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Solid-state NMR and SAXS studies provide a structural basis for the activation of α B-crystallin oligomers

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Abstract

The small heat shock protein α B-crystallin (α B) contributes to cellular protection against stress. For decades, high-resolution structural studies on oligomeric α B have been confounded by its polydisperse nature. Here, we present a structural basis of oligomer assembly and activation of the chaperone using solid-state NMR and small-angle X-ray scattering (SAXS). The basic building block is a curved dimer, with an angle of $\sim 121^\circ$ between the planes of the β -sandwich formed by α -crystallin domains. The highly conserved IXI motif covers a substrate binding site at pH 7.5. We observe a pH-dependent modulation of the interaction of the IXI motif with β 4 and β 8, consistent with a pH-dependent regulation of the chaperone function. N-terminal region residues Ser59-Trp60-Phe61 are involved in intermolecular interaction with β 3. Intermolecular restraints from NMR and volumetric restraints from SAXS were combined to calculate a model of a 24-subunit α B oligomer with tetrahedral symmetry.

Small heat shock proteins (sHSPs) help to maintain protein homeostasis by interacting with unfolded, aggregated or misfolded proteins to prevent cell damage^{1–3}. The ATP-independent chaperone α B-crystallin (α B, 20 kDa, 175 residues) is a paradigm example⁴. α B was originally found in the eye-lens as the B-subunit of α -crystallin, a protein essential for maintaining eye-lens transparency. In recent years, the list of biological roles for α B has grown substantially, including involvement in the regulation of the ubiquitin-proteasome pathway as well as

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Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

AUTHOR CONTRIBUTIONS

S.J. contributed to all aspects of the manuscript; P.R. performed solution NMR experiments and helped to write the manuscript; B.B. performed structure calculations; S.M. did solid-state NMR and SAXS measurements as well as data analysis; R.K. contributed to modeling of C-terminal intermolecular interactions; J.R.S. prepared samples; V.A.H. contributed to assignment strategies, was involved in structure calculations and helped write the manuscript; R.E.K. contributed to the interpretation of results and wrote the manuscript; B.J.v.R. contributed to solid-state NMR measurements, discussed the results and helped to write the manuscript; H.O. designed experimental strategies, contributed to the interpretation of results and wrote the manuscript.

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apoptosis⁵. Dysfunctions of α B in humans are associated with the occurrence of neurodegenerative diseases like Alzheimer's disease, Alexander's disease, myopathies and cataracts^{6–11}. In the brain of patients with Alexander's disease, the insoluble cell fraction contains protein fibers (Rosenthal fibers) coprecipitated with α B phosphorylated at Ser59, whereas unphosphorylated α B remains in the soluble fraction⁷. A missense mutation, R120G, in α B is associated with desmin-related cardiomyopathy^{8,9}. The mutations D140N and Q151X are associated with congenital cataracts and myopathy, respectively^{10,11}. A decreased concentration of α B in the cerebrospinal fluid was found to be associated with multiple sclerosis; therapeutic application of α B was shown to reverse the typical symptoms in mice by an involvement in the regulation of caspase 3 levels in the apoptotic pathway^{12,13}. Hypoxia, a condition often found in diseased states that include cancer and ischemia, induces the overexpression of α B¹⁴. Hypoxia is accompanied by acidosis, and it has been shown that α B binds cardiac-muscle proteins such as desmin and titin more tightly at acidic pH, presumably to confer protection to these proteins under conditions of cardiac ischemia¹⁴.

