Droplet-Microarray Based on Nanosensing Probe Patterns for Simultaneous Detection of Multiple HIV Retroviral Nucleic Acids

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ABSTRACT Multiplexed detection of viral nucleic acids is important for rapid screening of viral infection. In this study, we present a molybdenum disulfide (MoS_2) nanosheet modified dendrimer droplet microarray (DMA) for rapid and sensitive detection of retroviral nucleic acids of human immunodeficiency virus-1 (HIV-1) and human immunodeficiency virus-2 (HIV-2) simultaneously. The DMA platform was fabricated by omniphobic-omniphilic patterning on a surface-grafted dendrimer substrate. Functionalized MoS_2 nanosheets modified with fluorescent dye labeled oligomer probes were pre-patterned on positively charged amino-modified omniphilic spots to form the fluorescence resonance energy transfer (FRET) sensing microarray. With the formation of separated microdroplets of sample on the hydrophobic–hydrophilic micropattern, pre-patterned oligomer probes specifically hybridized with the target HIV genes and detached from MoS_2 nanosheet surface due to weakening of the adsorption force, leading to fluorescence signal recovery. As a proof of concept, we used this microarray with a small sample size (<150 nL) for simultaneous detection of HIV-1 and HIV-2 nucleic acids with limit of detection (LOD) to 50 pM. The multiplex detection capability was further demonstrated for simultaneous detection of 5 viral genes (HIV-1, HIV-2, ORFlab and N genes of SARS-COV-2, and M gene of Influenza A). This work demonstrated the potential of this novel MoS_2-DMA FRET sensing platform for high-throughput multiplexed viral nucleic acid screening.

KEYWORDS: Droplet microarray (DMA), 2D MoS_2 nanosheets, Fluorescent resonance energy transfer (FRET), HIV nucleic acids, Omniphobic

1. INTRODUCTION

Human immunodeficiency virus (HIV) is a lentivirus that causes HIV infection leading to acquired immunodeficiency syndrome (AIDS).[1-2] Generally, HIV family contains two major types

of viruses, HIV type-1 (HIV-1) and HIV type-2 (HIV-2). The two types of HIV can infect host through different pathological mechanisms and involve different clinical treatments. It is reported that co-infections of the two HIV virus may complicate the management of HIV infections.[3-4] An effective screening detection of the two HIV viruses in early stage, may provide important evidences for conducting clinical strategies and public health security in early time.[5-7] In addition to HIV, there are many other emerging viral infections, including Coronavirus Disease 2019 (COVID-19) caused by infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), requiring fast and efficient detection methods. The droplet microarray sensing platform in this paper can be easily adapted to detection of multiple viral genes for early diagnostics of other emerging viral infection diseases including COVID-19.

Nucleic acid testing and antibody testing are currently the dominating methods for HIV diagnostics. Since the host needs time to develop an immune response, antibody testing fails to detect early HIV infection. Retroviruses, such as HIV, infect patients by inserting their DNA within the genome of the host. Retroviral DNAs are important biomarkers existing in every infected host cells, which trigger pathological processes of HIV infection at an early stage. Thus, detection of integrated retroviral DNAs is important for early estimation of the incidence and prevention of viral transmission. Polymerase chain reaction (PCR) is considered as a standard genetic testing for HIV nucleic acid detection.[8-10] Besides, other biosensing methods are also developed for HIV nucleic acid detection, such as electrochemiluminescence,[11] colorimetry,[12] electrochemical,[13] and fluorescence based sensing.[14] However, most of the current methods face the disadvantages of being time consuming, requiring large volume of samples and lacking multiplexed capability. Therefore, it is of high importance to develop a new sensing platform that

is highly sensitive, fast, compatible with low sample/reagent consumption and capable of multiplexed detection.

Droplet microarray (DMA) based platform is a potential solution to meet these requirements. The DMA is a miniaturized high-throughput platform with individual droplet size down to nanoliters, which has been used in various biological applications such as cell screening, drug testing, lipidoids synthesis and biofilm patterning.[15-18] However, the application of DMA on parallel biosensing has not been explored yet. A small droplet microarray with defined location of separated droplets is of high interest for high-throughput biosensing. Each droplet represents an ultra-small liquid reservoir without solid walls where liquid is confided by either hydrophobic or omniphobic surface properties.[19] As a biosensing substrate, it could greatly reduce the cost of each sensing spot, while enabling the formation of ultra-small droplets. DMA platform is usually based on a solid substrate, such as a glass surface coated with a nanoporous polymer layer functionalized to form either superhydrophobic/hydrophilic or omniphobic/omniphilic micropatterns via UV-induced thiol-yne or thio-ene reactions.[20-21] DMA platform will generate an interface which is easily to graft functional nanomaterials to for biosensing purpose. In this paper, we further develop a droplet microarray platform based on dendrimer omniphobic-omniphilic coating with pre-patterned nanosensing probes as a parallel biosensing platform. Dendrimers are monodispersed and multivalent molecules with highly branched structures, which have been used for many biomedical interfaces due to their polyvalency, self-assembling, electrostatic interactions, chemical stability and low cytotoxicity.[22-23] Dendrimer-based DMA platform will generate an interface which is easy to be grafted with functional nanomaterials for biosensing purpose.

Nanomaterials based fluorescence resonance energy transfer (FRET) is a sensing mechanism based on energy transfer from donor to acceptor which is used in many biosensing and bioanalysis

applications.[24-25] The sensing performance of FRET assay is mainly determined by the design of donor and acceptor pairs. Recently, two-dimensional molybdenum disulfide (MoS_2) nanosheets have aroused a lot of interests for biosensing due to its high surface-to-volume ratio, unique optical properties, good biocompatibility and good quenching capability.[26-28] Compared with current ganic quenchers, MoS_2 nanosheets also showed advantages on the protecting the loaded genes from enzymatic cleavage.[29-30] Currently, nanomaterials-based FRET assays are mainly used in bulk solution to detect various biomolecules. The application of nanomaterials-based FRET in droplet microarray has not been explored yet.

In this paper, a droplet-microarray with MoS_2 FRET nanoprobe patterns (MoS_2-DMA) was developed for multiplexed HIV gene biosensing, which provided a solid substrate-based FRET array sensing platform. In this design, dendrimer-based micro-patterning was applied for DMA fabrication. MoS_2 nanosheets were then adsorbed on dendritic spots as acceptors for FRET assays. The MoS_2-DMA FRET sensing platform was established by further adsorption of fluorescent dye-labelled oligonucleotide probes on MoS_2 nanosheet coated hydrophilic spot surfaces as donors of FRET assays, which led to the "off" status of the fluorescence signal due to the energy transfer from dyes to MoS_2 nanosheets. In presence of HIV target nucleic acids, the specific hybridization between sensing probes and target nucleic acids would detach the probes from MoS_2 nanosheet surface, therefore triggering the "turn-on" of the fluorescence signal. As a proof-of-concept, we used this MoS_2-DMA FRET sensing platform to realize simultaneous sensing of multiple samples of HIV genes with good sensitivity and specificity. The limit of detection (LOD) for HIV1 and HIV2 genes are 1.24 nM and 1.26 nM, respectively. Our MoS_2-DMA FRET sensing platform combines droplet microarray with nanomaterials-based FRET sensing, open the possibility for

high-throughput multiplexed viral gene screening and rapid diagnostics for emerging viral infection diseases such as COVID-19.

2. EXPERIMENTAL SECTION

2.1 Materials

MoS_2 material was purchased from Nanjiang NKNANO Ltd, China. The oligonucleotides were synthesized and purified by Sangon Biotech. Co., Ltd., Shanghai, China. The sequences of oligonucleotides applied are as follows: capture sequence HIV-1 (FAM-5' CTGGGATTAAATAAAATAGTAAGAATGTATAGC-3'),[8] capture sequence HIV-2 (Cy5-5'-AAAGGACCAGGCGCAACTAAATTCA-3'), target sequence HIV-1 (5'-GCTATACATTCTTACTATTTTATTTAATCCCAG-3'), target sequence HIV-2 (5'-TGAATTTAGTTGCGCCTGGTCCTTT-3'), four-bases mismatched sequence to probe 1 (5'-GCTAAACATCCTAACTATTTAATTTAATCCCAG-3), four-bases mismatched sequence probe 2 (5'-TGAAGTTCGTTGCACCTGATCCTTT-3'), target sequence hepatitis B virus (HBV) (5'-CTGGATCCTGCGCGGGACGTCCTT-3'), target sequence hepatitis C virus (HCV) (5'-CACGCCCAAATCTCC-3'). All the synthesized sequences were dissolved in DNAse/RNAse free ultrapure distilled water as stock solution and kept in -20^0C. Normal human serums was purchased from Thermo Fisher Scientific, US. The glass substrates were purchased from Marienfeld superior Ltd, Germany. 4-(Dimethylamino)pyridine was purchased from Novabiochem Ltd, Germany. Ethanol/water (1:1, v/v) and acetone were purchased from Merck Millipore Ltd, Germany. N,N'-diisopropylcarbodiimine was purchased form Alfa Aesar Ltd, Germany.

