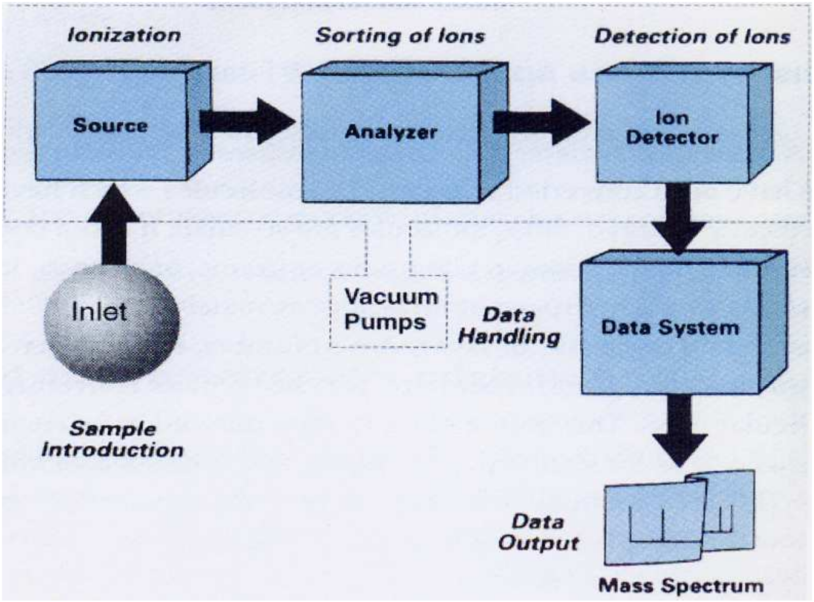
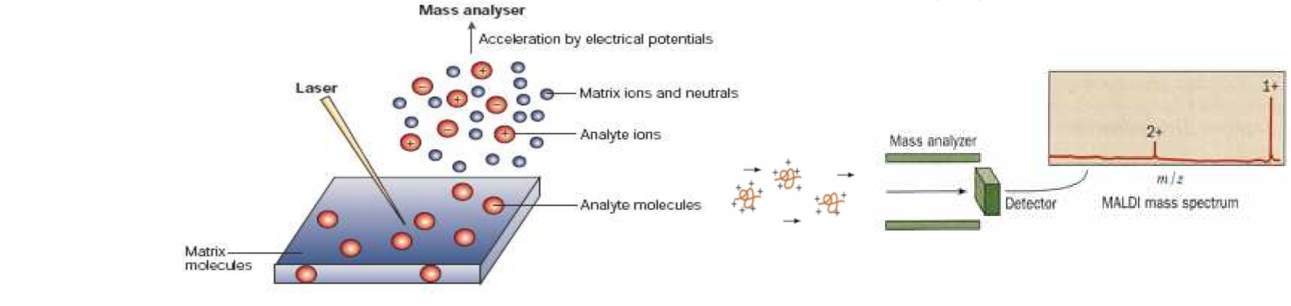
Masna spektroskopija proteinov in njena uporaba v proteomiki

Shema masnega spektrometra

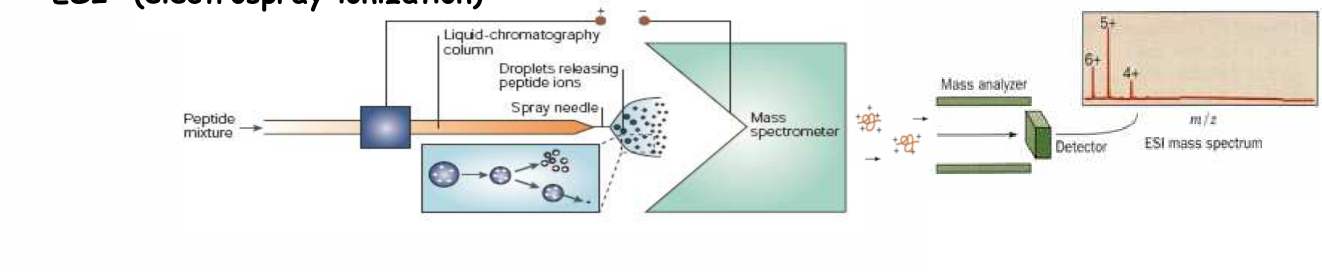


‘Mehke’ ionizcijske metode
ð uporaba MS za analizo proteinov

MALDI (matrix-assisted laser desorption/ionization) (variacija je SELDI)



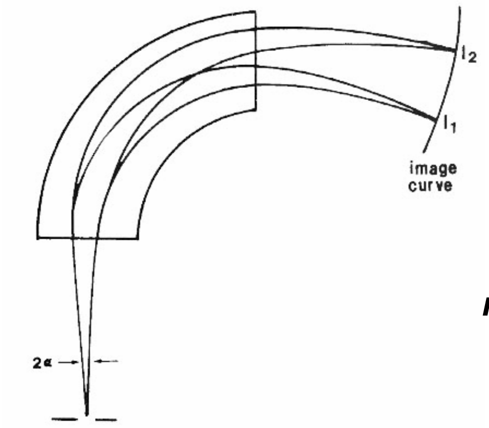
ESI (electrospray ionization)



Masni analizatorji

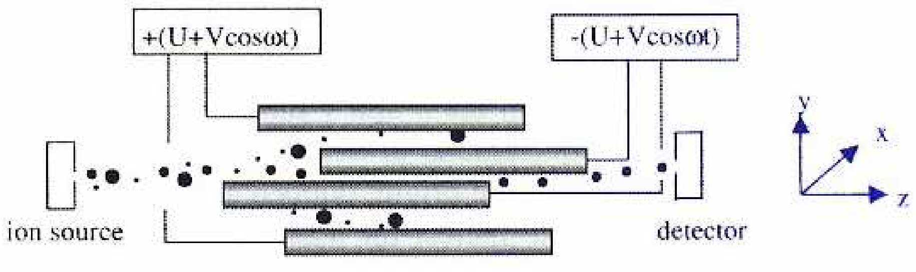
- Magnetni sektorski
- Kvadrupolni
- TOF
- Ionska past
- FT-ICR

