Supplementary Information

Fluorescence array-based sensing of nitroaromatics using conjugated polyelectrolytes

Jiatao Wu,[a,b] Chunyan Tan,*[a,b] Zhifang Chen,[a,b] Yu Zong Chen,[c] Ying Tan,*[a,b] and Yuyang Jiang[b,d]

[a] *Department of Chemistry, Tsinghua University, Beijing 100084 P. R. China*

[b] *The Ministry-Province Jointly Constructed Base for State Key Lab- Shenzhen Key Lab of Chemical Biology, The Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055 P. R. China*

[c] *Shenzhen Kivita Innovative Drug Discovery Institute, Shenzhen, 518055 China*

[d] *Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Beijing 100084 P. R. China*

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1. Experimental details

1.1. Materials

Bis(triphenylphosphine) palladium chloride ($PdCl_2(PPh_3)_2$), tetrakis(triphenylphosphine) palladium ($Pd(PPh_3)_4$), and cuprous iodide (CuI) were purchased from Aladdin Chemical Co. (Shanghai, China) and used as received. Boron tribromide (BBr_3), tetrabutylammonium fluoride (TBAF), 1,6-dibromohexane, and 1,4-dimethoxybenzene were purchased from JK Chemical (Beijing, China) and used without further purification. 2,5-dihydroxybenzoic acid (DHB) and silver trifluoroacetate were purchased from Sigma-Aldrich (Shanghai, China) and used as received. 4-nitrobenzoic acid (C-1), 3-nitrobenzoic acid (C-2), 4-nitrophenol (C-3), 2-methyl-4,6-dinitrophenol (C-4), 2,4-dinitrophenol (C-5), picric acid (C-6), 4-nitrobenzenesulfonic acid (C-7), 2,4-dinitrobenzenesulfonic acid (C-8), 2,4,6-trinitrobenzensulfonic acid (C-9) were purchased from ENERGY CHEMICAL (Shanghai, China) and used without further purification. The water used in all experiments was prepared in a SG water purification system and displayed a resistivity of $\geq$ 18.2 $M\Omega\cdot cm^{-1}$.

1.2. General methods

^1H and ^1C NMR spectra were recorded on a Bruker 400 MHz spectrometer, and chemical shifts were reported in parts per million using tetramethylsilane (TMS) as internal reference. The MALDI experiments were conducted on a 4700 proteomics analyzer MALDI-TOF/TOF-MS (Applied Biosystems, Framingham, MA, USA), equipped with a 337 nm nitrogen laser. Fluorescence spectra were recorded on a SPEX Fluorolog-3-TCSPC spectrometer with 1 cm path length cuvette, while absorption spectra were recorded on a Beckman DU 800 spectrophotometer. Nitroaromatics (NACs) arrays were measured on 96-well plates (300 µL Corning) using Tecan M1000 Pro plate reader.

1.3. Mass spectrometry

For all of the MALDI experiments, DHB was used as the matrix (M) and silver trifluoroacetate was used as cationization reagent (CR). All MALDI mass spectra were collected using linear ion mode.

The polymer samples were prepared at a concentration of 5 mg/mL, matrix solutions were prepared at a concentration of 10 mg/mL and cationization reagent solutions were prepared at a concentration of 0.1 mol/L. These solutions were mixed in the given volume mixing ratio ($V_{(polymer)}$:$V_{(M)}$:$V_{(CR)}$=5:5:1). 1 μL of the solution was hand-spotted on a stainless steel target and allowed to dry by air. All spectra data were taken from signal averaging of 300 laser shots and further processed using Data Explorer 4.5 (Applied Biosystems).

