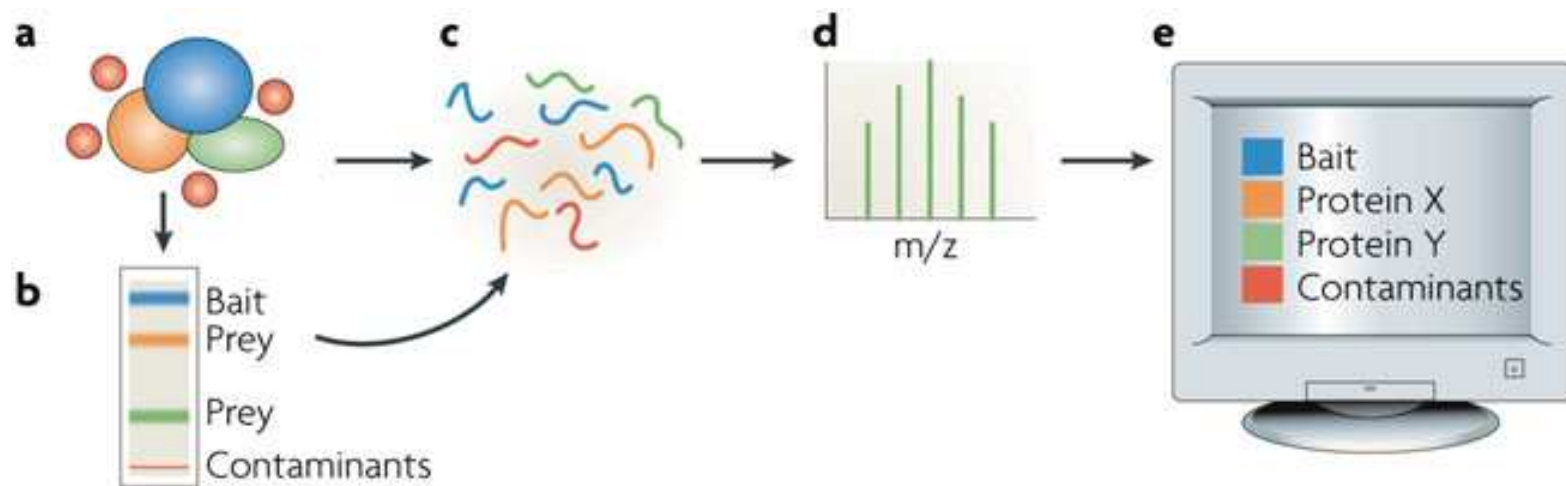


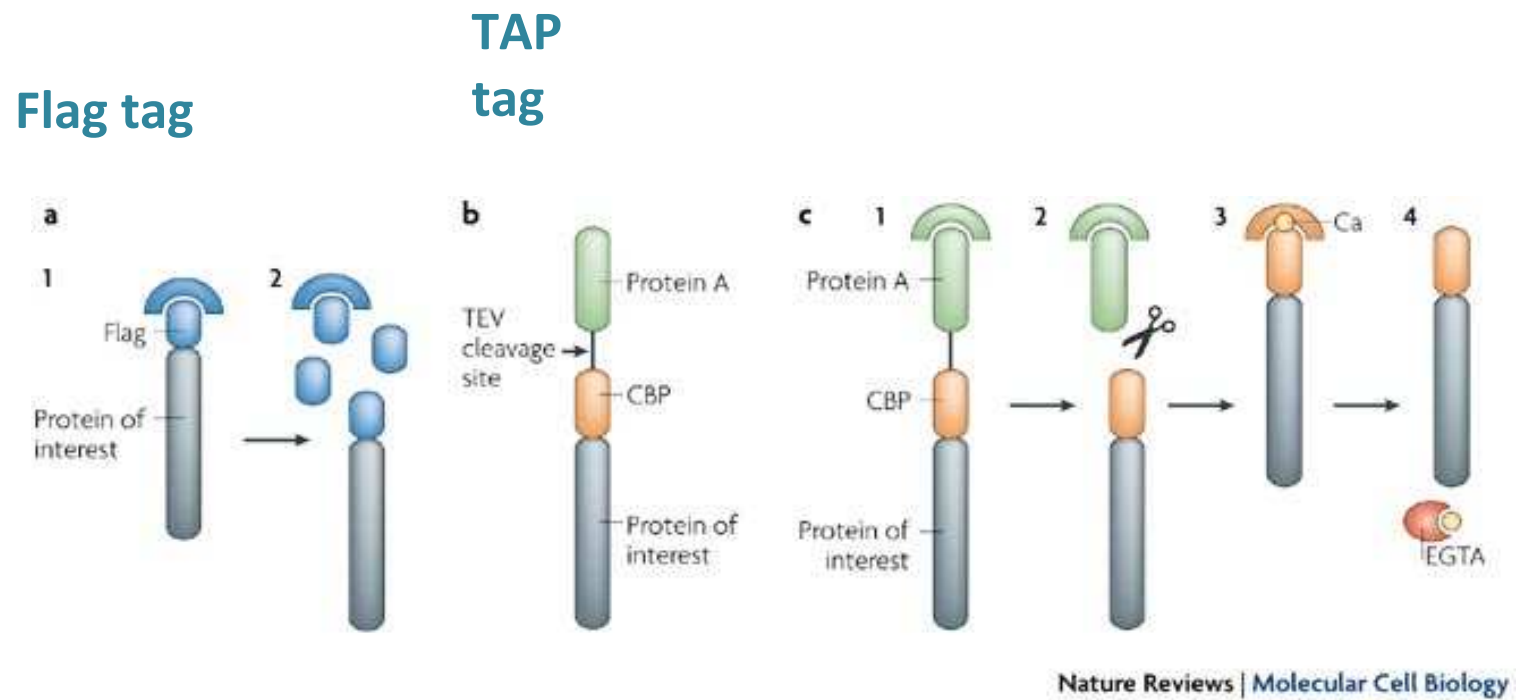
Analiza proteinskih kompleksov z masno spektrometrijo

