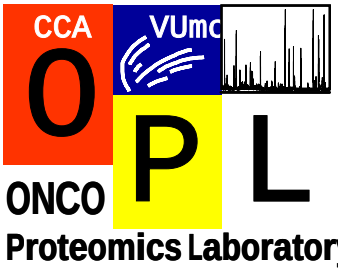
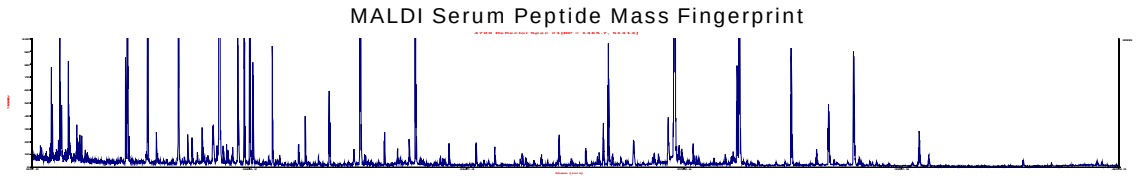


OncoProteomics Laboratory Annual Report 2006

Voorwoord



Dit is het eerste jaarverslag van het nieuwe OncoProteomics Laboratorium (OPL) dat gehuisvest is in het al even nieuwe gebouw voor kankeronderzoek van de VUmc Cancer Center Amsterdam (CCA). Het OPL is 13 april 2006 van start gegaan met de installatie van de eerste van twee geavanceerde, complementaire tandem massa spectrometers en met de aanstelling van het eerste personeel (hoofd, Dr. Connie R. Jimenez; biochemisch analist, Dr. Jaco Knol en massa spectrometrist, Dr. Sander Piersma). In april was het echt pionieren in het nieuwe CCA gebouw dat toen nog niet officieel bewoond werd en waar bijvoorbeeld nog geen internet aansluitingen waren. Dat veranderde gelukkig in mei toen de eerste CCA-V-ICI researchers het pand in trokken.
Binnen een maand na de installatie van de MALDI-TOF/TOF en eerste training van het personeel werden in mei al de eerste eiwit profielen in serum gemeten en de eerste eiwitten geïdentificeerd. Een vliegende start dus. Het installatie traject van de LTQ-FTMS met toebehoren (nanoLC en chip-interface) nam bij elkaar wat langer in beslag (mei-juni), en werd gevolgd door een periode van training op de 3 apparaten en technische validatie van de totale set-up. De eerste in-depth analyse van een complex pre-klinische monster mbv van de nanoLC-FTSM setup werd succesvol in september gemeten. Sindsdien zijn we met beide platforms in de lucht en lopen er verscheidene projecten op het terrein van cancer proteomics en biomarker discovery in veelal klinische monsters. In oktober werd het OPL versterkt met een computer scientist (Dr. Thang Pham) die mbv geavanceerde algoritmes en machine learning biomarker patronen destilleert uit de complexe massaspectrometrie datasets.
In dit verslag van het eerste half jaar van het OPL, zijn de (opstartende) onderzoeksactiviteiten van het OPL beschreven.

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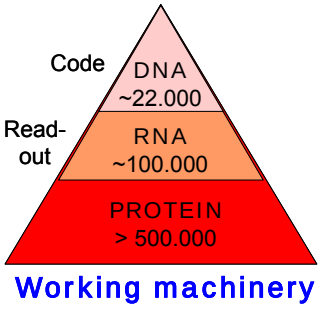
Contents

1. Introduction

The OncoProteomics Laboratory (OPL) has been founded in April 2006 together with the establishment of the cancer research building of the VUmc-Cancer Center Amsterdam (CCA). The OPL was created to provide a state-of-the-art proteomics infrastructure and knowledge center for CCA/V-ICI researchers.

Proteomics is a relatively new field to create a link between genomic information and biological function through global studies of protein expression, protein modification and protein-protein interactions Until now, proteomics has been a technology-driven science The emphasis in the coming years will be on applying proteomics to the understanding of biological function in healthy organisms and in disease.



Mission of the OPL
The mission of the OPL is to develop and implement innovative proteomics technologies and data analysis methods to improve diagnostics and treatment of cancer.

To this end, a major focus is on developing and implementing robust strategies for biomarker discovery in tumor tissue, in biofluids such as blood-serum/plasma that can be collected non-invasively, as well as on proximal fluids such as CSF and nipple aspirate fluid. In addition, cancer cell conditioned media and tumor secretomes hold great promise for discovery of candidate biomarkers. The samples are profiled using two complementary platforms for the discovery of diagnostic, predictive and drug response patterns and biomarkers: 1. An automated magnetic bead peptide capture method coupled to high-throughput MALDI-TOF/TOF mass spectrometry and 2. Label-free (nano)LC-LTQ-FT mass spectrometry of sub-fractionated samples for in-depth profiling.

Additional tasks of the OPL are facilitation and coordination of collaborative proteomics research projects with CCA-V-ICI researchers (and beyond), training and to obtain funding. Guidelines for the sample and project submission procedures can be found in appendix 1.

OPL research
The projects of the OPL broadly can be subdivided into 3 categories: 1. collaborative research projects (the majority with CCA-V-ICI researchers), 2. OPL core research (proteomics research to test and set up methods important for cancer biomarker discovery as well as oncoproteomics research projects initiated by the OPL) and 3. service projects (protein identification work on a fee-per-sample basis).

Information exchange

- In weekly lunch meetings (every Friday), proteomics data are discussed with the people working in the lab.
- Every first Friday of the month, the Friday lunchtime meeting is a joint meeting with the proteomics people from MCN-VU.
- Every other week on Friday afternoon, a proteomics data analysis meeting takes place. with collaborators from FEW-VU computer science and mathematics (including a joint post-doc)
- In monthly meetings (every 4th Tuesday lunch time starting in jan 2007) cancer proteomics topics of general interest will be discussed in a broader group of CCA-V-ICI researchers.
- All OPL people attend and participate in the weekly Friday morning department seminars of Medical Oncology.

