Site-specific protein-polymer conjugate micelles with dramatically enhanced pharmacology

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Protein drugs are increasingly used in the clinical treatment of various diseases including cancer and infectious diseases because of their specificity and high biological activity [1]. Nevertheless, the efficacy of protein drugs is constrained by their relatively short *in vivo* circulation half-life [2]. As a result, frequent administration at high concentrations is often required, leading to undesirable side effects and poor patient compliance [3].

Conjugating hydrophilic and stealth polymers to proteins has long been proven effective for improving the *in vivo* properties including circulation half-life, biodistribution, and even final therapeutic efficacy [4]. In the past decades, many synthetic polymers like poly(ethylene glycol) (PEG), poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA), poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) and biopolymers like polypeptides and polysaccharides have been employed for the purpose [5]. For instance, two PEGylated recombinant human interferon alpha (IFN- α), PEG-Intron from Schering-Plough and PEGASYS from Roche [6,7], are FDA-approved long-acting IFN- α . PEG-Intron is composed of IFN- α 2b and a linear PEG of 12 kDa, while PEGASYS consists of IFN- α 2a and a branched PEG of 40 kDa. However, both products are non-specifically labeled. As a result, the non-specific PEGylation leads to a heterogeneous product mixture with dramatically reduced bioactivity. For example, PEGASYS was inclusive of at least 9 positional isomers with significantly different bioactivities, respectively [8]. Hence, site-specific conjugation between protein and polymer represents an emerging direction in the development of long-acting protein drugs. On the other hand, the polymer conjugation onto protein usually leads to the decrease of bioactivity largely due to the physical shielding effect. Furthermore, this effect would grow with the increase in the chain length of conjugated polymer, even though a

longer conjugated polymer usually means a better *in vivo* pharmacokinetics behavior [9]. Thus, it falls into a dilemma of how to balance the bioactivity and *in vivo* pharmacokinetics.

In a recent publication of *Journal of the American Chemical Society* [10], Prof. Weiping Gao from Tsinghua University, put forward such a novel strategy called “Site-specific *in situ* polymerization induced self-assembly, SI-PISA”, to simultaneously solve the site-specific conjugation issue and the contradiction between bioactivity and *in vivo* pharmacokinetics. For the first time, they combined the polymerization induced self-assembly (PISA) and site-specific *in situ* growth (SIG) to grow an amphiphilic block copolymer (POEGMA-PPHMA) directly from the C-terminus of IFN- α , forming IFN-POEGMA-PPHMA conjugate micelles *in situ* via the supramolecular self-assembly mechanism (Fig. 1). The self-assembled protein-polymer conjugate micelles possessed a uniform size around 100 nm, which was confirmed by several methods including dynamic light scattering (DLS), atom force microscopy (AFM) and cryo-transmission electron microscopy (Cryo-TEM). It is noteworthy that the *in vitro* bioactivity of the micelles was 21.5-fold higher than that of PEGASYS. Moreover, the micelles also exhibited a longer *in vivo* half-life of 83.8 h, which was 100- and 1.7-fold longer than those of IFN- α (0.8 h) and PEGASYS (49.5 h), respectively. The combination of high bioactivity and long circulation half-life ended almost completely inhibition effect on the growth of subcutaneous xenografts of human ovarian tumor in nude mice without obvious systemic toxicity, while PEGASYS and IFN- α had much weaker inhibition effect toward tumor growth. These encouraging results may provide a promising possibility for the final treatment of cancer and viral diseases.

Therefore, these findings indicate that SI-PISA strategy may open up new opportunities for the development of

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Figure 1 Scheme for the synthesis of an IFN-POEGMA-PHPMA micelle via site-specific *in situ* polymerization induced self-assembly (SI-PISA).

next-generation long-acting protein drugs. This work may also inspire more advanced chemistry technologies addressing unmet medical need in clinic.

Received 7 September 2018; accepted 7 September 2018;
published online 13 September 2018

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