Prospective Detection Methods for Tau Protein:

An Important Biomarker for Alzheimer's disease

A project submitted by

Sumaiya Afra

ID: 17146058

To The Department of Pharmacy

In partial fulfillment of the requirements of the degree of

Bachelor of Pharmacy (Hons.)

November 2021

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help

Student's Full Name & Signature:

Sumaiya Afra
17146058

Approval

The thesis titled “Prospective Detection Methods for Tau Protein: An Important Biomarker of Alzheimer’s Disease” submitted by Sumaiya Afra (17146058) of Spring 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 22.11.2021.

Examining Committee:

Supervisor:

_____

Professor Dr. Hasina Yasmin
Deputy Chair, Department of Pharmacy
Brac University

Program Coordinator:

_____

Dr. Zara Sheikh
Program Coordinator, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

_____

Professor Dr. Eva Rahman Kabir
Chairperson, Department of Pharmacy
Brac University

Ethics Statement

This study does not involve any kind of animal or human trial.

Abstract

Tau protein is one of the keystones in the diagnosis of Alzheimer's disease. The modern detection techniques have come up to visualize, track and assess tau pathology and have further contributed with information about tau deposition in human brain. The commercially available techniques include PET scan, digitalized enzyme-linked immunosorbent assay, Biolayer interferometry. However, each of the techniques has its own limitations such as tracers binding to off target, higher chances of cross reaction. This report has detailed the information about the active field of ongoing research methods to detect tau protein such as Surface plasmon resonance, Quartz crystal microbalance, Surface-enhanced Raman scattering based sandwich assay along with their selectivity. Hence, all of these techniques can be used to detect the inhibitors that target tau protein deposits or phosphorylates.

Keywords: Alzheimer's disease; Tau protein; Surface plasmon resonance; enzyme-linked immunosorbent assay; Quartz crystal microbalance.

Dedication

Dedicated to my parents, who have sacrificed their worldly happiness in fulfilling my ones to their best and to my beloved sibling and friends and my project supervisor, Professor Dr. Hasina Yasmin.

Acknowledgement

I am grateful to the Almighty for giving me the strength to stay on course with determinations through all the hardships. It is only possible with His blessings and mercy that I am able to have come this far today.

Secondly, I would like to express my special appreciation and gratitude to my supervisor, Professor Dr. Hasina Yasmin, for her guidance from day one of my Bachelor's program. It would not have been possible to complete this paper without her suggestions and generous support.

I am immensely grateful to Professor Dr. Eva Rahman Kabir, (Chairperson, Department of Pharmacy, Brac University) who has been very kind to give her time and valuable suggestions that led to the different guidelines for conducting this study.

Finally, I would like to express my gratitude to my parents who keep inspiring me to go beyond my limits. I would not have come this far without their constant prayers and unconditional love. I would also like to thank my beloved friends and siblings and all the people who, whenever needed, have helped me with their utmost abilities.

Table of Contents

Declaration ii
Approval iii
Ethics Statement iv
Abstract v
Dedication vi
Acknowledgements vii
List of Figures x
List of acronyms xii
Chapter1: Introduction 1
1.1 Alzheimer Disease 1
1.2 Tau protein 4
1.3 Role of Tau protein in AD 5
1.4 Detection of Tau Protein 6
1.5 Purpose of the study 6
1.6 Aim of the study 7
Chapter 2: Tau Protein 8
2.1 Tau Protein 8
2.1.1 Structure 9
2.1.2 Classification 9
2.1.3 Distribution of Tau 11
2.2 Tau Protein in AD 11
2.2.1 Hyper phosphorylation of Tau Protein 13
2.2.2 Acetylation of Tau Protein 15
2.2.3 Self Aggregation of Tau Protein 15
Chapter 3: Current detection methods for Tau protein 17
3.1 Introduction 17
3.2 Positron emission tomography (PET) 17

3.2.1 Mechanism of action of PET tracer 18
[18F]FDDNP 19
[11C]PBB3 19
Quinoline Derivatives 20
Tauvid 21
3.3 Digitalized ELISA 23
3.3.1 Mechanism 23
3.3.2 Single molecule array Simoa™ 25
3.3.3 Sensitivity and specificity of the technique: 25
3.4 Biolayer Interferometry (BLI) 26
3.4.1 Detection of tau441 protein by the BLI biosensor 27
3.4.2 Selectivity of BLI biosensor 29
Chapter 4: Ongoing research on new detection methods for tau protein 30
4.1 Introduction 30
4.2 Surface Plasmon Resonance (SPR) 30
4.2.1 Mechanism of SPR assay: 31
4.2.2 Advantages of SPR technique 34
4.3 Quartz Crystal Microbalance (QCM) 35
4.3.1 Detection of Tau protein 37
4.3.2 Sensitivity of QCM 39
4.4 Surface-enhanced Raman scattering (SERS) based sandwich assay 39
4.4.1 Detection of Tau protein 40
4.4.2 Specificity & sensitivity of SERS: 42
Chapter 5: Conclusion and Future Prospects 44
References 46

List of Figures

Figure 1: Approximated life risk of AD at age 45 and 65 (Vermunt et al., 2019)..... 2
Figure 2: Alzheimer's disease continuum (Vermunt et al., 2019)...... 3
Figure 3: Number and age of people 65 or older with Alzheimer's dementia in 2020 (Hebert et al., 2013)...... 4
Figure 4: Tubulin binding domains (blue) used to hold microtubule with tau. Interaction of tau molecule with microtubule and function of axonal transport can be controlled by phosphorylation (pink) (Barbier et al., 2019)...... 5
Figure 5: (A) Tau eases microtubule stabilization within the cells (B) Tau's role is depleted in patients with Alzheimer's disease (Kent et al., 2020).*.....* 6
Figure 6: Linear diagram of the longest isoform of human tau protein (Pedersen & Sigurdsson, 2015)...... 9
Figure 7: The operational domains of the longest isoform of tau containing 441 amino acids are shown in here. It consists of the projection domain which comprises an acidic and a proline-rich region that combines with cytoskeletal elements to establish the spacing between the microtubules in axons. The C-terminal region is used for microtubule binding area (Luna-Munoz et al., 2013)...... 11
Figure 8 : Kinases regulate the stabilization of microtubule-associated tau protein. Aberrant hyper phosphorylation of protein leads to greater depolymerization of microtubule resulting to intractable cytoplasmic tau oligomers, which then aggregate to form protomers. Later two protomers coiled around each other to form paired helical filaments, which then gathered and eventually formed neurofibrillary tangles (Mamun et al., 2020)...... 14
Figure 9: Self aggregation of Tau (A. D. C. Alonso et al., 2001a)...... 16
Figure 10: Mechanism of PET tracer..... 18
Figure 11: Chemical structure of [11C]PBB3 (Wang & Edison, 1910)...... 19
Figure 12: Chemical structure of [18F]THK5117 and its S-enantiomer [18F]THK5351 (Wang & Edison, 1910)...... 20
Figure 13: Chemical structure of Tauvid (Jie et al., 2021)...... 21
Figure 14: Schematic diagram of digital ELISA system (S. H. Kim et al., 2012)...... 23
Figure 15: Mechanism of Digitalised ELISA method...... 24

Figure 16: Illustrates the BLI biosensor technique (Bio-layer Interferometry (BLI) Technology for Coronavirus Research - Coronavirus, n.d.). 27
Figure 17: BLI sensing strategy for Tau protein detection. 27
Figure 18: Detection method of tau441 protein. 28
Figure 19: A. schematic diagram of SPR process B. Variation of the angle before and after the biomolecule is bound in SPR C. A sonogram of the biomolecule adsorbed onto the surface of the sensor in real time (Sci-Hub | Surface plasmon resonance biosensors for detection of alzheimer's biomarkers; an effective step in early and accurate diagnosis. Biosensors and Bioelectronics, 112511 | 10.1016/j.bios.2020.112511, n.d.). 31
Figure 20: Schematic overview of SPR assay for Tau protein detection (Vu Nu et al., 2018). 31
Figure 21: Mechanism of SPR assay. 32
Figure 22: Three techniques of SPR platform a) direct detection b) label free sandwich assay c) multi-walled carbon nanotube-labeled sandwich assay (Lisi et al., 2017). 33
Figure 23: (A) Illustrates the QCM biosensor at which the quartz crystal is coated with two gold electrodes on either side. (B) Illustrates the basic operation of QCM (Migó et al., n.d.). 35
Figure 24: Direct assay method. 37
Figure 25: Sandwich-based assay. 38
Figure 26: SERS biosensor for Tau protein detection (*Sci-Hub | Biosensors for detection of Tau protein as an Alzheimer's disease marker. International Journal of Biological Macromolecules | 10.1016/j.ijbiomac.2020.06.239*, n.d.). 40
Figure 27: Detection mechanism of Tau protein using SERS method. 42
Figure 28: SERS intensity of IgG, Tau and BSA complexes (Zengin et al., 2013). 42

List of acronyms

AD- Alzheimer's disease

CSF- Cerebrospinal fluid

MAPs- Microtubule-Associated Proteins

MT- Microtubule

MTOC - microtubule-organizing center

PHFs- Paired helical filaments

NFTs- Neurofibrillary tangles

SPR- Surface plasmon resonance

QCM- Quartz Crystal Microbalance

BLI- Biolayer interferometry

ELISA- Enzyme-linked immunosorbent assay

PET- Positron emission tomography

Chapter1: Introduction

1.1 Alzheimer Disease

Alzheimer's disease (AD) is the most common cause of dementia, accounting for up to 80% of all diagnoses. Dementia is a psychiatric illness in which two or more cognitive domains, such as memory, speech, executive and visuospatial control, personality, and cognition, continue to deteriorate which impairs one's ability to conduct instrumental functions and/or accomplish the most fundamental duties of daily life (Weller & Budson, 2018). According to recent study, Dementia affects 40 million individuals around the world, and the number is expected to rise every 20 years until 2050 (Yiannopoulou & Papageorgiou, 2020). As dementia is most commonly affects elderly people of around 60 years, since dementia mostly affects people over the age of 60, the growing expansion of lifespan has resulted in a rapidly increasing number of patients with dementia, primarily Alzheimer's disease, prompting an increase in research focusing on the disease's treatment. Despite all of the hard effort that went into science, there are currently no successful treatment options for the disease (Yiannopoulou & Papageorgiou, 2020). According to a statistics from the Established Populations for Epidemiologic Study of the Elderly, approximately 491,000 persons aged 65 and up in the United States are expected to have Alzheimer's dementia by 2020.

The Framingham Heart Study data were used to calculate the lifetime risk of Alzheimer's dementia based on gender and age. In Figure 1, it shows that the study proves that life risk for people at age 45 was estimated around 20% for women and 10% for men for Alzheimer's dementia (AD). However, AD was comparatively greater among both the gender at age 65 (Hebert et al., 2013).

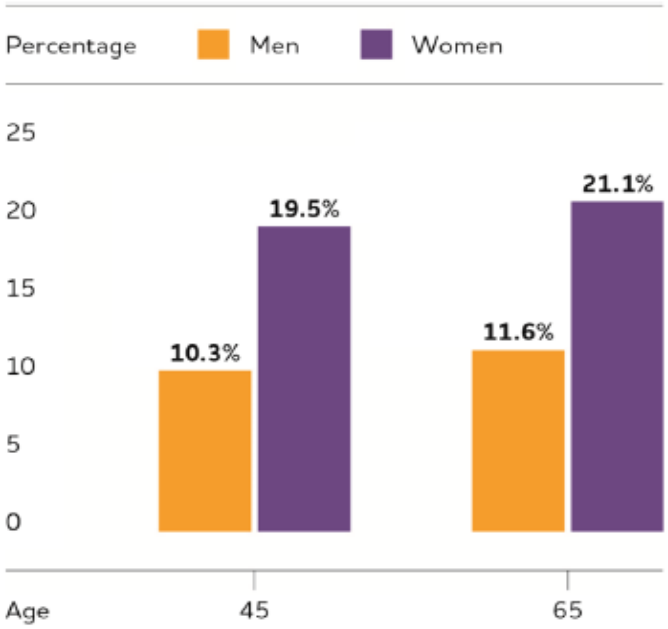

Figure 1: Approximated life risk of AD at age 45 and 65 (Vermunt et al., 2019)

1.1.1 Disease progression:

In the Alzheimer's disease continuum, it consists of three wide stages: Preclinical AD, mild cognitive impairment due to AD and dementia due to AD (described in figure 2). The dementia stage is further divided into mild, moderate and severe stages, which contemplate the extent to which the indications can influence the individual's daily activities. Moreover, the amount of time each individual go through each stage in continuum depends upon age, genetics, gender and many other factors (Vermunt et al., 2019).

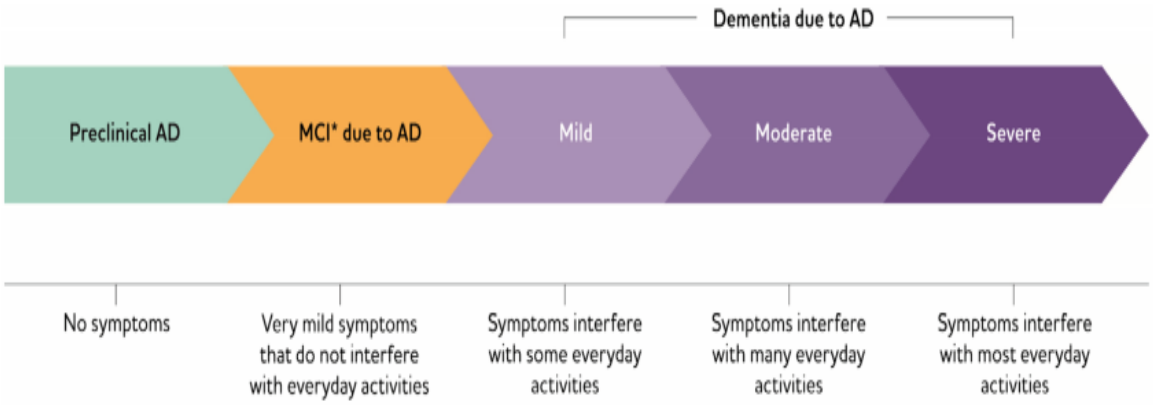

Figure 2: Alzheimer's disease continuum (Vermunt et al., 2019).

1.1.2 Prevalence:

The prevalence of AD indicates the quantity and percentage of individuals in a community suffering from Alzheimer's dementia at any given period. Millions of Americans are suffering from Alzheimer's or other brain disorders. In the United States, the total number of people living with Alzheimer's disease is increasing as the country's population of people aged 65 pursues to grow. Therefore, these will elevate quickly in the future when the population of America happens to expand from 56 million in 2020 to 88 million by 2050.

In 2020, approximate populations 5.8 million Americans age 65 and older are with Alzheimer's dementia. 80% are of age 75 or older as shown in figure 3

Statistics shows that out of the total U.S. population, the proportion of people living with Alzheimer's dementia is directly proportional to the age

- 10% of age 65 and older
- 3% of people age 65-74,
- 17% of people age 75-84

- 32% of people age 85 and older are living with Alzheimer's dementia (Hebert et al., 2013).