2. Organisation of the OPL

The OncoProteomics Laboratory is a facilitating center where most projects are shaped in close interaction with the collaborators. Moreover, a substantial part of the activities is core research to develop and implement proteomics methods of general interest for cancer researchers and clinicians.
Head of the unit is a scientist/coordinator (1 fte, CR Jimenez) who is leading a team of scientists (3 fte, a mass spectrometrist, S. Piersma; a computer scientist, T. Pham; and a biochemical technician, J. Knol) as well as co-workers on temporary projects and PhD students, post-docs and technicians on collaborative projects.
The OPL is housed at the Dept. of Medical Oncology. The head OPL is assisted by a 'Program-Advisory-Committee' with representatives from the major CCA/V-ICI departments (Medical Oncology, Hematology, Pathology, KNO, Molecular, Cellular Biology and Immunology, and Epidemiology and Biostatistics) who meet every 2 months with the head of the OPL to discuss progress, developments and assist in prioritizing projects. Furthermore, the head OPL is assisted by Cluster I in organizational and financial matters. See figure 1 for an outline of the organizational structure. Currently there has been no substantial secretarial assistance.

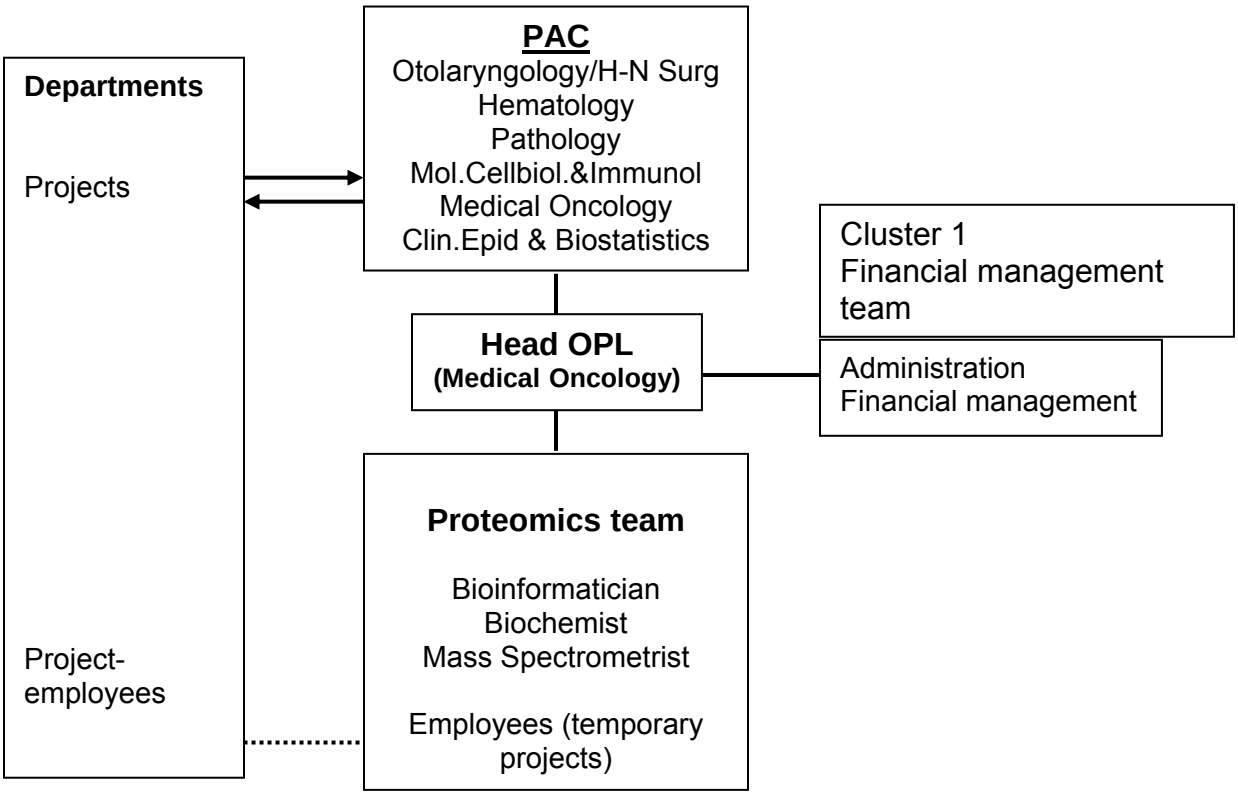


Figure 1. Schematic representation of the organisation structure of the OPL.

3. Infrastructure

The OPL is housed on the first floor of the CCA building with two laboratory spaces: a small protein chemistry lab (CCA 1-52) and a mass spectrometry lab (CCA 1-47). The lab spaces were experienced overall as adequate in 2006. The only minor point is that the MS lab is very noisy. In 2007 an effort may be made to put isolating shields around the instruments. For now, ear plugs have been the short-term solution.

