Supporting Information

A single-molecule homogenous immunoassay by counting spatially "overlapping" two-color quantum dots with wide-field fluorescence microscopy

Xiaojun Liu[‡, a], Conghui Huang[‡,a], Chenghua Zong[‡, a], Aiye Liang[b], Zhangjian Wu[a], Yusu Zhang[a], Qingquan Zhang[a], Wenfeng Zhao[a], Hongwei Gai[*, a]
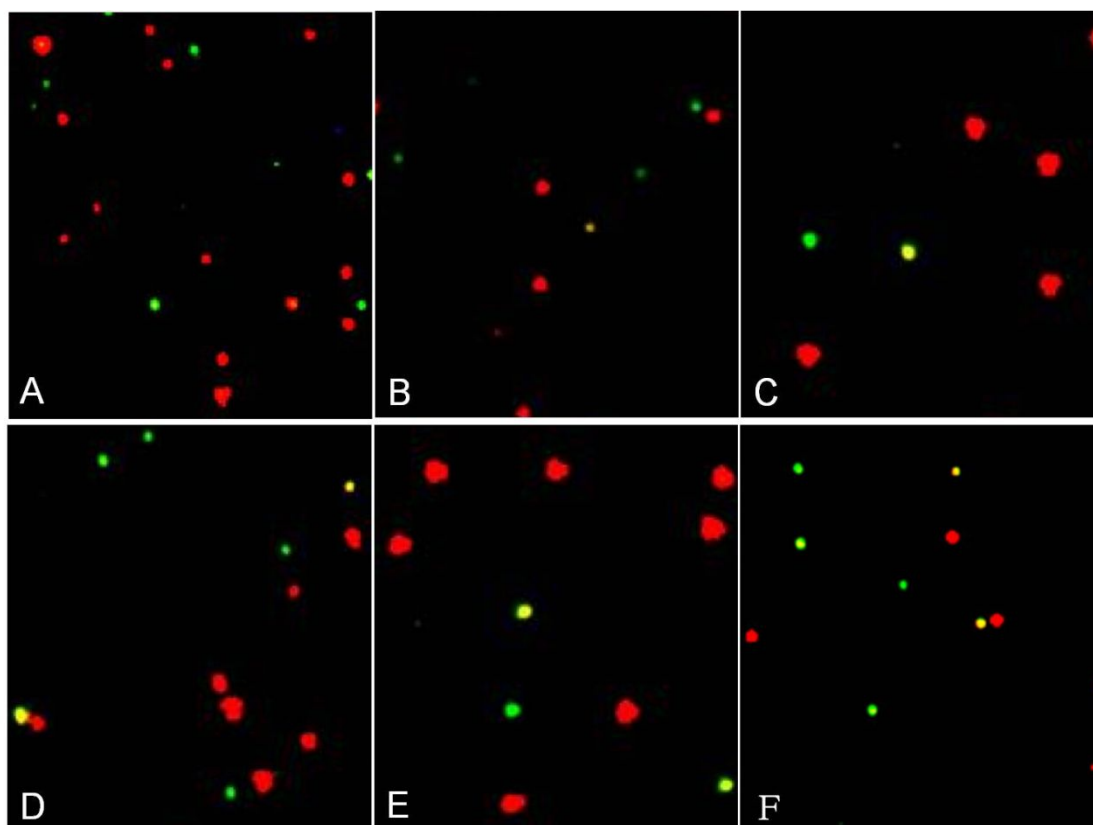
[a] School of Chemistry and Materials Science, Jiangsu Key Laboratory of Green Synthesis for Functional Materials, Jiangsu Normal University, Xuzhou, Jiangsu, 221116, China.

[b] Department of Physical Sciences, Charleston Southern University, Charleston, South Carolina, United States

Data processing

Homogenous immunoassay was done in triplicate. Randomly selected images from 3 glass slides were zoomed in with Image J, and the yellow and red dots from these images were manually counted. The counting stopped until the number of the red dots was over 100. Then the ratio of the yellow spots to the red spots (or the green spots), defined as the immunocomplex rate (IR), was calculated. The error bars referred to the standard error of the mean of the triplicate experiments.

