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Caged protein nanoparticles for drug delivery

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Caged protein nanoparticles possess many desirable features for drug delivery, such as ideal sizes for endocytosis, non-toxic biodegradability, and the ability to functionalize at three distinct interfaces (external, internal, and inter-subunit) using the tools of protein engineering. Researchers have harnessed these attributes by covalently and non-covalently loading therapeutic molecules through mechanisms that facilitate release within specific microenvironments. Effective delivery depends on several factors, including specific targeting, cell uptake, release kinetics, and systemic clearance. The innate ability of the immune system to recognize and respond to proteins has recently been exploited to deliver therapeutic compounds with these platforms for immunomodulation. The diversity of drugs, loading/release mechanisms, therapeutic targets, and therapeutic efficacy are discussed in this review.

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Introduction

Nanoparticles have the potential to address important issues related drug delivery, such as (i) reducing drug toxicity, (ii) protection from drug degradation/sequestration, (iii) increasing circulation times, (iv) targeting, and (v) increasing bioavailability [1–3]. Key particle variables, including size, surface charge, geometry, and the susceptibility to opsonization, all affect pharmacokinetic profiles [4]. Additionally, small size distributions of the nanomaterial allow for uniformity in drug dosing, with the size range of ~25–50 nm being optimal for receptor-mediated endocytosis (RME) and membrane wrapping kinetics of target cells [5]. Also crucial for drug delivery success using nanoparticles is the capability to functionalize with multiple elements in a uniform and precise manner.

Conventional materials investigated for drug delivery include synthetic polymeric and liposomal nanoparticles [3]. These, however, may have limitations such as wide size distributions, difficulty in site-specific functionalization, low drug loading, and instability. Caged proteins represent a class of nanomaterial that may address many of these concerns [2,6–8]. Since the earliest example in drug delivery [9], advances have been made in understanding the architecture, assembly, physical properties, and biomedical applicability of these nanoparticulate systems. Recent progress towards drug delivery using caged protein scaffolds will be covered in this review.

Advantages of caged protein nanoparticles for drug delivery

Caged protein complexes are hollow structures comprised of self-assembling protein subunits that produce nanocapsules with a nearly monodisperse size distribution. The individual asymmetrical subunits may comprise a single protein, as with pyruvate dehydrogenase E2 [10], or multiple proteins, such as with cowpea mosaic virus (CPMV) [11]. Typical sizes of protein nanocages range from 10 to 100 nm, and they display repetitive symmetrical elements, both of which are ideal structural elements for RME [5].

Protein cages are often produced in living hosts (e.g. *Escherichia coli*, plants, mammalian cells), and therefore the tools of protein engineering may be applied to impart functional elements at three distinct interfaces (i.e. internal, external, and inter-subunit) [7]. This permits fine control over surface charge, drug encapsulation, ligand display, and particle stability. Classes of protein cages used in drug delivery include those derived from viruses, enzymes, the ferritin superfamily, and heat shock proteins [6,7]. Although virus-like particles (VLPs) have found early applications as vehicles for gene therapy [12] and vaccines for infectious agents [13], this review will focus on delivery of therapeutic drugs.

