Self-Assembled Peptide- and Protein-Based Nanomaterials for Antitumor Photodynamic and Photothermal Therapy

Manzar Abbas, Qianli Zou, Shukun Li, and Xuehai Yan*

Tremendous interest in self-assembly of peptides and proteins towards functional nanomaterials has been inspired by naturally evolving self-assembly in biological construction of multiple and sophisticated protein architectures in organisms. Self-assembled peptide and protein nanoarchitectures are excellent promising candidates for facilitating biomedical applications due to their advantages of structural, mechanical, and functional diversity and high biocompatibility and biodegradability. Here, this review focuses on the self-assembly of peptides and proteins for fabrication of phototherapeutic nanomaterials for antitumor photodynamic and photothermal therapy, with emphasis on building blocks, non-covalent interactions, strategies, and the nanoarchitectures of self-assembly. The exciting antitumor activities achieved by these phototherapeutic nanomaterials are also discussed in-depth, along with the relationships between their specific nanoarchitectures and their unique properties, providing an increased understanding of the role of peptide and protein self-assembly in improving the efficiency of photodynamic and photothermal therapy.

1. Introduction

Proteins are ubiquitous and important biomolecules in all domains of organisms, ranging from unicellular prokaryotes to unicellular and multicellular eukaryotes. In living organisms, proteins serve as chief actors to perform the functions specifically encoded in genes through enzymes, cell signaling proteins, ligand binding proteins, and structural proteins. Importantly, most proteins tend to self-assemble and function in the form of self-assembled supramolecular architectures. Self-assembly not only endows the proteins with structural functions such as improved stability and mechanical strengths, but also plays a key role in regulating their biological functions. Natural proteins consist of one or more linear polymer chains built from up to 20 kinds of amino acids. Hence, proteins are characterized by their sequence of amino acids and their three-dimensional structures resulted from specific folding. Usually, short amino acid chains containing approximately less than 50 amino acids and lacking a stable three-dimensional structure are defined as peptides. Due to their simpler linear structures, peptides are easier to manipulate and synthetically easier accessible. In addition, specifically designed peptides have the ability to mimic certain properties of proteins, especially the self-assembly behavior. Therefore, both peptides and proteins are attractive self-assembling building blocks for constructing biomaterials with well-ordered structures and diverse functions.

Self-assembly of peptides and proteins is determined by non-covalent interactions, mainly π-effects, van der Waal forces, ionic attraction, hydrophobic effect, and hydrogen bonding. Often a change in the non-covalent interactions of peptides and proteins can be controlled by varying the sequences of amino acids and by manipulating the environmental parameters. Based on such a change, the self-assembly of peptides and proteins can be elaborately controlled to organize diverse supramolecular nanostructures. As the physical, chemical, and biological properties as well as the functionalities of peptides and proteins are highly correlated with their supramolecular nanostructures, great attention has been paid to self-assembly of peptides and proteins, and diverse nanostructures of peptide and proteins such as nanotubes, nanobelts, fibrils, nanovesicles, gels, and nanocages have been created. These self-assembled nanostructures usually possess diverse functions due to three aspects: (i) Many peptides and proteins are naturally functional as individuals, and their activities can be further improved by self-assembly due to enhanced collective behavior; (ii) The self-assembly also provides nanostructures with new collective properties and functions, that are not owned by their building blocks; (iii) The self-assembled nanostructures can be endowed with more functions by incorporation of new functional molecules. With respect to the diverse functions, self-assembly of peptides and proteins has been proposed for various applications. Additionally, the inherent biological origin of peptides and proteins makes the self-assembled nanostructures of peptides and proteins promising candidates for...
biomedical applications.\cite{18} A successful example is the clinical approval of protein-bound paclitaxel, which is a nanodrug prepared from the nanostructures of human serum albumin (HSA) and the chemotherapeutic agent paclitaxel.\cite{19} Self-assembled peptide and protein nanomaterials as drug delivery vehicles for chemotherapy and vaccine engineering have recently been reviewed.\cite{20} However, fabrication of peptide and protein nanomaterials for biomedical applications in a controllable and predictable approach has not been fully achieved yet, because elaborate manipulation of the non-covalent interactions and the corresponding nanostructures of peptides and proteins is still a challenge in supramolecular chemistry. As a consequence, deeper understanding of the self-assembling mechanisms and the complex structural features is still needed in designing self-assembled peptide and protein nanomaterials for biomedical applications, especially for the novel and emerging scopes of cancer therapy, such as phototherapies.

Phototherapies, including photodynamic therapy (PDT) and photothermal therapy (PTT), are attractive non-invasive techniques for treating cancers.\cite{21} Comprehensively, two major steps are involved in phototherapies, first is the delivery of a phototherapeutic agent to tumors and the following is the irradiation of the tumor sites with specific light to activate the phototherapeutic agent. Because the phototherapeutic agents have to be inherently non-toxic in dark and the light-induced toxicity can be limited to a confined area through direct light irradiation, phototherapies have no significant systemic toxic side effects, providing phototherapies better spatial selectivity and invasiveness than chemotherapy and radiotherapy. In PDT, under illumination, the phototherapeutic agents, also known as photosensitizers, react with molecular oxygen and convert it to reactive oxygen species (ROS), such as singlet oxygen, which can destroy the tumor cells and tissues through oxidative stress leading to tumor ablation.\cite{22} Since its first clinical approval in 1993 in Canada for the prophylactic treatment of bladder cancer, PDT has become the most site specific remedy applicable for cancers and has been clinically approved for the treatment of various cancers, such as head and neck cancer, oesophageal cancer, endobronchial cancer, gastric cancer, cervical cancer, and papillary bladder cancer.\cite{23} PDT has also been clinically applied together with surgery and other traditional treatments as a part of synergistic antitumor therapies.\cite{24} Despite such successful applications, PDT is still considered as an alternative or supporting remedy due to its limitations, mainly the limited penetration of visible light and the lack of tumor selectivity of photosensitizers. With the development of nanotechnology, nanomaterial-based PDT has been regarded as a promising method to overcome the limitations of current PDT.\cite{25} Nanoparticles not only can improve the solubility of photosensitizers but also provide a platform to encapsulate new light-absorbing molecules, especially those that can absorb near-infrared (NIR) light, as candidates for PDT. Moreover, nanoparticles have enhanced tumor selectivity either through the enhanced permeability and retention (EPR) effect or through surface modification using targeting ligands.\cite{26}

