

Bioinspired synergy sensor chip of photonic crystals-graphene oxide for **multiamines** recognition

Wanjie Ren^{1,2}, Meng Qin¹, Xiaotian Hu^{1,2}, Fengyu Li^{1,*}, Yuanfeng Wang³, Yu Huang¹, Meng Su^{1,2}, Wenbo Li^{1,2}, Xin Qian^{1,2}, Kang-lai Tang⁴ and Yanlin Song¹

¹Key Laboratory of Green Printing, Institute of Chemistry, Chinese Academy of Sciences (ICCAS), Beijing Engineering Research Center of Nanomaterials for Green Printing Technology, Beijing National Laboratory for Molecular Sciences (BNLMS), Beijing 100190, China

²University of Chinese Academy of Sciences, Beijing 100049, China

³Key Laboratory of Evidence Science, China University of Political Science and Law, Beijing, 100088, China

⁴Sports Medicine Center, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
E-mail: forrest@iccas.ac.cn

General

Methylamine, β -phenylethylamine, 1,5-Diaminopentane, Epinephrine Bitartrate and Spermidine were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD. Ethylamine, Dopamine hydrochloride, Norepinephrine, Histamine, Serotonin, Tyramine and Spermine were purchased from J&K Scientific Ltd. The artificial urine (pH 5.1) was purchased from Dongguan City Xinheng Technology Co. LTD. Drug amines (stock solution, 100 $\mu\text{g/mL}$) were supplied by criminal evidence samples from evidence laboratory with legitimate sources. The stock solution of drug amines was mixed into the artificial urine to prepare the drug analysis samples. All reagents were used as received. PBS (pH 7.4) was used as solvent when there no special indications. Scanning Electron Microscope (SEM) images were taken by scanning electron microscope (JEOL GmbH, JSM-7500F, Japan). Fluorescence measurements were performed by Hitachi F-4500 Fluorescence Spectrophotometer. The reflection spectrums were measured by angle-resolved fiber optical spectrometer (R1 series, shanghai Ideaoptics Instrument Co., Ltd). Photographs were taken by camera 60D (Canon Co., Ltd). Raman measurements were performed by confocal laser Raman spectrometer (HORIBA, LabRAM HR Evolution, France) with a laser excitation wavelength of 532 nm. UV-vis spectra were performed by Ocean Optic HR 4000 fiberoptics UV-vis spectrometer. Fluorescent images were taken by ChampChemi Professional + Image station and analyzed by software Lane 1D v4.0. (ChampChemi, Inc., China). The quantification of the images was carried out by NIH ImageJ.

Synthesis of colloidal particles and graphene oxide (GO)

The poly(styrene-methyl methacrylate-acrylic acid) [poly(St-MMA-AA)] colloidal particles were synthesized by one-step emulsion polymerization.¹

GO was synthesized using our previous method.² The graphite flakes (3 g) were firstly preoxide by concentrated H_2SO_4 (40 mL), $\text{K}_2\text{S}_2\text{O}_8$ (2.5 g) and P_2O_5 (2.5 g) at 80°C for 5 h. The mixture was cooled to room temperature and diluted with deionized water (1 L). And then it was vacuum filtered and washed with deionized water (1 L) to remove the acid. The solid was dried at room temperature for 24 h. After preoxide the graphite, concentrated H_2SO_4 (120 mL), KMnO_4 (9 g) and H_2O_2 (30%, 15 mL) were used to further oxide the graphite, and HCl (10%) solution was used to remove the residual metal oxides, followed by deionized water to remove the acid. GO aqueous dispersion was finally obtained after gentle stirring.

Determination of fluorescent molecule concentrations

To determine the concentration of fluorescent molecules used in the sensing platform, we carried out the quenching experiments with the amount of GO (250 $\mu\text{g/mL}$) constant (Fig. S4). The concentration of the fluorescent molecules was

determined when the fluorescent intensity was quenched about 50%. The concentration of R6G, RB and AO were about 48, 75 and 87 μM respectively.

Preparation of the PCs microplate and fabrication of the sensing platform

To achieve the PCs microplate, the microplate was first treated by air-plasma (80 w, 200 s) to get the hydrophilic properties, and then three different poly(styrene/methyl methacrylate/acrylic acid) [poly(St-MMA-AA)] latex particles with diameters of about 200, 230 and 250 nm were selectively added into the holes (1.5% w/w, 50 μL) and assembled in the constant temperature and humidity test chamber (60°C temperature, 60% relative humidity) for 24 h.

After got the PCs microplate, 25 μL GO solutions (5 mg/mL) were dropped into the holes and freeze-dried (Fig. 1a: top). Then the solution of fluorescent molecules (the volume is 25 μL , the concentration of R6G, RB and AO are about 48, 75 and 87 μM respectively) was spotted onto the GO aerogel layer respectively (AO, R6G and RB were separately added when the PCs is PC₁, PC₂ and PC₃ respectively) and freeze-dried again to keep the structure of the GO aerogel.

Spectroscopic characterization of GO aerogel complexes

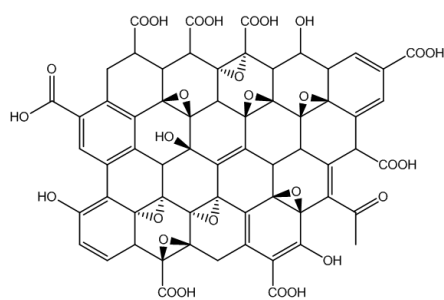
UV-vis spectra and Raman spectra was used to characterize GO aerogel complexes. The fluorophore solution was first spotted onto GO aerogel and then freeze-dried to keep the structure of GO aerogel. The GO aerogel complexes were then grounded into powers for Raman testing, and the powers were dissolved into water to proceed with UV-vis testing.

Amines identification by the sensing platform

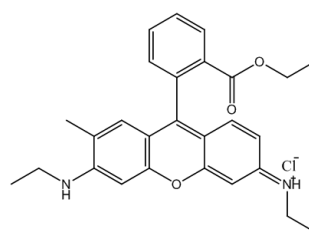
The integrated sensor with different fluorophores (AO, R6G and RB) and PCs (PC₁, PC₂ and PC₃) were used to the multi-analyte testing, the aqueous solutions (500 μL) of 10 biogenic amines and 7 drug amines were added into the sensing platform respectively. Fluorescence responses were recorded in 3 channels (CH 1: 535 nm, CH 2: 570 nm, CH 3: 605 nm) by Fluorescence scanner (ChampChemi Professional + Image station). The background deducting operation means that the values of control sample were subtracted from the values of those with amine compounds before the analysis.