Andreja Bratovš, Matja Zalar, Tamara Marić

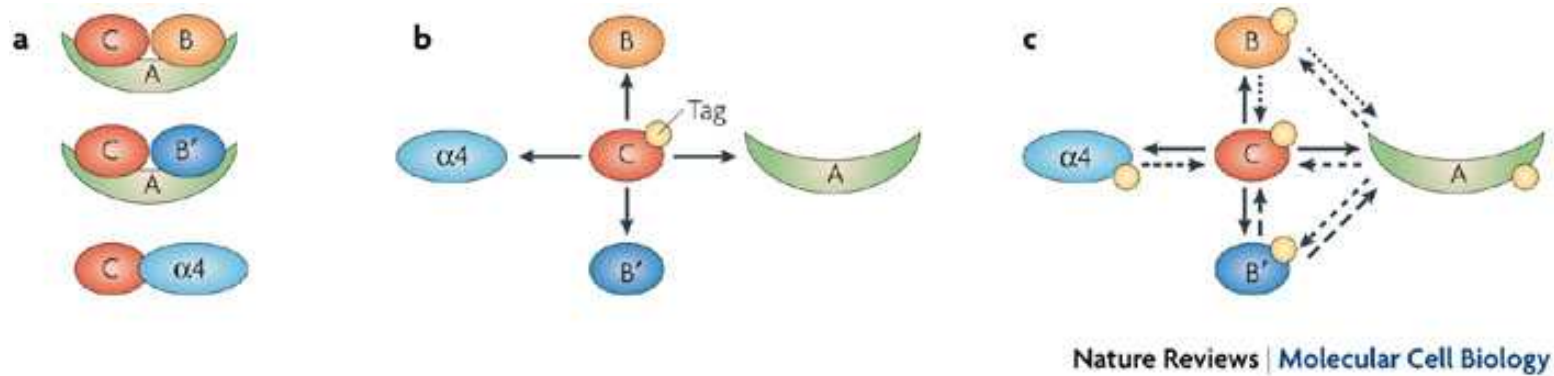
AP-MS



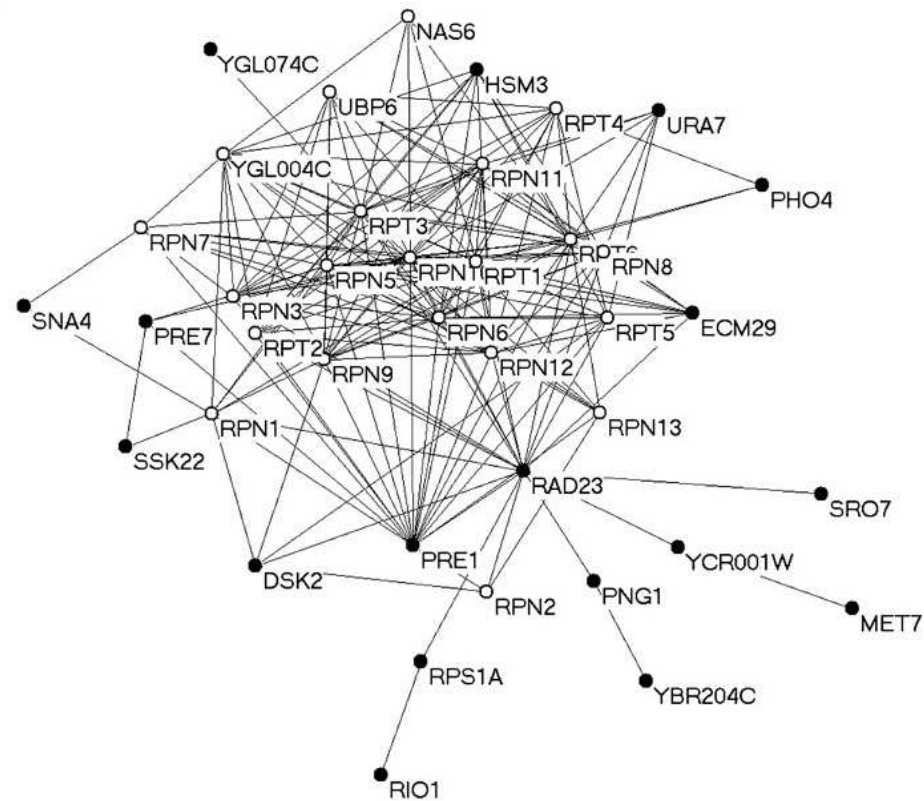
Afinitetne oznake

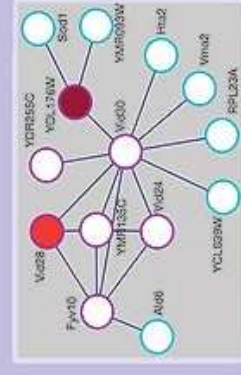
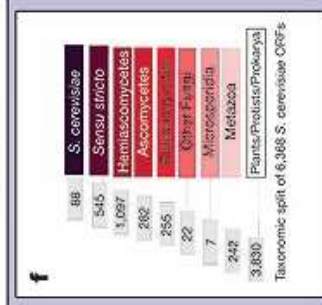


