Single-step, Single-column Protein Purification and Labelling for Fluorescence Studies

A Novel, Solid-Phase Click Reaction Applied to an Intrinsically Disordered Protein with Applicability to any Protein, and any Probe

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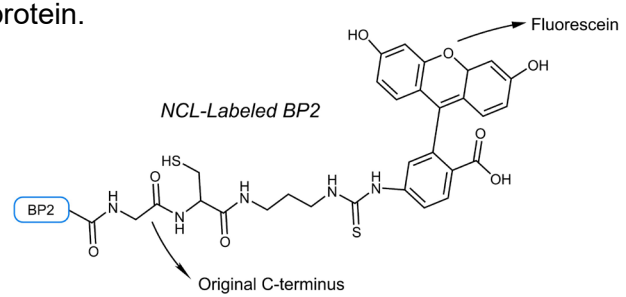
One of the major requirements of biophysical, medicinal research is that proteins of interest are free of impurities and are tagged with fluorescent labels- ideally with multiple labels attached via orthogonal chemistries.

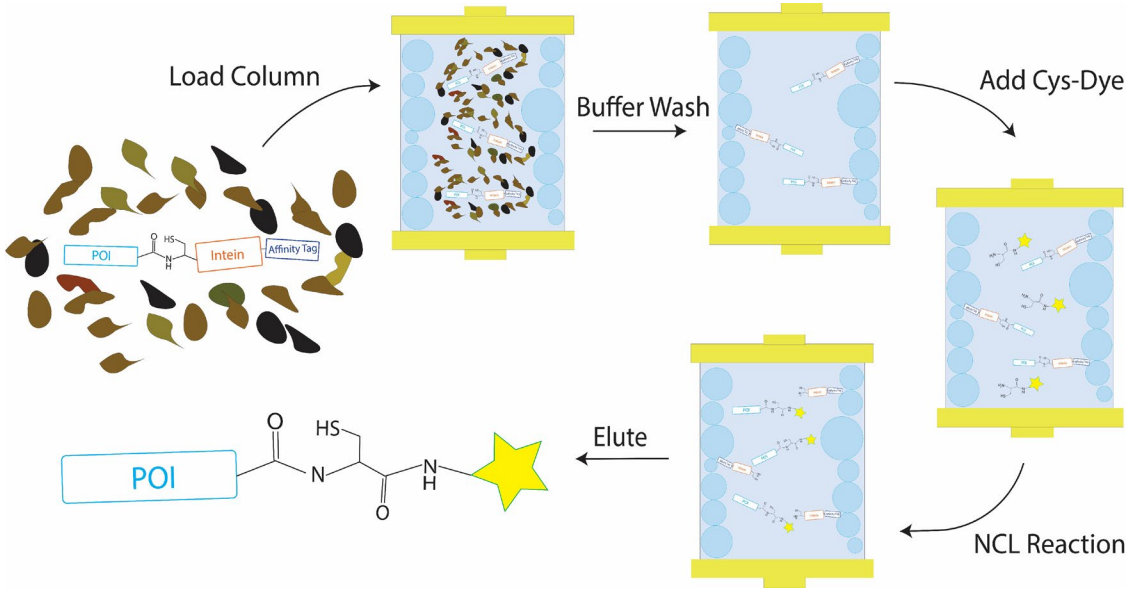
2 problems are often encountered in pursuit of this goal:

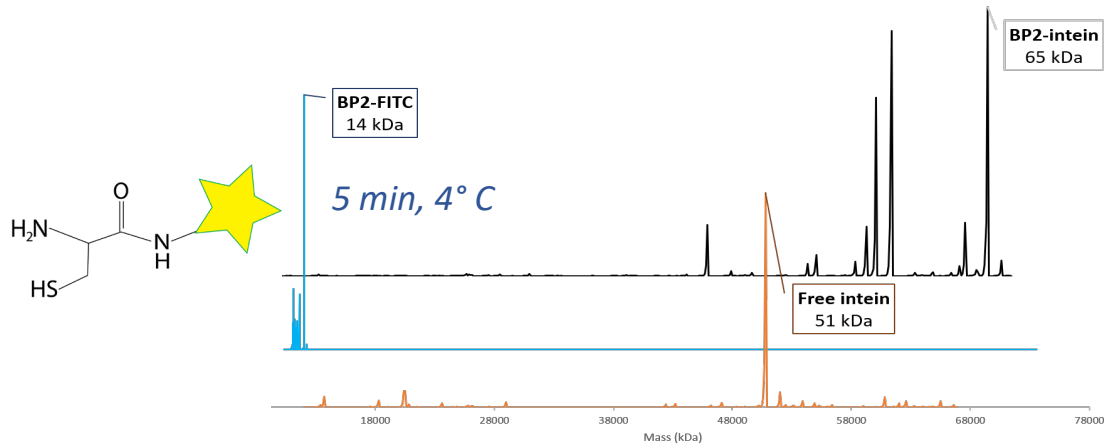
1. **Waste** of dyes, enzymes, protein, and time, with most protocols having **1-week prep time**
2. **Low yield** (<10%) of dual-labelled protein product, with low selection of dyes having orthogonal chemistries

The technique takes advantage of native chemical ligation (NCL) and requires no uncommon reagents or instrumentation. We demonstrate the efficacy of this method on the intrinsically disordered eukaryotic initiation factor 4E binding partner 2 (4E-BP2). The results highlight the quality of the novel sample preparation technique for IDPs in particular, as well as help reveal new dynamic information about the disordered C-terminal region of this protein.

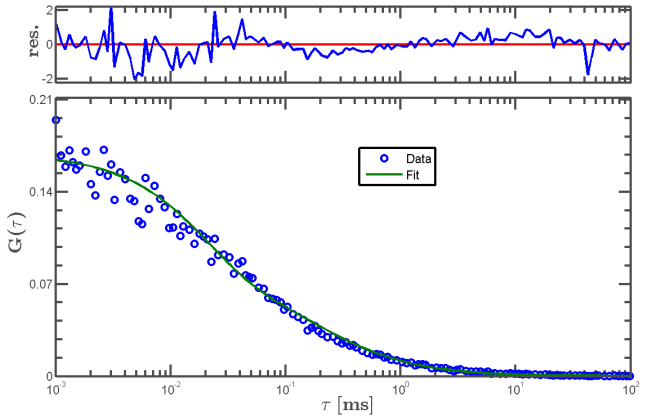


Above. Structure of BP2-FITC synthesized using the NCL purification and labeling technique presented here.





Left. Cys-dye used to catalyze NCL reaction, purifying & labeling protein in one step. **Right.** LCMS analysis of column elution fractions. Black: buffer wash; Blue: NCL reaction elution; Orange: intein elution.



Above. FCS of BP2-FITC from 5 minute incubation of BP2-intein on Ni-NTA beads with Cys-FITC. Expected R_H is 22-25 A; Data shows R_H 23.75 A, indicating the presence of BP2-FITC after NCL.

This novel technique:

1. Is **highly efficient** of dyes**,** has no enzyme requirement, minimal mutations**,** with **1 hour total prep time** from cell lysate
2. Gives **quantitative yield** of pure & labeled protein**,** with **complete orthogonality** to other dye chemistries

The NCL reaction is robust and rapid with IDPs, most likely due to their highly flexible and unhindered structure. This makes this technique especially efficient for preparation of pure & labeled IDPs, which comprise a rapidly growing and key field in structural biology.

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