## fizikalna kemija makromolekula

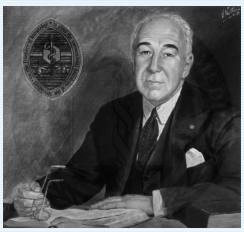
makromolekule

sintetski polimeri

biološki (prirodni) polimeri

fizikalna kemija proteina

**Edwin J. Cohn**



Simoni R D et al. J. Biol. Chem. 2002;277

Topljivost i kiselo-bazna svojstva proteina

Cohn, E. J., Hendry, J. L., and Prentiss, A. M., "Studies in the Physical Chemistry of the Proteins. V. Molecular Weights of the Proteins", *J. Biol. Chem.* 63 (1925) 721-766.

Cohn, E. J., "The Properties and Functions of Plasma Proteins with consideration of the Methods for their Separation and Purification", *Chem. Rev.* 28 (1941) 395.

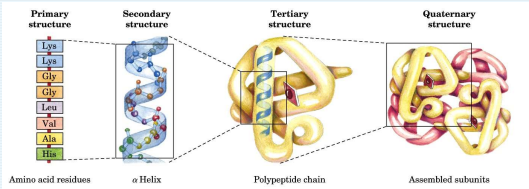


jbc

## Klasični način opisa proteina:

- Primarna struktura - redoslijed (sekvencija) aminokiselina
- Sekundarna struktura – konformacija peptidnih lanaca
- Tercijarna struktura
- Kvaterna struktura

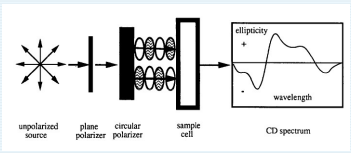
## Razine strukture proteina




## Spektroskopsko istraživanje sekundarne strukture proteina

- Cirkularni dikroizam (*Circular dichroism*, CD)
- Infracrvena (IR) i Raman spektroskopija
- Nuklearna magnetska rezonancija (NMR)

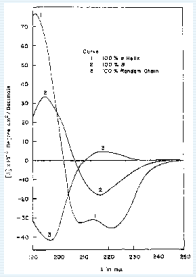
## Fizikalni principi CD-a




- Kiralne ili asimetrične molekule daju CD spektar zato jer različito apsorbiraju lijevo i desno polariziranu svjetlost i zato se smatraju "optički aktivnim"

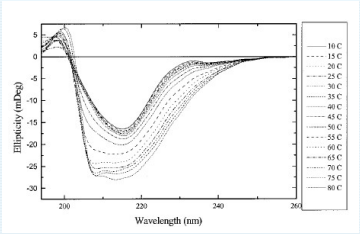
## Sekundarna struktura proteina iz CD spektara

- različita vrsta proteina daje različite CD spektre



## Sekundarna struktura proteina iz CD spektara

- CD spektri su posebno korisni za određivanje temperaturne ovisnosti sekundarne strukture proteina.




## Sekundarna struktura proteina iz CD spektara

ostale informacije:

- (1) interakcije protein - ligand;
- (2) termodinamika smatanja (*folding*) proteina;
- (3) promjene konformacije i agregacija proteina;
- (4) međuprodukti smatanja;
- (5) kinetika smatanja proteina.

N. J. Greenfeld, Applications of circular dichroism in protein and peptide analysis, *Trends in analytical chemistry*, vol. 18, no. 4, 1999

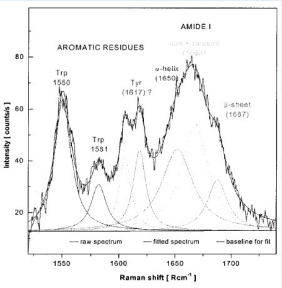
## Sekundarna struktura proteina iz IR i Ramanskih spektara (vibracije!)

- amidne vrpce se najčešće koriste za istraživanje strukture proteina

Principal Amide I Frequencies Characteristic of Protein Secondary Structures

| Conformation | $H_2O$ | $D_2O$ |
|---|---|---|
| $\alpha$-helix | 1650–1657 | 1647–1654 |
| Antiparallel $\beta$-sheet | 1612–1640; 1670–1690 (weak) | 1628–1635 |
| Parallel $\beta$-sheet | 1626–1640 | |
| Turn | 1655–1675 1680–1696 | |
| Unordered | 1640–1651 | 1643 |

## Sekundarna struktura proteina iz IR i Ramanskih spektara (vibracije!)




- Ramanski spektri daju informacije o aromatskim ostacima u području oko 1620 $cm^{-1}$

## Sekundarna struktura proteina pomoću NMR spektroskopije

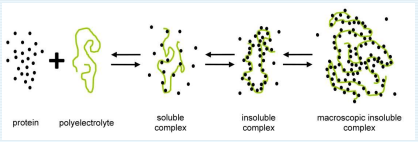
- Određivanje sekundarne strukture pomoću NMR spektroskopije ne zahtijeva potpunu trodimenzijsku strukturnu analizu kao što zahtijeva rendgenska kristalografija.
- Poznavanje kemijskih pomaka amida i protona su u principu sve što je potrebno.
- Spektroskopija NMR je najsnažnija i najtočnije metoda određivanja sekundarne strukture proteina bez trodimenzijske strukture.

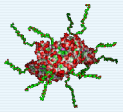
## Interakcija proteina sa...

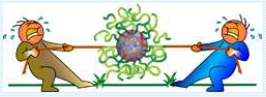
- ... polielektrolitima
- ... polisaharidima
- ... DNA
- ... itd

- Primjena!!

