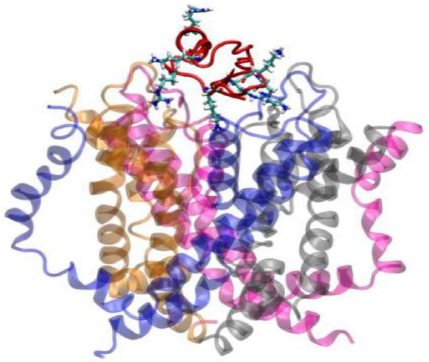


Was binding of free amino acids an early innovation in the evolution of allostery?

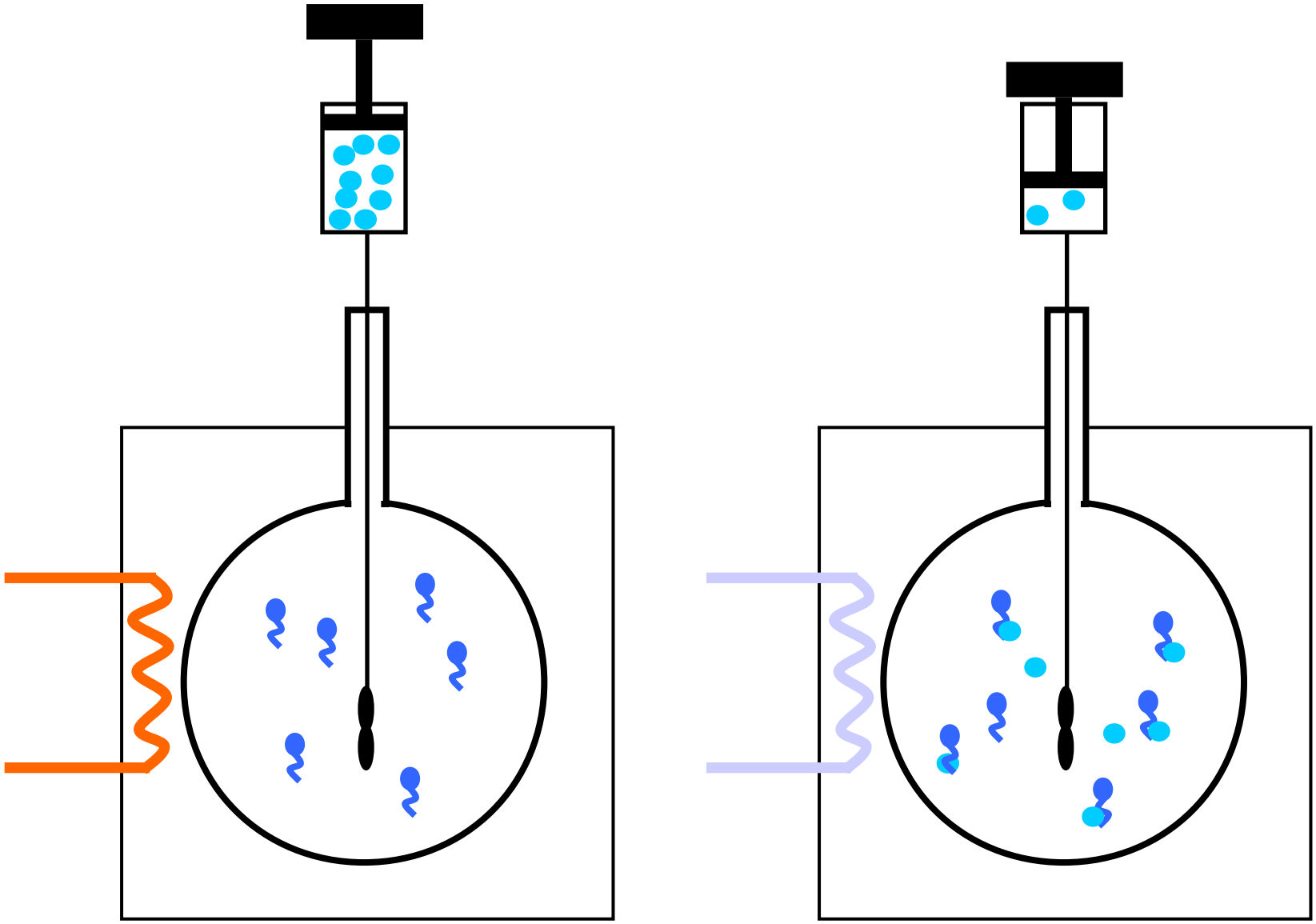
Rüdiger Ettrich

Institute of Nanobiology and Structural Biology
Academy of Sciences of the Czech Republic

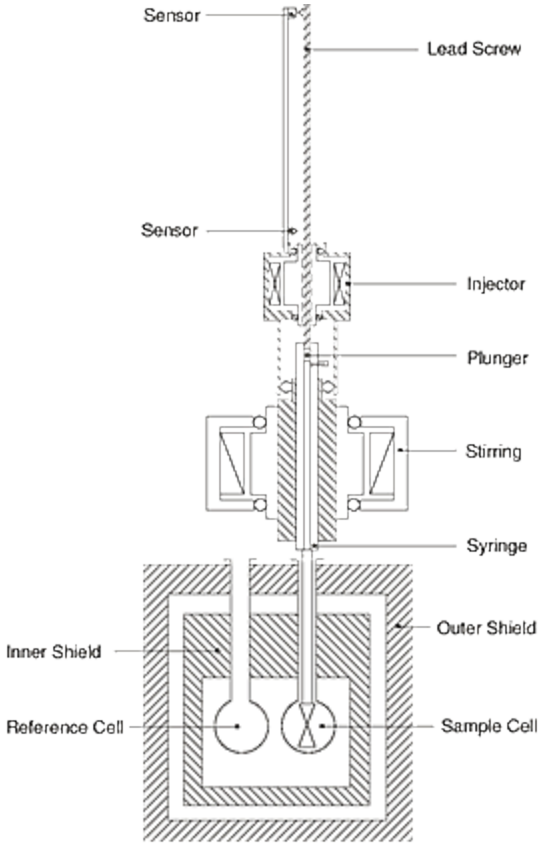
and

Faculty of Sciences, University of South Bohemia

Isothermal Titration Calorimetry (ITC)

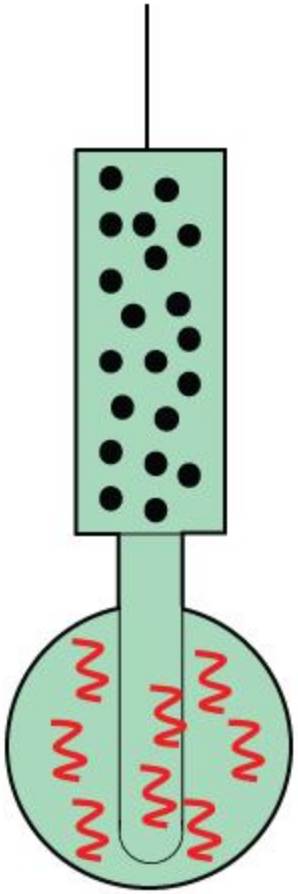


Isothermal Titration Calorimetry (ITC)



Taken from Micro Cal website

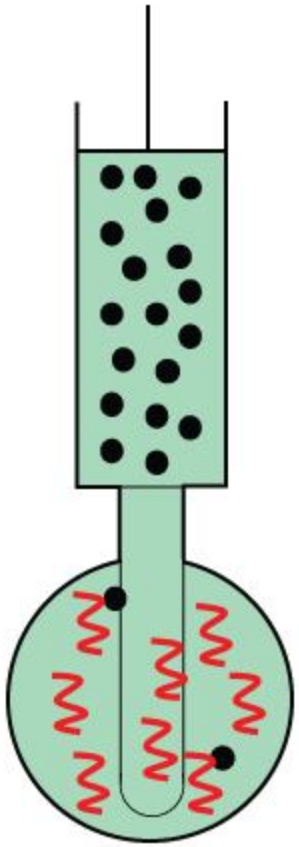
ITC - before titration



Ligand - in syringe

Protein (macromolecule) – in cell

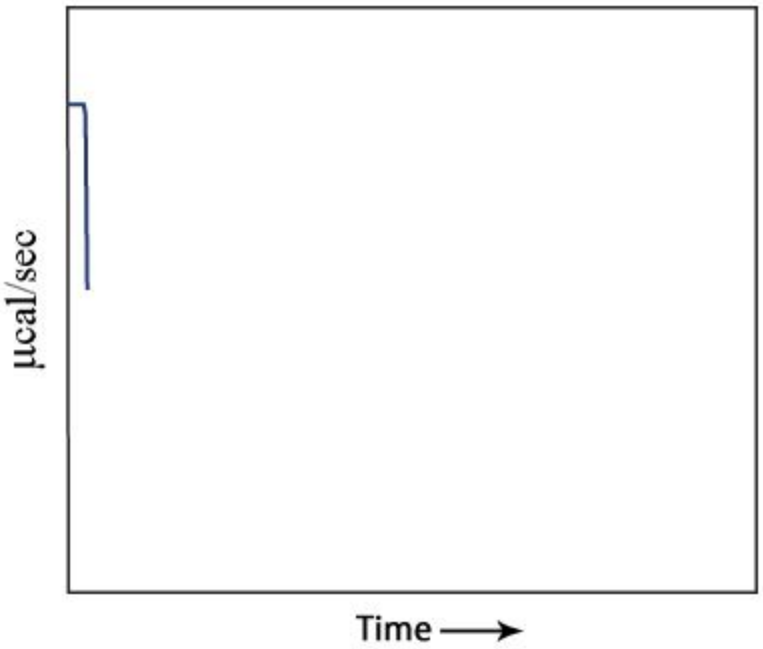
Titration Begins: First Injection



Ligand in syringe

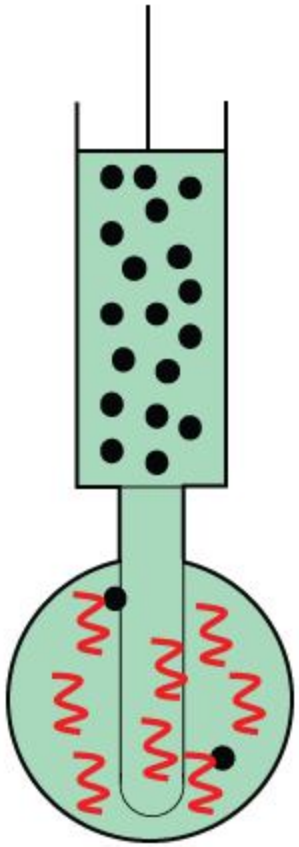
Protein in cell

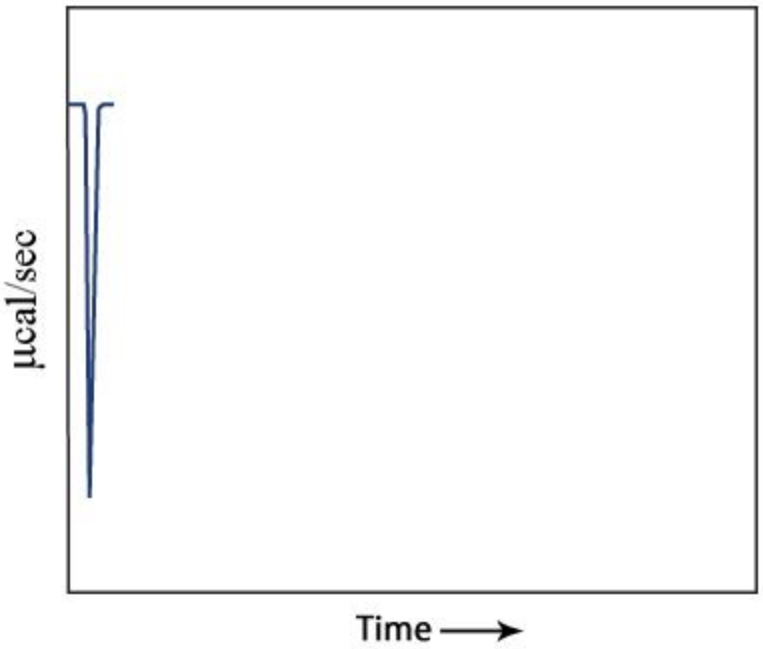
Protein-ligand complex



As the first injection is made, all injected ligand is bound to protein

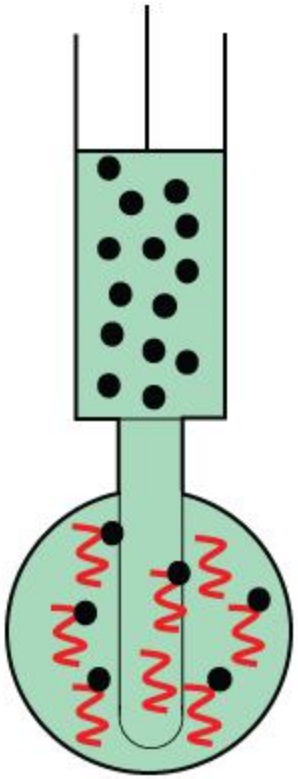
Return to Baseline

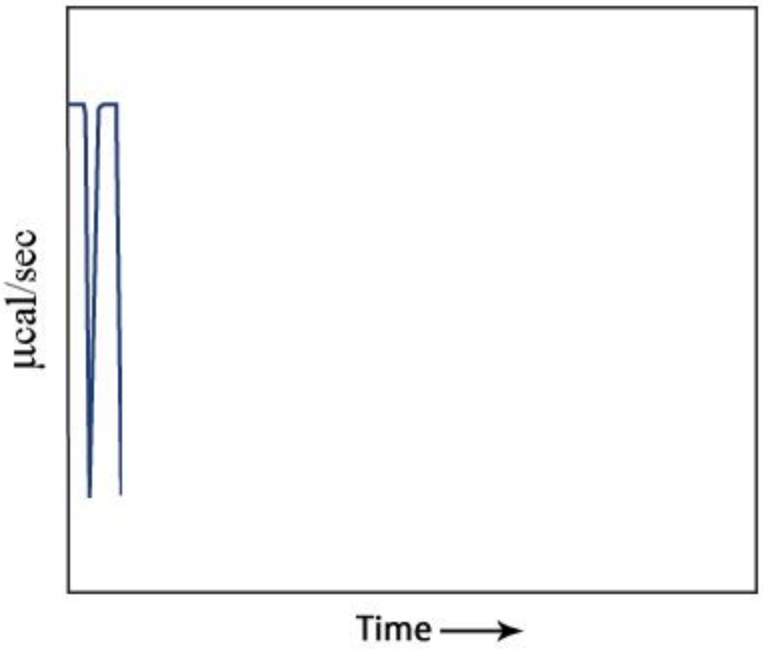




The signal returns to baseline before the next injection

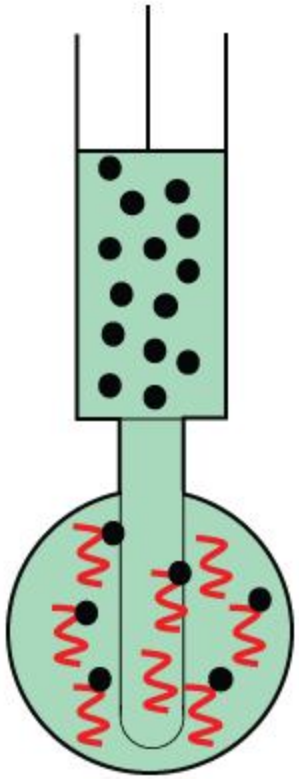
Second Injection

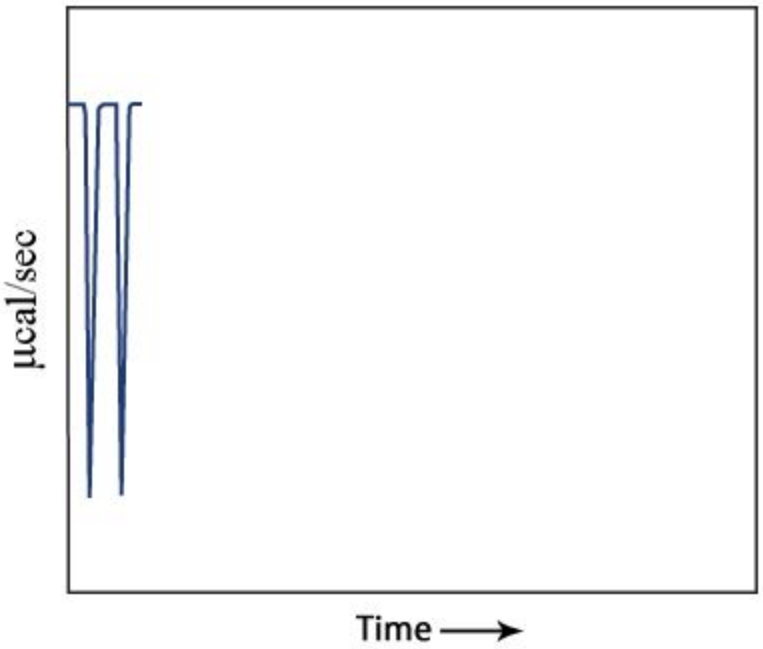




As a second injection is made, again all injected ligand becomes bound to the protein

