Origin of Proteolytic Stability of Peptide-Brush Polymers as Globular Proteomimetics

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ABSTRACT: Peptide-brush polymers (PBPs), wherein every side-chain of the polymers is peptidic, represent a new class of proteomimetic with unusually high proteolytic resistance while maintaining bioactivity. Here, we sought to determine the origin of this behavior and to assess its generality via a combined theory and experimental approach. A series of PBPs with various polymer backbone structures were prepared and examined for their proteolytic stability and bioactivity. We discovered that an increase in the hydrophobicity of the polymer backbones is predictive of an elevation in proteolytic stability of the side-chain peptides. Computer simulations, together with small-angle X-ray scattering (SAXS) analysis, revealed globular morphologies for these polymers, in which pendant peptides condense around hydrophobic synthetic polymer backbones driven by the hydrophobic effect. As the hydrophobicity of the polymer backbones increases, the extent of solvent exposure of peptide cleavage sites decreases, reducing their accessibility to proteolytic enzymes. This study provides insight into the important factors driving PBP aqueous-phase structures to behave as globular, synthetic polymer-based proteomimetics.

INTRODUCTION

Peptides are relatively short chains of amino acids that play crucial roles in human physiology, for example, as hormones, and represent some of the most promising candidates for therapeutics because of their biocompatibility, high selectivity, predictable metabolism, and a high degree of modularity in molecular design.1−5 However, the development of therapeutic peptides presents established challenges that include their lack of cell penetration, lack of multivalency, low molecular weight, and short half-life in biological systems due to proteolytic digestion and clearance.4,5 Indeed, digestive, or hydrolytic proteases, are ubiquitous, from intracellular organelles (lysosomes) to organs and tissues including in the digestive system and blood.6,7

The design of proteolytically stable peptides typically relies on chemical modification of the amino acid composition and sequence.8−11 Approaches include cyclization, implementation of α-amino acids, conjugation with polyethylene glycol, N-methylation, and substitutions, rendering peptides unrecognizable by, or inaccessible to, the active sites of proteases.12 Moreover, peptidomimetic strategies are time-consuming and often alter or decrease bioactivity compared to the original peptide sequence.13 It is therefore desirable to employ simple, generalizable modifications amenable to arbitrary peptide sequences that render them resistant to degradation without altering their bioactivity.

Peptide-brush polymers (PBPs), where every repeating unit consists of a peptide side-chain, represent an important class of cylindrical polymer brushes.14−19 These can be accessed via either graft-through polymerization of sequence-defined peptide-based monomers14,20−24 or graft-from polymerization of N-carboxyanhydrides on macroinitiators.15,16 In particular, our lab has observed that norbornenyl-modified peptides polymerized via ring-opening metathesis polymerization (ROMP) yield brush polymers with exceptional resistance to proteolytic digestion while retaining bioactivity.20,21 Herein, we direct our attention to the structure−property relationship of this class of brush polymer, wherein every monomer is peptide-modified and incorporated via graft-through polymerization, and ask specific questions: (i) Is the observed proteolytic stability a general phenomenon or one that is specific to certain privileged polymer backbone chemistries? (ii) What are the conformations adopted by the resulting polymers relative to one another? (iii) What structural and chemical parameters of the polymer backbones elicit proteolytic stability of the side-
chain peptides? Understanding the structure of this type of brush polymer provides insight into their interactions with proteases and the design of multivalent peptide−polymer conjugates with tunable proteolytic stability and bioactivity that represent a novel class of synthetic, polymer-based proteomimetic, or protein-like polymer.

■ RESULTS AND DISCUSSION

To explore the role of polymer backbones in conferring proteolytic stability, we first designed PBPs with four different polymer backbones but with the same peptide sequence (Figure 1). Four peptide monomers were prepared by solid-phase peptide synthesis, giving rise to peptide acrylamide (PepAm), peptide methacrylamide (PepMAm), peptide norbornylamide (PepNorAm), and peptide norbornylimide (PepNorIm) (Figures 1, and S1). In each case, the peptide sequence used was a protease substrate (GPLGLAGGWGERDGS) for the zinc metalloproteinase, thermolysin (Figure 1a,b). Electrospray ionization mass spectrometry (ESI-MS) and reverse-phase high-performance liquid chromatography (RP-HPLC) verified the identity and purity of the monomers (Figures S2−6). The RP-HPLC elution time of the monomers increases in the order of PepAm, PepMAm, PepNorAm, and PepNorIm, suggesting an upward trend in their hydrophobicity (Figure S2). This increase in the hydrophobicity of the polymer backbones was, in turn, predicted by all-atom explicit solvent MD simulations, specifically, the interaction energies between PBP backbone atoms and between PBP molecules (Table S6).

