Spin-label EPR on Disordered and Amyloid Proteins

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APPENDIX A

SUPPORTING INFORMATION ON THE MODEL OF ONE Aβ PER MICELLE DESCRIBED IN CHAPTER 2

In chapter 2, the effect of the membrane mimicking detergent SDS on the aggregation of the Aβ peptide is investigated. We propose that at SDS concentrations above the critical micelle concentration (CMC) only one species is present, which we assign to a monomeric, micelle bound form of Aβ. If Aβ in this form is indeed monomeric, non-diamagnetically diluted samples should show the same spectrum as the diamagnetically diluted samples investigated in chapter 2. The figure shows that this is the case, confirming the one Aβ per micelle model.

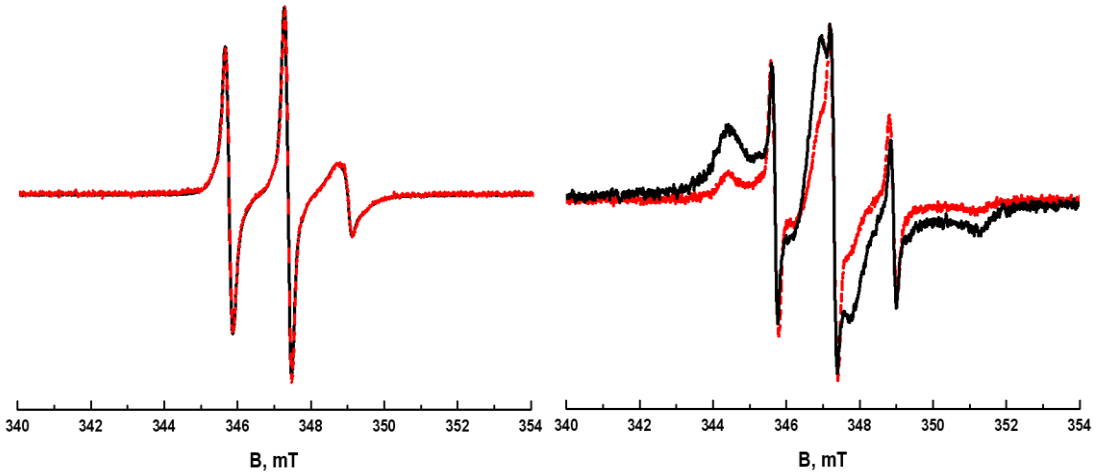

Figure A.1 Room temperature continuous wave EPR spectra of SL-Aβ40 comparing diamagnetically diluted (red line) with non-diamagnetically diluted (black line) samples. Left: Spectra of SL-Aβ40 at high SDS concentrations with D/P of 130.9 (SDS = 72 mM). The spectra consist of one mobility component with a rotation-correlation time of 0.93 ± 0.03 ns. Black and red spectra are identical within the signal to noise. Right: Spectra of SL-Aβ40 in the absence of SDS, i.e., D/P = 0. The spectra consist of at least three mobility components, fast, medium, and slow. The corresponding rotation-correlation times and the contributions of each component are given in chapter two of this thesis. Given in black is the non-diluted SL-Aβ40 at a D/P = 0. Similar to the red spectrum, the black spectrum consists of at least three mobility components. The contribution of the fast component to the spectrum of the non-diluted SL-Aβ40 is smaller than that of the diamagnetically diluted SL-Aβ40. Also, the slow component has a larger contribution to the spectrum of the non-diluted SL-Aβ40, compared to that of the diamagnetically diluted SL-Aβ40. We attribute the overall broadening in the right part of the figure to spin-spin interaction. Such a broadening is absent in the spectra given on the left, confirming the one Aβ per micelle model described in chapter 2.

APPENDIX B

THE TRIANGULATION APPROACH USED IN CHAPTER 6: CALCULATING THE POSITION OF RESIDUE 4 OF CP29

In chapter 6, the N-terminus of the light-harvesting protein CP29 is studied. The triangulation approach is used to determine the position of the end (residue 4) of the N-terminus, which is explained in this appendix. The distances between residue 4 and residues 101 and 205, and between residue 4 and a chlorophyll as obtained from a time-resolved FRET study [1] define the position.

