Supporting Information

for

A Heteromultivalent Host-Guest Chemical Nose for Cell Recognition and Discrimination

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Table of Contants

1. Materials and Methods

1.1. Materials

All the reagents and solvents were commercially available and used as received unless otherwise specified purification. Iodomethane, ammonium hexafluorophosphate (NH_4PF_6) and tetrabutyl ammonium chloride hydrate((*n*-butyl) NCl) and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were obtained from Sigma-Aldrich Co., Ltd. Fluorescein (Fl) was obtained from TCI Development Co. Ltd. Aluminum phthalocyanine chloride tetrasulfonic acid ($AlPcS_4$) was obtained from Frontier Scientific. Fetal bovine serum (FBS) and Dulbecco's modified eagle medium (DMEM) were purchased from Thermo Fisher Scientific. Penicillin streptomycin sol was purchased from Gibco. Guanidinium-modified calixarenes (GC4A and GC5A) and ammonium-modified calixarenes (QC4A and QC5A) were synthesized according to the previous literatures,[1-3] ammonium-modified calixarene Hela, HepG2, MIHA cell lines were obtained from BeNa Culture Collection, HCT-15, SW480, HT29 cell lines were obtained as a gift from the Yi-Liang Li group (Institute of radiation medicine, China academy of medical science), 293FT, 293T cell lines were obtained as a gift from the Guang Yang group (College of Pharmacy, Nankai University), HK-2 cell line was obtained as a gift from the Yue-Bing Wang group (School of Medicine, Nankai University). The HEPES buffer solution (pH 7.4, 10 mM) was prepared like follows: 2.38 g HEPES was dissolved in 0.9 L ultra-pure water and titrated to pH 7.4 with NaOH, and then made up the volume to 1 L with ultra-pure water.

1.2. Apparatus

NMR data were recorded on a Bruker AV400 spectrometer and Zhongke-Niujin BIXI-I 400 spectrometer. Mass spectra were recorded on an Agilent 6520 Q-TOF LC/MS. Steady-state fluorescence measurements were recorded in a conventional quartz cell (light path 10 mm) on a Cary Eclipse

equipped with a Cary single-cell peltier accessory and Microplate Reader Accessory. Dynamic light scattering, and zeta potential measurements were examined on NanoBrook 173plus and ZETAPALS/BI-200SM equipped with a digital correlator at 532 nm.

1.3. Preparation of coassemblies.

CAs (GC4A, GC5A, QC4A and QC5A) and CD were dissolved in methanol and chloroform at a concentration of 1.0 mM, respectively. The mixed organic solution of CAs and CD in a ratio of 1:1 or 2:1 was dried under high vacuum for 4 h to yield a thin film in a glass vial. HEPES buffer (pH 7.4, 10 mM) was added and the solution was sonicated at 80 °C for 3 h to make the CA-CD coassemblies. The sensor units were prepared by simply mixing the coassembly and dye.

1.4. Fluorescence titrations

Fluorescence titrations of CA-CD coassemblies were performed in HEPES buffer (pH 7.4, 10 mM). The complexation of the coassembly with reporter dye (FI) were measured by direct fluorescence titrations. A mixed solution containing known amounts of assembly and reporter dye was sequentially injected into 2.50 mL reporter dye solution in a quartz cuvette. The dye concentrations in mixed solution and cuvette are the same to keep dye concentration constant in the course of titrations. The fluorescence intensity was measured (λ_{ex} = 510 nm for FI) before the first addition and after every addition until a plateau was reached.

The complexation of the coassembly with cell line was measured by competitive fluorescence titrations. All cells were cultured in 90 mm Petri dishes as the standard protocol and then were harvested by centrifuging and resuspended in DMEM for counting. A mixed solution containing known amounts of reporter dye, coassembly and cells was sequentially injected into 2.50 mL reporter dye and coassembly constant in the course of titrations. The fluorescence intensity was measured (λ_{ex} = 510 nm for FI) before the first addition and after every addition.

The fitting of data from direct host-guest titrations were performed in a 1:1 nonlinear manner, the fitting of data from competitive titration of cells and coassemblies were performed in a *N*:1 manner, and the fitting modules were downloaded from the website of Prof. Nau's group (http://www.jacobs-university.de/ses/wnau) under the column of "Fitting Functions".[3] The number of cell was divided by Avogadro constant to give the molar amount. The binding sites on the cell surface were assumed as identical and independent, and the affinity of any site does not depend on whether or not the other sites are occupied.

