MD vs MS – PNP

PRIMJER INTERPRETACIJE MS EKSPERIMENATA MD SIMULACIJAMA

Heksamer *vs.* dimer - purinska nukleozidna fosforilaza (PNP) iz bakterije *E. coli*

RAZUMIJEVANJE REZULTATA MASENE SPEKROMETRIJE (MS) NA MOLEKULARNOJ RAZINI

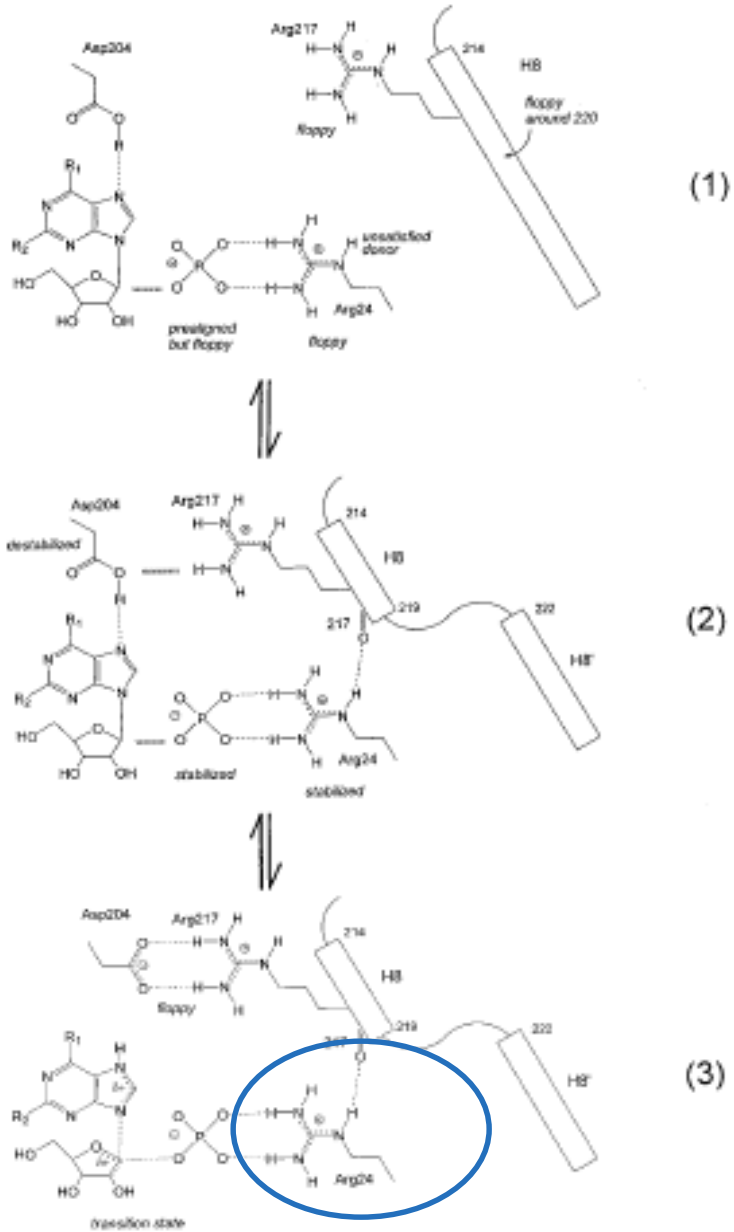
Sustavi koji su praćeni s MS i MD :

WT

1. APO
2. BINARNI KOMPLEKS SA FOSFATOM
3. TERNARNI KOMPLEKS (FOSFAT I NUKLEOZID)

R24A

1. APO
2. BINARNI KOMPLEKS SA FOSFATOM
3. TERNARNI KOMPLEKS (FOSFAT I NUKLEOZID)

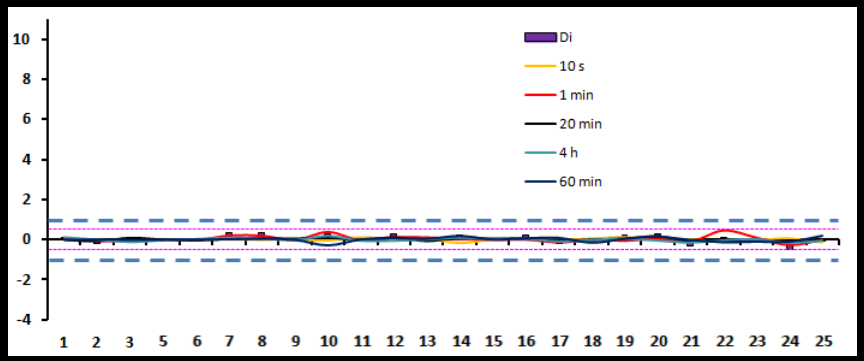


Scheme 6. Possible catalytic mechanism of *E. coli* PNP involving protonation of the base at position N7. Asp204 must be in the acid form prior nucleoside binding. Note that the mechanism allows large variations at the six-membered ring of the base, whereas replacement of N7 by C-H leads to complete loss of activity. This is actually observed in experiment (the enzyme allows 6-oxo- as well as 6-aminopurine nucleosides as substrates, and even cleaves benzimidazole nucleosides, whereas it is inactive towards 7-deazapurine nucleosides).

