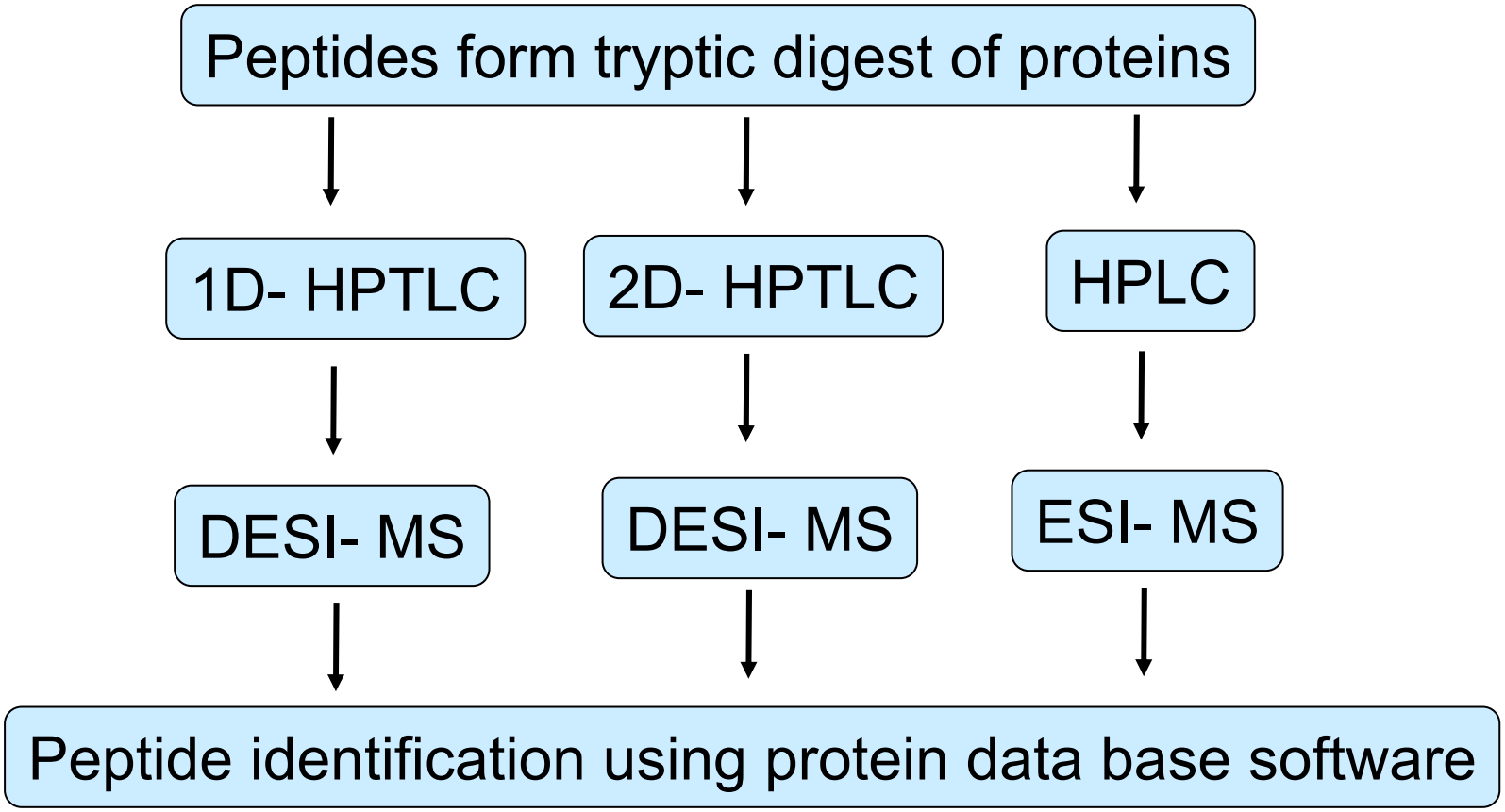
International Symposium for Thin Layer Chromatography

