Zeolitic Imidazolate Framework Antibody Conjugates in Bioanalytical Applications

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Improvements in bioanalytical technologies are driven by evolving threats and challenges to the global community. These challenges include emerging diseases, the need for substance screening and increasing environmental pollution. Here, we present a new bioanalytical concept that employs nanosized Metal-Organic Framework (MOF) particles as labels for antibody-based analytical methods, including enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA). A strategy for covalently linking antibodies to form stable, colloidal Zeolitic Imidazolate Frameworks (ZIFs) conjugates is described. The proof of principle for their use in LFIA is presented. In particular, ZIF-antibody conjugates displayed excellent behaviour in dispersion and good mobility via lateral flow on a solid substrate while retaining full selectivity of the antibody. Moreover, protein aggregation of the antibodies was prevented, suggesting protection against degradation in an exoskeleton-like manner. Our study could inspire future work to address global bioanalytical and diagnostic challenges.
Zeolitic Imidazolate Framework Antibody Conjugates in Bioanalytical Applications

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Abstract: Improvements in bioanalytical technologies are driven by evolving threats and challenges to the global community. These challenges include emerging diseases, the need for substance screening and increasing environmental pollution. Here, we present a new bioanalytical concept that employs nanosized Metal-Organic Framework (MOF) particles as labels for antibody-based analytical methods, including enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA). A strategy for covalently linking antibodies to form stable, colloidal Zeolitic Imidazolate Frameworks (ZIFs) conjugates is described. The proof of principle for their use in LFIA is presented. In particular, ZIF-antibody conjugates displayed excellent behaviour in dispersion and good mobility via lateral flow on a solid substrate while retaining full selectivity of the antibody. Moreover, protein aggregation of the antibodies was prevented, suggesting protection against degradation in an exoskeleton-like manner. Our study could inspire future work to address global bioanalytical and diagnostic challenges.  

Introduction  
The confrontation with global infections requires the development of robust, sensitive and selective analytical and bioanalytical tools with good response time. Antibodies can provide the required selectivity in fast analytical approaches. Yet, antibodies, in their natural habitat, are generally protected from external factors, such as ultraviolet radiation, dehydration, and high temperatures. Therefore, the use of antibodies in specifically robust assays require them to be protected by a pseudo-skeleton.\textsuperscript{1}  
Metal organic frameworks (MOFs) have emerged as ideal host materials for a wide range of low molecular weight substances and biomacromolecules. MOFs are crystalline porous compounds based on metal ions or clusters connected by organic ligands, which can be designed into many different
architectures and tailored for specific uses by adjusting their topology, pore size and/or chemical composition.\cite{2, 3}

In recent years, nanoscale MOFs (NMOFs), are being developed for bio-applications as they have much larger effective surface areas than traditional MOFs, rendering them improved chemical stability, more efficient surface modification capabilities and, in consequence, enhanced biological activity of the respective composites. The particle size of MOFs is determined by the nucleation and growth rates in their synthesis; higher nucleation rates result in smaller particles. Therefore, the synthetic conditions for the preparation of NMOFs must favour nucleation over growth.\cite{3} NMOFs have the potential of forming stable colloids in aqueous media required for bioanalytical applications. Colloidal suspensions occupy an intermediate place between solutions and suspensions; a colloidal system can flow like a liquid and therefore can be used in bioanalytical and diagnostic technologies like enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA).\cite{3} See Figure 1.

Figure 1. Nanoparticulate ZIF-90 used in bioanalytical and diagnostic technologies: enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA).

Despite the great advantages of NMOFs, they suffer from weak resistance to moisture or aqueous environment, owing to the facile decomposition of the metal-ligand bonds. Metal-ligand bonds can be significantly strengthened by the use of ligands rich in nitrogen, such as imidazoles, which, on deprotonation with a base, form powerful imidazolate anions.\cite{4} Zeolitic Imidazolate Frameworks (ZIFs) are a sub-family of MOFs formed by a self-assembly approach which consists of M–Im–M units (with e.g. M = Zn$^{2+}$, Co$^{2+}$, and Im = imidazolate linkers).\cite{5} Nanoscale ZIFs (NZIFs) display properties combining the advantages of both zeolites and NMOFs, such as ultrahigh surface areas, unimodal micropores, high crystallinities, abundant functionalities, and exceptional thermal and chemical stabilities.\cite{5}

We are here most interested in those NZIFs that can form colloids and present large cavities connected through small windows, such as ZIF-8\cite{3, 6} and ZIF-90.\cite{7} ZIF-8 chemical structure is [Zn(2-mim)$_2$] where 2-mim is 2-methylimidazolate while ZIF-90 is [Zn(i2ca)$_2$] where i2ca is imidazolate-2-carboxaldehyde. Both
ZIFs present a sodalite type architecture where Si/Al is replaced with tetrahedral Zn(II) in ZIFs and O is replaced by their corresponding imidazolate linkers.[3, 7] ZIF-8[3, 6, 8] and ZIF-90[7] have been demonstrated to have permanent porosity, high thermal stability, and remarkable chemical resistance to boiling water, alkaline aqueous solutions, and organic solvents.[6]

Initial research on the synthesis of ZIF-8 crystals performed in dimethylformamide (DMF) resulted in micrometre-to-millimetre scale crystals.[3] Cravillon et al. found that excess ligand was essential for promoting faster nucleation and stabilizing the initial colloids. The combination of excess ligand, methanol as solvent, and room temperature has since formed the basis for most syntheses of nanoscale ZIF-8 crystals.[9, 10]

Modulators are used in the synthesis of ZIFs as capping ligands. Modulators have two opposing effects on the size of the ZIF particles: i) they can increase the crystal size by competing with the organic linker, reducing the number of nucleation points, and ii) they can decrease the crystal size by limiting crystal growth. Cravillon et al. evaluated the effects of different modulators (formate, 1-methylimidazole, and n-butylamine) in the synthesis of ZIF-8 in methanol, and in case of n-butylamine, attributed the formation of small nanoparticles to the higher basicity of n-butylamine.[11]

Shin et al. observed that more reactive metal precursors such as Zn(NO₃)₂ led to faster nucleation and, consequently, to smaller ZIF-8 particles than using less reactive salts such as Zn(CH₃COO)₂.[8] It has been established, that reaction time, molar ratio, and reagent concentration are critical parameters in tuning the size and homogeneity of the desired colloidal ZIF-8 nanocrystals.[3]

Yaghi et al. demonstrated that the reactive aldehyde functionalities in ZIF-90 were able to react with the primary amino group of ethanolamine forming an imine in high yield and this without altering the original structural integrity. The formation of this new ZIF, termed ZIF-92, allows for chemical transformations to take place in precise and well-defined spatial arrangements. In a similar way, Liu et al. formed an imine with pentafluorobenzylamine leading to synthesis of superhydrophobic ZIF-90. While the ZIF-90 morphology was conserved, pore aperture of the fluorinated ZIF-90 was severely constricted.[12]

Strategies for preserving the functions and conformations of biological macromolecules with MOFs/ZIFs as exoskeletons have been performed by infiltration and encapsulation strategies.[1, 13, 14] Storage of biospecimens in ZIF-8 has been achieved by encapsulation with comparable preservation efficacy to the refrigeration method (freezing liquid samples at −20°C).[13, 15] Recently, a new strategy to form bio-MOFs has evolved, that of post-synthetic modification. In this strategy, a reactive functional site is elicited in the MOF and covalently linked to a reactive functional group in a biomolecule, termed conjugation. In proteins and antibodies such reactive functional groups are amino or carboxyl groups, and thiols from reduced cysteins.

The ability to form a conjugate between a protein, e.g. an antibody, and another entity, e.g. a molecular label, or gold or latex nanoparticles, is key for many applications in the life sciences.[16] For example, such conjugates are critical for diagnostic[17, 18, 19, 20] and therapeutic[21, 22] applications.

The strategy most predominantly used to date for post-synthetic modification of MOFs is the treatment of a carboxylic acid moiety with carbodiimide reagents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to form an activated ester intermediate; this intermediate on
reaction with a primary amine results in the formation of a covalent amide link between the MOF and the protein/antibody.\textsuperscript{[18, 21, 23]} In some cases, the carboxylic acid originates from the residues of glutamic or aspartic acid in the protein/antibody;\textsuperscript{[18]} in other cases it was induced in the MOF.\textsuperscript{[21, 23]} Alternatively, glutaraldehyde has been used as a crosslinker between the amine functionality installed in the MOF and an amine of a bacteriophage.\textsuperscript{[19]} This post-synthetic strategy activating a carboxylic acid with EDC has been widely used to conjugate DNA to MOFs, DNA requiring prior integration of a carboxylic acid.\textsuperscript{[24]} Antibodies have been also immobilized on MOFs via Zn–S bonds to cysteine residues.\textsuperscript{[25]}

Luminescent metal-organic framework (L-MOF) sensor materials based on fluorescence are applied for a wide range of analytical and bioanalytical procedures.\textsuperscript{[1, 26]} Fluorescence is one of the most desirable transduction mechanisms for its relative ease of use with affordable equipment, technical simplicity, and broad adaptability. Constraining analytes into the L-MOF cavity with appropriate pore dimensions can lead to the pre-concentration of the analytes resulting in high sensitivity and selectivity in fluorescence sensing.\textsuperscript{[4]} The fluorescence probe interaction with analytes like phthalates,\textsuperscript{[26]} or antibodies,\textsuperscript{[4, 27]} results in competitive absorption, in which the analytes compete with the luminescence probe material for excitation energy leading to apparent emission quenching. We have recently published a luminescence ZIF application which we referred to as Z8P using colloidal ZIF-8 with a covalently linked fluorescent pyrene tag for the sensitive detection of phthalate plasticizers which can act as endocrine-disrupting chemicals.\textsuperscript{[26]}

The potential of MOFs conjugated to antibodies as bioanalytical probes has been demonstrated.\textsuperscript{[18, 19]} IRMOF-3 conjugated to a bacteriophage as a fluorescence biosensor for the detection of \textit{S. arlettae} showed good selectivity over \textit{S. aureus} and \textit{E. coli},\textsuperscript{[19]} while a fluorescence BioMOF sensor conjugated to an anti-\textit{S. aureus} antibody for the detection of \textit{S. aureus} achieved selectivity over \textit{S. arlettae} and \textit{E. coli}.\textsuperscript{[18]}

The use of antibodies in immunoassays, including ELISA and LFIA, encompasses a broad variety of applications.\textsuperscript{[28, 29, 30]} Immunoassays are antibody-based analytical methods for qualitative or quantitative analysis.\textsuperscript{[31, 32]} Based on specific antigen-antibody interactions, immunoassays are used for diagnosis, pharmacokinetic studies by drug monitoring, environmental analysis,\textsuperscript{[33]} food surveillance,\textsuperscript{[34]} and quality control of commercially available products\textsuperscript{[35, 36, 37]}.

ELISA is a laboratory method carried out on microtiter plates which reaches very high throughput. LFIA is a strip-based platform, well-known from the pregnancy test. It can be used as point-of-care diagnostic assay where the results needed are mostly qualitative (yes/no) or semi-quantitative. It is a fast, portable, easy to use and cheap to produce assay at low cost, with long shelf life requiring no refrigeration for storage. Therefore, LFIA are very well adapted for use in developing countries, small ambulatory care settings, remote regions and battlefields.\textsuperscript{[32, 38]} For these applications, highly specific monoclonal or polyclonal antibodies are required. Many such antibodies are available, offering the opportunity to tune the analytical method towards biological and chemical analytes.\textsuperscript{[39]} Binding of these specific antibodies can be followed, even in complex mixtures\textsuperscript{[40, 41]}, by labels, many of them being particulate matter, often called “beads”. So, there are bead-based ELISAs,\textsuperscript{[42, 43]} and lateral flow immunoassays (LFIA)\textsuperscript{[16, 44, 45, 46, 47]} that employ nanoparticles\textsuperscript{[17, 48]} such as gold,\textsuperscript{[45, 49, 50]} and latex,\textsuperscript{[51]} covalently linked (conjugated) to the antibodies. On the other hand, employment of unconjugated NMOFs has been described.\textsuperscript{[42, 43, 46, 47]}

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The use of conjugated NMOFs bears the potential to expand the application range combining the versatility of structure and functionality of NMOFs with the selectivity and affinity of antibodies in stoichiometric and stable reagents. Nanoparticulate systems that form colloidal solutions are best for these applications since their behaviour might be close to a solute, in order to obtain highly reproducible results (high precision).

We set up clear goals we wanted to achieve when designing the antibody-conjugated MOF for proof of concept. 1) We needed the antibody-conjugated MOF to have good mobility so that it could be used in ELISA and LFIA tests; 2) it had to retain the crystal structure of the MOF while conserving the binding activity of the antibody; 3) The link between MOF and antibody had to be relatively long, flexible and the installed covalent link had to be robust against cleavage by chemical attack, and 4) the nature of the link should be such, that it could be confirmed by LCMS following digestion of the antibody-conjugated MOF.

We selected ZIFs, and specifically nano-ZIFs as they are the ideal MOFs for forming a good conjugate with antibodies, based on the properties previously discussed. For the antibody we selected to study IgG as they are commercially available, inexpensive, and as monoclonals of high purity. A strong and robust linker requires a strong covalent bond between ZIF and the antibody. From the potential 22 amino acids that make up natural proteins such as antibodies, only lysine presented the desired properties: lysine is ubiquitous and one of the more highly represented amino acids in all antibodies, increasing the probabilities of covalently bonding to a ZIF. Lysine has a long and flexible N-butyl chain with a primary amino group at the end. Primary amino groups are ideal functionalities as they are not so prone as acidic groups to ionisation in a normal pH range, which would prevent a conjugation reaction. A strong, flexible and robust link between the antibody and ZIF could be easily achieved if we could form an initial imine between the lysine and an aldehyde installed on the ZIF, like in ZIF-90; this imine could be later reduced to -CH$_2$-NH- obtaining the desired 6-member chain flexible link. We had previously investigated the experimental conditions to form an imine bond between a ZIF-8/ZIF-90 hybrid and a fluorescent probe 1-amino-pyrene that we named Z8P,[26]. Based on this we could directly adopt the experimental conditions for the present research project.

Scheme 1. We show here an overview of the process of covalently binding ZIF nanoparticles with free aldehyde functionality to antibodies. On the left, TEM images of the resulting nanoparticulate ZIF-90 is shown. On the right, a scheme of the procedure to covalently bind this ZIFs to IgG antibodies via Schiff base conjugation (a) and selectively reduce the imine (b) to obtain a flexible alkyl secondary amine linker (c)
Our synthetic strategy to obtain the antibody-conjugated MOF with the desired properties is illustrated in Scheme 1. We therefore propose to use a new post-synthetic modification strategy to form a ZIF-90 bioconjugate with an exemplary antibody (IgG type). We will refer to this conjugate as Z90-IgG.

Using the same post-synthetic modification, we also achieved conjugation of Z8P, our fluorescent, pyrene-tagged ZIF-8/ZIF-90 hybrid, to an IgG antibody. We will refer to this conjugate as Z8P-IgG.

We will demonstrate that nanosized Z90-IgG and Z8P-IgG conserve their colloidal properties making them ideal candidates to be used in ELISA and LFIA. We propose that the fluorescence tag in Z8P-IgG could simplify the enzyme-amplified ELISA to a more robust, enzyme-free assay. Accordingly, the synthesis of these conjugates is briefly outlined, followed by their first application in a non-competitive indirect ELISA and an LFIA.

Results and Discussion

ZIF-90 nanoparticles were synthesized in methanol using sodium methoxide as a modulating agent acting as a base to deprotonate the imidazole-2-carboxaldehyde to the respective imidazolate bridging ligand (i2ca), resulting in the formation of nanoparticles in the size range of 50-150 nm\(^2\)\(^3\), as confirmed by transmission electron microscopy (TEM; Figure S3 and Figure 2 for ZIF-90 and Figure S24 a-c for Z8P)\(^3\) The corresponding powder X-ray diffractograms (PXRD; SI Section 5.4 and Figure S14) are consistent with a sodalite-like crystalline structure (ZIF-8 and ZIF-90).

Figure 2. TEM images of nanoparticulate ZIF-90.

a) and d): The particles show a polydispersity in sizes between 30 to 350 nm, averaging 150 nm.
b) The rhombic dodecahedra characteristic for SOD ZIF systems can be identified on almost all the particles. d) The additional EDS analysis image confirms the presence of Zn atoms within the particle (purple).
We modified a ZIF-8/ZIF-90 hybrid to be fluorescent by including an aminopyrene core. For this, a fluorescent imine is formed by reacting 1-aminopyrene (1-AP) with Imidazol-2-carboxaldehyde (i2ca) via Schiff base condensation. Then the hybrid ZIF is precipitated obtaining the fluorescently modified ZIF (Z8P). The details for the synthesis can be found in the SI Section 4.1.

The ligand-to-metal stoichiometry of each ZIF was confirmed by HPLC (SI Section 5.2, Figure S13) and supported by Zn²⁺ analysis by ICP-OES (SI Section 5.3). Notably, the final composition of Z8P is consistent with 2-mem having been added in excess as required to achieve the desired nanostructure. HPLC analysis supports that most of the imidazole moieties in the structure are i2ca which are those that include the aldehyde functionality. This high share of i2ca in Z8P offers, though unintentionally, suitable binding sites for the antibodies.

The i2ca linker in the ZIF-90 and in Z8P concomitantly increases the hydrophilicity of the ZIF, facilitating its dispersion in aqueous solutions. Following this protocol, the ZIF-IgG conjugates Z90-IgG and Z8P-IgG have been produced.

The structural integrity of the ZIF-IgG conjugates is critical to their viability. PXRD and TEM are consistent with the ZIF structure and particle size being retained upon the formation of both ZIF conjugates (TEM: Figure S24; PXRD: Figure S14). Based on PXRD investigation we could even demonstrate that the crystal structures and corresponding particle sizes of ZIF-IgG conjugate remain intact for up to 120 hours at 4°C (SI Section 8, Figure S22).

Further structural characterization by LCMSMS proved the successful covalent conjugation between the ZIF i2ca moiety and the antibody though this robust link [-CH₂-NH-CH₂]₄⁻. To confirm the formation of this strong link, the ZIF conjugates were acid digested (SI Section 9.2) in order to cleave all amide bonds of the peptide chains in the antibody. If the antibody was successfully covalently bound though a lysine to the ZIF, this cleavage would result in the release of L-lysines covalently bound via an-NH-CH₂-to imidazole-as depicted in Scheme1c.

A compound in agreement with this structure was detected in its free form via liquid chromatography high-resolution mass spectrometry (LC-HRMS) (Figure 3; see Figure S23, and SI-9.2). The compound with a mass-to-charge ratio of 227.1503 was present in both ZIF-IgG conjugates but absent in all experimental controls (individually digested IgG and ZIFs without conjugation). All major MS/MS fragments could be assigned to substructures that explained the putative molecular structure consistently Thus, covalent
bonding of an imidazole carboxaldehyde moiety to an amino acid residue of the antibody is well supported by the present results.

The first important property to be conserved during conjugation of ZIFs to antibodies is the binding properties of the conjugated IgGs in terms of affinity and selectivity. The second important property of the conjugates is that they should be highly mobile in dispersion (for ELISA applications) as well as mobile in lateral flow on a solid matrix (for LFIA applications, e.g. on PVDF membrane). A third feature to be retained is the fluorescent properties of Z8P in the respective conjugates to employ them in fluorescence immunoassays and/or fluorescence quenching assays.

Figure 4. Proving the integrity of the ZIF-IgG conjugate: Results from a non-competitive competitive ELISA. The target is an immobilized mouse IgG, signal (OD) development results from an HRP-labelled anti-sheep (secondary) antibody. Top: Unmodified anti-mouse IgG (from sheep) as reference. Rising concentrations results in the typical sigmoidal curve for immunoassays. Bottom: Results when replacing free IgG by conjugate systems Z8P-IgG and Z90-IgG, respectively. Binding with increasing concentrations is observed (no full sigmoidal curve recorded). OD for the blank (absence of conjugates) is indicated by the dotted horizontal line (OD = ca. 0.26).
We tested the response of Z8P-IgG against various concentrations of dimethyl phthalate (DMP) in order to check if fluorescence quenching was conserved in comparison to the unconjugated Z8P, (SI Section 5.1). The presence of antibodies around the Z8P nanoparticles proved not to interfere with this mechanism, Z8P-IgG was still sensitive to quenching by short-chained phthalates; the fluorescence intensity as a function of the concentration of dimethyl phthalate was determined. A response comparable to the one reported previously by us for this system was recorded\(^2\) (Figure S9).

A model non-competitive indirect ELISA was conceived to determine the residual activity of IgG in the obtained ZIF-IgG conjugates. (SI Section 7.3).

