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Perspective

Evolving Experimental Techniques for Structure-Based Drug Design

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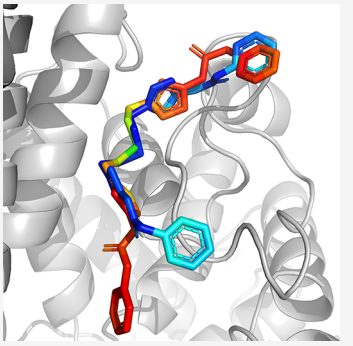
ABSTRACT: Structure-based drug design (SBDD) is a prominent method in rational drug development and has traditionally benefitted from the atomic models of protein targets obtained using X-ray crystallography at cryogenic temperatures. In this perspective, we highlight recent advances in the development of structural techniques that are capable of probing dynamic information about protein targets. First, we discuss advances in the field of X-ray crystallography including serial room-temperature crystallography as a method for obtaining high-resolution conformational dynamics of protein-inhibitor complexes. Next, we look at cryogenic electron microscopy (cryoEM), another high-resolution technique that has recently been used to study proteins and protein complexes that are too difficult to crystallize. Finally, we present small-angle X-ray scattering (SAXS) as a potential high-throughput screening tool to identify inhibitors that target protein complexes and protein oligomerization.



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INTRODUCTION

The development of new drugs to treat human disease has become increasingly expensive, with cost estimates ranging from $985 million to over $2.6 billion.[1,2] This has enhanced the need for high-throughput, low-cost screening methods that can be adapted for broad use. Out of this need, protein structure-based drug design (SBDD) has evolved into a prominent method in drug development, taking advantage of the advancement of computational methods for high-throughput ligand docking.[2−4] This method, which relies on the use of a high-quality 3D structure of the protein target, has been further enhanced by the rapid growth of the Protein Data Bank (PDB). The PDB has grown over the past decade to over 190000 macromolecular 3D structures deposited as of May 2022, compared to less than 90000 structures available at the end of 2012.[5] In this Perspective, we review emerging experimental techniques in protein structural biology that can be used to drive rational drug design. First, we discuss high-resolution techniques including room-temperature X-ray crystallography methods that can probe protein structural dynamics not readily detected by traditional cryocooling methods and single-particle cryogenic electron microscopy (cryoEM), which can resolve structures of membrane proteins and large protein complexes not amenable to protein crystallization. Then, we highlight small-angle X-ray scattering (SAXS) as a potential high-throughput screening tool to measure the ability of inhibitors to influence protein conformational changes and oligomerization.

X-RAY CRYSTALLOGRAPHY

The majority of protein atomic models obtained experimentally have been determined using macromolecular X-ray crystallography, which constitutes greater than 85% of the structures deposited in the PDB. Hence, SBDD has been accomplished primarily using protein structures obtained by cryogenic X-ray crystallography. In this method, protein crystals are grown from homogeneous protein preparations in a laborious process that relies on a trial-and-error screening of hundreds to thousands of crystallization conditions to identify a condition yielding protein crystals that diffract to high resolution.[6] Ligands, such as small-molecule drugs, can be introduced to the protein through cocrystallization or by soaking into preformed protein crystals.[7] Traditionally, the crystals are then manually harvested, transferred into a cryoprotectant solution, and finally preserved in liquid nitrogen. X-ray diffraction patterns from the crystals are then collected at either a home-source or a synchrotron, typically under cryogenic conditions to protect against radiation damage. With advancements in the automation of the data collection process at synchrotron light sources around the world, this technique is capable of producing much higher

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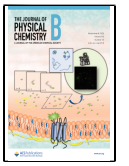

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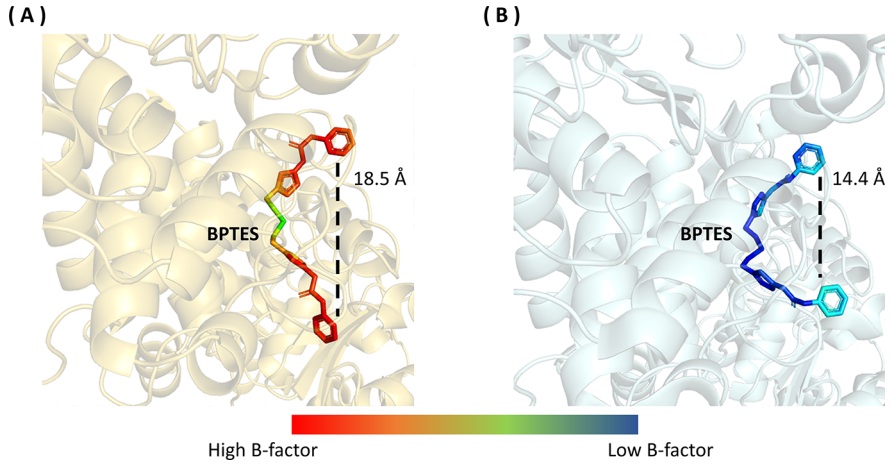

Figure 1. Comparing the serial room-temperature and cryo-cooled X-ray crystal structures of BPTES bound to GAC. (A) The serial room-temperature crystal structure of BPTES bound to GAC (light yellow) adopts an extended, semilinear conformation. (B) The cryo-cooled crystal structure of BPTES (PDB ID 4JKT) bound to GAC (light cyan) forms a "cup-like" orientation. BPTES is color coded by B-factor. B-factors represented by blue to green colors suggest regions of little movement, while red and orange colors suggest regions of greater movement. The distance between the terminal rings of BPTES are measured in Å and are indicated in the figure.

throughput than cryoEM and consistently results in higher resolution atomic models, with about 55% of all cryoEM maps deposited to the PDB in 2021 achieving a resolution of better than 3.5 Å compared to approximately 98% of X-ray crystallography structures.[5] However, cryogenic X-ray crystallography works best with large (100 μm or larger), single crystals, which can be difficult to grow; thus, protein crystallization is often the bottleneck for X-ray crystallography studies. Additionally, freezing the crystals at cryogenic temperatures traps the protein in a single discrete conformation and removes the flexibility that proteins maintain within the crystal lattice.[8] The use of cryoprotectants to protect the crystal from the freezing process can also impact the quality of diffraction, limiting the final resolution of the protein structure.

