JINXED: Just in time crystallization for easy structure determination of biological macromolecules

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Macromolecular crystallography (MX) is a well-established method in the field of structure biology and has led to the majority of known protein structures to date. After focusing on static structures, the method is now developing towards the investigation of macromolecular structural dynamics, e.g. by looking at protein-ligand or enzyme-substrate interactions. These experiments often require multiple handling steps of the sensitive protein crystals, e.g. for ligand soaking and cryo-protection, which can cause significant crystal damage and a decrease in data quality. These handling steps are avoided in time-resolved experiments based on serial crystallography (SX) at room-temperature, where the reaction is triggered within the crystals prior to X-ray probing – either optically or chemically. For the latter, the use of micron-sized crystals is necessary to ensure short diffusion times of ligands and quick saturation within each crystal. Despite crystal size, certain crystal morphologies e.g. small solvent channels can prevent sufficient ligand diffusion. Presented here is a method combining protein crystallization and data collection in a novel one-step-process to overcome the aforementioned challenges. We successfully performed corresponding experiments as a proof-of-principle with hen egg white lysozyme and crystallization times of only a few seconds. This method called JINXED (Just in time crystallization for easy structure determination) uses the mixing setup of the CFEL TapeDrive 2.0 with an adapted nozzle for sample delivery and promises to result in high-quality data due the avoidance of crystal handling [1]. It could enable time-resolved experiments with crystals containing small solvent channels by adding potential ligands to the crystallizing agent or the protein, simulating traditional co-crystallization approaches. Furthermore, JINXED avoids several sample delivery challenges such as crystal settling or line clogging since all samples are in liquid form and can therefore be injected by auto-samplers connected to conventional HPLC systems. In conjunction with upcoming online and automated SX data processing, this method will offer the best of both serial and single crystal MX: Detailed insights into protein-ligand dynamics with a high level of automation – the future of high-throughput ligand screenings?

![Figure 1](image_url)

**Figure 1.** Drawings of a) sample environment overview with TapeDrive nozzle, tape, sample line and X-ray beam, b) TapeDrive nozzle with inner (left) and outer mixing (right) channel, c) JINXED principle with TapeDrive nozzle incorporating the protein solution channel (yellow) and crystallizing agent channel (blue), mixing within the sample line on the tape results in protein crystallization, and d) a possible high-throughput setup showing two automated micro-dispensing systems for samples (e.g. protein mixed with compound) and crystallization agents, the sample delivery system CFEL TapeDrive 2.0 including the TapeDrive nozzle (TDN), X-ray beam and detector.

[1] Henkel, A., Galchenkova, M., Maracke, J., Yefanov, O., Hakanpää, J., Mesters, J.R., Chapman, H.N., Oberthür, D. (2022) bioRxiv 2022.10.26.513656