2.2 Characterization

The morphology of MoS_2 nanosheets was observed by using a JEOL-2100F transmission electron microscope installed with an Oxford Instruments EDS system (200 kV). Size distribution and ζ potential of the MoS_2 nanosheets were measured by a Zetasizer Nano Z system (Malvern Instruments Ltd). The optical absorbance spectra of the nanosheets were obtained with a UV−Vis spectrophotometer (Ultrospec 2100 pro, Harvard BioScience, Inc.). DMA surface and MoS_2 modified DMA surface were characterized by a JEOL Field Emission Scanning Electron Microscopy system. Atomic force microscopy characterization was performed on a Bruker Dimension ICON AFM machine with standard tapping mode. Raman spectrum of dendritic spots was measured by a Renishaw Micro-Raman Spectroscopy System. Microscopy image of the droplet microarray was obtained by using a Keyence BZ-9000 microscope. Fluorescence spectrum was characterized by FLS920 photoluminescence spectrometer (Edinburgh, UK).

2.3 Dendrimer-based DMA fabrication

Dendrimer has been used to modify glass slides for DNA microarray fabrication.[31] Here, a modified method was developed to fabricate dendrimer-based DMA. Vacuum plasma is a process of gas ionization in a vacuum chamber which is often used for surface modification. The glass slides were firstly cleaned by vacuum oxygen plasma treatment for 10 min and then silanized with triethoxyvinylsilane at 80°C overnight. The slides were washed with ethanol and dried with compressed air. After silanization, the slides were modified with thioglycerol (3-mercaptopropane-1,2-diol). The cleaned slides were covered with 300 μL thioglycerol solution and exposed to UV light (260 nm, 120 s) at an intensity of 3 mW/cm^2 (OAI Model 30, San Jose, USA). 112 mg 4-dimethylaminopyridine and 250 μL pentenoic acid were mixed and dissolved in 90 mL acetone as an esterification solution. Then the slides were placed into the esterification solution for an

overnight treatment at room temperature. The modification and esterification processes were repeated three times, resulting in a surface with abundant terminal alkenyl groups ready for patterning via the photo-click thiol-ene reaction.

The patterning was performed as follows. 300 μL *1H,1H,2H,2H*-perfluorodecanethiol (PFDT) solution (10 vol%) was used to cover the slide. The surface was then exposed to UV light (260 nm, 60 s, 3 mW/cm^2) through a quartz photomask (Rose Photomasken, Bergisch Gladbach, Germany). The hydrophobic boundary region was obtained after the fluorination process. Then the slides were covered with 1.2 M cysteamine hydrochloride ethanol solution and irradiated with UV light (260 nm, 60 s, 3 mW/cm^2) to functionalize the reactive spots with hydrophilic amino groups. The patterned slides were washed with ethanol and dried by compressed air for the following MoS_2 nanosheet coating.

2.4 Establishment of MoS_2-DMA FRET sensing platform

The MoS_2-DMA FRET sensing platform for single type of viral nucleic acid detection was prepared as following. Generally, MoS_2 nanosheets dispersed in ethanol solution were sonicated for 20 mins for exfoliation. The well dispersed nanosheets were then dialyzed with a filter membrane with molecular weight cutoff of 3.5KD for at least 1 day to remove the lithium hydroxide. The dispersion solution was then sonicated for 15 mins and then centrifuged at 2000 rpm for 5 min to eliminate the aggregated particles. Afterwards, the nanosheets dispersion was collected and sonicated for additional 10 min. The glass slides were cleaned by sonication in ethanol for 15 min and dried with compressed air. 100 nL suspension of MoS_2 nanosheets (200 μg/mL) in a mixture of ethanol and water (8:2) were printed into each hydrophilic spot by using a non-contact liquid dispenser (I-DOT; Dispendix GmbH, Stuttgart, Germany), followed by a slight

shaking for 5 mins. The droplets were then evaporated and kept at room temperature for 1-2 days. Then 1.7 mL water solution were used to clean the fabricated 14 × 14 array with gentle washing. To explore the optimal ratio of MoS_2 nanosheets and fluorescence dye-labeled probe solution for optimal quenching efficiency, a 150 nL fluorescence dye-labeled probe solutions with a series of concentration (0, 10, 20, 30, 40, 50 and 60 nM) was then printed into each spot by the I-DOT dispensing system, and then incubated in the dark environment at a controlled humidity for 1h at room temperature. DMA slide was raised and placed into a 100 mm petri dish with 4 mL phosphate-buffered saline (PBS) and equipped with a wet humidifying pad in the lid to prevent the evaporation during using. The fluorescence images were then captured with excitation at 488 nm and 668 nm for fluorescein amidite (FAM) and cyanine dye 5 (Cy5) labeled probes, respectively, to check the quenching effect. The array for final testing for HIV-1 or HIV-2 nucleic acid detection with a maximum quenching efficiency were prepared with an optimal ratio of MoS_2 nanosheets (100 nL suspension of 200 μg/mL) and fluorescence dye-labeled probe solution (150 nL of 50 nM).

The MoS_2-DMA FRET sensing platform for simultaneous detection of multiple HIV nucleic acids was prepared as following. A 4x4 droplet microarray is separated into HIV-1 detection section, HIV-2 detection section, HIV-1+HIV-2 detection section as well as 2 negative control spots. HIV-1 detection section included 3 testing spots and 1 positive control spot. The testing spot in HIV-1 detection section was prepared by filling hydrophilic spots with a 150 nL PBS solution of FAM-labeled probes (50 nM) on the MoS_2 coated spot surfaces. The positive control spot was prepared by printing with a 150 nL PBS solution of FAM labeled probes (50 nM) on the surface of a hydrophilic dendrimer spot without MoS_2 nanosheets. HIV-2 detection section also included 3 testing spots and 1 positive control spot. The testing spot in HIV-2 detection section was prepared by filling MoS_2 coated spots with a 150 nL PBS solution of Cy5-labeled probes (50 nM). The

positive control spot was prepared by printing 150 nL PBS solution of Cy5-labeled probes (50 nM) into a hydrophilic dendrimer spot without MoS_2 nanosheets. The HIV-1+HIV-2 detection section included 5 testing spots and 1 positive control spot. The testing spot in HIV-1+HIV-2 detection section was prepared by printing 150 nL PBS solution containing mixture of FAM-labeled probes (25 nM) and Cy5-labeled probes (25 nM) into the MoS_2 coated spot surfaces. The positive control spot was prepared by printing 150 nL PBS solution of FAM-labeled probes (25 nM) and Cy5-labeled probes (25 nM) onto the surface of a hydrophilic dendrimer spot without MoS_2 nanosheets. The two negative spots were prepared by printing 150 nL PBS solution into a MoS_2 coated spot. All the printed spots were then dried for further usage.