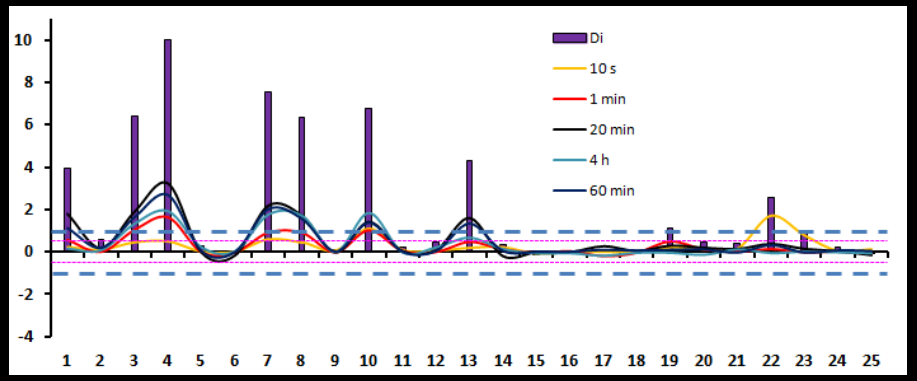
REZULTATI H/D IZMJENE PRAĆENE MASENOM SPEKTROMETRIJOM

Unliganded vs. binary complex with phosphate

A) R24A mutant of *E. coli* PNP protein

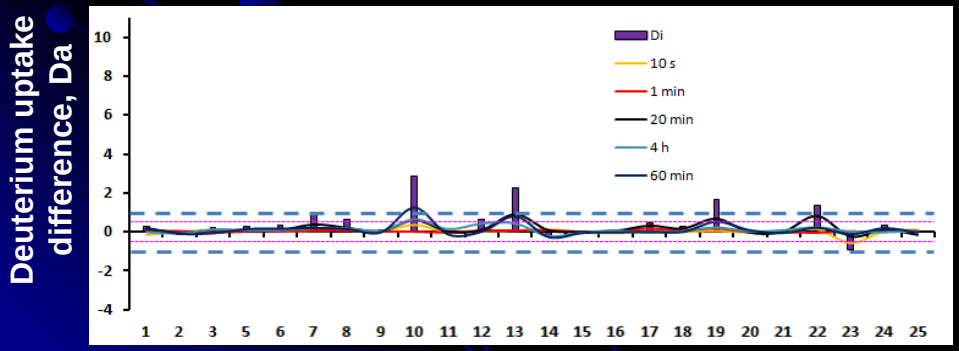


B) Wild type *E. coli* PNP protein

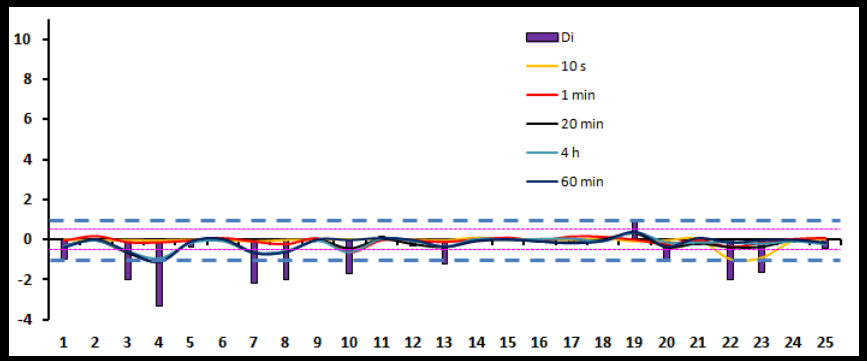


Binary complex with phosphate vs. ternary complex with phosphate and formyc

C) R24A mutant of *E. coli* PNP protein

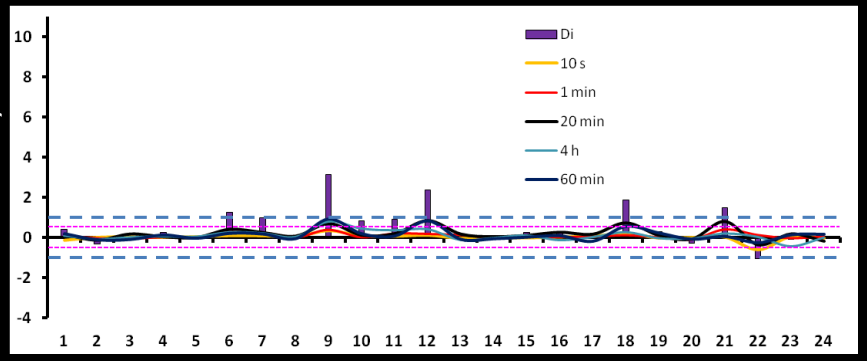


D) Wild type *E. coli* PNP protein

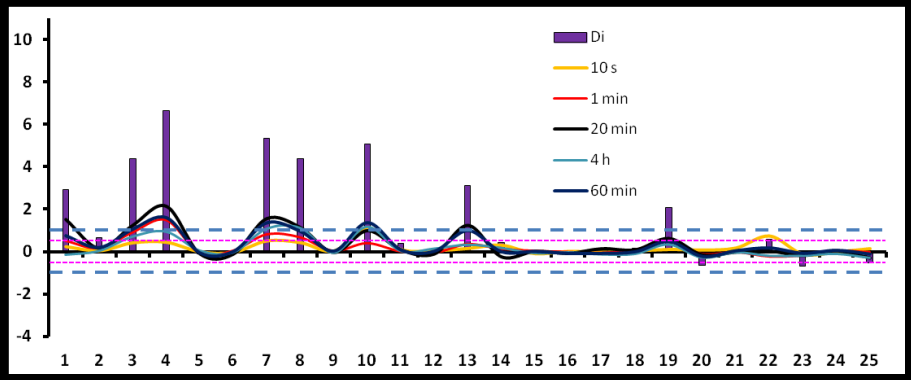


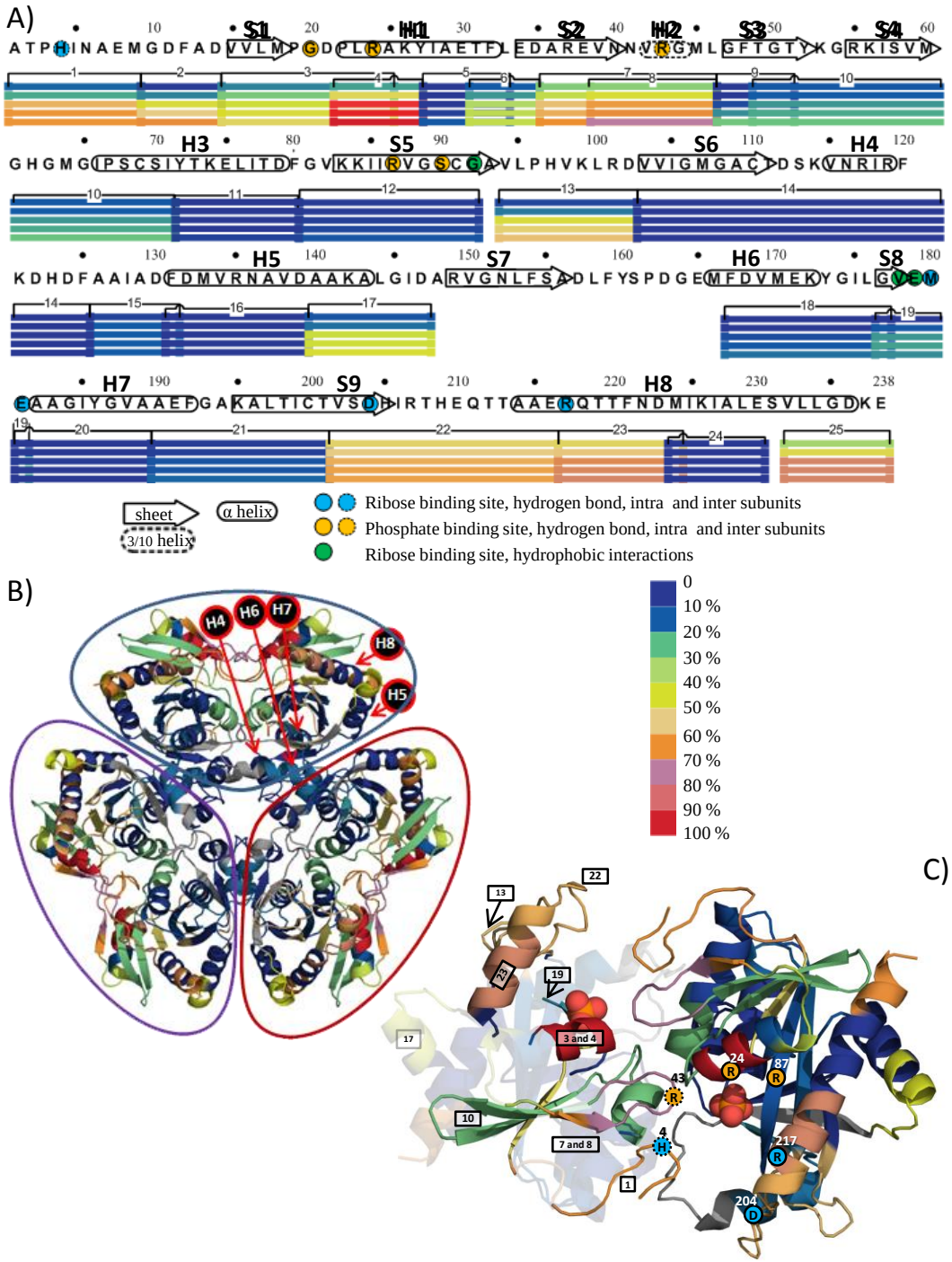
Unliganded vs. ternary complex with phosphate and formycin A

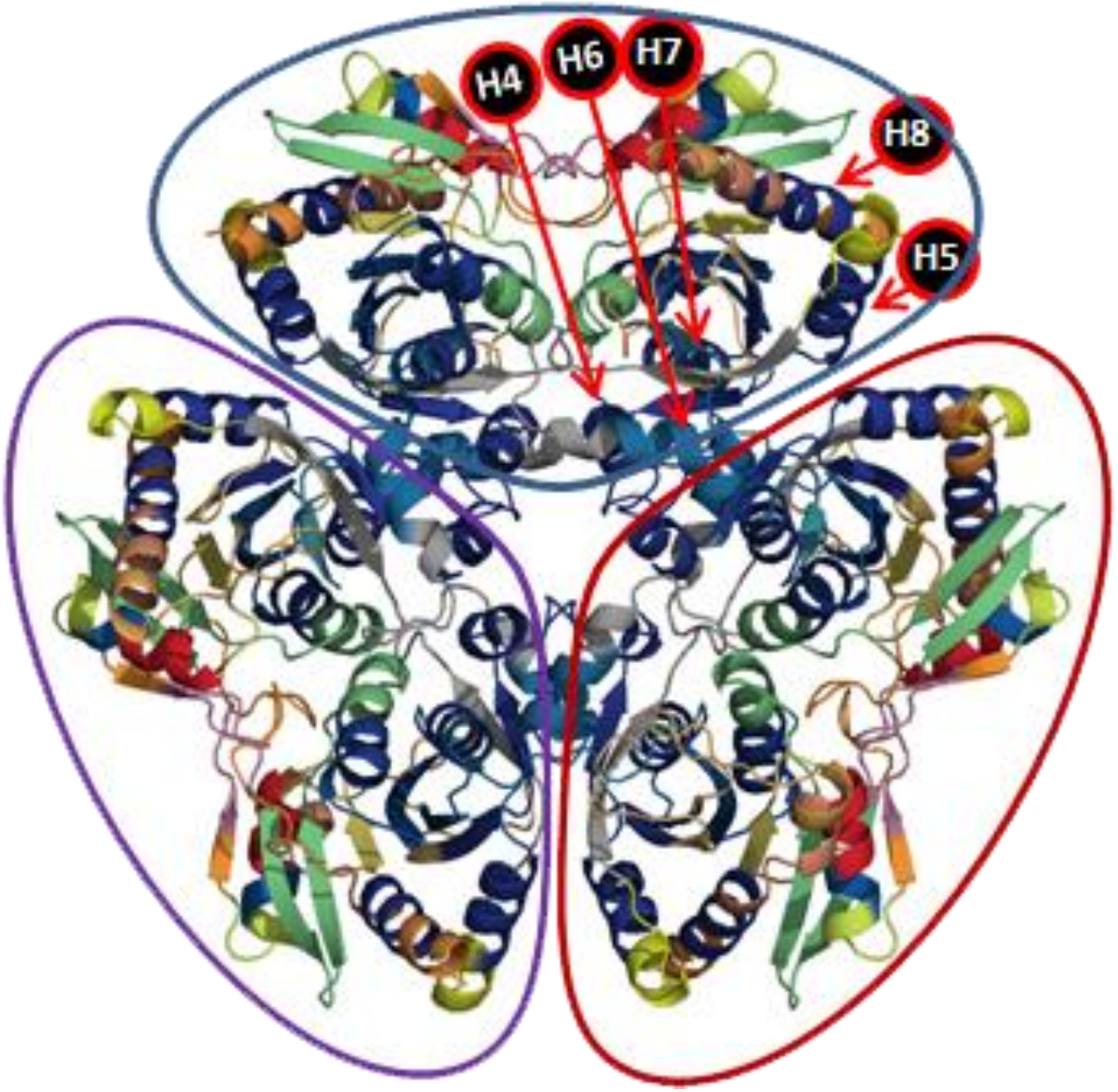
E) **R24A mutant of *E. coli* PNP protein**



F) **Wild type *E. coli* PNP protein**




A)
S1
H1
S2
H2
S3
S4
H3
S5
S6
H4
H5
S7
H6
S8
H7
S9
H8
sheet
α helix
3/10 helix
Ribose binding site, hydrogen bond, intra and inter subunits
Phosphate binding site, hydrogen bond, intra and inter subunits
Ribose binding site, hydrophobic interactions
B)
0
10 %
20 %
30 %
40 %
50 %
60 %
70 %
80 %
90 %
100 %
C)
3 and 4
7 and 8


H4
H6
H7
H8
H5

ZAKLJUČCI MS EKSPERIMENATA:

0. Generalno, **unutrašnjost heksamera je vrlo rigidna**, **fleksibilne regije su na površini**

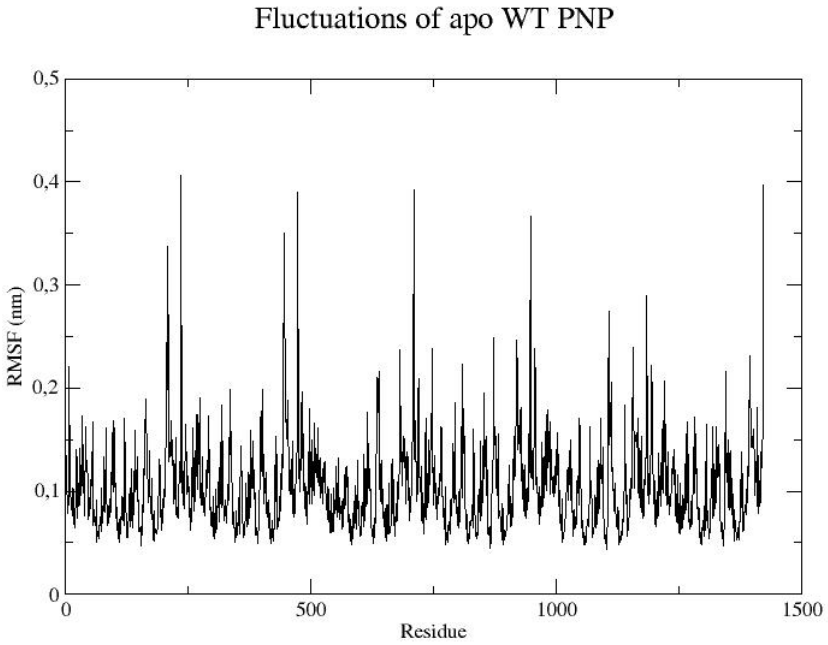
1. **Nije uočena veća razlika** u MS spektrima između **APO struktura** WT i R24A

