

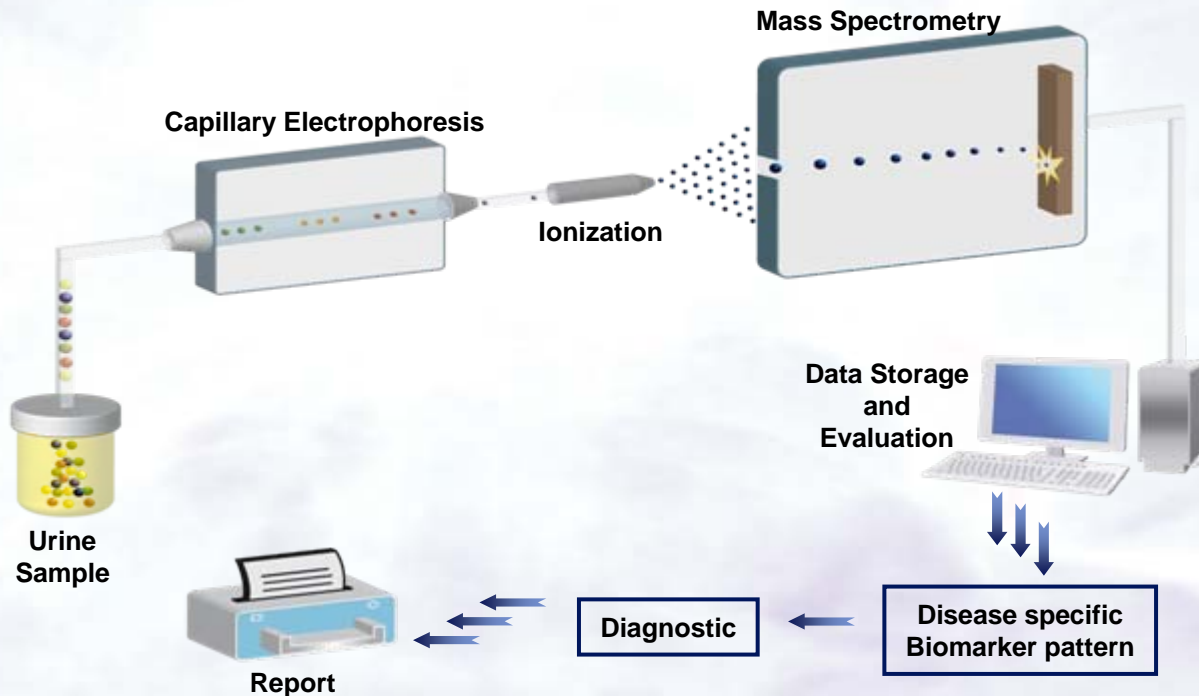
Clinical Proteomics

Ingenious HyperCare

Harald Mischak

WP I.3 Establishment of an Integrated Proteomics Platforms

Proteomics Technology platform: CE/MS Technologie
Capillary Electrophoresis coupled to Mass Spectrometry



Separation and analysis of proteins and peptides (>1,000)

Run time ~60 min

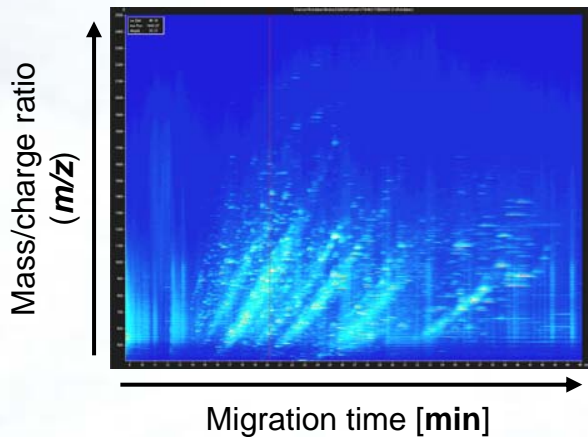
CE

- fast
- robust
- inexpensive
- reproducible

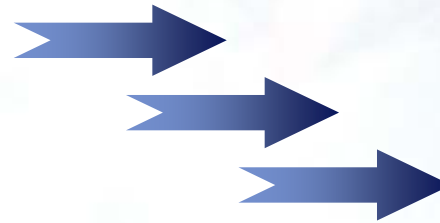
MS

- resolution
- scan speed

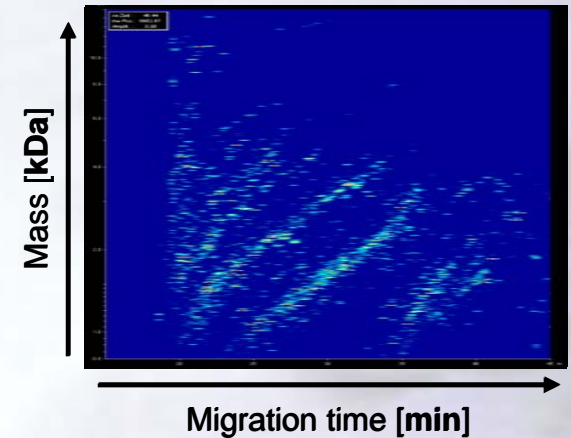
Evaluation and further processing of raw data