1.4. Sensor array detection

Conjugated polyelectrolytes (CPEs) were used at a concentration of 10-100 μM in ultrapure water. Two different experiments were performed, the first in which the concentration of NACs was known, 1 μM for each nitroaromatic, and second, the solutions were normalized such that A_{300} = 0.05 and the concentrations varied. The samples were measured in 96 well polypropylene plates with an excitation at 420nm. Six wells of a standard 96 well microtiterplate were filled with a stock solutions of CPEs, which the ultraviolet absorption of CPEs were fixed at A_{420} = 0.1. The emission spectrum was measured at 500 nm with the first well loaded with only CPEs in water for a measurement of I_0. The percent quenching was calculated with the equation $(I_0$ - $I)/I_0 \times 100)$ % quenching, where I_0 is the fluorescence intensity without added NACs. Fluorescence data collection was carried out in six replicates and the data were subjected to linear discriminant analysis (LDA) using R software. Confidence ellipse was fitted by MATLAB 7.0 with 95% confidence interval.

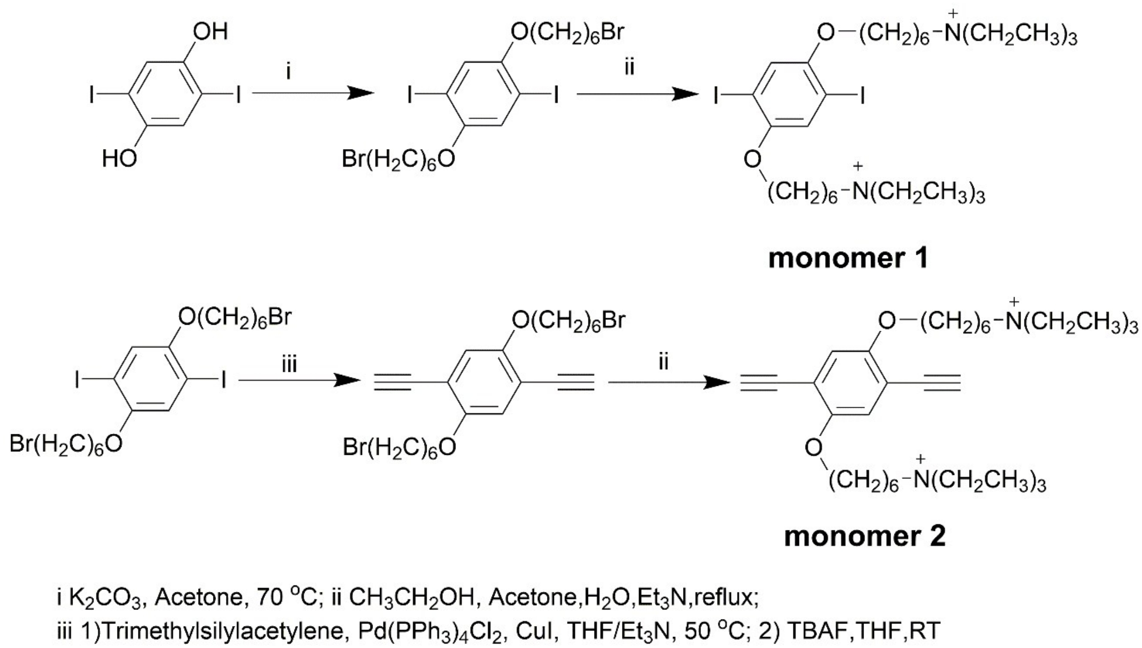
1.5. Unknown samples discrimination

Unknown samples were identified according to their canonical scores or their placement in this 2-D space. The array response to each unknown (A_{300}=0.05) was compared with the classification data, and unknowns were identified according to their placement in this 2-D space figure with a 95% confidence interval.

1.6. Data analysis method

Fluorescence intensity of each CPE-NAC solution was collected through plate reader. A training matrix (six CPEs × nine NACs × six replicates) was generated using I_0/I values for further computational analysis and were processed through LDA using R software (i3863.0.3). In the analysis, all of the variables were used in the complete mode, and the tolerance was set to 0.001. The raw fluorescence intensity patterns were transformed into canonical score patterns in which the within-class variance to between-class variance was minimized according to preassigned grouping. Canonical scores were calculated by LDA using R software for identification of the nine NACs.

