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Copper Binding and Subsequent Aggregation of α -Synuclein Are Modulated by N-Terminal Acetylation and Ablated by the H50Q Missense Mutation

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S Supporting Information

ABSTRACT: The Parkinson's disease-associated protein α -synuclein exhibits significant conformational heterogeneity. Bacterially expressed α -synuclein is known to bind to copper, resulting in the formation of aggregation-prone compact conformations. However, *in vivo*, α -synuclein undergoes acetylation at its N-terminus. Here the effect of this modification and the pathological H50Q mutation on copper binding and subsequent conformational transitions were investigated by electrospray ionization–ion mobility spectrometry–mass spectrometry. We demonstrate that acetylation perturbs the ability of α -synuclein to bind copper and that the H50Q missense mutation in the presence of N-terminal acetylation prevents copper binding. These modifications and mutations prevent the formation of the most compact conformations and inhibit copper-induced aggregation.

α -Synuclein (α -syn) is a highly conserved presynaptic protein associated with Parkinson's disease (PD) and other neurodegenerative disorders.¹ Despite α -syn being one of the most abundant proteins in the brain, its precise function is poorly understood, although it is known to play a role in maintaining synaptic integrity and function.² Fibrillar aggregates of α -syn are the primary constituents of Lewy bodies, intracytoplasmic inclusions that are a pathological hallmark of PD.³ This highly ordered, fibrillar form of α -syn is in contrast to the usual disordered monomeric form of the protein.⁴ It is known that α -syn can exhibit significant conformational heterogeneity,⁵ with collapsed conformers of α -syn linked to its aggregation.⁶ Certain environmental conditions are capable of inducing such conformations, including the presence of various divalent metal ions, including Cu^{2+} .⁷

Epidemiological studies imply that a high level of exposure to metals can act as a potential risk factor in certain nonfamilial forms of PD,¹² and alterations in metal homeostasis have been associated with many neurodegenerative diseases, including PD.¹³ Increased levels of iron in the substantia nigra of PD patients are well documented,¹⁴ with some studies finding an increase in the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratios of patients.^{15,16} Abnormal distributions of copper in PD patients have also been observed.¹⁷ The maintenance of metal homeostasis is vital for health as various metals play essential roles as cofactors in many biological processes. For example, the enzyme tyrosine hydroxylase, responsible for catalyzing the first step in the conversion of tyrosine to dopamine, requires ferrous iron to do so.⁸ A possible

role for α -syn in iron homeostasis has been suggested because of a putative iron response element identified in the 5'-untranslated region of α -syn mRNA.⁹ Iron response elements are a mechanism of post-transcriptional control of gene expression, commonly found in proteins involved in iron homeostasis, such as transferrin receptor protein 1 and ferritin.¹⁰ It has also been proposed that α -syn may have ferrireductase activity, reducing Fe^{3+} to Fe^{2+} , requiring copper bound to the protein to cycle between its oxidized and reduced forms, and hence binds these metals.¹¹

Polyvalent metals such as Cu^{2+} are known to enhance α -syn aggregation, leading to increased rates of fibril formation *in vitro*.⁶ Mapping studies employing circular dichroism, calorimetric titrations, nuclear magnetic resonance, and electron paramagnetic resonance spectroscopy have demonstrated the population of a collapsed species of α -syn in the presence of this metal.^{18–20,41} While most metal– α -syn interactions are nonspecific or low-affinity, this protein has a high binding affinity for Cu^{2+} . Evidence suggests α -syn is capable of binding Cu^{2+} at three specific sites:²¹ a high-affinity N-terminal site where Cu^{2+} is anchored by the freely available amino-terminal nitrogen, a lower-affinity site anchored by the imidazole ring of the histidine at position 50, and a low-affinity site at the C-terminus of the protein. Therefore, a paradoxical situation exists between the ability of α -syn to undergo metal-induced aggregation *in vitro* and its potential role as a metal binding protein *in vivo*.