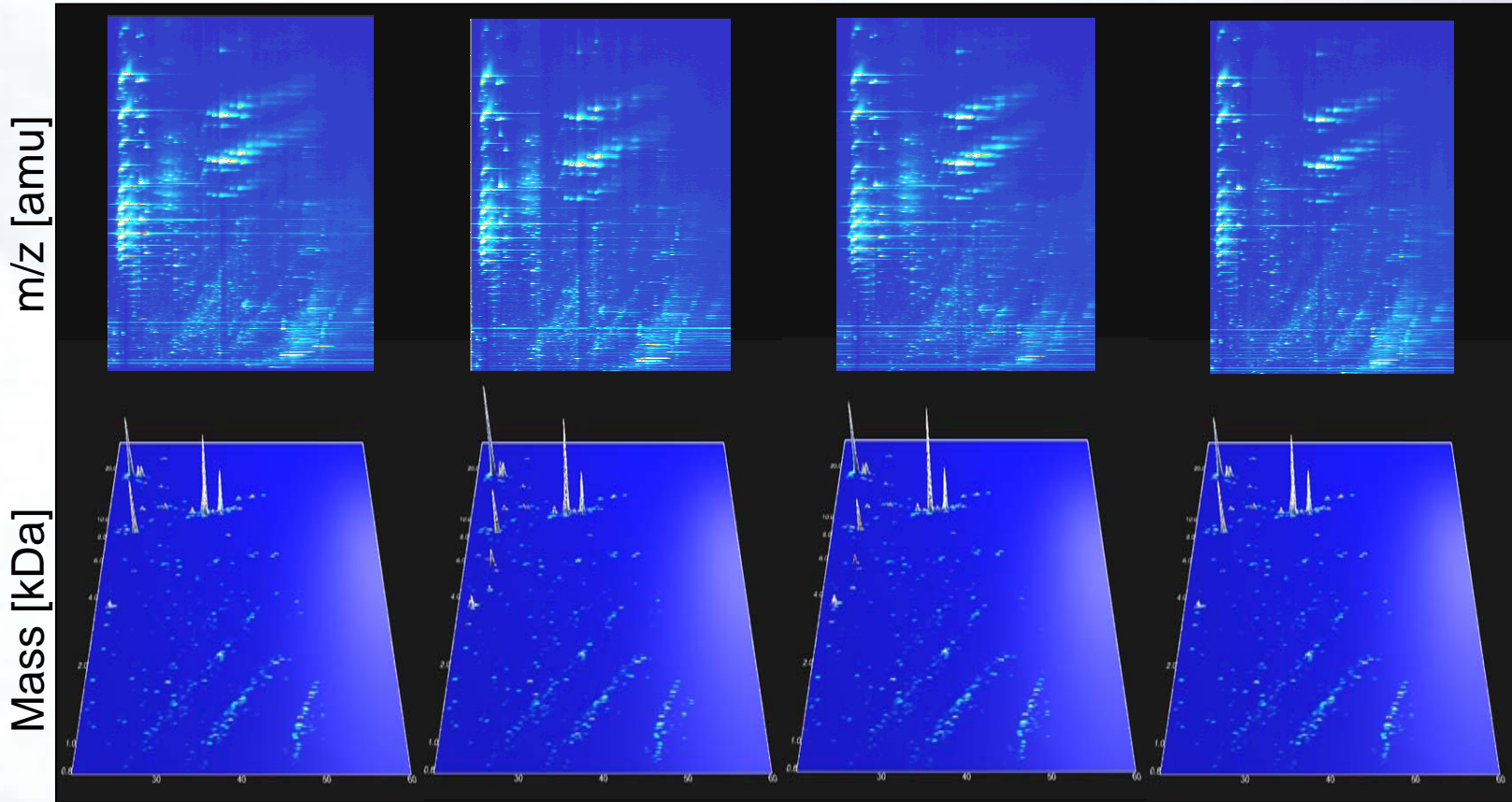
Proprietary Software Packages



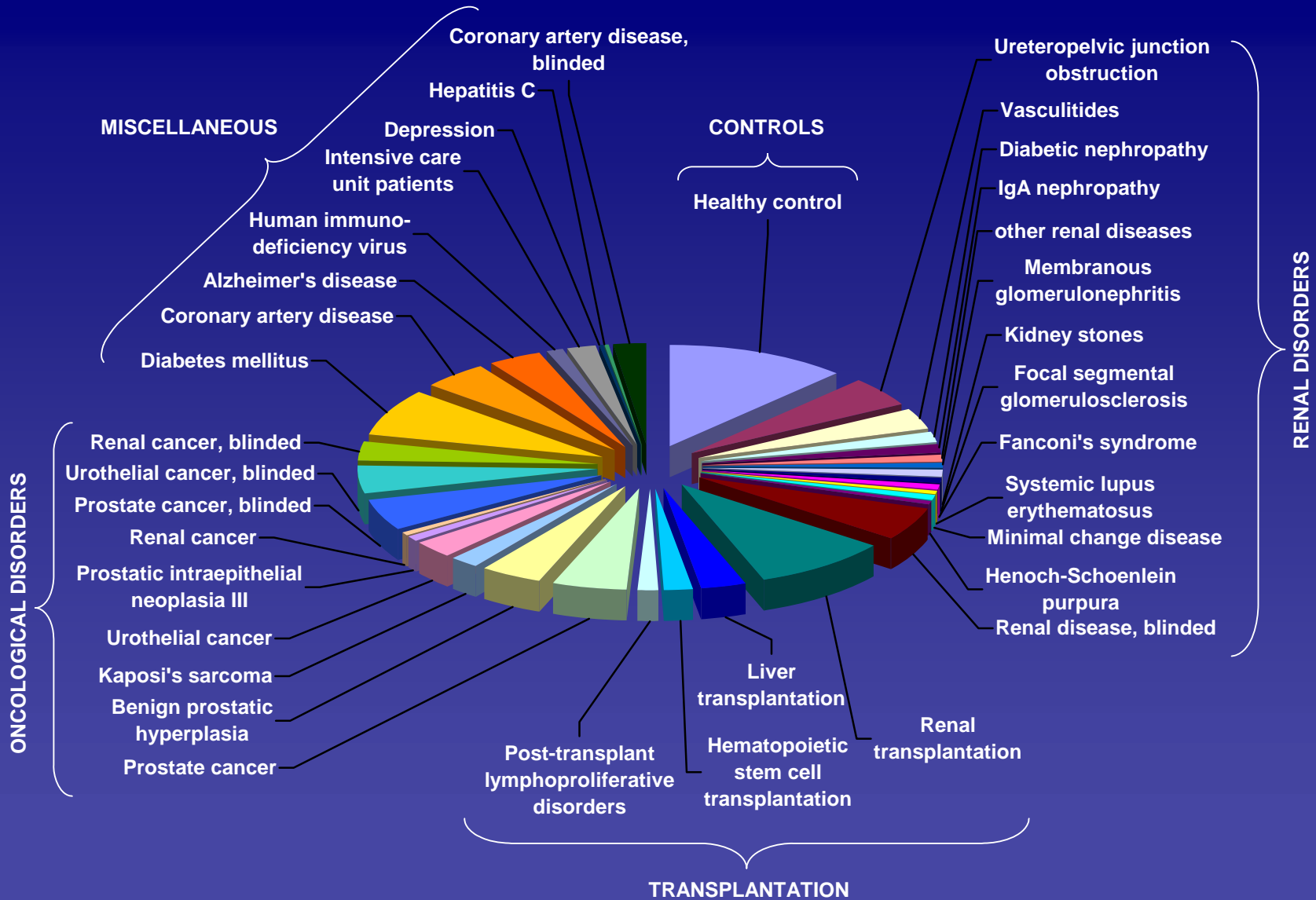
- Identifikation of relevant signals
- Determination of Charge and Mass
- Normalization and Calibration



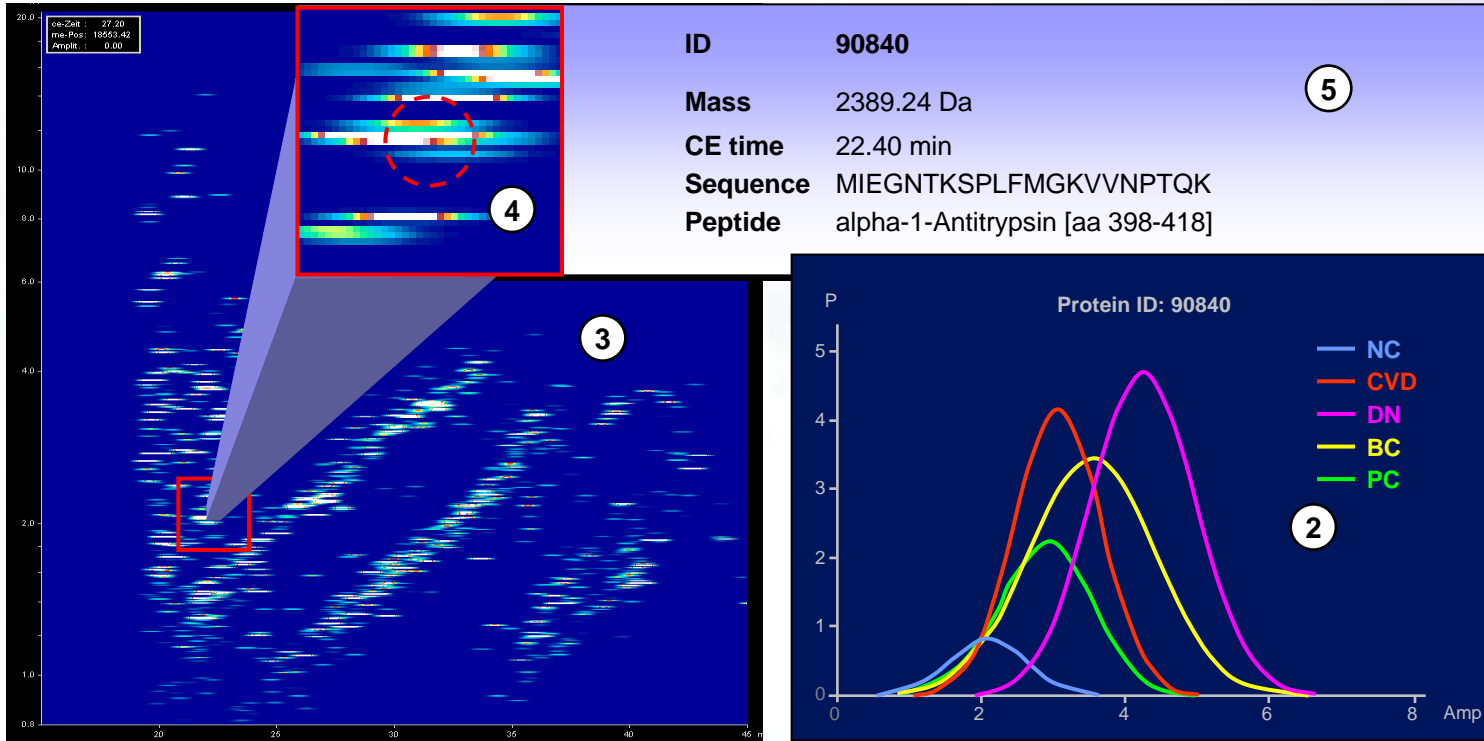
Reproducibility: Identical sample prepared and analyzed independently



