
Coronary heart disease related proteomics

Jaakko Niemi

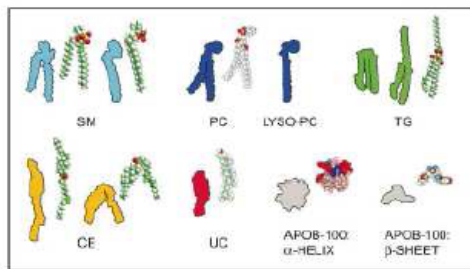
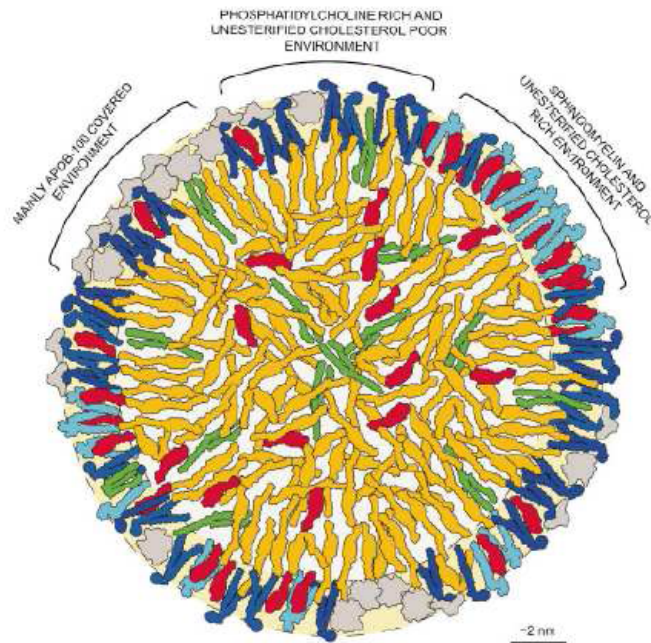
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- Shotgun proteomics
- SEQUEST algorithm
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Coronary heart disease

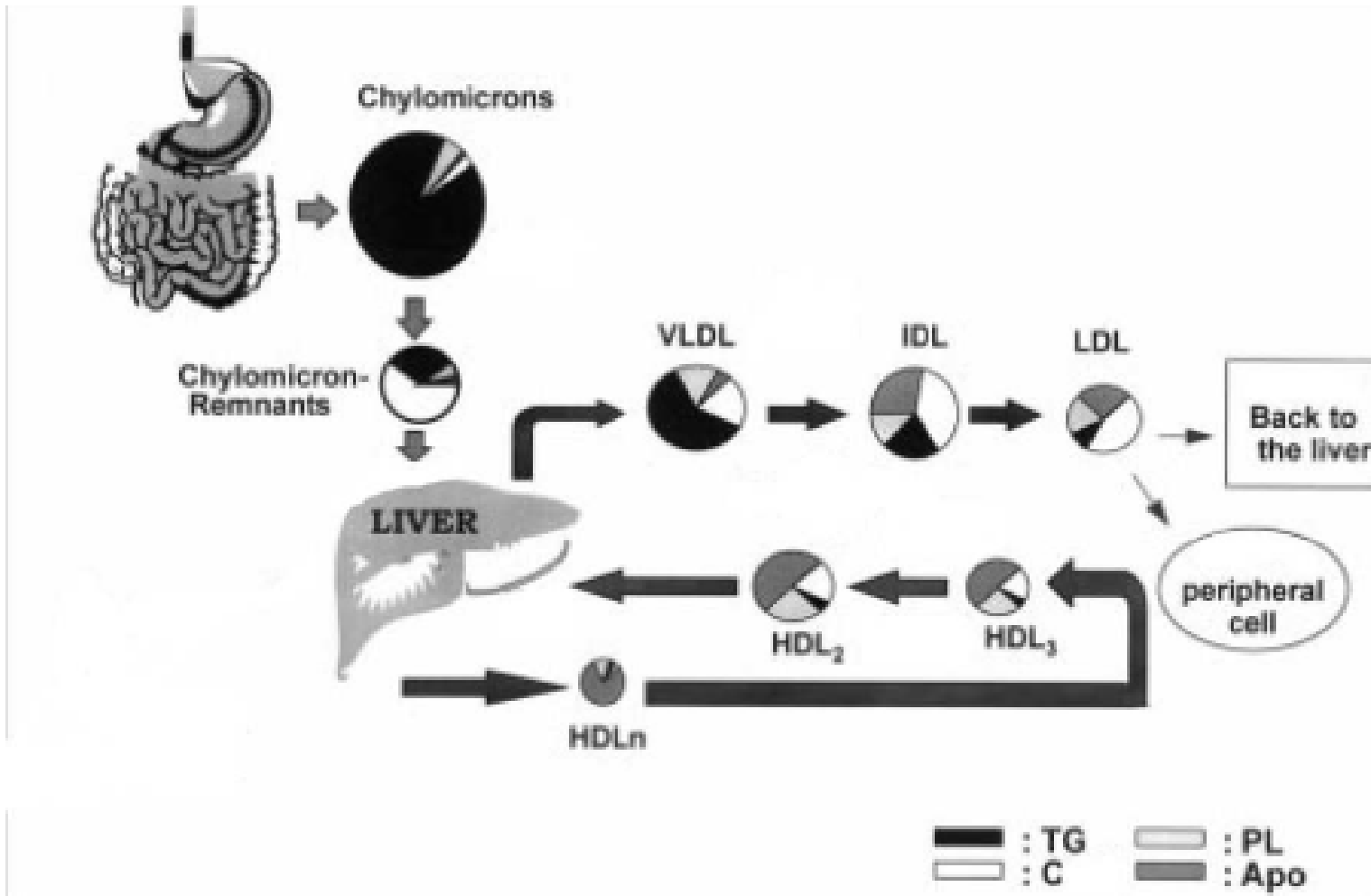
- the most common cause of death in the Western World
- annual costs 360 billion US dollars (in USA alone)
- lipoprotein particles have a major role in the development and progression of the disease