Identifikacija kompleksov PP2A

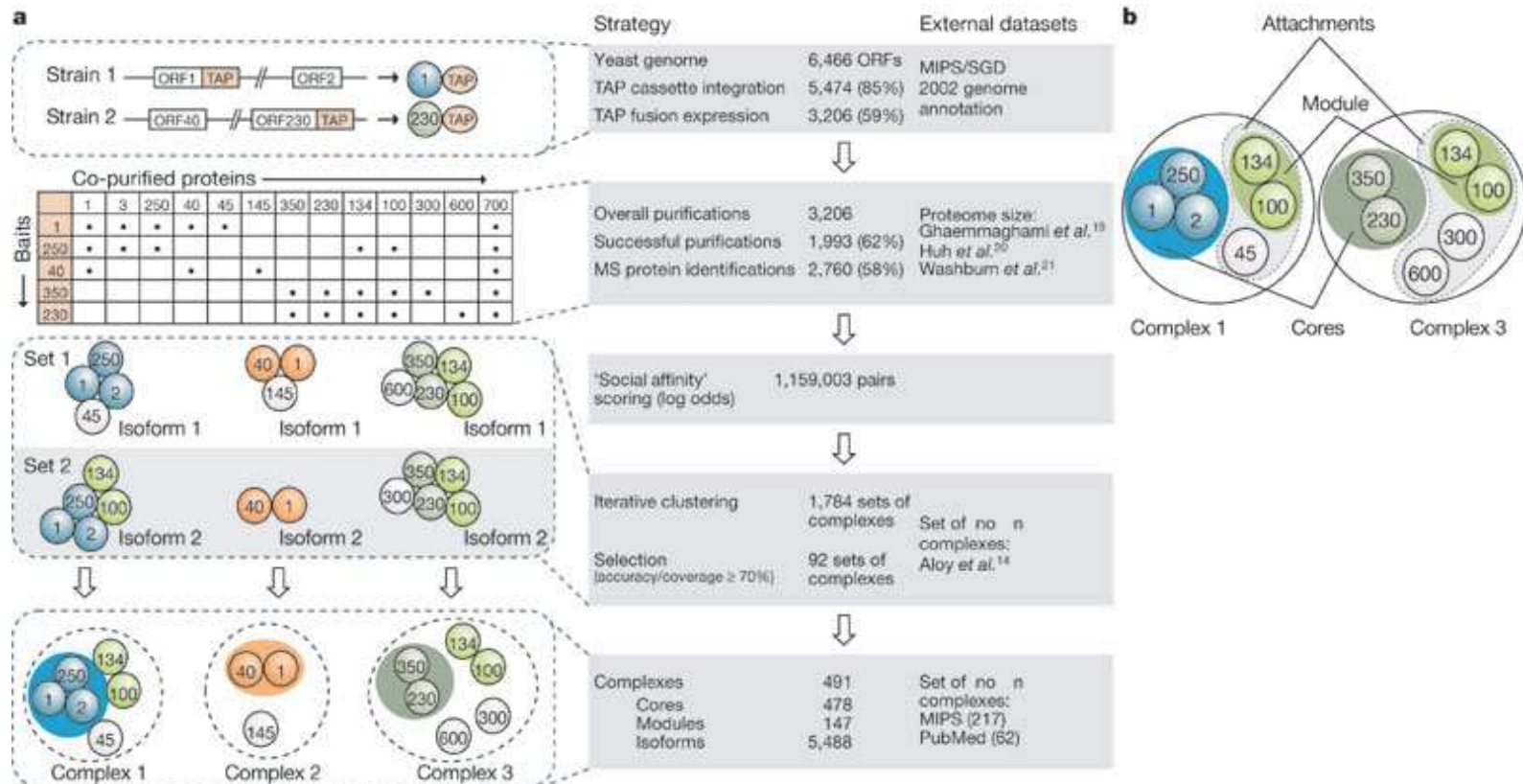


Graph-clustering algorithm





Socio-afinitetni indeks

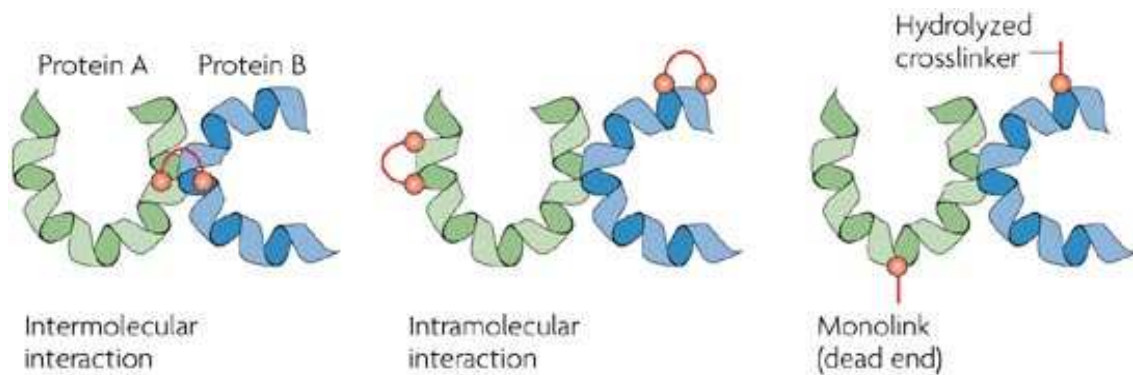


Primer čiščenja proteinskega kompleksa, ki vsebuje CDC45

- 1 Izkoriščamo biokemijske lastnosti proteinskega kompleksa
