Korean J. Chem. Eng., 35(9), 1765-1778 (2018)
DOI: 10.1007/s11814-018-0102-0
pISSN: 0256-1115
eISSN: 1975-7220

INVITED REVIEW PAPER

Recent advances in protein-based nanoparticles

Eun Jung Lee†

Department of Chemical Engineering, School of Applied Chemical Engineering,
Kyungpook National University, Daegu 41566, Korea
(*Received 8 March 2018 • accepted 13 June 2018*)

Abstract−Certain naturally occurring proteins consist of a number of subunit building blocks that are capable of self-assembling to form nanoscale particles with highly organized, symmetrical, and homogeneous structures. These protein-based nanoparticles have high surface/volume ratios and other favorable properties, including mono-dispersibility, high stability, low toxicity, biocompatibility, biodegradability, and capacity for easy genetic and chemical modification. Thus, these particles have attracted considerable research attention and have been manipulated for various applications in different fields. This review describes the engineering of existing protein nanoparticles, with a particular focus on scientific advances in diverse applications, including bioassays, molecular imaging diagnostics, drug delivery, biocatalysis, and materials science. In addition, barriers for the widespread industrial use of such nanoparticles and outlook for the design and creation of novel self-assembled protein-based nanoparticles are considered.

Keywords: Protein-based Nanoparticles, Functionalization, Diagnostics, Drug Delivery, Biocatalysis, Materials Science

INTRODUCTION

Nanoscale materials have attracted considerable research attention owing to their high surface/volume ratios, potential for surface engineering and multi-functionalization, and intrinsic properties [1-9]. A variety of nanoparticles have been developed to date, including liposomes, metal (Au, Ag, Cu) nanoparticles, silica nanoparticles, polymer micelles, quantum dots, carbon nanotubes, dendrimers, and protein-based nanoparticles. Numerous studies have focused on various applications of nanoparticles in nano/biotechnology, synthetic biology, medicine, energy, catalysis, mechanics and materials science through modification or conjugation with functional moieties [10-19].

Among these nanoparticles, protein-based nanoparticles are derived from naturally occurring products. They have highly organized, symmetrical, and homogeneous structures, formed through self-assembly of protein building blocks. These protein nanoparticles have high surface/volume ratios and other favorable properties, including mono-dispersibility, high stability, low toxicity, biocompatibility, biodegradability, and ability for easy genetic and chemical modification. A more detailed description of protein nanoparticles and their intrinsic advantages is outlined in the first section of this review. Therefore, protein nanoparticles have been extensively investigated as materials in various fields.

This review mainly focuses on recent studies that have investigated the applications of protein nanoparticles in various fields. Surface engineering and functionalization through genetic and chemical modification of protein nanoparticle are discussed. Specific applications of these engineered protein nanoparticles, including bioassays, molecular imaging diagnostics, drug delivery, biocatalysis and materials science, among other applications, are highlighted. The final part presents a summary and perspective of recent advances in protein nanoparticles involving *de novo* design technology.

PROTEIN-BASED NANOPARTICLES AND THEIR INTRINSIC ADVANTAGES

Protein-based nanoparticles are derived from natural sources, such as *Escherichia coli*, yeast, viruses as well as plant and mammalian cells. Various naturally occurring protein nanoparticles, including ferritin, *Methanococcus jannaschii* small heatshock protein (sHsp), *Aquifex aeolicus* lumazine synthase (AaLS), *Bacillus stearothermophilus* E2 protein, cowpea chlorotic mottle virus (CCMV), cowpea mosaic virus (CPMV), cucumber mosaic virus (CMV), hepatitis B virus (HBV), bacteriophage MS2, bacteriophage M13, bacteriophage Qβ, DNA binding protein (Dps), tobacco mosaic virus (TMV), *Thermoplasma acidophilum* proteasome (PTS), and major vault protein (MVP), have been discovered (Fig. 1) [20-31]. These protein nanoparticles play important roles in cellular regulation, including ion homeostasis, protection and transport of nucleic acids, endocytosis, catalysis, and protein folding. They also exist in diverse shapes—spherical (e.g. ferritin, sHsp, CCMV, CPMV, CMV, bacteriophage MS2, AaLS, and Dps), disk/ring (PTS and initially self-assembled TMVs), rod (full-structure TMV and bacteriophage M13), and ellipsoid (MVP)]—and range in size from 8 to 100 nm [20,32-38].

Compared with synthetic nanoparticles, protein nanoparticles offer many advantages. The main advantages are their highly symmetrical bottom-up structure and a very uniform size distribution [7,8]. Protein nanoparticles are comprised of self-assembling protein subunits produced in living host; thus they have a monodisperse size with perfect reproducibility. These properties of nanoparticles make it possible to easily and precisely control the function of devices

†To whom correspondence should be addressed.
E-mail: eunjunglee@knu.ac.kr

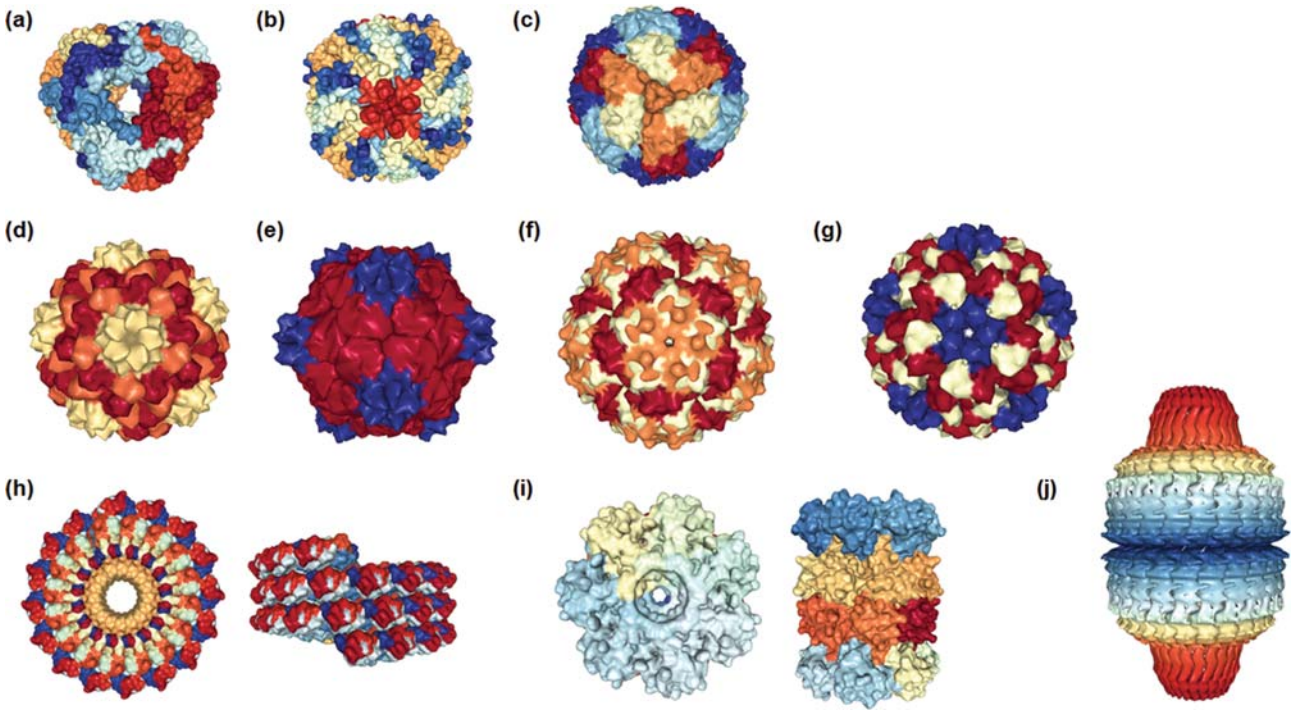

Fig. 1. Examples of protein-based nanoparticles. Structural illustration of (a) *Methanococcus jannaschii* small heatshock protein (sHsp, PDB 1SHS), (b) Human ferritin heavy chain (PDB 2FHA), (c) *Aquifex aeolicus* lumazine synthase (AaLS, PDB 1HQK), (d) Cowpea chlorotic mottle virus (CCMV, PDB 1CWP), (e) Cowpea mosaic virus (CPMV, PDB 1NY7), (f) Bacteriophage MS2 (PDB 1AQ3), (g) Cucumber mosaic virus (CMV, PDB 1F15), (h) Tobacco mosaic virus (TMV, PDB 3J06), (i) *Thermoplasma acidophilum* proteasome (PTS, PDB 1PMA), and (j) Major vault protein (MVP, PDB 2QZV).

and systems for various applications.

Given that protein nanoparticles are synthesized from biological building blocks (i.e., amino acid), they also have biocompatibility, biodegradability and low toxicity [4,5,9], making them more suitable for biomedical applications compared to inorganic nanoparticles. Furthermore, they retain excellent capacity for receptor-mediated endocytosis (RME), membrane wrapping kinetics of target cells, and enhanced permeability and retention effect (EPR, accumulation in tumor tissue through leaky tumor blood vessels) [1,6].

In addition, three distinct regions (interior, exterior and subunit interface), available for functionalization, of protein nanoparticles can be used to tune the properties of nanoparticles [1,6,20]. The surface of protein nanoparticles is decorated with functional ligands, and the inner cavity of protein nanoparticles is used for encapsulation of drug or enzyme, providing protection against environmental influences. Protein nanoparticles are assembled by noncovalent interaction between their monomer subunits; thus their interface can be engineered to change the permeability of pores or the stability of protein nanoparticles.

Ease of functionalization at precisely known locations through chemical or biological modification techniques is also an attractive property of protein nanoparticles [32,39,40]. Three-dimensional structures of most protein nanoparticles have been discovered; thereby it is possible to modify them at a desired location. Moreover, because protein nanoparticles are composed of many copies of the same protein subunits, single modifications are displayed identically in a controlled manner around the entire nanoparticle.

The remarkable characteristics of these protein nanoparticles have stimulated considerable research into applications in biological analysis, drug delivery and biocatalysis; such nanoparticles have also been used as templates for advanced electronic and energy materials.

BIOASSAYS AND MOLECULAR IMAGING DIAGNOSTICS

Biomolecules in nature are typically measured on a nano- to micro scale. For instance, the width of DNA is 2.5 nm and protein molecules measure approximately 1-20 nm. Thus, detection at the molecular or single-cell level is made possible by the development of probes based on nanomaterials. In addition, the high surface area-to-volume ratio and surface functionality of these nanomaterials has led to the development of highly sensitive and selective detection strategies. A number of biofunctional materials, such as antigens, antibodies and aptamers, have been displayed on the surface of protein nanoparticles, and small molecules, such as fluorescent dyes and metals, have also been loaded onto protein nanoparticles to create new and multiple functionalities.