Magnetni sektorski analizator

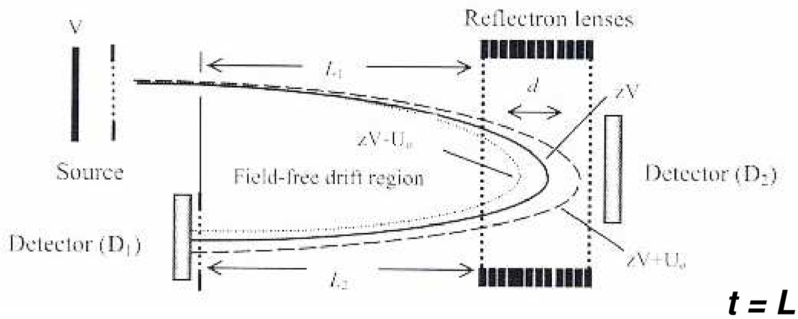


$m/z = B^2R^2/2V$

Kvadrupolni analizator

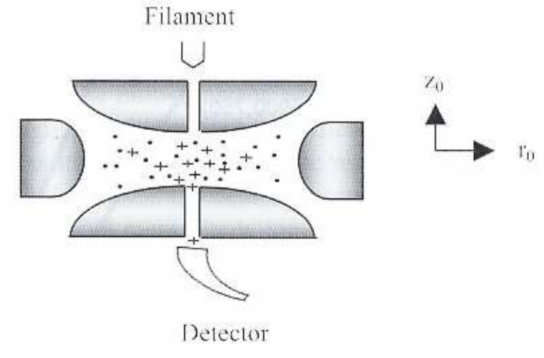


Analizator na čas preleta ionov (TOF)

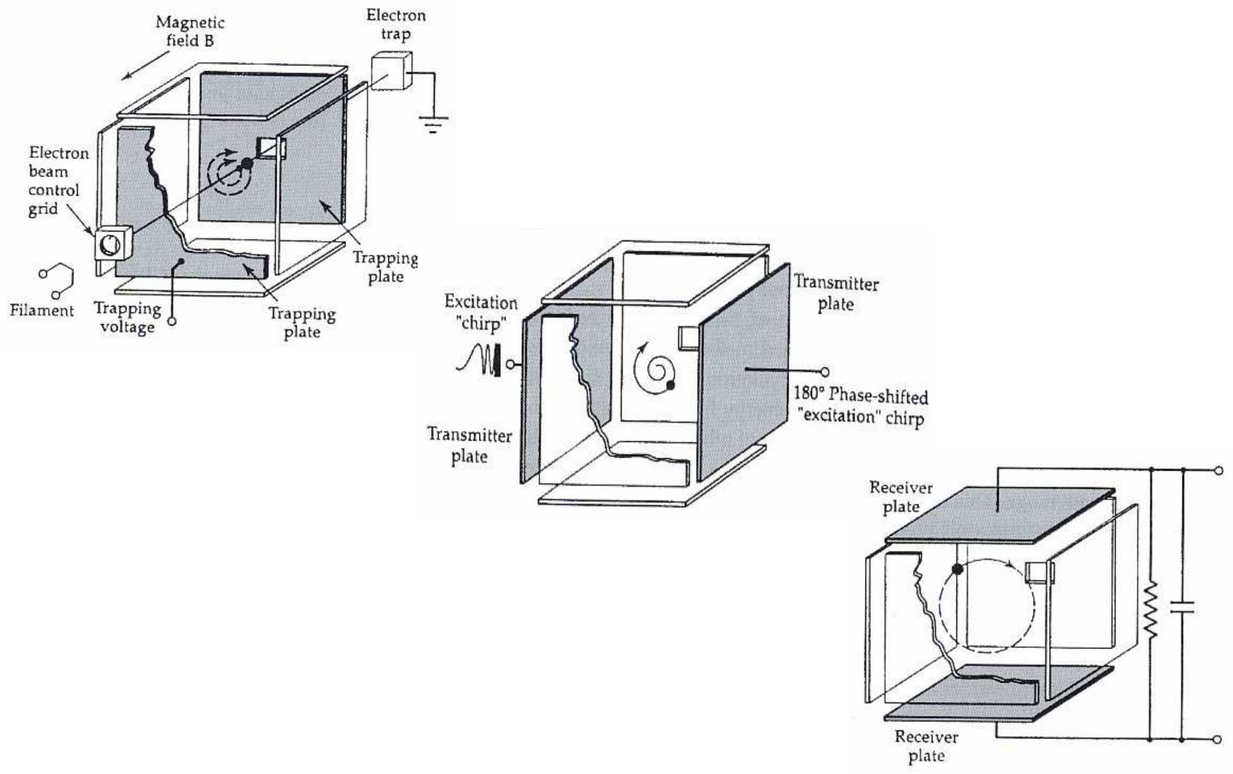


$$t = L(m/2zV)^{1/2}$$

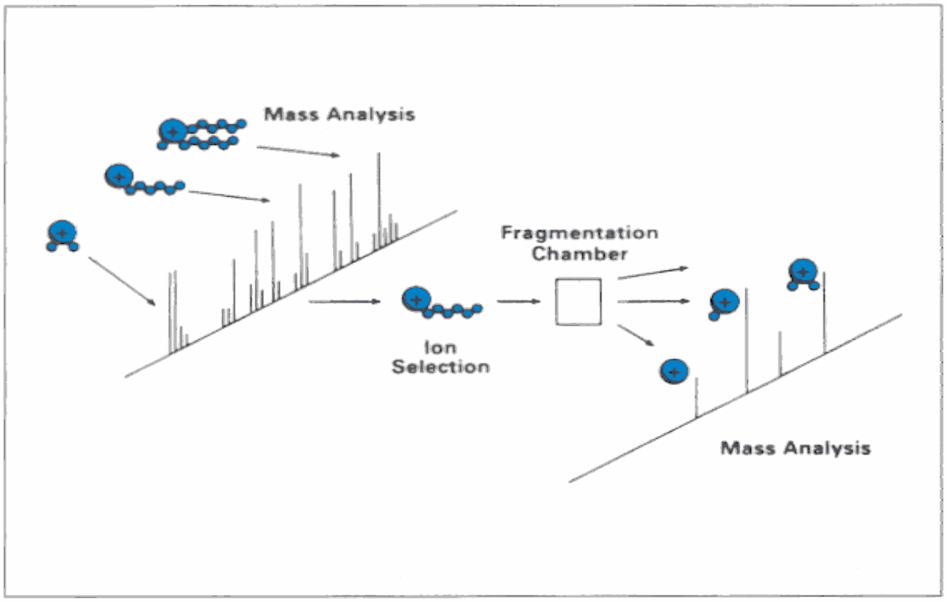
Ionska past



Fourier transform ionsko ciklotronski resonančni masni analizator (FT-ICR)

