Microchim Acta (2016) 183:1501–1506
DOI 10.1007/s00604-016-1762-2

SHORT COMMUNICATION

MoS_2 based digital response platform for aptamer based fluorescent detection of pathogens

Pargat Singh[1,2] · **Ritika Gupta**[1] · **Monika Sinha**[1] · **Rajesh Kumar**[2] · **Vijayender Bhalla**[1]

Received: 17 November 2015 / Accepted: 19 January 2016 / Published online: 27 January 2016

Abstract The authors describe a method for detecting the pathogen S. typhimurium with a 'yes-or-no' kind of response. A fluorescein-tagged aptamer (Apt-FAM) probe was used for the specific recognition of pathogens, and molybdenum(IV) disulfide nanosheets (MoS_2-Ns) were used as a primary binder of free (unbound) ssDNA that quenches its fluorescence. If, however, target pathogens are present, Apt-FAM probe binds to it and fluorescence is restored. The method displays a detection limit of 10 CFU·mL^{-1}, is selective over *E. coli* and *P. vulgaris*, and therefore represents a promising on-site screening tool for microbes.

Keywords S. typhimurium · Quenching · Fluorescence recovery · Nanosheets · Bacteria sensor · Bioassay · Water analysis

Introduction

The testing of food and water for the infectious disease-causing organism has been an important issue to ensure its safety and security for human consumption. Several infections occur through ingestion of contaminated water, and the cases of water borne illness are considerable healthcare burden on developing nations [1]. The incidence of these infections particularly typhoid fever account for 17–33 million cases each year and 600,000 deaths [2]. Besides, long term exposure of these microorganisms prompts severe chronic conditions such as ocular failure and kidney damage [3]. Water quality evaluation and the rapid diagnostics of pathogenic microorganisms remain a great challenge to ensure maintenance of public health, and prevention of bioterrorism [4]. As waterborne pathogens travel through the environment, they dilute to low but clinically significant concentrations creating a huge detection challenge [5].

2-D inorganic nano transition metal dichalcogenides (TMDs) have attracted attention due to their widespread applications in the field of catalysis, energy storage and electronic devices [6]. Particularly, MoS_2-Ns aggrandize TMD's due to its exemplary electronic, optoelectronic and energy harvesting properties [7–10]. Single layer MoS_2-Ns can act as quencher of fluorescence [11] which opens new analytical opportunities. Besides fluorescence quenching, MoS_2-Ns surface is also known to bind ssDNA molecules via van der Waals forces of attraction between nucleobases and the basal planes of MoS_2-Ns [12]. In the case of ssDNA aptamer, its binding to target molecule induces rigid structure, effectively preventing interactions of nucloebases with MoS_2-Ns surface [13]. This makes aptamer-MoS_2-Ns interactions weak and thus results in restoration of quenched fluorescence.

Herein on the basis of above mentioned thought, the design of rapid pathogen detection system is reported. We utilize Salmonella typhimurium as a model pathogen for the development of the assay platform. The assay employs MoS_2-Ns that act as a primary weak binder for free ssDNA and quenches its fluorescence. If, however, the specific pathogen is present in solution the Apt-FAM probe is unable to bind with MoS_2-Ns and fluorescence is restored. Scheme 1 briefly illustrates the operational mechanism of the assay.

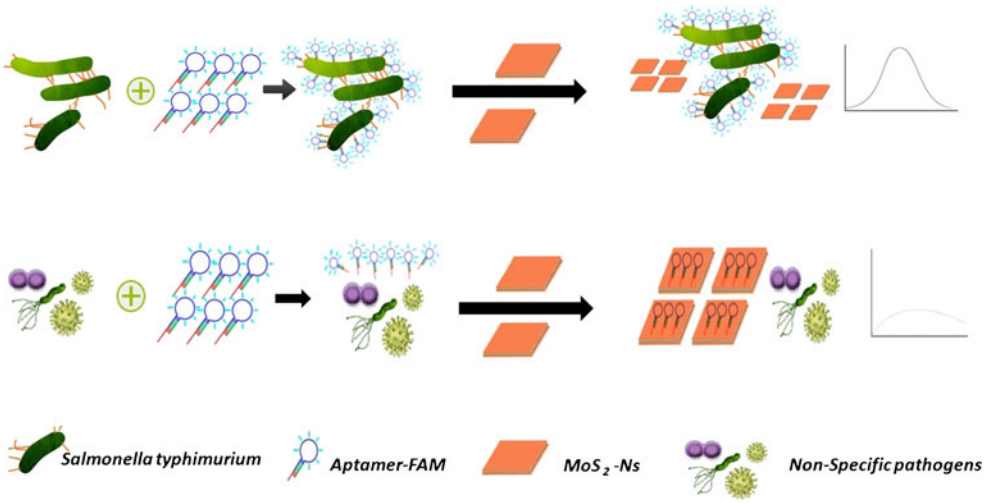
Pargat Singh and Ritika Gupta contributed equally to this paper.