2.5 MoS_2-DMA FRET platform for gene detection

For the testing with the platform for single type of viral nucleic acid detection, 150 nL HIV-1 or HIV-2 target nucleic acid solutions with various concentrations of 0, 10, 20, 30, 40, 50 and 60 nM were printed on testing spots and kept at 37℃ for incubation with 1 h in dark environment. The specificity testing was conducted by using the four bases mismatched probe sequences under the same conditions. To perform simultaneous detection of HIV-1 and HIV-2 target nucleic acids, 150 nL sample solutions including target HIV-1 probe (50 nM), target HIV-2 probe (50 nM), four-bases mismatched HIV-1 probe (50 nM), four-bases mismatched HIV-2 probe (50 nM), HIV-1+HIV-2 target probes (25 nM+25 nM) and four-bases mismatched HIV-1 probe+ four-bases mismatched HIV-1 probe (25 nM+25 nM) were printed into testing spots and kept at 37℃ for incubation with 1 h in dark environment. The fluorescence images were then captured with excitation at 488 nm and 668 nm for FAM and Cy5 labeled probes, respectively. All the droplets were firstly focused to obtain the clear edges in bright field images, and then switched to

fluorescence excitation mode to take florescence images. For concentration recovery test of the platform, complexed samples with different ratio target Probe 1 and target Probe 2 (concentration ratio as: 1:1, 1:2, 1:4, 4:1, 2:1) were applied. All the concentrations used were in the detectable range determined in this work. These complexed HIV targets were added into 100-fold-diluted human serum samples and then detected with the sample protocol above. All the fluorescence images were measured and analyzed by ImageJ and each spot average fluorescence intensity was obtained based on the defined square area.

3. RESULTS AND DISCUSSION

3.1 Mechanism of MoS_2-DMA FRET sensing platform

The MoS_2-DMA platform fabrication process is shown in Fig. 1a. Briefly, a glass slide is firstly silanized using triethoxyvinyl silane to modify the surface with alkene groups. Then, the surface is modified using the thiol-ene reaction with thioglycerol, followed by the esterification process with 4-pentenoic acid in a sequential 2-step process to modify the surface with a layer of dendrons with a high-density of terminal double bounds. After the dendrimer layer is generated, the surface is patterned by another thiol-ene reaction firstly with a fluorinated thiol (PFDT) to generate hydrophobic barriers and then with cysteamine to generate amino-functionalized hydrophilic spots in defined locations. Since cysteamine has a $pKa_{2(NH2)}$ of 10.75,[32] amino-functionalized hydrophilic spots will be protonated (NH_3^+) at pH=7. The MoS_2 nanosheets show negative charges (Fig. S1), which are then printed into the amino-functionalized hydrophilic spots to adsorb on the spots, forming the MoS_2-DMA platform. The droplet microarray can be formed on the fabricated MoS_2-DMA platform (Fig. 1b). The platform is fabricated on a 7.5 cm × 2.5 cm glass slide with low intrinsic fluorescence. The microarray platform is divided into three arrays

and each array contains 14 × 14 hydrophilic square spots (1 mm x 1mm), separated by 0.5 mm omniphobic barriers. This portable DMA slide contained 588 individual microreservoirs filled with 150 nL solutions for nucleic acid sensing.

The fluorescence resonance energy transfer (FRET) sensing mechanism of MoS_2-DMA platform for viral nucleic acid detection is shown in Fig. 1c. Fluorescein amidite (FAM)-labelled capture oligonucleotide probes of HIV-1 (capture Probe-1) and cyanine dye 5 (Cy5)-labelled capture oligonucleotide probes of HIV-2 (capture Probe-2) are absorbed onto MoS_2 nanosheets. MoS_2 nanosheets are able to adsorb nucleic acids via Van der Waals force between nucleobases and the basal plane of MoS_2 nanosheets which have been reported in both theoretical calculations and experiments.[33-34] The fluorescence of the capture probes adsorbed on MoS_2 nanosheets was quenched via energy transfer from the fluorescent dye to MoS_2 nanosheets. When target HIV-1 nucleic acids (target Probe-1) or HIV-2 nucleic acids (target Probe-2) bind to the absorbed fluorescence-labelled capture probes on MoS_2 nanosheets, the hybridized double stranded nucleic acid complex detaches from MoS_2 nanosheets due to the weaker affinity, leading to the recovery of green fluorescence signal (FAM dye) for HIV-1 and red fluorescence (Cy5 dye) for HIV-2. In hybridized nucleic acids, the nucleobases are surrounded by the dense phosphate layer, which weakens the interaction between MoS_2 and hybridized nucleic acids.[30, 35] It was shown that the adsorption energy between ssDNA and MoS_2 nanosheets was higher (>0.2 eV) than that for dsDNA (<0.05 eV).[35] With addition of non-target nucleic acid, fluorescently labelled capture probes still attach on MoS_2 nanosheets without fluorescence signal recovery.

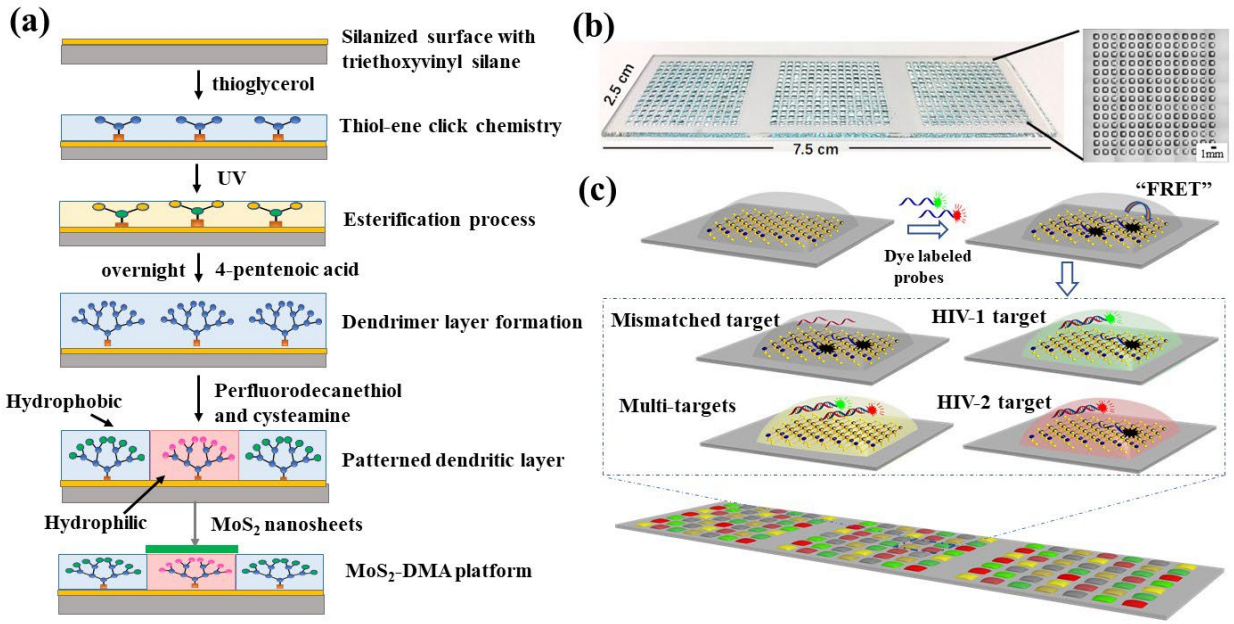

Figure 1. (a) Fabrication process of MoS_2-DMA sensing platform based on the patterned dendritic slides with an array of omniphilic spots on an omniphobic surface. (b) Droplet arrays formed on the fabricated MoS_2-DMA platform; Volume of the droplets is 150 nL. (c) Sensing mechanism on the MoS_2-DMA platform for the simultaneous detection of multiple target probes on the example of the detection HIV-1 and/or HIV-2 DNA sequences.

3.2 Establishment of MoS_2-DMA FRET platform

To establish MoS_2-DMA FRET platform, small MoS_2 nanosheets were firstly prepared by a sonication assisted exfoliation approach from bulk MoS_2 powder. The average particle size of MoS_2 powder is about 1 mm and there is obvious deposition in the aqueous solution (Fig. S2). The exfoliated MoS_2 showed a 2D layer structure with an average size around 90 nm (Fig. 2a and Fig. 2b). MoS_2 nanosheets showed a wide absorption spectral range from UV to the near infrared (NIR) in the UV-Vis absorbance spectrum. The overlap of the absorption spectrum of MoS_2 nanosheets with the emission spectra of FAM and Cy5 dyes could ensure the feasibility of the FRET process (Fig. 2c). MoS_2 nanosheets were then coated on the fabricated amino-functionalized hydrophilic

spots to form MoS_2-dentrimer interface. Before coating, amino-functionalized hydrophilic spots show smooth surface in the AFM image (Fig. 2d). The cysteamine modified hydrophilic spot surface was also characterized by Raman spectroscopy (Fig. S3). The characteristic bands of cysteamine appeared at 1047 cm^{-1}, 1239 cm^{-1}, 1359 cm^{-1} and 1420 cm^{-1}, which matched with the reference.[36] The stability of the adsorption of the nanosheets to the surface was proved by multiple washing, followed by AFM imaging. MoS_2 coated spot surface showed abundant nanosheets with sizes ranging from 50 nm to 200 nm attached on the dendritic surfaces in the AFM image (Fig. 2e), forming a stable MoS_2 nanolayer with thickness of 60 ~100 nm (Fig. 2f).