As is common for most sHSPs, α B is a polydisperse, supramolecular complex with a variable number of subunits (~24–32) (ref. ¹⁵) whose inherent dynamics is an important feature and prerequisite of its function. For decades, high-resolution structural studies on functional, full-length α B and mammalian sHSPs in general have been confounded by this polydisperse nature. α B contains a conserved, ~90-residue α -crystallin domain with an immunoglobulin fold, flanked by variable N- and C-terminal extensions. Intermolecular interactions of the N and C termini with the α -crystallin domain are responsible for the formation of the higher-order oligomers¹⁶. The segment containing the phosphorylation site Ser59 (Ser59-Trp60-Phe61), for example, was found to alter the oligomerization behavior if mutated or inverted¹⁷. Crystal structures of monodisperse sHSPs (Supplementary Figs. 1a,b and 2) and, after deposition of these coordinates, structures of short constructs comprising the conserved α -crystallin domain of rat HSP20 obtained at pH 6.5 and human α B obtained at pH 9.0 became available^{18–21}. In most of these structures, the α -crystallin domain forms a dimer of 7-stranded β -sandwiches (β 2– β 9). From previous NMR studies, it is known that residues 64–152 of α B form a 6-stranded β -sandwich and a dimer interface comprised of an antiparallel arrangement of the long β 6+7 strands from two protomers²². Electron spin resonance (EPR) analysis using spin labels had given a preliminary indication of this type of arrangement in the dimer interface as well²³. First indications of the oligomer structure came from electron microscopy studies, which showed that α B has a variable quaternary structure with a roughly spherical shape and a central cavity²⁴. More recently, single-particle reconstruction from negative-stain electron microscopy at 20-Å resolution showed that a dominant population of α B forms a hollow sphere-like particle with 24 subunits and tetrahedral symmetry²⁵.

Here, we have performed structural investigations on human full-length α B-crystallin (HspB5) using a combination of magic angle spinning (MAS) solid-state NMR, small-angle X-ray scattering (SAXS) and computational methods to determine the structure of the α -crystallin domain dimer within α B oligomers at pH 7.5 as well as its intersubunit interactions involving parts of the N- and C-terminal regions. The C-terminal residues Arg157–Arg163, including the IXI motif, bind into a presumed substrate binding groove, suggesting an autoinhibitory function. NMR spectra reveal a modulation of this interaction by pH changes, providing a structural mechanism for the known pH-dependent activation of α B.

RESULTS

Atomic-level structure of the homodimeric α -crystallin domain

We performed the MAS NMR investigations on full-length human α B (Fig. 1a and Supplementary Fig. 2) precipitated by PEG at pH 7.5. Initial solution- and solid-state NMR studies provided resonance assignments for the α -crystallin domain and parts of the C terminus,

defining the secondary structure²² (Supplementary Fig. 2). We expanded resonance assignments to 139 of 175 residues using a novel assignment procedure²⁶ (BMRB 16391). NMR spectra collected at pH 7.5 reveal a structurally homogeneous part of the protein comprising α -crystallin domain residues 71–150, with the exception of Ser76, Val77 and Asn78 in the β 3 strand, which each show two backbone ^{15}N signals. We performed structure calculations^{27,28} for residues 69–150 using 540 inter-residue NMR-derived distance restraints per protomer (Table 1 and Supplementary Table 1). We obtained structure-defining restraints from a variety of spectra (see Supplementary Table 2). Intramolecular $\text{C}\alpha$ - $\text{C}\alpha$ and $\text{H}\alpha$ - $\text{H}\alpha$ correlations define the topology of two antiparallel β -sheets, β 3- β 9- β 8 and β 4- β 5- β 6+7 (Supplementary Fig. 3). We obtained the $\text{C}\alpha$ - $\text{C}\alpha$ restraints from 3D NCACX spectra²⁹ of $^{13}\text{C}/^{15}\text{N}$ -labeled αB made from 1,3- and 2- ^{13}C -labeled glycerol (Supplementary Fig. 3.) and the $\text{H}\alpha$ - $\text{H}\alpha$ restraints from CHHC spectra³⁰, using a sample containing 20% $^{13}\text{C}/^{15}\text{N}$ -labeled and 80% unlabeled protein, using deuteration at the exchangeable sites to suppress sequential transfer. The homodimer is unambiguously defined by 26 intermolecular correlations derived from oligomers prepared from mixtures of differently labeled αB , using either 2- ^{13}C - or 1,3- ^{13}C -labeled glycerol and $^{14}\text{NH}_4\text{Cl}$ for expression mixed with αB made from ^{13}C -depleted glucose and $^{15}\text{NH}_4\text{Cl}$ (Supplementary Fig. 3). All ^{13}C - ^{15}N cross-peaks observed in spectra recorded from these samples reflect intermolecular correlations. The dimer interface is comprised of the antiparallel β 6+7 strands from each protomer, with Glu117 adjacent to the two-fold axis. This topology is consistent with solution NMR data from the homodimeric $\alpha\text{B}10.1$ construct (residues 64–152)²².

