Serotonin transporter: Recent progress of in silico ligand prediction methods and structural biology towards structure-guided in silico design of therapeutic agents

AUTHOR(S):

Nagayasu, Kazuki

CITATION:

Nagayasu, Kazuki. Serotonin transporter: Recent progress of in silico ligand prediction methods and structural biology towards structure-guided in silico design of therapeutic agents. Journal of Pharmacological Sciences 2022, 148(3): 295-299

ISSUE DATE:

2022-03

URL:

http://hdl.handle.net/2433/283248

Journal of Pharmacological Sciences 148 (2022) 295–299

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Review

Serotonin transporter: Recent progress of *in silico* ligand prediction methods and structural biology towards structure-guided *in silico* design of therapeutic agents

Kazuki Nagayasu*

Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan

ARTICLE INFO

Article history:
Received 30 August 2021
Received in revised form
21 December 2021
Accepted 6 January 2022
Available online 13 January 2022

Keywords:
Serotonin transporter
Machine learning
Virtual screening
Major depressive disorder

ABSTRACT

Serotonin transporter (SERT) is a membrane transporter which terminates neurotransmission of serotonin through its reuptake. This transporter as well as its substrate have long drawn attention as a key mediator and drug target in a variety of diseases including mental disorders. Accordingly, its structural basis has been studied by X-ray crystallography to gain insights into a design of ligand with high affinity and high specificity over closely related transporters. Recent progress in structural biology including single particle cryo-EM have made big strides also in determination of the structures of human SERT in complex with its ligands. Moreover, rapid progress in machine learning such as deep learning accelerates computer-assisted drug design. Here, we would like to summarize recent progresses in our understanding of SERT using these two rapidly growing technologies, limitations, and future perspectives.

1. Introduction

Serotonin transporter (SERT) is a membrane transporter which terminates neurotransmission of serotonin, a monoaminergic neurotransmitter, through its reuptake.[1] This transporter as well as its substrate have long drawn attention as a key mediator and drug target in a variety of diseases including mental disorders such as major depression, schizophrenia, obsessive-compulsive disorder, anxiety disorders, and drug addiction.[2–6] Accordingly, its structural basis has been studied by X-ray crystallography to gain insights into a design of ligand with high affinity and high specificity over closely related transporters such as dopamine transporter and noradrenaline transporter. Although the structures of these transporters for monoaminergic neurotransmitters have been investigated using bacterial homologues such as LeuT,[7,8] recent progress in structural biology including single particle cryo-EM have made big strides also in determination of the structures of human SERT in complex with its ligands. Moreover, rapid progress in machine learning such as deep learning accelerates computer-assisted drug design.[9,10] In line with this we recently reported that quantitative prediction model of pharmacological activity with high accuracy and high generalizability can be constructed with graph convolutional neural networks, a technique in deep learning and found that a compound predicted to act on SERT by our prediction model are found to be high affinity ligand against SERT in “real” assay and act as antidepressant in rodent,[11] indicating plausibility of the prediction model. In this review, we would like to summarize recent progresses in our understanding of SERT using these two rapidly growing technologies, limitations, and future perspectives.

2. Identification of new SERT ligand through *in silico* ligand prediction with pharmacological validation in “real” assay

2.1. Application of machine learning for activity prediction from chemical structure

Recent rapid growth in machine learning, especially deep learning, enables us to data-driven extraction of features from images, texts, audio, and networks.[9,12,13] Importantly, these extracted features surpass traditional features made by human in classification of images, texts, and many other types of inputs. Moreover, these extracted features can be used for generation of

* Fax: +81 75 753-4548.
E-mail address: nagayasu@pharm.kyoto-u.ac.jp.
Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2022.01.004