In the case of adding aqueous solution, small droplets were generated on the hydrophilic spots due to the difference in dewettability of the hydrophilic spots and omniphobic barriers. The geometry of formed droplet array on cysteamine DMA and MoS_2 nanosheets coated DMA was shown on Fig. 3a. The coating of MoS_2 nanosheets did not change surface hydrophilicity and droplet geometry much. To test the quenching ability of MoS_2 coated DMA, 150 nL of solutions of dye labeled DNA probes were printed into the cysteamine DMA spots and MoS_2 nanosheets modified DMA spots by a non-contact disperser and dried at 37°C with slight shaking. The successful attachment of the fluorescence-labelled DNA probes on MoS_2 nanosheets was confirmed by AFM analysis (Fig. 3b). The droplets with fluorescence-labelled capture Probe-1 and capture Probe-2 (50 nM) showed strong fluorescence signals on cysteamine DMA spots but very weak fluorescence signals on MoS_2 nanosheets (100 μg/mL) coated DMA spots (Fig. 3c). This demonstrated the quenching ability of MoS_2 nanosheets coated DMA on fluorescence-labelled DNA probes. However, the fluorescence signal on MoS_2 nanosheets (100 μg/mL) coated DMA spots was not fully quenched. The ratio between MoS_2 nanosheets and fluoresce-labelled

capture probes needs to be further adjusted to ensure a low fluorescence background before detection.

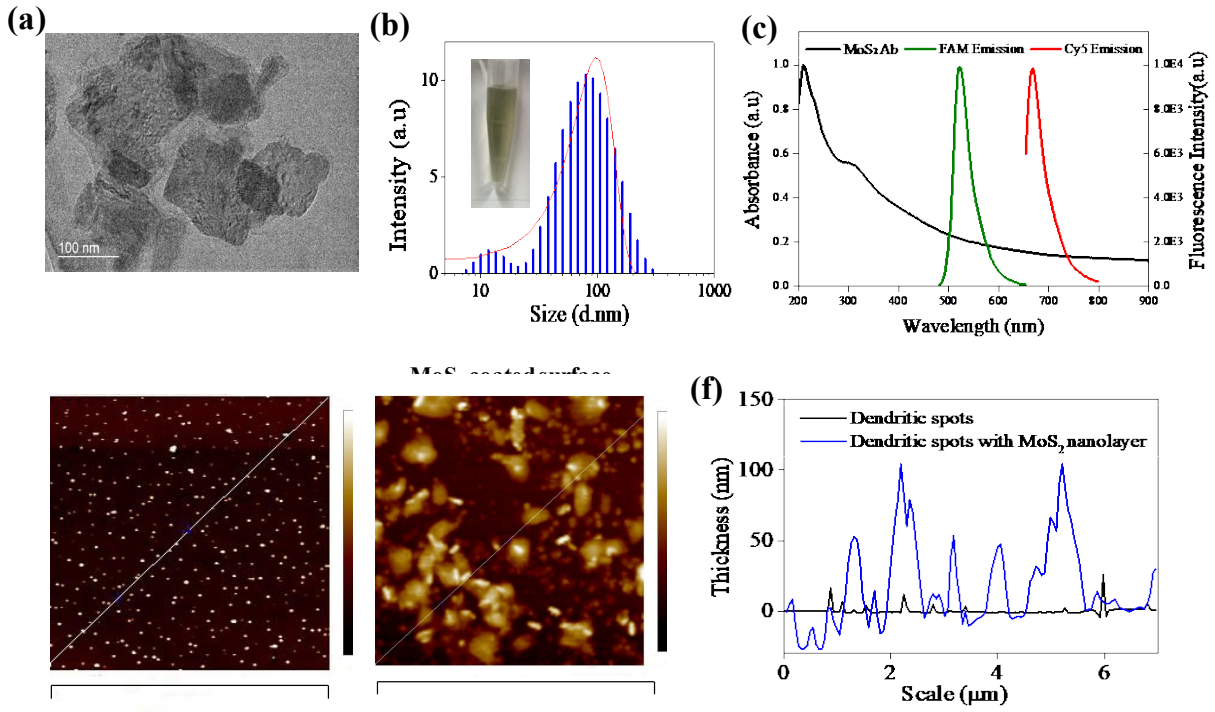

Figure 2 (a) TEM images of the MoS_2 nanosheets; (b) Size distribution of the MoS_2 nanosheets measured by DLS; (c) UV-vis absorbance of MoS_2 nanosheets and the fluorescence emission spectra of FAM and Cy5 dyes; (d) AFM image of cysteamine modified dendrimer spot surface; (e) AFM image of MoS_2 nanosheet coated spot surface; (f) Surface profiles of cysteamine modified dendrimer spot surface and MoS_2 nanosheet coated spot surface.

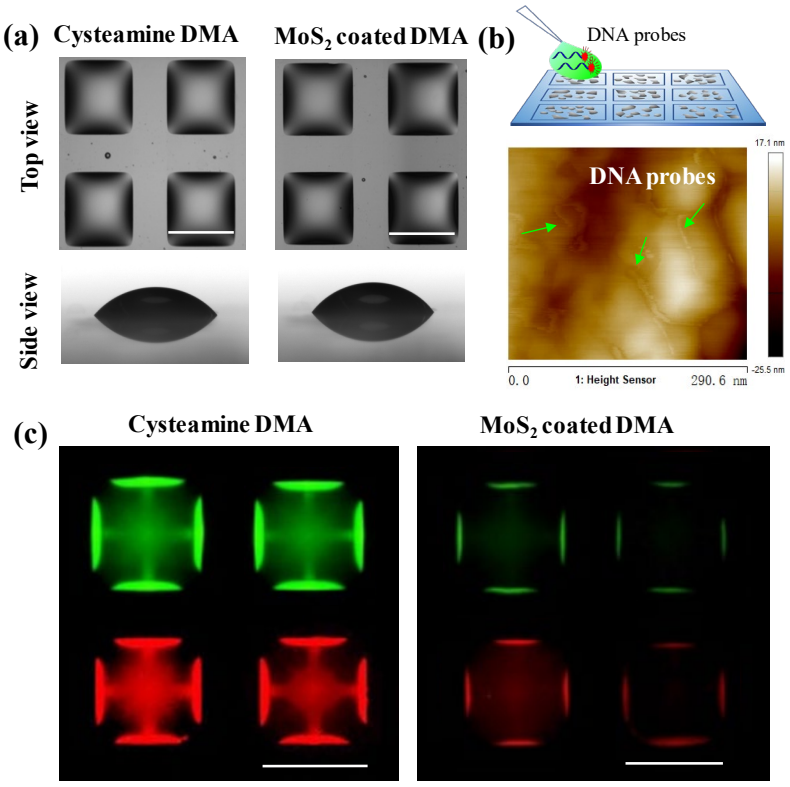

Figure 3 (a) Geometries of droplets (150 nL) formed by non-contact liquid dispenser on the cysteamine modified (left) and MoS_2 nanosheets-coated spots (right). (b) Scheme of printing fluorescence labeled capture probes on MoS_2 nanosheets-coated spot surface to form FRET sensing system and AFM image of fluorescence-labelled capture probes adsorbed on MoS_2 nanosheets coated spot surface. (c) Fluorescence images of labelled capture probes on cysteamine DMA and MoS_2 nanosheets-coated DMA. The scale bar in the above images is 1 mm.

