The structure and oxidation of the eye lens chaperone αA-crystallin

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The small heat shock protein αA-crystallin is a molecular chaperone important for the optical properties of the vertebrate eye lens. It forms heterogeneous oligomeric ensembles. We determined the structures of human αA-crystallin oligomers by combining cryo-electron microscopy, cross-linking/mass spectrometry, NMR spectroscopy and molecular modeling. The different oligomers can be interconverted by the addition or subtraction of tetramers, leading to mainly 12-, 16- and 20-meric assemblies in which interactions between N-terminal regions are important. Cross-dimer domain-swapping of the C-terminal region is a determinant of αA-crystallin heterogeneity. Human αA-crystallin contains two cysteines, which can form an intramolecular disulfide bond in vivo. Oxidation in vitro requires conformational changes and oligomer dissociation. The oxidized oligomers, which are larger than reduced αA-crystallin and destabilized against unfolding, are active chaperones and can transfer the disulfide to destabilized substrate proteins. The insight into the structure and function of αA-crystallin provides a basis for understanding its role in the eye lens.

The small heat shock proteins (sHsps) αA- and αB-crystallin are major constituents of the vertebrate eye lens. They ensure lens transparency and prevent lens proteins from aggregating. Mutations in both α-crystallins result in cataracts and in a variety of eye disorders, emphasizing their importance for the lens. Beside the commonality of the processes in which αA- and αB-crystallin are involved, there are differences in their distribution in the lens and expression patterns. Specifically, αA-crystallin is predominantly expressed in the eye lens. In vitro studies point towards mechanistic differences between αA- and αB-crystallin in suppressing the aggregation of model substrates, and mutations of conserved residues have different impacts on the two crystallins.

Human αA-crystallin, a 19.9 kDa protein with 173 residues, consists of three structurally distinct regions: the conserved α-crystallin domain (ACD, residues 61–145) flanked by the N-terminal region (NTR, residues 1–60) and the short, flexible C-terminal region (CTR, residues 146–173). The ACD adopts a β-sandwich fold composed of two anti-parallel sheets of three and four β-strands, respectively. It dimerizes through the interaction of the β6+7-strands of two adjacent protomers (‘β7-interface dimer’). αA-crystallin assembles into polydisperse oligomers with extensive size heterogeneity and a constant exchange of subunits between oligomers. There is, as yet, no structural information available for full-length αA-crystallin or for αA-crystallin in any oligomeric form. Consequently, the structural elements critical for assembly and those conferring plasticity to the oligomeric assembly are poorly understood. The involvement of the NTR in oligomer formation is indicated by the shift of the average oligomer ensemble to smaller species, dimers and/or tetramers, upon its truncation. Studies on C-terminal truncation mutants of αA-crystallin from different organisms display significant disparities, and the role of the CTR in oligomer formation remains ill defined. The CTR of αA-crystallin exhibits greater overall flexibility than that of αB-crystallin, including the segment containing the conserved IIXI motif, which promotes oligomer formation by binding into the β4/β8 groove within the ACD of a neighboring protomer.

A characteristic of human αA-crystallin is the presence of two cysteines in its ACD, the invariant C131 (found in most species) and an additional cysteine at position 142 that is also found in αA-crystallin from primates and zebrafish. The role of the CTR in oligomer formation is indicated by the shift of the average oligomer ensemble to smaller species, dimers and/or tetramers, upon its truncation. Studies on C-terminal truncation mutants of αA-crystallin from different organisms display significant disparities, and the role of the CTR in oligomer formation remains ill defined. The CTR of αA-crystallin exhibits greater overall flexibility than that of αB-crystallin, including the segment containing the conserved IIXI motif, which promotes oligomer formation by binding into the β4/β8 groove within the ACD of a neighboring protomer.

With aging, the amount of αA-crystallin increases up to 90% and it becomes a major constituent of high molecular weight aggregates, concomitant with an age-dependent loss of the chaperone activity of α-crystallin. αA-crystallin is undetectable in cataractous lenses. Despite their importance, the structural and functional consequences of αA-crystallin oxidation are as yet unknown. Here we present the architecture and plasticity of human αA-crystallin oligomers as well as the structural and functional consequences of its oxidation. The structures of human αA-crystallin assemblies and pseudoatomic models of a 16-meric assembly reveal the domain-swapping of the CTR to be a key determinant
of αA-crystallin heterogeneity. Formation of the intramolecular disulfide bond leads to distinct oligomers that are chaperone active and can transfer their intramolecular disulfides to destabilized substrate proteins.

**Results**

**Oligomer architecture and conformational heterogeneity of reduced αA-crystallin.** To determine the oligomer architecture of reduced human αA-crystallin, we employed single-particle cryo-EM (Extended Data Fig. 1). The initial analysis of ~74,000 projection images revealed two distinct populations, one containing round particles with three-, four- and five-fold symmetries and diameters varying between 6 and 16 nm (Extended Data Fig. 1b), and the other comprising elongated structures with two-fold symmetry and a nearly uniform long axis of 13–14 nm (Extended Data Fig. 1c). The two populations seemed to represent end- and side-on projections of a barrel-like architecture with varying subunit stoichiometries. On this basis, we established a three-dimensional (3D) reconstruction procedure that allowed us to assign ~80% of the particles to 12-, 16- and 20-meric assemblies with abundances of ~36, 27 and 19% and calculate the corresponding 3D models (Fig. 1) at resolutions of 9.2, 9.8 and 9.0 Å, respectively (Extended Data Fig. 1h and Table 1).

According to the reconstructed EM volumes (Fig. 1), all three assemblies form hollow, barrel-like structures with a recurring unit resembling a tilted ‘Z’ comprising two substructures that are connected in the mid-plane of the barrel. Each of these substructures is large enough to accommodate an αA-crystallin dimer, implying that the Z-shaped structures represent tetramers (dimers of dimers), which serve as the building blocks of the oligomers. Notably, in the average structures of the 12- and 16-mers, adjacent tetramers are not connected in the equatorial plane of the barrel (Fig. 1a,b) whereas there is a well-resolved density bridging neighboring tetramers in the 20-mer (Fig. 1c).

To elucidate the structural variability of αA-crystallin oligomers, each oligomer population was subjected to 3D sampling and classification, which revealed that the density corresponding to an αA-crystallin dimer remains almost invariant within all three populations (Extended Data Fig. 2). In contrast, significant heterogeneity exists in areas where adjacent tetramers come together in apical and equatorial regions, which most likely harbor the N and/or C termini. The oligomers differ in the density connecting the tetramers in the equatorial plane: this density is lacking in all sub-ensembles of the 12-mer population, while it is present in ~30% of the 16-mer population and in all sub-ensembles of the 20-mer population (Extended Data Fig. 2 and Supplementary Table 1). The observed heterogeneity is suggestive of dynamic inter-subunit interactions involving N- and/or C-terminal regions. This conformational heterogeneity, together with very similar projection views of different oligomers, presumably limits the resolution of the reconstructions.

