The Promise and Potential of Metal-Organic Frameworks and Covalent Organic Frameworks in Vaccine Nanotechnology

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Abstract: The immune system's complexity and ongoing evolutionary struggle against deleterious pathogens underscore the value of vaccination technologies, which have been bolstering human immunity for over two centuries. Despite noteworthy advancements over these 200 years, three areas remain recalcitrant to improvement owing to the environmental instability of the biomolecules used in vaccines—the challenges of formulating them into controlled release systems, their need for constant refrigeration to avoid loss of efficacy, and the requirement that they be delivered via needle owing to gastrointestinal incompatibility. Nanotechnology, particularly Metal-Organic Frameworks (MOFs) and Covalent Organic Frameworks (COFs), has emerged as a promising avenue for confronting these challenges, presenting a new frontier in vaccine development. Although these materials have been widely explored in the context of drug delivery, imaging, and cancer immunotherapy, their role in immunology and vaccine-related applications is a recent yet rapidly developing field. This review seeks to elucidate the prospective use of MOFs and COFs for biomaterial stabilization, eliminating the necessity for cold chains, enhancing antigen potency as adjuvants, and potentializing needle-free delivery of vaccines. It provides an expansive and critical viewpoint on this rapidly evolving field of research and emphasizes the vital contribution of chemists in driving further advancements.

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1. Introduction to vaccines and immuno-therapeutics and current limitations in the landscape of vaccine technology

Vaccination and immunotherapy aim to train or remodel the host's immune system and have made remarkable progress in controlling and treating infectious diseases, cancers, and other immune-related diseases.[1] Vaccines alone have saved millions of lives annually and have provided trillions of dollars in global economic benefits.[2] The SARS-CoV-2 pandemic highlighted the effectiveness of vaccination in preventing death, and comprehensive data exists to show significant protective effects for the elderly and people with preexisting conditions.[3] The pandemic also demonstrated the effectiveness of a new class of clinically approved mRNA-based vaccines, which proved much more protective than other COVID-19 vaccines.[4] Despite this significant advancement, vaccines face critical issues that have persisted for nearly two centuries, including high costs associated with cryogenic transport,[5] variable potency,[6] very limited shelf-life,[7] and painful injections.[8] As an example, most vaccines contain fragile biomacromolecules—proteins or RNA—that make room temperature storage impossible, necessitating a complex and costly "cold-chain" infrastructure that adds logical barriers to the deployment of vaccines in most of the world.[9] This has led to five of the top ten causes of death in low-income countries being vaccine-preventable diseases, underscoring the pressing need for significant enhancements in vaccine technology and administration.[10] With a few exceptions, immunotherapeutic drugs must be introduced directly into the blood, skin, or muscle.[11] Injection is known to reduce patient compliance with vaccination substantially and

correlates strongly with parental resistance to childhood vaccination.[12,13] This needle phobia is a severe downside of prophylactic vaccination; disregarding it would be a mistake. Finally, the biomolecules used in vaccines are also difficult to modify and enhance their stability or potency without altering their function. These issues make it clear why this problem has persisted—how do you adjust the stability and strength of a drug that is inherently fragile and difficult to modify?

To better understand how vaccines can be improved and how that can be accomplished via new chemistries, we will provide key immunology concepts tailored for chemists, engineers, and material scientists interested in this field. It is essential to acknowledge that immunology is a complex and rapidly advancing discipline, with diverse viewpoints on the crucial factors contributing to a robust and protective immune response against specific diseases. Consequently, while we may present general principles, we ask the reader to appreciate that numerous exceptions exist. We direct the reader to very accessible texts on immunology should this review motivate them to learn more.[14] With that in mind, vaccines are generally designed to generate antibodies, particularly Immunoglobulin G (IgG), which selectively target and strongly bind to specific antigens on a particular pathogen. Generally, antigens are proteinaceous biomacromolecules displayed on the pathogen's surface or are toxins released by the pathogen. When the body encounters a pathogen, an antigen-presenting cell (APC), usually a dendritic cell (DC), breaks down the pathogen and presents small fragments, or antigens, on its surface. The APC then carries this antigen to a local draining lymph node, where T-cells interact with the APC to coordinate the production of antibodies by B-cells against the antigen. These antibodies then opsonize (coat) the pathogen, rendering it unable to enter cells and/or marking it for uptake and destruction by other immune cells. While this seems simple, there is no assurance that an antigen administered to a person will generate a significant, much less protective, immune response at all. Reactions can range from being too mild or nonexistent to being too strong, which may result in severe symptoms.

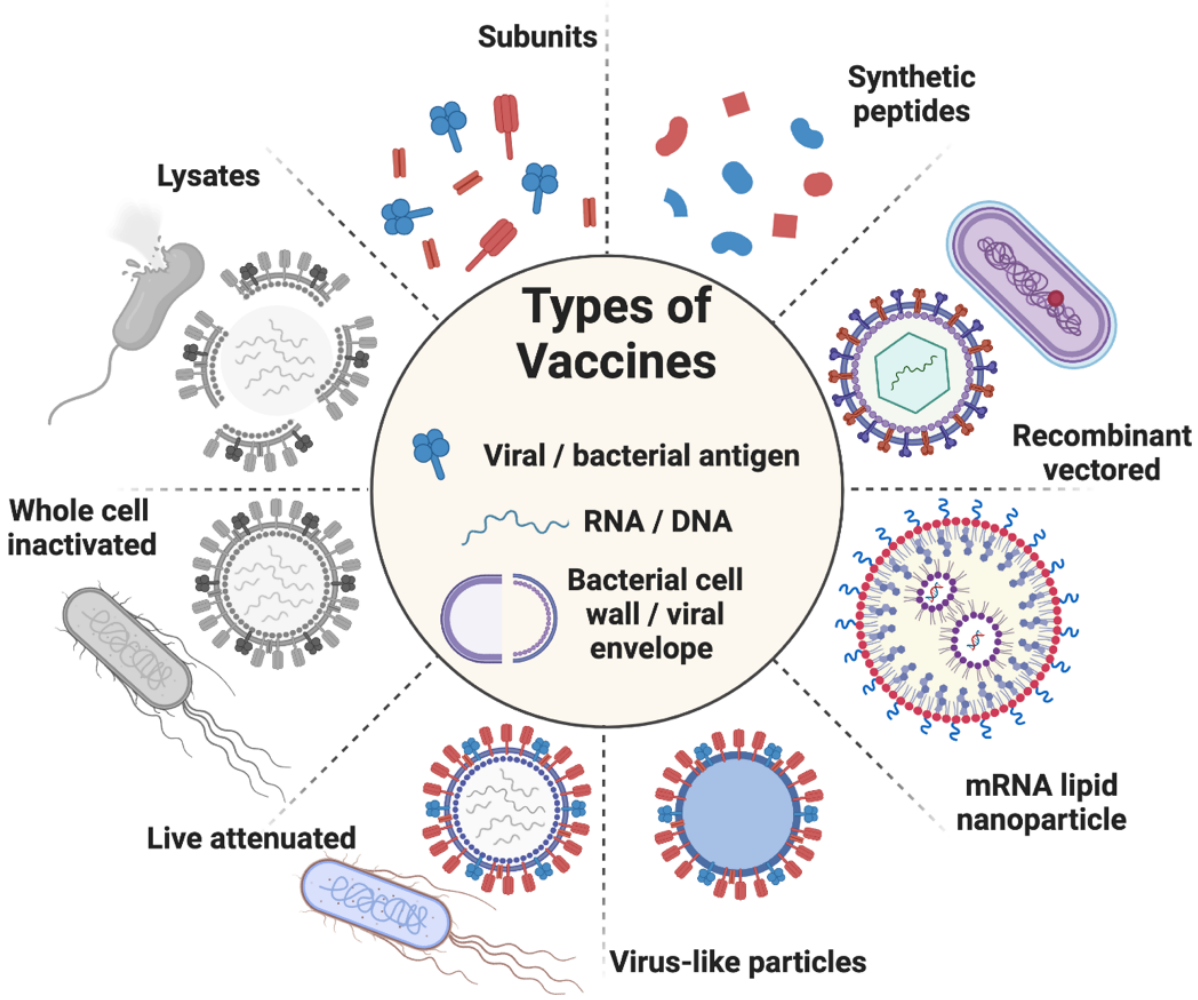

Figure 1. Schematic diagram of different types of vaccines based on the type of antigen used.

Based on this simplified and brief discussion, we can better understand why vaccines are prepared in so many different ways; for instance, as illustrated in **Figure 1**, the intact pathogen can be formulated in a weakened (live attenuated) or dead (whole cell inactivated/lysate) form. This approach is beneficial because it provides the immune system with every possible antigen found in the original disease.[15] A downside is that live attenuated vaccines can cause illness in immunocompromised patients, and inactivating a pathogen reduces its potency.[16] A second method is to use only one or several known antigens in a subunit or synthetic peptide vaccine. This approach has recently gone further by delivering the DNA (recombinant vectored)[17] or RNA (mRNA lipid nanoparticle)[18-20] required for host cells to produce the antigen inside the patient's body.[21] Subunit approaches that use proteins or nucleic acids reduce the side effects of whole-cell vaccines, but it requires identifying an antigen that elicits a strong immune response.[22] Most individual proteins instigate a weak immune response and would not provide

adequate protection as a vaccine formulation. As indicated above, our immune system has evolved to require *multiple or persistent stimuli* before a strong immune response is launched; therefore, it frequently needs additional stimulation to help adjuvant the immune response. Several methods exist to adjuvant the immune response, and we direct the reader to one of several of reviews that illustrate how adjuvants work and which is best for the specific immune response one is investigating.[23-27] One way to adjuvant an immune response is to use biomacromolecules that resemble those from infectious organisms.[28] Consequently, DNA strands based on the bacterial CpG motif, which can profoundly affect how strongly an immune response is and what type of immune response occurs, will frequently appear in this manuscript. Another way to adjuvant an immune response is to use metal-based crystalline materials like alum, which is believed to activate the NLRP3 inflammasome and, more controversially, is believed to act as an antigen depot.[29,30] A “depot” in this context means it slowly releases antigens to local APCs over several days providing immune cells within the lymph node the necessary time to create antibodies that are very specific and tightly binding against the antigen.[31] These higher affinity antibodies, which are more effective at neutralizing antigens, are created by B-cell refinement in germinal centers (GC) (**Figure 2**). GC development is important and takes time; this process is one of the reasons vaccines take several days to begin working, as they tune the immune system toward making antigen-specific antibodies. It is also a reason many vaccines are delivered in multiple doses—so that the GC process can obtain more antigens to continue the process of “training” B-cells to create the best antibodies possible.[32] That said, creating new materials that can act as a depot can be tricky as the skin or muscle is a togh environment, and this depot must ensure that the antigen will remain unbothered for days or weeks at a time, all the while slowly releasing it, feeding this GC response.

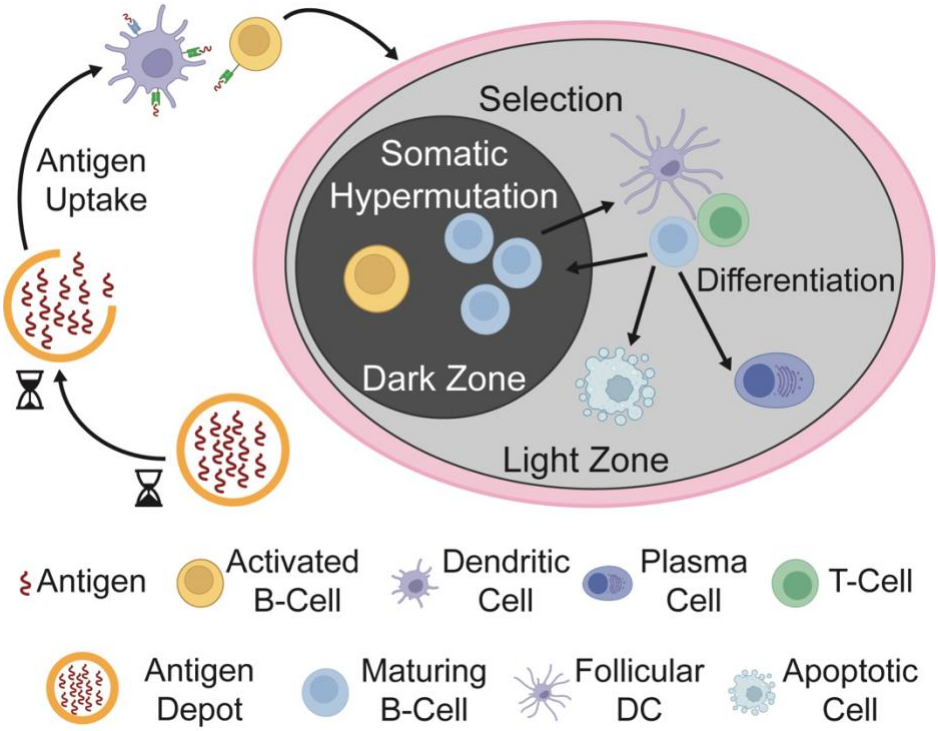

Figure 2. B-cell refinement in GC's enhanced by extended antigen presentation. B-cells will undergo stochastic mutations—called somatic hypermutation—in their B-cell receptor in the dark zone to create a large pool of potential antibodies that can recognize the antigen. These B-cells move to the light zone where antigen-presenting cells that constantly collect antigens from a depot work with T-cells to identify B-cells that have made a matching B-cell receptor. B-cells that do not match antigens undergo apoptosis. B-cells that match antigens in the light zone return to the dark zone to undergo more mutations, with the objective of making their working B-cell receptor bind antigens even stronger. The B-cells go back and forth between these two zones to create B-cell receptors, which will become IgG, that binds antigen very strongly and specifically. Because this process takes time, a steady supply of antigens in the GC is needed to create higher affinity antibodies from B-cells, creating a stronger defense against pathogens.