In addition to α -syn being the main constituent of Lewy bodies, genetic evidence also points to the involvement of the protein in PD. Multiplications and mutations of SNCA, the gene encoding α -syn, have been shown to cause familial forms of PD. Most recently identified has been the H50Q missense mutation in SNCA exon 4, reported by two research groups in 2013.^{22,23} This mutation has been shown to accelerate fibril formation of the protein,²⁴ reduce its solubility,²⁵ and increase its level of secretion and toxicity in cell culture.²⁶ This mutant is of interest in relation to copper binding as the loss of the histidine imidazole ring would presumably affect α -syn's copper binding ability. However, studies investigating copper binding of α -syn *in vitro* where the H50 residue has been mutated have found negligible effects on copper binding; this is contrary to what would be predicted by the removal of a copper-anchoring site.²⁷ The effect of the H50Q familial mutation on copper binding was

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investigated by Proukakis et al., who found the mutant was able to bind to copper, but in a manner different from that of the wild-type (WT) protein, with binding only involving the high-affinity N-terminal site. These results about the role of H50 (refs 23–27) were gained using unmodified α -syn, which lacks N-terminal acetylation. This leaves the question of how this physiologically relevant N-terminal modification affects metal binding and aggregation of this mutant.

In vivo α -syn is known to be constitutively acetylated at its N-terminus, a common modification of cytosolic proteins, particularly those that retain their initiating methionine residue.²⁸ α -Syn was first identified as being acetylated when it was purified from Lewy bodies using liquid chromatography and tandem mass spectroscopy,²⁹ which has since been confirmed in α -syn isolated from brain tissue homogenate³⁰ and erythrocytes.³¹ In recent years, a number of *in vitro* studies have aimed to elucidate the effect of acetylation on α -syn. Such studies have shown that acetylation results in an increase in the lipid binding affinity of α -syn,³² alters its transient secondary structure,³³ and decreases aggregation rates.³⁴ N-Terminal acetylation of α -syn is an important modification in relation to its Cu^{2+} binding as the transfer of an acetyl group to the copper-anchoring amino-terminal nitrogen has the potential to alter the protein's Cu^{2+} binding ability. It has recently been demonstrated that N-terminally acetylated α -syn lacks its high-affinity N-terminal Cu^{2+} binding site and undergoes altered fibril formation in the presence of this metal.³⁵

To date, the effect of the familial H50Q mutation on Cu^{2+} binding of biologically relevant N-terminally acetylated α -syn has not been investigated. Prior studies of the WT N-terminally acetylated form have successfully used ensemble-based spectroscopy techniques to probe structural changes caused by metal binding.³⁵ However, the changes to the individual conformational states of α -syn have not been investigated. In this study, electrospray ionization–ion mobility spectrometry–mass spectrometry (ESI–IMS–MS) has been used to observe the copper binding and subsequent conformational transitions of individual conformational states of unmodified and acetylated wild-type and H50Q α -syn, using conditions suitable to maintain protein–metal complexes in the gas phase. ESI–IMS–MS has been utilized because of its ability to interrogate dynamic ensembles of the same mass by separating extended and collapsed conformations.³⁶ Changes in solution conformation are detected in the gas phase as changes in collisional cross-sectional area (CCS). This method also allows the binding of ligands to specific conformational states to be determined.³⁷

To address the Cu^{2+} binding properties of N-terminally acetylated α -syn, modified protein was produced through co-expression of the α -syn plasmid with the pNatB plasmid.³⁸ Although α -syn is described as a predominantly natively unstructured protein, it is comprised of a range of co-populated conformational families under equilibrium, which have been investigated using ESI–IMS–MS by various groups.^{39–42} Here, both extended and compact conformational states were observed for unmodified and acetylated WT and H50Q α -syn, equivalent to those previously reported.⁴³ Figure S1 shows the Driftscope plot and overlaid mass spectrum of each of the four proteins. Individual CCS values were determined for each charge state ion by fitting the arrival time distributions (ATDs) to the minimal number of Gaussian distributions, as shown in Figure S2a. The center CCS of each of these individual distributions was plotted against charge state, shown in Figure S2b. Conformational families, with multiple populations of both extended and

compact conformations, can be observed. A primarily extended population is seen in charge state ions +8 to +15, while a subpopulation of more compact conformations is seen predominantly at charge state ions +6 to +9, with multiple overlapping features in both series. An increase in CCS is seen through the extended series, presumably as a result of Coulombic repulsion between charges, resulting in more open structures. Compared to that of unmodified WT α -syn, neither N-acetylation nor H50Q mutation of the protein resulted in significant changes in observed CCSs or observable changes in the relative populations of the extended and compact conformations, demonstrating that the unbound metal free conformational states of these proteins are comparable.

