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# The Application of Analytical Techniques to Alpha-Synuclein in Parkinson's Disease

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## Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor symptoms such as tremors, rigidity, and bradykinesia, as well as non-motor symptoms including cognitive impairment and mood disorders. A hallmark of PD is the accumulation of alpha-synuclein, a presynaptic neuronal protein that aggregates to form Lewy bodies, leading to neuronal dysfunction and cell death. The study of alpha-synuclein and its pathological forms is crucial for understanding the etiology of PD and developing effective diagnostic and therapeutic strategies. Analytical techniques play a pivotal role in elucidating the structure, function, and aggregation mechanisms of alpha-synuclein. Biochemical methods such as Western blotting and enzyme-linked immunosorbent assay (ELISA) are employed to detect and quantify alpha-synuclein in biological samples, offering insights into its expression levels and post-translational modifications. Imaging techniques like immunohistochemistry and positron emission tomography (PET) allow for the visualization of alpha-synuclein aggregates in tissue samples and *in vivo*, respectively, facilitating the study of its spatial distribution and progression in PD Spectroscopic methods, including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry, provide detailed structural information on alpha-synuclein and its isoforms, aiding in the identification of conformational changes associated with aggregation. Emerging techniques such as cryo-electron microscopy (Cryo-EM) and single-molecule fluorescence enable high-resolution structural analysis and real-time monitoring of alpha-synuclein aggregation dynamics, respectively. The application of these analytical techniques has significantly advanced our understanding of the pathophysiological role of alpha-synuclein in PD. They have contributed to the identification of potential biomarkers for early diagnosis and the evaluation of therapeutic interventions targeting alpha-synuclein aggregation. Despite technical limitations and challenges in clinical translation, ongoing advancements in analytical methodologies hold promise for improving the diagnosis, monitoring, and treatment of

Parkinson's disease through a deeper understanding of alpha-synuclein pathology.

## Keywords

Parkinson's Disease, Alpha-Synuclein, Techniques

## 1. Introduction

Chronic diseases like Parkinson's Disease (PD) involve long-term, progressive degeneration, impacting quality of life and requiring ongoing management and research [1] [2]. According to **Figure 1**, PD is a chronic and progressive neurodegenerative disorder primarily affecting motor function due to the degeneration of dopamine-producing neurons in the substantia nigra, a region of the brain crucial for movement regulation [3]-[5]. Chronic diseases such as PD and tuberculosis require special care and treatment [6]. The hallmark symptoms of PD include tremors at rest, bradykinesia (slowness of movement), muscle rigidity, and postural instability. Non-motor symptoms, which can significantly affect the quality of life, encompass cognitive impairment, mood disorders such as depression and anxiety, sleep disturbances, and autonomic dysfunction [1] [7]. These symptoms collectively lead to a gradual decline in the ability to perform daily activities, thereby necessitating comprehensive care and management. Parkinson's disease is the second most common neurodegenerative disorder, affecting approximately 1% of the population over the age of 60 [8]. Its prevalence increases with age, highlighting the growing burden of the disease in aging populations worldwide. The impact of PD extends beyond the individual, affecting families and caregivers and posing significant socioeconomic challenges [9]. The direct and indirect costs associated with PD, including medical care, lost productivity, and long-term care, impose a substantial economic burden on healthcare systems globally. Understanding the pathophysiology of PD and developing effective diagnostic and therapeutic strategies is, therefore, of paramount importance.

Alpha-synuclein is a small, soluble protein predominantly expressed in the brain, particularly in the presynaptic terminals of neurons [10]. It plays a vital role in synaptic function, including the regulation of neurotransmitter release and vesicle recycling. Although its precise physiological functions are not entirely understood, alpha-synuclein is believed to be involved in maintaining synaptic plasticity and neuronal health. However, in the context of Parkinson's disease, alpha-synuclein undergoes pathological changes that contribute to neurodegeneration. In Parkinson's disease, alpha-synuclein misfolds and aggregates into insoluble fibrils, forming the core component of Lewy bodies—abnormal protein inclusions found in the cytoplasm of neurons [11]. These aggregates disrupt normal cellular functions, leading to mitochondrial dysfunction, oxidative stress, impaired proteostasis, and ultimately, neuronal death. The aggregation process of alpha-synuclein is

a critical pathogenic event in PD, and it is implicated in the spread of pathology across different brain regions [12]. Understanding alpha-synuclein aggregation mechanisms and its toxic effects on neurons is essential for developing targeted therapeutic interventions. As explained in **Figure 2(a)**, the Presynaptic terminal of a dopaminergic neuron represents the well-known hallmark of Parkinson's disease: *α*-synuclein is an important protein for presynaptic dopaminergic vesicle release. **Figure 2(b)** The loss of normal function of this protein promotes the accumulation of dopamine in the cytoplasm that, together with *α*-synuclein oligomers is toxic to neurons (DAT, dopamine active transporter; VMAT, vesicular monoamine transporter)

