INTERACTIONS OF OLIVE OIL POLYPHENOLS WITH Aβ OLIGOMERS

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ABSTRAK

Neurotoksisitas terutama mempengaruhi patogenesa penyakit Alzheimer (AD). Mekanisme yang diprediksi adalah pembentukan dan pengendapan plak beta amiloid (Aβ) dalam keadaan agak terlarut dalam jaringan otak. Senyawa polifenol yang dihasilkan oleh minyak zaitun telah dihubungkan dengan treatment dari pembentukan plak Aβ dengan cara mengikat dan menghambat pembentukan agregat oligomerik beracun yang terlarut. Studi ini bertujuan untuk menganalisis afinitas pengikatan Aβ (11-42)- oligomer dengan beberapa senyawa polifenol dan menentukan senyawa yang paling mampu untuk menghambat agregasi Aβ-oligomer. Metode komputasi digunakan untuk memprediksi kemampuan senyawa polifenol untuk berikatan dengan Aβ (11-42)-oligomer. Metode ini termasuk docking, visualisasi 3D, dan analisis struktural. Hasil menunjukkan hubungan positif yang kuat antara server docking dan skor afinitas pengikatan yang dihasilkan. Hasil lebih lanjut diilustrasikan polifenol dengan kelompok O-H tinggi dari struktur kimia mereka berkorelasi dengan ikatan afinitas yang lebih tinggi untuk Aβ (11-42)-oligomer. Hesperidin memiliki jumlah kelompok O-H tertinggi dalam studi dan juga memiliki salah satu nilai ikatan afinitas tertinggi di ketiga server docking. Hal ini menunjukkan hubungan timbal balik antara struktur polifenol dan ikatan afinitas yang dihasilkan.

Key words: Alzheimer, Senyawa polifenol, beta amyloid, docking, visualisasi 3D, Hesperidin.

INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder affecting an estimated 36 million people worldwide (Lundkvist et al., 2014). Clinical manifestations commence with discrepancies in memory but inevitably progress to irreversible cognitive decline. A key neuropathological feature of AD is the accumulation of amyloid plaques in the parenchyma of the neocortex, amygdala and hippocampus (Huang et al., 2000). The main constituent of these plaque deposits is the ~4 kDa amyloidbeta peptide (Aβ), which is produced through the sequential cleavage of amyloid precursor protein (APP) via βsecretase and γsecretase.

Current treatment for AD provides a delayed progression of symptomatology at various stages of the disease; However such treatments do not stop the advancement of AD. As such, novel therapeutics for the treatment and prevention of AD are urgently required. Since the initiation of amyloid formation by Aβ is dependent on interactions involving the central hydrophobic region (residues 17 to

20) (Hilbich et al., 1992), many researchers have focused their attention on blocking this region. Docking programs are now extensively used to test for ligandpeptide binding properties and experimental research is only conducted after extensive in silico experimental research has produced satisfactory results. Thus, the focus of attention for many researchers in AD therapeutics is the use of small molecules to target the misfolding and/or aggregation phase of amyloidogenesis, with the aid of computational software. The screening process of computational docking helps narrow down compounds that have structural compatibility with the Aβ fibre pharmacophore (Jiang et al., 2013).

The most extensively studied small molecules that impact Aβ aggregation are the polyphenols (Gauci et al., 2011). Though polyphenols have been shown to inhibit Aβ aggregation, it is still not clear whether they can reach the brain in sufficient concentrations or in a form capable of acting on Aβ oligomers in vivo (Singh et al., 2008). Hence it is vital to do further research on their intracellular and molecular targets to determine the role of polyphenols in neuroprotection. More research is also needed to investigate the pharmacokinetics of polyphenols.

