Supplementary Information for

Single Nucleotide Variant Discrimination by Toehold Exchange Spherical Nucleic Acids Modulated on Hierarchical Molybdenum Disulfide Acanthosphere

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Experimental details

Materials and Reagents: Hexaammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) and thiourea were purchased from Sigma-Aldrich. Surfactant polyvinylpyrrolidone (PVP, Mw = 55000) was obtained from Energy Chemical company (China). Oligonucleotides used (Table S1) were obtained from Sangon Biotech Co., Ltd (Shanghai, China) and with HPLC purification. All nucleic acid stock samples (100 μM) used were dissolved in ultrapure water. DNA sequences (Table S1) were designed with the help of Integrated DNA Technologies (IDT). Exo III (*E. coli*) and 10×NEBuffer™ 1 were purchased from New England Biolabs (China). Tris-acetic acid-magnesium (1×TAMg) buffer (45 mM Tris-acetic acid and 7.6 mM magnesium acetate, pH 8.0) was used for all nucleic acids self-assemblies. All reagents were used as received without further purification. Healthy human serum was supplied by the Affiliated Hospital of Nantong University.

Methods and Apparatus: Oligonucleotide quantification was carried out using UV1800PC spectrophotometer (Shanghai, China). Fluorescence signals were recorded on a RF-5301PC (Shimadzu) spectrofluorophotometer at ambient temperature. For all measurements the fluorescence intensities were recorded at 517 nm with an excitation wavelength of 485 nm. Centrifugea (Microfuge® 20, BECKMAN, USA) was used to precipitate nucleic acids modified MoS_2. The DNA probes were annealed on an Applied Biosystems 96-well thermocycler. Syngene gel imaging system was used to image native polyacrylamide gel electrophoresis (PAGE) under UV light. The morphology of the MoS_2 superstructure was studied by a field-

emission scanning electron microscopy (FESEM, Hitachi, SU8010). Transmission electron microscopy (TEM) images were observed using a FEI Tecnai G2 F20. X-ray photoelectron spectra (XPS) were produced on a Thermo escalab 250Xi instrument equipped with an Al Kα source (hv =1486.6 eV). X-ray diffraction (XRD) characterization of MoS_2 superstructure was carried out on a Bruker D8 Advance with a Cu Kα radiation (λra 1.5418 Å).

Native PAGE analysis: All the oligonucleotides and reaction results were analyzed with 8% native PAGE. The electrophoresis was conducted in 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0). A constant voltage of 100 V was used for 1.5 h of electrophoresis before the gels were stained with 4S Red for imaging. And the strength of the band was calculated based on the gray scale of the PAGE image with software Image J.

The synthesis of the MoS_2 Superstructures: The MoS_2 superstructures were developed *via* a hydrothermal preparation process. Typically, 588 mg of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 1080 mg of thiourea were dispersed into 40 mL of deionized water with the assistance of 600 mg PVP. Then, the mixture was transferred into a 50 mL Teflon-lined stainless steel autoclave and the hydrothermal reaction was carried out at 220 ºC for 18 h. The obtained precipitates were washed several times with mixed water/ethanol (v/v, 50/50) to obtain the MoS_2 product. The MoS_2 sample was dried at 80 ºC for 6 h before thermal treatment in a tube furnace at 700 ºC for 2 h in Ar, thus achieving the final MoS_2 superstructures. For FESEM observation, the MoS_2 sample was dispersed in DI water and dripped on silica wafer. For TEM analysis, the MoS_2

superstructure was dispersed in a mixture of DI water and ethanol, and the prepared sample was loaded on copper mesh and dried at 60 ºC for 20 min before observation.

MoS_2 acanthospheres absorption: Substrate with different poly C lengths (Poly-5, Poly-10, Poly-15 and Poly-20) and blocker (Substrate S for PAGE, denoted as S) were annealed from 95 ℃ to 4 ℃ at a concentration of 1 μM. The resulting Poly-5, Poly-10, Poly-15 and Poly-20 toehold exchange probes were mixed with 400 μL MoS_2 (500 μg ml^{-1}) in the dark at room temperature for 3h. The samples were then washed six times by centrifugation at 12000 rpm for 10 min. The supernatant was collected and monitored by fluorophotometer to evaluate the quenching efficiency. Both the probe annealing and MoS_2 adsorption process were conducted in 1 × TAMg buffer.