1.5. Cell culture

All cell lines were maintained in DMEM with 10% FBS (v/v). The cells were incubated in a humidified atmosphere of 5 % CO_2 at 37 °C. Cells were washed with PBS buffer, trypsinized with trypsin and collected in the DMEM media (with no FBS) for further experiments.

1.6. Experimental procedure for cell identification

All cells were cultured in 90 mm Petri dishes as the standard protocol and then were harvested by centrifuging and resuspended in DMEM for counting. A total of 15000 cells were transferred to each well of a black 96-well plate to interact with the reporter dye and CA-CD coassembly. The mixtures were incubated for 20 min. Six repeated experiments were performed. All the fluorescence intensity change ratios were calculated by $(I-I_0)/I_0$ or $(I-I_0)/(I'-I_0)$, where I is the fluorescence intensity of each kind of coassembly-dye after adding the test cell sample, and I_0 is the fluorescence intensity of the relative coassembly-dye alone, I' is the fluorescence intensity of dye alone. The obtained raw data were analyzed by Past3 with 90% confidence ellipses.

1.7. Experimental procedure for cancer cell discrimination in the cancer cell/normal cell mixtures

The HepG2 cells (cancer) and MIHA (normal) were cultured in 90 mm Petri dishes as the standard protocol and then harvested by centrifuging and resuspended in DMEM for counting. The normal cells were mixed with

different amounts of cancer cells in ratios of 0%, 30%, 70% and 100% (percentages stand for the amount of cancer cells). All the mixed cell samples were measured using the same procedure and experimental conditions as in the cell identification part.

2. Supportive results and the original data of discrimination

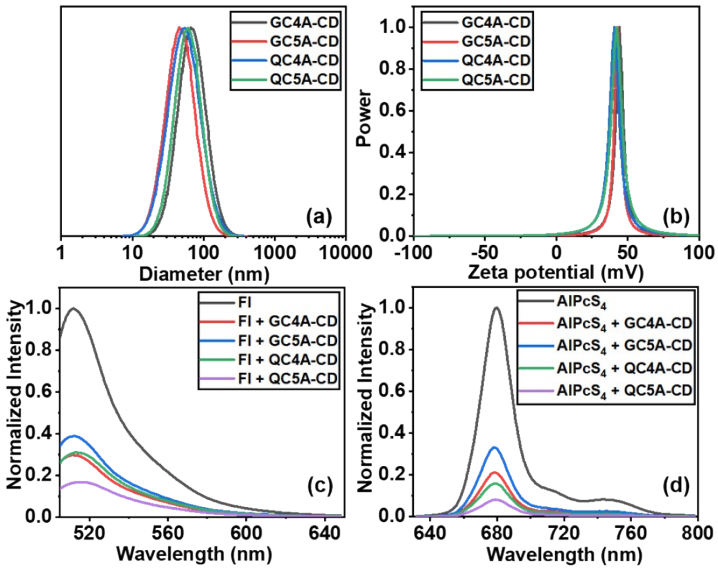

Fig. S1 (a) Dynamic light scattering data and (b) Zeta potentials of coassemblies GC4A-CD, GC5A-CD, QC4A-CD and QC5A-CD. Fluorescence quenching of (c) FI (1.0 μM) or (d) $AlPcS_4$ (1.0 μM) by the host-guest complexation of different CA-CD coassemblies (CA-CD: 1.0/1.0 μM). All experiments were in HEPES buffer (10 mM, pH = 7.4) at 25 °C. $\lambda_{ex\text{-}FI}$ = 490 nm, $\lambda_{ex\text{-}AlPcS4}$ = 607 nm.

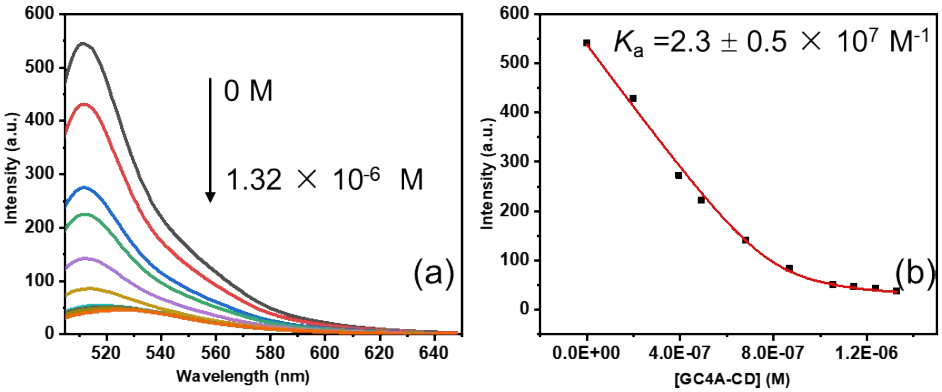

Figure S2. (a) Fluorescence titration of GC4A-CD and Fl (0.8 μM) in HEPES buffer (10 mM, pH 7.4) at 25 °C, λ_{ex} = 490 nm, (b) the associated titration curve at λ_{em} = 510 nm was fitted according to a 1:1 binding stoichiometry based on the concentration of GC4A unit.

