Supplementary Materials For

Fluorogenic 2D glycosheet for the simultaneous identification of human- and avian-receptor specificity in influenza viruses

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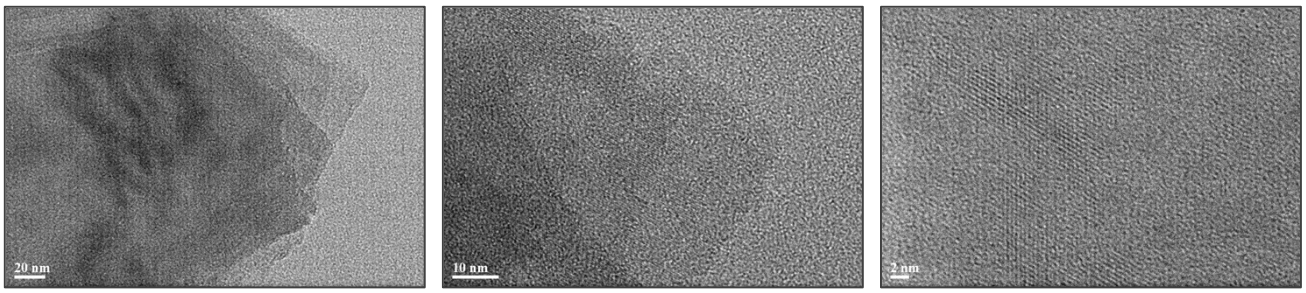


Fig. S1. Transmission electron microscopy of 2D MoS_2 recorded on JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 kV.

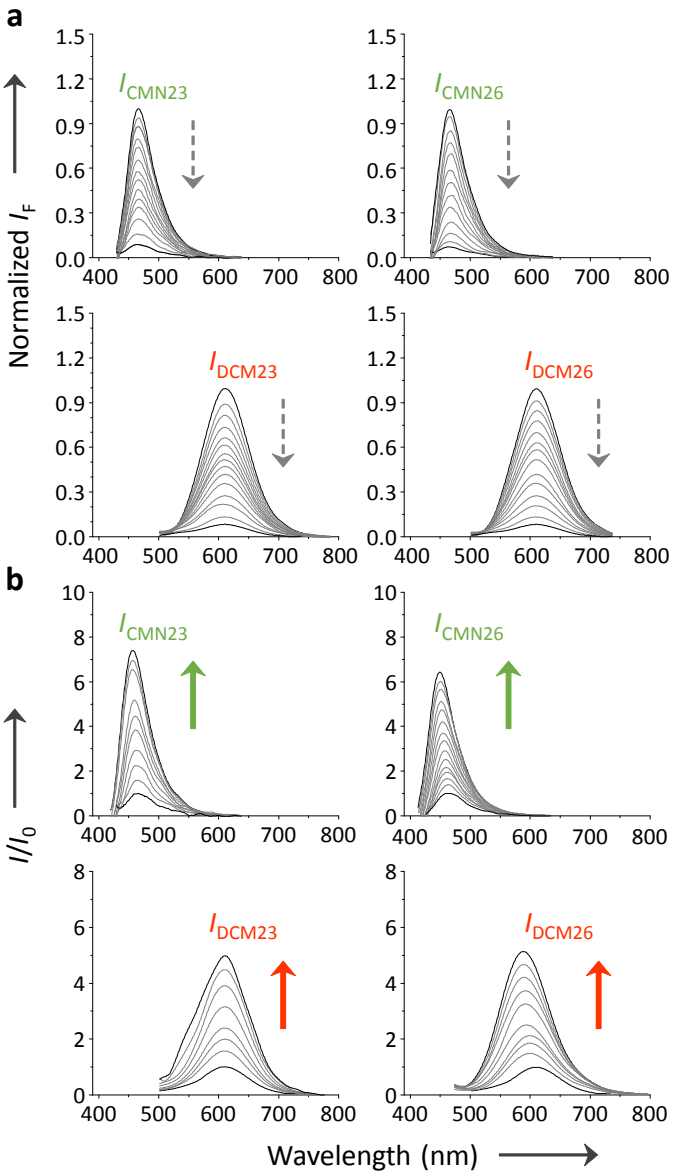


Fig. S2. (a) Fluorescence titration of **CMN23**, **CMN26**, **DCM23** and **DCM26** (1 µM) in the presence of increasing 2D MoS_2 (0-135 µg mL^{-1} and 0-370 µg mL^{-1} for **DCM** and **CMN**, respectively). (b) Fluorescence titration of **CMN23**/2D MoS_2 composite (1 µM/200 µg mL^{-1}), **CMN26**/2D MoS_2 composite (1 µM/200 µg mL^{-1}), **DCM23**/2D MoS_2 composite (1 µM/90 µg mL^{-1}) and **DCM26**/2D MoS_2 composite (1 µM/90 µg mL^{-1}) in the presence of increasing H10N8 (0-35.8 HAU 50 $µL^{-1}$), H1N1pdm09 (0-35.8 HAU 50 $µL^{-1}$), H10N8 (0-17.9 HAU 50 $µL^{-1}$) and H1N1pdm09 (0-25.6 HAU 50 $µL^{-1}$), respectively. The fluorescence measurements were carried out in phosphate buffered saline (0.01 M, pH 7.4) with an excitation of 430 nm for both **DCM** and **CMN** probes.