In PTT, the light energy absorbed by a photothermal agent, usually a light-absorbing nanomaterial, is converted to heat which is directly responsible for the ablation of tumor cells.\cite{27} Various nanomaterials with NIR absorbance have been investigated for PTT, mainly gold nanoparticles,\cite{28} carbon nanostructures,\cite{29} and light-absorbing polymers.\cite{30} However, PTT has not been clinically approved, most likely because the long-term safety of these nanomaterials has not yet been demonstrated. Excellent PTT agents should possess the characteristic features of strong absorbance in the NIR region, enhanced selective tumor accumulation, high light-to-heat conversion yield, as well as good biocompatibility and biodegradability. Clearly, there are some common features of the phototherapeutic agents required for PDT and PTT, such as the NIR absorbance,\cite{31} selective tumor accumulation, and the high biocompatibility and biodegradability. There are also different requirements for PDT and PTT. For example, for PDT, the encapsulated photosensitizer should be quickly released from the nanoparticle as monomers inside cells to recover its ROS generation ability; while for PTT, the loaded light-absorbing molecules need to be kept in an aggregated state to obtain a high light-to-heat conversion efficiency.

For improving the therapeutic efficacy of phototherapies, self-assembled peptide and protein nanostructures that are biocompatible and biodegradable and have the ability to be constructed in a controllable way to fulfill the specific demands of PDT and PTT, are receiving enormous comprehensive interest. Here, we highlight recent advances in the design of peptide- and protein-based nanomaterials for PDT and PTT (Figure 1). We first classify the peptide and protein nanostructures according to their kinds of building blocks. Then, we put emphasis on the non-covalent interactions involved in the self-assembling process of these building blocks and on the impact of the changes in non-covalent interactions on the formed supramolecular nanostructures. In some cases, the light-absorbing molecules not only present as loaded drugs but also participate in self-assembly and provide non-covalent interactions for the formation of the nanostructures. Based on the discussions of the properties, functions, and applications as phototherapeutic agents of the self-assembled peptide- and protein-based nanomaterials, we have attempted to provide a deep understanding of the relationship between the supramolecular nanostructure and the therapeutic efficiency. The challenges and future perspectives
of self-assembly of peptides and proteins in the area of PDT and PTT are finally presented.

2. Self-Assembled Peptide-Based Nanomaterials for PDT and PTT

Peptides, being composed of amino acids, are biological building blocks in nature. Amino acids contain amine and carboxylic acid moieties. The variations in the polarity of the side chains, charge, hydrophobicity, and size, give rise to the various physicochemical properties of amino acids. Hence, the number and sequence of amino acids in peptides are mainly responsible for the self-assembling behavior of peptides.[32] The flexibility in the structure and nature of peptides provides the possibility to fabricate specific types of nanomaterials with controllable characteristics.[33] Various types of peptide building blocks, such as cyclic peptides,[34] aromatic dipeptides,[35] amphiphilic peptides,[36] and polypeptides[37] have been developed for the construction of supramolecular nanostructures. Self-assembled peptide nanoparticles receive a lot of attention in the biomedical field, especially drug delivery and other potent techniques for cancer treatments, because peptide-based nanoparticles show advantages of biocompatibility, bioavailability, and bio-safety.[38] Moreover, the interactions between peptides and light-absorbing molecules provide a chance to adjust the physicochemical properties of the photosensitizers and photothermal agents,[39] leading to the formation of nanoparticles with controllable phototherapeutic effects. Therefore, peptide-based nanomaterials hold high potential for photodynamic and photothermal applications.

2.1. Short Peptide-Based Nanomaterials for PDT and PTT

Short peptides composed of several amino acids are excellent building blocks for nanoparticles due to their tunable structures by design, and easy accessibility by synthesis.[40] Self-assembling short peptides are usually amphiphilic peptides that exhibit both hydrophobic and hydrophilic character. Hence, hydrophobic domains in the formed nanomaterials are applicable for loading of hydrophobic photosensitizers through non-covalent interactions. When self-assembled peptide-based nanomaterials are applied in vivo, disassembly of these nanomaterials is possibly provoked before they can reach and accumulate in tumors due to dilution by body fluids, interactions with complex physiological components, and degradation by enzymes. To address this concern, several ways to stabilize the assembled nanomaterials have been suggested: (i) to introduce cysteine residues into the peptides in order to lock the peptides into the assembled state and to stabilize the nanomaterials via intermolecular disulfide bond formation;[41] (ii) to integrate amino acids containing highly hydrophobic residues to strengthen the hydrophobic interaction and facilitate the formation of the stabilized nanomaterials in a low concentration;[42] (iii) to on demand use amino acid sequences that are not specifically responsive to enzymes. For example, it has been reported that self-assembly of a short peptide with sequence of Ac-Ala-Ala-Val-Val-Leu-Leu-Trp-Glu-Glu forms vesicles with an average diameter of 120 nm.[43] These vesicles were further stabilized by introducing multiple cysteine residues in the hydrophobic part of the peptide and the formation of intermolecular disulfide linkers. A hydrophobic photosensitizer, Zinc-phthalocyanine, was successfully loaded in the vesicles in the presence of 10-fold molar excess of the short peptide. Under light irradiation, the peptide vesicles containing Zinc-phthalocyanine showed significant photocytotoxicity to COS-7 cells. In contrast, the peptide vesicles without zinc-phthalocyanine and the control group of free zinc-phthalocyanine had no significant cytotoxicity, suggesting that the peptide vehicles enhanced the internalization of the photosensitizer. Micelles self-assembled from a surfactant-like tetra-tail amphiphilic peptide containing four highly hydrophobic fatty tails and a targeting sequence of Arg-Gly Asp (RGD) also exhibited the ability as drug delivery vehicles for loading of photosensitizers.[44] Due to the presence of RGD, the micelles could be recognized and internalized specifically by HeLa cells, leading to improved intracellular concentration of the payload.