Monomers were subjected to either ROMP for PepNorAm and PepNorIm or photoinduced reversible addition−fragmentation transfer radical polymerization (photo-RAFT) for PepAm and PepMAm (Figure S7). The excellent functional group tolerance of ROMP and photo-RAFT enabled the preparation of poly(PepNorIm), poly(PepNorAm), poly(PepMAm), and poly(PepAm) with similar degrees of polymerization as well as similarly narrow molecular weight distributions (Table S1). Nuclear magnetic resonance (NMR) spectroscopy confirmed the disappearance of monomer signals in the final solution (Figures S8−11). Additionally, organic-phase gel permeation chromatography (GPC) revealed that the molecular weights of all PBPs are on par with their theoretical values, as evidence for controlled ROMP and photo-RAFT polymerizations in each case (Figure S12 and Table S6).

Figure 1. Polymer backbone structures of peptide-brush polymers (PBPs) determine the proteolytic stability of the peptide side-chains. (a) Chemical structures of the set of PBPs (DP = 15). (b) Chemical structure of the peptide (amino acid sequence: GPLGLAGGWGERDGS) used in this study. This peptide is a thermolysin substrate that is selectively cleaved between glycine and leucine with the cleavage site highlighted in orange. (c) Gel permeation chromatography (GPC) traces of PBPs using phosphate-buffered saline (PBS) as the mobile phase. The molecular weights of polymers in PBS are similar to their theoretical values, indicating the predominance of single polymer chains (see Table S1 for detailed molecular weight information). (d) Schematic illustration of proteolytic digestion of peptide-brush polymers in the presence of thermolysin using a representative polymer poly(PepNorAm), and thermolysin and the free peptide revealed by HPLC (Figures S20−24). The molar concentrations of peptide substrate and thermolysin are 200 and 0.05 μM, respectively. The material poly(PepNorIm) exhibits the highest proteolytic stability among all polymers. Data displayed as mean ± standard deviation of three independent experiments. (f) Catalytic efficiency (kcat/Km) of thermolysin in proteolysis of PBPs derived from nonlinear Michaelis−Menten Kinetics (see Figure S25 and Table S3 for details). The catalytic efficiency of the thermolysin-induced cleavage of poly(PepNorIm) is markedly lower than all other polymers, indicative of the slowest proteolysis of poly(PepNorIm) against thermolysin.

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Aqueous-phase GPC was then used to assess the molecular weights of PBPs in phosphate-buffered saline (PBS) (Figure 1c). The molecular weights of PBPs agree well with their theoretical, targeted molecular weights (Table S1), supporting that PBPs are single-chain particles and are not aggregated in aqueous solution (i.e., PBS). Dynamic light scattering analysis further corroborated these observations, showing no aggregates over 10 nm in hydrodynamic diameter for all PBPs (Figure S13).

The nanoscale morphology of these PBPs was further examined using small-angle X-ray scattering (SAXS). Background-subtracted SAXS patterns for all PBPs displayed a distinct shoulder at intermediate values of \( q \) that is characteristic of compact, globular objects (Figure S14a,b). Indeed, the shape of these SAXS patterns qualitatively resembles the shape of the scattering pattern of the known globular protein bovine serum albumin.\(^{26-28}\) Kratyk plots of each PBP revealed bell-shaped curves with a clear local maximum, suggesting that each brush polymer has a compact structure generated by folded chains (Figure S14c).\(^{26,29}\) This conclusion was further supported by calculating the Stokes ratio, \( \rho = R_g/R_h \), for each PBP (Table S2). Generally, for the PBPs, \( \rho \) was approximately 0.776, consistent with the expected behavior for spherical particles, including globular proteins.\(^{30,31}\)

