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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF MEMBRANE PROTEINS

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Abstract P-11: Microscale Thermophoresis of Mycobacterial Cytochrome P450 with Azole Drugs

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Background: Cytochrome P450 family members are found in most organisms where they are involved in the metabolism and synthesis of steroids, bile acids, unsaturated fatty acids, phenolic metabolites as well as exogenic chemicals. Drugs targeting cytochrome P450 have been shown to inhibit the growth of *Mycobacterium tuberculosis*, the causative agent of one of the deadliest diseases – tuberculosis. Recently, we showed that CYP124, CYP125, and CYP142 can bind and metabolize a panel of human immunoactive oxysterols *in vitro* (Varaksa *et al*., 2021) and one of them (CYP124) can metabolize antituberculosis drugs (Bukhdruker *et al*., 2020). Thus, inhibition of cytochrome P450 is a promising strategy for the development of new anti-tubercular drugs.
The existing methods used to assess protein-ligand interactions for cytochromes P450 (spectral titration and Surface Plasmon Resonance) have a number of limitations. In this regard, we used an alternative approach for this purposes – microscale thermophoresis (MST) which was not previously used for proteins of the cytochrome P450 superfamily
Methods: Here we show that MST can be used to determine the micromolar-range dissociation constants (Kd) of membrane-associated mycobacterial cytochrome CYP124 with small-molecule azole drugs. CYP124 was fluorescently labeled with Cy3-NHS and MST curves were collected at Monolith NT.115 instrument (blue/green channel, NanoTemper Technologies) in presence of various concentrations of azole compounds: econazole,

ketoconazole, itraconazole, and miconazole. The experimental results were approximated by the second-order bimolecular binding equation as well as by the Hill-Langmuir equation.
Results: Therefore, MST is a valuable method for the assessment of cytochrome P450 binding to their ligands for cases when traditional approaches are not applicable. The binding regime of CYP124 with azole derivatives was characterized by the structure of the CYP124 complex with carbethoxyhexyl imidazole solved with ~1Å resolution.

Key Words: tuberculosis • cytochrome P450 • microscaled thermophoresis

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