Crystallography

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Structural analysis of CrtM, an enzyme involved in staphyloxanthin formation in *Staphylococcus aureus*, as a new drug target for antibiotic therapy

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Staphylococcus aureus produces hospital- and community-acquired infections, with methicillin-resistant *S. aureus* posing a serious public health threat. The golden carotenoid pigment of *S. aureus*, staphyloxanthin, promotes resistance to reactive oxygen species and host neutrophil-based killing, and early enzymatic steps in staphyloxanthin production resemble those for cholesterol biosynthesis (1). We determined the crystal structures of *S. aureus* dehydrosqualene synthase (CrtM) at 1.58 Å, finding structural similarity to human squalene synthase (SQS). CrtM crystallizes in the $P3_221$ space group and there is one molecule per asymmetric unit. The overall fold shows clear similarity to that seen in human squalene synthase, as can be seen in the superposition (Fig. 1A and 1B).

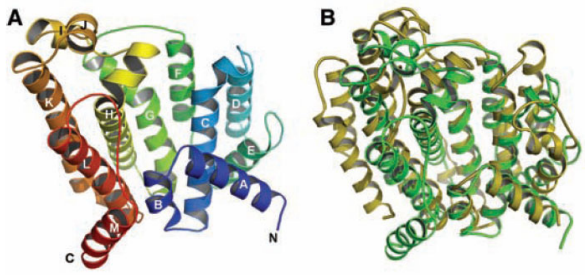

Figure 1.X-ray crystallographic structures. (A) X-ray structure of *S. aureus* CrtM. (B) Superposition of CrtM and human squalene synthase structures.

CrtM is all helical and the central cavity would possess two FPP binding sites to capable of accommodating the C_{30} product dehydrosqualene. *Thiolo*-farnesyl diphosphate (FsPP), a substrate-analog inhibitor of CrtM, which bind in the large central cavity of CrtM. Their diphosphate headgroups interacting with three Mg^{2+}, which in turn interact with Asp residues in the two conserved DXXXD repeats (Fig. 2A) seen in many prenyl synthases. Based on the structural similarity of CrtM and SQS, the SQS inhibitors developed as cholesterol-lowering drugs also had activity against *S. aureus* CrtM. All three phosphonosulfonates: BPH-652, BPH-698 and BPH-700, yielded well-resolved $2F_o$-F_c densities and the refined structures obtained are shown in Figures 2C-D, superimposed on the FsPP structure. BPH-652, a cholesterol lowering drug already tested in humanseffectively blocks staphyloxanthin biosynthesis *in vitro* (IC_{50} ~100 nM), resulting in colorless bacteria with diminished virulence that are cleared *in vivo* by the innate immune system, opening up a new approach to the development of highly specific anti-infectives against *S. aureus* (2).

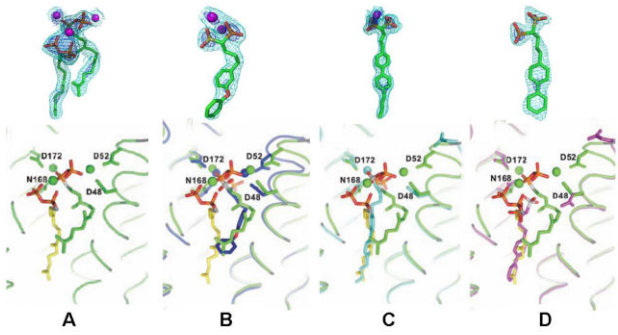

Figure 2. Electron densities and binding molds of substrate-analog inhibitor (A) and phosphonosulfonates (B-D).

Reference:

[1] G. Y. Liu *et al.*, J. Exp. Med. 202, 209-15 (2005).
[2] C. I. Liu *et al., Science* 319, 1391-4 (2008).

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