Unveiling the biological interface of protein complexes by mass spectrometry-coupled methods

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Abstract

Most biomolecules become functional and bioactive by forming protein complexes through interaction with ligands that are diverse in size, shape, and physicochemical properties. In the complex biological milieu, the interaction is ligand-specific, driven by molecular sensing and recognition of a binding interface localized within a protein structure. Mapping interfaces of protein complexes is a highly sought area of research as it delivers fundamental insights into proteomes and pathology and hence strategies for therapeutics. While X-ray crystallography and electron microscopy still serve as a gold standard for structural elucidation of protein complexes, artificial and static analytic nature thereof often results in a non-native interface that otherwise might be negligible or non-existent in biological environment. In recent years, the mass spectrometry-coupled approaches, chemical crosslinking (CLMS) and hydrogen-deuterium exchange (HDMS), have become valuable analytic complements to traditional techniques. These methods explicitly identify hot residues and motifs embedded in binding interfaces, in particular, for which the interaction is predominantly dynamic, transient, and/or caused by an intrinsically disordered domain. Here we review the principal role of CLMS and HDMS in protein structural biology with a particular emphasis on the contribution of recent examples to exploring biological interfaces. In addition, we describe recent studies that utilized these methods to expand our understanding of protein complex formation and related biological processes and to increase probability of structure-based drug design.

1. Introduction

Viability of all forms of life is crucially reliant on homeostasis of protein interactome. Therefore, elucidation of networks involving protein-protein or protein-ligand interactions is a cornerstone for understanding the complexity of biological processes. Additionally, comprehensive informatics of protein interactions serves as a useful template for drug discovery and drug repositioning (Payandeh and Volgraf 2021; Scott et al., 2016; Wang et al., 2021). A multitude of methods have been developed to study the protein interactome with high throughput and accuracy (Rao et al., 2014). While the protein interactome illustrates the global network and relation of proteins and ligands, mechanistic insight at molecular level into each set of protein interactions further requires high-resolution technologies capable of probing an array of critical motifs and residues, defined herein as an interface, that are collectively responsible for accurate molecular recognition and energetic stabilization.

Elementary bioanalytic methods readily available in most molecular biology laboratories are size exclusion chromatography (SEC) and native PAGE which examine if biomolecules under investigation do recognize each other, albeit with little information of binding interfaces. These methods macroscopically detect a change in mobility of biomolecules through a matrix, attributed by size increase and charge variation upon molecular association. Quantitative measurements of kinetics and thermodynamics parameters involved in molecular interactions require real-time techniques. Surface plasmon resonance (SPR) and bio-layer interferometry (BLI) employ sophisticated optical biosensors that monitor minute changes of resonance signals, or light interference quantitatively dependent upon intermolecular binding events (Dysinger and

King 2012; Rich and Myszka 2011). Data processing from repetitive measurements in different concentrations of an analyte yields the rates of association and dissociations as well as the strength of the interaction in terms of affinity (K_D). Microscale thermophoresis (MST) technique is quick to derive $K_D$from variations of a molecular movement of a capillary-filled, fluorescently labeled ligand throughout microthermal gradient, affected by its association with an analyte and subsequent changes in physical properties (Seidel et al., 2013). Isothermal titration calorimetry (ITC) is a unique method that investigates the interaction between biomolecules without labeling or immobilization (Pierce et al., 1999). By measuring differential changes in the heat supplied to the sample cell, as compared to the blank cell, in which two binding partners are incrementally mixed, ITC provides useful parameters related to the bimolecular interaction including binding stoichiometry, enthalpy and entropy changes as well as affinity. Each quantitative method has its advantages, and might serve as a standard technique depending on the amount, purity, and physicochemical properties of biomolecules under investigation. Despite being more informative than SEC and PAGE, these methods are still far from defining the binding interface which is mainly responsible for various interaction parameters obtained in the measurements. Structural information of a binding interface at atomic resolution can be derived by implementing X-ray crystallography, nuclear magnetic resonance spectroscopy (NMR), or cryo-electron microscopy (Cryo-EM). While X-ray crystallography is the most predominant in accessibility and practice thus far, the size of the crystallographic interface is usually smaller and the interface water content is higher in general compared to the interface under native environment, referred to herein as 'biological interface', due to non-physiological but crystallization-favored conditions inflicted on a protein complex (Schreiber 2020). Recent advances of NMR and Cryo-EM in instrumentation and software reliably extract structural information of protein complexes in pseudo-physiological conditions which can then be processed to reconstruct preexisting interfacial crystal contacts into a near-native state or even newly visualize the binding interfaces not resolved in X-ray crystallography. Cyro-EM and NMR provide unique and additional advantages of facile sample preparation and delivery of structural dynamics data, respectively. Still, variable and dynamic natures of binding interfaces embedded in protein complexes complicates the interpretation of spectroscopic data with reasonable resolution and fidelity, rendering mechanistic details of many protein interactions as yet to be explored.

Aside from the high resolution, another critical attribute expected from structural information of protein complexes is the high fidelity which presents the most probable and representative snapshot of biological interfaces under in-solution native environment, contrasting to a static condition in which X-ray crystallography and electron microscopy are generally best suited. Despite high resolution provided by static methods, however, a static interface features non-physiological water content and accessibility due to non-specificity caused by invasive conditions such as a wide range of pH, high ion strength, high protein concentration, all of which are detrimental to obtaining high-fidelity interfacial landscape. In addition to such empirical artifacts, a static interface often loses fidelity due to inherent natures of certain protein interactions which could be dynamic, transient, weak, or mediated by an intrinsically disordered domain that adopts no static conformation. Thus, in combination of X-ray crystallography and electron microscopy, supplementary methods to obtain structural data from the native solution-phase are of great necessity to probe a biological interface with both high resolution and fidelity.

Chemical crosslinking (CLMS) and hydrogen-deuterium exchange (HDMS), both of which are coupled to mass spectrometry, have become unique and complementary analytic methods to address abovementioned unmet needs in structural biology. CLMS and HDMS are particularly valuable for unveiling hot spots in interfaces that otherwise would be cryptic or of poor fidelity, thus enabling the construction of a new interfacial landscape of an unknown protein complex or reconstitution of a preexisting protein interface, and subsequently aid in the structure-based design of novel modulators of binding interfaces with a high success rate (Arkin et al., 2014; Chen et al., 2012). In this review, we briefly describe how CLMS and HDMS map the interfaces of protein complexes with high fidelity and their integration with other structural information to yield native-like high-order structures assembled via biological interfaces. We then discuss recent efforts that successfully employed CLMS and/or HDMS to reveal dynamic, transient, or intrinsically disordered interfaces found in various protein complexes that had been challenging to probe via X-ray crystallography

and electron microscopy.

Mass spectrometry-coupled techniques, CLMS and HDMS, provide novel means of landscaping a biological interface. In contrast to abovementioned spectroscopic techniques focusing on electronic or electromagnetic properties of respective atoms, CLMS and HDMS delve into reciprocal behavior of residues with other residues or matters surrounding them under a native condition. The resultant mass changes are detected to calculate corresponding inter-residue distances or solvent accessibility. Although structural information obtained from CLMS and HDMS is generally intermediate in resolution, it provides improved fidelity by repositioning known residues in a static interface or identifying unknown residues residing in a dynamic, transient, or disordered interfacial region.

2. Mapping biological interfaces by CLMS

A crosslinking reagent makes a short covalent bridge between two biomolecules in proximity, i.e. freezing them, that occurs when they are bound through specific molecular contacts, thereby usefully aiding the identification of an unknown binding partner interacting with a host protein, especially in proteome-wide studies (Figure 1) (Götze et al., 2019; Holding 2015; Piersimoni and Sinz 2020). Due to poor site-specific reactivity and/or lack of highly sensitive analytical tools, residue-level elucidation of a binding interface via chemical crosslinking had not been possible until controlled crosslinking chemistry and high-sensitivity mass spectrometry emerged into practice (Mishra et al., 2020; Yang et al., 2016).

Among a variety of chemical reactivity available, to date, a photo-activated functionality such as diazirine provides the most effective resolution of crosslinks due to *in situ* initiation and the fast kinetics of chemical steps. Additionally, a carbene formed from diazirine upon UV irradiation shows an equivalent crosslinking reactivity to all proteinogenic amino acids, avoiding biased estimation in the following mass spetrometric analyses. An unnatural amino acid containing a photo-activated residue (UAA) can be genetically and site-specifically inserted into a protein or a photo-activated moiety can be synthetically incorporated into a peptide or a small molecule (Chen et al., 2020; Pham et al., 2013; Yang et al., 2016). Alternatively, a natural amino acid residue such as amine or thiol in a protein is derivatized with N-hydroxysuccinimide (NHS) ester in a heterobifunctional linker that also contains a photo-activated group for a subsequent crosslinking reaction to a binding partner.

A conventional homobifunctional linker bearing NHS esters is still widely applicable not only for its ease of manipulation but also for newly introduced functionalities like MS-cleavable moiety, isotope labeling, or well-defined spatial constraints which, to a greater extent, facilitate automated mass data analyses and computational modeling with high confidence (Iacobucci et al., 2018; Ihling et al., 2020; Rappsilber 2011). For more details about the workflow of CLMS, crosslinking chemistries, and data processing which are beyond the scope of this review, interested readers are referred to several recent reviews (Chavez et al., 2019; Holding 2015; Piersimoni and Sinz 2020).

2.1. Interfaces revealed by UAA incorporation and crosslinking

Incorporation of a photo-reactive UAA into a target protein in live cells followed by controlled crosslinking *in situ* provides a great advantage of identifying target protein-specific binding partner(s) in a native environment (Figure 2A). Importantly, it circumvents the need for target protein extraction and purification in aqueous solution before crosslinking, which is almost impossible for some proteins, especially those in cellular membranes like G protein-coupled receptors (GPCRs). GPCRs are the largest family of integral membrane proteins with a characteristic structure comprising seven transmembrane helix folds, three intracellular loops together with the N-terminus, and three extracellular loops together with the C-terminus (Cvicek et al., 2016). Membranous and flexible natures of GPCRs usually compromise the sample preparation for classical structure analyses that require a substantial sample amount and purity. Even though dozens of GPCR structures have been determined, most of them are believed to represent a single snapshot of GPCR that otherwise would wiggle vigorously in a native environment.

CXC chemokine receptor 4 (CXCR4) is a pathologically and clinically important GPCR in cancers, inflam-

mation, and viral infection. To probe the binding site of T140, a 14-residue cyclic peptide HIV-1 entry blocker, CXCR4 was site-specifically mutated with a photoactivatable UAA, p-benzoyl-l-phenylalanine (BzF), at various positions one-by-one in live cells (Grunbeck et al., 2011). Co-incubation of the fluorescently labeled T140 with CXCR4-transfected cells was followed by UV exposure to trap a CXCR4-T140 complex. A series of experiments for each CXCR4 mutant showed that the crosslinked complex was formed only when BzF had been incorporated at Phe189. Based on a characteristic 3 Å-long reactivity distance of BzF as well as the positional information gained from the crosslinking analyses, molecular modeling could successfully derive a more accurate model of the CXCR4-T140 interface modified from available crystal structures. Similarly, strategic incorporation of photo-crosslinking UAA allowed identification of contact residues of CCR5 in complex with a small molecule HIV-1 entry inhibitor, maraviroc (Grunbeck et al., 2012). Newly discovered contact residues previously not recognized in the computational modeling could serve as a cue to reconstruct the interface taking the dynamic allosteric binding of maraviroc into account (Grunbeck et al., 2012). Although the UAA-mediated crosslinking studies mentioned above lacked the mass spectrometric analysis, a genetically well-defined position of crosslinking and the ability to incorporate the UAA in any position of interest could make a significant contribution to remodeling GPCR-ligand structures to display the biological interface. The most attractive aspect of the method in studying the structural biology of GPCRs lies in that important contact sites can be identified even when GPCR behaves dynamically in a native cellular environment in response to different biological stimulation. By analyzing 25 different mutants of the angiotensin II type 1 receptor (AT1R) containing a photo-reactive UAA, Gagnon et al. demonstrated that AT1R exhibits structurally differential binding modes, i.e., distinct conformations or residues, with an intracellular β-arrestin regulator depending on the presence of an extracellular ligand, angiotensin II (Gagnon et al., 2019).

