The researchers observed significant negative effects on cancer cell division and proliferation in cells expressing $PKM2^{C326S}$. These cells showed an increased percentage of polyploidy cells and a higher frequency of giant multinucleated cells (**Fig. 2(b)**). Time-lapse microscopy revealed a 1.5-fold increase in cytokinesis failure in $PKM2^{C326S}$-expressing cells. Furthermore, $PKM2^{C326S}$ failed to interact with the spindle checkpoint protein BUb3, and cells showed reduced proliferation rates. Similar effects were observed in cells with H_2S depletion, supporting the specific role of sulfhydration in these processes.

Perhaps most significantly, in a mouse xenograft model, tumor growth was completely suppressed in the $PKM2^{C326S}$ group (**Fig. 2(c)**). While there were no significant differences in mouse body weight between groups, there was a dramatic reduction in tumor bioluminescence signals in the $PKM2^{C326S}$ group, indicating strong anti-tumor effects.

These comprehensive results demonstrate that H_2S-mediated sulfhydration of PKM2 at C326 is a crucial mechanism regulating cancer cell metabolism. Blocking this modification through the $PKM2^{C326S}$ mutation leads to the stabilization of PKM2 tetramers, enhanced oxidative phosphorylation, reduced nuclear translocation and transcriptional activity, impaired cell division, and suppressed tumor growth. These findings suggest that targeting PKM2 sulfhydration could be a promising therapeutic approach for cancer treatment, particularly by rewiring glucose metabolism from aerobic glycolysis to oxidative phosphorylation. The study provides both mechanistic insights into cancer metabolism and potential therapeutic strategies for future drug development.
(Reported by Chun-Hsiang Huang)

This report features the work of Hui-Chun Cheng and her collaborators published in Nat. Commun. ***15****, 7463 (2024).*

TPS 07A Micro-focus Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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Structure of the Prc–NlpI–MepS Complex: Elucidating the Regulatory Mechanism of Bacterial Cell Walls

This study reveals how the adaptor protein NlpI regulates the activity and cellular levels of the cell wall endopeptidase MepS, which facilitates peptidoglycan remodeling and maintains cell wall integrity during bacterial growth and development.

Peptidoglycan (PG) is vital for protecting bacterial cells from osmotic pressure. It consists of linear glycan strands of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM), linked to short peptide chains. The main cross-linking of the short peptide chains is the 4–3 linkage between D-Ala and meso-diaminopimelic acid (DAP), forming a net-like structure that prevents osmotic rupture. During cell growth, the net-like structure must be cleaved to incorporate new PG strands, a process facilitated by several endopeptidases, including MepS, MepM, and MepH. In *Escherichia coli* (*E. coli*), these three endopeptidases are essential for cell wall expansion and their absence leads to abnormal cell shapes and lysis.

NlpI is an outer membrane-anchored lipoprotein found in Gram-negative bacteria (*e.g.*, *E. coli*) and plays multiple roles in cell division, cell wall metabolism, virulence, and host interactions. It interacts with various hydrolases and associates with the PG synthesis machinery, influencing the stability of cell envelope components. As an adaptor protein, NlpI can bind to three endopeptidases—MepS, MepM, and PBP4—and facilitates the formation of trimeric complexes (*e.g.*, MepS-NlpI-PBP4). In addition, NlpI helps localize these enzymes, connecting PG hydrolysis to expansion. Reconstitution experiments show that NlpI organizes PG multienzyme complexes, suggesting it aids in integrating hydrolases and synthases during PG expansion.

MepS is abundant during the log phase of cell growth but declines in the stationary phase. Its protein levels within the cell are regulated by the periplasmic PDZ-protease Prc (also called tail-specific protease), in complex with the adaptor NlpI. Without NlpI, Prc cannot effectively degrade MepS, highlighting NlpI's crucial role in MepS recruitment. Mutants lacking Prc or NlpI show increased MepS levels, leading to long filaments and growth defects in low-osmolarity conditions.

