Supporting Information

High-Performance Binary Protein Interaction Screening in a Microfluidic Format

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Material and Methods

Primer Sequences for linear templates

S100A1 primer 5':

TTGTTTAACTTTAAGAAGGAGATATACCATGATGGCAAGTATGACAGGGGGT

CAACAAATGGGAATGGGCTCTGAGCTGG

S100A1 primer with c-Myc tag 3' (reverse complement):

GTAGCAGCCTGAGTCGTAAATCTTCCTCACTTATCAATTTTTGTTC CTTTCTTATAATGCCAACTTTGTAC

S100B primer 5':

TTGTTTAACTTTAAGAAGGAGATATACCATGATGGCAAGTATGACAGGGGGTCAACAAATGGGAATGTCT

GAGCTGGAGAAGG

S100B primer with c-Myc tag 3' (reverse complement)**:**

GTAGCAGCCTGAGTCGTAAATCTTCCTCACTTATCAATTTTTGTTC GCAATGCTTTCTTATAATGCC

Extension PCR specific for the in vitro expression system

Primer 5'

GATCTTAAGGCTAGAGTACTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATT

TTGTTTAACTTTAAGAAGGAGATATACC

Primer 3' (reverse complement)**:**

CAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAAGGGGTTATGCTAGTTTTTTTTTTTTTTTTTTTTTT

TTTTTTGTAGCAGCCTGAGTCG

Primer Sequences for the p-SNAP fusion templates

SbfI S100A1 Serine Linker primer 5':

CAATAGCCTGCAGGTGGTAGCGGCAGCGGTAGCATGGGCTCTGAGCTGG

S100A1 His-Tag BamHI primer 3' (reverse complement)**:**

GATAACGGATCCGTGATGGTGATGGTGATG CTTTCTTATAATGCCAACTTTGTAC

SbfI S100B Serine Linker primer 5':

CAATAGCCTGCAGGTGGTAGCGGCAGCGGTAGCATGTCTGAGCTGGAGAAGG

S100B His-Tag BamHI primer 3' (reverse complement)**:**

GATAACGGATCCGTGATGGTGATGGTGATGGCAATGCTTTCTTATAATGCC

eGFP SbfI 5':

CAATAGCCTGCAGGTATGGTGAGCAAGGGCGA

eGFP His-Tag BamHI 3' (reverse complement):