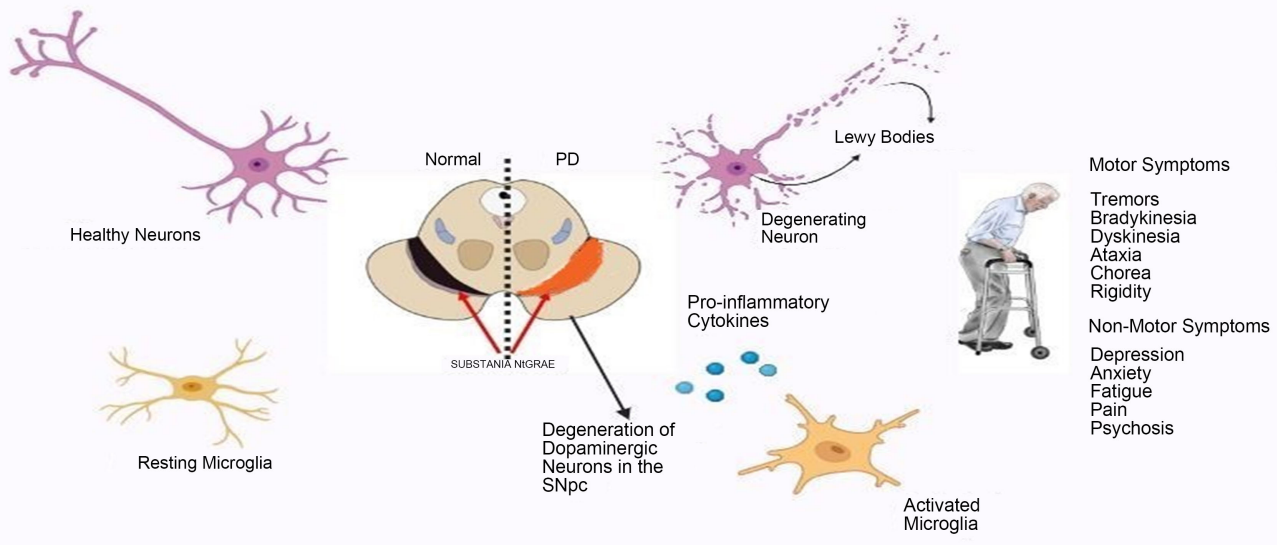


**Figure 1.** Principal hallmarks of Parkinson's disease.

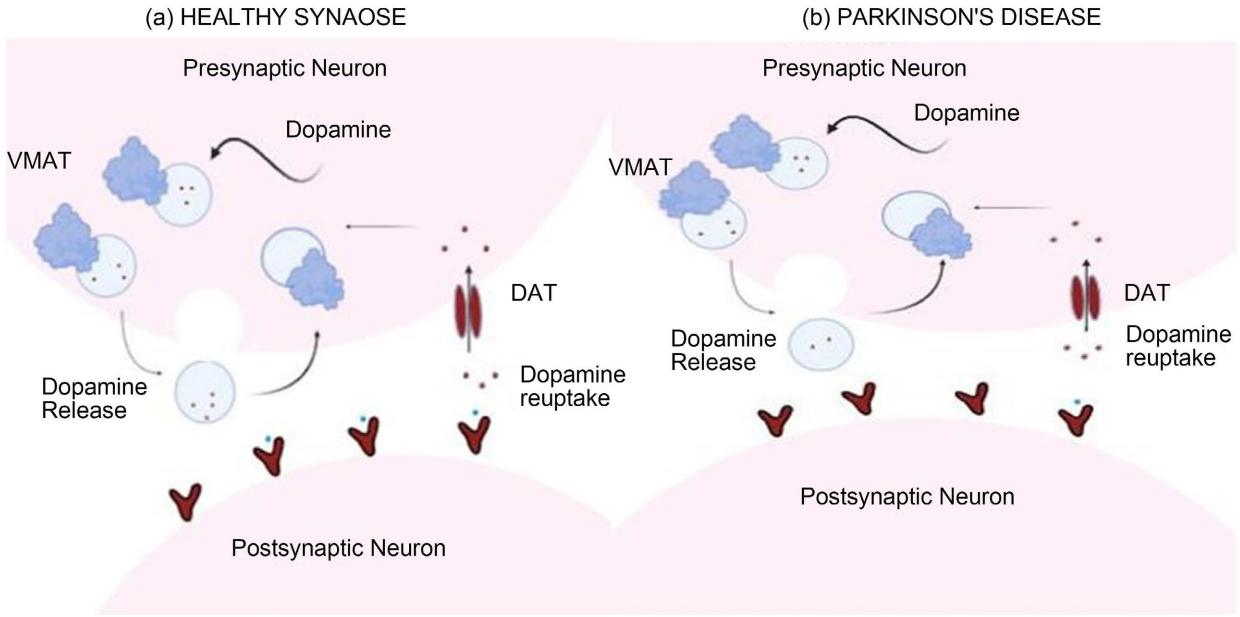


**Figure 2.** Role of normal and mutated *α*-synuclein in synapse.

The primary aim of this review is to explore the various analytical techniques used to study alpha-synuclein in the context of Parkinson's disease. These techniques encompass biochemical, imaging, and spectroscopic methods that provide insights into the structure, function, and aggregation dynamics of alpha-synuclein. By reviewing these techniques, we aim to highlight their contributions to advancing our understanding of alpha-synuclein biology and pathology in PD. Additionally, this paper seeks to assess the impact of these analytical techniques on both the understanding and treatment of Parkinson's disease. Through detailed analysis of alpha-synuclein, researchers have identified potential biomarkers for early diagnosis and progression monitoring. Furthermore, these techniques have facilitated the evaluation of therapeutic strategies aimed at preventing or reversing alpha-synuclein aggregation. By examining the current state of research and future directions, this paper aims to underscore the significance of these analytical tools in the ongoing battle against Parkinson's disease. This review provides a comprehensive overview of the critical role of alpha-synuclein in Parkinson's disease and the analytical techniques employed to study it, emphasizing their importance in improving diagnosis, understanding disease mechanisms, and developing effective treatments.

Parkinson's disease is a neurodegenerative disorder characterized by the accumulation of alpha-synuclein (*α*-syn) protein aggregates in the brain. Understanding the role of *α*-syn in PD has been a significant research focus, with various analytical techniques employed to study its structure, aggregation, and pathological effects. Mass spectrometry has been crucial in characterizing *α*-syn and its post-translational modifications. A study by [13] used mass spectrometry to identify oxidative modifications in *α*-syn, providing insights into how these modifications contribute to protein aggregation and PD pathogenesis. NMR spectroscopy has been instrumental in studying the structural properties of *α*-syn. Research by [14] utilized NMR to elucidate the conformational changes of *α*-syn in different environmental conditions, revealing the structural transitions that promote aggregation. Cryo-EM has advanced the visualization of *α*-syn fibrils at near-atomic resolution. In a landmark study, [15] employed cryo-EM to determine the high-resolution structure of *α*-syn fibrils, uncovering the molecular architecture that underlies their pathogenicity in PD. Immunohistochemical techniques have been widely used to localize *α*-syn in brain tissues. A study by [16] used immunohistochemistry to demonstrate the presence of *α*-syn aggregates in Lewy bodies, the hallmark pathological feature of PD SPR has been used to study the binding interactions of *α*-syn with other molecules. Research by [17] utilized SPR to investigate the interaction between *α*-syn and lipid membranes, shedding light on the initial steps of *α*-syn aggregation in PD.

These studies highlight the diverse analytical techniques employed to understand the role of *α*-syn in Parkinson's disease. Mass spectrometry, NMR spectroscopy, cryo-EM, immunohistochemistry, and SPR contributed to a deeper understanding of *α*-syn's structure, aggregation, and pathological impact. Future

research will continue to build on these findings, leveraging advanced analytical techniques to uncover new therapeutic targets for PD.

## 2. Alpha-Synuclein: Structure and Function

Alpha-synuclein (SNCA) is a 140-amino acid protein encoded by the SNCA gene. It comprises three distinct regions: the N-terminal domain, the non-amyloid-$\beta$ component (NAC) region, and the C-terminal domain [1] [18]. The N-terminal domain (residues 1 - 60) is amphipathic and contains imperfect repeats of an 11-amino acid sequence, facilitating its interaction with lipid membranes. The NAC region (residues 61 - 95) is hydrophobic and is critical for the protein's aggregation propensity. The C-terminal domain (residues 96 - 140) is highly acidic and unstructured, providing solubility to the protein and mediating interactions with other proteins and metals [19]. In its physiological state, alpha-synuclein is predominantly located in the presynaptic terminals of neurons, characterized by synaptic vesicles. It plays several roles in synaptic function, including regulating neurotransmitter release, synaptic plasticity, and recycling synaptic vesicles. Alpha-synuclein is also involved in maintaining the integrity of the neuronal membrane and may participate in the regulation of membrane curvature [20]. Additionally, it has been implicated in modulating dopamine synthesis and storage, highlighting its relevance to dopaminergic neurons, which are critically affected in Parkinson's disease. Alpha-synuclein is a pivotal protein associated with neurodegenerative diseases, particularly Parkinson's disease [21]. Understanding its structure and function is crucial for elucidating its role in these conditions. Table 1 summarizes key aspects of alpha-synuclein's structure and function.