Photo-reactive UAA incorporation technique in live cells readily allows investigation of a dynamic interface hidden in the transmembrane domain which is much more challenging to recombinantly prepare *in vitro*than the extracellular domain. CGRP is a ligand that binds to the CLR/RAMP1 receptor. While the crystal structure of the extracellular domain of CLR/RAMP1 in complex with CGRP and corresponding contact residues had been available, the interface located in the transmembrane domain remained to be probed. Crosslinked residue screening by photo-reactive UAA crosslinker incorporation at multiple potential contact sites in the CLR transmembrane helix domain identified major contact residues, providing insight into the extent of CGRP penetration into the transmembrane core of CLR (Simms et al., 2018). It should be noted that, besides GPCR, most protein complexes recombinantly expressible in E. coli or mammalian cells can benefit from techniques reviewed above for investigation of biological interfaces regardless of their size, cellular location, and complexity (Bridge et al., 2019; Owens et al., 2019; Rubino et al., 2020).

Since biochemical data obtained from the UAA-mediated crosslinking are an array of band shift patterns resulting from various crosslinked-target proteins or fragments in the immunoblotted gel electrophoresis, the output is generally moderate or low in three-dimensional conformation and resolution. The crosslinking data can be best utilized when integrated with other structural information to give a refined structure with high resolution and fidelity. For example, the class B corticotropin-releasing factor receptor type 1 (CRF1R) was mapped by genetically incorporating an UAA crosslinker systematically to characterize the difference in a conformational change and an interfacial landscape, when CRF1R was stimulated by an agonist or antagonist. Differences in band shift patterns were evident in western blotting, and the inter-residue distance constraints, a characteristic of the crosslinked chemical structure, estimated for all of the crosslinked residues were applied to computationally generate conformational models of the agonist- and the antagonist-bound CRF1R complex. Extensive sampling of conformations led to optimized structure models for both complexes, revealing that CRF1R adopted distinct binding interfaces and local conformations to engage the agonist and the antagonist, respectively (Seidel et al., 2017). Remarkably, the predicted models have been found to be very compatible with the high-resolution cryo-EM structures obtained years later (Liang et al., 2020; Ma et al., 2020). A unique hormone-binding motif of the insulin receptor could be recognized from the crosslinking data in conjunction with the preexisting crystal structure of the apo-insulin receptor. As was expected from the crystal contacts, a photo-reactive UAA incorporated in the typical β-strands 2 and 3 in the L1 domain of the insulin receptor α-subunit was found to be crosslinked to the other α-subunit in an

apo-state. However, in a holo-state where the insulin receptor was bound to the insulin, the crosslinks were made to the insulin, implying a dynamic interface change caused by the induced fit of the insulin (Whittaker et al., 2012). Similarly, a recent study performed a crystal structure-based photo-crosslinking analysis of the macromolecular complex of nuclear pore proteins, Nup82, Nup116, and Nup159. Of note, crosslinking patterns demonstrated that, in comparison to the crystal interfaces, the interfaces of Nup82 in contact with Nup116 and 159 were significantly different in areas and residues involved (Shin and Lim 2020). Interestingly, some contact residues in the interface engaging Nup82 and Nup116 in the ternary complex seemed to lose contacts when Nup82 and Nup116 formed the binary complex by themselves without Nup159, indicating a dynamic nature of the interface not locally isolated but wriggling in conjunction with overall structural changes. Abovementioned and related studies are summarized in the header row of 'photo-crosslinking UAA in live cells' (Table 1).

2.2. Interfaces revealed by UAA incorporation and crosslinking coupled to MS

While the UAA-mediated photo-crosslinking technique facilitates a scan of interfacial residues on one side of the biomolecular complex, specific locations of the crosslinks made at the other binding molecules remain elusive. This is because photo-reactive UAA can be site-specifically incorporated in the target protein but its reactivity upon irradiation towards a binding partner protein is non-specific. Analytic improvements made through enzymatic fragmentation of the crosslinked complex followed by fingerprinting by high-resolution mass spectrometry opened up a new arena of probing two-sided binding interfaces of a protein complex with better resolution and fidelity (Figure 2B). For example, to investigate the mechanistic details of lipopolysaccharide transport through the integral membrane protein LptD/E complex, LptE was site-specifically modified with an UAA photo-crosslinker at various positions. The covalent intermolecular complexes LptD/E formed upon irradiation were observed in Western blotting and then subjected to tryptic digestion followed by high-resolution two-dimensional MALDI-TOF/TOF spectrometry. The UAA incorporated at the 150^{th} position of LptE was found to be linked to S538 in LptD, specifically suggesting that a putative extracellular loop of the LptD β-barrel spanning a residue S538 was a major motif of interaction with LptE (Freinkman et al., 2011). In case a ligand is a small molecule that is amenable to derivatization with a photo-reactive group, the interface mapping can be performed without generating multiple protein mutants bearing a photo-reactive UAA. Diazirine-bearing cholesterol acting as a photoactivatable crosslinker as well as a cofactor for the glycine receptor was employed to investigate how the landscape encompassing contact residues between the cholesterol and the glycine receptor would vary depending on a cholesterol concentration. Crosslinks obtained after trypsin digestion were analyzed by the multidimensional MS to identify specific amino acids of the glycine receptor linked to the cholesterol with high sensitivity and subsequently map the cholesterol-protein interface altering as a function of cholesterol composition (Ferraro and Cascio 2018). A bifunctional UAA bearing a diazirine, for photo-crosslinking, and a terminal alkyne group, for bioorthogonal tagging, has been reported (Yang et al., 2020). A ligand harboring the bifunctional UAA captured a target protein by photo-crosslinking and then could be tagged with an affinity motif such as biotin by alkyne-azide click chemistry. This strategy might enable the enrichment of ligand-target peptide conjugates after digestion and purification for high resolution MS, allowing efficient mapping of biological interfaces on both sides of a ligand and a target.

Although the mass spectrometry greatly enhances the abundance of structural information obtained from the crosslinking, it often suffers from a high rate of false-positive hits and from missed true-positive hits due to complicated mass spectra to interpret, in particular when the photo-crosslinking is performed under living conditions, i.e., with high contamination backgrounds. To increase the confidence level of data analyses, a novel photo-reactive UAA bearing a transferable chemical label has been developed. The UAA introduced site-specifically to a target protein, upon interaction with a binding partner, makes a crosslink and then undergoes an oxidative cleavage leaving the label on the interface of a binding partner. This technique enables the identification of true-positive mass fragments with high confidence and the exact contact residues on both sides of interfaces simultaneously (He et al., 2017; Yang et al., 2016; Yang et al., 2017).

As demonstrated in studies reviewed above, the UAA-mediated crosslinking method genetically installs

a reactive chemical handle directly into a predicted interfacial residue of interest, and induces interfacial crosslinkage of a protein complex in a controlled manner. As it require no exogenous chemical reagents at the time of crosslinking, diffusional and steric limitations that otherwise would adversely affect data fidelity can be avoided. Despite such unique advantages it provides, however, some cares need to be taken to address a potential risk of poor expression yield of UAA-incorporated proteins and/or structural perturbation of a native interface by UAA mutation. Abovementioned and related studies are summarized in the header row of 'photo-crosslinking UAA' (Table 1).

2.3. Interfaces revealed by a chemical crosslinker coupled to MS

A bifunctional crosslinker with a specific reactivity towards amino acid residues or naturally occurring bonds in biomolecules can be usefully employed to 'freeze' a protein complex in the solution phase. The technique avoids the need for mutagenesis or derivatization of interacting molecules which sometimes interferes with a natural binding mode of interaction, and necessitates integrative analyses in tandem with mass spectrometry due to rich but complex dataset generated upon crosslinking. A chemical crosslinker is generally applied to a purified biomolecular complex to eventually obtain comprehensive distance information between residues or bonds thereof (Figure 2B). The intrinsically disordered domain (IDD) of a protein has little propensity to form a fixed or ordered conformation. Whereas some IDDs are involved in functional molecular interaction, their flexible structure or transient binding behavior often limits the investigation of binding interfaces by conventional spectroscopic methods. A chemical linker approach has proven effective to address this issue. The tumor suppressor p53 which is known for its inherent structural flexibility and resistance to crystallization was subjected to the chemical crosslinking using an amine-reactive bifunctional linker coupled to mass spectrometry. The analyses uncovered novel information regarding the dimer-dimer interface of the p53 tetramer as well as a large degree of flexibility of the C-terminal domain and DNA-dependent conformational changes (Arlt et al., 2017; Arlt et al., 2015).

Moreover, amine-specific linkers with different reactive groups and spacer distances were utilized to dissect the flexible or disordered interfaces of macromolecular complexes such as MICAL3, ELKS, and Rab8A; SLAIN2, CLASP2, and ch-TOG (Liu et al., 2017); NLRP1 and DPP9 (Hollingsworth et al., 2021); Rpn2 and Rpn13 (Gong et al., 2020). Recent developments in linker chemistry have produced crosslinker reagents that are cleavable, photo-activatable, or heterobifunctional, permitting controlled crosslink reactions, low background, improved sensitivity (Beard et al., 2021; Krist and Statsyuk 2015), and even in a native cellular environment (Xie et al., 2021). For instance, a crosslinker bearing a photoreactive moiety as well as a reducible spacer have been developed. A bait protein labeled with the crosslinker at a defined residue is photo-crosslinked to a prey protein after which a reductive removal of a bait protein leaves a thiol-containing fragment of the crosslinker onto the target that can be located by MS (Horne et al., 2018; Mintseris and Gygi 2020). This technique improves MS sensitivity by eliminating the background from bait peptides and, depending on the site of crosslinker labeling and spacer length, would provide structural information surrounding the interfacial region.

A substantial amount of interfacial information attained in these biological environments was completely new or contradictory to information previously obtained from the crystallographic or cryo-EM structures, enabling remodeling or refinement by the combinatorial data processing. The technique also successfully probed interfacial residues responsible for protein oligomerization, which had been poorly resolved by crystallography (Karagöz et al., 2017). Abovementioned and related studies are summarized in the header rows of 'photo-crosslinker' and 'chemical crosslinker' (Table 1).

3. Mapping biological interfaces by HDMS

Hydrogens in amides constituting a protein backbone undergo continuous exchange with hydrogens in a solvent. The exchange rate of each amide hydrogen varies significantly, depending on solvent accessibility (Figure 1). By incubating a protein complex in a solvent containing excess deuterium oxide (D_2O), the exchange rate can be quantitatively measured by a mass increase of the complex, a basic principle of hydrogen-deuterium exchange coupled to mass spectrometry (HDMS). Regional mass changes measurable by proteolytic frag-

ments of the complex followed by high-quality MS can be translated into residue- or peptide-level solvent accessibilities which are useful to infer overall protein conformation and, in particular, potential interfacial areas where the exchange rate differs in a bound state relative to an unbound state.

In comparison to CLMS, HDMS is more applicable to any size of a protein complex and involves relatively simple data interpretation. On the other hand, HDMS does not freeze the complex but monitors the time course of changes which might not be significant for unstructured or transient interfaces where H-D exchange is too fast to detect. For more details about the work-flow of HDMS and data processing which are beyond the scope of this review, interested readers are referred to several reviews elsewhere (Anderson et al., 2018; Masson et al., 2019; Zhang et al., 2012).

3.1. Antibody-epitope interface

Success factors for discovery of biopharmaceuticals include understanding the epitope-efficacy relationship in addition to other parameters such as affinity and manufacturability (Puchades et al., 2019; Sun et al., 2021; Zhu et al., 2019). HDMS is robust, high in throughput (Qi et al., 2021), ensuring quick and efficient determination of binding regions in a near-native physicochemical condition — a feature very attractive to the fast-paced pharmaceutical R&D sector. In this regard, HDMS has made significant contributions to probing binding interfaces of antibody-antigen complexes, i.e., epitope mapping, in industry as well as academia (Figure 3A) (Masson et al., 2017).

