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Review

Protein-functionalized nanoparticles: Emerging strategies in drug delivery

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ABSTRACT

The constantly developing field of protein-functionalized nanoparticles seems to be the future of medicine, as was proved by the Nobel Prize in 2022 for the development of "click chemistry". This method offers enormous possibilities for developing strategies for functionalizing nanoparticles with proteins. We scrutinize this field by exploring the latest advancements, methodologies, and applications in imaging, diagnostics, cancer treatment, and drug delivery. The research involves a comprehensive review of diverse strategies for functionalization, including in situ surface modification, post-synthesis techniques, and emerging click chemistry covalent and non-covalent coupling methods. Additionally, our review involves the importance of active targeting uptake. The flexibility and adaptability of PFNPs present encouraging prospects for numerous medical domains, notwithstanding the various obstacles encountered in implementing the solutions discussed. Our review highlights the need for further development to increase the universal applications of protein-functionalized nanoparticles while summarizing the remarkable advancements in this field.

1. Introduction - the idea of functionalization

Different molecules can be used to exploit the functionalization of the NP's surface to enhance biocompatibility; among these, PEG is one of the most used *in vitro* and *in vivo*. Functionalization gives more functional groups for the immobilization of probes and the reduction of nonspecific adsorption. In addition, it offers high sensitivity, selectivity, a high signal-to-noise ratio, and a low limit of detection to biosensors, etc. [1].

2. Passive and active targeting - uptake

Over 100 years ago, the German scientist Paul Ehrlich proposed the concept of creating "magic bullets" to address cancer-related issues [2]. In 1908, he was honored with the Nobel Prize alongside Ilya Mechnikov in recognition of their work on immunity [3]. Since then, continuous technological advancements and the current trend of personalized treatment have enabled the identification of numerous physicochemical parameters that can influence the pharmacokinetics and pharmacodynamics of chemotherapeutic agents, as well as their modification through drug carriers. A thorough investigation of the mechanisms governing biological processes in cancer cells, along with ongoing research and enhancement of already utilized nanoparticles, allows for the delivery of therapeutic agents more safely and effectively. Currently, we can distinguish two strategies for targeted drug delivery: passive targeting and active targeting.

2.1. Passive targeting uptake

The first reports that certain macromolecules preferentially accumulate in tumors date back to the 1990s. At that time, Matsumura and Maeda demonstrated that poly(styrene-co-maleic acid) preferentially accumulates in the tumor interstitium and remains there for an extended period [4]. Today, it is known that this phenomenon, termed the enhanced permeability and retention (EPR) effect, is associated with specific tumor conditions, such as inflammation and hypoxia. In these

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situations, increased angiogenesis occurs, resulting in new blood vessels with endothelium characterized by increased permeability compared to healthy vessels (Fig. 1b). This condition leads to the accumulation of large particles and nanocarriers in the interstitial tissue [5]. This phenomenon is related to the result of many factors that influence the final concentration of the substance in the tumor. We can point out complex biological processes among them, including angiogenesis, vascular endothelial permeability, lymphangiogenesis, diverse genetic profiles of cancer cells, tumor environment, and hemodynamic regulation. All of these mechanisms may function differently, depending on the patient and the type of cancer.

On the other hand, it has also been shown that the biological and physicochemical properties of the nanocarrier materials used, including size, charge, and shape, influence the distribution, permeability, and accumulation [6]. In terms of size, nanoparticles in the 20–200 nm range cross the 100–800 nm endothelial gaps of tumour vessels most efficiently. In contrast, larger constructs are excluded, and ultrasmall (<10 nm) constructs are rapidly cleared renally [7]. Considering surface charge, slightly negative or near-neutral ζ-potentials can minimize serum-protein adsorption and prolong circulation. While highly positive carriers exhibit faster opsonization and enhanced electrostatic interactions with cell membranes [7]. What's important is that the protein corona can influence nanoparticle surface charges [8]. As mentioned, the shape of NPs can also be a critical factor; high-aspect-ratio (rod or worm-like) nanocarriers exhibit enhanced margination and longer blood residence times compared with spheres. But, still, spheres are internalized more rapidly once they are at the target site [9].

2.2. Active targeting uptake

The phenomenon of active drug targeting relies mainly on the use of cell surface molecules. For this purpose, targeted drug carriers are used and modified in such a way that they can be specifically captured by the receptors and bound to the antigens of target cancer cells via the ligands on the surface of the nanoparticles [10]. The effectiveness and efficiency of this process depend on the proper biodistribution of the drug, which is influenced by the method of administration, the types of ligands used, and the processes involved in ligand-receptor coupling [11]. Among the molecules used as substrates are antibodies, proteins, amino acids, peptides, sugars, and small molecules such as vitamins (Fig. 1a and c). These modified molecules target other proteins, sugars, or lipids present on the surface of cells in diseased tissues due to overexpression [12].

3. Methods of functionalization

The functionalization of nanoparticles with proteins refers to the process of attaching molecules or functional groups to the surface of nanoparticles to modify their properties (wettability, stability, biochemical affinity, loading capacity, cell adhesion, intracellular delivery, toxicity) and enhance their performance in specific applications [15]. It offers a promising way of improving drug delivery systems (targeting ability, efficacy, or biocompatibility). Here, we concentrate on two functionalization strategies: in situ surface functionalization and post-synthesis surface functionalization. In situ surface functionalization involves modifying the surface of nanoparticles simultaneously with their synthesis. Functionalizing agents are added to the reaction mixture at the beginning of nanoparticle synthesis, allowing for the incorporation of functional groups. This method enables precise control of the process, leading to improved integration of functional groups. So, this is useful for creating polymeric nanoparticles with specific functional groups for subsequent protein attachment. Targeted drug delivery (nanoparticles can be targeted to particular ligands, improving drug transport to specific cells or tissues) or diagnostic imaging (uniformly functionalized nanoparticles can enhance the specificity and sensitivity of imaging agents) are examples of possible applications of this method [15]. For instance, thiolated ligands can be used to functionalize gold nanoparticles. During the first stage, the $AuCl(PPh_3)$ particle is liberated, followed by the replacement of remaining phosphine ligands as PPh_3 (assisted by gold complexes in solution). The final stage consists of the reorganization and completion of the thiol-based ligand shell [16]. It was revealed that metallic nanoparticle carriers are efficient in wound management. A powerful tool for establishing physical barriers and fostering an environment that promotes wound healing is hydrogel, a polymer with a three-dimensional lattice. It permits effective control over hemostasis, exudation, accelerated wound closure, and diminished scar formation [17].

Following the synthesis of the nanoparticle core, post-synthesis surface functionalization involves modifications to the surface of preformed nanoparticles. This technique makes greater flexibility possible when selecting functionalizing agents and modifying surface characteristics. This approach makes use of several mechanisms, involving:

- Covalent attachment – This method entails the formation of strong and stable covalent bonds between the functional groups and the nanoparticle surface; it ensures durable and stable modifications, which are crucial for numerous applications. Techniques usually include carbodiimide chemistry, silane coupling, or click chemistry.
- Non-covalent attachment – this process relies on weaker interactions, such as hydrophobic interactions, electrostatic forces, or hydrogen bonding; it offers the advantage of reversibility.
- Ligand exchange – this method involves replacing existing ligands on the nanoparticle surface with new functionalizing molecules, allowing modifications to the surface properties of metal nanoparticles.

  The advantages of this method of functionalization include versatility (allowing for the use of a wide range of functionalizing agents that may not be compatible with synthesis conditions), sequential functionalization (diverse functional groups can be added to the nanoparticle surface, allowing the creation of multifunctional nanoparticles), and adjustability.

  Post-synthesis surface functionalization is widely used in the development of nanoparticle-based drug delivery systems. Research into the application of nanocarriers in the delivery of cancer-fighting drugs has been a promising research field. However, their cytotoxic effects on cells, low uptake efficiency, and therapeutic resistance limited their therapeutic use. One of the aims is to find an effective antitumor drug delivery system [18]. It has to consist of spatial placement (the potential to target) and temporal delivery of the drug (controllability of the release) [19]. Nanoparticulate drug delivery systems (NPDDSs) demonstrate great potential as DDSs due to their nano size, increased surface-to-volume ratio, and advantageous physicochemical properties. They can modulate the pharmacodynamics and pharmacokinetic profiles of drugs and improve their therapeutic index [20]. Both in situ and post-synthesis surface functionalization methods have their advantages and are often used in complementarity to achieve the desired nanoparticle properties. To compare in situ and post-synthesis surface functionalization, this article lists distinguishing characteristics: timing and location of the process, prospects such as controlling the processes, stability, durability, speed, efficiency, and applications.
- Timing and location - during in situ surface functionalization, agents are added to the reaction mixture at the beginning, and this process occurs simultaneously with the synthesis of nanoparticles. In the case of post-synthesis functionalization, agents are added to pre-formed nanoparticles to modify their surface properties, which occurs after synthesis.
- Control over the processes - in situ functionalization permits precise control over the surface properties of nanoparticles. It can be useful for achieving uniform particles that behave similarly. Post-synthesis functionalization ensures greater flexibility and versatility and also allows modifications to be made after the synthesis of nanoparticles.

