Ph.D. Open Seminar

Department of Chemistry, IISER Bhopal

Lysine Directed Single-site Precision Engineering of Native Proteins

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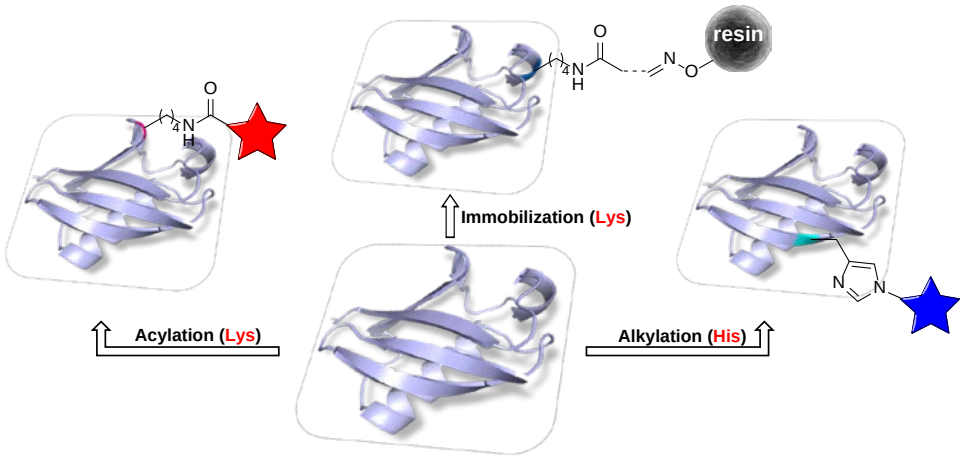
Date: 03/01/2020 **Time:** 11:00am **Venue:** AB2 (401)

Abstract: Proteins are versatile biomolecules with extraordinary diversity in their structure and function. Chemical methods for protein engineering and attachment of synthetic probes provide insights into their features.[1]The single-site modification of proteins with engineered unnatural amino acids, peptide fragments, or recognition motifs for enzymes have made remarkable contributions. However, these methods do not extend to a vast library of native proteins. Hence, chemical platforms for precision engineering of proteins are highly desirable. During my Ph.D., I developed a modular linchpin directed modification (**LDM**)platform for the chemoselective and site-selective transformation of Hisresidue (**LDM$_{K-H}$**).[2]Later, we extended the technology to address the single-sitetargeting of a high-frequency Lysresidue (**LDM$_{K-K}$**).[3]Besides, we learned that the chemoselectivity of an electrophile is a non-conserved parameter in protein chemistry. However, such promiscuous electrophiles can be regulated through proximal control to render the precision engineering of proteins.[4]Ourdisruptive chemical technologies are operationally simple for the non-experts. Besides, we have developed highly efficient purification protocols to give analytically pure bioconjugates.

The mild physiological reaction conditions of LDM empower it for the engineering of proteases, an enzyme with high self-degradation propensity.[5]It delivers single-site ordered immobilization of a serine protease to give enhanced thermal stability, reduced auto-digestion, and recyclability. Besides, LDM$_{K-H}$ and LDM$_{K-K}$ provide a convenient route for the conjugation of a probe to a Fab and monoclonal antibody. It delivers trastuzumab-doxorubicin and trastuzumab-emtansine conjugates (antibody-drug conjugate, ADC) with selective antiproliferative activity toward Her-2 positive SKBR-3 breast cancer cells.



Scheme 1. Modular platform for precision engineering ofproteins.

References

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2) Adusumalli, S. R.; **Rawale, D. G.**; Singh, U.; Tripathi, P.; Paul, R.; Kalra, N.; Mishra, R. K.; Shukla, S.; Rai, V. *J. Am. Chem. Soc.***2018**, *140*, 15114.
3) [#]Adusumalli, S. R.; [#]**Rawale, D. G.**; Thakur, K.; Landa, P.; Kalra, N.; Shukla, S.; Rai, V. *Manuscript submitted*.
4) **Rawale, D. G.**; Thakur, K.; Sreekumar, P.; Adusumalli, S. R.; Rai, V. *Manuscript under preparation*.
5) **Rawale, D. G.**; Gupta, M.; R.; Rai, V. *Manuscript under preparation*.