

Rapid one-step biotinylation of biological and non-biological surfaces

Stephen Henry^{1*}, Eleanor Williams¹, Katie Barr¹, Elena Korchagina², Alexandr Tuzikov², Natalia Ilyushina³, Sidahmed A. Abayzeed⁴, Kevin F. Webb⁴, Nicolai Bovin^{1,2*}

¹AUT Centre for Kode Technology Innovation, School of Engineering, Computer & Mathematical Sciences, Auckland University of Technology, Auckland, New Zealand.

²Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation

³FDA CDER, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA

⁴Optics & Photonics Research Group, School of Electrical & Electronic Engineering, University of Nottingham, United Kingdom

Supplementary Note 5 Coating of gold SPR chip with biot-CMG₂-DOPE, and interaction of modified chip with avidin

To study the interaction of biot-CMG₂-DOPE with surfaces in real time, we used a bespoke surface plasmon resonance (SPR) instrument ¹. The instrument used in the experiment features a high sensitivity of $\sim 1 \times 10^{-7}$ refractive index units (RIU), providing a detection limit approximately two orders of magnitude superior to current commercial systems. The SPR sensing surface was a glass slide coated with a thin film of pure gold (50 nm). A custom flow cell/handling system was used to expose the bare gold to biot-CMG₂-DOPE (1:50 in PBS from 1mg/mL solution = 9.6 μ M) at a flow rate of 500 μ L/min (8.33 μ L/s). The flow cell was perfused at this steady rate for 10 min, at which time the flow was halted and the SPR signal was monitored for 2 hours. Binding of biot-CMG₂-DOPE to the gold surface was measured at 10Hz, as an increase in refractive index at the gold surface - detectable as a shift in the SPR resonance angle (Figure s5-1). The rising phase of surface binding was fit, using MatLAB, to a monoexponential equation of the following form:

(Eq 1) $R = S(1 - e^{-\tau t})$ where R is the measured index (in RIU), S is the steady-state binding maximum, t is time, and τ is the binding rate constant.

For the binding of biot-CMG₂-DOPE to gold, the fitted time constant of binding at room temperature was 127.66s, reaching a steady state of 0.847×10^{-3} RIU by 360s. Binding remained extremely stable over more than 2 hours of continuous monitoring. The SPR sensor surface was then washed for 60 minutes with PBS, throughout which surface binding appeared stable.

Subsequently, to verify functional biotinylation, avidin ("Bio-Ultra", Sigma-Aldrich, 1.5×10^{-10} M in PBS) was added to the chamber at the same constant flow rate. Binding of avidin to the biot-CMG₂-DOPE coated surface was monitored for one hour, and the rising phase of the response (representing specific binding) was fit to Eq1 to extract a time constant of 230.78s, and a steady-state alteration in refractive index of 1.77×10^{-3} RIU.

[1] Abayzeed, S.A., et al. Responsivity of the differential-intensity surface plasmon resonance instrument. *Sensors and Actuators B: Chemical* **235**, 627-635 (2016):.

