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Going Nuclear with Amino Acids and Proteins – Basic Biochemistry and Molecular Biology Primer for the Technologist

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Abstract

In recent years, there has been an influx of new tracers into the field of nuclear medicine and molecular imaging. Most of these tracers that have been FDA approved for clinical imaging exploit various mechanisms of protein biochemistry and molecular biology to bring about their actions, such as amino acid metabolism, protein folding, receptor-ligand interactions, and surface transport mechanisms. In this review, we attempt to paint a clear picture of the basic biochemistry and molecular biology of protein structure, translation, transcription, post-translational modifications, and protein targeting, in the context of the various radiopharmaceuticals currently used clinically, all in an easy-to-understand language for entry level technologists in the field. Tracer characteristics, including indications, dosage, injection-to-imaging time, and the logic behind the normal and pathophysiologic biodistribution of these newer molecular tracers, are also discussed.

Key Words: proteins; biochemistry; molecular biology; molecular imaging; molecular therapy

Proteins are the fundamental building blocks of every cell *(1)*. They are made up of specific sequences of amino acids joined by peptide bonds and are arranged end-to-end in long chains called polypeptides. Two amino acids joined by a peptide bond is called a dipeptide, three amino acids linked by a peptide is a tripeptide, and so on, with an 8 amino acid sequence making up an octapeptide as seen with the radiotracer **111In-Octreotide** *(2)*. The sequence of amino acids in proteins are determined by the genetic code of the DNA. The gene sequence in the DNA is transcribed into the messenger RNA (mRNA) in the nucleus by an enzyme called RNA polymerase, with the help of a host of assisting enzymes called transcription factors. The initial mRNA contains sequences that code for the protein (exon) along with non-coding regions (introns) which are processed (spliced) to obtain the final mRNA with the correct sequence coding for the given protein. One gene may code for multiple proteins, whereby the same gene sequence is spliced out in a variety of different patterns to yield function proteins of differing sequences (alternative splicing). This increases the diversity and the coding capacity of the genes. However, aberrant splicing reactions can result in disease conditions like beta thalassemia, which is a severe blood disorder characterized by abnormal formation of hemoglobin *(3)***.**

Once spliced, the processed mRNA is then exported from the nucleus into the cytoplasm. Upon reaching the cytoplasm, the ribosomes (protein producing molecular machines) hop onto the mRNA in search of a specific three-nucleotide sequence called the start codon that will act as a cue for the ribosome to start building the polypeptide chain based on the subsequent nucleotide sequences. In eukaryotes (organisms with an intact nucleus, which includes everything from amoeba, worms, birds, and plants to humans), the start codon usually codes for the amino acid methionine *(4)*. In prokaryotes (unicellular organisms without a nucleus where the DNA is floating in the cytoplasm, which includes members such as bacteria and archaea), it is a modified version of methionine (formyl methionine) *(5)*. The nucleotide sequence in the mRNA is read in triplets (codon) and each codon codes for an amino acid. However, one amino acid may be called upon by different codons with differing nucleotide sequences (degeneracy of the codon), and this property of the genetic code makes it more fault-tolerant for point mutations. As the ribosomes move down the mRNA, reading the codons, the amino acids are brought to the ribosomes by specific transfer RNAs (tRNAs) which carry the corresponding amino acid and have matching anticodon nucleotide sequences that can correctly base pair (form covalent bond) to the codon on which the ribosome sits at any given moment. The new amino acid is then added to the methionine (or to formyl methionine) in a condensation reaction where a molecule of water is removed to form a peptide bond (-CONH-)

between the terminal carbon atom of the methionine (C-terminal) and the amino terminal of the next amino acid (N-terminal) (Fig. 1) *(6)*.

The previous tRNA (which brought in the methionine) is released and the ribosome along with the new tRNA now carrying the two amino acids (dipeptide) then proceeds to the next codon. The process is repeated to generate a tripeptide, tetrapeptide, pentapeptide, and so on, until a long polypeptide protein chain is created as prescribed by the genetic code. Once the ribosome reaches the stop codon with a sequence that does not code for an amino acid and no tRNAs are recruited, the ribosomes recruit a release factor enzyme that cause the hydrolysis of the final C-terminal group of the polypeptide that attaches it to the ribosome, thus resulting in the release of the full-length polypeptide chain. There are multiple ribosomes hurtling down the mRNA doing the translation simultaneously, one behind the other, resulting in many polypeptides being synthesized from an mRNA and thereby increasing the 'yield' of the protein product manyfold. Once the protein chain is translated, it must then be folded in precise three-dimensional conformations for it to become functionally active. This folding process is done co-translationally by special molecular chaperone proteins that guide the nascent polypeptide chain to fold into its secondary, tertiary, and quaternary structures *(7)*. The precise folding of the long polypeptide chain is important for correct forming of the protein's active site where the catalyzing reaction occurs or to stably incorporate the necessary ion or chemical group (cofactor) to achieve its designated biological task *(8)*. The secondary structure of the protein is formed by the meticulous folding of the peptide chain into a helix or a pleated sheet. This process is mediated by the specific phi and psi torsion angles of the amino acids that would result in hydrogen bonding of the adjacent groups of the amino acids in the vicinity. The order of the amino acids specified by the genetic code dictates this folding process which would result in an energetically favorable (less entropy) stable conformation (Fig. 2) *(9)*.

The chain of helices and sheets are further folded in the three-dimensional space and stabilized by hydrogen bonds and ionic interactions between atoms within the chain and also with that of the watery (aqueous) environment in the cytoplasm (Fig. 3).

Once the chain is correctly folded into its tertiary structure, it may then need to join cooperatively with one or more folded peptide chain to form the final functional quaternary structure (e.g., hemoglobin is made up of 4 folded polypeptide subunits with an iron group stabilized in the middle of each subunit) (Fig. 4) *(10,11)*. Similarly,

an antibody protein, such as the one used in non-Hodgkin's lymphoma radioimmunotherapy agent **90Y-Ibritumomab tiuxetan** (section 3.d), is made up of four different polypeptide chains (primary structure) that are folded into beta barrels (secondary structures). This folding in turn makes it possible for those from the same and adjacent chains to form bonds with each other called disulfide bridges (tertiary structure), thus giving the antibody molecule its final three-dimensional 'Y' shape (quaternary structure) *(12)*.

If the proteins are not correctly folded, it may be destroyed by specialized enzymes called proteasomes *(13)*. In some pathological situations, this misfolding of proteins may result in aberrant protein aggregates such as those seen in Alzheimer's disease, where the proteins amyloid beta and tau are incorrectly folded resulting in sticky tar-like plaques in the neuronal tissue of the brain and further leading to inflammation and associated pathology *(14)*. These misfolded protein aggregates in the affected neurons of the brain are the targets of the Alzheimer's detection agents **18F-Florbetaben**, **18F-Florbetapir**, **18F-Flutemetamol** (section 2.a), all which detect amyloid beta plaques *(15)*, and **18F-Flortaucipir** (section 2.b) which detects misfolded tau protein tangles (Fig. 5) *(16)*.

