# GPF Symposium 2019

## Mapping Cellular Microenvironments: Proximity Labeling and Complexome Profiling

## Abstracts

**Julien Béthune**

## Next-generation BioID assays for the analysis of dynamic protein complexes

Heidelberg University Biochemistry Center (BZH)

Since its initial description in 2012, BioID has established itself as a robust and viable alternative to the classical AP-MS approach for the identification of protein-protein interactions. Fused to a protein of interest, the abortive biotin protein ligase BirA* mediates the non-specific biotinylation of vicinal proteins in living cells allowing their facile isolation and identification. Recently, we have engineered split-BioID, a protein-fragments complementation assay that greatly enhances the resolution of BioID by allowing the analysis of context-dependent protein complexes. Applied to the miRNA silencing pathway, split-BioID let us identify a novel miRISC-associated factor that promotes miRNA-mediated translation repression. We are further developing more versatile assays with the goal to obtain smaller labeling enzymes with improved labeling kinetics and that can be used in a split assay format.

**Isabell Bludau**

## Complex-centric proteome profiling by SEC-SWATH-MS

Institute of Molecular Systems Biology, ETH Zürich

Proteins are major effectors and regulators of biological processes and can elicit multiple functions depending on their interaction with other proteins. Therefore, it is of central interest in systems biology to determine the interactions and cooperation of proteins as a function of cell state. With the goal to enable the parallel detection of hundreds of protein complexes in a single operation, we have therefore developed an integrated experimental and computational technique (Heusel & Bludau at al., 2019). The method consists of size exclusion chromatography (SEC) to fractionate native protein complexes, SWATH/DIA mass spectrometry to precisely quantify the proteins in each SEC fraction, and the computational framework *CCprofiler* to detect and quantify protein complexes by error-controlled, complex-centric analysis using prior information from generic protein interaction maps. Application of our workflow to the HEK293 cell line proteome delineates 462 complexes composed of 2,127 protein subunits and further provided insights on novel sub-complexes and assembly intermediates of central regulatory complexes. We have recently extended our workflow to study rearrangements of protein complex assemblies across different cell states, providing insights into assembly changes that are not captured by full proteome analyses. Furthermore, we currently extend our workflow to take advantage of available peptide-level information in the SEC-SWATH-MS data to investigate proteoform-specific complex integration. We expect our workflow to enable novel insights into the interplay between different protein variants and their impact on protein interactions and functionality on an unprecedented, system wide scale.

Moritz Heusel*, Isabell Bludau*, George Rosenberger, Robin Hafen, Max Frank, Amir Banaei-Esfahani, Ben Collins, Matthias Gstaiger, Ruedi Aebersold *Complex-centric proteome profiling by SEC-SWATH-MS* **Molecular Systems Biology** Jan 14;15(1):e8438. (2019)

**Daniel Kownatzki-Danger**

## Analysis of native cardiac protein complexes by Complexome Profiling

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Cardiac contraction and relaxation depend on cyclic increases versus decreases of the intracellular calcium concentration, respectively. Hence, we hypothesize that stoichiometric control of intracellular Ca cycling through calcium uptake versus release from the sarco/endoplasmic reticulum (SER) is co-regulated during contraction (calcium-activated calcium release) and relaxation (spontaneous calcium leak).
Here, a mass spectrometry-based method described as *complexome profiling*, originally established to identify OXPHOS protein supercomplexes and assembly factors, is used to elucidate the composition of essential protein complexes important for the intracellular Ca cycling in ventricular cardiomyocytes. Digitonin solubilized, enriched membrane fractions of isolated ventricular cardiomyocytes from wildtype and phospholamban knockout mice were loaded and separated by blue native gradient gel electrophoresis (BN-PAGE). Gel lanes were cut and trypsin digested followed by mass spectrometry (LC-MS/MS).
Hierarchical clustering and analysis of migration patterns confirmed distinct groups of co-migrating proteins, most prominently OXPHOS complexes, which were used for quantitative validation. Importantly, at higher molecular weight dual Ca transport complexes comprised of the ryanodine receptor (RyR2) calcium release channel, the SER calcium ATPase SERCA2a, and each regulatory protein subunits were identified. Finally, combined with phospholamban knockout, complexome profiling enabled the close-to-native analysis of previously unknown supramolecular protein complexes comprised of dual RyR2-SERCA2a transporters, changes in abundance due to phospholamban deficiency, and translation of the concept to patient samples.

**Ralph Kehlenbach**

## Proteomic mapping of proteins at the inner nuclear membrane

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Several hundred proteins have been shown to reside at the inner nuclear membrane (INM), but the interaction partners or even the function of many of these proteins remain unknown. Using a modified APEX2 (enhanced ascorbate peroxidase 2)-approach with rapamycin-dependent targeting of the peroxidase to a protein of interest, we now searched for proteins that are in close proximity to VAPB, a protein that has previously been localized to the endoplasmic reticulum (ER) but is also found at the INM. In combination with stable isotope labeling with amino acids in cell culture (SILAC), we confirmed many of the established interaction partners of VAPB at the ER and identified new ones at the level of the INM.

