Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information for:

Peptide Microarray-Based Fluorescence Assay for Simultaneously Detecting Matrix Metalloproteinases

Zhen Lei,[a,b] Jiaxue Gao,[a,b] Xia Liu,[a,b] Dianjun Liu[a] and Zhenxin Wang[a]*

[a]State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, P. R. China. E-mail: wangzx@ciac.ac.cn

[b]University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing, 100049, P. R. China

Contents

Additional experimental section

Fabrication of Peptide Microarray Peptide substrates in spotting buffer (0.3 M PBS (pH 8.5, 0.2 M NaCl) with 20 μg/mL BSA and 35% (v/v) glycerol) with desired concentrations were spotted onto the aldehyde 3-D glass slides using a SmartArrayer 136 system (CapitalBio Ltd., Beijing, China). After an overnight incubation under vacuum at 30 °C, the slides were rinsed with 50 mL phosphate buffer (pH 7.5, 50 mM) supplemented with 1% (w/v) BSA, and then incubated in blocking buffer (pH 7.5, 50 mM PB, 0.15 M NaCl containing 1% (w/v) BSA and 1% (v/v) ethanolamine) for 1 h to inactivate remaining free aldehyde groups on the slide surface. After the blocking reaction, the slides were subjected to a series of washing steps: (1) 30 mL Milli-Q water for 3 min (3 times), (2) 30 mL of washing buffer (pH 7.5, 20 mM Tris, 0.15 M NaCl, 10 mM EDTA with 0.1% Triton X-100) for 10 min (2 times), and (3) 30 mL of TCNB buffer (50mM Tris, 10mM $CaCl_2$, 150mM NaCl and 0.05% Brij-35, pH 7.5) for 10 min (2 times), respectively. After dried by centrifugation (300 g), the peptide microarray was divided into 12 independent subarrays by a PTFE masker, and employed to detect the activities of MMPs.

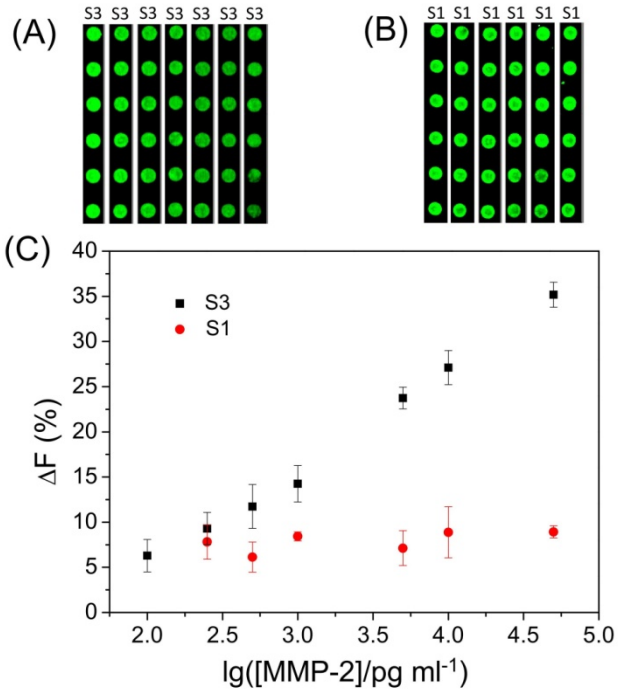

Figure S1 (A and B) Fluorescence images of subarrays and fluorescence intensity changes (ΔF%) of substrate S3 and S1 cleaved by various concentrations of MMP-2.

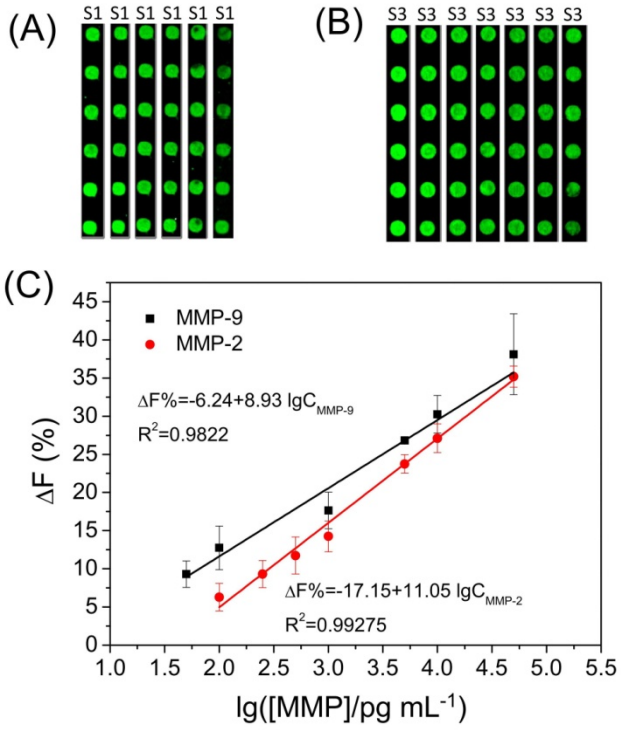

Figure S2 Fluorescence images of subarrays (A, MMP-9 and B, MMP-2) and (C) the fluorescence intensity change (ΔF%) of substrate S3 as a function of logarithm of MMP concentration.