The calculations yielded a curved dimer of the α -crystallin domain β -sandwich as the recurring building block of the oligomer (PDB 2KLR). The dimer is shown in Figure 1b–d together with interdimer interactions involving residues 59–61 (β 2a) and 157–163 (IXI motif). The backbone r.m.s. deviation for the ten lowest-energy structures in the ensemble is 1.36 Å for the dimer (0.87 Å if only the secondary structure is considered) and 1.14 Å when superposing monomers (Table 1 and Supplementary Table 1). A distinctive feature of the dimer is its curvature, with an average angle of $\sim 121^\circ$ between the two β 4- β 5- β 6+7 planes (Fig. 2a). The concave face is notable for its network of ionic interactions, especially across the dimer interface (Fig. 2b). In particular, the residue pairs Glu99-His119 and Glu117-His101 form potentially pH-dependent electrostatic interactions on both sides of the dimer interface that may contribute to the observed curvature (Fig. 2b). Intermolecular cation- π interactions between Phe118 and Arg116', identified using the program CAPTURE³¹, may contribute to dimer stability. The Hsp20 α -crystallin domain has a similar array of histidines and glutamates and is curved in a crystal structure solved at pH 6.5 (ref. ²¹) (Fig. 2c,d). In contrast, an X-ray structure of the human αB α -crystallin domain determined at pH 9.0 is virtually flat²¹, implying a pH dependence for this feature. A key difference between our curved dimer and the flat dimer observed at pH 9.0 involves Arg107, which forms a salt bridge across the dimer interface with Asp80' (Fig. 1d), whereas in the X-ray structure²¹ (PDB 2WJ7), Arg107 forms an intramolecular salt bridge with Glu88. The curved shape of the dimer is consistent with SAXS data obtained on the excised α -crystallin domain dimer $\alpha\text{B}10.1$ (ref. ²²) in solution (Supplementary Fig. 4). Monomers from the X-ray structures of isolated mammalian α -crystallin domains²¹ (PDB 2WJ7 and 2WJ5) superimpose with high accuracy (r.m.s. deviation 1.4 Å, Supplementary Fig. 1c), except for the tip of the loop connecting β 5 and β 6+7.

Intermolecular contacts within αB oligomers

The MAS NMR spectra contain multiple NMR signals, indicating diverse environments, for the putative β 2 strand observed in other sHSPs, which we instead call heterogeneity region 1 (HR1, residues Glu67–Leu70)²². We could not extract a consistent set of long-range restraints for HR1. In a recent X-ray structure of the α -crystallin domain from αB^{21} , two of five protomers in the unit cell contain a β 2 strand that aligns antiparallel to β 3 of the same protomer. In our

