Beginn der Vorlesung

- 740.750 Einführung in die Pharmazeutische Analytik + 725.042, Analytische Grundvorlesung I
  - wöchentlich; Di, Mi, Do; 11:30 - 13:00, L.EG.220
- Teil 1: Einführung und Gravimetrie
- Website (Skripten und Infos) http://www.uibk.ac.at/acrc/mitarbeiter/bonn/lehre.html
- Fragen bezgl. Vorlesung
  - Email -> christoph.woelger@uibk.ac.at

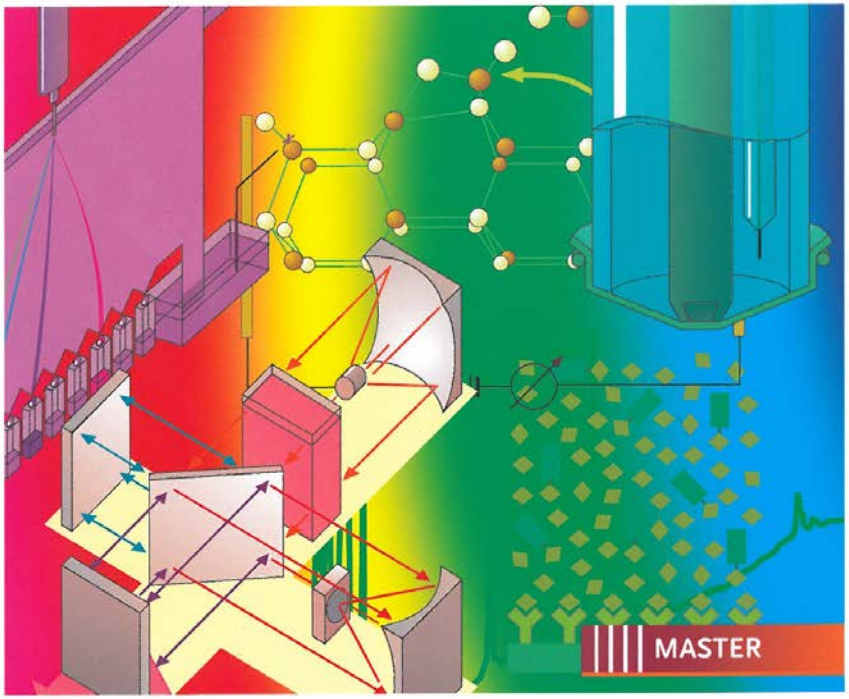
Georg Schwedt

WILEY-VCH

Analytische Chemie

Grundlagen, Methoden und Praxis

Zweite, vollständig überarbeitete Auflage



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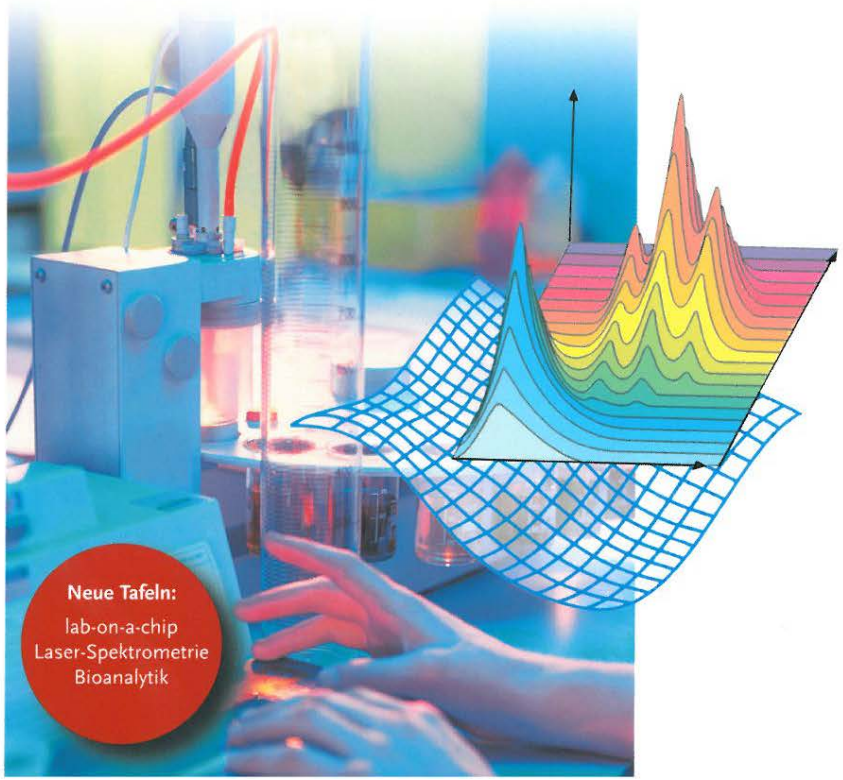
Georg Schwedt

Taschenatlas der Analytik

117 Farbtafeln von Joachim Schreiber
3., überarbeitete und erweiterte Auflage



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Analytische Grundvorlesung I

Quantitative Analyseverfahren

- Gravimetrie

  Fällung eines Niederschlages – Wägung und Berechnung

- Maßanalyse

  Säure-Base Titrationen, Komplexbildungstitrationen, Fällungstitrationen, Redoxtitrationen

❖ Optische Analyseverfahren - Spektroskopie

Lambert-Beersches Gesetz, Photometrie, Fluoreszenzspektroskopie,

UV, IR-Spektroskopie

❖ Trennoperationen

Fällung, Destillation, Verteilung, Ionenaustausch etc.

❖ Chromatographie

Theorie, Papierchromatographie, Dünnschichtschromatographie, Flüssigkeitschromatographie, Gaschromatographie, Apparaturen, Detektionssysteme

❖ Elektrophorese

Grundlagen, Gelelektrophorese, Kapillarelektrophorese

Was ist Analytische Chemie?

Chemische Analytik ist die Wissenschaft von der Gewinnung und verwertungsbezogenen Interpretation von Informationen über stoffliche Systeme mit Hilfe naturwissenschaftlicher Methoden. (IUPAC 1974)

<u>Der analytische Prozess (1)</u>

Problem

Hypothese

Analyseverfahren

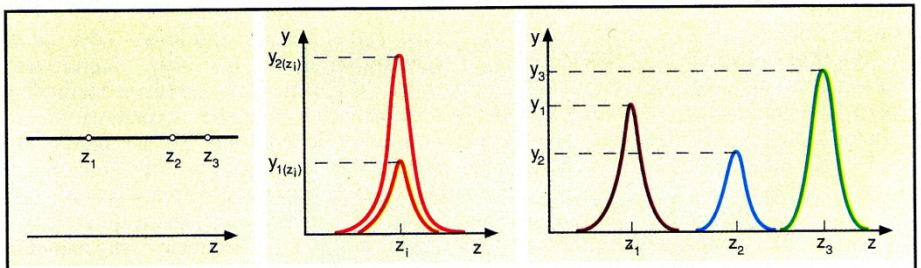
Informationen

Verwertung

Grundoperationen („unit operations“)

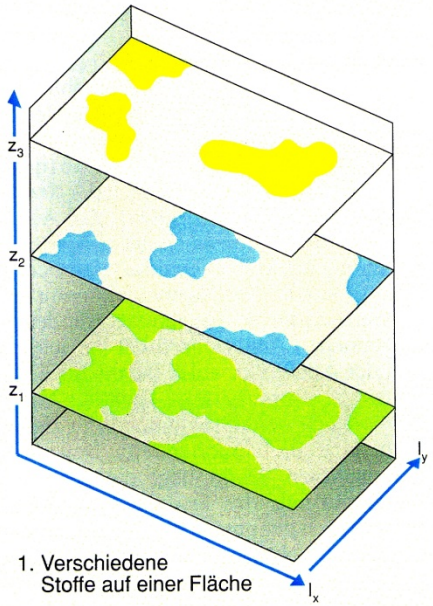
Jede Analyse besteht aus folgenden Teilschritten (Grundoperationen):

- Probenaufnahme (sampling)
- Probenvorbereitung (Trocknen, Lösen, Trennen)
- Messung (Messwert)
- Berechnung (Analysenwert) und Bewertung

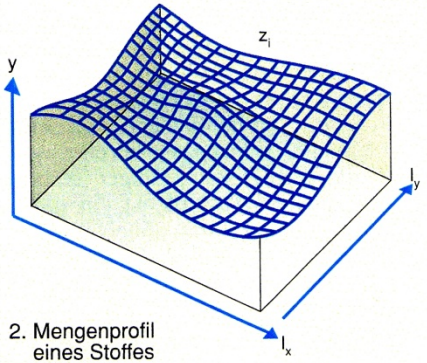


1. Qualitativ
2. Quantitativ
3. Qualitativ und quantitativ

A. Gehaltsanalyse

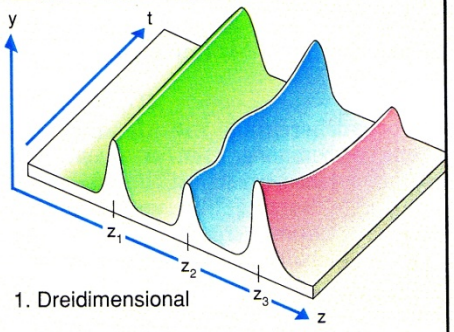


1. Verschiedene Stoffe auf einer Fläche

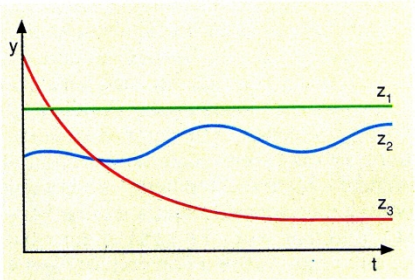


2. Mengenprofil eines Stoffes

B. Verteilungsanalyse

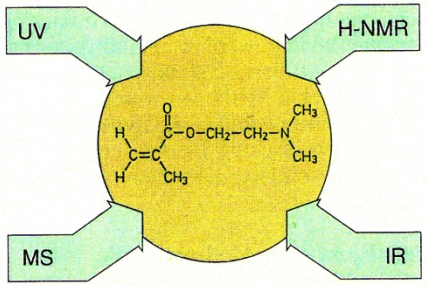


1. Dreidimensional

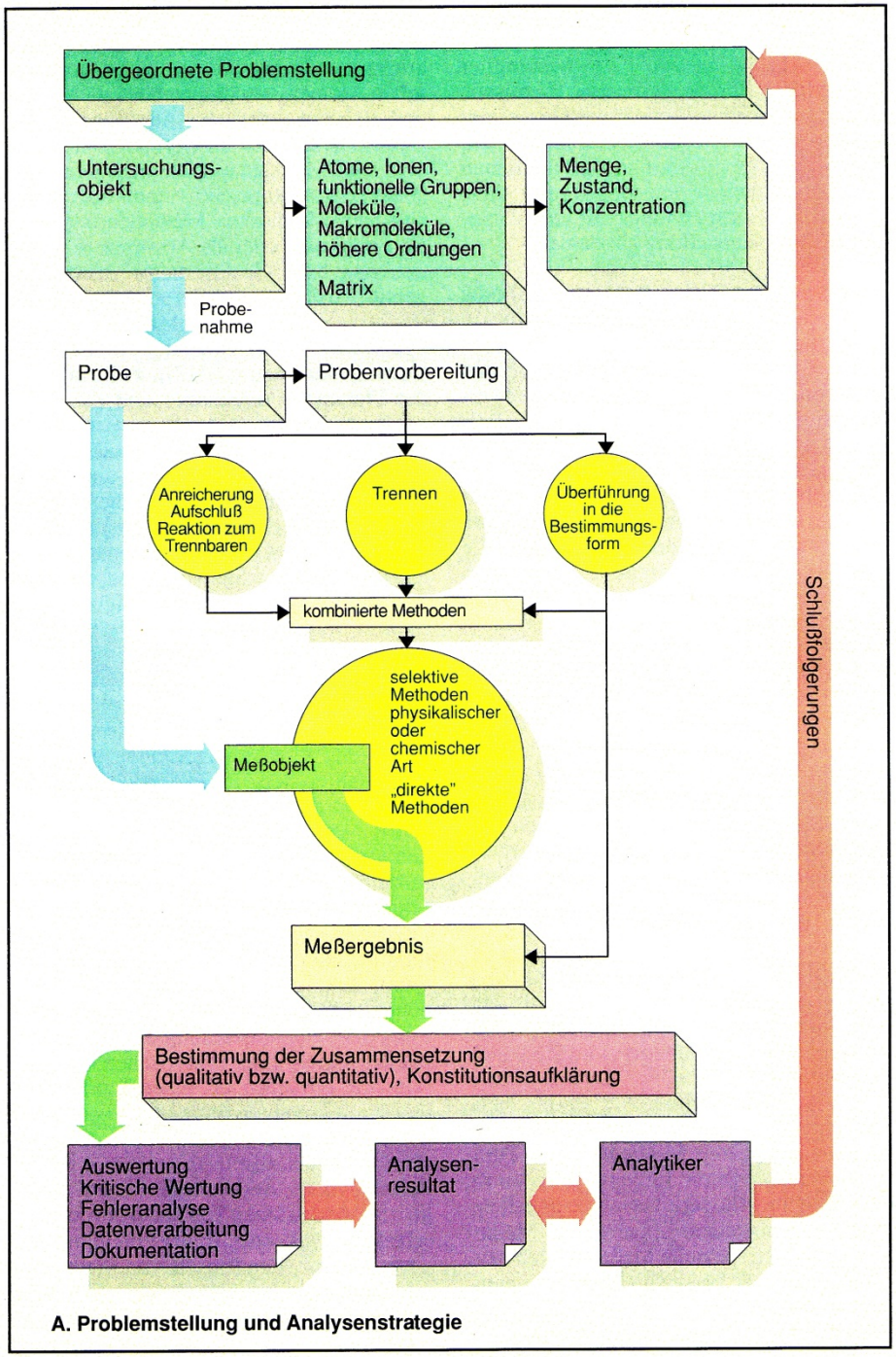


2. Zweidimensional

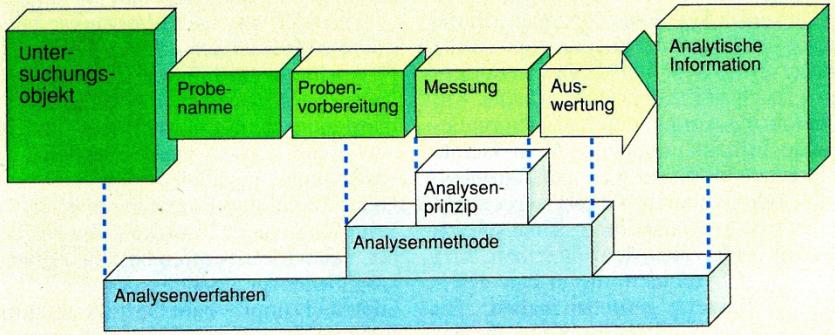
C. Prozessanalyse



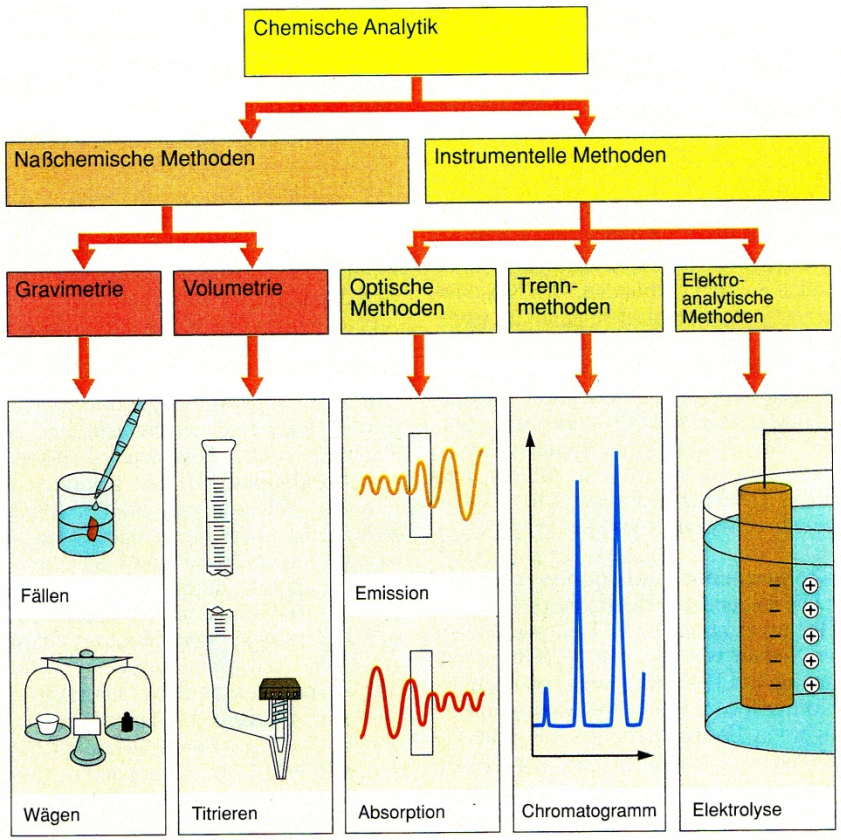
D. Strukturanalyse



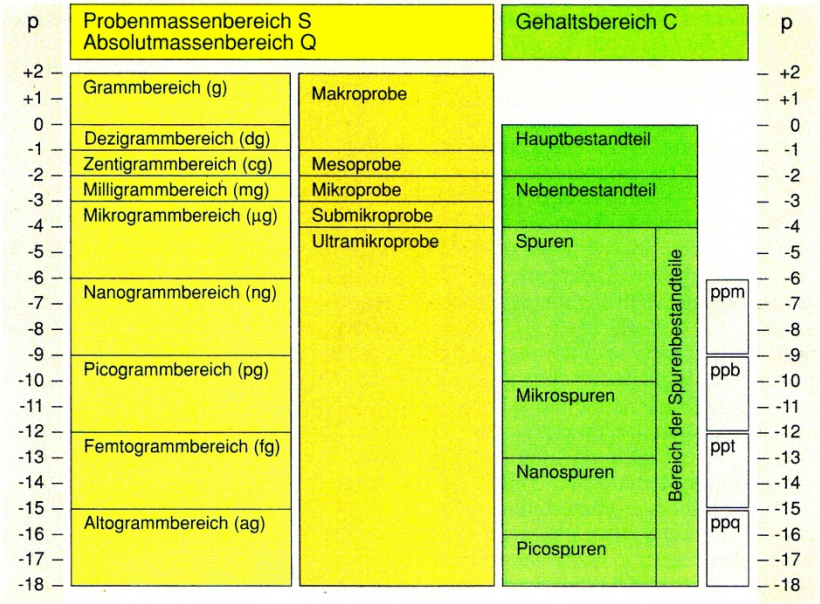
A. Problemstellung und Analysenstrategie



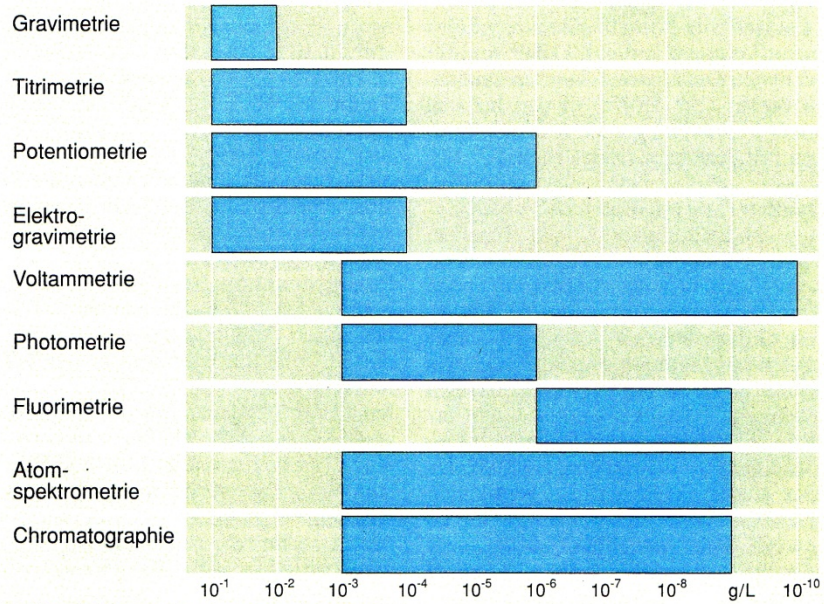
A. Analysenprinzip, Analysenmethode und Analysenverfahren



B. Systematik der Analysenmethoden



A. Arbeitsbereiche



B. Vergleich von Analysenmethoden

Arzneimittelanalysen

- Reinheit, Qualitätskontrolle

- Von Reinsubstanzen

- Von Gemischen in der Pharmazie

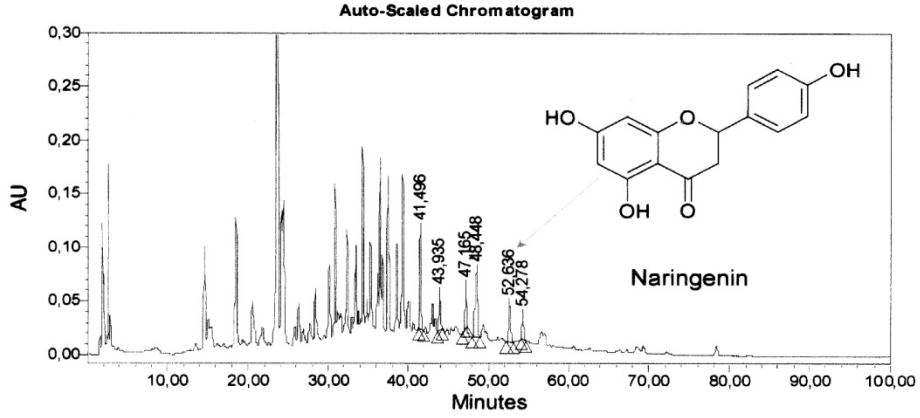
  Zunehmend von Bedeutung : Phytochemische Präparate

Current Date 13.03.2001

Sample Information

SampleName	Sinupret 00113011	Sample Type	Unknown
Vial	2	Date Acquired	21.02.2001 19:34:42
Injection	1	Acq Method Set	dwell 150 min
Injection Volume	20,00 ul	Processing Method	Naringenin
Channel	996	Date Processed	28.02.2001 15:21:10
Run Time	160,0 Minutes		

Channel Description PDA 190.0 to 400.0 nm at 1.2 nm



Holunder (*sambuci flos*)

Sinupret©

Medizinische Analysen – Diagnostik

- Pharmakologische Wirkung

  Wirkstoffanalytik

  Abbauprodukte, Blut

  Kinetik der Wirksubstanz

- Blut, Harn
- Genomics, Proteomics, Metabolomics

Umweltanalysen

- Luft, Wasser, Boden

  Schadstoffanalytik, z.B. Dioxine

Lebensmittelanalytik

- Inhaltsstoffe in Lebensmitteln
- z.B. Wein – Resveratrol

Kriterien für die Wahl des Analysenverfahrens

- Geforderte Information
- Genauigkeit, Präzision
- Probemenge
- Voraussichtlicher Gehalt der Probe
- Empfindlichkeit
- Selektivität
- Qualitative Zusammensetzung
- Analytische Operationen
- Ausrüstung
- Zeit
- Kosten

Probemenge – Gehalt - Arbeitsbereich

- Probemenge, Probenbereich (sample size) **P** :

  Menge (meist der Masse) der Analysenprobe

  Makrobereich: $m > 100$ mg

  Halbmakrobereich: $100 \text{ mg} > m > 10$ mg

  Mirkobereich: $m < 10$ mg

- Gehalt, Gehaltsbereich (content) **G** :

  Gehalt der Probe an der bestimmten Komponente

  Hauptbestandteil : $wi > 10\%$

  Nebenbestandteil: $10\% > wi > 1\%$

  Spurenbestandteil: $wi < 1\%$

❖ Arbeitsbereich **A**:

Menge (Masse) der Komponente i, welche mit der Methode bestimmt werden kann

$$A = G \times P$$

❖ Nachweisgrenze, Bestimmungsgrenze:

Untergrenze des Arbeitsbereichs (3s – Regel!)

Größen und Einheiten – heutige und frühere Bezeichnungen

heutige Bezeichnungen	frühere Bezeichnungen
Masse m in kg oder g	Masse, Gewicht, Menge, Gewichtsmenge, Grammenge in kg oder g
Stoffmenge n (X), n (eq) in mol	Menge, Molmenge, Molzahl, Anzahl Mole als Grammatom, Grammion, Grammolekül (Mol), Grammäquivalent (Val)
Molare Masse M (X), M (eq) in g/mol	Atomgewicht, Atommasse, Molekulargewicht, Molgewicht, Molmasse, Äquivalentgewicht, Äquivalentmasse, Formelgewicht, Formelmasse in g, g/Mol, g/Val, als relative Größen ohne Einheit
Massenkonzentration β in g/l	Konzentration, Gehalt in g/l
Stoffmengenkonzentration c (X) in mol/l	Konzentration, Gehalt; molare Konzentration oder Molarität in Mol/l, Zeichen: M
Äquivalentkonzentration c (eq) in mol/l	normale Konzentration oder Normalität in Val/l, Zeichen: N
Massenanteil w in g/g	Gewichtsprozent (Gew.-%), Massenprozent, Massenbruch
Stoffmengenanteil x in mol/mol	Molprozent (Mol-%), Molenbruch, Molgehalt, Atomprozent

Institute of Analytical Chemistry

Head of the Institute
o.Univ.Prof. Mag. Dr. Dr.hc.
Günther Bonn

Vice-Head
Univ.Prof. Mag. Dr.
Christian Huck

Spectroscopy
Univ.Prof. Mag. Dr. Christian Huck

Electroanalytics
ao.Univ.Prof. MMag. Dr.
Dagmar Obendorf

Development of Stationary Phases
PD. Mag. Dr. Rania Bakry

Capillary Electrophoresis
ao.Univ.Prof. Mag. Dr.
Andreas Zemann

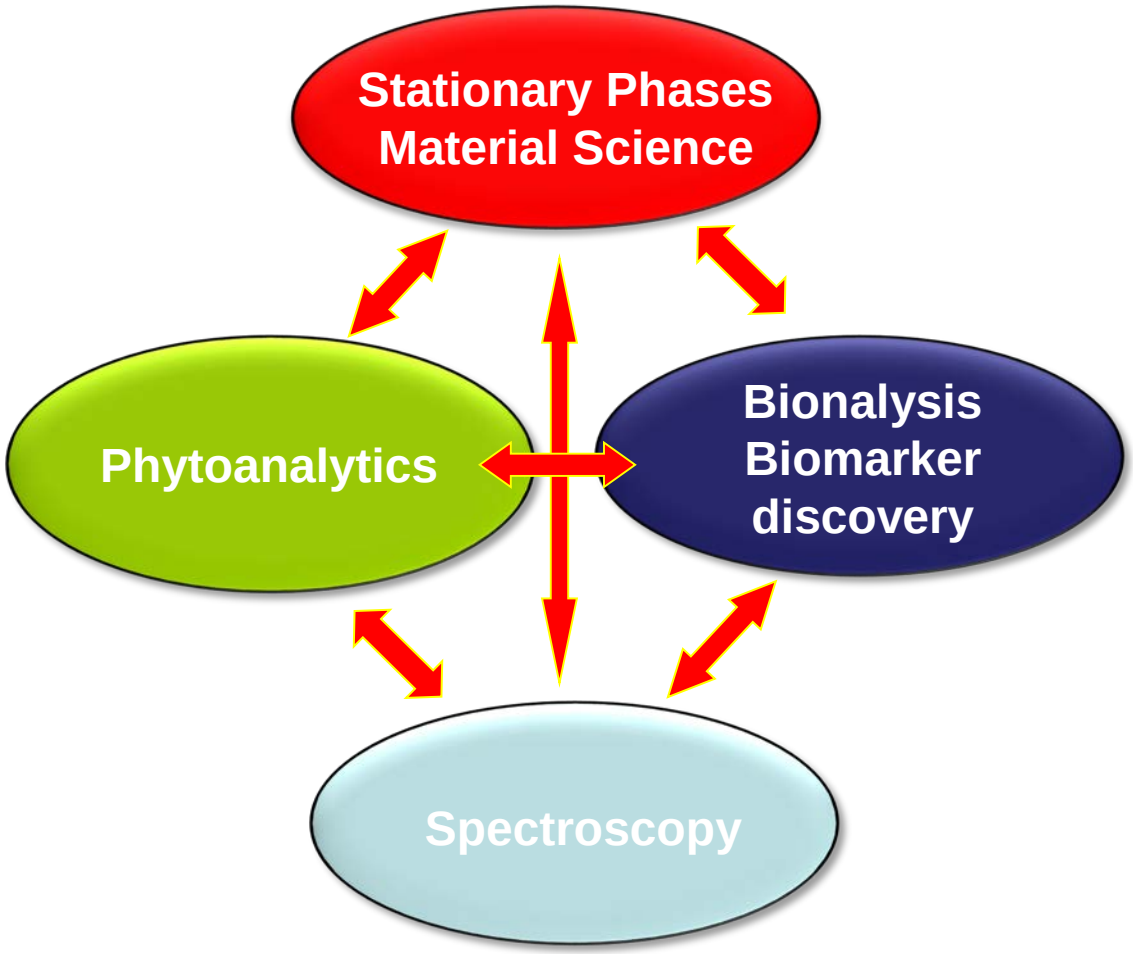
Bioanalysis
Ass.-Prof. DI (FH) Dr.
Matthias Rainer

Phytoanalytics
o.Univ.Prof. Mag. Dr. Dr.hc. Günther Bonn
Univ. Prof. Mag. Dr. Christian Huck

~ 40 co-workers

ACRC TOPICS / Synergies

Innovations in Analytical Chemistry

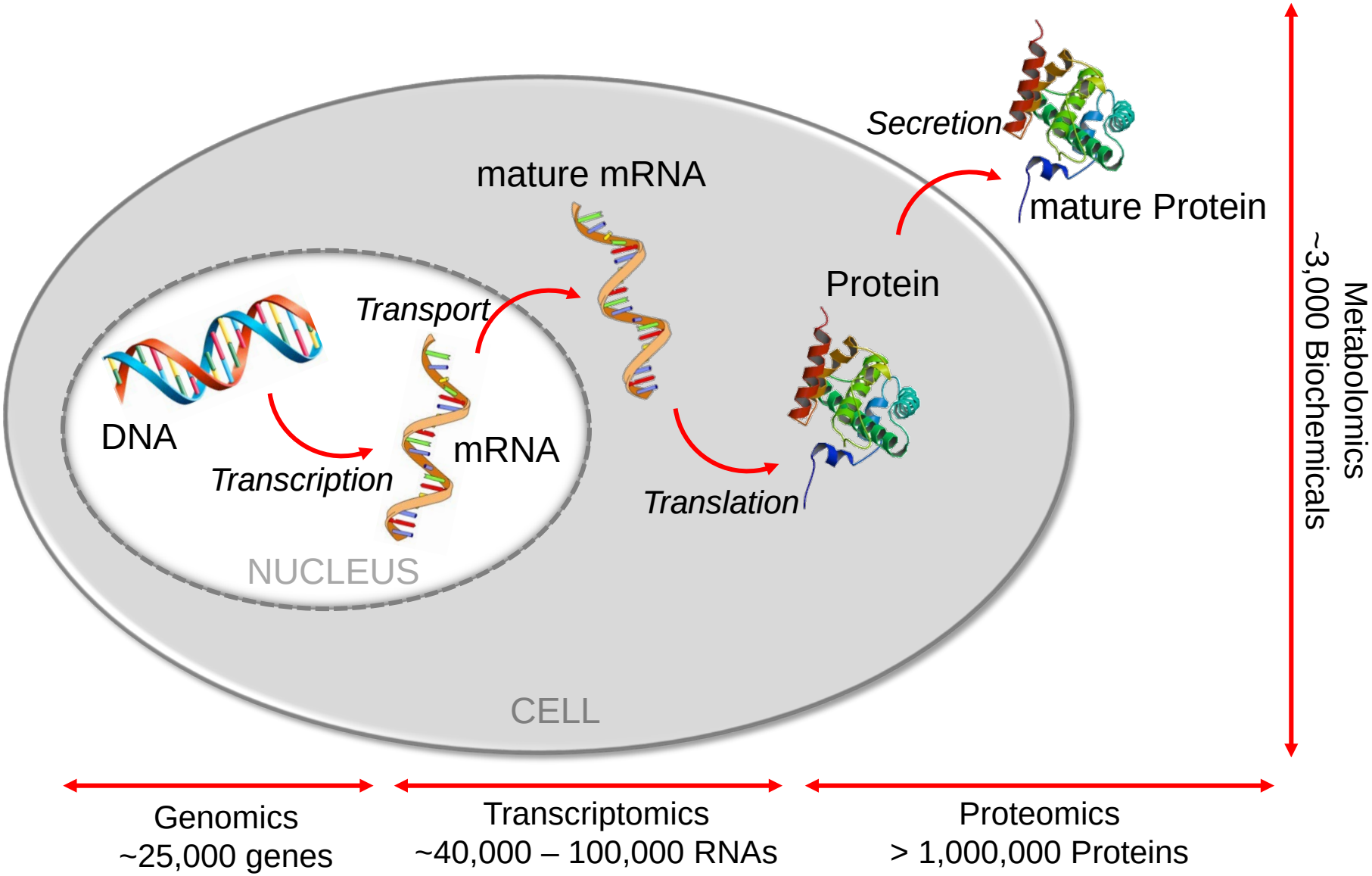


Application Fields

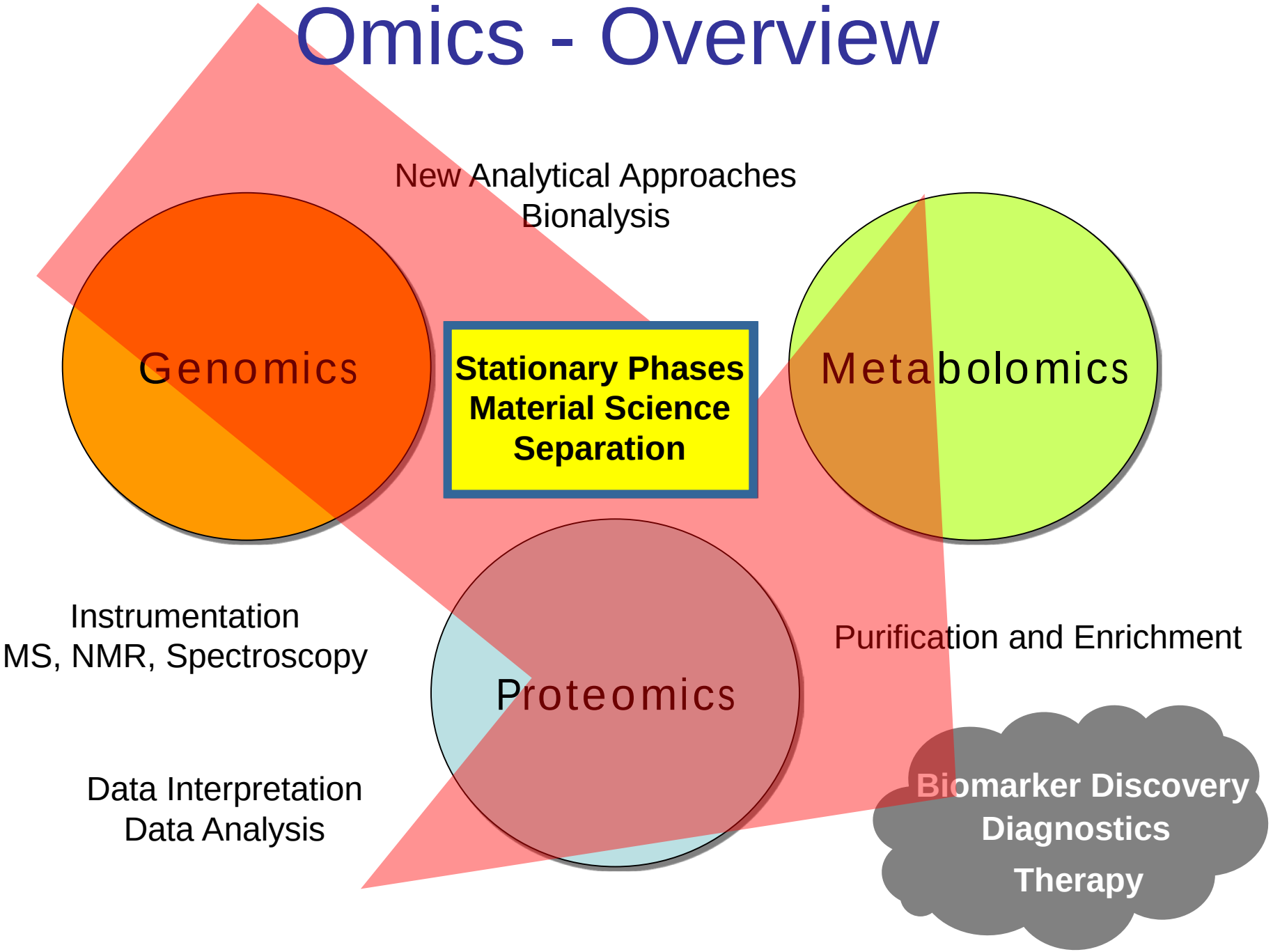
Bioanalysis
Biomarker
Diagnostics

Phytoanalysis
Metabolomics

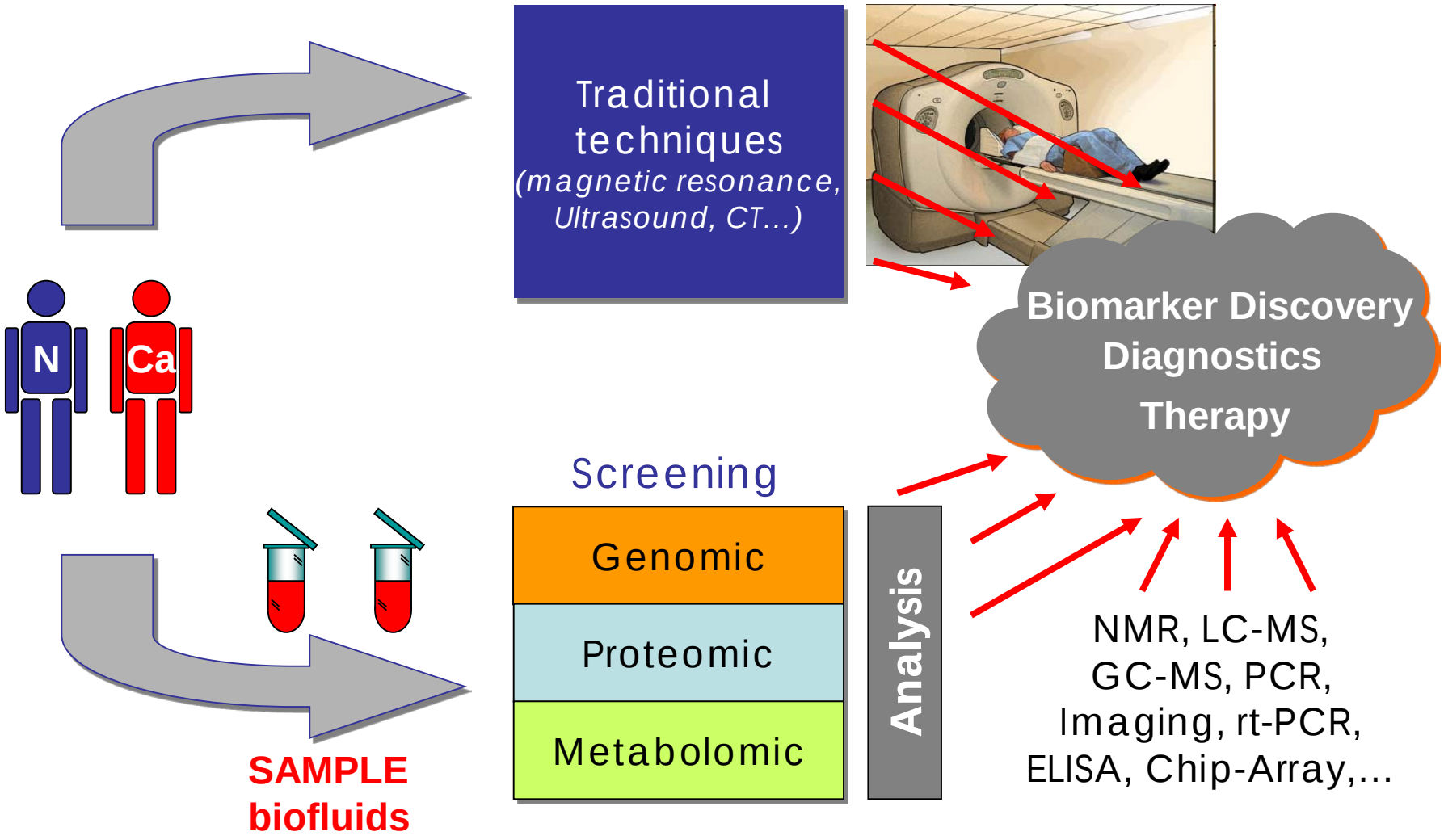
Omics - Overview



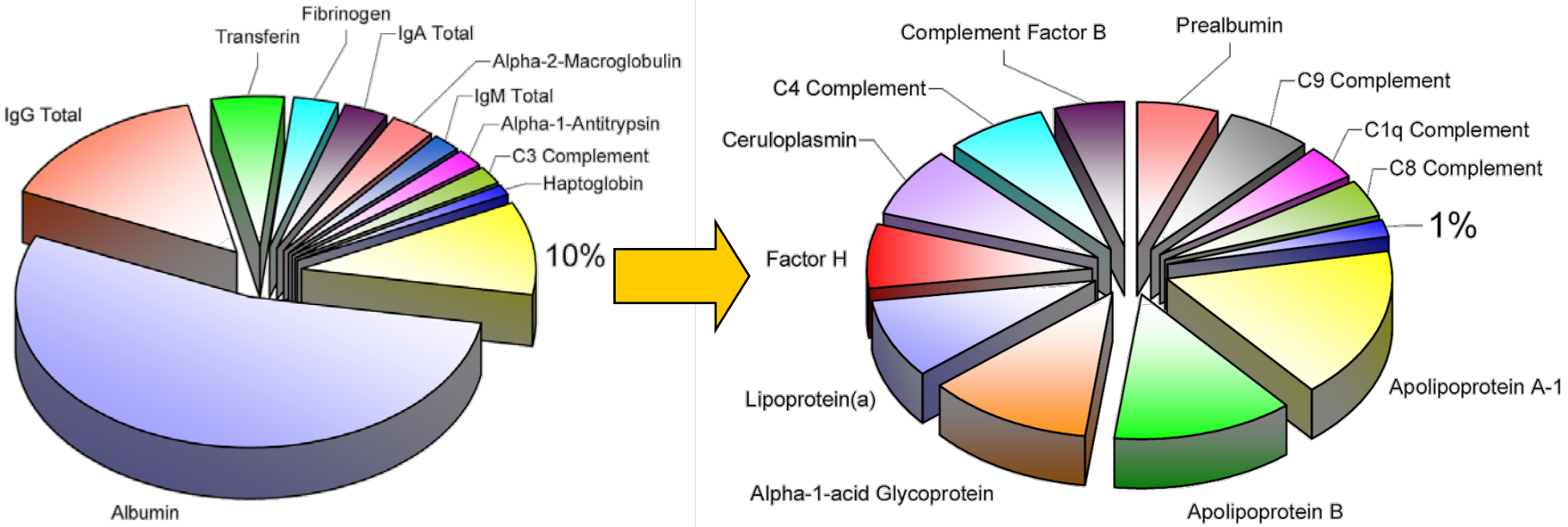
Omics - Overview



Omics - Overview



Complexity of Human Serum



90%

10%

22 proteins are approx. 99% of the whole serum proteome

→ Reducing the complexity by pre-analytical approaches!