In particular, exceptional applicability of HDMS to biomacromolecules is useful for mapping an epitope for an antigen-antibody complex which is usually as large-sized as more than 100 kDa — far beyond a measurable limit of NMR analysis. Huang et al. subjected a homotrimeric cytokine (90 kDa), TL1A, complexed with three antibodies (450 kDa) at an equimolar binding stoichiometry to HDMS for the epitope mapping. The analysis allowed residue-level determination of a discontinuous epitope within a predicted binding interface of TL1A and its cognate receptor DR3, rationalizing a strong potency of the antibody as a TL1A antagonist (Huang et al., 2018). The epitope mapping by HDMS is less idiosyncratic and resource-intensive, permitting comparative epitope analyses for a high-molecular-weight antigen targeted by two distinct antibodies. For instance, the epitopes of a large homotrimeric food allergen, cashew 11S globulin allergen (286 kDa), bound to either of two monoclonal antibodies, were mapped by the solution-phase HDMS (Zhang et al., 2011). Due to the large size and complexity of the antigen-antibody complex, the epitope mapping was inaccessible by NMR or X-ray crystallography. Desirably, HDMS was readily applicable to the complex, yielding comparative epitope analyses between two different antibodies in parallel, and required less overall effort than the conventional epitope mapping strategies such as mutagenesis and peptide scanning (Gershoni et al., 2007; Ozohanics and Ambrus 2020; Ständer et al., 2021).

One of the novel characteristics of immunoglobulin G (IgG) is the FcRn-mediated recycling that is responsible for long circulation half-lives of IgG. Understanding their structural mechanisms of interaction is crucially important for optimizing pharmacokinetics of therapeutic antibodies (Liu 2018). As revealed in the X-ray crystallography (Oganesyan et al., 2014), the Fc region was mainly involved in FcRn interaction. However, a lack of structural information on a full-length IgG (150 kDa) complexed with FcRn (50 kDa, extracellular domain) in solution raised a possibility of potential binding interfaces located at the F_{ab} region. Jensen et al. studied the interaction between a full-length human IgG1 and human FcRn via HDMS, and identified several loci at the F_{ab}region which were substantially protected from hydrogen/deuterium exchange in the presence of FcRn. Some of the loci were found even in V_H and V_L domain remote from the Fc region, implying a landscape of the FcRn-IgG binding interface extended throughout the whole IgG structure or conformational dynamics of the F_{ab} region relevant to the FcRn binding (Jensen et al., 2015). Future studies might provide supplementary CLMS datasets that include defined distance information between FcRn and the protected loci of IgG detected by HDMS, and therefore should scrutinize either possibility unambiguously.

A unique analytic feature of HDMS is its ability to directly monitor the interfacial dynamics at various protein concentrations in solution. Antibodies are prone to reversible self-association when highly concentrated (Yadav et al., 2011). Arora et al. investigated two antibody samples at low and high concentrations by

HDMS in parallel, and could define major interfacial hot spots in CDRs that are related to the concentration-dependent reversible self-association (Arora et al., 2015). Furthermore, the HDMS results demonstrated that C_{H1}-C_{H2} interface at the hinge region, distal to the self-association interface, exhibited significant local backbone flexibility in a concentration-dependent manner, presumably attributed by long-range, distant dynamic coupling effects. Abovementioned and related studies are summarized in Table 2.

3.2. Oligomer interface

Protein oligomerization occurs in biological systems, with about 30-35% of total proteins involved (Gabizon and Friedler 2014; Kumari and Yadav 2019). Thus, the ability to regulate protein oligomerization by introducing mutations in oligomerization interfaces or engaging a specific modulator should prove useful for studying the biological role of protein oligomerization of interest (Kumari and Yadav 2019). On the one hand, there is a variety of circumstances in which a process of protein folding is incomplete or interrupted, eventually leading to formation of pathological or pathogenic protein oligomers (Figure 3B). For instance, the development of cognitive disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) is typically accompanied by the formation and accumulation of amyloid fibrils in the patient's brain (Araki et al., 2019; Brown et al., 2020; Salahuddin et al., 2021). In this sense, artificial or natural modulators capable of inhibiting or reversing aberrant protein oligomerizations would be of great prophylactic or therapeutic measures to treat relevant diseases (Galzitskaya 2019; Lee et al., 2017). Above all, understanding the underlying mechanisms of protein misfolding is one of the primary steps for the development of novel therapeutic strategies. In the study using HDMS by Stephens et al., the unstructured amyloid protein, α-synuclein (aSyn) involved in PD, exhibited the highest solvent protection at the C-terminus, indicative of a fold at the C-terminal domain that had been presumed to play a role in modulating aggregation but without structural evidences (Stephens et al., 2018).

Aggregation of tau into the paired helical filament (PHF) is a characteristic feature of AD, and the way Cys-mediated disulfide bond is formed, either intermolecularly or intramolecularly, serves as a critical element in tau fibrillation. To understand a molecular feature of aggregation-resistance of tau conformer, Jebarupa et al. synthesized the intramolecular Cys cross-linked tau monomer by oxidation and analyzed the rearranged conformational dynamics by HDMS. As a result, they found that, due to induced intramolecular H-bonding, the oxidized tau exhibited increased conformational rigidity and reduced accessibility in the core of the oligo-inducing interface that otherwise would have made an intermolecular H-bonded β-sheet formation and subsequently tau fibrillation (Jebarupa et al., 2019). Other than the disulfide bond, aggregation propensity of tau was investigated in the context of its extent of phosphorylation by Zhu et al. Time-resolved electrospray ionization (TRESI) mass spectrometry in combination with hydrogen/deuterium exchange (TRESI-HDX) is responsive to dynamic, temporary, and weak hydrogen bond interactions, as well as solvent accessibility, both of which are influenced by residual structure — biases in their native conformational ensembles of intrinsically disordered proteins. Authors used TRESI-HDX to characterize the native structural ensembles of a full-length tau, one of the main amyloidogenic species in AD, offering a detailed picture of the conformational changes that occur upon hyperphosphorylation by a kinase GSK-3β (Zhu et al., 2015). Increased deuterium uptake of the hexapeptide motif (H2) of hyperphosphorylated tau sampled appropriately such that aggregates were not significantly populated at this time pointed to a dominant role for H2 in GSK-3β-mediated increases in tau amyloidogenic propensity, consistent with the conclusion of a previously reported loss-of-function mutagenesis study (von Bergen et al., 2000).

Aggregation-prone apolipoprotein E4 (ApoE4) is a major risk factor for AD and cardiovascular diseases. Huang et al. coupled HDMS to gas-phase electron-transfer dissociation fragmentation (Zehl et al., 2008) to achieve single amino acid resolution and specify residues responsible for self-association of ApoE4. Despite the lack of a determined crystal structure as a reference due to a high tendency of ApoE4 to aggregate, the method could tune the analytic concentrations appropriately and identify 15 residues in the C-terminal domain deemed critically situated in ApoE4 oligomerization interface (Huang et al., 2011).

Amyloid fibrils formed by β-2-microglobulin (β2m) are an inevitable symptom of kidney failure-induced dialysis in patients' joints. Borotto et al. used HDMS to reveal a few structural insights into metal-induced amyloid development of β2m. They reported that the Cu(II) binding to Asp59 is required for the formation

of amyloid-competent dimers, as well as cis-trans isomerization of the His31-Pro32 amide bond essential for the formation of the amyloidogenic conformer. In contrast, Ni(II) only binds to His31 and does not cause structural changes favorable for producing oligomers or amyloids. Interestingly, the dimer formation was observed in response to Zn(II) binding in a similar fashion with Cu(II)-induced β2m dimerization, but did not lead to the pathway to the amyloid. Focused investigation into the dimer interfaces by HDMS found out the Zn(II)-induced dimer interface quite different from the Cu(II)-induced dimer interface in terms of stability and local interfacial areas involved (Borotto et al., 2017). Abovementioned and related studies are summarized in Table 2.

3.3. Protein-lipid interface

Membrane proteins are notoriously insusceptible to high-purity preparation for structural analyses due to their lipophilic nature and propensity to deformation when not in contact with membranes. Detergent micelles and vesicles are usually employed as standard formulations to isolate membrane proteins in a stable and functional state, thus allowing HDMS to probe conformational dynamics and molecular interactions of membranes proteins. Readers are referred to recent review articles that discussed the accomplishments of HDMS in structural biology of membrane proteins (Kaiser and Coin 2020; Martens and Politis 2020). Here we focus on the role of HDMS in exploring a heterogeneous interface between a membrane protein and phospholipids surrounding it. Hydrogen-deuterium exchange analysis is one of the least invasive methods that requires neither reactive chemicals nor harsh conditions, and thus readily operational under any preparative condition optimized for specific membrane proteins that behave in a reversible (peripheral protein) or permanent (integral protein) manner (Figure 3C). Moreover, neither high sample purity nor large sample quantity is essential for HDMS owing to recent advances in instrumentation and data processing software (Martens et al., 2019).

The power of HDMS, free of labeling or crosslinking, was well utilized in the study of transmembrane regions within an integral membrane protein. A GPCR model, β_2-adrenergic receptor (β_2AR) was prepared in micelles for HDMS analyses, and the overall HDMS profile showed that the transmembrane regions surrounded by lipids was much lower in deuterium exchange than the exposed regions, correlating well with the predicted seven-transmembrane structure of GPCRs (Duc et al., 2015). In another study, transporter proteins prepared in nanodiscs with different lipid compositions were analyzed by HDMS for changes in conformational equilibrium (Martens et al., 2018). It was found out that the charge-conserved, lipid-contacting interfaces of transporters were responsible for the conformational shift whose equilibrium was significantly affected by the lipid composition.

Tumor suppressor phosphatase and tensin homolog (PTEN) interacts with cell membranes in a switchable manner depending on dynamic conformational ensembles affected by phosphorylation (Jang et al., 2021). In addition to active dynamics, PTEN possesses intrinsically disordered tails at both termini which are considered important as membrane binding elements but not crystallizable. HDMS could unveil a novel mechanism of membrane interaction by PTEN, a behavior specifically driven by the membrane-binding interface at the N-terminal tail in which the extent of deuterium exchange was significantly dependent upon co-incubation of the lipid vesicles interacting with PTEN (Masson et al., 2016). Similarly, HDMS was employed to map the interface of sphingosine kinase 1 (SK1) and membrane vesicles. SK1 is an enzyme that catalyzes the conversion of sphingosine in membranes to sphingosine-1-phosphate (S1P). The study identified a positively charged motif on SK1 responsible for electrostatic interactions with membranes, and further demonstrated a contiguous interface, comprising an electrostatic site and a hydrophobic site, that interacted with membrane-associated anionic phospholipids (Pulkoski-Gross et al., 2018). More recently, a 390-kDa heterotetrameric lipid kinase Vps34 examined by HDMS has revealed its so-called 'aromatic finger' that interacts directly with lipid membranes and regulates the catalytic activity. A decreased rate of deuterium exchange in the finger region in the presence of lipid vesicles served as a signature for its defined role. Abovementioned and related studies are summarized in Table 2.

4. Probing biological interfaces by integrative approaches

While CLMS and HDMS may stand alone as the techniques to map the biological interface, their integration aided by computational modeling with the dataset obtained from X-ray crystallography, EM, or NMR can significantly improve spatial resolution and coverage of binding interfaces to provide comprehensive illustrations. For example, Zhang et al. first implemented CLMS and HDMS together to extract previously unknown information on the epitope and paratope interface of a programmed cell death-1 (PD-1) and the corresponding antagonistic antibody. In the next round of refinement, the suggested critical binding residues and distance restraints were utilized to build high-confidence binding models through molecular docking onto the crystal structure (Zhang et al., 2020). While the epitope-paratope relationships revealed by the approach were generally comparable with those assigned by the crystal structure, one cryptic loop in PD-1 that had not been crystallographically resolved due to its flexible nature was found to be a non-epitope. The study demonstrated a complementary role of CLMS and HDMS for accurate and detailed examination of a binding interface. The same research group described a similar integrated approach combining CLMS, HDMS, and molecular docking to probe the binding interface of interleukin 7 (IL-7) complexed with its receptor IL-7Rα. While the predicted model was generally in accordance with the crystal structure, the approach newly discovered the C-terminal binding region of IL-7, highlighting the value of integrative approaches to obtain a high-confidence structural model (Zhang et al., 2019).

