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Aqueous Phase Nitric Oxide Detection by an Amine Decorated Metal-Organic Framework

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Selective and sensitive aqueous phase nitric oxide (NO) detection has been demonstrated by implementing an unsophisticated approach of ligand modulation in a porous, robust metal-organic framework (MOF). The detection is achieved through deamination by NO in an amine decorated luminescent MOF. This is the first report of employing a physiologically stable, functionalized MOF as a NO sensor.

Nitric oxide (NO) has been widely recognized as a vital biological signalling agent and identified as a key neurotransmitter.[1] It has also been found to be involved as a protective and regulatory agent in several physiological and pathological processes like cellular adhesion, renal functioning, leukocyte adhesion inhibition and bronchodilation. Despite reports of diverse functionality of NO in biological systems,[2] the mechanisms of such activity are not completely understood. NO has high diffusivity and its short lived nature makes it a difficult molecule to investigate with respect to its reactivity pathways.[3] To enhance our insightful understanding of *in vivo* NO functioning a number of techniques have been resorted to including chemiluminescence, colorimetry, electron paramagnetic resonance and electrochemistry.[4] Fluorescence-based methods score over others in higher sensitivity to analyte, non-invasive nature, quick response, ease of sample processing and simplicity in real-time monitoring when coupled to microscopic imaging.[5] To exploit these advantages, artificial fluorescent probes targeting such biologically relevant species and incorporating all the attributes are desired.

The conventional fluorometric strategies for NO recognition by synthetic molecules have focused on *ortho-diamine* probes and metal based complexes. The diamine appended systems have relied on NO activated triazole formation to disengage the photoinduced electron transfer (PET) process,[6] while those based on metal-centred complexes have utilized the reductive nature of NO towards the multivariate oxidation states of metal.[7] Despite considerable progress in the development of *in vivo* targeted synthetic probes, there exist a few intrinsic constraints. The poor water solubility of organic reporter molecules hinders their applicability and the limited compatibility of inorganic complexes to endogenic conditions limits their utility. In pursuit of overcoming these shortcomings, development of relevant newer materials and/or improved synthetic modulations is an active aspect of current research in this domain.

Metal-Organic Frameworks (MOFs) have emerged as an exciting class of porous materials, which are constructed from organic linkers and inorganic cationic nodes.[8] The possibility of tuning the pore surface actuates the fabrication of a host system sought for selective molecular recognition. The porous nature improves the extent of host-guest interactions by virtue of pre-concentration and serves to examine the confinement effect on the target molecule.[9] The ordered accessible channel enhances molecular diffusivity to hasten the response time. Owing to the chemical and thermal stability of such ordered networks, certain MOFs can be utilized in environments with diverse chemical and/or physical extremities. Inclusion of all these attributes has made MOFs as a potential candidate for sensing and biomedical applications.[10] The feasible accessory of luminescence as a signal transduction mechanism affords a notable subordinate classification of luminescent metal-organic frameworks (LMOFs).[11] LMOFs furnish this material as a probe to selectively recognize variety of molecules and ions by a detectable luminescence response.
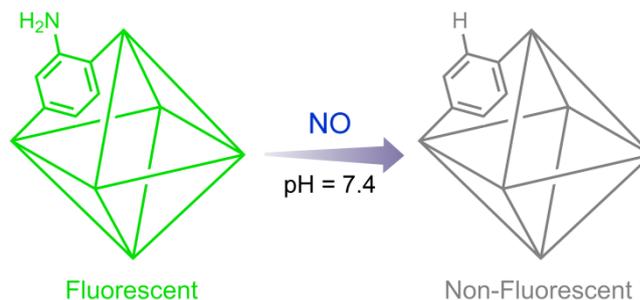
Scheme 1 Schematic illustration of nitric oxide detection by a functionalized MOF.

MOFs have found utility as NO storage and delivery agents by rational application of the porous character of these materials.[12] Yet the extension of the commonly applied approaches for NO detection to MOF-based sensors has been rare. The only known report has employed the reduction of the Cu^{2+} metal centre by NO as a function of fluorescence change.[13] Although the mechanism in play affords the formation of Cu(I) metal centres, MOFs based on Cu(I) and carboxylate anions are not known to be stable in aqueous phase and fluorescence response may arise from the disintegration of the ordered framework in such cases. It is therefore important to develop MOF systems which can be stable in different solvent environments. The superior approach to