The surface of the wells of a microtiter plate were coated with the model target, a mouse IgG antibody. Uncoated adsorptive areas of the microwell were blocked with casein. The analytical step is the subsequent binding of a “primary” anti-mouse IgG (polyclonal, produced in sheep, therefore cheap) to the mouse IgG target on the microwell walls. In a conventional ELISA the binding is quantified by adding an anti-sheep antibody that is labelled with an enzyme (often horseradish peroxidase, HRP) the substrate (here: 3,3′,5,5′-tetramethylbenzidine) turnover of which results in a blue colour and a measurable optical density (OD) at 450 nm. Determination of the OD of various concentrations results in a characteristic sigmoidal calibration curve (Figure 4, top and SI Section 7.2). Employing the conjugates of the same anti-mouse IgG and the ZIFs (Z90-IgG and Z8P-IgG) in this model ELISA resulted in the expected sigmoidal curves (Figure 4, bottom and SI Section 7.3). This proves that the antibodies in Z90-IgG and Z8P-IgG retained their binding properties.

To turn the enzyme immunoassay into a fluorescence immunoassay based on the inherent fluorescent properties of Z8P, the model non-competitive indirect ELISA was re-run (SI Section 7.4.1 and SI Section 7.4.2) but at higher concentrations than that used on the equivalent ELISA developed in Figure 4. These higher concentrations affect only the target (mouse IgG) and the primary (Z8P-IgG), keeping every other constituent of the ELISA with the same concentration as before. The reason for this increment in concentration is due to the inherent sensitivity of the fluorescence of the conjugated ZIF is significantly inferior when compared to the sensitivity offered by the TMB revealing methodology. Because of this increment in concentration when it comes to the TMB revealing methodology the system quickly reaches saturation of the detector (Figure 5a) at concentrations above 350 ng/mL, with saturation of the photometer (OD above 3.8).

Fluorescence measurements (excitation at 335 nm) on this system resulted in low fluorescence emission intensities (Figure 5b “Pre wash”), and after a washing step to remove loosely bound Z8P particles, the assay gave even lower intensities (Figure 5b “Post wash”). We believe this is because the fluorescence of the aminopyrene moieties in the Z8P particles are quenched by the contact of the antibodies surrounding the particles on their surface. The quenching can be substantially overcome by subjecting the Z8P-IgG to acidic digestion (addition of citric acid). this will result in the disintegration of the ligands in Z8P and the additional hydrolysis of the imine bond releasing the highly fluorescent free aminopyrene residues. This acid treatment results in a significant increase in the fluorescence emission as depicted in Figure 5b.

Finally, a Z8P-IgG was tested for its suitability for applications in LFIA. To do so, another Z8P-IgG conjugate (Z8P-IgG HRP) needed to be synthesised to covalently incorporate the HRG colour tag on the
IgG. To achieve this objective, we employed a commercially available anti-sheep IgG-HRP conjugate. The label HRP can be detected extremely sensitively via its substrate reaction using 3,3',5,5'-tetramethylbenzidine (TMB): an intense blue colour is formed even when only traces of labelled antibody are present. The synthesis of Z8P-IgG HRP is analogous to Z8P-IgG, the one with unlabelled sheep IgG (SI Section 10.1).
Figure 5. a): Non-competitive indirect ELISA calibration curves for Z8P-IgG (anti-mouse, from sheep) using high concentrations. The detector reaches saturation at concentrations above 350 ng/mL. The OD signal for the blank (coating antibody + blocking) is 0.72 ± 0.04. b): Fluorescence emission for the non-competitive indirect assay employing Z8P-IgG obtained with an excitation wavelength of 335 nm. All the data points are blank-corrected (the mean intensity for the blank was subtracted from all points; the blanks’ mean intensity was 168 ± 26 for pre wash fluorescence, 143 ± 54 for post wash and 356 ± 5 after disintegration of the ZIF (by citric acid).
Figure 6 Experimental set-up to demonstrating the aptitude of ZBP-IgG for the bioanalytical application lateral flow immunoassays (LFIA). a) Assay. The protocol begins by activating the PVDF membrane through submersion in pure ethanol followed by rinsing in deionized water. After air drying, a solution containing sheep IgG as target is placed in a central part of the strip, the later capture zone. The PVDF strip with the immobilized sheep IgG is then submerged in a casein solution to block all remaining active sites on the strip, and afterwards allowed to dry. A varying volume of the ZBP-IgG-HRP dispersion is pipetted on the elution starting point and allowed to dry. Then the absorption pad is attached to the strip. Subsequently, the ZBP-IgG-HRP is eluted by adding enough of a mobile phase (borate buffer) to the strip that the phase’s front-line flows across the capture zone into the absorption pad. This is followed by drying the strip in air. b) Revelation of mobility of the composite and retention of binding specificity of the antibody: The capturing of ZBP-IgG by the target (sheep antibody) in the capture zone is visible by the colour of the ZIF particles. For stronger evidence that the labelled antibody is binding selectively to the immobilized target, the HRP enzyme linked to ZBP-IgG is revealed via its reaction with the added substrate TMB. c) Proof of intact ZIF label. The area to which the antibody has bound is cut out and digested in H$_2$SO$_4$ to decompose the ZIF and liberate the pyrene. The supernatant is transferred to a black microwell and fluorescence of the solution measured. The emission spectra show that fluorophore is released in amounts related to the volume of conjugate employed (PVDF and TMB samples that were not run through the assay serve as negative control).
Polyvinylidene fluoride (PVDF) strips were identified as a suitable solid matrix for ZIF particle mobility studies. After preparation of the capture zone (washing with ethanol and water) of the strips, IgG (polyclonal, from sheep) used as a model target was immobilized by applying 1 µL of serum to the capture zone and subsequent blocking of free adsorptive sites with casein. Two experiments were performed with either 10 µL or 1 µL of Z8P-IgG-HRP (anti-sheep, from donkey, HRP-labelled) applied at the starting point of the PVSF strip then by the action of the mobile phase (borate buffer), Z8P-IgG-HRP was eluted from the starting point and flown across the capture zone where anti-sheep IgG bound to the immobilized target sheep IgG. Surplus of the non-bound Z8P-IgG HRP conjugate), Z8P-IgG-HRP ended up in the absorption pad located on the opposite side of the strip (Figure 6a). Dipping into a TMB solution revealed the presence of the enzyme (bound to the Z8P-IgG) in the capture zone (and in the adsorption pad), a mechanism that can be used to detect the presence of a target analyte (here: sheep IgG) (Figure 6b). To prove that the Z8P had also become immobilised in the capture zone, this zone was cut and digested with sulphuric acid, to release the fluorophore 1-AP. Dependent on the amount of Z8P-IgG employed (and thus captured), fluorescence could be measured at wavelengths specific to the 1-aminopyrene moiety of the ZIF in the Z8P-IgG-HRP (SI Section 10.2).

We report here the preparation of antibody conjugates with nanoparticulate Zeolitic Imidazolate Frameworks containing an aldehyde handle able to form an imine with lysine amino groups of antibodies, followed by subsequent reduction to form a stable secondary amine as the covalent link. To make the conjugates suitable as novel sensor molecules for bioanalytical applications the ZIF conjugated antibody contains a fluorescence tag (aminopyrene). The potential of the ZIF-IgG conjugate has been evaluated via a non-competitive indirect ELISA and an LFIA. The ZIF-antibody conjugates display excellent behaviour in dispersion and good mobility via lateral flow on a solid substrate, retaining the selectivity of the antibody.

This is the first time that for a ZIF-antibody conjugate, the formation of a stable covalent linkage is demonstrated while retaining all the properties of its constituent parts, ZIFs and immunoglobulins. In addition to preserving the mobility of the nanoparticulate entity the conjugation also prevents protein aggregation of the antibodies and, like an exoskeleton, might elicit protection against degradation. These composites expand drastically the potential of ZIFs to be applied as highly selective antibody conjugates in a variety of analytical and diagnostic applications. Avoiding the development steps in the enzymatic reaction of ELISAs, this development paves the way to better portable formats, shortening time-to-result, a feature gaining importance in the confrontation with global diseases and pandemics

**Experimental Section**

See Supplementary Information.

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The authors declare no conflict of interest.

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References
[1] S. Dutta, J. Kim, P. H. Hsieh, Y. S. Hsu, Y. V. Kaneti, F. K. Shieh, Y. Yamauchi, K. C. W. Wu, Small Methods 2019, 3, 1900213.
[2] S. R. Batten, N. R. Champness, X. M. Chen, J. Garcia-Martinez, S. Kitagawa, L. Ohrstrom, M. O’Keeffe, M. P. Suh, J. Reedijk, CrystEngComm 2012, 14, 3001-3004.
[3] J. Troyano, A. Carne-Sanchez, C. Avci, I. Imaz, D. Maspol, Chem Soc Rev 2019, 48, 5534-5546.
[4] W. P. Lustig, S. Mukherjee, N. D. Rudd, A. V. Desai, J. Li, S. K. Ghosh, Chem Soc Rev 2017, 46, 3242-3285.
[5] B. L. Chen, Z. X. Yang, Y. Q. Zhu, Y. D. Xia, Journal of Materials Chemistry A 2014, 2, 16811-16831.
[6] K. S. Park, Z. Ni, A. P. Cote, J. Y. Choi, R. Huang, F. J. Uribe-Romo, H. K. Chae, M. O'Keeffe, O. M. Yaghi, Proc Natl Acad Sci U S A 2006, 103, 10186-10191.
[7] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[8] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[9] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[10] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[11] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[12] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[13] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[14] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[15] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[16] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[17] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[18] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[19] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[20] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[21] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[22] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[23] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[24] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
[25] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, J Am Chem Soc 2008, 130, 12626-12627.
A. Chapartegui-Arias, J. A. Villajos, A. Myxa, S. Beyer, J. Falkenhagen, R. J. Schneider, F. Emmerling, ACS Omega 2019, 4, 17090-17097.

J. Yang, Y. W. Yang, Small 2020, 16, e1906846.

I. A. Darwish, Int. J. Biomed. Sci. 2006, 2, 217.

D. Bidwell, A. Buck, H. Diesfeld, B. Enders, J. Haworth, G. Huld, N. Kent, C. Kirsten, P. Mattern, E. Ruitenber, Bull. World Health Organ. 1976, 54, 129-139.

M. Seydack, Biosens Bioelectron 2005, 20, 2454-2469.

S. Sakamoto, W. Putalun, S. Vimolmangkang, W. Phoolcharoen, Y. Shoyama, H. Tanaka, S. Morimoto, J Nat Med 2018, 72, 32-42.

J. Yang, Y. W. Yang, Small 2020, 16, e1906846.

I. A. Darwish, Int. J. Biomed. Sci. 2006, 2, 217.

D. Bidwell, A. Buck, H. Diesfeld, B. Enders, J. Haworth, G. Huld, N. Kent, C. Kirsten, P. Mattern, E. Ruitenber, Bull. World Health Organ. 1976, 54, 129-139.

M. Seydack, Biosens Bioelectron 2005, 20, 2454-2469.

S. Sakamoto, W. Putalun, S. Vimolmangkang, W. Phoolcharoen, Y. Shoyama, H. Tanaka, S. Morimoto, J Nat Med 2018, 72, 32-42.

K. M. Koczula, A. Gallotta, Essays Biochem 2016, 60, 111-120.

A. Bahlmann, J. J. Carvalho, M. G. Weller, U. Panne, R. J. Schneider, Chemosphere 2012, 89, 1278-1286.

Y. Xiong, Y. K. Leng, X. M. Li, X. L. Huang, Y. H. Xiong, Trac-Trend Anal Chem 2020, 126, 115861.

E. Watanabe, S. Miyake, Y. Yogo, J Agric Food Chem 2013, 61, 12459-12472.

C. Wang, J. Gao, H. Tan, ACS Appl Mater Interfaces 2018, 10, 25113-25120.

X. J. Wang, X. Y. Wang, Y. Han, H. Li, Q. Kang, P. C. Wang, F. M. Zhou, AcS Appl Nano Mater 2019, 2, 7170-7177.

S. Aoyama, K. Monden, Y. Akiyama, M. Yamada, M. Seki, Anal Chem 2019, 91, 13377-13382.

N. Arifin, N. A. Yusof, J. Abdullah, S. F. Abd Rahman, N. H. Ahmad Raston, N. Kusnin, S. Suraiya, J Sensors 2020, 2020, 1-10.

M. Tian, W. Y. Xie, T. Zhang, Y. Liu, Z. S. Lu, C. M. Li, Y. S. Liu, Sensor Actuat B-Chem 2020, 309, 127728.

B. X. Zhao, Q. Huang, L. N. Dou, T. Bu, K. Chen, Q. F. Yang, L. Z. Yan, J. L. Wang, D. H. Zhang, Sensor Actuat B-Chem 2018, 223, 223-229.

D. Tang, Y. Cui, G. Chen, Analyst 2013, 138, 981-990.

M. Saleh, H. Soliman, O. Haenen, M. El-Matbouli, J Fish Dis 2011, 34, 845-852.

C. Parolo, A. de la Escosura-Muniz, A. Mercoci, Biosens Bioelectron 2013, 40, 412-416.

A. Raysyan, I. A. Galvidis, R. J. Schneider, S. A. Eremin, M. A. Burkin, J. Pharm. Biomed. Anal. 2020, 189, 113450.

J. Cravillon, C. A. Schröder, R. Nayuk, J. Gummel, K. Huber, M. Wiebcke, Angewandte Chemie International Edition 2011, 50, 8067-8071.

R. E. Morris, ChemPhysChem 2009, 10, 327-329.

Table of Contents

Caption: Metal-Organic Framework particles as labels for antibody-based analytical methods as a new ‘Swiss knife’ for global bioanalytical and diagnostic challenges.
Supplementary Information

Zeolitic Imidazolate Framework Antibody Conjugates in Bioanalytical Applications

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Nomenclature and abbreviations used

| Abbreviation | Meaning |
|--------------|---------|
| 1-AP         | 1-Aminopyrene |
| 2-mim        | 2-Methylimidazolate |
| CBZ          | Carbamazepine |
| DMP          | Dimethyl phthalate |
| DMSO         | Dimethylsulphoxide |
| EDS          | Energy-dispersive x-ray spectroscopy |
| ELISA        | enzyme-linked immunosorbent assay |
| FTIR         | Fourier-transform infrared spectroscopy |
| HPLC         | High-performance liquid chromatography |
| HRP          | Horseradish peroxidase |
| i2ca         | Imidazol-2-carboxaldehyde when referring to neutral species or Imidazolate-2-carboxaldehyde when referring to anionic species |
| ICP-OES      | Inductively coupled plasma atomic emission spectroscopy |
| IgG          | Immunoglobulin type G |
| MeONa        | Sodium methoxide |
| Na₂HPO₄      | Disodium hydrogen phosphate |
| NaBH₃CN      | Sodium cyanoborohydride |
| NaH₂PO₄      | Sodium phosphate monobasic |
| OD           | Optical density |
| PBS (buffer) | Phosphate-buffered saline solution |
| PFA          | Perfluoroalkoxy alkane |
| PVDF         | Polyvinylidene fluoride |
| PXRD         | Powder x-ray diffractometry |
| Sheep IgG    | Immunoglobulin type G from sheep |
| S-TEM        | Scanning transmission electron microscopy |
| TEM          | Transmission electron microscopy |
| TMB          | 3,3',5,5'-tetramethylbenzidine |
| TRIS (buffer)| tris(hydroxymethyl)aminomethane |
| Z8P          | Pyrene-modified ZIF-8/ZIF-90 hybrid |
| Z8P–IgG      | Z8P IgG antibody conjugate |
| Z90–IgG      | ZIF-90 IgG antibody conjugate |
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1 Chemical and Reagents

1.1 Reagents for ZIF synthesis

Zinc nitrate hexahydrate (CAS: 10196-18-6) was purchased from Roth (100% purity); 2-methylimidazole (CAS: 693-98-1) was purchased from Acros Organics (100% purity); 1-Aminopyrene (CAS: 1606-67-3; 95% purity) and Imidazolate-2-carboxaldehyde (CAS: 10111-08-7; 97% purity) were purchased from Sigma-Aldrich. Sodium methoxide (CAS: 124-41-4; 95% purity) was purchased from Merck. Sodium cyanoborohydride solution (CAS: 25895-60-7) 5.0 M in 1 M NaOH was purchased from Sigma-Aldrich. Methanol and dimethylformamide HPLC grade where purchased at CHEMSOLUTE.

1.2 Reagents for HPLC

All HPLC solvents used were of high purity HPLC grade. Water was acquired from a Milli-Q® Direct Water Purification System [18.2 MΩ•cm (25°C)]. Methanol for HPLC was purchased from CHEMSOLUTE as HPLC grade and TFA was purchased from Sigma Aldrich as Reagent-Plus 99%.

- Na$_2$HPO$_4$ (Reagent grade, CAS: 10028-24-7)
- NaH$_2$PO$_4$ (Reagent grade, CAS: 10049-21-5)

1.3 Reagents for ICP-OES

HNO$_3$ 65% (grade for analysis EMSURE® Reag. PhEur, ISO, Merck)

Ultrapure deionized water (18.2MΩ cm$^{-1}$, 25°C) was used for dilutions (Milli-Q Gradient, Merck Millipore).

The external standard solution of a Zn is directly traceable to the NIST SRM 3168a (Certipur® Certified Reference Material, Zn(NO$_3$)$_2$ in HNO$_3$ 2-3% Suprapur® Merck, Germany).

1.4 Reagents for LC/MS-MS

All solvents and additives (acetonitrile, ammonium formate, formic acid) were of LC-MS grade (CHEMSOLUTE$_5$).

Water was obtained from an ultrapure water purification system (Elga).

1.5 Reagents for buffers

All reagents for buffer preparation, and casein, were purchased from Merck.

- Di-sodium tetraborate decahydrate (Ph Eur,BP,JP,NF grade, CAS: 1303-96-4)
- Sodium chloride (Reagent grade, CAS: 7647-14-5)
- Sodium azide solution (10% w/v in water, CAS: 26628-22-8)
- NaH$_2$PO$_4$·2H$_2$O (Reag. Ph Eur grade, CAS: 10049-21-5)
- Na$_2$HPO$_4$·2H$_2$O (Ph Eur,BP,USP grade, CAS: 10028-24-7)
- C$_4$H$_7$NO$_3$ (TRIS, Ph Eur grade, CAS: 77-86-1)
- Sodium dihydrogen citrate (Reagent grade, CAS: 18996-35-5)
- KH$_2$PO$_4$ (molecular biology grade, CAS: 7778-77-0)
- K$_2$HPO$_4$ (Analysis grade, CAS: 7758-11-4)
- C$_6$H$_7$KO$_2$ (potassium sorbate, Ph Eur,BP,NF,FCC,E 202 grade, CAS: 24634-61-5)
- 0.05% (v/v) Tween™ 20 solution (CAS: 9005-64-5)
- Tetrabutylammonium borohydride (synthesis grade, CAS: 33725-74-5)
- 3,3',5,5'-tetramethylbenzidine (TMB, analysis grade, CAS: 54827-17-7)
- Casein (technical grade, CAS: 9000-71-9)

1.6 Antibodies

HRP-conjugated donkey anti-sheep-IgG (A3415), 1.8 mg/mL, in buffer (50 mM Na₂HPO₄, 20 mM Na₂HPO₄, TRIS HCl 0.1 M, pH 7.4, azide 0.02% w/v), was purchased from Sigma-Aldrich.

Anti-mouse IgG from sheep (R1256P), 2 mg/ml, in PBS buffer, was purchased from Acris Antibody GmbH (Herford, Germany).

Mouse anti-CBZ IgG (AK2512/01), 1.75 mg/mL, in PBS buffer, ordering code BAM-mab 01 (CBZ) was obtained from BAM.¹

1.7 Solvent for NMR

d6-DMSO (CAS: 2206-27-1, 99.9%) in 0.75 ml ampules was purchased from Sigma-Aldrich

2 Preparation of buffers

2.1 Preparation of the phosphate buffer for HPLC (pH 8.8)

1.75 g of Na₂HPO₄ (9.83 mmol) and 16.29 mg of NaH₂PO₄ (0.12 mmol) were weighed using a 5-figure balance, transferred to a 1 L HPLC glass solvent bottle and added 1 L of water obtained from a Milli-Q® Direct Water Purification System [18.2 MΩ•cm (25°C)], measuring the added volume with a 1 L graduated glass cylinder. After complete dissolution, the buffer was filtered through a 0.45 μm nylon membrane (47 mm diameter) using a 1 L solvent vacuum microfiltration apparatus to remove any undissolved crystal. The pH was determined to be 8.60 using a pH meter, and the buffer was ready to use.

2.2 Preparation of the buffers for immunoanalysis

2.2.1 Borate buffer

9.64 g (48 mmol) of di-sodium tetraborate decahydrate and 8.29 g (142 mmol) of sodium chloride were weighed and transferred to a 1 L HPLC glass solvent bottle. Using a 1L graduated glass cylinder 1 L of ultrapure water, obtained from a Milli-Q® Direct Water Purification System [18.2 MΩ•cm (25°C)], was added to the bottle. After full dissolution 1 mL of a sodium azide solution (10% w/v in water) was added and mixed. The final pH of the buffer was measured and determined to be pH 9.05.