Lipoprotein structure and main classes

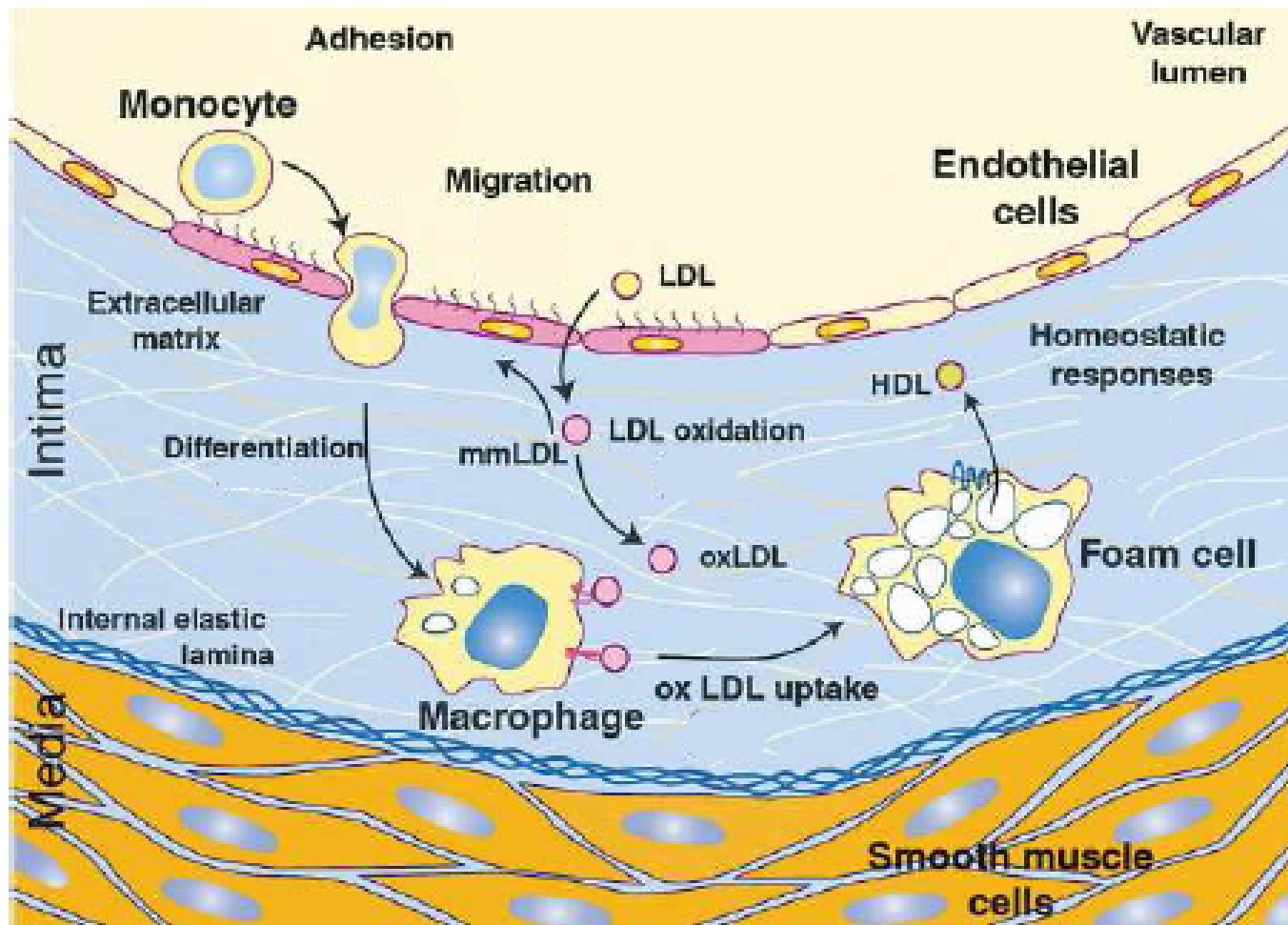


Lipoprotein Fraction	Density range, g/ml	Diameter range, nm	Average C, %	Average TG, %	Average Lipid, %	Average Protein, %
CM	<0.960	80-1200	4	88	99	1
VLDL	0.960-1.006	30-80	23	56	92	8
IDL	1.006-1.019	25-35	43	29	89	11
LDL	1.019-1.063	16-25	58	13	79	21
HDL ₂	1.063-1.125	8.5-12	41	16	67	33
HDL ₃	1.125-1.210	4-8.5	35	13	48	52

