Therapeutic Protein PEPylation: The Helix of Nonfouling Synthetic Polypeptides Minimizes Antidrug Antibody Generation

Yingqin Hou,† Yu Zhou,† Hao Wang,† Jialing Sun,‡ Ruijue Wang,† Kai Sheng,† Jingsong Yuan,† Yali Hu,‡ Yu Chao,§ Zhuang Liu,§ and Hua Lu*†

†Beijing National Laboratory for Molecular Sciences, Center for Soft Matter Science and Engineering, Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, People’s Republic of China
‡Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, People’s Republic of China
§Institute of Functional Nano & Soft Materials (FUNSOM), Collaborative Innovation Center of Suzhou Nano Science and Technology, Soochow University, Suzhou, Jiangsu 215123, China

Supporting Information

ABSTRACT: Polymer conjugation is a clinically proven approach to generate long acting protein drugs with decreased immune responses. Although poly(ethylene glycol) (PEG) is one of the most commonly used conjugation partners due to its unstructured conformation, its therapeutic application is limited by its poor biodegradability, propensity to induce an anti-PEG immune response, and the resultant accelerated blood clearance (ABC) effect. Moreover, the prevailing preference of unstructured polymers for protein conjugation still lacks strong animal data support with appropriate control reagents. By using two biodegradable synthetic polypeptides with similar structural compositions (l-P(EG3Glu) and dl-P(EG3Glu)) for site-specific protein modification, in the current study, we systematically investigate the effect of the polymer conformation on the in vivo pharmacological performances of the resulting conjugates. Our results reveal that the conjugate L20K-IFN, interferon (IFN) modified with the helical polypeptide l-P(EG3Glu) shows improved binding affinity, in vitro antiproliferative activity, and in vivo efficacy compared to those modified with the unstructured polypeptide analogue dl-P(EG3Glu) or PEG. Moreover, L20K-IFN triggered significantly less antidrug and antipolymer antibodies than the other two. Importantly, the unusual findings observed in the IFN series are reproduced in a human growth hormone (GH) conjugate series. Subcutaneously infused L20K-GH, GH modified with l-P(EG3Glu), evokes considerably less anti-GH and antipolymer antibodies compared to those modified with dl-P(EG3Glu) or PEG (dl20K-GH or PEG20K~G). As a result, repeated injections of dl20K-GH or PEG20K~G, but not L20K-GH, result in a clear ABC effect and significantly diminished drug availability in the blood. Meanwhile, immature mouse bone marrow cells incubated with the helical L20K-GH exhibit decreased drug uptake and secretion of proinflammatory cytokines compared to those treated with one of the other two GH conjugates bearing unstructured polymers. Taken together, the current study highlights an urgent necessity to systematically reassess the pros and cons of choosing unstructured polymers for protein conjugation. Furthermore, our results also lay the foundation for the development of next-generation biohybrid drugs based on helical synthetic polypeptides.

INTRODUCTION

Therapeutic proteins are important biologics that frequently exhibit high potency and selectivity. However, their clinical use has been hampered by their rapid renal clearance, susceptibility to proteolysis, and strong immunogenicity.1−3 Particularly, the generation of antidrug antibodies (ADAs) has been a serious hurdle for many protein drugs.4 One proven strategy to overcome these limitations is to covalently conjugate the protein of interest to polymers such as poly(ethylene glycol) (PEG), a process known as PEGylation, which can lead to significantly increased hydrodynamic volume, in vivo stability, and circulation half-life.5−10 However, there is mounting evidence that PEGylated proteins tend to show poorer binding affinity and biological activity than their unconjugated equivalents.11,12 Furthermore, although one of the initial purposes of PEGylation is for reduced ADA generation, PEG is known to elicit anti-PEG antibodies that adversely accelerate the blood clearance of the PEGylated proteins or nanoparticles, known as the ABC effect. As evidence, reduction in the therapeutic efficacy of many PEGylated proteins, such as uricase, asparaginase, and interferon (IFN), has been found to strongly correlate with the occurrence of the anti-PEG immune response that they induce.13 More worrisome is the fact that...
the percentage of healthy adults carrying pre-existing anti-PEG antibodies has increased sharply from 0.2% to 42% during the past three decades, likely because of their daily exposure to PEG-containing commodities. Thus, a pressing need in this field is seeking new polymers beyond PEGylation.

