Application of Polymer Brush-Shells on Nanoparticles for Controlling Interaction with Serum

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Abstract

The interaction of nanoparticles (NPs) with biological environments triggers the formation of a protein corona (PC), which significantly influences their behaviour *in vivo*. This review explores the evolving understanding of PC formation, focusing on the opportunity for decreasing or suppressing protein-NP interactions by macromolecular engineering of NP shells. The functionalization of NPs with a dense, hydrated polymer brush-shell is a powerful strategy to impart stealth properties in order to elude recognition by the immune system. While poly(ethylene glycol) (PEG) has been extensively used for this purpose, concerns regarding its stability and immunogenicity have prompted exploration of alternative polymers. The stealth properties of brush shells can be enhanced by tailoring functionalities and structural parameters, including molar mass, grafting density and polymer topology. Determining correlations between these parameters and biopassivity has enabled to obtain polymer-grafted NPs with high colloidal stability and prolonged circulation time in biological media.

KEYWORDS: polymer brushes; nanoparticles; macromolecular engineering; surface properties; protein corona; protein adsorption.

1. Introduction

The fate of inorganic and organic nanoparticles (NPs) dispersed in a physiological milieu is strongly determined by the formation of a protein corona (PC) that alters the surface composition and morphology of NPs, influencing their function, bio-distribution, and degradation. The formation of nanoparticle-protein corona complexes was first discovered by Cedervall *et al.* over ten years ago.[1] Since then, the understanding of this phenomenon has greatly evolved and stimulated further developments in nanomedicine.

The generation of a PC on NPs has crucial implications on the opsonization process, during which phagocytic cells can recognise and remove foreign bodies.[2] Opsonization can trigger cellular uptake, recognition by the immune system and other non-specific processes (*e.g.*, aggregation and precipitation) that undermine the stability of NPs.[3-5] In addition, the interactions between NP surface and proteins in the PC can mask chemical and biological functionalities on NPs, thus affecting their targetability and circulation time—essential features for drug-delivery systems and therapeutics aimed at accumulating carriers in specific tissues.[6-8]

The formation of a PC is governed by hydrophobic, van der Waals, electrostatic, and hydrogen bonding interactions between NPs and proteins in serum.[9] It is also influenced by various parameters, including the composition of biological media, the nature and properties of NPs. The process of protein deposition onto NPs occurs according to the so-called "Vroman effect", which involves a competitive adsorption of proteins that dynamically interchange on the substrate.[10-11] Proteins presenting high affinity towards NPs form a "hard corona", where they are tightly and irreversibly bound to the nanomaterial surface (Figure 1). Proteins with lower affinity are loosely bound to the NPs and/or weakly interact with proteins in the hard corona, developing an outer layer known as "soft corona". Proteins in the hard corona has a long residence time, which facilitates

their identification and isolation, whereas the identity and dynamics of proteins in the soft corona is more elusive.[1, 12-15] Human serum albumin (HSA) is the most abundant protein in serum, however it displays low binding affinity towards inorganic NPs and therefore it has been rarely identified in coronas.[16] In contrast, a less abundant protein with high affinity toward NPs such as apolipoprotein AI is classified as a hard corona protein.[17] The composition of the PC is further influenced by the physiological media through which NPs circulate, thus it provides a biological fingerprint of their migration path.[7] The nature of adsorbed proteins profoundly affects the fate of NPs, as "dysopsonins" (*e.g.*, HSA and lipoproteins) prolong the circulation time of NPs in blood, while "opsonins" (*e.g.*, immunoglobulins, complement proteins, coagulation proteins) promote recognition by the immune system.[4]

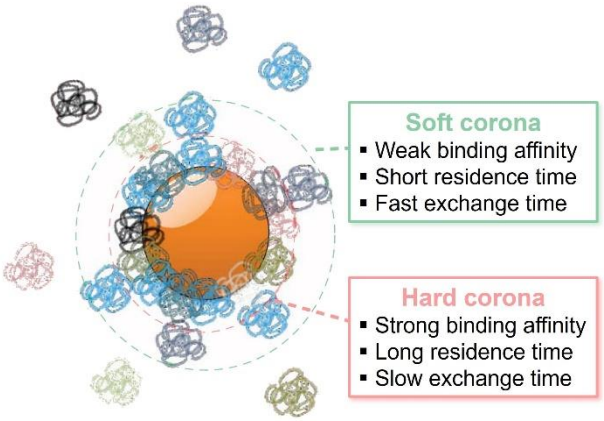

Figure 1. Nanoparticle-protein corona complexes: main characteristics of hard and soft coronas. Adapted with permission from reference [15]. Copyright (2016) Future Medicine.

The functionalization of NPs with polymer shells is a common strategy aimed at decreasing or suppressing protein binding, and conferring a stealth character, finally ensuring that NPs elude opsonization by immune cells.[5, 13] In particular, the fabrication of densely-grafted polymer brush shells is one of the most effective methods to modulate the interfacial properties of NPs and their interaction with serum proteins.[18] Neutral, hydrophilic poly(ethylene glycol) (PEG) grafts have been extensively exploited to build

highly hydrated, sterically blocking, thick shells that hinder hydrophobic interactions of NPs with biomolecules. However, mounting evidence of incomplete suppression of protein binding and PEG brush detachment from NP surface, together with pressing concerns over the immunogenicity of PEGylated drugs prompted researchers to develop alternatives.[19]

This review underscores the fundamental role of engineering NPs with dense polymer brush shells to make them capable of eluding protein interactions, thus potentially increasing their therapeutic efficacy.

We first introduce the most relevant factors determining the formation and composition of a PC and the response of the immune system to circulating NPs. Then, the ability of dense brush shells to shield NPs from non-specific protein adsorption and cellular uptake is described. Generally, a concise overview of PEG and alternative polymers used to fabricate brush shells onto NPs will be provided, highlighting the correlation between various structural parameters and biopassivity, and focusing on the emergence of topology as a critical tool to regulate NP fate within biological environments.

2. PC Formation and NP Interaction with the Immune System

2.1 Factors Determining PC Formation and Composition

The adsorption of proteins on NPs is influenced by the physicochemical and morphological properties of nanomaterials, including their size, shape, composition, and surface charge (Figure 2).[20] Lundqvist *et al*. analysed the effect of polystyrene (PS) NP size on PC composition, and compared bare PS NPs to amine-modified and carboxyl-modified PS NPs of analogous size to study the influence of positive and negative surface charges, respectively, on the PC. They observed that for 50-nm NPs, ~35-40% of proteins were found in all PCs irrespectively of their surface charge. However, ~35% of proteins

were strongly dependent on surface composition. Differences in PC composition were even more significant for PS NPs with larger size.[15] This study clearly showed that the size and surface functionalities of NPs are critical factors that alter the type of biomolecules in the corona, thereby potentially affecting the biological response to NPs, since different proteins perform distinctive biological functions.

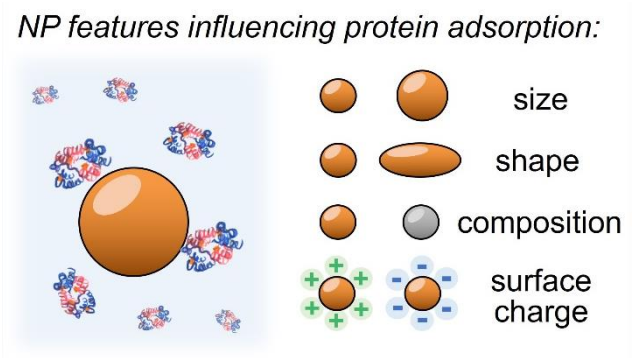

Figure 2. Properties of NPs that influence the adsorption of proteins and the formation of PCs.

This was confirmed for core-shell NPs constituted by an Au core and an amphiphilic block copolymer-based shell. The latter comprised both long, hydrophobic alkyl groups and charged functionalities in the side chains, either negatively charged (phosphonate groups, $-PO(OH)_2$) or positively charged (trimethylammonium groups, $-N(CH_3)_3$). Positively charged NPs were internalized by cells to a larger extent and with higher rates compared to negatively charged counterparts. The greater uptake for positively charged NPs was further associated to enhanced citotoxicity.[21]

The process of protein adsorption onto NPs is also significantly influenced by physiological factors, such as temperature, time of exposure to biological media, and shear forces in the blood stream. Despite the latter can have important implications for intravenous injection of NPs, only few studies addressed the impact of shear stress on PC. PEGylated liposomes incubated with circulating serum were shown to feature a larger variety of proteins and more negative charge when compared to similar liposomes

incubated under static conditions.[22] For PS NPs, a larger amount of proteins was recorded on the corona formed upon exposure to blood serum circulating at flow rates consistent with capillaries, veins and arteries. In addition, blood flow was observed to cause conformational changes to certain proteins in the corona, thus further acting on the biological impact of protein-NP complexes.[23]

Another factor to be considered for the effective translation of NP formulations from preclinical to clinical trials is represented by interspecies differences in PC composition. Large variations in PC profiles were identified on SiO_2 NPs functionalized with PEG and transferrin, upon incubation in the presence of either human plasma collected from healthy volunteers or mouse plasma. In this type of NPs, transferrin act as a ligand and promote the recognition by transferrin receptors in cancer cells. Thus, the NPs can serve for active targeting in cancer therapies. Upon exposure to mouse plasma, the PC contained great proportions of fibrinogen and serotransferrin, whereas exposure to human plasma resulted in the prevalence of immunoglobulins. Thus, the biological response observed for mouse models might be not representative, and adverse effects such as disseminated intravascular coagulation or vascular thrombosis could be overestimated due to the abundance of fibrinogen in mouse PC. These results highlighted that human plasma needs to be considered for preclinical studies.[24]

2.2 PC implications on interactions of NPs and immune system

The immune system is composed of lymphoid organs, cells, humoral factors, and cytokines, and it has the vital role of defending the organism by identifying and eliminating foreign bodies. The immune system is divided in two subsystems that operate together, undertaking different tasks. The innate immune system provides the first, rapid and non-specific defence against infectious agents, responding through physical and

chemical barriers.[25-26] The adaptive immune system consists of antigen-specific reactions through T and B lymphocytes. Adaptive immunity has a delayed maximal response upon antigen exposure. However it is characterized by immunologic memory, which results in increasingly rapid and more effective response upon repeated antigen exposure.