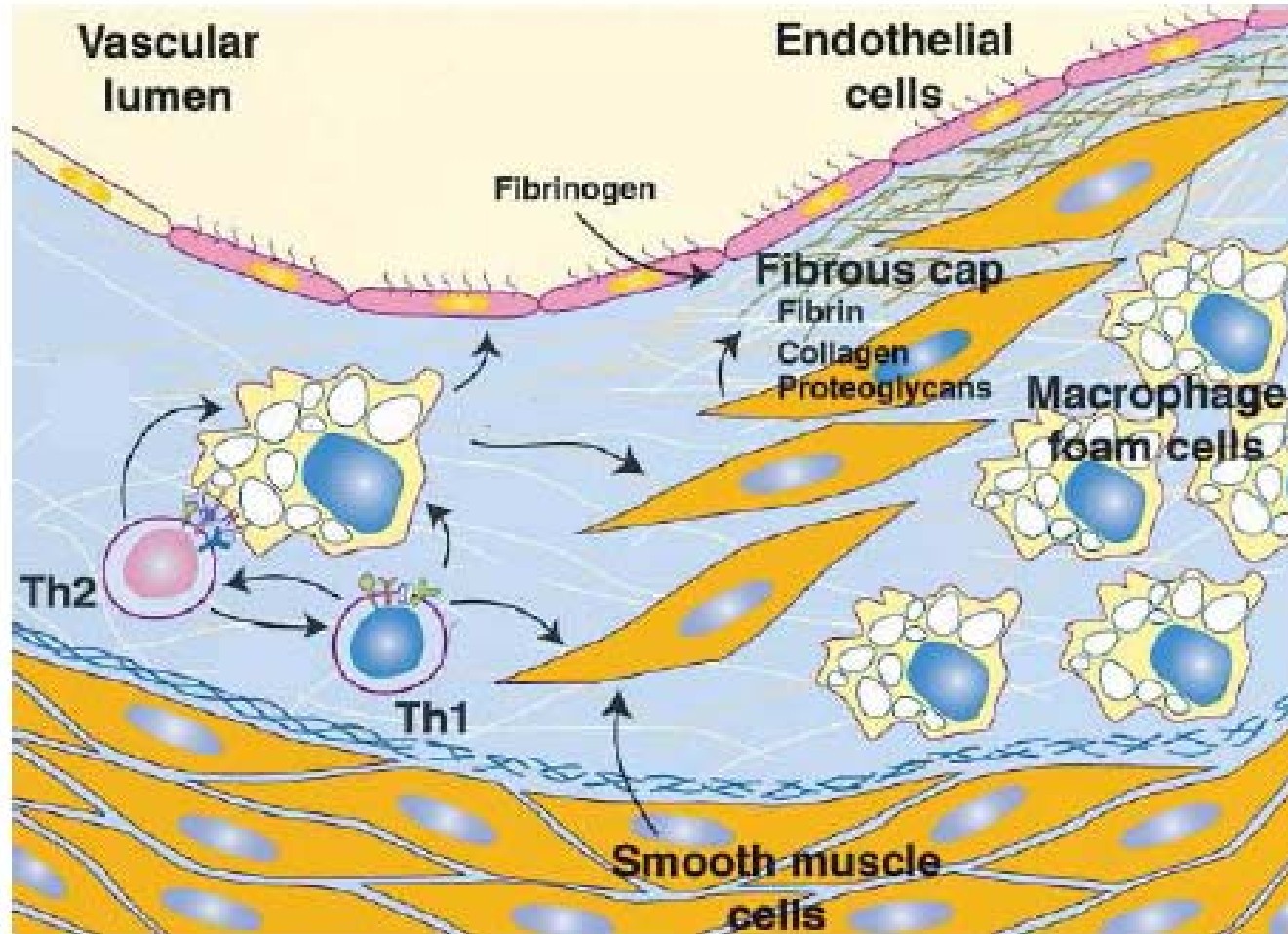
Lipoprotein metabolism



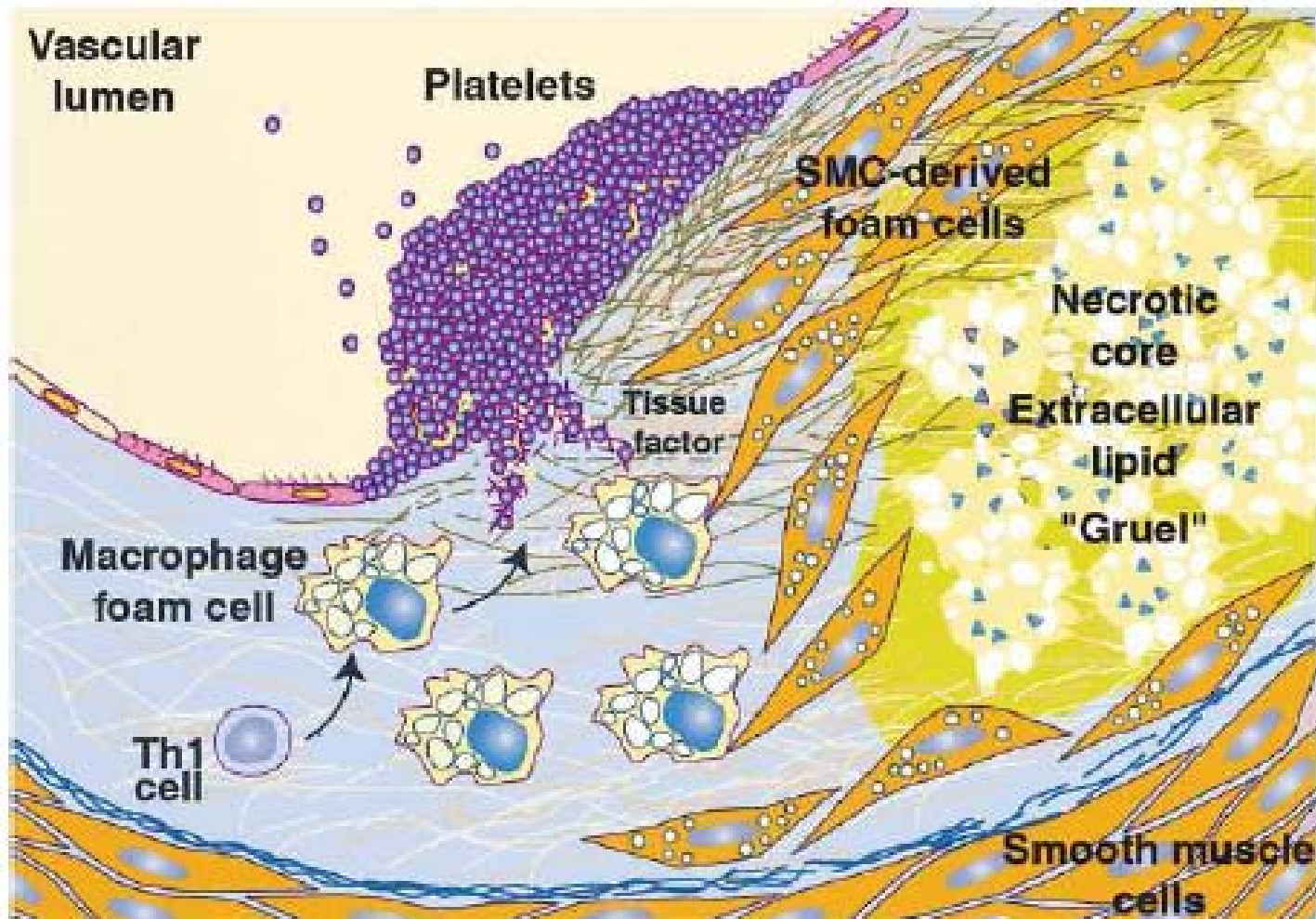
Development of a foam cell



Progression of an atherosclerotic lesion

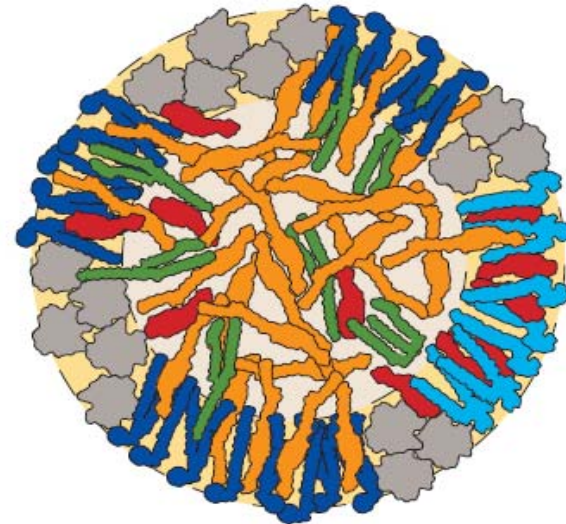