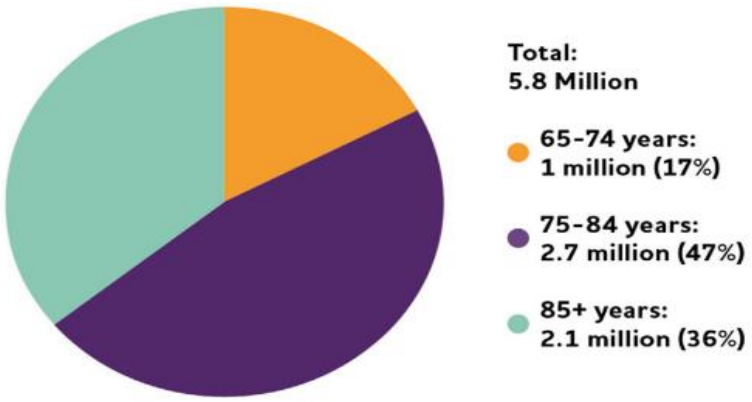

Figure 3: Number and age of people 65 or older with Alzheimer's dementia in 2020 (Hebert et al., 2013).

1.2 Tau protein

Tau is a long chain protein which is highly soluble and is essentially found in neuronal cells (Weingarten et al., 1975). Its primary role is to grip the microtubules (MT) tight and stable, ease neuronal transportation and to preserve the cellular consistency and stability. There are various types of tau post translational changes such as phosphorylation through protein kinases, are one the reason for tau aggregation and causing a distance between the microtubules, which leads to cell death and cytotoxicity (A. D. Alonso et al., 2010). However, the tau aggregates can proliferate from one cell to another resulting to loss of structure or function of neuron (neuro degeneration) (De Calignon et al., 2012). Hence, tau protein is a useful biomarker for disease identification, diagnosis and therapeutic development (Ziu et al., 2020).

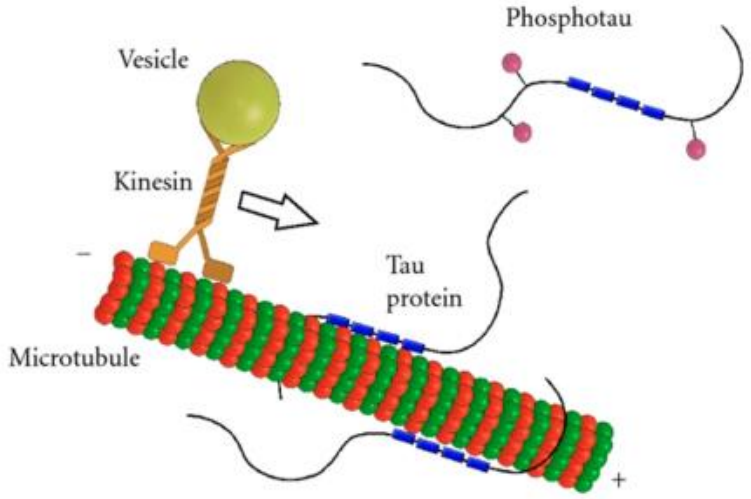

Figure 4: Tubulin binding domains (blue) used to hold microtubule with tau. Interaction of tau molecule with microtubule and function of axonal transport can be controlled by phosphorylation (pink) (Barbier et al., 2019).

1.3 Role of Tau protein in AD

The function of tau protein is to hold tight and stabilize microtubules (MT), decrease the number of quick changes like microtubule disintegration. Tau holds tubulin through microtubule-binding domains with a single tau molecule conjugated to several tubulin dimers. Lack of Microtubule or faulty microtubule assembly are the common cause of AD (Kent et al., 2020).

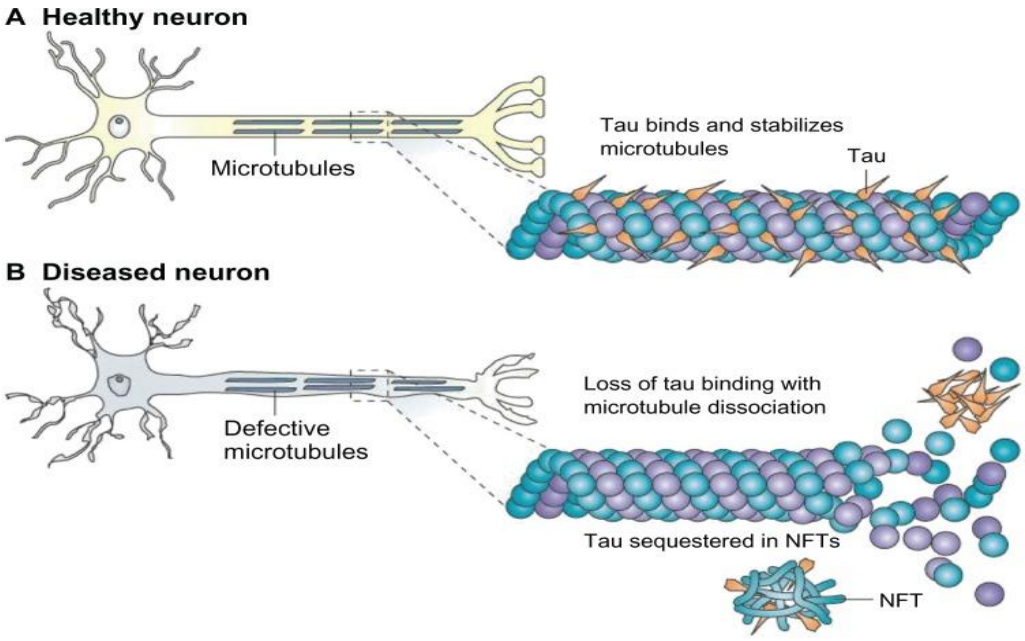

Figure 5: (A) Tau eases microtubule stabilization within the cell. (B) Tau's role is depleted in Alzheimer's patients (Kent et al., 2020).

1.4 Detection of Tau Protein

Tau protein is one of the biomarker for AD. There are various prospective detection methods that are used for tau protein. These methods are present in the specialized hospitals to diagnose, scan the patient's brain. However, there are many other potential methods found in the ongoing field of research in order to deplete the drawbacks of the present methodologies. Therefore, in this article we are going to focus on some prospective detection methods of tau protein.

1.5 Purpose of the study

Alzheimer's disease is a neurodegenerative disease. Tau protein plays vital role in AD. In normal condition, tau protein is used to stabilize the microtubule, ease neuronal transport and maintain

cell integrity. Lack of Microtubule or faulty microtubule assembly are the common cause of AD (Kent et al., 2020). As tau protein is a biomarker of AD, therefore it is necessary to study and find out the efficient and potential detection methods for this protein.

1.6 Aim of the study

The aim of the study is to know the importance of tau protein in AD along with the current detection methods for tau protein and to know about the ongoing research programs going on for further potential detection methods of tau protein.

Chapter 2: Tau Protein

2.1 Tau Protein

The tau gene has 16 exons and is centered over 100kb on the heavy chain of chromosome 17 at band position 17q21. Exon1 undergoes transcription but not translation as it is a component of the promoter. Fundamental exons include exon 1, 4, 5, 7, 9, 11, 12, and 13. In the brain of elderly person, exon 2,3 and 10 are alternately braided and manifested in which exon 2 can emerge independently whereas exon 3 is dependent on exon 2. Alternate intertwining of exon 2, 3 and 10 in the central nervous system leads to the emergence of the six tau isoforms that are overexpressed throughout neurodevelopment (Sergeant et al., 2005a).

Tau phosphorylation is the prime regulator of microtubules. The smallest tau protein is present in the fetus, while the lengthiest isoforms are seen adulthood. In AD, the mean number of phosphate groups for every tau protein molecule increases, which leads to separation of proteins from microtubule (Ebneth et al., 1998). Therefore, hyperphosphorylated tau molecules results in:

- synaptic dysfunction
- impaired degradation through autophagy or within the proteasome
- increased agglomeration
- interactions with other compounds are disrupted.

The key contributor for the formation of neurofibrillary tangles that contains paired, coiled and linear filaments of tau depositions are the inappropriately hyperphosphorylated tau (Kolarova et al., 2012a).

2.1.1 Structure

Tau is hydrophilic, unstructured and complex protein (Mandelkow & Mandelkow, 2012). According to biophysical studies, tau is a prototypical “natively unfolded” protein. Tau refers to a class of protein called as Microtubule-Associated Proteins (MAPs). Due to its secondary structure, it is found out that tau is heat resistant and is barely influenced by the acid treatment without losing their function. The protein is mostly found in the axons of the CNS which are subdivided into six isoforms by alternative splicing (Goedert et al. 1989). The structure varies by the exclusion or inclusion of two near-amino-terminal inserts of 29 residues each, coded by exons 2 and 3, and by one of the repeats (R2, 31 residues) in the carboxyl-terminal half (mandelkow2020).

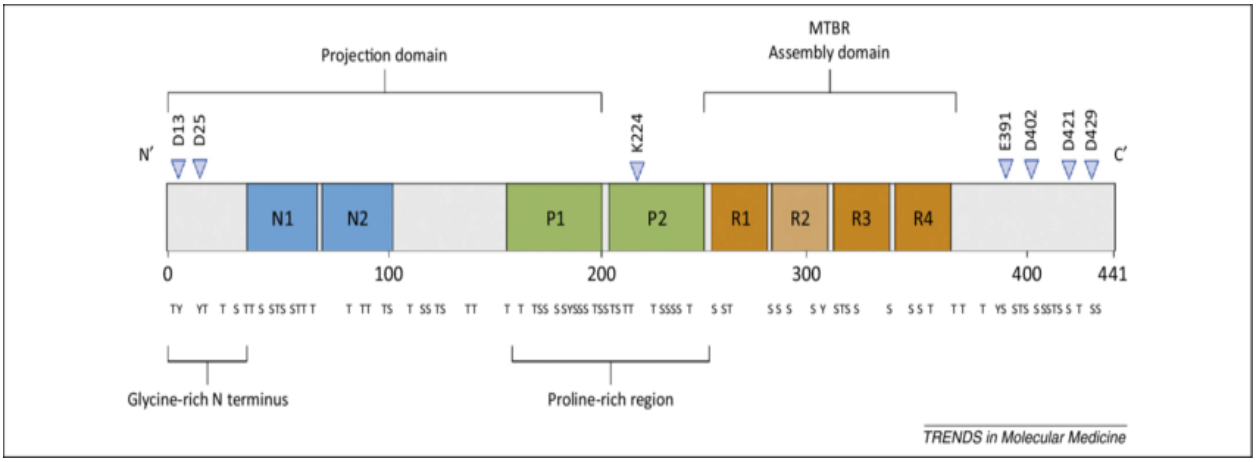

Figure 6: Linear diagram of the lengthiest isoform of human tau (Pedersen & Sigurdsson, 2015).

2.1.2 Classification

Tau proteins are distinguished based on the composition of three (3R) or four (4R) tubulin binding domains (repeats, R) of 31 or 32 amino acids in the C-terminal part of tau protein and

one (1N), two (2N), or no inserts of 29 amino acids each in the N-terminal portion of the molecule. The Presence and lack of sequence encoded by exon 2, 3 or 10 determines the length of an isoform, which ranges between 352 to 441 amino acid sequences. Incorporation of the faulty gene encoding exon 10 causes the expression of tau consisting four microtubule binding repeats (MTBRs) (4R tau: 0N4R, 1N4R, 2N4R), whereas omission of exon10 leads to binding products expressing tau with three MTBRs (3R tau: 0N3R, 1N3R, 2N3R) (M. Goedert & Jakes, 1990). These six isoforms are often labeled as τ3L, τ3S, τ3, τ4L, τ4S, and τ4 (A. D. C. Alonso et al., 2001b).

Primary structure sequence for tau protein contains of a half-N-terminal acidic part led by a proline-rich subunit and the C-terminal tail, which is the fundamental component of the protein. Exon 2 and 3 codes for the polypeptide sequence that gives acidity property to tau while exon10 codes a positively charged region that correlates to tau's fundamental characteristics. At the other hand, N terminal containing an isoelectric point of 3.8 along with a proline rich region having pI of 11.4 and the C terminal is positively charged with pI of 10.8. Hence it is said that tau protein is described as a dipole containing two domain of opposing charges. Each of the isoforms serves a distinct physiological function and is distinctively expressed during the growth of the brain. For example:

- isoform containing 3R and no N-terminal insert is found in the fetal stage
- isoform containing one or two N-terminal insert are found during the adulthood phase

However, tau protein is mostly found in the neurons although it is found in the oligodendrocytes. Another MAP2 is a microtubule-binding protein which is present in the somato-dendritic portion of neuron, whereas MAP4 is widely distributed (Ebneth et al., 1998) (Kolarova et al., 2012a).

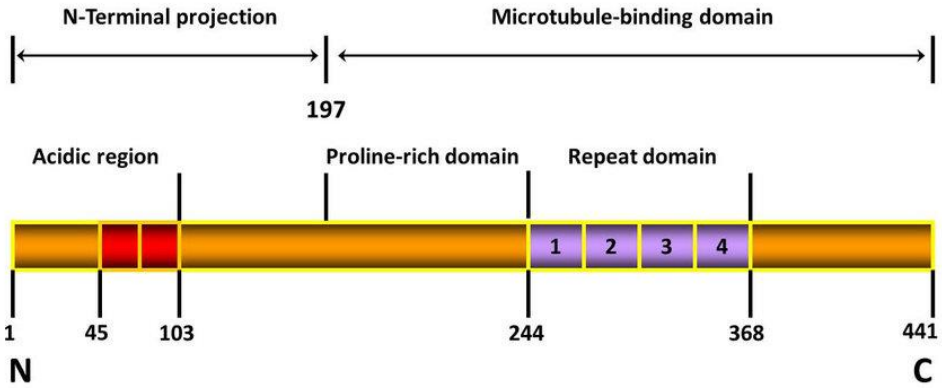

Figure 7: The operational domains of the lengthiest isoform of tau containing 441 amino acids are shown in here. It consists of the projection domain which comprises an acidic and a proline-rich region that combines with cytoskeletal elements to establish the spacing between the microtubules in axons. The C-terminal region is used for microtubule binding area (Luna-Munoz et al., 2013).

2.1.3 Distribution of Tau

Tau is primarily present in the brain, especially in the neurons. They are predominantly immature in neurons but as they develop, they become axonal with greater- molecular weight isoforms and lesser phosphorylation (Drubin and Kirschner 1986; Kosik et al. 1989). Lower concentration of tau are found in other neuronal compartments such as in nucleus, dendrites and in other brain cells, particularly in oligodendrocytes even after maturation. Moreover, tau can be present in wide variety of cell types, including muscle fiber, where tau clusters in addition to body myositis (Mandelkow & Mandelkow, 2012).

2.2 Tau Protein in AD

Normally, tau protein's function is to uphold the cytoskeleton in a regular manner in the axonal process. However, in AD this role of tau protein is lessened due to the capacity of the protein to

attach to the microtubule is lost. This uncommon behavior is facilitated by the epigenetic alteration and misfolding in the regular tau protein configuration resulting to the conversion of abnormal cluster formation into fibrillary structure inside the neuron. (Carrell & Gooptu, 1998). Hence, the transformed form of tau protein is spread and concentrates in the somatodendritic region and unhealthy neurons. Changing the sequence of tau protein can harm the stability of microtubule. For example, overexpression and mislocalization can rise the intracellular entry of tau concentration or may prohibit the plus-end-directed transport of vesicles into the microtubule via kinesin thereby making the plus-end-directed transport more robust which influences the dissemination of mitochondria by forming clusters nearby to microtubule-organizing center (MTOC). The lack of of mitochondria and endoplasmic reticulum in the periphery compartment in the axons can result to reduced glucose and lipogensis as well as ATP production and Ca2+ regulation (Futerman & Banker, 1996), resulting in a distal degeneration process known as "dying back" of axons (Kolarova et al., 2012a).

