

Synthesis and Optimization of Charged Polypeptides via *N*-Carboxyanhydride Polymerization for the Study of Polyelectrolyte Complex Phase Behavior

Ritu RITU, Jürgen ALLGAIER, Karoline RENGEL, Ralf BIEHL, Andreas STADLER,
Ekaterina BUVALAIA and Stephan FÖRSTER*

Forschungszentrum Jülich GmbH, JCNS-1, Jülich, Germany

Synthetic polypeptides are versatile model systems for investigating electrostatically driven self-assembly, polyelectrolyte complexation, and phase behavior in biomolecular and soft matter systems. Oppositely charged polypeptides such as polyarginine and poly(aspartic acid) provide well defined platforms for studying complex coacervation and liquid-liquid phase separation. However, commercially available materials are expensive, limited in molecular weight range, and often lack comprehensive characterization.

Here, we report the synthesis and optimization of charged polypeptides via *N*-carboxyanhydride (NCA) ring-opening polymerization. NCA monomers derived from protected ornithine and aspartic acid were synthesized under rigorously anhydrous conditions, with high monomer purity being essential for controlled polymerization. Polymerization conditions were optimized by varying the initiator type, concentration, solvent, and reaction time to obtain polymers with controlled degrees of polymerization and narrow molecular weight distributions.

Polyarginine was prepared by deprotection followed by guanidinylation of polyornithine, whereas poly(aspartic acid) was obtained through deprotection and neutralization of the corresponding precursor, yielding water soluble polyanionic chains. The polymers were characterized by gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR) spectroscopy to determine molecular weights and confirm chemical structures. Chiral purity was assessed by ¹³C NMR. These well-defined charged polypeptides provide model systems for investigating polyelectrolyte complex phase behavior, including liquid-liquid phase separation as models for membraneless organelles and solid-phase assemblies analogous to biological materials such as collagen.

E-mail of the corresponding author: s.foerster@fz-juelich.de