To determine the degree of binding of Cu^{2+} to α -syn, mass spectra were recorded at a 1:1 protein:metal ratio immediately upon the addition of CuCl_2 . Figure 1 shows resulting spectra for

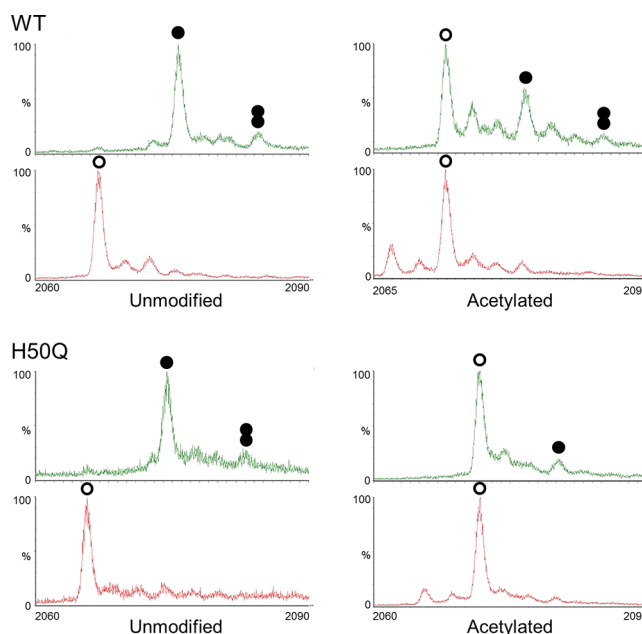


Figure 1. Binding of Cu^{2+} to unmodified and acetylated WT and H50Q α -syn at the +7 charge state ion. Spectra acquired at a protein concentration of 10 μM with CuCl_2 ratios of 1:0 (red) and 1:1 (green). Unbound forms of α -syn marked with an empty circles, the number of bound Cu^{2+} ions marked with filled circles. The x axis represents m/z , and the y axis represents relative abundance.

the +7 charge state ion of unmodified and acetylated WT and H50Q α -syn in apo and holo forms. While the +7 charge state ion has been highlighted here to illustrate Cu^{2+} binding, the same pattern of Cu^{2+} binding was seen across all charge states of the protein, with binding to each individual charge state ion shown in Figure S3.

All charge states of unmodified α -syn were able to bind one or two Cu^{2+} ions, indicating copper binding sites are available in all monomer conformations present under these conditions. This demonstrates, in agreement with α -syn–peptide binding studies,⁴⁴ that initial Cu^{2+} binding is determined at the primary structural level, as the extended, disordered state is capable of binding Cu^{2+} ions without a pronounced conformational change. At the concentration of copper tested here, the entire population of unmodified α -syn was found in its holo form, demonstrating a high affinity for this metal. In contrast to the case for the unmodified protein, in the presence of equimolar Cu^{2+} the

majority of acetylated α -syn remains in its apo form, with smaller amounts binding either one or two copper ions. These results, in agreement with previous studies,³⁵ indicate that acetylation weakens the ability of α -syn to bind Cu^{2+} , presumably as a result of the N-terminal binding site becoming unavailable upon acetylation. In agreement with others, we found that the unmodified H50Q mutation does not appear to hinder copper binding, indicating that the H50 residue does not play a prominent role in the binding of copper to the unmodified protein. However, the previously unstudied acetylated H50Q mutant shows greatly impaired copper binding, with only a small proportion binding to one Cu^{2+} ion, indicating that the histidine at position 50 plays a key role in the binding of copper to acetylated α -syn.