Human Urinary LMW Proteome database

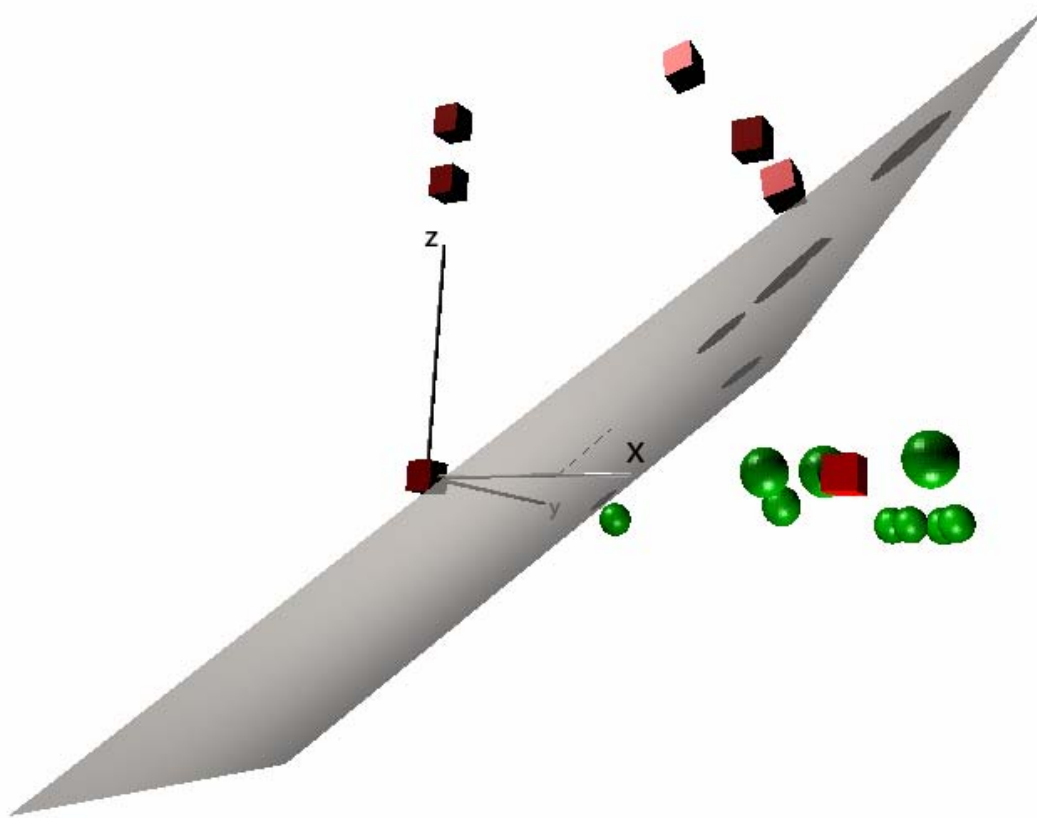


Definition und identification of relevant Biomarkers



| Statistics for Marker ID 90840 | | | | | 1 |
|-------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------|----------|
| Normal Control | Cardiovascular Disease | Diabetic Nephropathy | Bladder Cancer | Prostate Cancer | |
| Amp 2,0 Frequency 28/267 | Amp 3,0 Frequency 22/40 | Amp 4,3 Frequency 25/34 | Amp 3,5 Frequency 20/30 | Amp 2,9 Frequency 49/153 | |

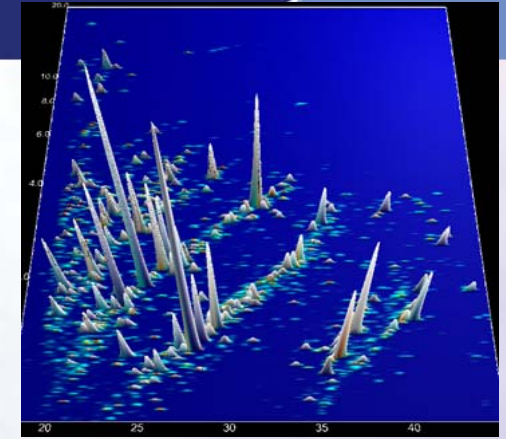
Classification using n-dimensional models



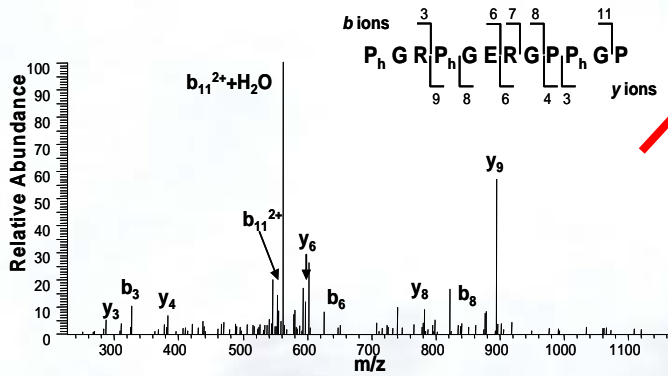
| |
|----------------------------|
| Age |
| Gender |
| Urinary albumin/creatinine |
| Cholesterol (mmol/l) |
| Creatinine (micromol/l) |

Clinical data
Patients history

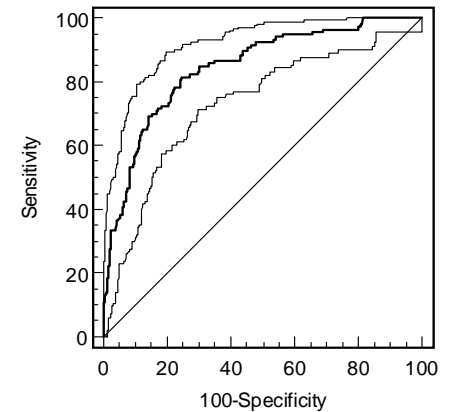
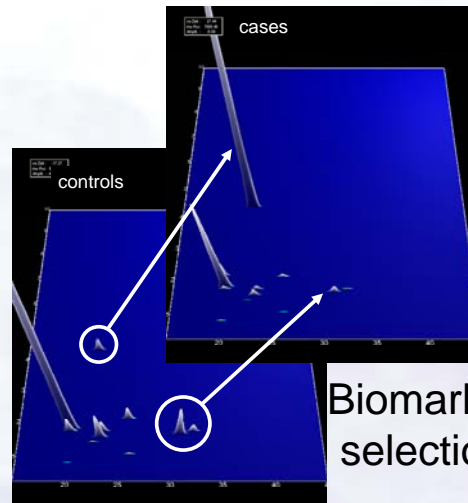
Database



