Towards applications of bioentities@MOFs in biomedicine

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ABSTRACT

Metal–organic frameworks (MOFs) combined with biomacromolecules, viruses and cells have emerged as novel biocomposites for application to drug delivery, biosensing, biospecimen preservation, and cell and virus manipulation. The integration of biological entities into MOF matrices generates MOF biocomposites with functional characteristics that cannot be observed in the separate components, such as enhanced chemical and thermal stability, resistance to proteases, MOF-conferred selectivity, and controlled release. In this review, we will discuss these functional properties and applications of the biocomposites obtained by the encapsulation of (i) proteins, (ii) carbohydrates, (iii) nucleic acids, and (iv) viruses or cells in a MOF matrix. Finally, we review the post functionalization of MOF-based drug carriers with lipids as a potential route to enhance the dispersion, stability in biological fluids, and blood circulation time of MOF-based drug delivery systems.

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1. Introduction

The development of new biocomposites is an area of current research interest at the intersection of material science, biology and biochemistry. Ideally a biocomposite will combine characteristics of the non-biological and biological components to access improved or new properties not observed for the individual entities [1]. This synergistic effect gives rise to novel properties that make biocomposites particularly interesting for application to biomedicine and biotechnology. Hitherto, biocomposites have been prepared using a variety of synthetic materials (i.e. liposomes, dendrimers, mesoporous silica, and nanoparticles) which act as hosts for biological guests [1–4]. Metal-organic Frameworks (MOFs), a class of porous, open framework materials synthesized via a building block approach, have emerged as a versatile platform for the control of their properties [7–9]. Moreover, both the pores and the mutability of the MOF building blocks enables molecular level containment and tracking of biological entities on the outer surface of MOFs either by a covalent attachment or through the adsorption induced by the electrostatic interactions [25]. Infiltration refers to the immobilization of biocomponents via a building block approach onto the outer surface of MOFs, which act as hosts for biological guests [1–4]. Metal-organic Frameworks (MOFs), a class of porous, open framework materials synthesized via a building block approach, have emerged as a versatile platform for the control of their properties [7–9]. Furthermore, external chemical stimuli such as pH can be used to dissolve MOFs allowing for triggered-release applications [15]. These selected examples show the versatility of MOF-based biocomposites can be designed for wide range of potential applications including: drug delivery, biospecimen preservation, biosensing, and drug and virus manipulation. Specifically, the integration of active pharmaceutical ingredients (APIs) within MOF materials allows common issues associated with the administration of free drugs to be overcome, including rapid biodegradation, systemic side effects, low specificity, poor solubility, and the inability of some biotherapeutics to cross cell membranes [11,12]. The use of MOFs to encapsulate biokineties such as vaccines, proteins, cells, and others, enhances their robustness when subjected to hostile environments during transport, handling, and storage (e.g. temperature) that can compromise their potency [16]. Finally, in the biosensing field, there have been extensive efforts to integrate MOF biocomposites in the design of diagnostic devices. In this respect, MOF-based biocomposites are typically employed as probe systems, as such materials are capable of carrying in one single particle both the biorecognition unit (enzymes, antibodies, etc) and large amount of signal molecules, which can be easily infiltrated within the porous network. This arrangement enhances the selectivity and sensitivity of detection of the target biomarker, which is attractive for the fabrication of new diagnostic technologies such as point-of-care (POC) tests [17–21]. The development of these technologies could afford the early clinical diagnosis and prompt treatment of several diseases.
biomacromolecules within the pore network of the material via diffusion processes [22–24,26].

Encapsulation strategies involve the growth of a MOF shell around a target bioentity and this methodology is the focus of the current review. A variety of bioentities that encompass a wide range in size from small proteins to larger cells and viruses have been successfully encapsulated by a MOF shell. This is because the encapsulation method is not limited by the pore size of the MOF [5,6,26–28], as the MOF grows around the bioentity. The integration of biomacromolecules within a MOF shell can be accomplished either by templating methods or via the one-pot synthesis of the MOF in presence of the biomolecule [5,29]. Typically, hard-templating and soft-templating strategies result in the biomacromolecules confined in a micrometric hollow MOF capsule [5,29]. For the hard-templating method the MOF shell is formed around a rigid material (i.e. silica nanoparticles) that could act as a sacrificial template; whereas in the soft-templating approach, around a rigid material (i.e. silica nanoparticles) that could act as a sacrificial template; whereas in the soft-templating approach, the MOF shell grows at the interface of vesicles, droplets, emulsions and cell walls [5,30–32]. The soft-template provides less control over the particle size and uniformity than the hard-templating strategy. Alternatively, one-pot encapsulation strategies are based on the heterogeneous nucleation of MOF crystallites around the target bioentity. This strategy will be discussed in detail in the following sections [5,6,26–28]. Indeed, we intend to provide a thorough overview of state-of-the-art applications of MOF-based biocomposites obtained by the one-pot encapsulation of biomacromolecules (proteins, polysaccharides and DNA), as well as complex bioentities (cells and viruses). When possible, we will include examples of biocomposites obtained through bioconjugation strategy for a comparison. Herein, we examine a wide variety of MOF-based composites classified in four different areas depending on the corresponding biological entity: (1) protein@MOFs, (2) carbohydrate@MOFs, (3) DNA@MOFs (4) cell&virus@MOFs. In each section we will discuss the potential applications of such biocomposites in different fields including drug delivery, biosensing and biobanking (Fig. 1). In Section 2 we discuss properties and relevant concepts to assess the suitability of these biocomposites for biomedical and biotechnological applications. Additionally, we include the post functionalization of MOF-based drug carriers with lipids (Section 7) as a potential route to enhance the colloidal stability in biological fluids and blood circulation time of MOF-based drug delivery systems. Finally, we conclude with perspectives and future opportunities related to each system.

2. Properties and relevant concepts

It is important to outline some key concepts of MOF biocomposite chemistry such as encapsulation efficiency (loading potential), protective capacity and residual activity, delivery/release processes, compatibility, and particle size. These criteria are often used to assess the properties and performance of biocomposites in the field of biomedicine. Some of these criteria are common to bioentity encapsulation more generally, while others are specifically relevant to using encapsulated biomacromolecules in drug or vaccine delivery.

2.1. Encapsulation efficiency and drug loading

Encapsulation efficiency is a crucial aspect of drug delivery systems used to ascertain the potential of carriers to deliver an API [33,34]. Given a certain amount of drug involved in a preparation method, the encapsulation efficiency provides quantitative information about the amount of drug successfully transferred into a particular carrier [35]. Moreover, it is a relevant parameter to consider when a specific dose of therapeutic must be delivered precisely to the site of action [36]. Finally, from an economic point of view, as many drugs are costly, it can determine whether a system will be commercialized or not [34,36] (see Section 2.6). It is also important in biosensing and biopreservation to understand the sensing performance or uptake of biotherapeutics.

The encapsulation efficiency (EE%) is the percentage obtained by taking the ratio between the mass of active ingredient (i.e. drugs, fragrances, proteins, pesticides, antimicrobial agents, etc.) integrated in a carrier (Mt) over its total mass used in the preparation of the composite (Mf) [35,37,38],

$$EE\% = \left( \frac{Mi}{Mf} \right) \times 100\%.$$  

Another important parameter to determine the capacity of the system to carry active pharmaceutical ingredients as a cargo is the drug loading (DL) [35]. The DL provides information about the ratio of the mass of drug (Md) and the mass of the vessel (Mv). This is an essential aspect to take into consideration in drug delivery systems as the use of excessive amounts of carrier could potentially increase side effects, such as toxicity and immune responses against the carrier [39]. Thus, DL can be calculated as...
where $Md$ is the mass of encapsulated active ingredient (e.g. drug molecule) and $Mv$ is the mass of encapsulating carrier.

While EE% can be used to study the effect of a preparation step for the composite on encapsulation of the active ingredient, DL% helps identify if an encapsulation method is effective for a specific application [35,40]. Both parameters, DL% and EE%, depend on the selected system and encapsulation method. However, we note that in drug delivery systems, the relevance of EE% or DL% is related on the aim of the study. For instance, the measurement of EE% should be the important criterion if the aim is to determine either the efficiency of an overall method or the optimization of the system [35]. Alternatively, DL% should be a central focus if the goal of the study is to evaluate a dosage of drug with respect to a desired pharmacokinetic profile [35,40].

2.2. Protection

The fragile nature of biomacromolecules and complex bioentities typically requires their manipulation under careful conditions, as when treatment conditions deviate from their ideal environment (e.g. increased temperature) conformational changes or disintegration can occur. A central challenge to preserve bioentity activity during handling, transport, and storage is the need for refrigeration, the so-called “cold chain” [6,41]. For example, protein-based therapeutics are prone to losing their effectiveness when handled outside of the temperature window where they are stable (typically 2–8 °C) [16,42]. The same applies to vaccines and virus-based therapeutics which require constant refrigeration to retain efficacy [6]. Similarly, the molecular conformation of enzymes is altered after exposure to high temperatures, organic solvents and mechanical stressors [43–45]. This unfolding process results in a considerable drop of the biocatalytic performance [44]. In the case of cells, additional aspects compromising their bioactivity include environmental factors such as cytotoxic compounds and radiation [46].

To overcome the fragile nature of bioentities including proteins, viruses and cells, researchers have focused on different approaches. For example, in the case of proteinaceous drugs, vaccine adjuvants or vaccines the application of a protective synthetic coating could provide a new tool for storage, handling and transportation [6,47]. In the case of living organisms such as cells, the fabrication of a tailored artificial exoskeleton can provide effective protection from environmental cytotoxic compounds, mechanical stress and radiation damage, thus facilitating storage, manipulation and transportation of cells for application in biomedicine and biotechnology [46].

Protection of bioentities is an important technological challenge and has motivated researchers to investigate different classes of materials for the fabrication of robust artificial biocomposites. Porous nanomaterials, in particular MOFs, are widely studied because of their high surface area, tunable morphology, and high affinity for protein conjugation [45]. In particular, MOFs have been considered attractive candidates as their structure, chemical properties, pore size and shape can be precisely tuned [48]. Additionally, there are an increasing number of MOFs that can be synthesized under biocompatible conditions, and moreover the encapsulated bioentity can be readily recovered upon applying external stimuli [19].

Although prior reports disclose biomolecule encapsulation in MOFs [26,28], the first systematic study comparing the biopreservation properties conferred by encapsulation was communicated by Falcaro and co-workers [27]. In that work, the authors compared the protection properties of ZIF-8 (sodalite, sod) versus other inorganic exoskeletons, including mesoporous SiO$_2$ and CaCO$_3$. To provide this comparison, the authors monitored the catalytic performance of horseradish peroxidase (HRP) upon exposure to denaturing conditions (e.g. boiling water or DMF). Unlike inorganic carriers, HRP@ZIF-8 retained most of the enzymatic activity. This study also experimentally correlated the pore size of a carrier with its protective properties (the smaller the pore the higher the protection) thus suggesting the tight encapsulation of the biomacromolecule within ZIF-8 yields superior protection (see Section 3.3). Additionally, the authors demonstrated that active proteins can be released from the MOF biocomposite under mildly acidic conditions. These data suggested the further investigation of MOFs for protection against physical and chemical stress (e.g. exposure of biocomposites to mechanical forces, organic solvents, chaotropic agents and temperature) [49]. Examples of these studies will be discussed in detail below, categorized for each type of bioentity [16,44,45].

2.3. Release

Pharmacokinetics describes the process of the uptake of drugs by the body, their biotransformation, distribution in the tissues, and elimination from the organism following a period of time after administration [50]. Pharmacokinetics is critical for the understanding of the therapeutic properties of a specific drug. Biodistribution refers to the distribution of chemicals to specific locations within the body [51]. This can be also associated to the spatial localization of a biocomposite (or a carrier) overtime within an animal or human body; thus, biodistribution is fundamental to identifying target organs and anticipating safety and efficacy [52]. By using nanocarriers, aspects of pharmacokinetics and biodistribution (e.g. the release profile of the drug, their accumulation in different tissues and the biocompatibility) can be precisely controlled by tuning the properties of the carrier material.

The release is characterized by a profile that determines the amount of drug that diffuses from the carrier into the surrounding environment as a function of time. Importantly, the release time can drastically influence the therapeutic effect of the same drug [53]. Depending on the therapeutic and the treatment, different release profiles are preferred. For example analgesics and anticoagulants typically require fast release [54]. Conversely, a slow release can be favoured in prolonged treatments as a replacement to administration via parenteral (non-oral) route which can be painful and problematic [8,55,56]. This is the case for protein-based treatments such as insulin, growth hormones or oxytocin, which require multiple injections causing the patients pain and discomfort [56,57]. An example of a successful commercial product is an injectable drug delivery system based on leuprolide hormone encapsulated in PLGA microspheres (Lupron Depot*) which provide prolonged release [58].

Although, drug release profiles of conventional macro-sized drug delivery systems are typically assessed by standard United States Pharmacopoeia (USP) methods, for micro- and nanoparticulate systems, standard tests are not available [59]. Thus, each micro- and nanoparticle-based drug carriers system requires an ad hoc testing regime for the evaluation of its specific drug release profile [59–62]. Examples of methods used are side-by-side diffusion cells with artificial or biological membranes, dialysis bag diffusion techniques, and agitation followed by centrifugation [59]. The latter is the most commonly employed for its simplicity and it can be combined with a dialysis technique using synthetic membranes for the separation of the nanoparticles from the release media. Typically, the collected release profiles show a biphasic behavior with an initial burst followed by a slower sustained release of the drug [59,63]. By tuning the material structure,
chemistry and drug location in the carrier, the burst effect can be minimized and a steady sustained drug release is obtained [59].

Following preliminary testing, deeper insight can be obtained by conducting preclinical studies, which are either carried out in vitro or in vivo on biological systems. For in vitro systems, drugs are tested in microorganisms, cells, tissues, or isolated organs in conditions that mimic their normal biological context [64]. In drug delivery, in vitro systems are interesting for early phase research studies [64]. Alternatively, in vivo systems require whole living animals to investigate the effect of selected therapeutics and administration methods. In vivo studies are considered crucial to determine the absence of side effects that cannot be observed or predicted by in vitro experiments [64].

Recent research has moved from regular drug delivery systems (DDS) [65] that exploit non-specific Fickian diffusion to stimuli-responsive materials using nanomaterials that can release therapeutics with simultaneous control over carrier localization, release time and dosage [65]. The release could be triggered by the local environment of the target cells/tissues (example of stimuli are pH, chemical environment, temperature), or it could be regulated using external controls (example of stimuli are light, magnetic field, temperature, ions, pressure) [15]. Within the broad range of nanomaterials, MOFs are ideal candidates as carriers: by selecting the proper building blocks and structure, it is possible to impart either regular DDS properties, or triggered-release responses either from local environments or from external controls. Indeed MOF-based systems can undergo structural modifications that release a payload under specific conditions including acidic pH [15,66], presence of certain anions [67–69], and irradiation with light [70–72].

2.4. Compatibility

When a MOF is used as a carrier to deliver a drug in the body, it can undergo a degradation process that will release not only the drug, but also the constituent building blocks (i.e. cations, ligands). Therefore, it is important to evaluate the toxicity of MOF components. An interesting approach was suggested by Horcajada et al. that recommended the selection of naturally occurring building blocks for the synthesis of biocompatible MOF (aka bioMOFs) [11,73]. For the choice of the ligand, endogenous biomolecules (amino acids, peptides, nucleobases, carbohydrates, porphyrins) or exogenous bioactive ingredients (nicotinic acid, curcumin, olsalazine and some dicarboxylic acids, including fumaric acid) have been recommended as ideal candidates [11]. For the choice of metal for the nodes, it is preferable to use those cations that part of the daily requirement of the human body [74]. However, each cation possesses its own degree of toxicity, therefore the median lethal dose (LD50) has been proposed as criteria to assess the compatibility of the specific cation. LD50 is the amount of compound that kills 50% of a given population within a selected time [74]. Based on this, the preferable metals for the construction of biocompatible MOFs are Mg\(^{2+}\) (LD50 MgSO\(_4\) = 50 00) > Ca\(^{2+}\) (LD50 CaCl\(_2\) = 1940) > Fe\(^{3+}\) (LD50 FeCl\(_3\) = 984) > Fe\(^{2+}\) (LD50 FeCl\(_2\) = 450) > Zn\(^{2+}\) (LD50 Zn(OAc)_2 = 100–600) [11,113].

Although a preliminary assessment can be conducted by considering the amount of MOF byproducts with respect to published cytotoxicity values, it is relevant to stress that reports on MOF biocomposites for biomedical aspects should involve in vitro and in vivo studies. While in vitro experiments can provide information on some cytotoxic aspects; however, it is not enough to simply assess the biocompatibility of the materials, since the behavior inside a living system can involve several important aspects including interferences, permanence in the circulatory system, accumulation in organs, immune response, etc. [75]. For this reason, a thorough investigation of the compatibility of the MOF-based biocomposites involving both in vivo and in vitro studies should be conducted.

2.5. Particle size

An appealing property of particulate DDS is their versatile administration that can include parenteral injection [75] and inhalation [76]. However, extensive studies have revealed the importance of the particle size and shape for blood circulation time, biodistribution, cellular internalization and compatibility [77]. For example the different mechanisms (phagocytosis, macropinocytosis, caveolar-mediated endocytosis or clathrin-mediated) of cellular internalization processes are particle size-dependent [78,79]. Particle size is also known to influence the efficiency of tumor-targeted drug delivery for cancer treatments, affecting circulation, biodistribution, tumor accumulation and penetration, cellular uptake and subcellular distribution. Studies have shown larger nanoparticles tend to be more capable of retention in tumor tissue when compared with smaller nanoparticles, but smaller particles present higher penetration efficiency in tumor tissues [80]. However, small particles (<5 nm) are quickly cleared from circulation through extravasation or renal clearance [77]. As size increases, particles seem to accumulate primarily in the liver, spleen and bone marrow. Typically, particles with dimensions in the 10 nm to 15 μm range are trapped by the spleen and then removed from circulation by cells of the reticuloendothelial system (RES). Additionally, when administered in vivo, specific proteins adsorb to surface of particles and this influences the accumulation in the RES. Particles larger than ~15 μm are typically removed from circulation by mechanical filtration in capillaries, and a high dose can be lethal [77].

The size range between 50 and 300 nm has been found to provide an optimal circulation half-life for the parenteral administration route [11,81,82]. Nanoparticles of sizes <150 nm can exit the blood vessels through openings (fenestrations) in the endothelial lining, potentially entering organs and tissues [83,84]. Based on this, MOF nanoparticles from tens to hundreds of nanometers are ideal nanocarriers for imaging agents and drug molecules [12,85], especially when administered with intravenous/subcutaneous injection methods [75]. However, “small” nanoparticles are not suitable for every application. For example, studies have shown that nanoparticles with sizes < 10 nm in diameter, when administered trans-dermally will not penetrate through the stratum corneum into viable human skin and will likely accumulate in the hair follicle openings [86]. Rather, it has been shown that bigger particles on the scale of 300 nm to 1.5 μm are effective for transdermal drug delivery [87]. Biodegradable microparticles (with sizes between 300 nm and 2 μm) have been shown to offer important advantages in the area of vaccine delivery and some formulations are in clinical trials [84,85,88]. Additional benefits can arise from having larger particles, such as an increased stability in buffer and reduced aggregation, [67,76,89,90] so there are tradeoffs required to produce an optimal DDS. Relevant considerations on the influence of particle size for DDS applications discussed in this section are schematized in Fig. 2, which shows different size-dependent processes and suggested DDS administration routes.

Although we have focused the discussion specifically on particle size, the particle’s shape and density can influence the retention and circulation time, biodistribution and mechanism of internalization [77,80,84]. For example, by controlling the aggregation of nanoparticles, clusters with inter-particle voids can be prepared. This strategy allows to modify the density of carriers and affords a deeper penetration (in the case of administration to the lungs) [84] and a higher retention in tumor tissues [80].

