Protein micro- and nano-capsules for biomedical applications†

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Micro- and nano-scale systems have emerged as important tools for developing clinically useful drug delivery systems. In this tutorial review, we discuss the exploitation of biomacromolecules for this purpose, focusing on proteins, polypeptides, nucleic acids and polysaccharides and mixtures thereof as potential building blocks for novel drug delivery systems. We focus on the mechanisms of formation of micro- and nano-scale protein-based capsules and shells, as well as on the functionalization of such structures for use in targeted delivery of bioactive materials. We summarise existing methods for protein-based capsule synthesis and functionalization and highlight future challenges and opportunities for delivery strategies based on biomacromolecules.

Key learning points
(1) Many therapeutically active compounds cannot be delivered using conventional strategies due to undesired pharmacological properties and/or interactions with cellular components during their delivery.
(2) To address this challenge, micro- and nano-scale systems have emerged as promising delivery systems enabling controlled transport and delivery, with protein-based systems being particularly attractive components in this context.
(3) The fabrication of protein or hybrid protein/polymer micro- and nanocapsules requires control of compartmentalisation on the micro and/or nano-scale. Several approaches are available to achieve this objective including emulsification and ultrasonic emulsification.
(4) A key challenge is to engineer the building blocks assembly pathways to encode the information required for the formation of capsules with desired properties. Chemical synthesis and functionalization approaches can play a key role in this area.
(5) While many challenges remain to fully realise the utility of biopolymer based delivery vehicles, their versatility, biocompatibility and low immunogenicity makes these materials a key addition to the palette of available delivery systems and open up new possibilities for biomedical applications. In addition, it is possible to obtain protein-based particles with a reduced size (<100 nm), as required for many applications, while keeping proteins fully functional and accessible as well as avert protein co-aggregation.

1. Introduction

To address the challenges associated with the controlled delivery of active components for biomedical applications, encapsulation methodologies with a high performance and tenability are required.1 A powerful motivation for this line of research is given by the observation that many clinically relevant drugs cannot be administered using conventional release methods due to unfavourable solubility and toxicology characteristics and inappropriate interactions with other chemical species during delivery.2–4 A class of materials that has emerged as being particularly promising in this context is that of biocompatible polymers, protein capsules and hybrid polymer–protein capsules. These structures consist of a polymeric shell with dimensions on the micro- or nanoscale encapsulating active species in their interior. Multiple synthetic pathways are available to generate these materials,3 and a crucial challenge is the engineering of the precursor components and the assembly pathway to generate capsules with the desired properties.4 For instance, chemical modification of precursor components can lead to several distinct capsule structures. Furthermore, significant structural complexity can be accessed by combining different materials in the core and shell parts of the capsules, for example in the form of hollow protein capsule...
shells, oil-filled protein capsule shells, water-filled protein capsule shells and protein coated nucleic acid particles. The chemical properties of the building blocks can be used to tune the material properties and structure of the final capsules. Protein-based capsules can offer significant advantages over purely synthetic systems, and the use of human proteins as the precursor materials often results in delivery materials with minimal immunogenicity. Several types of protein-based capsules, their derivatives, and other biocompatible protein complexes have gained prominence for the specific delivery of drugs to sites of disease while sparing healthy tissues. Protein modification and/or coupling with other functional molecules as well formulations of different types of proteins can be used and enhance the versatility of this class of materials. For instance, the engineering of functionalized protein capsules, with an external hydrophobic layer, can allow the incorporation of specific functionalities at the surface of the capsule to include specificity to a predetermined target, enhance transdermal transport properties, encode a release switch mechanism or even enhance the biological effect of the active species delivered through the capsules, for example by displaying antigens that will result in an increased immune response in the case of vaccines. The present tutorial review focuses on the synthesis pathways available for the generation of protein capsules with controlled properties. We discuss the most prevalent approaches, their advantages and limitations and highlight the key challenges and future opportunities in this area.

