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In Silico Approaches for Addressing Challenges in CNS Radiopharmaceutical Design

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Abstract

Positron emission tomography (PET) is a highly sensitive and versatile molecular imaging modality that leverages radiolabeled molecules, known as radiotracers, to interrogate biochemical processes such as metabolism, enzymatic activity, and receptor expression. The ability to probe specific molecular and cellular events longitudinally in a non-invasive manner makes PET imaging a particularly powerful technique for studying the central nervous system (CNS) in both health and disease. Unfortunately, developing and translating a single CNS PET tracer for clinical use is typically an extremely resource intensive endeavor, often requiring synthesis and evaluation of numerous candidate molecules. Existing experimental approaches and methods begin to address this challenge by working to predict likelihood of success prior to costly *in vivo* PET studies, however, most require significant investment of resources and possess substantial limitations. In the context of CNS drug development, significant time and resources have been invested into development and optimization of computational methods, particularly involving machine learning, to streamline the design of better CNS therapeutics. Analogous efforts developed and validated for CNS radiotracer design are however conspicuously limited. In this perspective article we overview the requirements and challenges of CNS PET tracer design, survey the most promising computational methods for *in silico* CNS drug design, and bridge these two areas by discussing the potential applications and impact of computational design tools in CNS radiotracer design.

Graphical Abstract

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Author Contributions

All authors contributed to writing this Perspective.

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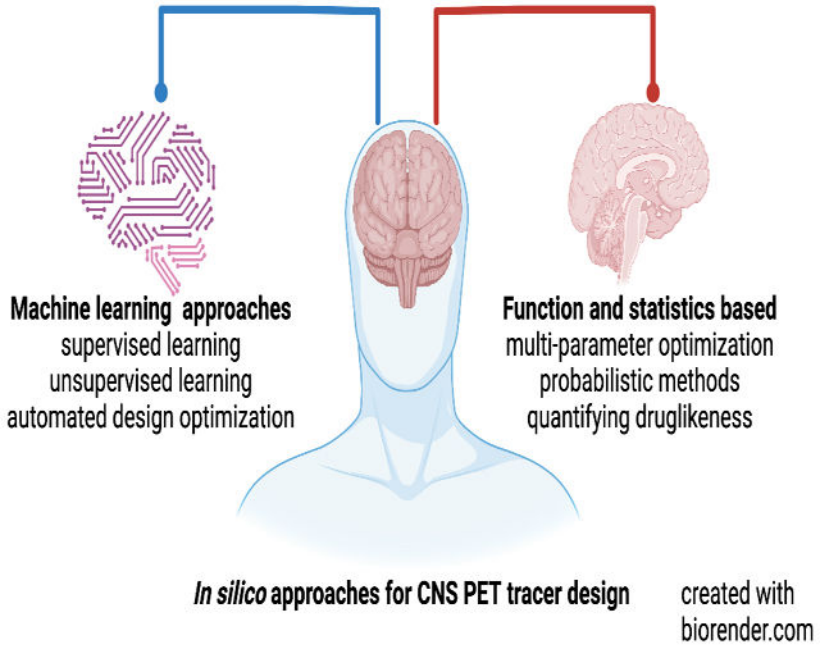

Keywords

positron emission tomography; radiochemistry; *in silico* ; machine learning; radiotracer design; methodology

1. Background and Significance

Radiochemistry can be broadly defined as the practice of appending radioactive isotopes to bioactive molecules. The resulting compounds, known as radiopharmaceuticals, are frequently used as molecular imaging tools and therapies in basic science and clinical research. One key application of radiochemistry entails radiolabeling molecules with short-lived positron (β^+) emitting radioisotopes (e.g. fluorine-18, carbon-11, gallium-68, copper-64). These radiopharmaceuticals, also referred to as positron emission tomography (PET) tracers, are subsequently administered to animals or human patients and detected via a PET scanner. PET imaging allows for extraordinarily sensitive and non-invasive *in vivo* visualization of many various cellular and biochemical processes including metabolism, enzymatic activity, biodistribution, and receptor occupancy. These attributes make PET a highly effective modality for detecting, monitoring, and studying pathological processes in numerous clinical contexts, such as cancer, heart disease, neurological disease, and infection.[1–3] More recently, the practice of pairing diagnostic PET agents with complementary probes labeled with therapeutic radionuclides has given rise to the rapidly growing area of theranostics for detecting and treating cancer.[4] The growing impact of PET imaging and radiotherapy is reflected in a recent uptick in approval of radiopharmaceuticals by the Food and Drug Administration (FDA): to date, twenty-four radiopharmaceuticals have been approved by the FDA, with eighteen of which having gained clearance in the past decade.[5]