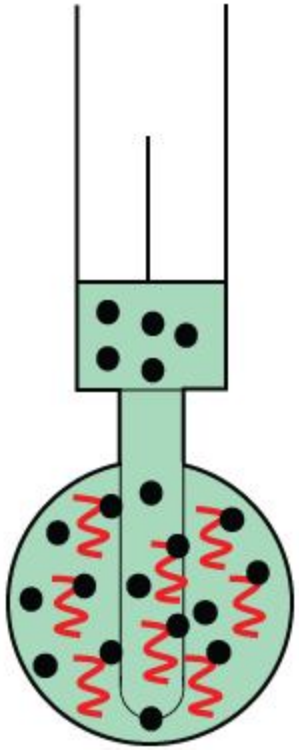
Second Return to Baseline

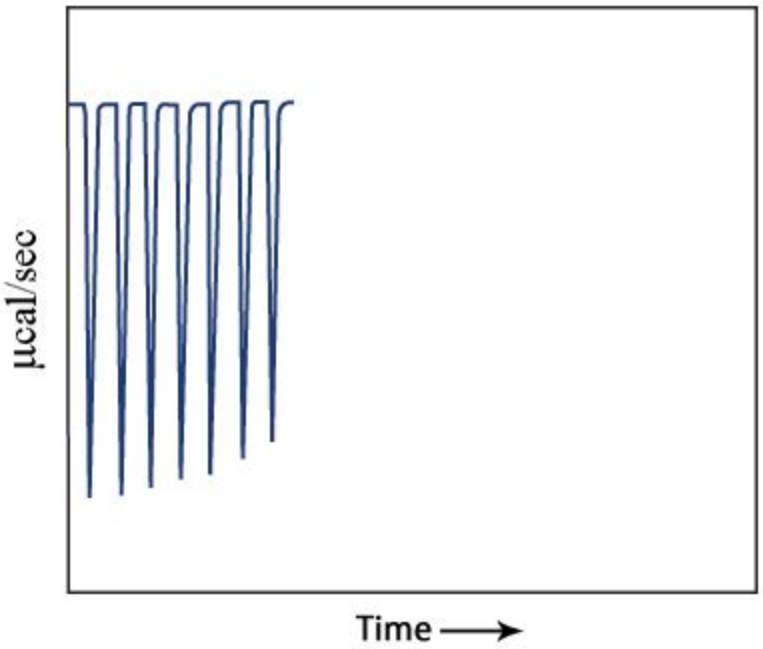




Signal again returns to baseline before next injection

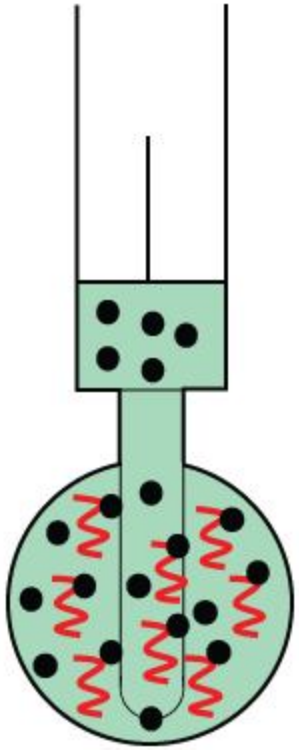
Injections Continue

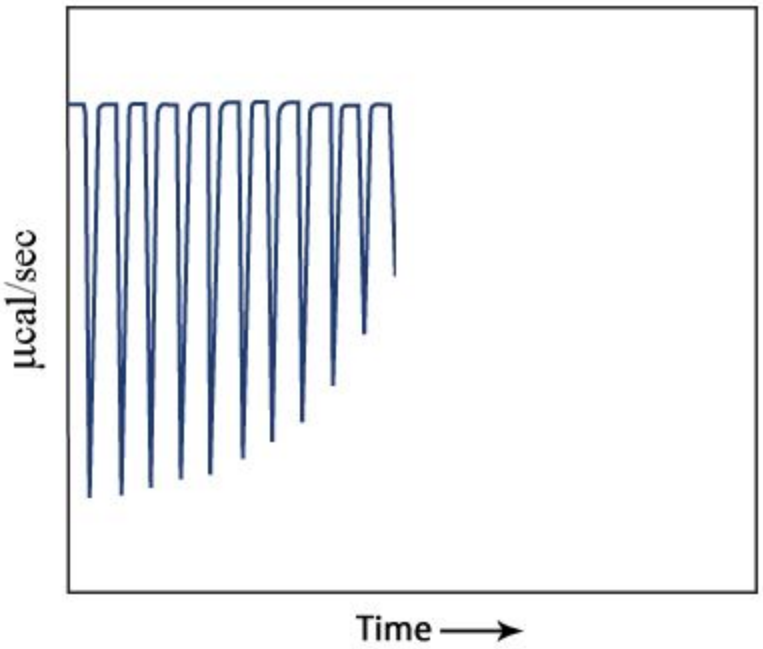




As the injections continue, the protein becomes saturated with ligand, so less binding occurs and the heat change starts to decrease

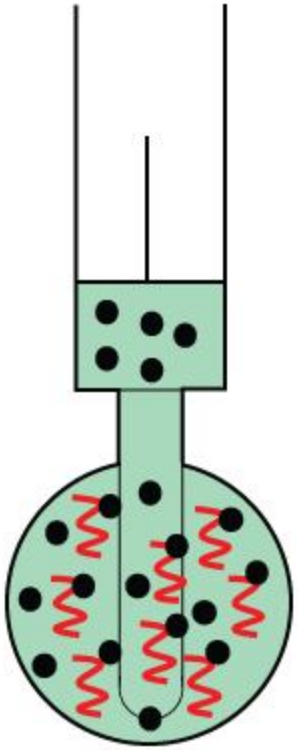
Injections Continue

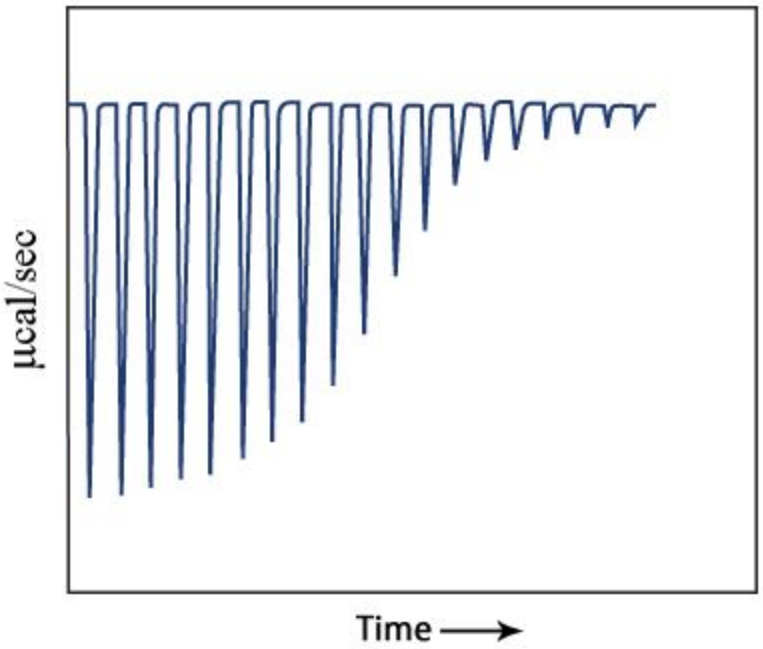




As the injections continue, the protein becomes less saturated with ligand so less binding occurs and the heat change starts to decrease

