

Supplementary for:

**Developed a new colorimetric bioassay for iodide determination based gold supported
iridium peroxidase catalysts**

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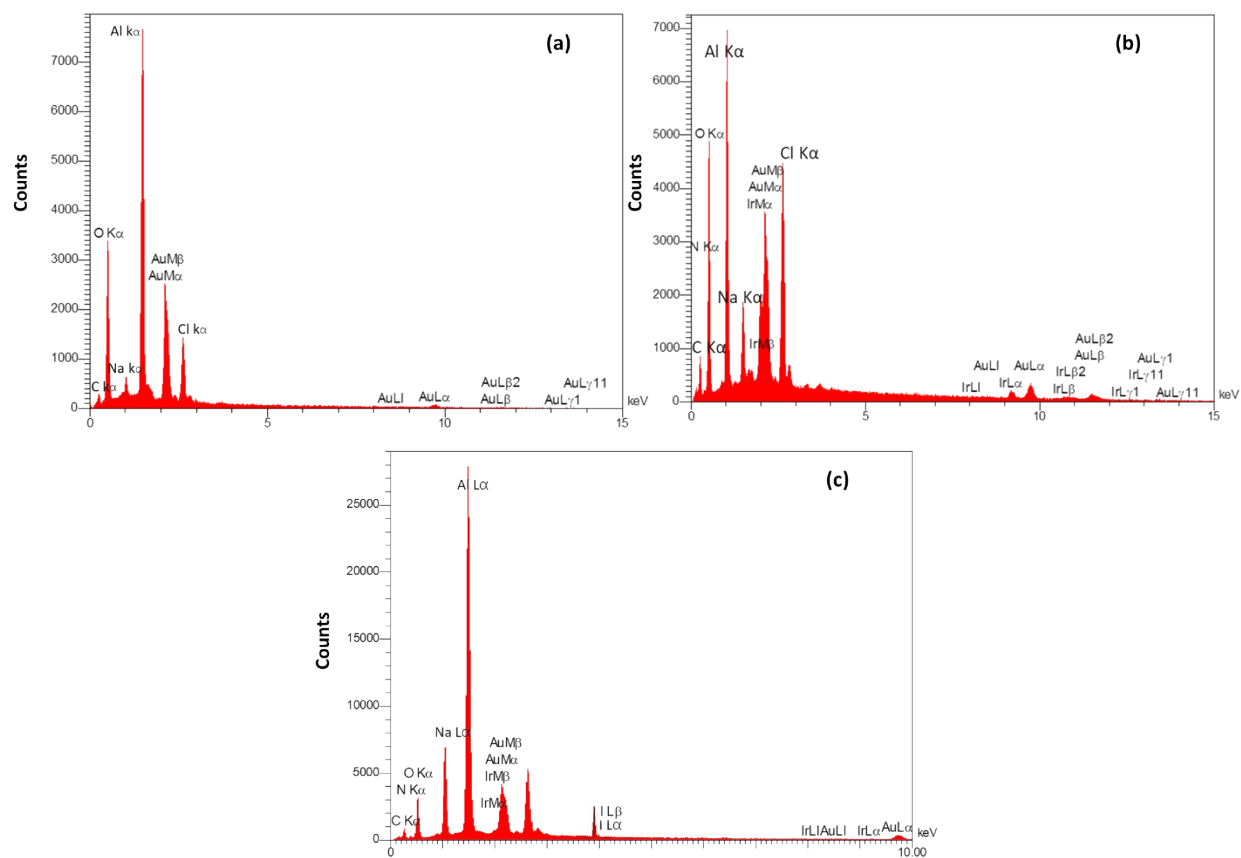


Fig. S1. EDS spectra of Au-Cy-PVA-NPs (a), AuNPs + Ir-NPs + HAuCl $_4$ + 3,4-diamiotolouene biomarker + H $_2$ O $_2$ (b) and AuNPs + Ir-NPs + HAuCl $_4$ + 3,4-diamiotolouene biomarker + H $_2$ O $_2$ + I $^-$ (c)