2. CPEs synthesis



Scheme S1 Synthesis of monomer 1 and monomer 2

1,4-bis((6-bromohexyl)oxy)-2,5-diiodobenzene. A solution of $Br(CH_2)_6Br$ (1.464g,6.0 mmol) and potassium carbonate (1.38g,10.0 mmol) in 150 mL acetone fitted with a condenser were degassed with argon for 10 minutes. Then 362 mg of 2,5-diiodohydroquinone (1.0 mmol) dissolve in 50 mL acetone was added slowly under argon. Then the reaction mixture was stirred at reflux for 20 hr. After cooling to room temperature, remove potassium carbonate through filtration. The reaction solvent was removed in vacuo. Then the remainders were partitioned between water and CH_2Cl_2. The organic solution was dried by adding anhydrous sodium sulfate, and then the crude product was purified by purified by column chromatograph with a mixture of CH_2Cl_2/hexane (v/v = 25/1) and white solid was collected and dried(yield: 344 mg 50 %).^{1}H NMR ($CDCl_3$, δ_{ppm}): 7.17 (s, 2H), 3.94 (t, 4H), 3.43 (t, 4H), 1.91 (m, 4H), 1.81 (m, 12H), 1.53 (t, 8H).

1,4-bis((6-bromohexyl)oxy)-2,5-diethynylbenzene. 4-bis((6-bromohexyl)oxy)-2,5-diiodobenzene (688 mg, 1 mmol) was dissolved in 25 ml of dry THF/Et_3N (3/1, v/v) in a Schlenk flask and degassed with argon for 15 minutes. Then 21 mg of $Pd(PPh_3)_2Cl_2$ (30 μmol) and 12 mg of CuI (63 μmol) were added, followed with the addition of 0.7 ml of trimethylsilylacetylene (5 mmol). The reaction was stirred at 50 ^oC for 22 hr. After filtration through a bed of celite, the solvent was removed in vacuo. The crude product was used for next step without further purification. The crude product obtained as was dissolved in 10 ml of THF. Tetrabutylammonium fluoride (5 ml of a 1 M solution in THF) was added and the resulting mixture was stirred at room temperature for 2 hr. Then the solution was diluted with 20 ml of ethyl ether, poured into a seperatory funnel and washed with water (30 ml × 1). The organic layer was collected and the solvent was removed in vacuo. The crude product was purified by column chromatograph. A slight yellow solid was obtained (yield: 242 mg, 50 %). ^{1}H NMR ($CDCl_3$, δ_{ppm}): 6.95 (s, 2H), 3.68 (t, 4H), 3.42 (t, 4H), 3.33 (s, 2H), 1.89 (m, 4H), 1.81 (m, 4H), 1.52 (m, 8H).

Monomer 1. 1,4-bis((6-bromohexyl)oxy)-2,5-diiodobenzene (688 mg, 1 mmol) was suspended in 40% trimethylamine in water(20 mL), ethanol(30 mL) and acetone (30 mL). The suspension was heated to 130 ^oC for 24 hours. The solution was concentrated to yield a solid, which was recrystallized from ethanol/ether (yield: 700 mg, 96.4%). ^{1}H NMR (CH_3DO, δ_{ppm}): 7.01 (s, 2H), 3.98 (t, 4H), 3.32 (m, 12H), 3.17 (m, 4H), 1.91 (m, 4H), 1.81(m, 4H), 1.73 (m, 4H), 1.58(m, 4H) 1.39 (t, 18H).

Monomer 2. 1,4-bis((6-bromohexyl)oxy)-2,5-diethynylbenzene (484 mg, 1 mmol) was suspended in 40% trimethylamine in water(20 mL), ethanol(30 mL) and acetone (30 mL). The suspension was heated to 130 ^oC for 24 hours. The solution was concentrated to yield a solid, which was recrystallized from ethanol/ether (yield: 485 mg, 92.1%). ^{1}H NMR (CH_3DO, δ_{ppm}): 7.01 (s, 2H), 3.98 (t, 4H), 3.76(s, 2H), 3.32 (m, 12H), 3.20 (m, 4H), 1.81 (m, 4H), 1.70(m, 4H), 1.63 (m, 4H), 1.48(m, 4H) 1.29 (t, 18H).