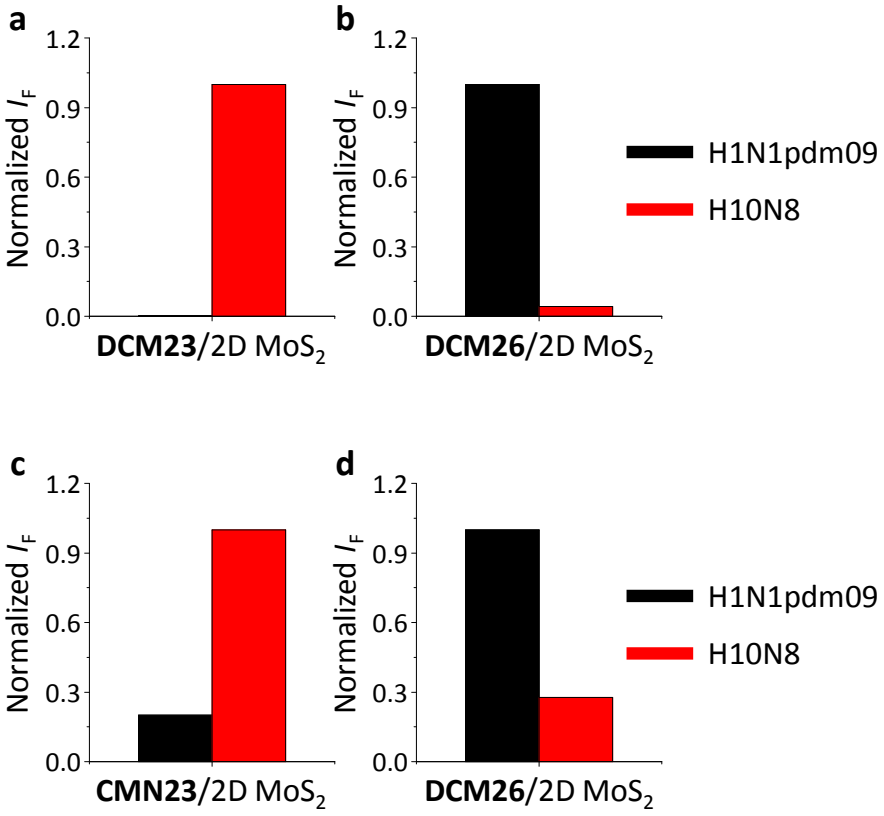


Fig. S3. Normalized fluorescence change of (a) **DCM23**/2D MoS_2 composite (1 μM/90 μg mL^{-1}), (b) **DCM26**/2D MoS_2 composite (1 μM/90 μg mL^{-1}), (c) **CMN23**/2D MoS_2 composite (1 μM/200 μg mL^{-1}) and (d) **CMN26**/2D MoS_2 composite (1 μM/200 μg mL^{-1}) in the presence of H1N1pdm09 and H10N8 (17.9 HAU 50 μL^{-1}) in phosphate buffered saline (0.01 M, pH 7.4) (excitation: 430 nm).

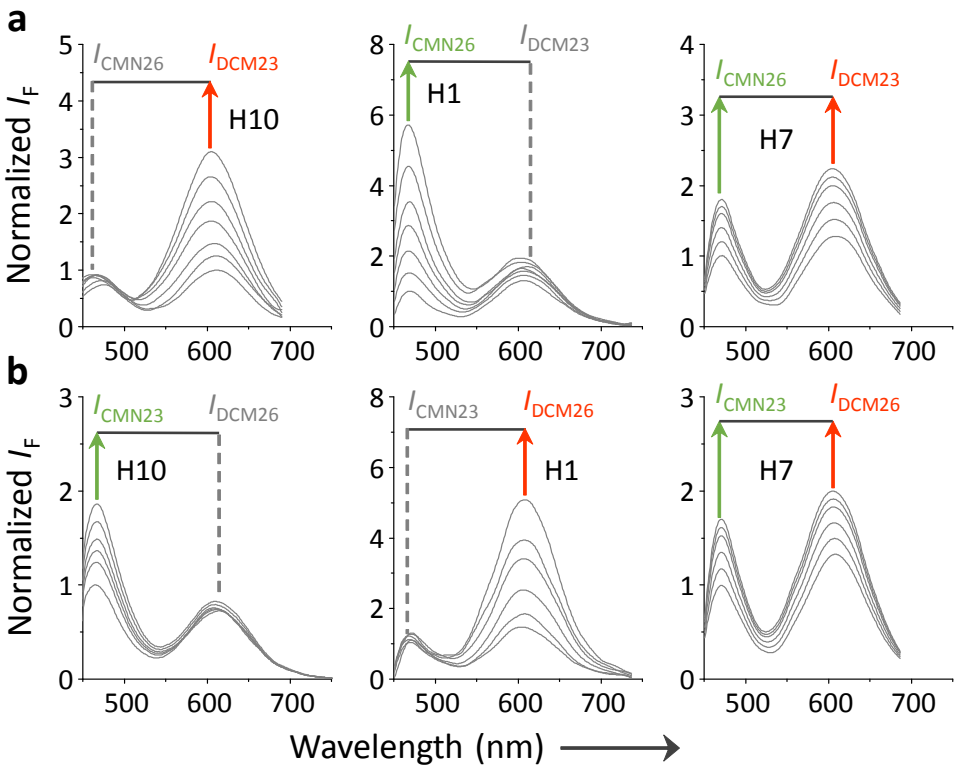


Fig. S4. Fluorescence titration of (a) **CMN26/DCM23** (1/2 μM) 2D glycosheet (2D MoS_2: 70 μg mL^{-1}) and (b) **CMN23/DCM26** (1/2 μM) 2D glycosheet (2D MoS_2: 70 μg mL^{-1}) with increasing H10N8 (A/Hunan/3-9/2007), H1N1pdm09 (A/California/08/2009) and H7N9 (A/Anhui/1/2013) in phosphate buffered saline (0.01 M, pH 7.4) (excitation: 430 nm). For the virus titers in detail, see Fig. 4 of main text.

