# Glycerol may jeopardize functional assays, lowering chances of success in drug design campaigns

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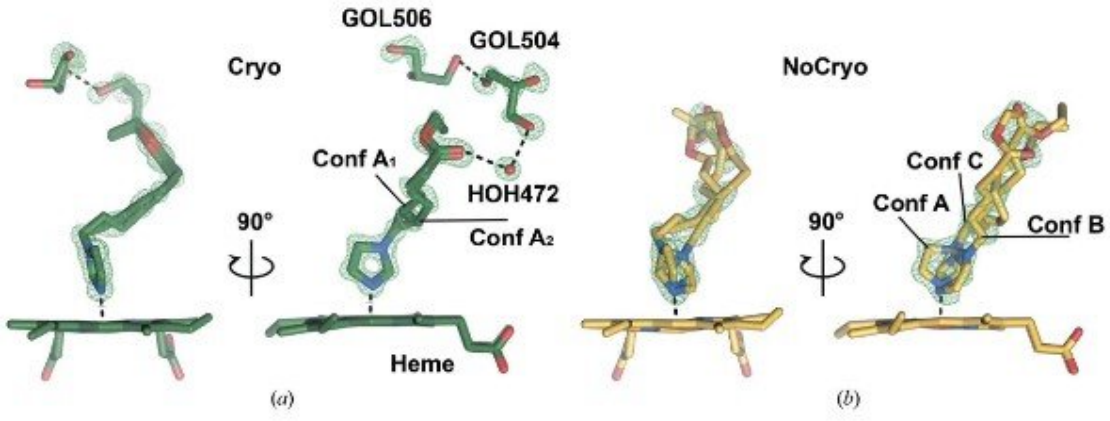


CHImi location in CYP124's active site: (a) crystal frozen using glycerol (Cryo); (b) no glycerol (NoCryo). Heme = cytochrome heme, HOH = water, GOL = glycerol molecules. Electron density maps are shown in green. Credit: *Acta Crystallographica Section D Structural Biology* (2023). DOI: 10.1107/S2059798322011019

New antitubercular drugs are vital due to the spread of resistant strains of Mycobacterium tuberculosis. A research team from Skoltech, MIPT, and the Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus (IBOCH NAS) has obtained ultra-high-resolution

structures of the M. tuberculosis cytochrome in complex with an inhibitor. The team's findings show that glycerol—a widely used additive to protein solutions—can compromise structural-functional studies and hinder drug development. The research was published in *Acta Crystallographica Section D Structural Biology*.

With tuberculosis remaining one of the deadliest human diseases and the number of drug-resistant strains growing every year, the development of new treatments becomes a compelling challenge for scientists worldwide.

According to one hypothesis, the P450 cytochromes are involved in drug resistance. However, the functions of most of the enzymes in this family are still unknown. Similar cytochromes exist in humans, too, and drug metabolism is one of their activities. M. tuberculosis infects human cells and manages to hide from the immune system inside macrophages like a spy. For the bacterium, the functioning of some cytochromes is the significant survival factor that determines its ability to evade the immune response.

It is important for drug developers to understand how cytochromes work. In various assays in the lab—from protein isolation to structural studies—scientists use glycerol to make the protein more stable during the experiment. The team questioned whether glycerol might affect the enzymatic activity.

Valentin Borshchevskiy, deputy director of the MIPT Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, explains, "Earlier we had solved the crystallographic structure of CYP124, one of the tuberculosis cytochromes complexed with the CHImi inhibitor, which serves as a prototype for new anti-tubercular drugs. We found glycerol molecules in the structure. To understand their effect on the enzyme, we determined the structure under the same

conditions but without glycerol. The resulting ultra-high resolution structure showed that the ligand has a different orientation and multiple conformations."

To check how glycerol affects ligand binding, the researchers performed functional tests based on spectrophotometric titration. The P450 cytochromes contain a prosthetic heme group with iron, which accounts for their red color and distinct absorption spectrum near ~450 nm (hence their name, P450). Binding small molecules affect the absorption spectrum—a change that can be registered by a spectrophotometer. The absorbance changes observed during equilibrium titrations of P450 with inhibitor plotted versus concentration allow calculation of the dissociation constant, a physicochemical value for binding specificity. The study demonstrated a tenfold decrease in affinity in the glycerol-containing buffer.

Natallia Strushkevich, an assistant professor at Skoltech Bio, comments, "We were lucky to obtain high-quality crystals for atomic analysis and confirm that adding glycerol to the solution affects the ability of cytochrome to bind inhibitors, which can present a problem for drug development."

The study showed that glycerol—a ubiquitous component in all biophysical structural studies—directly affects experimental results, causing misinterpretations and misleading researchers, thus putting drug design at risk. It is worthwhile to consider the effects of additional factors, such as glycerol, and carefully plan the experiment.

**More information:** Sergey Bukhdruker et al, Structural insights into the effects of glycerol on ligand binding to cytochrome P450, *Acta Crystallographica Section D Structural Biology* (2023). DOI: 10.1107/S2059798322011019

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