Supporting Information for:

Protein Cross-Linking Capillary Electrophoresis for Protein-Protein Interaction Analysis

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Supporting Information:

Figure S-1. Free solution electrophoresis of chaperone complexes Hsp70-488-Bag3 (A) and Hsp90-488 homodimer (B). The electrophoresis buffer was 10 mM, pH 10 borate.

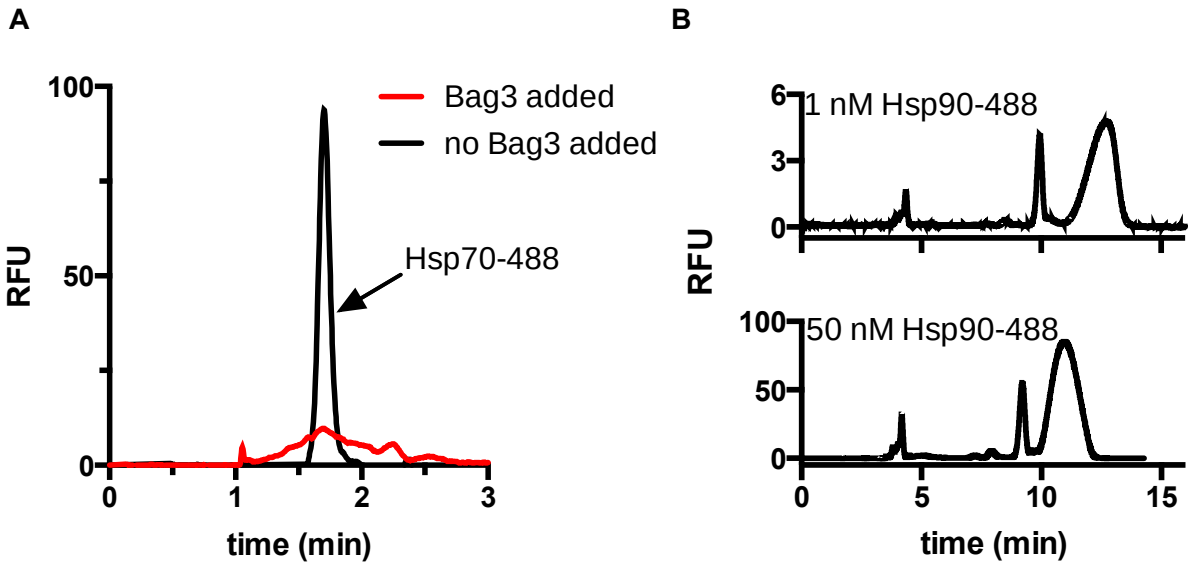

Table S-1. Resolution of free proteins from protein complexes.

Complex	**Resolution**
Hsp70-Bag3	1.0
Hsp90 homodimer	2.0
Lysozyme-anti-lysozyme	1.1

Figure S-2. Capillary gel electrophoresis separation of FITC-lysozyme immunocomplex.

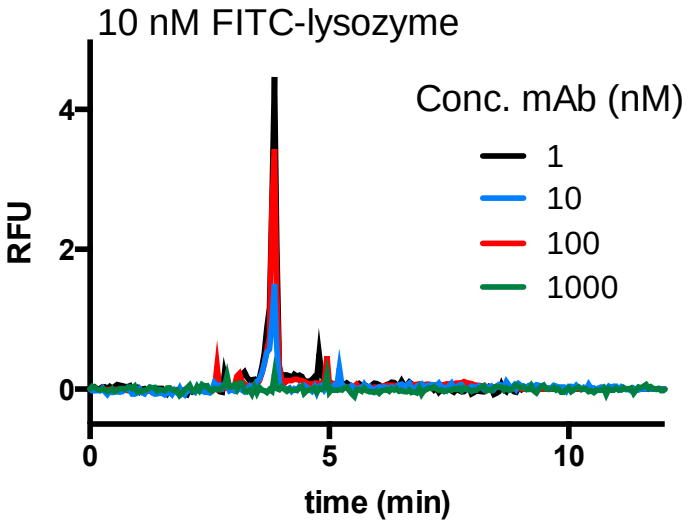

Table S-2. Dependence of 25 nM Hsp70-488 and 100 nM Bag3 on Hsp70-Bag3 complex detected on concentration of formaldehyde and cross-linking reaction time. Error is range of two trials.