Interpretation of HDMS data using a predetermined crystal structure as a template provides greater insight into reversible changes in regional flexibility of binding interfaces. A junctional epitope antibody VHH6, specifically recognizing a neo-epitope created only at the junction in which IL-6 and gp80 are interlocked, was considered a molecular clamp as shown in the ternary crystal structure. To understand the effect of VHH6 clamping on the structural flexibility of the junctional interface of IL-6-gp80, Adams et al. compared the amounts of deuterium exchange therein in the presence or absence of VHH6 (Adams et al., 2017). The presence of VHH6 increased the rigidity in the local region spanning the junction, stabilizing a transient interface between IL-6 and gp80.

Likewise, advances in CLMS techniques are better refining a low-resolution interface solved by other methods into medium- to high-resolution details. By applying multiple orthogonal crosslinking chemistries to a target protein complex, Mintseris and Gygi could attain a higher crosslinking density and improved sequence coverage (Mintseris and Gygi 2020). Self-consistent analytic results could be mapped onto cryo-EM models to define the interaction interface with high resolution and confidence.

A dramatic contribution of HDMS in harmony with CLMS to modeling the interface of an unstable macromolecular complex was presented by Shuka et al (Shukla et al., 2014). While the human $\beta_2$adrenergic receptor (β_2AR) and β-arrestin-1 had crystal structures reported individually, the β_2AR-β-arrestin-1 complex was intolerant to experimental conditions in X-ray crystallography or EM with only low-resolution map for the overall conformation available. Remarkably, constraints provided by HDMS and CLMS were mapped onto the preexisting data in an integrative manner, resulting in the three-dimensional reconstruction of the complex that displayed an unexpectedly extensive interface and a crucial involvement of the finger loop in β-arrestin-1 in the complex formation. More recently, such a combined approach investigated the mechanism of client binding of the periplasmic chaperone SurA and identified dynamic inter-domain interfaces that underwent substantial structural reorganization in response to the substrate binding interface being occupied by a client OmpX (Calabrese et al., 2020).

5. Conclusions and Perspectives

Efforts to map a proteome-wide PPI network have made significant contributions to our basic understanding of biological systems (Rual et al., 2005). Scientists increasingly have deeper and comprehensive insights into aberrant PPIs occurring in various pathological conditions to strategically come up with effective therapeutic avenues (Ryan and Matthews 2005). To this end, rational design of a potent modulator that best counteracts abnormal PPIs would require detailed knowledge on a binding interface of PPI with high fidelity comprising not only atomic resolution but also “reality” existing in native environments. CLMS and HDMS discussed herein are not competent enough for producing atomic resolution of an interface comparable to that by X-ray crystallography and EM. However, in terms of reality, they are especially useful in extracting

structural information in biological, native environment including contact residues at interfaces, inter-residue distances, peptide-level solvent accessibilities, and temporal dynamics of interfacial areas, from which refined and near-physiological interfacial landscape can be remodeled preferably using a preexisting structure as a template. Exciting computational advances in data processing and structural biology are greatly improving the fidelity of integration of data obtained from both convention methods and CLMS/HDMS. High-fidelity information of an interface thus acquired would serve as an excellent cornerstone, increasing the likelihood of successful rational drug discovery. Designer agonists or antagonists that recognize hot residues in an interface responsible for a targeted PPI in a specific pathological condition are expected to outperform conventional binders with regard to both potency and efficacy.

Limitations of CLMS and HDMS remain to be overcome in future advances. Specifically, CLMS users often suffer from excessive crosslinking condition optimization and non-specific reactions that would compromise precise measurement of spatial distances. Back exchange of deuterium in HDMS hampers the reliable interpretation of solvent accessibility dataset, necessitating effective sample treatment protocols by tricky trial and error approach. Developments of new crosslinkers with residue-specificity, bifunctionality, and/or reduced background mass signals are expected to address the current challenges of CLMS (Ding et al., 2016; Leitner et al., 2014; Lössl et al., 2014; Schneider et al., 2018). Developments of improved workflows in HDMS should prove to be innovative in the coming era (Hamuro and Zhang 2019; Lau et al., 2019; Oganesyan et al., 2018). Together with novel MS instrumentation and computational data processing techniques, all the efforts will continue to advance CLMS and HDMS as excellent polishing tools for high-fidelity structural determination of protein complexes and binding interfaces thereof, thereby fueling the growth of structural proteomics and biology, and, further, the advent of structure-based drug design regime with exceptional reliability and probability (Figure 4).

Declaration of Competing Interests

The authors declare that they have no competing interests that can influence the work reported in this review article.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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References

Adams, R., Burnley, R. J., Valenzano, C. R., Qureshi, O., Doyle, C., Lumb, S., Del Carmen Lopez, M., Griffin, R., McMillan, D., Taylor, R. D., Meier, C., Mori, P., Griffin, L. M., Wernery, U., Kinne, J., Rapecki, S., Baker, T. S., Lawson, A. D., Wright, M., Ettorre, A., 2017, Discovery of a junctional epitope antibody that stabilizes IL-6 and gp80 protein:protein interaction and modulates its downstream signaling,*Scientific Reports* .7:37716.

Adhikari, J., Zhao, H., Fernandez, E., Huang, Y., Diamond, M. S., Fremont, D. H., Gross, M. L., 2021, Hydrogen–deuterium exchange mass spectrometry identifies spatially distinct antibody epitopes on domain III of the Zika virus envelope protein, *Journal of Mass Spectrometry* .56(1):e4685.

Anderson, K. W., Gallagher, E. S., Hudgens, J. W., 2018, Automated Removal of Phospholipids from Membrane Proteins for H/D Exchange Mass Spectrometry Workflows, *Analytical Chemistry* .90(11):6409-6412.

Araki, K., Yagi, N., Aoyama, K., Choong, C. J., Hayakawa, H., Fujimura, H., Nagai, Y., Goto, Y., Mochizuki, H., 2019, Parkinson's disease is a type of amyloidosis featuring accumulation of amyloid fibrils of α-synuclein, *Proceedings of the National Academy of Sciences* .116(36):17963-17969.

Arkin, M. R., Tang, Y., Wells, J. A., 2014, Small-molecule inhibitors of protein-protein interactions: progressing toward the reality, *Cell Chemical Biology* .21(9):1102-1114.

Arlt, C., Flegler, V., Ihling, C. H., Schäfer, M., Thondorf, I., Sinz, A., 2017, An Integrated Mass Spectrometry Based Approach to Probe the Structure of the Full-Length Wild-Type Tetrameric p53 Tumor Suppressor,*Angewandte Chemie International Edition* .56(1):275-279.

Arlt, C., Ihling, C. H., Sinz, A., 2015, Structure of full-length p53 tumor suppressor probed by chemical cross-linking and mass spectrometry,*Proteomics* .15(16):2746-2755.

Arora, J., Hickey, J. M., Majumdar, R., Esfandiary, R., Bishop, S. M., Samra, H. S., Middaugh, C. R., Weis, D. D., Volkin, D. B., 2015, Hydrogen exchange mass spectrometry reveals protein interfaces and distant dynamic coupling effects during the reversible self-association of an IgG1 monoclonal antibody, *MAbs* .7(3):525-539.

Beard, H. A., Korovesis, D., Chen, S., Verhelst, S. H. L., 2021, Cleavable linkers and their application in MS-based target identification, *Molecular Omics* .17(2):197-209.

Borotto, N. B., Zhang, Z., Dong, J., Burant, B., Vachet, R. W., 2017, Increased β-Sheet Dynamics and D-E Loop Repositioning Are Necessary for Cu(II)-Induced Amyloid Formation by β-2-Microglobulin,*Biochemistry* .56(8):1095-1104.

Bridge, T., Shaikh, S. A., Thomas, P., Botta, J., McCormick, P. J., Sachdeva, A., 2019, Site-Specific Encoding of Photoactivity in Antibodies Enables Light-Mediated Antibody-Antigen Binding on Live Cells, *Angewandte Chemie International Edition* .58(50):17986-17993.

Brown, M. R., Radford, S. E., Hewitt, E. W., 2020, Modulation of β-Amyloid Fibril Formation in Alzheimer's Disease by Microglia and Infection, *Frontiers in Molecular Neuroscience* .13:609073.

Calabrese, A. N., Schiffrin, B., Watson, M., Karamanos, T. K., Walko, M., Humes, J. R., Horne, J. E., White, P., Wilson, A. J., Kalli, A. C., Tuma, R., Ashcroft, A. E., Brockwell, D. J., Radford, S. E., 2020, Inter-domain dynamics in the chaperone SurA and multi-site binding to its outer membrane protein clients, *Nature Communications* .11(1):2155.

Chavez, J. D., Mohr, J. P., Mathay, M., Zhong, X., Keller, A., Bruce, J. E., 2019, Systems structural biology measurements by in vivo cross-linking with mass spectrometry, *Nature Protocols* .14(8):2318-2343.

Chen, L., Morrow, J. K., Tran, H. T., Phatak, S. S., Du-Cuny, L., Zhang, S., 2012, From laptop to benchtop to bedside: structure-based drug design on protein targets, *Current Pharmaceutical Design* .18(9):1217-1239.

Chen, L., Zhu, C., Guo, H., Li, R., Zhang, L., Xing, Z., Song, Y., Zhang, Z., Wang, F., Liu, X., Zhang, Y., Ma, R. Z., Wang, F., 2020, Epitope-directed antibody selection by site-specific photocrosslinking,*Science Advances* .6(14):eaaz7825.

Coales, S. J., Tuske, S. J., Tomasso, J. C., Hamuro, Y., 2009, Epitope mapping by amide hydrogen/deuterium exchange coupled with immobilization of antibody, on-line proteolysis, liquid chromatography and mass spectrometry, *Rapid Communications in Mass Spectrometry* .23(5):639-647.

Comamala, G., Wagner, C., de la Torre, P. S., Jakobsen, R. U., Hilger, M., Brouwer, H.-J., Rand, K. D., 2020, Hydrogen/deuterium exchange mass spectrometry with improved electrochemical reduction enables comprehensive epitope mapping of a therapeutic antibody to the cysteine-knot containing vascular endothelial growth factor,*Analytica Chimica Acta* .1115:41-51.

Cvicek, V., Goddard, W. A., 3rd, Abrol, R., 2016, Structure-Based Sequence Alignment of the Transmembrane Domains of All Human GPCRs: Phylogenetic, Structural and Functional Implications, *PLOS Computational Biology* .12(3):e1004805.

Ding, Y. H., Fan, S. B., Li, S., Feng, B. Y., Gao, N., Ye, K., He, S. M., Dong, M. Q., 2016, Increasing the Depth of Mass-Spectrometry-Based Structural Analysis of Protein Complexes through the Use of Multiple

Cross-Linkers, *Analytical Chemistry* .88(8):4461-4469.

Duc, N. M., Du, Y., Zhang, C., Lee, S. Y., Thorsen, T. S., Kobilka, B. K., Chung, K. Y., 2015, Effective application of bicelles for conformational analysis of G protein-coupled receptors by hydrogen/deuterium exchange mass spectrometry, *Journal of the American Society for Mass Spectrometry* .26(5):808-817.

Dysinger, M., King, L. E., 2012, Practical quantitative and kinetic applications of bio-layer interferometry for toxicokinetic analysis of a monoclonal antibody therapeutic, *Journal of Immunological Methods* .379(1-2):30-41.

Ferraro, N. A., Cascio, M., 2018, Cross-Linking-Mass Spectrometry Studies of Cholesterol Interactions with Human α1 Glycine Receptor,*Analytical Chemistry* .90(4):2508-2516.

Freinkman, E., Chng, S. S., Kahne, D., 2011, The complex that inserts lipopolysaccharide into the bacterial outer membrane forms a two-protein plug-and-barrel, *Proceedings of the National Academy of Sciences* .108(6):2486-2491.

Gabizon, R., Friedler, A., 2014, Allosteric modulation of protein oligomerization: an emerging approach to drug design, *Frontiers in Chemistry* .2:9.