CE-MS peptidome profile



Sequence information



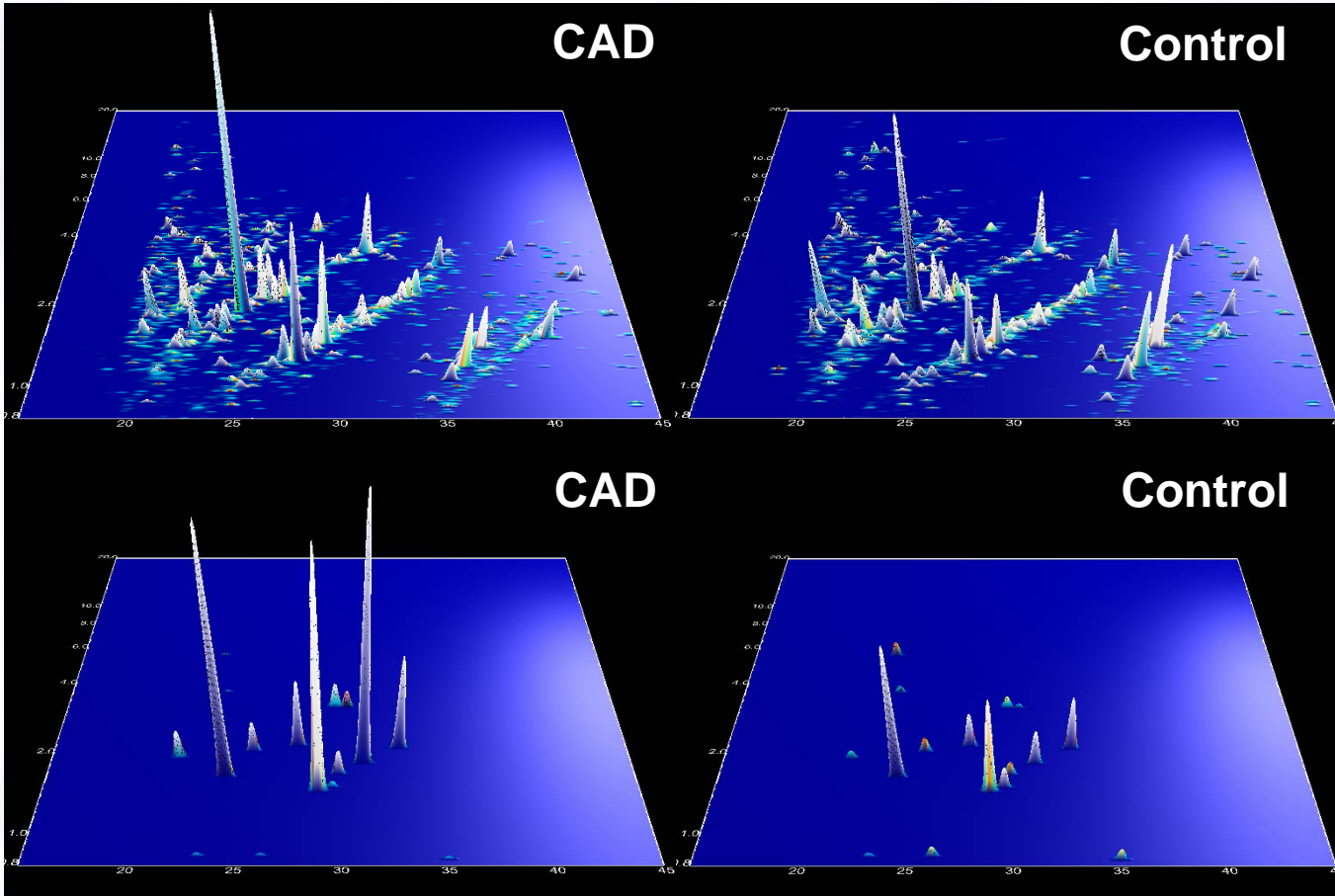
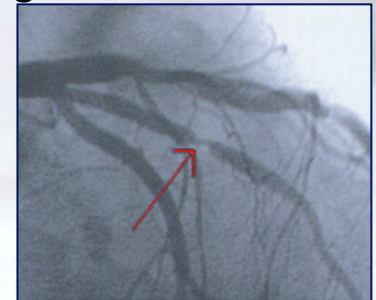
Statistics

WP A2.2 Inflammation and oxidative stress in hypertension: Proteomic biomarkers for CAD

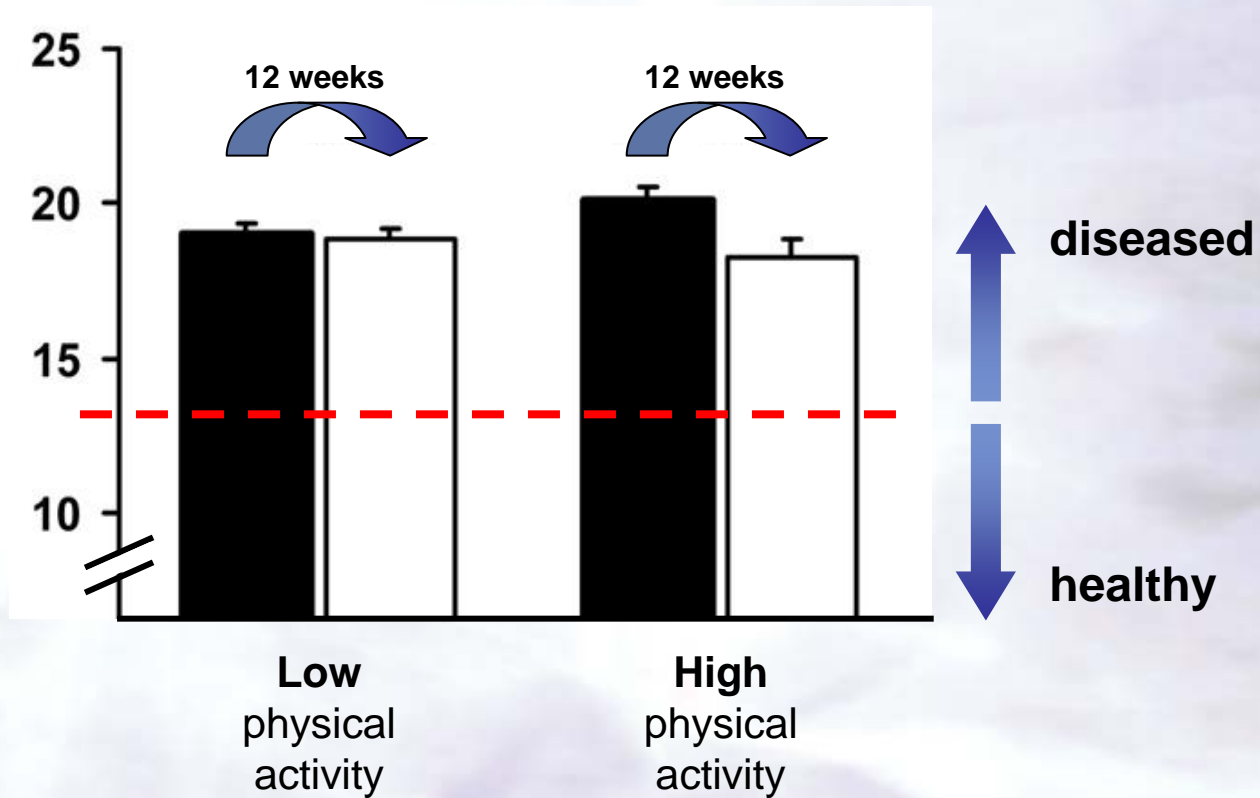


Multicenter
(Germany, UK,
USA, Australia)