The Lee group was the first to report a protein nanoprobe system, specifically using protein nanoparticles that detected a GAD65 (65 kDa glutamate decarboxylase)-specific autoantibody, an early marker of type 1 diabetes [41]. They also reported various applications of protein nanoparticles in bioassay, biocatalyst, molecular imaging, vaccine, and drug delivery, and introduced the 'proteinticle', which is a nanoscale protein particle that is self-assembled inside

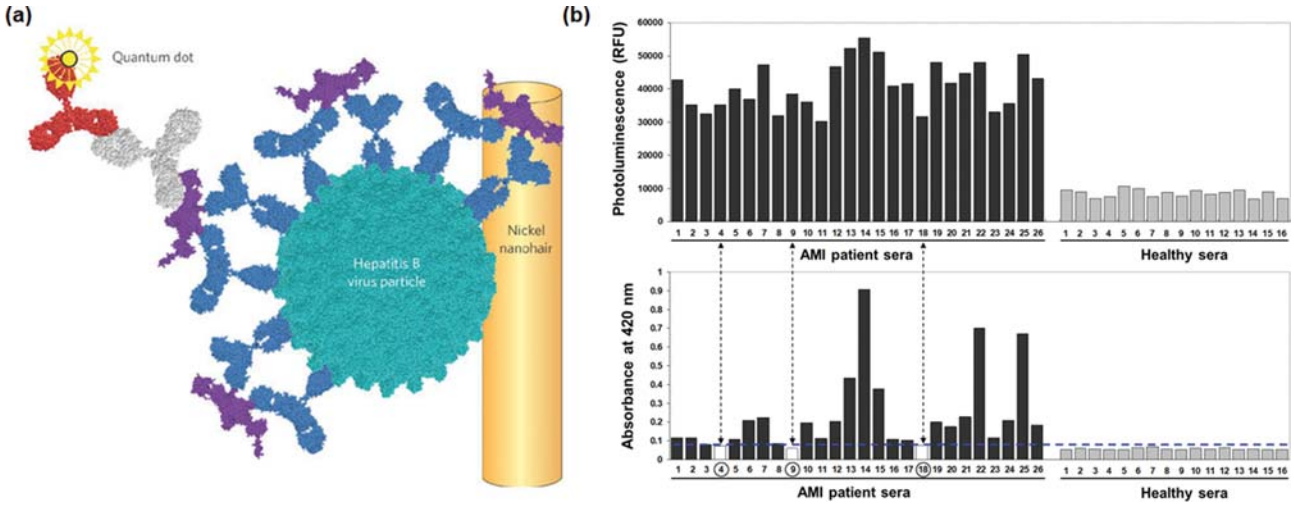

Fig. 2. 3D protein nanoparticle-inorganic hybrid systems for bioassays. (a) Schematic of the diagnostic assay based on chimeric HBV nanoparticles with nickel nanohairs. Fc domain of anti-troponin I antibodies (blue) binds to the protein A domain displayed on HBV chimeric nanoparticles. Troponin I (disease marker, magenta) binds to the antibodies and detection is achieved with another primary (gray) and secondary antibodies conjugated with quantum dots (orange). (b) Detection of troponin I in AMI patients and healthy sera using 3D protein nanoparticle-inorganic hybrid assay (upper panel) and a conventional ELISA assay (lower panel). The 3D protein nanoparticle-inorganic hybrid assay clearly detected troponin I in all patients, whereas the conventional ELISA assay failed to detect three patients (4, 9, 18). Horizontal dotted line: clinical cutoff signal. Schematic diagram and graph reproduced with the permission from [42] and [55].

cells with constant 3D structure and surface topology [41-54]. The nanoprobe system substantially enhanced sensitivity by displaying GAD65 on the surface of a ferritin nanoparticle in a homogeneous and stable conformation that facilitated autoantibody binding. This ferritin nanoparticle-based nanoprobe system is able to detect GAD65 at attomolar levels. The Lee group also developed a three-dimensional (3D) protein nanoparticle-inorganic hybrid system consisting of hepatitis B virus (HBV) nanoparticles containing nickel nanohairs as shown in Fig. 2(a) [42,55]. The 3D structure was created through interactions between a histidine tag on the protein nanoparticles and the nickel nanohairs, and the protein A domain on the protein nanoparticles and Fc domain of antibody, providing the controlled orientation of densely immobilized antibodies and the three-dimensional manner of protein capture. This 3D diagnostic assay system was applied to the detection of the acute myocardial infarction (AMI) marker, troponin I, and showed an increase in sensitivity up to seven orders of magnitude greater than that of conventional diagnostic immunoassays. Furthermore, troponin I in AMI patient sera was successfully detected using the 3D diagnostic assay system; remarkably, the assay system detected troponin I in all patients, whereas the conventional assay failed to detect three patients (4, 9, 18). (Fig. 2(b)). A similar 3D protein nanoparticle-inorganic hybrid system composed of vinylated ferritin nanoparticles and polyacrylamide (PAA), termed ferritin-based nanoprobe (FBNP) hydrogel, was developed by applying a simple one-step copolymerization process [43]. The FBNP hydrogel has a 3D matrix structure with a large surface area, and high porosity and water content. In addition, the amount and distribution of ferritin nanoparticles within the hydrogel could be easily controlled, and the stability of ferritin within the hydrogel was shown to be significantly enhanced. This FBNP hydrogel has been successfully applied to the highly specific, multiplex diagnosis of acquired immune deficiency syndrome (AIDS) and Sjögren's syndrome.

Ferritin, originally identified as an iron storage protein, can be mineralized by incorporating a variety of metals and minerals within its inner cavity [56-60]. Therefore, by combining the specificity of the bio-functional materials on the outer surface of ferritin with encapsulated metal within the inner cavity, it is possible to use ferritin in targeted molecular imaging approaches, such as near-infrared fluorescence (NIRF) imaging, positron emission tomography (PET), and magnetic resonance imaging (MRI) [61-65]. One example of such applications is ferritin multi-functionalized with the tumor targeting peptide, RGD4C, and Cy5.5 and ^{64}CU for PET and NIRF imaging [63]. This multi-modality, integrated into a hybrid form of ferritin nanoparticles, was generated through three different strategies: genetic modification (RGD4C) and chemical modification (Cy5.5) of the surface, and mineralization through encapsulation of ^{64}CU in the interior cavity via metal-binding channels. We also evaluated combinations of different ferritin nanoparticles, prepared using pH-induced assembly and disassembly—a facile method for functional hybridization. These nanoparticles were shown to have excellent cancer cell-targeting profiles *in vitro* and *in vivo*, and their potential suitability as multimodal imaging probes has been verified.

A highly modular 'bio-click' strategy for decorating protein nanoparticles was developed by the Chen group [66-68]. Specifically, they reported a sortase A-mediated ligation method for generating diverse post-translational modifications of *Bacillus stearothermophilus* E2 nanoparticles (Fig. 3(a)) [66,67]. Sortase A is a bacterial transpeptidase from *Staphylococcus aureus* that catalyzes the condensation reaction between a C-terminal LPXTG motif and an N-terminal polyglycine tag to generate a native amide bond. Therefore, the ligation provides a site-specific modification of targeted

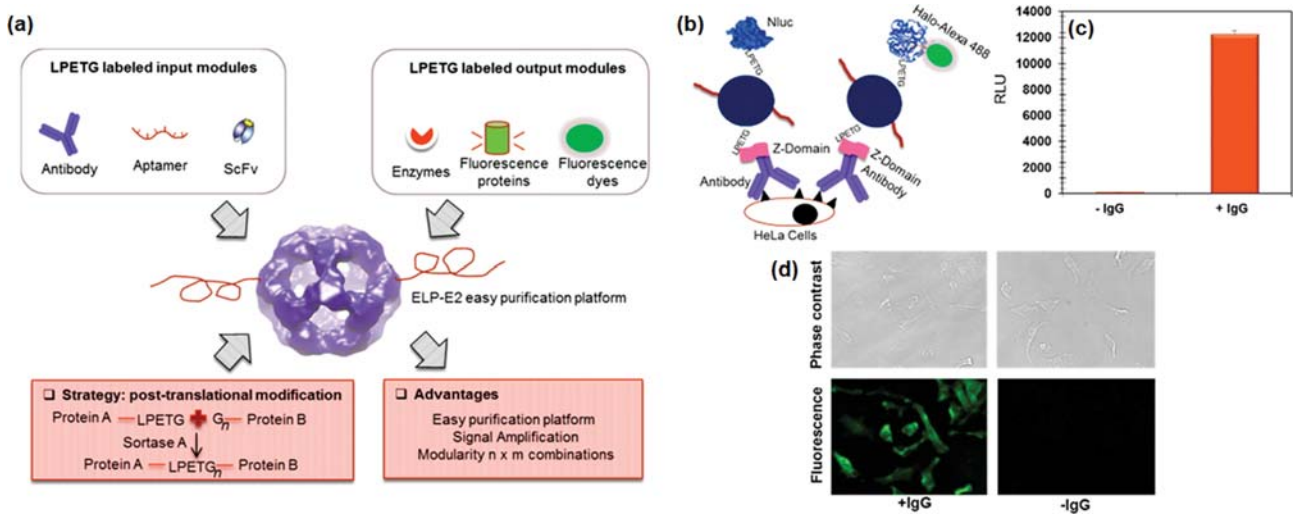

Fig. 3. A bio-click strategy to decorate protein nanoparticles for bioassay and molecular imaging with modularity, signal amplification and easy purification. (a) Schematic of sortase A (SrtA)-mediated modulation of E2 nanoparticles. (b) Schematic of MUC1 detection on HeLa cells using multi-decorated E2 nanoparticles. Result of MUC1 detection on HeLa cells using multi-decorated E2 nanoparticles (c) with Nluc as detection signal and (d) with Alexa 488 as the detection signal. Reprinted with permission from [66].

moieties with a minimal impact on functionalities. Furthermore, because the system is modular, the E2 protein nanoparticles can be decorated with a variety of functional moieties, including antibodies, enzymes, aptamers and fluorescent proteins/dyes, for a wide range of molecular targeting and detection applications. These nanoparticles are easily purified within 1 hour using relatively mild conditions and offer excellent signal amplification, providing highly specific and sensitive immunoassays and molecular imaging for cancer. For instance, E2 nanoprobe was engineered to contain a Z-domain for antibody capture as the input module and nanoluciferase (Nluc) or Alexa 488 as the output module for the detection of HeLa cells (Fig. 3(b)). Using the E2 nanoprobe with anti-MUC1 antibody, overexpressed MUC1 protein on HeLa cells was significantly detected through Nluc signal or Alexa 488 (Fig. 3(c) and (d)).

Rod-shaped protein nanoparticles, such as the coat proteins of M13 bacteriophage and TMV, are also efficient materials for use in biosensing applications, given their high surface area-to-volume ratios. For example, the red fluorescent protein, R-phycoerythrin (R-PE), or R-PE-conjugated antibody, has been attached to TMV within hydrogel microparticles using a bioorthogonal tetrazine (Tz)-trans-cyclooctene (TCO) cycloaddition and strain-promoted alkyne-azide cycloaddition (SPAAC) reaction [69]. This study demonstrated that TMV nanorods provide a high capacity—2400-times greater than native TMVs—and offer less hindrance for protein conjugation and detection. In the case of the coat protein of M13 bacteriophage, Brasino et al. demonstrated an ultrasensitive immunoassay based on dual-modified, filamentous nanorods [70]. These nanorods consist of a genetically fused antibody-binding ZZ domain on the minor coat protein pIII and multiple biotin sites on the major coat protein pVIII for binding avidin-linked, horseradish peroxidase (HRP)-conjugated enzymes. These dual-modified, filamentous nanorods containing anti-recombinant human tumor necrosis factor alpha (rTNFα) antibodies and HRP-conjugated enzymes showed significantly enhanced (3- to 4-fold) colorimetric detection at all antigen concentrations compared with antibodies alone.

Five protein nanoparticles (DPS, human ferritin, HBV, TMV and PTS), all completely different in size, shape and surface structure, have been genetically surface-modified to contain the SPA_B domain (B domain of staphylococcal protein) for binding the Fc region of IgG, enabling efficient immobilization of antibodies [44]. In this application, protein nanoparticles were conjugated with SPAB at the C-terminus, both N- and C-termini or the surface loop, creating high-density regions ('hot spots') for antibody binding. The dissociation constant (K_D) of the protein nanoparticle for IgG binding was estimated to be 1-3-times lower than previously reported values. Moreover, these studies revealed that the surface density and distribution, and particularly the presence of hot spots of SPA_B, which depended on the size, shape and number of subunits, as well as the surface structure of protein nanoparticles, are determinants of the effectiveness of immobilized antibodies. Using a similar approach, Kang and coworkers developed a polyvalent antibody-binding nanoplatform for *in vivo* diagnostics using nanoparticles based on the AaLS [71]. This nanoplatform, termed ABD-AaLS nanoparticles, is composed of genetically conjugated AaLS and antibody-binding Z domains (ABD). When formulated as complexes with anti-HER2 or anti-CD44 antibody, ABD-AaLS complexes specifically recognized and bound target SKBR3 or SCC7 cancer cells, respectively. This system may provide a new and effective molecular probe nanoplatform for target-specific cell imaging or drug delivery.

DRUG DELIVERY

As noted in the Introduction, protein nanoparticles offer advantages of biocompatibility, low toxicity, structural stability, uniform size distribution, easy manipulation by genetic or chemical strategies, and ability to encapsulate small molecules in the inner cavity. For drug-delivery applications, protein nanoparticles have the addi-

tional advantage of being able to overcome biological barriers and accumulate in cancer tissue through the passive targeting mechanism termed the enhanced permeability and retention (EPR) effect [2,72-77]. Moreover, active targeting to particular tissues can be achieved through modification with bio-functional moieties that enable molecular recognition or trigger drug release in the target tissue. Therefore, drug delivery using protein nanoparticles offers improved efficacy of drug delivery, leading to excellent therapeutic effects with minimal toxicity.

