

Conformational Studies of A β (1-12) with Metals and Small Molecules Suggests Plausible Disruption of Aggregation

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Abstract: *Alzheimer's disease (AD), the most common form of senile dementia, is associated with the progressive accumulation of plaques and tangles within the neuronal cell. The plaques are composed of amyloid (A β) peptide fragments with a high propensity for aggregation. Metal ion binding to A β peptide has been known to alter the aggregation of the peptide and to be involved in the pathogenicity of Alzheimer's. Earlier investigations suggest that the N terminal hydrophilic region of the peptide exhibit preferential ligand binding capabilities and, thus initiate the onset of aggregation events. The complexation events with metals like zinc, copper, aluminium, and small molecules like betaine and curcumin, reported in this paper provide a molecular level appreciation of the binding characteristics of the A β (1-12) peptide fragments and the variations thereof. Our data show notable conformational changes induced due the binding of these ligands, suggesting plausible clues to explore these molecules as potential inhibitors and neuro-protective agents for AD.*

Keywords: *Alzheimer's disease, A β peptide, Conformation, metals, Circular Dichroism*

1. INTRODUCTION

Alzheimer's disease (AD) is the most common form of neurodegenerative disorder [1, 2] characterized by the formation of extracellular deposits composed of Amyloid beta peptides (A β) [3] and, a large number of helically wound filaments in the cytoplasm of neuronal cell bodies, called neurofibrillary tangles [4]. The A β is a 39-43 residue peptide formed by proteolytic cleavage of the larger amyloid precursor protein (APP) [5]. Studies have revealed that the backbone structure of the monomeric A β (1-40) is generally a random coil, with the C-terminal hydrophobic regions showing due propensity to adopt β -structures [6]. The A β (1-28), A β (1-39), A β (1-42) and A β (29-42) are also known to form independent aggregates (7). It has been reported that the N terminal region of the A β peptide spanning residues 1-16 lie between the alpha and beta secretase cleavage sites (as depicted in Fig 1) and is crucial for neurotoxic effects (8). There is substantial evidence that the peptide fragment A β (17-42) is non amyloidogenic in nature [9].

The increased concentrations of metal ions such as copper iron and zinc, in the brains of Alzheimer's disease patients [10] has led researchers to study the effect of these ligands on the progression of AD. It has been well elucidated that metal ions play an important role in altering

the conformation of the A β peptide [11]. Of the metals, zinc and aluminium have been known to induce toxicity [12-13]. Reports highlight that, drastic conformational changes have been observed with the Al³⁺ peptide complexes [14]. Interestingly, it has been reported that betaine reverses the effect of Al³⁺ induced aggregation [15]. Investigations have also suggested the reversal of Al³⁺ triggered aggregations with boron in the form of borosilicates [16]. Inhibitory effects with platinum, curcumin, and nicotine have also been studied to explore their utilities in preventing aggregation and accumulation of fibrillar deposits [17-19].

There has been substantial evidence that the metal coordination site occurs in the N terminal region, with E11 and the 3 histidines playing an important role in ligand binding. [20] However, our earlier investigations on the shorter fragments with Aluminium via NMR, have revealed the not so prominent involvement of the histidines [21]. Thus, appreciating the fact that, the creation of smaller fragments is a consequential event during AD, a detailed investigation of the shorter fragment (1-12) of the N terminal appeared essential towards understanding its interactions with metal ions and small molecules. Further, it was supposed to be an interesting proposition to observe the binding patterns of the ligands to this truncated 1-12 peptide, especially in the absence of the 2 crucial Histidines (H13 and H14). With this background, Circular Dichroism (CD) studies on the 1-12 peptide fragments have been carried out, to understand the variations in the binding patterns of the residues with the ligands, and their potential exploitation as suitable inhibitors and metal chelators to control the progression of AD.