In recent years, researchers have investigated a wide range of alternative conjugation partners, including zwitterionic polymers, polyglycerol, glycopolymers, and oligo-EGylated poly(meth)acrylates, with varying degrees of success. Despite the potential of these methods, the lack of biodegradability has remained a central problem. Synthetic polypeptides have been increasingly considered as a biodegradable and biocompatible alternative to PEG with great clinical promise. There has been evidence that the genetic fusion of therapeutic proteins/peptides to intrinsically disordered polypeptides, such as XTEN, PAS, and elastin-like polypeptides (ELP), can lead to improved pharmacological performance in vivo. We envisage that the chemical modification of proteins by synthetic polypeptides, which we call PEPylation, could open up enormous possibilities.

Particularly, the chemical diversity of synthetic polypeptides has been greatly expanded by incorporating noncanonical amino acids via ring-opening polymerization (ROP) of α-amino acid N-carboxyanhydrides (NCA) and utilizing D-amino acids. Notably, during the preparation of this manuscript, Jiang et al. reported the nonspecific grafting of zwitterionic polypeptides to uricase, which showed extraordinarily low immunogenicity and outstanding safety profile in vivo. Their work underscored the exceptional clinical potential of PEPylation.

When surveying the aforementioned polymers for protein modification, one can easily draw the conclusion that unstructured and flexible polymers (e.g., PEG) have long been the preferred conjugation partners due to their ability to augment the hydrodynamic volume of the modified protein and provide an excellent stealth effect that minimizes renal toxicity.
IgG (C) and IgM (D) contents in the sera immunized with various polymer-IFN conjugates; for each polymer-of-interest, the ELISA plates were coated with wt-IFN and then incubated with 10^4-fold (for IgG) or 500-fold (for IgM) prediluted sera in PBS. (C) Antipolymer ELISA assays using free DL-P(EG3Glu) (E) or PEG (F) as the competition agent; sera immunized with DL20K-IFN or PEG20K−IFN (week 4) were prediluted 200-fold and incubated with the corresponding free polymer at gradient concentrations. Immunization protocol: rats were s.c. infused with L20K-IFN, DL20K-IFN, or PEG20K−IFN at a weekly dose 0.2 mg/kg for 4 weeks; sera were drawn from the rats (n = 3) every week starting from week 0. For ELISA analysis, after sera incubation and washing, all plates were incubated with antimouse IgG-HRP or IgM-HRP, and analyzed by TMB solution (CWBIO). TWEEN was excluded from the washers in all antipolymer ELISA studies. Data are expressed as mean ± SD.

**RESULTS**

**Synthesis and Characterization of Different IFN−Polymer Conjugates.** Recombinant IFN, an antiviral and antitumor cytokine, was selected as our first model drug. For a fair comparison, we synthesized two chemically similar but conformationally varied polypeptides (Scheme 1). 4,35 Specifically, monomer γ-(2-(2-(2-methoxyethoxy)ethoxy)ethyl L-glutamate NCA 39 (L-EG3GluNCA) was polymerized by trimethylsilyl phenylsulfide (PhS-TMS) to yield phenyl thioester-functionalized L-P(EGGlu) (Scheme 1). Similarly, DL-P(EGGlu) was produced from a racemic mixture of DL-EGGluNCA. The molecular weights (MW) of both polymers were carefully controlled to be ∼20 kDa, in line with many clinically approved PEG conjugates. Gel permeation chromatography (GPC) indicated that the two polymers had a similar MW ≈ 22−23 kDa and narrow dispersity (D) below 1.05 (Figure S1). 1H NMR spectroscopy showed that the two polymers differed in the chemical shift of the α-H due to the different α-C chirality (Figure S2). As expected, circular dichroism (CD) spectroscopy revealed that α-helices constituted more than 90% of L-P(EGGlu), whereas DL-P(EGGlu) was unstructured as designed (Figure S2). Subsequently, we conjugated each synthetic polypeptide to an IFN mutant bearing a N-terminal cysteine (Cys-IFN) via native chemical ligation, thereby forming two PEPylated IFNs denoted as L20K−IFN and DL20K−IFN (Scheme 1). We also generated PEG20K−IFN as a positive control by attaching a thioester-functionalized PEG (MW ∼ 20 kDa) to IFN via the same method (Scheme 1 and Figure S4).

