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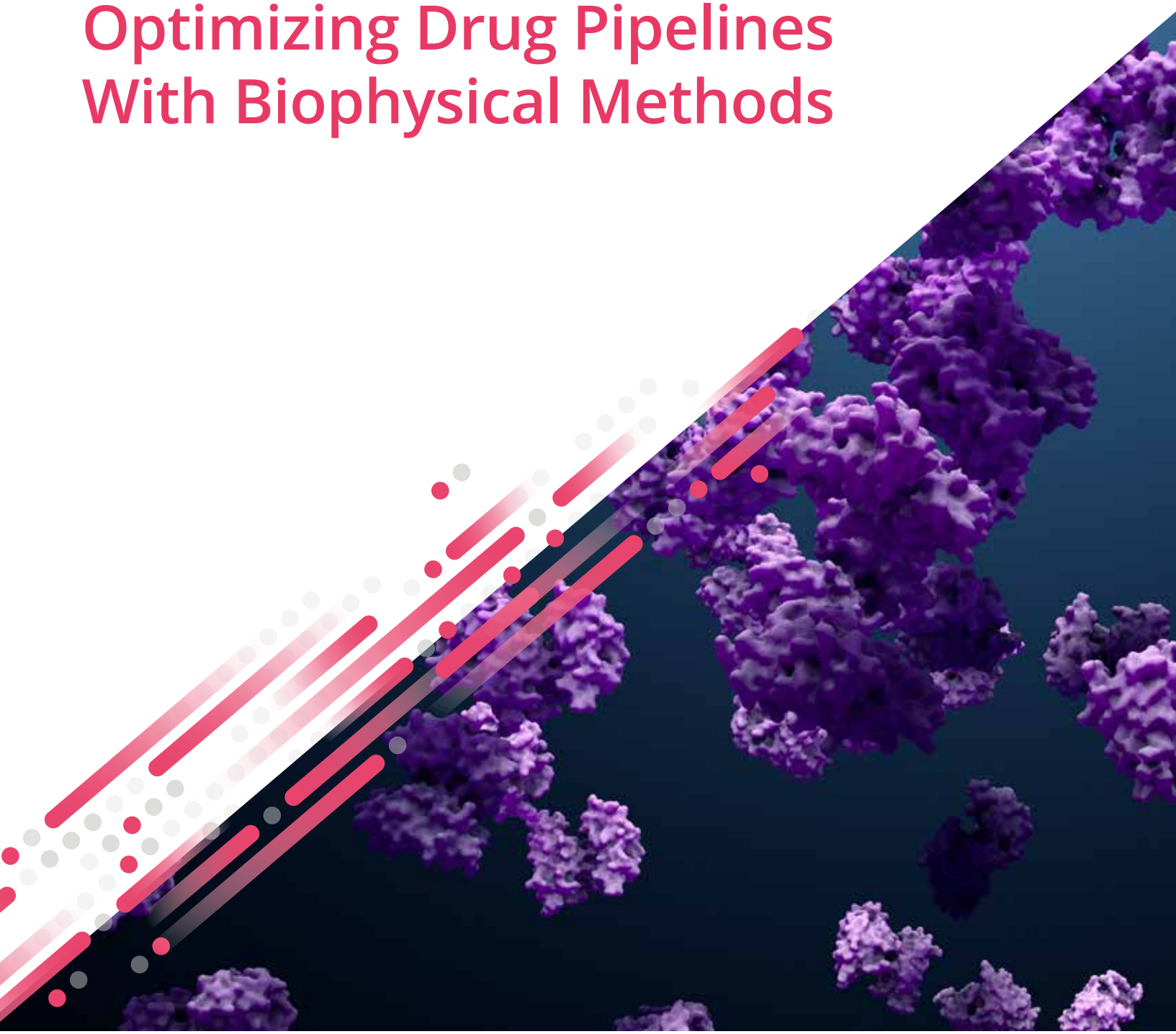
Optimizing Drug Pipelines With Biophysical Methods



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Optimizing Drug Pipelines With Biophysical Methods

Experts from WuXi AppTec explain the importance and benefits of using biophysical methods at every step of drug discovery. The ability to streamline and outpace current methods is enticing, and while opportunities are huge within many steps of the pharmaceutical process, challenges do remain.

"Biophysics is important to every step of the drug discovery process," says Moran Jerabek-Willemsen. "Biophysical methods are at the heart of modern drug discovery. They validate protein quality control (QC) and improve sample quality and target validation. These methods can be used in screening, hit validation, hit-to-lead and lead-to-candidate development, ultimately optimizing asset pipelines."

