# 16th Fall Meeting
# Netherlands Proteomics Platform

## Next-generation proteomics approaches

Netherlands Proteomics Platform

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## Afternoon session: Oratie Symposium

## Translational OncoProteomics

VUmc Cancer Center Amsterdam

**14 December 2016**
**Auditorium, Vrije Universiteit**
**Amsterdam, The Netherlands**

**16th Fall Meeting of the Netherlands Proteomics Platform**
**14 December 2016 - Auditorium**
**Vrije Universiteit, Amsterdam**

Netherlands Proteomics Platform

# NPP Program
# 'Next-generation proteomics applications'

**9.00-9.30** **Registration and coffee**

9.30- 9.35 Welcome
*Connie Jimenez (NPP steering committee, VUmc)*

9.35- 9.45 Update EuPA
*Meike de Wit (NPP representative EuPA Young Proteomics Investigator Club)*

9.45-10.15 Drug Resistance Assessed by Multi-Proteomics Approaches
*Simone Lemeer, UU, Utrecht*

10.15-10.45 HLA-peptidomics, applications in T-cell mediated therapy
*Peter van Veelen, LUMC, Leiden*

**KEY NOTE LECTURE**
**10.45-11.45 Proteogenomics of cancer**
***Bing Zhang, Baylor College of Medicine, Houston, Texas***

**11.45-13.00 LUNCH BREAK**

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VUmc Cancer Center Amsterdam

# Oratie-symposium, VU auditorium

*Chair Prof. dr. Henk Verheul, Dept. Medical Oncology, VUmc*

13.00-13.25 Proteomics as a cornerstone in translational colorectal cancer research
*Gerrit Meijer, NKI, Amsterdam*

13.25-13.50 Proteomics of genetically engineered mouse models for breast cancer
*Sven Rottenberg, NKI, Amsterdam/ University of Bern*

13.50-14.15 Phosphoproteomics: towards personalized precision medicine
*Henk Verheul, VUmc, Amsterdam*

**KEY NOTE LECTURE**

**14.15-15.15 Genotypic Variability and the Quantitative Proteotype**
***Ruedi Aebersold, ETH, Zurich***

15.15- 15.45 Coffee/ tea break

## Oratie, VU Aula

**15.45- 16.30 Inaugural Lecture "Translational OncoProteomics"**
***Connie Jimenez***

16.30 18.00 Reception, Foyer, VU

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# Drug Resistance Assessed by Multi-Proteomics Approaches

***Simone Lemeer,*** *Utrecht University, Utrecht*

## ABSTRACT

Despite initially high response rates to the small molecule kinase inhibitor lapatinib in ERBB2 overexpressing breast cancer, the acquisition of drug resistance frequently occurs. Here, we used an established BT-474 cell line model of lapatinib resistance and employed explorative mass spectrometry to profile the proteome, kinome and phosphoproteome changes in an effort to systematically investigate initial inhibitor response and concomitant kinome reprogramming and signaling rewiring in resistance. The resulting dataset, which collectively contains quantitative data for > 7,800 proteins, > 300 protein kinases and > 15,000 phosphopeptides enabled deep insight into signaling recovery and molecular reprogramming upon resistance. Our data-driven approach confirms previously described mechanism of resistance (e.g. AXL overexpression and PIK3 reactivation), reveals novel, pharmacologically actionable targets (e.g. CDK1 and the spliceosome) and furthermore suggests a great heterogeneity in molecular resistance drivers, which drive a multitue of phenotypical changes. Furthermore, we identified an extensive and exclusively phosphorylation-mediated reprogramming of glycolytic activity which is further supported by widespread changes of corresponding metabolites and an increased sensitivity towards glycolysis inhibition. Collectively, this in depth, multi proteomic analysis offers a new and comprehensive perspective on the molecular mechanisms of resistance which points to new therapeutically relevant markers and treatment options for lapatinib sensitive and resistant ERBB2 overexpressing breast cancer.