Gagnon, L., Cao, Y., Cho, A., Sedki, D., Huber, T., Sakmar, T. P., Laporte, S. A., 2019, Genetic code expansion and photocross-linking identify different β-arrestin binding modes to the angiotensin II type 1 receptor, *Journal of Biological Chemistry* .294(46):17409-17420.

Galzitskaya, O. V., 2019, Oligomers Are Promising Targets for Drug Development in the Treatment of Proteinopathies, *Frontiers in Molecular Neuroscience* .12:319.

Gershoni, J. M., Roitburd-Berman, A., Siman-Tov, D. D., Tarnovitski Freund, N., Weiss, Y., 2007, Epitope mapping: the first step in developing epitope-based vaccines, *BioDrugs* .21(3):145-156.

Gong, Z., Ye, S. X., Tang, C., 2020, Tightening the Crosslinking Distance Restraints for Better Resolution of Protein Structure and Dynamics, *Structure* .28(10):1160-1167.e1163.

Götze, M., Iacobucci, C., Ihling, C. H., Sinz, A., 2019, A Simple Cross-Linking/Mass Spectrometry Workflow for Studying System-wide Protein Interactions, *Analytical Chemistry* .91(15):10236-10244.

Gramlich, M., Hays, H. C. W., Crichton, S., Kaiser, P. D., Heine, A., Schneiderhan-Marra, N., Rothbauer, U., Stoll, D., Maier, S., Zeck, A., 2021, HDX-MS for Epitope Characterization of a Therapeutic ANTIBODY Candidate on the Calcium-Binding Protein Annexin-A1, *Antibodies (Basel)* .10(1).

Grunbeck, A., Huber, T., Abrol, R., Trzaskowski, B., Goddard, W. A., 3rd, Sakmar, T. P., 2012, Genetically encoded photo-cross-linkers map the binding site of an allosteric drug on a G protein-coupled receptor,*ACS Chemical Biology* .7(6):967-972.

Grunbeck, A., Huber, T., Sachdev, P., Sakmar, T. P., 2011, Mapping the ligand-binding site on a G protein-coupled receptor (GPCR) using genetically encoded photocrosslinkers,*Biochemistry* .50(17):3411-3413.

Guan, X., Noble, K. A., Tao, Y., Roux, K. H., Sathe, S. K., Young, N. L., Marshall, A. G., 2015, Epitope mapping of 7S cashew antigen in complex with antibody by solution-phase H/D exchange monitored by FT-ICR mass spectrometry, *Journal of Mass Spectrometry* .50(6):812-819.

Hamuro, Y., Zhang, T., 2019, High-Resolution HDX-MS of Cytochrome c Using Pepsin/Fungal Protease Type XIII Mixed Bed Column, *Journal of the American Society for Mass Spectrometry* .30(2):227-234.

Hauseman, Z. J., Harvey, E. P., Newman, C. E., Wales, T. E., Bucci, J. C., Mintseris, J., Schweppe, D. K., David, L., Fan, L., Cohen, D. T., Herce, H. D., Mourtada, R., Ben-Nun, Y., Bloch, N. B., Hansen, S. B., Wu, H., Gygi, S. P., Engen, J. R., Walensky, L. D., 2020, Homogeneous Oligomers of Pro-apoptotic BAX Reveal Structural Determinants of Mitochondrial Membrane Permeabilization, *Molecular Cell* .79(1):68-83.e67.

He, D., Xie, X., Yang, F., Zhang, H., Su, H., Ge, Y., Song, H., Chen, P. R., 2017, Quantitative and Comparative Profiling of Protease Substrates through a Genetically Encoded Multifunctional Photocrosslinker,*Angewandte Chemie International Edition* .56(46):14521-14525.

Holding, A. N., 2015, XL-MS: Protein cross-linking coupled with mass spectrometry, *Methods* .89:54-63.

Hollingsworth, L. R., Sharif, H., Griswold, A. R., Fontana, P., Mintseris, J., Dagbay, K. B., Paulo, J. A., Gygi, S. P., Bachovchin, D. A., Wu, H., 2021, DPP9 sequesters the C terminus of NLRP1 to repress inflammasome activation, *Nature* .592(7856):778-783.

Horne, J. E., Walko, M., Calabrese, A. N., Levenstein, M. A., Brockwell, D. J., Kapur, N., Wilson, A. J., Radford, S. E., 2018, Rapid Mapping of Protein Interactions Using Tag-Transfer Photocrosslinkers,*Angewandte Chemie International Edition* .57(51):16688-16692.

Huang, R. Y., Garai, K., Frieden, C., Gross, M. L., 2011, Hydrogen/deuterium exchange and electron-transfer dissociation mass spectrometry determine the interface and dynamics of apolipoprotein E oligomerization, *Biochemistry* .50(43):9273-9282.

Huang, R. Y., Krystek, S. R., Jr., Felix, N., Graziano, R. F., Srinivasan, M., Pashine, A., Chen, G., 2018, Hydrogen/deuterium exchange mass spectrometry and computational modeling reveal a discontinuous epitope of an antibody/TL1A Interaction, *MAbs* .10(1):95-103.

Iacobucci, C., Götze, M., Ihling, C. H., Piotrowski, C., Arlt, C., Schäfer, M., Hage, C., Schmidt, R., Sinz, A., 2018, A cross-linking/mass spectrometry workflow based on MS-cleavable cross-linkers and the MeroX software for studying protein structures and protein–protein interactions, *Nature Protocols* .13(12):2864-2889.

Ihling, C. H., Springorum, P., Iacobucci, C., Hage, C., Götze, M., Schäfer, M., Sinz, A., 2020, The Isotope-Labeled, MS-Cleavable Cross-Linker Disuccinimidyl Dibutyric Urea for Improved Cross-Linking/Mass Spectrometry Studies, *Journal of the American Society for Mass Spectrometry* .31(2):183-189.

Iversen, R., Mysling, S., Hnida, K., Jørgensen Thomas, J. D., Sollid Ludvig, M., 2014, Activity-regulating structural changes and autoantibody epitopes in transglutaminase 2 assessed by hydrogen/deuterium exchange, *Proceedings of the National Academy of Sciences* .111(48):17146-17151.

Jang, H., Smith, I. N., Eng, C., Nussinov, R., 2021, The mechanism of full activation of tumor suppressor PTEN at the phosphoinositide-enriched membrane, *iScience* .24(5):102438.

Jebarupa, B., Mathew, B., Srinivasu, B. Y., Sasikumaran, A., Joseph, S., Mandal, A. K., Thomas, T., Mitra, G., 2019, Understanding molecular features of aggregation-resistant tau conformer using oxidized monomer,*Biochimica et Biophysica Acta* .1863(6):993-1005.

Jensen, P. F., Larraillet, V., Schlothauer, T., Kettenberger, H., Hilger, M., Rand, K. D., 2015, Investigating the interaction between the neonatal Fc receptor and monoclonal antibody variants by hydrogen/deuterium exchange mass spectrometry, *Molecular & Cellular Proteomics* .14(1):148-161.

Kaiser, A., Coin, I., 2020, Capturing Peptide-GPCR Interactions and Their Dynamics, *Molecules* .25(20).

Karagöz, G. E., Acosta-Alvear, D., Nguyen, H. T., Lee, C. P., Chu, F., Walter, P., 2017, An unfolded protein-induced conformational switch activates mammalian IRE1, *Elife* .6.

Kim, M., Sun, Z.-Y. J., Rand, K. D., Shi, X., Song, L., Cheng, Y., Fahmy, A. F., Majumdar, S., Ofek, G., Yang, Y., Kwong, P. D., Wang, J.-H., Engen, J. R., Wagner, G., Reinherz, E. L., 2011, Antibody mechanics on a membrane-bound HIV segment essential for GP41-targeted viral neutralization, *Nature Structural & Molecular Biology* .18(11):1235-1243.

Krist, D. T., Statsyuk, A. V., 2015, Catalytically Important Residues of E6AP Ubiquitin Ligase Identified Using Acid-Cleavable Photo-Cross-Linkers, *Biochemistry* .54(29):4411-4414.

Kumari, N., Yadav, S., 2019, Modulation of protein oligomerization: An overview, *Progress in Biophysics and Molecular Biology* .149:99-113.

Lau, A. M. C., Ahdash, Z., Martens, C., Politis, A., 2019, Deuteros: software for rapid analysis and visualization of data from differential hydrogen deuterium exchange-mass spectrometry,*Bioinformatics* .35(17):3171-3173.

Lee, S. J., Nam, E., Lee, H. J., Savelieff, M. G., Lim, M. H., 2017, Towards an understanding of amyloid-β oligomers: characterization, toxicity mechanisms, and inhibitors, *Chemical Society Reviews* .46(2):310-323.

Leitner, A., Joachimiak, L. A., Unverdorben, P., Walzthoeni, T., Frydman, J., Förster, F., Aebersold, R., 2014, Chemical cross-linking/mass spectrometry targeting acidic residues in proteins and protein complexes, *Proceedings of the National Academy of Sciences* .111(26):9455-9460.

Li, J., Wei, H., Krystek, S. R., Bond, D., Brender, T. M., Cohen, D., Feiner, J., Hamacher, N., Harshman, J., Huang, R. Y. C., Julien, S. H., Lin, Z., Moore, K., Mueller, L., Noriega, C., Sejwal, P., Sheppard, P., Stevens, B., Chen, G., Tymiak, A. A., Gross, M. L., Schneeweis, L. A., 2017, Mapping the Energetic Epitope of an Antibody/Interleukin-23 Interaction with Hydrogen/Deuterium Exchange, Fast Photochemical Oxidation of Proteins Mass Spectrometry, and Alanine Shave Mutagenesis,*Analytical Chemistry* .89(4):2250-2258.

Liang, Y. L., Belousoff, M. J., Zhao, P., Koole, C., Fletcher, M. M., Truong, T. T., Julita, V., Christopoulos, G., Xu, H. E., Zhang, Y., Khoshouei, M., Christopoulos, A., Danev, R., Sexton, P. M., Wootten, D., 2020, Toward a Structural Understanding of Class B GPCR Peptide Binding and Activation, *Molecular Cell* .77(3):656-668.e655.

Liu, L., 2018, Pharmacokinetics of monoclonal antibodies and Fc-fusion proteins, *Protein Cell* .9(1):15-32.

Liu, Q., Remmelzwaal, S., Heck, A. J. R., Akhmanova, A., Liu, F., 2017, Facilitating identification of minimal protein binding domains by cross-linking mass spectrometry, *Scientific Reports* .7(1):13453.

Lössl, P., Kölbel, K., Tänzler, D., Nannemann, D., Ihling, C. H., Keller, M. V., Schneider, M., Zaucke, F., Meiler, J., Sinz, A., 2014, Analysis of nidogen-1/laminin γ1 interaction by cross-linking, mass spectrometry, and computational modeling reveals multiple binding modes,*PLOS ONE* .9(11):e112886.

Ma, S., Shen, Q., Zhao, L. H., Mao, C., Zhou, X. E., Shen, D. D., de Waal, P. W., Bi, P., Li, C., Jiang, Y., Wang, M. W., Sexton, P. M., Wootten, D., Melcher, K., Zhang, Y., Xu, H. E., 2020, Molecular Basis for Hormone Recognition and Activation of Corticotropin-Releasing Factor Receptors, *Molecular Cell* .77(3):669-680.e664.

Martens, C., Politis, A., 2020, A glimpse into the molecular mechanism of integral membrane proteins through hydrogen-deuterium exchange mass spectrometry, *Protein Science* .29(6):1285-1301.

Martens, C., Shekhar, M., Borysik, A. J., Lau, A. M., Reading, E., Tajkhorshid, E., Booth, P. J., Politis, A., 2018, Direct protein-lipid interactions shape the conformational landscape of secondary transporters, *Nature Communications* .9(1):4151.

Martens, C., Shekhar, M., Lau, A. M., Tajkhorshid, E., Politis, A., 2019, Integrating hydrogen-deuterium exchange mass spectrometry with molecular dynamics simulations to probe lipid-modulated conformational changes in membrane proteins, *Nature Protocols* .14(11):3183-3204.