An advantage of short peptide-based nanomaterials is that the sequence of peptides can be tailored to be functional.[45] It has been demonstrated that nanoparticles self-assembled from a photosensitizer-peptide conjugate containing a metalloproteinase-2 (MMP-2)-sensitive sequence can be applied as a drug delivery system for targeted PDT.[46] The conjugate PpIX-R_oGpLGLAGE_o was composed of protoporphyrin (PpIX) as the photosensitizer, R_o as cell-penetrating peptide, GpLGLAG as
the MMP-2-sensitive peptide, and E₈ as the masking peptide. In normal tissues, the cell-penetrating peptide is blocked by the masking peptide through electrostatic attraction. In tumor extracellular matrix, where overexpressed MMP-2 protein exists, the masking peptide is removed by MMP-2, resulting in recovery of the function of the cell-penetrating peptide. In vivo results demonstrated that the conjugate efficiently accumulated at the tumor sites. Such a (MMP-2)-sensitive peptide was also found effective in designing drug delivery systems for combined PDT and gene therapy and for aggregation-induced emission-guided PDT. The application of a cationic nuclear localization peptide sequence in fabrication of drug delivery systems has also been recently demonstrated.

Among self-assembling short peptides, diphenylalanine (FF) is a representative molecule with numerous well-defined nanostructures. FF has hydrophobic and hydrophilic moieties which are key parameters for molecular assembly. Other than self-assembly by itself, FF also shows the function as a co-assembling peptide for adjusting the self-assembly of various functional molecules, providing an easy and efficient method to modulate the morphology and property of these functional molecules. Recently, our group demonstrated, that short peptide-modulated self-assembly of photosensitizers is an elegant protocol for fabrication of nanoparticles for enhanced PDT. We demonstrated that both a diphenylalanine (H-Phe-Phe-NH₂·HCl, CDP) derived from FF and an amino acid derivative (9-fluorenylmethoxycarbonyl-L-lysine, Fmoc-L-Lys) can induce the self-assembly of Chlorin e6 (Ce6) to form well-ordered nanoparticles. Because both CDP and Fmoc-L-Lys are cationic amphiphilic molecules with aromatic groups, while Ce6 is an anionic molecule with aromatic systems, the formation of the nanoparticles is induced by several non-covalent interactions including π-stacking, electrostatic interaction and hydrophobic effect. The obtained representative nanoparticles of Fmoc-L-Lys/Ce6 (FCNPs) and CDP/Ce6 (CCNPs) showed sizes of 200 nm and 100 nm, respectively.

Figure 2. Amphiphilic dipeptide- or amino-acid-tuned self-assembly of photosensitizers for PDT. A) Schematic depiction of the self-assembly process. B) Internalization of the assembled FCNPs by MCF7 cells. The red staining is from the photosensitizer while nuclei are stained by blue and cell membrane is stained by green. C) In vitro cytotoxicity and photocytotoxicity of FCNPs. D) Fluorescence images of tumor-bearing mice showing in vivo distribution of FCNPs and free Ce6. E) Tumor growth curves of the mice in different groups. Reproduced with permission. Copyright 2016, Wiley-VCH.
Moreover, the size and surface charge of the nanoparticles can be easily tuned by changing the ratio between peptides and photosensitizers. Importantly, these nanoparticles are responsive to stimuli such as pH, detergents and enzyme, facilitating selective release of photosensitizers in the tumor microenvironment. When incubated with MCF-7 cells, FCNPs and CCNPs were efficiently internalized (Figure 2B). According to the IC50, the nanoparticles showed an approximately 4-fold increased photocytotoxicity as compared to the control group of Ce6 (Figure 2C). The selective accumulation of FCNPs and CCNPs in tumors was evident from in vivo fluorescence images obtained at 24 h post-injection (Figure 2D). After PDT treatment, the mice showed no significant organ damage and no variation in body weight, demonstrating the short peptide-tuned nanostructures of Ce6 are highly biocompatible.

Besides the preparation of peptide-based nanoparticles in solution, another alternative method is construction of functional nanoparticles in situ in living systems.[50] The self-assembly of peptide-photosensitizer complexes in vivo has been demonstrated by a responsive building block (Figure 3A), in which a photosensitizer, purpurin-18 (P18), is covalently linked to a targeting ligand of RGD through an enzyme responsive peptide linker, Pro-Leu-Gly Val-Ary-Gly (PLGVRG).[51] The formed P18-PLGVRGRGD is soluble in physiological solution. Under in vivo condition, P18-PLGVRGRGD passively diffused in the living system and targeted to cancer cells through its RGD ligand. Then, the PLGVRG linker was selectively cut by an overexpressed enzyme, gelatinase. The removal of several hydrophilic amino acids generated a new molecule which can self-assemble in physiological conditions (Figure 3B). Hence, nanofibers were formed in situ and such fibrous nanostructures exhibited an enhanced photoacoustic signal and PTT efficacy (Figure 3C). In situ formation of peptide-based nanostructures also showed a high efficiency in photoacoustic detection of bacterial infection,[52] demonstrating that the in vivo self-assembly of peptides and other functional groups has high potential as a novel strategy for cancer diagnostics and therapeutics.

2.2. Polypeptide-Based Nanomaterials for PDT and PTT

Drug delivery vehicles prepared from polypeptides have received great attention for delivery of photosensitizers due to their built-in biological nature. One example is the delivery of porphimer sodium by poly (ethylene glycol)-grafted poly L-lysine (PLL-g-PEG).[53] Porphimer sodium is a clinically approved photosensitizer for PDT. Though it has been proven efficient for various cancers, including esophageal cancer, early non-small cell lung cancer, and Barrett’s esophagus, still it has side effects such as prolonged phototoxic reaction of skin.[54] Patients need to avoid sunlight for as long as a month. When porphimer sodium was mixed with PLL-g-PEG in aqueous solution, PLL-g-PEG/porphimer sodium complexes with a size of 29.2 nm were obtained. The formation of the complexes is mainly through the electrostatic interaction between the positively charged amino groups of PLL-g-PEG and the negatively charged carboxylic groups of porphimer sodium. An in vivo study in mice revealed that the rate of porphimer sodium accumulation in tumor tissues was enhanced by formulation in PLL-g-PEG. Moreover, suppressed photosensitive reactions were found in the mice treated by PLL-g-PEG/porphimer sodium. It should be noted that PLL-g-PEG is highly hydrophilic. Hence, it disperses in water as a single molecule whether it loads with or without...
porfimer sodium. To form amphiphilic molecules, conjugation of water-soluble peptides to hydrophobic photosensitizers has been proposed. When four segments of poly L-lysine (PLL) were conjugated to a hydrophobic porphyrin core, they generated an amphiphilic molecule with the ability to form micelles.\(^{[55]}\) The formed micelles were further applied as drug delivery vehicles for delivery of doxorubicin, suggesting a possible method for combined chemotherapy and PDT.

