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EARLY DETECTION OF PROTEIN AGGREGATION IN NEURODEGENERATIVE DISEASE BY ANALYTICAL METHOD

BASTIA S[1], GAWADE V[1*] AND JAGTAP R[2]

1: Department of Quality Assurance, SSR College of Pharmacy, Sayli, Silvassa, U.T of Dadra Nagar Haveli-396230

2: Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, India

3: Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, India

***Corresponding Author: Dr. Vaibhav Gawade: E Mail: gawadevaibhav6060@gmail.com**

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ABSTRACT

Protein mis-folding and aggregation is important in detecting early stage of neurodegenerative disease such as Parkinson and Alzheimer disease. Early detection may help in accurate treatment of the patient. With proper treatment, most individuals with neurodegenerative disease can lead long productive lives.Many a disease are associated with the aggregated protein and this then gets transferred from one region to another leading to lifetime impairment. This review article mainly focuses on the early detection of the accumulated proteins in the brains with the help of analytical techniques. When protein aggregates reach a certain level in the cell, they become toxic. The accumulation of abnormal proteins causes neurons to lose their structure and/or function over time, eventually leading to their death. Prion, Alzheimer's, Parkinson's, and Huntington's diseases are only a few of the diseases linked to protein aggregation. As a result, dysmnesia, mental retardation, and apathy are common. Much quantitative analytical technique can be used to detect aggregated protein by different mechanisms based on the methods used. Therefore, a basic understanding on neurodegenerative diseases which are caused due to aggregation of different types of protein in the brain can now be identified by analytical techniques in prior so that they can be treated

and it won't spread. So, we have covered all the analytical techniques which can be used in this field by determining whether he/she is suffering from neurodegenerative disease so that they can be treated on time.

Keyword: Neurodegenerative disease, dysmnesia, analytical, protein, aggregation

INTRODUCTION

Neurodegenerative disease is age-dependent hereditary or sporadic condition which is recognized with progressive damage to the structural and functional system of neurons and can further lead to neuron death **[1]**. It is the main reason of debilitating and incurable disease which occurs when a particular part of the brain and spines is dealing with neuronal loss **[2]**. This can lead to many common and uncommon disease like Alzheimer disease, Parkinson disease, Prion disease,Huntington disease, Multiple atrophy, Fronto-temporal dementia **[3]**. This disease is manifest dementia resulting in memory loss, multiple cognitive abnormalities and intellectually disability that affects the patient's way of life **[4]**. Many other disease is connected with specific aggregated protein **[5]**. The accumulated protein can be alpha-synuclein, amyloid β, huntingtin, TDP-43, or tau **[2]**. This aggregated protein then gets transmitted from one specific region to another in a familiar pattern inside the anatomic region which are connected to each other **[3]**. The spreading of tau protein is of two typest-tau, p-tau. They both are of dissimilar shapes in both the frontal and temporal cortex [6]. However, they also get accumulated in some different areas of the brain like basal ganglia and sub-thalamus **[7]**. The Amyloid β gets accumulated in the brain from a decade before the patient will reach to dementia stage of neurodegenerative disease **[8]**. The Alphasynuclein being a non-β amyloid protein for a neurodegenerative disease that gets accumulated pre-synoptically in the brain that are responsible for interruption of chemical release among neurons **[9, 10]**. Normally 90% TDP-43 proteins are corely localised predominately in nucleus and some of them shutters to cytoplasm which help to regulate mRNA processing, stability and miRNA biogenesis, biologically process its own translocation and expression. In abnormal condition this protein mis localised to cytoplasm **[11, 12]**. This review article mainly focusses on the early detection of the accumulated proteins in the brains by analytical techniques like UV spectrophotometer, Fluorimetry and SPE-LC-MS/MS**.** There are many Analytical methods to analyse protein aggregates depending on different separation and detection method such as SDS-PAGE, capillary electrophoresis, field

flow fraction (e.g., AF4), microscopic methods (e.g., Light, electron atomic force microscopy), static light scattering, dynamic light scattering, analytical ultracentrifugation, light obscuration, coulter counter, UV-Vis spectroscopy, turbidity/ opalescence/clarity (visually or instrumentally), circular dichroism, fluorescence spectroscopy, FTIR, immunochemical method, ultrastructural imaging method, dyes and fluorescent process, NMR, turbidity and light scattering, ion-mass spectrometry, size exclusion chromatography, electrophoresis, except amyloid configuration that are insoluble which cannot be detected by direct analysis **[13]**.