2.2.2 Phosphate buffered saline (PBS)

1.56 g (10 mmol) of NaH₂PO₄·2H₂O, 12.46 g of Na₂HPO₄·2H₂O (70 mmol) and 8.47 g of NaCl (142 mmol) were weighed, transferred to a 1 L HPLC glass solvent bottle, 1 L of ultrapure water was added and the pH determined to be pH 7.60.
2.2.3 TRIS buffer

1.21 g of C₄H₁₁NO₃ (TRIS, 10 mmol) and 8.77 g of NaCl (150 mmol) were weighed and, as described in SI-2.2.2, used to prepare 1 L of buffer, pH 8.50.

2.2.4 Citrate buffer

47.10 g of sodium dihydrogen citrate (220 mmol) were weighed and, as described in SI-2.2.2, used to prepare 1 L of buffer, pH 4.00. For reasons of stability, the buffer was kept in the fridge at 4°C.

2.2.5 Wash buffer

6.124 g of KH₂PO₄ (45 mmol), 65.32 g of K₂HPO₄ (375 mmol), 0.225 g of C₆H₇KO₂ (potassium sorbate, 1.5 mmol) were weighed and, as described in SI-2.2.2, used to prepare 1 L of buffer. 30 ml of a 0.05% (v/v) Tween™ 20 solution using a 100 mL graduated glass cylinder was added. The final pH of the buffer was measured and determined to be pH 7.60.

2.2.6 TMB solution

102.93 mg of tetrabutylammonium borohydride (8 mmol) and 480.7 mg of 3,3’,5,5’-tetramethylbenzidine (TMB, 40 mmol) were weighed using a 5-figure analytical balance, transferred to a 200 mL new glass bottle and 50 mL of Milli-Q water added. For stability, the buffer was kept in the fridge at 4°C.

3 Analytical Investigations: Instruments and Methods

3.1 FTIR:

Fourier-transform infrared spectra were recorded on a Nicolet FT-IR NEXUS instrument with a Golden Gate-Diamond-ATR Unit and a DTGS KBr detector. For each measurement 32 sample scans and 32 background scans were recorded. The spectral range was from 200 cm⁻¹ to 4000 cm⁻¹ with a resolution of 4 cm⁻¹.

3.2 TEM:

Transmission electron microscopy (TEM) images were obtained in a Talos F200S Microscope (Thermo Fisher Scientific) by using a 200 kV microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. A Ceta 16M camera (TEM mode) and a HAADF detector (STEM mode) were used to capture the images.

Additionally, a side-entry retractable Energy Dispersive X-ray Spectroscopy (EDS) detector were also used in the STEM mode to collect detailed elemental information.

The specimens were prepared by dropping sample solutions onto a 3 mm copper grid (Lacey Carbon, 400 mesh) and leaving them to air-dry at room temperature.
3.3 NMR:

Nuclear magnetic resonance measurements were performed on a 400 MHz NMR spectrometer (Mercury-400 BB) at 29.0 °C, with a relaxation delay of 2.000 sec, a pulse of 45.0°, an acquisition time of 2.274 sec and 16 repetitions. Solvent was deuterated DMSO.

3.4 Bragg-Brentano diffractometry:

Powder X-ray diffractometry (PXRD) patterns were collected with Cu Kα radiation (λ = 1.50406 nm) on a D8 Advanced diffractometer (Bruker AXS, Germany) equipped with a LYNXEYE detector. Samples were measured in reflection geometry in a 2Θ range from 4° to 50° with a step size of 0.009° with spinning setup.

3.5 Fluorescence spectrometry:

Fluorescence spectra were obtained on an all-in-one microplate reader Synergy H1 with dispenser (BioTek). The software used was Gen5. The spectra was acquired from 450 to 600 nm, with a step of 2 nm, measurement time of 10 ms for the particle characterization, and of 50 ms for the fluorescence ELISA, with delay between measurements of 100 ms. The excitation wavelength was 335 nm. The samples were handled in non-binding or high-binding polypropylene 96-well black microtiter plates from Greiner Bio-One.

3.6 HPLC:

High performance liquid chromatography analysis of the Z8P samples were performed using a modular Agilent 1100 Series HPLC system composed of a high pressure binary pump, auto-sampler with injector programming capabilities, Peltier type column oven with 6 µL heat exchanger and a Diode Array Detector with a micro flow cell (1.6µL, 6mm pathlength) to reduce peak dispersion when using short columns as in this case. The flow-path was connected using 0.12 mm ID stainless steel tubing to minimize peak dispersion. ChemStation software was used to process the HPLC data.

3.6.1 HPLC method

HPLC column was Excel C18-PFP with 2 µm particle size (4.6 mm internal diameter × 50 mm length) manufactured by ACE. The C18-PFP stationary phase was prepared by covalently bonding a pentafluorophenyl octadecylsilane to pure silica. The HPLC column is manufactured to be stable up to pH 10 and 1000 bar back pressure.

HPLC conditions:

Solvent A = Phosphate buffer 10 mmol, pH=8.8
Solvent B = MeOH
0-4 min; 10-95 % B; run time of 7 min; post time of 2 min.
Flow rate was 2 mL/min; column temperature of 45 °C; 1 µL injection.

The chosen wavelength for 2-methylimidazole was 210 nm (8 nm bandwidth), for imidazolate-2-carboxaldehyde 280 nm (8 nm bandwidth) and for 1-aminopyrene 338 nm (8 nm bandwidth), all having a reference of 550 nm (100 nm bandwidth, Figure S10).
3.7 **ICP-OES:**

For inductively coupled plasma optical emission spectrometry an Agilent 5110 VDV ICP-OES instrument was employed (Agilent Technologies, USA). The emission line of zinc at wavelength 202.548 nm at radial mode was used. Detailed operating conditions are listed in Table S7.

3.8 **Absorbance spectrometry:**

Absorbance, with measurements in microtiter plates usually designated as optical density (OD), was measured on a SpectraMax Plus384 microplate reader (Molecular Devices, Ismaning, Germany) at 450 nm, referenced to 620 nm. All calibration standard solutions were determined in triplicate. SoftMax®Pro Software (v.5.3, Molecular Devices) was used for data acquisition.

3.9 **LC-MS:**

Liquid chromatography-mass spectrometry analysis was performed on a 1290 UHPLC system (Agilent, Germany) coupled to a 6600 TripleTOF mass spectrometer (SCIEX, Germany).

3.9.1 **LCMS method**

Samples were separated on a 2.1 mm × 75 mm × 1.8 µm BEH amide column (Waters, Germany) using a hydrophilic liquid interaction chromatography (HILIC) mode.

- **Solvent A:** acetonitrile/water 50:50, 10 mM ammonium formate, 0.125% formic acid.
- **Solvent B:** acetonitrile/water 95:5, 10 mM ammonium formate, 0.125% formic acid.
- **Solvent gradient (%B):** 0 min 100%, 1 min 100%, 10 min 0%, 12 min 0%, 12.1 min 100%, 15 min 100%.
- **Flow rate:** 0.5 mL/min. Column temperature: 35 °C.

Positive and negative electrospray ionization (ESI+/−) was carried out at 320 °C using capillary voltages of 5500 V and 4500 V, respectively. Mass spectrometric detection was performed in full-scan mode (m/z 50-600) at a scan rate of 4 s⁻¹ using data-dependent MS/MS acquisition (DDA Top-4). For data analysis, raw data files were converted to mzML format and imported into an R workspace. Peak pre-processing was performed using package xcms[2]. For MS/MS fragment substructure annotation, competitive fragmentation modelling was used[3] (CFM-ID).
4 Synthesis and characterisation of ZIFs

4.1 Preparation of the 1-aminopyrene-functionalized nanoparticulated ZIF-8/ZIF-90 hybrid (Z8P)

16.08 mg (0.075 mmol) of 1-aminopyrene and 47.28 mg (0.493 mmol) of Imidazolate-2-carboxaldehyde were accurately weighed and transferred into a sealable glass flask. 5 mL of methanol was added using a 10 mL pipette, the glass flask was sealed with an aluminium cap and afterwards heated at 80°C for 30 min with stirring. After cooling the solution to ambient temperature, 40.4 mg (0.493 mmol) of 2-methylimidazole was added to the solution as well as 250 µL of a nearly saturated solution of MeONa in methanol (0.25 g/mL) using a 1 mL pipette. Obtaining a well dissolved solution of MeONa requires heavy stirring or ensuring that the solution is being stirred while pipetting.

After the addition of MeONa, the solution was stirred for 1 minute. Finally, 5 ml of a Zn(NO₃)₂·6·H₂O solution was added using a 10 ml micropipette. This Zn(NO₃)₂ solution was prepared by weighting 146 mg of Zn(NO₃)₂·6·H₂O (0.494 mmol) and transferring it to a glass vial, then adding 10 ml of methanol with a 10 ml pipette and mixing by shaking gently.

Almost immediately after the addition of the Zn salt solution, a yellow solid appeared dispersed in the solution. Stirring was stopped. The mixture was left undisturbed for 4 hours. A yellow solid sedimented to the bottom of the vial.

The sedimented mixture was redispersed in the solvent with a short burst of vigorous agitation and aliquots were quantitatively transferred into a 2 mL Eppendorf tube. The suspended solid was then sedimented via centrifugation (15000 rpm, 3 mins), the supernatant removed with a pipette and discarded. The process was repeated until all the reaction volume had been centrifuged. All the solid residue remained at the bottom of the Eppendorf.

Fresh methanol was added to fill up the Eppendorf tube, the solid residue was then redispersed by agitation, the suspended solid was then sedimented via centrifugation (15000 rpm, 3 mins) and the coloured supernatant methanolic solution was removed. This procedure was repeated 5 additional times, until the methanolic solution became colourless. The solid residue was finally dried overnight at 5 mbar and 75 °C and let to cool down under vacuum.

For the three syntheses performed the final masses of Z8P obtained were: S1= 31.24 mg; S2= 30.02 mg; S3= 29.54 mg. When compared with the initial 119.8 mg of total mass weighted for synthesis (16.08 mg of 1-aminopyrene, 47.28 mg of Imidazolate-2-carboxaldehyde, 40.4 mg of 2-methylimidazole and 16.04 mg of Zn²⁺ [amount of Zinc coming from 73 mg of Zn(NO₃)₂·6·H₂O]) it can be estimated that the yield of the synthesis, expressed in %mass, is of around 25% of reacted mass.

WARNING: When handling Z8P solid to weight for HPLC analysis it must be kept away from humidity as much as possible.
4.2 Preparation of the nanoparticulated ZIF-90

94.56 mg (0.984 mmol) of **imidazolate-2-carboxaldehyde** was weighed, transferred to a sealable glass flask with an aluminium cap. 5 mL of methanol was added, using a 10 mL pipette. The flask was sealed and then heated to 80°C until total dissolution (5 min) was achieved.

After the addition of MeONa, the solution was stirred for 1 minute. Finally, 5 ml of a Zn(NO$_3$)$_2$·6·H$_2$O solution was added using a 10 ml micropipette. This Zn(NO$_3$)$_2$ solution was prepared by weighting 146 mg of Zn(NO$_3$)$_2$·6·H$_2$O (0.494 mmol) and transferring it to a glass vial, then adding 10 ml of methanol with a 10 ml pipette and mixing by shaking gently.

Almost immediately after the addition of the Zn salt solution, a white solid precipitated. The suspension was left undisturbed for 4 hours without stirring. The white solid sedimented to the bottom of the vial.

The mixture was redispersed with a short vigorous agitation and aliquots were transferred into a 2 mL Eppendorf tube. The suspended solid was then sedimented via centrifugation (15000 rpm, 3 mins), the supernatant removed with a pipette and discarded. The process was repeated until all the reaction volume had been centrifuged; all the solid residue remained at the bottom of the Eppendorf.

Then fresh methanol was added to fill the Eppendorf tube, the solid residue was then redispersed by vigorous shaking, the suspended solid was then sedimented via centrifugation (15000 rpm, 3 mins) and the methanolic supernatant removed. This procedure was repeated 5 additional times. The solid residue was finally dried overnight at 5 mbar and 75°C, let to cool down under vacuum.

**WARNING**: When handling ZIF-90 solid to weight for HPLC it must be kept away from humidity as much as possible.

4.3 Preparation of ZIF-8

This synthetic procedure was the same as in SI-4.2, with the following difference:

82.20 mg (0.984 mmol) of **2-methylimidazole** (2-mim) was used instead of imidazolate-2-carboxaldehyde. No heating is required to dissolve 2-mim. It dissolves almost immediately.
4.4 Structural characterization of ZIFs

4.4.1 FTIR characterization of the synthesised ZIFs

We performed FTIR measurements, in order to verify that the aldehyde functionality is incorporated in the Z8P. We used ZIF-8 as a negative control and ZIF-90 as positive control for comparison. For the measurements of each sample, a small amount of the solid powder was deposited on the diamond accessory sample holder.

All the characteristic peaks for each of the samples are consistent with the published spectra\(^4\). The band corresponding to the aldehyde functionality on the Imidazolate-2-carboxaldehyde moiety (broad band at 1670 nm\(^5\)) can only be observed on the ZIF-90 and the modified ZIF-8, Z8P and is not present in the unmodified ZIF-8 (Figure S2).

![FTIR spectra](image)

**Figure S2** FTIR spectra for a) Z8P, b) ZIF-90 and c) Z8P. The broad band at 1670 nm corresponds to the free aldehyde functionality of the Imidazolate-2-carboxaldehyde on the ZIF structure. The ZIF-90 has all the imidazole constituents with the aldehyde functionality, thus having the most intense band.
For the ZIF-8 particles, there are four characteristic peaks at 1600, 1145, and 995 cm\(^{-1}\), assigned to vibrations of C=N, and C=N in the imidazole ring, respectively. The intense peaks at 1457 and 1382 cm\(^{-1}\) correspond to the entire ring stretching. Several spectral bands seen in the range 1350 to 900 cm\(^{-1}\) can be ascribed to the in-plane bending of the ring and the peaks at 760 and 690 cm\(^{-1}\) are associated with aromatic sp\(^2\) C-H bending. Finally, a strong band at 421 cm\(^{-1}\) was observed that corresponds to the Zn-N stretching as the zinc atoms in the ZIF-8 structure connect to nitrogen atoms of the 2-mim linker during the formation of ZIF-8.

| Peak position (cm\(^{-1}\)) | Vibration assigned to                      |
|-----------------------------|------------------------------------------|
| 1670                        | free aldehyde on i2ca                     |
| 1600                        | C=N imidazole ring                       |
| 1457                        | imidazole ring stretching                 |
| 1382                        | imidazole ring stretching                 |
| 1145                        | C-N imidazole ring                       |
| 995                         | C-N imidazole ring                       |
| 760                         | aromatic sp\(^2\) CH bending              |
| 690                         | aromatic sp\(^2\) CH bending              |
| 421                         | Zn-N stretching                           |

4.4.2 Characterization of the ZIF particles and conjugate particles via TEM

Size and shape of the nanoparticles were analysed via TEM with respect to reported ZIFs\(^{[6]}\). The TEM analysis were complemented with EDS.\(^{[7]}\)

To prepare the Z8P sample, a dispersion of the Z8P ZIF in methanol (1 mg/mL) was prepared by weighing 4.97 mg of Z8P in a glass vial and adding 5 ml of methanol with a 10 ml pipette. After dispersion by ultrasonication for 10 min, 10 μL aliquot of the methanol solution of the Z8P dispersion was deposited on a copper ultrathin C film on Lacey Carbon support film with a 400 mesh that was carefully deposited on top of a small glass crystallizer. The excess of solvent was left to evaporate open to the atmosphere, and the sample was then ready to be analysed.

To prepare the ZIF-90 sample, a dispersion of the ZIF-90 in methanol (1mg/mL) was prepared by weighting 5.24 mg of ZIF-90 glass vial and adding 5 ml of methanol with a 10 ml pipette; the rest of the process mirrors the TEM sample preparation described above for Z8P.
The ZIF-antibody conjugates were also analysed by TEM measurements, complemented with S-TEM and EDS (Figure S24) in Section SI-9. To prepare the sample, 100 μL of a borate buffer dispersion of Z8P-IgG (0.75 mg/mL, SI-6.1) was transferred to a 2 mL Eppendorf tube with a 200 μL pipette and centrifuged at 15000 rpm for 3 min. The supernatant was carefully removed and the sedimented solid was then redispersed in fresh methanol (200 μL). A 10 μL aliquot was deposited on a copper ultrathin C film on Lacey Carbon support film with a 400 mesh. This film was carefully deposited on top of a small glass crystallizer. The excess of solvent was left to evaporate, and the sample was then ready to be analysed by TEM.

4.5 Synthesis of the imine formed by Schiff condensation of 1-AP and i2ca

To study the imine formation, 200 mg of 1-AP (0.8755 mmol) and 86.06 mg of i2ca (0.875 mmol) were transferred into an aluminium-capped sealable glass flask. 10 mL methanol was added. The solution was then heated at 80 °C for 30 min with slow stirring (300 rpm) on a magnetic stirrer. The imine precipitated as an intense yellow-mustard solid. After the reaction was completed, the suspension was allowed to cool down to room temperature while stirring. The suspension was transferred quantitatively to a 15 mL Falcon tube and the solid sedimented via centrifugation (4000 rpm, 5 min). The supernatant was carefully removed and discarded. At this stage there maybe residual starting materials.

The wash workup is as follows. 5 mL of a fresh methanol was added to the sedimented residue and redispersed by strong agitation; the suspension was the centrifuged to sediment the solid. After removing the supernatant, the open Falcon tube was subjected to vacuum for 3 hours at 5 mbar and 60°C in a vacuum oven. Some impurities can be observed on the NMR measurements (Fig S6.2) which may have resulted from the preparation method to obtain imines

**Caution:** Perform the wash procedure with methanol only once to prevent the imine from dissolving in methanol, hence reducing the final yield of the imine.
4.6 Characterisation of 1-AP, i2ca and the imine formed by Schiff condensation of 1-AP and i2ca

4.6.1 Characterization by 1H NMR

$^1$H NMR was used to confirm the structures of 1-AP and i2ca and to characterise the structure of the imine and assess its purity.

All samples were prepared using the same protocol: a small amount of each analyte (12.54 mg for i2ca, 13.54 mg for 1-AP and 11.54 mg for the imine) was transferred into a 5 mL glass vial, then the contents of a 0.75 mL d$_6$-DMSO ampule was quantitatively transferred into the glass vial, and after achieving complete dissolution, the solution was transferred using a Pasteur pipette into an NMR tube.

![Figure S4](image)

$^1$H NMR spectra for Imidazolate-2-carboxaldehyde (i2ca)
Figure S5  $^1$H NMR spectra for 1-aminopyrene (1-AP)
Figure S6.1  $^1$H NMR spectra for the imine formed between i2ca and 1-AP
Interpretation of NMR characterisation of the imine:

**i2ca:** ¹H NMR (DMSO, 400 MHz): δ 7.308 and 7.531 (2 d, 2H, H3 & H4 respectively; H3 and H4 are two unresolved doublets, theoretical calculations set the J value at approximately 4 Hz), 9.647 (s, 1H, H1), 13.501 (s br, 1H, H2; interchangeable proton)

**1-AP:** ¹H NMR (DMSO, 400 MHz): δ 6.31 (s, 2H, NH₂), 7.38 (d, J = 8.3 Hz, 1H, H2), 7.72 (d, J = 8.8 Hz, 1H, H4), 7.85 (t, J = 7.7 Hz, 1H, H6), 7.89 (d, J = 8.8 Hz, 1H, H3), 7.90 (d, J = 9.3 Hz, 1H, H9), 7.96 (d, J = 8.4 Hz, 1H, H2), 8.00 (d, 1H, H5), 8.27 (d, 1H, J = 9.2, H9)

**Imine:** ¹H NMR (DMSO, 400 MHz): δ 7.2-7.6 (2 d, 2H, H3 &H4 i2ca*), 8.038-8.326 (m, 8H), 8.760 (s, 1H, Imine H1), 8.823 (d, 1H, J = 9.2, H9 1-AP), 13.283 (s br, 1H, H2 i2ca)

In the ¹H NMR spectrum for **1-AP** matches the one reported on the literature⁹ (See Figure S5), and it has a strong singlet that can be seen at 6.311 ppm, corresponding to the 2 protons of the amino group of aminopyrene.

In the ¹H NMR spectrum of the **imine** (Figure S6.1) this peak at 6.311 ppm is almost completely gone (a very small peak can be seen), thus indicating the significant reduction of the amino group. When comparing the spectra of **i2ca** and the **imine**, the peak corresponding to the proton on the aldehyde functionality on **i2ca** can be seen at 9.647 ppm as a singlet. This singlet is also almost completely gone in the imine, instead a new singlet appears at 8.760 ppm, corresponding to the proton on the carbon of the C=N bond of the **imine**.
We can also see that the imine is not completely pure (Figure S6.2), since there is around 1% of i2ca and around 7.5% of 1-AP present on the mixture. This is most likely indicative of the imine not fully washed with the methanol, as well as maybe suffering small amounts of hydrolysis with the water molecules present on the system.

4.6.2 Characterization of imine by PXRD

Other evidence of the formation of the imine is supported by comparing the PXRD analysis of 1-AP, i2ca and the Imine (Figure S8). The new imine has a different crystallographic structure to that of the precursors.

