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Editorial

Special Issue: Advances of Peptide Engineering

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Peptides have been gaining increasing attention for their applications in various fields, such as medical, biotechnological, and nanotechnological fields. Peptides are promising compounds for use in all-round engineering scenes because they confer several advantages: (i) peptides can be designed to form secondary structures, such as helices, strands, sheets, and turns by arranging amino acids to mimic naturally-occurring functional small proteins; (ii) peptides can be prepared in large quantities by well-established chemical synthetic procedures; (iii) peptides are stable against oxidative degradation and dryness compared with proteins; (iv) peptides can be site-selectively modified with functional groups, such as unnatura1 amino acid residues and fluorophores, by chemical treatments; (v) peptides can be produced to be multifunctional molecules by combining the properties including membrane-spanning, hormonal, inorganic-compound precipitating, or self-assembling.

This Special Issue of *Processes*, entitled "Advances of Peptide Engineering", presents novel examples of the current trends and developments in the fields of peptide engineering and addresses the solutions for biological/chemical/medical/industrial concerns encountered. The accepted manuscripts are 11 original research papers and 3 reviews, which are summarized below.

In the topics of synthesis and purification processes in peptide engineering, one research paper was accepted. K. Usui et al. [1] developed an easier process of synthesis, deprotection, reduction, cleavage, and purification for amyloid beta peptide (Aβ) (1–40), using standard 9-fluorenylmethyloxycarbonyl (Fmoc)-protected amino acids and peptide synthesis resin that provides higher yields of Aβ (1–40) than conventional protocols. Their proposed process method will contribute to various fields using "difficult sequence" peptides, such as pharmaceutical and materials science fields.

In the topics of purification processes in peptide engineering, one research paper was accepted. J. Kuwahara [2] exhibited the extraction of type I collagen from inedible tilapia scales, using a dilute acetic acid solution and ultrafine bubbles of carbon dioxide for 5 h. An environmentally friendly extraction method was proposed for effective extraction of type I collagen from tilapia scales. The extraction processes will contribute to the development of medicinal materials, cosmetics, and health products.

In the topics of control of peptide self-assembly, two research papers were accepted. K.-Y. Tomizaki et al. [3] demonstrated that tandem-homodimers (TDs) of a β-sheet-forming short peptide (original monomer), in which the monomer units are duplicated in series and joined via an amino alkanoic acid linker, prevented the random-to-β structural transition of the original monomer. This research could allow the design of a new class of protein/peptide fibrillogenesis modulators.

K. Matsuura et al. [4] demonstrated a novel strategy to construct peptide nanocapsules (artificial viral capsids) decorated with enzymes via interactions between His-tag and Ni-NTA. They synthesized a β-annulus peptide bearing Ni-NTA at the C-terminus, which is directed towards the exterior surface of the artificial viral capsid. The Ni-NTA-displayed

Citation: Usui, K.; Tomizaki, K.-y. Special Issue: Advances of Peptide Engineering. *Processes* **2021**, *9*, 1096. https://doi.org/10.3390/pr9071096

Received: 18 June 2021
Accepted: 18 June 2021
Published: 24 June 2021

capsids were complexed with recombinant horseradish peroxidase with a C-terminal His-tag, which was expressed in *Escherichia coli*.

In the topics of polymeric materials, one research paper was accepted. T. Sawada et al. (T. Serizawa lab.) [5] demonstrated a simple and convenient method to prepare soft polymeric composite nanoparticles based on the specific interaction of peptides with synthetic polymers. Morphological observations of the composite nanoparticles indicated softness and high applicability as carriers for biomedical utilization. The findings expand the applicability of polymer-binding peptides for the future construction of biomedical materials.

In the topics of analytical systems using peptide, two research papers were accepted. H. Miyazaki et al. (K. Usui lab.) [6] developed a chromophore-based solid phase peptide reaction assay in vitro, using peptides immobilized on magnetic beads (C-SPRA-MB). They also developed synthesis protocol of lysine (Lys) and cysteine (Cys) immobilized on magnetic microbeads. C-SPRA-MB may be useful for effective prediction of their skin sensitization potential in the process of compound screening in pharmaceutical and material fields.

Y. Mimura et al. (Y. Imai lab.) [7] developed peptide–pyrene organic luminophores containing the 2-aminoisobutyric acid (Aib) units. The Aib peptide–pyrene exhibited excimer CPL (circularly polarized luminescence) in organic solvent upon increasing the number of pyrene units. This system may be of assistance in designing future materials using peptide–pyrene organic luminophores for CPL.