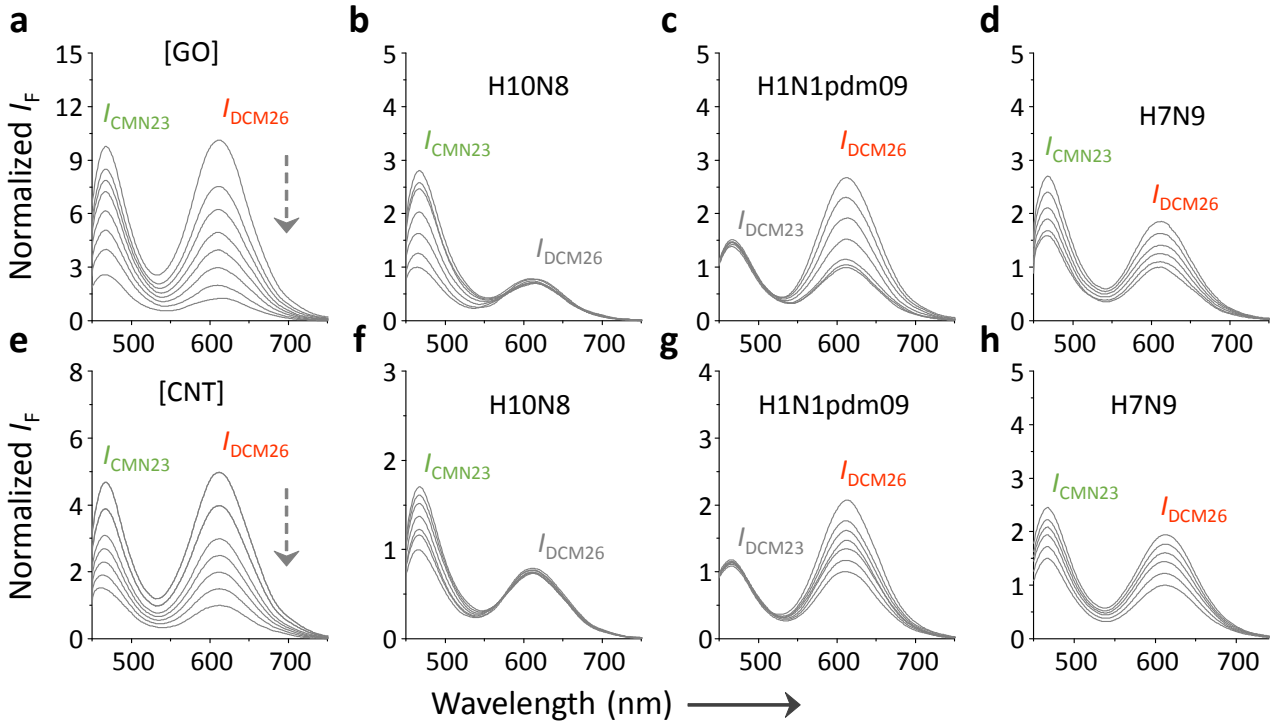


Fig. S5. Fluorescence titration of **CMN23/DCM26** (1/2 μM) in the presence of (a) increasing graphene oxide (GO; 0-40 μg mL^{-1}) and (e) carbon nanotube (CNT; 0-4.8 μg mL^{-1}). Fluorescence titration of (b-d) **CMN23/DCM26** (1/2 μM) 2D glycosheet (based on 2D GO: 40 μg mL^{-1}) and (f-h) **CMN23/DCM26** (1/2 μM) 2D glycosheet (based on 1D CNT: 4.8 μg mL^{-1}) with increasing H10N8, H1N1pdm09 and H7N9 in phosphate buffered saline (0.01 M, pH 7.4) (excitation: 430 nm). For the virus titers in detail, see Fig. 6 of main text.

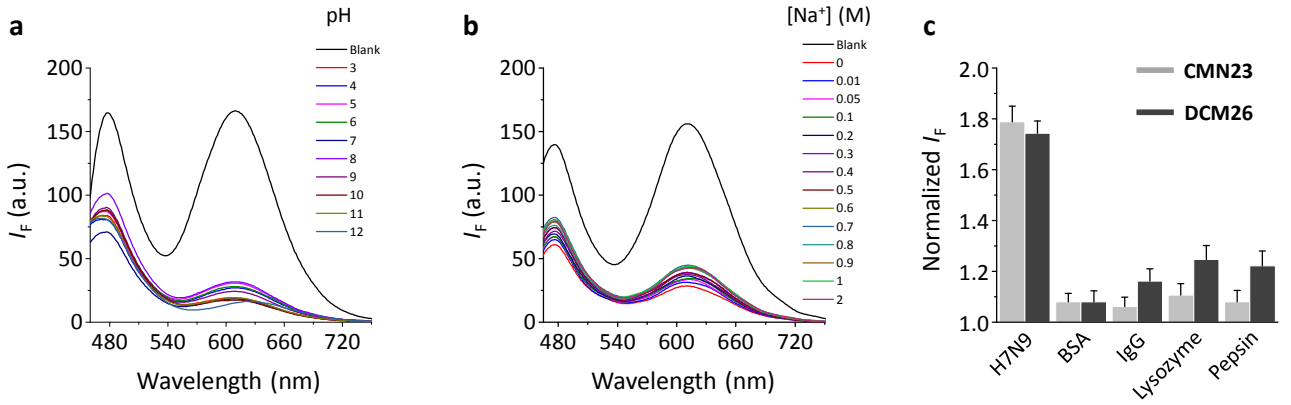


Fig. S6. Fluorescence spectra of **CMN23/DCM26** (1/2 μM) 2D glycosheet (2D MoS_2: 120 μg mL^{-1}) with increasing (a) pH and (b) sodium ion strength (where blank is the fluorescence spectrum of **CMN23/DCM26**) in phosphate buffered saline. (c) Normalized fluorescence intensity of **CMN23/DCM26** (1/2 μM) 2D glycosheet (2D MoS_2: 70 μg mL^{-1}) in the presence of H7N9 (23 HAU 50 μL^{-1}) and proteins including bovine serum albumin (BSA), IgG, lysozyme and pepsin (500 μM).

