

# Microfluidic Separation and Capture of Analytes for Single-Molecule Spectroscopy

Samuel Kim, Bo Huang, and Richard N. Zare

## Supplementary Information

### 1. Experimental Section

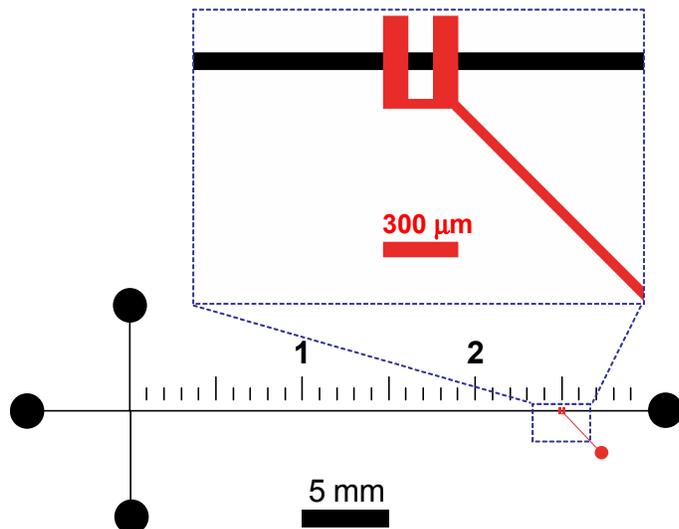
Streptavidin was labeled in the following procedure. Freshly prepared 1 M sodium bicarbonate solution (5  $\mu$ L) was added to aqueous solution of streptavidin (50  $\mu$ L, 1 mg mL<sup>-1</sup>, Molecular Probes, S-888) to adjust the pH to 8.3. Then, Alexa Fluor 647 amine-reactive dye (succinimidyl ester, <1  $\mu$ L, Molecular Probes, A-20006) in DMSO was added, and the mixture was incubated for 1 h at room temperature with vortexing every 10 min. No further purification step was performed because the microchip CE was capable of separating labeled proteins from free dye molecules.

The microfluidic channel was made using a multilayer soft lithography technique as described elsewhere.<sup>1</sup> In brief, PDMS prepolymer components (RTV615A and B, purchased from GE) were mixed at 1:10 mass ratio, poured on the master, and spincoated to create a 40- $\mu$ m thick membrane. After allowing incomplete curing (8 min at 70 °C), the valve structure molded previously was aligned and placed on the top of the channel structure. Then, more mixed prepolymer was poured on the master for a proper thickness (5 mm) and cured for 40 min at 70 °C. The lower layer of the channel structure is prepared by spincoating a diluted PDMS prepolymer mix (1:2 PDMS:cyclohexane by mass) on a coverslip (No. 1.5, VWR). The cured channel structure was cut and removed from the master, punched, and placed on the lower layer. Reservoirs were placed at four ends of channels, and the whole assembly was cured for 20 min at 120 °C for permanent bonding.

For the capillary electrophoresis experiments, a separation buffer (20 mM Hepes at pH 7.5, 0.1% *n*-dodecyl- $\beta$ -D-maltoside and 0.01% sodium dodecyl sulfate) was used. The sample was diluted into the separation buffer by a factor of 100 and added to the sample reservoir (#2). All other reservoirs were filled with a separation buffer. In the electrokinetic injection procedure, the following voltages were applied using a homemade high-voltage power supply for 17 s: 1=1kV, 2=700V, 3=0V, 4=700V. After the injection, the fluorescence measurement and separation procedure were initiated simultaneously. Voltages for separation step were 1=700V, 2=1kV, 3=700V, 4=0V.

A confocal microscope based on an inverted microscope (TE300, Nikon, USA) was used for fluorescence detection. A 638 nm laser beam (RCL-638-025, CrystaLaser, USA) was guided and spatially filtered by a single mode optical fiber (OZ Optics, Canada), and then collimated with an objective lens (5X, Zeiss) before being sent microscope. The laser beam was focused by a water immersion objective (Plan Apo, 60X, NA 1.20, Nikon, USA) after passing through a dichroic mirror (520-633 custom made, Omega Optical, USA). The fluorescence signal was collected by the same objective, passed through a 50- $\mu$ m pinhole, filtered by a bandpass filter (670DF40, Omega Optical, USA) and imaged onto an avalanche photodiode (SPCM AQR15, EG&G, Canada). The photon arrival time was recorded with a counter/timer data acquisition card (PCI-6602, National Instruments, USA). For PCH analysis, the photon arrival time data was analyzed using a data analysis program written in C and fitted to a PCH model using Igor software as previously described.<sup>2</sup>