Table 1: Diseases related with aggregating protein

Diseases	Aggregating protein
Alzheimer Disease	Amyloid β peptide, Tau protein
Parkinson Disease	Alpha-synuclein
Huntington's Disease	Huntingtin
Frontotemporal Dementia	Tau protein
Prion Disease	Prion protein
Serpinopathies	Neuroserpin
Familial amyloidotic polyneuropathy	Transthyretin apolipoprotein AI, gelsolin

Analytical methods used in early detection of protein aggregation in neuro-degenerative disease

Size exclusion chromatography:

It refers asa type of quantitative analytical technique in which the aggregated protein is detected by simple principle of molecular sieving or chromatography of gel permeation. Grant Henry Lathe and Colin R Ruthven developed these techniques. As per the matrix starch gels are usedby Ruthven and Lathe, Jerker Porath along with Per Flodin after used dextran gels as stationary phase. Here the column is bound with micro sized stationary phase particle which also consist of small pores, where the smaller sized proteins get stucked in between the pores and the larger protein gets elute out faster with the mobile phase The eluent fraction is further collected and examined by spectroscopic techniques **[14]**. Aggregation number of the protein can be analysed by attaching it with refractive index, quasielastic light scattering spectroscopy, multiangle static light scattering and electron microscopy **[15]**. SER-LC/UV/RI is the fast, reliable and accurate method to control the molecular weight and stoichiometry of protein as well as the reduced RNase **[16]**. Thomas J. Esparza reported that SEC was used to determine the size of the amyloid β aggregated by injecting 1 mL of aggregate in supradex 200 10/300 GL column using mobile phase as PBS containing 0.05% BSA filtered solution at 0.5 mL/min flow rate along with 23 elution fraction was collected and analysed **[17]**. T yang and *et*

al reported that SEC was used to show that larger soluble oligomer of Amyloid β protein in Alzheimer patient's brain are less neuro active than smaller oligomer by using superdex 75 column at 0.8 ml/min rate of flow with mobile level of 50 mM ammonium acetate pH 8.5 by size exclusion chromatography **[18]**. Anujkumar Das reported a developed method for quantifying proteins that interacts with neurotoxic forms of Aβ using Superdex 75 10/300 column with mobile phase 20 mM tris-hydrochloride, pH 8.0 200 mM sodium chloride at 0.7-0.9 ml/min flow rate at 280 nm detected by Nanodrop 1000 spectrophotometer (Thermo scientific) **[19]**.

Electrophoresis:

Electrophoresis is a method by that energized protein molecules are actually moved by way of a solvent by an electric area. Proteins may be divided by electrophoresis that is actually a delicate analytical application. Nearly all Protein has a net cost at any pH apart from the isoelectric thing of theirs and then migrate based on the charge density of theirs. The coming factors define the dependency of a protein's mobility: strength in field, net cost of proteins, protein's shape and dimension form, ionic toughness, as well as the matrix qualities over what the proteins migrates (like as, pore size, viscosity) **[20]**. Agarose as well as polyacrylamide are 2 assistance matrices widely utilized in electrophoresis. Given matrices work as permeable press along with act similar to a molecular filter. Agarose features a pore of big external, so it is ideal in sorting nucleic acids as well as big complexes of proteins. Polyacrylamide features a minor pore size also it is perfect for sorting out vast majority of proteins and smaller nucleic acids. A number of polyacrylamide gel electrophoresis types of (PAGE) are present, also every type is able to offer various information types about proteins of decreasing sodium and interest **[21]**. Denaturing dodecyl sulfate (SDS) PAGE by means of a process of irregular buffer is actually by far the utmost popular electrophoresis strategy along with splits proteins chiefly through mass. Non-denaturing PAGE, likewise, known as indigenous PAGE, splits proteins based on the ratio of mass/charge theirs. 2-D PAGE splits proteins through indigenous isoelectric thing under the very initial dimension as well as mass in the next capacity. SDS-PAGE splits proteins chiefly via mass as the detergent of ionic extent SDS denatures, also fixes to proteins to cause them to become evenly adversely exciting **[22]**. As soon as divided using electrophoresis, proteins may be recognized in a gel through different spots, shiftedupon a detection membrane by western blotting in addition also eliminated as well as mined for the evaluation through mass

