Sortase Allows for Efficient Segmental Labeling of Large Intrinsically Disordered Regions.

Kristina Boyko, Erin Rosenkranz, Derrick Smith, John Antos*, Sergey Smirnov*

Western Washington University, Chemistry Department

NMR spectroscopy is a tool of choice for studying function and dynamics of proteins, especially those containing intrinsically disordered regions (IDRs).[1] However, NMR spectra of large uniformly labeled IDRs can be obscured by severe spectral overlap, which led to development of highly sophisticated NMR schemes.[2] Segmental isotopic labeling allows simplification of the spectra by presenting only NMR signatures of smaller target portions within a large, unlabeled IDR context. Existing segmental labeling techniques have various limitations. To augment the repertoire of existing methods, we developed an application that utilizes sortase ligation enzymes[3] for segmental labeling of IDRs. We employed, for the first time, and optimized this approach for segmental labeling of plant villin, which contains a large IDR and a folded headpiece domain (HP). We will also present solutions to the problems that we encountered.

Sortase Ligation: An orthogonal labeling methodology

- Many important biological proteins contain large IDRs (200+ aa), such as plant villin, supervillin, and dematin[4,5].
- By exploiting sortase mediated ligation (SML) in combination with other methods (e.g. expressed protein ligation, EPL) we can develop samples that contain smaller, easy-to-assign, labeled segments within larger, IDR contexts (Figure 1).

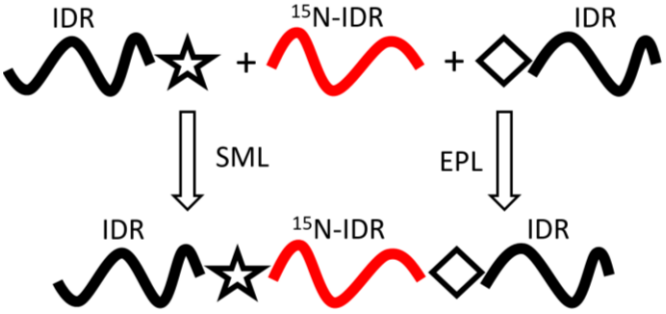

Figure 1. The combination of SML (star) and EPL (diamond) maximizes the yield of segmentally labeled ligation product by exploiting the strengths of both approaches, while negating their individual weaknesses.

SML: Suitable for minimally modified IDR substrates

- SML does not require a cysteine at the ligation site.
- SML can be implemented *in vitro* using recombinant polypeptides and an LPXTG ligation site, which often can be obtained with just one or two point mutations.
- High yield: ~50% percent ligation product over 2 hours.

Future Work

- Assign backbone amide resonances of the large IDRs of dematin and plant villin utilizing SML/EPL segmental labelling as well as traditional NMR assignment methods.
- Use the assignments to characterize the function of dematin and plant villin IDRs (e.g. binding).

Sortase-mediated segmental labelling of plant villin IDR

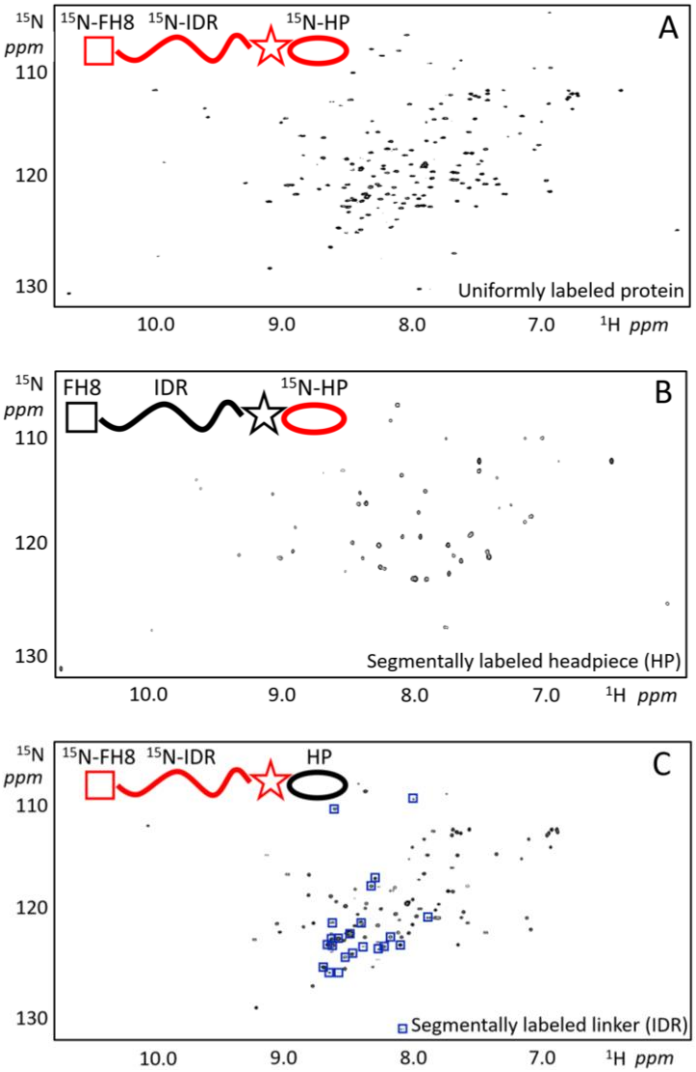

Figure 2. ^{15}N-HSQC spectra of 100+ C-terminal residues of native sequence from plant villin-4 fused with N-terminal FH8 tag. The star indicates the SML site. 36 peaks were assigned to the selected 36-residue fragment of the disordered linker (22 peaks shown boxed at this sensitivity threshold) by subtracting the FH8 signature (spectrum not shown). Through SML we were able to unburden the ^{15}N-HSQC spectra in the ^{1}H chemical shift range of 8.00-8.50 ppm for *segmental assignment* of NMR resonances within the target IDR.

References

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