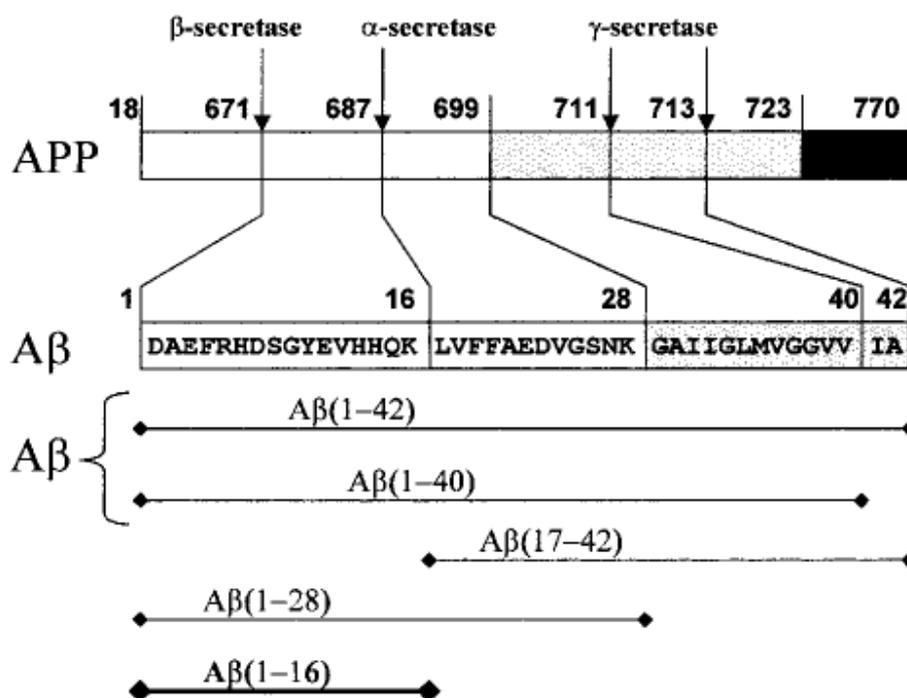


Figure 1. The secretase cleavage sites are indicated, namely the α , β and γ secretase cleavage sites (Courtesy: *Biochemical and Biophysical Research Communications*, 285, (4) 959-964 (2007))

2. MATERIALS AND METHODS

The customized A β (1-12) fragment with the sequence DAEFRHDSGYEV and having a molecular weight of 1423 Da, was purchased from M/s USV Peptides with >95% purity (HPLC grade). Various concentrations of metals were prepared in Milli-Q water and used for the interaction studies with peptide. The concentration of aluminium and zinc were maintained at 0.01mM, 0.1mM, 1mM and 10mM; while, the copper concentrations were recorded at 10nM, 100nM, 200nM, 400nM and 500nM respectively (as the noise became significant at higher concentrations). Due to solubility issues, studies with curcumin were done only at 1 μ M and 0.1mM concentrations respectively. The concentrations of betaine were kept at 1 μ M, 0.01mM, 0.1mM, 1mM and 10mM respectively. Recordings were also done using a 1:1 aluminium-peptide at 0.1mM concentration, with varying ratios of 0.01mM, 0.1mM and 1mM betaine solutions, to check the effect of small molecule on the peptide-aluminium complex. All studies were carried

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out using a working stock concentration of 0.1mM of peptide (dissolved in purified Milli-Q water). However, for all the experiments related to the binding of metal ions and small molecules, the peptide concentration was kept constant at 0.1mM. The pH was monitored and maintained at 6.2 throughout the titrations with and without addition of metallic salts and small molecules.

CD spectra were recorded using a JASCO J-715 spectro-polarimeter. Cuvettes with path length of 0.2 cm were used for spectral recording in the range 200 to 250nm with sampling points at every 0.5nm. The base line subtraction was done with milli Q water as blank. The plots were recorded (for 4scans) and raw CD data was converted to molar ellipticity. The data points were collected and secondary structure content determined using the K2D3 software [22]. The data for the native peptide (without any ligand) which served as a reference were recorded at concentrations of 0.01mM, 0.05mM, 0.1mM and 0.5mM, respectively.

3. RESULTS AND DISCUSSION

The CD spectra of the native A β (1-12) peptide at various concentrations are plotted in Fig. 2. The spectra and the estimated secondary structure values tabulated in Table 1 clearly highlight that, the peptide exhibits a tendency towards aggregation patterns (as suggested by the decreasing values of α helical geometries and increasing values of β sheet contents).