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There are a rich variety of methods described in the literature for the preparation of protein capsules and shells. One of the most widely adopted methods to obtain micro- to nanoscale capsules is based on micro-emulsion technology. This approach builds on the use of two immiscible fluids, commonly an oil phase and an aqueous phase, the former typically carrying the active substance (a hydrophobic substance for encapsulation in the organic phase and a hydrophilic substance for accumulation in the protein shell) to be encapsulated and the latter one carrying the protein precursor molecules used to form the shell. A micro-emulsion formed from these two immiscible phases acts as a template for the synthesis of the capsules around the interface of the phases. This approach allows phase separation and a range of related physical or chemical phenomena to be exploited, including variations in surface tension, repulsive and attractive forces or specific chemical interactions. The properties of the capsules are closely controlled by the sizes and characteristics of the template emulsions, and a wide range of approaches for the controlled generation of micro-emulsions have been exploited. These include bulk single emulsions, bulk double emulsions, polymerization techniques, phase separation coacervation technique, spray drying and spray congealing, solvent extraction, microfabrication, and homogenization and ultrasonic emulsification. These methods result in protein capsules with fundamentally different properties, including size and stability. When devising new synthetic strategies or evaluating existing ones it is key to carefully evaluate the impact of core functionalization on the thermodynamic and kinetic stability of the resulting protein capsules. A significant influence on the stability of the final structures originates from the properties of the materials that are used as precursor components in the synthesis. For emulsification techniques that rely on cavitation, notably sonication-based approaches, sonochemical processes can contribute to the cross-linking of the capsule proteins without the need for specific chemical cross-linkers. In the following section we discuss existing emulsification methods that are used for protein capsule formation.

2. Current methods for protein micro/nanocapsules production

In order to act effectively as delivery agents, protein micro- and nano-capsules should satisfy key criteria in respect of:

(a) Stability of the preparation after synthesis with a life compatible with clinical practice.
(b) Controllable particle size and monodispersity in biological media used for injection.
(c) Biocompatibility and controllable biodegradability as well as controllable release rate of the active reagent.
(d) Targeted delivery to specific sites of disease requires amenability to chemical surface functionalization.

A range of approaches have been developed to fulfil these criteria and are depicted in Fig. 1.

Single and double emulsion techniques

To synthesise micro- or nano-containers from biopolymers by using single emulsions (Fig. 1a), the precursor molecules, commonly proteins, are dissolved or dispersed in the aqueous medium followed by emulsification in a non-aqueous medium such as oil. Next, to ensure structural integrity, chemical or physical cross-linking of the dispersed droplet is carried out (Fig. 1b). The cross linking can be achieved either intrinsically by means of heat, or by using the chemical cross-linkers, including glutaraldehyde, formaldehyde or diacid chloride etc. Intrinsic cross-linking has the advantage of minimizing potential toxicity and undesired reactivity with other chemical components resulting from the presence of chemical cross linkers.

More complex structures can be obtained through the use of double emulsions (Fig. 1c). Similarly to the case of single emulsions, the aqueous protein solution is dispersed in a lipophilic organic continuous phase. The primary single water-in-oil emulsion obtained in this manner is then added to an aqueous solution of a second biopolymer that will then form the outer shell of the capsule. This process hence results in the formation of a double emulsion. The biggest advantage of the capsules created by the double emulsion method over capsules created via the single emulsion method is the possibility of controlling independently the inner and outer surface of the capsule. Capsules created via such a double emulsion method allow encapsulation of water-soluble species and are therefore well suited for the delivery of water-soluble drugs, peptides, proteins and design of vaccines.

Phase separation coacervation technique

Creation of protein capsules of specific sizes can also be achieved by phase separation in solution rather than emulsification. Colloidal systems, including polymer solutions, have the propensity to undergo coacervation where a homogeneous solution phase separates into two liquid phases, one of which is essentially pure solvent, and the other contains the solute. In the context of the creation of protein-based capsules, this phenomenon is exploited based on the principle of decreasing the solubility of the biopolymer (protein) to induce the formation of a protein rich phase, called the coacervates (Fig. 1d). In this method, the drug particles to be delivered are initially dispersed in a solution of the protein and then an incompatible polymer is added to the system leading to the phase separation of the protein and subsequent accumulation of the drug into the protein rich phase. In order to enhance the stability of the capsules formed through coacervation, in some cases, for unstable proteins, a cross-linking agent is used prior to adding the incompatible polymer.

