widely used to mimic the crowded environments in cells and shown to greatly promote the biomolecular folding primarily through steric repulsion. Nevertheless, the crowder size dependence has not yet been fully investigated as the role of small molecules in intracellular crowding remains unclear. Cells contain not only macromolecules but also the small solutes like inorganic ions, amino acids and various metabolites. The latter, despite small in individual size, are undoubtedly more abundant and thus they potentially contribute greatly to intracellular crowding. In this work, the crowder size dependence of crowding effect on RNA tertiary structure has been studied by single molecule FRET spectroscopy. The distinct variations in folding/unfolding rate constants along with the predominantly entropic origin revealed in the temperature dependence indicate the crowding effect as major contribution of structure stabilization by both polymer and small molecule crowders. Furthermore, the crowder size dependence is quantitatively described by the physical model where osmotic pressure and excluded volume are considered. Our study provides both experimental and theoretical evidence for small molecule crowding and suggests even stronger crowding effect for small molecule at constant concentration.

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