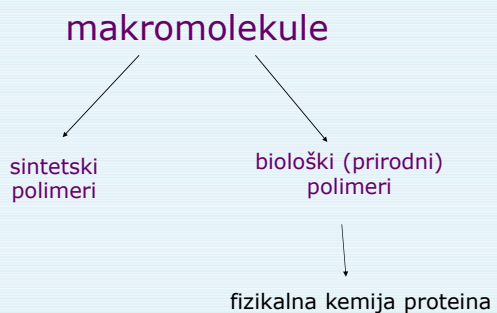


## fizikalna kemija makromolekula



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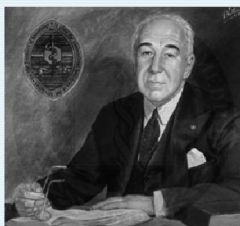
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### Edwin J. Cohn



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.

©2002 by American Society for Biochemistry and Molecular Biology

*jbc*

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## Klasični način opisa proteina:

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

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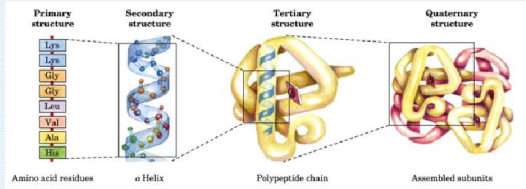
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## Razine strukture proteina




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## Spektroskopsko istraživanje sekundarne strukture proteina

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

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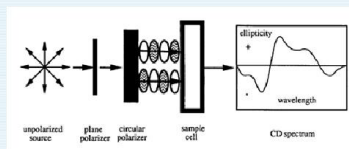
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## Fizikalni principi CD-a



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

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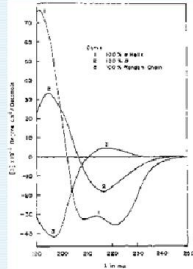
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## Sekundarna struktura proteina iz CD spektara

- različita vrsta proteina daje različite CD spektre



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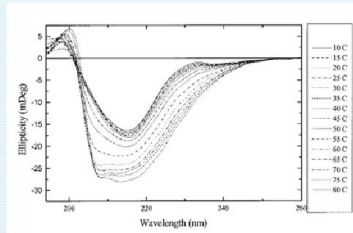
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## Sekundarna struktura proteina iz CD spektara

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



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## Sekundarna struktura proteina iz CD spektara

ostale informacije:

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

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

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## 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 <sub>2</sub> O	D <sub>2</sub> O
$\alpha$ -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

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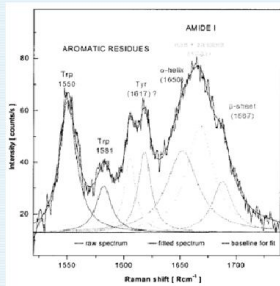
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## Sekundarna struktura proteina iz IR i Ramanskih spektara (vibracije!)



- Ramanski spektri daju informacije o aromatskim ostacima u području oko 1620 cm<sup>-1</sup>

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## 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.

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## Interakcija proteina sa...

- ... polielektrolitima
- ... polisaharidima
- ... DNA
- ... itd
  
- Primjena!!

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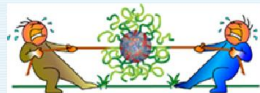
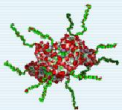
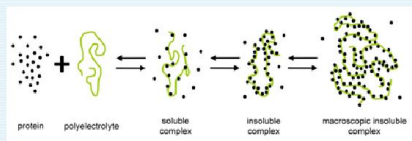
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## Kompleksi protein-polielektrolit



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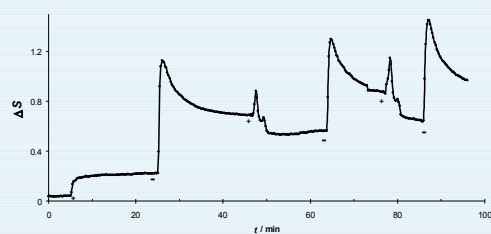
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## Karakterizacija polielektrolitno-proteinskih višeslojeva pomoću optičke reflektometrije