Amphiphilic polypeptides can be designed by incorporation of amino acids with hydrophobic side chains. For example, polymeric micelles have been constructed by self-assembly of PEG-polypeptide hybrid triblock copolymers of poly(ethylene glycol)-b-poly(L-lysine)-b-poly(L-leucine) (PEG-PLL-PLLeu).\(^{[56]}\) In these micelles, PLLeu formed the hydrophobic core while PEG formed a hydrophilic shell. The self-assembly of PEG-PLL-PLLeu in the presence of indocyanine green (ICG) formed PEG-PLL-PLLeu-ICG micelles (Figure 4A). The association of ICG occurs through its binding to the hydrophobic core by hydrophobic interaction and to the hydrophilic shell by electrostatic attractive interaction. ICG is a clinically approved cyanine dye for medical diagnostics with an absorption peak located at about 800 nm. Due to its absorption in the near-infrared range, ICG has been proposed for phototherapy. However, its instability in aqueous solution and its short in vivo half-life (150 to 180 seconds) hampers its biomedical applications for tumor therapy.\(^{[57]}\) When free-dissolved ICG was kept in dark for 5 weeks, its fluorescence was almost totally lost. Under the same condition, PEG-PLL-PLLeu-ICG micelles only lost about 30% fluorescence intensity, suggesting the enhanced aqueous stability of ICG in the formulation of polypeptide micelles. PEG-PLL-PLLeu-ICG micelles also showed enhanced cellular internalization as compared to free ICG, due to the electrostatic interaction between positively charged PLL segments and negatively charged cell membranes and the hydrophobic interaction of PLLeu segments with cell membranes. In vitro photothermal results revealed that the cells incubated with PEG-PLL-PLLeu-ICG micelles and irradiated with NIR light were efficiently killed (Figure 4B). In vivo analysis demonstrated that PEG-PLL-PLLeu-ICG had impressive ability to passively target to tumors through the EPR effect.

In order to reveal the impact of non-covalent interactions on the photostability of photosensitizers, two copolymers, poly(ethylene glycol)-block-poly(L-lysine) (PEG-b-PLL) and poly(ethylene glycol)-block-poly(4-vinylpyridine) (PEG-b-P4VP) were synthesized.\(^{[58]}\) The self-assembly of tetrakis(4-sulfonato-phenyl) porphyrin (MgTPPS) with PEG-b-PLL through electrostatic interaction between the anionic sulfonate groups and the cationic lysine groups formed electrostatic micelles, while the self-assembly of MgTPPS with PEG-b-P4VP through coordination interaction between the central metal of porphyrin and pyridine groups formed coordination micelles. In aqueous solutions, the fast demetallation of MgTPPS occurs due to the replacement of the magnesium ion by H\(^+\). However, the hydrolytic stability of MgTPPS was found enhanced inside both the electrostatic and the coordination micelles. Moreover, the photostability of MgTPPS in the formulation of micelles was found enhanced due to less accessibility for oxygen in the micellar cores, suggesting that the non-covalent interactions between peptides and photosensitizers are efficient in controlling the properties of photosensitizers. The non-covalent interaction between PEG-b-PLL and a series of poly(benzyl ether) dendrimer porphyrins (DPs) has also been reported.\(^{[59]}\) Polyion micelles were obtained through electrostatic interaction between the positively charged PLL segment and the negatively charged periphery of DPs. The properties of the micelles, including fluorescence lifetime, oxygen consumption ability, cellular uptake, and photocytotoxicity, are highly dependent on the generation of DPs.

**Figure 4.** Self-assembled polypeptide micelles for PTT. A) Structure of the polypeptide and loading and delivery of ICG by the self-assembled micelles. B) Fluorescence images of H460 cells incubated with the ICG-containing micelles and treated with 808 nm laser irradiation. Live and dead cells were indicated by calcein AM (green) and propidium iodide (red), respectively. Reproduced with permission.\(^{[56]}\) Copyright 2013, American Chemical Society.
In the complex in vivo environment, the complexes assembled through non-covalent interactions between peptides and photosensitizers usually disassemble and release the photosensitizers as free photosensitizers. An advantage of polypeptides as drug delivery vehicles is that the side chains of polypeptides can be tailored by multi-functional groups. The use of PLL as a polymeric backbone for construction of traceable probes for photodynamic therapy has been proposed. In this strategy, PLL was functionalized by four functional groups: diisopropylaminoo (DPA) as a pH-responsive group; phthethorbide A (PheA) as an aggregation-caused quenching (ACQ) photosensitizer; tetraphenylsilole (TPS) as a fluorogen with aggregation-induced emission (AIEgens); PEG for further conjugation to a targeting ligand. Self-assembly of the designed probe in neutral aqueous solution forms nanoparticles with a size of 115 nm through non-covalent interactions, mainly hydrophobic interaction and π-stacking from DPA, PheA, and TPS. Under irradiation, the nanoparticles show aggregation-induced green fluorescence from TPS, while red fluorescence and ROS from PheA are quenched. In contrast, the size of the nanoparticles decreases to 15 nm at pH 5.0 due to the increased hydrophilicity of protonated DPA at such acidic condition. Moreover, green fluorescence is highly quenched due to disassembly of NPs, and the red fluorescence from PheA is recovered along with the recovery of the photocytotoxicity of PheA. On the basis of its pH-dependent self-assembly behavior, the probe was demonstrated traceable in PDT: the decreased green fluorescence and increased red fluorescence indicate, that the nanoparticles are internalized by lysosomes (pH 5.0); the recovery of green fluorescence indicates that the lysosomal membrane is destroyed by ROS and the probe is leaked to the cytosol. Therefore, the probe design represents a novel strategy for traceable cancer therapy.

As mentioned earlier, in vivo self-assembly provides an elegant method to in situ construction of nanostructures. Since ROS involved in PDT are chemically reactive species, they can be applied as a trigger for in vivo self-assembly through inducing new chemical bonds. To demonstrate this concept, a recombinant elastin-like polypeptide containing periodic cysteine residues and Ce6 was designed. When the water-soluble Ce6-containing peptide was intratumorally injected and illuminated by light, the ROS generated from Ce6 induced in situ crosslinking of the cysteine thioles and the formation of a stable hydrogel. The hydrogel structures were found highly stable in subcutaneous tumor xenografts and were efficient in improving intratumoral retention of various payloads.

