Supplementary Material (ESI) for Chemical Communications
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Supplementary Information

Graphene oxide-enabled tandem signal amplification for sensitive SPRi immunoassay in serum

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1. Preparation of antibody-functionalized rGO

GO was synthesized by oxidizing natural graphite using the modified Hummers method, as previously reported in reference. As-prepared GO was dispersed in water, followed by strong sonication for 1h and unexfoliated graphene oxide was removed using centrifugation. 0.1 mL of ca. 0.1 mg mL^{-1} homogeneous GO suspension was added into 1 mL PBS buffer (pH 8.0) containing 1 mg mL^{-1} anti-Rabbit IgG and was stirred at 4℃ for 24 h. After centrifugation, the supernatant was discarded and the precipitation was washed with PBS buffer containing 10 mg mL^{-1} Bovine serum albumin (BSA) for three times, and finally dispersed in 1 mL PBS buffer containing 10 mg mL^{-1} BSA.

2. Preparation of polymer brush on gold surface

The brush is prepared according to our previous study with minor modification. Briefly, a clean gold chip (SPRi SpotReady^{TM} chip with 4×4 spots array) with a compact cysteamine self-assembled monolayer was immersed in 10 mL tetrahydrofuran (THF) solution containing 77 μL of triethylamine (TEA, 99.5%) and 64 μL of 2-

bromoisobutyryl bromide (BIB, 98%) for 2 h incubation with gentle orbital rotation. The SI-ATRP growth solution was prepared by mixing 8 mL of DI water, 8 mL of methanol (HPLC grade), 4 mL of OEGMA (typical M_n=360), and 100 μL of GMA (97%). After bubble the solution with pure nitrogen for 15 min, 2, 2'-bipyridyl (Bipy, ≥99%, 100 mg) and copper (II) bromide ($CuBr_2$, 99.999%, 70 mg) were dissolved in the monomer solution with the assistance of sonication. The initiator-attached gold chip was placed into the deoxygenated solution in a 50 mL centrifuge tube. A 1.0 mL 50 mg mL^{-1} ascorbic acid solution was rapidly added into the solution to start the polymerization. After 12 h growth, the chip was pulled out and rinsed with methanol and dried under gentle nitrogen flow for sequential use.

3. SPRi chip preparation and detection

200 μg mL^{-1} anti-AFP monoclonal antibody in PBS buffer was dropped to cover each brush-tethered gold spot. The chip was placed in a drying cabinet for 8 h, followed by intensive washing with 0.05 M Tris buffered saline (TBS) containing 0.05% Tween 20. SPRi measurements were performed on GWC imager II system (GWC Technologies Inc., WI USA) equipped with an 800 nm p-polarized monochromatic light source. During the detection, the sensing spots were firstly allowed to incubate with sample solutions with different target concentrations in 10% human serum for 1 h, followed by washing with 0.01 M PBS at a constant rate of 300 μL min^{-1}. After a stable baseline in PBS, 5 μg mL^{-1} anti-AFP polyclonal antibody (developed in Rabbit) solution was flowing on the sensing surface for 15 min, followed by flowing anti-Rabbit IgG functionalized GO solution at 30 μL min^{-1} for 10 min. For silver deposit enhancement, acetic buffer (0.2M acetic

acid/sodium acetate buffer, pH 5.2) was firstly flowing to obtain a stable baseline. Freshly prepared silver enhancer solution (adding 0.1 mL of 2.4 mg mL^{-1} silver nitrate and 0.1 mL of 1 mg mL^{-1} hydroquinone into 3 mL acetic buffer, pH 5.2) was then flowing for 5 min, followed by rinsing with acetic buffer. The *in situ* images and sensorgrams on each gold spots were collected by the V++ software. After the detection, the intensity of reflected light was converted to real reflectivity using a neutral density filter coupling with s-polarized light.