Despite a recent surge in approval and clinical use of radiopharmaceuticals, design and translation of novel, clinically impactful small molecule PET tracers remains hampered by an intrinsically resource-intensive development process with limited throughput and a high rate of attrition (Figure 1).[6–10] Each step in the radiopharmaceutical development process presents unique, nontrivial challenges: lead compound identification is typically followed by multi-step synthesis and characterization of both the non-radioactive reference standard and one or more radiosynthetic precursors. Although currently available tools such as *in vitro* assays and high throughput screening (HTS) methods are useful in the preliminary design of candidate molecules and de-risking candidates early on by screening individual aspects of *in vivo* success (e.g. affinity, metabolism[11,12], PgP-efflux[13–15], etc.), comprehensive evaluation of novel tracer candidates ultimately requires radiolabeling and preclinical *in vivo* imaging studies.[16–18] To that end a suitable radiosynthetic methods must be developed and applied to radiolabeling the precursor molecule, often requiring prolonged screening and optimization of reaction conditions to yield specialized automated synthetic methods for reproducibly producing high quality radiopharmaceuticals. The development of radiolabeling methods, requires the need to handle radioactivity while minimizing radiation dose to the chemist; these considerations necessitate specialized equipment and mean that radiosynthesis must either be automated or performed at a small scale if performed manually, effectively negating application of traditional high throughput reaction optimization approaches to radiosynthesis. Cumulatively, applying *in vitro* drug design methodology in conjunction with the unique challenges presented by radiosynthesis creates a workflow that is extremely resource intensive, which can be a particularly challenging hurdle in the context of academic research.

While presently available de-risking approaches are useful in the early stages of tracer development, an appreciable number of tracer candidates are still carried through and ultimately fail during *in vivo* evaluation after significant investment of resources, due to factors that are challenging to accurately predict *in vitro*, such as metabolic stability, biodistribution, and the kinetics of uptake and efflux.[7,8] Lead molecule design and optimization is particularly challenging for tracers targeting biomarkers within the central nervous system (CNS), where the stringent nature of the blood-brain barrier (BBB) adds additional considerations and further reduces likelihood of success.[19,20] It is thus imperative to establish fast, resource-efficient methods that complement currently available approaches to effectively identify promising tracer candidates and determine appropriate radiolabeling conditions for radiopharmaceutical synthesis. Such methods would both enhance efficiency and productivity in industrial research settings, as well as improve efficiency and accessibility in academic research settings which typically have different infrastructure, resource pools, and end goals. Ultimately, this would help drive a surge in design and translation of clinically impactful CNS PET tracers.

While a wealth of published research focuses on *in silico* computational and modeling approaches for therapeutic small molecule development, unique aspects of radiochemistry necessitate the creation and validation of analogous yet specialized methods to augment radiotracer design and radiosynthetic method optimization.[7,8,21–25] Machine learning (ML), a computational approach that applies various automated algorithms to data of interest to form models and uncover underlying patterns, has a rapidly growing number of applications

in therapeutic design and synthetic methodology; however, this exciting area of research has not yet been extensively explored in radiochemistry.[21,25,26,27] Broadly, ML approaches can be divided into three categories: supervised, unsupervised, and reinforcement learning.[26] Selection of the appropriate approach for a given application is largely context dependent, and combinations of all three are often highly effective. In supervised learning, datasets are further sub-divided into discrete training and test sets. ML algorithms are given the training set, which consists of data with inputs (features) and corresponding desired outputs or labels (a class or a numerical target for a classification or regression task, respectively).[26] Different algorithms and internal variables (hyperparameters) can be evaluated by either internal cross-validation (performance on a randomly selected subset) of the training set, or application of the resultant model to the test set. Upon final selection of a model and associated algorithm, good practice dictates that the performance of the system be confirmed by assessing a validation set, a separate dataset comprised of data similar but distinct from both training and test sets to which the models and algorithms have never been exposed. Unsupervised learning, in contrast, leverages a variety of algorithms to extract attributes from, visualize clusters, detect anomalies, and identify internal associations in large unlabeled datasets.[26] This approach can identify high dimensional patterns beyond the readily observable and transform the data to make those patterns apparent. Reinforcement learning is not an analytical method but rather a vehicle for exploring and extrapolating upon known data: In reinforcement learning systems, the learning actor, an agent representing the iteratively evolving algorithm, interacts with an environment in which a number of choices are available.[26] In this process, the actor is rewarded or penalized based on its choice and, over many choices, ultimately develops a policy in which rewards are maximized and penalties minimized, as knowledge of the environment is accumulated.[26]

Various aspects of radiopharmaceutical science, including clinical radiology and nuclear medicine (e.g. image recognition and enhancement), are beginning to utilize ML.[28,29] Despite success in these areas, there is a conspicuous lack of ML utilization in the earliest stages of the radiopharmaceutical development process.[28] We have previously explored in depth the significant potential impact of ML in optimizing radiosynthetic methodology.[21] This perspective article will identify the current state-of-the-art and prominent challenges in radiotracer design, summarize key relevant applications of ML in therapeutic drug design, and bridge these two topics by exploring the immediate potential applications and benefits of incorporating ML in CNS radiotracer design.

