Advances and Perspectives of Peptide and Polypeptide-Based Materials for Biomedical Imaging

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Biomedical imaging is an effective tool to reveal internal structures beneath the skin. Such tools are of great importance for disease diagnosis and treatment. Various small molecules, polymers, and micro- or nanomaterials have been applied as imaging agents for contrast enhancement. Peptide- and polypeptide-based materials have been designed and developed for enhanced and multifunctional imaging, due to their excellent biocompatibility and diverse biofunctionality. This review aims to summarize recent advances in peptide- and polypeptide-based materials for X-ray computed tomography (CT), magnetic resonance imaging (MRI), fluorescence imaging (FLI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and multimodal imaging. In addition to biomedical imaging, peptide- and polypeptide-based materials have also been endowed with therapeutic functions for disease theranostics. This review is concluded with a perspective on future peptide- and polypeptide-based materials for biomedical imaging.

1. Introduction

Prior to biomedical imaging assays, diseases were identified by auscultation, palpation, and percussion; the diagnosis was based on the physician’s personal experience. It is, however, difficult to obtain patients’ pathological information accurately by aforementioned traditional approaches. Emerging biomedical imaging technologies revolutionized clinical diagnostics as this approach allows convenient analysis and visualization of pathological changes in a noninvasive way. The use of X-rays can be seen as one of the earliest examples of biomedical imaging technology: X-rays were discovered by Wilhelm Conrad Röntgen in 1895 and applied in clinic by John Hall-Edwards in 1896; a new era for disease diagnosis and treatment had begun.[1,2] The development of chemical science and modern engineering in the 20th century allowed the development of various techniques for biomedical imaging.[3–5] For example: Structural and anatomical information of patients can be obtained through X-ray computed tomography (CT) and magnetic resonance imaging (MRI). Positron emission tomography (PET), single-photon emission computed tomography (SPECT), and fluorescence imaging (FLI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and multimodal imaging.

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functional and biocompatible imaging platforms with targeting capabilities for the effective diagnosis and treatment. Over the past few years, due to the excellent biocompatibility and diverse physiochemical properties, peptides and polypeptides have been widely studied for biomedical applications, including biomedical imaging.\textsuperscript{[25–31]} Furthermore, the structural diversity of peptides and polypeptides allows self-assembly into various well-organized nanoarchitectures with tunable physiochemical properties.\textsuperscript{[12–37]} In addition, peptides and polypeptides can endow existing contrast agents with targeting and responsive capability toward specific lesions.\textsuperscript{[27]} In this review, recent advances in different biomedical imaging modalities are summarized and perspectives of peptide/polypeptide-based materials for biomedical imaging are discussed.

2. Peptide- and Polypeptide-Based Materials for Biomedical Imaging

Recently developed peptide/polypeptide-based materials have made remarkable developments for their use as next-generation biomedical imaging platform. Peptides or polypeptides can be synthesized and self-assembled into nanoparticles with tunable shape and physiochemical properties. These nanoparticles are capable of generating imaging signals (such as fluorescence) or can be loaded with contrast agents that have an enhanced imaging efficiency and a reduced cytotoxicity, compared with the traditional contrast agents. In addition, peptides can be used to functionalize existing contrast agents or imaging platforms. This equips these traditional contrast agents with the ability to target specific lesions and allows responsive capability. Peptides and polypeptides with a diverse variety of functions provide excellent biocompatibility, specific bioactivity and flexible modifiability for peptide/polypeptide-based materials. These advantages enable peptide/polypeptide-based imaging materials with reduced contrast-agent toxicity, specific disease targeting, and the capability to achieve multimodal imaging.

This section summarizes the design, preparation, and application of various peptide/polypeptide-based materials for CT, MRI, FLI, PET, SPECT, and for multimodal imaging (Scheme 1). In addition, peptide/polypeptide-based materials discussed in this review are summarized in Table 1.

2.1. Peptide/Polypeptide-Based Materials for CT

CT is a biomedical imaging technique that uses X-rays to reveal the internal structures of the human body. This technique is based on the attenuation of X-rays that traverse the patient’s body. These attenuated X-rays are detected and converted into visual images.\textsuperscript{[38–40]} Contrast agents can be used to increase CT attenuation to improve the visualization of a specific region of interest. Such agents are often contained with iodinated compounds or are bismuth- or gold-based materials.\textsuperscript{[38,41]} However, these materials do not have the ability to target specific cells, and generally require a large dosage to be used efficiently. In addition, commonly used CT contrast agents can induce allergic reactions or cause severe side effects, such as contrast-induced nephropathy.\textsuperscript{[42]} Such disadvantages can be overcome by endowing these contrast agents with peptides with disease-targeting capabilities, to significantly reduce the required dosage of CT contrast agents.\textsuperscript{[23]} More specifically, the RGD peptide “Arg-Gly-Asp” and the cRGDfK cyclic peptide “c(Arg-Gly-Asp-D-Phe-Lys)” (cRGD) have a high-affinity toward \(\alpha_v\beta_3\) integrin, an essential cell-adhesion receptor that is often overexpressed in tumor cells and neovasculatures.\textsuperscript{[43]} Therefore, RGD and cRGDfK have been widely applied for targeted CT imaging of tumors. An example of such an approach was recently reported by Zhong and co-workers; biodegradable iodine-rich polymersomes (IPs) were functionalized with cRGD and used for targeted in vivo CT imaging of tumors (Figure 1).\textsuperscript{[44]} CT images of several types of malignant tumors at different time points after the administration of cRGD-IPs were obtained; results showed enhanced contrast at tumor site within 8 h, compared with polymersomes that were not functionalized with cRGD, which indicated the tumor-targeting function of cRGD. In addition, in vivo CT imaging showed sufficient contrast in 1–4 h with cRGD-IPs, better than clinically used CT contrast agent iohexol, which may attribute to longer retention times and relatively large size of the cRGD-IP. Indeed, cRGD-IPs are a promising nanoplatform for the early diagnosis of various malignancies for CT imaging. cRGDs have also been functionalized on other polymer nanoparticles to allow efficient CT imaging or usage as a theranostic platform.\textsuperscript{[45,46]} Through the tumor-targeting ability of cRGD
Table 1. Summary of peptides and polypeptides applied in biomedical imaging with different sequences/structures, functions, contrast agents, dosages, and imaging modalities.