Electronic supplementary material The online version of this article (doi:10.1007/s00604-016-1762-2) contains supplementary material, which is available to authorized users.

✉ Vijayender Bhalla
vkbhalla@imtech.res.in

[1] CSIR-Institute of Microbial Technology, Chandigarh 160036, India

[2] UIET-Panjab University, Chandigarh 160014, India



Scheme 1 Schematic illustration of binding of Apt-FAM probe with pathogen and MoS_2 Ns. In the presence of target pathogen assay shows fluorescence whereas in the absence quenching pre-dominates

Experimental

Reagents and materials

Molybdenum(IV) disulfide powder (<2 μm), poly-D-lysine, ammonium sulfate, EDTA, PBS buffer reagents (KCl, NaCl, KH_2PO_4, Na_2HPO_4) were purchased from Sigma Aldrich (www.sigmaaldrich.com). Aptamer specific to target pathogen (S. typhimurium cells) was identified from literature [14] and got synthesized from Sigma. The sequence was dissolved in deionized water and the stock was prepared. Maxisorp flat and round bottom polystyrene microtiter black plates were products of Nunc, Thermo Scientific (Denmark) (www.thermoscientific.com). Goat anti-rabbit secondary antibody HRP labelled was from Merck genei, Bangalore, India. Polyclonal antibodies against S. typhimurium were produced in-house (details provided in Supplementary information). Water used for making buffers and MoS_2-Ns synthesis was purified with Milli-Q ultra pure system (Millipore, India) having a resistivity ≥18 MΩ·cm.

Apparatus

Biotek synergy h1 hybrid plate reader was used throughout for fluorescence studies. UV-Vis spectra obtained using Shimadzu UV-1800 spectrophotometer. High resolution transmission electron microscopy was carried out using Hitachi (H-7500). Ultrasonication was performed using VCX750 from Sonics and Materials Inc., USA.

Pathogenic strains

Salmonella typhimurium, *Escherichia coli* and Proteus vulgaris were procured from in-house MTCC, IMTECH Chandigarh, India (www.mtcc.imtech.res.in) with strain number 3232, 6198, 744 respectively. All details pertaining to preparation of pathogenic strains are provided in supporting information.

Synthesis of MoS_2-Ns

MoS_2-Ns for pathogen sensing were prepared according to mixed-solvent exfoliation method with little modifications [15]. Briefly, 35 mg of MoS_2 powder was added to 25 mL of ethanol-water (45 % *v*/v) solution. The mixture was sonicated for 7 h, and then dispersion was centrifuged at 5000 rpm (2348 rcf) for 30 min. The colloidal supernatant was collected and was characterized by transmission electron microscopy and UV-Vis spectroscopy.

Concentration optimization and specificity check of apt-FAM probe

The concentration optimization and specificity check was performed using well established platform of binding assay by employing a microtiter black well plate that was initially coated with 100 $\mu g \cdot mL^{-1}$ poly-D-Lysine, overnight at 4 °C. 100 μL of S. typhimurium cells (10^9 $CFU \cdot mL^{-1}$) was then added to each well and the plate was then incubated for 2 h at 37 °C. After washing with 1X PBS, different concentrations of Apt-FAM probe (20 nM–100 nM) was added and incubated for 30 min. Fluorescence intensity was then recorded 494 nm (excitation) and 525 nm (emission) after multiple washings to remove unbounded Apt-FAM probe. For specificity analysis, 100 μL of each of the different pathogenic strains at a concentration of 10^5 $CFU \cdot mL^{-1}$ were coated on a poly-D-Lysine coated black microtitre plate. The plate was then incubated for 30 min with the optimized concentration of Apt-FAM probe followed by washing and fluorescence measurements.