We next examined the susceptibility of the peptide side-chains to proteolytic degradation in the presence of the zinc metalloproteinase, thermolysin. For the parent, the free oligopeptide sequence, ESI-MS analysis confirmed that thermolysin selectively cleaves the amide bond between the C-terminus of glycine (G) and the N-terminus of leucine (L), leading to two fragments, GPLG and LAGGWGERDGS (Figures S15−17). Fast proteolysis kinetics of the free peptide was demonstrated by HPLC analysis, where 93% of the original peptide signal diminished after 10 min (Figures S18−20). The proteolysis kinetics of PBPs was then studied under the same conditions (Figure 1d). While PBPs generally exhibited modest proteolytic stability compared to the free thermolysin substrate peptide (Figure 1e), poly(PepNorIm\(_{15}\)) in particular exhibited especially slow digestion kinetics (Figures 1e and S21−24). The catalytic efficiency (\( k_{\text{cat}}/K_m \)) of thermolysin in cleaving PBP substrates decreases in the order of poly(PepAm\(_{15}\)), poly(PepMAm\(_{15}\)), poly(PepNorAm\(_{15}\)), and poly(PepNorIm\(_{15}\)) (Figures 1f and S25 and Table S3). This decrease in catalytic efficiency of thermolysin for these substrates is predicted by an increase in hydrophobicity of the polymer backbone and, hence, an increase in globularity of the aqueous, dissolved PBP single chains.

To further probe the structural origin of the proteolytic resistance of PBPs, we next conducted all-atom explicit solvent MD simulations, where one PBP chain was dissolved in an aqueous solution with 0.14 M NaCl to mimic the experimental PBS environment. Each simulation lasted 250 ns, with the periodic annealing applied for equilibration in the first 200 ns and the last 50 ns of production simulations for data collection. These simulations provided insight into the morphology of PBPs and the distribution of cleavage sites (glycine and leucine) within the polymer structures. The equilibrated PBPs exhibit globular micellar-like structures, with hydrophobic polymer backbones surrounded by hydrophilic peptides,
generally consistent with conformations collected from experimental SAXS data (Figures 2a and S26 and Table S4). The side-chain peptides are structureless as evidenced by the predominance (>56%) of random coil secondary structures for all PBPs (Table S5). The flexibility of PBPs was also examined through a calculation of root-mean-square deviation (RMSD, Figure S26). Polymer backbones exhibit small RMSDs of around 0.2 nm, similar to folded proteins,32,33 with pendant peptides displaying higher flexibility with RMSDs of approximately 0.7 nm, comparable to intrinsically disordered proteins.34

Inspection of the PBP cleavage sites (orange beads in Figure 2a) indicates that for poly(PepNorIm15), they are partially embedded in the interior of the globular structure, whereas the cleavage sites of the other three PBPs are more distributed. Using a protein-sized probe with a radius of 3.14 nm,20 the exposure of the PBP cleavage sites, described in terms of solvent-accessible surface area (SASA), was found to decrease in the following order: poly(PepAm15) > poly(PepMAm15) > poly(PepNorAm15) > poly(PepNorIm15) (Figure S2 and Table S6). In contrast, the SASA of the free peptide is much larger (Table S7). These simulation results indicate that an increase in polymer backbone hydrophobicity is a predictive driving force, accounting for peptide side-chains condensing in proximity to the polymer backbones, leading to less exposure of peptide cleavage sites. Specifically, poly(PepNorIm15) displayed the highest backbone hydrophobicity, driving a larger fraction of the cleavage sites to the core of the collapsed, globular structure. The limited exposure of peptide cleavage sites for poly(PepNorIm15) is evidenced by the strong primary correlation peak at a distance of approximately 4.2 Å on the radial distribution function between the cleavage sites (Figure 2c).