All purified IFN conjugates exhibited a narrow size distribution based on SDS-PAGE analysis (Figure 1A). L20K−IFN coated with the corresponding polymer-GH conjugate. (E)}
IFN and DL20K-IFN shared an almost identical apparent MW, whereas PEG20K-IFN appeared to electrophoresize slightly slower than its PEPylated counterparts but was still comparable (Figure 1A). CD spectroscopy suggested that PEG20K-IFN and DL20K-IFN were similar in helicity as wt-IFN, whereas L20K-IFN produced a stronger helical signal intensity (Figure 1B). A thermo-fluoro assay indicated that L20K-IFN possessed a higher Tm, and therefore greater thermostability, than both DL20K-IFN and PEG20K−IFN (Figure 1C). All conjugates were shown to be significantly more resistant to proteolysis than wt-IFN in trypsin digestion assays (Figure S5).

Surface plasmon resonance (SPR) found the K_D values for the binding of L20K−IFN, DL20K−IFN, and PEG20K−IFN to human IFNAR2 were 5.8, 19.6, and 15.9 nM, respectively (Table 1 and Figure S6). Thus, L20K−IFN appeared to be ~3–4 fold more efficient in its receptor interaction than DL20K−IFN or PEG20K−IFN. Consistently, an in vitro viability assay demonstrated that the IC50 values of L20K−IFN, DL20K−IFN, and PEG20K−IFN against Daudi cells, an IFN-sensitive human cancer cell line, were 36, 160, and 190 pg/mL, respectively (Table 1). This implied that L20K−IFN could induce a significantly more potent antitumor effect than DL20K−IFN or PEG20K−IFN does.

In Vivo Pharmacological Performances of IFN Conjugates. We next measured the pharmacokinetic parameters of the IFN variants in female Sprague–Dawley rats. As shown in Figure 1D and Table 1, the elimination half-lives (t_1/2) of L20K−IFN, DL20K−IFN, and PEG20K−IFN were 9.6, 7.8, and 9.8 h, respectively, all significantly longer than the 0.5 h t_1/2 of wt-IFN. Interestingly, L20K−IFN was slightly but consistently longer-lived than DL20K−IFN (P value < 0.05; reproducible in at least two independent experiments with different batches of materials). This was further evidenced by the greater AUC of L20K−IFN than that of DL20K−IFN (Table 1). The in vivo efficacy of the conjugates was further evaluated in two murine models, one bearing OVCAR-3 tumor xenografts and the other xenografts derived from a prostate cancer patient (PDX) (see Materials and Methods). In both cases, administration of L20K−IFN, which carried the helical L-P(EG3Glu), led to significantly slower tumor growth (Figure 1A). The superior antitumor efficacy was further confirmed by the reduced tumor cell proliferation activity according to Ki-67 staining (Figure S7). No body weight loss was observed in either model during the treatment with L20K−IFN, suggesting that the drug was well tolerated under the regimen that we employed (Figure S8).
Antibody Generation Triggered by IFN Conjugates.
To investigate the immune response of the conjugates, Sprague−Dawley rats were randomly grouped and subcutaneously administrated with L20K-IFN, DL20K-IFN, or PEG20K-IFN at a weekly dose of 0.2 mg/kg. Interestingly, sera from the mice immunized with L20K-IFN showed significantly lower levels of anti-IFN IgG and IgM than those receiving DL20K-IFN or PEG20K-IFN (Figure 2A−B). Serial dilution of sera from week 4 revealed that L20K-IFN produced ∼50−100 fold lower anti-IFN IgG and ∼5−10 fold lower IgM titers than those immunized with DL20K-IFN or PEG20K-IFN (Figure S9). In addition, injection with DL20K-IFN or PEG20K-IFN appeared to also induce a detectable amount of antipolymer antibodies, particularly IgM (Figure 2C−D). The specificity of the antipolymer antibodies in DL20K-IFN and PEG20K-IFN sera was further validated by the corresponding polymer competition (Figure 2E−F). Strikingly, we discovered that L20K-IFN exhibited almost no detectable effect on the serum level of antipolymer IgG or IgM in the immunized rats.