| Peptide/polypeptide | Sequences/structures | Functions | Contrast agents | Dosages[^1] | Imaging modalities | References |
|---------------------|----------------------|-----------|-----------------|-------------|-------------------|------------|
| cRGDfK              | Cyclo-(Arg-Gly-Asp-D-Phe-Lys) | αvβ3 integrin binding for targeted imaging | Iodine-functionalized trimethylene carbonate | 500 mg [I] kg⁻¹ (Balb/c mice) | CT [44] |
| LyP-1               | Cys-Gly-Asn-Lys-Arg-Thr-Arg-Gly-Cys | p32 protein binding for targeted imaging | Bi₂S₃ nanoparticles | ≈1028 mg kg⁻¹ (Balb/c mice) | CT [48] |
|                     |                      |           | Bi nanoparticles | ≈7.2 mg kg⁻¹ (Balb/c mice) | CT [49] |
|                     |                      |           | Fe₃O₄ nanoparticles | ≈0.0448 mg [Fe] kg⁻¹ (C57BL/6 mice) | MRI [64] |
| TAT                 | Tyr-Gly-Arg-Lys-Arg-Arg-Arg-Arg-Arg-Arg-Arg | Blood–brain barrier penetration for targeted imaging | Gd³⁺ | ≈3.145 mg [Gd] kg⁻¹ (C57BL/6 mice) | MRI [68] |
| GB111-NH            | Carbobenzoxy-Phe-Lys | Cysteine cathepsins binding for targeted imaging | Iodinated nanoparticles/Au nanoparticles | ≈5.60 mg kg⁻¹ (Balb/c mice) | CT [51, 52] |
| RVRR                | Arg-Val-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg | Cleaved by furin for targeted and enhanced imaging | Olsalazine | ≈278 mg kg⁻¹ (NU/J nude mice) | CEST MRI [61] |
| DEVD                | Asp-Glu-Val-Asp | Cleaved by caspase 3/7 for targeted and enhanced imaging | Gd³⁺ | ≈0.0827 mg kg⁻¹ (Harlan rats) | MRI [62] |
| AANK                | Ala-Ala-Asn-Lys | Cleaved by legumain for activatable imaging | Cy5.5 fluorophore | ≈0.436 mg kg⁻¹ (nu/nu mice) | NIR-FLI [86] |
| CREKA               | Cys-Arg-Glu-Lys-Ala | Fibrin–fibronectin complexes recognition for targeted imaging | Manganese ferrite nanoparticles | 5.0 mg [Fe+Mn] kg⁻¹ (Balb/c mice) | MRI [63] |
| CGGTTGAKRRMQYNRR    | Cys-Gly-Cys-Thr-Cys-Arg-Arg-Ala-Lys-Arg-Arg-Met-Gln-Tyr-Asn-Arg-Arg | Bacteria-targeted imaging | Mn²⁺ | ≈0.0528 mg [Mn] kg⁻¹ (Balb/c mice) | MRI [66] |
| angiopep 2          | Thr-Phe-Phe-Tyr-Gly-Ser-Arg-Gly-Lys-Arg-Asn-Phe-Arg-Thr-Glu-Uty-Thr-Glu-Lys-Arg | Blood–brain barrier penetration for targeted imaging | Gd³⁺ | ≈3.145 mg [Gd] kg⁻¹ (nude mice) | MRI [67] |
| KREVRGP             | Lys-Asp-Glu-Val-Asp-Gly-Phy | Cleaved by cysteine cathepsins for targeted and enhanced imaging | Cy5 fluorophore | ≈1.016 mg kg⁻¹ (Balb/c mice) | NIR-FLI [83] |
| GLPGLPGKGG          | Gly-Pro-Leu-Gly-Leu-Pro-Gly-Lys-Gly-Gly | Cleaved by matrix metalloproteases for activatable imaging | 3-amino-1,2,4-triazole-fused 1,8-naphthalimide (ANNA) fluorophore | 10 mg [Fe] kg⁻¹ (Balb/c mice) | NIR-FLI [85] |
| AF7P                | Cys-Cys-His-Asp-Thr-His-Leu-His-Asn-Thr-Lys-Asp-Thr-Leu | Matrix metalloproteinase 14 recognition for targeted imaging | Ag₃S quantum dots | 2.5 mg kg⁻¹ (Balb/c mice) | NIR-FLI [92] |
| Cyclic octapeptide  | Cyclic (D-Cys-Cly-Arg-Asp-Ser-Pro-Cly-Lys) | Phosphorylated Annexin A2 binding for targeted imaging | Cyprate | ≈3.526 mg kg⁻¹ (C57BL/6 mice) | NIR-FLI [93] |
| TPP                 | Thr-Lys-Asp-Asn-Leu-Leu-Arg-Asn-Glu-Uty-Arg | Membrane heat shock protein 70 recognition for targeted imaging | ⁸⁹Zr | ≈177.6 MBq kg⁻¹ (Balb/c mice) | PET/CT [112] |
| Bombsin             | Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met | Gastrin-releasing peptide receptor binding for targeted imaging | ⁶⁸Ga | 1.85 MBq kg⁻¹ (patients) | PET/CT [113] |
| D²FQAVGH-StaL       | D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu | Gastrin-releasing peptide receptor binding for targeted imaging | ⁶⁸Cu | 168-960 MBq kg⁻¹ (ICR SCID mice) | PET/CT [114] |
| FS6                 | Trp-His-Asp-Met-Glu-Trp-Trp-Tyr-Leu-Gly | Vascular endothelial growth factor receptor 1 binding for targeted imaging | ¹¹¹In | ≈740 MBq kg⁻¹ (nude mice) | SPECT [123] |
| RDH6                | D-(Arg-Glu-Phe-Val-Phe-Phe-Leu-Tyr) | Human epidermal growth factor 2 binding for targeted imaging | ⁹⁹mTc | ≈1480 MBq kg⁻¹ (NOD SCID mice) | SPECT/CT [124] |
| Peptide/polypeptide | Sequences/structures | Functions | Contrast agents | Dosages$^a$ | Imaging References modalities |
|---------------------|----------------------|-----------|----------------|-------------|-----------------------------|
| cNGR                | Cyclic (Cys-Asn-Gly-Arg-Cys-Gly-Arg-Cys-Gly-Gly-Lys) | Tumor endothelial cell binding for targeted imaging | Au-Gd clusters | $\approx 7.88 \text{ mg [Au]}/20.12 \text{ mg [Gd] kg}^{-1}$ (Balb/c mice) | CT/MRI [135] |
| GGNYTCEVTREGTIIELK  | Gly-Gly-Asn-Tyr-Thr-Cys-Glu-Val-Thr-Glu-Leu-Thr-Arg-Glu-Gly-Thr-ile-Glu-Leu-Lys | Delaying the clearance for enhanced imaging contrast | $^{99m}$Tc/Fe$_3$O$_4$ | $1580 \text{ MBq kg}^{-1}/(10 \text{ mg [Fe] kg}^{-1})$ (Balb/c mice) | SPECT/CT and MRI [140] |
| G$^2$F-Pip-RSGGGKCy | Gly-D-Phe-Pip-Arg-Ser-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Lys-Cys | Cleaved by thrombin for targeted and activatable imaging | Cy5.5 fluorophore/Au | $100 \text{ mg kg}^{-1}$ (Balb/c mice) | NIR-FLI/CT [142] |
| Poly (lysine)        |                        | Efficiently loading contrast agents, reducing dosage, improving biocompatibility, decreasing toxicity of contrast agents | Triiodobenzoic chloride | $500 \text{ mg kg}^{-1}$ (Balb/c mice) | CT [54] |
|                     |                      | Efficiently loading functional molecules for combining therapy and multimodal imaging | Fe$_3$O$_4$ nanoparticles | $\approx 94 \text{ mg kg}^{-1}$ (Balb/c mice) | MRI [73] |
|                     |                      |                                           | $^{18}$ F | $\approx 148 \text{ MBq kg}^{-1}$ (Balb/c mice) | PET [115] |
|                     |                      |                                           | Fe$_3$O$_4$/Au nanocomposites | $\approx 36 \text{ mg kg}^{-1}$ [Fe]/$8 \text{ mg kg}^{-1}$ [Au] (Balb/c mice) | MRI/CT [144] |
| Poly (glutamic acid) |                        | Improving in vivo stability and biocompatibility of contrast agents | Mn$^{2+}$ | $\approx 12.375 \text{ mg [Mn] kg}^{-1}$ (Balb/c mice) | MRI [69] |
|                     |                      | Efficiently loading functional molecules for combining therapy and multimodal imaging | Gd$^{3+}$ | $\approx 240 \text{ mg kg}^{-1}$ (Balb/c mice) | MRI [71] |
|                     |                      |                                           | Fe$_3$O$_4$ nanoparticles | $\approx 0.616 \text{ mg [Fe] kg}^{-1}$ (Balb/c mice) | MRI [72] |
|                     |                      |                                           | $^{99m}$Tc | $\approx 19 \text{ MBq kg}^{-1}$ (New Zealand rabbits) | SPECT/CT [130] |
|                     |                      |                                           | Fe$_3$O$_4$/Au nanocomposites | $\approx 36 \text{ mg kg}^{-1}$ [Fe]/$8 \text{ mg kg}^{-1}$ [Au] (Balb/c mice) | MRI/CT [144] |
| Poly (aspartic acid) |                        | Improving in vivo biocompatibility of contrast agents | Gd$^{3+}$ | $\approx 7.8625 \text{ mg [Gd] kg}^{-1}$ (Balb/c mice) | MRI [70] |
| Poly (thyroxine)    |                        | Improving in vivo stability and biocompatibility of contrast agents; Efficiently loading contrasts agents for multimodal imaging and radioisotopes for radiotherapy | $^{125}$I/$^{131}$I | $500 \text{ mg [I] kg}^{-1}/160 \text{ MBq kg}^{-1}$ (Balb/c mice) | CT and SPECT/CT [131] |
| Poly ($\gamma$-benzyl glutamate) |                        | Improving biocompatibility of contrast agents; Loading functional molecules for multimodal imaging | $^{111}$In/Gd$^{3+}$ | $\approx 74 \text{ MBq kg}^{-1}$ (Balb/c mice) | SPECT/CT and MRI [145] |

$^a$(Dosage refers to the administrated dosages of imaging platforms or molecular probes if not specifically noted.)
peptides, polymer nanoparticles could specifically accumulate at tumor sites, and further loading of anticancer drug made these particles efficient platforms to combine imaging and therapeutic function.

In addition to the well-known RGD peptides, other recognition peptides have been used to functionalize different platforms with tumor-targeting abilities for CT imaging. For example, homing peptide LyP-1 (Cys-Gly-Asn-Lys-Thr-Arg-Gly-Cys); this peptide has shown specific homing to tumor lymphatic vessels, and is capable of targeting p32 proteins, which are overexpressed in certain cancer cells.[51] More specifically, LyP-1 was used to functionalize Bi$_2$S$_3$ nanoparticles, which are well-suited for CT imaging of breast cancer.[48] In another work by Li and co-workers, LyP-1-labeled semimetal Bi nanoparticles (Bi-LyP-1) were designed for dual-modal imaging and synergistic therapy for breast cancer.[49] These LyP-1-functionalized nanoparticles have high tumor accumulation properties and lead to enhanced CT contrast. In vivo CT imaging showed clear signal enhancement at tumor site 5 min after injection of peptide-functionalized nanoparticles, demonstrating enhanced targeting capability of LyP-1. Another property of these Bi-LyP-1 is that they significantly inhibit tumor growth through photothermal and radiotherapy, as the Bi nanoparticles can absorb both near infrared (NIR) laser and X-ray irradiation, combining imaging and therapy in one single nanoparticle.

Zhang and co-workers reported gold nanoparticles functionalized with a trans-activator transcription (TAT) peptide (Au@TAT) for mesenchymal stem-cells tracking.[50] The positively-charged surface from TAT peptide improved the internalization of gold nanoparticles into stem cells. This led to the enhanced attenuation of these stem cells, hence enabling the visualization and location using a CT scan. Stem cells were successfully tracked in mice using in vivo CT imaging and bioluminescence imaging within 10 days; in addition, using 3D CT imaging, the spatial distribution of transplanted stem cells in lungs was obtained after 7 days; this method could be used to study mechanism of stem-cell therapy.