**Table 1.** Summary of alpha-synuclein's structure and function [22].

| Aspect | Description | Significance |
|---|---|---|
| **Primary Structure** | Composed of 140 amino acids, including highly conserved regions. | Essential for maintaining protein function and interactions. |
| **Secondary Structure** | Predominantly unstructured in its native state, with tendencies to form alpha-helices in membranes. | Contributes to the protein's flexibility and interaction with cellular membranes. |
| **Tertiary Structure** | Lacks a well-defined tertiary structure in solution, exhibiting intrinsic disorder. | Impacts its ability to interact with other proteins and lipids. |
| **Quaternary Structure** | Aggregates into fibrils and oligomers, particularly in disease states. | Linked to pathological aggregation in neurodegenerative diseases, such as Parkinson's disease. |
| **Functional Role** | Involved in synaptic vesicle regulation, neurotransmitter release, and neuronal signaling. | Critical for normal dopaminergic neuron function and synaptic plasticity. |
| **Pathological Aggregation** | Misfolds into insoluble aggregates, forming Lewy bodies in Parkinson's disease. | Drives neurodegenerative processes and contributes to disease progression. |

Under pathological conditions, alpha-synuclein undergoes a conformational change from its native, largely unstructured form to misfolded beta-sheet-rich structures [23]. This misfolding leads to the self-assembly of alpha-synuclein monomers into oligomers, protofibrils, and insoluble fibrils. Several factors contribute to this aggregation process, including genetic mutations (such as A53T, A30P, and E46K) and post-translational modifications (e.g., phosphorylation, ubiquitination, and truncation). Environmental factors, oxidative stress, and interactions with metals and other proteins also promote the aggregation of alpha-synuclein [24]. The NAC region is particularly crucial in this process, acting as a core nucleation site for fibril formation. The aggregation of alpha-synuclein into fibrils leads to the formation of Lewy bodies and Lewy neurites, which are pathological hallmarks of Parkinson's disease. Lewy bodies are intracellular inclusions composed primarily of aggregated alpha-synuclein, along with other proteins such as ubiquitin, neurofilaments, and heat shock proteins. These inclusions are found in the cytoplasm of neurons and are most prevalent in the substantia nigra, a region critical for motor control. The formation of Lewy bodies is believed to play a central role in the pathogenesis of Parkinson's disease. The presence of these aggregates disrupts normal cellular functions through several mechanisms. They impair proteostasis by overwhelming the cellular protein degradation machinery, leading to the accumulation of damaged proteins [25]. Lewy bodies also induce mitochondrial dysfunction and oxidative stress, contributing to neuronal damage and cell death. The spread of misfolded alpha-synuclein between cells is thought to propagate the disease pathology throughout the brain. The toxic effects of alpha-synuclein aggregates are not limited to the cells they form. Soluble oligomeric forms of alpha-synuclein, which precede fibril formation, are particularly neurotoxic. These oligomers can disrupt cellular membranes, leading to ion imbalance and cellular toxicity. They also interfere with synaptic function, impairing neurotransmission and contributing to neurodegenerative processes. Understanding the molecular structure and physiological functions of alpha-synuclein and the mechanisms underlying its pathological aggregation is crucial for developing targeted therapeutic strategies for Parkinson's disease [26]. Therapeutic approaches aimed at preventing alpha-synuclein aggregation, promoting the clearance of aggregates, and protecting neurons from their toxic effects hold promise for altering the course of the disease and improving patient outcomes [27] [1]. Structural features of the alpha-synuclein monomer (**Figure 3**). A structure of the full-length, membrane-bound form of alpha-synuclein (SNCA) protein reveals a conformation in which the N-terminal two-thirds of the protein forms a broken, amphipathic alpha-helix. This structured portion of the protein is responsible for membrane binding, and residues at the very N-terminus are essential for this process. In the nuclear magnetic resonance structure of SNCA, the negatively charged C-terminal tail remains flexible and disordered. The positions of point mutations associated with Parkinson's disease are indicated with arrows and in pink. All mutations are heterozygous, except for p.A53V, which is homozygous.

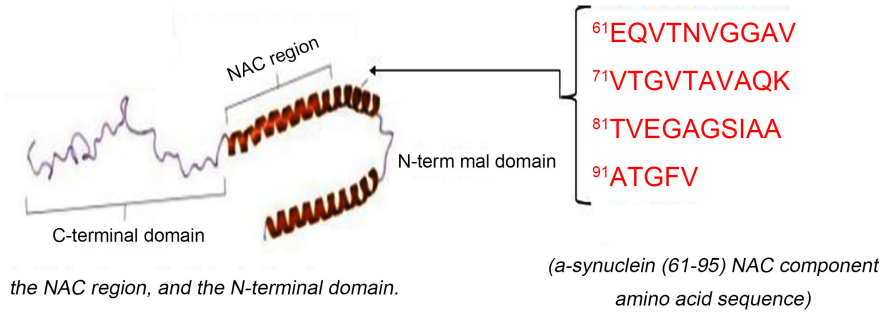


**Figure 3.** Structural features of the alpha-synuclein monomer.

## 2.1. Analytical Techniques for Studying Alpha-Synuclein

Western blotting is a widely used technique for detecting specific proteins within a complex mixture [28]. The process involves separating proteins by gel electrophoresis based on their molecular weight, transferring them onto a membrane, and probing the membrane with specific antibodies against the target protein. The antibody-protein complex is then visualized using various detection methods, such as chemiluminescence or fluorescence [29]. Western blotting is particularly valuable for detecting and quantifying alpha-synuclein in various biological samples, such as brain tissue extracts and cerebrospinal fluid. By using antibodies specific to alpha-synuclein, researchers can identify its different forms, including monomers, oligomers, and aggregated species. This technique allows for the analysis of alpha-synuclein expression levels, post-translational modifications, and the presence of pathological aggregates associated with Parkinson's disease. ELISA is a highly sensitive and specific technique used to detect and quantify proteins in biological samples [30]. The method involves immobilizing an antigen (in this case, alpha-synuclein) on a solid surface, such as a microplate, and then probing it with a specific antibody. A secondary antibody conjugated to an enzyme is used to produce a measurable signal, typically a color change, which is proportional to the amount of target protein present. ELISA is employed to measure alpha-synuclein levels in various biological fluids, such as blood and cerebrospinal fluid [31]. Its high sensitivity and specificity make it an excellent tool for detecting low concentrations of alpha-synuclein, which is crucial for early diagnosis and monitoring of Parkinson's disease progression. ELISA can also be used to distinguish between different alpha-synuclein isoforms and detect post-translational modifications [32].