spectrometry. Proteins gel electrophoresis is actually, consequently, an essential stage in most types of proteomics evaluation **[23]**.

Analytical Ultracentrifugation:

Analytical ultracentrifugation is a versatile tool used for protein analysis. Schachman 1992, reported analytical ultracentrifugation for characterisation of protein in history **[25, 26, 27]**. Two ultracentrifuse, XLA & XLI are introduced by Beckman-coulter instruments which has integrated absorbance & inference optics. The inference optics has a lot of advantage in protein analysis such as change in protein conformation and aggregation can be analysed without worrying that the UV absorbance will hinder the protein absorbance **[28, 29]**. Kelly K. Arthur has reported the protein detection aggregated through analytical ultracentrifugation of sedimentation velocity. In this stock solution of dimer and monomer were combined to get 3 % dimer in total 0.5 mg/ml mAb concentration. 20 mM sodium phosphate buffer was utilized as diluent. The buffer of this type was used to achieve perfect sedimentation at mAb concentration run was carried out at Beckman XLI analytical centrifuge at 280 nm. Rotor speed was 40000 rpm along with run temperature 20ºC. The result was defined to contain 96.5%monomer and 3.5% dimer. As per monomer the molecular weight was 150 kDa of with sedimentation coefficient 6.5 S and the dimer'smolecular weight was 300 kDa and sedimentation constant was 9.5S **[30]**.

Ion Exchange Chromatography:

The chromatography exchange ofion was developed in 1960 for charged biomolecules such as proteins, nucleic acid, peptides etc. This is highly selective separating technique based on charged amino acid. Ion exchange chromatography are of two types: In cation exchanger the positively exciting protein binds to adversely charged resin while in anion exchanger the negative ions binds to immobilized functional group with positive charge. Neutral proteins which are not charged or have same charge as that of the medium are not attracted and are easily washed. The bounded protein can be extracted by eluting by increasing the buffer concentration between protein and ion exchange resin **[31]**.

Dyes And Extrinsic Fluorescent Probes:

A young German chemist Paul Bottiger invented congo red in 1884 **[32]**. A renowned stain congo red is utilized to check protein deposit presence such as amyloid protein in tissue and helps in investigating neurological since last 100 yrs. The stained dye reveals the fibril protein constituent under electron microscopy. Firstly, Congo red was used to observe fall in casein brought general

amyloid plaque in mice with dye treatment. Sometimes the congo red is used to prevent amyloid β peptide accumulation along with its neurotoxic influence **[33]**. Congo red binds to β sheet in amyloid fibrils that cause characteristic shift in absorbance from 490-540 nm but it was not observed for prefibrillar. ThT is also used to detect the amyloid fibrils by binding to prefibrillar aggregates that has β sheet binding site. The β sheet structure has less binding site than that of fibrils so fluorescence is less but observable. ThT can easily indicate the toxic protofibril and fibrillar but cannot detect pre fibrillary oligomer that does not have β sheet structure **[34]**.