- 1 Prisotnost CDC45 v posameznih frakcijah preverjajo z imunoblottingom



Vezave prečnih povezovalcev

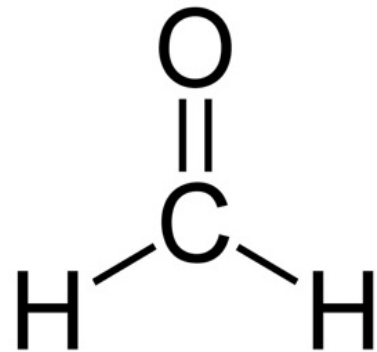


Nature Reviews | Molecular Cell Biology

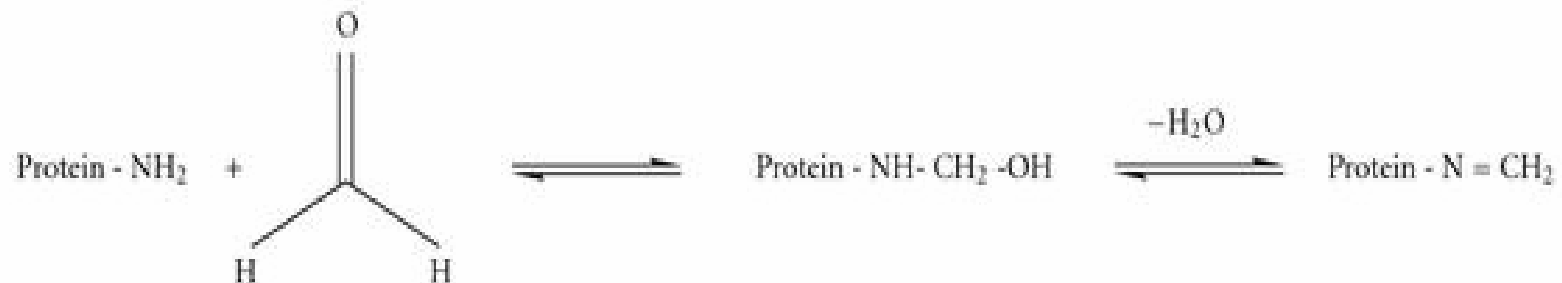
Za določanje strukture kompleksov so relevantne
le intermolekularne povezave