spectra, residues that precede HR1 (Ser59, Trp60, and Phe61) show intermolecular contacts to $\beta 3$ (see Supplementary Fig. 3f,h), indicating a ' $\beta 2a$ ' strand that is involved in the formation of quaternary structure. At opposite edges of the dimer, a putative substrate binding site^{32,33} formed by the groove between $\beta 4$ and $\beta 8$ contains small pockets that can accommodate aliphatic side chains (Fig. 3a). Indeed, the NMR data reveal intermolecular contacts between the IXI motif (Ile159-Pro160-Ile161) and residues of $\beta 4$ and $\beta 8$ (Leu89-Val93 and Ile133-Ser138; Fig. 3b and Supplementary Fig. 2g,h). We used 12 intermolecular ^{13}C - ^{15}N constraints to dock the C-terminal residues to the α -crystallin domain using Sybil (Tripos). In the resulting model, residues Arg157-Arg163 cross the binding site in an extended conformation, with the side chains of Ile159 and Ile161 residing in the two prominent hydrophobic pockets (Fig. 3a).

Model of a 24-mer αB -crystallin oligomer

The approach we have used to determine the structures shown in this study is shown schematically in Supplementary Figure 5. Using the dimer structure and intermolecular interactions obtained from MAS solid-state NMR and the shape of αB determined from SAXS, interactive modeling with Chimera^{34,35} and energy minimization with XPLOR-NIH³⁶ allowed us to obtain a structural model of a representative αB oligomer.

SAXS data obtained at pH 7.5 for oligomeric αB in solution were used to calculate six bead models, assuming tetrahedral symmetry, consistent with previous electron microscopy studies²⁵. χ^2 versus normalized spatial discrepancy plots generated with DAMAVER³⁷ indicate high similarity among the six reconstructions and a reasonable fit with the experimental SAXS curve (Supplementary Fig. 6a). We averaged the initial six bead models and transformed them into a volumetric map (Fig. 4a) that has a diameter of 14.5–16 nm. The individual bead models (Supplementary Fig. 6b) generated using the program GASBOR³⁸ have extrusions emanating from the central body, which we interpret as flexible C-terminal residues 166–175 of the oligomer³⁹. In individual models, these extrusions create a maximum diameter D_{max} of 19 nm. The cited range of αB oligomers is 24–32 subunits¹⁵, and a heterogeneous mixture exists in solution. Reasoning that larger species are assembled by combining fragments of 24-mers, we undertook to model the smallest species. αB_{69-175} has been shown to form dimers and trimers⁴⁰, indicating that the N-terminal domain is crucial for the formation of higher-order assemblies. Hence, the C-terminal domain alone is not responsible for the formation of higher-order oligomers. Accordingly, adjacent dimers in the recurring hexameric substructure (Fig. 4, red and blue) were connected by satisfying the observed C-terminal interactions. Using P_{432} symmetry, an oligomer may contain 24 or 48 subunits, with the asymmetric unit being the monomer or the dimer, respectively. Using P_{23} symmetry, the symmetry operations allow 12, 24 or 36 subunits in the oligomer, with the smallest asymmetric unit being the monomer, dimer or three monomers (trimer), respectively. We chose P_{23} symmetry because of both its congruence with our data (that is, a dimer is the smallest building block) and its agreement with the recently reported electron microscopy structure²⁵. We therefore constructed an atomic model of a 24-mer on the basis of the dimer structure, the intermolecular N- and C-terminal interactions observed by NMR and the volumetric data, applying P_{23} symmetry (Fig. 4a,b). We kept the core of each dimeric unit rigid, whereas we considered the loop connecting the α -crystallin domain with the IXI motif, docked in the $\beta 4/\beta 8$ substrate binding site, to be flexible. An electron density calculated at 20-Å resolution from the resulting molecular oligomer model (Fig. 4b) shows a similar molecular organization to that of the recently reported electron microscopy structure²⁵. We consider this solution as one species in a heterogeneous ensemble of oligomers. Variable conformations of the flexible C-terminal extensions (residues Lys166–Lys175) and variable intermolecular interactions may contribute to the observed heterogeneity.

