Electronic Supplementary Material (ESI) for Analyst.

An ultrasensitive electrochemical sensor for phospholipase C via signal amplification based on breathing ATRP and its application

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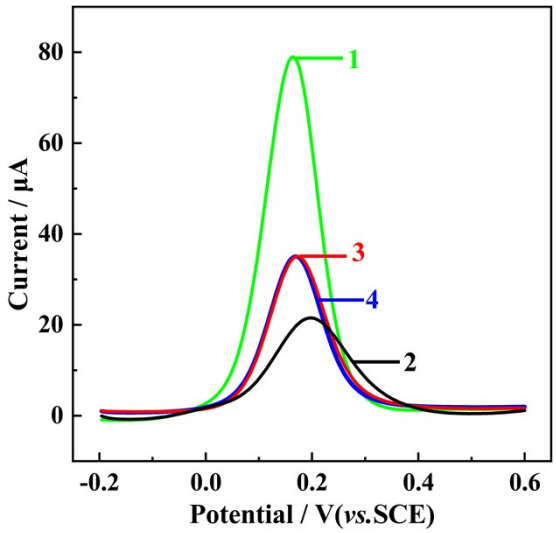

Fig.S 1 DPV results of Cys/Au (curve 1, curve 2) and PE/Cys/Au electrode (curve 3, curve 4) before (curve 1, curve 3) or after (curve 2, curve 4) soaking in $ZrOCl_2$ solution.

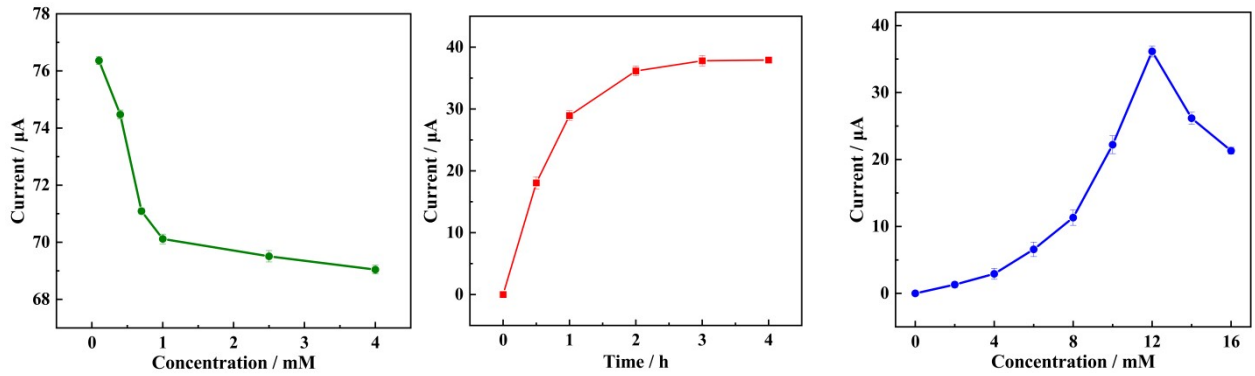

Fig.S 2 Selection of Cys concentration (A), polymerization time (B) and FcMMA concentration (C).

Table S1 Analytical performance of different methods for PLC detection.

Methods	Materials	Linear response range	LOD	Ref.
Fluorometry	11-mercaptoundecanoic acid−gold nanodot−liposome hybrids	5-300 U/L	2 U/L	1

Fluorometry	water-soluble conjugated polyelectrolyte -lipid complex	0-100 μmol/L	1 nmol/L	2
Enzyme catalysis	phosphomolybate complex	10-10^6 nmol/L	10 nmol/L	3
LC-MS assay	1-palmitoyl-2-oleoyl-diacylglycerol	1-10 U/mL	1 U/mL	4
Electrochemistry	PFcMMA/Zr^{4+}/PE/Cys/Au electrode	1-40 U/L (0.78-31.01 nmol/L)	0.27 U/L (0.45 nmol/L)*	This Work

* The value conversion was calculated according to the information provided by the reagent company

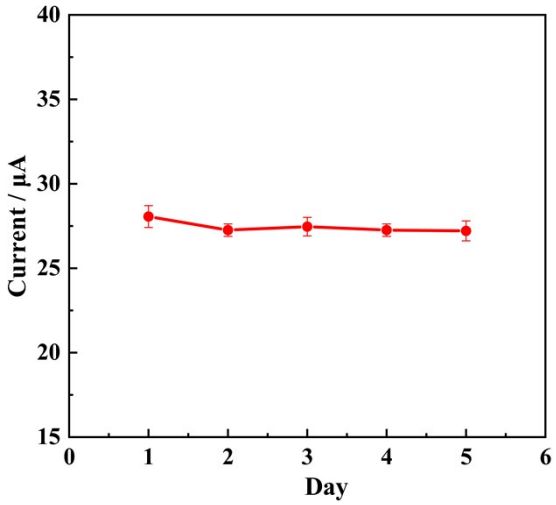

Fig.S 3 Relationship of SWV peak current of PFcMMA/Zr^{4+}/PE/Cys/Au electrode with days.

Table S2 Determination of PLC in three cell extracts by Elisa kit and PFcMMA/Zr^{4+}/PE/Cys/Au

cells	ELISA kit (U/L) [mean±SD (n=3)]	PFcMMA/Zr^{4+}/PE/Cys/Au electrodes				
		Direct detection (U/L) [mean±SD (n=3)] and RSD	Standard addition method			
			Added (U/L)	Found (U/L) [mean±SD (n=3)]	Recovery (%)	RSD (%)

MCF-7	1.380±0.051	1.406±0.038	2.73%	0.5	1.870±0.034	98.0%	1.80%
				1.0	2.388±0.033	100.7%	1.39%
				1.5	2.899±0.045	101.2%	1.57%
				2.0	3.336±0.050	97.8%	1.48%
				2.5	3.854±0.053	98.9%	1.36%
MDA-MB-231	1.519±0.067	1.581±0.053	3.46%	0.5	2.036±0.062	103.5%	3.05%
				1.0	2.495±0.067	97.6%	2.66%
				1.5	3.015±0.062	99.8%	2.05%
				2.5	4.093±0.062	103.0%	1.53%
				5.0	6.723±0.256	104.1%	3.92%
MCF-10A	0.730±0.088	0.666±0.013	1.73%	0.5	1.234±0.026	100.9%	2.14%
				1.0	1.743±0.036	101.3%	2.06%
				1.5	2.289±0.018	103.9%	0.80%
				2.0	2.808±0.056	103.9%	2.04%
				2.5	3.199±0.036	98.7%	1.13%

Reference

1. Chen, W.Y.; Chen, L.Y.; Ou, C.M.; Huang, C.C.; Wei, S.C.; Chang, H.T., Synthesis of fluorescent gold nanodot−liposome hybrids for detection of phospholipase C and its Inhibitor. *Anal. Chem.* **2013,** 85, 8834-8840.
2. Liu, Y.; Ogawa, K.; Schanze, K.S., Conjugated polyelectrolyte based real-time fluorescence assay for phospholipase C. *Anal. Chem.* **2008**, 80, 150-158.
3. Durban, M. A.; Bornscheuer, U.T., An improved assay for the determination of phospholipase C activity. *Eur. J. Lipid Sci. Tech.* **2007,** 109, 469–473.
4. Murakami, C.; Mizuno, S.; Kado, S.; Sakane, F., Development of a liquid chromatography-mass spectrometry based enzyme activity assay for phosphatidylcholine-specifific phospholipase C. Anal. Biochem. **2017,** 526, 43-49.