Molecular imaging through CT is often considered difficult due to low sensitivity. However, this can be improved by applying targeting peptides which could specifically target to certain biomolecules. Blum and co-workers reported iodinated nanoscale activity-based probes (IN-ABPs) for CT tumor molecular imaging; these probes were functionalized with carboxbenzoxo-Phe-Lys.[51] The targeting ability of these nanoparticles is based on carboxbenzoxo-Phe-Lys; this peptide can selectively bind to cysteine cathepsin, an enzyme that is expressed in some cancers cells. In vivo CT imaging showed a clear tumor accumulation of IN-ABPs 24 h after injection. A limited amount of accumulation was observed for nontargeted probes. Quantitative analysis showed enhanced tumor CT contrast of IN-ABPs compared with nontargeted probes. These data show the enhanced specificity that these targeting peptides can provide. It was further shown that IN-ABPs are able to monitor the cathepsin activity while using CT imaging; this approach can also be applied in the diagnosis of cancer and other diseases that are characterized by elevated cathepsin levels, such as vulnerable atherosclerotic plaques.[51]

Subsequently, Blum and co-workers further developed a new type of cathepsin-targeting CT imaging probe (ABPs) that is based on gold nanoparticles.[52] A library of ABPs was developed to screen and optimize the biochemical properties by varying the peptides/PEG-ratio and the nanoparticle size. The resulting optimized gold nanoparticle-ABPs showed accumulation properties at the tumor site, as evidenced by enhanced CT signals obtained in a mice tumor model. In addition, probes with targeting peptide showed an enhanced CT contrast at tumor site 72 h after injection, compared with nontargeted probes at different dosages, which demonstrated the efficient targeting capabilities of this cathepsin-targeting peptide. It was discovered that the covalent linkage between targeting peptide and targeted enzyme was a key factor that leads to increased imaging sensitivity, which may provide a strategy for CT molecular imaging.

In addition to improved targeted imaging, polypeptides were also used to enhance the biocompatibility of CT contrast agents. Iodinated materials are commonly used contrast agents for CT imaging; however, these suffer from fast renal clearance and acute renal toxicity, which could lead to severe renal damage and even contrast-induced nephropathy.[42,53] Polypeptide with intrinsic biocompatibility is favorable for CT imaging to reduce the adverse effects caused by clinically-used iodinated contrast agents.

Recently, Du and co-workers reported a polypeptide-based renoprotective angiographic polymersomes (RAPs) for blood pool CT imaging (Figure 2).[54] RAPs were prepared via post-polymerization self-assembly. Here, a block copolymer was used that consists of a Lys-triodobenzoic chloride block, that allowed CT imaging, and a renoprotective Lys-phenylboronic acid pinacol ester block, that acts as a reactive oxygen species (ROS) scavenger. An excess of ROS can be caused by the high dose of iodinated contrast agents and are harmful for the kidneys. In vivo CT angiography showed longer vascular enhancement times when using RAPs, five-times longer than that observed for the commonly used iohexol CT contrast agent. Furthermore, slower renal
clearance was observed of RAPs due to the large size and long blood circulation time which slowed down the metabolism of this contrast agent, allowing a reduced dose for adequate CT contrast. In vivo antioxidative activity assessment of RAPs and histopathology studies indicated excellent renal protective functions of these polymersomes. Longer retention time were also observed within the liver; however, no clear hepatic dysfunction and inflammation were observed, indicating a low liver toxicity of RAPs. In summary, RAPs demonstrated a new strategy to increase the imaging quality for CT while reducing the risk of contrast-induced nephropathy.

2.2. Peptide/Polypeptide-Based Materials for MRI

During an MRI scan, a strong external magnetic field and a radio-frequency magnetic field are applied. Magnetization and relaxation of H\(^+\)-ions within patient’s body are recorded, to construct a visual MRI.\(^{[55]}\) Contrast agents such as gadolinium(III), manganese(II), and superparamagnetic iron oxide (SPIONs) are used to change the relaxation rate of nearby H\(^+\), leading to enhanced contrast between different tissues.\(^{[56, 57]}\) However, commonly used MRI contrast agents suffer from poor biocompatibility and lack specificity. Fortunately, bioactive peptides and polypeptides could be introduced with MRI contrast agents to improve the biocompatibility and enhance imaging contrast of specific lesion sites.

A versatile strategy to enhance signal intensity is based on targeted accumulation and intracellular self-assembly. Intracellular self-assembly strategy enables to construct supramolecular nanostructures through in situ self-assembly at specific intracellular sites.\(^{[58]}\) Compared to traditional “always on” contrast agents, whose imaging signals could be detected through the whole imaging process, intracellular self-assembly platforms could keep the signal “off” until they reach the targeted physiological tissues or organs.\(^{[29, 58, 59]}\) This activatable characteristic of this intracellular self-assembly platform significantly enhances the signal-to-noise ratio for more accurate and efficient imaging. In particular, responsive peptides are suitable to construct intracellular self-assembly imaging platforms. For example, peptide IETD (Ile-Glu-Thr-Asp) is substrate for caspase 8 and caspase 8 is activated in apoptotic cells.\(^{[60]}\) Over-expressed enzymes and over-accumulated metabolites in lesions provide triggers to change the chemical structure of responsive peptides and activates the self-assembly process and imaging signals.\(^{[29]}\) In
addition, responsive peptide-functionalized imaging platforms have been designed to increase the retention within lesions for smart and activatable MRI.\(^{[29]}\)

Recently, Bulte and co-workers reported an anticancer agent–peptide conjugated probe for activatable MRI and drug release in tumor cells.\(^{[61]}\) Here, anticancer agent olsalazine (Olsa) was used as it allows distinct contrast in “chemical exchange saturation transfer magnetic resonance imaging” (CEST MRI). The positively charged cell penetrating peptide RVRR (Arg-Val-Arg-Arg) was selected to functionalize contrast agents. This peptide could be cleaved by the upregulated enzyme furin in many malignancies. In addition, a condensation reaction is initiated after cleavage by glutathione (GSH), which is over-accumulated in tumor cells. This process results in the self-assembly of Olsa-RVRR into large intracellular nanoparticles by \(\pi-\pi\) interaction, a process that “activated” the Olsa-RVRR probes. Here, intracellular self-assembly resulted in longer retention times and remarkable enhancement in the CEST MRI signals. In vivo CEST MRI showed that signals at tumor site reached maximum enhancement in 2 h, and enhanced signals could be detected within 24 h. However, enhanced CEST signals of free Olsa could be detected only in 4 h. In addition, in vivo studies with different tumor models showed that the OlsaCEST signal and antitumor therapeutic effect were 6.5- and 5.2-fold increased, respectively, compared with probes that did not contain RVRR peptides. Similarly, Nejadnik et al. reported a caspase-3-sensitive nanoaggregation MRI probe (C-SNAM) that was designed for the detection of stem-cell apoptosis in arthritic joints (Figure 3).\(^{[62]}\) C-SNAM was constructed by the conjugation of the responsive peptide DEVD (Asp-Glu-Val-Asp) and Gd-chelate MRI contrast agent. The DEVD peptide enables the detection of apoptosis as it can be cleaved by caspase-3, a common cell apoptosis biomarker. This cleavage process and the reduction of GSH allows C-SNAM to form a macrocyclic molecule by condensation reaction. This allows further self-assembly into Gd nanoaggregation for enhanced MRI signals and retention effect. In vivo MRI showed that, apoptotic adipose-derived stem cells were detected with enhanced MRI signals 30 min after administration. However, no clear signal enhancement was observed in the case of viable adipose-derived stem cells, indicating the specific accumulation of C-SNAM. These responsive peptide-functionalized platforms with activatable function could greatly enhance the retention at lesions and thereby increase the signal intensity, enabling the detection of abnormal activities and diseases.

In addition, this activation strategy can be applied to improve the sensitivity of \(^{19}\)F MRI. For example, an AANK peptide (Ala-Ala-Asn-Lys) functionalized probes were reported for the detection of legumain activity in zebrafish by \(^{19}\)F MRI.\(^{[63]}\) Here, the responsive peptide AANK served as a substrate for legumain, an asparaginyl endopeptidase that is expressed in several inflammatory diseases. Intracellular self-assembly was induced by GSH reduction; disassembly was induced by legumain hydrolysis.

**Figure 3.** Responsive peptide-functionalized platform for MRI. a) Schematic illustration of C-SNAM and b) corresponding mechanism of stem-cell apoptosis detection in vivo. Reproduced with permission.\(^{[62]}\) Copyright 2015, American Chemical Society.
These processes allowed the $^{19}$F signals to be switched “off” and “on” and enabled the accurate monitoring of the legumain activity. Enhanced $^{19}$F MRI signals at tumor regions in zebrafish were observed after injection with this probe, whereas no $^{19}$F MRI signals from other region of the tumor-bearing zebrafish or the healthy zebrafish were found. With high tumor-to-normal tissue ratio, this activatable strategy is promising for the design of new $^{19}$F MRI platforms to detect diseases with a high sensitivity.

Targeting peptide functionalization is another strategy to increase the MRI signals at lesion sites. For example, the tumor-targeting peptide LyP-1 can be used to functionalize silica-coated Fe$_3$O$_4$ nanoparticles. It was reported that such nanoparticles allow pancreatic cancer detection with MRI.\cite{64} The targeting capability of LyP-1 allowed specific accumulation at the tumor site. This led to enhanced MRI contrast, as observed in an orthotopic pancreatic cancer mouse model. More recently, the tumor-targeting peptide CREKA (Cys-Arg-Glu-Lys-Ala) was used to functionalize manganese ferrite nanoparticles. This composition was used for metastases detection by MRI.\cite{65} CREKA peptides can specifically recognize fibrin–fibronecin complexes, which are overexpressed on tumor vessels and in tumor stroma. In addition, these nanoparticles utilize the mild acidity and elevated H$_2$O$_2$ levels in the microenvironment of the tumor to trigger the release of Mn$^{2+}$, enhancing the T1-weighted MRI signals. In vivo MRI signal enhancement at tumor site was observed in 120 min, compared with nanoparticles without CREKA peptide functionalization, which confirmed the targeting capability of CREKA peptide. As a result, this approach led to an increased detection sensitivity of metastases, a minimum limit of 0.39 mm was achieved.