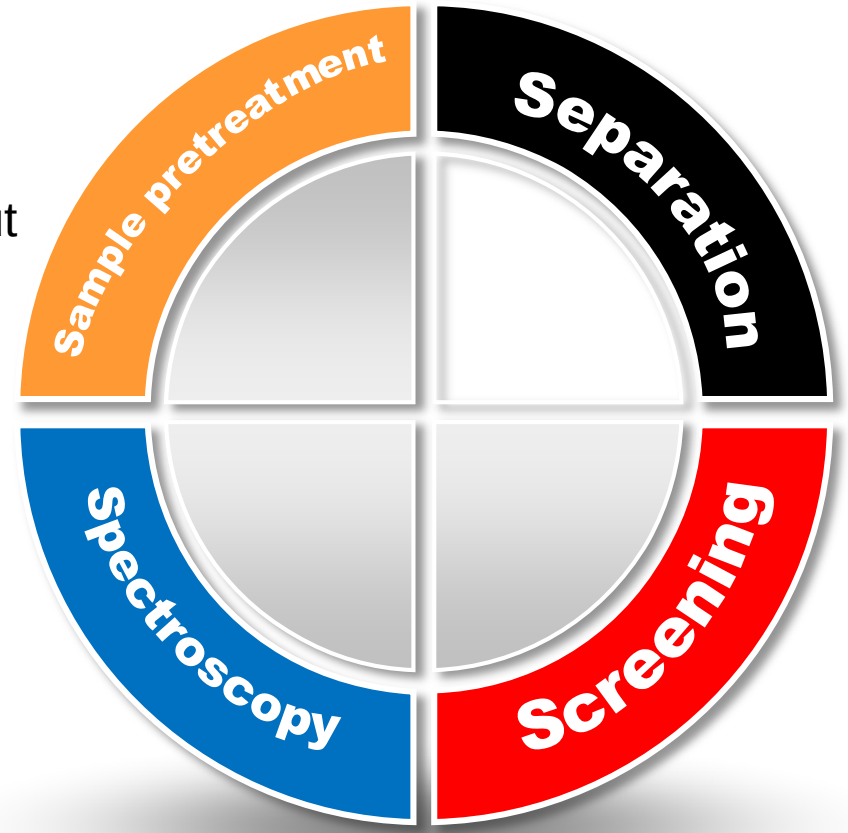
Analytical Approaches

Innovations

Enrichment

Desalting

High-sample througput



LC, LC-MS/MS

µ-LC, µ-LC-MS/MS

CE, CE-MS

CEC

Near-infrared

Mid-Infrared

Imaging/mapping

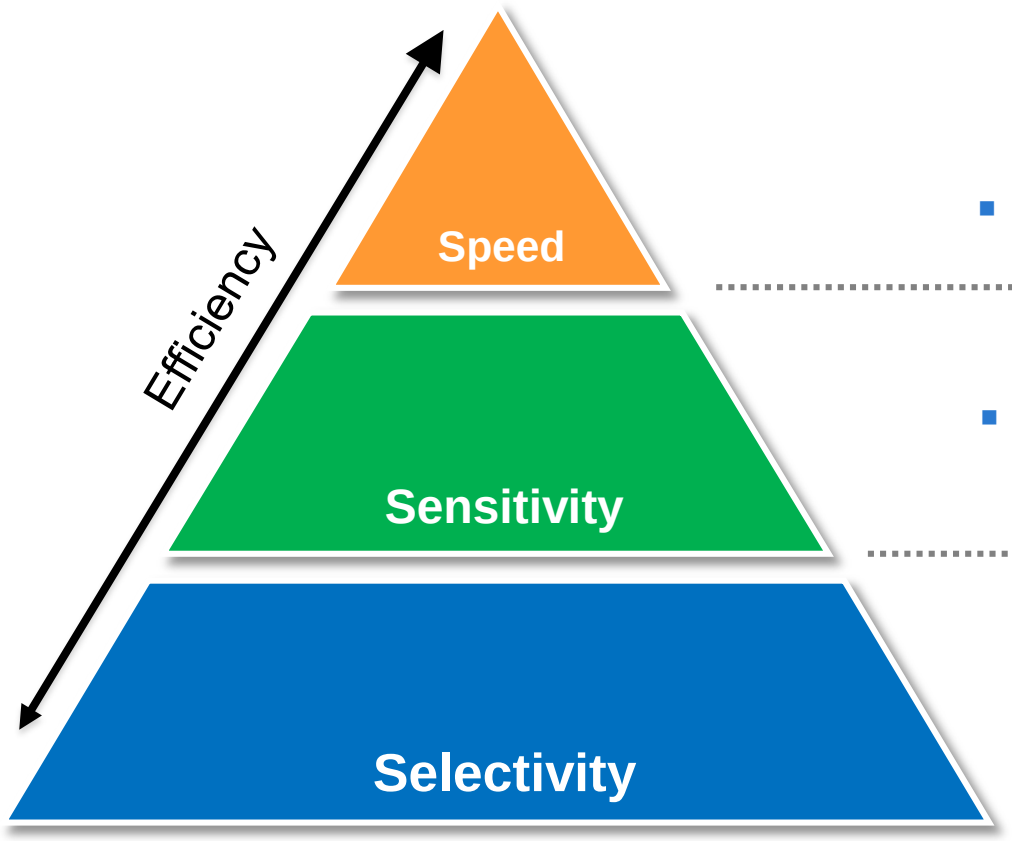
MALDI-TOF-MS/MS

Matrixfree-MALDI

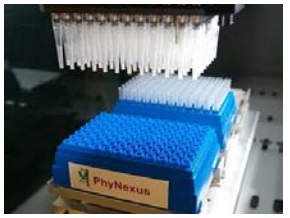
MELDI-TOF

MALDI-imaging/mapping

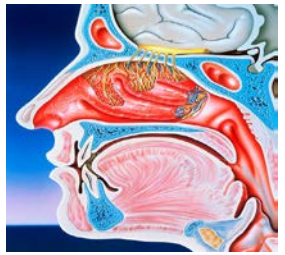
Why Analytical Innovations?



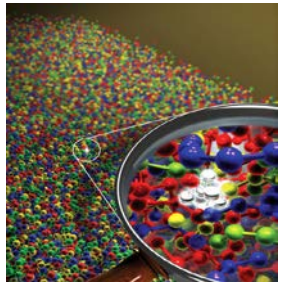
- High sample throughput



- Improved detection limit

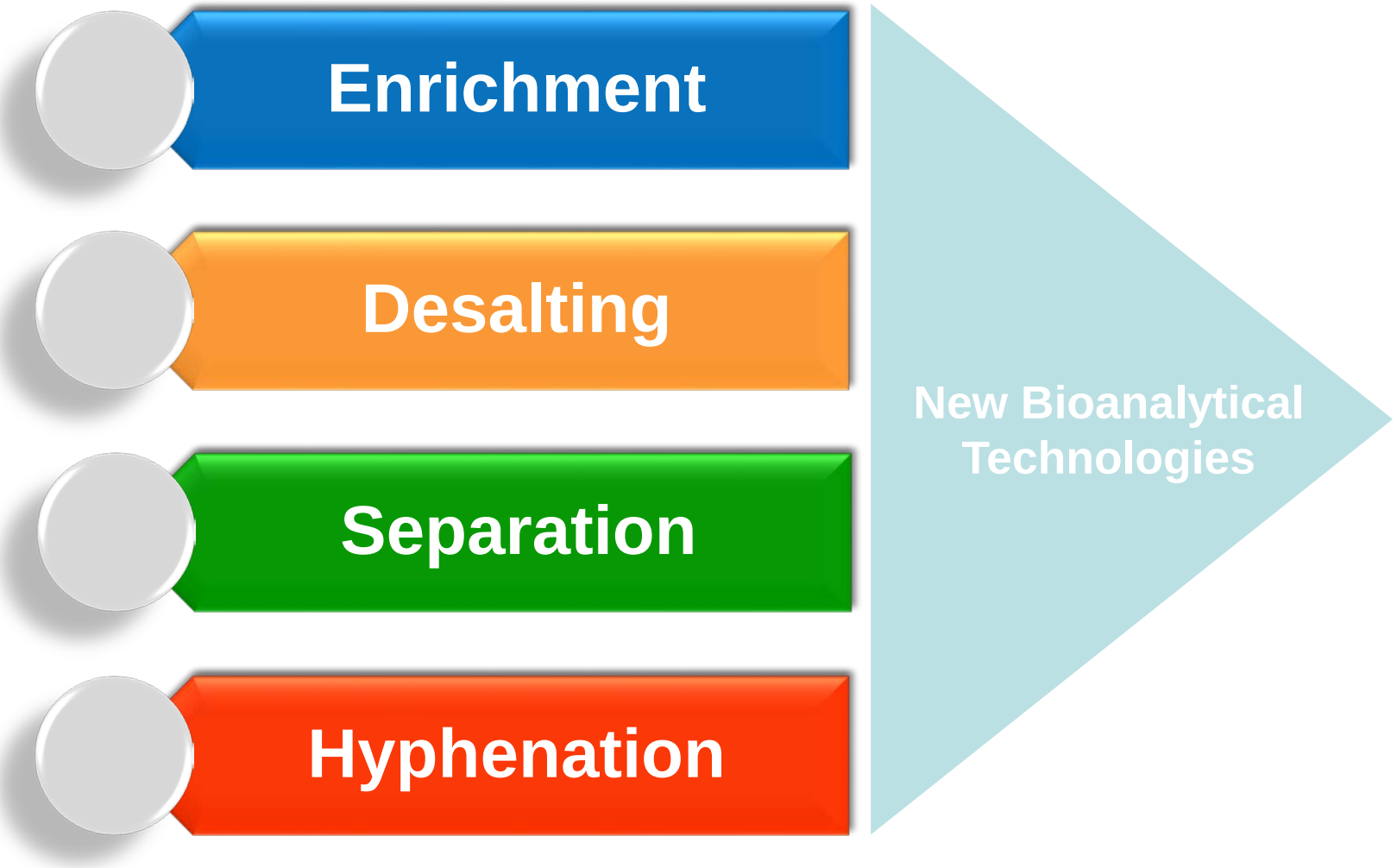


- Needle in haystack



„To see what one could not see before“

Highly Efficient Enrichment and Separation of Biomolecules



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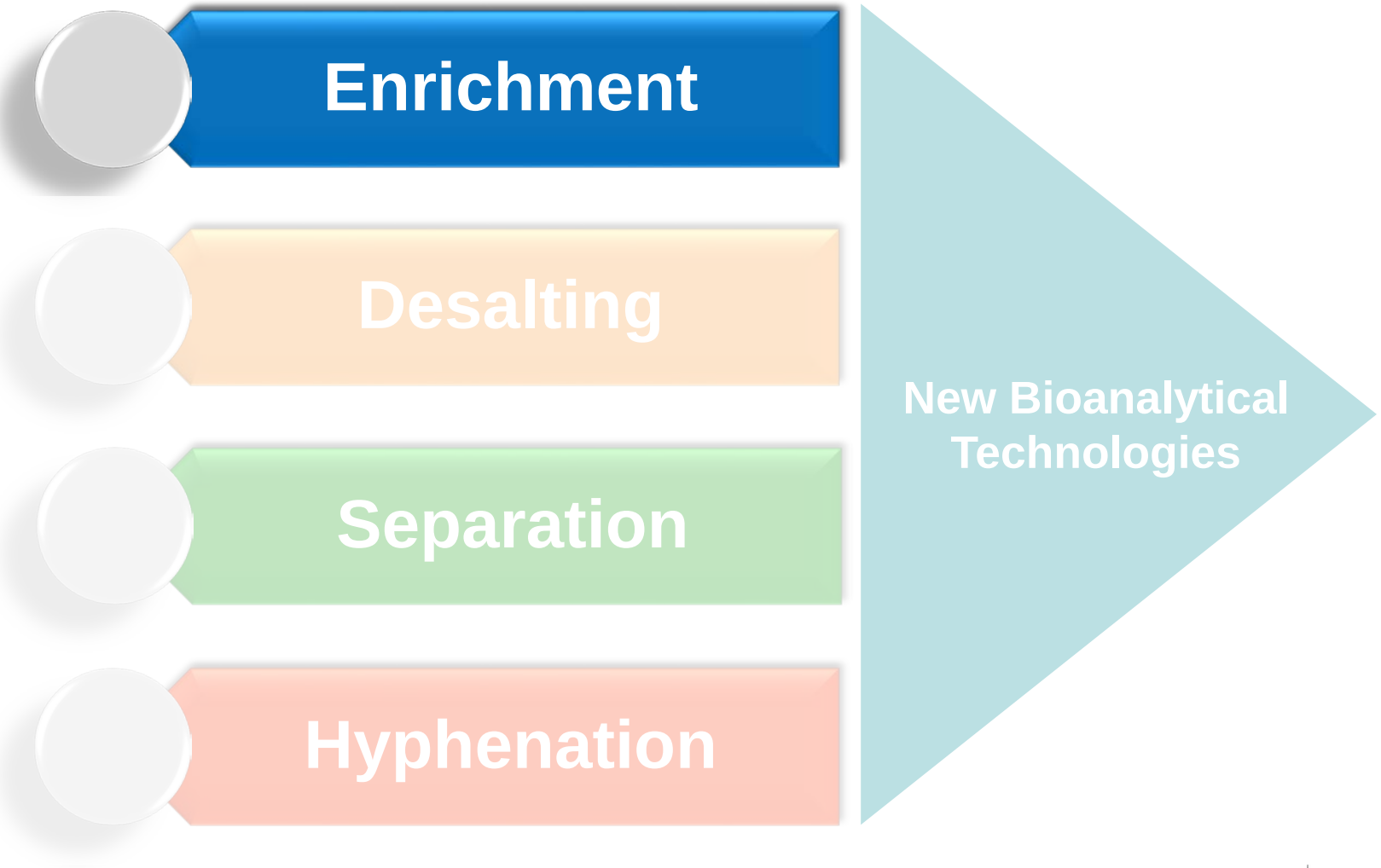
Center of Competence and Excellence
Leopold-Franzens University of Innsbruck, Austria

Highly Efficient Enrichment and Separation of Biomolecules



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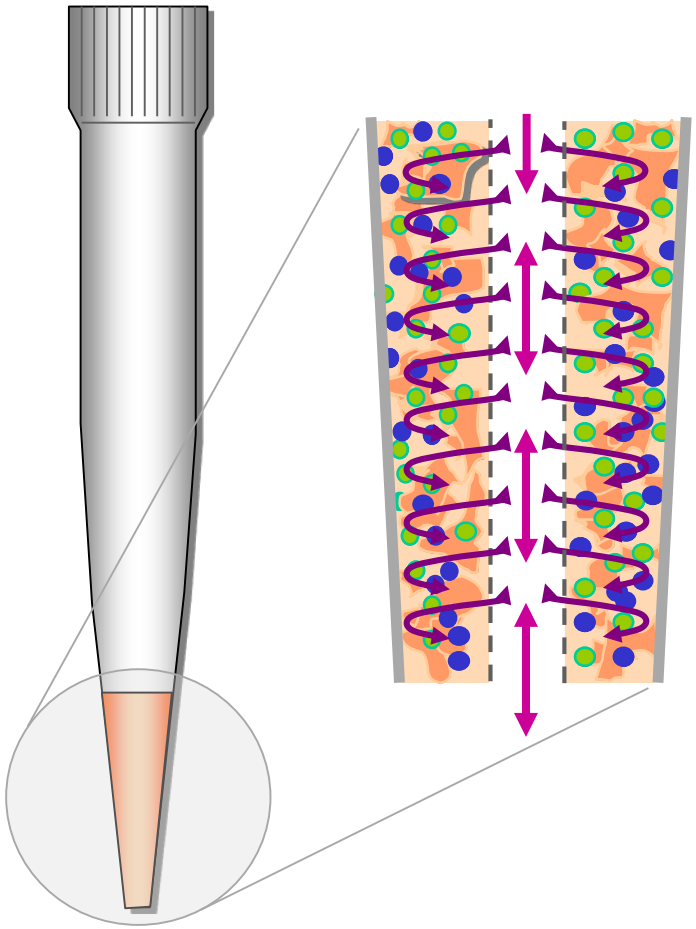
Leopold-Franzens University of Innsbruck, Austria

Preparation of - Hollow Monolith™

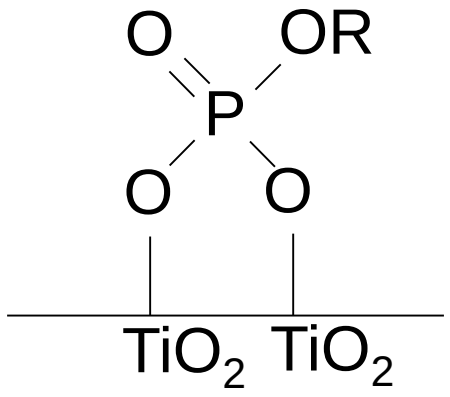


Hollow Monolith™

ZrO_2 < 100 nm

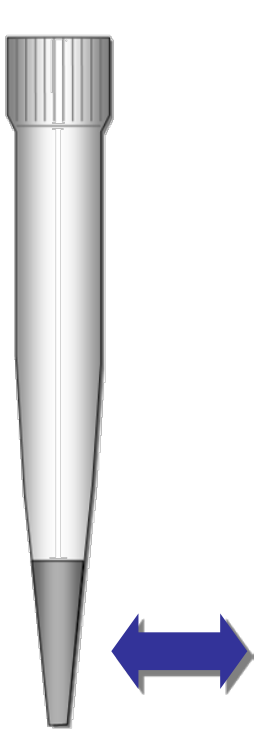
TiO_2 < 100 nm

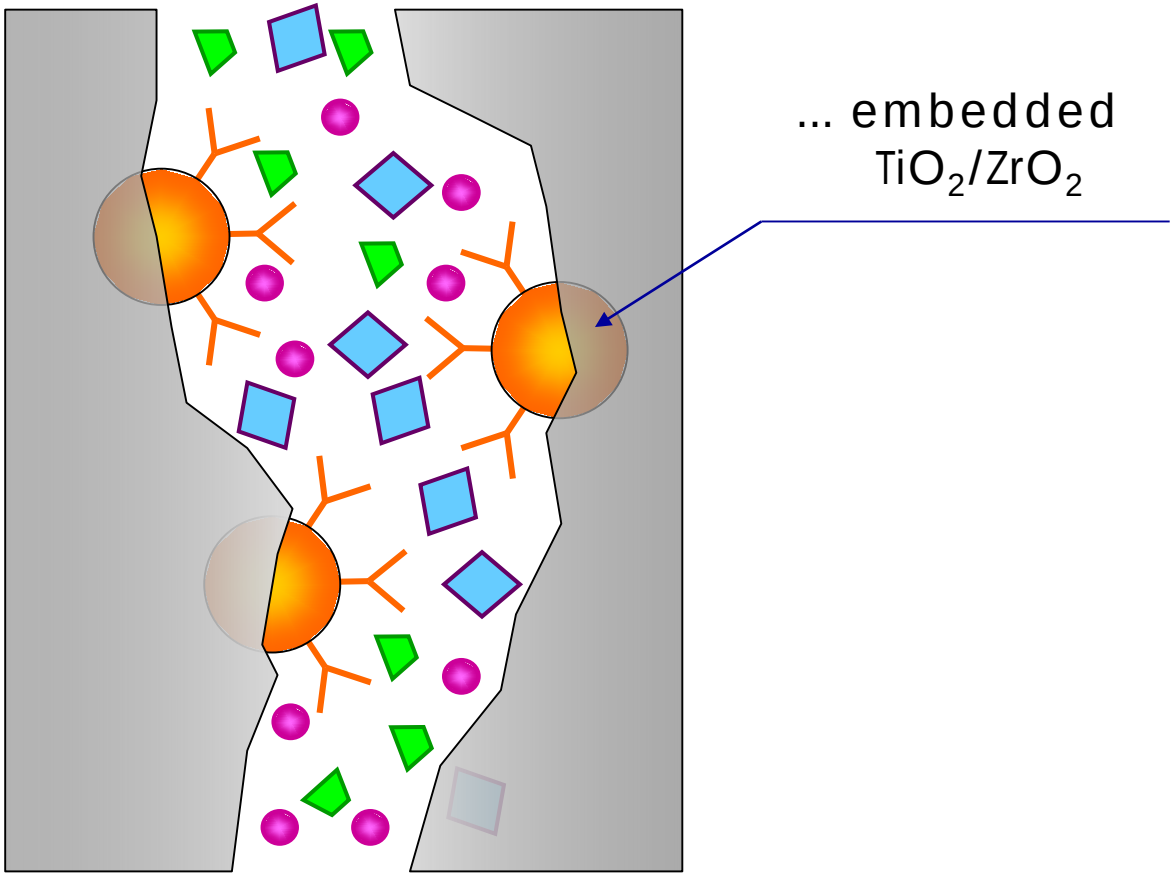
poly(divinylbenzene)



Mechanism: Bridging Bidentate

Enrichment of Phosphopeptides





... Phosphorylated Peptides

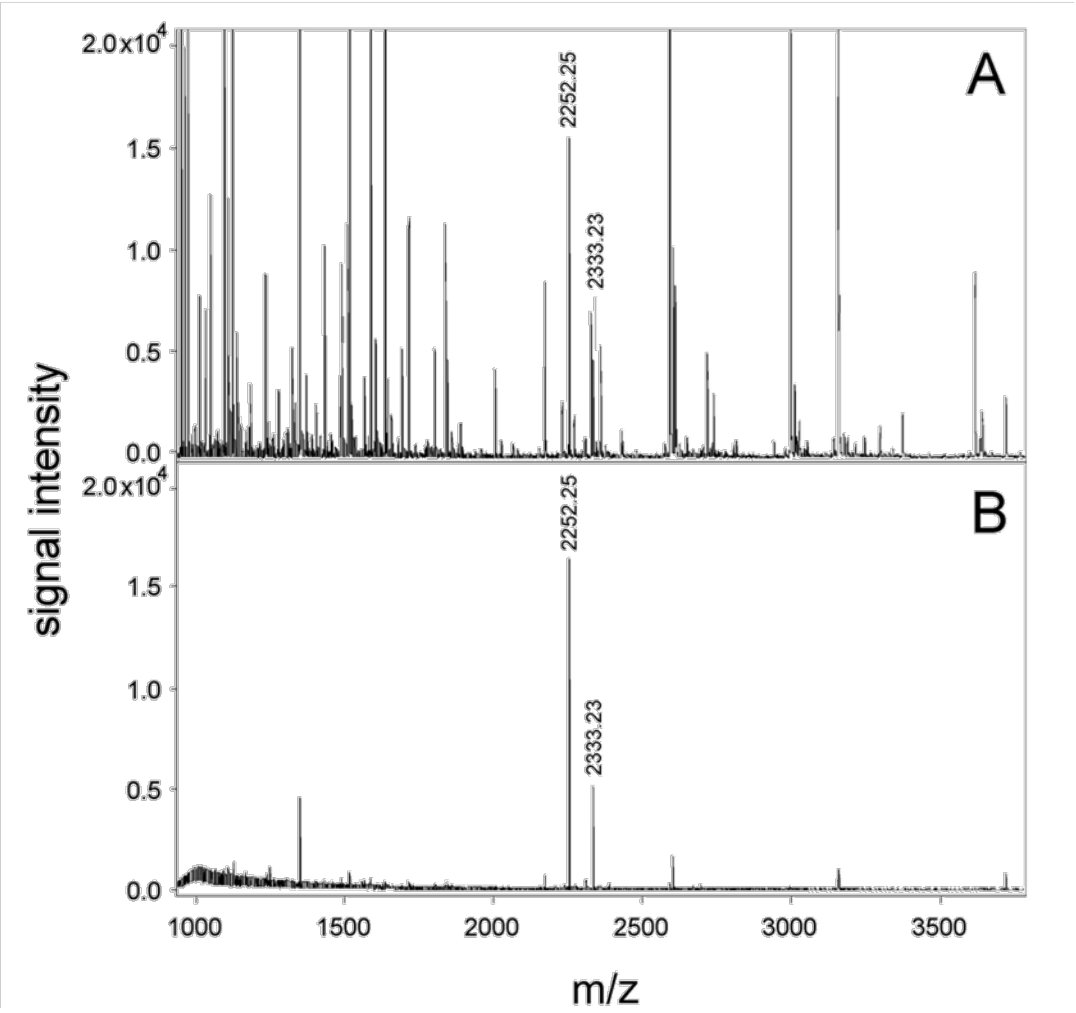
Enrichment of *in vitro* phosphorylated ERK1 digest

MALDI MS spectra:

1.) before enrichment (A)

2.) after enrichment with poly(DVB)-TiO_2/ZrO_2 tips (B)

Signals at *m/z* 2252.25 and *m/z* 2332.23 correspond to phosphorylated peptides

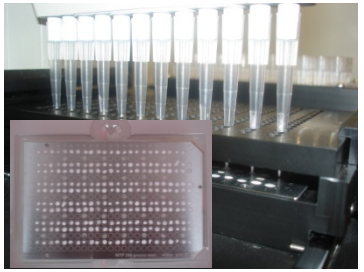


Collaboration with Prof. Lukas Huber, Biocenter - Innsbruck

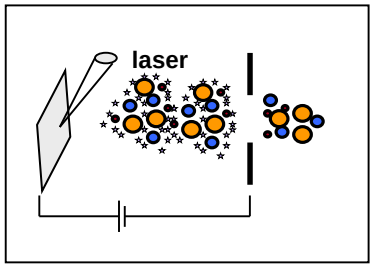
Automation of Sample Preparation

1. sample loading

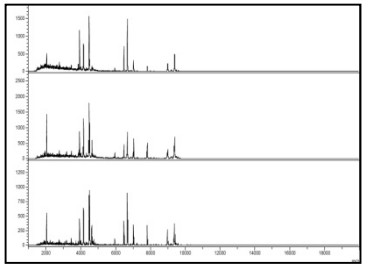
2. sample spotting



3. sample analysis



4. data processing

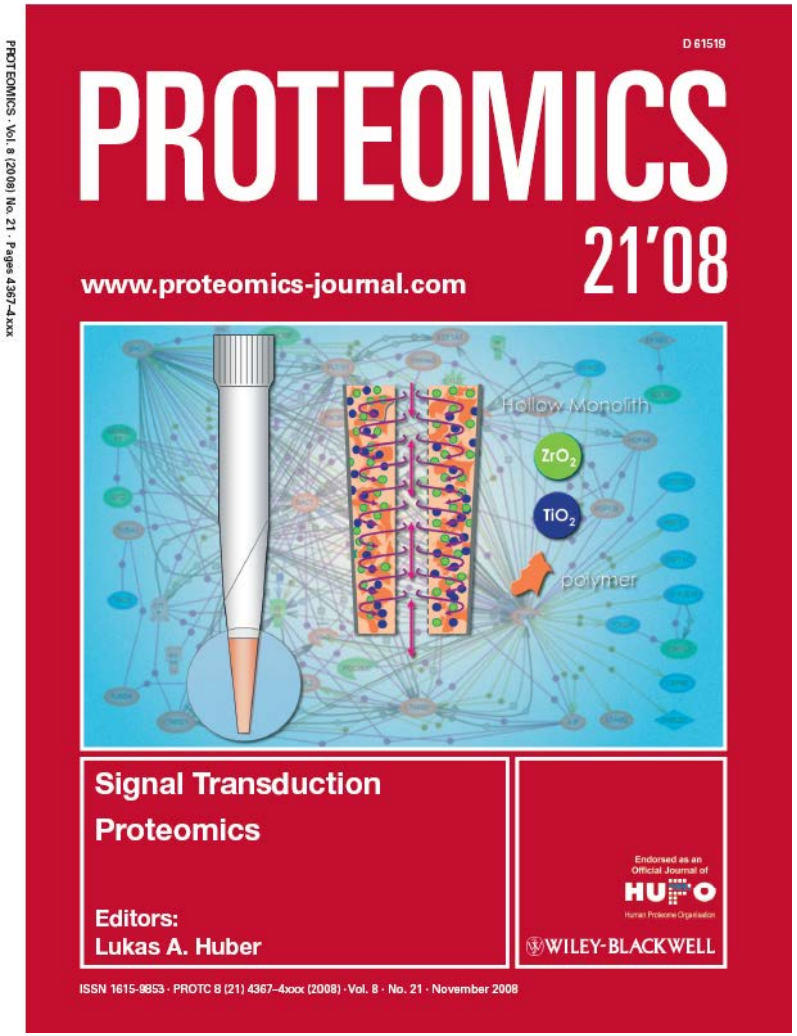


- **Specific enrichment**
- **Purification**
- **Desalting**

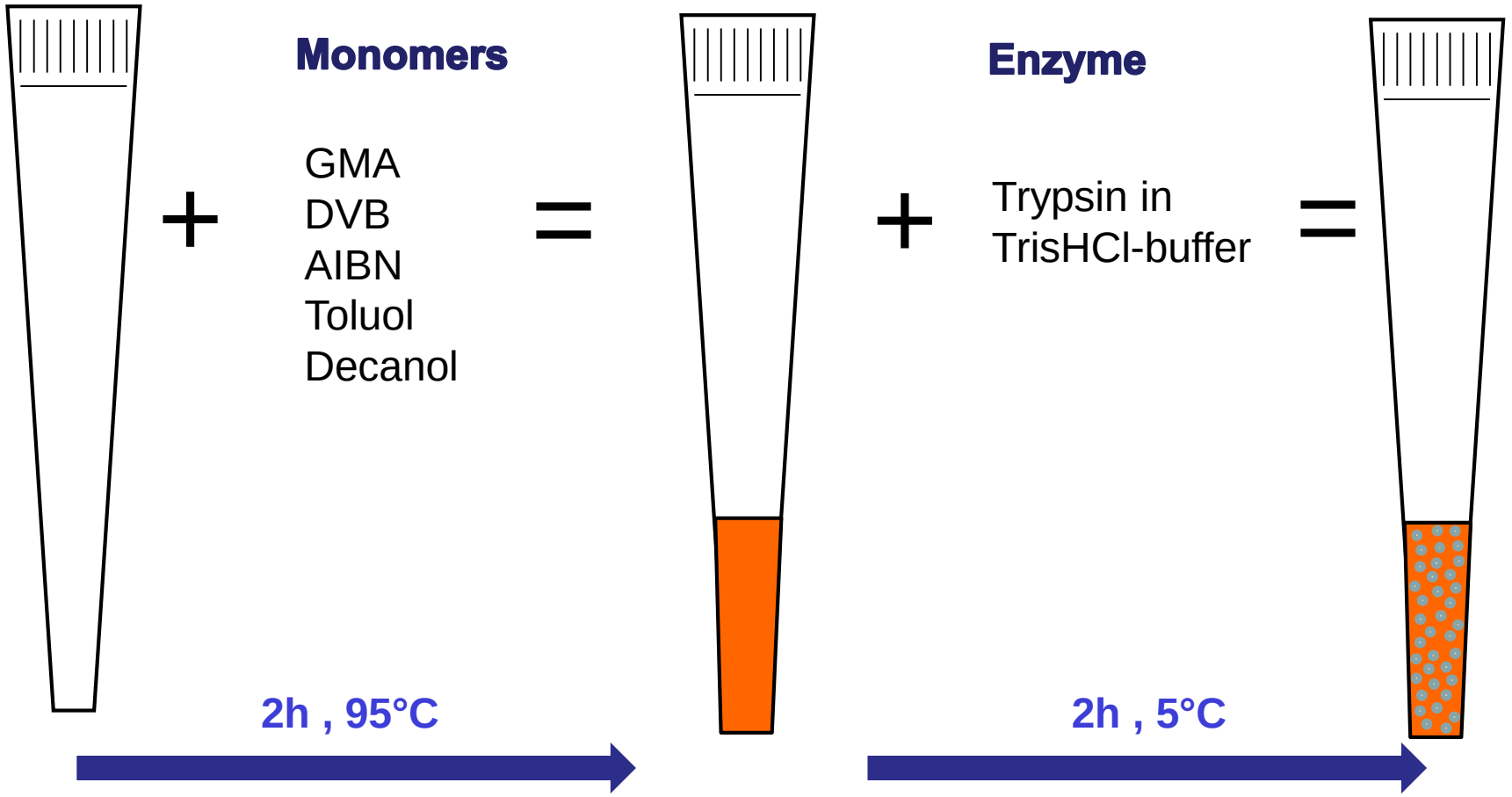
A collaboration with PhyNexus Inc., San Jose, CA, USA

PhyNexus

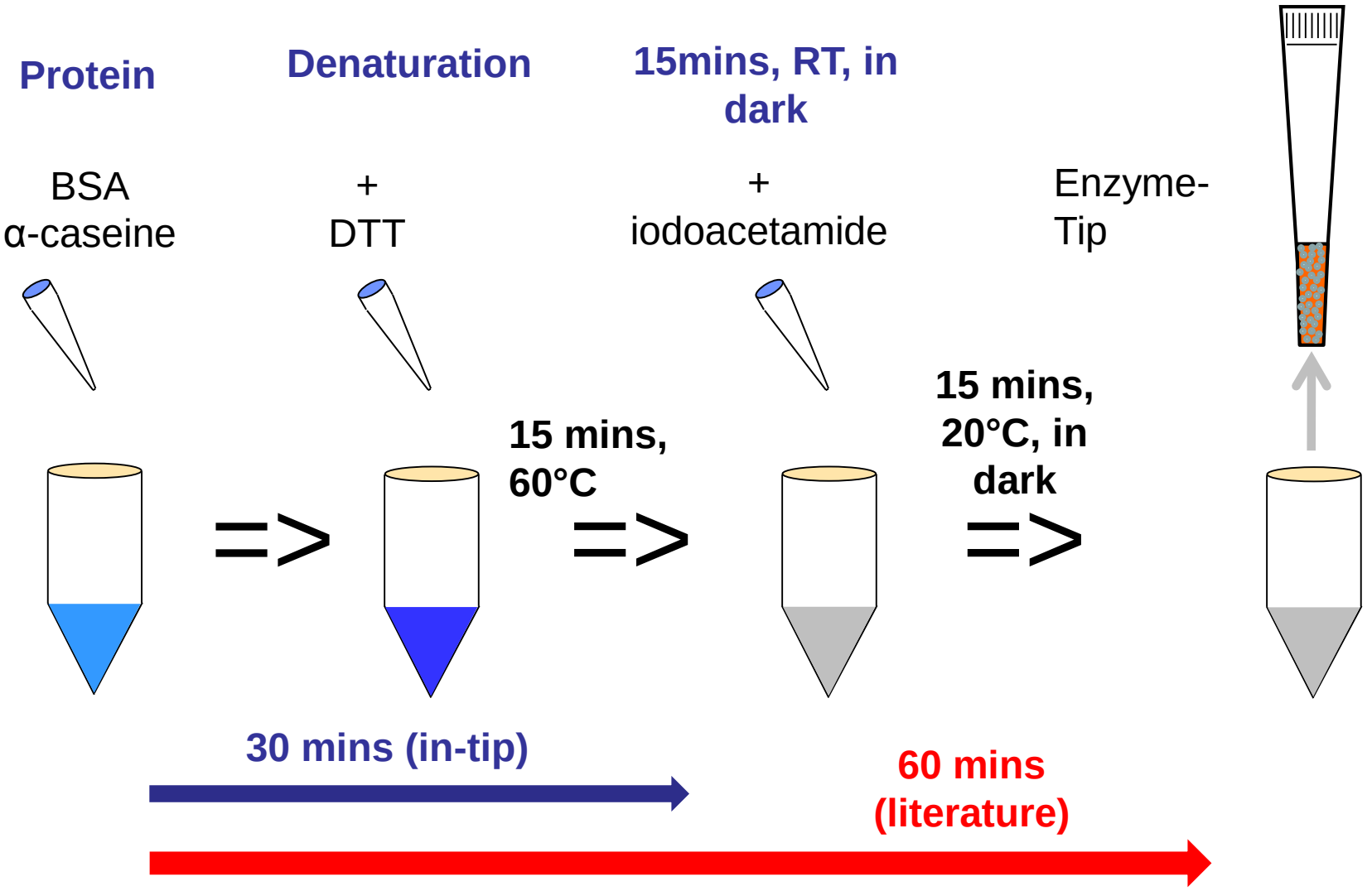
Proteomics Cover



Monolithic Extraction Tips for Enzymatic Digestion



Monolithic Extraction Tips for Enzymatic Digestion



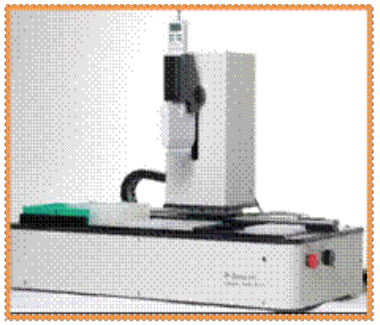
Microwave-Assisted In-Tip Digestion

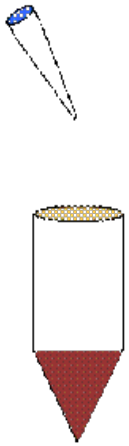
Denaturation

- Bovine Serum Albumin
- Myoglobin
- α-Casein
- Milk
- .....

Enzyme-Tip

Microwave In-Tip-Digestion





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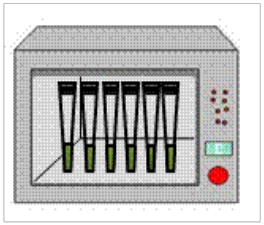

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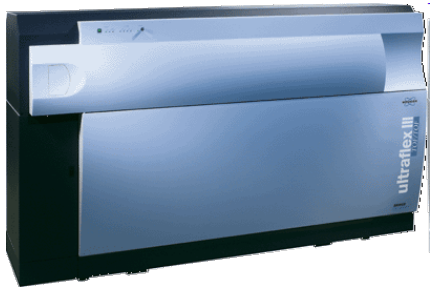
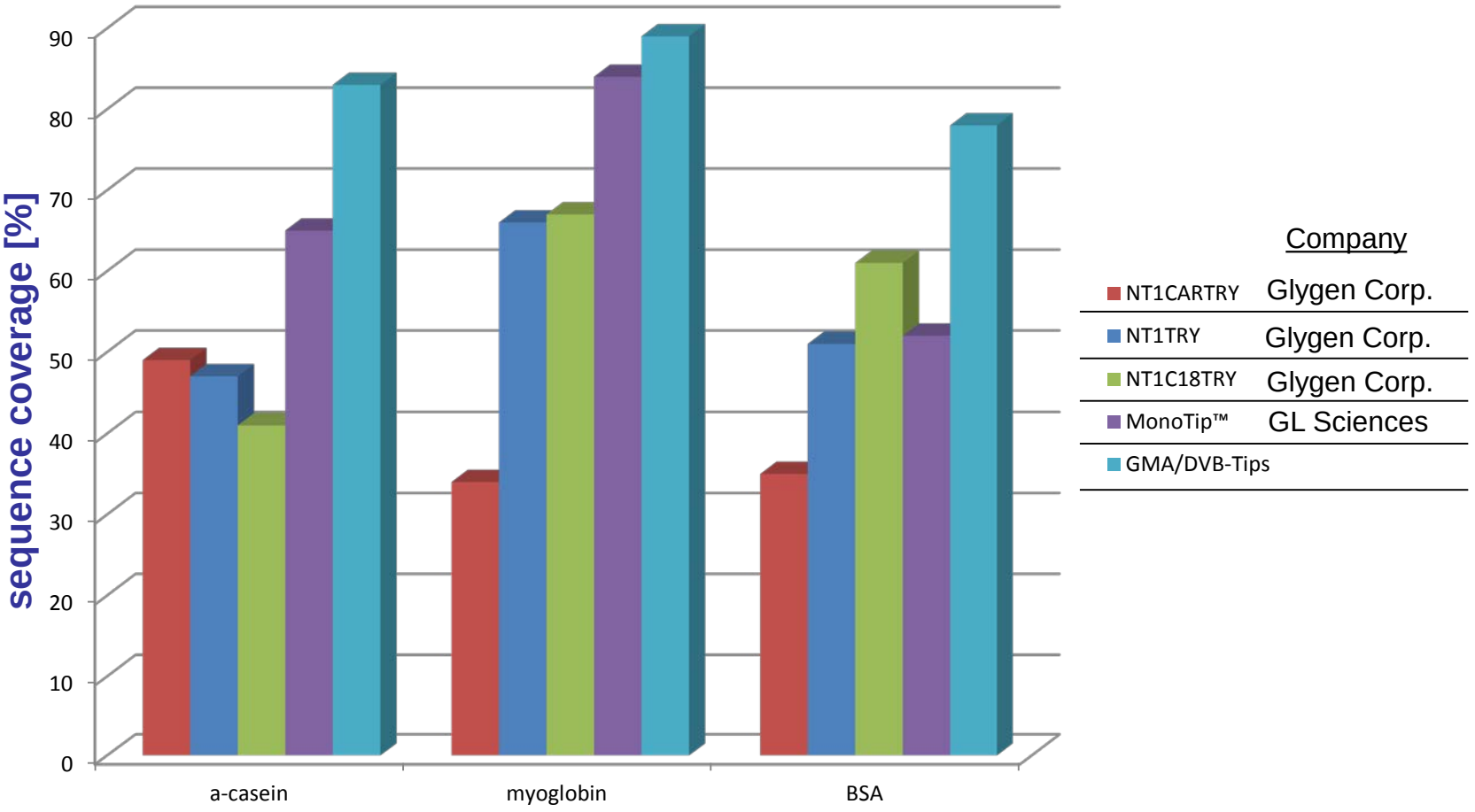
Eluting and MALDI TOF MS



Total Time 45 min!