Prečni povezovalci

- formaldehid

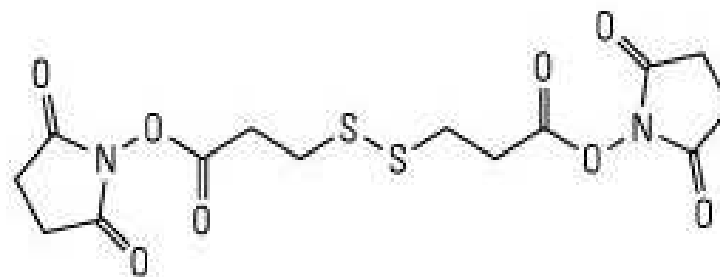


- 1 Vmesnik dolžine 2,3 Å
- 1 Zelo specifične vezave
- 1 Reagira s primarnimi amino skupinami, reakcija je reverzibilna
- 1 Uporaba *in vivo*



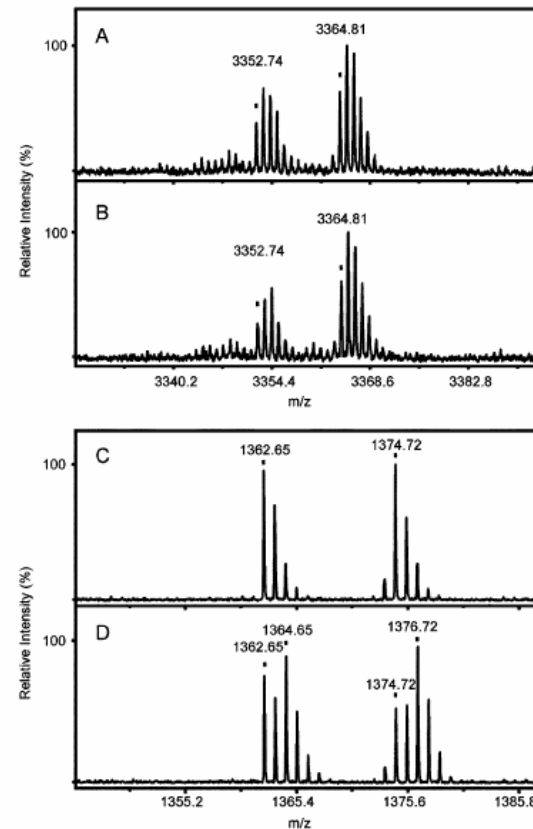
DSP- di-tiobis- sukcinimidil propionat

- 1 Reagira s primarno aaminsko skupino
- 1 Disulfidno vez v vmesniku se lahko cepi z DDT
- 1 Prehaja celično membrano
- 1 Uporaba *in vivo*

