



## Recent advances in NMR-based structural characterization of $\alpha$ B-crystallin and its potential role in human diseases

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**Abstract**  $\alpha$ B-crystallin ( $\alpha$ BC) is a member of a small heat-shock protein (sHSP) superfamily and plays a predominant role in cellular protein homeostasis network by rescuing misfolded proteins from irreversible aggregation.  $\alpha$ BC assembles into dynamic and polydisperse high molecular weight complexes containing 12 to 48 monomers; this variable stereochemistry of  $\alpha$ BC has been linked to quaternary subunit exchange and its chaperone activity. The chaperone activity of  $\alpha$ BC poses great potential as therapeutic agents for various neurodegenerative diseases. In this mini-review, we briefly outline the recent advancement in structural characterization of  $\alpha$ BCs and its potential role to inhibit protein misfolding and aggregation in various human diseases. In particular, nuclear magnetic resonance (NMR) spectroscopy and its complimentary techniques have contributed much to elucidate highly-dynamic nature of  $\alpha$ BCs, among which notable advancements are discussed in detail. We highlight the importance of resolving the structural details of various  $\alpha$ BC oligomers, their quaternary dynamics, and structural heterogeneity.

**Keywords**  $\alpha$ B-crystallin, small heat-shock protein, chaperone, protein structure, NMR spectroscopy

### Introduction

Advancement of our understanding to life and

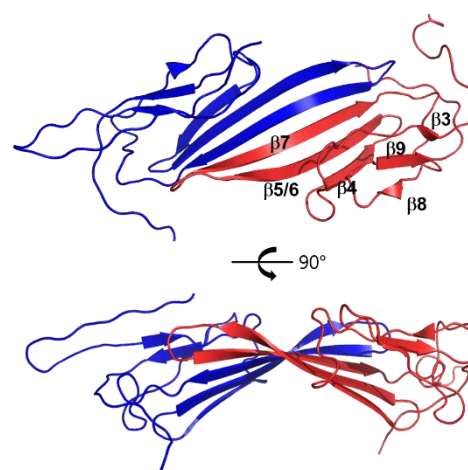
development of medicinal technology revolutionize our life in many aspects. As a result, the life expectancy of humans has been dramatically increased over a few decades.<sup>1</sup> However, this is accompanied with the increase of various aging-associated diseases including neurological diseases, cardiovascular diseases, cancers, and musculoskeletal diseases.<sup>2</sup> In particular, neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) causes long-term illness and severe economic burdens not only for patients and their relatives but also for the related societies.<sup>3</sup> The recent report estimated that approximately 44 million people worldwide are suffering from AD or the related dementia, and this number will be tripled by 2050.<sup>4</sup> One of the main pathogenic features for AD and the related dementia is the accumulation of misfolded and unmitigated proteins (e.g. amyloid-beta and tau protein), which initiate the cascade of forming plaques and fibrils in and around brain and neuronal cells, and ultimately prompt neuronal cell death.<sup>5-7</sup> For clearance of plaques and fibrils, cells have developed various protein homeostasis mechanisms,<sup>8-10</sup> among which molecular chaperones such as heat shock proteins (HSPs) play key roles in maintaining structural integrity of proteins and rescuing misfolded proteins from aggregation.<sup>11-13</sup> Especially,  $\alpha$ B-crystallin, one of the essential small heat shock proteins (sHSPs), serves as the first line of defense against the intracellular misfolded protein aggregation.<sup>14-18</sup> The name 'crystallin' comes from its dominant presence in the

