

## Conference Report

Molecular and Chemical Mechanism in Epigenetics – Swiss Summer School 2015  
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About forty participants, including eight invited international speakers, gathered in the Grand Hôtel Kurhaus in the peaceful village of Arolla, with a magnificent view on the majestic Mont Collon, for the first Swiss summer school in ‘Molecular and Chemical Mechanisms in Epigenetics’. This summer school, organized by Prof. Beat Fierz (EPFL) and Prof. Thomas Schalch (University of Geneva) focused on chemical methods to probe and dissect epigenetic processes, in combination with structural analyses of molecular complexes involved in epigenetic regulation as well as large-scale chromatin organization.



Heinz Neumann

On the first day of the meeting, chemical methods to synthesize chromatin carrying homogenous post-translational modification (PTM) patterns took center stage. Such reagents are of prime importance for functional *in vitro* studies of epigenetic mechanisms. **Heinz Neumann** (University of Göttingen, Germany) develops methods to expand the genetic code using evolved tRNA synthetase / tRNA pairs that accept non-natural amino acids and can incorporate those into proteins at designated positions. This chemical biology method is very powerful to study epigenetic processes such as PTMs, but also spectroscopic probes or chemical crosslinkers can readily be incorporated into histones or chromatin associated proteins. Neumann illustrated this approach by discussing his work on the effect of H3K56 acetylation, which he showed to result in more open and dynamic nucleosomes. Genetic code expansion is however not solely limited to *in vitro* studies, beautifully illustrated by his work on genetically incorporated photocrosslinkers that allowed

to investigate chromatin compaction in yeast cells. Finally, Neumann discussed interaction-mapping of histones with specialized binding proteins that dis- and reassemble chromatin during transcription, rendered possible by his powerful approach.

Protein semisynthesis, in particular expressed protein ligation (EPL), represents a complementary approach to synthetic chromatin. **Beat Fierz** (EPFL) presented key examples of mechanistic insights into epigenetic processes obtained by EPL approaches. After discussing his research on the functions of histone ubiquitylation, he focused on single-molecule imaging of epigenetic signaling mechanisms. Combinations of histone PTMs are ‘read’ by specific protein factors. For a combinatorial readout, multivalent interactions are of key importance. The Fierz laboratory thus conceived a methodology to directly observe such multivalent interactions of individual fluorescently labeled readers with immobilized synthetically modified chromatin. This method was applied to measure multivalent interactions of heterochromatin protein 1 (HP1) with chromatin that was specifically methylated using EPL. It turns out that multivalency not only increases the duration a factor stays bound to its chromatin substrate, but also leads to faster binding rates for multivalent factors.

A major aspect of chemical biology is the ability to employ small molecule chemistry to modulate and dissect biological pathways in living cells and whole organisms. In his lecture, **Raphael Rodriguez** (Curie Institute Paris, France) presented several intriguing stories on the discovery of small-molecule probe compounds and the identification of their targets. Lamin mutations are involved in diseases (laminopathies), such as the accelerated-aging disease Hutchinson-Gilford progeria syndrome. Biochemically, these mutations result in a disordered nuclear lamina, and thus to an irregular shape of the nucleus in affected cells. A small molecule screen for restoration of nuclear shape yielded the histone acetyltransferase inhibitor ‘Remodelin’ as an effective agent. Attaching an azide-moiety to the molecule, Rodriguez and colleagues could then employ click-chemistry for pulldowns of its protein target, which was revealed as the acetyltransferase NAT10, which is crucial for the maintenance of nuclear shape. In a second intriguing story Rodriguez described the elegant total synthesis of a cytotoxic natural product, Marmycin A, and the subsequent identification of its mode of function: Despite its structural similarity to DNA intercalators, Marmycin A was found to accumulate in lysosomes and trigger cell death. Finally, Rodriguez discussed highly innovative chemical biology application to target cancer stem cells.

In his lecture on Tuesday morning, **Matthew Fuchter** (Imperial College London, United Kingdom) focused on medical applications and discussed the development of epigenetic drugs, including new approaches to combat malaria. Such new compounds might serve as potent alternatives to current frontline drugs against which resistance has become a major issue. After a comprehensive introduction into medicinal chemistry, Fuchter illustrated the pitfalls of developing a chemical probe with a pertinent case study from his laboratory, where the analysis of a popular lysine methyltransferase (KMT) inhibitor, chaetocin, revealed that this molecule has a rather nonspecific activity profile in cells. KMTs are an interesting drug target, both for cancer therapy, as well as to inhibit the reproduction of the malaria