Immunohistochemistry (IHC) is a technique used to visualize the distribution and localization of specific proteins within tissue sections [33]. The method involves fixing and embedding tissues, sectioning them, and then probing with antibodies specific to the target protein. The antibody-protein complex is detected using chromogenic or fluorescent labels, allowing microscopic visualization. IHC is extensively used to study alpha-synuclein pathology in brain tissues. By using antibodies against alpha-synuclein, researchers can visualize Lewy bodies and neurites, which are hallmark features of Parkinson's disease [34]. This technique

provides valuable insights into the spatial distribution and progression of alpha-synuclein aggregates within different brain regions, aiding in understanding disease mechanisms [6] [35]. PET is a non-invasive imaging technique that uses radiolabeled tracers to visualize and measure biological processes *in vivo*. Tracers are designed to bind specifically to the target of interest, and their distribution and concentration are detected using a PET scanner, providing detailed images of the target within the body. Recent advancements have led to the development of PET tracers that can bind to alpha-synuclein aggregates. This allows for the non-invasive imaging of alpha-synuclein pathology in living patients, providing insights into the distribution and progression of the disease. PET imaging holds promise for early diagnosis, disease progression monitoring, and evaluating therapeutic interventions' efficacy targeting alpha-synuclein [36].

NMR spectroscopy is a powerful technique used to determine the structure and dynamics of proteins at the atomic level [37]. It involves placing a sample in a strong magnetic field and detecting the interaction of nuclear spins with radiofrequency pulses, providing detailed information about the protein's structure and interactions. NMR spectroscopy has been instrumental in studying the structural properties of alpha-synuclein, particularly its intrinsically disordered nature and the conformational changes associated with aggregation [38]. This technique allows researchers to investigate the detailed structural features of monomeric and oligomeric forms of alpha-synuclein, enhancing the understanding of the aggregation process and its pathological implications. Mass spectrometry (MS) is a technique used to identify and quantify proteins based on their mass-to-charge ratio. It involves ionizing the protein sample, separating the ions based on their mass-to-charge ratio, detecting them, and providing detailed information about the protein's composition and modifications. MS is widely used to analyze alpha-synuclein in biological samples, identifying its various isoforms and post-translational modifications [39]. This technique provides precise information about the molecular weight and structure of alpha-synuclein species, aiding in the characterization of pathological forms and understanding their role in Parkinson's disease. **Table 2** summarizes key techniques used in studying alpha-synuclein.

Cryo-EM is an advanced imaging technique that allows for the visualization of macromolecules at near-atomic resolution [41]. It involves rapidly freezing the sample to preserve its native structure and imaging it using an electron microscope, providing high-resolution structural information. Cryo-EM has revolutionized the study of alpha-synuclein fibrils, enabling the detailed visualization of their structural features at high resolution. This technique has provided unprecedented insights into the organization and morphology of alpha-synuclein aggregates, contributing to the understanding of their formation and pathological impact on Parkinson's disease. Single-molecule fluorescence techniques involve the detection and analysis of individual fluorescently labeled molecules, allowing for the observation of dynamic processes at the single-molecule level [42]. This approach provides high sensitivity and temporal resolution. Single-molecule

fluorescence techniques are used to study the real-time dynamics of alpha-synuclein aggregation. By observing individual alpha-synuclein molecules, researchers can gain insights into the kinetics and mechanisms of aggregation, identifying intermediates and transient species that are critical to understanding the aggregation process and developing therapeutic interventions. A variety of analytical techniques are employed to study alpha-synuclein, each providing unique insights into its structure, function, and pathological aggregation [43]. These techniques collectively enhance our understanding of alpha-synuclein biology and its role in Parkinson's disease, paving the way for the development of targeted diagnostic and therapeutic strategies.

**Table 2.** Summarizes key techniques used in studying alpha-synuclein [40].

| Analytical Technique | Description | Applications |
|---|---|---|
| **Western Blotting** | Detects and quantifies alpha-synuclein by separating proteins via gel electrophoresis and probing with specific antibodies. | Determines protein expression levels, identifies post-translational modifications, and compares expression in disease versus control samples. |
| **Enzyme-Linked Immunosorbent Assay (ELISA)** | Uses antibodies immobilized on a plate to quantify alpha-synuclein levels in biological samples. | Provides precise measurements of alpha-synuclein concentration in tissue extracts, cerebrospinal fluid, or serum. |
| **Immunohistochemistry (IHC)** | Employs antibodies to visualize the localization of alpha-synuclein in tissue sections using microscopy. | Identifies the distribution and abundance of alpha-synuclein in brain tissue, helping to reveal pathological features such as Lewy bodies. |
| **Positron Emission Tomography (PET)** | Utilizes radiolabeled tracers to non-invasively visualize and measure alpha-synuclein *in vivo*. | Assesses alpha-synuclein deposition and distribution in the brain, aiding in diagnosis and monitoring of neurodegenerative diseases. |
| **Nuclear Magnetic Resonance (NMR) Spectroscopy** | Uses magnetic fields to study the structure and dynamics of alpha-synuclein in solution. | Allows observation of protein dynamics, conformational flexibility, and interactions with other molecules. |
| **Cryo-Electron Microscopy (Cryo-EM)** | Visualizes protein structures at near-atomic resolution by imaging frozen-hydrated samples. | Useful for studying large protein complexes and fibrils associated with pathological aggregation. |

## 2.2. Applications of Analytical Techniques in Parkinson's Disease Research

Analytical techniques have significantly enhanced our understanding of the pathophysiology of Parkinson's disease, particularly the mechanisms underlying alpha-synuclein aggregation [21]. Techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy and cryo-electron microscopy (Cryo-EM) have elucidated

the structural transitions of alpha-synuclein from its native, unstructured form to aggregated beta-sheet-rich fibrils. NMR provides detailed information on the conformational changes and dynamics of alpha-synuclein at the atomic level, revealing key regions involved in aggregation. Cryo-EM has visualized the high-resolution structure of alpha-synuclein fibrils, uncovering their complex architecture and how specific mutations and post-translational modifications promote aggregation [44]. These insights are crucial for identifying potential therapeutic targets to inhibit or reverse aggregation. Mass spectrometry and Western blotting have been instrumental in identifying the various forms of alpha-synuclein present in Parkinson's disease [45]. These techniques allow for the detection and quantification of monomeric, oligomeric, and fibrillar forms of alpha-synuclein, as well as their post-translational modifications such as phosphorylation, ubiquitination, and truncation. Identifying these pathological forms is essential for understanding their distinct roles in disease progression and for developing specific biomarkers for diagnosis and monitoring [46].