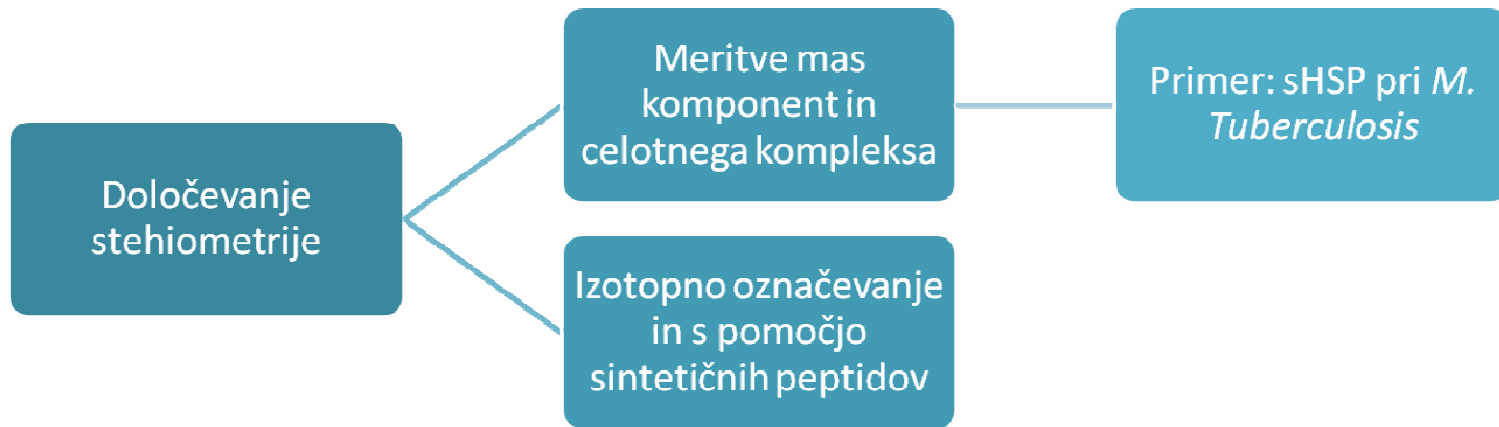


Določevanje tipa vezave prečnega povezovalca

- 1 Uporaba izotopno označenih prečnih povezovalcev
- 1 Eksperiment izvajamo v H_2O^{16} in mešanici $\text{H}_2\text{O}^{16}/\text{H}_2\text{O}^{18}$
- 1 Ločimo med crosslink in monolink vezavo

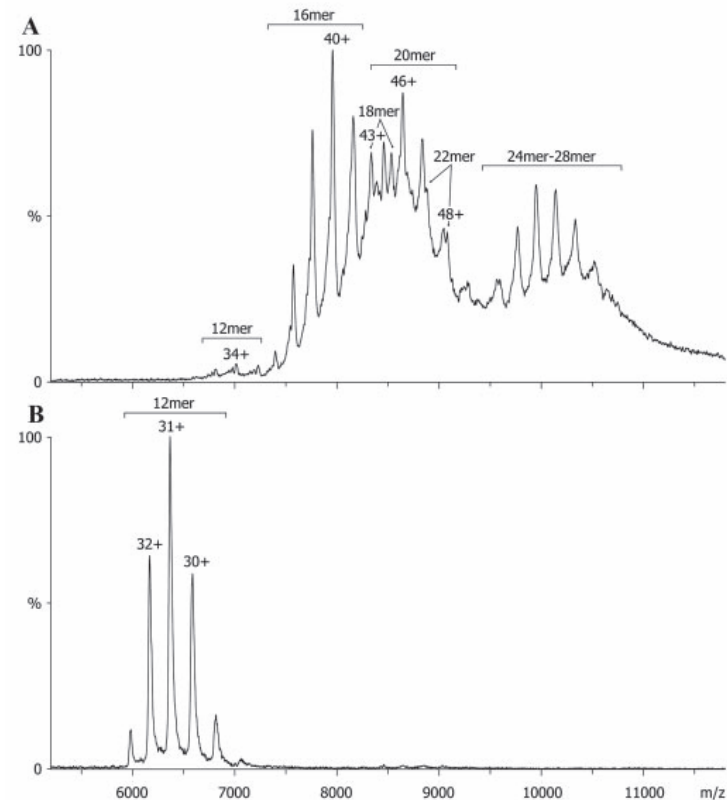


Stehiometrija kompleksa

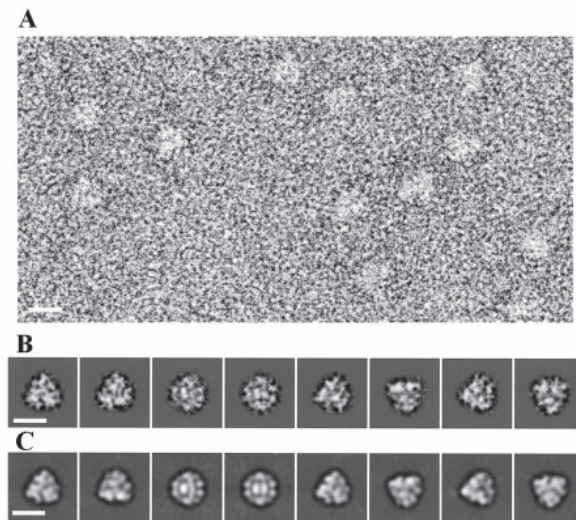


Stehiometrija sHSP-jev

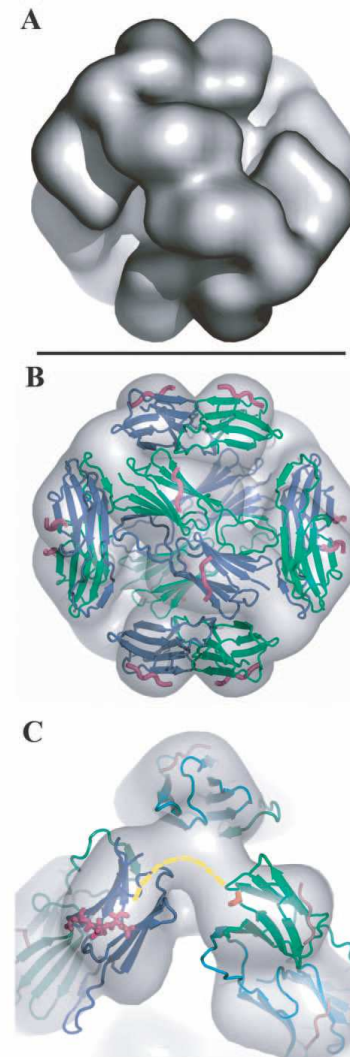
- Small heat shock proteina, arc2 in arc1
- Analiza pokazala stabilnost dimerov
- Drugačno obnašanje arc1 v primerjavi z arc2
- Analiza vrha pri arc1 razkrila, da gre za dodekamer