Helsinki, 11th – 13th June 2008

HPTLC/ DESI- MS for the analysis of proteins and peptides

Michael Schulz
PLS R&D LSS SIS

HPTLC/ DESI- MS of peptides from tryptic digest



HPTLC/ DESI- MS of peptides from tryptic digest

Tryptic digest, HPTLC and staining performed by Merck KGaA

HPTLC/ DESI- MS and HPLC/ ESI- MS performed by Organic and Biological Mass Spectrometry Group, Chemical Science Division, Oak Ridge National Laboratory, Oak Ridge

Lit.: Using HPTLC/ DESI- MS for peptide identification in 1D separations of tryptic protein digests, Anal Bioanal Chem (2008) 391: 317-324

Experimental / HPTLC

Stationary phases:

ProteoChrom® HPTLC Silica gel F254s, from Merck KGaA
Format 20x10 cm
Layer thickness 100 µm
Special binder composition

ProteoChrom® HPTLC Cellulose, from Merck KGaA
Format 10x10 cm
Layer thickness 100 µm
High layer density

Mobile phases:

2-butanol/pyridine/acetic acid/ water (30/20/6/24) (v/v/v/v) and
2-butanol/pyridine/ammonia/ water (39/34/10/26) (v/v/v/v)

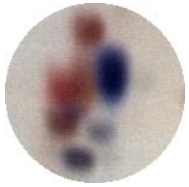
Instruments:

ATS4, Normal flat bottom chamber, TLC plate heater and DigiStore 2 from CAMAG (Muttenz, Switzerland)
DS 20 from DESAGA (Nürnbrecht, Germany)

Experimental / HPTLC

Staining:

Ninhydrin: The plate is sprayed with ninhydrin solution and heated up to 120°C for 2 min



ProteoChrom® Color: The sheet is spayed with ProteoChrom® Color Reagent. After drying at room temperature the sheet is sprayed with ninhydrin solution and heated up to 120°C for 1-2 min

Experimental / HPLC

Instrument:	Agilent 1100 Series Capillary LC System
Column:	Acclaim PepMap 100 C18 column (1 mm x 150 mm; 3 µm particle size; Applied Biosystems)
Mobile phase:	water (0.1% formic acid) (A) and 80% acetonitrile/20% water (0.1% formic acid) (B). (Gradient elution: 0 min with 96% A : 4% B; 30 min to 80% A : 20 % B; 20 min 80% A : 20 % B).