A number of drug-encapsulation and -delivery strategies based on protein nanoparticles have been reported to date. The natural affinity of protein nanoparticle for metals and nucleic acids, as well as the potential of protein nanoparticles to undergo structural changes in response to environmental factors (e.g., pH, salt and urea concentration, osmotic pressure), offers a strategy for drug loading and release. Covalent attachment through side residues of amino acids (i.e., lysine, tyrosine, cysteine, aspartate and glutamate) on protein nanoparticles is also commonly used for encapsulation of drugs, as well as additional functionalizations, such as targeting moieties for specific delivery, cell-penetrating peptides that allow access to the cytosol of the cell, enzymatic cleavage sites for controlled release, and a 'stealth' layer for improved pharmacokinetics. In recent years, insertion of the non-native amino acids homopropargylglycine (HPG) and azidohomoalanine (AHA), called click chemistry, and genetic fusion of a scaffold moiety to protein nanoparticles, are commonly used for site-specific attachment of drugs or functional moieties.

Most virus-like particles (VLPs), such as CCMV, CPMV, HBV and the bacteriophage Qβ, among others, have the inherent ability to transfer nucleic acids through their 'cationic patch'. This natural patch has been used to load nucleic acids in VLPs through electrostatic interactions between the positively charged patches in VLPs and the negative charges of the nucleic acid [78-89]. For instance, HBV nanoparticles modified with an RGD peptide efficiently encapsulated siRNAs into their inner cavity via electrostatic interactions and transferred them to target tumor cells [88,89]. These nanocages, which delivered red fluorescent protein (RFP)-specific siRNAs, substantially suppressed RFP expression in tumor-bearing mice through both the targeting effect of the multivalent RGD and the shielding effect of the HBV shell. In another instance, nucleic acid-conjugated drugs were encapsulated to CPMV, CMV, or Qβ nanoparticles [78,80]. For example, the drug-RNA-conjugated form of CMV was shown capable of encapsulating up to 1500 molecules of the anticancer drug, doxorubicin (dox), in its interior [80]. These CMV have also been decorated with fatty acids for tumor-targeting, and the resulting protein nanoparticles were shown to exhibit enhanced tumor targeting and tumor-growth inhibition with low cardiotoxicity in OVCAR-3 tumor cells. Similarly, vault protein nanoparticles can be encapsulated with hydrophobic drugs by virtue of their ability to pack lipoprotein complexes via the vault-interaction domain [90]. In this application, all-trans retinoic acid (ATRA), a highly insoluble and toxic hydrophobic drug, was stored in a vault through lipid bilayer nanodisks, and the resulting nanoparticles were shown to exert a substantial cytotoxic effect on HepG2 cells compared with drug alone.

As described above, ferritin has an affinity for metal cations because of the eight hydrophilic and hydrophobic channels (~3-4 Å wide) on its surface [56,91,92]. These features have been exploited to deposit and accumulate metal-based or metal-complexed drugs in the central cavity of ferritin [93,94]. For example, dox pre-complexed with Cu(II) was much more efficiently incorporated in the inner cavity of ferritin (up to ~74 wt%) than uncomplexed dox (14.14 wt%) [93]. pH- or urea-concentration–dependent, reversible structural changes that result in disassembly/assembly of ferritin have also been exploited for drug loading. Liang et al. loaded dox into the inner cavity of ferritin through urea concentration-dependent disassembly/assembly [95]. Remarkably, dox-loaded ferritin delivered high doses of dox to tumor cells with less toxicity than free dox; it also significantly inhibited tumor growth with a single injection and produced longer median survival times than clinically approved liposomal dox (Doxil).

Insertion of non-native amino acids or a scaffold moiety into protein nanoparticles has also been investigated for attachment of drugs. The reaction used to incorporate non-native amino acids into protein nanoparticles, called click chemistry, is biorthogonal, rapid, and highly specific. These strategies rely on reactions between azide and alkyne groups using copper(I) as a catalyst, referred to as copper(I)-catalyzed azide-alkyne cycloaddition (CuAAc), or between azide and alkyne groups without copper(I) in the presence of L-azidohomoalanine (AHA) [96-102]. Click chemistry, first investigated by the Finn group, has received considerable attention for its application to engineering of various protein nanoparticles (e.g., HBV, MS2, Qβ and CPMV) for drug-delivery systems [96,97,99]. Genetic fusion of scaffold moieties (e.g., attaching an siRNA-binding peptide to HBV and ferritin, an affinity tag for infectious HIV proteases to AaLS, and hydrophobic pockets for loading hydrophobic drugs to E2) have also been demonstrated [45,103,104]. A very recent study reported protein nanoparticles for photothermal therapy that densely display numerous small gold dots (1-3 nm) and tumor targeting moieties on their surface (Fig. 4(a)) [47]. Both polytyrosine peptides with a high reduction potential to cause Au^{+} reduction and EGFR-binding peptides for targeting of EGFR-expressing tumor cells are genetically fused to HBV nanoparticles, resulting in the formation of PGCS-NPs (proteinticle/gold core/shell nanoparticles) (Fig. 4(b)). PGCS-NPs showed a strong photothermal effect upon NIR laser irradiation, resulting in significant inhibition of tumor growth without causing any gross histological lesions in other major organs (Fig. 4(c)). In the case of intravenous injection of 20-nm gold NP into healthy mice, a dark brownish discoloration of the excised livers was observed, showing the extensive liver damage. However, remarkably, no accumulation of gold and visible change was observed in excised livers at all the time point for three weeks (1 day, 7 days, 14 days, and 21 days) after intravenous injection of PGCS-NP (Fig. 4(d)). This same group subsequently demonstrated a theragnostic agent based on PGCS-NPs, termed SPAuNCs (superparamagnetic gold-nanoparticle clusters), that exploited gold magnetism [48]. SPAuNCs enable T2-weighted magnetic resonance imaging and magnetic hyperthermia therapy under alternating magnetic fields.

Protein nanoparticles can be made inherently effective as pharmacologically active therapeutic agents through simple genetic engineering without any additional processing [49,105-108]. For instance,

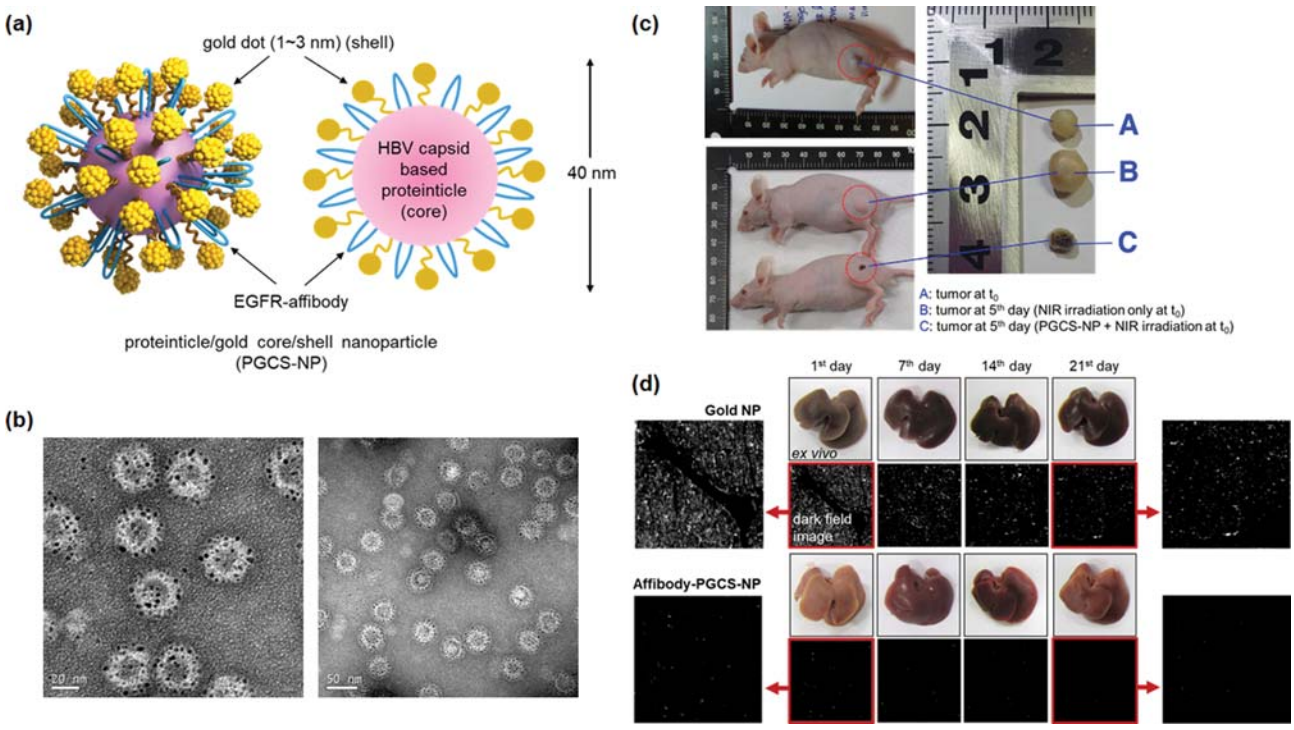

Fig. 4. Engineered PGCS-NP (proteinticle/gold core/shell nanoparticles) for photothermal therapy. (a) Schematic and (b) TEM image of PGCS-NP with EGFR-binding peptides and dotted small gold NPs. (c) Photothermal therapeutic effect of PGCS-NP for cancer. Representative pictures of MDA-MB-468 tumor-bearing mice and excised tumors after *in vivo* photothermal therapy. (d) Biocompatibility of PGCS-NP compared with 20-nm gold NPs. Representative macroscopic and dark field images of the excised livers of intravenously injected PGCS-NP and 20-nm gold NP in healthy mice. Reprinted with permission from [47].

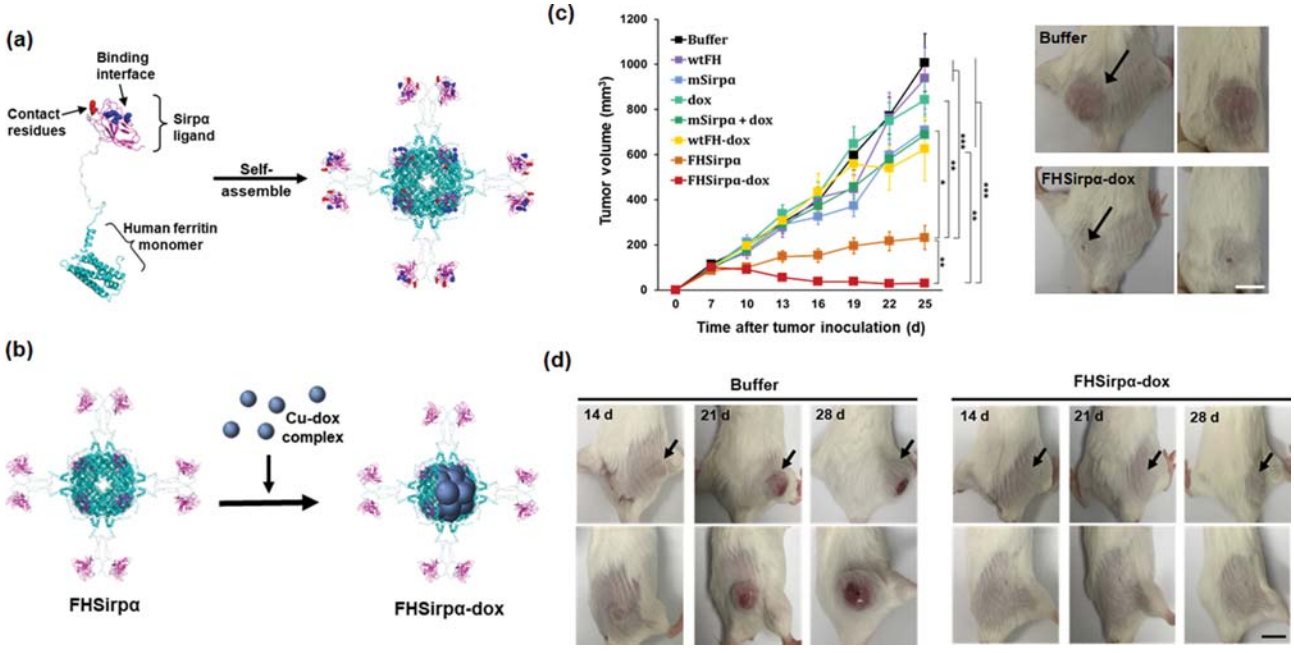

Fig. 5. Designed protein nanoparticles for cancer immunotherapy. Schematic of (a) genetic engineering of ferritin nanoparticles with immune check-point inhibitor (i.e. Sirpα, CD47 antagonist) and (b) encapsulation strategy of immunogenic cell death inducer (i.e. dox) into Sirpα-decorated ferritin (FHSirpα). (c) Anti-tumor effect of FHSirpα-dox in CT26.CL25 tumor bearing mice. Representative pictures in right panel are mice treated with FHSirpα-dox or buffer. (d) Durable anti-tumor responses of FHSirpα-dox. Representative pictures of mice re-challenged with CT26.CL25 tumor in the opposite flank of the primary CT26.CL25 tumor. Primary tumors in the left flank of CT26.CL25-bearing mice were surgically removed and the same CT26.CL25 tumor cells were subcutaneously injected into the right flank. Scale bars: 1 cm. Reprinted with permission from [107].

