Journal of the Korean Magnetic Resonance Society **2019**, *23*, 26-32
DOI 10.6564/JKMRS.2019.23.1.026

Recent advances in NMR-based structural characterization of αB-crystallin and its potential role in human diseases

Srinivasan Muniyappan and Jin Hae Kim*

Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology, Daegu 42988, Republic of Korea

Received Mar 18, 2019; Revised Mar 19, 2019; Accepted Mar 20, 2019

Abstract αB-crystallin (αBC) 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. αBC assembles into dynamic and polydisperse high molecular weight complexes containing 12 to 48 monomers; this variable stereochemistry of αBC has been linked to quaternary subunit exchange and its chaperone activity. The chaperone activity of αBC poses great potential as therapeutic agents for various neurodegenerative diseases. In this mini-review, we briefly outline the recent advancement in structural characterization of αBCs 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 αBCs, among which notable advancements are discussed in detail. We highlight the importance of resolving the structural details of various αBC oligomers, their quaternary dynamics, and structural heterogeneity.

Keywords αB-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.[1] However, this is accompanied with the increase of various aging-associated diseases including neurological diseases, cardiovascular diseases, cancers, and musculoskeletal diseases.[2] 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.[3] 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.[4] 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.[5-7] For clearance of plaques and fibrils, cells have developed various protein homeostasis mechanisms,[8-10] 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.[11-13] Especially, αB-crystallin, one of the essential small heat shock proteins (sHSPs), serves as the first line of defense against the intracellular misfolded protein aggregation.[14-18] The name 'crystallin' comes from its dominant presence in the

*Address correspondence to: **Jin Hae Kim,** Department of New Biology, Daegu Gyeongbuk Institute of Science &T echnology, Daegu 42988, Republic of Korea, Tel: 82-53-785-1770, E-mail: jinhaekim@dgist.ac.kr

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, α-crystallins, which includes αA- and αB-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.[19-23] Notably, this functionality of α-crystallins is not limited to eye lens; α-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.[24-27] Particularly, αB-crystallin (αBC) plays a pivotal role in clearing pathological aggregation of amyloids which are closely associated with human diseases such as AD, PD and multiple sclerosis.[26,28-31] αBC is a prominent member of sHSP family, consisting of the variable N- and C-terminal domains and the conserved α-crystallin domain (ACD) between the two terminal domains. αBC assembles into highly dynamic and polydisperse oligomeric complexes containing between 12 to 48 monomers.[32-36] The variable stoichiometry of the αBC complex is correlated with dynamic subunit exchange. Notably, the quaternary dynamics and polydispersity of αBC are essential for its chaperone function,[27,30,37] yet this structural heterogeneity of αBC 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 αBC.[27-30,32,34-36,38-40] These efforts indeed contributed much to come up with several structural models for αBC, yet there are still significant inconsistencies between them. Therefore, we discuss here recent advances in structural characterization of αBCs and its physiological implications, particularly regarding connectivity with various aging-related human diseases.

Subunit exchange and quaternary dynamics of full-length human αBC

As discussed previously, it has been a great challenge to obtain atomic-resolution information of human αBCs due to its polydisperse supramolecular nature. Therefore, researchers employed diverse and complimentary tools to study high-resolution structural features of human αBC over a decade. More recently, Jehle *et al.* resolved the full-length human αBC structure by using solid-state NMR and SAXS.[40] They have identified that the curved ACD domain, with an angle of ~121° between the planes of the β sandwich, acts as a basic building block for oligomeric assembly of human αBC (Figure 1). It has been noted that the residues Ser59-Phe61 of the N-terminal domain are involved in intermolecular interaction with β3 of the ACD domain and plays a crucial role in formation of higher-order assemblies.[40] 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 αBC.[40]

In contrast, Jovcevski *et al.* employed native mass

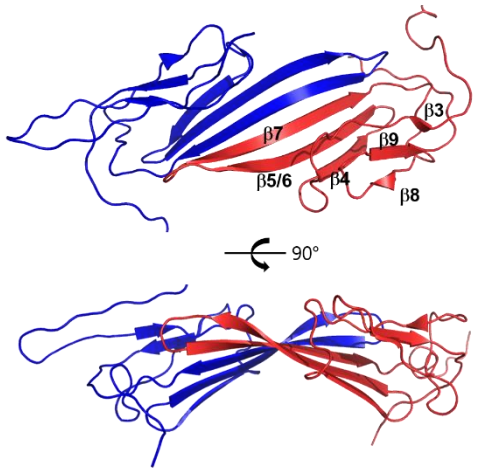

Figure 1. Conformational model of αBC dimer in its 24-mer state (PDB 3J07).[40] Each monomer is coloured blue or red, and the N- and C-terminal regions are removed for clear view (the residues 57-157 are only shown here). Note that two monomers adopt different conformations due to quaternary heterogeneity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