Once the protein is correctly folded, it either stays in the cytoplasm or is exported outside of the cell for its correct destination in the body. This process is guided by the types of amino acid residues called signal sequences in the N-terminal region of the polypeptide chain (e.g., hormones such as insulin are produced by the pancreas and are secreted into the blood for blood sugar regulation, while digestive enzymes are secreted by stomach cells into the gut for the task of digestion) *(17)*.

Some proteins are shunted to the cell surface to be part of the cell membrane to act as switches (a.k.a. "receptors") for transmitting a signal into the cell's nucleus. The signal starts a specific cellular function based on environmental cues or based on another specific outside protein, such as a hormone or neurotransmitter, binding to it *(18)*. These receptors have extremely specialized functions, such as in the case of 'cluster of differentiation' (CD) receptor protein CD20 (detected by the tracer **90Y-Ibritumomab tiuxetan** (section 3.d)) on the surface of B lymphocytes helping to produce antibodies *(19)*, or the CD206 protein (bound by the lymphoscintigraphy mannose sugar tracer **99mTc-Tilmanocept** (section 3.b)) on the surface of macrophages scavenging sugar molecules from pathogens *(20)*. Sometimes a receptor is synthesized in many almost-identical forms (a.k.a. subtypes) to achieve a variety of functions in different organs by the same activator ligand, as is the case of

somatostatin receptors (SR), SR1, SR2 (bound by neuroendocrine tumor theranostic agent **68Ga/177Lu/64Cu-DOTATATE, -DOTATOC** and **111In-Octreotide**), SR3 (bound by **111In-Octreotide**), SR4, and SR5 (bound by **68Ga/177Lu/64Cu-DOTATATE**, **-DOTATOC,** and **111In-Octreotide**), all of which, in response to somatostatin, inhibit a variety of cell growth and other activities from their cell surface locations depending on the organ (or cancer) in which they are present (section 3.c) *(21)*. Such versatile protein receptors are sometimes localized to the cytoplasm itself, such as the estrogen receptors (bound by the breast carcinoma estrogen-like synthetic tracer **18F-Fluoroestradiol**) (section 3.a) that would need the activating estradiol molecule (serving as a ligand) to cross the cell membrane and bind to the receptors. This causes the receptors to physically move into the nucleus and start the transcription of a multitude of genes regulating many important body functions, such as cell proliferation and bone health *(22)*.

Not all surface proteins take up the role of being receptors for cell signaling activities. Some function as carriers of other molecules across the cell membrane, such as the dopamine active transporter (DaT) helping to transport (reuptake) the secreted excitatory neurotransmitter dopamine back into the neuron *(23)*. Also taken up into intact neurons in the brain is the Parkinson's disease tracer **123I-Ioflupane** (section 4.a), which is analogous to dopamine *(24)*. Similarly, the uptake-1 transporter helps to reuptake the neurotransmitter norepinephrine back into the neuron. This reaction also occurs with the neuroendocrine tumor theranostic agent **123 / 131I-MIBG** (section 4.b), which is analogous to norepinephrine *(25,26)*.

Cell surface protein transporters act as transporters of not only neurotransmitters but also for a variety of other biomolecules, including amino acids, which are needed for protein synthesis. In humans, there are 10 different types of amino acid transporters. Some of these, including L-type amino acid transporter (LAT-1) and Alanine-Serine-Cysteine transporter 2 (ASCT2), in increased numbers are seen transporting large amounts of amino acids, such as Leucine, into the cells for increased protein synthesis due to high demand in a cancerous state *(27)*. A similar reaction occurs with the artificial non-metabolizable amino acid leucin analogue tracer 18F-F**luciclovine** (section 1.a) for prostate cancer imaging *(28)*.

Some proteins on the other hand act as enzyme catalysts. A protein enzyme catalyzes a reaction by creating a conducive and protective environment in its active site that will produce an ideal condition for the chemical reaction to proceed (decrease of activation energy for starting the chemical reaction, resulting in the

products becoming more stable than the reactants) (Fig. 6) *(29)*. These principles govern all the chemical reactions that take place in our body for it to work in a complex chemical environment. An example of a cell surface transport protein that also act as an enzyme is the prostate specific membrane antigen (PSMA) seen on the normal prostate cells, along with some other organs including the kidneys, small intestine, and nervous system. PSMA enzymatically acts on dietary folic acid (Vitamin B9) and on neurotransmitter N-Acetylaspartylglutamic acid to releases the amino acid glutamate, which helps mobilize calcium to support normal cell growth in the prostate as well as maintain neuronal functions in the brain *(30,31)*. The increased presence of PSMA on prostate cancer cells is the target for imaging agents **68Ga-PSMA11** and **18F-Piflufolastat** approved by the FDA in 2020 and 2021, respectively (section 4. c).

1. Molecular Tracer using Amino acid Metabolism

a. **18F-Fluciclovine (Axumin®)** *(32)***:** This is a synthetic amino acid that resembles leucine and is labeled with the radionuclide 18F. It was approved by the FDA in 2016 for imaging of prostate cancer recurrence with increased blood levels of prostate specific antigen (PSA). Fluciclovine (18F) is carried across the cancer cell's membrane by amino acid transporters LAT-1 and ASCT2, which are seen in higher amounts in the cancer cells. The driving force for this tracer uptake, apart from the increased need of the cancer cells for amino acids for protein building, are the androgens (male sex hormone produced from the testicles) which in general are dangerous proponents of the cancer growth. One of the treatments of prostate cancer is "biochemical castration" with drugs so that this hormone production from the male gonads are shut down. However, nature may eventually bypass this in some patients by producing androgen from sources outside the testicles or through DNA changing mutations, eventually resulting in recurrence of prostate cancer and metastasis. This condition is clinically called metastatic castration-resistance prostate cancer, abbreviated as mCRPC. Fluciclovine (18F), once transported into the prostate cancer cells, gets trapped in the cells. Being an artificial amino acid, it cannot be used for protein building by the cells t-RNAs and ribosomes (Table 1).

2. Molecular Tracers detecting Protein (Mis)Folding

a. **18F-Florbetapir (Amyvid®), 18F-Florbetaben (Neuraceq®), 18F-Flutemetamol (Vizamyl®)** *(33-35)***:** All of these tracers bind to the misfolded β-amyloid proteins forming plaques in the brain of Alzheimer's disease

patients, specifically plaques that appear in the grey matter of the outer cerebral cortex, where beta protein should not be present (Table 2).

b. **18F-Flortaucipir (TAUVID®)** *(36)***:** Flortaucipir binds to misfolded tau protein clumps called neurofibrillary tangles (NFT) in the brain of Alzheimer's disease patients (Table 2).

