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Ernest Laue is Professor of Structural Biology at the Department of Biochemistry, University of Cambridge. His research has focused on the development of new NMR methods and structural studies of protein complexes, in particular those involved in the assembly and control of chromatin structure. His group developed new ways to record NMR data that utilise pulsed field gradients and they have been particularly interested in developing new methods to process multidimensional (3D/4D) NMR spectra. During the course of these studies they realized that they could exploit new methods for processing NMR spectra, such as maximum entropy, in order to record NMR experiments more efficiently using selective (exponential) data sampling. As a result of their expertise in software development for NMR, his group were asked to set up and establish the CCPN project (<http://www.ccpn.ac.uk>) – a highly collaborative open source software development project, which involved many of the major NMR groups around the world – to coordinate the provision of a well-connected suite of software for NMR data analysis and structural studies.

His group currently focuses on biochemical and structural studies of the Nucleosome Remodelling and Deacetylase (NuRD) complex, which plays a key role in controlling chromatin structure during the early stages of stem cell differentiation. In addition to carrying out structural studies, they are attempting to understand how the complex controls chromatin structure *in vivo*. Around ten years ago they realized that one could take advantage of two key developments – single-molecule super-resolution fluorescence microscopy to image proteins at near molecular resolution *in vivo*, and high throughput DNA sequencing to determine the DNA sequence of single cells – to develop an NMR inspired approach to study nuclear architecture and to calculate 3D structures of chromosomes and intact genomes in single cells.

Abstract: Single Cell Hi-C and Single Molecule Imaging to Study Nuclear Architecture

Using data from this approach we have been able to calculate the first 3D structures of entire mammalian genomes in haploid embryonic stem cells, at present at a scale of ~25 kb (Stevens *et al.*, *Nature*, 2017; Lando *et al.*, *Nature Protocols*, 2018; Lando *et al.*, *Nucleus*, 2018). We can now see that the structures of chromosomes, individual topological-associated domains, loops, and the way they interact with each other, varies very substantially from cell to cell. Conversely, A and B compartments, lamina-associated domains, and active enhancers and promoters are organized in a consistent way on a genome-wide basis in every cell, suggesting that they could drive chromosome and genome folding. Mapping genome-wide ChIP- and RNA-seq data onto the single cell structures suggested that genes regulated by pluripotency factors and the NuRD complex cluster, a hypothesis that we were able to confirm using single molecule super resolution microscopy.

During this time, single-molecule imaging has also become a powerful method for tracking proteins and studying their dynamics in living cells. Currently, however, there is no generally applicable method to distinguish whether a protein is moving as part of one or several different complexes. We have also developed a novel FRET based approach, where a photo-stable acceptor dye is placed close proximity to a photo-activatable donor fluorophore. Transfer of energy via FRET increases the photo-stability of the donor fluorophore, thereby significantly increasing the length of time those molecules can be tracked in live mammalian cells. By tagging two different proteins the longer tracks can also be used to distinguish whether a protein is present in a particular complex, as opposed to being on its own or in other complexes (Basu *et al.*, *Nature Commun.*, 2018). We anticipate that this approach to directly image and map the localization of particular protein complexes will be very useful when combined with our approach for single-cell genome structure determination.