Watching Catalytic Single-Chain Polymeric Nanoparticles at Work

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Folding of single synthetic polymer chains into nanoparticles has emerged as a novel approach to attain defined polymer architectures.[1] Functional catalytic single-chain polymeric nanoparticles (SCPNs) have gained a lot of research interest since they resemble a first step to access artificial enzymes. Our group has developed a strategy to achieve water-soluble SCPNs that can perform catalysis in aqueous solution.[2] While several examples of SCPNs with efficient catalysis in water have been reported, they are still far from satisfactory to reach the performance exhibited by enzymes. In order to optimize the SCPN platform further, it is crucial to obtain more detailed understanding about the systems that are already established. However, approaches that could directly monitor the catalysis of SCPNs are yet to be introduced.

Single-molecule fluorescence microscopy (SMFM) has emerged as potential approach for studying the process of catalysis, especially for investigating enzymatic reactions. Using fluorogenic substrates, enzymatic reactions can be observed in real-time with single-turnover resolution.[3] Regarding certain similarity between the catalytic SCPNs and enzymes, we envision that SMFM could be a powerful tool for investigating the catalytic SCPNs in detail. Here we show our initial effort in applying SMFM on catalytic SCPNs, including the progress in molecular design of SCPNs, optimization of substrates, as well as practical protocols for performing SMFM experiments. These results will lay the foundation for the further investigation of the SCPN catalysis in single-molecule level. We further highlight the current limitations of the system and provide an outlook for future research.

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[2] Liu, Y. *et al., Journal of the American Chemical Society* **2015**, 137(40), 13096-13105. *Modular Synthetic Platform for the Construction of Functional Single-Chain Polymeric Nanoparticles: From Aqueous Catalysis to Photosensitization.*

[3] Turunen, P. *et al., FEBS Letters* **2014**, 588, 3553-3563. *Single-enzyme kinetics with fluorogenic substrates: lessons learnt and future directions.*.

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