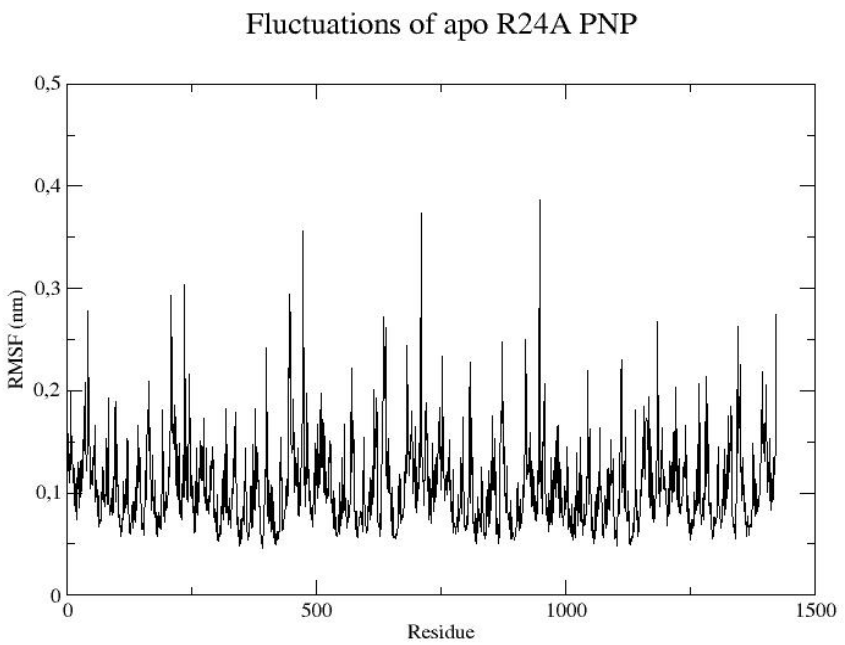
2. **Najveća razlika** je između **binarnih kompleksa** WT i R24A, pri čemu se binarni kompleks R24A ponaša se slično APO strukturama

3. **Ternarni kompleksi** WT i R24A se međusobno **razlikuju**

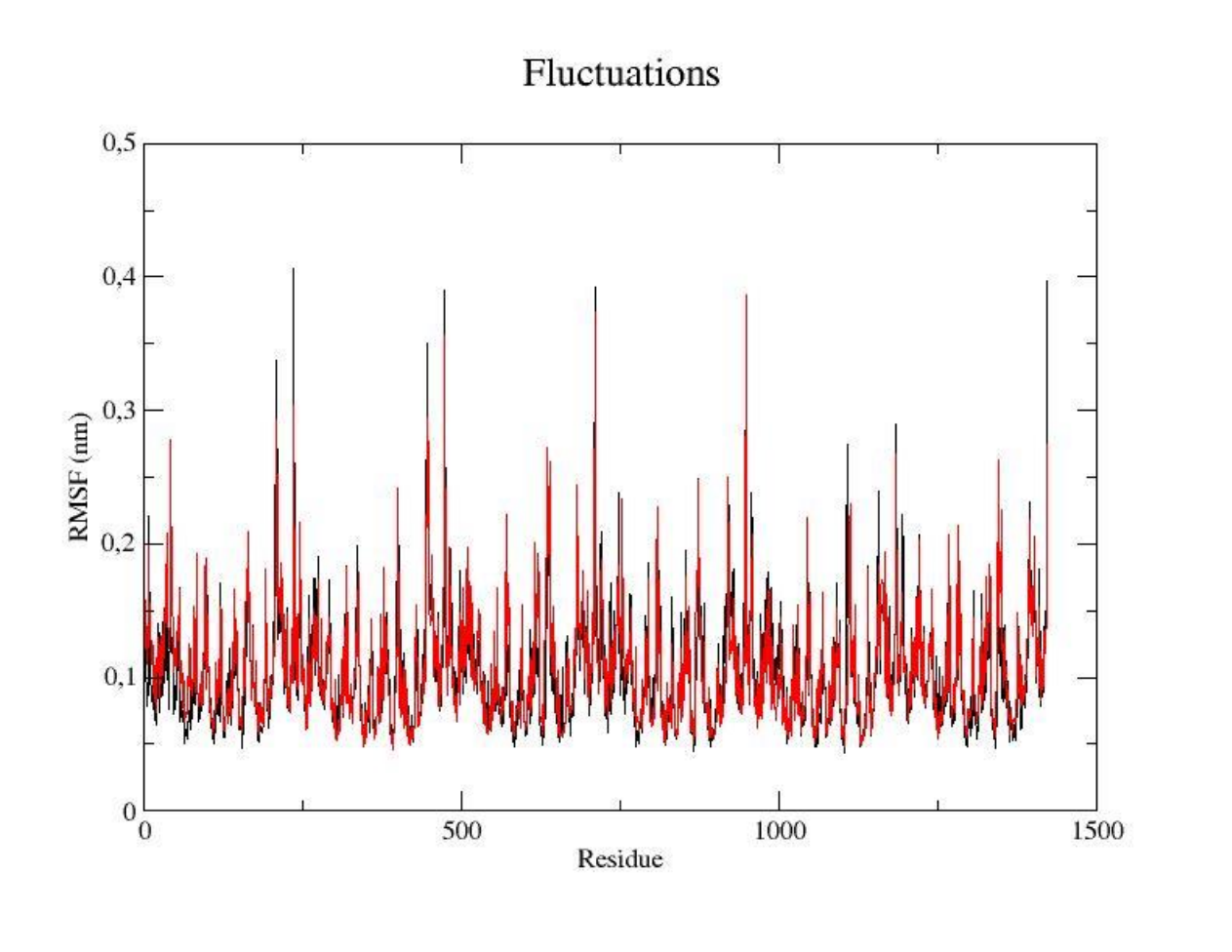
REZULTATI MD SIMULACIJA

FLUKTUACIJE



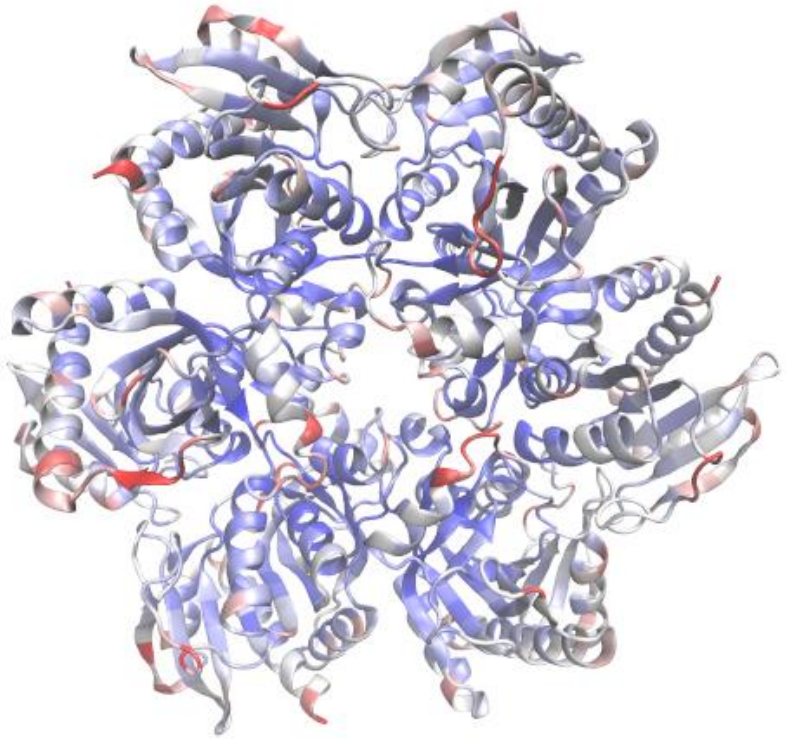


APO – WT vs R24A

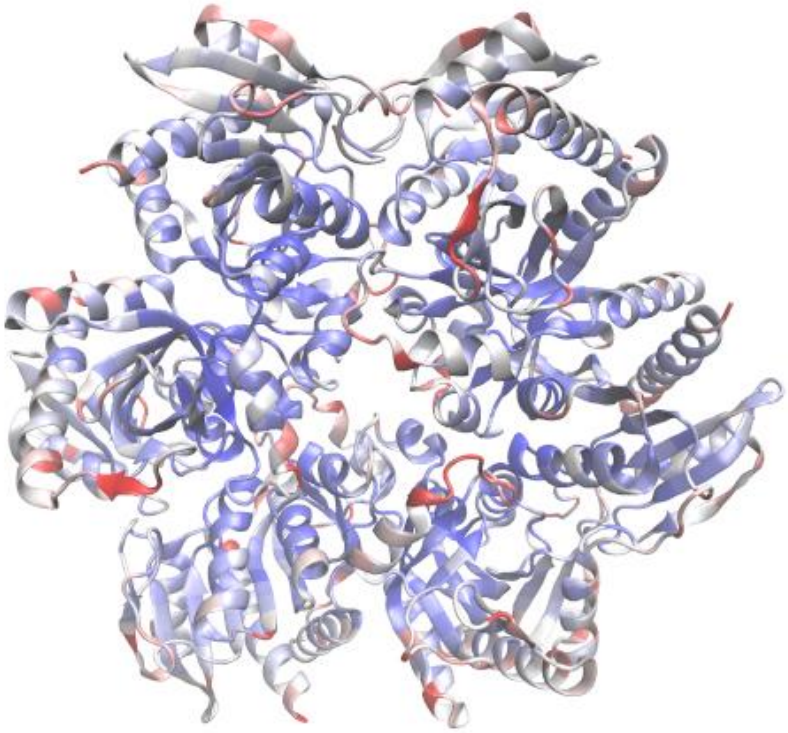


WT – crno
R24A - crveno

FLUKTUACIJE TIJEKOM MD SIMULACIJA (crveno 3 A, plavo 0.2 A)