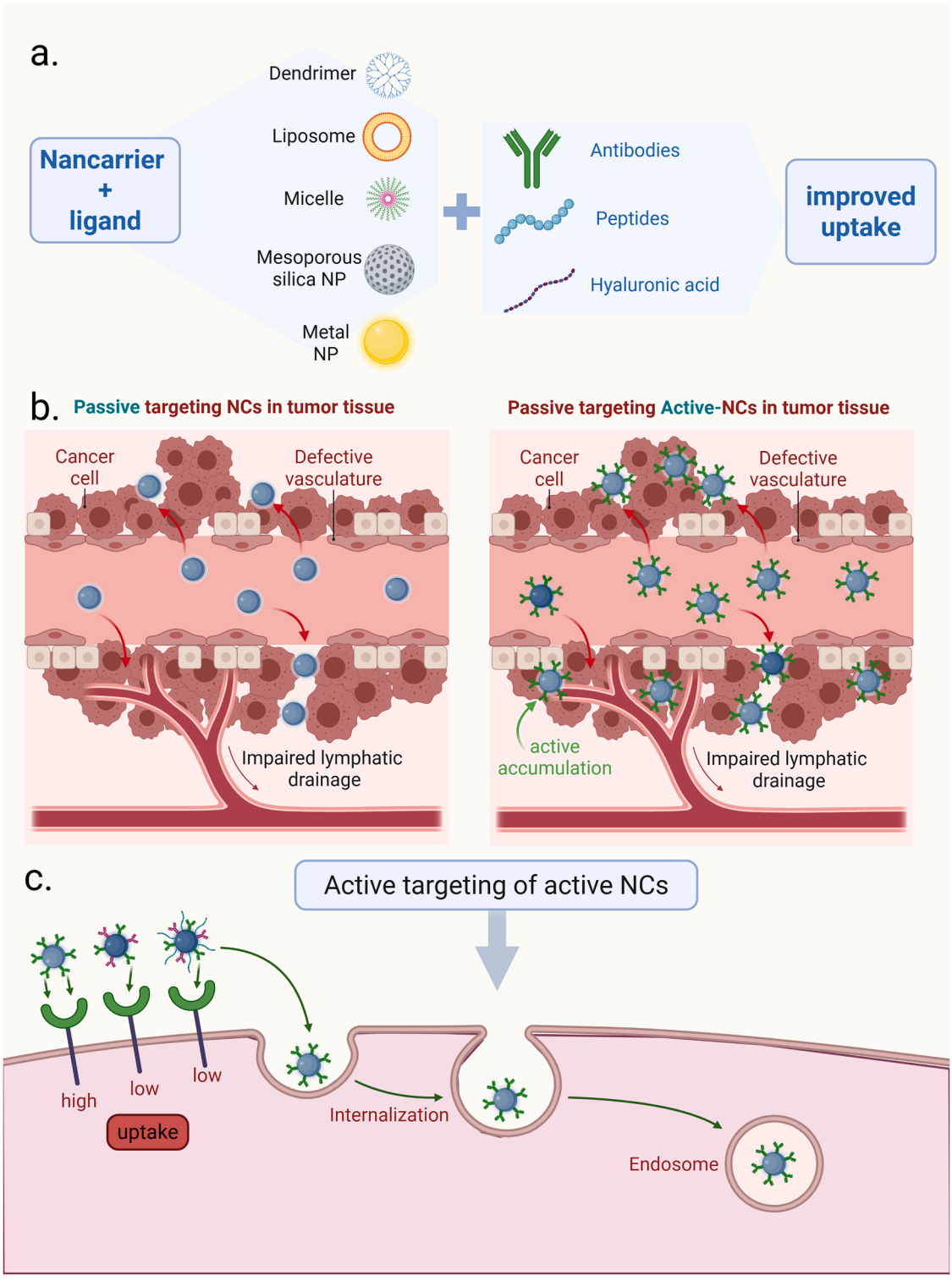

Fig. 1. Schematic overview of passive versus active nanocarrier (NC) targeting in solid tumours. (**a**) Common nanoplatform cores—dendrimers, liposomes, polymeric micelles, mesoporous silica, and metallic nanoparticles—can be surface-decorated with affinity ligands (e.g., antibodies, tumour-homing peptides, hyaluronic acid) to create "active-NCs" with improved uptake; (**b**) In passive targeting, drug-loaded NCs accumulate in the tumour interstitium through the enhanced permeability and retention (EPR) effect driven by leaky vasculature and ineffective lymphatic drainage. The same EPR effect still operates for active-NCs, but ligand decoration promotes additional active accumulation within the tumour mass. (**c**) Ligand–receptor recognition at cancer-cell membranes triggers receptor-mediated endocytosis, yielding high intracellular uptake and endosomal trafficking of the nanocarrier and its therapeutic payload [13,14]. Created in BioRender. Kulbacka, J. (2025) https://BioRender.com/o7p8nmu.

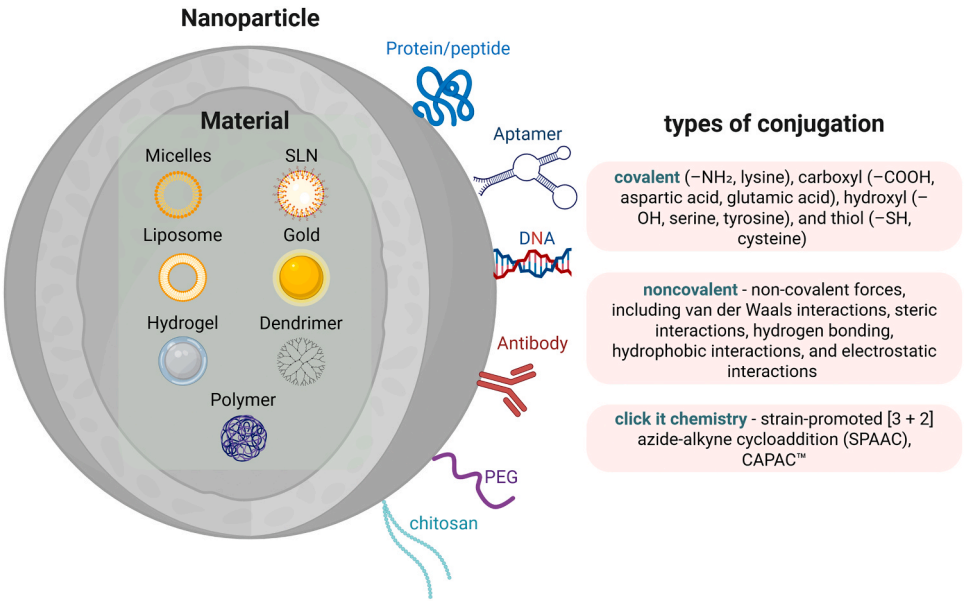

Fig. 2. Overview of nanoparticle materials and conjugation strategies for protein/peptide and antibody functionalization in drug delivery systems, including various materials, e.g., micelles, liposomes, hydrogels, polymers, solid lipid nanoparticles (SLNs), gold nanoparticles, and dendrimers. Conjugation types are categorized as covalent (e.g., lysine, thiol), noncovalent (e.g., hydrogen bonding, electrostatic interactions), and click chemistry (e.g., SPAAC). These functionalized nanoparticles enable targeted and efficient delivery of drugs. (Created in BioRender. Kulbacka, J. (2025) https://BioRender.com/x87g192).

- Stability and durability - in situ functionalization typically results in more stable and durable functionalization because the functional groups are incorporated directly into the surface of the nanoparticle during their formation. However, the stability of post-synthesis functionalization can be more variable because functional groups can be added in a separate step, and they can be exposed to environmental factors.
- Speed and efficiency - in situ functionalization is faster and more efficient because it eliminates the need for additional steps compared to post-synthesis, which can require extra time and phases.
- Applications- functionalization *in situ* is very good for applications requiring precise control over the surface or uniform and stable functionalization (drug delivery systems). In the post-synthesis case, it provides more flexibility, allowing for the customization of nanoparticles. It can be useful in imaging or therapeutic interventions because of the need for specific functional groups' interaction with target molecules [16−20].

4. Click-chemistry

4.1. Introduction to click-chemistry

In 2022, a Nobel Prize in Chemistry was awarded to Professors K. Barry Sharpless, Morten Meldal, and Carolyn Bertozzi. Their work involved developing a new approach to chemical transformations known as "click chemistry." Undoubtedly, this idea, first described by Sharpless and colleagues in 2001, has had a major impact on chemistry and chemical biology. The concept itself is relatively uncomplicated: creating a vast majority of complex structures of organic molecules, such as proteins, nucleic acids, and polysaccharides, from only a few smaller subunits through a few chemical reactions. It is like building something from a set of building blocks. The word „click" refers to satisfaction, which comes from snapping two pieces together, much like connecting two different objects through a luggage strap connection. For a reaction to be considered useful in click chemistry, it should be simple, modular, rapid, efficient, clean, and water-compatible. Moreover, the bonds created between molecules ought to be irreversible, and it should be possible to carry out this reaction in physiological conditions [21,22].