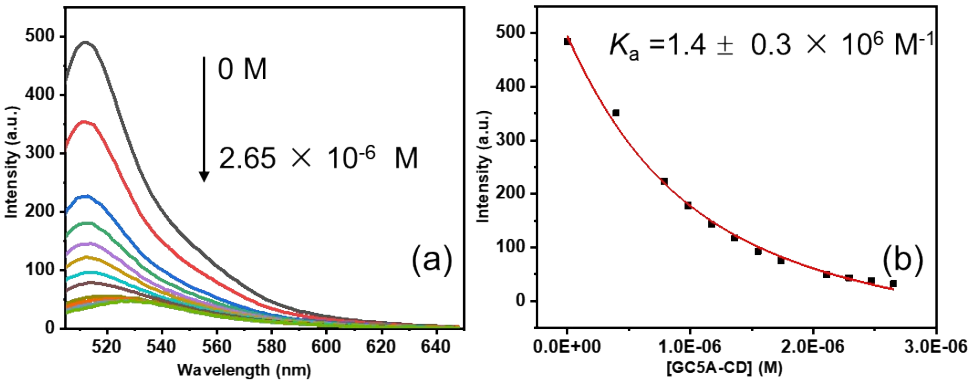

Figure S3. (a) Fluorescence titration of GC5A-CD and Fl (1 μM) in HEPES buffer (10 mM, pH 7.4) at 25 °C, λ_{ex} = 490 nm, (b) the associated titration curve at λ_{em} = 510 nm was fitted according to a 1:1 binding stoichiometry based on the concentration of GC5A unit.

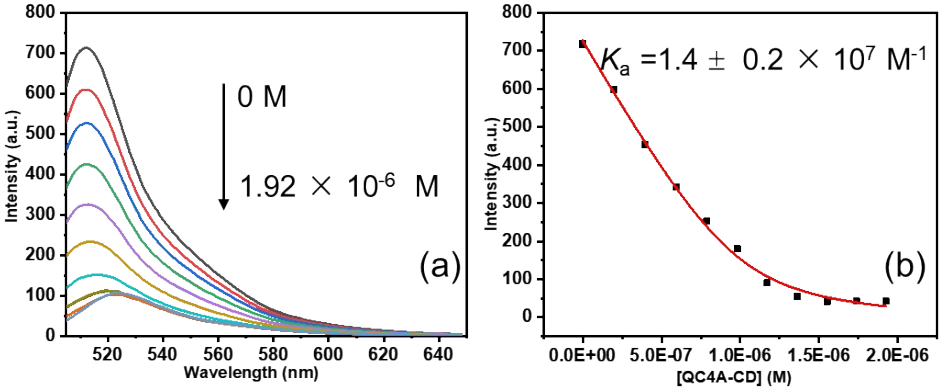

Figure S4. (a) Fluorescence titration of QC4A-CD and Fl (0.8 μM) in HEPES buffer (10 mM, pH 7.4) at 25 °C, λ_{ex} = 490 nm, (b) the associated titration curve at λ_{em} = 510 nm was fitted according to a 1:1 binding stoichiometry

based on the concentration of QC4A unit.

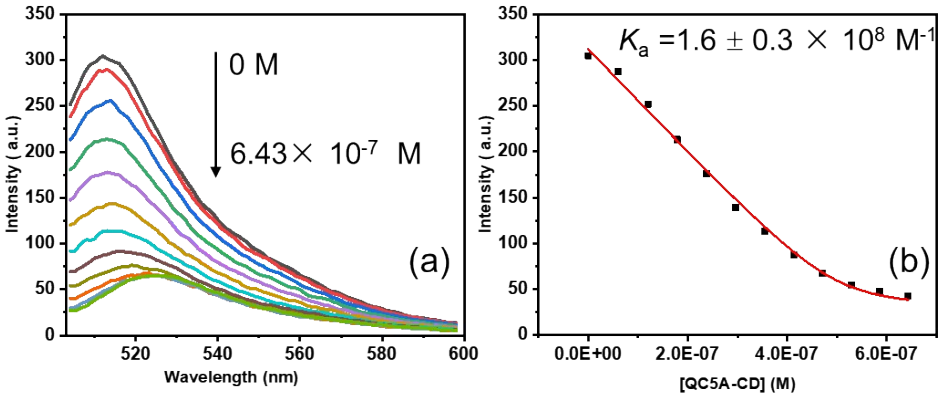

Figure S5. (a) Fluorescence titration of QC5A-CD and Fl (0.8 μM) in HEPES buffer (10 mM, pH 7.4) at 25 °C, λ_{ex} = 490 nm, (b) the associated titration curve at λ_{em} = 510 nm was fitted according to a 1:1 binding stoichiometry based on the concentration of QC5A unit.