APO - **WT**

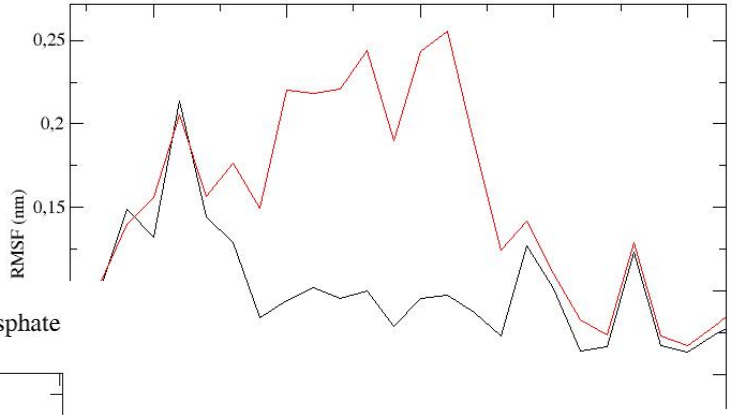


APO – **R24A**

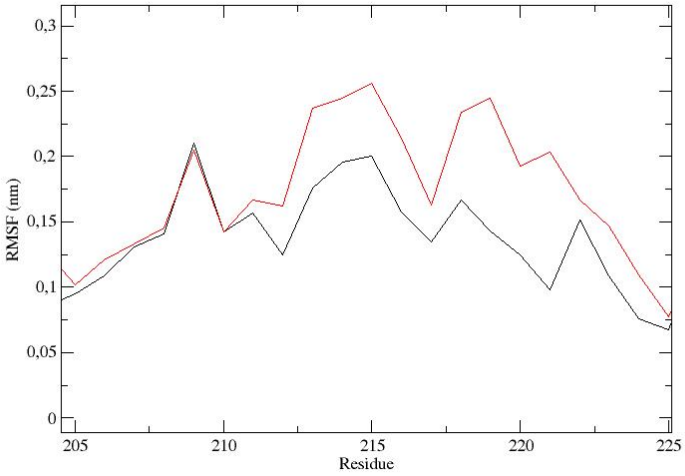
USPOREEDBA S MS EKSPERIMENTIMA:

0. Generalno, **unutrašnjost heksamera je vrlo rigidna**, **fleksibilne regije su na površini**

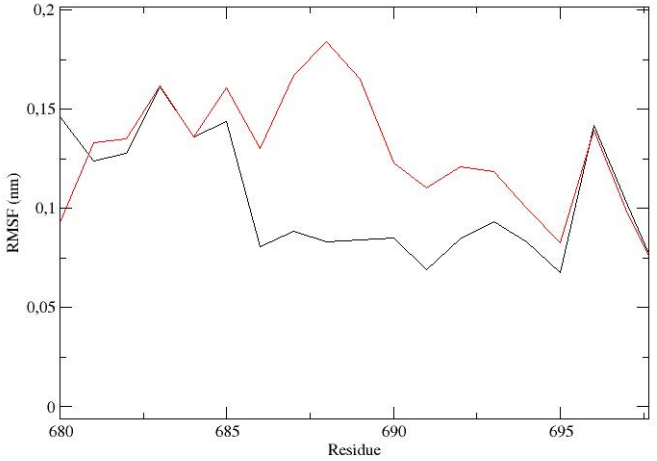
1. **Nije uočena veća razlika** u MS spektrima između **APO struktura** WT i R24A

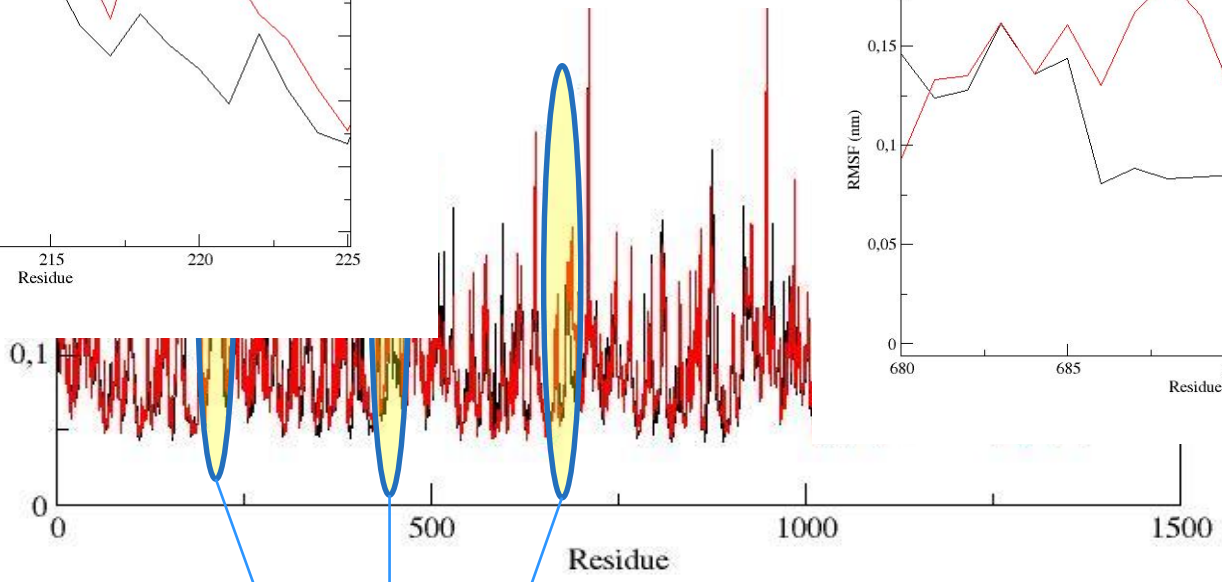


Fluctuations of R24A PNP binary complex with phosphate



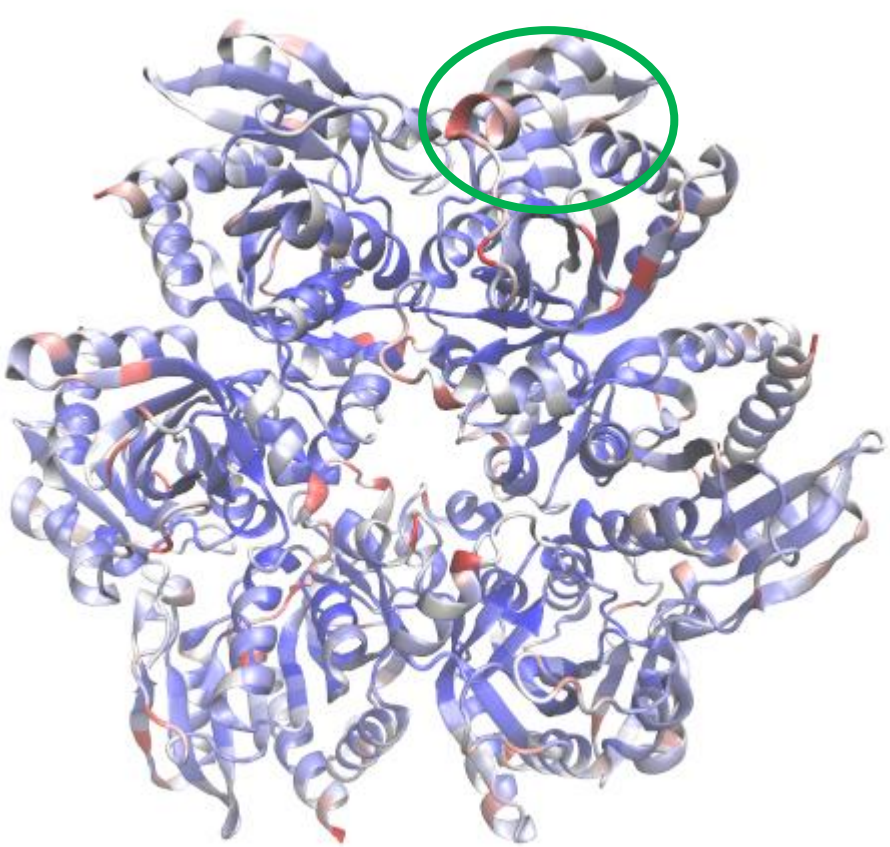
Fluctuations of R24A PNP binary complex with phosphate



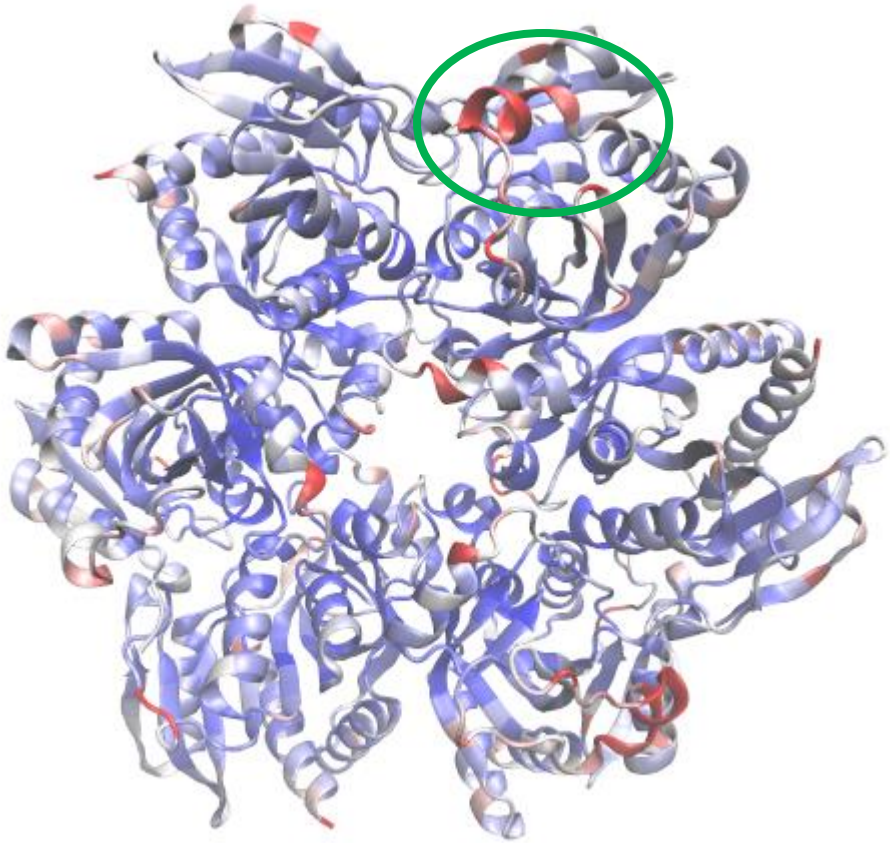


Amino kiseline - 212-222
(212-222, 449-459, 686-696)

