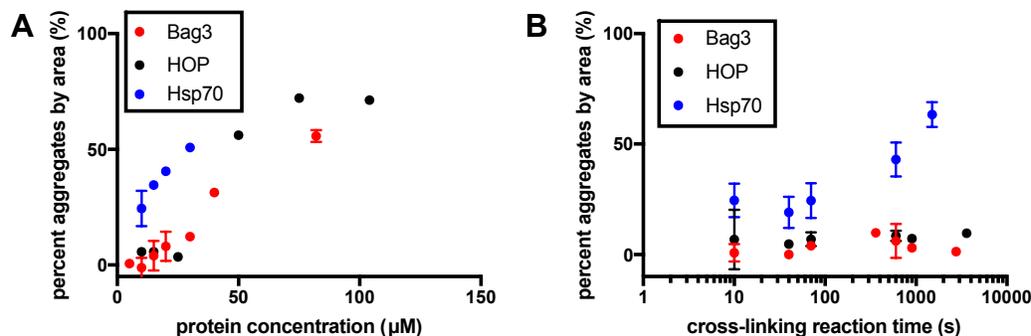


## Supplemental Information

**Table S1.** Molecular weight and protein function of proteins investigated

Protein or Peptide	molecular weight (kDa)
chaperone and co-chaperone proteins	
heat shock protein 70 (Hsp70)	70
heat shock organizing protein (HOP)	63
bcl2 associated athanogene 3 (Bag3)	62
heat shock protein 90 (Hsp90)	83
c-terminus of Hsp70 interacting protein (CHIP)	35
transcription factor related proteins and peptides	
KIX domain of CREB-binding protein (KIX)	12
E2A17	2.3
c-myb	3.2

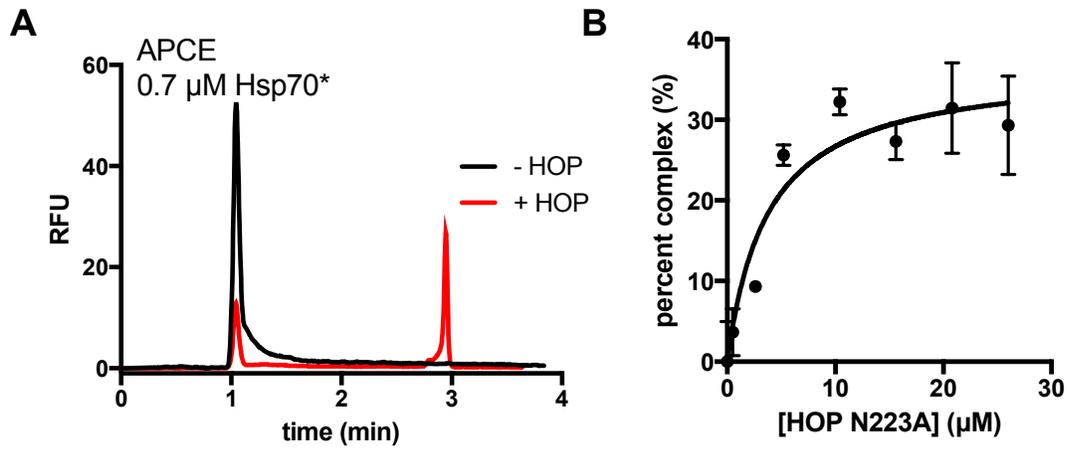
**Figure S1.** High molecular weight aggregates of Bag3, HOP, and Hsp70 observed by glutaraldehyde by size exclusion chromatography. Effect of protein concentration (A) on observation of high molecular weight aggregate after 10 s of glutaraldehyde cross-linking. Effect of increasing cross-linking reaction time on high molecular weight aggregates (B) protein concentration was 10  $\mu$ M.



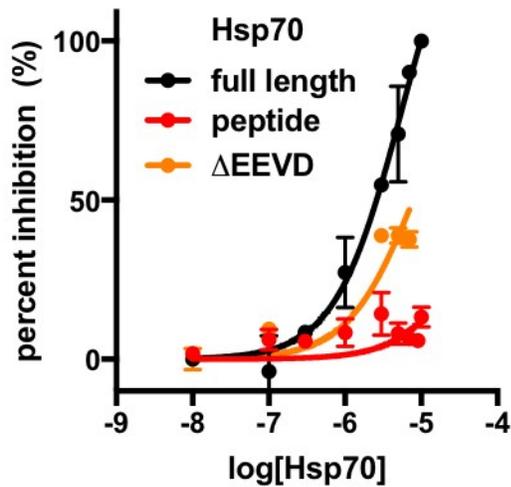
**Table S2.** Comparison of PXCE and Literature  $K_d$  values for  $\mu$ M to nM PPIs.