Ultrastructural Imagining Methods:

The methods of high-resolution ultra-structural like as electron microscopy (EM) along withatomic force microscopy (AFM) are intended for the detection of insoluble amyloid proteins. This method focuses on the morphological or structural base determination of proteins. The first-ever structural demonstration method used to detect amyloid protein was electron microscopy **[35]**. It determined the presence of amyloid protein by its long, unbranched fibrils like structure under the microscope **[36]**. The method is based on the examination of aggregated protein species such as prefibrillar and fibrillar. High-voltage concentrated electron beams utilized in this method, which is allowed to pass over the sampling in vacuoas well as the results to produce an image depending on scattering of the electron beam. The use of heavy metals for negative staining likeas to the uranyl acetate and its application through evaporation to the specimen's coveringi.e. rotary shadowing helps to enhance imaging of proteins. To determine specific epitopes, EM systemsmight be shared with antibodies that are categorized by gold particles **[37]**. Whereas this negative staining technique has its own limitations of resolution that is 25 Å.[4] The cryo-EM are used for examination of unstained and unfixed proteins, in such cases staining protocols such as pH, ionic strength, dehydration, high vacuum, intense radiation are also considered for sensitive biological samples **[38, 39]**. Also, it uses a low-to-moderate radiation dose for the determination of proteins which are preserved at -180 °C. This technique also used in the detection of amyloid fibrils of Aβ, α SN, yeast prion, along with the protein polymers in serpinopathies **[40]**. The primary non amyloid collective structures, e.g., αSN is possible to detect by combining EM with AFM **[41]**. A sub nanometer 100kV electron beam is used to scan the specimen of protein in an organized manner, that help in the look over of protein by EM with systematic abilities. The resulting distributed electron is verified for its loss of energy which is

directly related to the particular exposed irradiated state mass **[42]**. This helps in the qualitative and quantitative determination of proteins. The 3D-structure's conception of a protein tester is attained through atomic force microscopy (AFM). The surface area of a sample is actually scanned sometimes by constantly in touch (constant mode) or perhaps as an oscillating probe (non-contact or perhaps tapping mode) with the nanometer radius of-curvature-sized impression assistance below the control of piezoelectrical. Rather than the buffer solution tapping mode is quite suitable for the protein testers **[42]**. The width of the aggregated protein specimen is been detected by EM whereas the height is been detected by AFM data. It is promising to revise the growth and morphology of nanostructures, aggregates, fibrils, and protofibrils, e.g., amyloid β by time-lapse studies, whereas the structural detection is been possible due to sub-nanometer resolutions. Compare to TEM and SEM, the AFM has a limited scan area and a slow scan speed i.e. several seconds vs minutes. It may similarly result in some nonrepresentative ultrastructural images if the number of exposures of detections is less.

Immunochemical Method:

Antibodies are widely used to quantitate and detect protein conformation relevance to neurodegenerative disease. The monoclonal antibody devises a single site (epitope) overthe protein along with that the identical monoclonal antibody is utilised mutually for capture as well as the protein's detection. Consequently, merely dimers or advanced order oligomers would show sufficient sites of binding to be apprehended as well as perceived. Developed order oligomers would produce equivalently durable signals which also rise with the concentration of protein oligomer **[43]**. Advantage of using this method is high specificity as 3 Aβ antibody (one for capture) and two for detection. Therefore it is observed that cerebrospinal fluid of Alzheimer patient has high number of Aβ aggregates than normal patient **[44]**. This method is also used to visualise αSN aggregates **[45]**. Detection is done by confocal laser or fluorescence spectroscopy. The antibodies additionally a quite valuable tool for isolating protein complex For eg. Characterisation of Aβ & αSN from cerebrospinal fluid **[46, 47]**.

Mass Spectrometry And Ion Mobility Mass Spectometry

In the molecules of mass spectrometry (MS) are actually divided as molecular ions under gas stage are built on mass-to-charge (m/z) proportions of their. The illustrations are possibly ionized as well as volatilized through laser which is matrix aided beam samples desorption ionization (MALDI) co-crystallized with molecules of matrix on