Plaque rupture and thrombosis



HDL particles

- "the good cholesterol"
- inversely related to CHD
- maintain reverse cholesterol transport
- may have anti-inflammatory and anti-oxidative properties

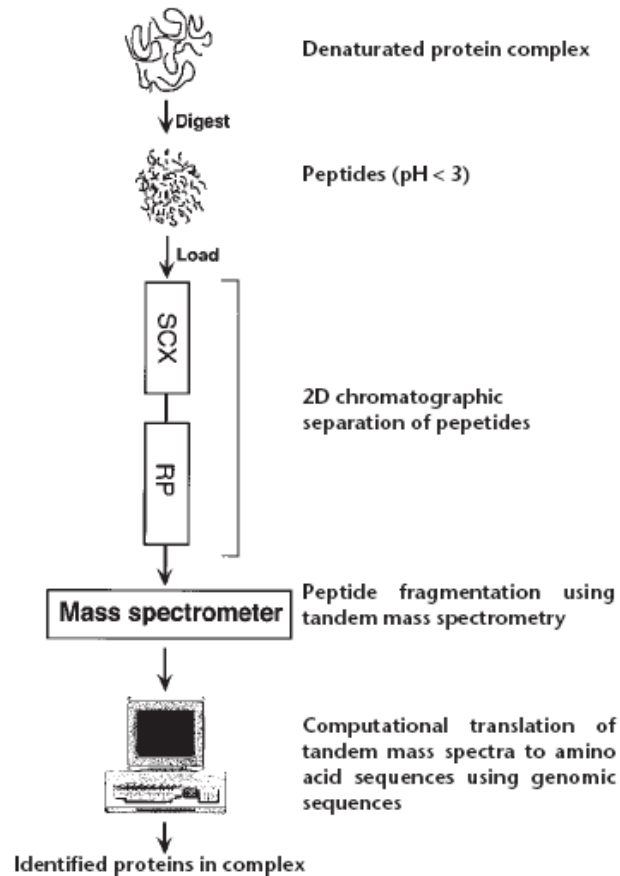


THE GOOD

Shotgun proteomics

- direct analysis of large protein complexes (DALPC)
- capable of identifying individual proteins in large protein complexes without first purifying each protein component to homogeneity