Protein Interaction	$K_d$ PXCE	$K_d$ Literature
c-Myb-KIX	$3.1 \pm 0.5 \mu\text{M}$	$1.6 \pm 0.2 \mu\text{M}$
Hsp70-HOP	$3.8 \pm 0.7 \mu\text{M}$	$2.7 \pm 0.4 \mu\text{M}^7$
Hsp70 homodimer	$1.2 \pm 0.3 \mu\text{M}$	$11 \mu\text{M}^{19}$
Hsp70-Bag3	$26 \pm 6 \text{ nM}$	$23 \pm 8 \text{ nM}^6$
Hsp90 homodimer	$2.1 \pm 0.3 \text{ nM}$	$1.29 \text{ nM}^{28} \quad 60 \pm 12 \text{ nM}^{29}$

**Figure S2.** APCE electropherograms (A) and saturation binding curve for Hsp70-HOP interaction without cross-linking (B).



**Figure S3.** Comparison of increasing concentrations of unlabeled Hsp70 constructs binding to 1  $\mu\text{M}$  HOPR77A in the presence of 100 nM Hsp70\*.



**Figure S4.** Separation of 11, 21, 32, 40, 63, 98, and 155 kDa protein ladder (BenchMark™ Fluorescent Protein Standard, Life Technologies, Carlsbad, CA) on low viscosity sieving matrix

at 1 kV/cm at 10 cm effective separation length.

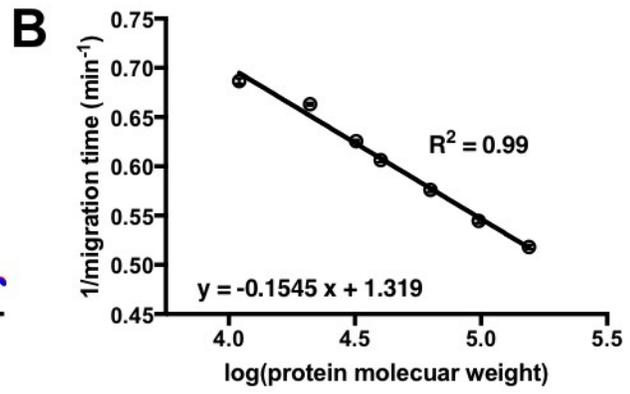
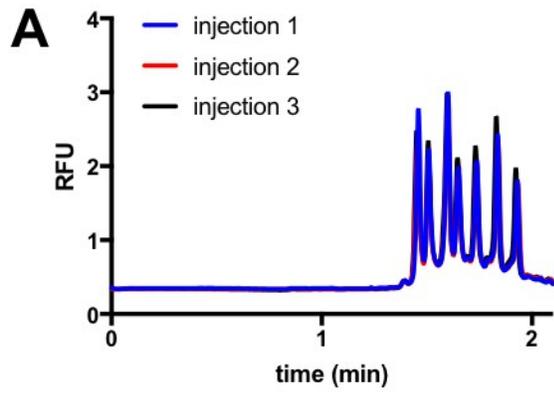
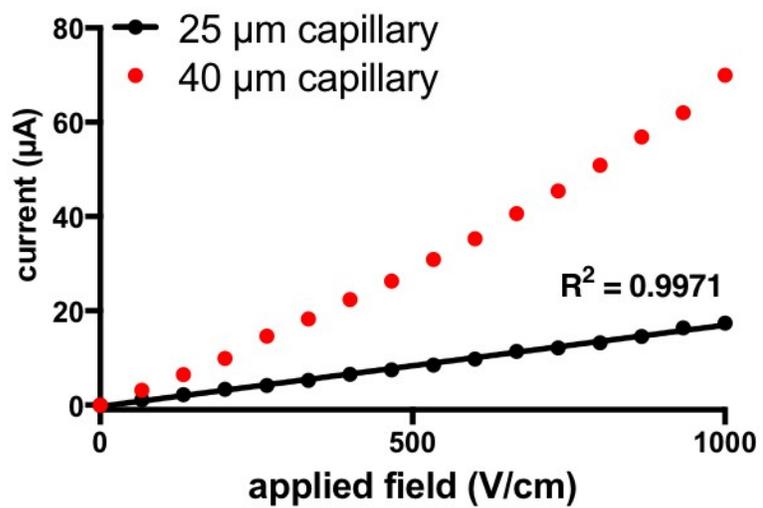


Figure S5. Ohm's plot of low viscosity sieving matrix in 40  $\mu\text{m}$  or 25  $\mu\text{m}$  capillary.



graphical abstract