Moreover, phosphorylated tau protein has strong affection towards kinesin so it is conveyed to the distant site of neuropile. This process explains how tangle pathogenesis in AD tends to originate distally and degrade backwards to the perikaryon. This helps to preserve the strength of microtubule by transferring hyperphosphorylated tau to the other regions of the cell at higher rate where they can form clusters. It is said that the unusual post-translational changes in the tau are the main reason of acquiring abnormal features which includes, abnormal phosphorylation (hyper phosphorylation), acetylation, glycation, ubiquitination, nitration, proteolytic cleavage (truncation), structural alteration and some other alterations (Martin et al., 2011). In the next subtopic we will focus on the hyper phosphorylation, acetylation and self aggregation of tau Protein.

2.2.1 Hyper phosphorylation of Tau Protein

The significant role of phosphorylated tau is to control the capability to attach microtubules and promote the assembly. A standard degree of phosphorylation is needed for the basic functioning of tau protein, whilst the hyper phosphorylation state diminishes its biological property. It is said that the longest tau isoform (containing 441 amino acids) contains about 80 potential serine or threonine phosphorylation sites (Sergeant et al., 2005b). Apart from Ser^{262}, Ser^{293}, Ser^{324}, and Ser^{356} in R1, R2, R3, and R4 domains (Drewes et al., 1995), (Dickey et al., 2007), majority of these potential sites are present nearby the microtubule binding region in the proline rich zone and at the end of C-terminal of tau (Sergeant et al., 2008), (Buée et al., 2000).

The disease condition causes up regulation of tau kinase(s) or down regulation of tau phosphatase(s) although these two possibilities are not completely exclusive (Buée et al., 2000), (Qtrojanowski & M-y Lee, n.d.). GSK-3β, cyclin-dependent kinase 5 (cdk5), cAMP-dependent protein kinase (PKA), and calcium/calmodulin-dependent kinase II (CaMK-II) are the kinases that are said to have the most essential part in phosphorylation of tau protein (Gong & Iqbal, 2008). GSK-3β has major crucial impact in managing tau phosphorylation in both healthy and diseased conditions as well as in phosphorylating tau on Ser^{199}, Thr^{231}, Ser^{396}, Ser^{400}, Ser^{404}, and Ser^{413}*in vivo* and *in vitro* that are highly phosphorylated in PHF-tau (Liu et al., n.d.). Phosphorylation of Thr^{231} leads to local conformational alteration that permits of GSK-3β or other kinases to phosphorylate tau more extensively. However, PP1, PP2A, PP2B, and PP2C gene can dephosphorylate tau protein in vitro have a complimentary and opposing impact. The action of PP2A has already been observed to be diminished in certain parts of the brain in AD sufferers (Liu et al., 2005).

Tau in the cytosol that has been inappropriately phosphorylated (AD P-tau) never attaches on tubulin or facilitates microtubule formation in the damaged neurons in AD (Kolarova et al., 2012c), (Dephosphorylation of Alzheimer Paired Helical Filaments by Protein Phosphatase-2A and â˜’2B (âˆ—) | Elsevier Enhanced Reader, n.d.). In fact, this protein inhibits the formation of microtubules and causes them to disorganize (Kolarova et al., 2012c). Therefore, these findings suggest that abnormal phosphorylation plays a vital role that results in aberrant aggregation of tau in AD.

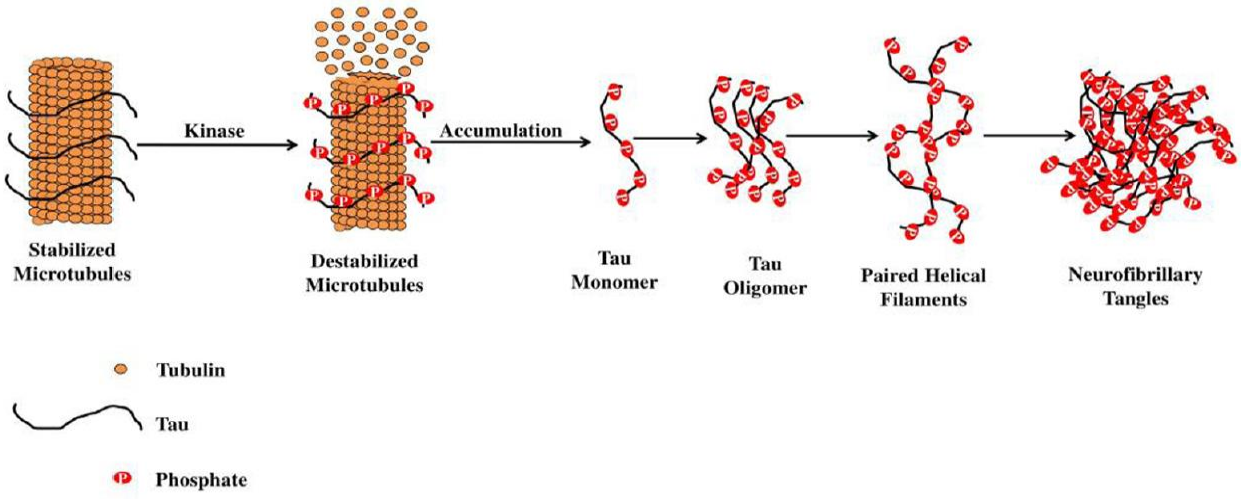

Figure 8 : Kinases regulate the stabilization of microtubule-associated tau protein. Aberrant hyper phosphorylation of protein leads to greater depolymerization of microtubule resulting to intractable cytoplasmic tau oligomers, which then aggregate to form protomers. Later two protomers coiled around each other to form paired helical filaments, which then gathered and eventually formed neurofibrillary tangles (Mamun et al., 2020).

2.2.2 Acetylation of Tau Protein

In the recent investigation, it is found out that tau acetylation is a form of post translational alteration that controls normal tau activity (Acetylation of Tau Inhibits Its Degradation and Contributes to Tauopathy | Elsevier Enhanced Reader, n.d.), (Cohen et al., 2011), (Kolarova et al., 2012b). In particularly, reversible lysine acetylation has identified as putative regulatory change linked to AD and other neurological illnesses. Abnormal acetylation may inhibit tau from attaching to microtubules as acetylation helps neutralizing the charges in the microtubule-binding domain. Hence, abnormal acetylation leads to tau malfunction and may play a vital part in tau accumulation in AD (Acetylation of Tau Inhibits Its Degradation and Contributes to Tauopathy | Elsevier Enhanced Reader, n.d.). According to recent study, acetylation of Lys^{280} may be an intermediary stage for creation of tangles (Kolarova et al., 2012c). Greater acetylation on Lys^{280} may disrupt collision of tau with microtubules and produces more cytoplasmic tau for abnormal PHF aggregation. $Lys^{280,}$ Which is found in the inter-repeat zone (^{275}VQIINKK^{280}), was recognized as one of three lysine groups which is most important in regulating tau-microtubular collisions (Cohen et al., 2011). The molecule of tau has plenty amount of phosphorylation sites among which majority of them are found in areas surrounding the microtubule-binding repetition (Buée et al., 2000), where Lys^{280} is present. Thus, phosphorylation of tau will disrupt the residues present for acetylation (Choudhary et al., 2009).

2.2.3 Self aggregation of Tau Protein

Extensive length of positively and negatively charged regions present in tau molecule make intermolecular hydrophobic interaction difficult (Iqbal et al., 2010). Only R2 (exon 10) and R3 (exon 11) in monomeric tau have the β-structure, which can assemble themselves into filamentous like substances and coassemble with heparin as a synthetic inducer (Michel Goedert

et al., n.d.). The availability of both N- and C-terminal that is located in microtubule binding region and inhibit the collision between these sticky domains hinders self-aggregation of Tau. A flexible structure of tau molecule caused by aberrant phosphorylation surrounding the N-terminal and C-terminal may unclip both the extremities from the microtubule binding region. Therefore, this allows self-collision between these sticky domains and produces paired helical filaments (PHF) / straight filaments (SF) as shown in figure 9 (A. D. C. Alonso et al., 2001a).

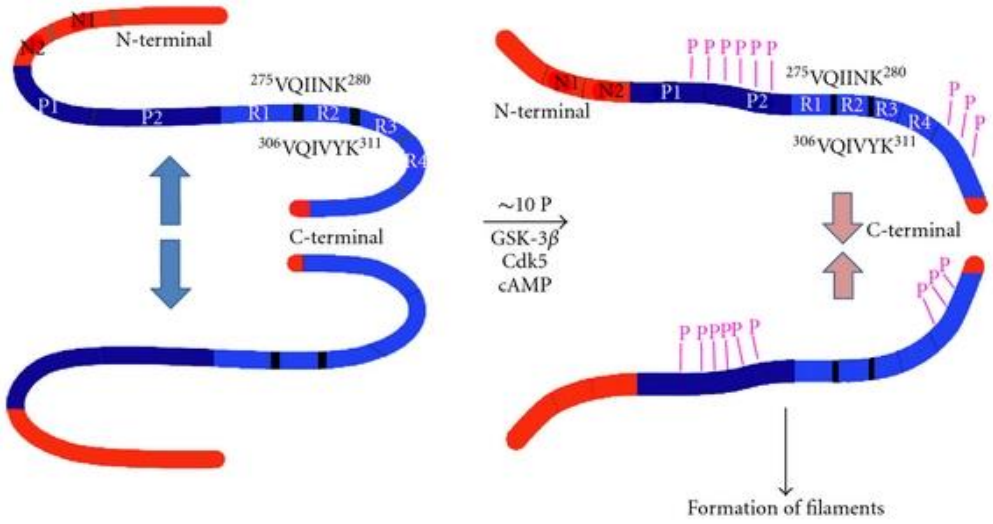

Figure 9: Self aggregation of tau (A. D. C. Alonso et al., 2001a).

Chapter 3: Current detection methods for Tau protein

3.1 Introduction

Alzheimer's dementia results from the gradual decline of brain cells. This deterioration may show up in a number of ways in brain scans. Therefore, the brain imaging can help to identify the degree of degeneration of the brain.

3.2 Positron emission tomography (PET)

Positron Emission Tomography (PET) is a non-invasive diagnosing imaging technique using isotope-labeled biomarkers that adhere to biomolecules with higher degree of precision and sensitivity. Various PET tracers attacking aberrant tau protein conformations that have developed in recent years, which helps the researchers to see tau aggregation in vivo (Yeung et al., 2017). As tau is a complicated protein with various forms that undergoes subsequent translational modications, tau PET tracers can link to single, several isoforms. Furthermore, tau is a cytoplasmic (intracellular) protein it needs the capability to pass across the plasma cell membrane and the blood-brain barrier (BBB). Several PET ligands have been used for imaging tauopathies in the brains of AD individuals, which includes [11C]PBB3, [18F]THK5105, [18F]THK5117, [18F]THK5351, [18F]T807, and [18F]T808 (Imaging of Tau Pathology in a Tauopathy Mouse Model and in Alzheimer Patients Compared to Normal Controls | Elsevier Enhanced Reader, n.d.-a), (Chien et al., 2014). Few of these PET tracers are now approved for therapeutic usage on individuals having tauopathies such as AD and healthy people. As a result, Tau imaging is essential for progression toward earlier and effective diagnosis of tau pathology along with monitoring disease progression therapeutic intervention tracking and drug development (Wang & Edison, 1910).

3.2.1 Mechanism of action of PET tracer

Alzheimer's disease is a neurodegenerative disorder caused by accumulation of hyperphosphorylated tau protein. Paired helical filaments (PHFs) are formed when hyperphosphorylated tau produces dimers which form cluster to form neurofibrillary tangles (NFTs) linked with neurodegeneration and extent of Alzheimer's symptoms (Bavaskar & Bhurat, 2021).

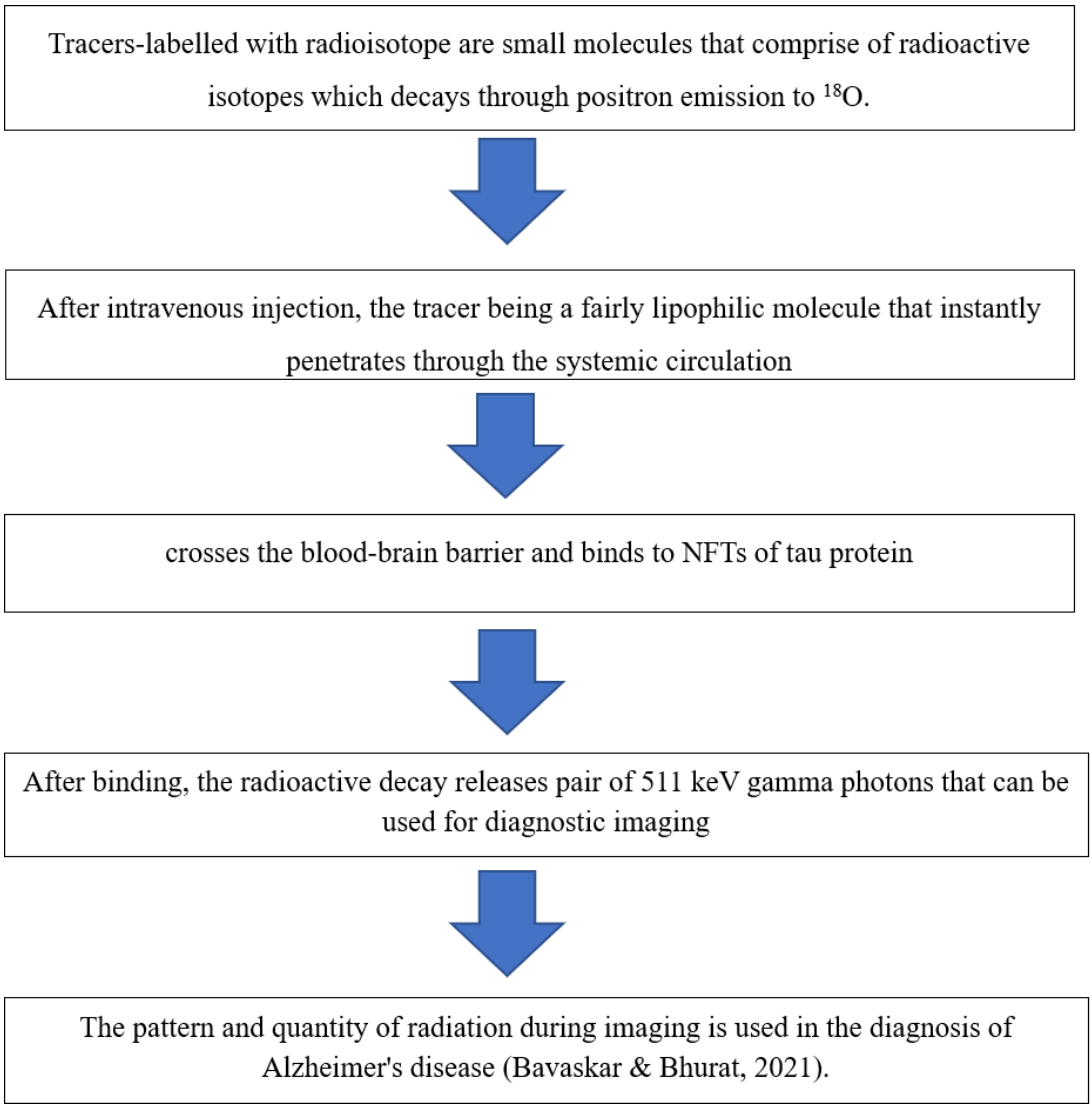

Figure 10: Mechanism of PET tracer

3.2.2 Commonly used PET tracers

[18F]FDDNP

Initially, 2-(1-{6-[(2- [fluorine-18] Fluoroethyl) (methyl) amino] -2-naphthyl}- ethylidene) malononitrile (FDDNP) is the PET tracer used to detect AD pathologies in actual humans (Mosconi et al., n.d.). FDDNP is not the sole tau tracer as it is seen in the brain, it attaches to neurofibrillary entangles and amyloid plaques (Smailagic et al., 2018).