**Coronary
angiography for
*gold standard***

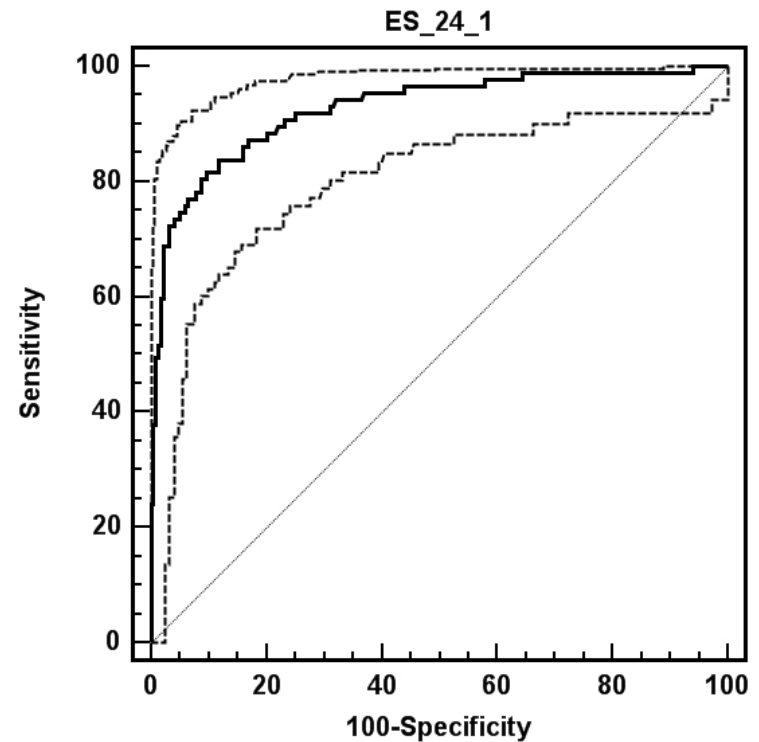


Assessment of therapy success



Coronary artery disease: further refinement

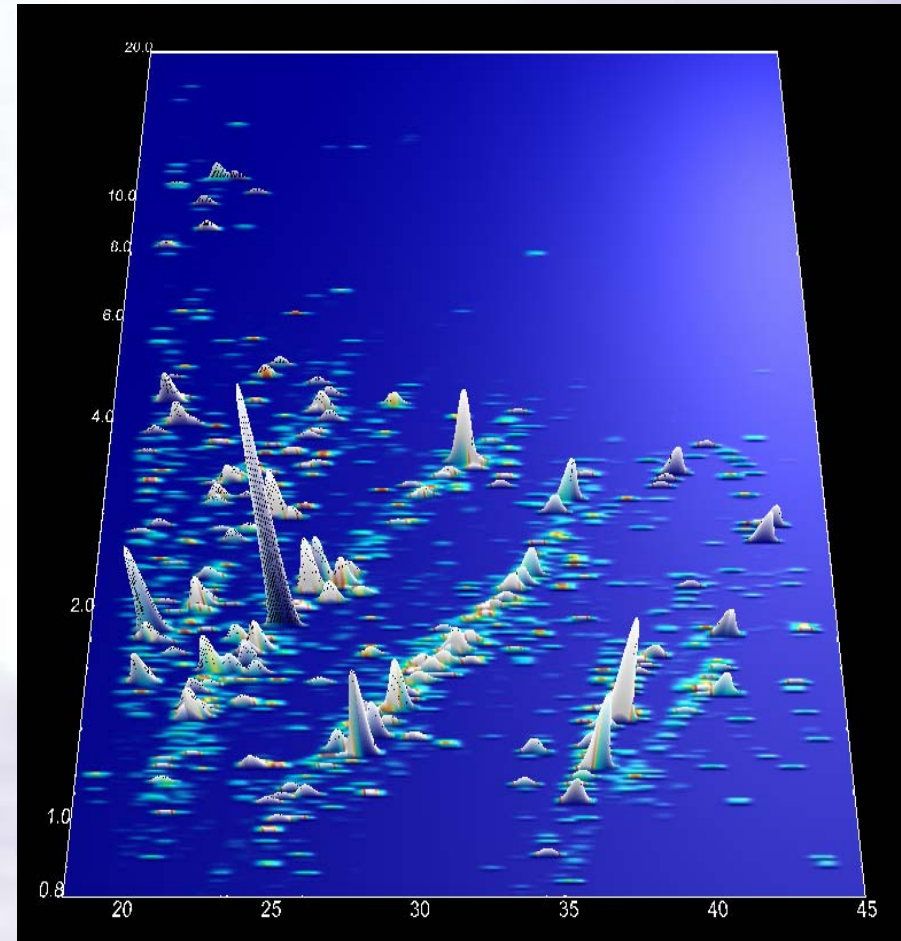
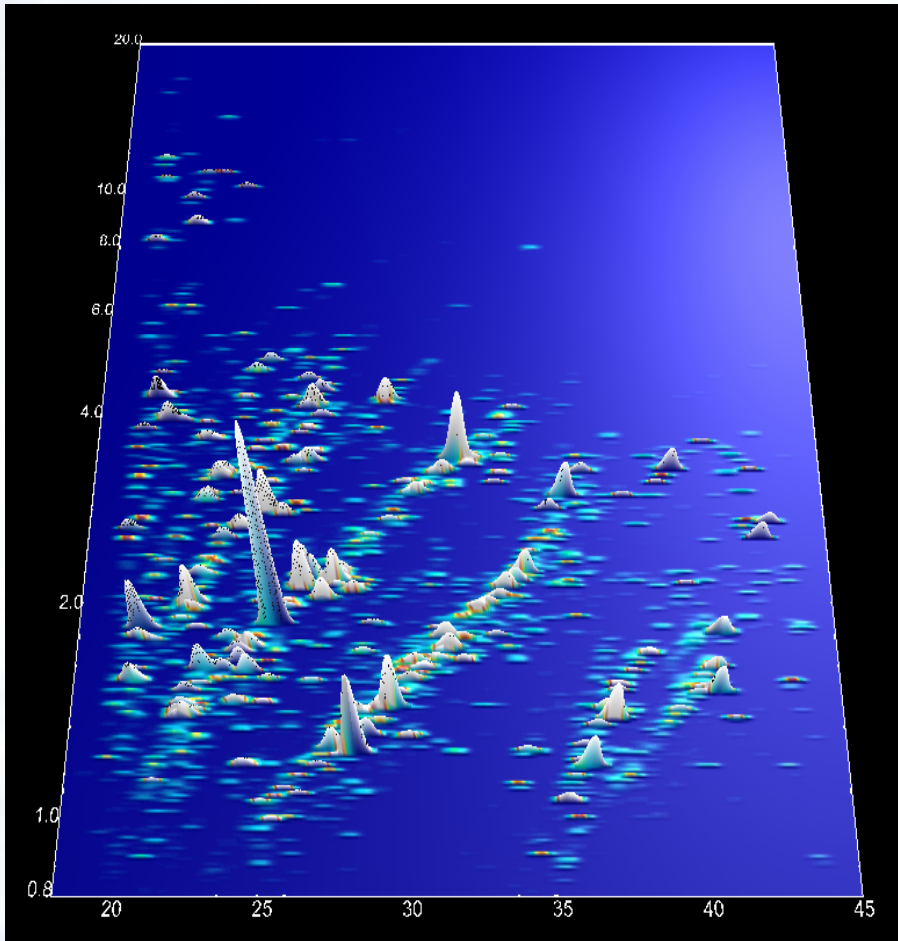
| centre | case | control | sums |
|------------------|------|---------|------|
| Denver (CO) | 18 | 15 | 33 |
| Erlangen (GER) | 0 | 30 | 30 |
| Glasgow (UK) | 151 | 32 | 183 |
| Freiburg (GER) | 35 | 24 | 59 |
| Grand Forks (ND) | 0 | 156 | 156 |
| Hannover (GER) | 0 | 73 | 73 |
| Chenove (F) | 0 | 52 | 52 |
| | 204 | 382 | 586 |