3. Self-Assembled Protein-Based Nanomaterials for PDT and PTT

Proteins such as HSA in the human body are natural vehicles for small molecules including drugs. Inspired by the interactions between protein molecules and drugs in vivo, protein molecules have been developed as drug delivery vehicles for photosensitizers. The conjugation of a heptamethine dye, CySCOOH, to HSA through amide coupling reaction formed HSA@CySCOOH conjugates. The conjugation induced an increase of only 1.2 nm to the size of HSA, indicating that no aggregation of HSA occurred. Compared to CySCOOH itself, the fluorescence originated from CySCOOH in HSA@CySCOOH was partly quenched with a quenching efficiency of 77%. The lower fluorescence efficiency benefits the conversion of light to heat. Within 24 h after injection of HSA@CySCOOH, the tumor-bearing mice exhibited an increase of the fluorescence and a photacoustic signal in the tumor site along with a gradual decrease of the fluorescence signal from other parts of the body, demonstrating that HSA molecules are efficient drug delivery vehicles for PTT. HSA molecules have also been investigated for PDT by conjugation with Ce6 through amide coupling reaction along with the conjugation with a targeting peptide of RGD through a PEG linker. Due to the presence of RGD, RGD-HSA-Ce6 showed higher cellular uptake than free Ce6 by A375 cells, that overexpressed ανβ3 integrin. In vitro PDT revealed that RGD-HSA-Ce6 showed higher photocytotoxicity to A375 cells than free Ce6. However, such a difference was not significant for 3T3 cells, which showed lower expression of ανβ3 integrin, suggesting that the modification of HSA by RGD can improve targeted delivery of photosensitizer to cancer cells.

Targeted delivery of photosensitizers by serum albumin for PTT has also been reported by utilizing bovine serum albumin (BSA) as drug delivery vehicles. The loading of a squaraine (SQ) dye by BSA followed by modification by folic acid (FA) through a coupling reaction formed adducts of SQ-BSA-FA. Molecular docking and molecular dynamics (MD) simulation revealed, that the hydrophobic and hydrogen bonding interactions were responsible for the selective binding of SQ to the hydrophobic domains of BSA. Interestingly, the fluorescence of SQ-BSA-FA increased up to 80 fold as compared to SQ without BSA. Moreover, the fluorescence of SQ-BSA-FA was highly stable in physiological solution and in the presence of nucleophilic cysteine or homocysteine. In vivo distribution study showed that FA played an important role in the accumulation of SQ-BSA-FA to tumor sites.

Since the pathways for generation of fluorescence and heat are different, the application of the same photosensitizer both for fluorescence imaging and PTT is a challenge. The loading by HSA was found to be an efficient method to tune the photophysical properties of IR825 dye (Figure 5A). The HSA-IR825 complexes were obtained by mixing of HSA with IR825
(pre-dissolved in methanol) at the molar ratios of 1:1. Interestingly, although free IR825 only had weak fluorescence either in methanol or water regardless of excitation wavelengths, HSA-IR825 showed fluorescence quantum yields of 40% and 0.33%, when excited at 600 nm and 808 nm, respectively. Such wavelength-dependent fluorescence properties enable the application of HSA-IR825 as an imaging-guided therapy agent for both fluorescence imaging and PTT. In vivo fluorescence imaging carried out upon 600 nm excitation demonstrated that the accumulation of HSA-IR825 in the tumor reached the maximum at 2 h after the injection due to the EPR effect. Irradiation of tumors by light of 800 nm carried on at 2 h after the injection of HSA-IR825 increased the tumor surface temperature rapidly to 50 °C, while the temperature of the control group irradiated by the same laser without HSA-IR825 showed little change (Figure 5B). The imaging guided PTT suggests, that self-assembly of photosensitizers and proteins is promising in providing the same agent for multiple biomedical applications.

3.2. Self-Assembled Protein Nanocages for PDT and PTT

Protein nanocages are special structures, in which self-assembly plays a key role in their reversible formation and dissociation. For example, the nanocage of ferritin, a protein for balancing the level of iron in most living organisms, is composed by the self-assembly of its subunits. Each ferritin nanocage possesses a nanostructure of a cage with external and internal diameters of 12 and 8 nm, respectively. Various materials including photosensitizers can be loaded by protein nanocages. For example, Cys-Asp-Cys-Arg-Gly Asp-Cys-Phe-Cys (RGD4C)-modified ferritins (RFRTs) were found efficient in loading of zinc hexadecafluorophthalocyanine (ZnF16Pc), a hydrophobic photosensitizer with a high singlet oxygen quantum yield. The association of RFRTs between ZnF16Pc was mainly based on the interaction between zinc and the metal binding sites at the interiors of ferritins. Though the loading rate was as high as 60%, the loading of ZnF16Pc showed no significant impact on the size of the nanocages. In vitro results carried out on U87MG.
cells demonstrated that P-RFRTs were internalized by the cell through the targeting interaction between the RGD ligand and αβ integrin. When tested on in vivo U87MG tumor models, P-RFRTs showed enhanced accumulation in tumors, a high rate of tumor inhibition, as well as minimal side effect to the skin and major inside organs. The large loading efficiency and high tumor accumulation rate favored the delivery of metal-containing photosensitizers by ferritin nanocages.

Other than the loading based on the interaction between metal ions and the metal binding sites, the loading of metal-free photosensitizers has also been realized by the nanocages of apoferritin, which is a form of ferritin without the iron. For example, Methylene blue (MB) was successfully encapsulated by the apoferritin nanocages synchronously in the disassembly and self-assembly processes of the nanocages by changing the pH values. When MB-loaded nanocages were incubated with MCF-7 cells, cellular uptake of the nanocages happened quickly in a few minutes and photocytotoxicity was efficiently induced by further irradiation. Due to its chemical instability in the biological environment, the direct in vivo use of MB is not successful. Hence, the loading of MB via self-assembly of apoferritin nanocages provides an effective route to the synthesis of stable nanocomposites of MB.