## HLA-peptidomics, applications in T-cell mediated therapy

***Peter van Veelen,*** *Leiden University Medical Center, Leiden*

T cells play a key role in cancer and autoimmunity. The peptide-specific interaction of T cells, through their T cell receptor, with their targets is mediated by a peptide presented in so-called Major Histocompatibility Complex (MHC) molecules, which present an array of peptides. These peptides represent the situation inside the cell. Knowledge of these central interactors, i.e. the peptides, is a major step to understanding the disease process and cure. Many diseases have a strong HLA-association, indicating the importance of T cells. In cancer T cells can be used to specifically target tumor cells. Some strategies to identify relevant peptides, and comprehensive HLA-peptidomics will be discussed, including possibilities, limitations and opportunities.

**KEYNOTE**

## Proteogenomic analysis of human cancer

***Bing Zhang,*** *Baylor College of Medicine, Houston, USA*

### ABSTRACT

This lecture will introduce proteogenomics, a new research field at the interface of genomics and proteomics. I will describe several bioinformatics approaches for proteogenomic data integration and demonstrate their applications using genomic and proteomic data generated from the TCGA (The Cancer Genome Atlas) and CPTAC (Clinical Proteome Tumor Analysis Consortium) projects.

**Biosketch:**
Bing Zhang is a Professor of Biomedical Informatics at the Baylor College of Medicine at Vanderbilt University. He received a Ph.D. in molecular genetics from the Chinese Academy of Sciences and completed postdoctoral training at the University of Tennessee, Knoxville and Oak Ridge National Laboratory and worked till 2016 Vanderbilt University Medical Center. Dr. Zhang's research interest lies in mining high-dimensional molecular data to gain novel biological insights. His current work focuses on two research areas: i) proteogenomics: filling the gap between genotypes and phenotypes using proteomics data; and ii) network medicine: understanding complex diseases through modeling and analyzing molecular networks.
A main obstacle to these advances is our ability to effectively manage, integrate, and interpret the large volume of heterogeneous data. The long-term goal of our research is to develop computational and statistical approaches that help translate multidimensional omics data into biological and clinical insights. Recent work in my group focus on the following three areas: 1) Proteogenomics; 2) Network medicine; and 3) Democratization of bioinformatics (http://www.zhang-lab.org). His group also makes methods developed in these areas available to the broad research community through user-friendly software tools. For example, their pathway analysis tool WebGestalt (URL: http://www.webgestalt.org) was accessed 55,449 times by 21,029 users and was cited in 267 scientific papers in 2014 and more recently, Netgestalt an integration framework that allows simultaneous presentation of large-scale experimental and annotation data from various sources in the context of a biological network to facilitate data visualization, analysis, interpretation, and hypothesis generation.

# Proteomics as a cornerstone in translational colorectal cancer research

***Gerrit Meijer,*** *Nederlands Kanker Instituut, Amsterdam*

## ABSTRACT

The clinical proteomics studies of diffferent subproteomes (cell surface, secretome, nuclear matrix, chromatin-binding fraction; 1-5) and total lysates (6) of colorectal cells and tissues have increased the insight into the tumor biology of colorectal cancer (CRC) and its development from precursor lesions. They provide a valuable addition to the existing large collections of DNA and RNA datasets, thereby contributing to the multi-omics perspective of CRC and its precursors. In addition, these studies provide biomarker efforts aimed at different biological mechanisms that may be translated to different clinical diagnostic approaches.
Moreover, these relatively small-scale clinical proteomics studies have shown the power of label-free proteomics and have paved the way for the current large-scale profiling and biomarker validation studies that are on-going in the context of colorectal cancer screenings markers (stool proteomics data for N=300 patients; submitted) and prognostic biomarkers for CRC subtypes (on-going).

**Selected references**

1. De Wit M, **Jimenez CR**, Carvalho B, Belien JA, Delis-van Diemen PM, Mongera S, Piersma SR, Vikas M, Navani S, Pontén F, Meijer GA, Fijneman RJ. Cell surface proteomics identifies glucose transporter type 1 and prion protein as candidate biomarkers for colorectal adenoma-to-carcinoma progression. Gut. 2012 61(6): 855-64.

2. Fijneman, RJA., de Wit, M., Pourghiasian, M.,Piersma, SR., Pham, TV., Warmoes, M., Lavaei, M., Piso, C., Delis-van Diemen, PM., Robanus-Maandag, EC., Smits, R., Fodde, R., van Hinsbergh, VWM., Meijer GA., and **Jimenez, CR**. Proteome profiling of mouse colon tumor proximal fluids reveals candidate biomarkers for colorectal cancer screening. 2010. Clinical Cancer Research. 18(9):2613-24.