Proteomics of Coronary Artery Disease

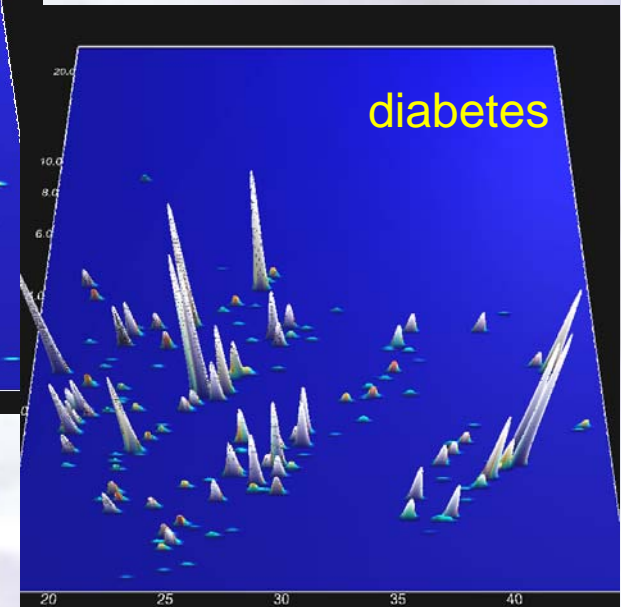
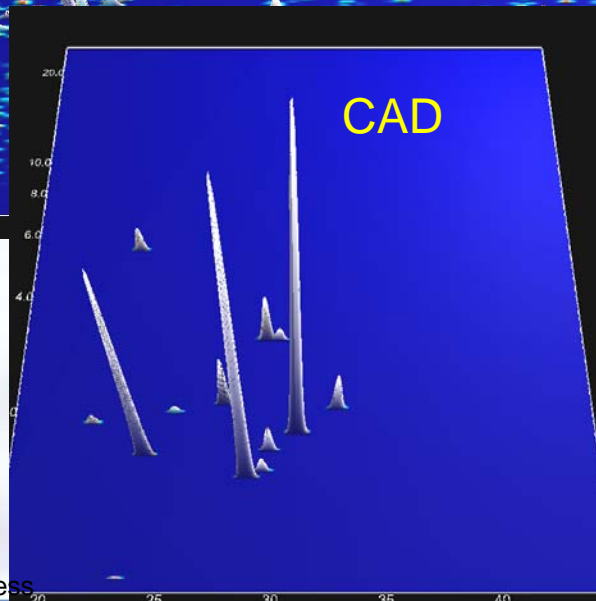
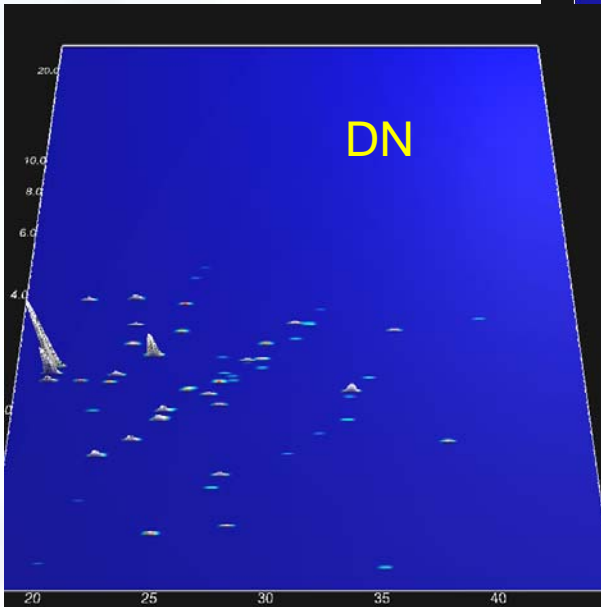
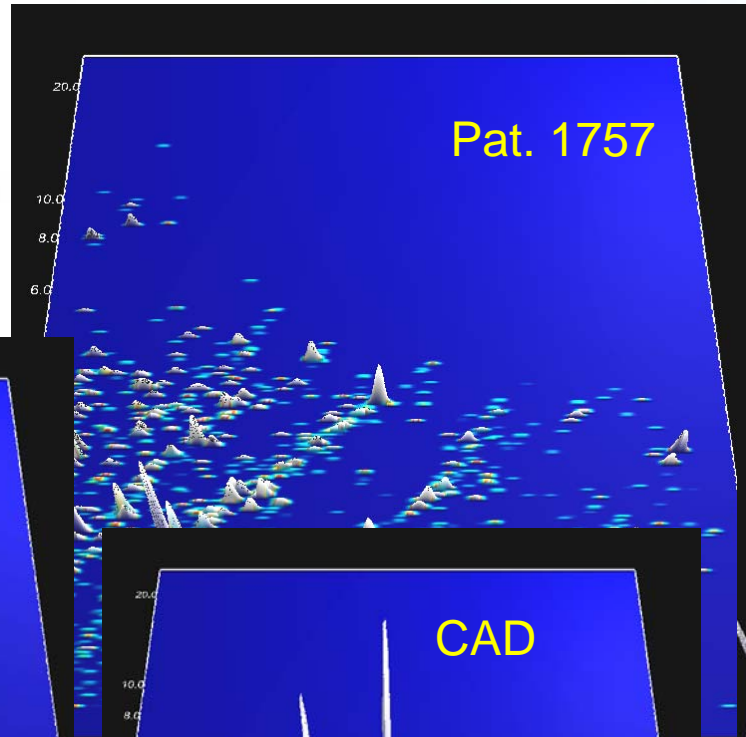
CAD

Controls

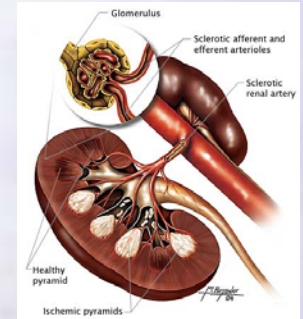
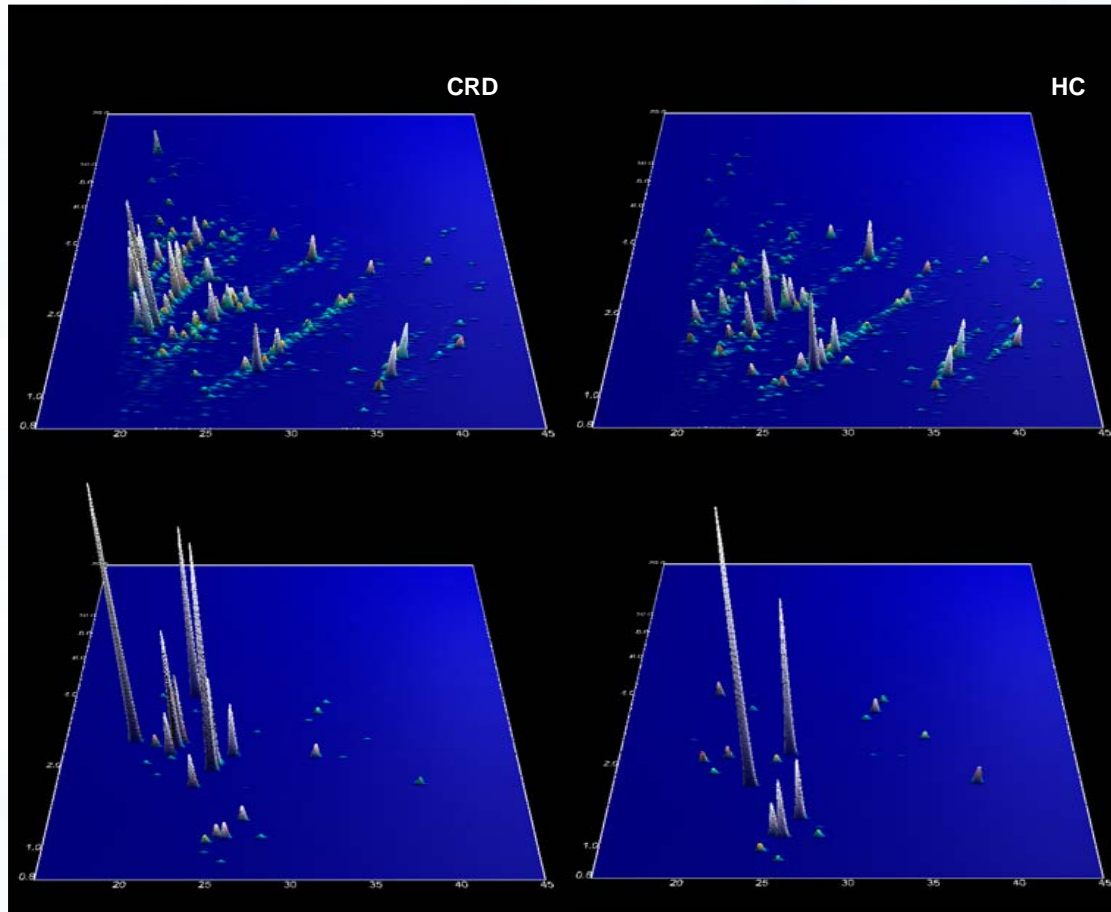


