

Antiparallel Arrangement of the Helices of Vesicle-Bound α -Synuclein

Malte Drescher,^{†,§} Gertjan Veldhuis,[‡] Bart D. van Rooijen,[‡] Sergey Milikisyants,[†]
Vinod Subramaniam,^{*,‡} and Martina Huber^{*,†}

*Department of Molecular Physics, Leiden University, P.O. Box 9504, 2300 RA Leiden, The Netherlands, and
Biophysical Engineering, MESA+ Institute for Nanotechnology and Institute for Biomedical Technology,
University of Twente, Zuidhorst ZH163, P.O. Box 217, 7500 AE Enschede, The Netherlands*

E-mail: v.subramaniam@utwente.nl; mhuber@molphys.leidenuniv.nl

α -Synuclein (α S) is a 140 residue protein, constituting the major component of Lewy bodies found in Parkinson's disease.^{1,2} Although the exact function of α S remains obscure, interaction of α S with synaptic vesicles has been suggested to be important for its physiological role, fostering interest in the structural properties of α S upon binding to membranes. Although α S has a random-coil configuration in solution,³ upon binding to membranes it adopts an amphipathic, α -helical structure involving residues 1–100.^{4–11} It was proposed that α S binds either as an extended, continuous helix (residues 1–100)^{11–13} or in a horseshoe-like conformation, consisting of helix 1 (residues 3–37), a bend around residue 42, and helix 2 (residues 45–92),¹⁴ with the two helices in an antiparallel arrangement but not touching.^{15,16} Most of the structural information available concerns studies of α S on micelles.^{14,17} Micelles, however, differ in important aspects from biological membranes. Micelles have typical diameters¹⁸ of 5 nm and therefore may be too small to accommodate α S in the extended conformation (around 15 nm for an extended helix of 100 residues).¹⁹ Therefore, micelles could enforce an antiparallel configuration of the helices even if that is not the preferred state of α S on a larger surface such as that of a biological membrane. In contrast, even small unilamellar vesicles (SUVs) have diameters⁴ in the order of 20 to 30 nm, providing a surface area that is large compared to the dimension of α S¹⁹ and a larger radius of curvature than micelles. Therefore, SUVs are more suitable models for biological membranes than micelles.

We present an investigation on the structure of α S on SUVs using a two-frequency, pulsed EPR method (double electron–electron resonance DEER or electron–electron double resonance ELDOR).^{20–24} To obtain long-range information on the conformation of α S bound to the vesicle surface, the distances between pairs of spin-labels introduced by site-selective cysteine mutagenesis were measured. The DEER method was chosen, because it allows the measurement of distances up to 8 nm in disordered materials²² and is not subject to limitations in the tumbling time and thereby the size of the complex. Four double-cysteine mutants, alkylated with MTSL (1-oxy-2,2,5,5-tetramethylpyrroline-3-methyl-methanethiosulfonate), were investigated: α S9/90, α S18/90, α S18/69, and α S9/69, each containing one label in the proposed helix 1, and a second label in helix 2 (see Figure 1). Distances were measured for α S mutants bound to vesicles and compared to those in the absence of vesicles, that is, for α S free in solution.

We show that α S adopts a two-helix, antiparallel arrangement on vesicles that are large enough to accommodate an extended helix of α S. This suggests that the bent structure is the preferred

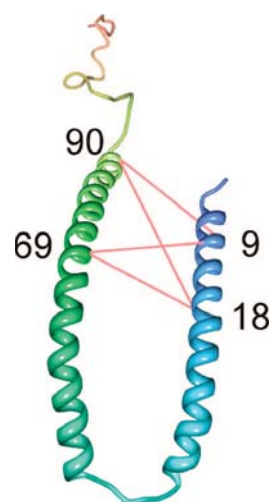


Figure 1. NMR-structure (1XQ8 of RCSB protein database)¹⁴ of α S bound to SDS micelles. Red lines indicate distances measured by DEER for α S bound to SUVs. Visualized by PDB Protein Workshop.²⁵

conformation of α S also on membranes, an indication that the horseshoe conformation is the physiologically relevant one.

Double-cysteine mutants were incubated with a 6-fold excess per cysteine of MTSL. Free label was subsequently removed using size-exclusion chromatography. The SUVs of POPG lipids [1-palmitoyl-2-oleoyl-*sn*-glycero-3-(phosphorac-(1-glycerol))] were produced by sonication in 10 mM Tris-HCl, pH 7.4, and mixed to obtain an α S/lipid ratio of 1:250.

DEER experiments were performed at $T = 40$ K using a Bruker Elexsys E680 X-band spectrometer modified as described previously.²⁶ Data were analyzed using DEERAnalysis 2006.1.^{27–29} Model free analysis revealed that the distance distributions could be well fitted by Gaussians. For details of sample preparation, measurements, and analysis, see Supporting Information.