Spray drying and spray congealing

These methods are based on microdroplet spray generation from a solution of the protein and the drug (Fig. 1e). The spray can then undergo solvent removal in spray drying or rapid cooling followed by freeze-drying in the case of spray congealing. One of
the major advantages of the process is the feasibility of operating under aseptic conditions due to the absence of a liquid phase, which could mediate contamination. This process has been used for protein loaded lipid capsules.\textsuperscript{25,26}

**Ultrasonic emulsification technique\textsuperscript{27}**

The creation of protein capsules \textit{via} the ultrasonic emulsification method involves mechanical mixing, by the action of high-intensity ultrasonic waves to a macroscopic oil/water interface. As a result, an oil-in-water emulsion is created in which the protein molecules, initially present in the aqueous phase, localize at the interface of the droplet. The preparation of capsules using ultrasound emulsification from aqueous protein solutions and immiscible hydrophobic solvents is a well-known technique and it has been the subject of recent reviews.\textsuperscript{22,28,29} Those reviews provide comprehensive information about the mechanism of formation of protein capsules.\textsuperscript{27}

The interfaces obtained through the emulsification methods described above can be used to direct polymerization of soluble precursor monomer molecules to form polymeric structures on the surface of the emulsion. This approach (Fig. 1b) is commonly applied to the generation of capsules from synthetic polymers, but also holds great potential in the area of protein-based capsules. Polymerization emulsification can be combined with the emulsification approaches described above, including, suspension, precipitation, emulsion and micellar polymerization processes. An alternative approach to stabilize protein-based capsules is to use them as templates to direct the polymerization of the synthetic polymers on their surface, leading to a coating that enhances the robustness of the capsules.
Microfabrication method

Monodispersed emulsions can be formed using the microfabrication method, which enables precise control of the particle size at micro/nano scale dimensions. The technique enables manipulation of ultralow interfacial tensions at the surface of emulsion droplets that results in a highly monodisperse emulsion. One of the most attractive features of the microfabrication method is precise manipulation of emulsion composition.

3. Properties of protein capsules

3.1 Size

The size of the capsules is a key parameter that affects the ability of such particles to interface with biological systems and influences the rate at which species can be released from protein capsules. In methods involving emulsification, the properties of the oil–water interface, including surface tension and viscosity, can be used to tune the size of the droplets which are used to template the capsules. In cases where the hydrophobic phase remains encapsulated in the final structure, its level of hydrophobicity can also influence the stability of the capsule. For instance it has been found that food oils yield more stable particles than dodecane, which is more hydrophobic. Furthermore, intrinsic differences between high viscosity food oils such as olive oil and low viscosity oils such as soya bean oil can be used to tune the size of the resulting capsules: capsules generated from a food oil with higher viscosity values leads to an increase in the size of the protein capsules, but commonly also reduces their stability and fraction of protein incorporated in such capsules.

A further parameter of interest is the ratio of hydrophobic solvent to water in the precursor mixture. Low fractions of the hydrophobic phase to the aqueous phase yield smaller capsules, which are less stable relative to bigger capsules formed with a higher organic solvent fraction. Moreover, when the capsules are used to carry a drug like small molecule, the chemical and physical nature of the encapsulated drug can affect not only the capsules' sizes, but also the hydrophobicity of the internal part of the capsule. For example, the size dependence of BSA protein (Bovine Serum Albumin) capsules has been shown to be affected by the length of encapsulated RNA molecules. These changes in size are shown in Fig. 3a. The results demonstrate that the size of the RNA loaded capsules increases with an increase of the number of nucleotides in the RNA chain, i.e. with an increase in the size of the encapsulated molecule. In parallel, the hydrophilicity of the inner part of the protein capsule increases upon encapsulation of a hydrophilic molecule such as RNA. For studies of changes in the environment within capsules, environmentally sensitive dyes such as Nile Red have proven to be very valuable due to their ability to change their fluorescence spectrum upon the change in solvent polarity. The red signal, indicating an hydrophilic environment, emits from the shell of unloaded capsules (Fig. 3b, top image). As soon as the protein spheres were loaded with a hydrophilic RNA molecule, the inner environment became more hydrophilic, and a red-shifted

![Fig. 2](image1.png)

