

Supporting Information for

Enzymatically modified peptide surfaces: Towards general electrochemical sensor platform for protein kinase catalyzed phosphorylations

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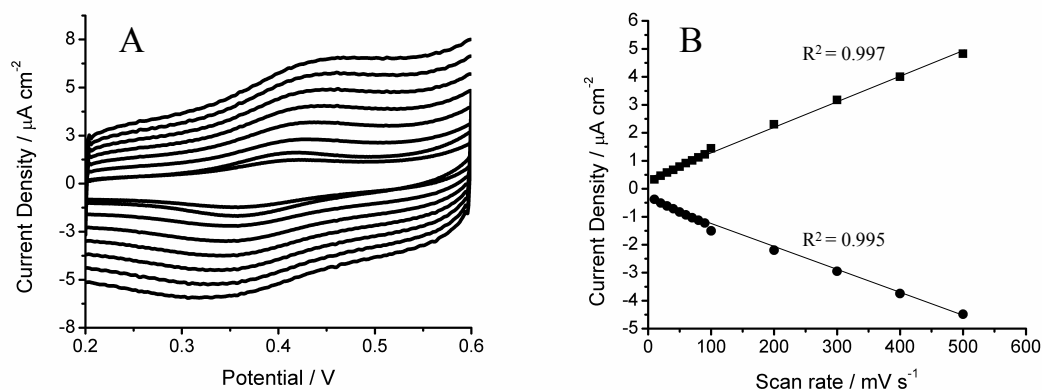


Figure S1. (A) Cyclic voltammograms of peptide modified Au electrode in the presence of Src (1 $\mu\text{g/ml}$) as a function of scan rate. (B) Plot of anodic and cathodic current density vs. scan rate. The current response is also dependent on the scan rate which is expected for the surface bound ferrocene-based film. At the sweep rates in 10 – 1000 mV range the linearity is maintained indicating the presence of surface bound Fc group and the absence of diffusive contributions. However, the changes in the potential with increasing scan rate suggest quasi-reversible behaviour in the system.

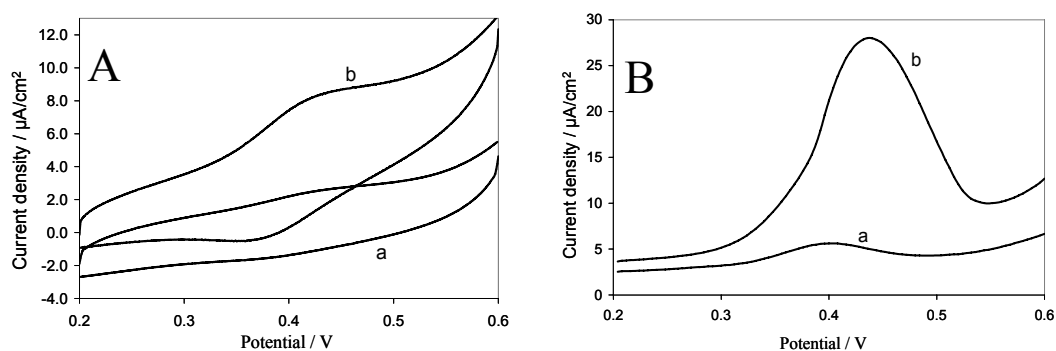


Figure S2. Cyclic voltammograms (A) and square-wave voltammograms (B) of 100 μM substrate peptide-modified gold surface electrodes for detection of Erk1 protein kinase ($10 \mu\text{g mL}^{-1}$). (a) in the absence of protein kinase and (b) in the presence of protein kinase. Measurements were taken in 0.1 M sodium phosphate buffer (pH 7.4) versus Ag/AgCl as reference electrode and Pt wire as counter electrode at 100 mV s^{-1} .

