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CONFOCAL RAMAN MICROSCOPY STUDIES OF REVERSIBLE PROTEIN ION-EXCHANGE INTERACTIONS WITHIN POROUS SILICA PARTICLES

Allison Jacobsen (Joel M. Harris, Maryam Zare)
Department of Chemistry

Protein purification, often accomplished via ion-exchange chromatography, is a necessary precursor for characterizing the structure and functions of proteins and developing pure products for research or clinical applications. A greater understanding of electrostatic protein interactions with charged surfaces is essential for improving ion-exchange materials, and few existing methods allow for the quantification of molecular populations at these interfaces. In this work, we investigate protein ion-exchange interactions with a model surface formed through engineering positively-charged sites into hybrid-supported phospholipid bilayers deposited in reversed-phase chromatographic silica particles. We utilize confocal Raman microscopy to detect the adsorption of bovine serum albumin to charged sites within the particles, which are formed through dispersing a small fraction of the cationic lipid 1,2-dipalmitoyl-3-trimethylammonium-propane (16:0 TAP) into a bilayer of the protein-repellent lipid dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). The positively-charged bilayers readily exhibit protein retention, detected through Raman scattering from the phenylalanine breathing mode of the protein. Quantitative determinations of protein coverage on the bilayer surface are accomplished through the use of the choline lipid headgroup as an internal standard. The reversibility of protein adsorption, demonstrated via spectroscopic data and the agreement of protein surface coverages with a Langmuir adsorption model, suggests the possible application of this material to ion-exchange processes. For a bilayer containing a moderate fraction of cationic lipid (25%), protein saturation is achieved with molecules spaced at a slightly greater distance than a close-packed monolayer, with 11-12 charged lipids found to collectively support the retention of each protein molecule. The approach described in this work readily lends itself to quantitative and molecular-level analyses of ion-exchange processes.