This project aims to analyse the binding affinity of Amyloid βeta (1142)-oligomer with several polyphenol compounds from olive oil and determine the most capable compound able to inhibit Aβoligomer aggregation. Establish anti-amyloidogenic properties and binding locations and predict affinities of polyphenols to Aβoligomers through the use of 3dimensional computational methods. This project will deduce the effectiveness of the predicted binding affinity of Aβ (1142)-oligomers with polyphenol compounds across the three docking servers. Finally, determine correlations between affinity score, Molecular Weight, Hydrogen Bond count and topology polar surface area of each ligand through statistical analysis.

METHOD

The following five steps were undertaken to predict the binding affinity of 60 polyphenolic compounds with Aβ (1142)oligomer, to determine and correlation between docking servers, to examine structural similarities and to determine the best regression model for binding affinity prediction.

Ligand Retrieval

The 3D structures of 60 polyphenolic compounds (ligands) were retrieved from the National Centre for Biotechnology Information (NCBIPubChem) in a spatial

data file (SDF). PubChem is a compound database containing authorised chemicaldescription information that has been successful in guiding scientists (Wang et al., 2014). The SDFs were then converted to protein data bank (PDB) format using the online Simplified Molecular Input Line Entry System (SMILES) translator. This conversion was necessary as SDFs are not recognised by most docking tools. SMILES aids in converting the 2D system molecules into 3D system molecules (Achary, 2014).

Docking

The second part of the project involved docking all 60 ligands with the Aβ-oligomer using three docking tools; PyRx 0.8 (Vina) with exhaustiveness of 8 and maximized dimensions (Angstrom), SwissDock server, and HexServer with default settings. These docking tools provided effective oligomerligand docking schematics, enabling full comprehension of the docking process and methods. In addition, they assisted in docking the ligand to create a set of grids used in describing the targeted protein. AutoDock Vina (0.8) is a virtual screening program that is used for computational drug discovery and uses the creation of screen libraries consisting of various compounds usable against possible drug targets (Dallkyan & Olson, 2015). The output files of AutoDock Vina were in SDF format for all 60 ligands. Only the highest binding affinities of the top 18 ligands were then converted into PDB format using Chem3D (Avogadro), also known as The Avogadro Project. Avogadro has been used to achieve crossplatform assignments in computational chemistry, bioinformatics, molecular modelling and materials science (Hanwell et al., 2012). SwissDock server and HexServer produced PDB files instantly for all 60 ligands.

Visualization

The 10 highest oligomerligand binding affinities from each docking tool were then visualized using Visual Molecular Dynamics (VMD 1.9.2). VMD is often described as a molecular visualisation program that is used for displaying, analysing and animating large biomolecular systems through the use of 3D graphics and a builtin script (Krone et al., 2012). PDB format was used to facilitate the processing of file editing in TextEdit (Macintosh system)/ Notepad (Windows system). In these textediting tools, the PDB text of the Aβ (1142)oligomer (2mxuabetafibril-oligomer.pdb) was then combined with the conformational data (PDB files) of each highest binding affinity of the 10 ligands from each docking tool. These combined

files were then visualized in VMD to see how those top 10 ligands interact with the oligomer with the representations of: style (NewRibbons) and colour (Secondary Structure) for the protein, style (VDW) for the ligand, and style (Licorice) for the residues within 2.5 Angstrom. Cyan colour and white background was used for the protein structure. This visualization provided vast amounts of data about binding, such as type of binding (i.e., hydrogen, van der Waals) and residues involved in the oligomerligand interaction. AutoDock Vina gave more accurate conformations in VMD, as such they have been used in this paper.

Structural Analysis

PubChem bioAssay was used to conduct a structural analysis in order to determine any type of relationship between the polyphenol compounds. Fifty eight compounds (ligands) were submitted for analysis using their compound identifier (CID) number. A dendrogram tree was obtained based on their molar refractivity and topological indices. Two ligands were unable to be submitted for structural analysis due to unidentified CID number. The non-included compounds were Hydroxytyrosol diglucoside and Hydroxytyrosol rhamnoside.

RESULTS AND DISCUSSION

A total of 60 ligands were analysed in the docking part of the study. The top 6 ligands and their VMD representation can be seen in Figures 1-6.