Sharpless and Medal separately invented a reaction considered a "classical click ligation." It is known as the copper-catalyzed azide-alkyne cycloaddition – also referred to as CuAAC. The use of this reaction in biological systems is limited due to copper's toxic properties. Bertozzi, however, resolved this issue by creating a two-component click ligation in which, instead of the catalyst, the ring strain of cyclic octane is used to achieve this cycloaddition. With this innovation, a new era of click chemistry emerged: click ligations can now be used in biological systems. Another copper-free reaction, the strain-promoted [3 + 2] azide-alkyne cycloaddition (SPAAC), has proven useful in living cells. Furthermore, SPAAC reactions have been shown to be significantly faster than the inverse electron demand Diels-Alder (iEDDA) reaction. It is a cycloaddition of s-tetrazine and trans-cyclooctene (TCO) derivatives, and it was invented by Blackman [21,22]

4.2. Metabolic engineering

Click chemistry provides researchers with the opportunity to modify living cells using biomolecules, such as lipids, proteins, and glycans, which contain chemical tags. Click chemistry enables scientists to introduce SPAAC and iEDDA chemical substrates, utilizing sugar analogs, into living cells. This branch of metabolic engineering can be referred to as metabolic glycoengineering. How can this be useful? It is possible to incorporate monosaccharides with those chemical tags into glycans, and then the chemical tags, such as alkene, azide, or alkyne, are presented on the cell surface. Reportedly, after approximately 14 days, azide groups presented on the cell surface are internalized and hydrolyzed. In spite of the fact that chemical tags gradually disappear from the surface of the cells, metabolic glycoengineering proves to be an effective way of cell labeling and functionalization via click chemistry [23].

Inventing click chemistry proved to be a breakthrough not only in biochemistry but also in drug delivery. It might give a new perspective on therapeutic options for many cancer patients. Firstly, tumor-specific cell labeling with azide groups could be used to target cancer cells. Ac4ManNAz proved to be effective in introducing azide groups onto the cell surface in vivo without signs of toxicity [23]. In addition, click chemistry has enabled the identification of new antitumor molecules such as P19G1. The scientists discovered P19G1 by synthesizing many

Erlotinib derivatives. Further experiments proved the inhibitory and antimetastatic properties of this molecule in A549 lung adenocarcinoma cells. P19G1 was tested in vivo and in vitro, and no toxic properties were observed. Click chemistry has proven to be far more efficient than traditional synthesis methods, and it could potentially enhance the process of creating new anticancer molecules [24]. Another case in which click chemistry proves its efficacy in oncology is Click Activated Protodrugs Against Cancer (CAPAC™). These prodrugs are activated at the tumor site, which magnifies their therapeutic effect. SQ3370 is one of them, and it contains tumor-localizing biopolymer (SQL70) and chemically attenuated doxorubicin (DOX), which exhibited promising outcomes in Phase 1 clinical trials [25].

4.3. Click chemistry in cell transplantation

Cell transplantation proved to be effective in the management of a wide variety of diseases. Several new clinical trials of cell-based therapy using mesenchymal stem cells have been conducted recently. Unfortunately, low engraftment rates and short survival durations fail to achieve satisfactory therapeutic effects. Recent in-vivo bioorthogonal "click" systems—most notably tetrazine–TCO and cyclopropene ligations—now enable proteins to be snapped onto pre-targeted nanoparticles inside the body, boosting tumor uptake while sparing healthy tissue [26,27]. Parallel advances in stimuli-responsive PFNPs use light-cleavable o-nitrobenzyl linkers, pH-labile hydrazones and redox-sensitive disulfides to trigger payload release with pinpoint spatiotemporal control [28,29]. Thus, the current attempts have been made to enhance the effects of therapy by functionalizing these cells. Click-chemistry creates an opportunity to track transplanted cells in vivo. With this method, it is possible to gain information about the proliferation, cell death, migration, and translocation of cells. It is crucial for developing an efficient therapeutic strategy [23].

4.4. Safety and regulatory limitations of click ligations

Although copper(I)-catalysed azide–alkyne cycloaddition (CuAAC) remains a work-horse ligation in nanomedicine manufacturing, residual copper poses reactive-oxygen-species (ROS) and hepatotoxicity risks in vivo. Concentrations $\geq$ 50 μM Cu(I) have been reported to compromise fibroblast viability within 24 h, while single-dose maximum tolerated doses in murine models rarely exceed 5 mg/kg. Strategies to mitigate this include the use of tris-(hydroxypropyltriazolyl)methyl-amine (THPTA) or bathocuproinedisulfonic acid to sequester free Cu(I) and rigorous diafiltration steps. Nevertheless, for the in-situ click assembly of protein-functionalized nanoparticles (PFNPs), bioorthogonal, catalyst-free alternatives such as strain–promoted azide–alkyne cycloaddition (SPAAC) or inverse–electron–demand Diels–Alder (iEDDA) reactions are preferred. Regulatory agencies are increasingly requesting quantitative data on residual metals and their clearance profiles, making catalyst-free ligations a more attractive option for late-stage translation. Table 1 also indicates the key limitations that allow residual metal limits and the current technology readiness level (TRL) for each click reaction. Cell transplantation proved to be effective in the management of a wide variety of diseases. Several new clinical trials of cell-based therapy using mesenchymal stem cells have been conducted recently. Unfortunately, low engraftment rates and short survival durations fail to achieve satisfactory therapeutic effects. Recent attempts have been made to enhance the therapeutic effects by functionalizing these cells. Click-chemistry creates an opportunity to track transplanted cells in vivo. With this method, it is possible to gain information about the proliferation, cell death, migration, and translocation of cells. It is crucial for developing an effective therapeutic strategy.

Table 1
Functionalization methods and their applications.

Target	Type of nanoparticle	Functionalization method	Targeting strategy	Application	Limitations	Clinical status	Reference
Breast cancer cells	Gold nanoparticles (AuNPs)	carbodiimide crosslinking chemistry – attaching Anti-ERα antibodies to the gold surface, PEGylation	Active (AuNPs functionalized with an anti-ERα (estrogen receptor alpha) antibody and BPE (1,2-bis(4-pyridyl) ethylene) Raman reporter (ERα-AuNPs))	Photothermal/ theranostic therapy cancer therapy	heterogeneous ERα expression; RES uptake and long-term Au retention; Limited light penetration	Pre-clinical (in vitro & mouse xenografts)	[49]
Cancer cells	Gold nanoparticles	Covalent coupling The azide group was covalently coupled to alkyne-functionalized NPs after incorporation into OmpA, and DNA-functionalization	Outer membrane vesicle-functionalized nanoparticles (OMV-NPs)	Cancer therapy Vaccine development	OMV immunogenicity; batch-to-batch reproducibility; scale-up of hybrid OMV-NPs	cancer vaccines entering Phase I	[53]
Prostate cancer	Gold nanoparticles	Gold nanoparticles functionalized with polyethylene glycol of different chain lengths	Passive (EPR strategy)	CT imaging and radiotherapy/ photothermal therapy	Variable EPR effect in humans; non-specific RES clearance; CT dose concerns	Early clinical; gold-silica nanoshell "AuroShell" trials (focal ablation, NCT02680535 etc.)	[54,55]
Lung cancer	Silica Nanoparticles	covalent coupling (site-specific conjugation via EGF functionalization), chitosan coating	Active (EGFR receptor targeting)	Drug delivery (EGFR)	Slow SiO_2 biodegradation; potential pulmonary toxicity	Pre-clinical (cell & mouse models only)	[56]
Gastric Cancer	Gold Nanoparticles	Covalent conjugation with polyethylene glycol (PEG), aptamer-based targeting	Active (HER2 receptor targeting via antibodies)	drug delivery and photothermal therapy	HER2 heterogeneity/ resistance; Au accumulation and immunogenicity; laser penetration limits	Pre-clinical	[57]
Glioblastoma	Solid lipid nanoparticles	Covalent conjugation (peptide bond), polyelectrolyte surface modification	Active (melanotransferrin targeting via antibodies)	Therapy of glioblastoma	OMV immunogenicity; batch-to-batch reproducibility; scale-up of hybrid OMV-NPs	cancer vaccines entering Phase I	[58,59]

5. Covalent and noncovalent coupling

The attachment of specific proteins to nanoparticles has brought significant advances in molecular and cellular biology. This innovation has improved drug delivery in vivo, medical, and tumor imaging, and opened up new ways to cross the blood-brain barrier [30−33].

5.1. Covalent coupling methods

In many areas of biochemistry, covalent conjugation is the preferred method for coupling specific proteins to a solid surface. Site-selective covalent conjugation ensures oriented and predictable conjugation of biomolecules, making it the method of choice to preserve protein conformation [34,35]. Covalent conjugation typically involves specific and reactive functional groups in proteins, such as amino (–NH_2, lysine), carboxyl (–COOH, aspartic acid, glutamic acid), hydroxyl (–OH, serine, tyrosine), and thiol (–SH, cysteine) [30,36]. One of the most important strategies is maleimide-thiol conjugation chemistry, which occurs between thiol groups in proteins, either naturally occurring or synthetically introduced, and maleimides on the nanoparticle surface [30]. This reaction can be carried out in an aqueous environment under mild conditions. Additionally, highly reactive maleimides exhibit good selectivity towards thiols at physiological pH. The resulting thioether bond is relatively stable [37].

5.2. Non-covalent coupling methods

Non-covalent interactions are also commonly used in the bioconjugation of nanoparticles. These reactions are mainly based on non-covalent forces, including van der Waals interactions, steric interactions, hydrogen bonding, hydrophobic interactions, and electrostatic interactions [38].