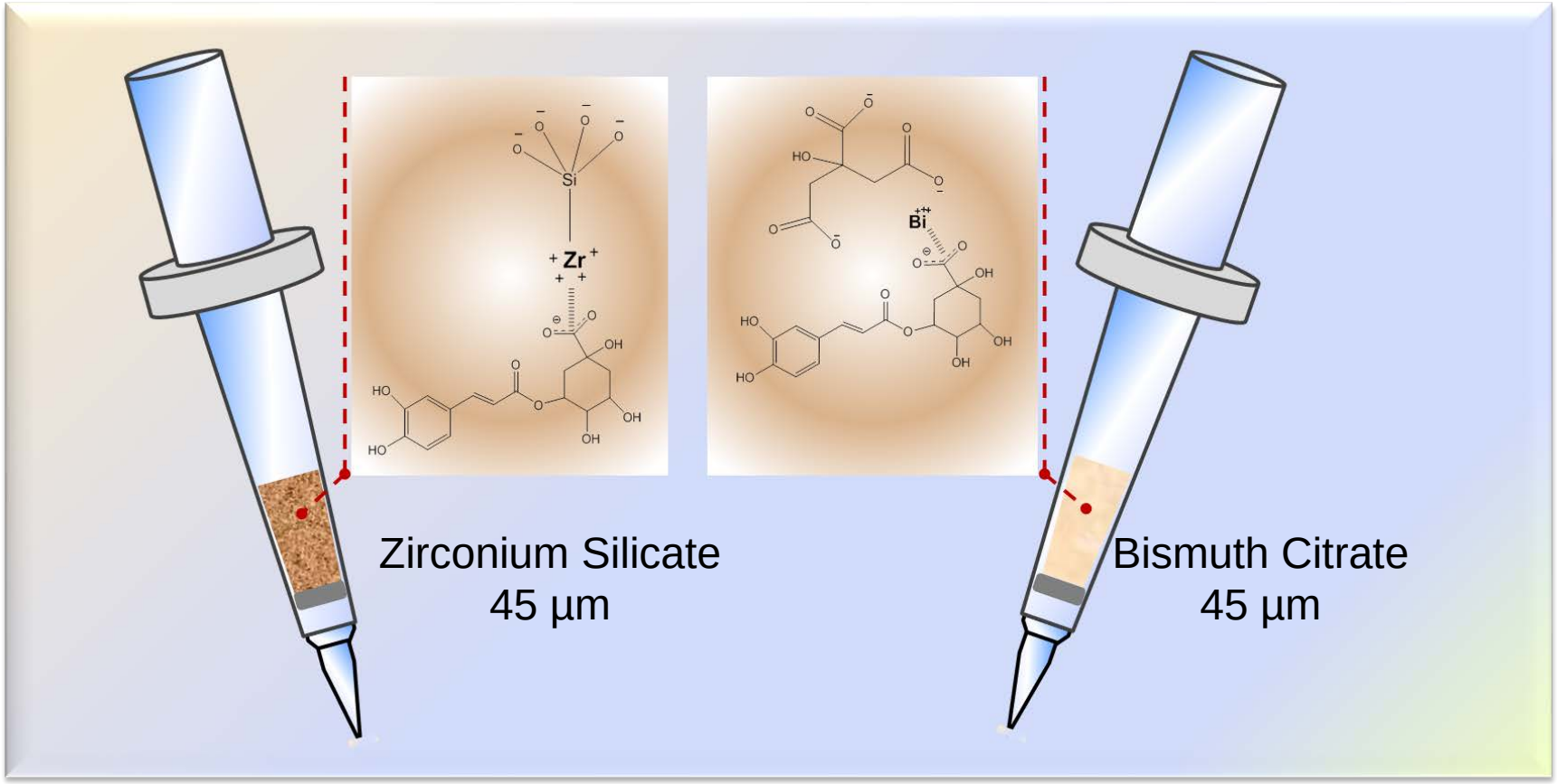
conventional digestion time: 6-10 h

Results - Comparison Study



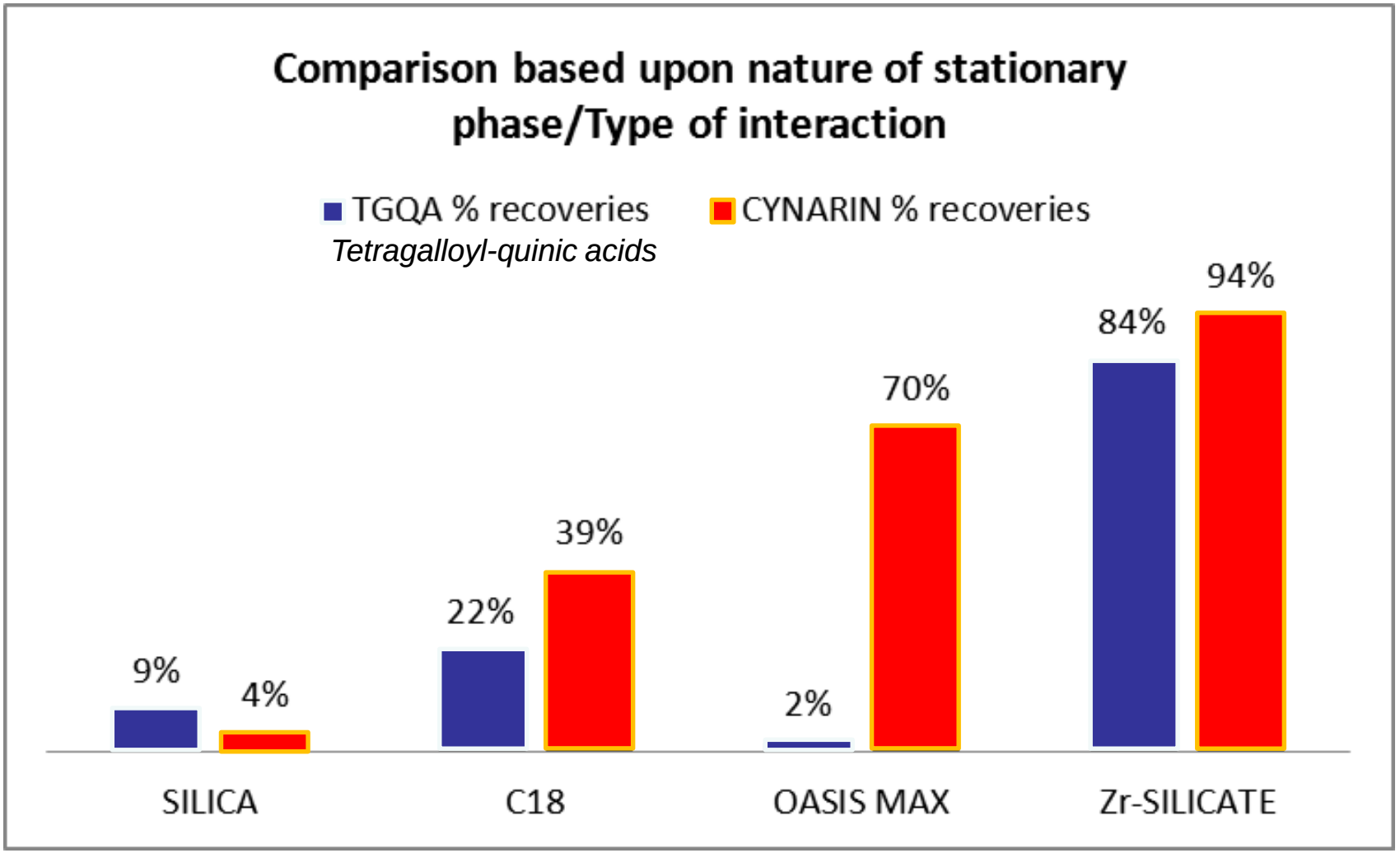
α-casein digested, eluted from enzyme-Tips with Tip-Technology

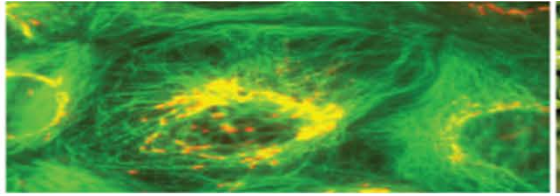
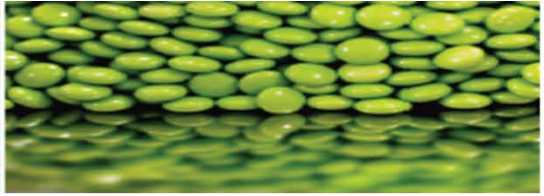
Schematic view of spin columns containing sorbents



Hussain, Shah, et al. Journal of Pharmaceutical and Biomedical Analysis (2013) in press

Recovery study of standards



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Pyrrolizidine alkaloids
Background

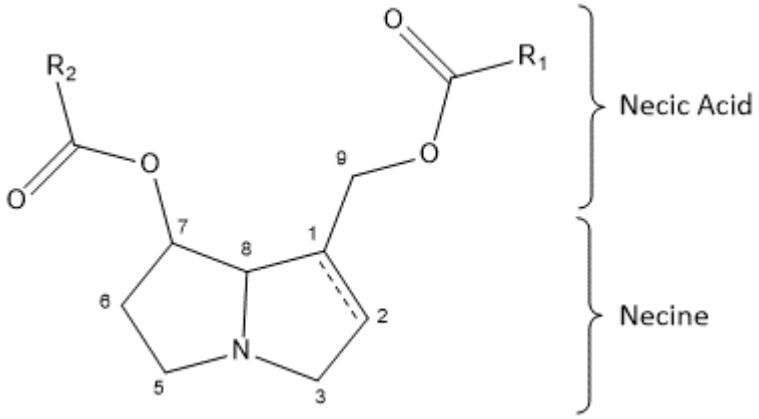
- Pyrrolizidine alkaloids (PA) are secondary plant metabolites (for plant protection)
- 400 different PAs in approximately 6000 plant species are known
  - → Contamination of plant products during harvest or through animals (for example bees)
  - → Examples for contaminated food: herbal teas, honey, salads etc.

- Problem: Hepatotoxic for animals as well as for humans
  - → Safety values for the maximum dose for drugs in Germany:
    - Application up to 6 weeks: 1 µg/day oral, 100 µg/day cortically
    - Application for more than 6 weeks: 0.1 µg/day oral, 10 µg/day cortically

Pyrrolizidine alkaloids

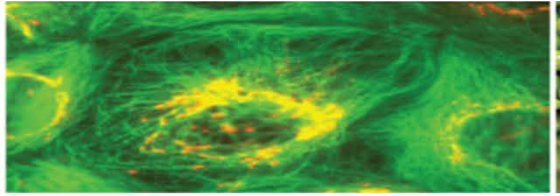
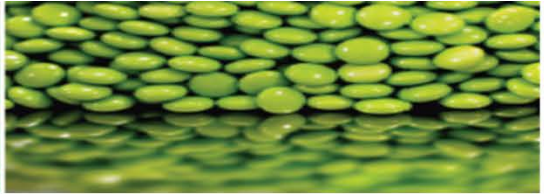
Toxicity

- General structure:



Nearly all types are in coexistence with their N-oxides

- Requirement(s) for **toxicity**:
  - 1,2-unsaturated necine
  - Esterification of at least one OH-group with a branched acid

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Pyrrolizidine alkaloids

Methods for detection

- HPLC-MS (LOD ~ 1 ppb)
- GC-MS (LOD = 3 ppb)
  → Otonecines and N-oxides (without derivatisation) not detectable
- Double antibody ELISA (LOD = 0.1 – 1.5 ppb)
  → Antibodys only against some specific PAs
- HPLC Evaporative Light Scattering Detection (ELSD) (LOD = 40 ppm)
- Nonaqueous capillary electrophoresis (NACE) MS (LOD < 7.5 ppm)
- Photometric detection with Ehrlich reagent (LOD = 10 ppm)

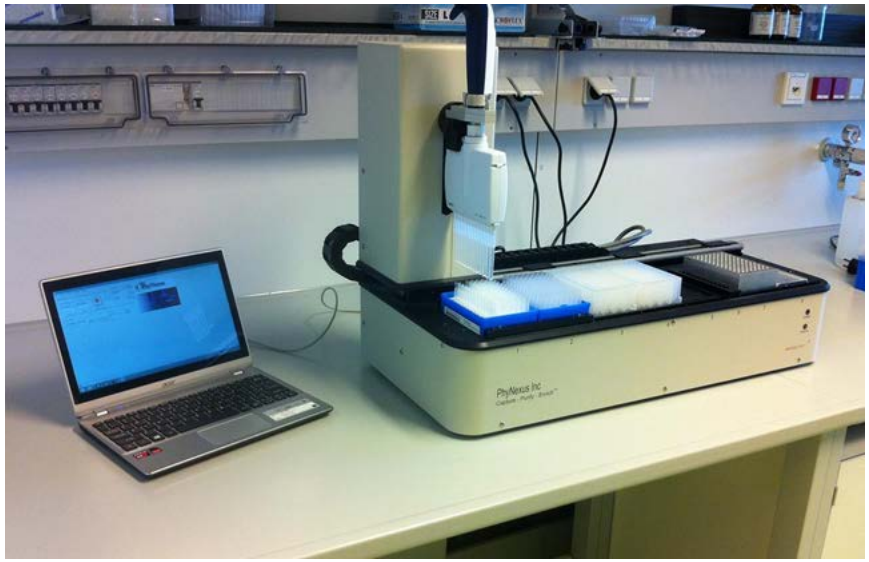
- General problem for PA analysis: Only about 25 standards are available
  → Validation is limited to a small spectrum of PAs

➡ Isolation of PAs out of plant material with countercurrent chromatography (CCC) to get more standards (Cooperation with Medical University of Lublin)

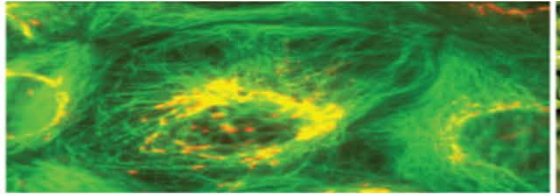
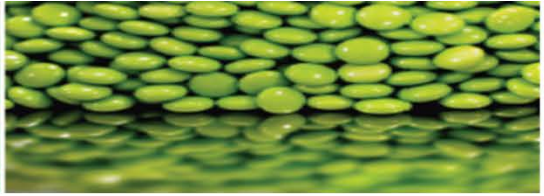
Pyrrolizidine alkaloids

Cation exchange

- Detection only possible in the low ppb-range (for GC- and HPLC-MS)
  → for lower concentrations and for separation from other plant substances enrichment is necessary
- Due to the chemical structure ion exchange is the enrichment method of choice
- Automation with PhyNexus MEA 2 possible



PhyTip with Toyopearl SP-650 resin

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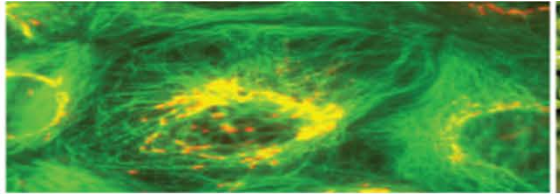
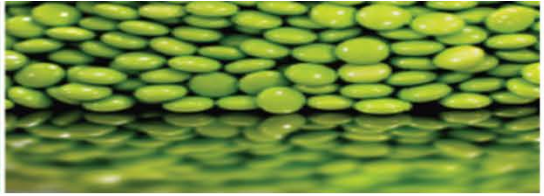
Pyrrolizidine alkaloids

Procedure

Extraction of the plant material

Enrichment of PAs with ion exchange

Detection with UPLC-MS

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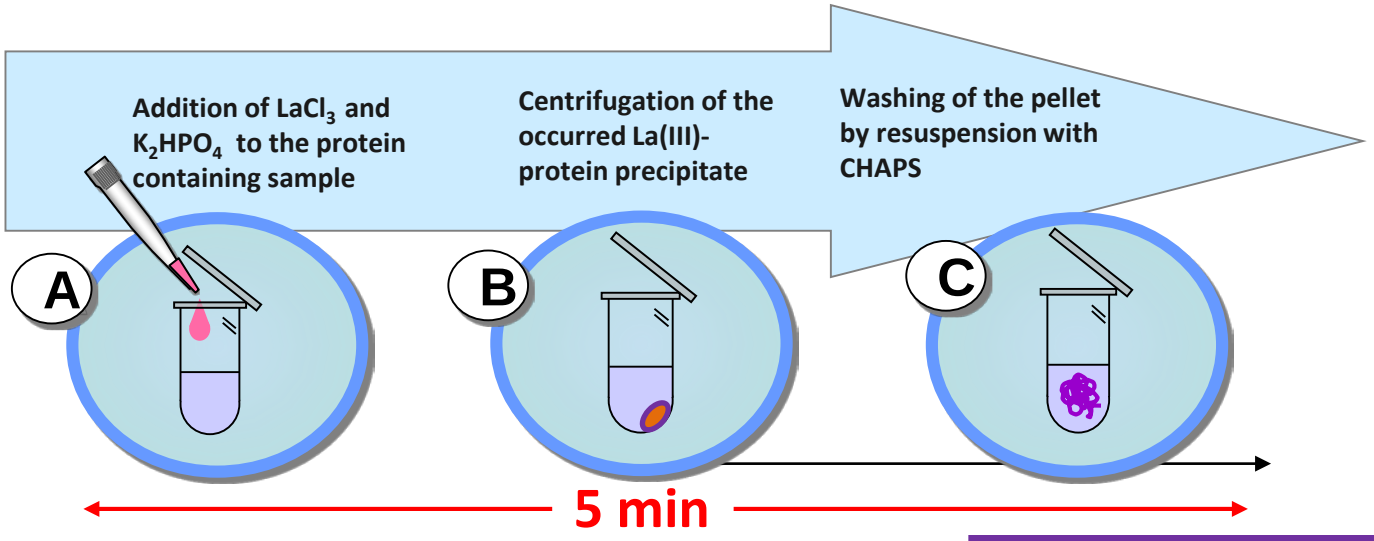
Pyrrolizidine alkaloids
Cation exchange – Comparison of commercial products

- In terms of enrichment of pyrrolizidine alkaloids different commercial materials were tested
- Best recoveries (**94-99%**) were achieved with a polystyrene-divinylbenzene resin functionalized with sulfonate groups
- Further approach
  - Polystyrene DVB resin in PhyNexus tips and automation of the cation exchange with PhyNexus MEA 2
  - Continuing comparison with other resins (in cartridges and tips)
  - Test of anion exchange resins and Immobilized Metal Ion Affinity Chromatography (IMAC)

Enrichment of Phosphoproteins by Precipitation using Trivalent Lanthanides

Highly Selective Precipitation of Phosphoproteins by Lanthanum



Dissolving of the pellet allows the recovery of the bound proteins

centrifugation

D

2 min Top-Down

50 min Bottom-Up

Enzymatic microwave-assisted digestion on pellet

Recovered proteins can be further analyzed by:

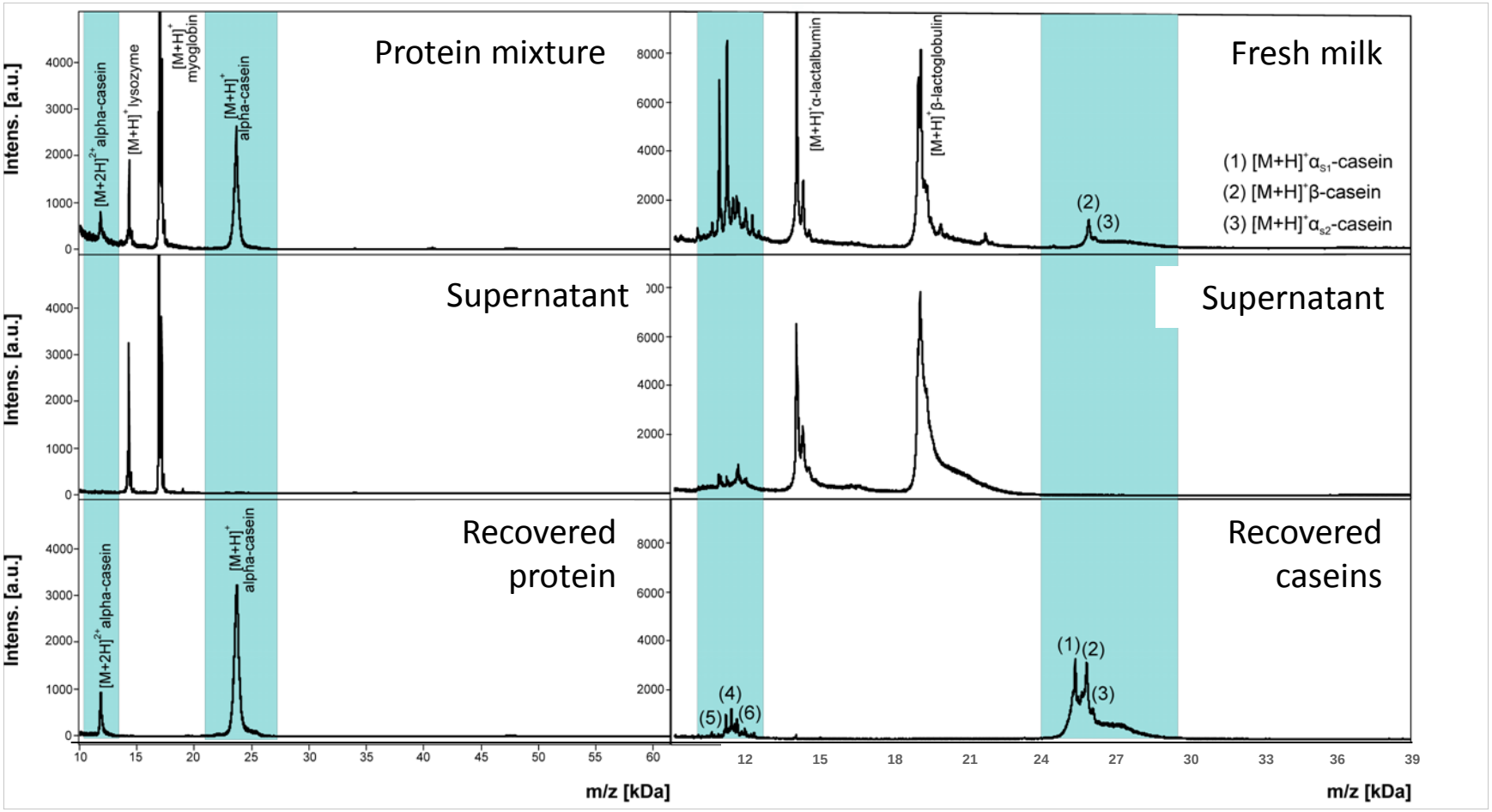
- **gel electrophoresis**
- **'top down' mass spec**

Obtained digest allows a fast Screening of the bound proteins by LC-MS:

→ peptide mass fingerprint

→ quantification

Highly Selective Precipitation of Phosphoproteins by Lanthanum

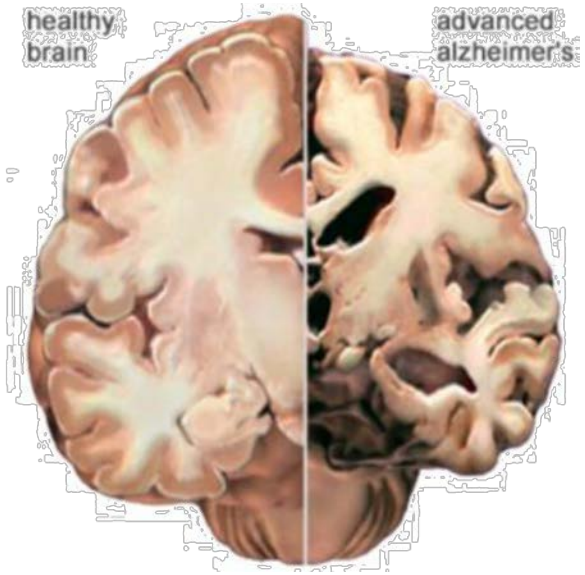


Recovery of intact proteins allows „top down“ protein analysis by LC-MS or gel electrophoresis

Highly Selective Precipitation of Phosphoproteins by Lanthanum

Analysis of Liquor for the Diagnosis of Alzheimer

- Alzheimer's disease is the most common form of dementia.
- Alzheimer's is a brain disorder destroying brain cells, causing problems with memory, thinking and behavior severe enough to affect work, lifelong hobbies or social life.
- An estimated 26.6 million people worldwide had Alzheimer's in 2006; this number may quadruple by 2050.



Analysis of Liquor for the Diagnosis of Alzheimer

Accession number	Protein	Hit	Phosphorylation	Glycosylation
P05067	Amyloid beta A4 protein	(Ray, 2000), (Grimmer, 2009)	+	+
P06396	Gelsolin	(Ray, 2000)	+	-
P01011	Alpha-1-antichymotrypsin	(Eriksson, 1995), (Gollin, 1992)	-	+
P02647	Apolipoprotein A-I	(Yin, 2009)	-	+
P02679	Fibrinogen	(Yin, 2009), (Ojien van, 2005)	-	+
P00738	Haptoglobin	(Yin, 2009)	+	+
P02766	Transthyretin	(Biroccio, 2006)	-	+
P10451	Osteopontin	(Wung, 2007)	-	+
Q14515	SPARC-like protein 1	(Yin, 2009)	+	+
P02649	Apolipoprotein E	(Corder, 1993),	+	+
P10909	Clusterin	(Yin, 2009)	-	+
Q9UBP4	Dickkopf-related protein 3	(Zenzmaier, 2004)	+	+
P36955	Pigment epithelium-derived factor	(Yin, 2009)	+	+
P02774	Vitamin D-binding protein	(Yin, 2009)	-	+
P01009	Alpha-1-antitrypsin	(Gollin, 1992)	-	+
P02763	Alpha-1-acid glycoprotein 1	(Merritt, 1988)	-	+
P04004	Vitronectin	(Rogers, 2000)	+	+
P01042	Kininogen-1	(Puchades, 2003)	+	+
P01034	Cystatin-C	(Mi, 2009)	+	-
P10645	Chromogranin A	(Lechner, 2004)	+	+

- CSF was analyzed by lanthanum (III) precipitation
- **53 phosphorylated and/or glycosylated proteins were identified** through peptide mass fingerprint
- **20 out of them are potential biomarkers for Alzheimer's disease**

→ Highly selective analysis

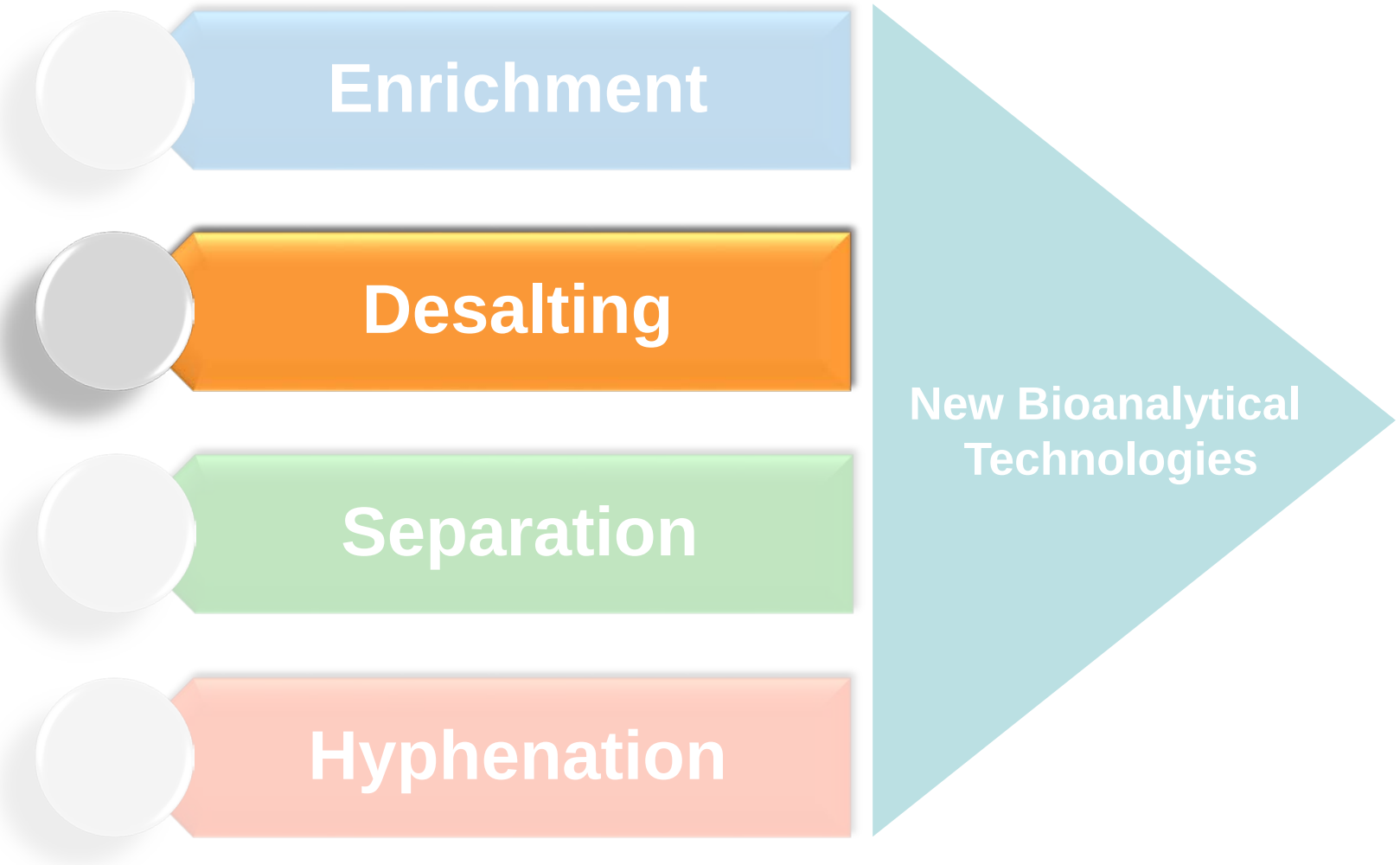
→ 20 biomarkers detected in the same analytical run

After the loading of 7 µl onto the trapping column with a flow rate of 20 µl/min for 4 min, the separation was performed under reversed phase conditions with solvent A 4 % acetonitrile (ACN) and 0.1 %(v/v) formic acid (FA) in water and solvent B 0.1 %(v/v) FA in 60 % ACN, at a flow rate of 300 nl/min and 60 °C. A linear gradient (300 min from 0 % B to 60 % B) was used.

Recovery of Phosphopeptides

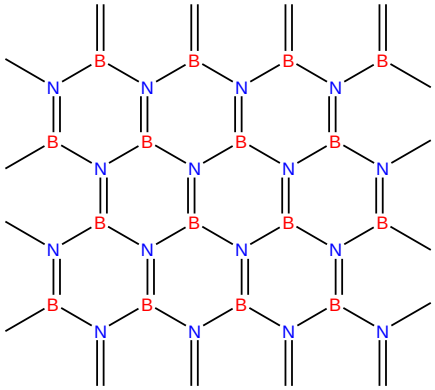
$[M+H]^+$ Da	Phosphopeptide Sequences[a]	Phospho-groups	$ErCl_3$	$HoCl_3$	$CeCl_3$	$LaCl_3$	$EuCl_3$	$TmCl_3$	$TbCl_3$	TiO_2
1254.52	EVVGSpAEAGVDAA (Ov-(340–352))	Mono	-	-	-	-	-	-	-	-
1331.53	EQLSpTSpEENSK (α-S2-(141–151))	Mono	-	-	-	-	-	-	-	-
1411.50	EQLSpTSpEENSK (α-S2-(141–151))	Di	-	-	-	-	-	-	-	-
1466.61	TVDMESpTEVFTK (α-S2-(153–164))	Mono	+	+	+	+	+	+	+	+
1594.70	TVDMESpTEVFTKK (α-S2-(153–165))	Mono	+	+	+	+	+	+	+	+
1660.79	VPQLEIVPNSpAEER α(-S1-(121–134))	Mono	+	+	+	+	+	+	+	+
1832.83	YLGEYLIVPNSpAEER (α-S1)	Mono	+	+	+	+	+	+	+	+
1847.69	DIGSESpTEDQAMEDIK (α-S1-(58–73))	Mono	-	+	+	-	+	+	-	-
1927.69	DIGSESpTEDQAMEDIK (α-S1-(58–73))	Di	+	+	+	+	+	+	+	+
1951.95	YKVPQLEIVPNSpAEER (α-S1-(119–134))	Mono	+	+	+	+	+	+	+	+
2061.83	FQSpEEQQQTEDELQDK (β-C-(33–48))	Mono	+	+	+	+	+	+	+	+
2088.89	EVVGSpAEAGVDAASVSEEFR (Ov-(340–359))	Mono	+	+	+	+	+	+	+	+
2432.05	IEKFQSpEEQQQTEDELQDK (β-C-(33–48))	Mono	+	+	+	+	+	+	-	-
2511.13	LPGFGDSpIEAQCGTSVNVHSSLR (Ov-(62–84))	Mono	-	-	-	-	-	-	-	-
2556.10	FQSpEEQQQTEDELQDKIHPF (β-C-(48-67))	Mono	+	+	+	+	+	+	-	+
2619.04	NTMEHVSpSpSpEESpIISQETYK (α-S2-(17–36))	Tetra	+	+	+	+	+	-	-	-
2678.01	VNELSpKDIGSpESpTEDQAMEDIK (α-S1-(52–73))	Tri	+	-	-	-	-	-	-	+
2703.50	LRLKKYKVPQLEIVPNSpAEERL(α-S1-(114–135))	Mono	+	+	+	+	+	-	+	+
2720.91	QMEAESpISpSpSpEEIVPNSVEAQK (α-S1-(74–94))	Penta	+	-	-	+	+	-	+	+
2747.10	NTMEHVSpSpSpEESpIISQETYKQ (α-S2-(17–37))	Tetra	+	-	-	-	-	-	-	-
2856.50	EKVNELSpKDIGSpESTEDQAMEDIK (α-S1-(50–73))	Di	+	+	+	-	+	-	-	+
2901.32	FDKLPGFGDSpIEAQCGTSVNVHSSLR (Ov-(59–84))	Mono	+	-	+	+	-	+	-	-
2935.15	EKVNELSpKDIGSpESpTEDQAMEDIK (α-S1-(50–73))	Tri	+	+	-	+	+	-	-	+
2966.16	ELEELNVPGEIVESpLSpSpSpEESITR (β-C-(17–40))	Tetra	-	-	+	-	-	-	-	-
3008.01	NANEEEYSIGSpSpSpEESpAEVATEEVK (α-S2-(61–85))	Tetra	+	+	+	+	+	-	+	+
3042.27	RELEELNVPGEIVESLSpSpSpEESITR (β-C-(16–40))	Tetra	+	+	+	+	+	-	+	-
3087.99	NANEEEYSIGSpSpSpEESpAEVATEEVK (α-S2-(61–85))	Penta	+	+	-	+	+	-	-	+
3122.27	RELEELNVPGEIVESpLSpSpSpEESITR (β-C-(16–40))	Tetra	+	+	+	+	+	-	+	+
3132.20	KNTMEHVSpSpSpEESpIISQETYKQEK (α-S2-(16–39))	Tetra	+	+	+	+	+	-	+	+
			23	**20**	**19**	**20**	**21**	**12**	**14**	**18**

Highly Efficient Enrichment and Separation of Biomolecules



ACRC
Institute of Analytical Chemistry and RadioChemistry
Center of Competence and Excellence
Leopold-Franzens University of Innsbruck, Austria

Boron Nitride, a Novel Material for the Enrichment and Desalting of Protein Digests and the Protein Depletion



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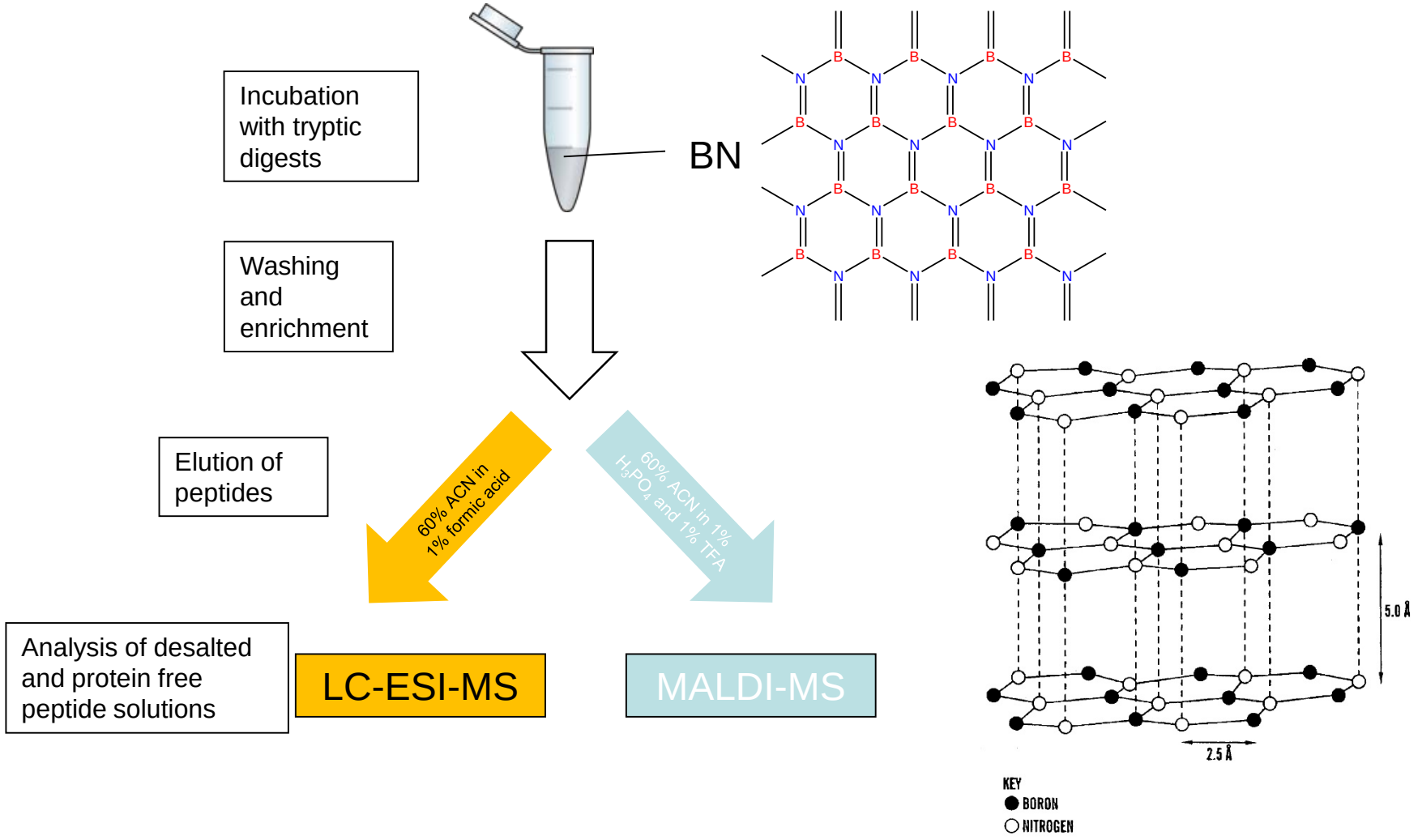
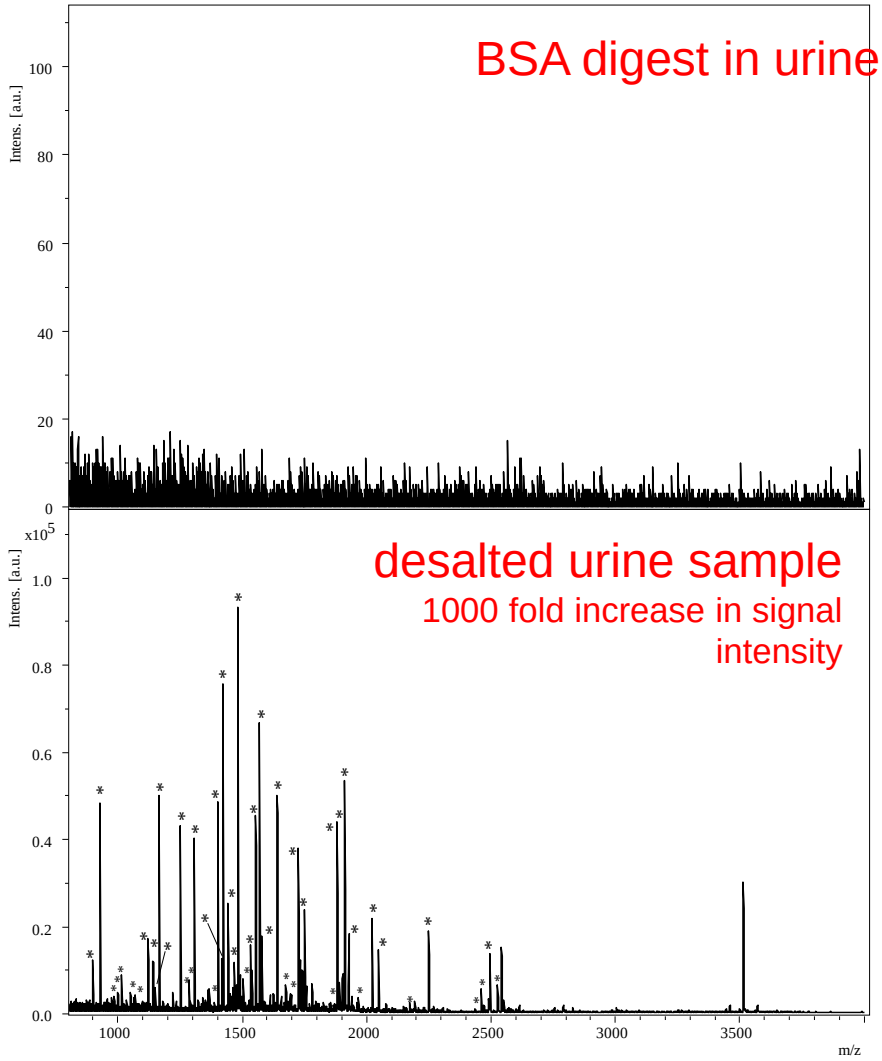
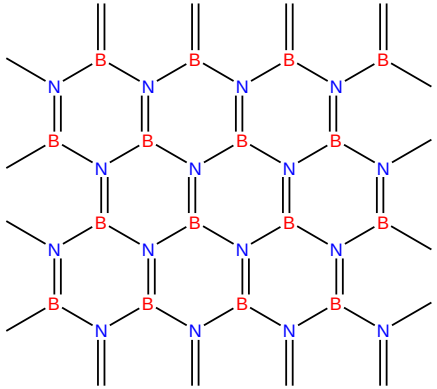


Boron Nitride, a Novel Material for the Enrichment and Desalting





MALDI Spectra:

The identified peptides are labeled with asterisk. Sequence Coverage of desalted BSA > 68 %

Bisphenol derivatives

	MW	pKa	log K_{ow}	R^2	LOD [ng/ml]	LOQ [ng/ml]
BADGE·2H$_2$O	376.44	14.7[b]	2.05[b]	0.999	27.0	82.0
BPF	200.23	7.5[a]	2.91[a]	0.999	27.0	82.0
BPA	228.29	9.6[a]	3.32[a]	0.999	33.0	99.0
BPZ	268.35	9.7[b]	4.53[b]	0.999	28.0	85.0
BADGE	340.41	-	4.02[a]	0.999	30.0	90.0

BPF

BPA

BPZ

BADGE

BADGE·2H$_2$O

Recovery for the enrichment of 5 Phenols

	Phenol [12.5 µg/ml]	**4-Nitrophenol** [12.5 µg/ml]	**2-Chlorphenol** [12.5 µg/ml]	**2-Nitrophenol** [12.5 µg/ml]	**Dimethylphenol** [12.5 µg/ml]
	87.51	94.74	108.08	91.29	100.71
	93.97	102.95	114.70	95.20	111.82
	91.36	101.91	113.24	96.88	105.84
Average	**90.95**	**99.87**	**112.01**	**94.46**	**106.13**
RSD	2.66	3.65	2.84	2.34	4.54

5 mg of BN was incubated with a phenol mixture and afterward eluted with 80% acetonitrile in water.

