FOOTPRINTING STRATEGY: LC-MS AND DATA ANALYSIS

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STRUCTURAL MASS SPECTROMETRY
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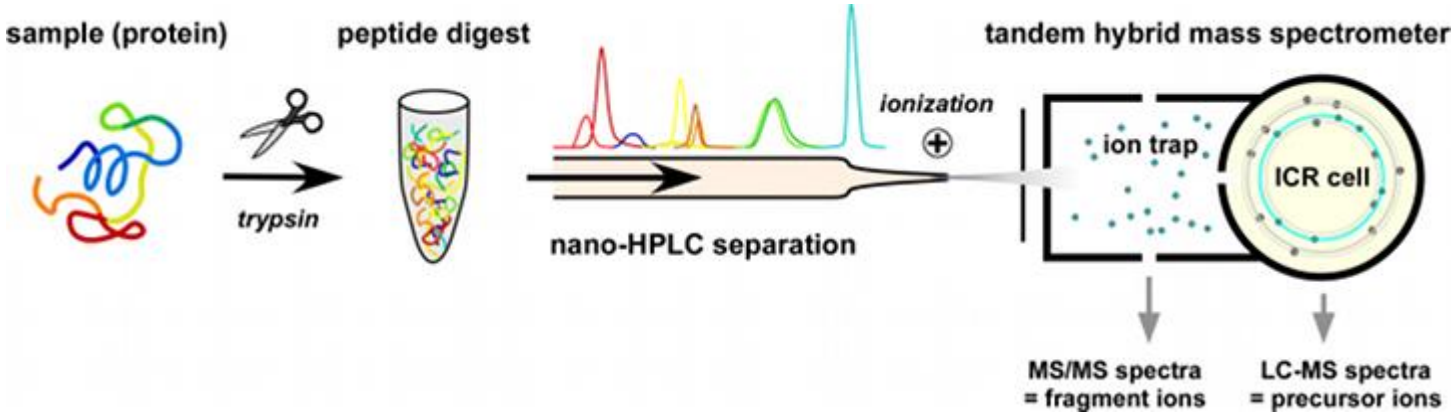
Sources: Mass Spectrometry Analysis of Protein-Protein Interactions and Dynamics, Chance, *Wiley*, New Jersey;

Takamoto & Chance, Ann. Rev. Biophysics and Biophysical Chem. 2006

OUTLINE

- LC-MS experimental set up
- MS/MS for identification of site of modification
- Data analysis: ProtMapMS software

HRF LC-MS ANALYSIS:



- LC-MS analysis – determination of peptide masses (*m/z*) and relative quantification of the extent of modification on the peptide level;

- LC-MS-MS analysis - peptide identification, determination of specific site of modification.