To prepare the samples for PXRD analysis, each powder was softly ground with an agar pestle on an agate mortar, the homogeneous fine powder was then placed on a Bragg-Brentano PVC sample holder with an opening of 0.8 cm in diameter, the cavity was filled with the powder and the top surface of the sample was compacted flat and flash with the top surface of the holder with the help of a glass slide.
**5 Additional analysis and characterisation of ZIF and ZIF conjugates**

We complement the studies and characterization for our ZIFs and ZIF-IgGs with more relevant analytical techniques: fluorescence of the Z8P-IgG conjugate particles (SI-5.1), organic composition of the ZIF-90 and Z8P particles (SI-5.2), metallic composition of the Z8P particles (SI-5.3), crystal structure of the ZIFs and ZIF conjugates (SI-5.4).

**5.1 Fluorescence characterization the conjugated Z8P particles versus DMP**

An experiment was designed using the analytical conditions developed in previous work.\(^{\text{[10]}}\) to assess the changes in fluorescence quenching of the conjugate, Z8P-IgG, when exposed to a model contaminant, dimethyl phthalate, DMP.

In each well of a black non-binding polypropylene 96-well black microtiter plate, 10 μL of a Z8P-IgG dispersion in borate buffer (0.75 mg/mL, SI-6.1), was pipetted. The respective aliquots of a DMP stock solution listed in Table S2 are added to each well and then brought to a final volume of 210 μL with borate buffer. Each well had a final concentration of 47 μg/mL of Z8P-IgG. The excitation wavelength was 335 nm, emission wavelength was observed at 445 nm. The gain was set to 100%.

**2 mg/mL DMP stock solution preparation:** 16.8 μL of the commercial DMP solution (d = 1.19 g/mL) was added to a sample glass vial containing 10 ml of borate buffer and homogenized with stirring.

**CAUTION:** DMP is an endocrine disrupting chemical, avoid skin contact and handle with care.
Figure S10  a): Fluorescence spectra for Z8P–IgG (with a constant concentration of 47 μg/mL) when dispersed in borate buffer with increasing concentrations of dimethyl phthalate (DMP, 0.0–1.0 g/mL). b): Modified Stern-Volmer fitting curve for the fluorescence quenching of Z8P–IgG versus the DMP concentrations expressed as g/L. The excitation wavelength was 335 nm, and the emission wavelength maximum was recorded at 445 nm. Each data point represents the mean of duplicate measurements.

Table S2  Fluorescence Intensity of Z8P-IgG and relative changes ($F_0/F_{DMP}$) observed to different concentrations of dimethyl phthalate. Final concentration for Z8P-IgG in borate buffer was 47 μg/mL in all wells. The fluorescence intensity was read at 445 nm and converted to the necessary mathematical form ($F_0/F_{DMP}$), where “$F_0$” is the intensity of fluorescence without the quencher (DMP) and “$F_{DMP}$” is the intensity of fluorescence with a specific concentration of the quencher, in order to apply the modified Stern-Volmer equation ($F_0/F_{DMP}$). Duplicates for each system were measured and the standard deviation (S.D.) calculated.

| DMP sample preparation | Fluorescence determination |
|------------------------|---------------------------|
| DMP conc. in borate buffer (g/L) | DMP stock in borate buffer; Aliquots taken (µL) | Fluorescence intensity ($F_{DMP}$) at 445 nm (counts) | $F_0/F_{DMP}$ | S.D. |
|-------------------------|----------------------------|-----------------------------------------------|----------------|------|
| 0                       | 0                          | 33114 ($F_0$)                                 | 1.00           | 0.000 |
| 0.19                    | 20                         | 30838                                         | 1.07           | 0.007 |
| 0.38                    | 40                         | 26660                                         | 1.24           | 0.013 |
| 0.57                    | 60                         | 23149                                         | 1.43           | 0.015 |
| 0.76                    | 80                         | 19471                                         | 1.70           | 0.013 |
| 0.95                    | 100                        | 15414                                         | 2.15           | 0.041 |
5.2 HPLC procedure to obtain the chemical composition of ligands in Z8P

To quantify the concentration of the organic ligands in Z8P, we selected phosphate buffer as the aqueous solvent (solvent A) for HPLC because it is the only buffer that is completely transparent at 210 nm, which was the wavelength required to detect the presence of i2ca by HPLC.

The pH was chosen considering the pKₐ values of the imidazole species (7.46 for 2-mim and for i2ca) to make them less-dissociated and therefore more lipophilic, increasing their retention time on reverse phase HPLC. It was possible to achieve baseline separation for both species at the selected pH of 8.8. (Figure S11d).

HPLC column: Excel C18-PFP with 2 µm particle size (4.6 mm internal diameter × 50 mm length) manufactured by ACE (SI-3.6).

Caution: This Excel C18-PFP column has been manufactured to be stable up to pH 10. However, to avoid unnecessary damage to the column and the HPLC system, both should be flushed after use for at least 20 minutes at a low flowrate with water/methanol to remove damaging residues from buffer which could crystallise in the HPLC equipment and column and also to avoid subjecting the silica-based column to damaging alkaline conditions.

The HPLC method is explained in SI-3.6.1.

![Figure S11. DAD absorption spectra for the analytes constituting the ZIFs of interest highlighting the wavelengths and wavelength range at which each ligand was determined](image-url)

Figure S11. DAD absorption spectra for the analytes constituting the ZIFs of interest highlighting the wavelengths and wavelength range at which each ligand was determined.
Figure S12. Chromatograms of all the species of interest (2-mim [a], i2ca [b], 1-AP [c], the digested Z8P [d], the mobile phase [e], and the mobile phase with TFA [f]). For all species all three chosen wavelengths were shown (from top to bottom: 338 nm, 280 nm and 210 nm). In Figure (d) the positions of all peaks on the digested ZIF was reported; the retention time for 2-mim was 1.53 min, for i2ca was 1.16 min, and for 1-AP was 5.63 min. TFA has two signals at 210 nm, one at 0.65 min and another one at 3.71 min.
5.2.1 Preparation of Standard solutions

Standards stock solutions for the quantification of imidazolate-2-carboxaldehyde (i2ca) and 1-aminopyrene (1-AP) by HPLC were prepared as follows.

Weigh 25 mg of the commercially available starting materials (97% purity for i2ca and 95% for 1-AP) and transfer each to a separate glass flask. Dissolve each in 8 mL of methanol. Full dissolution is only achieved by heating each of them to 60 °C in the sealed glass flask. Once dissolved, allow the solution to cool down to room temperature. Quantitatively transfer the content of the flask to a 10 mL graduated flask. Use 1 mL methanol to clean the glass flask and transfer this rinse solution to the 10 mL graduated flask. Make the solution up to 10 mL volume with methanol. One additional standard solution for each species was independently prepared to confirm that the working standard can be reproducibly prepared.

The stock standard (Stock 1) which will be later used to prepare the set of calibration standard solutions and the duplicate independent standard (Stock 2) were prepared as follows:

For i2ca: Stock 1: 24.20 mg (0.249 mmol) in 10 mL methanol. This stock was used in the preparation of the linearity standard solutions. Stock 2: 25.87 mg (0.261 mmol) in 10 mL methanol was independently prepared, and used to prepare Std 5bis in Table S3.

For 1-AP: Stock 1: 25.70 mg (0.112 mmol) in 10 mL methanol. This stock was used in the preparation of the linearity standard solutions. Stock 2: 25.03 mg (0.109 mmol) in 10 mL methanol was independently prepared, and used to prepare Std 5bis in Table S3.

Table S3 Procedure to prepare the Standard solutions in methanol (mg/mL) used for the calibration curve. The calibration standard solutions Std 1 to Std 8 were prepared by dilution from Stock 1 of 2-mim, i2ca or 1-AP while Std 5b were prepared in duplicate from the independent Stock 2.

| Concentration of analyte [2-mim, i2ca or 1-AP] (mg/mL) | Stock Solution (2.5 mg/mL) [2-mim, i2ca or 1-AP] aliquot added of each analyte (µL) | Aliquot added of methanol to bring solution to 1000 µL (µL) |
|--------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------|
| Blank                                                  | 0                                                                               | 1000                                            |
| Blank                                                  | 0                                                                               | 1000                                            |
| Std 1                                                  | 0.4                                                                            | 160                                            | 840                                            |
| Std 2                                                  | 0.7                                                                            | 280                                            | 720                                            |
| Std 3                                                  | 1.0                                                                            | 400                                            | 600                                            |
| Std 4                                                  | 1.3                                                                            | 520                                            | 480                                            |
| Std 5                                                  | 1.6                                                                            | 640                                            | 360.                                           |
| Std 5 Bis                                              | 1.6                                                                            | 640                                            | 360.                                           |
| Std 5 Bis                                              | 1.6                                                                            | 640                                            | 360.                                           |
| Std 6                                                  | 1.9                                                                            | 760                                            | 240                                            |
| Std 7                                                  | 2.2                                                                            | 880                                            | 120                                            |
| Std 8                                                  | 2.5                                                                            | 1000                                           | 0                                              |
5.2.2 Validation of the HPLC method: Linearity

Linearity tests for each compound were carried out using a series of 8 calibration standard solutions (Std 1 to Std 8) ranging from 0.4 to 2.5 mg/mL. Each calibration standard solution was prepared by pipetting the indicated volumes on Table S3 from a 2.5mg/mL (stock 1), then adding the corresponding methanol aliquot to accurately make a total volume of 1.000 μL.

The concentration of each working standard solution was calculated from the known concentration of Stock 1 or Stock 2. The peak area obtained by HPLC for each working standard solution was plotted against the known concentration of the calibration standard solution used. The concentrations range chosen demonstrates good linearity for all the analytes (Figure S12).

Once linearity has been demonstrated as part of the validation of the HPLC method, all subsequent analyses can be performed by using just one working standard solution prepared at the expected concentration ensuring that the concentration of the working standard and sample solutions are within the demonstrated linearity range. It is desirable to independently weigh 2 working standards to demonstrate that they have been accurately prepared. It is best to prepare the working standard directly and not by dilution of a stock solution. It is recommended to freshly prepare the working standard every time.

Figure S13. Linear calibration curves for each of the analytes of interest (2-mim, top left; i2ca, top right; 1-AP, bottom).
5.2.3 Preparation of Z8P acid digestion sample solutions

This protocol was done for each Z8P batch in triplicate. Approximately 4 mg of Z8P were weighed, transferred to a glass vial and dispersed in 1.5 ml of MeOH. Then 150 µL of concentrated trifluoroacetic acid (TFA) measured with a 500 µL glass syringe with metallic needle was added.

**Note**: after adding the TFA to the methanolic dispersion of the ZIF, the solution will turn to an intense yellow-mustard.

After complete dissolution of the ZIF, an additional 1.3 ml of MeOH is added, then 1.8 mL of a 1 M NaOH solution in water was added using a 2.5 mL automatic pipette, to neutralize the solution. We made sure, using a laser pointer, that there were no colloids present in the solution. We also made sure that the pH of the solution remained slightly acidic (around pH 3-5) using pH paper to avoid any reprecipitation. After cooling, the solution was transferred to a 5 mL volumetric flask and the volume was adjusted with a Pasteur pipette using deionized water as solvent.

**Note**: after 2-3 min of time the colour of the solution will turn from an intense yellow-mustard to almost completely transparent and colourless.

1 mL volume was transferred to a 2 mL HPLC glass vial using a 1 mL pipette. Each sample was injected on to the HPLC column in duplicate.

The results of the HPLC quantification are shown on Table S6, using the peak areas obtained with the HPLC method previously described to calculate their concentration in relation to the peaks areas obtained from the working standard solutions of known concentration. We decided to calculate the final composition of the Z8P ZIF in relation to Zn$^{2+}$ ions. We believe that this assist in a clearer understanding of the relative composition.

| Compound | Mass (mg) | Volume (ml) | Concentration (mg*mL$^{-1}$) | Peak area (counts) | wavelength |
|----------|-----------|-------------|------------------------------|--------------------|------------|
| 2-mim_inj.1 | 19.67 | 20 | 0.9835 | 2319 | 210 nm |
| 2-mim_inj.2 | 19.67 | 20 | 0.9835 | 2299 | 210 nm |
| Average | | | **0.9835** | **2309** | |
| % Rel.St.Dev. | | | | | 0.6 |
| i2ca_inj.1 | 12.15 | 10 | 1.215 | 4010 | 280 nm |
| i2ca_inj.2 | 12.15 | 10 | 1.215 | 4089 | 280 nm |
| Average | | | **1.215** | **4049.5** | |
| % Rel.St.Dev. | | | | | 1.4 |
| 1-AP_inj.1 | 11.05 | 10 | 1.105 | 1334 | 338 nm |
| 1-AP_inj.2 | 11.05 | 10 | 1.105 | 1312 | 338 nm |
| Average | | | **1.105** | **1323** | |
| % Rel.St.Dev. | | | | | 1.2 |

*Table S4: Peak areas determined by HPLC for each of the working standard solutions for each of the 3 compounds. Two different solutions from two independent weights where used.*
Table S5. Peak areas obtained by HPLC for the sample solution of 2-minm, i2ca and 1-AP from the digestion of Z8P. Sample solution were injected in duplicate.

| Sample     | Mass (mg) | Volume (ml) | Concent. (mg/mL) | 2-min Peak Area (nm) | i2ca Peak Area (nm) | 1-AP Peak Area (nm) |
|------------|-----------|-------------|------------------|----------------------|---------------------|---------------------|
| Z8P S1_1_inj.1 | 3.20      | 5.00        | 0.64             | 205.00               | 1214.00             | 35.83               |
| Z8P S1_1_inj.2 | 3.20      | 5.00        | 0.64             | 207.00               | 1225.00             | 36.64               |
| **Average**   |           |             |                  | **206.00**           | **1219.50**         | **36.24**           |
| % Rel.St.Dev. | 0.69      | 0.64        |                  | 1.58                 |                     |                     |
| Z8P S1_2_inj.1 | 3.22      | 5.00        | 0.644            | 202.00               | 1197.00             | 34.62               |
| Z8P S1_2_inj.2 | 3.22      | 5.00        | 0.644            | 206.00               | 1228.00             | 36.46               |
| **Average**   |           |             |                  | **204.00**           | **1212.50**         | **35.54**           |
| % Rel.St.Dev. | 1.39      | 1.81        |                  | 3.66                 |                     |                     |
| Z8P S1_3_inj.1 | 3.61      | 5.00        | 0.722            | 226.00               | 1340.00             | 40.46               |
| Z8P S1_3_inj.2 | 3.61      | 5.00        | 0.722            | 232.00               | 1377.00             | 41.39               |
| **Average**   |           |             |                  | **229.00**           | **1358.50**         | **40.93**           |
| % Rel.St.Dev. | 1.85      | 1.93        |                  | 1.61                 |                     |                     |
| **Average: 6 values** |           |             |                  | **213.00**           | **1263.50**         | **37.57**           |
| % Rel.St.Dev. | 5.9       | 6.0         |                  | 7.2                  |                     |                     |
| Z8P S2_1_inj.1 | 4.23      | 5.00        | 0.846            | 296.00               | 1526.00             | 46.92               |
| Z8P S2_1_inj.2 | 4.23      | 5.00        | 0.846            | 308.00               | 1593.00             | 48.22               |
| **Average**   |           |             |                  | **302.00**           | **1559.50**         | **47.57**           |
| % Rel.St.Dev. | 2.81      | 3.04        |                  | 1.93                 |                     |                     |
| Z8P S2_2_inj.1 | 3.23      | 5.00        | 0.646            | 245.00               | 1185.00             | 30.82               |
| Z8P S2_2_inj.2 | 3.23      | 5.00        | 0.646            | 247.00               | 1196.00             | 32.27               |
| **Average**   |           |             |                  | **246.00**           | **1190.50**         | **31.55**           |
| % Rel.St.Dev. | 0.57      | 0.65        |                  | 3.25                 |                     |                     |
| Z8P S2_3_inj.1 | 3.79      | 5.00        | 0.758            | 264.00               | 1380.00             | 42.00               |
| Z8P S2_3_inj.2 | 3.79      | 5.00        | 0.758            | 271.00               | 1413.00             | 43.26               |
| **Average**   |           |             |                  | **267.50**           | **1396.50**         | **42.63**           |
| St.Dev.       | 1.85      | 1.67        |                  | 2.09                 |                     |                     |
| **Average: 6 values** |           |             |                  | **271.83**           | **1382.17**         | **40.58**           |
| % Rel.St.Dev. | 0.21      | 0.00        |                  | 9.4                  | 12.1                | 18.2                |
| Z8P S3_1_inj.1 | 3.78      | 5.00        | 0.756            | 297.00               | 1331.00             | 42.78               |
| Z8P S3_1_inj.2 | 3.78      | 5.00        | 0.756            | 301.00               | 1349.00             | 43.60               |
| **Average**   |           |             |                  | **299.00**           | **1340.00**         | **43.19**           |
| % Rel.St.Dev. | 0.95      | 0.95        |                  | 1.34                 |                     |                     |
| Z8P S3_2_inj.1 | 4.13      | 5.00        | 0.826            | 319.00               | 1431.00             | 47.41               |
| Z8P S3_2_inj.2 | 4.13      | 5.00        | 0.826            | 337.00               | 1524.00             | 48.85               |
| **Average**   |           |             |                  | **328.00**           | **1477.50**         | **48.13**           |
| % Rel.St.Dev. | 3.88      | 4.45        |                  | 2.12                 |                     |                     |
| Z8P S3_3_inj.1 | 3.95      | 5.00        | 0.79             | 309.00               | 1397.00             | 46.94               |
| Z8P S3_3_inj.1 | 3.95      | 5.00        | 0.79             | 317.00               | 1437.00             | 48.44               |
| **Average**   |           |             |                  | **313.00**           | **1417.00**         | **47.69**           |
| % Rel.St.Dev. | 1.81      | 2.00        |                  | 2.22                 |                     |                     |
| **Average: 6 values** |           |             |                  | **313.33**           | **1411.50**         | **46.34**           |
| % Rel.St.Dev. | 4.6       | 4.9         |                  | 5.5                  |                     |                     |
### Table S6a  Calculation of the concentration of 2-min, i2ac & 1-AP as % g reactant per g Z8P. Zn2+ content is estimated as 100-concentration of 2-min, i2ac & 1-AP

| Sample            | 2-mim | i2ca | 1-AP | Estimated Zn2+ |
|-------------------|-------|------|------|----------------|
|                   | %/g g Z8P | %/g g Z8P | %/g g Z8P | (100 - % ligands) |
| Z8P S1_1_inj.1    | 13.6  | 56.9 | 4.7  | 24.8           |
| Z8P S1_1_inj.2    | 13.8  | 57.4 | 0.0  | 28.8           |
| **Average**       | **13.7** | **57.2** | **2.3** | **26.8**       |
| **St.Dev.**       | 0.7   | 0.6  | 141.4| 10.6           |
| Z8P S1_2_inj.1    | 13.4  | 55.8 | 4.5  | 26.4           |
| Z8P S1_2_inj.2    | 13.6  | 57.2 | 0.0  | 29.2           |
| **Average**       | **13.5** | **56.5** | **2.2** | **27.8**       |
| **St.Dev.**       | 1.4   | 1.8  | 141.4| 7.1            |
| Z8P S1_3_inj.1    | 13.3  | 55.7 | 4.7  | 26.3           |
| Z8P S1_3_inj.2    | 13.7  | 57.2 | 0.0  | 29.1           |
| **Average**       | **13.5** | **56.5** | **2.3** | **27.7**       |
| **St.Dev.**       | 1.9   | 1.9  | 141.4| 7.1            |
| **Average: 6 values** | **13.6** | **56.7** | **2.3** | **13.8**       |
| **% Rel.St.Dev.** | 0.9   | 0.7  | 2.4  | 96.0           |
| Z8P S2_1_inj.1    | 14.9  | 54.1 | 4.6  | 26.3           |
| Z8P S2_1_inj.2    | 15.5  | 56.5 | 0.0  | 28.0           |
| **Average**       | **15.2** | **55.3** | **2.3** | **27.2**       |
| **% Rel.St.Dev.** | 2.8   | 3.0  | 141.4| 4.3            |
| Z8P S2_2_inj.1    | 16.2  | 55.0 | 4.0  | 24.8           |
| Z8P S2_2_inj.2    | 16.3  | 55.5 | 0.0  | 28.2           |
| **Average**       | **16.2** | **55.3** | **2.0** | **26.5**       |
| **% Rel.St.Dev.** | 0.6   | 0.7  | 141.4| 8.9            |
| Z8P S2_3_inj.1    | 14.8  | 54.6 | 4.6  | 25.9           |
| Z8P S2_3_inj.2    | 15.2  | 55.9 | 0.0  | 28.8           |
| **Average**       | **15.0** | **55.3** | **2.3** | **27.4**       |
| **% Rel.St.Dev.** | 1.9   | 1.7  | 141.4| 7.6            |
| **Average: 6 values** | **15.5** | **55.3** | **2.2** | **27.0**       |
| **% Rel.St.Dev.** | 4.1   | 0.0  | 8.4  | 17.3           |
| Z8P S3_1_inj.1    | 16.7  | 52.8 | 4.7  | 25.7           |
| Z8P S3_1_inj.2    | 17.0  | 53.5 | 0.0  | 29.5           |
| **Average**       | **16.8** | **53.2** | **2.4** | **27.6**       |
| **% Rel.St.Dev.** | 0.9   | 0.9  | 141.4| 9.7            |
| Z8P S3_2_inj.1    | 16.4  | 52.0 | 4.8  | 26.8           |
| Z8P S3_2_inj.2    | 17.4  | 55.4 | 0.0  | 27.3           |
| **Average**       | **16.9** | **53.7** | **2.4** | **27.0**       |
| **% Rel.St.Dev.** | 3.9   | 4.5  | 141.4| 1.3            |
| Z8P S3_3_inj.1    | 16.7  | 53.1 | 5.0  | 25.3           |
| Z8P S3_3_inj.2    | 17.1  | 54.6 | 0.0  | 28.3           |
| **Average**       | **16.9** | **53.8** | **2.5** | **26.8**       |
| **% Rel.St.Dev.** | 1.8   | 2.0  | 141.4| 7.9            |
| **Average: 6 values** | **16.9** | **53.6** | **2.4** | **27.2**       |
| **% Rel.St.Dev.** | 0.2   | 0.6  | 2.5  | **17.5**       |
| **Ave. 3 Z8P samples** | **15.31** | **55.18** | **2.31** | **22.67**       |
| **% Rel.St.Dev.** | 10.8  | 2.9  | 4.5  | 33.7           |

\[
\text{% Analyte g/g Z8P} = 100 \times \frac{\text{PA}_{\text{Analyte in Z8P sol.}} \times \text{conc. Std sol. (mg Analyte/mL)}}{\text{PA}_{\text{Analyte in Std sol.}} \times \text{conc Std solution (mg Z8P/mL)}} \times 1000 \text{mg/g (Z8P)}
\]