a goal metal plate or perhaps through the liquid sample's electrospray ionization (ESI) from direct injection or perhaps as a result of an internet separating feature. MS is thoroughly utilized to characterize as well as keep track of the ionisable development of non-covalent oligomers along with combinations in vitro & yields accurate details on cumulative stoichiometry along with distribution of mass **[48, 49]**. Moreover; MS may be utilized for the post translational modifications mapping, where also for semiquantitative protein's characterization in complicated biological fluids. MS has an extremely high perception along with demands very little content (down to a couple of microliters during the concentration of nanomolar). Desorption of laser or maybe the ejection of area layer through beams of clustered ion (the latter the majority of helpful for molecules <2000 molecular mass) might additionally be carried out straight on the tissues (cells imaging MS or perhaps cells imaging secondary ion MS)[50,51]comprising samples of neuro-pathological that contain A build up. Nevertheless, the evaluation calls for molecules in answer which might present a dilemma mean while to the aggregate of amyloidogenic proteins along with impulsive out of alternative during time. By MALDIMS it's doable to learn insoluble species though the given particular method isn't immediately suitable with internet powerful techniques for learning the folding intermediates growth. The procedure for ventilation out down a model in a blend with an optional matrix of organic and natural acids additionally is susceptible to interrupt noncovalent centres. Last but not least, MALDI generally produces monovalent ions ensuing in wide, inaccurately definite mass peaks to the enormous proteins although the multivalent ions produced by ESI outcome in far additional accurate mass assignments **[49]**. In ESI the evaluation of cost declare division allows the estimation of the public of different conformational states in a certain proteins as proven for monomeric SN conformers **[50]**. Now, MS was coupled over a priorphase of electrophoretic separating stage gas in a blended strategy known as gas phase electrophoretic molecular evaluation or maybe ion mobility spectrometry MS (IMS MS).At this point, the ESI created ions are actually separated and charge-reduction based on the dimensions of theirs reliant electrophoretic mobility of atmosphere or maybe a gas like helium **[51]**.

Nuclear Magnetic Resonance

NMR was discovered in 1945 is one of the most valuable method for elucidating the protein conformation hence can be used as one of the method for early detection of neurodegenerative disease by analysing

aggregated protein's conformation **[52]**. NMR is used to study the intramolecular dynamics in macromolecules like protein and nucleic acid and their reaction kinetics and protein foldings. When the sample is radiated the energy level gets excited which leads to transition in energy level where the energy difference is equivalent to frequency. This works on the principal that certain atomic nuclei are intrinsically magnetic in nature. Due to which the energy level get splitted by this magnetic field which leads to spinning of proton. For further development of NMR pulsed fourier transform NMR and multi-dimentional NMR spectroscopy is used **[53]**. The unique fingerprints of protein delivered by the NMR spectra recommend chemical shift inheritantly carry enough information to regulate at high resolution **[54]**. The structural informational in the chemical shift is different from that of the NoE. Heteronuclei are immersed in the protein solution by isotopic labelling to allow fast prepation of sample for analysis. Till now proteins upto 30 kDa can be determined. Recent advancement in analysis in chemical shifts have allowed their value to be utilized to attain information about number of definite types of protein conformations as they use dihydral angle but they cannot match the standard of high resolution structure of protein obtained by NoE and RDC **[54, 56]**.

CONCLUSION

This review article mainly focuses on the early detection of the accumulated proteins in the brains by analytical techniques like UV spectrophotometer, Flourimetry and SPE-LC-MS/MS

There are many Analytical methods to analyse protein aggregates depending on different separation and detection method such as SDS-PAGE, capillary electrophoresis, field flow fraction (e.g., AF4), microscopic methods (e.g., Light, electron atomic force microscopy), static light scattering, dynamic light scattering, analytical ultracentrifugation, light obscuration, coulter counter, UV-Vis spectroscopy, turbidity/ opalescence/clarity (visually or instrumentally), circular dichroism, fluorescence spectroscopy, FTIR , immunochemical method, ultrastructural imaging method, dyes and fluorescent process, NMR, turbidity and light scattering, ion-mass spectrometry, size exclusion chromatography, electrophoresis, except amyloid configuration that are insoluble which cannot be detected by direct analysis

REFERENCES

[1] Magalingam, KB., Radhakrishnan, A., Ping, NS., Haleagrahara, N. *Current concepts of neurodegenerative mechanisms in Alzheimer's disease*. BioMed research international. 2018.**4**(11):p.175-180

[2] Yacoubian, TA. *Neurodegenerative disorders: Why do we need new therapies?* drug discovery approaches for the treatment of neurodegenerative disorders, Academic Press, 2017.**1**(8): p.1-16.