WT – crno
R24A - crveno

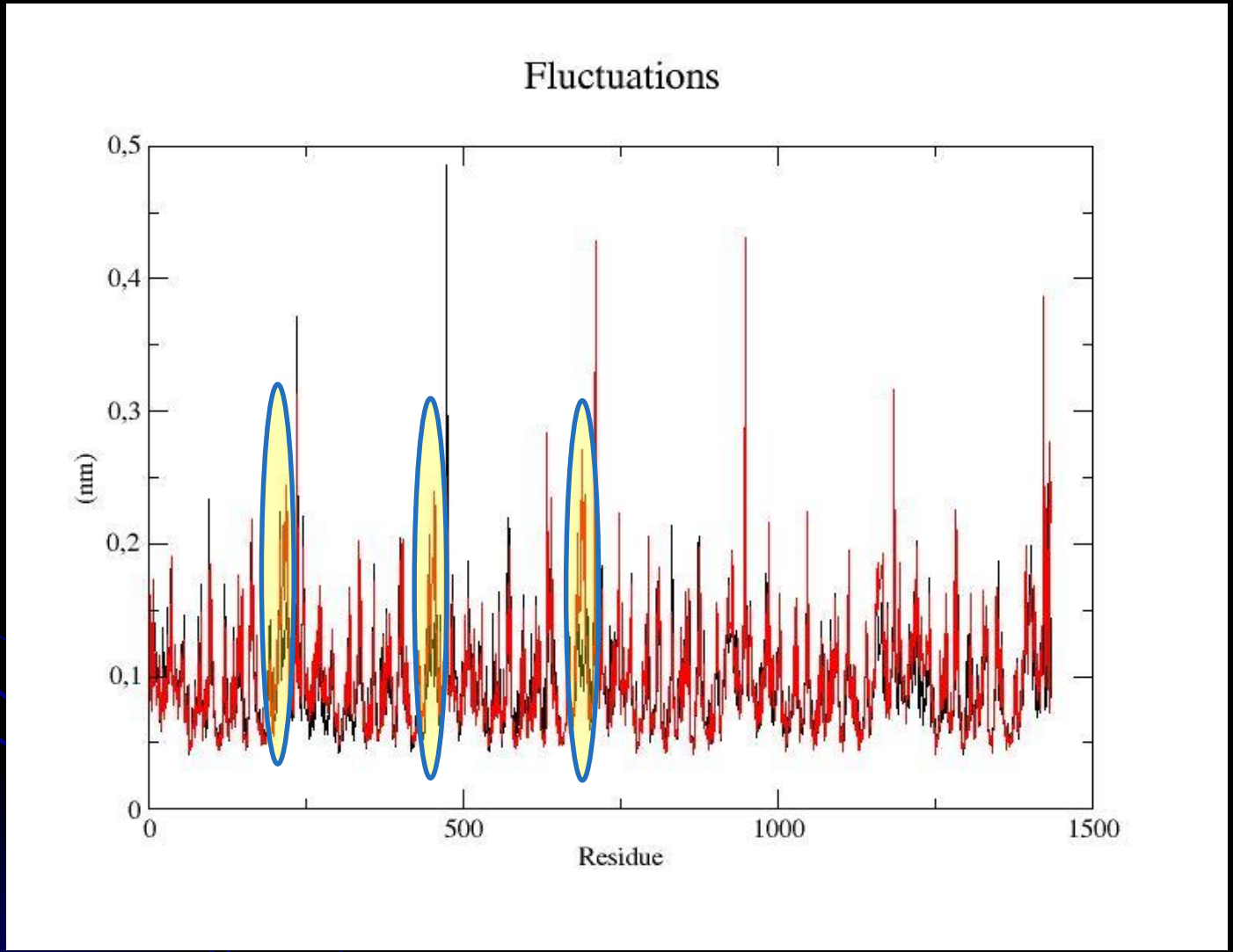


binarni- **WT**

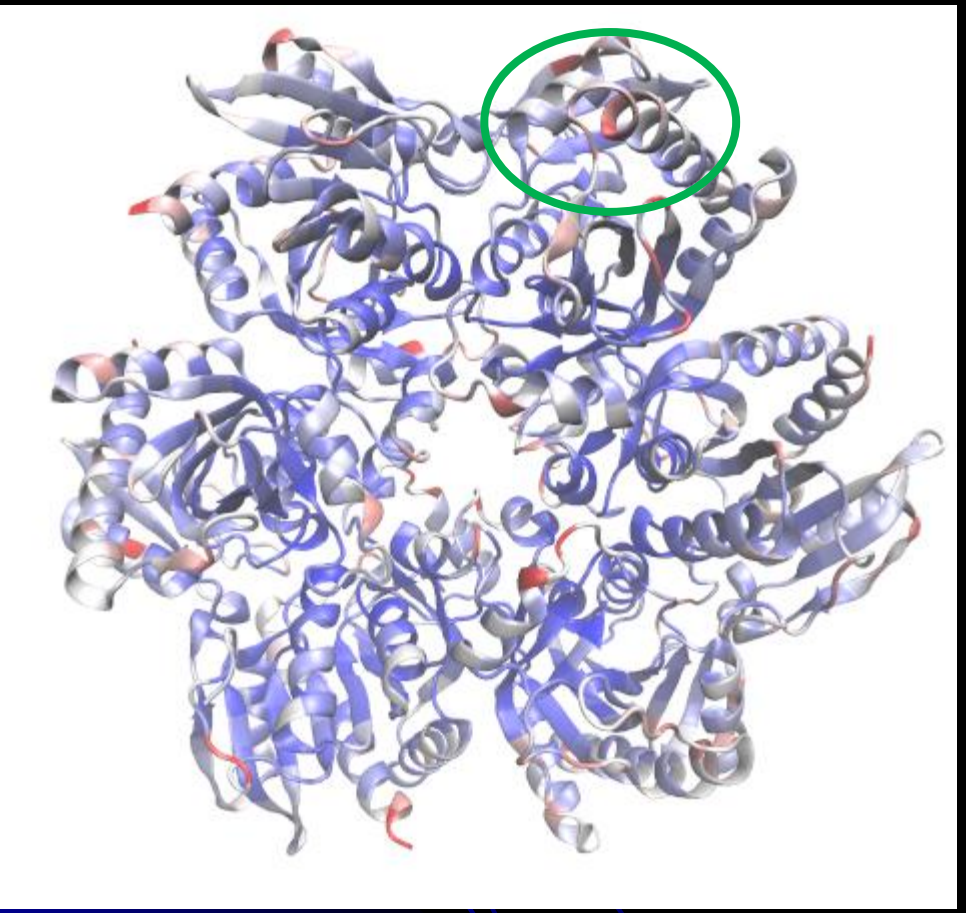


binarni– **R24A**

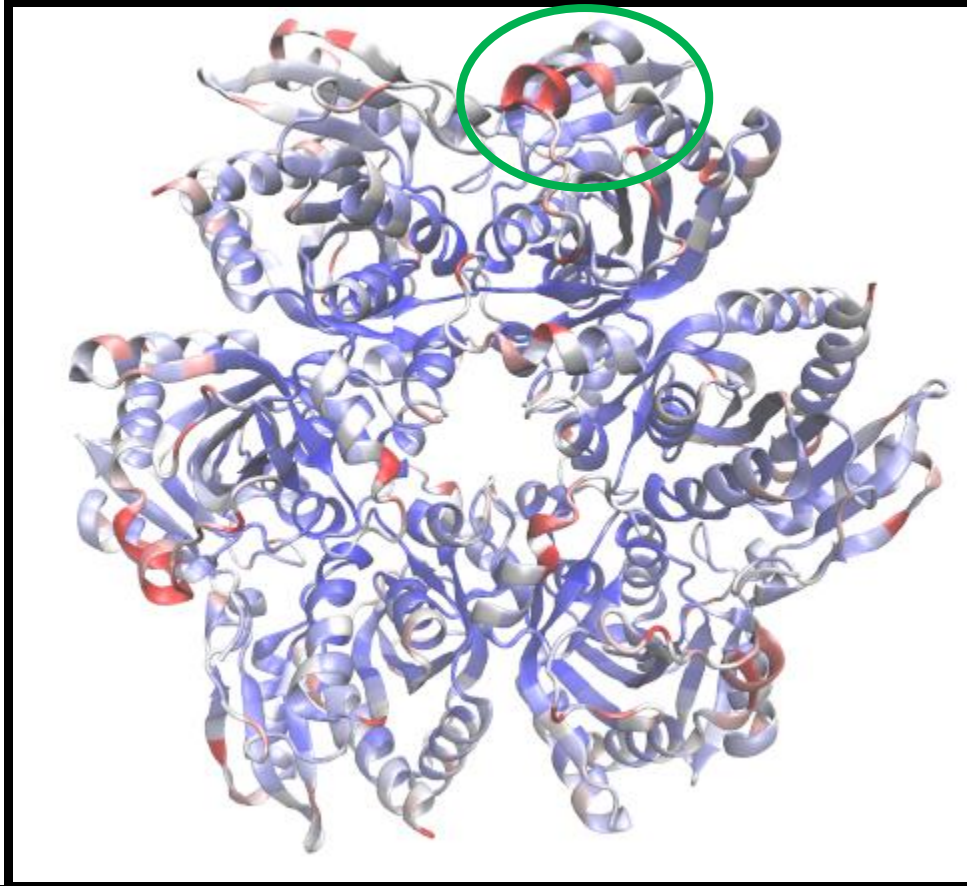
Ternarni kompleks– WT vs R24A



WT – crno
R24A - crveno



Ternarni - **WT**



Ternarni – **R24A**

REZULTATI ANALIZE FLUKTUACIJA

0. Općenito, enzim je vrlo rigidan, fleksibilne regije su na površini

1. Apo strukture se slično ponašaju, odnosno imaju slične fluktuacije

2. Najveća razlika u fluktuacijama binarnih kompleksa WT i R24A je u fluktuiranju rezidua 212-222; u 3 aktivna mjesta ti rezidiu značajno jače fluktuiraju kod R24A mutanta.