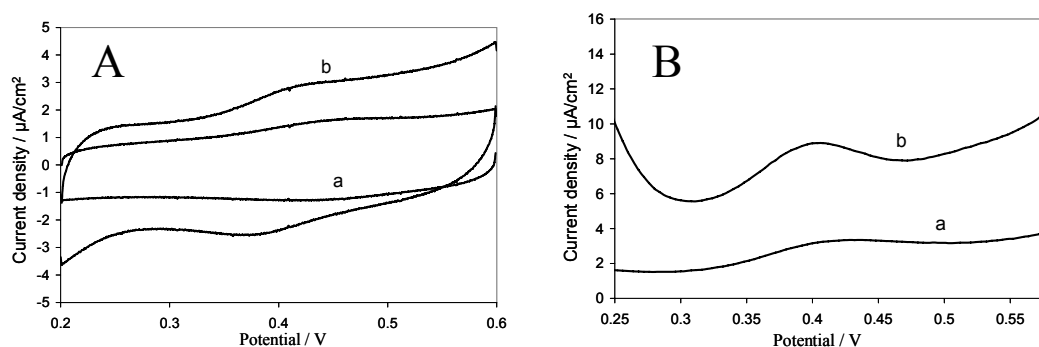


Figure S3. Cyclic voltammograms (A) and square-wave voltammograms (B) of 100 μM substrate peptide-modified gold surface electrodes for detection of CDK2/cyclin A protein kinase ($10 \mu\text{g mL}^{-1}$). (a) in the absence of protein kinase and (b) in the presence of protein kinase. Measurements were taken in 0.1 M sodium phosphate buffer (pH 7.4) versus Ag/AgCl as reference electrode and Pt wire as counter electrode at 100 mV s^{-1} .

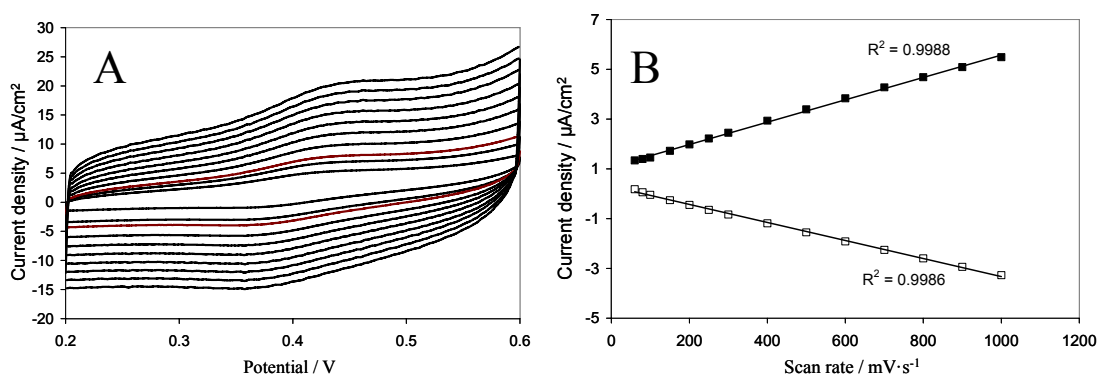


Figure S4. (A) Cyclic voltammograms of peptide modified Au electrode in the presence of Erk1 kinase (10 µg mL⁻¹) as a function of scan rate. (B) Plot of anodic and cathodic current density vs. scan rate.

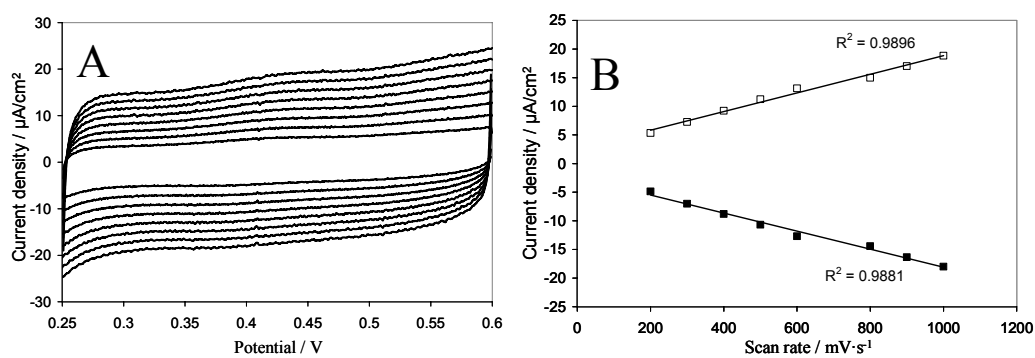


Figure S5. (A) Cyclic voltammograms of peptide modified Au electrode in the presence of CDK2/cyclin A kinase ($1 \mu\text{g mL}^{-1}$) as a function of scan rate. (B) Plot of anodic and cathodic current density vs. scan rate.