Analytical techniques are at the forefront of biomarker discovery and validation for Parkinson's disease [47]. Enzyme-linked immunosorbent Assay (ELISA) and mass spectrometry are used to quantify alpha-synuclein levels in biological fluids such as cerebrospinal fluid (CSF) and blood [48]. These techniques have enabled the identification of alpha-synuclein isoforms and post-translational modifications that correlate with disease states. For instance, elevated levels of phosphorylated alpha-synuclein in CSF are considered a potential biomarker for Parkinson's disease. The sensitivity and specificity of these techniques are critical for validating biomarkers that can reliably distinguish between Parkinson's disease and other neurodegenerative disorders. Imaging techniques such as positron emission tomography (PET) and immunohistochemistry (IHC) are pivotal in the early detection and monitoring of Parkinson's disease progression [49]. PET imaging with alpha-synuclein-specific tracers allows for the non-invasive visualization of alpha-synuclein aggregates *in vivo*, providing valuable information on the distribution and progression of pathology in the brain. IHC enables the visualization of alpha-synuclein aggregates in post-mortem brain tissue, offering insights into the spatial and temporal patterns of disease progression [50]. These techniques aid in early diagnosis and in tracking the effectiveness of therapeutic interventions over time.

Understanding the aggregation mechanisms of alpha-synuclein has led to the development of therapeutic strategies aimed at preventing or reducing aggregation. Analytical techniques play a crucial role in evaluating these therapeutic approaches [51]. For example, compounds designed to inhibit alpha-synuclein aggregation can be screened and optimized using in vitro assays monitored by techniques such as NMR and mass spectrometry. These methods provide detailed information on how potential therapeutics interact with alpha-synuclein and affect its aggregation state. The efficacy of therapeutic interventions targeting alpha-synuclein can be evaluated using a combination of biochemical, imaging, and spectroscopic techniques. Western blotting and ELISA are employed to measure

changes in alpha-synuclein levels and their modified forms in response to treatment [52]. PET imaging is used to monitor the *in vivo* reduction of alpha-synuclein aggregates over time, providing a non-invasive means to assess therapeutic impact [53]. Single-molecule fluorescence techniques allow real-time monitoring of alpha-synuclein aggregation dynamics, offering insights into the mechanisms by which treatments exert their effects. These comprehensive evaluations are essential for advancing therapeutic candidates from preclinical studies to clinical trials. The application of analytical techniques in Parkinson's disease research has profoundly enhanced our understanding of alpha-synuclein pathology, facilitated the development of diagnostic biomarkers, and advanced therapeutic strategies targeting alpha-synuclein aggregation [54]. These techniques are indispensable for driving progress in diagnosing, monitoring, and treating Parkinson's disease.

### 2.3. Challenges and Future Directions

One of the primary technical challenges in studying alpha-synuclein is the sensitivity and specificity of analytical techniques [55]. While widely used, techniques such as Western blotting and ELISA can sometimes lack the necessary sensitivity to detect low-abundance forms of alpha-synuclein, particularly in early disease stages [56]. Additionally, cross-reactivity and non-specific binding can lead to false positives, complicating the interpretation of results and hindering accurate diagnosis and monitoring. *In vivo* applications of analytical techniques, such as positron emission tomography (PET) imaging, face significant challenges. The development of highly specific and sensitive tracers for alpha-synuclein is ongoing. However, current tracers may still lack the precision needed to distinguish between pathological and non-pathological forms of the protein. Furthermore, the resolution of imaging techniques may not yet be sufficient to detect early, subtle changes in alpha-synuclein aggregation, limiting their utility in early diagnosis and monitoring of disease progression [57].

Translating findings from alpha-synuclein research into clinical practice remains a significant challenge. Despite promising advances in identifying biomarkers and potential therapeutic targets, there is often a gap between laboratory research and the development of clinically applicable diagnostic tools and treatments [58]. Ensuring that analytical techniques are robust, reproducible, and scalable for clinical use is crucial for bridging this gap. The clinical translation of new diagnostic and therapeutic approaches involving alpha-synuclein also involves navigating complex regulatory and ethical landscapes. Regulatory approval processes for new biomarkers and treatments can be lengthy and require extensive validation [59]. Additionally, ethical considerations around patient consent, data privacy, and the use of new technologies must be carefully addressed to ensure responsible and equitable implementation in clinical settings.

Future research directions include advancing analytical methodologies to overcome current technical limitations. Innovations in high-resolution imaging, such as improved PET tracers and advanced microscopy techniques, hold promise for

more precise *in vivo* detection of alpha-synuclein [60]. Additionally, developing more sensitive and specific biochemical assays, leveraging techniques like mass spectrometry and single-molecule analysis, will enhance the detection and characterization of alpha-synuclein. A comprehensive understanding of alpha-synuclein and its role in Parkinson's disease will benefit from integrating multi-disciplinary approaches. Combining biochemistry, molecular biology, imaging, and computational modeling insights can provide a more holistic view of alpha-synuclein dynamics and pathology. Collaborative efforts across different fields will be essential for developing more effective diagnostic tools and therapeutic strategies. Significant challenges remain in the study of alpha-synuclein and its application to Parkinson's disease research, ongoing advancements in analytical techniques and multi-disciplinary collaborations offer promising avenues for future progress [61] [62]. Overcoming technical and clinical translation barriers will be crucial for successfully developing and implementing novel diagnostic and therapeutic approaches.

## 3. Conclusions

The study of alpha-synuclein, a protein central to the pathophysiology of Parkinson's disease, has been significantly advanced through various analytical techniques. Biochemical methods like Western blotting and ELISA have been crucial in detecting and quantifying alpha-synuclein. At the same time, imaging techniques such as immunohistochemistry and PET imaging have provided insights into the spatial distribution and progression of alpha-synuclein pathology. Spectroscopic methods, including NMR and mass spectrometry, have shed light on alpha-synuclein's structural details and aggregation mechanisms. Emerging techniques like cryo-electron microscopy and single-molecule fluorescence are further pushing our understanding boundaries.

Analytical techniques are indispensable for advancing Parkinson's disease research. They offer the precision and detail needed to unravel the complex biology of alpha-synuclein, from its regular physiological roles to its pathological aggregation. These methods enable the identification of biomarkers for early diagnosis and monitoring of disease progression, providing a basis for developing targeted therapies. The ability to visualize and quantify alpha-synuclein in various biological contexts is crucial for understanding its role in disease and for designing effective interventions.

Looking ahead, the role of alpha-synuclein analysis in Parkinson's disease is poised to become even more pivotal. Advancements in analytical methodologies will likely lead to more sensitive and specific detection techniques, improving early diagnosis and enabling the monitoring of disease progression with greater accuracy. As understanding of alpha-synuclein's structure and aggregation mechanisms deepens, new therapeutic targets will emerge, paving the way for treatments that can more effectively halt or reverse disease progression. Multi-disciplinary approaches integrating biochemistry, imaging, and computational modeling

will provide a comprehensive understanding of alpha-synuclein dynamics, enhancing the development of diagnostic tools and therapeutic strategies. The continued refinement and application of analytical techniques are essential for translating research findings into clinical practice, ultimately improving the diagnosis, monitoring, and treatment of Parkinson's disease. The future of Parkinson's disease research and therapy is intimately tied to our ability to analyze and understand alpha-synuclein, making these analytical advancements critical for addressing this debilitating neurodegenerative disorder.

## Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

## References

[1] Okpokoro, E., Lesosky, M., Osa-Afiana, C., Bada, F., Okwor, U., Odonye, G., *et al.* (2023) Prevalence and Risk Factors for Mycobacterium Tuberculosis Infection among Health Workers in HIV Treatment Centers in North Central, Nigeria. *The American Journal of Tropical Medicine and Hygiene*, **109**, 60-68. https://doi.org/10.4269/ajtmh.22-0531

[2] Jumare, J., Dakum, P., Sam-Agudu, N., Memiah, P., Nowak, R., Bada, F., *et al.* (2023) Prevalence and Characteristics of Metabolic Syndrome and Its Components among Adults Living with and without HIV in Nigeria: A Single-Center Study. *BMC Endocrine Disorders*, **23**, Article No. 160. https://doi.org/10.1186/s12902-023-01419-x

[3] Khan, A.U., Akram, M., Daniyal, M. and Zainab, R. (2018) Awareness and Current Knowledge of Parkinson's Disease: A Neurodegenerative Disorder. *International Journal of Neuroscience*, **129**, 55-93. https://doi.org/10.1080/00207454.2018.1486837

[4] Castonguay, A., Gravel, C. and Lévesque, M. (2021) Treating Parkinson's Disease with Antibodies: Previous Studies and Future Directions. *Journal of Parkinson's Disease*, **11**, 71-92. https://doi.org/10.3233/jpd-202221

[5] Gannon, J., Bach, K., Cattaruzza, M.S., Bar-Zeev, Y., Forberger, S., Kilibarda, B., *et al.* (2023) Big Tobacco's Dirty Tricks: Seven Key Tactics of the Tobacco Industry. *Tobacco Prevention & Cessation*, **9**, 1-9. https://doi.org/10.18332/tpc/176336

[6] Okpokoro, E., Okwor, U., Osa-Afiana, C., Odonye, G., Bada, F., Igbinomwanhia, V., *et al.* (2022) Tuberculosis Infection Control Practice among Antiretroviral (ART) Clinics in North Central Nigeria. *Safety and Health at Work*, **13**, S108. https://doi.org/10.1016/j.shaw.2021.12.1092

[7] Chiò, A., Canosa, A., Calvo, A., Moglia, C., Cicolin, A. and Mora, G. (2021) Developments in the Assessment of Non-Motor Disease Progression in Amyotrophic Lateral Sclerosis. *Expert Review of Neurotherapeutics*, **21**, 1419-1440. https://doi.org/10.1080/14737175.2021.1984883

[8] Conway, K.A., Lee, S., Rochet, J., Ding, T.T., Williamson, R.E. and Lansbury, P.T. (2000) Acceleration of Oligomerization, Not Fibrillization, Is a Shared Property of both $\alpha$-Synuclein Mutations Linked to Early-Onset Parkinson's Disease: Implications for Pathogenesis and Therapy. *Proceedings of the National Academy of Sciences*, **97**, 571-576. https://doi.org/10.1073/pnas.97.2.571

[9] Marino, B.L.B., de Souza, L.R., Sousa, K.P.A., Ferreira, J.V., Padilha, E.C., da Silva, C.H.T.P., *et al.* (2020) Parkinson's Disease: A Review from Pathophysiology to Treatment. *Mini-Reviews in Medicinal Chemistry*, **20**, 754-767.

https://doi.org/10.2174/1389557519666191104110908

[10] Cardinale, A., Calabrese, V., de Iure, A. and Picconi, B. (2021) Alpha-Synuclein as a Prominent Actor in the Inflammatory Synaptopathy of Parkinson's Disease. *International Journal of Molecular Sciences*, **22**, Article No. 6517. https://doi.org/10.3390/ijms22126517

[11] Serratos, I.N., Hernández-Pérez, E., Campos, C., Aschner, M. and Santamaría, A. (2021) An Update on the Critical Role of $\alpha$-Synuclein in Parkinson's Disease and Other Synucleinopathies: From Tissue to Cellular and Molecular Levels. *Molecular Neurobiology*, **59**, 620-642. https://doi.org/10.1007/s12035-021-02596-3

[12] Brás, I.C. and Outeiro, T.F. (2021) Alpha-Synuclein: Mechanisms of Release and Pathology Progression in Synucleinopathies. *Cells*, **10**, Article No. 375. https://doi.org/10.3390/cells10020375

[13] Theillet, F., Binolfi, A., Bekei, B., Martorana, A., Rose, H.M., Stuiver, M., *et al.* (2016) Structural Disorder of Monomeric $\alpha$-Synuclein Persists in Mammalian Cells. *Nature*, **530**, 45-50. https://doi.org/10.1038/nature16531

[14] Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M.R. and Südhof, T.C. (2010) $\alpha$-Synuclein Promotes SNARE-Complex Assembly *in Vivo* and *in Vitro*. *Science*, **329**, 1663-1667. https://doi.org/10.1126/science.1195227

[15] Zhu, M., Li, J. and Fink, A.L. (2003) The Association of $\alpha$-Synuclein with Membranes Affects Bilayer Structure, Stability, and Fibril Formation. *Journal of Biological Chemistry*, **278**, 40186-40197. https://doi.org/10.1074/jbc.m305326200

[16] Spillantini, M.G., Schmidt, M.L., Lee, V.M., Trojanowski, J.Q., Jakes, R. and Goedert, M. (1997) $\alpha$-Synuclein in Lewy bodies. *Nature*, **388**, 839-840. https://doi.org/10.1038/42166

[17] Watanabe-Nakayama, T., Ono, K., Itami, M., Takahashi, R., Teplow, D.B. and Yamada, M. (2016) High-Speed Atomic Force Microscopy Reveals Structural Dynamics of Amyloid $\beta_{1\text{-}42}$ Aggregates. *Proceedings of the National Academy of Sciences*, **113**, 5835-5840. https://doi.org/10.1073/pnas.1524807113

[18] Gunning, D. (2016) Cultivating *Salicornia europaea* (Marsh Samphire). Irish Sea Fish. Board.

