Supplemental Information

Title: Time resolved single-step protease activity quantification using nanoplasmonic resonator sensors

Author: C. Sun, K.H Su, J. Valentine, X. Zhang, et. al.

Peptide synthesis

R6G-Ava-HSSKLQLAAAC-NH_2 401 mg (0.277 mmol) of Rink Amide AM polystyrene resin (loading 0.69 mmol/ g) was added to a 12 mL fritted syringe and swollen with NMP (4 mL). The Fmoc protecting group was removed by treatment with 1:2:2 piperidine/NMP/CH_2Cl_2 solution (3 mL) for 30 min, and the resin was filtered and washed with NMP (3 x 3 mL) and CH_2Cl_2 (3 x 3 mL). To load the α-amino acid residues, the resin was subjected to repeated cycles of coupling conditions (method A or method B), followed by washing (5 x 3 mL NMP, 5 x 3 mL CH_2Cl_2), Fmoc deprotection [treatment with 1:2:2 piperidine/NMP/CH_2Cl_2 solution (3 mL) for 30 min], and washing again with NMP (5 x 3 mL) and CH_2Cl_2 (5 x 3 mL). The first α-amino acid residue was loaded by addition of a preformed solution of Fmoc-Cys(Trt)-OH (1.17 g, 2.00 mmol), PyBOP (1.04 g, 2.00 mmol), and HOBt (270 mg, 2.00 mmol) in 1:1 NMP/CH_2Cl_2 (2 mL) onto the resin and the resulting slurry was stirred for 5 min on a wrist-action shaker, followed by addition of *i*-Pr_2EtN (0.55 mL, 4.0 mmol). The reaction was allowed to proceed for 5 h. The resin was then filtered, washed (5 x 3mL NMP, 5 x 3 mL CH_2Cl_2), and dried under high vacuum. The loading of Cys was determined to be 0.60 mmol/g (78% yield). Successive couplings were achieved either by method A or method B.

Method A consists of addition a preformed solution of Fmoc-protected amino acid [Fmoc-Cys(Trt)-OH (1.17 g, 2.00 mmol), Fmoc-Ala-OH (622 mg, 2.00 mmol), Fmoc-Leu-OH (707 mg, 2.00 mmol), Fmoc-Gln(Trt)-OH (1.22 g, 2.00 mmol), Fmoc-Ser(*t*Bu)-OH (767 mg, 2.00 mmol), and Fmoc-His(Trt)-OH (1.24 g, 2.00 mmol)], PyBOP (1.04 g, 2.00 mmol), and HOBt (270 mg, 2.00 mmol) in NMP/CH_2Cl_2 (1:1, 2 mL), followed by addition of *i*-Pr_2EtN (0.55 mL, 4.0 mmol). The reactions were allowed to proceed for at least 4 h. Method B consists of subjection of the resin to a a 0.4 M solution of the suitably protected acid [Fmoc-Lys(Boc)-OH (375 mg)], which had been pre-activated by incubation with DIC (130 μL, 0.84 mmol) and HOBt (108 mg, 0.800 mmol) in DMF (2 mL) for 10 min. The coupling was allowed to proceed for 4 h. After each coupling the resin was filtered and washed (NMP: 5 x 3mL, CH_2Cl_2: 5 x 3 mL), followed by removal of the Fmoc protecting group. After coupling and deprotection of the final α-amino acid residue, the Ava linker was added by subjection of the resin to a 0.4 M solution of Fmoc-S-Ava-OH (272 mg, 0.800 mmol) which had been pre-activated by incubation with DIC (120 μL, 0.80 mmol) and HOBt (108 mg, 0.800 mmol) in N-methylpyrrolydinone (1 mL) for 10 min. The coupling was allowed to proceed overnight. The resin was filtered and washed (5 x 3mL NMP, 5 x 3 mL CH_2Cl_2), the Fmoc protecting group was removed, and the resin washed again. The rhodamine group was incorporated by adding a 0.4 M solution of rhodamine 19 (412 mg, 0.8 mmol), which had been pre-activated by incubation with DIC (130 μL, 0.84 mmol) and HOBt (108 mg, 0.800 mmol) in NMP (2 mL) for 10 min. The reaction was allowed to proceed for 6 h, the coupling procedure was repeated once more and the reaction was allowed to proceed overnight. The substrate was cleaved from the resin by incubation with a solution of 94:2:2:2

TFA/triisopropylsilane/H_2O/ethanedithiol (3 mL) for 2 h, purified using preparatory C18 reverse-phase HPLC (CH_3CN/H_2O-0.1% TFA, 5-95% for 50 min, 20 mL/min, 220/254/280 nm detection for 100 min, t_R = 24.3 min), and lyophilized. MS (MALDI), *m/z* calcd for $C_{78}H_{116}N_{19}O_{17}S$: 1622.85. Found: *m/z* 1623.90.