GTAGCAGCCTGAGTCGTTATTAGTGATGGTGATGGTGATG

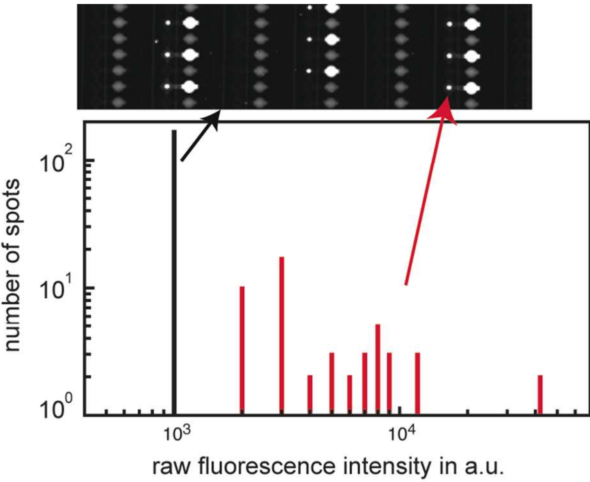
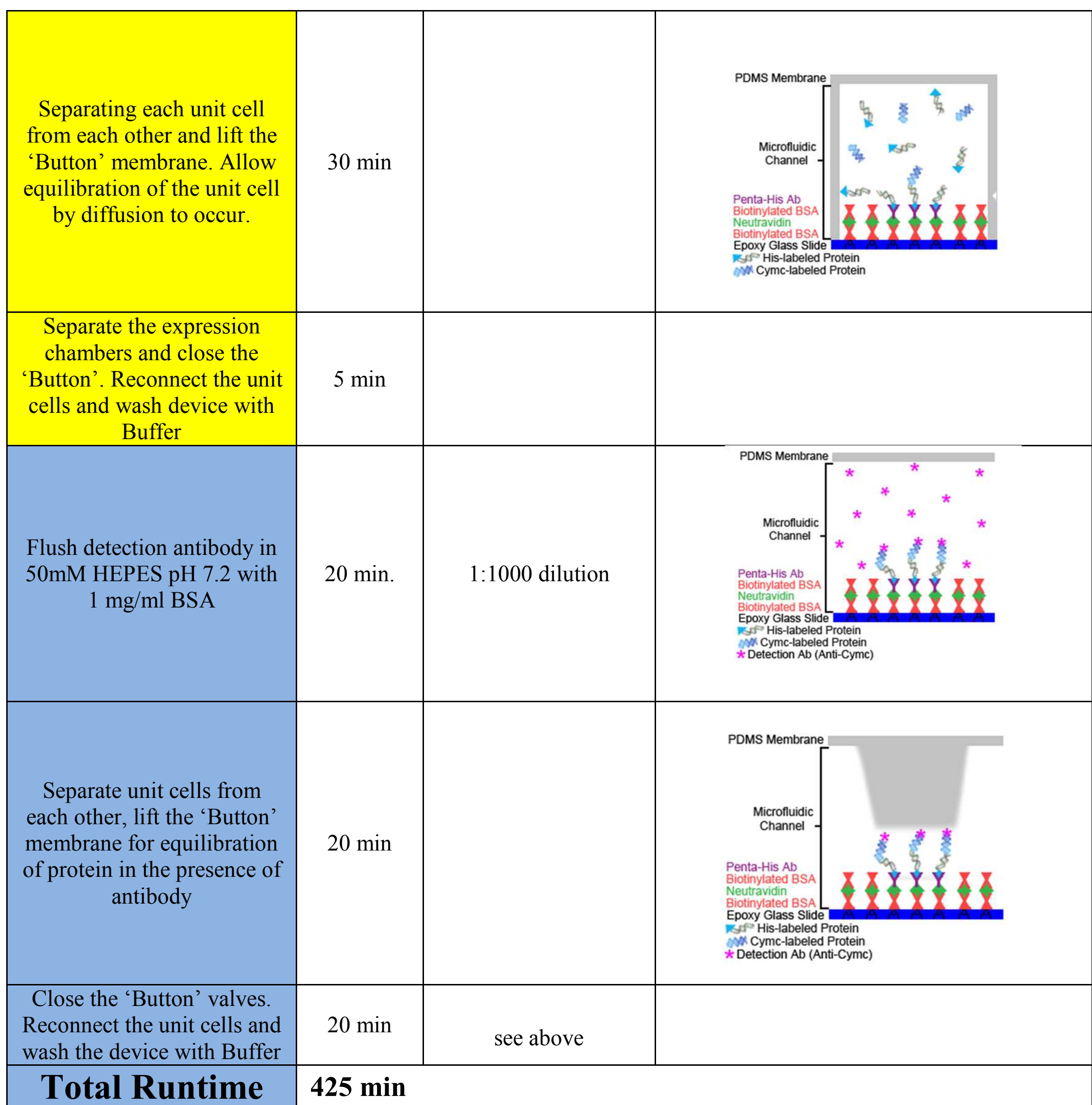


Figure SI-1. *In vitro* expression and pull-down of S-GFP-H on microfluidic chip with parallel micro-architecture. The histogram shows the raw fluorescence signal of S-GFP-H after pulled down on a surface with BSA-BG anchor in a ratio of 1:50. The red arrow points on the fluorescence image to a unit cell with expressed and pulled down GFP, were the black arrow points to a control chamber without GFP cDNA template.