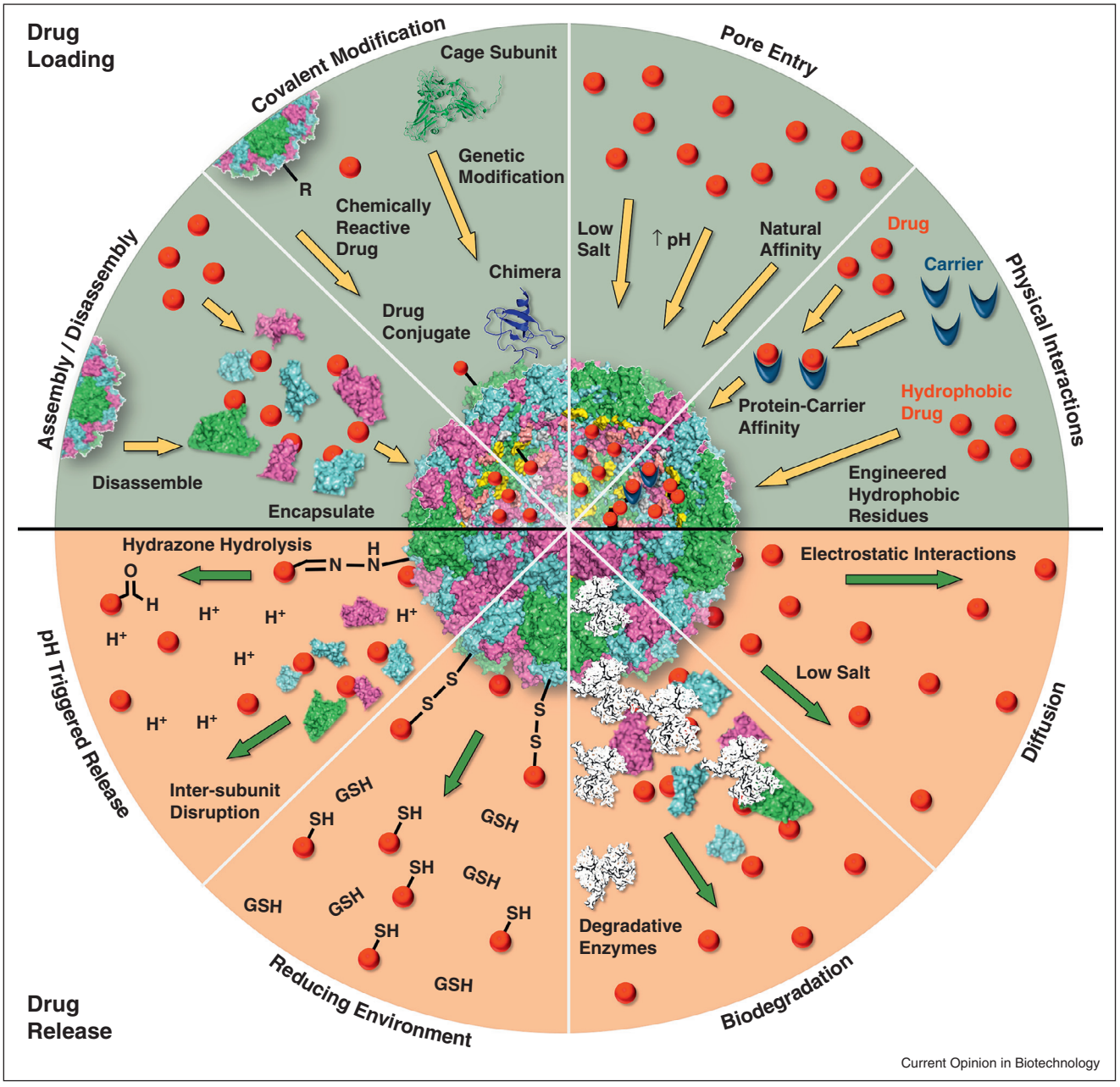
Drug loading and release

Key aspects in nanoparticle drug delivery technologies are the containment and release of drugs from within the particle, and these mechanisms are often related. The strategies available for a particulate system are dictated by its structure and dynamics, the type of drug loaded, and environment the nanoparticle is expected to encounter. Many avenues exist for functionalizing a protein cage, and the primary approaches are described below (Figure 1).

Protein engineering of nanoparticles

One main advantage of using protein nanoparticles over other systems is the fine precision afforded by genetic engineering of functional sites at distinct locations on the

Figure 1



Summary of methods explored for drug loading (upper panel) and drug release (lower panel) in protein nanoparticle cages. R: any reactive amino acid side chain; GSH: glutathione. Representative protein cage scaffold is cowpea chlorotic mottle virus (PDB code 1CWP, assembly from VIPERdb; http://viperdb.scripps.edu/).

nanoparticles, such as introduction of non-native amino acids [8]. For instance, a single cysteine point mutation introduces an exact number of new attachment sites, providing unique positions for drug conjugation and additional control in loading amounts [10]. The physicochemical character of the nanoparticle's hollow interior cavity can also be re-engineered to accommodate non-native hydrophobic [14•] or charged molecules [15•]. Knowledge of the protein crystal structure allows recombinant incorporation of peptides and/or entire proteins as N-terminal or C-terminal chimeras or within loop regions [16–18,19••,20]. Recombinant incorporation of peptide/protein therapeutics may prove to be one of the most effective loading mechanisms; once the genetic code for