References

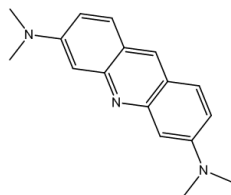
- (1) Wang, J. X.; Wen, Y. Q.; Ge, H. L.; Sun, Z. W.; Zheng, Y. M.; Song, Y. L.; Jiang, L., *Macromol. Chem. Phys.* **2006**, 207, 596-604.
- (2) Li, W.; Li, F.; Li, H.; Su, M.; Gao, M.; Li, Y.; Su, D.; Zhang, X.; Song, Y., *ACS Appl. Mater. Inter.* **2016**, 8, 12369-12376.



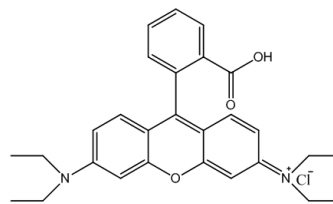
GO



R6G



AO



RB

Figure S1 Structures of graphene oxide and fluorescent molecules.

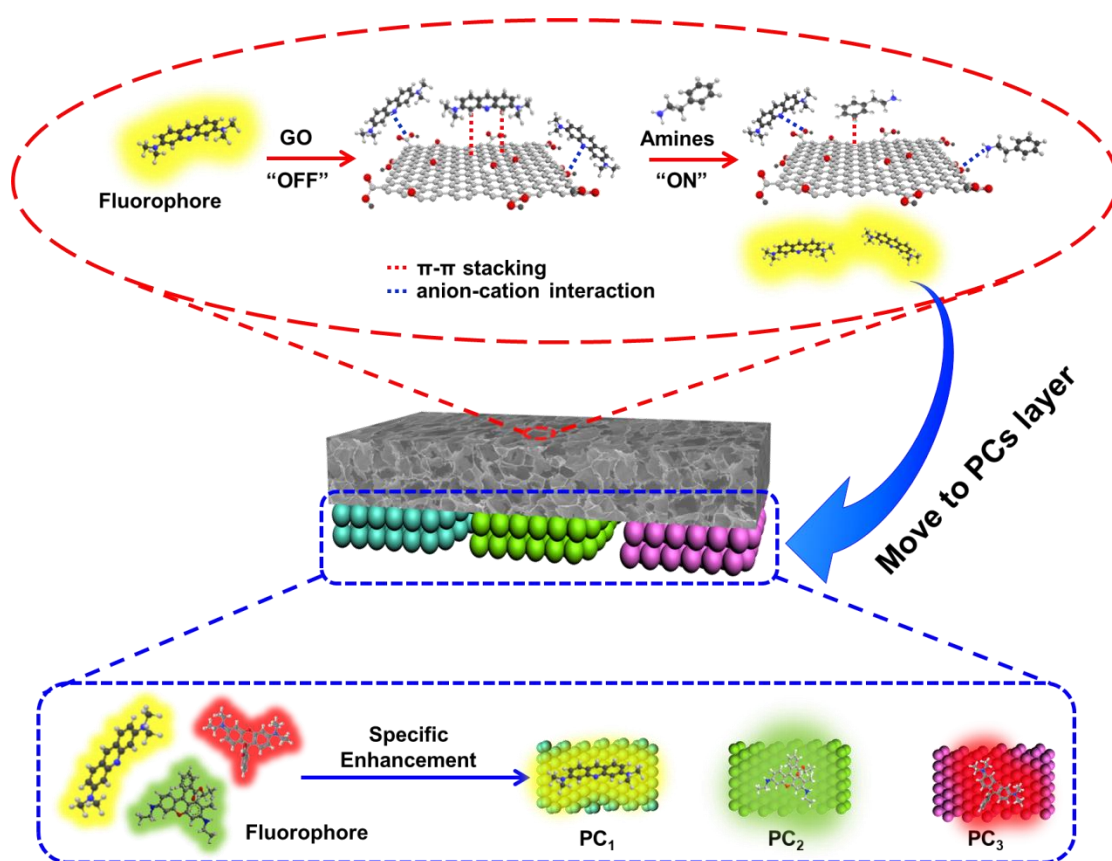


Figure S2 Sensing principle of the integrated sensor.

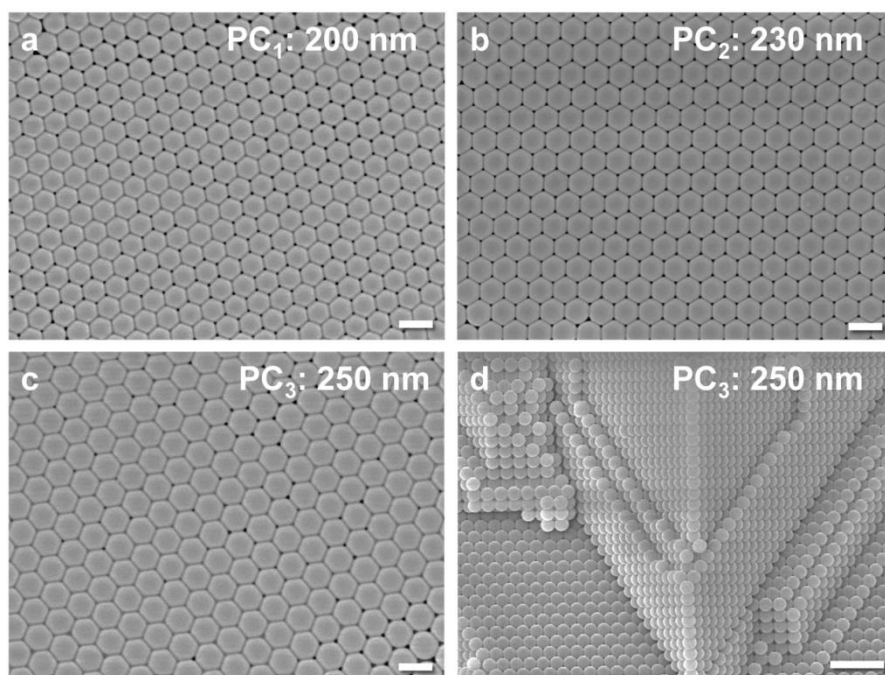


Figure S3 Top-view and side-view SEM images of PCs in the photonic crystal microplate assembled from various colloidal particles (The scale bar in **a**, **b** and **c** is 300 nm; The scale bar in **d** is 1 μm. **d** is the side-view of **c**).