To overcome the loss of information from cryo-cooling and the need to produce large, single crystals, investigators have turned to room temperature crystallography, which has been repopularized with the advent of serial crystallography at X-ray Free Electron Lasers (XFELs). XFELs deliver extremely high X-ray flux in ultrashort pulses (10s of femtoseconds), which enables collection of high quality, near damage-free diffraction patterns even from microcrystals that are 10 μm or smaller.[9] The XFEL pulse, however, destroys the microcrystal, so fresh sample needs to be provided for each subsequent X-ray pulse. This is primarily achieved through the use of a Gas Dynamic Virtual Nozzle (GDVN) which uses a helium gas sheath to produce a thin liquid jet (<10 um) to carry fresh crystals for each XFEL pulse.[10] During each X-ray interaction, an individual microcrystal is randomly oriented and gives rise to a partial diffraction pattern, and data from many, randomly oriented crystals are combined to give a full data set. Microcrystals are typically grown via batch crystallization, and crystal seeding is becoming increasingly common to boost crystal density and quality.[11]

Due to the achievements of serial crystallography at XFELs and advancements in third-generation synchrotron sources, microfocus beams, and fast frame-rate detectors, much effort has been expended to develop serial crystallography at synchrotrons. Different sample delivery methods are needed to accommodate the longer X-ray exposure times required at synchrotrons to accrue sufficient flux to produce high quality diffraction patterns. There are two main categories of sample delivery methods: moving target and fixed target. The moving target approach has many varieties, including viscous jets and tape-drive methods, but both serve to continuously supply new crystals to the X-ray interaction region.[12,13] The fixed target approach involves pipetting, or directly growing, microcrystals onto different types of sample supports. There are many types of fixed target chips, including those made from silicon, polymer, and polyimide, but they largely employ the same approach: a microfocused X-ray beam raster scans across the support and collects hundreds to thousands of diffraction images.[14−17] Some fixed target approaches also involve oscillating the chip to maximize the amount of information collected from each crystal. The scaling, filtering, and merging of these diffraction patterns result in a complete data set. This high-throughput technique is ideal for small crystals (10s of micron) and typically only requires a small amount of sample (~10 μL of crystals), which makes it an ideal method for SBDD and initial screening of drug binding.

The use of room-temperature, fixed-target serial crystallography has been successful in identifying structural changes in inhibitor compounds that help explain differences in their potency that eluded detection by traditional cryo-cooled crystallography. For example, the mitochondrial enzyme glutaminase C (GAC) is overexpressed in a variety of cancer cells in order to satisfy the "glutamine addiction" exhibited by many types of cancer. This has resulted in an increased interest in glutaminase as a potential therapeutic drug target, with several drugs already in clinical trials. One class of glutaminase inhibitors is based on the lead compound BPTES and consists of linear molecules with flexible linker domains and terminal rings. Despite the overall structural similarities between compounds within this class, they display a range of potencies. X-ray structures obtained at cryogenic temperatures were unable to distinguish the binding mode between members of this inhibitor class despite significant differences in their potency for inhibiting GAC, making it challenging to use these structures to identify the chemical determinants for potency and thus for the rational design of better drug candidates. However, by using serial room-temperature crystallography, we

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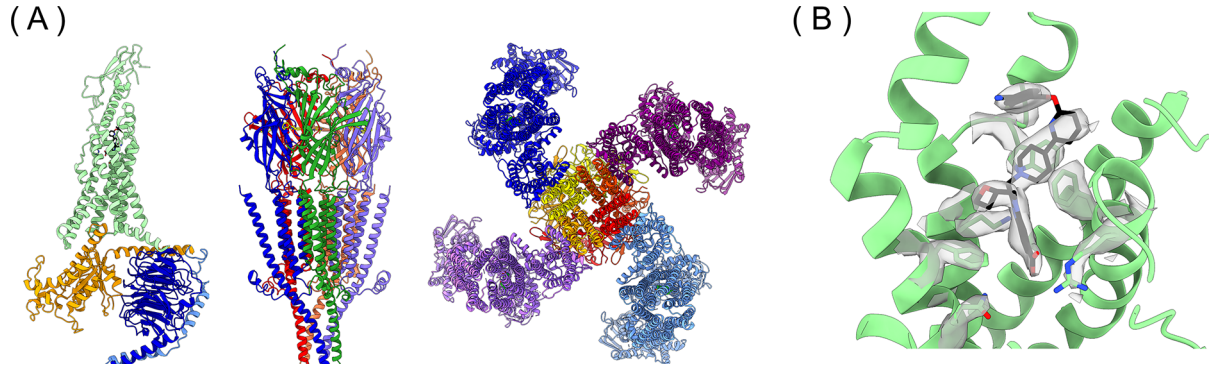

Figure 2. (A) Examples of high-resolution structures of protein-inhibitor complexes obtained using cryoEM. From left to right, the GLP-1R:Gs PF inhibitor complex, the serotonin receptor bound to granisetron (2.9 Å, PDB ID: 6NP0), and the pancreatic β-cell KATP channel bound to glibenclamide (3.63 Å, PDB ID: 6BAA). (B) Atomic model from a 3.2 Å cryoEM structure of the glucagon-like peptide-1 receptor (GLP-1R):Gs complex bound to a small molecule inhibitor (PF 06882961), obtained with a 200 kV electron microscope (PDB ID: 7LCI, EMDB: 23274). The cryoEM map is shown within 2.5 Å of key residues and the inhibitor.

recently were able to identify a new conformation of BPTES bound to GAC, showing a disrupted hydrogen bond and an increased amount of flexibility in the binding site that could explain its decreased potency relative to other drug candidates of the same inhibitor class (Figure 1).

In addition to identifying conformational changes in inhibitors, room-temperature crystallography can also be used to identify potential allosteric drug binding sites in a protein target for future drug design. These previously undetected "hidden" sites can be potentially targeted to modulate GPCR signaling by allosterically altering receptor conformation without binding to the orthosteric site. In addition, these sites can also be used to develop strategies to target proteins that were previously deemed "undruggable". For example, KRAS is commonly mutated in cancers and is the driving force behind over 90% of pancreatic cancers. This protein was previously deemed to be undruggable. However, recent efforts to design inhibitors that covalently attach to KRAS(G12C) mutants, which are GTP hydrolysis-defective, constitutively active, and present in some lung and pancreatic cancers (i.e., the "G12C" inhibitors), resulted in the identification of a newly appreciated binding pocket that lies between the switch II region and the nucleotide binding site. These inhibitors have recently been examined in clinical trials and have shown some exciting success. For proteins that have previously been studied using cryo-cooled crystallography, revisiting their structures at room temperature can allow for the identification of unique conformational dynamics in the protein and possibly yield new locations of allosteric modulation sites. Since crystallization conditions for these proteins are already known, single large crystals can be readily grown from purified protein via hanging or sitting drop crystallization and then examined at room temperature without the use of cryoprotectant. These large crystals are selected using crystal loops and protected by a long clear polyester capillary tube (MiTeGen) that slides over the crystal. Using this technique and a microfocused X-ray beam, large single crystals can be vector-scanned multiple times at different starting points around the crystal. This allows for the collection of numerous slices across the length of a single crystal and helps prevent radiation damage while benefiting from room-temperature data collection.

The other major advantage of room-temperature serial crystallography is the ability to probe dynamics and capture intermediate conformational states with time-resolved crystallography studies. There are two main types of stimuli for time-resolved experiments: light-activated reactions and ligand-binding studies, and both techniques can be performed at XFELs or synchrotrons. Light-activated reactions have been successfully observed, particularly at synchrotrons, and can be used in combination with viscous jets or fixed target sample delivery methods. The major limitation to this technique is that most proteins (>99%) are not light-active.