Different strategies have been employed to tune the MOF particle size [91,92]; for example, the crystal growth kinetics can be
influenced by adjusting the reaction parameters (e.g. type and amount of solvents, reagent concentrations, temperature, pH) [67,93], or the introduction of auxiliary additives (coordination modulators, surfactant-mediated synthesis, etc) [12,75,94–96]. These methods can be used to prepare MOF particles that can be post-

**Table 1**

<table>
<thead>
<tr>
<th>Biotherapeutic</th>
<th>MOF</th>
<th>Biotherapeutic wt%</th>
<th>Biotherapeutic/MOF cost ratio</th>
<th>Cost of MOF/total (%)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>ZIF-8</td>
<td>7.1</td>
<td>30</td>
<td>3.2</td>
<td>[103]</td>
</tr>
<tr>
<td>CS</td>
<td>ZIF-8</td>
<td>10.9</td>
<td>28</td>
<td>3.4</td>
<td>[103]</td>
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<tr>
<td>Insulin</td>
<td>ZIF-8</td>
<td>5.2</td>
<td>245</td>
<td>0.4</td>
<td>[100]</td>
</tr>
<tr>
<td>DS</td>
<td>ZIF-8</td>
<td>6.5</td>
<td>832</td>
<td>0.1</td>
<td>[103]</td>
</tr>
<tr>
<td>Insulin</td>
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<td>1168</td>
<td>0.09</td>
<td>[101]</td>
</tr>
<tr>
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<td>1195</td>
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<td>[97]</td>
</tr>
<tr>
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<td>15433</td>
<td>0.007</td>
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</tr>
<tr>
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<td>751116</td>
<td>0.0001</td>
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<td>[103]</td>
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<tr>
<td>Drug</td>
<td>MOF</td>
<td>Drug wt%</td>
<td>Drug/MOF cost ratio</td>
<td>Cost of MOF/total (%)</td>
<td>Refs.</td>
</tr>
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<td>[103]</td>
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<td>[105]</td>
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<td>0.3</td>
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Aspects disregarded in this preliminary material assessment costs include: the yield of the MOF synthesis, the excess of precursors often used (e.g. ligand), the energy involved of the process, solvents used in the synthesis, cleaning procedures, the operator and equipment cost, and the disposal of waste.

Fig. 2. Considerations of particle size for DDS applications. On the righthand-side different size-dependent processes are indicated (mechanism of internalization, penetration in the lungs, extravasation from endothelial fenestrations, etc). On the left side suggested DDS administration routes are represented, based on the available studies.

Information collected from several sources [69,70,76,81,83–86] for studies performed on particulate systems for drug delivery and biomedical use, based on different compositions (polymeric particles, liposomes, Au NP and MOF NPs). These studies were typically conducted on spherical particles. We note that this schematic is to be considered only as a general guide to the influence of particle size on the design of the carrier and not as a strict rule, since these processes are also affected by particle shape, density, composition and surface functionalization.
functionalized and post-infiltrated with biomacromolecules. However, for the control over the particle size of bioentities-MOF prepared via one-pot methods (e.g. encapsulation), the research is in its infancy. Pioneering work by Carraro et al. [97], used flow setup for the continuous production of BSA@ZIF-8 nanoparticles in the 40–100 nm range. The fluidic system was also used to control the particle size of antiprtypsin@ZIF-8 composites. However, this flow setup uses ethanol as a quenching agent which might prevent using the approach for a number of clinical biotherapeutics. Indeed, the exposure of fragile biomacromolecules to non-native conditions (e.g. presence of organic solvents, high temperature, pH changes), can result in a drastic activity loss [27]. Thus, biocompatible synthetic approaches for the precise engineering of bioentities-MOF particles, including particle size, morphology and crystallinity, would significantly progress the use of MOFs in biomedicine.

2.6. Material cost

As active pharmaceutical ingredients can be expensive, DDSs aim to increase efficiency by enhancing the bioavailability and efficacy of therapeutics thus reducing the amount of drug used [98]. The cost-effectiveness of nanoparticle-based DDS also requires careful assessment prior to commercialization [99]. With the purpose of stimulating advances in the area of MOF-bio-composites as DDSs, in this section we propose preliminary considerations on the costs of the material components.

Considering selected bio-composites reported in the literature, we will limit our discussion to the ratio between the cost of the MOF carrier to the value of the biomacromolecule-based therapeutic guest per gram of biocomposite (i.e. MOF component + biomacromolecule component). In one gram of biocomposite, the amount of biomacromolecule was calculated based on the reported loading (wt%) while the cost of the components are the prices reported in the supplier website. In the case of the MOF component, for the sake of simplicity, we used the chemical formula of the MOF to calculate the stoichiometric amount of the MOF precursors (e.g. ligand and metal salt). We selected examples based on three different MOFs: ZIF-8, ZIF-90 and MAF-7, due to their widespread use, and list the results in Table 1.

Currently, the most widely used azolate based framework for the encapsulation of protein is ZIF-8; this is considerably cheaper than ZIF-90 (36 times) and MAF-7 (87 times) because of the higher cost of imidazole-2-carboxaldehyde (ZIF-90) and 3-methyl-1,2,4-triazole (MAF-7) when compared to 2-methylimidazole (ZIF-8). Using ZIF-8, Carraro et al. encapsulated α1-antitrypsin with a loading of 4.2 wt% [97]. For this DDS, the therapeutic cost is 1195 times the cost of ZIF-8 matrix. Chen et al. and Hoop et al. prepared insulin@ZIF-8 with a protein loading of 5.2 and 20.7 wt%, providing a therapeutic cost that can be estimated as 245 and 1168 times the cost of ZIF-8, respectively [100,101]. Cheng et al. encapsulated gelonin into ZIF-8 with a 41 wt% thus, in this case the ratio of the therapeutic cost to that of ZIF-8 is 751,116 [102]. This observation highlights that, when a high-value protein is used, the cost of ZIF-8 bio-composites depends almost entirely on the cost of the encapsulated therapeutic.

Feng et al. have reported the encapsulation of polyclonal antibodies into ZIF-8 and ZIF-90 particles, allowing for a cost comparison of the respective ZIFs [45]. In the case of the encapsulation of Human immunoglobulin G (H-IgG) into ZIF-8, the loading was 37 wt% and the antibody cost 15,433 times the ZIF-8 component price. Using ZIF-90 as matrix, the H-IgG loading was 53 wt% and the antibody cost to ZIF-90 cost ratio is 827. From this comparison it is evident that the ZIF-90 matrix, even if more expensive than ZIF-8, accounts for only 0.1% of the final cost of the biocomposite.

A study by Velásquez et al allows the comparison of the three different ZIFs with a different class of therapeutics (carbohydrates). In this work hyaluronic (HA) acid and dermatan sulfate (DS) were encapsulated in ZIF-8, ZIF-90 and MAF-7 [103]. In the case of ZIF-8, the HA loading was 7.1 wt% and the DS loading was 6.5 wt%. In the final bio-composites, the cost of HA and DS are 30 and 832 times the cost of ZIF-8, respectively. We note that even with for an inexpensive therapeutic (HA is ca. 50 times cheaper than DS), the cost-effectiveness of ZIF-8 as carrier could be attractive given that it shows appropriate release properties. For ZIF-90 and MAF-7, we observe that the cost-effectiveness of ZIF-8 as carrier could be attractive given that it shows appropriate release properties. For ZIF-90 and MAF-7, we observe that the cost-effectiveness of the MOF is more reasonable only when the more expensive therapeutic (DS) is encapsulated. In this case, the therapeutic cost is 19 and 41 times the cost of ZIF-90 and MAF-7, respectively. Conversely, in the case of HA bio-composites, the costs of ZIF-90 and MAF-7 matrices are comparable to or higher than the cost of the therapeutic component which is driven by the lower biotherapeutic costs and the lower loading levels with respect to other examples.

For comparison, we report in Table 1 some examples of the encapsulation of small molecule therapeutics (e.g. Doxorubicin [DOXO] and 5-fluouracil [5-FU]) into ZIF-8 and ZIF-90 [104,105]. As in the previous cases, the cost-effectiveness of ZIF-8 is higher than ZIF-90 and it is more evident in the case of the encapsulation of high-value therapeutics (e.g. DOXO is 1400 times more expensive than 5-FU).

Overall, this summary highlights that ZIF-8 can be considered as a cheap carrier and, even with a low loading of therapeutics, the cost ZIF-8 will only marginally affect the overall cost of the bio-composite. For ZIF-90 and MAF-7 this cost evaluation should be assessed on a case by case basis.

3. Protein@MOFs for biomedical applications

3.1. One-pot encapsulation strategies for Protein@MOF bio-composites

Proteins have been shown to trigger the rapid growth of a MOF shell around their surface leading to the formation of protein@MOF bio-composites [5]. The main advantage of this method is that proteins of any size and shape can be integrated into MOFs as the pore network dimensions do not place a restriction on the size of the guest biomacromolecule. The first report describing the one-pot encapsulation of proteins within a MOF was published by Ge and co-workers [26]. The authors demonstrated the successful encapsulation of cytochrome c (Cyt c) within two different zinc-based zeolitic imidazolate frameworks sod-ZIF-8 and sod-ZIF-10 (sod = sodalite). Polyvinylpyrrolidone (PVP) was employed as a coprecipitating agent as it has a strong affinity for proteins [106], and facilitated the dispersion and stabilization of the protein in methanol. Furthermore, PVP is known to attract and coordinate metal cations [107,108], increasing their local crystallization facilitator [107,108]. This was supported by previous reports where PVP was used to control the encapsulation of inorganic nanoparticles within ZIFs shells [109–111]. Thus, in the formation of MOF biocomposites, when PVP and Cyt c was added to a solution of 2-methyl imidazole (HmIM) and Zn+2 in methanol, the rapid formation of a Cyt c@ZIF-8 biocomposite was observed. This co-precipitation method, was improved by Tsung and co-workers who used PVP to induce the encapsulation of catalase (CAT) within ZIF-8 and ZIF-90 in aqueous media, demonstrating that organic solvents are not needed (this strategy is termed de novo approach) [28,112].

Then, Falcão, Doonan, and co-workers demonstrated that additives (e.g. PVP) are not required as biomacromolecules (e.g. HRP,
BSA and DNA) can act as heterogeneous nucleation seeds and trigger the MOF formation in aqueous media [27,113]. This additive-free approach is termed biomimetic mineralization due to its similarity to natural biomineralization processes in which proteins spontaneously induce the formation of minerals (Fig. 3).

More recently, researchers demonstrated that the kinetics of the MOFs-shell formation is protein-dependent [114]. Under identical conditions, the time required for the formation of the biocomposite with a target protein varies from seconds to hours, and, in case of certain proteins, encapsulation does not occur. The authors analyzed the electrostatic (isoelectric point and zeta potential) and hydrophobicity properties of several proteins (Fig. 4a). This work revealed that only negatively charged molecules (isoelectric point (pI) < c.a. 7) triggered the spontaneous formation of ZIF-8 biocomposites. The related computational investigation disclosed how negative charges can increase the local density of Zn²⁺ of around the target biomolecule; this was considered the reason for the subsequent growth of the MOF-shell (Fig. 4a). The authors showed that positively charged proteins, that did not trigger the spontaneous biomimeralization (e.g. hemoglobin), became effective MOF seeds when surface functionalized (e.g. succinylation) with negatively charged chemical groups (Fig. 4b). This fact underscores the importance of the electrostatic interactions between the target biomolecule and Zn²⁺ ions to induce the growth of the MOF-shell.

Inspired by studies that demonstrate the role of cysteine (Cys) in the accumulation of metal cations around metalloproteins [115–117], Ouyang and co-workers [118] used a PVP/cysteine-based approach for the one-pot encapsulation of biomolecules with positive surface charges such as myoglobin (MB, pI = 7.6). In this water-based synthesis, the local concentration of Cys around the PVP-protein complex depends on the hydrogen-bonding interactions between Cys and the amido groups of PVP. Then, as for metalloproteins, the abundance of Cys around the PVP-protein complex enhanced the concentration of Zn²⁺ ions via mercaptide bond formation, leading to the rapid crystallization of ZIF-8 around PVP/cysteine modified-MB.

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**Fig. 3.** Biomimetic mineralization strategy for the encapsulation of proteins within a ZIF-8 matrix.

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**Fig. 4.** (a) plot of the calculated isoelectric point for different proteins (BSA, pepsin, Hb, and Mb) before and after the surface modification. (b) Schematic representation of the biomimetic mineralization of haemoglobin (Hb). The native Hb does not undergo biomimetic mineralization under standard conditions, but after being chemically modified via acetylation or succinylation process the encapsulation process take place due to the increase of the negative charge on the protein surface. Adapted with permission from ref. [114] Copyright 2018 Royal Society of Chemistry. Mechanisms of nucleation and growth of BSA@ZIF-8 biocomposites obtained varying the ligand:metal ratios: (c) HmIM:Zn = 4:1, (d) HmIM:Zn = 35:1. Reprinted with permission from ref. [119]. Copyright 2020 American Chemical Society.
Cui and co-workers demonstrated that positively charged biomacromolecules (ovalbumin OVA, Poly-1-lysine, PLL; lysozyme, Lyz) were successfully encapsulated within ZIF-8, when poly(ethylene glycol) is present during the mineralization process [96]. By changing the amount of PEG (40 kDa), the process yielded the formation of OVA@PEG@ZIF-8, PLL&PEG@ZIF-8 and Lyz&PEG@ZIF-8 with average particle sizes of 400, 360 and 200 nm, respectively. The authors attribute the successful encapsulation of positively charged biomacromolecules to the hydrogen-bonding interaction between PEG and proteins. Moreover, the presence of this additive permits the re-dispersion of the resultant nanoparticles in aqueous media and enhances the colloidal dispersity and stability of the biocomposites in cell media [96]. The PEG-based strategy affords the encapsulation of proteins with isoelectric point < 7, [114] providing a versatile method for the preparation of protein@ZIF-8 composites.

The mechanism of the encapsulation of BSA within ZIF-8 was revealed by Patterson and co-workers [119], who used cryo-transmission electron microscopy (cryo TEM) to monitor the structural evolution of the biocomposite. This study reveals that the encapsulation of proteins within ZIF-8 follows a nonclassical crystallization pathway which is characterized by aggregation of highly hydrated amorphous particles comprised of: (1) Zn^2+-mIM and (2) Zn^2+-mIM-BSA (Fig. 4c). Aggregation of these two types of amorphous particles is largely dependent on their electrostatic interactions that control the ZIF-8 nucleation and growth mechanisms. The authors suggested that the mechanism of BSA@ZIF-8 formation depends on the ligand:metal ratio and for the 4:1 ratio, the protein promotes the crystallization of ZIF-8 (sod) analogously to natural biominerallization processes (Fig. 4c). Indeed, for 4:1 ligand:metal ratio the Zn^2+-mIM does not spontaneously self-assemble; however, when BSA is added to the MOF precursor solution, the protein, concentrates cations to the protein surface, thus the local supersaturation favors ZIF-8 (sod) crystallization around protein particles (Fig. 4c). As a result, BSA@ZIF-8 (sod) crystalline particles are formed. Conversely, for high ligand:metal ratios (35:1) amorphous aggregates interact with crystalline ZIF-8 (sod); then large aggregates undergo dissolution–recrystallization to form BSA@ZIF-8 (Fig. 4d).

Finally, a recent report published by Hu et al. [120] demonstrated that the mIM:Zn^2+ ratio can be easily adjusted when using a microfluidic flow reactor to synthesise ZIF-8-based biocomposites. This approach allows for fine control over the number of structural defects in the resultant material (see Section 3.4.1) and thus influences the mass transfer within the porous MOF bio-composite. When compared with a Cyt @ZIF-8 biocomposite obtained via a batch procedure, the PXRD pattern of the Cyt @ZIF-8 biocomposite prepared using the microfluidic device reveals a reduction on the crystallinity with peaks shifted to low angles. This increase in the cell parameters was attributed to the defects generated during the synthesis.

In the following sections we discuss the current applications of protein@MOF in biomedicine for drug delivery, biobanking and biosensing (Fig. 5). For clarity, we include selected examples of protein-on-MOF biocomposites.

3.2. Protein@MOFs as DDS

Proteins play a key role in the metabolic functions of the cells, including gene regulation, signaling, and immune response. The malfunction or deficiency of specific proteins can lead to the development of chronic diseases such as diabetes mellitus, Parkinson and Alzheimer [121,122]. One possible treatment for such diseases is the administration of protein therapeutics. For example, insulin administration is one of the most effective therapies for the treatment of diabetes mellitus type I and type II [55]. Compared to smaller synthetic drugs, proteins present unique properties including high specificity and potency, and reduced side effects (e.g. low toxicity) [123,124]; however, their use as therapeutics is limited. A significant reason for this is that native proteins can decompose upon exposure to mechanical stress and mild temperatures [123,124]. Furthermore, once introduced in a biological system, therapeutics can lose their theoretical efficacy because of fast renal clearance, difficulties in crossing cell membranes and instability in serum [125], where the decomposition is carried out by proteolytic agents [55].

A promising strategy to enhance protein stability and tissue penetration/intracellular delivery is to integrate them within MOF carriers (Table 2). Indeed it has been shown that the on-demand release of encapsulated species from MOFs can be regulated by the application of specific internal or external stimuli, such as changes in the pH, or coordinating ions [15]. ZIFs have been widely investigated for drug delivery systems because: 1) they can be easily synthesized at room temperature in aqueous media, which are ideal synthetic conditions for the encapsulation of a variety of drugs (from small synthetic drugs to large biomacromolecule-based therapeutics) [15,103]; 2) the coordination bonds between Zn^2+ and azolate linkers can be cleaved at slightly acidic pH, via chelating agents or anions with high affinity for Zn^2+, thus chemical-stimuli responsive drug carriers can be designed (e.g. pH responsive ZIF carriers for intracellular delivery) [15]; 3) the low cytotoxicity of ZIFs allows for the delivery of therapeutic doses sufficient to treat some diseases [11,13,74,101].

A pioneering study by Qu and co-workers [126] demonstrated the feasibility of ZIF-8-based nanocarriers for intracellular delivery of a model vaccine. The authors encapsulated ovalbumin (OVA, a protein antigen capable of inducing a humoral and cellular immune response) within ZIF-8 via the de novo approach. The resultant OVA@ZIF-8 biocomposite was further functionalized by the adsorption of an immune adjuvant (cytosine-phosphate-guanine oligodeoxynucleotides, CpG ODNs) to afford a core–shell composite OVA@ZIF-8-CpG with average particle size of 200 nm (Table 2). This design permits the co-delivery of the antigen and the immune adjuvants within the cell. Thus, once the OVA@ZIF-8-CpG are internalized in the cell, the acidic environment of lyso/
endosomes triggers the cytosolic release of OVA. This study demonstrated that the formation of a MOF-shell around OVA enhanced the in vivo protection against blood proteases and the on-demand release of OVA triggers a systemic immune response.

Other studies on MOF-based drug carriers have focused on improving the cell uptake and enhancing the stability of MOF bio-composites in cell media. For instance, Chu and co-workers [127] prepared BSA@ZIF-8 nanoparticles (92 nm) post-functionalized with a polyvinylpyrrolidone (PVP) coating. Live-cell studies confirmed the rapid cellular uptake of PVP-coated BSA@ZIF-8 NPs, where the nano-carriers were successfully transported from endo-lysosomes into the cytosol affording an efficient intracellular co-delivery of multiple active proteins. Similarly, Fang and co-workers [128] reported the encapsulation of chloroquine diphosphate (CQ, an autophagy inhibitor), within ZIF-8 nanoparticles decorated with poly(ethylene glycol)-folic acid (FA-PEG). The collected data demonstrated that ZIF-8 NPs coated with FA-PEG are more effectively internalized by human cervical cancer cells (HeLa, cancer cells) than embryonic kidney cells (HEK293, healthy cells). The authors suggested that this specificity can be attributed to the presence of folic acid and folate receptors on the surface of HeLa cells.

Alternative methods for the regulation of cellular uptake and target efficacy include embedding of drug carrier NPs in extracellular vesicles or membranes [129]. In this regard, Zheng and co-workers [102] reported the encapsulation of gelonin (a ribosome-inactivating polypeptide used as an apoptotic agent), within ZIF-8 nanoparticles (ca. 80 nm). The resultant biocomposites were embedded within an extracellular vesicle to enhance the specific endocytosis by homotypic cells. Vesicle gelonin@ZIF-

<table>
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<tr>
<th>Ligand</th>
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<th>MOF</th>
<th>Biotherapeutic</th>
<th>Particle size</th>
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<td>[131]</td>
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<td>[127]</td>
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<td>OVA</td>
<td>65.2 nm</td>
<td>oral administration of antigens</td>
<td>[139]</td>
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Table 2: Protein@MOFs as DDS.
8 biocomposites improves the specificity of the treatment and allowed for a systemic drug administration without compromising the integrity of toxin gelonin. This strategy has been further applied for the localized treatment of malignant tumors by bioactive MOF composites capable of producing cytotoxic agents on demand (Table 2). For instance, Cheng et al. [130] designed a MOF nano-biocomposites for the spatio-temporally controlled production of cytotoxic 1O2 species under near-infrared irradiation (NIR) regardless the hypoxia environment of tumor tissues. The bioactive composite was obtained by the co-encapsulation of catalase (CAT) and Al(III) phthalocyanine chloride tetrasulfonic acid (AlPcS4), which acts as photosensitizer (PS), within a ZIF-8 shell. Subsequently, the resultant MOF biocomposites were coated with a cancer cell membrane (Mem) leading to Mem-on-CAT&PS@ZIF-8 NPs of ca. 110 nm. The Mem coating on CAT&PS@ZIF-8 possessed adhesion properties towards tumor cells, which is beneficial for targeting cancer cells. Thus, once the nanocarrier was internalized, CAT catalyzed the decomposition of endogenous intracellular H2O2 into H2O and O2, which increased the level of O2 within the hypoxic tumor cells. Then, the resultant O2 was transformed by the PS into 1O2 upon NIR irradiation, this highly reactive species reacts with biological molecules causing lethal damage to the cells. By contrast, Qu and co-workers [131] designed a bioactive MOF biocomposite for starvation-activated cancer therapy capable of aggravating hypoxia in tumor microenvironments (Table 2). Glucose oxidase (GOx) and tirapazamine (TPZ), which is an anticancer prodrug that can be transformed into a cytotoxic radical under hypoxia conditions [132], were co-encapsulated in ZIF-8 and the resultant particles were coated with an erythrocyte membrane. The 120 nm membrane-coated GOx&TPZ@ZIF-8 particles were used for their synergistic actions against tumor cells: GOx was used to consume glucose (nutrient for cells) and intratumorally O2 (enhance hypoxia), then the simultaneous production of gluconic acid promote the MOF dissolution, leading to the release of TPZ, which is transformed into a highly cytotoxic radical under the aggravated hypoxic microenvironment.