This hostile environment, which contains avaricious macrophages and other immune cells ready to destroy anything foreign residing in these tissues, makes creating slow-release and extended-release formulations with biomacromolecules difficult. Biomolecule-based prophylactics are delicate, and functionalizing them via bioconjugation can alter their immunogenicity.[33] Further, a slow-release depot must actively preserve the biomolecule as body temperature or the presence of proteases increases the chances of degradation, denaturation, and misfolding, resulting in loss of function, making long-lasting depot implants challenging. [34,35]

Finally, most immunotherapeutic drugs require multiple doses through needle and syringe, requiring technical skills that cannot be quickly transferred to the general population or volunteers.[36] Disposable needles result in biohazardous waste, and their accidental (or intentional) reuse spreads blood-borne pathogens worldwide.[37] While intramuscular injections are more straightforward than intravenous injections, they are prone to neurovascular injuries.[38,39] On the other hand, subcutaneous injections can result in the permeation of formulations parallel to the subcutaneous region instead of perpendicular, depending on the speed of administration.[39] This can result in the formation of misshapen depots of drugs or vaccines in the layers of skin, resulting in a different release pattern than anticipated. The general aversion to needles—especially in children—may deter vaccinating via both these administration routes.[40] Therefore, an active area of research is the development of syringe-free drug delivery systems that can achieve both burst- and slow-release profiles.[34,41,42]

This review analyzes literature reports from 2015 to 2023 that explore the use of MOFs and COFs in addressing challenges related to vaccine development. We examine how these materials can stabilize biomaterials, boost antigen potency, enable needle-free delivery, and enhance immunity against pathogens. We also provide insights into this rapidly developing field of research and how chemists can contribute to its progress. Additionally, we aim to explain fundamental concepts in immunology in a way that's easily understandable to those not in the field. Our discussion focuses on vaccine development for infectious diseases, but it’s worth noting that MOFs and COFs are increasingly used in cancer immunotherapy. We encourage readers to explore other high-quality reviews on this topic.[43-49] While both research areas differ in some fundamental ways, their combination produces a valuable repository of knowledge that helps us understand how reticular frameworks can be developed as biofriendly therapeutics and prophylactics, and how they behave *in vitro* and *in vivo*.

2. Unique structural features of MOFs/COFs

Metal-organic frameworks (MOFs) and covalent organic frameworks (COFs)—often clubbed together as reticular frameworks—have gained immense attention in the fields of materials chemistry and physics and have recently made significant inroads in biomedical applications.[50-52] Reticular synthesis connects rigid molecular building blocks through strong bonds to create predetermined, highly ordered 2D or 3D open molecular frameworks.[53] [54] These materials have high surface areas and porosities, and their tunable and well-defined structures allow for many routes of modification, making them applicable across multiple fields of science, medicine, and engineering.

MOFs are made from metal ions/nodes coordinated to organic linker molecules to make extended network structures with high porosity. They are currently amongst the most extensively studied materials, with well over 96,000 structures reported in the Cambridge Structural Database by 2019. The strong coordination bonds between the metal nodes or clusters and ligands are a source of establishing rigidity and direct the well-defined geometric structures within this class of materials.. The main source of structural variation in the array of MOFs published so far stems from the differences in (i) secondary building units that form the metal ion clusters and (ii) organic linkers connecting these clusters. Coordination numbers denote the number of linkers coordinated to each cluster, which can be as high as 66.[56] In all these geometry variants, metals of similar valency can replace one another to make isoreticular MOFs. This has been well-demonstrated in literature, as popular linkers like 1,4-benzenedicarboxylic acid (BDC) and 2,5-dihydroxy-1,4-bezenedicarboxylic acid (DHBDC) form many MOFs with different metals.[57-59]

An entire subsection of research in this field is dedicated to tuning pore sizes. One of the most popular approaches for such work is utilizing the chemistry and framework of existing MOFs and swapping out the linkers with their longer counterparts to extend the pore size. While the description makes the task sound facile, increasing pore size also results in a higher chance of pore collapsibility upon activation, making this a synthetic challenge.[60] Prominent examples would include the IRMOF and UiO series, which were built on the extension of linker length—while retaining the symmetry—from their parent MOFs IRMOF-1 (or MOF-5) and UiO-66, respectively.[61-63] Alternatively, changing the substituents on a linker instead of elongating the carbon chain can also result in different geometries and pore volumes,[64,65] which has downstream effects on the chemical properties and selectivity of the MOFs.[66-69] A variety of applications emerge from these properties; some of the popular fields of investigation include catalysis,[70,71] gas storage and separation,[72,73] drug delivery,[74,75] and sensing.[76-78]

COFs have also gained prominence as a closely related porous material being extensively investigated in various fields such as storage and separation of gases or other guest molecules,[79-81] catalysis,[82,83] sensing and detection,[76] photocatalysis, membrane technology,[84] and drug delivery.[85-87] COFs consist entirely of organic building units resulting in 2D and 3D crystalline open frameworks through the interactions between organic precursors, resulting in covalent bonds to afford porous organic materials with tunable pore sizes.[88, 89] The directionality and excellent covalent bond strength enable the creation of unique organic structures with rigid, low-density, exceptional stability, and high resistance. In addition, these materials possess permanent and well-defined porosity, further enhancing their versatility for numerous applications.[90] COFs are primarily based on light elements such as C, H, B, N, and O. Currently, more than 500 COFs with >18 topologies have been reported.[50] 2D COFs are formed by covalently

linking planar monomer units into extended sheets. These sheets, in turn, stack together through π-stacking interactions, resulting in a highly anisotropic crystalline porous structure.[91] This robustness allows COFs to withstand harsh conditions such as high temperature, moisture, and chemical environments.[92] The design of the COF's topology is decided by the geometric arrangement of its building blocks. In contrast, the formation of long-range ordered network structures depends upon the reversibility of covalent bond formation.

Organic chemists have long been successful at controlling covalent bond formation in zero-dimensional structures with polymer chemists able to extend the covalent chemistry to one-dimensional structures; however, higher dimensional structures were far more difficult to prepare, leading Nobel laureate Roald Hoffman to observe that "in two- or three-dimensions, it was a synthetic wasteland."[93] This changed when Yaghi and his colleagues achieved a breakthrough in 2005 by successfully linking small symmetric organic building blocks into an extended porous crystalline covalent organic framework (COF) using dynamic covalent chemistry.[94] Unlike other organic materials, higher dimensional COFs require in-situ crystallization owing to their insoluble and non-melting nature. That said, this area is developing quickly and a recent approach to address difficulties in solution processing employing pore engineering techniques now allows the production of flexible crystalline films with enhanced mechanical properties.[95]

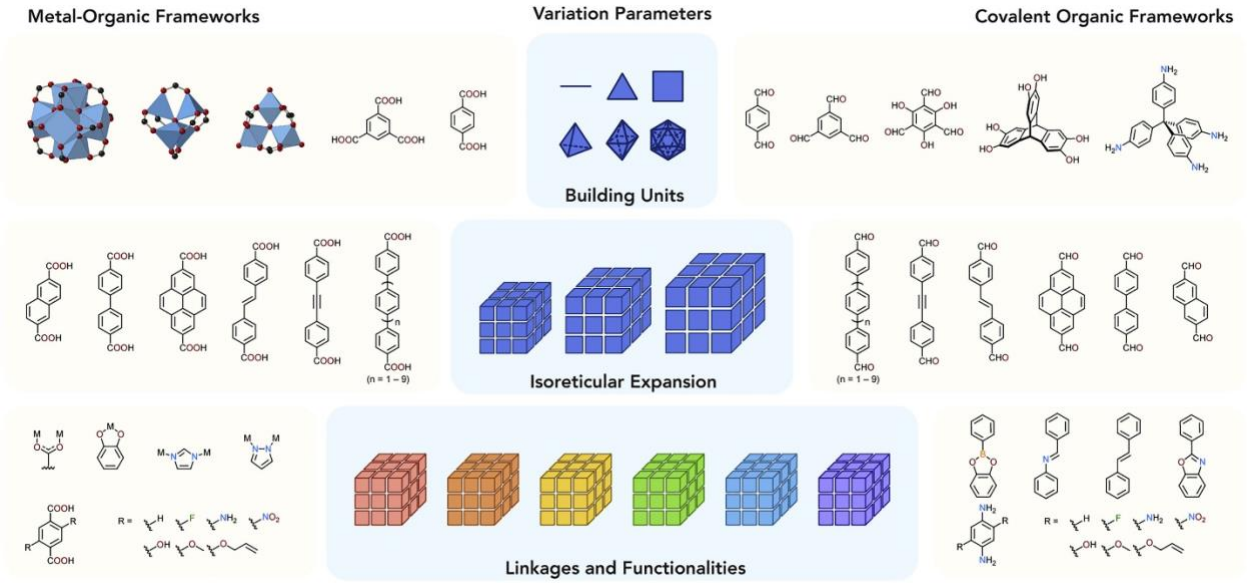

Figure 3. Scheme of various MOF and COF building units and linkers, and a schematic representation of isoreticular expansion and linkage-dependent properties. Figure adapted with permission from Lyu et al.[96] (Copyright 2020, Elsevier)

3. Advantages of using MOFs/COFs to fill the gap in current vaccine technology

While this review particularly focuses on MOFs/COFs for vaccine application, it is worth noting that there exists significant efforts in integrating reticular frameworks with antibodies[97] and bioactive materials for diagnostic imaging,[98-101] diagnostic chips,[102-104] and antibacterial materials.[105-107] Drug and cargo delivery is an expansive area of research,[108-110] as the large surface area and loading efficiency of MOFs/COFs makes them an ideal nanocarrier.[86] Supplying chemotherapeutics with MOFs,[111] or even using the MOF to sensitize crucial pathways which facilitate immunotherapy-based treatments has been an approach several researchers have made considerable progress.[112] It is worth noting that the features which make these reticular frameworks great candidates for all the aforementioned biomedical applications can be exploited to advance the next generation of vaccines, overcoming many issues with the current technology. This section discusses four main properties that allow them to excel as a candidate material in this area — thermodynamic stability, kinetic lability, synthetic functionalizability, and needle-free delivery**.**

3.1 Thermodynamic stability of MOFs

The thermodynamic stability of MOFs is well known. Thermogravimetric Analysis (TGA) studies show that most MOFs can resist temperatures over 100 °C — well past biologically relevant temperatures or temperatures inside shipping containers.[113,114] More detailed analysis can be carried out by coupling TGA with Differential Scanning Calorimetry (DSC), as data on loss of mass and heat flow are collected in tandem. Mu et al. used TGA-DSC on a library of MOFs, elucidating the impact of coordination environment and metal content on its thermal stability.[115] MOFs can confer their thermodynamic stability to biomaterials by inhibiting conformational changes in protein structure that lead to aggregation and denaturation.[116] Considerable effort has been extended to protect colloidally stable vaccines from denaturation by loading them post-synthetically in porous materials, as illustrated in **Figure 4A**. Early computational work by Dill and coworkers found[117] that encapsulating a protein into a “generic box” increased the stability of their folded tertiary structures by as much as 15 kcal/mol by “eliminating some expanded configurations of the unfolded chain and shifting the equilibrium from the unfolded state toward the native state”. Eggers and Valentine found[118] that encapsulating enzymes into the pores of microporous glass nanoparticles increased their denaturation temperature by as much as 32 °C. Some of the earliest work on stabilizing enzymes within porous materials like zeolites includes work by Balkus and co-workers, who evaluated the activity of trypsin when immobilized in various reaction conditions.[119] They also concluded that the protein size had an inverse relationship to their immobilization efficiency. However, from the thermal stability standpoint, having tiny proteins in large pores may not be ideal either. If the diameter of the protein and pore are similar, then the tight confines within a pore prevent proteins from sampling other conformations, allowing them

to experience much higher temperatures before undergoing an irreversible conformational change, rendering them inert.[120] Thermal preservation with reticular materials can address the cold chain problem resulting in room-temperature stable vaccine formulations. Of course, placing a protein post-synthetically within a MOF or COF necessitates the material be made with pores complementary to the protein inside. This is complicated with zeolites or microporous glass nanoparticles where pore sizes are difficult to tune; however, this level of precision has been achieved through the synthetic versatility of MOFs and COFs.[110,121-124]

