# Fabrication of Core-Shell Protein Condensates in *E. coli* Utilizing Self-Assembling Peptide Tags

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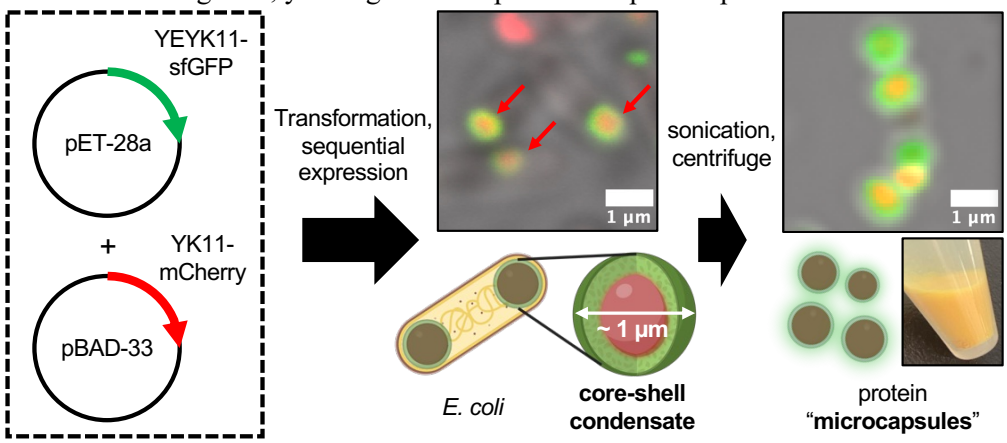
Proteins are attracting attention as next-generation functional materials due to their complex functionality, stimuli responsibility and biocompatibility. However, assembling them into uniformly sized microparticles with maintaining their structure and activity remains highly challenging. Moreover, conventional methods, where proteins were produced in *Escherichia coli (E. coli)*, extracted, purified and processed externally, were too costly for large-scale applications. One of the most promising approaches to address these challenges is to complete the processing of the materials directly within *E. coli*.

Our group has developed self-assembling peptide tags, YEYK-tags and YK-tags. When fused with the green fluorescent protein sfGFP, these peptide tags enable β-sheet-based self-assembly, forming aggregates or droplets within mammalian cells[1,2]. Herein, we aimed to fabricate protein microparticles in *E. coli,* utilizing self-assembling peptide tags. First, we fused YK-tag and YEYK-tag to fluorescent proteins sfGFP and mCherry and expressed them in *E. coli*. Both fusion proteins successfully formed condensates within *E. coli*. Next, genes of YK11-mCherry and YEYK11-sfGFP were introduced in pBAD-33 and pET-28a vectors respectively, and their expression was sequentially induced. As a result, core-shell shaped protein condensates were formed in *E. coli,* with YK11-mCherry (red) serving as the core and YEYK11-sfGFP (green) forming the shell. To note, these condensates were fluorescence active, meaning that the proteins were properly folded. Besides, these condensates remained stable even after sonication-based cell lysis and could be easily separated via centrifugation, yielding "microcapsule"-like protein particles.




[1] Miki T., Mihara H. et al. **2021** *Nat. Commun.* 12(1), 3412. [2] Miki T., Mihara H. et al. **2024** *Nat. Commun.* 15(1), 8503.