Structure-based Drug Design

High-throughput techniques enable structure guided drug discovery against the inflammatory target NLRP3

Andrew Thompson\textsuperscript{1*}, Tryfon Zarganis-Tzitzikas\textsuperscript{1}, Martin Lowe\textsuperscript{2}, Elena Fonfria\textsuperscript{2}, Emma Murphy\textsuperscript{1}, Frank von Delft\textsuperscript{1}, Paul Brennan\textsuperscript{1}

\textsuperscript{1}Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford, Oxford OX1 3QU, UK

\textsuperscript{2}Exscientia, The Schrödinger Building, Oxford Science Park, Oxford OX4 4GE, UK

\*Current address: Chemical Biology Division, WEHI, Parkville 3052, VIC, AUS. Email: thompson.a@wehi.edu.au

Keywords: structure guided drug discovery, high-throughput, protein science, crystallography, NLRP3, neuroinflammation

The NLRP3 inflammasome is a key regulator of the pyroptosis response in the innate immune system. It is through this highly regulated signalling cascade that macrophages respond to danger and damage associated molecules. Consequently, NLRP3 mutants are associated with inflammatory diseases. Targeting NLRP3 with small molecule inhibitors is an attractive strategy for the treatment of cancer and neurodegenerative diseases such as Alzheimer’s disease [1]. Currently, no NLRP3 inhibitors have reached the clinic, causing the hunt for alternate scaffolds to garner broad pharmaceutical investment.

Progress in the structural investigation of NLRP3 has been challenging due to its inherent instability and membrane association, impeding the development of structure-guided drug discovery pipelines that can consistently deliver high-resolution structural information. In recent years, several high impact structures of the various domains and binding partners of NLRP3 have revealed its biological mechanism [2]. Among these studies, it was confirmed that the NACHT nucleotide hydrolysis domain is the target for publicly available small molecule inhibitors, however the highest published resolution of this domain is 2.8Å [3], limiting structure-guided optimisation of inhibitors. In collaboration with the pharmaceutical company Exscientia, the Oxford Drug Discovery Institute aimed to improve upon this resolution by developing an in-house structure guided drug discovery pipeline.

Here, we describe the use of high-throughput cloning, expression, purification and crystallography techniques for the development of a structure-guided drug discovery pipeline against the NACHT domain of NLRP3 (Fig. 1). Broad and unbiased assessment of compounds and the N- and C-terminal boundaries for the NLRP3 NACHT domain constructs greatly influenced the crystallisation habit and the resulting resolution. Critically, the assessment of >450 crystals of NLRP3 was performed with the use of the semi-automated crystal harvesting robot: Crystal Shifter (Oxford Lab Tech) [4] in combination with unattended data collection and automated structure solution pipelines developed at Diamond Light Source. Using this approach, 8 high resolution structures of NLRP3 bound to lead molecules were solved (1.9-2.5Å), guiding medicinal chemistry outputs that are now patented. The use of formalised high-throughput processes was crucial to overcoming the technical challenges of working with an unstable protein. The techniques and mindset employed in this program are broadly applicable to many structure-guided drug discovery projects where higher throughput and resolution would be beneficial.

Figure 1. A workflow for the high-throughput development of a structure guided drug discovery pipeline. Construct design was performed to enumerate potentially soluble and crystallisable NLRP3 constructs. Through a combination of high-throughput PCR based cloning and gene synthesis, many proteins were expressed and purified at a small scale. Promising constructs were scaled up and purified in parallel. All observed crystals were harvested and sent for automated unattended data collection and analysis at Diamond Light Source. Critically, this process is iterative, with crystallography outputs guiding future construct design.