Percent complex (%)					
formaldehyde concentration (%w/v)	**Reaction time (min)**				
	0.5	**5**	**10**	**30**	**60**
0.050	20 ± 3	21 ± 8	30 ± 17	38 ± 3	29 ± 6
0.50	19 ± 5	40 ± 13	60 ± 9	74 ± 9	46 ± 13
1.0	22 ± 9	46 ± 24	73 ± 10	73 ± 6	24 ± 3
2.5	31 ± 2	62 ± 17	73 ± 2	75 ± 8	44 ± 5

Table S-3. Dependence of 50 nM Hsp90-488 on complex detected on concentration of formaldehyde and cross-linking reaction time. Error is range of two trials.

Percent complex (%)				
formaldehyde concentration (%w/v)	**Reaction time (min)**			
	0.5	**5**	**10**	**60**
0.050	80 ± 5	77 ± 1	81.2 ± 0.4	71.4 ± 0.9
0.50	77 ± 5	74 ± 6	73.3 ± 0.6	74.6 ± 0.8
1.0	73 ± 2	79 ± 10	72 ± 3	73 ± 6.5
2.5	78 ± 6	73 ± 7	64 ± 4	58 ± 4

Table S-4. Dependence of 10 nM FITC-lysozyme and 20 nM antibody on complex detected on concentration of formaldehyde and cross-linking reaction time. Error is range of two trials.

Percent complex (%)				
formaldehyde concentration (%w/v)	**Reaction time (min)**			
	0.5	**5**	**10**	**60**
0.050	27 ± 15	39 ± 1	34 ± 7	24 ± 3
0.50	33 ± 15	37 ± 8	37 ± 12	39 ± 11
1.0	39 ± 15	35 ± 1	40 ± 9	34 ± 11
2.5	42 ± 24	27 ± 8	28 ± 12	23 ± 4

Figure S-3. Saturation binding assays for Hsp70-488-Bag3 at different 1% formaldehyde reaction times. All samples contain 25 nM Hsp70-488, error bars are standard deviation (n = 3).

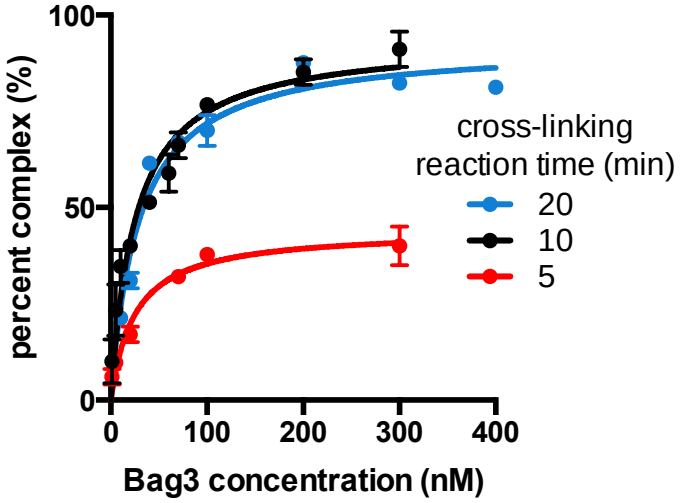

Table S-5. Dependence of measured K_d of Hsp70-488-Bag3 interaction on cross-linking reaction time using 1% formaldehyde.

Cross-linking reaction time (min)	$K_{d,\ Hsp70\text{-}Bag3}$ (nM)
5	25 ± 8
10	25 ± 5
20	29 ±6

Figure S-4. Calorimetric isothermal-titration measurement of FITC-lysozyme interaction with anti-lysozyme.

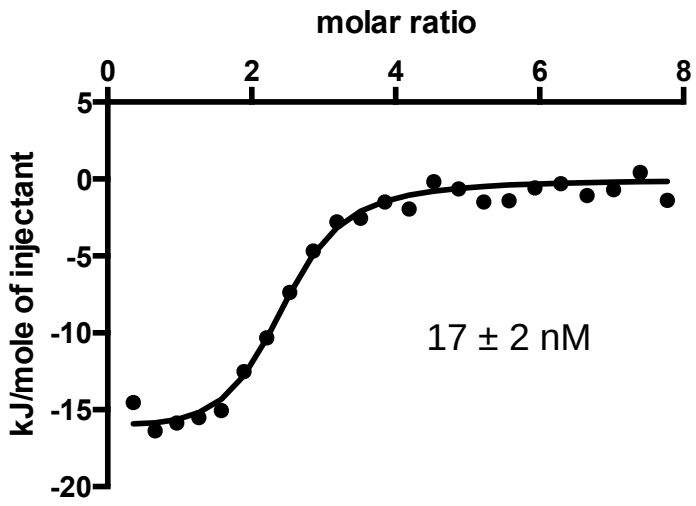

Figure S-5. FCPIA data of small molecule Hsp70-Bag3 inhibitors JG-98, JG-231 and JG-311. Error bars are range of two trials.