4. Characterizations

Angular SPR measurements were carried out on Autolab SPRINGLE system (Echo Chemie B.V., Netherlands) as reported previously; TEM images were obtained on a JEM-1400 Transmission Electron Microscopy system (JEOL, Japan) operated at 100 kV. Atomic force microscopy (AFM) image was obtained on a Nanoman AFM (Veeco metrology group, USA) with tapping mode at ambient temperature.

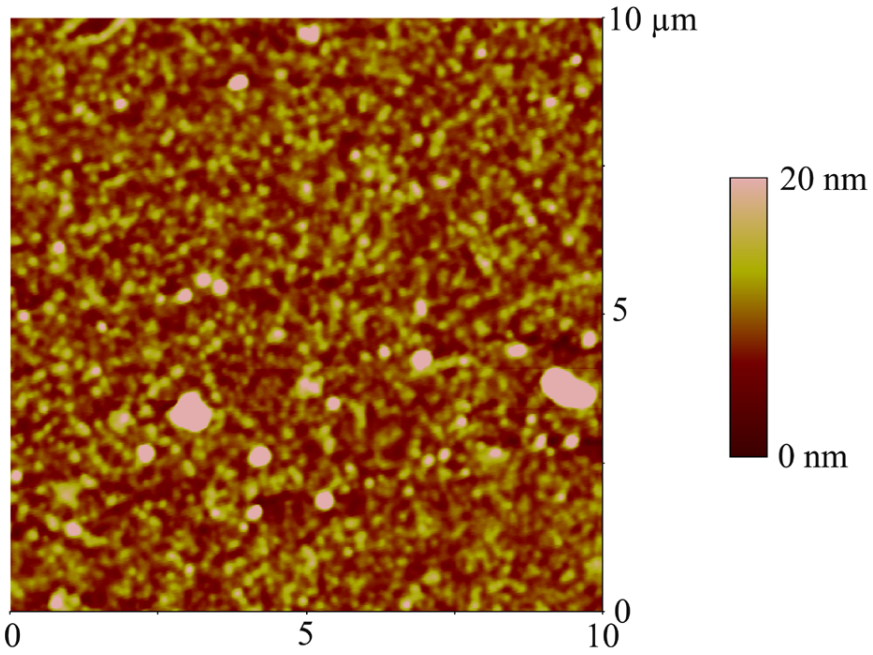

Fig. S1 AFM image of POEGMA-*co*-GMA brush on SPRi gold chip

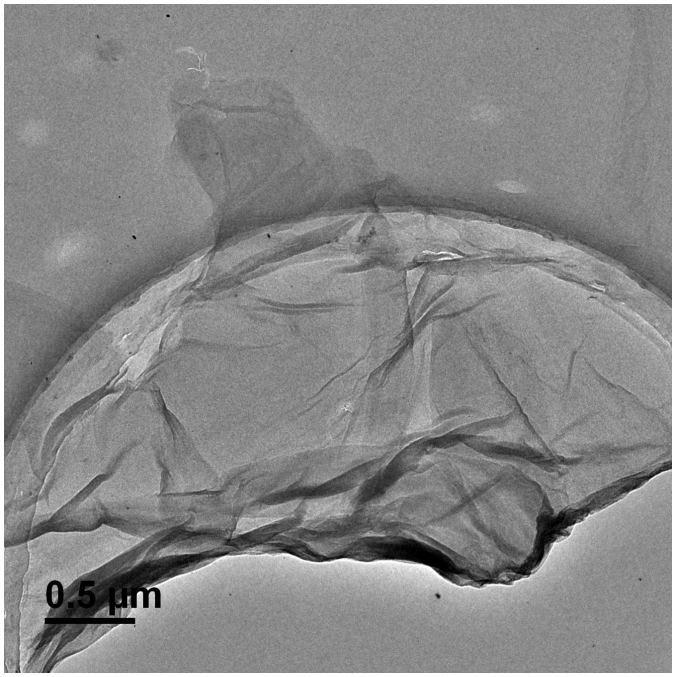

Fig. S2 TEM image of GO

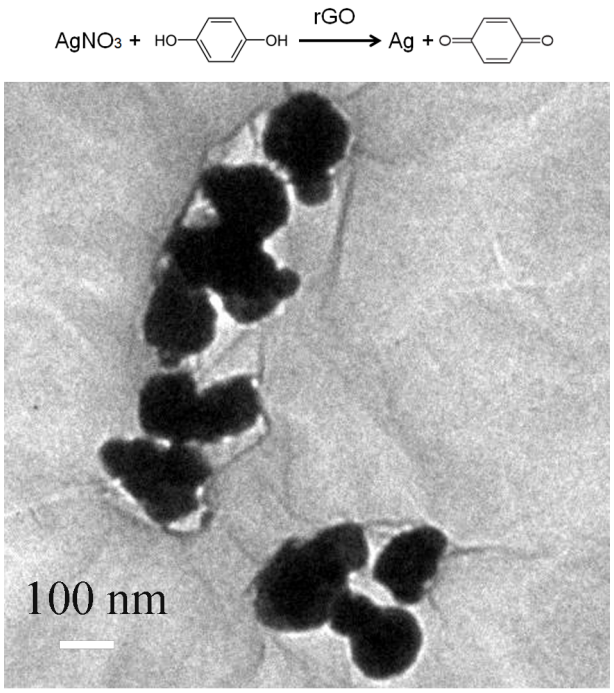

Fig. S3 Reaction of rGO-catalyzed silver deposition from silver growth solution and TEM image of rGO with deposited silver particles

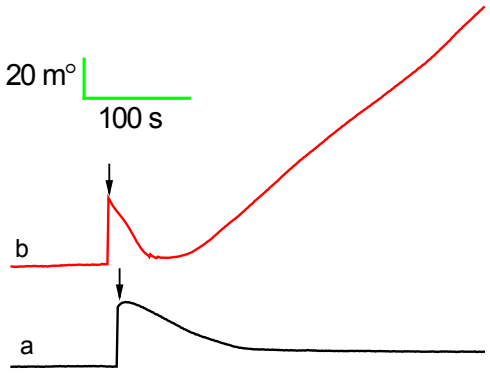

Fig. S4 SPR response of silver enhancer solution on clear gold surface (red) and on POEGMA-co-GMA brush modified gold surface (black). The arrows indicate the time on which the acetic buffer was changed to silver enhancer solution.