We next performed MARTINI coarse-grained, explicit solvent MD simulations on PBP–thermolysin complexes in aqueous solutions with 0.14 M NaCl for poly(PepNorIm15) and poly(PepMAm15) (Figures S27 and S28). The obtained interaction energies between PBPs and thermolysin showed that the cleavage sites of poly(PepNorIm15) negligibly interact with thermolysin active sites (−0.02 ± 0.02 kJ/mol), whereas much stronger interactions (−0.9 ± 0.7 kJ/mol) exist between poly(PepMAm15) cleavage sites and the thermolysin active site (Figure 2d,e). This is in line with experimental observations that poly(PepNorIm15) shows a lower PBP–thermolysin affinity than poly(PepMAm15) (Figure 1e,f and Table S3). The weaker interactions between poly(PepNorIm15) cleavage sites and thermolysin active sites (lower poly(PepNorIm15)–thermolysin affinity) can be ascribed to the limited exposure of the poly(PepNorIm15) cleavage sites (Figure 2b). For each PBP–thermolysin complex, the simulations last 18 μs, the last 9 μs of which was performed using the GoMartini 2.2 protein model35 to investigate a possible structural change in protein structure upon the binding of PBP to thermolysin (Figures S29.
and S30). Detailed calculations before and after the PBP–thermolysin binding event reveal that the structures of the protein and the PBPs were preserved, as evidenced by negligible changes in the RMSD of the backbones of the whole protein and its active site alone (Figures S30a,b) as well as the radius of gyration and the end-to-end distance of the PBP chains (Figure S30c−f). Therefore, the exposure of the PBP cleavage sites obtained by the all-atom simulations in the absence of protein (Figure 2b) might be employed in ranking the interactions between PBP cleavage sites and protein active sites (Figure 2d), which are associated with the proteolytic resistance of PBPs against proteases (Figure 1e,f). The structure–property relationship is thus established that an increase in PBP backbone hydrophobicity leads to decreased exposure of the PBP cleavage sites, which decreases their interaction with proteases, giving rise to enhanced proteolytic stability.

To experimentally test the predominance of the hydrophobic effect in driving protease stability of peptide side-chains in PBPs, we prepared a close structural analogue of poly(PepNorIm) using an oxanorbornene-based monomer with a bridging oxygen instead of a methylene (Figures S31−34). This poly(Oxa−PepNorIm) displays a similar polymer backbone rigidity but has a higher hydrophilicity compared to poly(PepNorIm).36,37 Proteolyis kinetics of poly(Oxa−PepNorIm) indicated that its stability is much lower than

Figure 4. Bioactivity of proapoptotic peptide-brush polymers before and after treatment with α-chymotrypsin. (a) Chemical structures of proapoptotic PBPs featuring a side-chain amino acid sequence: KLA\text{LAKLAKLAKLAK}, which can induce apoptosis in cancer cells. (b) Percentage of apoptotic cells analyzed by an annexin V/propidium iodide (PI) staining assay. HeLa cells were incubated with poly(MAm−KLA) (P1), poly(NorIm−KLA) (P2), TAT−KLA, and the respective chymotrypsin-pretreated materials at a concentration of 25 μM for peptides for 24 h. Data are presented as mean ± standard deviation (n = 3). Results shown for two-tailed student’s t test, ****: P < 0.0001, **: P < 0.01, N.S.: statistically nonsignificant difference. (c) Cell viability assay of HeLa cells incubated with original KLA-containing materials and chymotrypsin-pretreated materials. The concentration of the X-axis was calculated regarding the side-chain peptides. Data displayed as mean ± standard deviation (n = 3). (d) Mitochondria function assay of HeLa cells using a live-cell confocal microscope and JC-1 probe. The cells were incubated with poly(MAm−KLA), poly(NorIm−KLA), and respective chymotrypsin-pretreated analogues at a concentration of 25 μM for peptides for 24 h. Prior to imaging, cells were stained with 2 μM of JC-1 probe (green, monomer, λ\text{ex/em} = 488 nm/510−550 nm; red, J-aggregates, λ\text{ex/em} = 488 nm/585−649 nm) and then Hoechst (blue, λ\text{ex/em} = 358 nm/461 nm). Scale bar, 20 μm.
poly(PepNorIm) (Figures S35 and S36). Moreover, the SASA of peptide cleavage sites in poly(Oxa−PepNorIm) was calculated to be 31 ± 15 nm², markedly higher than that of poly(PepNorIm) (10 ± 10 nm²). These results support the dominant role of the PBP backbone hydrophobicity in determining the proteolytic stability of PBPs.