Synthesis of and Immune Responses Triggered by Different GH−Polymer Conjugates. To test whether our findings observed in the IFN conjugates were also applicable to other therapeutic proteins, we selected human growth hormone (GH) as our second example and engineered the protein with an N-terminal cysteine (Cys-GH), similar to that in Cys-IFN. We next covalently tethered L-P(EG3Glu), DL-P(EG3Glu), and PEG separately to Cys-GH to generate three conjugates denoted as L20K-GH, DL20K-GH, and PEG20K-GH, respectively (Scheme 1 and Figure 3A). Trypsin digestion revealed that L20K-GH was significantly more resistant to proteolysis than DL20K-GH and PEG20K-GH (Figure 3B). Furthermore, injection with L20K-GH provoked substantially less production of anti-GH IgG and IgM antibodies in rats from week 2, compared to treatment with DL20K-GH or PEG20K-GH (Figure 3C−D). Serial dilution of sera from week 4 revealed that L20K-GH produced ∼100 fold lower anti-GH IgG and ∼20-fold lower IgM titers than those immunized with DL20K-GH or PEG20K-GH (Figure S10). The same trend was observed when we measured the levels of antipolymer IgG and IgM following the immunization (Figure 3E−F and Figure S11). To examine the ABC effect, we measured the blood concentration of GH at selected time points after the first and third injection of each conjugate. The results demonstrated that infusions of L20K-GH led to very similar blood levels of GH during the first 12 h and generated almost no ABC effect in 24 h (Figure 3G, statistically insignificant). In sharp contrast, both DL20K-GH and PEG20K-GH caused a characteristic ABC effect after the third injection (Figure 3H−I). In fact, our ELISA kit failed to detect blood GH at 24 h following the administration of DL20K-GH or PEG20K-GH (Figure 3G−I). As a result, the AUC0−24h of L20K-GH were comparable after the first and third injection (100% vs 112%), whereas the AUC0−24h of both DL20K-GH and PEG20K-GH after the third infusion were only ∼6% of those after the first drug infusion (Table S1).

BMDC Uptake and Activation. During antibody production, the antigens are usually internalized, fragmented...
in lysosome, and displayed on the cell surface by dendritic cells (DCs) to trigger downstream T cell and B cell response. To understand the different antibody responses triggered by the conjugates, we sought to examine the very first DC internalization step. For this, we incubated the GH conjugates with freshly induced immature mouse bone marrow-derived dendritic cells (BMDCs), which are widely used for the assessment of antigen presenting.43 Flow cytometric analysis found clear evidence for the internalization of DL20K-GH and PEG20K–GH into BMDCs after 12 h of incubation, whereas the uptake level of L20K-GH was considerably lower (Figure 4A). Consistently, treatment of BMDCs with L20K-GH resulted in appreciably less secretion of proinflammatory cytokines, including interleukine-6 (IL-6, Figure 4B), interferon-γ (IFN-γ, Figure 4C), and tumor necrosis factor (TNF, Figure 4D), compared to the other two GH conjugates carrying unstructured polymers.