CPEs synthesis. The backbone of P1 and P2 is synthesized via Sonogashira protocol.[1, 2] P3 was provided by Prof. Kirk S. Schanze in University of Florida.[3] The synthesis of P4, P5 and P6 were referred to the method previously reported.[4] The backbone of P1 and P2 is synthesized as follow. A solution of monomeric building blocks of diacetylenes (0.2 mmol) and monomeric building blocks of bis(iodobenzenes) (0.2 mmol) in 20 mL of dry THF/Et_3N (V/V = 2/1) fitted with a condenser were degassed for 30 minutes. Then 12 mg of $Pd(PPh_3)_4$ and 5.3 mg of CuI were added under argon protection. The reaction mixture was stirred at 50 ^oC for 24 hr. The obtained reaction solution was poured into 300 mL of ethyl ether, and the precipitation were further purified by two repeated cycles of dissolution in THF and precipitation into ethyl ether and light yellow solid was collected and dried.

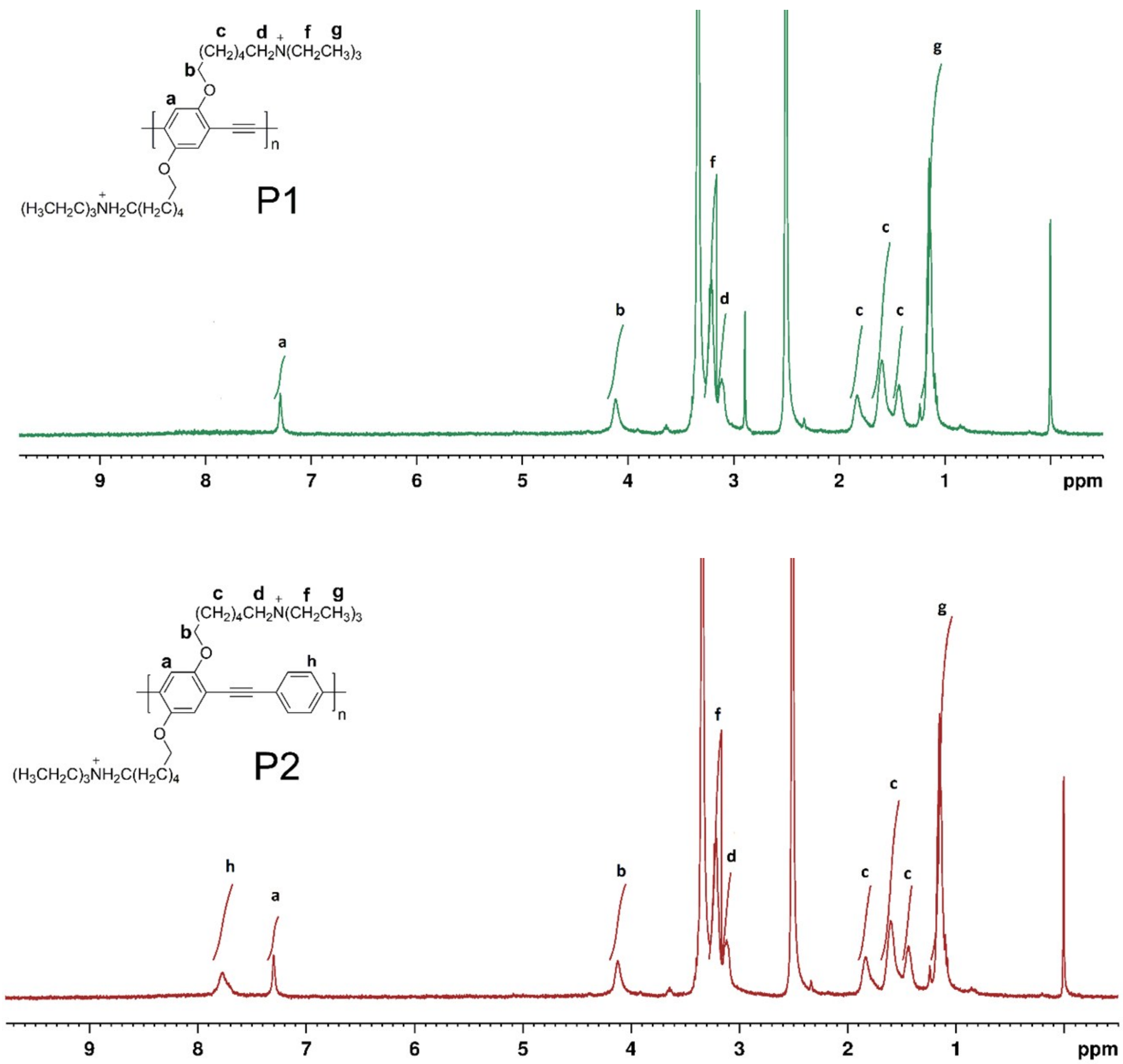


Fig. S1. ^{1}H NMR characterization of P1 and P2

3. Training matrix

Table S1. Training matrix of fluorescence response patterns obtained by six-CPE sensor array against NACs at 1 μM concentration.

	P1	P2	P3	P4	P5	P6
C-1	16.14149	0.410266	60.75646	-0.19711	12.2807	24.69136
C-1	15.51699	9.178514	60.8473	2.1682	30.07519	4.938272
C-1	14.07732	-7.12718	60.07928	-9.13272	30.07519	25.92593
C-1	13.06192	1.059059	57.61004	-6.11038	22.80702	-17.284
C-1	15.99847	3.186719	59.39384	6.30749	15.03759	-9.87654
C-1	19.30686	3.659002	54.11677	5.978975	31.82957	-17.284
C-2	41.73802	23.89904	78.79274	-1.89107	34.87179	34.14634
C-2	41.95335	29.12499	83.75178	4.387292	51.79487	29.26829
C-2	35.52422	15.73546	85.2208	-4.23601	52.05128	39.02439
C-2	40.44604	22.47997	84.20584	0.226929	38.20513	13.41463