short ferritin (sFn; lacking helix E and the loop of ferritin), designed to contain two therapeutic proteins—protein C domain of γ-carboxyglutamic acid of (PC-Gla) and thrombin receptor agonist peptide (TRAP) for an antiseptic response—have been developed [105]. These nanoparticles, modified using a single genetic engineering step, exhibited reduced sepsis-induced organ damage and septic mortality in *in vivo* models. Protein nanoparticles have also been used as immune-modulating agents owing to their ability to recognize and interact with the immune system. In particular, protein nanoparticles accumulate in lymph nodes through passive targeting and interact with many immune cells in the lymph. A recent study used four different protein nanoparticles, DPS, PTS, HBV and ferritin, to efficiently deliver tumor-specific antigens (TSAs) to immune cells in lymph nodes [49]. In this application, the model TSA, which elicits humoral and cellular immune responses, was genetically incorporated into ferritin nanoparticles which exhibit preferential lymph node targeting. The TSA-modified ferritin strongly induced TSA-specific $CD8^+$ T cell proliferation and inhibited the growth of TSA-expressing tumor growth. More recently, ferritin nanoparticles loaded with both an immune check-point inhibitor (Sirpα, CD47 antagonist) and immunogenic cell death inducer (dox) were developed for cancer immunotherapy (Fig. 5(a) and (b)) [107]. These nanoparticles block the CD47 ('Don't eat me' signal molecule) on the surface of tumor cells and trigger immunogenic cell death, which elicits extensive immune responses during cell dying. Therefore, they enhanced phagocytosis of tumor cells and resulted in cross-priming of tumor-specific T cells and successful inhibition of tumor growth, completely eradicating tumors in three different tumor-bearing mouse models [CT26.CL25 colon carcinoma (Fig. 5(c)), B16.OVA melanoma, CT26 colon carcinoma]. Remarkably, a second challenge of nanoparticle-treated mice with the same tumor cells after removal of the primary tumor resulted in no tumor growth in all mice, suggesting the development of durable, tumor-specific responses (Fig. 5(d)).

Testing of protein nanoparticles for drug delivery has moved beyond basic laboratory studies and into preclinical and clinical stages. For example, Qβ nanoparticles, loaded with tumor peptide antigen from Melan-A/MART-1 of melanosomes and CpG-oligonucleotides (CpG-ODN), termed MelQbG10, have been used as a vaccine [79,109]. MelQbG10 induces activation of Melan-A/MART-1-specific T cells through efficient display of Melan-A/MART-1 peptides on antigen-presenting cells (APCs) in stage III-IV melanoma patients. MelQbG10 was tested in recently completed clinical Phase II studies; other protein nanoparticle are also being investigated in preclinical and clinical studies for use as vaccines and cancer immunotherapeutics [108,110-119].

BIOCATALYSIS

In general, catalytic reactions occur at the surface of the reactant compounds, and their reaction rate is proportional to their surface area. Nanoparticles support a much faster rate than larger, micrometer- or millimeter-size particles owing to their higher surface/volume ratio. Therefore, protein nanoparticles are desirable as biocatalysts because, using simple genetic and chemical modifications, they can be made to display enzymes at a high concentration on their surface in a homogeneous and stable conformation. Moreover, they protect enzymes against degradation by proteases, thermal denaturation and oxidative damage, among other environmental influences, through encapsulation of enzymes in their inner cavity. For example, aspartate dipeptidate peptidase E (PepE), luciferase (Luc), and green fluorescent protein (GFP) have been packaged inside Qβ nanoparticles using a bifunctional mRNA bridge [120,121]. The bridge has both a hairpin structure for binding to the interior of the Qβ nanoparticle and an RNA aptamer for binding to an arginine-rich peptide (Rev) derived from HIV-1. Conjugation of the Rev peptide-fused enzyme inside of Qβ nanoparticles was shown to increase the overall stability of the enzyme. This strategy not only protects incorporated enzymes against thermal degradation, protease attack and hydrophobic adsorption, it also simplifies the production of fragile or difficult-to-purify enzymes.

The Douglas group has developed a nano-bioreactor platform for encapsulation of a wide range of enzymes, including hydrogenase, alcohol dehydrogenase D (AdhD), tetrameric β-glucosidase (CelB), ATP-dependent galactokinase (GALK), dimeric ADP-dependent glucokinase (GLUK), influenza and fluorescent proteins [122-126]. These nano-bioreactors, which are based on P22 nanoparticles, are composed of a helix-turn motif on a scaffold protein (SP) together with a genetically fused enzyme and a capsid coat protein (CP). Co-expression of enzyme-fused SP and CP directs assembly of the P22 protein nanoparticles, and enzyme-fused SP is incorporated into the interior of the assembled nanoparticles. In particular, a multi-enzyme system composed of CelB, GALK and GLUK was developed using CP and enzymes-fused SP (Fig. 6(a)) [125]. These three enzymes in a coupled cascade of a synthetic metabolic pathway are densely packed inside P22 nanoparticles (Fig. 6(b) and (c)). This multi-biocatalytic platform shows a two-fold faster metabolic rate than the two-enzyme GLUK-CelB system in P22 nanoparticles (Fig. 6(d)). A recent study by the Douglas group demonstrated encapsulation and protection of an active hydrogen-producing and oxygen-tolerant [NiFe]-hydrogenase for the production of hydrogens, which are sustainable fuels [127]. Because the hydrogenase was protected against thermal and proteolytic effects, and its quaternary structure was stabilized inside of P22 nanoparticles, it exhibited a 100-fold increase in activity compared with the free hydrogenase.

A bacterial microcompartment (BMC) nanoparticle has also been engineered for coupled cascades of a synthetic metabolic pathway to create an ethanol-producing bioreactor [128]. In this study, BMC-targeting peptides were fused with both a pyruvate decarboxylase (Pdc) and an alcohol dehydrogenase (Adh), which are then encapsulated within a BMC. The resulting BMC-based nanobioreactor efficiently converted pyruvate to ethanol; moreover strains containing the modified BMC bioreactor produced higher levels of ethanol than free enzymes.

Strategies for controlling the number of encapsulated enzymes within the nanoparticle have been investigated using AaLS-based nanoparticles. For example, a modified AaLS was generated by introducing negatively charged residues onto its surface, resulting in an increase in the net negative charge of its surface and 5- to 10-fold higher loading capacity of positively charged enzymes than native AaLS. This modification enabled the toxic enzyme, HIV protease,

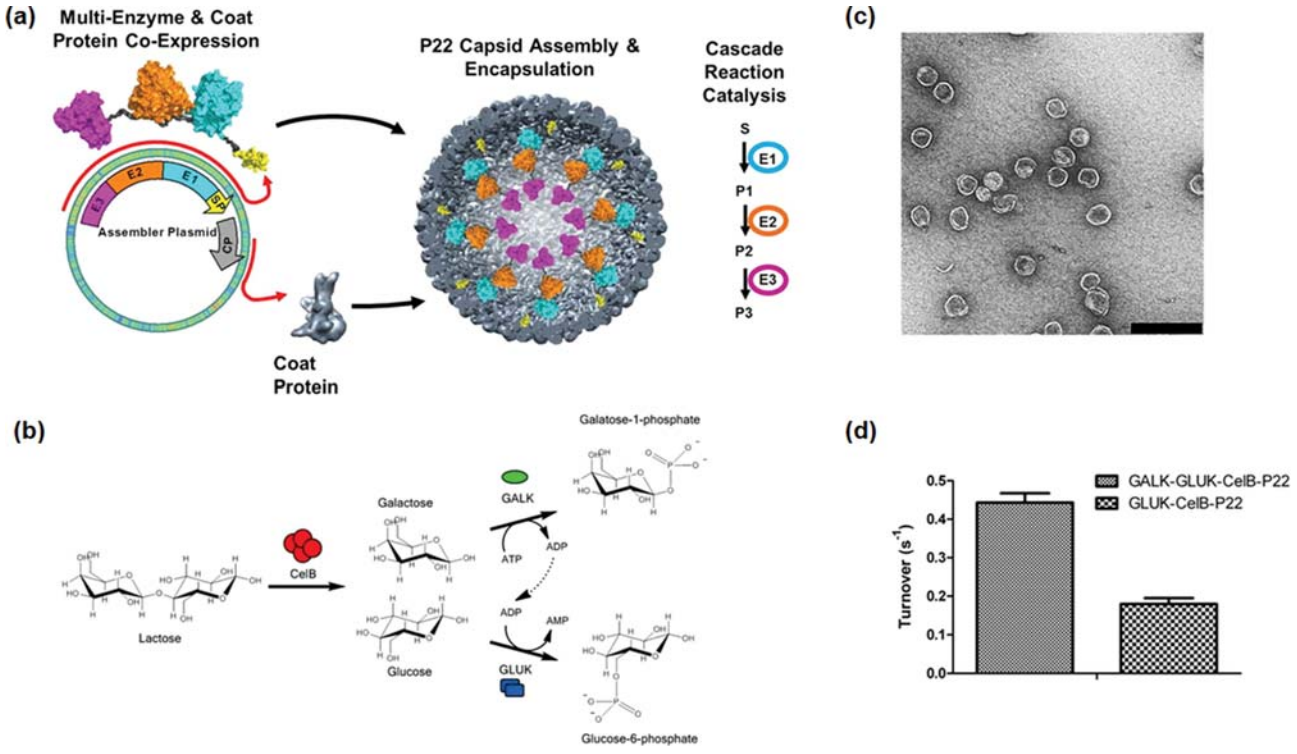

Fig. 6. Nano-bioreactor platform for encapsulation of a variety range of enzymes. (a) Schematic of multi-enzyme encapsulation systems [CelB, ATP-dependent galactokinase (GALK), and the dimeric ADP-dependent glucokinase (GLUK)] in P22 nanoparticles and (b) their coupled cascade of a synthetic metabolic pathways. (c) TEM image of multi-bioreactor platform-based P22 nanoparticles. (d) Catalytic activity of multi-bioreactor platform-based P22 nanoparticles. Turnovers for the conversion of lactose to G6P and G1P are observed with only ATP as a phosphate source. Reprinted with permission from [125].