3.3 Optimal quenching efficiency of MoS_2-DMA FRET platform

To obtain a high quenching efficiency, a reasonable ratio between FRET donor and accepter was a prerequisite. Here, we firstly incubated 50 nM fluorescence-labelled capture probes in 150 nL droplets on hydrophilic spots modified with a series of MoS_2 nanosheet concentrations (0 to 250 μg/mL) to explore the optimal ratio between fluorescence labelled probes and MoS_2 nanosheets (Fig. 4a). Upon the increase of concentrations of MoS_2 nanosheets printed on the spot surface, the fluorescence signals were gradually decreased for both capture Probe-1 and capture

Probe-2 (Fig. 4b and Fig. 4c). The quantitative analysis showed that the fluorescence intensity of spots decreased with the increase of concentrations of MoS_2 nanosheets and reached a lowest value at around 200 μg/mL MoS_2 nanosheets for 50 nM concentration of fluorescence labelled DNA probes (Fig. S4). The quenching efficiency (Q_e) was calculated by the equation $Q_e = (F_0 - F_m) / F_0$, where the original fluorescence intensity of the fluorescence-labelled capture probes on the hydrophilic spots without MoS_2 was defined as F_0, the fluorescence intensity of the dye-labeled probes on the MoS_2 coated spots was defined as F_m. It was observed that the optimal ratio of MoS_2 nanosheets (100 nL of 200 μg/mL) and fluorescence-labeled capture probes (150 nL of 50 nM) could reach a high quenching efficiency over 95%, which was used for further sensing experiments (Fig. 4d). The quenching capability of MoS_2 nanosheets (100 nL of 200 μg/mL) against FAM-labelled capture Probe-1 and Cy5-labelled capture Probe-2 (150 nL of 50 nM) was also confirmed by fluorescence spectroscopy measurement in solution (Fig. S5). This MoS_2-DMA sensing platform showed good stability and the quenching efficiency for both capture Probe-1 and capture Probe-2 kept a high value (>90%) during 1h, which covered the whole detection period (Fig. S6).

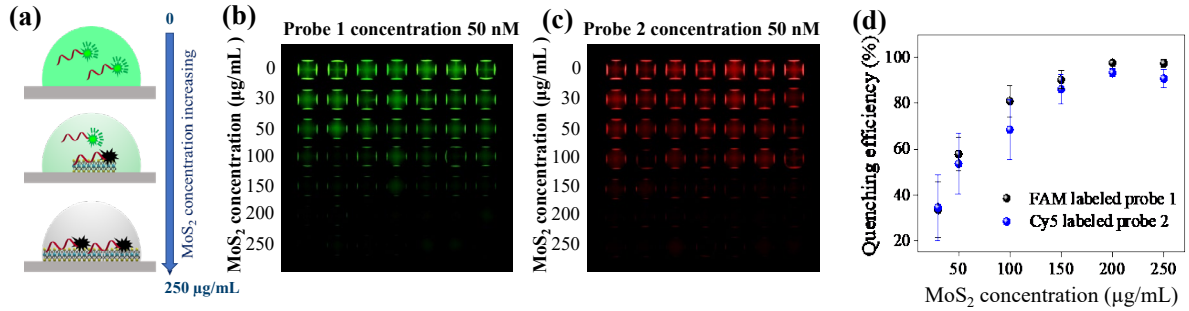

Figure 4 (a) Scheme of modulating ratio between fluorescence-labeled capture probes and MoS_2 nanosheets to achieve an optimal quenching efficiency. 50 nM fluorescence-labeled probes incubated in 150 nL droplets on hydrophilic spots modified by MoS_2 nanosheets with different concentrations (0 – 250 μg/mL) in 100 nL droplets; (b) Fluorescence images of 150 nL FAM-labeled capture Probe-1 (50 nM) in various concentrations of MoS_2 nanosheets coated hydrophilic spots; (c) Fluorescence images of 150 nL Cy5-labeled capture Probe-2 (50 nM) in various

concentrations of MoS_2 nanosheets coated hydrophilic spots; (d) Quenching efficiency of the two fluorescence-labelled probes, capture Probe-1 and capture Probe-2, in spots modified with solutions of MoS_2 nanosheets of variable concentrations.

3.4 Detection of HIV-1 and HIV-2 nucleic acids via the MoS_2-DMA FRET platform

The established MoS_2-DMA sensing platform was then tested for detection of single type of HIV-1 or HIV-2 single-stranded target nucleic acids. In practical applications, one more step is needed to separate double-stranded DNA to single-stranded DNA either by thermal denaturation or by adding helicase to break the bonds.[37] To establish the sensing platform for detection of target Probe-1, MoS_2 nanosheets (100 nL of 200 μg/mL) was firstly printed into hydrophilic spots and then air-dried. Capture Probe-1 (150 nL of 50 nM) was then printed on the MoS_2 nanosheets coated spots and incubated for 1h in the dark at room temperature under controlled humidity. The sensing platform was placed in a home-made humid chamber to keep the stability of the volume during the sensing process (Fig. S7a). Top and side views of the droplets were then captured using a digital camera at various time points within 24 hours (0 h, 0.5h, 1 h, 4 h and 24 h) to calculate the droplet volume (Fig. S7b). The approximate volume of droplets was calculated via the volume formula for a spherical cap: $(\frac{\pi h}{6})(3a^2+ h^2)$, in which a represented half of the side length of the spot size, and h represented the height of the droplet.[15] The droplets did not show obvious volume change even at 24 h (Fig. S7c), which indicated the stability of the volume during the sensing process. The established MoS_2-DMA FRET platform without addition of any target did not show any detectable fluorescence signals (Fig. 5a). Target Probe-1 of 150 nL with various concentrations (10 nM, 20 nM, 30 nM, 40 nM and 50 nM) were then printed into the hydrophilic spots, followed by 1h incubation in dark environment under controlled humidity at 37ºC, leading to the recovery

of green fluorescence (Fig. 5a). Using similar steps of establishing the platform for detection of target Probe-1, the sensing platform for detection of target Probe-2 was established. Here, MoS_2 nanosheets (100 nL of 200 μg/mL) was firstly printed on hydrophilic spots and air-dried. Capture Probe-2 (150 nL of 50 nM) was then printed on MoS_2 nanosheets-coated hydrophilic spots. Recovery of red fluorescence signals was also observed in the presence of target Probe-2 with various concentrations (10 nM, 20 nM, 30 nM, 40 nM and 50 nM) (Fig. 5b). The quantitative analysis showed that the fluorescence intensity of the sensing spots increased gradually with the increase of the concentrations of target Probe-1 and target Probe-2 and reached 5.84 and 6.03 times of control signal at 50 nM of target probes, respectively (Fig. 5c). The relative fluorescence recovery rate was defined as $(F_r - F_m) / F_m$, in which F_r is the recovered fluorescence intensity of the hydrophilic spots in the presence of target probes, and F_m is the original fluorescence intensity of the hydrophilic spots of the MoS_2-DMA platform. Generally, the relative fluorescence recovery rate increased consistently with the increase of logarithmic concentrations of target probes (Fig. 5d and 5e). The LOD for target Probe-1 and target Probe-2 with the fluorescence microscopy images analysis was calculated to be 1.24 nM and 1.26 nM, respectively. To improve the sensitivity of our platform, a miniaturized photodetector was put closely on the backside of the glass substrate of our platform in a closed dark chamber to measure the fluorescence signals. It was observed that the LOD could be further improved to 50 pM for both HIV-1 and HIV-2 target probes (Fig. S8). Our current platform used amplification-free sensing approach. With the adoption of amplification step in the droplet such as isothermal amplification, it is expected that the sensitivity could be further improved in the future.

The response time of this MoS_2-DMA FRET sensing platform for HIV nucleic acids detection was around 60 mins (Fig. S9). To determine the specificity of MoS_2-DMA FRET sensing platform

for detection of HIV nucleic acids, different groups of nucleic acids including target Probe-1, target Probe-2, four bases mismatched HIV-1 probes, four bases mismatched HIV-2 probes, target probes of hepatitis B virus (HBV) and target probes of hepatitis C virus genotype 1b (HCV) were used for sensing with the same condition (150 nL at 50 nM). The recovered fluorescence signals of target Probe-1 and target Probe-2 were 6.10 and 5.63 times that of the mismatched probes, and 5.04~5.85 times those of HBV and HCV target probes, respectively (Fig. 5f).