**Pseudoatomic model of the αA-crystallin 16-mer.** To obtain pseudoatomic models of the αA-crystallin 16-mer, we subjected full-length proteins to cross-linking and mass spectrometry. Using the cross-linker bis(sulfosuccinimidyl)suberate (BS3), we identified numerous intra- and intermolecular cross-links (Extended Data Figs. 3 and 4 and Supplementary Table 2). The data demonstrated the structural similarity of human αA-crystallin in its ACD and CTR to zebrafish and bovine αA-crystallin, as all distances between corresponding residue pairs resolved in the respective crystal structures were below 30 Å—approximately the upper distance limit dictated by the utilized cross-linker (Extended Data Fig. 4f).

The volume of the equatorial inter-tetramer density present in some 16-mer subpopulations is just large enough to accommodate the CTR (Extended Data Fig. 2). Together with its positioning, we concluded that the variability within this area stems from 3D domain-swapping of the CTR: in structural classes containing the equatorial inter-tetramer density, the IPV (the sequence of the IXI motif in αA-crystallin) motif binds intermolecularly into the β4/β8 pocket of an adjacent protomer (3D domain-swapped configuration), while in classes lacking the density, it binds intramolecularly into the β4/β8 pocket of the same polypeptide chain (non-3D domain-swapped configuration). This view is supported by the occurrence of the CTRs in swapped and non-swapped configurations in bovine and zebrafish αA-crystallin ACD crystal structures, respectively13,16. Thus, we generated pseudoatomic models of the αA-crystallin 16-mer with the CTRs in both configurations using (1) shape and symmetry constraints from the cryo-EM envelopes differing in the equatorial inter-tetramer density, (2) the crystal structures of truncated versions of bovine and zebrafish α-crystallins as templates and (3) intra- and intermolecular distance restraints from cross-linking. During modeling using molecular dynamics flexible fitting, a homology-modeled structure for the NTR (residues 1–60) was used that contained three short helices connected by flexible loops (Extended Data Fig. 5a,b). The structures of the central ACD (residues 61–145) and part of the CTR (residues 146–166) were derived from homology modeling based on the above-mentioned crystal structures. Residues 167–173 were not included in the model due to their flexibility13. The fitting procedure resulted in an ensemble of solutions with the NTRs of both apical (M8) and...
In both 16-mer models (Fig. 2a,b), the CTRs of Map are in a non-3D formation, the CTR is barely involved in inter-subunit interactions. In contrast to the prevailing contribution of the NTR to oligomer T13 (Supplementary Table 2), which are all satisfied in our models.

To distinguish whether the unbound CTRs are in proximity of the ACD of the same protomer (non-3D domain-swapped) or an adjacent one (3D domain-swapped), we incubated spin-labeled 15N-αAred sample (15N-αAred-IPSL) with 15N-αAred-IPSL. In the case where all CTR interactions (apical ACD of the same protomer) are preserved, the chemical exchange between bound and unbound CTRs was fast on the NMR timescale. However, complete chemical exchange between bound and unbound CTRs does not occur in the 3D domain-swapped state, where the distance between the apical ACD and CTR is too large compared to the length of the CTR.

3D domain-swapping of the C-terminal region in the αA-crystallin ensemble. The 12- and 20-meric αAred assemblies share the modular architecture of the 16-mer (Fig. 3a,b). In all three cases, the tetramers have the same curvature. The ACD positions within the tetramers are identical. In the apical regions, the CTRs do not swap domains due to the large distance between adjacent protomers, as for the 16-mer. However, the CTRs of all Map are in the non-3D domain-swapped state in the 12-mer, while those of the 20-mer connect neighboring tetramers by domain-swapping (Fig. 3).

Table 1 | Cryo-EM data collection and validation statistics for αA-crystallin oligomer reconstructions

|                  | 12-mer (D3) (EMD-4895) | 16-mer (D4) (EMD-4894, PDB 6T1R) | 20-mer (D5) (EMD-4896) |
|------------------|------------------------|---------------------------------|------------------------|
| **Data collection and processing** |                        |                                 |                        |
| Molecular mass (kDa) | 238.9                  | 318.5                           | 398.2                  |
| Magnification     | 37,000                 | 37,000                          | 37,000                 |
| Voltage (kV)      | 300                    | 300                             | 300                    |
| Electron exposure (e− Å−2) | 30                     | 30                             | 30                     |
| **Defocus range (μm)** | 1.2-2.5               | 1.2-2.5                         | 1.2-2.5                |
| **Pixel size (Å)** | 1.35                   | 1.35                            | 1.35                   |
| **Symmetry imposed** | D3                     | D4                              | D5                     |
| Initial particle images (no.) | 74,068                | 74,068                          | 74,068                 |
| Final particle images (no.) | 26,596                | 19,783                          | 14,336                 |
| **Relative abundance (%)** | 35.9                   | 26.7                            | 19.4                   |
| Map resolution (Å) | 9.2                    | 9.8                             | 9.0                    |
| FSC threshold     | 0.143                  | 0.143                           | 0.143                  |
| Dimensions (width x height, in Å) | 10.8×13.6             | 10.9×13.8                       | 12.0×13.7              |

**Validation**

|                | MolProbity score | Clashscore | Ramachandran plot |
|----------------|------------------|------------|-------------------|
| Favored (%)   | –                | 92         | –                 |
| Allowed (%)   | –                | 8          | –                 |
| Disallowed (%)| –                | 1          | –                 |

*Relative abundance with respect to the total number of images in the initial cryo-EM dataset.

equatorial (Map) protomers adopting a variety of possible conformations (Extended Data Fig. 5c,d), consistent with their flexibility. Although no consensus structure could be derived for the NTR, its integration during the fitting process was crucial because it restricted the positioning of the central ACD and CTR. The best structures were selected based on r.m.s. deviation (r.m.s.d.), stereochemistry and cross-correlation with respect to the cryo-EM density and further energy minimized.

In the final pseudo-atomic models of the αAred 16-mer (Fig. 2) that fit best into the EM map from all possible models and fulfill cross-linking restraints, all parts of the polypeptide chain are accommodated within the electron density. The models reveal that two protomers form a β7-interface dimer. Interactions between N-termini mediate the association of two dimers across the equator to form a tetramer (equatorial N-terminal interface, eq-NI; Fig. 2a,d), which is the recurring unit of the oligomer. Further N-terminal interactions between apical protomers of the tetramers (apical N-terminal interface, ap-NI) serve to form the 16-mer (Fig. 2a). The close proximity of the N-terminal segments is corroborated by intermolecular cross-links involving residues M1, K11 and T13 (Supplementary Table 2), which are all satisfied in our models. In contrast to the prevailing contribution of the NTR to oligomer formation, the CTR is barely involved in inter-subunit interactions. In both 16-mer models (Fig. 2a,b), the CTRs of Map are in a non-3D domain-swapped configuration as the distance between apical protomers is too large compared to the length of the CTR to permit an intermolecular IXI-β4/β8 interaction. On the other hand, although the distance between Map of neighboring tetramers supports this interaction in both potential directionality of the IPV sequence, the CTR contributes to the assembly by 3D domain-swapping in only ~30% of the 16-mer population (Fig. 2b,f). 3D domain swapping creates an interface (equatorial C-terminal interface, eq-CI) in which the CTRs of Map from neighboring tetrameric units are in close proximity, consistent with the observed intermolecular cross-link K166-K166 (not used as a modeling constraint), and interact through electrostatic interactions involving residues downstream of the IXI motif (Fig. 2b,f).

