Incorporating HT-SAXS into Drug Discovery Pipelines

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High-throughput (HT) methods for discovering single-target protein and nucleic acid ligands are well established and readily utilized for drug discovery. However, important biological outcomes are mediated by multi-component assemblies and dynamic macromolecular architectures. HT approaches to monitor ligand impact on functional assemblies and to assess chemical selection of architectural states remain underdeveloped. Here, we have incorporated HT-SAXS into a classic fragment screening pipeline and use this approach to identify chemical allosteric effectors targeting structural states of the mitochondrial and cell death protein, Apoptosis-Inducing Factor (AIF). AIF allosterically switches from monomer to dimer upon engaging NADH in a charge-transfer complex. This dimer-monomer exchange is proposed to regulate AIF's functional transition from supporting mitochondrial import to facilitating PARP-1-dependent cell death (parthanatos). Application of time-resolved HT-SAXS to lead fragment binders from differential scanning fluorimetry (DSF) screening identified and ranked three chemotypes allosterically stabilizing monomeric or dimeric AIF. Secondary screening with focused fragment libraries enriched with these chemotypes has produced optimized scaffolds targeting AIF's NADH binding site. Our results demonstrate how incorporation of HT-SAXS into fragment screening protocols customizes ligand development to macromolecular architecture, assembly, and allostery.