2. Design and Optimization of CNS Penetrant Small Molecules

2.1. Approaches and Challenges in CNS PET Tracer Design.

Given the costly and time-intensive radiopharmaceutical development process and the high attrition rate of candidate small molecules, early identification of promising candidates is a challenging yet crucial aspect of novel radiopharmaceutical translation. This challenge is particularly salient when designing PET tracers for use within the CNS, where the stringent and complex nature of the BBB presents a significant obstacle (Figure 2).[19,20] Generally, a successful CNS PET tracer exhibits high binding potential (BP, as often defined as B_{max}/K_D, with preferred values being ≥10), high quantities of tracer present

in the brain, in addition to selective and specific binding to the target of interest.[30] The amount of tracer present within the brain is impacted by factors including permeability across the BBB (ideally without active transporter [e.g. via P-glycoprotein {PgP}, breast cancer resistant protein {BCRP}] mediated efflux), metabolic stability, and plasma protein binding (PPB).[19,20,31,32] Key pitfalls in radiotracer design include insufficient BP, high PPB, low/no passive BBB permeability (CNS uptake), active CNS efflux, high non-specific binding (NSB), and/or significant off-target binding. Off-target binding typically manifests as non-selective or non-specific binding. *Non-selective* binding refers to binding of tracer to known receptors other than the target of interest and can be screened for through *in vitro* assays. *Non-specific* binding (NSB) is a highly prevalent, imaging-specific phenomenon that occurs when radiotracer binding is non-saturable (or cannot be blocked) and is observed as high background signal unrelated to target biomarker expression. NSB is extremely hard to predict without *ex vivo* tissue work or *in vivo* imaging studies but is generally thought to be correlated with high lipophilicity.[8] These nuanced considerations in tracer design have been discussed at length in the literature and careful consideration has gone into leveraging this collective knowledge to establish best practices for radiopharmaceutical design; unfortunately, current workflows still demand synthesis and evaluation of numerous molecules to translate a single clinical radiopharmaceutical.[6–8,30,33] Appreciable physiological differences between mice and higher species such as non-human primates and humans (e.g. rodents express higher levels of promiscuous PgP, while primates have higher levels of BRCP) further limit the extent to which *in vitro* work and rodent models can de-risk a given compound prior to costly primate studies and clinical translation.[19,20,31,32]

In light of these numerous challenges, novel methods that allow researchers to identify and prioritize a refined group of only the most promising candidate molecules for synthesis and evaluation may significantly enhance the success rate of radiopharmaceutical development. While numerous *in silico* methods have been established for CNS drug development, there are relatively few analogous methods for radiopharmaceutical design.[34,35] In one early effort, Pfizer leveraged computed physicochemical attributes (e.g., cLogP, polar surface area [tPSA], pKa, molecular weight [MW]) from a large library of well-characterized CNS therapeutic small molecules to develop mathematical algorithms to predict *in vivo* behavior and absorption, distribution, metabolism, and excretion (ADME) properties of 62 successful and 15 unsuccessful CNS radiotracers.[34,36,37] While this method is intuitive and straightforward to implement, the underlying dataset (containing few negative controls) limits the utility of the method for CNS tracer design. More recently, a second method from AstraZeneca attempted to differentiate successful CNS tracers from those limited by high NSB using *in vitro* measurements (binding affinity, NSB in brain tissue, and target protein expression) to calculate the predicted fraction of target-bound radiotracer in the brain.[35] This tool effectively screened out tracers with high NSB, but the need for *in vitro* data limits the generalizability and throughput of this method.

The general principles, concepts, and workflow of these early efforts demonstrate the potential promise of well-designed *in silico* methods for CNS tracer design, while the method design and execution highlight significant challenges that must be addressed. The Pfizer methodology highlights the importance of careful dataset assembly, as the training

set used to develop this method is comprised solely of marketed small molecule therapeutic CNS drugs and drug candidates, as opposed to known CNS tracers.[34] Given the appreciable differences in target parameters for orally administered CNS therapeutics in milligram scale doses vs. intravenously administered radiotracers (typically in nanogram-microgram scale doses), leveraging any given principle or concept to develop methods for CNS PET tracer design necessitates a robust underlying dataset of known, well-characterized CNS PET radiotracers.[7,8] While further research is needed to fully elucidate and quantify the fundamental differences between therapeutics and tracers, building methods for tracer design from therapeutic molecule data risks biasing results towards molecules ideal for use as drugs but not necessarily as radiotracers. Accordingly, thoughtful compilation of suitable datasets is a key hurdle in method development: therapeutic drug development tools rely on libraries of hundreds to tens of thousands of well-characterized small molecules for design and validation, while the number of existing molecules that have been radiolabeled and published as PET radiotracers is orders of magnitude smaller in comparison.[22–24,34–39] Notably, bias against publishing negative results makes it especially challenging to identify unsuccessful tracer candidates for characterization and incorporation into both training/test and validation datasets. The Pfizer and AstraZeneca methods were both limited in this sense, since validation sets for both methods represented a relatively narrow subset of successful and unsuccessful tracers, only including successful tracers and unsuccessful candidates with prohibitively high NSB.[34,35] While high NSB is a common pitfall in CNS tracer design, *in silico* tools must be designed and validated to examine other key considerations including passive CNS uptake and active efflux through assessment of both successful *and* unsuccessful tracers. A major hurdle and important component in addressing some of the above shortcomings is assembling requisite large and diverse datasets that include extensive *in vivo* data on known successful and unsuccessful CNS tracers: effective development and implementation of any novel approaches inspired by the work and concepts described herein will require cross-validation with *in vivo* data on CNS tracers.

