

Metal-Organic Frameworks for Cell and Virus Biology: a Perspective.

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ABSTRACT: Metal-Organic Frameworks (MOFs) are a class of Coordination Polymers, consisting of metal ions or clusters linked together by chemically mutable organic groups. In contrast to zeolites and porous carbons, MOFs are constructed from a building block strategy that allows for molecular level control of pore size/shape and functionality. An area of growing interest in MOF chemistry is the synthesis of MOF-based composite materials. Indeed, recent studies have shown that MOFs can be combined with biomacromolecules to generate novel biocomposites. In such materials the MOF acts as a porous matrix that can encapsulate enzymes, oligonucleotides, or even more complex structures that are capable of replication/reproduction (i.e. viruses, bacteria, and eukaryotic cells). The synthetic approach for the preparation of these materials has been termed 'biomimetic mineralization', as it mimics natural biomineralization processes that afford protective shells around living systems. In this perspective, we will focus on the preparation of MOF biocomposites that are comprised of complex biological moieties such as viruses and cells, and canvass the potential applications of this encapsulation strategy to cell biology and biotechnology.

Biomineralization is a natural process whereby inorganic materials (biominerals) are grown on living organisms.¹⁻⁴ A variety of natural systems including fungi, mollusks³, diatoms, radiolarians, and mammalian have developed methods to introduce or improve mechanical support, motility, protection, and sensing, through engineered biominerals; examples include carapaces, frustules, skeletons, and spikes.^{2,4,5} Biomineralization is exploited in all the taxonomic kingdoms (Animalia, Archaea, Bacteria, Fungi, Plantae, and Protista) since the beginning of the Cambrian geological period (more than 500 millions of years ago).^{6,7} Remarkably, organisms are able to promote and regulate the formation of biominerals with molecular level precision.⁴ For example, the shape of hydroxyapatite in bones and in tooth enamel is a biomineralization process.⁸

Inspired by nature, scientists have explored and developed an understanding biomineralization strategies in the laboratory.⁹ By applying self-assembly strategies, a number of hybrid systems have been synthesized where inorganic or organic materials are formed on a variety of biological moieties ranging from proteins to living cells. In this perspective we will refer to biomimetic mineralization as a process that produces synthetic coatings on living systems that would not otherwise occur naturally.¹⁰ This approach offers many opportunities for multidisciplinary research,

with respect to cells a hardened encasing could facilitate control of cell behavior,¹¹ and provide enhanced resistance toward unfavorable environments (e.g. heat, UV radiation, mechanical stress, lytic agents, enzymatic inhibitors, etc.).^{11,12} Furthermore, if the coating is porous it can function as a per-selective barrier for the transport of biologically relevant substrates,¹³ or act as a matrix to encapsulate enzymes.^{14,15}

Silicon dioxide (SiO₂) has been widely studied for coating cells via a biomimetic mineralization process.¹⁶⁻¹⁹ These studies include yeast cells,²⁰⁻²² eukaryotic cells,^{19,23-25} bacteria,^{26,27} and also other bio-entities such as viruses.²⁸ The exploration of SiO₂ was motivated by the desire to trigger and control the formation of synthetic inorganic coatings under physiological conditions.²⁹ This field is now attracting trans-disciplinary research groups who are working in areas such as the design of biosensors, bioreactors, and biomedical devices.^{24,30-32}

Metal-organic Frameworks (MOFs)³³ represent a class of materials that are now being investigated as coatings for cells and other complex bio-entities such as viruses. MOFs are constructed via a molecular building block approach, from organic links and inorganic nodes (metal ions or clusters), that offers a high level of control over their chemical

composition and functionality, structure topology, pore size and shape as well as crystal morphology.^{34,35} Furthermore, many MOFs are stable in a variety of solvents, including water, over a wide temperature range and can be prepared under physiological conditions.^{33,36,37}

Here we will highlight strategies for the biomimetic mineralization of MOFs on cells and canvas the current and potential applications for these advanced, functional cell coatings.

A recent discovery in MOF chemistry was the observation that they could encapsulate biomacromolecules under mild conditions.^{12,38} To date, the most extensively studied MOF material for this process is ZIF-8 ($[\text{Zn}(\text{C}_4\text{H}_5\text{N}_2)_2]$, ZIF = Zeolitic Imidazolate Framework),^{39,40} a three-dimensional framework of sodalite (**sod**) topology constructed from Zn(II) ions and 2-methylimidazole (2mIm).⁴¹ The biomimetic mineralization of ZIF-8 can be performed within minutes in aqueous solution without the need of heating, organic solvents and/or compatibilization agents.⁴² Typically, the target biomacromolecules and 2mIm ligands are dissolved in aqueous solution, followed by the addition of the metal solution at room temperature.³⁷ The biomolecules hitherto incorporated within MOFs include globular proteins like albumin, enzymes such as horseradish peroxidase or urease, hormones (insulin) and oligonucleotides (DNA).¹² Biomimetic mineralization has also been performed on surface patterned proteins,⁴³ viruses,⁴⁴ and living cells,¹³ exemplifying the versatility of this technique.