Table S1. Summary of SPR-based immunoassay performance

platform	Amplification method	target	Detection limit	Dynamic range	Ref
SPRi	Enzymatic deposit	VEGF	1 pM	n.a.	Ref. 3
SPRi	Enzymatic deposit	thrombin	500 fM	n.a.	Ref. 3
SPR	polymerization	CT	6.27×10^{-15} mol/cm^2	8.23×10^{-15} - 3.61×10^{-12} mol/cm^2	Ref. 8
SPR	Biological binding	CEA	3 ng/mL	3-400 ng/mL	Talanta 2011,86, 377
SPR	Ag@Au nanoplate	streptavidin	n.a.	n.a.	Ref. 9
SPR	Direct detection	troponin T	100 ng/mL	100 ng/mL-50 μg/mL	Anal. Chim. Acta 2011, 703, 80
SPR	Direct detection	thrombin	50 nM	50-200 nM	Biosens. Bioelectron. 2011, 26, 4832
SPRi	Direct detection	CEA	50 ng/mL	50 ng/mL-above	Ref. 5
SPRi	Direct detection	AFP	20 ng/mL	20 ng/mL-above	Ref. 5
SPRi	Direct detection	HBsAg	100 ng/mL	100 ng/mL-above	Ref. 5
SPRi	Tandem signal amplification	AFP	100 pg/mL	100 pg/mL-above	Present work