Target detection by the TESNA platform: All fluorescence measurements were performed in 1×NEBuffer™ 1 (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0, diluted with 1 × TAMg buffer or complex samples human serum or cell lysate). Different concentrations of target (ranging from 1 pM to 100 nM) were incubated with the TESNA system in 100 μL 1×NEBuffer™ 1 containing 3 U mL^{-1} Exo III for 1 h and then their fluorescence was measured with an excitation wavelength of 485 nm.

Cell lysate preparation and biosample detection: Human cervical carcinoma cells (HeLa) were cultured in RPMI 1640 medium which was supplemented with 10 % fetal calf serum, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin in a humidified 5 % CO_2 incubator at 37 ℃. HeLa cells (1×10^6 mL^{-1}) were lysed via low-frequency

ultrasonic wave. To conduct the target spiking and detection analysis, the probe absorbed MoS_2 was added into 100 μL 1×NEBuffer™ 1 solution (diluted with cell lysate or human serum) containing 3 U ml^{-1} Exo III. After different concentrations of target were added, the fluorescence was recorded after incubation for 1 h at room temperature.

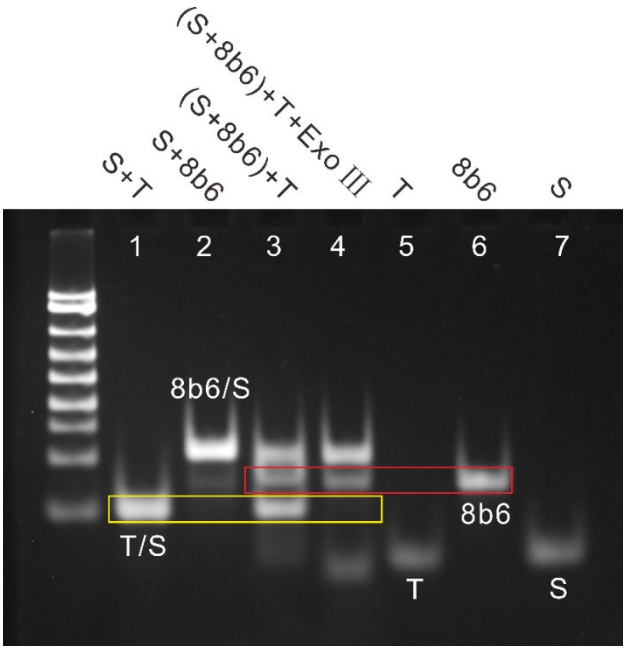

Fig. S1 8% native PAGE validation of toehold exchange probe-based hybridization reaction. Equivalent of substrate (Substrate S, used for PAGE, without poly C tail) and Target (T) or 8b6 at a concentration of 1 μM were annealed from 95 ℃ to 4 ℃ in 1 × TAMg buffer to get T/S or 8b6/S duplex. Then 10 μL 10×NEBuffer™ 1 was mixed with 90 μL above anealed duplex for further reaction or PAGE analysis. Lane 3: 800 nM Target was added to 8b6/S; Lane 4: 800 nM Target and 3 U mL^{-1} Exo III were added to 8b6/S. After reaction for 30 min, the resutls was analyzed with 8% native PAGE. The electrophoresis was conducted in 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0). A constant voltage of 100 V was used for 1.5 h of electrophoresis before the gels were stained with 4S Red for imaging.