C-2	37.85696	19.96125	83.2265	2.950076	34.10256	23.17073
C-2	40.29736	23.16594	83.99217	1.2548	51.28205	27.80488
C-3	0.325309	1.244795	14.74688	-2.77008	16.06218	1.538462
C-3	3.948751	3.528411	16.90163	-1.45429	1.036269	-16.9231
C-3	7.542165	4.858281	7.505607	-5.60942	-12.9534	-60
C-3	7.276913	1.741817	9.836591	-6.30194	4.663212	-18.4615
C-3	2.342225	3.680652	11.13425	-9.34903	-4.40415	-46.1538
C-3	-1.55147	3.250795	7.777956	-9.8338	15.54404	-15.3846
C-4	40.68354	45.48809	82.25708	10.52632	35.43046	43.36283
C-4	38.77676	48.15489	81.87633	16.06648	23.84106	16.81416
C-4	36.50758	42.46703	81.60219	16.34349	18.21192	53.09735
C-4	35.7326	45.02067	80.52087	29.08587	9.933775	28.17
C-4	37.90143	45.16335	80.96253	24.37673	18.87417	22.12389
C-4	40.20963	45.24208	77.15504	32.68698	6.291391	33.62832
C-5	34.87454	37.12966	75.70617	12.38671	43.40528	-4.34783
C-5	37.83457	36.87534	75.73567	17.46224	36.21103	-18.8406
C-5	41.65428	41.04183	71.31794	15.34743	53.23741	-34.7826
C-5	39.79554	38.67249	71.06719	16.97885	39.08873	-17.3913
C-5	36.99814	40.85534	75.6693	17.82477	51.31894	-14.4928
C-5	33.88011	39.06243	74.84328	18.67069	23.98082	-10.1449
C-6	50.10756	44.73672	69.45859	33.83743	33.50384	28.07018
C-6	47.44168	49.13181	75.35846	39.69754	50.12788	45.02924
C-6	51.73577	43.62148	76.00094	34.73535	36.82864	36.54971
C-6	50.42393	47.05661	75.71104	38.32703	29.92327	20.46784
C-6	50.99338	47.59305	73.85411	42.91115	44.75703	28.07018
C-6	51.24225	47.56953	75.14691	40.59546	46.03581	28.07018
C-7	17.87052	11.52639	64.1996	3.148528	39.38619	16.4557
C-7	20.14988	13.53305	64.3992	7.665982	31.71355	17.72152
C-7	20.86803	12.52496	58.38723	4.791239	35.54987	29.11392
C-7	16.26769	13.12411	56.84631	2.464066	36.31714	16.4557
C-7	11.69858	15.84403	61.58084	3.627652	39.8977	26.58228