## Table 1 | Cryo-EM data collection and validation statistics for αA-crystallin oligomer reconstructions

|                  | 12-mer (D3) (EMD-4895) | 16-mer (D4) (EMD-4894, PDB 6T1R) | 20-mer (D5) (EMD-4896) |
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| **Data collection and processing** |                        |                                 |                        |
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| Voltage (kV)      | 300                    | 300                             | 300                    |
| Electron exposure (e− Å−2) | 30                     | 30                             | 30                     |
| **Defocus range (μm)** | 1.2-2.5               | 1.2-2.5                         | 1.2-2.5                |
| **Pixel size (Å)** | 1.35                   | 1.35                            | 1.35                   |
| **Symmetry imposed** | D3                     | D4                              | D5                     |
| Initial particle images (no.) | 74,068                | 74,068                          | 74,068                 |
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| **Relative abundance (%)** | 35.9                   | 26.7                            | 19.4                   |
| Map resolution (Å) | 9.2                    | 9.8                             | 9.0                    |
| FSC threshold     | 0.143                  | 0.143                           | 0.143                  |
| Dimensions (width x height, in Å) | 10.8×13.6             | 10.9×13.8                       | 12.0×13.7              |

**Validation**

|                | MolProbity score | Clashscore | Ramachandran plot |
|----------------|------------------|------------|-------------------|
| Favored (%)   | –                | 92         | –                 |
| Allowed (%)   | –                | 8          | –                 |
| Disallowed (%)| –                | 1          | –                 |

*Relative abundance with respect to the total number of images in the initial cryo-EM dataset.*
In vitro formation of an intramolecular disulfide bond in human αA-crystallin. In agreement with the literature, in an Ellman’s assay of αA-crystallin we detected 0.93 ± 0.008 mol (SH) per mol (protein), corresponding to one accessible cysteine residue in vitro. The presence of only one reactive cysteine is puzzling at first glance, considering that the cysteines C131 and C142 of human αA-crystallin form an intramolecular disulfide bridge in vivo. However, when performed in the presence of urea, the Ellman’s assay detected 1.92 ± 0.070 mol (SH) per mol (protein) for αA-crystallin. Thus the second cysteine is not readily accessible under native conditions.

In our pseudo-atomic model of αA-crystallin, the cysteines are located on the adjacent antiparallel β8- and β9-strands and point in opposite directions (Fig. 3d). Their Cα atoms are ~6 Å apart, which is within the Cα-Cα distance range of 3.8–6.8 Å usually observed for disulfide bond conformations in proteins, but farther than the mean Cα-Cα distance of ~4.6 Å found in cross-strand disulfides. The formation of a cross-strand disulfide bond between C131 and C142 would require rotation of the cysteine side chains towards each other, resulting in significant conformational changes upon oxidation of αA-crystallin.

To study the formation of an intramolecular disulfide bond we performed redox reactions with glutathione (Fig. 4). At −149 mV, 50% of the protein was oxidized to the intramolecularly cross-linked species (αAox), while the remaining fraction consisted of intermolecularly disulfide-linked dimers (~20%) and trimers (~14%). On quantification of the ratio of αAox and αAred, the equilibrium constant of the redox reaction for intramolecular disulfide bond formation, $K_{eq}$, was determined to be $4.34 \times 10^{-4}$, corresponding to a redox potential of the intramolecular disulfide of ~135 mV (Fig. 4c).

For comparison, the above value is between the redox potentials of the catalytic disulfides in bacterial DsbA and DsbC, oxidoreductases with a strained conformation in the oxidized state. The low reaction rate and the formation of a substantial amount of intermolecular disulfide-bridged species suggest a thermodynamically unfavorable conformational state for intramolecular disulfide formation also in the case of αA-crystallin. Thus, a denaturant should facilitate oxidation. Indeed, in the presence of urea, the disulfide-linked oligomers were abolished and half-maximal oxidation was achieved at ~−222 mV (Fig. 4d,f), again implying that destabilization leads to the accessibility of both cysteines. As a consequence, we prepared αAox by incubation with GSSG in the presence of urea. According to Ellman’s assays this preparation contains no free sulfydryls: ~0.03 ± 0.037 mol (SH) per mol (protein). We also performed titration reactions with αAox (Fig. 4b,e). The results indicate that, after 20h, the forward and reverse reactions are indistinguishable and thermodynamic equilibrium was attained (Fig. 4c,f).

Structural impact of the intramolecular disulfide bond on human αA-crystallin. CD spectroscopy showed that oxidation does not lead to pronounced changes in the secondary structure (Extended Data Fig. 7a). However, differences became apparent in the environment of phenylalanine, tyrosine and tryptophan residues (Extended Data Fig. 7b). Given that ~60% of the Phe and Tyr residues, as well as the single Trp, are located within the NTR, altered tertiary interactions within the NTR upon oxidation appear likely.

**Fig. 2 | Pseudoatomic models of the human αA-crystallin (reduced) 16-mer.** a, Top and side views of the cryo-EM map of αA-crystallin 16-mer (reduced) superimposed with the atomic model (ribbon representation) containing the CTRs of apical (Meq) and equatorial (eq) protomers in a non-3D domain-swapped conformation. ap-NI, apical N-terminal interface; eq-NI, equatorial N-terminal interface (black dashed ellipse). b, 16-mer containing the CTRs of Meq in a 3D domain-swapped conformation. eq-CI, equatorial C-terminal interface (black solid ellipse).

![Image](image_url)
EM images of negatively stained αA-crystallin revealed that the protein assembles into oligomers that are more polydisperse and larger than observed for αA-red (Fig. 5a). The average oligomer size shifted from ~13.5 nm for αA-red to ~17.7 nm for αA-crystallin (Fig. 5b). In agreement, size exclusion chromatography (SEC) experiments indicated an increase in molecular mass from 380 kDa for αA-red to 770 kDa for αA-crystallin (Extended Data Fig. 7c) and sedimentation velocity analytical ultracentrifugation (aUC) experiments showed an increase in the sedimentation coefficient (s_{20,w}) from 14S to 25S (Extended Data Fig. 7d).

The projections seen in EM images of negatively stained αA-crystallin particles were either round or slightly elongated (Fig. 5a) and resembled projections seen in αA-red samples. A preliminary 3D reconstruction of a 32-mer calculated from class averages of 1,500 single-particle images (Extended Data Fig. 7c), without employing any starting model, revealed a hollow, slightly elongated assembly with D2 symmetry (Fig. 5c and Extended Data Fig. 7f). Notably, the assembly contains the characteristic Z-shaped tetramers as seen in the oligomers of αA-red (Fig. 1), but it is expanded through the insertion of further building blocks apparently composed of dimers (Fig. 5c).