Hydroxyltyrosol diglucoside

Hydroxyltyrosol diglucoside attained binding affinity scores of 5.7, 8.19 and 66.22 for Vina, Swissdock and Hex Server, respectively. Figure 1 shows the ligand interacting with the Aβ (1142) oligomer through Glycine, Leucine and Glutamine residues. Hydrogen bonding occurs between the amine and carboxylic groups of LEU17 with the hydroxyl groups from the ligand's glucoside and hydroxyltyrosol regions. Van der Waal interactions also occurred between methyl groups from LEU34 and the aromatic ring in the hydroxytyrosol region of the ligand. There is also Hbond interaction in similar fashion like LEU17 with the ligands glucoside regions. Studies from Serpell (2000) found that electrostatic interactions involving His-Asp/Glu salt bridges along with hydrophobic contacts in the region LEU17ALA21 stabilises an antiparallel arrangement of βstrands. Furthermore there are suggestions that hydrophobic residue, such as LEU17 is important in interactions in

the intersheet direction of abetafibril formation.

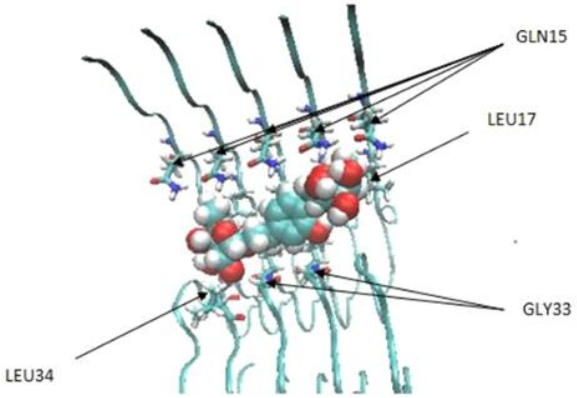

Figure 1. Hydroxyltyrosoldiglucoside interacts with Aβoligomer through glutamine (GLN), leucine (LEU) and glycine residues (GLY).

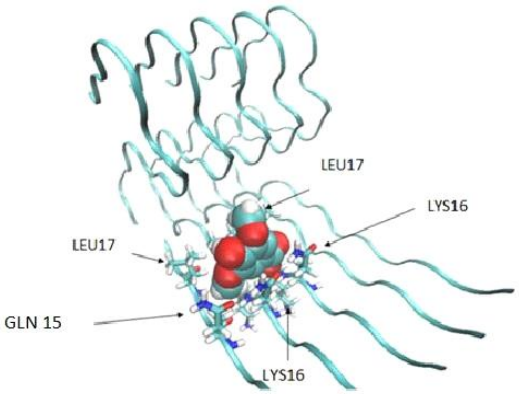

Figure 2. Three dimensional representation of Gentisic acid interacting with residues; leucine (LEU), lysine (LYS) and glutamine (GLN) originating from Aβoligo

Within the three docking servers used; Swissdock, Vina and Hex, Gentisic acid (Figure 2) showed to have the predicted binding affinities of: 6.2, 4.7, 39.43 to Aβ (1142)oligomer. This polyphenol interacted with Aβ oligomer residues; Leucine (LEU17), Lycine (LYS16) and Glutamine (GLN15). Studies have shown that these three residues enhance the fibrillation process of Aβ oligomer. Gentisic acid is a polyphenol composed of three OH groups on the aromatic ring, providing a three-dimensional conformation that is able to interact with amyloidogenic aromatic residues (Garai & Frieden, 2013).

Neonuezhenide

For docking servers Vina, Hex and Swiss Dock, Neonuezhenide attained binding affinity scores of 7.10, 82.93 and 8.87, respectively. As shown in figure 3, LEU17, LEU34, GLN15 and ILE32 residue groups interacted with Aβ (11-42)-oligomer. Isoleucine and leucine contains methyl groups, hydrophobic side chain and would usually been seen buried inside Aβ (1142)-oligomer. With the combination of hydrophobic and electrostatic interactions, it can determine the level

of the fibrils aggregating. The hydrophobicity of the residues is vital as it is a dominant factor in determining the amyloidogenesis of the protein.

