

Oral Contributions

[MS21] Instrumentation at X-ray FELs and MX beamline for structural dynamic studies

Co-Chairs: Dave Stuart (UK), Sebastien Boutet (US)

[MS21-01] Serial Femtosecond Crystallography of Membrane Proteins with a Lipidic Cubic Phase injector

Uwe Weierstall¹, Daniel James¹, Dingjie Wang¹, John C.H. Spence¹, R.B. Doak¹, Petra Fromme², Martin Caffrey³, Vadim Cherezov⁴

¹Arizona State University, Department of Physics, USA

²Arizona State University, Department of Chemistry and Biochemistry, USA

³Trinity College Dublin, IE

⁴Dept. of Molecular Biology, The Scripps Research Institute, USA

The progress in structure determination of Membrane Proteins and GPCRs in particular is limited by the difficulty of preparing large amounts of homogenous and stable samples and growing sufficiently large crystals for collecting high-resolution diffraction data at available synchrotron microfocus beamlines. Ultimately the achievable resolution from small, well ordered protein crystals is limited by radiation damage. The emerging method of serial femtosecond crystallography (SFX) which uses ultrashort X-ray pulses from an X-ray free electron laser to outrun radiation damage, has been shown recently to achieve atomic resolution from microcrystals in a liquid jet [1], [2].

This approach obviates the need for larger crystals and eliminates radiation damage, addressing the most significant barriers to high-resolution structure determination success with small crystals. Protein crystals grown in LCP often have limited size and are therefore ideal for SFX experiments. Unfortunately, the injector developed for SFX can only be used with low

viscosity liquids [3] and a new approach is needed to generate a stream of the gel-like LCP with tens of micrometer diameter. A new LCP injector setup, allowing the collection of data from a contiguous

stream of nanocrystals embedded in LCP, has been built and tested. SFX is based on hundreds of thousands diffraction patterns, collected from a continuous stream of fully hydrated nano/microcrystals embedded in LCP or buffer liquid using hard X-ray femtosecond pulses. An overview of current injection devices with an emphasis on the LCP injector and recent results will be presented.

[1] L. Redecke, K. Nass, D. P. DePonte, T. A. White, D. Rehders, A. Barty, F. Stellato, M. Liang, T. R. M. Barends, S. BOUTET, G. J. Williams, M. Messerschmidt, M. M. Seibert, A. Aquila, D. Arnlund, S. Bajt, T. Barth, M. J. Bogan, C. Caleman, T. C. Chao, R. B. Doak, H. Fleckenstein, M. Frank, R. Fromme, L. Galli, I. Grotjohann, M. S. Hunter, L. C. Johansson, S. Kassemeyer, G. Katona, R. A. Kirian, R. Koopmann, C. Kupitz, L. Lomb, A. V. Martin, S. Mogk, R. Neutze, R. L. Shoeman, J. Steinbrener, N. Timneanu, D. Wang, U. Weierstall, N. A. Zatsepin, J. C. H. Spence, P. Fromme, I. Schlichting, M. Duszynko, C. Betzel, and H. N. Chapman, "Natively Inhibited Trypanosoma brucei Cathepsin B Structure Determined by Using an X-ray Laser," *Science*, vol. 339, no. 6116, pp. 227–230, Jan. 2013.

[2] S. Boutet, L. Lomb, G. J. Williams, T. R. M. Barends, A. Aquila, R. B. Doak, U. Weierstall, D. P. DePonte, J. Steinbrener, R. L. Shoeman, M. Messerschmidt, A. Barty, T. A. White, S. Kassemeyer, R. A. Kirian, M. M. Seibert, P. A. Montanez, C. Kenney, R. Herbst, P. Hart, J. Pines, G. Haller, S. M. Gruner, H. T. Philipp, M. W. Tate, M. Hromalik, L. J. Koerner, N. van Bakel, J. Morse, W. Ghonsalves, D. Arnlund, M. J. Bogan, C. Caleman, R. Fromme, C. Y. Hampton, M. S. Hunter, L. C. Johansson, G. Katona, C. Kupitz, M. Liang, A. V. Martin, K. Nass, L. Redecke, F. Stellato, N. Timneanu, D. Wang, N. A. Zatsepin, D. Schafer, J. Defever, R. Neutze, P. Fromme, J. C. H. Spence, H. N. Chapman, and I. Schlichting,

“High-resolution protein structure determination by serial femtosecond crystallography.” *Science*, vol. 337, no. 6092, pp.362–364, Jul. 2012.

[3] U. Weierstall, J. C. H. Spence, and R. B. Doak, “Injector for scattering measurements on fully solvated biospecies,” *Rev Sci Instrum*, vol. 83, no. 3, pp. 035108–035108, Mar. 2012.

Keywords: FEL free electron lasers, lipid mesophases, membrane protein X-ray crystal structure determination