2.2. *In Silico* Computational Tools for Design of Small Molecule CNS Therapeutics.

A number of user-friendly tools and methodologies have been developed for *in silico* delineation of desirable physicochemical property ranges and values for predicting CNS uptake and ‘druglikeness’ of therapeutic small molecules.[22–24,36,40,41] By taking a range of approaches from statistical function-based assessments to ML, these methods use easily measured or calculated physicochemical properties to identify promising therapeutic candidates with high likelihood of *in vivo* utility. It is critical to note that, given the staggering complexity of *in vivo* biodistribution and metabolism, particularly in the CNS, conception of tools capable of predicting every facet of *in vivo* function is highly unlikely. However, studies in therapeutic drug development demonstrate that simple computational parameters and more complex aggregate functions based on these parameters have utility as *in silico* surrogates for key aspects of *in vivo* behavior (e.g., passive CNS uptake vs. active protein mediated transport across the BBB, metabolism).[23,24]

A seminal Pfizer study examined 119 CNS marketed drugs and 108 Pfizer clinical candidates and crafted a set of desirability functions based upon six fundamental physiochemical properties.[36,37] These functions, combined into a facile algorithm dubbed

Multi-Parameter Optimization (MPO), used trends in physicochemical properties gleaned from the dataset to delineate ideal property ranges for each parameter and assess overall likelihood of suitability for use as a CNS drug. Notably, this work was also the foundation for the CNS PET tool from Zhang and colleagues, described above. Ghose *et al.* built on the original MPO work to develop a Technically Extended MPO (TEMPO), defining improved boundaries for ideal properties and considering an expanded set of eight parameters.[40] Further modifying the basic concept of MPO, Gunaydin proposed a probabilistic variation, pMPO, that took a different approach to analyzing five physiochemical parameters in order to describe likelihood of CNS uptake.[41]

Beyond MPO-inspired methods, several other tools have been developed to aid in drug design. Bickerton and co-workers leveraged the distributions of 8 physicochemical properties for 771 orally absorbed small molecules to develop an algorithm called the Quantitative Estimate of Druglikeness (QED).[22] While this method did not focus exclusively on CNS drugs, the general approach is readily applicable to focus specifically on the CNS. SwissADME is a web-based drug design tool with a user-friendly interface and suite of calculated parameters comprehensively predicting key ADME properties for *in vivo* behavior. This method leverages WLOGP and tPSA as measures of lipophilicity and polarity to predict CNS uptake.[42] Most recently, Gupta and colleagues published two papers, the BBB Score and Brain Exposure Efficiency (BEE) score, to distinguish “preferentially CNS and non-CNS active” molecules, and active transport across the BBB (influx, efflux), respectively.[23,24] Cumulatively, these assorted methods provide a robust foundation and excellent source of inspiration for development of similar *in silico* tools specialized for CNS tracer design. While appreciable differences in lead molecule criteria mean that these tools cannot be directly applied to CNS tracer development, a combination of well-constructed datasets as well as inspiration taken from the general approaches and principles described above will provide an extremely strong basis for future method development for CNS tracer design.

2.3. ML Approaches to Small Molecule Design for CNS Therapeutic Development.

Supervised ML serves as a natural extension of these statistical and function-based approaches: ML is well-suited for interpreting data in high-dimensional information spaces that extend beyond what can easily be visualized, allowing simultaneous evaluation of numerous variables and interaction terms beyond one or two dimensions. This has been successfully applied to many individual aspects of therapeutic drug development, such as predicting sites for and products of metabolism.[12,43] Several datasets have been generated for CNS drug development and numerous supervised learning algorithms applied to predicting penetrance across the BBB, and partitioning in the CNS vs. periphery.[44–52] While these predictive models consider several traditional physicochemical descriptors (e.g. MW, HBD/HBAs, rotatable bonds) as input descriptors for determining CNS uptake, the methodology used to acquire the experimental data to categorize individual datapoints imposes key limitations on the applicability of the corresponding dataset to building models for predicting CNS uptake of radiopharmaceuticals in humans. Some of these models explicitly define CNS uptake based on observed pharmacological effect within the CNS (i.e. ‘sedative’ or ‘antidepressant’ effects), failing to account for CNS penetrance that occurs

without corresponding effect or below therapeutic threshold, and failing to distinguish CNS uptake from central effects occurring downstream of target-ligand interactions that take place outside of the brain in the periphery.[53,54] Still other models determine experimental data for brain uptake by administering the compound of interest to rodents, sacrificing the animals post-injection, and determining the equilibrium concentration of analyte in the brain or the brain/blood partition.[55] Because the conditions under which brain uptake is measured (i.e., a static timepoint *ex vivo*) and the appreciable physiological differences between mice and higher order species, these datasets intrinsically limit the generalizability of the resulting ML model built from this data.[19,20,31,32] Without information sets directly relevant to CNS radiopharmaceuticals, there will be a persistent discrepancy between application of such models and *in vivo* results.