A more widely applicable approach is probing ligand-binding on the millisecond to second scale using microfluidic mixers. Many proteins have been shown to be able to undergo catalysis and ligand binding in crystallo, suggesting that protein conformational dynamics within the crystal can yield important mechanistic insight. Mix-and-inject serial crystallography (MISC) was first pioneered at XFELs with the use of flow-focused diffusive mixers, which use an external ligand containing sheath flow to thin down a central microcrystal stream, enabling rapid diffusion of a ligand into the protein crystals to initiate the reaction.[18,19] To help drive rapid mixing, the ligand needs to be relatively small and highly soluble. This technique has been used successfully to monitor the binding of ceftriaxone and sulbactam, an antibiotic and an inhibitor, to β-lactamase from *Mycobacterium tuberculosis* (BlaC) and has helped to reveal important information about how this reaction proceeds differently in the various protein subunits.[20,21] There have also been efforts to perform time-resolved mixing experiments at synchrotrons. Fixed target approaches rely on ligand-containing picoliter-sized droplets to be sprayed onto crystals, while others employ a MISC style and use a microfluidic mixer in conjunction with a tape-drive to carry freshly mixed crystals into the X-ray interaction region.[13,22] It is difficult to use viscous jets in combination with rapid mixing experiments, as diffusion is much slower in viscous media. MISC represents the most promising technique for mixing drug candidates with protein microcrystals for the unprecedented high-resolution characterization of intermediate conformational states. This information will be vital in understanding drug binding and the subsequent inhibition mechanisms, which can help drive future drug development.

CRYOGENIC ELECTRON MICROSCOPY (CRYOEM)

The resolution revolution of single-particle cryogenic electron microscopy (cryoEM) has produced protein structural models

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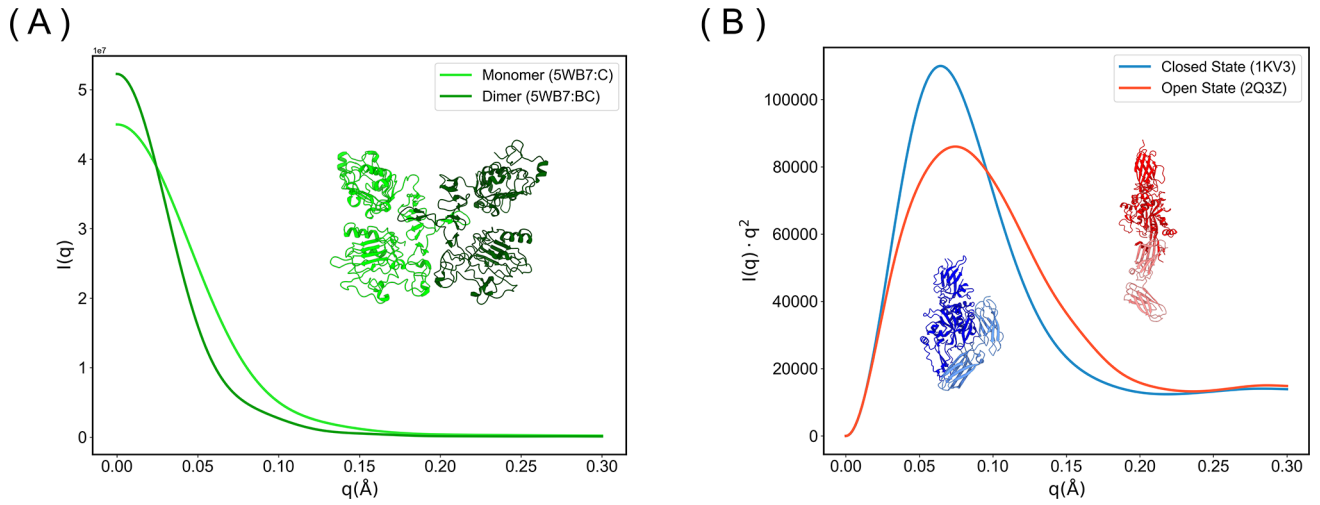

Figure 3. Theoretical SAXS profiles of two potential protein targets, determined using the fast X-ray scattering (FoXS) web server. Panel A shows a Lin-Lin plot for the scattering of the EGFR monomer and dimer (green and dark green, respectively, PDB ID: 5WB7). Panel B shows a Kratky plot of the X-ray scattering of tissue transglutaminase (tTG) in the open (red, PDB ID: 2Q3Z) and closed (blue, PDB ID: 1KV3) conformational states.

for several challenging drug targets including large protein complexes and membrane proteins such as ion channels, transporters, and receptors (Figure 2A).[23−27] One example is the determination of the structure of a GPCR/G-protein complex of the glucagon-like peptide-1 receptor (GLP-1R) bound to a small molecule inhibitor (PF 06882961).[28] Using a 200 kV microscope, Zhang et al. were able to solve a 3.2 Å structure of the inhibitor bound complex, allowing for the generation of a high-quality atomic model and demonstrating that this approach can be broadly applicable to other protein targets for SBDD (Figure 2B). CryoEM also offers the opportunity to observe high-resolution conformational dynamics, which can play an important role in SBDD, especially in the discovery of hidden allosteric binding sites on protein targets.[29−31]

Although cryoEM has the potential to accelerate SBDD for difficult protein targets, it currently faces some limitations. Despite the rapidly increasing ability of cryoEM to obtain high-resolution structures, true atomic level information (i.e., <2 Å) is still largely out of reach in practical applications. Indeed, there have been cases in which proteins with highly symmetric structures have been solved to very high resolution, including a 1.15 Å reconstruction of human apoferritin.[32] However, the vast majority of available cryoEM structures are within the 2.5 to 4.0 Å range, which can sometimes contain local resolutions that are too low to unambiguously place drugs and amino acid side chains into the cryoEM density map. Furthermore, with single-particle analysis limited to samples with a molecular mass of approximately 40 kDa and larger, this technique is not ideal for analyzing lower molecular mass proteins such as small GTPases that fall in the 19 to 25 kDa range.[33] Additionally, as is the case with X-ray crystallography, cryoEM faces challenges in dealing with the conformational flexibility of intrinsically disordered proteins, which have gained significant interest as potential therapeutic drug targets.[34−38] These limitations, however, could potentially be overcome through technical advances in the development of electron detectors and phase plates.[39−41]

Unlike X-ray crystallography, the lower throughput of cryoEM makes it less attractive for drug discovery pipelines. In the case of X-ray crystallography, once a crystallization condition is known for a given protein target, crystallization of protein−drug complexes can be done rapidly and with high-throughput, given the availability of crystallization robots that set up 96-well trays. Additionally, the data processing pipeline in X-ray crystallography is well-defined and for proteins with known structures or proteins highly similar to previously solved protein structures, phasing by molecular replacement can enable structure determination from a set of diffraction images within minutes. Automated cryoEM data processing driven by machine-learning approaches is increasingly becoming a reality; however, it still requires the use of expensive multi-GPU workstations for fast processing.[42−44] The raw micrographs and the processed data also demand a significant amount of storage space, on the order of terabytes, whereas a completely processed crystallography data set typically requires no more than a few gigabytes of storage.[45,46] These challenges could be overcome by decreases in the cost of high-speed computer storage and processing as well as advances in computer hardware such as GPUs and SSDs.