The application of ferritin nanocages for simultaneous fluorescence/photoacoustic imaging-guided PTT has also been investigated by means of the IR820 dye-loaded ferritin (DFRT) nanocages. DFRT nanocages were prepared by loading of IR820 in the process of pH-induced self-assembly of ferritin subunits. Interestingly, the quenching efficiency of IR820 in DFRT is about 20% upon 550 nm excitation, but almost about 80% upon 770 nm excitation. Moreover, such wavelength-dependence of fluorescence was also confirmed in the loading of ICG by ferritin nanocages, suggesting that ferritin nanocages are promising drug delivery vehicles for photosensitizers in preparation of multifunctional diagnosis and therapy agents.

The capsid of cowpea chlorotic mottle virus (CCMV) is another naturally occurring protein nanocage. CCMV is a positive, single-strand RNA plant virus with inner and outer diameters of 18 and 28 nm, respectively. In the presence of high ionic strength at neutral pH, CCMV capsids disassemble to positively charged protein dimers. After removal of RNA, self-assembly of the protein dimers can be triggered by adding polyanionic species or by reducing the pH to 5. The reversibility of the self-assembly of CCMV capsids has been used to encapsulate photosensitizers. The self-assembly of CCMV protein with a water soluble zinc Pc (ZnPc) at neutral pH formed nanoparticles of 19 nm, in which about 192 ZnPc molecules were encapsulated by one nanoparticle. The formation of the nanoparticles is due to the interaction between negatively charged photosensitizer and positively charged CCMV protein. Further analysis of the nanoparticles revealed, that they contained a core of 10 nm diameter (Figure 6). The cores were formed by self-assembly of ZnPc. Hence, the self-assembly of photosensitizer and CCMV protein provides a method to prepare monodisperse, uniform organic photoactive nanospheres.

The self-assembly of photosensitizer and CCMV protein can be further tuned by changing the structure of the photosensitizer. It has been reported, that the negatively charged photosensitizer dendrimers can be applied as templates for the self-assembly of the CCMV protein into nanoparticles. The results reveal, that the self-assembly of the CCMV protein

Figure 6. Formation and structures of monodisperse dye nanospheres in a protein cage. A) Illustration of the self-assembly of ZnPc and CCMV protein. B) Cryo-electron microscopy 3D-reconstructed model of ZnPc organization within a CCMV protein. Reproduced with permission. Copyright 2014, Royal Society of Chemistry.
is most efficient in the presence of dendrimers or dendrimer dimers with 16 negative charges. The dendrimer-induced self-assembly of CCMV protein allows a control of the structures and properties of the obtained nanomaterials by precisely tailoring the structure of the dendrimer.

### 3.3. Self-Assembly of Proteins as Drug Delivery Vehicles for PDT and PTT

Though some proteins, such as serum albumins, exist as monomers in nature, their self-assembly can be induced by chemical methods and the formed nanoparticles can be applied as drug delivery vehicles once stabilized. Ethanol diffusion, along with crosslinking by glutaraldehyde, is one of the methods to prepare stable nanoparticles of HSA. Such HSA nanoparticles have been investigated as drug delivery vehicles for two well-known photosensitizers, 5,10,15,20-tetrakis-(m-hydroxyphenyl) porphyrine (mTHPP) and 5,10,15,20-tetrakis(m-hydroxyphenyl)chlorin (mTHPC).[79] The nanoparticles loaded with mTHPP and mTHPC showed diameters of 189.9 nm and 211.6 nm, respectively. Both mTHPP and mTHPC loaded HSA nanoparticles showed a small singlet oxygen quantum yield of 0.03 though the singlet oxygen quantum yields of mTHPP and mTHPC are 0.63 and 0.65, respectively, suggesting that both the characteristic peaks of Ce6 and the broad absorption band of PpY. The incorporated Ce6 was further labeled by simply mixing PpY-BSA-Ce6 nanoparticles with GdCl3. As Gd<sup>3+</sup> offers contrast in MR imaging, the obtained PpY-BSA-Ce6-Gd nanoparticles were demonstrated to be a multifunctional agent for fluorescence imaging, MR imaging, PDT, and PTT. Hence, the stabilization of hydrophobic photothermal agents by protein not only improves the stability of the agents but also provides binding sites for other functional groups, allowing the incorporation of multifunctional biomedical agents.

In living systems, spontaneous self-assembly involving biomacromolecules including proteins and polypeptides is ubiquitous.[85] We have demonstrated that the self-assembly of proteins and polypeptides along with reversible crosslinking is an efficient protocol to achieve robust well-defined protein-based colloidal spheres as versatile drug delivery vehicles for both biomacromolecules and small molecules.[86] The colloidal spheres were constructed from the electrostatic self-assembly of negatively charged HSA and positively charged poly L-lysine (PLL), followed by the intermolecular disulfide recombination of HSA molecules in the presence of dithiothreitol as a reducing agent. The size of these colloidal spheres is tunable in the range of nanoscale to microscale by varying the parameters involved in preparation, such as concentration and ratio of HSA and PLL. A wide range of functional materials with distinct properties of surface charge, water-solubility, and molecular weight, including Rhodamine 6G, flavin adenine dinucleotide, doxorubicin, Nile red, dextran, and catalase were found efficiently encapsulated within the colloidal spheres in the process of colloid formation, and the encapsulated catalase still remained bioactive. Moreover, the colloidal spheres can be easily modified by surface polyethylene glycol coupling and the pegylated nanoparticles were capable of encapsulating various photosensitizers including Ce6, protoporphyrin IX, and verteporfin (Figure 7A).[87] Importantly, the Ce6-encapsulated nanospheres were demonstrated to be responsive to changes in pH, redox potential, and proteinase concentration, resulting in multitriggered rapid release of Ce6 in tumors (Figure 7B and C). Therefore, these protein-based nanospheres are excellent tumor-responsive nanocarriers for PDT.