[11C]PBB3

11C-pyridinyl-butadienyl-benzothiazole 3 ([11C] PBB3) was one of the first tau PET tracer to be described as to detect a wide spectrum of tau plaques. [11C]PBB3 has effectively showed the expansion of brain tauopathis during the shift from normal aging to intermediate AD, indicating that tau PET imaging can be used as a quantitative indicator of progression of the disease. (Wood, 2013). As a result, it supports the idea that tau lesions are highly linked to neuronal malfunction than Aβ plaques (Imaging of Tau Pathology in a Tauopathy Mouse Model and in Alzheimer Patients Compared to Normal Controls | Elsevier Enhanced Reader, n.d.-b).

..

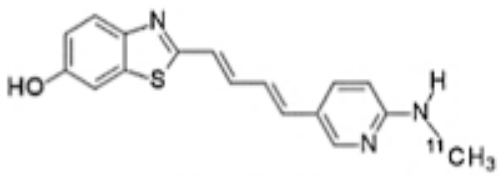

Figure 11: Chemical structure of [11C]PBB3 (Wang & Edison, 1910).

Quinoline Derivatives

The three quinolone derivatives of quinoline compounds including [18F]THK-523, [18F]THK-5105 and [18F]THK-5117 showed selectivity and greater affinities linking onto tau than Aβ on patients brain regions in vitro (R Harada et al., 2018).

[18F]THK5351 is a single S-enantiomer of [18F]THK5117 that was said to enhance the pharmacokinetics of aryl quinoline derivatives. [18F]THK5351 had a stronger attraction for linking hippocampal homogenates from AD brains and dissociated from white matter tissue faster compared to [18F]THK5117, as per the researchers (Wang & Edison, 1910).

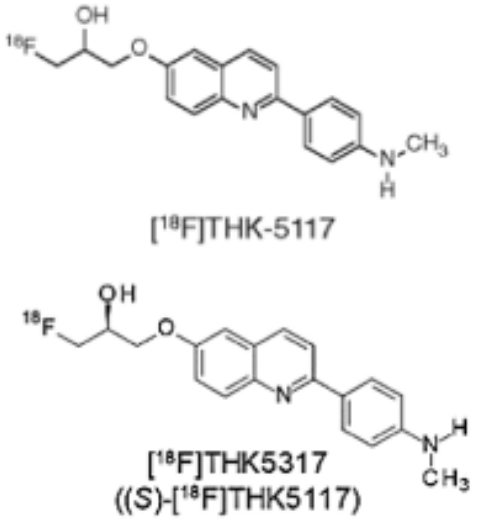

Figure 12: Chemical structure of [18F]THK5117 and its S-enantiomer [18F]THK5351 (Wang & Edison, 1910).

The quantity of tracer binding to human brain is closely associated with the amount of tau plaques. [18F]THK5351 had quicker kinetics and reduced accumulation in the subcortical white matter, indicating that it is an effective PET tracer for preliminary diagnosis of neurofibrillary disease in AD individuals (Ryuichi Harada et al., 2016).

Tauvid

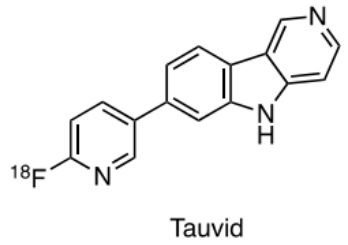

Figure 13: Chemical structure of Tauvid (Jie et al., 2021).

Tauvid is also known as [18F]Flortaucipir, [18F]AV-1451, and [18F]T807. Its IUPAC name is 7-(6-[18F]fluoropyridine-3-yl)-5H-pyrido [4,3-b]indole. On 28th May, 2020 Avid Radiopharmaceuticals launched TAUVID™ (Bavaskar & Bhurat, 2021), the first FDA approved PET tracer for monitoring tau disorder in AD (Jie et al., 2021). Tauvid is a tiny indole molecule produced with a radioactive fluorine isotope with molecular weights of 262.27. It is utilized as a biomarker in positron emission tomography (PET) scanning of Alzheimer's patient. Flortaucipir F-18 interacts to the tau plaques after penetrating the blood-brain barrier, a marker whose occurrence corresponds with disease development. Although flortaucipir F-18 has minimal levels of background binding across the brain, it does have off-target binding to monoamine oxidase MAO-A and MAO-B, as well as sites with significant quantities of melanin, neuromelanin, and iron. Pathological version of tau protein forms inside neurons in AD patient, resulting in neurofibrillary tangles. After flortaucipir (^{18}F) is injected intravenously, it binds to region of the brain containing tau protein misfolding. The brain can be then examined using a PET scan to diagnose the disease progression (Bavaskar & Bhurat, 2021).

Tauvid is used to evaluate the concentration and spreading of neurofibrillary tangles in the brain of patients suffering cognitive impairment who are examined for AD by PET. Tauvid should not

be recommended for the diagnosis of chronic traumatic encephalopathy (CTE) patients. The capability of tauvid binding may be hampered due to the changes in the tau conformation and distribution, therefore it is not recommended for chronic traumatic encephalopathy (CTE) patients (Jie et al., 2021).

3.2.3 Advantages and Disadvantages of PET tracers

Advantages:

- Has the ability to detect Alzheimer's disease progression
- Has decreased radioactivity exposure
- Also has affinity for other isoforms of tau
- [18F]AV-1451 is highly selective, specific, lipophilic along with greater affinity for PHF-tau binding (Wang & Edison, 1910).

Disadvantages:

- There is a greater chance of cross- reaction occurring, as the suggested PET ligands were developed to focus on β -pleated sheets, a fundamental protein feature found in tau and Aβ (Wood, 2013).
- Radioactive half- life of [11C] is very small
- Few tracers bind to "off-target"(Wang & Edison, 1910).
- As tau being present in the intracellular site it is more difficult to create a radio ligand (Lemoine et al., n.d.) meaning that the ligand need to have the ability to penetrate by the plasma membrane along with the blood-brain barrier to get access to tau protein (Wang & Edison, 1910).

3.3 Digitalized ELISA

Digital ELISA, also known as Single molecule array. Tau protein was evaluated using digital ELISA to regulate the NFTs and is used for the initial diagnosis of AD. In this technique, antibody/antigen complex was established on microbeads at single-molecule level (Ono et al., 2014).

Digital ELISA is a revolutionary approach that is approximately 1000 times more accurate compared to traditional ELISA methodology (Sci-Hub | Use of high-sensitivity digital ELISA improves the diagnostic performance of circulating brain-specific proteins for detection of traumatic brain injury during triage. Neurological Research, 1–8 | 10.1080/01616412.2020.1726588, n.d.).

3.3.1 Mechanism

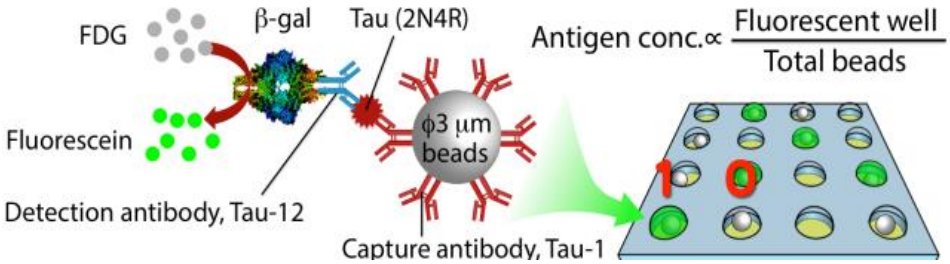

Figure 14: Schematic diagram of digital ELISA system (S. H. Kim et al., 2012).

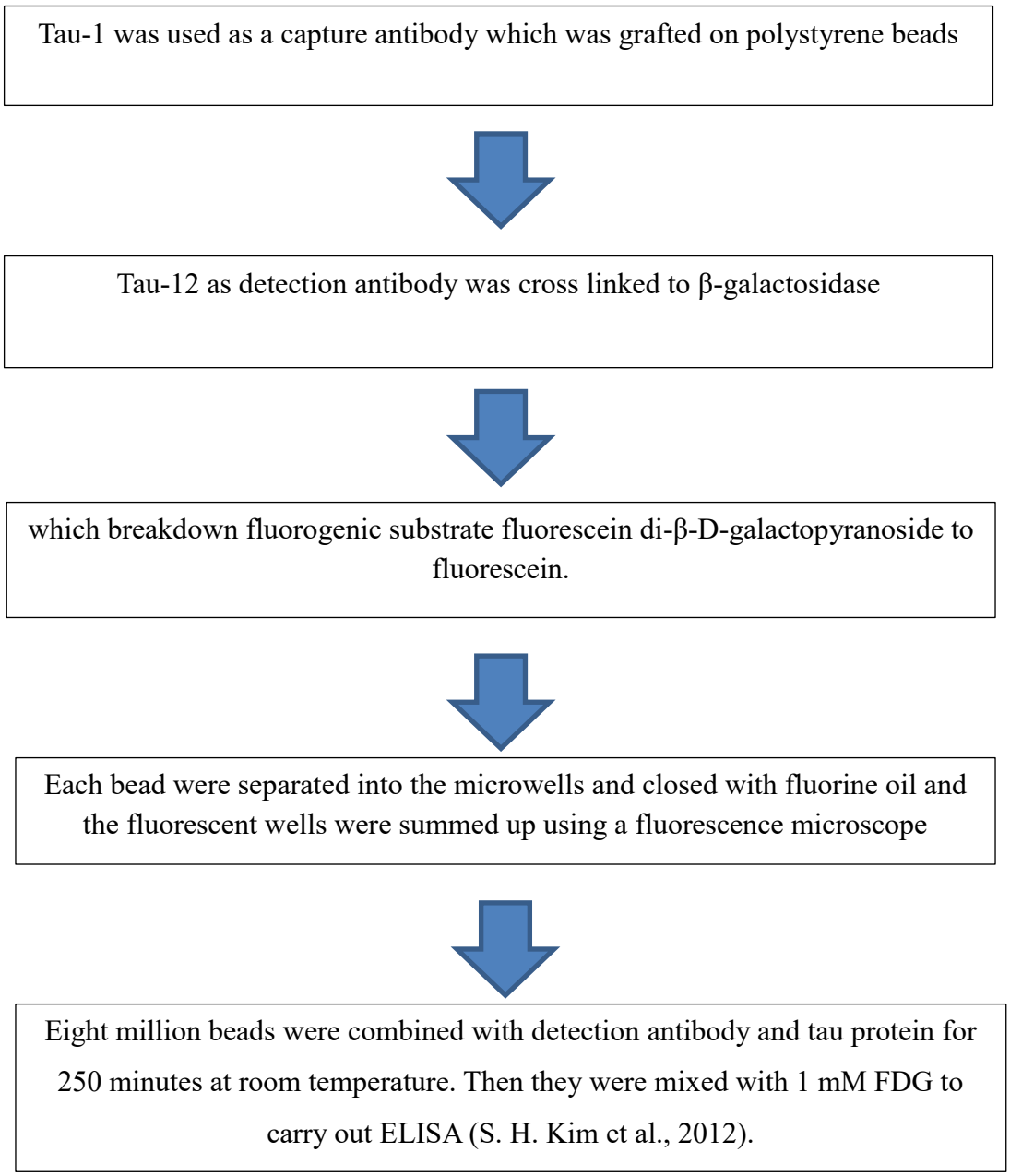

Figure 15: Mechanism of digitalised ELISA method.

3.3.2 Single molecule array Simoa™

Simoa™ is a commercially accessible technique used to evaluate the mid region of tau protein isoforms (Danni Li & Mielke, 2019). The Simoa™ technology is the center of Quanterix's platform that enables the identification and quantification of biomarkers that were previously difficult to quantify (The Scientific Principle of Single-Molecule Array Technology, n.d.).

A researcher named Zetterberg et al. were the first to apply Simoa technique to analyze both normal and p-tau protein in CSF and plasma samples. Since then, the business Quanterix has industrialized the assay technology. Additionally, Tatebe et al. also applied this technique to discover tau phosphorylated at Thr181 with an outstanding limit of detection of 0.0090 pg/mL by acquiring the plasma sample. (Arbaciauskaite et al., 2021).

3.3.3 Sensitivity and specificity of the technique:

- Digitalised ELISA has the capability to detect very low quantity of protein when contrasted to conventional ELISA, due to two factors:

  1. Simoa™ has great sensitivity to enzyme label

  2. the low background signal that may be achieved by digitizing protein detection (The Scientific Principle of Single-Molecule Array Technology, n.d.).

- Single molecule sensors are digital by character: every molecule emits a countable signal. As a result, detecting the presence and absence of signal is much easier than detecting the actual number of signal. In short, counting is easier than integrating (The Scientific Principle of Single-Molecule Array Technology, n.d.).

3.4 Biolayer Interferometry (BLI)

BLI is a powerful optical technique to learn about the molecular collisions without any need for marked agents for the identification of certain biomarkers like low molecular weight compounds, protein, cells and many others (Mechaly et al., 2016)**.** BLI is generally used for the serological testing for SARS-CoV-2 for the use of virus nucleocapsid.

BLI consist of a biosensor which are immersed into the microplate well holding pure or complex mixtures, engaging to extremely equidistant, user-friendly experiment. The interference pattern of the white light that is reflected on the reusable fiber optic-based sensor must be measured (Do et al., 2008). The interaction of a ligand bound on the surface of the biosensor tip with an element in sample causes the biosensor tip's optical thicknesses to develop, leads to alteration of the wavelength which is a quantitative measurement of the changes in biological layer thickness (*BLI Technology | Sartorius*, n.d.).

The fluctuation in the interference pattern can be observed as the amount of molecules linked to the biosensor tip varies, which can be calculated in real time as shown in figure16 (Do et al., 2008).

On the Octet system, only molecules that can attach to or dissociate from the biosensor can alter the interference pattern and produce a reaction profile. However, the interference pattern in unaffected by the loose unbound molecules or by variations in the refractive index or changes in the flow rate. This is the most uncommon property of BLI which allows it to perform in crude samples for protein binding, quantification, affinity, and kinetics applications (*BLI Technology | Sartorius*, n.d.).

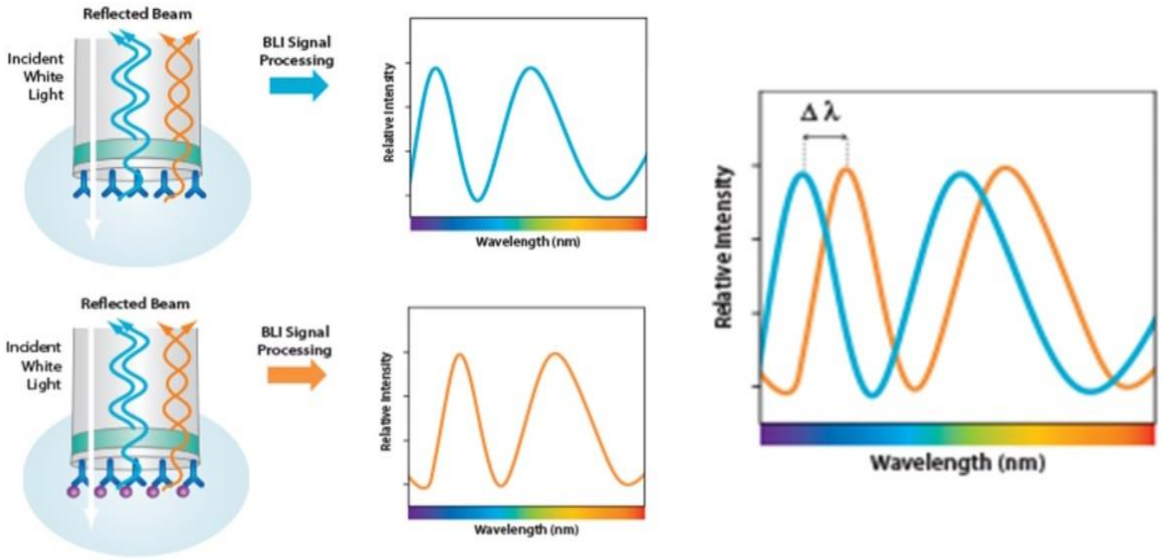

Figure 16: Illustrates the BLI biosensor technique (Bio-layer Interferometry (BLI) Technology for Coronavirus Research - Coronavirus, n.d.).