When NPs are circulating in the bloodstream, they can induce, inhibit or alter the response of the innate immune system.[27] NPs are generally recognized as foreign material and processed by the innate immune system. Its response is initiated by the binding of pattern recognition proteins and receptors, which recognize molecular patterns commonly found on pathogens (pathogen-associated molecular patterns, PAMPs) or molecules released from damaged cells (damage-associated molecular patterns, DAMPs). Typical molecular motives include charge clusters, neutral sugars, and vicinal hydroxyl groups or acetyl groups that can be present on NPs.[28-29] The presence of these motives is influenced by the composition of the PC, which in turn determines the interactions of NPs with the immune system.[30]

Three possible scenarios can be generally identified: 1) the PC masks the NP surface eluding recognition by immune cells or factors (*i.e.*, dysopsonic effect); 2) the PC facilitates recognition and elimination by immune cells, as in the case of NPs opsonised with immunoglobulins or complement components; and 3) plasma proteins in the PC present altered folding, thus being recognised by innate immune cells as danger signals, inducing an inflammatory response.[31-32]

In the innate immunity, the complement system plays a crucial role in opsonisation processes, and inflammatory and adaptive immune responses. The complement system consists of over 35 inactivated proteins that can get activated when adsorbed onto a surface. Complement activation ultimately induces phagocytosis by macrophages and adverse reactions of clinical concern. NPs can activate the complement system through

any of the three activation pathways. These are the classical pathway, initiated by antigen-antibody reactions, the antibody-independent alternative pathway, activated by polysaccharides, and the lectin pathway, stimulated by mannose-containing proteins and carbohydrates.[27] NP size, morphology, and surface physicochemical properties regulate the complement activation pathway. By modulating the surface chemistry of nanomaterials, it is therefore possible to modify the immune response, as it was shown, for instance, for 20-nm silica NPs bearing amine, carboxylic acid, oxazolines, and alkane surface functionalities. NP surfaces rich in acid groups had higher level of complement proteins, which resulted in increased production of pro-inflammatory cytokines. In contrast, amine rich surfaces led to increased expressions of anti-inflammatory markers.[33]

3. Polymer Brush Shells on NPs

3.1 Main features, functions, and fabrication methods of polymer brush shells

The fabrication and engineering of a polymer brush shell on NP cores is a prominent strategy to increase colloidal stability and prevent recognition and clearance, by suppressing or altering protein binding. Recently, the design of polymer shells for NPs has been guided by the so-called "Whitesides rules", established by systematically comparing the chemical structures of self-assembled monolayers on NPs and their efficiency in suppressing protein adsorptions. These rules suggest four characteristics of protein-resistant monolayers at the molecular level: 1) presence of polar functional groups (*i.e.*, hydrophilicity); 2) presence of hydrogen bond acceptor groups; 3) absence of hydrogen bond donor groups; and 4) absence of net charge.[34-37] Overall, an effective polymer shell should provide a hydration layer that hinders protein adsorption.

Tethering of polymer chains to the surface of NPs creates covalent chemical bonds that provide more effective stabilization in comparison with physically deposited polymeric

coatings. Thus, the generation of a dense polymer brush shell that fulfils the Whitesides rules would ensure enthalpic and entropic stabilization of NPs, preventing protein adsorption and shielding the core from the surrounding environment.

Polymer brushes can be generated onto NPs by means of three different strategies: 1) The "grafting to" method, whereby polymers with a reactive chain end are assembled onto the surface of NPs forming a brush layer; 2) the "grafting from" technique encompassing the *in situ* growth of polymer chains from initiator-functionalized NPs, through surface-initiated polymerizations (SIP);[38] and 3) the "grafting through" method, where monomer units are anchored to the NP surface and copolymerized with free monomers in solution (Figure 3).[39] The main advantage of the grafting to method is that polymer chains can be fully characterized prior to their attachment to the surface. However, it results in lower density of polymeric grafts in comparison to the grafting from technique, mainly because grafted chains limit polymer diffusion and their steric hindrance restrict further chain attachment. Similarly, the grafting through approach is self-limiting in its nature. Nevertheless, the properties of the polymer layer can be adjusted to some extent by changing the density of surface-bound monomers and the polymerization conditions. Overall, the grafting from method is the most frequently employed as it gives access to higher film thickness and grafting densities. Continuous developments in surface-initiated reversible deactivation radical polymerization (SI-RDRP) techniques has led to increasingly high versatility, easiness, and precise control over the architecture, composition, and molar mass of polymer brushes prepared via grafting from.[38]

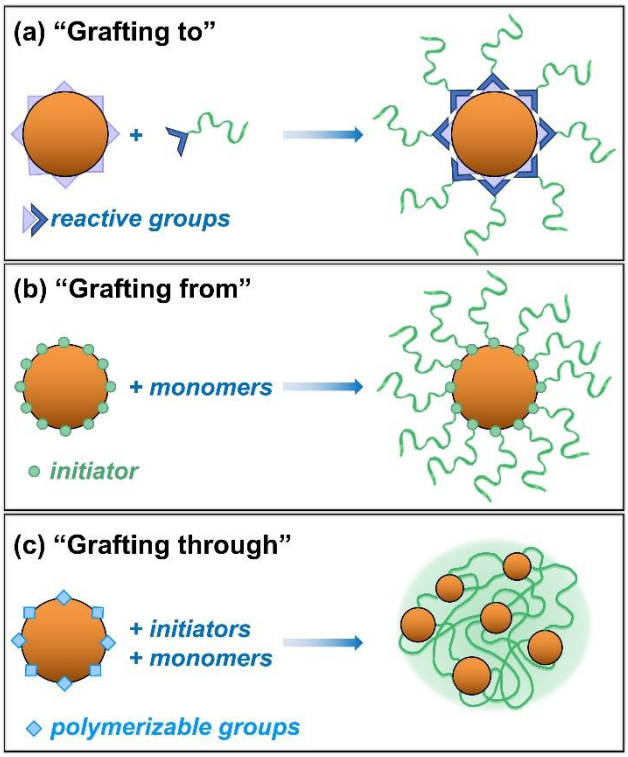

Figure 3. Approaches to graft polymers onto NPs: (a) grafting to, (b) grafting from, and (c) grafting through.

A dense hydrated brush shell acts as a steric-osmotic repulsive interface that counteract attractive non-specific colloidal interactions, impeding aggregation, protein binding and cell uptake. Interactions between colloidal particles are classically described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which considers attractive van der Waals forces promoting aggregation, and repulsive electric double layer forces favouring colloidal stabilization.[40-41] The presence of a hydrophilic polymer brushes can introduce a steric stabilization, which results from two effects known as entropic spring and osmotic pressure repulsion. The first indicates the rise of entropically unfavoured configurational restrictions when polymers are compressed to small volumes, while the second derives from high local concentrations of polymer chains with low concentrations of solvent molecules.[42] The steric repulsion provided by hydrophilic brushes not only prevent NP aggregation but also impede protein adsorption. Thus, the brush conformation is critical to impart a stealth behaviour to the NPs: If polymer grafts are sparse, the resulting

mushroom-like conformation cannot effectively prevent nonspecific binding onto the NP surface (Figure 4).[3] It should be noted however that the achievable grafting density is limited by the minimum exclusion volume and intramolecular repulsive interaction energies of the polymer.

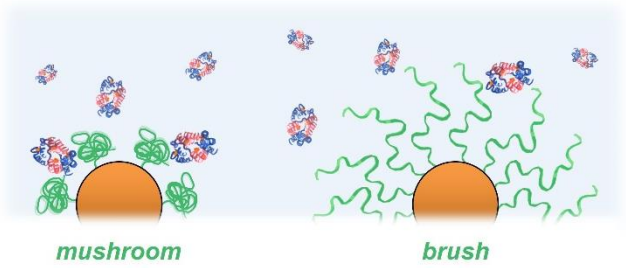

Figure 4. Correlation between protein interaction and conformation of polymer chains grafted onto NPs with different grafting densities.

A wide range of polymers has been used to generate polymer brush shells onto NPs to suppress or modulate protein adsorption. The most used and relevant polymers are described in the following paragraphs and their structure is shown in Figure 5.

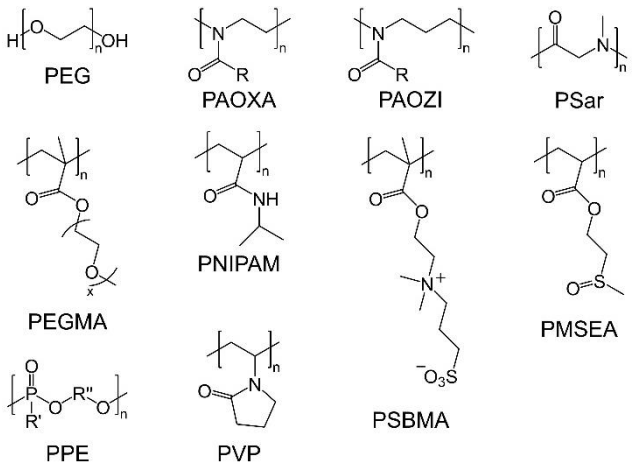

Figure 5. Structures of representative polymers employed to fabricate brush shells on NPs.

3.2 Poly(ethylene glycol) (PEG)

PEG is a hydrophilic, biocompatible polymer, approved for biomedical applications by the Food and Drug Administration (FDA), and it represents the gold standard for NP coating. When PEG chains are grafted onto NPs to yield a brush conformation, the resulting highly hydrated and neutral shell significantly reduces protein adsorption, and NPs acquire stealth properties with low levels of cell uptake and macrophage-driven clearance.[43-44] PEG has been shown to improve the colloidal stability of micelles, liposomes, dendrimers, and several inorganic and polymeric NPs, both during storage and *in vivo* applications.[19]

The antifouling properties of PEG-brush shells depend on several features, such as PEG molar mass and surface grafting density.[16, 45] The effect of PEG grafting density on Au NPs has been systematically studied in connection to NP size (Figure 6).[46] Increasing PEG grafting density suppressed protein adsorption and changed the nature of adsorbed proteins. For a fixed grafting density, smaller NPs resulted in increased protein adsorption, likely due to curvature-dependent steric effects, whereas polymer chains were more "spread out" on small NPs. The efficiency of macrophage uptake of NPs was found to be dependent on adsorbed serum proteins, however for grafting densities exceeding ~0.64 chain/nm^2 serum-independent uptake was observed.

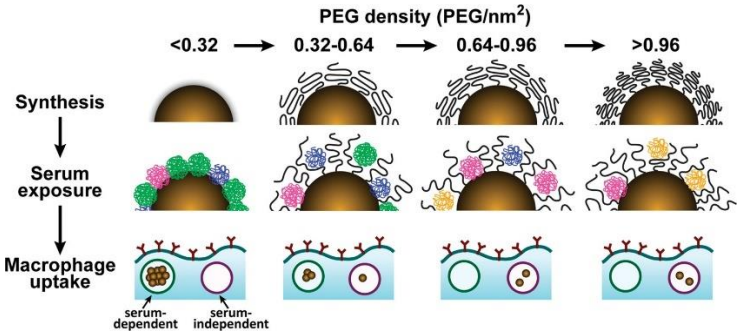

Figure 6. Increasing the density of grafted PEG chains modifies the nature of adsorbed proteins and reduces the total protein adsorption on gold NPs. Macrophage uptake depends on the adsorbed serum proteins for relatively low PEG grafting densities. Reprinted with permission from reference [46]. Copyright (2011) ACS Publications.

While high grafting density is key to steric stabilization, low-density PEG grafts can undergo conformational fluctuations that slows down the kinetic of protein adsorption. Thus, Zhou *et al*. designed poly(lactic-*co*-glycolic acid) (PLGA) NPs with a densely-grafted PEG inner layer and a second outer layer with variable grafting density. This was achieved by mixing methoxy-terminated and maleimide-terminated PEG chains in the first shell, followed by addition of methoxy-PEG-thiol, thus exploiting the thiol-maleimide reaction to form the outer layer. The grafting density of this second layer depended on the relative amount of maleimide-terminated PEG in the inner layer. While high density of the inner shell was crucial, longer NP circulation times were measured when the density of the outer layer was close to the mushroom-to-brush transition, as chain dynamics contributed to reduce protein interactions (Figure 7).[47]

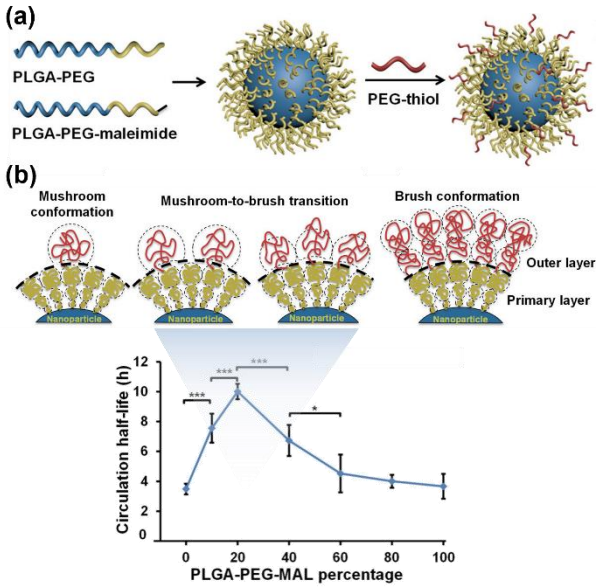

Figure 7. (a) General strategy used to generate a double PEG layer on NPs. (b) Variation in NP circulation time in blood as a function of the percentage of maleimide (MAL) functionalized PEG chains introduced in the primary layer, which in turn determined the grafting density of the outer layer and its conformation. Adapted with permission from reference [47]. Copyright (2018) ACS Publications.