Up to now, we have examined selected examples for cancer treatment; however, MOF-biocomposites can be applied to other pathologies, including protein disorder-related diseases. Willner’s group [100], developed a MOF-based glucose-responsive carrier for the controlled release of insulin for diabetic treatments. Glucose-responsive properties were obtained by co-encapsulation of insulin and GOx in ZIF-8 (In&GOx@ZIF-8). GOx was used to catalyze the transformation of glucose and O2 into gluconic acid and H2O2. The acidified microenvironment induced the degradation of the ZIF-8 matrix, thus liberating insulin (Fig. 6a). As H2O2 could inhibit the enzymatic function of GOx and acts as a cytotoxic agent, catalase (CAT) was co-embedded in ZIF-8 to decompose H2O2 into H2O and O2. By varying the concentration of glucose, it was shown that enzymatic cascade GOx/CAT could be activated or inhibited, thus controlling the insulin release on-demand (Fig. 6b, c). More recently, Tang and co-workers [87] used In&GOx@Co-ZIF-8 (Co-ZIF-8 = ZIF-8 doped with Co2+ ions) for the fabrication of a stimuli-responsive transdermal insulin delivery system. However, in this case the MOF matrix presents catalase-like activity due to the presence of Co2+ions as inorganic nodes, thus, the Co-ZIF-8 served as the catalase substituent in the multi-enzyme cascade process to achieve the controlled release of insulin and the simultaneous decomposition of H2O2. Falcaro and Doonan reported a systematic study on the effect of the crystalline phase on the insulin release properties [89]. In this work, the authors showed that different Zn2mIM-based crystals (dia = diamondoid, sod = sodalite, U13, and ZIF-CO3-1) can be prepared by varying the relative amount of ligand, metal, and biomacromolecule. Surprisingly, for certain synthetic conditions, the CO32− dissolved in water was co-assembled into a MOF structure named ZIF-CO3-1 (aka ZIF-C) [133]. The author examined encapsulation efficiency (EE%) and drug release kinetic of selected In@ZIFs composites upon applying an acidic stimuli (pH 5.5). A phase-dependent release profile was observed and the complete release of insulin under acidic conditions (pH 5.5) was achieved between 40 and 300 min depending on the crystalline phase of the MOF.

The stimuli-responsive release properties of ZIFs are not limited to pH-changes. Recent studies have demonstrated that ZIF-8 can be slowly degraded in phosphate buffered saline media (PBS) (Fig. 7a, b) [67]. The degradation takes place due to the affinity of the phos-
phosphate groups for the Zn$^{2+}$ cations, which leads to the formation of amorphous zinc phosphate (Fig. 7c). The biodegradability of ZIFs in presence of phosphate groups has inspired the development of ATP-responsive carriers based on ZIF-90 for the cytosolic delivery of Cas9, which is a RNA-guided endonuclease protein used to edit the genome of mammalian cells [134]. ZIF-90 is structurally analogous to ZIF-8; however, it is composed of Zn$^{2+}$ ions interconnected by imidazole-2-carboxaldehyde (HICA). This material was used by Mao and co-workers [134] for the encapsulation of RNase A and genome-editing Cas9 nuclease (protein) CRISPR/Cas9 (Fig. 8a). The competitive coordination of ATP towards Zn$^{2+}$ cations triggers the release of the cargo, where the RNase A and CRISPR/Cas9 genome editing is selectively delivered within the cells, due to the higher concentration of ATP in the cytosol (1–10 mM) than in the extracellular environment (<0.4 mM) (Fig. 8b).

Recent studies have demonstrated that the one-pot encapsulation of biomacromolecules within hybrid matrices can be extended to carboxylated-ligand based MOFs. To overcome the low solubility of this class of ligands, pioneering studies have focused on small carboxylic acids. For instance, Yang and Sun used gallic acid (GA) and Fe$^{3+}$ ions for the encapsulation of model proteins (BSA-Paclitaxel, OVA) in Fe-GA network [135,136]. The resultant nanobiocomposites were applied to localized photothermal therapy for tumor cell treatment (Table 2).

Liu and co-workers [137] developed a drug carrier comprised of meso-2,6-diaminopimelic acid (DAP) interconnected by Mn$^{2+}$ ions for targeted drug delivery of OVA. It is worth to mention that in this material, the DAP functioned as both ligand and adjuvant. Thus, the resultant nanoparticles OVA@Mn-DAP (ca. 150 nm) ensured the administration of an antigen protein and permitted the co-delivery of the adjuvant, which improved the cancer immunotherapy preventing the growth of melanoma tumors (Fig. 8c). The in vivo bioaccumulation of the biotherapeutics was tracked over time by magnetic resonance (MR) and fluorescence imaging. This study demonstrated that the retention of the biotherapeutics (OVA and DAP) in the lymph nodes increases when using OVA@Mn-DAP as a drug vehicle, in comparison to the administration of the free species (OVA and DAP) (Fig. 8d).

Similarly, Zhang and co-workers [138] reported the use of a lanthanide-based MOF carrier, obtained by guanine monophosphate (GMP) interconnected thorough Eu$^{3+}$nodes, for the encapsulation of OVA (Table 2). The resultant biocomposite was coated with an oligonucleotide capable of inducing a strong cellular immune response in cancer cells, enabling the intracellular co-delivery of OVA and tumor-associated antigens (TAAS). A further interesting example was reported by Sung, Chang, and co-workers who encapsulated OVA within MIL-53(Al)-NH$_2$ studying the system for oral administration of vaccines [139]. The MIL-53(Al)-NH$_2$ shell was found to protect the protein antigen against the harsh conditions of the intestinal tract, and, at the same time, was acting as an adjuvant for a long-lasting immune response. Next, to facilitate the permeation of the biocomposite through the mucosa barrier, the authors embedded OVA@Al-MOF particles.
within yeast-derived capsules. In vivo studies revealed that this co-ensembled arrangement functioned as “Trojan Horse”-like platform, allowing for the transepithelial transport of OVA@Al-MOF.

3.2.1. Summary and future outlook
MOFs have emerged as a new tunable platform for the design of protein-based drug delivery systems (DDS). One-pot encapsulation methods typically result in high EE% of model and clinical biotherapeutics in MOFs. By carefully choosing the target biological system (e.g. cancer cells) and the MOF (e.g. ZIFs), the DDS can be engineered toward its selective degradation (e.g. acidic pH in proximity of cancer tissues). In case of proteins@ZIFs, it was shown how these systems are responsive to specific chemical stimuli (e.g. presence of glucose for insulin release). Furthermore, the particle size of protein@MOF biocomposites are in the nano- to micro-meter range, which is suitable for administration routes ranging from intravenous injection to transdermal delivery (e.g. microneedles). Functionalisation of the MOF surface can improve the targeting properties or the circulation time of the DDS. Thus, by controlling degradation conditions, particle size, and surface chemistry both delivery and biodistribution can be optimized. In addition, the typical cost of the MOF material is marginal with respect to the price of the encapsulated biotherapeutic.

3.3. Protein@MOFs for biopreservation

Proteins are prone to denaturation and bioactivity loss upon their exposure to environmental stressors. Indeed the relatively “fragile” nature of biomacromolecules is the major issue that hampers the extended use of proteins as biotherapeutics, as well as in the development of new biosensing devices [123]. For example, after the production of protein-based therapeutics their storage, packaging, and transportation is needed before reaching the patient [16], and the bioactivity of the therapeutic has to be preserved along this journey. Lyophilization, or freeze-drying, is one of the most used strategies to preserve proteins in solid state [16]. However, a considerable amount of therapeutic proteins are formulated as aqueous solutions, which typically requires storage and transportation at low temperatures (aka “cold chain”) to improve their shelf-life. With respect to vaccines, the World Health Organization (WHO) suggest their storage at a temperature that ranges from 2 °C to 8 °C [16,42]. Thus, infrastructure required for the cold-chain increases the shipping costs, hampers the distribution of vaccines to geographically remote places and their storage in the absence of dedicated facility. Several strategies have been reported to address those problems, including lyophilization, spray-drying, vacuum foam, and protein immobilization using polymers or hydrogels [16]. An emerging protocol is the encapsulation of proteins within MOFs as this was shown to enhance the stability of the biomolecule against harsh conditions including high temperatures, organic solvents, mechanical stress, and presence of proteolytic/chaotropic agents. In this section, we highlight the recent progress of MOF-based biocomposites for biobanking.

The pioneering work by Falcaro’s group [27], demonstrated the bio-preservation capabilities of MOF-based biocomposites obtained via the biomimetic mineralization approach. In this study, the authors compared the enzymatic activity of free HRP and HRP@ZIF-8 upon exposure to inhospitable environments, including the presence of a proteolytic agent (trypsin), and boiling solvents (water and DMF) (Fig. 9a). According to this study, in presence of trypsin, HRP encapsulated within a ZIF-8 exoskeleton retained 88% of its enzymatic activity for the conversion of pyrogallol to purpurogallin, whilst the free enzyme exhibited only a 20% conversion. The protective properties of ZIF-8 were compared to other porous carriers such as CaCO3 and mesoporous SiO2. For this purpose, the authors incubated free HRP, HRP@ZIF-8, HRP@CaCO3, and HRP@SiO2 in boiling water for 1 h. The free enzyme lost its enzymatic activity, while HRP@CaCO3 and HRP@SiO2 only retained 39% and 65% of the bioactivity, respectively. By contrast, the HRP encapsulated within ZIF-8 preserved an 88% initial activity (Fig. 9a). The authors stated that the superior stability afforded by the ZIF-8 exoskeleton compared with CaCO3 and SiO2 is directly related to the tight encapsulation of the enzyme within the MOF architecture, where the biomolecules are enclosed in pockets slightly larger than the macromolecule's size. This fact was corroborated by the SAXS analysis on ZIF-8-based biocomposites, where the results revealed the presence of pocket in the MOF matrix that is 10–30% larger than the radius gyration of the encapsulated biomacromolecule. This arrangement inhibits the unfolding of the enzyme allowing bioactivity preservation [140].

More recently, Singamaneni and co-workers [44] demonstrated the practical use of ZIF-8 biocomposites for the preservation of biomarkers to improve their stability during transport, storage, and handling. In this study neutrophil gelatinase-associated lipocasin (NGAL, a protein present in blood after acute kidney injury) [141] and serum/plasma CA-125 (a tumor marker from ovarian cancer cells) [142] were used as biospecimens. The preparation of biomarkers@ZIF-8 was successfully conducted in different biological fluids such as urine, serum, plasma, and blood. Subsequently, the samples were supported on paper substrates and stored in a dry state. The samples were shipped from Missouri to California and sent back to test bioactivity of the encapsulated biospecimen (Fig. 9b). This study demonstrated that the biomacromolecules conformation is preserved at room temperature and 40 °C.

The same research group demonstrated that this strategy could be applied to preserve the biological activity of insulin (In) [143]. In this study, the authors compared the biological activity of free In and In@ZIF-8 after being exposed to various stressors, including high temperatures (25, 40 and 60 °C) for one week, mechanical agitation (200 rpm for 48 h), and the incubation in organic solvents (ethyl acetate). (Fig. 9c). The immunoassay and the spectroscopic analysis demonstrated the preservation of the biological activity of the In released from the ZIF-8 biocomposite after being stored in dry state at high temperatures (>95% at 25 °C and 40 °C, >80% at 60 °C). By contrast, free In stored under the same temperatures exhibited a decrease in its biological activity (~70%, ~60% and ~50% at 25 °C, 40 °C and 60 °C, respectively). A similar approach was employed by Chen and co-workers [45] who tested the stability of polyclonal antibodies including human immunoglobulin G (IgG), polyclonal antibody (H-IgG) and goat anti BSA IgG (G-IgG) encapsulated within two different MOF matrices (ZIF-8 and ZIF-90). To evaluate the protection effect of the MOF matrix on G-IgG@ZIF-90 and G-IgG@ZIF-8 biocomposites, the samples were exposed to a series of perturbation environments that would typically lead to denaturation of proteins (i.e. high temperatures, organic solvents, and mechanical pressure). Subsequently, the bioactivity of the encapsulated and free G-IgG was assessed by enzyme-linked immunosorbent assay (ELISA) test. The results showed that G-IgG released from the MOF matrix retained its binding capability (>90%), and showed low aggregation (13–25%) after being exposed at 75 °C for 20 min. By contrast, the free antibody, stored under the same conditions (75 °C), lost its initial binding activity (~10%) and presented severe aggregation (88%) (Fig. 9d). These results highlight the thermal protection of MOF matrices for antibodies.

The versatility of ZIFs was further confirmed by Ouyang and coworkers [118] who encapsulated HRP, Cyt c, ribonuclease A (RNase A), Ribonuclease B (RNase B) and trypsin, using PVP and cysteine as additives (vide supra). Then, the protection capabilities of the MOF shell were verified by exposing the resultant biocomposites to high temperatures, high concentrations of urea, and proteases.
The bioactivity preservation of proteins through encapsulation within MOFs is not limited to drug delivery applications. Recent studies have demonstrated that this approach can be easily integrated in the design of protein-based nanodevices, where the biorecognition capabilities or biocatalytic activity are threatened by the operating conditions. For instance, the development of technologies based on enzymes immobilized on plasmonic nanostructures opens the possibility of using the photothermal effect of the nanomaterial to enhance the catalytic activity of conventional enzymes by controlling the local temperature close to the biomolecule [144]. However, most of the enzymes present low thermostability. Thus, to overcome this issue, Singamaneni and co-workers showed that the stability of thermophilic enzymes (i.e. HRP) supported on Au nanorods (AuNRs) can be improved by encapsulation within a ZIF-8 shell [144]. The catalytic activity of the AuNR-HRP@ZIF-8 biocomposite was evaluated before and after the exposure to denaturing conditions, including high temperatures, toluene, and the presence of proteases. Some of the results indicate that the non-encapsulated system AuNR-HRP presents a catalytic activity below 5% after exposure to high temperatures. Conversely, AuNR-HRP@ZIF-8 preserves 85% of the original biological activity after exposure to 55 °C for 7 days.

More recently, Wang and co-workers [145] employed ZIF-8-based biocomposites to maintain the biorecognition capabilities of the anti-CD-146 antibody supported on an atomic force microscope (AFM) tip. This antibody-conjugated nanostructure (AFM tip/anti-CD-146) serves as a nanoscale test to study the recognition capabilities of anti-CD-146 towards CD-146, which is an antigen typically expressed on the surface of melanoma cells. To this end, the authors monitored the binding force and binding frequency of AFM tip/anti-CD-146 before and after being exposed to harsh reaction conditions. The results showed that the AFM tip functionalyzed with anti-CD-146 does not recognise CD-146 after being stored at 50 °C for 3 days. The binding capacity of the AFM tip/anti-CD-146 coated with a ZIF-8, exposed to the same conditions (50 °C/3 days), were tested after the removal of ZIF-8 exoskeleton by rinsing the tip with an acid solution. The collected data demonstrated that the ZIF-8 exoskeleton preserves the recognition capabilities of anti-CD-146 under denaturing conditions. Moreover, such results demonstrate that the encapsulation in ZIFs and the subsequent degradation MOF shell do not affect the antibody conformation.

3.3.1. Summary and future outlook

MOFs represent a promising material for biospecimen preservation due to their unique protection properties and on-demand degradability. In particular, biomacromolecules@MOF was found to be an effective strategy for rapid encapsulation at room temperature by adding the MOF precursors to a water-based solution of the biospecimen. Hormones, enzymes, biomarkers, vaccines, anti-
bodies have been encapsulated in ZIF-8 and protected from temperature, solvents, and mechanical stress. After their release from the ZIF matrix, their activity was consistently superior to the free biomacromolecules. Indeed, it has been shown that MOF coatings present superior protective properties than other inorganic coatings such as CaCO$_3$ and SiO$_2$. The remarkable biopreservation capabilities of MOF biocomposites have been applied in the cold-chain-free transport of biotherapeutics, this permits the handling, transport, and storage of biospecimens without the need for refrigeration, thereby reducing the shipping costs. Additionally, recent studies have demonstrated that the protective properties of MOF biocomposites can be easily applied in the design of protein-based nanodevices, where the biorecognition capabilities or bio-catalytic activity of the macromolecule are threatened by the operating conditions.

3.4. Protein@MOFs and Protein-on-MOFs for biosensing

As defined by IUPAC, a biosensor is a self-contained integrated device which provides selective quantitative or semiquantitative analytical information by using a biological recognition element in direct spatial contact with a signal transducer [146]. A biosensor can use a biological response mediated by enzymes, immunosystems, or cells into a quantified processable signal. The biological recognition unit acts as a chemical receptor that responds to a target analyte; this response is transformed by the transducer into a processable electrochemical, colorimetric or optical signal [145–149]. Although different sensing, transduction and integration methods are available, sensitivity and reproducibility remains the major challenges in current diagnostic technologies [145–149]. This stimulates research in different directions, including new sensing technologies, where the main goals are fast, accurate, precise, and reproducible responses [147–151]. Improved biosensors will facilitate early diagnoses and prompt treatments. To this end, protein-based MOF biocomposites have emerged as alternative materials for the design of new, highly sensitive, and cost-effective biosensors [17–21]. The protein, which acts as a biorecognition element, can be found either embedded (protein@MOFs) in or bioconjugated to MOFs (protein-on-MOFs) [18]; whilst the signal molecules can be located within the pore network of the MOF. Therefore, the use of MOF-based composites as detection probes permits the colocalization, of the biorecognition element and a large number of signaling elements in one single particle. So far, MOF biocomposites have been extensively studied for sensing a wide variety of analytes ranging from small molecules (glucose, H$_2$O$_2$, phenol, etc.), to large biomolecules such as antigens, biomarker, infectious agents and exosomes [17–21].

3.4.1. Applications of protein@MOF biocomposites for small molecule detection

In biochemistry, an analyte with molecular weight below 1000 Da is classified as a small molecule [148]. In particular, most of the available studies report the use of protein@MOF composites for the detection of small molecules such as H$_2$O$_2$ and glucose. Protein@MOFs as H$_2$O$_2$ sensors

Hydrogen peroxide H$_2$O$_2$ is a major biological reactive oxygen species obtained as a by-product of numerous metabolic reactions. Although H$_2$O$_2$ plays an important role in cellular signaling processes, H$_2$O$_2$ is prone to produce hydroxyl radicals, which are strong oxidants capable of reacting with many biological molecules causing cell and tissue damaging. Therefore, it is important to develop adequate analytic techniques able to detect H$_2$O$_2$ in living organisms [152].

A pioneering report by Ge and Liu and co-workers [26] in 2014 suggested the use protein@MOF biocomposites in biosensing by demonstrating that the biocomposite obtained from the encapsulation of Cyt c within ZIF-8 crystals can be used as a fluorometric sensor to detect H$_2$O$_2$, methyl ethyl ketone peroxide (MEKP), and tert-butyl hydroperoxide (TBHP) in solution. The authors used N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red, fluorogenic probe) as a signal molecule, since in presence of the target peroxides the Cyt c catalyzes the oxidation of Amplex Red to yield a fluorescent phenoxazine (i.e. resorufin) [153] (Scheme 1a).

