

Supporting Information:

Sensing of C-Reactive Protein Using an Extended-Gate Field-Effect Transistor with a Tungsten Disulfide-Doped Peptide-Imprinted Conductive Polymer Coating

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S1. Experimental

S1.1 Reagents.

Three C-reactive protein peptides, KESDTSYVSLKAPL (pK), ICTSWESASGIVEF (pI), RALKYEVQGEVFTK (pR), were ordered from Yao-Hong Biotechnology Inc. (HPLC grade, New Taipei City, Taiwan). Aniline (AN), tungsten disulfide (WS₂ 90 nm avg. particle size (SEM)), recombinant C-reactive protein (CRP, #C1617) expressed in *E. coli*, and human serum (#H4522-20ML) from human male AB plasma that was of USA origin and sterile-filtered were acquired from Sigma-Aldrich Co. (St. Louis, MO). M-Aminobenzenesulfonic acid (MSAN) was acquired from Acros Organics, New Jersey). All chemicals were used as received unless otherwise mentioned.

S1.2 Electropolymerization of WS₂-doped peptide K-imprinted poly(AN-co-MSAN)s on electrodes.

The peptide KESDTSYVSLKAPL (pK) was added to an equimolar solution of AN and MSAN at various concentrations (0–0.5 wt%) in 57 mM of DI water, as shown in Scheme 1 [1]. Indium tin oxide (ITO) glasses (from RuiLong glass in 200 × 370 × 0.7 mm³, with surface resistivity 7 Ω/sq) were cut to 1.0 × 2.0 cm² in size and were immersed in the monomer/WS₂/template mixture for electropolymerization (1.0 × 1.0 cm²). Tungsten disulfide nanoflakes were then added to the monomer mixtures at concentrations of 0–0.5 wt% to prepare the WS₂-doped peptide-imprinted or non-imprinted poly(AN-co-MSAN)-coated electrodes. These electrodes were then washed in 10 mL of 5 vol% ethanol at 130 rpm for 10 min on an orbital shaker (OSR201-1, GenePure technology, Taichung, Taiwan), and this procedure was repeated using a pure DI water wash. The same washing procedure was used to remove any bound CRP during the reusability studies. The electrodes were then connected to a potentiostat (CHI 400C, CH Instruments, Inc., Austin, TX), and cyclic potential (−0.6 to 0.6 V vs. Ag/AgCl at 0.1 V/s scan rate) was applied [1]. The counter and reference electrodes were platinum wire and Ag/AgCl (RE-1B, ALS Co. Ltd., Tokyo Japan).

S1.3 Characterization of peptide K-imprinted poly(AN-co-MSAN) conductive films with and without doping with WS₂ nanoflakes.

The electrochemical reactions between the target molecules and electrodes were controlled and monitored with a potentiostat (608-1A, CH Instruments, Inc., Austin, TX). The working, counter, and Ag/AgCl reference electrodes were immersed in a 20 mL solution containing 125 mM KCl, 5 mM K₄Fe(CN)₆, and 5 mM K₃Fe(CN)₆. The potential was scanned from -0.6 V to 0.6 V at 100 mV/s unless otherwise mentioned, and the effects of the peptides or proteins on the peak currents for the ferri-/ferrocyanide system were recorded [2,3]. AC impedance was characterized (ZENNIUM/IM6, Zahner-electrik GmbH & Co KG) for electrodes coated with WS₂/pKIP or NIPs/WS₂ [4]. Measurements were made with buffer, 1.0 pg/mL of pK, or CRP.

S1.4 CRP measurements in diluted human serum samples on the FET platform

Scheme 1 shows the setup of the FET platform. For the MOS transistor (CD4007UBE, Texas Instruments Incorporated, Dallas, Texas, USA), the gate-to-source voltage V_{GS} was applied at a constant voltage source of 1.0 V generated by the dc power supply (GPC-30300, GW Instek, Taipei, Taiwan) in series with the potential difference between the WE and RE electrodes; the drain-to-source voltage V_{DS} was applied by a voltage source V_D generated by the dc power supply (GPC-30300, GW Instek, Taipei, Taiwan), with voltages ranging from 0.5 V to 5 V and increasing at 0.5 V increments every 10 minutes. The constant voltage source $V_G = 1.0$ V prevented the MOS transistor from being biased at the cut-off region. The potential difference between WE and RE heavily depends on two factors: the doping conditions of the electrodes and the concentration of the solution. The gate-to-source voltage V_{GS} of the MOS transistor was adjusted accordingly based on the specific doping level of the electrodes and on the concentration of the solution. Therefore, we were able to measure the drain-to-source current I_{DS} of the MOS transistor with a digital multimeter (GDM-7145, GW Instek, Taipei, Taiwan) and complete the transfer characteristics resulting from the triode region to the saturation region of the MOS transistor for specific electrode-doping conditions or solution concentrations.