3. Kod ternarnih kompleksa je uočena ista razlika kao i kod binarnih

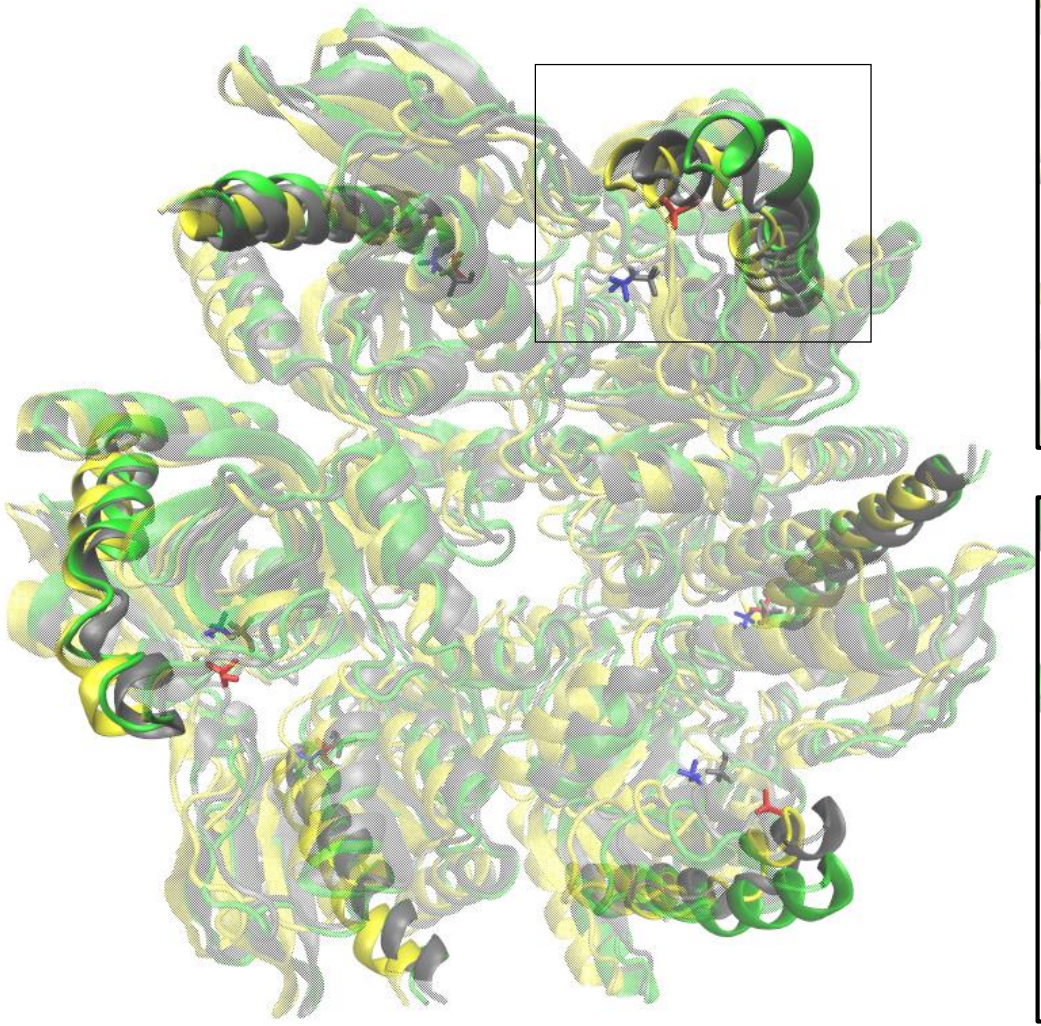
VEZANJE FOSFATA

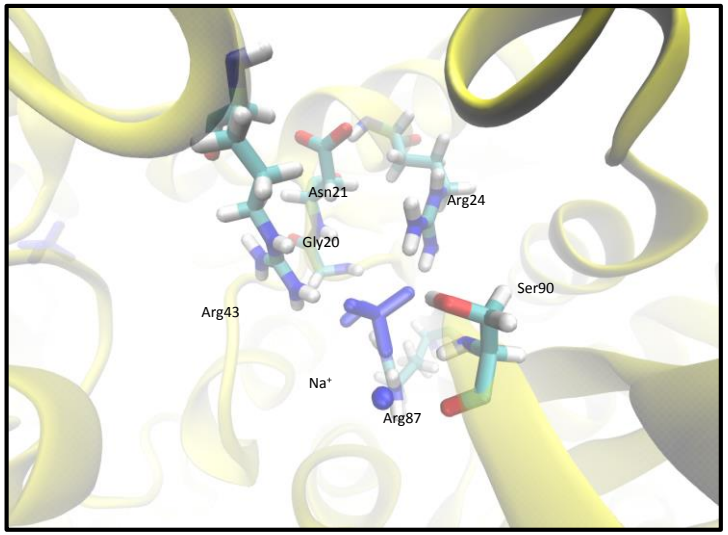
VEZANJE FOSFATA

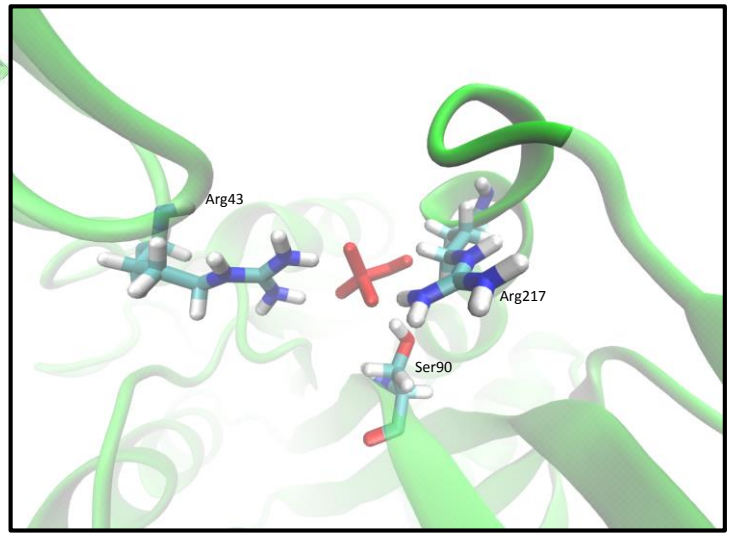
BINARNI KOMPLEKS

protein: zeleno R24A, žuto WT, sivo kristalna struktura

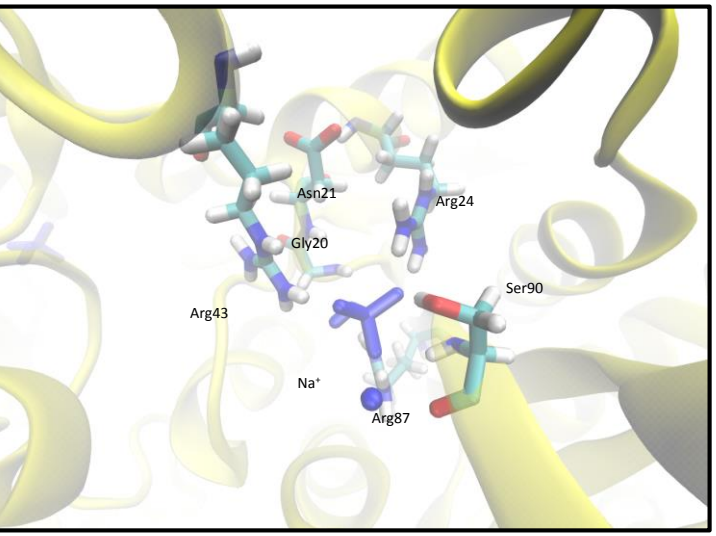
fosfat: crveno R24A, plavo WT, sivo kristalna struktura