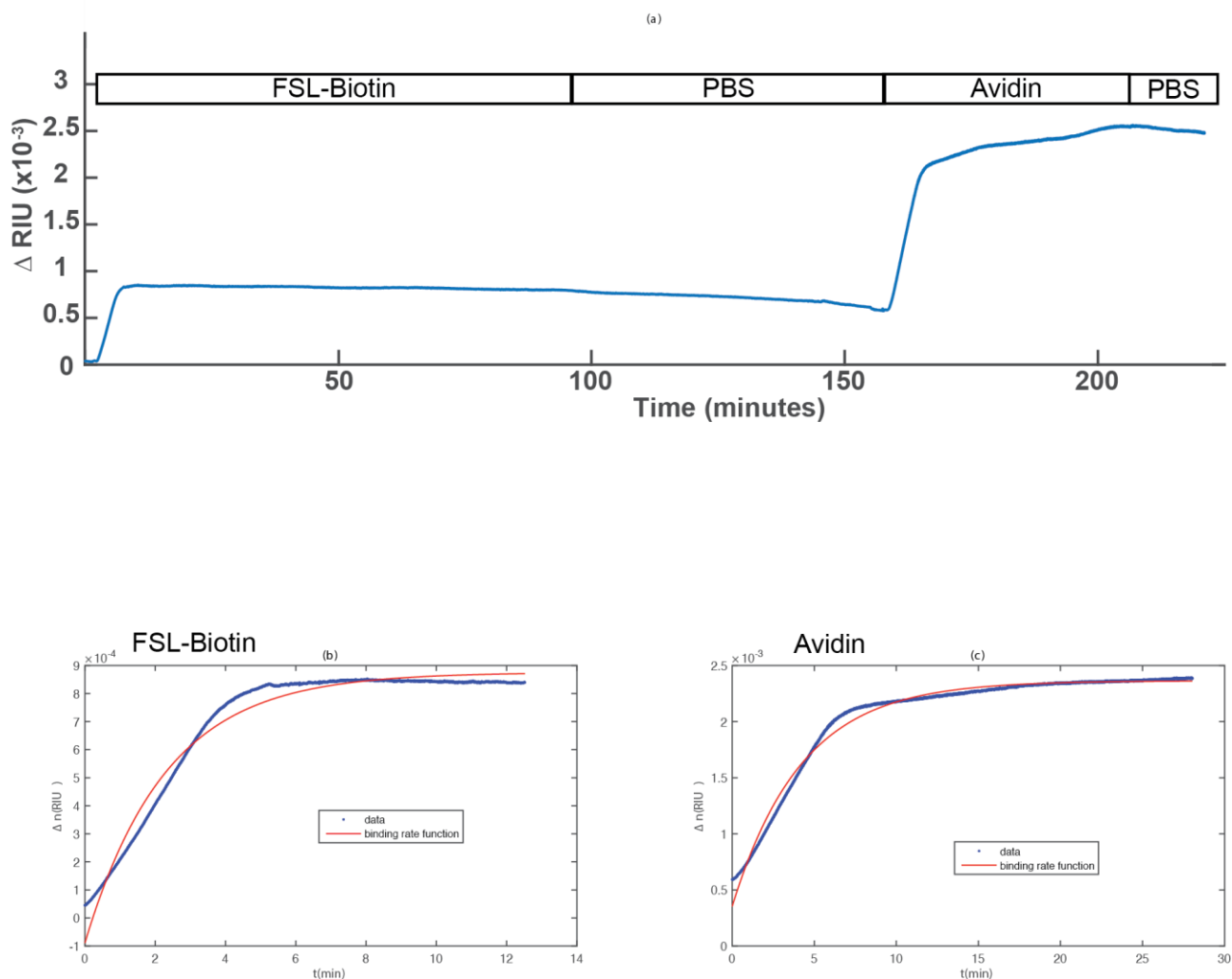


Figure s5-1. Coating of gold SPR chip with biot-CMG₂-DOPE, and interaction of modified chip with avidin. System configuration - from reference [1]: A differential-intensity surface plasmon resonance sensing platform was employed, adopting a prism-based Kretschmann-Raether configuration. A 633 nm linearly-polarised laser is coupled through a prism (BK7, $n = 1.515$) to the sensor-sample interface. A cylindrical lens is inserted into the beam path so that the light beam is focused into a line on the sensor chip. The angle of convergence of the light beam, inside the prism, is $\sim \pm 3^\circ$. Refractive index matching oil (Refractive Index 1.5150) was used between the prism and the sensor chip. The illumination resonance angle was set to $\sim 72^\circ$ to excite surface plasmons on the surface of the chip. Since the incident angle is greater than the critical angle, total internal reflection occurs at the sensor-sample interface. The reflected light is collimated using a second cylindrical lens and detected using a CMOS camera (μ Eye, IDS GmbH, Germany). Signal processing was performed via MatLAB, using a differential-intensity analysis approach which is described in the cited reference.

Stock solutions: biot-CMG₂-DOPE ($M_w = 2079.28$ g/mol, 1:50 in PBS from 1 mg/mL stock = $9.6 \mu\text{M}$); avidin ($M_w = 66\text{kDa}$, 1:100 in PBS from 1 mg/mL stock = 1.5×10^{-10} M).

(a) experimental curve for binding of biot-CMG₂-DOPE to the gold chip, followed by washing and binding of avidin to the formed biotinylated surface; (b) and (c) the respective fitted curves, which we contemplated placing together into an inset figure to show the comparison of rising phases.