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$_2$O.
**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.
**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 K₄Fe(CN)₆/K₃Fe(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) 5 suggested in SPR spectra.

Control experiments (Fig. S5) were performed also through surface plasmon resonance (SPR) measurements by incubating 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).

<table>
<thead>
<tr>
<th>Au chips</th>
<th>Contact angel /° (before polymerization)</th>
<th>Contact angel /° (after polymerization)</th>
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</thead>
<tbody>
<tr>
<td>1#</td>
<td>29.7±0.2</td>
<td>29.3±0.7</td>
</tr>
<tr>
<td>2#</td>
<td>30.1±0.4</td>
<td>52.9±0.9</td>
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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.