Electrostatic interactions, based on the attraction or repulsion of electrical charges, enable the functionalization of nanoparticles with proteins using simple methods such as surface adsorption. A wide range of proteins can adsorb easily through strong electrostatic interactions with the nanoparticle surface [39]. Conjugation via electrostatic attraction is a simple and easy method, as it does not require additional chemical reagents or catalysts. However, the resulting conjugates are generally considered less stable than those formed through covalent crosslinking due to possible dissociation when their charges are screened, such as in high-salt environments [40]. Hydrophobic interactions can also be utilized for bioconjugation. The hydrophobic binding sites of proteins may interact with lipophilic drugs, facilitating the encapsulation of these hydrophobic bioactives in the core of a protein nanoparticle, leaving the hydrophilic surface in contact with the aqueous medium [41]. Consequently, research has focused on developing new methods to induce protein self-assembly triggered by hydrophobic drug incorporation, serving as a drug delivery system [42].

The biotin-avidin interaction is another popular strategy for highly stable non-covalent bioconjugation. The avidin-biotin interaction is one of the strongest non-covalent interactions in nature. Compared to other covalent and non-covalent interactions, the avidin-biotin system offers several significant benefits, including signal amplification, efficient performance, high stability, and the ability to utilize highly diluted primary antibodies [43]. The π-π interactions, a subtype of non-covalent forces, play a crucial role in nanoparticle functionalization, particularly in stabilizing conjugates through aromatic stacking. These interactions occur between aromatic rings in biomolecules and π-electron-rich surfaces of nanomaterials, such as graphene-based nanoparticles or functionalized gold nanoparticles. π-π coupling enhances binding specificity and stability, offering a versatile approach for drug delivery and biosensing applications. Compared to electrostatic and hydrophobic interactions, π-π stacking provides an additional stabilization mechanism, particularly in aqueous environments, making it a valuable tool in nanoparticle bioconjugation strategies [44]. Both covalent and non-covalent methods of conjugating nanoparticles with proteins are significant. Covalent bonds are stronger and more stable, which can prevent the displacement of functionalized proteins by serum proteins after in vivo administration or their removal during purification [45]. Non-covalent bonds offer simpler methods for producing ready-to-use nanoparticles, providing good binding stability and specific targeting [40]. Fig. 1 below summarizes the types of nanoparticles, their functionalization, and types of conjugation.

6. Selectivity and applications of functionalization

A significant development in nanomedicine, protein-functionalized nanoparticles have immense promise for (1) targeted drug delivery, (2) imaging and diagnostics, and (3) biosensing and detection. The ability to functionalize nanoparticles with specific proteins enables the creation of multifunctional platforms capable of precise interactions with biological targets. Selectivity is paramount in biomedical applications to ensure the accurate targeting of diseased tissues or cells while minimizing off-target effects. Selectivity is achieved through receptor-specific interactions, ligand-mediated targeting, and functional surface modifications, facilitating tumor localization via the enhanced permeation and retention (EPR) effect. Stability under physiological conditions is crucial, as extreme pH levels can induce protein denaturation or conformational changes, thereby affecting functionality. Proteins like albumin and casein further enhance tumor targeting through receptor binding and stimuli-responsive drug release, positioning PFNPs as promising tools for advanced theranostic applications in cancer nanomedicine [46,47].

In addition, in targeted drug delivery systems, pH can affect the PFNPs' drug release kinetics. The relevance of pH-responsive drug release mechanisms in improving the efficacy and selectivity of PFNP-based drug delivery platforms has been discussed by Jokerst et al. (2011). By incorporating pH-sensitive linkers or coatings onto PFNPs, researchers can achieve controlled release of therapeutic agents in response to the acidic pH environment of tumor tissues, minimizing off-target effects and improving treatment outcomes [46]. The pH of the medium affects the surface charge of nanoparticles, which, in turn, influences the adsorption of proteins onto nanoparticle surfaces. At pH values above the protein's isoelectric point (pI), proteins tend to be negatively charged and may undergo electrostatic repulsion with negatively charged nanoparticles. Contrarily at pH levels below a protein's isoelectric point, proteins acquire a positive charge, promoting their attachment to negatively charged nanoparticles via electrostatic forces. The pH conditions also play a crucial role in optimizing the stability and aggregation tendencies of PFNPs in biological fluids. Smith et al. explored how pH influences the colloidal stability of protein-coated QDs used for cellular imaging. Their findings revealed that variations in pH affect the surface charge and aggregation behavior of QDs, potentially impacting their cellular uptake and imaging efficiency [48].

In targeted drug delivery, they enhance therapeutic efficacy and reduce side effects by directing drugs specifically to diseased cells. Elzoghby et al. discuss hybrid protein-inorganic nanoparticles designed for tumor-targeted drug delivery. These nanoparticles benefit from the specificity of the protein-ligand interaction, enabling them to effectively target cancer cells and increase the uptake of therapeutic agents in tumors, while reducing off-target effects. This hybrid approach combines the stability and controlled release properties of inorganic nanoparticles with the targeting capabilities of proteins, resulting in highly effective drug delivery systems [49]. Oxidized SWCNTs functionalized with cisplatin, an anticancer drug, and epidermal growth factor (EGF) were also used to demonstrate targeted drug delivery in vivo. When EGF-SWCNT was injected into mice with head and neck squamous cell carcinoma, the nanoparticles targeted EGF receptors overexpressed on tumour cells, resulting in slower tumour growth [50].

PFNPs are also being developed as advanced tools for medical

imaging and diagnostics, providing enhanced contrast and specificity in various imaging modalities. Functionalization of nanoparticles with proteins enhances their ability to precisely target specific cells or molecular processes while also enabling the visualization of these processes. Oliveira et al. studied carbon nanomaterials functionalized with proteins for use in biomedical imaging. By incorporating specific proteins, these nanomaterials demonstrated enhanced biocompatibility and improved targeting for imaging techniques, including fluorescence imaging, magnetic resonance imaging, and computed tomography. The functionalization of the proteins facilitates selective binding to target tissues, thereby increasing contrast and imaging specificity [51].

In biosensing and detection, they offer highly sensitive and selective detection of biomolecules, pathogens, and environmental contaminants. Nagaraju *et al.* noticed that the application of protein-functionalized carbon nanotubes (CNTs) in biosensing. Protein functionalization provides CNTs with selectivity, enabling the detection of specific biomolecules, such as glucose, cholesterol, and various disease-related proteins. These PFNP-based biosensors offer high sensitivity and fast response times, making them valuable tools for diagnostic applications and environmental monitoring [52]. Table 1 below summarizes various functionalization methods of nanoparticles, highlighting their targeting strategies and applications, such as gold nanoparticles functionalized with antibodies or polyethylene glycol for cancer therapy and imaging, offering promising advancements in precision medicine [49], [53].

7. Protein corona

It is crucial to understand how engineered nanomaterials interact with cells, organisms, and biological macromolecules in order to develop sustainable nanotechnologies [60]. Furthermore, protein caps on the surface of nanoparticles frequently determine their biological response because they alter their conformation and/or dynamically exchange with other proteins. Nanoparticles that get integrated into biological systems are practically always coated in biofluids. Therefore, to build up the selective transfer of nanoscale objects to a particular compartment of the body, one must comprehend the phenomena of conformational change and protein displacement at the interface [61, 62]. From a nano-medical perspective, competitive binding and protein displacement, known as the Vroman effect, are also very significant because they determine the selective transport of nanoscale objects to specific compartments of the body [63,64]. Dawson et al. have delineated the thermodynamics and kinetics of protein binding to polymeric nanoparticles in human plasma using a vast number of analytical methods [65,66]. They have delineated the presence of two forms of protein coronas on nanoparticle surfaces: a soft corona and a hard corona. Dawson and colleagues further identified nanoparticle size and surface chemistry as factors that determine the nanoparticle-protein corona structure. In a more recent publication, scientists determined plasma-derived protein coverage on the surface of polystyrene nanoparticles and silica nanoparticles to be long-lived enough that they, not the surface of the nanomaterial, are most likely to be perceived by the cell. Observe that protein-nanoparticle interaction is a function of their surface curvature. Extremely small nanoparticles have been found to inhibit protein adsorption under certain conditions. The phenomena described by L. Vroman [63] allow dynamics of biomolecules when in contact with nanoscale objects of interest for both in vitro and in vivo experiments to be modelled and predicted. Additionally, Norde's protein study showed that biomolecular reorganization upon treatment using functional materials is defined by the surface shape and material charge [67]. The important characteristics of nanoparticles are their small size and extremely high surface-area-to-volume ratios, and the importance of surface cannot be overemphasized when considering these particles. The peculiarity of this solid-liquid interface comes from the high surface area of nanoparticles, which tends to preferentially adsorb chemicals or biomolecules to decrease their surface energy [68]. The preference of a nanomaterial for biomolecules to adsorb is an integrated function of many adsorption sites on nanoparticle surfaces adjacent to amino-acid residues of proteins and not an individual and distinctive adsorption site.

