CPPC 2021

sciforum

Abstract

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

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† Presented at the First Canadian Peptide and Protein Community Virtual Symposium, 27–28 May 2021; Available online: https://cppc2021.sciforum.net/

Published: 27 May 2021

Abstract: The protocol to purify and fluorescently label proteins typically includes lengthy purification steps and stochastically governed labelling methods. When multi-labelled proteins are required, poor yields, waste of valuable reagents, and lengthy sample preparation times are generally inevitable. In addition, many fluorophore combinations are incompatible, which makes the task of efficiently producing multi-labelled, high-purity proteins very difficult.

Here, we describe a novel method that enables the preparation of purified and labelled proteins in only a few hours. Our protocol gives high yield of the desired protein product using minimal mutations, high specificity of labelling, and ease of chemically orthogonal attachment of other fluorescent probes in later steps. This technique takes advantage of native chemical ligation in a single-step, in one liquid-chromatography column, by the addition of one reagent, with the ability to purify and label any protein using any desired fluorescent probe.

We demonstrate the efficacy of this method on the eukaryotic initiation factor 4E binding partner 2 (4E-BP2). This translational regulation protein is intrinsically disordered and is implicated in autism and neurodegenerative diseases. Proof-of-principle biophysical experiments such as Förster resonance energy transfer (FRET)and fluorescence correlation spectroscopy (FCS) were performed on the 4E-BP2 protein labelled using our new method. The results highlight the quality of the novel sample preparation technique, as well as help reveal new dynamic information about the disordered C-terminal region of this protein.