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Guiding Interfacial Assembly of Semi-crystalline Block Copolymer Nanoparticles toward Design of Protocells for *in vitro* Enzymatic Synthesis

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Extended Abstract

Micelles from amphiphilic poly(ethylene oxide)-b-poly(ε-caprolactone) (PEO-PCL) block copolymers have been intensively studied in the field of drug delivery. When dispersed in water, a selective solvent, these block-copolymers self-assemble into nanostructured phases such as spherical or cylindrical micelles with a semi-crystalline core, or vesicles. However, the structural polymorphism and high tendency towards secondary aggregation strongly restrict their efficiency as drug carriers. On the other side, such amphiphilic semi-crystalline organic assemblies represent an effective alternative to inorganic particles in stabilization of Pickering emulsions and show promising potential in designing of bio-compatible materials with a high interfacial area such as emulsions, scaffolds, and protocells for enzymatic synthesis.

Assembly of PEO-PCL at toluene/water interface has been studied as a function of the molecular architecture and molecular weight, as well as under effects of external triggers such as emulsification method, and temperature. The resulting emulsions have been analysed in terms of the emulsion type and kinetic stability, droplet size distribution as well as thermo-responsive behaviour. We show that the interfacial curvature can be tuned by molecular architecture and by temperature trigger, which affects both the solubility of the PEO block and crystallization of the PCL block, so that the phase inversion between direct and inverse emulsion is possible.

The system was optimized to produce biomimetic micro-reactors for *in vitro* enzyme compartmentalization. Protocells in a form of double emulsion droplets (water-in-oil-in-water) have been prepared using selected PEO-PCL block copolymers which stabilize inverse and direct emulsions, so that one gene is statistically encapsulated into one double emulsion droplet. After enzyme compartmentalization droplets can be sorted by flow cytometry according to the activity of produced enzyme. These model reactors exhibit enhanced stability and reduced leakage of the components due to the crystalline-like state of the shells, and possess an advantageous bio- and thermal-degradability of the interfacial PEO-PCL layer. Therefore, these systems have high potential in directed enzyme evolution.