Experimental / MS

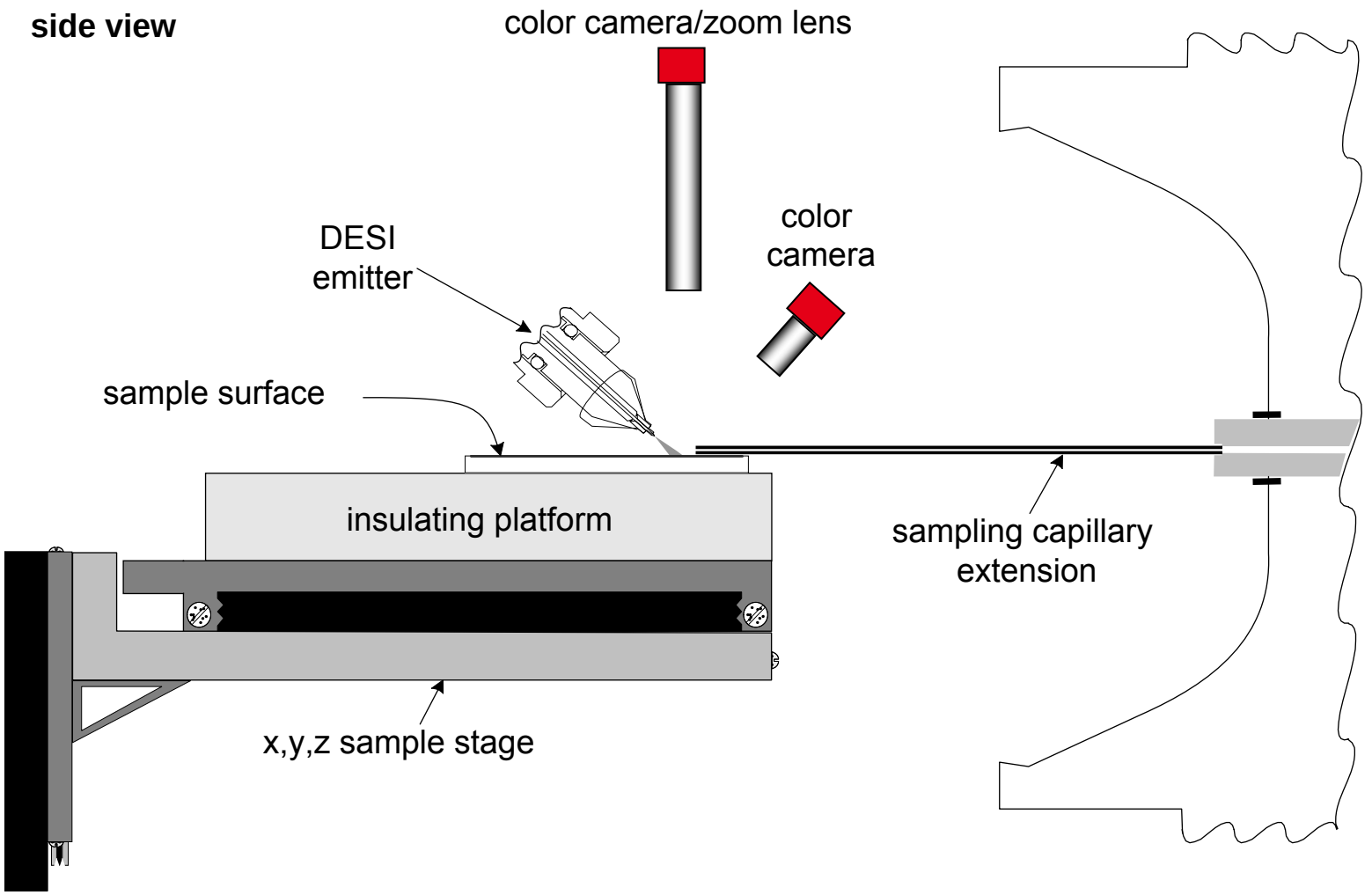
Instruments

MS: LCQ Deca ion trap or
LTQFT Ultra Hybrid Mass Spectrometer (Thermo Scientific, San Jose, CA, USA)
DESI Spray emitter: MicroIonSpray II (MDS Sciex, Concord, Ontario, Canada)

