Signed details of the excellence in research work for which the Sun Pharma Research Award is claimed, including references and illustrations. The candidate should duly sign on the details.

Research work for Sun Pharma Research Award 2021: A comprehensive technological platform for precision engineering of homogeneous antibody-fluorophore conjugates to assist directed image-guided tumor surgery. Besides, the substitution of fluorophore with a toxin to render novel antibody-drug conjugates for directed cancer chemotherapeutics.

Abstract: Is it even possible to imagine precision therapeutics with small molecules? The lack of answer to this question has inhibited the growth of covalent inhibitors while keeping the non-covalent inhibitors as a route of choice. Such an expedition would require a molecule to target a specific protein at a single site with absolute precision. The state-of-the-art drugs target at least 30-100 known and several unknown proteins. In such a scenario, *precision therapeutics generally depend on biologics*. In recent years, we developed chemical technologies enabling the synthesis of homogeneous antibody-drug conjugates for targeted cancer chemotherapeutics. However, we also realize that such routes could address only a few diseases. Besides, it would not be affordable for many patients across the world. *Hence, our ambition involves solving this mammoth challenge by developing chemical technologies for precision engineering of proteins.* Its success would require the reagent to selectively target a single-site in a single protein and regulate its activity favorably.

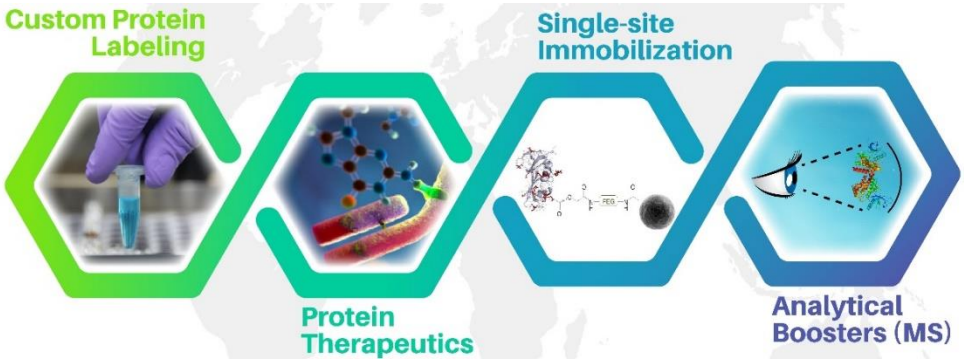

Figure 1: Combination of our technologies empowering (1) Directed therapeutics, (2) Antibody-drug conjugates, (3) Precision therapeutics by covalent inhibitors.

Summary: The chemical toolbox for enabling protein-based therapeutics requires the precise covalent attachment of tags to these biomolecules. In this perspective, we have been investing efforts to develop chemical technologies that can empower precise control over the site of bioconjugation. The critical barrier involves the simultaneous deconvolution of multiple challenges associated with reactivity and selectivity. *Due to these reasons, a large segment of the Scientific community believed this to be an unsolvable problem.*

In this perspective, we have developed a DisINtegrate or DIN theory that allows us to create new reactivity landscapes on a protein's surface. Overall, it enabled the development of methods for (a) utilization of N-terminus as a reactivity hotspot, (b) single-site tagging of Lys or His, (c) N-Gly residue-specific labelling (Gly-Tag®), and (d) modular linchpin directed modification (LDM®) for labelling single His or Lys residue. The LDM platform delivers single-site installation of various probes in native proteins. The user-friendly protocols result in analytically pure labelled proteins. The structure, enzymatic activity, binding to receptors, and downstream signalling pathways are typically unaffected. Importantly, these technologies allow access to homogeneous antibody-drug conjugates (ADCs) for directed cancer chemotherapeutics. Besides, we also developed Maspecter® MS sensitivity boosters for unambiguous characterization of the ADCs. The latter can solve the significant challenges associated with critical quality attributes and accelerate the ADCs towards IND and clinical stages.

Besides, understanding principles to regulate specificity at different levels of biological complexity empowers our current efforts to develop covalent inhibitors for identified kinase targets.

