A Quartz Crystal Microbalance-based Molecular Ruler for Biopolymers

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Electronic Supporting Information:

Two models of QCM were used here, namely Q-Sense E4 (Q-Sense, Gothenburg, Sweden) and a home-built QCM with a control software purchased from Resonant Probes GmbH (Goslar, Germany). Experiments were typically run at both machines and analysis indicated no significant differences.

Chemicals were purchased from various sources. Anhydrous N, N□-dimethylformamide (DMF), succinic anhydride, 4-(dimethylamino)pyridine (DMAP) were purchased from Aldrich. Goat anti-Rabbit IgG and Rabbit IgG were purchased from Bioss (Beijing, China). N-ethyl-N-[3-(dimethylamino)-propyl]-carbodiimide (EDC) and Hydroxy-2,5-dioxopyrolidine-3-sulfonicacid sodium salt (NHSS) were purchased from Medpep (Shanghai, China). Glycine (Gly) was purchased from Dingguo (Beijing, China). The 5'-thiol modified stem-loop DNA and its complementary strand were both purchased from Takara Biotechnology Co., Ltd.(Dalian, China) and used without further purification. The sequences were 5'- HS-(CH2)6-ggccgtTACTCCCTTCCTCCCCGCacggcc-3' and 5'-GTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATA-3' (Lowercase letters indicates the pairing bases). All DNA solutions were diluted to the concentration of 1 μM with PBS buffer before immobilization and hybridization. 6-Mercapo-1-hexanol (MCH) was diluted to 1 mM with PBS before use. The 11-mercaptoundecyl-tri(ethylene glycol) self-assembled monolayer (EG-SAM) chips were obtained from HRBio (Beijing, China).

IgG and anti-IgG. The terminal hydroxyl groups of EG-SAM were converted to terminal carboxyl groups by immersing the chips into a DMF solution containing succinic anhydride (10 mg mL^{-1}) and DMAP (15 mg mL^{-1}) for 12 hours at room temperature. The chips were then rinsed with DMF, methanol several times to remove salt particles and dried with nitrogen. The functionalized QCM chip was placed in a QCM sensor chamber and the temperature was set to 25 ℃. The QCM was operated in a flow-through mode. A baseline was established by passing PBS buffer (10mM, pH = 7.4) at a speed of 40 μL min^{-1}. The carboxyl groups were activated by a 5 min injection of an aqueous mixture of EDC (0.1 M) and NHSS (0.2 M). IgG was then pumped through sensor chamber by a peristaltic pump, series concentrations of IgG were as follows: 1, 10, 25, 50, 100 and 250 μg mL^{-1}. PBS buffer was finally passed through to establish the second stable baseline. The remaining active carboxyl groups were deactivated by ethanol amine (EtAmine at 1M, pH = 8.5). Anti-IgG was then introduced to the IgG surface (immobilization step with 25 μg mL^{-1}) at different concentrations, 50, 25, 12.5, 6.26 and 1 μg mL^{-1}. The sensor surface was then regenerated by washing with Gly (100 mM, pH = 2.0) to remove anti-IgG.

DNA immobilization and hybridization. A QCM chip was first loaded to a QCM sensor so that

its absolute resonating frequency was obtained. Then, PBS (pH 7.4) solution was pumped into the QCM chamber (S1 in Figure 2). After a stable baseline was established, a solution of 1 μM stem-loop DNA in PBS (0.1M PB, 1M NaCl, pH 7.4) was introduced and the immobilization of DNA on the chip was monitored online. Then, the DNA solution was replaced by PBS, and a solution of 1 mM MCH in PBS was pumped into the chamber and incubated for 20 min. The MCH solution was replaced by PBS to obtain a stable baseline (S2 in Figure 2), and the frequency shift induced by DNA immobilization was recorded as Δf_{im}. After that, a solution of 1 μM complementary strands in PBS was introduced and the DNA hybridization was also monitored online. Finally, PBS was introduced to obtain a baseline after 20 minutes of hybridization (S3 in Figure 2), the corresponding frequency shift was denoted as Δf_{hy}.