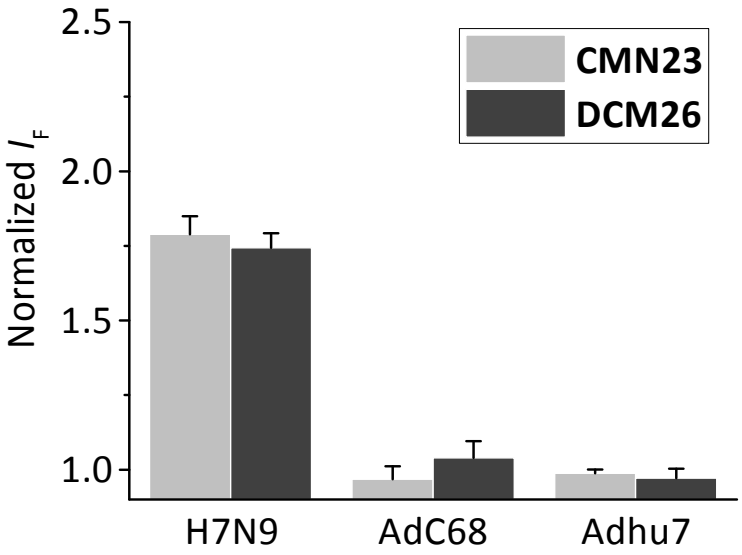


Fig. S7. Normalized fluorescence intensity of **CMN23/DCM26** (1/2 μM) 2D glycosheet (2D MoS_2: 70 μg mL^{-1}) in the presence of H7N9 (25 HAU 50 μL^{-1}), AdC68 (simian adenovirus, 4.73×10^9 virus particles mL^{-1}) and Adhu7 (human adenovirus, 3.1×10^9 virus particles mL^{-1}). The fluorescence was measured in phosphate buffered saline (0.01 M, pH 7.4) (excitation: 430 nm).

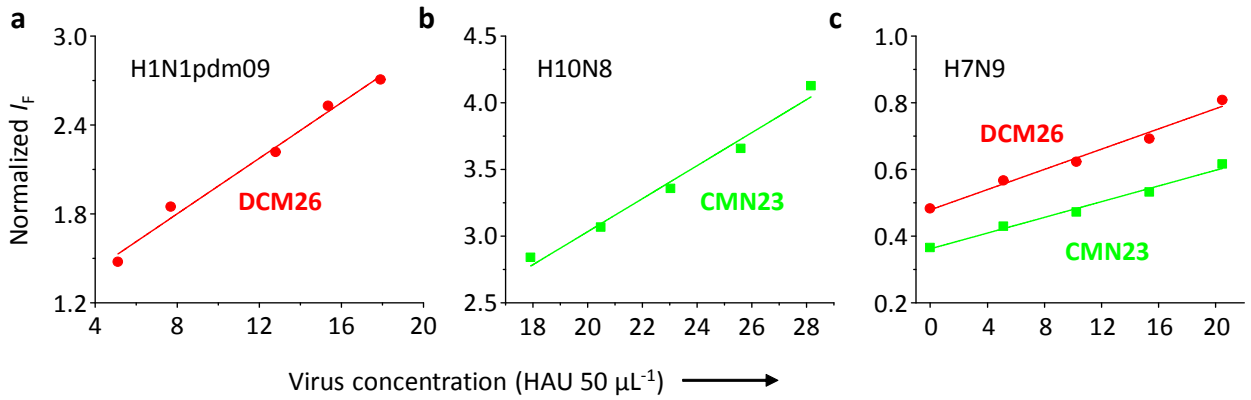


Fig. S8. Plotting the fluorescence intensity of **CMN23**/**DCM26** (1/2 µM) 2D glycosheet (2D MoS_2: 70 µg mL^{-1}) with increasing (a) H1N1pdm09, (b) H10N8 and (c) H7N9. The fluorescence was measured in phosphate buffered saline (0.01 M, pH 7.4) (excitation: 430 nm).

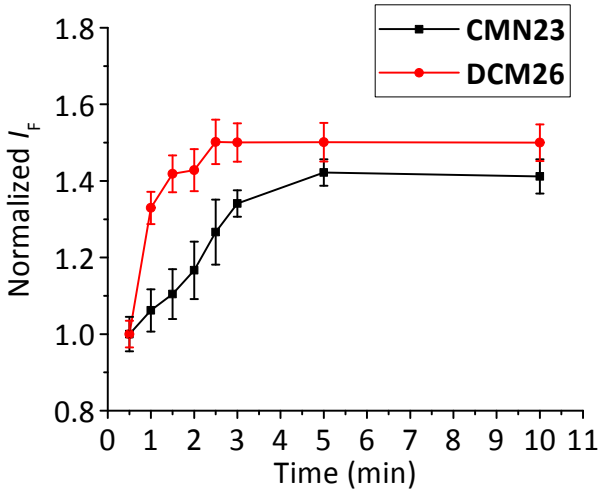


Fig. S9. Plotting the fluorescence intensity of **CMN23**/**DCM26** (1/2 μM) 2D glycosheet (2D MoS_2: 70 μg mL^{-1}) in the presence of H7N9 (25 HAU 50 μL^{-1}) with time. The fluorescence was measured in phosphate buffered saline (0.01 M, pH 7.4) (excitation: 430 nm).

Table S1. Comparison of the sensing performance of the 2D glycosheet for influenza viruses with previously developed techniques.

