Small peptide-based nano delivery systems for cancer therapy and diagnosis

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**Abbreviations:** Ac, Acetyl; A, Alanine; ACP, Anti-cancer peptides; AIE, Aggregation induced emission; AMC, 7-amino-4-methylcoumarin; BBB, Blood-brain barrier; C, Cysteine; CCP, cyclic citrullinated peptide; CESs, carboxylesterase; CMC, Critical micelle concentration; CPP, Cell penetrating peptide; DNPs, Dipeptide nanoparticles; DOX, Doxorubicin; DTX, Anticancer drug, docetaxel; D, Aspartic acid; E, Glutamic acid; EPR, Enhanced permeability and retention; AF, Dehydrated phenylalanine; FAP, Fibroblast activation protein; F, Phenylalanine; FITC, fluorescein isothiocyanate; G, Glycine; GLOBOCAN, Global Cancer Observatory; H, Histidine; HA, Hemagglutinin peptide; HB, hypocrellin B; HSA, Hierachial Self Assembly; Irpc, cyclometalated iridium(III) complex; I, Isoleucine; K, Lysine; L, Leucine; MMP-2, Matrix metallo-protease peptide; M, Methionine; MNR, Multifunctional peptide nanorods; MOP, Multi-strategy peptide; MSH, Melanocyte-stimulating hormone; NBD, Nitrobenzoxadiazole; NIR, Near Infra-red; NLS, Nuclear localization sequence; NPs, Nanoparticles; NSAIDS, Non-steroidal anti-inflammatory drugs; N, Aspargine; PA, Peptide Amphiphile; PDT, Photodynamic therapy; PEG, Polyethylene glycol; PET, Photon emission topography; POD, Peroxidase; P, Proline; PpIX, Protophorphyrin IX; PS, photosensitizer; Q, Glutamine; R, Arginine; RCC, Renal cell carcinoma; RDG, reduced density gradient; SIRT-5, Sirtuin-5; S, Serine; SOD, Superoxide dismutase; SPV, Self-assembling peptide nanovesicles; SPECT, Single photon emission commuted tomography; STED, Stimulated emission depletion; STORM, Stochastic optical reconstruction microscopy; T, Threonine; TAMRA, 5-carboxytetramethylrhodamine; TCASS, Tumor-selective cascade activatable self-detained system; TER, Tumor specific excretion-retarded V, Valine; W, Tryptophan.
Abstract

Developing nano-biomaterials with tunable topology, size, and surface characteristics has shown tremendously favorable benefits in various biological and clinical applications. Among various nano-biomaterials, peptide-based drug delivery systems offer multiple merits over other synthetic systems due to their enhanced bio and cytocompatibility and desirable biochemical and biophysical properties. Currently, around 100 peptide-based drugs are clinically available for numerous therapeutic purposes. In conjugation with chemotherapeutic moieties, peptides demonstrate a remarkable ability to reduce nonspecific drug effects by improving drug targetability at cancer sites. This review encompasses a wide-ranging role played by different peptide-based nanostructures in cancer theranostics. Section 1 introduces the rising concern about cancer as a disease and further describes peptide-based nanomaterials as biomedical agents to tackle the ailment. The subsequent section explores the mechanistic pathways behind the self-assembly of peptides to form hierarchically distinct assemblies. The crux of our review lies in an exhaustive exploration of the applications of various types of peptide-based nanostructures in cancer therapy and diagnosis.

Significance Statement

Peptide-based drug delivery systems possess superior biocompatibility, biochemical, and biophysical properties compared to other synthetic alternatives. The development of these nanobiomaterials with customizable topology, size, and surface characteristics have shown promising outcomes in biomedical contexts. Peptides in conjunction with chemotherapeutic agents exhibit the ability to enhance drug targetability at cancer sites, reducing nonspecific drug effects. This comprehensive review emphasizes the pivotal role of diverse peptide-based nanostructures as cancer theranostics, elucidating their potential in revolutionizing cancer therapy and diagnosis.

1. Introduction

Cancer is undoubtedly one of the most heterogeneous and fatal diseases, with a poor prognosis and an extremely high mortality rate, thereby terrifying human health globally. A total of 19.3 million cancer cases were reported globally for the year 2020 by the Global Cancer Observatory (GLOBOCAN). India stood second in the number of new cancer cases after China and the United States of America, with an estimated rise of 57.5 % in cancer incidences in 2040 from...
2020 (Sathishkumar et al., 2022). Factors impeding the success of anticancer drug delivery involve limited site-specific targetability, inadequate cellular uptake, and the rise of multidrug resistance. Also, the treatment regimen varies for individuals, depending on the type of cancer, the stage of diagnosis, and the patient’s tolerance (Ding and Guo, 2022). Irrespective of the availability of highly efficacious anticancer drugs, related side effects and chronic illnesses associated with various anticancer therapies remains a prime concern for the healthcare sector (Sim, 2022). In recent years, we have observed a remarkable rise in the emergence of various cancer-targeting nanotherapeutic modalities (Ganji et al., 2023; Kaur et al., 2023). In contrast to conventional anticancer therapy, nanotherapeutics are renowned for improving the drug's solubility, enhancing blood circulation time, and achieving targeted accumulation at tumor sites due to the EPR effect (Nakamura et al., 2016). Fabricating nano-biomaterials with varying topology, size, and surface characteristics has greatly benefited various biological and clinical applications of the materials (Patra et al., 2018). Nano-derived drug delivery platforms have appreciable capability to inculcate drug molecules in their matrix for site-specific delivery, with enhanced stability, extended half-life, reduced toxicity, and sustained release potential. Approximately 100 peptide-based drugs are available for various therapeutic purposes in clinics. When combined with chemotherapeutic moieties, peptides remarkably reduce nonspecific drug delivery and related adverse effects by improving drug targetability (Cooper et al., 2021). When combined/conjugated with various ligands, viz., lipids, polymers, aptamers, and other peptide moieties, peptide structures can be functionally expanded to achieve the requisite specificity and targetability. Peptide nanosystems are expanding as a supreme class of self-assembling bio-inspired systems projected for multiple biomedical applications (Huo et al., 2023).

Different peptide sequences possess the ability to self-assemble to form a variety of molecular and morphologically distinct structures. Anticancer peptides consist of small peptide sequences depicting typical pathways and mechanisms of action. ACPs can act as an alternative to conventional chemotherapeutic agents (Zhang et al., 2023). Besides, peptide-drug conjugates have been widely explored for treating various cancer types owing to their high specificity and lower antigenicity (Araste et al., 2018). Peptide-based therapeutic systems include tumor housing peptides, peptide selective altered pathological pathways, cell-penetrating peptides, etc. The tumor microenvironment is associated with physiological alterations and has aberrant expression of multiple proteins (Baghban et al., 2020). Tumor-homing peptide-based therapeutics can
recognize these sites and bind to them selectively to be taken up by the tumor cells (Kondo et al., 2021). These types of systems can be explored to deliver therapeutic and diagnostic agents specifically to tumor sites. CPPs are short, basic, and positively charged sequences that can pass through the cell membrane, thereby delivering the drug inside the cell (Raucher, 2019; Kondo et al., 2021). Previous literature suggests that conjugating drug molecules with CPP could augment their uptake in the cells, eventually improving therapeutic efficacy (Guidotti et al., 2017).