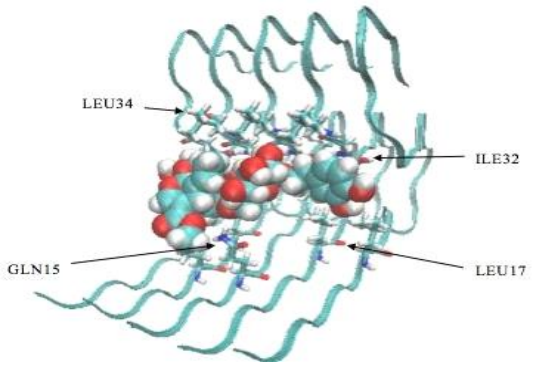

Figure 3. Three dimensional representation of Neonuezhenide interacting with Aβ amyloid oligomer residues; Isoleucine (ILE), leucine (LEU) and glutamine (GLN).

Just like the other residues, glutamine (GLN15) contains hydrogen bonding via its amide group and ester region of Neonuezhenide. A hydrolysis of amide group on glutamine (NH2) and OH group forms glutamine 15 (Schmid et al., 2011). With hydrogen bonding in between side chains and amide chains, poly glutamine can aggregate into beta sheets, which will result to polar zippers. The polar zippers can join specific transcription factors to different DNA segments and this can increase nonspecific affinity of affected proteins. Furthermore glutamine secretase can influence glutamine supply to neurons that can result to failures in the synaptic connectivity and transmission, which will affect brain functions (Nawrot et al., 2013).

Isoverbascoside

Isoverbascoside attained binding affinity scores of 8.75, 8, and 79.11 for Swissdock, Vina and Hex Server, respectively. The interaction between isoverbascoside with Aβ-(1142)-oligomer was represented in VMD as shown in Figure 4. Isoverbascoside interacts with beta amyloid fibril through 4 residues of the protein, leucine (LEU17 and LEU34), isoleucine (ILE32), and glutamine (GLN15). Leucine and isoleucine residues are hydrophobic therefore hydrogen bonds is the major interaction between beta amyloid fibrilisoverbascoside. Glutamine as polar residue also interacts with this ligand through hydrogen bonds by donating and accepting a proton.

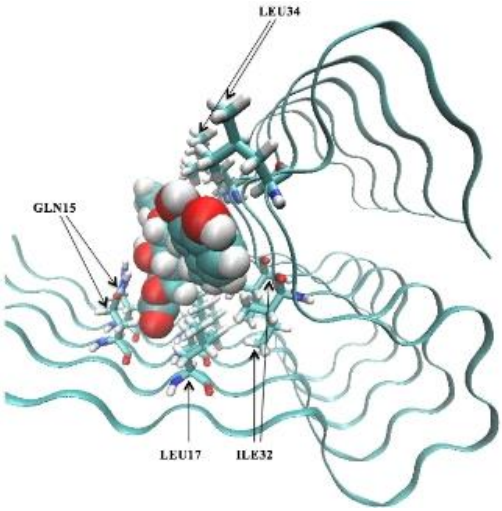

Figure 4. Three dimensional representation of Isoverbascoside interacting with Aβ amyloid oligomer residues; Leucine (LEU), isoleucine (ILE), and glutamine (GLN).

The side chains of ILE32 and LEU34 interaction with the aromatic rings is the most important hydrophobic which forced the layer of β2 sheet to be contorted, and partially opened the tightly compacted structure of the crossβ subunit (Buchete & Hummer, 2007). The compactness of the hydrophobic core comprising the β2 portion of the fibril can be a crucial stabilization element in aggregation and elongation of Aβ aggregates. According to Doig et al. (2002), NmethylLEU34 alter the fibril morphology and resulting in the reduction of the toxicity. Nmethylated derivatives of beta (2535) prevent the aggregation and inhibit the toxicity of the wild-type peptide by blocking hydrogen bonding on the outer edge of the assembling amyloid.