3. Albrethsen J, Knol JC, Piersma S, Pham TV, de Wit M, Mongera S, Carvalho B, Verheul HM, Fijneman RJ, Meijer GA, **Jimenez CR**. Sub-nuclear proteomics in colorectal cancer: Identification of proteins enriched in the nuclear matrix fraction and regulation in adenoma to carcinoma progression. Mol Cell Proteomics**.** 2010. 9(5):988-1005.

4. de Wit M, Kant H, Piersma SR, Pham TV, Mongera S, van Berkel MP, Boven E, Pontén F, Meijer GA, **Jimenez CR***, Fijneman RJ*. Colorectal cancer candidate biomarkers identified by tissue secretome proteome profiling. J Proteomics. 2014 Mar 17;99:26-39. *Shared senior authorship.

5: Knol JC, de Wit M, Albrethsen J, Piersma SR, Pham TV, Mongera S, Carvalho B, Fijneman RJ, Meijer GA, **Jiménez CR**. Proteomics of differential extraction fractions enriched for chromatin-binding proteins from colon adenoma and carcinoma tissues. Biochim Biophys Acta. 2014 May;1844(5):1034-43.

6. Snoeren N, Emmink BL, Koerkamp MJ, van Hooff SR, Goos JA, van Houdt WJ, de Wit M, Prins AM, Piersma SR, Pham TV, Belt EJ, Bril H, Stockmann HB, Meijer GA, van Hillegersberg R, Holstege FC, **Jimenez CR**, Fijneman RJ, Kranenburg OW, Rinkes IH. Maspin is a marker for early recurrence in primary stage III and IV colorectal cancer. Br J Cancer. 2013 Sep 17;109(6):1636-47.

# Proteomics of genetically engineered mouse models for breast cancer

***Sven Rottenberg**, NKI, Amsterdam/ University of Bern*

## ABSTRACT

Proteomics of a genetic mouse model for human BRCA1-deficient breast cancer in conjunction with network-based analysis has identified a new BRCAness signature that can identify patients with human genetic breast cancer (1) and revealed the impact of cisplatin in vivo (2). Secretome proteomics identified potential candidate biomarkers for non-invasive detection (3).

The tissue BRCAness signature shows high value as prognostic signatures for breast cancer. Based on these results a follow-up grant was obtained to validate the signature in a panel of state-of-the-art patient-derived xenograft models of triple negative breast cancer with known BRCA and homology-recombination deficiency status. Predictive potential will be validated in clinical trial material derived of patients treated with PARP inhibitors. These analyses that now also include phosphoproteomics are on-going.

### References

1. Warmoes, MO. Jaspers, JE.,Pham TV., Piersma SR, Massink MPG, Waisfisz Q., Rottenberg S, Boven, E., Jonkers, J., **Jimenez, CR.** Proteomics of mouse BRCA1-deficient mammary tumors identifies DNA repair proteins with diagnostic and prognostic value in human breast cancer. Mol Cell Proteomics. 2012. 11(7):M111.013334.

2. Warmoes M, Jaspers JE, Xu G, Sampadi BK, Pham TV, Knol JC, Piersma SR, Boven E, Jonkers J, Rottenberg S, Jimenez CR. Proteomics of genetically engineered mouse mammary tumors identifies fatty acid metabolism members as potential predictive markers for cisplatin resistance. Mol Cell Proteomics. 2013 May;12(5):1319-34.

3. Warmoes M, Lam SW, der Groep PV, Jaspers JE, Smolders YH, de Boer L, Pham TV, Piersma SR, Rottenberg S, Boven E, Jonkers J, van Diest PJ, Jimenez CR. Secretome proteomics reveals candidate non-invasive biomarkers of BRCA1 deficiency in breast cancer. Oncotarget. 2016 Aug 23.