Although various groups are working towards making peptide nanoparticles more sustainable in biomedical applications, it is obligatory to encourage the development of new strategies for building a better understanding of small peptide-derived nano-pulpits for predicting proper prognosis, imaging, and therapy of cancer. Thereby, we here present an exhaustive description of the design principles and mechanism of synthesis and self-assembly of peptides to obtain various nano-systems as potential cancer theranostic agents (Figure 1). Encompassing our prime objective, the next section (Section 2) includes an introduction to different types of peptide-nanostructures and the design principles and factors controlling peptide self-assembly. Subsequently, Section 3 intends to cover the application of peptide-based self-assembled nanoplatorms as therapeutics and diagnostics in the field of cancer. Our review further focuses on deciphering the advantages that peptide-based nanostructures carry for being considered as outstanding drug delivery systems as well as remarkable diagnostic systems for anticancer therapy.

2. Different types of peptide nanostructures

Peptide-based nanostructures are nano-sized entities formed by the spontaneous self-assembly of varying amino acids sequence. Peptides can undergo gradual assembly due to their unique chemical properties and inter- and intramolecular interactions, resulting in the formation of varied morphologies such as nanofibers, nanotubes, nanospheres, micelles, hydrogels, nanosheets, etc. Such nanostructures hold great potential in fields including medicine, nanotechnology, and materials science (Harish et al., 2022).

2.1 Design principles and factors controlling peptide self-assembly: Since the breakthrough of nanotechnology in drug delivery, several drug delivery systems, viz., lipid nanoparticles, polymeric nanoparticles, micelles, dendrimers, and organic and inorganic nanoparticles, have been explored for facilitating efficient and safe delivery of drugs to the brain (Mistretta et al.,
2023). However, different nanoparticulate systems carry certain drawbacks, including limited drug loading, hindered stability, higher cellular toxicity, and abrupt drug release (Patel et al., 2013; Kumar et al., 2023). Self-assembled peptides have emerged as a highly appealing class of nanomaterials, finding their applications in tissue regeneration, drug delivery, disease diagnosis, etc. (Sun et al., 2016). Peptide self-assembly results from the interplay among twenty essential amino acids that can be modulated depending on the number, type, sequence, and side chain groups. Attributed to their variegated physicochemical characteristics, peptides have the ability to get molded into miscellaneous nanostructures, including nanotubes, nanovesicles, nanoparticles, nanosheets, nanobowls, hydrogels, etc. (Chibh et al., 2021, 2022, 2023; Mészáros et al., 2023). The consecutive building units of a peptide, i.e., amino acids with varying functional side chains, control the molding and conformations of self-assembled peptide sequences. Long-range self-assembled peptide-based nanoparticles are obtained by mediating non-covalent interactions between the repeating amino acid units, usually via π–π stacking, H-bonding, and hydrophobic interactivity (Rho et al., 2019). Due to the chiral nature of peptides, self-assembling peptides exhibit chiral selectivity, giving rise to hierarchical self-assembly. During self-assembly, peptides grow into diverse 2° structures (viz., α-helices, random turns, β-sheets), eventually resulting in the formation of heterogeneous architectonics (viz., fibrillar, tubular, rod-shaped, coiled-coil nanostructures). Non-covalent interaction generates higher degree of composite architectures ranging in mesoscopic size range (Gatto et al., 2022). As presented in Figure 2, the hierarchical assembly design of peptides has been depicted to initially begin with a primary peptide sequence, extrapolating to complex quaternary self-assembled architectonics (Sinha et al., 2021).

2.2 Different types of peptide nanostructures: Peptides are widespread front-line warriors when it comes to constructing self-assembled nanostructures possessing potential biomedical applications. Ranging from 0D nanoparticles, peptides have the tendency to self-assemble into higher hierarchical orders, giving 1D, 2D, and 3D conformations (Manna et al., 2021).

2.2.1 0D Peptide nanostructures: Unlike bulk materials with three dimensions (length, width, and height), 0D nanostructures exist as individual particles or clusters without any extended dimension. The extremely small size of 0D nanostructures and their high surface-to-volume ratios endow them with a higher number of reactive sites per unit mass, thereby making them a
prototype nanomaterial (Wang et al., 2020). Self-assembled peptide nanoparticles and nanospheres usually comprise the category of 0D peptide nanostructures. Such nanostructures are formed due to non-covalent π-π and hydrophobic interactions between different peptide sequences (Wang et al., 2021). Hydrophobic interactions perform a significant part in the self-assembly of spherical nanoparticles. Following the fundamentals to achieve least entropy and increased stability, the hydrophobic part of an amphiphile aggregates to form hydrophobic centres, while the hydrophilic arms assemble themselves on the outer side, creating a shell morphology for enhancing contact with water (Zhang et al., 2022). The properties and applications of 0D nanostructures often differ significantly from their bulk counterparts due to quantum confinement effects, surface effects, and size-dependent properties. Goel et al. has communicated the self-assembly of the β-alanine homotetramer (βA–βA–βA–βA) in an aqueous medium to form nanovesicles. These nanovesicles were further loaded with L-DOPA for their drug delivery applications. Computational analysis for exploring the conformation of the tetrapeptide indicated the formation of 8-helix type of 2˚ structure backbone stabilized by two intramolecular hydrogen bonds, which were responsible for the self-assembly of tetrapeptide into 0D nanovesicles (Goel et al., 2015). Guo et al., synthesized spherical dipeptide, YF, nanospheres with hydrodynamic diameters in the order of ~60 nm. This 0D dipeptide nanospheres was then loaded with clofarabine (an adenosine analogue drug), the aptamer AS1411 (a breast cancer cell targeting ligand), influenza hemagglutinin peptide (HA) (for endosomal escape), and doxorubicin (an anthracycline anticancer drug) via simple π-π stacking interactions. The resultant system was capable of showing enhanced cancer cell uptake, higher anticancer efficacy (in vitro), lysosomal escape, and augmented therapeutic effects in the human breast cancer (MCF-7) cell tumor-bearing mice (Guo et al., 2021). Rizvi et al. synthesized NIR-responsive fluorescent dye conjugated self-assembled RGD-functionalized proapoptotic peptide nanospheres demonstrating an average diameter of 30–40 nm. The proapoptotic peptide sequence that was selected for this particular study (KLAK) demonstrated the capability to derange the mitochondrial membrane. RGD peptides conjugated to the nanospheres were intended to specifically target overexpressed αvβ3-integrin receptors on glioblastoma cells. The as-formed 0D nanospheres depicted enhanced apoptosis and anticancer efficacy in both cellular and animal glioblastoma models (Rizvi et al., 2022).
In another set of studies, Jiang et al. fabricated a switchable, photosensitizer loaded iRGD-derived peptide amphiphilic system, which demonstrated the formation of self-assembled spherical nanovesicles for tumor targeting applications. It was further reported that modification of iRGD (internalizing RGD, CRGDKGPDC), a tumor-housing cyclic nonapeptide with hydrophilic R-rich sequence, a short P sequence, and hydrophobic alkyl units successively led to the formation of spherical assemblies, as demonstrated in Figure 3. Modified iRGD nanospheres were further loaded with HB (a photosensitizer isolated from Hypocrella bambusae) to induce photodynamic effects for attaining anticancer properties (Jiang et al., 2018).

