Supplementary Material for

Highly Sensitive Protein Detection Based on a Novel Probe with Catalytic Activity Combined with a Signal Amplification Strategy: Assay of MDM2 for Cancer Staging

Hao Li[a], Haona Xie[a], Yue Huang[a], Bing Bo[a, c], Xiaoli Zhu[b], Yongqian Shu[c], Genxi Li[a, b, *]

[a] *Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, P. R. China*

[b] *Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, P. R. China*

[c] *Department of Oncology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, P. R. China*

* Corresponding author at: Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, PR China. Fax: +86 25 83592510.

2.1 Materials and Chemicals

The MDM2-binding peptide (11-mercaptoundecanoic acid (MUA)-TSFAEYWNLLSP, lyophilized powder, purity>95%) was custom-synthesized by Chinaoligopeptide Co, Ltd. (Shanghai, China). DNA sequence (5'-GTGCTACGTGCTGCCTAG-C_6-SH-3') and complementary sequence (5'-CTAGGCAGCACGTAGCAC-3') were custom-synthesized by Takara Co, Ltd. (Dalian, China). Recombinant human murine double minute 2 (hMDM2) (>98%) was purchased from ProSpec-Tany TechnoGene Ltd. Benzylviologen (BV), cucurbit[8]uril (CB[8]) and methylene blue were purchased from Sigma-Aldrich. All the other reagents were of analytical-grade. The stock solution of DNA was prepared by diluting to 0.4 μM with 10 mM Tris−HCl buffer, pH 7.4. Equal molar ratio of the two complimentary DNA strands were heated at 95 ℃ for 2 minutes and slowly cooled to room temperature to produce double-strand oligonucleotides. The stock solution of the peptide was prepared by dissolving the powder to 5 μM with 10 mM phosphate buffer solution (PBS) (pH 7.4). The standard sample of MDM2 was prepared by dissolving the protein with 10 mM PBS (pH 7.4) and then diluting it to desired concentrations with the same buffer. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of 18 MΩ·cm. Tissue samples of non-small cell lung cancer patients were obtained from Department of Oncology, the First Affiliated Hospital of Nanjing Medical University after elective cesarean, which were approved by the local hospital ethical committees. Immediately after biopsy, the tissue samples were sliced,

lysed and fractioned using a Nuclear Extract Kit (Active Motif, CA), the nuclear fraction was retained and 100× diluted with 10 mM PBS (pH 7.4) for MDM2 quantification. Using the above Nuclear Extract Kit, control nuclear fraction was extracted from a human non-small cell lung cancer A549 cell line (Type Collection of Chinese Academy of Science), the MDM2 expression of which is silenced using MDM2 siRNA (Sant Cruz Biotech, Inc.) following the manufacture's instruction. The cell line was cultured in RPMI 1640 medium (Gibco co.) supplemented with 8 mM L -glutamine (Gibco co.) and 5% fetal calf serum (FCS) (Hyclone co.) and maintained in a humidified atmosphere with 5% CO_2 at 37°C.

2.2 Preparation of the Probe.

The probe with catalytic activity was prepared as below. Briefly, BV and CB[8] powder (equal molar amounts) were dissolved in 10 mM PBS (pH 7.0) to 750 μM followed by moderate vortex-agitating overnight. This solution was prepared freshly each time and brought to experimental application immediately subsequent to the agitation process.

2.3 Electrode Treatment and Modification.

3 mm diameter gold disk electrode was firstly immerged in piranha solution (30% H_2O_2, 70% concentrated sulfuric acid) for 5 min, followed by rinsing with double-distilled water. Then the electrodes were polished in sequence with 1 μm and 0.3 μm alumina slurry. Ultrasonicating in ethanol and water was subsequently used to

remove residual alumina powder. Finally, the electrodes were dipped in nitric acid (50%) for 30 min, followed by electrochemical cleaning using 0.5 M H_2SO_4.

After being dried with mild stream of nitrogen, the electrode #1 was soaked in the peptide assembly solution (2.5 μM substrate peptide and 5mM TCEP in 10mM PBS, pH 7.4) at 4 ℃ for 16 h, where TCEP was adopted to prevent disulphide formation between peptides. Then, the electrode was dipped in 100 μL 9-mercaptononanol (MNH) solution (1 mM MNH in 10 mM PBS, pH 7.4) at room temperature for 3 h. Finally, non-specific absorbed MNH was removed by thorough rinsing of the modified electrode, which was subsequently dried under mild stream of nitrogen.

The electrode #2 was soaked in DNA assembly solution (0.2 μM pretreated dsDNAs, 10 mM TCEP and 0.1 M EDTA in 10 mM Tris−HCl buffer, pH 7.4) at room temperature for 16 h to form a self-assembled monolayer of dsDNAs on the electrode surface, followed by rinse with double-distilled water.

