Supporting Information For

Super-resolution imaging of cancer-associated carbohydrates using aptamer probes

Yingying Jing,[a,b] Junling Chen,[c] Lulu Zhou,[a] Jiayin Sun,[a] Mingjun Cai,[a] Yan Shi,[a] Yongmei Tian,[d] Jing Gao,*[a] and Hongda Wang*[a,e]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China.

[b] University of Science and Technology of China, Hefei, Anhui, 230027, P. R. China.

[c] Wuhan University of Science and Technology, Wuhan, Hubei, 430081, P. R. China.

[d] College of Public Health, Zhengzhou University, Zhengzhou, Henan, 450001, P. R. China.

[e] Laboratory for Marine Biology and biotechnology, Qing dao National Laboratory for Marine Science and Technology, Wenhai Road, Aoshanwei, Jimo, Qingdao, Shandong 266237, P. R. China.

E-mail: gaojing@ciac.ac.cn;

hdwang@ciac.ac.cn;

Fax: +86 431 85262453;

Tel: +86 431 85262453

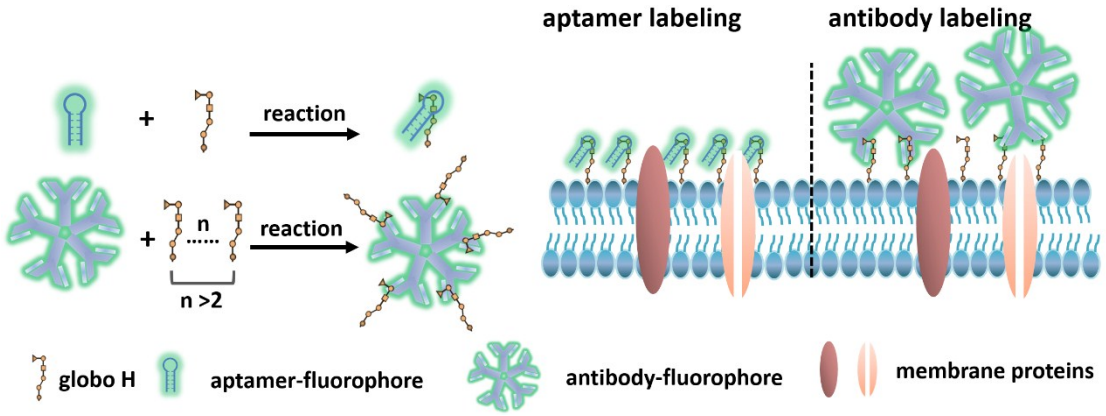

Fig.S1 Schematic of the recognition principle of aptamer and antibody in solution or on cell membrane. The aptamer is a monovalent DNA chain which has only one binding site to link globo H either in solution or on the cell membrane. However, the antibody (Mbr1, a kind of IgM) has multiple recognition sites that may cause globo H to cross-link, and steric hindrance caused by the large size of the antibody can lead to incomplete labeling on cell membrane.

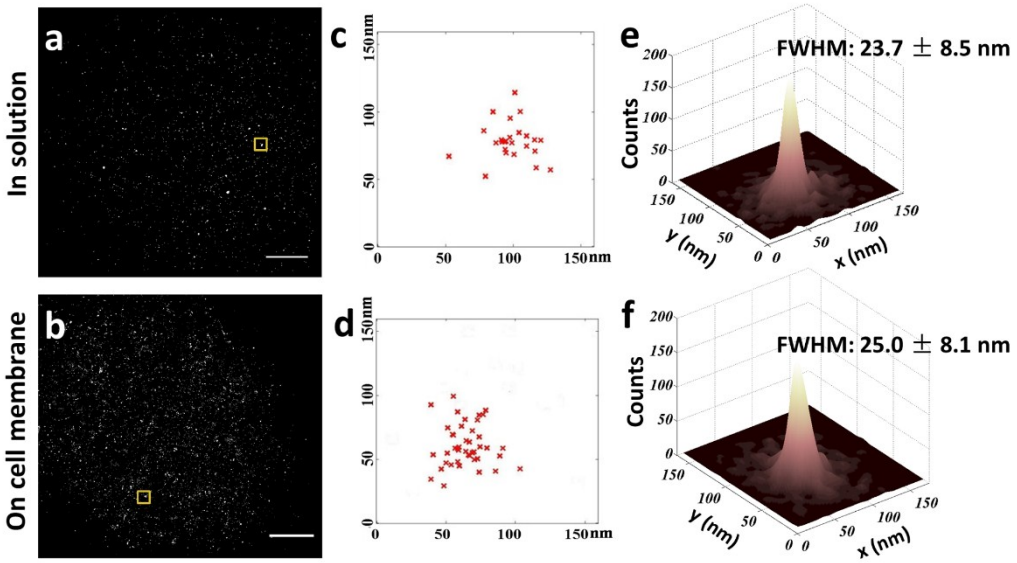

Fig.S2 Measurements of the resolution of TAMRA-conjugated aptamer at a low concentration. (a-b) Representative dSTORM images of TAMRA-conjugated aptamers in solution (a) and on MCF-7 cell membranes (b). Scale bars, 5 μm. (c-d) Repetitive localizations of a single TAMRA-aptamer molecule shown as crosses which were boxed in (a-b). (e-f) The three-dimensional Gaussian profile of localizations from 50 individual fluorescent molecules in three independent experiments. The full-width at half-maximum (FWHM) determined by Gaussian fitting represents a resolution of 23.7 ± 8.5 nm for fluorescent probes in solution and 25.0 ± 8.1 nm on cell membrane.