Masson, G. R., Burke, J. E., Ahn, N. G., Anand, G. S., Borchers, C., Brier, S., Bou-Assaf, G. M., Engen, J. R., Englander, S. W., Faber, J., Garlish, R., Griffin, P. R., Gross, M. L., Guttman, M., Hamuro, Y., Heck, A. J. R., Houde, D., Iacob, R. E., Jørgensen, T. J. D., Kaltashov, I. A., Klinman, J. P., Konermann, L., Man, P., Mayne, L., Pascal, B. D., Reichmann, D., Skehel, M., Snijder, J., Strutzenberg, T. S., Underbakke, E. S., Wagner, C., Wales, T. E., Walters, B. T., Weis, D. D., Wilson, D. J., Wintrode, P. L., Zhang, Z., Zheng, J., Schriemer, D. C., Rand, K. D., 2019, Recommendations for performing, interpreting and reporting hydrogen deuterium exchange mass spectrometry (HDX-MS) experiments,*Nature Methods* .16(7):595-602.

Masson, G. R., Jenkins, M. L., Burke, J. E., 2017, An overview of hydrogen deuterium exchange mass spectrometry (HDX-MS) in drug discovery, *Expert Opinion on Drug Discovery* .12(10):981-994.

Masson, G. R., Perisic, O., Burke, J. E., Williams, R. L., 2016, The intrinsically disordered tails of PTEN and PTEN-L have distinct roles in regulating substrate specificity and membrane activity, *Biochem J* .473(2):135-144.

Mintseris, J., Gygi, S. P., 2020, High-density chemical cross-linking for modeling protein interactions, *Proceedings of the National Academy of Sciences* .117(1):93-102.

Mishra, P. K., Yoo, C. M., Hong, E., Rhee, H. W., 2020, Photo-crosslinking: An Emerging Chemical Tool for Investigating Molecular Networks in Live Cells, *Chembiochem* .21(7):924-932.

Oganesyan, I., Lento, C., Wilson, D. J., 2018, Contemporary hydrogen deuterium exchange mass spectrometry, *Methods* .144:27-42.

Oganesyan, V., Damschroder, M. M., Cook, K. E., Li, Q., Gao, C., Wu, H., Dall'Acqua, W. F., 2014, Structural insights into neonatal Fc receptor-based recycling mechanisms, *Journal of Biological Chemistry* .289(11):7812-7824.

Owens, T. W., Taylor, R. J., Pahil, K. S., Bertani, B. R., Ruiz, N., Kruse, A. C., Kahne, D., 2019, Structural basis of unidirectional export of lipopolysaccharide to the cell surface,*Nature* .567(7749):550-553.

Ozohanics, O., Ambrus, A., 2020, Hydrogen-Deuterium Exchange Mass Spectrometry: A Novel Structural Biology Approach to Structure, Dynamics and Interactions of Proteins and Their Complexes, *Life (Basel)* .10(11).

Parker, B., Goncz, E., Krist, D. T., Statsyuk, A., Nesvizhskii, A. I., Weiss, E., 2018, LiF-MS: Mapping unstructured peptide-protein interactions using Ligand-Footprinting Mass Spectrometry,*bioRxiv* .361857.

Payandeh, J., Volgraf, M., 2021, Ligand binding at the protein-lipid interface: strategic considerations for drug design, *Nature Reviews Drug Discovery* .20(9):710-722.

Pham, N. D., Parker, R. B., Kohler, J. J., 2013, Photocrosslinking approaches to interactome mapping, *Current Opinion in Structural Biology* .17(1):90-101.

Pierce, M. M., Raman, C. S., Nall, B. T., 1999, Isothermal titration calorimetry of protein-protein interactions,*Methods* .19(2):213-221.

Piersimoni, L., Sinz, A., 2020, Cross-linking/mass spectrometry at the crossroads, *Analytical and Bioanalytical Chemistry* .412(24):5981-5987.

Przygońska, K., Poznański, J., Mistarz, U. H., Rand, K. D., Dadlez, M., 2018, Side-chain moieties from the N-terminal region of Aβ are Involved in an oligomer-stabilizing network of interactions, *PLOS ONE* .13(8):e0201761.

Puchades, C., Kűkrer, B., Diefenbach, O., Sneekes-Vriese, E., Juraszek, J., Koudstaal, W., Apetri, A., 2019, Epitope mapping of diverse influenza Hemagglutinin drug candidates using HDX-MS, *Scientific Reports* .9(1):4735.

Pulkoski-Gross, M. J., Jenkins, M. L., Truman, J. P., Salama, M. F., Clarke, C. J., Burke, J. E., Hannun, Y. A., Obeid, L. M., 2018, An intrinsic lipid-binding interface controls sphingosine kinase 1 function, *Journal of Lipid Research* .59(3):462-474.

Qi, H., Ma, M., Hu, C., Xu, Z. W., Wu, F. L., Wang, N., Lai, D. Y., Li, Y., Zhang, H., Jiang, H. W., Meng, Q. F., Guo, S., Kang, Y., Zhao, X., Li, H., Tao, S. C., 2021, Antibody Binding Epitope Mapping (AbMap) of Hundred Antibodies in a Single Run, *Molecular & Cellular Proteomics* .20:100059.

Rao, V. S., Srinivas, K., Sujini, G. N., Kumar, G. N., 2014, Protein-protein interaction detection: methods and analysis,*International Journal of Proteomics* .2014:147648.

Rappsilber, J., 2011, The beginning of a beautiful friendship: cross-linking/mass spectrometry and modelling of proteins and multi-protein complexes, *Journal of Structural Biology* .173(3):530-540.

Rich, R. L., Myszka, D. G., 2011, Survey of the 2009 commercial optical biosensor literature, *Journal of Molecular Recognition* .24(6):892-914.

Rual, J. F., Venkatesan, K., Hao, T., Hirozane-Kishikawa, T., Dricot, A., Li, N., Berriz, G. F., Gibbons, F. D., Dreze, M., Ayivi-Guedehoussou, N., Klitgord, N., Simon, C., Boxem, M., Milstein, S., Rosenberg, J., Goldberg, D. S., Zhang, L. V., Wong, S. L., Franklin, G., Li, S., Albala, J. S., Lim, J., Fraughton, C., Llamosas, E., Cevik, S., Bex, C., Lamesch, P., Sikorski, R. S., Vandenhaute, J., Zoghbi, H. Y., Smolyar, A., Bosak, S., Sequerra, R., Doucette-Stamm, L., Cusick, M. E., Hill, D. E., Roth, F. P., Vidal, M., 2005, Towards a proteome-scale map of the human protein-protein interaction network,*Nature* .437(7062):1173-1178.

Rubino, F. A., Mollo, A., Kumar, S., Butler, E. K., Ruiz, N., Walker, S., Kahne, D. E., 2020, Detection of Transport Intermediates in the Peptidoglycan Flippase MurJ Identifies Residues Essential for Conformational Cycling, *Journal of the American Chemical Society* .142(12):5482-5486.

Ryan, D. P., Matthews, J. M., 2005, Protein-protein interactions in human disease, *Current Opinion in Structural Biology* .15(4):441-446.

Salahuddin, P., Fatima, M. T., Uversky, V. N., Khan, R. H., Islam, Z., Furkan, M., 2021, The role of amyloids in Alzheimer's and Parkinson's diseases, *International Journal of Biological Macromolecules* .190:44-55.

Schneider, M., Belsom, A., Rappsilber, J., 2018, Protein Tertiary Structure by Crosslinking/Mass Spectrometry, *Trends in Biochemical Sciences* .43(3):157-169.

Schreiber, G., Protein–protein interaction interfaces and their functional implications, Protein–Protein Interaction Regulators2020.

Scott, D. E., Bayly, A. R., Abell, C., Skidmore, J., 2016, Small molecules, big targets: drug discovery faces the protein-protein interaction challenge, *Nature Reviews Drug Discovery* .15(8):533-550.

Seidel, L., Zarzycka, B., Zaidi, S. A., Katritch, V., Coin, I., 2017, Structural insight into the activation of a class B G-protein-coupled receptor by peptide hormones in live human cells, *Elife* .6.

Seidel, S. A., Dijkman, P. M., Lea, W. A., van den Bogaart, G., Jerabek-Willemsen, M., Lazic, A., Joseph, J. S., Srinivasan, P., Baaske, P., Simeonov, A., Katritch, I., Melo, F. A., Ladbury, J. E., Schreiber, G., Watts, A., Braun, D., Duhr, S., 2013, Microscale thermophoresis quantifies biomolecular interactions under previously challenging conditions, *Methods* .59(3):301-315.

Shin, G., Lim, S. I., 2020, Site-specific proximity ligation provides molecular insights into biologically relevant interfaces of protein-protein interaction, *Biochem Biophys Res Commun* .533(4):932-937.

Shukla, A. K., Westfield, G. H., Xiao, K., Reis, R. I., Huang, L.-Y., Tripathi-Shukla, P., Qian, J., Li, S., Blanc, A., Oleskie, A. N., Dosey, A. M., Su, M., Liang, C.-R., Gu, L.-L., Shan, J.-M., Chen, X., Hanna, R., Choi, M., Yao, X. J., Klink, B. U., Kahsai, A. W., Sidhu, S. S., Koide, S., Penczek, P. A., Kossiakoff, A. A., Woods Jr, V. L., Kobilka, B. K., Skiniotis, G., Lefkowitz, R. J., 2014, Visualization of arrestin recruitment by a G-protein-coupled receptor,*Nature* .512(7513):218-222.

Simms, J., Uddin, R., Sakmar, T. P., Gingell, J. J., Garelja, M. L., Hay, D. L., Brimble, M. A., Harris, P. W., Reynolds, C. A., Poyner, D. R., 2018, Photoaffinity Cross-Linking and Unnatural Amino Acid Mutagenesis Reveal Insights into Calcitonin Gene-Related Peptide Binding to the Calcitonin Receptor-like Receptor/Receptor Activity-Modifying Protein 1 (CLR/RAMP1) Complex, *Biochemistry* .57(32):4915-4922.

Ständer, S., L, R. G., Scarselli, M., Norais, N., Rand, K., 2021, Epitope Mapping of Polyclonal Antibodies by Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS), *Analytical Chemistry* .93(34):11669-11678.

Stephens, A. D., Nespovitaya, N., Zacharopoulou, M., Kaminski, C. F., Phillips, J. J., Kaminski Schierle, G. S., 2018, Different Structural Conformers of Monomeric α-Synuclein Identified after Lyophilizing and Freezing, *Analytical Chemistry* .90(11):6975-6983.

Suchanek, M., Radzikowska, A., Thiele, C., 2005, Photo-leucine and photo-methionine allow identification of protein-protein interactions in living cells, *Nature Methods* .2(4):261-268.

Sun, H., Ma, L., Wang, L., Xiao, P., Li, H., Zhou, M., Song, D., 2021, Research advances in hydrogen-deuterium exchange mass spectrometry for protein epitope mapping, *Analytical and Bioanalytical Chemistry* .413(9):2345-2359.

Uhrik, L., Hernychova, L., Muller, P., Kalathiya, U., Lisowska, M. M., Kocikowski, M., Parys, M., Faktor, J., Nekulova, M., Nortcliffe, C., Zatloukalova, P., Ruetgen, B., Fahraeus, R., Ball, K. L., Argyle, D. J., Vojtesek, B., Hupp, T. R., 2021, Hydrogen deuterium exchange mass spectrometry identifies the dominant paratope in CD20 antigen binding to the NCD1.2 monoclonal antibody, *Biochemical Journal* .478(1):99-120.

von Bergen, M., Friedhoff, P., Biernat, J., Heberle, J., Mandelkow, E. M., Mandelkow, E., 2000, Assembly of tau protein into Alzheimer paired helical filaments depends on a local sequence motif ((306)VQIVYK(311)) forming beta structure, *Proceedings of the National Academy of Sciences* .97(10):5129-5134.

Wang, X., Ni, D., Liu, Y., Lu, S., 2021, Rational Design of Peptide-Based Inhibitors Disrupting Protein-Protein Interactions,*Frontiers in Chemistry* .9:682675.

Whittaker, J., Whittaker, L. J., Roberts, C. T., Jr., Phillips, N. B., Ismail-Beigi, F., Lawrence, M. C., Weiss, M. A., 2012, α-Helical element at the hormone-binding surface of the insulin receptor functions as a signaling element to activate its tyrosine kinase, *Proceedings of the National Academy of Sciences* .109(28):11166-11171.