pH-dependent activation of α B chaperone function

The activation of cellular protection systems against stress is often associated with a decrease of pH^{2,41}. For example, α B binds cardiac-muscle proteins actin and desmin more strongly at acidic pH, preventing damage to these proteins during cardiac ischemia accompanied by acidification¹⁴. We investigated the effect of pH on α B and particularly on a substrate binding site between β 4 and β 8 (Fig. 5a) by NMR and SAXS. In ¹³C-¹³C correlation spectra collected at pH 7.5 (Fig. 5b–d), we observed single cross-peaks for Ile133 (β 8), Ile159 and Ile161. At pH 6.5, the involved isoleucines and threonines (Thr132 and Thr134 in β 8 and Thr158 and Thr162 of the C terminus) show chemical-shift perturbations or doubling of intraresidual cross-peaks (for example, at ~59–63 p.p.m., ~36–39 p.p.m. or 26 p.p.m. in F₁ and at 69–72.5 p.p.m. or 9.5–27 p.p.m. in F₂), indicating a pH-dependent change in the substrate binding groove (Figs. 3a and 5a). The radius of gyration (R_g) determined by SAXS is 5.4 nm at pH 7.5 and 6.7 nm at pH 6.5; $D_{\max}$ increases from 19 nm at pH 7.5 to 30 nm at pH 6.5 (Supplementary Fig. 7a,b), indicating a substantial change in quaternary structure of the α B oligomer. Such a change may be predominantly due to release of the IXI motif from the β 4/ β 8 site.

Our results provide a structural explanation for the autoinhibitory function of the C-terminal IXI motif and its role in balancing homeostasis of active and inactive binding sites under varying cellular conditions¹⁴. At physiological pH conditions, the IXI motif binds nearly quantitatively into the substrate binding groove, as indicated by the strong restraints and the single set of chemical shifts observed for Thr132, Thr134, Ile159 and Ile161 at pH 7.5. Thus, hydrophobic substrates must compete with the C terminus for binding, whereas this interaction is modulated at lower pH, very likely exposing a population of binding sites in the oligomer. The role of the IXI motif in regulating the chaperone activity is supported by the effects of mutating both isoleucines (Ile159 and Ile161) to glycine, which substantially enhances the chaperone activity of α B *in vitro*¹⁶, and by the truncation Δ Glu151, which causes myofibrillar myopathies¹¹, presumably by generating a constitutively active form with exposed hydrophobic surfaces and without the highly soluble C-terminal tail.

Cellular conditions thus modulate the dynamic structure of α B to adjust its functional role according to physiological needs. We have shown that a drop in pH results in observable changes in a substrate binding site of α B. Protein phosphorylation is another way to modulate protein function. Along these lines, the observed intermolecular interactions involving residues Ser59-Trp60-Phe61 (β 2a) in one dimer and β 3 in another contribute to a structural basis for understanding the effects of phosphorylation at Ser59 (ref. ⁷), which is also expected to alter the oligomerization behavior of α B¹⁷.

DISCUSSION

The polydisperse human α B oligomer has been refractory to structural analysis for decades. Structures have been solved for isolated α -crystallin domains from human α B and rat HSP20 (ref. ²¹) and for three sHSPs that form monodisperse multimers^{18–20} (Supplementary Figs. 1a,b and 2) and electron spin-label studies have been performed on several sHSPs²³. These reveal a conserved β -sandwich topology but do not explain the nature of heterogeneity in the native oligomer. Here, application of MAS NMR and SAXS to α B provides four new insights. First, the independently determined MAS NMR structure reveals a highly curved dimer as the fundamental building block of α B oligomers at pH 7.5; an array of histidines and glutamates on the concave face of the dimer may constitute a pH switch that controls the curvature and, ultimately, the quaternary structure (Fig. 2a,b). Second, experimentally observed distance restraints and chemical-shift changes among residues in the N or C termini and the α -crystallin domain provide atomic-level details of the quaternary structure of α B oligomers and its modulation upon activation. Third, NMR signal heterogeneity observed for residues Ser59–Leu70 indicate multiple environments for this segment within oligomers. Notably, this segment

is important for the oligomerization of α B and in the formation of hetero-oligomers with related sHSPs¹⁷. Fourth, SAXS measurements reveal large increases in both the R_g and D_{max} with a drop in pH from 7.5 to 6.5. We propose that release of the C-terminal IXI motif from the substrate binding cleft contributes to the more expanded oligomeric structure, activating α B as a chaperone.