The chemical structure, size, shape, and surface properties of nanoparticles control the protein adsorption onto the nanoparticles and, consequently, the interaction of the nanoparticles with cells and tissues. Nanomaterials bound with proteins can affect physiological and pathological changes, including macrophage uptake, blood coagulation, protein aggregation, and complement activation. Still, the mechanisms that lead to these changes remain only partially understood [69]. Thus, development in this field, based on the exploration of methodologies to follow changes in biofluids at interfaces, is crucial for nanomedicines and pharmacology. Morphology, surface characteristics, and dimensions of a nanoparticle are all critical determinants of its in vivo biodistribution. The effects of size have been extensively studied for spherically shaped particles, and some general trends have been established. Particles with a diameter of less than 5 nm are quickly eliminated from the circulatory system via extravasation or urinary excretion. As the particle diameter increases from the nanometre size range to ~15 μm, they are primarily deposited in the liver, spleen, and bone marrow. The biodistribution of nanoparticles within a particle diameter range of ~10 nm to 15 μm is highly inhomogeneous.

The bionano interface comprises dynamic physicochemical interactions, kinetics, and thermodynamic exchanges between nanomaterial surfaces and the surfaces of biological components (e.g., proteins, membranes, phospholipids, endocytic vesicles, organelles, DNA, and biological fluids) [70]. To continue down this path, we must get to know the dynamic forces and molecular elements that govern these interactions. It is impossible to describe with certainty all the biophysical and chemical interactions occurring at the interface. Still, we are at the point where the accumulated knowledge has begun to form a conceptual framework for further exploration [64].

Norde hypothesized that two classes of proteins exist: "hard" and "soft" [71]. Hard proteins exhibit strong internal order and undergo minimal structural alterations, which contribute little to the adsorption process. These proteins adsorb on hydrophobic surfaces, whereas on hydrophilic surfaces, they adsorb only if they are electrostatically attracted. "Soft" proteins, which have lower structural stability, adsorb onto hydrophilic, electrostatically repellent surfaces, even under seemingly unfavourable conditions. These proteins have a large driving force for adsorption as a result of their structural rearrangements [72–74]. Following these findings, it is crucial to analyze how the surface modification of functional, non-toxic materials affects the reorganization and conformational changes of proteins.

It is not the nanoparticles themselves, but the nanoparticle-protein system, that accurately mirrors the carriers' intrinsic characteristics and therapeutic behaviour once inside the body. Thus, a detailed understanding of the mechanisms of nanoparticle-protein complex formation is crucial for anticipating and controlling the nanoparticle pathway in vivo, including its biodistribution, bioavailability, and toxicity. Existing models of correlation between biological response and therapeutic effect, which take into account the action of the protein corona on nanoparticles, are more precise than existing models that primarily consider the physicochemical properties of the nanoparticles used. Using insights from these correlation models, researchers can engineer nanomaterials that reveal which protein-binding motifs are needed to create next-generation drug-delivery systems or diagnostic tools [75,76].

Model protein corona, particularly proteins that shape cell internalization and downstream biological effects, making them valuable probes for identifying receptors that are over-expressed on the target cells as a result of finding new pharmacological therapies [77]. Additionally, the presence of various types of proteins adsorbed on nanoparticles provides detailed information on biological fluids and blood components that nanoparticles encounter during their passage through the body. This information can be used to study and diagnose

physiological changes in the body associated with protein modification and structural changes, such as cancer formation and the prognosis of the progression of many diseases [7]. Extracellular vesicles, which carry a variety of biological materials, including proteins, lipids, nucleic acids, and metabolites, exhibit a similar effect. Because vesicles have a distinctive molecular signature that reflects their origin, they have become potential biomarkers of diseases, including diabetes and its complications.

Caracciolo [7,78] noted that different disease types change the interaction with the nanocarrier. Changes in plasma-protein levels, alterations in protein conformation, and shifts in body temperature, which are directly related to the state and type of disease, can alter the composition of proteins adsorbed by nanoparticles. This discovery is a new direction in the design of safe and highly effective DDS circuits for a tailored drug-delivery platform [79]. To improve the precise prediction of in vivo biological results and practical applications of the protein corona in pharmaceutical sciences, a complete evaluation of the nanoparticle-protein interaction under engineered biomimetic conditions should be carried out [80]. Table 2 below summarizes key aspects of protein corona formation on nanoparticles, its role in biological interactions, and its implications for drug delivery and nanomedicine.

7.1. Corona composition varies across nanoparticle types

Another aspect is how corona composition varies across nanoparticle types. The composition of the protein corona is highly material-specific. On surfactant-free gold nanocrystals, serum albumin dominates and displaces classical opsonins (C3, IgG), helping the particles evade complement and extend circulation times —an effect now traced to strong Au–S/Au–N coordination with albumin and annexins [83]. In contrast, dextran-coated superparamagnetic iron-oxide nanoparticles (SPIONs) utilize appropriate proteins depending on their surface chemistry, e.g., carboxylated SPIONs activate the alternative pathway. They can bind C3b indirectly, amplifying macrophage phagocytosis and even triggering adaptive anti-leukaemia immunity, whereas aminated SPIONs bind C3b directly via the lectin pathway and push macrophages toward an "exhausted" phenotype [84,85]. In the case of inorganic oxides, the surface curvature is of high importance. Proteomics of 10–100 nm silica nanoparticles reveals that high curvature (< 30 nm) selectively enriches apolipoproteins, while larger particles accumulate coagulation factors. This size-dependent corona was recently linked to inducing faster exocytosis of 100 nm SiO_2 from intestinal cells [86]. Among soft, ionizable lipid nanoparticles (LNPs), multi-omics screens revealed a strong correlation between HDL-enriched coronas (ApoA-I/ApoA-II) and high mRNA-delivery potency. HDL outperforms the canonical ApoE biomarker and its abundance shifts with donor physiology (lean vs obese plasma) [87]. Interestingly, poly(lactic-co-glycolic acid) (PLGA) carriers behave differently again: in plasma, they load dysopsonins such as clusterin and ApoA-I, but in cerebrospinal fluid, the corona flips to transferrin + transthyretin, doubling association with neurons and glia while PEGylation suppresses this effect [88]. Finally, regarding cadmium-telluride quantum dots, the corona is rich in immunoglobulins and acute-phase proteins, which exacerbate M1 polarization and ROS production in macrophages, and promote pyroptosis pathways (NLRC4 → NLRP1/NLRP3) [89]. Thus, these studies underscore the importance of corona composition in protein-functionalized nanocarriers for engineering surfaces that can finally recruit protective dysopsonins (e.g., albumin, clusterin, HDL) rather than attempting to abolish adsorption altogether.

Table 2
Protein corona on nanoparticles [44,81,82].

Factor	Description	Impact on Nanoparticles
Formation mechanism	Proteins in biological fluids adsorb onto the nanoparticle surface via electrostatic, hydrophobic, van der Waals, and π-π interactions.	Alters nanoparticle surface properties, affecting stability, aggregation, and biodistribution
Hard vs. soft corona	A hard corona consists of tightly bound proteins, while a soft corona is formed by loosely associated proteins in dynamic exchange.	A hard corona is more stable, whereas a soft corona undergoes rapid exchange in biological environments.
Protein composition	It depends on the nanoparticle material, surface charge, size, and exposure conditions (e.g., plasma, serum, or cellular environments).	Determines cellular uptake, immune recognition, and bioavailability.
Biological Identity	The protein corona modifies the nanoparticle's synthetic identity, influencing its interaction with cells and tissues.	Impacts nanoparticle clearance, toxicity, and targeting ability.
Influence on drug delivery	The corona can act as a barrier or mediator for drug release, affecting therapeutic efficiency	It may enhance or reduce nanoparticle targeting efficiency depending on the corona's composition.
Clearance and immune response	Corona composition determines nanoparticle recognition by the immune system (opsonization vs. stealth effect).	Stealth coatings (e.g., PEGylation) reduce immune recognition, while opsonins promote clearance by macrophages.
Nanoparticle surface engineering	Surface functionalization (e.g., PEG, zwitterionic coatings, or specific ligands) modulates corona formation and stability.	It helps tailor nanoparticle behavior for specific biomedical applications.

8. Conclusions

Protein-functionalized nanoparticles (PFNPs) are a very dynamically developing field, as evidenced by the increasing number of scientific papers published on this topic. PFPNs provide possibilities that traditional drug delivery methods, such as biocompatibility, precise targeting, and versatility, cannot offer. By using the self-assembly properties of proteins, it is possible to develop solutions to contemporary medical problems that use complex nanoparticles.

Proteins offer numerous opportunities to create innovative, nanostructured materials that will lead to further development in this field. This review illustrates the swift advancements made in recent years. In comparison, significant challenges such as the cytotoxic effect on cells and low cellular uptake efficiency remain before these tools become commonplace. However, the versatility and multitude of methods at our disposal, which can be used to adjust the properties of nanoparticles, make it possible to resolve current difficulties in the future. The use of protein-functionalized nanoparticles will have a significant impact on the development of unmet fields, including diagnostics, cancer therapy, imaging, and drug delivery. The hybrid combination of functionalized nanoparticles with therapeutic agents used in tumor-targeted drug delivery seems to be particularly promising. These functionalization approaches demonstrate notable strengths, e.g., their modular chemistry, which permits high-affinity, receptor-specific targeting while simultaneously accommodating imaging or photothermal cargos for theranostic solutions. Yet, their clinical translation remains constrained by target heterogeneity, off-target RES sequestration, and unresolved scale-up and batch consistency issues that, together, can decrease the efficacy of these nanoplatforms.