The overall architecture of the αA-crystallin 32-mer implies altered residue and/or subunit proximities and consequently an altered cross-linking behavior compared to αA-red. Due to the lack of quantitative cross-linking data, we only tentatively compared both patterns in Supplementary Table 3, which included those observed for αA-red but also indicated differences between the two redox states. As such, many cross-links observed...
only in αAox involved residues located within the NTR as well as within the β4-, β6+7- and β9-strands (including K145), suggesting alteration of their relative positions and/or enhanced side chain accessibilities in αAox (Extended Data Fig. 4b).

The intramolecular disulfide affects local structural dynamics of αA-crystallin. To further test how intramolecular disulfide formation affects the structure and dynamics of αA-crystallin, we performed hydrogen-deuterium exchange coupled to mass spectrometry (H/DX-MS) (Fig. 6a and Supplementary Fig. 1). In agreement with previous studies\(^6\), peptides from the NTR were characterized by a moderate protection at short D,O exposure times, but became increasingly deuterated at longer exposure, consistent with the dynamic nature of this region sampling different conformations. In general, the peptides from the ACD showed lower exchange. The β5- and β6+7-strands (F93–E102 and Y109–R119, respectively) were most strongly protected, while the CTR exchanged readily, consistent with high accessibility/flexibility. Thus, the degree of exchange in αAox was similar to that observed for αAred, but specific differences existed. In αAox, the N-terminal stretch comprising residues D2–F10 exhibited increased protection. The β6+7-strand (Y109–R119), the C-terminal region of the β8-strand (L133 and S134) and the β9-strand (L139–G143), as well as the loop connecting the latter, became deprotected upon disulfide formation, with F141 (β9-strand, neighboring C142) showing the strongest deprotection. These results suggest that the introduction of the intramolecular disulfide affects dynamics, solvent exposure and the hydrogen bonding network around the sites of disulfide formation (Fig. 6b).

To test oligomer stability, we performed aUc experiments in the presence of urea. With increasing urea concentrations, both αAox and αAox oligomers dissociated successively. A dissociated species with a sedimentation coefficient of ~2S was observed at 4.5 M urea for αAox and at 3.5 M urea for αAox (Extended Data Fig. 8a). Similarly, urea-induced unfolding transitions monitored by intrinsic fluorescence revealed cooperative unfolding with midpoints at 3.8 and 2.7 M urea for αAox and αAox, respectively (Extended Data Fig. 8b).

Chaperone activity of oxidized αA-crystallin. To compare αAox and αAox functionally, we performed in vitro aggregation assays using the cellular tumor antigen p53 (p53) and malate dehydrogenase...
The homology-modeled structure of the NTR contains three short helices connected by flexible loops, consistent with the propensity of NTRs to adopt secondary structure elements. These regions are highly dynamic and exist as ensembles of heterogeneous conformations. In our model, representing one of several possibilities, the N-terminal interactions occur mainly between the loops connecting helices α2 and α3. The amphiphilic helix α2 (residues 20–27) covering the conserved phenylalanine-rich sequence RLFDQXFG14 dictates the position of the interacting loop regions in equatorial protomers. This motif was implicated in contributing to the higher order subunit assembly, oligomer stability and dynamics.

The CTR of αA-crystallin occurs in non-3D and 3D domain-swapped configurations, as previously captured in crystals of truncated forms of zebradish-αα and bovine αA-crystallin, respectively. We show that both configurations coexist in solution. The transition between the two states requires the dissociation of the IPV motif from the β4/β8 groove. However, NMR studies on human αB-crystallin and Hsp27 show that the IXI motif is highly dynamic in solution and not rigidly bound to the protein scaffold. In αA-crystallin, the enhanced dynamics of the CTR are likely to facilitate domain-swapping.

In human αA-crystallin, the interplay between the geometric constraints imposed by the assembly architecture and the hinge loop connecting the CTR to the ACD is likely to dictate the propensity for domain-swapping. In all three assemblies, distance constraints preclude intermolecular binding of the CTR in apical protomers. Our reconstructions are of similar dimensions but differ in their number of subunits, leading to closer packing of protomers; that is, equatorial inter-protomer distances decrease gradually from 12-mer to 20-mer. Consequently, in equatorial protomers of the 12-mer, the non-3D domain-swapped configuration is favored, as a flexible chain of a given length is less likely to span large distances relative to its own length, resulting in folding back of the chain on itself (non-3D domain-swapped configuration). Shorter distances promote domain-swapping in all equatorial protomers of the 20-mer population. In the 16-mer population, both configurations coexist; nevertheless, the 3D domain-swapped state might impose more strain on the hinge region, and is thus less favored.

Despite the high similarity at the sequence level and virtually the same monomer length, αA- and αB-crystallin form different geometric bodies utilizing the same type of interactions: the β7-interface mediates dimerization and oligomerization as a determinant of ensemble heterogeneity. The recurring unit of αA-red oligomers is a tetramer in which two β7-interface dimers associate at the equator of the barrel-shaped assembly through N-terminal interactions. Further N-terminal interactions at the poles mediate the formation of higher-order assemblies by linking tetrameric units. A tetrameric building block is consistent with previous studies.

**Discussion**

Human αA-crystallin exists in heterogeneous ensembles of oligomers of varying subunit stoichiometries. The atomic models determined by combining data from cryo-EM, X-ray crystallography, cross-linking/mass spectrometry, NMR and molecular modeling reveal the roles of the NTR and CTR in oligomerization and C-terminal domain-swapping as a determinant of ensemble heterogeneity. The recurring unit of αA-red oligomers is a tetramer in which two β7-interface dimers associate at the equator of the barrel-shaped assembly through N-terminal interactions. Further N-terminal interactions at the poles mediate the formation of higher-order assemblies by linking tetrameric units. A tetrameric building block is consistent with previous studies.

**Fig. 5** | Oligomer architecture of oxidized human αA-crystallin. a, EM images of αAred (left) and αAox (right) oligomers negatively stained with 2% uranyl acetate. Scale bars, 50 nm. Note the increased oligomer size and polydispersity in αAox. b, Size distributions of the oligomers of αAred (black bars) and αAox (gray bars). The average oligomer size is shifted ~17.7 nm in αAox relative to its own length, resulting in folding back of the chain on itself. c, Different views of the 3D reconstruction of a 32-meric assembly of αAox. Scale bar, 10 nm. Dimeric building blocks are indicated by dashed ellipses.
of oligomers. This suggests that local conformational changes and/or partial unfolding occur, putting the two cysteines in the β8- and β9-strands in an appropriate spatial proximity. It has been suggested that partial unfolding of monomers upon dissociation may be a common property of human sHsps and partly unfolded monomers may exist within larger oligomers52,53. Upon removal of urea, αAox reassembles into oligomers that are distinct from those of αAred. Such oligomers harbor subunits, which are locally more dynamic in their β7-, β6+7- and β8-9-strands.