2.2.2 1D Peptide nanostructures: As stated above, biomolecular self-assembly can be controlled by either intra- and inter-molecular interactions (viz., hydrogen bonding, electrostatic interactions, π-π interactions, etc.) or external factors (viz., altered pH conditions, temperature conditions, solvent systems, etc.). Hydrogen bonds formed between a hydrogen atom and a highly electronegative atom drive the unidirectional nucleation of biomolecules, thereby resulting in the generation of long-range 1D nanostructures (Wang et al., 2019). Nanofibers constitute the most prominent class of 1D peptide nanostructures, which acquire a β-sheet like 2° structure to expand further into nanofibrillar entities (Tarvirdipour et al., 2020). The generation of amyloid fibrils has been known to be the most pronounced case of 1D self-assembly. Amyloids are protein/peptide aggregates formed due to the oligomerization of amyloidogenic peptides/proteins detected in some degenerative and chronic pathologies (Gatto et al., 2022). A minimalistic amyloid model was developed by Brahmachari et al. in which the self-assembly of the shortest functional unit, FF, into nanofibrillar structures was reported (Brahmachari et al., 2017). Min et al. reported nondestructive self-assembly of Y-rich peptide nanofibers, which were further conjugated with silver nanoparticles and carbon nanotubes to result in effective nanoelectrodes functionalized with biological molecules (Min et al., 2018). Cormier et al. developed self-assembled nanofibers from the peptide RADA16-1 to explore the self-healing properties of the nanofibers. The alternating hydrophobic and charged subunits in this case were responsible for the formation of β-strands, further giving rise to a hydrophobic core and a hydrophilic surface where two β-strands stacked into a basic fibril unit (Cormier et al., 2013).

2.2.3 2D Peptide nanostructures: 2D nanostructures include planar lattice geometry expanding in 2D space. The innate chiral property of amino acids initiates 1D elongation and restricts their growth in the lateral dimension. Peptide helices have been used to further develop 2D
morphologies (Merg et al., 2019). Dai et al. depicted the self-assembly of the amyloid-forming heptapeptide, KLVFFAK to form 2D nanosheets in aqueous solution (Figure 4), which were distinct from amyloid fibrils. They observed a 2D expansion of amyloid, i.e., a fibril axis (axis a’) and a perpendicular zipper axis (axis b’). During a classic amyloid fibril assembly, the fibril expanded as β-sheets via H-bonding, and perpendicularly, β-sheets were paired into “steric zippers” with hydrophobic complementary interfaces composed of side chains along a side of the β-sheets, as demonstrated by molecular docking simulation studies (Dai et al., 2015). Lee et al., sorted out a peptide sequence (YFCFY) that was transformed from monomer to dimer by the formation of a disulfide bridge between the C subunits. This disulfide linkage initiated and stabilized the helix formulation, which eventually led to the formation of 2D macroscopic flat sheets (Lee et al., 2016).

2.2.4 3D Peptide nanostructures: Peptides form an essential class of 3D self-assembled structures in the form of hydrogels, which are primarily used for drug delivery and wound healing. An adequate hydrophobic/hydrophilic balance and the occurrence of large aromatic constituents at the N-terminal region of the peptide hydrogelators favor the self-assembly of peptides into 3D hydrogels (Oliveira et al., 2022). By virtue of various non-covalent interactions, 2’ peptide structures (viz., β-pleated sheet, α-helix, coiled-coil, etc.) further undergo hierarchical self-assembly to form 3D hydrogels (Das and Das, 2021). Unique characteristics attributed to the distinctive peptide sequences and the nature of interactions thereof provide the peptide hydrogels with stimuli responsiveness, viz., pH responsiveness, enzyme responsiveness, and redox responsiveness. Stimuli-responsiveness is an add-on feature that can promote targeted and site-specific drug delivery by using peptide nanocarriers (Chen et al., 2022; D’Souza et al., 2022). Recently, Li et al. developed a self-assembled hydrogel system consisting of a pH-responsive ionic-complementary octapeptide, FOE (FOFOFRFE). FOE was observed to self-assemble into firm hydrogel at pH 7.4 by the virtue of an interplay between non-covalent, hydrophobic forces among its subunits. The formed hydrogel displayed good injectability and was further loaded with DOX to induce anticancer effects (Li et al., 2022). Peptide-based self-assembled hydrogels have been largely explored for their repair and regeneration properties in traumatic brain injuries. In contrast to α-peptide hydrogels, which are susceptible to proteolytic degradation, metabolically more stable β-peptide hydrogels are believed to serve as a favorable option for providing a sustained and long-term effect in neural tissue engineering (Cheng et al.,
2013; Mehrban et al., 2015). In a remarkably innovative set of studies, Tao et al. fabricated mini hydrogelators by conjugating forky peptides with non-steroidal anti-inflammatory drugs, which could self-assemble to generate supramolecular hydrogels in the aqueous environment. The forky segment was composed of three adjacent E-residues, possessing the ability to chelate zinc ions to form hydrogel complexes. As the zinc ion concentration is known to be elevated in prostate cancer, harnessing this alteration, Tao et al., developed an in situ drug delivery system that was able to form supramolecular hydrogel in response to higher zinc ion levels at the cancer site. The anticancer drug, docetaxel was further loaded into the forky peptide-NSAID conjugate system, which demonstrated significant anticancer efficacy in prostate cancer cells (DU-145 cells and PC-3) (Tao et al., 2019).

3. Peptides nanoparticles as therapeutic delivery agents in cancer

Peptide nanoparticles have emerged as promising delivery agents for cancer diagnostics and therapeutics because of their efficient drug loading capacity and targetability towards cancer tissues. Due to the EPR effect, peptide nanoparticles can be made to accumulate inside the tumor tissues. The unique cell targeting capabilities of these peptide nanoparticles offer the potential for precise and efficient delivery of therapeutic agents specifically to different types of tumor tissues. PDT has proven to be an effective treatment tool with minimal invasiveness and high therapeutic selectivity and potency. In a PDT procedure, a photoresponsive molecule or photosensitizer produces reactive oxygen species (ROS), inducing cell death (Abrahamse and Hamblin, 2016). The photosensitizers are generally activated by irradiating a particular wavelength of light to generate ROS (Yaraki et al., 2022).

3.1 Self-assembled peptides for drug delivery: Different types of peptide-based nanosystems, such as nanotubes, nanofibers, and hydrogels, are being used for drug delivery (Fan et al., 2017). The inherent biocompatibility exhibited by peptide-based nanomaterials makes them suitable for drug delivery based applications. Out of several nanostructures, self-assembled peptide nanosystems are gaining increased attention for their application as anticancer drug delivery agents to aid cancer therapy outcomes. The current section describes different types of peptide-based nanosystems for their applicability in anti-cancer drug delivery.