This work inspired the development of other protein@MOF biosensors for the detection of H$_2$O$_2$. For instance, Yang and co-workers [154] designed a colorimetric biosensor encapsulating bovine hemoglobin (Bhb) in ZIF-8 particles: H$_2$O$_2$ was detected by using 4-aminotripyrine (AAP) as signal molecules, where the peroxidase-like activity of BHb@ZIF-8 was used to perform the catalytic co-oxidation of phenol and AAP in presence of H$_2$O$_2$ (Scheme 1b) [155]. According to the reported results, the catalytic activity of this system was 423% higher than that observed in the free Bhb. A possible explanation could be the ability of most MOFs to uptake/concentrate hydrophobic molecules [156]. The BHb@ZIF-8 sensor showed faster catalytic response (4 min), than the free enzyme (15 min), and a wide linear range (0–800 μM) for H$_2$O$_2$, with a limit of detection (LOD) of 1 μM (Table 3). This example confirms the importance of both protein and MOF matrix as a strategy to prepare biosensors with enhanced properties (i.e. response time and LOD). The applications of MOF biocomposites for the detection of H$_2$O$_2$ have been extended to the development of electrochemically-based sensors. Liu and co-workers [157] reported the electrochemical detection of H$_2$O$_2$, using HRP@ZIFs nanocomposites supported on graphene oxide (GO). This HRP@ZIFs-on-GO multicomposite material was immobilized on ITO electrode using polyethyleneimine. This arrangement provides to the electrode a high sensitivity towards the target analyte (Table 3).

Protein@MOFs as glucose sensors

Glucose is a biomarker for diabetes and thus the development of novel sensors is an active field of research. Diabetes disease causes abnormal levels of insulin in the body, due to either a malfunction of the pancreas (diabetes type 1) or the ineffective use of insulin by cells (diabetes type 2). Insulin is the hormone that regulates the level of glucose in the blood, thus mild alterations in insulin levels or action will lead to hypoglycemia or hyperglycemia conditions, which can cause severe health issues including tissue damage, kidney failure, blindness, among others [158]. Consequently, regular glucose monitoring in diabetic patients is important to avoid further health complications [158]. The use of MOF-based biocomposites for the enzymatic detection of glucose has been extensively explored mostly as colorimetric or electrochemical sensors [21].

Liu and co-workers reported the first example of a colorimetric glucose biosensor based on the co-encapsulation of multiple enzymes (GOx and HRP) in ZIF-8 particles [159]. This multienzyme system (GOx&HRP@ZIF-8) operates via a biocatalytic cascade process: 1) GOx, in presence of O$_2$ oxidase glucose to yield gluconic acid and H$_2$O$_2$; 2) HRP consumes H$_2$O$_2$ for the oxidation of ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) into ABTS$^*$ (Scheme 1c) [160]. The latter is a chromogenic agent that absorbs light at 415 nm and thus the production can be monitored via UV–vis spectroscopy (Fig. 10a). The reported LOD was 0.5 μM, demonstrating higher sensitivity than the other colorimetric glucose sensors reported in the literature. Additionally, irrespective of interfering compounds (e.g. fructose, maltose) GOx&HRP@ZIF-8 showed specificity towards glucose detection.
It is worth mentioning that the close spatial location of GOx and HRP in a porous microenvironment can facilitate the molecular diffusion enhancing the efficiency of the enzymatic cascade reaction.

Recent studies further supported the importance of the spatial distribution of the enzymes within the MOF for enhanced multi-enzyme cascade catalysis [162]. To this end, Jiang and co-workers [163] demonstrated that the compartmentalization of GOx&HRP multi-catalytic system within ZIF-8 is an effective strategy to improve the sensitivity and increase the linear range of colorimetric biosensors for glucose detection (Table 3). The compartmentalization of the enzymes was achieved by mixing sodium deoxycholate (NaDC), HRP, and the Zn²⁺ precursor. This mixture resulted in HRP encapsulated in a hydrogel coating. Then, a second solution containing both HmIM and GOx was added to this mixture. The authors suggested that the hydrogel allowed for the spatial separation between enzymes and served as a soft template to form hollow ZIF-8 spheres denoted as H-ZIF-8. These H-ZIF-8 capsules serve to separate the enzymes in different compartments, where the HRP is located within the central cavity, whilst the GOx is supported onto the outer region of the H-ZIF-8 sphere. As a consequence, the controlled spatial localization of enzymes promotes the efficient diffusion of products from HRP to GOx pulling the equilibrium towards the product formation. The authors demonstrated the feasibility of this compartmentalized system GOx-on-HRP@H-ZIF-8 as a glucose colorimetric biosensor for point-of-care (POC) testing devices.

Similarly, Chen and co-workers [164] showed that the combined immobilization of GOx&HRP within ZIF-8 can be applied in the design of a portable lab-on-chip device for glucose detection. The colorimetric biosensor was fabricated using a polypropylene membrane as support and polydopamine/polyethyleneimine (PDA/PEI) to fix composite in microfluidic channels (Table 3).

Although, most of the GOx@Zn(mIM)₂ biocomposites used for glucose detection focused on crystalline sod-ZIF-8, Ge and co-workers [165] prepared amorphous am-GOx@Zn(mIM)₂ biocom-
posite and compared the performance of \( \text{am-Gox@Zn(mim)}_2 \) with \( \text{sod-Gox@Zn(mim)}_2 \) for glucose detection. The authors found that the am-Gox@Zn(mim)_2 nanocomposites (particle size 150 nm) are 20 times more active than its crystalline counterpart (Gox@ZIF-8) and as active as the free Gox in solution. The authors attribute the remarkable enzymatic activity to the presence of coordination defects in am-Zn(mim)_2 created during the encapsulation process. This was supported by the presence of mesopores (1–10 nm) in the am-Zn(mim)_2, which significantly improved the mass transfer towards the embedded enzyme and, thereby enhancing the enzymatic response. The am-Gox@Zn(mim)_2 particles were used as a probe for the in situ determination of glucose within living cells, and the results demonstrated a high fluorescence sensitivity for the determination of intracellular glucose (Table 3). In fact, this technique was able to discriminate among different cell types based on their glucose uptake capabilities. The determination of this cellular metabolite permits the distinction between cancerous and normal cells, providing an attractive platform for applications on early diagnosis of tumors. Such results are in agreement with a recent study published by Hu et al. [120], who reported the synthesis of different enzyme@ZIF-8 based biocomposites (enzyme = Cyt c, HRP, and Gox) using a microfluid flow reactor. The authors demonstrated that this synthetic procedure allows for the continuous change in the concentration of ZIF-8 precursors, which modifies the mIM:Zn^{2+} ratio in the laminar flow and leads to the formation of coordination defects. These defects create mesopores that facilitate the mass transport through the MOF shell, which improves the enzymatic activity of the biocomposites in comparison with their analogues obtained by batch synthesis. It should be noted that all multi-enzyme biosensors based on the catalytic oxidation of glucose by Gox give rise to an acidic microenvironment within the MOF biocomposite that could affect the stability MOFs. This is the case for ZIF-8, which degrades under acidic conditions [103]. This aspect is a limit for the reusability of Gox@ZIF-8 biosensors. Alternatively, Zhao et al. [166] described the preparation of Gox@ZIF-8 coated with Fe-polydopamine (Fe-PDA) to afford an Fe-PDA-on-Gox@ZIF-8 composite. The Fe-PDA coating enhances the stability of the nanoreactor under acidic conditions, and mimicked the peroxidase activity, acting as H_{2}O_{2} scavenger. The recyclability test performed on Fe-PDA-on-Gox@ZIF-8 demonstrated that this system maintained 85% initial activity after being reused 4 times.

So far, we have been discussing systems based on Zn(mim)_2; however, recent reports have shown that the enzymatic detection of glucose can be achieved by immobilization of a biorecognition element on the outer surface of a preformed MOF with peroxidase-mimicking activity. This approach could exploit the MOF to reduce issues related to the production of intermediates during multi-enzyme cascade reactions. To prove this hypothesis, Zhu and co-workers [161] reported the fabrication of colorimetric glucose biosensor based on grafting (covalent immobilization) of Gox onto Fe-MIL-88B-NH_2, a MOF that shows a peroxidase-like activity (Fig. 10c). In this catalytic process, first Gox catalyzes the glucose oxidation to yield gluconic acid and H_2O_2, then Fe-MIL-88B-NH_2 consumes H_2O_2 to produce OH, which oxidized the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB), into a green–blue colored ox-TMB intermediate (\( \lambda_{\text{max}} \) 652 nm) (see Scheme 1e). This Gox-on-Fe-MIL-88B-NH_2 biosensor displayed a linear response range of 1–500 \( \mu \)M, with a LOD of 0.478 \( \mu \)M (Table 3). When compared with the free enzyme system, Gox-on-Fe-MIL-88B-NH_2 showed higher tolerance to temperature, pH changes, and reusability (tested up to five cycles). Fe-MIL-88B was also employed by Zhao et al. [167] for the fabrication of enzymatic glucose sensor. In that work, the synthesis of Fe-MIL-88B was performed in the presence of lauric acid, to induce the formation of hierarchical porous material HP-Fe-MIL-88B (pore size distribution centered at 2, 4 and 8 nm). Then, the MOF matrix was post functionalized with boronic acid (BA) to facilitate the grafting Gox on the Fe-MOF surface. The glucose response of the hierarchical porous biocomposite Gox-on-HP-Fe-MIL-88B-BA (PSD centered at 4 and 8 nm; PSD = pore size distribution) and microporous Gox-on-Fe-MIL-88B-BA (PSD < 2 nm) was assessed by UV–vis spectroscopy using TMB as chromogenic substrate (Scheme 1e). The results indicated that hierarchically porous Gox-on-HP-Fe-MIL-88B-BA provides a 10-times faster catalytic response to glucose than Gox-on-Fe-MIL-88B-BA. The authors suggested that this difference can be attributed to the improved mass transfer in hierarchical porous systems.

Other MOFs that have been explored for the development of enzymatic glucose sensors are lanthanide-based coordination polymers. The fluorescence properties of such MOF materials together with the quenching effect of H_{2}O_{2} released during the enzymatic oxidation of glucose is attractive for the fabrication of all-in-one fluorescent probes. For instance, Gao et al. [168] used fluorescent properties of lanthanide-based coordination polymers and carbon dots (CDs) to prepare a ratiometric biosensor for glucose detection in serum. This biosensor was obtained via co-encapsulation of Gox and CDs within a coordination polymer (CP) comprised of Tb^{3+} ions interconnected by adenosine monophosphate linkers (Fig. 11a). Then biocomposite was further functionalized with carboxyphenylboronic acid (CPBA). The complexation of this compound to the free coordination sites of Tb^{3+}-
enhances the fluorescence of this lanthanide inhibiting the quenching effect of water molecules (Fig. 11a). Interestingly, the \( \text{H}_2\text{O}_2 \) released during the glucose oxidation induced the dequenching of CPBA, leading to quenching of \( \text{Tb}^{3+} \) fluorescence (Fig. 11b–e). Importantly, this process did not influence the fluorescent response of CDs, that could be employed as a reference for the ratiometric sensing of glucose. The simultaneous readings of two different wavelengths, the constant fluorescence of the internal standard (CDs response) and MOF response on glucose concentration (\( \text{Tb}^{3+} \) emission), was shown to minimize the effect of external interferences derived from the instrument, background, and environment. 

For this glucose sensor, the authors reported a linear response range of 0.5–300 \( \mu \text{M} \) and LOD of 80 \( \text{nM} \).

The use of MOF biocomposites for glucose detection has been extended in electrochemical biosensing (Tables 3 and 4). In 2013, Mao and co-workers \[169\] screened different zeolitic imidazolate frameworks (\( \text{ZIF}-7, \text{ZIF}-8, \text{ZIF}-67, \text{ZIF}-68, \) and \( \text{ZIF}-70 \)) as MOF supports to co-immobilize methylene green (MG) and glucose dehydrogenase (GDH) via bio-conjugation approach. Since \( \text{ZIF}-70 \) exhibited the highest loading capacity, the dehydrogenase-based electrochemical sensor was fabricated by depositing MG-on-\( \text{ZIF}-70 \) and GDH-on-\( \text{ZIF}-70 \) onto glassy carbon electrodes. According to the reported data, this biosensor was highly specific towards glucose with a linear response range within 0.1–2 \( \text{mM} \). Similarly, Patra et al. reported the development of glucose amperometric biosensor based on \( \text{GOx-on-MIL}-100(\text{Fe}) \) supported on a carbon electrode functionalized with PtNPs \[170\]. The sensitivity for this system is 71 \( \text{mA M}^{-1} \text{cm}^{-2} \) with a limit of detection of 5 \( \mu \text{M} \), and response time <5 s. More recently, Dong and co-workers \[171\] reported a glucose biosensor fabricated by the co-encapsulation of NiPd NPs and \( \text{GOx} \) within \( \text{ZIF}-8 \) nanoflowers. The resultant \( \text{GOx-NiPd@ZIF-8} \) biosensor combined the peroxidase-like activity of NiPd NPs with the enzymatic activity of \( \text{GOx} \). The authors demonstrate the applicability of \( \text{GOx-NiPd@ZIF-8} \) for both colorimetric and electrochemical detection of glucose. The performance of this \( \text{GOx-NiPd@ZIF-8} \) biosensor as a colorimetric glucose sensor was assessed using \( \alpha \)-phenylenediamine (OPD) as chromogenic substrate, which in presence of \( \text{H}_2\text{O}_2 \) is catalytically oxidized to yield a 2,3-diaminophenazine (PDA, \( \lambda_{\text{max}} = 417 \text{ nm} \)) (Scheme 1d). This system presents a linear response within the range of 0.01–0.3 \( \text{mM} \). Based on the competitive reactions of oxygen reduction and glucose oxidation, \( \text{GOx-NiPd@ZIF-8} \) was tested for the electrochemical detection of glucose. In this case, the authors detected an increment in the cathodic peak centered at \(-0.45 \text{ V} \) when the concentration of \( \text{H}_2\text{O}_2 \) rose from 0 to 10 \( \text{mM} \). However, the linear response reported for this electrochemical sensor is in the 0.01–0.3 \( \text{mM} \) range.

3.4.2. Protein@MOF and protein-on-MOF biocomposites in immunoassays

Early detection of specific biomarkers in biological fluids is crucial for a prompt medical diagnosis and successful therapeutic process. Immuno-diagnostic tests are attractive systems for early detection. In particular the enzyme-linked immunosorbent assay (ELISA), which is a biochemical method designed to detect and quantify targeted biomacromolecules (examples of antigens are proteins, carbohydrates, nucleic acids, viruses). ELISA uses antibodies as biorecognition elements, and an enzyme, that can be either directly or indirectly coupled to the antibody, provides the signal response when the antigen–antibody complex is formed \[149\] (Fig. 12a). Due to the extraordinary specificity of the antibodies, this analytical method has been extensively applied in food and environmental analysis, and medical diagnosis. \[149\] Nevertheless, the synthesis and purification of enzyme-antibodies conjugates requires laborious protocols. As result, a loss of biorecognition properties (antibody) and catalytic activity of the tag (enzyme) can be observed, and the sensitivity can be compromised \[172,173\]. These issues, combined with the low concentration of biomarkers and infection agents, limit the practical application ELISA tests at the POC tests \[147–151\]. A strategy to improve the sensitivity of immune-diagnostic tests is to increase either the antibody concentration or the number of signaling elements attached to the antibody. However, these changes can affect the background and the specificity of the method. An emerging strategy to improve sensitivity, stability, and selectivity is the immobilization of antibodies on MOFs, where the resulting antibody-on-MOF biocomposites can be used as probes (Fig. 12b). Indeed, both signaling and bio-recognition components can be co-localized in the same MOF-based particle. These systems as are further discussed in the next sections.

Colorimetric and fluorometric immunoassays

Tan and co-workers \[174\] reported the integration of a rabbit anti-mouse immunoglobulin G antibody (RlgG) on a Cu-MOF. The Cu-MOF network not only preserved the bioactivity of the antibody under operational conditions, but also due to its peroxidase-like activity, this material replaced the natural enzyme and acted as
the signal amplification unit. The RlgG-on-Cu-MOF system responded selectively towards mlgG, even in the presence of interfering proteins (GOx, BSA, HRP, etc.). The authors suggest that the lack of enzyme-antibody conjugate enhances the capture efficiency of the biosensor RlgG-on-Cu-MOF towards the antigen mlgG, thereby improving the detection sensitivity of immune-assay (reported of 0.34 ng/mL) (Table 5). This work demonstrates the feasibility of MOF composites with enzyme-like properties as replacements of natural enzyme-antibody conjugates. As, unlike traditional ELISA tests, this RlgG-on-Cu-MOF biosensor offers an easier and less costly detecting procedure.

Li and co-workers [175] reported the bioconjugation of secondary antibodies (Ab2) on the outer surface of HKUST-1 (Cu-BTC; BTC = 1,3,5-benzenetricarboxylic acid), to develop a fluorogenic click immunoassay for the detection of hepatitis B virus antigen (HBSAg) in clinical serum samples. This sandwich-type assay is composed of a microplate with immobilized antibodies (Ab1) that can create a complex with biomarker antigen (HBSAg); then the secondary antibodies attached to the MOF surface particles (Ab2-on-HKUST-1) will selectively bind to the immobilized HBSAg (Fig. 13a). In this system, HKUST-1 acts as an Ab2 carrier and as signal probe. Finally, Cu(II) in HKUST-1 can be reduced to Cu(1), which can catalyze the azide-alkyne cycloaddition reaction between an alkynyl and fluorogenic molecules of azide to yield the corresponding triazole, and produce a fluorescence signal. The formation of multiple triazole molecules within the porous MOF network produced such a strong fluorescence signal with a reported detection limit below 11.2 pg mL⁻¹ (Fig. 13b,c). Wang et al. [176] also reported a MOF-based probe for the design of a sandwich-type colorimetric immunoassay for prostate-specific antigen detection (PSA). The MOF-probe was prepared through the co-immobilization of: (i) Ab2 (biorecognition element) and (ii) methyl yellow (MY; signal molecule) on ZIF-8 nanoparticles. The resultant system Ab2&MY-on-ZIF-8 acts as signal tag for the specific recognition of PSA. The latter can be quantified by the colorimetric signal generated by the allochroic MY molecules released from the biocomposite of upon applying acidic conditions (λmax 510 nm). According to the authors the encapsulation of MY within the ZIF-8 probe reduces the leakage of the signal molecules, which permits the signal amplification (Table 5). The overexpression of carcinoembryonic antigen (CEA) is closely associated with colorectal cancer and it serves as tumor biomarker. Therefore, it is highly important the development of sensitive analytic techniques capable of detecting low concentrations of CEA in body fluids. With this aim in mind, Tan and co-workers [177] reported the one-pot co-immobilization of alkaline phosphatase (ALP) and anti-CEA antibody in a Zn²⁺-adenine coordination polymer (ZnCPs). The role of ALP in the resultant anti-CEA-on-ALP@ZnCPs biocomposite was to induce a catalytic cascade reaction to yield a colored product. This process take place in presence of ascorbic acid 2-phosphate (AAP), Fe³⁺, and 1,10-phenanthroline ( Phen) in 2 steps: first the ALP hydrolyses AAP producing ascorbic acid (AA); second, AA...
reduces Fe$^{3+}$ into Fe$^{2+}$, which is then coordinated by Phen forming the orange-colored complex $[\text{Fe(Phen)}_3]^{2+}$. The CEA concentration was determined by monitoring the intensity of this colored complex in a sandwich-assay configuration. The reported detection limit for this system is 21.1 pg/mL. The authors claimed that this biosensor is more cost-effective than traditional enzyme-labeled antibody immunoassays. Moreover, the integration of ALP&anti-CEA within the ZnCPs enhanced the stability of the biorecognition element. An alternative strategy to improve the sensitivity of immunoassays was reported by Tang and co-workers [178] for the colorimetric detecting alfa-fetoprotein (AFP, tumor biomarker). The authors used ZIF-67 as a MOF template for the construction of hollow metal-polydopamine framework (MPDA) particles as an efficient material for the surface adsorption of secondary antibodies (Ab2) and large amount of the signal molecules (thymolphthalein dye; TP) (Fig. 13 d–h). According to the authors, this configuration enhanced the signal amplification when compared with conventional colorimetric immunoassays (Table 5).

