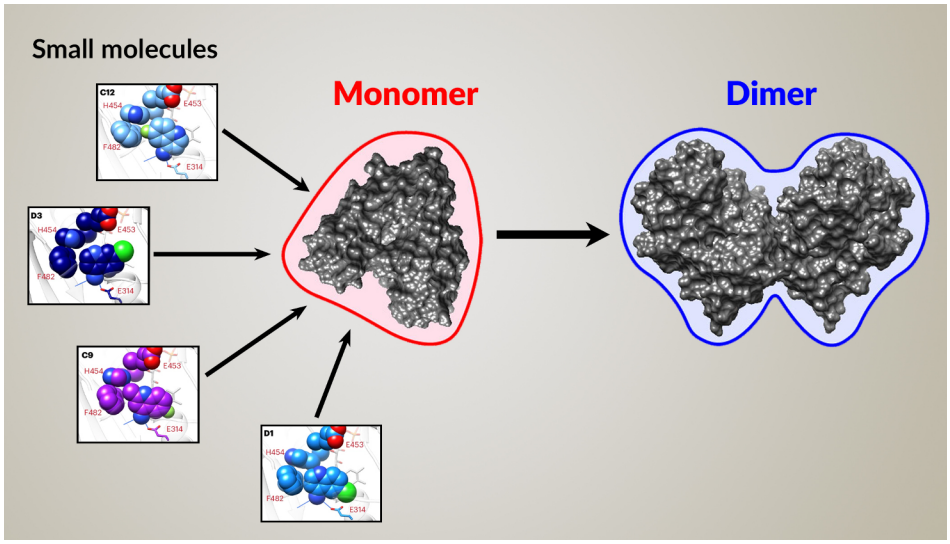
ADVANCED LIGHT SOURCE

BIOLOGICAL SCIENCES

Time-Resolved SAXS Screen of Small-Molecule Drug Candidates



When screening small molecules as potential drug candidates, it's important to understand how they affect a target protein's biological function, which is usually connected to its physical arrangement. In this case, researchers were looking for small molecules that would enable the transformation of a protein from a monomer into a dimer.

Scientific Achievement

Time-resolved, high-throughput, small-angle x-ray scattering (SAXS) at the Advanced Light Source (ALS) improved the screening of small-molecule drug candidates, providing insight into how they stimulate structural transitions in protein targets.

Significance and Impact

The work will speed the discovery of treatments designed to activate biomolecular dynamics associated with desired therapeutic outcomes.

The drug-discovery pipeline

Drug discovery relies on the identification of small molecules capable of interacting with protein targets ("hits"). However, many drug-discovery workflows don't evaluate the impact of hits upon protein form and function until later development phases. Early access to conformational information has the potential to focus hits toward mechanistically important structural states. Moreover, many targets of biological importance exist in more than one conformational state, with important biology associated with transitions between them.

Here, researchers present a discovery pipeline that integrates time-resolved, high-throughput, small-angle x-ray scattering (TR-HT-SAXS). They applied this new workflow to an important mitochondrial protein called apoptosis-inducing factor (AIF). By monitoring AIF states over time, TR-HT-SAXS can identify small molecules that modify the protein's function, enabling researchers to target the most promising compounds in drug screens.