The redox potential of the intramolecular disulfide bridge in human αA-crystallin is comparable to that determined for thiol-disulfide oxidoreductases42,43. In the presence of urea, the intramolecular disulfide is formed at −220 mV, which is even below the estimated redox potentials of −204 mV and −217 mV at the nuclear and cortical regions, respectively, of the young lens43,44, thus enabling the formation of the intramolecular disulfide bridge in vivo, for an unfolded or destabilized chain. Although it is delicate to deduce the redox potential of a disulfide bond in vivo from the redox potential determined in vitro under equilibrium conditions in dilute solutions, the mere existence of the intramolecular disulfide in αA-crystallin in vivo hints at certain similarities of its redox properties in vitro and in vivo.

The intramolecular disulfide bridge in human αA-crystallin is a cross-strand disulfide. Such disulfides are often reactive redox-based conformational switches due to their strained conformation45. Although the edge strand β8 might tolerate the conformational changes/distortions caused by the disulfide bond to a certain extent, the diminished stability of αAred against urea-induced dissociation and unfolding compared to αAox supports a strained structure.

Interestingly, the two cysteines in human αA-crystallin are conserved among primates. In vivo, introduction of additional cysteine residues may be detrimental, as naturally occurring arginine to cysteine mutations of human αA-crystallin are associated with cataracts5. Together with the general evolutionary selection against cysteines, this suggests that the cysteines of human αA-crystallin must serve a function in the eye lens. This notion appears contradictory to the increase of intramolecular disulfides concomitant with a decrease in chaperone activity of αA-crystallin during aging and cataractogenesis52. It should, however, be noted that mere coincidence of these processes has been demonstrated, but not a direct causality. The precise relationship between cysteine oxidation and cataractogenesis needs to be further clarified.

αAox is able to transfer its intramolecular disulfide to destabilized substrates; it has redox properties intermediate between disulfide oxidases DsbA and DsbC. Given that it constitutes ~15–20% of eye lens proteins, this corresponds to an intracellular concentration of 3–4 mM. The lenticular glutathione concentration is on the order of ~3.7 mM in the outer cortical regions and ~2.8 mM in the nuclear regions of young lenses45,46. It is therefore likely that the redox state of the eye lens is not solely dictated by the glutathione system, but αA-crystallin itself will be an integral co-determinant of the lenticular redox system and a yet unknown player in lenticular redox homeostasis. It could well be that the preferential oxidation

### Fig. 6 | Dynamics of oxidized human αA-crystallin.

a. Relative fractional deuterium uptake of all peptides detected in H/DX-MS experiments. The deuterium behavior for early timepoints (10 s and 60 s) of the exchange reaction is shown. Peptides ordered by their midpoint; the peptide start and end amino acid positions are indicated at the abscissa. Note that the uptake pattern, overall, is well conserved among αAox and αAred. Values plotted are means of n = 3 technical replicates and the error bars reflect the corresponding s.d.

b. Differences in amide hydrogen protection in αAox and αAred mapped onto the model of a non-3D domain-swapped monomer of αA-crystallin. Differences in deuterium uptake were obtained by the difference in local relative deuterium uptake (ΔD = DαAox − DαAred), averaged using the algorithm of DynamX 3.0 (Waters). Regions in αAox with unchanged protection from deuteration are colored white, with decreased protection red and increased protection blue. Regions with insufficient coverage are colored according to the domain color code.
of αA-crystallin prevents the formation of non-native disulfide bonds in other crystallins and thus their aggregation in the eye lens. Further in vivo studies are required to address this issue.

Taken together, our structural analysis of αA-crystallin revealing the assembly principles of its oligomer ensembles, together with the properties of αA \text{red} and αA \text{ox}, provides a framework for understanding its role in the normal lens and in cataractogenesis.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41594-019-0332-9.

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Methods

Cloning and protein purification. Wild-type human α-crystallin was recombinantly produced in E. coli at 20 °C. The cells were harvested by centrifugation and disrupted in the presence of protease inhibitor mix G (Serva). The first purification step was anion exchange chromatography (Q-Sepharose FF). After fraction pooling, urea was added to a final concentration of 4.5 M, then cation exchange (SP-Sepharose FF) and gel filtration chromatography (Superdex 75) were performed. After an additional high-resolution anion exchange chromatography step, urea was removed by dialysis against PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.76 mM KH2PO4, pH 7.4), 1 mM EDTA and 1 M DTT. As a final polishing step, a gel filtration run (Superdex 200; PBS, 1 mM EDTA, 1 mM DTT) was performed, yielding ααααα preparations of >95% homogeneity as judged by SDS–PAGE. Aliquots were stored at −80 °C in gel filtration buffer. Before all experiments, protein aliquots were thawed and incubated for 3 h at 37 °C to ensure proper thermal equilibration. If not stated otherwise, all experiments were performed at this temperature. Protein solution concentrations were verified by the refractive index (RI) detector of the HPLC system used, which relies on the optical density at 200 nm for the RI detection. All samples were incubated for 16 h. Spectra were measured in triplicates using a Jasco FP-6500 spectrofluorimeter (Jasco) connected to a thermostat. Helma TS 10 mm × 2.0 mm fluorescence ultra-micro cuvettes (Helma) were used for fluorescence measurements. The excitation wavelength was set to 295 nm and emission spectra were recorded from 395 nm to 450 nm. During incubation and measurement, the samples were kept at 20 °C. The excitation wavelength of 295 nm was chosen to avoid interference with the corresponding buffer signal. For each spectrum, at a given concentration of urea (denoted as [urea]), the intensity averaged emission wavelength $\lambda_P (\text{max})$ was calculated. The fraction of native folded protein $f_{\text{native}}$ was calculated for the measurement range between 0 M urea and 7.5 M urea as $f_{\text{native}} = \frac{C_{\text{refl}}}{C_{\text{refl}} + C_{\text{den}}}$ and plotted versus denaturant concentration.

CD spectra in the near (290–320 nm) and far (180–260 nm) UV ranges were measured using a Jasco J-710 (Jasco) or a Chirascan (Applied Photophysics) CD spectrophotometer equipped with a thermostatted cuvette holder set to 20 °C. Near-UV CD spectra were recorded at a protein concentration of 100 μM and far-UV CD spectra were measured at 40 μM in 20 mM KH2PO4/KOH, pH 7.4, 1 mM EDTA. To record near-UV spectra, a Q5 cuvette was used for far-UV spectra, a detachable-window Q5 0.2 mm cuvette (both Helma).

