WHITE PAPER

Precision by Design: Drug Innovation through Structure-based Drug Design (SBDD)

Structure-based drug design (SBDD) is a valuable tool in drug discovery. After the development of crystallography in the mid-20th century, it gained momentum in the 1990s with the first FDA-approved drug developed using structural information, the HIV protease inhibitor saquinavir.[1] Since then, thanks to the advances in several fields, including computational chemistry and structural biology, it has become a powerful approach in modern drug discovery.

SBDD is a rational and data-driven approach, providing several advantages over other strategies:

- **Insights into the mechanism of compounds:** It provides detailed information about how a compound interacts with its target, unravelling key interactions responsible for the compound activity as well as the mechanism of action. This informs decisions for continuing with the lead optimization process.
- **Identifying new interactions:** Analyzing the 3D structure of the binding site enables the identification of previously unknown binding pockets and side chain interactions, which allow the opportunity to improve compound affinity and selectivity.
- **Focus efforts and expedite timelines:** Given its structural rationale, SBDD prioritizes compounds based on their fit in binding sites, therefore increasing the probability of success. It also filters out poor hits earlier in the process, significantly shortening drug discovery timelines.

However, SBDD also presents some challenges and limitations:

- **Limited availability of high-quality structural data:** The main limitation of SBDD is the dependency on accurate 3D structures. Despite the advances in crystallography, some targets remain challenging to crystallize . In these instances, NMR or cryo-EM may provide structural information, although typically at a lower resolution than crystallography.
- **High upfront cost:** While the SBDD approach can reduce downstream costs, there's a substantial upfront investment needed in infrastructure, materials and expertise, which can limit the adoption by smaller organizations.
- **Specialized expertise and infrastructure:** A successful structure-based drug design program requires a multidisciplinary team with expertise in structural biology, medicinal chemistry and computational modelling, among others. Coordination is crucial to obtain quality hits in a time-efficient manner.

To illustrate how structure-based drug design can address common challenges and significantly accelerate the path from discovery to the clinic, the following two case studies highlight successful applications of SBDD in real-world drug discovery programs.

Artemis

Artemis is an endonuclease involved in DNA repair as well as the development of B and T lymphocytes.2 It is part of the non-homologous DNA end joining major pathway and forms a complex with a protein kinase catalytic subunit. Mutations in the gene lead to severe combined immune deficiency, making the patients hypersensitive to radiation. Radiotherapy is one of the standard treatment options for some solid malignant cancers. Therefore, Artemis was found to be an attractive target for the development of therapeutics to manage various B-cell and T-cell tumors. For approximately six years, multiple labs attempted to provide high-quality protein, but progress was slow due to several challenges, including low protein production for subsequent testing and the lack of experimental protein structures. As part of the CBC NExT program, Curia was assigned the goal of solving the Artemis production and crystallization barriers which had prevented the application of a SBDD approach to develop a potent, selective inhibitor suitable for proof-of-concept studies.

The starting point for crystallography was limited, since the Apollo Nuclease was only 32% identical to Artemis (Figure 1a). For production, three different constructs were designed, of which one proved successful. Production was followed by crystallization screening, where more than 7,000 conditions were tested, and the hits were analyzed (Figure 1b). The first crystal structure of Artemis was obtained with a resolution of 1.97 Å. This crystal structure led to the first accurate model to guide structure-based drug design.

