

# Supporting Information

## Species-Specific Structural and Functional Divergence of $\alpha$ -crystallins: Zebrafish $\alpha$ Ba- and rodent $\alpha$ A<sup>ins</sup>-crystallin encode activated chaperones

*Hanane A. Koteiche<sup>1</sup>, Derek P. Claxton<sup>1</sup>, Sanjay Mishra, Ezelle T. McDonald and Hassane S. Mchaourab\**

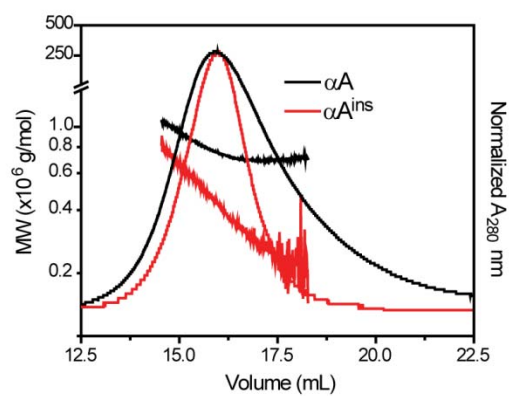
Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, United States

\*Corresponding author.

Department of Molecular Physiology and Biophysics  
741 Light Hall, 2215 Garland Ave, Nashville, TN 37232  
Telephone: (615) 322-3307  
Email: [hassane.mchaourab@vanderbilt.edu](mailto:hassane.mchaourab@vanderbilt.edu)

H	aA	1	MDVTIQHPWFKRTLGPFFYPSRLFDQFFGEGLEFYDLLPFLSSTISPYRQ
Zf	aA	1	MDIAIQHPWFRRTLGL--YPTRLFDQFFGEGLEFDYDLFPFTTSTVSPYYRH
R	aA <sup>ins</sup>	1	MDVTIQHPWFKRALGPFFYPSRLFDQFFGEGLEFYDLLPFLSSTISPYRQ
H	aA	51	SLFRTVLDS---GISE-----VRSDRDKVFIF
Zf	aA	49	SLFRNILDSSNSGVSE-----VRSDREKFTVY
R	aA <sup>ins</sup>	51	SLFRTVLDS---GISELTMHWFVMHQPHAGNPKNNPGKVRSDRDKVFIF
H	aA	75	LDVKHFSPEDLTVKVQDDFVEIHGKHNERQDDHGYISREFHRRYRLPSNV
Zf	aA	76	LDVKHFSPDELSVKVTDYVEIQGKHGERQDDHGYISREFHRRYRLPSNV
R	aA <sup>ins</sup>	99	LDVKHFSPEDLTVKVLDFVEIHGKHNERQDDHGYISREFHRRYRLPSNV
H	aA	125	DQSALSCSLSADGMLTFCGPKIQTGLDATHAERAIPVSREEKPTSAPSS
Zf	aA	126	DQSAITCTLSADGLLTLCGPKT-SGIDAGRGDRTIPVTREDKSNSGSSS
R	aA <sup>ins</sup>	149	DQSALSCSLSADGMLTFSGPKVQSGLDAGHSERAI PVSREEKPSAPSS
H	aB	1	MDIAIHHPWIRRRPFFPFHSPSRLFDQFFGEHLLESDFPTSTSLSPFYLR
Zf	aBa	1	MEISIQHPWYRRPLFPGFEPYRIFDQYFGEHLSDSD--PFSFPYTMFYR
Zf	aBb	1	MDIAINPP-FRRILFPIFFPRRQ----FGEHITEAD-----VISLSYSQ
H	aB	51	PPSFLRAPSWFDTGLSEMRLEKDRFSVNLVDVKHFSPEELKVKVLGDVIEV
Zf	aBa	49	-PYLWRFPSSWWDSGMSEMRQDRDRFVINLVDVKHFSPELTVKVNEDFIEI
Zf	aBb	40	RSSFLRSPSWMESGVSEVKMEKQFSLSLVDVKHFAPEELSVKIIIGDFIEI
H	aB	101	HGKHEERQDEHGFI SREFHRKYRIPADVDPLTITSSLSSDGVLTVNGPRK
Zf	aBa	99	HGKHDERQDDHGIVAREFFRKYKIPAGVDPGAI TSSLSSDGVLTINTLRH
Zf	aBb	91	HAKHEDRQDGHGFVSREFLRKYRVPVGVDPASITSSLSSDGVLTVTGPLK
H	aB	151	QVSGPER--TIPITREEKPAVTAAPKK
Zf	aBa	149	QLDILER--SIPIICGEKPPA----QK
Zf	aBb	141	LSDGPERTIAIPVTRDDKTTV-AGPQK

**Figure S1.** Sequence alignments of  $\alpha$ A and  $\alpha$ B crystallins. Multiple sequence alignments were performed with ClustalW and minor manual adjustment using sequence from the NCBI protein database. Top: human  $\alpha$ A (AAB33370.1), zebrafish  $\alpha$ A (AAK61363.1) and rat  $\alpha$ A<sup>ins</sup> (NP\_001276666.1). Bottom: human  $\alpha$ B (ACP18852.1), zebrafish  $\alpha$ Ba (NP\_571232.1) and zebrafish  $\alpha$ Bb (NP\_001002670.1). Highlighted red residues indicate consensus sites of phosphorylation in the human  $\alpha$ -crystallins. The gray shaded region outlines the approximate boundaries for the  $\alpha$ -crystallin domain derived from de Jong and colleagues<sup>7</sup>.



**Figure S2.** MALS analysis of  $\alpha A$  and  $\alpha A^{\text{ins}}$ . Rat  $\alpha A^{\text{ins}}$  forms smaller oligomers on average than  $\alpha A$ , and is more polydisperse as suggested by the steep slope of molecular weights sampled across the elution peak. The analysis was performed as described in Experimental Procedures.