Ph.D. Open Seminar

Department of Chemistry, IISER Bhopal

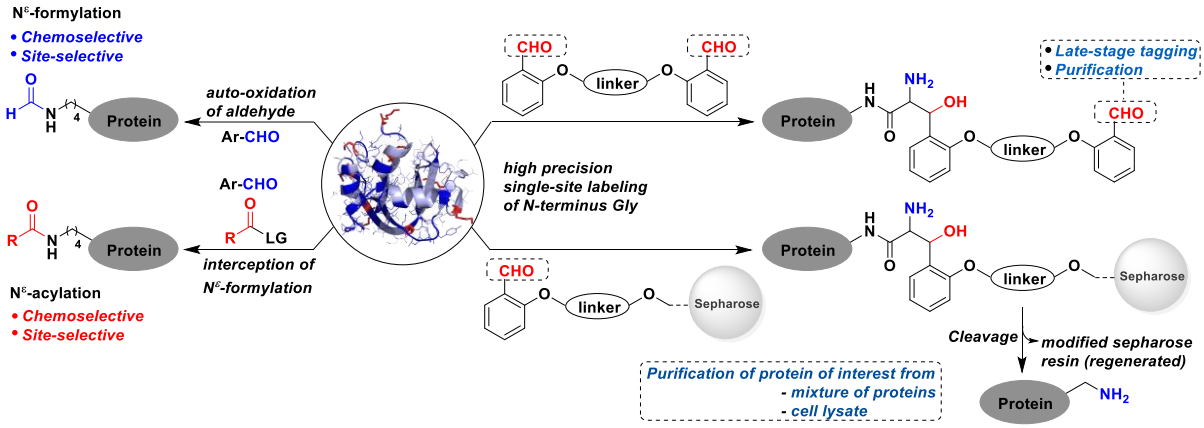
Title of Thesis: "Aldehyde enabled site-selective protein modification and purification"

Speaker: Landa Purushottam (Thesis advisor Dr. Vishal Rai) **Roll No.:** 1220207

Date: August 14, 2018 (Tuesday) **Time:** 12:00 PM **Venue:** AB2-401

The protein labeling tools enable a multitude of biophysical investigations, cell imaging, and protein-based therapeutics.[1] A chemical technology that can deliver single-site labeling of endogenous proteins would be ideal to meet these requirements. The challenge in the identification of a site with unique reactivity emerges from the abundance of functional groups with similar reactivity. It is complicated further by the presence of multiple copies of each residue.[2] We have developed chemical approaches that offer single-site installation of formyl[3] group to the ε-amine of native proteins under physiological conditions. The formylation methodology is enabled by aldehyde auto-oxidation re-routing, regulated formate generation, and reversible N-terminus protection. The interception of the formylation route delivers site-selective acylation. Further, we developed the site-specific labeling of N-terminus Gly in proteins with remarkable efficiency and selectivity.[4,5] The method thrives on an intramolecular H-bond acceptor that renders an aminoalcohol through C-C bond formation under physiological conditions. It differentiates N-terminus Gly as a unique target amongst other proteinogenic amino acids. The chemical platform administers an orthogonal aldehyde group primed for late-stage tagging with an affinity tag, ^{19}F NMR probe, and a fluorophore.

The analytically pure proteins are indispensable for probing their structure, post-translational modifications, and function. The affinity tag-based approach is widely accepted for the purification of proteins. We have developed a methodology for covalent, selective, and reversible immobilization of N-terminus Gly containing proteins.[6] The functionalized resin is used to capture the protein of interest (POI) selectively, leaving the other proteins in solution. Subsequently, we can release the POI along with the recovery of resin. The technique is anticipated to purify N-terminus Gly containing proteins from a mixture of proteins and the cell lysate.



[1] Krall, N.; da Cruz, F. P.; Boutureira, O.; Bernardes, G. J. L. *Nat. Chem.* **2016**, *8*, 103-113.
[2] Chilamari, M.; **Purushottam, L.**; Rai, V. *Chem. Eur. J.* **2017**, *23*, 3819-3823.
[3] (a) **Purushottam, L.**; Adusumalli, S. R.; Chilamari, M.; Rai, V. *Chem. Commun.* **2017**, *53*, 959-962. (b) Chilamari, M.; **Purushottam, L.**; Rai, V. PCT Int. Appl. **2018**, WO 2018047197 A1 20180315.
[4] **Purushottam, L.**; Adusumalli, S. R.; Singh, U.; Gujrati, M.; Rawale, D.; Mishra, R. K.; Rai, V. *Manuscript under revision.*
[5] **Purushottam, L.**; Rai, V. PCT Int. Appl. **2018**, WO 2018104962 A1 20180614.
[6] **Purushottam, L.**; Unnikrishnan, V. B.; Gujrati, M.; Adusumalli, S. R.; Rawale, D.; Mishra, R. K.; Rai, V. *Manuscript under preparation.*