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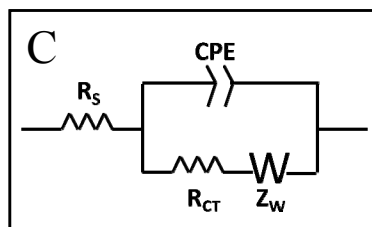
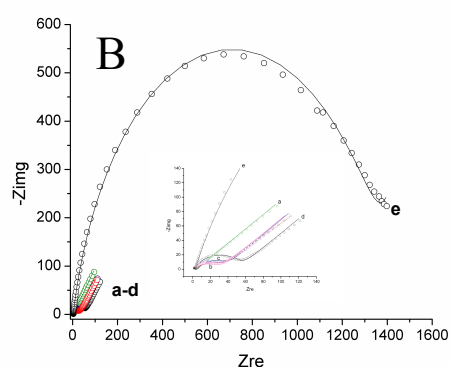
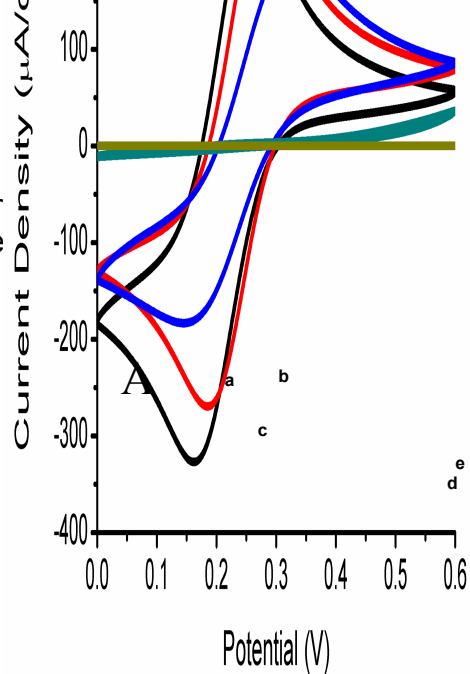


Figure S6. Surface characterization of the peptide biosensor in a 0.1 M sodium phosphate buffer (pH 7.4) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe. (A) Cyclic voltammograms (B) EIS spectra: (a) Bare Au electrode, (b) modification with NHS-ester, (c) immobilization of peptide substrate, d) after back-filling and e) following the phosphorylation reaction. (C) equivalent circuit used to fit the impedance spectra.

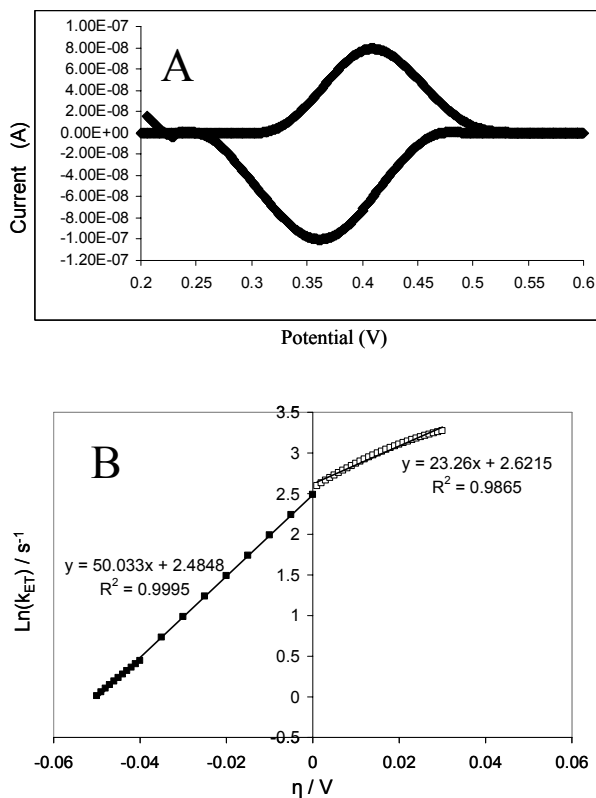


Figure S7. (A) Normalized background corrected cyclic voltammogram of current vs. potential. (B) Tafel plot for determination of k_{ET} rate for peptide films on Au surface following the Src-catalyzed phosphorylation. Tafel plots were used to obtain the electron-transfer rates, k_{ET} , for the corresponding peptide films.¹