Although many examples have now been reported, the mechanism of MOF biomimetic mineralization is far from being fully understood. The role of protein constituents were considered in previous work and it was found that amino acids can play a role in the formation of ZIF crystals.⁴⁵ In a different preliminary study where bovine serum albumin (BSA) was used for the preparation of BSA@ZIF-8 biocomposites, it was reported that twenty-two Zn(II) cations and thirty-one 2mIm ligands are attracted by one single BSA molecule;¹² in this provisional model, the BSA protein acts to concentrate the MOF precursors and facilitate the nucleation and growth of the ZIF-8 crystals on its surface in a similar process to the growth of MOFs around ceramic and inorganic nanoparticles.⁴⁶ The comparative size of BSA (c.a. 6 nm) and ZIF-8 pores (c.a. 1 nm in diameter) suggests that the protein is too large to be encapsulated within the pore network. Evidence for encapsulation of BSA within the ZIF-8 crystal lattice as opposed to being hosted within its pores was provided by small-angle X-ray scattering (SAXS). Interpretation of the SAXS data indicated the presence of mesopores (c.a. 7 nm in width) large enough to encapsulate the 6 nm protein within the ZIF-8 bulk crystals.¹²

VERSATILITY OF THE BIOMIMETIC MINERALIZATION APPROACH

The first reports of MOF biomimetic mineralization described encapsulation of biomacromolecules;¹² however,

the concept has now been extended to more complex systems. Our groups were the first to demonstrate the successful coating of ZIF-8 on viruses⁴⁴ and yeast cells.¹³ For example, growth of a ZIF-8 shell on yeast cells was carried out by first dispersing the cells in an aqueous solution of 2mIm followed by the addition of an aqueous solution of Zn(II). The thickness of the ZIF-8 shell was found to be ≈ 100 nm. It is noteworthy that the shell thickness could be increased between 100-250 nm via sequential coating steps (**Figure 1a**). We found that the mechanical constraints imposed by the ZIF-8 coating prevented the yeast cells from reproducing. However, cell metabolic processes continued as the porous shell facilitated the transfer of small molecules like glucose and oxygen to the cell. Accordingly, the encapsulated yeast cells survived for several days. With respect to viral encapsulation the MOF shell protected the virus from chemical and thermal treatment that would normally lead to degradation. The MOF shell allowed for the diffusion of small molecules to the surface of the virus for the purpose of bioconjugation reactions while protecting the biological moiety from degradation. Molecular transport through the MOF coating was observed to be size selective. For example, molecules vital to cell life diffused through the MOF shell; however, large biomacromolecules, such as lyticase that would normally lead to their death were prevented from accessing the cell membrane.^{47,48} Accordingly, this concept provides a new strategy for protecting cells in media that contain cytotoxic agents. Another feature of the biomimetic mineralization approach is the facile removal of the MOF shell 'on demand'. Addition of ethylenediaminetetraacetic acid (EDTA), a well-known complexation agent for Zn, or mildly acidic pH, degrades the MOF shell, and the cells or biomacromolecules are recovered and restored to their full functionality.^{12,13,44}

MOF-COATED CELLS: TOPOLOGY, POROSITY, AND FUNCTIONALISATION

MOFs are highly tailorable materials and, in principle, it is possible to tune their composition, porosity, topology, chemical functionality, and structural defects. Here these aspects are discussed in detail.

Composition/topology

The physical properties of MOFs are largely determined by the combination of metals (or metal clusters) and ligands that compose their structures. For example, $\text{Cu}_3(\text{BTC})_2$, (BTC = 1,3,5-benzenetricarboxylic acid), also known as HKUST-1, is a 3D material of **pto** topology synthesized by mixing Cu(II) salt with BTC;⁴⁹ however, if the ditopic organic ligand 1,4-benzenedicarboxylic acid (BDC) is employed, the 2D **sql** net MOF Cu(BDC) is formed.⁵⁰ In general a wide variety of different topologies can be generated through judicious selection of the metal and organic

building blocks (**Figure 1b**). On the other hand, it is possible to synthesize structurally different MOFs from the same starting materials by modifying the reaction conditions. For example, MIL-53(Cr)⁵¹ (a **gui** net) and MIL-101(Cr)⁵² (a **mtn-e** net) are both synthesized from Cr(III) and BDC. At the moment, for the practical application of MOFs to biological systems, there are two salient questions: is the biomimetic mineralization process feasible for the particular MOF (i.e. can the desired MOF be synthesized in biologically compatible conditions)? And, is the MOF, or its precursors, cytotoxic? For the first case, biomimetic mineralization has been tested only for a few MOFs (e.g. ZIF-8 and Tb/Eu based terephthalate).^{12,43} However, it is possible that new MOF candidates for biomimetic mineralization could be identified in the future, thus extending the present list to other systems. For the second question, a significant number of MOFs are composed of precursors that can damage living biological systems such as cells. For example, there are several reports stating low cytotoxicity for selected carboxylate-based MOFs,⁵³⁻⁵⁵ whereas ZIF-8 can be toxic towards certain cellular lines above a certain concentration.^{53,56,57} For example, Yu *et al.*⁵⁸ tested the effect of ZIF-8 nanotubes using HeLa cell cultures and a 75% viability was detected for 10 µg/mL of MOFs. Zheng *et al.* reported an EC₅₀ (half maximal effective concentration referring to cell viability) on HeLa cells of 63.8 µg/mL using 100 nm ZIF-8 particles.⁵⁹ For the same cell line Horcajada *et al.* reported 100 µg/mL using 90 nm ZIF-8 particles. Junior's group reported full viability at a concentration of 25 µg/mL using NCI-H292, HT-29, and HL60 cell lines with 100 nm ZIF-8 crystals.⁵⁵ Although pioneering studies have been conducted, for a better understanding of the effects of MOFs on different cell lines it is crucial that studies of multivariable systems (e.g. concentration and particle size) are conducted.