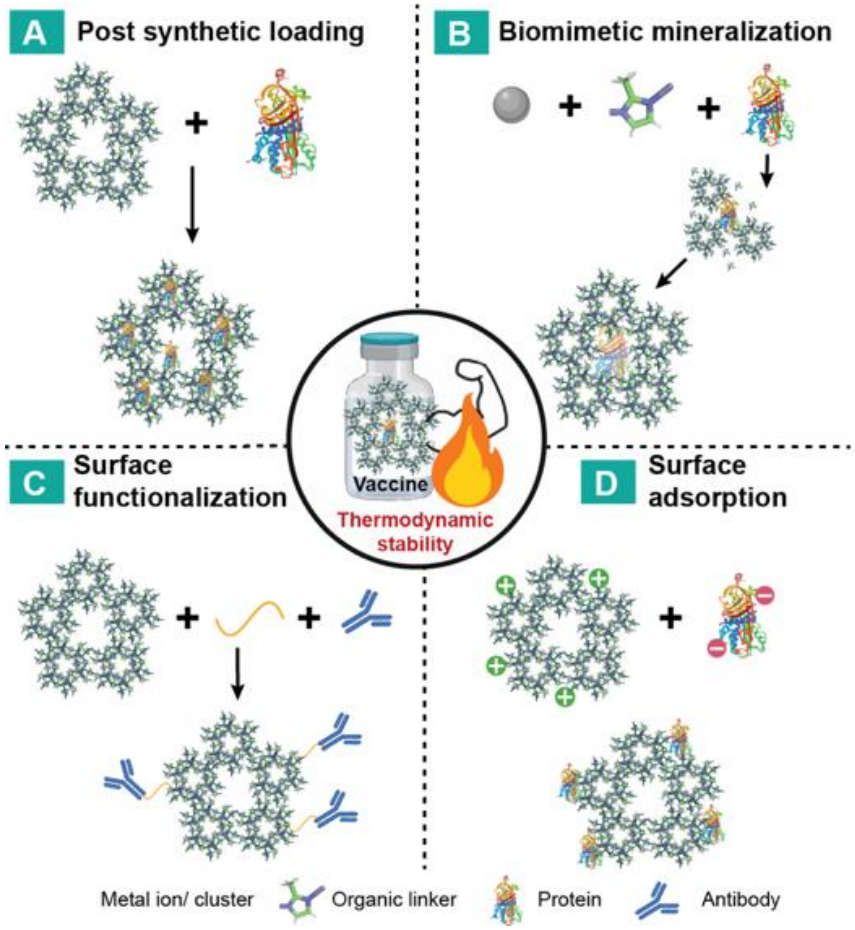

Figure 4: Scheme of the four methods by which MOFs can carry vaccines. A) Post-synthetic loading, where antigens/biomolecules are loaded into the already-synthesized MOFs via infiltration. B) Biomimetic mineralization is a process where MOF constituents are incubated along with the antigens/biomolecules through one-pot synthesis. Here biomolecules act as nucleating agents facilitating MOF growth on the surface of biomolecules, eventually encapsulating inside the porous framework. C) Surface functionalization, where antigens/biomolecules are attached to the surface through linker molecules using chemical reactions. D) Surface adsorption, where antigens/biomolecules are loaded onto the surface of MOFs via simple electrostatic or hydrophobic reactions.

Accordingly, MOFs can immobilize fragile antigens to make thermodynamically stable vaccine formulations, leading to room temperature storage and avoiding the cold chain.
More recently, Falcaro and Doonan demonstrated the nucleation and growth of zeolitic imidazolate framework-8 (ZIF-8) and other MOFs on proteins, enzymes, and DNA via a "biomimetic mineralization" process illustrated in **Figure 4B**. Biomimetic mineralization describes the triggered formation of MOF particles or films by biomacromolecules or more complex biological entities (e.g. a virus or cell).[125] These materials are not trapped within a pore but have been mineralized within the lattice structure. They first showed that proteins induce MOF formation and that mineralized enzymes retained their activity after exposure to temperatures that would ordinarily deactivate them.[122] This stabilization was experimentally proven by Murty *et al*., who optimized a method to detect proteinaceous material inside MOF scaffolds using small-angle X-ray scattering.[126] They showed that the native fold of mineralized proteins was retained inside ZIF when exposed to temperatures that unfold and denature the unencapsulated protein.

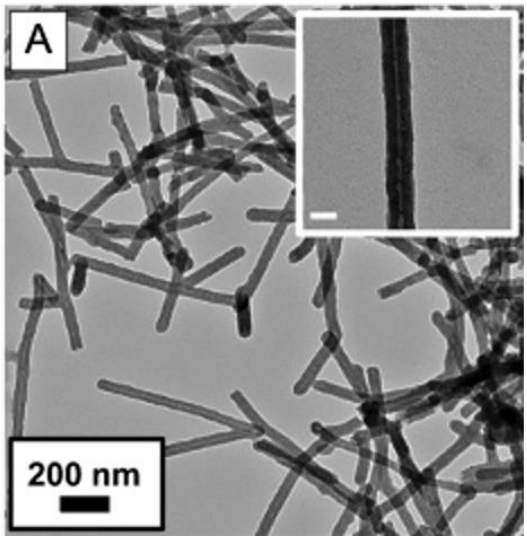

Figure 5. The first report of biomimetic mineralization of a virus using MOFs. TEM of as-synthesized TMV@ZIF through biomimetic mineralization process where the TMV (lighter region) can be seen inside the center of the ZIF shell (darker region) in the inset. Inset scale bar: 50 nm. Reprinted with permission from John Wiley and Sons.[127]

We demonstrated the ability of MOFs to stabilize whole viruses using biomimetic mineralization. Specifically, we encapsulated infectious Tobacco Mosaic Viruses (TMV) within a ZIF-substrate (TMV@ZIF) and showed that the morphology of the virus remained intact after encapsulation in ZIF via TEM (**Figure 5**).[127] ZIF-8 growth occurs regardless of the surface charge or if the biomaterial is functionalized with polyethylene glycol (PEG) polymers — an advantage that we illustrated[36] with liposomes where they were

successfully encapsulated regardless of being cationic, neutral, anionic, or PEG-ylated. The ZIF-8 shell remains porous post-encapsulation, and the MOF can be activated with organic solvents without damaging the underlying protein, as we showed small molecules could travel through the coating and conjugate to the exterior surface of TMV. In follow-up work, we showed that ZIF-8 mineralization protects TMV from high temperatures and organic solvents that would otherwise destroy the virus.[128] We exploited the kinetic lability of the MOF (*vide infra*) by removing the ZIF-8 coating to assess the integrity of the protein surface using an enzyme-linked immunosorbent assay (ELISA). We found that the TMV's surface epitopes were unaffected by the growth of ZIF-8, which was interesting because the surface of the protein was catalyzing the MOF formation. Indeed, when the virus was inside the MOF, it was far more resilient against extreme temperatures, denaturants, and organic solvents. Further, the encapsulated virus retained its ability to infect plants. This suggests that the RNA contained within the virus remained intact and that the MOF coating did not impede immune recognition. When we assessed how the TMV@ZIF performed *in vivo,* we found that the MOF-coated virus produced an even more robust immune response than the pristine virus.[128] Our subsequent studies have shown that we can preserve highly delicate vaccine systems like proteoliposomes—liposomes with fragile membrane proteins embedded in their lipid bilayer—by coating them in different polymorphs of ZIF.[129] ZIF coatings effectively shield the membrane proteins, enabling them to maintain activity even in conditions that otherwise destroy them. We went so far as to show that ZIF-encapsulated proteoliposomes could be shipped by standard mail in the US postal service, with only limited denaturation (20%) over three months. In contrast, the control pristine proteoliposomes were destroyed (only 15% of the activity compared to the pristine proteoliposome activity)within 12 hours at room temperature. Finally, we have shown that ZIF encapsulation of whole-cell organisms preserves complex cell-surface proteins that are known virulence factors of deadly *E. coli* infection.[130]

We do note that MOFs have competition in this area—many systems, including sugars and polymers, likewise confer thermal stability by trapping proteins inside a glassy matrix.[131] Sugar and polymer excipients use lyophilization or spray-drying to encapsulate proteins.[132,133] The slow drying of the two heterogeneous macromolecules makes phase separation of the commixture difficult to avoid—this phase separation results in small islands of protein aggregates within the amorphous structural interfaces.[134] The slow evaporative process also results in a concentration of the salt buffer, which can lead to protein unfolding. Choi *et al.* found[135] that the hygroscopic nature of the resulting sugar matrices might lead to rehydration of the sugar glass followed by structural changes in the sugar matrix. Finally, MOFs have a key advantage over sugar-based excipients—they are kinetically labile.[136]

3.2 Kinetic lability

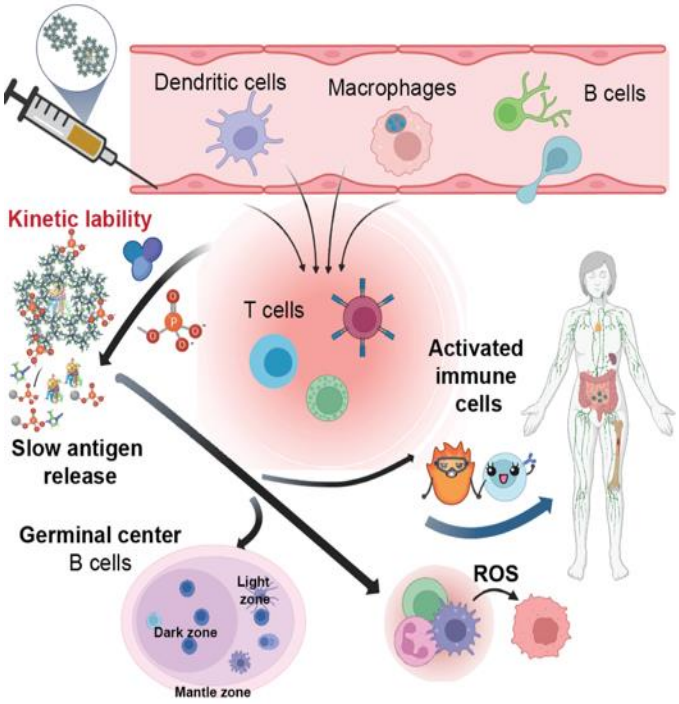

Figure 6. Schematic illustration of using kinetic lability of MOFs for vaccine purposes. The kinetic lability of MOFs facilitates the gradual dissolution of the MOF shells upon administration into the body after exposure to plasma proteins and biological anions like PO_4^{3-}. This gradual dissolution leads to the slow release of the encapsulated antigens/biomolecules. This depot ultimately strengthens both the humoral and cellular immune responses through activating immune cells (T-cells), germinal center formation (B-cell maturation), and production of ROS, which helps modulate immunosuppression/activation of macrophages and DCs.

Kinetic lability is the ability of (typically) inorganic compounds, like MOFs, to be interrupted rapidly by chelators or inorganic ions that can outcompete the endogenous ligand. For example, a basic ligand that becomes protonated in an acidic environment or a metal being stolen from the structure by a high-affinity chelator. Traditionally, this is considered a weakness of MOFs that people have been working to improve; however, it is an exploitable benefit in drug delivery for immune response.[137] MOFs like ZIF-8 can completely degrade in the presence of biological fluids and common laboratory buffers, and studies show that they can degrade over a day to one or two weeks.[138-140] Even UiO-66, which has a storied—albeit overstated—reputation as chemically inert, degrades in different buffers within hours or days.[136] Slow but steady kinetic degradation allows for the delivery and release of material without concern for MOF bioaccumulation in the body, something seen with other bio-persistent delivery vehicles like gold or silica nanoparticles.[141] Additionally, the kinetic lability of MOFs allows for tuning the

pharmacokinetics of biomacromolecules that traditionally cannot be formulated into slow-release polymers without altering their function.[142,143] The controllable release potential of MOFs leads to another benefit of their use, the depot effect. Depots of material can be built that slowly release antigens as they degrade *in vivo,* helping to promote a strong humoral immune response, as illustrated in **Figure 6**.[130]

COFs are often created via reversible condensation reactions that expel water to create the final connected structure.[93] In the presence of water, this process is reversed, albeit slowly, returning COFs back to their monomeric form. The *in vivo* degradation kinetics of COFs are less understood than MOFs, though work is proceeding in this area. The timing of this degradation is important because materials that build depots lasting too long—many weeks or months—can worsen the immune response by exhausting the local T-cell population.[144]

3.3 Functional modification

One of the classical reasons for using reticular platforms is their ability to be modified and highly tuned for many purposes, as elaborated on in these reviews.[145,146] More specifically, surface modification can increase therapeutic function by boosting colloidal stability[147] or intracellular delivery.[148,149] The same principles have been exploited for immunological purposes. For example, attaching an antigen or adjuvant to the surface or targeting material to increase cellular uptake may be more effective (**Figure 4C**). Specifically, combining an antigen—or multiple antigens—and an adjuvant in a single formulation will ensure the co-delivery of adjuvants and antigens to the same cell, thus producing a more uniform and likely improved immune response.[150,151]

Multiple groups have reported using functional groups to attach proteins to the surface as a method of post-synthetic loading. The Morris group modified the UiO-66 family of MOFs with folic acid and then showed the binding of BSA and covid spike proteins. While using it as a capture and cleaning system, this approach could be used for antigen presentation.[152] Further modification of MOFs can be used for targeting. For example, antibodies that target cancer cells have been chemically conjugated onto the surface of MOFs, which allows the MOFs to bind only to cells expressing the protein of interest.[153] A similar approach could target immune cells so that antigen is delivered specially to DCs, improving immune response. The size and surface tunability also contribute to uptake and can be used to control how a cell internalizes a MOF-based vaccine.[154]

Finally, it is worth noting that MOFs are easily scalable and are already manufactured at large volumes. Falcaro and Doonan have demonstrated that flow synthesis can be

applied to ZIF-8 biocomposites in size as a controllable method, demonstrating the potential for efficient manufacturing of MOF-based vaccines.[155]

3.4 Needle-free delivery

Two areas in non-traditional, skin-mediated delivery modalities have emerged using MOFs—microneedles and biolistic delivery. Microneedles are arrays of tiny projections that painlessly pierce the skin, allowing the introduction of materials, either drugs or antigens, through these tiny holes. They can be manufactured in various ways, including 3D printing.[156] In a short span of time, this area of research has made great progress, with microneedle-based influenza vaccines making it to Phase 1 clinical trials.[157] Even though microneedle-based vaccine candidates have been developed for influenza,[158] rotavirus,[159] measles, rubella,[160] COVID-19,[161,162] and hepatitis B,[163] to name a few, the number of studies that focus on protecting these antigens from their outside for extended shelf stability are much fewer in number. PLGA,[41] PMA,[164] and MOF-based systems have been published in only the last few years, but this shift is indicative of the need to develop delivery tools that address several drawbacks of the current vaccine technology at once. MOF-loaded microneedles have been prepared and demonstrated for insulin release. Briefly, the insulin was mineralized inside ZIF-8, which was then incorporated into microneedles for intradermal controlled insulin release.[165] Additionally, microneedles have been used as a potent vaccine platform to deliver antigens and improve immune response by increasing cargo availability and extending its presentation.[162] So far, MOF microneedles have yet to be used in vaccine applications; though, this is an interesting area for extended-release, pain-free vaccine delivery.