Fluorescence quenching by MoS_2 and development of colloidal assay

To study the fluorescence quenching behaviour different concentrations of MoS_2-Ns (100 μL) ranging from 3 $\mu g \cdot mL^{-1}$ to 18 $\mu g \cdot mL^{-1}$ were incubated with 50 μL of Apt-FAM probe for

5 min at 37 °C. The optimum concentration of MoS_2-Ns which show maximum quenching was used for the detection of pathogens in a colloidal solution format without immobilization of cells. Different concentrations of S. typhimurium 10–10^8 CFU·mL^{-1} (100 μL) were incubated with 50 μL of the optimized concentration of Apt-FAM probe in PBS buffer pH 7.4 for 30 min at 37 °C in a black well plate. Then, 100 μL of the optimized concentration of MoS_2-Ns was added followed by incubation for 5 min. The fluorescence readings were obtained using excitation wavelength of 494 nm and an emission of 519 nm. The same protocol was used for different pathogenic strains in order to justify selectivity and specificity of the assay.

Results and discussions

Characterization of MoS_2-Ns

Figure 1 shows UV-Vis spectrum and TEM micrographs of MoS_2-Ns. UV-Vis spectra of colloidal suspension showed absorption peaks around 612 nm and 672 nm that can be attributed to characteristic A and B direct excitonic transitions, with energy split from valence band spin orbital coupling. These results are in agreement with previously reported results and indicate the presence of dispersed layered MoS_2-Ns [16]. High resolution transmission electron microscopy was carried out to characterize the surface morphology of the exfoliated MoS_2-Ns that clearly shows the formation of nanosheets during the process with an average width of around 200 nm.

MoS_2-Ns and apt-FAM based detection of pathogens

The concentration optimization of APT-FAM probe was performed using the well established binding assay carried out by immobilization of cells on a microtiter plate. Fixed concentration of S. typhimurium cells were coated on plate and the concentration of Apt-FAM probe was varied. An optimum 50 nM concentration of the Apt-FAM probe was used in our experiments as it gives sufficient fluorescence window for pathogen detection as shown in Fig. S1 (Supporting information). This platform of binding assay was just used for optimization purposes and the MoS_2-Ns/Apt-FAM assay is capable of detection of free live pathogen in solution.

For the development of a colloidal assay, the MoS_2-Ns were initially kept in a hot air oven for 2–3 h at 60 °C and afterwards volume makeup was carried out by using deionized water. This was done to remove ethanol which is an important recipe for exfoliation during MoS_2-Ns synthesis. The presence of ethanol could lead to precipitation of ssDNA molecules and hence it's important to remove it before experimentation. Figure 2 shows fluorescence quenching of 50 nM Apt-FAM probe at different concentrations of MoS_2-Ns in solution. An increase in concentration of MoS_2-Ns provides a decrease in fluorescence intensity, and quenching up to 82 % was observed at 18 μg·mL^{-1}. This quenching mechanism can be explained by weak van der Waals forces between nucleobases of Apt-FAM probe and surface of MoS_2-Ns, which dominates on increasing MoS_2-Ns concentration [17]. As the Apt-FAM probe approaches near the surface of MoS_2-Ns, hairpin structure of Apt-FAM probe tends to open, and nucleobases get stacked over the surface of MoS_2-Ns. As reported earlier [18], transition metal ions possess intrinsic fluorescent quenching properties for organic dye molecules. The Apt-FAM probe conformation brings organic fluorescent dye molecule and MoS_2-Ns closer to each other and leads to quenching. Thus, it is clear from above discussion that MoS_2-Ns act as an effective fluorescence quencher for Apt-FAM probe. The kinetics of quenching process with time was also studied. The fluorescence intensity quenches exponentially within 5 min as shown in Fig. S2 (Supporting information) reaching a saturation value. This exponential behaviour is due to the availability of large surface area of MoS_2-Ns for quenching Apt-FAM probe fluorescence intensity.