images and texts similar to, but different from, original inputs.[14] From the pharmacological point of view, classification of images and generation of new images corresponds to classification (i.e. active or inactive) of drugs and generation of new drug-like compounds, respectively. Therefore, it is possible that activity prediction of any compound for target protein can be achieved through utilization of machine learning if sufficient amount of assay data is available. In this line, ChEMBL (https://www.ebi.ac.uk/chembl/) is a database curating pharmacological/biological activity of small molecules from published articles.[15] This database (ChEMBL 28) contains over 17 millions activity data of around 2 millions compounds for 14 thousands targets. More importantly, quantitative activity data (i.e. IC_{50} of 1 nM, EC_{50} of 10 nM and so on) is available in ChEMBL, which is necessary for quantitative prediction of activity. Recently we reported a method for quantitative prediction of virtually any compounds for more than 100 target proteins including SERT by using ChEMBL.[11] We also found that a compound, predicted to inhibit SERT by our method, strongly inhibit SERT in cell culture and show antidepressant-like efficacy in mice.[11] In that report, we used graph-convolution neural networks for feature extraction from compound structures by using assay data deposited to ChEMBL.

2.2. Usefulness of graph-convolution neural network for feature extraction from chemical structures

Graph-convolution neural networks is highly similar to convolutional neural networks, a deep learning method often used in visual recognition tasks.[16] In convolutional neural networks, each pixel is assumed to have a fixed number of neighborhoods. For example, each pixel is adjacent to four and six pixels in 2D and 3D images, respectively. Although some reports have constructed prediction models by applying (conventional) convolutional neural networks to 2D image of chemical structures, their prediction is not quantitative but qualitative, or their prediction accuracy is not high.[17,18] Chemical structures can be considered as a network with vertices of atoms and edges of bonds. Difference in atom composition as well as bonding pattern characterizes chemical compounds and ultimately determines biological activity. Thus, feature extraction from networks is thought to play an important role in activity prediction of chemical compounds. As mentioned above, conventional convolutional neural networks assumed that each node has a fixed number of neighborhoods. In chemical compounds, however, each atom has a variable number of neighborhoods, which cannot be processed by conventional convolutional neural networks. Graph-convolution neural network is developed to apply convolution to networks with a variable number of neighborhoods by Duvenaud et al.[19] By using graph-convolution neural networks, Pande and colleagues in Stanford university constructed quantitative prediction model for lipophilicity and quantum mechanics with higher accuracy than conventional method, indicating usefulness of graph-convolution neural networks.[10] According to these reports, we applied graph-convolution neural networks to quantitative prediction of pharmacological activity. In ChEMBL 25, there are activity data of 7886 compounds for SERT. We split these data into three parts, training dataset, validation dataset, and test dataset. Training dataset was used for construction of prediction model, whereas validation dataset was not directly used for model construction, but was used for searching best hyperparameters such as learning rate, model structures, and so on. Then, goodness of model was evaluated in test datasets. As a result, we found that mean absolute error of our model, an index of prediction accuracy in quantitative prediction, was 0.56 ± 0.011 (as pIC50) for compounds in test dataset. Although the constructed model successfully predicts activity of compounds which are not directly used for model construction, it is possible that the model performance is not high for compounds outside of training, validation, and test datasets. Thus, we virtually screened 1.7 million compounds from ChEMBL whose activity on SERT was not reported. Through visual inspection, we selected a compound designated as CHEMBL1377753 because of its high predicted activity for SERT and similar lipophilicity to marketed SSRIs. We measured the inhibition activity of this compound in HEK293T cells heterologously expressing human SERT.[20] We found that measured IC_{50} of CHEMBL1377753 was 6.24 nM and was quite similar to predicted IC_{50} (10 nM). Moreover, we found that this compound acutely induced dose-dependent antidepressant-like effect in mouse tail-suspension test, a widely used behavioral paradigm for screening of antidepressants,[6] without affecting general locomotor. These results indicate that the constructed model has high generalizability to compounds outside of the dataset used for model construction in terms of ligand prediction for SERT.

2.3. Importance of validation of prediction in “real” assay

Considering the huge number of deposited assay data in public domain including ChEMBL and PubChem (https://pubchem.ncbi.nlm.nih.gov/), one may think that there is no need to do more “real” assays. It is partly true; specifically for proteins which are heavily investigated as a drug target. However, for other vast majority of proteins, it is not. Even though ChEMBL and similar databases have huge number of assay data, only a few target proteins have been associated with compounds more than 500, which is on par with minimum number of assay data we have successfully build a prediction model with moderate accuracy.[11] Therefore, it should be emphasized that pharmacological “real” assay for a broad range of proteins is necessary for perfecting in silico drug design and ultimately simulating in vivo efficacy. Moreover, synthetically accessible chemical space is estimated to be in order of 10^{33-60}, which is too large to perform “real” assay.[21,22] Thus, it would be too optimistic to consider that our “real” assays have sufficiently cover the diversity of this huge chemical space, even for target proteins heavily studied.