Prognosis of CAD in blinded, prospective samples (CACTI), N = 38

Diabetes: $p < 0,001$
CRD: $p < 0,001$
CAD: $p = 0,0016$

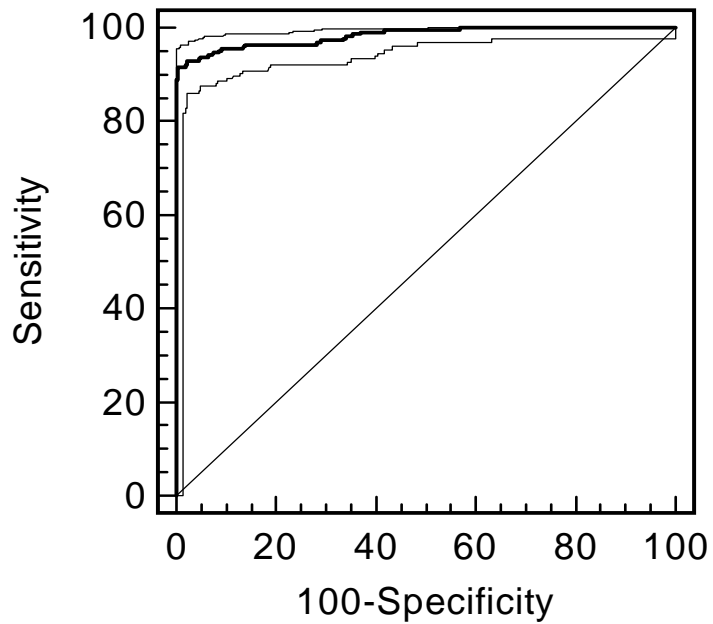


WP B2.2 Chronic renal disease: proteomic analysis

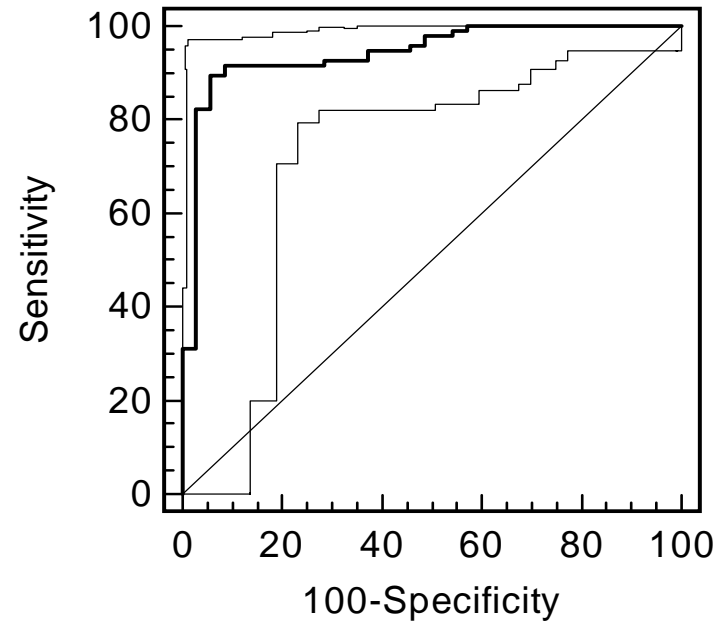


ROC Analysis of CKD diagnosis

A Trainingset



B Testset

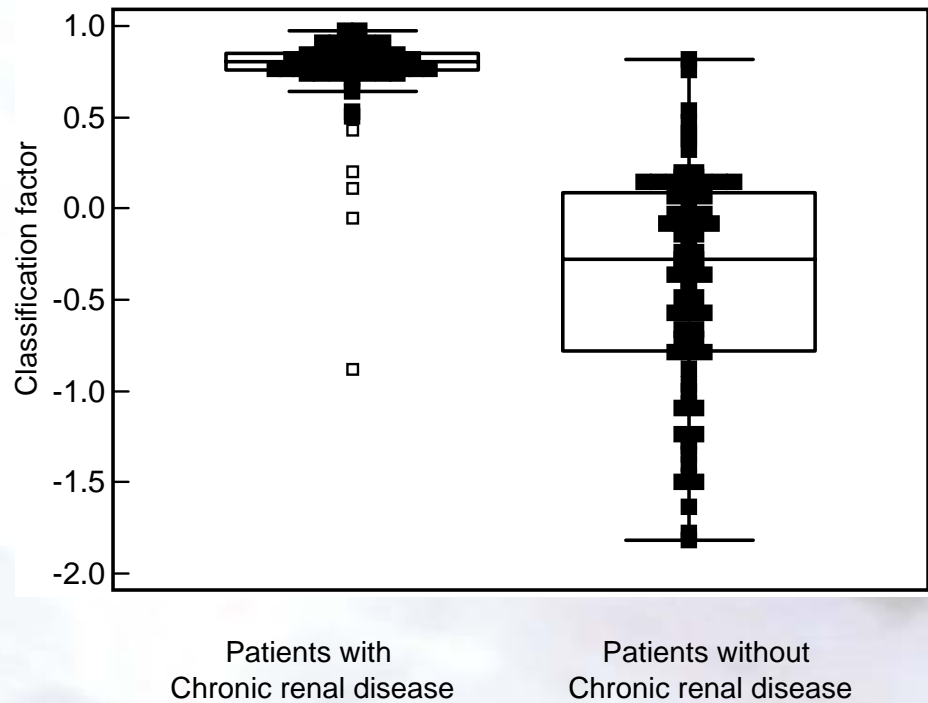
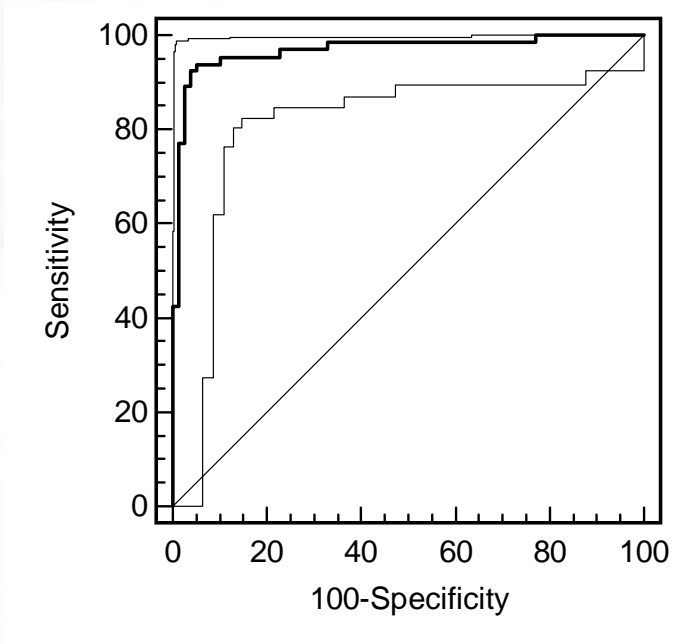


Urinary proteomics: Validation for CKD detection

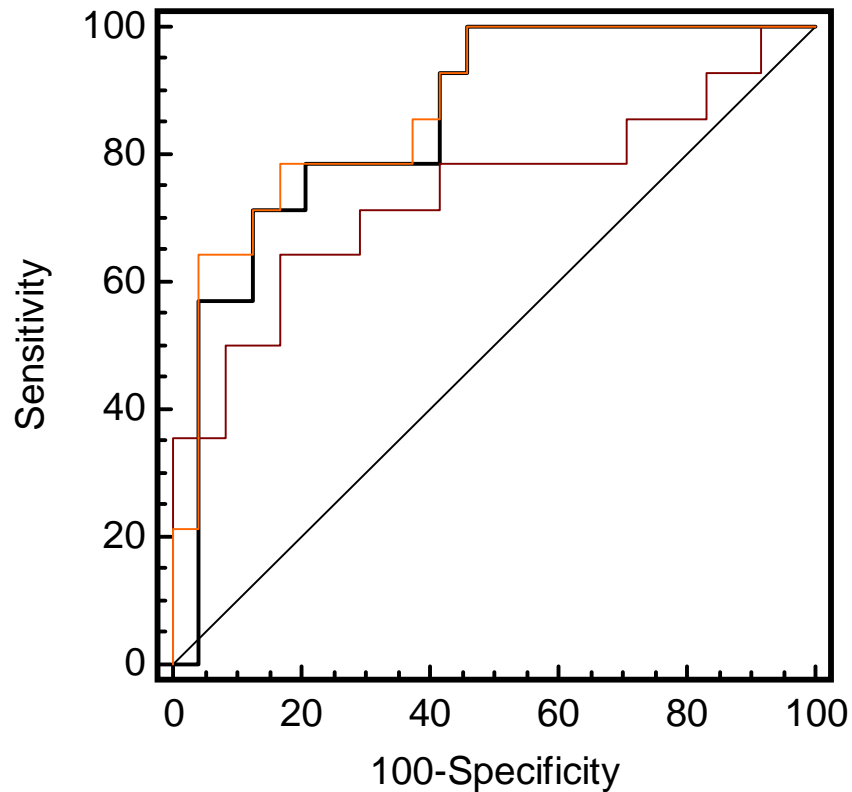
- Urinary biomarkers and biomarker models specific for chronic renal disease were evaluated in a blinded assessment using 148 prospectively collected samples from 3 clinical centers
- All samples were analyzed, 145 gave acceptable data, these were evaluated blinded using appropriate biomarker model