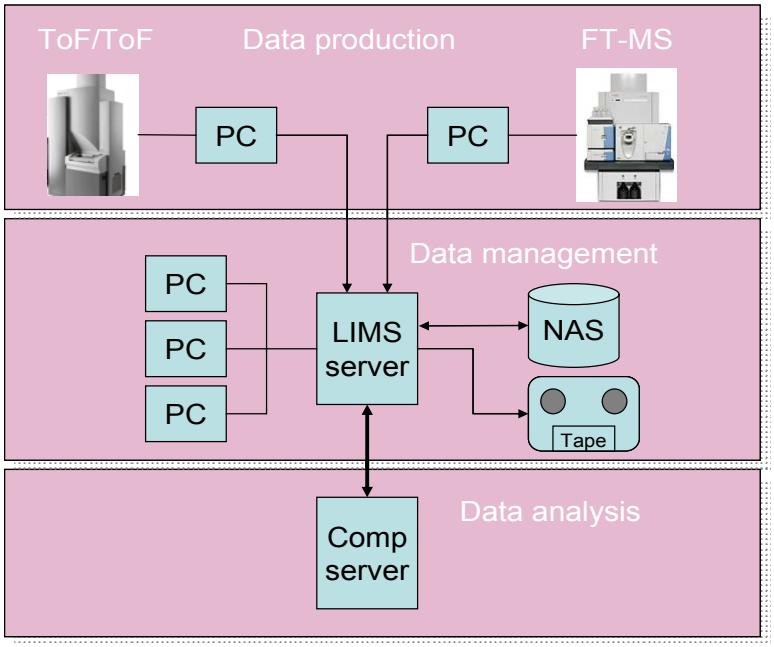
The OPL office space is very limited: a single small room that is shared by the 4 core people (head, 2 staff and technician). Two technicians on collaborative projects share a desk in the room next door (courtesy Henk Broxterman). As the OPL is expanding in 2007 with visiting foreign post-docs, masters students and a technician, more space is needed to accommodate everyone together on the first floor.

Instrumentation

Instrument	Company	Price kEuro incl.
LTQ-FTMS & KingFisher	ThermoElectron	
2D LC system	Dionex	
NanoMate Triversa	Advion Biosciences	
4800 MALDI-TOF/TOF	Applied Biosystems	
Liquid Handling system	Contribution Hamilton	
Scaffold	Proteome Software	
UV cell nanoLC	Dionex	
TOTAAL		**1.545K**
*Nog te besteden aan software en hardware in 2007:		**89.8K**

Upgrade of the IT infrastructure of the OPL in 2007

LC-FTMS experiments create large volume datasets (~ 0.5-1 Gb/ sample), therefore the data production per day may get as high as 10 Gb. In a typical profiling experiment we may need to compare series of up to100 LC-FTMS runs, which calls for separate LIMS and data analysis servers. A computation cluster is preferred over a single server. For data storage NAS local storage is more economical and we will opt for a solution of tape storage for finished projects and long-term storage. With the money that remains from the CCA start-up grant, we may cover the hardware expenses but not a Laboratory Information Management System (LIMS).



4. Research strategy and running projects

I. OPL core research:

Development of robust automated mass spectrometry-based methods for cancer biomarker discovery

The lack of reliable, robust and easily assessable biomarkers greatly hampers cancer management. Proteins are ideal biomarkers as they can be immuno-stained in routine paraffin-embedded specimen and immuno-detected in blood using conventional ELISA. In recent years, proteomics has raised the hope of identifying novel biomarkers for cancer diagnosis and detection. This hope is based on the ability of proteomics technologies such as mass spectrometry, to identify hundreds of proteins in complex biofluids

A major focus of the OPL is on targeted MS-based proteomics for biomarker discovery in tumor tissue and biofluids. The samples are profiled using two complementary platforms: 1. An automated magnetic bead-based peptide capture method coupled to high-throughput MALDI-TOF-MS and 2. Peptide separation by nano-liquid chromatography (LC) coupled to FTMS for in-depth profiling of fractionated samples (Fig 1). Cancer-related proteins may provide novel drug targets and candidate biomarkers for development into non-invasive (multiplex) antibody-based assays.

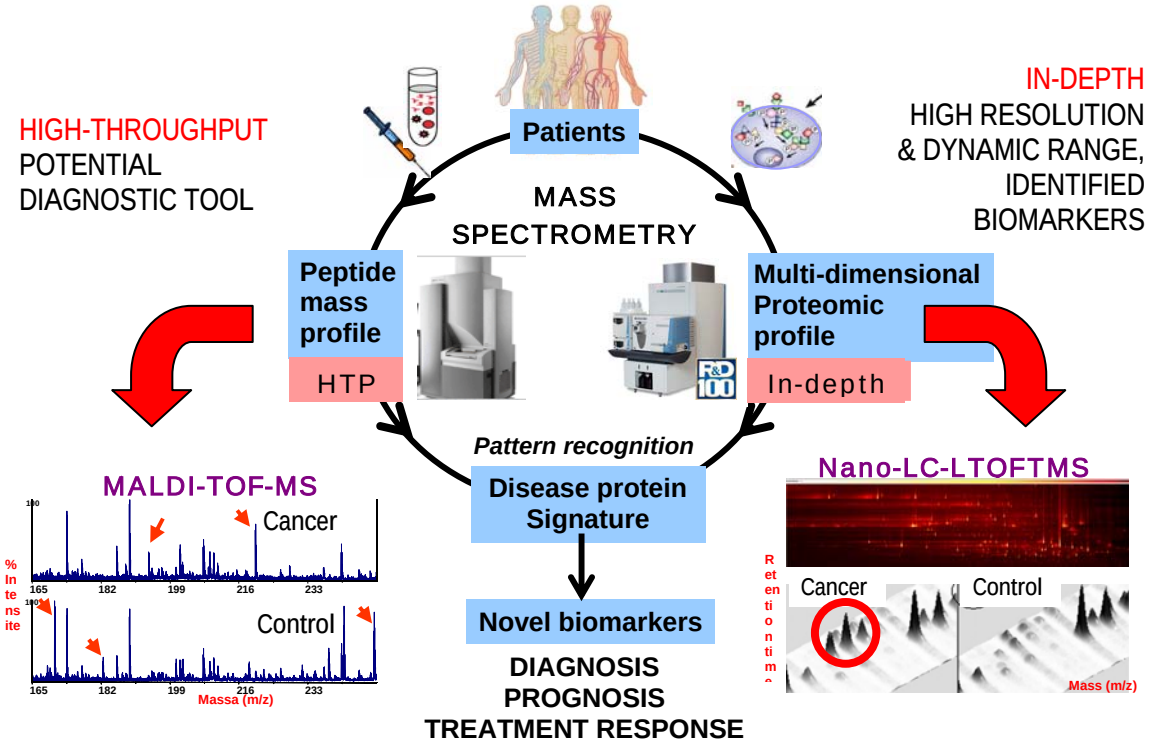


Fig 1. Complementary mass spectrometry-based proteomics approaches for discovery of cancer signatures and biomarkers at the OPL in the VUmc-Cancer Center Amsterdam.

