SUPPORTING INFORMATION FOR

On-Chip Synthesis of Protein Microarrays from DNA Microarrays Via Coupled In Vitro Transcription and Translation for Surface Plasmon Resonance Imaging Biosensor Applications

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PCR Amplification of Encoding DNA

PCR amplification was performed in order to add amino linkers to the encoding DNA templates. A 50 uL PCR reaction mixture containing 0.2 uM of two primers, 0.2 ug of the DNA vector and the enzyme master mix (Qiagen) was amplified for 30 cycles. Green fluorescent protein (GFP) expression vector was based on pIVEX 2.3d (Roche). Luciferase expression vector was kindly provided by professor Jennifer Prescher at University of California, Irvine. The PCR product was purified with QIAquick PCR Purification Kit (Qiagen) and eluted in water. The primers used were:

5' -/5AmMC12//iSp18//iSp18/GATCTCGATCCCGCGAAATTAATAC - 3'
5' - AAAAAACCCCTCAAGACCCGTTTAG - 3'

The forward primer was modified with a 5'- amine linker (5AmMC12) and two internal spacers (iSp18) (IDT). The concentrations of purified encoding DNA were measured by Nanodrop 2000 spectrometer (Thermo Scientific).

Microarray Fabrication

The 16 element microarrays were created on the SF-10 glass substrates (Schott Glass). First, gold film spots (1 mm diameter, 45 nm thickness) with 1 nm underlayer of chromium were deposited using a Denton DV-502A metal evaporator. The slides were then immersed in a 1mM ethanolic solution of 11-amino-1-undecanethiol hydrochloride (MUAM, Dojindo) for at least 12 h. After rinsing with ethanol, water and drying with N_2, the slides were exposed to 2mg/mL poly-L-glutamic acid (PGlu, MW=2,000-15,000, Sigma) in PBS buffer and incubated at room temperature for 1 h to form an electrostatically adsorbed pGlu monolayer. After a second rinsing and drying step, a PBS buffer that contained PCR amplified encoding dsDNA(5 μM) or N-(5-Amino-1-carboxypentyl)iminodiacetic acid (NTA, Dojindo, 100 μM), together with 75 mM 1-ethyl-3-(3-(dimethylamino) propyl)carbodiimide hydrochloride (EDC; Pierce) and 15 mM N-hydroxysulfosuccinimide (NHSS; Pierce), was spotted onto each microarray element (0.5 μL per spot) and incubated at room temperature for 12 hours to covalently link the dsDNA or NTA to the pGlu and the pGlu to the MUAM monolayer. After another rinsing and drying step, 1mM $CuSO_4$ aqueous solution was spotted onto the detector NTA elements and the slides were incubated on a shaker at room temperature for 30 min, followed by blocking with 3% BSA on a shaker for 15 min and washing with 1x PBS buffer.