C-7	14.10283	12.30147	61.89222	10.13005	25.57545	13.92405
C-8	32.37006	19.34727	67.00985	31.27985	37.80161	-11.6402
C-8	35.596	21.2372	67.61721	32.85578	30.8311	-31.746
C-8	38.21938	24.12116	63.45456	33.09456	15.81769	-9.52381
C-8	36.61666	23.75427	60.95104	33.33333	38.3378	-20.6349
C-8	33.53828	24.73976	66.11362	30.22923	31.36729	-41.2698
C-8	28.87359	21.86433	65.34331	30.99331	19.57105	-22.963
C-9	76.10861	66.21073	93.45402	40.78254	70.78086	30
C-9	76.67056	65.16686	94.36342	43.34086	70.27708	-13.3333
C-9	77.47733	66.56039	92.13281	39.50339	69.01763	28.33333
C-9	77.00996	65.88163	91.75532	45.89917	70.27708	33.33333
C-9	75.91387	64.90461	93.80577	42.51317	75.81864	26.66667
C-9	75.29071	65.37769	93.96877	46.87735	64.98741	-6.66667

Table S2. Training matrix of fluorescence response patterns obtained by six-PPE sensor array (A_{420}=0.1) against nine NACs (A_{300}=0.05)

	P1	P2	P3	P4	P5	P6
C-1	46.91355	20.14826	88.68053	16.99127	74.9643	47.43548
C-1	47.60584	20.47008	91.69224	1.61182	73.78886	46.84744
C-1	48.81734	19.77943	93.1299	9.301545	80.18236	48.6769
C-1	44.75976	18.78141	93.49358	3.626595	71.92134	51.06174
C-1	46.54817	19.43591	92.85146	4.231028	75.85411	41.62039
C-1	49.69369	24.65015	92.7094	11.98791	77.47995	47.04345
C-2	72.96395	48.38693	95.59267	39.06916	80.50327	59.09456
C-2	73.95948	47.94212	95.12436	35.39823	77.0307	59.25529
C-2	73.9827	49.24587	92.72037	26.58145	78.75189	55.42459
C-2	73.41383	48.98512	92.39255	42.74009	79.39607	50.12055
C-2	73.28612	47.70694	93.93277	35.2999	76.69854	54.43343
C-2	72.51988	46.77642	93.75585	33.56277	80.5536	53.79052
C-3	44.80615	36.78269	77.87731	12.14393	48.13953	18.4257
C-3	42.16322	37.83284	82.18941	12.27511	45.41004	18.95517
C-3	41.0801	35.77581	82.89368	9.557721	48.31089	15.81363
C-3	40.59134	35.28024	83.76475	13.71814	44.23501	16.3431
C-3	40.75426	36.39725	81.36159	12.25637	46.29131	17.3844
C-3	42.03047	40.22812	81.76932	13.56822	15.49572	11.01306
C-4	93.10798	86.32544	98.71495	69.65075	90.744	67.98277
C-4	93.28552	86.5391	98.7466	73.38638	88.45372	64.71644
C-4	93.38849	86.64831	98.41109	70.55703	87.46443	61.37832
C-4	93.37429	87.01391	98.39843	66.62246	88.44017	54.66619
C-4	93.44175	86.65306	98.59467	73.34218	86.32606	60.58866
C-4	93.40269	87.30355	98.63265	68.08134	90.82532	59.58363
C-5	91.31916	85.31034	98.20271	67.5162	92.36682	63.44302
C-5	91.22377	85.31969	98.6256	62.03024	90.67055	62.80096
C-5	90.76043	85.03459	98.71018	66.69546	93.37397	62.43981

