Electronic Supplementary Material (ESI) for RSC Advances

Supporting information

Macroinitiator Triggered Polymerization for Versatile Immunosensing

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Fig. S1 ^{1}H NMR spectra and peak assignments of P(AA-AM) (A) and P(AA-AM)-NHS-Br (B) dissolved in D_2O.

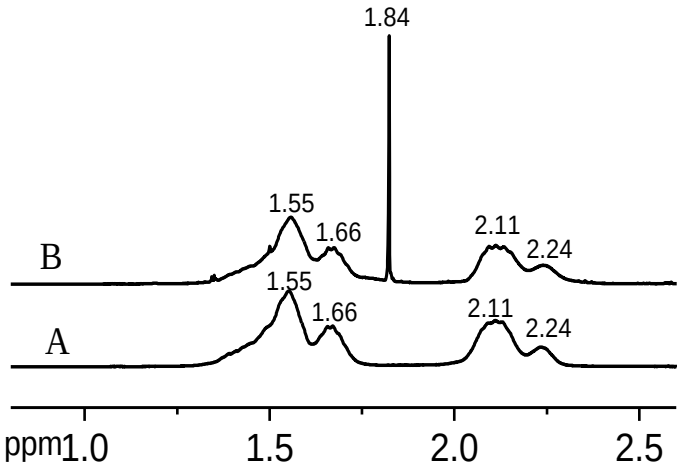

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Fig. S2 UV/Vis absorbance spectra of P(AA-AM)-NHS-Br (a) and P(AA-AM)-streptavidin (b) in 0.1 M PBS (pH 7.2).

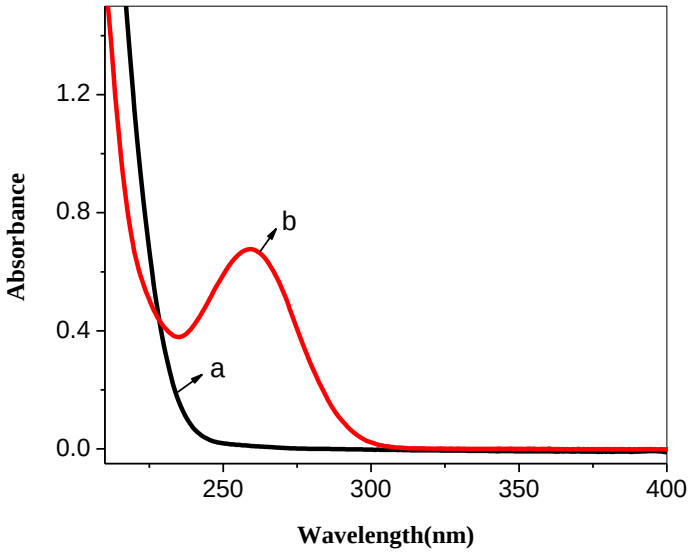

Fig. S3 ATR-IR spectra of PHEMA film formed on the substrate surface (a) without any modification, modified with macroinitiator with sandwiched immunoassay (b) before and (c) after polymerization.