Another factor that has been recently reported to critically affect the physicochemical properties of polymer-grafted NPs and their interactions with biological entities is the polymer architecture. The next section of this review describes the observed correlations between brush topology and stealth properties of NPs, both for PEG and other polymer shells.

Despite the extensive use of PEG for stealth NP design, PEG possesses several drawbacks including low stability in biological milieus, and adverse biological effects, such as causing cell cycle arrest and DNA damage and reducing cellular uptake of NPs.[9, 48] Moreover, opsonization by the third protein of the complement system can still occur on PEGylated NPs, leading to uptake by human macrophages. In addition, PEG can undergo oxidative degradation, thus exposing the NP core to the biological environment, and facilitating NP recognition by the immune system.[49] Further concern is posed by the increasing production of anti-PEG antibodies as a consequence of the *in vivo* accumulation of the polymer. Anti-PEG antibodies can recognize PEGylated NP formulations, severely compromising their efficacy.[16] These reasons have prompted the scientific community to develop alternatives to PEG for fabricating stealth NPs.

3.3 Alternatives to PEG

A wide range of hydrophilic polymers are exploited to regulate pharmacokinetics and recognition features of nanoparticle formulations. These polymers share similarities with PEG, *i.e.*, they are uncharged, hydrophilic, and flexible, and they can benefit from lower cost, and higher chemical versatility compared to PEG, while providing longer circulation times to NPs when attached to their surface.

Poly(oligo(ethylene glycol)) methyl ether methacrylate (POEGMA).

The comb-shape polymer POEGMA consists of hydrophilic PEG oligomeric chains grafted to a hydrophobic poly(methacrylate) backbone (Figure 5). In aqueous solutions, POEGMA exhibits a more compact conformation than PEG with similar molecular weight. Importantly, the translation of the PEG motif from long backbones to relatively short side chains was proved to result in reduced immunogenicity after repeated administration.[50-52] The principal drawback of POEGMA is the hydrophobic non-degradable methacrylic backbone which can limit its widespread use in biomedicine.[53]

When employed for generating polymer-peptide conjugates, POEGMA resulted in reduced steric hinderance relative to PEG, promoting increased cellular uptake of the biomolecules.[54] The non-fouling properties of POEGMA brushes in serum were reported for a variety of flat surfaces functionalized via a grafting from approach, using surface-initiated atom transfer radical polymerization (SI-ATRP). The resistance to protein adsorption varied with the thickness and grafting density of PEOGMA brushes, with low fouling behaviour observed already at relatively low grafting densities, which was explained with the large footprint of the polymer. Resistance to lysozyme, fibronectin, bovine serum albumin (BSA), and undiluted fetal bovine serum (FBS) solutions was determined for POEGMA brushes with five ethylene glycol units in the side chains and thicknesses exceeding 10 nm.[51-52]

POEGMA brush shells were generated on magnetic Fe_3O_4 NPs via SI-ATRP achieving grafting density as high as 0.7 chains nm^{-2}. The dense shell strongly decreased the uptake of NPs by macrophage cells.[55] Similarly, block copolymers of poly(glycidyl methacrylate) and POEGMA grafted from superparamagnetic iron oxide nanoparticles (SPIONs) conferred high dispersibility and stability in aqueous solutions to the NPs, while also avoiding macrophage uptake in *in vitro* studies, as a result of the outer PEGMA shell. Finally, POEGMA-grafted-SiO_2 NPs with a grafting density of approximately 0.5

chains nm^{-2} and POEGMA molecular weight of 50 kDa displayed very low adhesion to a human epidermal cell line and to soft epithelial tissue samples.[56]

Poly(2-alkyl-2-oxazoline)s (PAOXAs) and poly(2-alkyl-2-oxazine)s (PAOZIs).

Poly(2-alkyl-2-oxazoline)s (Figure 5) are biocompatible, hydrophilic polymers, generally synthesized with pre-determined molecular weights and narrow molecular weight distributions via cationic ring opening polymerization (CROP). By varying the polymerization initiator, terminating agent and the alkyl group in the oxazoline monomer, various functionalities can be precisely installed in PAOXA chains. The hydrophilicity of PAOXAs decreases with increasing the length of the alkyl side chains. Poly(2-methyl-2-oxazoline) (PMOXA) and poly(2-ethyl-2-oxazoline) (PEOXA) are comparable to PEG in terms of water-solubility, biocompatibility and ability to repel proteins.[9, 37, 57] PAOXAs feature enhanced chemical stability compared to PEG, due to the polyamide backbone which is less prone to hydrolytic and oxidative degradation than the ether functionalities in PEG. Comparison of PEG and PMOXA brushes grown from macroscopic surfaces with identical grafting density and dry thicknesses revealed similar protein repellent properties upon exposure to physiological media, indicating that brush-protein interactions are mainly dictated by conformational entropy, and they are independent on the polymer nature. However, PMOXA-coated surfaces exhibited better oxidative stability and retained antifouling behaviour upon long-term exposure.[58-59]

The protein adsorption in serum was compared for 10-30 nm poly(organosiloxane) (POS) NPs with PEG and PEOXA shells. Thiol-terminated polymer chains were attached to the NP surface by reacting with maleimide functionalities, via a grafting to approach. The PEOXA shell provided comparable stabilization as PEG, with diminished protein interactions (Figure 8), significantly decreasing non-specific cellular uptake by

macrophage-like and endothelial cells.[60] Poly(2-isopropyl-2-oxazoline) (PiPOXA) and PEOXA brush shells with high grafting densities of 0.9-1.3 chains nm^{-2} were fabricated on ~10 nm SPIONs by grafting to using a nitrocathecol linker.[61] While high grafting density was paramount to achieve stealth behaviour, a small number of albumin protein inevitably adsorbed, as measured by isothermal titration calorimetry (ITC).[16, 62] This was also verified for similar NPs with PEG and poly(N-isopropyl acrylamide) (PNIPAM) shells. Interestingly, the lower critical solution temperature (LCST) of PAOXA-grafted NPs was at least 10 °C lower than the values measured for the corresponding free polymer solutions. When exposed in serum at temperatures above their LCST, the NPs started to aggregate, loosed their stealth properties and were recognized by HeLa cells.[61]

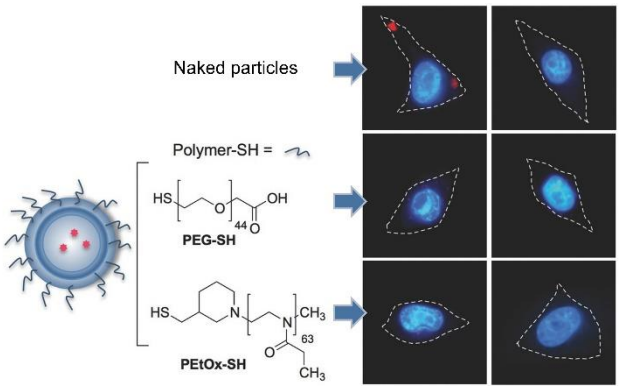

Figure 8. Uptake of PEG- and PEOXA-*g*-poly(organosiloxane) NPs in ISO-HAS1 cells after exposure for 60 min. in serum-free (left) and in serum-containing medium (right). Scale bar 10 µm. Adapted with permission from reference [60]. Copyright (2016) Wiley-VCH.

Recently, PLGA NPs modified with mannose-functionalized diblock-polyoxazolines (poly(2-butyl-2-oxazoline)-*block*-PMOXA) were employed to build a nanovaccine platform capable of generating antigen-specific T-cell responses that control tumor growth. Their efficacy was greater than for analogous nanovaccines based on PEG.[63] It should be noted however that Tavano *et al.* reported evidences of human complement

activation by PMOXA-coated NPs, leading to NP recognition by leukocytes and monocyte-derived macrophages. This contrasts with the reported stealth behaviour in murine and mouse models, and highlights the need for interspecies studies.[49]

Poly(2-alkyl-2-oxazine)s (PAOZIs, Figure 5) represent intriguing alternatives to PAOXAs, as they can exhibit comparable hydrophilicity, while showing higher main-chain flexibility than corresponding PAOXAs. In particular, poly(2-methyl-2-oxazine) (PMOZI) brushes hydrate similarly to the most hydrophilic poly(2-methyl-2-oxazoline) (PMOXA) simultaneously featuring a more flexible chain.[64] Morgese *et al.* studied the protein resistance of macroscopic SiO_2 surfaces coated with poly(L-lysine) (PLL)-*g*-PEG, PLL-*g*-PMOXA, PLL-*g*-PEOXA, and PLL-*g*-PMOZI when subjected to human serum (HS) and FBS. PMOZI brushes surpassed the other substrates, reducing protein fouling by ~97% and preventing unspecific cell adhesion.[65] Subsequent studies found even greater protein resistance for poly(2-ethyl-2-oxazine) (PEOZI) brushes, likely due to larger steric effects and flexibility with increasing the side-chain length.[66]

Polymeric NPs including PAOZI-based coatings have been fabricated by exploiting different nanoprecipitation strategies.[67] Crystallisation-driven self-assembly of PiPOXA-*b*-PMOZI block copolymers enabled to prepare rod-shaped NPs with a PMOZI shell. The elongated NPs tested *in vitro* showed excellent biocompatibility and suppression of non-specific cellular adhesion.[68]

Poly(meth)acrylamides (P(M)As).

Polyacrylamides are hydrophilic polymers that have received increasing attention, owing to their biocompatibility, and antifouling properties. PNIPAM (Figure 5) is a thermoresponsive polymer with a LCST of T = 32 °C. This property influences the colloidal stability and protein adsorption of NPs coated with PNIPAM shells, depending

on the temperature of incubation in serum. For PNIPAM-*g*-SPION, a significant increase in protein binding was observed when raising temperature above the LCST.[16] Different PC composition was observed for PS-PNIPAM core-shell NPs incubated in human plasma at 25 °C and above 37 °C. In particular, the NPs incubated at the lower temperature showed an enrichment in apolipoprotein J (*i.e.*, clusterin) in their PC, which resulted in strongly decreased uptake by different cell lines.[69]

Poly(*N*-(2-hydroxyethyl)acrylamide) (PHEAA) was grafted from polystyrene NPs via photopolymerization to yield colloidally stable dispersions in model media and in FBS. Very few albumin units were found to be entrapped in the PEAA shell, highlighting the stealth properties of the modified NPs. Relevantly, block copolymers of polyacrylic acid and PHEAA grafted from PS NPs via a photoiniferter approach provided showed a significant reduction of BSA adsorption only when PHEAA composed the outer shell layer.[70] In other studies, block copolymers comprising a block of poly(*N*-(2-hydroxypropyl) methacrylamide (PHPMA) were employed to fabricate NPs that efficiently hindered interactions with different protein types.[71-72]

It is important to emphasize that PEAA and PHPMA have the advantage of bearing hydroxyl groups in the side chains for further functionalization or conjugation with biomolecules, thus serving as potential drug carriers.[73] Noteworthy, PHPMA demonstrated an excellent preclinical efficacy as a carrier for chemotherapeutic drugs, however the toxicity of acrylamide monomers raise concerns over the extensive use of PAs in therapeutics.[43]

Polypeptoids.