1. Pattern diagnostics by MALDI-TOF-MS-based high-throughput peptide profiling

Recent studies have established distinctive serum polypeptide patterns through MS and bioinformatics that reportedly correlate with clinically relevant outcomes. Moreover, they provided a direct link between peptide marker profiles of cancer and differential protease activity, suggesting that the patterns may have clinical utility as surrogate markers for detection and classification of cancer.

We have shown that automated magnetic-particle-assisted peptide capture coupled to MALDI-TOF-MS provides a fast and reproducible profiling platform for measuring peaks in the low molecular mass range of the serum and CSF proteomes (Fig. 2; Jimenez et al., in press & submitted). Advantages of the method are: it is high-throughput (HTP): ~100 samples can be processed and measured in less than a day, and it uses only little material (for blood-serum 20 μl is enough). In addition, the method is also being applied to the analysis of endogenous peptides and small proteins in proximal fluids such as CSF, tumor secretomes and cancer-cell conditioned media.

Treatment response monitoring. We are currently examining the alterations in the serum profiles across sample sets from healthy subjects and cancer patients in phase I/II trials in order to assess the feasibility of the current platform in combination with decision algorithms to detect biomarker signatures associated with different cancer types, and with drug response (prediction) (collaboration G. Giaccone, K. Hoekman, MedOnc).

Quality control screening tool. MALDI-TOF-MS-based profiling provides a rapid screening tool to drive decisions on sample selection prior to more in-depth analysis by (2D)LC-FTMS. Peptides indicative of serum and CSF sample quality have been identified (Jimenez et al., submitted and in prep). Therefore, MALDI-TOF-MS may be used as an upfront quality control step. Multivariate analysis of the data sets would help reveal potential outliers as a result of either sample handling or intrinsic patient variability. This would aid in the selection of a smaller sample subset for in-depth comparative analysis using lower throughput LC-MS based strategies (see under 2).

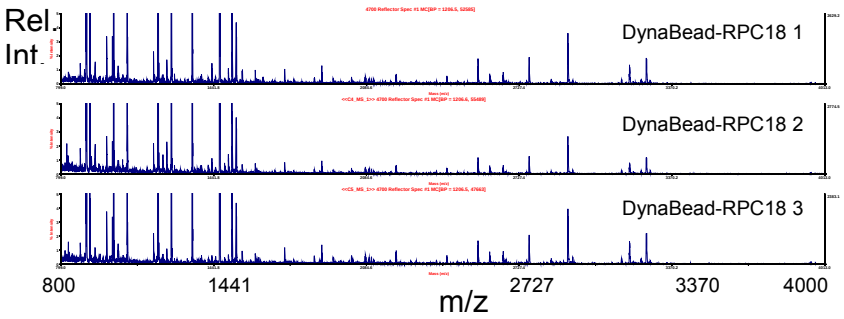


Fig. 2. Serum peptide capture using magnetic beads off-line coupled to peptide profiling by 4800 MALDI-TOF/TOF in reflectron mode.
The result of 3 independent sample processings of control serum is shown for C18 DynaBeads. Intra-exp %CV of relative peptide intensities is 5-20% and inter-exp %CV 10-25%.

TUMOR TISSUE High-throughput MALDI-TOF-MS-based peptide profiling may also be employed to get profiles of tissue homogenates after a simple desalting step (eg using Dynabeads on the KingFisher). An alternative that also preserves spatial information is to make cryo-sections and place those directly on the MALDI sample stage. After deposition of the UV-absorbing matrix proteins may be directly ionized from the tissue (spatial resolution ~ 100 μm). This avenue will explored in more detail in 2007 by Dr. Sander Piersma who has ample experience with imaging mass spectrometry.

2. Biomarker discovery and cancer mechanisms by targeted LC-FTMS-based proteomics.
For in-depth analysis of peptides and proteins in patient samples, we focus on sub-proteomes (looking at less to see more). Which sub proteome depends on the sample type and research question. Moreover, we focus on sub-proteomes that are amendable for analysis in a single dimension nano-liquid chromatography (LC) separation followed by on-line detection of the peptides in an FTMS instrument (LTQ-FTMS). Together this will ensure a large dynamic range of detection (~ 10^6) at reasonable throughput. Because this approach yields identified proteins, the data can be used for pathway analysis and candidate biomarkers can be more easily coupled to antibody-based screening.

BIOFLUIDS For nanoLC-FTMS profiling of biofluids such as plasma and CSF, a first focus is on native peptides and protein <30 kD instead of tryptic peptides, because the low molecular weight proteome of plasma has great diagnostic potential. Moreover, this selection also increases the sensitivity of the analysis as very high abundant plasma proteins such as albumin and IgG are removed. We are currently optimizing existing methods to reproducibly isolate the low molecular weight proteome while minimizing protein losses. Other sub-proteomes of interest in biofluids are

the glycoproteome ('sialome') and the diagnostic cargo on abundant proteins (the 'albuminome') and platelet-derived releasates and microparticles.