Shotgun proteomics



- 2D Chromatography (charge and hydrophobicity)
 - Strong cation exchange (SCX)
 - Reversed-phase (RP)
- SEQUEST algorithm for identifying proteins in MS spectra

SEQUEST

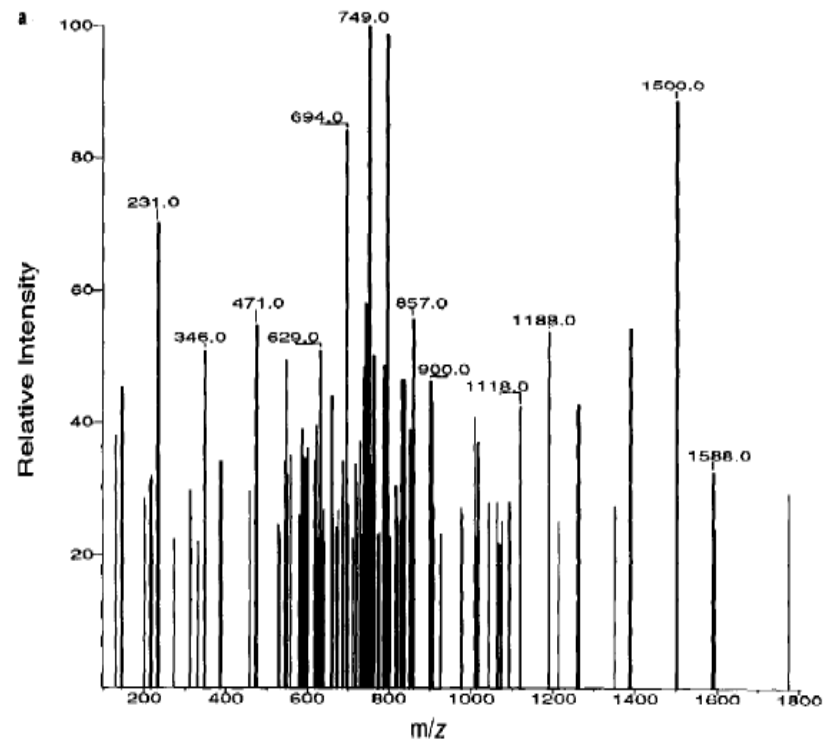
- tandem mass spectrometry data analysis method used for protein identification
- identifies collections of tandem mass spectras comparing these spectras to the protein sequences found in protein databases
- identifies each tandem mass spectrum individually.

SEQUEST

- Step 1: Tandem mass spectrometry data reduction
- Step 2: Searching amino acid sequences from protein database
- Step 3: Scoring method and theoretical spectra construction
- Step 4: Cross-correlation analysis

Step 1: MS data reduction

- Aim: to eliminate noise and to reduce the number of ions to be considered
- all but the 200 most abundant ions are removed
- the remaining ions are renormalised