**DISCUSSION**

The conjugation of polymers to a protein has been demonstrated to extend its half-time by increasing its hydrodynamic volume and mitigating the ADA generation.1 However, the role that the secondary conformation of a polymer plays in the resultant protein conjugate has been very rarely investigated, as unstructured polymers have been the heavily favored choice in past studies. Notably, the polypeptide–uricase conjugate reported by Jiang focused on the zwitterionic side chain without studying the secondary conformation effect.36 We speculated that peptide-based drugs and biomaterials covalently modified with α-helical polypeptides could exhibit improved proteolytic and thermal stability, binding, as well as other biological functions over those conjugated with disordered polymers.37,44–47 To ascertain whether this is the case, however, one needs to employ polymers that only differ in conformation. Gratifyingly, controlled NCA ROP and chemoselective labeling collaboratively enabled us to generate protein conjugates that shared almost identical modification sites and MWs, and were attached to nearly the same number of polypeptides with highly similar chemical compositions.34 As a result, the secondary conformation of the tethered polypeptides became the only major variable. This was corroborated by the GPC curves of the polymers and the narrow size distributions of the resultant conjugates on the SDS-PAGE gel (Figure S1, Figures 1A and 3A). Of note, due to the distinct chemical structures of PEG and our P(E,G,Glu), the migration of those conjugates in SDS-PAGE gel might not completely correlate their MWs, however, the only major variable. This was corroborated by the GPC results shown (Figure S1). The results lent further evidence to the generality of the helix effect. Moreover, the above study help ruling out the possibility of D-amino acid-induced antibody production in the cases of DL20K-IFN and DL20K-GH. Although the exact mechanistic role of helicity remains insufficiently explored, a number of reasons may count for the unexpected findings. First of all, the helical 1-P(E,G,Glu) seems to provide better antifouling property than DL-P(E,G,Glu) and PEG, and thus minimizing nonspecific internalization with cells and proteins. Our initial investigation provided preliminary evidence of conformation-dependent internalization and activation of immature BMDCs for those examined protein–polypeptide conjugates. In fact, this helical conjugation enhanced antifouling and anticell adhesion was also observed when the polypeptides were anchored on gold surfaces.56 Second, helical polypeptides are well-known more proteolytic stable (Figure 3B) than those unstructured peptidyl analogues, which may lead to inefficient fragmentation and MHC presentation after BMDC internalization. More rigorous experimental and modeling studies are currently ongoing to fully uncover the molecular mechanism of the unusual helical conformation effect.

**CONCLUSIONS**

In conclusion, we generated synthetic polypeptides that only differed in conformation and compared their effects on the in vivo therapeutic and immunological properties of the protein drugs to which they were conjugated. Compared with the unstructured DL-P(E,G,Glu) or PEG, the covalent attachment of the helical 1-P(E,G,Glu) to therapeutic proteins (namely, IFN and GH) led to substantial improvement in a variety of pharmacological properties, such as binding affinity, stability, and in vivo efficacy. Most interestingly, the helical 1-P(E,G,Glu)-conjugated IFN and GH elicited a significantly milder immune response and exhibited a much weaker ABC effect than those modified with unstructured polymers. Thus, the helical nonfouling polypeptides that we employed could be excellent alternatives to PEG for mitigating the antibody response to repeatedly administered therapeutic proteins, though whether similar benefits apply to more immunogenic foreign proteins requires further validation. Moreover, our results suggested that the helical conformation of the synthetic nonfouling polypeptides played an important role in minimizing/delaying this antibody response. Taken together, the current study highlighted an urgent necessity to systemically reassess the pros and cons of choosing unstructured polymers for protein conjugation. Furthermore, our results also recognition and the generation of anti-PEG antibodies.13,48 In the clinic, the anti-IFN neutralizing antibodies has previously been observed in nonresponding patients and believed to be the major reason for their development of resistance.49 In this regard, it was remarkable that the administration of L20K-IFN provoked substantially lower production of anti-IFN, as well as antipolymer IgG and IgM, than DL20K-IFN or PEG20K–IFN (Figure 2). Importantly, similar results were also obtained from the GH conjugates, indicating that the benefits we observed were independent of the modified protein (Figure 3). We also synthesized a left-handed helical polypeptide α-P(E,G,Glu) (~23 kDa) and produced two conjugates, D20K-IFN and D20K-GH (data not shown). We discovered that both D20K-IFN and D20K-GH, similar to L20K-IFN or L20K-GH, showed almost no antibody response after repeated administration (data not shown). The results lend further evidence to the generality of the helix effect. Moreover, the above study help ruling out the possibility of D-amino acid-induced antibody production in the cases of DL20K-IFN and DL20K-GH. Although the exact mechanistic role of helicity remains insufficiently explored, a number of reasons may count for the unexpected findings. First of all, the helical 1-P(E,G,Glu) seems to provide better antifouling property than DL-P(E,G,Glu) and PEG, and thus minimizing nonspecific internalization with cells and proteins. Our initial investigation provided preliminary evidence of conformation-dependent internalization and activation of immature BMDCs for those examined protein–polypeptide conjugates. In fact, this helical conjugation enhanced antifouling and anticell adhesion was also observed when the polypeptides were anchored on gold surfaces.56 Second, helical polypeptides are well-known more proteolytic stable (Figure 3B) than those unstructured peptidyl analogues, which may lead to inefficient fragmentation and MHC presentation after BMDC internalization. More rigorous experimental and modeling studies are currently ongoing to fully uncover the molecular mechanism of the unusual helical conformation effect.

