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# Protein nanoparticles as multifunctional biocatalysts and health assessment sensors

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The use of protein nanoparticles for biosensing, biocatalysis and drug delivery has exploded in the last few years. The ability of protein nanoparticles to self-assemble into predictable, monodisperse structures is of tremendous value. The unique properties of protein nanoparticles such as high stability, and biocompatibility, along with the potential to modify them led to development of novel bioengineering tools. Together, the ability to control the interior loading and external functionalities of protein nanoparticles makes them intriguing nanodevices. This review will focus on a number of recent examples of protein nanoparticles that have been engineered toward imparting the particles with biocatalytic or biosensing functionality.

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## Introduction

Protein nanoparticles (PNPs) are made of multiple protein-based building blocks assembled either by protein–protein or protein–nucleic acid interactions [1]. It is well known that immobilization of functional biomolecules either onto the exterior or the inside of PNPs improves their stability and activity [2]. This feature when combined with the ability of PNPs to tolerate extreme conditions make PNPs an ideal platform for a wide range of bionanotechnology applications in biomedicine [2–4] and biocatalysis [5,6].

Although most PNPs used today are based on biologically evolved protein assemblies such as viral capsids [5,7] or multimeric enzymes [6,8], there is a move toward engineering these protein nano-assemblies based on *in silico* design [9,10••]. The ability to provide multi-functionalization is often highly challenging as the modifications may result in a loss of nanoparticle self-assembly or protein functionality. In this review, we will discuss recent progress in PNP functionalization and their corresponding applications in biocatalysis and biomedical diagnostics and imaging. Table 1 summarizes the different types of PNPs reviewed in this paper and their corresponding applications.

## Virus-like particles

Virus-like particles (VLPs) are naturally occurring multimeric PNPs derived from viruses. Unlike intact viruses, VLPs are derived only from the noninfectious protein capsids and provide the benefit of mass-production using simple, recombinant DNA technology [11]. Similar to other protein-based materials, VLPs can be modified with a wide range of cargos such as drugs, enzymes, or other biomolecules using either genetic manipulation, post-translational conjugation, and/or encapsulation [5,12]. Traditionally, modified VLPs have been widely used for vaccine design and drug delivery because of their biocompatibility and stability [7]. However, efforts toward the use of engineered VLPs as nanoscale bioreactors are gaining traction due to their ability to tolerate harsh conditions [13,14].

In addition to direct tethering, biomolecular cargos can also be encapsulated within some VLPs by exploiting their ability to undergo a conformational change upon exposure to thermal or pH variations. One example is the bacteriophage P22, which self-assembles into a 58 nm capsid based on the interaction between the coat proteins (CP) and a helix-turn motif on the scaffold proteins (SP) [12]. Heating of P22 VLPs results in an irreversible change in the structure as some of the SP subunits are released, leading to an expanded shell of 64 nm. Further heating to 75 °C results in the formation of a 'Wiffleball' structure with an almost two-fold increase in the effective volume and multiple 10 nm holes within the icosahedral structure (Figure 1a) [12]. Cowpea Chlorotic Mottle Virus (CCMV) is a second VLP that offers reversible assembly and allows facile incorporation of cargos to the inside based on electrostatic interactions between the positively charged N-terminus and the negatively charged cargo. The assembly/disassembly pathway can be easily controlled by pH and ionic strength (Figure 1b) [16].