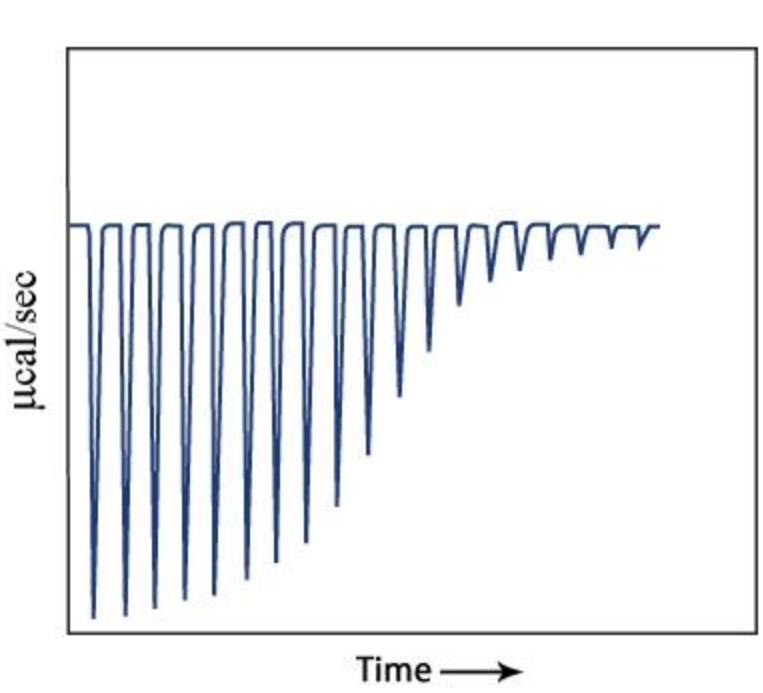
End of titration

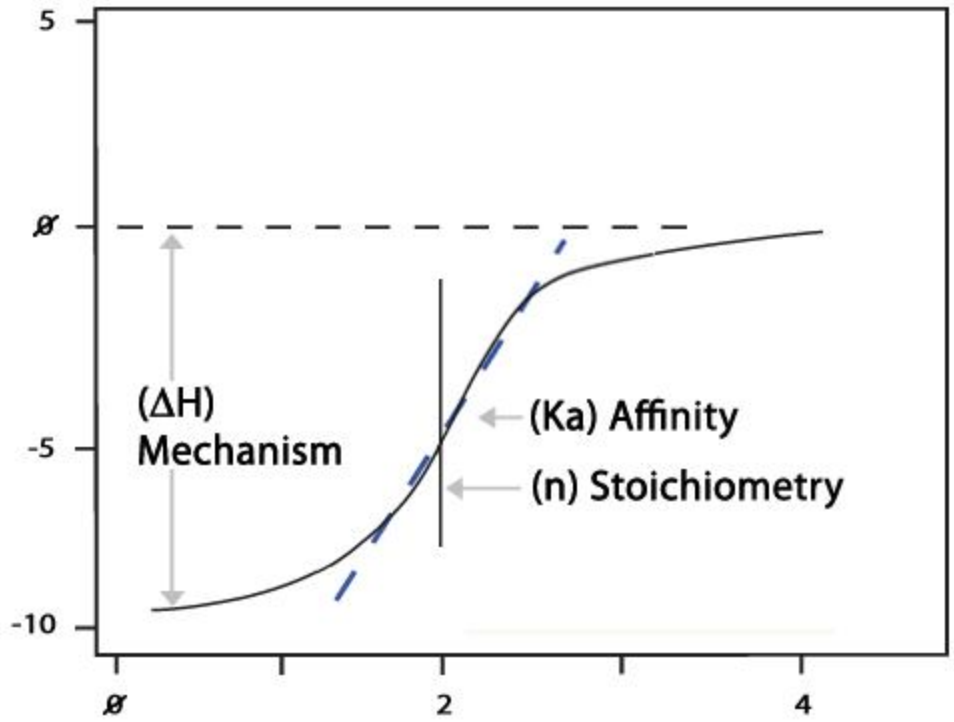




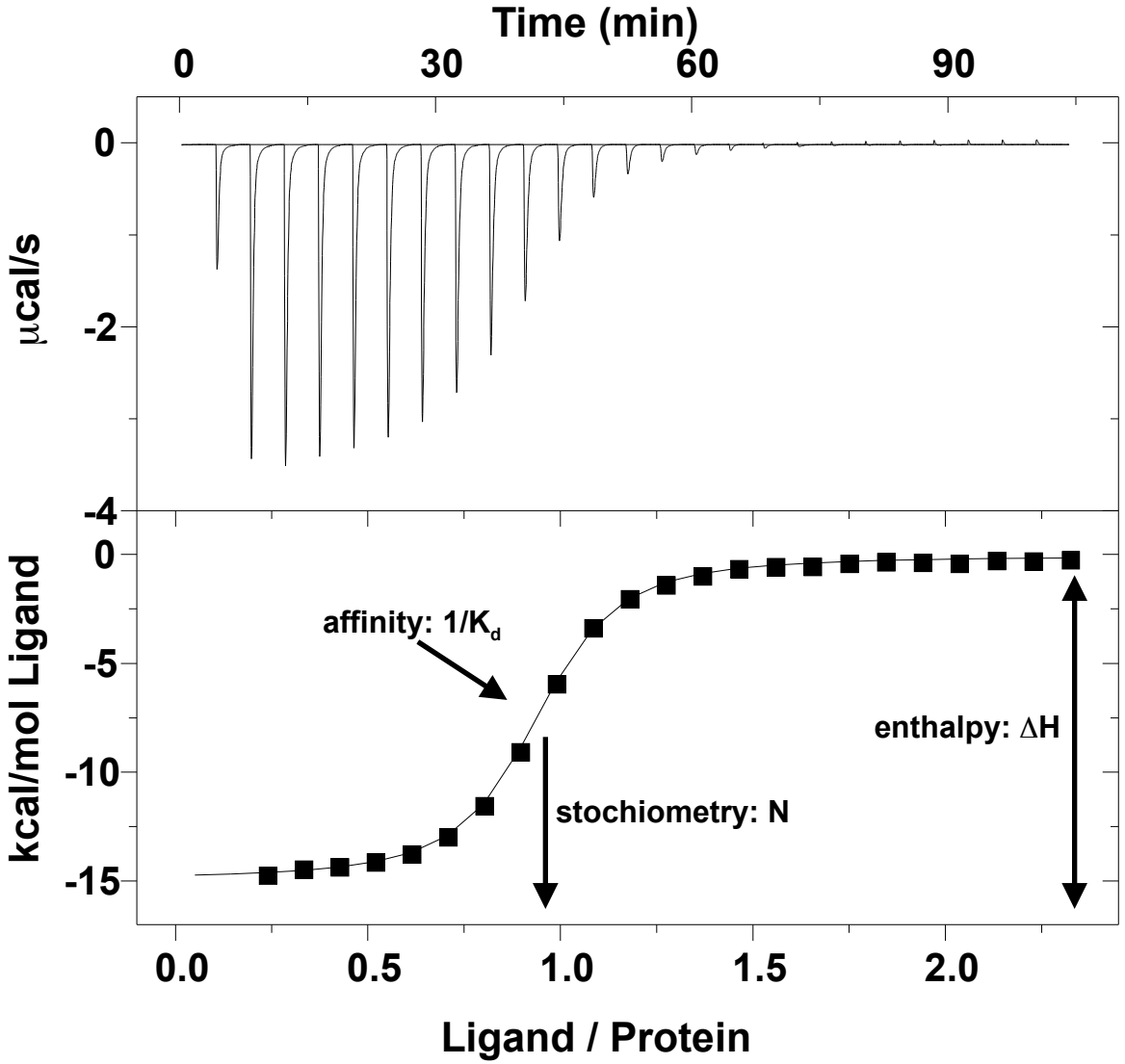
When the protein is saturated with ligand, no more binding occurs, and only heat of dilution is observed

ITC - fitting the data





Isothermal Titration Calorimetry (ITC)

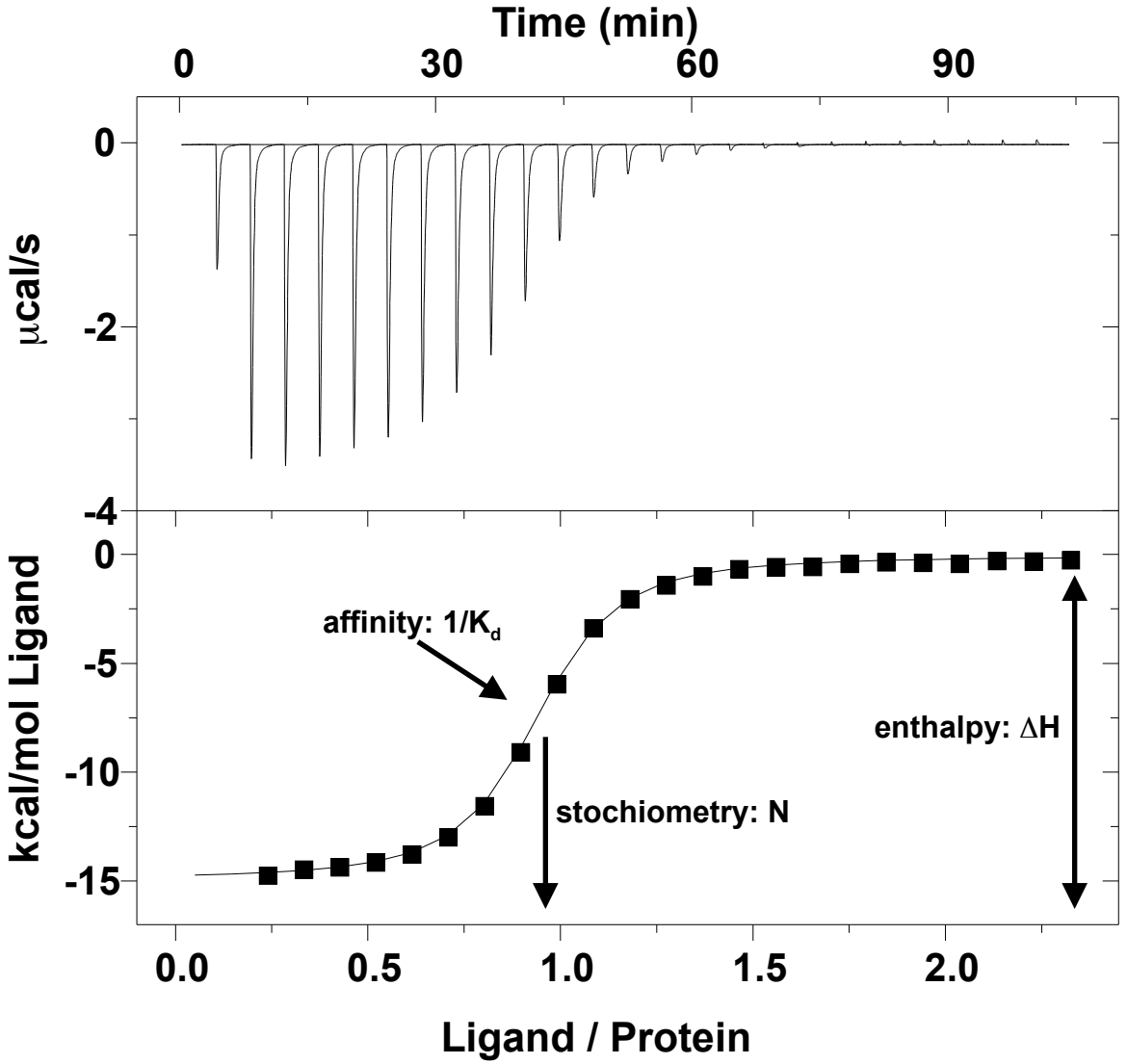


ITC
Theoretical Basis

$$\Delta G = -RT \ln K_B$$

$$\Delta G = \Delta H - T\Delta S$$

Isothermal Titration Calorimetry (ITC)

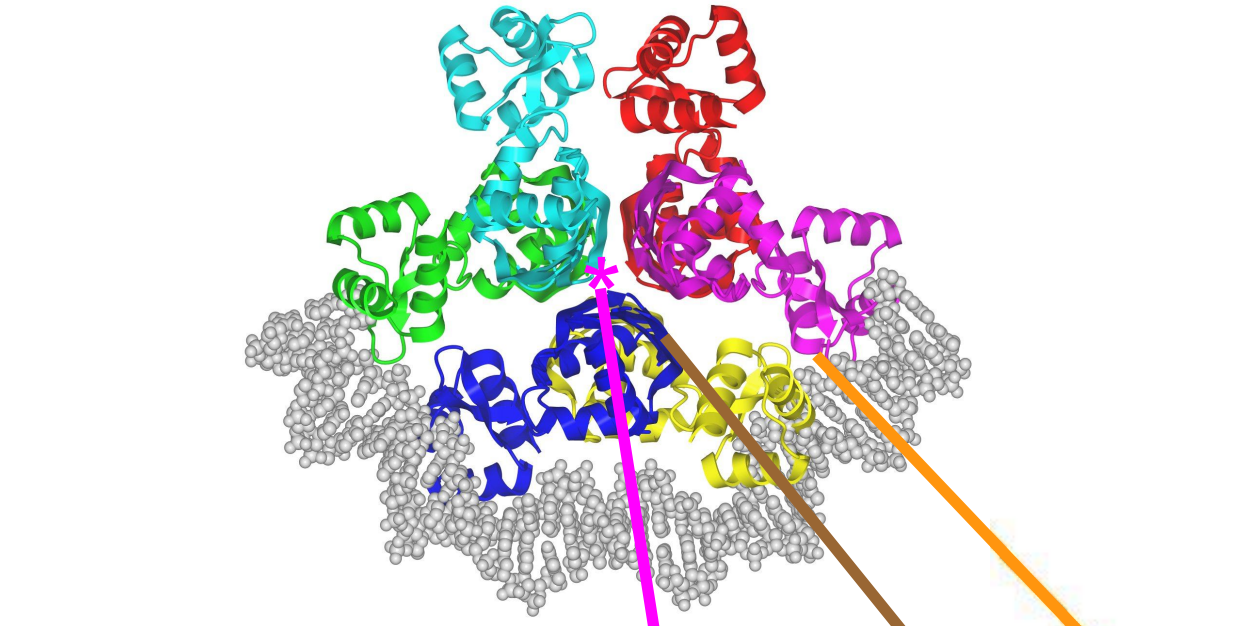


ΔG =- 14.1

ΔH =- 19.9

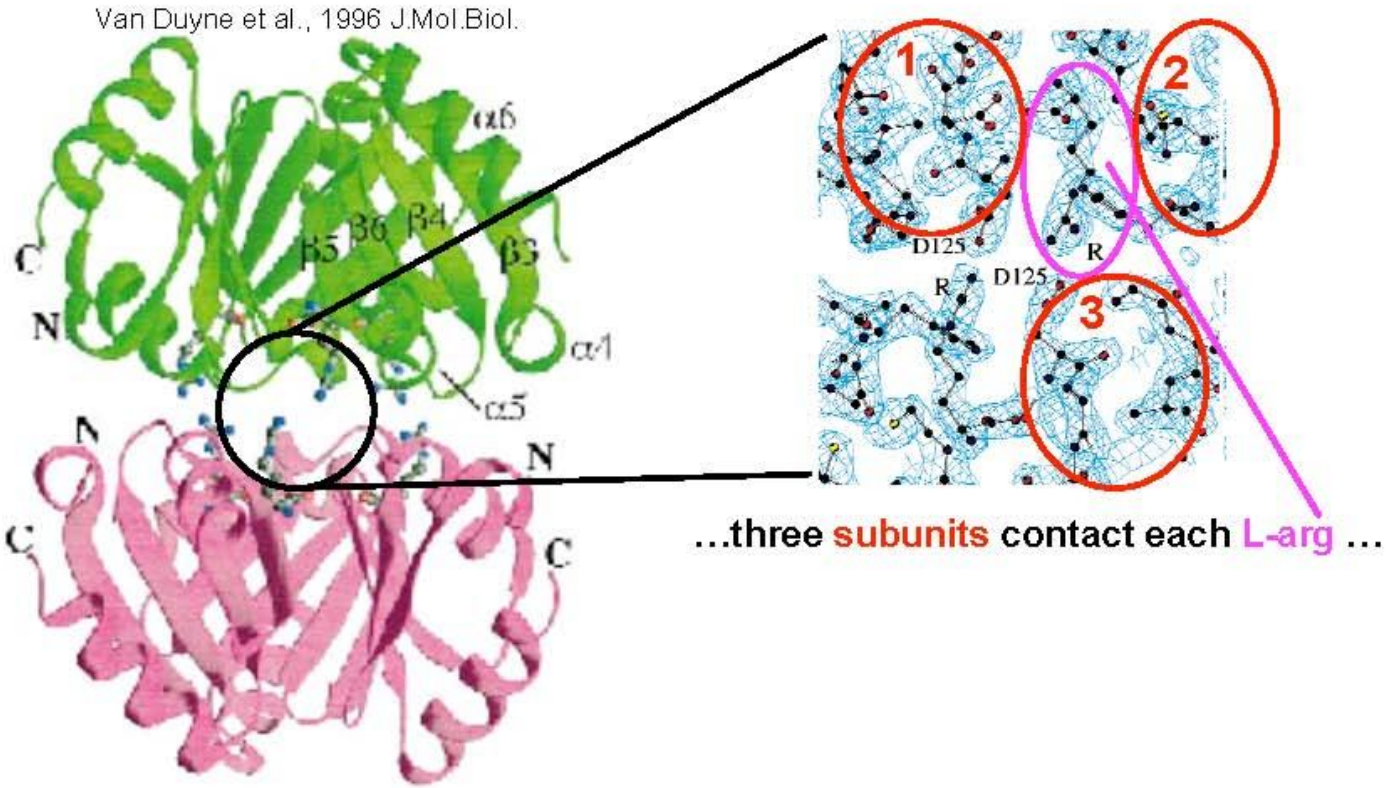
$T\Delta S$ =- 5.8

ArgR action at a distance controls L-arginine metabolism



Grandori et al., 1995 J. Mol. Biol.
Sunnerhagen et al., 1997 Nature Str. Biol.
Szwajkajzer et al., 2001 J. Mol. Biol.
Jin et al., 2005 J. Mol. Biol.

Two ArgRC trimers sandwich six L-arginines …



…three **subunits** contact each **L-arg** …

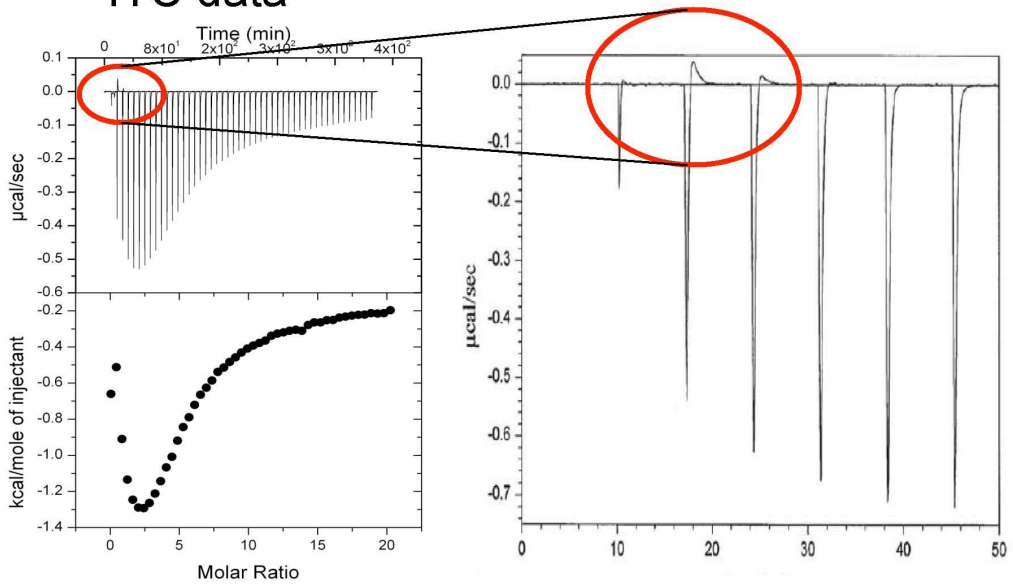
… but apo- and holo-ArgRC structures are essentially identical.