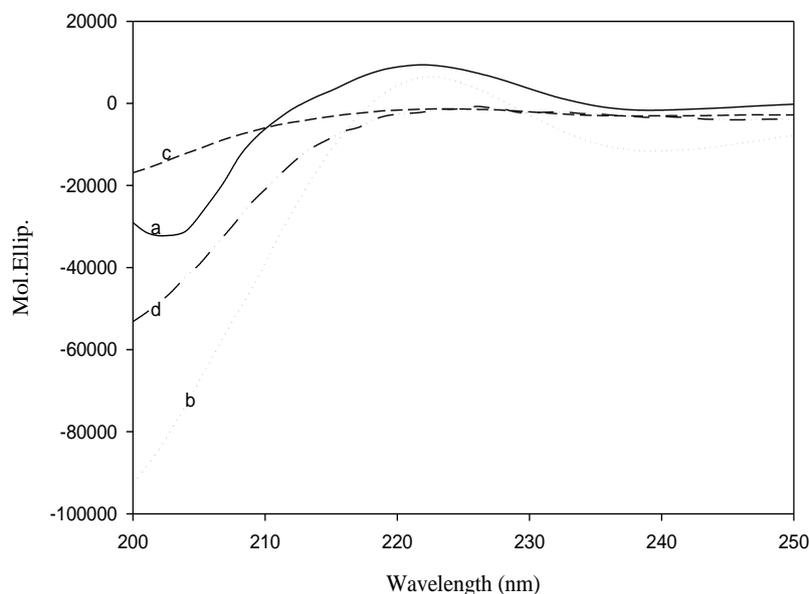


Figure 2. CD Spectra of native A β (1-12) at different concentrations a)0.5mM b) 0.1mM c) 0.05mM d) 0.01mM. The units for molar ellipticity are given in deg. cm² dmol⁻¹

3.1. Interaction of A β (1-12) Peptide With Ligands

An overlay of the CD spectra of A β (1-12) peptide fragment with ligands like aluminum, copper, zinc, curcumin and betaine at concentrations showing maximum variation with respect to native conformation is shown in Fig 3. The spectra highlights that, the negative band was around 205 nm for all curves indicating marked changes in the peptide conformations. It is interesting to note that the change in negativity with respect to native (Fig.3a) is maximum in case of the copper and aluminium complexes as shown in spectra c and f respectively. This suggests that copper and aluminium are able to induce secondary structural changes in the native peptide. Similarly for zinc and betaine complexes, the molar ellipticity values increase at 200nm (Fig.3b and 3d). Likewise, it is interesting to note that the molar ellipticity values decreases drastically in case of curcumin, in comparison to the native at 200nm. The aluminium induced aggregation is commensurate to the observations made in various literatures [14, 15, 23-25]. The estimated secondary structure content (Table 1) showed slight increase in the percentage of β sheet content with increase in the concentration of zinc, while the α -helical values showed large increase at lower concentration of zinc. A higher propensity for alpha helix at low zinc concentration possibly indicates that the metal may behave like a neuroprotective substance. This is in

accordance with an earlier review that low levels of zinc might reduce toxicity of A β [26]. Our results with copper (Fig. 2c) indicate that the maximum changes in β sheet content have been observed at 10nM copper; the metal seems to induce considerable amount of α -helical conformation at copper concentration of 100nM and above. Of course, the recordings of the CD spectra became difficult at higher concentrations of copper attributed to noise.

The CD spectra of the peptide with curcumin showed marked decrease in the negative peak around 200nm and marginal increase in the positive band around 220nm, the secondary structure estimated from the spectral changes on addition of 1 μ M and 0.1mM of curcumin is given in Table 1. The different percentages of the helical and beta sheet contents observed at varying concentrations of betaine are given in Table 1. The estimated secondary structure content of helix for the peptide-betaine complex showed drastic increase from about 40% (as in native form) to 70% (at 0.1mM betaine), suggesting that betaine could alter the progression towards the polymeric forms.

Additionally, in order to explore the betaine induced reversal of the conformational changes of the peptide by aluminium, as reported in earlier studies [15], the metal-bound forms of A β (1-12) in complex with 0.1mM aluminium, were titrated with increasing concentrations of betaine. The estimated secondary structure from the spectra (Table 1) shows that though the β sheet content was reduced by only 4%, the helical content drastically increased by 72% on gradual addition of betaine. This clearly suggests that betaine could facilitate the reversal of aluminium induced aggregation of the peptide. It should however be noted that in all the above experiments, the estimates of the secondary structure content from the CD spectra (Table 1) are only indicative of the possible structural changes and does not refer to any absolute conformational values.