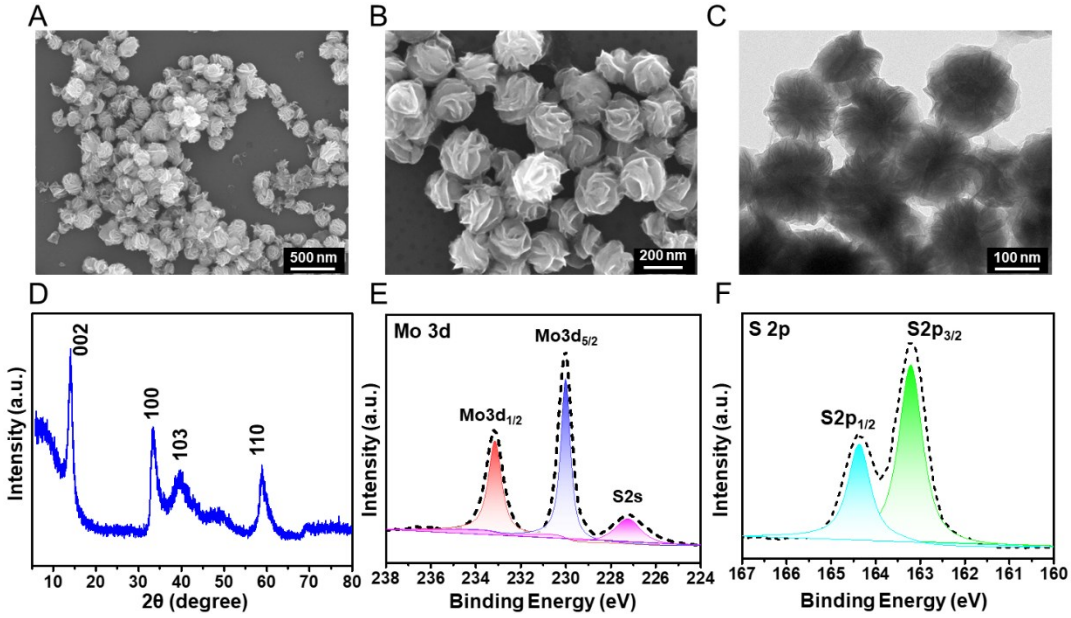

Fig. S2 Characteristics of the MoS_2 superstructures. (A) and (B) SEM images at low magnifications. (C) TEM images of MoS_2 superstructures at low magnifications. (D) XRD pattern of the MoS_2 superstructures. XPS spectra of the MoS_2, (E) Mo 3d, and (F) S 2p.