[19] Ramis, R., Ortega-Castro, J., Vilanova, B., Adrover, M. and Frau, J. (2021) $Cu^{2+}$, $Ca^{2+}$, and Methionine Oxidation Expose the Hydrophobic $\alpha$-Synuclein NAC Domain. *International Journal of Biological Macromolecules*, **169**, 251-263. https://doi.org/10.1016/j.ijbiomac.2020.12.018

[20] Bozelli, J.C., Kamski-Hennekam, E., Melacini, G. and Epand, R.M. (2021) $\alpha$-Synuclein and Neuronal Membranes: Conformational Flexibilities in Health and Disease. *Chemistry and Physics of Lipids*, **235**, Article ID: 105034. https://doi.org/10.1016/j.chemphyslip.2020.105034

[21] Srinivasan, E., Chandrasekhar, G., Chandrasekar, P., Anbarasu, K., Vickram, A.S., Karunakaran, R., *et al.* (2021) Alpha-Synuclein Aggregation in Parkinson's Disease. *Frontiers in Medicine*, **8**, Article ID: 736978. https://doi.org/10.3389/fmed.2021.736978

[22] Albert, K., Kälvälä, S., Hakosalo, V., Syvänen, V., Krupa, P., Niskanen, J., *et al.* (2022) Cellular Models of Alpha-Synuclein Aggregation: What Have We Learned and Implications for Future Study. *Biomedicines*, **10**, Article No. 2649. https://doi.org/10.3390/biomedicines10102649

[23] Menon, S., Armstrong, S., Hamzeh, A., Visanji, N.P., Sardi, S.P. and Tandon, A. (2022) Alpha-Synuclein Targeting Therapeutics for Parkinson's Disease and Related

Synucleinopathies. *Frontiers in Neurology*, **13**, Article ID: 852003. https://doi.org/10.3389/fneur.2022.852003

[24] Bisi, N., Feni, L., Peqini, K., Pérez-Peña, H., Ongeri, S., Pieraccini, S., *et al.* (2021) *α*-Synuclein: An All-Inclusive Trip around Its Structure, Influencing Factors and Applied Techniques. *Frontiers in Chemistry*, **9**, Article ID: 666585. https://doi.org/10.3389/fchem.2021.666585

[25] Huiting, W. and Bergink, S. (2020) Locked in a Vicious Cycle: The Connection between Genomic Instability and a Loss of Protein Homeostasis. *Genome Instability & Disease*, **2**, 1-23. https://doi.org/10.1007/s42764-020-00027-6

[26] Bernal-Conde, L.D., Ramos-Acevedo, R., Reyes-Hernández, M.A., Balbuena-Olvera, A.J., Morales-Moreno, I.D., Argüero-Sánchez, R., *et al.* (2020) Alpha-Synuclein Physiology and Pathology: A Perspective on Cellular Structures and Organelles. *Frontiers in Neuroscience*, **13**, Article No. 1399. https://doi.org/10.3389/fnins.2019.01399

[27] Teil, M., Arotcarena, M., Faggiani, E., Laferriere, F., Bezard, E. and Dehay, B. (2020) Targeting *α*-Synuclein for PD Therapeutics: A Pursuit on All Fronts. *Biomolecules*, **10**, Article No. 391. https://doi.org/10.3390/biom10030391

[28] Rashed, M.A.S., Abou-Deif, M.H., Khalil, K.M. and Mahmoud, F.E.S. (2021) Expression Levels of Heat Shock Proteins through Western Blot and Real-Time Polymerase Chain Reaction in Maize. *Jordan Journal of Biological Sciences*, **14**, 671-676.

[29] Hang, Y., Boryczka, J. and Wu, N. (2022) Visible-Light and Near-Infrared Fluorescence and Surface-Enhanced Raman Scattering Point-of-Care Sensing and Bio-Imaging: A Review. *Chemical Society Reviews*, **51**, 329-375. https://doi.org/10.1039/c9cs00621d

[30] Xiong, Y., Leng, Y., Li, X., Huang, X. and Xiong, Y. (2020) Emerging Strategies to Enhance the Sensitivity of Competitive ELISA for Detection of Chemical Contaminants in Food Samples. *TrAC Trends in Analytical Chemistry*, **126**, Article ID: 115861. https://doi.org/10.1016/j.trac.2020.115861

[31] Chang, C., Yang, S., Yang, C., Chang, C. and Wu, Y. (2020) Plasma and Serum Alpha-Synuclein as a Biomarker of Diagnosis in Patients with Parkinson's Disease. *Frontiers in Neurology*, **10**, Article No. 1388. https://doi.org/10.3389/fneur.2019.01388

[32] Petricca, L., Chiki, N., Hanna-El-Daher, L., Aeschbach, L., Burai, R., Stoops, E., *et al.* (2022) Comparative Analysis of Total Alpha-Synuclein (*α*syn) Immunoassays Reveals That They Do Not Capture the Diversity of Modified *α*syn Proteoforms. *Journal of Parkinson's Disease*, **12**, 1449-1462. https://doi.org/10.3233/jpd-223285

[33] Xue, Z., Wu, Y., Gao, Q., Zhao, L. and Xu, Y. (2020) Automated Classification of Protein Subcellular Localization in Immunohistochemistry Images to Reveal Biomarkers in Colon Cancer. *BMC Bioinformatics*, **21**, Article No. 398. https://doi.org/10.1186/s12859-020-03731-y

[34] Koss, D.J., Erskine, D., Porter, A., Palmoski, P., Menon, H., Todd, O.G.J., *et al.* (2022) Nuclear Alpha-Synuclein Is Present in the Human Brain and Is Modified in Dementia with Lewy Bodies. *Acta Neuropathologica Communications*, **10**, Article No. 98. https://doi.org/10.1186/s40478-022-01403-x

[35] Moors, T.E., Maat, C.A., Niedieker, D., Mona, D., Petersen, D., Timmermans-Huisman, E., *et al.* (2021) The Subcellular Arrangement of Alpha-Synuclein Proteoforms in the Parkinson's Disease Brain as Revealed by Multicolor STED Microscopy. *Acta Neuropathologica*, **142**, 423-448. https://doi.org/10.1007/s00401-021-02329-9

[36] Zoey, F.L., Palanivel, M., Padmanabhan, P. and Gulyás, B. (2021) Parkinson's Disease: A Nanotheranostic Approach Targeting Alpha-Synuclein Aggregation. *Frontiers in*

*Cell and Developmental Biology*, **9**, Article ID: 707441.
https://doi.org/10.3389/fcell.2021.707441

[37] Reif, B., Ashbrook, S.E., Emsley, L. and Hong, M. (2021) Solid-State NMR Spectroscopy. *Nature Reviews Methods Primers*, **1**, Article No. 2.
https://doi.org/10.1038/s43586-020-00002-1

[38] Stephens, A.D., Zacharopoulou, M., Moons, R., Fusco, G., Seetaloo, N., Chiki, A., *et al.* (2020) Extent of N-Terminus Exposure of Monomeric Alpha-Synuclein Determines Its Aggregation Propensity. *Nature Communications*, **11**, Article No. 2820.
https://doi.org/10.1038/s41467-020-16564-3

[39] Azevedo, R., Jacquemin, C., Villain, N., Fenaille, F., Lamari, F. and Becher, F. (2022) Mass Spectrometry for Neurobiomarker Discovery: The Relevance of Post-Translational Modifications. *Cells*, **11**, Article No. 1279.
https://doi.org/10.3390/cells11081279

[40] Oliveira, L.M.A., Gasser, T., Edwards, R., Zweckstetter, M., Melki, R., Stefanis, L., *et al.* (2021) Alpha-Synuclein Research: Defining Strategic Moves in the Battle against Parkinson's Disease. *NPJ Parkinson's Disease*, **7**, Article No. 65.
https://doi.org/10.1038/s41531-021-00203-9