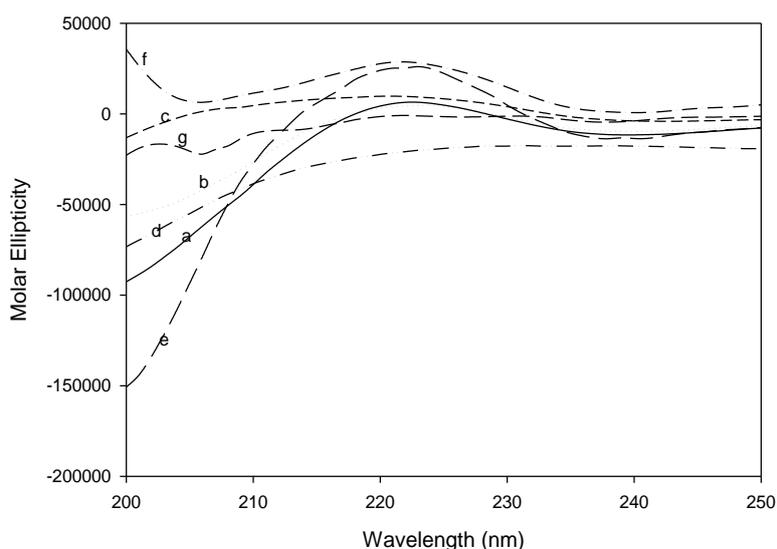


Figure3. Spectra of a) 0.1mM Native peptide; b) 0.1mM peptide with 0.01mM Zn²⁺; c) 0.1mM peptide and 100nM Cu²⁺; d) 0.1mM peptide and 0.1mM betaine; e) 0.1mM peptide and 0.1mM curcumin; f) 0.1mM peptide with 10mM Al³⁺; g) 0.1mM peptide with 0.1mM Al³⁺ and 0.1mM betaine.

Table 1. Fraction of α helix and β sheet in the native A β (1-12) with varying ligand concentrations. The metal/ligand concentrations taken for interaction studies are indicated in bold. The % change of the secondary structural elements with ligands is indicated in parenthesis.

Native A β (1-12)	Concentration of peptide								-
	0.01mM		0.05mM		0.1mM		0.5mM		
	α	β	α	β	α	β	α	β	
	93.64	0	92.53	0.01	39.91	0.01	5.52	9.75	
Aluminum	Concentrations of metal								-
	0.01mM		0.1mM		1mM		10mM		
	α	β	α	β	A	β	α	β	
	31.26 (8)	1.17 (1.1)	21.24 (18)	3.6 (3.5)	3.68 (36)	12.39 (12.3)	0 (39)	28.45 (28.4)	

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Zinc	Concentrations of metal									
	0.01mM		0.1mM		1mM		10mM			
	α	β	α	β	A	β	α	β		
	71.78 (31)	(0.04) (0.03)	65.5 (25)	0.04 (0.03)	10.4 (29)	7.04 (7.0)	1.51 (38)	9.01 (9.0)		
Copper	Concentrations of metal									
	10nM		100nM		200nM		400nM		500nM	
	α	β	α	β	A	β	α	β	α	β
	0 (39)	21.76 (21.7)	82.3 (82)	0.01 (0)	88.01 (48)	0.01 (0)	88.01 (48)	0.01 (0)	80.5 (40)	0.02 (0.01)
Curcumin	Concentrations of ligand									
	1 μ M		0.1mM							
	α	β	α	β						
	5.4 (34)	9.31 (9.3)	6.13 (33)	10.95 (10.9)						
Betaine	Concentrations of ligand									
	1 μ M		0.01mM		0.1mM		1mM		10mM	
	α	β	α	β	α	β	α	β	α	β
	32.64 (8)	1.17 (1.16)	40.98 (1)	0.58 (5.7)	70.58 (30)	0.28 (.27)	56.5 (17)	0.12 (.11)	27.81 (10)	0.02 (1.01)
Aluminum and Betaine	Concentration of Al and Peptide at 0.1mM		Concentration of Betaine							
			0.01mM		0.1mM		1mM			
	α	β	α	β	A	β	α	β		
	21.24 (18)	3.6 (3.5)	93.56 (53)	0 (0)	93.35 (30)	0 (0)	92.53 (30)	0.01 (0)		

