The Effect of Oxidized Dopamine on the Structure and Molecular Chaperone Function of the Small Heat-Shock Proteins, αB-Crystallin and Hsp27

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Supplementary Figure 1. The effect of modification of α Bc and Hsp27 by DA_{ox} on their chaperone activity against reduced and carboxymethylated α -lactalbumin (RCM α LA). α Bc:DA_{ox} (A) or Hsp27:DA_{ox} (B) were prepared at a 1:0, 1:1 and 1:5 molar ratio of sHsps:DA_{ox}. Then, 30 μ M RCM α LA was incubated with 10 μ M of the respective sHsps:DA_{ox}, and allowed to aggregate, in 50 mM phosphate, 150 mM NaCl, 5 mM MgCl₂ at 37 °C, pH 7.4 with no shaking. To separate the soluble and insoluble components, the incubation was centrifuged at 13,500 rpm for 15 min. 25 μ L of the supernatant was taken and flash frozen. The samples were then loaded on SDS-PAGE. *SDS-PAGE gels*. The bands represent the soluble component of RCM α LA taken every 45 min until 9 h, and a final time point at 24 h. *Graphs:* The band intensity of RCM α LA was quantified using ImageJ software. The data were normalized against time 0 h.



Supplementary Figure 2. The effect of modification of α Bc by a 10, 20 and 30 molar excess of DA_{ox} on its chaperone activity against reduced and carboxymethylated α -lactalbumin (RCM α LA). α Bc:DA_{ox} was prepared at a 1:0, 1:10, 1:20 and 1:30 molar ratio of sHsps:DA_{ox}. Then, 30 μ M RCM α LA was incubated with 10 μ M of the respective sHsps:DA_{ox} and allowed to aggregate in 50 mM phosphate, 150 mM NaCl, 5 mM MgCl₂ at 37 °C, pH 7.4 with no shaking. To separate the soluble and insoluble components, the incubation was centrifuged at 13,500 rpm for 15 min. 25 μ L of the supernatant was taken and flash frozen. The samples were then loaded on SDS-PAGE. *SDS-PAGE gels:* The bands represent the soluble component of RCM α LA taken every 45 min until 9 h, and a final time point at 24 h. *Graph:* The band intensity of RCM α LA was quantified using ImageJ software. The data were normalized against time 0 h.



Supplementary Figure 3. Densitometry of sHsp monomeric band intensity upon incubation with amorphous or amyloid fibrillar aggregating target proteins. ImageJ software was used to quantify the band intensity of the sHsps:DA_{ox} in the chaperone assays of Figures 1A, 1B, 1C and 1D, and Supplementary Figures 1A and 2B.



Supplementary Figure 4. HMW DA_{ox} -modified αBc species are not affected by the presence of excess DTT. 15 μM of αBc was modified with a five-fold molar excess of DA_{ox} . Prior to SDS-PAGE, the samples were additionally treated with a one, five or 10-fold molar excess of DTT.



Supplementary Figure 5. ANS fluorescence of DA_{ox} -modified sHsps. ANS fluorescence spectra of 20 μ M α Bc: DA_{ox} (A) or Hsp27: DA_{ox} (B) after modification with zero, equivalent or five-fold excess of DA_{ox} in the presence of 100 μ M ANS in 10 mM phosphate buffer, pH 7.4 at room temperature. The displayed spectra are an average of three. The spectrum of 10 mM phosphate, pH 7.4, with 100 μ M ANS was subtracted from each spectrum.

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	Native (Da)	Methylated (Da)	Number of methyl groups added
αBc	20,158.4	20,466.7	22
Hsp27	22,649.4	22,874.7	16

N.B. Dimethylation of the N-terminal amino group or of one lysine residue results in the addition of 28 Da in mass. Human αBc contains 10 lysine residues and one N-terminal amino group. Hsp27 contains seven lysine residues and one N-terminal amino group.