To interrogate whether the subtle difference in the polymer backbone observed between the norbornene imido and amide backbones is a general phenomenon, two more peptide substrates were designed and examined for their stability against α-chymotrypsin (Figures 3a,b and S37−48) and pepsin (Figures 3c,d and S57−64). These are common digestive proteases and belong to the subsets of serine proteases and aspartyl proteases, respectively.38,39 In light of the prevalence of α-chymotrypsin and pepsin in human digestive systems, stability against these proteases would have promise in the oral administration of therapeutic peptides and peptideomimetics. Studies of proteolysis kinetics revealed that the stability of PBPs with norbornene imido backbones consistently outperforms other PBPs (Figures 3b,d, S49−56, and S65−69 and Tables S9−10). Moreover, the proteolytic stability of all PBPs is noticeably higher than the corresponding peptide monomers (Figures 3b,d and S56). These results are in good agreement with our experimental observation in the case of thermolysin (Figure 1). Given that the investigated peptide cleavage sites range from nonpolar (glycine−leucine, Figure 1b) to charged (arginine−glycine, Figure 3a), and to modestly polar (methionine−serine, Figure 3c) amino acids, this suggests that polymer backbone effects on PBP peptide side-chain proteolytic stability are a general trend, with hydrophobic residues proximal to the backbone resulting in the largest protective effect. Furthermore, peptide-brush polymers and free peptides were exposed to fetal bovine serum. Here, again, peptide-brush polymers with the norbornene imido backbone significantly outperformed free peptides in preserving the integrity of amino acid sequences (Figure S70).

Since the proteolytic stability of PBPs with a norbornenyl imido backbone is higher than other analogues, we reasoned that the poly(NorIm)-based PBPs would exhibit preservation of bioactivity to a greater extent than other PBPs in probe−containing environments (Figure 4). To test this hypothesis, we designed two proof-of-concept PBPs with different backbones, poly(MAm−KLA) and poly(NorIm−KLA), consisting of the proapoptotic peptides (amino acid sequence: KLAKLAKKLKLAKL, abbreviated as KLA) (Figures 4a and S71−79). The KLA peptide is well-known for promoting apoptosis in cancer cells via disruption of mitochondrial membranes but does not penetrate cells alone.40,41 Frequently, the activity of peptides that are otherwise unable to penetrate cells are assessed when they are conjugated to a cell-penetrating peptide such as the TAT sequence (YGRKKRRQRRR).42 Therefore, we prepared TAT−KLA to compare its bioactivity, that is, proapoptotic efficacy, with poly(MAm−KLA) and poly(NorIm−KLA) (Figure S80).

To evaluate the impact on cancer cells (HeLa cell line), we performed an annexin V/propidium iodide (PI) staining to assay for the percentage of apoptotic cells upon incubation with poly(MAm−KLA), poly(NorIm−KLA), TAT−KLA, and KLA as well as their respective analogues, which were pretreated with α-chymotrypsin (Figures 4b and S81−83). Notably, both poly(MAm−KLA) and poly(NorIm−KLA) demonstrated higher proapoptotic efficacy than that of TAT−KLA, as evidenced by higher percentages of apoptotic cells in the cases of poly(MAm−KLA) and poly(NorIm−KLA). The enhanced bioactivity of PBPs compared to free peptides and TAT−KLA can be attributed to superior cell uptake of the polymers, which stems from the display of multiple cationic residues.12 The multivalent display of proapoptotic peptides on the intact polymer scaffold induces apoptosis by colocalization with mitochondria and disruption of mitochondrial membranes.3,4,43 Chymotrypsin pretreatment of poly(MAm−KLA) and TAT−KLA led to a marked decrease in their proapoptotic efficacy, in contrast to poly(NorIm−KLA), which retained much of its bioactivity (Figure 4b). These differences can be attributed to differences in degrees of proteolysis of the samples upon incubation with chymotrypsin. Additionally, the cytotoxicity of KLA-containing materials to cancer cells was evaluated (Figure 4c). The half-maximal effective concentrations (EC ₅₀) of original poly(NorIm−KLA) and poly(MAm−KLA) were similarly higher than that of TAT−KLA. However, upon pretreatment with chymotrypsin, poly(NorIm−KLA) preserved its EC ₅₀ value, while the EC ₅₀ values of poly(MAm−KLA) and TAT−KLA dramatically increased, agreeing with the cell apoptosis assay results (Figure 4b).

To further confirm that the mechanism of cell death was apoptosis, we performed a mitochondrial function assay using the turn-on JC-1 probe to evaluate the mitochondrial membrane potential, a key feature of mitochondria health (Figures 4d and S84). The JC-1 probe is a green-fluorescent carbocyanine dye that can form red-fluorescent JC-1 aggregates after accumulation in healthy mitochondria with a normal membrane potential (Figure 4d).44 As expected, HeLa cells incubated with original poly(NorIm−KLA) and poly(MAm−KLA) showed weak red fluorescence, similar to cells treated with TAT−KLA, indicating that the mitochondrial membrane was disrupted by the KLA-containing materials. In contrast, chymotrypsin-induced digestion of poly(MAm−KLA) and TAT−KLA resulted in a significant loss of their proapoptotic function, as evidenced by the appearance of strong red fluorescence, which is associated with healthy mitochondria (Figures 4d and S84). The behavior of poly(NorIm−KLA) was unaltered after enzyme treatment, because poly(NorIm−KLA) exhibits high proteolytic stability.