Compact conformations of α -syn are known to be aggregation-prone, and the equilibrium of conformations can be shifted toward the compact states under certain conditions. Polyvalent metal ions have the propensity to shift the conformational equilibrium and trigger structural rearrangements. Ion mobility spectra were acquired to investigate changes in α -syn conformations that occur upon Cu^{2+} binding. CCSs were calculated for each charge state of unmodified and acetylated WT and H50Q α -syn, in the presence and absence of Cu^{2+} , with the results for the +7 charge state ion shown in Figure 2 (taken as being representative of the compact

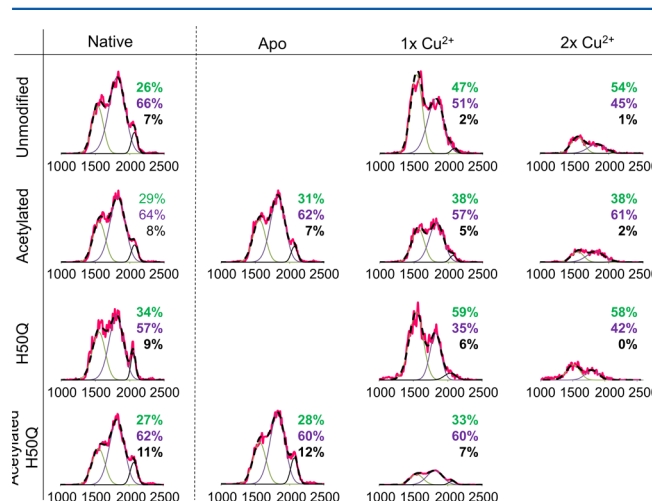


Figure 2. Arrival time distributions and experimental collisional cross sections of the +7 charge state ion of unmodified and acetylated WT and H50Q α -syn. Apo and Cu^{2+} -bound forms resulting from mixing 10 μM α -syn with equimolar CuCl_2 . The percentages of alternate populations of differing CCSs were calculated using Gaussian fitting, indicated by the green, purple, and black curves.

conformations). Figure S5 details the ATD of all charge states of the four proteins in the absence or presence of equimolar copper. Gaussian fitting was performed on each of the ATDs, and the areas under each curve were used to gain estimates of the population of each conformational state. The ATDs of the +7 charge state ion of all four unbound proteins have three distinct conformational distributions, populated to similar extents, with a most compact distribution centered at a CCS of 1608 \AA^2 , an intermediate conformation centered at a CCS of 1840 \AA^2 , and an extended state centered at a CCS of 2079 \AA^2 .

When unmodified α -syn is bound to one Cu^{2+} ion, an overall shift toward the more compact conformations can be observed (from 26 to 47% of the +7 charge state ion) with a concurrent

reduction in the proportions of the most extended and intermediate states. Binding of a second Cu^{2+} ion results in a further increase in the proportion of compact conformations to 54%. Similar shifts to the more compact states are observed across the +8 to +12 charge state ions as shown in Figure S5. When Cu^{2+} binds to the unmodified H50Q mutant, the same pattern of conformational change occurs, indicating that H50 is not directly involved in copper binding in the unmodified proteins. When Cu^{2+} binds to the acetylated proteins, the shift to the more compact conformations is not observed. In contrast to the unmodified proteins, a larger proportion of the +7 charge state ion remains in the more extended states, demonstrating that acetylated α -syn undergoes reduced conformational changes upon Cu^{2+} binding.

The aggregation propensity of each of the proteins in the presence and absence of Cu^{2+} was investigated using thioflavin T fluorescence. The lag time was determined by calculating the intercept between the maximal derivative and the pretransitional baseline. Figure 3 shows the mean and standard deviation

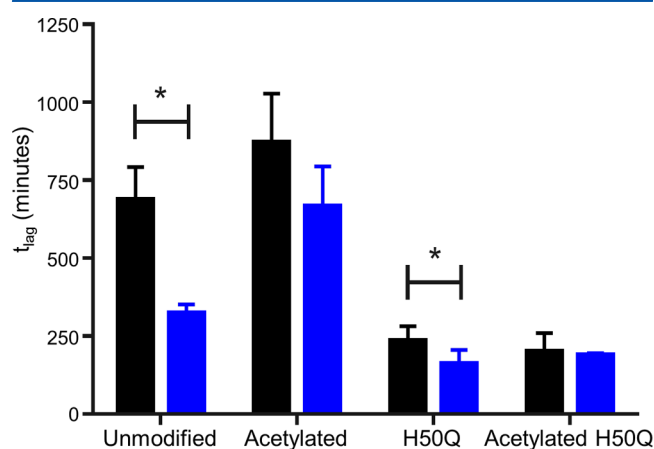


Figure 3. Copper-induced aggregation of α -syn as monitored by thioflavin T. α -Syn was incubated at 70 μM alone (black) or in the presence of equimolar CuCl_2 (blue). Statistical significance determined by the Kruskal–Wallis with Conover–Inman post hoc analysis test ($P \leq 0.05$).