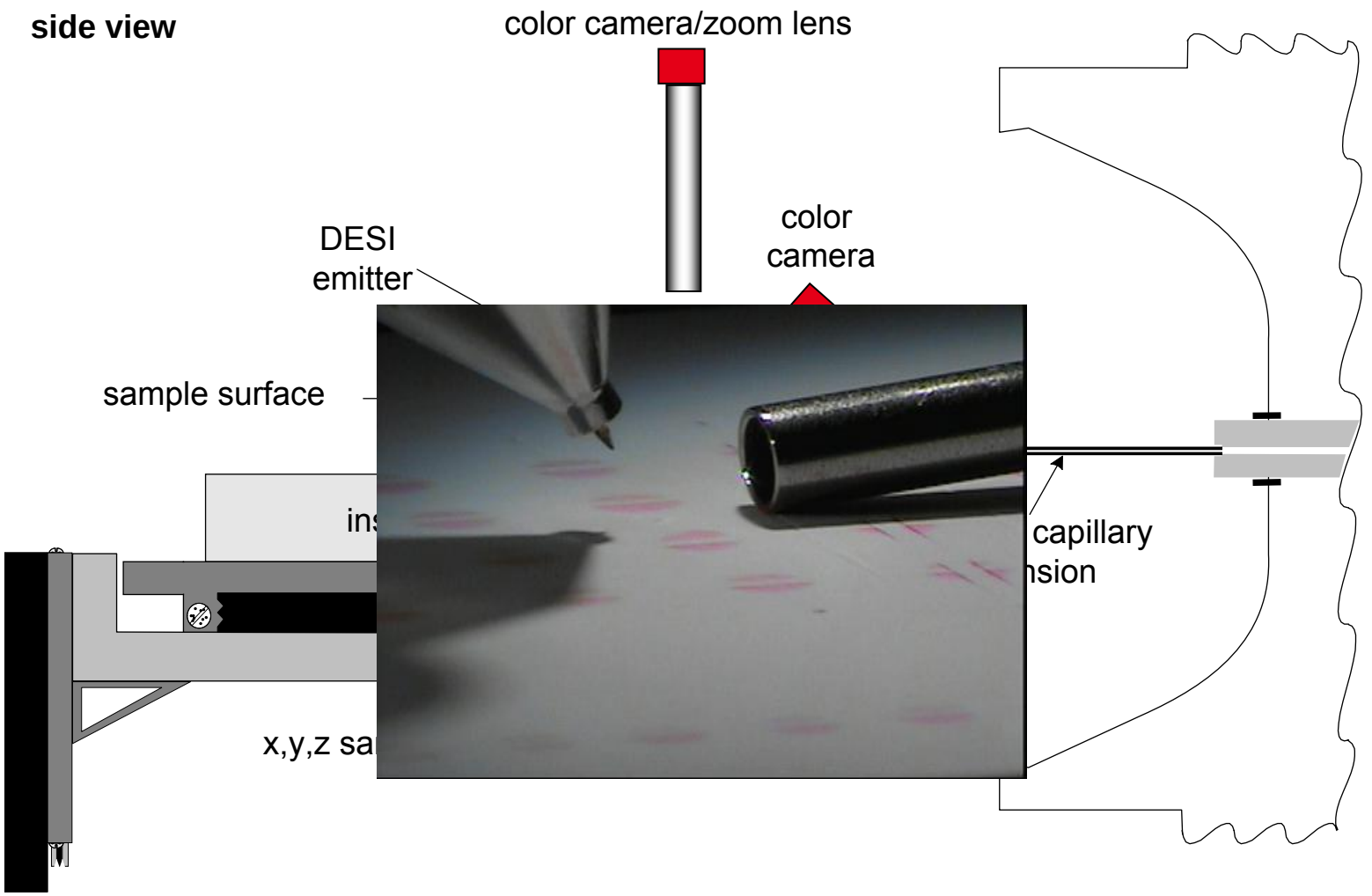
DESI conditions

Solvent: H_2O
Solvent flow rate: 5 µL/min
Spray voltage: 4 kV
Gas flow (N_2): 1,7 l/h
Scan rate: 100 µm/s
AGC on

Experimental / MS



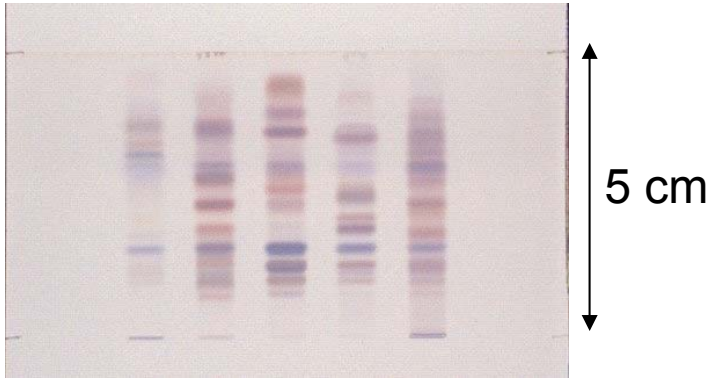
Experimental / MS



1D- HPTLC/ DESI- MS

ProteoChrom® Cellulose

Lys Myo Cyt Cas BSA

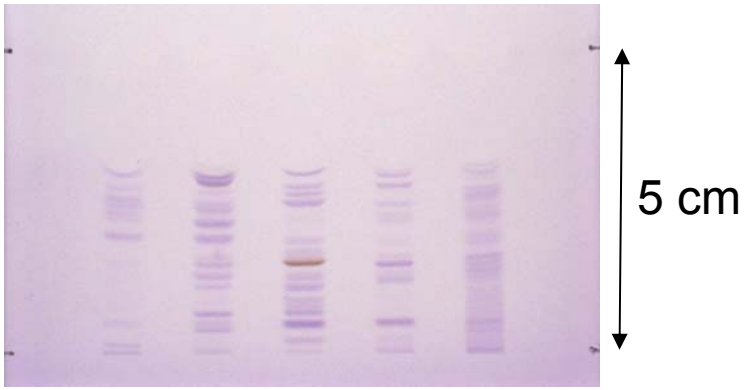


2-butanol/pyridine/acetic acid/water
(30/20/6/24) (v/v/v/v)