With AFP as an example, the partial screenshots for counting and the statistics result of the triplicate experiments are given as following:
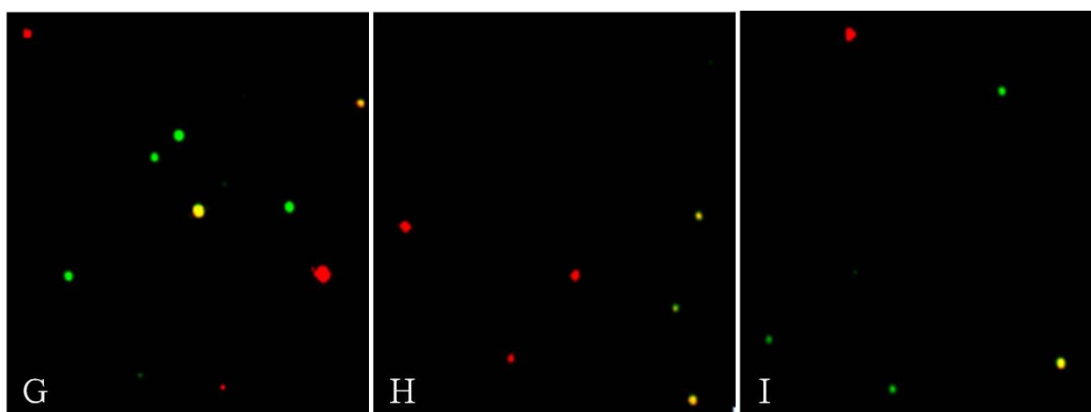
Figure S1. Representative images of anti-AFP A-QDs in the presence of AFP with different concentrations: (A) 0 pM, (B) 12.50 pM, (C) 25.00 pM, (D) 50.00 pM, (E) 75.00 pM, (F) 100.00 pM, (G) 150.00 pM, (H) 200.00 pM, (I) 300.00 pM.

Concentration (pM)	duplicate sample	Number of Red Dots	Number of Yellow Dots	IR (%)	Average of IR	RSD	IR substrated by blank
0.00	1	289	31	10.7			
	2	356	38	10.7	10.8	0.2	0.0
	3	301	33	11.0			
12.50	1	180	29	16.1			
	2	165	26	15.8	16.2	0.5	5.4
	3	155	26	16.8			
25.00	1	171	36	21.1			
	2	160	32	20.0	21.1	1.1	10.3
	3	153	34	22.2			
50.00	1	152	45	29.6			
	2	149	45	30.2	30.3	0.8	19.5
	3	154	48	31.2			
75.00	1	145	59	40.7			
	2	139	57	41.0	41.0	0.4	30.2
	3	140	58	41.4			
100.00	1	132	67	50.8			
	2	137	67	48.9	49.6	1.0	38.8
	3	149	73	49.0			
150.00	1	141	80	56.7			
	2	125	70	56.0	56.8	0.8	46.0
	3	139	80	57.6			
200.00	1	128	61	47.7			
	2	132	62	47.0	47.9	1.1	37.1
	3	108	53	49.1			
300.00	1	117	45	38.5			
	2	113	42	37.2	37.8	0.7	27.0
	3	130	49	37.7			
400.00	1	141	43	30.5			
	2	135	43	31.9	30.8	0.9	20.0
	3	143	43	30.1			
600.00	1	128	34	26.6			
	2	116	29	25.0	25.2	1.2	14.4
	3	149	36	24.2			
800.00	1	151	30	19.9			
	2	198	44	22.2	21.1	1.2	10.3
	3	174	37	21.3			

Table S1 Statistics result of AFP standard curve.

Concentration (pM)	triplicate sample	Number of Red Dots	Number of Yellow Dots	IR (%)	Average of IR	RSD	IR substrated by blank
	1	265	37	14.0			
0.00	2	322	46	14.3	14.1	0.2	0.0
	3	342	48	14.0			
	1	160	42	26.3			
10.42	2	175	45	25.7	26.3	0.6	12.2
	3	152	41	27.0			
	1	163	49	30.1			
20.83	2	160	47	29.4	29.4	0.7	15.3
	3	143	41	28.7			
	1	151	54	35.8			
41.67	2	147	50	34.0	35.1	1.0	21.0
	3	135	48	35.6			
	1	156	63	40.4			
62.50	2	135	52	38.5	39.4	0.9	25.3
	3	122	48	39.3			
	1	130	56	43.1			
83.33	2	141	63	44.7	44.1	0.9	30.0
	3	150	67	44.7			
	1	136	73	53.7			
125.00	2	138	76	55.1	54.5	0.7	40.4
	3	124	68	54.8			
	1	112	69	61.6			
166.67	2	120	75	62.5	61.7	0.8	47.6
	3	115	70	60.9			
	1	129	66	51.2			
250.00	2	137	72	52.6	52.2	0.9	38.1
	3	125	66	52.8			
	1	134	58	43.3			
333.33	2	119	52	43.7	44.3	1.3	30.2
	3	142	65	45.8			
	1	135	46	34.1			
500.00	2	130	44	33.8	34.2	0.5	20.1
	3	141	49	34.8			
	1	142	37	26.1			
666.67	2	157	41	26.1	25.6	0.8	11.5
	3	138	34	24.6			

Table S2 Statistics result of CEA standard curve.