Porosity

The building block approach to MOF synthesis allows for control of pore size and volume (**Figure 1c**). This represents an advantage of using MOFs for the encapsulation of biological moieties as this is not possible for other artificial coatings such as silica and metal/polymers. Careful choice of the metal and ligand precursors can yield a MOF of specific pore size, affording a perm-selective shell that can sieve substrates of a defined molecular size. For example, the network pore aperture can be tuned to transport gases, essential ions, and nutrients while shielding the system from larger cytotoxic agents such as enzymes. However, to protect the cells from small ions, such as heavy metals (e.g., Hg, Pb, Cd, that are notoriously prone to bind and block sulfur sites on peptides)^{60,61} and organic aromatic pollutants,^{46,62} different strategies are required. One possibility would be to prevent the transport of specific metal ions to cells by chemically modifying the MOF pores with functional groups that have a high affinity for them.⁶³⁻⁶⁵

Functionalization

Incorporating ligands with specific functional groups into the MOF architecture is a common strategy employed to tailor the performance characteristics of the material.⁶⁶ A large variety of functional organic ligands can be accessed either by one-pot assembly, post synthetic modification,⁶⁷ or by linker exchange via the so-called Solvent Assisted Ligand Exchange (SALE) approach.^{33,67-69} In this way the functional space encompassing the biomolecule can be tailored to maximize compatibility.⁷⁰⁻⁷³

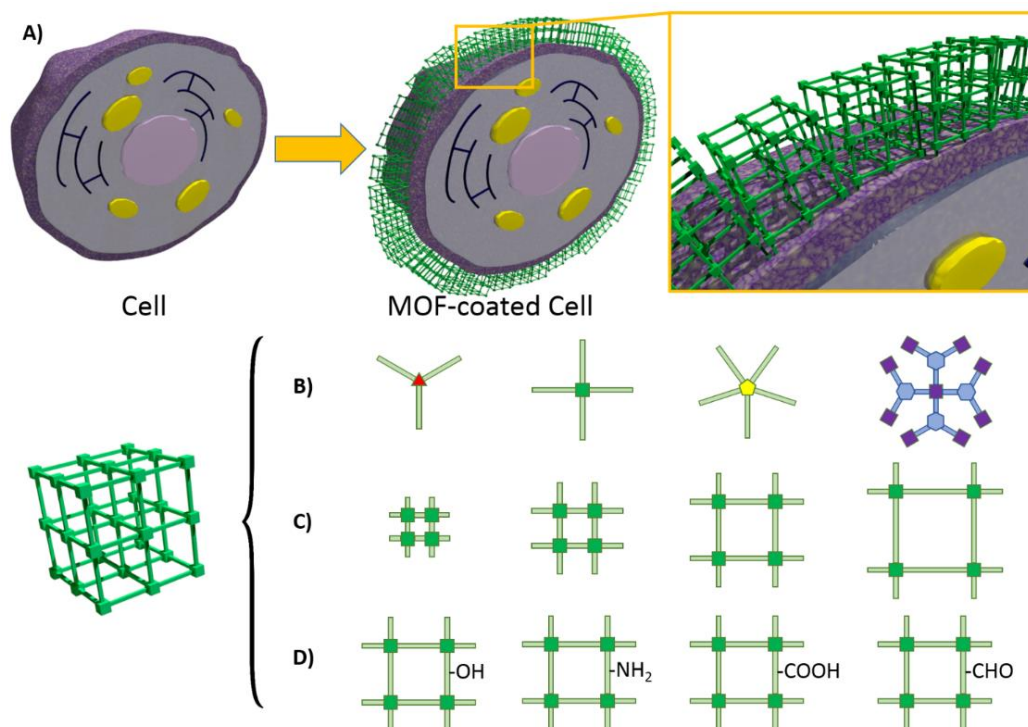


Figure 1. Biomimetic mineralization (A) of the desired cell can be conducted with different MOF compositions and topologies (B), and/or with MOFs having different porosity (C), and/or with MOFs bearing different functional groups (D) useful for subsequent post-synthesis chemistry.

Hydroxyl, amino, carbonyl and carboxylic acid groups can react with biological materials to form esters, ethers, amides, and imines (**Figure 1d**). In addition to covalent interactions, amine and carboxylic acid groups can modulate the pH at the bio-interface, thus permitting its regulation in a range compatible with cell activity.⁷⁴

Defects in the coating

Although the MOF pores are often considered responsible for the perm-selective properties, interstices between the MOF crystals could dictate the final properties. An example of this system is provided by the growth of ZIF-8 on cells.¹³ It is noteworthy that glucose (ca. 8 Å in dimension)⁷⁵ is unlikely to efficiently diffuse through the framework based on the ZIF-8 static pore aperture size of 3.4 Å (**Figure 2a**).⁷⁶ However, when glucose was added to the culturing media, the yeast cells were found to be metabolically active despite the presence of a ZIF-8 shell. An interpretation of this data is that, rather than homogeneous coverage, the ZIF-8 coating is a polycrystalline thin film and that glucose is able to percolate through defects such as interstices between crystals (**Figure 2b**). The important role of defects was further inferred in a study that investigated β -galactosidase (β -Gal) ZIF-coated cells. In this case, the MOF-coated β -Gal film processed lactose (ca. 11 x 6.2 x 7.4 Å), whereas β -Gal@ZIF-8 particles did not.¹⁴ Nevertheless, these putative defects in the MOF coatings, are of a size range that blocked the diffusion of Lyticase and protected yeast cells from lysis. Considering the molecular size and weight of Lyticase (ca. 5.3 nm and 54.6 kDa) such size exclusion properties could be expected. Further experimental data indicated that the same ZIF-8 coating also protected yeast cells from Filipin III antifungal drug,⁷⁷ a molecule with dimensions ca. 1 x 1.3 x 1.9 nm; thus the mechanism of diffusion clearly requires further study. Indeed, if defects are controlling the transport of molecules to the cell, new opportunities for overcoming the limitations imposed by the intrinsic pore size of the material can be explored.