3.1.1 Nanotubes/rods as drug delivery agents: Peptide nanotubes offer a novel scaffold to transport drugs to specific sites throughout the body. They have received increased attention in
drug delivery based applications. The nanoscale size and tubular shape make them the ideal carriers for interacting with and exhibiting a safe passage across the cell membrane (Zhang et al., 2021). A large number of drugs can be easily entrapped within these nanotubes, and hence, these can be potentially utilized to deliver drugs into the intracellular space to target cell organelles. Several studies showed that diphenylalanine and its analogs self-assembled into various types of peptide nanotubes. Diphenylalanine (FF) is an aromatic dipeptide identified from the core aggregating motif of the β-amyloid (Aβ-42) polypeptide, which can self-assemble into different nanostructures. Porter et al. described the use of FF nanotubes for delivering sodium fluorescein. These nanotubes showed sustained release of sodium fluorescein in acidic conditions at around pH 5.5 (Porter et al., 2020). Also, several small linear peptide sequences have been reported that can self-assemble into nanotubes and nanofibre/nanorods. For reference, KLVFF, a small sequence obtained from Aβ peptides, could also self-assemble into nanotubes (Krysmann et al., 2008). Due to the hydrophobic nature of KLVFF nanotubes, they have been shown to exhibit great potential to be used as suitable carriers for hydrophobic drugs. Similarly, sequences like DFNK and DFNKF, have been observed to attain nanofibrillar morphologies based on their self-assembly in the varying pH conditions. Presence of aromatic groups and charged side chains in these sequences are responsible for driving their self-assembly (Reches et al., 2002; Reches and Gazit, 2006). Apart from the linear peptides, cyclic dipeptides also undergo self-assembly to form peptide nanotubes (Hamley, 2014). Cyclic peptide nanotubes possess novel characteristics like accurate diameter controls that can be modified by varying the lengths and sequences of the peptides (Chapman et al., 2012). For instance, cyclic peptide nanotubes obtained from E and C amino acids have been utilized in drug delivery. These self-assembled nanotubes modified with polyethylene glycol were claimed to have a superior doxorubicin encapsulation ratio. Moreover, in vitro studies demonstrated that in contrast to only DOX, drug-loaded polyethylene glycol-modified nanotubes exhibited higher cytotoxicity with enhanced internalization of drug in human breast cancer MCF-7/ADR cells. These results show that polyethylene glycol-modified nanotubes with doxorubicin can be effective in multidrug-resistance tumor therapy (Wang et al., 2014). Larnaudie et al. designed poly(2-hydroxypropyl methacrylamide) (pHPMA) conjugated cyclic peptide (CPAETC) core-shell self-assembled nanotubes for efficient delivery of highly potent organoiridium drugs. These drug-carrying nanotubes depicted improved cellular killing in human ovarian cancer cell line in comparison to drug alone (Larnaudie et al., 2018). Song et al.
developed an amphiphilic peptide, RH$_3$F$_8$ based on the oligo-phenylalanine residues (Figure 5). Arginine is a vital amino acid in cell-penetrating peptides (Wang et al., 2014). Histidine instigates endo-lysosomal escape due to its proton sponge effect (Yu et al., 2011). The phenylalanine group provides the sequence with the characteristics of improved hydrophobicity responsible for a firm self-assembly along with significantly high drug loading (Ren et al., 2021). RH$_3$F$_8$ self-assembled into nanorods and remained stable in water for many days. These positively charged peptide nanorods were shown to possess a high curcumin-carrying capacity (Song et al., 2017). Cheng et al. developed chimeric peptide (C$_{16}$-K(PpIX)-PKKKRKV-PEG$_8$ nanorods consisting of hydrophobic alkyl chains and photosynthesizer, PpIX for inducing a synergistic plasma membrane and nucleus targeting delivery. The hydrophobic alkyl chain (C$_{16}$) present in the chimeric peptide sequence enabled plasma membrane selective targeting, and the NLS heptapeptide (PKKKRKV), helped nuclear translocalization. The presence of the NLS peptide and alkyl chain could further increase cellular uptake due to necrosis and enhanced membrane permeability. These positively charged, self-assembled nanorods facilitated higher nuclear localization upon light irradiation (Cheng et al., 2019). Chen et al. described multifunctional core-shell peptide derived nanorods (MNR) for selective targeting of carcinoma cells. MNR consisted of a DOX-loaded peptide (DOX-KGFRWR) core and a shell made from a polypeptide-(PEG) derivative (PPD). PPD was made up of pH-responsive polyglutamic acid, a stealth, long-chain PEG group, and a galactosamine containing target head. Occurrence of K and R residues rendered the core of MNR with a positive charge, whereas the negative charge of PPD helped in the formation of stable nanorod like structures (Cheng et al., 2019). Arul et al. designed different peptide nanorods containing FF as building blocks for intracellular drug delivery. A peptide sequence (OMe-FF-TA-FF-OMe) was fabricated with two FF units connected by a linker, terephthaldehyde (TA). These self-assembled nanorods were observed to successfully encapsulate DOX for delivery to the cells, highlighting their potential as drug delivery vehicles (Arul et al., 2021).

3.1.2 Nanovesicles/spheres as anticancer drug delivery agents: In a polypeptide chain, the hydrophilic and hydrophobic groups present in the constituent amino acid chain determine the self-assembling behavior of the polypeptide. Haridas and group described different sequences of linear and dendritic peptides that showed self-assembly to form spherical nanovesicles (Figure 6). The formation of these peptides into spherical vesicles was reported in 1:1
CHCl₃/MeOH. These spherical vesicles showed excellent encapsulation of rhodamine B and could penetrate the cell membrane (Haridas, 2021). In an interesting piece of work, Song et al. described the significance of the 1° and 2° structures of the constituent amphiphilic peptide sequences, towards their self-assembly to form various nanostructures (Song et al., 2017). Chauhan and co-workers reported a variety of dehydrated derivatives of dipeptide nanovesicles denoted as FΔF, RΔF, EΔF, and MΔF as drug delivery nanosystems, where F, R, E, and M represent the corresponding amino acids. These nanovesicles possessed good encapsulation efficiency for vitamins, anticancer drugs, and antibiotics. Alam et al. designed nanovesicles (~40 nm) from the self-assembly of the dipeptide, MΔF, in methanol that demonstrated sustained release behaviour of curcumin from the vesicles for over 36 hours (Alam et al., 2012). These curcumin-loaded nanovesicles inhibited tumor growth in B16F10 melanoma bearing Balb/c mice. As discussed in the previous section, smaller dipeptides like FF can self-assemble into nanorods and nanotubes. Naskar and co-workers prepared stable peptide nanovesicles (320 ±50 nm) at different pH ranging from 2 to 12. These nanovesicles were prepared from dipeptides containing E residue at the C-terminus and a lipoamino acid at the N-terminus. The vesicles were then encapsulated with DOX which was released under the stimulus of calcium ions (Naskar et al., 2011). Hell et al. successfully developed self-assembled vesicles from a short acetylated peptide, Ac-AAVVLLWEE, which were then loaded with a hydrophobic photosensitizer, zinc-phthalocyanine. When exposed to light, the zinc-phthalocyanine-containing vesicles significantly exhibited photocytotoxicity to COS-7 cells. The absence of significant cytotoxicity in the control groups containing only bare vesicles and the free zinc-phthalocyanine suggested that the peptide-based vehicles boosted PDT by improving cellular uptake of the photosensitizer (van Hell et al., 2010). Liang and coworkers created extremely stable SPVs by using an EGFR-binding, PA. The generated EGFR-targeted SPVs were capable of effectively encasing therapeutic cargos as well as increasing medication or plasmid DNA delivery efficiency to tumor locations. Consequently, this peptide amphiphile could work as a versatile tool for the delivery of anticancer medications (Liang et al., 2016). Dube et al. have shown the self-assembly of Fmoc-Trp (Boc)-OH into nanospheres for the delivery of different bioactive molecules. The self-assembling nature of Fmoc-Trp (Boc)-OH was driven by the Fmoc group present at the N-terminus of the amino acid (Dube et al., 2017). These nanospheres demonstrated significant drug-carrying and drug-releasing capacities for the anticancer drug doxorubicin (DOX). DOX-loaded Fmoc-Trp(Boc)-
OH vesicles have shown strong cytotoxicity toward C6 glioma cells (Dube et al., 2017). Mishra et al. reported the self-assembly of anionic and cationic spherical nanoparticles generated from EΔF and KΔF, respectively. The vesicles formed by the amphiphilic dipeptides were non-toxic and claimed to be very effective in encapsulating different small bioactive molecules (Mishra et al., 2008). It was observed that insulin was efficiently trapped in the cationic dipeptide vesicles prepared from KΔF but not in the anionic EΔF vesicles. This study represented a classic example wherein the encapsulation of the drug molecules was controlled depending on the charge carried by the dipeptide vesicles. Kim et al. described the glutathione-responsive release of an anticancer drug from vesicles made of PAs carrying a TAT CPP (RKKRRQRRR) (Kim et al., 2020). The PA-TAT peptide was created based on the position and amount of disulfide linkages in the PAs. The position of C in the molecular structure was responsible for a change in the structure of self-assembled PAs. The presence of C at the N-terminus of the hydrophilic PA resulted in the formation of vesicles, whereas 2D nanosheets were obtained by placing C between the pyrenyl and alkyl chain of the hydrophobic part (Wang et al., 2021).