PA stand for Peak Area

* PA Analyte in Z8P sol. * conc. Std sol. (mg Analyte/mL) * 1000 mg/g (Z8P)
* PA Analyte in Std sol. * conc Std solution (mg Z8P/mL) * 1000 mg/g (Analyte)
| MW (g/mol) | 82.1 | 96.09 | 217.27 | 65.38 |
|-----------|------|-------|--------|-------|
| Sample    | 2-mim | i2ca  | 1-AP   | Estimated Zn$^{2+}$ |
|           | mmol/g Z8P | mmol/g Z8P | mmol/g Z8P | mmol/g Z8P |
| ZBP S1_1_inj.1 | 1.66 | 5.92 | 0.22 | 3.79 | 2.00 |
| ZBP S1_1_inj.2 | 1.68 | 5.98 | 0.22 | 3.67 | 2.08 |
| Average   | 1.67  | 5.95  | 0.22  | 3.73 | 2.04 |
| % Rel.St.Dev. | 0.7  | 0.6   | 1.6   | 2.2  | 2.8  |
| ZBP S1_2_inj.1 | 1.63 | 5.80 | 0.21 | 4.04 | 1.84 |
| ZBP S1_2_inj.2 | 1.66 | 5.95 | 0.22 | 3.74 | 2.04 |
| Average   | 1.64  | 5.88  | 0.21  | 3.89 | 1.94 |
| % Rel.St.Dev. | 1.4  | 1.8   | 3.7   | 5.4  | 7.1  |
| ZBP S1_3_inj.1 | 1.62 | 5.80 | 0.22 | 4.02 | 1.84 |
| ZBP S1_3_inj.2 | 1.67 | 5.96 | 0.22 | 3.72 | 2.05 |
| Average   | 1.65  | 5.88  | 0.22  | 3.87 | 1.95 |
| % Rel.St.Dev. | 1.9  | 1.9   | 1.6   | 2.2  | 3.0  |
| Average: 6 values | 1.65 | 5.90 | 0.22 | 3.83 | 1.98 |
| Std.Dev.  | 1.3   | 1.4   | 2.3   | 4.2  | 5.4  |
| ZBP S2_1_inj.1 | 1.82 | 5.63 | 0.21 | 4.03 | 1.85 |
| ZBP S2_1_inj.2 | 1.89 | 5.88 | 0.22 | 3.55 | 2.19 |
| Average   | 1.85  | 5.76  | 0.22  | 3.79 | 2.02 |
| % Rel.St.Dev. | 2.8  | 3.0   | 1.9   | 8.9  | 11.8 |
| ZBP S2_2_inj.1 | 1.97 | 5.73 | 0.18 | 3.80 | 2.03 |
| ZBP S2_2_inj.2 | 1.98 | 5.78 | 0.19 | 3.67 | 2.12 |
| Average   | 1.98  | 5.75  | 0.19  | 3.73 | 2.07 |
| % Rel.St.Dev. | 1.9  | 1.9   | 2.1   | 5.6  | 7.5  |
| Average: 6 values | 1.89 | 5.75 | 0.21 | 3.78 | 2.03 |
| Std.Dev.  | 1.4   | 1.6   | 7.4   | 4.9  | 6.5  |
| ZBP S2_3_inj.1 | 1.81 | 5.68 | 0.21 | 3.96 | 1.89 |
| ZBP S2_3_inj.2 | 1.85 | 5.82 | 0.22 | 3.68 | 2.08 |
| Average   | 1.83  | 5.75  | 0.22  | 3.82 | 1.99 |
| % Rel.St.Dev. | 1.9  | 1.7   | 2.1   | 5.2  | 6.9  |
| Average: 6 values | 1.89 | 5.75 | 0.21 | 3.78 | 2.03 |
| Std.Dev.  | 4.0   | 1.6   | 7.4   | 4.9  | 6.5  |
| ZBP S3_1_inj.1 | 2.04 | 5.50 | 0.22 | 3.93 | 1.92 |
| ZBP S3_1_inj.2 | 2.07 | 5.57 | 0.22 | 3.78 | 2.02 |
| Average   | 2.05  | 5.53  | 0.22  | 3.85 | 1.97 |
| % Rel.St.Dev. | 3.9  | 4.5   | 2.1   | 12.8 | 17.1 |
| ZBP S3_2_inj.1 | 2.00 | 5.41 | 0.22 | 4.10 | 1.81 |
| ZBP S3_2_inj.2 | 2.12 | 5.76 | 0.23 | 3.41 | 2.31 |
| Average   | 2.06  | 5.59  | 0.22  | 3.76 | 2.06 |
| % Rel.St.Dev. | 3.9  | 4.5   | 2.1   | 12.8 | 17.1 |
| ZBP S3_3_inj.1 | 2.03 | 5.52 | 0.23 | 3.87 | 1.95 |
| ZBP S3_3_inj.2 | 2.08 | 5.68 | 0.24 | 3.55 | 2.19 |
| Average   | 2.06  | 5.60  | 0.23  | 3.71 | 2.07 |
| % Rel.St.Dev. | 1.8  | 2.0   | 2.2   | 6.1  | 8.1  |
| Average of 6 | 2.06 | 5.57 | 0.23 | 3.77 | 2.03 |
| % Rel.St.Dev. | 3.9  | 4.5   | 2.1   | 12.8 | 17.1 |
| Ave.3 Z8P samples | 1.87 | 5.74 | 0.22 | 3.80 | 2.01 |
| % Rel.St.Dev. | 10.8 | 2.9   | 4.3   | 0.8  | 1.5  |

Table S6b  Calculation of the concentration of 2-min, i2ac, 1-AP and Zn$^{2+}$ as mmol reactant/g Z8P.

Analyte mmol/g Z8P = \( \frac{\text{PA \text{ Analyte in Z8P sol.}} \times \text{conc. Std sol. (mg \text{ Analyte} /mL)} \times 1000 \text{ mg/g (Z8P)} \times 1000 \text{ mmol/mol (Analyte)}}{\text{PA \text{ Analyte in Std sol.}} \times \text{conc Std solution (mg \text{ Z8P} /mL)} \times 1000 \text{ mg/g (Analyte)} \times \text{MW g/mol (Analyte)}} \)
5.3 Quantification of Zn$^{2+}$ content on Z8P by ICP-OES

ICP-OES (inductively coupled plasma atomic emission spectroscopy) was used in order to obtain the quantitative content of Zn$^{2+}$ in ZIF samples. ZIF must undergo a wet digestion procedure before the samples can be analysed by ICP-OES.

5.3.1 Suitable disintegration procedure for Z8P analyses by ICP-OES

The disintegration procedure is performed as follows. The samples were divided into four synthetic batches. The solution for each batch was independently prepared in duplicate and each solution was measured five times by ICP-OES.

1. Around 1 to 3 mg of each sample of Z8P was weighted
2. The sample was dissolved in 2 mL of HNO$_3$ 30 %v/v. Concentrated HNO$_3$, previously purified by sub-boiling in a PFA device.
3. The disintegrated ZIF sample in concentrated HNO$_3$ was diluted with ultrapure deionized water to a volume of 10 mL and mixed well.

**Note:** When weighting the samples for ICP quantification, the weighted amounts were very small (1,115 mg and 1,118mg for sample 1; 2.863 mg and 2,865 for sample 2; 0.76 mg and 0.78 mg sample 3) and they were dissolved it to 10 ml as final volume. This dilution magnifies any error that is carried from the whole process.

ICP determination of a Zn$^{2+}$ in the samples was performed against an external standard solution of a Zn$^2+$ which is directly traceable to the NIST SRM 3169 (Certipur© Certified Reference Material, Merck, Germany).

5.3.2 ICP-OES parameter for analysis of disintegrated Z8P

The operating conditions for the ICP-OES (Agilent 5110 VDV) are listed on Table S7. The quantitation of Zn$^{2+}$ in samples are summarized in Table S8.

*Table S5a Operational conditions for the ICP OES analysis*

| Parameter                        | Operating condition |
|----------------------------------|---------------------|
| RF power (kW)                    | 1.2                 |
| Auxiliary gas flow rate (L·min$^{-1}$) | 1.00               |
| Plasma gas flow rate (L·min$^{-1}$) | 12.00              |
| Nebulizer gas flow rate (L·min$^{-1}$) | 0.70               |
| Viewing height (mm)              | 8                   |
| Pump speed (rpm)                 | 15                  |
| Stabilization time (s)           | 30                  |
| Integration time (s)             | 5                   |
| Replicates                       | 5                   |
| Viewing mode                     | Radial              |
| Wavelength (nm)                  | 202.548 (Zn II)     |
5.3.3 ICP Determination of Zn$^{2+}$: Results

The results of the ICP determination of the Zn content are as shown on Table S8. It reinforces the results of the composition obtained via HPLC on Figure S13.

Table S7b  Results for zinc determination in Z8P by an ICP-OES method. Uncertainties were reported as the combined standard deviation from two weighing and five repetitions.

| Z8P Sample | Zn w/w % | RSD * | Zn$^{2+}$ (g/g ZIF) | Zn (µmol/g ZIF) |
|------------|----------|-------|---------------------|---------------|
| A          | 20.8     | 0.9   | 0.208               | 3.181         |
| B          | 18.2     | 0.98  | 0.182               | 2.784         |
| C          | 19.5     | 0.69  | 0.195               | 2.983         |
| D          | 16.6     | 3.59  | 0.166               | 2.539         |

5.3.4 Summary of HPLC and ICP-OES results

Table S8  Tabulation of reactants added to form target Z8P versus concentration of bound reactants in target Z8P determined by HPLC, both referred to g target Z8P. This table is illustrated on Figure S13.

| Z8P S1 | MW (g/mol) | Added reactants to synthesise target Z8P |
|--------|------------|----------------------------------------|
|        | Reagent    | mg added | mmols Reactant per g target Z8P | % mol/mol Reactants per g target Z8P |
|        | 2-mim      | 82.1     | 40.400 | 16.26 | 37.8 |
|        | i2ca       | 96.09    | 47.280 | 16.26 | 37.7 |
|        | 1ap        | 217.27   | 16.080 | 2.45  | 5.7 |
|        | Zn$^{2+}$  | 65.38    | 16.043 | 8.11  | 18.8 |
|        | Total      |          | 119.803 | 43.068 | 100.0 |

Weight of target Z8P after wash (mg) 30.267

Zn$^{2+}$ = 100-%ligands

| Z8P S1 | MW (g/mol) | Bound reactants in target Z8P Composition obtained by HPLC |
|--------|------------|----------------------------------------------------------|
|        |            | g Reactant per g target Z8P | mmols Reactant per g target Z8P | % mol/mol Reactants per g target Z8P |
|        | 2-mim      | 82.1     | 15.31 | 1.87  | 16.05 |
|        | i2ca$^{free}$ | 96.09   | 55.18 | 5.74  | 49.43 |
|        | Imine      | 217.27   | 4.69  | 0.22  | 1.86 |
|        | Zn$^{2+}$  | 65.38    | 24.81 | 3.80  | 32.66 |
|        | Total      |          | 11.619 | 100.00 |

[i] = [1-AP] = [i2ca$^{bound}$]  
[i2ca$^{free}$] = [i2ca$^{total}$] - [1-AP]
Figure S14  **Graphical representation of the results of the chemical composition of Z8P determined by HPLC expressed in %mol/mol reactants per g of target Z8P as calculated on Table S8.** After reaction of 120 mg of reactants (2-mim, i2ca, 1-AP and Zn2+) and extensive washing of the excess of reactants and intensive drying (5 mbar, 70 °C for 2 days), an average of 30 mg of target Z8P is obtained, resulting on an approximate yield of 25% in mass. Zn2+ values calculated from the HPLC data was obtained by deducting the total percentage (%g/g Z8P) for 2-mim, i2ca and 1-AP from the total (100%), assuming all the non-accounted for mass on the Z8P comes from Zn2+. The calculated ratio of the structural constituents for the ZIF is compared to the expected levels based on: the crystal structure of the corresponding Z8P which are stoichiometrically formed by 2 imidazole bridging species per one Zn2+ metallic core. Using the concentration values from Table S6b and dividing the total mols for i2ca and 2mim by the mols of Zn2+, we obtain a value of 2.01 very close to the stoichiometric composition of the crystal.

**Note 1:** We assume that 1 eq. of the imine is formed quantitatively from 1 eq. of 1-ap added, reacting with 1 eq. of added i2ca

**Note 2:** 2-mim needs to be added in excess to form controlled nanosized Z8P (50-200 nm)
5.4 Powder X-Ray diffractograms for the ZIFs, both conjugated and unconjugated

Both conjugated and unconjugated ZIFs were analysed by X-ray powder diffraction with Bragg-Brentano symmetry to determine the crystal structure of the obtained ZIFs. The parameters for all diffractograms are: 2θ 5-50°; step size 0.02°; integration time 6 sec.

These measurements demonstrate that the ZIFs conserve its characteristic SOD (sodalite) crystalline structure even after the conjugation process has taken place.

5.4.1 Preparation of PXRD sample of non-conjugated ZIFs

To prepare the non-conjugated ZIF samples, each ZIF powder was softly ground in an agate mortar with an agate pestle, then placed on a Bragg-Brentano PVC sample holder with an opening of 0.8 cm in diameter. The cavity of the sample holder was filled with the powder and with the help of a glass slide, the top surface of the sample was compacted flat and flash with the top surface of the holder.

5.4.2 Preparation of PXRD sample of conjugated ZIFs

For the conjugated ZIFs, 400 μL of a freshly prepared ZIF solution (SI-6.1) from each conjugate (a total mass of 0.3 mg for each measurement), was transferred to an Eppendorf tube, centrifuged and all excess supernatant to 50 μL carefully removed with a pipette. This remaining 50 μL suspension was placed on a zero-background silicon monocrystalline (SIOV) sample holder using a 100 μL pipette. The measurement was started immediately, with the sample still wet.
Figure S16  Powder diffractograms for ZIF-90 (a), Z8P (b), Z90-IgG conjugate (c) and Z8P-IgG conjugate (d). All samples were measured in Bragg-Brentano symmetry. The position of all diffractions, as well as the relative intensity between a), b), c) and d) match the expected sodalite structure, showing that they all were isostructural. The most relevant Miller indices are shown.[11]
6 Conjugation of ZIFs with sheep IgG

In this section we present the two-step protocol to covalently bind the free amino functionality of the lysine residue \((\text{CH}_2)_4\text{NH}_2\) of the antibody tested to the free aldehyde functionality part of the imidazole \(2\text{-carboxaldehyde}\), included on the ZIFs of interest (ZIF-90 and the fluorescently modified ZIF-8/ZIF-90 hybrid, Z8P).

In SI-9 we discuss how we demonstrate that this binding has been successfully achieved.

6.1 General procedure scheme

![Schematic representation of the conjugation reaction between the ZIFs and the antibodies.](image)

**Figure S17**  Schematic representation of the conjugation reaction between the ZIFs and the antibodies. In the first step the dispersed particles were mixed in solution with the antibodies. The free amino functionalities from lysine residues side chains in of the antibodies can react with the aldehyde functionality on the ZIF to form an imine bond. Sodium cyanoborohydride was added in the second step to selectively reduce the imine bond to an amine, rendering the conjugation irreversible under assay conditions.

6.1.1 Conjugation of ZIF-90 and Z8P with anti-mouse IgG protocol

1) Weight 1.5 mg of the ZIF (ZIF-90 or Z8P) on a 5-figure balance and transfer it to a small glass vial with a plastic cap.
2) Add 1.5 mL of a borate buffer solution (see SI-2.2.1), measured with a 2.5 mL pipette.
3) Seal the glass vial with the cap, and sonicate for 15 min.
4) Add 50 μL of a 2 mg/mL solution of **sheep anti-mouse IgG** (see SI-1.6) to this dispersion without any previous purification step using a 100 μL pipette.
5) Add a magnetic stirrer bar, seal the glass vial, and stir the mixture for 15 min at room temperature.
6) Add 50 μL of a commercially prepared aqueous solution of **Na[BH$_3$(CN)]** (5 M) in 1 M NaOH using a 200 μL pipette and allow to react for an additional 4 hours at room temperature.

**Warning:** Exercise extreme care when handling Cyanoborohydride. Always wear gloves, glasses and work in a properly enclosed fume hood.

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6.1.1.2 **Protocol for washing the freshly prepared ZIF-90-IgG or Z8P-IgG**

The next steps were designed to remove any anti-mouse IgG not covalently bonded to ZIF-90 or Z8P. The washing protocol of this conjugation reaction is as follows:

1) Transfer the 1.5 mL suspension to a 2 mL Eppendorf tube with the help of a 1 mL pipette and centrifuge it at 15000 rpm for 5 min.
2) Carefully remove the supernatant taking care not to disturb the solid residue and transfer it to a common container labelled as “Wash 1”. This wash is reserved for later use, in case the ELISA experiment fails suggesting unsuccessful binding between the IgG and the ZIF. “Wash 1” will help to investigate if the free IgG in the wash was active or inactive. “Wash 1” is disposed of, after successful completion of ELISA experiments.

**Warning:** The very first supernatant of “Wash 1” contain cyanide derivatives. Extreme care must be exercised when dealing with and disposing of “Wash 1”.

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3) Add 1 mL of borate buffer to the Eppendorf tube to wash the solid residue.
4) Sonicate the suspension for 2 min to ensure full dispersion of the solid particles.
5) Centrifuge the dispersion at 15000 rpm for 5 min.
6) Remove the supernatant carefully avoiding contact with the solid residue and transfer to a separately container labelled as “Wash 2”. Wash-2 is also reserved for later use, in case the ELISA experiment fails suggesting unsuccessful binding between the IgG and the ZIF. “Wash 2” will further help to investigate if the free IgG in the wash was active or inactive. “Wash 2” is disposed of, after successful completion of ELISA experiments.
7) Add 1 mL of TRIS buffer (see SI-2.2.3) to the residue.
8) Redisperse the solid residue by vigorous agitation, and centrifuge at 15000 rpm for 5 min.
9) Carefully remove this TRIS buffer supernatant and discard it.
10) Repeat steps 7-9 three more times to wash out any IgG not properly conjugated to the ZIF
11) Perform steps 7-9 two additional times, this time with borate buffer
12) Disperse the properly washed solid residue containing the ZIF conjugate in 2 mL of borate buffer. This is the ZIF-IgG stock solution (0.75 mg/ml) used in all the ELISA, LFIA, LC/MS and PXRD characterization.

**Addendum:** The amount of washing steps was developed so that only the covalently bound antibody remains on the ZIF particles.

### 7 Studies on conjugated antibody activity

In this section we study the residual activity of the antibodies bound to the nanoparticulate ZIFs. We show the viability of the ZIFs as fluorescent bio labels on a series of non-competitive ELISA experiments.