**Lena Munzel**

## Mapping the interactors of yeast PROPPINs

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β-propellers that bind polyphosphoinositides, also called PROPPINs, are a conserved family of phosphatidylinositol-3-phosphate and phosphatidylinositol-3,5-bisphosphate binding proteins which are involved in the process of autophagy. Structurally they belong to the family of WD40-repeat proteins which are known to serve as platforms for multiple protein-protein interactions.
Most of the WD40-repeat proteins, including PROPPINs, comprise seven WD40-repeats each folding into a four-stranded antiparallel β-sheet (blade) which are further organized into a β-propeller architecture. The phosphoinositide binding of PROPPINs is mediated by two basic pockets at the circumference of the β-propeller which are formed by two conserved arginines. Due to their preference for phosphatidylinositol-3-phosphate and phosphatidylinositol-3,5-bisphosphate, PROPPINs are enriched at endosomes, vacuoles and autophagic membranes. So far only little is known about their function especially at endosomes. PROPPINs do not have an intrinsic enzymatic activity but seem to act as scaffolds. Uncovering their functions at the different compartments therefore depends on the identification of interactors.
Without success, a variety of techniques, including recombinant pull down assays and co-immunoprecipitations followed by mass spectrometry analysis, were applied to identify the interacting proteins of the three yeast PROPPINs (Atg18, Atg21 and Hsv2). One major problem is to preserve the integrity of PROPPIN complexes upon cell lysis. This can be overcome by the use of *in vivo*-labeling approaches like the proximity-dependent biotin identification (BioID) assay. Based on the protocol of Opitz et al. (2017) the BioID assay was optimized, validated using known interactors of the three yeast PROPPINs and compared to standard methods like co-immunoprecipitations.

**Uwe Schulte**

## High-resolution complexome profiling by cryo-slicing blue native gel electrophoresis coupled to LC-MS/MS (csBN-MS)

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Proteins exert their biological function through interactions with other proteins, either in dynamic protein assemblies or in the framework of stably formed protein complexes. The latter can be effectively resolved by their molecular size using native polyacrylamide gel electrophoresis (BN-PAGE), and analysis of such gel separations by liquid chromatography coupled mass spectrometry for identification of protein complexes (BN-MS) is well established. Application of this approach, however, has remained restricted to abundant, rather well-characterized protein entities (mainly from mitochondria).

We have developed an optimized complexome profiling approach comprising preparative-scale BN-PAGE separation, sub-millimeter sampling of broad gel lanes by cryotome slicing and comprehensive mass spectrometric analysis with accurate label-free protein quantification (csBN-MS). This approach allows for complexome profiling with unprecedented comprehensiveness (some thousand proteins) and effective size resolution (<5% difference of molecular weights) as demonstrated in a benchmarking study of rat brain mitochondria, and, more recently, application to preparations enriched for mouse kidney endosomes and brain plasma membrane. A range of examples will be presented specifically high-lighting the importance of effective size resolution, achievable sensitivity and coverage as well as potential challenges like reproducibility, dynamic range and, ultimately, determination of subunit composition.

**Oliver Valerius**

## The RACK1-proxiOME captured with BioID

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Eukaryotic *Receptor for Activated C Kinase-1* (RACK1; named Asc1 in *Saccharomyces cerevisiae*) is a 7-bladed Gβ-like WD40-repeat protein serving as a signaling scaffold at the head region of the ribosomal 40S subunit (*hr40S*). Its physical incorporation into the small ribosomal subunit places RACK1/Asc1 at the cellular site of mRNA translation-initiation close to the mRNA entry and exit tunnels of translating 80S ribosomes. We identified Asc1-proximal proteins beyond its physical ribosomal neighbors by using a quantitative SILAC-LCMS based BioID approach. *In vivo* labeling of Asc1-birA*-proximal proteins with biotin enabled the enrichment and identification of proteins involved in the regulation of general ribosome activity, homeostasis and cycle, but also in the respective adjustment of nuclear activities. Cellular adaptation through Asc1-mediated signal transduction (e.g. phosphorylation of shuttling proteins) might coordinate ribosomal with nuclear activities. Further BioID baits (e.g. Asc1-variants with amino acid exchanges at phospho- or ribosome-binding sites, or Asc1-proximal proteins), BioID2/turboID (smaller and faster biotin ligases), and split-BioIDs (Asc1 in combination with chosen Asc1-BioID candidates) will be used to confirm Asc1-proximal proteins and to reveal context-specific *hr40S* microenvironments.