3. Molecular tracers targeting cell surface protein receptors

a. **18F-Fluoroestradiol (CERIANNA®)** *(37)***:** Fluoroestradiol is indicated for use in PET imaging for the detection of estrogen receptor (ER)-positive lesions in patients with recurrent or metastatic breast cancer. Fluoroestradiol (18F) is a synthetic estrogen analog that migrates to the estrogen receptor proteins in the cytoplasm of breast cancer cells (Table 3).

b. **99mTc-Tilmanocept (Lymphoseek®)** *(38)***:** 99mTc-Tilmanocept is indicated for use with or without scintigraphic imaging for lymphatic mapping using a handheld gamma probe in order to locate lymph nodes draining from a primary solid tumor site as a component of intraoperative management. It is also indicated with or without scintigraphic imaging for guiding a sentinel lymph node biopsy using a handheld gamma probe in patients with clinically node negative squamous cell carcinoma of the oral cavity, breast cancer, or melanoma. 99mTc-Tilmanocept is made up of many DTPA (kidney imaging agent) and mannose (a type of sugar) units, linked together on a carbohydrate dextran backbone to form a giant molecule which is able to bind to the CD20 receptor protein on the surface of macrophages (a type of disease fighting blood cell constantly patrolling the blood and lymph nodes). When injected under the skin, 99mTc-Tilmanocept drains into the lymph and accumulates in the macrophages in the sentinel lymph node and beyond (Table 3).

c. **68Ga/177Lu/64Cu-DOTATATE (NETSPOT®/ LUTATHERA®/ Detectnet®)**, **-DOTATOC,** -**DOTANOC**, and **111In-Octreotide (Octreoscan®)** *(39-42)***:** These molecular agents bind to the protein receptors on the surface of somatostatin receptors-containing neuroendocrine tumors. This binding is selective for the type of somatostatin receptor, with radiolabeled DOTATATE and DOTATOC binding to receptor subtypes 2 and 5 (SSTR2, SSTR5), while 111In-Octreotide binds to all three somatostatin receptor subtypes (SSTR2, SSTR3, and

SSTR5). The radiolabeled DOTATATE fits the classical definition of a theranostic radiopharmaceutical, where the same agent can be used for both diagnostic (68Ga-DOTATATE) and therapeutic (177Lu/ 64Cu-DOTATATE) purposes (Table 3).

d. 90Y-Ibritumomab tiuxetan (Zevalin®) *(43)***:** This antibody radiopharmaceutical binds to the CD20 receptor protein molecules on mature and malignant B-lymphocytes, a type of disease fighting cells in the blood, while sparing the immature and the parent cells in the bone marrow. Although the function of CD20 receptor on B-cells is unknown, it is thought to play a role in calcium entry across the B-cell membrane and maintaining the required amounts of calcium inside the cell for activation in the disease-fighting process, including antibody production. The big advantage of anti CD20 antibodies is they attack B-cells that are only malignant and not the precursor cells which prevents the patient from losing all B-cells, avoiding even more negative outcomes. The beta radiation from the Y-90 isotope then destroys the cancerous B-cells (Table 3).

4. Molecular Imaging tracers targeting cell surface protein transporters

a. 123I-Ioflupane (DaTscan®) *(44)***:** 123I-Ioflupane binds to presynaptic dopamine transporters, abbreviated as DaT, seen in the striatal region of the brain. A defining feature of Parkinson's disease is a marked reduction in the dopamine secreting neurons in this portion of the brain. Dopamine, once secreted into the synaptic gap between the pre-synaptic and post-synaptic neurons (neurons sending and receiving the nerve signals, respectively), is reabsorbed back into the pre-synaptic neuron through the dopamine transporter proteins on the pre-synaptic neuron endings, thus preventing continuous excitation. The nerve signals are related to higher order functions of the brain, including that of movement and coordination. In the absence or destruction of these neurons, movement disorders typical to that of Parkinson’s syndromes will result. By utilizing a tracer that binds to the pre-synaptic dopamine transporters, a quantitative measure and spatial distribution of these transporters, and hence the dopamine secreting neurons, can be obtained (Table 4).

b. 123/131I-Iobenguane (AdreView®/ AZEDRA®) *(45)***:** Radiolabeled iobenguane, a.k.a. meta-iodobenzylguanidine (MIBG) is structurally similar to the ‘fight-or-flight’ hormone norepinephrine. Once secreted

into the synapse gap between two neurons to facilitate neurotransmission, norepinephrine is taken back up into the pre-synaptic neurons through transporters called uptake 1. Uptake-1 is normally found in tissues, but is overexpressed in certain neuroendocrine tumors, such as pheochromocytomas and neuroblastomas. Since MIBG resembles norepinephrine, it will be taken up in neuroendocrine tumor cells along with the neurotransporter in a higher amount. The radiolabeled MIBG is also a theranostic radiopharmaceutical, where the same agent can be used for both diagnostic (123I-MIBG) and therapeutic (131I-MIBG) purposes (Table 4).

c. 68Ga-PSMA11, 18F-Piflufolastat (PYLARIFY®) *(46,47)***:** Both these agents attach to the outer portion (extracellular domain) of the prostate specific membrane antigen (PSMA) found in elevated levels (one-hundred to one-thousand fold) on the surface of 95% of the prostate cancer cells *(48)*. PSMA is also normally seen at low levels in the kidneys, liver, tear and salivary glands, spleen, and the nervous tissue. Once the radiotracer binds to the surface membrane protein, PSMA along with the attached radiotracer is transported into the cell, thus trapping the tracer within the cancer cell *(49)* (Table 4).

Conclusion: If the past decades in nuclear medicine saw bold moves in physics and instrumentation, the future landscape is evolving to be the scintillating age of molecular and systems biology. The present and future growth of molecular imaging and therapy is based on utilizing the intricate biological and biochemical pathways of the human body. In order for the nuclear medicine technologist to appreciate the interactions of the radiopharmaceuticals within the body to delineate the best information possible, a good understanding of the underlying mechanisms is required. This knowledge should be grounded in sound fundamentals of the molecular processes in the living systems, which will enable the technologist to integrate the theory and processes into the realm of applied clinical practice. This will also enable a new generation of professionals who eagerly seek out cutting-edge knowledge and challenges, which may elude the average health care professional, leading the profession to the 21st century.

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Key Points:

QUESTION: How do the newly approved radiopharmaceuticals exploit the protein biochemistry and molecular biology to bring out various pathologies in the human body?

PERTINENT POINTS: Recently approved molecular tracers target various aspects of protein biochemistry and molecular biology including amino acid metabolism, protein (mis)folding, cell surface protein receptors, and protein transporters to bring out the various pathologies.

IMPLICATIONS FOR PATIENT CARE: A sound knowledge in the biological and biochemical basis for the biodistribution of the radiopharmaceuticals helps the technologist to anticipate any issues, optimize data acquisition, and perform quality control to bring out the clinical information in the best possible way.

References:

1. Whitford D. *Proteins : structure and function. Hoboken, NJ: J. Wiley & Sons; 2005. xiv, 528 p. p.*

2. Bauer W, Briner U, Doepfner W, et al. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci*. 1982;31(11):1133-40.

3. Thalassemia major: molecular and clinical aspects. NIH Conference. *Ann Intern Med*. 1979;91(6):883-97.

4. Merrick WC. Overview: mechanism of translation initiation in eukaryotes. *Enzyme*. 1990;44(1-4):7-16.

5. Schmitt E, Guillon JM, Meinnel T, Mechulam Y, Dardel F, Blanquet S. Molecular recognition governing the initiation of translation in Escherichia coli. A review. *Biochimie*. 1996;78(7):543-54.