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ocular lens and formation of a crystalline state to maintain clarity of an eye lens. There are various isoforms of crystallins in a human eye lens; among them,  $\alpha$ -crystallins, which includes  $\alpha$ A- and  $\alpha$ B-crystallins, act as chaperones to prevent structural deformation and aggregation of other crystallins. Abnormal accumulation of crystallin aggregates in eye lens causes cataract in human.<sup>19-23</sup> Notably, this functionality of  $\alpha$ -crystallins is not limited to eye lens;  $\alpha$ -crystallins are present in various non-lenticular tissues and inhibit pathological aggregation of various proteins, which are associated with a number of neuropathological protein folding diseases.<sup>24-27</sup> Particularly,  $\alpha$ B-crystallin ( $\alpha$ BC) plays a pivotal role in clearing pathological aggregation of amyloids which are closely associated with human diseases such as AD, PD and multiple sclerosis.<sup>26,28-31</sup>  $\alpha$ BC is a prominent member of sHSP family, consisting of the variable N- and C-terminal domains and the conserved  $\alpha$ -crystallin domain (ACD) between the two terminal domains.  $\alpha$ BC assembles into highly dynamic and polydisperse oligomeric complexes containing between 12 to 48 monomers.<sup>32-36</sup> The variable stoichiometry of the  $\alpha$ BC complex is correlated with dynamic subunit exchange. Notably, the quaternary dynamics and polydispersity of  $\alpha$ BC are essential for its chaperone function,<sup>27,30,37</sup> yet this structural heterogeneity of  $\alpha$ BC has been a great challenge for its structural characterization in an atomic resolution. Despite this difficulty, however, many researchers have utilized various complimentary techniques, such as X-ray crystallography, solution-state nuclear magnetic resonance (NMR) spectroscopy, magic-angle spinning (MAS) solid-state NMR, neutron scattering, small angle X-ray scattering (SAXS), and cryogenic electron microscopy (cryo-EM), to unveil structural heterogeneity and quaternary structural organization of  $\alpha$ BC.<sup>27-30,32,34-36,38-40</sup> These efforts indeed contributed much to come up with several structural models for  $\alpha$ BC, yet there are still significant inconsistencies between them. Therefore, we discuss here recent advances in structural characterization of  $\alpha$ BCs and its physiological implications, particularly regarding connectivity with various aging-related human diseases.

### Subunit exchange and quaternary dynamics of full-length human $\alpha$ BC

As discussed previously, it has been a great challenge to obtain atomic-resolution information of human  $\alpha$ BCs due to its polydisperse supramolecular nature. Therefore, researchers employed diverse and complimentary tools to study high-resolution structural features of human  $\alpha$ BC over a decade. More recently, Jehle *et al.* resolved the full-length human  $\alpha$ BC structure by using solid-state NMR and SAXS.<sup>40</sup> They have identified that the curved ACD domain, with an angle of  $\sim 121^\circ$  between the planes of the  $\beta$  sandwich, acts as a basic building block for oligomeric assembly of human  $\alpha$ BC (Figure 1). It has been noted that the residues Ser59-Phe61 of the N-terminal domain are involved in intermolecular interaction with  $\beta$ 3 of the ACD domain and plays a crucial role in formation of higher-order assemblies.<sup>40</sup> Notably, whereas the C-terminal domain alone is not directly responsible for the formation of higher-order oligomers, this study showed that the residues Arg157-Arg163 within this domain may bind into the presumed substrate binding groove of  $\alpha$ BC.<sup>40</sup> In contrast, Jovcevski *et al.* employed native mass



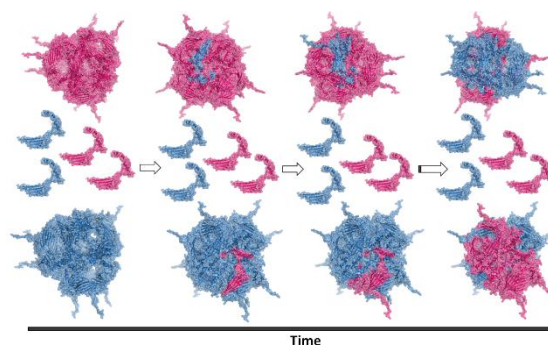
**Figure 1.** Conformational model of  $\alpha$ BC dimer in its 24-mer state (PDB 3J07).<sup>40</sup> Each monomer is coloured blue or red, and the N- and C-terminal regions are removed for clear view (the residues 57-157 are only shown here). Note that two monomers adopt different conformations due to quaternary heterogeneity of  $\alpha$ BC.

spectrometry to analyze the impact of mutations in the N-terminal domain, and found that this domain is not a major contributor to overall  $\alpha$ BC oligomer stability.<sup>41</sup> Moreover, truncated human  $\alpha$ BC (57–157) forms a stable dimer and the chaperone activity of this construct was fully preserved as observed in full-length human  $\alpha$ BC.<sup>41</sup> The detailed structural features and oligomeric dynamics of  $\alpha$ BC need to be further investigated in order to resolve this inconsistency and to reveal the precise role of oligomeric polydispersity in performing its chaperone functions.

