Supporting Information

Electrochemically Controlled RAFT Polymerization for Highly Sensitive Electrochemical Biosensing of Protein Kinase Activity

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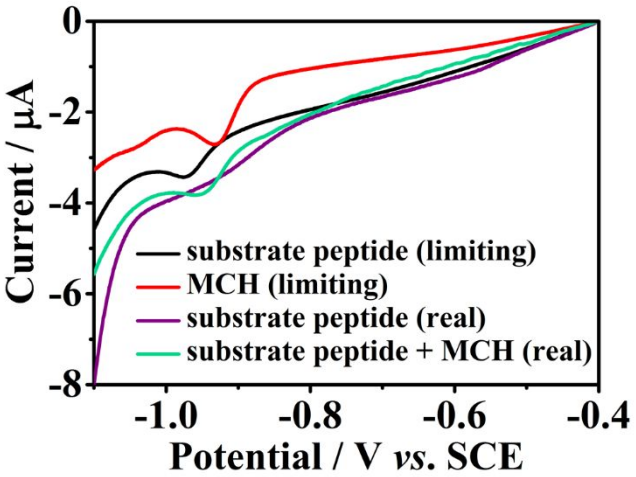

Figure S-1: LSV curves of differently modified electrodes. Scan rate: 20 mV s^{-1}; quiet time: 1.0 min. "limiting" represents the maximal surface coverage of substrate peptide or MCH, "real" represents the real surface coverage of substrate peptide or substrate peptide/MCH of the gold electrode modified as described in the Experimental Section. Surface coverage (Γ_m) can be calculated according to $\Gamma_m = Q_m/FA\nu$, where Q_m, F, A and ν represent the integral value of the reductive desorption wave (A V), the Faraday constant (96485.33 C mol^{-1}), the electrochemical surface area (0.07 cm^2), and the scan rate (0.02 V s^{-1}). The Γ_m values of substrate peptide (limiting), MCH (limiting), substrate peptide (real), and substrate peptide + MCH (real) are calculated to be 2.10×10^{-10} mol cm^{-2}, 3.84×10^{-10} mol cm^{-2}, 1.30×10^{-10} mol cm^{-2}, and 2.64×10^{-10} mol cm^{-2}, respectively. Assuming that the surface areas occupied by a substrate peptide or a MCH molecule is *a* and *b* respectively, then $2.10a = 3.84b$. As calculated, the surface area occupied by the substrate peptide + MCH (real) is $3.72b$. Thus, ~96.9% (i.e., $3.72b/3.84b$) of the electrode surface is occupied by the substrate peptide and MCH (real).

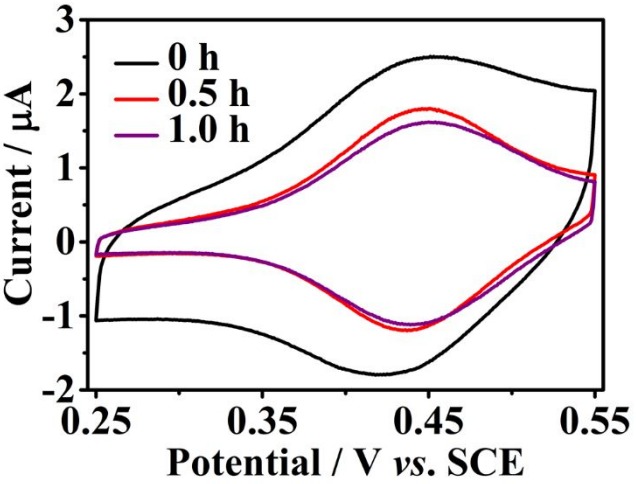

Figure S-2: Effect of MCH blocking time on the charge transfer of the Fc-SLGGGGC-modified gold electrode. Fc-SLGGGGC: 1.0 μM; modification time: 1.0 h; MCH: 2.0 mM; supporting electrolyte: 0.5 M $LiClO_4$; scan rate: 0.1 V s^{-1}; quiet time: 30 s. The blocking of the electrode surface leads to a decrease of the

oxidation peak current by 1.04% (0.5 h) and 7.8% (1.0 h), respectively. The small decrease in oxidation peak current may be ascribed to the substitution of some of the pre-immobilized Fc-modified peptides by MCH molecules during the MCH blocking. With this in mind, the blocking of the electrode surface with MCH has little effect on the charge transfer between the surface-tethered Fc tags and the electrode surface.