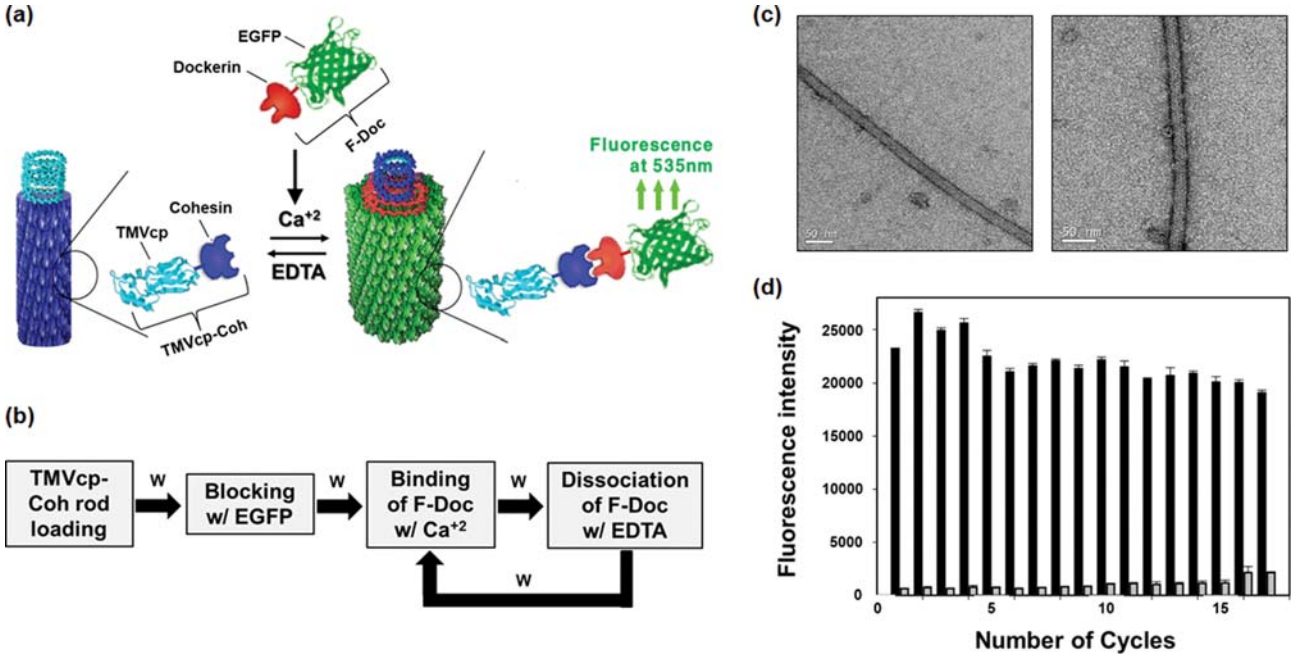

Fig. 7. Biocatalyst platform-based TMV nanorod for multi-cyclic enzyme reactions. (a) Schematic illustration and (b) procedure of reversible biocatalyst platform by Ca^{2+}-dependent interaction of cohesin and dockerin. w: a washing step. (c) TEM images of TMVcp-Coh in pH 5.9 (right panel) and pH 7.5 after the rod-shaped nanostructures are formed at pH 5.9 (left panel) (d) Multi-cyclic operation activity of biocatalyst platform-based TMV nanorod. Fluorescence emission signals of F-Doc which are bound to TMVcp-Coh rod are measured in multi-cyclic operations through binding and dissociation between TMVcp-Coh and F-Doc according to the described procedure (b). Reprinted with permission from [50].

and GFP to be encapsulated within AaLS nanoparticles through electrostatic interactions [104,129]. Moreover, the yield and packing density of enzymes could be controlled by modulating the AaLS/enzyme ratio. CCMV has also been used to encapsulate a precise number of proteins in their interior via heterodimeric coiled-coil motifs [130,131]. *Pseudozyma antarctica* lipase B (PalB) or enhanced green fluorescent protein (EGFP) with negatively charged E-coils was incorporated into positively charged K-coils in the inner surface of CCMV. Prior to assembly, enzymes were attached to dimers of CCMV through the coiled-coil motif, and the number of encapsulated enzymes was fine-tuned by controlling the ratio of wild-type capsid protein to capsid protein-enzyme complex. With this strategy, it is possible to increase encapsulation efficiency and enzyme loading in a precisely controlled fashion; the resulting CCMV-encapsulated PalB exhibited a five-fold increase in reaction rate compared with free PalB.

A TMV-based nanorod-type biocatalyst generated using the cohesin and dockerin domains of bacterial cellulosome as socket and plug, respectively, for multi-cyclic enzyme reactions has also been demonstrated (Fig. 7(a)) [50]. Cohesin sockets were incorporated into the TMV coat protein and were densely immobilized on the surface of TMV (TMVcp-Coh). The dockerin plug was fused to the model fluorescent reporter EGFP (F-Doc); thus, dockerin-fused EGFP was also densely displayed on the TMV nanorod. As the dockerin binds to cohesin in the presence of Ca^{2+}, the specific binding between TMVcp-Coh and F-Doc is dissociated by chelating Ca^{2+} using EDTA (Fig. 7(b)). In particular, TMVcp-Coh is successfully assembled into rod-shape structure under original acidic conditions as well as neutral pH (reversible reaction condition) (Fig. 7(c)). Association and dissociation of TMVcp-Coh with F-Doc are simply repeated by modulating the Ca^{2+} concentration, and multiple cycles (up to 17) of plugging in and out of the F-Doc to TMVcp-Coh were successfully performed through reversible cohesin-dockerin interactions (Fig. 7(d)). As another example, TMVs were engineered for a biocatalyst application by insertion of a cysteine residue on the surface [132]. This TMV mutant was linked to polyethylene glycol (PEG)-biotin through this cysteine and a maleimide linker, enabling conjugation of streptavidin-tagged enzymes (i.e., horseradish peroxidase and glucose oxidase). Because of their substantial increase in surface area and steric accessibility of immobilized enzyme, these TMV-based bioreactors achieved catalytic activities 45-fold higher than those of free enzyme.

MATERIALS SCIENCE AND OTHER APPLICATIONS

Nanoscale materials science is another field that could benefit from protein-based nanoparticles. Naturally occurring protein nanoparticles exist in a wide variety of shapes, sizes and functionalities, with highly symmetrical and complex architectures. These highly organized structures, formed through self-assembly of building blocks, provide homogeneous templates for materials science applications. Moreover, by making only a single modification in the basic building block, it is possible to engineer the elaborate bottom-up structure of the entire particle in a controlled nanoscale manner. This capability allows for the design of appropriate template materials to suit the synthetic needs of specific materials science applications.

Silver metal nanoparticles have been synthesized through diffusion and reduction of Ag^+ within the inner cavity of ferritin [133, 134]. Ag^+ ions diffuse into the ferritin cavity through channels with three-fold symmetry, and are reduced upon addition of $NaBH_4$. The initial amount of Ag^+ within the ferritin cage was shown to determine the final size (1 or 4 nm) of the silver nanoparticles. The synthesized silver-containing ferritin nanoparticles exhibited excellent stability, showing no UV/Vis spectral changes in solution for one month; in contrast, previous conventional silver nanoparticles were unstable and readily aggregated in solution at high concentrations. Using a similar approach, researchers prepared homogeneous gold-silver alloy nanoparticles through diffusion of a mixture the metal ions Ag^+ and $AuCl_4^-$ [135,136]. These Au-Ag alloy nanoparticles are initially generated through reduction of the metal ion mixture to form a nucleation center; subsequent agglomeration of reduced metal ions causes the center to ultimately grow to fill the ferritin inner cavity. The resulting alloy nanoparticles, which possess different properties depending on metal ion mixing ratios, can be used for biomineralization research and fabrication of multi-composite nanoelectronic devices.

TMV is a very attractive bio-template for use in fabricating nanorod structures. Metallized TMV nanorods with uniform and densely packed gold coating have been synthesized through controlled $AuCl_4$-addition, $NaBH_4$-reduction cycles [137]. In addition, molecular wrapping of these nanorods with poly-L-lysine was shown to confer high homogeneity and stability to the nanorod suspension. Metallic nanorods as well as silicon oxide nanorods have been prepared using TMV as a bio-template [138]. In particular, pretreatment with aniline to TMV allowed growth of thick (>20 nm) silica layer coating, whereas unmodified TMV produced growth of thin (~1 nm) silica layer coating. Moreover, these silicon oxide nanorods with a thick silica layer exhibited both high stability and increased affinity for nucleating metals, allowing multilayer deposition of materials over the bio-template to create novel composites.

Building blocks created by cross-linking protein nanoparticles or through combination with synthetic polymers can produce regular and periodic 2D or 3D array architectures [12,139-141]. Kostiainen et al. reported well-defined micrometer-sized hierarchical supramolecular complexes using self-assembly and photo-triggered disassembly of CCMV nanoparticles [142]. Anionic CCMVs containing cationic dendrons self-assemble into hierarchical supramolecular complexes through electrostatic interactions. The size of hierarchical complexes was controlled by varying the concentration of salt and dendrons, the latter of which act as a molecular 'glue'. Notably, because dendrons are photosensitive, irradiation with UV light results in the cleavage of self-assembled complexes. This reversible self-assembly/disassembly strategy has also been applied to superparamagnetic ferritin, providing magnetic 3D crystals.

Other studies have demonstrated array architectures through combination with synthetic polymers. These studies have reported the controlled synthesis of tubular nanostructures encapsulating size-controllable, uniform 1D arrays of ferritin nanoparticles using a coaxial electrospinning method [143]. Coaxial electrospinning of the polyelectrolyte, poly(2-acrylamino-2-methyl-1-propane sulfonic

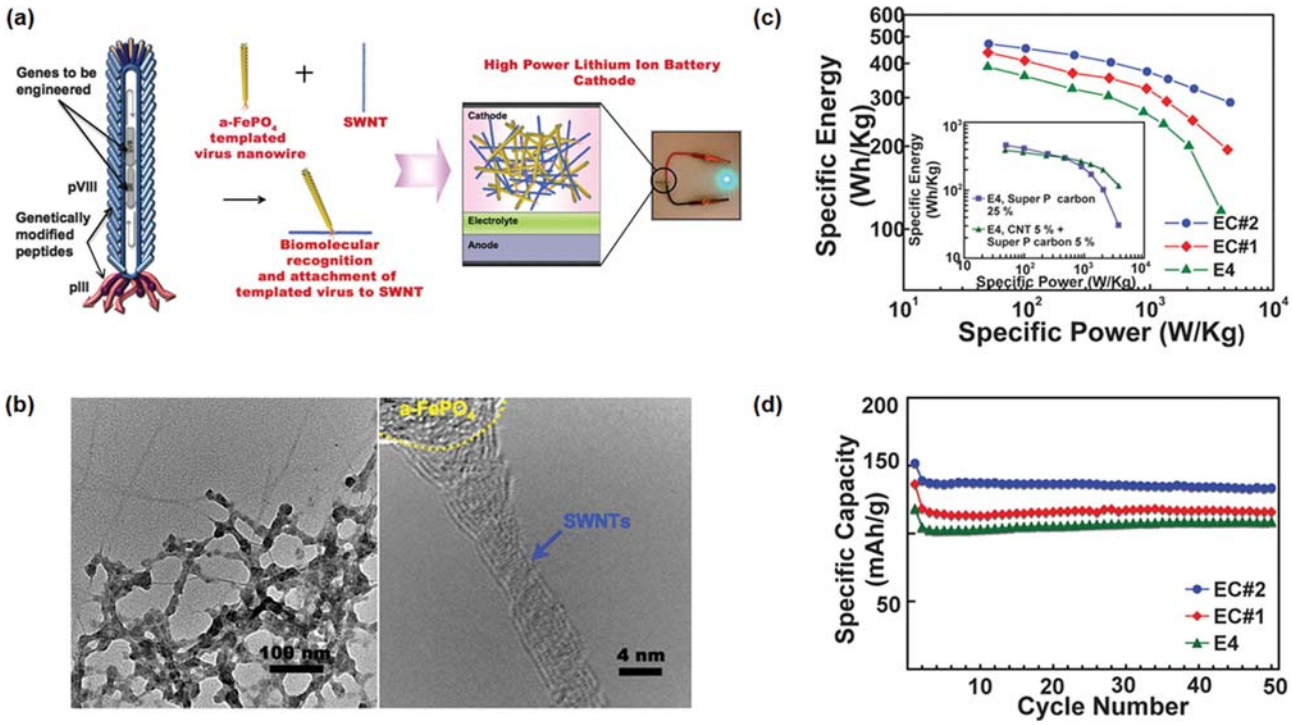

Fig. 8. Modified protein nanoparticles for battery electrodes. (a) Schematic diagram and representative picture of multi-functional coat protein of M13 bacteriophage with peptides capable of nucleating amorphous iron phosphate (a-$FePO_4$) and single-walled carbon nanotubes (SWNTs)-binding peptides for fabricating high-power lithium-ion battery cathodes. Modified coat protein of M13 bacteriophage used as a positive electrode in a lithium-ion battery using lithium metal foil as a negative electrode to power a green LED. TEM image (b) and electrochemical properties (c), (d) of multi-functional coat protein of M13 bacteriophage/SWNT hybrid nanostructures. EC#2 represents a modified two-gene system with the strongest binding affinity to SWNTs; EC#1 is a modified two-gene system with moderate binding affinity; and E4 is a modified one-gene system with no insert on pIII. Reprinted with permission from [146].

acid) (PAMPS), and ferritin with glycerol, which acts as a stabilizer, was shown to produce a thin, accurately controlled width (40 nm) of nanofiber structure containing 1D arrays of ferritin in the core. Given that precise control of tubular nanostructures containing an inner 1D particle array is key to determining the efficiency and performance of nanodevices, and further considering that the core of ferritin can be substituted with metal or semiconductor nanoparticles, this strategy can be successfully applied to a variety of nanodevices, such as nanobiosensors and batteries.