ENHANCING THE FUNCTIONALITY OF MOF CELL COATINGS WITH ENZYMES

Enzyme-functionalized MOF cell coatings were recently reported as a method for enhancing the bioactive properties of these systems. For example, a MOF biocomposite film was prepared by combining ZIF-8 with an enzyme exogenous to a cell system; this bioactive porous shell was

used to convert substances in the environment into nutrients for cells. We provided a proof-of-concept study in which a lactose-based MOF biocomposite could be exploited to produce glucose.¹⁴ This research is in its infancy and additional examples should be investigated; however, it is possible that other enzymes can be exploited for new bio-catalytic processes that might not be accessible in native (i.e. non-genetically modified) cells. Nevertheless, it is important to consider the current limitation of this method: the biocomposite shell does not allow for cell replication as the porous film acts as a mechanical barrier. Therefore, the cell colony will eventually die unless the MOF coating is removed.

Looking at the future of this field, we envisage four main strategies for the preparation of enzyme-functionalized MOF cell coatings; these are grafting, infiltration, biomimetic co-mineralization, and biomimetic post-replication

Grafting

By exploiting the presence of functional groups such as carboxylic acids or amines, it is possible to externally decorate MOF coatings with enzymes (**Figure 3a-b**). This method has been extensively used in literature to graft trypsin,⁷⁰ β -glucosidase,⁷² hydrolase,⁷³ and streptavidin⁷⁸ on MOFs. Two general strategies are used for immobilization:

1. *amide bond formation* between the carboxyl functionality of the ligand and an amino group from the peptide (this reaction can also be performed with linkers with inverted positions) mediated by EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) or DCC (2,3-dicyclohexylcarbodiimide);⁷⁰
2. *imine formation* between the amino groups, originating both from the MOF ligand and the peptide, using the homobifunctional linker glutaraldehyde.⁷²

Both of these methods are efficient; however, binding enzymes to the MOF surface does not offer significant protection from the environment media (e.g. in the presence of proteolytic agents, enzymes will be degraded).

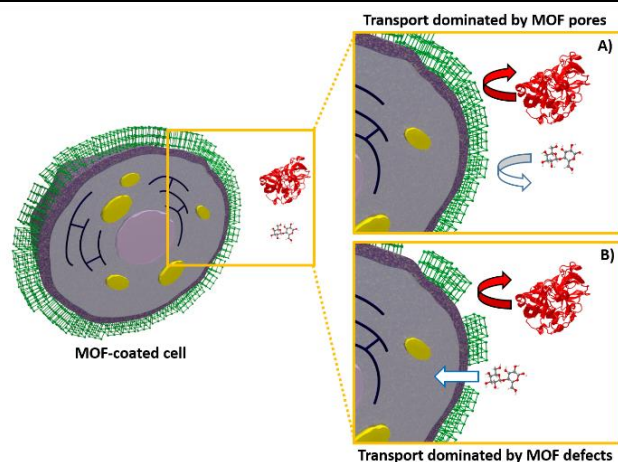


Figure 2. Cell coated with MOFs; a) the MOF coating is grown and the mass transport to the cell predominantly occur via diffusion through the intrinsic pores in MOFs (both nutrients and proteolytic agents can be blocked); b) MOF coating with defects allows nutrients diffusing through while protecting the cell from proteolytic agents.