ProteoChrom® Color

ProteoChrom® Silica gel 60 F254s

Lys Myo Cyt Cas BSA



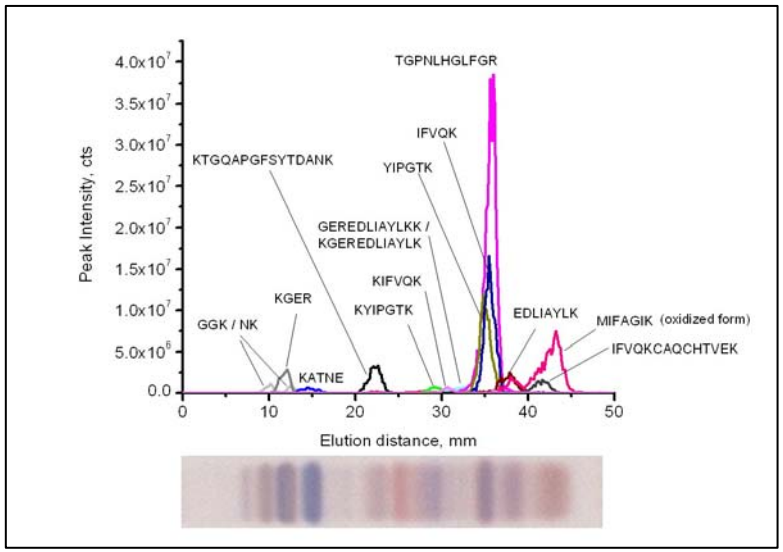
2-butanol/pyridine/ammonia/water
(39/34/10/26) (v/v/v/v)

Ninhydrin

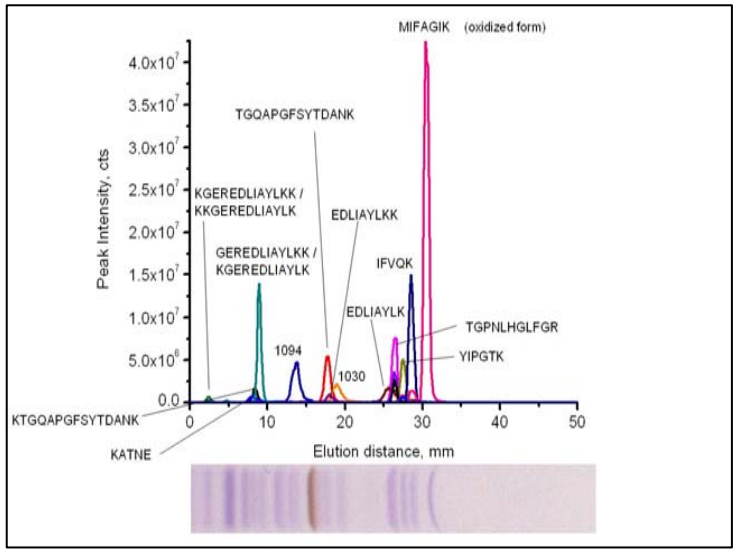
1D- HPTLC/ DESI- MS

Tryptic digest of Cytochrome C separated on:

ProteoChrom® Cellulose



ProteoChrom® Silica gel 60 F254s



Mass spectrometer:	LCQ Deca ion trap
Application volume:	7 µl
Concentration:	2 mg/ml
Total amount of protein:	~ 14 µg

1D- HPTLC/ DESI- MS

Tryptic digest of Cytochrome C separated on:

ProteoChrom® Cellulose

GDVEKGKKIF VQKCAQCHTV
EKGGKHKTGP NLHGLFGRKT
GQAPGFSYTD ANKNKGITWG
EETLMEYLEN PKKYIPGTKM
IFAGIKKKGE REDLIAYLKK ATNE

Sequence coverage 72%

ProteoChrom® Silica gel 60 F254s

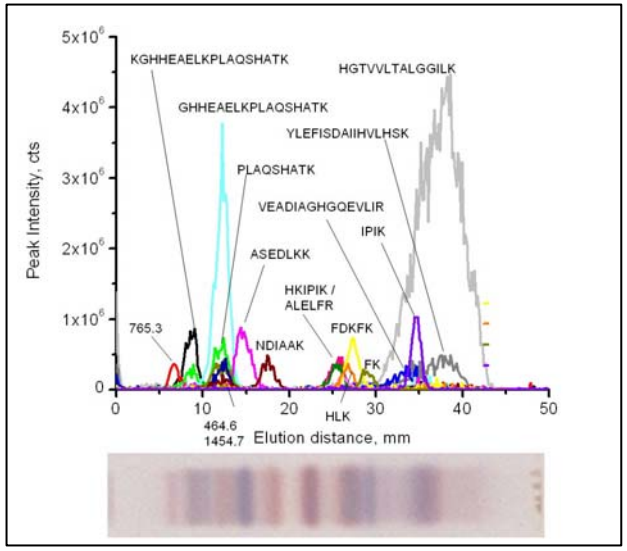
GDVEKGKKIF VQKCAQCHTV
EKGGKHKTGPNLHGLFGRKT
GQAPGFSYTD ANKNKGITWG
EETLMEYLEN PKKYIPGTKM
IFAGIKKKGE REDLIAYLKK ATNE

Sequence coverage 58%

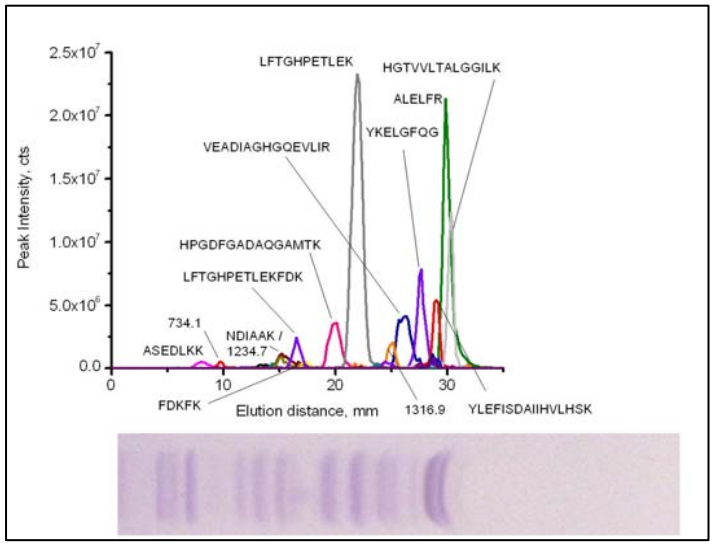
1D- HPTLC/ DESI- MS

Tryptic digest of Myoglobin separated on:

ProteoChrom® Cellulose



ProteoChrom® Silica gel 60 F254s



Mass spectrometer:	LCQ Deca ion trap
Application volume:	7 µl
Concentration:	2 mg/ml
Total amount of protein:	~ 14 µg

1D- HPTLC/ DESI- MS

Tryptic digest of Myoglobin separated on:

ProteoChrom® Cellulose

GLSDGEWQQV LNVWGKVEAD IAGHGQEVLI
RLFTGHPETL EKFDKFKHLK TEAEMKASED
KKHGTVVLT ALGGILKKKG HHEAELKPLA
QSHATKHKIP IKYLEFISDA IIHVLHSKHP
GDFGADAQGA MTKALELFRN DIAAKYKELG
FQG

Sequence coverage 68%

ProteoChrom® Silica gel 60 F254s

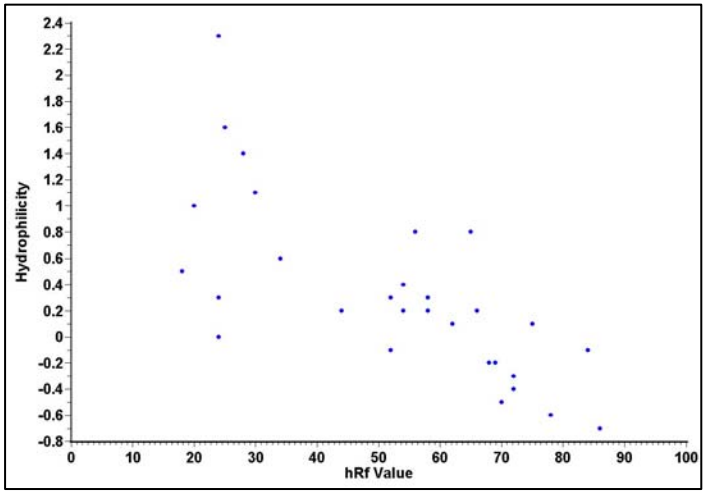
GLSDGEWQQV LNVWGKVEAD IAGHGQEVLI
RLFTGHPETL EKFDKFKHLK TEAEMKASED
LKKHGTVVLT ALGGILKKKG HHEAELKPLA
QSHATKHKIP IKYLEFISDA IIHVLHSKHP
GDFGADAQGA MTKALELFRN DIAAKYKELG
FQG