[3] Davis, AA., Leyns, CE., Holtzman, DM. *The intercellular spread of protein aggregates in neurodegenerative disease*. Annual review of cell and developmental biology. 2018.**6**(34):p.545-68.

[4] Gitler, AD., Dhillon,P., Shorter, J. Neurodegenerative disease: models, mechanisms, and new hope, Disease models and mechanism, 2017.**10**(5): p.499–502.

[5] Pedersen, JT., Heegaard, NH. *Analysis of protein aggregation in neurodegenerative disease*. Anal. Chem. 2013.**85**(9): p.4215–4227

[6] Mukaetova-Ladinska EB, Abdel-All Z, Mugica ES, Li M, Craggs LJ, Oakley AE, Honer WG, Kalaria RN. Tau proteins in the temporal and frontal cortices in patients with vascular dementia. Journal of Neuropathology & Experimental Neurology. 2015 Feb 1; 74(2):148-57.

[7] Wolfe MS. The role of tau in neurodegenerative diseases and its potential as a therapeutic target. Scientifica. 2012; 2012:p.1-20

[8] Palmqvist, S., Schöll,M., Strandberg, O., Mattsson,N. Earliest accumulation of β-amyloid occurs within the default-mode network and concurrently affects brain connectivity. Nature communications. 2017. 8(1): 1214-1228.

[9] Bendor, JT., Logan,TP. The function of α-synuclein. Neuron. 2013. 79(6): p. 1044-66.

[10] Polymeropoulos, MH., Lavedan,C., Leroy,E., Ide SE. Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science. 1997. 276(5321): 2045-7.

[11] Hans, F., Eckert, M., Zweydorf, F., Gloeckner, CJ., Kahle, PJ. Identification and characterization of ubiquitinylation sites in the TAR DNA-binding protein of 43 kDa (TDP-43). Journal of Biological Chemistry, 2018.293(41):16083-99.

[12] Wang, IF., Wu, LS., Shen, CJ. TDP-43: an emerging new player in neurodegenerative diseases. Trends in molecular medicine. 2008. 14(11): p. 479-85.

[13] Mahler HC, Friess W, Grauschopf U, Kiese S. *Protein aggregation: pathways, induction factors and analysis*. Journal of pharmaceutical sciences. 2009. 98(9):p.2909-34.

[14] Hong, P., Koza,S., Bouvier, ES. *A review size-exclusion*

chromatography for the analysis of protein biotherapeutics and their aggregates. Journal of liquid chromatography & related technologies. 2012.**35**(20):p.2923-50.

[15] Nagy, K., Vékey, K. Separation methods. In Medical Applications of Mass Spectrometry, Elsevier 2008.**31**(7): p.61-92.

[16] Walsh, DM., Lomakin, A., Benedek, GB. *Amyloid β-protein fibrillogenesis detection of a protofibrillar intermediate*. Journal of Biological Chemistry, 1997. **272**(35): p.22364-72.

[17] Wen, J., Arakawa, T., Philo, JS. *Size-exclusion chromatography with on-line light-scattering, absorbance, and refractive index detectors for studying proteins and their interactions*. Analytical biochemistry. 1996. **240**(2): p. 155-66.

[18] Laganowsky, A., Reading, E., Hopper, JT. Mass spectrometry of intact membrane protein complexes. Nature protocols. 2013.8(4): p.639-647.

[19] Yang,T., Li, S., Xu,H., Walsh,DM., Selkoe, DJ. L*arge soluble oligomers of amyloid β-protein from Alzheimer's brain are far less neuroactive than the smaller oligomers to which they dissociate*. Journal of Neuroscience. 2017.**37**(1):p.152-63.

[20] Laganowsky, A., Reading, E., Hopper, JT. Mass spectrometry of intact membrane protein complexes. Nature protocols. 2013.8(4):639.