In the topics of enzymatic mechanisms and inhibitors using peptide, one research paper and two reviews were accepted. Y. Yamawaki et al. (T. Kato lab.) [8] synthesized unnatural amino acid, 2-amino-4-ethylhexanoic acid (AEH), with a branched side chain using diethyl acetamidomalonate as a starting material and prepared a novel peptidyl 7-Amino-4-methylcoumarin (AMC) substrate. Substrate incorporating AEH with a high hydrophobicity greatly affected the activity of chymotrypsin by steric hindrance based on the branched side chain of AEH residue. Substrate incorporating unnatural amino acid can investigate the preference of chymotrypsin for the substrate in detail.

From the field of the protease inhibitor, Y. Tsuda et al. [9] presented a novel class of active site-directed plasmin (Plm) inhibitors containing tranexamic acid, which generally binds to the lysine binding site (LBS), not to the active site. Along with the elucidation of X-ray crystal structure of the new type inhibitors in the complex with μPlm, the further optimization of the series for both activity and selectivity led to the second-generation inhibitors.

M. Mizunuma et al. (Y. Chuman lab.) [10] developed the novel methods for the identification of Ser/Thr phosphatases, especially small C-terminal domain phosphatase 1 (Scp1), using the peptide-displayed phage library with AlF_4^-/BeF_3^- which they termed as "phosphorylation mimic phage display (PMPD) method". These methods are powerful tools, not only to clarify the complicated biological functions of Ser/Thr phosphatases but also to develop novel therapeutic drugs for several diseases, including cancer.

In the topics of cellular engineering using peptide, one research paper was accepted. Y. Futaki et al. (Y. Hirano lab.) [11] described the induction of a cell aggregate of the newly discovered $(Lys\text{-}Pro)_{12}$(KP24) peptide. Cell aggregation can affect cell–cell interactions, being more representative of the normal tissue microenvironment. Therefore, 3D cell culture technologies have been developed, however the general method for cell aggregate is a physical method. In any case, no chemical method has been discovered yet. The paper attempted to develop a new method to solve these problems.

In the topics of DDS using peptide engineering, two research papers and one review were accepted. M. Kitamatsu et al. [12] investigated the water solubility of the conjugate of the boron cluster (BSH) and the cell-penetrating peptide (CPP). It has been demonstrated that the water solubility of the BSH-CPP is improved by interaction with cyclodextrin and/or the incorporation of an ethylene glycol linker into the conjugate. The improvement may be useful for pharmaceutical application of boron neutron capture therapy (BNCT).

A. Kashiwada et al. [13] designed and characterized the melittin mimetic pH-selective lytic polypeptide LPE and its retro isomer (rLPE). Then the rLPE polypeptide and stearic acid conjugate (rLPE-St) anchoring liposomes were developed and could be activated by an acidic condition to the hydrophobic segment of the rLPE polypeptide, and, in the process, incorporate into the bilayers, supporting liposomal contents release. Their results provided useful insights onto the ongoing efforts and design considerations of unique and effective self-lytic liposomes with a pH-selectivity for the development of efficient therapeutic drug or gene delivery systems.

For the development of next-generation therapeutic systems using extracellular vesicles, exosomes, I. Nakase [14] has developed techniques to enhance cellular uptake and cytosolic release by the modification of bio-functional peptides (e.g., arginine-rich cell-penetrating peptides, artificial leucine-zipper peptides, pH-sensitive fusogenic peptides) on exosomal membranes. In the review paper of I. Nakase [14], each advanced experimental technique for exosome-based intracellular delivery by effective usages of not only functional peptides but also other active target systems, such as e.g., gene-tethering technologies, was introduced.

The articles in this Special Issue highlight various topics of peptide synthesis process, peptide purification process, peptide self-assembly, polymeric materials, analytical system, enzymatic mechanisms, inhibitors, cellular engineering, and DDS in biological/chemical/medical/industrial fields. Such knowledge, methods, tools, materials, and concepts described in these papers hold promise for the expansion of peptide engineering. The papers from this Special Issue can be accessed at the following link: https://www.mdpi.com/journal/processes/special_issues/Peptide_Engineering.

Conflicts of Interest: The authors declare no conflict of interest.

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