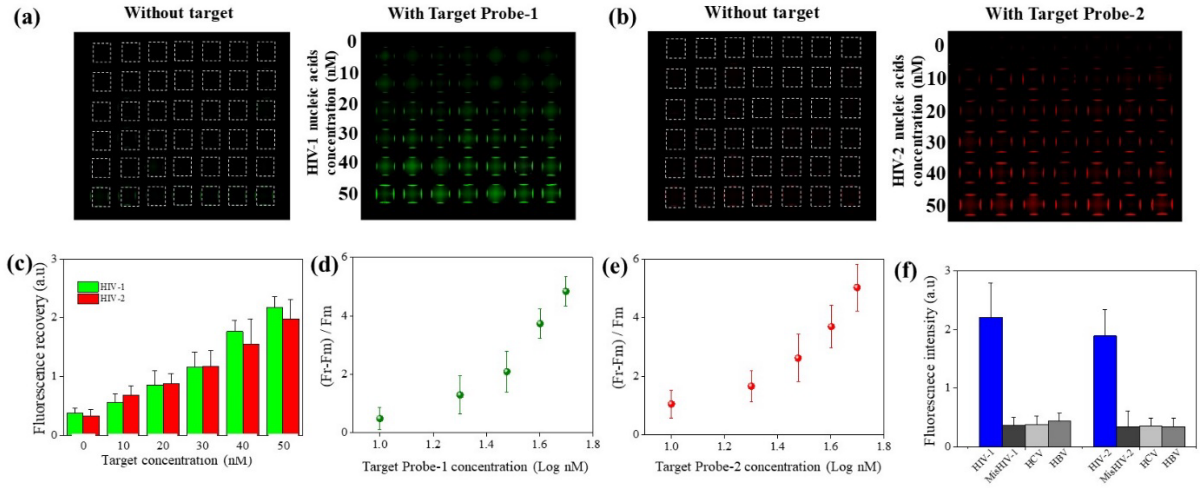

Figure 5 (a) Fluorescence intensity of MoS_2-DMA FRET sensing platform in presence of target Probe-1 with concentration range from 10 nM to 50 nM. (b) Fluorescence intensity of MoS_2-DMA FRET sensing platform in presence of target Probe-2 with concentration range from 10 nM to 50 nM. (c) Quantitative analysis of fluorescence intensity of the sensing spots and concentrations of target probes. (d) The diagram of relative fluorescence recovery rate change with logarithmic concentration of target Probe-1. (e) The diagram of relative fluorescence recovery rate change with logarithmic concentration of target Probe-2. (f) Specificity testing of MoS_2-DMA FRET sensing platform for HIV-1 and HIV-2 target probes using the four bases mismatched probes, HBV target probes, and HCV target probes as controls.

For simultaneous detection of dual HIV nucleic acids, a 4x4 MoS_2 coated droplet microarray was prepared. This sensing platform is composed of HIV-1 detection section, HIV-2 detection section, HIV-1+HIV-2 detection section as well as negative control section (Fig. 6a). In HIV-1

detection section, 50 nM target Probe-1 led to obvious green fluorescence signal recovery. In contrast, 50 nM four bases mismatched HIV-1 probes and PBS solution did not have obvious recovery fluorescence signals (Fig. 6b). The similar results were also observed in HIV-2 detection section. 50 nM target Probe-2 led to obvious red fluorescence signal recovery. 50 nM four bases mismatched HIV-2 probes and PBS solution did not lead to recovery of the fluorescence signal. In the HIV-1+HIV-2 detection section, mixed sample of target Probe-1 (25 nM) and target Probe-2 (25 nM) led to the appearance of yellow fluorescence, indicating the capability of simultaneous detection of different genes in the same sample, in our example both HIV-1 and HIV-2 nucleic acids. In contrast, target Probe-1 (25 nM) only leads to green fluorescence signal change and target Probe-2 (25 nM) only leads to red fluorescence signal change, which indicated the capability of this sensing platform to differentiate HIV-1 and HIV-2 nucleic acids. Moreover, the mixed sample of 4 bases mismatched HIV-1 probes (25 nM) and 4 bases mismatched HIV-2 probes (25 nM) probes did not lead to either green or red fluorescence signal recovery, which demonstrated the specificity of this sensing platform. We also proved repeatability of the test results in 6 independent experiments (Fig. 6c).

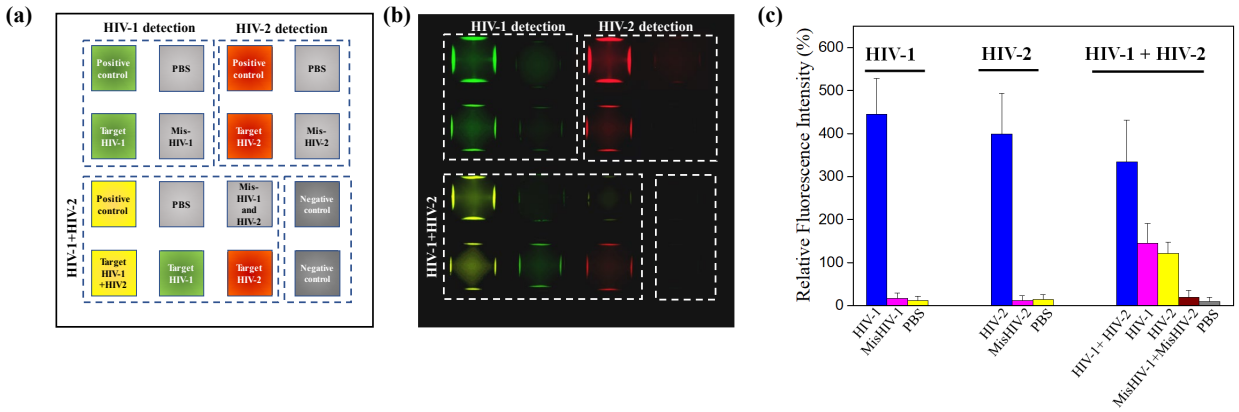

Figure 6 (a) Schematic view of the MoS_2-DMA sensing platform for detection of both HIV-1 and HIV-2 nucleic acids; (b) Fluorescence microscopy image of the MoS_2-Droplet Microarray for detection of both HIV-1 and HIV-2 nucleic acids; see (a) for droplet identification; (c) Repetitive

experiments' analysis of the sensing platform for detection of dual HIV-1 and HIV-2 nucleic acids. The error bar represents the relative fluorescence intensity (RFI) ± standard deviation of RFI. ($P < 0.001$ for target probe detection of HIV-1, HIV-2, HIV-1+HIV+2, $P>0.05$ for mismatched nucleic acids detection. Student's unpaired two-tailed t-test, $n = 6$)

In practical application, it is very important to apply simple facility-free methods for rapid sample delivery. We developed a simple "brushing droplet" method to quickly generate hundreds of separated sample droplets by brushing bulk aqueous sample solution on our MoS_2-DMA platform. The MoS_2-DMA platform is composed of micropattern of hydrophilic square spots separated by hydrophobic borders. MoS_2 nanosheets with various capture oligo have been modified on the hydrophilic spots. When the bulk sample solution was brushed on the surface of the microarray surface, multiple and separated sample micro-droplets will be quickly generated through the effect of discontinuous dewetting (Refer to the video in Supplementary Materials).

In order to explore the ability of the MoS_2-DMA biosensing platform to quantitatively detect mixed HIVs' target probes in complex samples, a variety of mixed molar ratios (1:1, 1:2, 1:4, 4:1, and 2:1; 1 represents 10 nM) of HIV-1 and HIV-2 target probes are spiked in human serum solution to simulate real clinical samples. Firstly, Calibration was performed with a series of concentrations of HIV-1 target probes and HIV-2 target probes to correlate the intensity of green and red recovered fluorescence signals with HIV-1 target probes and HIV-2 target probes, respectively (Fig. 10a). Then samples with various molar ratios of HIV-1 and HIV-2 target probes were then added to the HIV-1 + HIV-2 detection area of the platform for detection. It was observed that the ratio of detected green fluorescence signal intensity to red fluorescence signal intensity matched well with the molar ratio of HIV-1 target probes to HIV-2 target probes (Fig. S10b). Based on the calibration curves, the detected concentrations of HIV-1 target probes to HIV-2 target probes in the complex samples could be calculated which also matched well with the real concentrations of

HIV-1 target probes to HIV-2 target probes (Table S1). The above results demonstrated that it was possible to detect concentrations of multiple HIVs in a complex sample by analyzing the fluorescence signal ratio. This MoS_2-DMA biosensing platform has the potential for simultaneous detection of multiple HIVs quantitatively in a complex clinical sample.

