Supplementary Material (ESI) for Lab on a Chip

Rapid, continuous purification of proteins in a microfluidic device using genetically-engineered partition tags

Supporting Information

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Supplementary Materials and Methods

Construction of plasmids. The open reading frames for green fluorescent proteins (AcGFP1) and glutathione S-transferase (GST) were amplified from pAcGFP1 (Clontech Laboratories, Mountain View, CA) and pDEST15 (Invitrogen, Carlsbad, CA) respectively using the primers in Table 1, below. The amplified products were then cloned into the bacterial expression vector pET151/D-TOPO (Invitrogen) according to the manufacture's manual. All plasmid constructs were verified by DNA sequencing. A diagram of the genetic constructs is shown in Figure S1, below.

Table S1. Sequences of Oligonucleotides Used in the Cloning of AcGFP amd GST Constructs.

Name	Sequences (5′→ 3′)[a]
$(WP)_4$-AcGFP1-1Amp-forward	**CCTTGGCCTTGGCCT**ATGGTGAGCAAGGGCG
$(WP)_4$-AcGFP1-1Amp-reverse	TGCGGGGGTCACTTGTACAGCTCATCCATGCCG
$(WP)_4$-AcGFP1-2Amp-forward	CACCATG**TGGCCTTGGCCTTGGCCTTGGCC**
$(WP)_4$-AcGFP1-2Amp-reverse	TGCGGGGGTCACTTGTACAGCTCATCCATGCCG
Y_3P_2-AcGFP1-forward	CACCATG**TACTACTACCCTCCT**ATAGCAAGGGCG
Y_3P_2-AcGFP1-reverse	GGATGAGCTGTACAAGTGACCCGCGAAA
Y_3P_2-AcGFP1-P_2Y_3-forward	CACCATG**TACTACTACCCTCCT**ATAGCAAGGGCG
Y_3P_2-AcGFP1-P_2Y_3-reverse	TCA**GTAGTAGTAAGGAGG**CTTGTACAGCTCATCCATGC
$(WP)_4$-GST-1Amp-forward	**CCTTGGCCTTGGCCT**ATGTCCCCTATACTAGG
$(WP)_4$-GST-1Amp-reverse	CCAGGACAACAACGCGGAACCAGATCCG
$(WP)_4$-GST-2Amp-forward	CACCATG**TCCCCTATACTAGGTTATTGG**
$(WP)_4$-GST-2Amp-reverse	TCAGCCACCGCAACAGCCAGGACAACAACGC
Y_3P_2-GST-forward	CACCATG**TACTACTACCCTCCT**ATGTCCCCTATACTAGG
Y_3P_2-GST-reverse	GTAGTAGTAAGGAGGCTAACGCGGAACCAGATCCG
Y_3P_2-GST-P_2Y_3-forward	CACCATG**TACTACTACCCTCCT**ATGTCCCCTATACTAGG
Y_3P_2-GST-P_2Y_3-reverse	TCA**GTAGTAGTAAGGAGG**ACGCGGAACCAGATCCG

[a] The sequences in bold code for the specific residues in the tags and the sequences in underline are for directional TOPO® cloning .