The peptide K-imprinted poly(AN-co-MSAN)-coated electrode was used as WE on the FET platform to measure different concentrations of CRP in 125 mM KCl, 5 mM K₄Fe(CN)₆, and 5 mM K₃Fe(CN)₆ buffer solution to construct a calibration curve based on the Hill equation (Fig. 3). Samples were incubated with the electrode for 10 minutes to reach equilibrium. Human serum from human male AB plasma was diluted into the same buffer for real samples. Human C-reactive protein enzyme-linked immunosorbent assay (ELISA) kit (#KHA0031, Invitrogen - Thermo Fisher Scientific) was purchased for the human male AB plasma measurements, and the protocol from the product sheet was followed (<https://www.thermofisher.com/elisa/product/CRP-Human-ELISA-Kit/KHA0031>).

S1.5 Data Analysis

All experiments were carried out in triplicate, and data are expressed as means \pm standard deviation.

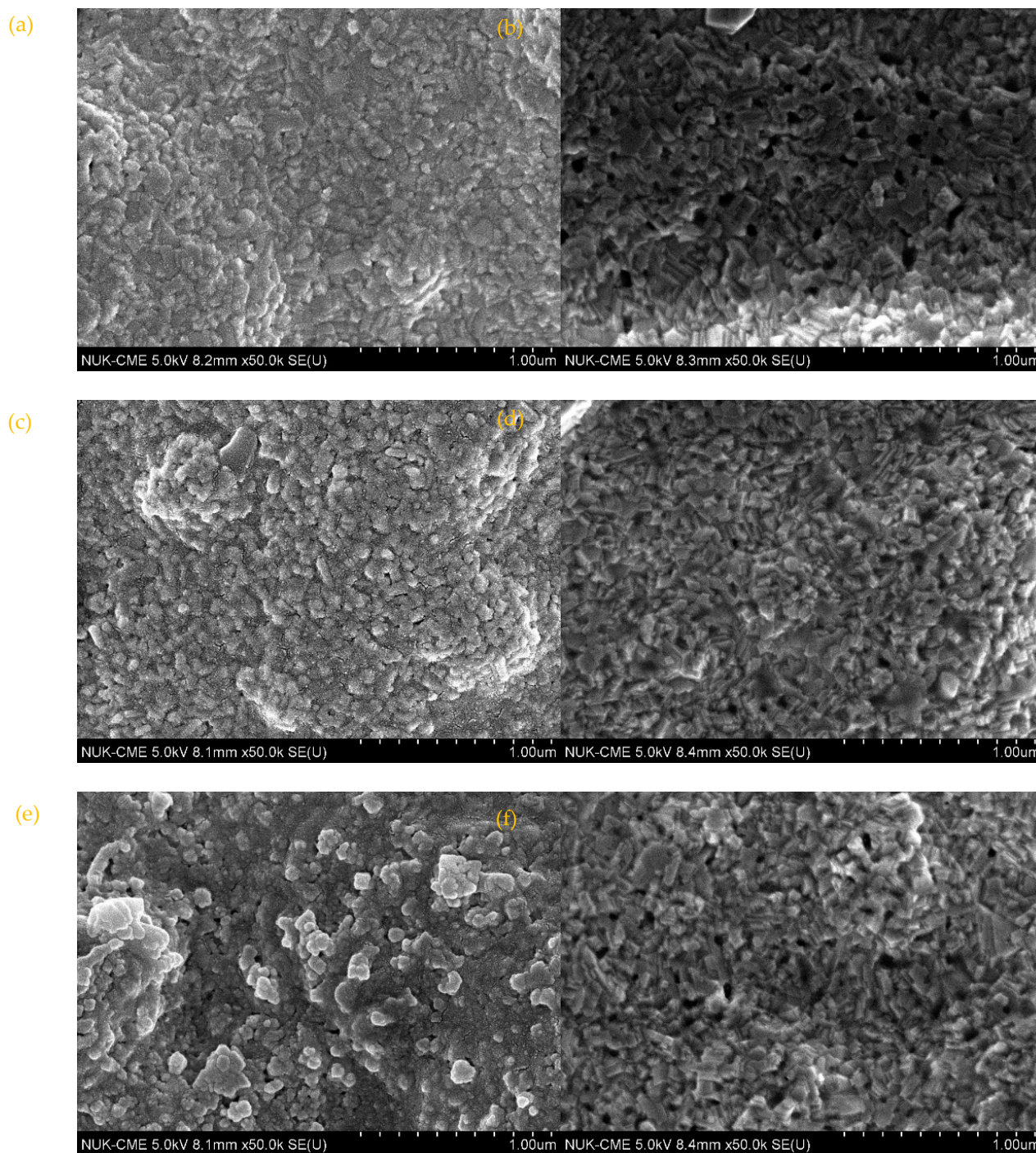


Figure S1. SEM images of 90 nm WS_2 -(0.5 wt%) doped (a) NIPs and (b) peptide K-imprinted poly(AN-co-MSAN)-coated electrodes before template removal ((a) and (b)), after template removal ((c) and (d)), and after rebinding peptide K ((e) and (f)).