[21] O'Malley, TT., Linse, S., Walsh, DM. Production and Use of Recombinant Aβ for Aggregation Studies. Methods Mol Biol, 2018.**1777**: p.307–320

[22] Coligan, J.E. Electrophoresis, *In Current Protocols in Protein Science*, John Wiley and Sons, Inc. New York 2002:p10.0.1-10.4.36

[23] Bollag, D.M., Rozycki, M.D. and Edelstein, S.J. Protein Methods,. Wiley-Liss, Inc. New York. 2002

[24] Hames, BD., Rickwood, D. *Gel Electrophoresis of Proteins: a Practical Approach*, Oxford University Press, New York. 1990 2nd edition.

[25] Cubillos-Rojas M, Schneider T, Sánchez-Tena S, Bartrons R, Ventura F, Rosa JL. Analysis of Protein Oligomerization by Electrophoresis. In Proteostasis. Humana Press, New York, NY. 2016: p.341-348

[26] Cantor, CR., Schimmel, PR.: *Techniques for the Study of Biological Structure and Function*, Biophysical Chemistry. Part II, W. H.

Freeman and Company: New York, 1980,1st edition.

[27] Brown, P.H., Balbo, A., Schuck, P. Characterizing Protein-Protein Interactions by Sedimentation Velocity Analytical Ultracentrifugation, Current Protocols in Immunology; John Wiley and Sons: New York, 2008, **81**:p.1−18.

[28] Cole, JL., Lary, JW., Moody TP, Laue TM. Analytical ultra-centrifugation: sedimentation velocity and sedimentation equilibrium. Methods in cell biology. 2008. 84: 143-79.

[29] Lebowitz, J., Lewis, MS., Schuck, P. Modern analytical ultracentrifugation in protein science: a tutorial review. Protein science. 2002, 11(9): p. 2067-79.

[30] Arthur, KK., Gabrielson, JP., Kendrick, BS., Stoner, MR. Detection of protein aggregates by sedimentation velocity analytical ultracentrifugation (SV-AUC): Sources of variability and their relative importance. Journal of pharmaceutical sciences. 2009. 98(10): p. 3522-39.

[31] Jadaun, GP ., Dixit,S., Saklani,V. HPLC for Peptides and Proteins: Principles, Methods and Applications. Pharmaceutical Methods. 2017, 8(1): p.14-18.

[32] Yakupova, EI., Bobyleva, LG., Vikhlyantsev, IM., Bobylev, AG. Congo Red and amyloids: history and relationship. Bioscience reports. 2019.39(1):BSR20181415.

[33] Frid, P., Anisimov, SV., Popovic, N. *Congo red and protein aggregation in neurodegenerative diseases*. Brain research reviews. 2007.**53**(1):135-60.

[34] Gregoire, S., Irwin, J., Kwon, I. Techniques for monitoring protein misfolding and aggregation in vitro and in living cells. Korean Journal of Chemical Engineering. 2012.29(6):p.693-702.

[35] Bladen, HA., Nylen,M.U., Glenner, GJ. *Theoretical study of octocalcium phosphate-hydroxyapatite (OCP-HA) interface model*, Ultrastruct.Res. 1966.**14**:p.449−459.

[36] Cohen, A. S., Calkins, E. The Amyloid State of Proteins in Human Diseases Nature 1959, **183**:p.1202−1203.

[37] Robinson, J. L., Geser, F., Stieber, A., Umoh, M., Kwong, L. K., Van Deerlin, V. M.; Lee, V. M., Trojanowski, J. Q. Acta Neuropathol. 2013.**125**: p.121−131.

[38] Lashuel, HA., Wall, JS. Methods for Studying and Structure–Function Relationships of Non-Fibrillar Protein Assemblies in Alzheimer's Disease

and Related Disorders Methods Mol. Biol. 2005, **299**:p.81−101

[39] Li, H., Rahimi, F., Sinha, S., Maiti, P. Amyloids and Protein Aggregation−Analytical Methods. In Encyclopedia of Analytical Chemistry; Meyers, R. A., Ed.; John Wiley & Sons Ltd.: Chichester, U.K., 2009: p.1−32.