The triangulation approach used in chapter 6: calculating the position of residue 4 of CP29

For the triangulation of the unknown position of residue 4 of CP29 we start from three known atom positions: A, B and C. To determine the position of residue 4, we have three experimental distances from atoms A, B and C to residue 4 that are measured either by DEER (table B.1) or by FRET.

The distances *a* and *b* between A respectively B and the residue 4 define the radius of spheres around A and B that intersect as a circle between A and B (figure B.1). The distance *c* (distance between C and residue 4) defines a sphere around C with a radius of *c*. This sphere can have two points of intersection with the circle between A and B, one of these points is the position sought. The atoms are selected such that the distance AB (r_{AB}) is longer than the distance AC (r_{AC}). The lines joining these three positions r_{AB}, r_{AC}, and r_{BC} (figure B.1) define the sides of a triangle.

Amongst the three experimentally determined distances (*a*, *b*, and *c*), two are obtained from DEER measurements: residue 4 to residue 101 (88 in *Spinach*) and residue 4 to residue 205 (191 in *Spinach*). The third distance is the distance between residue 4, labeled with a fluorescents dye [1], and a chlorophyll taken from FRET data reported earlier [1].

The FRET distance of 1.8 nm refers to a distance between residue 4 and one of the 13 chlorophylls in the CP29 protein. To account for this, all 13 chlorophylls are considered individually and the coordinates of their central Mg-atom are combined with the other two C_α- positions. We exclude only those chlorophylls whose Mg atoms are farther than 30 Å from the C_α- atoms of residue 101 (88 in *Spinach*) or of residue 205 (191 in *Spinach)*, because if these distances are larger, the chlorophylls are located on the other, luminal side of the protein and therefore cannot contribute to FRET with residue 4. Eight chlorophylls remain, which are listed in table B.2.

Table B.1 Distance parameters of the doubly spin-labeled CP29 (for details see chapter 6) derived from the analysis of DEER data selected and labeled as used in the present model. Given are: <r>, distance in nm; S(r), the width of the distance distribution in nm, % shows the contribution of each peak. The width of the distance distributions reflects the conformation distribution of the N-terminus as well as the distribution of the conformations of the spin-label linkers.

	short (s)			**medium (m)**			**long (l)**		
mutant	**$\langle r_s \rangle$ nm**	**S (r_s) nm**	**%**	**$\langle r_m \rangle$ nm**	**S (r_m) nm**	**%**	**$\langle r_l \rangle$ nm**	**S (r_l) nm**	**%**
4/97C	3.19	0.84	28	4.27	0.67	20	4.94	0.98	52
4/101C	1.96	1.28	35	3.53	0.68	22	5.14	0.95	43
4/205C	3.14	0.84	15	3.69	0.34	2	5.06	0.76	39

