Proteomics and Mass Spectrometry Laboratory

Department for Biomedical Research, Murtenstr. 28, 3008 Bern

UNIVERSITÄT BERN

The core facility team

The Proteomics and Mass Spectrometry Core Facility (PMSCF) of the University of Bern consists of a small team of dedicated scientists. Our mission is to provide proteomics and mass spectrometry services to anyone in Life Science wishing to identify and quantify peptides/proteins in their samples.

Our wetlab team receives samples and prepare them. When ready, our instrument scientist injects them in the instrument, which will deliver a set of data (chromatograms, spectra etc.) that needs interpreting.

The core of the *bioinformatics* work in our group consists in **confidently identifying and quantifying peptides and proteins**, producing **visualisation of the results** and when appropriate also **differential expression between groups.** We also provide bioinformatics support for downstream analysis such as **gene set enrichment**, STRING **network integration**, feature reduction such as least absolute shrinkage and selection operator (**Lasso**) etc.

Anne-Christine is the local computational scientist and will be your main port of call and guide through your bioinformatics project in our group.

Prof. Dr. phil. nat Manfred Heller

Head of core facility

M.Sc. Sophie Braga Lagache

Senior assistant

Natasha Buchs

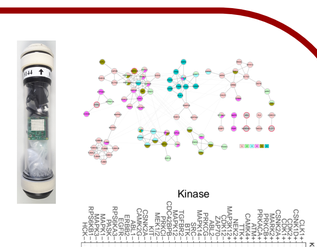
Lab assistant

Dr. phil. nat Anne-Christine Uldry

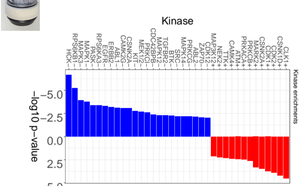
Computational scientist

Examples of past bioinfomatics student projects

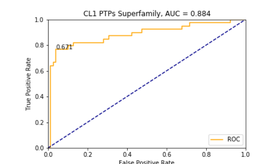
*) Blood transport study 2022 [1]: how the mode of transport of blood samples affects the proteome of circulating extracellular vesicles; proteomics coupled to acceleration sensor measurements as well as application of Lasso algorithm



*) Kinase Activity Enrichment Analysis 2021 [2] docker tool: phosphosites detected by mass spectrometry are investigated by re-purposing the gene set enrichment tool setrank in order to detect kinase activity in sample groups



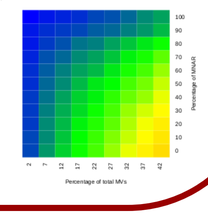
*) Master project 2021 (L. Kadamala Samuel): training of neural networks in order to predict phosphotase substrates from short AA motifs



*) 4 weeks project 2020 (V. Paukku): exploring the output of a variety of gene set enrichment tools

*) Master project 2019 (M. Jornod): development of software tools (python) for the Identification of cross-linked peptides

*) 4 weeks project 2018 (M. Jornod): exploring the effect of different imputation procedures on synthetic data



Present research activities:

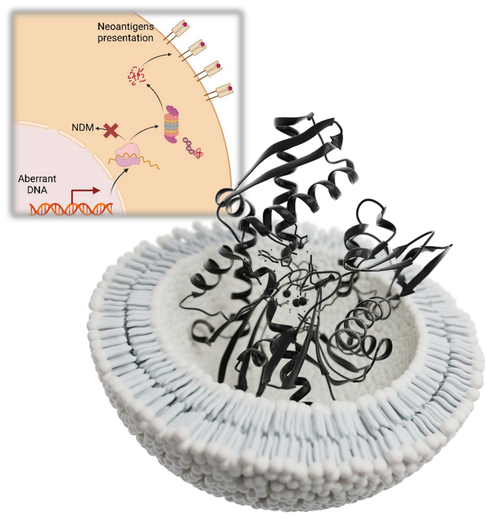
Immunopeptidomics

Alexandra joined our team end of 2022 to work on a proteogenomic project which consists in collecting cancer neoantigens and study them by mass spectrometry.

The idea is that we should be able to promote MHC-I presentation of neoantigens at the cell surface by inducing NMD inhibition, thereby potentially triggering an adequate immune response. Identifying these presenting peptides is therefore a crucial step towards a potential treatment.

Alexandra E. Burger

PhD student



Bioinformatics involved:

- Proteogenomics: using PacBio Iso-Seq data in order to produce a suitable protein database for the identification of neoantigens by mass spec (database search)
- *De novo* identifications to look for potential mutations and boost identifications
- Validation of presenting candidate peptides, including using *a priori* knowledge of immunopeptidomics

Extra-cellular vesicles

Extracellular vesicles (EVs) are lipid-bound enclosures that contain a variety of cellular components (metabolites, proteins etc.), and which the cells naturally secrete throughout their life cycle and death.

As the cells release EVs, many of them will be found circulating in the blood stream, and as such they serve as long distance messengers to all parts of the organism. EVs have indeed been shown to play roles in a variety of processes including signaling and cancer growth, and are now widely recognized as **potential disease biomarkers and treatment targets**.

Methods have been developed to collect peripheral blood or urine from donors and extract circulating EVs from it, and to analyze them by mass spectrometry.

