

**Non-peptide guided auto-secretion of recombinant proteins
by super-folder green fluorescent protein in *Escherichia coli***

Zhen Zhang¹, Rongxing Tang¹, Dewu Zhu¹, Wenfeng Wang¹, Li Yi¹, Lixin Ma^{1*}

¹Hubei Collaborative Innovation Center for Green Transformation of Bio-resources,
Hubei Key Laboratory of Industrial Biotechnology, College of Life Sciences, Hubei
University, Wuhan, People's Republic of China

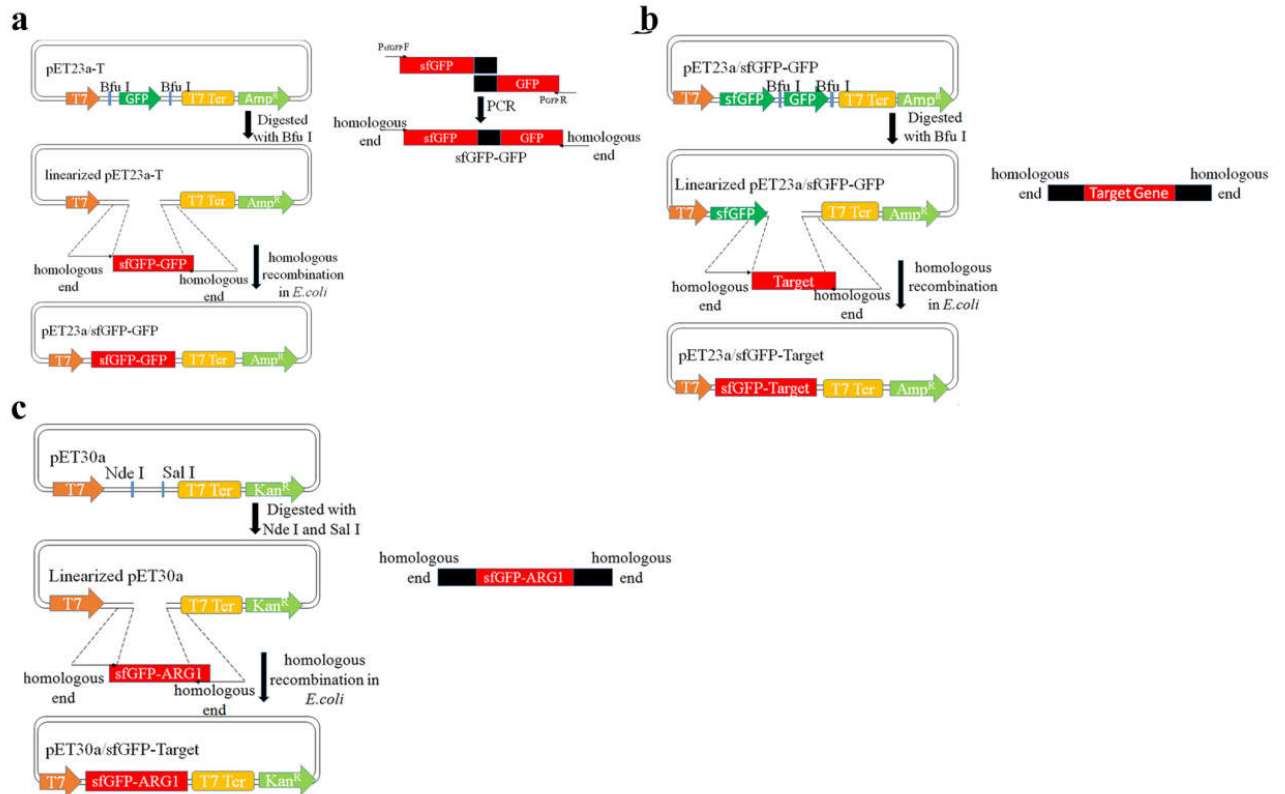
*Corresponding author

Prof. Lixin Ma

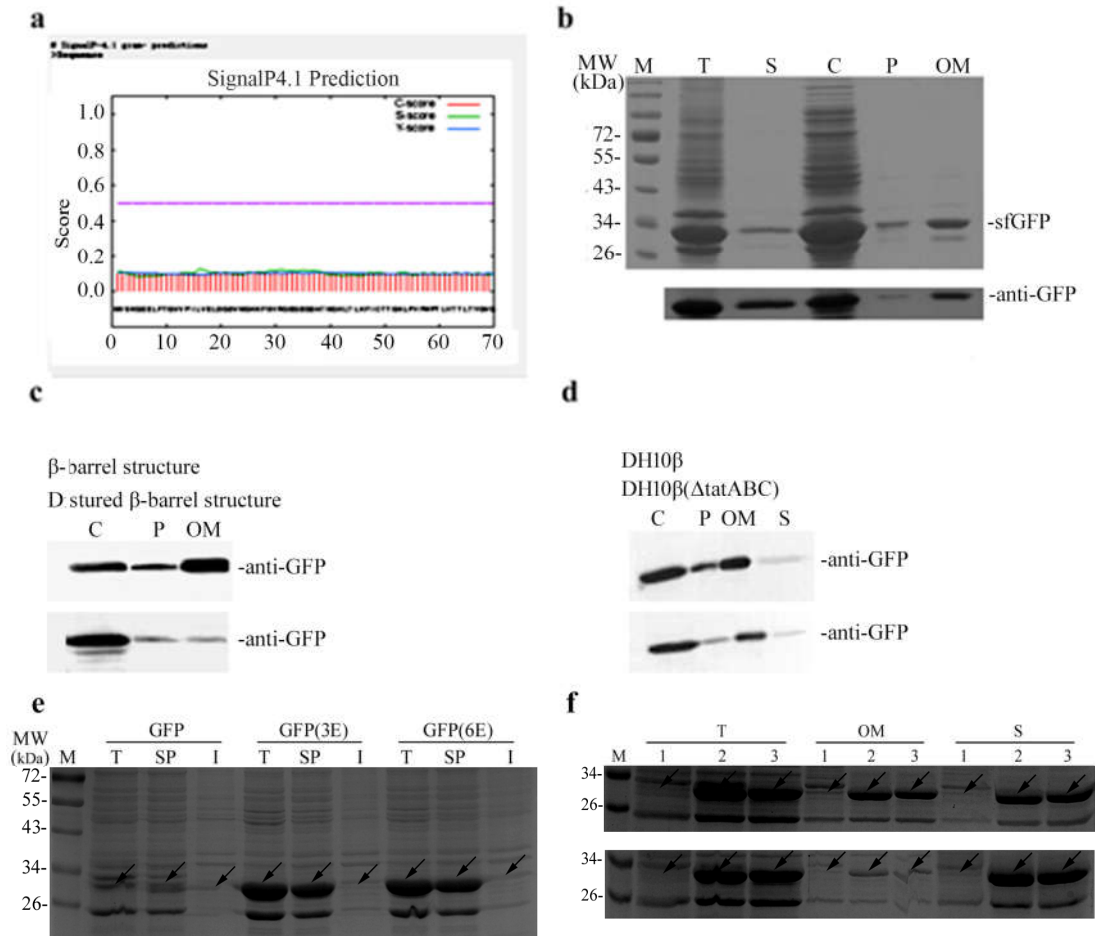
Department of Biochemistry and Molecular Biology

E-mail addresses: malixing@hubu.edu.cn

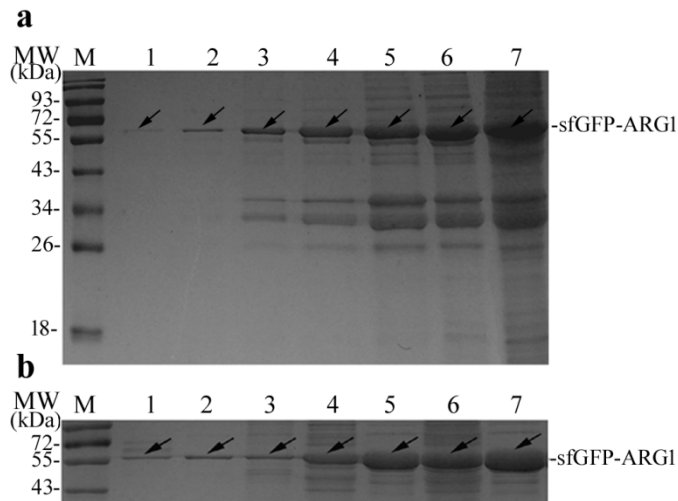
Supplementary Figures



Supplementary Figure 1. Constructs in the study. (a) Schematic diagram of pET23a/sfGFP-GFP vectors; (b) Schematic diagram of pET23a/sfGFP-Target vectors; (c) Schematic diagram of pET30a/sfGFP-ARG1 vectors.



Supplementary Figure 2. sfGFP analysis in this study. (a) Signal peptide prediction of sfGFP; (b) SDS-PAGE and Western blot analysis of the expression of sfGFP (without His-Tag) in *E. coli*(*Rosetta Blue*); (c) Western blot analysis of the secretion of N-terminal sequence deleted sfGFP in *E. coli*(*Rosetta Blue*); (d) Western blot analysis of the auto-secretion expression of sfGFP in DH10β and DH10β(ΔtatABC) strain; (e) SDS-PAGE analysis of the expression of wtGFP and wtGFP mutants in *E. coli*(*Rosetta Blue*); (f) Sub-fraction analysis of wtGFP and wtGFP mutants in *E. coli*(*Rosetta