# Phosphoproteomics: towards personalized precision medicine

***Henk Verheul,*** *Medische Oncologie, VU Medisch Centrum, Amsterdam*

## ABSTRACT

Protein phosphorylation by kinases plays a key role in crucial changes in the activity and/or wiring of intracellular signaling pathways and cell-transforming machineries. Cell/tissue-wide analysis of these phosphorylations using 'phosphoproteomics' may uncover information not available from genomics.
For successful phosphoproteome analyses, phosphopeptides need to be enriched prior to analysis by nanoLC-MS/MS. The OPL has implemented and benchmarked two robust and reproducible modalities for phosphopeptide capture: (i) titanium oxide for global capture of phosphopeptides with pSer, pThr or pTyr residues (1), and (ii) a pTyr-specific antibody for selective capture of pTyr residues (2). In recent years, successful downscaling of these methods (3) has enabled phosphoproteomic analyses of tumor biopsies in clinical studies. Moreover, a dedicated integrative data analysis strategy has been developed to identify hyperactive phosphokinases in individual tumor samples. Recent studies show the power of phosphoproteomics in various cancer cell lines studies (4-5) and patient-derived xenograft models, in which we "driver kinases" that are thought to play a central role in oncological transformation/progression.

We foresee that this approach will be used in the future to complement genomic information in the setting of patient selection for targeted therapies and personalized medicine. Tumor biopsies are being collected in multiple clinical trials at the VUmc Department of Medical Oncology and by the national Center for Personalized Cancer Treatment. In the coming years, the value of tumor biopsy phosphoproteomics for patient selection for targeted therapy will be established.

### References

1. Piersma SR, Knol JC, de Reus I, Labots M, Sampadi BK, Pham TV, Ishihama Y, Verheul HM, Jimenez CR. Feasibility of label-free phosphoproteomics and application to base-line signaling of colorectal cancer cell lines. J Proteomics. 2015 Sep 8;127(Pt B):247-58.

2. Van der Mijn JC, Labots M, Piersma SR, Pham TV, Knol JC, Broxterman HJ, Verheul HM, Jiménez CR. Evaluation of different phospho-tyrosine antibodies for label-free phosphoproteomics. J Proteomics. 2015 Sep 8;127(Pt B):259-63.

3. Mariette Labots, Johannes C. van der Mijn, Robin Beekhof, Sander R. Piersma, Richard R. de Goeij – de Haas, Thang V. Pham, Jaco C. Knol, Henk Dekker, Nicole C.T. van Grieken, Henk M.W. Verheul, Connie R. Jiménez; Phosphoproteomics scaled-down to biopsy level for analysis of individual tumor biology and treatment selection. In revision for J. Proteomics

4. De Vries-van Leeuwen IJ, da Costa Pereira D, Flach KD, Piersma SR, Haase C, Bier D, Yalcin Z, Michalides R, Feenstra KA, Jiménez CR, de Greef TF, Brunsveld L, Ottmann C, Zwart W, de Boer AH. Interaction of 14-3-3 proteins with the estrogen receptor alpha F domain provides a drug target interface. Proc Natl Acad Sci U S A. 2013 May 28;110(22):8894-9.

5. Van der Mijn JC, Broxterman HJ, Knol JC, Piersma SR, De Haas RR, Dekker H, Pham TV, Van Beusechem VW, Halmos B, Mier JW, Jiménez CR, Verheul HM. Sunitinib activates Axl signaling in renal cell cancer. Int J Cancer. 2016 Jun 15;138(12):3002-10.

6. Nagel R, Stigter-van Walsum M, Buijze M, van den Berg J, van der Meulen IH,Hodzic J, Piersma SR, Pham TV, Jiménez CR, van Beusechem VW, Brakenhoff RH. Genome-wide siRNA Screen Identifies the Radiosensitizing Effect of Downregulation of MASTL and FOXM1 in NSCLC. Mol Cancer Ther. 2015 Jun;14(6):1434-44.