Recently, Wang and co-workers reported a polymer–peptide–porphyrin conjugate for MRI-guided sonodynamic therapy.\cite{66} Here, peptide PLGVRG, KLVFF, CGGGTGRAKRRMQYNRR, and porphyrin conjugated GGGG were designed and synthesized for specific cleavage by gelatinase, in situ self-assembly, bacteria-targeting and Mn$^{2+}$ loading, respectively. These peptides were conjugated with polymer backbone and self-assembled into nanoparticles. In vivo MRI showed enhanced signals in the inflammatory area of mice within 3 days after injection. In addition, compared with mice treated with PBS or CGGG–porphyrin conjugates, peptide-based nanoparticles exhibited clear accumulation in the inflammatory area, which indicated the effective diagnosis combining bacteria-targeting, gelatinase-responsive and in situ self-assembly functions.

MRI can also be used for the diagnosis and treatment of brain tumors. However, the blood–brain barrier complicates the administration of contrast agents and medicine. Li and co-workers reported gold nanoparticles that were able to cross the blood–brain barrier and target glioma. These nanoparticles were functionalized with peptide angiopep 2.\cite{67} This allowed penetration of the blood–brain barrier and can be used to guide brain tumor surgery. Interestingly, these nanoparticles can self-assemble in the acidic environment of the brain tumor and amplify the MRI signals. The position of glioma was located by T2-weighted MRI and T1-weighted MRI was applied to observe the T1 signal enhancement from these nanoparticles. Compared with T2-weighted images, the tumor boundary was clearly visualized 30 min after injection in T1-weighted images, and could also be observed 24 h after administration. Another work showed that tumor-targeting TAT peptide functionalized gold nanoparticles were able to deliver Gd$^{3+}$ chelates to malignant gliomas in mice with enhanced MRI contrast.\cite{68} TAT peptide (Tyr-Gly-Arg-Lys-Lys-Arg-Gln-Arg-Arg-Ard-Arg) was selected to functionalize gold nanoparticles to allow them to cross the blood–brain barrier and target the brain tumor for glioma imaging and therapy.

High molecular weight polypeptides can also be used for targeted and enhanced MRI imaging. The biocompatibility and loading capability enable polypeptide-assemblies to load contrast agents that amplify the MRI signals. Kataoka and co-workers reported a polypeptide MRI platform that confined Mn$^{2+}$ within a calcium phosphate crystal.\cite{69} Here, poly(ethylene glycol)(PEG)–poly(glutamic acid) block copolymers were used. The interactions between polypeptide’s glutamic acid groups and Ca$^{2+}$ contributed to the size control of the nanoparticles. This greatly improved the stability of this imaging platform. In vivo MRI showed clear enhancement in MRI signals at tumor site within 6 h after administration. In addition, tumor-to-normal tissues contrast ratio with this nanoplatfrom was significantly enhanced within 4 h, compared to contrast agents Gd-chelate and Mn$_2$O$_3$. In addition, the liver metastasis of tumor was detected by MRI with this method, due to the disintegration of pH-sensitive calcium phosphate and the release of Mn$^{2+}$ in the low pH of the tumor’s microenvironment.

The potential toxicity of Gd is a major drawback for MRI. The confined Gd within the self-assembled polypeptide nanoparticles can reduce the risk of Gd remaining in the human body. Mi et al. reported a polypeptide-based polymer micelle for MRI-guided gadolinium neutron capture tumor therapy.\cite{70} Polymer micelles were endowed with an MRI contrast agent and used in MRI and MRI-guided gadolinium neutron capture tumor therapy. In this work, Gd-diethyleneetriamnientopentaacetic acid (DTPA) was incorporated in these polymer micelles, and the imaging capability of polymer micelles was examined on a mouse tumor model. Clear contrast enhancement at tumor site was observed in 4 h with these polymer micelles, showing improved MRI contrast than free Gd-DTPA.

Du and co-workers reported a poly(glutamic acid)-based block copolymer vesicles that contained the Gd$^{3+}$ chelator DTPA for enhanced tumor MRI contrast and drug delivery.\cite{71} The DTPA groups were present in the inner corona of the vesicles that were effectively chelated with Gd$^{3+}$. As a result, these nanoparticles afforded an 8-fold enhancement in the T1 relaxivity. In addition, the toxicity of the Gd$^{3+}$ ions was minimized by chelating within the vesicles. Anticancer drug loading (doxorubicin hydrochloride (DOX-HCl)) in the outer corona further equipped these imaging polymersomes with the possibility of targeted tumor therapy.

SPIONs can also be applied as MRI contrast agents—these are generally less toxic than Gd$^{3+}$–based contrast agents. For example, Du and co-workers reported a superparamagnetic poly(glutamic acid)-based polymersome for MRI with extremely short T2 relaxation times (Figure 4).\cite{72} Here, nano-sized Fe$_3$O$_4$ was loaded within the coronas of a polymersome by in situ chemical precipitation of Fe$^{2+}$ and Fe$^{3+}$. These particles were examined in vivo; time-dependent MR images revealed negative contrast enhancement of these polymersomes in 60 min, while using a very low iron concentration. More recently, Cui and co-workers reported polypeptide nanoparticles for T2-weighted MRI and
MRI-guided chemo-ferroptosis combination therapy. Here, Fe₃O₄ and cisplatin were introduced into nanoparticles through electrostatic interaction with poly(glutamic acid) and poly(lysine). In vivo MRI imaging on tumor-bearing mice showed enhanced T2 contrast at tumor site after injection with polypeptide nanoparticles. Quantification of T2 signals also confirmed enhanced MRI with best enhancement during 2-4 h after injection. In addition, hematoxylin and eosin (H&E) staining of organs, as well as immunohistochemical analysis of blood from mice showed low systematic toxicity of the applied polypeptide nanoparticles. SPIONs can be coated with peptides in situ; this is an efficient strategy to improve the biocompatibility, the targeting ability, and the in vivo particle stability. For example, Paramelle and co-workers designed a library of 21 peptides and ligands, and identified highly stable self-assembled peptide monolayers on the surface of ultra-small superparamagnetic iron oxide nanoparticles (USPIONs) (Figure 5). Eighty-six different peptide-coated USPIONs were prepared; subsequently, their specificity, toxicity, and MRI contrast-enhancement were evaluated on mice liver tumors. As a result, selected USPIONs that were coated with single or mixed peptides exhibited an optimized target-to-noise ratio and tumor-to-liver contrast, which are promising for the early cancer diagnosis by MRI.

2.3. Peptide/Polypeptide-Based Materials for FLI

FLI is a noninvasive imaging technique to visualize physiological activities within living organisms. However, the low tissue penetration properties of photons within the UV–vis spectral window limit its clinical applications. NIR-FLI is a rapidly evolving imaging technology for clinical transformation. Photons within NIR window (NIR-I and NIR-II window) provide low absorption, scattering, and exponentially decreased biofluorescence of tissues, leading to excellent penetration capabilities. This significantly enhanced the signal intensity and reduced the background noise. In 2019, the first clinical trial of FLI in humans, for guided liver tumor surgery using both visible and NIR window, was reported; showing the potential of clinical application of NIR-FLI. Lack of targeting capability is a limitation for the applicability of fluorophores for FLI. Various targeting and responsive peptides are designed to functionalize fluorophores to perform specific imaging and activatable imaging by FLI. In addition, peptide- and polypeptide-based platforms with tunable fluorescence or loaded fluorophores are capable of loading drugs to realize real-time treatment and FLI monitoring.

The activatable strategy is feasible in FLI to improve the imaging specificity and accuracy due to the “fluorescence quenching effect”. Conjugation of a quencher and a fluorophore with a linker is a direct and effective strategy to introduce an activatable performance into an FLI platform. With the cleavage of the linker, fluorescence quenching effects will be relieved and fluorescence signals will be turned on. As many responsive peptides are cleavage substrates for specific enzymes, they are well suited to be designed as cleavable linkers for activatable FLI platforms. Recently, Bogyo and co-workers reported the AND-gate FLI by conjugating a quencher and a fluorophore with a cleavable linker.
probes. The fluorophore was designed in the middle of probe and was functionalized with two responsive peptides that linked quenchers to silence fluorescence emission, which could be cleaved by multiple specific enzymes to activate fluorescence signals in targeted site. Peptides, Lys-Phe and Lys-Asp-Glu-Val-Asp-Gly-Pro, were chosen to be specifically and orthogonally cleaved by caspase 3 and cysteine cathepsins, which are overexpressed in tumor cells. In vivo and ex vivo FLI of tumor-bearing mice showed enhanced accumulation at tumor site 2 h after injection with dual-responsive peptide-based probes, compared with probes with single responsive peptide. These results indicated that the dual-responsive peptide-based probes could significantly improve the imaging specificity and sensitivity. Unlike the single quencher strategy, this FLI probe with two cleavage sites for two different and orthogonal enzymes showed higher specificity and accuracy of targeted tumor imaging due to the fluorescence signals, which can only be activated if both enzymes are present at the same cell.