Careful cultivation of relevant datasets will address this issue by enabling cross-validation of any future methods with *in vivo* data. The quality of datasets will be directly correlated to the potential impact and power of emerging methods. Design of large robust and diverse datasets including experimental data on aspects if *in vivo* performance such as CNS uptake, PPB, NSB, and active efflux will allow for development and assessment of methods for predicting these same features. Finally, care should be taken to build datasets in such a way that accounts for differences across species when compiling available *in vivo* data: while human data represents the gold standard and is, by definition, available for successful tracers, oftentimes unsuccessful tracers are rightfully terminated prior to translation. In these cases, any conclusions drawn from this data should be carefully examined for relevance before inclusion in these datasets. For example, while CNS uptake into the brain in rodent models is generally representative of passive permeability across the BBB, known inter-species differences in efflux pump expression (e.g., PgP, BCRP) means that the presence or absence of active efflux in rodents should not be extrapolated to higher species. Although assembling such datasets will certainly be both time and potentially resource intensive, the immense potential impact of novel approaches to CNS tracer design merits meeting this challenge.

Following the construction of an appropriate dataset and modeling through ML, a second challenge arises in interpreting the resulting model for optimizing prospective tracers. One strength of MPO scoring methods is that they enable facile interpretation of how various properties can impact the overall score of a tracer candidate.[22–24,36,41] However, this often comes at the cost of overall accuracy in correctly categorizing test compounds.[26] While ML models typically display increased accuracy, this comes at the cost of interpretability. Notable exceptions are linear-model based-ML classification and regression methods.[26] These models indicate the relative importance of variable terms from the magnitude of the variable's coefficients, while decision tree-based algorithms can be interpreted by visualizing the model's decision tree to elucidate how predictions were determined. While the most complex algorithms, such as neural networks, are considered "black boxes" in that they are difficult to interpret, the relative importance of descriptor variables can also be evaluated by permutation importance. In this statistical approach, all the values for a given variable are randomly permuted. A model is retrained on this single mixed variable dataset and the resulting drop (or lack thereof for low importance variables) in performance accuracy indicates how an input variable determines the outcome;[56] however,

little indication of a target value or range of values for a property is provided to optimize towards, making it challenging to use from a human chemist perspective. Despite this, these high accuracy, low transparency models hold tremendous power as *in silico* surrogates for *in vivo* behavior.

It is readily apparent that large libraries of molecules can be rapidly screened to identify promising tracer candidates likely to meet a desired biochemical outcome, without the necessity of (radio)synthesis and preclinical evaluation (Figure 3). The less apparent but more powerful application arises in utilizing these models in a reinforcement learning system to "evolve" from the original candidate and allow generation of novel candidates optimized to satisfy the model (i.e., if lipophilicity is a problem for BBB penetrance, increase lipophilicity to achieve BBB crossing). In recent years, programmatic exploration of chemical space to generate novel molecules has been intensely studied for pharmaceutical development.[57] In these instances, automatic manipulation of text-based (SMILES[58–60], SMARTS[61], SELFIES[62]) or graph-topology[63,64] (scaffold[65] or fragment[66] based) representations of molecules has been demonstrated to produce novel molecules and provide the basis for molecular manipulation choices available to a reinforcement learning agent. Multiple generative adversarial models for reinforcement have been previously demonstrated, in which one model competes to produce a novel molecule that is indistinguishable from a set of molecules with desired properties, in an effort to trick a second model trained to identify dissimilar molecules.[57] More recently, reports have detailed approaches for generating novel, similar molecules from seeds (initial lead molecule).[63,64,66] In a particularly salient example, Zhou *et. al.* demonstrated the application of a deep learning neural network, known as a deep Q network, to estimate the future reward for a given choice by an agent in combination with reinforcement learning to maximize multiple properties, such as similarity to the seed molecule (maximizing Tanimoto similarity), while simultaneously optimizing drug-likeness (maximizing QED[22]).[64] This approach mirrors how a chemist would intuitively adjust a molecule, albeit with the key benefit of being able to iteratively evaluate produced molecules upon generation *in silico*. Functionally, this implies that, upon development of a model that can accurately predict an objective function (i.e. BBB permeability or binding affinity) for a novel chemical entity, further iterative structural optimization can rapidly occur *in silico*. Molecules that display desired *in vitro* properties but suffer poor *in vivo* performance in other aspects can be automatically manipulated in both predictable and non-intuitive ways towards achieving desirable *in vivo* performance. Even molecular scaffolds that have been discarded due to poor permeability or binding potential may find renewed interest after *in silico* modification. In short, development of predictive models for radiochemistry affords not only enhanced predictivity for molecule translation success, but also an unprecedented opportunity to automatically generate and optimize novel structures without laborious synthesis and evaluation of each candidate.