aggregation lag time of five repeats; raw data are shown in Figure S6. In agreement with others, we have found that Cu^{2+} decreases the aggregation lag time of unmodified α -syn and that acetylation hinders this effect. The H50Q mutant displays an aggregation propensity higher than that of the WT protein, with a further reduction in lag time in the presence of Cu^{2+} . The previously unstudied acetylated H50Q mutant shows the same increased aggregation propensity as the unmodified mutant, but in this case, no further decrease in lag time is observed upon the addition of Cu^{2+} , consistent with the impaired Cu^{2+} binding and lack of conformational change seen with this protein by ESI–IMS–MS.

In summary, it has been demonstrated that binding of Cu^{2+} to unmodified α -syn can induce a conformational change to a more compact state⁴¹ which is prone to aggregation,⁶ potentially playing a role in the pathogenesis of PD. Results presented here demonstrate that the biologically relevant, acetylated α -syn undergoes altered conformational changes upon Cu^{2+} binding compared to the better studied unmodified protein, with an observable decrease in the binding affinity for Cu^{2+} . It is an important observation that α -syn is N-terminally acetylated²⁹ *in vivo*, and the effect of this modification needs to be considered

when investigating binding of metal to this protein. Acetylation *in vitro* perturbs the ability of α -syn to bind Cu^{2+} and attenuates the metal-induced formation of the most compact conformations. In agreement with others,³⁵ we have shown that Cu^{2+} has a weakened effect on the aggregation kinetics of acetylated α -syn, which can be explained by the reduced shift toward the aggregation-prone, compact state as observed by ESI-IMS-MS. As α -syn is acetylated *in vivo*, if Cu^{2+} is indeed involved in the pathogenesis of PD via interactions with α -syn, it may occur through mechanisms other than the formation of an aggregation-prone conformation.

Results presented here are the first, to the best of our knowledge, describing the effect of acetylated on H50Q α -syn copper binding. We have demonstrated that while the H50Q mutation does not appear to alter the Cu^{2+} binding of unmodified α -syn, the acetylated H50Q mutant has greatly impaired Cu^{2+} binding. This demonstrates the importance of the N-terminus and the role of H50 in the Cu^{2+} binding site of α -syn and suggests that the proposed C-terminal binding region has little influence. If Cu^{2+} binding is important for α -syn's physiological function, such as its suggested role as a ferrireductase, then the familial H50Q mutation may result in loss of function due to the protein not being able to bind Cu^{2+} in the usual fashion. These results highlight the need to take into account post-translational modifications when investigating the metal binding propensity of α -syn and its mutants.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.6b00708.

Relevant methodological details, Driftscope plot and overlaid mass spectrum of each of the four proteins (Figure S1), arrival time distribution for each charge state ion of unmodified α -syn (Figure S2), α -syn spectra in the absence and presence of equimolar Cu^{2+} (Figure S3), charge state distribution (Figure S4), arrival time distribution for each charge state ion of all α -syn species studied here (Figure S5), and copper-induced aggregation of α -syn as monitored by thioflavin T (Figure S6) (PDF)

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Author Contributions

R.J.M. performed all experimental procedures. R.J.M. and D.P.S. analyzed the data. D.P.S. conceived the experiments.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