Biolistic delivery is another form of needle-free delivery that uses pressurized gas to fire material at high speeds so that it enters the skin like micro-bullets.[34] Jet injectors, which pressurize a liquid solution of drug and fires it into the skin at high velocity, were discontinued for human use due to the risk of cross-contamination. This occurred because fluid could squirt back out of the skin onto the jet injector nozzle, which was caused by the high velocity required for the delivery modality.[166] However, by delivering solid, rigid particles using a gas, this problem can be circumvented since the delivery velocity can be lower, no liquid is required, and the rigidity of the particles can help with skin penetration. Moreover, using MOFs as these rigid carriers provides a dual benefit of protecting the biomaterial that needs to be delivered. By coating liposomes with ZIF-8, Kumari *et al*. showed that a ZIF-8 shell, only 100 nm thick, could increase the fracture strength of the liposome by more than 300 orders of magnitude, allowing them to be shot into the subcutaneous layer of porcine skin. This demonstrated the remarkable protection of MOFs while exploiting the benefits of kinetic lability to remove the coating and showing that the liposomes were still intact after delivery.[36] While other materials like PEG-crosslinked hydrogels have also been demonstrated as possible solid carriers for vaccine

biomaterials,[167,168] MOFs help combine kinetic lability with biolistic delivery in a unique way that allows the carrier gas to be used as a reagent to control drug release rates. Wijesundara *et al*. demonstrated that tuning biomolecule release half-life with MOFs is possible—burst or slow-release kinetics were achieved *in vivo* using different gases to deliver the MOFs into the skin (**Figure 7A**). Specifically, the group demonstrated this gas-controlled delivery method using ZIF-coated OVA in mice (**Figure 7B**) and DNA plasmid delivery into plants (**Figure 7C**), highlighting this approach's versatility. Carbon dioxide as the carrier gas induced carbonic acid formation around the MOF in the tissue, which promoted the rapid dissolution of the ZIF coating and release of all cargo within a few hours.[34] In contrast, regular air did not affect MOFs once in the skin, where they dissolved and slowly released cargo in the presence of biological anions (**Figure 7D**). Both examples provide a template for MOF biolistic delivery that could be applied to vaccine administration.

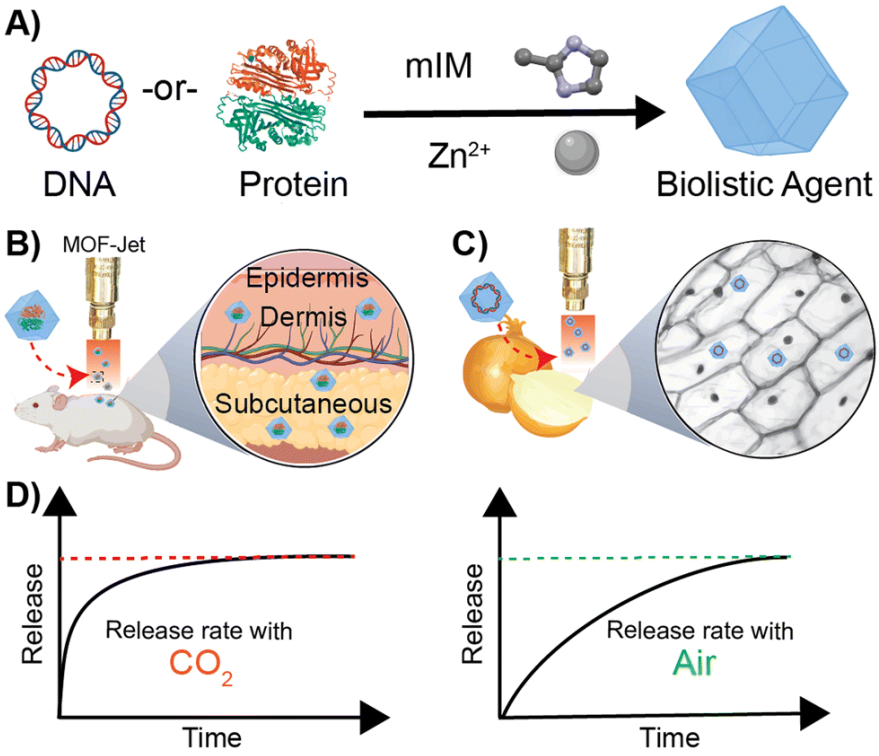

Figure 7: Schematic representation of biomimetic mineralization with ZIF-8 for biolistic delivery. (A) synthesis of DNA-or-Protein loaded ZIF-8 crystals from 2-methyl imidazole and zinc acetate dihydrate in aqueous conditions at room temperature. (B) Biolistic delivery of protein-loaded ZIF-8 crystals into animal skin. (C) Biolistic delivery of DNA-loaded ZIF-8 into plant tissue. (D) Respective release profiles of the encapsulated biomolecule when biolistically delivered with CO2 and air as the propellent. Reprinted with permission from Wijesundara *et al.*[34] Copyright 2021 American Chemical Society.

Intranasal and pulmonary delivery is another less-invasive delivery modality that has seen a piqued interest from researchers and clinicians alike, owing to its ease of administration and potential enhanced effect by tapping into mucosal immunity. However, there are no subunit vaccines on the market that can be delivered intranasally; only inactivated and

whole cell preparations of intranasal vaccines have made their way to the clinic so far.[169] This trend suggests the need for developing techniques that can enhance or boost the immunogenicity of subunit formulations in a manner that can withstand the mucosal environment, or provide a depot that can increase the bio-residency long enough to elicit a potent immune response. Fernández-Paz *et al*. were amongst the first to deliver a MOF in an *in vivo* model into the lungs – they delivered microspheres made of MIL-100 coated in mannitol intratracheally into rats.[170] These results were impressive as they were able to deliver up to 8 mg into the lungs and, according to histopathological analyses, without damaging the tissues. More recently, Kumari *et al.* demonstrated how coating liposomes in ZIF and delivering them intranasally into mice can extend the bio-residency half-life of the liposomes by over four times.[171] Further, they were able to deliver up to 1 mg of uncoated ZIF-8 directly into the intranasal cavity and observed no tissue damage to the delicate turbinate structures in the sinuses, nor loss of pulmonary function or elevation of liver and kidney enzymes, showing this route as being highly compatible with MOF delivery. These efforts—although nascent and still in the stage of fundamental discovery—show potential for the possible marriage of all aforementioned benefits of MOFs as vaccine carriers along with being able to exploit them as a tool for mucosal delivery.

4. Protection and delivery of biomaterials via encapsulation/biomimetic mineralization in MOFs

Biomimetic mineralization and encapsulation offer an attractive avenue for vaccine development, as they can protect larger biomaterials like liposomes, lipid nanoparticles, proteins, viruses, or bacteria within a MOF. This is not possible through post-synthetic modification. Preserving antigen integrity is crucial, so the mineralization procedures should be carried out under biofriendly conditions. While ZIF-8 is the most widely used MOF for biomimetic mineralization, recent studies have also explored optimizing biofriendly conditions for other MOFs.[116,170,172-176] This section showcases various examples of encapsulating proteins, nucleic acids, spores, viruses, and bacteria under biofriendly conditions, demonstrating the broad range of vaccine models that can be developed.

It is worth noting that ovalbumin (OVA) is frequently employed as a model antigen since its immune responses in murine models are very well reported, and many commercially available kits exist to analyze an antigen-specific response.[2,177-180] This is also convenient; it allows us to compare different materials and adjuvants more directly. Note that despite the similarities, many of these papers incorporate interesting orthogonal strategies to enhance the immunogenicity of their vaccine model.

The first use of MOFs as a protective scaffold to produce a vaccine formulation was prepared by co-precipitating the antigen (OVA) with an adjuvant (CpG) electrostatically coated to the surface of ZIF-8.[2] Before biofriendly biomimetic mineralization strategies were developed, MOF-forming reactions to encapsulate proteins or enzymes were carried out in organic solvents, including methanol, which denatures proteins. To overcome this solvent limitation, the OVA protein had to be initially coated in polyvinyl pyrrolidone (PVP) to keep it folded in methanol.[120] This protein and polymer composite was added to methanol, zinc (II), and methylimidazole, where the ZIF-8 formed indiscriminately, and the antigen was coprecipitated with empty ZIF. The mixture of antigen-containing and empty MOFs was then coated in CpG on the surface. Now that we better understand ZIF degradation in tissue, it is very likely that electrostatically bound materials dissociate and diffuse from the injection site once inside tissues, where the ZIF begins to degrade. Nevertheless, this early report was foundational, establishing MOFs as potential vaccine carriers.

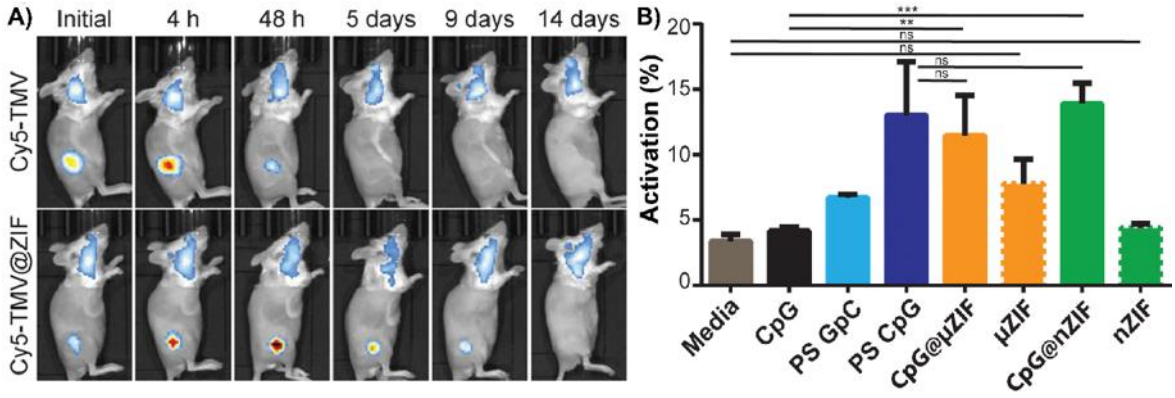

Figure 8: *In vivo* studies of TMV and CpG encapsulated in ZIF. (A) Mice were shaved on their torso and limbs and injected subcutaneously with Cy5-TMV (TMV conjugated to fluorescent dye Cy5) and Cy5-TMV@ZIF. The mice were imaged periodically until all the fluorescence from the injected material disappeared. Reprinted with permission from Luzuriaga *et al*.[128] Copyright 2019 American Chemical Society. (B) Murine splenocytes were incubated with CpG, phopshorothioated backbone CpG (PS CpG), CpG encapsulated in ZIF and relevant controls for 48 h. Flow cytometry measured the activated B-cell percentage (CD19+, CD80+, and CD86+) after stimulation. Reprinted with permission from Brohlin *et al*.[181] Copyright 2022 American Chemical Society.

