Molecular basis of ubiquitin modification by SdeA from *Legionella pneumophila*

With the support by the National Natural Science Foundation of China, the research group led by Prof. Feng Yue (冯越) at the Beijing Advanced Innovation Center for Soft Matter Science and Engineering, Beijing Key Laboratory of Bioprocess, Beijing University of Chemical Technology, uncovered the structural basis of a non-canonical ubiquitination, which was published in *Nature* (2018, 557(7707): 674—678). This paper was also featured by News & Views in the same issue of *Nature*.

Ubiquitination is one of the most prevalent protein modifications in eukaryotic cells, which regulates a wide array of essential cellular processes. Canonical ubiquitination is a three-enzyme cascade (E1, E2 and E3), finally transferring ubiquitin (Ub) to a lysine residue of the substrate, during which process G76 of Ub is involved and ATP is required. However, recent studies identified that the SidE family effectors from *Legionella pneumophila* could catalyze Ub transfer to several endoplasmic reticulum (ER)-associated human proteins, in a unique approach obviously different from the canonical ubiquitination pathway.

As the representative member of the SidE family members, SdeA contains a deubiquitinase/DUB domain ($SdeA^{DUB}$), a PDE domain ($SdeA^{PDE}$), an mART domain ($SdeA^{mART}$) and a C-terminal domain (CTD, $SdeA^{CTD}$). During the ubiquitination process, the R42 residue of Ub is first ADP-ribosylated with nicotinamide adenine dinucleotide (NAD^+) by $SdeA^{mART}$, and then the phosphodiester bond of the ADPrUb is cleaved by $SdeA^{PDE}$ to make PrUb, which can either remain by itself or be linked via a phosphodiester bond to the hydroxyl group of serine residues of the substrates or SdeA itself, also catalyzed by $SdeA^{PDE}$. However, the mechanism of these modifications awaits structural investigation.

Feng's group solved the crystal structures of SdeA in its ligand-free, Ub-bound, and Ub-NADH-bound states, respectively. In the $SdeA^{231—1190}$ structure, $SdeA^{mART}$ and $SdeA^{PDE}$ interact primarily through hydrophobic interactions and form a catalytic core, which sits on top of $SdeA^{CTD}$. They found that unlike $SdeA^{PDE}$, which is fully active as a single domain in processing ADPrUb to PrUb and catalyzing ubiquitination, $SdeA^{mART}$ needs to be stabilized by $SdeA^{PDE}$ to be active. In the $SdeA^{231—1190}$-Ub structure, the ARTT (ADP-ribosyltransferase toxin turn-turn) and PN (phosphate-nicotinamide) loops, and the α-helical lobe of $SdeA^{mART}$ together contribute to Ub recognition. In the $SdeA^{231—1190}$-Ub-NADH complex structure, NADH in the cavity forms a ring-like conformation.

Together with the structural and biochemical results, Feng's group proposed that during the catalytic process, Ub^{R72} might function as a "probe", together with Ub^{R74}, by anchoring Ub on $SdeA^{mART}$. After cleavage of the nicotinamide group from NAD^+, the strains in the highly folded structure of the intermediate would be alleviated, which might destabilize the binding of Ub^{R72}, causing its leaving. This in turn possibly facilitates the approaching of Ub^{R42} to the active site and the subsequent modification of Ub^{R42}. In all, their study provides unprecedented mechanistic insight into the structure and function of SdeA and serves as an important foundation for the further studies of the phosphoribosyl-ubiquitination.

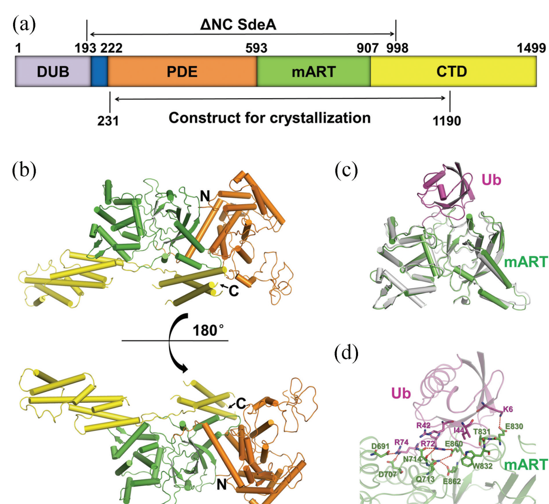

Figure The structures of SdeA and its complexes with ubiquitin. (a) Domain architecture of SdeA; (b) overall structure of SdeA; (c) and (d) the structure of $SdeA^{mART}$-Ub complex.