Risk associated with bisphenols

- The French Agency for Food, Environmental and Occupational Health & Safety showed that there are ‘recognized’ effects in animals (effects on reproduction, on the mammary gland, on metabolism, the brain and behaviour) and other ‘suspected’ effects in humans (on reproduction, metabolism and cardiovascular diseases). These effects could be observed, even at low levels of exposure, during sensitive phases of an individual’s development.
- The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions of the Expert Committee on Assessment of the risks related to chemical substances relating to the risks associated with BPA for human health, and on toxicological data and data on the use of bisphenols M, S, B, AP, AF, F and BADGE.
- Endokrine Disruptoren

  Tolerable Daily Intake (TDI) of **50 µg BPA/kg** body weight (b.w.)/day as set by EFSA in 2006.

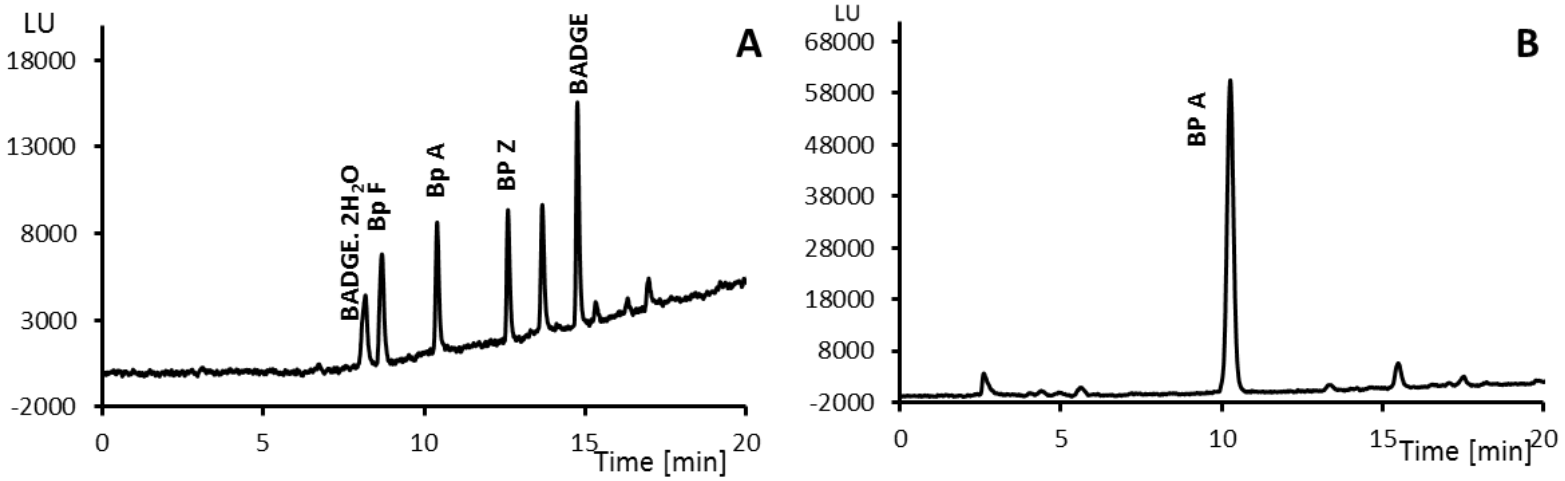
  The migration limit of BPA set by European Union of **600 µg/kg** food.

  Tolerable Daily Intake (TDI) of **150 µg** BADGE and its hydrolytic products/kg body weight (b.w.)/day.

  The migration limit of BADGE and its hydrolytic products **9000 µg/kg** food.

  http://www.efsa.europa.eu/en/topics/topic/bisphenol.htm

Analysis of 5 bisphenol derivatives after enrichment with BN by LC and fluorescence detector



HPLC-FLD chromatogramms of (A) standard solution containing 5 bisphenol derivatives [100 ng/mL] and (B) bisphenol leached from baby bottle after enrichment with h-BN-60.

Chromatographic conditions: Shimadzu LC-10 Acvp, Phenomenex Luna 5u C18 250*4,6 mm, isocratic 3 min 30% B, gradient 30-70% B in 15 min, flow rate 1mL/min, 50°C, inj.: 30 µL,FLD: 230/303 nm

Determination of bisphenol derivatives

	Found ± SD [ng/ml] Recovery rates ± SD [%]				
	BADGE·2H$_2$O	**BPF**	**BPA**	**BPZ**	**BADGE**
river water[a]	nd (92.13 ± 2.69)	nd (102.62 ± 3.73)	nd (107.14 ± 6.50)	nd (104.95 ± 1.92)	nd (98.74 ± 2.57)
drinking water[a]	nd (90.16 ± 2.40)	nd (98.56 ± 3.51)	nd (99.16 ± 1.60)	nd (101.14 ± 2.58)	nd (96.68 ± 3.35)
cola[b]	4.63 ± 0.14 (103.41 ± 4.39)	2.23 ± 0.21 (96.98 ± 2.39)	10.34 ± 0.41 (96.93 ± 5.72)	nd (92.46 ± 6.58)	1.49 ± 0.04 (96.57 ± 3.84)
apple juice[b]	1.93 ± 0.15 (96.43 ± 3.63)	3.11 ± 0.30 (90.77 ± 3.63)	nd (84.22 ± 2.20)	nd (98.74 ± 1.23)	1.36 ± 0.22 (98.22 ± 2.71)
lemon soda[b]	8.14 ± 0.09 (82.46 ± 3.01)	1.40 ±0.07 (84.45 ± 5.44)	nd (97.60 ± 1.81)	nd (87.61 ± 2.94)	5.70 ± 0.04 (94.00 ± 2.61)
citrus soft drink[b]	5.32 ± 0.71 (103.90 ± 2.58)	nd (97.82 ±5.82)	1.04 ± 0.31 (98.31 ± 5.67)	nd (98.05 ± 4.04)	1.56 ± 0.042 (99.63 ± 4.42)
herbal soda[b]	nd (100.45 ± 5.15)	nd (99.07 ± 5.09)	nd (104.43 ± 6.20)	nd (105.07 ± 7.21)	nd (108.42 ± 4.83)
energy drink[b]	4.46 ± 0.07 (98.54 ± 9.21)	1.87 (91.55 ± 4.21)	5.57 ± 0.81 (93.55 ± 5.84)	nd (100.76 ± 2.04)	nd (103.60 ± 1.91)
canned mushroom liquid[b]	20.42 ± 1.19 (99.06 ± 6.88)	nd (91.35 ±0.79)	8.60 ± 0.37 (98.47 ± 8.37)	nd (103.71 ± 6.17)	1.81 ± 0.12 (99.63 ± 0.62)
pickled cucumber liquid[b]	1.81 ± 0.15 (97.53 ± 1.12)	2.23 ± 0.21 (96.02 ± 2.45)	125.00 ± 2.18 (109.12 ± 0.77)	nd (103.36 ±3.80)	1.44 ± 0.06 (92.67 ± 0.71)
pickled onion liquid[b]	2.98 ± 0.08 (105.80 ± 3.90)	nd (96.53 ± 8.20)	13.45 ± 0.63 (92.03 ± 4.70)	nd (97.91 ± 3.12)	1.74 ± 0.00 (95.20 ± 0.63)
urine[b]	nd (100.45 ± 3.13)	nd (105.67 ± 0.68)	nd (92.80 ± 2.52)	nd (105.26 ± 4.00)	nd (103.70 ±3.45)

Leaching of BPA from polycarbonate products

	leached BPA [ng/ml]	**± SD (%)**
Babybottle 1. treatment	20.79	1.05
Babybottle 2. treatment	19.82	0.72
muffin form	2.26	0.07
Siringe	0.85	0.03

Treatment conditions: boiling water for 1 h

Peking University

Health Science Center

University of Innsbruck

Institute of Analytical Chemistry and Radiochemistry
Horvath Lab of Bioseparation

Chinese - Austrian Center for Biomarker Discovery at Peking University Health Science Center

TEACHING COURSES

• **Fundamentals** - separation sciences e.g. electrophoresis, HPLC, capillary electrophoresis

• mass spectrometry, LC-MS, MALDI

• biomarker discovery

• including practical exercises

Lecturers from Austria, PUHSC and guests; **Duration** per year: 6 – 14 days

SCIENTIFIC PROJECTS

• **Development of new biomarker methods** as well as new materials for separation and MELDI

• **Development of disease related biomarker discovery methods including bioinformatics e.g**. liver cancer and prostate cancer

• **Application studies**

• **Natural Product Proteomics** (Phyto-Proteomics)

FELLOWSHIP PROGRAMS - Exchange on different levels

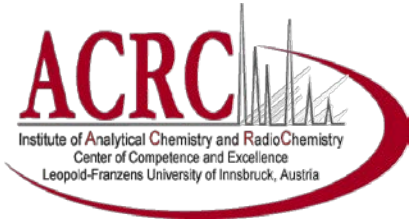
Academics, Students and Non-academic positions – technicians

Participation at the International Proteomics Conference, Seefeld Austria

Duration: 2 weeks, 3 months, up to 3 years (PH.D.) fellowships

EURASIA-PACIFIC UNINET

Collaborations



Prof. Günther Bonn

WHO – Lyon, France

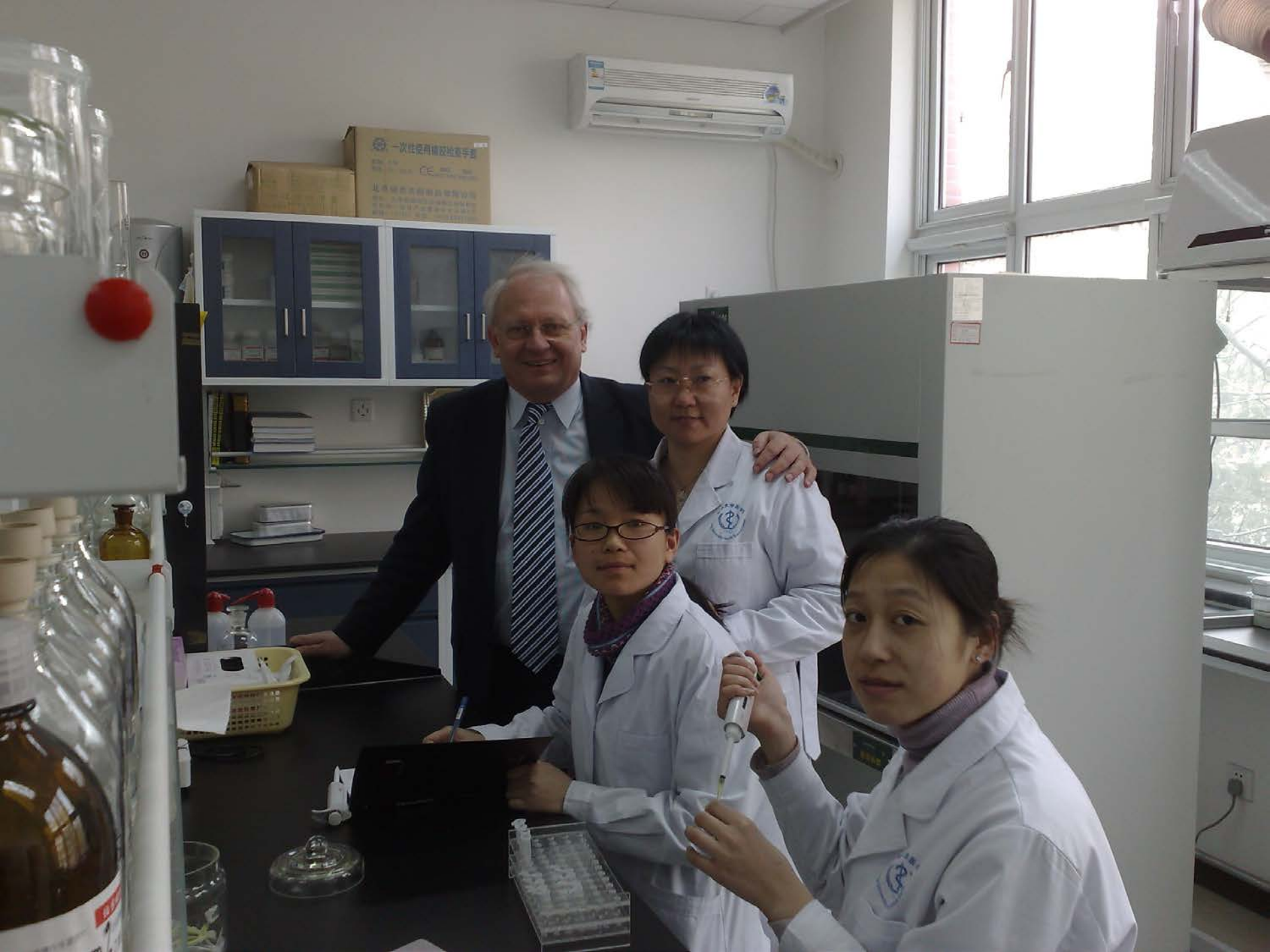
北京大学医学部
Peking University
Health Science Center

Prof. Ke Yang

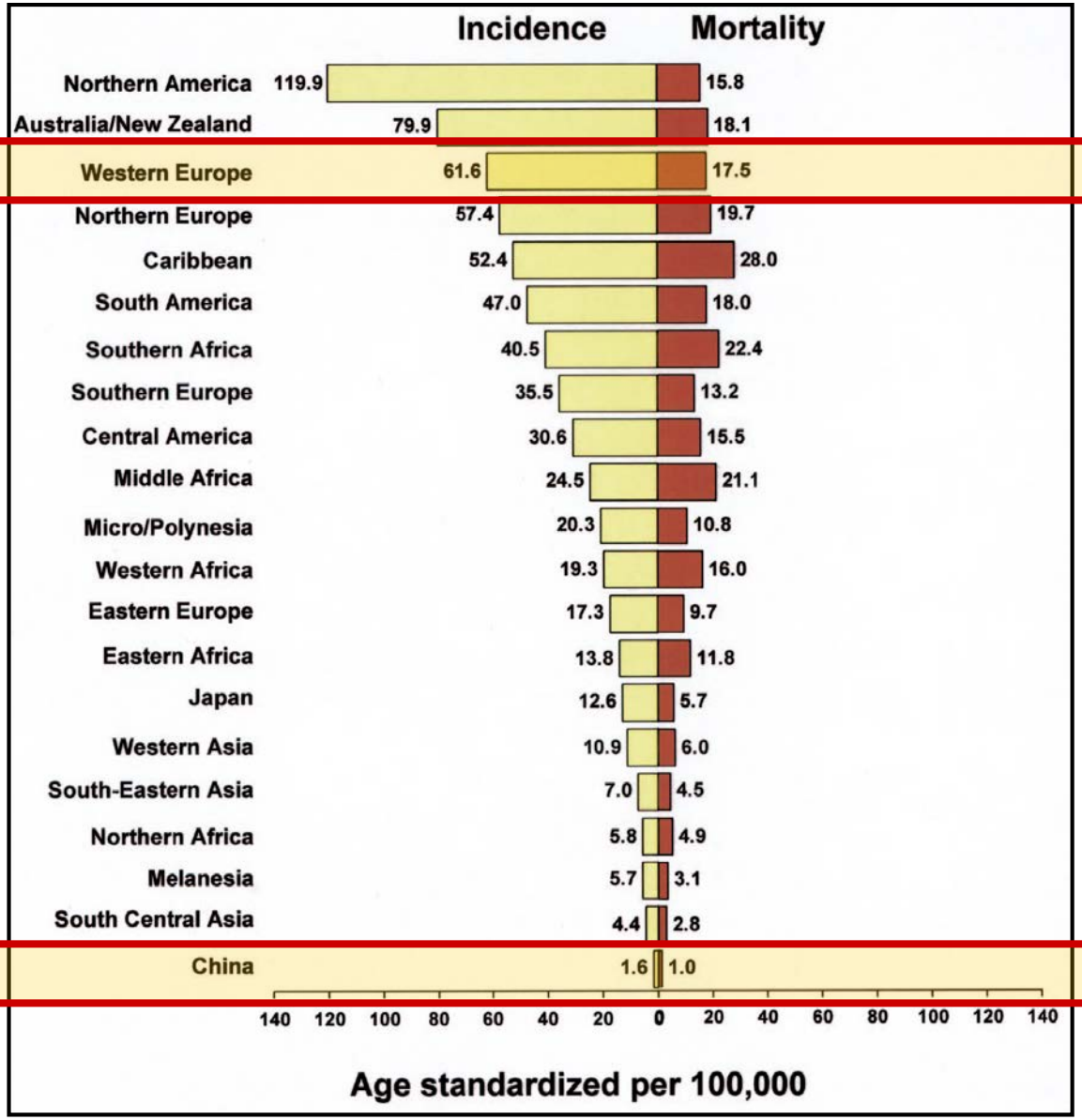
Prof. Siqi Liu


北京大学国学院
国际合作交流中心


中奥生物标志物研究中心
Sino-Austrian Bio-Marker Center



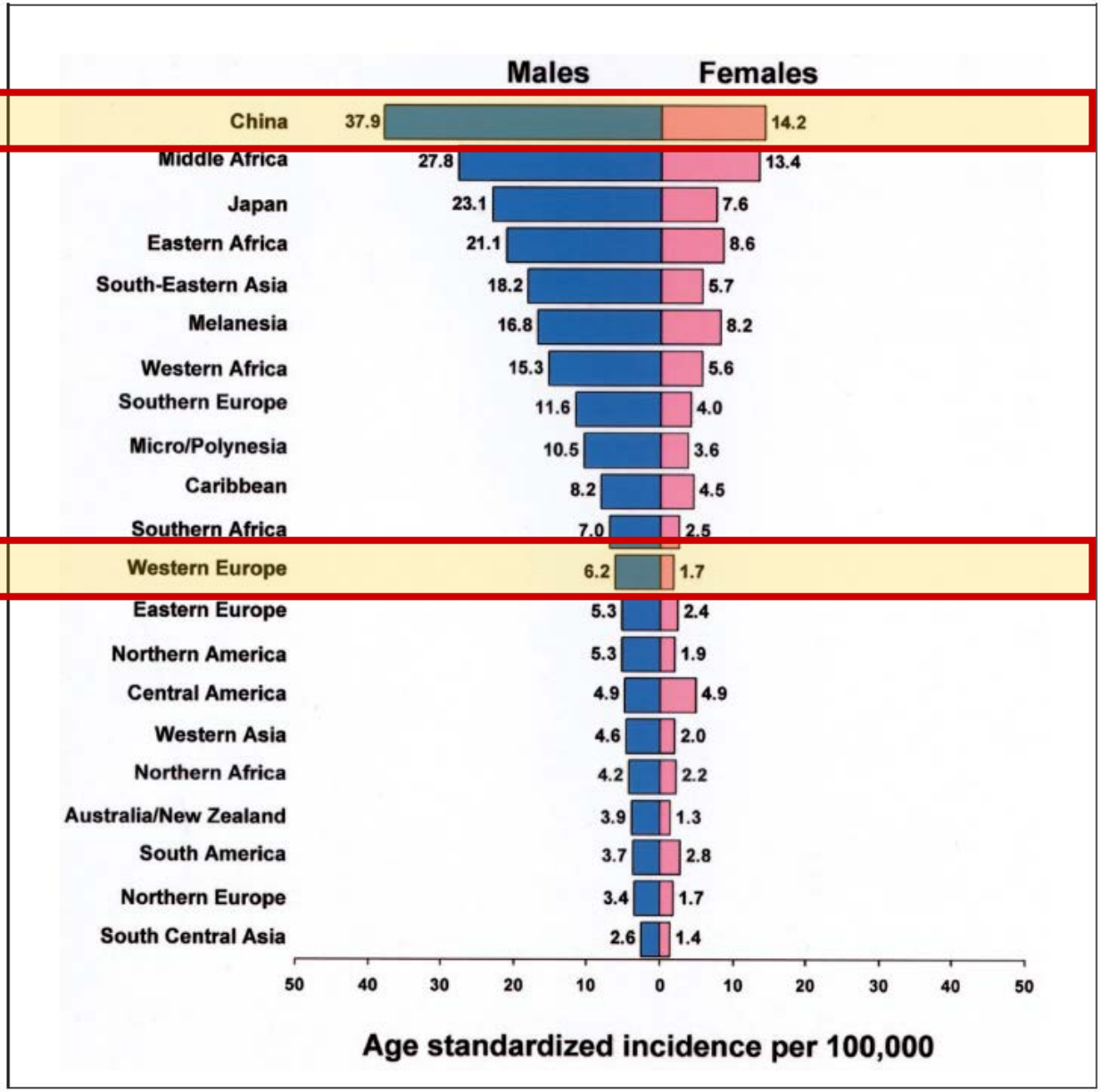
Prostate Cancer



D. Max Parkin, Freddie Bray, J. Ferlay and Paola Pisani *CA Cancer J Clin* 2005;55;74-108

Age-standardized Incidence and Mortality Rates for Prostate Cancer. Data shown per 100,000.

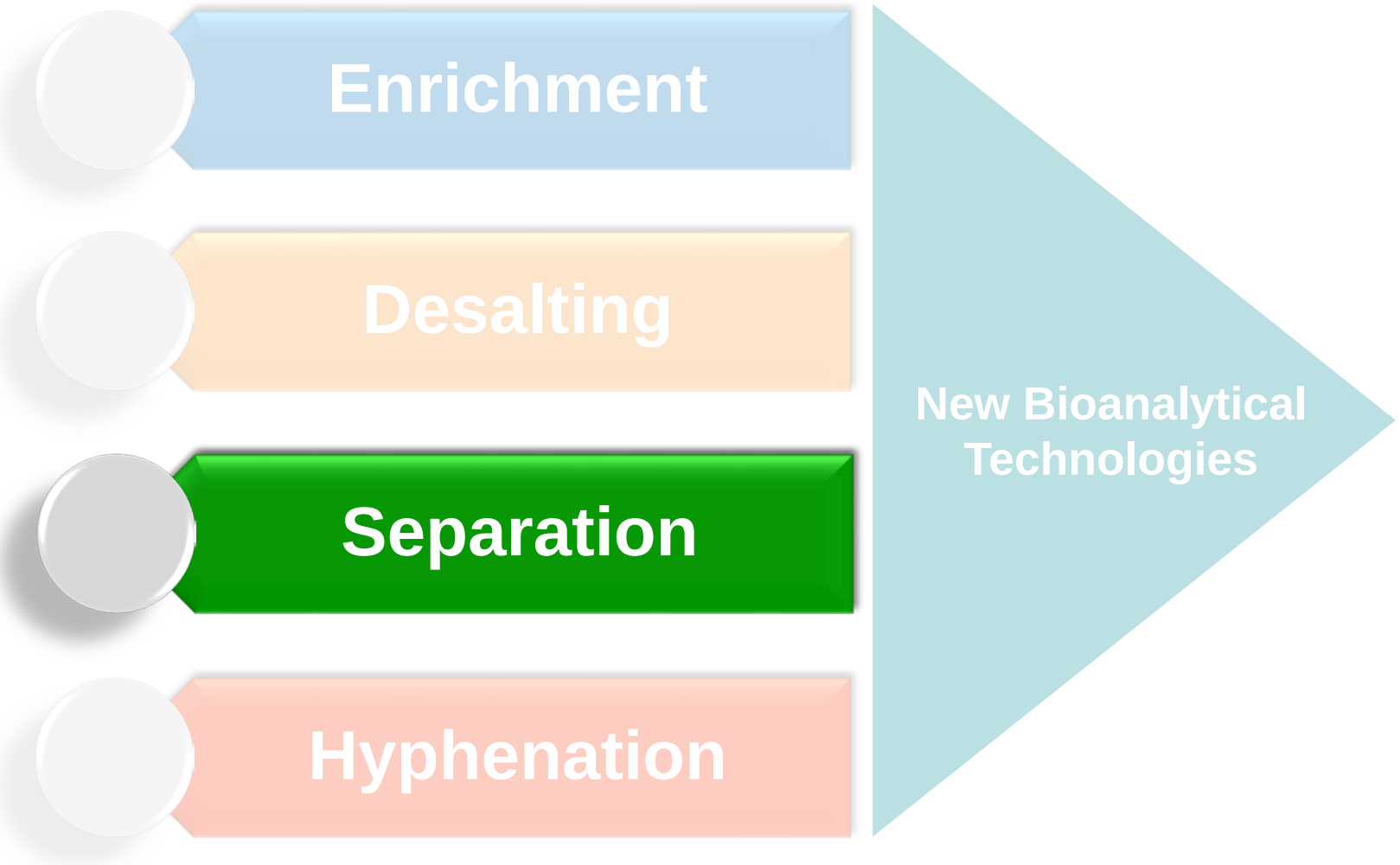
Liver Cancer



D. Max Parkin, Freddie Bray, J. Ferlay and Paola Pisani *CA Cancer J Clin* 2005;55;74-108

Age-standardized Incidence and Mortality Rates for Liver Cancer. Data shown per 100,000.

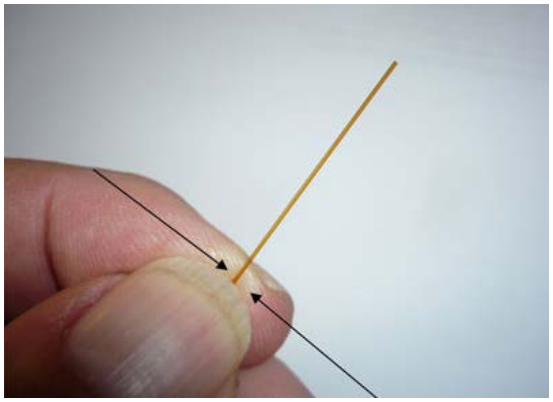
Highly Efficient Enrichment and Separation of Biomolecules



Stationary Phases – Formats – Strategies

HPLC - column

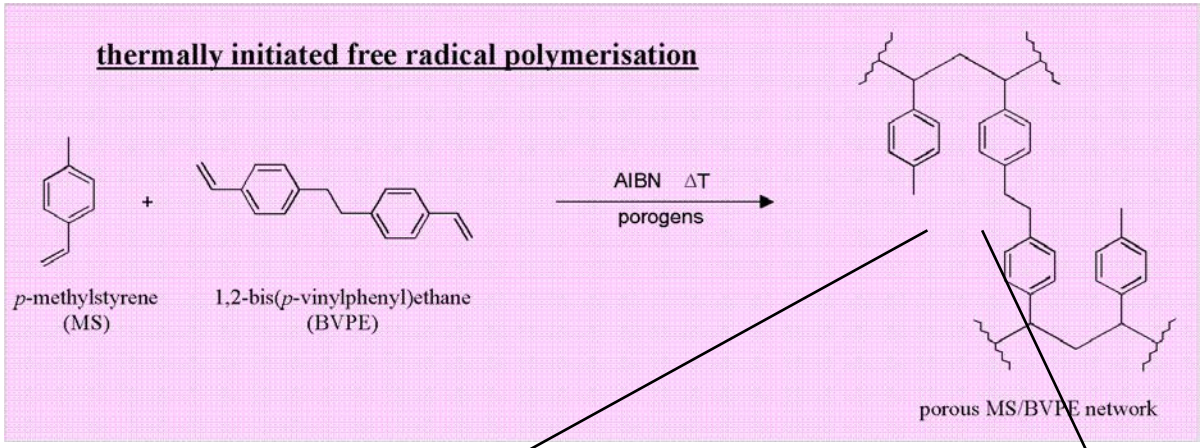
µ-LC - capillary



Chip - LC

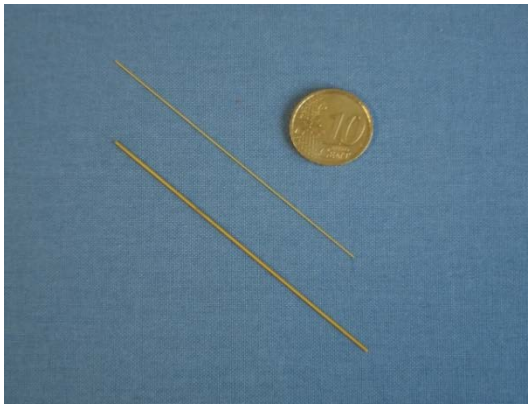
Disc - technology

MONOLITHIC MS/BVPE



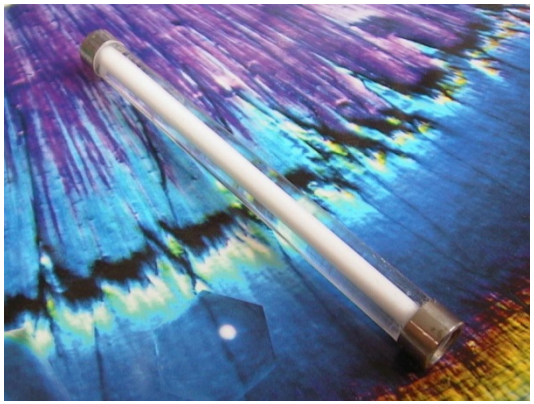
- highly crosslinked
- high crosslinking homogeneity
  due to non-conjugated crosslinker
- minimised swelling
- high pressure stability

→ **Applicable to HPLC**



capillary columns
different dimension

80 x 0.2 mm
80 x 0.533 mm

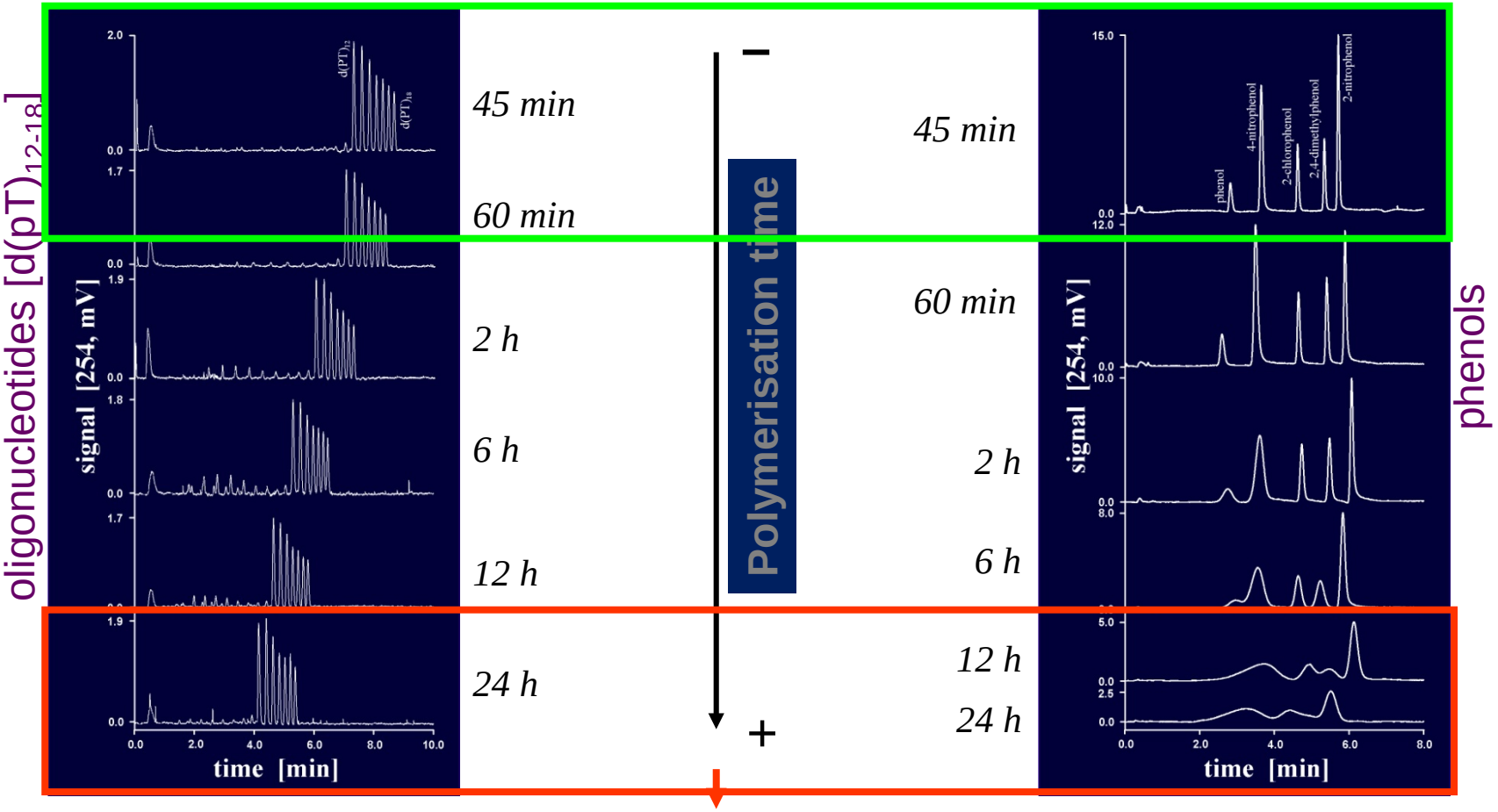


conventional HPLC columns

90 x 3.0 mm

Monolithic Separation

Impact on the Separation Efficiency of Monolithic MS/BVPE

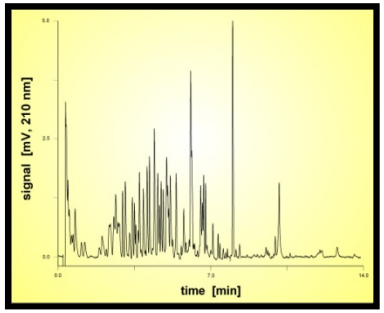


Achievable also with commercial monolithic organic capillary columns

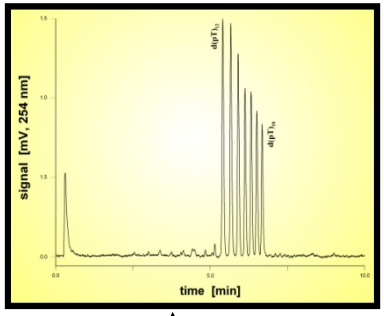
Extended Applicability of the shortly Polymerised Monoliths

Wide variety of BIOMOLEUCLES

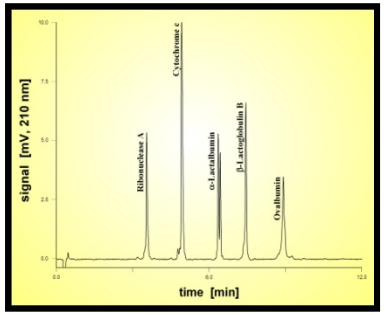
SMALL MOLEUCLES of different polarity



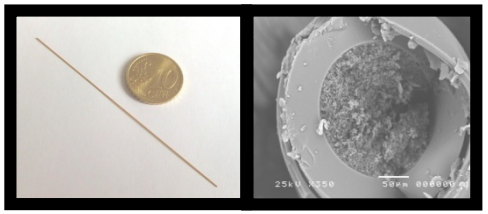
Peptides



Oligos

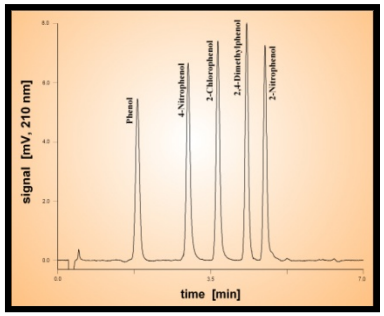


Proteins

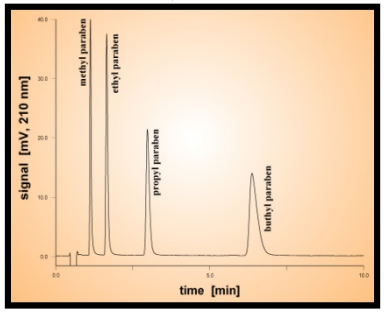


DIONEX

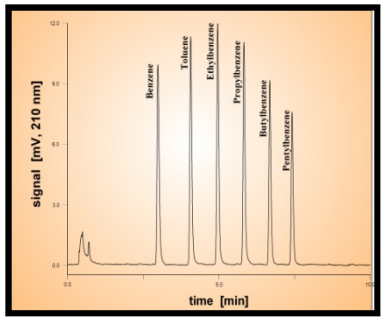
ThermoFisher SCIENTIFIC



Phenols



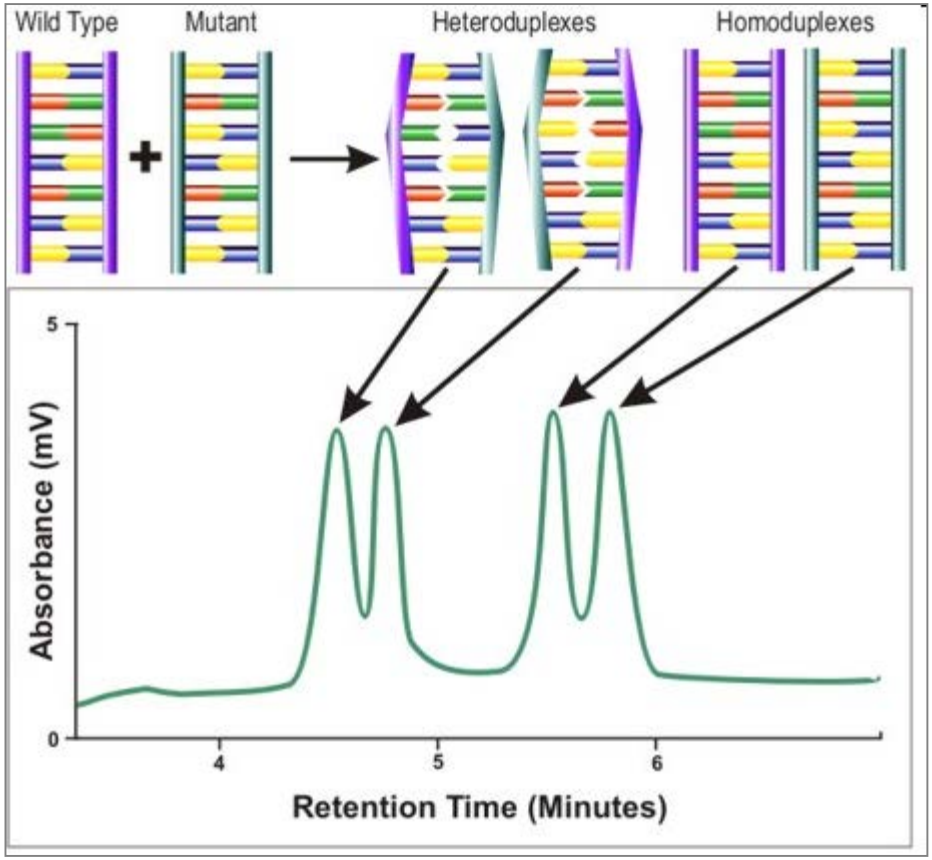
Parabens

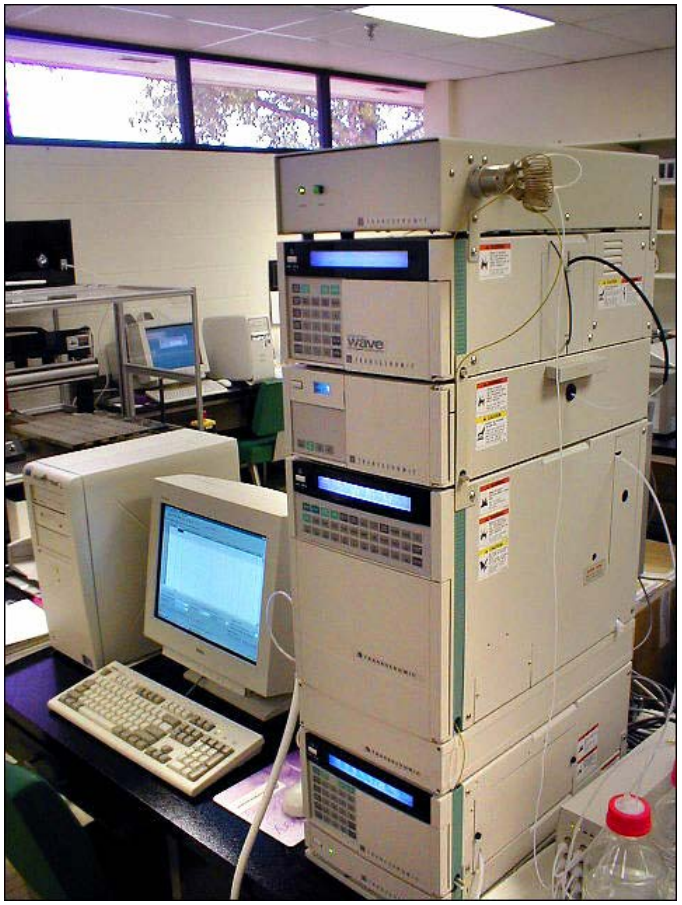


Alkylbenzenes

→ Separation of biopolymers as well as small molecules of various polarity can be separated on the same monolithic capillary column

Transgenomic Wave-System - Mutation Analysis



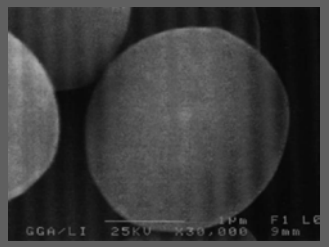


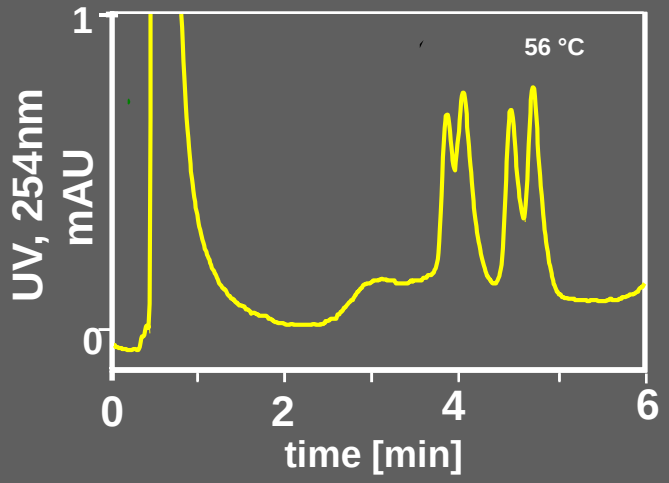
Transgenomic Wave HPLC-System

Bonn, Gunther; Huber, Christian; Oefner, Peter. ***Separation of nucleic acid fragments with alkylated nonporous polymer beads.*** *PCT Int. Appl. (1994), 30 pp.*