4. CONCLUSION

While the native conformation of the peptide fragment is unstructured and near random coil, it is likely that it adopts an ordered structure when complexed with metals/ligands. The changes were expectedly predominant in the case of aluminium and copper. NMR studies have also revealed the binding of aluminium to the A β (1-12) fragment at the N terminal region [21]. The alpha helical content in case of the peptide-betaine complex shot up to 70% indicating that the small molecule could disrupt the progressions towards the aggregated forms.

In summary, our CD studies on the A β (1-12) peptide fragment and its complexes with metal ions (aluminium, zinc and copper) and small molecules (betaine and curcumin) has indeed provided indications of the conformational changes the peptide undergo during the binding of various ligands. Further, the results with betaine offer due insights into the probable use of betaine and betaine like structures as potential drug candidates for AD. Similarly, analogs of curcumin could also be exploited towards designing lead compounds to tackle the onset of AD.

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REFERENCES

- [1] Dennis J. Selkoe, Alzheimer's disease: Genes, Proteins, and Therapy. *Physiol Rev* 81 (2), 741-766, (2001)
- [2] Matthew L. Hemming, Joshua E. Elias Steven P. Gygi, Dennis J. Selkoe, Identification of β -Secretase [BACE1] Substrates Using Quantitative Proteomics. *PLoS ONE* 4 (12), 8477 (2009).