3. Conclusion

Cumulatively, the work described here illustrates the high potential for optimized *in silico* methodology, particularly applications of ML initially established for therapeutic drug development, to streamline development of successful CNS radiopharmaceuticals. Extending and tailoring the concepts described above will accelerate and improve design,

development, and translation of clinically impactful CNS radiopharmaceuticals. Statistical function-based and ML-based approaches have complementary strengths and shortcomings, necessitating further investigation of both approaches and their potential synergy for future applications in radiopharmaceutical development. While statistical methods are highly transparent (i.e., they provide important insights into key properties and trends impacting *in vivo* behavior and thus enable facile method optimization), ML methods are comparatively opaque, often a "black box," but offer an automated, scalable, and potentially less biased approach. Combining such methods with reinforcement learning models will provide the foundation for simulation-based optimization of novel entities, enabling time- and resource-efficient design of novel CNS radiotracers.

Realizing the potential of these techniques will require overcoming a number of key challenges. Development of an applicable large and diverse dataset (of both positive and negative controls) is of paramount importance in both establishing powerful new approaches to CNS tracer design and cross validating these approaches with *in vivo* data to establish their utility. Effectively meeting these challenges, particularly in assembling viable databases to drive this important work, is best achieved through a concerted and coordinated effort between radiopharmaceutical scientists worldwide across both industry and academia. Such collaborative efforts will allow experts in the field to address the immediate resource demands and logistical challenges of assembling such a database, as well as future hurdles. Looking forward to applications in human health and translational medicine, the biases, trustability, and ethics of applying machine learning, as well as the logistics of implementing good machine learning practices (cGMLP) in compliance with the regulatory environment, must be considered.[67] Importantly, the potential benefit of these applications sufficiently warrants addressing these complex issues: Used in conjunction with *in silico* approaches for other aspects of radiopharmaceutical science (e.g., radiosynthesis and image analysis), *in silico* tools for design and structural optimization of CNS PET tracers hold the potential to rapidly revolutionize design of novel radiopharmaceuticals, which will in turn undoubtedly have a positive impact on clinical nuclear medicine.

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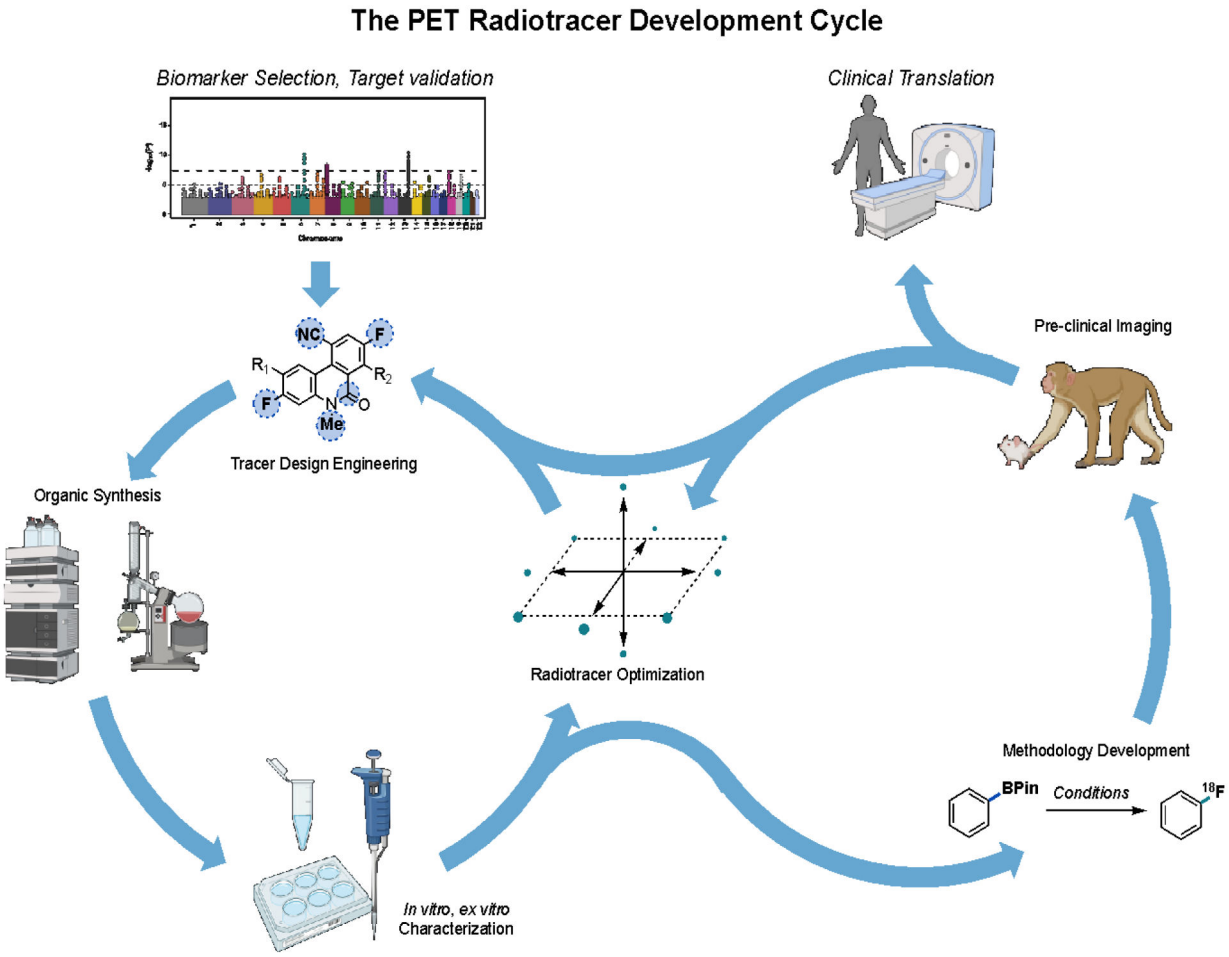