2.4 MDM2 detection.

Firstly, the above prepared peptide modified electrode, i. e., the electrode #1 was interacted with standard or biological samples containing MDM2 for 2 h at room temperature. After that, the peptide-modified electrode was thoroughly rinsed with double-distilled water, followed by being dipped in 5% Tween-20 for 30 min to exclude non-specific adsorption. Then, the MDM2-bound electrode was incubated with solution containing the probe (750 μM probe in 10 mM PBS pH 7.0) at 4 ℃ for 16 h to label the MDM2-free peptides. After this step, the peptide-modified electrode

could be used to optimize the labeling conditions. The electrode was then subject to potentiostatic electrolysis at -1.0 V for 1 min in 1 ml buffer solution (10 mM PBS pH 7.0), thus the probes were collected in this solution. The DNA-modified electrode, i. e., the electrode #2 was then dipped in this solution and exposed to UV radiation (PLS-LAM500 high-voltage mercury lamp, Perfectlight Co, Ltd.) for 40 min. Finally, the DNA-modified electrode was thoroughly rinsed with double-distilled water and immersed in a 0.1 mM methylene blue solution for 10 min, after gentle rinse, the DNA-modified electrode was ultimately ready for measurements.

2.5 Electrochemical Measurements.

Electrochemical measurements were carried out on a CHI660D Potentiostat (CH Instruments) with a conventional three-electrode system: the modified electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode. Cyclic voltammograms (CVs) and square wave voltammograms (SWVs) were recorded in 10 mM PBS, pH 7.4, which was deoxygenated by purging with nitrogen gas and maintained under this inert atmosphere during the electrochemical measurements. Experimental parameters are as follows. Cyclic voltammetry: scan range, -0.9 ~ 0.3 V and -0.45 ~ -0.2 V, scan rate, 0.1 V/s. Square wave voltammetry: scan range, -0.9 ~ -0.2 V and -0.6 ~ 0.0 V, step potential, 5 mV, frequency, 15 Hz, amplitude, 25 mV. The data were obtained from at least three times of repetition of independent experiment.

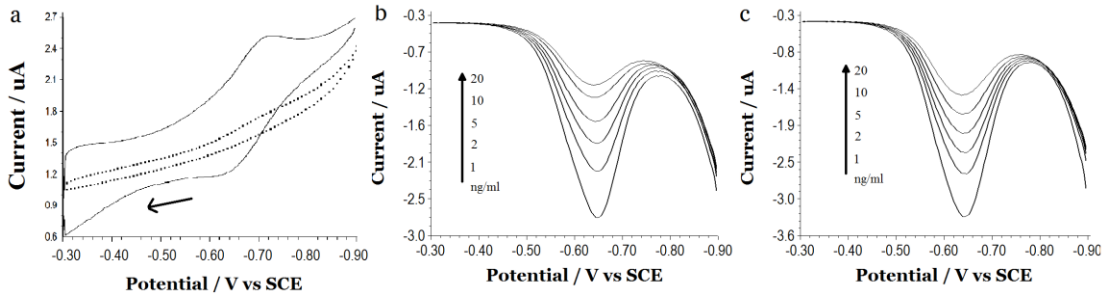
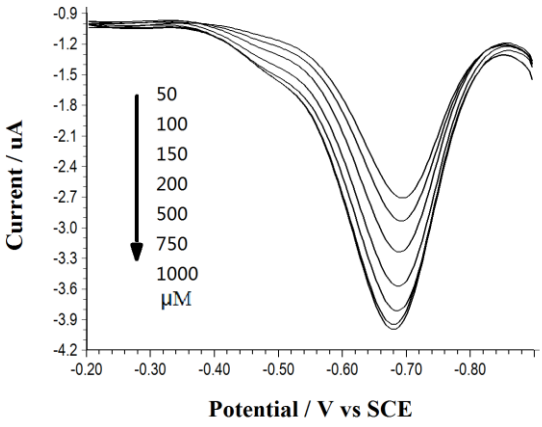


Figure S1. (a) CVs obtained at the peptide-modified electrode (solid line) and bare electrode (dotted line) after incubation with 750 μM probe. The arrow marks the scan direction. Scan rate: 0.1 V/s. (b) SWVs obtained at the peptide-modified electrode, which has been treated first with different concentrations of MDM2, and then with 750 μM probe. (c) SWVs of probes electrochemically detached from the peptide-modified electrode previously treated as described in panel (b).



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2 Figure S2. SWVs obtained at the peptide modified electrode after incubation with

3 different concentrations of the probes.

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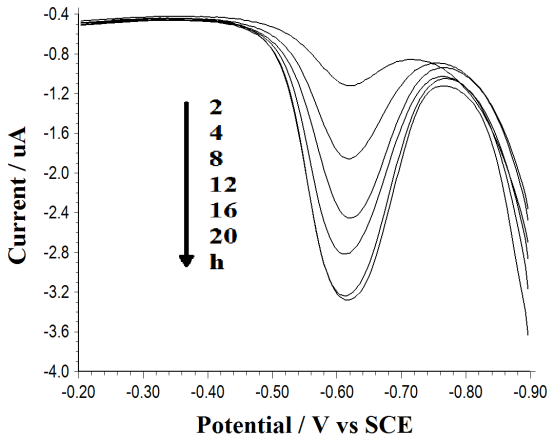

Figure S3. SWVs obtained at the peptide modified electrode after incubation with 750 μM probe for different hours.

