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Probing Large Intrinsically Disordered Regions through Novel Sortase-Mediated Ligation

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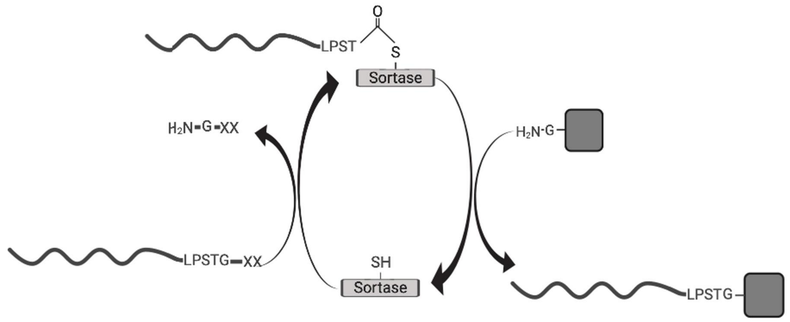
Abstract

In the realm of proteins, it is widely accepted that structure informs function. However, there are many proteins that contain intrinsically disordered regions (IDRs). These regions are areas in which the protein lacks defined structure, and IDPs are also often unstable, which complicates structural studies. NMR spectroscopy is an established method for probing protein structure and has been applied to that end in small IDRs. However, larger IDRs often have spectral overlap that makes data difficult to interpret. Furthermore, low-concentration samples limit spectral clarity. One method to address these difficulties is to use sortase ligation and segmental labeling, which increases protein yield and eliminates spectral overlap while maintaining the relevant polypeptide environment. We applied this method in order to investigate the conformational changes incurred by headpiece phosphorylation in dematin. Dematin is an important structural protein in red blood cells, which consists of a folded 68 residue headpiece domain and a large 315 residue IDR. **To probe conformational changes induced by covalent modification of the headpiece, we created segmentally labeled samples for use in ^{15}N-HSQC NMR spectroscopy.** Fragments of the IDR were created with N-terminal sortase site (LPSTG), and both domains include FH8 and 6xHis tags for purification and a TEV cleavage site for removal of the tags after purification. A S381E headpiece mutation was used as a phosphorylated mimic. To create complete constructs, an isotopically labeled fragment is linked to the remaining domain via sortase-mediated ligation. **This ongoing work shows the efficacy of this novel approach for preparing high-yields of segmentally labeled samples and promises to create new methodology for investigating large IDRs through heteronuclear NMR spectroscopy.**

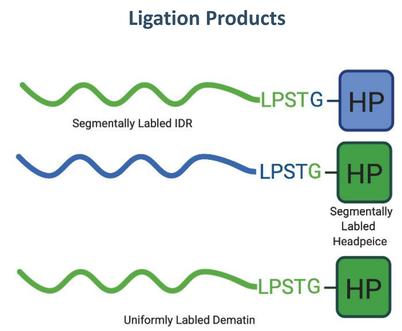
Sortase Mediated Ligation

- Polypeptides can be ligated together via a sortase mediated reaction that involves transpeptidation.
- Minimal point mutations can be used to introduce the sortase site (LPXTG) into polypeptide fragments
- This reaction is consistent and fast, and can be applied *in vitro*