SMALL ANGLE X-RAY SCATTERING (SAXS)

Small angle X-ray scattering (SAXS) is a solution-based X-ray scattering technique that is capable of yielding low-resolution (10−15 Å) structural information while providing valuable insights into a variety of biophysical properties of a protein (overall shape, oligomeric state, intrinsic stability, flexibility of proteins and protein complexes) which can play an important role in human disease.[47,48] One of the primary advantages of SAXS is that the sample preparation is straightforward. A pure protein sample is simply analyzed in its storage buffer, which allows the molecule to explore its entire range of motion and exist in a variety of conformational states. Any partners, such as native ligands or potential drug candidates, can be simply added to the solution to see their effect on the overall conformation of the protein. Advances in the automation of SAXS sample loading and data collection at synchrotron sources enables high-throughput measurements with the capability to screen nearly 100 samples in less than 5 h.[49,50] Thus, SAXS can be more high-throughput than high-resolution structural techniques, as there is virtually no bottleneck in sample preparation, which is advantageous for drug discov-

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ery.[49] Although SAXS is not suitable for generating high-resolution structural models, it can readily integrate high-resolution data using hybrid modeling approaches to investigate protein structure in a near-native environment.[51]

Although SAXS has not yet been widely applied in drug screening, it has been used to investigate protein targets that are important to human diseases, including the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase that is frequently mutated and overexpressed in cancers.[52−54] Activation of the receptor occurs when it binds to one of its several extracellular ligands, leading to its dimerization and autophosphorylation, resulting in the activation of downstream signaling partners such as Akt and MAPK, which both contribute to cell proliferation and survival.[55−58] Interestingly, the structure of the activated EGFR dimer is dependent on the ligand, with EGF inducing a long-lived symmetric dimer and epiregulin inducing a short-lived asymmetric dimer.[59] Using SAXS, Hu et al. investigated the effect of extracellular mutations on the structure of EGFR dimers and found that they resulted in enhanced dimerization upon binding of ligands that normally induce weaker dimers, suggesting that these mutations impair ligand discrimination and increase the amount of active EGFR dimers.[60] Peptides that disrupt EGFR dimerization have recently been investigated as a potential treatment option for drug-resistant cancers, suggesting that disrupting EGFR dimerization may be a viable approach for future drug development.[61,62] SAXS is well suited to play an important role as a high-throughput screening method for EGFR dimerization, providing a direct structural readout of a drug candidate's ability to impact dimer formation. To demonstrate this, the theoretical X-ray scattering profiles of the EGFR monomer and dimer (PDB ID: 5WB7 chains B and C) were determined using the Fast X-ray Scattering (FoXS) web server (Figure 3A). These scattering profiles were then used to calculate the radius of gyration, R_g, a parameter that is extracted from the lowest angle part of the scattering profile via Guinier analysis and can be used to describe the size of a molecule in solution.[50] Using this approach, the R_g of the EGFR monomer was found to be ∼27 Å and the R_g of the EGFR dimer was ∼39 Å, demonstrating a simple and direct readout for EGFR dimerization. In addition to detecting oligomerization, SAXS can also be employed to investigate protein−protein interactions that play an important role in human disease.[63−65] For example, C-terminal peptide fragments of G alpha subunits have been used to modulate GPCR signaling by competing with the native G-proteins and blocking GPCR signaling.[66,67] This presents an opportunity for using SAXS as a high-throughput approach for screening peptides for the disruption of GPCR/G-protein complexes.

SAXS can also be used to measure protein conformational changes that are associated with disease states and has the potential to screen conformational state inhibitors that are able to trap a protein target in a desired conformation. Many proteins exist in multiple conformations, dependent on the environmental conditions or ligand binding, and this has previously been shown to play an important role in several diseases, including Alzheimer's disease and liver disease.[68,69] For example, the enzyme tissue transglutaminase (tTG) is frequently overexpressed in cancer and has been implicated in a number of diseases, including celiac disease and neurodegenerative disorders.[70−72] tTG can adopt two strikingly distinct conformations designated as open- and closed-state conformations that are regulated by calcium and guanine nucleotide binding, respectively.[73,74] The transitions between these two conformational states involve large-scale rearrangements of the C-terminal beta barrels, resulting in a displacement of greater than 100 Å. Previous studies have shown that nucleotide binding-defective mutants, which can only adopt the open state, are cytotoxic to cancer cells that overexpress tTG.[75−77] This finding led to the development of conformational state inhibitors of the enzyme, which are able to trap tTG in an open state conformation and have been effective in killing cancer cells.[78] To demonstrate that this conformational change can be detected by SAXS, theoretical scattering profiles were calculated using FoXS and the previously published crystal structures of the open and closed state of tTG (PDB ID 2Q3Z and 1KV3, respectively).[73,74] By again comparing the R_g of the open state (∼37 Å) and the closed state (∼28 Å), the two species can be distinguished with relative ease (Figure 3B). The data of Figure 3B are displayed as Kratky plots (scattering intensity times angle squared as a function of scattering angle (or q)). Kratky plots are a useful tool for exploring or comparing the size and compaction of different samples.[50]

SAXS is also capable of studying samples that have a high degree of flexibility including proteins that have flexible linker regions, have been unfolded or denatured, or are intrinsically disordered (IDPs).[79,80] Proteins with high levels of flexibility are difficult to study using cryoEM due to the averaging out of dynamic motion during particle alignment and can be extremely difficult to crystallize due to the large conformational landscape these proteins occupy. SAXS can be used to describe the conformational ensemble of a protein in solution and is particularly powerful when paired with computational techniques to sample the conformational space of an input protein structure.[79,81] Drugs that bind to IDPs are likely to push the conformational equilibrium of the IDP toward a distinct state compared to that for IDP alone in solution, resulting in changes to the scattering profile.[37,82,83] SAXS can also provide structural information about protein unfolding and therefore could be used to identify compounds that destabilize or prevent the correct folding of a protein, as well as the degree to which the drug denatures the target.[84,85]

In addition to providing steady-state structural information on a drug target, SAXS is also amenable to time-resolved studies.[86] This allows for the investigation of intermediate structural states induced by several biological phenomena, including temperature or pH changes, photoexcitation, or ligand binding by biomolecules such as proteins and nucleic acids. Intermediate conformational states have recently been investigated as a potentially untapped opportunity to find new inhibitors for several important drug targets.[87−90] Time-resolved SAXS (TR-SAXS) has the potential to probe conformational intermediates and could be used to investigate the effect of drug binding on the longevity of intermediate states.[91,92] Because of the demands of both the experimental setup and the data analysis required for dynamic studies, which often relies on small signals, TR-SAXS is not yet ideal for use as a high-throughput approach. Once drug targets are more narrowed down, however, TR-SAXS is well suited to help elucidate the mechanism of the drug through its ability to capture intermediate conformations, providing a foundation for TR-SAXS as a drug-screening method.