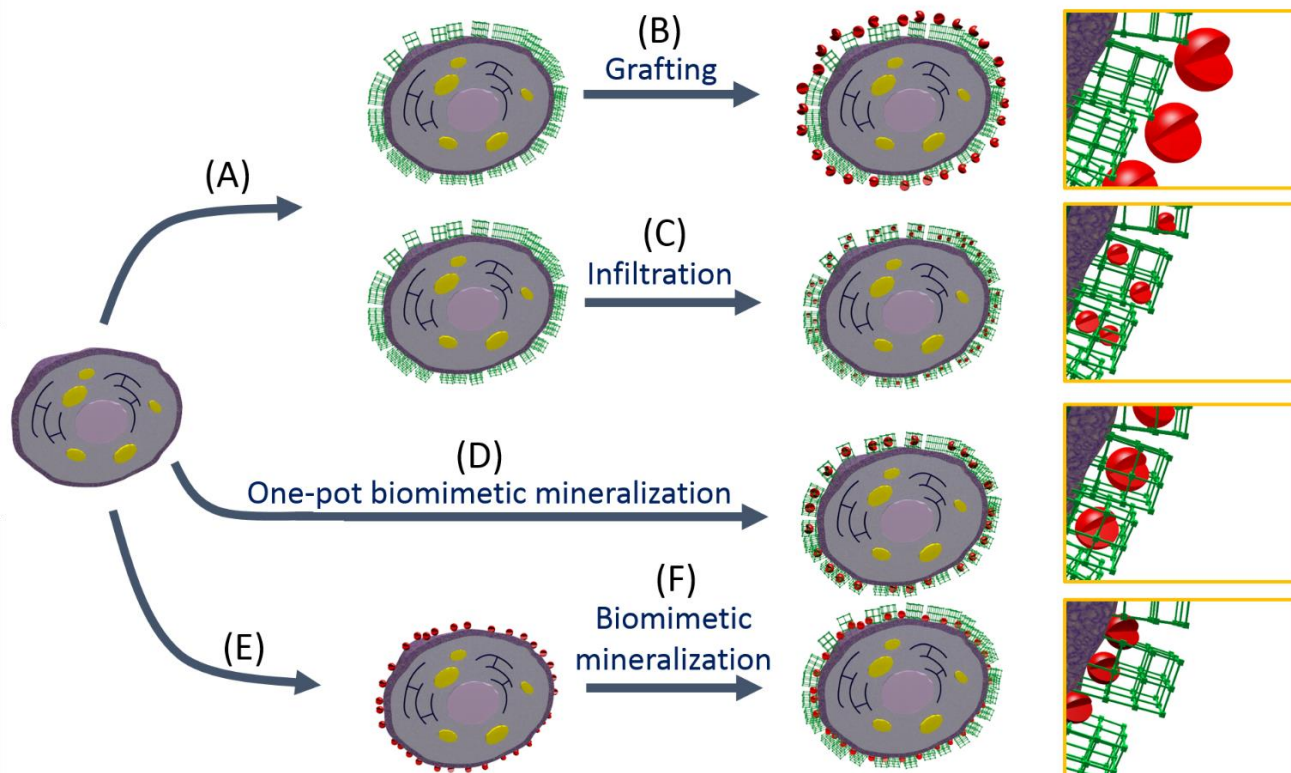


Figure 3. A cell can be coated with a MOF via biomimetic mineralisation (A), then enzymes can be grafted to the surface of the framework (B). If the framework pore and pore aperture size are large enough, enzymes can be introduced into the MOF via infiltration (C). A biomimetic mineralisation procedure in the presence of enzyme results in a shell where the biomacromolecules are encapsulated in a single step (D). Finally, enzymes can be directly immobilized on the surface of the cell (E) followed biomimetic mineralization to afford a cell/enzyme system (F)

Infiltration

In this process the enzyme is impregnated into the MOF pores, thus the size of the enzyme and the MOF pore aperture need to be compatible (**Figure 3c**). Given that the majority of MOFs are microporous this can not be considered a general strategy. Nevertheless, biomacromolecules such as Cytochrome *c*,^{79,80} MP-11 (microperoxidase),⁸⁰⁻⁸² Cutinase,⁸³ OPAA (organophosphorus acid anhydrolase),^{84,85} GOx (glucose oxidase),⁸⁶ Lipase,⁸⁷ and HRP (horseradish

peroxidase),^{80,86} have been successfully infiltrated into MOF pores.⁸⁸

Biomimetic Co-Mineralization

Another strategy for functionalizing the MOF coating is biomimetic co-mineralization. In this case the initial mixture of MOF precursors is enriched with an enzyme that is co-mineralized and integrated in the MOF coating of the

cell (**Figure 3d**). Similar to the classic mineralization of biomolecules, we believe it should be possible to encapsulate bio-macromolecules larger than the intrinsic framework pore overcoming the limitations of the infiltration technique. This method has not been explored yet but offers great opportunity for expanding the functional space of cell/MOF systems.

Biomimetic Post-Replication

We have previously shown that protein films can seed MOF growth.³¹ Similarly, protein-coated cells can be used to trigger biomimetic mineralization (**Figure 3e-f**). By means of stable electrostatic interactions, or covalent bonding, enzymes can be deposited on the outer surface of the cell and then exposed to MOF precursors. This method could possibly solve issues where the biomimetic mineralization does not occur naturally on a specific type of cell. Indeed this approach has been recently exploited for the synthesis of beta-galactosidase/ZIF-8 coatings on yeast cells.¹⁴

APPLICATIONS: MOF-AIDED THERAPIES

Breaking the “Cold Chain”

Most proteins denature rapidly when exposed to temperatures outside their operational range, a process that is thought to occur through physical changes in the protein’s global conformation.

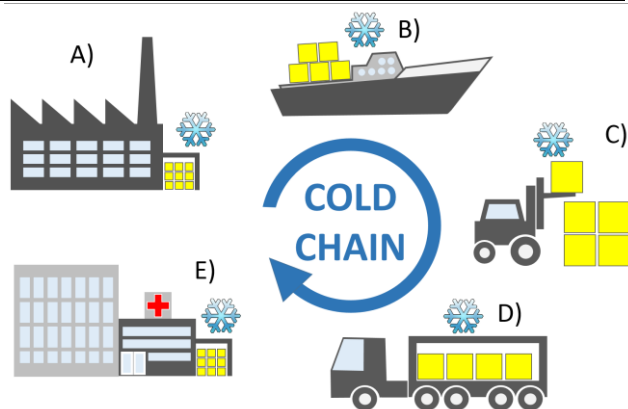


Figure 4. The cold chain schematically represented is a chain of custody ensuring that constant and uninterrupted refrigeration occurs from (A) manufacture, (B) shipment, (C) distribution, (D) delivery, and (E) at the clinic itself. Each point is a potential source of failure. Local infrastructure can cause problems at points (D) and (E) in particular.