In 2017, the structure of the Prc-NlpI complex (PDB ID 5WQL) was determined by X-ray crystallography, revealing a symmetric NlpI homodimer attached to two bowl-shaped Prc proteins. NlpI, an adaptor protein with four tetratricopeptide repeats (TPRs), interacts with Prc through TPR2, forming an extensive electrostatic network. The unliganded PDZ domain of Prc has a misaligned conformation, which rearranges upon ligand binding, activating its proteolytic activity (**Fig. 1(a)**).[1] However, two important issues regarding the regulation mechanism of PG expansion have not been resolved, namely how NlpI regulates MepS to affect their activities and how NlpI modulates the protein levels of MepS in the presence of the Prc protease. To investigate the mechanisms underlying these two important issues, Shiou-Ru Tzeng (National Taiwan University) and her collaborators U-Ser Jeng (NSRRC) and Chun-Hsiang Huang (NSRRC) employed various experimental techniques, including nuclear magnetic resonance (NMR), biological small-angle X-ray scattering (BioSAXS), protein crystallography (PX), and other biochemical analysis methods. Specifically, X-ray diffraction and scattering data were acquired using the beamlines **TPS 05A**, **TPS 07A**, and **TPS 13A** at the NSRRC.

To elucidate how NlpI regulates MepS, NMR experiments were performed (**Fig. 1(b)**) and demonstrated that the mature full-length MepS (mMepS) can utilize its intrinsically disordered N-terminal region to interact with the adaptor protein NlpI. The crystal structure of the NlpI–mMepS complex reveals a heterohexameric assembly, where a homodimer of NlpI binds to four MepS molecules (**Fig. 1(c)**). This structural arrangement facilitates the colocalization and cooperative function of multiple MepS molecules, enhancing their avidity for PG binding and hydrolysis. Notably, upon binding to NlpI, the disordered N-terminal region of MepS undergoes a transition to an ordered state (**Fig. 1(d)**), promoting the dimerization of MepS. This structural insight provides a mechanistic understanding of how NlpI regulates the activity of MepS through modulating its oligomerization state and cooperative interactions with the PG substrate.