As most abundant proteins in various connective tissues of mammalian bodies, collagen proteins such as type 1 collagen have long been known to undergo self-assembly to form fibrous hydrogels by non-covalent interactions.[88] However, such collagen hydrogels have poor mechanical properties, disabling in vivo biomedical applications. Recently, our group illustrated the biomineralization triggered self-assembly of collagen protein (Figure 8A).[89] When collagen proteins were mixed with chloroauric acid (HAuCl₄) in acid solution, electrostatic interaction between the positively charged collagen chains and anionic clusters ([AuCl₄]⁻) promoted the rapid formation of collagen protein-based hydrogels. Subsequent formation of gold nanoparticles (AuNPs) through chemical reduction of anionic clusters significantly enhanced the mechanical properties of the gel. Such enhancement was demonstrated due to the presence of AuNPs as cross-linkers. The rheological properties revealed that the collagen-based hydrogels containing in situ formed AuNPs were suitable for in vivo injection with good shear-thinning and self-healing behavior. The collagen-based hydrogels

---

**Reference:**
[79] The nanoparticles loaded with mTHPP and mTHPC showed diameters of 189.9 nm and 211.6 nm, respectively.

[85] We have demonstrated that the self-assembly of proteins and polypeptides along with reversible crosslinking is an efficient protocol to achieve robust well-defined protein-based colloidal spheres as versatile drug delivery vehicles for both biomacromolecules and small molecules.

[86] The colloidal spheres were constructed from the electrostatic self-assembly of negatively charged HSA and positively charged poly L-lysine (PLL), followed by the intermolecular disulfide recombination of HSA molecules in the presence of dithiothreitol as a reducing agent.

[87] Importantly, the Ce6-encapsulated nanospheres were demonstrated to be responsive to changes in pH, redox potential, and proteinase concentration, resulting in multitriggered rapid release of Ce6 in tumors.

[88] However, such collagen hydrogels have poor mechanical properties, disabling in vivo biomedical applications.

[89] When collagen proteins were mixed with chloroauric acid (HAuCl₄) in acid solution, electrostatic interaction between the positively charged collagen chains and anionic clusters ([AuCl₄]⁻) promoted the rapid formation of collagen protein-based hydrogels.
containing AuNPs showed good photothermal properties due to the presence of AuNPs, which are commonly utilized as photothermal agents. More importantly, various photosensitizers such as meso-tetra-(N-methyl-4-pyridyl) porphine tetrachloride (TMPyP), can be incorporated into hydrogels, yielding collagen-based hydrogels containing both AuNPs and TMPyP as injectable nanomaterials for combined PTT and PDT. Intriguingly, the release of TMPyP from the hydrogels sustained for 120 h after tumor injection of the hydrogels (Figure 8B and C). Hence, the tumors can be exposed to irradiation for several times after one injection. In vivo results showed that our “one injection, multiple-treatment” strategy was efficient in inhibition of the tumor growth.

3.4. Drug-Induced Self-Assembly of Proteins

The binding of paclitaxel (PTX) with HSA via hydrophobic interaction has generated a FDA approved nanodrug, protein-bound paclitaxel.[19] Similarly, ICG can also bind to HSA by hydrophobic interaction. Taken together, multi-component nanoparticles containing both PTX and ICG were obtained simply by mixing the components, PTX, ICG, and HSA, in aqueous solution (Figure 9A).[90] The formed HSA-ICG-PTX nanoparticles exhibited a size of 90 nm, similar to the size of PTX-HSA nanoparticles, while the size of ICG-PTX nanoparticles was only 7–8 nm, suggesting that self-assembly of HSA and enlargement of the size was mainly induced by PTX (Figure 9B). In vitro
Figure 8. An injectable collagen-based hydrogel containing gold nanoparticles based on a biomineralization-triggered self-assembly. A) Schematic diagram of the fabrication of the hydrogel. B) Fluorescence images of tumor-bearing mice after injection of the TMPyP-loaded hydrogel (up) or the control group of TMPyP (bottom). C) Fluorescence images of the organs harvested at 120 h after in vivo injection of the TMPyP-loaded hydrogel (up) or the control group of TMPyP (bottom). Reproduced with permission.[89] Copyright 2016, Wiley-VCH.

results suggested that PTX in the formulation of HSA-ICG-PTX was still effective in killing cells. Importantly, HSA-ICG-PTX nanoparticles showed selective accumulation in metastatic tumors spread into lungs, although the size of the tumors was less than 1 mm. The combined chemotherapy and PTT using HSA-ICG-PTX nanoparticles delayed the animal death of the mice in a lung metastatic tumor model. The PTX-induced self-assembly strategy was also investigated in construction of nanoparticles for combined chemotherapy and PDT.\(^{(91)}\)

The nanoparticles were prepared by mixing PTX with Ce6-modified HSA and RGD-modified HSA. The self-assembled HSA-Ce6-PTX-RGD nanoparticles showed enhanced accumulation in tumors of mice bearing subcutaneous αβ3-integrin-positive U87MG tumors. In vivo combination therapy by using HSA-Ce6-PTX-RGD nanoparticles plus laser irradiation demonstrated synergistic effects in inhibition of tumor growth.

The self-assembly of Ce6 and HSA without the addition of PTX was achieved by synthesis of conjugates of Ce6-HSA.\(^{(92)}\) Self-assembly of the Ce6-HSA conjugates in aqueous solution yielded nanoparticles with a size around 90 nm. As compared to free Ce6, Ce6-HSA nanoparticles showed prolonged clearance kinetics within blood, an enhanced accumulation to tumors, as well as a better tumor inhibition. Recently, it was revealed that self-assembly of Ce6 and BSA via hydrophobic interaction in aqueous solution formed Ce6-BSA nanoparticles with a mean hydrodynamic diameter of 38 nm.\(^{(93)}\) Further mixing the Ce6-BSA nanoparticles with graphene oxide (GO) in aqueous solution yielded Ce6-BSA-GO nanohybrids with a mean hydrodynamic diameter of 112 nm. As compared to the Ce6-BSA nanoparticles, Ce6-BSA-GO nanohybrids showed quenched fluorescence and singlet oxygen generation activities in solution, demonstrating, that the nanohybrids have the capability to protect Ce6. Intriguingly, Ce6-BSA-GO nanohybrids showed enhanced cellular uptake and in vitro release of Ce6, leading to an improved PDT efficiency as compared to free Ce6 and Ce6-GO.