3D model arc1



3D rekonstrukcija arc1 s pomočjo negativnega barvanja elektroske mikroskopije



Dinamika kompleksa

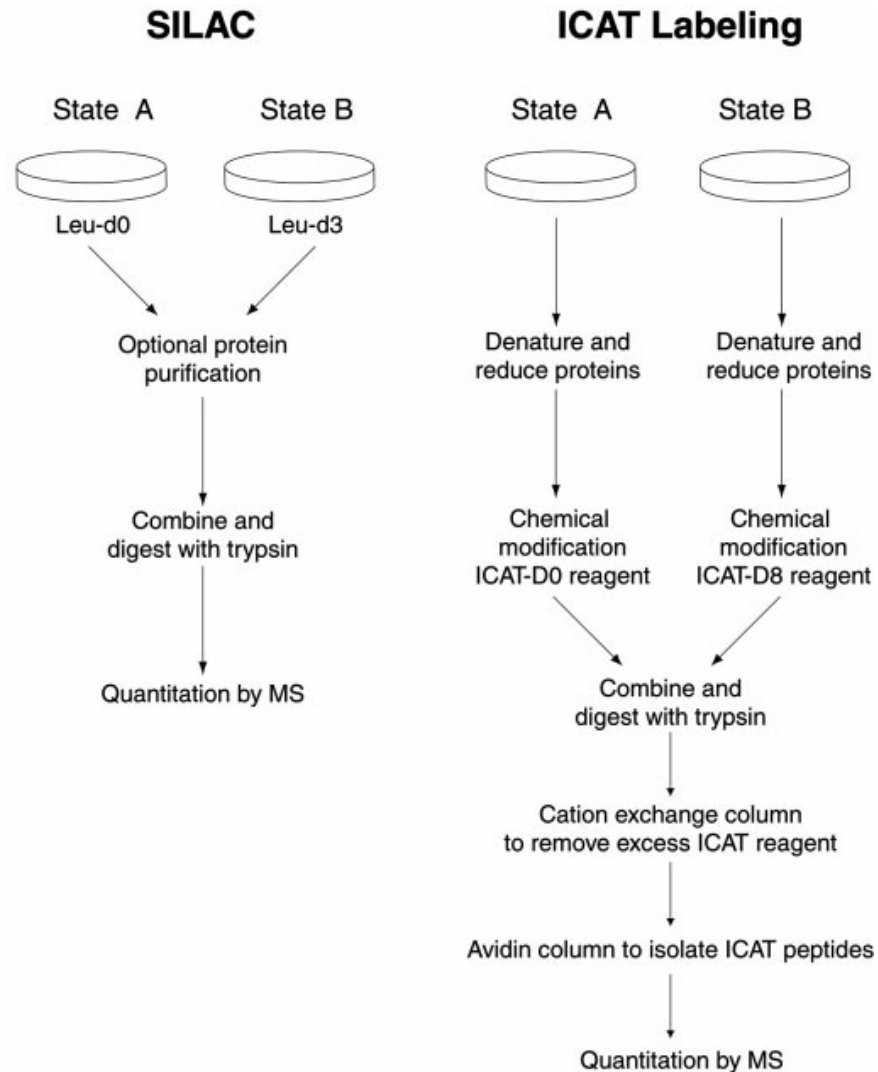
- MS nam omogoča, da s posebnimi metodami opazujemo dinamiko kompleksa (celična signalizacija)