Employing fluorescence resonance energy transfer (FRET) is another strategy for the design of activatable FLI platforms. When a fluorophore and a quencher are arranged in close proximity (<10 nm), energy from excitation photons absorbed by fluorophore will mostly transfer to quencher by resonance rather than emission, resulting in fluorescence quenching. Responsive peptide is introduced in the design of FLI platforms with FRET for cleavage to activate fluorescent signals. Gao and co-workers reported a protease-activated fluorescent platform based on FRET between a fluorophore and a Fe₃O₄ nanocrystal for tumor pH mapping (Figure 6). A responsive peptide substrate, Gly-Pro-Leu-Gly-Leu-Pro-Gly-Lys-Gly-Gly, served as a linker between the Fe₃O₄ quencher and the pH-sensitive fluorophore. The responsive peptide substrate could response to protease and be cleaved to remove FRET and activate fluorescence signals. In addition, a gastric-cancer-specific monoclonal antibody was further covalently attached to the peptide for targeting of cancer cells. Based on pH response of the fluorophore to the tumor microenvironment, the resulting probe was successfully used to image subcutaneous tumor in vivo at different time intervals within 60 min post-injection. In addition, pH-mapping images of tumor were obtained with pH value from 6.6 to 7.6. In addition, the tumor size on the pH-mapping images at 60 min was comparable with the extracted tumor, which indicated the accurate response of this responsive peptide substrate.

Intracellular and in situ self-assembly of responsive peptide functionalized FLI probes could enhance the fluorescence signals through accumulation and retention at the lesion site, thereby enhancing the specificity during FLI. Responsive peptides for cleavage have also been designed into intracellular and in situ self-assembly FLI platforms. Rao and co-workers used a responsive peptide into a fluorescence platform to control cyclization-mediated in situ self-assembly, and to activate the fluorescence signals. This probe consisted of a Cy5.5 fluorophore moiety, for cyclization and self-assembly, and a peptide moiety, DEVD (Asp-Glu-Val-Asp), for cleavage by caspase-3/7 which is activated in apoptotic cells. With the GSH-mediated reduction and caspase-3/7-mediated cleavage, this responsive peptide-functionalized platform was cyclized and formed nanoggregates in situ self-assembly, resulting in enhanced fluorescence signals. In vivo FLI showed significantly enhanced fluorescent signals at tumor site of mice that treated with

![Figure 6](https://www.advancedsciencenews.com)
DOX. In addition, the signals reached maximum 1 h after administration, and a 1.6-fold enhancement of fluorescent signals could be detected at tumor site after 4 h. However, no clear fluorescent signals were found with saline-treated mice. These data indicated the high specificity of DEVD and the efficient accumulation in DOX-induced apoptotic cells of this imaging platform. This strategy combines the advantages of small molecular probes with advantages of nano-sized probes: enabling activatable and enhanced imaging of enzyme activity in vivo. Wang and co-workers reported a strategy of cancer-associated fibroblasts imaging by the in situ formation of nanoﬁbers from a molecular peptide–cyanine platform.\(^8\) The responsive peptide GPA (Gly-Pro-Ala) could be specifically cleaved by fibroblast activation protein-α on fibroblasts, which induced the cleaved residue KLVF (Lys-Leu-Val-Phe-Phe) to assemble into β-sheet nanoﬁbers. As a result, a 5.5-fold signal enhancement in the tumor was obtained compared with molecule probes that did not aggregate. In addition, the selective assembly of the platform resulted in a signal intensity over four and ﬁvefold higher in tumor than in the liver and kidney, indicating the speciﬁcity of GPA and enhancement of tumor-to-normal tissue ratio. More recently, Wang et al. also reported a new molecular peptide–cyanine platform with tunable thananostics.\(^8\) This peptide–cyanine platform was modularly designed with a recognition moiety (AVPIAQK) for X-linked inhibitor of apoptosis protein, a responsive moiety (DEVD) for cleavage by caspase-3/7, and a self-assembly moiety (KLVFFAECG or GCKLVFFAECG) for in situ self-assembly. Especially, after responsive moiety was cleaved, self-assembly moiety with two cyanine substitutions could yield enhanced photothermal conversion efﬁciency while exhibiting high ﬂuorescence quantum yields for one cyanine substitution, due to different aggregation patterns of cyanine. In vivo FLI for tumor-bearing mice showed an enhanced ﬂuorescent signal at tumor site, which could be maintained for 48 h. In addition, enhanced ﬂuorescent signals were only observed at tumor based on in vitro FLI imaging of mice organs, demonstrating effective tumor-targeting capability.

Although responsive peptides with cleavage function have been widely used in the peptide functionalized imaging platforms, most of their designs were based on one single enzyme, which may lead to unsatisfactory performance at lesion sites that have a complex microenvironment. To improve upon this, Bogyo and co-workers reported a method to identify optimal peptide sequences for imaging platform design.\(^8\) They designed a combinatorial library for tumor extract screening, and optimal peptide sequence was identiﬁed with high tumor speciﬁcity. The introduction of combinatorial library and screening technology did not only simplify the design and optimization of peptide imaging probes but also ensured the targeting function of specific lesions.

In addition to the responsiveness of peptides to enzymes, peptides can be responsive at a low pH of the tumor microenvironment—an approach that can be applied to target tumor lesions. Zhang and co-workers reported a pH low insertion peptide (pHLIP) functionalized photosensitizer platform for tumor-targeted imaging and photodynamic therapy.\(^6\) pHLIPs are able to spontaneously change conformation and insert into membrane of tumor cell at acidic tumor microenvironment. In vivo FLI of tumor-bearing mice showed enhanced fluorescent signals and reached maximum at 6 h after administration. However, no clear accumulation of nontargeted platforms at tumor site, which demonstrated the targeting capability of pHLIPs. As a result, with low pH at tumor lesions, pHLIP functionalized photosensitizers were found superior targeted accumulation, which could also beneﬁt photodynamic therapy.

Targeting peptide functionalization is another common strategy to design targeted imaging platforms. Recently, CREKA peptide–modiﬁed chitosan nanoparticles were reported for NIR imaging and combinational therapy.\(^9\) CREKA peptide could speciﬁcally recognize ﬁbrin–ﬁbronectin complexes, which were uniquely overexpressed on tumor vessels and in tumor stroma. In vivo NIR-FLI of tumor-bearing mice showed that NIR signals accumulation at tumor site was observed after nanoparticles with or without CREKA modiﬁcation. In addition, quantiﬁcation of NIR signals showed that tumor accumulation efﬁciency of CREKA-modiﬁed nanoparticles was 24-fold than that of unmodiﬁed nanoparticles, indicating superior targeting capability of CREKA.

Even, targeting peptide, responsive peptide, and cell-penetrating peptide could be designed in one system to perform a more accurate diagnosis. Wang et al. reported a membrane-penetrating TAT-peptide decorated activatable NIR-II nanoplaform for neuroblastoma diagnosis.\(^8\) This imaging platform consisted of several moieties: an AF7P peptide moiety for targeting the matrix metalloproteinase 14, a responsive peptide moiety containing TAT peptide, and an NIR absorber A1094 to block NIR emission of Ag2S quantum dots (QDs) through FRET effect. When the imaging platform was targeted to neuroblastoma cells, responsive peptide would be cleaved by matrix metalloproteinase 14; TAT peptide would be exposed to help penetrate cell membrane; and FRET effect would be eliminated to activate ﬂuorescence. The in vivo NIR-FLI imaging of tumor-bearing mice showed that NIR signals were observed 5 min after the injection, which indicated the activatable property of this imaging platform. In addition, due to stable NIR signals with high tumor-to-normal tissue ratio, the imaging-guided surgery was successfully conducted with more recognized tumor number and larger tumor volume, which indicated the high accuracy and sensitivity of this imaging platform. This strategy would be promising for the more precise diagnosis and surgery using NIR-FLI.

One disadvantage of peptide in clinical applications is their poor in vivo stability, due to its potential biodegradability. However, modiﬁcation of peptide may overcome this drawback. Recently, Achilefu and co-workers reported a cyclic octapeptide–modiﬁed NIR platform for cancer detection and imaging-assisted surgery.\(^9\) This cyclic octapeptide was modiﬁed from a targeting linear hexapeptide (Gly-Arg-Asp-Ser-Pro-Lys). Cyclized with disulﬁde bond between D-L Cys, octapeptide was endowed with enhanced stability and could selectively bind to phosphorylated Annexin A2, which was expressed in some tumor regions. This cyclic octapeptide–modiﬁed imaging probe was evaluated in different solid tumor model in mice, including 4T1 subcutaneous/orthotopic mammary tumor, EMT6 mammary carcinoma, patient-derived breast cancer xenograft, HT1080 ﬁbrosarcoma, BXCPC-3 pancreatic adenocarcinoma, delayed brain-tumor glioblastoma, and MMTV-PyMT spontaneous breast cancer. In vivo NIR-FLI showed enhanced ﬂuorescent signals at tumor sites in different tumor animal models, indicating this probe’s targeting...
ability and detectability for a wide range of solid tumors. In addition, fluorescence-guided surgery with this NIR-FLI probe was successfully conducted. This work provided a promising strategy for tumor detection with cyclic octapeptide.