spectrometry to analyze the impact of mutations in the N-terminal domain, and found that this domain is not a major contributor to overall αBC oligomer stability.[41] Moreover, truncated human αBC (57–157) forms a stable dimer and the chaperone activity of this construct was fully preserved as observed in full-length human αBC.[41] The detailed structural features and oligomeric dynamics of αBC 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 αBC oligomers by using deuteration-assisted small-angle neutron scattering (DA-SANS) and electrospray ionization (ESI) native mass spectrometry (nMS).[37] 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 αBC. Consistently, it was shown that the chaperone activity of αBC is regulated by monomeric or dimeric subunits.[37]

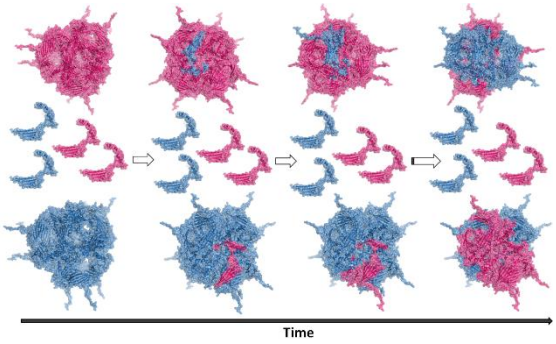

Figure 2. Schematic representation of subunit exchange and quaternary dynamics of full-length human αBC (PDB 3J07), which is based on DA-SANS and ESI-nMS studies.[37] The 24-mer complexes of αBC are shown in red and blue (left; before subunit exchange), while subunit exchange facilitates subunits to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

Model for capturing amorphous and amyloid clients

Previous studies have shown that human αBC is able to interact with a wide range of client proteins including amorphous and amyloid aggregates. It was suggested that αBC employs distinctive mechanisms to capture different types of aggregates. Mainz *et al.* monitored the interaction between the human αBC oligomers and aggregation-prone amorphous and amyloid clients with NMR spectroscopy.[30] They have investigated the binding mode of amorphously-aggregating lysozyme with the αBC oligomers, and observed the perturbation of NMR signals originating from the loop region preceding to β8 (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 maintaining the quaternary structure of αBC.[34] They have postulated either that the observed signal changes arose from the direct binding of lysozyme to the hydrophobic β4-β8 binding groove of human αBC 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 αBC oligomers. However, N-terminal truncated human αBC oligomers was ineffective in inhibiting aggregation of reduced lysozyme, which suggests that human αBC oligomers interacts with amorphously aggregates of lysozyme through its N-terminal domain. Also, they investigated the binding mode of amyloid peptide $A\beta_{1\text{-}40}$ with αBC oligomers, and observed the signal changes at the residues located around the β4-β8 binding groove of human αBC 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 $A\beta_{1\text{–}40}$. 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 $A\beta_{1\text{–}40}$ S26C variant. The significant paramagnetic relaxation effects were observed at the β4-β8 binding region of αBC by the MTSL-tagged $A\beta_{1\text{–}40}$. In addition, N-terminal truncated αBC suppresses the amyloid formation as much as wild-type αBC does, which suggests that the αBC oligomer interacts with amyloid

aggregates through its β4-β8 binding groove. Together, Mainz *et al.* proposed the model that the N-terminal domain of αBC 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 β4-β8 binding groove of αBC oligomer is enough to block aggregation of $A\beta_{1-40}$. The concerted model for αBC-mediated inhibition mechanisms for amorphous and amyloid aggregation is depicted in Figure 3.[30]

Role of αBCs in Alzheimer's disease and other disease models

Previous studies revealed that αBCs 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. αBCs are expressed in various tissues such as eyes, skeletal muscle, kidney, and oligodendrocytes in the central nervous system.[24,25,42,43] Also, expression of αBCs 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.[25,38,44,45] The recent findings suggest that αBCs affect the elongation phase of Aβ fibril growth and prevent the further shedding and secondary nucleation.[26,30,46-51] Researchers are therefore trying to explore the concept of therapeutic potential of αBCs in AD treatment. Recently, αBC-based therapeutic peptides were developed and tested with various human disease models including neuropathies.[52-56] Notably, it was shown that αBCs exert protective effects for cancerous cells and enhance their survival.[55,57,58] The role of αBCs in cancerous cells needs to be further examined, particularly in order to evaluate their potential applications as therapeutics.[55]