3. Towards structure-guided *in silico* screening with minimal validation in “real” assay

3.1. Rapid progress in structural biology provides deep insights into how SERT recognizes ligands

Considering the broadness of chemical space, predictability of prediction model for compounds outside of the chemical diversity used in model construction explicitly and implicitly will be limited. From this perspective, virtual screening and subsequent molecular dynamics simulation of target proteins and possible ligands to seek chemical structures fit to possible ligand binding pockets are promising to overcome above mentioned limitation.[23–25]

Until recently, it was, however, far from realistic to gain a number of 3-D structures of target proteins, especially membrane proteins, which is indispensable for molecular dynamics simulation. In 2005, Gouaux and colleagues reported the crystal structure of LeuT, a bacterial orthologue of neurotransmitter sodium symporters (NSSs) such as SERT.[7] However, NSSs consist of other transporters like dopamine transporter (DAT) and GABA transporter than SERT and overall sequence identity between LeuT and NSSs is around 20%. Therefore, the 3-D structure of mammalian SERT had long been awaited to gain mechanistic insights into ligand selectivity among NSSs. After more than 10 years, Coleman et al. reported crystal structure of human SERT in complex with its ligands, citalopram, paroxetine, and subsequently sertraline and fluvoxamine, although it derived from not wild-type but

thermostable mutant.[26,27] Whilst X-ray crystallography have revealed structures of a number of proteins and large protein complexes such as ribosomes,[28–30] there has been a rapid surge of single particle analysis of cryo-EM in structural biology, which does not require a crystallizing protein, one of the highest barriers for membrane proteins. In line with this, Coleman and Yang et al. successfully resolved the structure of wild-type SERT using cryo-EM in 2019.[31] Although it derives from wild-type SERT truncated with N- and C-termini, analyses of serotonin uptake and ligand binding suggest it has similar function to full-length wild-type SERT as well as ts2 thermostable mutant.[26,31] This series of work from Gouaux and colleagues provides deep insights into not only the structural detail of human SERT but also how antidepressants act on this protein.[26,27,31,32] More recently, Coleman and colleagues reported the structure of human SERT in complex with vilazodone, a combined SERT inhibitor and 5-HT_{1A} receptor partial agonist.[33] Interestingly, vilazodone is a non-competitive SERT inhibitor while all other selective serotonin reuptake inhibitors are competitive inhibitors, indicating a possible therapeutic efficacy of vilazodone through different mode of action. Cryo-EM structure of SERT with vilazodone and imipramine suggest that imipramine binds to the central sites similar to other SERT inhibitors whereas vilazodone binds to the allosteric sites located to the extracellular "vestibule".[33] They also demonstrated that binding of vilazodone concentration-dependently delayed the dissociation of prebound imipramine or escitalopram from SERT. These data suggest that the allosteric sites may be a promising drug target enhancing efficacy of antidepressants. Although above mentioned reports have revealed structure of mammalian SERT, structures of mammalian DAT and norepinephrine transporter is still unknown as far as we know. Because almost all DAT ligands have addictive property like cocaine and amphetamines,[34,35] high-resolution 3-D structure of mammalian DAT will be of high importance for *in silico* screening to seek drugs for mental illnesses with minimal risk of addiction.

Many proteins in cell present as a complex with other proteins. Previous reports have identified syntaxin 1A, SCAMP2, M6B, and cGMP-dependent protein kinase I as an associated protein with SERT. More importantly, association with these proteins modulates function of SERT.[36–39] Recent proteomic analyses of protein complex affinity purified for SERT in the mouse brain have revealed more than 300 proteins are potentially associated with SERT.[40,41] Quinlan et al. also used immunoprecipitants from SERT-KO mice, compared the results with SERT-WT mice, and identified 459 proteins associated with SERT.[41] These reports strongly suggest that SERT is associated with more proteins in vivo than we expected and possible regulation of its function by these bound auxiliary proteins. From this perspective, cryo-EM analysis of immunoprecipitated SERT complex from the brain will provide deep insights into how SERT function is regulated in the brain in health and disease, as reported in the cryo-EM analysis of 3-D structure of AMPA receptor complex with auxiliary subunits in the hippocampus of mice.[42]

