Impact of Salt Identity, Concentration, and Buffer Composition on Hierarchically Structured Protein Fibers from Recombinant Tobacco Mosaic Virus Self-Assembling Capsids

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Biological organisms have mastered the self-assembly of proteins into multifunctional materials, achieving remarkable properties through complex molecular organization at the macroscopic scale. However, replicating this level of hierarchical organization in man-made materials remains a persistent challenge. Tobacco Mosaic Virus coat proteins (TMVcp) can self-assemble into different conformations (helical rods, disks, stacked disks) through variation of solution ionic strength and pH. Recent data from the Blum group suggests that TMVcp are able to form macroscale hierarchically structured protein fibers. The objective of this study was to investigate the effect of exogenous salt concentration and buffer identity on the hierarchical structure of the TMVcp fibers. Four salts were introduced at concentrations ranging from 1mM to 100mM and six buffer systems were evaluated, varying in both cation and anion identity. The fibers were characterized through Polarized Light Microscopy (PLM), which revealed the presence of birefringence that indicates a hierarchical structure, and Small Angle Xray Scattering (SAXS), which was used to characterize the organization and spacing between the helical rods within a fiber. Results indicate an optimal added salt concentration range which significantly enhanced birefringence and internal hierarchical order. Notably, citrate-based buffers demonstrated superior self-assembly efficiency compared to other buffer systems, as evidenced by distinct features in the SAXS spectra. Furthermore, Scherrer analysis confirmed that the rods pack into an hexagonal lattice, a consistent feature across all fibers formed with 25mM added salt, regardless of salt identity. This work highlights the influence of ionic strength and buffer composition on the hierarchical structure of the TMVcp fibers, opening new pathways for tuning the self-assembly of viral capsids via environmental control.