**REFERENCES**

1. S. Bhardwaj, S. K. Tripathi, and J. Sagar, Journal of Liposome Research, 2001, 11, 399.
2. P. L. F. Ferreira, T. W. L. da Silva, and N. C. L. Teixeira, Journal of the American Chemical Society, 2002, 124, 8659.
lay the foundation for the development of next-generation biohybrid drugs based on helical synthetic polypeptides.

**ASSOCIATED CONTENT**

* Supporting Information
  The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.8b00548.

**REFERENCES**

(9) Dumas, A.; Spicer, C. D.; Gao, Z.; Takehana, T.; Lin, Y. A.; Yasukohchi, T.; Davis, B. G. Self-ligated Suzuki-Miyaura coupling of site-selective protein PEylation. *Angew. Chem., Int. Ed.* 2013, 52 (14), 3916–3921.

(10) Pasut, G.; Veronese, F. M. State of the art in PEylation: the great versatility achieved after forty years of research. *J. Controlled Release* 2012, 161 (2), 461–472.

(11) Fishburn, C. S. The pharmacology of PEylation: Balancing PD with PK to generate novel therapeutics. *J. Pharm. Sci.* 2008, 97 (10), 4167–4183.

(12) Gauthier, M. A.; Klok, H. A. Polymer-protein conjugates: an enzymatic activity perspective. *Polym. Chem.* 2010, 1 (9), 1352–1373.

(13) Zhang, P.; Sun, F.; Liu, S. J.; Jiang, S. Y. Anti-PEG antibodies in the clinic: Current issues and beyond PEylation. *J. Controlled Release* 2016, 244, 184–193.

(14) Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew. Chem., Int. Ed.* 2010, 49 (36), 6288–6308.

(15) Liu, S. J.; Jiang, S. Y. Chemical conjugation of zwitterionic polymers protects immunogenic enzyme and preserves bioactivity without polymer-specific antibody response. *Nano Today* 2016, 11 (3), 285–291.

(16) Keefe, A. J.; Jiang, S. Poly(zwitterionic)protein conjugates offer increased stability without sacrificing binding affinity or bioactivity. *Nat. Chem.* 2012, 4 (1), 59–63.

(17) Frey, H.; Haag, R. Dendritic polyglycerol: a new versatile biocompatible-material. *Rev. Mol. Biotechnol.* 2002, 90 (3–4), 257–267.

(18) Mancini, R. J.; Lee, J.; Maynard, H. D. Trehalose glycopolymers for stabilization of protein conjugates to environmental stressors. *J. Am. Chem. Soc.* 2012, 134 (20), 8474–8479.

(19) Liu, M.; Johansen, P.; Zabel, F.; Leroux, J. C.; Gauthier, M. A. Semi-permeable coatings fabricated from comb-polymers efficiently protect proteins in vivo. *Nat. Commun.* 2014, 5, 5526.

(20) Gao, W. P.; Liu, W. G.; Mackay, J. A.; Zalutschy, M. R.; Toone, E. J.; Chilkoti, A. In situ growth of a stoichiometric PEG-like conjugate at a protein’s N-terminus with significantly improved pharmacokinetics. *Proc. Natl. Acad. Sci. U. S. A.* 2009, 106 (36), 15231–15236.

(21) Deming, T. J. Synthesis of side-chain modified polyglycerides. *Chem. Rev.* 2016, 116 (3), 786–808.

(22) Talelli, M.; Vicent, M. J. Reduction sensitive poly(l-glutamic acid) (PGA)-protein conjugates designed for polymer masked-unmasked protein therapy. *Biomacromolecules* 2014, 15 (11), 4168–4177.