■ ADDITIONAL TECHNIQUES AND CONCLUSIONS

In addition to the techniques described above, Nuclear Magnetic Resonance (NMR) can be used to investigate the

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structural basis of protein−ligand and protein−protein interactions.[93] This technique can provide dynamic structural information for small proteins, typically less than about 30 kDa, and requires large quantities of pure protein. NMR is well suited to study intrinsically disordered proteins, as the protein structure is presented as a dynamic series of probabilistic conformations. Protein structural models can also be obtained using computational methods, including homology modeling, bioinformatics, thermodynamics, and more recently artificial intelligence and machine learning.[94−96] Although computational models can provide insight for proteins with an unknown tertiary fold, the accuracy of these predictions are case by case dependent and often must be verified by experimental data.

In this perspective, we highlighted recent advances in structural biology that can be used to better inform the structure guided drug discovery effort. Although the immediate future of the field is likely to continue to be dominated by cryo-cooled crystallography, emerging techniques such as room temperature crystallography, cryoEM, and high-throughput SAXS offer exciting possibilities for providing unique insights into the dynamic motion of proteins and identify important details in the effects of drug binding. These techniques are all amenable to time-resolved studies, allowing for the discovery of new protein conformational states that can be investigated for SBDD.

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Notes

The authors declare no competing financial interest.

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Lois Pollack: Lois Pollack received an undergraduate degree in Physics from Brandeis University and a Ph.D. in Experimental Condensed Matter Physics from MIT. As a postdoc, she came to Cornell to work with Bob Richardson in the low temperature physics group. After many years as an ultralow temperature physicist, she joined Sol Gruner's group in Cornell's physics department, where she retrained in biophysics. She develops new methods for using X-rays to explore the dynamic structural ensembles of biological macromolecules, including RNA, DNA, proteins, and their complexes. She joined the Cornell faculty in 2000 and is presently the John Edson Sweet Professor of Engineering in the School of Applied and Engineering Physics at Cornell.

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REFERENCES

(1) Wouters, O. J.; McKee, M.; Luyten, J. Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009−2018. *JAMA* **2020**, *323* (9), 844−853.

(2) Kiriiri, G. K.; Njogu, P. M.; Mwangi, A. N. Exploring different approaches to improve the success of drug discovery and development projects: a review. *Future Journal of Pharmaceutical Sciences* **2020**, *6* (1), 27.

(3) Fan, J.; Fu, A.; Zhang, L. Progress in molecular docking. *Quantitative Biology* **2019**, *7* (2), 83−89.

(4) Śledź, P.; Caflisch, A. Protein structure-based drug design: from docking to molecular dynamics. *Curr. Opin. Struct. Biol.* **2018**, *48*, 93−102.

(5) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28* (1), 235−242.

