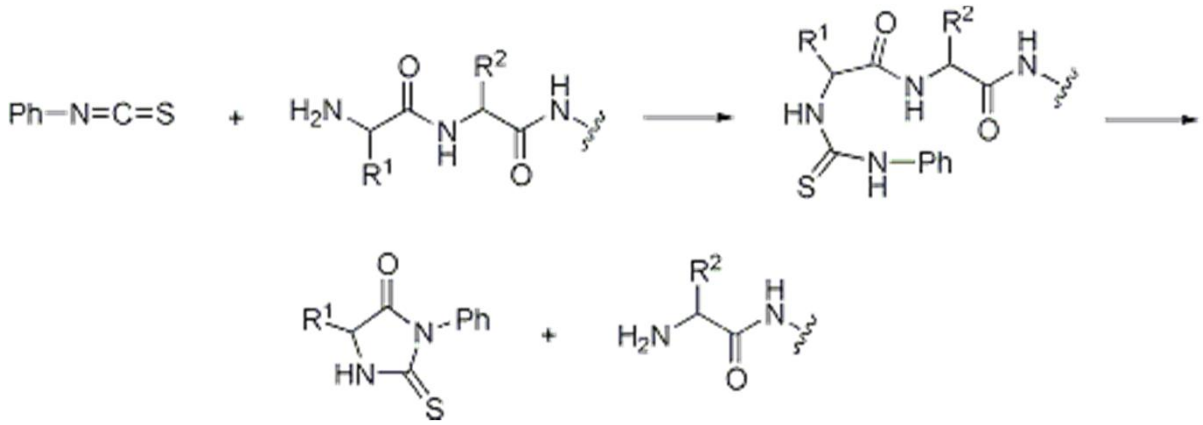
Edman Degradation

Cyclic degradation of peptides based on the reaction of phenylisothiocyanate with the free amino group of the *N*-terminal residue such that amino acids are removed one at a time and identified as their phenylthiohydantoin derivatives:



P. Edman, *Acta Chem. Scand.* **4,** 283 (1950).

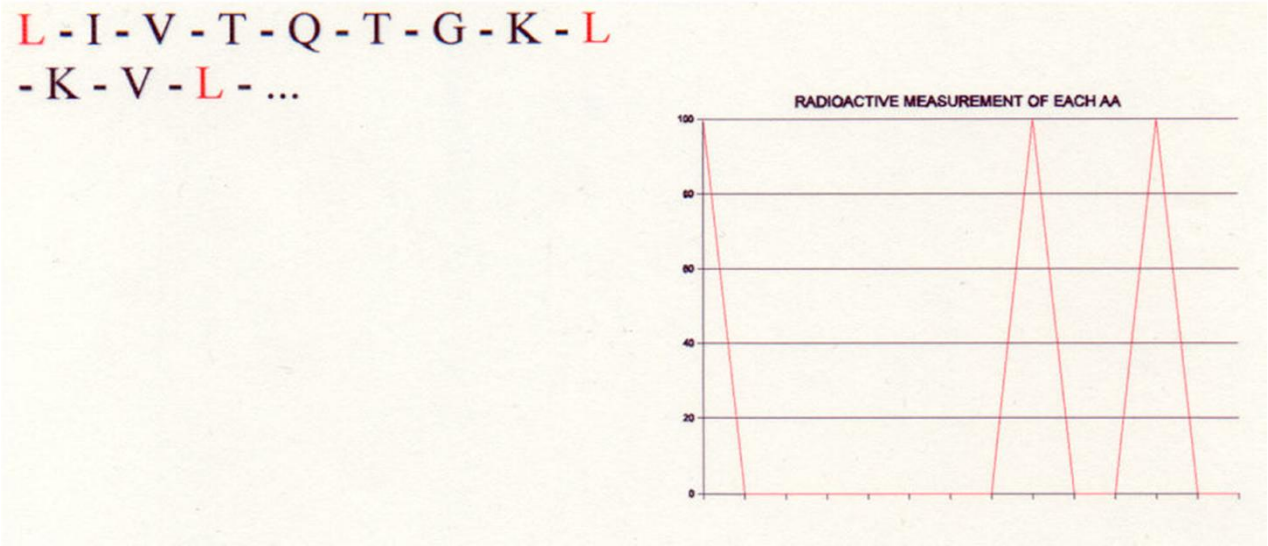
MICROSEQUENCING

BASED ON CHEMICAL REACTION OF THE
N-TERMINAL AMINO ACID
(EDMAN DEGRADATION)

CAN BE PERFORMED MANUALLY (MANUAL SEQUENCING)
--USAGE OF SEVERAL SAMPLES AT THE SAME TIME
4 – 5 AMINO ACIDS PER DAY

THOUGH USUALLY PERFORMED BY AUTOMATED
INSTRUMENTATION (=MICROSEQUENCING)

EXCEPTION: RADIOSEQUENCING
(RADIOSEQUENCING = SOME PARTICULAR AMINO ACIDS ARE
RADIOACTIVELY LABELLED)



ADVANTAGES OF AUTOMATED SEQUENCING

- EFICIENCY IS EXTREMELY HIGH (~ 97%)

(HOWEVER, ONLY APPROXIMATELY 60% OF THE TOTAL SAMPLE IS USED)

- FAST SEQUENCING CYCLE TIMES
  (~ 20 - 30 MINUTES / AA)

- HIGH SENSITIVITY (LESS THAN 10 PMOL)

C-TERMINAL SEQUENCING

→ EXTREMELY RARELY USED
(NOT BASED ON EDMAN CHEMISTRY)

CHEMICAL OR ENZYMATICAL REACTION
(CARBOXYPEPTIDASES)

BLOCKAGE OF THE N-TERMINAL AMINO ACID:

N-TERMINAL ENDING OF A PROTEIN IS OFTEN BLOCKED BY:

ACETYLATION
FORMYLATION
PYROLYZATION (GLUTAMATE)
NATURAL CYCLIZATION

ETC. SEVERAL BLOCKAGES KNOWN
CAUSED *IN VIVO AND/OR IN VITRO*

TO GO AROUND

- DIGESTION OF THE BLOCKED PROTEIN
- ISOLATION OF THE PEPTIDES
  (SPECIFIC ISOLATION OF N- OR C- TERMINAL PEPTIDES)
- MASS SPECTROMETRIC ANALYSIS AND SEQUENCING
- NORMAL SEQUENCING OF THE ISOLATED PEPTIDES == IDENTIFICATION OF THE PROTEIN

PREPARING THE SAMPLE FOR MICROSEQUENCING

- HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY)
- HPCE (HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS)
- HPEC (HIGH PERFORMANCE ELECTROPHORESIS CHROMATOGRAPHY)
- PAGE (POLYACRYLAMIDE GEL ELECTROPHORESIS)

HPLC

MICROBORE CHROMATOGRAPHY (i.d. 2.1 - 1.1 mm)

- GF, SAX, SCX, RP, HIC ...

(ADVANTAGES: FAST 20 - 30 MIN, HIGH SENSITIVITY)

HPCE

- HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS

HPEC

- HIGH PERFORMANCE ELECTRO CHROMATOGRAPHY

PAGE

- ELECTROBLOTTING

BLOT ⇨ PVDF/NC

☞ THE BLOTS MAY BE STAINED WITH SEVERAL DYES

☞ YOU MAY SEND A MEMBRANE BY MAIL

☞ YOU MAY KEEP YOUR MEMBRANE FOR "EVER"