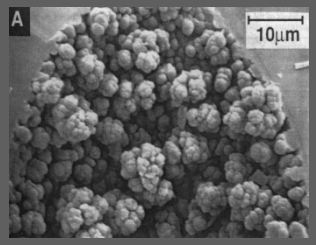
Mutation Detection by Denaturing HPLC using Packed Columns and Monolithic Capillaries

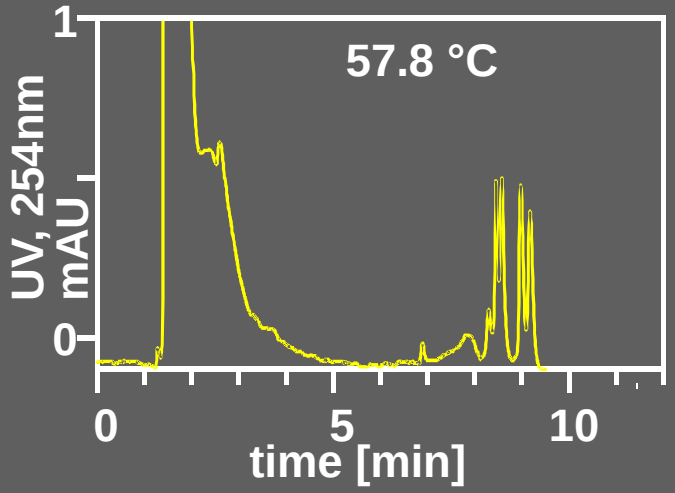
Packed Column





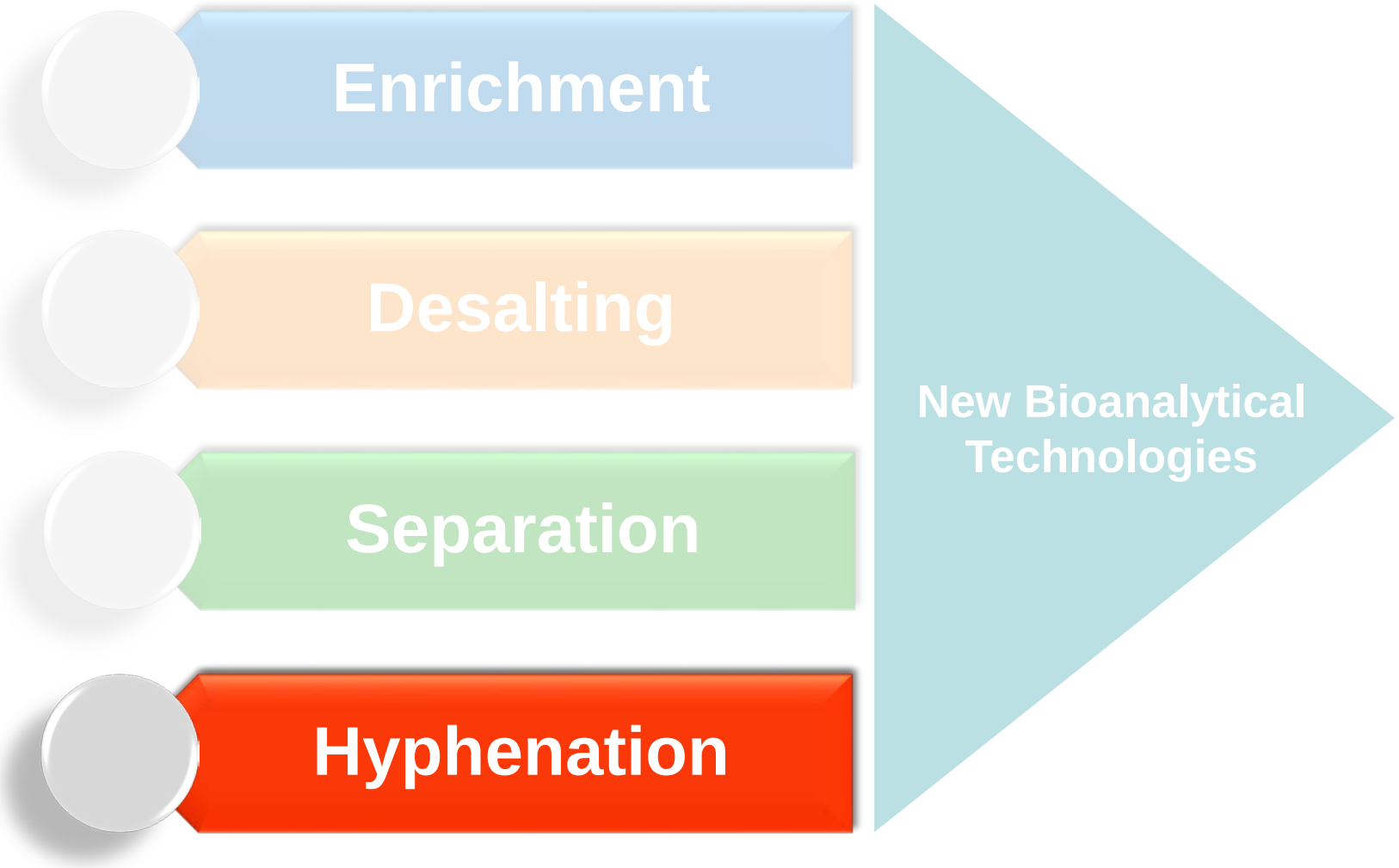
Monolithic Capillary





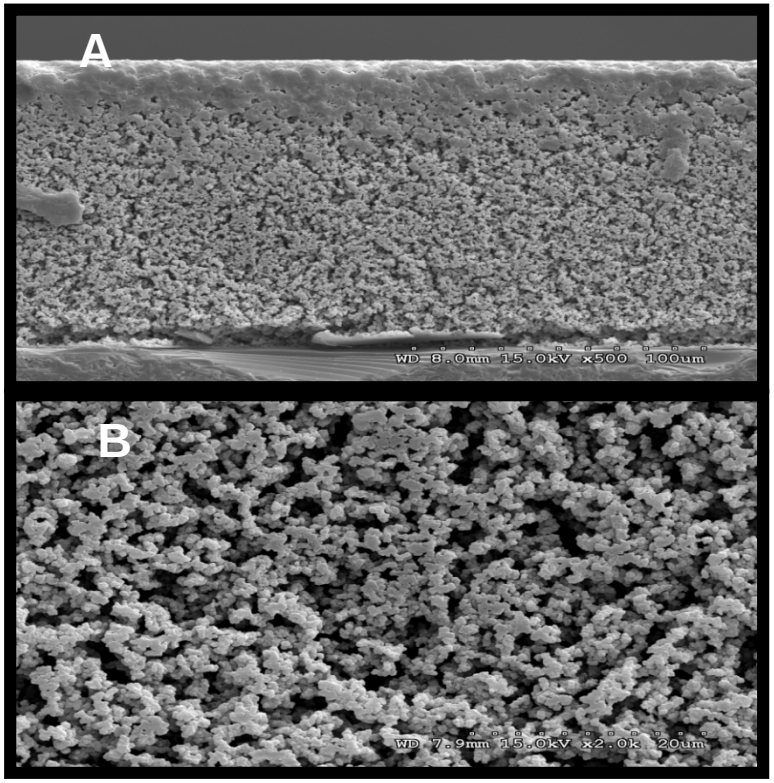
column:	50 x 4.6 mm I.D.	→	50 x 0.2 mm I.D.
flow rate:	1 ml/min	→	3.0 µl/min
injection vol.:	5 µl	→	500 nl

Highly Efficient Enrichment and Separation of Biomolecules

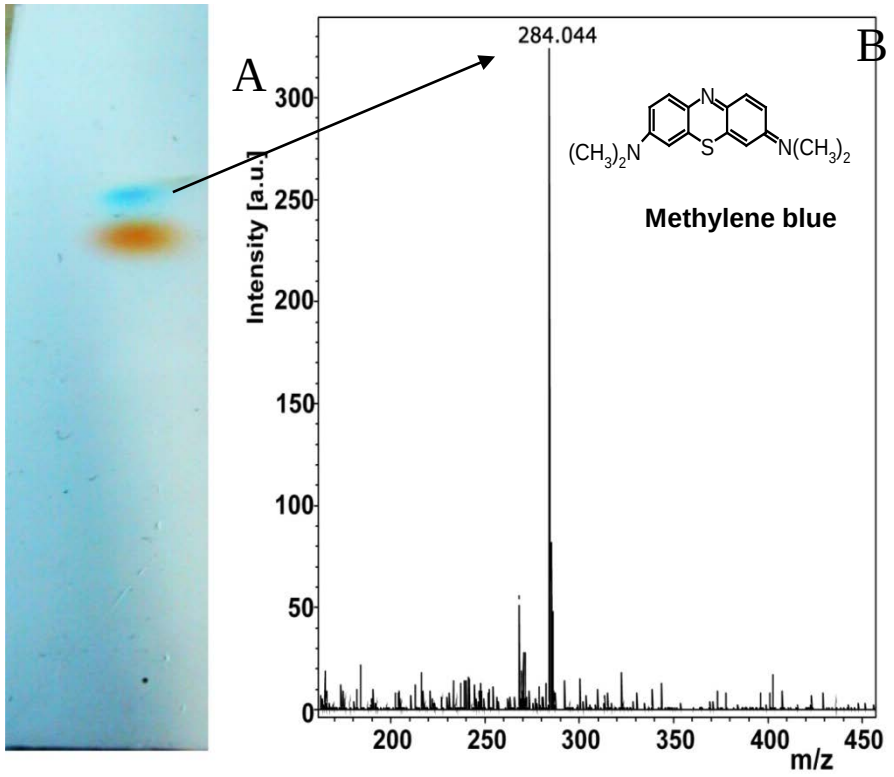


ACRC
Institute of Analytical Chemistry and RadioChemistry
Center of Competence and Excellence
Leopold-Franzens University of Innsbruck, Austria

Monolithic Porous Polymer Layer For Thin-Layer Chromatography Coupled with MALDI- TOF-MS



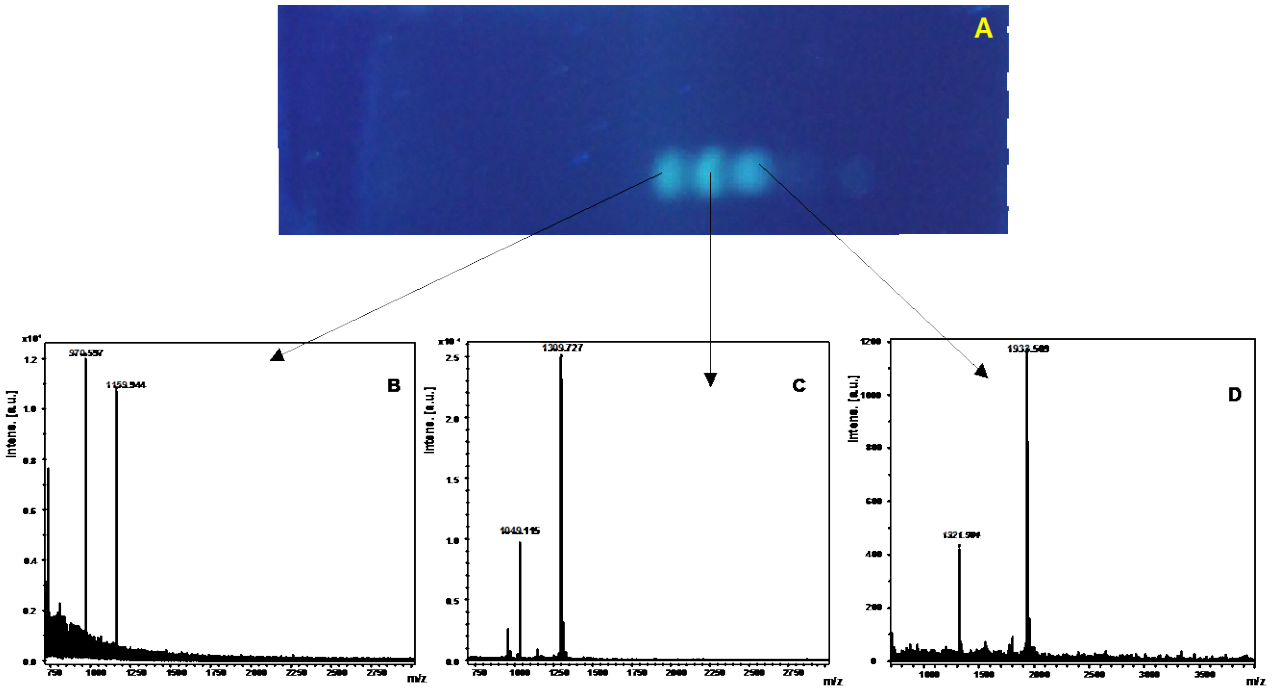
SEM micrographs of porous structure of 150 µm thick poly(butyl acrylate-*co*-ethylene dimethacrylate) monolith attached to a glass plate



TLC separation of methylene blue and methyl red on monolithic layer attached to a glass plate using ethyl acetate-ethanol-water mixture (6:4:3) as the mobile phase (A) and MALDI spectrum from the spot of methylene blue obtained "from-plate" without using any matrix (B) .

Collaboration with Prof. F. Svec, Lawrence Berkeley National Lab

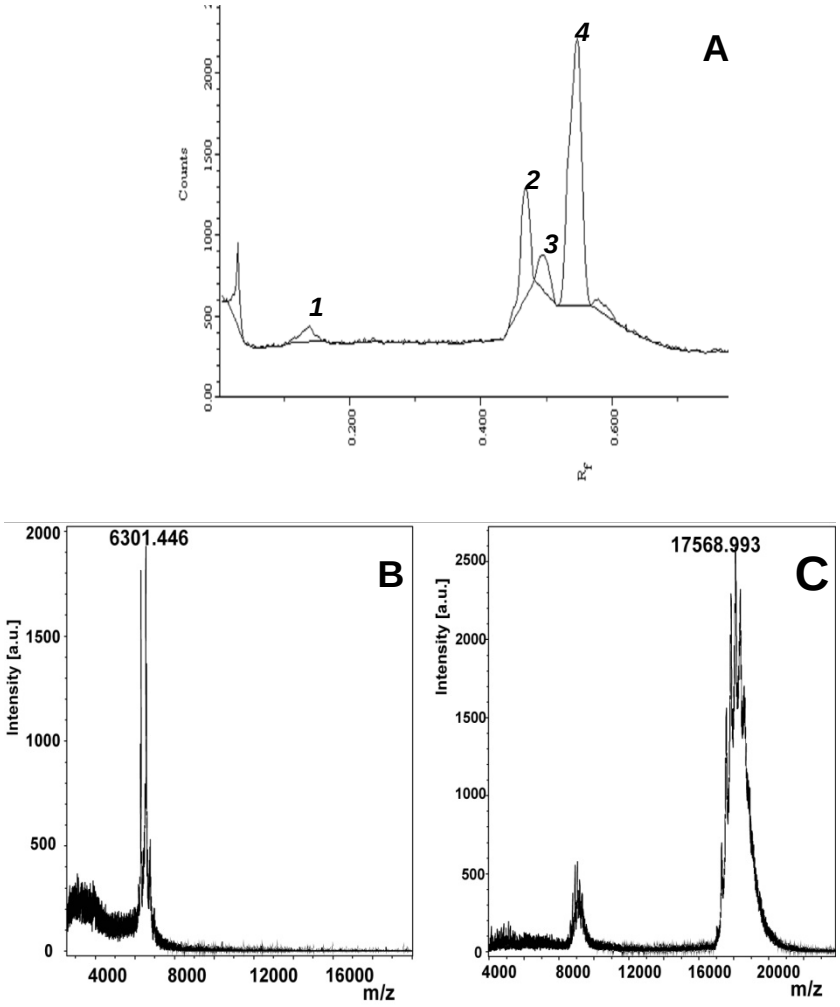
Monolithic Porous Polymer Layer For Thin-Layer Chromatography Coupled with MALDI- TOF-MS



TLC separation of mixture of peptides labelled with fluorescamine on 150 µm thick poly(butyl acrylate-*co*-ethylene dimethacrylate) monolithic layer attached to a glass plate using 0.1% TFA in 45% aqueous acetonitrile as the mobile phase (A). Sample volume 0.5 µL MALDI-TOF MS spectra of fluorescently labelled [Sar1,Ile8]-angiotensin II (B), angiotensin II (C), and neurotensin (D) obtained "from-plate" using HCCA as matrix.

Collaboration with Prof. F. Svec, Lawrence Berkeley National Lab

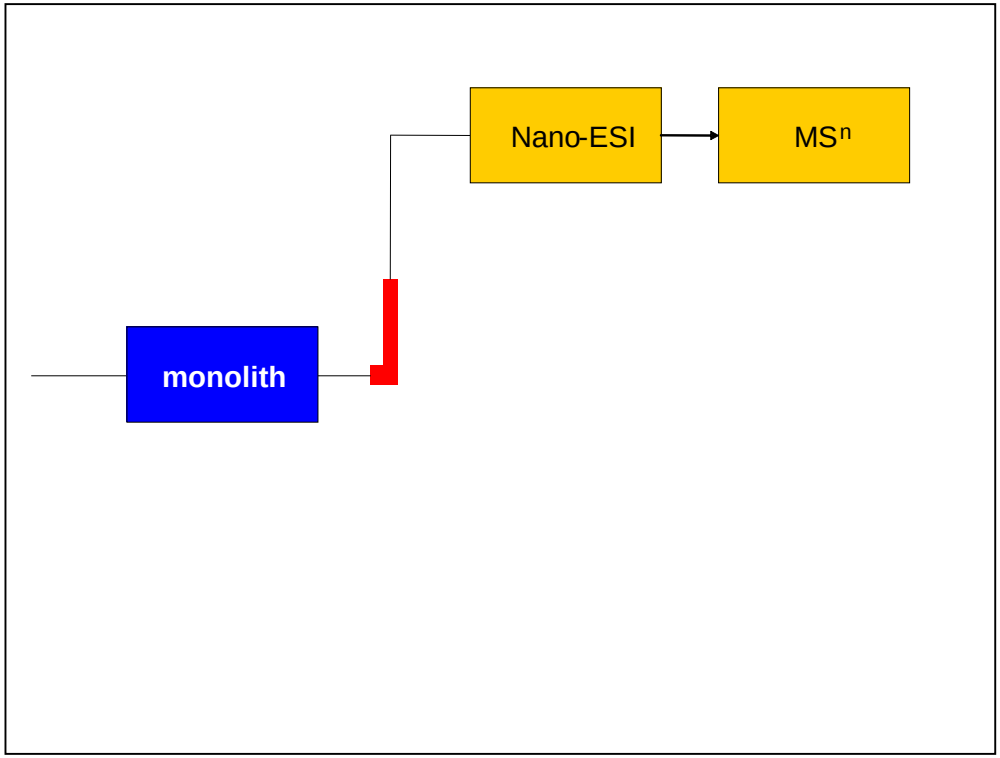
Monolithic Porous Polymer Layer For Thin-Layer Chromatography Coupled with MALDI- TOF-MS



TLC separation of mixture of proteins labelled with fluorescamine (A). Sample volume 0.5 µL. Peaks: Insulin (1), cytochrom c (2), lysozyme (3), and myoglobin (4). MALDI-TOF MS spectra of fluorescently labelled insulin (B), and myoglobin (C) obtained “from-plate” using sinapinic acid as matrix.

Structural Elucidation with Hyphenated techniques for Phyto-Metabolomics

µ-HPLC-ESI-MS



Electrospray Ionisation (ESI-MS) – online

→ *Increase speed of separation in case of MS instruments with high scan rates*

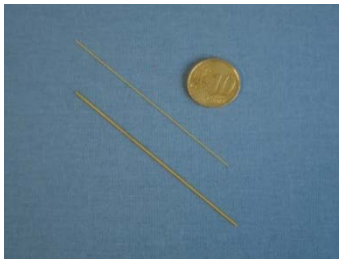
Qualitative Analysis of Flavonoids using µLC- ESI MS and MS^n of Heartsease (Viola tricolor L.)

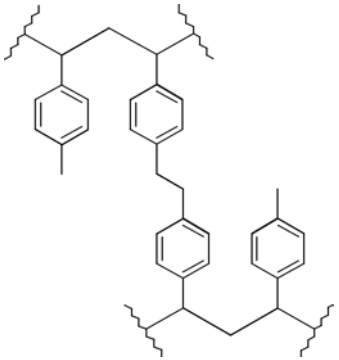
Heartsease has a long history in phytomedicine, it´s biological activities are attributed to its secondary plant metabolites, antioxidant flavonoid compounds

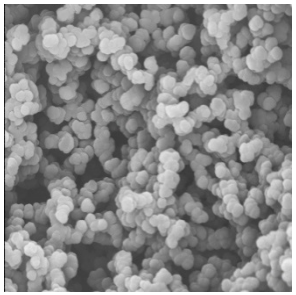
It has been utilized to treat:

- various skin disorders
- upper-respiratory problems
- as a diuretic
- prophylaxis and treatment of cardiovascular problems
- complications of diabetes
- Inflammations
- immune disorders
- liver problems

Separations were carried out using ***p-methylstyrene 1,2-bis(p-vinylbenzylchloride)*** monolithic capillary column





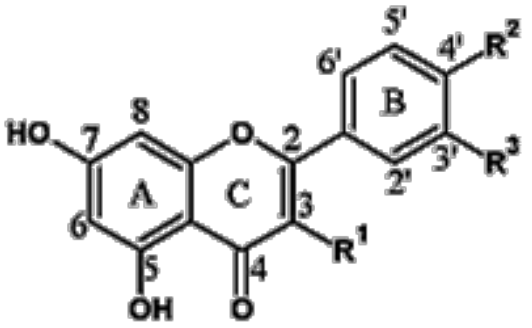


Horvath Laboratory of Bioseparation Scienc
Innsbruck, Austria

Prof. Andras Guttman

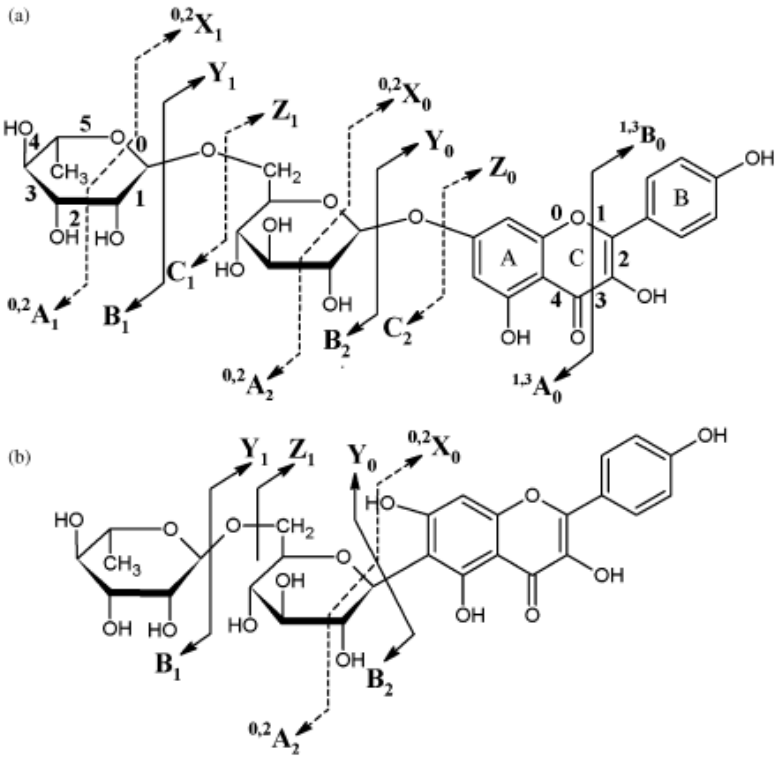
HLBS
Csaba Horvath Laboratory of Bioseparation
Innsbruck, Austria

Qualitative Analysis of Flavonoids of Heartsease (Viola tricolor L.)



	MW	R^1	R^2	R^3
apigenin	270	H	OH	H
luteolin	286	H	OH	OH
kaempferol	286	OH	OH	H
chrysoeriol	300	H	OH	OCH_3
diosmetin	300	H	OCH_3	OH
quercetin	302	OH	OH	OH
isorhamnetin	316	OH	OH	OCH_3

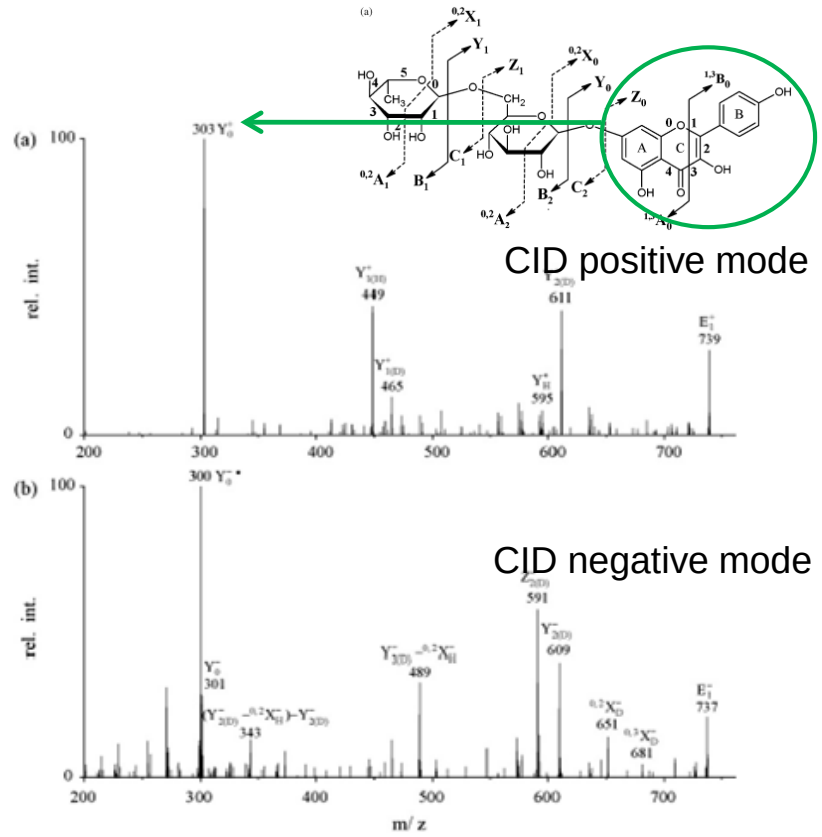
Core structure of flavonoid aglycones



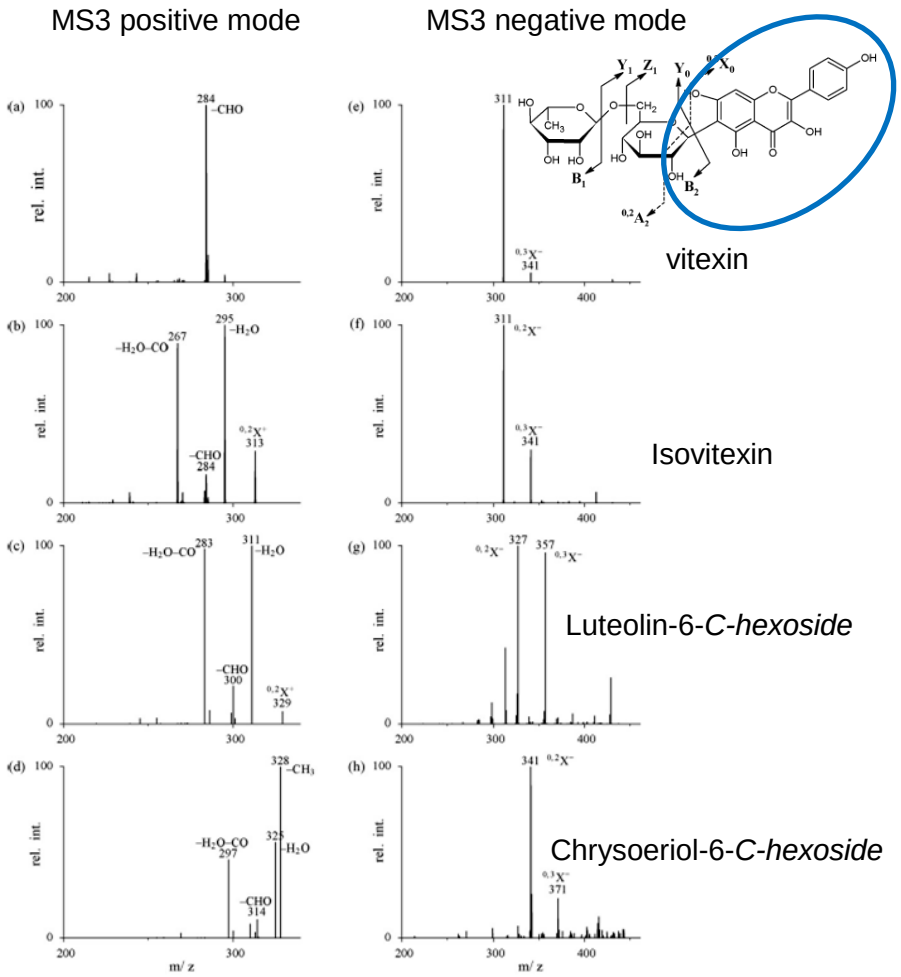
Fragment nomenclature applied for
(a) O-glycosides
(b) C,O-glycosides and C-glycosides

Vukics V; Toth B Hevesi; Ringer T; Ludanyi K; Kery A; Bonn G K; Guttman A, Journal of chromatographic science (2008), 46(2), 97-101.

Qualitative Analysis of Flavonoids of Heartsease (Viola tricolor L.)



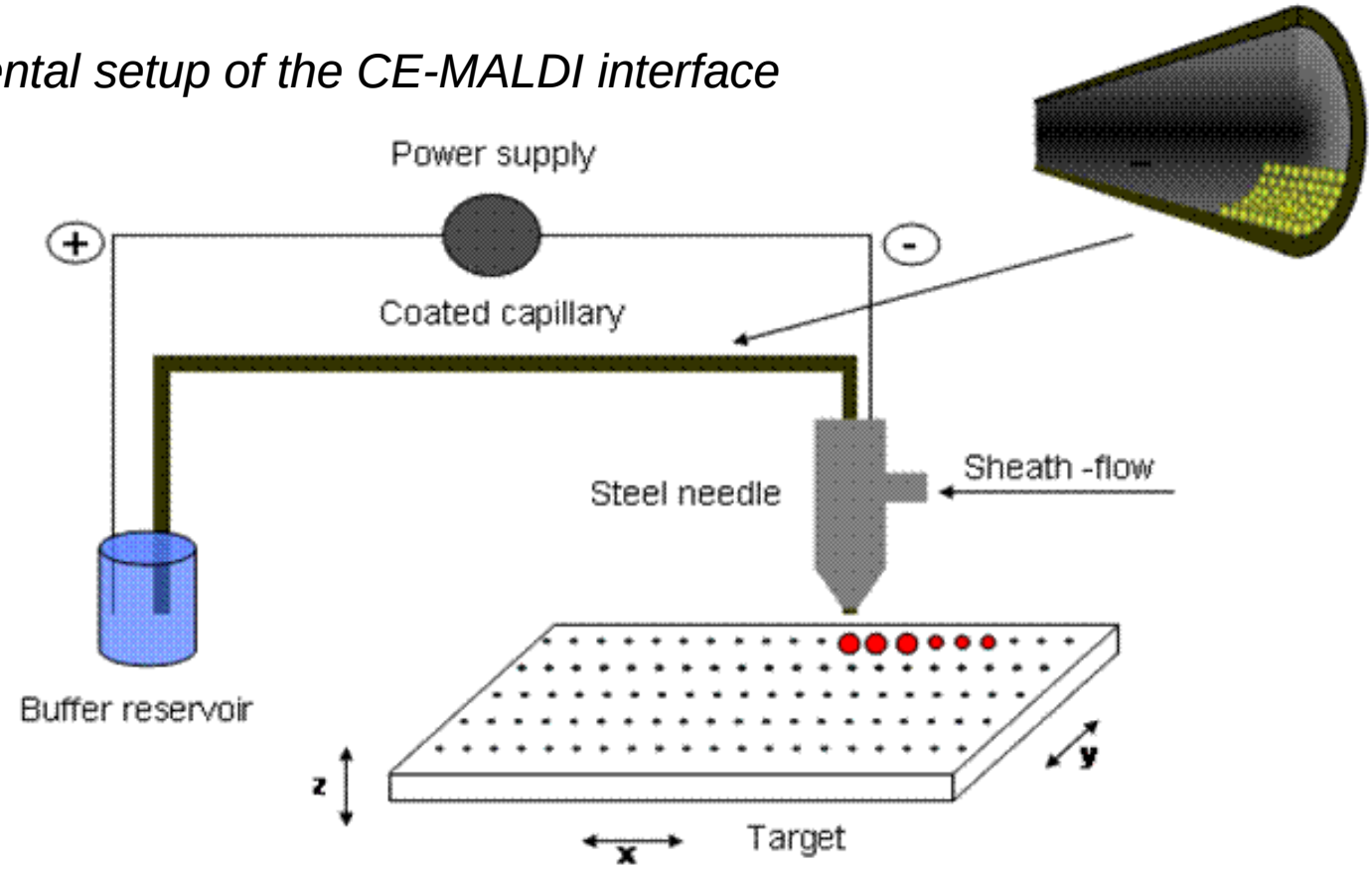
Fragment spectra of an O-glycoside
(Quercetin-3-O-deoxyhexosylhexoside-7-O-deoxyhexoside



Fragment spectra of C-glycosides
Positive (a-d) and negative (e-h) ion MS3 spectra
of [M+H]+→0,2X+

Hyphenation of CE with MALDI

Experimental setup of the CE-MALDI interface

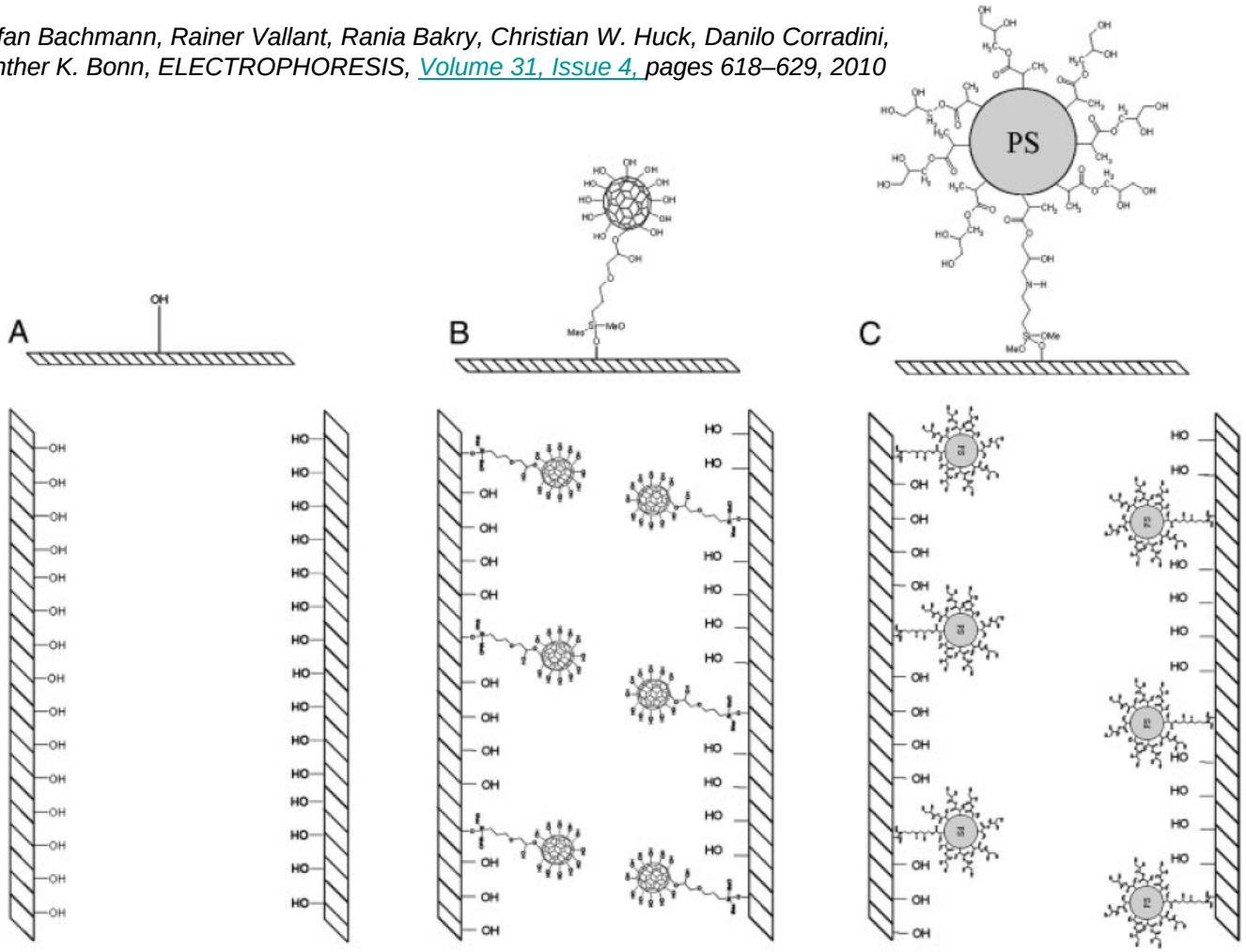


Institute of Chemical Methodologies

Collaboration with Prof. Danilo Corradini

CE coupled to MALDI with novel covalently coated capillaries

Stefan Bachmann, Rainer Vallant, Rania Bakry, Christian W. Huck, Danilo Corradini, Günther K. Bonn, ELECTROPHORESIS, Volume 31, Issue 4, pages 618–629, 2010



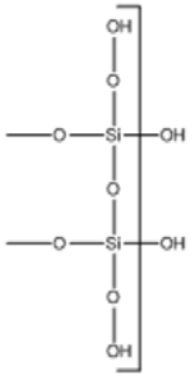
fused silica

fused silica with attached fullerenol

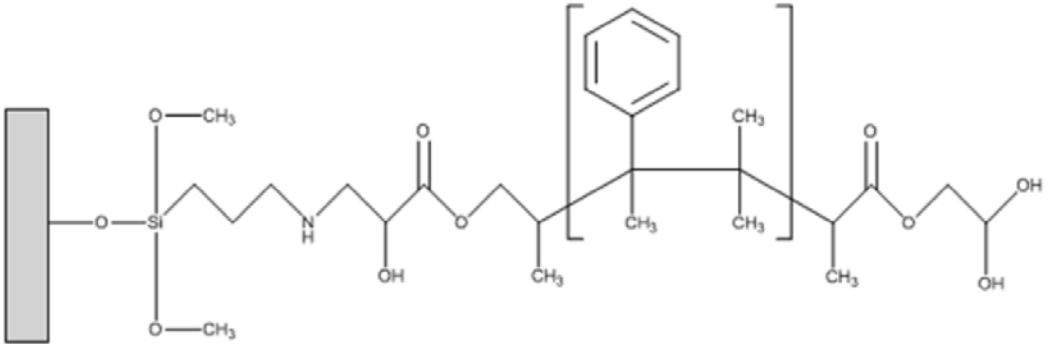
fused silica latex diol

Characterization of coated capillaries for CE by infrared spectroscopy

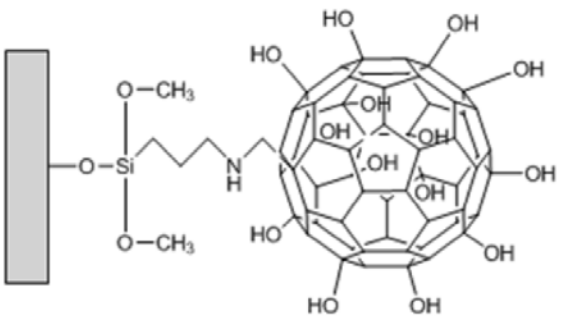
fused silica Polymicro capillary (uncoated)

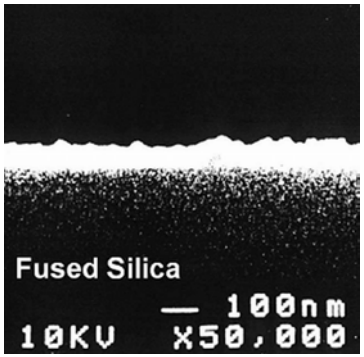


fused silica Polymicro capillary (latex diol coated)

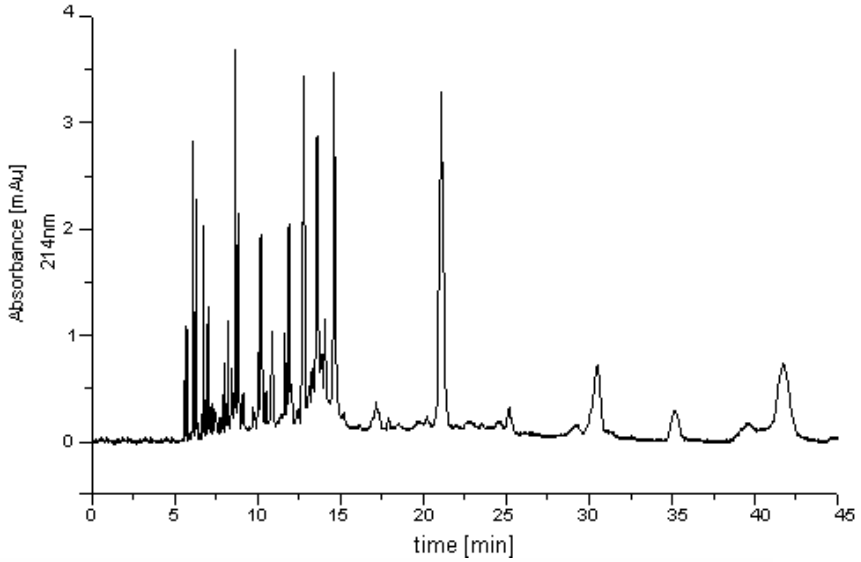


fused silica Polymicro capillary (fullerenol coated)



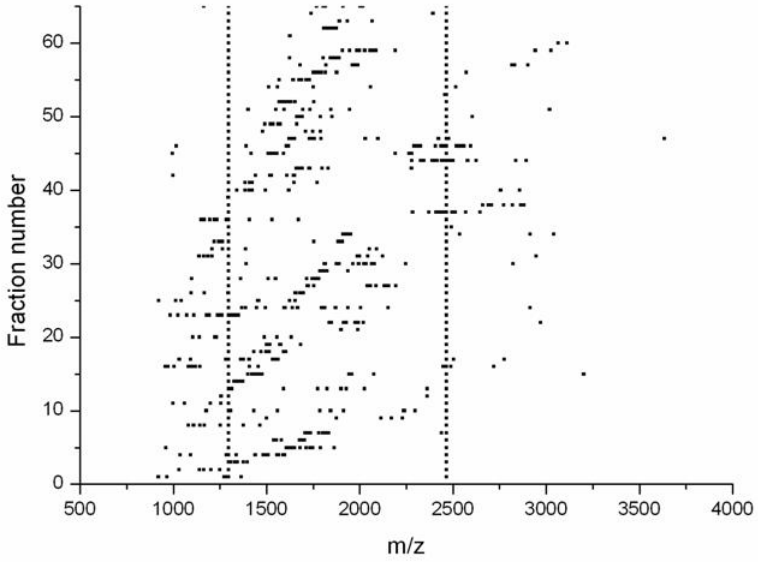


Hyphenation of MALDI with CE



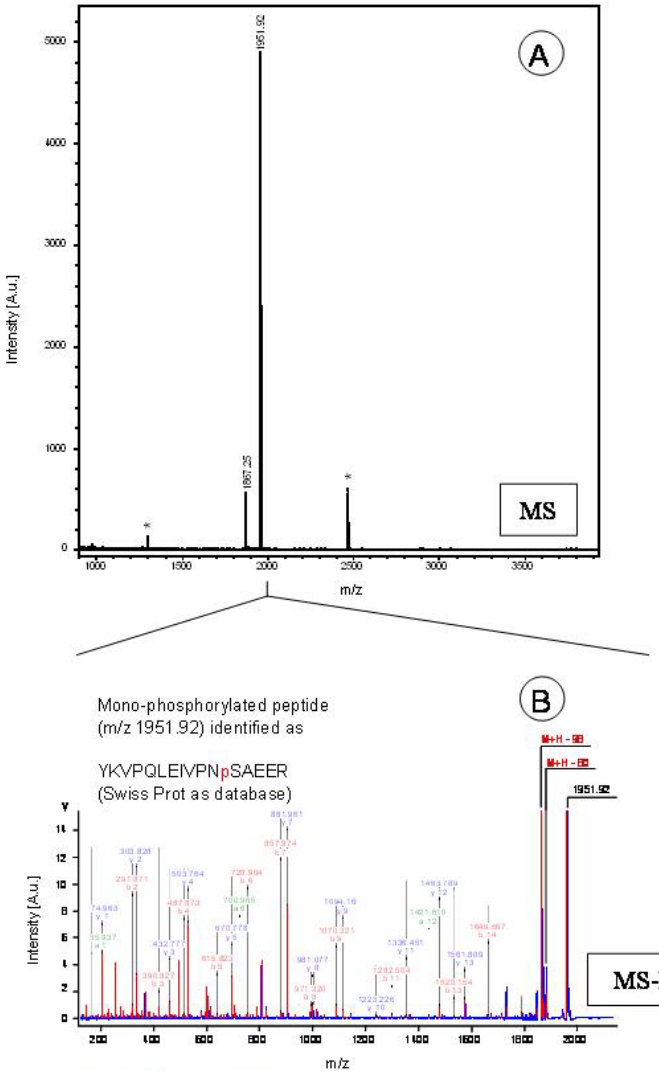
Electropherogram for the separation of α–casein tryptic digest obtained with latex diol.