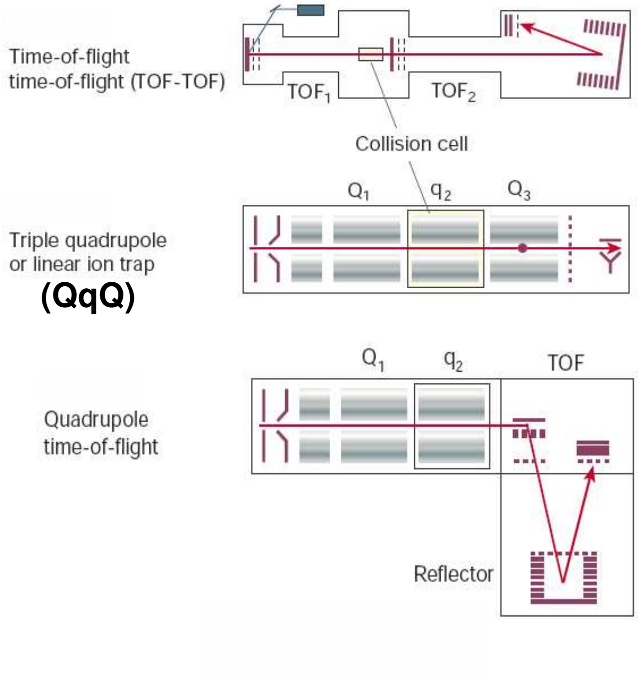


Tandemska MS



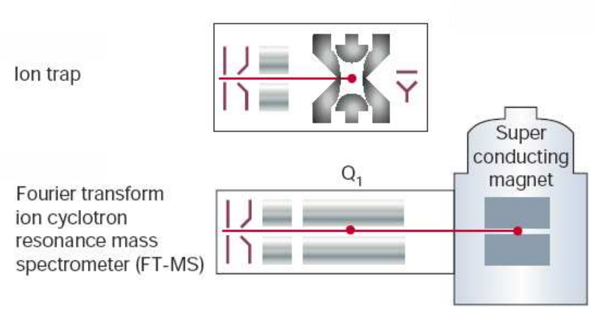
Sistemi za tandemsko MS

MS^2-in space



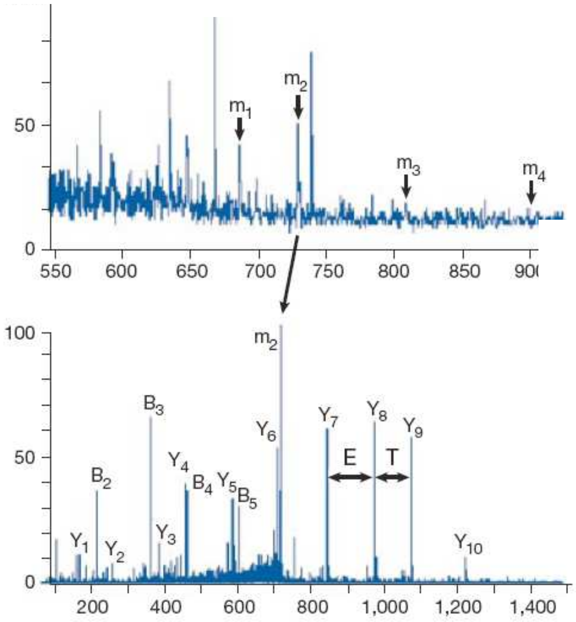
(QqQ)

MS^n-in time

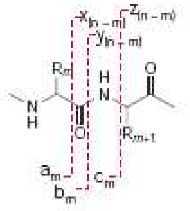


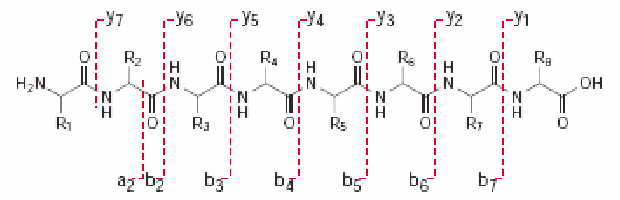
Aebersold & Mann (2003) Nature 422, 198.

Določanje aminokislinskega zaporedja v proteinih z MS



Roepstorff-Fohlmann-Biemann nomenclature

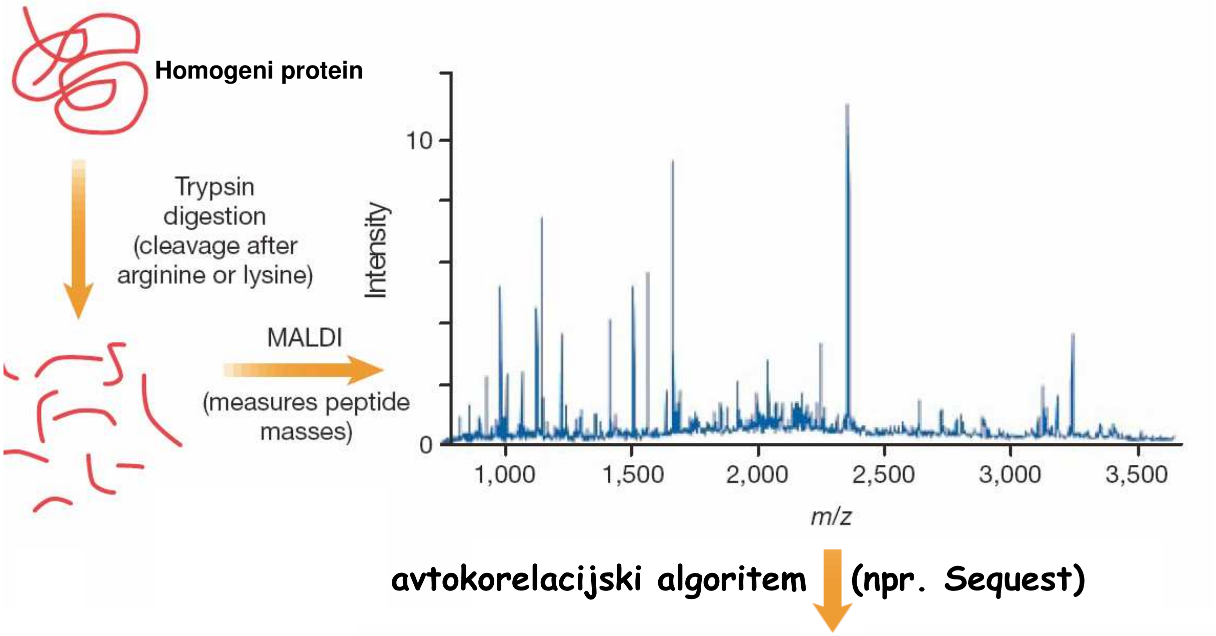




Interpretacija masnih spektrov:

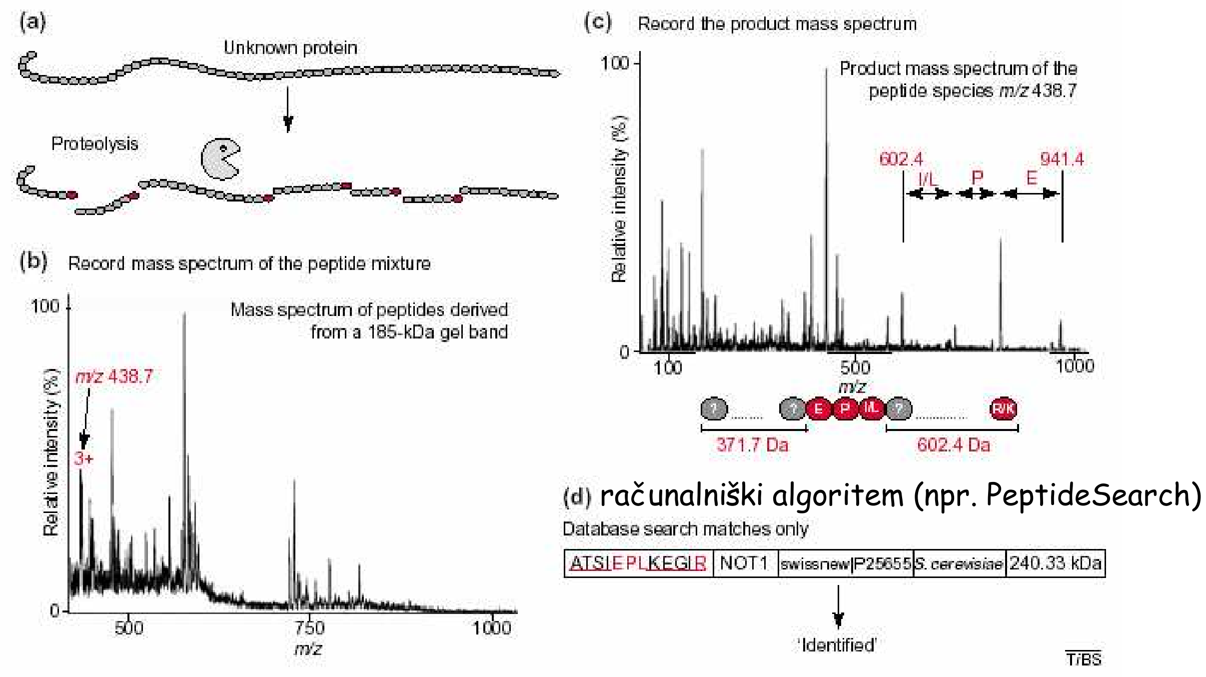
- *de novo* sekveniranje
- avtokorelacijski algoritmi (npr. Mascot)

Določitev mas peptidnih fragmentov proteina: “prstni odtis” proteina



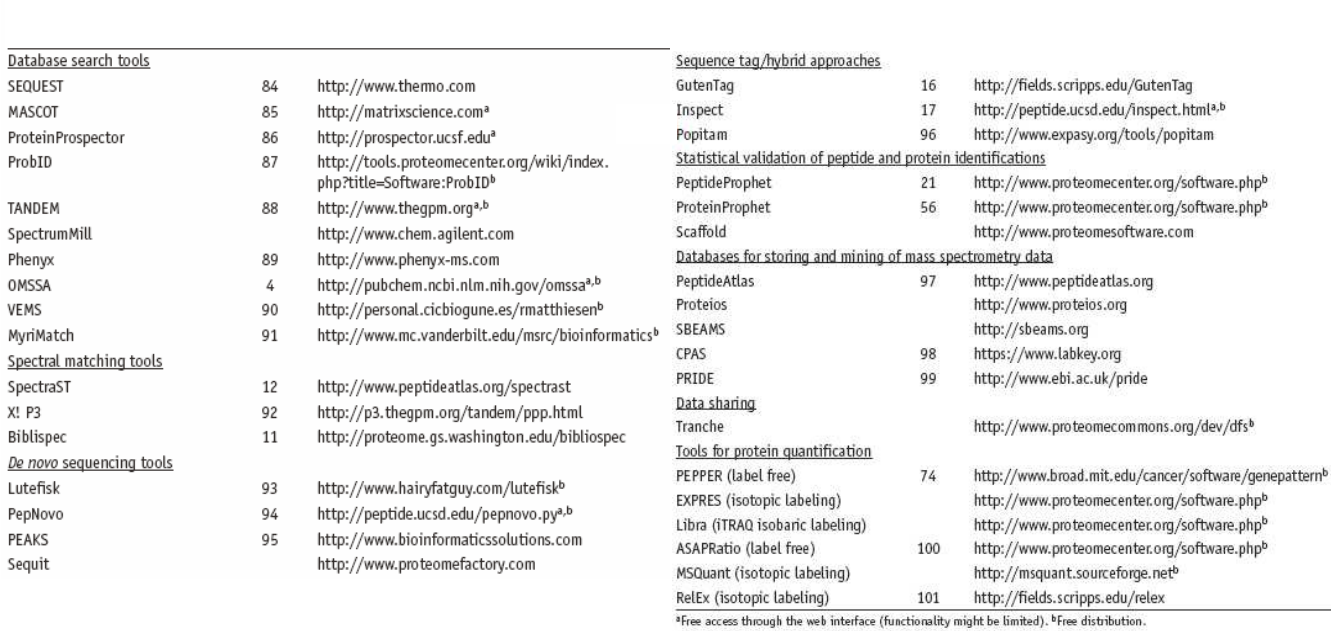
List of measured peptide masses matched against theoretically predicted peptides of proteins in databases

Identifikacija proteina preko t.i. “peptidne značke”



Rappsilber & Mann (2002) TIBS 27, 74.