Conclusions and future directions

Despite evident physiological importance, structural characterization of αBC and its relationship with

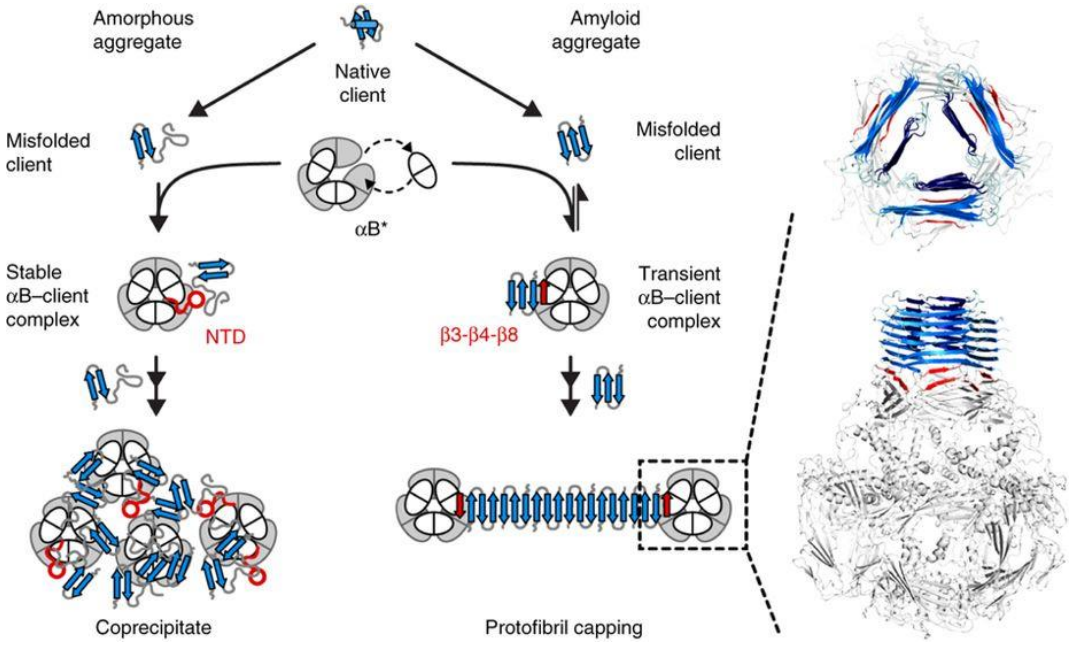

Figure 3. Model for capturing amorphous and amyloid clients by full-length human αBC. For interaction with amorphous aggregates, the N-terminal domain (NTD) of αBC plays an essential role, while the β4-β8 binding groove of αBC constitutes the major interface to interact with amyloid aggregates. Reprinted by permission from Nature.[30]

functional aspects is still elusive. It appears critical to elucidate which structural elements of αBC 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).[59] 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 αBCs is closely correlated with its specificity for certain aggregation intermediates of the aggregation pathways. Although it is indeed consistent with the finding that αBCs exert its activities for multiple intermediate states of aggregation-prone clients, more studies to reveal the mechanistic details regarding this observation are necessary.[60] Once theses 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 αBC as novel therapeutics for various aging-related diseases will be significantly facilitated.

Acknowledgements

This research was supported by the National Research Foundation (NRF) funded by the Ministry of Science & ICT (NRF-2018R1C1B6008282).