TUMOR TISSUE If tumor tissue is available at large enough quantity (tens of mgs), we will isolate tumor sub-proteomes for nanoLC-FTMS analysis, again to enable detection of low abundant proteins while maintaining throughput. To this end, we isolate sub-cellular fractions as the starting material for our discovery. Sub-cellular fractions of special interest for cancer proteomics are: 1. cell surface/ plasma membrane to provide candidate biomarkers for molecular imaging and 2. sub-nuclear fractions (chromatin-binding fraction and the nuclear matrix) to learn more about mechanisms of chromosomal instability, chromatin regulation and identify cancer-related biomarkers. 3 In vitro generated tumor secretomes to identify candidate biomarkers that have an increased chance to be detected in serum as well.
In hybrid core-collaborative projects, we started exploring various protocols for subproteome capture using colorectal tumor tissue as the model. For this cancer, frozen tissue is available for proteomics and along with extensive knowledge of the chromosomal and transcriptome aberrations in adenoma to carcinoma progression (on-going work in the Tumor Profiling Unit of Prof. Gerrit Meijer).

DATA ANALYSIS We are developing robust data analysis methods for label-free quantitation of peptide ion abundance, because this approach represents a promising (and economical) avenue for quantitation at high sensitivity. In LC-MS-based profiling additional dimension of separation allows for profiling of much larger number of peptides in complex mixtures. We are implementing open-source tools as well as home-made algorithms (Horizon Breakthrough project granted to Drs. Marchiori and Jimenez) for the analysis of LC-MS datasets.
Moreover, we are evaluating data mining tools for pathway analysis to go from HTP data to new molecular knowledge about cancer mechanisms.

II. Running projects

Proteomics applied to model systems (in cell culture or animal studies) is well established and can solve a range of questions related to protein expression levels, composition of protein complexes, proteolytical processing, post-translational modifications as well as (subcellular) localization and secretion to the medium. Therefore these types of projects have started in 2006 immediately after the instruments were up- and running.

a. OPL core research 2006

A list of specified OPL core projects can be found in Table 1. Core projects are typically initiated by the OPL but may be carried out jointly with personel from collaborators.

Table 1. OPL-core projects

Name	Department	Project
Jimenez/ Hoekman	Onc	Method development HTP serum peptide profiling
Jimenez/ Hoekman	Onc	Investigation of pre-analytical variables in serum sample handling
Jimenez/ Teunissen	Onc/ MCBI	Method optimization HTP CSF peptide profiling and CSF pre-analytics
Jimenez/ Teunissen	Onc/ MCBI	Method comparison capture of the low molecular weight proteome of serum (CSF)
Marchiori/ Jimenez	Onc/ FEW-VU	Comparison and implementation of LC-MS data analysis tools and algoritms

b. Collaborative projects CCA-V-ICI:

Table 2 summarizes the list of on-going collaborative projects with CCA-V-ICI researchers that have been initiated in 2006.

Table 2. collaborative projects CCA/V-ICI

Name	Department	Project
Fijneman, de Wit	Onc/ PA	A proteomics based search of cell surface markers for colon cancer.
Lankelma	Onc	Quantitative urinary peptide measurements by fluorogenic labeling
Sminia, Reijneveld	RadTher, Neurol.	Proteomic of brain tumors to identify targets for therapy
Middeldorp, Klijn	Pathologie	Exosome proteomics in EBV+ tumor immune escape)
Giaccone, Voortman	Onc	fase 1B studie met Velcade/ Gemcitabine/ Cisplatin
Bitter	Med.Microbio	Mycobacterium mutant screening
Van Dongen	KNO	Determination of number of labels on antibodies

c. Collaborative projects VU/VUmc:
Table 3 summarizes the list of non-cancer collaborative projects with researchers from ICEN (cell biology) and with FALW-VU (molecular neurobiology and plant physiology)

Table 3. collaborative projects VU/Vumc		
Name	**Department**	**Project**
Teunissen, Jimenez	MCBI	Biomarkers for axonal damage in MS, CSF proteomics
VU		
Klychnikov/ Li (CMSB)	MCN-VU	Synapse phosphoproteomics
De Boer	VU	13-3-3 interactors & interaction with new anti-cancer agent from plant fungus

d. External collaborative projects:
Table 3 shows the list of external collaborative projects.

Table 4. External collaborative projects		
Name	**Department**	**Project**
Niclou	NorLux Neuro-Oncol	Membrane proteomics of differentially invasive brain tumor sferoids
Van Diepen	Univ.London	Proteomics of PTEN interactors

e. Service projects:
Table 4 lists the service projects in which routine proteomics tasks were performed on a fee-for-sample basis without special input of intellectual property in the project (other than doing the job well). In these types of projects, in publications only acknowledgement for mass spectrometry services is requested.

Table 5 Service projects		
Name	**Department**	**Project**
Ligtenberg	Med. Microbio	Massabepaling synthetische peptiden
Luurtsema	NucMed/PET	Structural analysis of verapimil metabolites
Extern		
Westerveen	ID Lelystad	Protein identification from 2D gel spots
Mohammadi	UvA	Protein identification from 1D gel bands
Kikkert	Sanguin	Protein identification from 1D gel bands

f. Projects completed in 2006
Table 6 contains the list of studies that were completed in 2006. The projects with Llamas/Bitter and Stienen resulted in a co-authorship (see publications) and the projects with Buermans/Simonides and Kruyt are currently chapters in dissertations and will be submitted for publication in 2007.