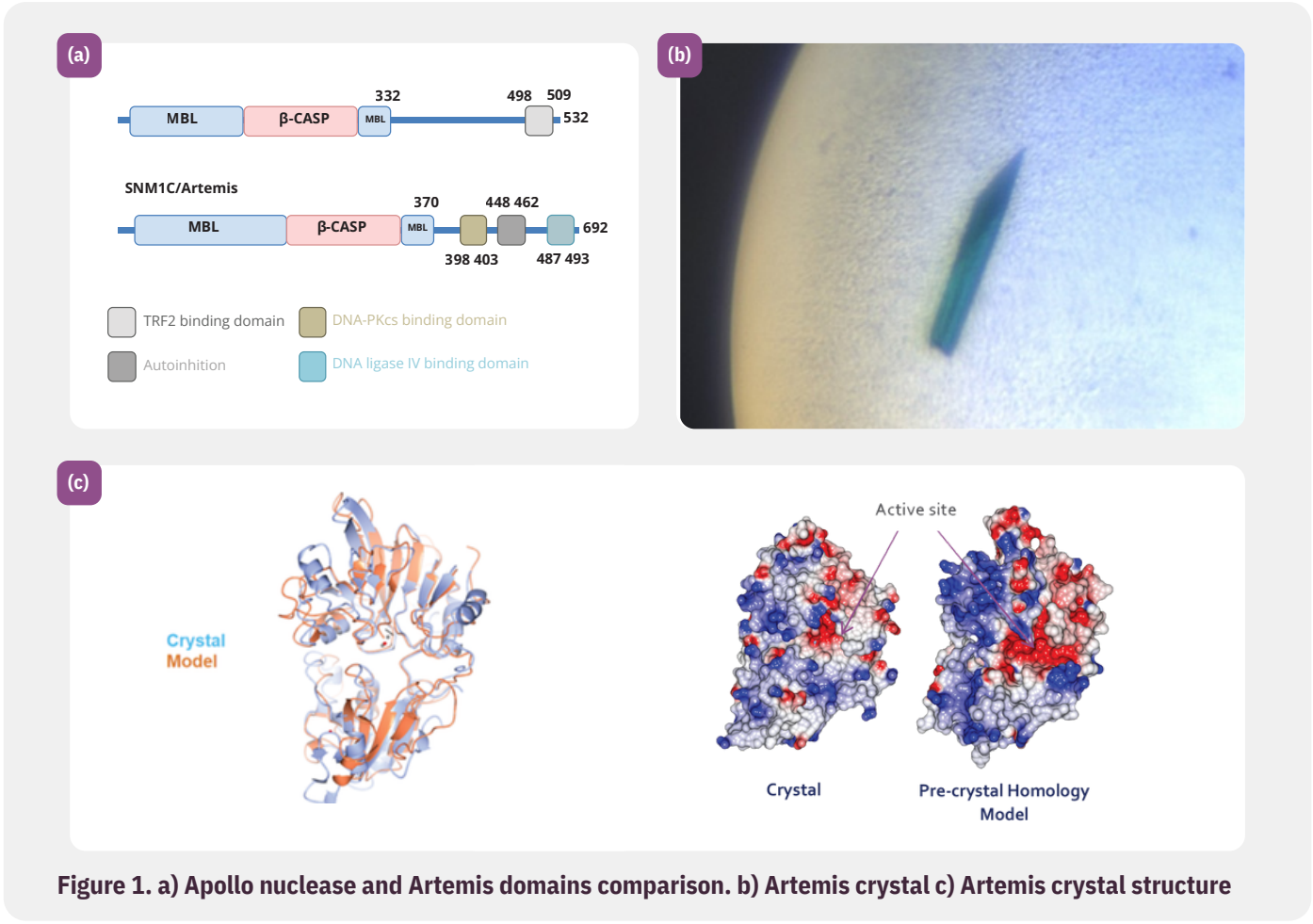


Figure 1. a) Apollo nuclease and Artemis domains comparison. b) Artemis crystal c) Artemis crystal structure

A high-throughput screening was performed using compounds from the Curia Compound Library Consortium (CLC) . This allowed the identification of a novel binding site (Figure 2) in Artemis distant from the metallo-enzyme active site.3 This binding site undergoes a small but significant backbone shift that favors non-metal-binding compounds distant from the enzyme active site.

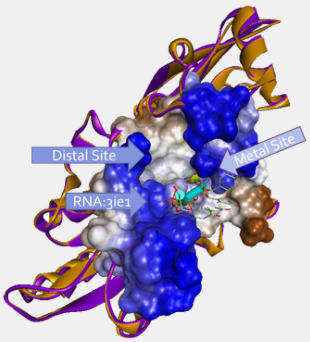


Figure 2. Apo Artemis structure (brown) overlapped with the homology model (purple)

The co-crystals of the early hits were shared with the computational and medicinal chemistry team and used to design more potent inhibitors. From the crystal structures, it was seen that three hits with unique scaffolds bound in the same pocket. This overlap allowed for hybrid designs of scaffolds that resulted in going from ~10 μM to 500 nM potency. Further study of the crystal structures revealed an opportunity to capture a new salt bridge interaction, resulting in a 10-fold improvement in potency. Co-crystals of new medicinal chemistry analogs provided structural insights that informed designs. This valuable information led to the improvement of inhibitors, with IC50 measurements of ~10 μM to low nM, in 12 months of synthesis.

This case study highlights the importance of having an integrated approach towards a structure-based drug design program:

- The combination of production of a high-quality protein, a novel HTS library and rapid co-crystallization of early hits elucidated a novel binding site in Artemis.
- Co-crystals of new medicinal chemistry analogs allowed a rapid improvement in the inhibition.
- Additionally, the structure-driven SAR identified novel combinations of scaffolds from the original hits.