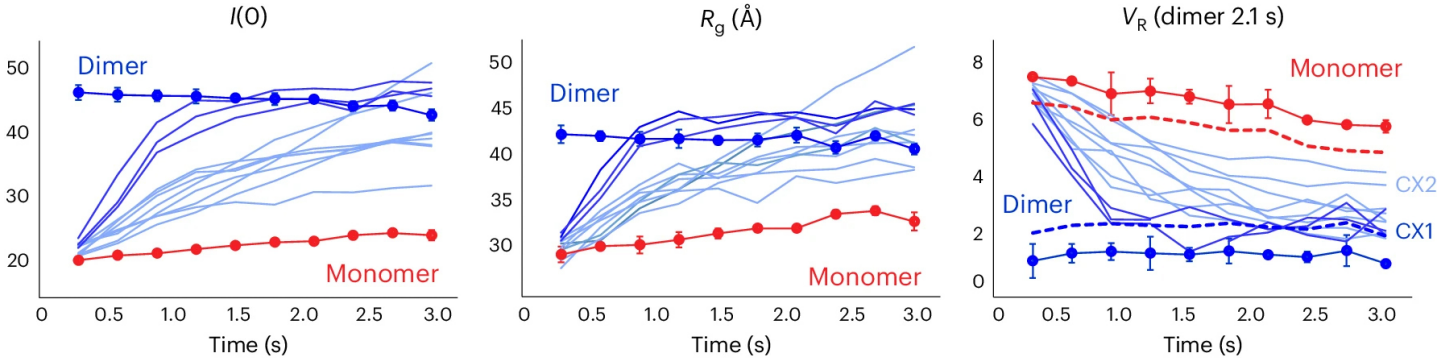
A mitochondrial protein pathway

AIF functions as part of a pathway for importing proteins into mitochondria. The donation of electrons to AIF (chemical reduction) precedes its dimerization, which then facilitates mitochondrial import. If AIF cannot do this efficiently, it results in symptoms associated with muscle degeneration or neurodegeneration in patients carrying certain AIF genetic mutations.

In this SAXS-augmented screening workflow, the researchers started out with a library of 2,500 compounds. They performed an initial screening that determined whether a molecule binds to AIF. They then advanced the top performers to the SAXS portion of the screening, to find out whether those molecules induce AIF dimerization.

SAXS captures proteins in transition

TR-HT-SAXS at ALS Beamline 12.3.1 (SIBYLS) can provide structural information on more than one state in a single screening experiment, provided that an appropriate conformational trigger is available. Targets that rearrange in response to oxidation-reduction processes are uniquely suited to monitoring by TR-HT-SAXS, as x-ray exposure can trigger conversion from oxidized states to x-ray-stimulated reduced states, allowing candidate molecules to interact with both.



Using a time-resolved approach, researchers were able to monitor, not just one AIF shape, but transitions to a biologically functional state, using parameters such as zero-angle scattering intensity *I*(0) (related to the molecular weight of the macromolecule), radius of gyration R_g (distribution of mass around an axis), and volatility of ratio V_R (the quantity visually displayed in the similarity matrices shown below). The connected red circles represent oxidized monomeric AIF, and the connected blue circles represent reduced dimerized AIF. The solid blue lines represent small molecules that stimulated a transition in AIF from the monomer to the dimer state, with the darker blue samples being the most effective.

Each small-molecule candidate was mixed with AIF in solution and repeatedly probed with x-rays. The scattering curves were then compared to each other and to reference curves for the AIF monomer and dimer. The results were summarized in SAXS similarity matrices, where the color of each cell indicates the similarity between two scattering curves.

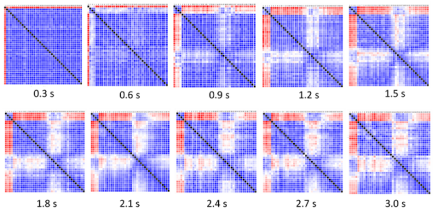
The full conformational landscape

Based on the results, the researchers sorted the small molecules into groups based on how closely each complex resembled the reference dimer structure. The highest-ranking group induced robust AIF dimerization, revealing—via a number of parameters—a rapid transition from monomer to dimer.

As a final step, the researchers crystallized the top small-molecule contenders and analyzed their atomic-level structures at the National Synchrotron Light Source II and at ALS Beamline 8.3.1. The structures provided further insight into how AIF operates and validated SAXS as a way to uncover structure–activity relationships.

Synchrotrons have a well-recognized role in drug development with crystallography. This work identifies a new potential role with x-ray scattering. The ALS upgrade (ALS-U) could make data collection possible on smaller sample sizes—a challenge where protein quantity is often limiting.

Importantly, this new workflow opens the way to early screening of the full conformational landscape and associated kinetic transitions of a target, expanding the range of accessible clinical targets and ligand binding sites.



Time-resolved SAXS data shown in a series of similarity matrices. In this presentation, each cell indicates (via color) how similar the curves in the corresponding row and column are. Blue indicates high similarity, and red indicates low similarity. The dimer reference curve is in the first row/first column, and the monomer reference curve is in the sixth row/sixth column. All other rows represent a given small molecule being compared to every other small molecule in the in the data set over the course of 10 measurements, 0.3 seconds apart. The black diagonal represents each SAXS curve compared with itself.

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