What is the allosteric mechanism?

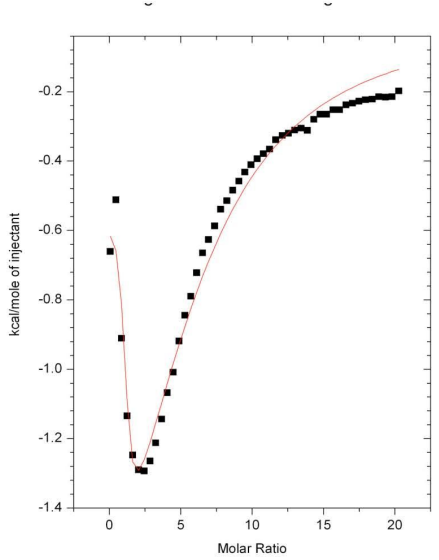
Symmetric ArgR (RC) hexamers binds six L-arg asymmetrically!

Jin et al., 2005 J. Mol. Biol.

ITC data



model-fitting

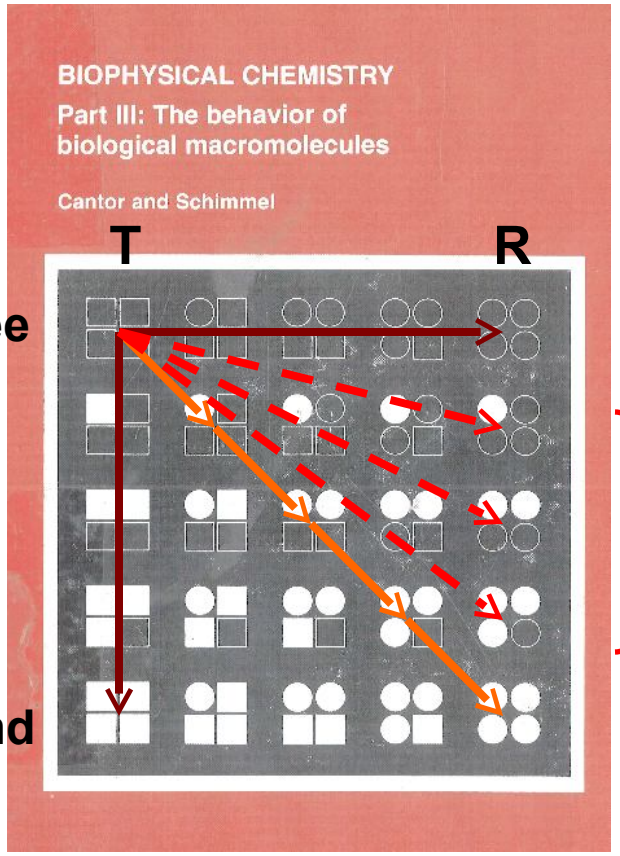


1 strong site with slow endotherm (~1 μM)
5 weaker, ~equivalent sites (~100 μM)

Negative cooperativity affords two action levels of response to L-arg

MD simulations to aid interpretation

Historical controversy in allosteric models hinges on symmetry



Ligand binding relaxes conformational constraints imposed upon monomers by subunit assembly.

Is conformational change sequential or concerted?

Monod, Wyman, Changeux (MWC): ***concerted***
partially-ligated R states are symmetric
positive cooperativity only

Koshland, Nemethy, Filmer: ***sequential***
partially-ligated R states need not be symmetric
positive and negative cooperativity

Perutz: correlated Hb structural changes with ligation
X-ray: structural change uncorrelated with ligation

Molecular dynamics (MD) purpose

- is to provide a molecular level picture of structure and dynamics

FOR MORE INFO...

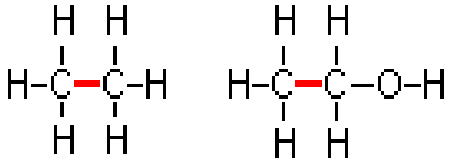
- P. Allen, D. J. Tildesley (1989) Computer simulation of liquids. Oxford University Press. ISBN 0198556454.
- D. C. Rapaport (1996) The Art of Molecular Dynamics Simulation. Cambridge University Press. ISBN 0521445612.
- Tamar Schlick (2002) Molecular Modeling and Simulation. Springer. ISBN 038795404X.

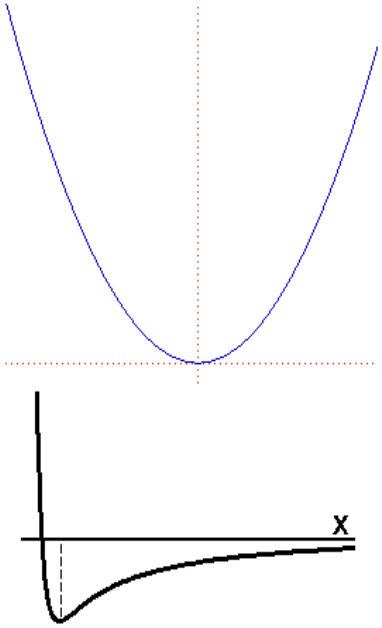
The basic principles

- Force Field
- Solving of classical (Newton's) equations
  - Variations of Verlet algorithm
    - Leap-frog algorithm

Force fields

- OPLSAA
- AMBER
- CHARMM
- GROMOS





$$U = \sum_{bonds} \frac{1}{2} k_r (r - r_0)^2$$

$$+ \sum_{angles} \frac{1}{2} k_\theta (\theta - \theta_0)^2$$

$$+ \sum_{torsions} \frac{V_n}{2} [1 + \cos(n\phi - \delta)]$$

$$+ \sum_{improper} V(improper\ torsion)$$

$$+ \sum_{elec} \frac{q_i q_j}{r_{ij}}$$

$$+ \sum_{LJ} \left[\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right]$$

LIST OF FORCE FIELD REFERENCES

http://msdlocal.ebi.ac.uk/docs/mmrefs.html

Verlet algorithm

$$\mathbf{r}(t+\Delta t) = \mathbf{r}(t) + \mathbf{v}(t)\Delta t + (1/2)\mathbf{a}(t)\Delta t^2 + (1/6)\mathbf{b}(t)\Delta t^3 + O(\Delta t^4) \quad (1)$$

$$\mathbf{r}(t-\Delta t) = \mathbf{r}(t) - \mathbf{v}(t)\Delta t + (1/2)\mathbf{a}(t)\Delta t^2 - (1/6)\mathbf{b}(t)\Delta t^3 + O(\Delta t^4) \quad (2)$$

Adding these two Tailor's

$$\mathbf{r}(t+\Delta t) = 2\mathbf{r}(t) - \mathbf{r}(t-\Delta t) + \mathbf{a}(t)\Delta t^2 + O(\Delta t^4) \quad (3)$$

$$\mathbf{a}(t) = -(1/m)\nabla V\left(\mathbf{r}(t)\right) \quad (4)$$

$$\mathbf{v}(t) = \frac{\mathbf{r}(t+\Delta t) - \mathbf{r}(t-\Delta t)}{2\Delta t}. \quad (5)$$

And if we put expression 4 into expression 3 we can use expression 3 with recent and previous positions to calculate the new position. Kinetic energy is calculate using expression 5.

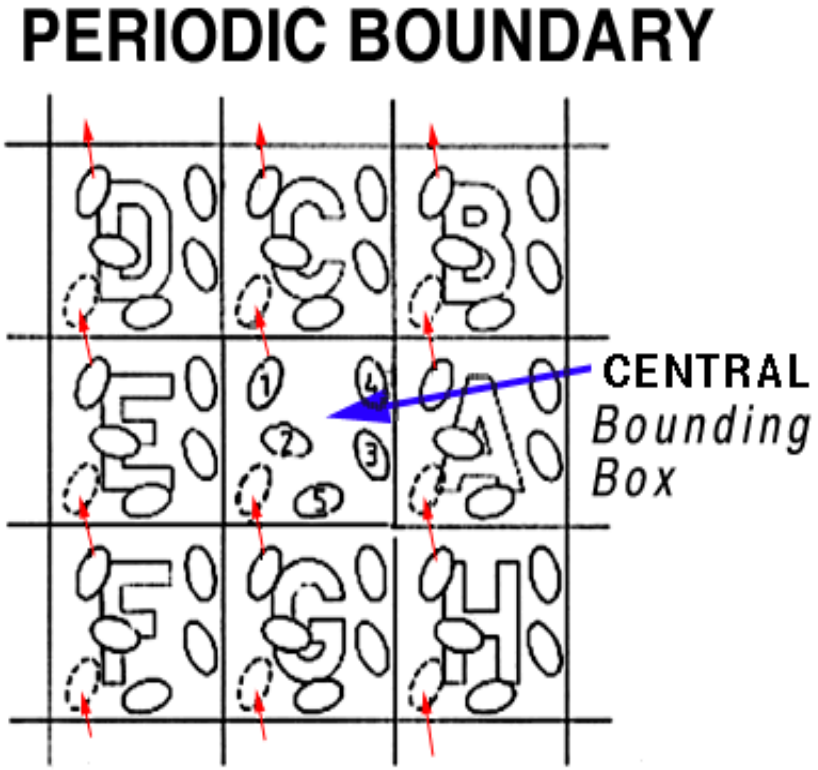
Advantages of MD

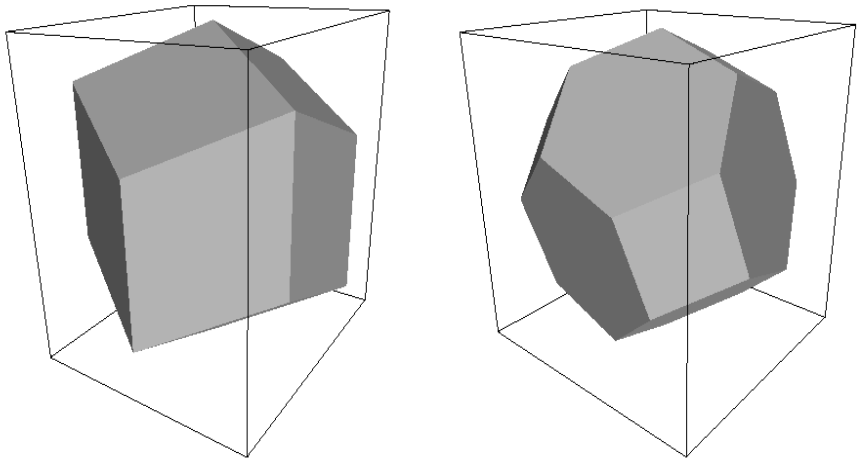
- Agreement between simulation and experiment is possible
- The only method which allows property/structure relationships observation at atomic level in nanosecond time scale
- Energy conservation
- Implicit inclusion of the thermodynamics

General MD protocol

- Setup of simulation box
- Read coordinates of the atoms, topologies of molecules which are in the simulation box and parameters
- Add solvent and ions
- Minimization of energy (solves coordinates collisions)
- Warm to desired temperature and equilibrate system
- Run dynamics and Analyze

Boundary conditions



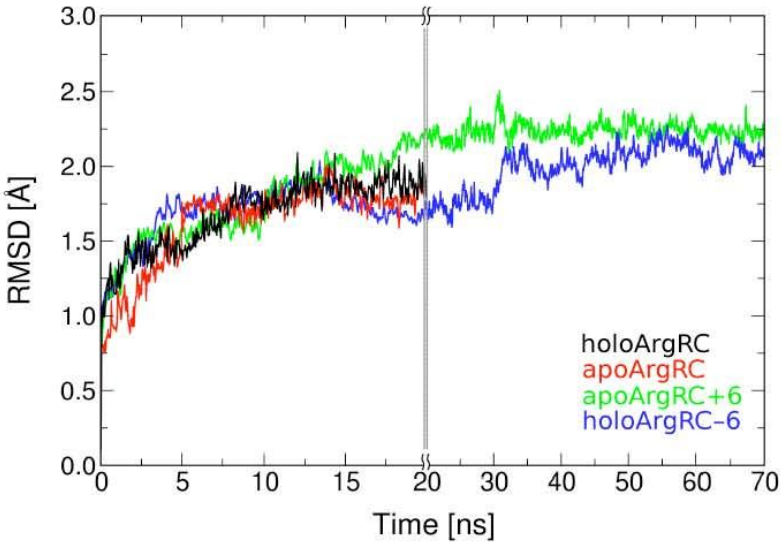


Box

Rhombic dodecahedron

Truncated octahedron

Full-atom simulations from apo- and holoArgRC crystal structures



numerous simulations also completed for:

apoArgRC+1arg

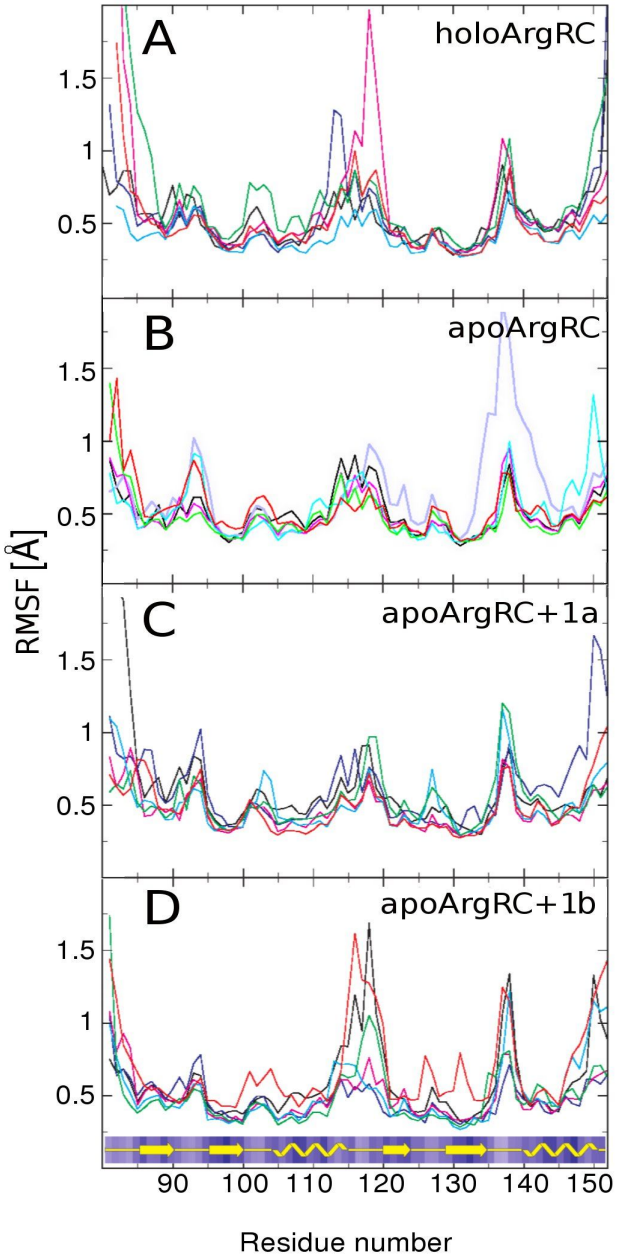
apoArgRC+2arg

holoArgRC-5arg

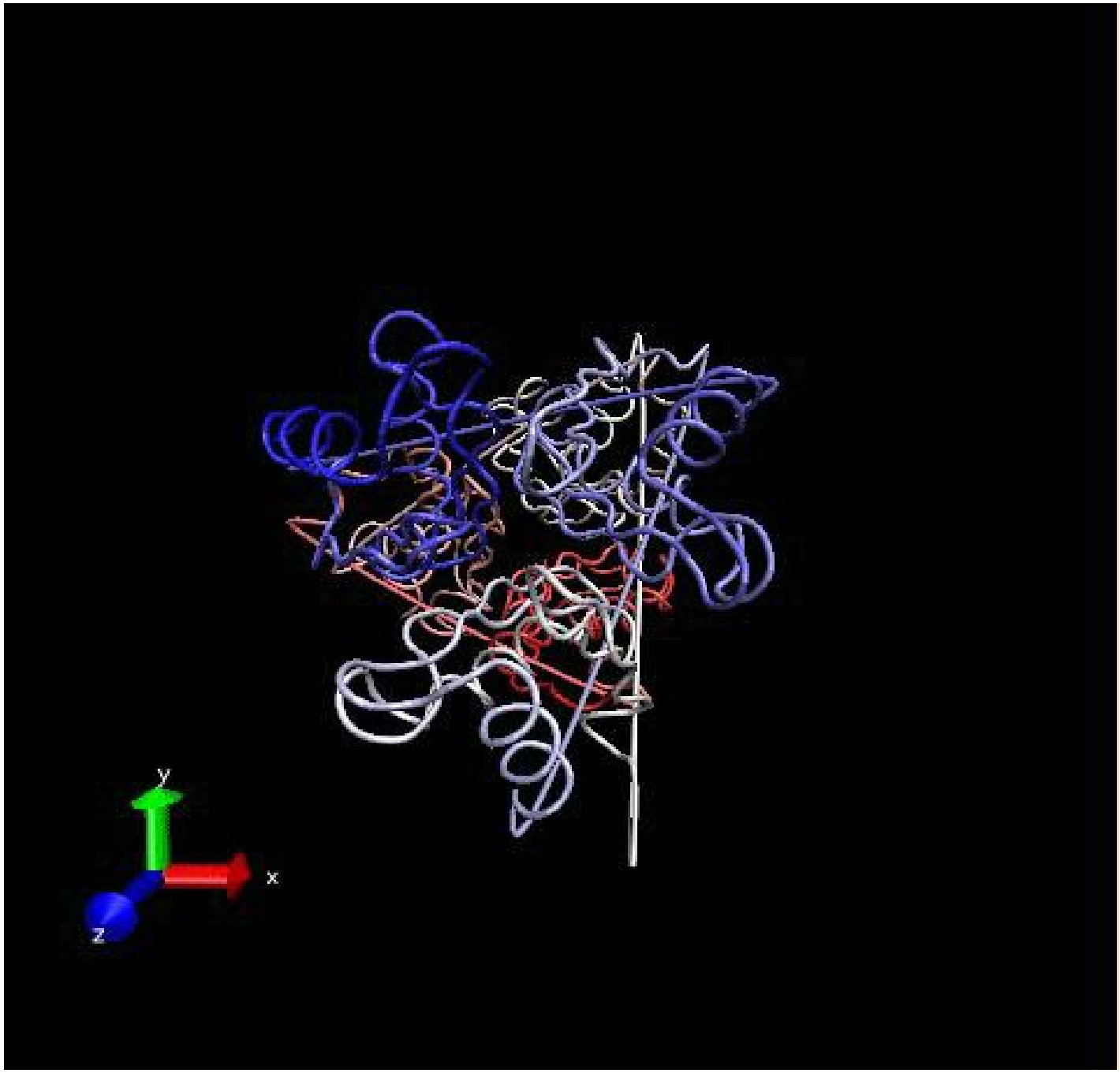
all ≥20 ns equilibrated

MW ~ 50,000 Da

Cα fluctuations

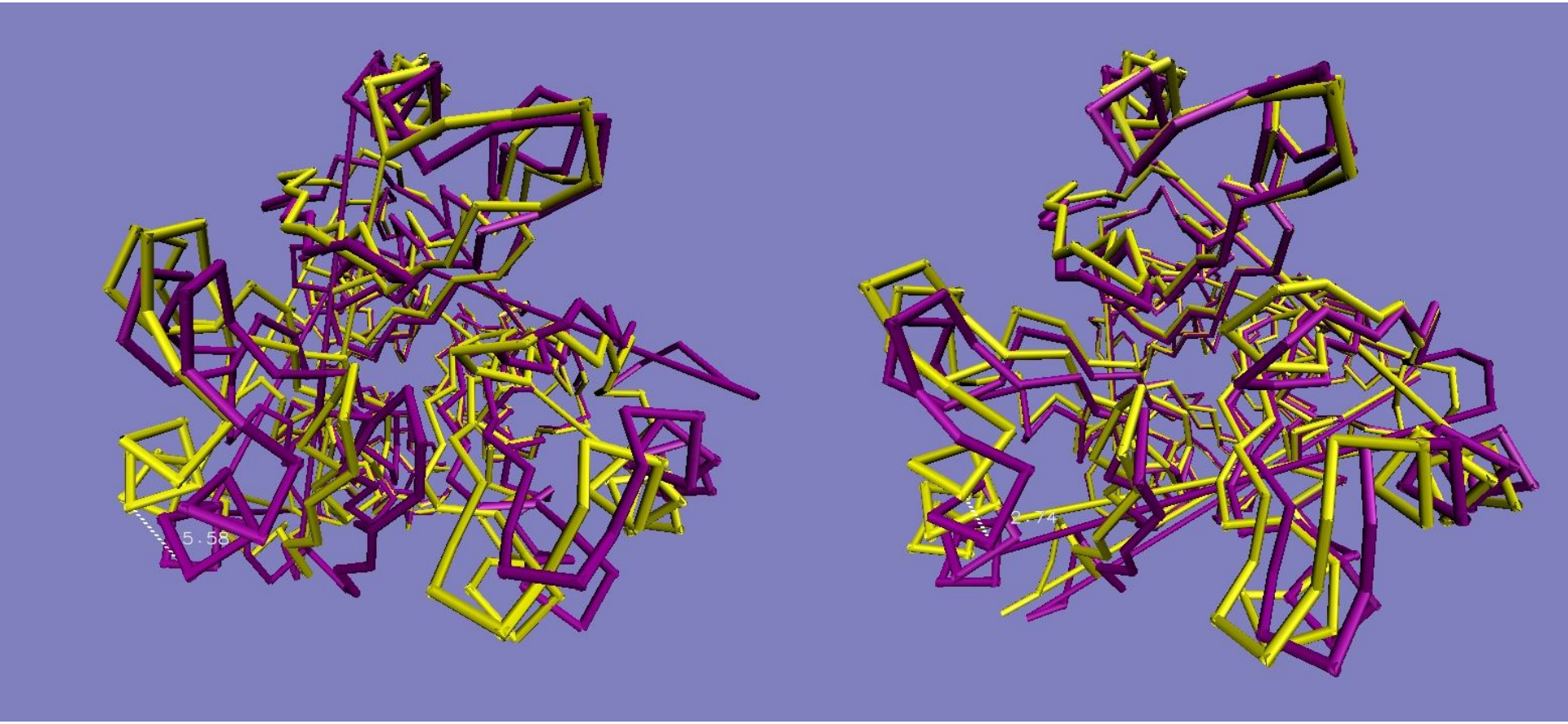


The maximum displacement of each Cα during the last 10 ns is reported (RMSF, Å).


y
x
z

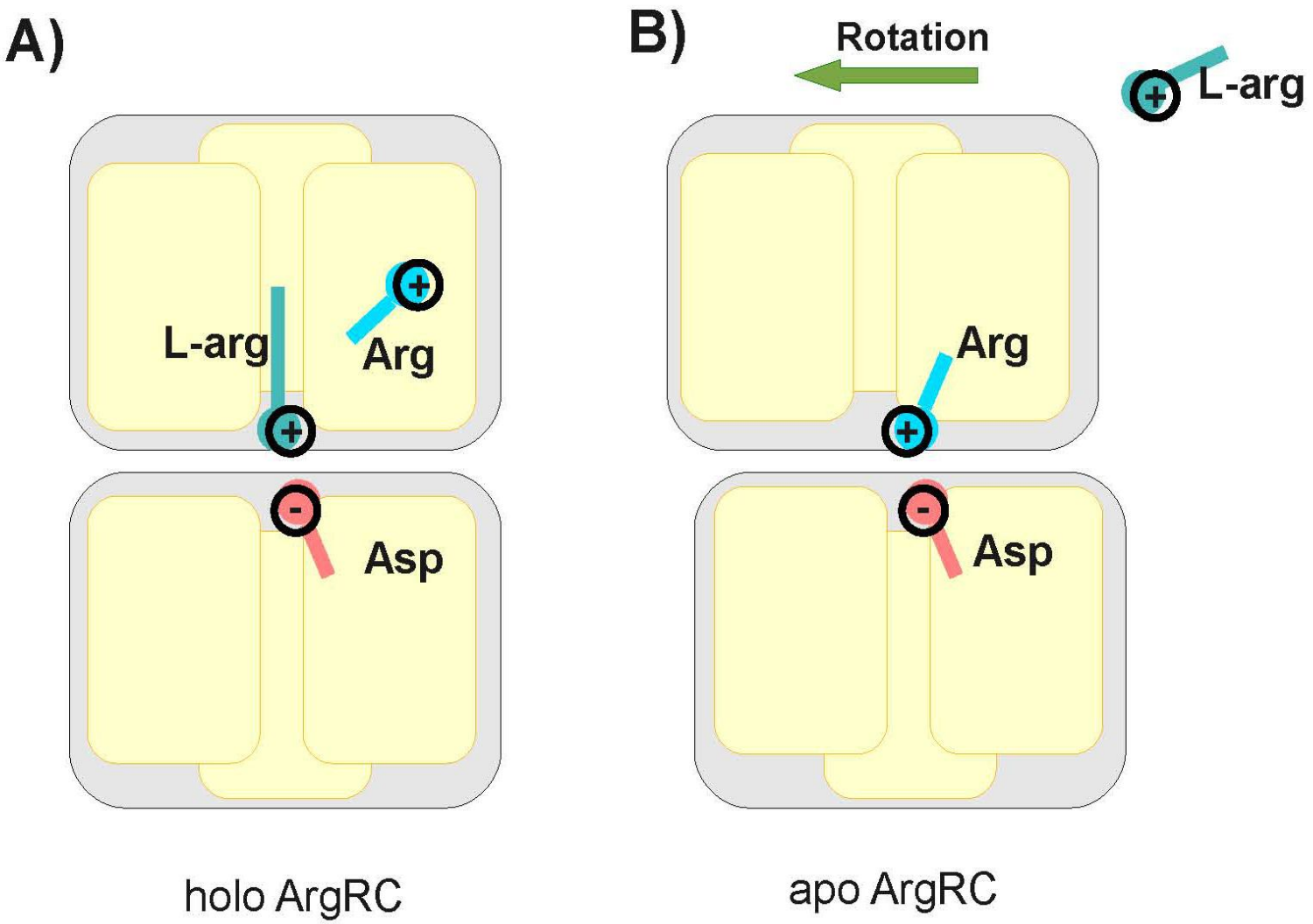
Oscillating motion of apoArgRC

Rotation of one trimer about the other by ~ 13 degrees in one direction only.



left: the maximum movement on the outer part of Calpha of ArgC is 5.7 A, corresponding to a rotation with an angle of 13 degrees as maximum value.

right: ArgC with the presence of one L-Arginine gives a value of maximum movement of 3.2 A and an angle of 7 degrees.



L-arg binds in transiently open pockets; stops rotation.

L-arg guanidino group substitutes for Arg110 in salt bridge to Asp128.

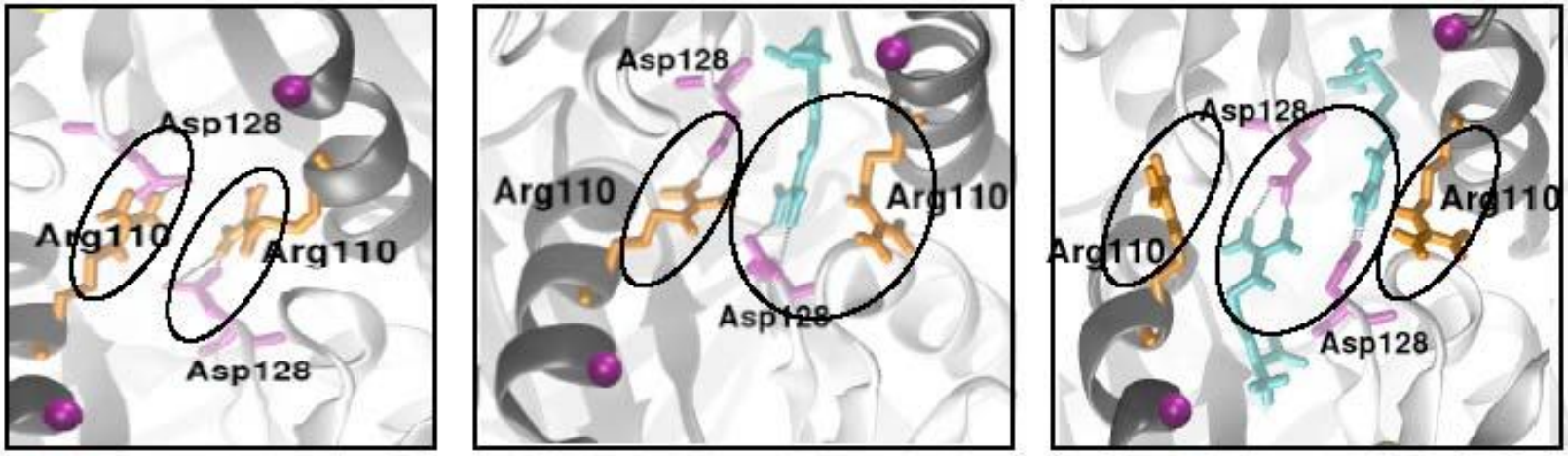
Other salt bridges remain intact until additional L-arg displaces them.