The Curia team of structural biologists, medicinal chemists, and computational chemists improved potency ~500-fold while creating novel chemical matter. Using this iterative structure-based drug design approach drove the discovery of potent Artemis inhibitors from construct design (Figure 3), showcasing the value of executing a structure-driven strategy, to fast-track drug discovery and invention within 18 months.

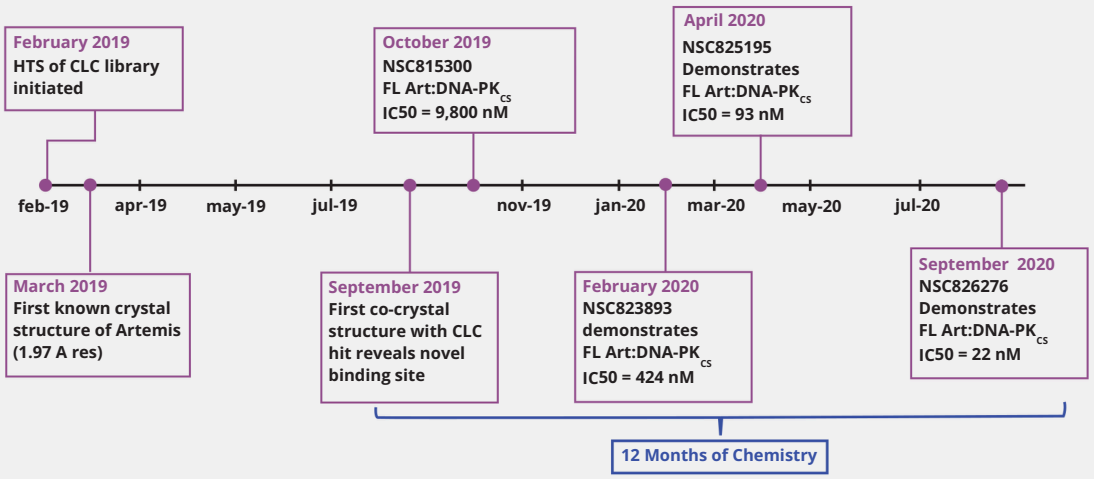


Figure 3. Timeline of the Artemis project

Nonretinoid Antagonists of Retinol-Binding Protein 4

Dry age-related macular degeneration (AMD) is the leading cause of blindness for individuals aged 55 or older. Although approved therapies already exist, they require regular intraocular injections and are associated with safety concerns. A program funded by the BPN was created to identify novel, orally administered non-retinoid RBP4 antagonists with an improved safety profile.

Dry AMD begins with abnormalities in the retinal pigment epithelium, such as the accumulation of cytotoxic lipofuscin, a byproduct of the visual cycle. An attractive treatment option involves inhibiting this accumulation to delay cone cell death by disrupting the retinol delivery that fuels the visual cycle. Retinol binds to serum retinol-binding protein 4 (RBP4), so inhibiting this complex should lead to a decreased uptake into the retina. There were preclinical proof-of-concept studies that confirmed the hypothesis. They were carried out with two compounds, fenretinide and A1120.

A1120 is a nonretinoid RBP4 ligand developed by Amgen (Figure 4a) for the treatment of diabetes and showed reduced serum RBP4 levels in the transgenic mouse model after administration. However, this compound suffered from poor metabolic stability. Therefore, A1120 was used as the starting point to develop novel antagonists with improved ADME characteristics.

The ensuing iterative structure-based design program utilized publicly available structures of RBP4. The A1120:RBP4 co-crystal structure (PDB code 3fmz) was particularly valuable. The medicinal chemistry plan involved multiple rounds of optimization, conducted in collaboration with Curia's CADD team (Figure 4b). Several significant alterations of different sections of the A1120 structure yielded highly potent RBP4 binders with improved ADMET properties.