On the other hand, Inoue *et al.* characterized the subunit exchange of human  $\alpha$ BC oligomers by using deuteration-assisted small-angle neutron scattering (DA-SANS) and electrospray ionization (ESI) native mass spectrometry (nMS).<sup>37</sup> They have observed increases in both the subunit exchange rate and monomer population over time and with temperature increase (Figure 2). They have proposed the model that the ‘transiently-liberated’ subunits of a large oligomeric complex mediate the subunit exchange, and this plays an important role in maintaining the chaperone activity of  $\alpha$ BC. Consistently, it was shown that the chaperone activity of  $\alpha$ BC is regulated by monomeric or dimeric subunits.<sup>37</sup>

### Model for capturing amorphous and amyloid clients

Previous studies have shown that human  $\alpha$ BC is able to interact with a wide range of client proteins including amorphous and amyloid aggregates. It was suggested that  $\alpha$ BC employs distinctive mechanisms to capture different types of aggregates. Mainz *et al.* monitored the interaction between the human  $\alpha$ BC oligomers and aggregation-prone amorphous and amyloid clients with NMR spectroscopy.<sup>30</sup> They have investigated the binding mode of amorphously-aggregating lysozyme with the  $\alpha$ BC oligomers, and observed the perturbation of NMR signals originating from the loop region preceding to  $\beta$ 8 (T124, T132 and I133) as well as the C-terminal IPI motif (T158, I159 and I161). The highly-conserved IPI motif at the C-terminal domain plays an important role for



**Figure 2.** Schematic representation of subunit exchange and quaternary dynamics of full-length human  $\alpha$ BC (PDB 3J07), which is based on DA-SANS and ESI-nMS studies.<sup>37</sup> The 24-mer complexes of  $\alpha$ BC are shown in red and blue (left; before subunit exchange), while subunit exchange facilitates subunits to be mixed as shown with both colors present for each complex (right).

maintaining the quaternary structure of  $\alpha$ BC.<sup>34</sup> They have postulated either that the observed signal changes arose from the direct binding of lysozyme to the hydrophobic  $\beta$ 4- $\beta$ 8 binding groove of human  $\alpha$ BC oligomer, or that these changes might arise indirectly from the global structural changes upon binding of lysozyme to the flexible N-terminal domain of human  $\alpha$ BC oligomers. However, N-terminal truncated human  $\alpha$ BC oligomers was ineffective in inhibiting aggregation of reduced lysozyme, which suggests that human  $\alpha$ BC oligomers interacts with amorphously aggregates of lysozyme through its N-terminal domain. Also, they investigated the binding mode of amyloid peptide  $A\beta_{1-40}$  with  $\alpha$ BC oligomers, and observed the signal changes at the residues located around the  $\beta$ 4- $\beta$ 8 binding groove of human  $\alpha$ BC oligomer (V91, V93, I124, S135, S136 and L137). This region corresponds to the hydrophobic edge of the ACD, indicating that it provides a possible binding mode for the amyloid client  $A\beta_{1-40}$ . Besides, this was further confirmed by introducing a paramagnetic label [S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate; MTSL] on the  $A\beta_{1-40}$  S26C variant. The significant paramagnetic relaxation effects were observed at the  $\beta$ 4- $\beta$ 8 binding region of  $\alpha$ BC by the MTSL-tagged  $A\beta_{1-40}$ . In addition, N-terminal truncated  $\alpha$ BC suppresses the amyloid formation as much as wild-type  $\alpha$ BC does, which suggests that the  $\alpha$ BC oligomer interacts with amyloid

aggregates through its  $\beta 4$ - $\beta 8$  binding groove. Together, Mainz *et al.* proposed the model that the N-terminal domain of  $\alpha$ BC oligomers plays a critical role in chaperoning aggregation-prone amorphous lysosome while its role to inhibit aggregation of  $A\beta_{1-40}$  is minimal; in contrast, the  $\beta 4$ - $\beta 8$  binding groove of  $\alpha$ BC oligomer is enough to block aggregation of  $A\beta_{1-40}$ . The concerted model for  $\alpha$ BC-mediated inhibition mechanisms for amorphous and amyloid aggregation is depicted in Figure 3.<sup>30</sup>