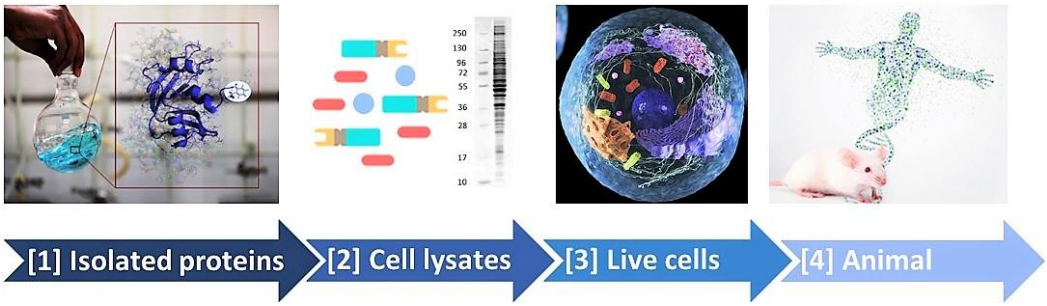

Figure 2: A systematic development of principles that can empower development of covalent inhibitors for precision therapeutics.

Our representative technological platforms empowering precision engineering of proteins

[1] LDM® platform

(For video abstract, see: https://www.youtube.com/watch?v=uWekVcL2yBU)

It is the first-ever modular chemical platform that enables chemoselective and site-selective labeling of native proteins. Initially, a reversible intermolecular reaction places the "chemical linchpins" globally on all the accessible Lys residues. These linchpins have the capability to drive site-selective covalent labeling of proteins. The linchpin detaches within physiological conditions and capacitates the late-stage installation of various tags. The chemical platform is modular, and the reagent design regulates the site of modification. The linchpin is a multitasking group and facilitates purification of the labeled protein eliminating the requirement of an additional chromatography tag. The methodology allows the labeling of a

single protein in a mixture of proteins. The precise modification of an accessible residue in protein ensures that their structure remains unaltered. The enzymatic activity of myoglobin, cytochrome C, aldolase, and lysozyme C remains conserved after labeling. Also, the cellular uptake of modified insulin and its downstream signaling process remain unperturbed. The linchpin directed modification (LDM) provides a convenient route for the conjugation of a fluorophore and drug to a Fab and monoclonal antibody. It delivers trastuzumab-doxorubicin and trastuzumab-emtansine conjugates with selective antiproliferative activity toward Her-2 positive SKBR-3 breast cancer cells. (For details, see: *J. Am. Chem. Soc.* **2018**, *140*, 15114-15123)

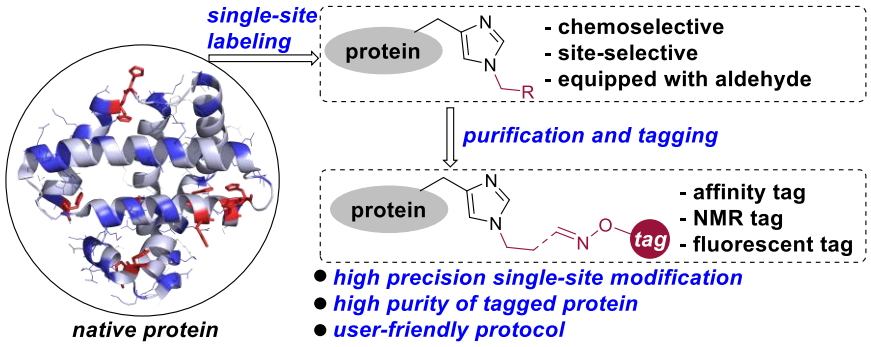

Figure 3: $LDM_{K\text{-}H}$ provides the first ever modular technology for single-site modification of a His residue in proteins, enzymes, and antibodies.

Subsequently, we extended the LDM platform's reach by developing a method that enables single-site labeling of a high-frequency Lys residue in the native proteins. At first, the enabling reagent forms stabilized imines with multiple solvent-accessible Lys residues chemoselectively. These linchpins create the opportunity to regulate the position of a second Lys-selective electrophile connected by a spacer. Consequently, it enables the irreversible single-site labeling of a Lys residue independent of its place in the reactivity order. The user-friendly protocol involves a series of steps to deconvolute and address chemoselectivity, site-selectivity, and modularity. Also, it delivers ordered immobilization and analytically pure probe-tagged proteins. The methodology also provides access to antibody-drug conjugate (ADC), which exhibits highly selective anti-proliferative activity towards HER-2 expressing SKBR-3 breast cancer cells. (For details, see: *Angew. Chem. Int. Ed.* **2020**, *59*, 10332-10336; for His and Asp, see *Chem. Sci.* **2021**, *12*, 6732-6736)