☞ YOU MAY PROCESS YOUR PROTEIN FROM THE MEMBRANE

PVDF CAN BE USED FOR SEQUENCING, NC NOT

AUTOMATED 2 - D, MICRO-GELS

GO SMALL, IT GIVES A HIGHER SENSITIVITY

⇨ YOU GET MORE INFORMATION FROM LESS SAMPLE

LIMITATIONS FOR SEQUENCE ANALYSIS

- ☞ SIZE OF THE PROTEIN (AD 100 kDa)
- ☞ UNPURE SAMPLE

  (BOTH RESULT IN HIGH BACKGROUND)

SPECIALITIES

- ☞ USING SPECIFIC N - OR C - TERMINAL - BINDING MEMBRANES (SOLID PHASE SEQUENCING)
- ☞ CLEAVAGE IN THE GEL

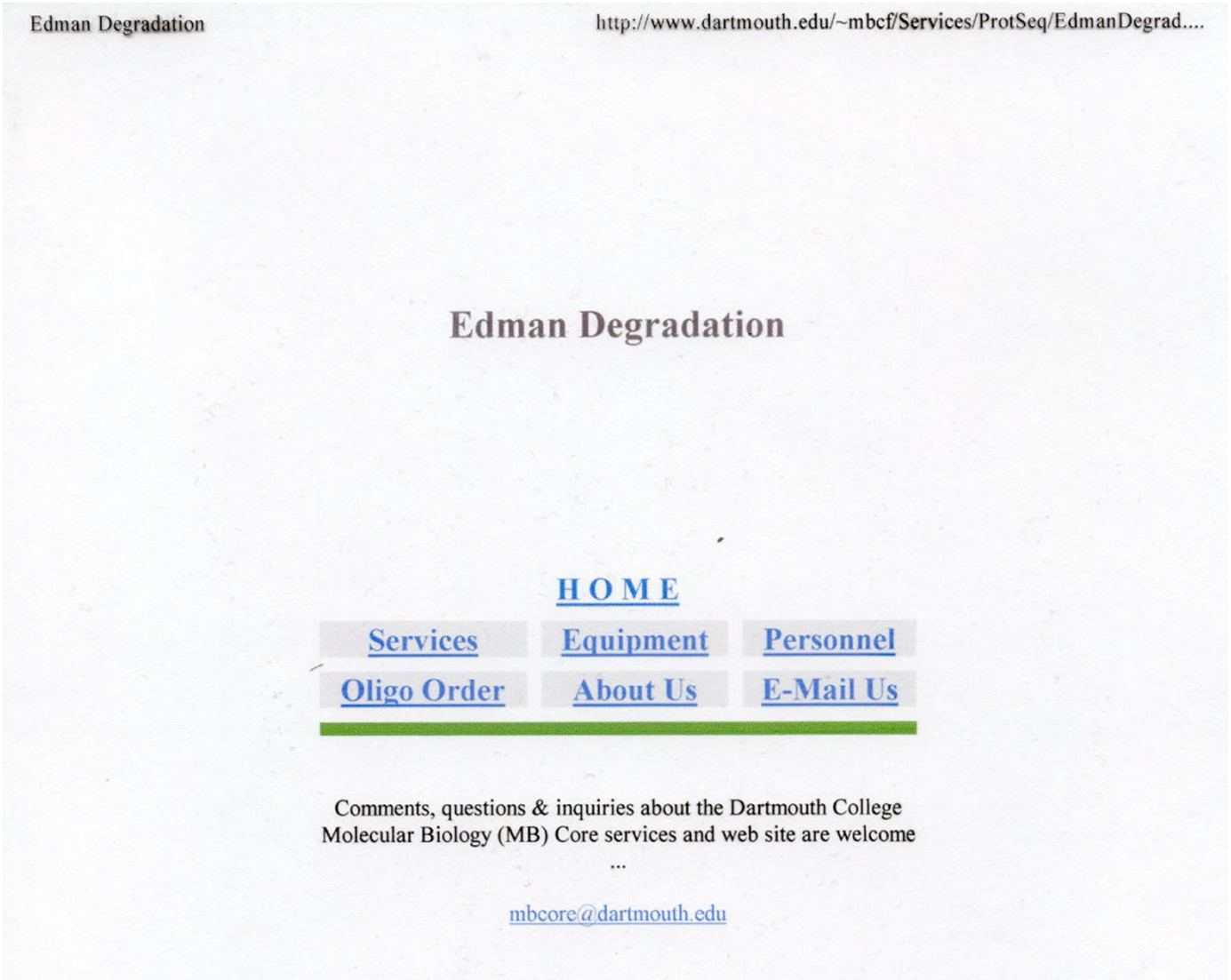
MICROSEQUENCING IS USUALLY DONE IN SPECIAL LABORATORIES FOR PROTEIN CHEMISTRY SERVICES