**CONCLUSION**

In summary, we have identified a key relationship between the polymer backbone and proteolytic stability of pendant peptides in densely functionalized peptide-brush polymers. Experimental and computational results show that polymer backbone hydrophobicity directly predicts the optimal performance of PBPs in terms of proteolytic resistance. Specifically, an increase in PBP backbone hydrophobicity drives the pendant peptide chains to condense at the core of the aqueous-phase structures, resulting in less surface exposure of peptide cleavage sites. This consequently weakens the interactions between peptide cleavage sites and protease active sites, which determines the elevated proteolytic resistance of PBPs as the hydrophobicity of the PBP backbone increases (e.g., poly(NorIm)). The impact of this proteolytic stability on the biological performance of PBPs was assessed, showing that proapoptotic PBPs with a more hydrophobic poly(NorIm) backbone, upon exposure to protease, retained a much higher degree of bioactivity than that of the PBP with a less hydrophobic poly(MAm) backbone. Notably, this type of bioconjugate stands out in terms of the inherently high percentage of active...
peptide by weight of PBP (>85%), compared to high-molecular-weight polyethylene glycol peptide conjugates, antibody fusions, or liposomal and other nanoscale formulations where carriers dominate the functional peptide. Therefore, these single-chain globular PBP materials are predominantly peptide-based by weight and by display of amino acid sequences on their exterior. Given the substantial promise of therapeutic peptides in biomedical applications, we envision that the mechanistic insights provided in this study will drive future development of proteolytically stable systems with high retention of bioactivity based on protein-like polymer structures consisting of densely functionalized polymer–peptide bioconjugates with multivalent peptide displays. Our lab is currently evaluating the efficacy of therapeutic peptide-brush polymers in relevant models of human disease.

**EXPERIMENTAL SECTION**

**Safety Statement.** No unexpected or unusually high safety hazards were encountered.

**Synthesis of Poly(PepMAm) and Poly(PepAm) via Photo-RAFT Polymerization.** Homopolymers of PepMAm and PepAm were achieved by photoinitiated reverse addition–fragmentation transfer (photo-RAFT) polymerization. In a typical aqueous photo-RAFT polymerization for making poly(PepMAm15), targeting a DP of 15, peptide methacrylamide monomer (30 mg, 15 equiv) was dissolved in 150 μL of PBS buffer. Then, water-soluble RAFT agent 4′-(((2-carboxyethyl)thio)carbonothioyl)thio)-4-cyanopentanoic acid (0.38 mg, 1.0 equiv) was added to the reaction mixture. Next, 19 μL of the SPTP stock solution (0.3 equiv., 6.0 mg in 1 mL of PBS buffer) was added. The solution was degassed by a N2 purge for 30 min and then placed into the photoreactor (λmax = 365 nm, 3.4 mW/cm²) for 12 h, after which the polymer solution was purified via dialysis against deionized water. Finally, the polymer product was obtained by lyophilization.

**Synthesis of Poly(PepNorIm) and Poly(PepNorAm) via ROMP.** Homopolymers of PepNorIm and PepNorAm were achieved by ring-opening metathesis polymerization (ROMP) under a N2 atmosphere in a glovebox. In a typical ROMP protocol for preparing poly(PepNorIm15), PepNorIm (20 mg, 15.0 equiv) was dissolved in 300 μL of degassed DMF (1 M LiCl). Next, 287 μL of the olefin metathesis initiator (IMesH2)(C₅H₅N)2(Cl)₂Ru=CHPh stock solution (1.0 equiv, 2 mg/mL in DMF) was quickly added to the monomer solution. The solution was left to stir for 5 h with full consumption of monomers. After the polymerization, the polymer solution was subjected to dialysis against deionized water. The solution was left to stir for 5 h with full consumption of monomers. After the polymerization, the polymer solution was subjected to dialysis against deionized water. Finally, the polymer product was obtained by lyophilization.