REVERSE-PHASE SEPARATION OF PEPTIDE MIXTURE: EXPERIMENTAL SET UP

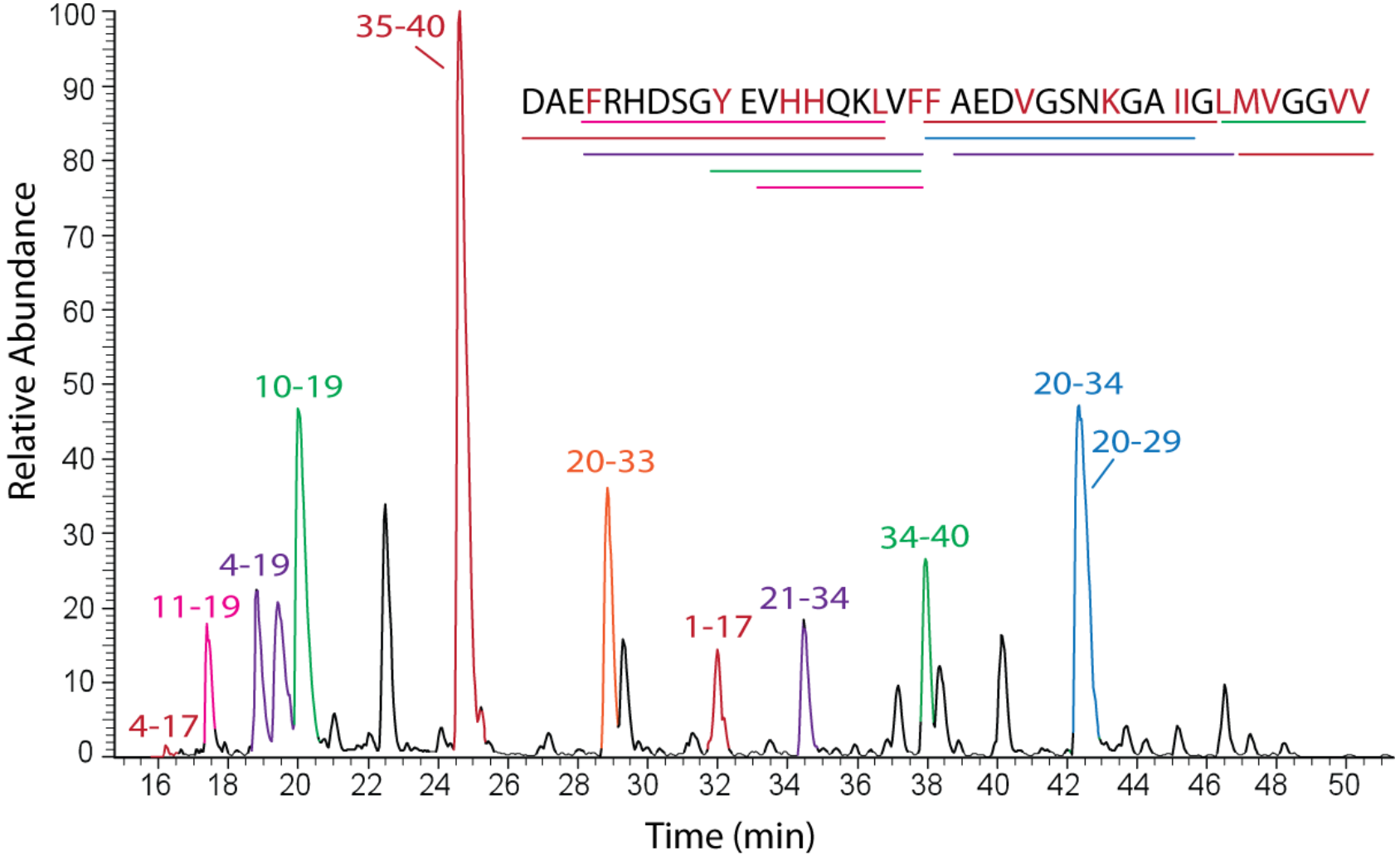
- nanoACQUITY UPLC (Waters, Co.) is coupled to MS
- Typical gradient for a 50K protein is 2-40% of ACN in 60 min, for large proteins and protein complexes (MAbs or MAb-antigen complexes) the gradient is 2-55% in 120 min.
- The lower the flow the greater the sensitivity (300 nl/min).
- C18 nano column: 75 um x 25 cm.
- 2-3 pmol of peptide mixture is loaded on column.

Provide peptide separation and resolution

MS AND MS/MS: EXPERIMENTAL SET UP

- Thermo Scientific, Inc. Orbitrap Elite MS
- nano-electrospray source with a capillary voltage of 2.4 kV
- A full MS scan is typically set for eluted peptides in the range of 380–1600 m/z and resolution (*R*) of 120,000
- Twenty data dependent MS/MS scans are set with a minimum signal threshold of 2000, collision energy of 35%, an isolation width of 2.5, and an activation time of 30 msec to generate a series of b- and y-ions as major fragments. *R* for fragment ions is set to 60,000

LC-MS: PEPSIN DIGEST CHROMATOGRAM OF AB40



SELECTED ION CHROMATOGRAMS (SIC) FOR 10-19:
VARIOUS MODIFICATION SPECIES ELUTE AT DIFFERENT RT