Hydrogen/deuterium exchange-mass spectrometry. H/D MS experiments were performed using an ACQUITY UPC LC-mass system-class with H/D technology (Waters). H/D kinetics were determined by measuring data points at 0, 10, 60, 600 and 7,200 s exposure to deuterated buffer at 25 °C. At each data point, 4 μl of a solution of 30 μM protein was diluted automatically 1:20 into PBS, 1 mM EDTA, 1 mM DTT or 1 mM trans-5,4-dihydroxy-1,2-dithiane, pH 7.5, with 0, 10, 60, 600 and 7,200 s exposure and incubation for 20 min at 20 °C. For redox titrations in the presence of urea, all redox buffers and the buffer for initial DTT removal contained 4.5 M urea. The quenched reactions were analyzed by loading 2 μg of total protein per lane onto gradient gels (TG Prime, 8–16%, Serva) using non-reducing sample buffer. The relative amount of remnant reduced and oxidized monomeric ααααα (fraction oxidized, O) was determined by densitometry using ImageJ. The equilibrium constant for formation of the intramolecular disulfide $K_{\text{eq}}$ was determined through nonlinear regression of the data using the function $O = 1 - \left( \frac{[\text{GSSG}]}{[\text{GSH}]} \right) / \left( K_{\text{eq}} + \frac{[\text{GSSG}]}{[\text{GSH}]} \right)$. To determine the corresponding redox potential at 43 °C and pH 7.4 from the Nernst equation, $E_{\text{m}} = \frac{\lambda_{\text{IR}}}{2} = \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} = \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2}$ with $\lambda_{\text{IR}} = \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2}$ and $\lambda_{\text{IR}} = \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2} + \frac{\lambda_{\text{IR}}}{2}$.

Fluorescence and cryo-electron microscopy. Near-UV CD spectra were recorded at a protein concentration of 100 μM and near-UV CD spectra at a protein concentration of 100 μM and $f_{\text{den}} = \frac{C_{\text{refl}}}{C_{\text{refl}} + C_{\text{den}}}$ and plotted versus denaturant concentration.

Aggregation assays. Chaperone assays and disulfide-transfer reactions were performed in parallel using MDH or human p53 as model substrates in the absence of reducing agent. MDH was diluted to 4 μM and 53 μM was diluted to 2 μM into PBS containing 1 mM EDTA on ice. Oxidized and reduced ααααα, oxidized and reduced ααααα and ααααα folded to a final concentration of 0.1 M and 0.1 M respectively. $f_{\text{native}}$ was calculated by dividing the deuterium level incorporated at a given timepoint (in Da) by the total number of backbone amide hydrogens in the peptide (this equals the number of amino acids, minus proline residues minus 1 for the N-terminal amide). All experiments were performed with triplicate determination at each timepoint.

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to glow-discharged Quantifoil R 2/1 holey carbon copper grids, incubated for 30 s, blotted and plunge-frozen in liquid ethane using a manual plunger. The samples were mounted into autoloader cartridges and transferred into a Titan Krios electron microscope (FEG) equipped with a K2 Summit direct detector (Gatan) and operated in energy-filtered transmission electron microscopy (EFTEM) mode at 300 kV. Automatic data acquisition was performed using the TOM toolbox39. A total of 2,334 movie images were collected at defocus values ranging from $-1.2 \mu m$ to $-2.3 \mu m$ and at a nominal magnification of $x=37,000$ ($1.35 \AA$ per pixel) in super-resolution mode ($0.675 \AA$ per pixel). The movies were recorded at dose rates of 7–8 electrons per pixel per second, with exposure times of 0.37–0.27 s per frame and a target total dose of 25–36 electrons per $A^2$. The frames were aligned, averaged and binned to a final pixel size of 1.35 $\AA$ per pixel. Well-separated particle images were selected manually and extracted into 200 200 200 pixel boxes using e2boxer of the EMAN2 software package40. Images were corrected for the contrast transfer function by phase flipping using Bsoft41. All subsequent image processing procedures were carried out within the IMAGIC5 suite42.

**Image processing and 3D reconstruction.** For processing the cryo-EM data, reference-free class averages were generated from 74,068 CTF-corrected and bandpass-filtered (20–160 Å) single-particle images. The class averages revealed almost spherical particles ranging in size between 6 and 16 nm, as well as elongated ones with a maximum length of 14 nm. The presence of two-, three-, four- and five-fold symmetries in projection images together with the variation of particle dimensions and shapes suggested the presence of multiple structures of a similar barrel-like architecture but of varying subunit stoichiometries. Based on this, three models, each consisting of a bundle of ‘pillars’ (three, four and five pillars), compatible with three-, four- and five-fold symmetries were generated as starting references. Reference-free class averages were sorted into initial particle subsets based on particle diameter and symmetry. Within each subset, an initial 3D reconstruction was calculated by projection matching cycles using the above ‘pillar-bundle’ models as starting models. In a next step, initial 3D reconstructions were used as references to align and sort all single-particle images of the cryo-EM data set into three final subsets in an iterative procedure. All particles were aligned independently by multi-reference alignment (MRA) to each of the three reference structures. Within each of the three particle sets, multivariate statistical analysis (MSA) was applied to generate class averages. The Euler angles of the class averages were assigned by angular reconstitution (AR)43. Subsequently, classes that mismatched with the corresponding model reconstructions were sorted out. This ‘purification’ step by MSA/AR was repeated until all remaining class averages agreed well with reference reprojections, resulting in three distinct particle subsets.

For 3D reconstruction, the final class averages were refined iteratively by six rounds of MRA and AR using starting models generated from the input class averages of the respective set. For the refinement, single-particle images of each class were aligned with the corresponding reprojection of the respective preliminary reference, while particles that were rotated by more than 9° as well as the 10% of images with the lowest cross-correlation coefficients were ignored. Euler angles of the class averages comprising the remaining particles were refined by AR and a new 3D reconstruction was calculated, which served as a reference for the subsequent refinement cycle. During refinement, filtering of input and output images as well of the reference models changed in every iteration starting from 20 Å and ending at 7 Å to sequentially allow more details to affect the alignment. Class averages and 3D reconstructions were masked with an adaptive soft edge mask unless when used for Fourier shell correlation (FSC) calculations. For resolution determination, the ‘gold standard’ 0.143 criterion was used44.

FSCs were calculated between the final reconstructions of independently processed half sets using the FSC validation server (http://pdbe.org/fsc) within the PDBe (Protein Data Bank in Europe)45. 3D reconstructions and atomic models were rendered using UCSF Chimera46.

**3D sampling and classification.** To analyze the conformational heterogeneity of human αA-crystallin oligomers, the particles from the final oligomer subsets were subjected to bandpass filtering (140–10 Å), normalization and 3D sampling, followed by rigid-body classification. Within each dataset, 1,000 from 3D reconstructions from randomly selected 1,000 projection images were generated (3D sampling of structure subensembles). The 3D reconstructions were analyzed by 3D MSA followed by clustering into 10 distinct subpopulations by 3D classification using IMAGIC5.

