Structure of *P. aeruginosa* Hot-dog Fold Thioesterase, PA1618

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The Hot-dog Fold superfamily enzymes are represented in all domains of life and are dominated by acyl-thioester hydrolases. Thioesterases of the Hot-dog family are required for recycling of the thioesters formed by the actions of ligases or synthases (Zhuang *et al*., 2008). In short, thioesters are critical substituents among cellular metabolites and thus the size (> 40,000 enzymes) of this family mirrors the demands of cellular processes and metabolic pathways. The hotdog-fold thioesterase is a small (~ 19 kDa) ubiquitous enzyme fold comprised of 5-7 antiparallel β-sheets (the bun) wrapped around an elongated α-helix (the sausage) (Leesong *et al*., 1996). The catalytic residues are shared between two subunits, thus the homodimer forms the minimal catalytic unit (figure 1a); although their physiological oligomeric state is often tetrameric (figure 1b) or hexameric. Hydrolysis of thioesters involves either general base catalysis or nucleophilic catalysis. However, little is known in terms of their physiological substrate and structural determinants for substrate specificity or promiscuity.

The Hot-dog fold thioesterase structure determined during the 2012 RapiData course was PA1618 from *P. aeruginosa*, a homolog of *E. coli* Hot-dog thioesterase, YdiI, which by bioinformatic and biochemical analysis has been shown to be involved in the menaquinone pathway of *E. coli*. Notably, this pathway does not exist in *P. aeruginosa*, thus the PA1618 biological role and substrate has yet to be determined. The native PA1618 structure was solved in the $C222_1$ space group by remote access to SSRL beamline 12-2 using MAD phasing. Diffraction data was collected to 1.73 Å resolution.

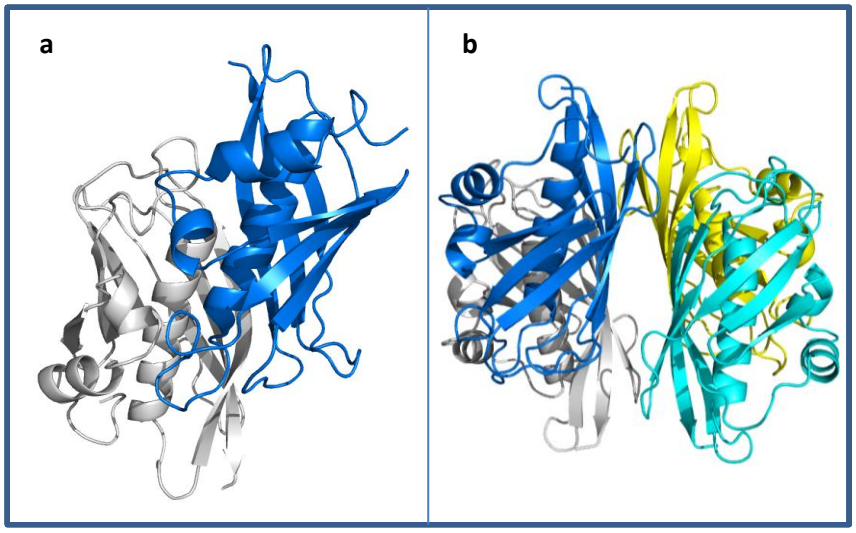

Figure 1. Native PA1618 structure solved during RapiData 2012. **a.** The dimer that forms the catalytic unit. **b.** The biological assembly of PA1618 is a tetramer.

Zhuang Z, et al. 2008. Divergence of function in the Hotdog-fold enzyme superfamily: the bacterial thioesterase YciA. *Biochemistry* **47**:2789–2796.

Leesong, M., B.S. Henderson, J.R. Gillig, J.M. Schwab and J.L. Smith (1996). Structure of a dehydratase-isomerase from the bacterial pathway for biosynthesis of unsaturated fatty acids: two catalytic activities in one active site. *Structure* **4**(3): 253-264.

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I attended the 2012 RapiData course as a 2nd year graduate student in the Allen laboratory at Boston University in the Molecular Biology, Cell Biology & Biochemistry program. Overall, the course was an amazing learning experience! It was incredible to be surrounded by a group of people with different levels of expertise and biological questions but coming together to share their interest and knowledge of X-ray crystallography. I had brought 96 crystals with me and the very first one yielded the winning crystal that diffracted to 1.73 Å, leading to the native structure of PA1618. For the following two days, Graeme Card and Clyde Smith (beamline specialists from SSRL) spent long hours helping me with data processing and refinement. The most memorable experience was when we encountered a problem in Phenix AutoBuild and realized that all we needed to do was walk over to Tom Terwilliger's station for help!