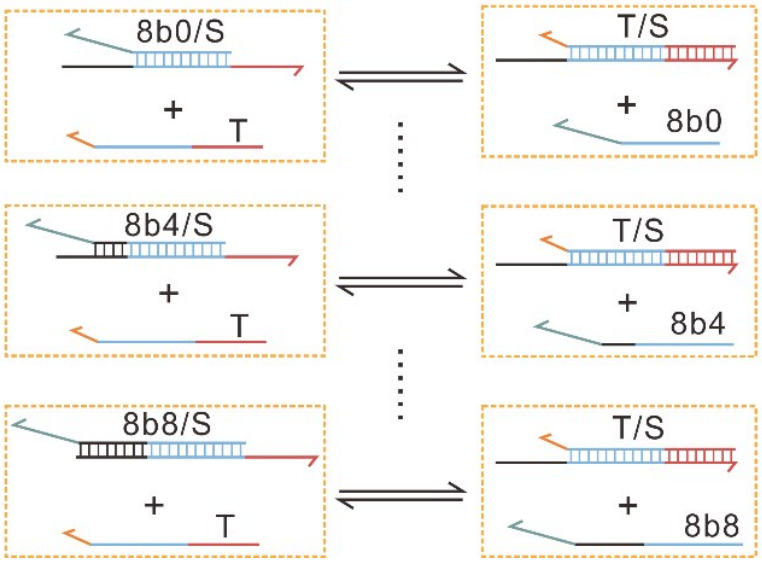

Fig. S3 Schematic illustration of the toehold mediated strand displacement reaction process with different dissociating toehold lengths (0, 2, 4, 5, 6, 7, 8). Firstly, Equivalent of substrate strand (S, 1 μM) was annealed with blocker (1 μM) 8b8, 8b7, 9b6, 8b5, 8b4, 8b2, 8b0, from 95 ℃ to 4 ℃ in 1 × TAMg buffer, respectively. All the blockers used here were appended with a 36 nt poly-T tail. Then 10 μL 10×NEBuffer™ 1 was mixed with 90 μL above anealed duplex for further reaction or PAGE analysis. Subsequently, we added T, M1, M5, M10, M15 and M19 to the above prefabricated toehold exchange probe system to a final concentration of 800 nM for hybridization at room temperature for 30 min. The binding of T with the initial toehold (red segment) will initiate the branch migration process, and the blocker will be released after spontaneously dissociating. T/S will be formed simultaneously. To protect target from the subsequent Exo III hydrolysis, we left a 6 nt overhang at the 3' of the T strand.

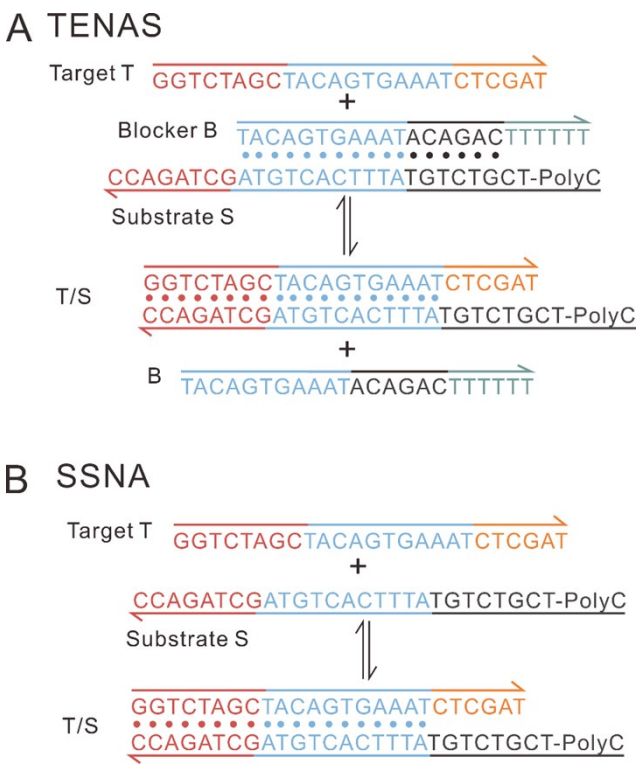

Fig. S4 Mechanism of TESNA (A) and SSNA (B). Fluorescence measurements were performed in 1×NEBuffer™ 1 (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0, diluted with 1 × TAMg buffer) containing 3 U mL^{-1} Exo III. Target (or spurious analogues) were incubated with the TESNA or SSVN system at a final concentration of 10 nM for 1 h and then their fluorescence was measured with an excitation wavelength of 485 nm.