**NMR spectroscopy.** αA-cryst., was $\text{[}^{15N}\text{C}_{\text{4}}\text{]}$-labeled upon recombinant expression and purified as described above. Purified protein was dialyzed against 10 mM HEPES/KOH (pH 7.4), 2 mM DTT, 1 mM EDTA. Solution-state NMR experiments were carried out using a Bruker Biospin Avance III spectrometer operating at a $\text{H}_2$ Larmor frequency of 950 MHz (22.3 T) using a CPTCI triple-resonance cryoprobe. All experiments were performed at 300 K in HEPES/KOH buffer containing 3% D$_2$O. For spin-labeling experiments, IPSL (Sigma-Aldrich; 50 mM stock dissolved in DMSO) was used. As only one cysteine residue is readily surface accessible in native αA-crystallin, the label most likely reacted with C14247. As a control, protein-bound IPSL was reduced with a 10 molar excess of freshly prepared ascorbic acid in HEPES/KOH buffer to yield the diamagnetic species. PREs arising from the spin label were determined using the ratio of peak intensities of the $\text{H}^\text{1}$/N-HSQC spectra obtained for the paramagnetic (oxidized) and the diamagnetic (reduced) state ($I_{\text{H}^\text{1}}, I_{\text{N}}$) in the absence and presence of 10 molar equivalents of ascorbic acid, respectively. Further experimental details are described in Supplementary Note 1.

**Crosslinking and mass spectrometry.** For cross-linking experiments, BS$	ext{3}$ cross-linker (Thermo Scientific), added to the protein upon continuous vortexing of the protein solution. The reaction mixture was incubated at 20°C for 1 h and quenched. The samples were loaded on gradient gels, which were run at a constant voltage of 200 V using MOPS-SDS running buffer. The protein in excised gel bands was alkylated with iodoacetamide and digested with trypsin (Thermo Fischer Scientific), following previously established protocols48. Peptides were separated by reverse-phase chromatography and analyzed by LC-MS/MS on an Orbitrap Fusion Lumos (Thermo Fisher Scientific) with a ‘high/ high’ acquisition strategy. The mass spectrometry raw files were processed into peak lists using MaxQuant (version 1.5.3.0)49 and cross-linked peptides were matched to spectra using Xi software (version 1.6.745)50. FDR was estimated using X!Tandem on 5% residue level51. Further experimental details are described in Supplementary Note 1.

**Model building.** Structural modeling of the human αA-crystallin 16-mer was based on homology models of either the non-3D domain-swapped structure of truncated αA-crystallin (αA$_{1–59}$) from zebrasfish (PDB 3N3E)52 or on the 3D domain-swapped structure of bovine truncated α-crystallin (αA$_{1–59}$) (PDB 3D1E)53 using theHamilton Modeller54. The N-terminal segment (αA$_{1–59}$) was modeled using I-Tasser55. Homology modeled ACD dimer structures were fitted as rigid bodies into the corresponding cryo-EM densities using the program colores of the Situs package56. The N-terminal modeled segment (αA$_{1–59}$) was placed randomly in various positions. The oligomers were energy minimized using the Sander module of the Amber software package (Amber16)57. Molecular dynamics flexible fitting was started from energy-minimized structures using the emap option in Sander58. For each of the initial placements of the N-terminal segments, the final flexibly fitted structure was evaluated based on r.m.s.d., stereochemistry and cross-correlation with respect to the cryo-EM density. The non-3D and 3D domain-swapped structures with low force field energy and best cross-correlation to the cryo-EM density were selected as best representative solutions. Further experimental details are described in Supplementary Note 1.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The cryo-EM density maps of αA-crystallin oligomers have been deposited in the EMBD under accession codes EMD-4895 (12-mer), EMD-4894 (16-mer) and EMD-4896 (20-mer). The coordinates for the 16-mer model were deposited in the wwPDB under accession number PDB 6T1R. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with dataset identifier PXD013587. The ‘$\text{H}^\text{1}$’ and ‘$\text{C}^\text{1}$’ chemical shifts of reduced αA-crystallin are available at the BioMagResBank (BMRB) with accession number BMRB-27109. All other data are available from the corresponding authors upon reasonable request.