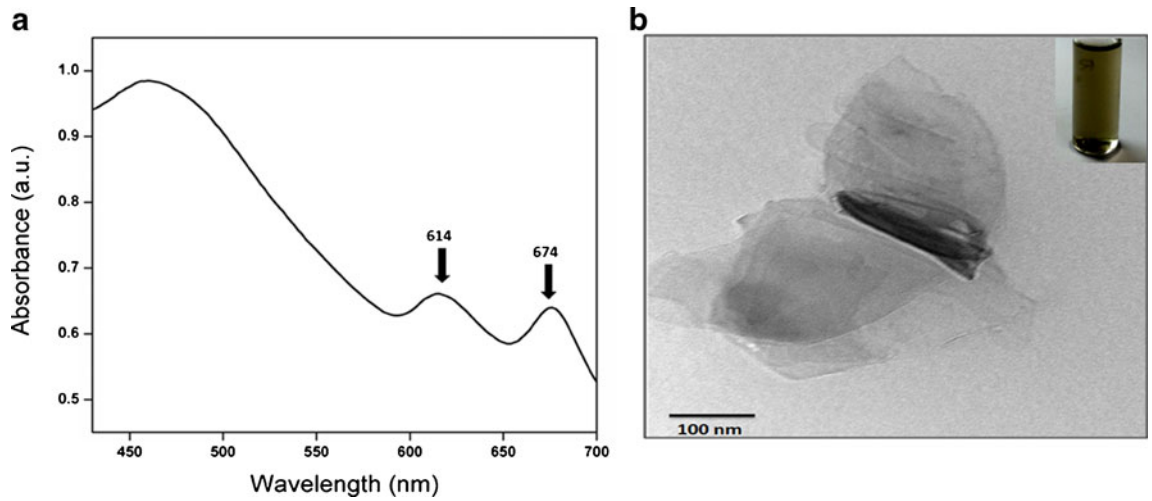


Fig. 1 **a** UV-Vis spectra of MoS2-Ns. **b** TEM micrograph of MoS_2-Ns. Inset shows snapshot of the sample

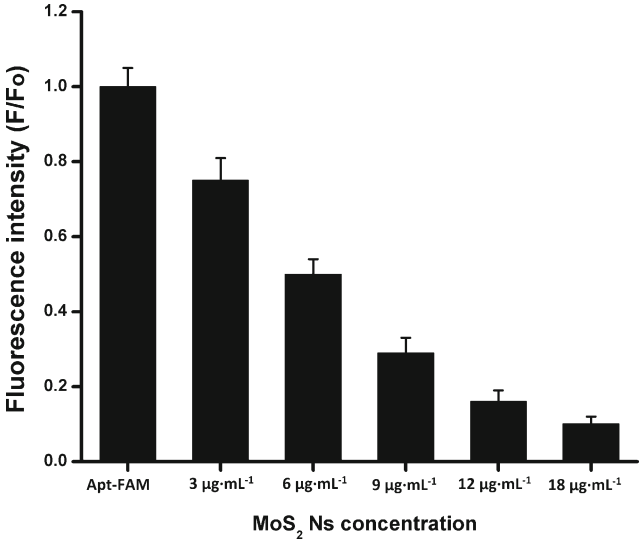


Fig. 2 Fluorescence Quenching of Apt-FAM probe upon addition of different concentrations of MoS_2-Ns

Figure 3 shows the response of the assay to the increasing concentration of pathogens in water. The fluorescence was found to restore by approximately 74 % to 84 % between 10 CFU·mL^{-1} to 10^8 CFU·mL^{-1} respectively. This fluorescence increase is due to the strong affinity of Apt-FAM probe with surface moieties of pathogen cell in comparison to weak interactions between MoS_2-Ns and Apt-FAM probe. As the Apt-FAM probe binds to the cell surface moieties the distance between organic dye molecule and MoS_2-Ns surface increases leading to increase in fluorescence. Interestingly, the assay does not show a clear linear response along the entire tested concentration range. On the other hand, it shows digital on/off response as it very efficiently detects the presence of a pathogen at low cell concentration of 10 CFU·mL^{-1}. The assay does not show much of a difference in fluorescence between 10 CFU·mL^{-1} to 10^4 CFU·mL^{-1}. However, we observe a linear trend between 10^4 CFU·mL^{-1} to 10^7 CFU·mL^{-1} indicating to us a good possibility of analytical interpretation in this particular range.

The authors were interested to find reasons for lack of linearity along all the tested concentrations. The addition of different concentrations of cells to bare Apt-FAM probe did not lead to any changes in the fluorescence intensity thus ruling out any influence of opacity (Fig. S3 Supporting information). Experiments performed using specific antibody probe shows interactions of MoS_2-Ns with S. typhimurium (Fig. S4 Supporting information). This explains the digital like response since the pathogen bound to the Apt-FAM probe can freely interact with MoS2-Ns. This leads to quenching which ideally increases with an increase in pathogen concentration. At a first glance, this seems to be a weakness of these kinds of a system that does not exactly predict the concentration of pathogens. But the ease to use and simple workflow makes it fit as a platform for a qualitative analytical device. Thus, the system can be used to detect the occurrence of harmful disease-causing microbes at very low concentrations. The sensitivity and time took to obtain results rival many of the reported systems for S. typhimurium (Table 1). As illustrated, the detection limit of MoS_2-Ns based fluorescent assay is much lower than that of previously reported methods.