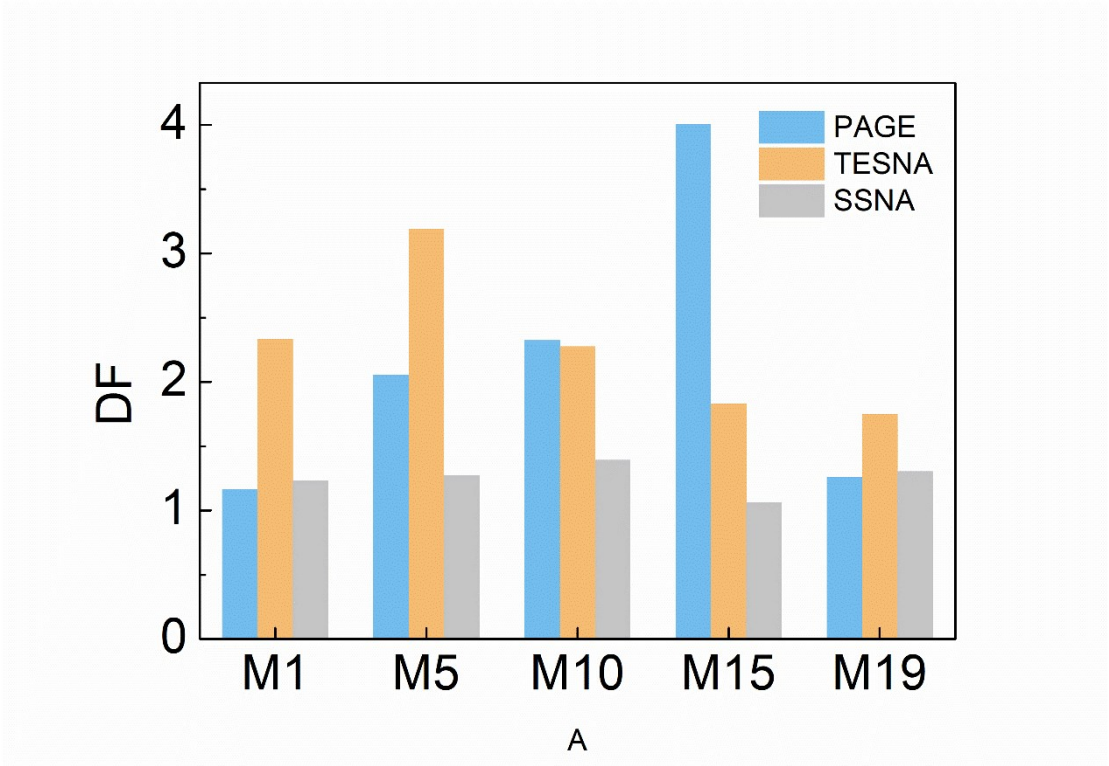

Fig. S5 DFs for mutations at different locations across the target strand derived from PAGE (Fig. 3A), TESNA and SSNA (Fig. 4F). For both PAGE and TESNA, they showed much higher DFs for all mutations than SSNA and the highest DFs for PAGE and TESNA located in the middle (M5 and M15) of the strand. DF for PAGE were in the range of 1.16-4.00, which increased from M1 (1.16) to M15 (4.00), and then decreased for M19 (1.26). In terms of TESNA, the DFs ranged from 1.75 to 3.19 and showed better DF for mutations in the terminals of the strand (M1 and M19) than PAGE. M5 exhibited the highest DF (3.19) for TESNA.