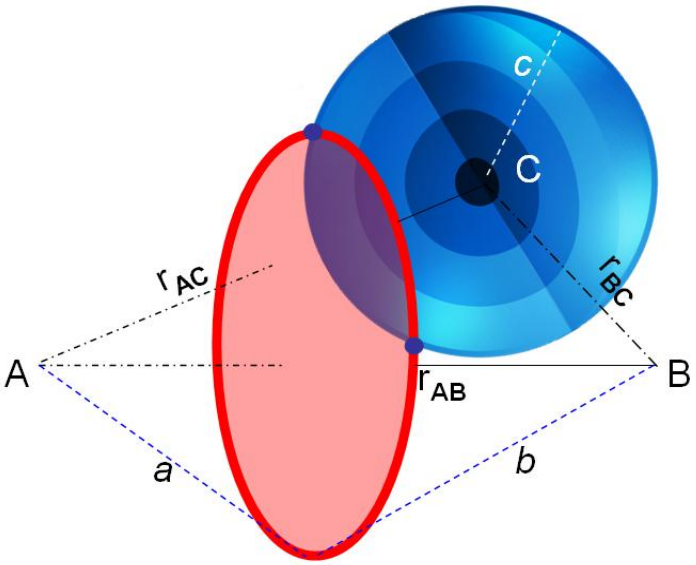

Figure B.1 Principle of the triangulation approach. For the triangulation we use three atom positions: A, B, and C. The coordinates of the three atom positions, C_α- for residues 101 (88 in *Spinach*), 205 (191 in *Spinach*), and the central Mg-atom of the chlorophylls are taken from the CP29 pdb file (access code: 3PL9) [2]. The vectors joining these three positions (r_{AB}, r_{AC}, and r_{BC}) define the sides of a triangle. The distances *a* and *b* between A or B to the residue of interest define spheres around A and B that intersect as a circle between A and B (shown as a red circle). The distance *c* defines a sphere around C with radius *c*. This sphere can have two intersections with the red circle (shown as blue dots), one of them the residue position sought. The sides were defined such that r_{AB} is longer than r_{AC}.

Table B.2 Distance combinations considered to determine the location of residue 4 in CP29. Given are the 72 possible combinations in two sections of the table, which comprise the five left-hand and the five right-hand columns each. In the first column, the number of the chlorophylls is given together with the atom label A, B, and C used in the computation. The second column gives the distances from DEER, either for the 4/101 or for the 4/205 mutant. The letters s, m, and l stand for short, medium and long distance derived from DEER analysis. The remaining three columns give the distances: *a*, *b*, and *c*. Nine rows describe all possible distance combinations for the chlorophyll in question.

section 1					section 2				
		a (Å)	**b (Å)**	**c (Å)**			**a (Å)**	**b (Å)**	**c (Å)**
	1: 101(s), 205(s)	18	31.4	19.6		1: 101(s), 205(s)	18	19.6	31.4
	2: 101(s), 205(m)	18	36.9	19.6		2: 101(s), 205(m)	18	19.6	36.9
CHL602	3: 101(s), 205(l)	18	50.6	19.6	**CHL610**	3: 101(s), 205(l)	18	19.6	50.6
	4: 101(m), 205(s)	18	31.4	35.3		4: 101(m), 205(s)	18	35.3	31.4
A: CHL	5: 101(m), 205(m)	18	36.9	35.3	**A: CHL**	5: 101(m), 205(m)	18	35.3	36.9
B: 205	6: 101(m), 205(l)	18	50.6	35.3	**B: 101**	6: 101(m), 205(l)	18	35.3	50.6
C: 101	7: 101(l), 205(s)	18	31.4	51.4	**C: 205**	7: 101(l), 205(s)	18	51.4	31.4
	8: 101(l), 205(m)	18	36.9	51.4		8: 101(l), 205(m)	18	51.4	36.9
	9: 101(l), 205(l)	18	50.6	51.4		9: 101(l), 205(l)	18	51.4	50.6
	1: 101(s), 205(s)	19.6	31.4	18		1: 101(s), 205(s)	31.4	19.6	18
	2: 101(s), 205(m)	19.6	36.9	18		2: 101(s), 205(m)	36.9	19.6	18
CHL603	3: 101(s), 205(l)	19.6	50.6	18	**CHL611**	3: 101(s), 205(l)	50.6	19.6	18
	4: 101(m), 205(s)	35.3	31.4	18		4: 101(m), 205(s)	31.4	35.3	18
A:101	5: 101(m), 205(m)	35.3	36.9	18	**A:205**	5: 101(m), 205(m)	36.9	35.3	18
B: 205	6: 101(m), 205(l)	35.3	50.6	18	**B: 101**	6: 101(m), 205(l)	50.6	35.3	18
C: CHL	7: 101(l), 205(s)	51.4	31.4	18	**C: CHL**	7: 101(l), 205(s)	31.4	51.4	18
	8: 101(l), 205(m)	51.4	36.9	18		8: 101(l), 205(m)	36.9	51.4	18
	9: 101(l), 205(l)	51.4	50.6	18		9: 101(l), 205(l)	50.6	51.4	18
	1: 101(s), 205(s)	18	19.6	31.4		1: 101(s), 205(s)	31.4	19.6	18
	2: 101(s), 205(m)	18	19.6	36.9		2: 101(s), 205(m)	36.9	19.6	18
CHL608	3:101(s), 205(l)	18	19.6	50.6	**CHL612**	3: 101(s), 205(l)	50.6	19.6	18
	4: 101(m), 205(s)	18	35.3	31.4		4: 101(m), 205(s)	31.4	35.3	18
A: CHL	5: 101(m), 205(m)	18	35.3	36.9	**A: 205**	5: 101(m), 205(m)	36.9	35.3	18
B: 101	6: 101(m), 205(l)	18	35.3	50.6	**B: 101**	6: 101(m), 205(l)	50.6	35.3	18
C: 205	7: 101(l), 205(s)	18	51.4	31.4	**C: CHL**	7: 101(l), 205(s)	31.4	51.4	18
	8: 101(l), 205(m)	18	51.4	36.9		8: 101(l), 205(m)	36.9	51.4	18
	9: 101(l), 205(l)	18	51.4	50.6		9: 101(l), 205(l)	50.6	51.4	18
	1: 101(s), 205(s)	19.6	31.4	18		1: 101(s), 205(s)	31.4	19.6	18
	2: 101(s), 205(m)	19.6	36.9	18		2: 101(s), 205(m)	36.9	19.6	18
CHL609	3:101(s), 205(l)	19.6	50.6	18	**CHL615**	3: 101(s), 205(l)	50.6	19.6	18
	4: 101(m), 205(s)	35.3	31.4	18		4: 101(m), 205(s)	31.4	35.3	18
A: 101	5: 101(m), 205(m)	35.3	36.9	18	**A: 205**	5: 101(m), 205(m)	36.9	35.3	18
B: 205	6: 101(m), 205(l)	35.3	50.6	18	**B: 101**	6: 101(m), 205(l)	50.6	35.3	18
C: CHL	7: 101(l), 205(s)	51.4	31.4	18	**C: CHL**	7: 101(l), 205(s)	31.4	51.4	18
	8: 101(l), 205(m)	51.4	36.9	18		8: 101(l), 205(m)	36.9	51.4	18
	9: 101(l), 205(l)	51.4	50.6	18		9: 101(l), 205(l)	50.6	51.4	18