3.1.3 Nanosheets: Self-assembled peptide nanosheets can improve the pharmacokinetic and pharmacodynamic properties of the drugs entrapped in them. In an interesting piece of work, Chibh et al. depicted the synthesis of pH-sensitive tetrapeptide that can self-assemble into 1D and 2D nanostructures. This tetrapeptide, Fmoc-HCKF-OH, could self-assemble into nanoshperes, nanofibers, and nanosheets depending on the pH of the solvent. All the peptide nanostructures were shown to be non-cytotoxic towards C6 cells and exhibited safe internalization into the glioma cells. Interestingly, the anticancer drug, DOX-loaded peptide nanostructures, showed shape-dependent cytotoxicity in C6 glioma cells based on their morphology (Chibh et al., 2022). Li et al. presented the co-assembly of Fmoc-LLL-OMe and meso-tetra(p-hydroxyphenyl) porphine (a porphyrin derivative) to form supramolecular peptide-nanoparticles for improved PDT (Figure 7) (Li et al., 2018).

3.1.4 Micellar delivery systems: Surfactant molecules get self-assembled into molecular clusters, mostly in spherical shapes, in water. This happens when the concentration of the surfactant is higher than a threshold concentration called the CMC. The self-assembled nanocluster shape comprises an inner hydrophobic tail inside the core and a hydrophilic head interacting with the water molecules. The hydrophilic head group can be made up of different kinds of amino acids. It has been reported that different kinds of polyamino acids can be assembled into micellar
structures. Polymeric micelles have certain disadvantages in loading and delivering bioactive molecules. They indeed suffer from disaggregation and release of the loaded drug molecules when the concentration of the micelle falls below CMC (Perumal et al., 2022). Furthermore, there are also reports that micellar structures undergo disintegration when they interact with mucus, epithelia, stratum corneum lipids, and sebum (Bae and Yin, 2008; Islam et al., 2020; Ghezzi et al., 2021). Zheng et al. reported a modified micellar delivery system using DSPE-PEG$_{2000}$ and a targeting peptide pep2 (RRGTFDNVWDTGTRVYD). The co-assembled micelle delivery system increased specificity and sensitivity towards the antigen, CD36 in tumor cells. The co-assembled micelle-based delivery further enhanced drug binding affinity to the CD36 receptor (Zheng et al., 2021). Mao et al. designed a tumor-homing ‘d’ (dextrorotatory isomer) amino acid carrying peptide, d-AE ($^{d}$F$^{d}$A$^{d}$L$^{d}$G$^{d}$E$^{d}$A), the enantiomer of l (levorotatory isomer) formed, $^{l}$F$^{l}$A$^{l}$L$^{l}$G$^{l}$E$^{l}$A developed from D-amino acids. The peptide d-AE modified PEG-PLA micellar system showed strong stability and improved tumor targeting efficiency in both in vitro and in vivo (Figure 8) conditions. The conjugation of D-amino acids to the peptide-forming micelle enabled its permeability across the BBB. The ‘d’ amino acid containing, FALGEEA modified micelles showed higher recognition abilities towards EGFR-positive tumor cells (Mao et al., 2017). Other studies described that peptide-loaded micelles could promote specific tumor recognition and accumulation (Liu et al., 2021). Barve et al. described enzyme-responsive micelles constructed from PEG and matrix metallo-protease-2 (MMP-2) peptide along with MMP-2 responsive peptide, PLGVRK. These micelles were able to load and release the anticancer drug cabazitaxel. Site-specific and targeted tumor drug delivery could be achieved by these structures when tumor tissue overexpressed, MMP-2 protease, would initiate the cleavage of the micelle and trigger release of cabazitaxel (Barve et al., 2020). Apart from the delivery and targeting of anticancer drugs to tumor cells, Liu et al. used two antigenic peptides E7 (GQAEPDRAHYIVTFCCCKCD) and OVA (SGLEQLESIINFEKL) as antigens to activate tumor-specific immune responses. Han and co-workers created an amphiphilic chimeric peptide [(Fmoc) 2 KH 7-TAT] that could self-assemble into micelles. The TAT peptide (YGRKKRRQRRR) is the first known CPP that was identified from HIV-1 protein-encoded proteins (Taylor and Zahid, 2020). The micelles achieved simultaneous co-delivery of medication and genes by utilizing the hydrophobicity of the micelle core and the cationic nature of TAT (Han et al., 2013). Taxol, an anticancer medication, can be combined with the CPP,
RLYMRYYSPTTRRYG, to create self-assembling taxol-peptide conjugates. These conjugates exhibited apparent cytotoxicity on their own and may also be used as drug carriers for DOX (Albanese et al., 2012; Tian et al., 2015).
3.1.5 Peptide hydrogels as cancer drug delivery moieties: Amino acids provide the structural foundation for creating peptide nanomaterials. The side chains of amino acids contain different charges, hydrophobicities, sizes, and polarities. It is possible to create new self-assembled peptides with distinct $2^\circ$ structures and novel physicochemical characteristics by modifying the number of hydrophobic and hydrophilic amino acid residues. Peptide-based hydrogels have been found to possess higher biodegradability, low bioaccumulation, and low toxicity. The EAK16 peptide was first identified in 1993 for the formation of nanofibers. Zhao et al. also described the construction of self-assembled hydrogels from EAK16. These peptides carry a charged group on one side and hydrophobic chains on the other. The hydrophobic side chains assembled into $\beta$-sheet-like structures inside the nanofibers, and the charged groups assembled outwards. Therefore, the complementary ionic forces present led to the formation of a stable and firm hydrogel-like system. Presence of strong H-bonding and ionic forces could help these hydrogels to stabilize at different pH, temperatures, and organic solvents (Zhao et al., 2010). An eleven residue containing $\beta$-sheet peptide, QQRFEWEFEQQ, self-assembled to form pH-responsive hydrogel using the ionizable side chains of E and R present in the peptide. These peptides were claimed to be soluble in neutral pH conditions and changed to a hydrogel structure when the pH was low. The hydrogel formation was due to the antiparallel-$\beta$-sheet tapes stacking together at lower pH values. Some other reports also suggested that $\beta$-hairpin peptides could self-assemble into various nanostructures at the water and air interfaces. A $\beta$-hairpin peptide consisting of VKVKVKVDPPTKVKVKV, was utilized to form stimuli-responsive hydrogels. The mechanism of the formation of hydrogels can be controlled by altering the pH of the solution (Schneider et al., 2002; Aggeli et al., 2003). Self-assembled injectable peptide hydrogels with high drug loading capacity could prove as potential prospects for applications in anticancer drug delivery. Hydrogels could aid in improving direct contact of the loaded chemotherapeutic drugs with the targeted cancer tissues at higher local concentrations, thereby improving therapeutic efficacy.