Figure 1:

The need for *in vivo* preclinical imaging studies, low throughput (screening 1–5 molecules at a time), and the iterative nature of tracer design pose hurdles to the clinical translation process. Graphic created in part with Biorender.com.

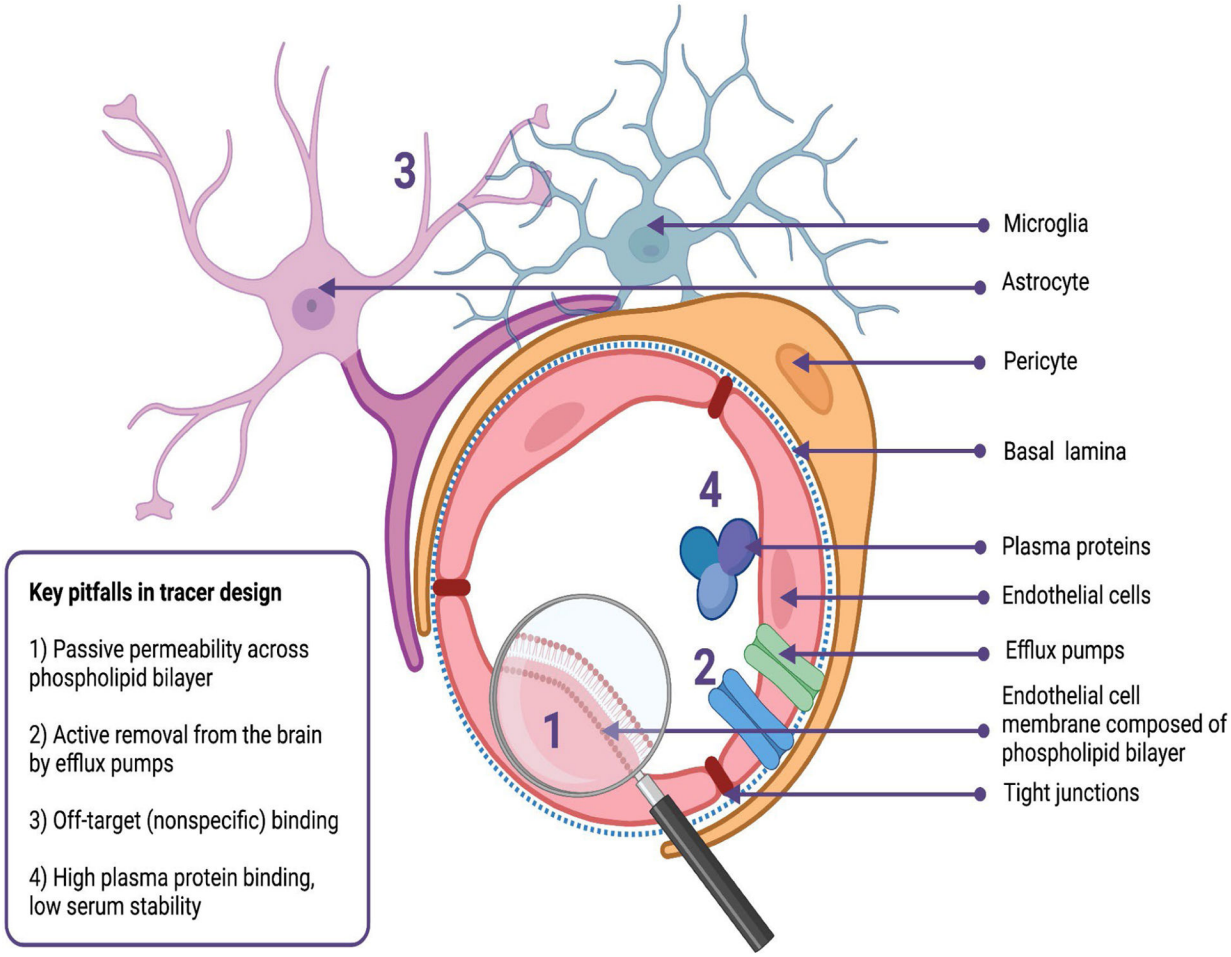

Figure 2:

The BBB is comprised of the endothelial cells making up the brain microvasculature which are interconnected via tight junctions, decorated with many influx and efflux transporters, and surrounded by a combination of basal laminae, pericytes, and extended astrocyte