**Fig. 2** Schematic representation of size and stability studies for protein aqueous microspheres as a function of peptide sequence, size of amphiphilic peptides, aqueous/organic solvent ratio and type of organic solvent. The scheme and microscopic image of a protein aqueous microsphere is shown in the left corner. Scheme: the protein is localised at the water/oil interface with hydrophobic residues (green) towards the oil phase and hydrophilic (red) towards the water. Microscopy image: Nile Red coloured protein microsphere filled with oil (green) in its inner part and protein (red) forming the shell of the sphere. Schematic representation based on data from reference.27

![Fig. 3](image2.png)

**Fig. 3** Length of encapsulated RNA influences the size of protein capsules. (a) Graphic represents size dependence of the protein capsules as a function of the length of encapsulated RNA chain. (b) Fluorescent microscopy images of Nile Red colored BSA spheres. Unloaded spheres are presented on the top and RNA loaded on the bottom side. (c) 3D confocal image of a fitc-BSA sphere loaded with Cy3RNA molecules. Scale bars = 1 micrometer.
emission signal of Nile Red dye, indicating a hydrophilic environment, was observed from the shell as well as from the inner part of the capsules (Fig. 3b, bottom image).

Furthermore, capsules can be formed from a mixture of two different proteins.\(^{12}\) Unlike in the case of capsule formation from a mixture of nucleic acids and proteins, where RNA loaded BSA spheres were created, the mixture of two different proteins forms uniform protein capsules where two proteins co-localize in the shell of the capsule.

An additional approach that enables control of the size of protein-based particles has been recently reported.\(^{33}\) Incorporation of PEGylated surfactants (polyethylene glycol-based surfactants) into BSA based protein particles leads to decrease in particle size from 200 to 100 nm. Particle formation in the presence of PEGylated surfactants is physically restricted by the steric effect of the polymer (PEG) resulting in decrease in the average protein particle size.

### 3.2 Stability

In general, an emulsion of oil and water, once formed, can be unstable with respect to separation into two bulk phases, a reflection of the fact that oil and water notoriously do not mix. The stability of such emulsions is dependent on the protein sequence and size, and may be enhanced by the presence of surfactant molecules that are added to the precursor mixture. Proteins are amphoteric enough to localize at the oil–water interface and stabilize the emulsion, leading to an increase in the lifetime of un-cross-linked oil-filled protein spheres and preventing the droplets from merging and reforming two bulk phases. Disulfide bonding has been thought to be the most prevalent contributor to protein capsule stability by formation of cross-linking between proteins. However, a recent report shows that the replacement of cysteine residues involved in disulfide bonding by hydrophobic residues can result in stable capsules (Fig. 2).\(^{27}\) Thus, stabilization of protein capsules may also be achieved through a hydrogen network formed at hydrophobic residues instead and/or in addition to covalent cross-linking. In addition, the length of amphiphilic peptides determines the final size and stability of the resulting protein capsules. A longer amphiphilic peptide yields a larger and more stable protein aqueous sphere (Fig. 2).\(^{27}\) Furthermore, it has been found that the inner hydrophobic parts when exposed to the oil interface may lead to a change of protein conformation, which results in enhanced capsule stability (Fig. 2).\(^{27}\)

The stability of mixed protein capsules can also be enhanced through the addition of a biocompatible synthetic polymer to the mixture of proteins. For example, the silk fibroin capsules, prepared by the phase separation method, were shown to be stabilised by polyvinylalcohol polymer (PVA).\(^{34}\) This stabilization can in part originate from the change in the secondary structure observed for silk fibroin following the addition of PVA. A further agent that has been shown to stabilize protein capsules is polyethylene glycol (PEG).\(^{15}\) In addition to the enhanced stability, the interactions of the capsules with biological systems are modified by pegylation. In particular, such capsules are often less immunogenic, resulting in the blocking and delaying of the first step in the phagocytic process, opsonisation. Thus, the pegylated capsule shells have an increased half-life in blood circulation; this effect has been observed to reach up to several orders of magnitude. A representative list of protein stabilizers is given in Table 1.