An innovative approach to chemistry, such as click chemistry, offers possibilities for forming covalent and non-covalent bonds within structures, as well as the ability to add functional groups to the surface of nanoparticles. This provides hope for the widespread use of this technology in various applications. The future challenges include developing GMP-compatible manufacturing routes for protein ligands, integrating AI-guided ligand design to overcome tumour-antigen heterogeneity, and engineering stimulus-responsive PFNPs that can adapt dynamically to the evolving tumour microenvironment. The future of protein nanotechnology in drug delivery is indeed bright, promising transformative

impacts on healthcare and patient outcomes.

CRediT authorship contribution statement

Agata Hutny: Writing – original draft, Methodology, Investigation, Data curation. **Anna Wietrzyk:** Writing – original draft, Methodology, Investigation, Data curation. **Julita Kulbacka:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Barbara Jachimska:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Karol Biliński:** Writing – original draft, Methodology, Investigation, Data curation. **Laura Jonderko:** Writing – original draft, Methodology, Investigation, Data curation. **Jarosław Kalinin:** Writing – original draft, Methodology, Investigation, Data curation. **Małgorzata Makieła:** Writing – original draft, Methodology, Investigation, Data curation.

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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

References

[1] G. Bashiri, M.S. Padilla, K.L. Swingle, S.J. Shepherd, M.J. Mitchell, K. Wang, Nanoparticle protein corona: from structure and function to therapeutic targeting, Lab Chip 23 (2023) 1432–1466, https://doi.org/10.1039/d2lc00799a.
[2] K. Strebhardt, A. Ullrich, Paul Ehrlich's magic bullet concept: 100 Years of progress, Nat. Rev. Cancer 8 (2008), https://doi.org/10.1038/nrc2394.
[3] MLA style: The Nobel Prize in Physiology or Medicine 1908. NobelPrize.org. Nobel Prize Outreach AB 2024. Fri. 14 Jun 2024. <⟨https://www.nobelprize.org/prizes/medicine/1908/summary/⟩> n.d.
[4] Y. Matsumura, H. Maeda, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs, Cancer Res 46 (1986) 6387–6392.
[5] V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect, Adv. Drug Deliv. Rev. 63 (2011) 131–135, https://doi.org/10.1016/j.addr.2010.03.011.
[6] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology, Adv. Drug Deliv. Rev. 66 (2014) 2–25, https://doi.org/10.1016/j.addr.2013.11.009.
[7] G. Caracciolo, D. Caputo, D. Pozzi, V. Colapicchioni, R. Coppola, Size and charge of nanoparticles following incubation with human plasma of healthy and pancreatic cancer patients, Colloids Surf. B Biointerfaces 123 (2014) 673–678, https://doi.org/10.1016/j.colsurfb.2014.10.008.
[8] S. Balog, M.S. de Almeida, P. Taladriz-Blanco, B. Rothen-Rutishauser, A. Petri-Fink, Does the surface charge of the nanoparticles drive nanoparticle–cell membrane interactions? Curr. Opin. Biotechnol. 87 (2024) 103128 https://doi.org/10.1016/J.COPBIO.2024.103128.
[9] K. Öztürk, M. Kaplan, S. Çalış, Effects of nanoparticle size, shape, and zeta potential on drug delivery, Int J. Pharm. 666 (2024) 124799, https://doi.org/10.1016/J.IJPHARM.2024.124799.
[10] M. Alavi, M. Hamidi, Passive and active targeting in cancer therapy by liposomes and lipid nanoparticles, Drug Metab. Pers. Ther. 34 (2019), https://doi.org/10.1515/dmpt-2018-0032.
[11] M.P. Monopoli, C. Åberg, A. Salvati, K.A. Dawson, Biomolecular coronas provide the biological identity of nanosized materials, Nat. Nanotechnol. 7 (2012) 779–786, https://doi.org/10.1038/nnano.2012.207.
[12] B. Yu, H.C. Tai, W. Xue, L.J. Lee, R.J. Lee, Receptor-targeted nanocarriers for therapeutic delivery to cancer, Mol. Membr. Biol. 27 (2010) 286–298, https://doi.org/10.3109/09687688.2010.521200.
[13] G. Su, H. Jiang, B. Xu, Y. Yu, X. Chen, Effects of Protein Corona on Active and Passive Targeting of Cyclic RGD Peptide-Functionalized PEGylation Nanoparticles, Mol. Pharm. 15 (2018) 5019–5030, https://doi.org/10.1021/ACS.MOLPHARMACEUT.8B00612/SUPPL_FILE/MP8B00612_SI_001.PDF.
[14] M.F. Attia, N. Anton, J. Wallyn, Z. Omran, T.F. Vandamme, An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites, J. Pharm. Pharmacol. 71 (2019) 1185–1198, https://doi.org/10.1111/JPHP.13098.
[15] Navarro-Tovar G., Salado-Leza D., Carreón-Álvarez C., Acosta-Ruelas B.J., Rodríguez-López J.L. Surface functionalization of nanoparticles: Structure determines function. Antimicrobial Activity of Nanoparticles: Applications in Wound Healing and Infection Treatment 2023:203–248. https://doi.org/10.1016/B978-0-12-821637-8.00004-3.
[16] G.H. Woehrle, L.O. Brown, J.E. Hutchison, Thiol-functionalized, 1.5-nm gold nanoparticles through ligand exchange reactions: scope and mechanism of ligand exchange, J. Am. Chem. Soc. 127 (2005) 2172–2183, https://doi.org/10.1021/ja0457718.
[17] Y. Wang, M. Zhang, Z. Yan, S. Ji, S. Xiao, J. Gao, Metal nanoparticle hybrid hydrogels: the state-of-the-art of combining hard and soft materials to promote wound healing, Theranostics 14 (2024) 1534–1560, https://doi.org/10.7150/thno.91829.
[18] T.A. Seidu, P.T. Kutoka, D.O. Asante, M.A. Farooq, R.N. Alolga, W. Bo, Functionalization of Nanoparticulate Drug Delivery Systems and Its Influence in Cancer Therapy, Pharmaceutics 14 (2022), https://doi.org/10.3390/pharmaceutics14051113.
[19] A.E. Dunn, D.J. Dunn, A. Macmillan, R. Whan, T. Stait-Gardner, W.S. Price, et al., Spatial and temporal control of drug release through pH and alternating magnetic field induced breakage of Schiff base bonds, Polym. Chem. 5 (2014) 3311–3315, https://doi.org/10.1039/C4PY00150H.
[20] N. Aminu, I. Bello, N.M. Umar, N. Tanko, A. Aminu, M.M. Audu, The influence of nanoparticulate drug delivery systems in drug therapy, J. Drug Deliv. Sci. Technol. 60 (2020) 101961, https://doi.org/10.1016/j.jddst.2020.101961.
[21] D. Bauer, S.M. Sarrett, J.S. Lewis, B.M. Zeglis, Click chemistry: a transformative technology in nuclear medicine, Nat. Protoc. 18 (2023) 1659–1668, https://doi.org/10.1038/s41596-023-00825-8.
[22] N.K. Devaraj, M.G. Finn, Introduction: click chemistry, Chem. Rev. 121 (2021) 6697–6698, https://doi.org/10.1021/acs.chemrev.1c00469.
[23] Y. Takayama, K. Kusamori, M. Nishikawa, Click chemistry as a tool for cell engineering and drug delivery, Molecules 24 (2019) 172, https://doi.org/10.3390/molecules24010172.
[24] Q. Cui, P. Song, T. Ma, Z. Wang, X. Lu, Y. Shi, et al., Discovery of a novel potent antitumor molecule, P19G1, by Erlotinib derivative libraries synthesized by modular click-chemistry, :153303382211096, Technol. Cancer Res Treat. 21 (2022), https://doi.org/10.1177/15330338221109649.
[25] Sangeetha Srinivasan, Nathan A. Yee, Michael Zakharian, Ma.ša Alečković, Amir Mahmoodi, Tri-Hung Nguyen, et al. SQ3370, the first clinical click chemistry-activated cancer therapeutic, shows safety in humans and translatability across species. bioRxiv [Preprint] n.d. https://doi.org/10.1101/2023.03.28.534654.
[26] A. Mishra, A. Carrascal-Miniño, J. Kim, T.M. de Rosales R, [68 Ga]Ga-THP-tetrazine for bioorthogonal click radiolabelling: pretargeted PET imaging of liposomal nanomedicines, RSC Chem. Biol. 5 (2024) 622–639, https://doi.org/10.1039/D4CB00039K.
[27] L. Chen, F. Jin, Q. Luo, Y. Luo, Q. Zhang, R. Li, et al., Novel Cyclopropene Probes as Chemical Reporters for Bioorthogonal Metabolic Labeling of Benzoylated Post-Translational Modification, Anal. Chem. 97 (2025) 6410–6417, https://doi.org/10.1021/ACS.ANALCHEM.4C04707/ASSET/IMAGES/LARGE/AC4C04707_0005.JPEG.
[28] Y. Liu, T. Wang, W. Wang, Photopharmacology and photoresponsive drug delivery, Chem. Soc. Rev. 54 (2025) 5792–5835, https://doi.org/10.1039/D5CS00125K.
[29] Y. Wu, J. Pei, H. Wang, M. Yu, D. Wang, Development of a KRP-based pH-responsive drug delivery system for solid tumors, Transl. Cancer Res 14 (2025) 3812–3821, https://doi.org/10.21037/TCR-2025-1151/COIF.
[30] A. Cox, D. Vinciguerra, F. Re, R.D. Magro, S. Mura, M. Masserini, et al., Protein-functionalized nanoparticles derived from end-functional polymers and polymer prodrugs for crossing the blood-brain barrier, Eur. J. Pharm. Biopharm. 142 (2019) 70–82, https://doi.org/10.1016/J.EJPB.2019.06.004.
[31] A. Fu, W. Hu, L. Xu, R.J. Wilson, H. Yu, S.J. Osterfeld, et al., Protein-functionalized synthetic antiferromagnetic nanoparticles for biomolecule detection and magnetic manipulation, Angew. Chem. 121 (2009) 1648–1652, https://doi.org/10.1002/ange.200803994.
[32] A. Natarajan, C.-Y. Xiong, C. Gruettner, G.L. DeNardo, S.J. DeNardo, Development of multivalent radioimmunonanoparticles for cancer imaging and therapy, Cancer Biother Radio. 23 (2008) 82–91, https://doi.org/10.1089/cbr.2007.0410.
[33] N. Sathyamoorthy, D. Magharla, S. Vankayalu, Effect of surface modification on the In vitro protein adsorption and cell cytotoxicity of vinorelbine nanoparticles, J. Pharm. Bioallied Sci. 9 (2017) 135, https://doi.org/10.4103/jpbs.JPBS_258_16.
[34] S. Avvakumova, M. Colombo, P. Tortora, D. Prosperi, Biotechnological approaches toward nanoparticle biofunctionalization, Trends Biotechnol. 32 (2014) 11–20, https://doi.org/10.1016/j.tibtech.2013.09.006.
[35] E. Harrison, J.W.J. Hamilton, M. Macias-Montero, D. Dixon, Peptide functionalized gold nanoparticles: the influence of pH on binding efficiency, Nanotechnology 28 (2017) 295602, https://doi.org/10.1088/1361-6528/aa77ac.
[36] A. Ghosh, M. Sharma, Y. Zhao, Cell-penetrating protein-recognizing polymeric nanoparticles through dynamic covalent chemistry and double imprinting, Nat. Commun. 15 (2024) 3731, https://doi.org/10.1038/s41467-024-48131-5.
[37] L. Martínez-Jothar, S. Doulkeridou, R.M. Schiffelers, J. Sastre Torano, S. Oliveira, C.F. van Nostrum, et al., Insights into maleimide-thiol conjugation chemistry: Conditions for efficient surface functionalization of nanoparticles for receptor targeting, J. Control. Release 282 (2018) 101–109, https://doi.org/10.1016/j.jconrel.2018.03.002.
[38] Karshikoff Andrey, Introduction. Non-Covalent Interactions in Proteins, WORLD Sci. (2021) 1–26, https://doi.org/10.1142/9789811228094_0001.
[39] N. Sathyamoorthy, D. Magharla, S. Vankayalu, Effect of surface modification on the In vitro protein adsorption and cell cytotoxicity of vinorelbine nanoparticles, J. Pharm. Bioallied Sci. 9 (2017) 135, https://doi.org/10.4103/jpbs.JPBS_258_16.