Javno dostopna orodja za analizo MS/MS podatkov

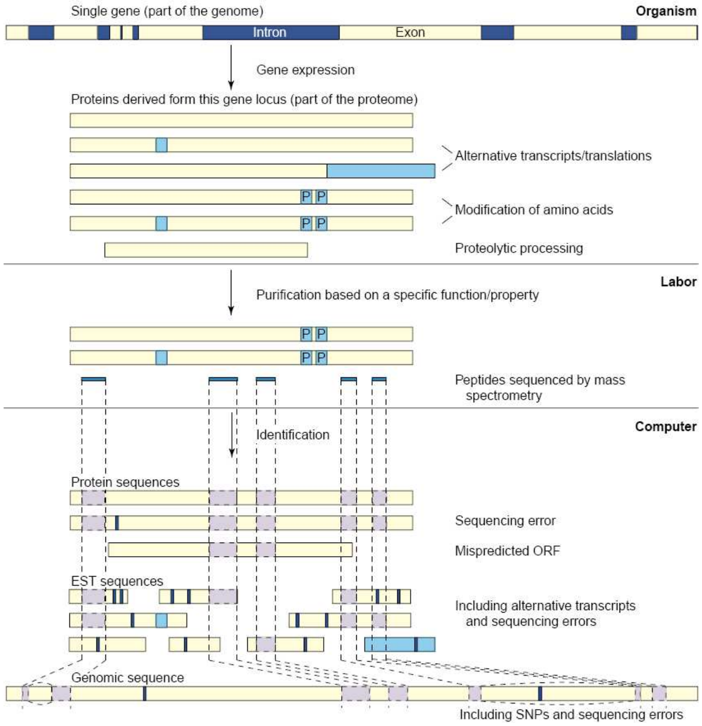


Database search tools		
SEQUEST	84	http://www.thermo.com
MASCOT	85	http://matrixscience.com[a]
ProteinProspector	86	http://prospector.ucsf.edu[a]
ProbID	87	http://tools.proteomecenter.org/wiki/index.php?title=Software:ProbID[b]
TANDEM	88	http://www.thegpm.org[a,b]
SpectrumMill		http://www.chem.agilent.com
Phenyx	89	http://www.phenyx-ms.com
OMSSA	4	http://pubchem.ncbi.nlm.nih.gov/omssa[a,b]
VEMS	90	http://personal.cicbiogune.es/rmatthiesen[b]
MyriMatch	91	http://www.mc.vanderbilt.edu/msrc/bioinformatics[b]
Spectral matching tools		
SpectraST	12	http://www.peptideatlas.org/spectrast
X! P3	92	http://p3.thegpm.org/tandem/ppp.html
Biblispec	11	http://proteome.gs.washington.edu/bibliospec
De novo sequencing tools		
Lutefisk	93	http://www.hairyfatguy.com/lutefisk[b]
PepNovo	94	http://peptide.ucsd.edu/pepnovo.py[a,b]
PEAKS	95	http://www.bioinformaticssolutions.com
Sequit		http://www.proteomefactory.com
Sequence tag/hybrid approaches		
GutenTag	16	http://fields.scripps.edu/GutenTag
Inspect	17	http://peptide.ucsd.edu/inspect.html[a,b]
Popitam	96	http://www.expasy.org/tools/popitam
Statistical validation of peptide and protein identifications		
PeptideProphet	21	http://www.proteomecenter.org/software.php[b]
ProteinProphet	56	http://www.proteomecenter.org/software.php[b]
Scaffold		http://www.proteomesoftware.com
Databases for storing and mining of mass spectrometry data		
PeptideAtlas	97	http://www.peptideatlas.org
Proteios		http://www.proteios.org
SBEAMS		http://sbeams.org
CPAS	98	https://www.labkey.org
PRIDE	99	http://www.ebi.ac.uk/pride
Data sharing		
Tranche		http://www.proteomecommons.org/dev/dfs[b]
Tools for protein quantification		
PEPPER (label free)	74	http://www.broad.mit.edu/cancer/software/genepattern[b]
EXPRES (isotopic labeling)		http://www.proteomecenter.org/software.php[b]
Libra (iTRAQ isobaric labeling)		http://www.proteomecenter.org/software.php[b]
ASAPRatio (label free)	100	http://www.proteomecenter.org/software.php[b]
MSQuant (isotopic labeling)		http://msquant.sourceforge.net[b]
RelEx (isotopic labeling)	101	http://fields.scripps.edu/relex

[a] Free access through the web interface (functionality might be limited). [b] Free distribution.

Nesvizhskii et al. (2007) Nature Methods 4, 787.

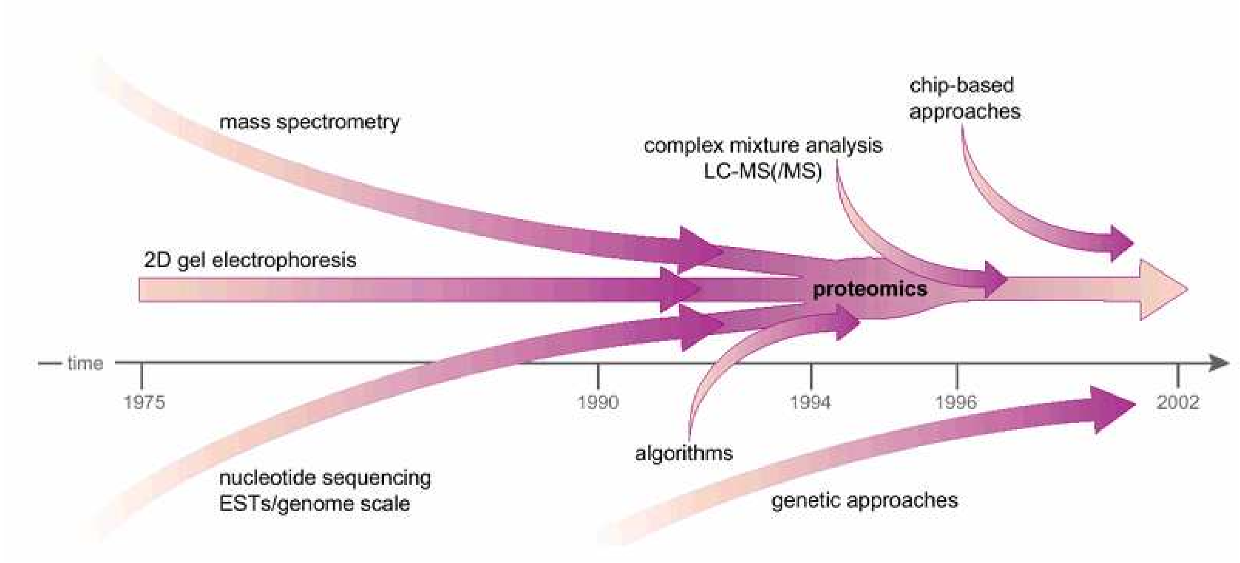
Pri interpretaciji MS rezulatatov je potrebno biti previden!