Recently, a new class of self-threaded fluorescent molecular probes was reported. A deep-red squaraine rotaxane dye was covalently modified with two peptides for a figure-eight topology. Structure, stability, and photophysical properties studies indicated that the trapped fluorophore and rigidified peptide loops could significantly enhance the imaging capability by promoting water solubility, eliminating probe self-aggregation, and increasing probe stability.

Excepted for peptide-functionalized fluorescent platforms, selected peptides could also be self-assembled into fluorescent nanoarchitectures without introducing organic dyes. Inspired by the fluorescence mechanism in natural fluorescent proteins, Fan et al. reported Trp–Phe dipeptide nanoparticles (DNPs) that shifted the intrinsic peptide fluorescence from ultraviolet to the visible range. Similar to natural fluorescent proteins, DNPs were prepared through self-assembly with Zn$^{2+}$ by π–π stacking and Zn$^{2+}$ coordination, which shift peptide’s intrinsic fluorescence signal from the ultraviolet to the visible range. Narrow emission bandwidth in visible range and remarkable photostability of DNPs were observed, which could be an effective agent for fluorescent biomedical imaging. Afterward, the fluorescence property of peptide assemblies was further optimized to NIR range, which could be more practical for in vivo imaging. Quantum-confined peptide assemblies of aromatic cyclodipeptides were reported subsequently (Figure 7). Dimerized peptides cyclo-Phe-Trp and cyclo-Trp-Trp were designed as QDs to self-assemble into quantum-confined nanostructures with similar preparation method as described earlier. Notably, the emission can be tuned from the visible region to the NIR region (420–820 nm) by modulating the self-assembly process. In vivo whole-body NIR-FLI demonstrated the potential and feasibility of fluorescent peptide nanoparticles for biomedical imaging applications. Furthermore, fluorescent cyclic peptide nanoparticles (f-PNPs) were designed for in vivo tumor imaging. f-PNPs were constructed with cyclic octa-peptide (cyclo-[D-Ala-L-Glu-D-Ala-L-Trp]$_2$) and Zn$^{2+}$ through self-assembly (Figure 7b). By the coordination with Zn$^{2+}$ and flat ring structure of the peptide, f-PNPs could produce fluorescence in the visible and NIR range by quantum confinement. Further embedded with the chemotherapeutic agent epirubicin, and decorated with RGD, f-PNPs were evaluated in an esophageal cancer mouse model and showed targeted tumor imaging and enhanced antitumor efficacy. These peptide self-assemblies with fluorescence emission, intrinsic biocompatibility, and underlying biodegradability represent a unique strategy for design of peptide-based imaging platform for FLI.

### 2.4. Peptide/Polypeptide-Based Materials for PET and PET/CT

PET has excellent imaging sensitivity to nanomolar or even picomolar due to the penetration depth of the detected signal (511 keV γ protons). However, the half-life of traditional radionuclides in PET is short, such as $^{18}$F, $^{15}$O, $^{13}$N, and $^{11}$C, which limits the longer time detection that is required for the rapid radiosynthesis. PET/CT is the first commercial and most successful multimodal imaging technology, both in clinical practice and preclinical drug discovery. Specific targeting is necessary in PET and PET/CT for specific molecular imaging. Although antibodies with a higher affinity and specificity have been successfully applied in PET and PET/CT, targeting peptides have the advantages of a low cost, are relatively facile to synthesize, and can potentially be produced on a large scale, and most importantly, are more biocompatible, which are more practical for functionalization of the radionuclides.

**Figure 7.** Fluorescent peptide assemblies for FLI. a) Schematic representing the process of cyclodipeptides self-assembly and extracted emission spectra of cyclodipeptides self-assembly. b) Schematic illustration of the synthesis of RGD-f-PNPs/EPI and fluorescence emission spectra of the RGD-f-PNPs with excitation wavelengths are 760 nm. Reproduced with permission. Copyright 2018, Springer Nature.
18F substitution of hydroxyl is the most widely used biological isostructural substitution due to similar electronegativity and steric hindrance.[99] In addition, the lowest positron energy and appropriate half-life of 18F endows 18F contrast agents with the best physical properties for imaging and offers the capability of longer-time imaging. Despite the success of [2-18F]-fluoro-2-deoxy-D-glucose in the clinic and an increasing number of 18F-labeled pharmaceuticals approved for clinical use, the targeting specificity and sensitivity of radiolabeled agents for PET imaging is still limited for some diseases.[103,104] Targeting peptides can be applied for PET platforms to enhance targeting specificity and sensitivity. Various targeting-peptide-functionalized 18F-labeling platforms have been developed for PET and PET/CT imaging and are tested in preclinical and clinical trials.[105–109] Rapid and facile 18F labeling is crucial for the 18F-labeled peptide imaging platforms for clinical use. Britton and co-workers reported 18F labeling strategies for unmodified peptides (Figure 8).[110] A direct prosthetic-group-free 18F-labeling of Leu-containing peptides method was developed by site-selective 18F-fluorination with a photo-activated hydrogen-atom abstracting agent and a fluorine atom donor. 18F-fluorinations of different Leu-containing dipeptides and tetrapeptides were compared. Unlike the multiple-step labeling for traditional 18F-fluorination, this streamlined process provided a direct method to provide 18F-labeled peptide PET platforms under mild aqueous conditions, enabling the rapid construction and discovery of peptide-based molecular imaging tools.

Notably, linkers are usually used to conjugate peptide moieties and imaging moieties to avoid the influence of imaging moieties on the bioactivity of peptide.[111] 89Zr-labeled peptide platform with a PEG linker was reported for tumor-specific PET/CT imaging.[112] A tumor cell–penetrating peptide TPP and a chelator for 89Zr was conjugated with PEG to construct PET platform. Stability of this PET imaging platform was improved with the link of PEG. Also, a favorable biodistribution provided rapid renal clearance, which further reduced the radiotoxicity. In vivo PET scan of tumor-bearing mice showed accumulated PET signals at tumor site, and the highest uptake of this PET platform was observed 24 h after administration. In addition, PET signals were observed in gall bladder within the first 3 h, which may indicate part of this PET platform is excreted via the hepatobiliary pathway. As a result, targeting specificity and rapid internalization of peptide-functionalized platform led to accumulation at tumor site and yielded a high tumor-to-normal tissue ratio. Chen and co-workers designed a 68Ga-labeled heterodimeric peptide Bombesin-RGD (68Ga-BBN-RGD) for breast cancer detection (Figure 9).[113] Bombesin is a peptide which could target gastrin-releasing peptide receptor (GRPR). With the targeting specificity of peptides, this dual-peptide tracer could specifically bind to GRPR and αβ3 integrin, both overexpressed in human breast cancer cells. In this study, 68Ga-BBN-RGD was evaluated to detect primary lesions and metastases of breast cancer in different breast cancer patients. In addition, 68Ga-BBN-RGD was found to be more sensitive for more detected tumor lesions, compared 68Ga-BBN, which could be attributed to the multivalency effect of BBN-RGD heterodimer. PET/CT scans were conducted on patients with different subtypes of breast cancer, newly diagnosed breast cancer patients, and patients with metastasis of breast cancer after administration of 68Ga-BBN-RGD. As a result, primary tumors and different metastases were detected with 68Ga-BBN-RGD, indicating the high sensitivity and targeting efficiency of peptide BBN-RGD. This PET imaging platform could be useful for the detection and prognosis of breast cancer. More recently, Hennkens and co-workers reported the 64Cu-labeled peptide D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH2 for PET imaging of prostate cancer.[114] This peptide could bind to GRPR, which endows the probe to specifically target GRPR-positive tumors. PET/CT imaging showed clearly visualization of tumors at various time points (1, 2, 24 h) with high tumor-to-normal tissue ratio. In addition, biodistribution studies showed that effective uptake and retention at tumor site and rapid renal clearance of peptide probes.

**Figure 8.** 18F-labeling strategy for peptide. a) Prosthetic-group-free 18F-labeling of Leu-containing peptides. b) Direct site-selective 18F-fluorination of various unprotected peptides. Reproduced with permission.[110] Copyright 2018, Wiley-VCH.
In addition to tumor imaging, radiolabeled polypeptide could be used for in vivo tracking of cargo delivery. Cheng and co-workers reported a fluorinated poly(lysine) for gene delivery, which could be tracked through PET imaging. Negatively-charged pDNA was loaded on positively-charged poly(lysine) through electrostatic interaction, and 18F was introduced into the fluorinated poly(lysine) through a facile halogen exchange. In vivo PET imaging was conducted within 6 h after injection of 18F-labeled fluorinated poly(lysine)/pDNA complex. Later, biodistribution of this complex was studied, suggesting rapid liver clearance, which may be due to the relatively low molecular weight of polypeptide in this study. In addition, in vitro degradation of fluorinated poly(lysine) was studied with simulated gastric fluid, simulated intestinal fluid and buffers at different pH, which showed that fluorinated poly(lysine) could be degraded in simulated intestinal fluid.

2.5. Peptide/Polypeptide-Based Materials for SPECT and SPECT/CT

\( \gamma \) Photon signals from 100 to 360 keV can be detected and transferred to generate images in SPECT. SPECT/CT is a more recent multimodal imaging technology with higher accuracy, less noise, broad spectrum, capability of quantification, and a low-dose CT radiation. Among them, \( {^{99m}}\text{Tc}, {^{123}}\text{I}, \) and \( {^{111}}\text{In} \) are the most commonly used radionuclides for SPECT and SPECT/CT in clinic. They have a longer half-life and are more practical in clinical applications for longer-time imaging and radiosynthesis. Among them, \( {^{99m}}\text{Tc} \) is most accessible radionuclide, known as the mainstay of nuclear medicine imaging. At present, SPECT is still the most commonly used nuclear medicine imaging method. However, similar to other imaging technologies, contrast agents for SPECT and SPECT/CT also lack targeting capabilities. The specific targeting ability of targeting peptide is essential for SPECT and SPECT/CT. In addition, polypeptide exhibits efficient loading of radionuclides, and can be used to construct imaging platforms to improve the biocompatibility of contrast agents for SPECT.