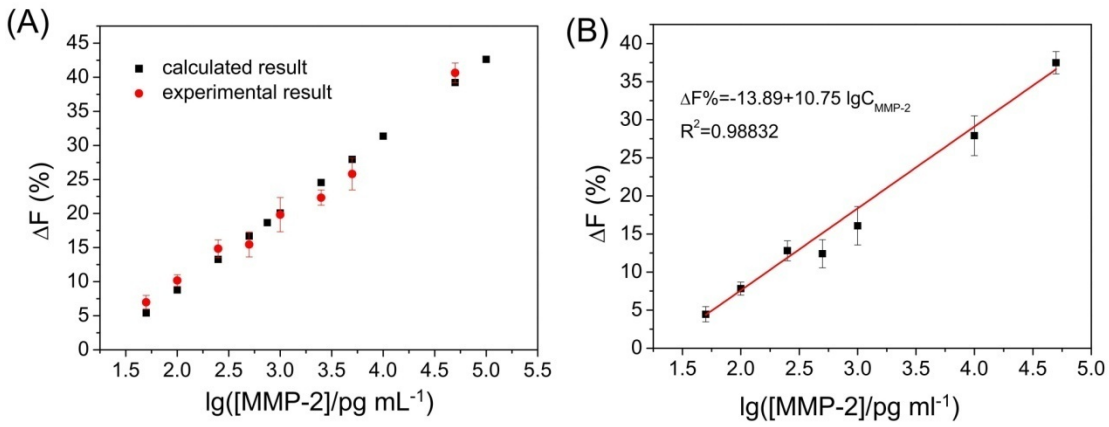

Figure S3 (A) The experimental and calculated fluorescence intensity changes (ΔF%) of substrate S3 responding to various MMP-2 concentrations in the mixture. (B) The actual fluorescence intensity change (ΔF%) as a function of logarithm of MMP-2 concentration in the MMPs mixture.

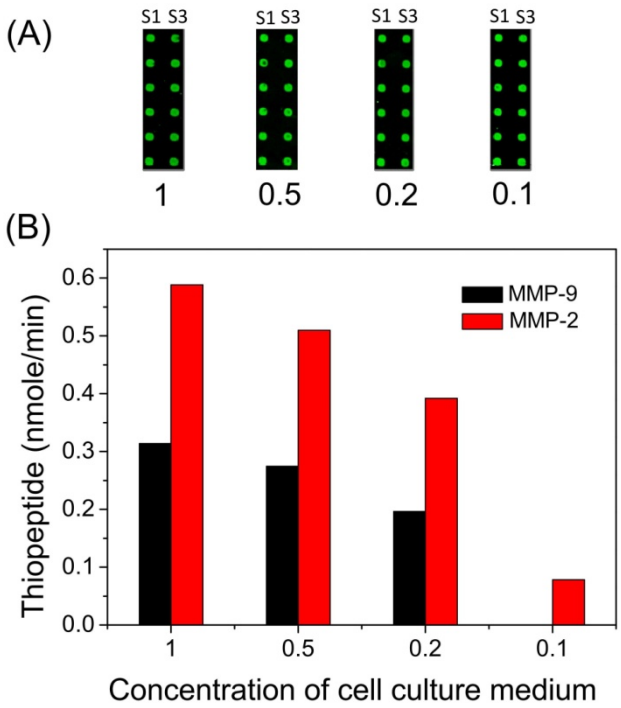

Figure S4 (A) The corresponding fluorescence images of subarrays in Figure 5A. (B) Activities of MMPs in the different concentrations of MDA-MB-231 cell culture medium determined by commercial kits.

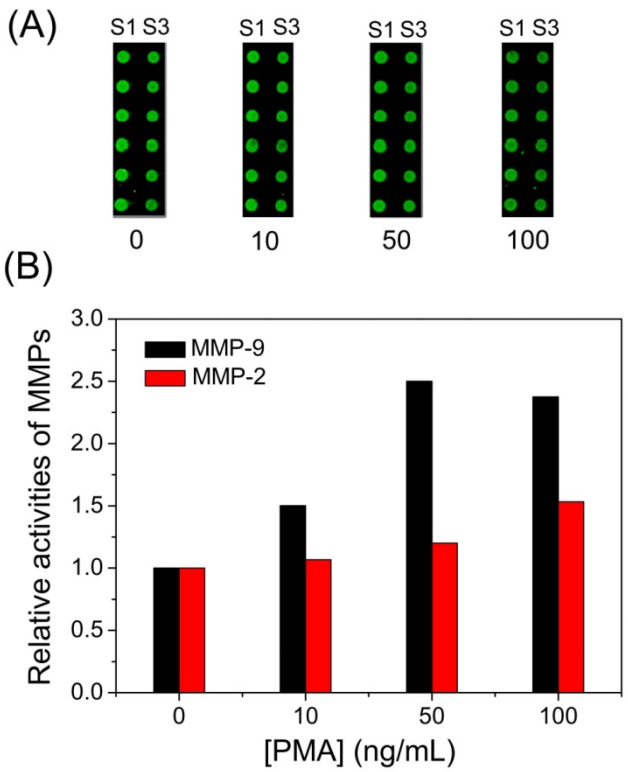

Figure S5 (A) The corresponding fluorescence images of subarrays in Figure 5B. (B) Relative activities of MMPs in the MDA-MB-231 cell culture medium stimulated with various concentrations of PMA determined by commercial kits.

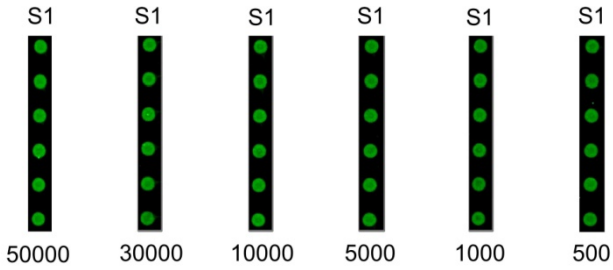

Figure S6 The corresponding fluorescence images of subarrays in Figure 6.