4. Self-assembled peptide nanostructures in cancer diagnosis

In the preceding sections of the review, different types of self-assembled peptide nanostructures and their mechanisms of formation are described. These self-assembled peptide-nanostructures are used for anticancer drug delivery-based applications. However, spotting cancer at an early
stage through fast diagnosis is very important for achieving successful therapy. Thus, in this concluding section, we present different types of cancer diagnosis and imaging modalities based on peptide nanostructures such as pH-responsive, enzyme-responsive nanoparticles aimed at optical and magnetic resonance-based imaging.

4.1 pH responsive peptide nanostructures: The tumor microenvironment plays an essential role in pH-responsive drug delivery and imaging as it has a slightly acidic pH. Live cell imaging is an optical method to examine living cells over a period of time using time-lapse microscopy. Jin and co-workers developed a naphthalene-based aggregation induced emission (AIE) compound using an aromatic peptide, FFK, conjugated with a pH responsive cyclometalated iridium (III) complex called Irpc (Figure 9). The compound exhibited stable fluorescence due to the self-assembling nature of the aromatic peptides (Jin et al., 2021). These compounds were self-assembled into large nanoparticles at high a pH of around 8. However, when these nanoparticles reached the lysosome, they transformed into smaller fiber-like structures. To increase the endocytic uptake and associated pH-triggered self-assembly, two aminosulfonic acids—(taurine), was attached to the C-terminal of NapFFK, obtaining a nonhydrolyzable iridium complex (Irpc) for long-term lysosome imaging (Figure 10) (Jin et al., 2021). In other work, Sun et al. developed an acid-activatable transmorphic nanosystem by using a tryptophan-glycine-porphyrin conjugate to make pH responsive fibers. Herein, tryptophan provided fluorescence, and glycine served as a pH-responsive unit. When these particles were exposed to the acidic pH of the tumor microenvironment, the units got protonated and due to strong hydrogen bonding, the particles got transformed into fibers, which demonstrated fluorescence at the tumor site (Sun et al., 2020).

4.2 Enzyme-responsive peptide nanostructures: The chemical interactions between molecules and peptides are generally influenced by enzymes in the body. Enzymes mediate the self-assembly of peptides and cause cytotoxicity in cancerous cells. Those enzymes, which are specific to certain diseases and are overexpressed under certain conditions, can be used to trigger self-assembly. For example caspase, cathepsin B, carboxylesterases (CESs), and enterokinases are overexpressed in different cancerous cells (Zhou et al., 2018; An et al., 2019; Cheng et al., 2019; He et al., 2020), etc. Supramolecular materials are often employed in nanotechnology research. Understanding their nanoscale structure and function is similarly crucial. Real-time imaging is restricted by the laborious sample preparation and long acquisition times of conventional optical imaging methods like stochastic optical reconstruction microscopy
(STORM) and stimulated emission depletion (STED)-based superresolution imaging (Dankovich and Rizzoli, 2021). An et al. developed a self-assembled peptide probe for image guided surgery that improved patients’ survival. As the NIR region is considered to be non-invasive and penetrating, they developed an NIR peptide probe that had three domains; 1) an RGD sequence which is specific for αvβ3 integrins in renal cell carcinoma (RCC); 2) an enzyme-responsive peptide linker, “PLGYLG” which can be cleaved by MMP-2/9, which is overexpressed in renal cell carcinoma; 3) a peptide motif, “YLGFFC”, for self-assembly. The peptide probe first identified the overexpressed αvβ3 integrin in renal cancer cells, then the up regulated MMP-2/9 present in the tumor microenvironment cleaved the peptide. The peptide exhibited tumor specific excretion-retarded (TER) and identification of renal cell carcinoma (RCC) (An et al., 2020). Yang and co-workers designed a SIRT-5 (sirtuin 5) responsive peptide. SIRT-5 (Sirtuin 5) is a mitochondrial enzyme involved in regulating various biological processes, such as ROS, fatty acid metabolism, and apoptosis. The peptide contained three parts: 1) a fluorophore NBD (nitrobenzoxadiazole) for imaging; 2) a phenylalanine-rich fragment; and 3) a switch module (succinylated lysine). The aromatic interaction of phenylalanine propelled the formation of fibers, and NBD (nitrobenzoxadiazole) showed fluorescence in a strong hydrophobic environment. The carboxyl group of the succinylated lysine helped in solubilization, and negative charge decreased the self-assembly process of the peptide. When these peptide nanoparticles got internalized into the cellular environment, they self-assembled. SIRT-5 (sirtuin 5) present in mitochondria hydrolyzed the succinylated lysine and made it positively charged, due to which the peptide self-assembled and formed fibers inside mitochondria. Thus by controlling the hydrophobicity and charges of the peptides, imaging of SIRT-5 (sirtuin 5) enzymes could be achieved (Yang et al., 2020). Other enzymes like alkaline phosphatases, CESs (carboxylesterases), enterokinase, and cathepsin B were also used to trigger the self-assembly of organelle-specific peptides in the intracellular milieu (Tan et al., 2021).

4.3 Radio imaging with self-assembled peptides nanostructures: Due to their specificity and diversity, nuclear isotope-labeled monoclonal antibodies have been created over the past few decades for diagnostic imaging and therapy (Reichert and Pavlou, 2004). Nuclear imaging continues to be the most effective clinical technique, despite the rapid advancement of several imaging modalities. Radiolabeling is a vital step in creating peptide probes for nuclear imaging. An exemplary targeting peptide should have high specific activity, physiological stability, and
the efficiency to be radiolabeled. Both PET (photon emission tomography) and SPECT (single photon emission computed tomography) have undergone extensive development and are frequently utilized in clinical settings (Juhász and Bosnyák, 2016). High sensitivity and a small amount of tracer molecule injection are two unmatched benefits that PET (photon emission tomography) and SPECT offer, as they represent two sensitive molecular imaging methods with picomolar-range sensitivity (Juhász and Bosnyák, 2016). In a peptide-based direct labeling method, the radionuclide is attached to a modified amino acid side chain utilizing a straightforward one-step procedure. The prosthetic group labeling method, which is frequently used to create peptide radiopharmaceuticals using radioactive iodine or radionuclides with lower atomic masses like 11C and 18F, is the second method for radiolabeling a peptide. This method involves preparing a radioactively labeled component, which is subsequently added to an amino acid side chain through a series of processes. The extracellular matrix is composed of integrin receptors, a family of heterodimeric glycoproteins. They are essential for the angiogenesis, or developing new blood vessels, within the tumor. As a result, integrin receptors have become yet another intriguing imaging target. The RGD peptide motif is the principal integrin-binding domain present in extracellular matrix proteins like fibronectin and vitronectin. Therefore, different isotope-labeled peptides such as, F-galacto-RGD 18F-AH111585, and cyclic 18F-FPP-[cyclo (RGDyK)]2 were designed for radio imaging (Beer et al., 2006; Kenny et al., 2008; Chin et al., 2012). Chen et al also described other imaging applications of linear, cyclic derivatives, and shortened versions of MSH (melanocyte stimulating hormone) peptides (Chen et al., 2001).