6. Forbes J, Krishnamurthy K. *Biochemistry, Peptide*. *StatPearls*. Treasure Island (FL)2021.

7. Ellis RJ. The general concept of molecular chaperones. *Philos Trans R Soc Lond B Biol Sci*. 1993;339(1289):257-61.

8. Hartley RW. Barnase and barstar: two small proteins to fold and fit together. *Trends Biochem Sci*. 1989;14(11):450-4.

9. Al Mughram MH, Herrington NB, Catalano C, Kellogg GE. Systematized analysis of secondary structure dependence of key structural features of residues in soluble and membrane-bound proteins. *J Struct Biol X*. 2021;5:100055.

10. Perutz MF, Rossmann MG, Cullis AF, Muirhead H, Will G, North AC. Structure of haemoglobin: a three-dimensional Fourier synthesis at 5.5-A. resolution, obtained by X-ray analysis. *Nature*. 1960;185(4711):416-22.

11. Bringas M, Petruk AA, Estrin DA, Capece L, Marti MA. Tertiary and quaternary structural basis of oxygen affinity in human hemoglobin as revealed by multiscale simulations. *Sci Rep*. 2017;7(1):10926.

12. Davies DR, Padlan EA, Segal DM. Three-dimensional structure of immunoglobulins. *Annu Rev Biochem*. 1975;44:639-67.

13. Arrigo AP, Tanaka K, Goldberg AL, Welch WJ. Identity of the 19S 'prosome' particle with the large multifunctional protease complex of mammalian cells (the proteasome). *Nature*. 1988;331(6152):192-4.

14. Garcia-Morales V, Gonzalez-Acedo A, Melguizo-Rodriguez L, et al. Current Understanding of the Physiopathology, Diagnosis and Therapeutic Approach to Alzheimer's Disease. *Biomedicines*. 2021;9(12).

15. Filippi L, Chiaravalloti A, Bagni O, Schillaci O. (18)F-labeled radiopharmaceuticals for the molecular neuroimaging of amyloid plaques in Alzheimer's disease. *Am J Nucl Med Mol Imaging*. 2018;8(4):268-81.

16. Wolters EE, Dodich A, Boccardi M, et al. Clinical validity of increased cortical uptake of [(18)F]flortaucipir on PET as a biomarker for Alzheimer's disease in the context of a structured 5-phase biomarker development framework. *Eur J Nucl Med Mol Imaging*. 2021;48(7):2097-109.

17. Lippincott-Schwartz J, Roberts TH, Hirschberg K. Secretory protein trafficking and organelle dynamics in living cells. *Annu Rev Cell Dev Biol*. 2000;16:557-89.

18. Nair A, Chauhan P, Saha B, Kubatzky KF. Conceptual Evolution of Cell Signaling. *Int J Mol Sci*. 2019;20(13).

19. Rizzieri D. Zevalin((R)) (ibritumomab tiuxetan): After more than a decade of treatment experience, what have we learned? *Crit Rev Oncol Hematol*. 2016;105:5-17.

20. Leong SP. Detection of melanoma, breast cancer and head and neck squamous cell cancer sentinel lymph nodes by Tc-99m Tilmanocept (Lymphoseek(R)). *Clin Exp Metastasis*. 2021.

21. Pauwels E, Cleeren F, Bormans G, Deroose CM. Somatostatin receptor PET ligands - the next generation for clinical practice. *Am J Nucl Med Mol Imaging*. 2018;8(5):311-31.

22. Katzenellenbogen JA. The quest for improving the management of breast cancer by functional imaging: The discovery and development of 16alpha-[(18)F]fluoroestradiol (FES), a PET radiotracer for the estrogen receptor, a historical review. *Nucl Med Biol*. 2021;92:24-37.

23. Bu M, Farrer MJ, Khoshbouei H. Dynamic control of the dopamine transporter in neurotransmission and homeostasis. *NPJ Parkinsons Dis*. 2021;7(1):22.

24. Tuma Santos CA, Wallace WD, Kim S, Vijayakumar V. Pitfalls and Artifacts of (123)I-Ioflupane SPECT in Parkinsonian Syndromes: A Quality Improvement Teaching Tool. *J Nucl Med Technol*. 2021;49(2):114-9.

25. Shapiro B, Gross MD. Radiochemistry, biochemistry, and kinetics of 131I-metaiodobenzylguanidine (MIBG) and 123I-MIBG: clinical implications of the use of 123I-MIBG. *Med Pediatr Oncol*. 1987;15(4):170-7.

26. Agrawal A, Rangarajan V, Shah S, Puranik A, Purandare N. MIBG (metaiodobenzylguanidine) theranostics in pediatric and adult malignancies. *Br J Radiol*. 2018;91(1091):20180103.

27. Broer S. Amino Acid Transporters as Targets for Cancer Therapy: Why, Where, When, and How. *Int J Mol Sci*. 2020;21(17).

28. Gusman M, Aminsharifi JA, Peacock JG, Anderson SB, Clemenshaw MN, Banks KP. Review of (18)F-Fluciclovine PET for Detection of Recurrent Prostate Cancer. *Radiographics*. 2019;39(3):822-41.

29. Schowen RL. How an enzyme surmounts the activation energy barrier. *Proc Natl Acad Sci U S A*. 2003;100(21):11931-2.

30. O'Keefe DS, Bacich DJ, Huang SS, Heston WDW. A Perspective on the Evolving Story of PSMA Biology, PSMA-Based Imaging, and Endoradiotherapeutic Strategies. *J Nucl Med*. 2018;59(7):1007-13.

31. Kaittanis C, Andreou C, Hieronymus H, et al. Prostate-specific membrane antigen cleavage of vitamin B9 stimulates oncogenic signaling through metabotropic glutamate receptors. *J Exp Med*. 2018;215(1):159-75.

32. Kogai T, Ohashi E, Jacobs MS, et al. Retinoic acid stimulation of the sodium/iodide symporter in MCF-7 breast cancer cells is mediated by the insulin growth factor-I/phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase signaling pathways. *J Clin Endocrinol Metab*. 2008;93(5):1884-92.

33. Furuya F, Shimura H, Suzuki H, et al. Histone deacetylase inhibitors restore radioiodide uptake and retention in poorly differentiated and anaplastic thyroid cancer cells by expression of the sodium/iodide symporter thyroperoxidase and thyroglobulin. *Endocrinology*. 2004;145(6):2865-75.

34. Kogai T, Kanamoto Y, Li AI, et al. Differential regulation of sodium/iodide symporter gene expression by nuclear receptor ligands in MCF-7 breast cancer cells. *Endocrinology*. 2005;146(7):3059-69.

35. Dohan O, De la Vieja A, Carrasco N. Hydrocortisone and purinergic signaling stimulate sodium/iodide symporter (NIS)-mediated iodide transport in breast cancer cells. *Mol Endocrinol*. 2006;20(5):1121-37.