Table S1. Training matrix of fluorescence response patterns of four-channel sensor array against four cell lines.

Cell	GC4A-CD	GC5A-CD	QC4A-CD	QC5A-CD
Hela	0.588599	0.949991	1.602744	2.312345
Hela	0.484689	0.830761	1.24521	2.434507
Hela	0.320261	1.167428	1.172542	2.236489
Hela	0.386919	0.822712	1.300400	2.266893
Hela	0.327764	0.895298	1.139461	2.040293
Hela	0.370478	0.906202	1.491494	2.134433
HepG2	2.029922	0.744563	0.822576	1.255235
HepG2	2.140861	0.681075	0.729405	1.285930
HepG2	2.125102	0.699147	0.878001	1.135568
HepG2	2.100695	0.772335	0.723276	1.131338
HepG2	2.358234	0.696127	0.746025	1.052034
HepG2	2.436431	0.610001	0.747039	1.013716
293FT	0.623114	0.887262	1.189855	4.149174
293FT	0.559300	0.827972	1.253409	4.367621
293FT	0.555044	0.903537	1.063400	4.058025
293FT	0.587349	0.775777	1.180402	4.333921
293FT	0.539116	0.727998	1.868957	4.350913
293FT	0.571237	0.866523	1.061029	4.518385
MIHA	0.590536	0.307201	0.542365	0.699923
MIHA	1.066960	0.319039	0.528210	0.570986
MIHA	2.176489	0.365707	0.612556	0.681085
MIHA	1.175362	0.422582	0.501962	0.567706
MIHA	1.619724	0.248863	0.570342	0.630786

MIHA	0.282565	0.372815	0.694683	0.307723

Table S2. Training matrix of fluorescence response patterns of six-channel sensor array against nine cell lines.

Cell	GC4A-CD	GC5A-CD	QC4A-CD	QC5A-CD	2GC4A-CD	2GC5A-CD
Hela	0.588599	0.949991	1.602744	2.312345	0.030204	0.345217
Hela	0.484689	0.830761	1.245210	2.434507	0.171471	0.352164
Hela	0.320261	1.167428	1.172542	2.236489	0.053807	0.351213
Hela	0.386919	0.822712	1.300400	2.266893	0.150551	0.374041
Hela	0.327764	0.895298	1.139461	2.040293	0.050321	0.412457
Hela	0.370478	0.906202	1.491494	2.134433	0.107770	0.037209
HepG2	2.029922	0.744563	0.822576	1.255235	0.064454	0.064099
HepG2	2.140861	0.681075	0.729405	1.28593	0.041973	0.047537
HepG2	2.125102	0.499147	0.878001	1.135568	0.038549	0.106363
HepG2	2.100695	0.772335	0.623276	1.131338	0.061258	0.068863
HepG2	2.358234	0.896127	0.646025	1.052034	0.069701	0.066667
HepG2	2.436431	0.610001	0.747039	1.013716	0.055299	0.116626
293T	0.350319	1.604493	1.494991	3.302236	1.619089	1.146545
293T	0.293805	1.447020	1.473624	3.384762	1.636001	1.131895
293T	0.328401	1.632700	1.434614	3.239771	1.782603	1.590462
293T	0.363780	1.505861	1.575183	3.195882	1.529765	1.328192
293T	0.369394	1.574436	1.456394	3.376865	1.747025	0.928508
293T	0.242841	1.421546	1.530411	3.332427	1.76038	1.110225
HK2	1.756096	3.727322	2.93875	3.590015	2.569466	0.199195
HK2	1.474286	3.413479	1.707817	3.509111	2.598109	0.323705
HK2	1.594911	2.817406	2.021141	3.052523	2.329145	0.319673
HK2	1.547225	2.734668	2.108228	2.636784	2.206010	0.102078
HK2	1.572473	2.551883	1.818042	3.756489	2.143670	0.108954
HK2	1.314365	2.401313	2.402445	3.742268	1.737617	0.283685
293FT	0.623114	0.887262	1.189855	4.149174	2.365045	2.539509
293FT	0.559300	0.827972	1.253409	3.367621	2.754154	2.180846
293FT	0.555044	0.903537	1.063400	4.058025	2.929971	2.585455
293FT	0.587349	0.775777	1.180402	4.333921	2.330639	1.923211
293FT	0.539116	0.727998	1.868957	4.350913	3.600618	1.994517
293FT	0.571237	0.866523	1.061029	4.518385	3.870044	2.174024
HT29	0.328491	0.218699	-0.13006	0.793421	0.230328	0.305150
HT29	0.222092	0.232052	-0.10009	0.828021	0.294333	0.250726
HT29	0.181798	0.707975	-0.02750	0.663714	0.392574	0.407965
HT29	0.245217	0.067184	0.097917	0.964959	0.326199	0.376270
HT29	0.308358	0.155323	0.163241	0.863500	0.338161	0.375017
HT29	0.366232	0.236444	0.176102	0.767046	0.370127	0.176923
SW480	0.376333	0.276771	0.357964	0.564021	0.396518	0.338881
SW480	0.145365	0.550167	0.168567	0.567818	0.483134	0.290569