4.4 Optical imaging with self-assembled peptide nanostructures: Optical imaging uses readily available tools at a low cost to enable real-time, non-invasive, and sensitive visualization. Just like radio-labeling, peptides can be labeled with fluorophores like fluorescein isothiocyanate (FITC), 5-carboxy-tetramethylrhodamine (TAMRA), and 7-amino-4-methylcoumarin (AMC). Bombesin and Alexa fluor 680 conjugates have been used for imaging lymph nodes by Cai et al., 2013. Octreotide with cyanine was used by Becker et al. for tumor imaging in 2001 (Alakshin et al., 2011). Similarly, many peptides are being used for imaging, like PSMA (prostate-specific membrane antigen) peptides with IR dye 800. which were prepared for prostate cancer imaging (Alakshin et al., 2011). Peptide-based nanostructures like micelles, nanotapes, and ribbons are being used with many radioactive probes for diagnosis and drug delivery (Tarvirdipour et al.,
Guo et al. developed a peptide nanostructure by stapling strategy and used it for real-time imaging. First, they screened CCP (cyclic citrullinated peptide) peptides specific for CD133$^+$ tissues and modified their structure with stapling techniques, which resulted in the formation of SCCP2 (stapled peptide). This SCCP2 resulted in higher stability and penetrability. Finally, they conjugated a NIR-II fluorescent probe to SCCP2, thus providing high-resolution, real-time imaging of tumors (Guo et al., 2021). In another work, Zheng et al developed peptide-cyanine conjugates, which intracellularly formed 1-D columnar structures. This peptide backbone was cleaved after entering the cell by caspase 3/7. The self-assembled structures with double cyanine included a more robust columnar structure and also showed an increase in photothermal effect than the structures formed by single cyanine-containing peptides (Zheng et al., 2021). A traditional fluorescent agent causes quenching when present in aggregated stage (Li et al., 2018). To resolve this problem, AIE fluorescence species were used for imaging purposes (Ma et al., 2017). Lu et al. used a fluorogen, TPE (tetraphenylethene) for imaging. They developed a peptide-TPE probe with four parts: 1) a targeting motif, “RGD”, 2) a hydrophilic motif, D6, 3) FAP (fibroblast activation protein)-alpha specific “GPA”, 4) a self-assembly motif, TPE-FF. This probe was then used for real time cell imaging (Lu et al., 2021). Gao et al. proposed an investigation that can be used for image-guided cancer surgery. They synthesized MPA-Ph-RFGYSAYPDSVPMMMS, peptide for the AIE active turn on probe. Here MPA-Ph-R served as a luminogen, FFG acted as a self-assembling peptide unit, and the YSAYPDSVPMMMS peptide served as an EphA2 protein-specific unit. This peptide was further used for tumor imaging of tissues overexpressing EphA2 protein clusters (Gao et al., 2020). Multimodality imaging by self-assembled peptide nanostructures was also carried out, which incorporated the advantages of various imaging techniques, thus providing highly sensitive and accurate imaging. Rizvi et al. developed a nanoprobe of cyclic heptapeptide, RGDKALK in which RGD was the tumor blood vessel homing motif and KALK was the mitochondrial targeting motif. This peptide was self-assembled to form a spherical nanostructure. These nanostructures showed dual imaging and improved apoptosis by destroying mitochondria (Rizvi et al., 2022).

4.5 Magnetic resonance-based imaging achieved by self-assembled peptide nanostructures: Magnetic resonance imaging provides high-resolution imaging in three-dimensions. It has the drawback of low sensitivity; therefore, contrast agents are used to differentiate normal tissue from cancerous tissue. MRI contrast agents are of two types: one type includes transverse
relaxation agents, and the other includes longitudinal relaxation agents. Longitudinal relaxation agents are mainly used for observation and anatomy. Gallo et al. developed a nanostructure of DTPA(Gd)-PEG8-(FY)3 or DOTA(Gd)-PEG8-(FY)3. Both of these peptides had approximately the same gelation tendencies. (FY)3 moiety provided beta-sheets like structure to the self-assembled molecule. Supramolecular contrast agents can be obtained in different ways: 1) by entrapping Gd(III) within the nanostructures, 2) by covalently linking with the hydrophobic part of the peptide, 3) by the covalent functionalization of the inorganic part of the structure on the outer surface. The characteristics of peptides affect the type of aggregated nanostructures. Aromatic peptides with Gd form water soluble nanostructures (Gallo et al., 2020). In the work described by Gu and co-workers, self-assembled peptide nanostructures coupled with iron particles were developed. They used PA for the nanostructure formation, which was represented as PA (Gu et al., 2018). Peptides with a hydrophobic tail attached to hydrophilic regions form PAs. These structures had amino acids linked to a hydrophobic alkyl chain, a beta-sheet sequence, and an epitope. Once exposed to an aqueous medium, they undergo a folding mechanism similar to protein folding in cells. As a result, they formed self-assembled structures like micelles, vesicles, nanotapes, nanotubes, and ribbons (Cui et al., 2009; Matson et al., 2011). An et al. developed a tumor-selective cascade activatable self-detained system (TCASS) for drug delivery and cancer imaging. TCASS had (i) a tumor-specific recognition motif (AVPIAQK), (ii) an enzymatically cleavable linker (DEVD), (iii) a self-assembling motif (KLVFFAECG), and (iv) a functional molecule (cyanine dye, Cy, or doxorubicin, DOX). In the presence of caspase-3/7, self-assembly was triggered, and fibrous structures with beta-sheet domains were obtained (An et al., 2019).

4.6 Photoacoustic imaging by self-assembled peptide nanostructures: Photoacoustic imaging represents a combination of the high contrast characteristics of optical imaging and the deep penetration of ultrasonic imaging (Steinberg et al., 2019). NIR dye is generally used in photoacoustic imaging (Cardinell et al., 2021). Photoacoustic imaging has the advantages of low autofluorescence, high penetration, and minimal light scattering. Usage of NIR dyes also serves the purpose of photosensitizers. Ren et al. developed a nanostructure through the covalent loading of porphyrin and tyroservatide, which caused enhanced cell apoptosis and histone acetylation. The PpIXFFYSV molecule is formed of three parts: 1) Porphyrin (PpIX), 2) FF for self-assembly, and 3) Tyroservatide-anticancer peptide (YSV), which forms nanorods. These
nanorods enhanced histone acetylation by inhibiting deacetylase (Ren et al., 2021). Xia et al. developed a nanogel of di-lysine with coordinated Fe. SiO$_2$ was added to the peptide nanogel to mimic the activity of superoxide dismutase (SOD) and peroxidase (POD). The addition of 2,2’-azinobis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) as a substrate to the above nanogel led to the conversion of O$_2$ to H$_2$O$_2$ in the tumor microenvironment, and then ABTS changed to its oxidized state. This was absorbed in the NIR region and thus helped in photoacoustic imaging (Xia et al., 2021).