**Evaluation of the Proteolytic Stability of Peptide-Brush Polymers.** In a typical thermolysin-induced cleavage experiment, the molar concentration of thermolysin was set to 0.1 μM. The concentration of side-chain peptides (GPLGL-AGGWGERDGS) varied in the range of 50–200 μM. In a typical experiment, poly(PepNorIm15) (1.65 mg, 1 μmol regarding the side-chain peptides) was dissolved in 5 mL of PBS solution, leading to a stock polymer solution with a peptide concentration of 200 μM. Next, 3.5 μL of thermolysin stock solution (1.0 mg/mL) was added to 1 mL of the polymer solution, which was subsequently stirred in a preheated oil bath at 37 °C. In this case, the molar ratio of peptide substrate to thermolysin was 2000:1. During the proteolysis reaction, aliquots were taken for HPLC analysis at predetermined time points. Each degradation experiment was repeated three times.

**All-Atom Explicit Solvent MD Simulations on Peptide-Brush Polymers in the Absence of Protein.** Classical all-atom explicit solvent MD simulations were performed to study the morphology of peptide-brush polymers in aqueous solution. The package GROMACS (version 2016.3) was used. The CHARMM 36m potential was employed for amino acids as well as Na⁺ and Cl⁻ ions, along with the recommended CHARMM TIP3P water model. The CHARMM 36m potential was improved from the previous version of CHARMM 36 to better describe disordered proteins, which is of strong relevance to the present work, where most of the peptides favor disordered structures (Table S5). The CHARMM General Force Field (CGenFF 4.0) was employed for PBP backbone atoms. For each simulation, one PBP chain was dissolved in a water box with an edge length of 12 nm to mimic the single-molecule state of PBPs in experiments. A NaCl concentration of 0.14 M was employed to mimic the PBS buffer. A periodic annealing simulation of 200 ns was conducted to fully relax the PBP chains before the production simulation, which lasted 50 ns. The solvent-accessible surface area (SASA) was calculated using the GROMACS program gmx sasa.

**MARTINI Coarse-Grained (CG) MD Simulation on PBP–Thermolysin Complexes.** The MARTINI force field parameters for the backbones of poly(PepNorIm15) and poly(PepMAm15) were missing from the original MARTINI potential and thus were developed here. The obtained parameters were able to reproduce the structures (radius of gyration and end-to-end distance, Figure S27) of both PBPs. In the PBP–thermolysin complex simulations, thermolysin was positioned at the center of a simulation box with an edge length of 16 nm and surrounded by 12 PBP chains. The ratio of 1 thermolysin to 12 PBP chains agreed with the experimental concentrations. A NaCl concentration of 0.14 M was employed. Each simulation lasted 18 μs, where the ElnEdyn 2.2 protein model was employed in the first 9 μs to preserve the protein structure upon the PBP binding. The GoMartini 2.2 protein model55 was applied in the last 9 μs to enable the flexibility of thermolysin. The interactions between the PBP cleavage sites and thermolysin active sites were calculated using the GROMACS program gmx gmx energy.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.1c01149.

Materials, instrumentation, experimental methods, computational methodology, supporting figures, and supporting tables (PDF)

The rotation animation of poly(PepNorIm15) structure generated by all-atom molecular dynamic simulation (MP4)

The rotation animation of poly(PepNorAm15) structure generated by all-atom molecular dynamic simulation (MP4)

The rotation animation of poly(PepMAm15) structure generated by all-atom molecular dynamic simulation (MP4)
The rotation animation of poly(PepAm15) structure generated by all-atom molecular dynamic simulation (MP4)

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**Author Contributions**

H.S., B.Q., M.O.d.l.C., and N.C.G. conceived and designed the project. H.S., W.C., M.P.T., J. O., and O.M.E. conducted the synthesis and characterizations of materials. N.H. and S.J.W. performed the small-angle X-ray scattering analysis of peptide-brush polymers. Q.P. performed all the simulations. W.C. and N.C.M. performed the cell study. M.O.d.l.C. and N.C.G. supervised the project. H.S., B.Q., and W.C. drafted the manuscript. All authors discussed the results and contributed to writing the manuscript.

**Notes**

The authors declare the following competing financial interest(s): H.S. and N.C.G. are named inventors on a patent application on the materials and polymers described in this work. N.C.G. is a co-founder of Grove Biopharma.

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