- Uversky, V. N. (2008) *Curr. Protein Pept. Sci.* 9, 507–540.
- Burré, J. (2015) *J. Parkinson's Dis.* 5, 699–713.
- Shults, C. W. (2006) *Proc. Natl. Acad. Sci. U. S. A.* 103, 1661–1668.
- Theillet, F.-X., Binolfi, A., Bekei, B., Martorana, A., Rose, H. M., Stuver, M., Verzini, S., Lorenz, D., van Rossum, M., Goldfarb, D., and Selenko, P. (2016) *Nature* 530, 45–50.
- Uversky, V. N., and Eliezer, D. (2009) *Curr. Protein Pept. Sci.* 10, 483–499.
- Uversky, V. N., Li, J., and Fink, A. L. (2001) *J. Biol. Chem.* 276, 44284–44296.
- Santner, A., and Uversky, V. N. (2010) *Metalomics* 2, 378–392.
- Fitzpatrick, P. F. (1989) *Biochem. Biophys. Res. Commun.* 161, 211–215.
- Febbraro, F., Giorgi, M., Caldarola, S., Loreni, F., and Romero-Ramos, M. (2012) *NeuroReport* 23, 576–580.
- Wang, J., and Pantopoulos, K. (2011) *Biochem. J.* 434, 365–381.
- Davies, P., Moualla, D., and Brown, D. R. (2011) *PLoS One* 6, e15814.
- Gorell, J. M., Peterson, E. L., Rybicki, B. A., and Johnson, C. C. (2004) *J. Neurol. Sci.* 217, 169–174.
- Chen, P., Miah, M. R., and Aschner, M. (2016) *F1000Research* 5.
- Dusek, P., Roos, P. M., Litwin, T., Schneider, S. A., Flaten, T. P., and Aaseth, J. (2015) *J. Trace Elem. Med. Biol.* 31, 193–203.
- Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G. P., Hebenstreit, G., and Youdim, M. B. H. (1988) *J. Neural Transm.* 74, 199–205.
- Riederer, P., Sofic, E., Rausch, W.-D., Schmidt, B., Reynolds, G. P., Jellinger, K., and Youdim, M. B. H. (1989) *J. Neurochem.* 52, 515–520.
- Kozłowski, H., Luczkowski, M., Remelli, M., and Valensin, D. (2012) *Coord. Chem. Rev.* 256, 2129–2141.
- Binolfi, A., Lamberto, G. R., Duran, R., Quintanar, L., Bertoncini, C. W., Souza, J. M., Cerveñansky, C., Zweckstetter, M., Griesinger, C., and Fernández, C. O. (2008) *J. Am. Chem. Soc.* 130, 11801–11812.
- Binolfi, A., Rodriguez, E. E., Valensin, D., D'Amelio, N., Ippoliti, E., Obal, G., Duran, R., Magistrato, A., Pritsch, O., Zweckstetter, M., Valensin, G., Carloni, P., Quintanar, L., Griesinger, C., and Fernández, C. O. (2010) *Inorg. Chem.* 49, 10668–10679.
- Drew, S. C., Ling Leong, S., Pham, C. L. L., Tew, D. J., Masters, C. L., Miles, L. A., Cappai, R., and Barnham, K. J. (2008) *J. Am. Chem. Soc.* 130, 7766–7773.
- Binolfi, A., Quintanar, L., Bertoncini, C. W., Griesinger, C., and Fernández, C. O. (2012) *Coord. Chem. Rev.* 256, 2188–2201.
- Appel-Cresswell, S., Vilarino-Guell, C., Encarnacion, M., Sherman, H., Yu, I., Shah, B., Weir, D., Thompson, C., Szu-Tu, C., Trinh, J., Aasly, J. O., Rajput, A., Rajput, A. H., Jon Stoessl, A., and Farrer, M. J. (2013) *Mov. Disord.* 28, 811–813.
- Proukakis, C., Dudzik, C. G., Brier, T., MacKay, D. S., Cooper, J. M., Millhauser, G. L., Houlden, H., and Schapira, A. H. (2013) *Neurology* 80, 1062–1064.
- Ghosh, D., Mondal, M., Mohite, G. M., Singh, P. K., Ranjan, P., Anoop, A., Ghosh, S., Jha, N. N., Kumar, A., and Maji, S. K. (2013) *Biochemistry* 52, 6925–6927.
- Porcari, R., Proukakis, C., Waudby, C. A., Bolognesi, B., Mangione, P. P., Paton, J. F. S., Mullin, S., Cabrita, L. D., Penco, A., Relini, A., Verona, G., Vendruscolo, M., Stoppini, M., Tartaglia, G. G., Camilloni, C., Christodoulou, J., Schapira, A. H. V., and Bellotti, V. (2015) *J. Biol. Chem.* 290, 2395–2404.
- Khalaf, O., Fauvet, B., Oueslati, A., Dikiy, I., Mahul-Mellier, A.-L., Ruggeri, F. S., Mbefo, M. K., Vercruysse, F., Dietler, G., Lee, S.-J., Eliezer, D., and Lashuel, H. A. (2014) *J. Biol. Chem.* 289, 21856–21876.
- Davies, P., Wang, X., Sarell, C. J., Drewett, A., Marken, F., Viles, J. H., and Brown, D. R. (2011) *Biochemistry* 50, 37–47.
- Bradshaw, R. A. (1989) *Trends Biochem. Sci.* 14, 276–279.
- Anderson, J. P., Walker, D. E., Goldstein, J. M., de Laat, R., Banducci, K., Caccavello, R. J., Barbour, R., Huang, J., Kling, K., Lee, M., Diep, L., Keim, P. S., Shen, X., Chataway, T., Schlossmacher, M. G., Seubert, P., Schenk, D., Sinha, S., Gai, W. P., and Chilcote, T. J. (2006) *J. Biol. Chem.* 281, 29739–29752.
- Ohrfelt, A., Zetterberg, H., Andersson, K., Persson, R., Secic, D., Brinkmalm, G., Wallin, A., Mulugeta, E., Francis, P. T., Vanmechelen, E., Aarsland, D., Ballard, C., Blennow, K., and Westman-Brinkmalm, A. (2011) *Neurochem. Res.* 36, 2029–2042.