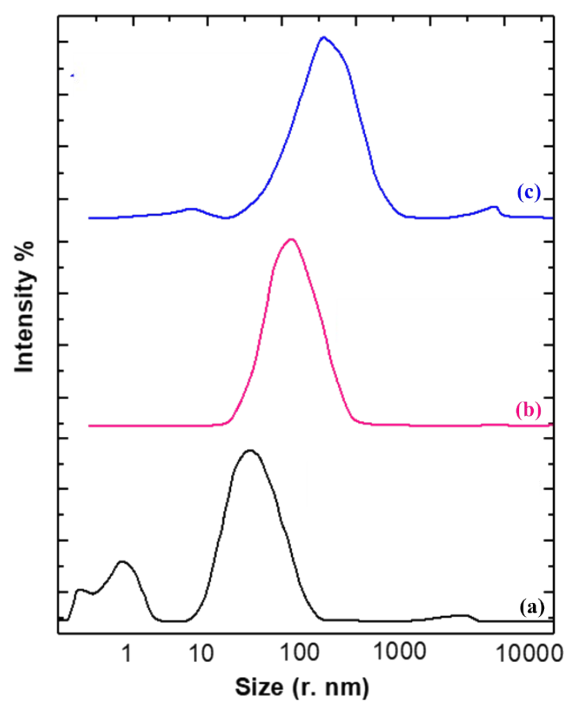


Fig. S2. DLS profiles of Au-Cy-PVA-NPs (a), AuNPs + Ir-NPs + HAuCl₄+ 3,4-diamiotolouene biomarker + H₂O₂ (b) and AuNPs + Ir-NPs + HAuCl₄+ 3,4-diamiotolouene biomarker + H₂O₂ + I⁻ (c)

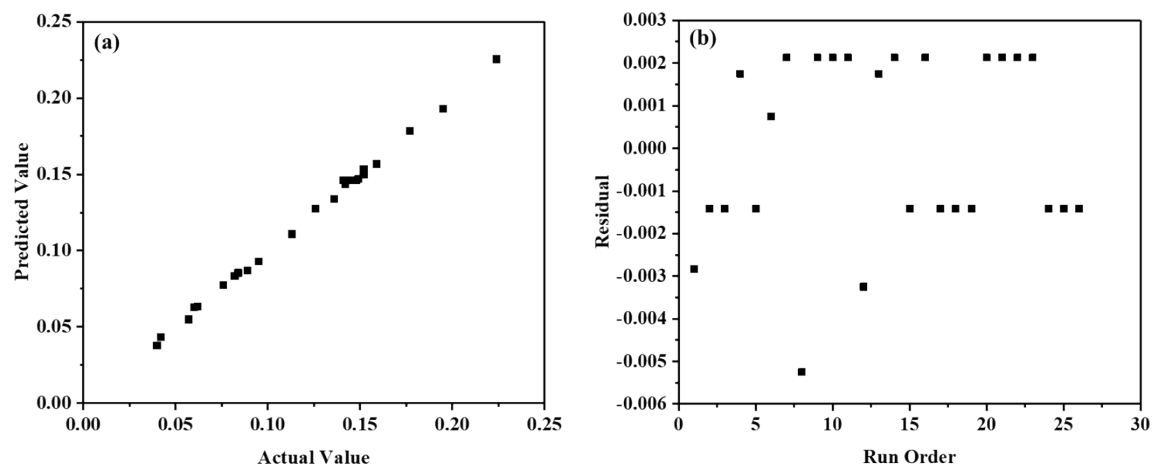


Fig. S3. Parity plot showing the correlation between the observed and predicted values (a), residual values vs. run number (b)

Table S1. The operating variables level, central composite design matrix and response.

Factor	Name		Units	Minimum	Maximum	Coded	Values	Mean
A	Au-NPs Volume		μL	500.00	1500.00	750.00	1250.00	1009.62
B	HAuCl ₄ Volume		μL	50.00	150.00	5.00	125.00	100.96
C	H ₂ O ₂ Volume		μL	50.00	150.00	75.00	125.00	100.96
D	3,4-diamiotolouene biomarker Volume		μL	75.00	175.00	100.00	150.00	125.96
E	Ir-NPs Volume		μL	75.00	175.00	100.00	150.00	125.96
Run	Block	Au-NPs Volume	HAuCl ₄ Volume	H ₂ O ₂ Volume	3,4-diamiotolouene biomarker Volume	Ir-NPs Volume	Observed Value	Predicted Value
1	Block 1	750.00	75.00	75.00	100.00	100.00	0.06	0.063
2	Block 1	1250.00	125.00	125.00	100.00	100.00	0.126	0.13
3	Block 1	1250.00	125.00	75.00	100.00	150.00	0.084	0.085
4	Block 1	1000.00	100.00	100.00	125.00	125.00	0.148	0.15
5	Block 1	750.00	125.00	125.00	100.00	150.00	0.076	0.077
6	Block 1	1000.00	100.00	100.00	125.00	125.00	0.147	0.15
7	Block 1	1000.00	100.00	50.00	125.00	125.00	0.089	0.087
8	Block 1	1000.00	100.00	100.00	125.00	125.00	0.141	0.15
9	Block 1	1000.00	100.00	100.00	125.00	175.00	0.152	0.15
10	Block 1	500.00	100.00	100.00	125.00	125.00	0.136	0.13
11	Block 2	1000.00	50.00	100.00	125.00	125.00	0.057	0.055
12	Block 2	1000.00	100.00	100.00	125.00	125.00	0.143	0.15
13	Block 2	1000.00	100.00	100.00	125.00	125.00	0.148	0.15
14	Block 2	1000.00	100.00	100.00	125.00	75.00	0.095	0.093
15	Block 2	1250.00	75.00	125.00	150.00	100.00	0.142	0.14
16	Block 2	1500.00	100.00	100.00	125.00	125.00	0.195	0.19
17	Block 2	1250.00	75.00	125.00	100.00	150.00	0.177	0.18
18	Block 2	750.00	125.00	75.00	150.00	150.00	0.082	0.083
19	Block 2	750.00	125.00	125.00	150.00	100.00	0.042	0.043
20	Block 2	1000.00	100.00	100.00	175.00	125.00	0.113	0.11
21	Block 3	1000.00	100.00	100.00	75.00	125.00	0.04	0.038
22	Block 3	1000.00	150.00	100.00	125.00	125.00	0.149	0.15