**Electrochemical immunoassays**

Electrochemical biosensors operate by converting a biological event into an electronic signal. These devices merge biological materials as sensitive components with electrodes as conversion

**Table 5**

Comparative overview of protein@MOF and protein-on-MOF biocomposites for biomacromolecules detection.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Molecule target</th>
<th>Biorecognition element</th>
<th>MOF</th>
<th>Synthetic strategy</th>
<th>Linear range (pM/L)</th>
<th>Detection limit (pg/mL)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPR colorimetric</td>
<td>anti-IgG</td>
<td>rabbit IgG</td>
<td>ZIF-8</td>
<td>Encapsulation</td>
<td>not clear</td>
<td>not clear</td>
<td>[187]</td>
</tr>
<tr>
<td>colorimetric</td>
<td>anti-IgG</td>
<td>RIgG</td>
<td>Cu-MOF</td>
<td>One-pot immobilization</td>
<td>1–100 ng/mL</td>
<td>0.34 ng/mL</td>
<td>[174]</td>
</tr>
<tr>
<td>sense-and-treat ELISA colorimetric</td>
<td>CEA</td>
<td>CEA Ab2</td>
<td>ZIF-8</td>
<td>Grafting</td>
<td>10–500 pg/mL</td>
<td>10 pg/mL</td>
<td>[188]</td>
</tr>
<tr>
<td>Fluorometric</td>
<td>hep-B virus antigen (HBsAg)</td>
<td>Ab2</td>
<td>ZIF-8</td>
<td>Grafting</td>
<td>0.001–1 ng/mL</td>
<td>0.67 pg/mL</td>
<td>[176]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>PSA antigen</td>
<td>Ab2</td>
<td>ZIF-8</td>
<td>Grafting</td>
<td>15–10,000 TCID$_{50}$ mL$^{-1}$</td>
<td>[179]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALV-f</td>
<td>Ab2</td>
<td>HKUST-1</td>
<td>Grafting</td>
<td>0.001–1 mg/mL</td>
<td>0.13 pg/mL</td>
<td>[180]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>PSA antigen</td>
<td>Ab2</td>
<td>HKUST-1</td>
<td>Grafting</td>
<td>1–400 ng/mL</td>
<td>0.2 ng/mL</td>
<td>[181]</td>
</tr>
<tr>
<td></td>
<td>CRP exosomes</td>
<td>Ab2</td>
<td>ZIF-67</td>
<td>Grafting</td>
<td>1.3×10$^{-2}$ to 2.6×10$^{-2}$ particles mL$^{-1}$</td>
<td>[183]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>insulin</td>
<td>Ab1</td>
<td>UIO-67</td>
<td>Grafting</td>
<td>0.0025 to 50 ng mL$^{-1}$</td>
<td>0.001 ng mL$^{-1}$</td>
<td>[189]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>insulin</td>
<td>Ab2</td>
<td>MPDA</td>
<td>Grafting</td>
<td>10–1000 pg/mL</td>
<td>2.3 pg/mL</td>
<td>[178]</td>
</tr>
<tr>
<td>colorimetric</td>
<td>AFP</td>
<td>Ab2</td>
<td>ZIF-8</td>
<td>Grafting</td>
<td>11.1 fM to 35.6 pM</td>
<td>4.4 fM</td>
<td>[190]</td>
</tr>
<tr>
<td>Fluorometric</td>
<td>cTnI</td>
<td>Ab2</td>
<td>ZIF-8</td>
<td>Grafting</td>
<td>0.05–100 ng/mL</td>
<td>21.1 pg/mL</td>
<td>[177]</td>
</tr>
<tr>
<td>colorimetric</td>
<td>CEA</td>
<td>CEA Ab2</td>
<td>ZnCPs</td>
<td>Encapsulation</td>
<td>0.05–100 ng/mL</td>
<td>21.1 pg/mL</td>
<td>[177]</td>
</tr>
</tbody>
</table>

Fig. 13. (a) Schematic representation of the synthesis of Ab2 immobilized onto a Cu-MOF, and the conceptual sandwich-type method for immunodetection of HBsAg. A fluorescence signal is detected at 395 nm due to the CuAAC click reaction catalyzed by the Cu-MOF. (b) Fluorescence emission spectra upon addition of different concentrations of HBsAg. (c) Selectivity response of the immunoassay against different protein biomarkers. Adapted with permission from ref. [175] Copyright 2020 American Chemical Society. (d,e) Schematic representation of the preparation procedure of MPDA@TP-Dab and MPDA@TP-linked immunoassay (MLISA) for alpha-fetoprotein (AFP) detection. (f) TEM image and HAADF-STEM image and elemental mapping for Co, N, and O of ZIF-67. (g) TEM image and photograph and HAADF-STEM image and elemental mapping for Co, N, and O of MPDA. (h) Photographs taken from the MLISA-based visual assay using different concentrations of AFP standards. Adapted with permission from ref. [178] Copyright 2018 American Chemical Society.
In this regard, Gui and co-workers [183] developed an electrochemical sensor for the detection of a specific avian retrovirus known as ALV-J (avian leukosis virus subgroup J). This immunosensor was comprised of two principal components: (1) the probe, which was made with hollow ZIF-8 structures, where the outer surface was functionalized with secondary antibodies (Ab2) and HRP. (2) The electrochemical-responsive interface fabricated using a glassy carbon electrode (GCE) functionalized with tannic acid (TA), Fe₃O₄ nanoparticles, and primary antibodies (Ab1). Here, tannic acid allowed for the in-situ reduction of graphene oxide to conductive graphene. The presence of reactive cis-diols in tannic acid were used for the grafting of Ab1 onto the GCE. The Fe₃O₄ nanoparticles mimicked the oxidation properties of HRP, which enhances the LOD of the immunosensor. The authors stated that the use of hollow ZIF-8 improved the electron transfer properties of the material, which enhanced the response of the immunosensor platform. The reported detection range was 152–10,000 TCID₅₀ mL⁻¹ (TCID₅₀ is the tissue culture infective dose), with a LOD = 140 TCID₅₀ mL⁻¹.

More recently, Ma and co-workers [180] reported the design of a sandwich electrochemical immunosensor based on Fe-MIL-88B-NH₂ decorated with gold nanoparticles and functionalized with secondary antibodies. This sensor was used for the detection of prostate-specific antigen (PSA) (Table 5).

Yang and co-workers [181] reported a simple electrochemical immunoassay for the detection of C-reactive protein which is a biomarker typically associate to coronary heart diseases. In this work, HKUST-1 particles were immobilized onto the surface of Au NPs. Subsequently, the resultant MOF-on-Au particles were used to immobilized signal antibodies (Ab2) onto the HKUST-1 surface, where the immobilization process was achieved by an adsorption strategy. The authors claimed that in this system, the Cu²⁺ ions from the HKUST-1 can be used to produce a signal transduction which allows the detection of the analyte. Furthermore, the authors reported the use of covalent organic frameworks (COFs) functionalized with platinum nanoparticles as a substrate to further increase the electronic conductivity. This strategy offers an excellent signal transduction platform for the detection of CRP. Indeed, under optimized experimental conditions this biosensor presented a linear detection range from 1 to 400 ng/mL and a LOD of 0.2 ng/mL.

Electrochemical biocomposite devices can be even extended to the detection of complex biomarkers such as exosomes (extracellular vesicles implicated in the transmission of disease states) [182]. In this regard, Gui and co-workers [183] developed an electrochemical sensor for early cancer diagnosis. To fabricate this sensor, ZIF-67 doped with ferrocene (Fc) was electrodeposited on an indium tin oxide (ITO) support. The resultant electrode was further functionalized with black phosphorous nanosheets (BPNSs) and a single-strand DNA aptamer labeled with methylene blue (MB). The latter presents high selectivity towards CD63 protein expressed on breast cancer MCF-7 cells. This aptasensor presents a dual redox signal response. The first one originates from the MB labeled on aptamer, hence, it changes in the presence of exosomes. The second one, that comes from the Fc, hardly changes, which allows the intrinsic self-calibration of the biosensor. The resultant device exhibits rapid response and high specificity towards breast cancer exosomes detecting from 1.3 × 10⁴ to 2.6 × 10⁵ particles mL⁻¹, with a LOD of 100 particles mL⁻¹.

3.4.3. Summary and future outlook

Protein-based MOF biocomposites are emerging materials as recognition elements for the preparation of cost-effective biosensors with sensitivity and selectivity towards a variety of analytes ranging from small molecules such as H₂O₂ and glucose to large biomacromolecules such as protein biomarkers. Both protein@MOF and protein-on-MOF configurations can be exploited for colorimetric, electrochemical and fluorometric biosensors. An important aspect of this class of composites is the facile co-immobilization of the biorecognition element and the signaling components within the same MOF matrix; this aspect is expected to facilitate the implementation of the assay in miniaturized biochips, reduce the time involved in the detection process, and enhance the LOD (Tables 3–5). Accordingly, the discussed strategies lay the foundation for the progress of MOF-based sensors for POC testing devices for real-time diagnostic analyses.

4. Carbohydrates and MOFs as drug delivery systems

Carbohydrates (CHs) are a class of biomolecules which provide several biological functions, including cellular and intracellular interactions in the form of cell surface receptors, signaling molecules, and bacterial adhesives [191]. In biomedicine, carbohydrate-based drugs have recently gained attention as potential treatments for cancer, diabetes, AIDS, influenza, bacterial infections, and rheumatoid arthritis [192–194]. Among the CH subset utilized in biotechnology and medicine, the most commercially important ones are glycosaminoglycans (GAGs) [195]. GAGs are unbranched high molecular weight polysaccharides constructed from amino sugars (D-glucosamine or D-galactosamine) and uronic acid (D-glucuronic or D-iduronic acid) [196]. GAGs and proteoglycans regulate important biological activities. For instance, they contribute significantly to the extracellular matrix organization, cellular signaling, as well as the regulation of cell growth and tissue maturation [197]. The application of GAGs in biomedicine includes their use as anticoagulant and anti-inflammatory agents, as well as in wound healing therapy. Moreover, GAGs-based therapeutics have been explored for the treatment of osteoarthritis, diabetes, viral and bacterial infections and more recently for cancer treatment, as tumor progression inhibitors [195–197].

However, the targeted-delivery of GAGs-based therapeutics presents challenges including structural fragility and low bioavailability because of their high molecular weight and charge [197]. For instance, Heparin (HP), which is the most employed carbohydrate-based drug for the treatment of thromboembolic and anti-inflammatory angiogenesis, suffers from pharmacokinetics issues such as poor bioavailability, fast serum clearance, and rapid degradation [195,197–199]. Another example is Hyaluronic acid (HA) that can be used for wound healing applications. HA is prone to lose its therapeutic properties due to the presence of hydrolytic enzymes (hyaluronidase) and reactive oxygen species (ROS) [200,201]. Thus, from an administration perspective, having access to a carrier that provides protection and tunable release profiles would enhance the efficacy of the therapies [202]. Both of these are desired properties for GAGs when used as wound healing agents (e.g. HA requires controlled and local delivery), and as an anticoagulant (e.g. HP if administered via the parenteral route could result in bleeding-related complications due to burst release) [203].

In this context, MOFs have the potential to become suitable carriers for carbohydrate-based drug therapeutics as: 1) MOFs have demonstrated protective properties for other biotherapeutics; and, 2) the encapsulation of CH-based drugs in MOFs can enhance the pharmacokinetics and bioavailability properties of these therapeutics [103,204,205]. However, research devoted to CHs@MOFs is in its infancy; thus, further exploratory and systematic studies about protective properties of CHs@MOFs and CHs-on-MOFs are required.
Fig. 14. CH@MOF and CH-on-MOF biocomposites and their applications in drug delivery systems. CH=carbohydrate

In this section, we will focus on the application of MOFs as drug delivery systems (DDS) for the delivery of CH-based therapeutics (Fig. 14). Additionally, there are several interesting studies where CHs are used as coatings for MOF carriers to improve the targeting ability and stability. Indeed, the CHs-on-MOF particles have shown interesting properties as stealth drug carriers for DDS. Thus, selected examples are included in this section.

4.1. MOF composites as DDS for carbohydrate-based therapeutics

As is the case for formation of protein biocomposites, the integration of a CH-based therapeutic in a MOF carrier can be achieved through a bioconjugation approach (adsorption, grafting) or via a one-pot embedding strategy. Regarding formation of CH-on-MOFs biocomposites for use as DDS, Vinogradov and co-workers [205] reported the adsorption of heparin on MIL-101(Fe). The biocomposite was prepared by adding MIL-101(Fe) into a heparin solution to yield HP-on-MIL-101(Fe). Subsequently, to extend the bioactivity of the composite, HP-on-MIL-101(Fe) was mixed with an alumina sol–gel matrix and streptokinase (SK, a protein-based thrombolytic medication) solution to create HP-on-MIL-101(Fe) + SK@alumina hybrid. The resulting material was applied to polytetrafluoroethylene (PTFE) vein implants (Fig. 15). The heparin adsorption occurred due to partial ligand loss from MIL-101(Fe) followed by heparin sulfate group chelation on iron at defect sites in the MIL-101(Fe) to give a declared encapsulation efficiency of 90% (average particle size was 163 nm). The cytotoxicity of HP-on-MIL-101(Fe) was found negligible up to 40 μM on HCT116 cells. Moreover, an anticoagulant test was carried out by immersing the composites in blood plasma which lead to the degradation of the MOF structure resulting in HP release, and providing good anticoagulant activity which was comparable to free HP. In addition, HP-on-MIL-101(Fe) coated with alumina-based sol–gel matrices (HP-on-MIL-101(Fe) + SK@alumina) could enhance the bioactivity of HP-on-MIL-101(Fe) and prevent the premature clogging of the thrombolytic coating. The authors suggested this bioactive nanocoating as a useful approach to reduce complications in PTFE vein implants [205].

Another bioconjugation approach for the preparation of CH-on-MOF biocomposites exploits a grafting strategy, which involves the formation of covalent bonds between a reactive group on the outer surface of the MOF and targeted biomolecule, (vide supra). For instance, Ghae and co-workers [204] grafted HA to the outer surface of a carboxylated ZIF (FZIF) constructed from zinc ions and 2-methyl-1H-imidazole-4,5-dicarboxylic acid (2MtIMDC). HA was cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysuccinimide (EDC/NHS). The average particle size of HA-on-FZIF was c.a. 45 nm (drug loading not disclosed). The solution was freeze-dried and a film of 300 μm thickness was obtained. The MOF-biocomposite film demonstrated superior mechanical properties when compared with a film made with pure MOF and this was attributed to the affinity of HA for the ZIF particles. The degradation tests for HA and HA-on-FZIF films were investigated by exposing the biocomposites to PBS solution, with and without hyaluronidase to promote the hydrolytic degradation of HA. The authors showed that the films degraded after 11 days; however, the contribution of phosphate ions to the MOF degradation was not disclosed. The MOF biocomposite films showed antibacterial properties and promoted fibroblast migration and proliferation. [204]

Recently, Astria et al. [206] encapsulated carbohydrates into MOFs using the biomimetic mineralization approach. The potential biomimetic mineralization of different carbohydrates in ZIF-8 (CHs@ZIF-8) was assessed by testing mono, di, oligo, and polysaccharides such as d-glucose, d-galactose, d-mannose, d-xyllose, d-glucitol, meglumine, methyl-β-d-glucopyranoside, N-acetyl-d-glucosamine, d-glucosamine, sucrose, maltodextrin, d-glucronic acid-ß-lactone, dextran, diethylaminoethyl dextran (DEAE-dextran), carboxymethyl dextran (CM-dextran) and cellulose as seeding agents for ZIF-8. To achieve this, each specific CH was mixed with HmIM and zinc acetate at room temperature (Fig. 16a) and the results showed that only negatively charged carbohydrates (i.e. CM-dextran, a dextran backbone substituted with carboxymethyl substituents imparting a polyanionic character) led to a MOF biocomposite (i.e. CM-dextran@ZIF-8), thus contradicting a previous finding [207]. A computational study supported the enrichment of Zn2+ around CM-dextran because of the COO− groups, thus providing the local conditions for triggering the self-assembly of the MOF around these CHs. [206]. The observations of the biomimetic mineralization role triggered by negatively charged biomacromolecules, and assembly of thereof, is in agreement with previous studies that investigated the relevance of electrostatics in the biomimetic mineralization process [114,208]. By optimizing the synthetic protocol, the CM-dextran@ZIF-8 biocomposite was obtained with a 100% encapsulation efficiency. By changing the concentration of a chelating agent (EDTA) in solution, tunable release profiles were measured for particles with 25 nm diameter. As CM-dextran is considered an inexpensive carbohydrate that closely mimics glycosaminoglycans (GAGs), CM-dextran has been widely used as a model GAG in drug delivery systems [209]. The study considered GAGs-based therapeutics; because of the negative charge of these carbohydrates, they were considered promising biomimetic mineralization agents [103]. In particular, the encapsulation of heparin (HA), hyaluronic acid (HA), chondroitin sulfate (CS), and dermatan sulfate (DS) within three different pH-responsive metal-azolate frameworks: ZIF-8, ZIF-90, and MAF-7 (Fig. 16b) was investigated. To further demonstrate the versatility of CHs@MOFs as DDS, two preclinical drugs based on GAGs and proteoglycans (GM-1111 and HepSyl) were also encapsulated in the metal azolate frameworks. GM-1111 is an anti-inflammatory drug designed for chronic rhinosinusitis and HepSyl is a synthetic proteoglycan for oncotherapy. The resultant GAG@MOFs present different properties in terms of cys-
tallinity, particle size, and spatial distribution of the cargo (Table 6). For instance, a crystalline powder was obtained from GAGs@ZIF-8 and GAGs@ZIF-90, whereas a gel was found in GAGs@MAF-7 [103]. The formation of metal–organic gels (MOG), as seen for the latter example, is typically attributed to the rapid formation of MOF nanoparticles (NPs) that aggregate through van der Waals interactions [210,211]. The EE% of GAG@MOF was evaluated using a carboxyl assay and UV–vis spectroscopy (Fig. 16 c, Table 6). The release profiles of each GAG@MOFs were studied in citrate buffer, showing that the release kinetics are system dependent (Fig. 16 d, Table 6). To further understand the distribution of HepSyl in the MOF shell, samples were assessed by confocal laser scanning microscopy (CLSM) and the results showed that while the proteoglycan is homogeneously distributed within ZIF-8 and MAF-7; in ZIF-90, HepSyl is located on the surface of the MOF particles. These observations showed that azolate-based MOFs could be employed for the encapsulation of GAGs and proteoglycan-based therapeutics, and furthermore that specific drug release properties could be designed by selecting the proper MOF matrix. These findings could pave the way for the delivery of clinical carbohydrate-based therapeutics by using MOF carriers [103].

4.2. Carbohydrates as protective coatings of MOF composites for DDS

Despite the advantageous properties MOFs as DDS, there are still some limitations that could restrict their applications, including rapid degradation, short circulation time, and poor colloidal stability in aqueous media [11,212]. To address these issues, MOF crystals have been coated with different materials such as polymers, proteins, lipids, cyclodextrins, and high molecular weight CHs [29,212–216]. The surface modification of MOFs with CHs can impart targeting delivery abilities and improve both the biodistribution and stimuli-responsive properties [217,218]. For example, it has been demonstrated that the use of HP improved the colloidal stability of the drug carrier, [218,219] while HA enhanced the protection of the MOF and improved the targeting properties of the carrier [212,217]. Moreover, as HA can be enzymatically degraded by hyaluronidase enzyme, the HA-based coating can be removed on demand once internalized within environments with high concentrations of hydrolytic enzymes (e.g. tumor cells) [212].

In 2015, Horcajada and co-workers [218] employed heparin as a coating agent to modify the outer surface of MIL-100(Fe) nanocarriers for the release of two model drugs (i) caffeine and (ii) furazan. Heparin was chosen as a coating agent due to its specific interaction with the surface of the MOF particle for the preparation of the HP-on-MIL-100(Fe) biocomposite. HP-on-MIL-100(Fe) DDS were prepared using two different approaches: 1) HP solution was added to a colloidal solution of MIL-100(Fe) NPs in ethanol, and then the HP-on-MIL-100(Fe) was loaded with a model drug (caffeine); or, 2) MIL-100(Fe) NPs were preloaded with furazan and then the resultant furazan@MIL-100(Fe) NPs were coated with rhodamine-labeled-heparin (Fig. 17 a). The HP-on-MIL-100(Fe) coating preserved the crystallinity and porosity of the starting MIL-100(Fe), and enhanced the colloidal stability in different media such as water, PBS, PBS containing albumin 5.4% w/v and Roswell Park Memorial Institute (RPMI) medium. When infiltrating caffeine, HP-on-MIL-100(Fe) showed a drug loading up to 42 wt% and release profile data showed only 20% of caffeine was released from HP-on-MIL-100(Fe) compared to uncoated MIL-100(Fe) (56%) in the first hour in PBS solution. Thus, this study demonstrated how the surface modification of MOF NPs with GAGs can improve
the properties of MOF-based DDS for biomedical applications [218].