36. Tanosaki S, Ikezoe T, Heaney A, et al. Effect of ligands of nuclear hormone receptors on sodium/iodide symporter expression and activity in breast cancer cells. *Breast Cancer Res Treat*. 2003;79(3):335-45.

37. Chai W, Yin X, Ren L, et al. Sodium/iodide symporter gene transfection increases radionuclide uptake in human cisplatin-resistant lung cancer cells. *Clin Transl Oncol*. 2015;17(10):795-802.

38. Guerrieri F, Piconese S, Lacoste C, et al. The sodium/iodide symporter NIS is a transcriptional target of the p53-family members in liver cancer cells. *Cell Death Dis*. 2013;4:e807.

39. Ohashi E, Kogai T, Kagechika H, Brent GA. Activation of the PI3 kinase pathway by retinoic acid mediates sodium/iodide symporter induction and iodide transport in MCF-7 breast cancer cells. *Cancer Res*. 2009;69(8):3443-50.

40. Liu Z, Xing M. Induction of sodium/iodide symporter (NIS) expression and radioiodine uptake in non-thyroid cancer cells. *PLoS One*. 2012;7(2):e31729.

41. Kim HW, Kim JE, Hwang MH, et al. Enhancement of natural killer cell cytotoxicity by sodium/iodide symporter gene-mediated radioiodine pretreatment in breast cancer cells. *PLoS One*. 2013;8(8):e70194.

42. Unterholzner S, Willhauck MJ, Cengic N, et al. Dexamethasone stimulation of retinoic Acid-induced sodium iodide symporter expression and cytotoxicity of 131-I in breast cancer cells. *J Clin Endocrinol Metab*. 2006;91(1):69-78.

43. Kogai T, Sajid-Crockett S, Newmarch LS, Liu YY, Brent GA. Phosphoinositide-3-kinase inhibition induces sodium/iodide symporter expression in rat thyroid cells and human papillary thyroid cancer cells. *J Endocrinol*. 2008;199(2):243-52.

44. Taki K, Kogai T, Kanamoto Y, Hershman JM, Brent GA. A thyroid-specific far-upstream enhancer in the human sodium/iodide symporter gene requires Pax-8 binding and cyclic adenosine 3',5'-monophosphate response element-like sequence binding proteins for full activity and is differentially regulated in normal and thyroid cancer cells. *Mol Endocrinol*. 2002;16(10):2266-82.

45. Schmutzler C, Winzer R, Meissner-Weigl J, Kohrle J. Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. *Biochem Biophys Res Commun*. 1997;240(3):832-8.

46. Bogazzi F, Bartalena L, Pinchera A, Martino E. Adjuvant effect of lithium on radioiodine treatment of hyperthyroidism. *Thyroid*. 2002;12(12):1153-4.

47. Romao R, Rubio IG, Tomimori EK, Camargo RY, Knobel M, Medeiros-Neto G. High prevalence of side effects after recombinant human thyrotropin-stimulated radioiodine treatment with 30 mCi in patients with multinodular goiter and subclinical/clinical hyperthyroidism. *Thyroid*. 2009;19(9):945-51.

48. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res*. 1997;3(1):81-5.

49. Rajasekaran SA, Anilkumar G, Oshima E, et al. A novel cytoplasmic tail MXXXL motif mediates the internalization of prostate-specific membrane antigen. *Mol Biol Cell*. 2003;14(12):4835-45.

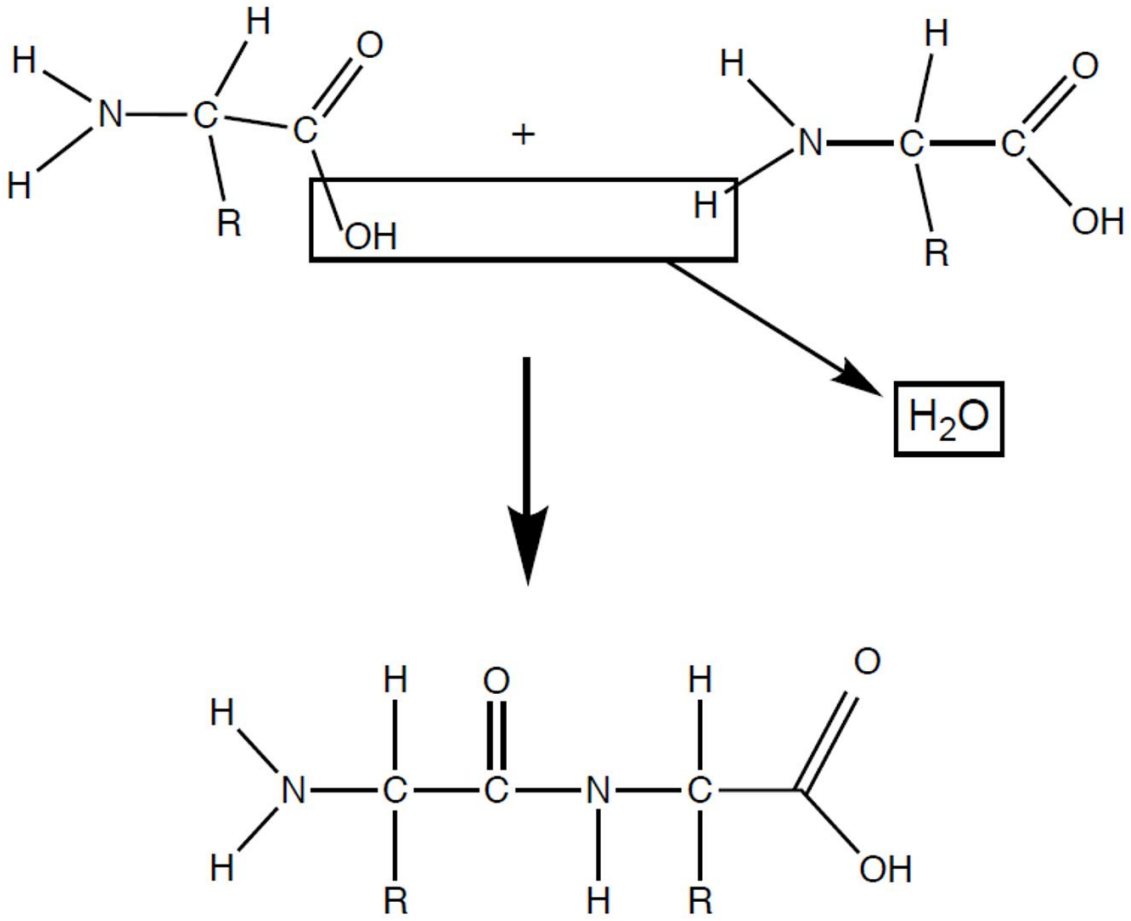

Fig 1. Condensation reaction. Two amino acids are joined together to form a peptide bond with the release of a water molecule.