[40] J. Nam, N. Won, J. Bang, H. Jin, J. Park, S. Jung, et al., Surface engineering of inorganic nanoparticles for imaging and therapy, Adv. Drug Deliv. Rev. 65 (2013) 622–648, https://doi.org/10.1016/j.addr.2012.08.015.
[41] X.-X. Xia, M. Wang, Y. Lin, Q. Xu, D.L. Kaplan, Hydrophobic Drug-Triggered Self-Assembly of Nanoparticles from Silk-Elastin-Like Protein Polymers for Drug Delivery, Biomacromolecules 15 (2014) 908–914, https://doi.org/10.1021/bm4017594.
[42] I.A. Hassanin, A.O. Elzoghby, Self-assembled non-covalent protein-drug nanoparticles: an emerging delivery platform for anti-cancer drugs, Expert Opin. Drug Deliv. 17 (2020) 1437–1458, https://doi.org/10.1080/17425247.2020.1813713.
[43] A. Jain, K. Cheng, The principles and applications of avidin-based nanoparticles in drug delivery and diagnosis, J. Control. Release 245 (2017) 27–40, https://doi.org/10.1016/j.jconrel.2016.11.016.
[44] R. García-Álvarez, M. Vallet-Regí, Hard and Soft Protein Corona of Nanomaterials: Analysis and Relevance, Nanomaterials (2021) 11, https://doi.org/10.3390/nano11040888.
[45] E.P. Ivanova, J.P. Wright, D.K. Pham, N. Brack, P. Pigram, Y.V. Alekseeva, et al., A comparative study between the adsorption and covalent binding of human immunoglobulin and lysozyme on surface-modified poly(*tert* -butyl methacrylate), Biomed. Mater. 1 (2006) 24–32, https://doi.org/10.1088/1748-6041/1/1/004.
[46] J.V. Jokerst, T. Lobovkina, R.N. Zare, S.S. Gambhir, Nanoparticle PEGylation for imaging and therapy, Nanomed. (Lond.) 6 (2011) 715–728, https://doi.org/10.2217/nnm.11.19.
[47] A.O. Elzoghby, A.L. Hemasa, M.S. Freag, Hybrid protein-inorganic nanoparticles: from tumor-targeted drug delivery to cancer imaging, J. Control. Release 243 (2016) 303–322, https://doi.org/10.1016/j.jconrel.2016.10.023.
[48] A.M. Smith, S. Nie, Chemical analysis and cellular imaging with quantum dots, Analyst 129 (2004) 672, https://doi.org/10.1039/b404498n.
[49] A. Kapara, V. Brunton, D. Graham, K. Faulds, Investigation of cellular uptake mechanism of functionalised gold nanoparticles into breast cancer using SERS, Chem. Sci. 11 (2020) 5819–5829, https://doi.org/10.1039/d0sc01255f.
[50] A.A. Bhirde, V. Patel, J. Gavard, G. Zhang, A.A. Sousa, A. Masedunskas, et al., Targeted Killing of Cancer Cells *in Vivo* and *in Vitro* with EGF-Directed Carbon Nanotube-Based Drug Delivery, ACS Nano 3 (2009) 307–316, https://doi.org/10.1021/nn800551s.
[51] S.F. Oliveira, G. Bisker, N.A. Bakh, S.L. Gibbs, M.P. Landry, M.S. Strano, Protein functionalized carbon nanomaterials for biomedical applications, Carbon N. Y 95 (2015) 767–779, https://doi.org/10.1016/j.carbon.2015.08.076.
[52] K. Nagaraju, R. Reddy, N. Reddy, A Review on Protein Functionalized Carbon Nanotubes, J. Appl. Biomater. Funct. Mater. 13 (2015) 301–312, https://doi.org/10.5301/jabfm.5000231.
[53] J.-H. Bong, A. Dombovski, R. Birus, S. Cho, M. Lee, J.-C. Pyun, et al., Covalent coupling of functionalized outer membrane vesicles (OMVs) to gold nanoparticles, J. Colloid Interface Sci. 663 (2024) 227–237, https://doi.org/10.1016/j.jcis.2024.02.137.
[54] D. Luo, X. Wang, S. Zeng, G. Ramamurthy, C. Burda, J.P. Basilion, Prostate-specific membrane antigen targeted gold nanoparticles for prostate cancer radiotherapy: does size matter for targeted particles? Chem. Sci. 10 (2019) 8119–8128, https://doi.org/10.1039/c9sc02290b.
[55] D. Sung, A. Sanchez, J.D. Tward, Successful salvage brachytherapy after infusion of gold auroshell nanoshells for localized prostate cancer in a human patient, Adv. Radiat. Oncol. 8 (2023) 101202, https://doi.org/10.1016/J.ADRO.2023.101202.
[56] E. Susnik, A. Bazzoni, P. Taladriz-Blanco, S. Balog, A.M. Moreno-Echeverri, C. Glaubitz, et al., Epidermal growth factor alters silica nanoparticle uptake and improves gold-nanoparticle-mediated gene silencing in A549 cells, Front. Nanotechnol. 5 (2023) 1220514, https://doi.org/10.3389/fnano.2023.1220514.
[57] J. Zhang, T. Zhao, F. Han, Y. Hu, Y. Li, Photothermal and gene therapy combined with immunotherapy to gastric cancer by the gold nanoshell-based system, J. Nanobiotechnology 17 (2019) 80, https://doi.org/10.1186/s12951-019-0515-x.
[58] Y. Kuo, I. Chao, Conjugation of melanotransferrin antibody on solid lipid nanoparticles for mediating brain cancer malignancy, Biotechnol. Prog. 32 (2016) 480–490, https://doi.org/10.1002/btpr.2214.
[59] Y.C. Kuo, I.H. Lee, Delivery of doxorubicin to glioblastoma multiforme in vitro using solid lipid nanoparticles with surface aprotinin and melanotransferrin antibody for enhanced chemotherapy, J. Taiwan Inst. Chem. Eng. 61 (2016) 32–45, https://doi.org/10.1016/J.JTICE.2015.12.012.
[60] P.C. Ke, S. Lin, W.J. Parak, T.P. Davis, F. Caruso, A decade of the protein corona, ACS Nano 11 (2017) 11773–11776, https://doi.org/10.1021/acsnano.7b08008.
[61] M. Mahmoudi, I. Lynch, M.R. Ejtehadi, M.P. Monopoli, F.B. Bombelli, S. Laurent, Protein−nanoparticle interactions: opportunities and challenges, Chem. Rev. 111 (2011) 5610–5637, https://doi.org/10.1021/cr100440g.
[62] E. González-García, M. Maly, F.J. de la Mata, R. Gómez, M.L. Marina, M.C. García, Factors affecting interactions between sulphonate-terminated dendrimers and proteins: a three case study, Colloids Surf. B Biointerfaces 149 (2017) 196–205, https://doi.org/10.1016/J.COLSURFB.2016.10.020.
[63] L. Vroman, A.L. Adams, G.C. Fischer, P.C. Munoz, Interaction of high molecular weight kininogen, factor xii, and fibrinogen in plasma at interfaces, Blood 55 (1980) 156–159, https://doi.org/10.1182/blood.V55.1.156.156.
[64] A.E. Nel, L. Mädler, D. Velegol, T. Xia, E.M.V. Hoek, P. Somasundaran, et al., Understanding biophysicochemical interactions at the nano–bio interface, Nat. Mater. 8 (2009) 543–557, https://doi.org/10.1038/nmat2442.