3.2. Current state of structure-guided in silico screening of SERT ligands and its limitation

By utilizing the unveiled structure of human SERT, molecular docking and molecular dynamics simulations were performed to seek novel ligands and binding mode of known ligands. Zhang et al. have reported the binding mode of vilazodone in the human SERT through molecular docking and molecular dynamics simulations.[43] In their prediction vilazodone binds to allosteric and orthosteric sites with its cyanoindole moiety and benzofurancarboxamide moiety, respectively. Although this binding mode is different from that obtained from cryo-EM structure where vilazodone predominantly binds allosteric site, it should be noted that cryo-EM structure was obtained in the presence of imipramine, a high affinity ligand to orthosteric site, leading to different binding mode.[33] Erol et al. identified several candidate SERT inhibitors through virtual screening of about 260,000 small molecules from a chemical database.[44] Of note candidate SERT inhibitors they identified have a distinct chemical structure from SSRIs; 3 out of 4 candidates have two aromatic rings in both ends which are connected by long flexible chains similar to the structure of vilazodone. Although they also performed molecular dynamics simulation of SERT in complex with candidate inhibitors, pharmacological validation and characterization of these candidates in real assay are needed. Moreover, selectivity over related proteins, DAT and NET as well as serotonin receptors in case of SERT, is also important. Although structures of mammalian DAT and NET are not currently available, the structures of several serotonin receptors, 5-HT_{1B}, 5-HT_2, and 5-HT_3, have been resolved.[45–48] Considering that these receptors are therapeutic targets of a variety of diseases including mental disorders, migraine, and nausea, virtual screening against these receptors using 3-D structures will greatly help to achieve labor- and cost-effective drug development.

3.3. In silico protein folding is a key component for realization of structure-guided in silico screening with minimal validation

Although cryo-EM have rapidly resolved 3-D structures of proteins, it is still unrealistic to determine 3-D structures of all human gene products, more than 20,000 at least. To this end, *in silico* protein folding gains much attention reflecting rapid growth of computational resources and machine learning techniques. Recently, DeepMind, a London-based artificial intelligence company, and Baker and colleagues developed *in silico* protein folding systems named Alphafold2 and RoseTTAfold, respectively.[49,50] Both methods vastly outperform previous methods and are highly accurate in predicting the position of backbone carbon (Cα) as well as all atoms. Although inputs to these folding systems are primary amino-acid sequences of interest, it should be noted that they used previously resolved 3-D structure of homologues for protein-of-interest, nonetheless their prediction is accurate for proteins with no structural homologues. Therefore, further experimental determination of 3-D structures of proteins are important because of not only determination of structure itself but also increasing accuracy of prediction in a future. Importantly, these *in silico* folding systems provide a structure "template" which accelerates or is necessary in some cases to solve 3-D structure from raw data of cryo-EM and X-ray crystallography experiments. Collectively, these advances in experimental structural biology and computational structural biology will synergistically accelerate our understanding of mechanistic basis of protein functions.

Despite huge effort in developing antidepressant with new mechanisms of action, almost all current antidepressants are still ligands for SERT, evidencing the critical role of this transporter in treatment of major depression. Meanwhile, recreational drugs and narcotics such as ketamine, psilocybin, and 3,4-methylene-dioxymethamphetamine have drawn attention as novel antidepressants overcoming drug-resistance and delayed efficacy onset from which current antidepressants suffer.[51,52] Because these drugs have addictive or psychedelic property, combinatorial approach using protein structures and machine learning-based technologies will speed up a design of robust antidepressant with minimal adverse effects.