Although protein-based nanoparticles have most commonly been used in bioassay, molecular imaging diagnostics, drug-delivery and biocatalysis applications, as well as uses in electronic devices, for example as bioenergy or battery, have also been studied.

Modified TMVs engineered to contain surface cysteine residues, as described above, have also been used as a template for conducting wire for use as battery electrodes [144,145]. In this application, TMV particles are immobilized onto gold surfaces through gold-thiol interactions, increasing the surface area by more than ten-fold. A subsequent reductive-deposition process produces a dense carpet of nickel and cobalt, yielding a metal-coated TMV wire up to 40 nm thick. This TMV based-electrode shows increased stability and voltage output in a battery system, resulting in enhanced total electrode capacity. A modified M13 bacteriophage coat protein was also developed as a cathode material for a lithium-ion battery (Fig. 8(a)) [146]. In this study, the major coat protein pVIII of M13 bacteriophage, genetically fused with peptides capable of nucleating silver nanoparticles, and amorphous iron phosphate (a-$FePO_4$), were produced on silver nanoparticles. The minor coat protein pIII was engineered to contain single-walled carbon nanotube (SWNT)-binding peptides. These nanorods were successfully coated with a-$FePO_4$ and tightly attached to SWNTs, producing a-$FePO_4$/SWNTs hybrid nanostructures (Fig. 8(b)). The dual-functional nanorods (EC#2) observed higher energy than the only a-$FePO_4$ coated nanorods (E4) or dual-functional nanorods with low binding affinity to SWNT (#EC1) and stable capacity retention up to 50 cycling, showing good electrochemical performance (Fig. 8(c) and (d)). This strategy allowed realization of nanoscale electrical wiring for high-power lithium-ion batteries; by contrast, conventional nanoscale wiring has poor electronic conductivity for lithium-ion batteries. As an additional example, ferritin nanocages immobilized on gold, SAM-modified gold, or indium tin oxide (ITO) surfaces have also been investigated as an electrode material [147-158], reflecting the oxidizing and reducing ability of their iron core.

In the bioenergy field, photocatalytic water-splitting systems based on the M13 bacteriophage coat protein have been developed by the Belcher group [159]. In this application, peptides with an affinity for iridium oxide (IrO_2) and a metal oxide catalyst are genetically inserted into the major coat protein pVIII; the photosensitizer Zn(II) deuteroporphyrin IX 2,4 bis-ethylene glycol (ZnDPEG) was also chemically grafted onto pVIII. Co-assembled

ZnDPEG and IrO_2 on pVIII was then immobilized on a porous polymer hydrogel, which not only provided effective light-driven water-oxidation, but also improved the structural stability and recycling ability of the nanostructures.

CONCLUSIONS AND PERSPECTIVES

Protein-based nanoparticles consist of subunit building blocks that interact through self-assembly to form highly organized and symmetrical structures. These protein nanoparticles have been of particular interest owing to their high surface/volume ratio as well as favorable properties, such as mono-dispersibility, high stability, low toxicity, biocompatibility, biodegradability, and capacity for easy genetic and chemical modification. Researchers have manipulated these protein nanoparticles to create molecular probes, drug-delivery carriers, biocatalysts and biotemplates, and have recently attempted to explore these protein nanoparticles as nanomaterials in a variety of nanodevices, such as batteries and electrodes.

Despite dramatic advances in scientific research on protein-based nanoparticles, there are still many obstacles standing in the way of their widespread use. In particular, although protein-based nanoparticles have been used extensively for molecular imaging and drug delivery, very few examples of plant-based protein nanoparticles (e.g., as vaccine systems) have reached the stage of clinical investigation. Before translation of preclinical results to the clinical stage, the general toxicity, hematotoxicity and immunogenicity of protein nanoparticles must be better defined, and their absorption, distribution, metabolism, and excretion (ADME) properties must be clarified in animal models and humans. In all fields, including molecular imaging and drug delivery, large-scale manipulation and low-cost production methods are still needed. Recombinant DNA technology and techniques for expressing recombinant protein in microbial hosts, such as *E. coli* or yeast, are well developed, but purification procedures, such as ultracentrifugation, ion exchange and affinity chromatography, will require additional optimization.

Finally, although protein-based nanoparticles can be easily engineered and repurposed for new- or multiple functions, these strategies are limited by the characteristics and structures of existing proteins. Recent studies have explored the intriguing idea of designing and creating novel, self-assembling multi-subunit proteins and protein nanoparticles to overcome these limitations. *De novo* protein engineering, computational interface design, and *in silico* techniques are under development by many pioneering groups, with the goal of designing a broad range of multi-subunit protein assemblies with different geometries and symmetries [160-173]. These efforts could ultimately result in the generation of novel protein-based nanoparticles with unexplored properties. Although these strategies are generally still in early, proof-of-concept stages, they represent a particularly promising avenue for investigating potentially new protein-based nanomaterials.

ACKNOWLEDGEMENTS

This research was supported by the Kyungpook National University Research Fund, 2017.

REFERENCES

1. E. J. Lee, N. K. Lee and I. S. Kim, *Adv. Drug Deliv. Rev.*, **106**, 157 (2016).
2. M. Fakruddin, Z. Hossain and H. Afroz, *J. Nanobiotechnol.*, **10**, 31 (2012).
3. J. Gao and B. Xu, *Nano Today*, **4**, 37 (2009).
4. L. Schoonen and J. C. van Hest, *Nanoscale*, **6**, 7124 (2014).
5. L. P. Herrera Estrada and J. A. Champion, *Biomater. Sci.*, **3**, 787 (2015).
6. N. M. Molino and S. W. Wang, *Curr. Opin. Biotechnol.*, **28**, 75 (2014).
7. B. J. Pieters, M. B. van Eldijk, R. J. Nolte and J. Mecinovic, *Chem. Soc. Rev.*, **45**, 24 (2016).
8. S. Howorka, *Curr. Opin. Biotechnol.*, **22**, 485 (2011).
9. S. Deshayes and R. Gref, *Nanomedicine (Lond)*, **9**, 1545 (2014).
10. S. K. Nune, P. Gunda, P. K. Thallapally, Y. Y. Lin, M. L. Forrest and C. J. Berkland, *Expert. Opin. Drug Deliv.*, **6**, 1175 (2009).
11. G. M. Whitesides, *Nat. Biotechnol.*, **21**, 1161 (2003).
12. S. Mann, *Nat. Mater.*, **8**, 781 (2009).
13. T. A. P. F. Doll, S. Raman, R. Dey and P. Burkhard, *J. R. Soc. Interface*, **10**, 20120740 (2013).
14. P. V. Kamat, *J. Phys. Chem. B*, **106**, 7729 (2002).
15. Y. Sun and J. A. Rogers, *Adv. Mater.*, **19**, 1897 (2007).
16. Z. Niu, J. He, T. P. Russell and Q. Wang, *Angew. Chem. Int. Ed. Engl.*, **49**, 10052 (2010).
17. Y.-L. Wu, N. Putcha, K. W. Ng, D. T. Leong, C. T. Lim, S. C. J. Loo and X. Chen, *Acc. Chem. Res.*, **46**, 782 (2013).
18. P. Cai, W. R. Leow, X. Wang, Y. L. Wu and X. Chen, *Adv. Mater.*, 29 (2017).
19. B. Hu, W. R. Leow, P. Cai, Y. Q. Li, Y. L. Wu and X. Chen, *ACS Nano*, **11**, 12302 (2017).
20. M. Uchida, M. T. Klem, M. Allen, P. Suci, M. Flenniken, E. Gillitzer, Z. Varpness, L. O. Liepold, M. Young and T. Douglas, *Adv. Mater.*, **19**, 1025 (2007).
21. T. Lin, Z. Chen, R. Usha, C. V. Stauffacher, J. B. Dai, T. Schmidt and J. E. Johnson, *Virology*, **265**, 20 (1999).
22. R. W. Lucas, S. B. Larson and A. McPherson, *J. Mol. Biol.*, **317**, 95 (2002).
23. J. A. Speir, S. Munshi, G. Wang, T. S. Baker and J. E. Johnson, *Structure*, **3**, 63 (1995).
24. R. Golmohammadi, K. Valegard, K. Fridborg and L. Liljas, *J. Mol. Biol.*, **234**, 620 (1993).
25. K. Schott, R. Ladenstein, A. Konig and A. Bacher, *J. Biol. Chem.*, **265**, 12686 (1990).
26. D. M. Lawson, P. J. Artymiuk, S. J. Yewdall, J. M. Smith, J. C. Livingstone, A. Treffry, A. Luzzago, S. Levi, P. Arosio, G. Cesareni, C. D. Thomas, W. V. Shaw and P. M. Harrison, *Nature*, **349**, 541 (1991).
27. K. K. Kim, R. Kim and S. H. Kim, *Nature*, **394**, 595 (1998).
28. R. A. Grant, D. J. Filman, S. E. Finkel, R. Kolter and J. M. Hogle, *Nat. Struct. Biol.*, **5**, 294 (1998).
29. C. M. Shepherd, I. A. Borelli, G. Lander, P. Natarajan, V. Siddavanahalli, C. Bajaj, J. E. Johnson, C. L. Brooks, 3rd and V. S. Reddy, *Nucleic Acids Res.*, **34**, D386 (2006).
30. G. Stubbs, *Philos Trans R Soc. Lond B Biol. Sci.*, **354**, 551 (1999).
31. Y. Zhu, B. Carragher, D. J. Kriegman, R. A. Milligan and C. S. Pot-