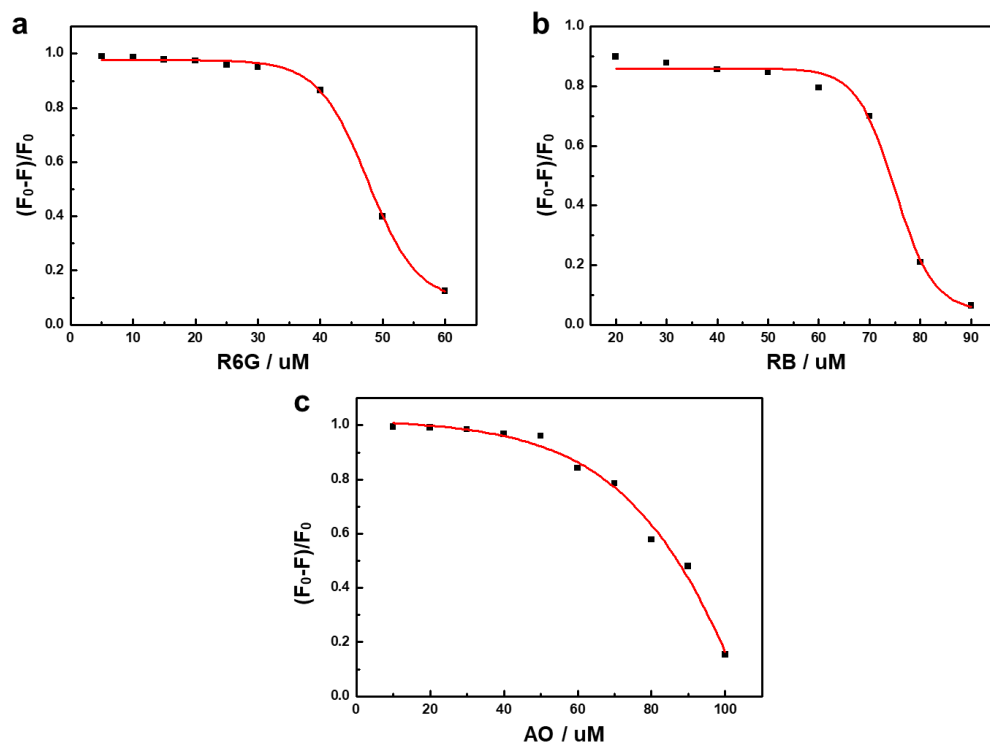


Fig. S4 Fluorescence-quenching curves used for determination of the concentration of fluorescent molecules. (a) Fluorescence-quenching curves used for determination of the concentration of R6G; (b) Fluorescence-quenching curves used for determination of the concentration of RB; (c) Fluorescence-quenching curves used for determination of the concentration of AO.

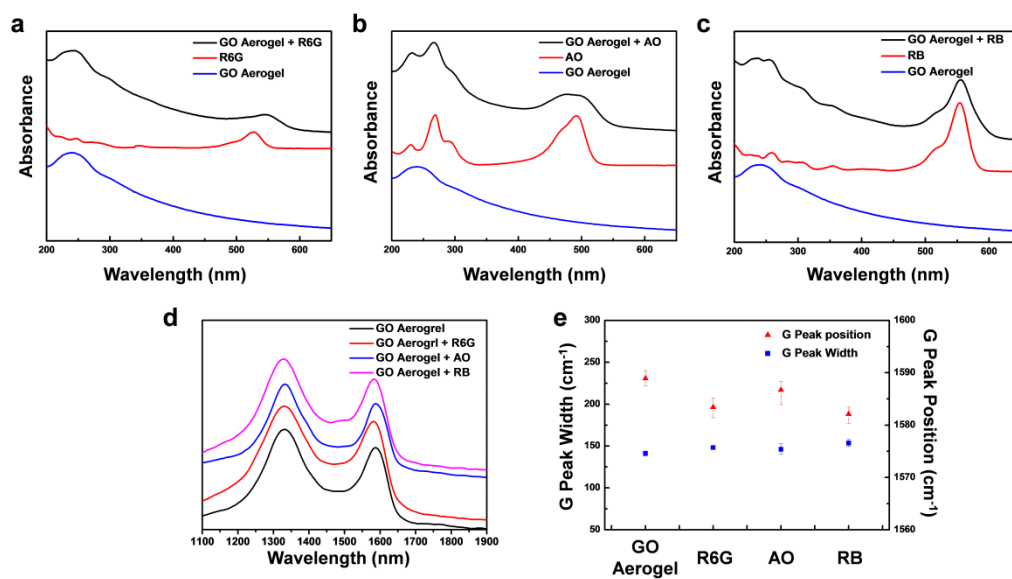


Figure S5 Spectroscopic characterization of GO aerogel complexes. (a)-(c) UV-vis spectra of different fluorescent molecules and GO aerogel complex. (d) Raman D and G bands from different GO aerogel complexes. (e) The change of G band width and position upon complexation with different fluorescent molecules.