4. Concluding remarks

Growth in machine learning technologies and structural biology have rapidly changed our understanding of SERT and methodology for identification of new ligands (Fig. 1). However, it is still

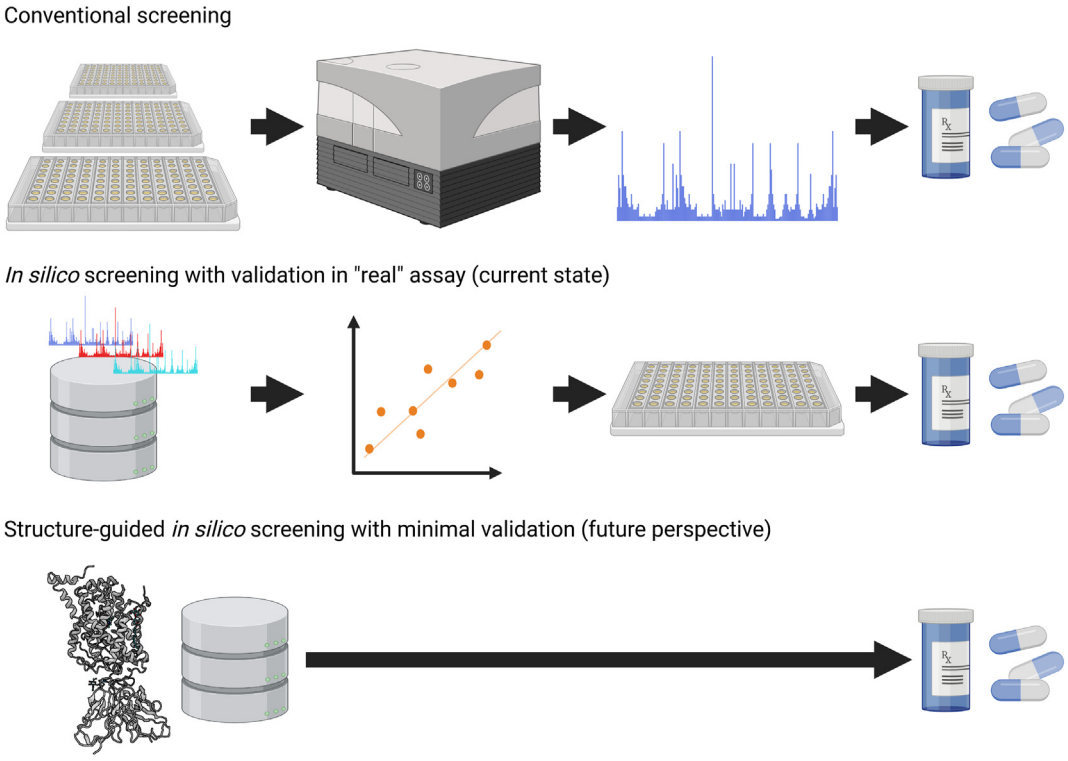

Fig. 1. Graphical summary of conventional screening, current hybrid screening, and structure-guided in silico screening in a future. (Top) Conventional screening requires a huge number of "real" assays to obtain hit compound for target protein. (Middle) Deep learning-based in silico screening utilized results of "real" assay deposited in databases such as ChEMBL and PubChem to find a hidden rule under an association between chemical structure and target protein. This method, however, necessitates pharmacological validation in "real" assay to some extent. (Bottom) Rapid progress in structural biology and in silico protein folding system will enable more accurate and far more comprehensive in silico screening, resulting in no or minimal requirement of validation in "real" assay. Illustrations were created with BioRender.com.

important to validate the prediction made *in silico* in "real" assay to some extent at least in the current state. We believe that structure-guided *in silico* screening with minimal validation will be realized through synergistic utilization of *in silico* protein folding system in a near future.

Declaration of competing interest

The author declares no competing interests.

Acknowledgement

We thank all our lab members, especially Miyuki Sakai and Prof. Shuji Kaneko. We thank Maiko Kitaichi-Nagayasu for helpful discussion. This work was partly supported by Grants-in-Aid for Scientific Research from JSPS (to K.N. (JP20H04774, JP20K07064), SENSHIN Medical Research Foundation, the Shimizu Foundation for Immunology and Neuroscience Grant, and the Uehara Memorial Foundation.