References

1. E. M. Crimmins, *Gerontologist* **55**, 901 (2015)
2. A. V. Belikov, *Ageing Res. Rev.* **49**, 11 (2019)
3. T. S. Dharmarajan and S. G. Gunturu, *Am. Health Drug Benefits* **2**, 39 (2009)
4. Alzheimer's Association, *Alzheimers Dement.* **12**, 459 (2016)
5. J. Hardy and D. J. Selkoe, *Science* **297**, 353 (2002)
6. Y. Huang and L. Mucke, *Cell* **148**, 1204 (2012)
7. S. M. Ward, D. S. Himmelstein, J. K. Lancia, and L. I. Binder, *Biochem. Soc. Trans.* **40**, 667 (2012)
8. R. J. Baranello, K. L. Bharani, V. Padmaraju, N. Chopra, D. K. Lahiri, N. H. Greig, M. A. Pappolla, and K. Sambamurti, *Curr. Alzheimer Res.* **12**, 32 (2015)
9. M. M. Wilhelmus, R. M. de Waal, and M. M. Verbeek, *Mol. Neurobiol.* **35**, 203 (2007)
10. P. Yan, X. Hu, H. Song, K. Yin, R. J. Bateman, J. R. Cirrito, Q. Xiao, F. F. Hsu, J. W. Turk, J. Xu, C. Y. Hsu, D. M. Holtzman, and J. M. Lee, *J. Biol. Chem.* **281**, 24566 (2006)
11. A. Ciechanover and Y. T. Kwon, *Front. Neurosci.* **11**, 185 (2017)
12. F. U. Hartl, A. Bracher, and M. Hayer-Hartl, *Nature* **475**, 324 (2011)
13. Y. E. Kim, M. S. Hipp, A. Bracher, M. Hayer-Hartl, and F. U. Hartl, *Annu. Rev. Biochem.* **82**, 323 (2013)
14. E. Basha, H. O'Neill, and E. Vierling, *Trends Biochem. Sci.* **37**, 106 (2012)
15. M. Haslbeck, T. Franzmann, D. Weinfurtner, and J. Buchner, *Nat. Struct. Mol. Biol.* **12**, 842 (2005)
16. H. S. McHaourab, J. A. Godar, and P. L. Stewart, *Biochemistry* **48**, 3828 (2009)
17. E. V. Mymrikov, A. S. Seit-Nebi, and N. B. Gusev, *Physiol. Rev.* **91**, 1123 (2011)
18. R. Van Montfort, C. Slingsby, and E. Vierling, *Adv. Protein Chem.* **59**, 105 (2001)
19. P. P. Fagerholm, B. T. Philipson, and B. Lindstrom, *Exp. Eye Res.* **33**, 615 (1981)
20. U. P. Andley, *Prog. Retin. Eye Res.* **26**, 78 (2007)
21. A. Tardieu, *Annu. Rev. Biophys. Biophys. Chem.* **17**, 47 (1988)