ter, *J. Struct. Biol.*, **135**, 302 (2001).
32. P. van Rijn and A. Boker, *J. Mater. Chem.*, **21**, 16735 (2011).
33. T. Douglas and M. Young, *Science*, **312**, 873 (2006).
34. E. M. Plummer and M. Manchester, *Wiley Interdiscip. Rev. Nanomed Nanobiotechnol.*, **3**, 174 (2011).
35. S. E. Aniagyei, C. Dufort, C. C. Kao and B. Dragnea, *J. Mater. Chem.*, **18**, 3763 (2008).
36. D. J. Kushner, *Bacteriol. Rev.*, **33**, 302 (1969).
37. J. Swift, C. A. Butts, J. Cheung-Lau, V. Yerubandi and I. J. Dmochowski, *Langmuir*, **25**, 5219 (2009).
38. A. Klug, *Philos Trans R Soc. Lond B Biol. Sci.*, **354**, 531 (1999).
39. S. Bhaskar and S. Lim, *NPG Asia Mater.*, **9**, e371 (2017).
40. M. Rother, M. G. Nussbaumer, K. Renggli and N. Bruns, *Chem. Soc. Rev.*, **45**, 6213 (2016).
41. S. H. Lee, H. Lee, J. S. Park, H. Choi, K. Y. Han, H. S. Seo, K. Y. Ahn, S. S. Han, Y. Cho, K. H. Lee and J. Lee, *FASEB J.*, **21**, 1324 (2007).
42. J. S. Park, M. K. Cho, E. J. Lee, K. Y. Ahn, K. E. Lee, J. H. Jung, Y. Cho, S. S. Han, Y. K. Kim and J. Lee, *Nat. Nanotechnol.*, **4**, 259 (2009).
43. E. J. Lee, K. Y. Ahn, J. H. Lee, J. S. Park, J. A. Song, S. J. Sim, E. B. Lee, Y. J. Cha and J. Lee, *Adv. Mater.*, **24**, 4739 (2012).
44. E. J. Lee, E. Lee, H. J. Kim, J. H. Lee, K. Y. Ahn, J. S. Park and J. Lee, *Nanoscale*, **6**, 14919 (2014).
45. E. J. Lee, S. J. Lee, Y. S. Kang, J. H. Ryu, K. C. Kwon, E. Jo, J. Y. Yhee, I. C. Kwon, K. Kim and J. Lee, *Adv. Funct. Mater.*, **25**, 1279 (2015).
46. K. C. Kwon, H. K. Ko, J. Lee, E. J. Lee, K. Kim and J. Lee, *Small*, **12**, 4241 (2016).
47. K. C. Kwon, J. H. Ryu, J. H. Lee, E. J. Lee, I. C. Kwon, K. Kim and J. Lee, *Adv. Mater.*, **26**, 6436 (2014).
48. K. C. Kwon, E. Jo, Y. W. Kwon, B. Lee, J. H. Ryu, E. J. Lee, K. Kim and J. Lee, *Adv. Mater.*, 29 (2017).
49. B. R. Lee, H. K. Ko, J. H. Ryu, K. Y. Ahn, Y. H. Lee, S. J. Oh, J. H. Na, T. W. Kim, Y. Byun, I. C. Kwon, K. Kim and J. Lee, *Sci. Rep.*, **6**, 35182 (2016).
50. H. J. Kim, E. J. Lee, J. S. Park, S. J. Sim and J. Lee, *J. Biotechnol.*, **221**, 101 (2016).
51. K. Y. Ahn, H. K. Ko, B. R. Lee, E. J. Lee, J. H. Lee, Y. Byun, I. C. Kwon, K. Kim and J. Lee, *Biomaterials*, **35**, 6422 (2014).
52. S. E. Kim, S. D. Jo, K. C. Kwon, Y. Y. Won and J. Lee, *Adv. Sci. (Weinh)*, **4**, 1600471 (2017).
53. S. E. Kim, B. R. Lee, H. Lee, S. D. Jo, H. Kim, Y. Y. Won and J. Lee, *Sci. Rep.*, **7**, 17327 (2017).
54. J. H. Lee, H. S. Seo, J. A. Song, K. C. Kwon, E. J. Lee, H. J. Kim, E. B. Lee, Y. J. Cha and J. Lee, *ACS Nano*, **7**, 10879 (2013).
55. W. Choe, T. A. Durgannavar and S. J. Chung, *Materials (Basel)*, 9 (2016).
56. H. N. Munro and M. C. Linder, *Physiol. Rev.*, **58**, 317 (1978).
57. B. Langlois d'Estaintot, P. Santambrogio, T. Granier, B. Gallois, J. M. Chevalier, G. Precigoux, S. Levi and P. Arosio, *J. Mol. Biol.*, **340**, 277 (2004).
58. H. A. Hosein, D. R. Strongin, M. Allen and T. Douglas, *Langmuir*, **20**, 10283 (2004).
59. P. Sanchez, E. Valero, N. Galvez, J. M. Dominguez-Vera, M. Marinone, G. Poletti, M. Corti and A. Lascialfari, *Dalton Trans*, 800 (2009).
60. R. M. Kramer, C. Li, D. C. Carter, M. O. Stone and R. R. Naik, *J. Am. Chem. Soc.*, **126**, 13282 (2004).
61. S. Aime, L. Frullano and S. Geninatti Crich, *Angew. Chem. Int. Ed. Engl.*, **41**, 1017 (2002).
62. M. Uchida, D. A. Willits, K. Muller, A. F. Willis, L. Jackiw, M. Jutila, M. J. Young, A. E. Porter and T. Douglas, *Adv. Mater.*, **21**, 458 (2009).
63. X. Lin, J. Xie, G. Niu, F. Zhang, H. Gao, M. Yang, Q. Quan, M. A. Aronova, G. Zhang, S. Lee, R. Leapman and X. Chen, *Nano Lett.*, **11**, 814 (2011).
64. S. Geninatti Crich, B. Bussolati, L. Tei, C. Grange, G. Esposito, S. Lanzardo, G. Camussi and S. Aime, *Cancer. Res.*, **66**, 9196 (2006).
65. F. Yan, Y. Zhang, H. K. Yuan, M. K. Gregas and T. Vo-Dinh, *Chem. Commun. (Camb)*, 4579 (2008).
66. Q. Sun, Q. Chen, D. Blackstock and W. Chen, *ACS Nano*, **9**, 8554 (2015).
67. Q. Chen, Q. Sun, N. M. Molino, S. W. Wang, E. T. Boder and W. Chen, *Chem. Commun. (Camb)*, **51**, 12107 (2015).
68. G. T. Hess, J. J. Cragnolini, M. W. Popp, M. A. Allen, S. K. Dougan, E. Spooner, H. L. Ploegh, A. M. Belcher and C. P. Guimaraes, *Bioconjugate Chem.*, **23**, 1478 (2012).
69. S. Jung and H. Yi, *Langmuir*, **30**, 7762 (2014).
70. M. Brasino, J. H. Lee and J. N. Cha, *Anal. Biochem.*, **470**, 7 (2015).
71. H. Kim, Y. J. Kang, J. Min, H. Choi and S. Kang, *RSC Adv.*, **6**, 19208 (2016).
72. R. N. Alyautdin, E. B. Tezikov, P. Ramge, D. A. Kharkevich, D. J. Begley and J. Kreuter, *J. Microencapsulation*, **15**, 67 (1998).
73. E. Garcia-Garcia, S. Gil, K. Andrieux, D. Desmaele, V. Nicolas, F. Taran, D. Georgin, J. P. Andreux, F. Roux and P. Couvreur, *Cell. Mol. Life Sci.*, **62**, 1400 (2005).
74. S. Chakrabarti and K. Ruud, *J. Phys. Chem. A*, **113**, 5485 (2009).
75. H. Meng, M. Xue, T. Xia, Z. Ji, D. Y. Tarn, J. I. Zink and A. E. Nel, *ACS Nano*, **5**, 4131 (2011).
76. S. Keereweer, I. M. Mol, J. D. Kerrebijn, P. B. Van Driel, B. Xie, R. J. Baatenburg de Jong, A. L. Vahrmeijer and C. W. Lowik, *J. Surg. Oncol.*, **105**, 714 (2012).
77. J. Fang, H. Qin, H. Nakamura, K. Tsukigawa, T. Shin and H. Maeda, *Cancer. Sci.*, **103**, 535 (2012).
78. I. Yildiz, K. L. Lee, K. Chen, S. Shukla and N. F. Steinmetz, *J. Controlled Release*, **172**, 568 (2013).
79. T. Storni, C. Ruedl, K. Schwarz, R. A. Schwendener, W. A. Renner and M. F. Bachmann, *J. Immunol.*, **172**, 1777 (2004).
80. Q. Zeng, H. Wen, Q. Wen, X. Chen, Y. Wang, W. Xuan, J. Liang and S. Wan, *Biomaterials*, **34**, 4632 (2013).
81. F. A. Galaway and P. G. Stockley, *Mol. Pharm.*, **10**, 59 (2013).
82. Y. Pan, T. Jia, Y. Zhang, K. Zhang, R. Zhang, J. Li and L. Wang, *Int. J. Nanomedicine*, **7**, 5957 (2012).
83. Y. Pan, Y. Zhang, T. Jia, K. Zhang, J. Li and L. Wang, *FEBS J.*, **279**, 1198 (2012).
84. M. Wu, T. Sherwin, W. L. Brown and P. G. Stockley, *Nanomedicine*, **1**, 67 (2005).
85. C. E. Ashley, E. C. Carnes, G. K. Phillips, P. N. Durfee, M. D. Buley, C. A. Lino, D. P. Padilla, B. Phillips, M. B. Carter, C. L. Willman, C. J. Brinker, C. Caldeira Jdo, B. Chackerian, W. Wharton and D. S. Peabody, *ACS Nano*, **5**, 5729 (2011).
86. M. Kwak, I. J. Minten, D. M. Anaya, A. J. Musser, M. Brasch, R. J. Nolte, K. Mullen, J. J. Cornelissen and A. Herrmann, *J. Am. Chem. Soc.*, **132**, 7834 (2010).