#### 7.1 Model non-competitive indirect ELISA

![Figure S18](image_url)  
**Figure S18** Schematic representation of the general non-competitive indirect ELISA procedure developed to study the antibody activity of the conjugate. The electrostatically charged surface of the high-binding microtiter plates (MTP) was coated with the mouse IgG and incubated on a plate shaker. After washing, the remaining binding sites on the MTP were blocked with casein to avoid any passive adsorption of the conjugates to the surface of the MTP ("non-specific binding"), which would lead to false positive results. After another incubation, the plates were washed again and dilutions of an anti-mouse IgG antibody (or a modified system including anti-mouse IgG) at various concentrations were added and the MTP incubated for 18 hours on a plate shaker. After an additional washing step, a solution with the HRP-labelled detection antibody (anti-sheep IgG) was added and incubated for 1 hour on a microplate shaker. After a final washing step, the substrate 3,3',5,5'-tetramethylbenzidine (TMB) was added to each well and incubated for 15 min on a microplate shaker, then 100 µL of 1 M sulphuric acid was added to each well and the optical density (OD) was measured. The response that should be obtained, should the system response correctly, would be a characteristic sigmoidal calibration curves, obtained by fitting a four-parameter logistic function to the data points.
The basic model to assess antibody activity by a non-competitive ELISA methodology is depicted in Figure S17. It is a layered sandwich system which has a base formed by:

1. **Target**: mouse IgG
2. **Primary [selective antibody]**: sheep anti-mouse IgG
3. **Detector**: HRP labelled donkey anti-sheep IgG

This sandwich system is used to characterize the activity response of the primary, analyte-selective antibody. This system will be later modified by switching the primary antibody for our synthesised ZIF-IgGs.

The system will be quantified via HRP (Horse Radish Peroxidase) redox reaction driven TMB colour development, in which HRP triggers the conversion of TMB (transparent) to its oxidized coloured product (first intense blue, then bright yellow after the addition of 1M sulfuric acid)[12]; the coloration is proportional to the amount of HRP present on the system which, in turn, is proportional to the amount of primary present on the system.

The calibrating solutions were prepared by making serial dilutions from a stock solution of the antibody species to be assessed (anti-mouse IgG or ZIF-IgG conjugate). The rest of the species taking part on the experiment should be maintained at constant concentrations. In order to compare accurately the amounts of soluble antibodies, each well was processed under the identical conditions, and 4 replicates of each measurement was performed.

### 7.2 Protocol of the non-competitive indirect ELISA for the anti-sheep IgG primary

In this version of the protocol we assess the activity of our base ELISA system [mouse IgG (target) / sheep anti-mouse IgG (from sheep, primary, selective antibody) / anti-sheep IgG-HRP (from donkey, enzyme-labelled detector antibody)].

The standard curve was prepared by making serial dilutions of one known concentration of the primary (sheep anti-mouse IgG) across a range of concentrations (0.25-750 ng/ml), while keeping every other parameter of the system constant. Each well was treated under the same conditions, and each measurement was done in quadruplicate.

For details on the antibodies see SI-1.6.

The protocol is performed at follows:

1) Coat a 96 well transparent high-binding microtiter plate (flat-bottom, Nunc MaxiSorp™) with the target mouse IgG (Capture antibody solution, SI-7.2.1) by transferring 200 µL of the capture antibody solution into each well using a 200 µL multichannel electronic pipette
2) Seal the plates with Parafilm® to prevent evaporation
3) Incubate for 4 h on a microplate shaker
4) Wash the wells of the microtiter plates with a 3-repetition cycle automatized microtiter washer using a PBS-based washing buffer (SI-2.2.6).
5) Transfer 200 µL of the casein solution (Casein blocking solution, SI-7.2.2) into each well using a 200 µL multichannel electronic pipette. This will ensure that the remaining binding sites on the MTP are blocked
6) Seal the plates with Parafilm® to prevent evaporation
7) Incubate for 1 h on a microplate shaker
8) Wash the plates again after this incubation, as in point 4
9) Add 200 µL of the sheep anti-mouse IgG solutions (sheep anti-mouse IgG solutions in borate buffer, SI-7.2.3) to the corresponding wells using a 200 µL pipette
10) Seal the plates with Parafilm® to prevent evaporation
11) Incubate for 18 hours on a microplate shaker
12) Wash the plates again after this incubation, as in point 4
13) Add 200 µL of the anti-sheep HRP-labelled IgG antibody (HRP-labelled detection antibody, SI-7.2.4) to each well using a 200 µL multichannel electronic pipette
14) Seal the plates with Parafilm® to prevent evaporation
15) Incubate for 1 hour on a microplate shaker
16) Wash the plates again after this incubation, as in point 4
17) Add 200 µL of the TMB solution (TMB-based substrate solution, SI-7.2.5) to each well using a 200 µL multichannel electronic pipette and incubate for 15 min on a microplate shaker. Each wells solution will turn from transparent to blue in colour
18) Add 100 µL of the stop solution (1 M sulphuric acid) to each well. The colour will change from blue to yellow
19) Measure the optical density (OD) of the wells (SI-3.8)

Characteristic sigmoidal calibration curves were obtained by fitting a four-parameter logistic function in Origin to the data points obtained from OD measurements. The sigmoidal shape of Standard calibration curves is expected when the binding occurs via intact antibody binding sites and not, e.g., due to non-specific binding. The calibration standard solutions were prepared by making serial dilutions from a stock solution of the antibody species to be assessed (anti-mouse IgG), while keeping the rest of the solution constituents at constant concentrations. In order to compare the amounts of soluble antibodies, each well was processed under the same conditions, and 4 replicates of each measurement were performed in.

7.2.1 Preparation of Capture antibody solution

1) Transfer 20 mL of borate buffer (see SI-2.2.1) to a long glass vial with a plastic cap using a 10 mL pipette
2) Transfer 1.5 µL of mouse IgG using a 5 µL pipette. Wash the tip by aspirating/dispensing the solution several times
3) Seal the glass vial and homogenize the mixture using a mechanical shaker

7.2.2 Preparation of Casein blocking solution

1) Weigh 20 mg of casein and transfer to a long glass vial with a plastic cap
2) Add 20 mL of PBS buffer (see SI-2.2.2) using a 10 mL pipette
3) Seal the glass vial and homogenize the mixture using a mechanical shaker
7.2.3 Preparation of sheep anti-mouse IgG solutions in borate buffer

7.2.3.1 Preparation of 3 stock solutions

Prepare in total 3 stock solutions, each to a total volume of 1 mL. Stock solution MS1 was prepared from the original stock solution of the sheep anti-mouse IgG (2 mg/mL). Stock solution MS2 was prepared by dilution from MS1; Stock solution MS3 was prepared by dilution from MS2.

MS1 was prepared as follows:

1) Transfer 1 mL of borate buffer inside a 1.5 mL Eppendorf tube
2) Remove 25 µL of the buffer using a 100 µL pipette
3) Transfer 25 µL of the original stock solution of the sheep anti-mouse IgG with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

MS2 was prepared as follows:

1) Transfer 1 mL of borate buffer inside a new 1.5 mL Eppendorf tube
2) Remove 100 µL of borate buffer with a 100 µL pipette
3) Transfer 100 µL of the MS1 stock solution with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

MS3 was prepared as described for MS2 but using MS2 as the stock solution

The concentration for the MS solutions will be:

- MS1 = 50 µg/mL;
- MS2 = 5 µg/mL;
- MS3 = 0.5 µg/mL;

7.2.3.2 Preparation of the dilution series for assessing antibody activity of anti-mouse IgG

We prepare 11 new solutions from these four stock solutions (MS1, MS2 and MS3) to varying concentrations for sheep anti-mouse IgG.

The procedure for each dilution series stock (number #) is performed as follows:

1) Transfer 1 mL of borate buffer into a new Eppendorf tube.
2) Using the suitable micropipette, remove from the Eppendorf tube a volume of borate buffer equivalent to the corresponding cell on the column named “aliquot volume from MS stock solution” as listed on Table S9.
3) Using the same micropipette, transfer the aliquot volume corresponding to the exact same cell as on step 3 from Table S9, with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

WARNING: In order to keep the antibodies stable during the whole process all solutions are kept on ice.
Table S6  Table for the preparation of the solutions for the sheep anti-mouse IgG solutions in borate buffer. The * represent the volume of buffer to be replaced by the stock solutions (MS1, MS2, MS3 and MS4)

| Dilution series stock # | MS Stock solution used | aliquot volume from MS stock solution* (µL) | Concentration in stock solution (ng/mL) |
|-------------------------|------------------------|---------------------------------------------|----------------------------------------|
| 1                       | MS1                    | 15                                          | 750.00                                 |
| 2                       | MS1                    | 10                                          | 500.00                                 |
| 3                       | MS1                    | 5                                           | 250.00                                 |
| 4                       | MS1                    | 2.5                                         | 125.00                                 |
| 5                       | MS2                    | 10                                          | 50.00                                  |
| 6                       | MS2                    | 5                                           | 25.00                                  |
| 7                       | MS2                    | 2                                           | 10.00                                  |
| 8                       | MS3                    | 10                                          | 5.00                                   |
| 9                       | MS3                    | 5                                           | 2.50                                   |
| 10                      | MS3                    | 2                                           | 1.00                                   |
| 11                      | MS3                    | 0.5                                         | 0.25                                   |

7.2.4 Preparation of the solution of HRP-labelled detection antibody

1) Transfer 10 mL of PBS buffer (see SI-2.2.2) to a long glass vial with a plastic cap using a 10 mL pipette
2) Transfer 1.5 µL of anti-sheep IgG HRP labelled using a 5 µL pipette. Wash the tip by aspirating/dispensing the solution several times.
3) Close the glass vial, and homogenize the mixture using a mechanical shaker

7.2.5 Preparation of the solution of TMB-based substrate

1) Transfer 22 mL of citrate buffer (see SI-2.2.4) to a long glass vial with a plastic cap using a 10 mL pipette

**WARNING:** each pipette tip was used only once, since the citrate buffer was cold after being kept in the fridge for stability purposes. Using the same pipette tip for transferring all 22 ml can confer significant error, as the shape of the plastic tip becomes deformed.

2) Add 8.5 µL of H₂O₂ (30% v/v) using a 10 µL pipette
3) Add 550 µL of the TMB solution (SI-2.2.7) using a 1 mL pipette
4) Close the glass vial, and homogenize the mixture using a mechanical shaker
Table S7  Optical density (OD) values obtained in the non-competitive indirect ELISA of the anti-mouse IgG with the associated standard deviation. These values are displayed graphically in Figure S18.

| Initial concentration for sheep IgG in Borate Buffer with NaCl (ng/mL) | Optical Density (OD) | Standard Deviation (S.D.) |
|-------------------------------------------------|----------------------|--------------------------|
| 750                                            | 3.400                | 0.013                    |
| 500                                            | 3.203                | 0.066                    |
| 250                                            | 2.866                | 0.159                    |
| 125                                            | 2.374                | 0.049                    |
| 50                                             | 2.002                | 0.029                    |
| 25                                             | 1.539                | 0.030                    |
| 10                                             | 0.798                | 0.031                    |
| 5                                              | 0.494                | 0.017                    |
| 2.5                                            | 0.307                | 0.014                    |
| 1                                              | 0.150                | 0.011                    |
| 0.25                                           | 0.049                | 0.040                    |

Table S8  Controls performed to check any cross reactivity in the indirect ELISA. All controls have been done with a very high concentration for all the compounds that could show cross-reactivity with the primary (anti-mouse IgG). Anti-sheep-HRP has some cross-reactivity against the mouse IgG deposited on the surface of the plate but this was not so strong as to interfere with the signal of the indirect ELISA. The ZIF-IgG conjugates, tested at very high concentrations, show a residual signal when no mouse IgG was present, thus showing non-interaction and potential false positives.

| Controls | Concentration of Z8P–IgG conjugate (ng/mL) | Concentration of anti-sheep–HRP (ng/mL) | Concentration of Casein (ng/mL) | OD   | S.D.  |
|----------|---------------------------------------------|-----------------------------------------|---------------------------------|------|-------|
| Mouse IgG + casein + anti-sheep IgG–HRP        | 0                                           | 200                                    | 1000                           | 0.191| 0.002 |
| Casein + ZIF–IgG conjugate + anti-sheep IgG–HRP | 1500                                       | 200                                    | 1000                           | 0.085| 0.007 |
|                                                   | 1125                                       | 200                                    | 1000                           | 0.079| 0.008 |
|                                                   | 750                                        | 200                                    | 1000                           | 0.067| 0.004 |
| Casein + anti-sheep IgG–HRP                      | 0                                           | 200                                    | 1000                           | 0.057| 0.003 |
Figure S19  Non-competitive indirect ELISA calibration curves for the system of interest. The optical density (OD) was plotted against the initial concentration for the sheep anti-mouse-IgG in ng/mL. Controls were performed to check if there was any cross-reactivity in the indirect ELISA (Table S11).

**Conclusion:** The activity of our primary (sheep anti-mouse IgG) is tested and proven. This is a suitable base system to modify it including our ZIF-IgG conjugates s primaries.

### 7.3 Protocol of the non-competitive indirect ELISA for the ZIF-IgG conjugates

In this version of the protocol we assess the residual activity of the antibodies bound to both our ZIF-IgG conjugates by a modified ELISA system [mouse IgG (target) / ZIF-IgG (Z8P-IgG and Z90-IgG, primary, selective antibody) / anti-sheep IgG-HRP (from donkey, enzyme-labelled detector antibody)].

The standard curve was prepared by making serial dilutions of one known concentration of the analyte (ZIF-IgG conjugates, both Z90-IgG and Z8P-IgG) across a range of concentrations (0-150 ng/ml), while keeping every other parameter of the system constant. Each well was treated under the same conditions, and each measurement was done in quadruplicate.

The protocol described in SI-7.2 was performed with the following modification:

**Step 9 in SI-7.2 protocol:** Add 200 µL of the ZIF-IgG conjugate solutions (ZIF-IgG solutions in borate buffer, SI-7.3.1) to the corresponding wells using a 200 µL pipette. Incubate for 18 hours.
7.3.1 Preparation of ZIF–IgG solutions in borate buffer:

7.3.1.1 Preparation of 4 stock solutions of ZIF-IgG

Prepare in total 4 stock solutions, each to a total volume of 1 mL. Stock solution MS1 was prepared from the initial stock solution of Z8P-IgG (0.75 mg/mL) and Z90-IgG (0.75 mg/mL); Stock solution MS2 was prepared by dilution from MS1; Stock solution MS3 was prepared by dilution from MS2; Stock solution MS4 was prepared by dilution from MS3.

MS1 was prepared as follows:
1) Transfer 1 mL of borate buffer (see SI-2.2.1) inside a 1.5 mL Eppendorf tube
2) Remove 10 µL of the buffer using a 100 µL pipette
3) Transfer 10 µL of the original stock solution of the ZIF-IgG (Z90-IgG or Z8P-IgG) with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

MS2 was prepared as follows:
1) Transfer 1 mL of borate buffer inside a new 1.5 mL Eppendorf tube
2) Remove 100 µL of borate buffer with a 100 µL pipette
3) Transfer 100 µL of the MS1 stock solution with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

MS3 was prepared as described for MS2 but using MS2 as the stock solution

MS4 was prepared as described for MS2 but using MS3 as the stock solution

The concentration for the MS solutions will be:

- MS1 = 7.5 µg/mL;
- MS2 = 0.75 µg/mL;
- MS3 = 0.075 µg/mL;
- MS4 = 0.0075 µg/mL.

7.3.1.2 Preparation of the dilution series for assessing antibody activity of ZIF-IgG

We prepare 23 new solutions from these four stock solutions (MS1, MS2, MS3 and MS4) to varying concentrations for ZIF-IgG (both Z90-IgG and Z8P-IgG).

The procedure for each dilution series stock (number #) is performed as follows:

1) Transfer 1 mL of borate buffer into a new Eppendorf tube.
2) Using the suitable micropipette, remove from the Eppendorf tube a volume of borate buffer equivalent to the corresponding cell on the column named “aliquot volume from MS stock solution” as listed on Table S12.
3) Using the same micropipette, transfer the aliquot volume corresponding to the exact same cell as on step 3 from Table S12, with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker
**WARNING:** In order to keep the antibodies stable during the whole process all solutions are kept on ice.

Table S9  Table for the preparation of the ZIF–IgG solutions in borate buffer. The * represent the volume of buffer to be replaced by the stock solutions (MS1, MS2, MS3 and MS4).

| Dilution series stock # | MS Stock solution used | aliquot volume from MS stock solution* (µL) | Concentration in stock solution (ng/mL) |
|-------------------------|------------------------|-------------------------------------------|----------------------------------------|
| 1                       | MS1                    | 20                                        | 150                                    |
| 2                       | MS1                    | 15                                        | 112.5                                  |
| 3                       | MS1                    | 10                                        | 75                                     |
| 4                       | MS1                    | 8                                         | 60                                     |
| 5                       | MS1                    | 6                                         | 45                                     |
| 6                       | MS1                    | 4                                         | 30                                     |
| 7                       | MS1                    | 2                                         | 15                                     |
| 8                       | MS2                    | 10                                        | 7.5                                    |
| 9                       | MS2                    | 8                                         | 6                                      |
| 10                      | MS2                    | 6                                         | 4.5                                    |
| 11                      | MS2                    | 4                                         | 3                                      |
| 12                      | MS2                    | 2                                         | 1.5                                    |
| 13                      | MS3                    | 10                                        | 0.75                                   |
| 14                      | MS3                    | 9                                         | 0.675                                  |
| 15                      | MS3                    | 6                                         | 0.45                                   |
| 16                      | MS3                    | 4                                         | 0.3                                    |
| 17                      | MS3                    | 2                                         | 0.15                                   |
| 18                      | MS4                    | 10                                        | 0.075                                  |
| 19                      | MS4                    | 8                                         | 0.06                                   |
| 20                      | MS4                    | 6                                         | 0.045                                  |
| 21                      | MS4                    | 4                                         | 0.03                                   |
| 22                      | MS4                    | 2                                         | 0.015                                  |
| 23                      | MS4                    | 0                                         | 0                                      |
Table S10  Optical Density (OD) values obtained on the non-competitive indirect ELISA for the ZIF conjugates.

| Z90-IgG |         |         |
|---------|---------|---------|
| Initial concentration for Z90−IgG in Borate Buffer with NaCl (ng/mL) | OD   | S.D.   |
| 150.00  | 0.979   | 0.040   |
| 112.50  | 0.850   | 0.025   |
| 75.00   | 0.646   | 0.069   |
| 60.00   | 0.568   | 0.044   |
| 45.00   | 0.511   | 0.006   |
| 30.00   | 0.429   | 0.016   |
| 15.00   | 0.363   | 0.025   |
| 7.50    | 0.300   | 0.018   |
| 6.00    | 0.313   | 0.014   |
| 4.50    | 0.285   | 0.020   |
| 3.00    | 0.296   | 0.009   |
| 1.50    | 0.288   | 0.007   |
| 0.75    | 0.277   | 0.027   |
| 0.68    | 0.259   | 0.009   |
| 0.45    | 0.284   | 0.016   |
| 0.30    | 0.269   | 0.003   |
| 0.15    | 0.284   | 0.012   |
| 0.08    | 0.275   | 0.009   |
| 0.06    | 0.284   | 0.016   |
| 0.05    | 0.263   | 0.017   |
| 0.03    | 0.261   | 0.007   |
| 0.02    | 0.259   | 0.003   |
| 0.00    | 0.277   | 0.007   |

| Z8P-IgG |         |         |
|---------|---------|---------|
| Initial concentration for Z8P−IgG in Borate Buffer with NaCl (ng/mL) | OD   | S.D.   |
| 150.00  | 1.224   | 0.096   |
| 112.50  | 0.966   | 0.053   |
| 75.00   | 0.719   | 0.047   |
| 60.00   | 0.664   | 0.020   |
| 45.00   | 0.544   | 0.060   |
| 30.00   | 0.451   | 0.043   |
| 15.00   | 0.391   | 0.029   |
| 7.50    | 0.321   | 0.008   |
| 6.00    | 0.316   | 0.030   |
| 4.50    | 0.289   | 0.020   |
| 3.00    | 0.280   | 0.012   |
| 1.50    | 0.275   | 0.013   |
| 0.75    | 0.265   | 0.014   |
| 0.68    | 0.267   | 0.025   |
| 0.45    | 0.262   | 0.002   |
| 0.30    | 0.264   | 0.009   |
| 0.15    | 0.275   | 0.009   |
| 0.08    | 0.281   | 0.013   |
| 0.06    | 0.276   | 0.010   |
| 0.05    | 0.258   | 0.009   |
| 0.03    | 0.260   | 0.016   |
| 0.02    | 0.304   | 0.079   |
| 0.00    | 0.285   | 0.042   |
Figure S20  Non-competitive indirect ELISA calibration curves for ZIF-IgG conjugates. The optical density (OD) was plotted against the initial concentration for the sheep anti-mouse-IgG in ng/mL (Table S13). Controls were performed to check if there was any cross-reactivity in the indirect ELISA. Binding with increasing concentrations is observed (no full sigmoidal curve recorded). OD for the blank (absence of conjugates) is indicated by the dotted horizontal line (OD = ca. 0.26).

