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# A novel protein purification strategy mediated by the combination of CipA and Ssp DnaB intein

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## Abstract

**Conventional purification methods including the affinity purification or the usage of self-aggregating tags suffered from many drawbacks such as the complicated steps, high cost and low efficiency. Moreover, the fusion tag usually had negative effects on the activity of the target protein. Here we propose a novel protein purification method which needs simple operation steps, and this method is mediated by the combination of CipA protein and a mini-intein (Synechocystis sp. PCC6803 DnaB, Ssp DnaB), depending on the assembly function of CipA and the self-cleavage function of Ssp DnaB. To realize the purification, CipA-DnaB-eGFP protein was expressed and assembled into protein crystalline inclusions (PCIs) in E. coli. Then, only cell lysis, cleavage and centrifugation steps were required to purify eGFP. Purified eGFP was in the supernatant with a purity of over 90%. The cleavage efficiency and the yield of eGFP reached 51.96% and 13.99 ± 0.88 mg/L fermentation broth, respectively. Furthermore, to broaden the application of this approach, three other proteins which were maltose binding protein (MBP), ketoisovalerate decarboxylase (Kivd) and alcohol dehydrogenase (AdhP) were purified with high cleavage efficiency. The purified Kivd and AdhP remained high specific activities. This work demonstrated an effective and convenient protein purification method.**