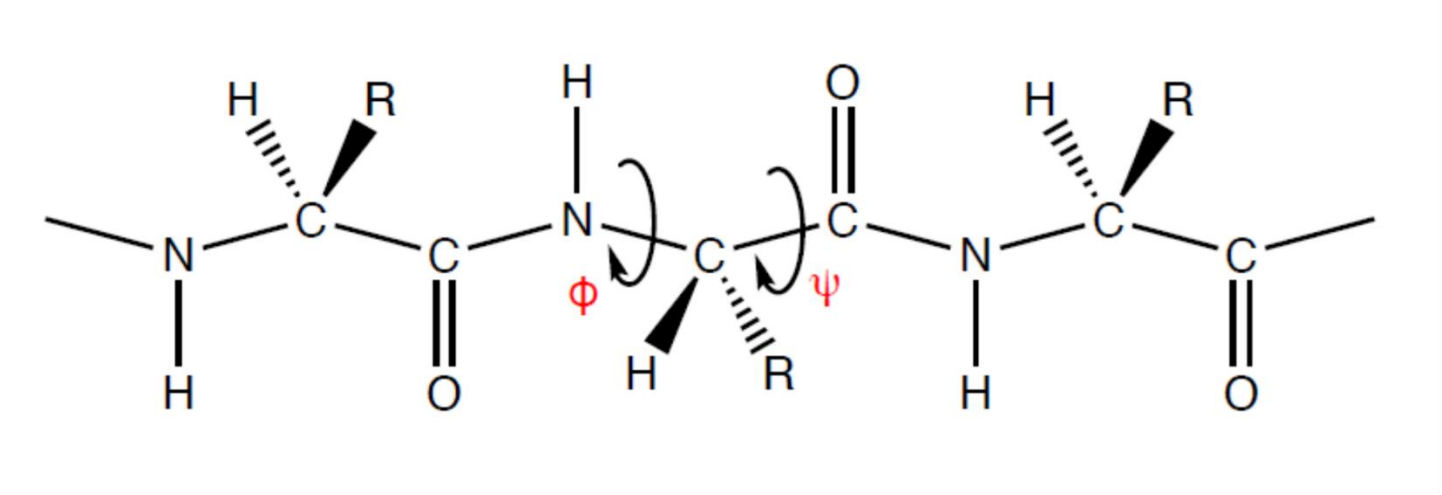

Fig. 2. Protein secondary structure formation. The precise folding of the polypeptide chain is achieved by the rotational angles (φ, ψ) of the backbone bonds flanking the central alpha carbon atom of each amino acid. These rotational angles are specific for each amino acid are instrumental in shaping the protein structure as prescribed by the genetic code.

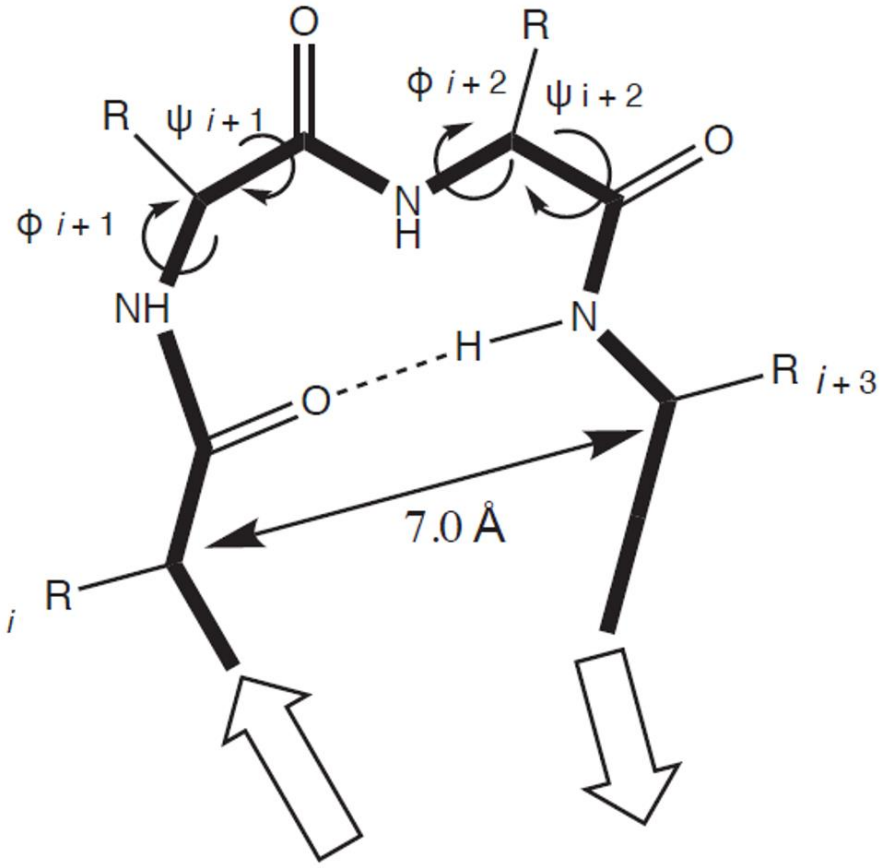

Fig. 3. Protein tertiary structure: The intra- and inter-molecular bonds help form and stabilize the precise three-dimensional protein structure into helices and sheets.