C-5	90.83197	85.09534	98.72427	65.78834	91.28015	66.53291
C-5	90.9955	85.69359	98.67494	64.44924	91.41267	54.69502
C-5	91.48269	86.47878	98.57626	63.84449	69.53353	55.25682
C-6	94.94334	93.52408	99.37436	84.4883	95.48328	80.55774
C-6	94.76766	93.17597	99.35981	84.71776	93.79774	76.9835
C-6	94.60577	93.10443	99.35254	85.45204	95.36476	80.63629
C-6	94.6471	92.70386	99.33799	85.86508	94.46932	80.71485
C-6	94.778	93.29041	99.36709	86.00275	95.27258	79.02592
C-6	94.98123	93.83882	99.35254	85.91097	88.72794	77.76905
C-7	71.25449	55.64494	95.45531	37.83098	85.70931	73.80451
C-7	71.84945	55.53899	95.00252	36.27131	80.43882	73.59398
C-7	72.12306	57.42652	91.59819	27.20348	81.42559	71.12782
C-7	71.67446	57.98768	91.26279	37.21436	83.07407	66.16541
C-7	71.24177	58.70973	93.1634	25.2811	78.46529	68.66165
C-7	70.92997	62.66138	92.64911	27.5662	84.32784	64.27068
C-8	87.09509	78.67633	98.29909	43.51554	90.70468	89.39176
C-8	87.29429	78.20638	98.18312	65.05895	91.73621	88.38282
C-8	87.29429	77.8226	96.59819	61.1647	91.87529	87.66215
C-8	86.87138	78.41394	96.3552	58.30654	89.01252	84.11646
C-8	87.11961	78.03015	97.38237	66.3094	91.73621	85.47132
C-8	86.66605	77.86959	97.28297	56.66309	91.5044	84.3759
C-9	98.97053	99.41635	99.83441	93.35562	96.49972	91.96694
C-9	98.94878	99.4307	99.83441	94.19139	96.40288	92.49587
C-9	99.02128	99.44984	99.84714	94.31676	96.48589	91.63636
C-9	99.05028	99.46419	99.84077	93.85708	96.52739	90.87603
C-9	99.05753	99.44506	99.82804	94.44212	95.91865	91.33884
C-9	99.1844	99.47376	99.84077	94.5257	96.59657	90.90909

Table S3. Training matrix of fluorescence response patterns obtained by six-PPE sensor array (A_{420}=0.1) against three unknown samples (A_{300}=0.05).

	P1	**P2**	**P3**	**P4**	**P5**	**P6**
A	98.80454	99.40763	99.81507	96.16766	97.31016	91.76265
B	42.05178	30.76349	74.59006	7.225549	46.76185	-2.75742
C	95.18986	92.38701	99.38355	83.47305	93.87544	73.71728
C-1	46.91355	20.14826	88.68053	16.99127	74.9643	47.43548
C-1	47.60584	20.47008	91.69224	1.61182	73.78886	46.84744
C-1	48.81734	19.77943	93.1299	9.301545	80.18236	48.6769
C-1	44.75976	18.78141	93.49358	3.626595	71.92134	51.06174
C-1	46.54817	19.43591	92.85146	4.231028	75.85411	41.62039
C-1	49.69369	24.65015	92.7094	11.98791	77.47995	47.04345
C-2	72.96395	48.38693	95.59267	39.06916	80.50327	59.09456
C-2	73.95948	47.94212	95.12436	35.39823	77.0307	59.25529
C-2	73.9827	49.24587	92.72037	26.58145	78.75189	55.42459