Since the radiopeptide \( {^{111}}\text{In}-\text{octreotide} \) was first introduced into clinical use for diagnosis of neuroendocrine tumors using SPECT, radiopeptides have been developed for imaging and diagnosis. Different radionuclide-labeled peptides with different targeting specificity have been reported for SPECT or SPECT/CT. Recently, \( {^{111}}\text{In} \)-labeled peptide probes for gastric cancer tumor SPECT imaging were reported. A novel targeting peptide F56 was designed to specifically bind to vascular endothelial growth factor receptor 1 (VEGFR1), which is overexpressed in gastric cancers. By conjugating with a chelator, this peptide platform was able to load \( {^{111}}\text{In} \) by radiolabeling and yielded high radiochemical purity and specific activity. Clear accumulation of \( {^{111}}\text{In} \)-labeled peptide platform at tumor site was observed in SPECT imaging and reached a maximum contrast 24 h after administration, which indicated its potential for early detection of VEGFR1 positive gastric cancer. Recently, Wang and co-workers designed a \( {^{99m}}\text{Tc} \)-labeled D-peptide for SPECT imaging of breast cancer (Figure 10). This D-peptide could specifically bind to human epidermal growth factor 2 (HER2), that is overexpressed in cancer cells. A retro-inverso D-peptide was designed to increase the metabolic stability of this radionuclide imaging platform. The SPECT imaging demonstrated that this targeting peptide functionalized platform could specifically target to HER2-positive tumors, with enhanced imaging contrast. In vivo SPECT/CT showed that enhanced SPECT signals were observed at tumor sites after injection of probes with D-peptide and retro-inverso D-peptide, compared with L-peptide; and SPECT quantification showed higher tumor uptake of \( {^{99m}}\text{Tc} \) with retro-inverso D-peptide platforms, which indicated the targeting capability of D-peptide and improved metabolic stability of retro-inverso D-peptide. It is worth noting that \( {^{99m}}\text{Tc} \) was not labeled on peptide but with a PEG linker, which improves the in vivo pharmacokinetics and prevents the impact on HER2-targeting activity of peptide. In vivo pharmacokinetics and imaging contrasts of retro-inverso D-peptide platforms with different lengths of PEG linker were compared; they found that tumor uptake would not be influenced by lengths of PEG; and SPECT signals in the liver would decrease with longer PEG linker. In addition to targeted tumor localization, targeting...
peptide functionalization of imaging platforms for SPECT and SPECT/CT are widely used to evaluate biodistribution, targeting efficiency, and therapeutic effect.\[125–129\]

Polypeptide imaging platforms with radionuclides loading are promising for SPECT and SPECT/CT imaging with improved biocompatibility and potential combination of treatment. Recently, Du and co-workers reported a bone-targeting polypeptide-based polymer vesicle for simultaneous imaging and effective malignant bone-tumor treatment (Figure 11).\[130\] Alendronic acid (ADA), which could efficiently target bone tissue by chelating divalent calcium ions in hydroxyapatite, was covalently connected to the glutamic acid side chains of polypeptide backbone. In addition, ADA units in polymer chain could chelate $^{99m}$Tc to turn the vesicles in an imaging platform for SPECT. Encapsulation of DOX-HCl further combined treatment ability into this vesicle. The distribution of DOX was dynamically monitored through SPECT/CT in a bone tumor rabbit model. In vivo SPECT/CT showed gradually accumulated SPECT signals from bone tissues 3 h after administration; whereas signals could be only observed in kidney and bladder of rabbits injected free $^{99m}$Tc, which indicated the bone-tissue imaging capability of these polypeptide-based vesicles. This design provides a promising strategy of theranostic polypeptide nanoplastform for combination of real-time SPECT imaging diagnosis, and effective treatment of diseases. Deng and co-workers reported a block copolyptide for SPECT/CT dual-modality imaging.\[131\] Different from the common strategy to only load radionuclides for SPECT, they modified polypeptide with both iodinated molecules for enhanced CT and $^{125}$I for SPECT. They utilized iodine-exchange reaction to easily label $^{125}$I on the iodinated side chain of polypeptide. With the further functionalization of cRGD, this polypeptide platform could target tumors for specific imaging. In vivo CT imaging showed enhanced contrast at tumor site within 8 h after administration; and accumulated SPECT signals were observed at tumor site, which reached a better contrast at 24 h. Both CT and SPECT/CT scans demonstrated efficient

![Figure 10](image10.png)

**Figure 10.** Tumor-targeting peptide-functionalized platform for SPECT/CT imaging. a) Chemical structures of H6, RDH6, and $^{99m}$Tc-radiolabeled complexes and b) SPECT/CT images of $^{99m}$Tc-radiolabeled complexes in a breast cancer mouse model. Reproduced with permission.\[124\] Copyright 2020, American Chemical Society.

![Figure 11](image11.png)

**Figure 11.** Polypeptide-based nanoplastform for SPECT/CT. a) Schematic illustration of bone-targeting polypeptide-based polymer vesicles for synchronous diagnosis and treatment of malignant bone tumor. b) In vivo SPECT/CT imaging of rabbits using $^{99m}$Tc-labeled vesicles. Reproduced with permission.\[120\] Copyright 2020, Elsevier.
tumor targeting and adequate tumor contrast of this platform. SPECT quantification showed that some of these platforms were accumulated in spleen and liver, which may be caused by sequestration of reticuloendothelial system. Also, H&E staining results showed negligible damage on major organs including spleen and liver, which indicated the excellent biocompatibility of this polypeptide-based platform. Furthermore, \(^{131}\)I could also be introduced into this polypeptide platform with iodine-exchange reaction, indicating the potential to combine enhanced imaging and radiotherapy.

2.6. Peptide/Polypeptide-Based Materials for Other Multimodal Imaging

In addition to PET/CT and SPECT/CT, nanoplatforms were designed for other multimodal imaging, such as FLI/CT, FLI/MRI, PET/FLI, MRI/CT, which could provide different functional or anatomical information for disease diagnosis. PET/MRI is another multimodal imaging modality that is already in clinical use.\(^{132}\) But PET/MRI is not comparable to PET/CT due to the high associated costs and complex operations. Other multimodal imaging technologies are also potential to combine anatomical information and functional information, or combine different functional information. However, new clinically applicable multimodal imaging techniques are still under development because of the limitation of FLI in clinical applications and the technical difficulties of integrating different imaging systems. Composite platforms with targeting specificity and different contrast agents for multimodal imaging are also currently under development. Peptide with targeting or responsive ability is a crucial portion to design platforms for multimodal imaging. Moreover, polypeptide-based platforms are capable to efficiently load various kinds of imaging agents, making them more feasible for multimodal imaging.

A common strategy for targeted multimodal imaging is to prepare contrast agents with different imaging modes, and to further functionalize them with targeting peptides afterward. Recently, Liu and co-workers reported a cRGD peptide-functionalized, NIR molecules TC-1, and SPIONs encapsulated nanoplatforms for photoacoustic imaging, FLI, and MRI multimodal imaging of brain tumor (Figure 12).\(^{133}\) Especially, through functionalization, nanoplatforms with separate region of SPIONs and TC-1 were realized, resulting in reduced fluorescence quenching, enhanced MRI signals, and, finally, optimized multimodal imaging. Enhanced signals at tumor site were observed by MRI within 24 h after administration, indicating the targeting capability of cRGD; and this nanoplatform could be used to monitor the therapeutic effects by visualizing the tumor size with FLI. You and co-workers reported targeting peptide-functionalized Ba\(_2\)GdF\(_7\) nanoparticles for tumor-targeting MRI/CT dual-modality imaging.\(^{134}\) Combining the enhanced CT contrast of barium and MRI contrast of Gd\(^{3+}\), Ba\(_2\)GdF\(_7\) nanoparticles were capable of improving imaging quality of both imaging modalities. In addition, functionalized with epidermal growth factor receptor-targeted peptides, Ba\(_2\)GdF\(_7\) nanoparticles were able to target and accumulate within tumor cells. Both enhanced MRI and CT contrasts at tumor tissue were observed within 24 h after administration of targeting peptide-functionalized platforms, compared with nontargeted ones, which demonstrated efficient targeted imaging with these peptide-functionalized platforms. In another work, cNGR peptide (cyclic NAc-Cys-Asn-Gly-Arg-Cys-Gly-Gly-Lys(Ac)-NH\(_2\))-functionalized GSH-coated Au:Gd nanoplates for CT/MRI dual-modality imaging were reported.\(^{135}\) GSH served as a protective agent and was functionalized with cNGR peptides, which could target tumor endothelial cells. With the biocompatibility of peptides and small size of Au: Gd clusters, this nanoplatform showed favorable pharmacokinetics and enhanced contrasts for in vitro and in vivo MRI and CT, which is promising for the detection of tumor angiogenesis. In addition, PET/FLI multimodal imaging was reported by loading fluorophores and radionuclides together within one system, and the RGD peptide functionalization provided targeting specificity to tumor site.\(^{136–138}\) Here, the first-in-