In view of their applications as delivery systems, an important parameter of interest of protein capsules is their ability to

### Table 1 List of main protein stabilizers

<table>
<thead>
<tr>
<th>Type of protein stabilizers</th>
<th>Protein stabilizers</th>
<th>Function</th>
<th>Protein storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoprotectants</td>
<td>DMSO, Ethylene glycol, Glycerol, 2-Methyl-2,4-pentadiol (MPD), Propylene glycol, Sucrose</td>
<td>Prevent formation of ice crystals</td>
<td>–20 °C</td>
</tr>
<tr>
<td>Antimicrobial agent</td>
<td>Sodium azide, Ethylenediaminetetraacetic acid (EDTA)</td>
<td>Inhibit microbial growth in protein solutions</td>
<td>4 °C</td>
</tr>
<tr>
<td>Metal chelators</td>
<td>Orthophenanthroline</td>
<td>Inhibit metal-induced oxidation of free thiols helping in maintaining the protein in a reduced state</td>
<td>4 °C</td>
</tr>
<tr>
<td>Reducing agents</td>
<td>Dithiothreitol (DTT), 2-Mercaptoethanol, Tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP)</td>
<td>Maintain the protein in a reduced state</td>
<td>–20 °C, 4 °C</td>
</tr>
</tbody>
</table>
interact with biological systems and in particular their biocompatibility. A key objective, therefore, is to develop chemistry, which is compatible both with the structural requirements as well as minimizing the toxicity of the capsules. The use of potentially toxic covalent cross-linkers, for example, will lead to capsule stabilization but, at the same time, can induce an increase in toxicity of the resulting capsule. In order to circumvent this limitation, for emulsification methods such as template based polymerization, suspension polymerization, etc., that commonly require the use of toxic chemical cross-linkers, the toxicity can be alleviated by removal of the unreacted cross-linkers post synthesis, leading in many cases to a reduction in toxicity of the protein capsules. This additional step is not required for synthesis methods that do not involve chemical cross-linkers, including double emulsification, solvent extraction, and ultrasonic emulsification. In such cases, capsules can be stabilized by native cross-linking in the form of covalent bonds in inter-molecular disulfide bonds between two cysteine residues in adjacent protein molecules or hydrogenic forces, or hydrogen bonding networks, that are strong enough to hold entire 3D protein capsules intact. The methods that lead to creation of such a native supportive system for the protein capsules are therefore of great interest due to the inherent non-toxicity and biocompatibility. In order to favor the creation of such native cross-linking networks some form of activation is commonly required. In the case of ultrasonic emulsification, the activation originates from the presence of superoxide radicals formed upon the collapse of cavitation bubbles, favoring S-S bond creation; a further effect of sonication is enhanced mass-transfer from the flows generated by exposure to ultrasound. The activation as well as capsule formation occurs in a one-step sonochemical reaction.

A summary of the different emulsification methods that have been used for the synthesis of different types of protein capsules and their basic properties are presented in Table 2. Many of the capsules synthesized by emulsification methods are found to be compatible with a role as delivery vehicles and in many cases are either less toxic or non-toxic. Remarkable differences from one emulsification method to another are found in the capsules’ stability and average size (Table 2).

The use of “free of cross-linkers” methods (methods that do not involve the use of chemical cross-linkers) is relatively new in the field of interfaces. Thus, it is of great importance to unveil the mechanism of protein capsules formation that does not use cross-linkers, in particular for those capsules formed using sonochemical procedures. The detailed mechanism of formation of protein capsules is discussed below.

4. Mechanism and structural changes accompanying the formation of protein capsules

In order to optimize rationally the properties of protein capsules, the mechanism leading to their formation is required. A significant effort has recently been focused in particular on the formation of cross linker free capsules, especially in the case of capsules that were formed sonochemically.

Insights into the structural changes in the protein following capsule formation have been gained by Fourier Transform Infra-Red spectroscopy (FTIR) and molecular dynamic simulations.

These studies showed that protein molecules accumulate at the oil/water interface and undergo conformational changes in their secondary and tertiary structures. In particular, an increase of β-sheet content was found in silk fibroin microspheres while molecular simulation has shown a twist of globular 3D structure (see protein conformation images on the top of the chart – Fig. 4). The proteins are able to adjust their globular structures upon binding to the interface by orienting the hydrophobic residues towards the organic phase and the hydrophilic residues towards water; this transition results in a new energetically favorable 3D structure. The increase in β-sheet content of the protein due to the ultrasonic spheridization is reminiscent of amyloid-like behaviour, when protein molecules aggregate to form densely hydrogen-bonded fibrillar structures.