**Conclusion:** A similar response to the one we obtained on our previous ELISA system (SI-7.2) can be observe, proving that the antibodies bound to the ZIF conjugates retain their activity.
7.4 Protocol of the non-competitive ELISA for the antibody conjugates Z8P-IgG / non-competitive fluorescent ELISA tandem

To study the viability of our fluorescently modified ZIF-8/ZIF-90 hybrid as a fluorescent bio label in immunoassays, we design a new ELISA by modifying our previous methodology, comparing the response for Z8P-IgG between non-competitive ELISA and non-competitive fluorescent ELISA.

In both these ELISAs the standard curve was prepared by making serial dilutions of one known concentration of the primary, Z8P-IgG, across a range of concentrations (0-1500 ng/ml), while keeping every other parameter of the system constant. Each well was treated under the same conditions, and each measurement was done in quadruplicate. Both ELISA protocols took place using the exact same concentration for all species. Two measurements took place in parallel:

- **Non-competitive ELISA**: On a high-binding transparent microtiter plate (SI-7.4.1), where OD was measured as a final step
- **Non-competitive fluorescent ELISA**: On a high-binding black microtiter plate (SI 7.4.2), fluorescence was measured as a final step

**Note**: the significant increase on the concentrations for our target and our primary is due to the fact that the fluorescence sensitivity is not high enough to work on the previously used range of concentrations (SI-7.3), thus we are forced to increase them to reasonably detect and quantify fluorescence.

The protocols area as follows:

### 7.4.1 Protocol of the non-competitive ELISA for the antibody conjugates Z8P-IgG

The protocol described in SI-7.2 was followed with the following modifications:

1) **Step 1 on SI-7.2 protocol**: Coat a 96 well transparent high-binding microtiter plates (flat-bottom, Nunc MaxiSorp™) with the target mouse IgG (Capture antibody solution, SI-7.4.1.1) by transferring 200 µL of the capture antibody solution into each well using a 200 µL multichannel electronic pipette and incubating for 4 h on a microplate shaker

2) **Step 9 in SI-7.2 protocol**: Add 200 µL of the ZIF-IgG conjugate solutions (ZIF-IgG solutions in borate buffer, SI-7.4.1.2) to the corresponding wells using a 200 µL pipette. Incubate for 18 hours.

#### 7.4.1.1 Preparation of Capture antibody solution

1) Transfer 20 mL of borate buffer (see SI-2.2.1) to a long glass vial with a plastic cap using a 10 mL pipette
2) Transfer 5.0 µL of mouse IgG using a 5 µL pipette. Wash the tip by aspirating/dispensing the solution several times
3) Close the glass vial and homogenize the mixture d using a mechanical shaker
7.4.1.2 Preparation of 4 stock solutions

Prepare in total 4 stock solutions, each to a total volume of 1 mL. Stock solution MS1 was prepared from the initial stock solution of Z8P-IgG (0.75 mg/mL); Stock solution MS2 was prepared by dilution from MS1; Stock solution MS3 was prepared by dilution from MS2; MS4 was prepared by dilution from MS3.

MS1 was prepared as follows:
1) Transfer 1 mL of borate buffer inside a 1.5 mL Eppendorf tube
2) Remove 100 µL of the buffer using a 100 µL pipette
3) Transfer 100 µL of the original stock solution of the Z8P-IgG with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

MS2 was prepared as follows:
1) Transfer 1 mL of borate buffer inside a new 1.5 mL Eppendorf tube
2) Remove 100 µL of borate buffer with a 100 µL pipette
3) Transfer 100 µL of the MS1 stock solution with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

MS3 was prepared as described for MS2 but using MS2 as the stock solution

MS4 was prepared as described for MS2 but using MS3 as the stock solution

The concentration for the MS solutions will be:

- MS1 = 75 µg/mL;
- MS2 = 7.5 µg/mL;
- MS3 = 0.75 µg/mL;
- MS4 = 0.075 µg/mL.

7.4.1.3 Preparation of the dilution series for Z8P-IgG

We prepare 23 new solutions from these four stock solutions (MS1, MS2, MS3 and MS4) to varying concentrations for Z8P IgG.

The procedure for each dilution series stock (number #) is performed as follows:
1) Transfer 1 mL of borate buffer into a new Eppendorf tube.
2) Using the suitable micropipette, remove from the Eppendorf tube a volume of borate buffer equivalent to the corresponding cell on the column named “aliquot volume from MS stock solution” as listed on Table S14.
3) Using the same micropipette, transfer the aliquot volume corresponding to the exact same cell as on step 3 from Table S14, with a new tip on the micropipette. Wash the tip by aspirating/dispensing the solution several times
4) Seal the Eppendorf tube and homogenise the solution using a mechanical shaker

**WARNING**: In order to keep the antibodies stable during the whole process all solutions are kept on ice.
Table S11  Table for the preparation of the ZIF–IgG solutions in borate buffer. The graphic representation of the results is in Figure S20. The * represent the volume of buffer to be replaced by the stock solutions (MS1, MS2, MS3 and MS4).

| Dilution series stock # | MS Stock solution used | aliquot volume from MS stock solution* (µL) | Concentration in stock solution (ng/mL) |
|------------------------|------------------------|--------------------------------------------|----------------------------------------|
| 1                      | MS1                    | 20                                         | 1500                                   |
| 2                      | MS1                    | 15                                         | 1125                                   |
| 3                      | MS1                    | 10                                         | 750                                    |
| 4                      | MS1                    | 8                                          | 600                                    |
| 5                      | MS1                    | 6                                          | 450                                    |
| 6                      | MS1                    | 4                                          | 300                                    |
| 7                      | MS1                    | 2                                          | 150                                    |
| 8                      | MS2                    | 10                                         | 75                                     |
| 9                      | MS2                    | 8                                          | 60                                     |
| 10                     | MS2                    | 6                                          | 45                                     |
| 11                     | MS2                    | 4                                          | 30                                     |
| 12                     | MS2                    | 2                                          | 15                                     |
| 13                     | MS3                    | 10                                         | 7.5                                    |
| 14                     | MS3                    | 9                                          | 6.75                                   |
| 15                     | MS3                    | 6                                          | 4.5                                    |
| 16                     | MS3                    | 4                                          | 3                                      |
| 17                     | MS3                    | 2                                          | 1.5                                    |
| 18                     | MS4                    | 10                                         | 0.75                                   |
| 19                     | MS4                    | 8                                          | 0.6                                    |
| 20                     | MS4                    | 6                                          | 0.45                                   |
| 21                     | MS4                    | 4                                          | 0.03                                   |
| 22                     | MS4                    | 2                                          | 0.015                                  |
| 23                     | MS4                    | 0                                          | 0                                      |
7.4.2 Protocol of the non-competitive ELISA for the antibody conjugates Z8P-IgG via non-competitive fluorescence immunoassay

We developed a non-competitive indirect immunoassay for the assessment of the activity of the Z8P-IgG conjugate and its fluorescence capabilities.

The protocol described in SI-7.2 was followed with the following modifications:

1) **Step 1 in the SI-7.2 protocol**: This experiment was performed on a 96 well black high-binding plate for fluorescence measurements (flat-bottom, Nunc MaxiSorp™)

2) **Step 1 in the SI-7.2 protocol**: the target mouse IgG is prepared differently (Capture antibody solution, SI-7.4.1.1)

3) **Step 9 in SI-7.2 protocol**: Add 200 µL of the ZIF-IgG conjugate solutions (ZIF-IgG solutions in borate buffer, SI-7.4.1.2) to the corresponding wells using a 200 µL pipette. Incubate for 18 hours.

4) After the incubation in **step 11 in SI-7.2 protocol**, the fluorescence of each well was measured.

5) After the measurement, a three-cycle washing step takes place as described in **step 4 in SI-7.2 protocol** and each well was filled with 200 µL of borate buffer (see SI-2.2.1)

6) The fluorescence was then measured again

7) After this measurement, a three-cycle washing step took place as described in **step 4 in SI-7.2 protocol**

8) Each well was filled with 200 µL of citrate buffer (see SI-2.2.4) to disintegrate the ZIFs and liberate the fluorophores.

9) The plate was incubated in a plate shaker for 30 min

10) Fluorescence was then measured again
**Table S12**  Fluorescence results from the non-competitive fluorescent ELISA for Z8P-IgG. The graphic representation of the results is in Figure S21.

| Concentration (ng/ml) | Pre-Wash emission intensity (counts) | Post-Wash emission intensity (counts) | Post-wash + post citric acid emission intensity (counts) |
|-----------------------|-------------------------------------|--------------------------------------|----------------------------------------------------------|
| 1500                  | 3793.50 ± 155.67                    | 2115.67 ± 153.28                    | 8887.25 ± 363.79                                         |
| 1125                  | 3153.00 ± 141.77                    | 1548.33 ± 23.12                     | 5804.00 ± 233.98                                         |
| 750                   | 2836.75 ± 146.33                    | 1542.75 ± 259.12                    | 5366.75 ± 423.67                                         |
| 600                   | 2607.25 ± 116.64                    | 1519.67 ± 180.78                    | 3826.75 ± 357.38                                         |
| 450                   | 2234.75 ± 73.25                     | 1037.50 ± 46.51                     | 3163.00 ± 62.23                                          |
| 300                   | 2061.00 ± 19.88                     | 969.75 ± 119.81                     | 2740.50 ± 151.25                                         |
| 150                   | 1833.75 ± 25.68                     | 978.25 ± 99.68                      | 2410.67 ± 135.61                                         |
| 75                    | 1797.00 ± 96.09                     | 950.24 ± 141.42                     | 2332.00 ± 26.87                                          |
| 60                    | 1719.00 ± 23.48                     | 684.25 ± 126.27                     | 2843.25 ± 344.27                                         |
| 45                    | 1677.67 ± 13.65                     | 612.67 ± 101.86                     | 2577.00 ± 124.83                                         |
| 30                    | 1677.33 ± 15.04                     | 610.69 ± 105.51                     | 2871.33 ± 602.85                                         |
| 15                    | 1632.67 ± 9.19                      | 609.26 ± 102.56                     | 2457.00 ± 220.01                                         |
| 7.5                   | 1623.50 ± 13.72                     |                                       |                                                          |

**Figure S22**  Fluorescence emission for the non-competitive indirect assay employing Z8P-IgG obtained with an excitation wavelength of 335 nm. All the data points are blank-corrected (the mean intensity for the blank was subtracted from all points; the blanks’ mean intensity was 168 ± 26 for pre wash fluorescence (blank = borate buffer), 143 ± 54 for post wash (blank = borate buffer) and 356 ± 5 after disintegration of the ZIF (by citric acid, blank = citric buffer).

**Conclusion**: Data suggests that the Z8P-IgG conjugate system can be used as a fluorescent bio label in immunoassays.
8 Studies on the decomposition of the ZIF conjugates

By determining the variation of the crystallite sizes over time, it was possible to demonstrate that the crystallinity structure of the ZIFs after the conjugation reaction has not been heavily affected.

8.1 PXRD measurements of the Z90-IgG and Z8P-IgG conjugates

To study how the crystallinity of the ZIFs (both Z8P and ZIF-90) could be degrading over time because of the action of the antibodies, we obtained x-ray diffractograms of a sample of the conjugates over time.

For each diffractogram, 400 μL aliquot (a total mass of 0.3 mg for each measurement) of the stock solution for each ZIF-IgG conjugate was freshly added to an Eppendorf tube, centrifuged (15000 rpm, 3 min) to reduce its volume to 50 μL by removing excess supernatant, and then this 50 μL dispersion was placed on a zero background silicon monocrystalline (SIOV) sample holder. The measurement was started with the sample still wet. The parameters for all diffractograms are: 2θ 5-50°; step size 0.02°; integration time 6 sec.

Figure S23 Stacked Diffractograms taken at the times shown from the start of the stability experiments (0 to 120 hours) for both conjugate systems. Z90-IgG conjugate to the left and Z8P-IgG to the right. The background was subtracted from the diffractograms.
To assess that the crystallinity of the system was not severely damaged over time we measured the crystallite size for each system, applying the Scherrer equation to the first three reflections, (110), (200) and (211), for all diffractograms.

The Scherrer equation is a formula that relates the size of sub-micrometre crystallites in a solid to the broadening of a peak in a diffraction pattern and was used in the determination of size of crystals in the powder.\textsuperscript{113} If the Scherrer value remains stable over time, this is an indication that there is no significant degradation of the ZIF particles in the conjugates.

\[
\tau = \frac{K \lambda}{\beta \cos \theta}
\]

Equation S1. Scherrer equation for calculating the crystallite size, where “\( \tau \)” is the mean size of the ordered (crystalline) domains, “\( K \)” is a dimensionless shape factor [we took \(1\)], “\( \lambda \)” is the x-ray wavelength (1.50406 nm), “\( \beta \)” is the line broadening at half the maximum intensity (FWHM) in radians, “\( \theta \)” the Bragg angle. We calculated all the FWHM for each diffraction peak after background subtraction and applying a Gaussian fitting model with Origin.

Table S13.1 Positions for each of the first diffractions for the ZIF−IgG conjugates and its value in radians for use on the Scherrer equation.

| Diffraction | Angle (°) | Angle (radians) | Cosen angle |
|-------------|-----------|-----------------|-------------|
| [1,1,0]     | 7.3       | 0.064           | 0.998       |
| [2,0,0]     | 10.4      | 0.091           | 0.996       |
| [2,1,1]     | 12.7      | 0.111           | 0.994       |

Table S14.2 FWHM for each of the first three diffractions for each conjugate (Z8P-IgG on top, Z90-IgG on bottom) for each of the degradation times studied. To calculate these values, we used the background subtracted diffractograms (Using “Diffrac.EVA”) and we fitted a Gaussian model using Origin.

| Decomposition time (hours) | Diffraction [1,1,0] | Diffraction [2,0,0] | Diffraction [2,1,1] |
|----------------------------|---------------------|---------------------|---------------------|
| 0                          | 0.222               | 0.192               | 0.247               |
| 12                         | 0.119               | 0.192               | 0.241               |
| 24                         | 0.220               | 0.192               | 0.243               |
| 48                         | 0.121               | 0.198               | 0.248               |
| 120                        | 0.157               | 0.153               | 0.247               |
### FWHM for each peak for Z90–IgG

| Decomposition time (hours) | Diffraction [1,1,0] | Diffraction [2,0,0] | Diffraction [2,1,1] |
|---------------------------|---------------------|---------------------|---------------------|
| 0                         | 0.201               | 0.209               | 0.291               |
| 12                        | 0.192               | 0.204               | 0.288               |
| 24                        | 0.195               | 0.210               | 0.316               |
| 48                        | 0.187               | 0.190               | 0.258               |
| 120                       | 0.174               | 0.197               | 0.251               |

*Table S15.3  Values for the Scherrer crystallite sizes for all the diffractions chosen on the stability times chosen for our two ZIF–IgG systems. This data was illustrated in Figure S23.*

### Scherrer crystallite size for Z8P–IgG

| Decomposition time (hours) | Diffraction [1,1,0] | Diffraction [2,0,0] | Diffraction [2,1,1] |
|---------------------------|---------------------|---------------------|---------------------|
| 0                         | 13.602              | 15.773              | 12.264              |
| 12                        | 25.394              | 15.724              | 12.585              |
| 24                        | 13.732              | 15.225              | 12.476              |
| 48                        | 24.890              | 15.748              | 12.195              |
| 120                       | 19.187              | 19.716              | 12.264              |

### Scherrer crystallite size for Z90–IgG

| Decomposition time (hours) | Diffraction [1,1,0] | Diffraction [2,0,0] | Diffraction [2,1,1] |
|---------------------------|---------------------|---------------------|---------------------|
| 0                         | 15.019              | 14.487              | 10.405              |
| 12                        | 15.707              | 14.828              | 10.517              |
| 24                        | 15.466              | 14.390              | 9.590               |
| 48                        | 16.145              | 15.940              | 11.754              |
| 120                       | 17.313              | 15.317              | 12.049              |
Figure S24  Scherrer crystallite sizes for the first three reflections for the studied ZIF–IgG conjugates, ZBP–IgG and Z90–IgG. No marked changes in crystallite size were observed in the conjugated ZIFs under storage at 4°C for 120 hours using this crystallographic method. The pronounced variation on the (110) reflection for Z8P–IgG does not seem to be representative for the system.

A significant crystallinity degradation over time could not be observed in the studied time frame, since both systems have a similar range of crystallite sizes for all diffractions and over time. The pronounced variation on the diffraction [1,0,0] for Z8P–IgG does not seem to be representative of the system, and was probably caused by the sample not being completely immobilized on the sample holder (was deposited on a water dispersion and started measurement immediately), thus generating the disparity on the first 30 mins of measurement (corresponding to the first 8-9°, approximately).
9 Studies on the ZIF-antibody bond

To study the nature of the bond between our ZIFs (ZIF-90 and Z8P) and the antibody (sheet anti-mouse IgG) we design a high-resolution mass spectroscopy methodology combined with TEM pictures.

9.1 TEM images of the ZIF-IgG conjugates

The protocol to characterize the particles via TEM is described in SI-4.4.2.

TEM images are consistent with the ZIF structure and particle size being retained upon formation of both ZIF conjugates. The antibodies appear as a rugged layer surrounding the ZIF particles.

Figure S25  TEM pictures for the ZIFs. A rhombic dodecahedra morphology can be observed for the Z8P-ZIF particles (pictures a-c), with some polydispersity in size, between 50 and 150 nm and averaging 100 nm. The pictures were complemented with EDS analysis (picture c), confirming that the signal for Zn was contained within the particles. For Z8P-IgG (pictures d-g) the particles were covered by a rugged layer of antibodies. The relative size of the whole system was in the same range as the one for the ZIF particles without the antibodies. The relative size of each entity was around 10 nm, with was consistent with the relative size of the antibodies themselves. S-TEM pictures were taken to detect the ZIF particles (picture e) and they were located within the yellow circles. EDS analysis complements the images (picture g). Most of the Zn signals were inside the rugged particles, which was consistent with the system description. There was also a not insignificant number of signals for zinc outside of the ruggedness, suggesting that after the conjugation procedure some of the ZIF have been damaged.
9.2 Study of the nature of the bond on the ZIF-IgG conjugates via HRMS

In order to confirm covalent conjugation through the imidazole carboxaldehyde moiety of the ZIF particles to a free amino group of the antibody, conjugates were digested (SI-9.2.1). Digestion results in the cleavage of most amide bonds in the protein and the potential release of L-lysine-bound Imidazolate-2-carboxaldehyde. The hydrolysate was then screened with liquid chromatography high-resolution mass spectrometry (LC-HRMS) (Figure S25). After developing the methodology to detect the fragment (SI-9.2), it revealed a potential lysine-imidazole derivative at a retention time of 558 s (Figure S26b). The compound was present in both ZIF-IgG conjugates and absent in all control experiments (individually digested IgG and ZIF without conjugation), thus supporting the notion of it being formed exclusively in ZIF–IgG conjugates.

9.2.1 Digestion of the conjugate

The protocol for digesting the samples was as follows:

To a distinct amount of sample (See Table S11 for more information) 1 mL of an aqueous solution of HCl 6N was added, the air was purged with the use of a Schlenk line with vacuum and Argon, and then heated to 107 °C for 24 h. After the digestion, the samples were cooled down and transferred to a 1.5 mL Eppendorf tube, and the solvent was removed using a SpeedVac.

Table S16. Amount for each species used in the digestion for subsequent mass spectrometric characterization.

| Sample                        | Amount of sample                                      |
|-------------------------------|-------------------------------------------------------|
| Sheep anti-mouse IgG          | 50 µL of a solution 2 mg/mL                           |
| ZIF-90                        | 0.4 mg                                                |
| Z8P                           | 0.4 mg                                                |
| ZIF-90–IgG conjugate          | 2 mg of conjugate                                     |
| Z8P–IgG conjugate             | 2 mg of conjugate                                     |
| Sheep IgG / i2ca              | 100 µg of IgG + 10 µg of i2ca in 0.5 mL of borate buffer |
| Sheep IgG / i2ca              | 100 µg of IgG + 1 µg of i2ca in 0.5 mL of borate buffer |
9.2.2 High-resolution mass spectrometry

First, the efficiency of this hydrolysis was tested using pure antibody instead of the conjugate. Extracted ion chromatograms (EICs) of protonated and deprotonated molecular peaks ([M+H]⁺, [M-H]) acquired in both positive and negative electrospray ionisation modes suggest the presence of 17 of the 20 proteinogenic amino acids in the sample (Figure S27), indicating efficient protein breakdown. Next, ZIF-IgG conjugates were hydrolysed and subjected to LC-HRMS. Chromatograms of hydrolysed ZIF-IgG conjugates contained approximately 140 chromatographically deconvoluted compounds (“features”) in the mass range of 50 to 600 Da (Figure S27). This shows extensive hydrolytic breakdown of conjugates into smaller molecules. In these chromatograms, ion traces of possible amino acid imidazole derivatives were checked using a target list of theoretical precursor ions (Table S18).

This approach revealed a potential lysine imidazole derivative at a retention time of 558 s (Figure S26b). The compound was present in both ZIF/IgG conjugates but in none of the control measurements (IgG alone, ZIFs alone), supporting the notion that it was formed exclusively in ZIF/IgG conjugates.