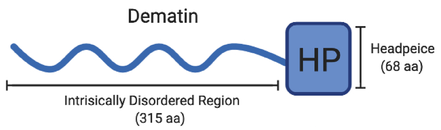
Segmental Labeling

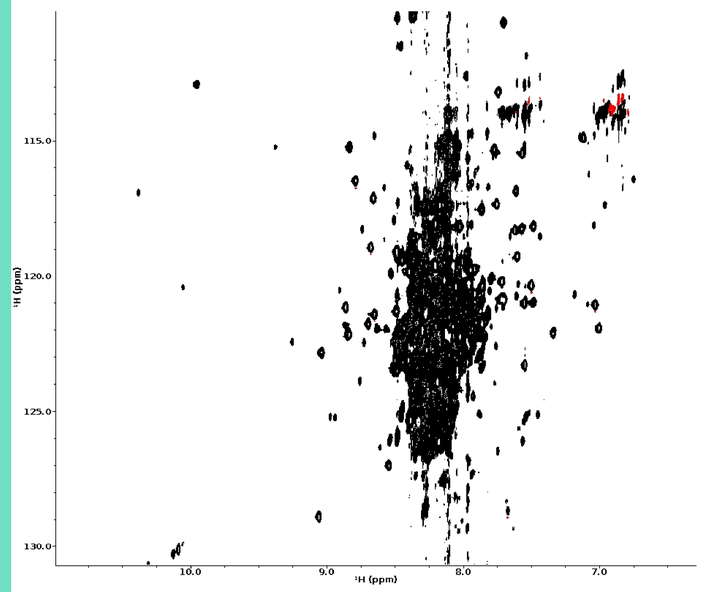
- Dematin fragments are expressed in *E.coli.*
- Expressing headpiece and IDR fragments separately allows us to increase product yields, as the smaller fragments are easier to express and react via SML
- Isolated fragments can then be ligated together via SML yielding desired constructs



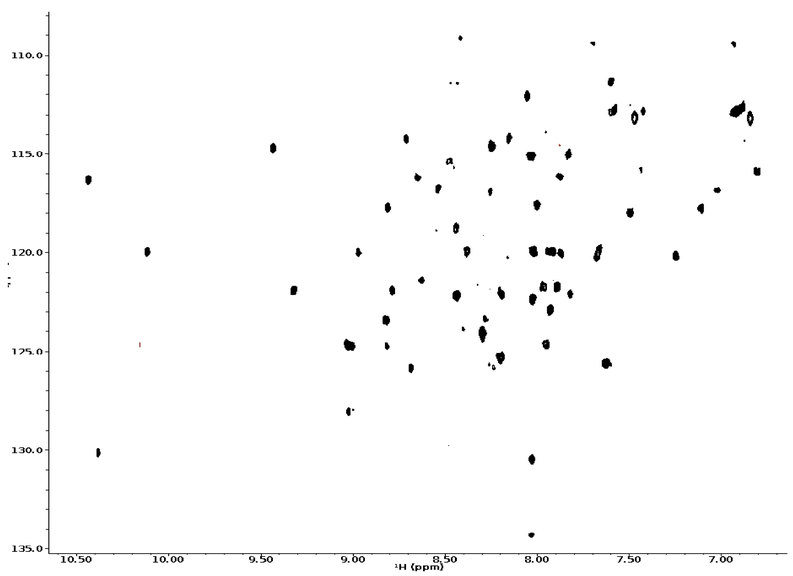
IDRs and Dematin: Functional Relevance

- 30% of eukaryotic proteins have large (>50 aa) IDRs
- IDR lack a single defined conformation
- Cannot be probed using other methods like X-ray crystallography due to lack of one conformation and instability
- NMR is the best method for probing IDR structure, but spectral overlap complicates characterization
- Performs vital biological functions such as actin binding & bundling [Villin, Dematin, other villin family proteins]
- Covalent modification of the headpiece inhibits dematin's abilityto bundle actin
- This mechanism is not well understood and must be studied





Full-size dematin with phosphorylation mimic, uniformly labeled with ^{15}N, recorded with 850 MHz NMR spectrometer at Univ. of British Columbia, Vancouver (BC)
This spectra is a prime example of how spectral overlap between the dematin headpiece and IDR obscures data and prevents peak assignment and interpretation.



Dematin headpiece in isolation with phosphorylation mimic, labeled with ^{15}N, recorded with 500 MHz NMR spectrometer at Western Washington University, Bellingham (WA)
Simplified spectra produced by labeled dematin headpiece in isolation. This is an example of how single-ligation segmental labeling can potentially lead to production of clarified NMR spectral data. We expect to produce similar spectra with full-sized dematin soon, as samples can now be produced at adequate concentrations for spectral clarity.

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