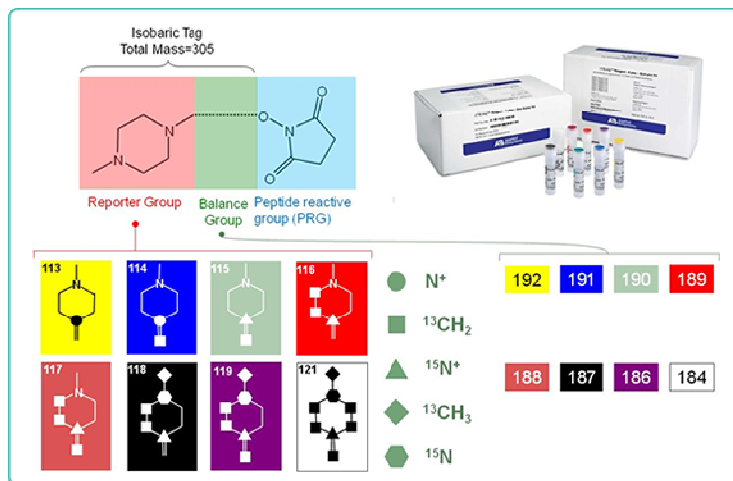
- Kvantitativna proteomika

- SILAC

- ICAT

- iTRAQ



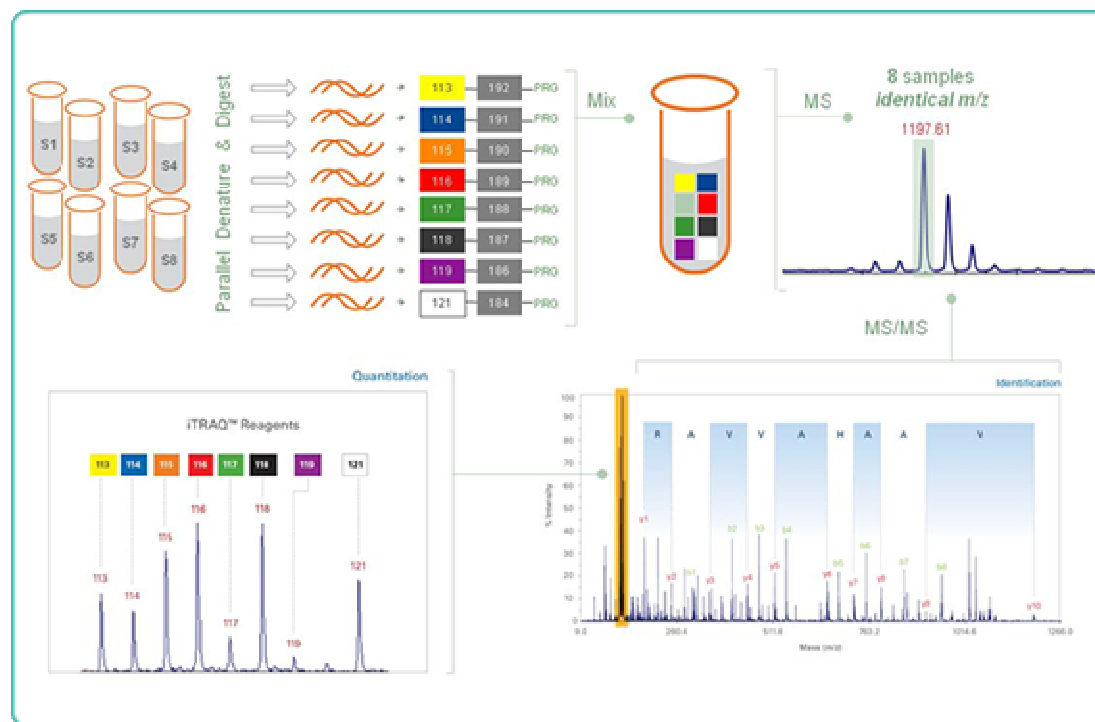


iTRAQ metoda

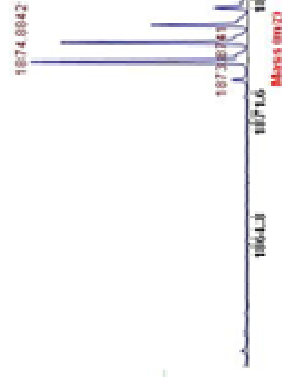
Do 8 različnih vzorcev ->

označen željen protein ->

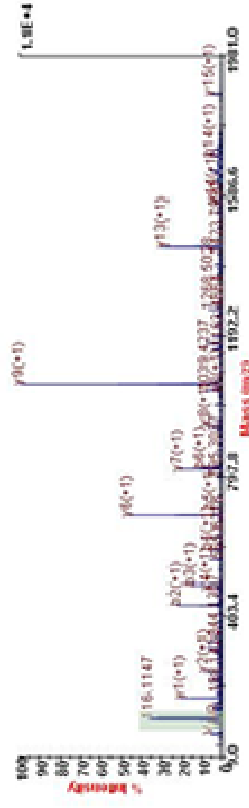
MS/MS -> kvantitativni rezultati



MS



MS/MS



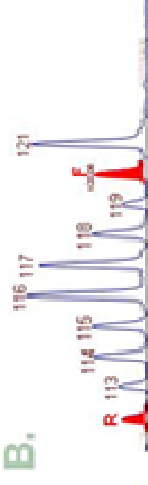
Reporter ions

A.



Expected Ratio:
1:1:1:1:1:1:1

B.



Expected Ratio:
1:2:2:5:5:2:1:5