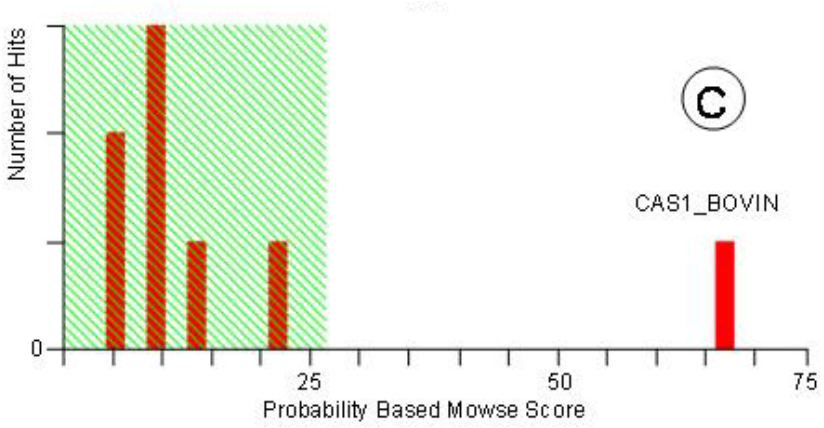
Conditions: *capillaries,* (65/57.5 cm eff., 75 µm ID, 360 µm OD); BGE, pH 2.3: 40 mM TEA titrated with phosphoric acid mixed with 20% (v/v) ACN.



2D Plot CE-MALDI –MS data obtained from the analysis of a tryptic digest of five proteins (myoglobin, cytochrom c, BSA, α-casein, ovalbumin).

Hyphenation of MALDI with CE





(A) MALDI-TOF mass spectrum of CE fraction no. 34. **(B)** The fragment ion spectrum of peptide 1951.92 Da. **(C)** The identification probability plot from the swiss prot database.
The internal standards are indicated with asterisks

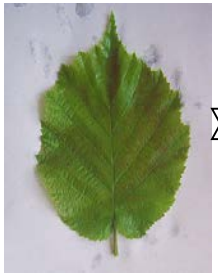
Institute of Chemical Methodologies

Collaboration with Prof. Danilo Corradini

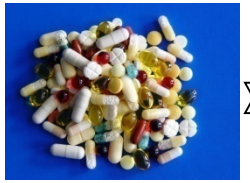

New Analytical Approaches
in “Phytoanalytics”

Analytical Chemistry in Phytopharmacy
Steps for Quality Control

plant → extraction → sample pretreatment → production → product → patient








Selective materials for sample pretreatment:	particles (spherical, irregular) monoliths SPE, columns, disks
Stationary phases for HPLC and µ-HPLC:	particles (different mechanisms) monoliths
Screening: MALDI targets/MELDI materials:	matrix free with matrix
Hyphenated techniques:	µ-HPLC-ESI-MS µ-HPLC-MALDI-TOF MS
Infrared Spectroscopy:	non-invasive quality control

Natural Products - Extraction and Purification - Strategies

Primula veris

Echinacea

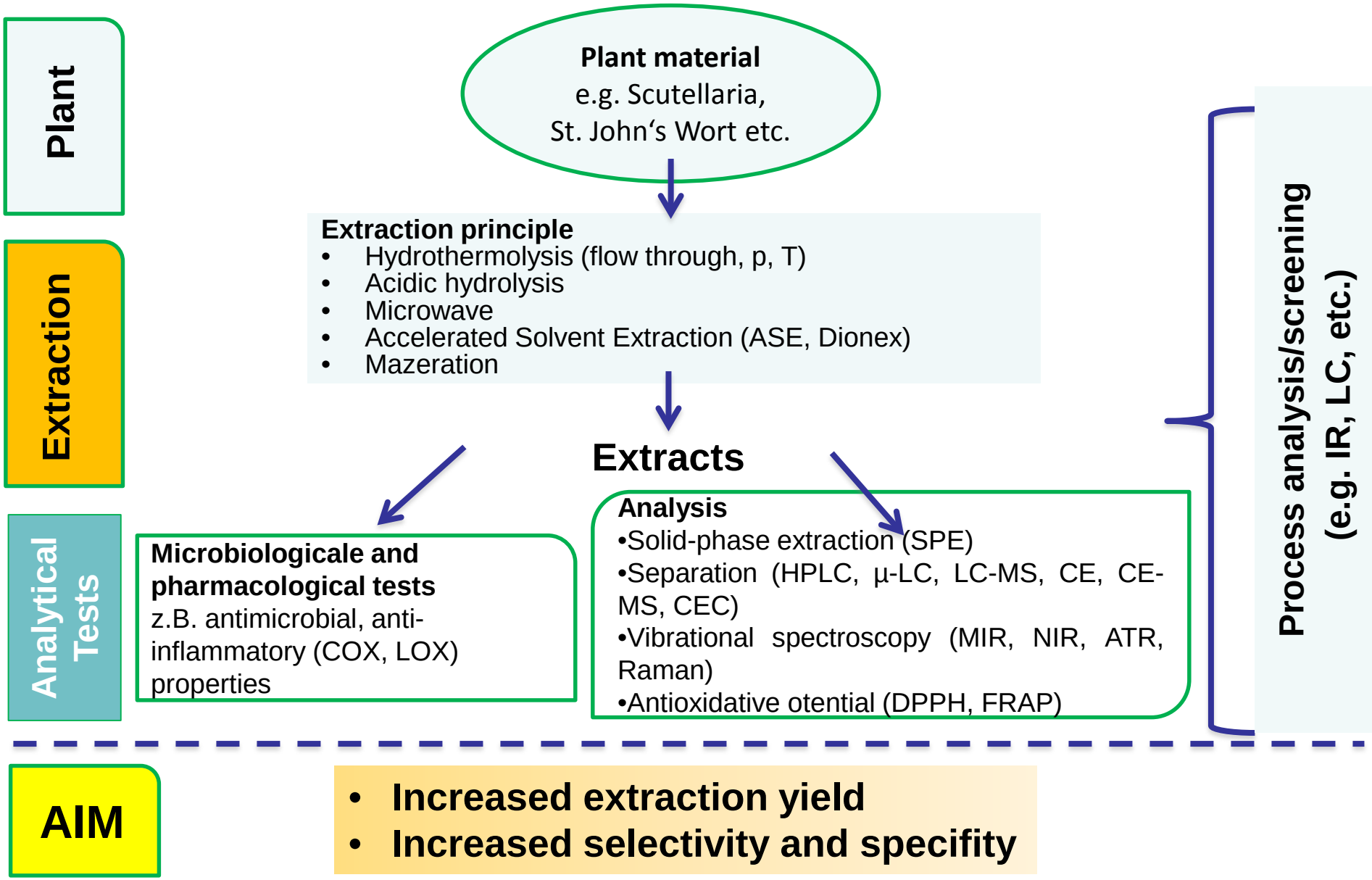
Pharmacological studies of single and synergetic effects

Quality Control

Extractives

- Flavonoids
- Carbohydrates
- Vitamines
- Tocopherols
- Carotenoids
- Polyphenols
- Resveratrol
- Naphtodianthrone
- Hypericin, Pseudohypericin
- Hyperforin
- Lectines
- **Proteins / Amino acids Phytoproteomics**
- Alkaloids

Extraction Scheme



Cooperation with Bionorica®

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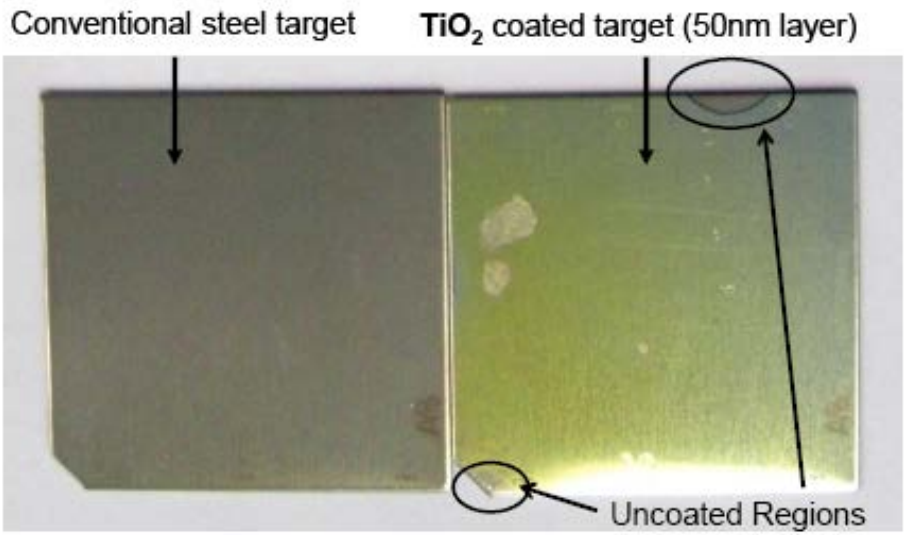
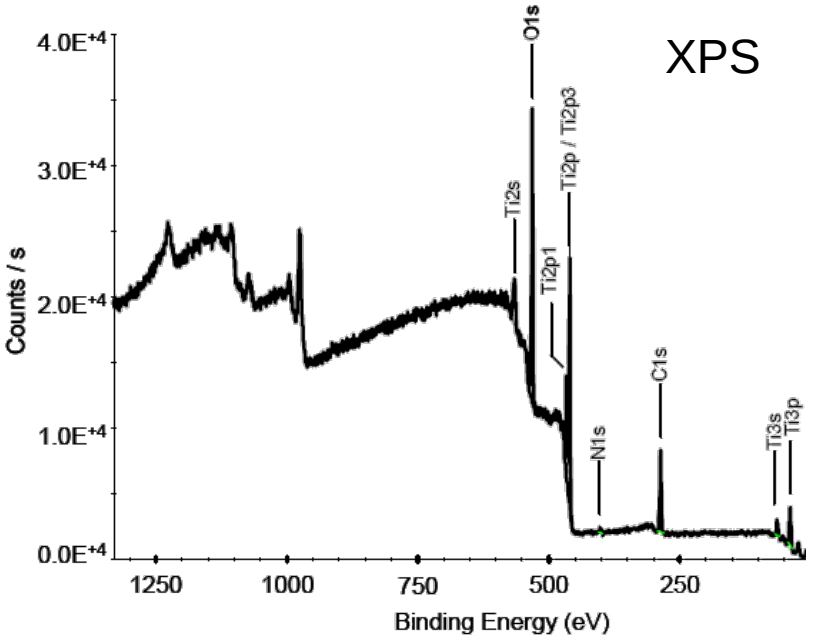
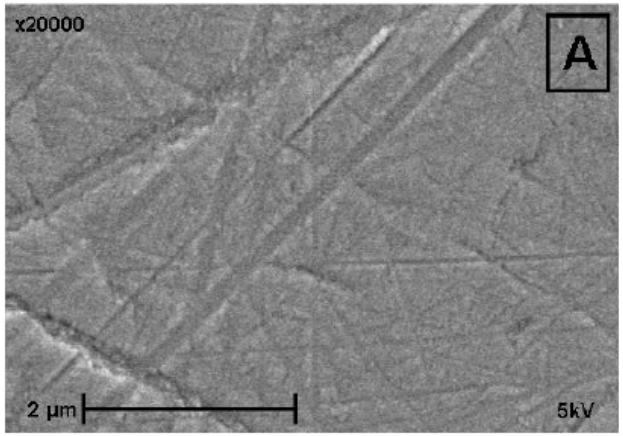
BIONORICA®
The phytoneering company

In the field of phytoanalytics
and antimicrobial activities of plants

TiO_2 coated Targets for matrix-free LDI MS







SEM picture

50 nm TiO_2 coating

TiO_2 coated Targets for matrix-free LDI MS

Analysis of Artichoke Samples

Cynara scolymus

Apigenin (A),

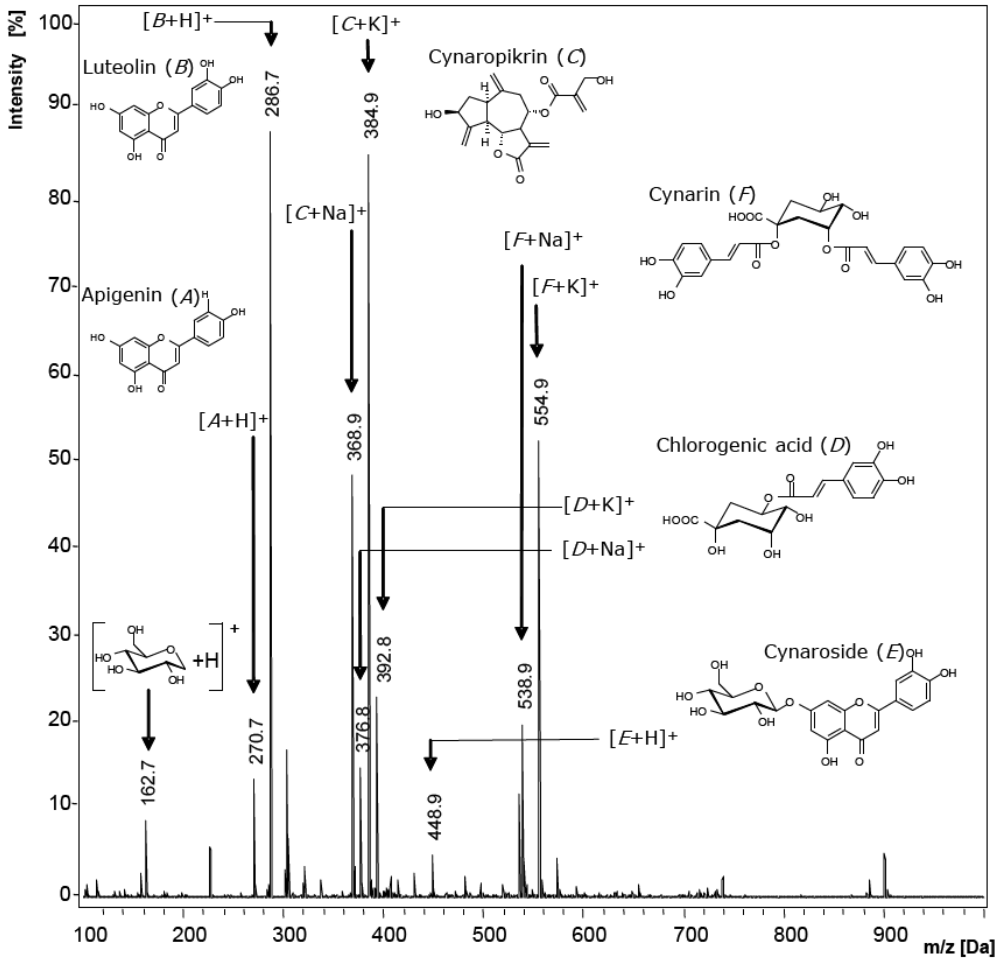
Luteolin (B),

Cynaropikrin (C),

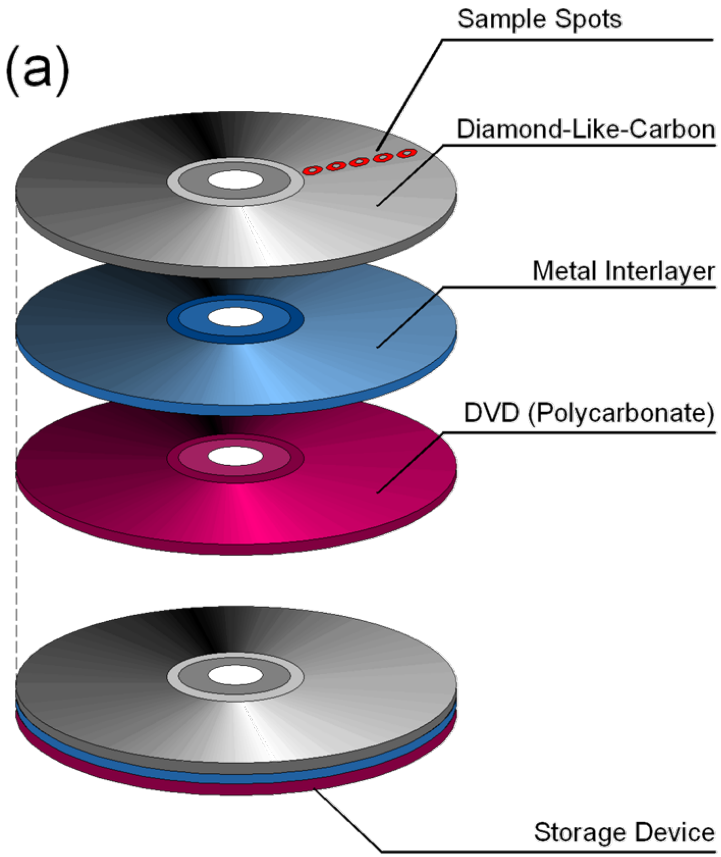
Chlorogenic acid (D),

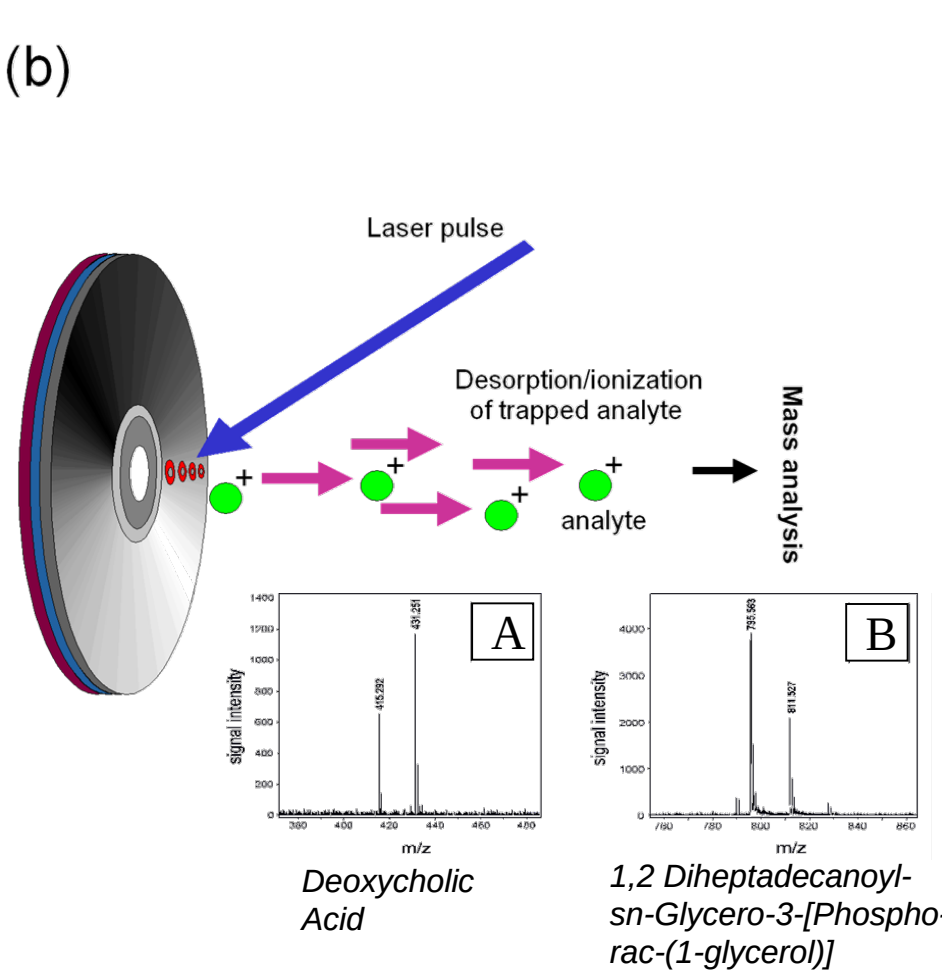
Cynaroside (E) and

Cynarin (F),



Matrix-free LDI MS for the Detection of Low Molecular Weight Compounds





Deoxycholic Acid

1,2 Diheptadecanoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)]

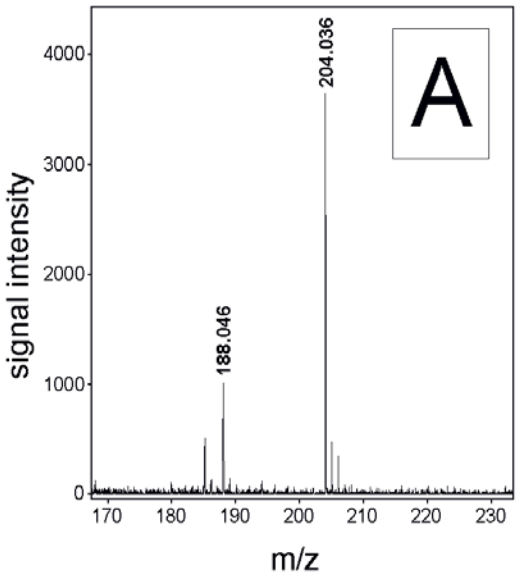
Collaboration with SONY

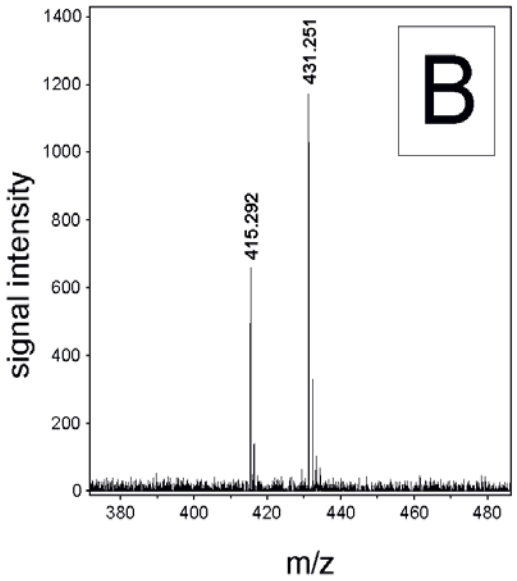
Laser Desorption/Ionization Mass Spectrometry on Diamond for the Application in Metabolomics

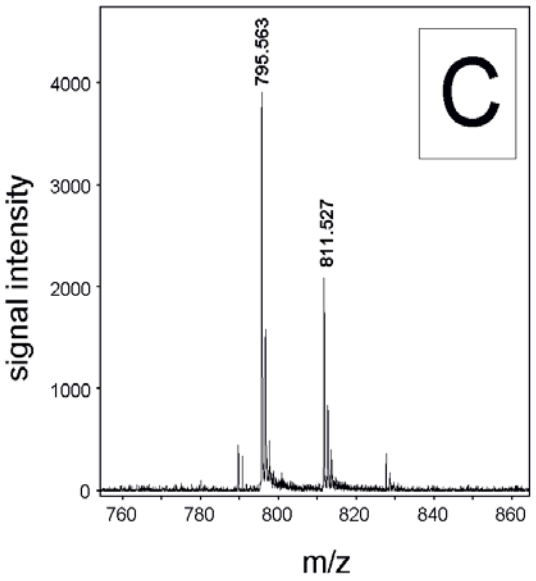
A.) Amino Acids
e.g. L-Phenylalanine

B.) Blood Metabolites
e.g. Deoxycholic Acid

C.) Lipids
e.g. 1,2 Diheptadecanoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)]







Na^+ and K^+ adducts.

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Invited Interview in Analytical Chemistry 2008

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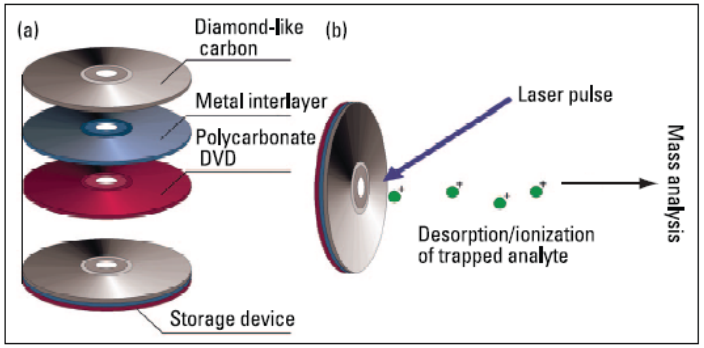
Published on Web 08/29/2008

RESEARCH PROFILE

Femtomolar sensitivity with matrix-free LDI MS

Scientists who want to analyze small molecules with MALDI MS have long been frustrated by unwelcome signals from the matrix. And until now, matrix-free LDI has not reached the femtomolar sensitivity required to, for example, perform clinical blood tests for metabolites. But Günther Bonn and colleagues at Leopold-Franzens University (Austria) report in a new *AC* paper (DOI 10.1021/ac801190e) that they have boosted sensitivity into the femtomolar range by replacing their MALDI target with a DVD coated with diamond-like carbon (DLC). "That it worked was really a surprise," says Bonn, who hopes to use the technology to create metabolomic tests for prostate cancer.

The porous silicon most commonly used as a target for matrix-free LDI is easily contaminated and can't be stored for layer, says Matthias Rainer, another group member. "Their electrical, thermal, and optical properties improve the efficiency of laser transfer, leading to high reproducibility."



(a) The matrix-free target is a polycarbonate DVD coated with an interlayer of molybdenum and topped with a layer of DLC. Even after the DVD is coated, it can store data. (b) A laser pulse desorbs and ionizes the sample from the carbon layer for analysis by MS.

Bonn wanted to use DVDs so he could burn analytical data onto their undersides. This technique is particularly useful, he points out, when hundreds of

The researchers also detected peptides that fall into the same mass range as vaporized matrix would and successfully measured the m/z of peptides in a BSA digest, an amino acid, a phospholipid, and an emulsifier found in the small intestine. The masses of these test compounds ranged from 100 to 1500 Da. "The different analytes reported here show the broader application of DLC," Rainer says. "This is important, as many matrix-free systems are reported to be viable for a fixed kind of analyte."

The group is trying to increase sensitivity even further by decreasing the thickness of the DLC layer and chemically modifying its surface. For example, they are applying aromatic compounds, such as sinapinic acid, to boost UV sensitivity and thereby expand the mass range. Before the technology can be commercialized, they must also

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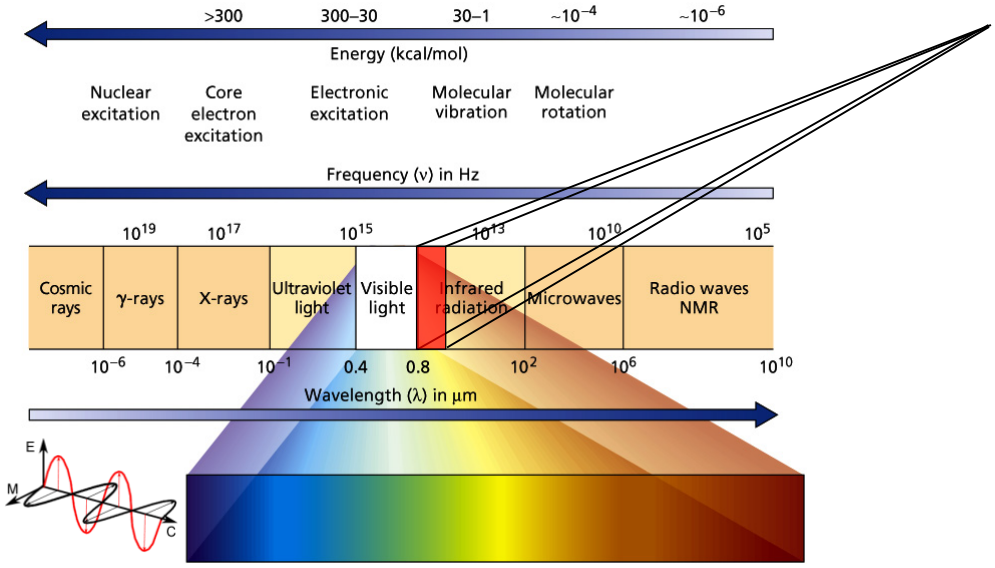
Basics of Near–infrared Spectroscopy (NIRS)



- **NIR wavelength range: 780 – 2500 nm (4000 – 12800 cm^{-1})**
- interaction between sample and electromagentic radiation
- Pre – condition: change of dipole moment
- overtone and combination bands appear
- use of chemometrical and statistical tools

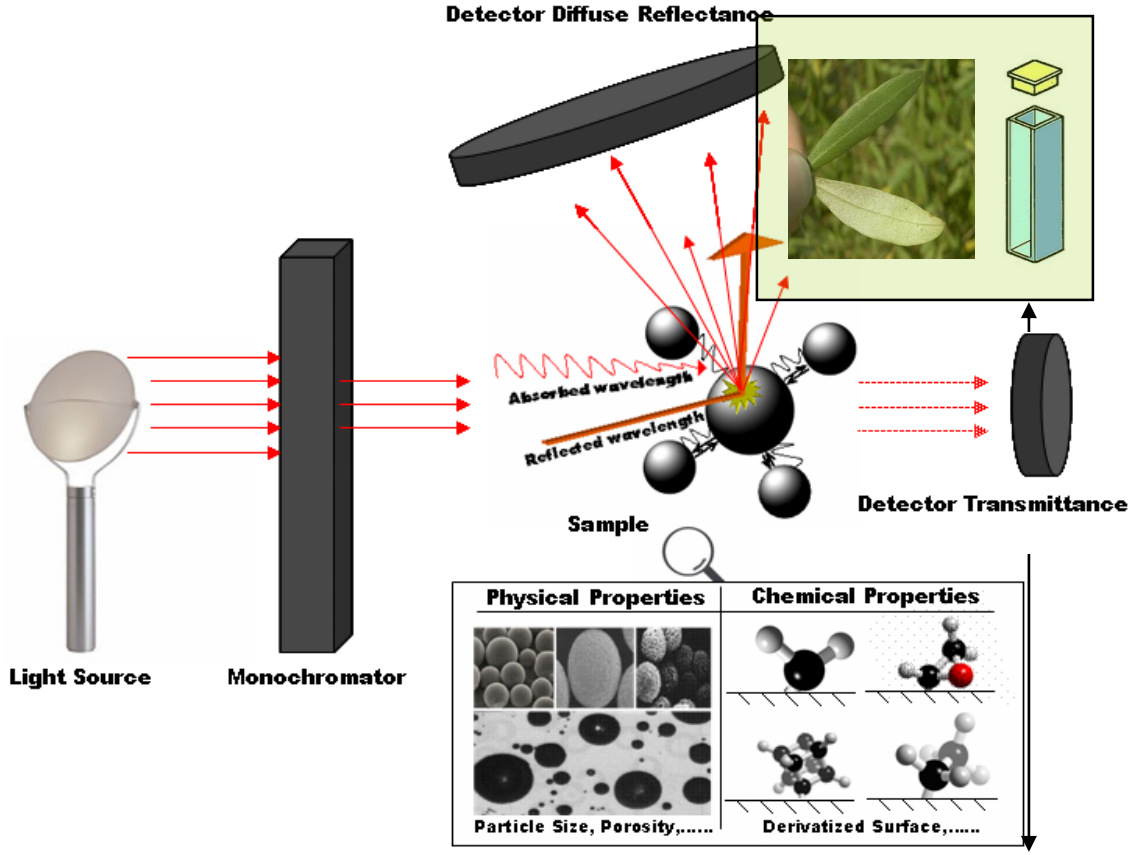
→ Absorption of NIR radiation by organic molecules → mainly O-H, C-H, N-H and C=O groups whose fundamental molecular stretching and bending absorb in the mid – IR region (400 – 4000 cm^{-1})

→ These overtone and combination bands are anharmonic → very complex and not direct interpretable as in other spectral regions → need for chemometric evaluation

→ Chemical (e.g. functional groups) as well as physical parametes (particle size, pore size, specific surface area) can be detected

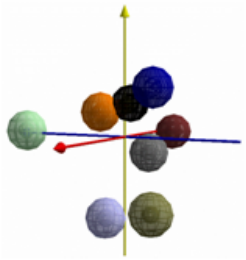
Basics of Near–infrared Spectroscopy (NIRS)

- NIRS Setup -

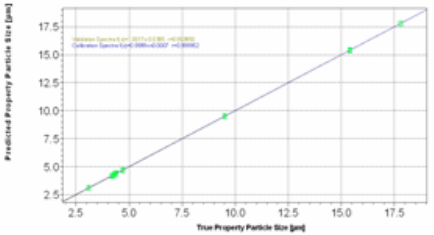


Determination of Parameters

Qualitative Analysis



Chemometrics / Multivariate Data Analysis

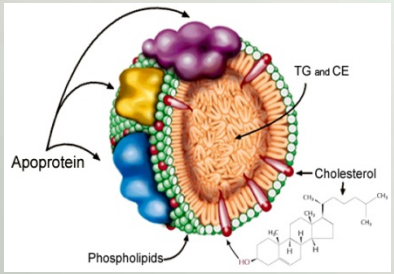


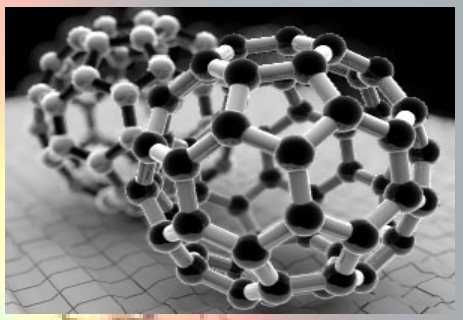
Quantitative Analysis

Lambert Beer´s law $$A = \varepsilon * c * d = -\log \frac{I}{I_0}$$

MIR + NIR

Phytomics	Bioanalysis	Pharmacy	Material Science
Chemical paramters	Tissue analysis	Chemical parameters	Morphological properties
Physical parameters	Blood ingredients	Physical parameters	Physical properties



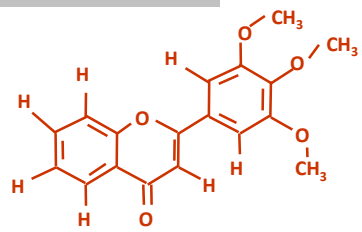


NIR Strategy of Analysis

Sinupret®

Optimization of the NIR-parameters

Optimization of the reference method



HPLC → Content of the leading compound 3‘,4‘,5‘-trimethoxyflavone → NIR

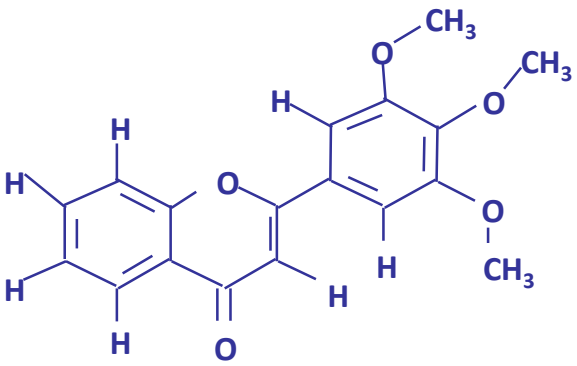
Optimization of the reference method

Content of *Flos Primulae veris*

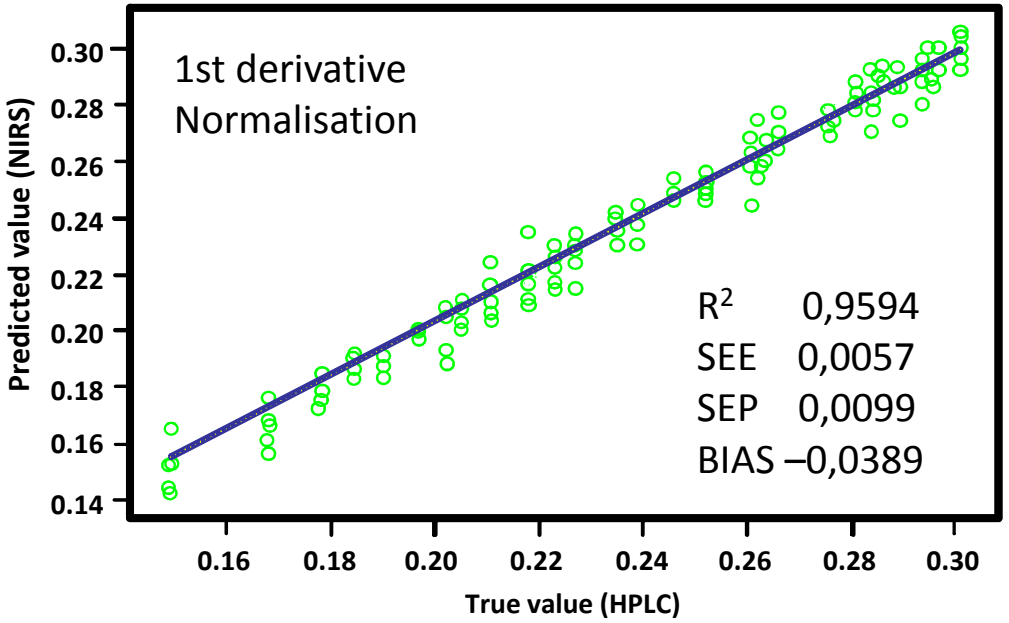
HPLC → Content of the other leading compounds

Content of other plants in Sinupret ®

NIR Strategy of Analysis

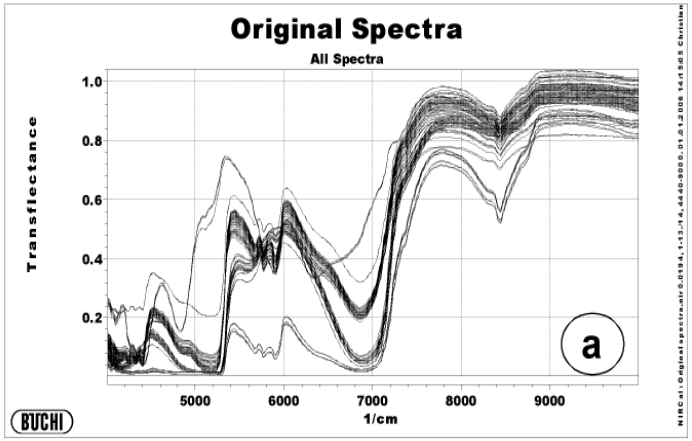


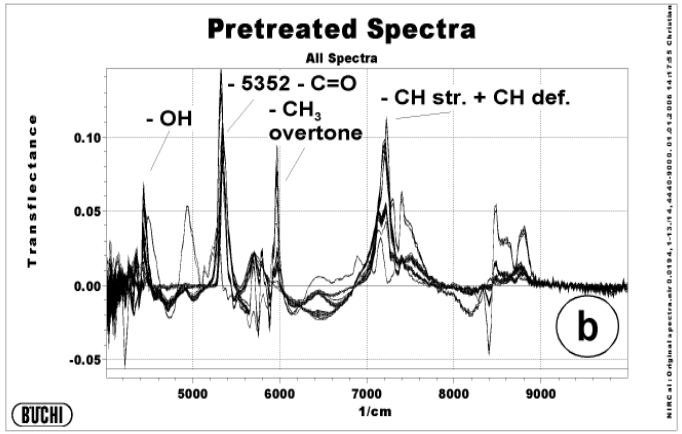
3‘,4‘,5‘- Trimethoxyflavone
Leading Compound



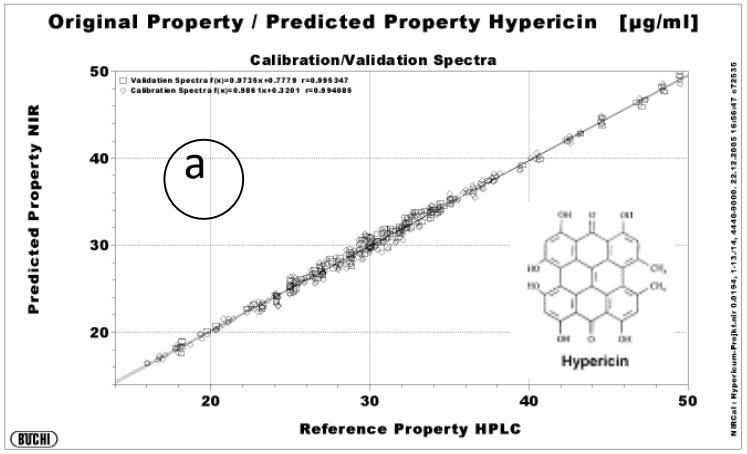
Charge	Area (µV*sec)	Primula (g/100g)	Conc. LC (ng/µl)	NIR-MW (ng/µl)	Water (%)	EtOH (%)
91102064 28.10.91	70490	0,493	0,219	0,219	80,38	15,7
91112182 05.11.91	71740	0,500	0,222	0,210	80,83	15,2
91112191 08.11.91	61346	0,442	0,197	0,230	80,25	15,7
91112302 22.11.91	66799	0,472	0,210	0,186	81,33	14,5
91112311 28.11.91	81551	0,555	0,245	0,193	79,69	15,7