Method	Glycan specificity	Detection time	LOD (HAU 50 μL^{-1})
SPR	No	1.5 h	0.128[1] (for H5N1)
HPLC	No	1 h	3.2[2] (for H1N1)
AuNP	No	30 min	1.6[3] (for H3N2)
QCM	No	30 min	0.01[4] (for H5N1)
2D Glycosheet	**Yes**	**5 min**	**0.02 (for H10N8)**

[1]*Sensors*, 2012, **12**, 12506.

[2]*J. Chromatography A*, 2011, **1218**, 2432.

[3]*Analyst*, 2015, **140**, 3989.

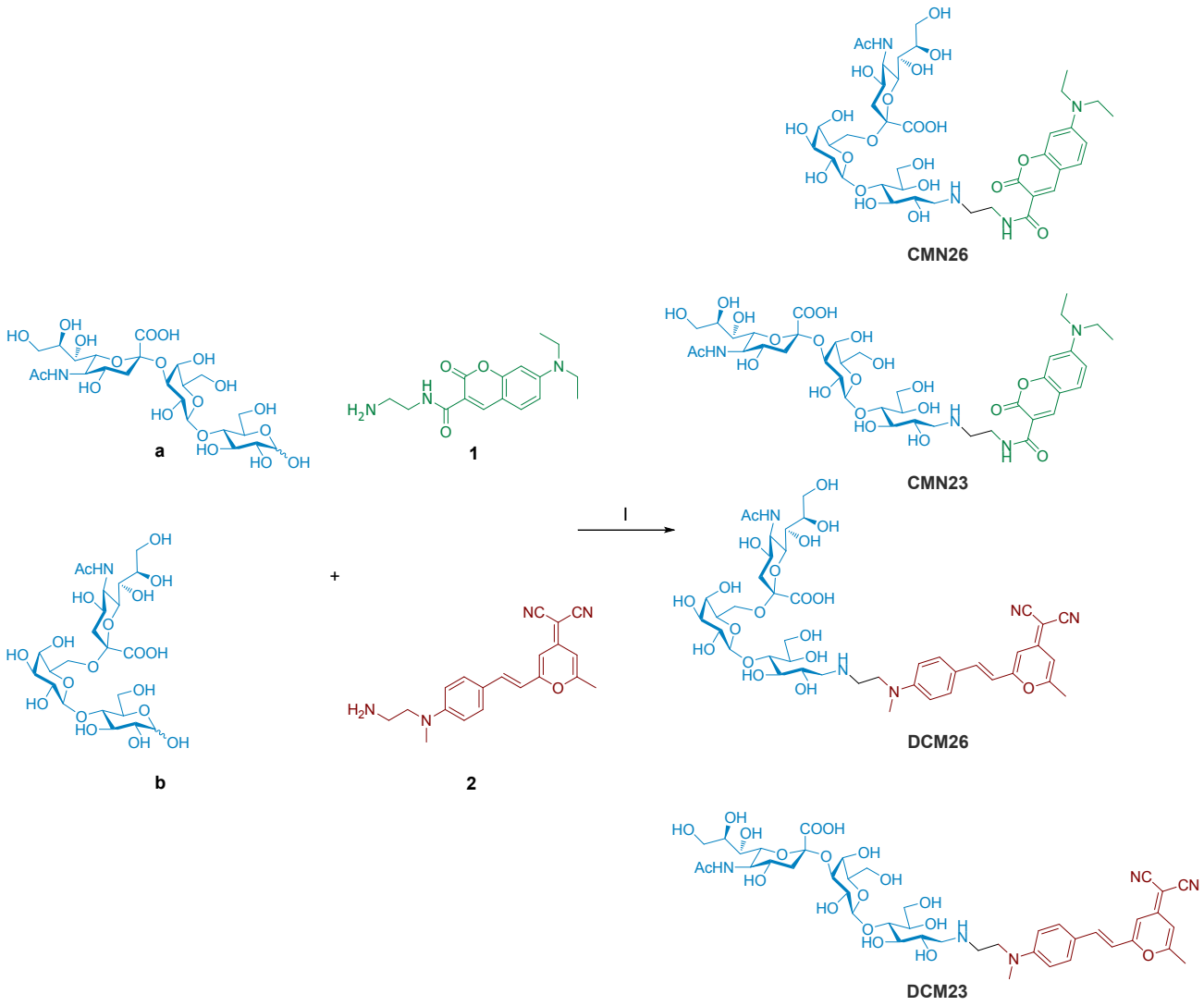
[4]*Biosens. Bioelectron.*, 2013, **42**, 148.

S2. Experimental section

General remarks. All purchased chemicals and reagents are of analytical grade. Solvents were purified by standard procedures. Graphene oxide (GO) and carbon nanotube (CNT) were purchased from XFNANO (China). Reactions were monitored by TLC (thin-layer chromatography) using E-Merck aluminum precoated plates of Silica Gel. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV). High performance liquid chromatography (HPLC) was carried out on an Agilent 1100 Series equipment. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. Raman spectra were obtained using a Renishaw InVia Reflex Raman system (Renishaw plc, Wotton-under-Edge, UK) employing a grating spectrometer with a Peltier-cooled charge-coupled device (CCD) detector coupled to a confocal microscope, which were then processed with Renishaw WiRE 3.2 software. The Raman scattering was excited by an argon ion laser (λ = 514.5 nm). Dynamic light scattering (DLS) was carried out on a Horiba LB-550 Dynamic Light Scattering Nano-Analyzer.