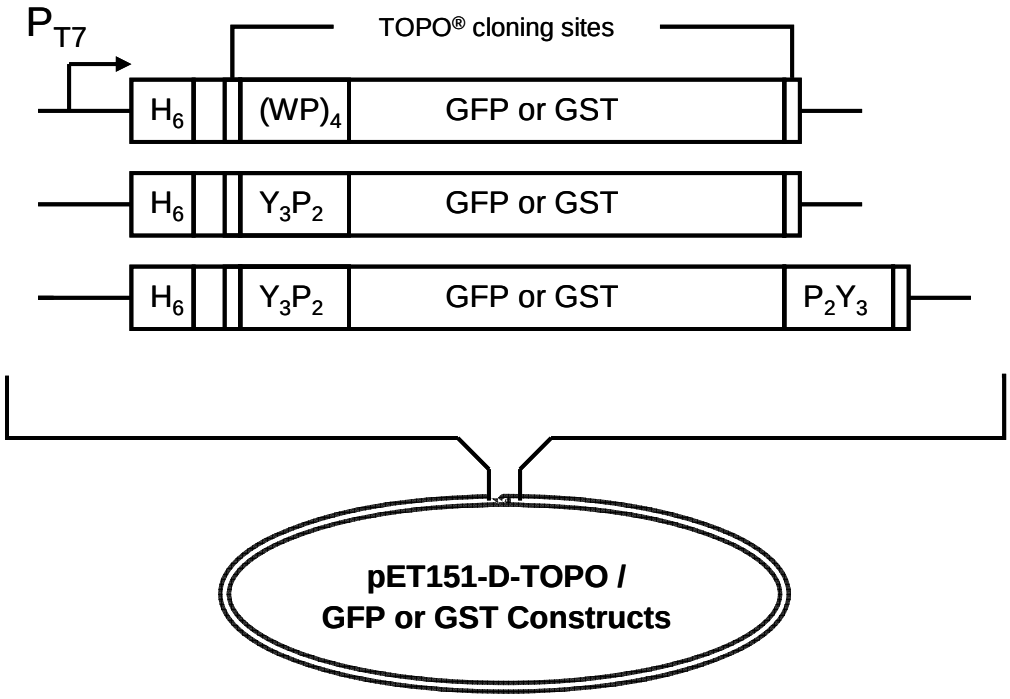

Figure S1: Genetic constructs used for creation of GFP or GST modified with partitioning tags.

Protein expression, cell lysate preparation, and protein purification. BL21 Star™(DE3) cells (Invitrogen) were transformed with the recombinant plasmids and grown and induced in Overnight™ Express Instant TB Medium (Novagen, Madison, WI) for 6 h at 37°C followed by 12h at 22°C. The cells were harvested by centrifugation (5000 × g for 5min) and resuspended in 1/10 culture volume of phosphate-buffered saline (PBS, pH 7.4). The cell lysate was prepared by sonicating the resuspended cells followed by collecting supernatant after centrifugation (10,000xg for 10 min). In experiments where affinity-purified proteins were tested, the supernatant was applied to Ni-NTA Spin Column (Qiagen, Valencia, CA) and interacting proteins were eluted from the column after washing steps according to the manufacture's instructions.

Supplementary Results

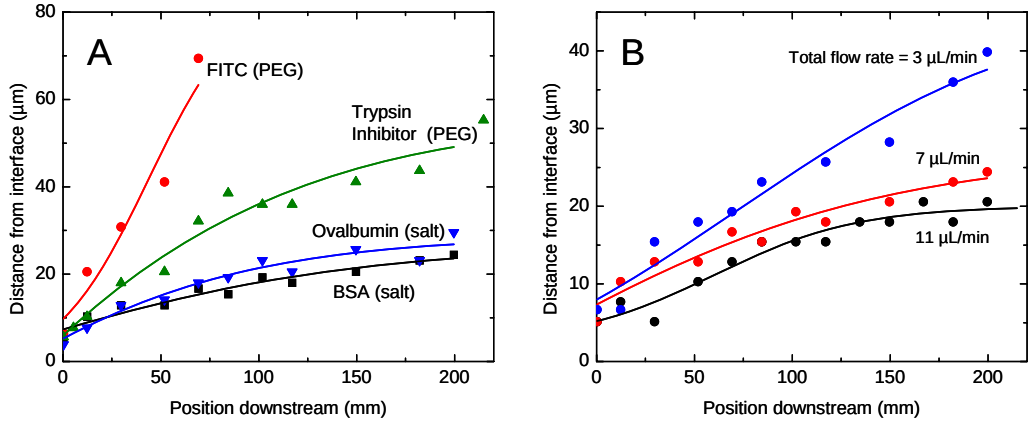

Figure S2: (A) spreading of different analytes as a function of position downstream, at a total flow rate of 7 µL/min. (B) Spreading of fluorescently labeled BSA (66 kDa) as a function of downstream position, at several different flow rates. Curves are drawn to guide the eye.