

141c Fundamental Studies of Polymer Physical Entanglement Interactions with Monodisperse, Random-Coil Protein Polymers

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We have used genetic engineering to create a series of non-natural, repetitive polypeptides (“protein polymers”) that are monodisperse, water-soluble, and possess a random-coil conformation. These protein polymers are produced with a precise sequence and molecular length by expression in *E. coli* bacteria. The “controlled cloning” method was used to create an artificial gene encoding multiple repeats of the DNA “monomer” sequence GAGTGSA. This DNA template was then inserted into, and expressed by, bacteria through the use of an expression vector containing an N-terminal decahistidine fusion tag. The desired protein was purified from the cell lysate by affinity chromatography on a nickel resin, and the fusion tag was later removed via cyanogen bromide cleavage. Pure, monodisperse proteins were produced with molecular weights of 9 kDa, 18 kDa, and 36 kDa. Amino acid analysis and MALDI confirmed the composition and size of the proteins. Circular dichroism spectroscopy was used to observe the (lack of) folded structure of the protein polymer in water over a range of temperatures. The creation of a series of protein-based polymers allows for the study of polymer physical properties using truly monodisperse polymers of tailored molecular weights. This will allow direct and quantitative comparisons to theoretical predictions of polymer physics. In particular, the overlap threshold concentration (c^*) can be determined as a function of protein polymer chain length and compared to theory. The radii of gyration for these protein polymers were also determined by multi-angle laser light scattering. Protein solutions were tested for potential LCST behavior using temperature-controlled visible spectroscopy. We will present the preliminary conclusions of this interesting fundamental study.