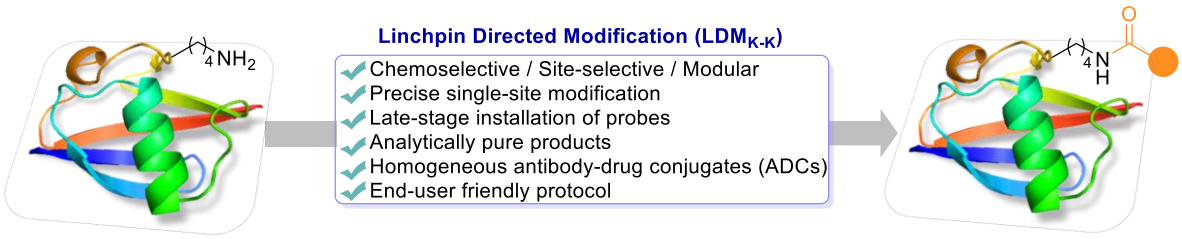

Figure 4: $LDM_{K\text{-}K}$ extends the modular technology for single-site modification of a high frequency Lys residue in proteins, enzymes, and antibodies.

[2] Gly-Tag® platform

This technology empowers site-specific labeling of natural or easy-to-engineer N-terminus Gly in proteins with remarkable efficiency and selectivity. The method generates a latent nucleophile from N-terminus imine that reacts with an aldehyde to deliver an aminoalcohol under physiological conditions. It differentiates N-Gly as a unique target amongst other proteinogenic amino acids. The method allows single-site labeling of proteins in isolated form and extends to lysed cells. It administers an orthogonal aldehyde group primed for late-stage tagging with an affinity tag, 19F NMR probe, and a fluorophore. A user-friendly protocol delivers analytically pure tagged proteins. The mild reaction conditions do not alter the structure and function of the protein. *The cellular uptake of fluorophore-tagged insulin and its ability to activate the insulin-receptor mediated signaling remains unperturbed.* (For details, see: *Nat. Commun.* **2019**, *10*, 2539.)

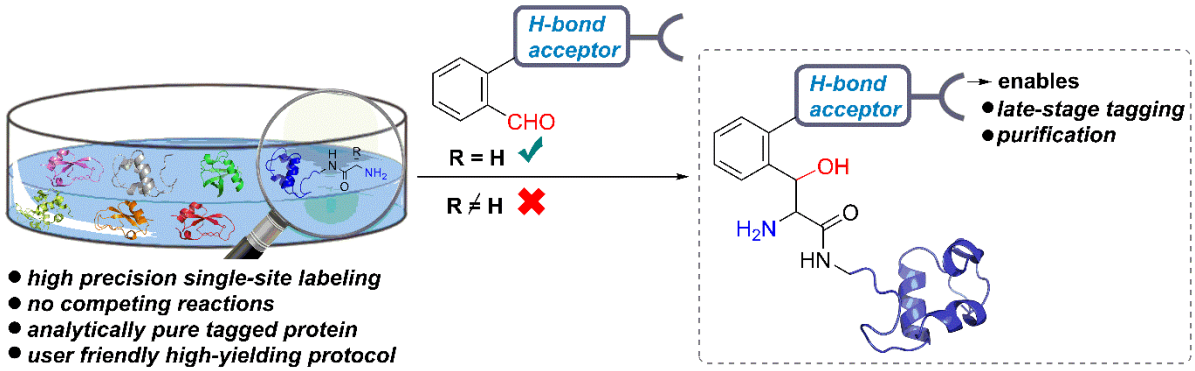

Figure 5: Gly-Tag provides the first ever chemical method for N-Gly-specific single-site targeting of proteins in a complex environment.