The multiplex capability of the MoS_2-DMA platform is based on combination of two multiplex approaches including multicolor fluorescence probes for detection of various viral genes of the same virus and spatially dividing the array into various sensing sections for different viruses. With the combination of these two approaches, the total number of genes of different viruses could be N x M, where N is the number of fluorescence colors and M is the number of sensing sections. For example, if 4 color fluorescence probes and 20 sensing sections are applied in the droplet array, the multiplex capacity of viral genes can reach 80. As a demonstration, we performed the experiments for 5 genes from 3 viruses including HIV-1 gene and HIV-2 gene for HIV, ORFlab and N genes of SARS-COV-2 for COVID-19 infection, and M gene of Influenza A with various concentrations (0.5 nM, 1 nM, 2 nM, 5 nM and 10 nM). The capture and target probes information are included in Table S2. Here, 2 fluorescence colors and 3 sensing sections were used. It was clearly observed that this MoS_2-DMA platform could detect all the 5 genes with concentration-dependent fluorescence signals (Fig. S7). As shown in Figure 1b, we have already fabricated the MoS_2-DMA platform with 588 droplets in a glass substrate. Using this combinational multiplex approach, our MoS_2-DMA platform is easily to be adapted for detection of more genes.

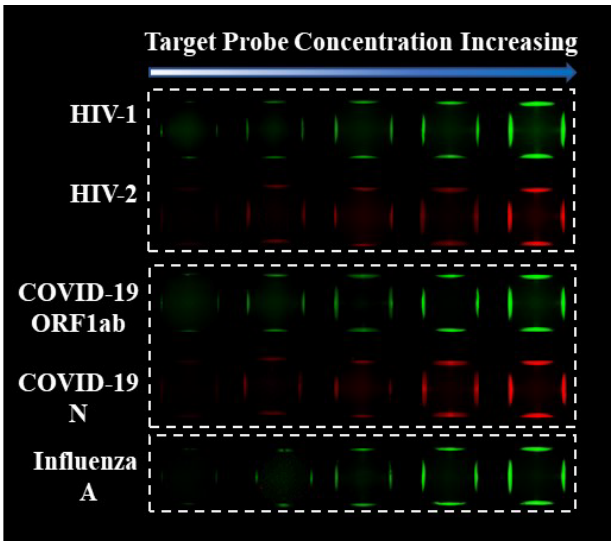

Figure 7 MoS_2-DMA FRET sensing platform for detection of 5 gene target probes of different viruses.

4. CONCLUSION

In this work, a novel MoS_2-DMA FRET sensing platform was developed for detection of multiple HIV retroviral nucleic acids based on combination of the droplet microarray platform and MoS_2 nanosheet-based FRET nanoprobes. MoS_2 nanosheets were used to coat hydrophilic dendrimer-modified spots separated by hydrophobic background as quenchers and down to 150 nL droplets containing fluorescence-labelled capture probe DNAs were then deposited into each MoS_2-coated spot as donors. "OFF" status of the sensor array was formed due to energy transfer from absorbed fluorescence-labelled capture probe DNAs to MoS_2 nanosheets, In the presence of target viral (HIV-1 or HIV-2) nucleic acids, fluorescence-labelled sensing probes were detached from MoS_2 nanosheets coated on the hydrophilic spots, leading to a fluorescence signal recovery with "ON" status. As a proof of concept, this sensing platform has been successfully used for detection of single type HIV-1 or HIV-2 nucleic acids with various concentrations, as well as simultaneous detection of both HIV-1 and HIV-2 nucleic acids with rapid response, good

sensitivity and specificity. This MoS_2-DMA FRET sensing platform successfully transferred solution-based FRET assay to a substrate-based microarray sensing platform with advantages of low sample consumption, parallel detection as well as spatial separation between the sensor microspots, which provides a potential for parallel multiplexed detection of various viral and bacterial pathogens in the future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Zeta potential and size distribution of MoS_2, Raman spectroscopy of cysteamine modified hydrophilic spot surface, quantitative analysis for correlation between probes concentration and MoS_2 nanosheets amount, fluorescence spectra of probes before and after coating on MoS_2 modified spots, quenching stability, fluorescence recovery response curve, concentration recovery test, droplet volume change, gene detection for different virus.

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Notes

The authors declare no competing financial interest

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ABBREVIATIONS

FRET, fluorescence resonance energy transfer; MoS_2: molybdenum disulfide; HIV: Human immunodeficiency virus; DMA: droplet microarray

REFERENCES

(1) Haase, A.T., Targeting Early Infection to Prevent HIV-1 Mucosal Transmission. *Nature* **2010**, *464,* 217-223.

(2) Campbell-Yesufu, O.T.; Gandhi, R.T., Update on Human Immunodeficiency Virus (HIV)-2 Infection. *Clin Infect Dis.* **2011**, *52*, 780-787.

(3) Nsagha, D.S.; Njunda, A.L.; Kamga, H.L.F.; Assob, J.C.N.; Bongkem, E.A., HIV-1/HIV-2 Co-infection among Voluntary Counselling and Testing Subjects at a Regional Hospital in Cameroon. *African Health Sciences* **2012,** *12,* 276-281.

(4) Wejse, C.; Patsche, C.B.; Kuhle, A.; Bamba, F.J.V.; Mendes, M.S.; Lemvik, G.; Gomes, V.F.; Rudolf, F., Impact of HIV-1, HIV-2, and HIV-1+2 Dual Infection on the Outcome of Tuberculosis. *International Journal of Infectious Diseases* **2015,** *32,* 128-134.

(5) Jones, D. M.; Padilla-Parra, S. Imaging Real-Time HIV-1 Virion Fusion with FRET-Based Biosensors. *Sci. Rep.***2015,** *5*, 1-10.

(6) Zhang, D.; Peng, Y.; Qi, H.; Gao, Q.; Zhang, C. Label-Free Electrochemical DNA Biosensor Array for Simultaneous Detection of the HIV-1 and HIV-2 Oligonucleotides Incorporating Different Hairpin-DNA Probes and Redox Indicator. *Biosens. Bioelectron.* **2010,** *25*, 1088-1094.

(7) Granade, T. C.; Kodani, M.; Wells, S. K.; Youngpairoj, A. S.; Masciotra, S.; Curtis, K. A.; Kamili, S.; Owen, S. M. Characterization of Real-Time Microarrays for Simultaneous Detection of HIV-1, HIV-2, and Hepatitis Viruses. *J. Virol. Methods* **2018,** *259*, 60-65.

(8) Lee, B.E.; Plitt, S.S.; Jayaraman, G.C.; Chui, L.; Singh, A.E.; Preiksaitis, J.K. Use of Quantitative HIV RNA Detection for Early Diagnosis of HIV Infection in Infants and Acute HIV Infections in Alberta, Canada, *Journal of Clinical Microbiology*, **2012**, *50,* 502-505.

(9) Vandamme, A.M.; Van Dooren, S.; Kok, W.; Goubau, P.; Fransen, K.; Kievits, T.; Schmit, J. C.; De Clercq, E.; Desmyter, J. Detection of HIV-1 RNA in Plasma and Serum Samples Using the NASBA Amplification System Compared to RNA-PCR, *Journal of Virological Methods* **1995**, *52*, 121-132.

(10) Henrich, T. J.; Gallien, S.; Li, J. Z.; Pereyra, F.; Kuritzkes, D. R. Low-Level Detection and Quantitation of Cellular HIV-1 DNA and 2-LTR Circles Using Droplet Digital PCR. *J. Virol. Methods* **2012,** *186*, 68-72.

(11) Babamiri, B.; Salimi, A.; Hallaj, R. A Molecularly Imprinted Electrochemiluminescence Sensor for Ultrasensitive HIV-1 Gene Detection Using EuS Nanocrystals as Luminophore. *Biosens Bioelectron.* **2018,** *117*, 332-339.

(12) Karami, A.; Hasani, M. A Palindromic-based Strategy for Colorimetric Detection of HIV-1 Nucleic Acid: Single-component Assembly of Gold Nanoparticle-core Spherical Nucleic Acids. *Analytica Chimica Acta* **2020,** *1102*, 119-129.