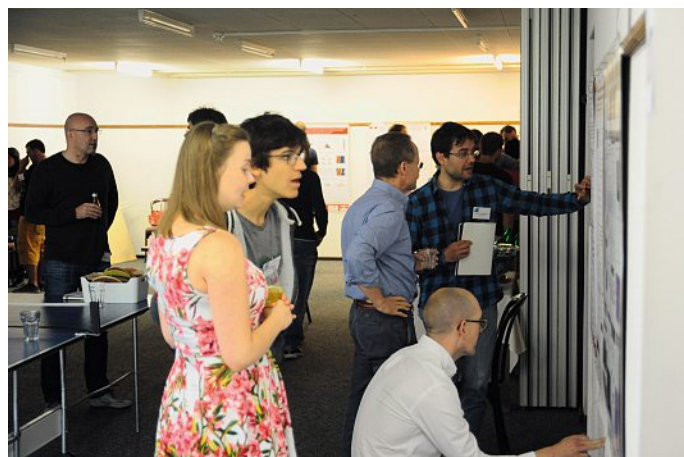
Matthew Fuchter

parasite. Here, exquisite specificity for the target enzyme is of prime importance to minimize side effects. The KMT inhibitor BIX-01294 and derivatives turned out to fulfill these prerequisites and showed great potency in cell and mouse experiments. This talk nicely illustrated the reach of epigenetic therapy, from cancer to parasitic diseases.

Molecular understanding of epigenetic mechanism requires insight into the structure of the, oftentimes very large, protein complexes involved in gene regulation. Furthermore, medicinal chemistry approaches greatly benefit from structural insight, for example into the enzyme active sites, the protein interaction motifs or allosteric regulatory positions. In a fascinating lecture, **John Schwabe** (University of Leicester, United Kingdom) provided in-depth insight into the structure and function of histone deacetylase (HDAC) co-repressor complexes. HDACs are numerous and belong to several enzyme families – they have wide ranging functions in transcriptional repression, in the DNA damage response and DNA replication. Furthermore, they represent a very important group of anticancer drug targets. Intriguingly, the activity of the enzymes critically depend on their complex member proteins, thus a deep understanding of the architecture of such complexes is paramount for the development of specific inhibitors. In his lecture, Schwabe described the discovery of a highly charged small molecule, inositol(1,4,5,6)-tetrakisphosphate (IP<sub>4</sub>), that forms an integral part in an important HDAC, the SMRT/NCoR complex. The complex is only stable if IP<sub>4</sub> is bound in a specific interface. This molecule, or the related IP<sub>6</sub>, which both occur naturally in mammalian cells, was further discovered to constitute a potent activator of several HDACs. This work demonstrates that small molecules modulate epigenetic enzymes directly *in vivo*.

On Tuesday afternoon, graduate students and postdocs presented their research during short research talks: **Yann Pierson** (EPFL) discussed the development of a FRET sensor to study DHFR inhibition in living cells. **Christiane Brugger** (University of Geneva) presented the structure of Clr3, a HDAC important for heterochromatin formation, followed by **Babatunde Ekundayo** (University of Geneva), who provided insight into chromatin fiber structure. **Kyle Douglass** (EPFL) combined super-resolution microscopic methods and theoretical considerations to reveal chromatin conformation in cells. Finally, the session was closed by **Aleksandra Vanceska** (EPFL) who studies telomeres, and in her work unveiled the crucial role of a specific protein, SMCHD1, for telomere maintenance.

On Wednesday the focus shifted to detailed biochemical and



Poster session

biophysical investigations of chromatin mechanisms. **Wolfgang Fischle** (Max Planck Institute of Göttingen, Germany) elegantly combines high-powered biochemistry with synthetic chromatin methods. In his lecture he discussed two case studies of protein ‘readers’ of the epigenetic histone PTM landscape. The ubiquitin ligase UHRF1 is recruited to chromatin *via* a reader domain specific for a heterochromatin-specific histone PTM. In the processing of reconstituting this interaction *in vitro*, the Fischle group discovered that the protein only interacts with its cognate PTM in the presence of a nuclear phosphoinositide. This regulation proceeds through an allosteric mechanism and underscores the importance for cofactors in regulating protein-protein interactions. Secondly, Fischle discussed multivalent chromatin interactions of HP1 and its role in higher order chromatin organization. Of note, in a fruitful collaboration, the Fischle and Neumann laboratories performed extensive crosslinking studies to demonstrate the ability of HP1 to engage multiple chromatin fibers simultaneously, thereby crossbridging chromatin domains and enacting longer range chromatin compaction.