(6) McPherson, A.; Gavira, J. A. Introduction to protein crystallization. *Acta Crystallogr. F Struct Biol. Commun.* **2014**, *70* (1), 2−20.
(7) Maveyraud, L.; Mourey, L. Protein X-ray Crystallography and Drug Discovery. *Molecules* **2020**, *25* (5), 1030.
(8) Fraser, J. S.; van den Bedem, H.; Samelson, A. J.; Lang, P. T.; Holton, J. M.; Echols, N.; Alber, T. Accessing protein conformational ensembles using room-temperature X-ray crystallography. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (39), 16247−16252.
(9) Chapman, H. N.; Fromme, P.; Barty, A.; White, T. A.; Kirian, R. A.; Aquila, A.; Hunter, M. S.; Schulz, J.; DePonte, D. P.; Weierstall, U.; et al. Femtosecond X-ray protein nanocrystallography. *Nature* **2011**, *470* (7332), 73−77.
(10) DePonte, D. P.; Weierstall, U.; Schmidt, K.; Warner, J.; Starodub, D.; Spence, J. C. H.; Doak, R. B. Gas dynamic virtual nozzle for generation of microscopic droplet streams. *J. Phys. D: Appl. Phys.* **2008**, *41* (19), 195505.
(11) Dods, R.; Båth, P.; Arnlund, D.; Beyerlein, K. R.; Nelson, G.; Liang, M.; Harimoorthy, R.; Berntsen, P.; Malmerberg, E.; Johansson, L.; et al. From Macrocrystals to Microcrystals: A Strategy for Membrane Protein Serial Crystallography. *Structure* **2017**, *25* (9), 1461−1468.
(12) Martin-Garcia, J. M.; Conrad, C. E.; Nelson, G.; Stander, N.; Zatsepin, N. A.; Zook, J.; Zhu, L.; Geiger, J.; Chun, E.; Kissick, D.; et al. Serial millisecond crystallography of membrane and soluble protein microcrystals using synchrotron radiation. *IUCrJ.* **2017**, *4* (4), 439−454.
(13) Beyerlein, K. R.; Dierksmeyer, D.; Mariani, V.; Kuhn, M.; Sarrou, I.; Ottaviano, A.; Awel, S.; Knoska, J.; Fuglerud, S.; Jonsson, O.; et al. Mix-and-diffuse serial synchrotron crystallography. *IUCrJ.* **2017**, *4* (6), 769−777.
(14) Roedig, P.; Vartiainen, I.; Duman, R.; Panneerselvam, S.; Stübe, N.; Lorbeer, O.; Warmer, M.; Sutton, G.; Stuart, D. I.; Weckert, E.; et al. A micro-patterned silicon chip as sample holder for macromolecular crystallography experiments with minimal background scattering. *Sci. Rep.* **2015**, *5* (1), 10451.
(15) Schulz, E. C.; Mehrabi, P.; Müller-Werkmeister, H. M.; Tellkamp, F.; Jha, A.; Stuart, W.; Persch, E.; De Gasparo, R.; Diederich, F.; Pai, E. F.; et al. The hit-and-return system enables efficient time-resolved serial synchrotron crystallography. *Nat. Methods* **2018**, *15* (11), 901−904.
(16) Gilbile, D.; Shelby, M. L.; Lyubimov, A. Y.; Wierman, J. L.; Monteiro, D. C. F.; Cohen, A. E.; Russi, S.; Coleman, M. A.; Frank, M.; Kuhl, T. L. Plug-and-play polymer microfluidic chips for hydrated, room temperature, fixed-target serial crystallography. *Lab Chip* **2021**, *21* (24), 4831−4845.
(17) Illava, G.; Jayne, R.; Finke, A. D.; Closs, D.; Zeng, W.; Milano, S. K.; Huang, Q.; Kriksunov, I.; Sidorenko, P.; Wise, F. W.; et al. Integrated sample-handling and mounting system for fixed-target serial synchrotron crystallography. *Acta Crystallographica Section D* **2021**, *77* (5), 628−644.
(18) Calvey, G. D.; Katz, A. M.; Pollack, L. Microfluidic Mixing Injector Holder Enables Routine Structural Enzymology Measurements with Mix-and-Inject Serial Crystallography Using X-ray Free Electron Lasers. *Anal. Chem.* **2019**, *91* (11), 7139−7144.
(19) Calvey, G. D.; Katz, A. M.; Schaffer, C. B.; Pollack, L. Mixing injector enables time-resolved crystallography with high hit rate at X-ray free electron lasers. *Structural Dynamics* **2016**, *3* (5), 054301.
(20) Olmos, J. L.; Pandey, S.; Martin-Garcia, J. M.; Calvey, G.; Katz, A.; Knoska, J.; Kupitz, C.; Hunter, M. S.; Liang, M.; Oberthuer, D.; et al. Enzyme intermediates captured "on the fly" by mix-and-inject serial crystallography. *BMC Biology* **2018**, *16* (1), 59.
(21) Pandey, S.; Calvey, G.; Katz, A. M.; Malla, T. N.; Koua, F. H. M.; Martin-Garcia, J. M.; Poudyal, I.; Yang, J. H.; Vakili, M.; Yefanov, O.; et al. Observation of substrate diffusion and ligand binding in enzyme crystals using high-repetition-rate mix-and-inject serial crystallography. *IUCrJ.* **2021**, *8* (6), 878−895.
(22) Mehrabi, P.; Schulz, E. C.; Agthe, M.; Horrell, S.; Bourenkov, G.; von Stetten, D.; Leimkohl, J.-P.; Schikora, H.; Schneider, T. R.; Pearson, A. R.; et al. Liquid application method for time-resolved analyses by serial synchrotron crystallography. *Nat. Methods* **2019**, *16* (10), 979−982.
(23) Renaud, J.-P.; Chari, A.; Ciferri, C.; Liu, W.-t.; Rémigy, H.-W.; Stark, H.; Wiesmann, C. Cryo-EM in drug discovery: achievements, limitations and prospects. *Nat. Rev. Drug Discovery* **2018**, *17* (7), 471−492.
(24) Van Drie, J. H.; Tong, L. Cryo-EM as a powerful tool for drug discovery. *Bioorg. Med. Chem. Lett.* **2020**, *30* (22), 127524.
(25) de Oliveira, T. M.; van Beek, L.; Shilliday, F.; Debreczeni, J. É.; Phillips, C. Cryo-EM: The Resolution Revolution and Drug Discovery. *SLAS DISCOVERY: Advancing the Science of Drug Discovery* **2021**, *26* (1), 17−31.
(26) Martin, G. M.; Kandasamy, B.; DiMaio, F.; Yoshioka, C.; Shyng, S.-L. Anti-diabetic drug binding site in a mammalian KATP channel revealed by Cryo-EM. *eLife* **2017**, *6*, e31054.
(27) Basak, S.; Gicheru, Y.; Kapoor, A.; Mayer, M. L.; Filizola, M.; Chakrapani, S. Molecular mechanism of setron-mediated inhibition of full-length 5-HT3A receptor. *Nat. Commun.* **2019**, *10* (1), 3225.
(28) Zhang, X.; Johnson, R. M.; Drulyte, I.; Yu, L.; Kotecha, A.; Danev, R.; Wootten, D.; Sexton, P. M.; Belousoff, M. J. Evolving cryo-EM structural approaches for GPCR drug discovery. *Structure* **2021**, *29* (9), 963−974.
(29) Do, P.-C.; Lee, E. H.; Le, L. Steered Molecular Dynamics Simulation in Rational Drug Design. *J. Chem. Inf. Model.* **2018**, *58* (8), 1473−1482.
(30) Lu, S.; Ji, M.; Ni, D.; Zhang, J. Discovery of hidden allosteric sites as novel targets for allosteric drug design. *Drug Discovery Today* **2018**, *23* (2), 359−365.
(31) Ferraro, M.; D'Annessa, I.; Moroni, E.; Morra, G.; Paladino, A.; Rinaldi, S.; Compostella, F.; Colombo, G. Allosteric Modulators of HSP90 and HSP70: Dynamics Meets Function through Structure-Based Drug Design. *Journal of medicinal chemistry* **2019**, *62* (1), 60−87.
(32) Yip, K. M.; Fischer, N.; Paknia, E.; Chari, A.; Stark, H. Atomic-resolution protein structure determination by cryo-EM. *Nature* **2020**, *587* (7832), 157−161.
(33) Henderson, R. The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules. *Q. Rev. Biophys.* **1995**, *28* (2), 171−193.
(34) Biesaga, M.; Frigolé-Vivas, M.; Salvatella, X. Intrinsically disordered proteins and biomolecular condensates as drug targets. *Curr. Opin. Chem. Biol.* **2021**, *62*, 90−100.
(35) Metallo, S. J. Intrinsically disordered proteins are potential drug targets. *Curr. Opin. Chem. Biol.* **2010**, *14* (4), 481−488.
(36) Hosoya, Y.; Ohkanda, J. Intrinsically Disordered Proteins as Regulators of Transient Biological Processes and as Untapped Drug Targets. *Molecules* **2021**, *26* (8), 2118.
(37) Ruan, H.; Sun, Q.; Zhang, W.; Liu, Y.; Lai, L. Targeting intrinsically disordered proteins at the edge of chaos. *Drug Discovery Today* **2019**, *24* (1), 217−227.
(38) Nwanochie, E.; Uversky, V. N. Structure Determination by Single-Particle Cryo-Electron Microscopy: Only the Sky (and Intrinsic Disorder) is the Limit. *International Journal of Molecular Sciences* **2019**, *20* (17), 4186.
(39) Danev, R.; Baumeister, W. Cryo-EM single particle analysis with the Volta phase plate. *eLife* **2016**, *5*, e13046.
(40) Zhang, Y.; Tammaro, R.; Peters, P. J.; Ravelli, R. B. G. Could Egg White Lysozyme be Solved by Single Particle Cryo-EM? *J. Chem. Inf. Model.* **2020**, *60* (5), 2605−2613.
(41) Nakane, T.; Kotecha, A.; Sente, A.; McMullan, G.; Masiulis, S.; Brown, P. M. G. E.; Grigoras, I. T.; Malinauskaite, L.; Malinauskas, T.; Miehling, J.; et al. Single-particle cryo-EM at atomic resolution. *Nature* **2020**, *587* (7832), 152−156.
(42) Li, Y.; Cash, J. N.; Tesmer, J. J. G.; Cianfrocco, M. A. High-Throughput Cryo-EM Enabled by User-Free Preprocessing Routines. *Structure* **2020**, *28* (7), 858−869.