## 2. Design of the double-T microchip



**Fig. S1.** Design of the capillary electrophoresis microchip. A blown-up view of the valve layer (red) is illustrated.

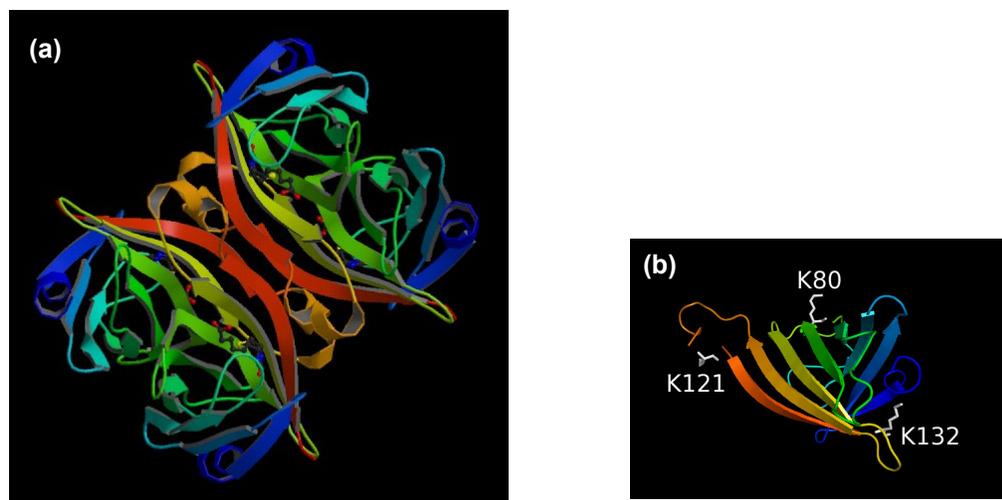
## 3. Table S1. PCH parameters obtained for a simple dye.

	Individual dye in a glass-bottom chamber		1:1 Mixture separated in a microchip	
	Cy5	A647	Cy5	A647
$\varepsilon$ (cpsm)	69,000 ( $\pm 3,000$ )	82,000 ( $\pm 3,000$ )	67,000 ( $\pm 3,000$ )	79,000 ( $\pm 3,000$ )
$N$	4.71 ( $\pm 0.05$ )	4.92 ( $\pm 0.05$ )	4.68 ( $\pm 0.05$ )	4.53 ( $\pm 0.05$ )

## 4. The location of lysine residues in streptavidin.

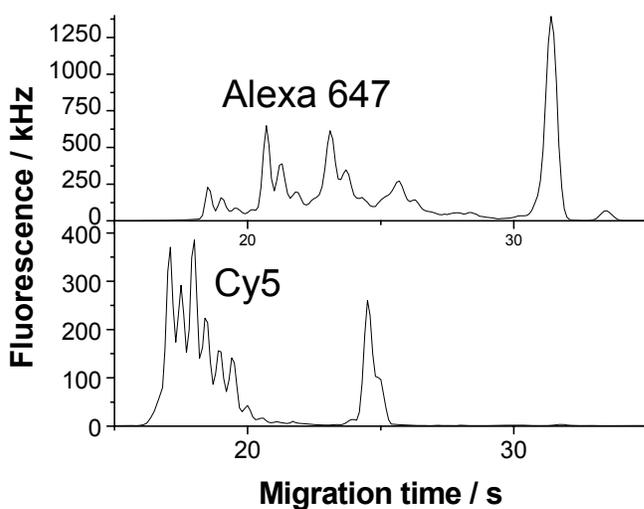
The entry 1STP in the RCSB Protein Data Bank has 121 residues (from 13 to 133) with three lysines (yellow) and one reactive N-terminus. For the case of the processed protein with 127 residues, two additional lysines (cyan) may be included into the “core” streptavidin structure.

```
>1STP: | PDBID | CHAIN | SEQUENCE
DPSKDSKAQV SAAEAGITGT WYNQLGSTFI VTAGADGALT GTYESAVGNA
ESRYVLTGRY DSAPATDGS TALGWTVAWK NNYRNAHSAT TWSGQYVGG
EARINTQWLL TSGTTEANAW KSTLVGHDTF TKVKPSAASI DAAKKAGVNN
GNPLDAVQQ
```



**Fig. S2.** (a) Assumed structure of tetrameric streptavidin from the RCSB Protein Data Bank.<sup>3</sup> (b) The subunit structure with three lysine residues highlighted (white). This image was rendered with the PyMOL software.<sup>4</sup>

### 5. Comparison of Cy5 and Alexa 647 labeling of streptavidin



**Fig. S3.** Electropherograms of streptavidins labeled with Cy5 and Alexa Fluor 647 succinimidyl esters.

### References

- 1 H. K. Wu, A. Wheeler, R. N. Zare, *Proc. Nat. Acad. Sci. U. S. A.* **2004**, *101*, 12809.
- 2 T. D. Perroud, B. Huang, M. I. Wallace, R. N. Zare, *ChemPhysChem* **2003**, *4*, 1121; B. Huang, T. D. Perroud, R. N. Zare, *ChemPhysChem* **2004**, *5*, 1523.
- 3 Image Gallery for the entry 1STP in the RCSB Protein Data Bank.  
<http://www.rcsb.org/pdb/explore/images.do?structureId=1STP> (accessed on May 16, 2007)
- 4 DeLano, W.L. *The PyMOL Molecular Graphics System* (2002) DeLano Scientific, Palo Alto, CA, USA.  
<http://www.pymol.org>