3.4.1 Detection of tau441 protein by the BLI biosensor

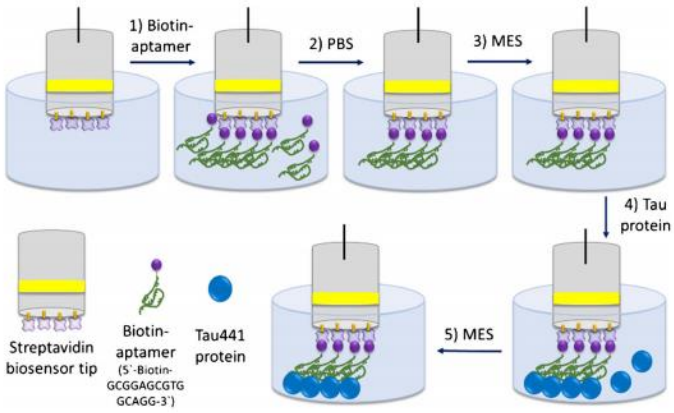

Figure 17: Tau protein identification using a BLI sensing technique (Ziu et al., 2020).

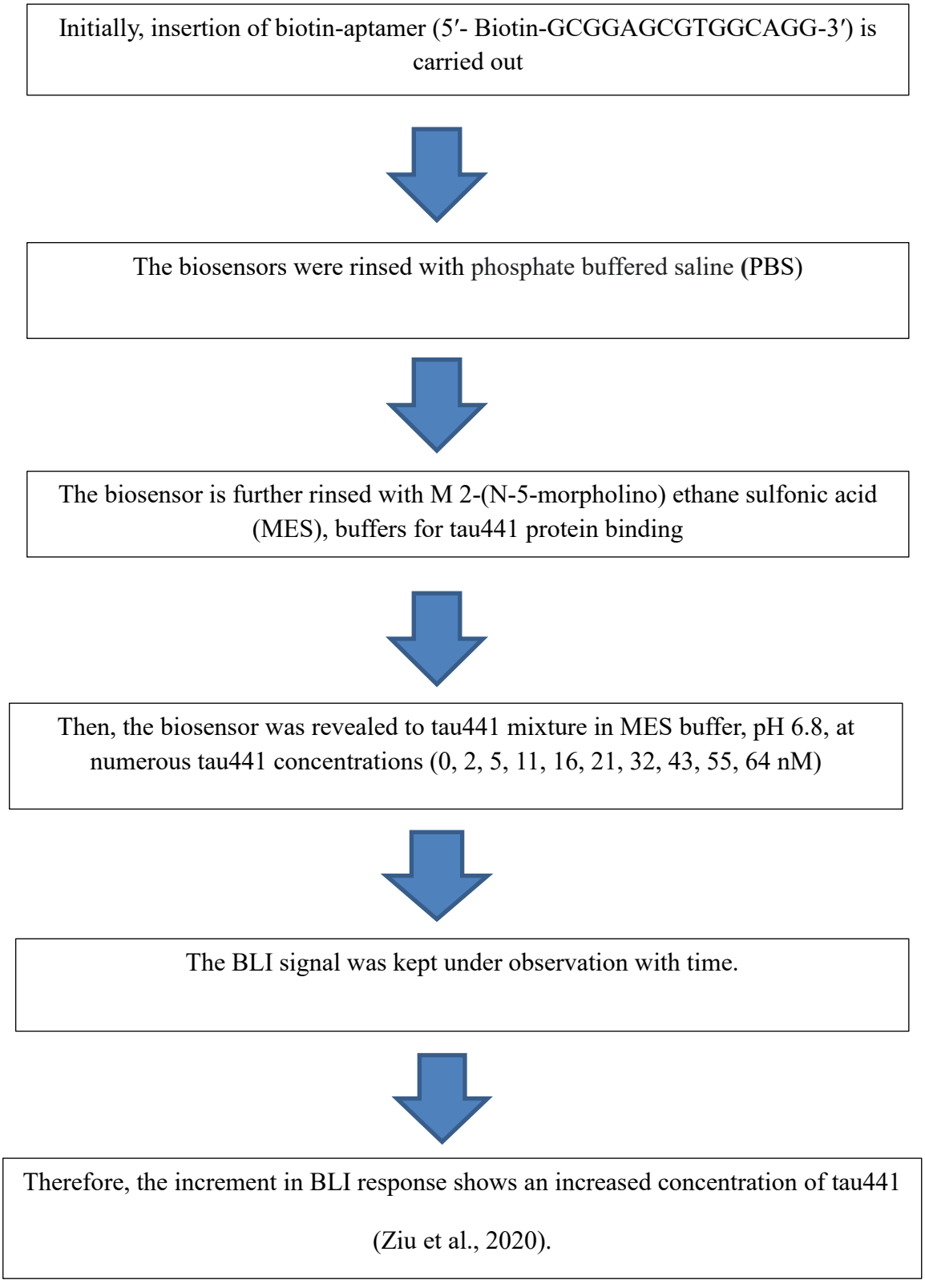

Figure 18: Detection method of tau441 protein.

3.4.2 Selectivity of BLI biosensor

1. The aptamer-based biosensor was tau441 specific with a very negligible non-specific binding to BSA as the tau441 biosensors selectivity was tested between bovine serum albumin (BSA) and MES buffer. Although, BSA protein was comparably similar to MES buffer yet had no impact on the biosensor.

2. The biosensor selectivity was further investigated in the vicinity of other neurodegenerative indicators such as Amyloid-β40 and α-synuclein. As a result, proving the biosensor specificity for tau441 indicators over other neural proteins is essential for the creation of a successful tauopathies biosensor (Ziu et al., 2020).

Chapter 4: New detection methods for tau protein

4.1 Introduction

Due to the fact that different detection methods are available, there is always a potential need for better and improved methodologies in order to deplete the drawbacks of the current methods mentioned in previous chapters. Hence, researchers are working to develop simpler and analytically low cost methodologies that can screen the target molecules in a very small period of time with excellent sensitivity and accuracy in the field of research.

4.2 Surface Plasmon Resonance (SPR)

SPR is an optical method for detecting collisions between two molecules on a thin gold film, one of which is mobile and the other static (Drescher et al., 2009).

SPR is referred when free mobile electrons present on the interface of a metal media vibrates after taking in the energy of the incident light. When a bond is formed between the target and biomolecules that are absorbed on the metal media's surface, a change in refractive index occurs, causing the resonate spectrum to shift, allowing for quantitative analysis of the target molecule (Lisi et al., 2017).

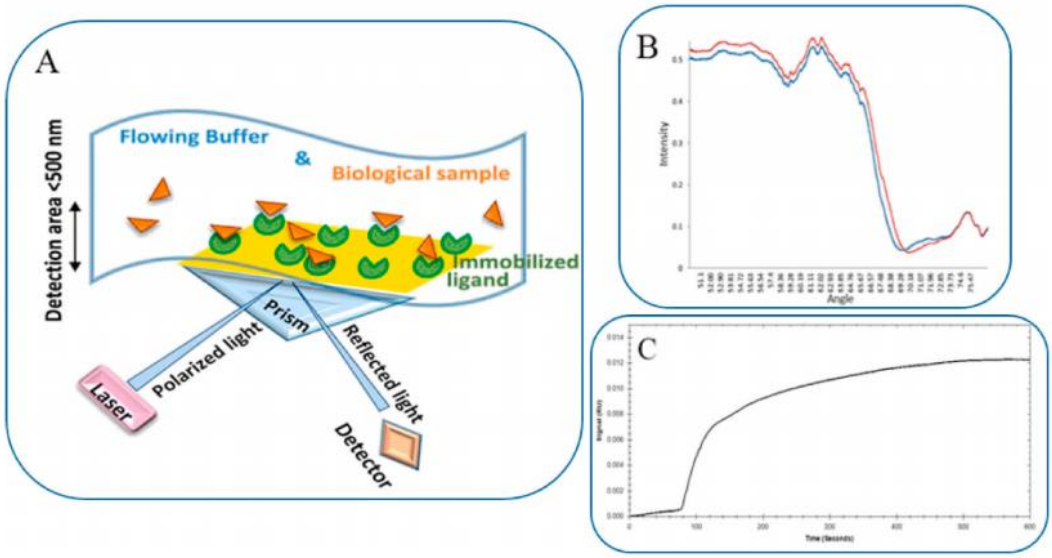

Figure 19: A. schematic diagram of SPR process B. Variation of the angle before and after the biomolecule is bound in SPR C. A sonogram of the biomolecule adsorbed onto the surface of the sensor in real time (Sci-Hub | Surface plasmon resonance biosensors for detection of alzheimer's biomarkers; an effective step in early and accurate diagnosis. Biosensors and Bioelectronics, 112511 | 10.1016/j.bios.2020.112511, n.d.).

4.2.1 Mechanism of SPR assay:

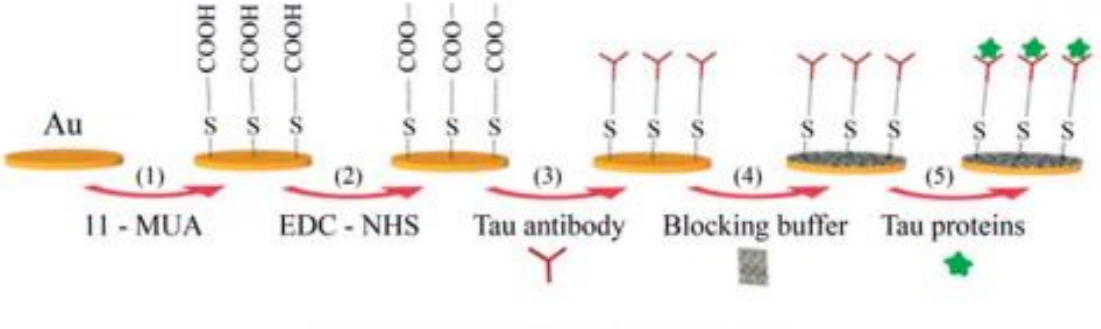

Figure 20: Schematic diagram of SPR assay for Tau protein detection (Vu Nu et al., 2018).

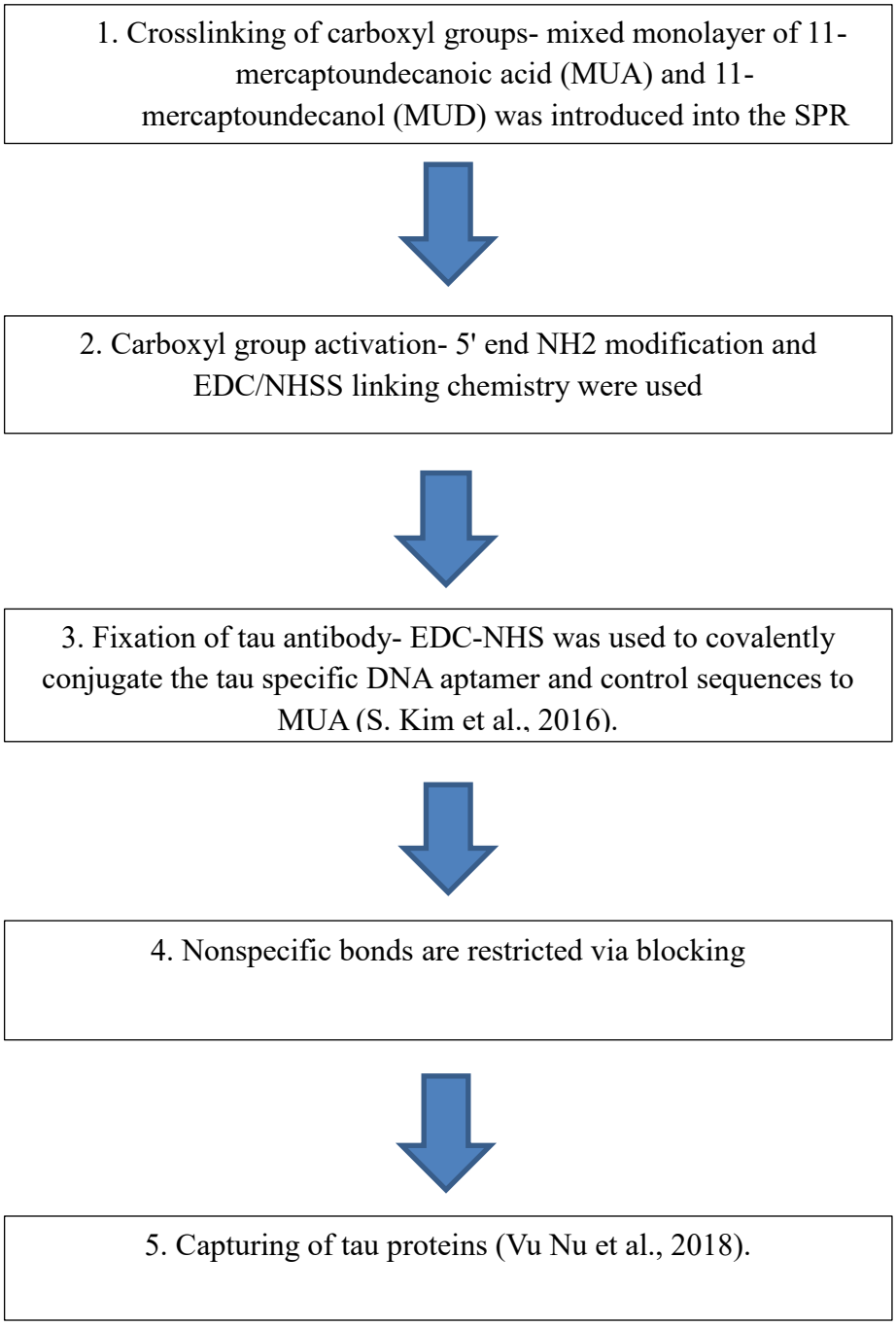

Figure 21: Mechanism of SPR assay.

A researcher named, Lisi et al, Focused on SPR platform and antibodies (Lisi et al., 2017) had enveloped three techniques of qualitative detection of Tau which includes a) direct detection b) unlabeled sandwich c) labeled sandwich and the corresponding linear ranges of Tau were 7–250 nmol/L, 2–25 nmol/L and 125–1000 nmol/L, and the Limit of detection (LODs) were 15, 2 and 0.125 nmol/L, respectively as shown in figure 21.