Figure 4a shows the area fluorescence scans of wells of the black microtitre plate in the presence and absence of pathogens. The change in colour indicates a change in fluorescence intensity on the surface of micro wells. This change in fluorescence at the surface of microwell expands the application of assay to use in modern smartphone based handheld

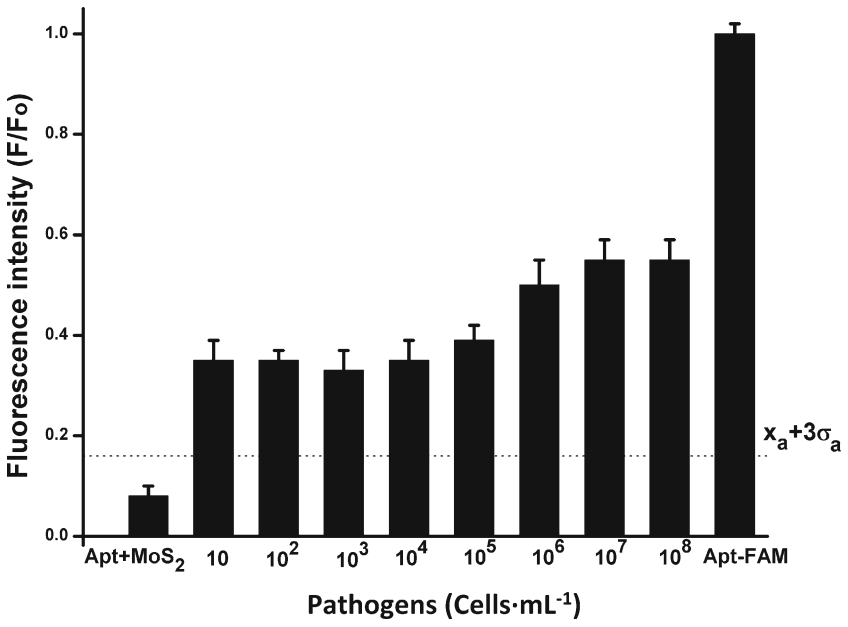


Fig. 3 Assay response to different concentrations of S.typhimurium cells in comparison to Apt-FAM + MoS_2-NS (Apt + MoS_2) and bare Apt-FAM. The error bar correspond to three independent experiments ($n = 3$). Line of LOD is given by ($x + 3\sigma$) rule

Table 1 Figures of merit of recently reported methods for determination of S. typhimurium

Method	Material	Linear range ($CFU{\cdot}mL^{-1}$)	Detection limit ($CFU{\cdot}mL^{-1}$)	Reference
Calorimetric	ELISA using HRP labelled antibody	9.2×10^3 -9.2×10^6	-	[19]
SPR	Gold surface for monitoring antigen antibody interactions	–	1×10^6	[20]
Electrical Impedance	Au Nps immunoreactions on GCE	10^3-10^7	5×10^2	[21]
Flow-Cytometry	CdTe Qds labelling of aptamer	3.8×10^4-3.8×10^7	5×10^3	[22]
Chemiluminescent	ELISA using HRP labelled antibody	9.2×10^3–9.2×10^6	-	[19]
Fluorescence	Graphene Oxide aptamer based assay	1×10^3 - 1×10^8	100	[23]
Fluorescence	MoS_2-Ns aptamer based assay	Digital "Yes or No" kind	10	This work

fluorescence assays, in which just a change in fluorescence at the surface area of micro well is detected to predict the results.

Specificity of assay

To demonstrate the specificity of assay for a single pathogen, we employed other commonly present pathogens in water as negative controls. Typically, E.coli, *P. Vulgaris* were used as non-target pathogens at a concentration of 10^5 and 10^7 $CFU{\cdot}mL^{-1}$. As shown in Fig. 4b, although the fluorescence regain is lesser as compared to Fig. 3, but it proves beyond doubt the specificity of method for S. typhimurium. The assay does not show any response to the presence of these non target pathogens and the response was similar to that of blank sample. The results clearly demonstrate that the method detects S. typhimurium with high specificity and the system is not affected by the presence of any other pathogen. This response is attributed to recognition ability Apt-FAM probe that