Poly(sarcosine) (PSar, Figure 5) is a nonionic, highly hydrophilic polypeptoid based on sarcosine, a natural amino acid that can be found in muscle tissues. PSar and its

derivatives are generally synthesized by ring opening polymerization (ROP) of N-carboxyanhydrides. PSar containing disulfide functionalities were synthesized by ROP of sarcosine-*N*-thiocarboxyanhydride and then grafted onto Au NPs and compared with analogous NPs presenting PEG grafts. PSar brushes with a molecular weight of ca. 5 kDa provided superior colloidal stability and protein resistance upon incubation in 10% FBS solution when compared to their PEG-coated counterparts.[74]

Interestingly, a more hydrophobic shell composed of random copolymers of PSar and poly(*N*-butylglycine), obtained by ROP of sarcosine- and *N*-butylglycine-*N*-thiocarboxyanhydrides with molecular weight of 13.6 kDa showed lower colloidal stability and increased accumulation in cells (Figure 9).

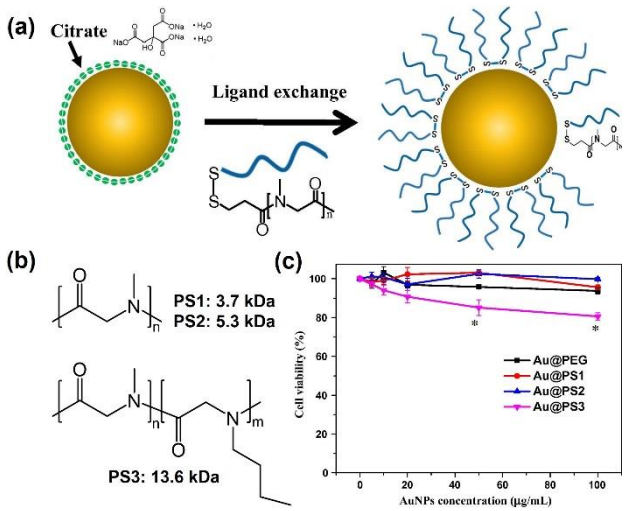

Figure 9. (a) Schematic illustration of surface modification of citrate capped AuNPs with disulfide functionalized PSar. (b) Structure and molecular weight of PSar polymers and PSar-poly(*N*-butylglycine) copolymers used for AuNPs' functionalization, and (c) NPs' uptake in endothelial cells as a function of the incubation time. Adapted with permission from reference [74]. Copyright (2016) Elsevier.

Molecular dynamic simulations were applied to rationalize the interaction of various polymers with HSA and revealed that polyalanine—isomeric to PSar—has a much higher propensity toward protein adsorption. This cannot be ascribed to larger interaction energy with the protein or different patterns of interaction with the surface amino acids, but it

was rather attributed to the different mode of interactions of the two polymers with water, whereby PSar shows a lower tendency to diminish surface exposure to water molecules by adsorbing proteins.[75]

Wang *et al.* designed a library of branched peptoid oligomers comprising two types of functional monomers with primary amine (6 or 12 units per oligomer) and triethyleneglycol side chains in different numeric ratios and arrangements. The oligomers were assembled on Au NPs exploiting the covalent binding of amines to Au surfaces and electrostatic interactions with citrate groups on Au. The oligomer providing better colloidal stability was attached with a grafting density of 0.13 chains nm^{-2} and a shell thickness of 1.5 nm. Oligopeptoid-coated NPs exhibited stealth properties and stability comparable to analogous NPs coated with PEG (2 kDa), both under molar salt concentration, following freeze/thaw treatment, and upon exposure to a wide range of pH.[76]

Relevantly, when PSar was grafted on liposomes, the accelerated blood clearance typically observed for PEGylated liposomes was significantly suppressed. In addition, PSar grafted from lipid nanoparticles (LNPs) provided a dense, stealth shell and higher transfection efficacy in comparison to PEGylated LNPs.[77]

Consecutive ROP and reversible addition-fragmentation chain-transfer (RAFT) polymerization enabled to build block copolymers that self-assembled into a variety of nanobjects, where the outer PSar layer ensured good colloidal stability in physiological media.[78] These results highlighted the versatility of polypeptoids and their compatibility with various synthetic methodologies that together with biopassivity and non-immunogenicity make these polymers highly promising replacements for PEG.[79]

Poly(phosphoester)s (PPEs).

Poly(phosphoester)s (Figure 5) are a class of water-soluble and degradable polymers with high chemical modularity owing to the presence of pentavalent phosphorous in the main chain, which allows for tuning the polymer properties by changing the lateral group. A seminal study compared the PC on 100-nm PS-based NPs with PEG (2 kDa and 5 kDa) and poly(ethyl ethylene phosphate) (PPEP, 7.6 kDa), prepared via anionic ROP of a cyclic phosphonates. A grafting to strategy was adopted by reacting *N*-hydroxysuccinimide-functionalized-polymers with amine groups on the surface of PS-based NPs, obtaining about 2000-4000 polymer chains per particles. Upon incubation with human plasma, all functionalized NPs exhibited a remarkable decreased in protein adsorption in comparison with bare PS NPs. Clusterin was found to be the main component of the PC on all functionalized NPs, which has a dysopsonizing function inhibiting macrophage uptake.[80] The effect of PPE hydrophilicity on the PC composition was subsequently investigated by grafting onto PS NPs random PPE copolymers based on 2-ethyl-2-oxo-1,3,2-dioxaphospholane and 2-*n*-butyl-2-oxo-1,3,2-dioxaphospholane. The increased hydrophobicity did not affect the amount of adsorbed proteins, however cellular uptake was strongly enhanced (Figure 10) as the composition of the PC changed with a decrease in the amount of adsorbed clusterin.[81] Hydrophilic PPE-grafted PS NPs showed also good blood compatibility irrespectively of the polymer molar mass.[82]

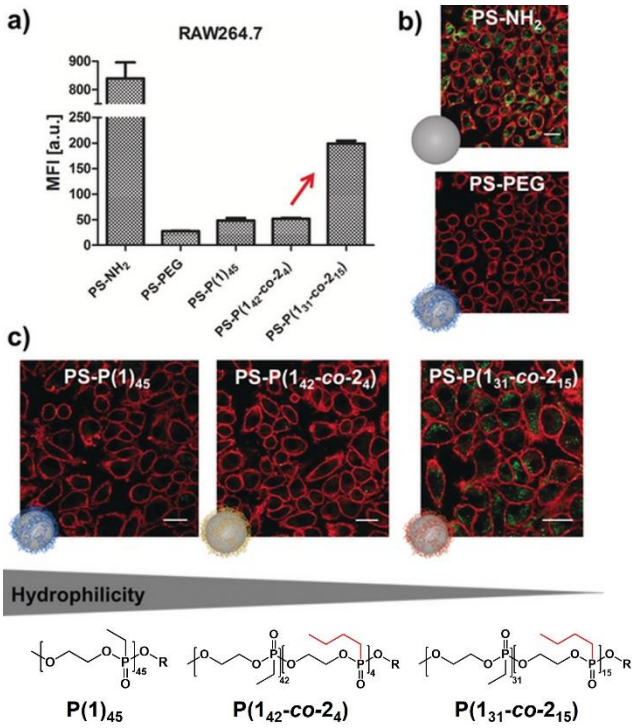

Figure 10. Cellular uptake of pristine and polymer-coated polystyrene NPs (PS NPs) after plasma incubation. (a) Flow-cytometry analysis. Values expressed as the mean ± SD of triplicates. (b) and (c) Confocal laser scanning microscopy images. The cell membrane was stained with CellMask Orange and pseudocolored in red; PS NPs were pseudocolored in green. Scale bars: 10 µm. These results demonstrated that a greater hydrophilicity decreases cellular uptake. Reprinted with permission from reference [81]. Copyright (2018) Wiley.

The role of PPE hydrophilicity was further investigated in block copolymers of poly(ε-caprolactone) (PCL) and PPEs with different pendant groups (2-methoxyethyl-2-oxo, 2-methyl-2-oxo, 2-ethyl-2-oxo, 2-propyl-2-oxo). A nanoprecipitation approach provided NPs with a size of ~40 nm and PPE shells. In 10% FBS solution, the NPs showed greater colloidal stability and stealth behaviour with increasing the PPE hydrophilicity. The cellular uptake also decreased with the polymer hydrophilicity, therefore mixed nanoparticles with both 2-methoxyethyl-2-oxo and 2-methyl-2-oxo groups in the PPE shell provided the best balance between stability and effective uptake by tumor cells, demonstrating that the fine tuning of polymer structure is key to therapeutic efficacy.[83]

Polyglycerols (PGs).

PGs are biocompatible and multihydroxy-functional polymers that can be synthesized with different architectures depending on the employed monomer and polymerization conditions. Hyperbranched PG (Figure 5) are obtained by anionic ROP of glycidol, while linear PGs are generally synthesized from protected glycidol derivatives and they have been extensively used for bioconjugation and for the fabrication of degradable biomaterials.[84] PG-grafted SPIONs were prepared by directly initiating the anionic ROP of glycidol from the hydroxyl groups on the SPION surface. The PG layer had a thickness of approximately 9 nm and provided excellent stability to the particles in physiological media.[85] When PG-grafted-SPIONs (with grafting densities of 2-7 PG monomers nm^{-2}) were compared to PEG-grafted-SPIONs (5 kDa, ~5 chains nm^{-2}), PG provided better protein resistance and lower macrophage uptake. These excellent antifouling properties were attributed to the hyperbranched structure of PG and the use of a grafting from approach that enabled the formation of a denser shell in comparison to that obtained by grafting to of PEG.[85] The presence of multiple -OH groups in PG was later exploited to introduce different functionalities, specifically carboxylate, sulfate and ammonium groups, to study their influence on PC composition. The introduction of sulfate at low density and carboxylate groups did not alter the antifouling behaviour even in 55% FBS. In contrast, high density of sulfate groups and ammonium functionalities resulted in protein association which led to high cellular uptake.[86]

Zwitterionic (ZI) polymers.

ZI polymers consist of positively and negatively charged groups linked by a small carbon chain, typically located in the side chains. Thus, ZI polymers present an overall neutral charge, and they are highly hydrophilic, due to the strong hydration of charged groups. Sulfobetaine methacrylate (SBMA, Figure 5) is one of the most employed ZI monomers,

which has been grafted from the surface of various NPs through SI-RDRPs. Depending on the molar mass and grafting density of PSBMA brushes, the hydrophilicity of the shell can largely change, thus affecting the stability of the NPs. PSBMA-grafted-SiO_2 NPs with core size of 50 nm and brush grafting density of 0.3-0.4 chains nm^{-2}, but with molar mass varying from 6 to 13 kDa, exhibited an upper critical solution temperature (UCST) in aqueous solution. The UCST increased with increasing the brush molar mass, due to enhanced intra- and interchain associations of the sulfobetaine groups.[87] Nevertheless, when incubated in BSA solution at 37 °C, which was higher than the UCST of all samples, the NPs showed long-term stability and resistance to protein adsorption.