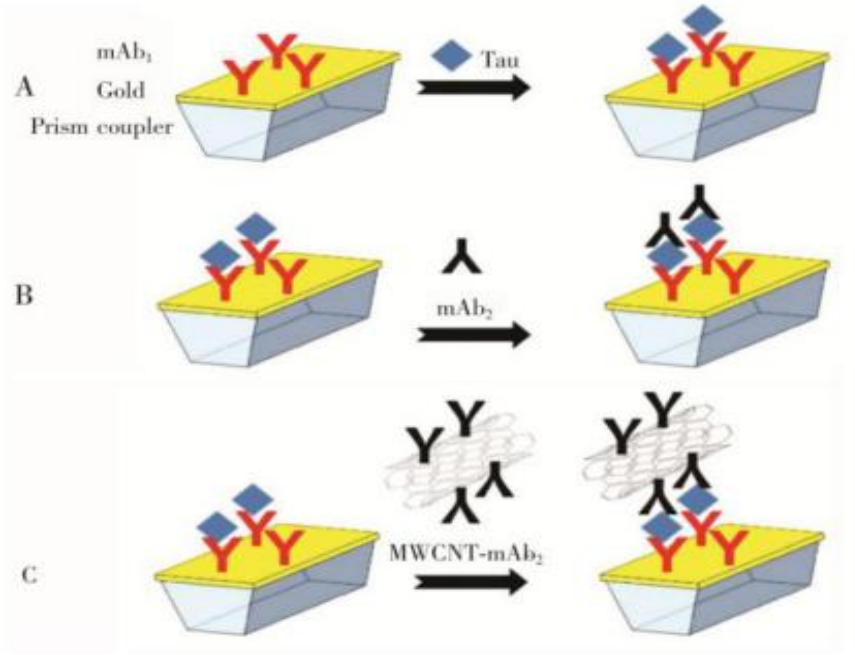

Figure 22: Three techniques of SPR platform a) direct detection b) label free sandwich assay c) multi-walled carbon nanotube-labeled sandwich assay (Lisi et al., 2017).

a) Direct detection method is where the primary monoclonal antibody (mAb1) directly conjugates with tau protein
b) Label free sandwich assay is where a secondary monoclonal antibody (mAb2) was used to further bind to tau protein which was already bound to primary monoclonal antibody (mAb1). Hence forming a sandwich.

c) Multi-walled carbon nanotube (MWCNT)-labeled sandwich assay- It is another step to b where MWCNT is bound to mAb2. A Multi-walled carbon nanotube (MWCNTs) was used to diagnose sensitive, rapid and selective tau protein in AD (Lisi et al., 2017). The metal nanoparticles, carbon-based nanomaterials, which includes graphene oxide and carbon nanotubes (CNTs) due to their distinctive properties like higher molecular mass, larger surface area, greater RI and stronger suitable adhesion of molecules can boost the SPR signals (Gupta et al., 2019). MWCNTs modified with tau protein-related secondary antibody and a sandwich method for the binding of secondary monoclonal tau antibody (mAb2) on MWCNTs interface in order to produce appropriate signals (Rezabakhsh et al., 2020).

4.2.2 Advantages of SPR technique

- SPR imaging technique allows real-time and tag free analysis for both control and experimental groups.
- Researchers have been able to produce accurate and effective methodologies for femto-molar series in diagnosis of AD markers in sufferers.
- Various proportions of AD antibodies or nucleic adjuvants can be immobilized on SPR gold chips with using SPR method using modified surface chemistry (Palchetti et al., 2019).

4.3 Quartz Crystal Microbalance (QCM)

The Quartz Crystal Microbalance (QCM) is a highly sensitive mass balance that detects changes in mass per unit area at the nanogram to microgram level. Quartz is a piezoelectric substance that is induced to vibrate at a specific frequency via supplying voltage using metal electrodes. The insertion or elimination of small quantity of mass from the electrode surface can cause a deflection in frequency of vibration. Therefore, the shift in frequency can be tracked in real time to learn about the biomolecular interaction and reactions occurring in the interface of the electrode such as film growth, oxidation, corrosion/decay, etc (Quartz Crystal Microbalance (QCM) - Nanoscience Instruments, n.d.-a).

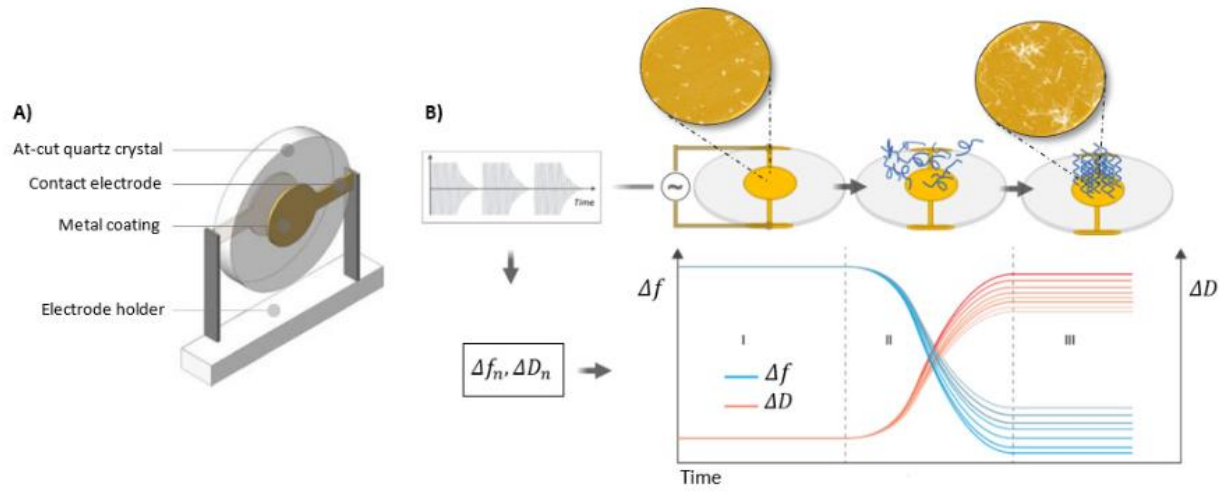

Figure 23: (A) Illustrates the QCM biosensor at which the quartz crystal is coated with two gold electrodes on either side. (B) Illustrates the basic operation of QCM (Migó et al., n.d.).

The quartz crystal was covered with two gold electrodes on either side as shown in fig23. In the Δf and ΔD graph, shows the basic operation which shows how the change in Δf and ΔD changes as the molecule mass binds to gold surface sensor. The clean surface and stable

baselines are shown in section I of the schematic adsorption mechanism; during adsorption, molecular variations in f and D are noticed (section II). The baselines are stabilized after complete adsorption on the surface (section III) of figure 23 (Migó et al., n.d.).

4.3.1 Detection of Tau protein

a) For Direct Assay

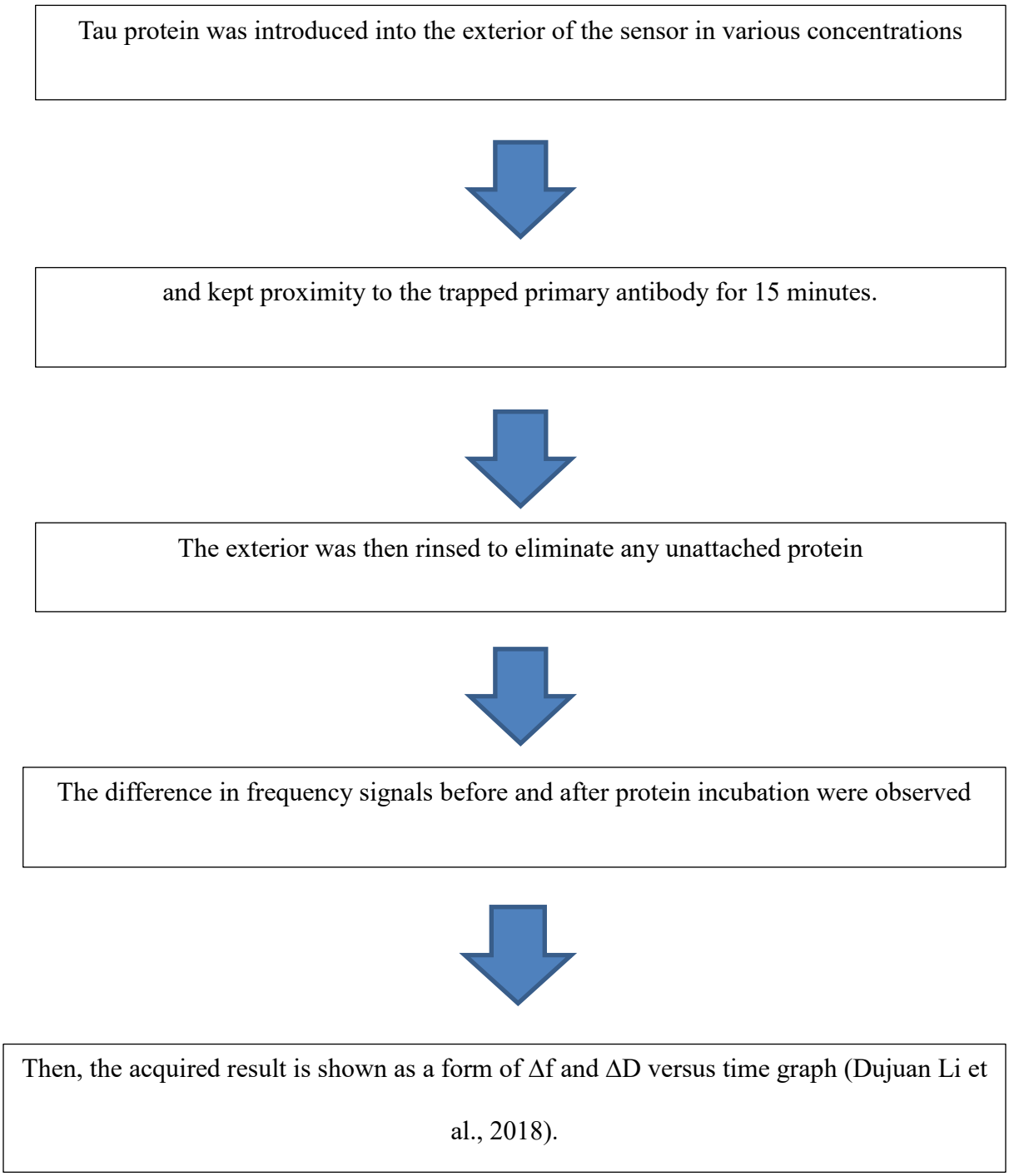

Figure 24: Direct assay method.

b) For sandwich-based assay

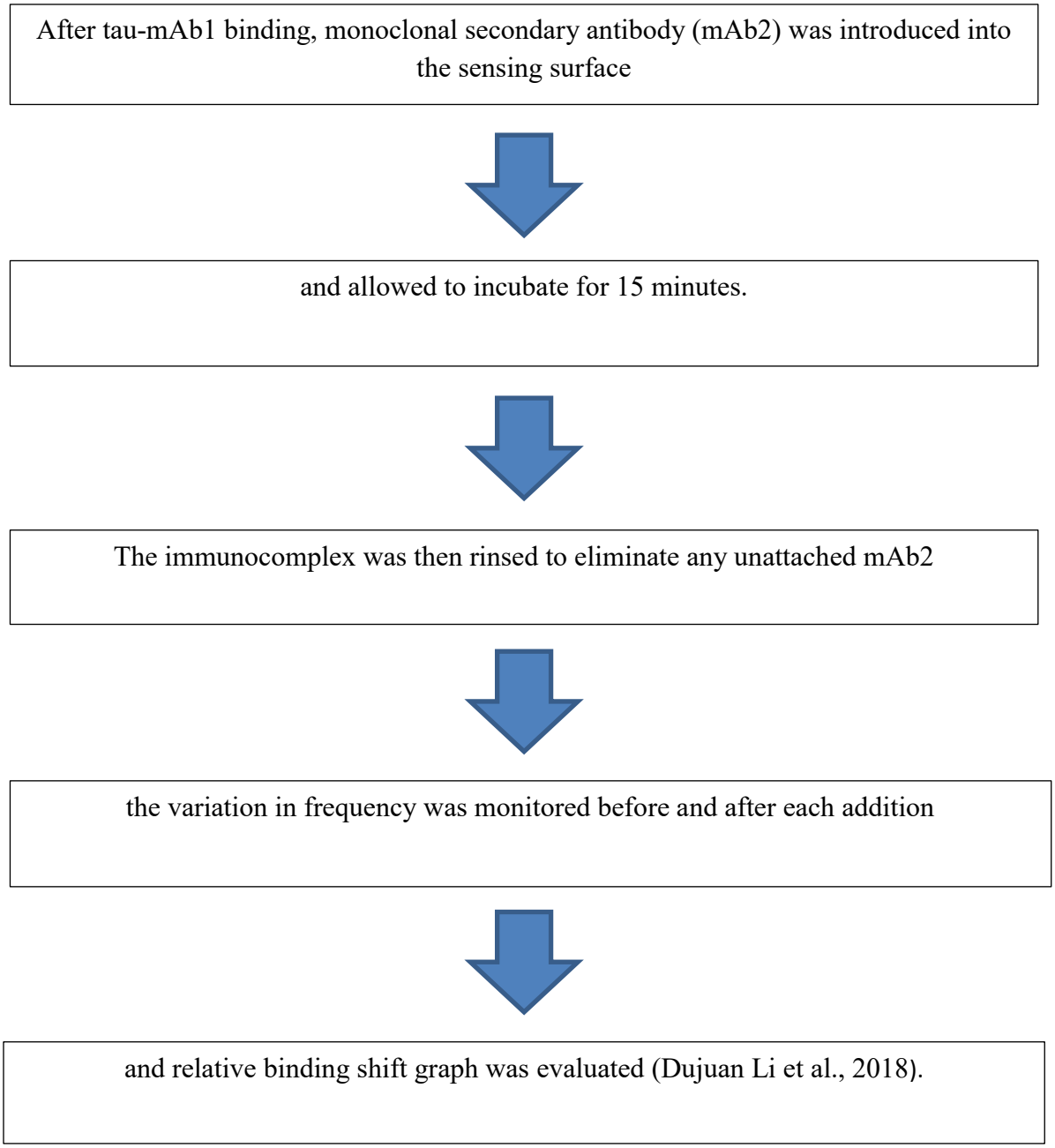

Figure 25: Sandwich-based assay.

4.3.2 Sensitivity of QCM

1. Because of its excellent resonator stability, the frequency alteration in QCMs can be detected on crystals with resolution of 1 Hz or lesser. As a result, measurement of nanogram-scale masses can be attained (Quartz Crystal Microbalance (QCM) - Nanoscience Instruments, n.d.-b).
2. The maximum thickness that can be detected ranging from hundreds of nanometers to a few microns, based upon the hardness of the surface (Quartz Crystal Microbalance (QCM) - Nanoscience Instruments, n.d.-b).
3. Sensitivity improves as the concentration of the injected analyte rises (Hadi Shinen et al., 2014).

4.4 Surface-enhanced Raman scattering (SERS) based sandwich assay

Surface-enhanced Raman spectroscopy (SERS), a biomolecular detecting technology that intensifies Raman scattering whenever the targeted substrate gets deposited onto the top of a coarse metal or using nanoparticles such as plasmonic- magnetic silica nanotubes, is based on Raman scattering, a phenomenon in which photons scatter inelastically. The Raman spectroscopy signals are significantly enhanced by the electromagnetic environment surrounding nanostructures and nanomaterials. SERS biosensors have proven to be precise yet promising technology for detecting solutes in solutions at low doses due to its high sensitivity. Hence, SERS biosensor was established for tau protein with limit of detection less than 25 femtomolar.