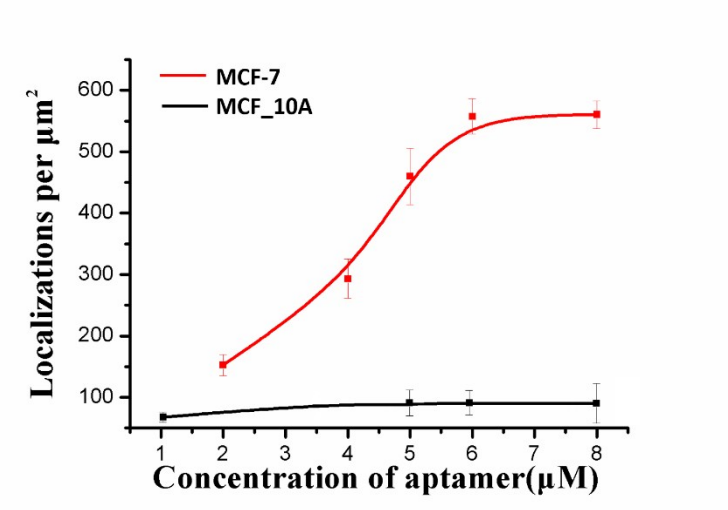

Fig.S3 The saturated concentration curve of aptamers on MCF-7 and MCF-10A cells by calculating localization per μm^2. Data shown are means ± standard deviation (s.d.). The results were obtained from ten cells in three independent experiments.

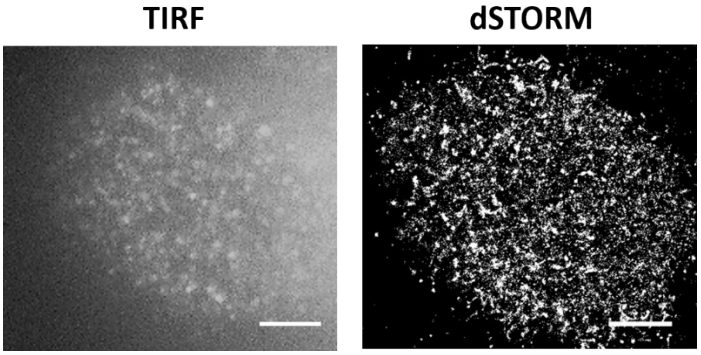

Fig.S4 Conventional TIRF image and the corresponding dSTORM image of globo H on MCF-7 cell membrane. The conventional TIRF image displays a diffraction-limited distribution of globo H on the cell membranes, whereas the dSTORM imaging improved the resolution substantially. Scale bars, 5 μm.

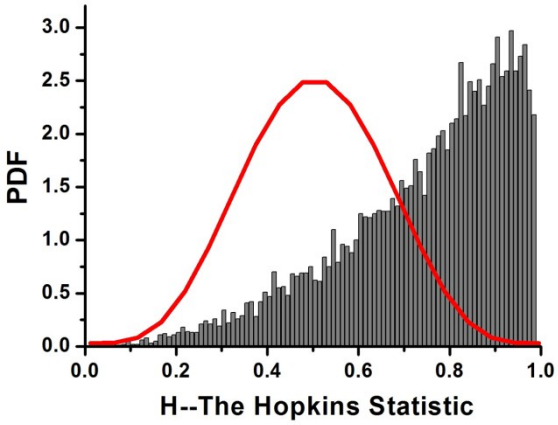

Fig.S5 Average of the Hopkins statistic for testing spatial randomness on the examined domains. Red curve shows a random distribution, with centering at the Hopkins statistic 0.5 on the x-coordinate. Clustered points will result in an H value close to 1.