Self-assembly of HSA with hydrophobic photosensitizers can be promoted by breaking the disulfide bond of HSA by reductants such as glutathione (GSH) and 2-mercaptoethanol. When the disulfide bond of HSA is broken, hydrophobic domains in HSA are exposed and more accessible to hydrophobic photosensitizers. For example, HSA-Ce6 nanoparticles can be prepared by self-assembly of HSA in the presence of Ce6 and GSH, mainly through hydrophobic interaction between HSA and Ce6 and intermolecular disulfide bond cross-linking of HSA (Figure 10A).\(^{(94)}\) The HSA-Ce6 nanoparticles showed excellent response to reduction and enhanced tumor accumulation as compared to free Ce6. Along with the well-known imaging by fluorescence, HSA-Ce6 nanoparticles could also be localized by photoacoustic and by magnetic resonance imaging, when Ce6 was chelated with Mn\(^{2+}\), lending the HSA-Ce6 nanoparticles a function as contrast agent for triple-modal imaging and photodynamic therapy (Figure 10B and C). Similarly, the self-assembly of HSA with IR780, a hydrophobic photosensitizer, in the presence of 2-mercaptoethanol produced spherical HSA-IR780 nanoparticles with a diameter of about 250 nm, and the nanoparticles were demonstrated efficient for tumor inhibition through combined PDT and PTT.\(^{(95)}\) Moreover, the injection of 2.5 mg kg\(^{-1}\) of IR780 caused immediate death to the mice, suggesting that IR780 is highly toxic. However, the injection of HSA-IR780 nanoparticles showed no significant side effects to the mice even at 10 times higher dosage, confirming that HSA-IR780 nanoparticles are biocompatible.

4. Conclusions and Future Perspectives

Here, we have presented a comprehensive overview of recent advances in the area of self-assembled peptide- and protein-based nanomaterials for phototherapies. To date, the self-assembly of peptides and proteins has been widely investigated and the constructed supramolecular nanostructures have been proved to be effective in enhancing the therapeutic efficacy of phototherapies. Due to the diversity and controllability of the building blocks of peptide and protein and the non-covalent interactions, there is plenty of room for manipulating the nanostructures to meet the requirements of efficient PDT and PTT. Regardless of the building blocks, formulating phototherapeutic agents by using self-assembled peptide and protein nanomaterials as drug delivery systems usually shows the merits of improving the solubility and stability of hydrophobic phototherapeutic agents in aqueous solution, decreasing the possible toxicity of the phototherapeutic agents in dark and the side effects of phototoxic skin reactions, and enhancing tumor accumulation through the EPR effect. Supramolecular nanoparticles of peptide and protein also provide a platform for incorporating multiple diagnostic functions such as fluorescence imaging, photoacoustic imaging, and MR imaging, simultaneously with phototherapies. With special design, self-assembled peptide- and protein-based nanomaterials could be further endowed with specific properties such as responsiveness to tumor microenvironment, enabling the realization of triggered release of photosensitizers from the nanomaterials for efficient PDT and traceable PDT. Intriguingly, amphiphilic dipeptides and even amino acids are found effective in modulating the self-assembly of photosensitizers, and the obtained nanoparticles are endowed with tunable size and surface charge and multiresponsiveness to pH, detergents, and enzyme, suggesting, that short peptide-modulated self-assembly of photosensitizers is a simple but efficient method to formulate and deliver photosensitizers. Other than the examples of pre-assembled nanoparticles, the example of in situ self-assembly toward enhanced PTT has also been demonstrated in vivo by using a water-soluble building block containing an enzyme-responsive peptide, providing an alternative way to explore self-assembling peptides for phototherapies. Moreover, a novel strategy for PDT, “one injection, multiple-treatment”, has also been demonstrated by using self-assembled collagen hydrogels.

Despite recent successful results regarding self-assembled peptide- and protein-based nanomaterials for phototherapies, there are still many challenges of these nanomaterials for clinical translation. An ideal phototherapeutic nanomaterial should possess the following features: (i) good biocompatibility and biodegradability while no dark toxicity and unwanted immunogenicity; (ii) stability in blood circulation, and selective tumor targeting and accumulation; (iii) strong absorption in NIR range to ensure efficient light absorbance and deep light penetration depth; (iv) large singlet oxygen quantum yield for
PDT or high light-to-heat conversion yield for PTT. To realize the goal of phototherapeutic nanomaterials with clinical value, more efforts are still needed concerning light-absorbing molecules, peptide and protein building blocks, and the pharmacokinetics of the nanomaterials. For example, part of the widely investigated NIR dyes, such as IR820 and IR825, are toxic. Though their toxicity is decreased when encapsulated by nanoparticles, the long-term safety has not been demonstrated, leading to a potential negative impact on the biocompatibility of the formed phototherapeutic nanomaterials. Also, most photosensitizers investigated for PDT can be activated only by visible light, other than NIR light, restricting the penetration depth of PDT. Hence, new photosensitizers that possess NIR absorbance along with high biocompatibility and biodegradability are urgently required. For PTT, another limitation is the relatively high light intensity (0.5–1.0 W cm\(^{-2}\) for the reviewed reports) that required to generate sufficient heat for tumor ablation, while the maximum exposure threshold for skin at the widely applied wavelength of 808 nm is about 0.3 W cm\(^{-2}\).[96] High-intensity light can induce undesired side effects to healthy cells and tissues that locate in the pathway of the light. Reduction of the light intensity relies on efficient delivery of photothermal nanomaterials that have enhanced NIR absorbance and high light-to-heat conversion yield to tumors.[97] Regarding the self-assembled nanostructures, the peptide and protein building blocks can be further tailored with anticancer, antimicrobial, and immunomodulatory activities by using the sequences of bioactive peptides. In such a design, the nanostructures will possess intrinsic functions other than only serve as nanocarriers for loading of the light-absorbing molecules, providing a novel approach of synergistic therapy. Moreover, the detailed pharmacokinetic/pharmacodynamic analysis is absent for most of the reported systems, though it is important not only for determining an optimized condition for the therapy and demonstrating the long-term safety of the phototherapeutic agents, but also for deeply understanding the in vivo behaviors of the self-assembled nanomaterials and providing fundamentals for further development of peptide- and protein-based nanomaterials in the area of phototherapies.

Acknowledgments

M.A. and Q.Z. contributed equally to this work. We acknowledge financial support from the National Natural Science Foundation of China (Project Nos. 21522307, 21473208, 51403214 and 91434103), the Talent Fund of the Recruitment Program of Global Youth Experts, and the Chinese Academy of Sciences (CAS, Project No. QYZDB-SSW-JSC034). X.Y. is greatly indebted to Prof. Möhwald for his long-term support.

Received: September 18, 2016
Revised: October 18, 2016
Published online: January 6, 2017
学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具