## Method

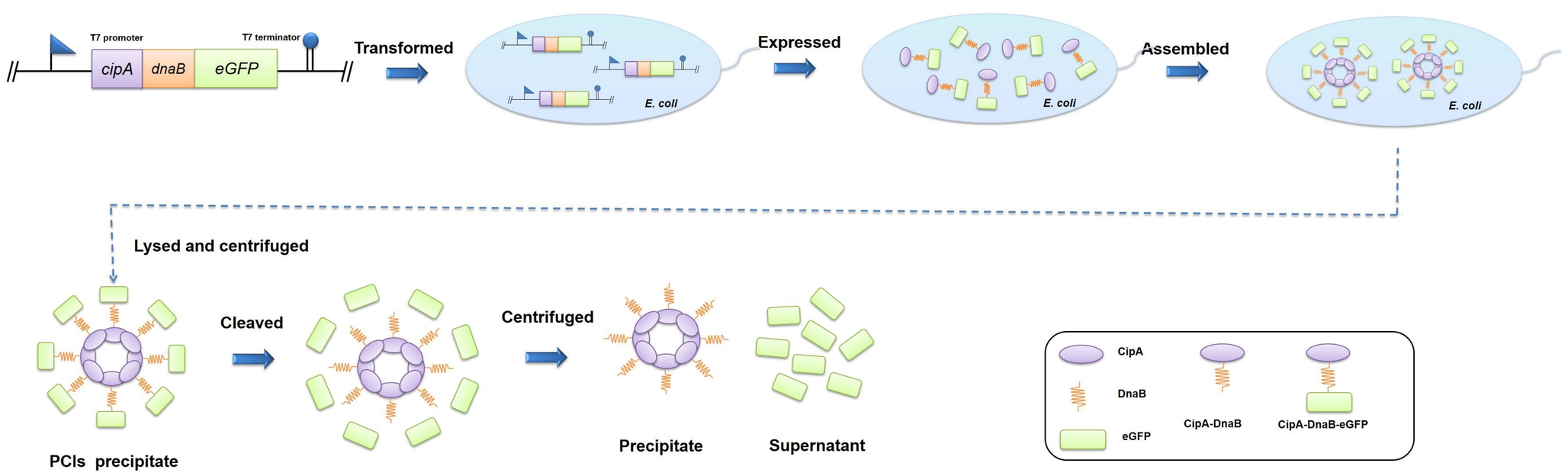


**Fig. 1. The whole purification process of the novel approach. The target protein is eGFP.**

## Experimental results

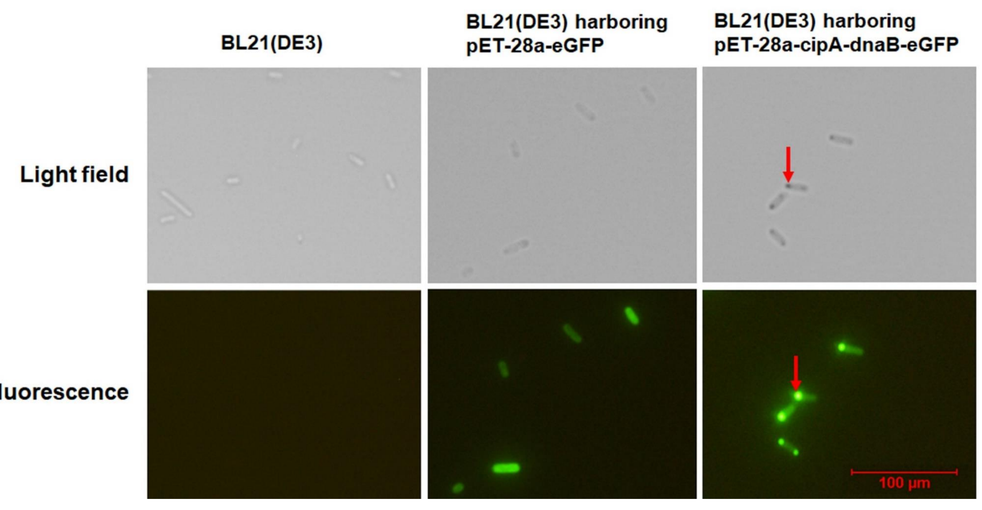


**Fig. 2. CipA scaffolds organizing eGFP into subcellular structures in E. coli. All the strains were cultured at 30 °C after induction. The pictures were taken by Fluorescence microscopy.**

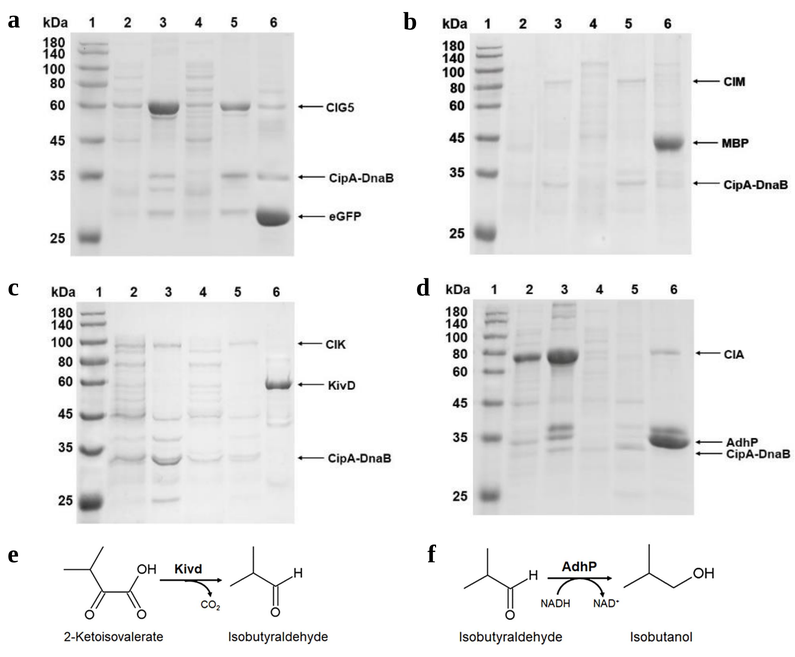


**Fig. 3 SDS-PAGE of (a) CipA-DnaB-eGFP (b) CipA-DnaB-MBP (c) CipA-DnaB-KivD and (d) CipA-DnaB-AdhP which were cleaved in a buffer (50 mM Tris-HCl, 1 mM DTT, pH 6.5), CipA-DnaB and eGFP， MBP, KivD, AdhP. Lane 1: 180 kDa protein molecular weight marker. Lane 2: the lysed mixture after ultrasonic treatment. Lane 3 and 4: the precipitate and the supernatant of the lysed mixture after centrifugation, respectively. Lane 5 and 6: the precipitate and the supernatant of the cleaved mixture after self-cleavage and centrifugation, respectively. (e) and(f), the reactions of Kivd and AdhP, respectively.**

## Conclusions

**This study reports a simple alternative protein purification approach which relied on the assemble function of CipA protein and the self-cleavage function of Ssp DnaB intein. Significantly, this approach is convenient and needs only centrifugation and self-cleavage steps. Considerable amount of tag-free eGFP was obtained using this approach after optimization of cleavage condition and linkers. In addition, purification of MBP, Kivd and AdhP expanded the application of this approach, and the purified enzymes have high specific activities. This novel protein purification approach could serve as a promising tool to purify the desired proteins industrially with low cost.**