References

1. Blakely RD, Berson HE, Fremeau RT, et al. Cloning and expression of a functional serotonin transporter from rat brain. *Nature*. 1991;354(6348):66–70. https://doi.org/10.1038/354066a0.
2. Ressler KJ, Nemeroff CB. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depress Anxiety*. 2000;12(SUPPL.1):2–19. https://doi.org/10.1002/1520-6394(2000)12:1+<2::AID-DA2>3.0. CO;2-4.
3. Green AR. Neuropharmacology of 5-hydroxytryptamine. *Br J Pharmacol*. 2006;147(SUPPL. 1):145–152. https://doi.org/10.1038/sj.bjp.0706427.
4. Liu Z, Zhou J, Li Y, et al. Dorsal raphe neurons signal reward through 5-HT and glutamate. *Neuron*. 2014;81(6):1360–1374. https://doi.org/10.1016/j.neuron.2014.02.010.
5. Ren J, Friedmann D, Xiong J, et al. Anatomically defined and functionally distinct dorsal raphe serotonin sub-systems. *Cell*. 2018;175(2):472–487. https://doi.org/10.1016/j.cell.2018.07.043. e20.
6. Nishitani N, Nagayasu K, Asaoka N, et al. Manipulation of dorsal raphe serotonergic neurons modulates active coping to inescapable stress and anxiety-related behaviors in mice and rats. *Neuropsychopharmacology*. 2019;44(4):721–732. https://doi.org/10.1038/s41386-018-0254-y.
7. Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E. Crystal structure of a bacterial homologue of Na+/Cl –dependent neurotransmitter transporters. *Nature*. 2005;437(7056):215–223. https://doi.org/10.1038/nature03978.
8. Krishnamurthy H, Gouaux E. X-ray structures of LeuT in substrate-free outward-open and apo inward-open states. *Nature*. 2012;481(7382):469–474. https://doi.org/10.1038/nature10737.
9. Lecun Y, Bengio Y, Hinton G. Deep learning. *Nature*. 2015;521(7553):436–444. https://doi.org/10.1038/nature14539.
10. Wu Z, Ramsundar B, Feinberg EN, et al. MoleculeNet: a benchmark for molecular machine learning. *Chem Sci*. 2018;9(2):513–530. https://doi.org/10.1039/c7sc02664a.
11. Sakai M, Nagayasu K, Shibui N, et al. Prediction of pharmacological activities from chemical structures with graph convolutional neural networks. *Sci Rep*. 2021;11(1):525. https://doi.org/10.1038/s41598-020-80113-7.
12. Oord A, Dieleman S, Zen H, et al. WaveNet: a generative model for raw audio. arXiv:1609.03499 [cs.SD].
13. Devlin J, Chang MW, Lee K, Toutanova K. BERT: pre-training of deep bidirectional transformers for language understanding. NAACL HLT 2019. In: *2019 conf north Am chapter assoc comput linguist hum lang technol - proc confvol*. 1. 2019:4171–4186. Mlm.
14. Goodfellow IJ, Pouget-Abadie J, Mirza M, et al. Generative adversarial nets. *Adv Neural Inf Process Syst*. 2014;3(January):2672–2680. https://doi.org/10.3156/jsoft.29.5_177_2.
15. Mendez D, Gaulton A, Bento AP, et al. ChEMBL: towards direct deposition of bioassay data. *Nucleic Acids Res*. 2019;47(D1):D930–D940. https://doi.org/10.1093/nar/gky1075.
16. Szegedy C, Ioffe S, Vanhoucke V, Alemi AA. Inception-v4, inception-ResNet and the impact of residual connections on learning. *31st AAAI Conf Artif Intell AAAI*. 2017:4278–4284, 2017.