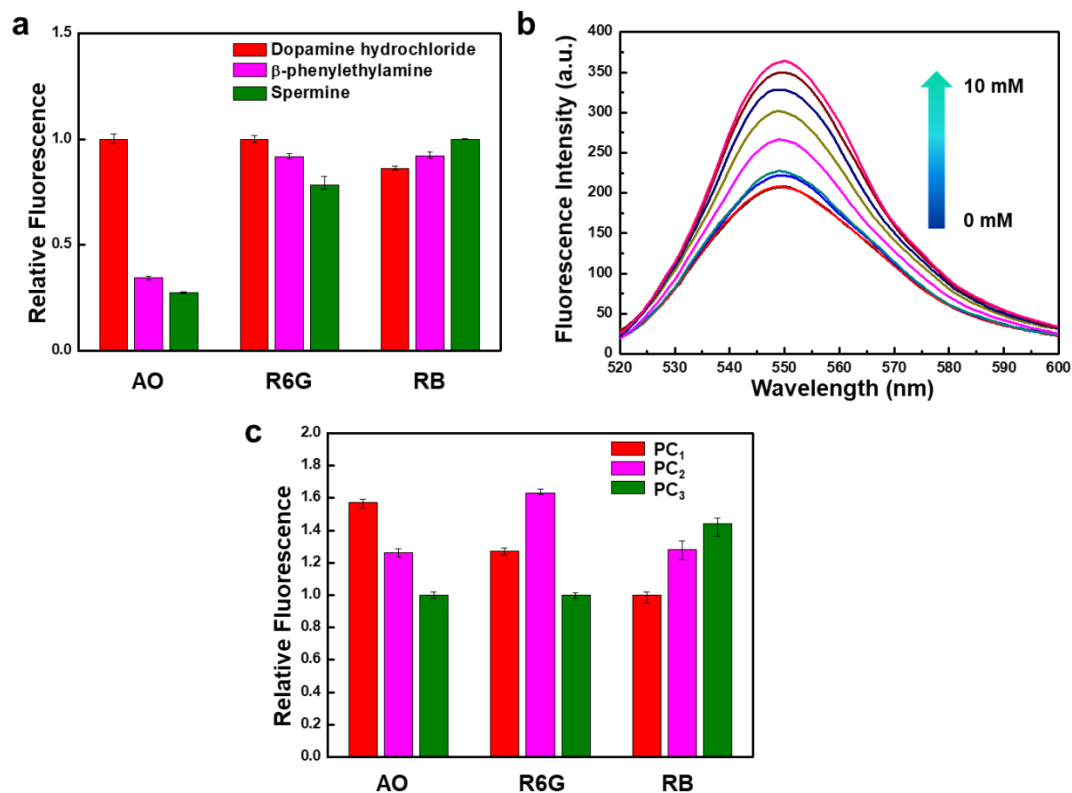


Figure S6 (a) Column diagram of the relative fluorescence after addition of different amines when the fluorophore is AO, R6G and RB respectively (The concentration of the solution is 2 mM). (b) Fluorescence change of the sensor without PCs (the fluorophore is R6G) with the concentration increase of dopamine (0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 mM). (c) Fluorescent intensity amplification by different PCs without any amines present (The concentration of AO, R6G and RB are 6, 0.8 and 3 μ M, respectively).

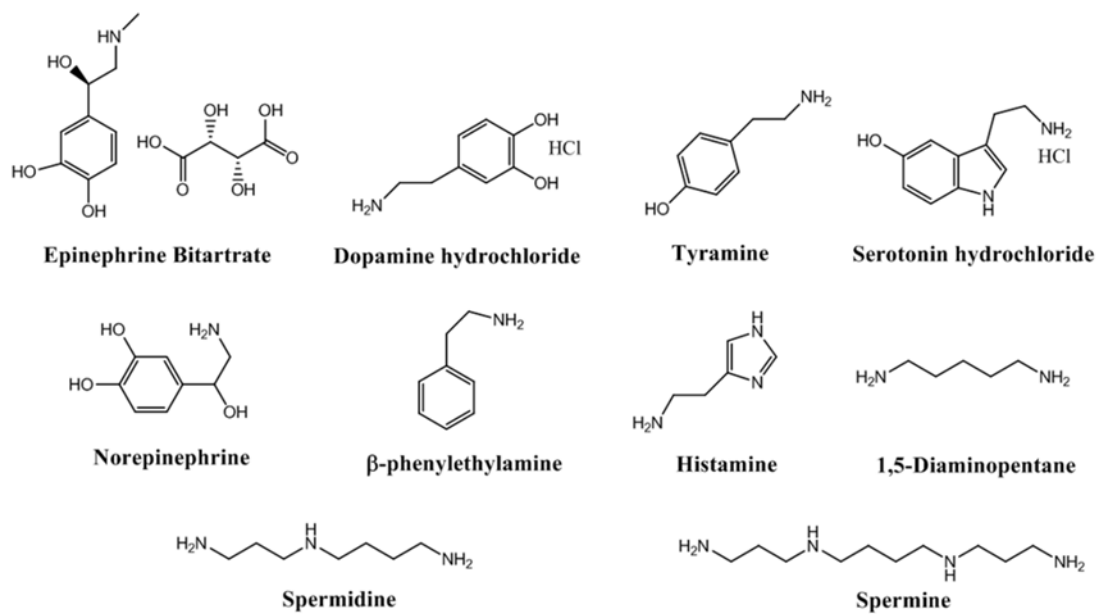


Figure S7 Structures of biogenic amines.

Table S1 Raw fluorescence responses in three channels of the bare GO sensor to biogenic amines.

	GO								
	AO			R6G			RB		
	CH ₁	CH ₂	CH ₃	CH ₁	CH ₂	CH ₃	CH ₁	CH ₂	CH ₃
PEA	11285	4006	3177	16739	5706	4254	10684	18972	15440
	11300	3785	3231	16374	5821	4526	8677	18658	15127
	11268	3790	3036	16791	5664	4909	9960	17352	14016
	11445	3583	3155	16991	5821	4356	10145	17173	13788
	11780	4186	3104	16669	5941	4414	11155	17966	14440
	11745	4130	3063	16781	5905	4833	9582	18516	14888
	11829	4030	3014	16056	5801	4800	8951	19080	15391
DA	11711	4042	3096	16758	5879	4632	9454	17512	14275
	12335	3837	2932	16069	5859	4042	10177	15937	12897
	12235	3655	2737	16190	5716	4770	9320	16240	13123
	12332	3457	2896	16553	5904	4370	9114	16349	13211
	12410	3892	2882	16222	5788	4604	9070	17035	13715
	12023	3960	2586	15720	5630	4910	9077	17527	14071
	11278	3896	2820	15246	5459	4515	6781	17617	14214
NP	4400	2168	1217	10226	1796	1411	3807	11455	5744
	4411	2035	1189	10079	2152	1559	3940	10707	5343
	4462	1987	1149	10215	2294	1496	4022	10249	5078
	4601	1921	1279	10139	2040	1785	3694	10634	5271
	4688	2310	1249	10361	2453	1724	4070	10674	5271
	4583	2206	1257	10280	2494	1592	3486	10899	5400
	4332	2150	1321	9914	2447	1694	3371	10907	5369
HA	12673	4545	3560	19055	6700	5370	10927	19410	15772
	11940	4280	3412	18510	6398	5321	11344	18619	15075
	11779	4119	3280	17805	5826	5238	11180	18127	14649
	12216	3958	3589	17735	5962	5051	10273	18418	14828
	12751	4487	3488	16605	5973	4451	9411	19318	15581
	12982	4508	3376	16599	5983	5205	9668	19600	15751
	12191	4369	3480	14986	5497	4682	8888	20177	16266
5-HT	13610	5077	3534	14061	3478	2980	4167	10844	6400
	13623	4942	3578	13689	3218	2857	4313	10841	6362
	13225	4974	3419	12624	2968	2294	3682	10700	6251
	13591	4736	3701	12496	3034	2461	3786	10473	6111
	14222	5223	3795	12333	3043	2801	4103	10472	6078
	14008	5275	3602	12304	3211	2736	4045	10842	6290
	13358	5331	4035	11244	2576	2835	3409	10959	6360
TA	10548	3795	2972	19035	6739	4606	9870	18011	14704
	11353	3786	2864	18822	6499	5329	10181	18185	14773
	11301	3680	2919	18448	6238	4771	8899	17743	14356
	11580	3707	3037	17831	5495	4736	8689	17312	13951