PVP<sup>+</sup>/BSA



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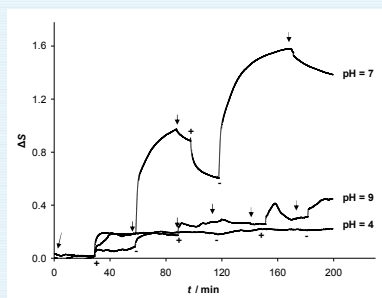
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### PAMA/BSA



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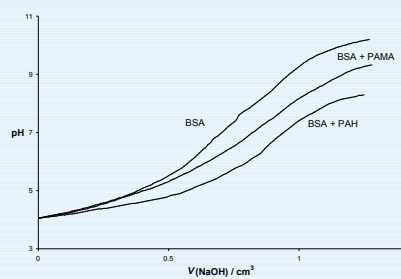
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### potenciometrijska titracija



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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.

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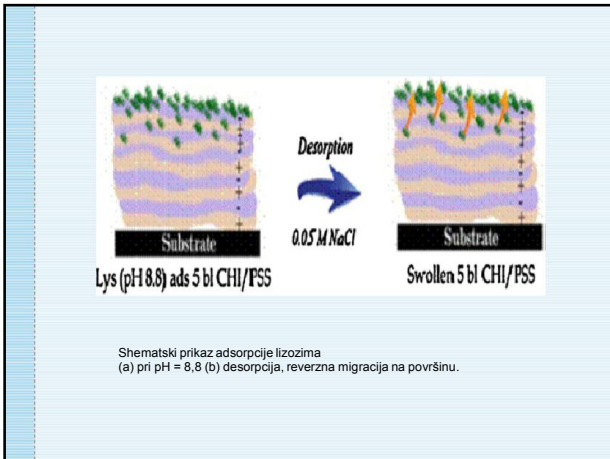
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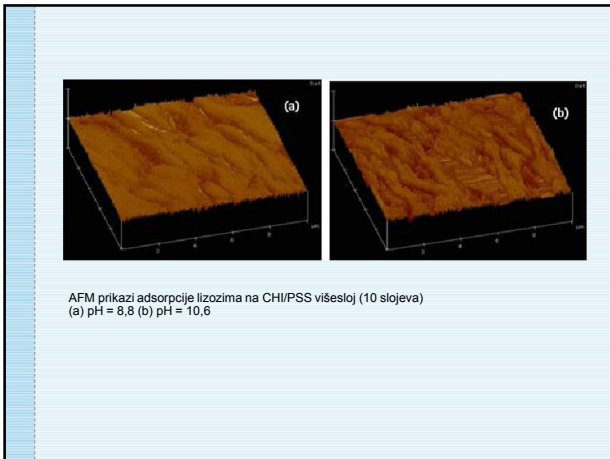
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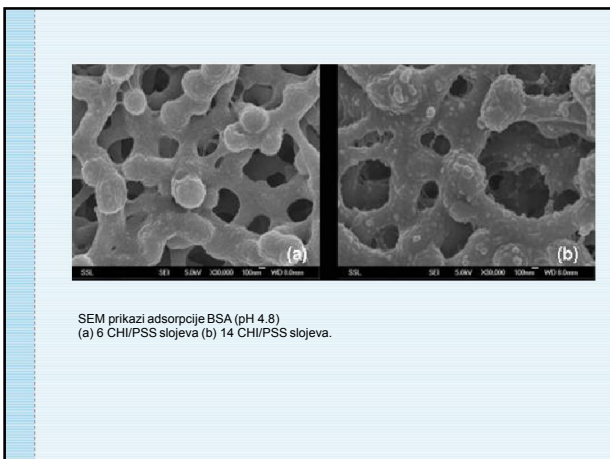
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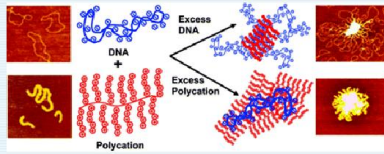
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## Polyelectrolyte – DNA complexes



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.

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