pK_a calculations

Methods and Applications

A.H. Juffer

The University of Oulu

Finland-Suomi

Definition of pK_a (1)

- The pK_a of a titrating site is defined as the pH for which the site is 50% occupied, that is
  - The pH for which the occupancy q is 0.5.

$$HA + H_2O \Leftrightarrow A^- + H_3O^+$$

Deprotonation reaction

Definition of pK_a (2)

$$HA + H_2O \Leftrightarrow A^- + H_3O^+$$

$$K_a = \frac{a_{A^-} a_{H_3O^+}}{a_{HA}} = \frac{a_{A^-}}{a_{HA}} a_{H_3O^+} = \frac{1-q}{q} a_{H_3O^+}$$

q is degree of protonation or occupancy:
Number of bound protons as a function of p*H*

Titration curve:

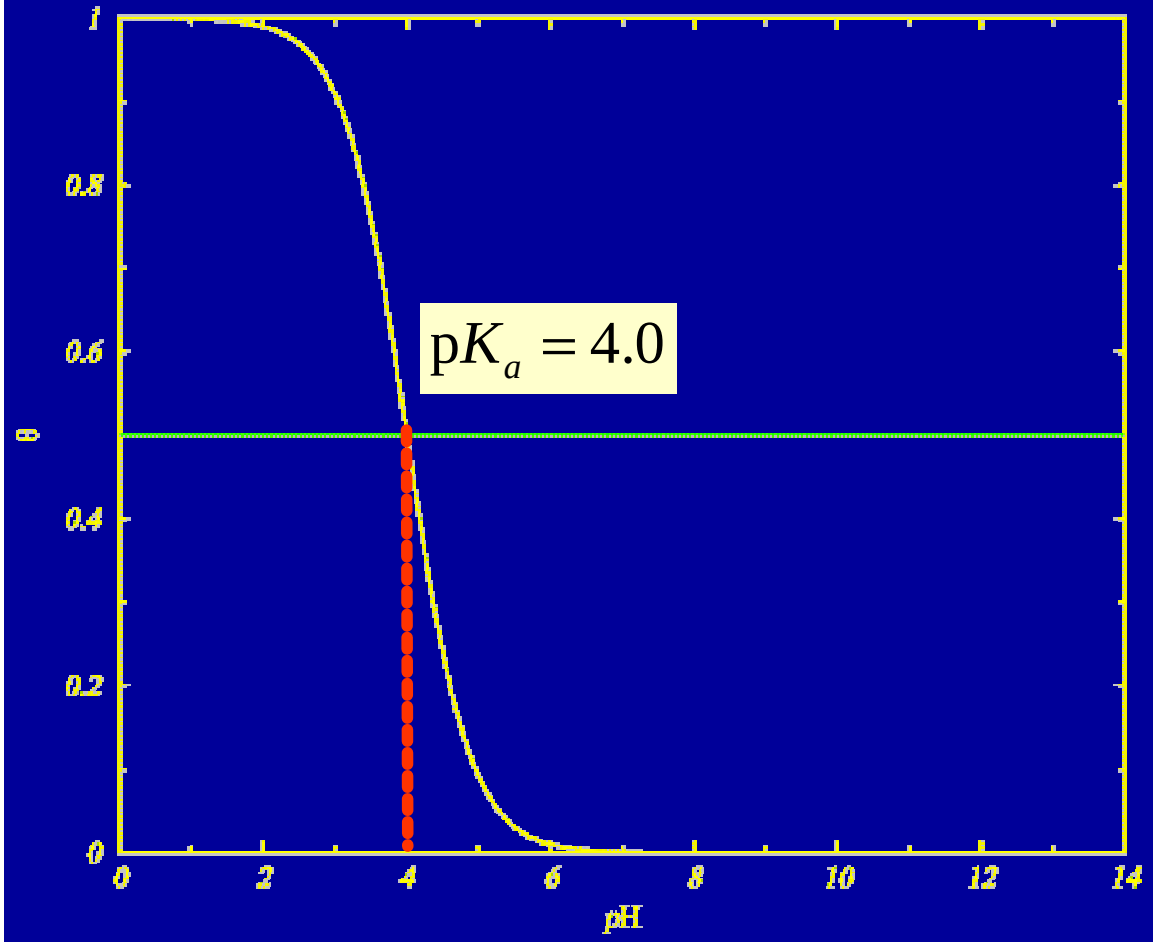
$$q(\mathrm{p}H) = \frac{1}{1 + e^{-\ln 10 (\mathrm{p}K_a - \mathrm{p}H)}}$$

$$\mathrm{p}H = -^{10}\log a_{H_3O^+}$$

$$\mathrm{p}K_a = -^{10}\log K_a$$

$$\Delta G^{\Theta} = -RT \ln K$$

Definition of pK_a (3)