[40] Schmidt, M., Sachse, C., Richter, W., Xu, C. Comparison of Alzheimer Aβ(1–40) and Aβ(1–42) amyloid fibrils reveals similar protofilament structures Proc. Natl. Acad. Sci. U.S.A. 2009, 106 (47), 19813−19818.

[41] Saibil,HR., Seybert, A., Habermann, A., Winkler, J. Solid-State NMR Studies of Amyloid Materials: A Protocol to Define an Atomic Model of Aβ (1–42) in Amyloid Fibrils Proc. Natl. Acad. Sci. U.S.A. 2012, **109** (37):p. 14906−14911.

[42] Meinhardt, J., Sachse, C., Hortschansky, P., Grigorieff, N., Fandrich, M. J. *Globular Tetramers of β2-Microglobulin Assemble into Elaborate Amyloid Fibrils*, Mol. Biol. 2009, **386** (3):p.869−877.

[43] Quintero-Ronderos,P., Arango, MT., Castiblanco, J., Correa, NE., Montoya-Ortíz G. Analysis of proteins and antibodies. In Autoimmunity: From Bench to Bedside. El Rosario University Press. 2013

[44] Funke, S. A., Birkmann, E.,Henke, F., Gortz, P. Biochem. Biophys. Evaluating Amyloid-β Oligomers in Cerebrospinal Fluid as a Biomarker for Alzheimer's Disease Res. Commun. 2007, **364** (4):p.902−907.

[45] Hubinger, S., Bannach, O., Funke, S. A., Willbold, D., Birkmann, E. *Detection of α-synuclein aggregates by fluorescence microscopy.* Rejuvenation. Res. 2012.**15** (2):p.213−216.

[46] Portelius, E., Brinkmalm, G., Tran, A., Andreasson, U. *Full-length and C-terminal neurogranin in Alzheimer's disease cerebrospinal fluid analyzed by novel ultrasensitive immunoassays*, Exp. Neurol. 2010.**223** (2):p.351−358.

[47] Ohrfelt, A., Zetterberg, H., Andersson, K., Persson, R. *Identification of Novel α-Synuclein Isoforms in Human Brain Tissue by using an Online Nano LC-ESI-FTICR-MS Method*, Neurochem. Res. 2011.**36** (11):p.2029−2042.

[48] Morris CB. Molecular Descriptor on the Basis of Structural Analysis in Ion Mobility-Mass Spectrometry (Doctoral dissertation, Vanderbilt University). 2019

[49] Heck, A. J., Heuvel, R.H. Investigation of intact protein complexes bymass spectrometryMass Spectrom. Rev. 2004.23 (5):p.368−389.

[50] Caprioli, R. M.; Farmer, T. B.; Gile, J. *MALDI Mass Spectrometry Imaging in Microscope Mode with Infrared Lasers: Bypassing the Diffraction Limits*, Anal. Chem. 1997.**69** (23):p.4751−4760.

[51] Luxembourg,SL., Mize, TH.; McDonnell, LA. *Imaging of Peptides in the Rat Brain Using MALDI-FTICR Mass Spectrometry ,* Anal. Chem.2004. **76** (18):p.5339−5344.

[52] Seng CO. *Studies on the Three-dimensional Structures of Proteins Using X-ray Crystallography* (Doctoral dissertation, Arizona State University) 2017.

[53] Poulsen, FM. *A brief introduction to NMR spectroscopy of proteins*.2019

[54] Gronwald, W., Kalbitzer, HR. *Automated structure determination of proteins by NMR spectroscop*y. Progress in Nuclear Magnetic Resonance Spectroscopy. 2004, **44**(1-2):p.33-96.

[55] Cavalli, A., Salvatella, X., Dobson.CM. *Protein structure determination from NMR chemical shifts*. Proceedings of the National Academy of Sciences. 2007. **104**(23): p.9615-20.

[56] Van der Wel PC. *Insights into protein misfolding and aggregation enabled by solid-state NMR spectroscopy*. Solid state nuclear magnetic resonance. 2017.**88**(6):p.1-4.