In contrast to the previous methodology, biomimetic mineralization occurs in water or biological buffers and is very gentle to proteins. Because the MOF growth is nucleated selectively on biomacromolecules, it is not necessary to treat or alter them further. Moreover, the MOF coating can also provide an adjuvanting or depot effect, improving the immunogenicity of the administered biomaterial. After administering TMV@ZIF into the skin of mice, we confirmed[128] that the mice not only created antibodies against TMV,

but the ZIF-8 was promoting an adjuvant effect all on its own as titers of anti-TMV IgG were higher for the TMV@ZIF group compared to the neat TMV group. We now attribute this adjuvant effect to the prolonged tissue residency of the TMV compared to the relatively brief period the TMV remains in the skin after injection. Specifically, we used a fluorescently labeled TMV to show that the TMV@ZIF takes about 12 days to fully clear from the injection site, whereas the uncoated TMV was cleared within 5 days (**Figure 8A**). Further, Brohlin *et al.* demonstrated it was possible to capture CpG inside ZIF-8 biomimetically and showed that it improves the stability of the unmodified phosphodiester bond. The unmodified CpG encapsulated in ZIF-8 showed significant enhancement of B-cell activation in splenocytes compared to the variant of CpG with a phopshorothioated backbone – which has been used so far as a workaround to protect from nucleases (**Figure 8B**).[181]

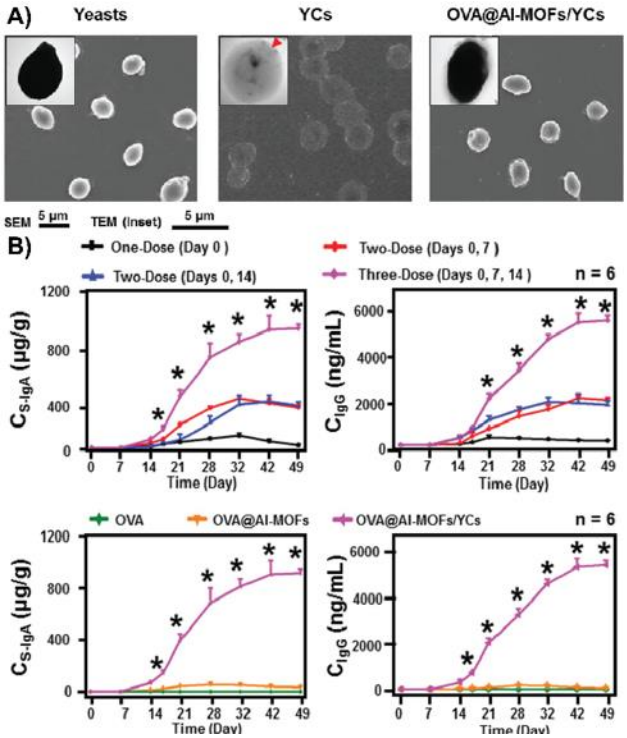

Figure 9. Yeast-derived capsules encapsulating OVA and Al-MOF for a combinatorial delivery approach. (A) SEM micrographs and TEM micrographs (inset) of yeast, yeast capsules (Ycs), and the OVA-Al MOF-yeast capsule composite (OVA@Al-MOF/YCs). (B) Mice were vaccinated with OVA@Al-MOFs/YCs in varying doses, and S-IgA and IgG were quantified in their fecal extracts and blood serum, respectively (top). To compare the potencies in formulation, mice were vaccinated in triplicate with OVA, OVA@AlMOFs, and OVA@Al-MOFs/YCs, and the same analysis of S-IgA and IgG was repeated (bottom). Reprinted with permission from John Wiley and Sons.[180]

Combining an adjuvant and the antigen within the same system can help improve immune responses. Yonggang Hu's group incorporated both spores of *Bacillus amyloliquefaciens*

as an adjuvant with OVA inside ZIF-8. They benchmarked the performance of the spores against alum and found the IgG titers with spores to be significantly higher. Since the spore-containing groups also had much higher IgG than the OVA and OVA@ZIF-8 groups, suggesting that spores but not ZIF-8 could induce a higher humoral immune response *in vivo.*[177] Hsing-Wen Sung and group developed a biomimetic mineralization technique to protect OVA with an aluminum MOF (Al-MOF) that uses 2-aminoterepthalic acid as a linker. This composite is stable at lower pH ranges but labile towards phosphates, which can help overcome the challenge of oral administration of vaccines, preventing gastrointestinal proteolysis. Al-MOF doubles up as the delivery vehicle and supplements the formulation as an adjuvant. Porous yeast-derived capsules were used to load the OVA@Al-MOF inside (**Figure 9A**). The authors propose this setup can act like a "Trojan Horse," which would aid in their uptake in specialized epithelial microfold cells—an important step in generating immunity from orally-administered vaccines. When tested *in vivo*, it was shown that the vaccine platform induced a potent and long-lasting immune response by yielding high levels of mucosal S-IgA and serum IgG antibodies (**Figure 9B**).[180]

As we progress and the examples become more complex, a critical concept in vaccine development is worth introducing. Generally, most vaccines promote strong antibody responses with the expectation that these antibodies will coat the pathogen, render it unable to infect cells, and help the rest of the immune system break it down.[182] This is a Th2 or humoral response and is strongly promoted by the adjuvant alum.[183] However, for intracellular pathogens like tuberculosis or cancers, the ideal is to use a type of T-cell called a cytotoxic T-cell to surveil the host's cells and kill the infected ones.[184] This is called a Th1 or cellular response; different types of adjuvants promote this. A Th1 response still produces IgG antibodies, albeit slightly different in structure and function. There are other types of responses; for instance, responses against pathogens that infect the mucosal membrane prefer a Th17 response.[185,186] To better understand the type of immune response, cytokines are measured, and the ones discussed in the following sections are summarized in **Table 1**. Cytokines are signaling proteins released by cells that act as messengers between different parts of the immune system. For a more detailed explanation about the types and functions of cytokines, the authors direct the reader to several reviews.[187-189]

Table 1: The following cytokines are frequently measured in these experiments to assess the type of immune response. This is not an exhaustive list.

TNF-α	Tumor necrosis factor; secreted primarily by macrophages directing a pro-inflammatory response. Typically associated with Th1 responses.[190,191]

IL-2	Interleukin-2; stimulates naïve CD4+ T-cells and promotes Th1 and Th2 biased immune response while it impedes Th17 biased response[192]
IFN-γ	Class II interferon; secreted by T-cells and NK cells directing a pro-inflammatory Th1 response[193]
IL-17	Interleukin-17; pro-inflammatory cytokine indicative of a Th17 immune response. Indispensable in mediating inflammatory and autoimmune responses, particularly in the defense against extracellular bacteria and fungi [194,195]
IL-4	Interleukin-4; produced by Th2 T-cells and regulates humoral adaptive immunity[196]
IL-6	Interleukin-6; cytokine supporting growth of B-cells and promoting a Th2 biased immune response, as well as a marker for inflammation[197,198]
IL-1β	Interleukin-1β; produced by macrophages and monocytes and some DCs, observed in Th17 biased response. pro-inflammatory mediator, instrumental in fever induction and inflammation.[199,200]

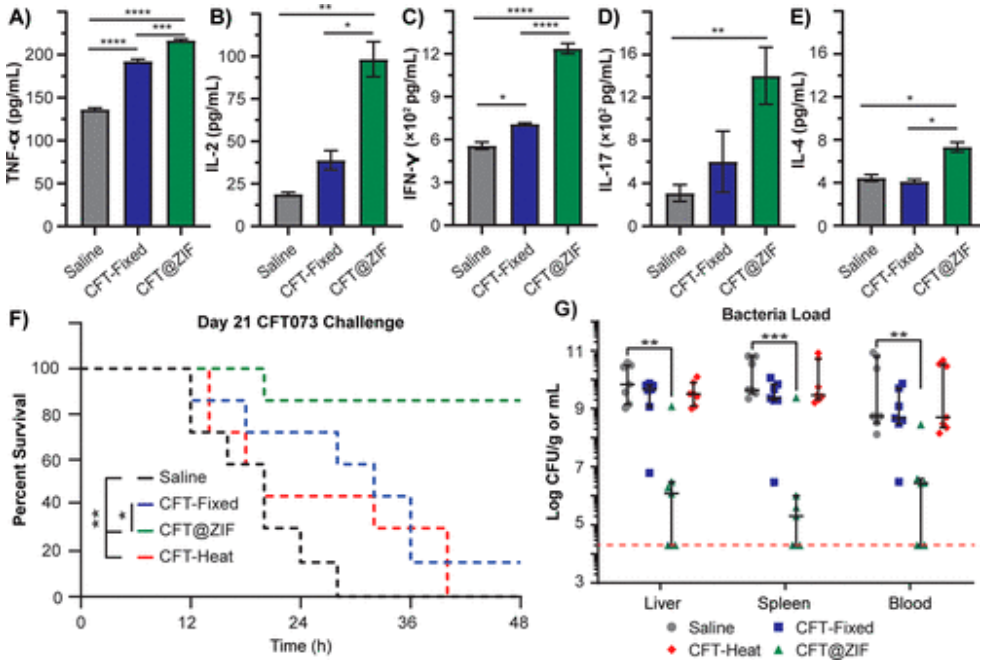

Figure 10: *In vivo* studies with uropathogenic *E. coli* encapsulated in ZIF. Mice were injected with CFT-Fixed or CFT@ZIF. On day 42, splenocytes were collected from immunized mice and incubated with CFT073. After 48 h, the supernatant was tested for (A) TNF-α, (B) IL-2, (C) IFN-γ, (D) IL-17, and (E) IL-4. Two cohorts of mice post-vaccination and boosters were injected intraperitoneally with a lethal dose of CFT073 at day 21 and monitored for 48 h. (F) Survival for each group over the course of 48 h. (G) Bacterial loads in the liver, spleen, and blood at the end point of the survival study.

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As discussed briefly in the introduction, vaccine formulations that depend on whole-inactivated pathogens have historically underperformed. This is unfortunate because many organisms—bacteria in particular—have so many antigens that rationally choosing the ones that will produce a strong and protective vaccine will ultimately be very labor intensive.[201] Using an intact organism with all its potential antigens seems to be the solution, but that has not worked well previously. One issue is that the inactivation procedure, which is necessary to kill the pathogen, so it does not cause disease itself, makes the material much less immunogenic.[202] Current inactivation methods often involve chemically altering surface antigens by crosslinking with formaldehyde or treatment with phenol, followed by lyophilization, lysis under alkaline conditions, or high heat.[203] These processes cause loss of tertiary structure and chemical changes in protein side chains[204] that may negatively impact immune memory by limiting antigen processing and presentation by T-cells,[205] enhancing antigen proteolytic degradation,[206] producing antibodies such as IgG with lower affinities, and increasing Th2 skewed T-cell responses.[207] We consider this area especially attractive for biomimetic mineralization-based preparation methods, as chemically modifying antigens or thermally treating bacteria is likely the cause of the routine underperforming of vaccines based on inactivated pathogens.[208,209] In summary, the gentle encapsulation retains antigen structural features important for generating an effective immune response with the additional benefits of stabilizing and providing a depot for the antigen.

We utilized a biomimetic mineralization approach to create a vaccine to inhibit sepsis, a deadly complication from recurrent or untreated bacterial infection. Uropathogenic *E. coli* (CFT073) was biomimetically mineralized in ZIF-8 (CFT@ZIF) and was tested for its effectiveness against more traditional forms of inactivation – heat-mediated inactivation (CFT-Heat) and formalin fixation (CFT-Fixed).[130] The process of encapsulating the bacteria in ZIF also killed them, thanks to the strongly bactericidal properties of zinc.[210,211] We confirmed that the encapsulation process protected the surface antigens while the "traditional" methods to inactive the bacteria resulted in the degradation of these same antigens. Upon challenge, not only did restimulated splenocytes of the CFT@ZIF group have significantly higher levels of TNF-α, IL-2, IFN-γ, IL-17, and IL-4 (**Figure 10A-E**), the bacterial load in the liver, spleen, and blood of mice from the ZIF-8 group was several orders of magnitude lower than the other groups (**Figure 10G**). The results shown by the cytokines and bacterial load reflected in the survivability of the mice, as the CFT@ZIF group far outperformed the CFT-Heat and CFT-Fixed groups (**Figure 10F**). We attributed the impressive results to the depot effect from the ZIF coating that promotes a stronger immune response because more B-cells can produce higher-affinity antibodies thanks to extended antigen presentation to the germinal center (GC). The traditional formulas

cleared the injection site faster, reducing presentation time and producing B-cells with less opportunity to mature. Additionally, the preservation of antigen by the MOF means the antigen being presented is closer to its native form, and the antibodies created match native bacteria better. A similar approach was recently published and adapted to develop a VLP-based vaccine for foot-and-mouth disease.[212] The VLP generated from the disease-causing virus was used to present antigens, while the ZIF-8 exterior's adjuvanting behavior boosted APC activation.

5. Protection and delivery of biomaterials via post-synthetic loading in MOFs

The previous section detailed growing MOFs on biomacromolecules, which is useful because this process occurs regardless of charge and molecular size, and tolerates many different buffers; however, a relatively limited number of MOFs will "biomimetically mineralize" on biomolecules. MOFs and COFs possess highly ordered porous surfaces with a large surface area. Small molecules have a long history of being effectively embedded into crystalline material by diffusion through the pores. Loading porous materials after they are synthesized (post-synthetic loading) is a versatile tool for smaller biomolecules since the MOF can be synthesized under relatively harsh conditions, rinsed to remove residual solvent, and then loaded by allowing antigens to diffuse through the pores. No examples of antigens loaded into the pores of MOFs have been reported, though it is clearly possible. Farha and coworkers have shown that nanosized NU-1003 can immobilize the enzyme organophosphorus acid anhydrolase (OPAA) through a simple co-incubation procedure.[213] These same authors have also shown that the enzyme is protected at elevated temperatures and in the presence of organic solvent tetrahydrofuran and urea.[214] Future research could extend this approach to antigens with similar size and charge to OPAA.