## Kompleksi protein-polielektrolit

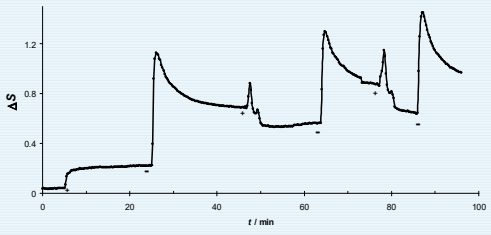


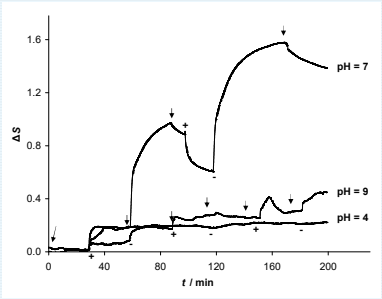


*Karakterizacija polielektrolitno-proteinskih višeslojeva pomoću optičke reflektometrije*

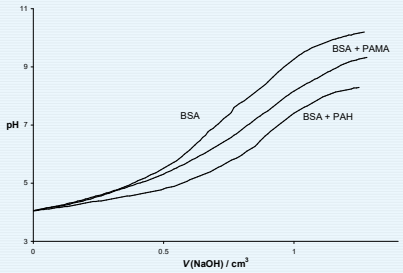
$PVP^+$/BSA




PAMA/BSA

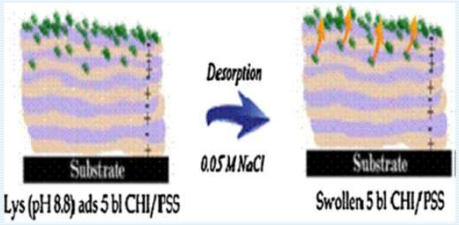



## potenciometrijska titracija

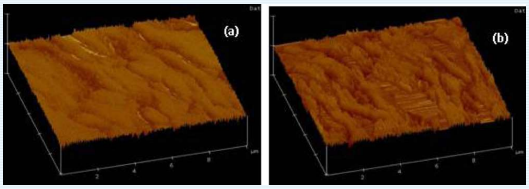



J. Mathew et al., *Fabrication of switchable protein resistant and adhesive multilayer membranes*, Colloids and Surfaces B: Biointerfaces 94 (2012) 118– 124

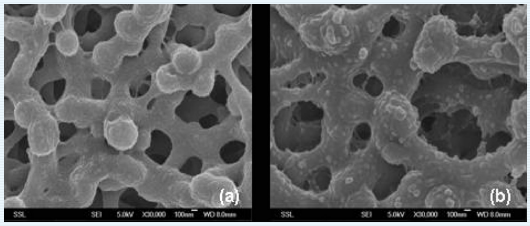
- Fabrication of protein adhesive and resistant surfaces based on chitosan/polystyrene sulfonate (CHI/PSS) multilayer membranes is presented. Adsorption behavior of bovine serum albumin (BSA) and lysozyme to CHI/PSS multilayer was studied.




Shematski prikaz adsorpcije lizozima
(a) pri pH = 8,8 (b) desorpcija, reverzna migracija na površinu.

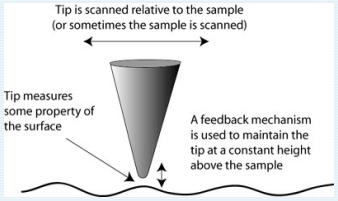



AFM prikazi adsorpcije lizozima na CHI/PSS višesloj (10 slojeva)
(a) pH = 8,8 (b) pH = 10,6




SEM prikazi adsorpcije BSA (pH 4.8)
(a) 6 CHI/PSS slojeva (b) 14 CHI/PSS slojeva.

## "Scanning Probe Microscopy" tehnike




## Atomic Force Microscopy (AFM)

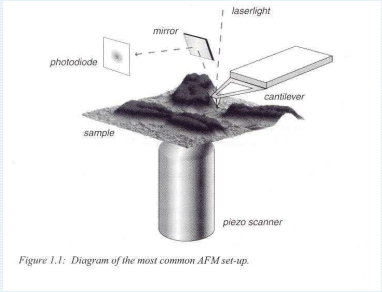
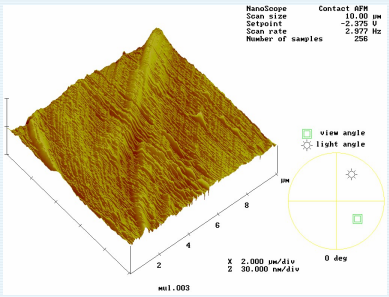



Figure 1.1: Diagram of the most common AFM set-up.

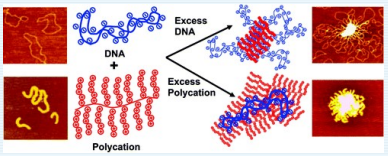
*Contact mode*
Kontaktni režim rada

*Tapping mode*
Tapkajući režim rada

## Atomic Force Microscopy (AFM)



Polyelectrolyte – DNA complexes




D. Störkle, S. Duschner, N. Heimann, M. Maskos, and M. Schmidt, Complex Formation of DNA with Oppositely Charged Polyelectrolytes of Different Chain Topology: Cylindrical Brushes and Dendrimers, *Macromolecules*, 2007, 40 (22), pp 7998–8006.

Seminarske prezentacije:

cca 10 min + diskusija

19. 5.
26. 5.
9. 6.

16. 6. predrok