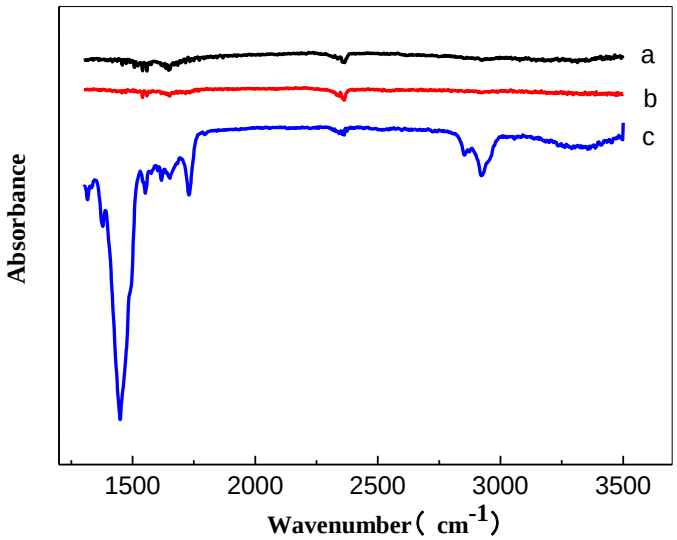

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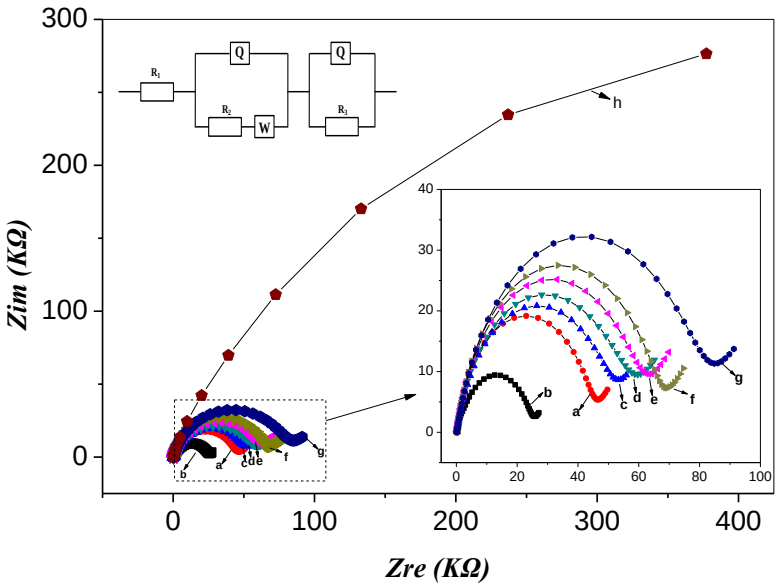
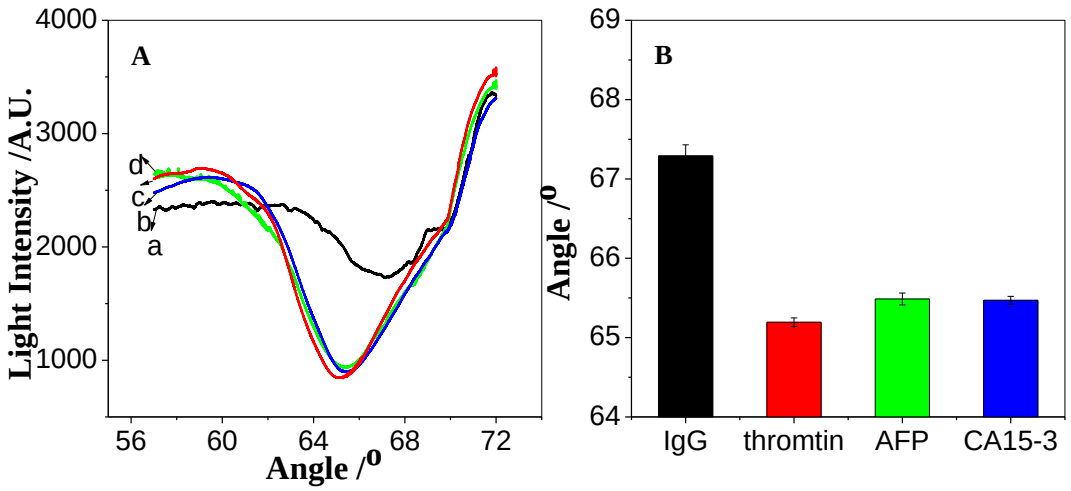
Fig. S4 Electrochemical impedance spectra of (a) MUA/Au, (b) EDC/MUA/Au, (c) Ab1/MUA/Au, (d) BSA/Ab1/MUA/Au, (e) Ag/BSA/Ab1/MUA/Au, (f) Ab2/Ag/BSA/Ab1/MUA/Au, (g) Macroinitiator/Ab2/Ag/BSA/Ab1/MUA/Au and (h) PHEMA/Macroinitiator/Ab2/IgG/Ab1/MUA/Au in 0.1 M pH 7.2 PBS containing 10 mM $K_4Fe(CN)_6$ /$K_3Fe(CN)_6$. Inset: the magnified electrochemical impedance spectra of substrate (a)-(g) and the equivalent circuit.



