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Biophysical Approach of Fragment Screening to inhibit the heme transfer system of Pathogenic bacteria

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Acquisition of iron from host sources is of vital importance to many pathogenic bacteria during the course of infection. The process of obtaining heme from the host by Gram-positive pathogens often involves binding of heme or hemoproteins by bacterial receptor proteins which then deliver the heme to a membrane transporter for translocation to the cytoplasm [1]. Interestingly, several pathogenic species of Gram-positive bacteria display surface proteins implicated in heme capture that contain more than one NEAT domain [2]. The heme capture protien has NTD, NEAT1, and NEAT2 domains. NTD has a hemoglobin-binding ability and both NEAT1 and NEAT2 has a heme-binding ability. However, their detail analysis and interaction among them is not done. We have studied the interaction with hemoglobin and its heme transfer system of Shr, and revealed that NTD can be a candidate of the target protein to inhibit the heme transfer.

Here we carried out the fragment-based drug screening to inhibit the interaction between hemoglobin and NTD. First hit screening of about 2000 compounds fragment library were conducted in surface plasmone resonance (SPR). After the 124 hit candidates were obtained in SPR screening, the competition assay of the candidates were carried out in SPR and we obtained 21 compounds that inhibit the interaction between hemoglobin and NTD. To validate the quality of the selected compounds in SPR, dose-dependent binding assay to NTD in SPR and thermal inhibition assay in isothermal titration calorimetry (ITC) were conducted [3]. In these hit validation, we obtained consequently some compounds that can inhibit specifically the PPI by binding to NTD.

[1] Nobles, C.L. and Maresso, A.W., The theft of host heme by Gram-positive pathogenic bacteria, *Metallomics*, 3:788-796, 2011.
[2] Andrade, M.A., Ciccarelli, F.D., Perez-Iratxeta, C. and Bork, P., NEAT: a domain duplicated in genes near the components of a putative Fe3+ siderophore transporter from Gram-positive pathogenic bacteria, *Genome Biology*, 3:research0047, 2002.
[3] Kobe, A., Caaveiro, J.M., Tashiro, S., Kajihara, D., Kikkawa, M., Mitani, T. and Tsumoto, K., Incorporation of rapid thermodynamic data in fragment-based drug discovery, *Journal of Medicinal Chemistry*, 56:2155-2159, 2013.