Hesperidin

Hesperidin attained binding affinity scores of 7.7, 79.25 and 8.90 for Vina, Hex Server and Swissdock, respectively. Figure 5 shows a VMD representation of hesperidin interacting with isoleucine, leucine and glycine on the Aβ (1142) oligomer. ILE32 interacts with hesperidin at three distinct locations. The alkyl group of two ILE32 residues can be seen interacting with the disaccharide portion of the ligand (rutinose). That is, the methyl groups of ILE32 interact with rhamnose and glucose, respectively. The third interaction of ILE32 is with the distal benzene ring on the ligand.

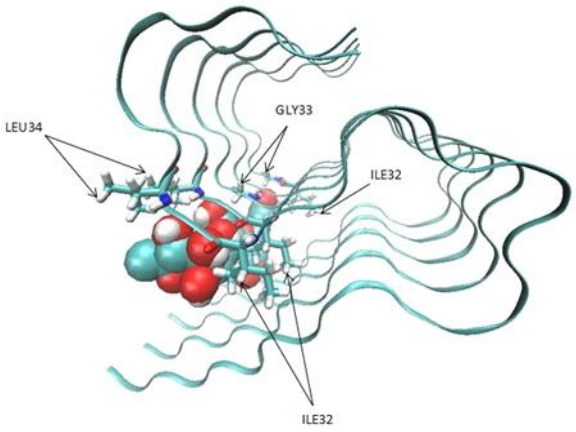

Figure 5. Three dimensional representation of Hesperidin interacting with Aβ amyloid oligomer residues glycine (GLY), leucine (LEU) and isoleucine (ILE).

Glycine (GLY33) interacts with the ligand at two distinct locations; the first is proximal to the disaccharide rutinose and the second is adjacent to the hydroxyl group on the distal benzene ring. GLY33 is a hydrophobic residue in the Aβ (1142) oligomer (Ahmed et al., 2010). Studies of Kanski et al. (2002) suggest that GLY33 may aid in the toxicity of the Aβ (1142) oligomer by contributing free radical propagation processes. Thus, disruption of GLY33 may reduce toxicity.

Ligstroside

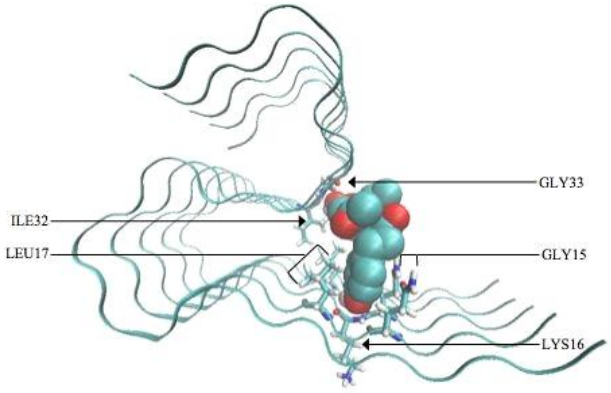

Figure 6 shows Ligstroside residues (GLY15, GLY33, LEU17, ILE32, GLN15 and LYS16) are the residues that interacts with Aβ oligomer. From the three docking servers (Vina, Swissdock and Hex), Ligstroside had binding affinities of 6.8, 8.68 and 75.12, respectively.

LYS16 mainly interacts via hydrogen bonding formation between lysines carboxylic and amine groups with the hydroxyl group attached to the aromatic ring of Ligstroside. ILE32 formed an interaction with Ligstroside via van der waal interactions, the methyl groups of ILE32 interacted with the aromatic, alkane and alkene regions of the ligand. ILE32 and GLY33 interaction has been studied and

there was interruption in the structure of Aβ oligomer, however this interruption cannot disaggregate the structure of Aβ oligomer (Crescenzi et al., 2002).