L-arg ligands compete with resident Arg residues

rotated apoArgRC
Arg-Asp salt bridges

non-rotated apoArgRC
+1 L-arg in open pocket
5 Arg-Asp salt bridges

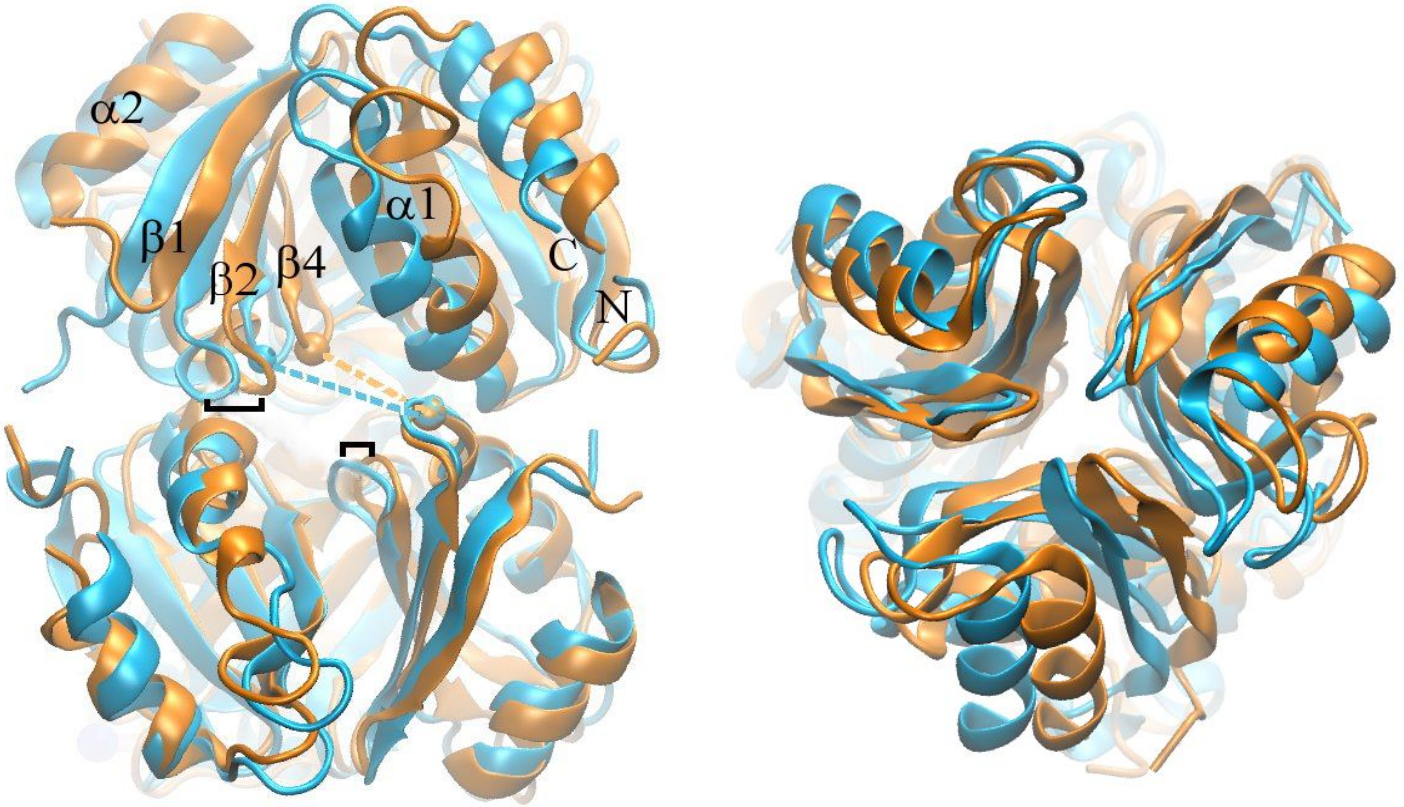
holoArgRC
+6 L-Arg
no Arg-Asp salt bridges



Promoted by Arg110-Asp128 salt bridges across the L-arg binding pockets.

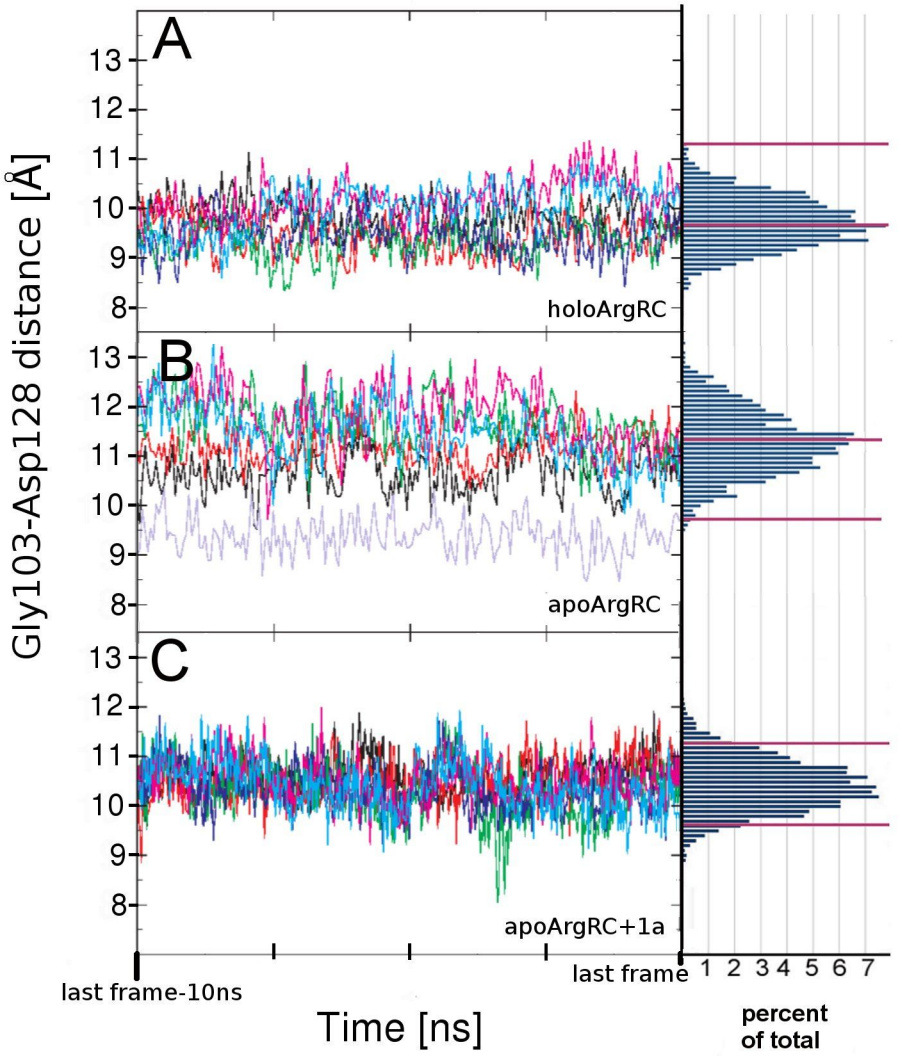
Arg110Ala mutation eliminates rotation; therefore Arg110 causes rotation.

ArgRC rotation



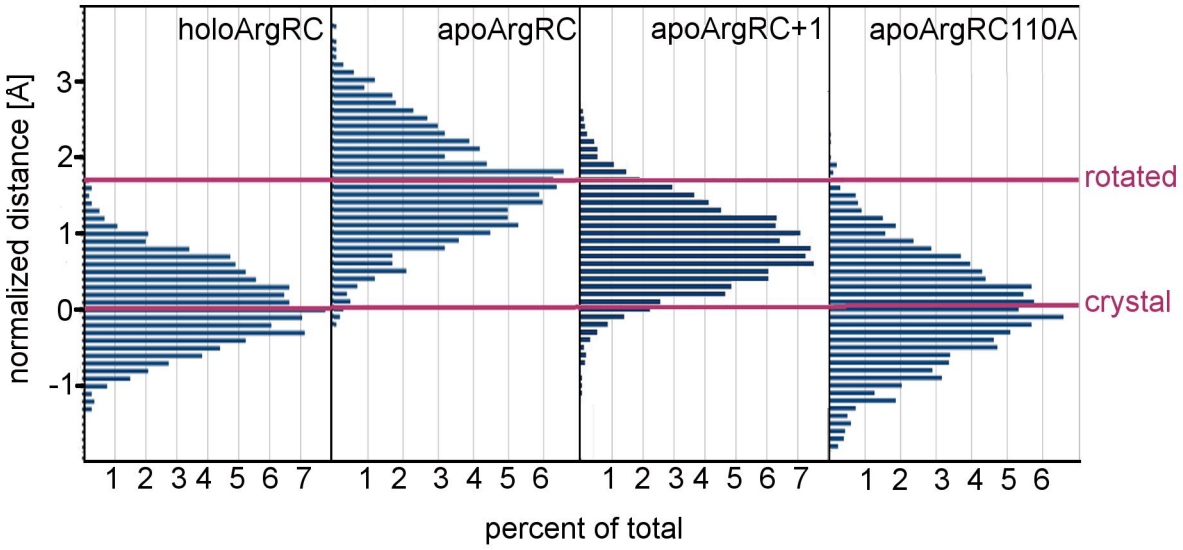
Overlay of average hexamer structures from the equilibrated part of the simulations showing the conformational shift from the holoArgRC (orange) structure that occurs uniquely in apoArgRC (blue).

Species distribution



Distances between Gly103 and Asp128 C_α atoms are averaged over the six pairs in the hexamer every 50 ps during the final 10 ns of each indicated simulation.

Species distribution



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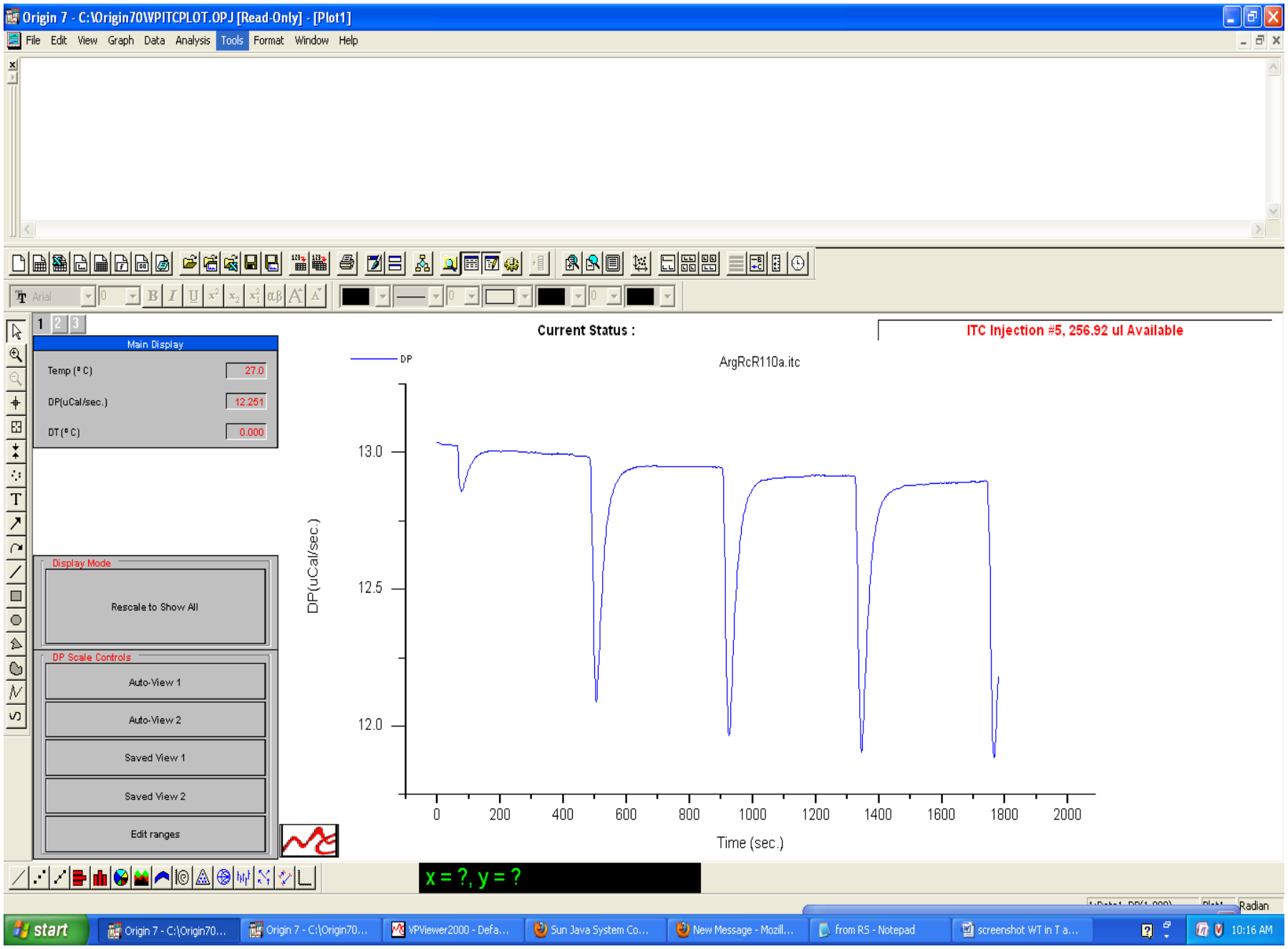
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DP(uCal/sec.) 12.251
DT (° C) 0.000
Display Mode
Rescale to Show All
DP Scale Controls
Auto-View 1
Auto-View 2
Saved View 1
Saved View 2
Edit ranges
Current Status :
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DP
ArgRcR110a.itc
DP(uCal/sec.)
13.0
12.5
12.0
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200
400
600
800
1000
1200
1400
1600
1800
2000
Time (sec.)
x = ?, y = ?
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start
Origin 7 - C:\Origin70...
Origin 7 - C:\Origin70...
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from RS - Notepad
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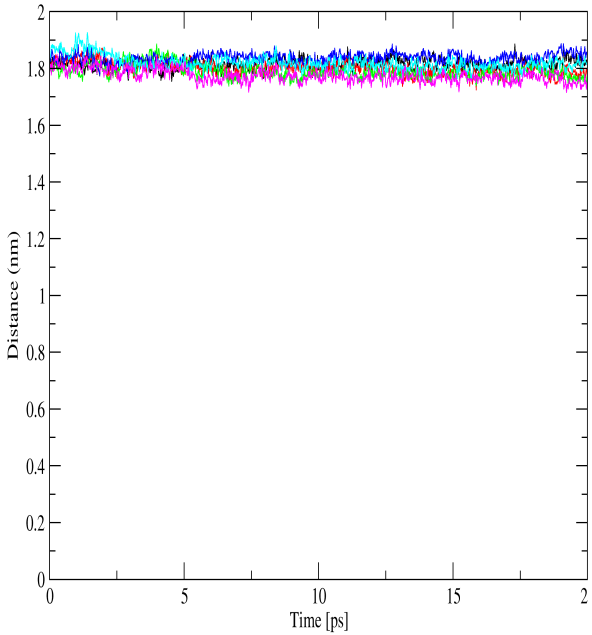
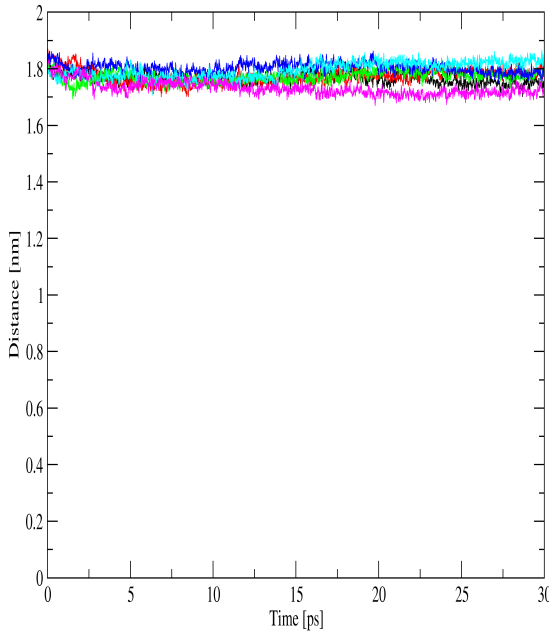
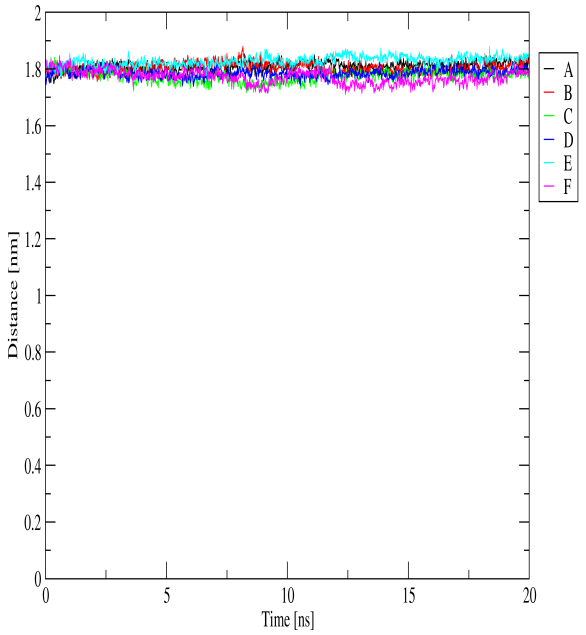
10:16 AM