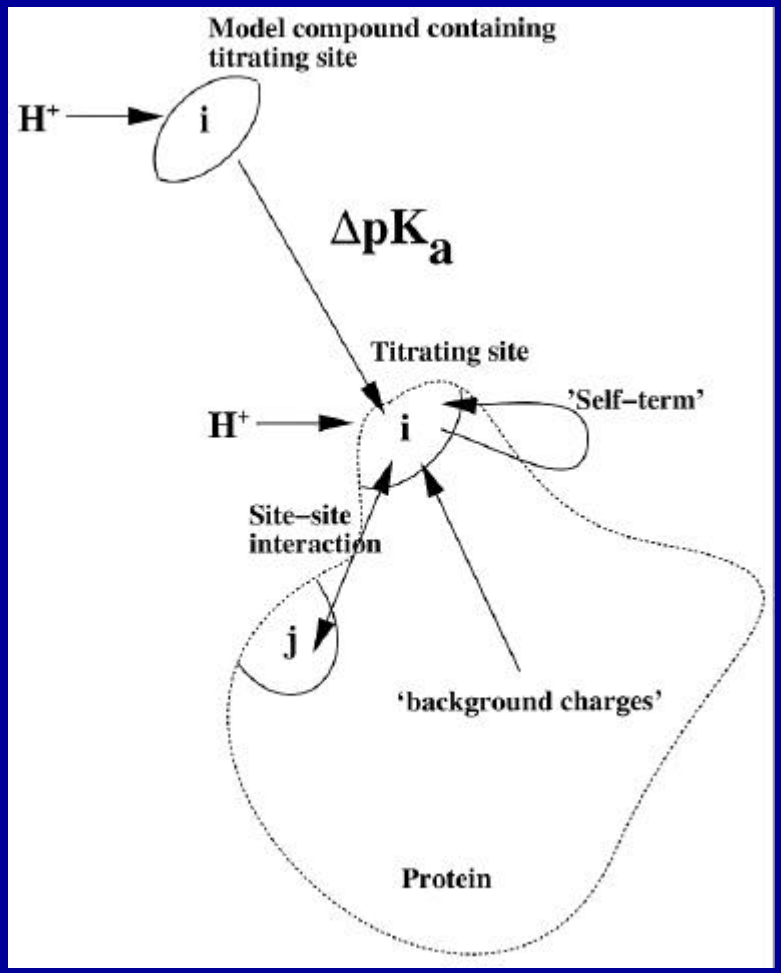


$$q(\mathrm{p}H) = \frac{1}{1 + e^{-\ln 10(\mathrm{p}K_a - \mathrm{p}H)}}$$

One state transition

Computer simulation:
Calculation of θ as a function of pH

Method of calculation: Shift in pK_a

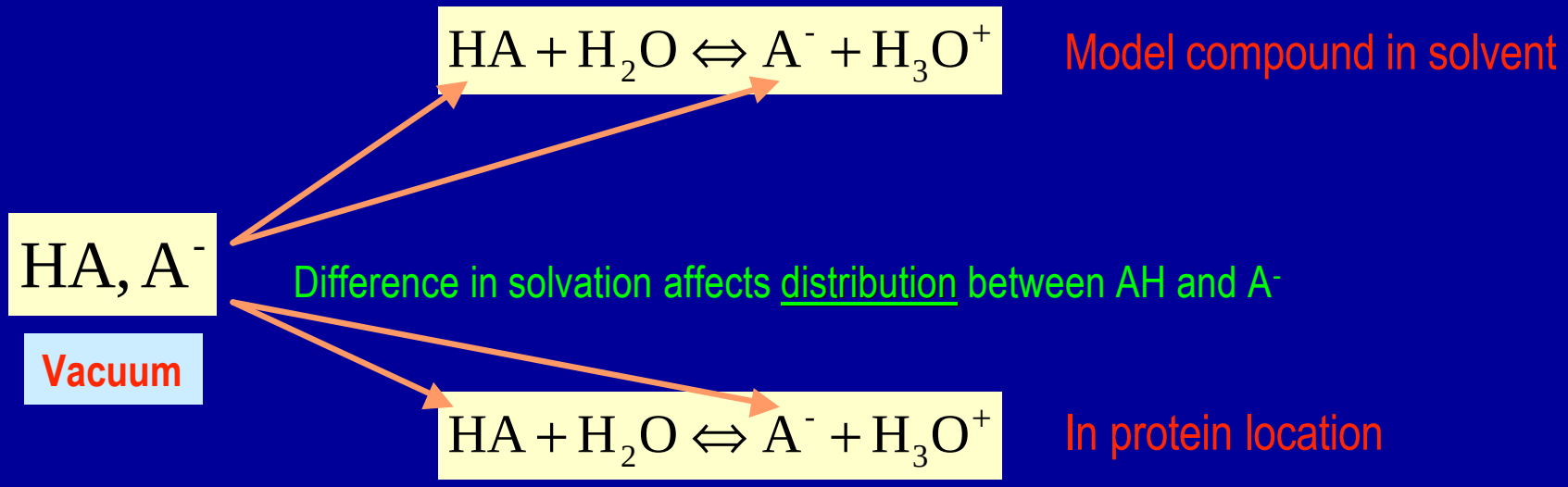


- Intrinsic pK_a: Transfer of site from model compound into protein location:
  - Self-term
  - Background charges
  - No site-site interaction
- Site-site interaction:
  - Sampling of accessible of protonation states as a function of p*H* => *Titration curve* *q*(p*H*)

$$\mathrm{p}K_a^{\mathrm{intr}} = \frac{\Delta\Delta G^{\Theta} + 2.30RT\mathrm{p}K_a^{\mathrm{m}}}{2.30RT}$$

