Invited Lecture

Time-resolved serial crystallography and spectroscopy on earth w/ the XFEL Hub at Diamond

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We have developed several, on-demand, sample-efficient delivery and reaction initiation strategies that use room temperature microcrystal slurries and serial crystallography methods for time-resolved studies [1-3]. As illustrated in Fig. 1 and 2, our drop-on-drop on tape strategy produces high spatial resolution over a wide range time points and can be applied at synchrotrons or at XFELs. However, it can be challenging to interpret electron density maps from reaction cycle intermediates, especially when mixtures of species are present in the data. Therefore, to help reduce ambiguity we and our collaborators have also pioneered strategies to simultaneously collect time-resolved serial crystallography (tr-SSX/tr-SFX) diffraction data in the forward direction, and X-ray emission spectroscopy (tr-XES) data at ~ 90º, using either XFEL (tr-SFX) or synchrotron (tr-SSX) sources. The resulting atomic and electronic structures are fully correlated and have been applied to a range of enzymes [1, 2, 4-9]. For instance, isopenicillin N synthase (IPNS) uses nonheme iron to catalyse the O_2-dependent conversion of its tripeptide substrate delta-(L-alpha-aminoadipoyl)-L-cysteinyl-D-valine (ACV) into isopenicillin N (IPN, the precursor of all penicillin/cephalosporin beta-lactam antibiotics). The unique four electron oxidation reaction leading to the beta-lactam bicyclic ring proceeds via two high-valent iron species, an Fe(III)-superoxo and a high-spin Fe(IV)=O oxyferryl species. These enable two sequential C-H bond cleavage steps that each exhibit large kinetic isotope effects (KIE). Our recent tr-SFX and tr-XES studies have characterised the Fe(III)-superoxo species and revealed unexpected, correlated motions throughout the whole protein caused by O_2 binding [4].

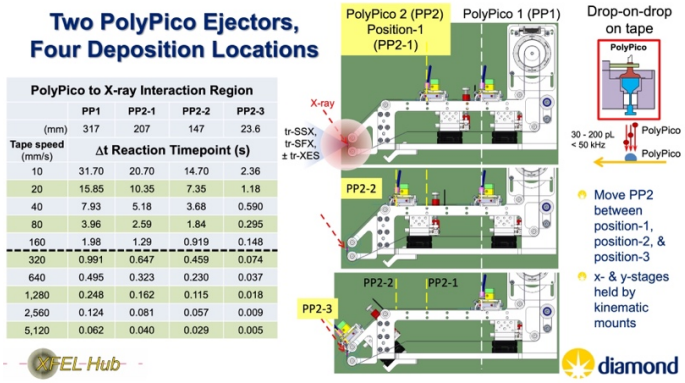

Figure 1. Schematic overview for time-resolved serial crystallography correlated with X-ray emission spectroscopy.

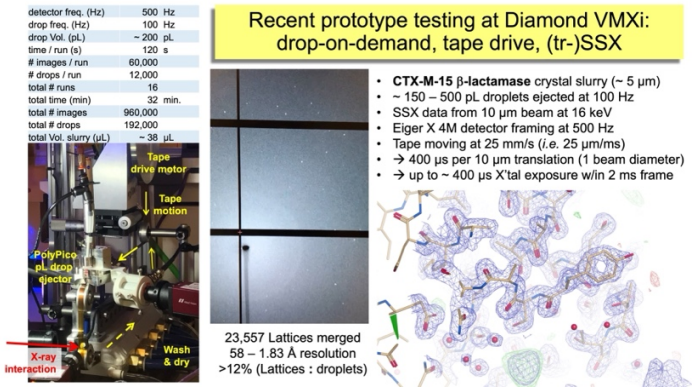

Figure 2. Our sample efficient, drop-on-demand on tape strategy provides high quality structures with no waste.

[1] Fuller, F.D., Gul, S., Chatterjee, R. et al., (2017) Nature Methods **14**, 443 – 449.

[2] Butryn, A., Simon, P.S., Aller, P. et al., (2021) Nature Communications **12**, 4461.

[3] Stubbs, J., Hornsey, T., Hanrahan, N., et al., (2024) IUCrJ **11**, 237 – 248.

[4] Rabe, P., Kamps, J. J. A. G., Sugherlin, K. D., Linyard, J. D., Aller, P. et al., (2021) Science Advances **7**, eabh0250.

[5] Lebrette, H,. Srinivas, V., John, J., Aurelius, O., et al., (2023) Science **382**, 109 – 113.

[6] Bhowmick, A., Hussein, R., Bogacz, I., Simon, P. S. et al., (2023) Nature **617**, 629 – 636.

[7] Nguyen, R. C., Davis, I., Dasgupta, M., Wang, Y., et al., (2023) J Am Chem Soc **145**, 25120 – 25133.

[8] John, J., Aurelius, O., Srinivas, V., Saura, P., et al., eLife **11**, e79226 (2022).

[9] Srinivas, V., Srinivas, V., Banerjee, R., Lebrette, H., Jones, J. C. et al., (2020) J Am Chem Soc **142**, 14249 – 14266.

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