	11637	3982	2920	15287	5287	4770	9523	17949	14563
	11699	3956	2957	15459	5340	4599	8728	17634	14175
	10888	3812	3548	14549	4905	4293	8509	18984	15325
PDA	16317	6475	5526	21402	7648	5905	11361	18606	15166
	18023	6406	5767	21231	7266	6054	10399	18830	15290
	17711	6535	5685	20501	6871	5894	10169	18885	15287
	17661	6469	5721	19356	6376	4816	9234	18855	15272
	17564	6686	5586	17167	6297	5695	10395	18988	15305
	16942	6620	5125	17863	6471	5222	9490	18959	15295
	16341	6416	5444	16238	5827	4916	8650	20228	16291
EB	14067	4883	3893	25942	8884	7028	10892	14647	12284
	15633	4893	3802	25455	8833	7133	9796	16001	13399
	15781	4769	4062	24716	7532	7103	10005	16457	13804
	16109	5007	4144	22863	6996	6315	9064	16619	13922
	15918	5167	4126	17171	6588	6861	8852	16468	13791
	15055	5172	4170	17797	6739	6061	9449	17196	14347
	13966	5116	4335	17095	6539	4494	9604	17706	14756
SID	19468	8330	7213	24036	7995	6430	10092	17093	13984
	19117	8133	7453	23320	7433	5994	9616	16769	13630
	19449	8314	7110	21779	6623	6014	9458	18185	14858
	19763	8008	7139	19412	6646	4766	8888	18463	15033
	18934	8200	7085	16971	6297	5491	9323	19261	15688
	17702	8075	6822	17503	6399	4755	8404	18981	15408
	17031	7975	7415	18234	6613	5451	7324	18717	15156
SI	18191	6948	6158	20096	8455	5353	9536	16297	13252
	17010	7168	5779	22295	8298	6368	10034	15702	12772
	18340	6762	5942	22225	6938	6107	11725	17223	14065
	18592	6929	5712	22589	7796	6186	9488	18503	15140
	18255	6742	5558	21492	7977	6374	7950	19549	16027
	16940	6699	5413	20789	8063	5055	6560	20072	16420
	16460	6483	5905	19727	7342	6361	6589	20234	16467

Table S2 Raw fluorescence responses in three channels of the integrated sensor to biogenic amines.

	GO								
	PC₁			PC₂			PC₃		
	AO			R6G			RB		
	CH ₁	CH ₂	CH ₃	CH ₁	CH ₂	CH ₃	CH ₁	CH ₂	CH ₃
PEA	112655	59426	47196	158709	57731	49753	100851	157174	143993
	109958	57665	46938	156189	55732	48890	100174	153495	135900
	108806	56829	44532	156183	55585	48271	98133	150742	131897
	106787	54517	44275	151435	55500	47697	96010	150741	130978
	105821	52464	44230	146390	54709	47198	94803	148254	128499
	105448	51562	43249	144744	54035	47025	93848	148086	127901
	104651	51407	42823	143053	53592	45998	93392	147639	127378
DA	122056	51431	37668	138235	47334	28405	88367	105247	89507
	119344	50065	37588	137059	46879	27230	88077	103929	89483
	110899	48265	37384	134537	45504	25661	87145	99323	87946
	104300	45968	37059	130100	45402	25259	85664	98497	87451
	101424	41946	35314	129803	44986	25078	82018	94672	86232
	100301	41724	35301	124430	43056	24367	78064	93499	86044
	97565	41032	34943	121196	42989	23533	77729	91106	85800
NP	22403	15609	11610	63952	18220	15038	34705	80188	46420
	20948	15490	10880	60870	15724	14207	32312	79714	46278
	20136	15398	10814	60768	15608	13482	31244	79577	46234
	19594	15365	9333	59872	15150	12798	29958	78498	46018
	19569	14773	9075	54267	14717	12157	29550	77990	45618
	19485	14597	9008	53889	14141	12048	28821	77860	44327
	19166	13974	8404	53455	13996	11516	28568	77664	41605
HA	139415	64073	47646	134819	59767	46529	99991	246240	174834
	134133	62634	46548	134123	59596	46038	99584	245832	168179
	132801	58571	45687	133363	59331	44836	99127	238666	167843
	130893	57784	44847	131578	58846	43835	98956	238422	166804
	130745	57089	44107	128493	58635	43360	98401	236799	165400
	123065	55612	43931	126347	58079	43225	97793	234852	163752
	122494	54293	43523	123780	57764	42759	97102	233465	163612
5-HT	92495	39770	38602	101463	26094	25600	36943	78159	49913
	92477	37835	38277	99199	25942	25371	35717	77807	49902
	89957	37536	37943	98108	25931	24312	34583	77367	47724
	85486	36772	36610	95682	25433	23971	34484	76492	47075
	84154	36600	35237	95028	25231	23697	33239	76489	45993
	83975	36499	34009	93246	25077	23689	33196	76191	45755
	81853	36379	33902	90598	24407	23679	32937	76140	45506
	104192	47898	40293	118879	41955	36812	49373	139734	109794
	100702	47406	40150	117735	39986	36722	49351	138606	108685
	98385	47211	39622	115664	39350	36157	48005	132246	108096