In the presence of vesicles, the DEER responses of all four mutants differed significantly from those in the absence of vesicles. This is illustrated in Figure 2, where DEER results are shown for α S18/69 (remaining mutants, see Supporting Information). In Figure 2a, the time traces are shown. The shape of the decays could be well simulated by the Gaussian distance distributions shown in Figure 2b. In the absence of vesicles the distribution was broad. Upon addition of vesicles, the distance distribution became much narrower and shifted by 0.4 nm to shorter distances. Table 1 lists the parameters of the distance distributions for all mutants.

In the absence of vesicles, the widths of the distributions were large (≥ 1.8 nm), as expected from the unstructured nature of α S in solution.³ Narrower distributions and a more differentiated set of distances in the presence of vesicles reveal that α S undergoes a

[†] Leiden University.

[‡] University of Twente.

[§] Present address: Department of Chemistry, University of Konstanz, 78457 Konstanz, Germany. E-mail: malte.drescher@uni-konstanz.de.

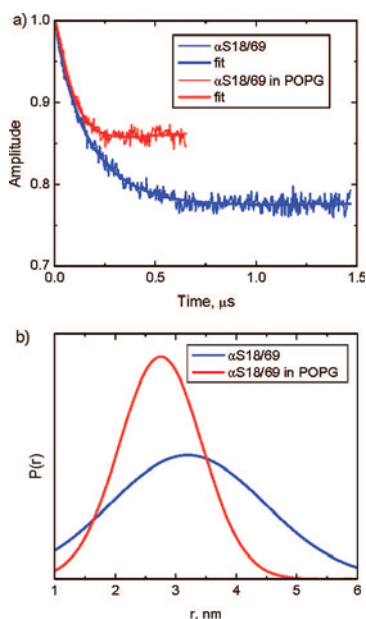


Figure 2. (a) DEER traces of α S18/69 after background correction in the presence (red, top trace) and absence (blue) of POPG vesicles and corresponding fits (solid lines) assuming a Gaussian distance distribution. (b) Distance distributions corresponding to the fits in panel a.

Table 1. Parameters of Gaussian Distance Distributions Obtained by DEER^a

α S double Cys mutant	in solution		with vesicles	
	distance (nm)	width of distribution (nm)	distance (nm)	width of distribution (nm)
9/90	3.1 ± 0.1	2.1 ± 0.2	3.2 ± 0.2	0.9 ± 0.3
18/90	3.1 ± 0.05	1.8 ± 0.15	3.4 ± 0.1	1.5 ± 0.3
18/69	3.2 ± 0.1	1.8 ± 0.2	2.8 ± 0.05	1.0 ± 0.3
9/69	2.8 ± 0.1	2.0 ± 0.2	3.2 ± 0.2	1.3 ± 0.3

^a Determination of errors: see Supporting Information.

structural transition to a more ordered state upon binding to the membrane, in agreement with the known transition of α S from randomly disordered to α -helical upon vesicle binding.^{4,6} The width of these distributions, although narrower than in the absence of vesicles, is still relatively large, indicating that there is a large range of conformations for the individual molecules. For vesicle-bound α S, the distances measured between strategically chosen points on the two proposed helices span 2.8–3.4 nm, which is consistent with the distances derived from the NMR structure on micelles.^{8,14} The four distances determined provide a redundant set that proves that the arrangement of the two helices must be antiparallel, as shown in Figure 1. A more refined model, along the lines of the study of Borbat et al. for α S on micelles,¹⁷ where triangulation in conjunction with rigid body refinement was possible using an extensive set of mutants,³⁰ is work in progress. The distance constraints obtained in the present study enable us to unambiguously determine that the vesicle-bound α S fits the “broken helix” model, where the first two-thirds of α S loop back upon themselves. We conclude that the horseshoe type conformation is the most likely conformation

of α S in the membrane-bound state. This result has implications for the conformation of α S in vivo, where α S interacts with synaptic vesicles with diameters around 40 nm.

Acknowledgment. We thank Frans Godschalk for stimulating discussions and experimental contributions. M.D. gratefully acknowledges financial support by Deutsche Forschungsgemeinschaft (DR 743/1-1). G.V. is supported by the Stichting Internationaal Parkinson Fonds. This work is part of the research program of the “Stichting voor fundamenteel onderzoek der materie (FOM)”, which is financially supported by the “Nederlandse Organisatie voor Wetenschappelijk onderzoek (NWO)”.

Supporting Information Available: Details of protein expression, sample preparation, measurements, and data analysis. DEER traces before background correction as well as corrected DEER traces for all double mutants.