The protein conformations observed within the β-sheet rich nanospheres might therefore have structural analogies with bona fide amyloid fibrils. The possibility to form protein capsules from proteins like silk fibroin and cysteine-free peptides indicates the presence of further driving forces in addition to covalent cross-linking, namely hydrophobic, electrostatic interactions and enhanced mass transport, induced by ultrasound in liquid media.

Interestingly, this approach is not restricted to proteins. Indeed, this strategy has been successfully used for the formation of nanospheres made of nucleic acids, DNA and RNA, where the stabilization of the spheres was proposed to be due to hydrogen bonding, hydrophobic and electrostatic interactions stabilized by counter ions (commonly Ca2+, Na+ and Mg2+).

The role of radicals in covalent cross-linking during ultrasonic capsule formation was studied by preventing the creation of radicals in the reaction cell through performing the synthesis under an inert (Ar) atmosphere. In all three cases where these phenomena were probed for proteins as well as for nucleic acid capsules, it was observed that capsules were successfully formed even under an inert atmosphere and without the presence of disulfide residues. These results demonstrated that capsule stabilization can occur even in the absence of covalent cross-linking. The formation of a hydrogen bonding network connecting proteins in the capsule can be promoted by organic solvents.

In this work, the formation of a core–shell structure made of Zein protein induced by the presence of citral (3,7-dimethyl-2,6-octadienal) in the precursor mixture was reported. The solvent ratio of binary solvents (ethanol 60–90% and citral) affects the formation of core–shell structures. It was shown that solvent polarity affected the ability of core material (zein protein) to form small diameter droplets inside the shell. The solvent with relatively high polarity (60% aqueous ethanol) increased interfacial tension, leading to solidification of entire zein spheres structure with no core. By contrast, low polarity of the solvent (90% aqueous ethanol) decreased interfacial tension, and allowed separation of the core material from
shell material, thus leading to zein core–shell structure formation.

The phenomenon of formation of hydrogen bonding networks makes the incorporation of two different proteins in a single capsule shell structure possible. The evidence for the presence of a dense network of hydrogen bonds was reported for the BSA-silk fibroin capsules.44 The protein capsule shell made of a mixture of BSA and silk fibroin proteins showed the characteristic FTIR absorption bands of the two proteins with spectra of varying intensities due to the composition and interactions of different groups of proteins, confirming the blending of the two proteins.

Another evidence of hydrophobic interactions in protein capsule shell structure was reported by Subirade and co-workers.45 The whey protein granular capsule shells were stabilized by adding calcium (Ca) alginate to the precursor protein solution. The strong hydrophobic interactions created between hydrophobic side-chains of whey protein buried in the core of the whey sphere and hydrophilic side chains facing alginate resulted in enhanced stabilization of protein capsule structure.

5. Incorporation of functionalities at the surface of the protein capsules

Tailoring of the surface chemistry of protein capsules allows for control over the pathway and targeting of the protein capsules inside the living systems as well as modification of the immunogenicity of the particles.46 However, incorporating specific surface modifications into the protein emulsions is challenging.