address the objective would be to append the appropriate functionality to the organic ligand. This could avoid the concerns regarding the stability of the probe system and also improve specificity to analyte response.
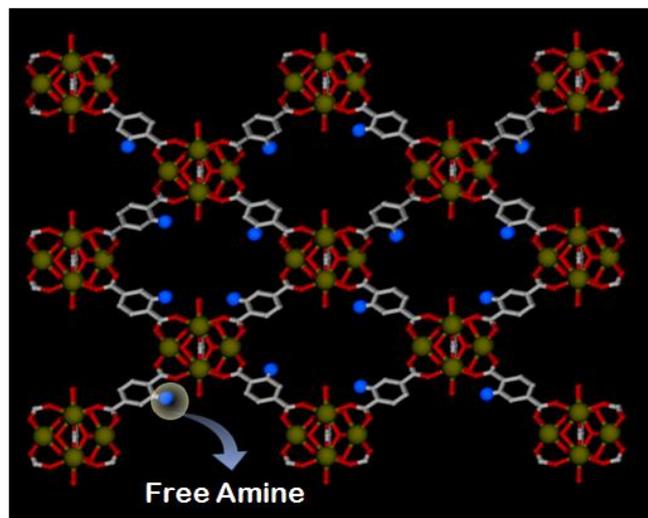
On these lines we sought to encompass feasible approaches into LMOFs by applying functional organic moieties for constructing such porous networked probes. Lately the potency of free primary aromatic mono-amine and *ortho-hydroxyamino* group as a NO sensory functional group under aerobic conditions by deamination mechanism has been reported.[14] The active moiety was tagged to a fluorophore and the alteration of photo-induced electron transfer (PET) process was corresponded to the deamination reaction. Enthused with this knowledge we sought to incorporate the free mono-amine aromatic group in to a small ligand of a chemically stable, luminescent and a porous MOF viz. UiO-66@NH₂ (**1-NH₂**),[15] for detection of NO (Scheme 1). In contrast to the reaction of NO with aromatic amines under inert conditions,[16] the present case involves deamination in a MOF as the reaction proceeds in aerated aqueous medium. The MOF is stable under physiological conditions for several hours, constituted of relatively non-toxic metal centres and bears free primary amine moieties as pendant groups in the porous channels (Fig. 1). The origin of fluorescence in this MOF is primarily from the organic ligand and thus seemingly the modulation of the active site as a consequence of the deamination reaction can be monitored in the fluorescence response directly without interference from any background metal based emission.

To examine the response of **1-NH₂** towards NO, the desolvated phase of the MOF (dispersed in HEPES buffer 10 mM, pH 7.4) was excited at 325 nm and the emission profile was recorded between 340-630 nm followed by NO addition. A smooth emission curve peaking at 432 nm was observed, which underwent a significant change upon treatment with NO (Fig. 2). Notably the fluorescence change is more than 80% with only 1.0 equivalent addition of NO. To ascertain the structural integrity of the probe compound and corroborate with the understood mechanism, powder X-ray diffraction (PXRD), ¹H-NMR,

Fourier-Transform infrared (FT-IR) spectroscopy, gas adsorption, scanning electron microscope (SEM) and ultraviolet reflectance experiments were performed before and after NO addition. The PXRD patterns confirmed the stability of the probe towards the target species under physiological conditions (Fig. S1). SEM images also assert the morphological stability of the probe after NO treatment (Fig. S19). ¹H-NMR of the digested samples of **1-NH₂** before and after NO treated phases (Fig. S3) along with the decrement of the N-H bending peak at 1650 cm⁻¹ in the FT-IR spectrum (Fig. S2) and the ~10 nm shift in the reflectance spectrum accords with the deamination mechanism (Fig. S5). Low temperature N_2 & CO_2 gas adsorption measurements were performed on both the compounds. As anticipated, N_2 adsorption (77 K) uptake amount subtly enhances in case of the NO-treated compound (Fig. S17). The CO_2 (195 K) uptake amount in case of the NO treated phase appreciably lowers from that of the parent compound (Fig. S18), which substantiates the correlation of lessening of active amine sites with the deamination reaction, since the effect of amino group on CO_2 adsorption in MOFs is well understood.[17]
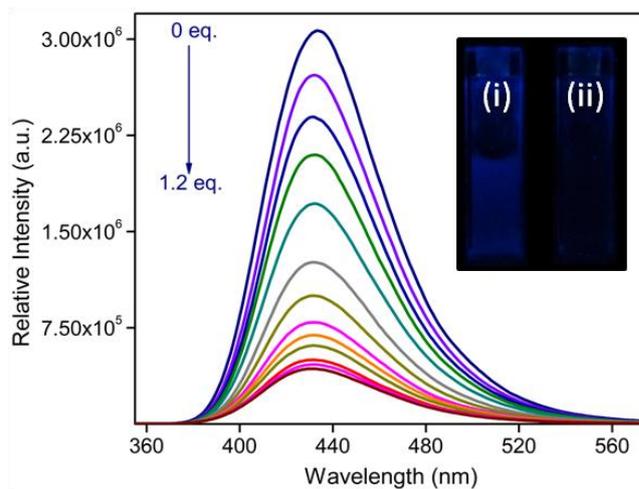
Fig. 2 Change in fluorescence intensity of **1-NH₂** upon incremental addition of 0.1 mM NO solution. Inset - fluorescence of **1-NH₂** (i) before and (ii) after NO addition.