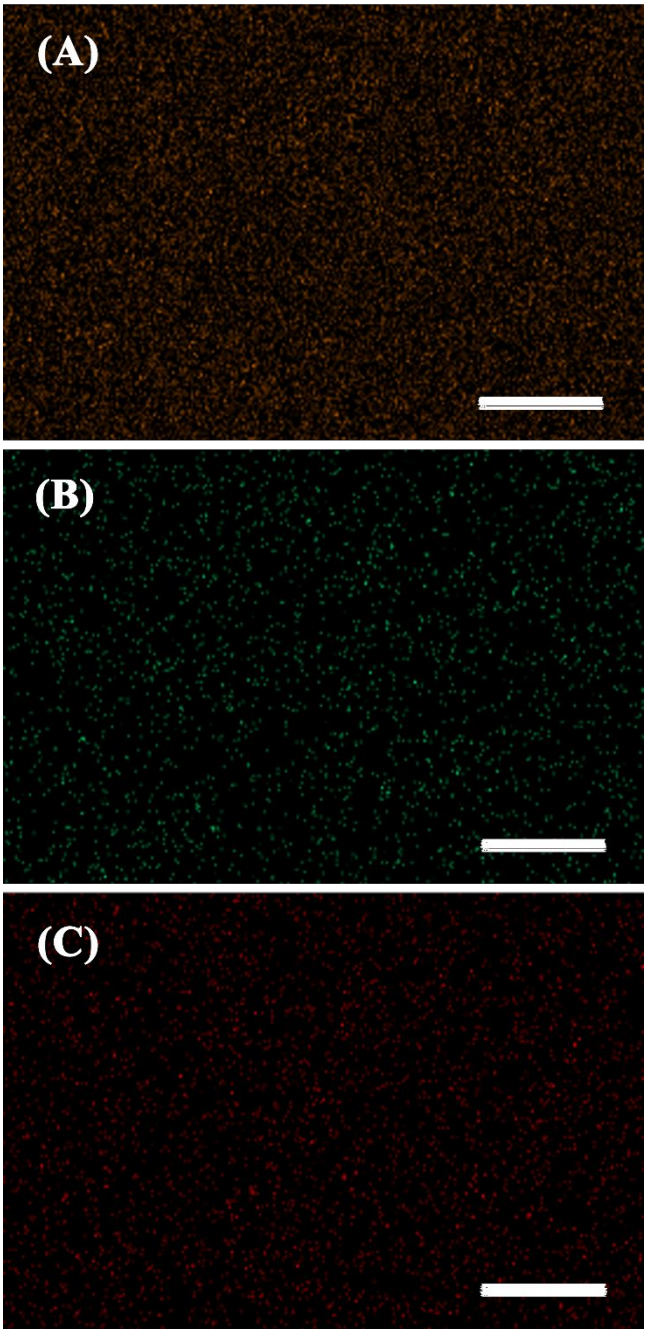

Figure S-3: The traces of P element (A), Br element (B), and Fe element (C). The scale bar is 1.0 μm.

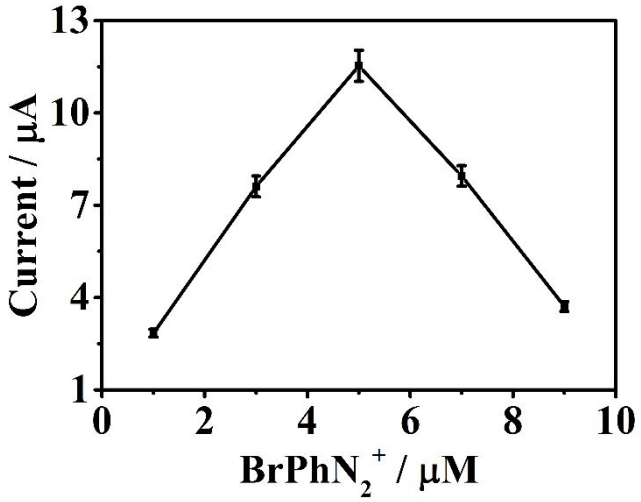

Figure S-4: Oxidation currents at ~0.3 V in the presence of different concentrations of $BrPhN_2^+$. PKA, 140 mU mL^{-1}. Error bars show the SDs of five independent assays.

Table S-1: Values of R_{ct}, *CPE*, R_s, and *W* of Differently Modified Electrodes

Electrode	R_{ct} (Ω)	*CPE* ($\times 10^{-6}$ S sec^n)	R_s (Ω)	*W* ($\times 10^{-4}$ S $sec^{0.5}$)
AuE	~143.7	~1.41	~152.9	~7.74
AuE/S	~772.5	~1.37	~162.3	~7.26
AuE/S/MCH	~1891	~0.81	~168.9	~7.06
AuE/S_P/MCH	~2117	~1.16	~157.2	~6.96
AuE/S_P/MCH/Zr^{4+}	~2568	~1.05	~156.9	~6.95
AuE/S_P/MCH/Zr^{4+}/CPAD	~2756	~1.12	~143.6	~6.80
AuE/S_P/MCH/Zr^{4+}/CPAD/Fc	~648.2	~1.43	~157.9	~7.54

Table S-2: Comparison of the Analytical Performance with Those of Other Methods

Method	Amplification	Linear range* (U mL^{-1})	LOD (mU mL^{-1})	Mode	Ref.
Electrochemical	DNA-AuNPs	0.03−40	30	Signal-on	6
Electrochemical	eATRP	0−0.14	1.63	Signal-on	8
Fluorometric	CdSe/ZnS QDs@SiO_2 NPs	0.01−40	4.0	Signal-on	9
Fluorometric	Metal nanoclusters	0.4−3.0	100	Signal-on	10
Fluorometric	Gold nanoclusters	0.01−40	4.0	Signal-on	11
PEC	ALP	0.05−100	170	Signal-on	15
ECL	AuNPs	0.01−10	5.0	Signal-on	17
Electrochemical	TiO_2 NPs	0−1.0	200	Signal-on	18
Electrochemical	RCA	5.0−500	500	Signal-on	29
Electrochemical	eRAFT polymerization	0−0.14	1.02	Signal-on	This work

*The normal range of extracellular PKA activity in the serum of healthy individuals is 0−10.6 mU mL^{-1}.

Table S-3: Detection of PKA Activity in Serum Samples

		Commercial ELISA kit		The as-fabricated biosensor	
Samples	PKA spiked (mU mL^{-1})	PKA detected (mU mL^{-1})	Recovery (%)	PKA detected (mU mL^{-1})	Recovery (%)
1	0	4.47			
2	70	72.65	97.56	73.02	98.05
3	140	147.53	102.12	148.16	102.55