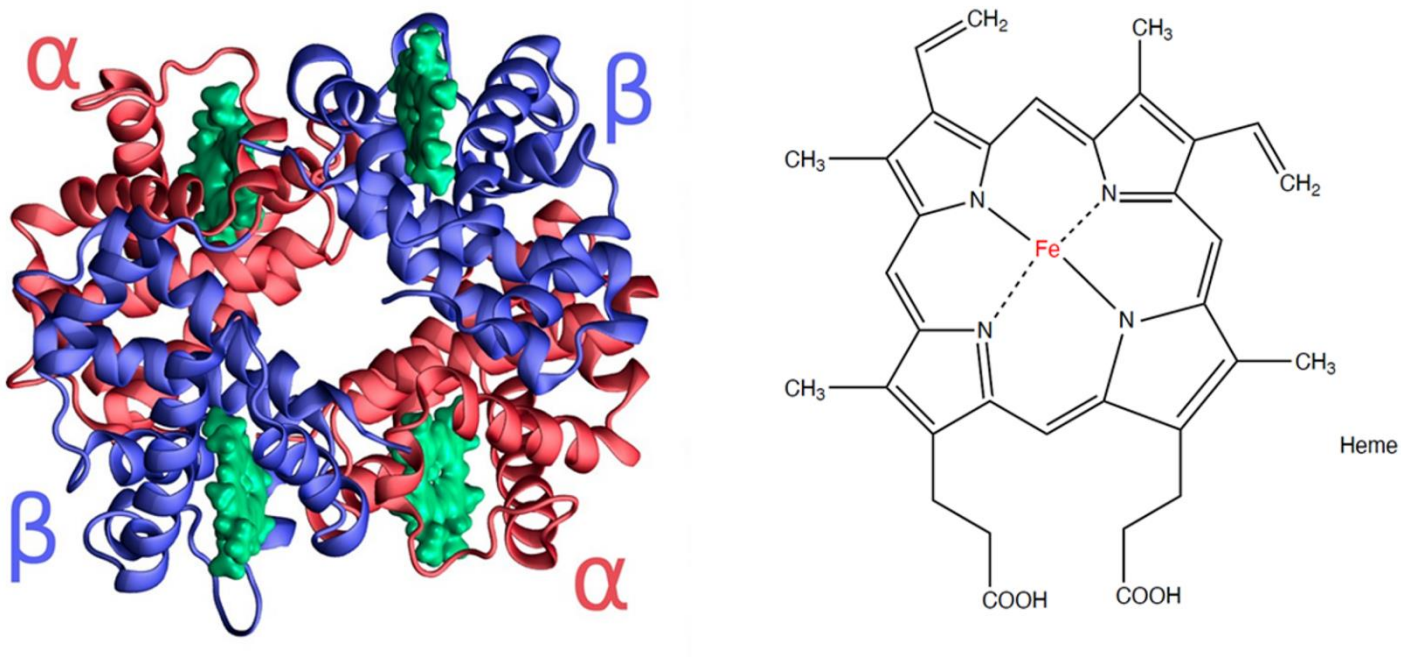

Fig. 4. Protein quaternary structure: The helices and the pleated sheets of different polypeptide chains may further associate together to form quaternary structures as in the case of hemoglobin molecule in the red blood cells. Each hemoglobin molecule is composed of four polypeptide subunits (two alpha chains and two beta chains), each stabilized by an ion group (heme) in the center.

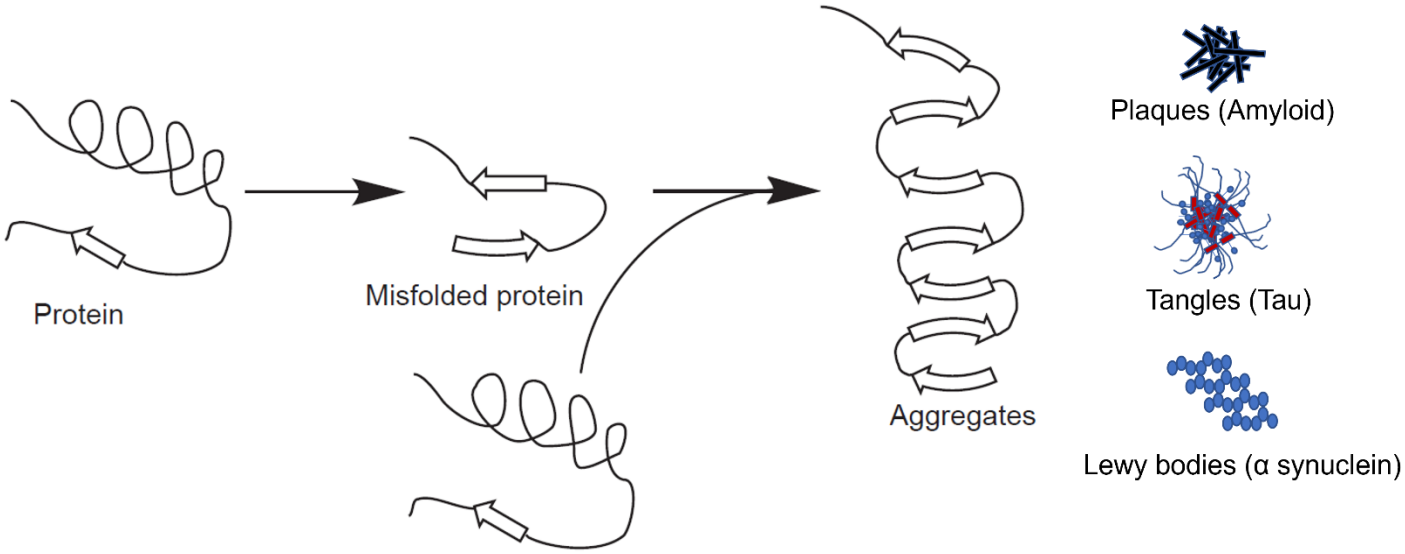

Fig. 5. Protein misfolding can lead to pathology. Correct folding of the proteins into its proper three-dimensional structure is important to function correctly. Incorrectly folded proteins are either destroyed by proteasomes or may form insoluble aggregates such as plaques, tangles, and lewy bodies that can lead to pathological conditions as in Alzheimer's disease.

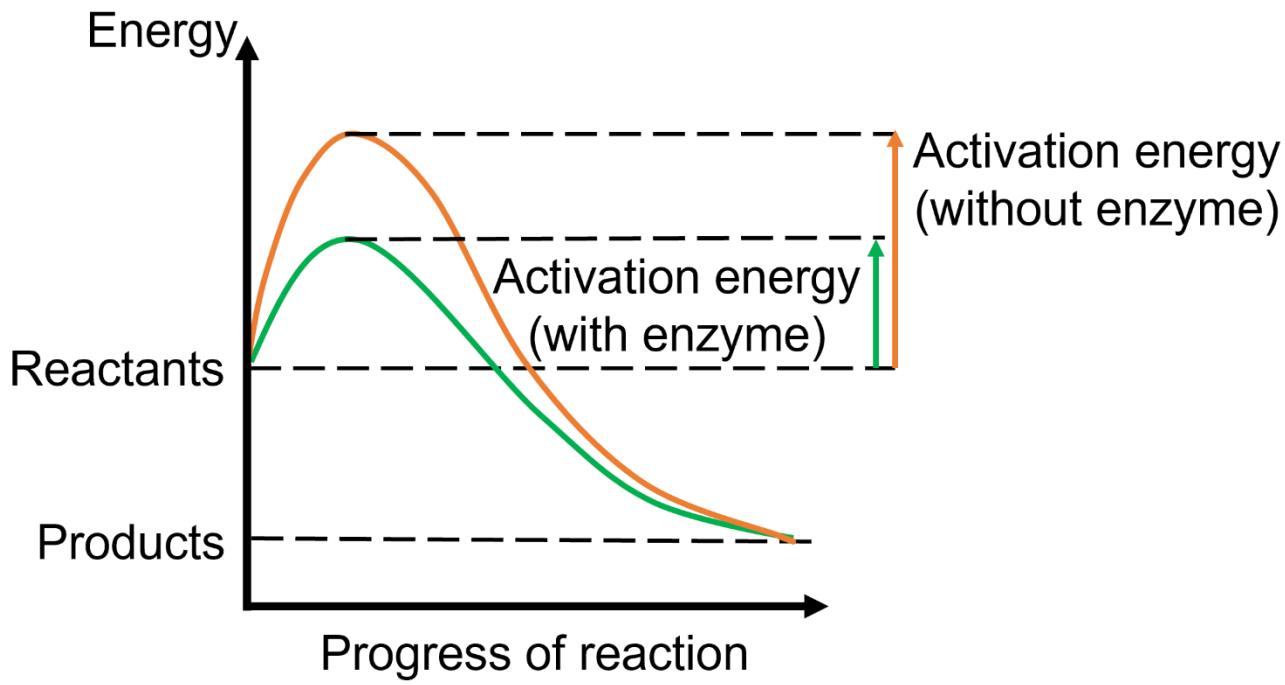

Fig. 6. Enzyme as catalysts. Enzymes catalyze chemical reactions by lowering the activation energy required for the reactants to progress through the steps of the chemical reaction. This includes a high energy transition state at the peak of the energy profile which is lower when enzymes are present – hence making it easier for the reaction to progress.