In conclusion, application of solid-state NMR and SAXS has provided high- and intermediate-resolution structural information on a dynamic protein assembly under conditions in which its biological function is known to be modulated. α B's chaperone function is implicated in diseases including multiple sclerosis, cancer, neurodegenerative diseases and cardiomyopathy. These and other diseases are connected to imbalanced protein homeostasis, making the responsible proteins potential therapeutic targets. The ability to obtain structural information on large dynamic protein assemblies while maintaining the ability to control relevant solution conditions such as pH represents a milestone for understanding both the function and dysfunction of sHSPs, in particular α B, and may help to guide future structure-based drug design efforts.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/nsmb/>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Structure of the αB-crystallin core domain dimer and its intermolecular interactions. **(a)** Domain architecture of small heat-shock proteins consists of a proline- and phenylalanine-rich N-terminal segment, a conserved α-crystallin domain and a C-terminal region that contains the conserved IXI motif. The heterogeneous region 1 (HR1), the IXI motif, the flexible C-terminal residues Lys166–Lys175 and three disease-related mutations involved in cataract and myopathy (R120G, D140N and Gln151-stop) are indicated. **(b)** Ribbon representation of the lowest-energy solid-state NMR structure of the αB-crystallin dimer (shown in red and blue, PDB 2KLR) and its intermolecular interactions with N- and C-terminal regions of other protomers (shown in gray, labeled C-ter and β2a). The side chains of residues Asp109, Arg116, Phe118 and Arg120 at the dimer interface are shown, and the sites of known disease-associated mutations D140N and Q151X are indicated separately. **(c)** Ionic interactions between Asp80' and Arg107 across the dimer interface in full-length αB and correlations of Asp80' Cγ with Ile114 Cβ and Arg107 Cβ and Cγ from a 2D ¹³C-¹³C correlation spectrum recorded from 2- [¹³C]-glycerol-labeled αB. **(d)** Stereo view of a superposition of the ten lowest-energy structures, showing the intermolecular interactions with N- and C-terminal regions in red and blue, respectively; see main text for details.

Figure 2.

Curvature of the α -crystallin domain dimer. **(a)** Side view of the α B-crystallin dimer (solid-state NMR, pH 7.5), showing the curvature of the extended β -sheet formed by the dimer interface. **(b)** Concave face of β 4- β 5- β 6+7 sheet of α B dimer (solid-state NMR, pH 7.5). A superposition of two conformers taken from the NMR ensemble is shown. **(c)** Side view of the α -crystallin domain dimer from HSP20 (X-ray, pH 6.5)²¹. **(d)** Concave face of β 4- β 5- β 6+7 plane of HSP20 (X-ray, pH 6.5), with multiple side chain conformations according to PDB 2WJ5 (ref. ²¹).

Figure 3.

A substrate binding site of α B-crystallin. **(a)** Model of the C-terminal IXI motif binding to a hydrophobic substrate binding groove of α B. A docking study was performed on the basis of 12 restraints from solid-state NMR data. The C-terminal sequence Pro155-Glu156-Arg157-Thr158-Ile159-Pro160-Ile161-Thr162-Arg163 is shown as sticks. In the surface plot, yellow represents hydrophobic residues; green, hydrophilic residues. **(b)** Contour plot of the amide-methyl region of a 2D TEDOR spectrum recorded from differentially labeled α B; the sample was obtained using a mixture of protomers expressed from 1,3- ^{13}C -glycerol/ $^{14}\text{NH}_4\text{Cl}$ and ^{13}C -depleted glucose/ $^{15}\text{NH}_4\text{Cl}$. Cross-peaks between amide nitrogens and methyl groups are observed for Ser135, Leu137, Lys90 and Val91 (on the α -crystallin domain), with Ile159, Ile161 and Thr162 (on the C terminus) defining the binding interface between the IXI motif and the hydrophobic groove formed by β 4 and β 8. Cross-peaks between the amide nitrogens of Val81 and Lys121 with the side chain of Ile114 are also observed.