TA	94728	47143	38758	113952	39324	35355	47154	128569	107948
	93391	45984	38409	111356	36239	34762	46970	127118	107802
	93356	44418	38165	108629	35327	34750	45687	124992	107224
	93216	44140	37513	106009	35276	34136	44725	120875	106180
PDA	136214	59785	45509	149456	57027	48856	127893	200110	172512
	125128	58252	45222	148630	56486	48598	127720	195768	171126
	124558	56221	45047	147890	55715	46340	127389	193533	169403
	121634	56045	43349	146561	55655	45962	125510	191589	162483
	120619	55551	43077	145977	54992	45281	124840	185985	162459
	114779	54747	42725	145692	54798	44006	124149	182581	160439
	112436	54629	42393	144994	53523	43632	123548	181195	159499
EB	121580	58895	46395	139220	47412	46418	96242	109967	103369
	117749	54623	45669	137788	47040	46393	94594	109417	100391
	116622	53788	43858	136842	46661	45485	92725	109368	96628
	115000	53537	43495	131618	45340	44979	90836	109280	96141
	114158	52683	42798	129047	45292	44938	90745	108826	95429
	113099	52507	41966	125262	44767	44676	84272	108709	95212
	110973	52039	41880	120550	44261	43913	82909	108408	95182
SID	141975	50311	30910	135494	47837	43293	117058	169940	128989
	141363	48465	29172	135449	47105	42801	111368	168906	128024
	130639	48139	29063	132260	45583	42611	106979	168228	125267
	128672	47530	29023	117098	42374	41958	106873	164969	123030
	127857	44091	27598	115410	41738	39759	102856	162291	122453
	127801	42655	26383	114226	41480	37472	101844	160705	121890
	122596	42403	25709	113648	39940	32887	101141	160265	119218
SI	119824	40425	30412	170568	37967	35913	110228	177737	139025
	118381	40380	29048	167417	37384	35707	109728	176197	136739
	117919	39577	27817	166466	37030	34715	109262	173355	130862
	114594	39319	27210	162526	36359	34414	108127	169710	130671
	112579	37833	25644	156915	35493	33929	107751	167243	130154
	112406	36306	24795	156523	34562	33332	106839	163859	129630
	106609	35521	24650	155331	34272	31702	103715	163667	125733

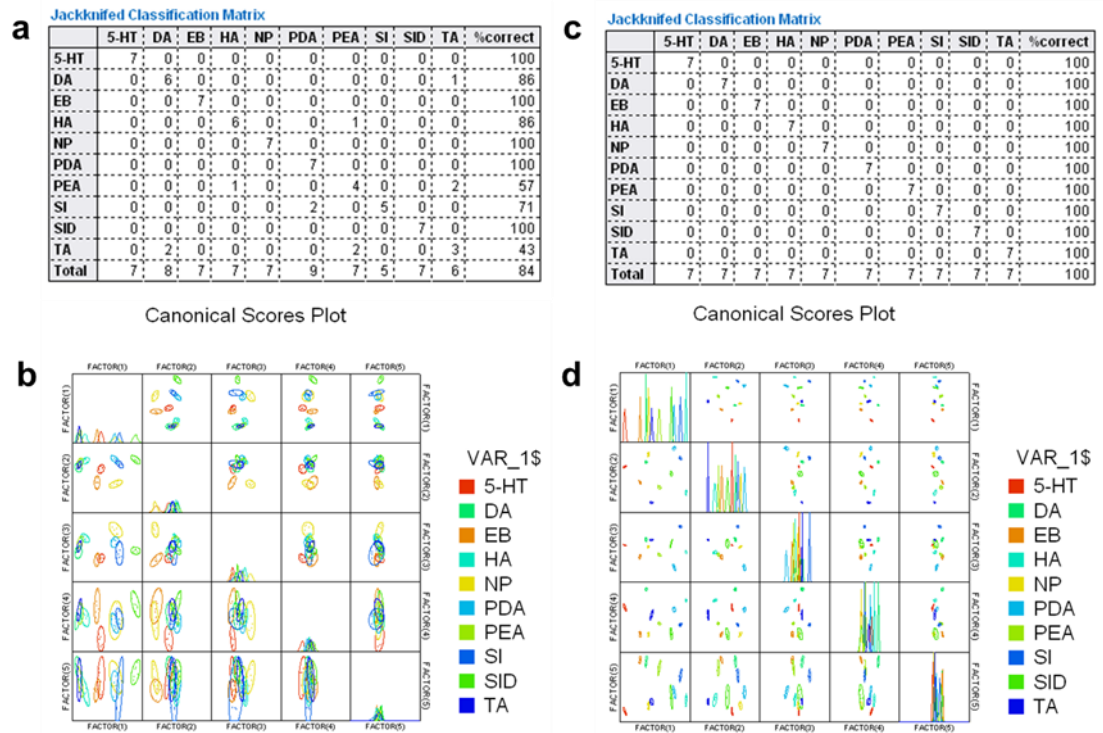


Figure S8 Jackknifed classification matrix and canonical scores plot. (a)-(b) sensors without PCs. (c)-(d) The integrated sensors.

Table S3 Raw fluorescence responses in three channels of the integrated sensor in the discrimination of drug amines.