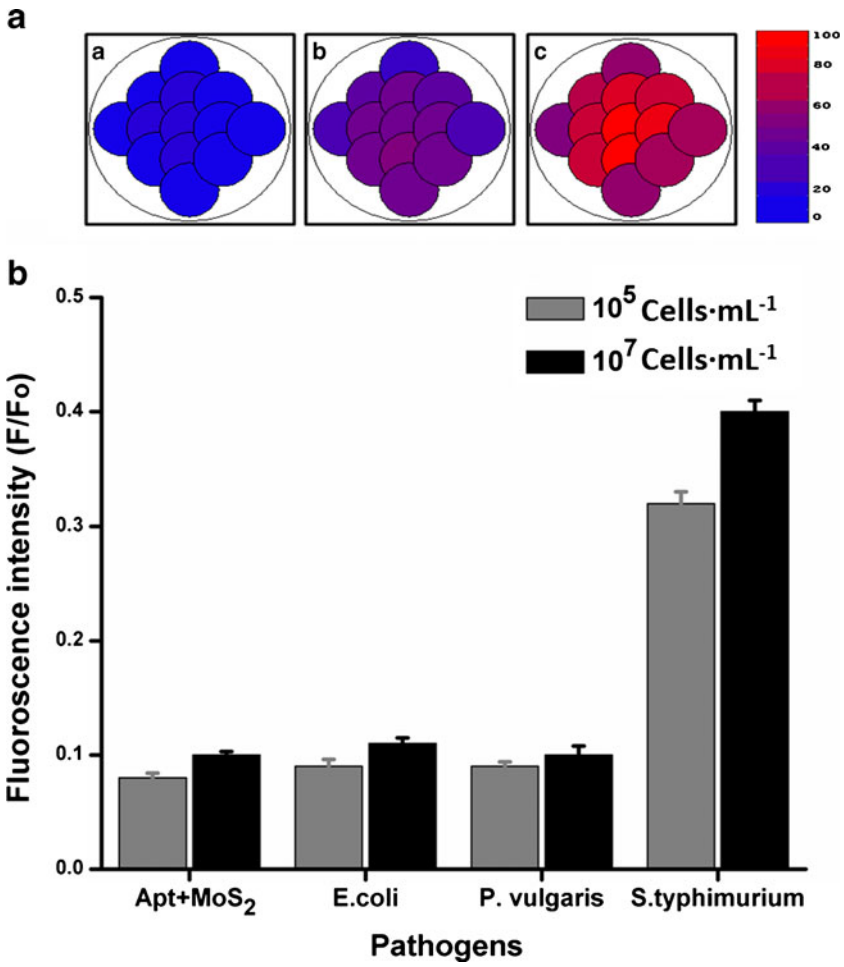


Fig. 4 **a** Color coded pictures for the area spectral scans of the microwells showing visible change in fluorescence intensity at the surface. The three presented pictures represent (*a*) absence of pathogen, (**b**) presence of pathogen, and (**c**) Apt-FAM probe at 50 nM concentration. 4 **b** Fluorescence intensity of Apt-FAM probe towards different pathogens

selectively binds to its target pathogen. Fig. S5 (Supporting information) shows fluorescence intensity of Apt-FAM probe with different pathogenic strains employing the well established binding assay performed using the coating of cells in a microtiter plate. The Apt-FAM probe clearly shows binding with S. typhimurium only. In order to simulate real-time conditions the experiment were also performed using tap water and skim milk. Assay shows a good response in both conditions Fig. S6 (Supporting information). A major limitation of the method is the low analytical interpretation capability. On a whole the MoS_2-Ns based assay shows high selectivity, simplicity and promising detection ability also in complex matrixes with a digital like response.

Conclusion

A digital MoS_2-Ns based assay for the detection of pathogens at very low concentrations is reported. MoS_2-Ns were synthesized by an easy mix solvent method and then used for effective quenching of Apt-FAM probe. In the presence of a target pathogen, Apt-FAM probe induced a rigid conformation with pathogen and thus resulting in restoration of fluorescence intensity. The method showed a digital like on/off response to the presence of target pathogen. The assay also shows excellent sensitivity and selectivity to target pathogen up to a concentration of 10 $CFU{\cdot}mL^{-1}$. Main advantages of method include opportunities to develop rapid, simple, low-cost technologies that can be conveniently executed using a handheld fluorescence detector for pathogen detection.

Acknowledgments The authors thank Director, IMTECH for generous startup funds. VB thanks DST for YS grant.

Compliance with ethical standards The authors declare that they have no competing interests.

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