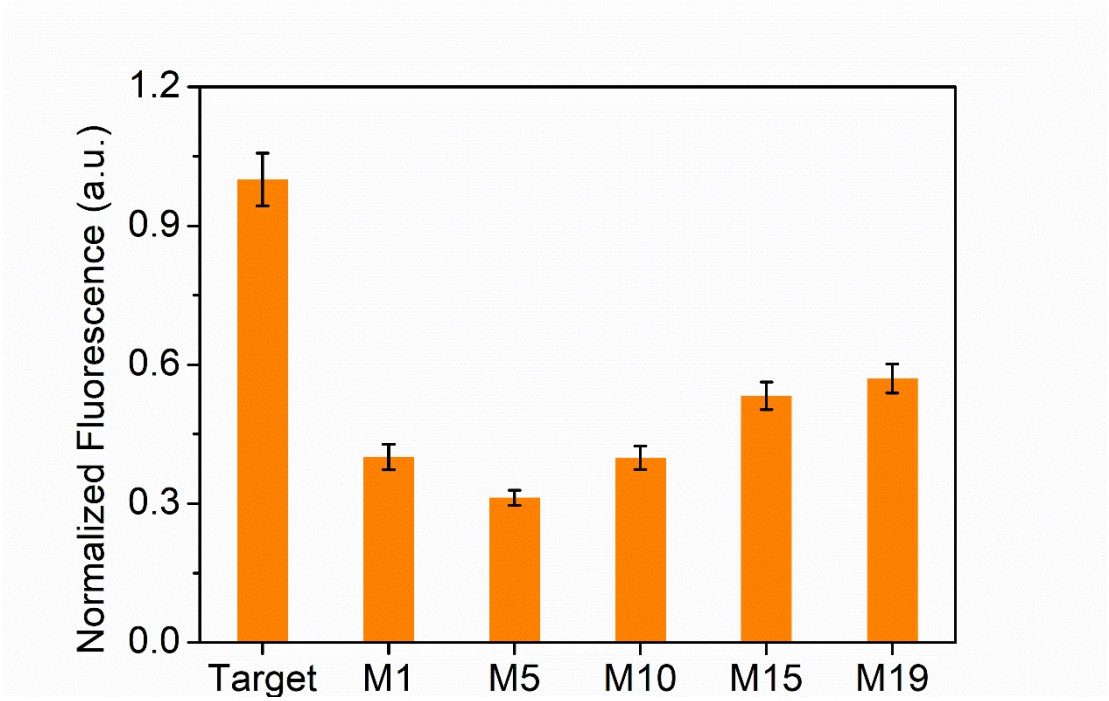

Fig. S6 Normalized fluorescence data for target and spurious analogues via TESNA under the interference of 0.1 mg mL^{-1} herring sperm genome fragments. Fluorescence measurements were performed in 1×NEBuffer™ 1 (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0, diluted with 1 × TAMg buffer) containing 0.1 mg mL^{-1} herring sperm genome fragments and 3 U mL^{-1} Exo III. Target (or spurious analogues) were incubated with the TESNA system at a final concentration of 10 nM for 1 h and then their fluorescence was measured with an excitation wavelength of 485 nm.

Table S1. All the oligonucleotides used in this work.

Name	Sequence (5'-3')	Length (nt)	Used in Figures
Target	GGTCTAGCTACAGTGAAATCTCGAT	25	Fig. 3, Fig. 4, Fig. S1，Fig. S6
M1	CGTCTAGCTACAGTGAAATCTCGAT	25	Fig. 3, Fig.4, Fig. S6
M5	GGTCAAGCTACAGTGAAATCTCGAT	25	Fig. 3, Fig.4, Fig. S6
M10	GGTCTAGCTTCAGTGAAATCTCGAT	25	Fig. 3, Fig.4, Fig. S6
M15	GGTCTAGCTACAGTCAAATCTCGAT	25	Fig. 3, Fig.4, Fig. S6
M19	GGTCTAGCTACAGTGAAAACTCGAT	25	Fig. 3, Fig.4, Fig. S6
Poly-5	CCCCCTCGTCTGTATTTCACTGTAGCTA GACC-FAM	32	Fig. 4
Poly-10	CCCCCCCCCCTCGTCTGTATTTCACTGTA GCTAGACC-FAM	37	Fig. 4

Poly-15	CCCCCCCCCCCCCCCTCGTCTGTATTTCACTGTAGCTAGACC-FAM	42	Fig. 4
Poly-20 (S) for fluorescence	CCCCCCCCCCCCCCCCCCCCTCGTCTGTATTTCACTGTAGCTAGACC-FAM	47	Fig. 4, Fig. S6
Substrate S for PAGE	TCGTCTGTATTTCACTGTAGCTAGACC	27	Fig. 3, Fig. S1
8b8	TACAGTGAAATACAGACGATTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	55	Fig. 3
8b7	TACAGTGAAATACAGACGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	54	Fig. 3
8b6	TACAGTGAAATACAGACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	53	Fig. 3, Fig. S1
8b5	TACAGTGAAATACAGATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	52	Fig. 3
8b4	TACAGTGAAATACAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	51	Fig. 3
8b2	TACAGTGAAATACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	49	Fig. 3
8b0	TACAGTGAAATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	47	Fig. 3
Blocker	TACAGTGAAATACAGACTTTTTT	19	Fig. 4, Fig. S6

Table S2. Standard addition analysis in human serum and cell lysate samples.

Sample	Added (pM)	Detected [a] (pM)	Recovery (%)
Cell lysate	300	323 ± 45	107.7
	800	755 ± 82	94.4
Human serum	300	330 ± 32	110.0
	800	869 ± 77	108.6

[a] Values represented mean ± SD (n = 3).