Symmetry in the hexamer

Distance monomer center of mass to hexamer center of mass







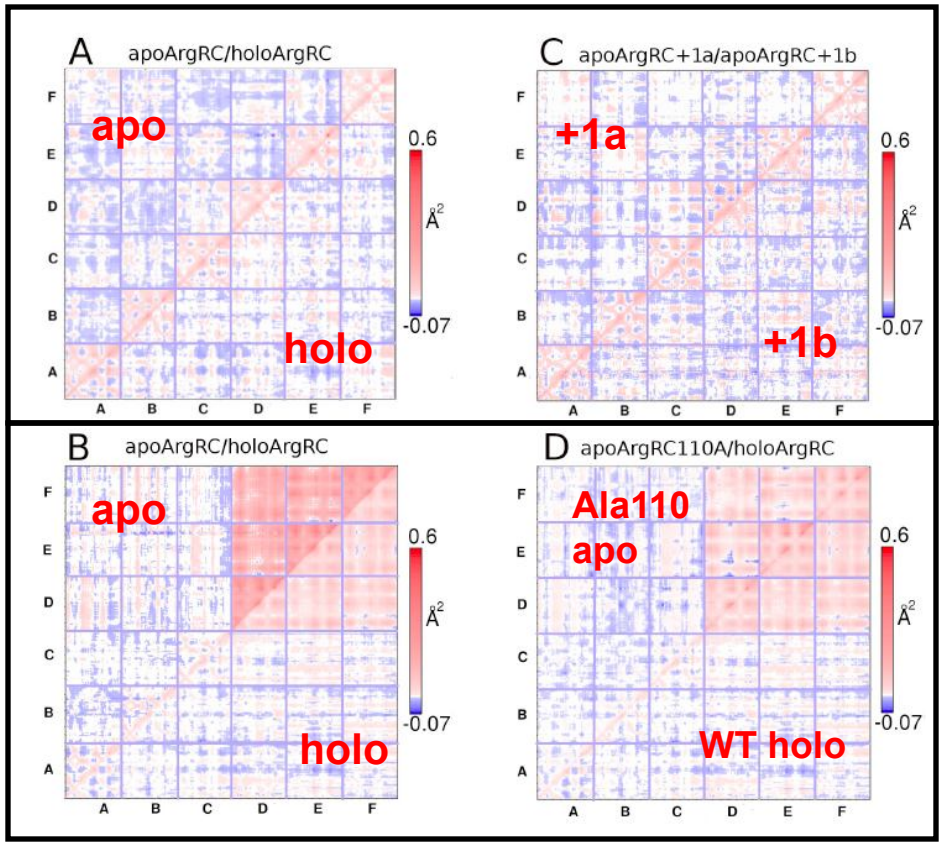
Apo
L-Arg

+1
Holo

Covariance analysis of C_α motions

hexamer as reference structure →

trimer as reference structure →



apoArgRC: rotational oscillation of trimers

holoArgRC with six L-arg: no rotation

apoArg110Ala mutant: no rotation

+1arg: no rotation but variable (a, b), intense, ***random***, motions of folded monomers

Combine calculated energy contributions

- TS: system configurational entropy

inter-trimer H-bond number

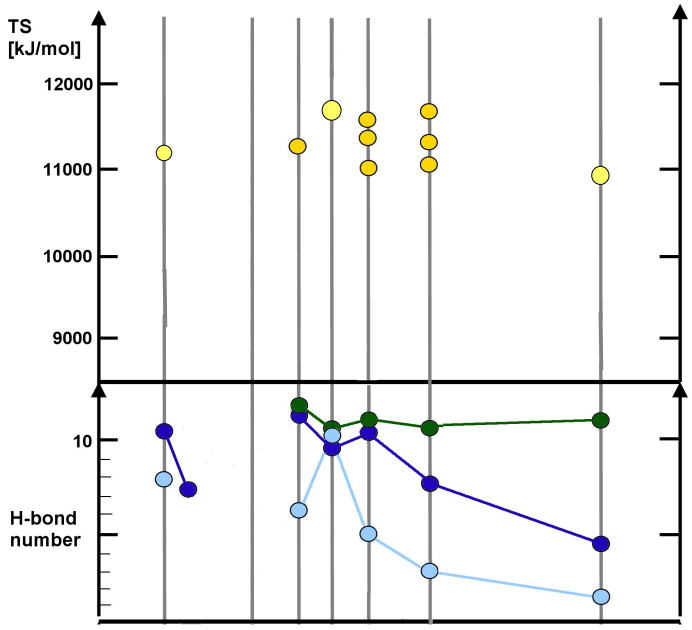
- persistent (>50% of time)
- total (excludes L-arg)
- total (includes L-arg)

also calculated from simulations:

per-ligand binding enthalpy (all 6 equal)

per-ligand binding free energy

(+1 L-arg = ~50% more favorable)



Contributions to enthalphy and entropy can be combined with experimental knowledge from ITC to describe the reaction coordinate and to estimate relative free energies

Combined energetic contributions

1 ApoArgRC oscillates between rotational conformers; non-rotated conformer (grey) transiently admits L-arg

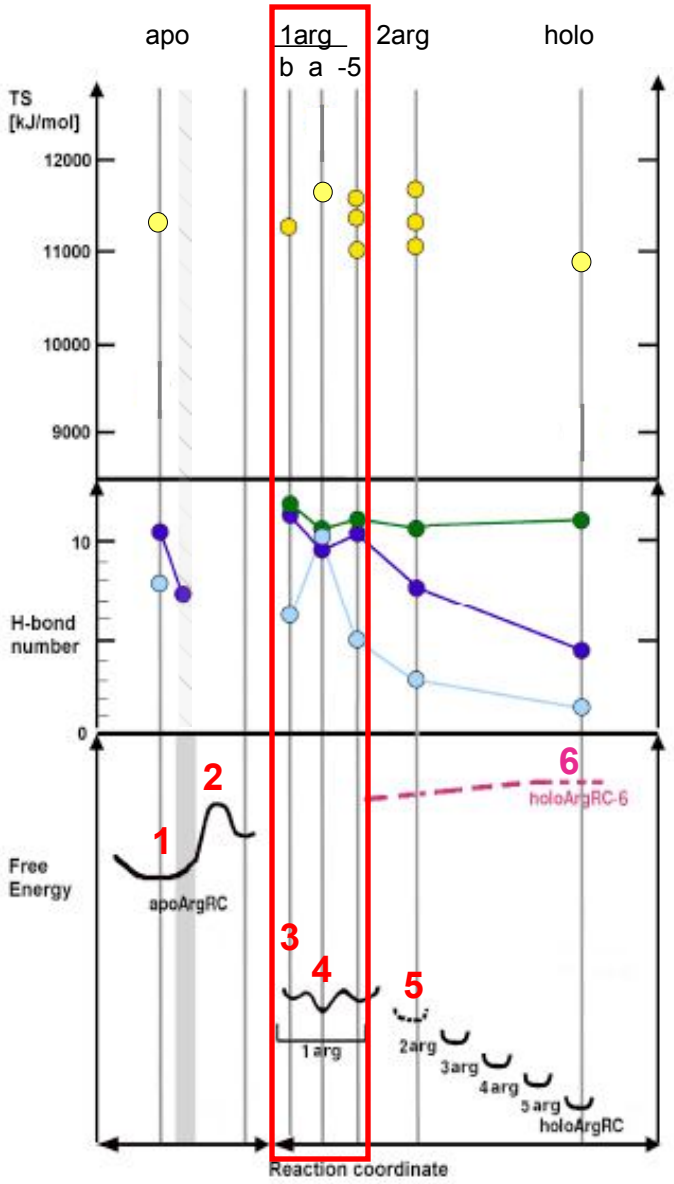
2 L-arg promotes barrier crossing (ITC endotherm) leading to binding-competent state

3 First L-arg replaces one Arg-Asp salt bridge
Stops oscillation

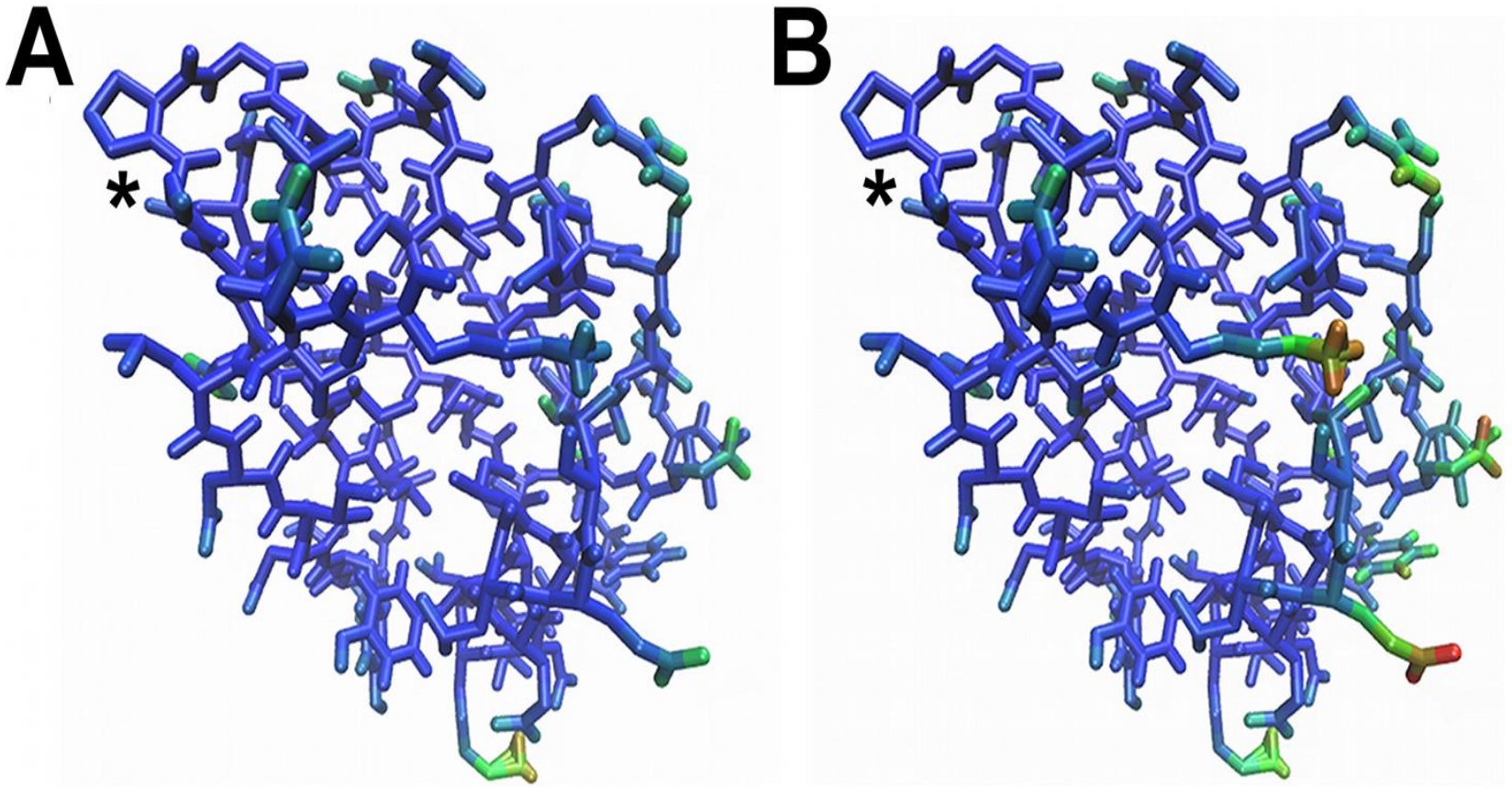
4 All H-bonds are persistent in +1a state
Cooperative, symmetric network
Paradoxically, entropy stays constant

5 Second L-arg compromises H-bond network
Less optimal L-arg affinity

6 HoloArgRC with 6 L-arg removed does not equilibrate - blocked by barrier?



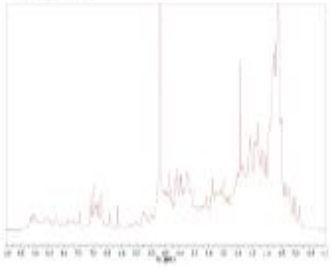
Motion gradient



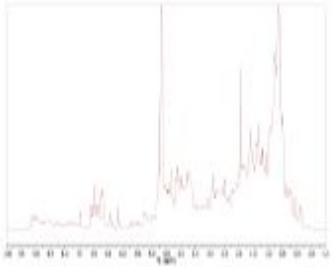
Macroscopic analog

With long shutter speed colored halos reveal motion (shadows are below the image only). The central attachment point of each balloon is not moving.