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Author contributions

C.J.O.K., J.B. and S.W. designed and conceived the research plan. C.P., B. Rockel and C.J.O.K. performed EM experiments and processed the data. C.J.O.K. carried out, with contributions from P.W.N.S., E.V.M. and M.H., the experiments for the biochemical and biophysical characterization. V.D. provided full-length human recombinant p53. M.S. and S.A. performed NMR experiments. M.S. and B. Reif analyzed the NMR data. J.Z. conducted cross-linking/mass spectrometry experiments. J.Z. and J.R. analyzed the cross-linking data. M.Z. performed molecular dynamics simulations and model building. C.J.O.K., J.B. and S.W. wrote the manuscript, with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Cryo-EM/3D reconstruction of human αA-crystallin oligomers. a, Cryo-EM micrograph of human αA-crystallin (reduced, αAred). Top- and side-views are highlighted by white and black circles, respectively. Scale bar: 50 nm. b, Reference-free 2D class averages of top-views (top and middle rows) with corresponding eigenimages indicating size variations and 3-, 4- and 5-fold symmetries (bottom row). c, Reference-free 2D class averages of side-views (top and middle rows) with corresponding eigenimages indicating 2-fold symmetry (bottom row). d–f, Characteristic final class averages (top row) with the corresponding 2D reprojections of the 3D model (bottom row) of the αA-crystallin 12-mer (d), 16-mer (e) and 20-mer (f). Box size in b–f, 17.3 nm. g, Angular distribution plots, that is the distributions of the Euler angles of the final class averages contributing to the 3D reconstructions of the αA-crystallin 12-, 16- and 20-mer. h, Fourier shell correlation (FSC) curves between maps from two independently refined half data sets of 12-, 16- and 20-mer populations. According to the 0.143 gold standard criterion, the resolutions for 12-, 16- and 20-mer 3D reconstructions are 9.2, 9.8 and 9.0 Å, respectively.
Extended Data Fig. 2 | Structural variability of human αA-crystallin (reduced) oligomers. a, c, e, Top and side views of the cryo-EM maps of αA-crystallin (reduced) 12-mer (a), 16-mer (c) and 20-mer (e) (mesh presentation) overlaid with the most important 3D eigenvector (red) indicating the positions of main variances (variance map). b, d, f, Representative 3D class averages of the 12-mer (b), 16-mer (d) and 20-mer (f). The map used for modeling of the 16-mer in 3D domain-swapped configuration is marked in (d) by an asterisk. Scale bar, 10 nm.
Extended Data Fig. 3 | Cross-linking of human αA-crystallin. a, Cross-linker titration of αA-crystallin, denaturing NuPAGE gel. Reduced (left) and oxidized (right) αA-crystallin were incubated for 1 h at room temperature with BS3 cross-linker at the indicated molar BS3:αA-crystallin ratios. Excised monomer (450:1, blue), dimer (450:1 and 900:1, red) and oligomer (450:1, green) gel bands for both αAred and αAox were digested with trypsin and further analyzed. Sequence coverages: αAred-monomer: 97.1%, αAred-dimer: 99.4%, αAred-oligomer: 100%, αAox-monomer: 83.2%, αAox-dimer: 94.8%, αAox-oligomer: 100%. b, Fragmentation spectrum of a cross-linked peptide with an intramolecular link between K70 and K99. c, Fragmentation spectrum of a cross-linked peptide with an intermolecular cross-link between M1 and M1.
Extended Data Fig. 4 | Cross-links observed in reduced and oxidized human αA-crystallin. a, Primary sequence of human αA-crystallin. BS3 reactive K, S, T, Y residues and the N-terminus are coloured red. b, Linkage maps comparing the cross-linked residue pairs observed in monomer, dimer and oligomer pools of αA red and αA ox. In total, 113 auto-validation cross-links are shown. Colour code: blue, shared cross-links between αA red and αA ox (44 shared cross-links, 39%); black, unique cross-links in αA ox (63 cross-links, 56%); orange, unique cross-links in αA red (6 cross-links, 5%). Colour code for the sequence regions of αA-crystallin: NTr (residues 1–60), sienna; ACD (residues 61-145), gray; CTr (residues 146-173), green. c, Histograms of Cα-Cα distances of cross-links observed in αA red. The distances were measured between corresponding residues resolved in the crystal structures of truncated versions of zebrafish (PDB 3N3E, left) and bovine (PDB 3L1E, right) αA-crystallin.
Extended Data Fig. 5 | Secondary structure prediction and modeling of the N-terminal region of human αA-crystallin. a, Summary of sequence-based secondary structure predictions of the NTR as obtained from 15 different web-based prediction programs. The predictions reproduce all β-strand segments (blue) present in metazoan sHsp structures. According to the predictions, the NTR most likely contains 3-4 α-helical segments (orange). b, A possible 3D structure model of the NTR of human αA-crystallin predicted using I-Tasser. c, Examples of possible conformations of the NTR of apical (Map) and d, equatorial protomers (Meq) obtained upon structure modeling by molecular dynamics flexible fitting. Although the positions of the three helices within the EM-density in both Map and Meq differ, their arrangement relative to each other is well preserved in comparison to the I-Tasser model mRMSD ~2 Å.
Extended Data Fig. 6 | Superposition of $^1$H,$^1$N correlation spectra of $^{15}$N-$\alpha$A$_{red}$ and $^{15}$N-$\alpha$A$_{red}$-IPSL. The superposition of $^1$H,$^1$N correlation spectra of $^{15}$N-$\alpha$A$_{red}$ (black) and $^{15}$N-$\alpha$A$_{red}$-IPSL treated with ascorbic acid (reduced) shows chemical shift perturbations for residues, for which we have observed an attenuation of the signal intensity for the oxidized $^{15}$N-$\alpha$A$_{red}$-IPSL sample. In particular, residues T153, A155, E156, T157 display significant chemical shift changes, consistent with the PRE results. At the same time, the chemical shifts of the C-terminal residues (T168, S169, A170, S172, S173) are not affected by the presence of the nitroxyl moiety.
Extended Data Fig. 7 | Impact of oxidation on αA-crystallin structure. a, b, Far-UV (a) and near-UV (b) CD spectra of αA<sub>red</sub> (black line) and αA<sub>ox</sub> (gray line). Note that the chemical microenvironment of tyrosins, phenylalanines and W9 are affected by oxidation. c, SEC elution profiles of αA<sub>red</sub> (black line) and αA<sub>ox</sub> (gray line) on a Superose 6 10/300 GL column. Inset: a segment of the calibration curve using the filtration standard mixture from BioRad. The calculated average molecular masses are 380 kDa for αA<sub>red</sub> and 770 kDa for αA<sub>ox</sub>, respectively (ThG: bovine thyroglobulin, 670 kDa; γG: bovine γ-globulin, 158 kDa). Note the peak broadening, that is increased polydispersity in αA<sub>ox</sub>. d, Analysis of αA<sub>red</sub> (black line) and αA<sub>ox</sub> (gray line) by sedimentation velocity aUC in a concentration range from 2 µM to 150 µM using SEDFIT. The inset shows the concentration dependence of the sedimentation coefficient. e, A set of the class averages used for the 3D reconstruction of αA<sub>ox</sub> 32-mer. f, 2D reprojections of the reconstructed 3D volume corresponding to the orientations of the class averages shown in (e). Box size in e and f. 26.7 nm.
Extended Data Fig. 8 | Impact of oxidation on \( \alpha A \)-crystallin stability. **a**, Oligomeric states of \( \alpha A_{\text{red}} \) (black circles) and \( \alpha A_{\text{ox}} \) (gray circles) in the presence of urea as determined by sedimentation velocity aUC at 20 °C. The oligomers of both proteins dissociate successively with increasing urea concentrations. Note that \( \alpha A_{\text{red}} \) and \( \alpha A_{\text{ox}} \) form a ~2S species at urea concentrations of 4.5 M and 3.5 M, respectively, suggesting destabilization of \( \alpha A_{\text{ox}} \) oligomers. **b**, Intrinsic fluorescence urea unfolding transitions for \( \alpha A_{\text{red}} \) and \( \alpha A_{\text{ox}} \) at 20 °C. The midpoints of the cooperative transition are at 2.7 M for \( \alpha A_{\text{ox}} \) and at 3.8 M urea for \( \alpha A_{\text{red}} \), indicating destabilization of the NTR in the case of \( \alpha A_{\text{ox}} \). The spectral settings of the fluorimeter were chosen to selectively assess the transition of W9 located within the NTR.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | αA-crystallin is capable of transferring disulfide bonds to MDH. 

a, Denaturing, non-reducing PAGE of samples withdrawn at the indicated timepoints (red arrows) from the aggregation assays in the presence of αAred and recombinant reduced E. coli DsbA as shown in Fig. 7a. Note that disulfide-bridged species of p53 are formed only in marginal amounts. 

b, Heat-induced aggregation of recombinant malate dehydrogenase (MDH, 4µM) in the presence of an equimolar amount of GSSG, αAred, αAox and reduced (DsbAred) or oxidized (DsbAox) E. coli DsbA. Note that the aggregation of MDH is fully suppressed in the presence of αAred and almost fully suppressed in the presence of αAox. 

c, Relative intensity of the MDH monomer band as a fraction of the initial intensity (amount of monomer) at the beginning of each aggregation kinetics experiment (t = 0 min). 

d, e, Denaturing, non-reducing PAGE of samples withdrawn at the indicated timepoints (red arrows) from the aggregation assays shown in (b). Experiments were performed in the presence of GSSG, αAox or DsbAox (d), in the absence of GSSG (MDH only) and in the presence of αAred or DsbAred (e). Note that disulfide-bridged species of MDH are formed in the presence of αAox.
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All relevant data are available from the corresponding authors upon request and/or included in the manuscript or Supplementary Information. The cryo-EM density maps of α-crystallin 12-, 16- and 20-mer have been deposited in the EMBD under the entry IDs EMD-4895, EMD-4894 and EMD-4896. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD013587 (Reviewer account details: Username: reviewer16270@ebi.ac.uk, Password: MSWfHQEQc). The 1H, 15N, 13C chemical shifts of reduced αA-crystallin are available at the BioMagResBank with the accession number BMRB-277109.
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| Sample size                  | Measurements were replicated as indicated in the figure legends. No statistical sample size estimation was performed. |
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| Data exclusions              | No data were excluded from analysis.                                                                                   |
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