17. Uesawa Y. Quantitative structure–activity relationship analysis using deep learning based on a novel molecular image input technique. *Bioorg Med Chem Lett*. 2018;28(20):3400–3403. https://doi.org/10.1016/j.bmcl.2018.08.032.
18. Cortés-Ciriano I, Kekulescope Bender A. Prediction of cancer cell line sensitivity and compound potency using convolutional neural networks trained on compound images. *J Cheminf*. 2019;11(1):1–16. https://doi.org/10.1186/s13321-019-0364-5.
19. Duvenaud D, Maclaurin D, Aguilera-Iparraguirre J, et al. Convolutional networks on graphs for learning molecular fingerprints. *Adv Neural Inf Process Syst*. 2015:2224–2232, 2015-Janua.
20. Ramamoorthy S, Bauman AL, Moore KR, et al. Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci U S A*. 1993;90(6):2542–2546. https://doi.org/10.1073/pnas.90.6.2542.
21. Bohacek RS, McMartin C, Guida WC. The art and practice of structure-based drug design: a molecular modeling perspective. *Med Res Rev*. 1996;16(1):3–50. https://doi.org/10.1002/(SICI)1098-1128(199601)16:1<3::AID-MED1>3.0. CO;2-6.
22. Polishchuk PG, Madzhidov TI, Varnek A. Estimation of the size of drug-like chemical space based on GDB-17 data. *J Comput Aided Mol Des*. 2013;27(8):675–679. https://doi.org/10.1007/s10822-013-9672-4.
23. Iwata H, Kanda N, Araki M, Sagae Y, Masuda K, Okuno Y. Discovery of natural TRPA1 activators through pharmacophore-based virtual screening and a biological assay. *Bioorg Med Chem Lett*. 2021;31(August 2020):1–5. https://doi.org/10.1016/j.bmcl.2020.127639.
24. Matsumoto S, Ishida S, Araki M, Kato T, Terayama K, Okuno Y. Extraction of protein dynamics information from cryo-EM maps using deep learning. *Nat Mach Intell*. 2021;3(2):153–160. https://doi.org/10.1038/s42256-020-00290-y.
25. Araki M, Matsumoto S, Bekker GJ, et al. Exploring ligand binding pathways on proteins using hypersound-accelerated molecular dynamics. *Nat Commun*. 2021;12(1):1–10. https://doi.org/10.1038/s41467-021-23157-1.
26. Coleman JA, Green EM, Gouaux E. X-ray structures and mechanism of the human serotonin transporter. *Nature*. 2016;532(7599):334–339. https://doi.org/10.1038/nature17629.
27. Coleman JA, Gouaux E. Structural basis for recognition of diverse antidepressants by the human serotonin transporter. *Nat Struct Mol Biol*. 2018;25(2):170–175. https://doi.org/10.1038/s41594-018-0026-8.
28. Schluenzen F, Tocilj A, Zarivach R, et al. Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. *Cell*. 2000;102(5):615–623. https://doi.org/10.1016/s0092-8674(00)00084-2.
29. Ban N, Nissen P, Hansen J, Moore PB, Steitz TA. The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. *Science*. 2000;289(5481):905–920. https://doi.org/10.1126/science.289.5481.905.
30. Wimberly BT, Brodersen DE, Clemons WM, et al. Structure of the 30S ribosomal subunit. *Nature*. 2000;407(6802):327–339. https://doi.org/10.1038/35030006.
31. Coleman JA, Yang D, Zhao Z, et al. Serotonin transporter–ibogaine complexes illuminate mechanisms of inhibition and transport. *Nature*. 2019. https://doi.org/10.1038/s41586-019-1135-1.
32. Coleman JA, Navratna V, Antermite D, Yang D, Bull JA, Gouaux E. Chemical and structural investigation of the paroxetine-human serotonin transporter complex. *Elife*. 2020;9:1–38. https://doi.org/10.7554/eLife.56427.
33. Plenge P, Yang D, Salomon K, et al. The antidepressant drug vilazodone is an allosteric inhibitor of the serotonin transporter. *Nat Commun*. 2021;12(1). https://doi.org/10.1038/s41467-021-25363-3.
34. Sora I, Hall FS, Andrews AM, et al. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc Natl Acad Sci U S A*. 2001;98(9):5300–5305. https://doi.org/10.1073/pnas.091039298.