(23) Lu, Y. J.; Mpong, G. N. N.; Liu, P.; Chan, C.; Cai, Z. L.; Weinrich, D.; Boyle, A. J.; Reilly, R. M.; Winnik, M. A. Synthetic polyglyceramide-based metal-chelating polymers and their site-specific conjugation to trastuzumab for auger electron radioimmunotherapy. *Biomacromolecules* 2014, 15 (6), 2027–2037.

(24) Song, Z.; Han, Z.; Lu, S.; Chen, C.; Chen, L.; Yin, L.; Cheng, J. Synthetic polypeptides: from polymer design to supramolecular assembly and biomedical application. *Chem. Soc. Rev.* 2017, 46 (21), 6570–6599.

(25) Kramer, J. R.; Ono, B.; Bustamante, C.; Bertozzi, C. R. Chemically tunable mucin chimeras assembled on living cells. *Proc. Natl. Acad. Sci. U. S. A.* 2015, 112 (41), 12574–12579.

(26) Schellenberger, V.; Wang, C. W.; Geething, N. C.; Spink, B. J.; Campbell, A.; To, W.; Scholle, M. D.; Yin, Y.; Yao, Y.; Bogen, O.; Cleland, J. L.; Silverman, J.; Stemmer, W. P. C. A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner. *Nat. Biotechnol.* 2009, 27 (12), 1186–1195.

(27) Hu, J.; Wang, G. L.; Liu, X. Y.; Gao, W. P. Enhancing pharmacokinetics, tumor accumulation, and antitumor efficacy by elastin-like polypeptide fusion of interferon alpha. *Adv. Mater.* 2016, 27 (45), 7320–7324.

(28) Lugubniuk, K. M.; Schaal, J. L.; Umstead, B.; Masria, E. M.; Li, X.; Banskota, S.; Arnold, S.; Feinglos, M.; D’Alessio, D.; Chilkoti, A. One-week glucose control via zero-order release kinetics from an injectable depot of glucagon-like peptide-1 fused to a thermosensitive biopolymer. *Nat. Biomed. Eng.* 2017, 1, 0078.
Manning, A. M.; Fotouhi, N.; Nash, H.; Vassilev, L. T.; Sawyer, T. K. Stapled alpha-helical peptide drug development: a potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy. Proc. Natl. Acad. Sci. U. S. A. 2013, 110 (36), E3445−E3454.

Moelling, R. E.; Cornejo, M.; Davis, T. N.; Del Bianco, C.; Aster, J. C.; Blacklow, S. C.; Kung, A. L.; Gilliland, D. G.; Verdin, G. L.; Bradner, J. E. Direct inhibition of the NOTCH transcription factor complex. Nature 2009, 462 (7270), 182−188.

Walenk, L. D.; Bird, G. H. Hydrocarbon-stapled peptides: principles, practice, and progress. J. Med. Chem. 2014, 57 (15), 6275−6288.

(47) Mochida, Y.; Cabral, H.; Miura, Y.; Albertini, F.; Fukushima, S.; Osada, K.; Nishiyama, N.; Kataoka, K. Bundled assembly of helical nanostructures in polymeric micelles loaded with platinum drugs enhancing therapeutic efficiency against pancreatic tumor. ACS Nano 2014, 8 (7), 6724−6738.

Kierstead, P. H.; Okochi, H.; Venditto, V. J.; Chuang, T. C.; Kivimae, S.; Frechet, J. M. J.; Szoka, F. C. The effect of polymer backbone chemistry on the induction of the accelerated blood clearance in polymer modified liposomes. J. Controlled Release 2015, 213, 1−9.

(49) van der Eijk, A. A.; Vrolijk, J. M.; Haagmans, B. L. Antibodies neutralizing peginterferon alfa during retreatment of hepatitis C. N. Engl. J. Med. 2006, 354 (12), 1323−1324.

(50) Zhang, C.; Yuan, J.; Lu, J.; Hou, Y.; Xiong, W.; Lu, H. From neutral to zwitterionic poly(alpha-amino acid) nonfouling surfaces: Effects of helical conformation and anchoring orientation. Biomaterials 2018, 178, 728−737.