THE HONG KONG POLYTECHNIC UNIVERSITY 香港理工大學
DEPARTMENT OF APPLIED BIOLOGY AND CHEMICAL TECHNOLOGY 應用生物及化學科技學系

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A study of the conformational change of lipid II flipping enzyme MurJ

Yuan-Yuan Zheng, Kwok-Yin Wong*
The Hong Kong Polytechnic University

Introduction

In 2017, the World Health Organization (WHO) mentioned that some pathogenic bacteria have become increasingly resistant to clinical antibiotics. The task of developing new therapeutic drugs is urgent, otherwise humans will soon have no antibiotics available.[1]

The peptidoglycan cell wall layer exists in almost all types of bacteria. Lipid II is the precursor of the PG cell wall. It is synthesized in the cytoplasm, and since the assembly of the cell wall is finally completed outside the cell membrane, it must be transferred outside the membrane before it can be polymerized into peptidoglycan.[2] Therefore, MurJ, as its transport enzyme candidate, has become a popular target for the development of new antibiotics in recent years.[3,4]

MurJ is essential for bacterial viability and reproduction as its depletion can causes an accumulation of lipid II at the cytoplasm following by the bacterial shape distortion and cell lysis.[5] Recent studies have reported the binding site and crystals structures with different conformations(Figure 1), but the energy of the flip movement and the change between different conformations are still unclear.[6]

A simple explanation from the literal meaning, hydrogen-deuterium exchange is the exchange of hydrogen atoms by deuterium atoms. This method has been used in recent years to study membrane protein dynamics, although it has been used to characterize soluble proteins for decades.[7] HDX-MS can provide very useful information, including the conformation changes, protein folding and unfolding pathways.[8]

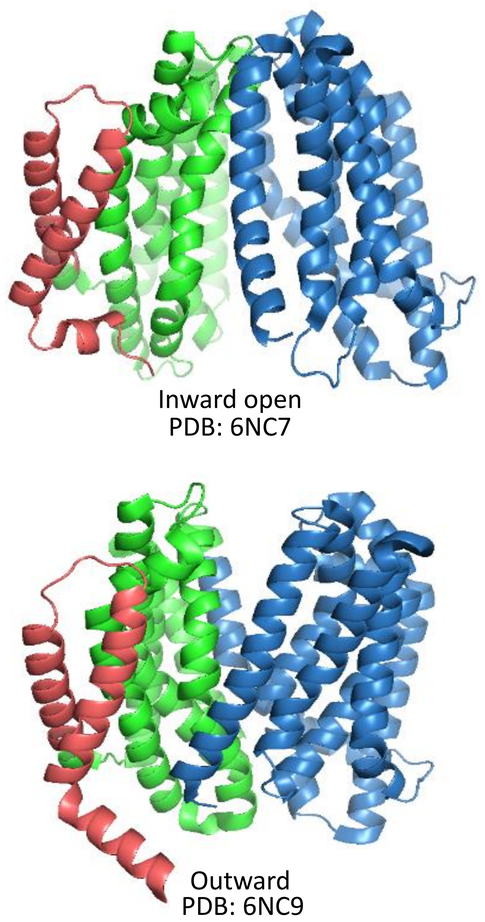

Figure 1. Crystal structures of MurJ. (**A**) Inward open confirmation (PDB: 6NC7). (**B**) Outward confirmation (PDB: 6NC9). PDB files are excerpted from Protein Data Bank (https://www.rcsb.org/).

Results and Dicussions

So far, we have designed and purified MurJ proteins from *Escherichia coli* and *Thermosipho africanus*. The proteins were purified and preliminarily confirmed by SDS-PAGE(Figure 2A). The required protein portion are then collected and concentrated, followed by mass spectrometry(Figure 2B) to further confirm the protein identification.

The theoretical molecular weight of these proteins is around 55-56 kD. There is a 'gel shifting' of the target protein band which may be due to the protein character and the detergents used during the purification process. Compared with the standard ladder, the protein band position was about 40 kD, which was consistent with the reported literature.[4,9]

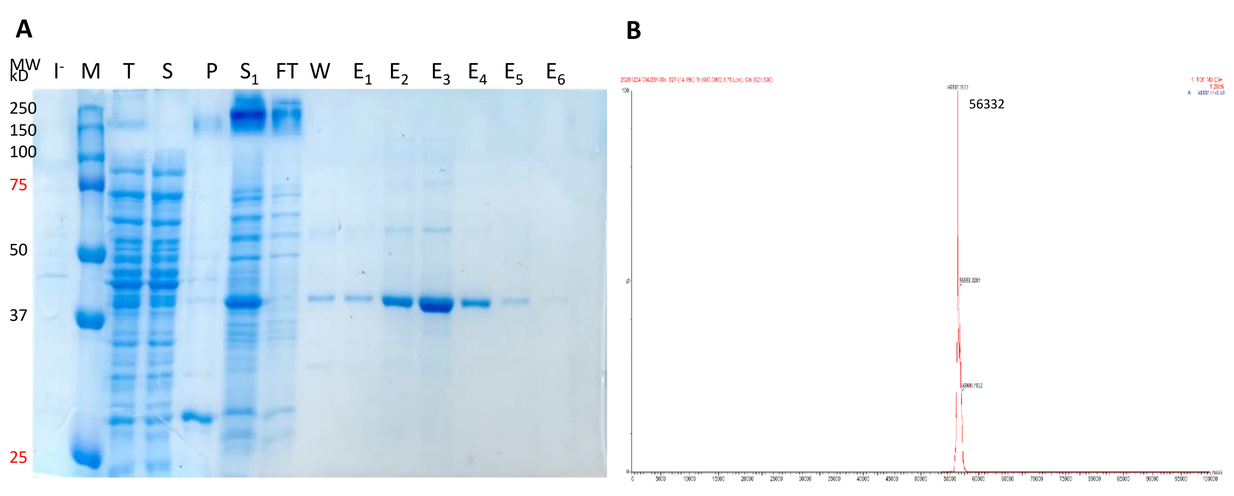

Figure 2. (**A**)SDS-PAGE images and (**B**)Mass spectrum of purified MurJ from *Escherichia coli*. I^--total lysis before IPTG induced, M-marker, T-total lysis after IPTG induced, S-supernatant, P-pellet after ultracentrifuge, S_1-supernatant after ultracentrifuge, FT-flowthrough, W-wash, E-eluate.

Summary

At present, the global situation of drug-resistant bacteria is serious, and the development of novel antibiotics is imminent. In recent years, protein MurJ has become a popular potential antibiotic target due to its widespread in bacteria and its importance in cell wall synthesis. So far, we have successfully expressed and purified MurJ proteins from *Escherichia coli* and *Thermosipho africanus*, which means we can move on to study the protein confirmation using HDX-MS. HDX-MS has been used in recent years to study membrane protein dynamics while it has been used to characterize soluble proteins for decades. It can provide very useful information including changes in protein conformation. Hope we can get some useful information in the future to provide some theoretical basis for the futher development of antibiotics targeting MurJ.

Reference

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Contact information

Yuan-yuan Zheng (Email: 19048675r@connect.polyu.hk); Kwok-Yin Wong* (Email: kwok-yin.wong@polyu.edu.hk)