Z MS lahko zanesljivo identificiramo le za produkt katerega gena gre!

Rappsilber & Mann (2002) TIBS 27, 74.

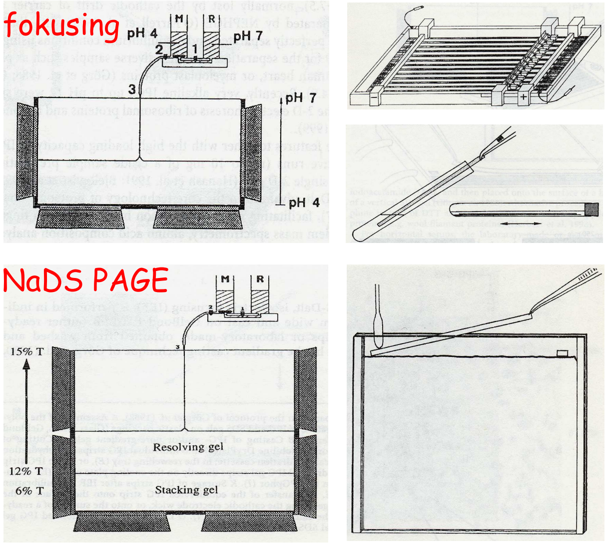
Proteomika


chip-based approaches
mass spectrometry
complex mixture analysis LC-MS(/MS)
2D gel electrophoresis
proteomics
time
1975
1990
1994
1996
2002
algorithms
nucleotide sequencing ESTs/genome scale
genetic approaches

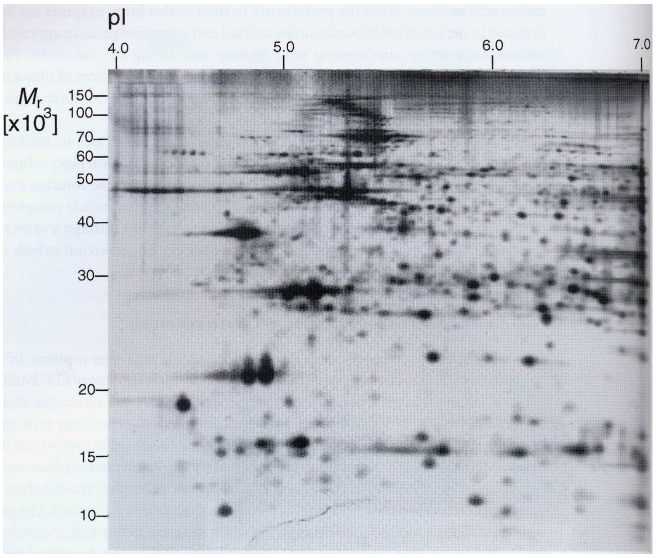
Klasična proteomika: ekspresijsko profiliranje z 2DE

Izoelektrični fokusing

NaDS PAGE

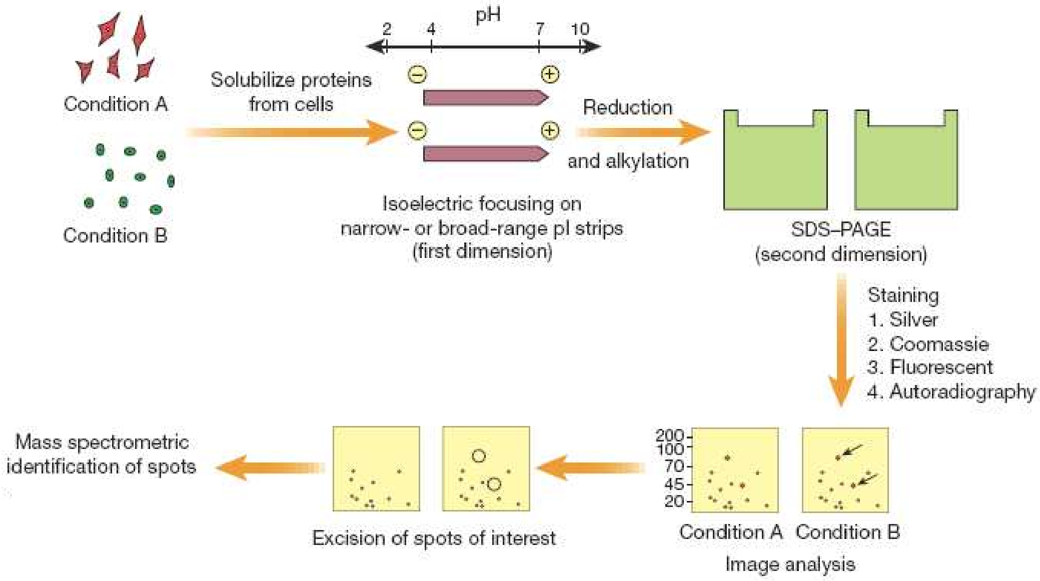


Klasična 2DE in IPG-Dalt



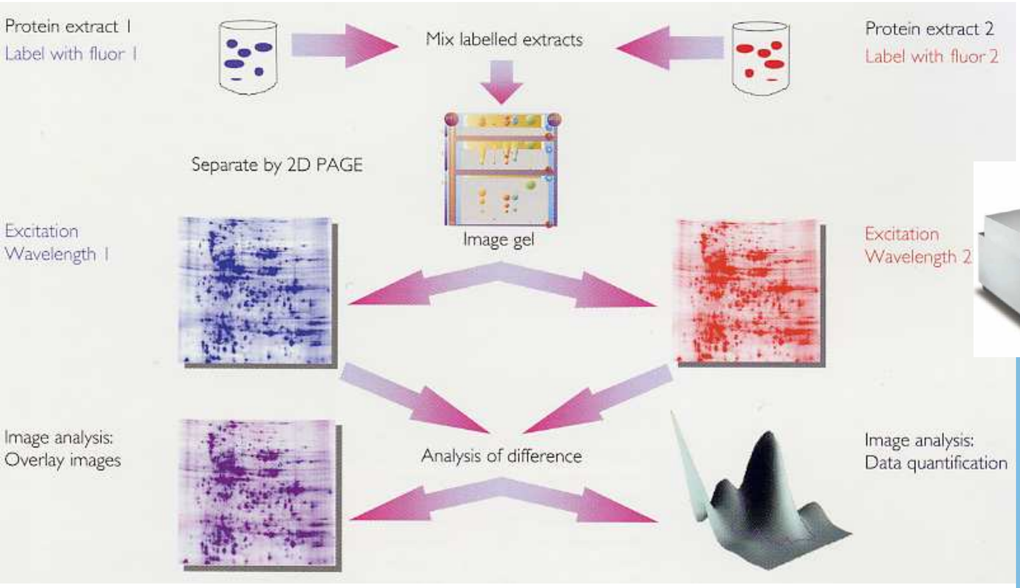
Loči lahko > 10.000 proteinov!