Another option is to physisorb antigens onto the outer surface of the MOF (**Figure 4D**), similar to how antigens are currently adsorbed onto the surface of crystalline alum in clinically used vaccines. Unlike alum, MOFs have a deep bench of crystal engineering techniques that allow them to be made in different sizes and morphologies. Different surface chemistries permit stronger or weaker binding, changing how the antigens are presented to the APCs. One possible downside is that MOFs ubiquitously have positively charged surfaces owing to exposed metal centers, so antigens that become cationic at physiological pH may not electrostatically bind well.

Hsing-Wen Sung's group beautifully demonstrated this concept by creating a spiky Al-MOF with OVA physisorbed onto the surface post-synthetically (**Figure 11A-B**). In this work, they synthesized a MOF of Al^{3+} and 2-aminoterephthalic acid, with tunable spike-like nanostructures on their surface. It was proven that these pollen-mimetic MOFs with tunable nanospikes have greater cellular attachment with faster and enhanced

phagocytosis in cells (**Figure 11C**), eventually resulting in a greater expression of pro-inflammatory cytokines. Overall, the authors have successfully shown that the system was able to promote and improve antigen-specific humoral immunity in vaccination through the quantification of OVA-specific IgG, IL-1β and IL-6 (**Figure 11D-G**).[215] Yanxin Qi and group incubated amine-functionalized Zr-based UiO-66 (UiO-AM) with OVA to make UiO-OVA.[216] In this case, the amine group helped to activate the complement system, which is a largely biochemical system works with the immune system to destroy foreign materials in the blood. This leads to an enhanced antigen-mediated immune response indicating the UiO-OVA system to be an effective immunomodulatory agent and a nano vaccine.

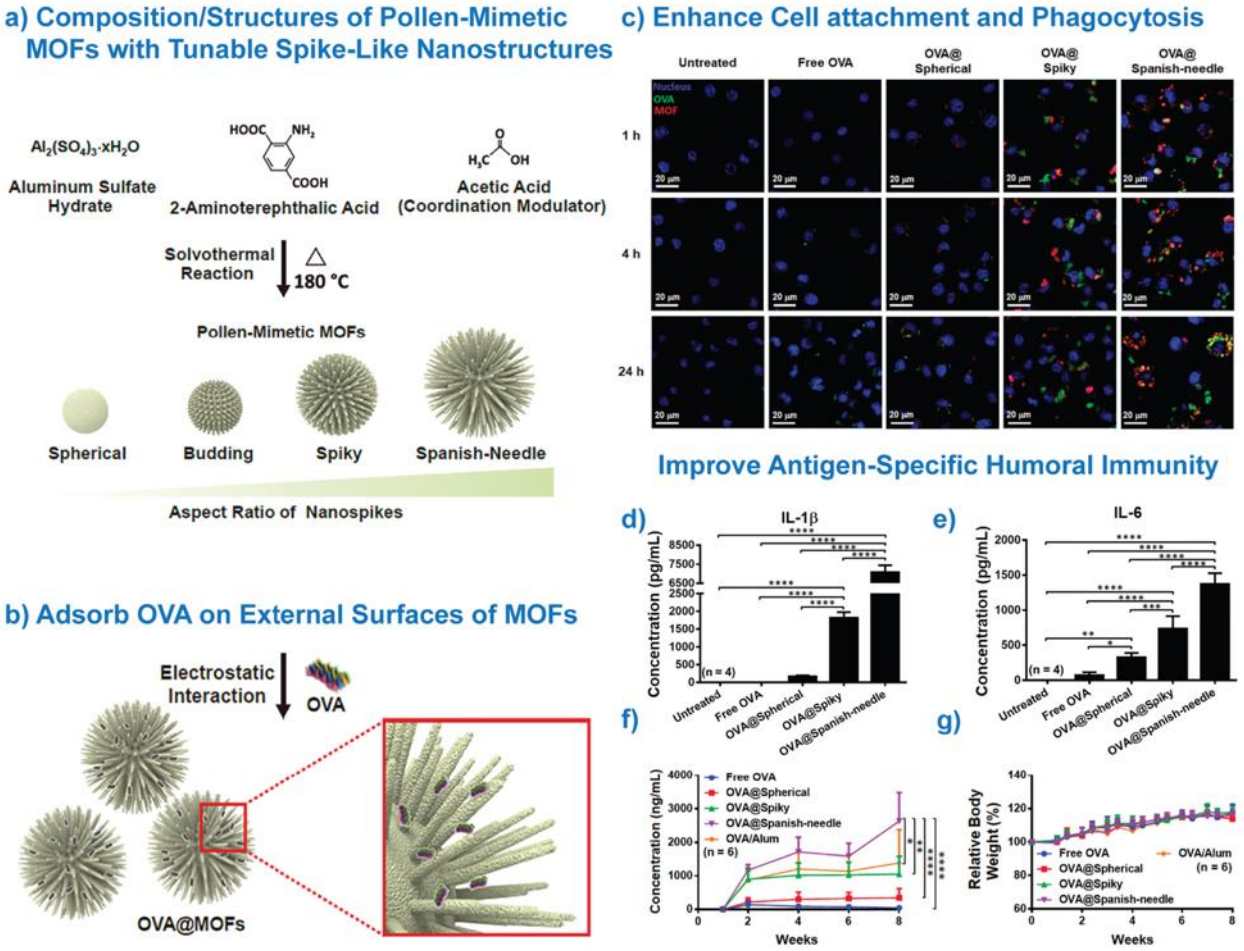

Figure 11: The composition and structures of pollen-mimetic MOFs designed to carry a model antigen (OVA) with tunable spike-like nanostructures along with the *in vivo* mechanism of these MOFs in vaccination. (A) Synthesis scheme of pollen-mimetic MOFs with tunable aspect ratios of nanospikes via a solvothermal reaction of aluminum sulfate hydrate, 2-aminoterephthalic acid, and acetic acid at 180 °C. (B) Post-synthetic loading of OVA onto the MOF surface via electrostatic interactions. (C) Spike-like MOF nanostructures physically interact with cell membranes, boosting cell attachment and

phagocytosis compared to other MOF shapes. Levels of (D) IL-1β and (E) IL-6 cytokines produced from J774A.1 cells treated with cell media, free OVA, and various shapes of OVA@MOFs for 24 h. (F) OVA-specific IgG in serum at predetermined times upon subcutaneous injection of free OVA, OVA@MOFs, and OVA/alum in mice. (G) Relative body weights after immunizations. Reprinted with permission from Chen *et al*.[215] Copyright © 2021, American Chemical Society.

In addition to antigens, adjuvants for generating immune responses such as CpG have been post-synthetically adsorbed to the surface of multiple MOFs, including Zr-based UiO-66 and iron-based MIL-101. Loading CpG onto MOF surfaces may have some benefits, including better cellular uptake. CpG-coated nano MOFs have shown they are rapidly uptaken by cells. In work by Zhang *et al*., CpG-coated MIL-101(Fe) is efficiently taken in by RAW264.7 cells compared to free CpG. The nanoconjugates were able to efficiently deliver the CpG to the endosomes of APCs, where it could interact with a CpG-sensing receptor called Toll-Like Receptor (TLR)-9, which induces an increased secretion of proinflammatory cytokines such as TNF-α and IL-6 as shown in **Figure 12**.[217]

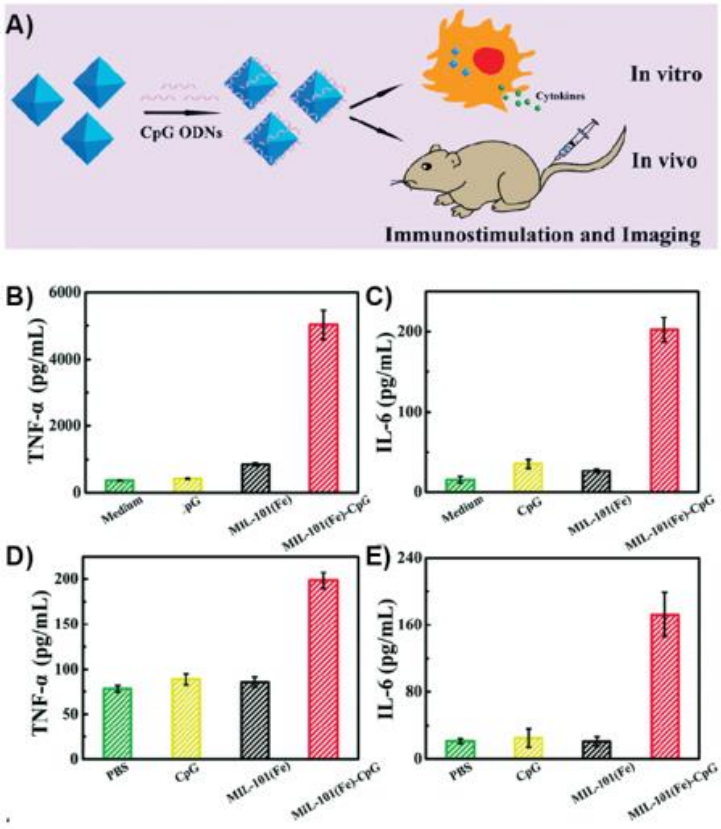

Figure 12: Immune response studies of CpG-coated MIL-101(Fe). (A) Schematic illustration of MIL-101-(Fe)-CpG synthesis and its use in immunostimulation and magnetic resonance imaging. (B) The secretion of TNF- α, and (C) IL-6 *in vitro* from cells stimulated by cell media, CpG, MIL-101-(Fe), and MIL-101-(Fe)-CpG in RAW 264.7. The secretion of (D) TNF-Iand (E) IL-6 in mice serum after stimulation with PBS, CpG, MIL-101-(Fe),

and MIL-101-(Fe)-CpG, respectively. Reprinted with permission from Royal Chemical Society.[217]

Surface loading of both antigen and adjuvant can be combined to elicit a potent cytotoxic T-cell-based cellular immune response. Yang *et al*. utilized[179] MIL-101-Fe-NH_2 to conjugate OVA to the surface via disulfide bonds using a thiol-disulfide exchange (**Figure 4C**). They adjuvanted their system with CpG, which was co-loaded into the positively charged MOF surface through electrostatic interactions (**Figure 4D**) as a stimulatory adjuvant (MOF-S-S-OVA@CpG). When delivered into cells, OVA was expected to be released into the cytosol of APCs thanks to the high concentration of the biological reductant glutathione. The hypothesis was that as cytoplasm contains higher levels of glutathione than the extracellular environment, disulfide bonds will be reduced and broken down by glutathione in the APCs. It is unclear how antigen presentation would be affected as a result of the antigen being released into the cytosol, as antigen presentation by APCs is a complex process that is not fully explained. Overall, this study showed that MOF-S-S-OVA and MOF-S-S-OVA@CpG induced a higher level of IFN-γ and TNF-α cytokines, indicating that chemically linked OVA could initiate stronger cellular immunity. MOF-S-S-OVA@CpG demonstrated the highest levels of IFN-γ and TNF-α, which could fight against viral infections. In addition, it also showed increased levels of IL-4 and IL-10, suggesting its potential to enhance both cellular and humoral immunity. The proportions of the memory T-cell in the splenocytes of immunized mice analyzed by the flow cytometry shows that the MOF-S-S-OVA@CpG group induced higher frequencies of effector memory T-cells, particularly CD8+ T-cells, compared to soluble OVA, a mixture of OVA with CpG, or a mixture of OVA with MOFs test groups. This suggests that the reductive-responsive co-delivery system can induce a potent immune memory response by aiding the immune system to fight off reinfections.

6. Protection and delivery of biomaterials via post-synthetic loading in COFs

COFs are organic polymeric materials that exhibit porosity and are constructed by linking organic units through labile covalent bonds. These bonds are labile by design, as COF synthesis is an inherently error-correcting process where bonds are broken and reformed—releasing and capturing water in the process—until the lowest energy structure is formed.[218] The absence of metal ions and the fact that COFs are primarily composed of C, H, B, N, and O, most reported COFs in biomedical applications have proven more biocompatible than MOFs.[219]

Designing and synthesis of porous materials with large and long-lasting pores has been a significant uphill battle.[220] Reasons for this are poor solubility of large organic molecules needed to create large pores and the tendency of the pores to collapse or the structure to be damaged during the solvent removal step.[221-223] That said, new advances in

supramolecular design have allowed larger pore COFs to be made and characterized. Diwakara *et al*. successfully synthesized a novel large pore 2D COF called PyCOFamide, which has a pore size larger than 6 nm in diameter, as observed experimentally. PyCOFamide's ability to maintain the stability of its structure after activation is attributed to the interlayer hydrogen bonding. This was proven by showing that the monomers that cannot form hydrogen bonds fail to produce crystalline COFs. This represents one of the largest pore sizes ever reported for a 2D COF. Further, they have shown that PyCOFamide can successfully encapsulate fluorescent proteins such as Superfolder green fluorescent protein (sGFP) and mNeonGreen (mNG) within their pores (**Figure 13**).[124] Mu *et al*. conducted a study in which they synthesized a series of COFs with record pore aperture values ranging from 7.7–10 nm by designing building blocks with large conformational rigidness, planarity, and suitable local polarity. All the resulting COFs have proved to possess high stability, permanent porosity, and high crystallinity. Further, the researchers also loaded tyrosinase (5.5×5.5×5.6 nm^3) into the COFs and demonstrated its protection from heat-induced denaturation while catalyzing the oxidation of paracetamol.[224] Therefore, these COFs hold great promise to act as vaccine delivery vehicles and be used in immunological applications within biomedicine.