Another carbohydrate that could be employed as a coating agent for MOF carriers is CM-dextran. In this realm, Nikitin and co-workers [220] described the use of a CM-dextran coating for antibody-on-MOF DDS in cancer therapy. The biocomposite was prepared by adding CM-dextran to a suspension of Fe₃O₄@MIL-100(Fe), a magnetic framework composites (MFC) [221] that possess dynamic localization properties [156,222] using an external magnetic field. Following coating doxorubicin/daunorubicin was loaded into the platform and coupled with an antibody. The CM-dextran coating avoided the aggregation of magnetic MOF particles, while providing a sustained release profile and maintaining the antibody specificity [220].

CH-coated MOFs were also tested for oral drug administration. Namazi and co-workers [223] used a pH-sensitive biopolymer, carboxymethylcellulose (CMC), to protect and control the release of ibuprofen encapsulated in Cu-MOF (IBU@Cu-MOF) for oral delivery. The CMC-coated IBU@Cu-MOF composite was prepared by mixing the dispersed IBU@Cu-MOF in water with CMC. The CMC-on-IBU@Cu-MOF biocomposite demonstrated better protection than uncoated Cu-IBU@MOF under simulated gastrointestinal conditions. In addition, CMC-on-IBU@Cu-MOF was reported to have low cytotoxicity and extended stability of drug dosing that leads to a controlled release in the gastrointestinal tract [223].

In terms of targeting properties, HA has been reported as a good targeting agent for cancer cells which over-express CD44 [224]. CD44 is a transmembrane glycoprotein that is highly expressed in cancer cells and which plays an essential role in cancer development and metastasis progression. [225]. Post-modification of MOF-based DDS with HA has been widely investigated to enhance the efficacy of cancer therapies [226]. For instance, a new promising phototheranostic platform HA-on-MIL-100(Fe) NPs infiltrated with
Indocyanine green (ICG) was developed for multimodal imaging-guided cancer photothermal therapy (PTT) by Liu and co-workers [227]. ICG is an FDA-approved near-infrared (NIR) organic dye for clinical application. However, its low aqueous solubility, low cancer specificity, and low sensitivity for cancer theranostics prevent its use in practical medical applications. To overcome these limitations, ICG-infiltrated within HA-on-MIL-100(Fe) NPs were prepared by the conjugating of MIL-100(Fe) NPs with HA in aqueous solution. Following this the porous biocomposite was loaded with ICG to yield a HA-on-ICG@MIL-100(Fe) biocomposite. The ICG drug

Table 6
Properties of GAG@MOF biocomposites formed with different metal-azolate framework.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Topology</th>
<th>Particle size</th>
<th>EE %</th>
<th>Time elapsed for 100% drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA@ZIF-8</td>
<td>sod</td>
<td>&lt;500 nm</td>
<td>60</td>
<td>40 min</td>
</tr>
<tr>
<td>HP@ZIF-8</td>
<td>sod</td>
<td>1 µm</td>
<td>100</td>
<td>40 min</td>
</tr>
<tr>
<td>CS@ZIF-8</td>
<td>sod</td>
<td>&lt;500 nm</td>
<td>100</td>
<td>1 h</td>
</tr>
<tr>
<td>DS@ZIF-8</td>
<td>sod</td>
<td>&lt;500 nm</td>
<td>60</td>
<td>1 h</td>
</tr>
<tr>
<td>GM-1111@ZIF-8</td>
<td>predominantly sod</td>
<td>500 nm–4 µm</td>
<td>60</td>
<td>20 min</td>
</tr>
<tr>
<td>HepSYL@ZIF-90</td>
<td>mix of sod and dia</td>
<td>5 µm–7 µm</td>
<td>50</td>
<td>1.5 h</td>
</tr>
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Fig. 17. (a) A schematic illustration of the external surface modifications of MIL-100(Fe) NPs with heparin. Reprinted with permission from ref. [218] (b) In vitro cellular uptake of HA-on-ICG@MIL-100(Fe) NPs investigated using CLSM and (c) flow cytometry analysis (solutions that contained 10 µg/mL of ICG, scale bar = 50 µm) Reprinted with permission from ref. [227] Copyright 2016 American Chemical Society.
loading reported for this system is 40 wt%. Additionally, the authors state that this system provides low cytotoxicity and photothermal stability under NIR light irradiation which is a desirable property for PTT. The cellular uptake was investigated using CLSM and flow cytometry analysis and showed the high targeting ability for CD44-positive MCF-7 cells/xenograft tumors (Fig. 17b, c). This demonstrates that ICG-infiltrated HA-on-MIL-100(Fe) NPs could be a potential theranostic platform for imaging-guided cancer treatment [227].

A dual chemo- and photodynamic therapy (PDT) system based on the PCN-224 MOF coated with HA was developed for advanced anticancer therapy by Ryu and co-workers [226]. HA-coated MOF NPs were prepared by dispersing Dox-loaded PCN-224 into an HA aqueous solution (HA-on-Dox@PCN-224) [226]. As in the case of Ghaee and co-workers [204], the interaction between HA and MOF NPs was attributed mostly to the coordination between carboxylate groups of HA and the cations of MOF surface. The formation of HA-on-PCN-224 enhances the targeting ability of the carrier towards CD44 as described previously. This feature could be used for selective drug delivery in tumor treatments. In fact, an in vitro experiment for the combined chemo- and PDT demonstrated Dox loaded HA-on-PCN-224 provided an enhanced cancer therapeutic effect under the chosen light irradiation conditions [226].

Another interesting example of using HA as a targeting agent in cancer therapy was reported by Yao and co-workers [228]. In this work, the authors encapsulated the antibacterial and anticancer drug Acriflavine (ACF) into MOF NPs (PCN-224) and coated them with immunologic adjuvant (CpG) and HA. This study again demonstrated a HA-mediated preferential cellular uptake behavior for targeting CD44 membrane in cancer cells [228].

For photodynamic therapy (PDT), Yu and co-workers [229] mixed Ce6 (a second-generation photosensitizer with antitumor activity) with zinc nitrate and HmIM to obtain Ce6@ZIF-8 that could concentrate the zinc ions in and around biomacromolecules (computational modelling that negatively charged carbohydrates assist structural formation and play a role in several functions). This molecule showed high affinity to MOFs, as this knowledge will progress the design new carbohydrate scaffolds with high affinity for biomimetic mineralization, and their role of carbohydrates for biomimetic mineralization, and their examples further exemplify the need to understand in detail the chemistry and biofunctionality of HA-on-MOFs [230].

In another example, Zhao and co-workers [230] demonstrated that an HA conjugated MOF could improve the targeting ability of the anticancer drug doxorubicin (HA-on-Dox@ZIF-8) for chemotherapy and MR imaging [230]. The synthesis of HA-on-Dox@ZIF-8 was carried out by loading the doxorubicin into ZIF-8 followed by coating Dox@ZIF-8 with polydopamine and conjugating with hyaluronic acid via Fe\(^{3+}\) mediated coordination reaction. HA-on-Dox@ZIF-8 provided pH-responsive release of DOX in acidic environments and targeted drug delivery towards CD44-rich membranes on PC-3 cells (prostate cancer cell line). Moreover, the authors suggested that this system could be used for theranostic (cancer therapy and magnetic resonance (MR) imaging) due to Fe\(^{3+}\) coordinated to the carboxylic groups of HA coating the Dox@ZIF-8 particles [230].

4.3. The role of carbohydrates in living cell encapsulation processes to form MOF biocomposites

We and others have postulated that carbohydrates also play a significant role in the encapsulation of living cells, such as yeast cells and bacteria, within MOF composites [231,232]. The bacterial cell wall contains a variety of glycoconjugates and polysaccharides that assists structural formation and play a role in several functions of the cell [233]. For instance, in gram-negative bacteria such as *Escherichia coli* (E. coli), peptidoglycans can be found in the cell wall and they contribute to the mechanical strength and maintenance of cell shape [233]. A peptidoglycan is comprised of glycan chains associated with short peptides [234]. In yeast cells, carbohydrates represent a large portion of the cell wall; indeed 85% of the yeast cell wall is made of polysaccharides [234], and in yeast and bacterial cells they contribute significantly to the overall negative surface charge [232,235,236]. For example, the yeast *Saccharomyces cerevisiae* has a negative charge due to ionized carboxylic and phosphoric acid groups on the surface [232,235]. Chen et al. studied the effect of different zinc precursors (zinc nitrate, zinc sulfate and zinc acetate) for the formation of a ZIF-8 exoskeleton on *S. cerevisiae*. The authors elegantly explained that yeast cells are enveloped by mannoprotein which consists of 90% (w/w) carbohydrate fraction and has negative charges due to the phosphodiester bridges [232]. Given that in the biomimetic mineralization process negatively charged carbohydrates enhance MOF crystallization [206], the presence of negatively charged carbohydrates likely facilitates the MOF shell formation on living cells [232].

In the case of *Micrococcus luteus* coated with ZIF-8, we note that this bacterium also has a membrane rich in peptidoglycans [231,237], which have a negative surface charge due to the presence of teichoic acid polymers [237]. Therein, the authors postulated that peptidoglycans and glycoproteins could enhance the MOF precursor concentration, which favours the formation of a ZIF-8 exoskeleton. Later it was confirmed by experimental and computational modelling that negatively charged carbohydrates could concentrate the zinc ions in and around biomacromolecules [206]. Thus, it was postulated that peptidoglycans and glycoproteins could enhance the precursor concentration, thereby triggering the formation of a ZIF-8 exoskeleton. Another study by Chen et al. reported the formation of a ZIF-8 shell on *E. coli*. According to the authors, the surface of this type of bacteria is negatively charged because of the constituent phosphate groups in lipopolysaccharides [232,238]. Therefore, the presence of lipopolysaccharides in the outer membrane of the cell was suggested to enhance the local concentration of zinc cations, thus facilitating the nucleation and growth of ZIF-8 particles. These examples further exemplify the need to understand in detail the role of carbohydrates for biomimetic mineralization, and their affinity to MOFs, as this knowledge will progress the design new protective and perm-selective artificial coatings for living cells for applications to biomedicine.

4.4. Summary and future outlook

Progress has been made combining carbohydrates (CHs) and MOFs for biomedical applications. CH MOF biocomposites were investigated for DDS and two main configurations have been tested: CHs-on-MOFs and CHs@MOFs. The first class, which involves CH coatings on MOFs, is the most extensively studied to improve stability, targeting properties and bio-distribution of pre-formed MOF-based DDS. More recently, it was found that large clinical CH-based therapeutics can be encapsulated within MOFs using a one-pot strategy [103,206]. Because of the large number of CHs-based therapeutics in the market and in clinical trial [239], additional work is needed to develop a better understanding of the advantages and limitations of these promising systems.

5. Nucleic acids@MOF composites

Nucleic acids including oligonucleotides (ODN), ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) provide promising tools for therapeutic targets [240,241]. Small interfering ribonucleic acid (siRNA) can knock down a gene in a sequence-specific manner and control a disease caused by the activity of one or several genes,
includes viral infections [242–246], dominant genetic disorders [247–249], autoimmune disease [250], and cancer [251–256]. Plasmid deoxyribonucleic acid (pDNA) encodes a wild-type gene to a “depot” organ where the subsequent secretion of the therapeutic protein from this depot has the potential to treat genetic diseases such as hemophilia [257]. The main hurdle for developing such promising therapeutics remains in overcoming the abyss of targeted delivery. To this end, both viral and non-viral vectors have been actively used to achieve this goal. Although viral gene delivery achieves high levels of gene expression, it has several disadvantages such as immunogenicity and oncogenicity that make it problematic for clinical trials [258]. Non-viral vectors have attracted much attention due to their universal application range and high safety level over viral vectors [259]. Among the different synthetic gene delivery vehicles, MOFs show great prospects due to the possibility of tuning their porosity and functionality by tailored molecular design [260]. Moreover, MOFs can overcome enzymatic and acidic digestion through endosomal escape and successfully maintain the physical and chemical integrity of the entrapped genetic materials [261]. In this section we will discuss relevant examples of the encapsulation of nucleic acids in MOF shells and the use of these biocomposites in gene expression, silencing and editing, for application in drug delivery and cell manipulation (Fig. 18).

The first examples of MOF biocomposites for the delivery of nucleic acids involved siRNA [262] and other oligonucleotides [263] bioconjugated to the MOF surface. Lin and co-workers explored multidrug resistance (MDR) gene-silencing by reporting the first example of nanoscale metal–organic frameworks (nMOFs) for the co-delivery of siRNAs and cisplatin [262]. They used Zr-based metal-organic frameworks (UiO-68), formed by heating a solution of ZrCl₄ and amino triphenyl dicarboxylic acid in DMF for 5 days at 80 °C, followed by loading Cisplatin prodrug via infiltration (Cis@UiO). MDR gene-silencing siRNAs were coordinated with metal ions on the UiO-68 nMOF’s surface through simple mixing in water to give (siRNA-on-Cis@UiO) with 128 ± 3 nm size. siRNA-on-Cis@UiO exhibited higher siRNA uptake compared to naked siRNA by 11-fold. The release profile of siRNA in phosphate buffered saline (PBS) was higher than siRNA release in water as phosphate ions coordinate with the dissociated Zr ions after internalization leading to the destruction of the endosomal structure. Cytotoxicity studies of SKOV-3 cells treated by siRNA-on-UiO showed no toxic effects.

The concept of lysosomal escape prompted researchers to test the possibility of more advanced gene editing technology using MOFs. For example, clustered Regularly Interspaced Short Palindromic Repeats or CRISPR-associated Cas genes are essential in adaptive immunity in selected bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material [264]. Consequently, this approach can make precise, targeted changes to the genome of living cells. However, for this technique to be effective, the Cas9 protein and sgRNA need to be perfectly protected from endosomal degradation. In 2018, Khashab and co-workers reported the first example of Crispr/Cas9 delivery by one-pot co-encapsulation into nanoscale zeolitic imidazolate framework-8 (Crispr/Cas9@ZIF-8, abbreviated as CC@ZIF-8) with a size of 100 nm and excellent loading efficiency of 17% [261]. Moreover, CC@ZIFs enhanced the endosomal escape through the protonation of the imidazole moieties (Fig. 19c). The stability study of CC@ZIFs indicated that <3% of Crispr/Cas9 was released under physiological conditions (pH 7) while >60% was released at acidic conditions due to ZIF degradation at low pH (Fig. 19d). The CC@ZIFs exhibit superior efficiency for knocking down gene expression (GFP), which reached up to 37% over 4 days in Chinese hamster ovary (CHO) cells [261]. Furthermore, Khashab and co-workers improved the targetability by using a cancer cell membrane coating strategy to enable cell specific delivery of Crispr/Cas9 [265]. Human breast adenocarcinoma cell membrane coated CC-ZIFs were prepared through coextrusion of CC@ZIFs and the MCF extracted membrane forming C²-ZIFMCF with an average size of 120 nm. C²-ZIFs were supported by TEM and Zeta potential measurements as the charge decreased from 8.83 ± 2.96 eV to −17.3 ± 2.82 eV after coating as a result of using the negatively charged cell membrane (Fig. 19e–f). C²-ZIFMCF efficiency for specific and targeted delivery has been demonstrated by ICP-MS, flow cytometric and CLSM where the cellular uptake of C²-ZIFMCF was higher in MCF-7 cells compared to other cell types due to the inherent homotypic binding phenomenon. Moreover, The C²-ZIFMCF efficiency for gene editing against EGFP expression showed a decrease of EGFP expression by 3-fold compared to MCF-7 treated by C²-ZIFMCF only which showed a 1-fold repression in the EGFP expression. This confirms the ability to improve the targetability of MOF systems by proper functionalization [263].

In 2019, Farha and co-workers reported the successful infiltration and delivery of unmodified siRNA, utilizing a mesoporous zirconium-based MOFs (NU-1000), to the cytoplasm [23]. After activation at 100 °C for 3 days, proton NMR demonstrated that residual solvent (i.e. DMF) was successfully removed thus allowing for the subsequent loading of the NU-1000 pores with siRNA. The capability of NU-1000 to protect siRNA from enzyme degradation was demonstrated as relevant siRNA bands were observed on a polyacrylamide gel after an enzymatic attack. The location of siRNA in the MOF particles was evaluated by using fluorescence-lifetime imaging microscopy (FLIM) indicating that some siRNA was located on NU-1000 surface, while the majority of siRNA was found inside NU-1000 pores. However, in vitro studies did not show an obvious change in the mCherry expression when HEK293-mC cells were incubated with siRNA@NU-1000, at another time although the mCherry expression was similar to siRNA@lipofectamine. They hypothesized that the inconsistent change in mCherry gene knockdown is due to siRNA@NU-1000 degradation in the endosomes. The authors overcame endosomal entrapment by employing cofactors that have the ability to open endosomes such as proton-sponges (PS) and membrane opening peptides. siRNA@NU-1000 incubated with either 5.3 μg of ammonium chloride (NH₄Cl) or...
0.04 PS resulted in decrease of mCherry expression to ca. 75% and 78% respectively compared to NU-1000 alone. Also, 0.4 lg of the amphipathic KALA cell-penetrating peptide incubated with siRNA@NU-1000 led to a decrease in mCherry signal to ca. 73% [23]. More recently, Horcajada and co-workers reported the first example of mRNA infiltration into biocompatible iron-based mesoporous MOFs [26]. In this work, iron (III) trimesate (MIL-100) and iron (III) aminoterephthalate (MIL-101_NH2) were used as hosts for the infiltration of non-specific siRNA (as a control) and miR-145 (an onco-suppressor miRNA, for therapeutic purposes). The siRNA and miR-154 infiltration was favored by the opposite charges that the nucleic acids and the MIL particles possess in water. To investigate the localization of the loaded nucleic acids, the authors studied N2 sorption isotherms: after the nucleic acid infiltration, the N2 uptake, calculated surface area and pore volume were lowered, suggesting successful nucleic acids entrapment within the pores. Compared to MIL-101_NH2, MIL-100 NPs showed better nucleic acid protection against enzymatic degradation and this behavior was associated with the smaller pore size. The authors proved also the successful delivery of RNA as RNA@bioMOF were internalized, while the bare RNA could not penetrate the tested cells. Finally, to evaluate the RNA@bioMOF’s therapeutic efficiency, SW480 cancer cells were incubated for 72 h with miR145@MIL-101_NH2 and miR145@MIL-100 and efficient transfection activities of the delivered miR145 were demonstrated [26].

As for DNA delivery, Deng, Zhou and co-workers reported in 2018 the reversible DNA infiltration into mesoporous MOFs [26]. Different lengths of single-stranded (ssDNA) were infiltrated into a series of MOFs (Ni-IRMOF-74-II to -V) with the same hexagonal topology and different pore size (2.2 nm, 2.9 nm, 3.5 nm and 4.2 nm) (Fig. 19a). The pore environment of the Ni-IRMOF-74 series reveal that MOF–ssDNA interaction and release are responsive to the DNA target (cDNA) existing in the cells (Fig. 19b). Furthermore, the Ni-IRMOF-74 series exhibits a better protection efficiency of ssDNA from degradation compared to UiO-66, UiO-67, and mesoporous silica, which were used as controls. Both Ni-IRMOF-74-II and Ni-IRMOF-74-III show excellent transfection efficiency in primary mouse immune cells (CD4 + T cells), 60% and 92% respectively, compared to the commercial agent, Neofect (50%) [26].

Early attempts at DNA one-pot encapsulation in MOFs and delivery were initiated by Shukla and co-workers and by Tang and co-workers in 2019 [268,269]. Shukla and colleagues reported a complete gene-set (6.5 kilo base-pairs) encapsulation using zeolitic imidazolate framework-8 (ZIF-8) following the biomimetic mineralization process (Fig. 20a) [268]. They confirmed that the DNA has been completely encapsulated using the propidium iodide (PI) dye commonly used for DNA staining. Fluorescence spectroscopy detected the emission peak of PI labeled DNA at 617 nm. The absence of any emission peak when the supernatant and plGFP@ZIF-8 were treated with PI confirms the successful encapsulation (Fig. 20 b, c). Furthermore, they demonstrated that the encapsulated plasmid (plGFP) remains functionally intact through detecting green fluorescent signals corresponding to GFP expression in human prostate cancer epithelial cells (PC-3) [268]. Tang et al. prepared pDNA (pEGFP, 4.7 kilo base-pairs) and ZIF-8 biocomposites following two encapsulation strategies: the biomimetic mineralization of pDNA@ZIF-8 and the coprecipitation of pEGFP-C1@ZIF-8-PEI synthesis (Fig. 20 d) [269]. In the presence of PEI (MW~25kD), pEGFP-C1@ZIF-8-PEI nanostructures exhibit higher loading (~3.4 wt%) and better stability against DNase degradation (~82.3%) due to the strong electrostatic interaction between PEI and pDNA. Cellular viability study of pEGFP-
C1@ZIF-8 and pEGFP-C1@ZIF-8-PEI shows the negative impact of PEI on cytotoxicity. Moreover, pEGFP-C1@ZIF-8-PEI exhibits higher cellular uptake than pEGFP-C1@ZIF-8, probably owing to the positive charge interaction with the negatively charged cell membrane, which further facilitates the internalization of the nanostructures. ZIF-8 was also utilized for gene and photodynamic therapy as a nanocarrier for chlorin e6 (PDT agent) functionalized DNAzyme, and Zn2+ ions to support the biocatalytic activity of the Zn2+ dependent DNAzyme [270]. Western blot analysis and qRT-PCR studies indicated successful DNAzyme-mediated cleavage reaction in MCF-7 cells treated by DNAzyme@ZIF-8 [270]. The EPR effect facilitates the delivery of Ce6-DNAzyme@ZIF-8 to the cancer cells without DNAzyme degradation followed by controlled release of Ce6-DNAzyme via ZIF-8 degradation in the cytoplasm. Surprisingly, the apoptotic ratio of the photo-irradiated Ce6-DNAzyme@ZIF-8 was higher than that of the gene therapy and PDT alone, (44.9%, 19.85% and 33.6%) respectively [270].