5. Conclusion

The case studies in this review demonstrate the potential utility of self-assembled peptides for more effective medication administration and diagnosis. Self-assembled peptide nanostructures possess several advantages, including easy cell penetration, endosomal escape, targeted delivery, and other benefits. Peptides, being involved in extracellular matrix signaling, play a significant role in drug delivery and diagnosis. Furthermore, self-assembled peptides acquire important properties of nanostructures, such as nanotubes and nanofibers, offering a wide range of possibilities. Over the years, peptide research has expanded beyond initial studies focusing on different nanostructures and self-assembly, leading to in vivo testing and exploration of various biological targets for medicinal purposes. Compared to block copolymers, self-assembled peptides exhibit greater biocompatibility and stability due to their resemblance to naturally occurring protein folding pathways, making them suitable for in vivo applications. However, to employ peptide nanostructures for clinical trials, it is crucial to control their binding, folding, and half-life by manipulating environmental factors such as pH, temperature, and molarity. For instance, Fmoc-HCKF-OH can self-assemble into nanospheres and nanosheets, depending on the solvent pH. Promisingly, published articles demonstrate the consolidation of numerous peptide therapies and diagnostics, providing hope for the approval of peptide-based delivery and diagnosis for medical applications. Consequently, research is underway using disease-specific models, and early-stage clinical trials have been conducted. Peptide-based self-assembled structures are predominantly used in cancer, endocrinology, and metabolic disorders, while applications in cardiovascular diseases, gastrointestinal tract disorders, bone ailments, dermatitis, and erectile dysfunction have also been reported. Peptides are increasingly recognized as essential components of drug delivery systems, and they serve as building blocks for new
biomaterials with vast potential in medicine, alongside their utilization as therapeutics and diagnostic agents.
Authorship contribution

Wrote or contributed to the writing of the manuscript: I.R. Singh, N. Aggarwal, S. Srivastava, JJ Panda, J. Mishra; Participated in research design: I.R. Singh, N. Aggarwal, JJ Panda, J. Mishra

Data availability

No data was used for the research described in the article.

6. References


diphenylalanine analogues with rigid or flexible chemical linkers. *Nanoscale Adv* 3:6176–6190, RSC.


Footnotes

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Declaration of competing interest

The authors declare that they have no competing financial interest
Figure captions

**Figure 1:** Peptide-based nano-pulpits as cancer theranostics.

**Figure 2:** Schematic diagram of different peptide assemblies and their structures. Reprinted (adapted) with permission from Sinha et al., 2021. Copyright (2021) American Chemical Society. Copyright 2021 American Chemical Society.

**Figure 3:** Schematic representation of iRGD-based peptide-amphiphile and the encapsulation of hypocrellin B (HB) in it. Reprinted (adapted) with permission from Jiang et al. 2018. Copyright 2018 American Chemical Society.

**Figure 4:** Structural model of KLVFFAK nanosheet. Reprinted with permission from, Li et al., 2015. Attribution-Noncommercial-Share Alike 4.0 International (CC BY-NC-SA 4.0).

**Figure 5:** (I) Schematic illustration of RH<sub>3</sub>F<sub>8</sub> nanorods as drug carriers. (II) Physical characterization of the RH<sub>3</sub>F<sub>8</sub> nanorods. (A) Size distribution profile of the nanorods. (B) Size changes of the nanorods as a function of storage time. (C) Field emission scanning electron microscopic image of the nanorods. (D) Transmission electron microscopic image of the nanorods. (E) Circular dichroism spectrum of RH<sub>3</sub>F<sub>8</sub> nanorods. (III) Drug release from RH<sub>3</sub>F<sub>8</sub>–Cur nanorods at pH 5.5 and 7.4. Reprinted (adapted) with permission from (Song et al. 2017), Publication Copyright 2021 American Chemical Society.

**Figure 6:** SEM images of different types of peptide vesicles. Reprinted (adapted) with permission from V. Haridas Publication Copyright 2021 American Chemical Society.

**Figure 7:** Anti-tumor efficacy of nanoPS (co-assembled porphyrin derivative (m-THPP) and Fmoc-L<sub>3</sub>-OMe). (A) A time-dependent fluorescence image of MCF-7 cancer-carrying mice at different durations; (B) The inhibition of tumor growth by the nanoPS; (C) The plot of body weight changes of the animals with respect to time; (D) Illustrative image of mice after various treatments taken at different time points using nanoPS. Reprinted (adapted) with permission from (Li et al., 2018). Copyright (2018) American Chemical Society.

**Figure 8:** Tumor targeting efficiency of modified d<sup>d</sup>F<sup>d</sup>A<sup>d</sup>L<sup>d</sup>G<sup>d</sup>E<sup>d</sup>A micellar system. (A) Fluorescence images of subcutaneous U87 xenograft-carrying mice taken at different time points. (B) Ex-vivo NIR images of different organs and tumors after 24 h of injection. (C) Semiquantitative ROI study of mean DiR-loaded micelle fluorescence intensity in tumor and
organ, 24 hr after injection. Reprinted (adapted) with permission from (Mao et al., 201). Copyright (2017) American Chemical Society.

**Figure 9:** a) Different structural arrangement of Irpc in solution determined at pH 7 and pH 4. The affinity of water at crucial group positions is represented by the related binding energy presented; molecules are drawn in stick models with CPK colors; the distance between two sulfur atoms is represented by a green dash line. b) Two Irpc molecules in stack conformation at pH 7 and pH 4 solution represented with the reduced density gradient in the isosurface map in solution; van der Waals effect is shown in green color, and the spiral flake-like green surface indicates strong π–π interactions. Adapted with permission from (Jin et al., 2021). Copyright (2023) John Wiley and Sons.

**Figure 10:** a) Fluorescent pictures of a single HeLa cell at passage 0 to 14 following a 20 min treatment starting with Irpc (2 μM) at passage 0. b) The total intensity of self-assembled Irpc in lysosomes from 1 to 15 cell generation. c) Overlap DIC and fluorescent pictures of multiple HeLa cells at passages 4, 9, and 14 after the same treatment as in (a). d) Fluorescent and DIC images of a HeLa cell taken at different times after being treated with Irpc (2 μM) for 20 min. Green color signifies the self-assemblies of Irpc. e) Merged fluorescent and DIC image of a multicellular HeLa spheroid after a four-day culture formed by HeLa cells pre-treated with Irpc (2 μM/20 min). f) Combined fluorescent and DIC pictures of a multicellular HeLa spheroid after incubation with Irpc for three days. g) The DIC image of a distributed spheroid that had been treated with Irpc for three days and then changed from U-dish to flat dish for two days; the fluorescent image of HeLa cells on the spreading edge is specified with a yellow border. Adapted with permission from (Jin et al., 2021). Copyright (2023) John Wiley and Sons.
Figure 1
Figure 2

Hierarchical design

Deterministic assembly

Finite
- 3-d cages

Indefinite
- 2-d lattices
- 1-d chains

Stochastic assembly

Quaternary structure

Tertiary structure

Secondary structure

Primary Structure
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8
Figure 9
Figure 10