35. Gether U, Andersen PH, Larsson OM, Schousboe A. Neurotransmitter transporters: molecular function of important drug targets. *Trends Pharmacol Sci*. 2006;27(7):375–383. https://doi.org/10.1016/j.tips.2006.05.003.
36. Quick MW. Regulating the conducting states of a mammalian serotonin transporter. *Neuron*. 2003;40(3):537–549. https://doi.org/10.1016/S0896-6273(03)00605-6.
37. Müller HK, Wiborg O, Haase J. Subcellular redistribution of the serotonin transporter by secretory carrier membrane protein 2. *J Biol Chem*. 2006;281(39):28901–28909. https://doi.org/10.1074/jbc.M602848200.
38. Fjorback AW, Müller HK, Wiborg O. Membrane glycoprotein M6B interacts with the human serotonin transporter. *J Mol Neurosci*. 2009;37(3):191–200. https://doi.org/10.1007/s12031-008-9092-4.
39. Steiner JA, Carneiro AMD, Wright J, et al. cGMP-dependent protein kinase iα associates with the antidepressant-sensitive serotonin transporter and dictates rapid modulation of serotonin uptake. *Mol Brain*. 2009;2(1):1–12. https://doi.org/10.1186/1756-6606-2-26.
40. Haase J, Grudzinska-Goebel J, Müller HK, et al. Serotonin transporter associated protein complexes are enriched in synaptic vesicle proteins and proteins involved in energy metabolism and ion homeostasis. *ACS Chem Neurosci*. 2017;8(5):1101–1116. https://doi.org/10.1021/acschemneuro.6b00437.
41. Quinlan MA, Robson MJ, Ye R, Rose KL, Schey KL, Blakely RD. Ex vivo quantitative proteomic analysis of serotonin transporter interactome: network impact of the SERT Ala56 coding variant. *Front Mol Neurosci*. 2020;13(June). https://doi.org/10.3389/fnmol.2020.00089.
42. Yu J, Rao P, Clark S, Mitra J, Ha T, Gouaux E. Hippocampal AMPA receptor assemblies and mechanism of allosteric inhibition. *Nature*. 2021;594(7863):448–453. https://doi.org/10.1038/s41586-021-03540-0.
43. Zhang Y, Zheng G, Fu T, et al. The binding mode of vilazodone in the human serotonin transporter elucidated by ligand docking and molecular dynamics simulations. *Phys Chem Chem Phys*. 2020;22(9):5132–5144. https://doi.org/10.1039/c9cp05764a.
44. Erol I, Aksoydan B, Kantarcioglu I, Salmas RE, Durdagi S. Identification of novel serotonin reuptake inhibitors targeting central and allosteric binding sites: a virtual screening and molecular dynamics simulations study. *J Mol Graph Model*. 2017;74:193–202. https://doi.org/10.1016/j.jmgm.2017.02.001.
45. Hassaine G, Deluz C, Grasso L, et al. X-ray structure of the mouse serotonin 5-HT3 receptor. *Nature*. 2014;512(7514):276–281. https://doi.org/10.1038/nature13552.
46. García-Nafría J, Nehmé R, Edwards PC, Tate CG. Cryo-EM structure of the serotonin 5-HT1B receptor coupled to heterotrimeric Go. *Nature*. 2018;558(7711):620–623. https://doi.org/10.1038/s41586-018-0241-9.
47. Peng Y, McCorvy JD, Harpsøe K, et al. 5-HT2C receptor structures reveal the structural basis of GPCR polypharmacology. *Cell*. 2018;172(4):719–730.e14. https://doi.org/10.1016/j.cell.2018.01.001.
48. Kimura KT, Asada H, Inoue A, et al. Structures of the 5-HT 2A receptor in complex with the antipsychotics risperidone and zotepine. *Nat Struct Mol Biol*. 2019;26(2):121–128. https://doi.org/10.1038/s41594-018-0180-z.
49. Tunyasuvunakool K, Adler J, Wu Z, et al. Highly accurate protein structure prediction for the human proteome. *Nature*. 2021;596(May). https://doi.org/10.1038/s41586-021-03828-1.
50. Baek M, DiMaio F, Anishchenko I, et al. Accurate prediction of protein structures and interactions using a three-track neural network. *Science (80-)*. 2021;373(6557):871–876. https://doi.org/10.1126/science.abj8754.
51. Sessa B, Nutt DJ. MDMA, politics and medical research: have we thrown the baby out with the bathwater? *J Psychopharmacol*. 2007;21(8):787–791. https://doi.org/10.1177/0269881107084738.
52. Nutt D. Help luck along to find psychiatric medicines. *Nature*. 2014;515(7526):165. https://doi.org/10.1038/515165a.