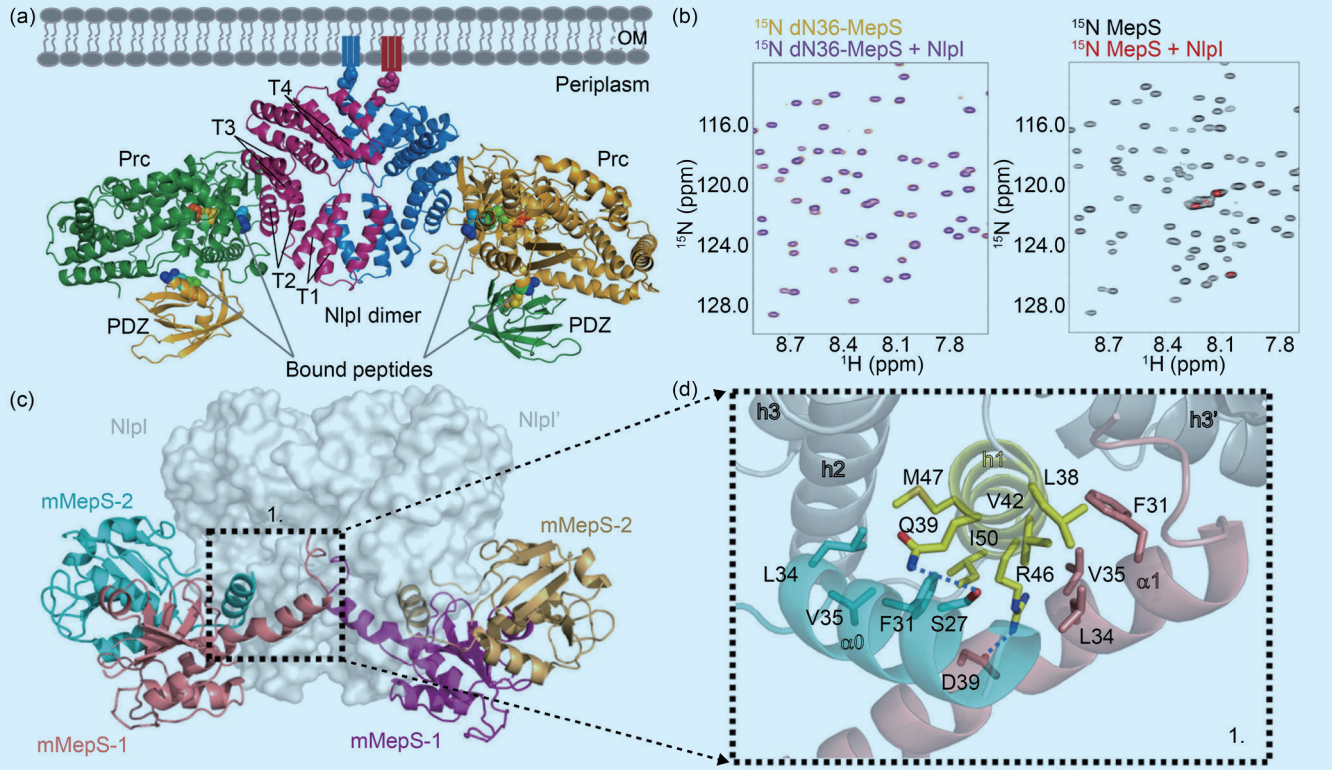

Fig. 1: (a) Overall structure of the NlpI–Prc complex shows a dimeric NlpI bound to two Prc proteins, represented in different colors. The PDZ domain of Prc and the four tetratricopeptide repeats (TPR1–4) of NlpI are labeled. The outer membrane (OM) and lipid anchors are depicted, with the first residues linked to the lipobox cysteine shown as spheres. Four co-crystallized substrate peptides are shown as rainbow-colored spheres. (b) NMR experiments were performed to study the interaction between NlpI and the mMepS proteins. ^{1}H-^{15}N TROSY-HSQC spectra were acquired for the truncated mutant dN36-MepS, which is devoid of the N-terminal 36 residues, and mMepS in the absence and presence of unlabeled NlpI dimer. (c) Overall structure of the NlpI-mMepS complex reveals a dimeric NlpI (grey surface) bound to four mMepS proteins (illustrated in different colors). (d) The interactions between NlpI and mMepS involve specific hydrophobic contacts. [Reproduced from Ref. 1 and Ref. 2]