Sequence coverage 62%

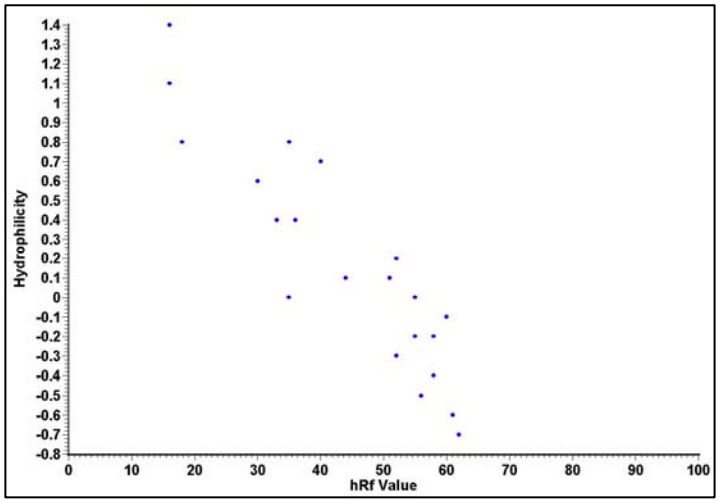
Sequence coverage 86%

1D- HPTLC/ DESI- MS

ProteoChrom® Cellulose

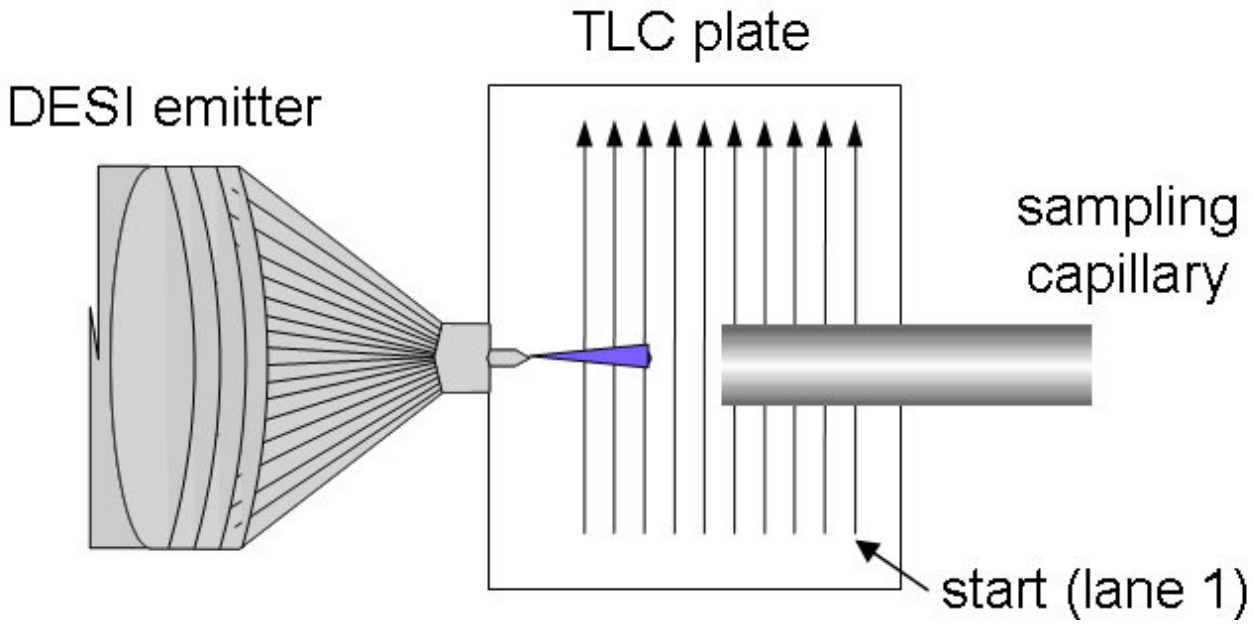


ProteoChrom® Silica gel 60 F254s



- Separation of peptides on Silica gel and Cellulose mainly according hydrophilicity