Table 6. Collaborative projects completed in 2006		
Name	**Department**	**Project**
Llamas/ Bitter	Med. Microbio	Proteomics of siderophores signaling pathways in Pseudomonas aeruginosa
Buermans, Simonides	Med. Physiol	Early hypertrophic myocardial proteome responses during pressure overload
Stienen	Med. Physiol	Changes in contractile function in Atrial Dilatation and Atrial Fibrillation
Chęcińska/ Kruyt	Onc	Proteomics of affinity-purified caspase-9 complexes in NSCLC cells

g. New projects (other than above) planned for 2007:
Many exploratory meetings between the head OPL and interested CCA-V-ICI researchers took place in 2006. Table 7 lists all the new projects planned for 2007. Some of them are pilot projects where the goal is to obtain data that can be the basis for a grant proposal. A major focus in 2007 will be on cancer cell and patient tissue analysis including subcellular fractions (see description core research).

Table 7. Core and collaborative projects starting in 2007		
Name	**Department**	**Project**
Jimenez/ Boven/ H.Meijers	Onc/ KlinGen	Proteomics in breast cancer
Jimenez	Onc	Clinical Neuroproteomics of Neurodegenerative Diseases, cNEUPRO (EU kp6 STREP)
Jimenez/ Broxterman/ Hoekman	Onc	Proteomics of platelet releasates for early detection of cancer
Jimenez/ Kruyt	Onc	Proteomics of NSCLC-conditioned media
Jimenez/ Meijer	Onc/ Pa	Tissue plasma membrane proteomics in CRC adenome-carcinome progression
Jimenez/ Meijer	Onc/ Pa	Nuclear proteomics in CRC adenome-carcinome progression
Fijneman/ Jimenez	Onc/ Pa	CRC secretome /blood proteomics for biomarker discovery in mouse model
Van Die/ Jimenez	MCBI	Glycan and glyco-peptide mapping in disease (incl cancer)
De Winter	KlinGen	Analysis of PTMs and prot-prot interactions in the Fanconi anemia pathway
Broxterman/ Hoekman	Onc	A phase II study of erlotinib and bevacizumab in NSCLC patients
Van Cruijsen/ Hoekman	Onc	Serum proteomic analysis as part of a phase I clinical evaluation of AZD2171
Verweij (CMSB)	MCBI	Serum profiling in rheuma artritis
Schuurhuis/Ossenkoppele	Hema	Characterization of the tumor resistance modulating microenvironment of AML
Schuurhuis/Ossenkoppele	Hema	Cancer stem cell proteomics in AML
Kruyt	Onc	Proteomics of affinity-purified Bcl complexes in lung cancer cells
Veerhuis	PA	Mapping van eiwitcomplex in Alzheimer brain
Van der Velden, Stienen	Med. Physiol	Myocardial proteomics
Van Dam	MCBI	CSF analysis for biomarker discovery in Parkinson-pilot HTP profiling
VU		
G. Smit (CMSB)	MCN-VU	Serum profiling in depression
M. Smit	Pharm.Chemie-VU	Phosphoproteomics of viral signaling networks in oncogenesis and metastasis

5. Milestones first half year:

1. Mass spectrometry platforms up and running
2. Highly capable, trained OPL personel
3. Reproducible, automated high-throughput proteomics workflow for serum analysis (manuscript accepted for publication)
4. Identification of QC peptides and signatures indicative of sample quality of serum (manuscript in prep).
5. Several series of serum samples collected in a standardized fashion in different phase I studies for high-throughput MALDI profiling
6. High-throughput peptide profiling work flow optimized for CSF and identification of QC peptides indicative of sample quality of CSF (manuscript in prep).
7. Qualitative in-depth profiling of biological and clinical samples using nanoLC-FTMS operational
8. Tissue bank of CRC available for tissue fractionation and sub-proteome analysis (important for core research/ method development).
9. Prototype tool developed and implemented for 1D- LC-MS data mining of mass spectra

6. Education

In 2006 several lectures (8 in total) were given to bachelors, masters and PhD students. In addition, two courses were organized: the 3 day graduate course 'Oncogenomics and Proteomics, a samples platter of applications and techniques' together with Dr. Bauke Ylstra and the under-graduate course: 'Proteomics data analysis mini-course', together with Dr. Elena Marchiori. Both courses attracted a considerable number of students.
The classes taught resulted in two stage-students that will start an internship in 2007 on platelet proteomics (supervisors Jimenez and Broxterman) and on LC-MS data analysis for biomarker discovery (supervisors Marchiori and Piersma).

Academic teaching		
Date	**Course**	**Class**
Dr. CR. Jimenez		
Jan06	ITCB bachelor course tumor biology:	Clinical proteomics
Mar06	Basis cursus Oncologie, Ellecom:	Proteomics: techniek en toepassing
Nov06	OAA Graduate course Oncogenomics*	Mass spectrometry-based proteomics for cancer biomarker discovery
Nov06	Bachelors Oncology	Short proteomics lecture and tour of lab
Nov06	Masters Oncology, Tumor Biology	Mass spectrometry-based proteomics for cancer biomarker discovery
Nov06	Masters Oncology, Therapeutics	Proteomics in cancer therapy
Nov06	Bachelors, Bioinformatics, FEW-VU	Overview proteomics
	Proteomics data analysis mini-course**	for cancer biomarker discovery
Dr. Sander Piersma		
Nov06	Bachelors, bioinformatics, FEW-VU	Protein identification strategies

*Course organized with Dr. Bauke Ylstra (PA, VUmc)
**Course organized with Dr. Elena Marchiori (FEW-VU)

Received training

Besides teaching, being in a brand new laboratory with advanced equipment, the OPL people received several trainings that are listed in the table below.