"Consistent results obtained with various biophysical techniques significantly increase the biological significance of the study and help to streamline the decision-making process," explains Jerabek-Willemsen, Head of Biophysics and Screening at WuXi AppTec HitS (CRELUX).

CRELUX is based in Martinsried, one of Munich's two science suburbs. Started in 2005, it has a 17-year history of working in protein production, crystallography, and biophysics.

Expertise And Experience

To offer expertise at any stage of the molecular discovery program, WuXi AppTec HitS has created a versatile toolbox combining different biochemical and biophysical methods and assays. It allows the team to investigate protein binding, thermodynamics, stoichiometry, protein quality, kinetics, and function. The different methods have varying sensitivities and throughput, so the WuXi AppTec team is capable to characterize both low numbers of compounds and large small molecule libraries up to 50,000.

"During project design we take a close look at the target protein and analyze which technology out of the available biophysical toolbox is suitable," explains Jerabek-Willemsen. "In many cases multiple technologies can be considered. In WuXi AppTec we put a lot of effort into designing the most appropriate protein construct for the requested application."

Feasibility studies during assay setup help to find the most suitable technology for the target protein and the study in hand. During the assay setup the company optimizes conditions using a well-characterized positive control with technologies such as differential scanning fluorometry (nanoDSF), microscale thermophoresis (MST), surface plasmon resonance (SPR) and further tools in its sizeable biophysical toolkit. The company's offering also includes technologies such as nuclear magnetic resonance spectroscopy (NMR), isothermal titration calorimetry (ITC), and dynamic light scattering (DLS), all of which play a pivotal role in studying the binding affinities, kinetics, and thermodynamics of a lead molecule-target interaction.

The company has 10 years of experience using MST and was the first contract research organization (CRO) to establish and integrate the technology into our drug discovery toolbox. It has characterized over 200 different protein targets using this technology.

"After completing the feasibility study, we would decide which assay should be used for screening, the decision being based on the assay performance and validation criteria such as the reproducibility, repeatability, and signal-noise ratio. In many cases, high-quality assays can be developed using different methods. Such a scenario is very helpful as we can select which methods will be used for screening and which methods should be used for orthogonal validation," explains Jerabek-Willemsen.

This improved speed, sensitivity, and throughput allows the biophysical screening of thousands of small molecules in a short time, providing pharmaceutical companies the ability to 'fail fast' at the point of identification and discovery.

"During assay setup we use a broad variety of different biophysical methods to quality control the target protein and to identify the most suitable assay conditions to reduce protein aggregation," he says.

Biophysical characterization reduces the possibility of aggregation and increases quality assurance. "In nanoDSF or standard DSF, the protein thermal stability is determined. A higher Tm and Tagg indicates a higher protein stability and integrity. Using the DLS polydispersity index the particle radius can be studied. DLS is useful in identifying conditions that favor the monodisperse state of the target protein," he explains.

Using binding assays such as MST and SPR, (compound-induced) aggregation can be determined. In addition, the use of binding assays allows WuXi AppTec to determine the binding capacity of a protein of interest, which provides important insight into the protein quality and feasibility for biophysical studies.

"At WuXi AppTec, we try from the beginning to

find the most promising biophysical solution to support our partner's need," explained Jerabek-Willemsen. "Antibodies, for example, work very nicely using SPR, while MST works very nicely for challenging protein complexes. The availability of a suitable protein tag, which can be used for fluorescence labelling or immobilization, needs to be carefully considered before a project is initiated. The combinations of MST and nanoDSF or SPR and nanoDSF are very useful in reducing assay setup time."

Fresh Opportunities To Characterize Challenging Drug Targets

Difficult drug targets, such as NLRP3, benefit from thorough characterization using biophysical methods.

NLRP3 is an intracellular sensor that detects a broad range of microbial motifs, endogenous danger signals and environmental irritants, resulting in the formation and activation of the NLRP3 inflammasome.

Upon its activation, NLRP3 triggers pyroptosis which triggers inflammatory reactions in the body. As such, NLRP3 inflammasome is directly related to the pathogenesis of several chronic diseases such as asthma, atherosclerosis, Alzheimer's disease and obesity.

The challenge with NLRP3 is that its activation results in its functionally critical polymerization. However, after trying 70 different constructs, WuXi AppTec was able to develop an activity-validated assay grade protein that can be used in various biophysical and biochemical assays.

To ensure that the protein is well-folded the team ran a nanoDSF experiment to study its unfolding behavior. The protein exhibited clear thermal stability and bound MCC-950, one of the most studied NLRP3 inhibitors, as expected. Next, they established a binding assay to allow its discovery partner to measure binding affinity with compounds and proteins. The team was able to establish a ready-to-go assay to support its client in profiling small molecules being evaluated for a complicated and challenging target.