In the context of protein based therapies, these denaturing temperatures can be as low as 8 °C and, consequently, a number of proteinaceous therapeutics currently on the market degrade when left at room temperature in as little as a few hours.^{89,90} This is highly undesirable, particularly

when a patient assumes that the drug or vaccine they are receiving is fully effective. In the context of vaccine therapies, degradation can have tragic consequences. For example, the recent outbreaks of Ebola and Zika have ravaged areas of the world with inadequate access to basic infrastructure.^{91,92} It is widely anticipated that vaccines for these drugs will be based upon attenuated viruses or use non-infectious recombinantly expressed Virus-Like Particles (VLPs). To the best of our knowledge, all commercially available VLPs and attenuated virus based vaccines—whether stable to lyophilization or not—require constant refrigeration from the time they are synthesized until just before being injected into the patient. As a consequence, these therapies are very challenging to deliver, store, and administer in the tropical and subtropical developing world where 24/7 access to electricity is not always available. Indeed, as much as 80% of the total cost of vaccines⁹³ is wrapped up in keeping them cold from supplier to manufacturer (the so-called cold-chain illustrated in **Figure 4**), this chain of custody is difficult to track and issues at any of the handoffs can compromise the therapeutic value of an entire shipment without anyone even realizing it. Failures in the cold chain result in the loss of nearly half of all global vaccines.⁹⁴ The global impact of eliminating this process will save hundreds of millions of dollars and increase access to therapies to countless people around the world.

1. *Therapies in MOFs:* MOF-based biomimetic mineralization can play an important role in overcoming the cold chain. It has been known for quite some time that encapsulation of proteins within a porous solid increases their thermal stability. Early work by Lyu and coworkers⁹⁵ found that micron sized ZIF crystals could be grown containing the enzyme Cyt c and the crystal coating increased the temperature of denaturation (T_m) of the enzyme, though the enzyme had to be coated in polyvinylpyrrolidone (PVP) for the synthesis to work. Recently we demonstrated coating enzymes in a polymer is unnecessary when synthetic conditions are tuned appropriately.¹² This paved the way for further exploration of the thermally protective effect of MOFs on a common virus,⁴⁴ which is non-infectious toward humans, as a prelude to vaccine based VLPs. In this case, the ZIF-8/virus biocomposite was fabricated by pre-mixing a solution of tobacco mosaic virus (TMV) with 2mM, followed by the addition of an aqueous Zn(II) acetate solution. The thermal stability of the resulting composite was then tested by boiling in water for 20 minutes. The framework was disassembled using the chelator EDTA, and pristine virus was recovered from otherwise highly denaturing conditions. With this background, it should be possible to extend the cryoprotective effects of the MOF scaffold to other common platforms for vaccine development, including common self-assembled proteinaceous VLPs engineered to display antigens in commercial vaccines against HPV (Cervarix and Gardasil) or Hepatitis B (Engerix, Recombivax HB). There are yet two outstanding questions: 1) Does the MOF coating/removal process change the surface of the proteins in such a way that antibody recognition will be impacted? 2) Will MOF formation

occur on the surface of smaller icosahedral or enveloped viruses? As enveloped viruses, a common type of virus where proteins imbedded in the lipid bilayer are superficially exposed,⁹⁶ emerge as attractive VLP platforms for vaccines,^{97,98} further investigation in these areas is necessary to fully determine the feasibility of this technology.

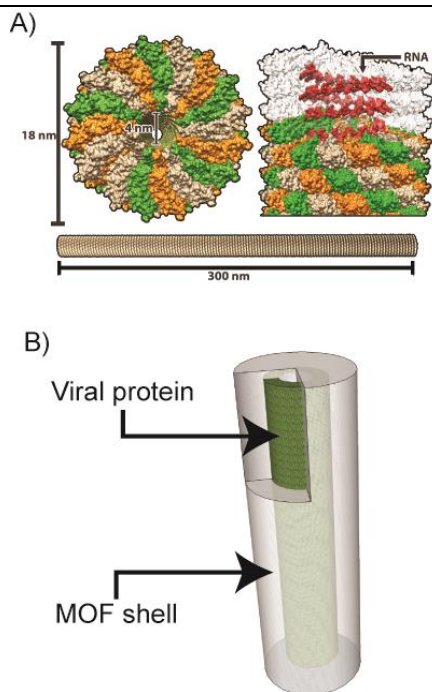


Figure 5. A) The Tobacco Mosaic Virus is a 300 nm long anisotropic viral nanoparticle with a diameter of 18 nm and an inner channel 4 nm in diameter. It is composed of 2130 coat proteins that self-assemble around a single strand of RNA. B) By growing a shell of ZIF-8 on the surface, we have found we can impart incredible stability to the viral nanoparticle in both organic solvents as well as boiling water.

2. *Possible Approaches to Access other Viral architectures:* We have shown that it is possible to form core shell structures on the anisotropic rod-shaped TMV (Figure 5), which contains only two or three solvent exposed tyrosine residues. Presumably, under ZIF-8 formation, these residues are deprotonated, imparting a negative charge to the surface of the virus. It is not clear what would happen if a virus containing other functionalities, were significantly smaller or isotropic were used, therefore it is worthwhile to speculate how such synthetic approaches might appear.

