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Conserved allostery in heme-copper oxidases

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Heme-copper oxidases (HCOs) are terminal components of the respiratory electron transport chain. Recent studies have shown the enzymes in the respiratory chain containing HCOs as a target of antibiotics.

Recently we identified novel inhibitors of mitochondrial cytochrome c oxidase (mtCcO), which is a mammalian HCOs, and determined mtCcO structure in complex with the inhibitors. These structures showed two inhibitors bound to the same site of mtCcO. The inhibitor binding pocket is distant from the ligand-binding sites, suggesting that the pocket is an allosteric site of mtCcO.

The allosteric site of mtCcO forms a narrow tunnel with four helices. Three of them are conserved in bacterial HCOs, but the other isn't. These led us to hypothesize that the allosteric inhibitory mechanism is conserved in bacterial HCOs and we could reasonably screen novel antibiotics from derivatives of mtCcO inhibitor.

To this end, we established a custom library by using the ligand-based virtual screening system, LAILAPS, with the mtCcO inhibitors as queries from commercially available compounds. The custom library has a high probability of binding to not only the allosteric site of mtCcO but also the homologous position in bacterial HCOs: *E.coli* ubiquinol oxidase (ecoUqO). As results of the enzymatic analysis with the library, we identified both improved mtCcO inhibitors and importantly ecoUqO specific inhibitors. Cryogenic electron microscopy shows the ecoUqO specific inhibitor binds to the corresponding allosteric site in ecoUqO. Finally, antibacterial activity analysis showed the inhibitor prevented the growth of UqO-dependent *E. coli* strain, whereas the effect was abrogated in UqO-independent strain, thus the growth inhibition is not a nonspecific effect.

From these results, we conclude that the allosteric site is conserved among HCOs and can lead to the development of specific antibiotics for bacterial HCOs. In this presentation, we will also mention the mechanism of conserved allostery which was unveiled by molecular dynamics analysis and resonance Raman spectroscopy.