22. A. Tardieu, *Int. J. Biol. Macromol.* **22**, 211 (1998)
23. M. Delaye and A. Tardieu, *Nature* **302**, 415 (1983)
24. T. Iwaki, A. Kume-Iwaki, and J. E. Goldman, *J. Histochem. Cytochem.* **38**, 31 (1990)
25. T. Iwaki, A. Kume-Iwaki, R. K. Liem, and J. E. Goldman, *Cell* **57**, 71 (1989)
26. S. L. Shammas, C. A. Waudby, S. Wang, A. K. Buell, T. P. Knowles, H. Ecroyd, M. E. Welland, J. A. Carver, C. M. Dobson, and S. Meehan, *Biophys. J.* **101**, 1681 (2011)
27. Z. Liu, C. Wang, Y. Li, C. Zhao, T. Li, D. Li, S. Zhang, and C. Liu, *J. Biol. Chem.* **293**, 14880 (2018)
28. Z. Liu, S. Zhang, D. Li, and C. Liu, *Protein Pept. Lett.* **24**, 315 (2017)
29. I. Lopez-Gonzalez, M. Carmona, L. Arregui, G. G. Kovacs, and I. Ferrer, *Neuropathology* **34**, 517 (2014)
30. A. Mainz, J. Peschek, M. Stavropoulou, K. C. Back, B. Bardiaux, S. Asami, E. Prade, C. Peters, S. Weinkauf, J. Buchner, and B. Reif, *Nat. Struct. Mol. Biol.* **22**, 898 (2015)
31. Z. Ren, M. Yang, Z. Guan, and W. Yu, *Curr. Alzheimer Res.* **16**, 39 (2019)
32. S. Jehle, P. Rajagopal, B. Bardiaux, S. Markovic, R. Kuhne, J. R. Stout, V. A. Higman, R. E. Klevit, B. J. van Rossum, and H. Oschkinat, *Nat. Struct. Mol. Biol.* **17**, 1037 (2010)
33. A. Laganowsky, J. L. Benesch, M. Landau, L. Ding, M. R. Sawaya, D. Cascio, Q. Huang, C. V. Robinson, J. Horwitz, and D. Eisenberg, *Protein Sci.* **19**, 1031 (2010)
34. A. J. Baldwin, G. R. Hilton, H. Lioe, C. Bagneris, J. L. Benesch, and L. E. Kay, *J. Mol. Biol.* **413**, 310 (2011)
35. A. J. Baldwin, H. Lioe, C. V. Robinson, L. E. Kay, and J. L. Benesch, *J. Mol. Biol.* **413**, 297 (2011)
36. S. P. Delbecq, S. Jehle, and R. Klevit, *EMBO J.* **31**, 4587 (2012)
37. R. Inoue, T. Takata, N. Fujii, K. Ishii, S. Uchiyama, N. Sato, Y. Oba, K. Wood, K. Kato, N. Fujii, and M. Sugiyama, *Sci. Rep.* **6**, 29208 (2016)
38. J. Lowe, D. R. Errington, G. Lennox, I. Pike, I. Spendlove, M. Landon, and R. J. Mayer, *Neuropathol. Appl. Neurobiol.* **18**, 341 (1992)
39. S. Jehle, B. van Rossum, J. R. Stout, S. M. Noguchi, K. Falber, K. Rehbein, H. Oschkinat, R. E. Klevit, and P. Rajagopal, *J. Mol. Biol.* **385**, 1481 (2009)
40. S. Jehle, B. S. Vollmar, B. Bardiaux, K. K. Dove, P. Rajagopal, T. Gonen, H. Oschkinat, and R. E. Klevit, *Proc. Natl. Acad. Sci. U. S. A.* **108**, 6409 (2011)
41. B. Jovcevski, J. Andrew Aquilina, J. L. P. Benesch, and H. Ecroyd, *Cell Stress Chaperones* **23**, 827 (2018)
42. R. A. Dubin, E. F. Wawrousek, and J. Piatigorsky, *Mol. Cell. Biol.* **9**, 1083 (1989)
43. J. Piatigorsky and G. J. Wistow, *Cell* **57**, 197 (1989)
44. H. Shinohara, Y. Inaguma, S. Goto, T. Inagaki, and K. Kato, *J. Neurol. Sci.* **119**, 203 (1993)
45. N. Tomokane, T. Iwaki, J. Tateishi, A. Iwaki, and J. E. Goldman, *Am. J. Pathol.* **138**, 875 (1991)
46. G. Esposito, M. Garvey, V. Alverdi, F. Pettirossi, A. Corazza, F. Fogolari, M. Polano, P. P. Mangione, S. Giorgetti, M. Stoppini, A. Rekas, V. Bellotti, A. J. Heck, and J. A. Carver, *J. Biol. Chem.* **288**, 17844 (2013)
47. T. P. Knowles, C. A. Waudby, G. L. Devlin, S. I. Cohen, A. Aguzzi, M. Vendruscolo, E. M. Terentjev, M. E. Welland, and C. M. Dobson, *Science* **326**, 1533 (2009)
48. A. Rekas, L. Jankova, D. C. Thorn, R. Cappai, and J. A. Carver, *FEBS J.* **274**, 6290 (2007)
49. T. M. Treweek, S. Meehan, H. Ecroyd, and J. A. Carver, *Cell. Mol. Life Sci.* **72**, 429 (2015)
50. C. A. Waudby, T. P. Knowles, G. L. Devlin, J. N. Skepper, H. Ecroyd, J. A. Carver, M. E. Welland, J. Christodoulou, C. M. Dobson, and S. Meehan, *Biophys. J.* **98**, 843 (2010)
51. K. J. Binger, H. Ecroyd, S. Yang, J. A. Carver, G. J. Howlett, and M. D. Griffin, *FASEB J.* **27**, 1214 (2013)
52. J. Bhattacharyya, E. G. Padmanabha Udupa, J. Wang, and K. K. Sharma, *Biochemistry* **45**, 3069 (2006)
53. Y. Kanno and H. Matsuno, *Curr. Pharm. Des.* **12**, 887 (2006)
54. E. F. Lim, S. T. Nakanishi, V. Hoghooghi, S. E. Eaton, A. L. Palmer, A. Frederick, J. A. Stratton, M. G. Stykel, P. J. Whelan, D. W. Zochodne, J. Biernaskie, and S. S. Ousman, *Proc. Natl. Acad. Sci. U. S. A.* **114**,

E1707 (2017)
55. V. S. Reddy and G. B. Reddy, *Curr. Mol. Med.* **15**, 47 (2015)
56. M. Raju, P. Santhoshkumar, and K. K. Sharma, *Biochim. Biophys. Acta* **1860**, 246 (2016)
57. S. K. Gruvberger-Saal and R. Parsons, *J. Clin. Invest.* **116**, 30 (2006)
58. J. V. Moyano, J. R. Evans, F. Chen, M. Lu, M. E. Werner, F. Yehiely, L. K. Diaz, D. Turbin, G. Karaca, E. Wiley, T. O. Nielsen, C. M. Perou, and V. L. Cryns, *J. Clin. Invest.* **116**, 261 (2006)
59. R. Bakthisaran, R. Tangirala, and C. M. Rao, *Biochim. Biophys. Acta* **1854**, 291 (2015)
60. S. P. Delbecq and R. E. Klevit, *J. Biol. Chem.* **294**, 3261 (2019)