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Hierarchical assembly of protein nanocrystals into macroscopic gels DANIEL GREENE, STANLEY SANDLER, NORMAN WAGNER, ABRAHAM LENHOFF, Univ of Delaware — From crystallization screens to downstream processing, protein gel phases are common during protein solution processing. While the structure of crystalline protein is well known, very little is known about the structure of these gel phases. We recently measured the microstructure of a salted-out ovalbumin dense phase and found that nanocrystalline protein clusters, which are only a few unit cells in size, percolate 5 micron gel beads. It is unclear if the behavior seen for ovalbumin is representative of a more general phenomenon. Here we present microstructural measurements on a salted-out monoclonal antibody (mAb) and salted-out ribonuclease-a that support this possibility. Using small-angle x-ray and neutron scattering (SAS) and transmission electron microscopy (TEM), we find both salted-out mAb and ribonuclease-a gels exhibit nanocrystalline regions. Within the mAb gel, the mAb aggregates into hollow tubular structures that are hundreds of nanometers long, have an inner diameter of approximately 15-20 nm and an outer diameter of approximately 20-30 nm. The SAS intensity from these structures contains a peak at high-q that is commensurate with scattering from idealized mAb nanocrystals that are 1-2 unit cells wide. Ribonuclease-a does not appear to from tubular structures, but the SAS intensity contains peaks at high-q that are consistent with the scattering from a nanocrystal 2-3 unit cells wide. Power-law scattering at low-q indicates the nanocrystals aggregate into a gel with fractal dimension 2.5. This research provides insight into the nanostructure and formation of protein gel phases.

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