Turning insights into medicines: Synchrotron radiation used for the structural analysis of proteins

CrystalsFirst, a biotech company specialized in fragment-based drug discovery (FBDD), performed protein crystallography at the P11 beamline of DESY's PETRA III. Using synchrotron radiation during this highly efficient campaign, delivered promising candidates for the inhibition of the protein kinase A (PKA) in just nine weeks.

CHALLENGE

The initial stage of drug discovery is often compared to the search for a 'needle in a haystack'. The 'haystack' is a library of small molecules which is screened against a protein target.
Companies normally use a traditional approach for drug screening, which doesn't include X-ray protein crystallography as a primary option. For example; in high throughput screenings (HTS) and DNA-encoded libraries (DELs) use hundreds of thousands or billions of molecules to screen for their binding to the protein of interest. But only focusing on the binding events in such campaigns comes with the downsides of low hit rates, often too large molecules as well as unknown binding mode.
One of the goals of CrystalsFirst is to prove that obtaining the structural information as a first step can indeed provide a much better starting point for further drug development. By incorporating what has been learned there, the chemical expansion of drug candidates is guided and improved by prior learnings. Hence, their fragment-based drug discovery (FBDD) approach to retrieve active molecules from vast chemical spaces containing billions of candidate molecules by starting with structural biology, is more effective than usual campaigns.

SAMPLE

The protein used in this experiment as a study case, is the protein kinase A (PKA). In general, protein kinases act as catalysts for the transfer of phosphate groups between molecules. They take part in important biochemical signaling pathways and cellular functions, i.e. necrosis and cell proliferation. Overexpression of PKA can lead to multiple diseases, including cancer. Hence researchers are always on the look to find new drug candidates that inhibit PKA activity, to treat or prevent PKA related diseases.

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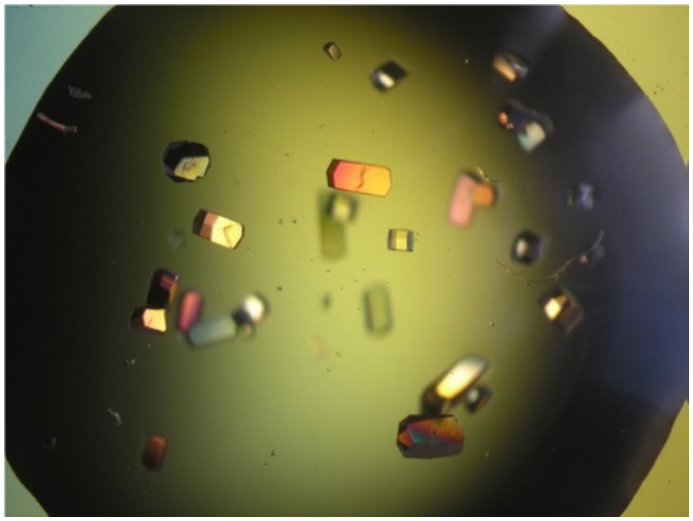

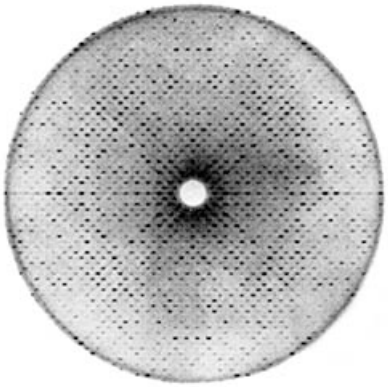

Figure 1: Left: Protein crystals used for protein crystallography. Right: Raw diffraction data from protein crystallography after data collection.

METHOD

X-ray crystallography allows weak bonds to be visualized within a resolution in the Ångström regime. The diffraction data shows the electron density distribution, from which the atomic structure can be extracted. Identifying such binding sites provides very valuable information for drug development, which is especially critical for diseases that are currently untreatable.

EXPERIMENT

CrystalsFirst makes use of fragment-based drug discovery. In this case, the drug discovery campaign started by selecting 4 small organic molecules called fragments, and implementing protein crystallography. From crystallographic data of PKA and the fragments, 3-D structures can be modeled, which show how the fragments bind to the protein. The protein crystallographic screening was performed on the P11 beamline at PETRA III during two steps of the drug development. At the beginning, four fragments were soaked into PKA crystals. After subsequent chemical modifications of the fragments and biological activity assessments, the most promising candidates were cocrystallized with PKA and examined at P11.

P11 was selected due to its a high throughput capability, using the high-brilliance beam produced by PETRA III. These features allow a large number of samples to be measured in very short timeframes, but also generate highest quality scattering data, which is ideal for fragment screening.

INSIGHTS AND ANALYSIS

X-ray crystallography provides 3-D models at an atomic resolution that depict the interactions of the ligand with the binding sites of the protein. Thus, false-positive hits can be avoided, as the high-resolution structure provides a clear view of whether the ligand is binding to the protein, and where it binds. Other screening techniques, i.e. HTS, can result in some false-positive hits that must be validated anyway, using X-ray protein crystallography.

The four starting fragments are only very weak binders (affinity ~ mM), so in a binding assay these fragments most likely wouldn't be identified as binders at all. But the crystallography data didn't only identify them as binders, but also gave insights on the intramolecular interactions as well as binding poses. This information was used in the following steps: CrystalsFirst generated a library of molecules, scored their binding, and filtered unpromising molecules out. In the end, 93 drug candidates were synthesized and their activities were tested by assays.

A little comparison with HTS is quite impressive at this stage. The 93 candidates have similar sizes as the candidates in HTS campaigns, but CrystalsFirst molecules are not coincidental hits. They were pre-selected on the basis of high crystallography data. As a consequence, 40% of the molecules showed activity in the assays, a much higher amount than in HTS campaigns.

Finally, the most promising candidates were cocrystallized with PKA and measured at P11. Here, the binding modes of the inhibitor and PKA could be brought into agreement with the binding modes of the four starting fragments.

BENEFITS

Thanks to the high-throughput capability of the P11 beamline and the high-brilliance beam provided by PETRA III, the company was able to test many samples in a short time. The high resolution obtained is an ideal starting point for the FBDD approach, allowing CrystalsFirst to observe even weak binders, but also by being able to identify binding poses and intramolecular interactions between the protein and the fragment. Besides, the subsequent analysis of the screening hits and expanding the molecules with new chemical groups to produce the first prototypes of the new drug, profited from the crystallographic data. All in all, the campaign was finished only in nine weeks, due to the effectiveness and efficiency of the CrystalsFirst approach and the access to PETRA III's brilliant synchrotron radiation.

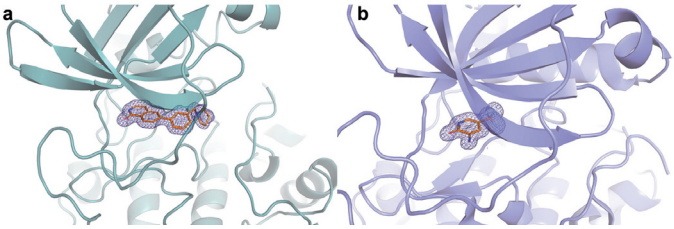

Figure 2: Electron density determined by protein crystallography of the most potent compound candidate/ compound with highest affinity (around 700 nM) (a) and the starting fragment used as a template (b).

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