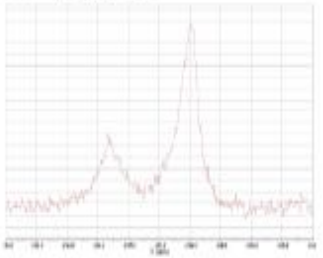
1H apo



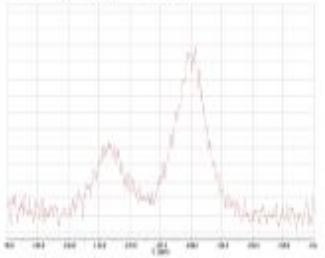
1H +1arg



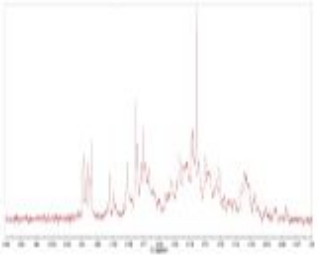
19F (Tyr) apo



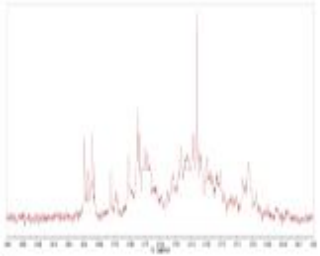
19F (Tyr) +1arg



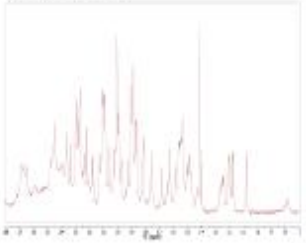
13C apo
(low field)



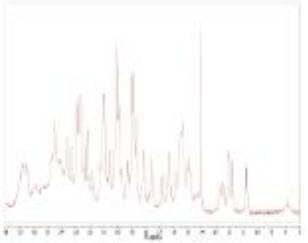
13C +1arg



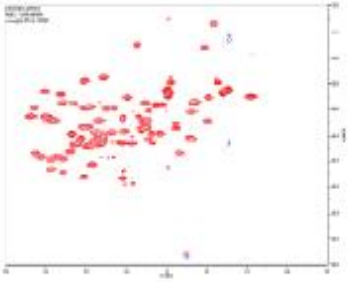
13C apo
(high field)



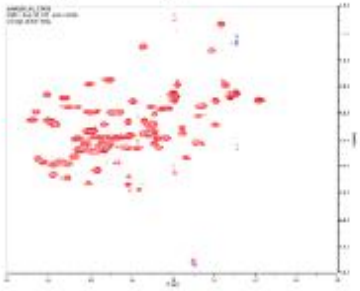
13C +1arg



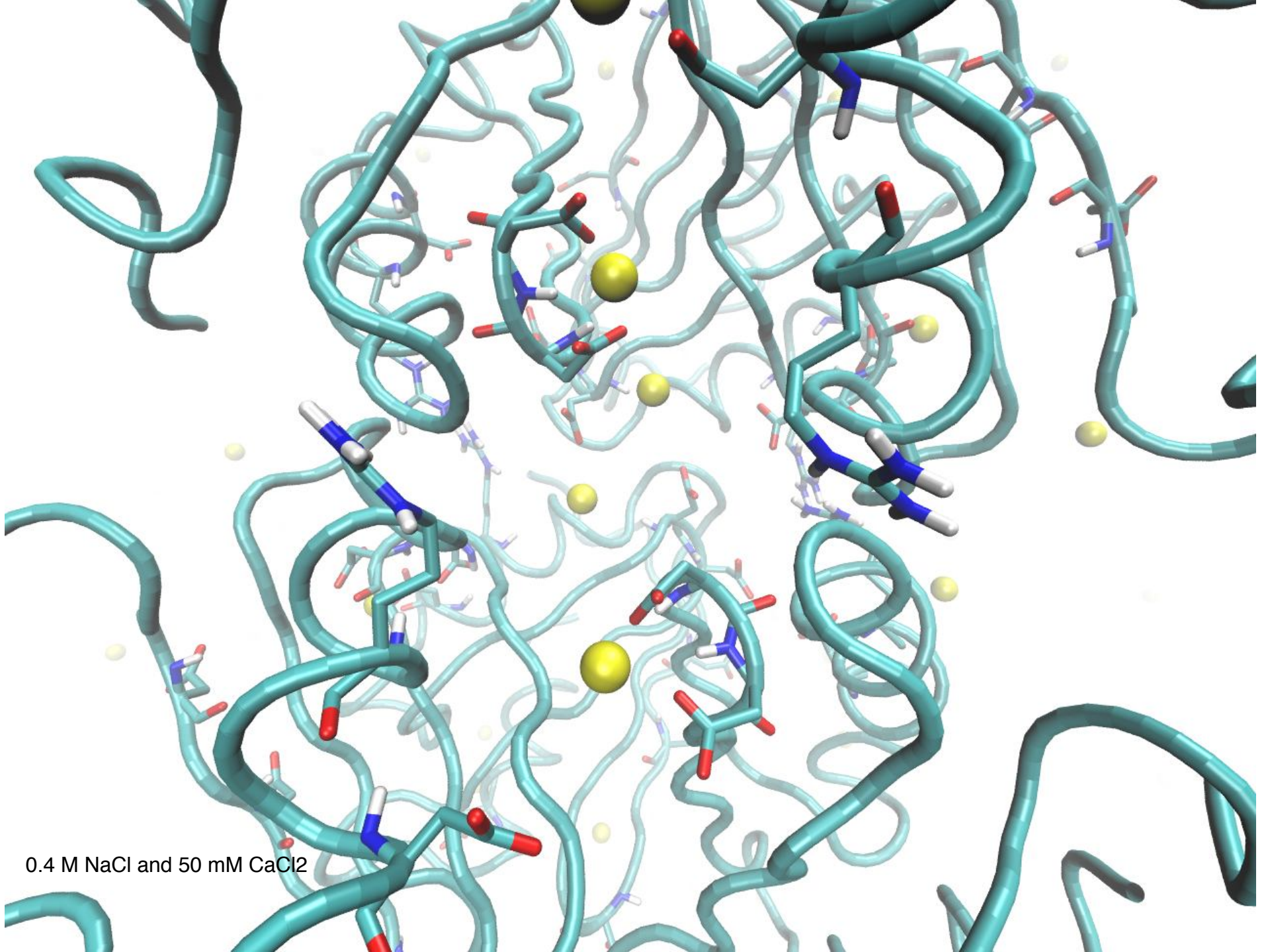
HSQC apo



HSQC +1arg



Allosteric relaxed-state structure. A. NMR data. The observed nucleus is indicated above each pair of spectra. The left spectrum of each pair is that of apoArgRC and the right panel is that of singly-liganded ArgRC. Sample preparation and final concentrations of protein and ligand are given in the Supplemental Information. **C. Macroscopic analog.** With long shutter speed colored halos reveal motion (shadows are below the image only). The central attachment point of each balloon (i.e., subunit; 10) is not moving.


0.4 M NaCl and 50 mM CaCl2

Origin 7 - C:\Origin70\VPITCPLOT.OPJ [Read-Only] - [Plot1]

File Edit View Graph Data Analysis Tools Format Window Help

1 2 3

Main Display

Temp (° C)	27.0
DP(uCal/sec.)	14.455
DT (° C)	0.000

Display Mode

Rescale to Show All

DP Scale Controls

Auto-View 1

Auto-View 2

Saved View 1

Saved View 2

Edit ranges

Current Status :

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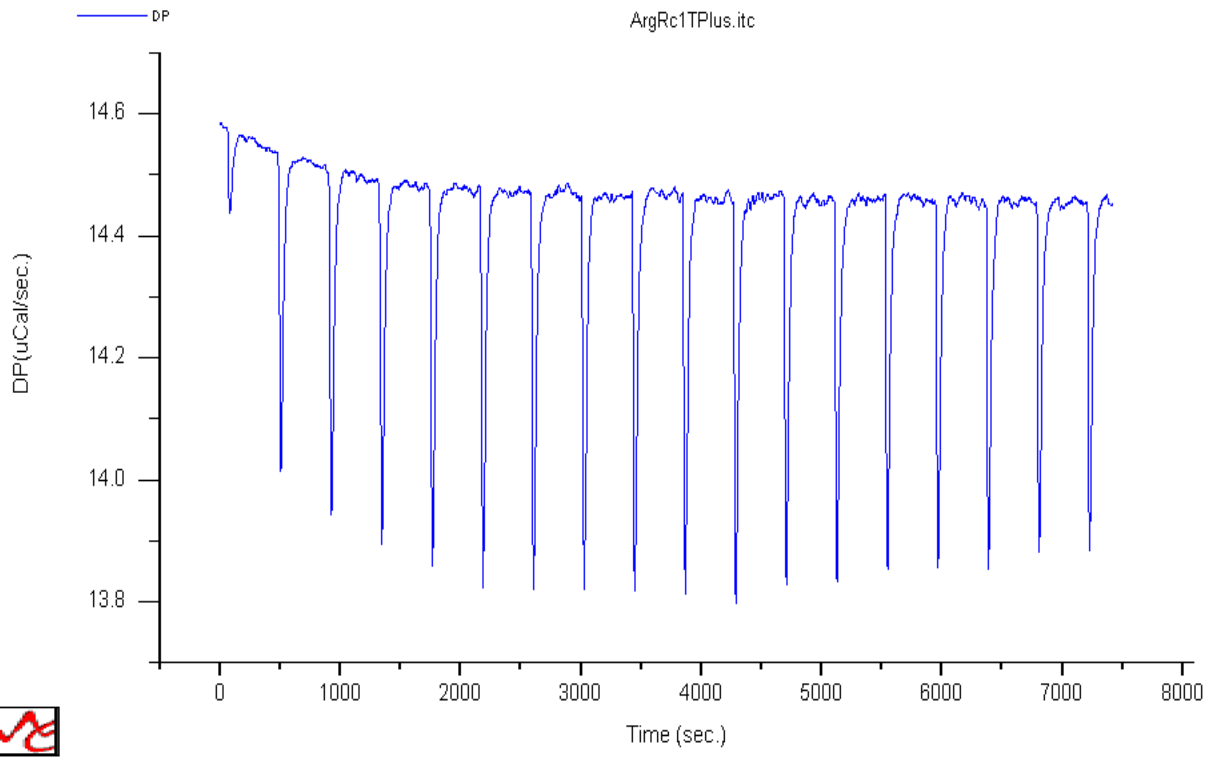


x = ?, y = ?

Radian

start | Origin 7 - C:\Origin70... | VPViewer2000 - Defa... | Sun Java System Co... | Downloads | New Message - Mozill... | Data (D:) | from RS - Notepad | 4:14 PM

Take home message

The singly-bound state can be pictured as a hexamer in which all subunits are anchored near the center, with thermal motion transferred to the periphery like a bouquet of balloons in strong wind.

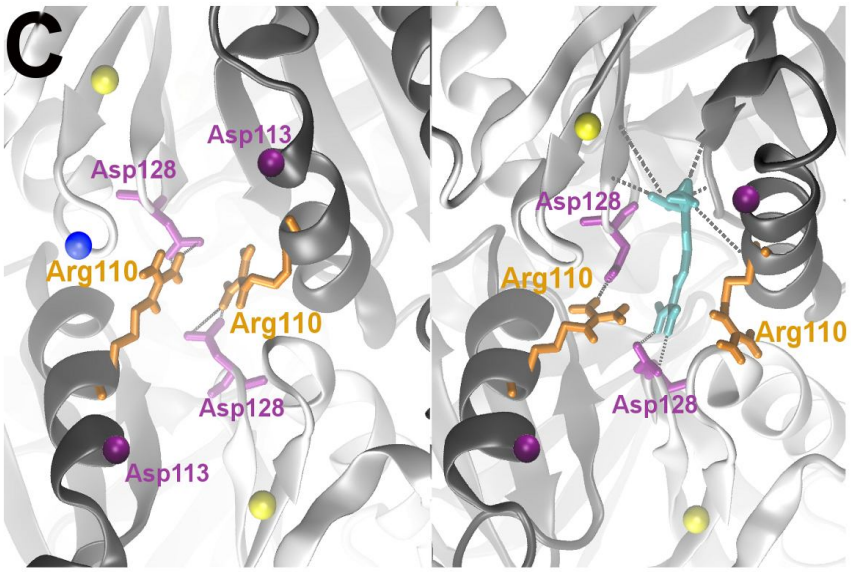
Addition of a second ligand forces a compromise among optimized interactions, reducing affinity, but distance fluctuation measurements indicate that the high symmetry of the singly-liganded state is preserved.

Partially-liganded states can be *structurally symmetric* despite being *conceptually asymmetric*

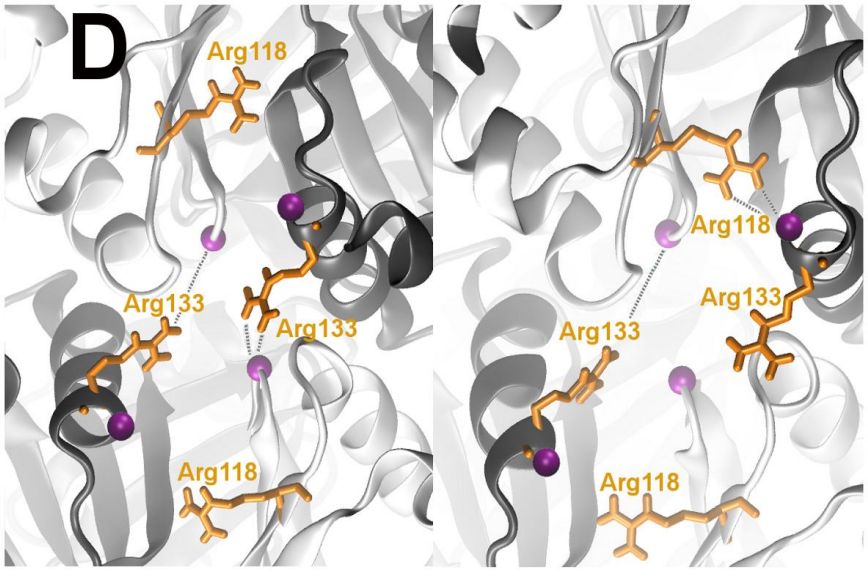
Symmetric relaxed states can be achieved even when binding is negatively cooperative (sequential)

The L-arg co-factor is not only an amino acid competing with Arg110 for Asp128, but also an ion. Therefore in high concentrations other ions can compete for the aspartic acids in the binding site .

Mycobacterium tuberculosis



C. EcoArgRC.
Left, apoArgRC. Right, +1arg state.



D. ApoMtArgRC.
Left, clockwise rotation. Right, counterclockwise rotation.

Publications

R Strawn, M Melichercik, M Green, T Stockner, J Carey, R Ettrich (2010) *Symmetric allosteric mechanism of hexameric E. coli arginine repressor exploits competition between L-arginine ligands and resident arginine residues PLoS Comput Biol. 2010 Jun 3;6(6):e1000801*

R Strawn, P Murthy, I Pelczer, R Ettrich, J Carey (2012) *Structural and thermodynamic evidence for allosteric relaxed-state symmetry* Submitted

R Strawn, T Stockner, M Melichercik, L Jin, J Carey, R Ettrich (2011) *Synergy of molecular dynamics and isothermal titration calorimetry in studies of allostery.* In: *Methods in Enzymology*, Biothermodynamics D Edited by:Michael L. Johnson, Jo M. Holt , Gary K. Ackers. 492:151-88.

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