	GO								
	PC₁			PC₂			PC₃		
	AO			R6G			RB		
	CH ₁	CH ₂	CH ₃	CH ₁	CH ₂	CH ₃	CH ₁	CH ₂	CH ₃
Control	238678	76643	56896	238491	111725	58502	127498	155663	129129
	235257	76610	56829	233374	109962	57553	125162	155488	129027
	234004	75577	56495	232804	105540	57168	125154	155326	127536
	233406	75086	56014	226565	102427	56655	123866	154576	126963
	230388	74419	55794	224941	100476	56616	123382	153216	125840
	230214	74022	55334	221863	100476	55470	122038	152902	122590
	228623	74004	55248	221369	100412	55249	121772	151634	120672
Noscapine	297322	93668	75895	277404	108014	67248	202380	223866	212726
	288630	93205	75140	268531	107435	67033	198458	217792	202320
	285159	93062	74534	260562	106413	66638	196254	216322	202242
	278753	92933	73118	259567	105547	66077	196045	213668	202150
	276224	92399	71614	255898	103713	65876	193939	211935	198092
	275167	91435	70759	247247	102867	65818	193853	209053	197593
	272573	91119	70188	246186	100395	65010	192847	208430	194277
Ethylmorphine	254455	88256	62867	218435	96445	58063	156841	180008	159399
	251177	88164	62572	217313	95881	58053	155622	179755	157143
	249052	87465	62149	215399	94192	57843	155602	177725	157115
	245010	86796	62065	214681	93132	57519	153907	177703	156854
	240401	86478	61159	213331	93128	57495	153625	177175	155613
	239225	85898	60896	212803	93034	57490	153249	175941	155083
	238066	85200	60762	211663	92478	57456	153175	171305	153801
Cocaine	235610	79537	61646	236471	93767	56892	149903	198389	164655
	230073	79536	60368	235427	93439	56419	149889	197779	163859
	229844	79217	59350	234422	93223	56288	149812	197570	162966
	229713	79051	58745	232233	93190	55707	149445	197266	160654
	228354	78551	58595	232109	92584	55561	148371	194810	160103
	226695	77417	58376	232061	91967	55556	146920	193498	159661
	224499	77375	58326	221946	90274	55230	146509	192458	158135
Caffeine	236890	81889	60058	229795	107473	61244	156549	188410	157572
	235592	81544	59742	225100	107050	61123	155903	187160	156350
	231921	81523	59512	223544	103123	60617	155619	185420	155671
	230866	81232	59357	220958	101865	60566	152686	184686	155369
	230429	81062	59309	219462	99816	60323	151583	183503	153431
	230366	80621	59293	219265	98534	60289	150745	183163	153067
	230322	80453	59252	219023	97997	60083	149862	180508	153022
Ketamine	245950	80534	59947	246233	95905	59704	149087	183969	158374
	242329	80101	59926	243408	95580	58847	147481	183881	156235
	242192	80098	59318	242615	95536	58309	146570	182574	152343

	241482	79752	59033	233609	94528	58063	146563	182114	152059
	240890	79668	58281	231592	92597	58042	143627	181494	151777
	237452	79557	57953	230358	92564	57380	141817	180219	150631
	237029	77474	57673	230336	92376	56156	141457	180115	149832
Metham- phetamine	228738	89469	56642	212730	94554	55947	156057	177398	172092
	227536	87249	56005	212414	94472	55803	153255	175952	171693
	226323	86085	55817	208816	94334	55195	153176	175374	170458
	226136	85921	54910	207555	93323	54889	151705	174665	169182
	224463	84634	54380	207002	92685	54778	150792	173213	166020
	221597	84143	54154	206001	92617	54519	150704	170999	165271
	219090	83236	53856	202691	92006	54498	150291	170263	162385
3,4-Methyl- Enedioxy- Metham- phetamine	264714	86686	68548	237750	92661	53463	144461	214226	169875
	263937	84924	68042	234075	92452	52400	143435	213343	169456
	254011	83906	67830	230634	92440	51877	143405	200577	168670
	249220	83097	66695	227835	92095	51619	143248	197646	168217
	248795	82493	66671	227240	91949	51381	142578	193909	167434
	246324	82488	65914	223361	91393	50840	141300	190300	165426
	237402	82247	64728	217618	91370	50395	140421	189343	165339

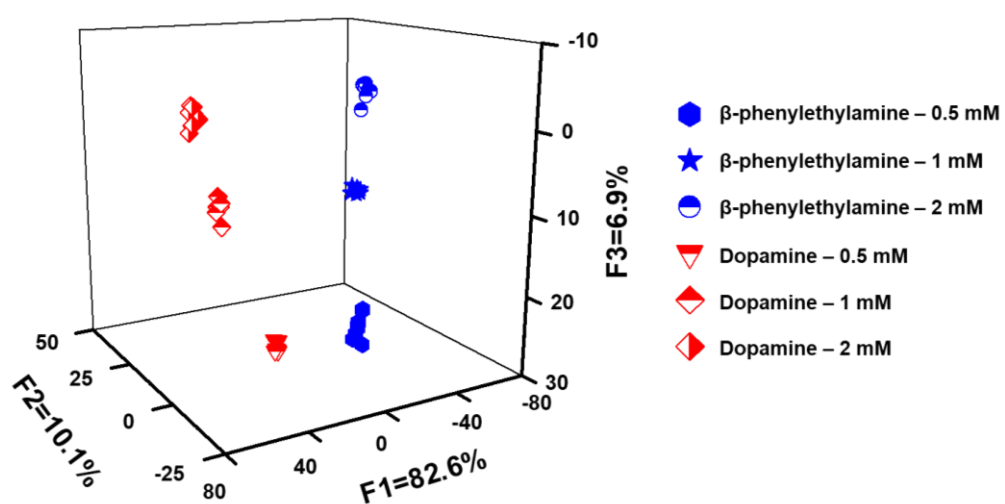


Fig. S9 Discrimination results (LDA) of β -phenylethylamine and dopamine in gradient concentrations.