Changing the surface properties of viral nanoparticles using synthetic bioconjugation strategies has emerged as a means of modifying their biodistribution, function, and pharmacokinetics.⁹⁹ One could thus envision a strategy to chemically modify the surface of the virus with functional groups that promote the formation of the framework. While no specific design rules exist for MOF growth on large biomolecules, it is hypothesized that a surface rich in histidine (imidazoles), glutamates, and aspartates (carboxylates), or tyrosines (phenoxy groups) would facilitate nucleation. In instances where the surface chemistry of the VLP seems to weakly facilitate MOF growth, the bioconjugation of short or medium chain anionic or histidine rich polymers on the surface is one possible route to facilitate more favorable growth. For instance, several strategies have been developed that place polymers on the surface of viral nanoparticles by either a graft-to or graft-from strategy.⁹⁹ These strategies have produced new hybrid bio-synthetic systems with enhanced pharmacokinetics and can modulate immune response.¹⁰⁰ On the other hand, polymeric growth from covalently bound sites on the surface of viral nanoparticles may interfere with antigen recognition. It is therefore important that this covalent attachment be reversible under physiological conditions. Several so-called traceless bioconjugation strategies,¹⁰¹ which accomplish just that, have thus recently been described.¹⁰²⁻¹⁰⁵ In this iteration, both the MOF coating and the bio-conjugated polymer would dissolve *in vivo* leaving pristine virus.

An articulated difficulty in very small isotropic materials is the very high stress-strain that could form from MOF shell growth on very small viral nanoparticles.¹⁰⁶ Polio virus and most human enteroviruses, for instance, are only 30 nm in diameter, which would produce immense strain on a MOF coating. It is presently not known if such a small core-shell structures could be constructed on a nanoparticle this small. While only core-shell syntheses have been reported on single viral nanoparticles, synthetic methods that encapsulate multiple viral nanoparticles into a larger crystal are possible, as has been done for enzyme work. One possible disadvantage to this approach is that these larger crystals do not form stable colloids and settle from solution rapidly,¹⁰⁷ making administration by intramuscular injection difficult. One possibility would be to use bioconjugation strategies to covalently tether multiple viral nanoparticles together via ester linkages to make larger ensembles,¹⁰⁸ as depicted in Figure 6, which are sufficiently stable in solution to permit administration.

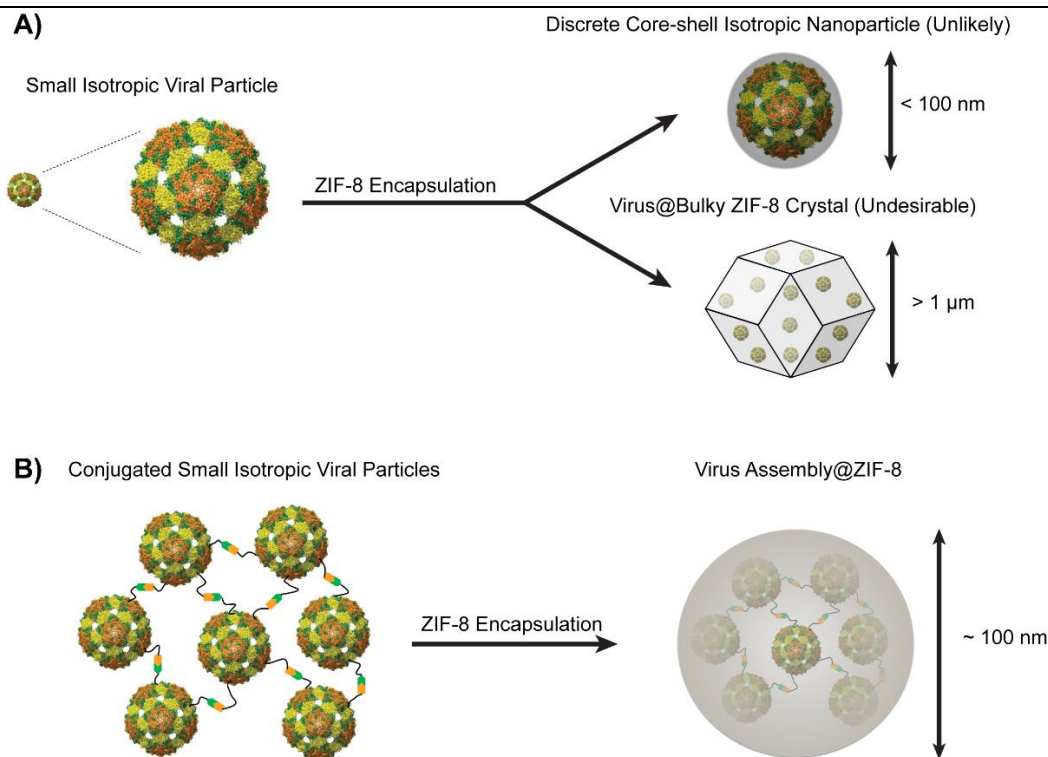


Figure 6. A cell can be covered with a MOF via normal biomimetic mineralisation (A), then if the MOF possesses a functional group, it can be used for a subsequent grafting with enzymes (B). Otherwise, if the framework pore and pore aperture size are big enough, enzymes can be infiltrated into the metal-organic shell (C). A biomimetic mineralisation procedure in the presence of enzyme results in a shell where the biomacromolecules are encapsulated in a single step (D). Finally, it is expected to immobilise enzymes directly on the surface of the cell (E) followed by performing the biomineralization in the obtained cell/enzyme system (F)

Finally, ZIF-8 has proven to be a reliable workhorse MOF in formation of coatings on viral capsids, though other linker and metal combinations could be explored. A limitation to working with biological material is that MOF synthesis would have to be conducted at room temperature and under aqueous environments. While this appears to be inescapable until water-phase stable MOF syntheses are expanded, the thermal and solvent stability imparted by the initial coating of ZIF-8 to proteins would permit linker and metal exchange above room temperature after their initial formation.