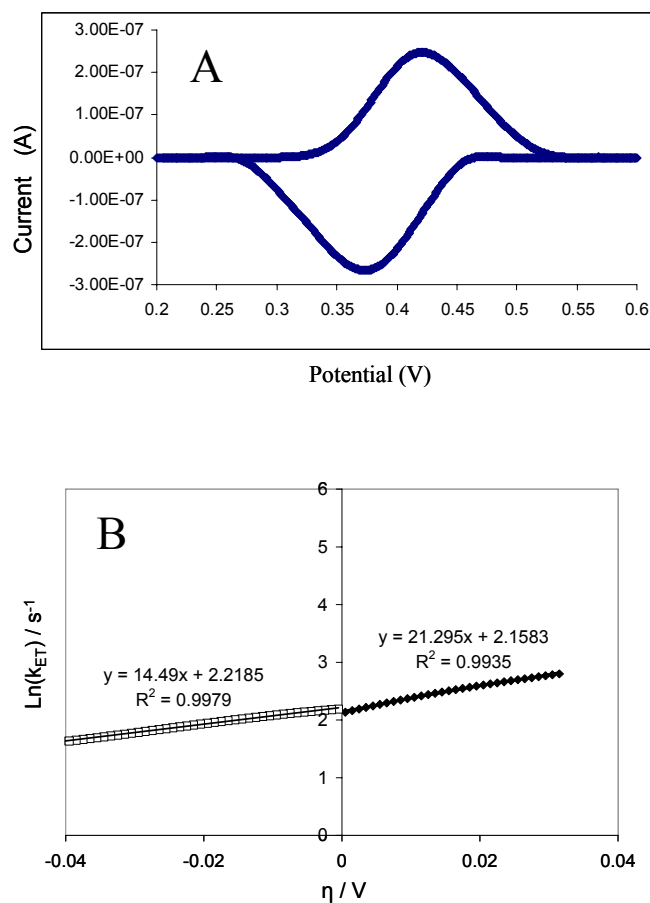


Figure S8. (A) Normalized background corrected cyclic voltammogram of current vs. potential. (B) Tafel plot for determination of k_{ET} rate for peptide films on Au surface following the Erk1-catalyzed phosphorylation.