The protein resistance of NPs decorated with different ZI polymer brushes were compared by grafting polymethacrylates or polymethacrylamides bearing SB, phosphorylcholine (PpC) or carboxybetaine (CB) functions (Figure 11) to CdSe/CdS/ZnS quantum dots via reactive vinylimidazole groups. Upon incubation in human serum, no PC could be detected on SB-coated NPs, while reversible adsorption of proteins or partial aggregation was identified for PpC- and CB-coated NPs. Moreover, the introduction of additional charged groups in SB-coated NPs led to protein aggregation, where the addition of neutral moieties such as biotin preserved the antifouling ability.[88] Nevertheless, ZI polymethacrylates with PC functionalities in the side chains were recently conjugated to lipids to form LNPs that demonstrated stealth behaviour and long-term stability in FBS solutions.[89]

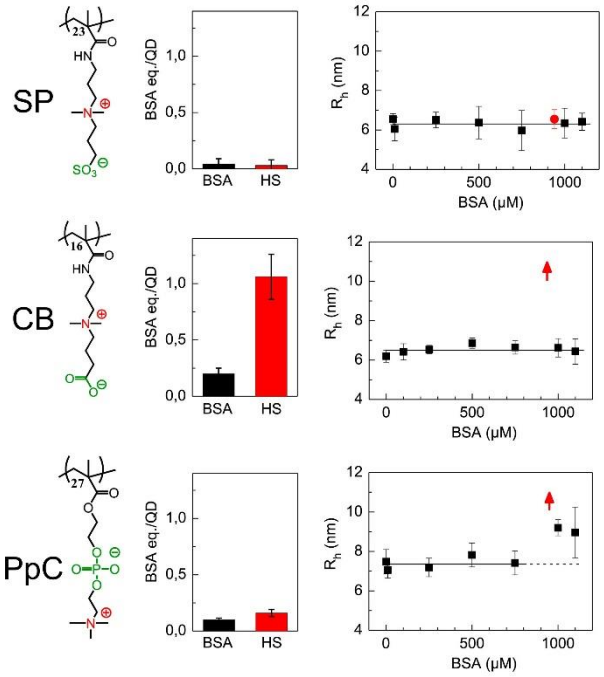

Figure 11. Structure of the sulfobetaine (SB), carboxybetaine (CB) and phosphorylcholine (PpC) polyzwitterions; average number of BSA equivalents adsorbed per QD within the hard corona, and QD hydrodynamic radius determined by fluorescence correlation spectroscopy as a function of BSA (black squares) concentration and whole human serum, HS (red circle). Red arrows reflect an aggregation of the QDs. Adapted with permission from reference [88]. Copyright (2019) Elsevier.

The water uptake of ZI polymers tend to increase with decreasing the linker length between opposite charges. This observation has guided the synthesis of a ZI monomer carrying a timethylamine N-oxide (TMAO) group in the later chain. TMAO is a small organic osmolyte present in saltwater fishes. The resulting polymer brushes grafted onto macroscopic surfaces showed excellent non-fouling properties. Moreover, PTMAO-conjugated proteins exhibited minimal immunogenicity and extended circulation time, indicating that PTMAO is a highly promising candidate that could be explored for stealth NP therapeutics.[90]

Polysaccharides.

Polysaccharides are natural polymers, biocompatible, biodegradable, and non-toxic. Chitosan, alginate, dextran, and hyaluronic acid are the most studied for fabricating polymer materials that find application in biomedicine.[91] Emulsion procedures have been largely employed to prepare core-shell NPs comprising a poly(alkylcyanoacrylate) core and chitosan or dextran-based shells arranged in a brush conformation. By increasing the length of dextran brushes, reduced interactions with complement proteins were observed upon exposure to human serum. Molar masses of at least 10 and 30 kDa were necessary to prevent complement activation on dextran and chitosan, respectively.[92] Importantly, the molecular features of dextran shells were found to strongly influence the extent of complement activation and its specific pathway. On the one hand, dense but thin shells (dextran molecular weight of 17.7 kDa) prevented adsorption of large proteins like immunoglobulins. On the other hand, long brushes (dextran molecular weight of 67 kDa) efficiently entrapped some proteins, shielding their interactions with complement proteins, in the case of sufficiently dense shells.[93] Similar NPs with heparin-based shells were effective even at lower polysaccharide molar mass, due to the inhibiting activity of heparin on complement system activation. Emulsion and nanoprecipitation techniques were also developed to produce poly(lactic acid) (PLA) NPs with more dense dextran-based shells, exploiting click reactions between α-alkyne PLA and N_3-functionalized dextran.[94]

Other polymers.

Poly(N-vinylpyrrolidone) (PVP, Figure 5) is a hydrophilic polymer that has been employed as a cryoprotectant for cells and lyoprotectant for proteins. A comparison of PVP (4.8 kDa)-coated and PEG (1.7 kDa)-coated PLA NPs prepared by emulsion polymerization revealed higher levels of opsonizing proteins in the PC of PVP-coated NPs, which led to more significant macrophage uptake.[95] However, when polymer NPs

were prepared by self-assembly of PCL-*b*-PVP block copolymers with different length of the PVP block, the latter was found to play a key role on the stealth properties of the nanoobjects. Increasing the molar mass of PVP from 3kDa to 12 kDa led to substantially reduced cytotoxicity and protein adsorption in BSA solutions.[96]

Polymers containing sulfoxide groups have recently gained attention for their hydrophilic nature. In particular, poly(2-(methylsulfinyl)ethylacrylate) (PMSEA, Figure 5) was employed for coating iron oxide NPs and the generated core-shell systems were studied in comparison to poly[(oligoethylene glycol)methyl ether acrylate] (POEGA). Polymer brush shells were fabricated on IONs via a grafting to strategy from diphosphonate-terminated polymers with a similar degree of polymerization. PMSEA was shown to significantly outperform POEGA in terms of NP colloidal stability in FBS, protein resistance and low-toxicity.[97]

4. The influence of polymer topology on stealth properties

Compared to their linear counterparts, star, cyclic, dendronized, and hyperbranched polymers have smaller hydrodynamic size, which has a critical effect on their transport in fluids and particularly through the body, if they are to be used for chemotherapies.[98] Architectural features of a polymer including its hydrodynamic size, molecular conformation, chain flexibility, and branching can influence its interaction with extracellular matrix and cells within tissues, as well as its removal from the body through the kidneys and intestines.[99] While considerable progress has been made in understanding the effects of polymer architecture on polymer drug-carriers, the relevance of topology for densely grafted polymer assemblies tethered to NPs has only emerged in recent years.[100]

The intense focus on PEGylated NPs and the drive to mitigate the drawbacks associated with PEG have spurred research into topological effects. The behaviour of PLA NPs obtained by self-assembly of PLA-PEG (5 kDa) copolymers with linear, linear-dendritic and bottlebrush architectures was evaluated in buffered media, and in the presence of HSA and FBS. PEG exposure increased with increasing the molecular weight of PEG for the linear architecture and was high for the other configurations. All NPs were colloidally stable even at elevated temperature, with the brush system showing enhanced performance. In all cases, the steric stabilization enabled to shield the NPs from proteins in HSA.[101] Topological effects of polysaccharide shells on polymeric NPs were also examined. When chitosan and dextran grafts were arranged in loops, an increase in protein adsorption and complement activation was measured with increasing the length of polysaccharide chains. This was attributed to the higher exposure and availability of reactive groups that could interact with proteins. In contrast, the brush configuration resulted in a lower number of exposed reactive groups, thus reducing protein adsorption.[92] Cyclic polymers are characterized by unique physicochemical properties that stems from their topology and from the absence of chain ends. Polymer micelles constructed from block copolymers comprising cyclic segments presented higher colloidal stability than their linear counterparts and enhanced drug encapsulation capability.[102] Cyclic PEOXA brushes on macroscopic surfaces exhibited a more compact conformation, smoother interface, higher swelling ratios and solvent uptake compared to their linear analogues.[103] In addition, the smaller hydrodynamic radius of ring PEOXAs enabled to generate denser brush layers which created a more efficient steric barrier that suppressed protein adsorption.[65]

To highlight the effect of polymer topology on the properties of core-shell systems silver NPs were functionalized with cyclic PEG (*c*PEG) and compared to Ag NPs functionalized

with linear PEG chains terminated with either hydroxyl or methoxy groups. The *c*PEG-Ag NPs exhibited lower and more uniform particle size. [104] On gold NPs, *c*PEG brushes formed a thicker layer than the linear counterparts, resulting in enhanced stabilization even when the NPs were frozen, lyophilized, and heated.[105]

Our group demonstrated that cyclic PAOXAs can form ultra-dense shells that protect NP cores from interacting with proteins. Linear and cyclic PAOXAs with different molar mass (5-6 kDa and 10-11 kDa) and bearing nitrocatechol anchors were used to functionalize SPIONs. Lower hydrodynamic radii and 70-80% higher grafting densities were measured for the NPs with cyclic brush shells. These thin and ultra-dense shells provided substantially enhanced long-term colloidal stability and effectively prevented BSA adsorption.[106] Further studies on SPIONs with dense shells constituted by linear or cyclic PEOXA brushes (Figure 12) by means of ITC and Dynamic Light Scattering (DLS) proved that cyclic PEOXA chains completely suppressed protein adsorption, outperforming their linear analogous which weakly interacted with HSA.[3, 16] The stealth behaviour was maintained when the shell hydration was decreased by raising the temperature to the LCST of the cyclic PEOXA brush. These findings demonstrated the great potential of polymer bushes with cyclic topology for engineering NPs and promote their long circulation time in physiological media.

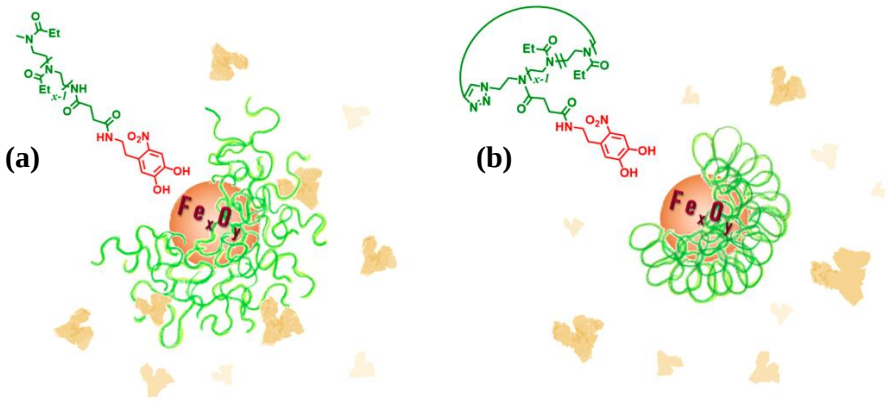

Figure 12. SPIONs coated with linear (a) and cyclic (b) polymer brushes. The cyclic topology effectively suppresses HSA from interacting with the NP core. Reprinted with permission from reference [3]. Copyright (2020) ACS Publications.

5. Conclusions and perspectives

The functionalization of NPs with dense, hydrated, and thick polymer brush shells represents the most convenient method for diminishing protein adsorption and increasing their stability and circulation time in biological media. While PEG has been the polymer of reference for providing nanocarriers with stealth properties, pressing concerns over PEG immunogenicity have spurred the investigation and development of alternative polymers. ZI polymers, polysaccharides, PPEs, PAOXAs, PGs and others have been used to confer NPs comparable or even better performance then PEG.

Furthermore, the ability to modulate polymer topology has expanded the synthetic toolbox for regulating the physicochemical interactions of NPs with the surrounding environment. The dispersity of polymer brushes is another parameters that can be modified to tailor technologically relevant properties of polymer-functionalized materials.[109] The distribution of chain length of polymer brushes, including both the main chain and the side chain, can substantially alter the hydration properties of the brushes.[100, 107] However, the impact of polymer brush dispersity on the response to biological environments remains underexplored. In perspective, the capability to finely control the

homogeneity of brush shells can represent another powerful means for regulating the interactions of nanocarriers with proteins and cells.