- (31) Bartels, T., Choi, J. G., and Selkoe, D. J. (2011) *Nature* 477, 107–10.
- (32) Dikiy, I., and Eliezer, D. (2014) *J. Biol. Chem.* 289, 3652–3665.
- (33) Kang, L., Janowska, M. K., Moriarty, G. M., and Baum, J. (2013) *PLoS One* 8, e75018.
- (34) Kang, L., Moriarty, G. M., Woods, L. A., Ashcroft, A. E., Radford, S. E., and Baum, J. (2012) *Protein Sci.* 21, 911–917.
- (35) Moriarty, G. M., Minetti, C. A. S. A., Remeta, D. P., and Baum, J. (2014) *Biochemistry* 53, 2815–2817.
- (36) Jenner, M., Ellis, J., Huang, W.-C., Lloyd Raven, E., Roberts, G. C. K., and Oldham, N. J. (2011) *Angew. Chem., Int. Ed.* 50, 8291–8294.
- (37) Hopper, J. T. S., and Oldham, N. J. (2009) *J. Am. Soc. Mass Spectrom.* 20, 1851–1858.
- (38) Johnson, M., Coulton, A. T., Geeves, M. A., and Mulvihill, D. P. (2010) *PLoS One* 5, e15801.
- (39) Bernstein, S. L., Liu, D., Wytenbach, T., Bowers, M. T., Lee, J. C., Gray, H. B., and Winkler, J. R. (2004) *J. Am. Soc. Mass Spectrom.* 15, 1435–1443.
- (40) Frimpong, A. K., Abzalimov, R. R., Uversky, V. N., and Kaltashov, I. A. (2010) *Proteins: Struct., Funct., Genet.* 78, 714–722.
- (41) Natalello, A., Benetti, F., Doglia, S. M., Legname, G., and Grandori, R. (2011) *Proteins: Struct., Funct., Genet.* 79, 611–621.
- (42) Phillips, A. S., Gomes, A. F., Kalapothakis, J. M. D., Gillam, J. E., Gasparavicius, J., Gozzo, F. C., Kunath, T., MacPhee, C., and Barran, P. E. (2015) Conformational dynamics of α -synuclein: insights from mass spectrometry. *Analyst* 140, 3070–3081.
- (43) Illes-Toth, E., Dalton, C. F., and Smith, D. P. (2013) *J. Am. Soc. Mass Spectrom.* 24, 1346–1354.
- (44) Brown, D. R. (2009) *Biochem. Biophys. Res. Commun.* 380, 377–381.