Wu, B., Peisley, A., Richards, C., Yao, H., Zeng, X., Lin, C., Chu, F., Walz, T., Hur, S., 2013, Structural Basis for dsRNA Recognition, Filament Formation, and Antiviral Signal Activation by MDA5,*Cell* .152(1):276-289.

Xie, Y., Chen, S., Li, Q., Sheng, Y., Alvarez, M. R., Reyes, J., Xu, G., Solakyildirim, K., Lebrilla, C. B., 2021, Glycan-protein cross-linking mass spectrometry reveals sialic acid-mediated protein networks on cell surfaces, *Chemical Science* .12(25):8767-8777.

Yadav, S., Sreedhara, A., Kanai, S., Liu, J., Lien, S., Lowman, H., Kalonia, D. S., Shire, S. J., 2011, Establishing a link between amino acid sequences and self-associating and viscoelastic behavior of two closely related monoclonal antibodies, *Pharmaceutical Research* .28(7):1750-1764.

Yang, T., Li, X., Li, X. D., 2020, A bifunctional amino acid to study protein–protein interactions, *RSC Advances* .10(69):42076-42083.

Yang, Y., Song, H., Chen, P. R., 2016, Genetically encoded photocrosslinkers for identifying and mapping protein-protein interactions in living cells, *IUBMB Life* .68(11):879-886.

Yang, Y., Song, H., He, D., Zhang, S., Dai, S., Lin, S., Meng, R., Wang, C., Chen, P. R., 2016, Genetically encoded protein photocrosslinker with a transferable mass spectrometry-identifiable label, *Nature Communications* .7:12299.

Yang, Y., Song, H., He, D., Zhang, S., Dai, S., Xie, X., Lin, S., Hao, Z., Zheng, H., Chen, P. R., 2017, Genetically encoded releasable photo-cross-linking strategies for studying protein-protein interactions in living cells, *Nature Protocols* .12(10):2147-2168.

Zehl, M., Rand, K. D., Jensen, O. N., Jørgensen, T. J., 2008, Electron transfer dissociation facilitates the measurement of deuterium incorporation into selectively labeled peptides with single residue resolution, *Journal of the American Chemical Society* .130(51):17453-17459.

Zhang, M. M., Beno, B. R., Huang, R. Y., Adhikari, J., Deyanova, E. G., Li, J., Chen, G., Gross, M. L., 2019, An Integrated Approach for Determining a Protein-Protein Binding Interface in Solution and an Evaluation of

Hydrogen-Deuterium Exchange Kinetics for Adjudicating Candidate Docking Models, *Analytical Chemistry* .91(24):15709-15717.

Zhang, M. M., Huang, R. Y., Beno, B. R., Deyanova, E. G., Li, J., Chen, G., Gross, M. L., 2020, Epitope and Paratope Mapping of PD-1/Nivolumab by Mass Spectrometry-Based Hydrogen-Deuterium Exchange, Cross-linking, and Molecular Docking, *Analytical Chemistry* .92(13):9086-9094.

Zhang, Q., Noble, K. A., Mao, Y., Young, N. L., Sathe, S. K., Roux, K. H., Marshall, A. G., 2013, Rapid Screening for Potential Epitopes Reactive with a Polycolonal Antibody by Solution-Phase H/D Exchange Monitored by FT-ICR Mass Spectrometry, *Journal of The American Society for Mass Spectrometry* .24(7):1016-1025.

Zhang, Q., Willison, L. N., Tripathi, P., Sathe, S. K., Roux, K. H., Emmett, M. R., Blakney, G. T., Zhang, H. M., Marshall, A. G., 2011, Epitope mapping of a 95 kDa antigen in complex with antibody by solution-phase amide backbone hydrogen/deuterium exchange monitored by Fourier transform ion cyclotron resonance mass spectrometry,*Analytical Chemistry* .83(18):7129-7136.

Zhang, Z., Zhang, A., Xiao, G., 2012, Improved protein hydrogen/deuterium exchange mass spectrometry platform with fully automated data processing, *Analytical Chemistry* .84(11):4942-4949.

Zhu, S., Liuni, P., Ettorre, L., Chen, T., Szeto, J., Carpick, B., James, D. A., Wilson, D. J., 2019, Hydrogen-Deuterium Exchange Epitope Mapping Reveals Distinct Neutralizing Mechanisms for Two Monoclonal Antibodies against Diphtheria Toxin, *Biochemistry* .58(6):646-656.

Zhu, S., Shala, A., Bezginov, A., Sljoka, A., Audette, G., Wilson, D. J., 2015, Hyperphosphorylation of intrinsically disordered tau protein induces an amyloidogenic shift in its conformational ensemble,*PLOS ONE* .10(3):e0120416.

Legends of Figures

Figure 1. Structural information obtainable from CLMS and HDMS. In CLMS, a crosslinking reagent makes a covalent, site-specific bridge between binding proteins. The crosslinked complex is subjected to mass spectrometry to provide contact residues or inter-residue distance. In HDMS, the hydrogen (H)-deuterium (D) exchange rate in amides of binding proteins varies depending on solvent accessibility. Relative to residues or areas in non-interfaces, those in binding interfaces generally exhibit lower D/H exchange, a mass change identifiable by mass spectrometry.

Figure 2. Crosslinking techniques employed in CLMS to unveil biological interfaces. (A) Photo-reactive unnatural amino acid (UAA) site-specifically incorporated into a predetermined position is irradiated by UV when proteins are interacting. The formation of a crosslink observed by gel electrophoresis or mass spectrometry indicates the UAA-incorporated site is within a potential binding interface. (B) A photo-activatable or chemical crosslinker with defined geometry and bifunctional, residue-specific reactivity forms a crosslink between interacting proteins. Mass analyses provides an abundance of inter-residue distance constraints surrounding interfacial regions.

Figure 3. Major protein complexes and their native interfaces investigated by HDMS. (A) Antibody-antigen complex. (B) Self-associated protein oligomers. (C) Membrane proteins associated with cell membranes.

Figure 4. Computer-aided, integrative structural modeling to provide near-physiological landscape of binding interfaces of proteins complexes with high resolution and fidelity. Design of PPI modulators based on biological interfaces thus obtained would allow new drug discovery with higher potency and efficacy.

Figure 1.

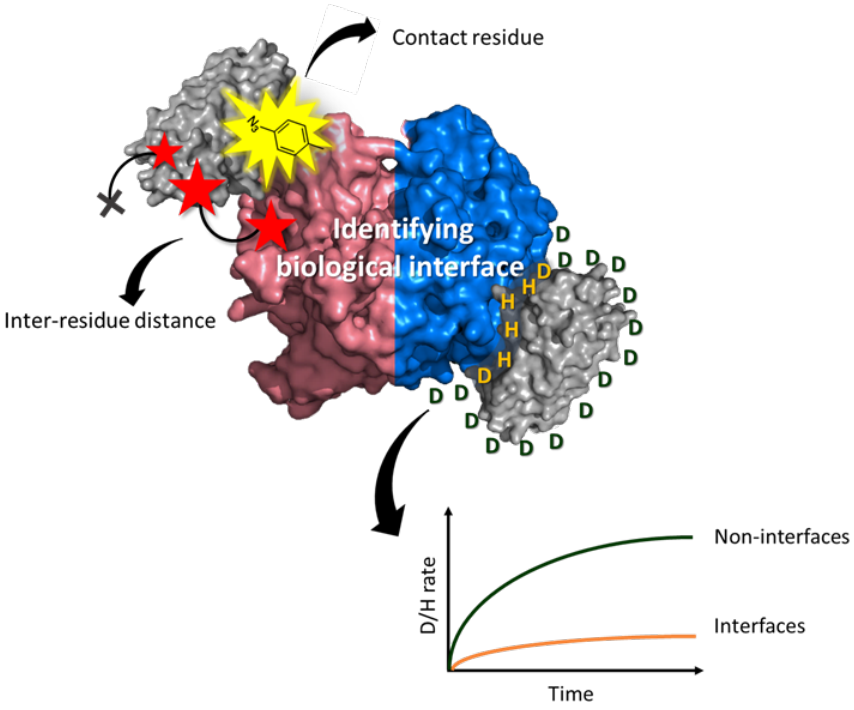

Figure 2.

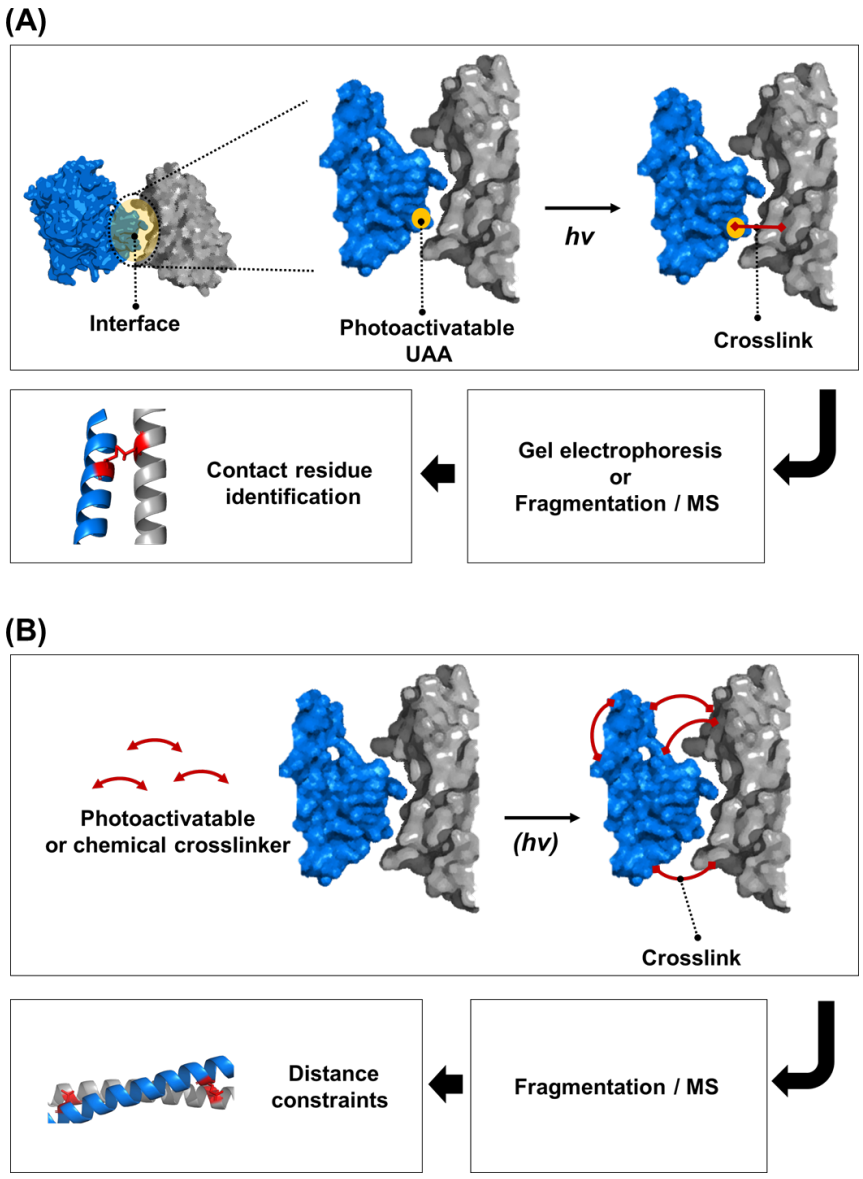

Figure 3.