87. K. Niikura, N. Sugimura, Y. Musashi, S. Mikuni, Y. Matsuo, S. Kobayashi, K. Nagakawa, S. Takahara, C. Takeuchi, H. Sawa, M. Kinjo and K. Ijiro, *Mol. Biosyst.*, **9**, 501 (2013).
88. K. M. Choi, S. H. Choi, H. Jeon, I. S. Kim and H. J. Ahn, *ACS Nano*, **5**, 8690 (2011).
89. K. M. Choi, K. Kim, I. C. Kwon, I. S. Kim and H. J. Ahn, *Mol. Pharm.*, **10**, 18 (2013).
90. D. C. Buehler, D. B. Toso, V. A. Kickhoefer, Z. H. Zhou and L. H. Rome, *Small*, **7**, 1432 (2011).
91. T. Takahashi and S. Kuyucak, *Biophys. J.*, **84**, 2256 (2003).
92. Y. H. Pan, K. Sader, J. J. Powell, A. Bleloch, M. Gass, J. Trinick, A. Warley, A. Li, R. Brydson and A. Brown, *J. Struct. Biol.*, **166**, 22 (2009).
93. Z. Zhen, W. Tang, H. Chen, X. Lin, T. Todd, G. Wang, T. Cowger, X. Chen and J. Xie, *ACS Nano*, **7**, 4830 (2013).
94. Z. Yang, X. Wang, H. Diao, J. Zhang, H. Li, H. Sun and Z. Guo, *Chem. Commun. (Camb)*, 3453 (2007).
95. M. Liang, K. Fan, M. Zhou, D. Duan, J. Zheng, D. Yang, J. Feng and X. Yan, *Proc. Natl. Acad. Sci. USA*, **111**, 14900 (2014).
96. N. F. Steinmetz, V. Hong, E. D. Spoerke, P. Lu, K. Breitenkamp, M. G. Finn and M. Manchester, *J. Am. Chem. Soc.*, **131**, 17093 (2009).
97. K. G. Patel and J. R. Swartz, *Bioconjugate Chem.*, **22**, 376 (2011).
98. J. K. Pokorski and N. F. Steinmetz, *Mol. Pharm.*, **8**, 29 (2011).
99. E. Strable, D. E. Prasuhn, Jr., A. K. Udit, S. Brown, A. J. Link, J. T. Ngo, G. Lander, J. Quispe, C. S. Potter, B. Carragher, D. A. Tirrell and M. G. Finn, *Bioconjugate Chem.*, **19**, 866 (2008).
100. S. Qazi, L. O. Liepold, M. J. Abedin, B. Johnson, P. Prevelige, J. A. Frank and T. Douglas, *Mol. Pharm.*, **10**, 11 (2013).
101. J. Lucon, M. J. Abedin, M. Uchida, L. Liepold, C. C. Jolley, M. Young and T. Douglas, *Chem. Commun. (Camb)*, **46**, 264 (2010).
102. L. O. Liepold, M. J. Abedin, E. D. Buckhouse, J. A. Frank, M. J. Young and T. Douglas, *Nano Lett.*, **9**, 4520 (2009).
103. D. Ren, M. Dalmau, A. Randall, M. M. Shindel, P. Baldi and S. W. Wang, *Adv. Funct. Mater.*, **22**, 3170 (2012).
104. B. Worsdorfer, K. J. Woycechowsky and D. Hilvert, *Science*, **331**, 589 (2011).
105. W. Lee, J. Seo, S. Kwak, E. J. Park, D. H. Na, S. Kim, Y. M. Lee, I. S. Kim and J. S. Bae, *Adv. Mater.*, **27**, 6637 (2015).
106. J. O. Jeon, S. Kim, E. Choi, K. Shin, K. Cha, I. S. So, S. J. Kim, E. Jun, D. Kim, H. J. Ahn, B. H. Lee, S. H. Lee and I. S. Kim, *ACS Nano*, **7**, 7462 (2013).
107. E. J. Lee, G. H. Nam, N. K. Lee, M. Kih, E. Koh, Y. K. Kim, Y. Hong, S. Kim, S. Y. Park, C. Jeong, Y. Yang and I. S. Kim, *Adv. Mater.* (2018).
108. J. A. Han, Y. J. Kang, C. Shin, J. S. Ra, H. H. Shin, S. Y. Hong, Y. Do and S. Kang, *Nanomedicine*, **10**, 561 (2014).
109. S. M. Goldinger, R. Dummer, P. Baumgaertner, D. Mihic-Probst, K. Schwarz, A. Hammann-Haenni, J. Willers, C. Geldhof, J. O. Prior, T. M. Kundig, O. Michielin, M. F. Bachmann and D. E. Speiser, *Eur. J. Immunol.*, **42**, 3049 (2012).
110. C. I. Champion, V. A. Kickhoefer, G. Liu, R. J. Moniz, A. S. Freed, L. L. Bergmann, D. Vaccari, S. Raval-Fernandes, A. M. Chan, L. H. Rome and K. A. Kelly, *PLoS One*, **4**, e5409 (2009).
111. U. K. Kar, J. Jiang, C. I. Champion, S. Salehi, M. Srivastava, S. Sharma, S. Rabizadeh, K. Niazi, V. Kickhoefer, L. H. Rome and K. A. Kelly, *PLoS One*, **7**, e38553 (2012).
112. N. M. Molino, A. K. Anderson, E. L. Nelson and S. W. Wang, *ACS Nano*, **7**, 9743 (2013).
113. J. S. Ra, H. H. Shin, S. Kang and Y. Do, *Clin. Exp. Vaccine Res.*, **3**, 227 (2014).
114. V. Juarez, H. A. Pasolli, A. Hellwig, N. Garbi and A. C. Arregui, *Open Virol. J.*, **6**, 270 (2012).
115. D. Franco, W. Liu, D. F. Gardiner, B. H. Hahn and D. D. Ho, *J. Acquir. Immune Defic. Syndr.*, **56**, 393 (2011).
116. R. Zhang, S. Zhang, M. Li, C. Chen and Q. Yao, *Vaccine*, **28**, 5114 (2010).
117. M. Kanekiyo, C. J. Wei, H. M. Yassine, P. M. McTamney, J. C. Boyington, J. R. Whittle, S. S. Rao, W. P. Kong, L. Wang and G. J. Nabel, *Nature*, **499**, 102 (2013).
118. A. Rynda-Apple, E. Dobrinen, M. McAlpine, A. Read, A. Harmsen, L. E. Richert, M. Calverley, K. Pallister, J. Voyich, J. A. Wiley, B. Johnson, M. Young, T. Douglas and A. G. Harmsen, *Am. J. Pathol.*, **181**, 196 (2012).
119. L. E. Richert, A. E. Servid, A. L. Harmsen, A. Rynda-Apple, S. Han, J. A. Wiley, T. Douglas and A. G. Harmsen, *Vaccine*, **30**, 3653 (2012).
120. J. K. Rhee, M. Hovlid, J. D. Fiedler, S. D. Brown, F. Manzenrieder, H. Kitagishi, C. Nycholat, J. C. Paulson and M. G. Finn, *Biomacromolecules*, **12**, 3977 (2011).
121. J. D. Fiedler, S. D. Brown, J. L. Lau and M. G. Finn, *Angew. Chem. Int. Ed. Engl.*, **49**, 9648 (2010).
122. A. O'Neil, C. Reichhardt, B. Johnson, P. E. Prevelige and T. Douglas, *Angew. Chem. Int. Ed. Engl.*, **50**, 7425 (2011).
123. D. P. Patterson, P. E. Prevelige and T. Douglas, *ACS Nano*, **6**, 5000 (2012).
124. D. P. Patterson, B. Schwarz, K. El-Boubbou, J. van der Oost, P. E. Prevelige and T. Douglas, *Soft Matter*, **8**, 10158 (2012).
125. D. P. Patterson, B. Schwarz, R. S. Waters, T. Gedeon and T. Douglas, *ACS Chem. Biol.*, **9**, 359 (2014).
126. D. P. Patterson, A. Rynda-Apple, A. L. Harmsen, A. G. Harmsen and T. Douglas, *ACS Nano*, **7**, 3036 (2013).
127. P. C. Jordan, D. P. Patterson, K. N. Saboda, E. J. Edwards, H. M. Miettinen, G. Basu, M. C. Thielges and T. Douglas, *Nat. Chem.*, **8**, 179 (2016).
128. A. D. Lawrence, S. Frank, S. Newnham, M. J. Lee, I. R. Brown, W. F. Xue, M. L. Rowe, D. P. Mulvihill, M. B. Prentice, M. J. Howard and M. J. Warren, *ACS Synth. Biol.*, **3**, 454 (2014).
129. B. Worsdorfer, Z. Pianowski and D. Hilvert, *J. Am. Chem. Soc.*, **134**, 909 (2012).
130. I. J. Minten, V. I. Claessen, K. Blank, A. E. Rowan, R. J. M. Nolte and J. J. L. M. Cornelissen, *Chem. Sci.*, **2**, 358 (2011).
131. I. J. Minten, L. J. Hendriks, R. J. Nolte and J. J. Cornelissen, *J. Am. Chem. Soc.*, **131**, 17771 (2009).
132. C. Koch, K. Wabbel, F. J. Eber, P. Krolla-Sidenstein, C. Azucena, H. Gliemann, S. Eiben, F. Geiger and C. Wege, *Front Plant Sci.*, **6**, 1137 (2015).
133. J. M. Domínguez-Vera, N. Gálvez, P. Sánchez, A. J. Mota, S. Trasobares, J. C. Hernández and J. J. Calvino, *Eur. J. Inorg. Chem.*, **2007**, 4823 (2007).
134. O. Kasyutich, A. Ilari, A. Fiorillo, D. Tatchev, A. Hoell and P. Ceci, *J. Am. Chem. Soc.*, **132**, 3621 (2010).

135. Y. Shin, A. Dohnalkova and Y. Lin, *J. Phys. Chem. C*, **114**, 5985 (2010).
136. S. Liu, G. Chen, P. N. Prasad and M. T. Swihart, *Chem. Mater.*, **23**, 4098 (2011).
137. K. M. Bromley, A. J. Patil, A. W. Perriman, G. Stubbs and S. Mann, *J. Mater. Chem.*, **18**, 4796 (2008).
138. E. S. Royston, A. D. Brown, M. T. Harris and J. N. Culver, *J. Colloid Interface Sci.*, **332**, 402 (2009).
139. M. A. Kostiainen, P. Hiekkataipale, A. Laiho, V. Lemieux, J. Seitsonen, J. Ruokolainen and P. Ceci, *Nat. Nanotechnol.*, **8**, 52 (2013).
140. G. M. Whitesides and B. Grzybowski, *Science*, **295**, 2418 (2002).
141. S. Zhang, *Nat. Biotechnol.*, **21**, 1171 (2003).
142. M. A. Kostiainen, O. Kasyutich, J. J. Cornelissen and R. J. Nolte, *Nat. Chem.*, **2**, 394 (2010).
143. M. S. Kim, K. M. Shin, S. I. Kim, G. M. Spinks and S. J. Kim, *Macromol. Rapid Commun.*, **29**, 552 (2008).
144. G. Konstantinos, M. Matthew, R. Elizabeth, N. C. James and G. Reza, *J. Micromech. Microeng*, **18**, 104003 (2008).
145. E. Royston, A. Ghosh, P. Kofinas, M. T. Harris and J. N. Culver, *Langmuir*, **24**, 906 (2008).
146. Y. J. Lee, H. Yi, W. J. Kim, K. Kang, D. S. Yun, M. S. Strano, G. Ceder and A. M. Belcher, *Science*, **324**, 1051 (2009).
147. N. L. Ritzert, S. S. Casella and D. C. Zapien, *Electrochem. Commun.*, **11**, 827 (2009).
148. S. S. Casella, N. L. Ritzert and D. C. Zapien, *Electroanalysis*, **21**, 811 (2009).
149. M. Tominaga, K. Miyahara, K. Soejima, S. Nomura, M. Matsumoto and I. Taniguchi, *J. Colloid Interface Sci.*, **313**, 135 (2007).
150. M. Tominaga, K. Soejima and I. Taniguchi, *J. Electroanal. Chem.*, **617**, 78 (2008).
151. M. Tominaga, K. Soejima, M. Matsumoto and I. Taniguchi, *J. Electroanal. Chem.*, **579**, 51 (2005).
152. M. Tominaga, A. Ohira, Y. Yamaguchi and M. Kunitake, *J. Electroanal. Chem.*, **566**, 323 (2004).
153. T. Masato and T. Isao, *Chem. Lett.*, **32**, 954 (2003).
154. K. C. Martin, S. M. Villano, P. R. McCurdy and D. C. Zapien, *Langmuir*, **19**, 5808 (2003).
155. T. D. Martin, S. A. Monheit, R. J. Niichel, S. C. Peterson, C. H. Campbell and D. C. Zapien, *J. Electroanal. Chem.*, **420**, 279 (1997).
156. B. L. Scott and D. C. Zapien, *Electroanalysis*, **22**, 379 (2010).
157. M.-S. Pyon, R. J. Cherry, A. J. Bjornsen and D. C. Zapien, *Langmuir*, **15**, 7040 (1999).
158. D. C. Zapien and M. A. Johnson, *J. Electroanal. Chem.*, **494**, 114 (2000).
159. Y. S. Nam, A. P. Magyar, D. Lee, J. W. Kim, D. S. Yun, H. Park, T. S. Pollom, Jr., D. A. Weitz and A. M. Belcher, *Nat. Nanotechnol.*, **5**, 340 (2010).
160. M. B. Dickerson, K. H. Sandhage and R. R. Naik, *Chem. Rev.*, **108**, 4935 (2008).
161. D. N. Woolfson and Z. N. Mahmoud, *Chem. Soc. Rev.*, **39**, 3464 (2010).
162. R. V. Ulijn and D. N. Woolfson, *Chem. Soc. Rev.*, **39**, 3349 (2010).
163. E. F. Banwell, E. S. Abelardo, D. J. Adams, M. A. Birchall, A. Corrigan, A. M. Donald, M. Kirkland, L. C. Serpell, M. F. Butler and D. N. Woolfson, *Nat. Mater.*, **8**, 596 (2009).
164. F. Boato, R. M. Thomas, A. Ghasparian, A. Freund-Renard, K. Moehle and J. A. Robinson, *Angew. Chem. Int. Ed. Engl.*, **46**, 9015 (2007).
165. S. Raman, G. Machaidze, A. Lustig, U. Aebi and P. Burkhard, *Nanomedicine*, **2**, 95 (2006).
166. Y. Yang, P. Ringler, S. A. Muller and P. Burkhard, *J. Struct. Biol.*, **177**, 168 (2012).
167. M. Gerstmayr, N. Ilk, I. Schabussova, B. Jahn-Schmid, E. M. Egelseer, U. B. Sleytr, C. Ebner and B. Bohle, *J. Immunol.*, **179**, 7270 (2007).
168. N. P. King, J. B. Bale, W. Sheffler, D. E. McNamara, S. Gonen, T. Gonen, T. O. Yeates and D. Baker, *Nature*, **510**, 103 (2014).
169. N. P. King, W. Sheffler, M. R. Sawaya, B. S. Vollmar, J. P. Sumida, I. Andre, T. Gonen, T. O. Yeates and D. Baker, *Science*, **336**, 1171 (2012).
170. J. B. Bale, R. U. Park, Y. Liu, S. Gonen, T. Gonen, D. Cascio, N. P. King, T. O. Yeates and D. Baker, *Protein Sci.*, **24**, 1695 (2015).
171. Y. T. Lai, D. Cascio and T. O. Yeates, *Science*, **336**, 1129 (2012).
172. Y. T. Lai, E. Reading, G. L. Hura, K. L. Tsai, A. Laganowsky, F. J. Asturias, J. A. Tainer, C. V. Robinson and T. O. Yeates, *Nat. Chem.*, **6**, 1065 (2014).
173. J. E. Padilla, C. Colovos and T. O. Yeates, *Proc. Natl. Acad. Sci. USA*, **98**, 2217 (2001).

Eun Jung Lee is an Assistant Professor in the Department of Chemical Engineering at Kyungpook National University in Korea. She received her B.S. (2007) and Ph.D. (2014) degree in Chemical and Biological Engineering at Korea University. She was a research professor in the Institute of Clean Chemical Engineering Systems at Korea University and a postdoctoral fellow in the Biomedical Research Institute at the Korea Institute of Science and Technology (KIST) before joining Kyungpook National University in 2017. Her research interests include nano-biomaterials, protein-based nanoparticle and hydrogel, nanomedicine, biosensor, drug delivery and cancer immunotherapy.