Results after unblinding

,crdbiomarker1' model:



Prognosis of Diabetic Nephropathy (6 years)



— CKD_model
— Urin Albumin [mmol/24h]
— CKD model * U-Albumin

AUC_Alb 0.738

AUC_CKD 0.851

AUC CKD+Alb 0.872

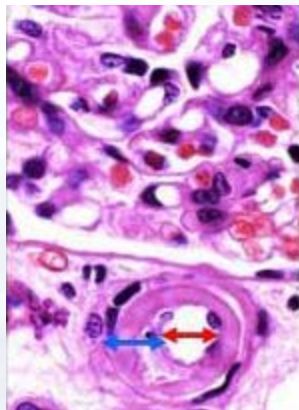
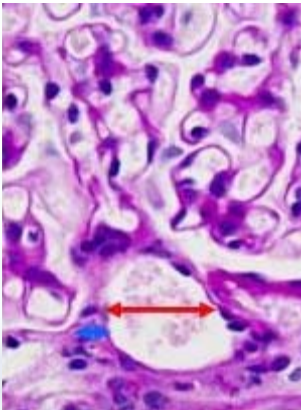
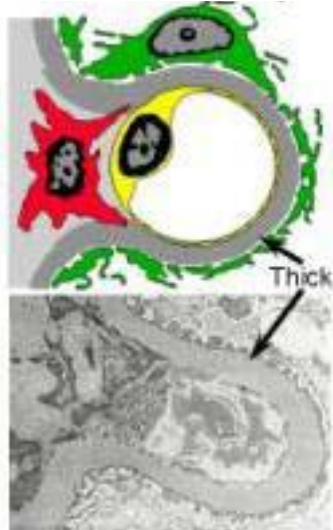
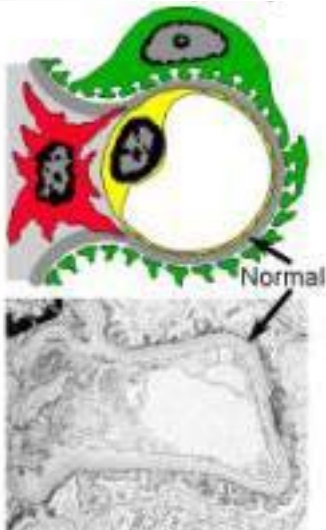
Collagen fragments represent the most abundant urinary peptides, several of these serve as specific biomarkers for distinct diseases

| Number of peptides | Protein name | Gene symbol | Proteins detected by: | |
|--------------------|-------------------------------------------------------|-------------------|---------------------------|-----------------------------|
| | | | Adachi <i>et al.</i> [21] | Castagna <i>et al.</i> [20] |
| 157 | Collagen alpha-1 (I) chain | <i>COL1A1</i> | Yes | No |
| 69 | Collagen alpha-1 (III) chain | <i>COL3A1</i> | Yes | No |
| 24 | Alpha-1-antitrypsin | <i>SERPINA1</i> | Yes | Yes |
| 24 | Collagen alpha-2 (I) chain | <i>COL1A2</i> | Yes | No |
| 19 | Hemoglobin subunit beta | <i>HBB</i> | Yes | Yes |
| 18 | Uromodulin | <i>UMOD</i> | Yes | Yes |
| 17 | Hemoglobin subunit alpha | <i>HBA1, HBA2</i> | Yes | No |
| 16 | Serum albumin | <i>ALB</i> | Yes | Yes |
| 14 | Fibrinogen alpha chain | <i>FGA</i> | Yes | No |
| 12 | Beta-2-microglobulin | <i>B2M</i> | Yes | Yes |
| 6 | Polymeric-immunoglobulin receptor | <i>PIGR</i> | Yes | Yes |
| 3 | Alpha-2-HS-glycoprotein | <i>AHSG</i> | Yes | Yes |
| 3 | Collagen alpha-1 (II) chain | <i>COL2A1</i> | No | No |
| 3 | Membrane associated progesterone receptor component 1 | <i>PGRMC1</i> | Yes | No |
| 3 | Osteopontin | <i>SPP1</i> | Yes | No |
| 3 | Transthyretin precursor (Prealbumin) | <i>TTR</i> | Yes | Yes |
| 2 | Alpha-1-microglobulin | <i>AMBP</i> | Yes | Yes |
| 2 | Apolipoprotein A-I | <i>APOA1</i> | No | Yes |
| 2 | CD99 antigen | <i>CD99</i> | No | No |
| 2 | Clusterin | <i>CLU</i> | Yes | Yes |

Increase in ECM and collagen in CKD and CAD

normal

CRD



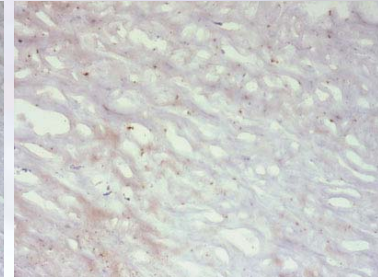
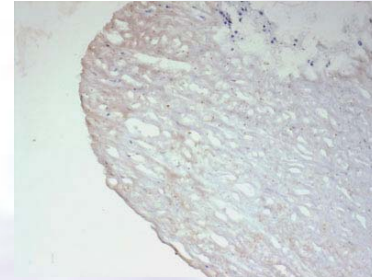
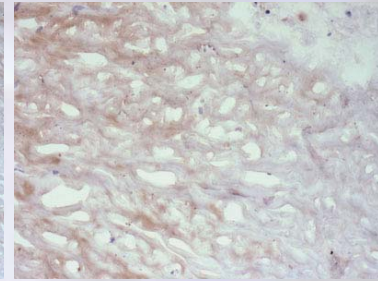
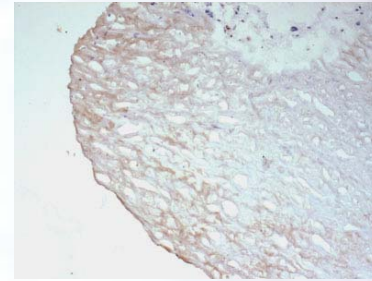
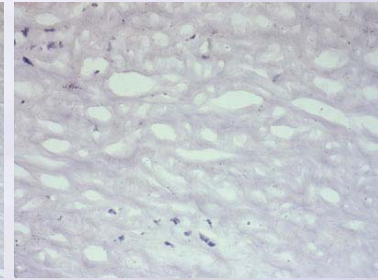
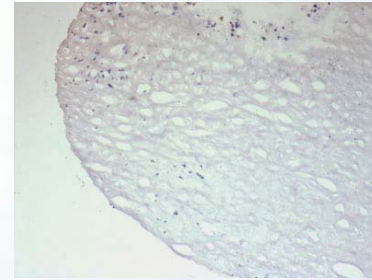
negative control

collagen type I

collagen type III

x100

x200



Summary

- We have developed a high-throughput proteomic platform that enables highly reproducible analysis of human urine samples and interpretation of data.
- Application of this platform resulted in the identification of urinary proteomic biomarker panels enabling (early) detection, prognosis, and assessment of therapy of a variety of diseases, with 80 - 95% accuracy.
- The biomarker panels could be validated in independent multi-centric blinded studies.
- Variability in single biomarkers is counteracted by diagnostic patterns that tolerate instability and inconsistency of individual polypeptides/biomarkers
- Urinary peptides to a large degree display and consequently enable assessment of (patho)physiological turnover of extracellular matrix
- CE-MS-based proteome analysis can be applied towards preclinical and clinical targets. In concert with data on e.g. physiology, histopathology, etc., proteome analysis enables assessment of disease on a molecular level, definition of new, more appropriate therapeutic targets.