[65] D. Walczyk, F.B. Bombelli, M.P. Monopoli, I. Lynch, K.A. Dawson, What the cell "sees" in bionanoscience, J. Am. Chem. Soc. 132 (2010) 5761–5768, https://doi.org/10.1021/ja910675v.
[66] T. Cedervall, I. Lynch, S. Lindman, T. Berggård, E. Thulin, H. Nilsson, et al., Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, Proc. Natl. Acad. Sci. 104 (2007) 2050–2055, https://doi.org/10.1073/pnas.0608582104.
[67] W. Norde, J. Lyklema, Why proteins prefer interfaces, J. Biomater. Sci. Polym. Ed. 2 (1991) 183–202, https://doi.org/10.1080/09205063.1991.9756659/ASSET//CMS/ASSET/CED98CC6-F798-4887-B20E-625DF529259E/09205063.1991.9756659.FP.PNG.
[68] X.-R. Xia, N.A. Monteiro-Riviere, J.E. Riviere, An index for characterization of nanomaterials in biological systems, Nat. Nanotechnol. 5 (2010) 671–675, https://doi.org/10.1038/nnano.2010.164.
[69] D. Dutta, S.K. Sundaram, J.G. Teeguarden, B.J. Riley, L.S. Fifield, J.M. Jacobs, et al., Adsorbed proteins influence the biological activity and molecular targeting of nanomaterials, Toxicol. Sci. 100 (2007) 303–315, https://doi.org/10.1093/toxsci/kfm217.
[70] G. Sahay, D.Y. Alakhova, A.V. Kabanov, Endocytosis of nanomedicines, J. Control. Release 145 (2010) 182–195, https://doi.org/10.1016/j.jconrel.2010.01.036.
[71] W. Norde, My voyage of discovery to proteins in flatland …and beyond, Colloids Surf. B Biointerfaces 61 (2008) 1–9, https://doi.org/10.1016/j.colsurfb.2007.09.029.
[72] C.A. Haynes, W. Norde, Globular proteins at solid/liquid interfaces, Colloids Surf. B Biointerfaces 2 (1994) 517–566, https://doi.org/10.1016/0927-7765(94)80066-9.
[73] B. Jachimska, K. Tokarczyk, M. Łapczyńska, A. Puciul-Malinowska, S. Zapotoczny, Structure of bovine serum albumin adsorbed on silica investigated by quartz crystal microbalance, Colloids Surf. A Physicochem Eng. Asp. 489 (2016) 163–172, https://doi.org/10.1016/j.colsurfa.2015.10.033.
[74] K. Kubiak-Ossowska, G. Burley, S.V. Patwardhan, P.A. Mulheran, Spontaneous Membrane-Translocating Peptide Adsorption at Silica Surfaces: A Molecular Dynamics Study, J. Phys. Chem. B 117 (2013) 14666–14675, https://doi.org/10.1021/jp409130s.
[75] C.D. Walkey, J.B. Olsen, F. Song, R. Liu, H. Guo, D.W.H. Olsen, et al., Protein Corona Fingerprinting Predicts the Cellular Interaction of Gold and Silver Nanoparticles, ACS Nano 8 (2014) 2439–2455, https://doi.org/10.1021/nn406018q.
[76] G. Miotto, M. Magro, M. Terzo, M. Zaccarin, L. Da Dalt, E. Bonaiuto, et al., Protein corona as a proteome fingerprint: The example of hidden biomarkers for cow mastitis, Colloids Surf. B Biointerfaces 140 (2016) 40–49, https://doi.org/10.1016/j.colsurfb.2015.11.043.
[77] R.R. Arvizo, K. Giri, D. Moyano, O.R. Miranda, B. Madden, D.J. McCormick, et al., Identifying new therapeutic targets via modulation of protein corona formation by engineered nanoparticles, PLoS One 7 (2012) e33650, https://doi.org/10.1371/JOURNAL.PONE.0033650.
[78] G. Caracciolo, Liposome–protein corona in a physiological environment: challenges and opportunities for targeted delivery of nanomedicines, Nanomedicine 11 (2015) 543–557, https://doi.org/10.1016/j.nano.2014.11.003.
[79] M.J. Hajipour, S. Laurent, A. Aghaie, F. Rezaee, M. Mahmoudi, Personalized protein coronas: a "key" factor at the nanobiointerface, Biomater. Sci. 2 (2014) 1210–1221, https://doi.org/10.1039/C4BM00131A.
[80] V. Mirshafiee, R. Kim, M. Mahmoudi, M.L. Kraft, The importance of selecting a proper biological milieu for protein corona analysis in vitro: Human plasma versus human serum, Int J. Biochem Cell Biol. 75 (2016) 188–195, https://doi.org/10.1016/J.BIOCEL.2015.11.019.
[81] F. Pederzoli, G. Tosi, M.A. Vandelli, D. Belletti, F. Forni, B. Ruozi, Protein corona and nanoparticles: how can we investigate on? Wiley Inter. Rev. Nanomed. Nanobiotechnol 9 (2017) e1467 https://doi.org/10.1002/WNAN.1467.
[82] R. Bilardo, F. Traldi, A. Vdovchenko, M. Resmini, Influence of surface chemistry and morphology of nanoparticles on protein corona formation, Wiley Inter. Rev. Nanomed. Nanobiotechnol 14 (2022) e1788, https://doi.org/10.1002/WNAN.1788.
[83] A.A. Ashkarran, S. Tadjiki, Z. Lin, K. Hilsen, N. Ghazali, S. Krikor, et al., Protein corona composition of gold nanocatalysts, ACS Pharm. Transl. Sci. 7 (2024) 1169–1177, https://doi.org/10.1021/ACSPTSCI.4C00028.
[84] Y. Li, W. Wu, Q. Liu, Q. Wu, P. Ren, X. Xi, et al., Specific surface-modified iron oxide nanoparticles trigger complement-dependent innate and adaptive antileukaemia immunity, 15:1 2024;, Nat. Commun. 15 (2024) 1–16, https://doi.org/10.1038/s41467-024-54810-0.
[85] Y. Portilla, V. Mulens-Arias, N. Daviu, A. Paradela, S. Pérez-Yagüe, D.F. Barber, Interaction of iron oxide nanoparticles with macrophages is influenced distinctly by "self" and "non-self" biological identities, ACS Appl. Mater. Interfaces 15 (2023) 35906–35926, https://doi.org/10.1021/ACSAMI.3C05555.
[86] L. Dietz, J. Simon, K.R. Speth, K. Landfester, V. Mailänder, Plasma protein corona on silica nanoparticles enhances exocytosis, Biomater. Sci. 13 (2025) 3532–3543, https://doi.org/10.1039/D4BM01189A.
[87] K. Liu, R. Nilsson, E. Lázaro-Ibáñez, H. Duàn, T. Miliotis, M. Strimfors, et al., Multiomics analysis of naturally efficacious lipid nanoparticle coronas reveals high-density lipoprotein is necessary for their function, 14:1 2023, Nat. Commun. 14 (2023) 1–16, https://doi.org/10.1038/s41467-023-39768-9.
[88] C. Rennie, N. Morshed, M. Faria, L. Collins-Praino, A. Care, Nanoparticle association with brain cells is augmented by protein coronas formed in cerebrospinal fluid, Mol. Pharm. 22 (2025), https://doi.org/10.1021/ACS.MOLPHARMACEUT.4C01179.
[89] N. Liu, Y. Liang, T. Wei, X. Huang, T. Zhang, M. Tang, Protein corona exacerbated inflammatory response in macrophages elicited by CdTe quantum dots, NanoImpact 33 (2024), https://doi.org/10.1016/j.impact.2024.100494.