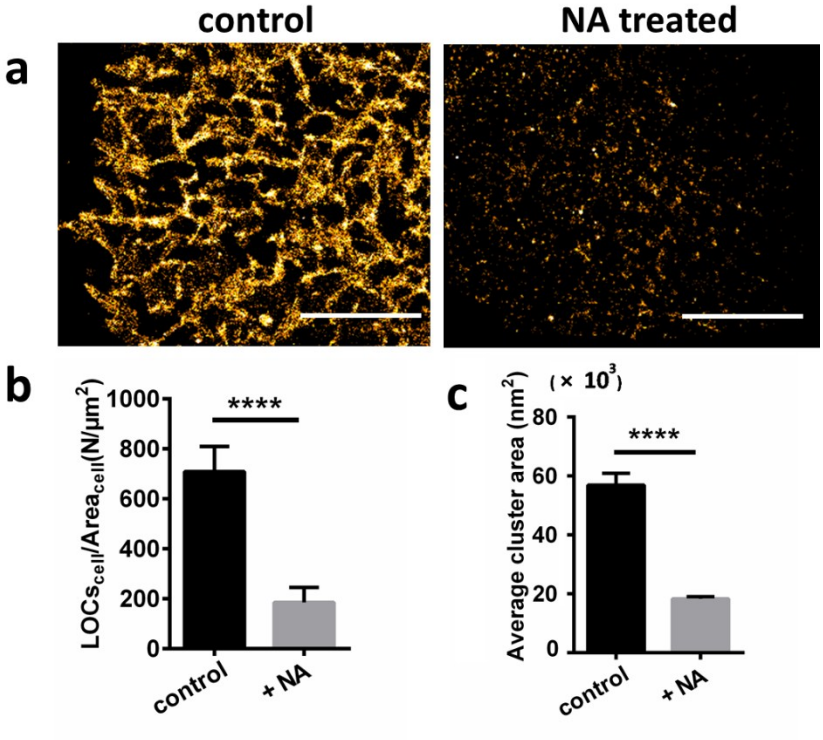

Fig.S6 Neuraminidase treatment reduced sia clusters. (a) dSTORM images of sia on the control and neuraminidase-treated cell membranes. Scale bars are 5 μm. (b-c) The number of localizations per μm^2 (b) and the average cluster area on the control and treated cell membranes. LOCs is the abbreviation for localization. Data shown are means ± standard deviation (s.d.). The statistical results were obtained from ten cells in three independent experiments. "****" means $P < 0.0001$. Analysis of variance was processed using the two-tailed unpaired t-test.

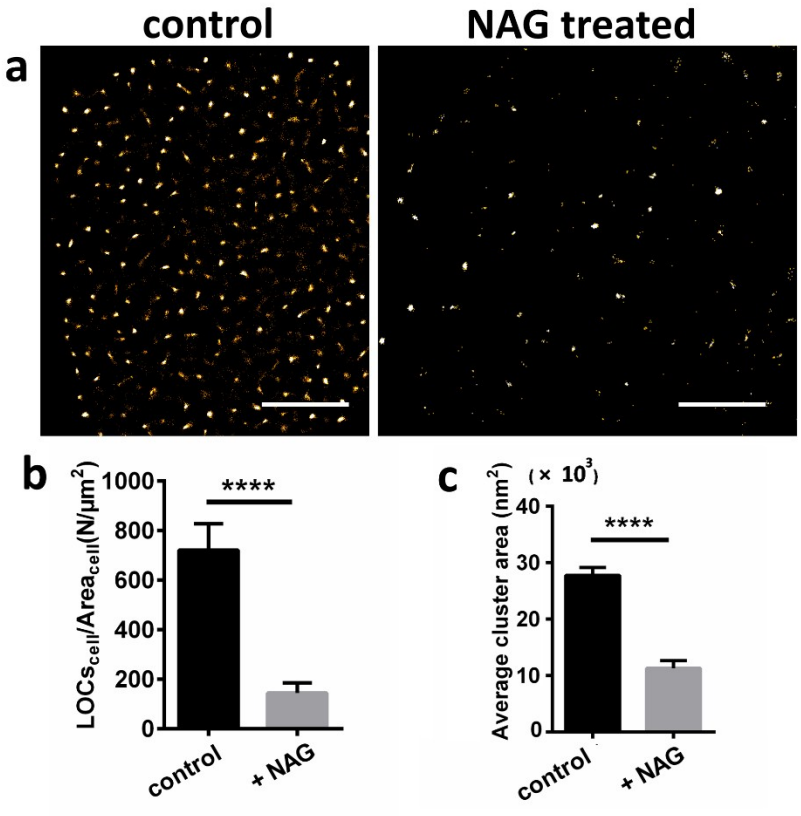

Fig.S7 Changes of GlcNAc after N-acetyl-β-D-glucosaminidase (NAG) treatment on the cell membranes. (a) dSTORM images of GlcNAc on the control and NAG-treated cell membranes. Scale bars are 5 μm. (b) The number of localizations per μm^2 of GlcNAc on the control and NAG-treated cell membranes. (c) The average area of these two types of clusters under the same conditions as (b). Data are the means ± standard deviation (s.d.), which were obtained from ten cells in three independent experiments. "****" means $P < 0.0001$. Analysis of variance was carried out by the two-tailed unpaired t-test.

Table S1. Quantitative characterization of globo H clusters.

Circularity		**Diameter**		**Area**	
scope	percentage (%)	scope (nm)	percentage (%)	scope (x10^3 nm^2)	percentage (%)
0.1-0.2	0.1	30-60	3.8	0.9-4	4.1
0.2-0.3	2.0	60-100	23.0	4-7	9.8
0.3-0.4	9.1	100-300	60.9	7-10	12.0
0.4-0.5	17.3	300-600	11.1	10-40	45.5
0.5-0.6	20.8	600-900	1.1	40-70	11.5
0.6-0.7	19.0	900-1100	0.09	70-100	6.0
0.7-0.8	16.1	>1100	0.03	100-400	10.6
0.8-0.9	10.1			400-1000	0.5
0.9-1	5.5				

Statistics were from ten cells in five independent experiments.