SW480	0.364377	0.51965	0.175215	0.506893	0.455815	0.077982
SW480	0.215778	0.220753	-0.25462	0.412433	0.448602	0.336529
SW480	0.542682	0.32499	-0.43756	0.449873	0.223983	0.397544
SW480	0.235488	0.091074	0.08265	0.388002	0.371213	0.032302
HCT-15	0.090543	0.300464	0.010079	0.742245	0.346820	-0.026500
HCT-15	0.335823	0.101635	0.039537	0.581794	0.353396	0.141675
HCT-15	0.200844	0.080514	0.128716	0.912417	0.200548	0.077838
HCT-15	0.613383	0.351321	0.206315	0.772251	0.384569	0.149517
HCT-15	0.224093	0.490243	0.493028	0.533526	0.359179	0.115765
HCT-15	0.346827	0.105982	-0.16565	0.784794	0.320998	0.200473
MIHA	0.590536	0.307201	0.542365	0.699923	1.574639	1.114801
MIHA	1.066960	0.319039	0.52821	0.570986	1.549927	1.639483
MIHA	2.176489	0.365707	0.612556	0.681085	1.525055	1.726613
MIHA	1.175362	0.422582	0.501962	0.567706	1.474043	1.618099
MIHA	1.619724	0.248863	0.570342	0.630786	1.444057	1.99111
MIHA	0.282565	0.372815	0.694683	0.307723	1.808747	1.849136

Table S3. Training matrix of fluorescence response patterns of four-channel sensor array against mixed cells.

Cell	GC4A-CD	GC5A-CD	QC4A-CD	QC5A-CD
HepG2	0.17817	0.459246	0.320079	0.301245
HepG2	0.179743	0.438031	0.363098	0.297185
HepG2	0.166869	0.436498	0.306899	0.268237
HepG2	0.169105	0.483064	0.330306	0.300075
HepG2	0.160223	0.499533	0.351409	0.303316
HepG2	0.176324	0.474267	0.382081	0.313853
30%MIHA+70%HepG2	0.249103	0.621979	0.341281	0.207005
30%MIHA+70%HepG2	0.264297	0.615464	0.308190	0.199511
30%MIHA+70%HepG2	0.210983	0.603420	0.300284	0.215393
30%MIHA+70%HepG2	0.263542	0.613294	0.343714	0.188086
30%MIHA+70%HepG2	0.261471	0.658920	0.361432	0.192412
30%HepG2+70%MIHA	0.364191	0.475383	0.287530	0.158671
30%HepG2+70%MIHA	0.378171	0.491625	0.271249	0.137338
30%HepG2+70%MIHA	0.288872	0.573099	0.203290	0.159390
30%HepG2+70%MIHA	0.376126	0.568935	0.264440	0.127580
30%HepG2+70%MIHA	0.319665	0.458849	0.296234	0.127906
30%HepG2+70%MIHA	0.318898	0.480886	0.226299	0.158745
MIHA	0.572468	0.224048	0.118340	0.132908
MIHA	0.301483	0.452268	0.112565	0.095931
MIHA	0.647582	0.437728	0.134530	0.114868
MIHA	0.630724	1.059922	0.149758	0.111975
MIHA	0.660339	0.374699	0.133644	0.120560
MIHA	0.562350	0.141897	0.178396	0.067860

References

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3. J.-H. Tian, X.-Y. Hu, Z.-Y. Hu, H.-W. Tian, J.-J. Li, Y.-C. Pan, H.-B. Li and D.-S. Guo, *Nat. Commun.*, 2022, **13**, 4293.