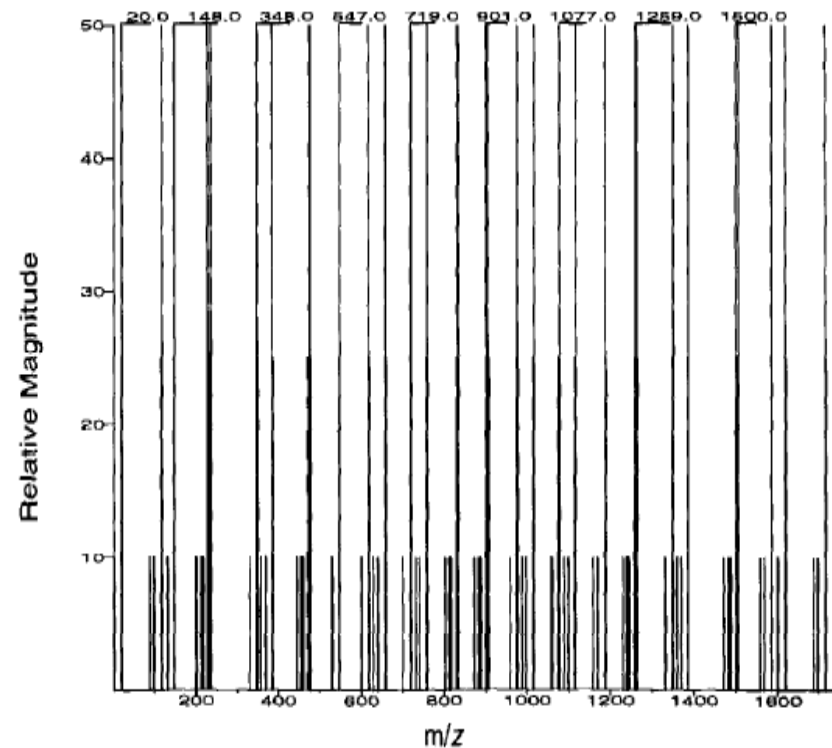
processed MS data for the peptide
DLRSWTAADTAAQISQ

Step 2: Search method

- possible amino acid sequences are identified in a protein database
- protein sequences are retrieved and scanned to find linear combinations of amino acids that match the mass of the peptide

Step 3: Scoring method

- The fragment ions are predicted for the sequences derived from database
- They are compared to the MS spectra using a scoring function
- Theoretical spectra is created for the top 500 best fit sequences



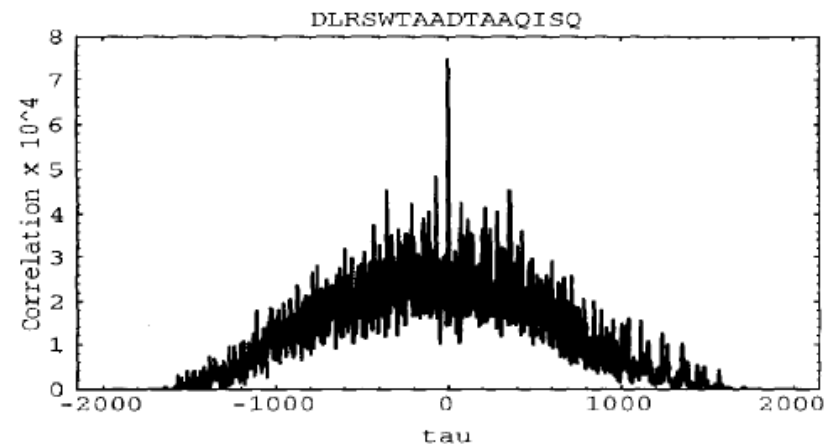
Theoretical spectra for the peptide
DLRSWTAADTAAQISQ

Step 4: Cross-correlation analysis

- Theoretical spectras are compared to the experimental spectra using cross-correlation
- The final score attributed to each candidate peptide sequence is the value of the function at $\tau=0$ minus the mean of the cross-correlation function over the range $-75 < \tau < 75$
- The scores are normalized to 1.0 and the peptide sequence with the biggest score is selected

$$R_{\tau} = \sum_{i=0}^{n-1} x[i]y[i + \tau]$$

x is theoretical spectra
y is experimental spectra
 τ is a displacement value



Cross-correlation function for the peptide
DLRSWTAADTAAQISQ

Application: HDL proteomics

- Quantify the protein composition of HDL
→ provide insights into the properties of HDL
- 33 subjects (7 CAD patients)
- Shotgun proteomics used for protein identification

Results (1 / 2)

- 48 proteins identified in HDL
 - 22 linked to cholesterol and lipoprotein metabolism
 - 23 acute-phase-response proteins
- Fundamental role for HDL in innate immunity

Results (2/2)

- HDL₃ from CAD patients is selectively enriched in some proteins related to inflammatory response
- Individual protein levels as markers of CAD?

Discussion

- HDL proteomics provides new information on HDL functions
- The degree of variation in the number and identity of HDL proteins large
- Future direction: find simple HDL biomarkers to predict CHD

Conclusions

- Lipoproteins are closely related to coronary heart disease
- Shotgun proteomics is a useful method to identify individual proteins in large protein complexes
- SEQUEST method can be used to identify proteins from experimental MS spectras
- Proteomics may provide new information on coronary heart disease