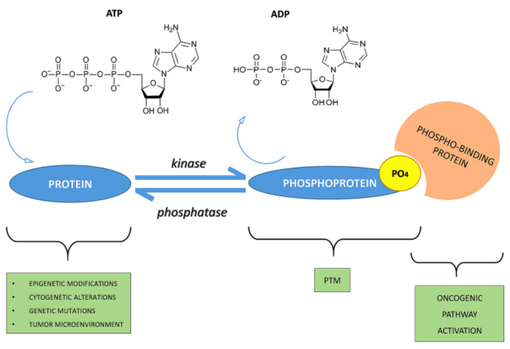
Bioinformatics involved:

- Data mining techniques (feature extractions etc)

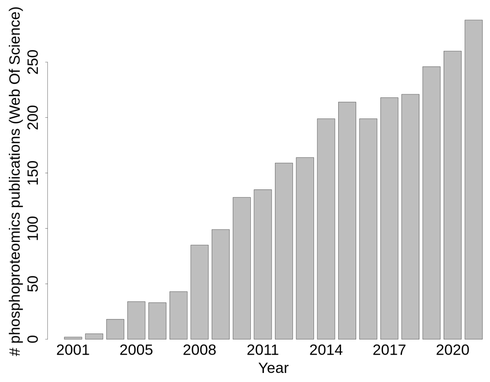
Hot topic: phosphoproteomics!

Phosphorylated peptides present special difficulties to mass spectrometers for a variety of reasons; providing solid identifications is a major bioinformatics challenge

1) Phosphorylation is a very common and all-important post-translational modification of proteins and is critical in many aspects of biology (picture from [3])

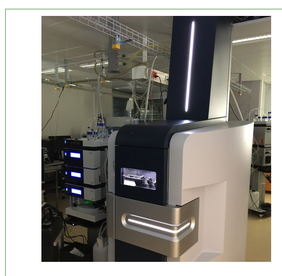


2) Differential phosphorylation levels for instance may be used to infer kinase activities and their potential mis-regulation. Reliable detection and quantification of phosphorylated peptides by mass spectrometry is therefore increasingly sought after by research groups [4]

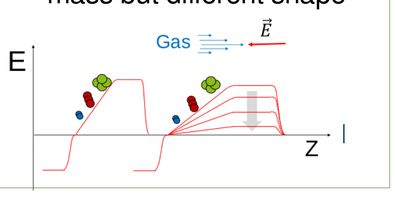


3) A new class of instruments (tims) using ion mobility has however lately opened up the possibility of separately fragmenting peptides carrying a phosphorylation on different sites due to an additional gas phase ion separation, globally leading to improved identifications

tims (**t**rapped **i**on **m**obility **s**pectrometry)

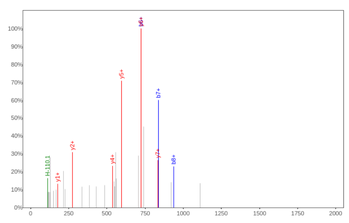


Ion mobility is used to separate ions of same mass but different shape



These 3 groups of ions (blue, red and green) will be measured separately, since they have a different ion mobility, characterised by the collision cross section (CCS) of the ions.

4) Yet controlling the quality of phosphopeptide identifications and quantification remains a challenge.



b+	b2+	#	Seq	#	y+	y2+
157.1084	79.0578	1	R	15		
270.1925	135.5999	2	L	14	1614.7636	807.8854
437.1909	219.0991	3	S	13	1501.6795	751.3434
538.2385	269.6229	4	T	12	1334.6811	667.8442
625.2706	313.1389	5	S	11	1233.6335	617.3204
722.3233	361.6653	6	P	10	1146.6014	573.8044
837.3563	419.1788	7	D	9	1049.5487	525.2780
936.4187	468.7130	8	V	8	934.5217	467.7645
1049.5027	525.2550	9	I	7	835.4533	418.2303
1177.5613	589.2843	10	Q	6	722.3692	361.6883
1234.5828	617.7950	11	G	5	594.3107	297.6590
1371.6417	686.3245	12	H	4	537.2892	269.1482
1499.7003	750.3538	13	Q	3	400.2303	200.6188
1596.7530	798.8802	14	P	2	272.1717	136.5895
		15	R	1	175.1190	88.0631

RLSTSPDVIQGHQPR

This peptide carries one phosphorylation PTM, but its localisation is uncertain!

Bioinformatics involved:

- CSS training and predictions, and inclusion of ion-mobility in phosphorylation validation
- Data curation, spectral library filtering
- Identification of kinase or phosphatase activities

[1] Effect of Sample Transportation on the Proteome of Human Circulating Blood Extracellular Vesicles (2022); Uldry A *et al;* Int. J.Mol. Sci. 2022,23, 4515; doi.org/10.3390/ijms23094515

[2] Inference of kinase-signaling networks in human myeloid cell line models by Phosphoproteomics using kinase activity enrichment analysis (KAEA) (2021); Hallal *et al*; BMC Cancer 21, 789; doi.org/10.1186/s12885-021-08479-z

[3] The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review) (2017); Ardito F *et al; I*nt J Mol Med. 2017;40(2):271-280; doi:10.3892/ijmm.2017.3036

[4] Computational systems approach towards phosphoproteomics (2022); Xiao D *et al*; Proteomicse 2200068; doi.org/10.1002/pmic.202200068