Fluorescence response to incremental treatment of NO was recorded and gradual quenching of the MOF fluorescence could be observed (Fig. 2). The fluorescence change at low concentration gives excellent linear fitting (R^2 = 0.9997) with a quenching constant of 4.15×10^5 M⁻¹ (Fig. S6). The high quenching constant value emphasizes the strong interactions between the highly diffusive NO and the MOF bearing pendant free amine. The LOD (limit of detection) for the probe was calculated to be 0.575 μM (Fig. S20, Table S1-S2) which is near the relevant cellular range of NO.[18] Additionally the response of NO towards an analogous MOF, bearing no free functionality viz. UiO-66 (**2**) was checked under similar conditions (Fig. S16). The fluorescence response was not perturbed considerably, which highlights the role of the primary amine group in the sensing process. Likewise, the response of NO towards the ligand only was recorded. The fluorescence response is not altered at such concentration which clearly emphasizes the superiority of an ordered framework for the present application. This result also

suggests that LMCT based fluorescence dominates in case of **1-NH$_2$**, which renders low concentration detection.[19]
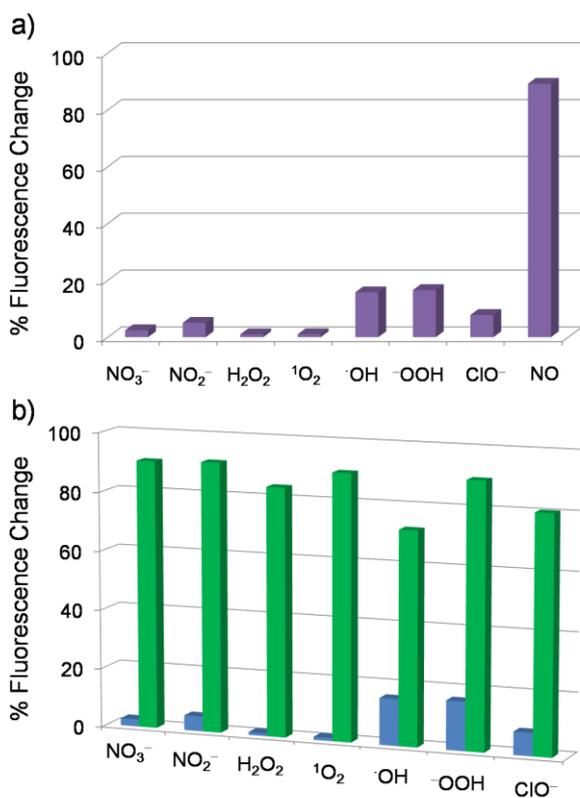
Fig. 3 a) The fluorescence change of **1-NH$_2$** towards various RNS/ROS analytes. b) Fluorescence change of **1-NH$_2$** in the presence of the particular RNS/ROS species (blue) followed by the NO addition (green).

Exclusivity in detection is imperative for a real-time sensor system. To validate this aspect, the responses of **1-NH$_2$** towards different possible competitive RNS and ROS species (2 equiv.) like H_2O_2, ClO^-, ^-OH, $^1O_2$, NO_3^- and NO_2^- were recorded. In a typical experiment the particular RNS/ROS species were added to the solution of **1-NH$_2$** in HEPES and allowed to equilibrate for 5 minutes. No substantial change was observed for any of the species (Fig. 3a), affirming the selective performance of the probe.

Encouraged from these results we sought to check the consistency of the performance in concurrent presence of the other RNS/ROS species. In this experiment, the particular RNS/ROS species was added to the solution containing **1-NH$_2$** and equilibrated for 5 min followed by recording the emission spectrum. To this solution NO was added and the response was monitored after equilibration. As anticipated, the presence of other RNS/ROS species does not alter the activity of NO towards the functionalized MOF compound (Fig. 3b). From the perspective of applicability in complex biological systems, this result renders the present MOF relevant for NO detection, avoiding off-target reactivity and false response.

In summary, we have presented a novel approach of regulating the ligand functionality in a MOF as a function to probe the presence of NO in aqueous environment. Hitherto only metal based emission in MOFs has been demonstrated as a tool to detect NO, but the aspect of functional pore surface seeks to be studied for better applicability. Deamination by NO in an amine decorated MOF has been tapped as a process to monitor its presence. The MOF performs the sensory activity in a selective manner, even in presence of potentially interfering RNS/ROS species. Although few organic compounds are known as NO sensors via deamination pathway, this report presents a new platform of stable MOFs for investigation of such reaction based sensors. Owing to accessible and ordered porosity, MOFs present a uniquely superior of class of sensors with enhanced host-guest interactions and tuneable functionalization of building blocks can render its use as a real-time sensor for variety of applications. We believe that the present work will stimulate the research in the field of MOF based sensors for NO detection and will propel the goal of attaining probes for relevant biological applications.

Notes and references

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† Electronic Supplementary Information (ESI) available: For synthesis details, PXRD patterns, ^{1}H-NMR, FT-IR spectra, solid-state reflectance spectra, gas adsorption profiles and photo physical studies of MOF. See DOI: 10.1039/b000000x/

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