Increased understanding of the PC has made clear that the corona represents a biological fingerprint of a NP, which can provide important information on the protein source. This observation has inspired the development of the concept of "personalized" PC, which was based on the recognition that changes in plasma protein concentrations and structures mediated by different diseases affect the formation of the PC on a NP. Thus, the PC can be exploited as a sensor array platform for the early detection and identification of diseases.[108-109] In this context, the ability to install selected functionalities onto NPs by engineering the composition of a polymer shell can become the key to enhance the diagnostic or therapeutic potential of NPs for biomedical applications. Tailored functionalities introduced within polymer shells can allow for controlling protein adsorption on NPs, rather than suppressing interactions. In turn, the ability to control the interaction with proteins can enable to direct NPs toward targeted locations, such as tumor cells. As an example, polymer nanocarriers coated with adsorbed mannosylated PPEs were designed to take advantage of mannose groups as targeting ligands for triggering cellular recognition by monocyte-derived dendritic cells. While PPE provided stealth properties, mannose moieties served as dendritic cell targeting units, which can potentially lead to precise drug delivery.[110] Furthermore, selected proteins can be pre-loaded onto the polymer shell of coated NPs to regulate their biological fate.[80]

In conclusion, the fine tuning of polymer shells opens promising avenues for advancing the diagnostic and therapeutic capabilities of nanoparticles in biomedical contexts, ultimately paving the way for more precise and effective drug delivery systems.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ACKNOWLEDGEMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 956544.

REFERENCES

1. Cedervall, T.; Lynch, I.; Lindman, S.; Berggård, T.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S., Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (7), 2050-2055.
2. Sanchez-Cano, C.; Carril, M., Recent Developments in the Design of Non-Biofouling Coatings for Nanoparticles and Surfaces. *Int. J. Mol. Sci.* **2020**, *21* (3), 1007.
3. Schroffenegger, M.; Leitner, N. S.; Morgese, G.; Ramakrishna, S. N.; Willinger, M.; Benetti, E. M.; Reimhult, E., Polymer Topology Determines the Formation of Protein Corona on Core–Shell Nanoparticles. *ACS Nano* **2020**, *14* (10), 12708-12718.
4. Richtering, W.; Alberg, I.; Zentel, R., Nanoparticles in the Biological Context: Surface Morphology and Protein Corona Formation. *Small* **2020**, *16* (39), 2002162.
5. Leitner, N. S.; Schroffenegger, M.; Reimhult, E., Polymer Brush-Grafted Nanoparticles Preferentially Interact with Opsonins and Albumin. *ACS Appl. Bio. Mater.* **2021**, *4* (1), 795-806.
6. Alberg, I.; Kramer, S.; Schinnerer, M.; Hu, Q.; Seidl, C.; Leps, C.; Drude, N.; Möckel, D.; Rijcken, C.; Lammers, T.; Diken, M.; Maskos, M.; Morsbach, S.; Landfester, K.; Tenzer, S.; Barz, M.; Zentel, R., Polymeric Nanoparticles with Neglectable Protein Corona. *Small* **2020**, *16* (18), 1907574.
7. Ke, P. C.; Lin, S.; Parak, W. J.; Davis, T. P.; Caruso, F., A Decade of the Protein Corona. *ACS Nano* **2017**, *11* (12), 11773-11776.
8. Pino, P. D.; Pelaz, B.; Zhang, Q.; Maffre, P.; Nienhaus, G. U.; Parak, W. J., Protein corona formation around nanoparticles – from the past to the future. *Mater. Horiz.* **2014**, *1* (3), 301-313.
9. Wang, M.; Gustafsson, O. J. R.; Siddiqui, G.; Javed, I.; Kelly, H. G.; Blin, T.; Yin, H.; Kent, S. J.; Creek, D. J.; Kempe, K.; Ke, P. C.; Davis, T. P., Human plasma proteome association and cytotoxicity of nano-graphene oxide grafted with stealth polyethylene glycol and poly(2-ethyl-2-oxazoline). *Nanoscale* **2018**, *10* (23), 10863-10875.
10. Gupta, M. N.; Roy, I., How Corona Formation Impacts Nanomaterials as Drug Carriers. *Mol. Pharmaceutics* **2020**, *17* (3), 725-737.
11. Vroman, L.; Adams, A. L., Findings with the recording ellipsometer suggesting rapid exchange of specific plasma proteins at liquid/solid interfaces. *Surf. Sci.* **1969**, *16*, 438-446.
12. Wang, H.; Lin, Y.; Nienhaus, K.; Nienhaus, G. U., The protein corona on nanoparticles as viewed from a nanoparticle-sizing perspective. *WIREs Nanomed. Nanobiotechnol.* **2018**, *10* (4).
13. García-Álvarez, R.; Vallet-Regí, M., Hard and Soft Protein Corona of Nanomaterials: Analysis and Relevance. *Nanomaterials* **2021**, *11* (4), 888.
14. Corbo, C.; Molinaro, R.; Parodi, A.; Toledano Furman, N. E.; Salvatore, F.; Tasciotti, E., The impact of nanoparticle protein corona on cytotoxicity, immunotoxicity and target drug delivery. *Nanomedicine* **2016**, *11* (1), 81-100.
15. Lundqvist, M.; Stigler, J.; Elia, G.; Lynch, I.; Cedervall, T.; Dawson, K. A., Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (38), 14265-14270.
16. Gal, N.; Schroffenegger, M.; Reimhult, E., Stealth Nanoparticles Grafted with Dense Polymer Brushes Display Adsorption of Serum Protein Investigated by Isothermal Titration Calorimetry. *J. Phys. Chem. B* **2018**, *122* (22), 5820-5834.

17. Winzen, S.; Schoettler, S.; Baier, G.; Rosenauer, C.; Mailaender, V.; Landfester, K.; Mohr, K., Complementary analysis of the hard and soft protein corona: sample preparation critically effects corona composition. *Nanoscale* **2015**, *7* (7), 2992-3001.
18. Milner, S. T., Polymer Brushes. *Science* **1991**, *251* (4996), 905-914.
19. Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S., Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. *Angew. Chem. Int. Ed.* **2010**, *49* (36), 6288-6308.
20. Weber, C.; Voigt, M.; Simon, J.; Danner, A.-K.; Frey, H.; Mailänder, V.; Helm, M.; Morsbach, S.; Landfester, K., Functionalization of Liposomes with Hydrophilic Polymers Results in Macrophage Uptake Independent of the Protein Corona. *Biomacromolecules* **2019**, *20* (8), 2989-2999.
21. Hühn, D.; Kantner, K.; Geidel, C.; Brandholt, S.; De Cock, I.; Soenen, S. J. H.; Rivera_Gil, P.; Montenegro, J.-M.; Braeckmans, K.; Müllen, K.; Nienhaus, G. U.; Klapper, M.; Parak, W. J., Polymer-Coated Nanoparticles Interacting with Proteins and Cells: Focusing on the Sign of the Net Charge. *ACS Nano* **2013**, *7* (4), 3253-3263.
22. Palchetti, S.; Colapicchioni, V.; Digiacomo, L.; Caracciolo, G.; Pozzi, D.; Capriotti, A. L.; La Barbera, G.; Laganà, A., The protein corona of circulating PEGylated liposomes. *Biochim. Biophys. Acta* **2016**, *1858* (2), 189-196.
23. Jayaram, D. T.; Pustulka, S. M.; Mannino, R. G.; Lam, W. A.; Payne, C. K., Protein Corona in Response to Flow: Effect on Protein Concentration and Structure. *Biophys. J.* **2018**, *115* (2), 209-216.
24. Solorio-Rodríguez, A.; Escamilla-Rivera, V.; Uribe-Ramírez, M.; Chagolla, A.; Winkler, R.; García-Cuellar, C. M.; De Vizcaya-Ruiz, A., A comparison of the human and mouse protein corona profiles of functionalized SiO_2 nanocarriers. *Nanoscale* **2017**, *9* (36), 13651-13660.
25. Murphy, K.; Weaver, C., *Janeway's immunobiology*. Garland science: 2016.
26. Pulendran, B.; Katsikis, P. D.; Schoenberger, S. P., *Crossroads between innate and adaptive immunity III*. Springer Science & Business Media: 2011; Vol. 780.
27. Parkin, J.; Cohen, B., An overview of the immune system. *The Lancet* **2001**, *357* (9270), 1777-1789.
28. Boraschi, D.; Italiani, P.; Palomba, R.; Decuzzi, P.; Duschl, A.; Fadeel, B.; Moghimi, S. M., Nanoparticles and innate immunity: new perspectives on host defence. *Semin. Immunol.* **2017**, *34*, 33-51.
29. Pondman, K.; Le Gac, S.; Kishore, U., Nanoparticle-induced immune response: Health risk versus treatment opportunity? *Immunobiology* **2023**, *228* (2), 152317.
30. Barbero, F.; Russo, L.; Vitali, M.; Piella, J.; Salvo, I.; Borrajo, M. L.; Busquets-Fité, M.; Grandori, R.; Bastús, N. G.; Casals, E.; Puntes, V., Formation of the Protein Corona: The Interface between Nanoparticles and the Immune System. *Semin. Immunol.* **2017**, *34*, 52-60.
31. Papini, E.; Tavano, R.; Mancin, F., Opsonins and Dysopsonins of Nanoparticles: Facts, Concepts, and Methodological Guidelines. *Front. immunol.* **2020**, *11*, 567365.
32. Owensiii, D.; Peppas, N., Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int. J. Pharm.* **2006**, *307* (1), 93-102.
33. González-García, L. E.; MacGregor, M. N.; Visalakshan, R. M.; Lazarian, A.; Cavallaro, A. A.; Morsbach, S.; Mierczynska-Vasilev, A.; Mailänder, V.; Landfester, K.; Vasilev, K., Nanoparticles Surface Chemistry Influence on Protein Corona Composition and Inflammatory Responses. *Nanomaterials* **2022**, *12* (4), 682.
34. Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M., A Survey of Structure−Property Relationships of Surfaces that Resist the Adsorption of Protein. *Langmuir* **2001**, *17* (18), 5605-5620.

35. Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlin, R. E.; Yan, L.; Whitesides, G. M., Surveying for Surfaces that Resist the Adsorption of Proteins. *J. Am. Chem. Soc.* **2000**, *122* (34), 8303-8304.
36. Wei, Q.; Becherer, T.; Angioletti-Uberti, S.; Dzubiella, J.; Wischke, C.; Neffe, A. T.; Lendlein, A.; Ballauff, M.; Haag, R., Protein Interactions with Polymer Coatings and Biomaterials. *Angew. Chem. Int. Ed.* **2014**, *53* (31), 8004-8031.
37. Komsthöft, T.; Bovone, G.; Bernhard, S.; Tibbitt, M. W., Polymer functionalization of inorganic nanoparticles for biomedical applications. *Curr. Opin. Chem. Eng.* **2022**, *37*, 100849.
38. Zoppe, J. O.; Ataman, N. C.; Mocny, P.; Wang, J.; Moraes, J.; Klok, H.-A., Surface-Initiated Controlled Radical Polymerization: State-of-the-Art, Opportunities, and Challenges in Surface and Interface Engineering with Polymer Brushes. *Chem. Rev.* **2017**, *117* (3), 1105-1318.
39. Datta, P.; Genzer, J., "Grafting through" polymerization involving surface-bound monomers. *J. Polym. Sci., Part A: Polym. Chem.* **2016**, *54* (2), 263-274.
40. Derjaguin, B.; Landau, L., Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Prog. Surf. Sci.* **1993**, *43* (1-4), 30-59.
41. Verwey, E. J. W., Theory of the Stability of Lyophobic Colloids. *J. Phys. Colloid Chem.* **1947**, *51* (3), 631-636.
42. Lane, L. A., Physics in nanomedicine: Phenomena governing the in vivo performance of nanoparticles. *Appl. Phys. Rev.* **2020**, *7* (1).
43. Hoang Thi, T. T.; Pilkington, E. H.; Nguyen, D. H.; Lee, J. S.; Park, K. D.; Truong, N. P., The Importance of Poly(ethylene glycol) Alternatives for Overcoming PEG Immunogenicity in Drug Delivery and Bioconjugation. *Polymers* **2020**, *12* (2), 298.
44. Pasut, G.; Veronese, F. M., State of the art in PEGylation: The great versatility achieved after forty years of research. *J. Controlled Release* **2012**, *161* (2), 461-472.
45. Banerjee, I.; Pangule, R. C.; Kane, R. S., Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. *Adv. Mater.* **2011**, *23* (6), 690-718.
46. Walkey, C. D.; Olsen, J. B.; Guo, H.; Emili, A.; Chan, W. C. W., Nanoparticle Size and Surface Chemistry Determine Serum Protein Adsorption and Macrophage Uptake. *J. Am. Chem. Soc.* **2012**, *134* (4), 2139-2147.
47. Zhou, H.; Fan, Z.; Li, P. Y.; Deng, J.; Arhontoulis, D. C.; Li, C. Y.; Bowne, W. B.; Cheng, H., Dense and Dynamic Polyethylene Glycol Shells Cloak Nanoparticles from Uptake by Liver Endothelial Cells for Long Blood Circulation. *ACS Nano* **2018**, *12* (10), 10130-10141.
48. Quach, Q. H.; Kong, R. L. X.; Kah, J. C. Y., Complement Activation by PEGylated Gold Nanoparticles. *Bioconjugate Chem.* **2018**, *29* (4), 976-981.
49. Tavano, R.; Gabrielli, L.; Lubian, E.; Fedeli, C.; Visentin, S.; Polverino De Laureto, P.; Arrigoni, G.; Geffner-Smith, A.; Chen, F.; Simberg, D.; Morgese, G.; Benetti, E. M.; Wu, L.; Moghimi, S. M.; Mancin, F.; Papini, E., C1q-Mediated Complement Activation and C3 Opsonization Trigger Recognition of Stealth Poly(2-methyl-2-oxazoline)-Coated Silica Nanoparticles by Human Phagocytes. *ACS Nano* **2018**, *12* (6), 5834-5847.
50. Qi, Y.; Simakova, A.; Ganson, N. J.; Li, X.; Luginbuhl, K. M.; Ozer, I.; Liu, W.; Hershfield, M. S.; Matyjaszewski, K.; Chilkoti, A., A brush-polymer/exendin-4 conjugate reduces blood glucose levels for up to five days and eliminates poly(ethylene glycol) antigenicity. *Nat. Biomed. Eng.* **2016**, *1* (1), 0002.

51. Joh, D. Y.; Zimmers, Z.; Avlani, M.; Heggestad, J. T.; Aydin, H. B.; Ganson, N.; Kumar, S.; Fontes, C. M.; Achar, R. K.; Hershfield, M. S.; Hucknall, A. M.; Chilkoti, A., Architectural Modification of Conformal PEG-Bottlebrush Coatings Minimizes Anti-PEG Antigenicity While Preserving Stealth Properties. *Adv. Healthc. Mater.* **2019**, *8* (8), 1801177.
52. Ozer, I.; Kelly, G.; Gu, R.; Li, X.; Zakharov, N.; Sirohi, P.; Nair, S. K.; Collier, J. H.; Hershfield, M. S.; Hucknall, A. M.; Chilkoti, A., Polyethylene Glycol-Like Brush Polymer Conjugate of a Protein Drug Does Not Induce an Antipolymer Immune Response and Has Enhanced Pharmacokinetics than Its Polyethylene Glycol Counterpart. *Adv. Sci* **2022**, *9* (11), 2103672.
53. Lutz, J. F., Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46* (11), 3459-3470.
54. Gunasekaran, K.; Nguyen, T. H.; Maynard, H. D.; Davis, T. P.; Bulmus, V., Conjugation of siRNA with Comb-Type PEG Enhances Serum Stability and Gene Silencing Efficiency. *Macromol. Rapid Commun.* **2011**, *32* (8), 654-659.
55. Hu; Neoh, K. G.; Cen, L.; Kang, E.-T., Cellular Response to Magnetic Nanoparticles "PEGylated" via Surface-Initiated Atom Transfer Radical Polymerization. *Biomacromolecules* **2006**, *7* (3), 809-816.
56. Cozens, E. J.; Kong, D.; Roohpour, N.; Gautrot, J. E., The physico-chemistry of adhesions of protein resistant and weak polyelectrolyte brushes to cells and tissues. *Soft Matter* **2020**, *16* (2), 505-522.
57. Khutoryanskiy, V. V., Beyond PEGylation: Alternative surface-modification of nanoparticles with mucus-inert biomaterials. *Adv. Drug Deliv. Rev.* **2018**, *124*, 140-149.
58. Konradi, R.; Acikgoz, C.; Textor, M., Polyoxazolines for Nonfouling Surface Coatings — A Direct Comparison to the Gold Standard PEG. *Macromol. Rapid Commun.* **2012**, *33* (19), 1663-1676.
59. Chen, Y.; Pidhatika, B.; Von Erlach, T.; Konradi, R.; Textor, M.; Hall, H.; Lühmann, T., Comparative assessment of the stability of nonfouling poly(2-methyl-2-oxazoline) and poly(ethylene glycol) surface films: An *in vitro* cell culture study. *Biointerphases* **2014**, *9* (3), 031003.
60. Koshkina, O.; Westmeier, D.; Lang, T.; Bantz, C.; Hahlbrock, A.; Würth, C.; Resch-Genger, U.; Braun, U.; Thiermann, R.; Weise, C.; Eravci, M.; Mohr, B.; Schlaad, H.; Stauber, R. H.; Docter, D.; Bertin, A.; Maskos, M., Tuning the Surface of Nanoparticles: Impact of Poly(2-ethyl-2-oxazoline) on Protein Adsorption in Serum and Cellular Uptake. *Macromol. Biosci.* **2016**, *16* (9), 1287-1300.
61. Kurzhals, S.; Gal, N.; Zirbs, R.; Reimhult, E., Controlled aggregation and cell uptake of thermoresponsive polyoxazoline-grafted superparamagnetic iron oxide nanoparticles. *Nanoscale* **2017**, *9* (8), 2793-2805.
62. Reimhult, E.; Schroffenegger, M.; Lassenberger, A., Design Principles for Thermoresponsive Core–Shell Nanoparticles: Controlling Thermal Transitions by Brush Morphology. *Langmuir* **2019**, *35* (22), 7092-7104.
63. Matos, A. I.; Peres, C.; Carreira, B.; Moura, L. I.; Acúrcio, R. C.; Vogel, T.; Wegener, E.; Ribeiro, F.; Afonso, M. B.; Santos, F. M., Polyoxazoline-Based Nanovaccine Synergizes with Tumor-Associated Macrophage Targeting and Anti-PD-1 Immunotherapy against Solid Tumors. *Adv. Sci* **2023**, *10* (25), 2300299.
64. Bloksma, M. M.; Paulus, R. M.; van Kuringen, H. P. C.; van der Woerdt, F.; Lambermont-Thijs, H. M. L.; Schubert, U. S.; Hoogenboom, R., Thermoresponsive Poly(2-oxazine)s. *Macromol. Rapid Commun.* **2012**, *33* (1), 92-96.

65. Morgese, G.; Verbraeken, B.; Ramakrishna, S. N.; Gombert, Y.; Cavalli, E.; Rosenboom, J. G.; Zenobi-Wong, M.; Spencer, N. D.; Hoogenboom, R.; Benetti, E. M., Chemical Design of Non-Ionic Polymer Brushes as Biointerfaces: Poly(2-oxazine)s Outperform Both Poly(2-oxazoline)s and PEG. *Angew. Chem. Int. Ed.* **2018**, *57* (36), 11667-11672.
66. Svoboda, J.; Lusiani, N.; Sivkova, R.; Pop-Georgievski, O.; Sedlacek, O., Antifouling Properties of Poly(2-Oxazoline)s and Poly(2-Oxazine)s: Direct Comparison of Polymer-Coated Surfaces with the Same Coating Parameters. *Macromol. Rapid Commun.* **2023**, *44* (17), 2300168.
67. Drago, S. E.; Craparo, E. F.; Luxenhofer, R.; Cavallaro, G., Development of polymer-based nanoparticles for zileuton delivery to the lung: PMeOx and PMeOzi surface chemistry reduces interactions with mucins. *Nanomedicine: NBM* **2021**, *37*, 102451.
68. Warne, N. M.; Elbourne, A.; Tran, M. P.; Finnegan, J. R.; Feeney, O. M.; Kempe, K., Length-tuneable biocompatible block copolymer nanorods with a poly(2-methyl-2-oxazine)-corona via heat-induced crystallisation-driven self-assembly. *Polym. Chem.* **2023**, *14* (24), 2916-2929.
69. Prawatborisut, M.; Oberländer, J.; Jiang, S.; Graf, R.; Avlasevich, Y.; Morsbach, S.; Crespy, D.; Mailänder, V.; Landfester, K., Temperature-Responsive Nanoparticles Enable Specific Binding of Apolipoproteins from Human Plasma. *Small* **2022**, *18* (3), 2103138.
70. Wang, Z.; Chen, K.; Hua, C.; Guo, X., Conformation Variation and Tunable Protein Adsorption through Combination of Poly(acrylic acid) and Antifouling Poly(N-(2-hydroxyethyl) acrylamide) Diblock on a Particle Surface. *Polymers* **2020**, *12* (3), 566.
71. Hemmelmann, M.; Mohr, K.; Fischer, K.; Zentel, R.; Schmidt, M., Interaction of pHPMA–pLMA Copolymers with Human Blood Serum and Its Components. *Mol. Pharmaceutics* **2013**, *10* (10), 3769-3775.
72. Zhang, X.; Niebuur, B.-J.; Chytil, P.; Etrych, T.; Filippov, S. K.; Kikhney, A.; Wieland, D. C. F.; Svergun, D. I.; Papadakis, C. M., Macromolecular p HPMA-Based Nanoparticles with Cholesterol for Solid Tumor Targeting: Behavior in HSA Protein Environment. *Biomacromolecules* **2018**, *19* (2), 470-480.
73. Yao, X.; Qi, C.; Sun, C.; Huo, F.; Jiang, X., Poly(ethylene glycol) alternatives in biomedical applications. *Nano Today* **2023**, *48*, 101738.
74. Chen, Y.; Xu, Z.; Zhu, D.; Tao, X.; Gao, Y.; Zhu, H.; Mao, Z.; Ling, J., Gold nanoparticles coated with polysarcosine brushes to enhance their colloidal stability and circulation time in vivo. *J. Colloid Interface Sci.* **2016**, *483*, 201-210.
75. Settanni, G.; Schäfer, T.; Muhl, C.; Barz, M.; Schmid, F., Poly-sarcosine and Poly(Ethylene-Glycol) Interactions with Proteins Investigated Using Molecular Dynamics Simulations. *Comput. Struct. Biotechnol. J.* **2018**, *16*, 543-550.
76. Wang, S.-T.; Zhang, H.; Xuan, S.; Nykypanchuk, D.; Zhang, Y.; Freychet, G.; Ocko, B. M.; Zuckermann, R. N.; Todorova, N.; Gang, O., Compact Peptoid Molecular Brushes for Nanoparticle Stabilization. *J. Am. Chem. Soc.* **2022**, *144* (18), 8138-8152.
77. Son, K.; Ueda, M.; Taguchi, K.; Maruyama, T.; Takeoka, S.; Ito, Y., Evasion of the accelerated blood clearance phenomenon by polysarcosine coating of liposomes. *J. Controlled Release* **2020**, *322*, 209-216.
78. Fokina, A.; Klinker, K.; Braun, L.; Jeong, B. G.; Bae, W. K.; Barz, M.; Zentel, R., Multidentate Polysarcosine-Based Ligands for Water-Soluble Quantum Dots. *Macromolecules* **2016**, *49* (10), 3663-3671.
79. Birke, A.; Ling, J.; Barz, M., Polysarcosine-containing copolymers: Synthesis, characterization, self-assembly, and applications. *Prog. Polym. Sci.* **2018**, *81*, 163-208.