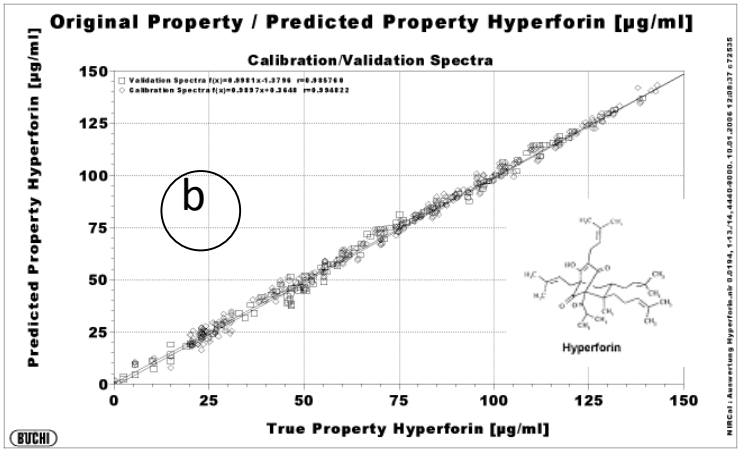
NIRS of Naphthodianthrones and Phloroglucines in St. John´s Wort





NIR spectra
(a) Original spectra, (b) pretreated spectra

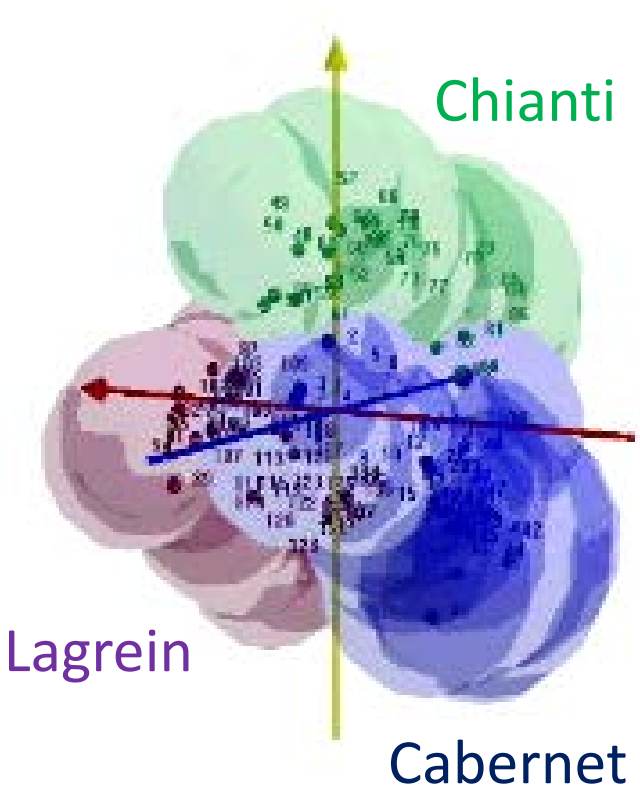




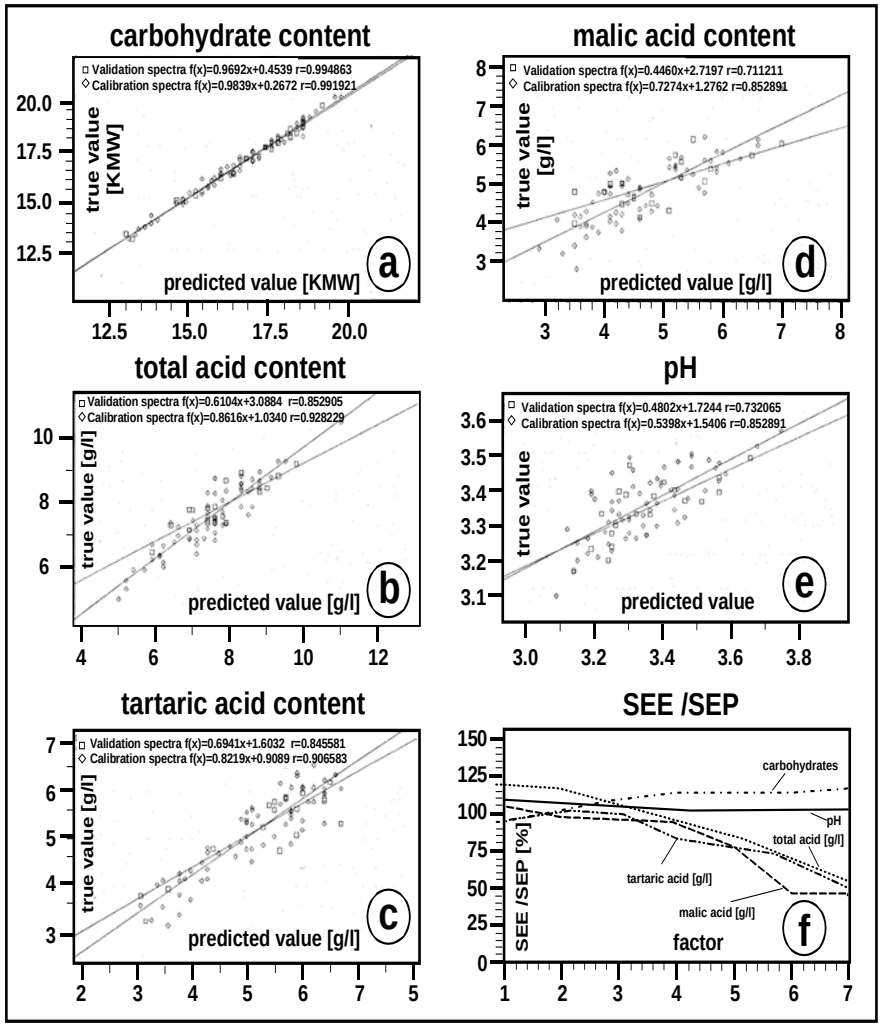
Predicted (NIRS) vs. True values (LC)
(a) Hypericin, (b) Hyperforin
(n=80). (a) R^2=0.99; SEP=0.68; (b) R^2=0.99; SEP=0.72

Wine Analysis using NIRS

Wine Analysis using NIRS



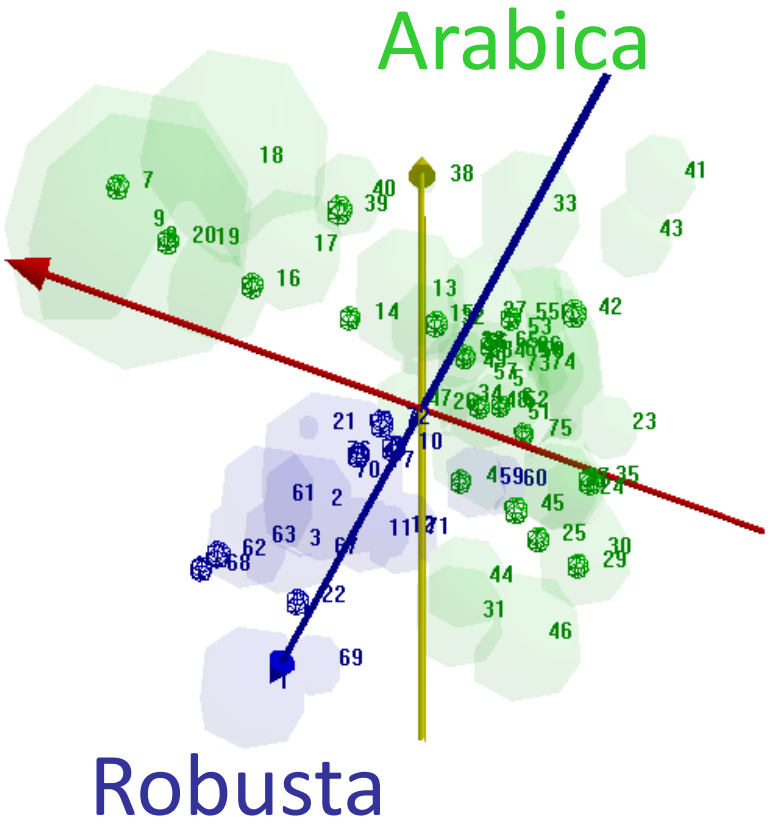
Factor plot of 141 spectra of different wines (Lagrein, Chianti, Cabernet Sauvignon). Conditions: Normalisiation, 1st derivative; wavenumer range, 4500 - 10000 cm^{-1}; thickness, 3 mm; scans, 10; temperature, 23°C.

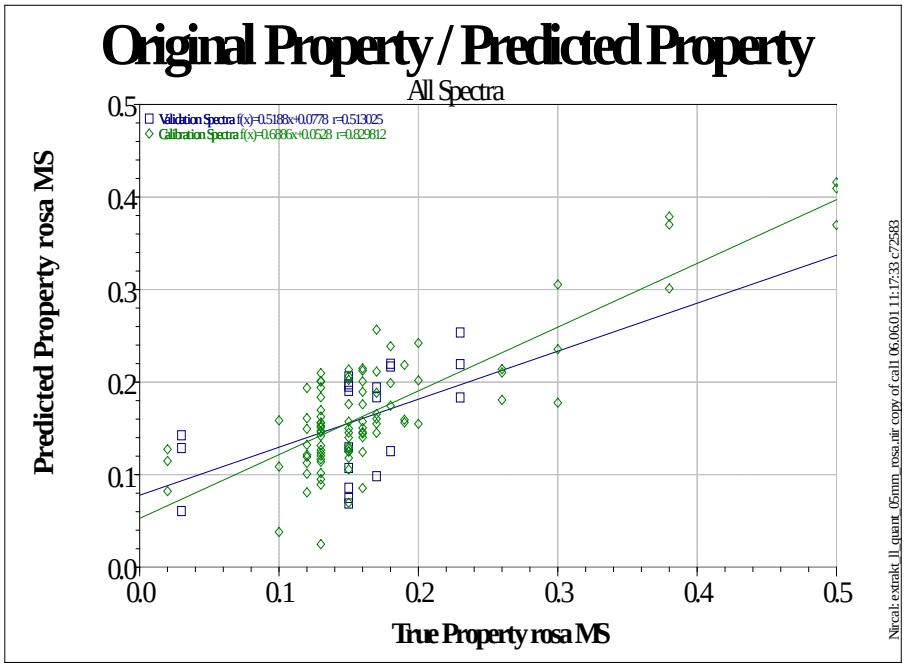


Michael A. Popp, Guenther Bonn, Christian Huck, Wolfgang Guggenbichler, **Method for classifying wine and coffee.** Patent No. WO 2002097431, Application Number WO 2002-EP4988

Coffee Analysis using NIRS

Quality Control of Coffee via NIRS





Quantitative analysis of caffeine and theophylline

C.W. Huck, W. Guggenbichler, G.K. Bonn, **Analysis of Caffeine, Theobromine and Theophylline in Coffee by Near Infrared Reflectance Spectroscopy (NIRS) Compared to High Performance Liquid Chromatography (HPLC) Coupled to Mass Spectrometry.** Anal. Chim. Acta, 538 (2005) 195

International Collaborations

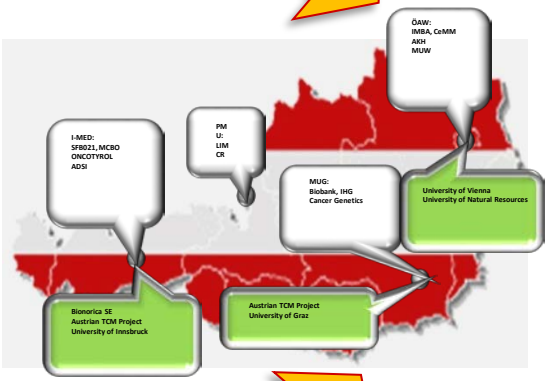
Prof. Günther Bonn

ACRC
Institute of Analytical Chemistry and RadioChemistry
Center of Competence and Excellence
Leopold-Franzens University of Innsbruck, Austria

BIOCENTER INNSBRUCK

Prof. Lukas Huber

adsi
Austrian Drug Screening Institute



International Agency for Research on Cancer
Centre International de Recherche sur le Cancer

WHO – Lyon, France

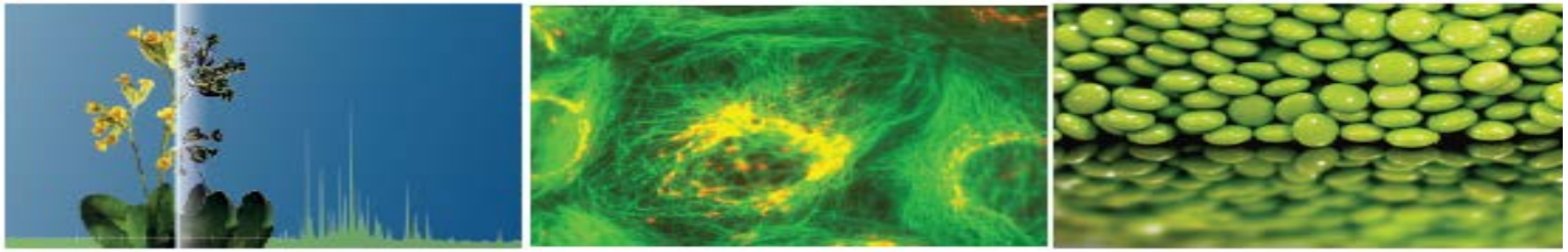
Sino-Austrian
Center for Biomarker Research

北京大学医学部
Peking University
Health Science Center

Prof. Ke Yang

מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

Prof. David Mirelmann



Austrian Drug Screening Institute (ADSI)

Prof. Lukas A. Huber
Scientific Director – Biological Division
Cell Biology, Biocenter
Innsbruck Medical University

Prof. Günther K. Bonn
Scientific Director – Analytical Division
Institute of Analytical Chemistry and Radiochemistry
University of Innsbruck

BMWF

ÖAW Österreichische Akademie der Wissenschaften

tirol Unser Land

Bionorica®

adsi
Austrian Drug Screening Institute

Tyrol as a region of cancer and phyto research: stepwise development

Projects of Bionorica SE and Bionorica Research

at the Universities and Medical Universities in Innsbruck, Graz and Vienna

Bionorica®

2003

Special Research Program SFB021: Cell Proliferation and Cell Death in Tumors

2004

Doctoral Program in Oncoscience

Austrian Proteomics Platform (APP)

2005

Formation of Biocenter Innsbruck

Oncology in the scientific focus at the I-MED

FP6-Project Growthstop

2006/7

Austrian TCM Project with The Chinese Academy of Traditional Chinese Medicine

Proposal for COMET Center ONCOTYROL

First „Clinical Trial Center" in Austria established at the I-MED

GEN-AU II

2008/9

Launch of the „ONCOTYROL – Center for Personalized Cancer Medicine"

4x START grants at the I-MED Biocenter

Drug Screening Platform Concept

GEN-AU III

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SFB021

4x START grants

Gen-Au:

adsi onco

oncotyrol

basic research | target identification | target validation | hit generation | lead development | lead optimization | pre-clinical development | clinical development

validated targets

validated hits

lead candidates

Projects Bionorica

Austrian TCM Projects

adsi phyto

Bionorica SE

Bionorica

Bionorica Research, Austri

academic research

industry-academia partnership

industrial development

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Academic Drug Screening Centres - USA

UNIVERSITY	CHEMICAL LIBRARY SIZE	DIRECTOR	WEBSITE
Broad Institute of MIT and Harvard	500,000	Michael Foley	www.broad.mit.edu/node/139
Columbia University	118,000	James Rothman	www.columbia.edu
Emory University	200,000	Ray Dingledine	www.pharm.emory.edu
Harvard Medical School	250,000	Caroline Shamu	iccb.med.harvard.edu
Johns Hopkins University	180,000	Min Li	www.hopkinschemcore.org
Rockerfeller University	46,000	J. Fraser Glickman	www.rockefeller.edu
Stanford University	130,000	David Solow-Cordero	htbc.stanford.edu
University of California	55,000	Scott Lokey	chemistry.ucsc.edu
University of Cincinnati	250,000	William L. Seibel	www.gri.uc.edu
University of Illinois	200,000	Carson Putt	www.scs.uiuc.edu/htsf
University of Kansas	110,000	Rathnam Chagaturu	www.hts.ku.edu
University of Michigan	56,000	Martha Larsen	www.umich.edu
University of Minnesota	5,000	Marc von Keitz	www.bti.umn.edu/htba
University of New Mexico	231,000	Larry Sklar	nmmlsc.health.unm.edu
University of Pennsylvania	218,000	Scott Diamond	www.seas.upenn.edu/~pcmd/hts
University of Pittsburgh	280,000	John Lazo	www.upddi.pitt.edu
University of Rochester	23,000	Alan V. Smrcka	www.urmc.rochester.edu/hts
University of Texas Southwestern Medical Center	200,000	Michael Roth	www.utsouthwestern.edu
University of Wisconsin Madison	105,000	F. Michael Hoffmann	www.hts.wisc.edu
Vanderbilt University	260,000	Charles David Weaver	www.vanderbilt.edu
Washington University	140,000	Jayne Marasa	mic.wustl.edu/Cores/HighThroughputCore
Yale University	30,000	Paul Fletcher	cgp.yale.edu/chemical/chem_info

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Academic Drug Screening Centers - Europe

	Library size
Medical Research Council Technology (Mill Hill, UK)	51-100.000
Imperial College Drug Discovery Centre (London, UK)	11- 50.000
Scottish Hit Discovery Facility (Dundee, UK)	51-100.000
Max Planck Institutes Chemical Genomics Centre (Dortmund, Germany)	11- 50.000
EMBL/DKFZ Chemical Biology Core Facility (Heidelberg, Germany)	51-100.000
USEF (Santiago de Compostela, Spain)	<10.000
HTS, Spanish National Cancer Research Centre (Madrid, Spain)	51-100.000
LiMoNe, K.U.Leuven (Leuven, Belgium)	11-50.000
IBITEC-S, CEA Saclay (Paris, France)	11-50.000
PCBIS, Universite Louis pasteur (Strasbourg, France)	11-50.000
CMBA (Grenoble, France)	11-50.000
Biomolecular Screening Facility, EPFL (Lausanne, Switzerland)	11-50.000
European Screening Port (Hamburg, Germany)	**151 - 300.000**

High-Content Screening prevails at European Academic Screening Centres

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Austrian Drug Screening Institute, Tirol

Comparison of early drug discovery approaches at industry and academia

	high throughput screening (industry)	high content screening (academia)
throughput	high to ultra-high	low to medium
content	low	high
format	1536- and 3456-well plates	96- and 384-well plates
assay	simple biochemical assays (cell-free)	complex biological assays (cell-cell interactions)
detection	absorbance, fluorescence, luminiscence	advanced biooptics, mass spectrometry
capacity	> 100.000 compounds/day	< 10.000 compounds/day
screen size	up to 2 millions compounds	< 100.000 compounds
compounds	own synthetic libraries	external libraries (synthetic and natural)
focus	selection of active compounds	understanding of the mode of action
purpose	generation of new drugs for profit	generation of new methods and research tools
IPR strategy	protection of active compounds	protection of methods and research tools
IPR owner	company	project owner
equipment	> 10.000.000 €	< 5.000.000 €

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ADSI management

scientific advisory board

Günther Bonn

ANALYTICAL DIVISION

- mass spectrometry
- HPLC
- LC-MS, MS/MS
- IR/NMR spectroscopy
- computational chemistry
- drug pull downs
- new materials for separation technology & enrichment
- advanced electrophoresis
- bioinformatics

Lukas Huber

BIOLOGICAL DIVISION

- biological tests
- immunological tests
- high content biooptics
- molecular screening
- structural biology
- cell –cell interaction assays
- drug signatures
  - Proteomics
  - Genomics

Management

Shareholder University of Innsbruck (100%)

CEO:

Dr. Laco Kacani

Scientific Directors:

Prof.Mag.DDr.h.c. Günther Bonn – Analytical Division

Prof.Dr. Lukas Huber – Biological Division

Scientific Board

Prof.Dr. Peter Boyle	IPRI , Lyon, France
Dr. Danilo Corradini	Institute of Chemical Methodologies, Chromatography and Capillary Electrophoresis Unit, Montelibretti, Italy
DDr. Friedrich Lottspeich	Max Planck Institute of Biochemistry, Martinsried, Germany
Prof.Dr. David Mirelman	Weizmann Institute of Science, Rehovot, Israel
Mag. Markus Pasterk	Biobanking and Biomolecular Research Infrastructure, Graz, Austria
Prof.Dr. Siqi Liu	Chinese Academy of Sciences, Bejing Institute of Genomics, Bejing, China

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Examples of Screening Projects

Screening in oncology – partnership with ONCOTYROL:

Treatment of prostate cancer, breast cancer, lymphoma & myeloma

Screening of natural compounds – partnership with phytopharmacy:

Treatment of inflammatory, immunological & infectious diseases

Screening for external partners – partnership with Austrian academy:

External projects – library screening, drug signatures, mode of action

ACRC
Institute of Analytical Chemistry and RadioChemistry
Center of Competence and Excellence
Leopold-Franzens University of Innsbruck, Austria

Extraction

Distribution

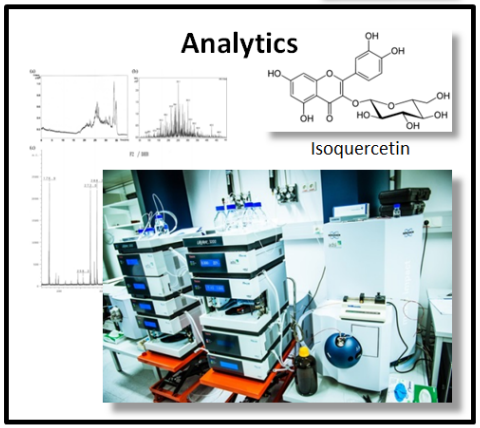
Analysis of compounds

Cell co-culture

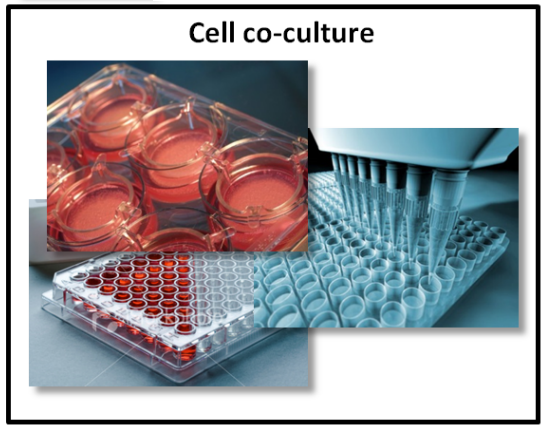
Projects:
Metabolic Syndrome
Inflammatory disease
Liver protection

Bionorica®

Target oriented cell-enzyme Models and specific cell- and co-cell culture models

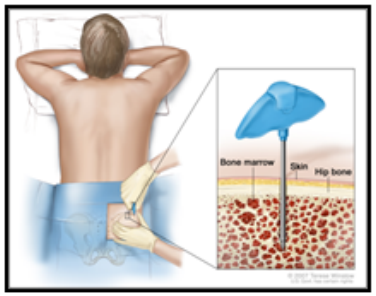


Analytics
Pharmacokinetics
Pharmacodynamics



Project Multiple Myeloma

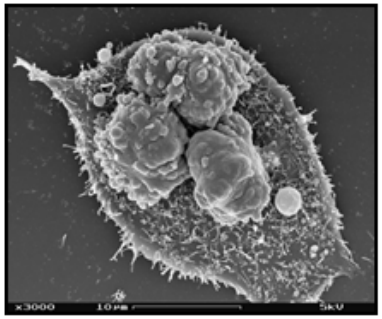
Collection of cells



Bone marrow aspirate

Cells of individual patients are collected and cultivated in Co-culture

Cell Co-cultures



Co-culture of tumor and bone marrow

Identification of the best combination of current therapeutic(s)

Development of new therapeutics

Development of new personalized diagnostics

Therapeutics

Plant Extracts

Chemical compound library

Marine extract library

Current therapeutics are tested to find ideal treatment

New substances and extracts are screened to find potentially new drugs

Equipment

Extraction

ASE 350

Rotavapor

Moisture Analyzer

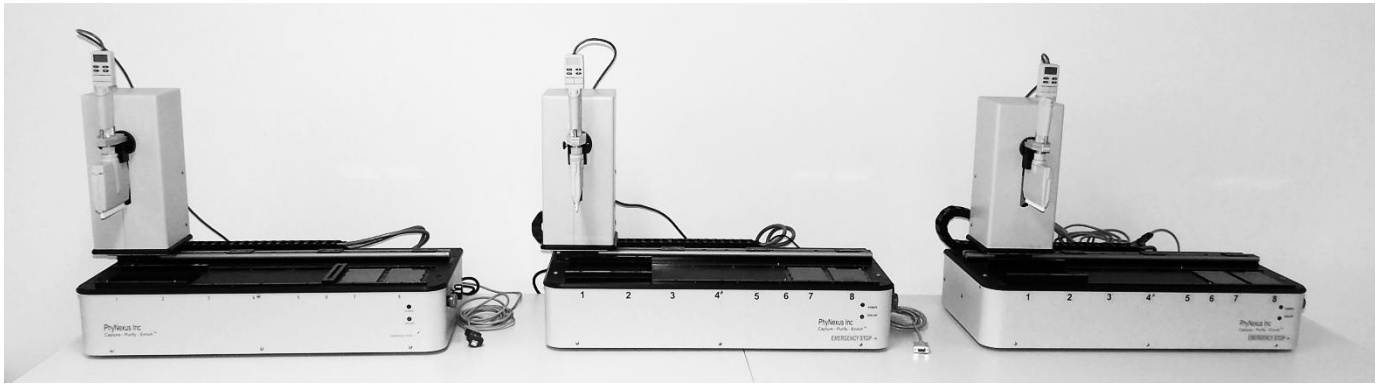
Aquasolv

CertoClav
Sterilization

Sample Preparation

Proteineer
Fractionation, LC-MALDI coupling

ImagePrep
MALDI Imaging



PhyNexus MEA System
liquid handling robotic system

Mass Spectrometry

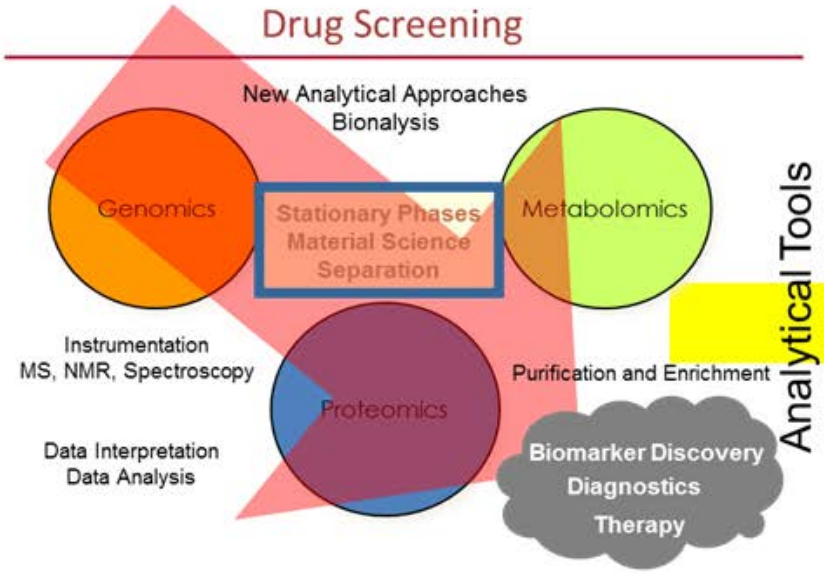
2x Maxis Impact

UPLC

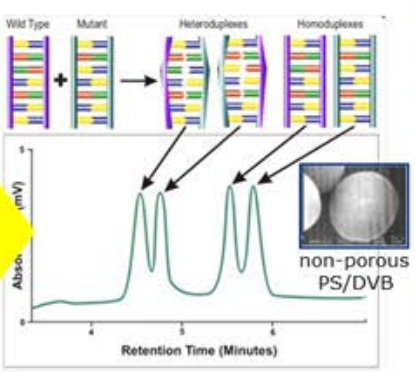
2D, analytical and nano

Autoflex Speed

Drug Screening



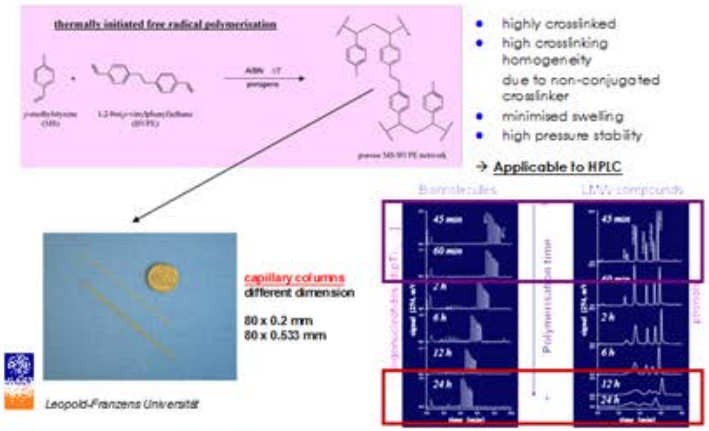
Transgenomic Wave-System - Mutation Analysis



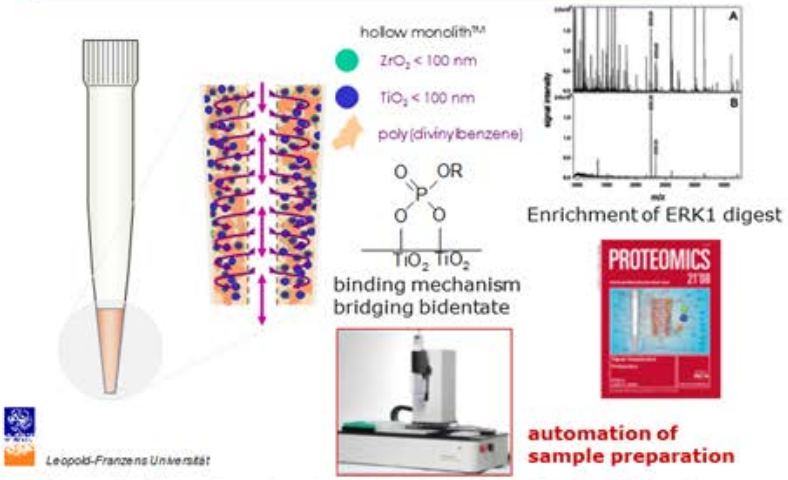
Transgenomic Wave HPLC-System

Bonn, Gunther; Huber, Christian; Oefner, Peter. ***Separation of nucleic acid fragments with alkylated nonporous polymer beads.*** *US Patent (1994)*

Stationary Phases for Separation Science



Preparation of hollow monolith™

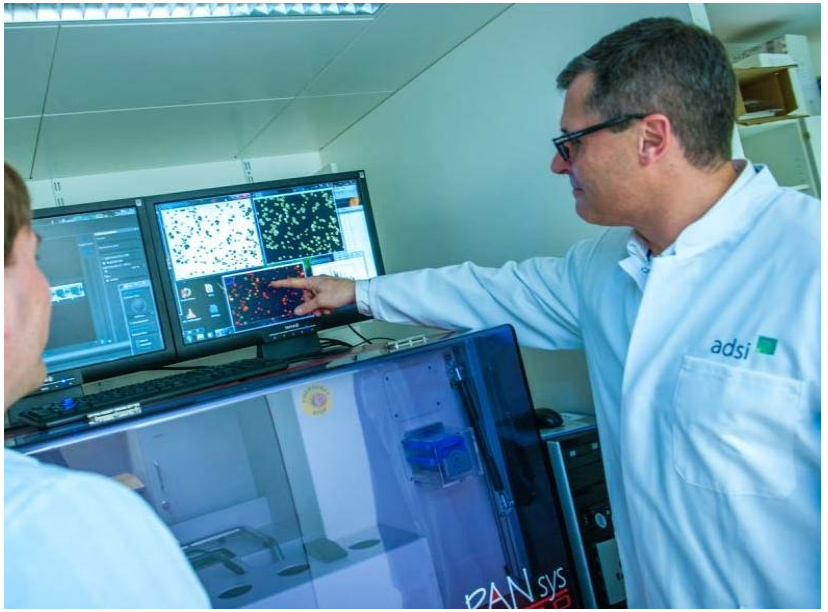


Trojer L. et al. ***Monolithic poly(p-methylstyrene-co-1,2-bis(p-vinylphenyl) ethane) capillary columns as novel styrene stationary phases for biopolymer separation.*** *Journal of Chromatography, A (2006), 1117(1), 56-66.*

Bonn, Alexander; Bonn, Guenther; Huck, Christian; Maerk, Bernhard; Sonderegger, Harald; Rainer, Mathias; Gjerde, Douglas T. ***Pipette tip containing particle-filled polymer monolith.*** *U.S. Pat. Appl. Publ. (2010)*

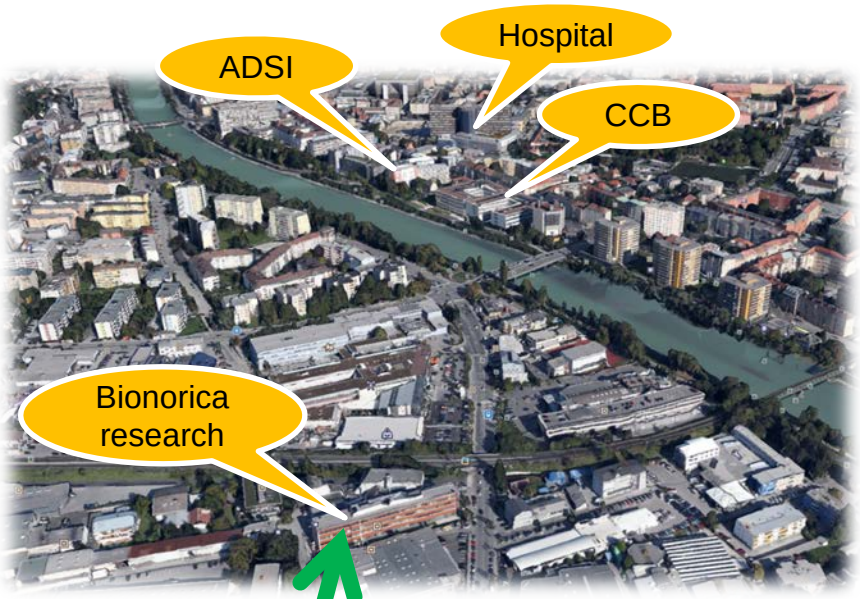
Developement of **co- culture testsystems**

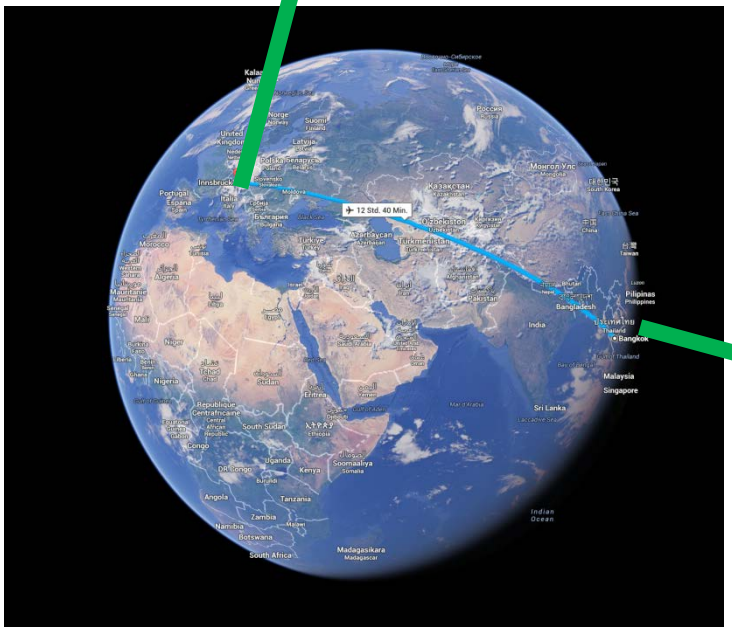
Guava easycyte 8HT Milipore
Cell sorter



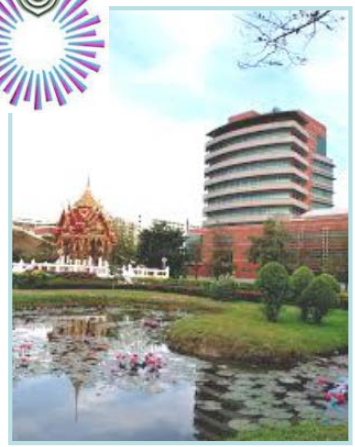
PanSys 4000
Fully automated cell culture system


Prof.Dr.Surapote Wongyai
UNIDO Expert in Herbal Medicinal Products Development, Trieste, Italy
Thai FDA Expert in ASEAN Harmonization of Traditional Medicine and Health Supplement (TMHS)
Founder Dean and Dean, Faculty of Oriental Medicine, Thailand





Association of Southeast Asian Nations (ASEAN), 10 Countries



Rangsit University
Faculty of Oriental Medicin
Bangkok, Thailand

Standardisation/Validation Traditional Medicine and Health Supplements Trainingcenter

Prof. Dr. Surapote

Trennung durch Fällung und Gravimetrie

Fällung

Ziel: Bildung einer neuen Phase

Parameter: Löslichkeit (KL, pH, Komplexbildung, Teilchengröße

Mitfällung, Adsorption

Filtration

Ziel: Phasentrennung

Anmerkung: gemeinsam mit Waschen

Waschen

Ziel: Entfernung der „Mutterlauge“

Parameter: Löslichkeit, Komplexbildung, Peptisation

Trocknen, Glühen

Ziel: stöchiometrische Zusammensetzung

Anmerkung: Analysenfunktion

Messung: Wägen

Ziel: Bestimmung des Messwertes

Anmerkung: Wägefehler

Berechnung und Bewertung

Ziel: Analysenwert,
Vertrauensbereich

Parameter: stöchiometrische
Faktoren

Gravimetrische Grundoperationen

- Lösen der Analysensubstanz
- Fällen eines Niederschlages
- Abtrennen des Niederschlages von der flüssigen Phase durch Filtrieren
- Auswaschen des Niedeschlages
- Trocknen und/oder Glühen bis zur Gewichtskonstanz
- Auswiegen der Wägeform des Niederschlages

Das Löslichkeitsprodukt

Kontakt von schwerlöslichen Verbindungen und H_2O

→Gleichgewicht ($v_{Auflösung} = v_{Wiederaussscheidung}$)

→Gleichgewicht d. festen Stoffes und d. gesättigten Lösung des festen Stoffes

$$AgCl_{(s)} \rightleftarrows Ag^{+}_{(aq)} + Cl^{-}_{(aq)}$$

$$K = \frac{[Ag^{+}] \bullet [Cl^{-}]}{[AgCl]}$$

Berechnung von K_L : $K \cdot [AgCl] = [Ag^+].[Cl^-]$ [ ] ... c

$K_L = [Ag^+].[Cl^-]$ c ... Ionenkonzentration

K_L oder L_p

$$Ba^{2+}_{aq} + SO_4^{2-}_{aq} \underset{c}{\overset{K_{rea}}{\rightleftharpoons}} BaSO_{4\ aq}$$

$\Delta G_f^{\theta}\ BaSO_{4\ aq}$	-722,874842 E_h
$\Delta G_f^{\theta}\ Ba^{2+}_{\ aq}$	-25,166661 E_h
$\Delta G_f^{\theta}\ SO_4^{2-}_{\ aq}$	-697,670227 E_h
$\Delta G_f^{\theta}\ BaSO_{4\ aq}$ reac	-0,037954 E_h
$\Delta G_f^{\theta}\ BaSO_{4\ aq}$ reac	-23,82 kcal/mol
K_{reac}	**$2{,}90 \cdot 10^{17}$**

$$Ag^{+}_{aq} + Cl^{-}_{aq} \underset{c}{\overset{K_{rea}}{\rightleftharpoons}} AgCl_{\ aq}$$

$\Delta G_f^{\theta}\ AgCl_{\ aq}$	-606,030719 E_h
$\Delta G_f^{\theta}\ Ag^{+}_{\ aq}$	-146,329850 E_h
$\Delta G_f^{\theta}\ Cl^{-}_{\ aq}$	-459,659021 E_h
$\Delta G_f^{\theta}\ AgCl_{\ aq}$ reac	-0,041848 E_h
$\Delta G_f^{\theta}\ AgCl_{\ aq}$ reac	-26,26 kcal/mol
K_{reac}	**$1{,}79 \cdot 10^{19}$**

Programm: Gaussian 09 C.01

Methode: Møller-Plesset Ströungstheorie (MP/2)

Basissatz: Karlsruhe double-ζ mit Polarisationsfunktionen
Small Core ECPs für Ag und Ba

Solvatisierung: Polarisierbares Kontinuumsmodell (PCM)

E_h: 627,5095 kcal/mol

R: 8,3145 J/mol K

T: 298,15 K

Oliver Lutz
17.10.2013

Fällungsreaktionen

Ionenprodukt:

- Ionenprodukt $< K_L$: Lösung nicht gesättigt

  → weitere Substanz kann gelöst werden

- Ionenprodukt $= K_L$: Lösung gesättigt

  Lösung im Gleichgewicht mit ungelöster Substanz

- Ionenprodukt $> K_L$: Lösung übersättigt

  → Fällung bis L erreicht ist

Löslichkeitsprodukte[a]

		pK_L
Ag	AgBr	12,4
	AgCl	10,0
	$AgCN^b$	11,4
	Ag_2CO_3	11,3
	Ag_2CrO_4	11,7
	AgI	16
	AgOH	7,7
	AgSCN	12
	Ag_2S	49
Al	$Al(OH)_3$	32,7
Ba	$BaCO_3$	8,16
	BaC_2O_4	6,77
	$BaCrO_4$	9,70
	$BaSO_4$	10
Ca	$CaCO_3$	7,92
	CaC_2O_4	8,07
	CaF_2	10,46
	$CaSO_4$	4,32
Cu	CuBr	7,4
	CuCl	6,0
	CuI	11,3
	$Cu(OH)_2$	19,8
	Cu_2S	46,7
	CuS	37–44
	CuSCN	10,8
Fe	$Fe(OH)_2$	13,5
	$Fe(OH)_3$	37,4
	FeS	18–21
Hg	Hg_2Cl_2	17,5
	HgS	52
K	$KClO_4$	2,05
	$K_2[PtCl_6]$	5,85

[a] aus [1]. Leider fehlt die Temperaturangabe; vermutlich beziehen sich die Werte auf 18–25 °C.
[b] $2\,[AgCN]_f \rightleftharpoons Ag^+ + [Ag(CN)_2]^-$

Löslichkeitsprodukte (Fortsetzung)

Mg	$MgCO_3$	3,7
	MgC_2O_4	4,1
	MgF_2	8,2
	$Mg(OH)_2$	10,9
	$MgNH_4PO_4$	12,6
Na	$NaHCO_3$	2,1
Pb	$PbCl_2$	4,8
	$PbCO_3$	13,5
	$PbCrO_4$	13,8
	PbF_2	7,5
	$Pb(OH)_2$	15,6
	$PbSO_4$	8,0
	PbS	28–29
Sn	$Sn(OH)_2$	15,3[a]
	$Sn(OH)_4$	56
	SnS	28
Zn	$ZnCO_3$	10,2
	$Zn(OH)_2$	16,8
	ZnS	23–25

[a] Originalangabe 25,3 [1], wahrscheinlich Druckfehler (vgl. [2]).