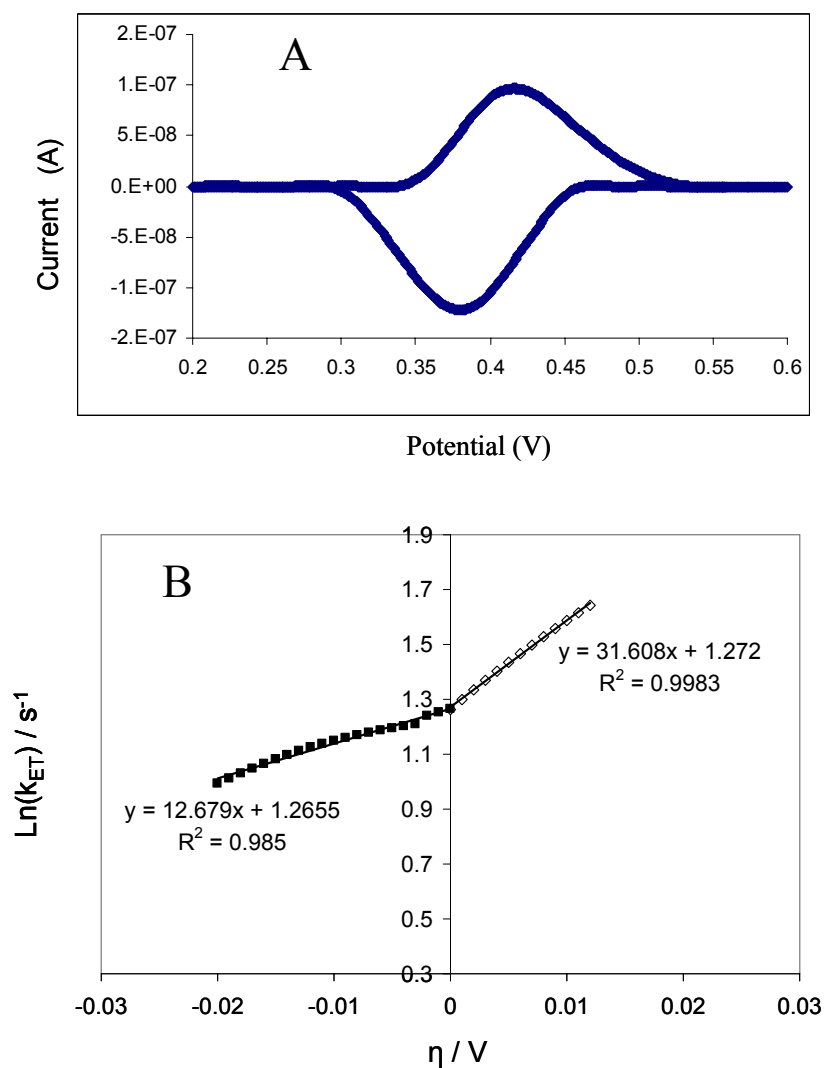


Figure S9. (A) Normalized background corrected cyclic voltammogram of current vs. potential. (B) Tafel plot for determination of k_{ET} rate for peptide films on Au surface following the CDK2/cyclin A-catalyzed phosphorylation.

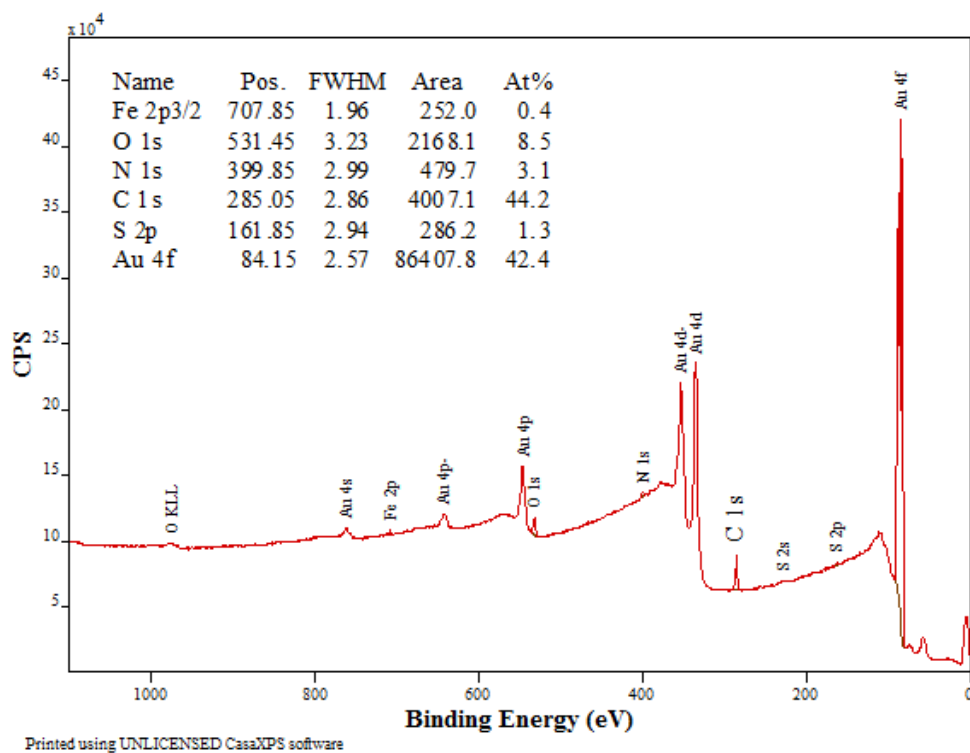


Figure S10. X-ray photoelectron spectroscopy survey scans of Au surface following the kinase-catalyzed peptide phosphorylation.