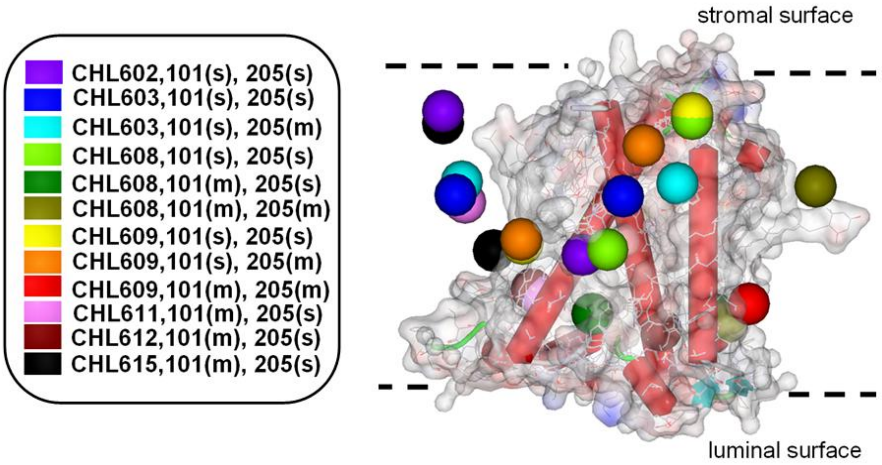

Figure B.2 Location of all geometrically possible positions for residue 4 within the structure of CP29. The orientation of CP29 is such that the membrane stromal surface is horizontal and follows the top surface of the protein. The positions are determined using the distances, which were measured with DEER (chapter 6) and FRET [1], as described in the text. Using the triangulation approach, 12 points of intersection are obtained. The intersection points are presented as spheres in different colors. Each color corresponds to one chlorophyll (CHL) number (see legend) and the distances from DEER, s: short, m: medium, and l: long. Six out of the 12 positions are physically possible positions for residue 4.

