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

Research work for Sun Pharma Research Fellowship 2024:
LDM® 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: Biopharma presents one of the most rapidly growing therapeutics segments. Antibody conjugates have a significant role in their exponential rise. Their critical quality attributes present a technological demand for a comprehensive platform for their precision engineering. In this perspective, we developed LDM® platform to enable the synthesis of homogeneous antibody-conjugates.

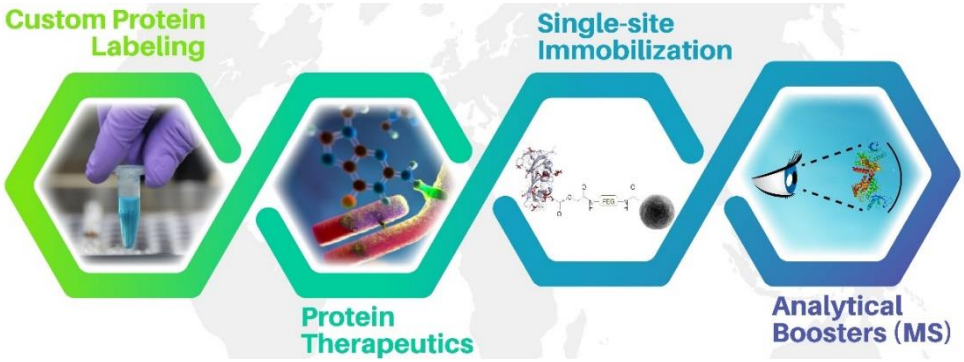

Figure 1: Combination of our technologies empowering homogeneous antibody-drug conjugates (ADCs) and antibody-fluorophore conjugates (AFCs).

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. Among other technologies, it enabled the development of modular linchpin directed modification (LDM®) for precise single-site labelling of His, Lys, Asp, and Glu residues. 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.

Representative tools from 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)

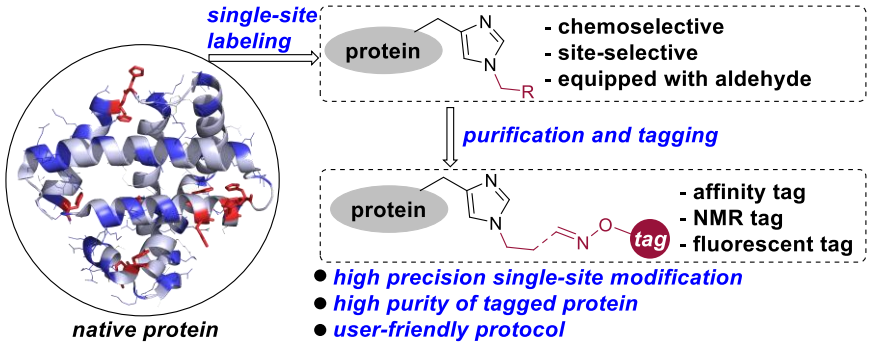

Figure 2: $LDM_{K\text{-}H}$ provides the first ever modular technology for single-site modification of a His residue in proteins, enzymes, and antibodies.

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.)

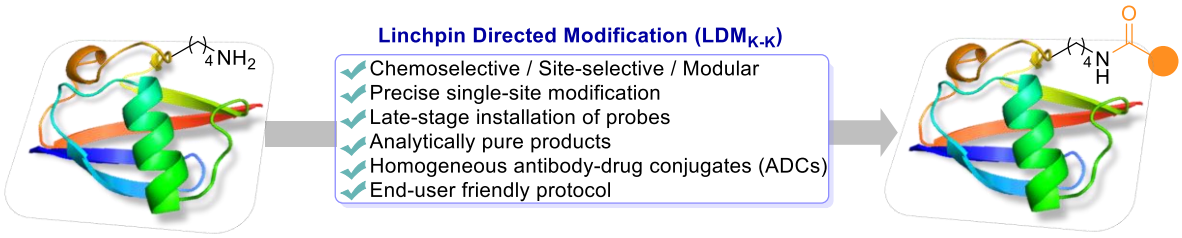

Figure 3: LDM$_{K-K}$ extends the modular technology for single-site modification of a high frequency Lys residue in proteins, enzymes, and antibodies.