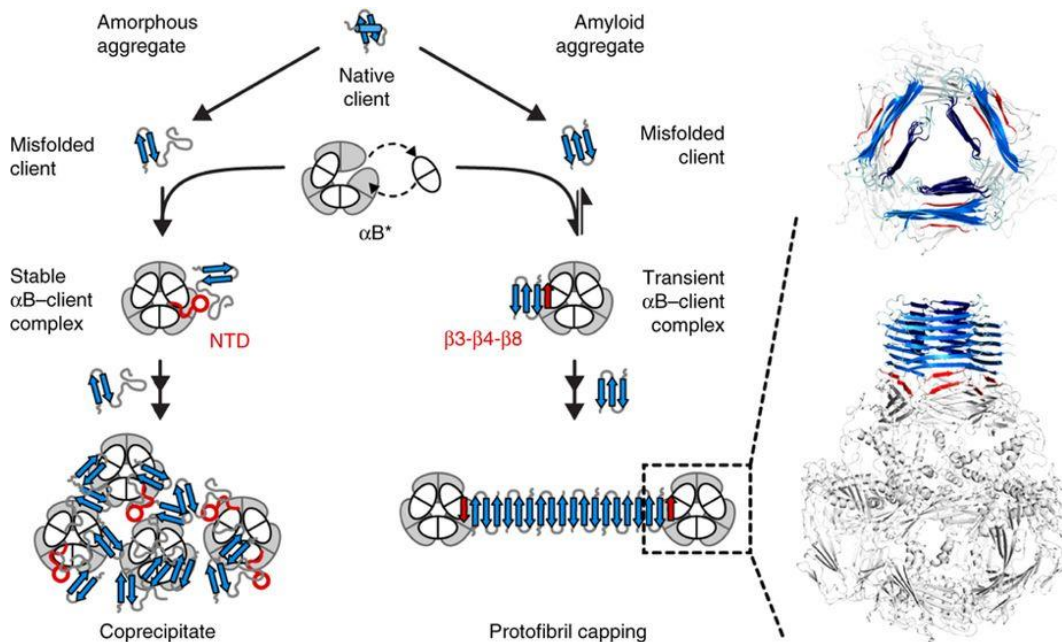
### Role of $\alpha$ BCs in Alzheimer's disease and other disease models

Previous studies revealed that  $\alpha$ BCs play an important role in clearing pathological protein aggregates such as cataract formation in eyes and clearance of fibrils in neurological diseases including AD and PD.  $\alpha$ BCs are expressed in various tissues such as eyes, skeletal muscle, kidney, and oligodendrocytes in the central nervous system.<sup>24,25,42,43</sup> Also, expression of  $\alpha$ BCs is enhanced in Rosenthal fibers in the astrocytes of

patients suffering from Alexander's disease, in ballooned neurons in several neurodegenerative diseases, and in the cerebral cortex of patients with Alzheimer's disease.<sup>25,38,44,45</sup> The recent findings suggest that  $\alpha$ BCs affect the elongation phase of  $A\beta$  fibril growth and prevent the further shedding and secondary nucleation.<sup>26,30,46-51</sup> Researchers are therefore trying to explore the concept of therapeutic potential of  $\alpha$ BCs in AD treatment. Recently,  $\alpha$ BC-based therapeutic peptides were developed and tested with various human disease models including neuropathies.<sup>52-56</sup> Notably, it was shown that  $\alpha$ BCs exert protective effects for cancerous cells and enhance their survival.<sup>55,57,58</sup> The role of  $\alpha$ BCs in cancerous cells needs to be further examined, particularly in order to evaluate their potential applications as therapeutics.<sup>55</sup>

### Conclusions and future directions

Despite evident physiological importance, structural characterization of  $\alpha$ BC and its relationship with



**Figure 3.** Model for capturing amorphous and amyloid clients by full-length human  $\alpha$ BC. For interaction with amorphous aggregates, the N-terminal domain (NTD) of  $\alpha$ BC plays an essential role, while the  $\beta 4$ - $\beta 8$  binding groove of  $\alpha$ BC constitutes the major interface to interact with amyloid aggregates. Reprinted by permission from Nature.<sup>30</sup>

functional aspects is still elusive. It appears critical to elucidate which structural elements of  $\alpha$ BC are responsible for its chaperone activities; particular focus needs to be made to appreciate heterogeneous quaternary structure. Notably, the quaternary polydispersity is also an important structural feature of other sHSP systems (e.g. Hsp27).<sup>59</sup> It is therefore tempting to speculate that the capability of sHSPs to form a range of dynamic oligomers is directly correlated with its wide substrate specificity, yet more experimental supports need to be added to validate this statement. For example, it is probable that quaternary dynamics of  $\alpha$ BCs is closely correlated with its

specificity for certain aggregation intermediates of the aggregation pathways. Although it is indeed consistent with the finding that  $\alpha$ BCs exert its activities for multiple intermediate states of aggregation-prone clients, more studies to reveal the mechanistic details regarding this observation are necessary.<sup>60</sup> Once these mysteries are resolved, we believe not only that our understanding to the quaternary assembly and architecture of proteins will be greatly advanced, but also that clinical application of  $\alpha$ BC as novel therapeutics for various aging-related diseases will be significantly facilitated.

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