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Binding Affinity Effects on Physical Characteristics of a Model Phase-Separated Protein Droplet SARA CHUANG, Princeton University, SALMAN BANANI, UT Southwestern Medical Center, MICHAEL ROSEN, UT Southwestern Medical Center, HHMI, CLIFFORD BRANGWYNNE, Princeton University — Non-membrane bound organelles are associated with a range of biological functions. Several of these structures exhibit liquid-like properties, and may represent droplets of phase-separated RNA and/or proteins. These structures are often enriched in multi-valent molecules, however little is known about the interactions driving the assembly, properties, and function. Here, we address this question using a model multi-valent protein system consisting of repeats of Small Ubiquitin-like Modifier (SUMO) protein and a SUMO-interacting motif (SIM). These proteins undergo phase separation into liquid-like droplets. We combine microrheology and quantitative microscopy to determine affect of binding affinity on the viscosity, density and surface tension of these droplets. We also use fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS) and partitioning experiments to probe the structure and dynamics within these droplets. Our results shed light on how inter-molecular interactions manifests in droplet properties, and lay the groundwork for a comprehensive biophysical picture of intracellular RNA/protein organelles.

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