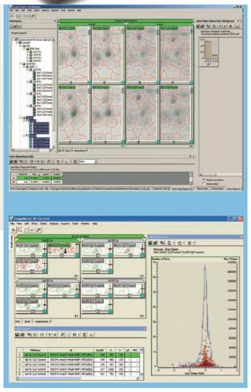
Diferenčni prikaz



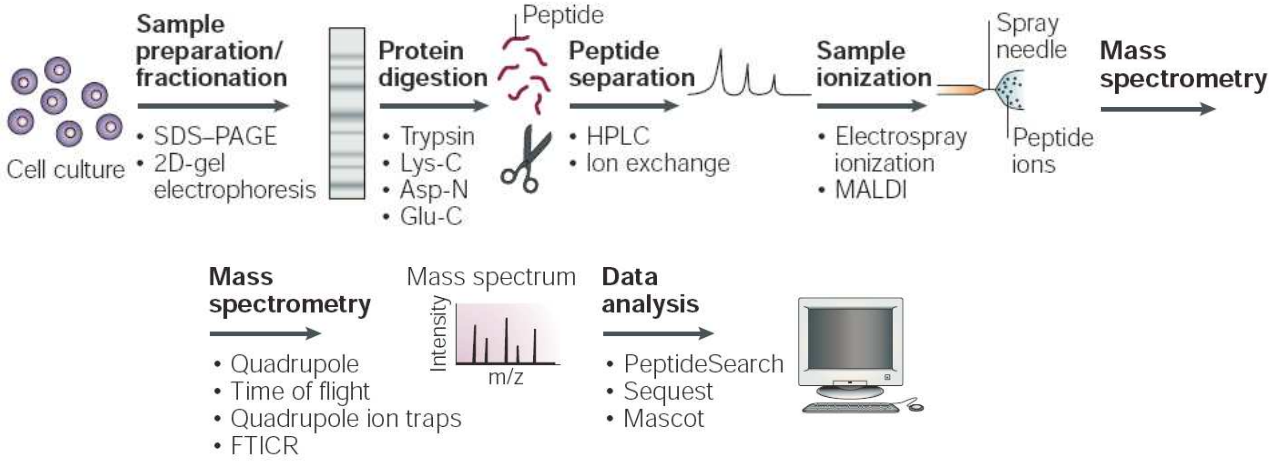
Pandey & Mann (2000) Nature 405, 837.

Diferenčna gelska elektroforeza (DIGE)



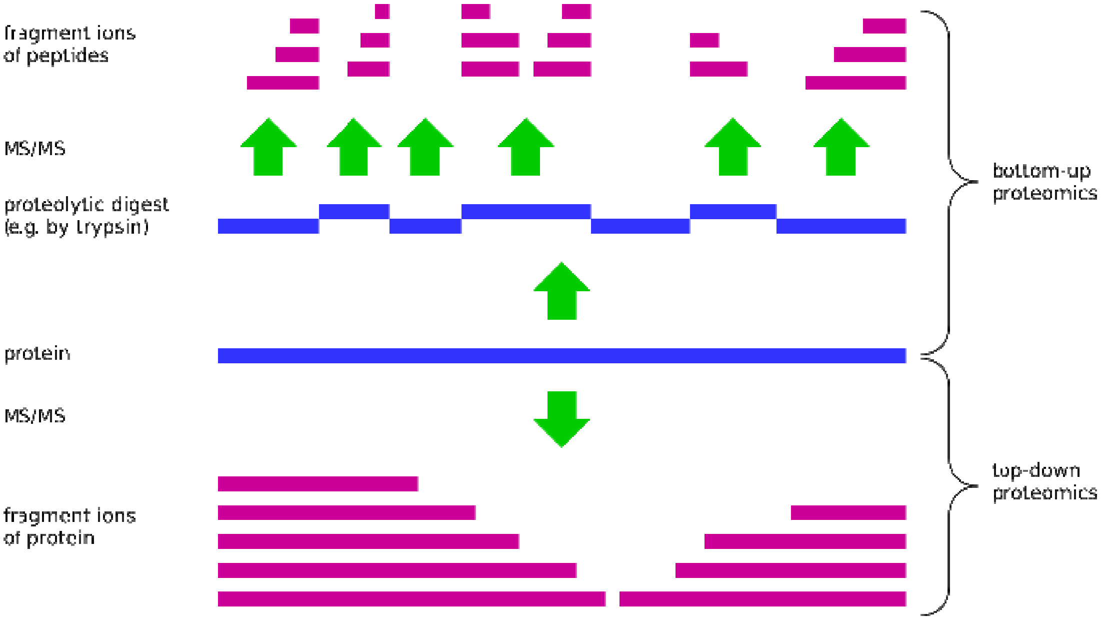


Klasičnen proteomski poskus na osnovi MS
(t.i. pristop “bottom-up”)



Steen & Mann (2004) Nature Rev. Mol. Cell Biol. 5, 699.

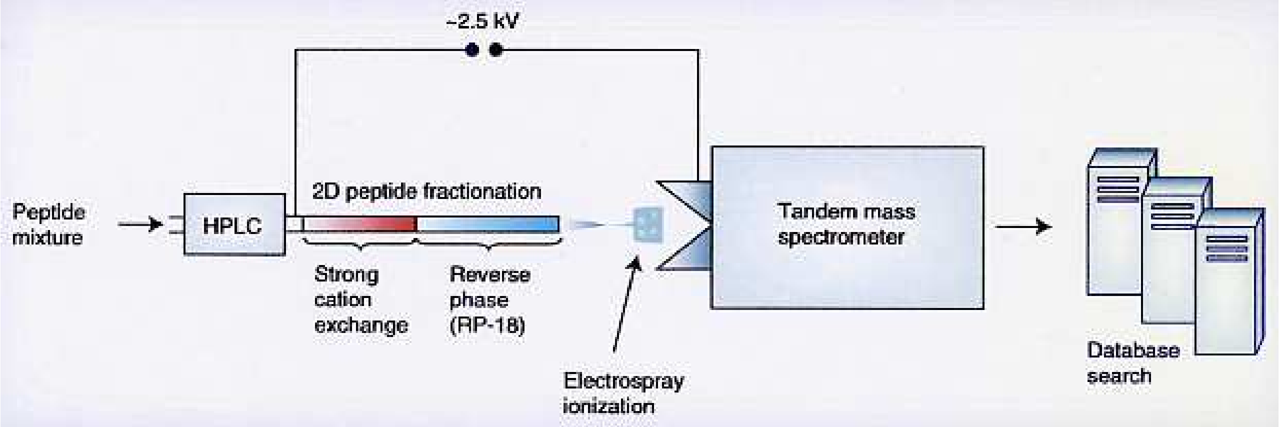
Proteomska pristopa na osnovi MS: “bottom-up” in “top down”