![Figure 12. Targeting peptide-functionalized nanoplatform for multimodal imaging. Schematic of RGD-functionalized, NIR fluorophore-loaded ultrasmall iron oxide nanoparticles for photoacoustic/FLI/MRI multimodal imaging. Reproduced with permission.\(^{133}\) Copyright 2019, Wiley-VCH.](https://www.advancedsciencenews.com/fig12.png)
human clinical trial of an ultrasmall inorganic nanoparticle imaging platform decorated with 124I-labeled cRGDY for optical/PET imaging has been reported, revealing the promising clinical applications of targeting peptide-functionalized platforms for multimode imaging.[139]

In addition, responsive peptides are also used in the design of platforms for multimodal imaging. Gao et al. reported a 99mTc-labeled Fe3O4 nanoparticle platform for SPECT/CT and MRI (Figure 13).[140] These nanoparticles were functionalized with a responsive peptide sequence, in which an RGD peptide was designed for tumor targeting and a self-peptide was linked with RGD through a disulfide bond. The self-peptide served as a “stealth coating” to deliver the nanoplatforms to the tumor site. Disulfide bond would be cleaved by GSH in the microenvironment of the tumor and crosslinks of the remaining residues would be induced, resulting in the aggregation of Fe3O4 nanoparticles, which enhanced the imaging of SPECT and MRI. Both SPECT and MRI showed that enhanced imaging contrasts were observed within 24 h after injection with responsive probes, better than probes without responsive peptide. Later, their group reported a dual-ratiometric fluorescence platform designed with FRET for FLI/MRI, which could be activated by the cleavage of a responsive peptide.[141]

FRET was also used in another work with a thrombin-activatable fluorescent peptide (Cy5.5-Gly-D-Phe-Pip-Arg-Ser-Gly-Gly-Gly-Lys-Cys) for thrombus imaging by FLI/CT.[142] Fluorescent peptide molecules were functionalized on SiO2-coated Au-nanoparticles, which provided an enhanced attenuation for CT contrast. Fluorescence emission signals were silenced by FRET with Au nanoparticles and could be activated through cleavage of responsive peptides by thrombin at the thrombotic lesion, which increased the target-to-normal tissue ratio for FLI. In addition, Au-nanoparticles and fluorescent peptide molecules tended to accumulate at the targeted thrombotic lesion, providing high contrasts for both CT and FLI. In vivo thrombus imaging was conducted at a in situ thrombotic mouse model. CT images and FLI images showed clear contrast enhancement of thrombus after administration of these nanoparticles. The bright-field images of thrombus further confirmed the precise imaging with CT and FLI, which indicated the responsive sensitivity of applied peptide toward thrombin. More recently, a responsive peptide carbobenzyloxy-Gly-Gly-Arg-OH was designed into an activatable NIR/photoacoustic imaging polymeric platform with cleavable ability to urokinase-type plasminogen for detection of breast cancer.[143] The specific cleavage of the responsive peptide enabled the distinction of invasive breast tumor from noninvasive breast tumor, which is promising for the evaluation and monitoring of malignant tumors.

Polypeptides that are highly biocompatible and have a high loading capability are suitable for the design of nanoplasforms for multimodal imaging. Fe3O4/Au nanocomposites were reported for MRI/CT, which is prepared through coprecipitation and layer-by-layer assembly with poly(glutamic acid) and polylysine.[144] Short T2 relaxation times and a good X-ray attenuation were obtained by optimizing molar ratio of Fe3O4 and Au. Cytotoxicity and hemolysis assays with these nanoparticles showed excellent biocompatibility and the negligible hemolytic activity, which indicated the improved biocompatibility due to the polypeptides. In addition to improved biocompatibility, with versatile modification of polypeptide, functional molecules could be further decorated, which represented potential for diagnosis and therapy of various diseases. Kataoka and co-workers reported a poly(benzyl glutamate)-based polymer linked with a chelator for 111In and Gd13+ (Figure 14).[145] Micelles were prepared from this polymer by self-assembly, which were able to be labeled with 111In for SPECT imaging or Gd13+ for MRI. In addition, the Gd-labeled micelle could be further labeled with 111In in a dually-labeled micelle. Clear SPECT contrast enhancement at tumor site with 111In-labeled micelles was observed 24 h after administration. Gd-labeled micelles showed clear MRI contrast at tumor site 24–72 h after administration. Also, similar kinetics and tumor-to-muscle ratio was found by MRI and SPECT with 111In /Gd-labeled micelles, which demonstrated the dual-modal imaging capability of this polypeptide-based system.

Recently, Sun and co-workers reported a poly(y-glutamic acid) coated Mn, Cu-carbon dots nanoparticles for MRI/FLI dual-modal imaging.[146] Realtime in vivo FLI of tumor-bearing mice was conducted after the injection of these nanoparticles, showing gradually accumulated fluorescent signals at tumor site with maximum intensity at 36 h after injection. The accumulation

Figure 13. Activatable peptide probe with FRET strategy for multimodal imaging. a) Schematic of 99mTc-labeled Fe3O4 nanoparticles. b) T2-weighted MR images, and c) SPECT/CT images of tumor-bearing mice after injections of the responsive probe. Reproduced with permission.[140] Copyright 2017, Wiley-VCH.
of these nanoparticles at tumor site could be attributed to the γ-glutamyl transpeptidase-mediated endocytosis and enhanced internalization and retention due to poly(γ-glutamic acid). In addition, in vivo MRI showed enhanced T1 signals at tumor site even 48 h after the injection, further demonstrating the long-time retention of these nanoparticles. Furthermore, the introduction of glucose oxidase and combination with anti-PD-L1 checkpoint blockade could be applied for combined starving-like therapy/photodynamic therapy/photothermal therapy and immunotherapy.

3. Conclusion and Perspective

In this review, we have summarized recent advances in peptide/polypeptide-based imaging platforms for diagnosis of various diseases and monitoring disease-related physiological activities. Peptide/polypeptide-based imaging platforms with excellent biocompatibility and loading capability could greatly reduce the potential side effects of contrast agents, and facilitate the function integration for multimodal imaging and theranostics. In addition, the functionalization of targeting and responsive peptides confers targeting specificity to contrast agents and imaging platforms, improving the accuracy of diagnosis. Taking advantage of the biological activity of peptides toward disease-related receptors, enzymes, metabolites, and microenvironments, visualized tracking and monitoring of pathological changes of diseases could be achieved for early detection and screening of related diseases.

Despite the significant progress that has been made so far, many challenges of peptide/polypeptide-based materials for biomedical imaging still need to be overcome, as follows: 1) The detection sensitivity of peptide-functionalized imaging platforms should be improved. One of the current strategies is to apply targeting peptide for imaging probes. However, biomolecular recognition based on targeting peptides suffer from relatively low affinity and specificity, compared with clinically used monoclonal antibodies. In addition, receptor expression may differ in subtypes of cancers, which limits the sensitivity of tumor imaging. One possible approach is coupling of identical/diverse targeting peptide sequences as homo-/hetero-multimer for one or more recognition sites of targeted cells. Multivalent effect of homo-/hetero-multimers could improve the detection sensitivity of targeting peptide-functionalized imaging platforms. In addition to targeting peptides, responsive peptide-functionalized imaging platforms also suffer from the specificity to microenvironments or enzymes. For example, certain responsive peptide may not be specific for one enzyme and could be responsive to several enzymes, which weakens the selectivity and accuracy of disease imaging. With the development of combinatorial peptide libraries and high-throughput screening technology, peptides with optimal selectivity to specific enzyme could be discovered.\textsuperscript{[147]}

In addition, multiple enzyme-response strategies could be applied in responsive peptide-functionalized imaging platforms,

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Figure 14. Polypeptide-based nanoplatforms for multimodal imaging: Schematic of poly(benzyl glutamate)-based polymer micelles labeled with $^{111}\text{In}$ or $^{111}\text{In/Gd}$ for SPECT/MRI. Reproduced with permission.\textsuperscript{[145]} Copyright 2015, American Chemical Society.
which could be triggered by multiple pathological parameters for enhanced detection sensitivity and selectivity. 2) The synthesis procedure of peptide/polypeptide-based materials needs to be optimized for future clinical application. The self-assembly process of peptide/polypeptide is usually concentration dependent, which could not proceed at high concentration. One strategy is ring-opening polymerization of N-carboxyanhydride-induced self-assembly (NCA-PISA).\textsuperscript{[148,149]} NCA-PISA enables self-assembly of polypeptides into nanostructures at high concentration and introduction of D-amino acids, in vivo stability of peptide/polypeptide could be one reason for the slow progress for preclinical or clinical trials. The poor in vivo biostability of peptide/polypeptide-based imaging materials have been developed and applied for live animal imaging, only a small portion have been applied for preclinical or clinical trials. The poor in vivo biostability of peptide/polypeptide could be one reason for the slow progress in clinic. Due to the potential biodegradability, peptide/polypeptide may be hydrolyzed and degraded by enzymes in the physiological environments without biological activity. One possible approach is to cyclize the peptides or replace L-amino acids with D-amino acids for longer in vivo circulation time. With cyclization and introduction of D-amino acids, in vivo stability of peptide/polypeptide-based imaging platforms would be enhanced to ensure bioactivity for more accurate and sensitive disease diagnosis.

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Conflict of Interest

The authors declare no conflict of interest.

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