80. Schöttler, S.; Becker, G.; Winzen, S.; Steinbach, T.; Mohr, K.; Landfester, K.; Mailänder, V.; Wurm, F. R., Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. *Nat. Nanotech.* **2016**, *11* (4), 372-377.
81. Simon, J.; Wolf, T.; Klein, K.; Landfester, K.; Wurm, F. R.; Mailänder, V., Hydrophilicity Regulates the Stealth Properties of Polyphosphoester-Coated Nanocarriers. *Angew. Chem. Int. Ed.* **2018**, *57* (19), 5548-5553.
82. Pelosi, C.; Constantinescu, I.; Son, H. H.; Tinè, M. R.; Kizhakkedathu, J. N.; Wurm, F. R., Blood Compatibility of Hydrophilic Polyphosphoesters. *ACS Appl. Bio. Mater.* **2022**, *5* (3), 1151-1158.
83. Wang, L.; Li, S.-Y.; Jiang, W.; Liu, H.; Dou, J.-X.; Li, X.-Q.; Wang, Y.-C., Polyphosphoestered Nanomedicines with Tunable Surface Hydrophilicity for Cancer Drug Delivery. *ACS Appl. Mater. Interfaces* **2020**, *12* (29), 32312-32320.
84. Thomas, A.; Müller, S. S.; Frey, H., Beyond Poly(ethylene glycol): Linear Polyglycerol as a Multifunctional Polyether for Biomedical and Pharmaceutical Applications. *Biomacromolecules* **2014**, *15* (6), 1935-1954.
85. Zhao, L.; Chano, T.; Morikawa, S.; Saito, Y.; Shiino, A.; Shimizu, S.; Maeda, T.; Irie, T.; Aonuma, S.; Okabe, H.; Kimura, T.; Inubushi, T.; Komatsu, N., Hyperbranched Polyglycerol-Grafted Superparamagnetic Iron Oxide Nanoparticles: Synthesis, Characterization, Functionalization, Size Separation, Magnetic Properties, and Biological Applications. *Adv. Funct. Mater.* **2012**, *22* (24), 5107-5117.
86. Zou, Y.; Ito, S.; Fujiwara, M.; Komatsu, N., Probing the Role of Charged Functional Groups on Nanoparticles Grafted with Polyglycerol in Protein Adsorption and Cellular Uptake. *Adv. Funct. Mater.* **2022**, *32* (22), 2111077.
87. Dong, Z.; Mao, J.; Yang, M.; Wang, D.; Bo, S.; Ji, X., Phase Behavior of Poly(sulfobetaine methacrylate)-Grafted Silica Nanoparticles and Their Stability in Protein Solutions. *Langmuir* **2011**, *27* (24), 15282-15291.
88. Debayle, M.; Balloul, E.; Dembele, F.; Xu, X.; Hanafi, M.; Ribot, F.; Monzel, C.; Coppey, M.; Fragola, A.; Dahan, M.; Pons, T.; Lequeux, N., Zwitterionic polymer ligands: an ideal surface coating to totally suppress protein-nanoparticle corona formation? *Biomaterials* **2019**, *219*, 119357.
89. Khunsuk, P.-o.; Pongma, C.; Palaga, T.; Hoven, V. P., Zwitterionic Polymer-Decorated Lipid Nanoparticles for mRNA Delivery in Mammalian Cells. *Biomacromolecules* **2023**, *24* (12), 5654-5665.
90. Li, B.; Jain, P.; Ma, J.; Smith, J. K.; Yuan, Z.; Hung, H.-C.; He, Y.; Lin, X.; Wu, K.; Pfaendtner, J.; Jiang, S., Trimethylamine *N*-oxide–derived zwitterionic polymers: A new class of ultralow fouling bioinspired materials. *Science Advances* **2019**, *5* (6), eaaw9562.
91. Skalickova, S.; Horky, P.; Mlejnkova, V.; Skladanka, J.; Hosnedlova, B.; Ruttkay-Nedecky, B.; Fernandez, C.; Kizek, R., Theranostic Approach for the Protein Corona of Polysaccharide Nanoparticles. *Chem. Rec.* **2021**, *21* (1), 17-28.
92. Bertholon, I.; Vauthier, C.; Labarre, D., Complement activation by core–shell poly (isobutylcyanoacrylate)–polysaccharide nanoparticles: influences of surface morphology, length, and type of polysaccharide. *Pharm. Res.* **2006**, *23*, 1313-1323.
93. Coty, J.-B.; Eleamen Oliveira, E.; Vauthier, C., Tuning complement activation and pathway through controlled molecular architecture of dextran chains in nanoparticle corona. *Int. J. Pharm.* **2017**, *532* (2), 769-778.
94. Laville, M.; Babin, J.; Londono, I.; Legros, M.; Nouvel, C.; Durand, A.; Vanderesse, R.; Leonard, M.; Six, J.-L., Polysaccharide-covered nanoparticles with

improved shell stability using click-chemistry strategies. *Carbohydr. Polym.* **2013**, *93* (2), 537-546.
95. Gaucher, G.; Asahina, K.; Wang, J.; Leroux, J.-C., Effect of Poly(N-vinyl-pyrrolidone)-block-poly(d,l-lactide) as Coating Agent on the Opsonization, Phagocytosis, and Pharmacokinetics of Biodegradable Nanoparticles. *Biomacromolecules* **2009**, *10* (2), 408-416.
96. Zhu, Z.; Xie, C.; Liu, Q.; Zhen, X.; Zheng, X.; Wu, W.; Li, R.; Ding, Y.; Jiang, X.; Liu, B., The effect of hydrophilic chain length and iRGD on drug delivery from poly(ε-caprolactone)-poly(N-vinylpyrrolidone) nanoparticles. *Biomaterials* **2011**, *32* (35), 9525-9535.
97. Qiao, R.; Fu, C.; Li, Y.; Qi, X.; Ni, D.; Nandakumar, A.; Siddiqui, G.; Wang, H.; Zhang, Z.; Wu, T., Sulfoxide-containing polymer-coated nanoparticles demonstrate minimal protein fouling and improved blood circulation. *Adv. Sci* **2020**, *7* (13), 2000406.
98. Takata, T.; Aoki, D., Topology-transformable polymers: linear–branched polymer structural transformation via the mechanical linking of polymer chains. *Polym. J.* **2018**, *50* (1), 127-147.
99. Fox, M. E.; Szoka, F. C.; Fréchet, J. M. J., Soluble Polymer Carriers for the Treatment of Cancer: The Importance of Molecular Architecture. *Acc. Chem. Res.* **2009**, *42* (8), 1141-1151.
100. Pester, C. W.; Benetti, E. M., Modulation of Polymer Brush Properties by Tuning Dispersity. *Advanced Materials Interfaces* **2022**, *9* (34), 2201439.
101. Aguilar-Castillo, B. A.; Santos, J. L.; Luo, H.; Aguirre-Chagala, Y. E.; Palacios-Hernández, T.; Herrera-Alonso, M., Nanoparticle stability in biologically relevant media: influence of polymer architecture. *Soft Matter* **2015**, *11* (37), 7296-7307.
102. Romio, M.; Trachsel, L.; Morgese, G.; Ramakrishna, S. N.; Spencer, N. D.; Benetti, E. M., Topological Polymer Chemistry Enters Materials Science: Expanding the Applicability of Cyclic Polymers. *ACS Macro Lett.* **2020**, *9* (7), 1024-1033.
103. Vagias, A.; Nelson, A.; Wang, P.; Reitenbach, J.; Geiger, C.; Kreuzer, L. P.; Saerbeck, T.; Cubitt, R.; Benetti, E. M.; Müller-Buschbaum, P., The Topology of Polymer Brushes Determines Their Nanoscale Hydration. *Macromol. Rapid Commun.* **2023**, *44* (9), 2300035.
104. Wang, Y.; Quinsaat, J. E. Q.; Li, F.; Isono, T.; Tajima, K.; Satoh, T.; Sato, S.-i.; Yamamoto, T., Size Control and Enhanced Stability of Silver Nanoparticles by Cyclic Poly(ethylene glycol). *Polymers* **2022**, *14* (21), 4535.
105. Oziri, O. J.; Maeki, M.; Tokeshi, M.; Isono, T.; Tajima, K.; Satoh, T.; Sato, S.-i.; Yamamoto, T., Topology-Dependent Interaction of Cyclic Poly(ethylene glycol) Complexed with Gold Nanoparticles against Bovine Serum Albumin for a Colorimetric Change. *Langmuir* **2022**, *38* (17), 5286-5295.
106. Morgese, G.; Shirmardi Shaghasemi, B.; Causin, V.; Zenobi-Wong, M.; Ramakrishna, S. N.; Reimhult, E.; Benetti, E. M., Next-Generation Polymer Shells for Inorganic Nanoparticles are Highly Compact, Ultra-Dense, and Long-Lasting Cyclic Brushes. *Angew. Chem. Int. Ed.* **2017**, *56* (16), 4507-4511.
107. Romio, M.; Grob, B.; Trachsel, L.; Mattarei, A.; Morgese, G.; Ramakrishna, S. N.; Niccolai, F.; Guazzelli, E.; Paradisi, C.; Martinelli, E., Dispersity within brushes plays a major role in determining their interfacial properties: The case of oligoxazoline-based graft polymers. *J. Am. Chem. Soc.* **2021**, *143* (45), 19067-19077.
108. Hajipour, M. J.; Laurent, S.; Aghaie, A.; Rezaee, F.; Mahmoudi, M., Personalized protein coronas: a "key" factor at the nanobiointerface. *Biomater. Sci.* **2014**, *2* (9), 1210.
109. Caracciolo, G.; Safavi-Sohi, R.; Malekzadeh, R.; Poustchi, H.; Vasighi, M.; Zenezini Chiozzi, R.; Capriotti, A. L.; Laganà, A.; Hajipour, M.; Di Domenico, M.; Di

Carlo, A.; Caputo, D.; Aghaverdi, H.; Papi, M.; Palmieri, V.; Santoni, A.; Palchetti, S.; Digiacomo, L.; Pozzi, D.; Suslick, K. S.; Mahmoudi, M., Disease-specific protein corona sensor arrays may have disease detection capacity. *Nanoscale Horiz.* **2019**, *4* (5), 1063-1076.
110. Simon, J.; Bauer, K. N.; Langhanki, J.; Opatz, T.; Mailänder, V.; Landfester, K.; Wurm, F. R., Noncovalent Targeting of Nanocarriers to Immune Cells with Polyphosphoester-Based Surfactants in Human Blood Plasma. *Adv. Sci* **2019**, *6* (22), 1901199.

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Application of Polymer Brush-Shells on Nanoparticles for Controlling Interaction with Serum

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