Analytically pure therapeutic proteins free of metal contaminants are indispensable. Hence, we developed a methodology where Gly-Tag enables metal-free protein purification and serves as a covalent affinity tag. A Gly-tag resin enables this robust platform for site-specific capture, enrichment, and release through chemically triggered C–C bond dissociation by resonance-assisted electron density polarization. We built the initial platform based on our residue-specific N-Gly labeling strategy. It enabled us to develop a Gly-capture reagent that delivered high efficiency even in the solid phase. The resulting functionalized Gly-tag purification resin facilitated selective capture of the protein of interest (POI) while leaving the other proteins in the solution. Importantly, we were successful in developing a methodology for C–C bond dissociation under mild conditions. This on-demand reversibility proved critical for the operation of the overall technology and enabled the release of the POI. Besides, the recovery and recycling of the Gly-tag purification resin allowed us to reduce the cost of operation. The technology facilitates N-Gly tagged proteins' separation and isolation from a mixture of proteins and cell lysates. (For details, see: *Chem. Sci.* **2020**, 11, 13137-13142)

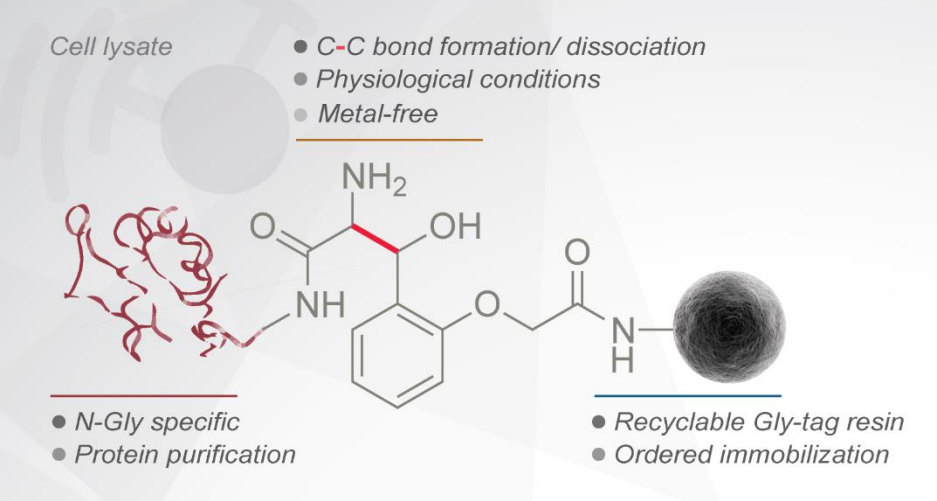

Figure 5: Gly-Tag for metal-free protein purification.

[3] Maspecter® technology

We have developed Maspecter sensitivity boosters that provide the MS detection of a peptide up to attomolar concentration. It enables a remarkable enhancement in the detection of peptides from a protein digest. The reagent exhibits excellent chemoselectivity in labeling the mixture of peptides and results in a predefined mass change. Its reaction with C-terminus lysine outcompetes all the other side reactions. In the absence of Lys, it exclusively reacts with the N-terminus α-amine. Besides, it ensures excellent coverage of fragments in the MS–MS and renders a simplified spectrum with y or b type ions. The protocol results greatly enhanced peptide mapping for cytochrome C, a monoclonal antibody (trastuzumab), and antibody-drug conjugates (trastuzumab-toxin conjugates). Moreover, it provides an excellent tool for unambiguous analysis of the protein and antibody bioconjugates. We demonstrate that it can establish the sites of modification with remarkably higher efficiency than the classical method. It extends remarkably well for the identification of labeled sites in the antibody–drug conjugates. (For details see: *Chem. Commun.* **2019**, *55*, 9979-9982; for extension to ADC analysis, see: *Angew. Chem. Int. Ed.* **2020**, *59*, 10332-10336)

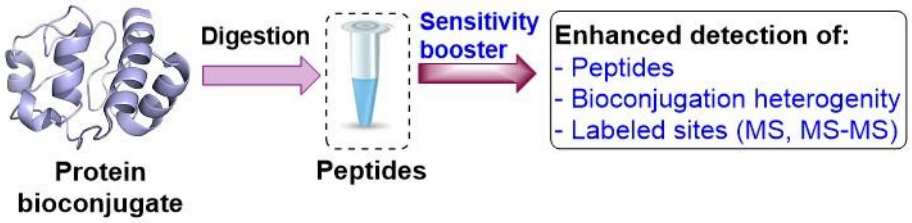

Figure 6: Maspecter reagents enable peptide detection at attomolar concentration. It empowers sequencing of antibody-drug conjugates and unambiguous analysis of site-of-drug-conjugation.