**KEY NOTE**

## Genotypic Variability and the Quantitative Proteotype

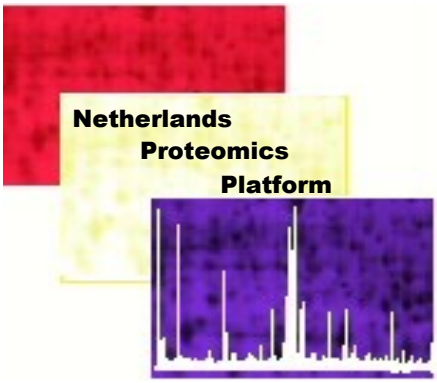
***Ruedi Aebersold,*** *ETH, Zurich*

The question how genetic variability is translated into phenotypes is fundamental in biology and medicine. Powerful genomic technologies now determine genetic variability at a genomic level and at unprecedented speed, accuracy and (low) cost and this technology is used extensively in the clinic, particularly in the field of oncology. Concurrently, life style monitoring devices and improved clinical diagnostic procedures generate an even larger amount of phenotypic information. To date the effects of genomic variability on the expressed information of the cell, and thus on the phenotype, have been mainly studied by transcript profiling.

The systematic determination of the effects of genomic variability on the proteotype (the acute state of the proteome of a cell) is a challenging analytical chemistry problem because a large number of proteins need to be precisely and consistently quantified across a large number of samples of a cohort. In contrast to the next generation sequencing techniques of genomics, at present there is no proteomic method known that consistently quantifies the entire proteome of a sample with a throughput that support cohort analyses. Yet, it is expected that proteomic analyses will be particularly informative because they reflect the biochemical state of the cell.

In this presentation we will discuss emerging computational and quantitative proteomic technologies to relate genotypic variation to the proteome. Proteomic data to support such correlations need to be quantitatively accurate, highly reproducible across multiple measurements and samples and generated at high throughput. Ideally, the data also would provide information about spatial arrangement of proteins in the cell. Data with these qualities can now be generated by the targeted proteomic methods selected reaction monitoring (SRM) and, at higher throughput, by SWATH-MS.
We will discuss the principles of these mass spectrometric methods, discuss the computational challenged they pose for data analysis and demonstrate with selected applications, using genetic reference strain compendia and clinical sample cohorts, their ability to determine the effect of genetic variability on the quantitative proteome, thus functionally connecting the genome to the proteome

**Biosketch** Ruedi Aebersold is a Swiss and Canadian scientist trained as a cell biologist at the Biocenter of the University of Basel. He completed his education at the California Institute of Technology. He holds an appointment as Professor at the ETH (Swiss Federal Institute of Technology) Zurich, with a joint appointment at the University of Zurich, Switzerland. He has served on the faculties of the Universities of British Columbia and Washington and co-founded the Seattle Institute for Systems Biology. He participates as a member of Scientific Advisory Boards for a number of academic and private sector research organizations and has served as senior editor for the journals *Molecular and Cellular Proteomics* and *Molecular Systems Biology*. He has co-founded several companies and was the chairman of the biology/disease related branch of the human proteome project of the world human proteome organization HUPO till December 2014. The research focus of his group is the proteome. The group has pioneered several and important and widely used development in proteomics, including stable isotope based proteome quantification, open access/open source software and statistical tools supporting proteomic analyses, targeted proteomics for the generation of accurately quantitative, reproducible datasets and chemical cross linking/mass spectrometry for the analysis of proteins in their cellular context. The work has been recognized with numerous awards and prizes. More than 40 trainees of the group have reached faculty status at leading research institutions in the US, Canada, Australia, Europe and China.




## Achtergrondinformatie over het Netherlands Proteomics Platform

Het Nederlands Proteomics Platform is eind 2000 opgericht door Connie Jimenez, met als doel kennis en informatie over proteomics technologie uit te kunnen wisselen tussen Nederlandse research groepen. Het NPP opereert onder de paraplu van de Nederlsandse Vereniging voor Biochemie en Moleculaire Biologie (NVBMB) en iedereen die geïnteresseerd is kan gratis lid worden, mits men lid is van de NVBMB. Studenten die lid zijn van de KNCV en jonger zijn dan 25 jaar kunnen kosteloos lid worden. Momenteel telt het Proteomics Platform Nederland ongeveer 100 leden.

Het proteomics platform houdt zich voornamelijk bezig met het organiseren van jaarlijkse bijeenkomsten waarbij informatieoverdracht via lezingen en workshops centraal staat.

*Het platform wordt gecoördineerd door de volgende mensen:*

Connie R. Jiménez (VUmc) (c.jimenez@vumc.nl ) (representeert het NPP in EuPA)
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**Thank you for your visit, we hope to welcome you again next year!**