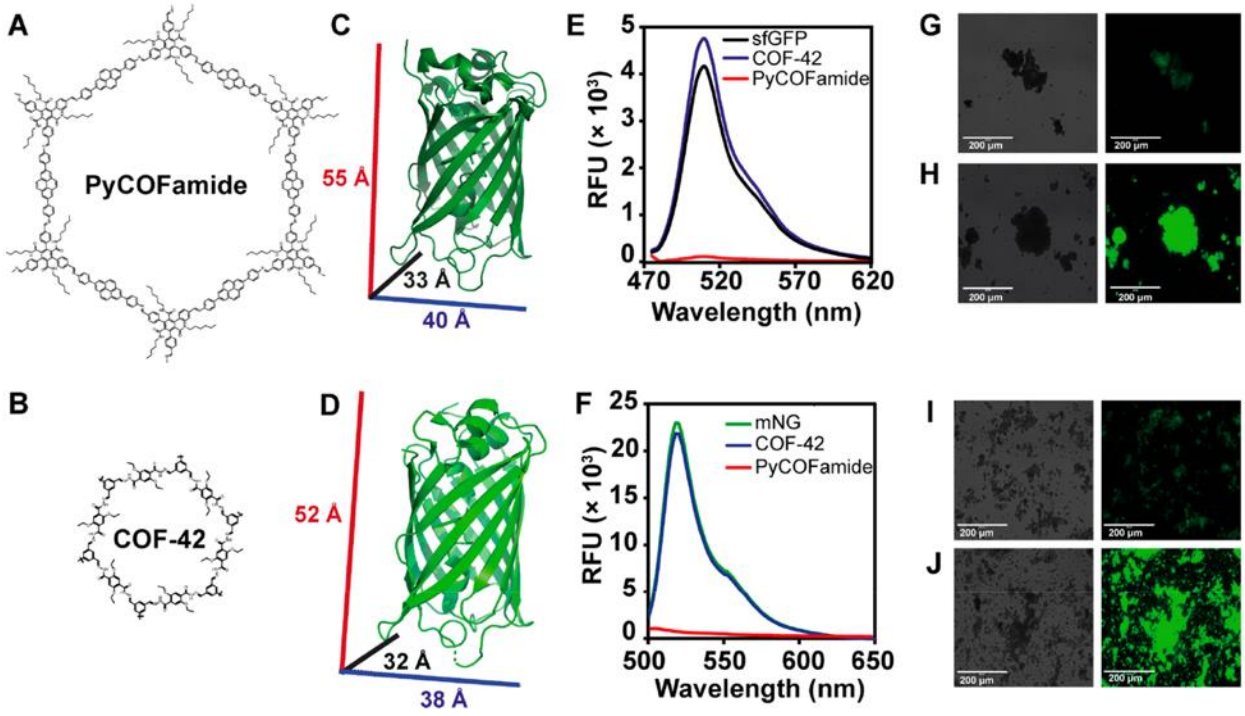

Figure 13: Structures of2D COFs and encapsulation of fluorescent proteins inside them. (A) PyCOFamide with >6 nm pore diameter and (B) COF-42 with 2.3 nm pore diameter. Dimensions of (C) sfGFP and mNG fluorescent proteins. Fluorescence sItra of (E) sfGFP and (F) mNG loaded PyCOFamide and COF-42 compared to the pristine proteins. Epifluorescence microscopic images of (G) sfGFP@COF42 and (H)

sfGFP@PyCOFamide. (I) mNG@COF-42 and (J) mNG@PyCOFamide solid formulations. Reprinted with permission from Diwakara *et al*.[124] Copyright © 2022, American Chemical Society.

While COFs have not been used to deliver antigens per se, we highlight an instance where COFs were used to adjuvant an immune response in a putative approach to photothermal cancer therapy. Zhang *et al.* synthesized a series of multienzyme-mimicking

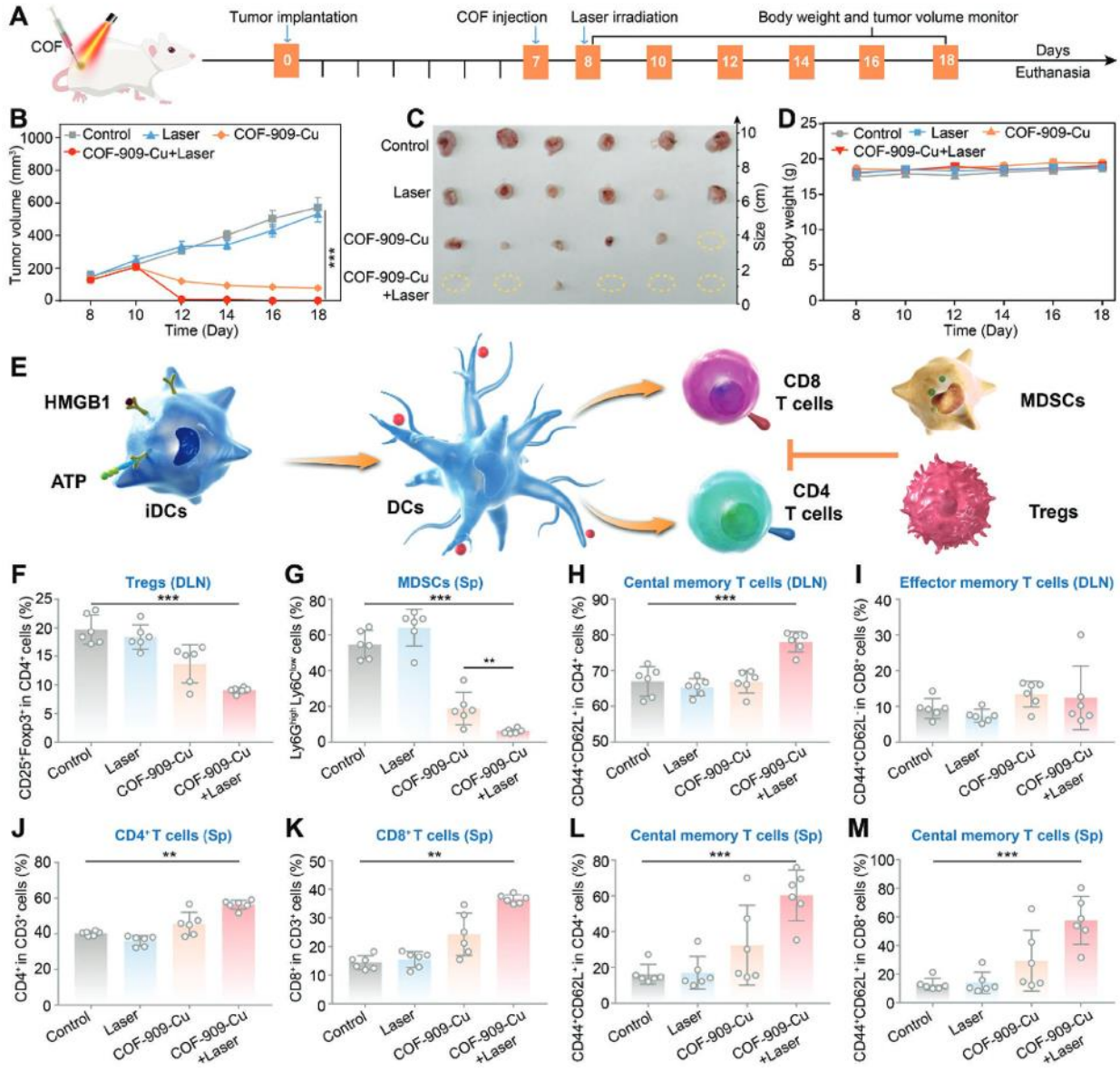

Figure 14. Incorporation of metals in COF-909 for photothermal therapy. (A) COF-909-Cu treatment schedule for antitumor immunotherapy. (B) Tumor volume, (C) tumor size, and (D) body weight of 4T1 tumor-bearing mice subjected to differentIeatments. (E) *In vivo* treatment mechanism of multienzyme-mimicking COF promoting DC maturation and remodeling the immunosuppressive tumor microenvironment. Quantification of (F)

regulatory T-cells ($CD4^+CD25^+FoxP3^+$) in the draining lymph node and (G) myeloid-derived suppressor cells ($Ly6G^{high}Ly6C^{low}$) in the spleen. (H-M) Number of central and effector memory T-cells of CD4+ and CD8+ T-cell subtypes in the lymph node and spleen. Reprinted with permission from John Wiley and Sons.[225]

COFs by incorporating metal ions such as Cu^{2+}, Fe^{3+}, and Ni^{2+} into the COF-909 backbone. This was done to fine-tune the photo-lifetime and light absorption properties to achieve excellent enzyme-mimicking catalytic performance. These catalytic properties include superoxide dismutase (SOD), peroxidase (POD), and glutathione peroxidase (GPx) activities. By testing these COF-metal complexes, it has been demonstrated that COF-909-Cu is an effective inducer of pyroptosis and can potentially enhance cancer immunotherapy.[225] This is the first example of a COF-based pyroptosis inducer holding great promise for a new generation of pyroptosis inducers to boost cancer immunotherapy (**Figure 14A**). They showed that COF-909-Cu heated by laser irradiation (808 nm) could eliminate almost all the tumors in 4T1-tumor-bearing mice models *in vivo* without exhibiting any abnormalities in spleen size (**Figure 14B-D**). The authors have demonstrated that by applying COF-909-Cu along with the laser, DC maturation reduces the proportion of myeloid-derived suppressor cells and regulatory T-cells (**Figure 14E**). These findings suggest that this approach effectively remodeled the tumor microenvironment, leading to an antitumor immune response. Further, they have shown that this system can enhance the immune memory effect of T-cells by assessing the proportion of central and effector memory T-cells using activation markers (**Figure 14F-M**). Although this study is not specifically focused on vaccines, the use of COFs is a relatively new field, and this research has the potential to provide a strong fundamental basis for vaccine development efforts with all the immunological data assessed.

7. Limitations and unrealized advantages of using reticular platforms

MOFs' disadvantages exist, though it is unclear how relevant those concerns are within the context of vaccine systems. Most skepticism for MOFs comes from metal toxicity concerns, which, further down the road, could also cause alarm in the general public.[226] This toxicity is aided by the ability of MOFs to escape the endo/lysosome, which enables cytosolic delivery of their metals.[154] It is important to understand, however, that the FDA-approved adjuvant alum is more cytotoxic than ZIF-8 that the same approximate dose used to vaccinate mice would be used in humans, which are comparatively gigantic.[227] The material needed is typically only 10-50 µg of antigen, meaning the MOF needed is typically well below a milligram. Further, vaccines are not injected into the blood, and most of their degradation occurs at the injection site. Nevertheless, metals can play a distinct role in immunology. Specifically, calcium, zinc, manganese, iron, aluminum, and potassium are all known to modulate the immune system, and several metals, including platinum and arsenic, are suspected to modulate the immune system, though it is unclear

how.[228] In this regard, MOFs' endo/lysosomal escape nature is useful, as they can deliver these metals inside the cell. As such, there are significant opportunities to utilize MOFs in immunology, where relatively small doses can be administered in a safe therapeutic window. Still, efforts have been directed toward developing biomimetic mineralization techniques using metals the body has a higher tolerance for, like iron.[172,229] Likewise for ligands, choosing endogenous ligands will help make the dissociation of MOFs inside biological systems more friendly to cells. Forgan and coworkers demonstrated this using a MOF made with zirconium and fumarate, a linker present in the Kreb's cycle.[230] Another under-explored approach is using mixed-metal MOFs since each metal is metabolized differently in the body, for example iron and nickel.[231]

COFs are also a promising alternative whose implementation can facilitate utilizing the benefits of external protection of biomacromolecules while being metal-free. Typically, the COFs must be made beforehand, and the biomolecules are added later using a post-synthetic loading approach. This is because many COFs require organic solvents for synthesis, which makes them unfit candidates for one-pot synthesis with native protein antigens, which denature in polar organic solvents. This strategy is more intricate and costly than one-pot synthesis, which restricts these complexes' mass production and clinical applications.[232] Recent work by Gao *et al.* does pave the way for the future; they developed a mechanochemical strategy for *de novo* encapsulation of an enzyme inside the COF TpPa-1, which also stabilizes the enzyme against acid, heat, or denaturing agents.[121] Further work will still be required to develop a library of structurally diverse, highly customizable COFs with suitable chemical and morphological properties to immobilize a variety of biomacromolecules, including protein drugs, antibodies, and other biomacromolecules. Also, COFs being primarily hydrophobic materials, often suffer from poor solubility in aqueous media. To overcome this issue, researchers must develop methods such as surface modifications to improve solubility and dispersion of COFs in water or biological media. Potential intercalation of by-products is another common issue with COF formulations. These by-products may leach into the surrounding environment, potentially impacting the desired therapeutic outcome. Developing robust purification methods and quality control measures are essential to minimize the risk of by-product intercalation ensuring the safety and efficacy of COF-based therapeutic formulations. While much is still unknown about COFs—particularly how they degrade *in vivo*—COFs nevertheless show promise as vaccine platforms and more research is needed to evaluate their potential and address the above-mentioned limitations fully.