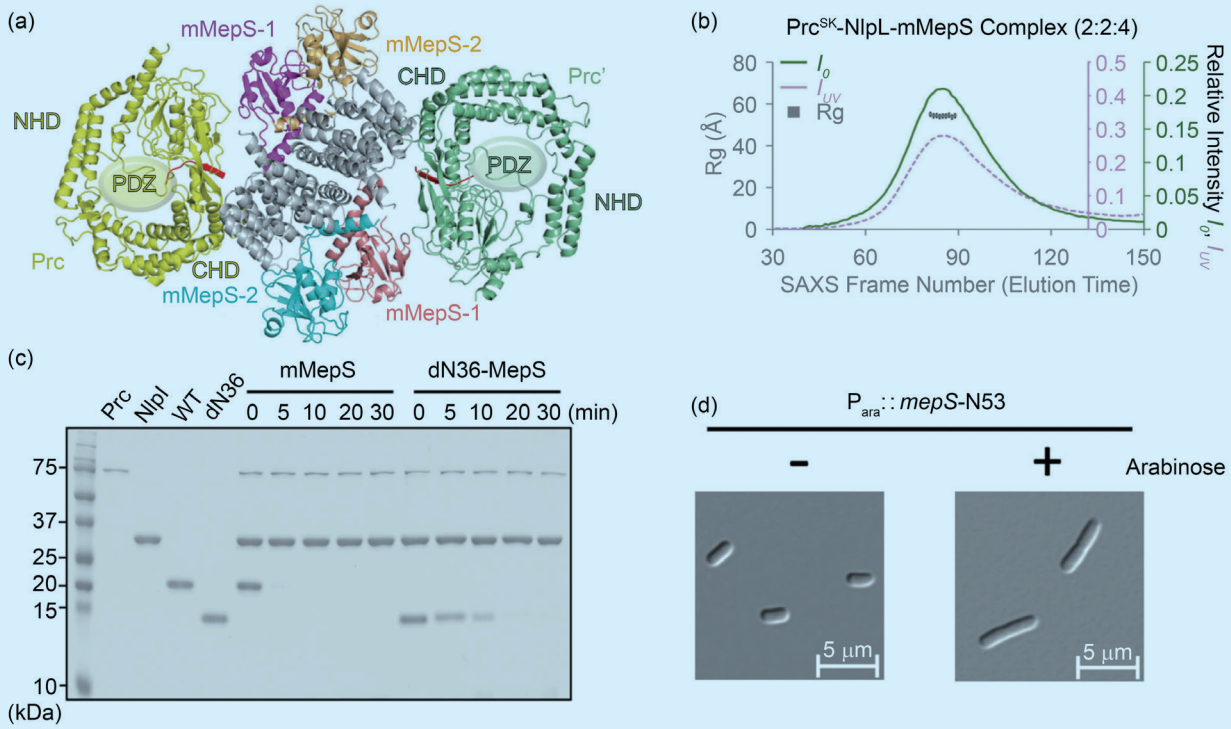

Fig. 2: (a) Overall structure of the Prc–NlpI-mMepS complex is shown, with NlpI in gray, Prc in yellow and pale green, and mMepS1 and mMepS2 in other colors. (b) A size-exclusion chromatogram of the Prc–NlpI–mMepS complex is presented, displaying the radius of gyration (*Rg*), zero-angle scattering intensity (I_0), and absorbance at 280 nm (I_{UV}). (c) *In vitro* degradation assays were conducted to examine the proteolytic activity of the Prc–NlpI system on mMepS and dN36-MepS. (d) The impact of overexpressing MepS-N53 on bacterial cell morphology was visualized using differential interference-contrast (DIC) microscopy. [Reproduced from Ref.1]

To understand how NlpI modulates the protein levels of MepS in the presence of the Prc protease, structural analysis was performed and revealed that the tail-specific protease Prc forms a 2:2:4 hetero-octameric complex with the adaptor protein NlpI and the endopeptidase mMepS (**Fig. 2(a)**). Size exclusion chromatography coupled with small-angle X-ray scattering (SEC-SAXS) experiments confirmed this 2:2:4 stoichiometry, ruling out the possibility that the observed stoichiometry was an artifact of crystal packing (**Fig. 2(b)**). Furthermore, the experimental data showed that the dN36-MepS significantly reduced its interaction with NlpI and decreased the degradation efficiency of mMepS by the Prc–NlpI system (**Fig. 2(c)**). This further confirms the important role of the N-terminal region in the recognition of mMepS by NlpI and the subsequent targeting of mMepS for Prc-mediated degradation. At the cellular level, overexpression of the N-terminal 53 residues of mMepS (MepS-N53) led to a significant change in bacterial morphology, resulting in the formation of long filamentous cells (**Fig. 2(d)**).

This study provides crucial structural insights into how the lipoprotein NlpI recruits and colocalizes the endopeptidase MepS, facilitating enhanced PG hydrolysis during bacterial cell growth. The binding of NlpI induces a disorder-to-order transition in the N-terminal region of MepS, promoting its dimerization and increasing enzymatic activity. Additionally, NlpI plays a pivotal role in targeting MepS for degradation by the protease Prc during the stationary phase, thus regulating the cellular levels of MepS and maintaining cell wall integrity. These findings advance our understanding of the molecular mechanisms underlying bacterial cell wall remodeling and highlight the functional versatility of NlpI in coordinating PG synthesis and degradation. (Reported by Chun-Hsiang Huang, NSRRC)

This report features the work of Shiou-Ru Tzeng and her collaborators published in Nat. Commun. ***15****, 5461 (2024).*

TPS 05A Protein Microcrystallography
TPS 07A Micro-focus Protein Crystallography
- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

TPS 13A Biological Small-angle X-ray Scattering
- BioSAXS
- Structural Transitions of Macromolecules in Solution

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