**Andrew Flaus** (NUI Galway, Ireland) moved the basic unit of chromatin, the nucleosome, and its components, the histones, into the limelight. The human genome, and that of most higher eukaryotes, encodes a large number of canonical histone isoforms as well as variants with unknown function. This raises the question of whether the histone isoforms and variants can be localized on the genome and contribute to the responsiveness of local chromatin environments. Nucleosomes are formed from four histone types, each of which appears in two copies. This allows a huge number of combinations, with possible distinct functions. Flaus then demonstrated that one H2A variant prefers to form homotypic nucleosomes avoiding combination with canonical isoforms and thereby forming special nucleosomes.

On Thursday, bridging the gap of molecular biochemistry and cell biology, **David Shore** (University of Geneva) presented his group’s results on the factors that determine nucleosome architecture and transcriptional output in yeast. Previously, it was thought that eukaryotic promoters display a stereotypical chromatin landscape characterized by a well positioned ‘+1’ nucleosome at the transcription start site and a nucleosome-free region on the actual promoter, thereby allowing DNA access for transcription factors. Revising this paradigm, Shore demonstrated the existence of an elusive, highly dynamic ‘fragile’ nucleosome upstream of the +1 nucleosome at a subset of genes, using micrococcal nuclease titration analysis combined with next-generation DNA sequencing (MNase-Seq). The positioning of nucleosomes at promoters, as well as the dynamic nature of ‘fragile’ nucleosomes actually depend on chromatin remodeling activities, as demonstrated by elegant experiments exploiting the



Anchor-Away technique, in which the enzymes in question are physically removed from the nucleus by rapid anchoring to the cytoplasm. These studies demonstrated that chromatin is highly dynamic and actively remodeled in cells, to finely regulate gene expression.

The investigation of dynamic regulatory processes on a cellular scale requires novel, highly specific imaging techniques. The next speaker, **Kerstin Bystricky** (University of Toulouse, France) presented a recently developed, powerful approach (called ANCHOR) to directly observe chromosome conformational changes and motion in living cells: The Bystricky laboratory discovered that certain bacterial DNA binding proteins (ParB) involved in chromosome segregation, which oligomerize to form large nucleoprotein complexes, can be used to label single genomic loci in eukaryotic cells. When tagged with a fluorescent protein, this system allows the visualization of one or several distinct chromatin loci. Measuring the distance between two marked loci in three dimensions, using advanced microscopy techniques, and combining them with chromosome conformation capture approaches, reveals the dynamic large-scale chromatin folding on a single cell level. Using ANCHOR, Bystricky could directly image such conformational rearrangements upon activation of estrogen-responsive genes in human breast cancer cells, thereby highlighting the importance of chromatin dynamic changes during transcription initiation.

Following these inspiring lectures, Thursday afternoon was dedicated to research presentations of students and postdocs: **Andrea Calligari** (EPFL) discussed single-particle tracking approaches to investigate transcription factor binding kinetics in living cells. **Sinan Kilic** (EPFL) further expanded on single-molecule investigations of chromatin binding proteins, followed by **Eddie Rodriguez-Carballo** (University of Geneva), who elaborated on the analysis of a higher-order chromatin folding at a key developmental gene cluster. Finally, **Sophie Vieweg** (EPFL)

presented her studies on combining EPL and biochemistry to investigate the molecular determinants of huntingtin aggregation.

The last lecture of this exciting summer school was given by **Thomas Schalch** (University of Geneva). Taking the audience back to structural biology, Schalch presented his latest results in understanding heterochromatin formation, structure and regulation on a molecular scale. The Schalch laboratory, using fission yeast as a model organism, was able to solve the molecular structures of a host of key proteins important in heterochromatin establishment. In particular this included proteins involved in the RNA interference pathway that plays a key role in initiation of silencing, as well as a crucial methyltransferase and HDAC/remodeling complex. Schalch's talk demonstrated how a large and possibly partially unstructured scaffold protein can bring together two enzymatic functions (in his example the HDAC and remodeling activity) to generate an integrated molecular regulatory machine. This underscores the importance of elucidating complex partnerships of chromatin factors to deepen our understanding of epigenetic gene regulation.

After a week of stimulating discussions and excellent lectures, the summer school drew to a close. But not without the announcement of the student award for the best poster presentation. The prize went to **Anna-Sophia Reis** (EPFL), for her poster on the development of an elegant approach to discover the interacting partners of shelterin, a telomere-associated protein complex.

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Swiss Summer School participants outside the Hotel Kurhaus, Arolla