https://doi.org/10.1021/acs.jpcb.2c04344
J. Phys. Chem. B 2022, 126, 6599−6607

(43) Kimanius, D.; Dong, L.; Sharov, G.; Nakane, T.; Scheres, S. H. W. New tools for automated cryo-EM single-particle analysis in RELION-4.0. *Biochem. J.* **2021**, *478* (24), 4169−4185.
(44) Stabrin, M.; Schoenfeld, F.; Wagner, T.; Pospich, S.; Gatsogiannis, C.; Raunser, S. TranSPHIRE: automated and feedback-optimized on-the-fly processing for cryo-EM. *Nat. Commun.* **2020**, *11* (1), 5716.
(45) Baldwin, P. R.; Tan, Y. Z.; Eng, E. T.; Rice, W. J.; Noble, A. J.; Negro, C. J.; Cianfrocco, M. A.; Potter, C. S.; Carragher, B. Big data in cryoEM: automated collection, processing and accessibility of EM data. *Curr. Opin Microbiol* **2018**, *43*, 1−8.
(46) Grabowski, M.; Langner, K. M.; Cymborowski, M.; Porebski, P. J.; Sroka, P.; Zheng, H.; Cooper, D. R.; Zimmerman, M. D.; Elsliger, M.-A.; Burley, S. K.; et al. A public database of macromolecular diffraction experiments. *Acta Crystallographica Section D* **2016**, *72* (11), 1181−1193.
(47) Meisburger, S. P.; Thomas, W. C.; Watkins, M. B.; Ando, N. X-ray Scattering Studies of Protein Structural Dynamics. *Chem. Rev.* **2017**, *117* (12), 7615−7672.
(48) Skou, S.; Gillilan, R. E.; Ando, N. Synchrotron-based small-angle X-ray scattering of proteins in solution. *Nat. Protoc.* **2014**, *9* (7), 1727−1739.
(49) Dyer, K. N.; Hammel, M.; Rambo, R. P.; Tsutakawa, S. E.; Rodic, I.; Classen, S.; Tainer, J. A.; Hura, G. L. High-Throughput SAXS for the Characterization of Biomolecules in Solution: A Practical Approach. In *Structural Genomics: General Applications*; Chen, Y. W., Ed.; Humana Press: Totowa, NJ, 2014; pp 245−258.
(50) Hura, G. L.; Menon, A. L.; Hammel, M.; Rambo, R. P.; Poole II, F. L.; Tsutakawa, S. E.; Jenney, F. E., Jr; Classen, S.; Frankel, K. A.; Hopkins, R. C.; et al. Robust, high-throughput solution structural analyses by small angle X-ray scattering (SAXS). *Nat. Methods* **2009**, *6* (8), 606−612.
(51) Gräwert, T. W.; Svergun, D. I. Structural Modeling Using Solution Small-Angle X-ray Scattering (SAXS). *J. Mol. Biol.* **2020**, *432* (9), 3078−3092.
(52) Sigismund, S.; Avanzato, D.; Lanzetti, L. Emerging functions of the EGFR in cancer. *Molecular Oncology* **2018**, *12* (1), 3−20.
(53) Normanno, N.; De Luca, A.; Bianco, C.; Strizzi, L.; Mancino, M.; Maiello, M. R.; Carotenuto, A.; De Feo, G.; Caponigro, F.; Salomon, D. S. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* **2006**, *366* (1), 2−16.
(54) Paez, J. G.; Jänne, P. A.; Lee, J. C.; Tracy, S.; Greulich, H.; Gabriel, S.; Herman, P.; Kaye, F. J.; Lindeman, N.; Boggon, T. J.; et al. EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy. *Science* **2004**, *304* (5676), 1497−1500.
(55) Kolch, W.; Halasz, M.; Granovskaya, M.; Kholodenko, B. N. The dynamic control of signal transduction networks in cancer cells. *Nature Reviews Cancer* **2015**, *15* (9), 515−527.
(56) Arteaga, C. L.; Engelman, J. A. ERBB receptors: from oncogene discovery to basic science to mechanism-based cancer therapeutics. *Cancer Cell* **2014**, *25* (3), 282−303.
(57) Thorpe, L. M.; Yuzugullu, H.; Zhao, J. J. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nature Reviews Cancer* **2015**, *15* (1), 7−24.
(58) Mattoon, D. R.; Lamothe, B.; Lax, I.; Schlessinger, J. The docking protein Gab1 is the primary mediator of EGF-stimulated activation of the PI-3K/Akt cell survival pathway. *BMC Biol.* **2004**, *2*, 24.
(59) Freed, D. M.; Bessman, N. J.; Kiyatkin, A.; Salazar-Cavazos, E.; Byrne, P. O.; Moore, J. O.; Valley, C. C.; Ferguson, K. M.; Leahy, D. J.; Lidke, D. S.; et al. EGFR Ligands Differentially Stabilize Receptor Dimers to Specify Signaling Kinetics. *Cell* **2017**, *171* (3), 683−695.
(60) Hu, C.; Leche, C. A.; Kiyatkin, A.; Yu, Z.; Stayrook, S. E.; Ferguson, K. M.; Lemmon, M. A. Glioblastoma mutations alter EGFR dimer structure to prevent ligand bias. *Nature* **2022**, *602* (7897), 518−522.
(61) Liu, Q.; Zhou, J.; Gao, J.; Ma, W.; Wang, S.; Xing, L. Rational design of EGFR dimerization-disrupting peptides: A new strategy to combat drug resistance in targeted lung cancer therapy. *Biochimie* **2020**, *176*, 128−137.
(62) Gerhart, J.; Thévenin, A. F.; Bloch, E.; King, K. E.; Thévenin, D. Inhibiting Epidermal Growth Factor Receptor Dimerization and Signaling Through Targeted Delivery of a Juxtamembrane Domain Peptide Mimic. *ACS Chem. Biol.* **2018**, *13* (9), 2623−2632.
(63) Bergendahl, L. T.; Gerasimavicius, L.; Miles, J.; Macdonald, L.; Wells, J. N.; Welburn, J. P. I.; Marsh, J. A. The role of protein complexes in human genetic disease. *Protein Sci.* **2019**, *28* (8), 1400−1411.
(64) Jiménez-García, B.; Pons, C.; Svergun, D. I.; Bernadó, P.; Fernández-Recio, J. pyDockSAXS: protein−protein complex structure by SAXS and computational docking. *Nucleic Acids Res.* **2015**, *43* (W1), W356−W361.
(65) Schindler, C. E.M.; de Vries, S. J.; Sasse, A.; Zacharias, M. SAXS Data Alone can Generate High-Quality Models of Protein-Protein Complexes. *Structure* **2016**, *24* (8), 1387−1397.
(66) Gallo, M.; Defaus, S.; Andreu, D. Disrupting GPCR Complexes with Smart Drug-like Peptides. *Pharmaceutics* **2022**, *14* (1), 161.
(67) Mannes, M.; Martin, C.; Triest, S.; Pia Dimmito, M.; Mollica, A.; Laeremans, T.; Menet, C. J.; Ballet, S. Development of Generic G Protein Peptidomimetics Able to Stabilize Active State Gs Protein-Coupled Receptors for Application in Drug Discovery. *Angew. Chem., Int. Ed.* **2021**, *60* (18), 10247−10254.
(68) Klementieva, O.; Willén, K.; Martinsson, I.; Israelsson, B.; Engdahl, A.; Cladera, J.; Uvdal, P.; Gouras, G. K. Pre-plaque conformational changes in Alzheimer's disease-linked Aβ and APP. *Nat. Commun.* **2017**, *8* (1), 14726.
(69) Paar, M.; Fengler, V. H.; Rosenberg, D. J.; Krebs, A.; Stauber, R. E.; Oettl, K.; Hammel, M. Albumin in patients with liver disease shows an altered conformation. *Communications Biology* **2021**, *4* (1), 731.
(70) Lesort, M.; Tucholski, J.; Miller, M. L.; Johnson, G. V. W. Tissue transglutaminase: a possible role in neurodegenerative diseases. *Progress in Neurobiology* **2000**, *61* (5), 439−463.
(71) Dieterich, W.; Ehnis, T.; Bauer, M.; Donner, P.; Volta, U.; Riecken, E. O.; Schuppan, D. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nature Medicine* **1997**, *3* (7), 797−801.
(72) Katt, W. P.; Aplin, C.; Cerione, R. A. Exploring the Role of Transglutaminase in Patients with Glioblastoma: Current Perspectives. *Onco Targets Ther* **2022**, *15*, 277−290.
(73) Pinkas, D. M.; Strop, P.; Brunger, A. T.; Khosla, C. Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol.* **2007**, *5* (12), e327−e327.
(74) Liu, S.; Cerione, R. A.; Clardy, J. Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99* (5), 2743−2747.
(75) Singh, G.; Zhang, J.; Ma, Y.; Cerione, R. A.; Antonyak, M. A. The Different Conformational States of Tissue Transglutaminase Have Opposing Affects on Cell Viability *. *J. Biol. Chem.* **2016**, *291* (17), 9119−9132.
(76) Datta, S.; Antonyak, M. A.; Cerione, R. A. GTP-binding-defective forms of tissue transglutaminase trigger cell death. *Biochemistry* **2007**, *46* (51), 14819−29.
(77) Colak, G.; Keillor, J. W.; Johnson, G. V. W. Cytosolic Guanine Nucledotide Binding Deficient Form of Transglutaminase 2 (R580a) Potentiates Cell Death in Oxygen Glucose Deprivation. *PLoS One* **2011**, *6* (1), e16665.
(78) Katt, W. P.; Blobel, N. J.; Komarova, S.; Antonyak, M. A.; Nakano, I.; Cerione, R. A. A small molecule regulator of tissue transglutaminase conformation inhibits the malignant phenotype of cancer cells. *Oncotarget* **2018**, *9* (76), 34379−34397.
(79) Kikhney, A. G.; Svergun, D. I. A practical guide to small angle X-ray scattering (SAXS) of flexible and intrinsically disordered proteins. *FEBS Lett.* **2015**, *589* (19, Part A), 2570−2577.