Wikimedia Commons

Ekspresijsko profiliranje z tehnologijo MudPIT (t.i. pristop "shotgun")

to improveme sensitivity, dynamic range & detection coverage.

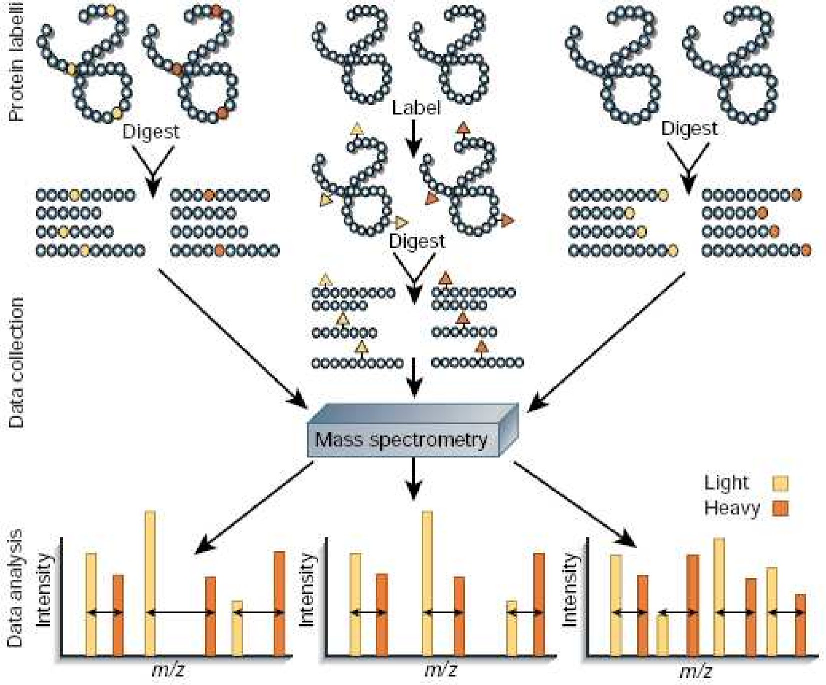


MudPIT combines multidimensional liquid chromatography on- or off-line with ESI/MS/MS (<u>shotgun sequencing</u>).

Identification of over 1000 unique proteins/day (yeast).
Detection of lower abundance proteins, small or positively charged & hydrophobic transmembrane proteins usually missed by 2DE.

Kvantitativna MS

SILAC **(ICAT, iTRAQ)**



Aebersold & Mann (2003) Nature 422, 198.

Seminarji/Literatura

Multidimenzionalna tehnologija za identifikacijo proteinov - MudPIT

Kislinger, T. and Emili, A. (2005): Multidimensional protein identification technology: Current status and future prospects. Exp. Rev. Proteomics 2, 27-39.

Kvantitativna proteomika na osnovi masne spektroskopije

Ong, S.-E. and Mann, M. (2005): Mass spectrometry-based proteomics turns quantitative. Nat. Chem. Biol. 1, 252-262.

Analiza proteinskih kompleksov z masno spektroskopijo

Gingras, A.-C., Gstaiger, M., Raught, B. and Aebersold, R. (2007): Analysis of protein complexes using mass spectrometry. Nat. Rev. Mol. Cell Biol. 8, 645-654.

Dvo-dimenzionalna gelska elektroforeza v proteomiki

Rabilloud, T. and Lelong, C. (2011): Two-dimensional gel electrophoresis in proteomics: A tutorial. J. Proteomics 74, 1829-1841.

Proteomska analiza post-translacijskih modifikacij

Kamath, K.S., Vasavada, M.S. and Srivastava, S. (2011): Proteomic databases and tools to decipher post-translational modifications. J. Proteomics 75, 127-144.

Zhao, Y. and Jensen, O.N. (2009): Modification-specific proteomics: Strategies for characterization of post-translational modifications using enrichment techniques. Proteomics 9, 4632–4641.

Witze, E.S., Old, W.M., Resing, K.A. and Ahn, N.G. (2007): Mapping protein posttranslational modifications with mass spectrometry. Nature Meth. 4, 798-806.

Proteomika membranskih proteinov

Gilmore, J.M. and Washburn, M.P. (2010): Advances in shotgun proteomics and the analysis of membrane proteomes. J. Proteomics 73, 2078-2091.

Upodabljanje tkiv s pomočjo masne spektroskopije

Amstalden van Hove, E.R., Smith, D.F. and Heeren, R.M.A. (2010): A concise review of mass spectrometry imaging. J. Chromatogr. A 1217, 3946–3954.

Seeley, E.H. and Caprioli, R.M. (2012): 3D Imaging by Mass Spectrometry: A New Frontier. Analit. Chem. 84, 2105−2110.

Cornett, D.S., Reyzer, M.L., Chaurand, P. and Caprioli, R.M. (2007): MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. Nat. Methods 4, 828-833.

Schwamborn, K. and Caprioli, R.M. (2010): Molecular imaging by mass spectrometry — looking beyond classical histology. Nat. Rev. Cancer 10, 639-646.

Masna spektroskopija v medicini

Hanash, L.A.S. and Taguchi, A. (2010): The grand challenge to decipher the cancer proteome. Nat. Rev. Cancer 10, 652-660.

Nomura, D.K., Dix, M.M. and Cravatt, B.F. (2010): Activity-based protein profiling for biochemical pathway discovery in cancer. Nat. Rev. Cancer 10, 630-638.

Melendez, L.M., Colon, K., Rivera. L., Rodriguez-Franco, E. and Toro-Nieves, D. (2011): Proteomic Analysis of HIV-Infected Macrophages. J. Neuroimmune Pharmacol. 6, 89-106.