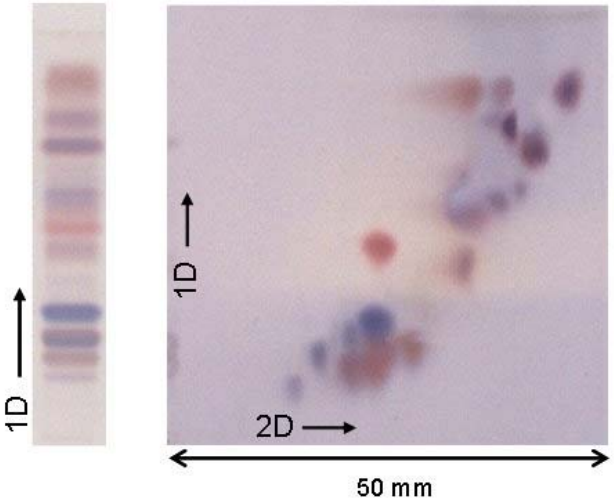
2D- HPTLC/ DESI- MS



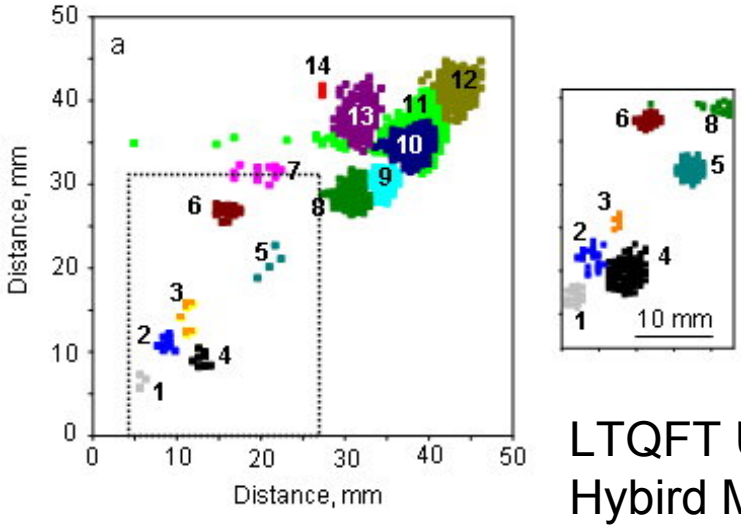
2D- HPTLC/ DESI- MS

Tryptic digest of Cytochrome C separated on:

ProteoChrom® Cellulose



Chemical image



LTQFT Ultra Hybird Mass Spectrometer

Application volume:	10 µl
Concentration:	2 mg/ml
Total amount of protein:	~ 20 µg

LCQ Deca ion trap Mass Spectrometer

2D- HPTLC/ DESI- MS

		HPLC/ ESI-MS	1D-HPTLC/ DESI-MS	2D-HPTLC/ DESI-MS
ProteoChrom® HPTLC Silica gel 60 F254s	Cytochrome C		58%	
ProteoChrom® Cellulose	Cytochrome C		72%	81%
Acclaim PepMap 100 C18	Cytochrome C	92%		
ProteoChrom® HPTLC Silica gel 60 F254s	Myoglobin		62%	
ProteoChrom® Cellulose	Myoglobin		68%	74%
Acclaim PepMap 100 C18	Myoglobin	84%		

Conclusions

- Limited separation efficiency of 2D- HPTLC
- Low DESI signal for peptides with low hRf- value
+ Direct sampling from the plate under ambient conditions
+ Lane scan and spot sampling mode possible
+ 2D-HPTLC/ DESI- MS Sequence Coverage is comparable to HPLC/ ESI- MS
+ Separation can be stored on the HPTLC plates
+ Numerous separations with every HPTLC run

Appreciation

- Merck KGaA
  Susanne Schorcht

- Organic and Biological Mass Spectrometry Group, Oak Ridge National Laboratory
  Sofie P. Pasilis
  Vilmos Kertesz
  Gary J. Van Berkel