https://doi.org/10.1021/acs.jpcb.2c04344
J. Phys. Chem. B 2022, 126, 6599−6607

(80) Rambo, R. P.; Tainer, J. A. Characterizing flexible and intrinsically unstructured biological macromolecules by SAS using the Porod-Debye law. *Biopolymers* **2011**, *95* (8), 559−571.
(81) Antonov, L. D.; Olsson, S.; Boomsma, W.; Hamelryck, T. Bayesian inference of protein ensembles from SAXS data. *Phys. Chem. Chem. Phys.* **2016**, *18* (8), 5832−5838.
(82) Fuertes, G.; Nevola, L.; Esteban-Martín, S. Perspectives on drug discovery strategies based on IDPs. In *Intrinsically Disordered Proteins*; Salvi, N., Ed.; Academic Press, 2019; Chapter 9, pp 275−327.
(83) Bhattarai, A.; Emerson, I. A. Dynamic conformational flexibility and molecular interactions of intrinsically disordered proteins. *Journal of Biosciences* **2020**, *45* (1), 29.
(84) Bowman, M. A.; Riback, J. A.; Rodriguez, A.; Guo, H.; Li, J.; Sosnick, T. R.; Clark, P. L. Properties of protein unfolded states suggest broad selection for expanded conformational ensembles. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (38), 23356−23364.
(85) Aznauryan, M.; Delgado, L.; Soranno, A.; Nettels, D.; Huang, J.-r.; Labhardt, A. M.; Grzesiek, S.; Schuler, B. Comprehensive structural and dynamical view of an unfolded protein from the combination of single-molecule FRET, NMR, and SAXS. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (37), E5389−E5398.
(86) Cho, H. S.; Schotte, F.; Stadnytskyi, V.; Anfinrud, P. Time-resolved X-ray scattering studies of proteins. *Curr. Opin. Struct. Biol.* **2021**, *70*, 99−107.
(87) Lu, S.; He, X.; Yang, Z.; Chai, Z.; Zhou, S.; Wang, J.; Rehman, A. U.; Ni, D.; Pu, J.; Sun, J.; et al. Activation pathway of a G protein-coupled receptor uncovers conformational intermediates as targets for allosteric drug design. *Nat. Commun.* **2021**, *12* (1), 4721.
(88) Gur, M.; Taka, E.; Yilmaz, S. Z.; Kilinc, C.; Aktas, U.; Golcuk, M. Conformational transition of SARS-CoV-2 spike glycoprotein between its closed and open states. *J. Chem. Phys.* **2020**, *153* (7), 075101.
(89) Roskoski, R. Small molecule inhibitors targeting the EGFR/ErbB family of protein-tyrosine kinases in human cancers. *Pharmacol. Res.* **2019**, *139*, 395−411.
(90) Miao, Y.; Huang, Y.-m. M.; Walker, R. C.; McCammon, J. A.; Chang, C.-e. A. Ligand Binding Pathways and Conformational Transitions of the HIV Protease. *Biochemistry* **2018**, *57* (9), 1533−1541.
(91) Pollack, L.; Doniach, S. Time-resolved X-ray scattering and RNA folding. *Methods Enzymol.* **2009**, *469*, 253−68.
(92) Lamb, J. S.; Zoltowski, B. D.; Pabit, S. A.; Crane, B. R.; Pollack, L. Time-Resolved Dimerization of a PAS-LOV Protein Measured with Photocoupled Small Angle X-ray Scattering. *J. Am. Chem. Soc.* **2008**, *130* (37), 12226−12227.
(93) Sugiki, T.; Furuita, K.; Fujiwara, T.; Kojima, C. Current NMR Techniques for Structure-Based Drug Discovery. *Molecules* **2018**, *23* (1), 148.
(94) Kuhlman, B.; Bradley, P. Advances in protein structure prediction and design. *Nat. Rev. Mol. Cell Biol.* **2019**, *20* (11), 681−697.
(95) Baek, M.; DiMaio, F.; Anishchenko, I.; Dauparas, J.; Ovchinnikov, S.; Lee, G. R.; Wang, J.; Cong, Q.; Kinch, L. N.; Schaeffer, R. D.; et al. Accurate prediction of protein structures and interactions using a three-track neural network. *Science* **2021**, *373* (6557), 871−876.
(96) Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; et al. Highly accurate protein structure prediction with AlphaFold. *Nature* **2021**, *596* (7873), 583−589.

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