foot processes. This complex system presents multiple challenges in CNS tracer design. Graphic created with Biorender.com.

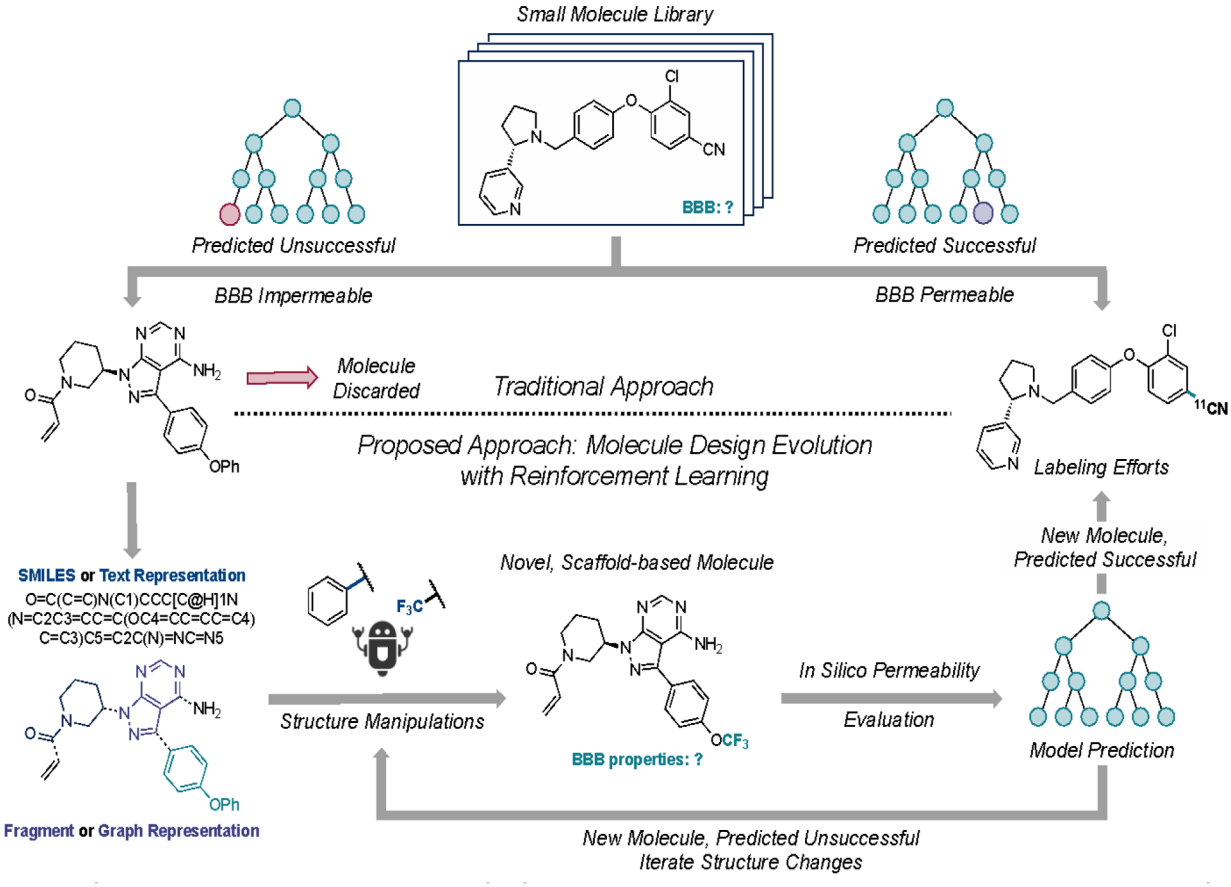

Figure 3:

Current in silico approaches in CNS tracer design categorize molecules as either successful or unsuccessful, leading to either termination of development or radiosynthesis and *in vivo* evaluation. Reinforcement learning approaches will allow for iterative *in silico* optimization of compounds categorized as unsuccessful, generating novel candidates with high likelihood of success.