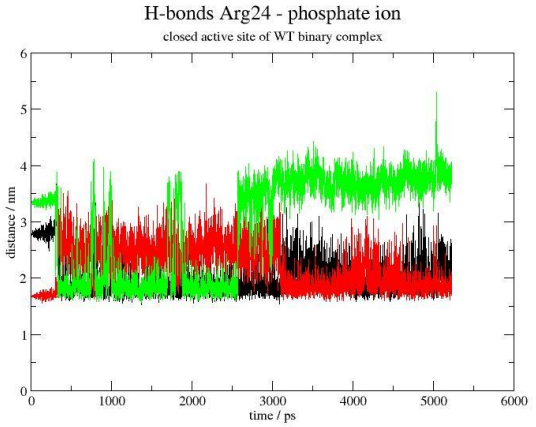


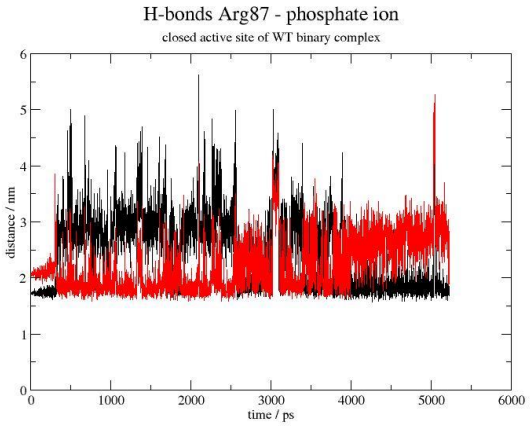


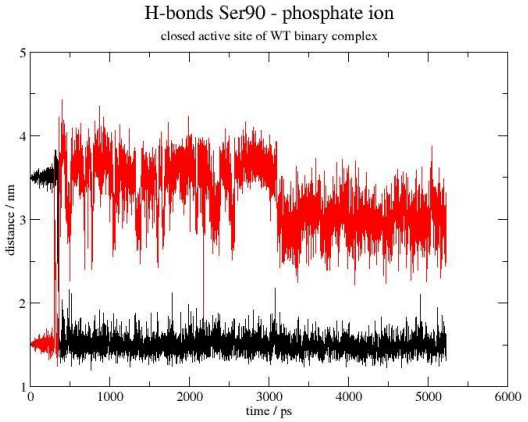


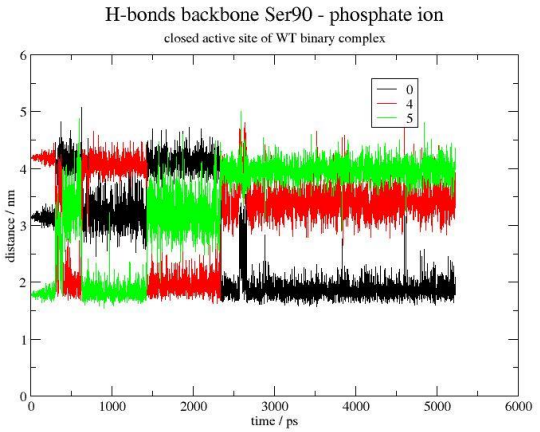
INTERAKCIJE FOSFATA TIJEKOM MD SIMULACIJE KOD **WT PNP**

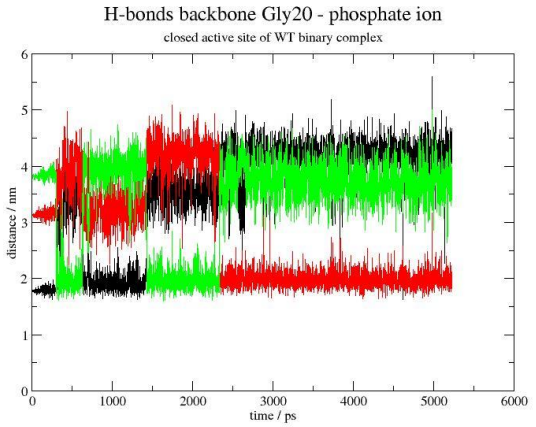


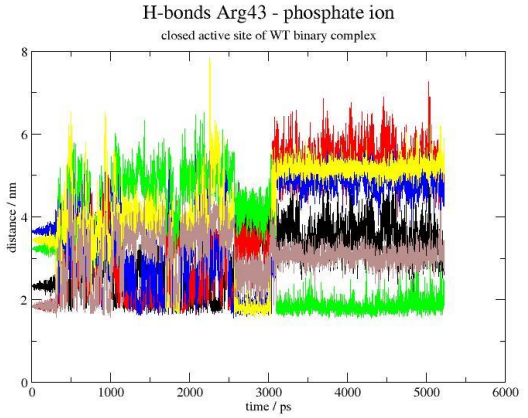




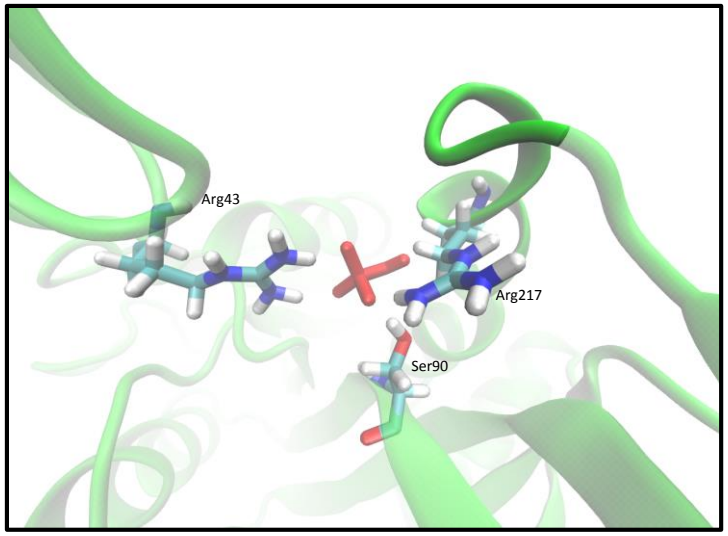


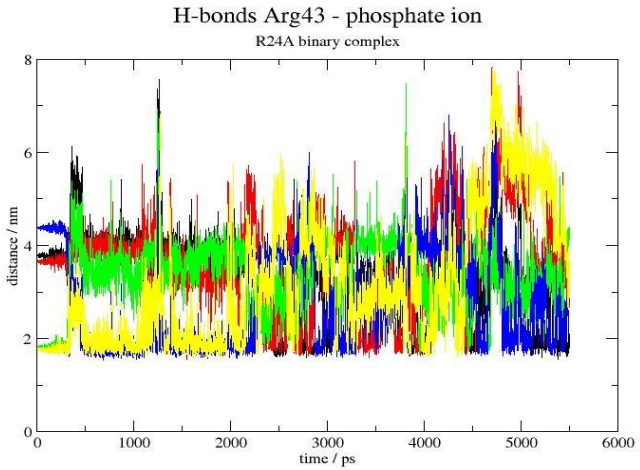


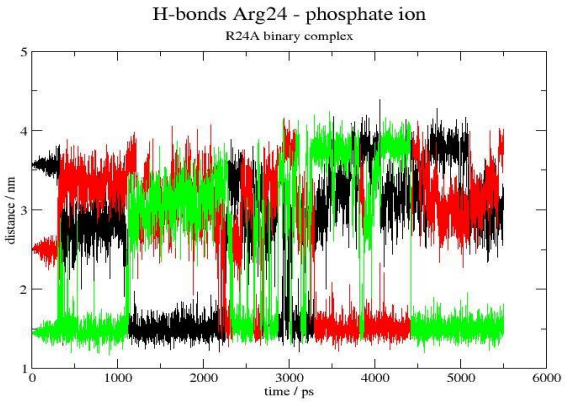


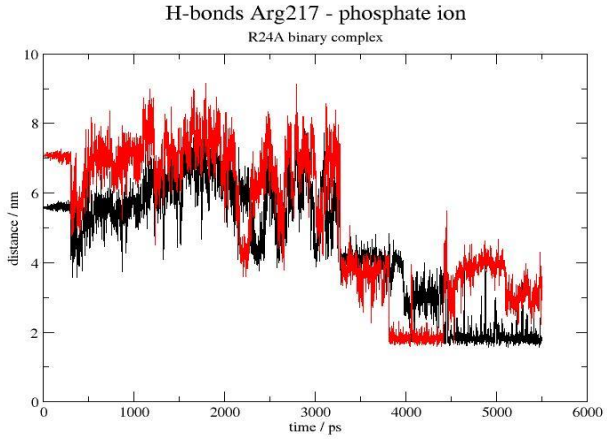


INTERAKCIJE FOSFATA TIJEKOM MD SIMULACIJE KOD **R24A mutanta PNP**







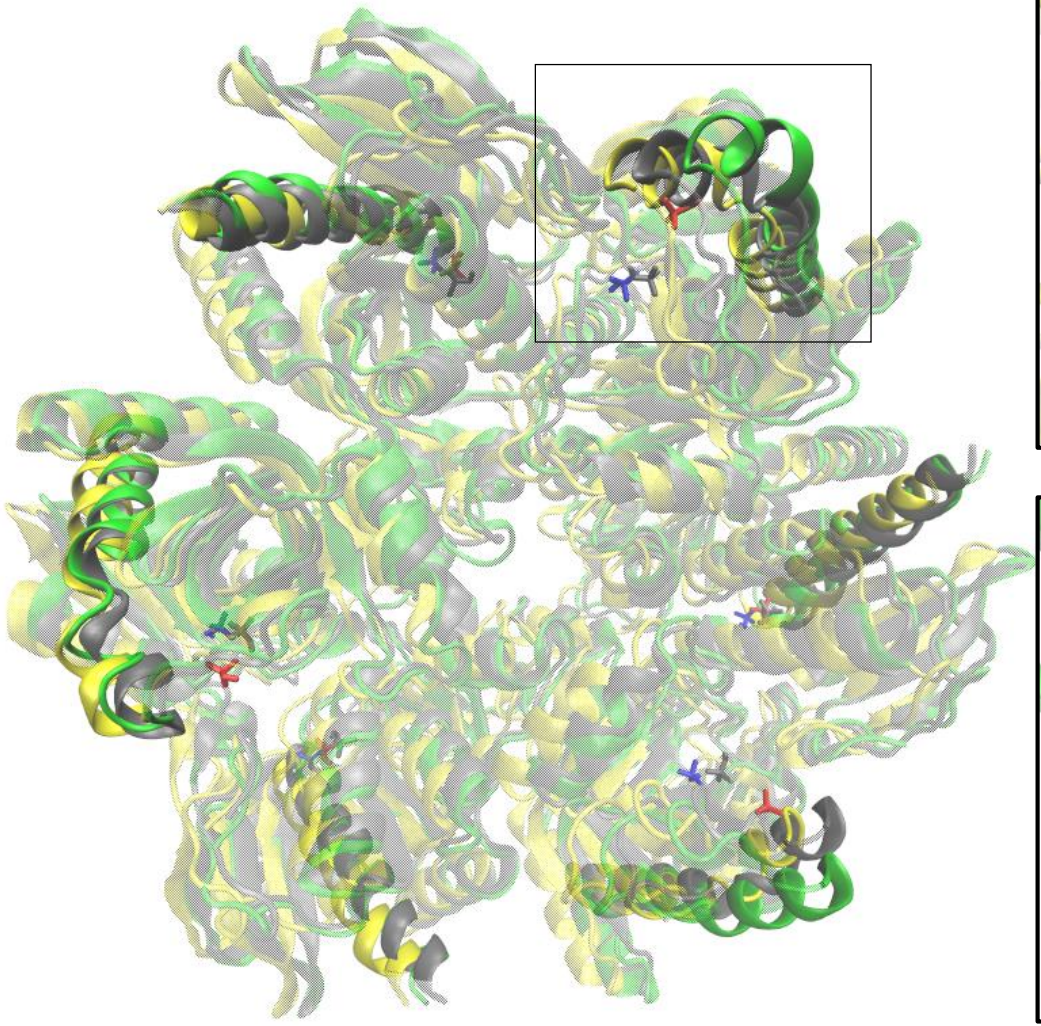


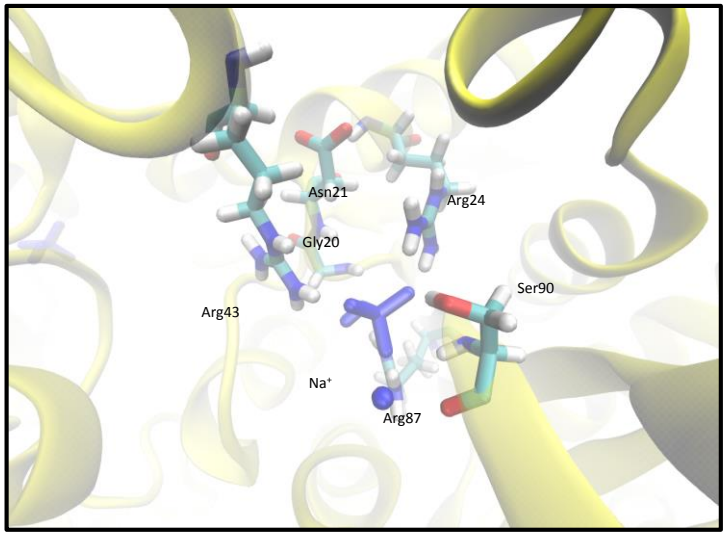
VEZANJE FOSFATA

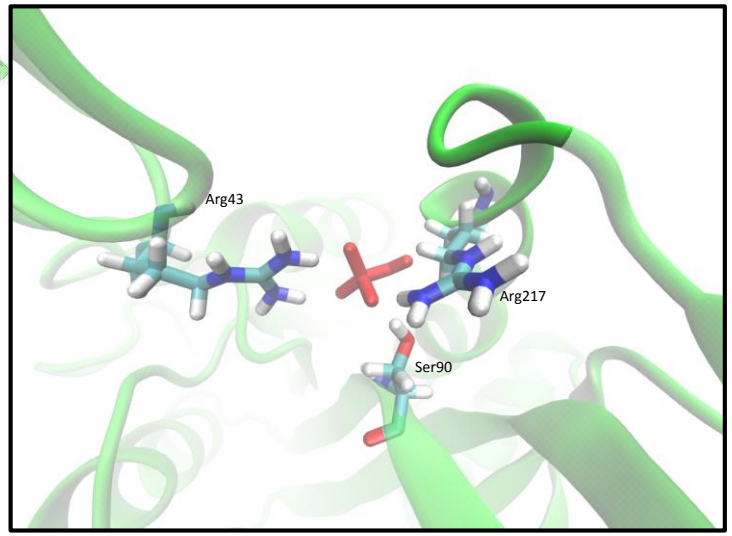
BINARNI KOMPLEKS

protein: zeleno R24A, žuto WT, sivo kristalna struktura

fosfat: crveno R24A, plavo WT, sivo kristalna struktura





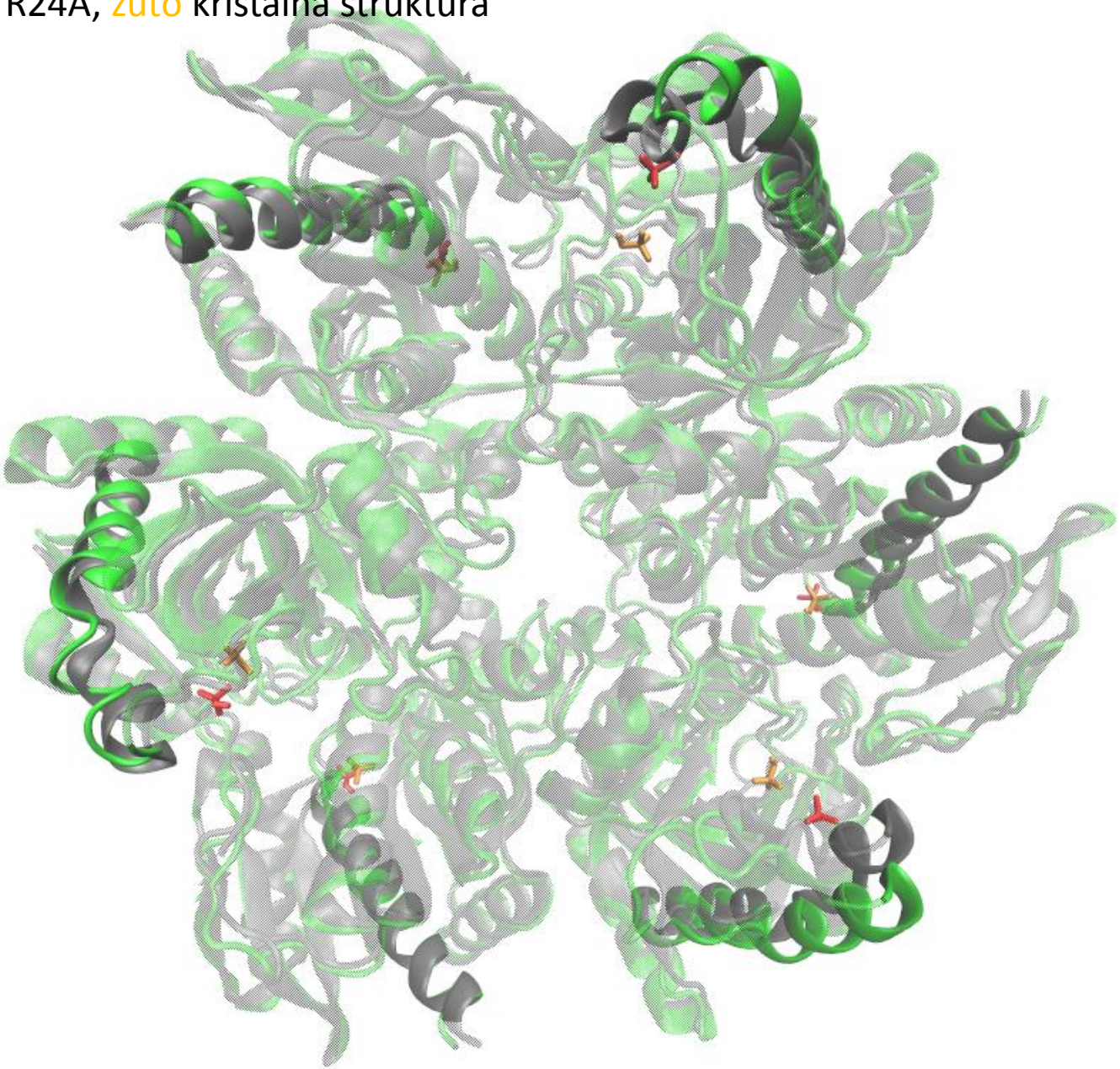


BINARNI KOMPLEKS

VEZANJE FOSFATA I KONFORMACIJSKA PROMJENA

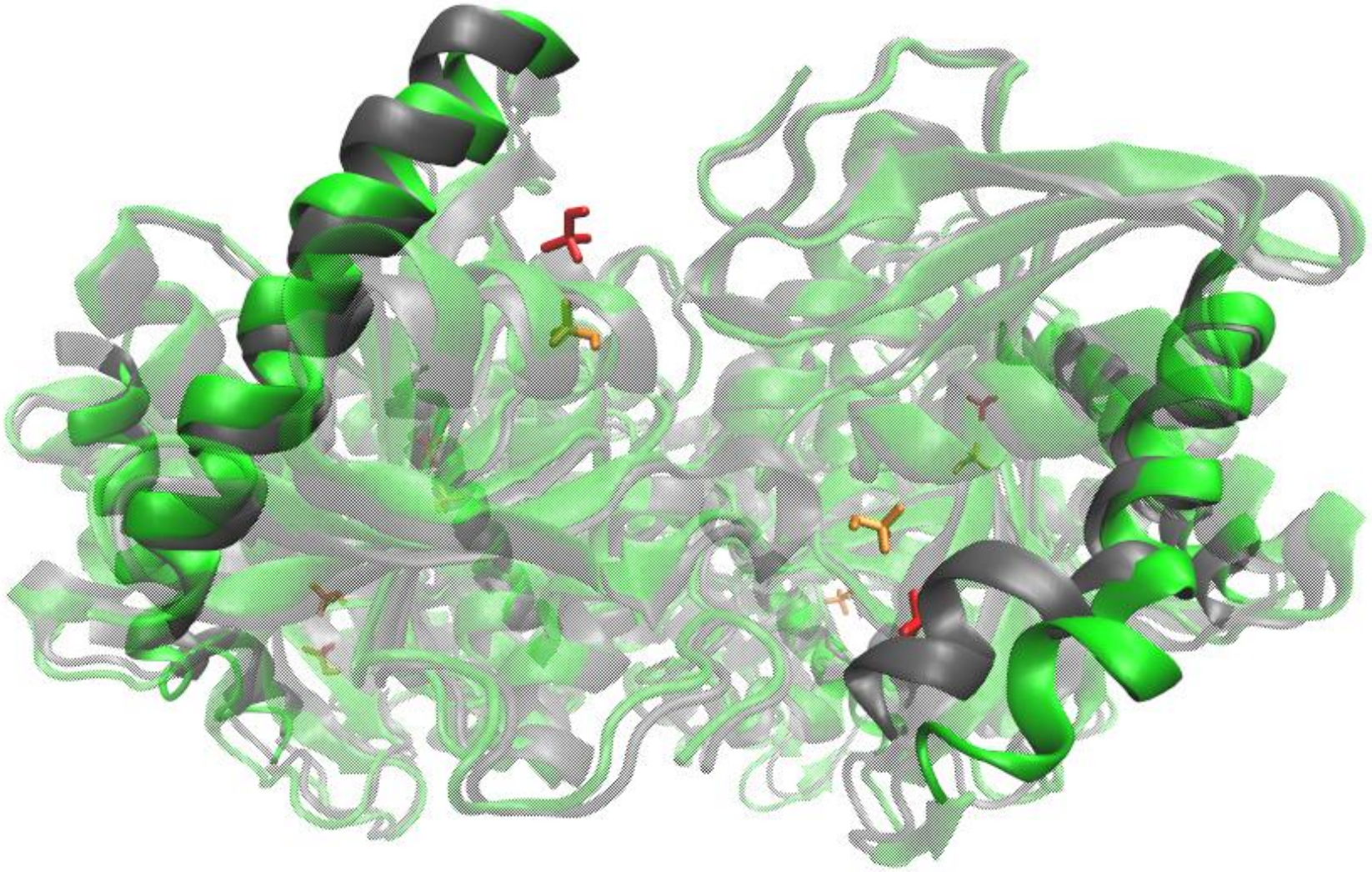
protein: zeleno R24A, sivo kristalna struktura

fosfat: crveno R24A, žuto kristalna struktura



KONFORMACIJSKA PROMJENA