Preparation of 2D MoS_2. 2D MoS_2 was obtained through sonication-assisted exfoliation of bulk MoS_2 crystals in a mixed aqueous solution of EtOH and water. MoS_2 (100 mg, Aladdin Reagent Inc.) was added to a 25 mL vial filled with 20 mL of EtOH/water (1:1, v/v). The sealed vial was sonicated for 8 h, and then the dispersion was centrifuged at 3200 rpm for 10 min to remove aggregates. After collecting the supernatant and drying at 60 ^{o}C in a drying oven, an army green powder was obtained. The obtained powder was dissolved in Milli-Q ultrapure water and sonicated for 0.5 h to provide a homogeneous stock solution.

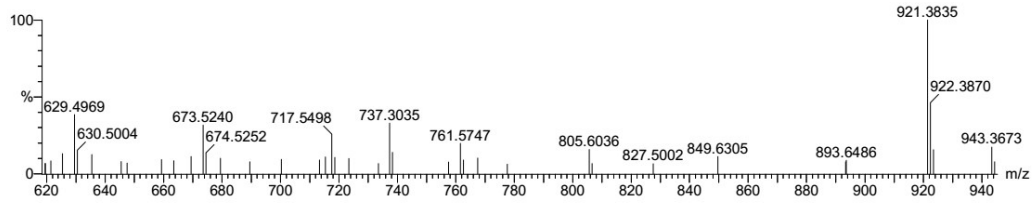
Fluorescence spectroscopy. In a typical fluorescence quenching assay, to a probe (**CMN** or **DCM** or mixed) solution in phosphate buffered saline (0.01 M, pH 7.4) was added low-dimensional material (2D MoS_2, GO or CNT pre-dispersed in deionized water) of different concentrations. Then, the mixture was shaken for 5 min and then the fluorescence was measured on a Varian Cary Eclipse fluorescence spectrophotometer with excitation of 430 nm. In a typical fluorescence recovery assay, to a 2D glycosheet solution in phosphate buffered saline (0.01 M, pH 7.4) was added viruses of different concentrations. Then, the mixture was shaken for 5 min and then the fluorescence was measured on a Varian Cary Eclipse fluorescence spectrophotometer with excitation of 430 nm.



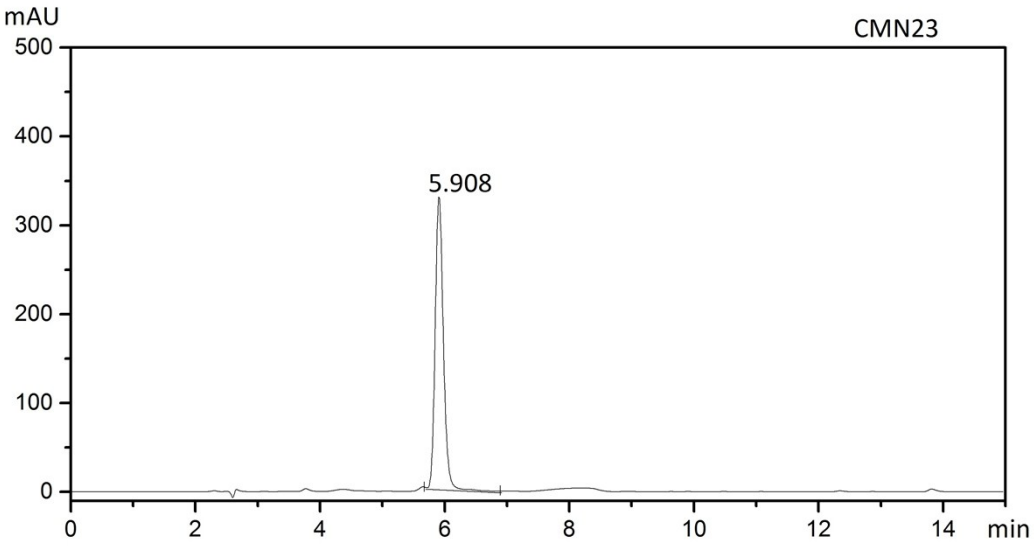
Scheme S1. Reagents and conditions: (I) $NaBH_3CN$ in $MeOH/CH_3Cl$.

General procedure for the synthesis of CMN probes. A suspension of **1** synthesized according to a previous report[1] (42.0 mg, 0.15 mmol) and glycan **a** (Neu5Acα2,3Gal-β1,4Glc) or **b** (Neu5Acα2,6Gal-β1,4Glc) (35 mg, 0.060 mmol) in 5 mL MeOH/$CHCl_3$ (1:1, v/v) was stirred at 50 °C. Then, cyanoborohydride (8 mg, 0.121 mmol) was added and the resulting mixture was stirred overnight. Then, solvent was removed in vacuum and the resulting residue was purified by column chromatography.

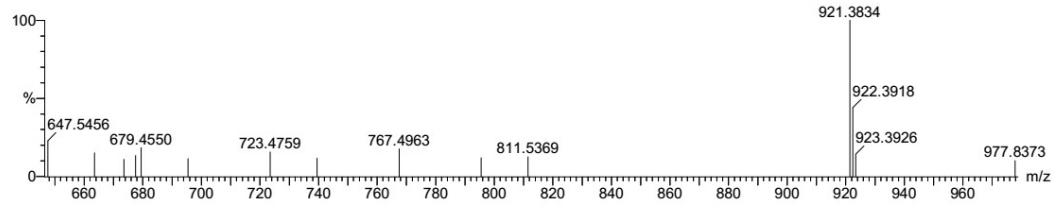
CMN23. Column chromatography (CH_2Cl_2/CH_3OH = 5:1 to 1:1, v/v) afforded **CMN23** as a yellow solid (32 mg, 52% yield). HR-ESI-MS: *m/z* $[M+H]^+$ calcd for $C_{39}H_{61}N_4O_{21}$ 921.3828, found 921.3835. HPLC: t_R = 5.908 min over 15 min of 1.0 mL min^{-1} mobile phase containing 85% methanol and 15% water, purity 99%.