C-2	73.41383	48.98512	92.39255	42.74009	79.39607	50.12055
C-2	73.28612	47.70694	93.93277	35.2999	76.69854	54.43343
C-2	72.51988	46.77642	93.75585	33.56277	80.5536	53.79052
C-3	44.80615	36.78269	77.87731	12.14393	48.13953	18.4257
C-3	42.16322	37.83284	82.18941	12.27511	45.41004	18.95517
C-3	41.0801	35.77581	82.89368	9.557721	48.31089	15.81363
C-3	40.59134	35.28024	83.76475	13.71814	44.23501	16.3431
C-3	40.75426	36.39725	81.36159	12.25637	46.29131	17.3844
C-3	42.03047	40.22812	81.76932	13.56822	15.49572	11.01306
C-4	93.10798	86.32544	98.71495	69.65075	90.744	67.98277
C-4	93.28552	86.5391	98.7466	73.38638	88.45372	64.71644
C-4	93.38849	86.64831	98.41109	70.55703	87.46443	61.37832
C-4	93.37429	87.01391	98.39843	66.62246	88.44017	54.66619
C-4	93.44175	86.65306	98.59467	73.34218	86.32606	60.58866
C-4	93.40269	87.30355	98.63265	68.08134	90.82532	59.58363
C-5	91.31916	85.31034	98.20271	67.5162	92.36682	63.44302
C-5	91.22377	85.31969	98.6256	62.03024	90.67055	62.80096
C-5	90.76043	85.03459	98.71018	66.69546	93.37397	62.43981
C-5	90.83197	85.09534	98.72427	65.78834	91.28015	66.53291
C-5	90.9955	85.69359	98.67494	64.44924	91.41267	54.69502
C-5	91.48269	86.47878	98.57626	63.84449	69.53353	55.25682
C-6	94.94334	93.52408	99.37436	84.4883	95.48328	80.55774
C-6	94.76766	93.17597	99.35981	84.71776	93.79774	76.9835
C-6	94.60577	93.10443	99.35254	85.45204	95.36476	80.63629
C-6	94.6471	92.70386	99.33799	85.86508	94.46932	80.71485
C-6	94.778	93.29041	99.36709	86.00275	95.27258	79.02592
C-6	94.98123	93.83882	99.35254	85.91097	88.72794	77.76905
C-7	71.25449	55.64494	95.45531	37.83098	85.70931	73.80451
C-7	71.84945	55.53899	95.00252	36.27131	80.43882	73.59398
C-7	72.12306	57.42652	91.59819	27.20348	81.42559	71.12782
C-7	71.67446	57.98768	91.26279	37.21436	83.07407	66.16541
C-7	71.24177	58.70973	93.1634	25.2811	78.46529	68.66165
C-7	70.92997	62.66138	92.64911	27.5662	84.32784	64.27068
C-8	87.09509	78.67633	98.29909	43.51554	90.70468	89.39176
C-8	87.29429	78.20638	98.18312	65.05895	91.73621	88.38282
C-8	87.29429	77.8226	96.59819	61.1647	91.87529	87.66215
C-8	86.87138	78.41394	96.3552	58.30654	89.01252	84.11646
C-8	87.11961	78.03015	97.38237	66.3094	91.73621	85.47132
C-8	86.66605	77.86959	97.28297	56.66309	91.5044	84.3759
C-9	98.97053	99.41635	99.83441	93.35562	96.49972	91.96694
C-9	98.94878	99.4307	99.83441	94.19139	96.40288	92.49587

C-9	99.02128	99.44984	99.84714	94.31676	96.48589	91.63636
C-9	99.05028	99.46419	99.84077	93.85708	96.52739	90.87603
C-9	99.05753	99.44506	99.82804	94.44212	95.91865	91.33884
C-9	99.1844	99.47376	99.84077	94.5257	96.59657	90.90909

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