BINARNI KOMPLEKS (zeleno R24A, sivo kristalna struktura)



REZULTATI MD SIMULACIJA:

1. analiza fluktuacija potvrđuje rezultate MS eksperimenata - **unutrašnjost heksamera je vrlo rigidna**, **fleksibilne regije su na površini**

2. uočena **razlika u vezanju fosfata** između R24A mutanta i WT

3. uočena **konformacijska promjena** iz „zatvorenog” u „otvoreno” vezno mjesto kod R24A mutanta u binarnom i ternarnom kompleksu

OPĆENITI ZAKLJUČCI:

0. vrlo dobro slaganje rezultata MS eksperimenata i MD simulacija

1. fleksibilnost - unutrašnjost heksamera je vrlo rigidna, fleksibilne regije su na površini

2. Arg24 je neophodan za produktivno vezanje fosfata, ali i za stabilizaciju „zatvorene" konformacije aktivnog mjesta (*možda je vezanje fosfata neophodno za stabilizaciju veznog mjesta, a vezanje fosfata ovisi o R24?)*

3. razlike u MS spektrima posljedica su različitog načina vezanja fosfata kod WT i R24A mutanta i konformacijske promijene uzrokovane mutacijom

EKSPERIMENTI SU POTVRDILI RAČUNALNE REZULTATE

ILI

RAČUNALNI REZULTATI OMOGUĆAVAJU INTERPRETACIJU EKSPERIMENTALNIH REZULTATA NA MOLEKULARNOJ RAZINI