Löslichkeit L

- Löslichkeit nennt man die maximale Menge eines Stoffes, die ein Lösungsmittel bei einer bestimmten Temperatur aufnehmen kann.

- Angabe der Löslichkeit:

  Mol/l (Molarität, molare Löslichkeit)

  Md/ 1000g Lösemittel (Molalität)

Literatur: Analytische Chemie

Latscha, Klein

Springer Verlag

Tabelle 11. Löslichkeit einiger Salze in Abhängigkeit von der Temperatur in g/100 g Lösung

Verbindung	0°C	20°C	30°C	40°C	100°C
NaCl	26,28	26,39	26,51	26,68	28,15
Na_2SO_4	4,5	16,1	28,8	32,5	29,9
Na_2CO_3	6,6	17,8	29,0	33,2	31,1
$MgSO_4$	20,5	26,2	29,0	31,3	40,6
KNO_3	11,6	24,1	31,5	46,2	71,1
$AgNO_3$	53,5	68,3	73,8	77,0	90,1
AgCl		$1{,}5 \cdot 10^{-4}$			$2{,}2 \cdot 10^{-3}$
AgHr		$1{,}3 \cdot 10^{-5}$			$3{,}7 \cdot 10^{-4}$
$Ca(OH)_2$		$1{,}2 \cdot 10^{-1}$			$6{,}0 \cdot 10^{-2}$
$Mg(OH)_2$		$8{,}5 \cdot 10^{-4}$			$4{,}0 \cdot 10^{-3}$
$CuSO_4$		$2{,}0 \cdot 10^{-1}$			$6{,}5 \cdot 10^{-2}$
$SrSO_4$		$1{,}2 \cdot 10^{-2}$			$1{,}8 \cdot 10^{-2}$
$BaSO_4$		$2{,}4 \cdot 10^{-4}$			$3{,}9 \cdot 10^{-4}$
$PbSO_4$		$4{,}4 \cdot 10^{-3}$			$6{,}0 \cdot 10^{-3}$

Tabelle 10. (aus dem Europäischen Arzneibuch EuAB)

Bezeichnung	Ungefähre Anzahl Volumenteile Lösemittel für 1 Masssenteil Substanz			
sehr leicht löslich	weniger als	1 Teil		
leicht löslich	von	1 Teil	bis	10 Teile
löslich	über	10 Teile	bis	30 Teile
wenig löslich	über	30 Teile	bis	100 Teile
schwer löslich	über	100 Teile	bis	1 000 Teile
sehr schwer löslich	über	1 000 Teile	bis	10 000 Teile
praktisch unlöslich	mehr als	10 000 Teile		

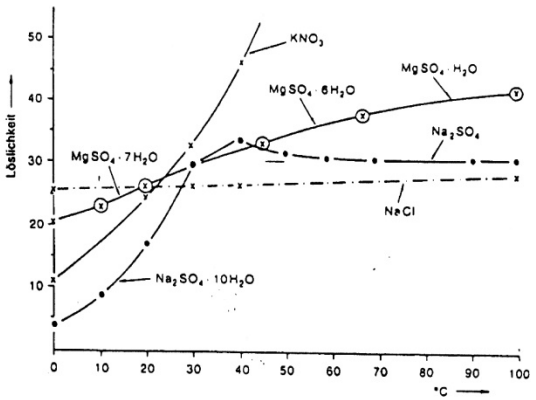


Abb. 21. Temperaturabhängigkeit der Löslichkeit einiger Salze. L = g/100 g Lösung

Anmerkung: Ca-Citrat ist ausnahmsweise in kaltem Wasser leicht löslich, aber in heißem schwer löslich

Tab. 1 Wichtige gravimetrische Verfahren [2, 5]

Ion	Fällungsform	Wägeform	Störungen
K^+	$K[B(C_6H_5)_4]$ [a]	$K[B(C_6H_5)_4]$	NH_4^+, Rb^+, Cs^+
Ag^+	AgCl	AgCl	Hg_2^{2+}
$M = Mg^{2+}, Zn^{2+}$	$MNH_4PO_4 \cdot 6\,H_2O$	$M_2P_2O_7$	alle Metalle außer Na
	$M(oxinat)_2 \cdot 2\,H_2O$	$M(oxinat)_2$	viele Metalle
Ca^{2+}	$CaC_2O_4 \cdot H_2O$	$CaCO_3$ (CaO)	alle Metalle außer Na Mg
$M = Ba^{2+}, Pb^{2+}$	MSO_4, $MCrO_4$	MSO_4, $MCrO_4$	(Ca, Sr)
Ni^{2+}	Ni(diacetyldioximat)$_2$	Ni(diacetyldioximat)$_2$	—
$M = Al^{3+}, Fe^{3+}$	$M(OH)_3 \cdot$ aq	M_2O_3	Schwermetalle
Si (IV)	$SiO_2 \cdot$ aq	SiO_2	Sn
Sn (IV)	$SnO_2 \cdot$ aq	SnO_2	Si, Sb
F^-	PbClF	PbClF	SO_4^{2-}, PO_4^{3-}
Cl^-	AgCl	AgCl	Br^-, I^-, CN^-, SCN^-
SO_4^{2-}	$BaSO_4$	$BaSO_4$	NO_3^-, ClO_3^-, PO_4^{3-}
PO_4^{3-}	$MgNH_4PO_4 \cdot 6\,H_2O$	$Mg_2P_2O_7$	CO_3^{2-}
	$(NH_4)_3[P(Mo_{12}O_{40})] \cdot$ aq [b]	$(NH_4)_3[P(Mo_{12}O_{40})]$ (300 °C)	(AsO_4^{3-})

[a] Die Kaliumbestimmung als $KClO_4$ ($K_L = 10^{-2}$) ist viel zu ungenau und sollte endlich aus den Lehrbüchern verschwinden.

[b] Statt NH_3 wird auch Oxin als Base empfohlen [19].

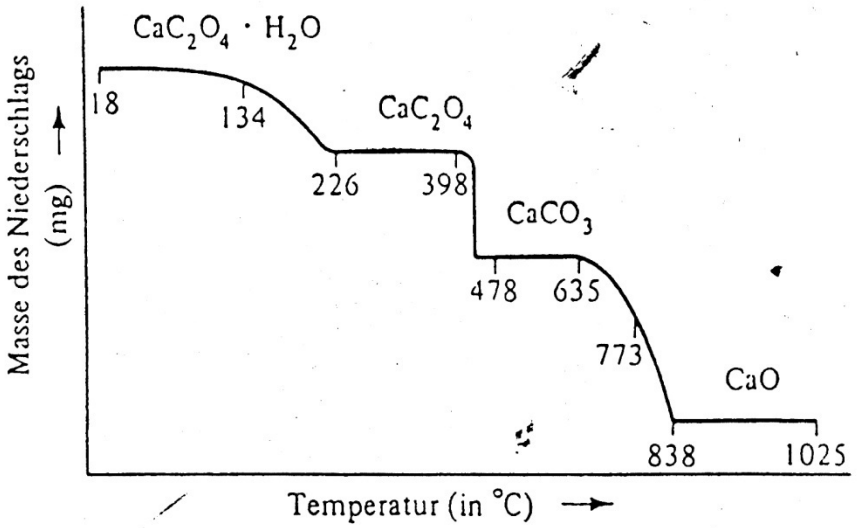


Bild 4-3 Thermogravimetrische Messung: Gewichtsverlust von $CaC_2O_4 \cdot H_2O$ beim Erhitzen. [Nach Cl. Duval, *Inorganic Thermogravimetric Analysis* (Elsevier, Amsterdam 1953) S. 36]

Gleichioniger Zusatz L_c

$$A + B \rightleftarrows C$$

Anteil Dissoziationsprodukte gering

Löslichkeit richtet sich nach dem Ion mit der geringeren Konzentration

$[A^+] > [B^-]$

$$L_c = [B^-] = \frac{K_L}{[A^+]} = \frac{K_L}{C^0{}_{A^+} + [A^+]} \sim \frac{K_L}{C^0 A^+}$$

$$K_L = [A^+] \bullet [B^-]$$

$$[B^-] = \frac{K_L}{[A^+]}$$

$C^0{}_A+$ ... Zugesetzte Menge A^+

$[A^+]'$ ... der durch Dissoziation entstandene Anteil

$(C^0{}_A+) >> [A^+]'$

$L_c < L$; Die Löslichkeit bei gleichionigem Zusatz nimmt ab

Beispiel: AgCl

$$C^0 Ag^+ = 10^{-2}\, mol/l$$

$$L_c = \frac{K_L}{C^0 Ag} = \frac{10^{-10}}{10^{-2}} = 10^{-8}\, mol/l$$

Fremdioner Zusatz L_a

Aktivitätskoeffizient = f (Ionenstärke)

⇨ Änderung der Löslichkeit eines Stoffes bei Fremdsalzzusatz

$K_L = a_{A^+} . a_{B^-} = [A^+] [B^-] . f_{A^+} . f_{B^-}$

$a = c.f$

$f = 1$

a ... Aktivität

c... Konzentration

f ... Aktivitätskoeff.

Löslichkeit bezieht sich auf stöchiometrische Konzentrationen

–

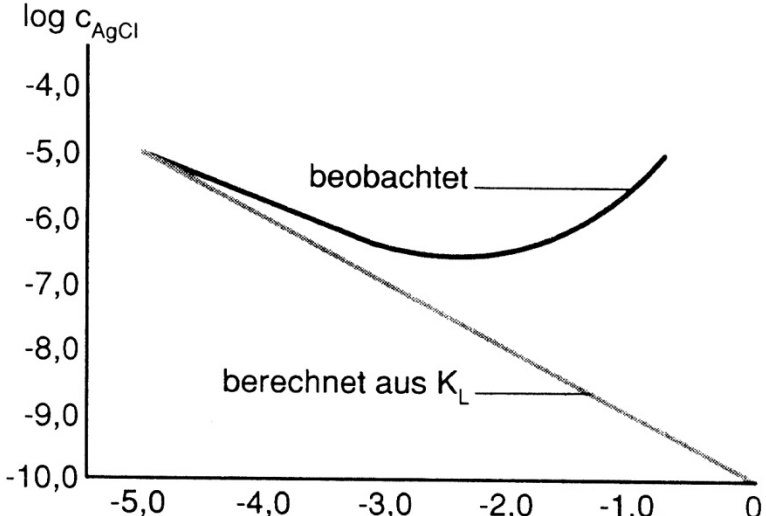
$$\rightarrow \quad K_L = L_a^2 \bullet f_{A^+} \bullet F_{B^-}$$

$$L_a = \sqrt{\frac{K_L}{f_{A^+} \bullet f_{B^-}}}$$

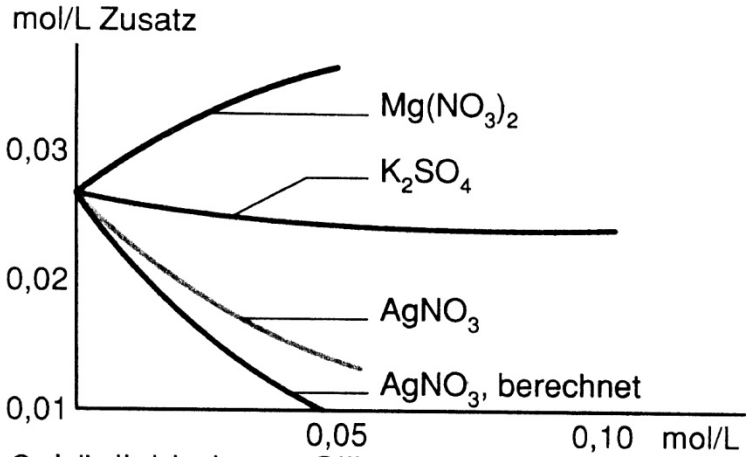
$$f < 1$$

$$L_a > L$$

→ Löslichkeit nimmt bei Anwesenheit von Fremdionen zu → Alkaliaufschluss



1. Löslichkeit von Silberchlorid



2. Löslichkeit von Silbersulfat

C. Beeinflußung der Löslichkeit

NIEDERSCHLAGSBILDUNG – MECHANISMUS

FÄLLEN

THERMODYNAMISCH = FÄLLUNG

PHASENBILDUNG

FÄLLUNG

INDUKTIONSPERIODE

(KRISTALLKEIME 10^9–10^{12} KEIME pro Mol

KEIMBILDNER

(IMPFKRISTALL, VERUNREINIGUNG, GLASWAND)

WIRKUNG: - VERRINGERUNG DER GRENZFLÄCHENSPANNUNG

- VERGRÖßERUNG DES TEILCHENRADIUS

KOLLOIDBILDUNG

$As_2O_3 + H_2S \not\rightarrow$ kein Niederschlag

trübe, gelbe Lösung As_2S_3

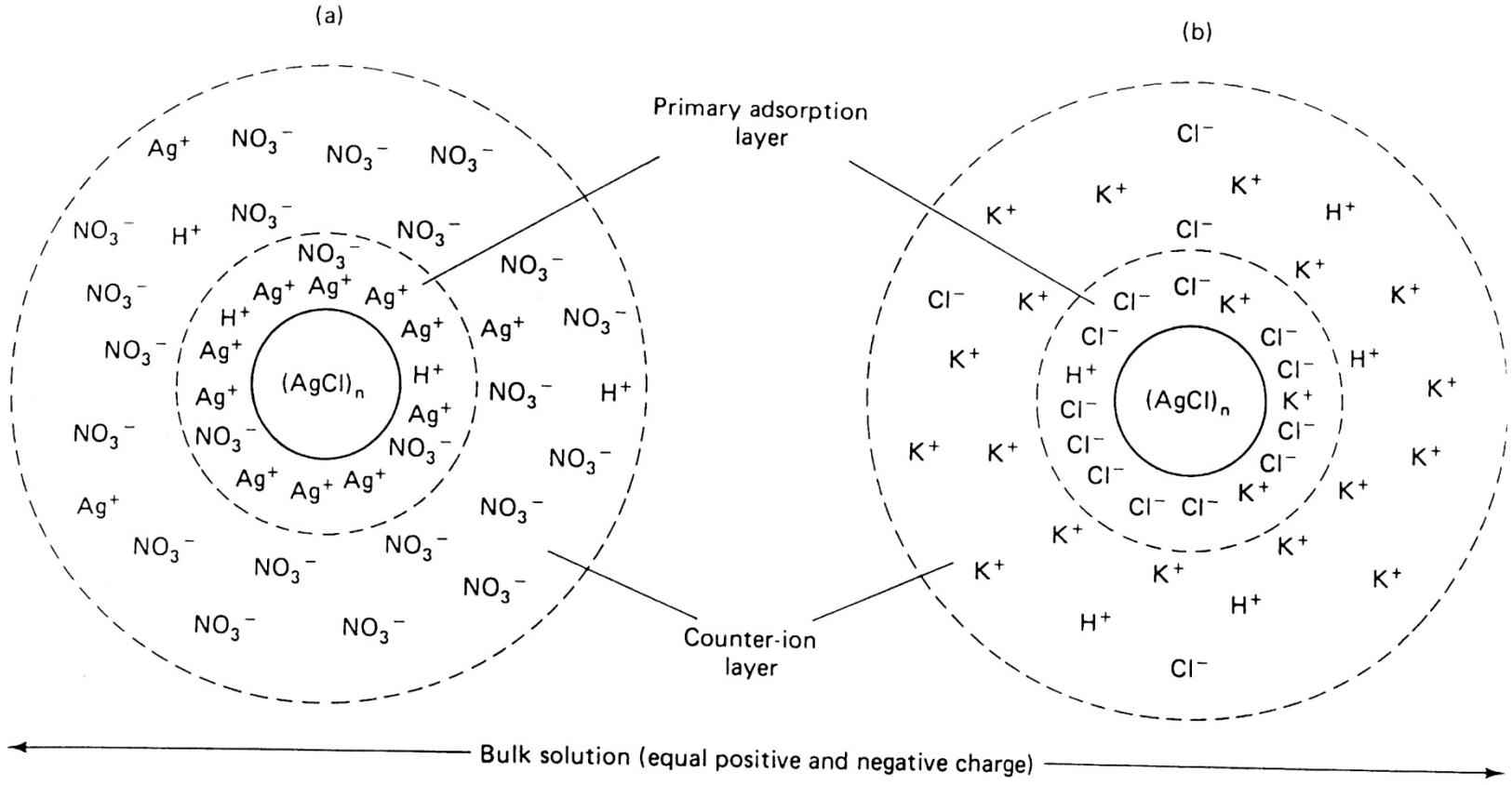
	TEILCHENGRÖßE [cm]
SUSPENSION	$\gg 10^{-5}$
KOLLOID	$10^{-5} - 10^{-7}$
LÖSUNG	$\leq 10^{-7}$

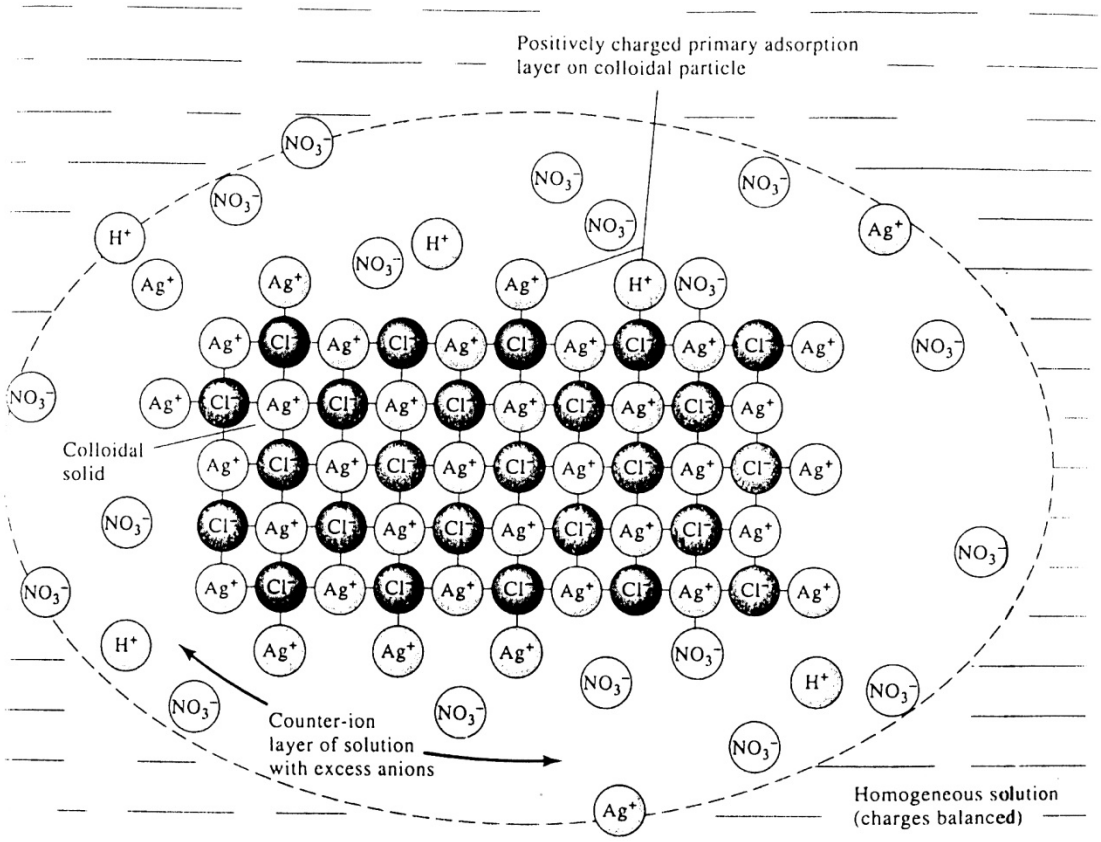
ABTRENNUNG: ZENTRIFUGIEREN
DIALYSE (Diffusion durch Membranen)

NICHT DURCH FILTER!

FÄLLUNG ODER KOAGULATION
DURCH SALZZUSATZ ("AUSSALZEN")

KOLLOID (SOL) $\underset{\text{Peptisation}}{\overset{\text{Koagulation}}{\rightleftharpoons}}$ Niederschlag (gel)


(a)
(b)
Primary adsorption layer
Counter-ion layer
$(AgCl)_n$
$(AgCl)_n$
Bulk solution (equal positive and negative charge)


Positively charged primary adsorption layer on colloidal particle
Colloidal solid
Counter-ion layer of solution with excess anions
Homogeneous solution (charges balanced)

MITFÄLLUNG

- EIGENIONEN
- FREMDIONEN

- ADSORPTION

  bei Niederschlägen mit aktiver Oberfläche

- OKKLUSION

  Einschluß von Fremdteilchen im Inneren des Kristalls

- INKLUSION (Mischkristallbildung)

  Fremdionen werden direkt in das Kristallgitter eingebaut

KOMPLEXBILDUNG

Niederschläge können durch Komplexbildung ganz oder teilweise wieder in Lösung gehen

$$[AgCl]_f + Cl^- \rightarrow [AgCl_2]^-_{gel}$$

ALTERUNG - REIFUNG

physikalisch - chemische Veränderung
nach Fällung als Niederschlag

- REKRISTALLISATION

instabile Kristallbezirke gehen in Lösung

- TEMPERUNG (bei höherer Temp.)

= Ordnungsprozeß durch Diffusion
innerhalb eines Kristalls
⇒ Fehlstellen werden beseitigt

- CHEMISCHE ALTERUNG

Modifikationsänderung d. Niederschlags

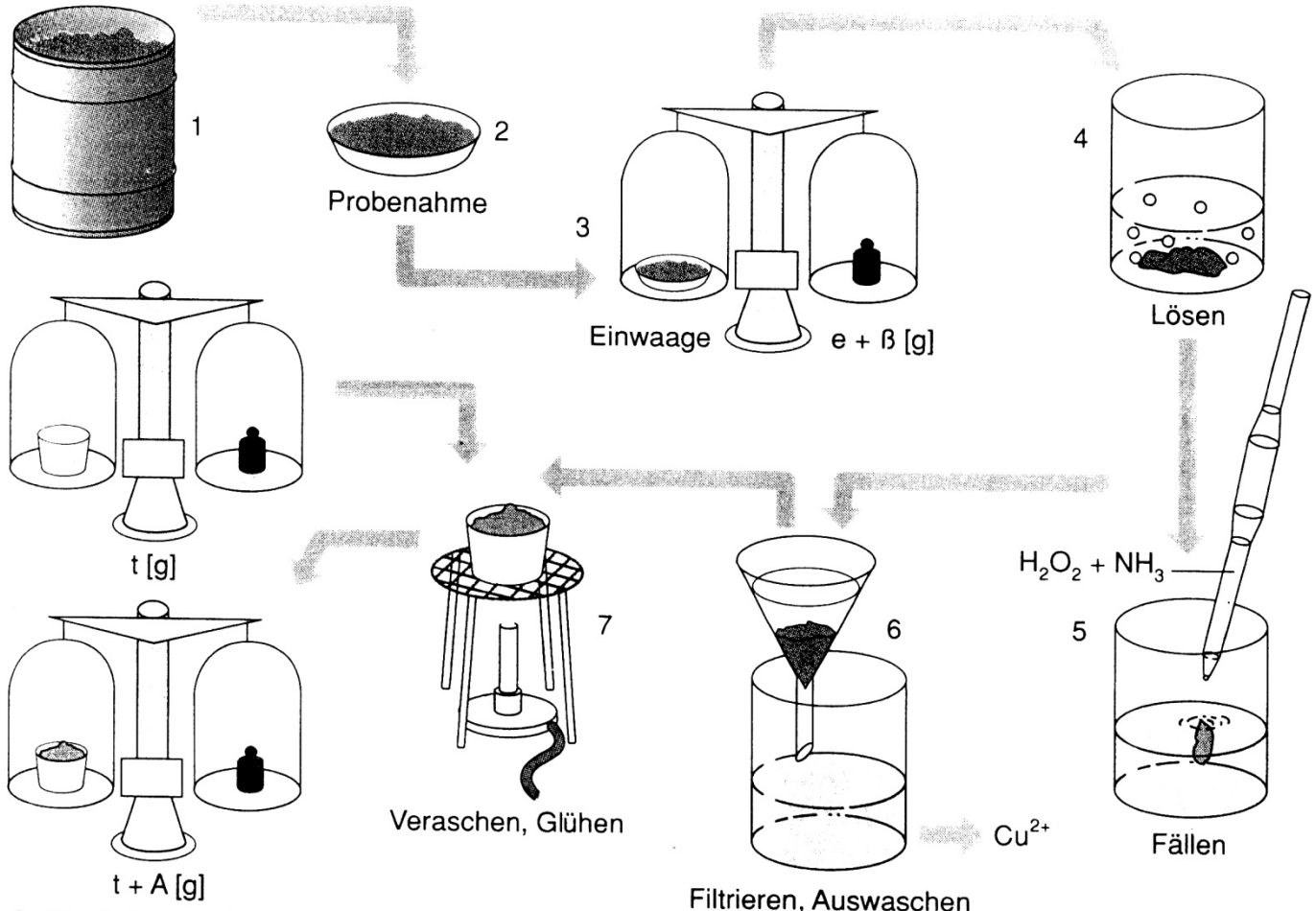
$[HgS] \longrightarrow [HgS]$

SCHWARZ ROT

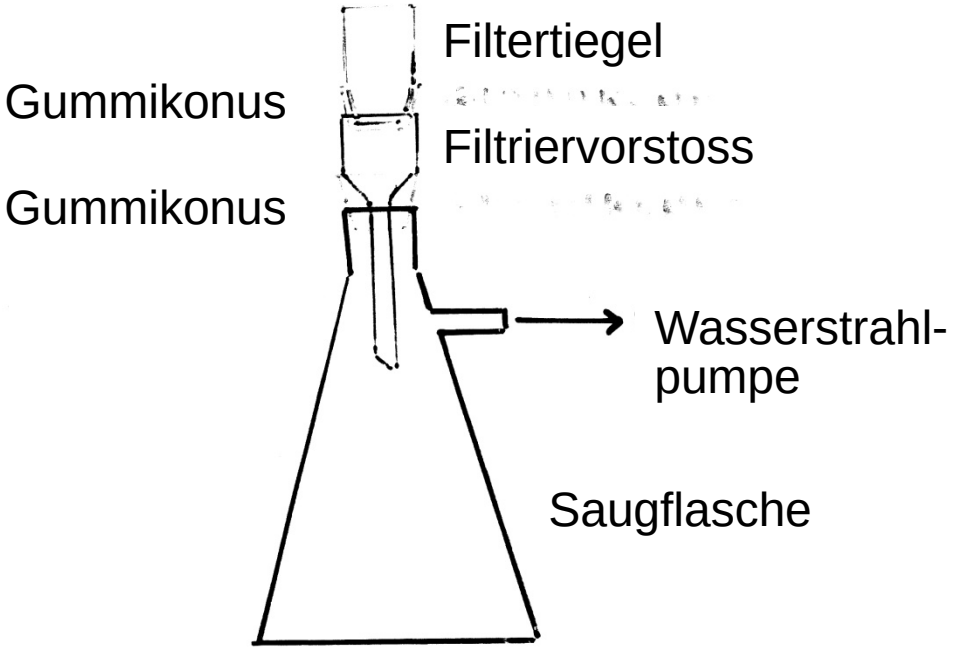
NACHFÄLLUNG

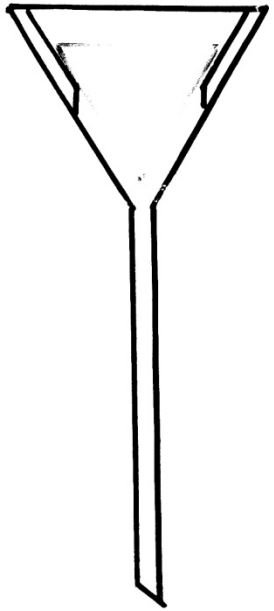
VORGÄNGE, DIE ZUR ÄNDERUNG DER ZUSAMMENSETZUNG DES NIEDERSCHLAGS FÜHREN

MgC_2O_4 / CaC_2O_4



A. Verfahren einer gravimetrischen Analyse


Filtertiegel
Gummikonus
Filtriervorstoss
Gummikonus
Wasserstrahl-
pumpe
Saugflasche



Gravimetrische Verfahren

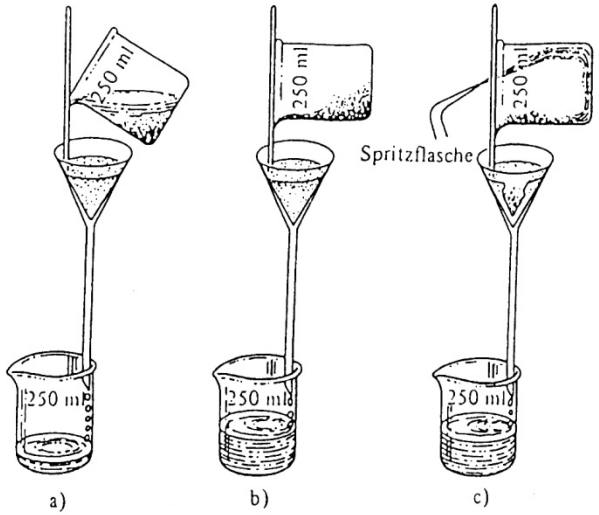


Bild 28-7 Dekantieren und Überführen eines Niederschlags in ein Filter.

a) Dekantieren, Hauptmenge des Niederschlags verbleibt im Becherglas.
b) Entlang eines Glasrührstabs wird die Hauptmenge des Niederschlags abgegossen.
c) Mit der Spritzflasche wird restlicher Niederschlag in den Trichter gespült (dazu kann auch ein Gummiwischer, oft scherzhaft „Atomwischer" genannt, erforderlich sein).

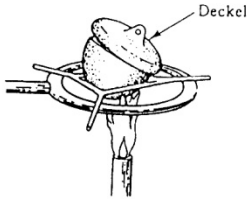


Bild 28-8
Veraschen eines Filters im Tiegel

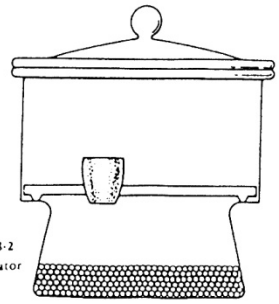
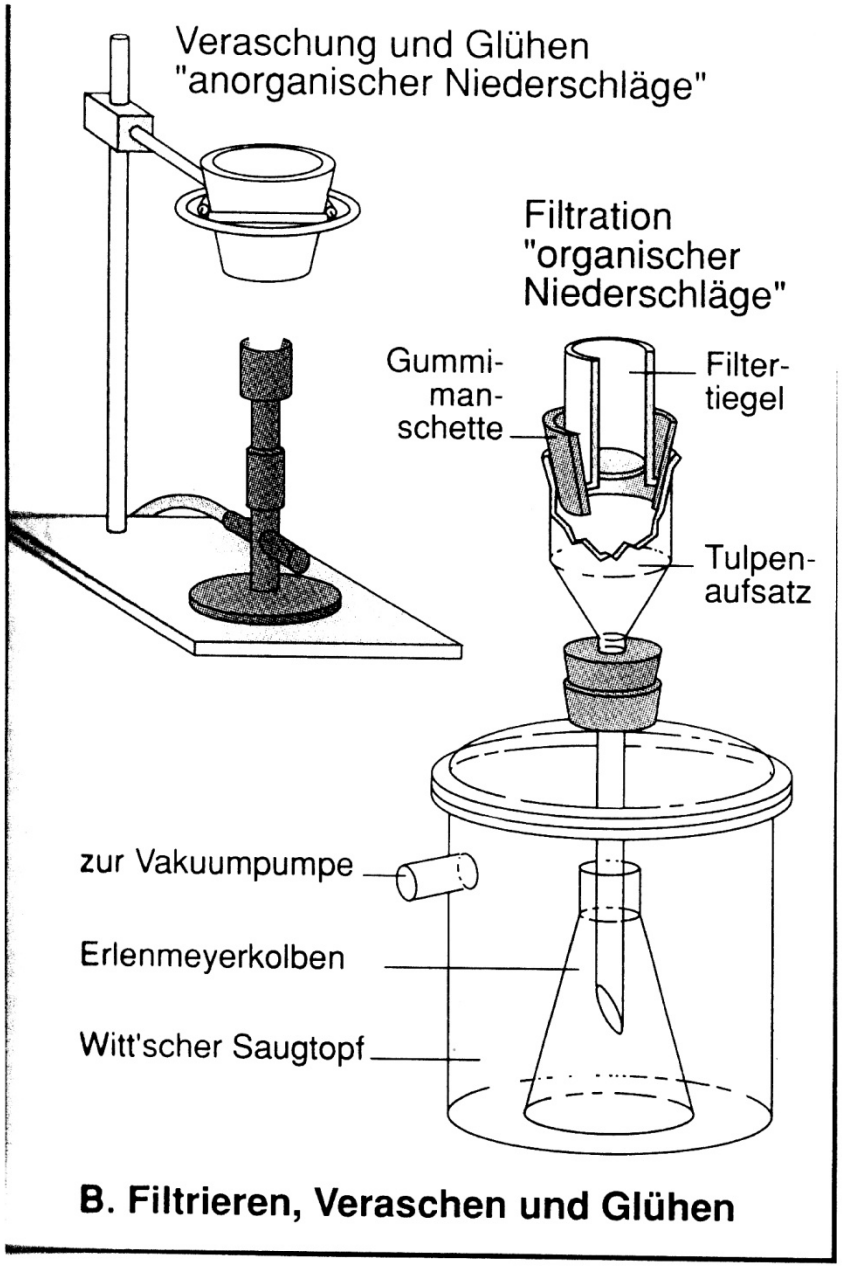


Bild 28-2
Exsiccator



B. Filtrieren, Veraschen und Glühen

Spezifikation von Papierfiltern und Filtertiegeln

Papierfilter Art	Typenbez.	Anwendung, Eigenschaft	Filtertiegel Glas	Porzellan
weich	Schwarzband	großporig grobkrist. Niederschlag	D 1	A 5
mittel	Weißband		D 3	A 3
hart	Blauband, Rotband	feinporig feinkrist. Niederschlag	D 5	A 1

Gebräuchliche Trockenmittel (nach [18])

Trockenmittel	Partialdruck[a] (mbar)	Anwendbar für	Nicht geeignet für
$CaCl_2$ gekörnt	0,20	Neutrale und saure Feststoffe (Gase)	NH_3, Br_2, HBr, HF; Alkohole, Amine
BaO	$1{,}6 \cdot 10^{-4}$	Anorg. und organ. Basen (NH_3, Amine), Alkohole	F_2, NO, Säuren
MgO	0,11		
$Al_2O_3 \cdot aq$	$4{,}1 \cdot 10^{-3}$		
NaOH geschmolzen	0,21	NH_3, Alkohole, organ. Basen	O_3, F_2, Säuren
KOH geschmolzen	$2{,}8 \cdot 10^{-3}$		
H_2SO_4 konz.	$2{,}8 \cdot 10^{-3}$	Neutrale und saure Verbindungen; O_2, N_2, CO, CH_4, Halogene, HCl, SO_2	HBr, HI, HF, NH_3, H_2S, PH_3, NO, NO_2, C_2H_2
P_4O_{10}	$3{,}4 \cdot 10^{-5}$	O_2, CO, C_2H_2, CS_2, CCl_4, Stickoxide	Halogene, HHal, NH_3, H_2S, Ether
Silicagel	$3{,}0 \cdot 10^{-2}$	universell	NH_3, HF, Halogene

[a] Wasserdampf-Partialdruck bei 25 °C (Reines Wasser: 31,3 mbar)

FILTER

ART	KENNUNG	POREN µm	GESCHW.	T	G	VERWENDUNG	BEISPIELE
PAPIER	BLAU	2-3	langsam	-	+	feinteilige N.	$BaSO_4$, $TiO_2.aq$
	WEISS	6-7	mittel	-	+	krist., körnige N.	$CaC_2O_4.aq$
	SCHWARZ	7-8	rasch	-	+	gelartige N.	$Fe(OH)_3$, $Al(OH)_3$
PORZELLAN	P1, A1	5-6	langsam	+	+	feinstkörnig	$BaSO_4$
	P2, A2	7-8	mittel	+	+	universell	$AgCl$, $Mg(NH_4)PO_4$
	P3, A3	10	rasch	+	+	grobkristallin	$PbSO_4$
GLAS	(G, D) 0	230		+	-		
	1	110	rasch	+	-	grobkristallin	
	2	50		+	-		
	3	15-40	mittel	+	-	feinkristallin	$Ni(DAD)_2$, $Mg(Ox)_2$
	4	5-15	langsam	+	-	sehr feint.	
	5	3-4					

Waagen

Waagentyp	Maximalgewicht	Auflösung
Analytische Waagen	150-200g	± 0,1mg
Semimicrowaagen	75-100g	± 0,01mg
Microwaagen	10-30g	± 0,001mg

➢Empfindlichkeit

Ausschlag bei einer Auflage von 0,1mg. Die Empfindlichkeit sinkt mit zunehmendem Gewicht. Die Grenze der Auflage ist erreicht, wenn die Waage nur mehr 40 % der Maximalempfindlichkeit hat.

➢Referenz

Urkilogramm in Paris aus Pt/Ir (1887)

➢Bauarten von Waagen

Oberschalige-, Unterschalige-, Elektronische, Digitalwaagen

➢Dämpfung

Luft-, magnetische Dämpfung

Fällungsreaktionen in der analytischen Chemie

- Sulfatfällung

  $SO_4^{2-} + Ba^{2+} \rightleftharpoons [BaSO_4)_f$ (Mitfällung!)

- Chloridfällung

  $Cl^- + Ag^+ \rightleftharpoons [AgCl]_f$

  lichtempfindlich (Ag!)

  rasch filtrieren, trocknen

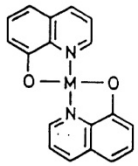
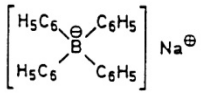
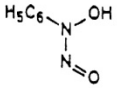
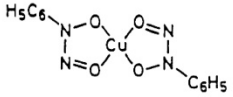
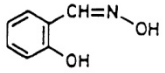
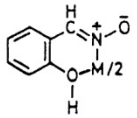
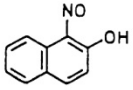
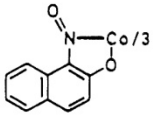
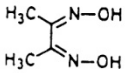
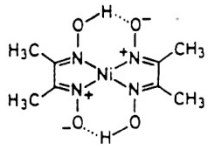
- Hydroxidfällung

  $M^{3+} + 3OH^- \rightleftharpoons [M(OH)_3]_f \quad ?^T \rightarrow M_2O_3$

- Phosphatfällung

  $M^{2+} + NH_4^+ + PO_4^{3-} \rightleftharpoons [MNH_4PO_4]_f$

Organische Fällungsreagenzien

Organische Verbindung	Komplex	Anwendung
		Mg^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Al^{3+}, Fe^{3+}, Sb^{3+} u.a.
8-Hydroxychinolin (Oxin)	M(II)-oxinat	
		K^+ (NH_4^+, Rb^+, Cs^+)
Natriumtetraphenylborat (Kalignost)		
		außer Cu noch für zahlreiche andere Metalle verwendbar
N-Nitrosophenylhydroxylamin (Kupferron)		
		Cu^{2+}, Ni^{2+}, Pb^{2+}, Bi^{3+}, Fe^{3+} u.a.
Salicylaldoxim	M(II)-Komplex	
		spezifisches Reagenz für Co(III)
α-Nitroso-β-naphthol	Co(III)-Komplex	
		spezifisches Reagenz für Ni(II)
Diacetyldioxim (Dimethylglyoxim)	Ni(II)-Komplex	

Anwendungsbereich - Gravimetrie

mg-Bereich

Vorteile:

Geringer app. Aufwand

Hohe Präzision

Nachteil:

Hoher Zeitaufwand

Keine Serienanalysen

Kaum automatisierbar

$$m(\text{gesuchte Substanz}) = \frac{M(\text{gesuchte Substanz})}{M(\text{Wägeform})} \cdot m(\text{Niederschlag}) .$$
$$= f_g \cdot m(\text{Niederschlag})$$

$$\text{Prozentgehalt(gesuchte Substanz)} = \frac{m(\text{gesuchte Substanz})}{m(\text{Probe})} \cdot 100$$

$$\text{Prozentgehalt(gesuchte Substanz)} = \frac{100 \cdot f_g \cdot m(\text{Auswaage})}{m(\text{Probe})}$$

m = Masse Gewicht f_g.... gravimetrischer Faktor

M = molare Masse g/ml

(Atomgewicht alt

Molgewicht)

3,1. Analytische und stöchiometrische

Gesucht	Gegeben	Faktor	lg
K_2O	K	1,2046	08 084
	$K(C_6H_5)_4B$	0,1314	11 874
	KCl	0,6317	80 056
	$KClO_4$	0,3399	53 142
	K_2PtCl_6 empirisch	0,1931	28 570
	K_2SO_4	0,5406	73 285
	$BaSO_4$	0,4036	60 597
	Pt empirisch	0,4810	68 217
	Pt empirisch [1]	0,4809	68 203
$K_2O \cdot Al_2O_3 \cdot 6SiO_2$	Al_2O_3	5,460	73 717
	KCl	3,733	57 210
	K_2O	5,910	77 153
	K_2SO_4	3,194	50 440
K_2SO_4	$K(C_6H_5)_4B$	0,2432	38 588
	$BaSO_4$	0,7466	87 311
	KCl	1,1687	06 770
	K_2O	1,850	26 713
La	La_2O_3	0,8527	93 079

[1]) Bei Gegenwart von Sulfat nach Finkeners Methode

Der Bariumgehalt einer Substanzprobe ergibt sich aus dem Verhältnis des gefundenen Wertes und der Einwaage.

Einwaage: 0,8450 g

$$w_{Ba} = \frac{0{,}2034\ \text{g}}{0{,}8450\ \text{g}} = 0{,}241 \triangleq 24{,}1\ \%$$

b. Auswaage an Fe_2O_3: 0,2345 g. Gesucht: m_{Fe}

$$m_{Fe} = \frac{2\,M(Fe)}{M(Fe_2O_3)} \cdot m(Fe_2O_3) = 0{,}6994 \cdot 0{,}2345\ \text{g} = 0{,}1640\ \text{g}$$

In einigen Fällen wird statt des exakten gravimetrischen Faktors ein *empirischer* Faktor verwendet, wenn der Niederschlag signifikant von der stöchiometrischen Zusammensetzung abweicht. Solche empirischen Werte sind allerdings mit gewisser Vorsicht zu betrachten.