Blue*). lane 1: wtGFP; lane 2: wtGFP(3E); lane 3: wtGFP(6E); T: total cell protein; SP: soluble part of total cell protein; I: insoluble part of total cell protein; S: extracellular protein; C: cytoplasmic protein; P: periplasmic protein; OM: outer membrane protein.



Supplementay Figure 3. SDS-PAGE analysis extracellular secretion of sfGFP-ARG1 in fed-batch fermentation condition. (a) SDS-PAGE analysis of unpurified sfGFP-ARG1; lane 1: unpurified supernatant sample after 6 hours fermentation; lane 2: unpurified supernatant sample after 14 hours fermentation; lane 3: unpurified supernatant sample after 18 hours fermentation; lane 4: unpurified supernatant sample after 28 hours fermentation; lane 5: unpurified supernatant sample after 34 hours fermentation; lane 6: unpurified supernatant sample after 40 hours fermentation; lane 7: unpurified supernatant sample after 45 hours fermentation; each sample was loaded with 20 μ L; (b). SDS-PAGE analysis of purified fusion sfGFP-ARG1; lane 1: purified supernatant sample after 6 hours fermentation; lane 2: purified supernatant sample after 14 hours fermentation; lane 3: purified supernatant sample after 18 hours fermentation; lane 4: purified supernatant sample after 28 hours fermentation; lane 5: purified supernatant sample after 34 hours fermentation; lane 6: purified supernatant sample after 40 hours fermentation; lane 7: purified supernatant sample after 45 hours fermentation; each sample was loaded with 5 μ L.