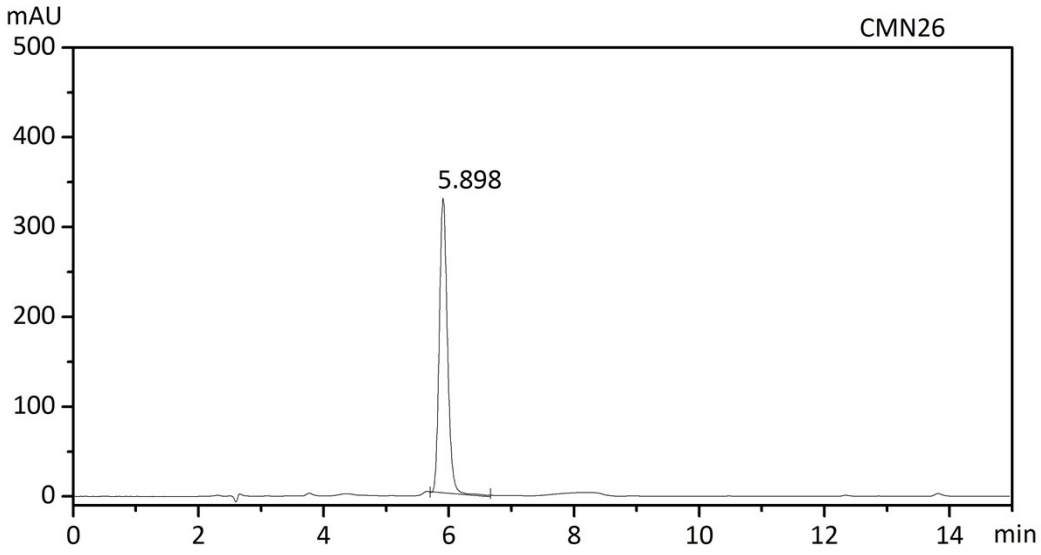
Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
921.3835	921.3828	0.7	0.8	11.5	8.1	0.0	C39 H61 N4 O21



CMN26. Column chromatography (CH_2Cl_2/CH_3OH = 2:1, v/v) afforded **CMN26** as a yellow solid (25 mg, 42% yield). HR-ESI-MS: *m/z* $[M+H]^+$ calcd for $C_{39}H_{61}N_4O_{21}$ 921.3828, found 921.3834. HPLC t_R = 5.898 min over 15 min of 1.0 mL min^{-1} mobile phase containing 85% methanol and 15% water, purity 96%.

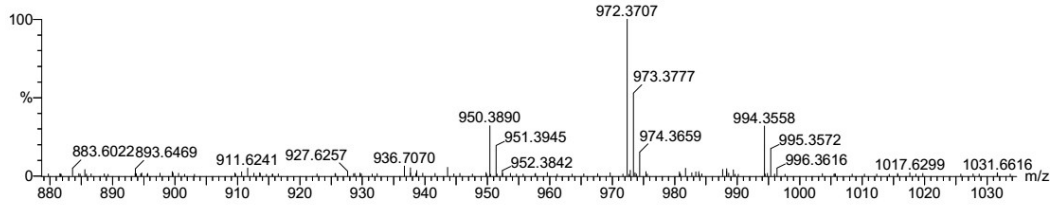


Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
921.3834	921.3828	0.6	0.7	11.5	11.1	0.0	C39 H61 N4 O21



General procedure for the synthesis of DCM probes. A suspension of **2** synthesized according to a previous report[2] (81 mg, 0.24 mmol) and glycan **a** (Neu5Acα2,3Gal-β1,4Glc) or **b** (Neu5Acα2,6Gal-β1,4Glc) (35 mg, 0.060 mmol) in 5 mL MeCN/$CHCl_3$ (1:1, v/v) was stirred at 50 °C. Then, cyanoborohydride (8 mg, 0.121 mmol) was added and the resulting mixture was stirred overnight. Then, solvent was removed in vacuum and the resulting residue was purified by column chromatography.

DCM23. Column chromatography (CH_2Cl_2/CH_3OH = 2:1, v/v) afforded **DCM23** as a red solid (32 mg, 52% yield). HR-ESI-MS: *m/z* $[M+H]^-$ calcd for $C_{43}H_{59}N_5O_{19}Na$: 972.3702, found 972.3707. HPLC: t_R = 9.001 min over 15 min of 1.0 mL min^{-1} mobile phase containing 85% methanol and 15% water, purity 97%.