HERE IS ONE LINK AMONG OTHERS:

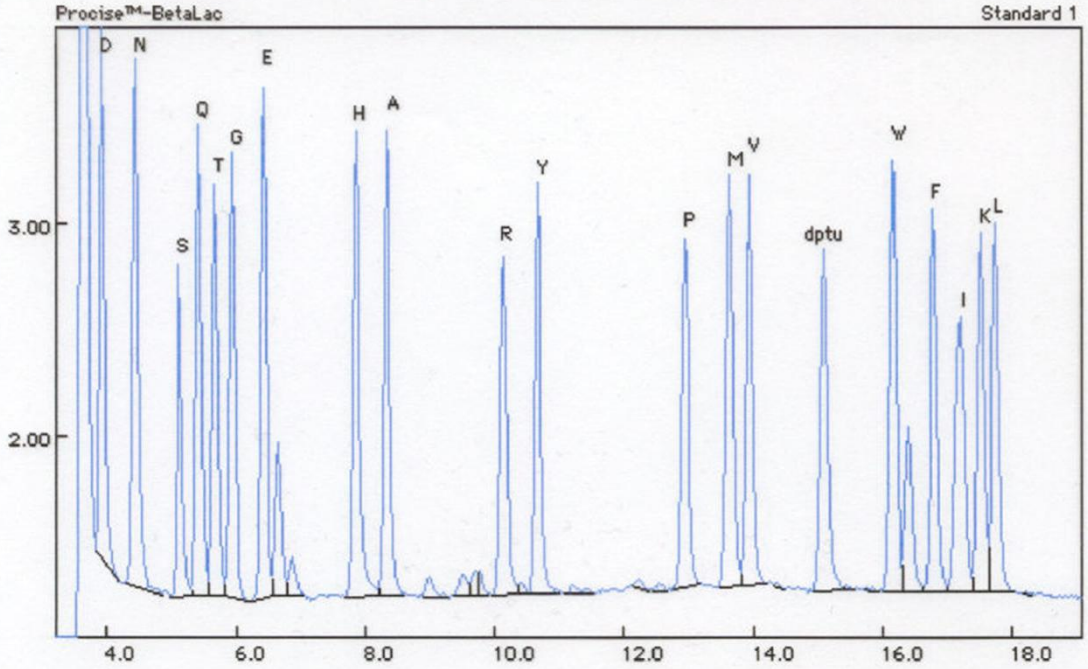
(THIS LINK OFFERS EVEN AN ANIMATION OF EDMAN DEGRADATION)

http://www.biotech.iastate.edu/facilities/protein/nsequence494.html

HERE ANOTHER:



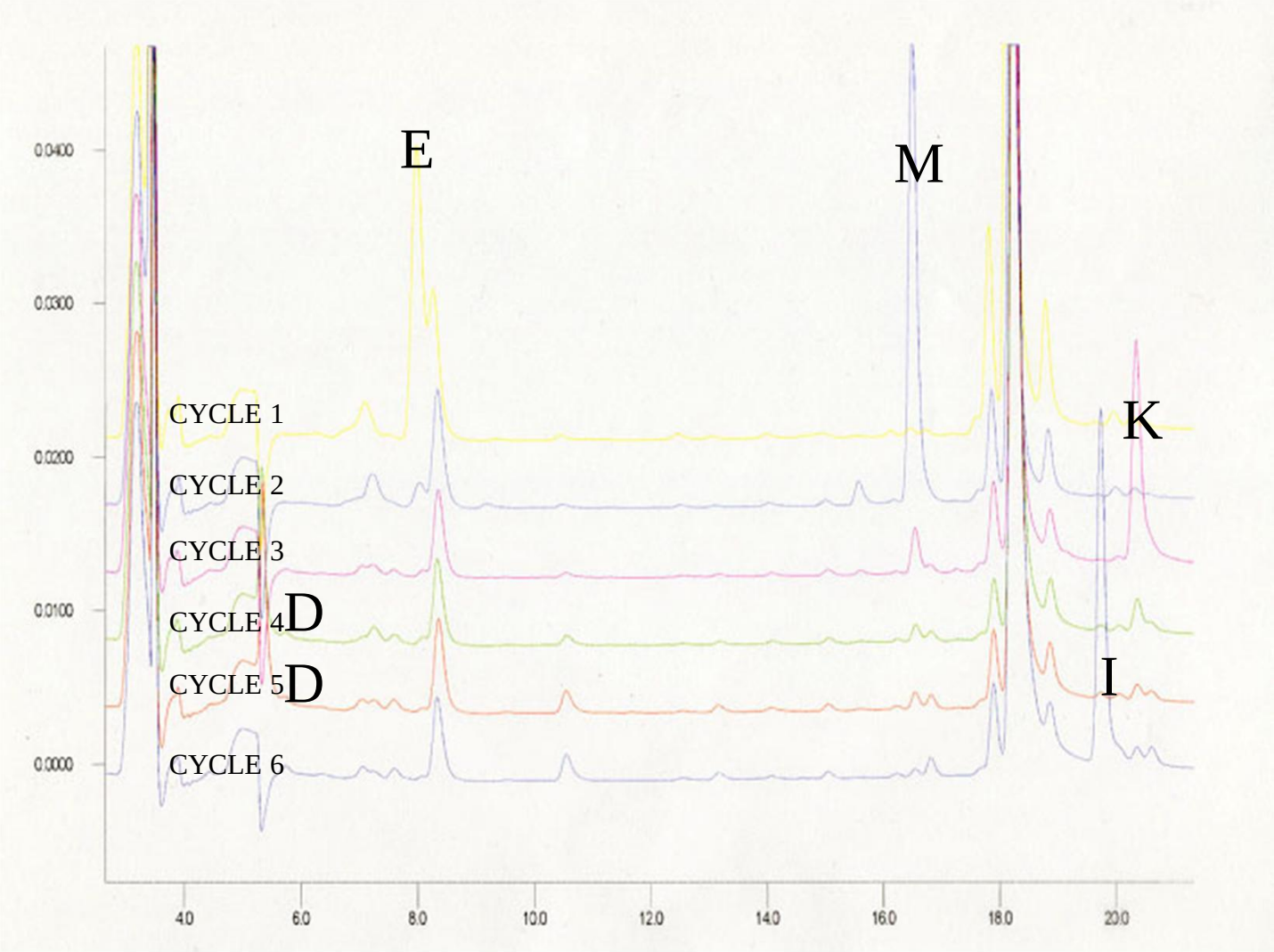
Protein microsequencing by Edman degradation



Dr. Simona Fontana, responsible of microsequencing service.

SEQUENCE ANALYSIS BY LIQUID CHROMATOGRAPHY

EACH CYCLE RESEMBLES ONE CHROMATOGRAPH RUN



A TYPICAL EXAMPLE:

AQUIRED SEQUENCE (6 FIRST AMINO ACIDS):

GLU-MET-LYS-ASP-ASP-ILE