Biophysical Screening Methods

Fragment-based drug discovery (FBDD) is a screening technique that has come of age within the last decade, leading to four FDA drug approvals for venetolcax, erdafitinib, vemurafenib, and pexidartinib.

Fragment screening has a number of key features. The first is the relatively small collection (< 5000) of low molecular weights compounds (< 20 heavy atoms). Fragments should be big enough for binding to the target protein and small enough to reduce unfavorable interactions. In FBDD a diverse set of 3000 fragments represents chemical space almost as effectively as 10 trillion diverse drug-sized molecules, explains Annika Niedner-Boblenz, scientific lead of biophysics and screening at WuXi AppTec HitS .

The other important feature of FBDD is the optimization of a fragment hit to a lead molecule after the screening is completed through structure-guided growth of the fragment or following an activity-based approach.

It is ideally suited to targeting protein-protein interactions (PPIs) as fragments bind to small pockets available on the protein surface. High hit rates of 3%-10% are an excellent predictor that high affinity, small molecule ligands can be identified. "These fragments display unique binding modes with the target. Through chemical enhancement there is the ability to optimize pharmacokinetic profiles simultaneously with potency as the fragment hits develop into clinical candidates," she says.

In another technique, researchers can use DNA-encoded libraries (DEL) to screen more than 65 billion chemical compounds in a single experiment. The compounds are conjugated to short DNA sequences that serve as identification barcodes. The target protein is immobilized on an affinity matrix and interacting chemical compounds are enriched. Through sequencing and decoding these hits are identified, and the hits undergo further hit validation.

The DEL method, which has been in use for 25 years but is only just becoming a mainstay of drug discovery, can identify lead candidates that bind to the target protein with nanomolar or even picomolar affinities. In comparison, FBDD yields initial hits that have micromolar affinities. However, "it can be an advantage to combine both screening technologies in order to identify promising drug candidates," explains Niedner-Boblenz.

In the future, Jerabek-Willemsen says, the team will be able to exploit screening synergies, for example a focused DNA-encoded library (DEL) screen in which the compounds are based on the results of Fragment Screening hits.

Discovery And Beyond

Other new strategies and technologies employing biophysical methods will be critical for future drug discovery and development. "Powerful new screening methods such as DNA-encoded library screening on living cells could significantly increase the success rate of drug discovery projects in the near future," says M. Jerabek-Willemsen.

"Increasingly we are designing drugs in silico and these drugs need to be biophysically validated. Biophysics will most likely remain an important tool in drug discovery, but the way biophysics is being implemented will need to be adapted to new drug discovery paradigms," he adds.

While a range of biophysical technologies, such as MST/TRIC (temperature-related intensity change), SPR, and nanoDSF can be utilized to support drug discovery programs, there are opportunities to further use biophysical methods to identify ligands for challenging drug targets.

In the coming years, the researchers at WuXi AppTec hope to see fully integrated and automated plate-based platforms that can screen larger compound libraries and enable biophysical HTS. They also hope to see biophysical screening in in vivo-like conditions, such as cell lysate and cell suspension in MST, TRIC and cell NMR. New strategies to purify membrane proteins in assay grade quality will make biophysics possible for as many important new targets as possible.

Machine learning and bioinformatics in general will play a huge role in the analysis and interpretation of biophysical, biochemical and structural data. Big data analysis for example enables the efficient screening of billions of small molecules by DNA Encoded Library Screenings in an unprecedented short time. And with the release of alpha fold and the according algorithm, a potentially disruptive new tool in the structural biology field came on the market. It will enable small molecule docking and structure-guided drug design for targets that were so far not amenable to any structural biology tool.

It is not only drug discovery where biophysics can be significant. Other pharmaceutical processes such as manufacturing of therapeutics proteins can benefit from control over certain biophysical properties, such as thermal stability, aggregation propensity and monodispersity of the target, for example in formulation development, as well as in manufacturing and quality controls.

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About WuXi AppTec

WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable global pharmaceutical and healthcare industry to advance discoveries and deliver groundbreaking treatments to patients. Through its unique business models, WuXi AppTec's integrated, end-to-end services include chemistry drug CRDMO (Contract Research, Development and Manufacturing Organization), biology discovery, preclinical testing and clinical research services, cell and gene therapies CTDMO (Contract Testing, Development and Manufacturing Organization), helping customers improve the productivity of advancing healthcare products through cost-effective and efficient solutions. WuXi AppTec's open-access platform is enabling more than 5,600 collaborators from over 30 countries to improve the health of those in need – and to realize the vision that "every drug can be made and every disease can be treated."

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