Courses/ training of OPL people	
April06	Basic training MALDI-TOF/TOF (Knol, Piersma, Jimenez)
May06	Basic training LTQ-FTMS (Knol, Piersma, Jimenez)
June06	LC training (Piersma)
June06	NanoMate training (Piersma)
June06	Hamilton training (Knol and Jimenez)
Oct06	Advanced training FTMS (Piersma)
Nov06	Advanced training MALDI-TOF/TOF (Knol, Piersma, Jimenez)
Aug-Dec06	STEP A, a basic statistics course (Jimenez)
Dec06	OAA graduate course Oncogenesis in colorectal cancer (Jimenez)

7. Input - output data

a. Staff:
The OPL core unit consists of 3 staff members (3 fte, WP3) and one technician (1 fte, NWP3). Furthermore 2 technicians (2x 0.6 fte, NWP3) are working at the OPL on temporary projects. Below follows the list of publications of the staff people.

b. Publications in international (refereed) journals

Dr. Connie Jimenez

1. **Jiménez**, CR, El Filali, Z, JC. Knol, Li KW, Hoekman, K, FAE Kruyt, Giaccone, G., Smit AB. Automated serum peptide profiling using novel magnetic C18 beads off-line coupled to MALDI-TOF mass spectrometry. (2006) Proteomics, provisionally accepted.
2. Elena Marchiori, Connie **Jimenez**, Mikkel West-Nielsen and Niels Heegaard. Robust SVM-based biomarker selection with noisy mass spectrometric proteomic data. In Applications of Evolutionary Computing. EvoBIO: Evolutionary Computation and Machine Learning in Bioinformatics. Springer, pp. 79-90, LNCS 3907, 2006.
3. Michel C. Van den Oever, Sabine Spijker, Ka Wan Li, Connie R. **Jiménez**, Eisuke Koya, Roel C. Van der Schors, Yvonne Gouwenberg, Rob Binnekade, Taco J. De Vries, Anton N. M. Schoffelmeer, and August B. Smit. A proteomics approach to identify long-term molecular changes in rat medial prefrontal cortex resulting from sucrose self-administration. J.Prot. Res. (2006) 5, 147-154.
4. Eiras S, Narolska NA, van Loon RB, Boontje NM, Zaremba R, **Jimenez** CR, Visser FC, Stooker W, van der Velden J, Stienen GJ. Alterations in contractile protein composition and function in human atrial dilatation and atrial fibrillation. J Mol Cell Cardiol. (2006) 41(3):467-77.
5. **Jiménez** CR, Li KW, Smit AB, Janse C. Auto-inhibitory control of peptidergic molluscan neurons and reproductive senescence. Neurobiol Aging. (2006)
6. Li, KW., **Jiménez**, CR, Hornshaw, MP; Van der Schors, RC, Schoffelmeer, ANM, Smit AB. Intermittent administration of morphine alters protein expression in rat nucleus accumbens. Proteomics (2006), 6-2003-2008.
7. María A. Llamas, Marion Sparrius, Roy Kloet, Connie R. **Jiménez**, Christina Vandenbroucke-Grauls, and Wilbert Bitter. Heterologous siderophores ferrioxamine B and ferrichrome activate signaling pathways in Pseudomonas aeruginosa. Journal of Bacteriology (2006) 188 (5), 1882-1891.
8. **Jiménez**, CR., S. Spijker, S. de Schipper, J. C. Lodder, C. Janse, W. P. M. Geraerts, J. van Minnen, N. I. Syed, A. L. Burlingame, A. B. Smit, K. W. Li. Peptidomics of a single identified neuron reveals diversity of multiple neuropeptides with convergent actions on cellular excitability. J. Neurosci. (2006) 26, 518-529.

Dr. Sander Piersma

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Dr. Thang Pham

1. Thang V. **Pham**, Arnold W. M. Smeulders: Metric tree partitioning and Taylor approximation for fast support vector classification. ICPR (4) 2006: 132-135.
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Thesis chapters:

1. Agnieszka Chęcińska, Giuseppe Giaccone, Jose A. Rodriguez, Frank A.E. Kruyt#, Connie R. **Jimenez #** Comparative proteomics analysis of caspase-9-interacting proteins in untreated and cytochrome-c-induced NSCLC cells
2. Henk P.J. Buermans, Marian Zuidwijk, René J.P. Musters, Cornelis van Hardeveld, Connie R. **Jiménez**, Frans C. Visser, Walter J. Paulus, Warner S. Simonides. Early adaptive and maladaptive hypertrophic myocardial proteome responses during pressure overload

Vakpublicatie:

CR. **Jimenez**, Proteomics in de Oncologie, April 2006, In : Kanker, tijdschrift van de Nederlandse Vereniging voor Oncologie

8. Indicators of esteem

a. Obtained grants

1. - title project: **Informatics Tool for Proteomic Biomarker Detection using large-scale nanoLC-FT Mass Spectrometry Data**
   - type grant (+ nummer): Horizon Breakthrough, Reg nr. 050-71-450
   - amount: 100K
   - post-doc 1,5 yrs
   - department: Collaboration FEW-VU and OncoProteomics Laboratory
   - projectleader(s): Drs. Elena Marchiori and Connie R. Jimenez

2. - title project: **Clinical Neuroproteomics of Neurodegenerative Disease cN*EU*PRO**
   - type grant (+ nummer): FP6-LIFESCIHEALTH-7 (STREP)
   - amount: 280K
   - technician 3 yrs
   - department: OncoProteomics Laboratory
   - projectleader(s): Dr. Connie R. Jimenez (Work Package Leader)

b. Participation and function in scientific societies

Function	Organisation
Dr. Connie Jimenez	
Coördinator	Netherlands Proteomics Platform
General Council Member	European Proteomics Association (EuPA)
Member	EuPA-HUPO interactions committee
Work Package Leader	EU kp6 STREP cNeuPro
Proteomics coördinator colon team	International Cancer Biomarker Consortium
Member	Human Proteome Organisation
Member	American Society for Mass Spectrometry
Member	Dutch Society for Biochemistry and Molecular Biology
Member	Dutch Society for Mass spectrometry
Dr. Sander Piersma	
Member	American Society for Mass Spectrometry
Member	Netherland Proteomics Platform
Member	Dutch Society for Biochemistry and Molecular Biology
Member	Dutch Society for Mass spectrometry
Dr. Thang Pham	
Member	Dutch Society for Pattern Recognition and Image Processing
Member	Netherlands Proteomics Platform

c. Presentations at (inter)national scientific conferences:

Conference	Date	Place	Type	Title
Dr. Connie Jimenez				
2nd Norwegian Proteomics Meeting	Aug 7-8	Bergen, Norway	Key-note	Mass spectrometry-based biomarker discovery
European Biomarker Summit	Sept 25-26	Prague	Lecture	Automated serum peptide profiling using novel C18 beads off-line coupled to MALDI-TOF mass spectrometry
2nd NUBIN biomarker symposium	June 19-20	Amsterdam	Invited Lecture	Mass spectrometry-based biomarker discovery
CCA Oncogenomics and proteomics symposium & Pre-Clinical Strategies in Radiation Oncology	6-Nov	Amsterdam	Lecture	MS-based proteomics for cancer biomarker discovery
HUPO 5th Annual World Congress	Oct 28- Nov 1	Long Beach, USA	Poster	Comparison of high-throughput serum peptide capture methods for peptide profiling by MALDI-TOF-MS
Biomarker discovery by mass spectrometry	May 18-19	Amsterdam	Poster	Comparison of high-throughput serum peptide capture methods for peptide profiling by MALDI-TOF-MS
Dr. Sander Piersma				
American Society for Mass Spectrometry				
Dr Thang Pham				
6th IEEE International Workshop on Visual Surveilla	13-May	Graz, Austria	Poster	Efficient projection pursuit density estimation for background
18th International Conference on Pattern Recognitic	Aug. 2023	Hong Kong	Oral	subtraction Metric tree partitioning and Taylor approximation for fast support vector classification
By collaborator (Sminia)				
3ed Int. Conference on Translational Research	March 12-15	Lugano, Switzerland	Oral	Proteomics of human brain tumors
By collaborator (Codrea/ Marchiori)				
Biomarker discovery by mass spectrometry	May 18-19	Amsterdam	Poster	Tools for visualization and processing of LC/MS datasets: a comparison from a user's perspective

d. Collaborations

International

- Dr. Matthew Fitzgibbon, Fred Hutchinson Cancer Research Center, Seattle, USA
- Dr. L. Pasa-Tolic, Mass Spectrometry Facility, Pacific Northwest National Laboratory, USA
- Dr. Frode Berven, Proteomics Unit, University of Bergen, Bergen, Norway
- Dr. Simone Niclou, NorLux Neuro-Oncology Laboratory, Luxembourg
- Prof. S. Gammeltoft, Department of Clinical Biochemistry, Glostrup Hospital, Denmark
- Prof. dr. AL Burlingame, Mass Spectrometry Resource, University of California San Francisco, USA
- Dr. Bogdan Budnik, Proteomics Unit, Childrens Hospital, Harvard, Boston, USA
- Dr. D. Gillooly (Invitrogen, Finland)
- Dr. M. Hornshaw (London, ThermoElectron)
- Dr. Matthias Glueckmann and Christie Hunter (Applied Biosystems)

National

- Dr. Monique Slijper, Dr. J. Krijgsveld, University of Utrecht, Netherlands Proteomics Center
- Prof. Dr. A.M. Deelder, Biomolecular mass spectrometry resource, LUMC, Leiden
- Dr. Rene Houtman, Dr. Rob Ruitenbeek, PamGene, Den Bosch
- Dr. Elena Marchiori, Dept. Computer Science, FEW, VU, Amsterdam
- Dr. Aad van der Vaart, Dept. Mathematics, FEW, VU, Amsterdam
- Dr. Jaap Herringa, Dept. Bioinformatics, FEW, VU, Amsterdam
- Dr. Ka Wan Li, Prof. dr. Guus Smit, Dept. Molecular & Cellular Neurobiology, VU

Appendix 1

VUmc-CCA

Proteomics and Mass Spectrometry

The OncoProteomics Laboratory (OPL) is accessible to any academic investigator from any institution as well as clients from industry.

Procedure for submitting samples

Guidelines for service:

- Prior to sending samples, investigators are encouraged to contact the OPL to discuss the required analysis. This is necessary to insure that the most efficient and cost-effective analytical methods are employed.
- Samples are normally analyzed in the order of their receipt, but special arrangements can be made for unstable samples.
- A Sample Submission Form should accompany each set of samples. The information requested on this form should be filled out completely to assure that the analyses could be carried out properly. Missing information from the form will result in delays in the requested analyses. If you are a new customer, please also fill out a customer information form.

Procedure for submitting projects

Guidelines for collaboration:

- Proteomics projects are generally done on a collaborative basis after contacting Dr. Connie Jimenez. In an exploratory meeting, it can be determined whether solving the research question can be done in a simple experiment or whether it requires a more elaborate (labor-intensive) approach.
- Project proposals are set up in collaboration between the collaborator and the Head OPL.
- The Head OPL submits a completed 'Project Submission' form and for finished projects a 'Project Report' to the OPL Program Advisory committee.
- Depending on the effort needed, the project is carried out by the OPL (eg., for a small pilot) or by the collaborator at the OPL, only after instruction and under the guidance of the OPL personel.
- A Project Submission Form should be in possession of the OPL prior to the desired start of the project. If you are a new collaborator, please also fill out a Collaborator Information Form.

NB. All forms can be down-loaded from our website and sent via email to Dr. Jimenez.

For more information please visit our website (www.oncoproteomics.nl) or contact:

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VUmc-Cancer Center Amsterdam
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands
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Email: c.jimenez@vumc.nl

To send samples, please use the above address (at the attention of Dr. Jaco Knol)