(13) Wang, Y.; Bai, X.; Wen, W.; Zhang, X.; Wang, S. Ultrasensitive Electrochemical Biosensor for HIV Gene Detection Based on Graphene Stabilized Gold Nanoclusters with Exonuclease Amplification. *ACS Appl. Mater. Interfaces* **2015,** *7*, 18872-18879.

(14) Liu, X.Y.; Zhou, X.M.; Xia, X.Y.; Xiang, H. Catalytic hairpin assembly-based double-end DNAzyme cascade-feedback amplification for sensitive fluorescence detection of HIV-1 DNA. *Analytica Chimica Acta* **2020**, *1096*, 159-165.

(15) Popova, A. A.; Demir, K.; Hartanto, T. G.; Schmitt, E.; Levkin, P. A. Droplet-Microarray on Superhydrophobic-Superhydrophilic Patterns for High-Throughput Live Cell Screenings. *Rsc Adv.* **2016,** *6*, 38263-38276.

(16) Tronser, T.; Demir, K.; Reischl, M.; Bastmeyer, M.; Levkin, P. A. Droplet Microarray: Miniaturized Platform for Rapid Formation and High-Throughput Screening of Embryoid Bodies. *Lab Chip* **2018,** *18*, 2257-2269.

(17) Benz, M.; Molla, M. R.; Böser, A.; Rosenfeld, A.; Levkin, P. A. Marrying Chemistry with Biology by Combining On-Chip Solution-Based Combinatorial Synthesis and Cellular Screening. *Nat. Commun.* **2019,** *10*, 1-10.

(18) Bruchmann, J.; Pini, I.; Gill, T. S.; Schwartz, T.; Levkin, P. A. Patterned SLIPS for the Formation of Arrays of Biofilm Microclusters with Defined Geometries. *Adv. Healthc. Mater.* **2017,** *6*, 1601082.

(19) Feng, W.; Ueda, E.; Levkin, P. A. Droplet Microarrays: From Surface Patterning to High-Throughput Applications. *Adv.Mater.* **2018,** *30*, 1706111.

(20) Feng, W.; Li, L.; Du, X.; Welle, A.; Levkin, P. A. Single-Step Fabrication of High-Density Microdroplet Arrays of Low-Surface-Tension Liquids. *Adv.Mater.* **2016,** *28*, 3202-3208.

(21) Geyer, F. L.; Ueda, E.; Liebel, U.; Grau, N.; Levkin, P. A. Superhydrophobic-Superhydrophilic Micropatterning: Towards Genome-on-a-Chip cell microarrays. *Angew. Chem. Int. Ed.* **2011,** *50*, 8424-8427.

(22) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. Starburst Dendrimers: Molecular-Level Control Of Size, Shape, Surface Chemistry, Topology, and Flexibility Form Atoms To Macroscopic Matter. *Angew. Chem. Int. Ed. Engl.* **1990,** *29*, 138-175.

(23) Esfand, R.; Tomalia, D. A. Poly (amidoamine) (PAMAM) Dendrimers: from Biomimicry to Drug Delivery and Biomedical Applications. *Drug Discov. Today* **2001,** *6*, 427-436.

(24) Tian, F.; Lyu, J.; Shi, J.; Yang, M. Graphene and Graphene-Like Two-Denominational Materials Based Fluorescence Resonance Energy Transfer (FRET) Assays for Biological Applications. *Biosens. Bioelectron.* **2017,** *89*, 123-135.

(25) Shi, J.; Tian, F.; Lyu, J.; Yang, M. Nanoparticle Based Fluorescence Resonance Energy Transfer (FRET) for Biosensing Applications. *J Mater Chem B* **2015,** *3*, 6989-7005.

(26) Liu, T.; Wang, C.; Gu, X.; Gong, H.; Cheng, L.; Shi, X.; Feng, L.; Sun, B.; Liu, Z. Drug Delivery with PEGylated MoS_2 Nano-Sheets for Combined Photothermal and Chemotherapy of Cancer. *Adv.Mater.* **2014,** *26*, 3433-3440.

(27) Shi, J.; Lyu, J.; Tian, F.; Yang, M. A Fluorescence Turn-on Biosensor Based on Graphene Quantum Dots (GQDs) and Molybdenum Disulfide (MoS_2) Nanosheets for Epithelial Cell Adhesion Molecule (EpCAM) Detection. *Biosens. Bioelectron.* **2017,** *93*, 182-188.

(28) Lu, Z.; Chen, X.; Hu, W. A Fluorescence Aptasensor Based on Semiconductor Quantum Dots and MoS_2 Nanosheets for Ochratoxin A Detection. *Sens. Actuators B Chem.* **2017,** *246*, 61-67.

(29) Lu, C.; Liu, Y.; Ying, Y.; Liu, J. Comparison of MoS_2, WS_2, and Graphene Oxide for DNA Adsorption and Sensing. *Langmuir* **2017,** *33*, 630-637.

(30) Oudeng, G.; Au, M.T.; Shi, J.Y.; Wen, C.Y.; Yang, M. One-step In-situ Detection of MiRNA-21 Expression in Single Cancer Cells based on Biofunctionalized MoS_2 Nanosheets, *ACS Applied Materials & Interfaces*, **2018**, *10*, 350-360.

(31) Le Berre, V.; Trevisiol, E.; Dagkessamanskaia, A.; Sokol, S.; Caminade, A.M.; Majoral, J.P.; Meunier, B.; Francois, J. Dendrimeric Coating of Glass Slides for Sensitive DNA Microarrays Analysis, *Nucleic Acids Research*, **2003**, *31*, e88.

(32) Atallah, C.; Charcosset, C.; Greige-Gerges, H. Challenges for Cysteamine Stabilization, Quantification, and Biological Effects Improvement. *J Pharm Anal* **2020,** 10.1016/j.jpha.2020.03.007

(33) Moses, P. G.; Mortensen, J. J.; Lundqvist, B. I.; Norskov, J. K. Density Functional Study of the Adsorption and Van der Waals Binding of Aromatic and Conjugated Compounds on the Basal Plane of MoS_2. *J. Chem. Phys.* **2009,** *130*, 104709.

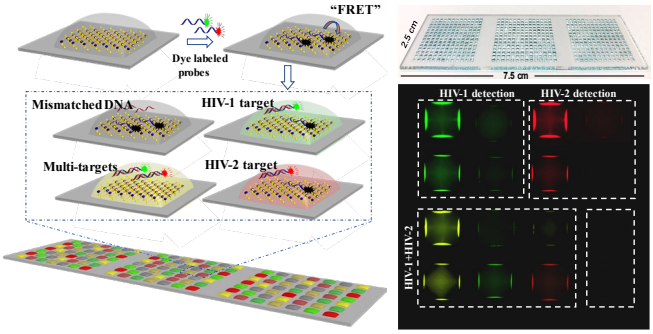
(34) Zhu, C.; Zeng, Z.; Li, H.; Li, F.; Fan, C.; Zhang, H. Single-Layer MoS_2-Based Nanoprobes for Homogeneous Detection of Biomolecules. *J. Am. Chem. Soc.* **2013,** *135*, 5998-6001.

(35) Jin, K.; Xie, L.; Tian, Y.; Liu, D. Au-modified Monolayer MoS_2 Sensor for DNA Detection. *The Journal of Physical Chemistry C* **2016,** *120*, 11204-11209.

(36) Ma, Q.; Zhang, H.; Liu, W.; Ge, J.; Wu, J.; Wang, S.; Wang, P. Surface-Enhanced Raman Scattering Substrate Based on Cysteamine-Modified Gold Nanoparticle Aggregation for Highly Sensitive Pentachlorophenol Detection. *RSC Adv.* **2016,** *6*, 85285-85292.

(37) Pyle, A.M. Translocation and Unwinding Mechanisms of RNA and DNA Helicases. *Annual Review of Biophysics* **2018,** *37*, 317-336.

TOC


"FRET"
Dye labeled probes
Mismatched DNA
HIV-1 target
Multi-targets
HIV-2 target
2.5 cm
7.5 cm
HIV-1 detection
HIV-2 detection
HIV-1+HIV-2