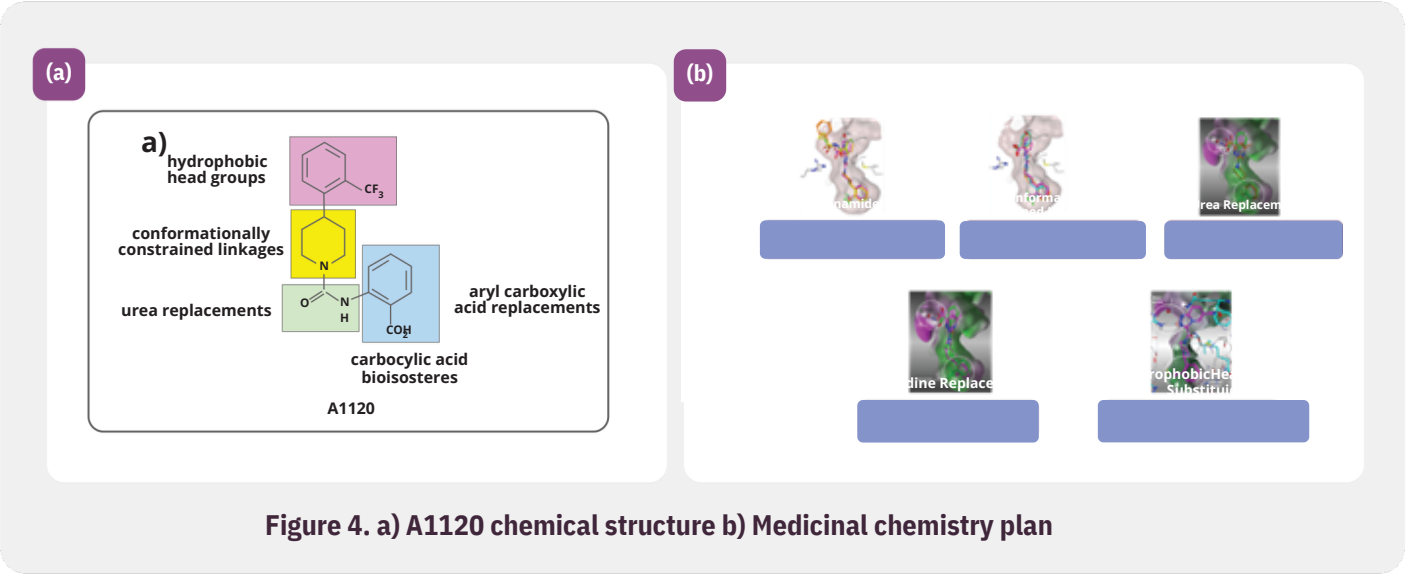


Figure 4. a) A1120 chemical structure b) Medicinal chemistry plan

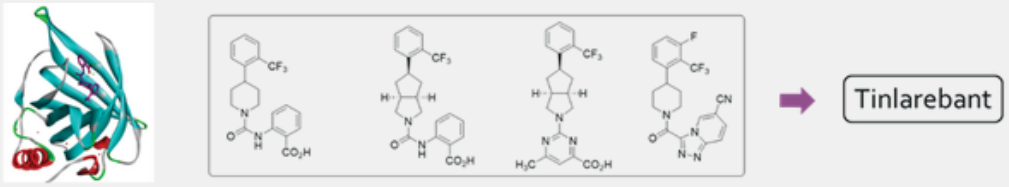
Overall, multiple series of compounds were designed, synthesized and tested for their DMPK characteristics. From those, two compounds showed particularly promising results in vitro and in vivo. Compound 31 showed an attractive PK profile based on:

- Very slow elimination from plasma with t1/2 = 22.0 h (iv) and 38.8 h (po)
- Very low clearance of 5.06 mL/h/kg and volume of distribution (156 mL/kg)
- Very high oral exposure with Cmax = 62.2 µM and AUCinf = 3,636 µM*h
- Very high bioavailability of >100% (possibly related to very low plasma clearance)

In addition, compound 91 numbers were the following:

- Slow elimination from plasma with t1/2 = 10.5 h
- Moderate clearance of 1134 mL/h/kg and volume of distribution (4281 mL/kg)
- Adequate oral exposure with Cmax = 175 ng/mL and AUClast = 1771 h*ng/mL
- Reasonable bioavailability of 42%

Both compounds were able to show increased reduction in serum RBP4 levels compared to A1120 (Figure 5).4 Continued optimization led to the discovery of Tinlarebant, currently in Phase III clinical trials for advanced dry AMD.



Compound	A1120 (3)	13	31	91
RBP4 SPA IC_{50} (nM)	15.0	72.7	12.8	5.7
RBP4 HTRF IC_{50} (nM)	122	294	43.6	16.1
HLM Stability (% Remaining)	3	100	94	100
CYP2C9 IC_{50} (μM)	>100	0.34	>34	>10
Reduction in Serum RBP4 Levels	75%	60%	95%	85%

Figure 5. Summary of A1120, compound 13, 31 and 91 performance

To summarize, A1120 (3) and RBP4 crystal structure 3fmz were used with structure-based drug design strategies to identify nonretinoid RBP4 antagonists with improved drug-like properties. Structural modifications of the A1120 template provided potent compounds with improved HLM stability and good in vivo efficacy.

Conclusion

Structure-based drug design has matured into a powerful and practical strategy for modern drug discovery, facilitating the identification and optimization of drug candidates, reducing trial-and-error approaches, shortening SAR development timelines and improving the likelihood of clinical success.