5.1. Summary and future outlook

MOFs nanoparticles are promising vectors for genetic materials encapsulation and delivery. The main challenges in genetic material delivery are poor cellular uptake and rapid degradation. Consequently, some of the main factors that should be considered for the design of superior genetic materials delivery platforms include: 1) high protection against enzymatic degradation in the case of MOF bioencapsulates synthesized via one-pot encapsulation methods, or coating with cofactors such as cell membranes as nucleic acids could stay on the outer surface of MOFs due to their complex nature, charge and size; 2) enhancing the cellular uptake by controlling MOF size, coating with positively charge materials or other materials that can selectively adhere to cell membranes such as antibodies; 3) enhancing endosomal/lysosomal escape by using cofactors such as proton sponges or membrane penetrating peptides. We believe that heterogeneous coordination-based delivery systems will play a major role in the future of the design and fabrication of the next generation smart materials for automated and controlled biomedical applications.

6. Encapsulation of cells and viruses in MOFs

Among the wide variety of MOF-biocomposites, the encapsulation of cells and viruses within a MOF has been demonstrated to improve stability during manipulation, handling or storage, suggesting that encapsulation of cells and viruses in MOFs could progress cell- and virus-based therapies, diagnostics and drug screening [6,48]. However, this remains one of the most underexplored fields, mostly due to the limitations related to maintaining cell viability or preventing the virus degradation, while working with biocompatible reagents and synthetic conditions [6].

In general, the advantages that a synthetic shell could offer are numerous. For example, single-cell coatings can provide effective protection from environmental factors such as cytotoxic compounds, mechanical stress and UV radiation, which would help in enabling potential applications for cultured cells. Applications such as cell therapy and tissue engineering require ex vivo handling and manipulation of cells in which mechanical stress can be an important hazard [46]. For example, one of the key applications of animal cells is bioprinting and it has been reported that the shear stress suffered during the extrusion of bio-inks is a limiting factor for the development of this technique [271]. Physical forces such as shear stress in the needle during intravenous injection, shear and extensional forces during in vitro applications (e.g. microfluidic or bioreactor systems) and during centrifugation, damage the plasma membrane of cells, reducing cell viability due to leakage of cytosolic components [272–274]. This is particularly important for mammalian cells, since animal cells do not possess a strong cell wall or exoskeletal shell to provide structural support. The possibility of overcoming this problem by protecting the cells through micro- and nano-encapsulation has been therefore of increasing interest in the research community.

Typical strategies involve microencapsulation in hydrogel systems, nanocoatings of soft polymers and hydrogels, polyelectrolytes, mineral shells and supramolecular metal–organic complexes [275,276]. Single cell-in-shell approaches based on materials such as metal-oxides and polymers have shown good results for applications in biocatalysis (mainly photosynthesis or organic transformations), probiotics (probiotic delivery) and cell therapy (immunotherapy, blood transfusion and stem-cell ther-
Various types of microbial and mammalian cells (including stem cells, red blood cells, fibroblasts, and endothelial cells) have been successfully encapsulated with these materials [277]. However, among the challenges that limit their application is the need for precise control over the perm-selective properties of the artificial exoskeletons and the necessity to recover the fully functional cells via the degradation of the synthetic shell under biocompatible conditions.

MOFs offer a number of unique features which make them applicable for live-cell encapsulation. For instance, an important aspect in the case of live-cell encapsulation is the preservation of cell’s normal functions; thus the protective coating should allow for the diffusion of nutrients and stimulants, while blocking potentially cytotoxic agents. In this context, the narrow pore distribution of MOFs provides a clear advantage over other materials (i.e. silica, other metal oxides and polymers) used as coatings [6]. In addition, biological entities can trigger the self-assembly of MOFs. For example, self-standing cell walls extracted from yeast cells have been shown to be a good template for the creation of different MOF microcapsules with size-selective permeability and different compositions (ZIF-8, Cu-BTC, MIL-53) by biomimetic mineralization [278]. Additionally, the building-block approach to MOF-coatings can also afford biocomposites with abiotic properties such as magnetism, electrical conductivity, multi-wavelength fluorescence, intracellular activity sensing, selective permeability and tunable cell mechanics, which do not occur naturally on most living organisms [279].

With respect to virus encapsulation and virus-like particles (VLP), it was suggested that MOFs could provide essential protection for virus-based therapies and vaccines which typically require constant refrigeration (i.e. a cold chain) to protect the biostructures (VLP), it was suggested that MOFs could provide essential protection for the prevention of viral diseases. To highlight the progress made so far, and to visualize more clearly the aspects where there is still opportunity for further development, we have summarized the discussed results in Table 7. From this summary, it can be seen that the synthetic strategies can be broadly divided in two: 1) biomimetic mineralization, and 2) encapsulation by depositing pre-synthesized MOF nanoparticles onto the living cell surface, with an optional additional step of cross-linking to promote NP-NP complexation. In this section we will mainly discuss MOF-encapsulated live cells and viruses with applications in biopreservation and cell and virus manipulation (Fig. 21). Although the scope of this review is focused on the applications of the bioentity@MOF composites, given the research on this topic is not widely developed we will divide the discussion into cells@MOF and virus@MOF composites, with each topic covered in subsections describing both preparation and potential applications.

6.1. Encapsulation of live cells within MOFs

In 2016, Falcaro and co-workers performed the first encapsulation of living cells in a MOF via self-assembly of zeolitic imidazolate framework-8 (ZIF-8) on the surface of Saccharomyces cerevisiae (baker’s yeast) and Micrococcus luteus (a bacterium) [231] (Fig. 22a). The synthesis was performed in water and the formation of the MOF occurred spontaneously. By comparing the viability of yeast cells cultured in the presence of free ZIF-8 particles with that of untreated cells (24 h incubation period), the authors found that the ZIF-8 particles did not adversely affect the viability of yeast cells. Critically, the viability before and after application of the MOF coating was also studied using two standard assays with fluorescein diacetate (FDA) and resazurin as indicators, showing no significant changes in the viability of coated yeast cells with respect to untreated cells. These results indicated that the ZIF-8 coating is non-toxic to yeast cells. The crystalline porous coating was found to form a nutrient permeable exoskeleton with perm-selective properties, which protected the yeast cells in presence of lyticase, a cytotoxic enzyme, whose size is larger than the MOF pores. The same ZIF-8 shell was also found to protect against filipin, a polyene macrolide antibiotic [280], showing that yeast cells could also be successfully shielded from large antifungal agents. Remarkably, after removing the ZIF-8 coating with EDTA, a normal reproductive rate of free yeast cells was measured (Fig. 22b). The authors postulated that the biomolecule-rich surface of living yeast cells and bacteria was the determining factor for the formation of a ZIF-8 shell [27]. In this context, negatively charged carbohydrates were found to trigger the MOF formation [103,206]; thus the glycoproteins and peptidoglycans in the cell membrane could locally increase the concentration of the MOF precursors (see Section 4.3). This hypothesis was supported by Chen et al. [232] who studied different precursors for the ZIF-8 coating (vide infra).

In follow-up work, Liang et al. explored the functionalization of the cellular yeast wall with β-galactosidase, and induced the formation of a ZIF-8 coating [281]. With this proof-of-concept experiment, Liang and co-workers demonstrated that the MOF coating could preserve the bioactive functionality of an enzyme immobilized on the surface of living cells (Fig. 22c) providing nutrients in an oligotrophic environment for yeast. Indeed, in a solution with lactose, β-galactosidase, which is not naturally present in yeast cells, was used to convert disaccharides into monosaccharides (a nutrient that yeast could metabolize). Experiments showed that the bioactive exoskeleton allowed the cells to survive for >7 days in a nutrient-deficient medium, where lactose was added instead of glucose. An interesting implication of this study is the demonstration that lactose, and the biocatalytically formed glucose, could diffuse through the porous coating and reach β-galactosidase and the yeast cells, respectively. The properties of this bioactive exoskeleton were further tested by exposing the coated cells to a culture medium containing lactose and cytotoxic enzymes such as lyticase or protease, to simulate a cytotoxic and nutrient-deficient environment (Fig. 22d). The MOF-coated cells were able to survive with only a minor reduction in viability (<30%) for 7 days in these extreme environments, while the naked yeast was lysed in a matter of hours. As in the previous report, the cells were confirmed to recover their full growth potential after the removal of the biocomposite shell.

A recent study by Chen et al. systematically investigated the influence of different zinc precursors on the cell viability for the biomimetic mineralization of ZIF-8 on E. coli and yeast cells [232]. Zinc nitrate, zinc acetate, and zinc sulfate were studied as precursors to obtain a ZIF-8 shell on both types of cells. The authors found that the three zinc salts and the organic ligand (2-methylimidazole) have low toxicity to these cells; however, for concentrations above 4 mM, zinc sulfate is the precursor that impacts cell viability the most.

Other hybrid materials have been explored taking advantage of the biomimetic mineralization process on live cells for applications in biocatalysis. Qiu et al. produced a special strain of E. coli (BL21 (DE3)/pCDFDuet-gdh-cr) capable of asymmetrically reducing tert-butyl 6-cyano-(5R)-hydroxyl-3-oxohexanoate ((5R)-1) to (3R,5R)-2, for biocatalysis [282]. In order to protect the bacteria...
Table 7
Summary of recent progress made on the encapsulation of live cells and viruses in MOF-based exoskeletons.

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<th>Type</th>
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<th>Additional agents</th>
<th>Synthesis method</th>
<th>Application/effect</th>
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<td>2-mIm</td>
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<td>Biomimetic mineralization</td>
<td>Protection</td>
<td>[231,232]</td>
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<td>Yeast</td>
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<td>Protection and lactose metabolism</td>
<td>[281]</td>
</tr>
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<td>Ln-MOF</td>
<td>Tb^{3+}, Eu^{3+}</td>
<td>mellitic acid</td>
<td></td>
<td>Encapsulation by pre-synthesized particles</td>
<td>Luminescent exoskeleton and organelles</td>
<td>[285]</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Trichoderma sp.</em></td>
<td>Ln-MOF</td>
<td>Tb^{3+}, Eu^{3+}</td>
<td>mellitic acid</td>
<td></td>
<td>Encapsulation by pre-synthesized particles</td>
<td>Luminescent exoskeleton and organelles</td>
<td>[285]</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Aspergillus niger</em></td>
<td>Ln-MOF</td>
<td>Tb^{3+}, Eu^{3+}</td>
<td>mellitic acid</td>
<td></td>
<td>Encapsulation by pre-synthesized particles</td>
<td>Luminescent exoskeleton and organelles</td>
<td>[285]</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>E. coli</em></td>
<td>ZIF-8</td>
<td>Zn^{2+}</td>
<td>2-mIm</td>
<td></td>
<td>Biomimetic mineralization + crosslinking</td>
<td>Protection and enhanced recovery for biocatalysis</td>
<td>[231,232]</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>E. coli</em> (BL21(DE3)/pCDFDuet-gdh-cr) strain</td>
<td>ZIF-8</td>
<td>Zn^{2+}</td>
<td>2-mIm</td>
<td>Activated carbon, glutaraldehyde</td>
<td>Encapsulation by pre-synthesized particles</td>
<td>Protection against ROS for artificial photosynthesis</td>
<td>[286]</td>
</tr>
<tr>
<td>Bacteria (anaerobic)</td>
<td><em>Morella thermoacetica</em></td>
<td>Zr_{x}O_{y}O_{z}[(BTB)]<em>{x}[(H</em>{2}O)]_{y}</td>
<td>Zr^{4+}</td>
<td>1,3,5- benzenetribenzoate (BTB)</td>
<td>Encapsulation by pre-synthesized particles</td>
<td>Protection, abiotic properties</td>
<td>[279]</td>
<td></td>
</tr>
<tr>
<td>Mammalian cell</td>
<td>HeLa, A549, HL-60</td>
<td>ZIF-8</td>
<td>Zn^{2+}</td>
<td>2-mIm</td>
<td>Tannic acid</td>
<td>Encapsulation by pre-synthesized particles + crosslinking</td>
<td>Protection, abiotic properties</td>
<td>[279]</td>
</tr>
<tr>
<td>Mammalian cell</td>
<td>HeLa, A549, HL-60</td>
<td>MIL-100 (Fe)</td>
<td>Fe^{3+}</td>
<td>trimesic acid</td>
<td>Tannic acid</td>
<td>Encapsulation by pre-synthesized particles + crosslinking</td>
<td>Protection, abiotic properties</td>
<td>[279]</td>
</tr>
<tr>
<td>Mammalian cell</td>
<td>Raw 264.7</td>
<td>UiO-66-NH_{2}</td>
<td>Zr^{4+}</td>
<td>2-amino terephthalic acid</td>
<td>Encapsulation by pre-synthesized particles + crosslinking</td>
<td>Protection, abiotic properties</td>
<td>[279]</td>
<td></td>
</tr>
<tr>
<td>Mammalian cell</td>
<td>HeLa</td>
<td>MET-3-Fe</td>
<td>Fe^{3+}</td>
<td>1H-1,2,3-triazole</td>
<td>Tannic acid</td>
<td>Encapsulation by pre-synthesized particles + crosslinking</td>
<td>Protection, abiotic properties</td>
<td>[279]</td>
</tr>
<tr>
<td>Virus</td>
<td>Tobacco mosaic virus (TMV)</td>
<td>ZIF-8</td>
<td>Zn^{2+}</td>
<td>2-mIm</td>
<td></td>
<td>Encapsulation by pre-synthesized particles + crosslinking</td>
<td>Protection, controlled drug delivery</td>
<td>[47,208,292]</td>
</tr>
</tbody>
</table>
and to create a recyclable robust matrix which could be used continuously, the authors combined ZIF-8 encapsulation of the bacteria, glutaraldehyde (GA) cross-linking and immobilization in activated carbon (AC). The authors define the activity of the biocomposite in terms of one unit of activity taken as “the amount of enzyme required for 1 μmol of (3R,5R)-2 formed per minute at 30 °C, pH 7.0”. Then, the “activity recovery” was defined as the immobilized cells’ percentage of activity, compared to the original activity of the free whole-cells (taken as 100%). The authors report an activity recovery of 82.6% under the optimized conditions for the synthesized biocomposites (AC-ZIF-8@E. coli-GA), with a very high conversion yield of 99.5%. When compared with the free cells and celite-polyethylenimine-GA immobilized E. coli (same special strain BL21(DE3)/pCDFDuet-gdh-cr), the improved stability and recyclability of AC-ZIF-8@E. coli-GA was attributed to the additional MOF exoskeleton.

Yan et al. capitalized on the combination of live-cell encapsulation with a chemical-loaded MOF for targeted cancer treatment [283]. In this work, the authors combined a specific strain of E. coli that can target tumor cells, E. coli (MG1655), with a ZIF-8 shell loaded with both an anti-cancer drug (doxorubicin, DOX), and a photosensitizer (chlorin e6, Ce6). The therapeutic-loaded ZIF-8 coating did not affect the targeting abilities, nor the viability of the bacteria. With this strategy, the authors were able to activate the therapeutic effect of the biocomposites (E. coli@ZIF-8/C&D) in mild near-infrared laser irradiation conditions. Both in vitro and in vivo tests demonstrated the efficacy of the method. Remarkably, E. coli@ZIF-8/C&D was shown to inhibit tumor growth in mice after only a one laser treatment (10 min, 660 nm, 200 mW cm⁻²). This innovative concept combines all of the elements described in this section, as well as the drug delivery strategies discussed in Sections 2 and 3.2.

Fig. 21. Encapsulation of live cells and viruses in MOFs and their applications in biobanking and cell and virus manipulation.

Fig. 22. (a) CLSM cross-section of the ZIF-8-coated yeast cells. Living yeast cells were labeled with FDA (green) and the ZIF-8 coatings were labeled by infiltration of Alexa Fluor 647 fluorescent dyes (red). (b) Yeast growth measurement before and after the removal of MOF coatings by EDTA for native (blue circles) and ZIF-8 coated yeast (red circles). Reprinted with permission from ref. [231]. Copyright 2016 John Wiley and Sons. Yeast@β-gal/ZIF-8 bioactive coating; (c) Schematic of the construction of the bioactive porous shell (β-gal/ZIF-8) for adaptive cell survival. (d) Relative cell viability (%) of the biocomposites in oligotrophic and inhospitable environments (cytotoxic enzymes and lactose-rich media). Adapted with permission from ref. [281]. Copyright 2017 John Wiley and Sons.
Following the demonstration of ZIF-8 shells on live yeast and bacteria, there have been more recent developments extending this concept to different cell types and other kinds of MOFs. In 2019, Brinker and co-workers presented the concept of “SupraCells”: a versatile encapsulation approach, by which living mammalian cells were instantly encapsulated within a nanoparticle-based exoskeleton thus preventing typical endocytotic nanoparticle (NP) internalization pathways [279] (Fig. 23a). Additionally, this method preserved cell viability and functions (e.g. metabolism), since the MOF NP-based coating did not disturb the permeability of nutrients, metabolites, and signaling molecules. The authors demonstrated these properties by preparing NP-exoskeletons made of ZIF-8, MIL-100 (Fe), UiO-66-NH2 and MET-3-Fe (see Table 7) and other inorganic coatings (mesoporous silica and iron oxide). NP-based exoskeletons were constructed via the sequential addition of a colloidal MOF solution and tannic acid to cell suspensions prepared in phosphate buffered saline (PBS) solution, in a three-step process: 1) MOF NP synthesis, 2) cell culture in colloidal NP solution and 3) incubation in tannic acid solution. By adjusting the incubation time in tannic acid between 30 s and 60 s depending on the MOF, it was possible to freeze the cellular internalization of the MOF nanobuilding blocks via tannic acid-mediated interparticle binding due to strong multivalent metal–phenolic complexation. Control experiments showed that without this crosslinking agent (tannic acid), in <5 min, the NPs were physically adsorbed onto the cellular surface and then internalized and accumulated around the nucleus. The SupraCell synthesis, initially applied to HeLa cells, was successfully extended to other mammalian cell lines such as adenocarcinomic human alveolar basal epithelial cell (A549 cells), human promyelocytic leukemia cells (HL-60 cells), and Raw 264.7 cell line, all of them yielding SupraCells with continuous MOF exoskeletons. The authors demonstrated increased resistance to various stressors such as osmotic pressure, pH, reactive oxygen species (ROS), UV irradiation, and toxic NPs for the SupraCell-MIL-100(Fe) (Fig. 23d). Additionally, the authors showed that the NP functionality can be tuned to provide the cells with abiotic properties including increased mechanical stability (Fig. 23 b,c), size-selective permeability, intracellular activity sensing, magnetism and electrical conductivity. For example, the NO sensing by luminescence quenching was shown for UiO-66-NH2 on Raw 264.7 cell line SupraCells. Electrical conductivity was also demonstrated by using metal–triazolate MOF (MET-3(Fe) NPs [284]) The authors suggested the use of this approach for cell-based sensing, regenerative medicine, immunotherapy, and tissue engineering.

A similar approach was used by Rosário et al., who synthesized a luminescent exoskeleton on live filamentous fungi using pre-synthesized lanthanide-MOF (Ln-MOF) NPs [285]. Fungal spores of Phialomyces macrosporus, Trichoderma sp. or Aspergillus niger were inoculated in a sterile solution containing glucose and a particulate dispersion of Ln-MOF NPs. The Ln-MOFs were prepared using Tb3+ and Eu3+ and mellitic acid (1,2,3,4,5,6-benzenehexacarboxylic acid) to give MOF particles in the 70–900 nm range. The fungi were cultured for two weeks under dark conditions, and
Ln-MOF decorated mycelia were obtained and analyzed with fluorescence microscopy and further physicochemical characterizations. SEM images reveal that particulate Ln-MOFs are accumulated uniformly on tubular cells, and firmly bound in the surrounding biopolymer matrix. The results show that the controlled deposition of Ln-MOFs on filamentous fungi imparts photoluminescent properties in living entities. The authors suggested that these findings may be relevant for the development of living imaging analysis and treatment of fungal infections.