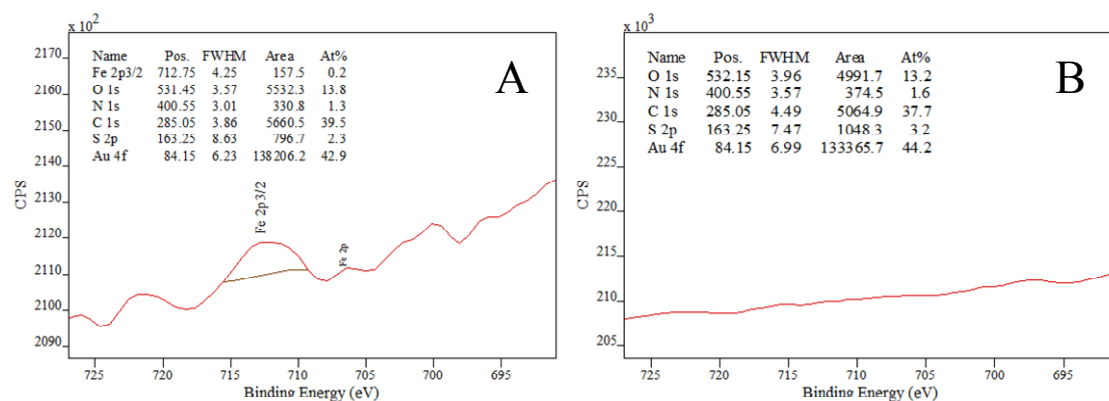


Figure S11. XPS survey scan of Src kinase assays on the flat Au substrates containing Src peptide substrate and Fc-ATP: A) in the presence and B) in the absence of Src protein kinase.

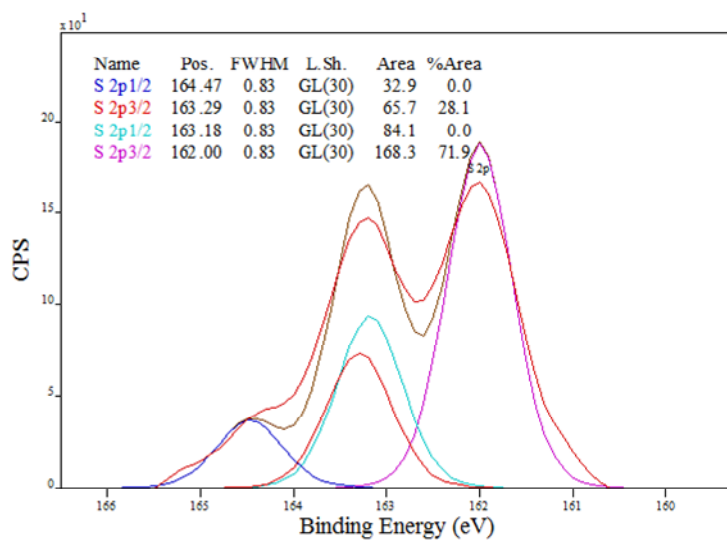


Figure S12. A typical S 2p core level of the peptide-immobilized Au surface following the Src-catalyzed phosphorylation reaction in the presence of Fc-ATP.

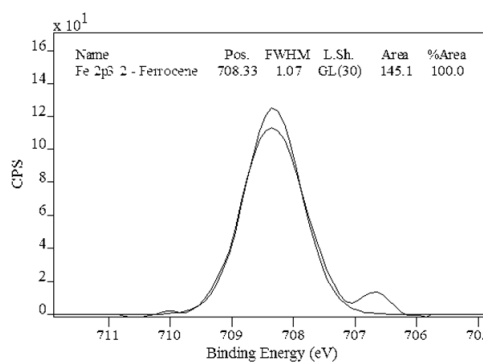


Figure S13. High resolution XPS scan of Fe 2p_{3/2} core level of the peptide-immobilized Au surface after Src kinase-catalyzed phosphorylation reaction in the presence of Fc-ATP.

References

- ¹ (a) G. A. Orlowski, S. Chowdhury and H.-B. Kraatz, *Langmuir*, 2007, **23**, 12765-12770.
- (b) G. A. Orlowski, S. Chowdhury and H.-B. Kraatz, *Electrochimica Acta*, 2007, **53**, 2034-2039.