

Supplementary Information: Estimation of Pfs Specific Activity

Materials. Mouse anti-polyhistidine and goat anti-mouse IgG conjugated to alkaline phosphatase were purchased from Sigma (St. Louis, MO). Glacial acetic acid, Tris base, acrylamide, Bis-acrylamide, methanol, and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ were purchased from Fisher Chemical (Fair Lawn, NJ). Sodium dodecyl sulfate (SDS), glycine, non-fat dry milk, and Tween 20 were purchased from BioRad (Hercules, CA).

Chip fabrication. The microfabrication process for the chips was reported previously¹. Briefly, 4" diameter silicon wafers were coated with 1 μm silicon nitride film, followed by deposition of 50 \AA chromium film, and finally, deposition of 2000 \AA gold film. The patterns were created by photolithography, and the photoresist removed using acetone. The chips contain two upper gold rectangular patterns (6 mm long \times 3 mm wide). The left upper pattern was where the alligator clip was attached. The upper patterns are each linked by gold lines to two lower gold rectangular patterns. The left lower gold pattern was where assembly took place, and two patterned areas were investigated: 8 mm long \times 0.5 mm wide (4 mm^2), and 8 mm long \times 4 mm wide (32 mm^2).

Pfs-chitosan conjugation, chip assembly, and disassembly. First, the chip was incubated in 1 % (w/v) BSA – PBS for 2 h, rinsed with de-ionized water, and set aside. The conjugate was prepared as described previously (see Materials and Methods section), and deposited onto the left gold electrode pattern by dipping the chip into the conjugate until the pattern was submerged and applying negative bias to the pattern (4 min at 3 A/m^2). This was done by connecting the cathode and anode (nickel chromium wire) using alligator clips to a DC power supply (Keithley 2400 SourceMeter). After deposition, the chip was rinsed with de-ionized water, and washed with gentle shaking 3 \times 5 min in 5 mL each of PBS buffer. The deposited conjugate was then resolubilized by washing the chip in 2 % (v/v) acetic acid. Next, the resolubilized conjugate samples were analyzed via Western blot.

SDS-PAGE and Western blot analysis. Sample buffer (0.0625 M Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 5% (v/v) β -mercaptoethanol, 0.025% (w/v) bromophenol blue) was mixed with the resolubilized conjugate samples 1:1 (v:v), and these mixtures were then heated at 92 – 95 $^\circ\text{C}$ for 10 minutes. Proteins were separated by SDS polyacrylamide gel electrophoresis using 12.5% acrylamide gels at 180 V for 1 hour using the BioRad Mini Protean 3 system, and blotted onto BioRad nitrocellulose membranes using a BioRad Trans-Blot semi-dry transfer cell and Bjerrum-Schafer-Nielsen transfer buffer (48 mM Tris, 39 mM glycine, 20% (v/v) methanol, 0.0375% (w/v) SDS) for 30 minutes at 15 V. Unbound membrane sites were blocked using 5% (w/v) non-fat dry milk in 20 mM Tris-HCl, pH 7.5, 500 mM NaCl. The membrane was first incubated for 2 hours at room temperature in mouse monoclonal anti-polyhistidine at 1:4,000 dilution. The membrane was then incubated at room temperature for 1 hour in goat anti-mouse IgG conjugated to alkaline phosphatase at 1:4,000 dilution. Both antibodies were diluted in 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1% (w/v) non-fat dry milk, 0.05% (v/v) Tween 20. Membranes were developed colorimetrically using Roche

NBT/BCIP stock diluted 1:50 (v:v) in 0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl, 0.05 M MgCl₂.

Estimations of Pfs specific activity. Pfs-chitosan conjugate was prepared and electrodeposited onto different electrode areas of microfabricated chips. Each deposited conjugate was resolubilized with dilute acid, and finally analyzed via Western blot. By comparing the Western blot band intensities of the resolubilized conjugate samples with that of a known quantity of purified Pfs standard, the mg Pfs in each sample (i.e. mg Pfs attached on each chip) was estimated. The mg Pfs attached was plotted against assembly area to generate a linear fit, which was extrapolated to 0.5 mm², the assembly (electrode) area inside the microchannel, to estimate the mg Pfs attached to the electrode inside the microchannel. The μmol SAH converted per minute was calculated from the % SAH conversion at 3 μL/min averaged over all reaction samples (3 μL/min × 3 min collection time = 9 μL reaction sample volume).

References

1. H. M. Yi, L. Q. Wu, R. Ghodssi, G. W. Rubloff, G. F. Payne and W. E. Bentley, *Analytical Chemistry*, 2004, **76**, 365-372.