- [3] Selkoe DJ Amyloid β -protein and the genetics of Alzheimer's disease. *JBiolChem*. 271 (31) 18295-18296 (1996)
- [4] Selkoe DJ, Cell biology of protein misfolding: The examples of Alzheimer's and Parkinson's diseases. *Nat Cell Biol*, 6 (11) 1054-1061 (2004)
- [5] Craig S. Atwood, Mark E. Obrenovich, Tianbing Liu, Hsien Chan, George Perry, Mark A. Smith, Ralph N. Martins, Amyloid- β : a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid- β , *Brain Research Reviews* 43 (1) 1–16 (2003)
- [6] Douglas V. Laurents, Paul M. Gorman, Meng Guo, Manuel Rico, Avijit Chakrabartty, and Marta Bruix. 6. Alzheimer's A β 40 Studied by NMR at Low pH Reveals That Sodium 4,4-Dimethyl-4-silapentane-1-sulfonate [DSS] Binds and Promotes β Ball Oligomerization. *J Biol Chem*, 280 (5), 3675-85 (2005)
- [7] Barrow CJ, Zagorski MG Solution structures of beta peptide and its constituent fragments: relation to amyloid deposition, *Science*, 253 (5016), 179-82 (1991).
- [8] Sergey A Kozin, Séverine Zirah, Sylvie Rebuffat, Gaston Hui Bon Hoa and Pascale Debey, Zinc Binding to Alzheimer's A β [1–16] Peptide Results in Stable Soluble Complex *Biochemical and Biophysical Research Communications*, 285, (4) 959-964 (2007)
- [9] E. Gowing, a E. Roher, a S. Woods, R. J. Cotter, M. Chaney, S. P. Little, and M. J. Ball, Chemical characterization of A beta 17-42 peptide, a component of diffuse amyloid deposits of Alzheimer disease. *The Journal of biological chemistry*, 269, (15) 10987–90 (1994)
- [10] Lovell, M.A., Robertson, J.D, Teesdale, W.J, Campbell, J.L &Markesbery, W.R, Copper, Copper, Iron and zinc in Alzheimer's disease senile plaques, *J Neurol Sci*, 158,(1) 47-5 (1998)
- [11] Ashley I. Bush The metallobiology of Alzheimer's disease, *TRENDS in Neurosciences*, 26(4), 207-14 (2003)
- [12] Religa D, Strozyk D, Cherny R.A, Volitakis, I, Haroutunian V, Winblad B, Naslund J & Bush A.I, Elevated cortical zinc in Alzheimer disease. *Neurology*, 67, (1) 69-75 (2006)
- [13] Brown A.M, Tummolo D.M, Rhodes K.J, Hofmann J.R, Jacobsen J.S & Sonnenberg-Reines J, Selective aggregation of endogenous beta-amyloid peptide and soluble amyloid precursor protein in cerebrospinal fluid by zinc, *J Neurochem*, 69, (3),1204-1212 (1997)
- [14] G. D. Fasman, A. Perczeland C. D. Moore Solubilization of β -amyloid-[1-42]-peptide: Reversing the β -sheet conformation induced by aluminum with silicates, *Proc. Natl. Acad Sci. USA*, 92, (2) 369-71, (1995).
- [15] Ramakrishna T, Vatsala S, Shobi V, Sreekumaran E, Madhav TR, Ramesh J, Easwaran KRK, Betaine reverses toxic effects of aluminium: Implications in Alzheimer's disease [AD] and AD- like pathology, *Current Science* .75 (11) 1153-1156 (1998)
- [16] Ramakrishna T, Vatsala S, Madhav TR, Sreekumaran E, Ramesh J, Easwaran KRK. Conformational Change in b-amyloid Peptide [1-40] with Aluminium: Reversal by Borate. *Alzheimer's Research*, 3 (5), 223-226, (1997)
- [17] Barnham KJ, Kenche VB, Ciccotosto GD, Smith DP, Tew DJ, Liu X, Perez K, Cranston GA, Johanssen TJ, Volitakis I, Bush AI, Masters CL, White AR, Smith JP, Cherny RA, Cappai R Platinum-based inhibitors of amyloid-beta as therapeutic agents for Alzheimer's disease, *Proc Natl Acad Sci*, 105, (19),6813-8, (2008)
- [18] Zhang J, Liu Q, Chen Q, Liu NQ, Li FL, Lu ZB, Qin C, Zhu H, Huang YY, He W, Zhao BL, Nicotine attenuates beta-amyloid-induced neurotoxicity by regulating metal homeostasis, *Faseb J*, 20, (8),1212-4, (2006)
- [19] Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, Chen PP, Kaye R, Glabe CG, Frautschy SA, Cole GM Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo, *J BiolChem*, 280(7)5892-901, (2005)
- [20] Syme CD, Viles JH, Solution 1H NMR investigation of Zn²⁺ and Cd²⁺ binding to amyloid-beta peptide [A β] of Alzheimer's disease, *Biochim BiophysActa*, 1764, (2)246-56 (2006)
- [21] Narayan P, Krishnarjuna B, Vishwanathan V, Jagadeesh Kumar D, Babu S, Ramanathan KV, Easwaran KR, Nagendra HG, Raghothama S, *ChemBiol Drug Des.*, Does Aluminium

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Bind to Histidine? An NMR Investigation of Amyloid β 12 and Amyloid β 16 Fragments
ChemBiol Drug Des., 82, (1).48-59 (2013)

- [22] Caroline Louis-Jeune, Miguel A. Andrade-Navarro, Carol Perez-Iratxeta, Prediction of protein secondary structure from circular dichroism using theoretically derived spectra , *Proteins: Structure, Function, and Bioinformatics*, 80,(2) 374–381 (2012)
- [23] Christopher Exley, Olga Korchazhkina The association of Aluminium and β Amyloid in Alzheimers Disease, *Aluminium and Alzheimers Disease: The Science that describes the Link*, (2001).
- [24] Christopher Exley, The coordination chemistry of aluminium in neurodegenerative disease, *Coordination Chemistry Reviews*, 256, 19–20, 2142–2146 (2012)
- [25] Chen.Y.R, Huang. H.B, Chyan. C.L, Shiao. M.S, Lin. T.H & Chen.Y.C The Effect of A β Conformation on the Metal Affinity and Aggregation Mechanism Studied by Circular Dichroism Spectroscopy, *J. Biochem*, 139(4) 733–740 (2006)
- [26] Christopher Exley, Nicholas C. Price, Sharon M Kelly and Derek Birchall, An interaction of β - amyloid with aluminium in vitro *FEBS* 324, 293-295. (1993)

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