Advanced SPR applications accelerate hit identification and validation in fragment-based drug discovery

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Over the past two decades, fragment-based drug discovery (FBDD) has emerged as a powerful way to quickly assess the druggability of new targets and identify potential leads. To date, six FDA-approved drugs were identified by FBDD, such as cancer drugs vemurafenib and erdafitinib. The advantage of FBDD is that it reduces the molecular complexity to be explored by orders of magnitude, providing excellent coverage of chemical space and a starting point for hit-to-lead optimization.

FBDD approaches focus on low-atomic weight molecules that provide broad coverage of chemical and property space. This leads to fragment libraries of a few thousand molecules, compared to 10^{60} [1], which is the estimated number of possible compounds with a molecular weight of 500 Da. Even compared to the average industrial-scale small molecule library with up to 10^6 molecules, fragment libraries vastly simplify screening.

My research group applies FBDD principles to disease-relevant protein-protein interactions involved in redox signaling, oxidative stress, and inflammation. These targets play important roles in diseases such as, multiple sclerosis, stroke, lung inflammation, fibrosis, rheumatoid arthritis, and some cancers.

There are two stages to FBDD: 1) fragment screening to identify initial hits, and 2) subsequent characterization and optimization of those hits into real leads. We use sensitive biophysical methods, like surface plasmon resonance (SPR) and ligand-based NMR, to screen our fragment library of around 2,500 molecules. At this stage we expect low-affinity hits that are in the high micromolar or low millimolar affinity range. We then validate the hits by more rounds of SPR testing or other assays, such as fluorescence polarization (FP). The best hits are characterized by structural methods, like X-ray crystallography, to define the binding mode to the target. We then optimize the hits in an iterative fragment-to-lead (F2L) cycle of design, synthesis, assay testing, and X-ray crystallography. The goal of this medicinal chemistry optimization process is to improve binding affinities and other drug-like properties.

SPR is one of the primary methods for the detection and characterization of fragment binding events due to its ability to detect low-affinity interactions of low molecular-weight compounds. SPR has numerous advantages for this application. It reduces overall sample consumption and is a label-free method, which simplifies workflows and avoids functional interference from labeling schemes. Further, it allows for fast, high-throughput screening and determination of accurate kinetics and binding affinity.

Modern SPR technologies, like the Octet® SF3 SPR System, have significantly streamlined workflows with sensitive and reliable methods for identifying fragment hits and ruling out false positives. Advanced injection features, like the OneStep® Injection on the Octet® SF3, where equilibrium binding constants (K_D) values are determined in a single injection instead of multiple fixed-concentration injections, have also had a notable impact on overall workflow efficiency.

Three of our recent publications demonstrate the utility of SPR in our process, where we used a Pioneer FE, the 1st generation instrument preceding the Octet® SF3 System. Our main targets are Kelch-like ECH-associated protein 1 (Keap1), which regulates the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor

and thereby the endogenous antioxidant response, and the superoxide-generating enzyme complex NADPH oxidase 2 (NOX2).

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase isoform 2 is a multi-subunit enzyme complex referred to as NOX2 that generates reactive oxygen species. Uncontrolled production of reactive oxygen species is linked to a variety of diseases, including diabetes and cancer. Thus, therapeutic strategies for regulating the function of NOX2 are of clinical interest. The catalytic activity of NOX2 requires an interaction between the p47phox and p22phox subunits, making p47phox a potential target.

In a 2020 study[2], we applied FBDD to developing novel protein-protein interaction inhibitors of the p47phox subunit of NOX2. We screened our 2,500-fragment commercial library by FP and thermal shift assay (TSA) and then validated the hits using a variety of SPR binding assays. Subsequent biostructural studies showed that two of our fragments bound to two separate binding sites on p47phox. Based on these observations, we designed a potent dimeric inhibitor, providing a new mode of inhibition for p47phox.

Another target involved in oxidative stress is Keap1. Keap1 is a repressor of Nrf2, a transcription factor and master cellular regulator of oxidative stress, so targeting the Keap1-Nrf2 interaction is one way to manage diseases involving oxidative stress. In a 2021 study[3], we developed potent inhibitors of the Keap1-Nrf2 interaction using a reverse approach called fragment-based deconstruction reconstruction (FBDR). We dissected known small-molecule inhibitors of the Keap1-Nrf2 interaction into 77 fragments and used those as a launch pad for screening and design of novel inhibitors with improved potency.

In a related study from 2022[4] targeting the same Keap1-Nrf2 interaction, we screened our commercial library of 2,500 fragments using the orthogonal methods FP, TSA, SPR, and validated the hits by saturation transfer difference (STD) NMR and dose-response SPR. Using structure-based drug discovery we optimized our top hits, producing a novel, high-affinity (K_i = 280 nM) noncovalent small-molecule inhibitor – a 1,700-fold increase in affinity compared to the original fragment hit.

These studies demonstrate the power of FBDD for designing new, highly potent drugs against clinical targets. Our work also highlights the importance of using orthogonal assays to screen and validate fragment hits. The weak affinities and unspecific nature of fragments make it challenging to reliably identify true hits from false positives. SPR technology is key to our triaging process, which relies on high-quality kinetic data as the basis for validating and advancing molecules for X-ray crystallography. Additionally, SPR counter-screens are one of our main strategies for successfully flagging false-positive hits.

My group continues to optimize methodologies around FBDD using the most advanced analytical assays with the ultimate goal of identifying small-molecule inhibitors with potential for pharmaceutical development.

References

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Anders received his PhD in medicinal chemistry from University of Copenhagen (UCPH) in 2009. Here, he developed compounds against PDZ domains involved in protein-protein interactions in the CNS. Particularly, his bivalent peptidomimetic PSD-95 inhibitors led to the founding of Avilex Pharma and clinical trial development. He did postdoc research first at UCPH and then at the Italian Institute of Technology, where he worked on covalent small-molecule inhibitors of enzymes involved in lipid metabolism and signaling. He now holds a position as associate professor and group leader at UCPH, where he uses fragment-based drug discovery to make small-molecule inhibitors of protein-protein interactions involved in oxidative stress and inflammation.