4.4.1 Detection of Tau protein

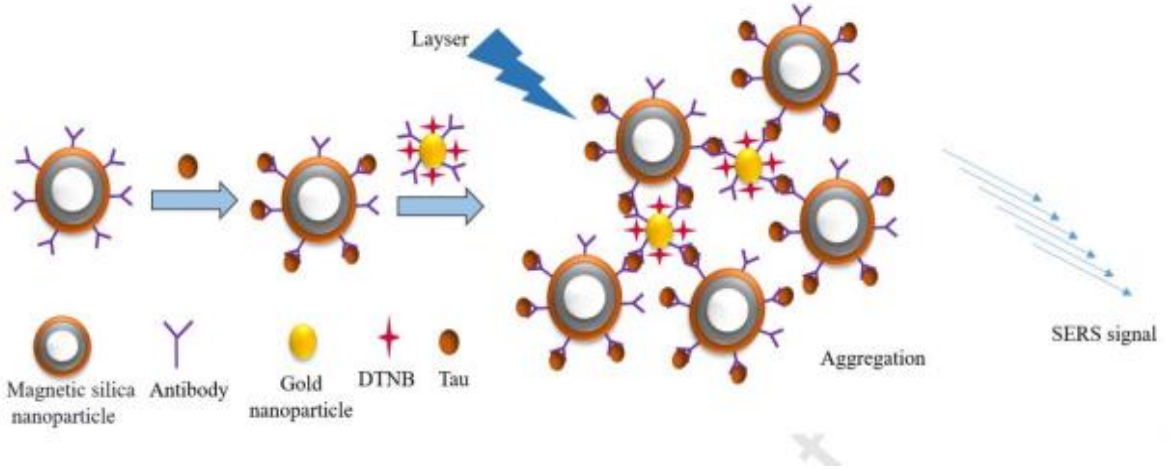

Figure 26: Tau protein detection using a SERS biosensor (*Sci-Hub | Biosensors for detection of Tau protein as an Alzheimer's disease marker. International Journal of Biological Macromolecules | 10.1016/j.ijbiomac.2020.06.239*, n.d.).

It is comprised of magnetic silica nanoparticles and monoclonal anti -tau as trapping reagents having high affinity to binding tau protein within the samples as well as functional gold nanoparticles with polyclonal anti -tau acting as SERS surface.

A EDC/NHS polyclonal anti-tau layer deposited on the interface of gold nanoparticles to activate them

Also, the Raman reporter 5, 5 -dithiobis (2 -dinitrobenzoic acid) (DTNB) has employed to coat gold nanoparticles surface

During low concentration tau protein samples, using polyclonal anti-tau as bioreceptor component enhances the signal strength

Collision between anti-tau and tau protein solution leads to nanoparticle accumulation

SERS signal can be seen which produces strong peak in the SERS spectrum due to DTNB absorption.

Peak height may fluctuate depending on tau concentration in the sample (Sci-Hub | Biosensors for detection of Tau protein as an Alzheimer's disease marker. International Journal of Biological Macromolecules | 10.1016/j.ijbiomac.2020.06.239, n.d.).

Figure 27: Detection mechanism of Tau protein using SERS method.

4.4.2 Specificity & sensitivity of SERS:

1. SERS, is an exceptional technique for the detection of relatively low concentration analyte (SERS: Materials, applications, and the future | Elsevier Enhanced Reader, n.d.).
2. A researcher named, Zengin et al. acquired SERS intensity from the response of IgG, Tau, BSA complexes which showed no detectable variation, indicating that BSA and IgG had least effect on tau detection (Zengin et al., 2013).

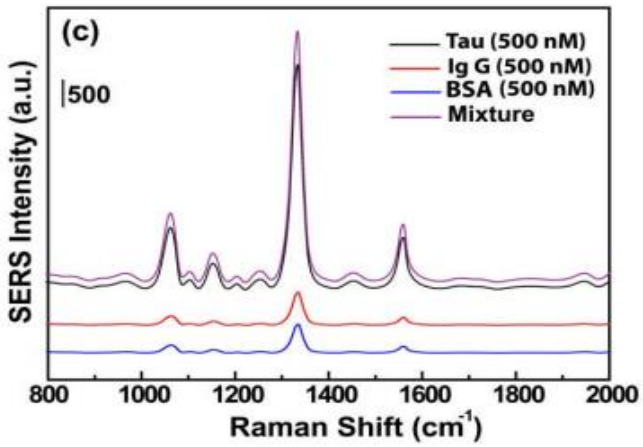

Figure 28: SERS intensity of IgG, Tau and BSA complexes (Zengin et al., 2013).

Chapter 5: Conclusion and Future Prospects

As dementia is most commonly affects elderly people of around 60 years and the most leading cause of dementia is Alzheimer's disease. Tau proteins are essential biomarker for the detection of AD and other forms of neurodegenerative diseases and to monitor the disease progression. This enables with various clinical trials for the initial detection of disease and to assess the efficiency of the drug for preventing its progression. Tau proteins are classified into six isoforms created via alternative splicing and are distinguished based on the composition of three (3R) or four (4R) tubulin binding domains (Goedert et al. 1989). Primary role of tau is to hold tight and stabilize the microtubules (MT) and is mostly distributed in the brain along the axons of the neuron. Also it is believed that aberrant posttranslational modifications which are hyperphosphorylation, acetylation, glycation, nitration and truncation are the major reason of tau pathology. Therefore, it can hamper the stability of microtubule. However, there are various current methodologies that are commercially available to diagnose tau pathology in AD. Multiple novel PET radiotracers are found that binds to tau protein for instance, Tauvid is a PET tracer that is first approved by FDA which is highly selective in nature. However, biosensors based technique like BLI have come up which are analytical low cost devices that can screen the target molecules in a very small period of time with excellent sensitivity and specificity in the field of research (Ameri et al., 2020). BLI is a label free technique used to determine the biomolecular collisions without the need of any tagged reagents. Likewise, Digital ELISA is also a commercially revolved method that is approximately 1000 times more accurate compared to traditional ELISA methodology. However, due to each of the techniques has its own limitations the researchers have come up with more simpler methodologies such as SPR, QCM, SERS that can easily detect the target molecules with excellent in the field of research. SPR biosensor has

the capability to detect even the tiniest mass changes on the gold sensor surface. QCM is a highly sensitive mass balance that detects variation in mass per unit area at the nanogram to microgram level whereas SERS-based biosensors tend to be used for detecting proteins even at as low as 25 femtomolar dose with high sensitivity. Therefore, all of these techniques could be used to track the inhibitors that target tau protein deposits or phosphorylates. A further enhancement of the sensor's sensitivity may be acquired by improving the aptamer pattern and by limiting the non-specific adsorption. Finally, precise detection of modified tau protein from cerebrospinal fluid and other biological fluids may help forecast the development and progression of dementia.

References

Acetylation of Tau Inhibits Its Degradation and Contributes to Tauopathy | Elsevier Enhanced Reader. (n.d.). Retrieved June 2, 2021, from https://reader.elsevier.com/reader/sd/pii/S0896627310006872?token=2A212C3F7AE1F7F9A1D42A64B27A9CC726F28A6F29E4E2F67E4DA3B9F6CE4C591F6366BDF2548F930DF23A2E567636E9&originRegion=eu-west-1&originCreation=20210601182708

Alonso, A. D. C., Zaidi, T., Novak, M., Grundke-Iqbal, I., & Iqbal, K. (2001a). *Hyperphosphorylation induces self-assembly of into tangles of paired helical filamentsstraight filaments*. www.pnas.orgcgidoi10.1073pnas.121119298

Alonso, A. D. C., Zaidi, T., Novak, M., Grundke-Iqbal, I., & Iqbal, K. (2001b). Hyperphosphorylation induces self-assembly of τ into tangles of paired helical filaments/straight filaments. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(12), 6923–6928. https://doi.org/10.1073/pnas.121119298

Alonso, A. D., Di Clerico, J., Li, B., Corbo, C. P., Alaniz, M. E., Grundke-Iqbal, I., & Iqbal, K. (2010). Phosphorylation of Tau at Thr212, Thr231, and Ser262 combined causes neurodegeneration. *Journal of Biological Chemistry*, *285*(40), 30851–30860. https://doi.org/10.1074/jbc.M110.110957

Ameri, M., Shabaninejad, Z., Movahedpour, A., Sahebkar, A., Mohammadi, S., Hosseindoost, S., Ebrahimi, M. S., Savardashtaki, A., Karimipour, M., & Mirzaei, H. (2020). Biosensors for detection of Tau protein as an Alzheimer's disease marker. *International Journal of Biological Macromolecules*, *162*, 1100–1108. https://doi.org/10.1016/j.ijbiomac.2020.06.239

Arbaciauskaite, M., Lei, Y., & Cho, Y. K. (2021). High-specificity antibodies and detection methods for quantifying phosphorylated tau from clinical samples. *Antibody Therapeutics*, *4*(1), 34–44. https://doi.org/10.1093/abt/tbab004

Barbier, P., Zejneli, O., Martinho, M., Lasorsa, A., Belle, V., Smet-Nocca, C., Tsvetkov, P. O., Devred, F., & Landrieu, I. (2019). Role of tau as a microtubule-associated protein: Structural and functional aspects. In *Frontiers in Aging Neuroscience* (Vol. 10, Issue JUL). Frontiers Media S.A. https://doi.org/10.3389/fnagi.2019.00204

Bio-layer Interferometry (BLI) Technology for Coronavirus Research - Coronavirus. (n.d.). Retrieved August 18, 2021, from https://www.creative-biostructure.com/coronavirus/bio-layer-interferometry-bli-p35.htm

BLI Technology | Sartorius. (n.d.). Retrieved August 18, 2021, from https://www.sartorius.com/en/applications/life-science-research/label-free-detection/bli-technology#id-523146

Buée, L., Bussière, T., Buée-Scherrer, V., Delacourte, A., & Hof, P. R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. In *Brain Research Reviews* (Vol. 33, Issue 1, pp. 95–130). Elsevier B.V. https://doi.org/10.1016/S0165-0173(00)00019-9

Carrell, R. W., & Gooptu, B. (1998). Conformational changes and disease - Serpins, prions and Alzheimer's. *Current Opinion in Structural Biology*, *8*(6), 799–809. https://doi.org/10.1016/S0959-440X(98)80101-2

Chien, D. T., Szardenings, A. K., Bahri, S., Walsh, J. C., Mu, F., Xia, C., Shankle, W. R., Lerner, A. J., Su, M.-Y., Elizarov, A., & Kolb, H. C. (2014). Early Clinical PET Imaging Results with the Novel PHF-Tau Radioligand [F18]-T808. *Journal of Alzheimer's Disease*, *38*, 171–184. https://doi.org/10.3233/JAD-130098

Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehman, M., Walther, T. C., Olsen, J. V., & Mann, M. (2009). Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science*, *325*(5942), 834–840. https://doi.org/10.1126/science.1175371

Cohen, T. J., Guo, J. L., Hurtado, D. E., Kwong, L. K., Mills, I. P., Trojanowski, J. Q., & Lee, V. M. Y. (2011). *The acetylation of tau inhibits its function and promotes pathological tau aggregation*. https://doi.org/10.1038/ncomms1255

De Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, D. H., Kopeikina, K. J., Pitstick, R., Sahara, N., Ashe, K. H., Carlson, G. A., Spires-Jones, T. L., & Hyman, B. T. (2012). Propagation of Tau Pathology in a Model of Early Alzheimer's Disease. *Neuron*, *73*(4), 685–697. https://doi.org/10.1016/j.neuron.2011.11.033

Dephosphorylation of Alzheimer Paired Helical Filaments by Protein Phosphatase-2A and âˆ'2B (â€—) | Elsevier Enhanced Reader. (n.d.). Retrieved May 31, 2021, from https://reader.elsevier.com/reader/sd/pii/S0021925818828496?token=13A7C35D1CE88600548E6343C59CD054BFF5B786613DA96C11DFA7DFF108D6A5C4D7A26B8D70A50C5DC32247FD29513B&originRegion=eu-west-1&originCreation=20210531115226

Dickey, C. A., Kamal, A., Lundgren, K., Klosak, N., Bailey, R. M., Dunmore, J., Ash, P., Shoraka, S., Zlatkovic, J., Eckman, C. B., Patterson, C., Dickson, D. W., Stanley Nahman Jr, N., Hutton, M., Burrows, F., & Petrucelli, L. (2007). The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. *The Journal of Clinical Investigation*, *117*. https://doi.org/10.1172/JCI29715

Do, T., Ho, F., Heidecker, B., Witte, K., Chang, L., & Lerner, L. (2008). A rapid method for determining dynamic binding capacity of resins for the purification of proteins. *Protein Expression and Purification*, *60*(2), 147–150. https://doi.org/10.1016/j.pep.2008.04.009

Drescher, D. G., Ramakrishnan, N. A., & Drescher, M. J. (2009). *Surface Plasmon Resonance (SPR) Analysis of Binding Interactions of Proteins in Inner-Ear Sensory Epithelia*. https://doi.org/10.1007/978-1-59745-523-7_20

Drewes, G., Trinczek, B., Illenberger, S., Biernat, J., Schmitt-Ulms, G., Meyer, H. E., Mandelkow, E.-M., & Mandelkow, E. (1995). *THE JOURNAL OF BIOLOGICAL CHEMISTRY Microtubule-associated ProteinlMicrotubule Affinity-regulating Kinase (pll0 m a r k) A NOVEL PROTEIN KINASE THAT REGULATES TAU-MICROTUBULE INTERACTIONS AND DYNAMIC INSTABILITY BY PHOSPHORYLATION AT THE ALZHEIMER-SPECIFIC SITE SERINE 262** (Vol. 270, Issue 13).

Ebneth, A., Godemann, R., Stamer, K., Illenberger, S., Trinczek, B., Mandelkow, E. M., & Mandelkow, E. (1998). Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: Implications for Alzheimer's disease. *Journal of Cell Biology*, *143*(3), 777–794. https://doi.org/10.1083/jcb.143.3.777

Futerman, A. H., & Banker, G. A. (1996). The economics of neurite outgrowth - The addition of new membrane to growing axons. In *Trends in Neurosciences* (Vol. 19, Issue 4, pp. 144–149). Elsevier Ltd. https://doi.org/10.1016/S0166-2236(96)80025-7

Goedert, M., & Jakes, R. (1990). Expression of separate isoforms of human tau protein: Correlation with the tau pattern in brain and effects on tubulin polymerization. *EMBO Journal*, *9*(13), 4225–4230. https://doi.org/10.1002/j.1460-2075.1990.tb07870.x

Goedert, Michel, Jakes, R., & Crowther, R. A. (n.d.). *E¡ects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau ¢laments*.