Step	Flush Time	Solution	Pictogram
Coating the glass surface with biotinylated BSA (Pierce,US)	45 min	1 mg/ml in 0.5 M HEPES pH 7.2	PDMS Membrane Microfluidic Channel Biotinylated BSA Epoxy Glass Slide
Wash with Buffer	20 min	50 mM HEPES (pH 7.2), 1mg/ml BSA	
Coat the surface with Neutravidin (Pierce, US)	45 min	0.5 mg/ml in 50 mM HEPES, pH 7.2	PDMS Membrane Microfluidic Channel Neutravidin Biotinylated BSA Epoxy Glass Slide
Wash with Buffer	20 min	see above	
Close ‘button’ membrane and passivate remaining surface area	45 min	biotinylated BSA 1mg/ml in 50 mM HEPES, pH 7.2	PDMS Membrane Microfluidic Channel Biotinylated BSA Neutravidin Biotinylated BSA Epoxy Glass Slide
Wash with Buffer	20 min	see above	
Lift ‘button’ membrane and introduce the pull-down Ab	45 min	1:100 dilution in 1 mg/ml BSA solution buffered in 50 mM HEPES 7.2	PDMS Membrane Microfluidic Channel Penta-His Ab Biotinylated BSA Neutravidin Biotinylated BSA Epoxy Glass Slide
Wash with Buffer	20 min	See above	
Close ‘button’ membrane and add the E.coli cell extract to start the protein expression	15 min	TNT-T7 transcripion S30 extract (Promega)	
Fill the expression chamber with E.coli and close them off. Incubate chip at 37°C	75 min		



Separating each unit cell from each other and lift the 'Button' membrane. Allow equilibration of the unit cell by diffusion to occur.	30 min		
Separate the expression chambers and close the 'Button'. Reconnect the unit cells and wash device with Buffer	5 min		
Flush detection antibody in 50mM HEPES pH 7.2 with 1 mg/ml BSA	20 min.	1:1000 dilution	
Separate unit cells from each other, lift the 'Button' membrane for equilibration of protein in the presence of antibody	20 min		
Close the 'Button' valves. Reconnect the unit cells and wash the device with Buffer	20 min	see above	
Total Runtime	**425 min**		

Table-S1. Flush steps during a PPI run on a first generation chip with IP chemical-architecture. The flow rate used during the runs was 0.5 uL per min. The devices were operated at 16 psi in the control channels and 5 psi for the flow lines. Green, red, yellow, and blue correspond to the four processes given in the main text.

First Flow Circuit				Second Flow Circuit		
Step	**Flush Time**	**Solution**	**Pictogram**	**Step**	**Flush Time**	**Solution**
Close 'button'. Coat the surrounding glass surface with BSA	20 min.	0.5 mg/ml in 50 mM HEPES, pH 7.2, 500 mM NaCl	PDMS Membrane; Microfluidic Channel; BSA; Epoxy Glass Slide	Start in vitro expression of bait and prey.	10 min	E.coli S 30 Extract (Promega)
Coat the surface with BG-BSA	20 min.	0.5 mg/ml in 50 mM HEPES, pH 7.2	PDMS Membrane; Microfluidic Channel; BG-BSA; BSA; Epoxy Glass Slide	Incubation Incubate chip at 37°C	50 min	
Wash	20 min.	50 mM HEPES, pH 7.2				
Separating each unit cell from each other and lift the 'Button' membrane. Allow diffusion of bait and prey over the pull down surface.	30 min.		PDMS Membrane; Microfluidic Channel; BG-BSA; BSA; Epoxy Glass Slide; Bait Protein			

Wash	20 min.	50 mM HEPES, pH 7.2		
Flush detection antibody in 50mM HEPES pH 7.2 with 1 mg/ml BSA and Separate unit cells from each other, lift the ‘Button’ membrane for equilibration of protein in the presence of antibody	30 min	1:1000 dilution		
Wash with button down	10 min	50 mM HEPES, pH 7.2, 0.05% Tween		
Total Time:	**150 min.**			

Table S2. Flush steps during a PPI run on a chip with SNAP chemical-architecture. The flow rate used during the runs was 0.5 uL per min. The devices were operated at 16 psi in the control channels and 5 psi for the flow lines. Green, red, yellow, and blue correspond to the four processes given in the main text.