[41] Namba, K. and Makino, F. (2022) Recent Progress and Future Perspective of Electron Cryomicroscopy for Structural Life Sciences. *Microscopy*, **71**, i3-i14.
https://doi.org/10.1093/jmicro/dfab049

[42] Ha, T., Kaiser, C., Myong, S., Wu, B. and Xiao, J. (2022) Next Generation Single-Molecule Techniques: Imaging, Labeling, and Manipulation *in Vitro* and *in Cellulo*. *Molecular Cell*, **82**, 304-314. https://doi.org/10.1016/j.molcel.2021.12.019

[43] Vidović, M. and Rikalovic, M.G. (2022) Alpha-Synuclein Aggregation Pathway in Parkinson's Disease: Current Status and Novel Therapeutic Approaches. *Cells*, **11**, Article No. 1732. https://doi.org/10.3390/cells11111732

[44] Lashuel, H.A. (2020) Do Lewy Bodies Contain Alpha-Synuclein Fibrils? And Does It Matter? A Brief History and Critical Analysis of Recent Reports. *Neurobiology of Disease*, **141**, Article ID: 104876. https://doi.org/10.1016/j.nbd.2020.104876

[45] Magalhães, P. and Lashuel, H.A. (2022) Opportunities and Challenges of Alpha-Synuclein as a Potential Biomarker for Parkinson's Disease and Other Synucleinopathies. *NPJ Parkinson's Disease*, **8**, Article No. 93.
https://doi.org/10.1038/s41531-022-00357-0

[46] Condrat, C.E., Thompson, D.C., Barbu, M.G., Bugnar, O.L., Boboc, A., Cretoiu, D., *et al.* (2020) Mirnas as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells*, **9**, Article No. 276.
https://doi.org/10.3390/cells9020276

[47] Anjo, S.I., dos Santos, P.V., Rosado, L., Baltazar, G., Baldeiras, I., Pires, D., *et al.* (2020) A Different Vision of Translational Research in Biomarker Discovery: A Pilot Study on Circulatory Mitochondrial Proteins as Parkinson's Disease Potential Biomarkers. *Translational Neurodegeneration*, **9**, Article No. 11.
https://doi.org/10.1186/s40035-020-00188-0

[48] Barkovits, K., Kruse, N., Linden, A., Tönges, L., Pfeiffer, K., Mollenhauer, B., *et al.* (2020) Blood Contamination in CSF and Its Impact on Quantitative Analysis of Alpha-Synuclein. *Cells*, **9**, Article No. 370. https://doi.org/10.3390/cells9020370

[49] Golan, H., Volkov, O. and Shalom, E. (2022) Nuclear Imaging in Parkinson's Disease: The Past, the Present, and the Future. *Journal of the Neurological Sciences*, **436**, Article ID: 120220. https://doi.org/10.1016/j.jns.2022.120220

[50] Rahayel, S., Mišić, B., Zheng, Y., Liu, Z., Abdelgawad, A., Abbasi, N., *et al.* (2021) Differentially Targeted Seeding Reveals Unique Pathological Alpha-Synuclein Propagation Patterns. *Brain*, **145**, 1743-1756. https://doi.org/10.1093/brain/awab440

[51] Davis, K.D., Aghaeepour, N., Ahn, A.H., Angst, M.S., Borsook, D., Brenton, A., *et al.* (2020) Discovery and Validation of Biomarkers to Aid the Development of Safe and Effective Pain Therapeutics: Challenges and Opportunities. *Nature Reviews Neurology*, **16**, 381-400. https://doi.org/10.1038/s41582-020-0362-2

[52] Nwabufo, C.K. and Aigbogun, O.P. (2022) Diagnostic and Therapeutic Agents That Target Alpha-Synuclein in Parkinson's Disease. *Journal of Neurology*, **269**, 5762-5786. https://doi.org/10.1007/s00415-022-11267-9

[53] Alzghool, O.M., van Dongen, G., van de Giessen, E., Schoonmade, L. and Beaino, W. (2022) *α*-Synuclein Radiotracer Development and *in Vivo* Imaging: Recent Advancements and New Perspectives. *Movement Disorders*, **37**, 936-948. https://doi.org/10.1002/mds.28984

[54] Polissidis, A., Petropoulou-Vathi, L., Nakos-Bimpos, M. and Rideout, H.J. (2020) The Future of Targeted Gene-Based Treatment Strategies and Biomarkers in Parkinson's Disease. *Biomolecules*, **10**, Article No. 912. https://doi.org/10.3390/biom10060912

[55] O'Hara, D.M., Kalia, S.K. and Kalia, L.V. (2020) Methods for Detecting Toxic *α*-Synuclein Species as a Biomarker for Parkinson's Disease. *Critical Reviews in Clinical Laboratory Sciences*, **57**, 291-307. https://doi.org/10.1080/10408363.2019.1711359

[56] Rideout, H.J., Chartier-Harlin, M., Fell, M.J., Hirst, W.D., Huntwork-Rodriguez, S., Leyns, C.E.G., *et al.* (2020) The Current State-of-the-Art of Lrrk2-Based Biomarker Assay Development in Parkinson's Disease. *Frontiers in Neuroscience*, **14**, Article No. 865. https://doi.org/10.3389/fnins.2020.00865

[57] Koga, S., Sekiya, H., Kondru, N., Ross, O.A. and Dickson, D.W. (2021) Neuropathology and Molecular Diagnosis of Synucleinopathies. *Molecular Neurodegeneration*, **16**, Article No. 83. https://doi.org/10.1186/s13024-021-00501-z

[58] Sobsey, C.A., Ibrahim, S., Richard, V.R., Gaspar, V., Mitsa, G., Lacasse, V., *et al.* (2020) Targeted and Untargeted Proteomics Approaches in Biomarker Development. *Proteomics*, **20**, Article ID: 1900029. https://doi.org/10.1002/pmic.201900029

[59] Hendrix, S.B., Mogg, R., Wang, S.J., Chakravarty, A., Romero, K., Dickson, S.P., *et al.* (2021) Perspectives on Statistical Strategies for the Regulatory Biomarker Qualification Process. *Biomarkers in Medicine*, **15**, 669-684. https://doi.org/10.2217/bmm-2020-0523

[60] Kaur, A., New, E.J. and Sunde, M. (2020) Strategies for the Molecular Imaging of Amyloid and the Value of a Multimodal Approach. *ACS Sensors*, **5**, 2268-2282. https://doi.org/10.1021/acssensors.0c01101

[61] MacDougall, G., Brown, L.Y., Kantor, B. and Chiba-Falek, O. (2021) The Path to Progress Preclinical Studies of Age-Related Neurodegenerative Diseases: A Perspective on Rodent and hiPSC-Derived Models. *Molecular Therapy*, **29**, 949-972. https://doi.org/10.1016/j.ymthe.2021.01.001

[62] Cassotta, M. (2022) The Future of Parkinson's Disease Research: A New Paradigm of Human-Specific Investigation Is Necessary… and Possible. *ALTEX*, **39**, 694-709. https://doi.org/10.14573/altex.2203161