TABLE 1: Clinical properties of current FDA approved molecular tracer using *amino acid metabolism*.

Radiopharmaceutical	18F-Fluciclovine (Axumin®)
Indications	Imaging in men with suspected prostate cancer recurrence based on elevated blood prostate specific antigen (PSA) levels following prior treatment
Adult administered dose	370 MBq (10 mCi)
Route of Injection	Intravenous
Injection to Imaging Time	4 – 10 minutes
Normal biodistribution	Pancreas, liver, bone marrow, muscle

TABLE 2: Clinical properties of current FDA approved molecular tracers detecting *protein (mis)folding*.

Radiopharmaceuticals	18F-Florbetapir (Amyvid®), 18F-Florbetaben (Neuraceq®), 18F-Flutemetamol (Vizamyl®), 18F-Flortaucipir (TAUVID®)
Indications	18F-Florbetapir/ Florbetaben/ Flutemetamol: Imaging of β-amyloid plaques in suspected Alzheimer's disease patients 18F-Flortaucipir: Imaging of aggregated tau neurofibrillary tangles in suspected Alzheimer's disease patients
Adult administered dose	18F-Florbetapir: 370 MBq (10 mCi) 18F-Florbetaben: 296 MBq (8 mCi) 18F-Flutemetamol: 185 MBq (5 mCi) 18F-Flortaucipir: 370 MBq (10 mCi)
Route of Injection	Intravenous
Injection to Imaging Time	18F-Florbetapir: 30-50 mins 18F-Florbetaben: 45-130 mins 18F-Flutemetamol: 80-100 mins 18F-Flortaucipir: 80-100 mins
Normal biodistribution	18F-Florbetapir/ Florbetaben/ Flutemetamol: Inner white matter of the brain (due to slower blood clearance from this region) 18F-Flortaucipir: some normal retention in choroid plexus, striatum, and brainstem nuclei

TABLE 3: Clinical properties of current FDA approved molecular tracers targeting cell surface *protein receptors*.

Radiopharmaceutical	18F-Fluoroestradiol (CERIANNA®)	99mTc-Tilmanocept (Lymphoseek®)	68Ga-DOTATATE (NETSPOT®), 68Ga-DOTATOC, 177Lu-DOTATATE (LUTATHERA®), 64Cu-DOTATATE (Detectnet®), 111In-Pentetreotide (Octreoscan®)	90Y-ibritumomab tiuxetan (Zevalin®)
Indications	Imaging for the detection of estrogen receptor (ER)-positive lesions as an adjunct to biopsy in patients with recurrent or metastatic breast cancer	Mapping of lymph nodes draining from the primary tumor site and to guide sentinel lymph node biopsy with an intraoperative gamma probe	68Ga-DOTATATE, -DOTATOC: To locate somatostatin receptor positive neuroendocrine tumors (NETs) in adult and pediatric patients 177Lu/ 64Cu-DOTATATE: Treatment of somatostatin receptor-positive gastroenteropancreatic neuroendocrine tumors (GEP-NETs), including foregut, midgut, and hindgut neuroendocrine tumors in adults.	Relapsed or refractory, low-grade or follicular B-cell non-Hodgkin's lymphoma (NHL)
Adult administered dose	111 - 222 MBq (3 - 6 mCi)	18.5 MBq (0.5 mCi)	68Ga-DOTATATE: 2 MBq/kg (0.054 mCi/kg) up to 200 MBq (5.4 mCi) 68Ga-DOTATOC: Adult - 148 MBq (4 mCi); Pediatric - 1.59 MBq/kg (0.043 mCi/kg) with a range of 11.1 MBq (0.3 mCi) to 111 MBq (3 mCi) 177Lu-DOTATATE: 7.4 GBq (200 mCi) every 8 weeks for a total of 4 doses 64Cu-DOTATATE: 148 MBq (4 mCi)	14.8 MBq per kg (0.4 mCi/kg). Dose adjustment needed if platelet counts are low.
Route of Injection	Intravenous	Subcutaneous, intradermal, subareolar, or peritumoral	Intravenous	Intravenous

		injection in 1 mL or less		
Injection to Imaging Time	80 minutes	10-15 minutes	68Ga-DOTATATE: 40 to 90 minutes 68Ga-DOTATOC: 60 minutes	Imaging not done usually
Normal biodistribution	Hepatobiliary system (excretion), intestines (excretion), heart, blood, uterus, kidney (excretion), and bladder (excretion).	Lymphatic channels draining the injection site	Pituitary, thyroid, spleen, adrenals, kidney, pancreas, prostate, liver, salivary glands	Significant marrow and splenic distribution of the radiopharmaceutical is seen without cold antibody pretreatment. Pretreatment with rituximab cold anti-CD20 antibody blocks the CD20 sites of the normal circulating B-cells in the spleen and bone marrow by binding to it, thereby allowing the following ‘hot’ antibodies to reach the tumor areas.

TABLE 4: Clinical properties of current FDA approved molecular tracers targeting cell surface *protein transporters*.

Radiopharmaceutical	123I-Ioflupane (DaTscan®)	123I-Iobenguane (AdreView®) 131I-Iobenguane (AZEDRA®)	68Ga-PSMA11 18F-Piflufolastat (PYLARIFY®)
Indications	Striatal dopamine transporter imaging to assist in the evaluation of adult patients with suspected Parkinsonian syndromes (PS).	123I-Iobenguane: detection of primary or metastatic pheochromocytoma or neuroblastoma 131I-Iobenguane: treatment of adult and children older than 12 years with Iobenguane scan positive, unresectable, locally advanced or metastatic pheochromocytoma or paraganglioma	Positron emission tomography of prostate-specific membrane antigen positive lesions in men with prostate cancer: • with suspected metastasis who are candidates for initial definitive therapy. • with suspected recurrence based on elevated serum prostate-specific antigen (PSA) level.
Adult administered dose	111 to 185 MBq (3 to 5 mCi)	123I-Iobenguane: 370 MBq (10 mCi) 131I-Iobenguane: 185 to 222 MBq (5 to 6 mCi) (dosimetric dose); 18,500 MBq (500 mCi) x 2 doses 90 days apart (therapeutic dose)	68Ga-PSMA11: 111 – 259 MBq (3 – 7 mCi) 18F-Piflufolastat: 333 MBq (9 mCi) is the recommended dose; acceptable range is 8 – 296 to 370 MBq (10 mCi)
;Route of Injection	Intravenous	Intravenous	Intravenous (bolus)
Injection to Imaging Time	3 - 6 hours	24 ± 6 hours	60 minutes

Normal biodistribution	Prominent comma-shaped striatal activity compared to surrounding brain tissue	Adrenals not always visualized (but activity less than liver), Liver, Heart (uptake inversely proportional to catecholamine levels), Bowel (large intestine), Salivary glands, Lung, Spleen, Urinary bladder, Uterine/neck muscles	Kidneys, salivary glands, small intestine, tear glands, spleen.