A thermostable organic-solvent-tolerant Lipase 42 (L42) was successfully crystallized under the condition of 0.1M MES monohydrate, 0.1M NaH_2PO_4, 0.1M KH_2PO_4 and 2.0M NaCl using counter-diffusion and sitting-drop vapor diffusion technique. Remarkably after data processing, counter-diffusion technique shows an enhancement of resolution at 2.0Å [1] as compared to sitting-drop method with only 2.4Å. There are several advantageous of counter-diffusion technique; it promotes slower and mild diffusion of protein and precipitant.

As a conclusion, a better L42 lipase resolution was achieved by counter-diffusion technique. Thus, by obtaining a better model of protein, deeper understanding on protein- solvent interaction could be obtained

[1] M.S. Khusaini, R.N.Z.R.A RAhman, M.S.M. Ali, T.C. Leow, A.B. Salleh, M. Basri. *Acta Cryst.* **2011** *F67*, 401-403.

Keywords: counter diffusion, vapour diffusion, organic solvent tolerant lipase

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A component of the xanthomonadaceae T4SS Combines a VirB7 motif with a N0 Domain

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Type IV secretion systems (T4SS) are used by Gram-negative bacteria to translocate protein and DNA substrates across the cell envelope and into target cells. Translocation across the outer membrane is achieved via a ringed tetradecameric outer membrane complex made up of a small VirB7 lipoprotein (normally 30 to 45 residues in the mature form) and the C-terminal domains of the VirB9 and VirB10 subunits [1]. Several species from the genera of *Xanthomonas* phytopathogens possess an uncharacterized type IV secretion system with some distinguishing features, one of which is an unusually large VirB7 subunit (118 residues in the mature form).

Here, we report the NMR and X-ray structures of the VirB7 subunit from *Xanthomonas citri* subsp. citri ($VirB7_{XAC2622}$) and its interaction with VirB9 [2]. The solution structure showed that $VirB7_{XAC2622}$ has an unfolded N-terminus and a unique C-terminal domain whose topology is strikingly similar to that of N0 domains found in proteins from different systems involved in transport across the bacterial outer membrane. We submitted the globular N0 domain of $VirB7_{XAC2622}$ to crystallization trials, that resulted in large plates which belong to space group $C222_1$ and diffracted up to 1.0 Å. Molecular replacement was performed using the solution structure of the globular domain as the search model, resulting, after refinement, in a model with R_{work} of 13.0% and R_{free} of 15.2%.

NMR solution studies showed that residues 27-41 of the disordered flexible N-terminal region of $VirB7_{XAC2622}$ interacts specifically with the VirB9 C-terminal domain, resulting in a significant reduction in the conformational freedom of both regions. We show that $VirB7_{XAC2622}$ oligomerizes through interactions involving conserved residues in the N0 domain and residues 42-49 within the flexible N-terminal region and that these homotropic interactions can persist in the presence of heterotropic interactions with VirB9. Finally, we propose that $VirB7_{XAC2622}$ oligomerization is compatible with the core complex structure in a manner such that the N0 domains form an extra layer on the perimeter of the tetradecameric ring.

[1] V. Chandran, R. Fronzes, S. Duquerroy, N. Cronin, J. Navaza, G. Waksman, *Nature* **2009**, *462*, 1011-1015. [2] D.P. Souza, M.O. Andrade, C.E. Alvarez-Martinez, G.M. Arantes, C.S. Farah, R.K. Salinas, *PLoS Pathogens* **2011**, *Accepted for publication*.

Keywords: structural biology, protein NMR, type IV secretion system

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The structure of a bacterial esterase essential for NO-stress response. Rafael M Couñago,

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Nitrosative stress is an important factor in host-pathogen interactions. *Neisseria gonorrhoeae* (ng) and *N. meningitidis* (nm) are closely related human pathogens that can pose significant health problems. Both bacteria display a series of mechanisms to cope with oxidative and nitrosative stress, which can come from the bacteria's own metabolism and the host's microenvironment, including the host's innate immune response. Previous work [1] has identified Esterase D (EstD) as essential for protection of *N. gonorrhoea* against nitrosative stress in vitro and for its intracellular survival in human primary cervical epithelial cells. EstD is a carboxylic ester hydrolase and is part of the NmlR regulon, which is upregulated during nitrosative stress and present in a number of pathogenic bacteria.

Here we present the structure of EstD from *N. meningiditis* (97% sequence identity to ngEstD) at 1.3 Å resolution. The enzyme displays the characteristic α/β hydrolase family fold and a catalytic triad formed by Ser, Asp and His residues. The identity of active site residues was further confirmed by mutagenesis studies. The structure of mnEstD also reveals that the "gate-keeper" Cys-54 residue is found at the enzyme's active site entrance. In the eukaryotic counterparts of Neisserial EstDs, oxidation of this residue by glutathione has been shown to modulate the enzyme's activity. Likewise, we show that wild-type mnEstD can be reversibly inhibited via oxidation of Cys-54 by glutathione and that an engineered Ala-54 mnEstD mutant is insensitive to glutathione challenge. Although common in eukaryotes, modulation of protein function by glutathione oxidation has been shown for only a handful of proteins in prokaryotes.

Ongoing experiments in *Neisseria* are to complement our biochemical and structural analysis of EstD and will provide a better understanding of how EstD's function protects these pathogens against nitrosative stress and the host's innate immune response.

[1] Potter et al., *J. Infect. Diseases* **2009**, *200*, 273-278.

Keywords: bacterial, esterase, crystallography

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Crystal structure of Clostridium perfringens Delta toxin and a model of its pore-form

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