The sandwiched immunoreaction and the polymerization initiated by the macroinitiator were also confirmed by electrochemical impedance spectroscopy (EIS) measurements (Fig. S4). The charge-transfer resistance (R_{ct}) was extracted with an equivalent circuit (inset in Fig. S4), which changed in the following order: EDC/NHS activated-MUA/Au (22.94 kΩ) < MUA/Au (43.68 kΩ) < Ab1/MUA/Au (48.16 kΩ) < Ag/Ab1/PAB/Au (58.67 kΩ)< Ab2/Ag/Ab1/PAB/Au (65.52 kΩ) < macroinitiator/Ab2/Ag/Ab1/PAB/Au (78.16 kΩ) << PHEMA/macroinitiator/Ab2/IgG/Ab1/MUA/Au (400.2 kΩ). These changes were attributed to (i) the presence of negatively charged MUA film atop Au resulted in significant increases in the charge-transfer resistance; (ii) the EDC activated MUA displayed electropositive, thus facilitating charge transfer and decrease the charge-transfer resistance;

(iii) the coupling of protein in MUA film which increased the charge-transfer resistance due to the insulation of the protein shell; (iv) the polymer growth which led to resist charge-transfer thus increase the R_{ct}.

Fig. S5 (A) SPR spectra of the Ab1/Au substrate immersed in (a)10 μg mL^{-1} IgG, (b) 50 μg mL^{-1} breast cancer antigen (CA15-3), (c) 50 μg mL^{-1} thrombin, and (d) 50 μg mL^{-1} α-fetoprotein (AFP), followed by BT-Ab2, macroinitiator incubation, and macroinitiator triggered AGET ATRP of HEMA for signal amplification detection. (B) The angle of the substrate after detecting different target proteins as (A) suggested in SPR spectra.



Control experiments (Fig. S5) were performed also through surface plasmon resonance (SPR) measurements by incubating of the Ab1-modified substrate with 50 μg mL^{-1} thrombin, 50 μg mL^{-1} breast cancer antigen (CA15-3), and 50 μg mL^{-1} α-fetoprotein (AFP), followed by same procedures including the second immunoreaction with Ab2, the coupling macroinitiator through streptavidin-biotin interaction and macroinitiator-triggered AGET ATRP. When the target changed to thrombin, AFP, and CA15-3, the resonance angle of 65.19°, 65.48° and 65.47° was very close to 65.25° for Ab1/MUA/Au chip. This confirmed that the macroinitiator could be loaded to the Au substrate through highly specific sandwich immunoreactions while not originated from physical absorption or cross-reaction.

Fig. S6 The SEM images of NHS-Br-coupled Ab2*/IgG/Ab1/Au substrate (A) and macroinitiator/Ab2/IgG/Ab1/Au (B).

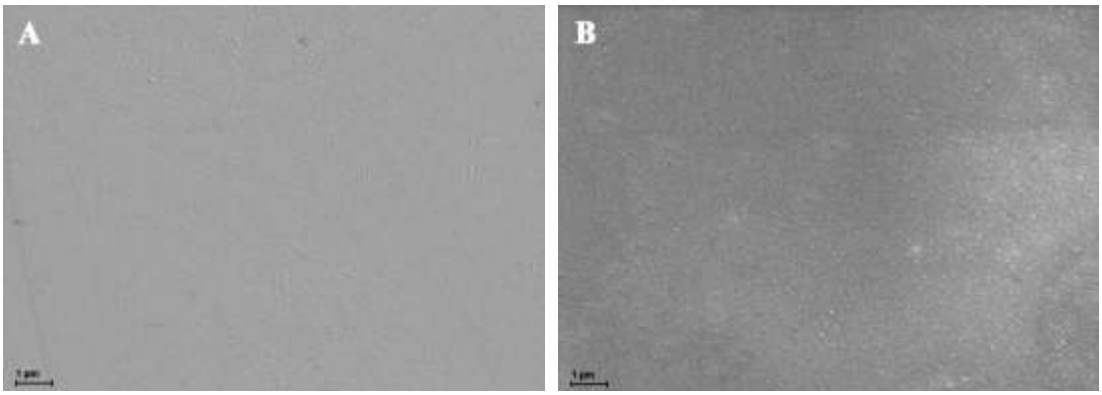


Table S1. The contact angle measurements of different Au chips, at IgG concentration of 100 μg mL^{-1}, polymerization time of 7 min. (mean ± SD, n=3).

Au chips	Contact angel /^{0} (before polyerization)	Contact angel /^{0} (after polyerization)
1#	29.7±0.2	29.3±0.7
2#	30.1±0.4	52.9±0.9

1#: Ab2/IgG/Ab1/Au substrate reacted to SA-labeled P(AA-AM) without NHS-Br coupling.

2#: Ab2/IgG/Ab1/Au substrate reacted with macroinitiator.