Structural Analysis

A total of 58 ligands were analysed in the structural analysis. Table 1 lists the ligands and their associated CID. The dendrogram shows that heavier polyphenols with high OH group counts have similar structural activity. Compounds which gave high affinity scores in the docking study displayed a large number of OH groups in their chemical structure and showed similar structural activity. For example high affinity polyphenols such as Hesperidin (CID: 10621), Apigenin6,8diCglycoside (CID: 52808805), Isoverbascoside (CID: 6476333) and Cyanidin3Orutinoside (CID: 29232) showed similar structural activity. Juxtapose, molecules with less to no hydroxyl groups such as 1,3Dimethylol2imidazolidinone (CID: 8704), Cinnamic acid (CID: 44539) and Hydroxytyrosol acetate (CID: 7174906) were clustered distal to the latter compounds; these compounds attained weak affinity scores from all three docking servers.

Table 1. Ligands analysed and their associated CID

CID	Ligand	CID	Ligand
10621	Hesperidin	72	3,4-Dihydroxybenzoic acid
5280443	Apigenin-6,8-di-C-glycoside	547	3,4-Dihydroxyphenylacetic acid
5291488	Luteolin-7-O-glucoside	1738	Homovanillic acid
5319116	Luteolin-4'-O-glucoside	370	Gallic acid
378305	Luteolin 7-O-rutinoside	3469	Gentisitc acid
5748483	Chrysoeriol-7-O-glucoside	10394	Propionic acid
5280805	Rutin	6508	Quinic acid
29232	Cyanidin-3-O-rutinoside	8655	Syringaldehyde
49852298	Luteolin-6-C-glucoside	10742	Syringic acid
25203368	Quercetin-3-O-glucoside	8468	Vanillic acid
442831	Acetoxypinoresinol	8704	1,3-Dimethylol-2-imidazolidinone
3010930	Hydroxypinoresinol	840	6-Deoxyhexopyranose
6476723	Neonuezhenide	6479876	caffeoyl glucuronide
91895359	Nuezhenide	131348	Cornuside
6476722	Oleoside dimethylester	169607	Elenolic acid
5281544	Oleuropein	71718370	Ligstroside aglycone
56842347	Oleuropeinglycon	14136859	Ligstroside
73399	Pinoresinol	89640	Loganic acid
24352	Propiopromazine	5458792	Oleaside A
443023	Syringaresinol	11652416	Oleocanthal
44429837	Acetoside	289	Cathecol
689043	Caffeic acid	16928	Homovanillyl alcohol
1794427	Chlorogenic acid	82755	Hydroxytyrosol
444539	Cinnamic acid	7174906	Hydroxytyrosol acetate
445858	Ferulic acid	N/A	Hydroxytyrosol diglucoside
6476333	Isoverbascoside/isoacteoside	5316821	Hydroxytyrosol glucoside
637542	o-Coumaric acid	N/A	Hyroxytyrosol rhamnoside
637542	p-Coumaric acid	10393	Tyrosol
637775	Sinapic acid	11195402	Tyrosol acetate
5281800	Verbascoside/acteoside	159278	Tyrosol glucoside

Thus, compounds that contain a high number of OH groups may suggest that OH groups are very important in the interaction process with the Aβoligomer and which research shows may prevent protein aggregation (Stefani & Rigacci, 2013). A limitation of this research was the omission of Hydroxytyrosol diglucoside and Hyroxytyrosol rhamnoside in the structural analysis. This was due to a lack of CID number in the PubChem database. However, as a result of the correlation between hydroxyl groups and high affinity, it is expected that both omitted compounds have similar structural activity to those compounds with high affinity scores.

CONCLUSION

Further statistical analysis needs to be conducted to confirm the predictive capabilities of the regression model by testing the model with an independent set of ligands (i.e., predict the binding affinities of the ligands using Mw and compare the results to actual binding affinities for Swissdock). Crossvalidation is a possible method to evaluate the predictive capabilities of the model by separating the available data into two parts. Modeling of the data uses one part only and the model designated for this part is then utilised to predict the values in the other part of the data. This can deduce the predictive accuracy of the model as good models should have high predictive accuracy.