References

- (1) Spillantini, M. G.; Schmidt, M. L.; Lee, V. M. Y.; Trojanowski, J. Q.; Jakes, R.; Goedert, M. *Nature* **1997**, *388*, 839–840.
- (2) Goedert, M. *Nat. Rev. Neurosci.* **2001**, *2*, 492–501.
- (3) Weinreb, P. H.; Zhen, W. G.; Poon, A. W.; Conway, K. A.; Lansbury, P. T. *Biochemistry* **1996**, *35*, 13709–13715.
- (4) Davidson, W. S.; Jonas, A.; Clayton, D. F.; George, J. M. *J. Biol. Chem.* **1998**, *273*, 9443–9449.
- (5) Perrin, R. J.; Woods, W. S.; Clayton, D. F.; George, J. M. *J. Biol. Chem.* **2000**, *275*, 34393–34398.
- (6) Lee, J. C.; Langen, R.; Hummel, P. A.; Gray, H. B.; Winkler, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16466–16471.
- (7) Ferreon, A. C. M.; Deniz, A. A. *Biochemistry* **2007**, *46*, 4499–4509.
- (8) Ulmer, T. S.; Bax, A. *J. Biol. Chem.* **2005**, *280*, 43179–43187.
- (9) Ramakrishnan, M.; Jensen, P. H.; Marsh, D. *Biochemistry* **2006**, *45*, 3386–3395.
- (10) Ramakrishnan, M.; Jensen, P. H.; Marsh, D. *Biochemistry* **2003**, *42*, 12919–12926.
- (11) Jao, C. C.; Der-Sarkissian, A.; Chen, J.; Langen, R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8331–8336.
- (12) Bussell, R.; Eliezer, D. *J. Mol. Biol.* **2003**, *329*, 763–778.
- (13) Bisaglia, M.; Tessari, I.; Pinato, L.; Bellanda, M.; Giraud, S.; Fasano, M.; Bergantino, E.; Bubacco, L.; Mammì, S. *Biochemistry* **2005**, *44*, 329–339.
- (14) Ulmer, T. S.; Bax, A.; Cole, N. B.; Nussbaum, R. L. *J. Biol. Chem.* **2005**, *280*, 9595–9603.
- (15) Bussell, R.; Ramlall, T. F.; Eliezer, D. *Protein Sci.* **2005**, *14*, 862–872.
- (16) Chandra, S.; Chen, X. C.; Rizo, J.; Jahn, R.; Sudhof, T. C. *J. Biol. Chem.* **2003**, *278*, 15313–15318.
- (17) Borbat, P.; Ramlall, T. F.; Freed, J. H.; Eliezer, D. *J. Am. Chem. Soc.* **2006**, *128*, 10004–10005.
- (18) Itri, R.; Amaral, L. Q. *J. Phys. Chem.* **1991**, *95*, 423–427.
- (19) Rhoades, E.; Ramlall, T. F.; Webb, W. W.; Eliezer, D. *Biophys. J.* **2006**, *90*, 4692–4700.
- (20) Milov, A. D.; Maryasov, A. G.; Tsvetkov, Y. D. *Appl. Magn. Reson.* **1998**, *15*, 107–143.
- (21) Larsen, R. G.; Singel, D. J. *J. Chem. Phys.* **1993**, *98*, 5134–5146.
- (22) Jeschke, G. *Chemphyschem* **2002**, *3*, 927–932.
- (23) Jeschke, G.; Polyhach, Y. *Phys. Chem. Chem. Phys.* **2007**, *9*, 1895–1910.
- (24) Schiemann, O.; Prisner, T. F. *Q. Rev. Biophys.* **2007**, *40*, 1–53.
- (25) Moreland, J. L.; Gramada, A.; Buzko, O. V.; Zhang, Q.; Bourne, P. E. *Bmc Bioinformatics* **2005**, *6*, n/a.
- (26) Steigmiller, S.; Börsch, M.; Gräber, P.; Huber, M. *Biochim. Biophys. Acta* **2005**, *1708*, 143–153.
- (27) Jeschke, G.; Koch, A.; Jonas, U.; Godt, A. *J. Magnet. Reson.* **2002**, *155*, 72–82.
- (28) Jeschke, G.; Chechik, V.; Ionita, P.; Godt, A.; Zimmermann, H.; Banham, J.; Timmel, C. R.; Hilger, D.; Jung, H. *Appl. Magn. Reson.* **2006**, *30*, 473–498.
- (29) Jeschke, G. *Macromol. Rapid Commun.* **2002**, *23*, 227–246.
- (30) Bhatnagar, J.; Freed, J. H.; Crane, B. R. *Two-Compon. Signaling Syst., Pt B* **2007**, *423*, 117–133.