Structural evidence for the compound could be obtained from both isotope pattern and MS/MS spectrum. First, isotopologue intensities matched calculated ones within instrument-limited error margins (<2%; Table S19). Second, all major MS/MS fragments could be assigned substructures that explained the putative molecular structure very consistently (Figure S26c).

Taken together, while only co-elution of a chemical standard or NMR could give definite structural evidence, covalent bonding of an Imidazolate-carboxaldehyde moiety to an amino acid residue of the antibody was well supported by the present results.

For mass spectrometric analysis (SI-3.9), hydrolysed samples were reconstituted in acetonitrile/methanol (75:25) containing 0.2% formic acid and injected into a 1290 UHPLC system.
Samples were separated on a 2.1 mm × 75 mm × 1.8 µm BEH Amide column (Waters, Germany) using a hydrophilic liquid interaction chromatography (HILIC) mode.
Figure S27  Mass spectrometric characterization of the assumed covalent bond in ZIF-IgG conjugates.  (a) Nontargeted (HILIC-)LC-ESI-HRMS of hydrolysis products; representative total ion chromatogram (TIC) and ion map, respectively, demonstrating sample complexity.  (b) Extraction of a putative lysine imidazolate derivative (C_{10}H_{18}N_{4}O_{2}, m/z 227.1503). Ion traces were detected in measurements of both ZIF conjugates but not in those of controls. (c) MS/MS spectrum of putative lysine derivative (precursor m/z 227.1503, isolation window 1 Da). MS/MS fragments were assigned most likely molecular structures based on competitive fragmentation modelling (CFM-ID). Deviations of theoretical from measured masses were indicated. Minor fragment annotation left out for clarity.
Figure S28 Extracted ion chromatograms for the 20 proteinogenic amino acids released from acidic hydrolysis of 0.01 mg IgG (anti-mouse, from sheep). Extracted ions ([M+H]^+, [M-H]) and mass windows are indicated.
| Name               | SMILES                                               | Formula  | Neutral mass | [M+H]+  | [M-H]   |
|--------------------|------------------------------------------------------|----------|--------------|---------|---------|
| Ala                | CC(C(=O)O)N                                         | C3H7NO2  | 89.0477      | 90.0550 | 88.0404 |
| Ala imidazole      | CC(C(=O)O)NCC1N=CC=N1                               | C7H11N3O2 | 169.0851     | 170.0924 | 168.0779 |
| derivative 1       |                                                     |          |              |         |         |
| Arg                | C(CC(C(=O)O)N)=C(N)N                                 | C6H14N4O2 | 174.1117     | 175.1190 | 173.1044 |
| Arg imidazole      | C(CC(C(=O)O)NCC1N=CC=N1)CN=C(N)N                    | C10H18N6O2 | 254.1491     | 255.1564 | 253.1418 |
| derivative 1       |                                                     |          |              |         |         |
| Arg imidazole      | C(CC(C(=O)O)O)CN=C(N)NCC1N=CC=N1                    | C10H18N6O2 | 254.1491     | 255.1564 | 253.1418 |
| derivative 2       |                                                     |          |              |         |         |
| Asn                | C(C(C(=O)O)N)=C(O)N                                  | C4H8N2O3 | 132.0535     | 133.0608 | 131.0462 |
| Asn imidazole      | C(C(C(=O)O)NCC1N=CC=N1)C(O)N                         | C8H12N4O3 | 212.0909     | 213.0982 | 211.0837 |
| derivative 1       |                                                     |          |              |         |         |
| Asn imidazole      | C(C(C(=O)O)N)=C(O)NCC1N=CC=N1                       | C8H12N4O3 | 212.0909     | 213.0982 | 211.0837 |
| derivative 2       |                                                     |          |              |         |         |
| Asp                | C(C(C(=O)O)N)=C(O)O                                   | C4H7NO4  | 133.0375     | 134.0448 | 132.0302 |
| Asp imidazole      | C(C(C(=O)O)NCC1N=CC=N1)C(O)O                         | C8H11N3O4 | 213.0750     | 214.0822 | 212.0677 |
| derivative 1       |                                                     |          |              |         |         |
| Cys                | C(C(C(=O)O)N)=S                                      | C3H7NO2S | 121.0197     | 122.0270 | 120.0125 |
| Cys imidazole      | C(C(C(=O)O)NCC1N=CC=N1)C(O)S                         | C7H11N3O2S | 201.0572    | 202.0645 | 200.0499 |
| derivative 1       |                                                     |          |              |         |         |
| Glu                | C(CC(=O)O)C(C(=O)O)=N                                | C5H9NO4  | 147.0532     | 148.0604 | 146.0459 |
| Glu imidazole      | C(CC(=O)O)C(C(=O)O)NCC1N=CC=N1                      | C9H13N3O4 | 227.0906     | 228.0979 | 226.0833 |
| derivative 1       |                                                     |          |              |         |         |
| Gln                | C(CC(=O)N)=C(O)N                                      | C5H10N2O3 | 146.0691     | 147.0764 | 145.0619 |
| Gln imidazole      | C(CC(=O)NCC1N=CC=N1)C(O)=N                           | C9H14N4O3 | 226.1066     | 227.1139 | 225.0993 |
| derivative 1       |                                                     |          |              |         |         |
| Gln imidazole      | C(CC(=O)N)C(C(=O)O)NCC1N=CC=N1                      | C9H14N4O3 | 226.1066     | 227.1139 | 225.0993 |
| derivative 2       |                                                     |          |              |         |         |
| Gly                | C(C(=O)O)N                                          | C2H5NO2  | 75.0320      | 76.0393  | 74.0248  |
| Gly imidazole      | C(C(=O)O)NCC1N=CC=N1                                | C6H9N3O2  | 155.0695    | 156.0768 | 154.0622 |
| derivative 1       |                                                     |          |              |         |         |
| His                | C1=NC(=N1)CC(C(=O)O)=N                              | C6H9N3O2  | 155.0695    | 156.0768 | 154.0622 |
| His imidazole      | C1=NC(=N1)CC(C(=O)O)NCC1N=CC=N1                     | C10H13N5O2 | 235.1069   | 236.1142 | 234.0996 |
| derivative 1       |                                                     |          |              |         |         |
| Ile                | CCC(C(C(=O)O)O)N                                     | C6H13NO2  | 131.0946    | 132.1019 | 130.0874 |
| Ile imidazole      | CCC(C(C(=O)O)O)NCC1N=CC=N1                          | C10H17N3O2 | 211.1321   | 212.1394 | 210.1248 |
| derivative 1       |                                                     |          |              |         |         |
| Leu                | CC(C(C(=O)O)O)N                                      | C6H13NO2  | 131.0946    | 132.1019 | 130.0874 |
| Leu imidazole      | CC(C(C(=O)O)O)NCC1N=CC=N1                           | C10H17N3O2 | 211.1321   | 212.1394 | 210.1248 |
| derivative 1       |                                                     |          |              |         |         |
| Lys                | C(CC(C(=O)O)O)N                                      | C6H14N2O2 | 146.1055    | 147.1128 | 145.0983 |
| Name                              | SMILES                                               | Formula      | Neutral mass | [M+H]^+ | [M-H]  |
|----------------------------------|------------------------------------------------------|--------------|--------------|----------|--------|
| Lys imidazole derivative 1       | C(CCNC1N=CC=N1)CC(C(=O)O)N                         | C10H18N4O2   | 226.1430     | 227.1503 | 225.1357|
| Lys imidazole derivative 2       | C(CCN)CC(C(=O)O)NCC1N=CC=N1                       | C10H18N4O2   | 226.1430     | 227.1503 | 225.1357|
| Met                              | C(=C=CC)N=C=CC(=C=O)N                              | C5H11NO2S    | 149.0510     | 150.0583 | 148.0438|
| Met imidazole derivative 1       | C(=C=CC)N=C=CC(=C=O)N NCC1N=CC=N1                  | C9H15N3O2S   | 229.0885     | 230.0958 | 228.0812|
| Phe                              | C1=CC=C(C=C1)CC(C(=O)O)N                           | C9H11NO2     | 165.0790     | 166.0863 | 164.0717|
| Phe imidazole derivative 1       | C1=CC=C(C=C1)CC(C(=O)O)N NCC1N=CC=N1               | C13H15N3O2   | 245.1164     | 246.1237 | 244.1092|
| Pro                              | C1CC(NC1)C(=O)O                                    | C5H9NO2      | 115.0633     | 116.0706 | 114.0561|
| Ser                              | C(C(C(=O)O)N)O                                     | C3H7NO3      | 105.0426     | 106.0499 | 104.0353|
| Ser imidazole derivative 1       | C(C(C(=O)O)NCC1N=CC=N1)O                           | C7H11N3O3    | 185.0800     | 186.0873 | 184.0728|
| Thr                              | CC(C=C=CC(=C=O)O)N                                 | C4H9NO3      | 119.0582     | 120.0655 | 118.0510|
| Thr imidazole derivative 1       | CC(C=C=CC(=C=O)O)N NCC1N=CC=N1O                    | C8H13N3O3    | 199.0957     | 200.1030 | 198.0884|
| Trp                              | C1=CC=C2C(=C1)C(=CN2)CC(C(=O)O)N                   | C11H12N2O2   | 204.0899     | 205.0972 | 203.0826|
| Trp imidazole derivative 1       | C1=CC=C2C(=C1)C(=CN2)CC(C(=O)O)N NCC1N=CC=N1      | C15H16N4O2   | 284.1273     | 285.1346 | 283.1200|
| Tyr                              | C1=CC=C(C=C1)C(=CO)N                              | C9H11NO3     | 181.0739     | 182.0812 | 180.0666|
| Tyr imidazole derivative 1       | C1=CC=C(C=C1)C(=CO)N NCC1N=CC=N1O                   | C13H15N3O3   | 261.1113     | 262.1186 | 260.1041|
| Val                              | CC(C(=C=CC(=O)O)N                                 | C5H11NO2     | 117.0790     | 118.0863 | 116.0717|
| Val imidazole derivative 1       | CC(C(=C=CC(=O)O)N NCC1N=CC=N1                     | C9H15N3O2    | 197.1164     | 198.1237 | 196.1092|

Table S19. Mass accuracy and isotope pattern analysis for putative lysine imidazole derivative at 558 s (m/z 227.1500)

| Isotope | m/z   | theoretical m/z | Error (mDa) | Intensity (%) | Theoretical intensity (%) | Difference (%) |
|---------|-------|-----------------|-------------|--------------|--------------------------|---------------|
| M       | 227.1500 | 227.1503       | 0.3         | 100          | 100                       | 0             |
| M+1     | 228.1533 | 228.1530       | 0.3         | 14.26        | 12.56                     | -1.69         |
| M+2     | 229.1567 | 229.1552       | 1.5         | 1.31         | 1.14                      | -0.17         |
10 Studies on the mobility of the ZIF-antibody conjugate

The mobility of the ZIF conjugates was important for their application in bioanalytical assays. The feasibility of our ZIF-IgG system as a mobile conjugate on a solid matrix for lateral flow assays is next tested. PVDF matrix is chosen from the candidates available; tests on nitrocellulose were performed but proved to be an unsuitable matrix given the strong interactions between the nitrocellulose and the conjugates.

In order to facilitate the proof of concept for the Z8P platform as a bio label, we create a new conjugate system, Z8P-IgG HRP, by changing the antibody conjugated to the ZIFs from sheep-anti-mouse IgG to HRP labelled donkey anti-sheep IgG.

For details on the antibodies see SI-1.6.

10.1 Conjugation reaction of Z8P particles to IgG–HRP

10.1.1 Process for the conjugation of ZIF-90 and Z8P with IgG-HRP

1) 1.5 mg of Z8P was carefully weighed on a 5-figure balance and transferred to a small glass vial with a plastic cap.
2) 1.5 mL of a borate buffer solution (see SI-2.2.1) was added, measured with a 2.5 mL pipette.
3) After sealing the glass vial with the cap, it was sonicated for 15 min.
4) 50 μL of an anti-sheep IgG-HRP solution (HRP conjugated to donkey anti-sheep IgG was added without any previous purification step using a 100 μL pipette to this dispersion.
5) A magnetic stirrer bar was added, the glass vial closed, and the mixture stirred for 15 min at room temperature.
6) 50 μL of a commercially prepared aqueous solution of Na[BH₃(CN)] (5 M) in 1 M NaOH was added using a 200 μL pipette and allowed to react for an additional 4 hours at room temperature.

**Warning:** Exercise extreme care when handling Cyanoborohydride. Always wear gloves, glasses and work in a properly enclosed fume hood.

10.1.1.2 Protocol for washing the freshly prepared Z8P-IgG HRP

Next step was designed to remove any anti-sheep IgG HRP not covalently bonded to Z8P. The washing protocol of this conjugation reaction is as follows:

1) Transfer the 1.5 mL suspension to a 2 mL Eppendorf tube with the help of a 1 mL pipette and centrifuge it at 15000 rpm for 5 min.
2) Carefully remove the supernatant taking care not to disturb the solid residue and transfer it to a common container labelled as “Wash 1”. This wash is reserved for later use, in case the ELISA experiment fails suggesting unsuccessful binding between the
IgG and the ZIF. “Wash 1” will help to investigate if the free IgG in the wash was active or inactive. “Wash 1” is disposed of, after successful completion of Elisa experiments.

**Warning:** The very first supernatant of “Wash 1” contains cyanide derivatives. Extreme care must be exercised when dealing and disposing with “Wash 1”.

3) Add 1 mL of borate buffer to the Eppendorf tube to wash the solid residue.
4) Sonicate the suspension for 2 min to ensure full dispersion of the solid particles.
5) Centrifuge the dispersion at 15000 rpm for 5 min.
6) Remove carefully the supernatant avoiding contact with the solid residue and transfer it to a separately container labelled as “Wash 2”.
7) Add 1 mL of TRIS buffer (see SI-2.2.3) to the residue.
8) Redisperse the solid residue by vigorous agitation, and centrifuge at 15000 rpm for 5 min.
9) Carefully remove this TRIS buffer supernatant and discard it.
10) Repeat steps 7-9 three more times to wash out any IgG not properly conjugated to the ZIF.
11) Perform steps 7-9 two additional times, this time with borate buffer.
12) Disperse the properly washed solid residue containing the ZIF conjugate in 2 mL of borate buffer. This is the **ZIF-IgG HRP stock solution** (0.75 mg/ml) used in all the ELISA analysis.

**Conclusion:** The amount of washing steps was developed so that only the covalently bound antibody remains on the ZIF particles.

### 10.2 Mobility studies of ZIF conjugates on a PVDF membrane

The mobility was studied on the commercial membrane TransBlot® Turbo™ Midi-size PVDF Membrane (Bio-Rad, Cat. # 10026933).

#### 10.2.1.1 Protocol for the PVDF mobility tests of Z8P-IgG

1) The **activation of the PVDF strip** is done by submerging the membrane in pure ethanol for 10 sec.
2) Remove the excess ethanol by rinsing it in deionized water for 3 sec.
3) Allow the strip to dry at open atmosphere.
4) Place 1 µL of a sheep anti-mouse IgG (2 mg/ml) solution on the immobilization point using a 5 µL micropipette operating in reversed mode.
5) Allow the PVDF strip with the immobilized sheep IgG dry.
6) Submerge the strip into a **casein** solution in PBS (1mg/mL, see SI-7.2.2) for ten seconds to block all active sites on the strip.
7) Allow the strip to dry at room temperature.
8) Dispense 10-30 μL of the Z8P-IgG HRP dispersion in borate buffer (0.75 mg/mL) on the elution starting point using a reverse micropipette.

9) Allow to dry

10) Attach an adsorption pad on the opposite side of the strip in relation to the elution starting point.

11) Elute the Z8P-IgG HRP using borate buffer until the deposited Z8P-IgG HRP reaches the adsorption pad (yellow colouring from the Z8P).

12) Allow dry.

The Z8P-IgG HRP can be seen by the naked eye on the capture zone and on the adsorption pad (yellow colouring), indicating antibody binding.

13) TMB solution is used to reveal the location of the HRP-labelled antibodies. This will trigger an intense blue coloration within minutes wherever IgG HRP labelled are present, with by extension shows were the ZIF-IgG HRP conjugates are present.

14) Cut out the relevant area of the strip and digest it in 200 μL of 1 M H₂SO₄ for 15 min using a mechanical shaker and a desk centrifuge to aid the process.

15) Pipetted the digestion solution into a 96-well black microtiter plate.

16) Measure the fluorescence of the solution measured.

**Note:** TMB and PVDF samples were also prepared as negative controls.

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**Figure S29** Outcome of the Lateral Flow Immunoassay built upon Z8P-IgG. Left: The Z8P-IgG can be seen by the naked eye in the capture zone and on the adsorption pad, indicating antibody binding and surplus antibody, respectively. Right: TMB solution was used to reveal the location of the HRP-labelled antibodies. In both pictures all amounts for all compounds of the system were kept constant (1 μL of a sheep IgG solution in PBS (2 mg/mL); casein solution 1 mg/mL in PBS) and the initial volume of Z8P-IgG dispersion increases from left to right from 10, 20 to 30 μL of the Z8P-IgG in borate buffer (1 mg/mL).
Figure S30  Experimental set-up to demonstrate the aptitude of Z8P-IgG for the bioanalytical application lateral flow immunoassay (LFIA). a) Assay. The protocol begins by activating the PVDF membrane through submersion in pure ethanol followed by rinsing in deionized water. After air drying, a solution containing sheep IgG as target is placed in a central part of the strip, the later capture zone. The PVDF strip with the immobilized sheep IgG is then submerged in a casein solution to block all remaining active sites on the strip, and afterwards allowed to dry. A varying volume of the Z8P-IgG-HRP dispersion is pipetted on the elution starting point and allowed to dry. Then the absorption pad is attached to the strip. Subsequently, the Z8P-IgG-HRP is eluted by adding enough of a mobile phase (borate buffer) to the strip that the phase’s front-line flows across the capture zone into the absorption pad. This is followed by drying the strip in air. b) Revelation of mobility of the composite and retention of binding specificity of the antibody: The capturing of Z8P-IgG by the target (sheep antibody) in the capture zone is visible by the colour of the ZIF particles. For stronger evidence that the labelled antibody is binding selectively to the immobilized target, the HRP enzyme linked to Z8P-IgG is revealed via its reaction with the added substrate TMB. c) Proof of intact ZIF label. The area to which the antibody has bound is cut out and digested in H2SO4 to decompose the ZIF and liberate the pyrene. The supernatant is transferred to a black microwell and fluorescence of the solution measured. The emission spectra show that fluorophore is released in amounts related to the volume of conjugate employed (PVDF and TMB samples that were not run through the assay serve as negative control).
References

[1] L. Oberleitner, U. Dahmen-Levison, L.-A. Garbe, R. J. Schneider, Analytical Methods 2016, 8, 6883-6894.
[2] R. Tautenhahn, C. Boettcher, S. Neumann, BMC Bioinformatics 2008, 9, 504.
[3] F. Allen, A. Pon, M. Wilson, R. Greiner, D. Wishart, Nucleic acids research 2014, 42, W94-W99.
[4] aC. Wu, Q. Liu, R. Chen, J. Liu, H. Zhang, R. Li, K. Takahashi, P. Liu, J. Wang, ACS applied materials & interfaces 2017, 9, 11106-11115; bH. Kaur, G. C. Mohanta, V. Gupta, D. Kukkar, S. Tyagi, Journal of Drug Delivery Science and Technology 2017, 41, 106-112.
[5] W. Morris, C. J. Doonan, H. Furukawa, R. Banerjee, O. M. Yaghi, Journal of the American Chemical Society 2008, 130, 12626-12627.
[6] J. Cravillon, R. Nayuk, S. Springer, A. Feldhoff, K. Huber, M. Wiebcke, Chemistry of Materials 2011, 23, 2130-2141.
[7] N. Lugg, G. Kothleitner, N. Shibata, Y. Ikuhara, Ultramicroscopy 2015, 151, 150-159.
[8] J. Troyano, A. Carné-Sánchez, C. Avci, I. Imaz, D. Maspoch, Chem Soc Rev 2019, 48, 5534-5546.
[9] E. Cunha, M. F. Proença, M. G. Pereira, M. J. Fernandes, R. J. Young, K. Strutyński, M. Melle-Franco, M. Gonzalez-Debs, P. E. Lopes, M. D. C. Paiva, Nanomaterials (Basel) 2018, 8.
[10] A. Chapartegui-Arias, J. A. Villajos, A. Myxa, S. Beyer, J. Falkenangen, R. J. Schneider, F. Emmerling, ACS Omega 2019, 4, 17090-17097.
[11] J. Cravillon, R. Nayuk, S. Springer, A. Feldhoff, K. Huber, M. Wiebcke, Chem Mater 2011, 23, 2130-2141.
[12] T.-T. Wang, K. Guo, X.-M. Hu, J. Liang, X.-D. Li, Z.-F. Zhang, J. Xie, Chemosensors 2020, 8, 10.
[13] P. H. Ho, F. Salles, F. Di Renzo, P. Trens, Inorganica Chimica Acta 2020, 500, 119229.