Figure 4.

Molecular organization of the oligomer. **(a)** A model of the α B oligomer is shown within the average volumetric map generated from SAXS data. Six bead models calculated using P_{23} symmetry with the program GASBOR were averaged using the program DAMAVER. The volumetric data (shown as light gray surface) were generated from the average bead model using the software SITUS⁴². The volumetric SAXS data were fitted with α B homodimers (shown as red and blue ribbons) using P_{23} symmetry and NMR-derived intermolecular distance constraints in an energy minimization protocol using the program XPLOR-NIH³⁶. **(b)** Predicted electronic density map calculated at 20-Å resolution from the molecular oligomer model using the program CHIMERA^{34,35}. **(c)** Arrangement of three dimers, connected by their C termini.

Figure 5.

pH-dependent modulation of a substrate binding site. (a) Substrate binding site between $\beta 4$ and $\beta 8$ of αB (red ribbons) and the C terminus from an adjacent dimer (blue ribbon). The isoleucine and threonine residues that show chemical-shift perturbation or peak doubling are shown as sticks. (b,c) Superposition of contour plots measured at pH 7.5 (blue) and pH 6.5 (red), showing the spectral region where cross-peaks between $\text{C}\delta$, $\text{C}\gamma 1$, $\text{C}\gamma 2$, $\text{C}\alpha$ and $\text{C}\beta$ of isoleucines (b) and threonines (c) are visible. Cross-peaks involving Thr158, Ile159, Ile161 and Thr162 in the IXI motif and Thr132, Ile133 and Thr134 in the substrate binding site are underlined and show peak doubling or chemical-shift perturbations upon a pH decrease from 7.5 to 6.5. (d) Cross-sections along F_2 from 2D ^{13}C - ^{13}C spectra recorded at pH 7.5 (blue) and pH 6.5 (red), extracted at the $\text{C}\alpha$ resonance of Thr134 (60.2 p.p.m.) and the $\text{C}\gamma 2$ resonance of Ile133 (18.3 p.p.m.), respectively.

Table 1 Structure statistics for the α -crystallin dimer

α B-crystallin dimer	
NMR distance and dihedral constraints (per dimer)	
Distance constraints	
Total NOE	1,086
Intra-residue	6
Inter-residue	1,080
Sequential ($ i - j = 1$)	258
Medium-range ($ i - j < 4$)	176
Long-range ($ i - j > 5$)	336
Ambiguous	258
Intermolecular	52
Hydrogen bonds	84
Total dihedral angle restraints	270
φ	136
ψ	134
Structure statistics (for dimer)	
Violations (mean $\pm$ s.d.)	
Distance constraints ($\text{\AA}$)	0.059 ± 0.047
Dihedral angle constraints ($^\circ$)	1.175 ± 0.880
Max. dihedral angle violation ($^\circ$)	4.472
Max. distance constraint violation ($\text{\AA}$)	0.322
Deviations from idealized geometry	
Bond lengths ($\text{\AA}$)	0.013 ± 0.001
Bond angles ($^\circ$)	1.269 ± 0.049
Impropers ($^\circ$)	1.810 ± 0.131
Average pairwise r.m.s. deviation ^a ($\text{\AA}$)	
Heavy	2.00 ± 0.32
Backbone	1.36 ± 0.37

See Supplementary Table 1 for detailed structure statistics.

^aPairwise r.m.s. deviation was calculated among ten refined structures. $\pm$, s.d.