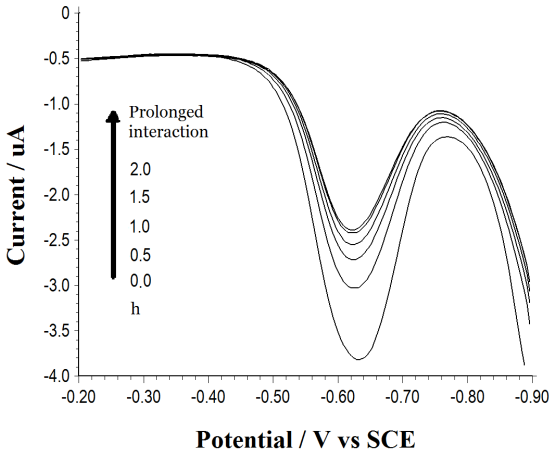

Figure S4. SWVs obtained at the peptide modified electrode to optimize the time of interaction between MDM2 and its binding peptides. The electrode has been treated first with 2 ng/ml MDM2 for different time, and then with 750 μM probe.

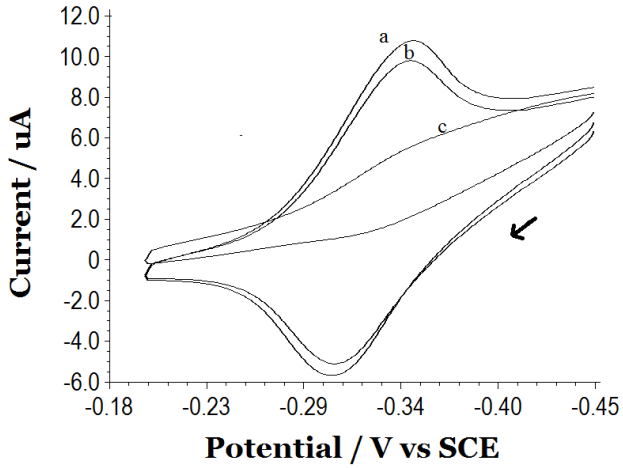

Figure S5. Cyclic voltammograms (CVs) obtained at the dsDNA modified electrode, which has (a) been treated with methylene blue without any pretreatment, (b) first undergone photo-cleavage in the absence of the probe, then been treated with methylene blue, and (c) first undergone catalytic photo-cleavage (that is to undergo photo-cleavage in the presence of the probe), then been treated with methylene blue. The arrow marks the scan direction. Scan rate: 0.1 V/s. Time of UV-radiation for photo-cleavage: 2 hours. For curve (c), the photo-cleavage is catalyzed by the probes electrochemically detached from a peptide-modified electrode fully labeled with 750 μM probe, and without previous incubation with any MDM2.

The result in Figure S5 (curve b compared with curve a) may manifest the interference of UV-radiation to our assay. Since the central aspect of our method is to use the catalytic activity of the probe to transduce the variance in the amount of MDM2 to the variance in the amount of residual dsDNAs after photo-cleavage, the precision of this method demands that no factors other than the catalytic activity of the probe can result in the cleavage of the dsDNAs. However, as shown in Figure S5,

curve b, prolonged UV-radiation alone can result in dsDNA cleavage even in the absence of the probe. Because the final readout is obtained by quantifying the amount of the remaining dsDNAs, this spontaneous cleavage by UV-radiation can make the readout appear to be smaller than its real value, namely, that resulted solely from the catalytic cleavage by the probe. Therefore the UV-radiation can lead to false negative of the readout, the time of UV-radiation must be optimized to check its interference (Figure S6).

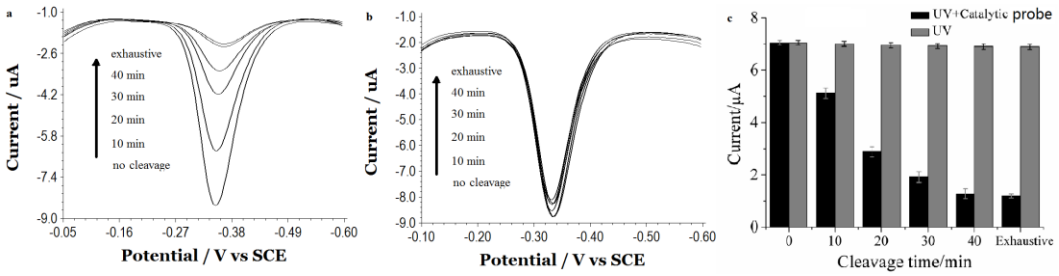

Figure S6. (a) SWVs obtained at the dsDNA modified electrode, which has first undergone catalytic photo-cleavage for different time and then been treated with methylene blue. The photo-cleavage is catalyzed by the probes electrochemically detached from a peptide-modified electrode fully labeled with 750 μM probe, and without previous incubation with any MDM2. (b) SWVs of control experiments. The photo-cleavage is done by UV radiation alone, without the catalysis of the probes. All the other procedures are the same as in (a). (c) Kinetics of photo-cleavage in the presence and absence of probes measured by the corresponding peak currents in panel (a) and (b).