Gong, C.-X., & Iqbal, K. (2008). Hyperphosphorylation of Microtubule-Associated Protein Tau: A Promising Therapeutic Target for Alzheimer Disease. *Current Medicinal Chemistry*, *15*(23), 2321–2328. https://doi.org/10.2174/092986708785909111

Hadi Shinen, M., Omran Essa, F., & Saieb Naji, A. (2014). *Study the Sensitivity of Quartz Crystal Microbalance (QCM) Sensor Coated with Different Thickness of Polyaniline for Determination Vapors of Ether, Chloroform, Carbon tetrachloride and Ethyl acetate*. *6*(3). www.iiste.org

Harada, R, Okamura, N., Furumoto, S., & Yanai, K. (2018). Imaging Protein Misfolding in the Brain Using β-Sheet Ligands. *Front. Neurosci*, *12*, 585. https://doi.org/10.3389/fnins.2018.00585

Harada, Ryuichi, Okamura, N., Furumoto, S., Furukawa, K., Ishiki, A., Tomita, N., Tago, T., Hiraoka, K., Watanuki, S., Shidahara, M., Miyake, M., Ishikawa, Y., Matsuda, R., Inami, A., Yoshikawa, T., Funaki, Y., Iwata, R., Tashiro, M., Yanai, K., … Kudo, Y. (2016). *F-THK5351: A Novel PET Radiotracer for Imaging Neurofibrillary Pathology in Alzheimer Disease*. https://doi.org/10.2967/jnumed.115.164848

Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology*, *80*(19), 1778–1783. https://doi.org/10.1212/WNL.0b013e31828726f5

Imaging of Tau Pathology in a Tauopathy Mouse Model and in Alzheimer Patients Compared to Normal Controls | Elsevier Enhanced Reader. (n.d.-a). Retrieved August 10, 2021, from https://reader.elsevier.com/reader/sd/pii/S0896627313006612?token=6A215F5C251C7F23DFD68602EE72F3BD2E57C857AF495CBD246A3EC26A11791DC7E407605CD475A649636CA1A34DB348&originRegion=eu-west-1&originCreation=20210810074519

Imaging of Tau Pathology in a Tauopathy Mouse Model and in Alzheimer Patients Compared to Normal Controls | Elsevier Enhanced Reader. (n.d.-b). Retrieved August 10, 2021, from https://reader.elsevier.com/reader/sd/pii/S0896627313006612?token=5928F7AAF53E63046FBF62FD8AB03399C12F879908E0F82E6D52A1371C1203854A6EC70215EDC4EFD955D65DCBF04E98&originRegion=eu-west-1&originCreation=20210810140407

Iqbal, K., Liu, F., Gong, C.-X., & Grundke-Iqbal, I. (2010). *Tau in Alzheimer Disease and Related Tauopathies*.

Jie, C. V. M. L., Treyer, V., Schibli, R., & Mu, L. (2021). *pharmaceuticals Tauvid^{TM}: The First FDA-*

Approved PET Tracer for Imaging Tau Pathology in Alzheimer's Disease. https://doi.org/10.3390/ph14020110

Kent, S. A., Spires-Jones, T. L., & Durrant, C. S. (2020). The physiological roles of tau and Aβ: implications for Alzheimer's disease pathology and therapeutics. *Acta Neuropathologica*, *140*, 417–447. https://doi.org/10.1007/s00401-020-02196-w

Kim, S. H., Iwai, S., Araki, S., Sakakihara, S., Iino, R., & Noji, H. (2012). Large-scale femtoliter droplet array for digital counting of single biomolecules. *Lab on a Chip*, *12*(23), 4986–4991. https://doi.org/10.1039/c2lc40632b

Kim, S., Wark, A. W., & Lee, H. J. (2016). Femtomolar Detection of Tau Proteins in Undiluted Plasma Using Surface Plasmon Resonance. *Analytical Chemistry*, *88*(15), 7793–7799. https://doi.org/10.1021/acs.analchem.6b01825

Kolarova, M., García-Sierra, F., Bartos, A., Ricny, J., & Ripova, D. (2012a). Structure and pathology of tau protein in Alzheimer disease. *International Journal of Alzheimer's Disease*, *2012*. https://doi.org/10.1155/2012/731526

Kolarova, M., García-Sierra, F., Bartos, A., Ricny, J., & Ripova, D. (2012b). Structure and pathology of tau protein in Alzheimer disease. In *International Journal of Alzheimer's Disease*. https://doi.org/10.1155/2012/731526

Kolarova, M., García-Sierra, F., Bartos, A., Ricny, J., & Ripova, D. (2012c). Structure and Pathology of Tau Protein in Alzheimer Disease. *International Journal of Alzheimer's Disease*, *2012*, 13. https://doi.org/10.1155/2012/731526

Lemoine, L., Gillberg, P.-G., Svedberg, M., Stepanov, V., Jia, Z., Huang, J., Nag, S., Tian, H., Ghetti, B., Okamura, N., Higuchi, M., Halldin, C., & Nordberg, A. (n.d.). *Comparative binding properties of the tau PET tracers THK5117, THK5351, PBB3, and T807 in postmortem Alzheimer brains*.

https://doi.org/10.1186/s13195-017-0325-z

Li, Danni, & Mielke, M. M. (2019). An Update on Blood-Based Markers of Alzheimer's Disease Using the SiMoA Platform. *Neurology and Therapy*, *8*, 73–82. https://doi.org/10.1007/S40120-019-00164-5

Li, Dujuan, Scarano, S., Lisi, S., Palladino, P., & Minunni, M. (2018). Real-time tau protein detection by sandwich-based piezoelectric biosensing: Exploring tubulin as a mass enhancer. *Sensors (Switzerland)*, *18*(4). https://doi.org/10.3390/s18040946

Lisi, S., Scarano, S., Fedeli, S., Pascale, E., Cicchi, S., Ravelet, C., Peyrin, E., & Minunni, M. (2017). Toward sensitive immuno-based detection of tau protein by surface plasmon resonance coupled to carbon nanostructures as signal amplifiers. *Biosensors and Bioelectronics*, *93*, 289–292. https://doi.org/10.1016/j.bios.2016.08.078

Liu, F., Iqbal, K., Grundke-Iqbal, I., Rossie, S., & Gong, C. X. (2005). Dephosphorylation of tau by protein phosphatase 5: Impairment in Alzheimer's disease. *Journal of Biological Chemistry*, *280*(3), 1790–1796. https://doi.org/10.1074/jbc.M410775200

Liu, F., Zaidi, T., Iqbal, K., Grundke-Iqbal, I., Merkle, R. K., & Gong, C.-X. (n.d.). *Role of glycosylation in hyperphosphorylation of tau in Alzheimer's disease*.

Luna-Munoz, J., R., C., M., C., Flores-Rodriguez, P., Avila, J., R., S., la Cruz, F. De, Mena, R., A., M., & Floran-Garduno, B. (2013). Phosphorylation of Tau Protein Associated as a Protective Mechanism in the Presence of Toxic, C-Terminally Truncated Tau in Alzheimer's Disease. In *Understanding Alzheimer's Disease*. InTech. https://doi.org/10.5772/54228

Mamun, A. Al, Uddin, M. S., Mathew, B., & Ashraf, G. M. (2020). Toxic tau: Structural origins of tau aggregation in Alzheimer's disease. In *Neural Regeneration Research* (Vol. 15, Issue 8, pp. 1417–1420). Wolters Kluwer Medknow Publications. https://doi.org/10.4103/1673-5374.274329

Mandelkow, E. M., & Mandelkow, E. (2012). Biochemistry and cell biology of Tau protein in neurofibrillary degeneration. *Cold Spring Harbor Perspectives in Medicine*, *2*(7), 1–26. https://doi.org/10.1101/cshperspect.a006247

Martin, L., Latypova, X., & Terro, F. (2011). Post-translational modifications of tau protein: Implications for Alzheimer's disease. In *Neurochemistry International* (Vol. 58, Issue 4, pp. 458–471). Elsevier Ltd. https://doi.org/10.1016/j.neuint.2010.12.023

Mechaly, A., Cohen, H., Cohen, O., & Mazor, O. (2016). A biolayer interferometry-based assay for rapid and highly sensitive detection of biowarfare agents. *Analytical Biochemistry*, *506*, 22–27. https://doi.org/10.1016/j.ab.2016.04.018

Migó, D., Wasilewski, T., & Suchy, D. (n.d.). *molecules Application of QCM in Peptide and Protein-Based Drug Product Development*. https://doi.org/10.3390/molecules25173950

Mosconi, L., Berti, V., Glodzik, L., Pupi, A., De Santi, S., & De Leon, M. J. (n.d.). *Pre-Clinical Detection of Alzheimer's Disease Using FDG-PET, with or without Amyloid Imaging*. https://doi.org/10.3233/JAD-2010-091504

Ono, T., Yamauchi, L., Miyasaka, T., Takashima, A., & Noji, H. (2014). Single-molecule assay for tau protein using digital elisa system. *18th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS 2014*, 2566–2567.

Palchetti, I., Compagnone, D., Chau, L.-K., Spoto, G., Bellassai, N., D'agata, R., & Jungbluth, V. (2019). Surface Plasmon Resonance for Biomarker Detection: Advances in Non-invasive Cancer Diagnosis. *Frontiers in Chemistry | Www.Frontiersin.Org*, *1*, 570. https://doi.org/10.3389/fchem.2019.00570

Qtrojanowski, J., & M-y Lee, V. (n.d.). *Phosphorylation of paired helical filament tau in Alzheimer's disease neurofThrfflary lesions: focusing on phosphatases*. https://doi.org/10.1096/fasebj.9.15.8529836

Quartz Crystal Microbalance (QCM) - Nanoscience Instruments. (n.d.-a). Retrieved September 15, 2021, from https://www.nanoscience.com/techniques/quartz-crystal-microbalance/#qcm

Quartz Crystal Microbalance (QCM) - Nanoscience Instruments. (n.d.-b). Retrieved September 16, 2021, from https://www.nanoscience.com/techniques/quartz-crystal-microbalance/?fbclid=IwAR1zgqwgH1ZFFk75ehdsrJ57UHQZT-UJdnTXviw4Mhlgs5DMITEUUP77fAs#sauerbrey

Rezabakhsh, A., Rahbarghazi, R., & Fathi, F. (2020). Surface plasmon resonance biosensors for detection of Alzheimer's biomarkers; an effective step in early and accurate diagnosis. *Biosensors and Bioelectronics, 167*(July), 112511. https://doi.org/10.1016/j.bios.2020.112511

Sci-Hub | Biosensors for detection of Tau protein as an Alzheimer's disease marker. International Journal of Biological Macromolecules | 10.1016/j.ijbiomac.2020.06.239. (n.d.). Retrieved September 19, 2021, from https://sci-hub.se/https://doi.org/10.1016/j.ijbiomac.2020.06.239

Sci-Hub | Surface plasmon resonance biosensors for detection of alzheimer's biomarkers; an effective step in early and accurate diagnosis. Biosensors and Bioelectronics, 112511 | 10.1016/j.bios.2020.112511. (n.d.). Retrieved August 18, 2021, from https://sci-hub.se/https://doi.org/10.1016/j.bios.2020.112511

Sci-Hub | Use of high-sensitivity digital ELISA improves the diagnostic performance of circulating brain-specific proteins for detection of traumatic brain injury during triage. Neurological Research, 1–8 | 10.1080/01616412.2020.1726588. (n.d.). Retrieved August 21, 2021, from https://sci-hub.se/10.1080/01616412.2020.1726588

Sergeant, N., Bretteville, A., Hamdane, M., Caillet-Boudin, M. L., Grognet, P., Bombois, S., Blum, D., Delacourte, A., Pasquier, F., Vanmechelen, E., Schraen-Maschke, S., & Buée, L. (2008). Biochemistry of Tau in Alzheimer's disease and related neurological disorders. In *Expert Review of*

Proteomics (Vol. 5, Issue 2, pp. 207–224). Future Drugs Ltd. https://doi.org/10.1586/14789450.5.2.207

Sergeant, N., Delacourte, A., & Buée, L. (2005a). Tau protein as a differential biomarker of tauopathies. In *Biochimica et Biophysica Acta - Molecular Basis of Disease* (Vol. 1739, Issue 2, pp. 179–197). https://doi.org/10.1016/j.bbadis.2004.06.020

Sergeant, N., Delacourte, A., & Buée, L. (2005b). Tau protein as a differential biomarker of tauopathies. In *Biochimica et Biophysica Acta - Molecular Basis of Disease* (Vol. 1739, Issue 2, pp. 179–197). https://doi.org/10.1016/j.bbadis.2004.06.020

SERS: Materials, applications, and the future | Elsevier Enhanced Reader. (n.d.). Retrieved September 19, 2021, from https://reader.elsevier.com/reader/sd/pii/S1369702112700172?token=F69ADEE2689388E678AE4DB8F59D8022B6C8D8B6A3A1F00B37C074C04EC6E38B34C5DA3758DEBB9BE03061C845E98335&originRegion=eu-west-1&originCreation=20210919131606

Shin, J., Kepe, V., Barrio, J. R., & Small, G. W. (2011). The Merits of FDDNP-PET Imaging in Alzheimer's Disease. *Journal of Alzheimer's Disease*, *26*, 135–145. https://doi.org/10.3233/JAD-2011-0008

Smailagic, N., Lafortune, L., Kelly, S., Hyde, C., & Brayne, C. (2018). F-FDG PET for Prediction of Conversion to Alzheimer's Disease Dementia in People with Mild Cognitive Impairment: An Updated Systematic Review of Test Accuracy. *Journal of Alzheimer's Disease*, *64*, 1175–1194. https://doi.org/10.3233/JAD-171125

The Scientific Principle of Single-Molecule Array Technology. (n.d.). Retrieved August 21, 2021, from https://www.news-medical.net/whitepaper/20190805/The-Scientific-Principle-of-Single-Molecule-Array-Technology.aspx

Vermunt, L., Sikkes, S. A. M., van den Hout, A., Handels, R., Bos, I., van der Flier, W. M., Kern, S., Ousset, P. J., Maruff, P., Skoog, I., Verhey, F. R. J., Freund-Levi, Y., Tsolaki, M., Wallin, Å. K., Olde Rikkert, M., Soininen, H., Spiru, L., Zetterberg, H., Blennow, K., … Coley, N. (2019). Duration of preclinical, prodromal, and dementia stages of Alzheimer's disease in relation to age, sex, and APOE genotype. *Alzheimer's and Dementia*, *15*(7), 888–898. https://doi.org/10.1016/j.jalz.2019.04.001

Vu Nu, T. T., Tran, N. H. T., Nam, E., Nguyen, T. T., Yoon, W. J., Cho, S., Kim, J., Chang, K. A., & Ju, H. (2018). Blood-based immunoassay of tau proteins for early diagnosis of Alzheimer's disease using surface plasmon resonance fiber sensors. *RSC Advances*, *8*(14), 7855–7862. https://doi.org/10.1039/c7ra11637c

Wang, Y. T., & Edison, P. (1910). *Tau Imaging in Neurodegenerative Diseases Using Positron Emission Tomography*. https://doi.org/10.1007/s11910-019-0962-7

Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., & Kirschner, M. W. (1975). A protein factor essential for microtubule assembly. *Proceedings of the National Academy of Sciences of the United States of America*, *72*(5), 1858–1862. https://doi.org/10.1073/pnas.72.5.1858

Weller, J., & Budson, A. (2018). *Current understanding of Alzheimer ' s disease diagnosis and treatment [version 1 ; referees : 2 approved] Referee Status : 7*(0), 1–9. https://doi.org/10.12688/f1000research.14506.1

Wood, H. (2013). *Alzheimer disease: [11C]PBB3—a new PET ligand that identifies tau pathology in the brains of patients with AD*. https://doi.org/10.1038/nrneurol.2013.216

Yeung, A., Goto, T. K., & Leung, W. K. (2017). The Changing Landscape of Neuroscience Research, 2006–2015: A Bibliometric Study. *A Bibliometric Study. Front. Neurosci*, *11*. https://doi.org/10.3389/fnins.2017.00120

Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and Future Treatments in Alzheimer Disease: An Update. *Journal of Central Nervous System Disease*, *12*, 117957352090739. https://doi.org/10.1177/1179573520907397

Zengin, A., Tamer, U., & Caykara, T. (2013). A SERS-based sandwich assay for ultrasensitive and selective detection of Alzheimer's tau protein. *Biomacromolecules*, *14*(9), 3001–3009. https://doi.org/10.1021/bm400968x

Ziu, I., Laryea, E. T., Alashkar, F., Wu, C. G., & Martic, S. (2020). A dip-and-read optical aptasensor for detection of tau protein. *Analytical and Bioanalytical Chemistry*, *412*(5), 1193–1201. https://doi.org/10.1007/s00216-019-02350-8