Yang, Yaghi and co-workers added pre-synthesized MOF \([\text{Zr}_6\text{O}_{4}\text{(OH)}_4(\text{BTB})_2\text{(OH)}_2(\text{H}_2\text{O})_6]; \text{BTB} = 1,3,5\)-benzenetribenzoate] NPs into the culture media of *M. thermoacetica*, an anaerobic bacteria [286] (Fig. 24). The exposure of the bacteria to the MOF colloidal solution allowed for the self-assembly of a flexible NP-monolayer (1–2 nm) that could adapt to the bacteria cell wall changes, including growth and reproduction. The authors, by adding an excess of MOF to the culture media, observed the formation of a coating on newly grown cell surfaces, allowing the extension of the artificial MOF-based exoskeleton over generations of the anaerobic bacteria (Fig. 24d). We note that this type of process is not likely to be possible via biomimetic mineralization of ZIF-8 as the reproduction or growth of yeast cells is temporarily blocked until the coating is removed. The protective effect of the MOF shell on the anaerobic bacteria was investigated by assessing cell viability after exposure to \(\text{O}_2\) and \(\text{H}_2\text{O}_2\) for a 1–3 day period (Fig. 24b). Compared to pristine bacteria in anaerobic environments, the results showed that MOF-protected bacteria in aerobic conditions have comparable cell viability. MOF-encapsulated live anaerobic bacteria displayed “active” protection which allows an enhanced artificial photosynthesis process, reduced death of the bacteria (5 times) in presence of \(\text{O}_2\), and enabled continuous production of acetate from \(\text{CO}_2\) fixation under oxidative stress. The authors attributed the protection against oxidative stress to catalytic activity (decomposition of the reactive oxygen species, ROS) by the MOF because of structural resemblance between the zirconium clusters of the MOF and active sites of zirconia. However, control experiments comparing the MOF-coated bacteria with bacteria cultured in the presence of zirconia nanoparticles, found no cytoprotective effect for the zirconia NPs. This result suggests that the proximity of the MOF catalytic active sites to the cell, as well as their accessibility (surface active sites) are important factors.

In recent years, interesting progress has been made on coating living cells with an artificial shell comprised of a self-assembled ferric ion-tannic acid complex that spontaneously forms in presence of tannic acid (TA) and Fe\(^{\text{III}}\). Studies performed by Caruso, Choi and co-workers, have demonstrated the feasibility and potential for protection of live yeast [287], adherent and cancerous mammalian cells, [288,289] and red blood cells (RBC) [290]. These coordination compound coatings, which lack the extended MOF periodic structure formed via coordinate bonds, showed interesting properties: 1) TA-Fe\(^{\text{III}}\) complexes formed a very thin layer and its thickness can be precisely controlled by changing the precursor concentration in the 5 to 20 nm range; 2) it is highly cyto-compatible; and, 3) it generates a protective and degradable nanoshell. In particular, experiments on red blood cells showed that the coating helped attenuate the antibody-mediated agglutination, while retaining the oxygen-carrying capability of RBCs after shell formation. The authors suggested these biocomposites could progress the development of universal blood. TA-Fe\(^{\text{III}}\) coatings were also applied to other mammalian cell lines (HeLa, NIH 3 T3, and Jurkat cells) [288]. Albeit not technically a MOF [291], we include this discussion about TA-Fe\(^{\text{III}}\) complexes in the present review as this could inspire the development of Fe-based MOF coatings for biomedicine.

### 6.2. Encapsulation of viruses within MOFs

The first report on virus-MOF composites was performed by Gassensmith and co-workers [292], and described the crystallization of a ZIF-8 shell on tobacco mosaic virus (TMV), a model virus (Fig. 25a). The authors achieved precise control over the thickness of the ZIF shell that could be tuned between 70 and 100 nm by changing the ligand to metal ratio (2-methylimidazole:Zn). The authors also exposed the resultant biocomposites to different environmental stressors, such as polar solvents and high temperature. The TMV@ZIF-8 composites were found to be stable in polar organic solvents (methanol and DMF) for 16 h, and for the thickest shell the biocomposites were also able to endure immersion in dichloromethane (DCM) for 16 h and boiling water for 20 min.
without structural degradation. Furthermore, the virus itself could be reclaimed undamaged after immersion in pure methanol overnight, by using an aqueous solution of EDTA to remove the ZIF-8 coating.

A comprehensive study performed subsequently by the same group analyzed the dominating factors that influence the final morphology of TMV@ZIF-8 biocomposites [208]. The authors performed an in-depth study of the formation mechanism of the core–shell biocomposites, by exploring different ligand to metal (L/M) ratio and precursor concentrations (Fig. 25c), as well as the effect of surface charge changes induced by bioconjugation reactions on the surface of the virus. The biocomposites were divided into two sets: small core–shell bionanoparticles (CSBN), which retained the underlying morphology of the virus (~300 nm × 18 nm rods), and multiple TMV particles entrapped in micrometer-sized rhombic dodecahedral (r.d.) single crystals of ZIF-8 (Fig. 25a,b). Interestingly, the CSBN morphology was found to form most favorably in reaction conditions in which the spontaneous formation of ZIF-8 was otherwise disfavored, yielding only amorphous granules without the TMV. Using a bioconjugation strategy, the authors functionalized the TMV exterior via a Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction to attach functionalized azides that could modify the electrostatic charge of the virus capsid. The results suggest that CSBN formation proceeds in very good yields (~90%) under most circumstances, unless the surface is highly positively charged, in which case the yield is still ~70%. Time-dependent SEM characterization of the different phases revealed that the growth of ZIF-8 on the surface of the TMV proceeds at different rates from the precursor solution and is probably induced by an elevated local concentration of zinc in the microenvironment around the TMV. This is in line with studies by Doonan and co-workers on the influence of electrostatic charge of proteins in biomimetic mineralization of ZIF-8 [114].

The most recent progress on virus-based MOF biocomposites was performed by the same team, in a 2019 publication in which they apply the optimized conditions already found for the encapsulation of TMV in ZIF-8 to further study the viability of MOF-encapsulated vaccines [47]. In this case, to evaluate the potential of ZIFs for vaccine preservation, TMV@ZIF composites were subjected to different stressors such as heat and denaturing solvents, then the ZIF-8 shell was removed and the protein was recovered and analyzed by ELISA to assess the integrity of the surface (Fig. 26a). Studies on N. benthamiana (tobacco) plants were performed in which the plants were inoculated and the evolution of the infection was assessed as well as a viral recovery study, measured by ELISA on harvested leaves (Fig. 26b). The study also found that the RNA of the encapsulated virus was still active, indicating that the TMV remains virulent and the RNA survives the encapsulation and release process. Finally, studies on live animal models shed light on the immune response toward these biocomposites, their biocompatibility, and the ZIF-8 shell decomposition profile and virus release in vivo (Fig. 26c–f). Importantly, the results of this study have shown that the crystalline porous coating grown on the viruses did not affect the structure of the proteins and their ensembles, and that it did not damage significantly the viral RNA. The results obtained from the in vivo studies on mice showed no alteration of tissue morphology at the injection site or distal organs (Fig. 26e). Additionally, no behavioral changes, illness or deaths were observed on the animals as a result of the TMV@ZIF administration. Based on these results, the authors propose that ZIF-8-based shells in combination with the highly tunable TMV platform could provide a safe and reliable method for the delivery of proteinaceous drugs.

6.3. Summary and future outlook

In this section we have discussed the most relevant results of live cell and virus encapsulation in MOFs. The progress made on these complex biocomposites has shown the potential of MOF-based artificial shells [6], and some systematic studies [208,279] have shed light on MOF formation mechanisms and assessed the protective properties. However, for a better understanding of these systems, further systematic studies should reveal the importance of the synthetic conditions on the protection of different cell types.
In particular, an important step is the extension to different cell types and MOFs [6]. As a future perspective for this field, MOF-coatings could be used to impart control over cell aggregation and coagulation, which are critical for applications such as 3D cell printing [276] in regenerative medicine.

Another important step would be a comparison of MOF coatings with more established materials. For example, typical materials used for single-cell encapsulation are polymers deposited using a layer-by-layer approach. For these polymers, the control over shell thickness seems superior to the current control over MOF exoskeletons [277]. For example, the thicknesses of the explored coatings have ranged between 70 and 900 nm [285] to ~50–80 nm [208,279] to 1–2 nm [286], but this is strictly dependent on the MOF chosen and deposition process used. Layer-by-layer MOF growth is also possible [293,294], which suggests that adoption of polymer deposition approaches could be employed if a suitable MOF material can be identified.

It is also important to note that most of the progress for MOF coatings has been made on cells with robust cell walls (e.g. fungi, bacteria). Although the encapsulation of bacteria can be useful for applications such as whole-cell biocatalysis, for other applications such as cell therapy and tissue engineering it is fundamental to develop this field toward cytoprotective and compatible coatings on mammalian cells [6,276]. The coating of mammalian cells, which are encased only in lipid bilayer membranes, remains a challenge due to their fragile nature. For this reason, they require more carefully selected precursors and processing [288] and handling conditions. Thus, coating mammalian cells with different MOFs, while preserving their viability, would significantly progress these composites toward application in regenerative medicine and cell therapy.

7. Lipids-on-MOFs: A surface functionalization approach for MOFs-based DDS

Lipids (e.g. fatty acids, sterols, glycerolipids and glycerophospholipids) play a key role in many biological processes such as cell metabolism and signaling. In addition, lipids are fundamental structural biological components as cell membranes are mainly composed of lipids (e.g. glycercophospholipids). In the field of drug delivery, lipids are particularly attractive because of their biocompatibility and amphiphilic properties [295]. The amphiphility of phospholipids permits the formation of supramolecular structures in solution, such as micelles and liposomes. The vesicular structure of liposomes is used for the integration of a wide variety of active pharmaceutical ingredients (APIs) including hydrophobic drugs. This strategy is commercially viable as it enhances the drug loading and bioavailability of several clinical therapeutics via different administration routes (e.g. oral, parenteral, topical) [295].

In the field of MOF-based drug delivery systems (DDS), lipids have been employed as functional coatings on MOF-based carriers (i.e. lipid-on-MOF). The main advantages that a lipid coating can impart to MOF-based DDS are the improved colloidal stability and biocompatibility, longer circulation time and easier cellular internalization. Pioneering reports on lipid-on-MOFs were provided in 2015 by Wuttke et al. [213] and Wang et al. [296]. Wang et al. synthesized a series of hydrophilic Zr-based MOFs (UiO-66, UiO-67, and BUT-30) crystals with different particle sizes (e.g. particles in the range of 20 to 30 nm, and from 200 to 500 nm range) [296] and high affinity for polar solvents (e.g. water) (Fig. 27a). The main goal of this study was to obtain a stable colloidal suspension of the hydrophilic MOFs in low-polarity media. To obtain stable colloidal suspension of MOF NPs, the crystals were sonicated in presence of 1.2-dieoleyl-sn-glycero-3-phosphate (DOPA). According to $^{31}$P($^1$H) magic angle spinning (MAS) NMR spectroscopy results, the MOF functionalization occurred due to the phosphates of DOPA coordinating to the zirconium oxide nodes on the external surface of the MOF nanoparticle. The DOPA-functionalized MOFs retained both the crystallinity and porosity of the pristine MOF. Following this approach, the authors showed that it was possible to tailor the stability of MOF colloids in low-polarity solvents using lipids (e.g. CHCl$_3$).
The study from Wuttke et al. on lipid-on-MOF systems demonstrated the potential use of such biocomposites in the field of drug delivery [213]. The authors prepared MIL-100(Fe) and MIL-101(Cr) nanoparticles (ca. 50 nm) and loaded a fluorescent cargo molecule (e.g. fluorescein and Atto663) in the porous framework. Then, the resultant dye@MOF particles were functionalized with a 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) bilayer via a controlled solvent-exchange deposition method: by increasing the water content in a DOPC-MOF ethanol/water dispersion, the DOPC molecules concentrated on the MOF surface leading to the formation of a lipid bilayer. The MOF structure was preserved after the coating process, and the DOPC-on-dye@MOF NPs were successfully dispersed in water showing improved colloidal stability that is a central aspect of DDS. In addition, the presence of the DOPC bilayer prevented the uncontrolled release of the cargo, an additional important property in DDS. The cellular uptake of DOPC-on-dye@MOF biocomposites, while uncoated particles leaked rapidly. The authors examined the controlled release of the cargo after cellular internalization by exposing the exosome-MOF biocomposites to an artificial lysosomal fluid (ALF), a solution that simulates the endocytosis environment inside cells. The enhanced osmotic pressure in the exosome coating caused by ALF lead to bursting of the exosome and the consequent calcine release. Finally, exosome-coated calcine@MIL-88A was incubated with HeLa cells: fluorescent microscopy studies confirmed the successful biocomposite internalization and calcine release. Motivated by these interesting results, the authors loaded an anti-cancer drug (suberoylhydroxamic acid, SBHA, loading = 2.3 wt%) into MIL-88A NPs and coated them with exosomes. Exosome-coated SBHA@MIL-88A were incubated with HeLa cells and the authors confirmed the apoptotic properties: after 3 days of incubation, the IC50 value (4.78 μg/mL) was found to be 3 times higher than the IC50 offree SBHA.

After these seminal works, the coating of MOF NPs with mono- and bi-layers of lipids was expanded and applied to different MOFs and lipids [300,301]. Yang et al. synthesized nanoparticles (<200 nm) of a porphyrinic Zr-MOF (PCN-223) and coated them with a continuous monolayer of DOPC exploiting the formation of Zr-O-P bonds on the MOF surface [301]. Then, the DOPC-on-PCN-223 was further coated with DOPCand cholesterol to self-assemble into a lipid bilayer on the MOF surface. The DOPC-on-PCN-223 biocomposites showed high colloidal stability in different chemical environments (including phosphate-based media that could degrade pure PCN-223), more favorable biocompatibility and cellular uptake. Furthermore, the DOPC coating ensured the intracellular stability of the biocomposite: the authors observed that the internalized NPs maintained their rod morphology in the cytoplasmic milieu of SMMC-7721 cells after 12 h of incubation. The intracellular stability was associated with the shielding properties of DOPC that protected the MOF particles from the phosphate ions available in the intracellular environment [67]. The authors suggested the use of these coated MOFs for bioimaging and photodynamic therapies to improve the stability of MOF systems during their imaging functions. In 2018, Zhu et al. investi-
gated a versatile and reversible strategy to decorate the surface of several different MOF microparticles (e.g. MOFs based on Al, Cr, Fe, Co, Cu, Zn, Zr, In and Eu) with a phenolic-inspired lipid molecule DPGG (1,2-dipalmityloxy-sn-glycero-3-galloyl) via the coordination of the galloyl head groups to the metal sites on the MOF surfaces [300] (Fig. 27b). After the self-assembly of the DPGG monolayer, the DPGG-on-MOF particles showed a hydrophobic character (i.e. hydrophobic tails of the lipid were oriented opposite to the MOF surface) and colloidal stability in non-polar solvents (Fig. 27c, d). The DPGG monolayer was dis-assembled by washing in weakly acidic water, since the metal-phenol complexation is pH sensitive, and this aspect could be exploited for triggered-release applications. Then, the authors demonstrated that DPGG-on-MOF biocomposites could serve as platform for the synthesis of a variety of materials via the successive functionalization of the lipid monolayer (e.g. silica coating via silanes condensation, addition of a second lipid to form a lipid bilayer on the MOF surface) and posit that these materials can be exploited for biomedical applications (e.g. multi-compartment biocarrier, targeted delivery and photothermal therapies).

In summary, lipid-on-MOF biocomposites represent a promising platform for the development of MOF-based DDSs. The rich chemical variability of lipids can be exploited to impart additional functionality to drug-loaded or bioentity-loaded MOF particles, ranging from the improvement of MOF DDS biocompatibility, bioavailability and blood-circulation time to active targeting properties. Furthermore, the lipid functionalization strategy could be applied to different MOF systems, thus expanding their field of application from drug delivery systems to other biotechnological platforms including bioimaging and biosensing.

8. Conclusions and perspectives

In this review we have highlighted the advantages and promising applications of different bioentities@MOFs, sorted mainly into four categories based on the bioentity: proteins, carbohydrates, nucleic acids and live cells and viruses. Additionally, we have discussed the use of lipids and carbohydrates-on-MOFs as a strategy to improve targeting, blood circulation time and compatibility of the biocomposites. We have discussed different synthetic approaches for the formation of these biocomposites, experimental techniques used for their characterization and their potential application in biomedicine. We have summarized aspects that demonstrate how MOFs are an effective platform for the protection and controlled release of protein-based therapeutics, and moreover demonstrated that MOFs are emerging materials for the delivery of nucleic acids- and carbohydrate-based drugs. Azolate-based MOFs (e.g. ZIF-8, ZIF-90 and MAF-7), which feature highly in this field, afford high encapsulation efficiency and a tunable release profile, along with favorable biocompatibility, which commend them as drug delivery systems. While these MOFs have led the way, considerable scope is available to extend the encapsulation process to other MOF matrices, perhaps containing bio-derived linkers [11,302] or even to metal-free systems that are assembled from organic molecular building blocks [303].

From a preliminary assessment of the cost of the materials involved in forming ZIF-biocomposites, the MOF component is typically inexpensive with respect to the therapeutic and can range between 0.01% and 5% with respect to the total cost of the final material (MOF + encapsulated bio-therapeutic), see Table 1. Additionally, post functionalization methods could further enhance specific desired properties, including blood circulation time, cell uptake, and targeted delivery.

Where possible, we have discussed further aspects relevant to biomedicine such as particle size, encapsulation efficiency, release properties, biocompatibility and therapeutic properties. Regarding the particle size of MOF-based biocomposites for drug delivery applications, only a few studies have investigated the effect on the biodistribution, biocompatibility and release kinetics of the cargo. However, it would be crucial to have comparative studies across different MOF carriers and several precisely controlled particle size distributions, as both the chemistry of the MOF particle and their size could influence the properties of both micro- and nano-MOF carriers. By controlling the chemistry, the particle size and the surface functionalization of MOFs, both the degradability and bioaccumulation of the carrier can be manipulated. For example, stimuli-responsive carriers have been designed where the degradation process is selectively triggered by the presence of glucose to achieve controlled insulin release.

Encapsulation of biomacromolecules within a MOF shell has been demonstrated to be an effective strategy for maintaining the bioactivity of a wide range of compounds including proteins, enzymes, antibodies and viruses, even after exposing them to denaturing conditions such as elevated temperature. Thus encapsulation has emerged as a promising technology for handling, transport, and storage of biospecimens without refrigeration, which is an attractive alternative for providing cold-chain-free transport of vaccines, and thereby reducing the shipping costs.

For other biomacromolecules such as enzymes, their embedding within a MOF shell can progress the fabrication of cost-effective biosensors for the detection of a wide variety of analytes, ranging from small molecules such as H2O2 and glucose, to large biomacromolecules including protein-based biomarkers and exosomes. Such platforms offer several benefits including high signal amplification, sensitivity, specificity, and reproducibility. The presented strategy is envisioned to facilitate the design of point-of-care testing devices and for real-time diagnostic analytical systems.

Finally, in this review we discussed the new trends in the encapsulation of live-cells and viruses using a MOF-based exoskeleton for the protection of bioentities. To date, most of the progress has been made on cells within robust walls (fungi, bacteria) and this could be useful for applications such as whole-cell biocatalysis. However, for applications of MOF protective coatings to cell therapy and tissue engineering, the development of protocols for the fabrication of cytoprotective and cytocompatible coatings on mammalian cells is needed. The promising results obtained for cells encapsulated in bioactive coatings suggest an opportunity to expand this research by combining the MOF shell with different biomacromolecules, which can endow the cells with new properties.

As a final remark, while the characterization of the bioentity and biomacromolecule@MOF composites has greatly improved, the use of in-situ analytical and structural characterization methods could further advance MOF biocomposites. This could shed light on critical aspects, such as the formation mechanisms of MOFs on bioentities and related MOF-to-biomacromolecule interactions. These fundamental insights will guide the use of other MOF materials as coatings for DDS, and will inform the methodologies employed to encapsulate particular classes of biomacromolecules and cells that are not robust enough for current approaches.

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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