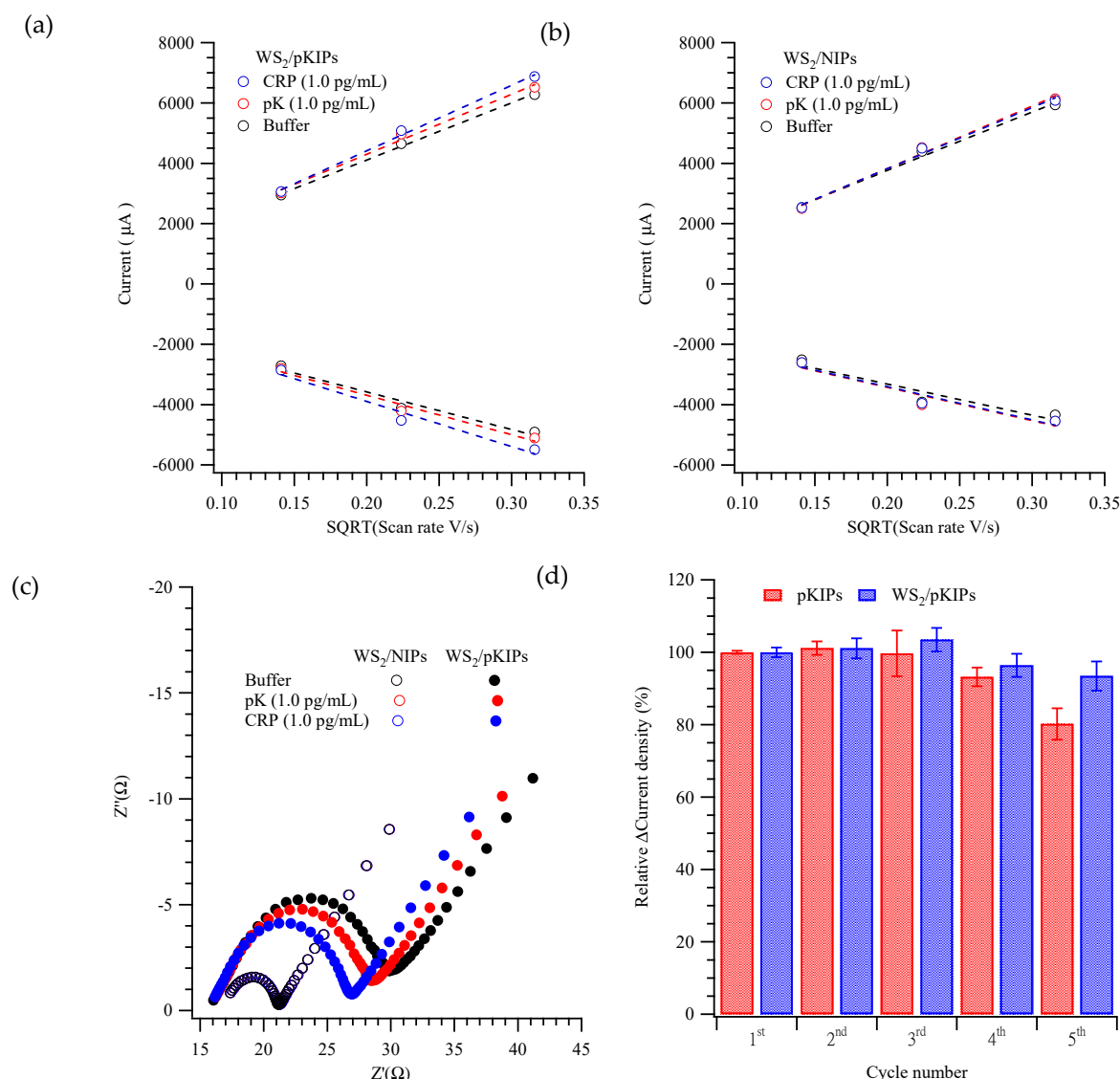


Figure S2. The electrochemical response of (a) WS₂/NIPs and (b) WS₂/pKIPs-coated electrodes in buffer, 1.0 pg/mL of pK, or CRP. The responses were then fit using the Randles–Sevcik equation for the solution at 25°C, $i_p = 268600n^{3/2} AD^{1/2}Cv^{1/2}$. (c) AC impedance measurements for WS₂/NIPs- and WS₂/pKIPs-coated electrodes in buffer, 1.0 pg/mL of pK, or CRP solutions. (d) The reusability of the WS₂/NIPs and WS₂/pKIPs-coated electrodes. The electrode was used to measure a 1.0 ng/mL solution of CRP and was then rinsed and reused for five cycles.

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