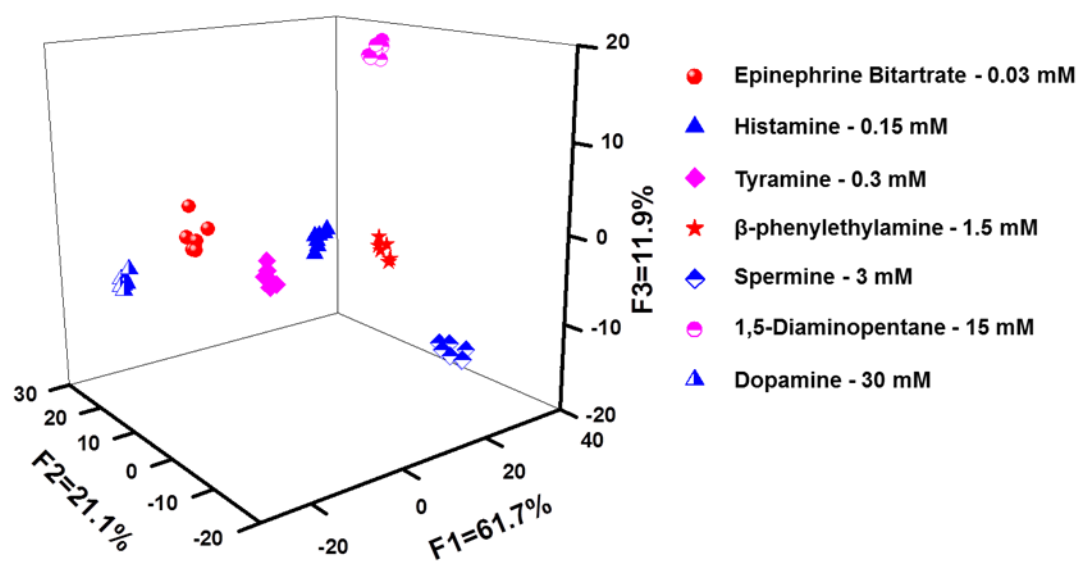


Figure S10 Discrimination results (LDA) of different biogenic amines with different concentrations (epinephrine bitartrate in 0.03 mM, histamine in 0.15 mM, tyramine in 0.3 mM, β -phenylethylamine in 1.5 mM, spermine in 3 mM, 1,5-Diaminopentane in 15 mM and dopamine hydrochloride in 30 mM).

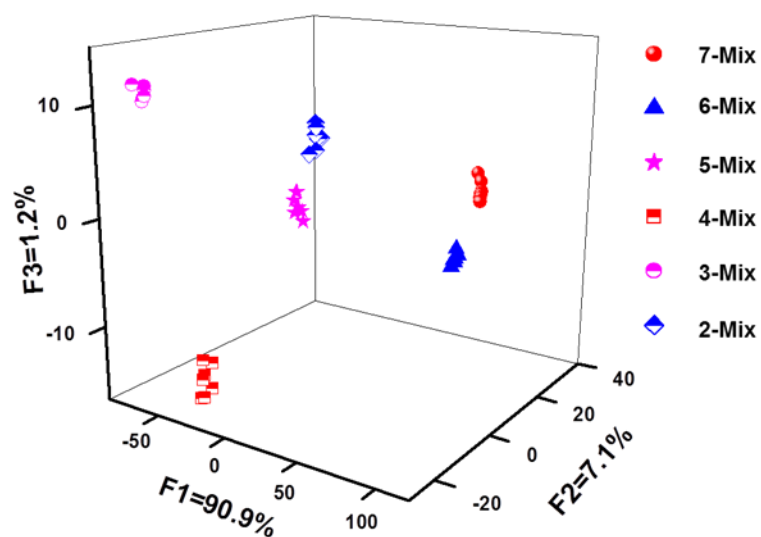


Figure S11 Discrimination results (LDA) of the mixture (2-Mix: mixture of β -phenylethylamine and 1,5-diaminopentane; 3-Mix: 2-Mix mix spermine; 4-Mix: 3-Mix mix tyramine; 5-Mix: 4-Mix mix histanmine; 6-Mix: 5-Mix mix epinephrine bitartrate; 7-Mix: 6-Mix mix dopamine. The final concentration of each amine is 1 mM).

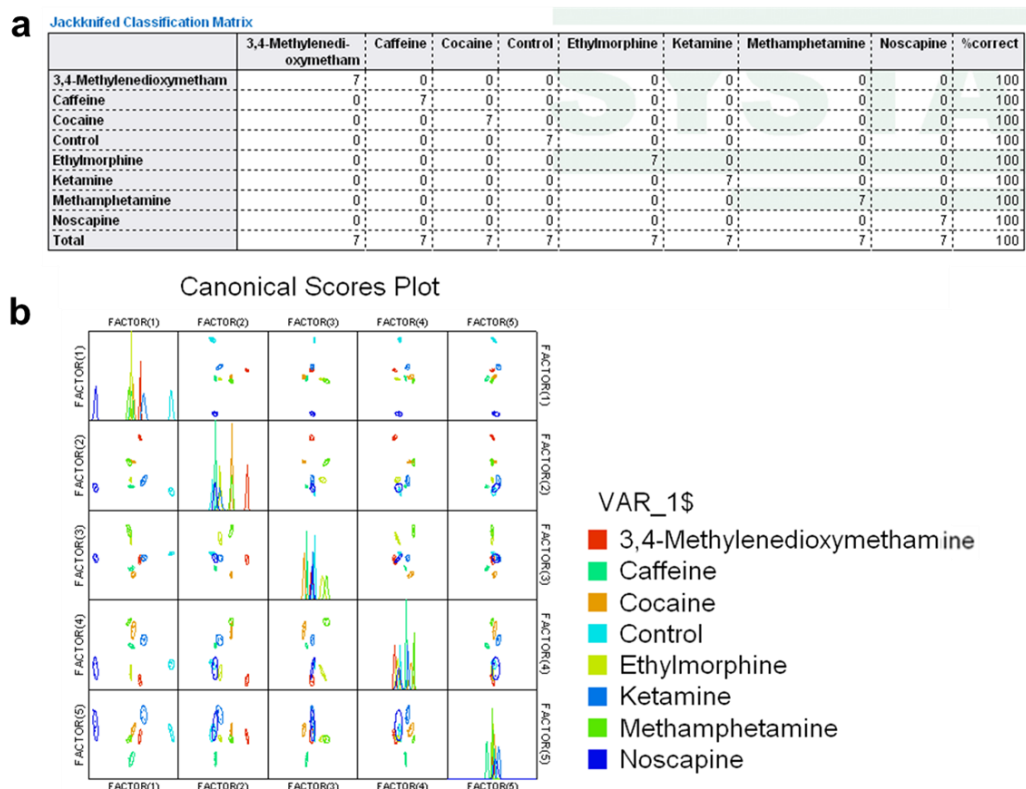


Fig. S12 Jackknifed classification matrix and canonical scores plot of the integrated sensors for the discrimination of different drug amines. (a) Jackknifed classification matrix of the integrated sensors for the discrimination of different drug amines; (b) Canonical scores plot of the integrated sensors for the discrimination of different drug amines.