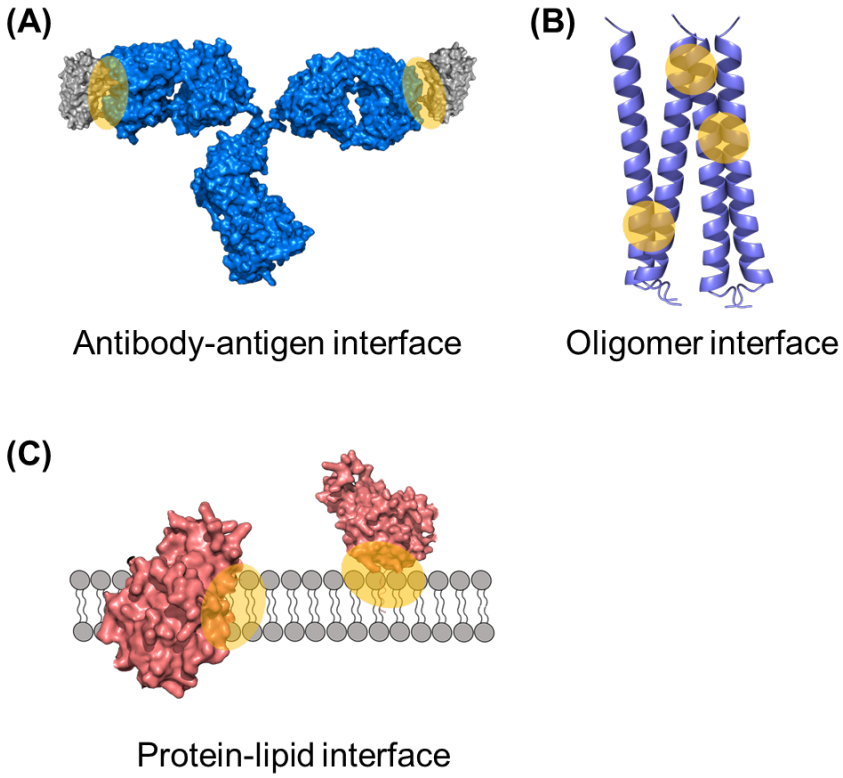

Figure 4.

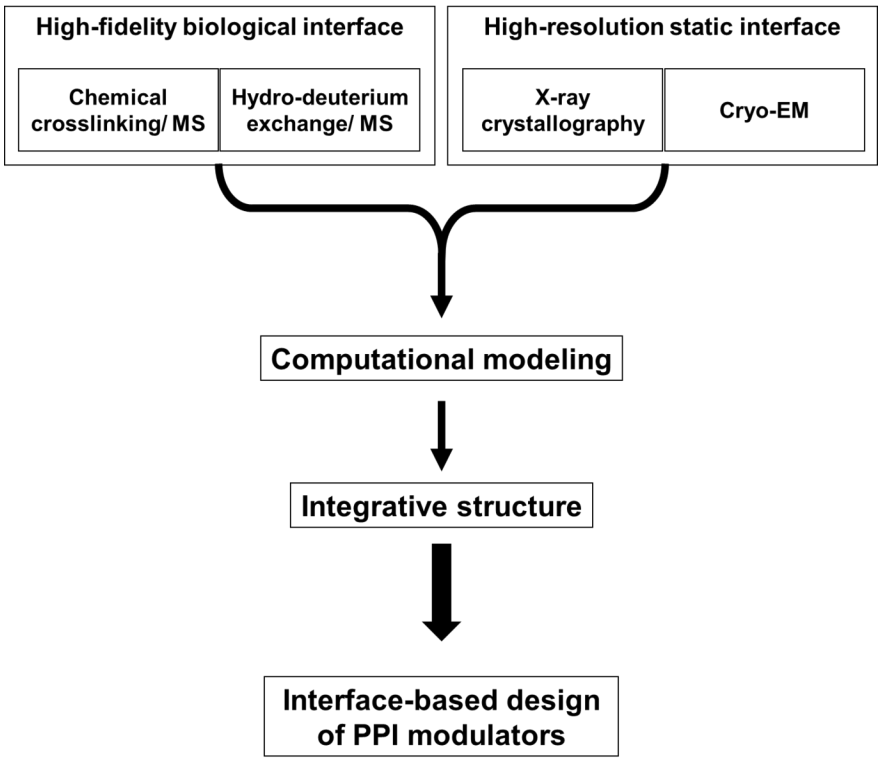

Table 1 . Examples of biological interfaces or interfacial residues uncovered by CLMS.

Crosslinking methods	Protein complex	Protein complex	Interaction	Findings	Ref.
Photo-crosslinking UAA in live cells (No MS involved)	CXCR4(GPCR)-T140	Protein- small molecule	Protein- small molecule	F189 is important for binding T140 ligand	(Grunbeck et al., 2011)
	CCR5(GPCR)-maraviroc	Protein- small molecule	Protein- small molecule	New contact residues identified	(Grunbeck et al., 2012)
	PGRMC1-Insig-1	Protein-protein	Protein-protein	New interfaces identified	(Suchanek et al., 2005)
	AT1R(GPCR)-arrestin	Protein-protein	Protein-protein	Ligand-dependent interface change, newly found intracellular loop engagement in the interface	(Gagnon et al., 2019)

Crosslinking methods	Protein complex	Protein complex	Interaction	Findings	Ref.
	Antibody-EGFR	Protein-protein	Protein-protein	Roles of interfacial residues in binding	(Bridge et al., 2019)
	MurJ-LipidII	Protein-lipid	Protein-lipid	Critical interfacial residues responsible for transient LipidII binding	(Rubino et al., 2020)
Photo-crosslinking UAA	LptD-LptE	Protein-protein	Protein-protein	Critical interfacial residues	(Freinkman et al., 2011)
	DegP-substrate	Protein-protein	Protein-protein	New substrates identified by a photoactivatable UAA at the substrate recognition site	(He et al., 2017)
	Histone H3-various ligands	Protein-protein	Protein-protein	Weak and transient PPIs identified	(Yang et al., 2020)
	HdeA dimer	Protein-protein	Protein-protein	pH-dependent changes in the dimer interface	(Yang et al., 2016)
	HdeA - DegP	Protein-protein	Protein-protein	HdeA binding to multiple interfacial regions in DegP	
	Rho-RTKN	Protein-protein	Protein-protein	RTKN interaction with RohA through a similar binding interface as that on PKN	
Photo-crosslinker, cleavable, MS label	MAP kinase - MKK4 peptide	Protein-peptide	Protein-peptide	Unique binding interface of an intrinsically disordered peptide within a folded protein domain	(Parker et al., 2018)

Crosslinking methods	Protein complex	Protein complex	Interaction	Findings	Ref.
	E6AP(E3) - UbcH7(E2)	Protein-protein	Protein-protein	Previously unrecognized catalytic residues of the E6AP ligase	(Krist and Statsyuk 2015)
Photo-crosslinker, heterobifunctional	SurA-Unfolded OmpX	Protein-protein	Protein-protein	Multi-site binding of OmpX to SurA	(Calabrese et al., 2020)
	Sky-OmpA	Protein-protein	Protein-protein	Unique residues and sub-regions involved in transient interaction between Skp and denatured OmpA	(Horne et al., 2018)
Photo-activatable cholesterol	GlyR - cholesterol	Protein-lipid	Protein-lipid	Unique cholesterol binding interfaces of GlyR Cholesterol concentration-dependent changes in the interface	(Ferraro and Cascio 2018)
Lys/Asp/Glu-cross-reactive chemical linker	Yeast proteasome	Protein-protein	Protein-protein	Dynamic and disordered interface of subunits (RPN2, RPN13)	(Mintseris and Gygi 2020)
	BAX oligomer	Protein-protein	Protein-protein	The unstructured N terminus and newly exposed α1 of BAX adjacent to one another in the oligomeric state	(Hauseman et al., 2020)
Heterobifunctional chemical crosslinker	RAP1B-Glycan	Protein-glycan	Protein-glycan	Different contact residues of RAP1B with glycan variants	(Xie et al., 2021)

Crosslinking methods	Protein complex	Protein complex	Interaction	Findings	Ref.
	MDA5 filament	Protein-protein	Protein-protein	Configuration of filaments	(Wu et al., 2013)
Amine-reactive chemical linker	p53 tetramer	Protein-protein	Protein-protein	DNA-dependent changes in the dimer-dimer interface	(Arlt et al., 2017)
	p53 tetramer	Protein-protein	Protein-protein	Flexible C-terminal	(Arlt et al., 2015)
	NLRP1-DPP9	Protein-protein	Protein-protein	CLMS data used for validation of the atomic model revealed by the cryo-EM	(Hollingsworth et al., 2021)
	SLAIN2, CLASP2, ch-TOG	Protein-protein	Protein-protein	SLAIN2 showed crosslink patterns contradictory to previous x-ray crystal structure	(Liu et al., 2017)
	hIRE1α LD oligomer	Protein-protein	Protein-protein	Critical residues in the oligomeric interface	(Karagöz et al., 2017)
Structure refinement from published dataset	Rpn2-Rpn13	Protein-protein	Protein-protein	The intrinsically disordered C-terminal interface of Rpn2	(Gong et al., 2020)

Table 2 . Examples of biological interfaces or interfacial residues uncovered by HDMS.

Type of interaction	Protein complex	Findings	Ref.
Antibody-antigen	Fab-GP41 MPER	Induction of conformational changes in both the MPER and paratope during the process of core epitope recognition	(Kim et al., 2011)
	IgG1-FcRn	Potential FcRn-binding interfaces in the Fab region	(Jensen et al., 2015)
	IgG4-TL1A	A discontinuous epitope within the predicted interaction interface of TL1A and DR3	(Huang et al., 2018)

Type of interaction	Protein complex	Findings	Ref.
	Fab-VEGF	The identified epitope overlapping with the binding interface of the biological receptor of VEGF	(Comamala et al., 2020)
	IgG-CD20 peptide	CDR3 (VH) functioning as the dominant antigen docking motif	(Uhrik et al., 2021)
	Fab-IL23	Peptide-level epitopes identified	(Li et al., 2017)
	Camelid single domain antibody-IL6/gp80 complex	The junctional epitope present only in the IL6/gp80 complex, not in the individual components	(Adams et al., 2017)
	IgG-fHbp	Four immunogenic regions located on the N- and C-termini of fHbp	(Ständer et al., 2021)
	IgG-transglutaminase 2	Main epitopes targeted by celiac disease autoantibodies	(Iversen et al., 2014)
	IgG-rAna o 2	Conformational and linear epitopes recognized respectively by two different antibodies	(Zhang et al., 2011)
	IgG-ANXA1	Interaction region within domain III of ANXA1 in Ca2+-bound conformation	(Gramlich et al., 2021)
	Antibody-Zika envelop	Three spatially distinct epitopes for the six mAbs	(Adhikari et al., 2021)
	Fab- cytochrome c	The epitope being in good agreement with the contact residues identified by the X-ray crystallographic structure	(Coales et al., 2009)
	IgG- rAna o 2	Five discontinuous, conformational epitopes	(Guan et al., 2015)
	IgG- rAna o 2	Four regions identified as potential epitopes	(Zhang et al., 2013)

Type of interaction	Protein complex	Findings	Ref.
	IgG1 self-association	Major protein-protein interfaces associated with the concentration-dependent self-oligomerization of IgG	(Arora et al., 2015)
Self-oligomer	Apolipoprotein E	Oligomerization via the C-terminal regions	(Huang et al., 2011)
	Tau	Hyperphosphorylation-induced interface formation in the microtubule binding repeat region	(Zhu et al., 2015)
	β-2-Microglobulin	The region spanned by Arg45-Leu54 serving as the interface for the dimer	(Borotto et al., 2017)
	α-synuclein	The C-terminus acting as a fold	(Stephens et al., 2018)
	Aβ	Sequence-specific engagement of side-chains of residues located at the N-terminal part in a network of oligomer-stabilizing interactions.	(Przygońska et al., 2018)
Protein-lipid	PTEN	The membrane-binding helix in the N-terminal of PTEN	(Masson et al., 2016)
	β2AR	The transmembrane domains embedded in bicellar region	(Duc et al., 2015)
	Homologous transporters: XylE, LacY and GlpT	Conserved networks of charged residues regulated by interactions with surrounding phospholipids, acting as molecular switches for the conformational transition	(Martens et al., 2018)
	PTEN	The salt bridge interactions between the basic residues and the lipids as a major driving force in stabilizing the protein-membrane interface	(Jang et al., 2021)

Type of interaction	Protein complex	Findings	Ref.
	Sphingosine kinase 1	A positively charged motif responsible for electrostatic interactions with membranes	(Pulkoski-Gross et al., 2018)

Graphical Abstract