While challenges remain, recent advances in structural biology and computational tools have significantly lowered these barriers. The two case studies presented in this white paper demonstrate how SBDD is already overcoming these limitations in practice. These examples underscore the transformative potential of SBDD, not only in terms of speed and efficiency, but also in enabling more informed decision-making throughout the drug research and development pipeline. As technologies like cryo-EM, AI-based structure prediction and molecular simulation continue to evolve, SBDD will play an even more central role in the discovery of next-generation therapeutics.

In an era where innovation, precision, and speed are more critical than ever, structure-based drug design offers a compelling framework for designing smarter, safer, and more effective medicines.

References

1.Wlodawer, A., & Vondrasek, J. (1998). INHIBITORS OF HIV-1 PROTEASE: a major success of Structure-Assisted drug design. Annual Review of Biophysics and Biomolecular Structure, 27 (1), 249–284. https://doi.org/10.1146/annurev.biophys.27.1.249

2. Yosaatmadja, Y., Baddock, H. T., Newman, J. A., Bielinski, M., Gavard, A. E., Mukhopadhyay, S. M. M., Dannerfjord, A. A., Schofield, C. J., McHugh, P. J., & Gileadi, O. (2021). Structural and mechanistic insights into the Artemis endonuclease and strategies for its inhibition. Nucleic Acids Research, 49 (16), 9310–9326. https://doi.org/10.1093/nar/gkab693

3. Karim, M. F., Liu, S., Laciak, A. R., Volk, L., Koszelak-Rosenblum, M., Lieber, M. R., Wu, M., Curtis, R., Huang, N. N., Carr, G., & Zhu, G. (2020). Structural analysis of the catalytic domain of Artemis endonuclease/SNM1C reveals distinct structural features. Journal of Biological Chemistry, 295 (35), 12368–12377. https://doi.org/10.1074/-jbc.ra120.014136

4. Cioffi, C. L., Racz, B., Varadi, A., Freeman, E. E., Conlon, M. P., Chen, P., Zhu, L., Kitchen, D. B., Barnes, K. D., Martin, W. H., Pearson, P. G., Johnson, G., Blaner, W. S., & Petrukhin, K. (2019). Design, synthesis, and preclinical efficacy of novel nonretinoid antagonists of Retinol-Binding protein 4 in the mouse model of hepatic steatosis. Journal of Medicinal Chemistry, 62 (11), 5470–5500. https://doi.org/10.1021/acs.jmedchem.9b00352

5. Cioffi, C. L., Racz, B., Freeman, E. E., Conlon, M. P., Chen, P., Stafford, D. G., Schwarz, D. M. C., Zhu, L., Kitchen, D. B., Barnes, K. D., Dobri, N., Michelotti, E., Cywin, C. L., Martin, W. H., Pearson, P. G., Johnson, G., & Petrukhin, K. (2015). Bicyclic [3.3.0]-Octahydrocyclopenta[c]pyrrolo Antagonists of Retinol Binding Protein 4: Potential Treatment of Atrophic Age-Related Macular Degeneration and Stargardt Disease. Journal of Medicinal Chemistry, 58 (15), 5863–5888. https://doi.org/10.1021/acs.jmedchem.5b00423

6. Cioffi, C. L., Dobri, N., Freeman, E. E., Conlon, M. P., Chen, P., Stafford, D. G., Schwarz, D. M. C., Golden, K. C., Zhu, L., Kitchen, D. B., Barnes, K. D., Racz, B., Qin, Q., Michelotti, E., Cywin, C. L., Martin, W. H., Pearson, P. G., Johnson, G., & Petrukhin, K. (2014). Design, synthesis, and evaluation of nonretinoid retinol binding Protein 4 antagonists for the potential treatment of Atrophic Age-Related Macular Degeneration and Stargardt Disease. Journal of Medicinal Chemistry, 57(18), 7731–7757. https://doi.org/10.1021/jm5010013

ABOUT CURIA

Curia is a global contract research, development, and manufacturing organization (CDMO) with over 30 years of experience. With an integrated network of 20+ facilities worldwide and a team of 3,000+ dedicated professionals, we specialize in partnering with biopharmaceutical customers to bring life changing therapies to market. Our offerings in small molecules, generic APIs, and biologics span discovery through commercialization, with integrated regulatory, analytical, and sterile fill-finish capabilities. Our scientific and process experts, along with our regulatory-compliant facilities, deliver a best-in-class experience across drug substance and drug product manufacturing. From curiosity to cure, we are your trusted ally in accelerating life-changing therapeutics.

Learn more at curiaglobal.com

curia