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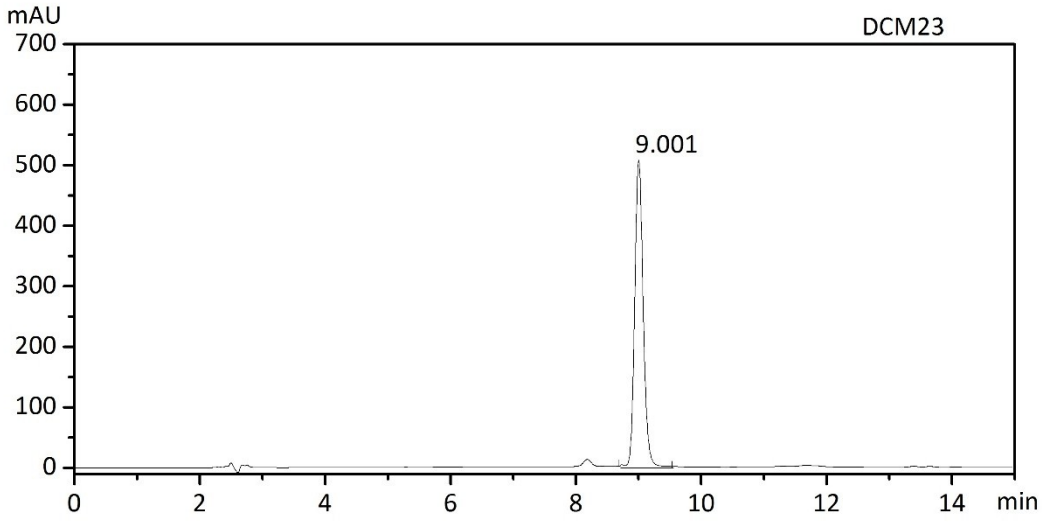
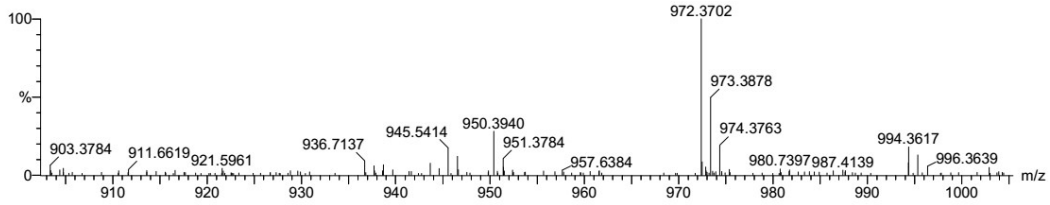
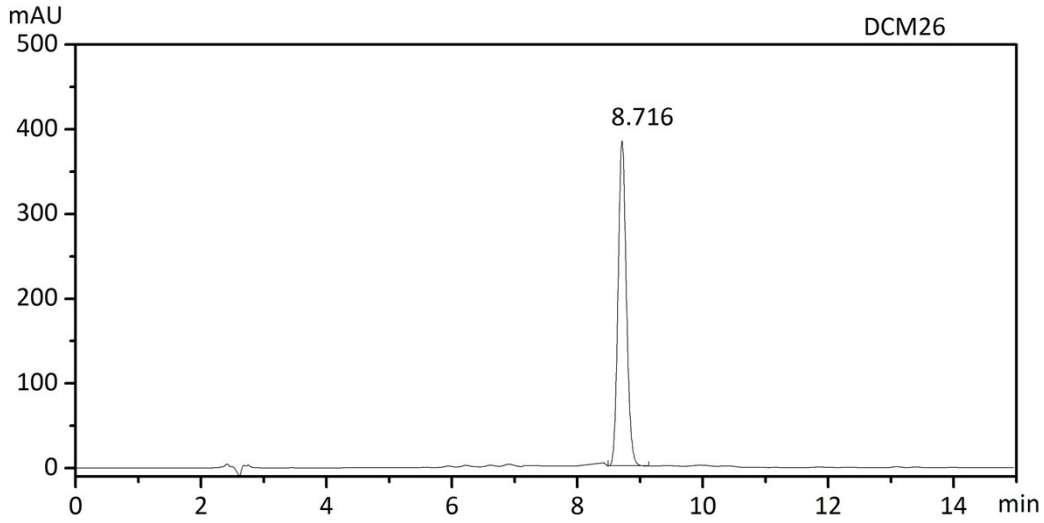
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
972.3707	972.3702	0.5	0.5	16.5	43.2	0.0	C43 H59 N5 O19 Na



DCM26. Column chromatography (CH_2Cl_2/CH_3OH = 2:1, v/v) afforded **DCM26** as a red solid (23 mg, 45% yield). HR-ESI-MS: *m/z* $[M+H]^-$ calcd for $C_{43}H_{59}N_5O_{19}Na$: 972.3702, found 972.3702. HPLC: t_R = 8.716 min over 15 min of 1.0 mL min^{-1} mobile phase containing 85% methanol and 15% water, purity 97%.



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
972.3702	972.3702	0.0	0.0	16.5	74.7	0.0	C43 H59 N5 O19 Na



Influenza A virus propagation and purification. A/California/08/2009 (H1N1pdm09) and A/environment/Dongting Lake/Hunan/3-9/2007 (H10N8) were kept in Institute Pasteur of Shanghai (Shanghai, China). The H7N9 "6+2" reassortant virus, with A/Anhui/1/2013 derived HA and NA in the background of A/Puerto Rico/8/1934, was kindly provided by Chen Ling (The First Affiliated Hospital of Guangzhou Medical University, China). All strains were amplified in the allantoic cavity of 10-day-old specific-pathogen-free chicken embryos and inactivated with 0.1% β-propiolactone as previously reported.[3] Experiments with avian influenza viruses were performed in biosafety level 3 facilities (BSL-3) at Fudan University (Shanghai, China) and others were in BSL-2 lab in Institute Pasteur of Shanghai. Inactivated virion particles were purified as follows: Allantoic fluid was centrifuged at 2000 rpm for 20 min to remove large fractions. Supernatant was further spun 1 h at 30000 rpm with 25% sucrose cushion and the pellet was resuspended in phosphate buffered saline (PBS) at 4 ºC overnight. The suspension was subjected to ultracentrifuge in 30–60% sucrose gradient at 20000 rpm for 1.5 h. The virion-containing band was collected, diluted with PBS and pelleted at 30000 rpm for 1 h. The sediment was rediluted in 500 μL of PBS and stored at -80 ºC.

S3. Additional references

1. H. Zhang, R. Liu, Y. Tan, W. H. Xie, H. Lei, H.-Y. Cheung and H. Sun, *ACS Appl. Mater. Interfaces*, 2015, **7**, 5438-5443.
2. D.-K. Ji, G.-R. Chen, X.-P. He and H. Tian, *Adv. Funct. Mater.*, 2015, **25**, 3483-3487.
3. F. Geeraedts, N. Goutagny, V. Hornung, M. Severa, A. De Haan, J. Pool, J. Wilschut, K. A. Fitzgerald and A. Huckriede, *PLoS Pathog.*, 2008, **4**, e1000138.