23	Block 3	1000.00	100.00	150.00	125.00	125.00	0.159	0.16
24	Block 3	750.00	75.00	125.00	150.00	150.00	0.152	0.15
25	Block 3	1250.00	75.00	75.00	150.00	150.00	0.062	0.063
26	Block 3	1250.00	125.00	75.00	150.00	100.00	0.224	0.23

Table S2. Sequential model sum of squares for CCD analysis

Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	0.38	1	0.38			
Linear vs Mean	0.016	5	3.294E-003	1.59	0.2079	
2FI vs Linear	0.028	10	2.790E-003	2.07	0.1336	
<u>Quadratic vs 2FI</u>	<u>0.013</u>	<u>5</u>	<u>2.673E-003</u>	<u>113.09</u>	<u>< 0.0001</u>	<u>Suggested</u>
Cubic vs Quadratic	7.698E-005	1	7.698E-005	7.47	0.0522	Aliased
Residual	4.120E-005	4	1.030E-005			
Total	0.44	26	0.017			

Table S3. Lack of fit tests for CCD analysis

Lack of Fit Tests						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	0.041	16	2.584E-003	250.88	< 0.0001	
2FI	0.013	6	2.240E-003	217.52	< 0.0001	
<u>Quadratic</u>	<u>7.698E-005</u>	<u>1</u>	<u>7.698E-005</u>	<u>7.47</u>	<u>0.0522</u>	<u>Suggested</u>
Cubic	0.000	0				Aliased
Pure Error	4.120E-005	4	1.030E-005			

Table S4. Model summary statistics for CCD analysis

Model Summary Statistics						
Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.045	0.2847	0.1059	-0.3213	0.076	
2FI	0.037	0.7669	0.4174	-10.1880	0.65	
<u>Quadratic</u>	<u>4.862E-003</u>	<u>0.9980</u>	<u>0.9898</u>	<u>0.9500</u>	<u>0.043</u>	<u>Suggested</u>
Cubic	3.209E-003	0.9993	0.9955		+	Aliased

Table S5. The results of ANOVA for the response surface quadratic model for the F⁻, Br⁻ and I⁻ by proposed colorimetric sensor

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.058	20	2.887E-003	122.14	< 0.0001	significant
A	1.740E-003	1	1.740E-003	73.64	0.0004	significant
B	4.232E-003	1	4.232E-003	179.04	< 0.0001	significant
C	2.450E-003	1	2.450E-003	103.65	0.0002	significant
D	2.664E-003	1	2.664E-003	112.73	0.0001	significant
E	1.625E-003	1	1.625E-003	68.73	0.0004	significant
AB	1.715E-003	1	1.715E-003	72.57	0.0004	significant
AC	3.204E-003	1	3.204E-003	135.57	< 0.0001	significant
AD	2.247E-003	1	2.247E-003	95.05	0.0002	significant
AE	4.137E-005	1	4.137E-005	1.75	0.2431	not significant
BC	2.612E-003	1	2.612E-003	110.51	0.0001	significant
BD	2.131E-005	1	2.131E-005	0.90	0.3860	not significant
BE	2.120E-003	1	2.120E-003	89.70	0.0002	significant
CD	1.593E-010	1	1.593E-010	6.738E-006	0.9980	not significant
CE	1.530E-003	1	1.530E-003	64.73	0.0005	significant
DE	1.548E-004	1	1.548E-004	6.55	0.0507	not significant
A ²	5.013E-004	1	5.013E-004	21.21	0.0058	significant
B ²	3.521E-003	1	3.521E-003	148.95	< 0.0001	significant
C ²	1.016E-003	1	1.016E-003	42.99	0.0012	significant
D ²	8.834E-003	1	8.834E-003	373.72	< 0.0001	significant
E ²	1.058E-003	1	1.058E-003	44.77	0.0011	significant
Residual	1.182E-004	5	2.364E-005			significant
Lack of Fit	7.698E-005	1	7.698E-005	7.47	0.0522	not significant
Pure Error	4.120E-005	4	1.030E-005			
Cor Total	0.058	25				

Table S6. Model summary statics

Std. Dev.	4.862E-003
Mean	0.12
C.V. %	4.03
PRESS	0.043
R-Squared	0.9980
Adj R-Squared	0.9898
Pred R-Squared	0.9500
Adeq Precision	42.923
-2 Log Likelihood	-246.05
BIC	-177.63
AICc	26.95