Instrument setup

Micro-region SERS measurements are performed on an inverted optical microscope (Axiovert 200, Zeiss) with matched high resolution grating spectrameter (Triax 550, Jobin Yvon). To excite the particles plasmons, the Nanoplasmonic Resonators (NPRs) were illuminated with a collimated laser beam (Frequency doubling YAG laser, 532 nm) through a right angle prism at an angle resulting in a total internal reflection (TIR) configuration. An evanescent electromagnetic wave is generated and used to excite the particle plasmons. The samples are attached to the bottom of the prism by applying index matching oil between the sample substrate and the prism. Such sample configuration effectively eliminates all stray scattering light due to surface defects and dust particles, leading to significantly reduced background signal. The excited collective electron oscillations within the particles then radiate electromagnetic waves of the same frequency into the far field, whereby the collection and spectral measurement takes place. The scattered light from the NPRs is then collected by a 50× long working distance objective. A holographic notch filter (532 nm, Kaiser Optical System) was placed in the beam path in order to remove the illuminating beam. The emerging light is then imaged onto the entrance slit of the grating spectrometer system with a liquid Nitrogen cooled charge coupled device (CCD) camera for spectrum analysis.

The extinction spectrum measurement was performed in a similar configuration. In this measurement, 150W Xenon white light source has been used as the illumination source and the collimated light was delivered through an optical fiber bundle.

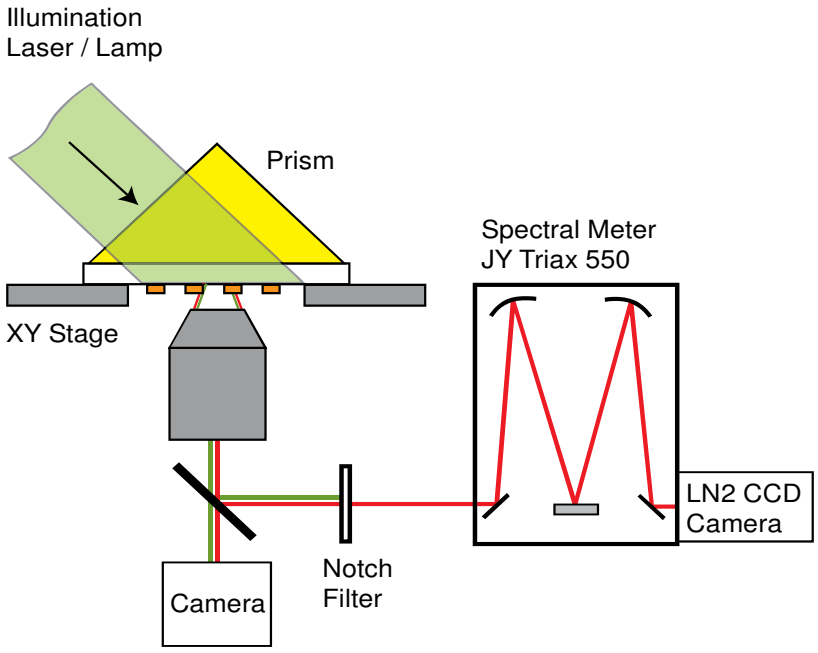

Figure S1. Schematic drawing of the optical spectrum measurement setup.

Estimation of the detection volume of NPR-based nano-sensor

To estimate the detection volume of the NPR-based nano-sensor, the diffusion length is first calculated as $L_D = \sqrt{Dt} \sim .5mm$ where D is estimated as $1\text{x}10^{-6}\ cm^2/s$ and t is $1800s$. The un-normalized detection volume is then determined as $V = (L_A + L_D)^2 L_D \sim 1\times10^{-7} L$ where L_A is the length of the NPR array ($15\mu m$). This volume must be normalized due to the fact that the molecules have an equal likelihood to

diffusion in any direction. The likelihood of these molecules coming in contact with the NPR array can be estimated based on the surface area of the array and the total surface area available for diffusion. This probability of molecules can diffuse to the NPR array is then given by: $W_{Det} = \frac{L_A^{\ 2}}{6L_D^{\ 2}} \sim 1.5x10^{-4}$ assuming the NPR array surface area of the array is much smaller than the available diffusion surface area. The total sampling volume is then calculated as $V_{Det} = W_{Det}V \sim 15pL$.