As mentioned earlier, in most cases, MOFs and COFs are rigid, crystalline solids that are not always colloidally stable in aqueous systems for prolonged periods, although methods to overcome this are promising.[95] Still, several groups have made efforts to improve these nanocarrier systems. For instance, Morris *et al.* explores an array of variables to

investigate what factors affect the colloidal stability of UiO-66.[233] They have synthesized nanoscale UiO-66 with a series of carboxylic modulators, R-COOH (R= H, CH3, CF3, and CHCl2). The stability of the synthesized MOFs in colloidal form was evaluated and results showed that the colloidal stability depended on the conditions of the modulators used during synthesis. Specifically, MOFs produced with modulators having lower pKa values and higher acid concentrations were found to be more colloidal stable. This was attributed to the replacement of terephthalic acid ligands by modulator molecules, ultimately enhancing the colloidal stability of UiO-66 nanoparticles. Their other conclusion is that stability improved with a drop in size — a phenomenon observed in other MOFs as well. However, controlling size beyond a certain point is not possible when the goal is to use these systems to trap and protect biomaterials that can range from a few nanometers in size to a whole micron.[233] A similar school of thought suggests that a protein corona formed from soluble proteins could improve the colloidal stability of these nanoparticles; however, that could prove to be a double-edged sword as it could affect uptake and toxicity in unpredictable ways and may also bear a closer resemblance to a pathogen activating the complement system.[234]The poor colloidal stability of MOFs and COFs in aqueous systems is also a significant concern. It would be very unlikely that an unstable colloid would be translated as a therapeutic as precipitation issues would prohibit exact knowledge of dosing.

8. Future direction: how research can be streamlined towards building more sophisticated systems

The existing research for MOF- and COF-based vaccines is quite expansive for model antigens like OVA or fluorescent proteins. However, there is a need for more work on utilizing antigens that build immunity against infectious diseases. Going beyond proof-of-concept research and demonstrating the difference in immunogenicity with and without protection from the framework is the direction this field is expected to take. Rigorous challenge experiments in animal models can help make a strong argument to push these reticular frameworks closer to clinical trials. This work—particularly the *in vivo* work—should be generally repeated in multiple independent trials, which fails to happen too frequently in the literature presently.

ZIF-8's lability and ease of degradation in the presence of albumin and phosphates may or may not be ideal for all vaccine applications. There is a piqued interest in exploring different MOFs and COFs that can serve a similar function to achieve a variety of pharmacokinetic profiles.[173, 235] While there is still plenty of scope for exploration in this area, some crucial groundwork has certainly been done to lay the foundation for future studies.[236] Mónica Giménez-Marqués' group has demonstrated the encapsulation of a wide array of proteins across different isoelectric points in the iron MOF MIL-100.[237] The comparative exploration of different MOFs and COFs is valuable, even from an

immunological standpoint. Although traditionally, MOFs have been assumed to be immunologically inert, the immunogenicity of certain metals and ligands can indeed impact the final delivery system. Hidalgo *et al.* compared the immunological profiles of Fe^{3+}-, Al^{3+}-, and Zn^{2+}-based nano-sized MOFs, which included extensive cytokine panels and studying their ROS production.[1] While this is a great start, the body of literature available which can help us understand the interaction of the MOF or COF itself with then immune system is still sorely lacking. We hope to see the community include MOF-only and COF-only controls in future papers on complex delivery systems, as it will help build a repository of knowledge that can help us predict the immunological behaviors of these materials depending on their size and mode of administration. Ultimately, with sufficient interest and research, the literature will help guide future researchers in choosing MOFs with different metals (or COFs for no metal at all) for different possible adjuvanting properties, which could help eliminate the use of highly reactogenic external adjuvants.

Given the rise of popularity in lipid nanoparticle vaccine technology post-COVID, we expect even greater interest in lipid-based vaccines for more diseases. Plagued by cold chain issues, these delicate formulations could use the robust exterior of MOFs to make their transport affordable. While the work of Herbert *et al.* lays a solid foundation by demonstrating proteoliposome encapsulation,[129] there still exists a need for follow-up research with clinically relevant lipid nanoparticles.

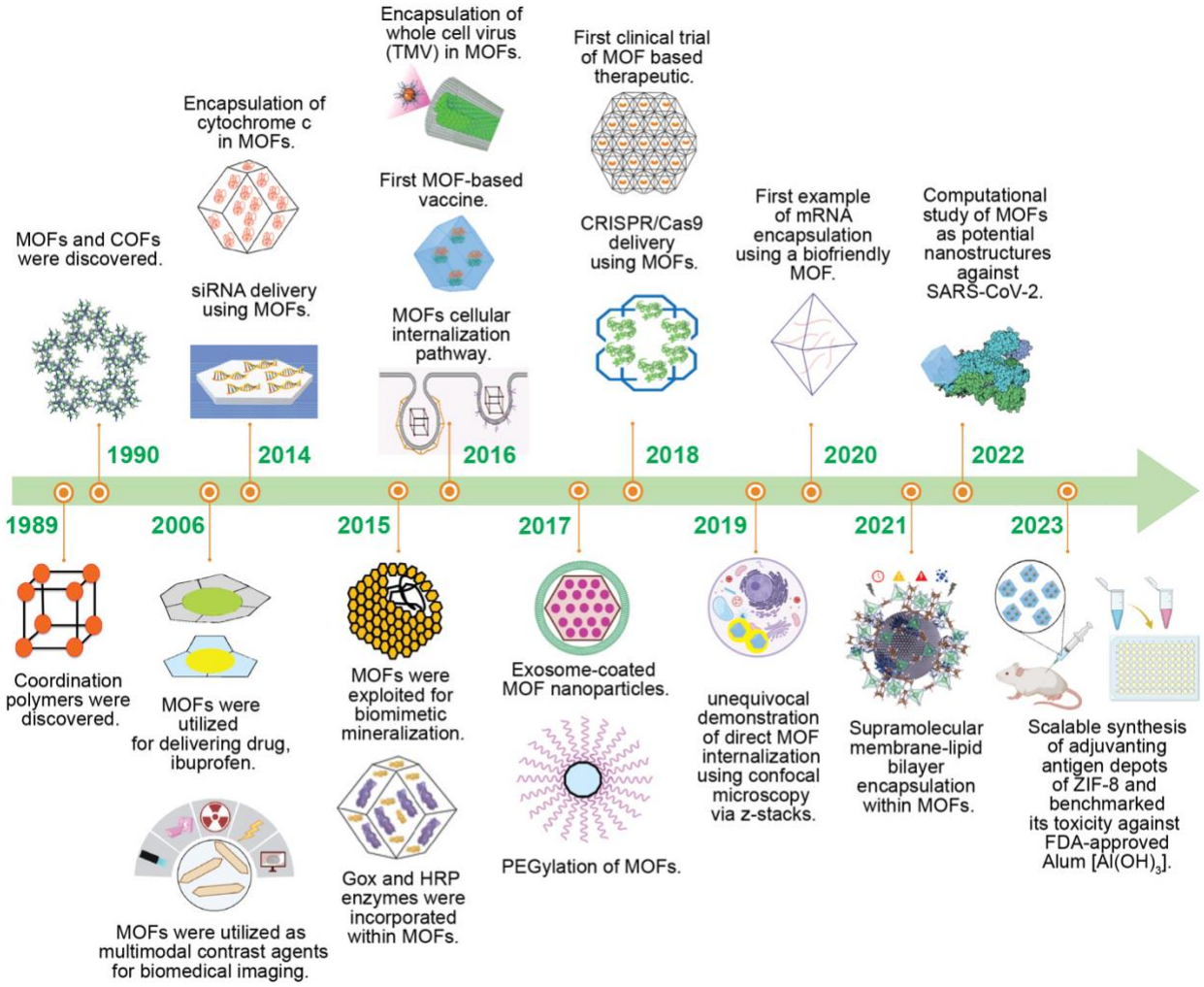

Figure 15. A timeline illustrating the significant achievements in MOFs regarding drug and vaccine delivery and diagnosis applications. Recreated with permission from Nisha Tyagi *et al*.[238] Copyright © 2023, Springer Nature Limited.

One of the more crucial factors to consider while developing MOF-based systems for clinical application is their biocompatibility. While there are several FDA-approved metal-based therapies for cancer—often used as a last resort—there is still considerable skepticism surrounding the use of metals in therapeutics aimed at healthy individuals. Patricia Horcajada's group pioneered these efforts — they studied the *in vivo* toxicity of three iron-based MOFs in mice.[239] Abramenko *et al.* and Ruyra *et al.* investigate the toxicity of nano-sized MOFs in embryo and adult zebrafish models.[240, 241] Despite being so thoroughly investigated on the application front, ZIF-8 still has a disproportionately small body of work concerning biocompatibility studies. Kumari *et al.* recently investigated the dose-dependent toxicity of ZIF-8 when administered intranasally, ensuring that doses up to 1 mg per mouse did not cause any significant change in their lung diffusing capacity

and serum protein/enzyme levels.[171] Extensive experimentation on larger mammals like dogs, monkeys, and pigs will also supplement future findings done on murine models. It will serve as necessary evidence for furthering MOFs in clinical trials. A detailed discussion of biocompatibility and toxicity of MOFs is beyond the discussion of this perspective, and readers are encouraged to refer to a comprehensive review on the topic published by Ettlinger *et al.*[242] and our recently published commentary on the clinical translation of MOFs (**Figure 15**). These sources are worth visiting and provide a more detailed perspective on the potential future of MOFs in clinical applications.

9. Conclusion

This review touches upon the tools MOFs and COFs provide us to deal with vaccine technology issues. These frameworks act as a protective coating around fragile biomaterials, help provide thermal and mechanical stability and a slow-release system. There are two main ways to develop these composites — *in situ* biomimetic mineralization of the biomaterial and either loading the material inside the pores or onto the surface of the already-synthesized MOF or COF. While the first technique helps us expand on the range of biomaterials that can be protected, the latter helps us exploit a range of protecting frameworks as it takes harsh synthetic conditions out of the equation. Composite systems have also utilized both strategies simultaneously when beneficial, especially for co-loading antigens and adjuvants on the same system. While reticular frameworks are undeniably beneficial and can address many problems we currently face, this field of research is still young, and more work and more hands are needed to bring these materials into the clinic. With that said, it is very promising to see that research in this area has also caught on broadly, and efforts to perform extensive biocompatibility studies and work on building safer MOF and COF formulations have already begun. We expect to see a sharp rise in not only such efforts but the demonstration of vaccine formulations that can be delivered in a painless, needle-free manner, and prevent an array of infectious diseases, rapidly expanding our knowledge repository in the future.

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Biographies

Prof. Jeremiah J. Gassensmith

In 2003, Jeremiah Gassensmith earned his BSc degree under the tutelage of Prof. Joseph Gajewski at Indiana University in Bloomington and his PhD from Prof. Bradley D Smith at the University of Notre Dame. After his PhD, he traveled to Northwestern University and studied under Prof. Sir J. Fraser Stoddart (Nobel Laureate, 2016). He joined the faculty of the University of Texas at Dallas in August 2013 to carry out independent research and is now an Associate Professor. His research group pioneered using MOFs as biodegradable and biocompatible nanomaterials that thermally protect proteins and lipid nanoparticles and inhibit antigen degradation by nuclease degradation. The material slowly dissolves in the injection site releasing the biotherapeutic for many days to weeks. These materials are being “nvestigated to "break the cold chain” in vaccines and as a non-metal-based biolistic delivery vehicle for transdermal delivery of therapeutic agents including DNA, RNA, lipid nanoparticles, and proteinaceous drugs. Jeremiah is a native of Oaklandon, Indiana, and is one of the few Hoosiers that knows how to cook a proper brisket.

Yalini H. Wijesundara

Yalini Wijesundara grew up in Kandy, Sri Lanka, and earned her B.Sc Chemistry (Hons) from the University of Peradeniya. She joined the Department of Chemistry and Biochemistry, University of Texas at Dallas, to pursue her PhD in Biochemistry. Currently, she is working on her research which is mainly focused on reticular frameworks-based biomaterials in drug delivery and vaccine formulations/applications under the guidance of Prof. Jeremiah J. Gassensmith.

Sneha Kumari

Sneha Kumari simultaneously completed her BE in Chemical Engineering (Hons) and MSc Chemistry (Hons) at the Birla Institute of Technology and Science Pilani, Pilani Campus, in 2020. For her Chemistry thesis project, she worked on porphyrin-functionalized gold nanoparticles for photodynamic therapy under the guidance of Prof. Surojit Pande. Sneha is currently pursuing her PhD under the tutelage of Prof. Jeremiah J. Gassensmith, where she is studying the use of zeolitic imidazolate frameworks for biomaterial protection and their syringe-free delivery *in vivo*.

Thomas S. Howlett

Thomas Howlett graduated with a B.S. in Biochemistry from the University of Texas at Dallas in 2018. During his undergraduate education, he worked on metal-organic frameworks under the guidance of Prof. Kenneth Balkus Jr. Currently, he is pursuing a PhD under Prof. Jeremiah Gassensmith, as he continues research on metal-organic frameworks for biomedical applications.

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