3. *Fabrication and delivery of MOF Vaccines:* Typical vaccine formulations contain an adjuvant, which is a component used to excite—or potentiate—the immune system into identifying the antigens in the vaccine. Typically, human vaccines contain adjuvants,¹⁰⁹ which are thought to potentiate the immune system by stimulating dendritic cells to release immune signals that promote antibody production. Aluminum salts are commonly employed though they are not universally effective. For instance, they show little efficacy potentiating the immune system towards malaria and tuberculosis vaccines;¹¹⁰ however, organic hydrocarbons have shown potential, such in the case of squalene, a natural, water-insoluble polyunsaturated hydrocarbon regarded as strong immune potentiator.¹⁰⁹ Combining the porosity of a colloidal suspension of a MOF-based vaccine

would permit an insoluble adjuvant to be loaded in the pores and surface defects. Indeed, it is quite possible that a single MOF crystal might contain an entire suite of vaccine adjuvants and VLPs, each one protected against thermal degradation and loaded with organic adjuvants.

Although the virus@MOF-based vaccine that we propose here has promising potential, there is still space for researchers to explore a rationalized approach to MOF-based vaccine design in regard to drug delivery. In this case, we need to consider the role of the MOF capsule — Will the MOF exterior degrade in the blood before trafficking to the liver, before any immune recognition takes place? Would we then need to consider a method of exfoliating the ZIF at the clinic before administration? If we do not remove the shell prior to administration, additional technical matters will need to be addressed, such as toxicity, bio-degradability, and drug loading efficiency.^{36,111} Apart from ZIF-8, MIL-series MOFs have also been widely studied owing to their high drug loading capacity and water stability.^{53,112} One added benefit to the virus@MOF-based vaccine being capable for solution-free storage, they can be readily prepared in powder formulation which favors tablet (oral) vaccines and transdermal delivery mechanisms like microneedles.^{113–115}

Many diseases and or physical defects due to injury result in the loss of specialized cells within organ systems and lead to organ system dysfunction.¹¹⁶ Parkinson's disease is a well-known example as it results in a progressive loss of dopaminergic neurons; however, other relevant examples are certain meniscal tears and spinal cord injury, insulin dependent diabetes mellitus (IDDM), multiple sclerosis (MS) and other autoimmune disorders.¹¹⁶ Cell therapy is a promising approach to replace, repair or enhance the biological function of damaged tissues or organs. However, this method can succeed only if the transplanted isolated cells are in sufficient number and quality to survive long enough to restore the needed biological function.¹¹⁷ Possible candidates used for cell therapy include autologous primary cells, cell lines, and stem cells.¹¹⁶ Organs and tissues treated in this way can show an improved efficiency when compared with conventional therapies such as transplantation.¹¹⁸ As biological products are banked, transported, and processed, there is a risk for contamination;¹¹⁹ accordingly the manufacturer must demonstrate that the biological product is safe, pure, and potent. In this regard, we believe MOFs could have the potential to limit external contaminations as we preliminary demonstrated that a protective cage with perm-selective properties can be prepared.

Another relevant problem in cell therapy concerns the safety of a transplanted cell population that is largely determined by the purity of the population.¹¹⁹ In stem cell biology, safety concerns are predominantly focused on contamination of a cell population by immature stem cells that can proliferate in an uncontrolled manner, forming tumors.^{120,121} A MOF coating could provide a tool for freezing the collected cells and allowing for their potential differentiation and functionality to be investigated. In this regard, the progress in engineering dye molecules for the staining (identification) of undifferentiated stem or tumor cells with increased efficiency and decrease cytotoxicity will play a crucial role. It is worth to highlight that the choice of the proper dye has to be compatible with a MOF coating and the related pore size or defects (vide supra). Finally microfluidics¹²² technology combined with the use of automated or semi-automated screening methods could make the cell encapsulation and screening an effective tool for cell therapy.^{123,124}

A further important aspect that should be investigated in detail is the relevance of MOF coatings for cell differentiation as it was established that surfaces with different morphology/chemical functionalization can have different effects on stem cells.¹²⁵

CONCLUSIONS AND OUTLOOK

The recent application of MOF biomimetic mineralization to living cells and viruses presents many exciting possibilities for cell biology and biotechnology. The encapsulation processes introduced in this perspective are gener-

ally facile and protect cells from inhospitable external environments that typically lead to cell death or Virus degradation. However, this research field is in its infancy and extension to different cells, viruses and MOFs are required to prove the versatility of this technique. Therefore, systematic studies varying the synthetic conditions for the MOF/living entities biocomposites are required to shade light on the synergistic effect between MOFs and cells.

In the near future, storage and transportation of valuable and fragile living organisms could become a real perspective. However, there are several challenges that should be addressed before MOF-coated cells can reach their full potential. For example, a precise understanding of the growth mechanism of MOFs on the cell surface (phospholipids, membrane proteins, glycosylated portions, etc.). Additionally, it will be important to investigate how different cell walls chemistry affects the MOF structure and morphology. Several possible biomineralization methods are listed here in order to promote the development of this research field and the majority of them are not explored yet (e.g. grafting, infiltration, biomimetic co-mineralization). Studies on the compatibility of MOFs with cells are also important as well new protocols MOFs preparation in physiological conditions.

Therefore, despite the exceptional properties demonstrated in pioneering studies, several challenges related to MOFs and their integration with cells need to be addressed before considering the commercial application of this approach.

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Suggested TOC