<table>
<thead>
<tr>
<th>Protein class</th>
<th>Protein type</th>
<th>Emulsification method</th>
<th>Stability</th>
<th>Capsules’ size</th>
<th>Toxicity</th>
<th>Compatibility as a “drug carrier”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globular</td>
<td>Bovine Serum Albumin (BSA)</td>
<td>Template based polymerization</td>
<td>High</td>
<td>200 nm–5 μm</td>
<td>Low</td>
<td>Compatible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coacervation</td>
<td>High</td>
<td>150–200 µm</td>
<td>Non-toxic</td>
<td>Compatible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single/Double emulsification</td>
<td>High</td>
<td>200 nm–1 µm</td>
<td>Non-toxic</td>
<td>Compatible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ultrasonic emulsification</td>
<td>High</td>
<td>100 nm–3 µm</td>
<td>Non-toxic</td>
<td>Compatible</td>
</tr>
<tr>
<td>Globular</td>
<td>Casein</td>
<td>Coacervation</td>
<td>High</td>
<td>150–200 µm</td>
<td>Non-toxic</td>
<td>Compatible</td>
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<td></td>
<td></td>
<td>Single emulsification</td>
<td>Average</td>
<td>2–50 µm</td>
<td>Non-toxic</td>
<td>Compatible</td>
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<tr>
<td></td>
<td></td>
<td>Suspension polymerization</td>
<td>Low</td>
<td>200 nm–1 µm</td>
<td>Low</td>
<td>Compatibility as a “drug carrier”</td>
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<tr>
<td></td>
<td></td>
<td>Ultrasonic emulsification</td>
<td>High</td>
<td>2–3 µm</td>
<td>Non-toxic</td>
<td>Compatible</td>
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<tr>
<td>Fibrous</td>
<td>Collagen</td>
<td>Phase separation</td>
<td>Average</td>
<td>1–2 mm</td>
<td>Average</td>
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<td></td>
<td></td>
<td>Coacervation</td>
<td>High</td>
<td>100–200 µm</td>
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<tr>
<td></td>
<td></td>
<td>Solvent extraction</td>
<td>Average</td>
<td>100 nm–1 µm</td>
<td>Non-toxic</td>
<td>Compatible</td>
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<tr>
<td></td>
<td></td>
<td>Template based polymerization</td>
<td>High</td>
<td>100 nm–1 µm</td>
<td>Non-toxic</td>
<td>Unknown</td>
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<tr>
<td>Globular alpha-protein</td>
<td>Hemoglobin (Hb)</td>
<td>Template based polymerization</td>
<td>High</td>
<td>1–5 μm</td>
<td>Low</td>
<td>Compatible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ultrasonic emulsification</td>
<td>Average</td>
<td>1–2 µm</td>
<td>Low</td>
<td>Unknown</td>
</tr>
<tr>
<td>Globular plasma-protein</td>
<td>Human Serum Albumin (HSA)</td>
<td>Template based polymerization</td>
<td>High</td>
<td>200 nm–5 µm</td>
<td>Low</td>
<td>Compatible</td>
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<td></td>
<td>Coacervation</td>
<td>High</td>
<td>500 nm–20 µm</td>
<td>Low</td>
<td>Unknown</td>
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<tr>
<td></td>
<td></td>
<td>Ultrasonic emulsification</td>
<td>High</td>
<td>1–3 µm</td>
<td>Low</td>
<td>Unknown</td>
</tr>
<tr>
<td>Fibrous</td>
<td>Insulin (INS)</td>
<td>Template based polymerization</td>
<td>High</td>
<td>200 nm–5 µm</td>
<td>Low</td>
<td>Compatible</td>
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功能性蛋白质微胶囊的构建可以从多种方法中进行选择，包括：

1. 从混合两种蛋白质的溶液中构建蛋白质微胶囊。
2. 将蛋白质微胶囊与生物相容性聚合物化学结合。
3. 将生物活性配体（如叶酸）附着到蛋白表面。
4. 通过将第二种蛋白质与主要蛋白质共组装来构建蛋白质微胶囊。

这些方法允许根据所需特性的构建微胶囊。这可以通过使用具有额外蛋白的另一种蛋白质来实现，从而保持微胶囊所需的特性。这种方法可以实现通过改变表面电荷来保持生物相容性并获得毒性。因此，蛋白质微胶囊有可能成为最具安全性和有效性之一的药物递送载体。

6. 未来方向和结论

蛋白质微胶囊领域在过去十年中取得了显著的发展。实际上，现在可以将多肽胶囊的尺寸和功能定义。这种方法使设计和组装蛋白质微胶囊成为可能，其可与特定疾病相关的药物递送、成像或疫苗接种。我们期待这些方法，特别是最近的创新，将能够在纳米和微型胶囊中显著扩展特定药物递送的应用。
Acknowledgements

U.S. is grateful to Professor Aharon Gedanken, Chemistry Department, Bar-Ilan University, Israel, for his support and supervision during her PhD research work. G.J.L.B. is a Royal Society University Research Fellow at the Department of Chemistry, University of Cambridge and an Investigador FCT at the Instituto de Medicina Molecular, Lisboa. We thank Nuno Azoia assistance with the preparation of the figures. The authors thank the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement NMP4-LA-2009-228827 NANOFOL.

References