REFERENCES

Achary, P. G. R. 2014. Simplified molecular input line entry systembased optimal descriptors: QSAR modelling for voltagegated potassium channel subunit Kv7. 2. SAR and QSAR in Environmental Research, 25(1), 7390.

Buchete, N. V., & Hummer, G. 2007. Structure and dynamics of parallel βsheets, hydrophobic core, and loops in Alzheimer's Aβ fibrils. Biophysical journal, 92(9), 30323039.

Dallakyan, S., & Olson, A. J. 2015. SmallMolecule Library Screening by Docking with PyRx. Chemical Biology: Methods and Protocols, 243250.

Garai, K., & Frieden, C. 2013. Quantitativ tyye analysis of the time course of AÎ² oligomerization and subsequent growth steps using tetramethylrhodamine-labeled AÎ². Proceedings of The National Academy Of Sciences, 110(9), 3321-3326.

Gauci, A. J., Caruana, M., Giese, A., Scerri, C., & Vassallo, N. 2011. Identification of polyphenolic compounds and black tea extract as potent inhibitors of lipid membrane destabilization by Aβ42 aggregates. Journal of Alzheimer's Disease, 27(4), 767.

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. 2012. Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. J. Cheminformatics, 4(1), 117.

Hilbich, C., KistersWoike, B., Reed, J., Masters, C. L., & Beyreuther, K. 1992. Substitutions of hydrophobic amino acids reduce the amyloidogenicity of Alzheimer's disease βA4 peptides. Journal of molecular biology, 228(2), 460-473.

Huang, X., Cuajungco, M. P., Atwood, C., Moir, R. D., Tanzi, R. E., & Bush, A. I. 2000. Alzheimer's disease, βAmyloid protein and zinc. The Journal of Nutrition, 130(5), 14881492. Retrieved from http://jn.nutrition.org/content/130/5/1488S.full

Jiang, L., Liu, C., Leibly, D., Landau, M., Zhao, M., Hughes, M.P., & Eisenberg, D.S. 2013. Structurebased discovery of fiberbinding compounds that reduce the cytotoxicity of amyloid beta. Elife, 16(2).

Krone, M., Stone, J. E., Ertl, T., & Schulten, K. 2012. Fast visualization of gaussian density surfaces for molecular dynamics and particle system trajectories. EuroVis 2012 Short Papers, 1, 6771

Lundkvist, J., Halldin, M. M., Sandin, J., Nordvall, G., Forsell, P., Svensson, S., Ekstrand, J. 2014. The battle of Alzheimer's disease – the beginning of the future. Unleashing the potential of academic discoveries. Frontiers in Pharmacology, 5(102), 16. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4023025/

Nawrot, M.K., Sykova, E., Chvatal, A., Verkhratsky, A., & Rodriguez, J.J. 2013. Astrocytes and glutamate homeostasis in Alzhemier's disease: a disease in glutamine synthetase, but not in glutamate transporter1, in the prefrontal cortex. ASN Neuro, 5(4).

Singh, M., Areseneault, M., Sanderson, T., Murth, V. & Ramassamy, C. 2008. Challenges for research on polyphenols from foods in alzheimer's disease: bioavailability, metabolism and cellular and molecular mechanisms. J. Agric. Food Chem. 56(13). 48554873

Stefani, M., & Rigacci, S. 2013. Protein folding and aggregation into amyloid: the interference by natural phenolic compounds. Int J Mol Sci, 14(6), 12411-12457.

Wang, J., Bi, W., Cheng, A., Freire, D., Vempati, P., & Zhao, W. 2014. Targeting multiple pathogenic mechanisms with polyphenols for the treatment of Alzheimer's diseaseexperimental approach and therapeutic implications. Front Aging Neuroscience, 6(42)