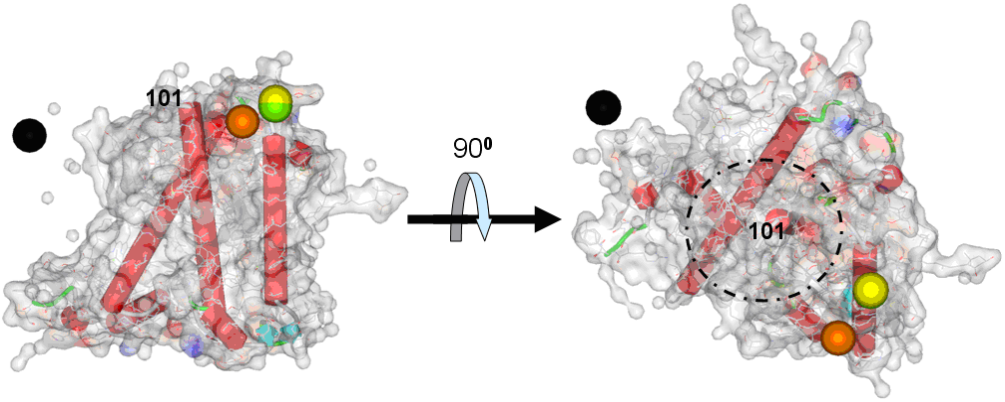

Figure B.3 The four most likely positions of residue 4, selected from those in figure B.2. The orientation of CP29 on the left is as in figure B.2. On the right, the view onto the stromal surface of the protein is shown. These positions are given as spheres with different colors. Three of these spheres (the green, yellow, and orange sphere) cluster in the area where helices B and C reach the protein stromal surface, and the black sphere is located farther from the stromal surface of the protein, close to the membrane surface. The projection of the protein is so that it is hiding two of the spheres representing the position of residue 4 (shown in yellow and green). The dotted circle indicates the possible location of residue 97 (see chapter 6 of this thesis).

Each of the DEER experiments probing the separation of residue 4 with residues 101 or 205 resulted in three distances, s, m, and l (see chapter 6 of this thesis). Combining the eight possible Mg atom positions and the three distances found for each DEER experiment results in the 72 combinations listed in table B.2. Amongst these 72 combinations, only 12 have points of intersection as defined in figure B.2. All these points are shown in figure B.2 as spheres, color coded to indicate the combination of distances that gives rise to these solutions. Because every combination gives rise to two intersection points, only six of these 12 solutions are physically meaningful, i.e., represent possible positions of residue 4. We discarded all points that were in the interior of the protein or the membrane, which leaves us with the four points discussed in chapter 6 of this thesis (figure B.3).

Reference List

[1] B.A.Berghuis, R.B.Spruijt, R.B.M.Koehorst, A.van Hoek, S.P.Laptenok, B.van Oort, H.van Amerongen, *European Biophysics Journal with Biophysics Letters* **2010,** *39* 631-638.

[2] X.Pan, M.Li, T.Wan, L.Wang, C.Jia, Z.Hou, X.Zhao, J.Zhang, W.Chang, *Nat.Struct.Mol.Biol.* **2011,** *18* 309-315.

LIST OF ABBREVIATIONS

Aβ	amyloid beta
AD	Alzheimer's disease
APP	amyloid precursor protein
αS	alpha synuclein
CMC	critical micelle concentration
cw	continuous wave
DEER	double electron electron resonance
DMSO	anhydrous dimethyl sulfoxide
DTT	DL-dithiothreitol
EPR	electron paramagnetic resonance
J	the exchange interaction
LHCII	light harvesting complex II
MTS	((1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate)
mw	microwave
PBS	phosphate-buffered saline
PD	Parkinson's disease
PSII	photosystem II
SDS	sodium dodecyl sulfate
ThioT	thioflavin T
TOAC	alpha-amino acid 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid
τ_r	rotation-correlation time