**Table 1**

**Size and origin of protein nanoparticles in this review**

| Protein cage | Species | Shape | Size (nm) | Applications | References |
|---|---|---|---|---|---|
| *Viral-like particles (VLPs)* | | | | | |
| P22 | *Salmonella typhimurium* | Icosahedral | 58–64 | Biocatalysis | [12,49•,50,51] |
| Qβ | *E. coli* | Icosahedral | 28 | Biosensor<br>Delivery | [14,18,39,58] |
| AP205 | *Acinetobacter* species | Icosahedral | 20 | Vaccine | [15] |
| CCMV | Cowpea plant | Icosahedral | 28 | Biocatalysis | [16,54,61] |
| HBV | Human | Icosahedral | 30–34 | Biosensor<br>Vaccine<br>Delivery<br>MR Imaging | [17,41,43,45,46,53,59] |
| Fd | *E. coli* | Filamentous | 7 × 880 | Biosensor | [35] |
| MS2 | *E. coli* | Icosahedral | 27 | Biosensor | [38] |
| TMV | Tobacco plant | Rod | 18 × 300 | Biosensor | [40,44,62] |
| M13 | *E. coli* | Filamentous | 7 × 880 | Biosensor | [42] |
| *Enzyme-derived PNPs* | | | | | |
| E2 | *Bacillus stearothermophilus* | Spherical | 24 | Biocatalysis<br>Biosensor<br>Delivery | [6,22,23,37] |
| Lumazine synthase | *Aquifex aeolicus* | Spherical | 15.4 | Biocatalysis<br>Biosensor | [8,21,24,25,55,56] |
| Ferritin | *Human* | Spherical | 12 | Delivery<br>MR Imaging | [20,28,57] |
| Ferritin | *E. coli* | Spherical | 12 | Photothermal<br>Therapy | [26,60] |
| Ferritin | *Pyrococcus furiosus* | Spherical | 12 | Biosensor<br>MR Imaging | [27] |
| Encapsulin | *Mycobacterium tuberculosis* | Spherical | 22 | Biocatalysis | [29] |
| Encapsulin | *Thermotoga maritime* | Spherical | 24 | Biosensor<br>Delivery | [30,36] |
| *Re*encapsulin | *Rhodococcus erythropolis* | Spherical | 28 | Biosensor | [31] |
| Pdu BMC | *Citrobacter freundii* | Spherical | 100–150 | Biocatalysis | [52,63] |
| *Synthetic PNPs* | | | | | |
| O3-33 | Synthetic | Octahedral | 13 | TBD | [9] |
| T3-10 | Synthetic | Tetrahedral | 11 | TBD | [9] |
| I53 | Synthetic | Icosahedral | 24–40 | TBD | [10••] |
| I52 | Synthetic | Icosahedral | 24–40 | TBD | [10••] |
| I32 | Synthetic | Icosahedral | 24–40 | TBD | [10••] |
| CCMV-avidin crystal | CCMV and avidin hybrid | Body-centered cubic | Unit cell: 35 | TBD | [32] |
| ATC cage | Hybrid from *E. coli* components | Spherical | 22.5 | TBD | [33] |

Some VLPs have the benefit of incorporating functional cargos into their interior through direct genetic fusions to termini oriented toward the interior, as in the case of Hepatitis B Virus (HBV) [17]. Another example is the bacteriophage Qβ, which utilizes the natural interaction between the RNA genome and the capsid for cargo encapsulation [13,18].

In addition to surface functionalization and cargo loading, the ability to immobilize PNPs onto surfaces has gained interest for biosensing applications. In the case of Tobacco mosaic virus (TMV), the 5′-end of TMV nanorods can be partially disassembled at neutral pH to expose its mRNA, which can be used for docking TMVs to hydrogel surfaces *via* DNA hybridization [19]. Additional functionalization was achieved by using bioorthogonal tetrazine (Tz)-trans-cyclooctene (TCO) 'click' chemistry to selectively attach R-Phycoerythrin (R-PE) to TMV nanorods immobilized on hydrogel microparticles. This technology allows higher capacity of protein conjugation to the surface of a hydrogel for improved and controlled protein conjugation and biosensing applications.

## Enzyme-derived PNPs

Another class of PNPs that is gathering more attention is based on multimeric enzymes that naturally self-assemble into nanocages of varying sizes [20,21,22•]. These nanostructures provide naturally evolved, confined reaction spaces for enhanced cellular metabolism and are ideal candidates as nanoreactors. The E2 core from the pyruvate dehydrogenase complex of *Bacillus stearothermophilus* is a frequently used PNP because of its thermophilic origin [23]. E2 is composed of 60 identical monomers that self-assemble into a highly stable cage-like structure with an outer diameter of ~24 nm [23]. The thermostability of E2 nanocages allows for simple purification by heating to