J. Phys. Chem. B. 101, 7664-7673, 1997

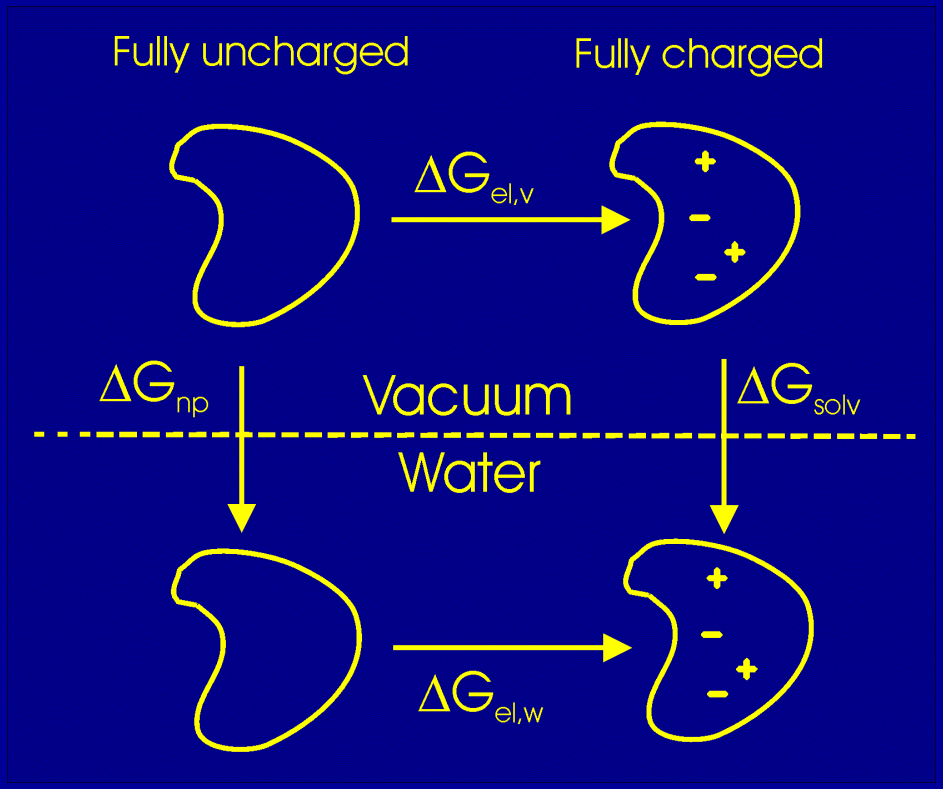
Method of calculation: pK_a^{intr}



$$\Delta\Delta G^{\Theta} = \Delta\Delta G^{v\rightarrow s}_{solv,A/AH} - \Delta\Delta G^{v-p}_{solv,A/AH}$$

$$\mathrm{p}K_a^{\mathrm{intr}} = \frac{\Delta\Delta G^{\Theta} + 2.30RT\mathrm{p}K_a^{\mathrm{m}}}{2.30RT}$$

Solvation free energy



J. Comput. Chem. 25, 393-411, 2004

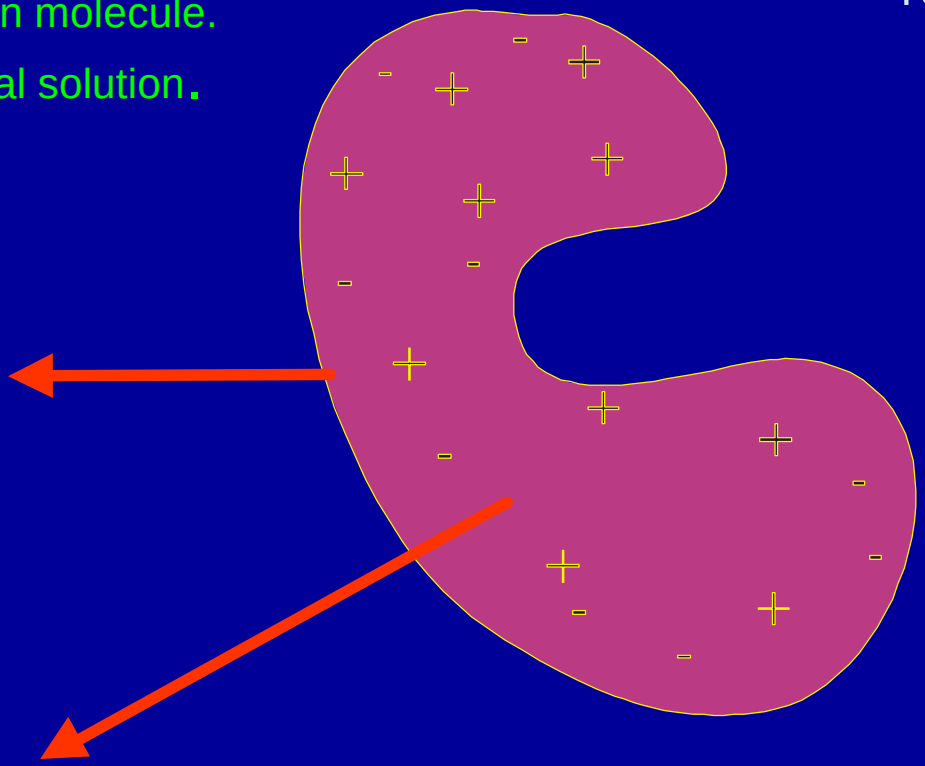
- Continuum electrostatics
- Nonpolar contribution: SAS
  - Cancels for difference between A- and AH

Continuum electrostatics

- Rigid protein molecule.
- No analytical solution.

Total electrostatic energy:

$$W_{el} = \frac{1}{2}\sum_i q_i f(\mathbf{r}_i)$$



Dielectric boundary

Solvent region

$$\nabla^2 f(\mathbf{r}) = k^2 f(\mathbf{r})$$

Inverse Debye Length

$$e_s, I$$

Protein

$$\nabla^2 f(\mathbf{r}) = -\sum_{i=1}^{N} \frac{q_i}{e_p e_0}$$

Partial charges

J. Comput. Phys. 97, 144, 1991

Site-site interactions

$$\langle q \rangle = \sum_s p_s \, s_s$$

Titrating curve: AVERAGE occupancy

s_s : Number of bound protons for protonation state s

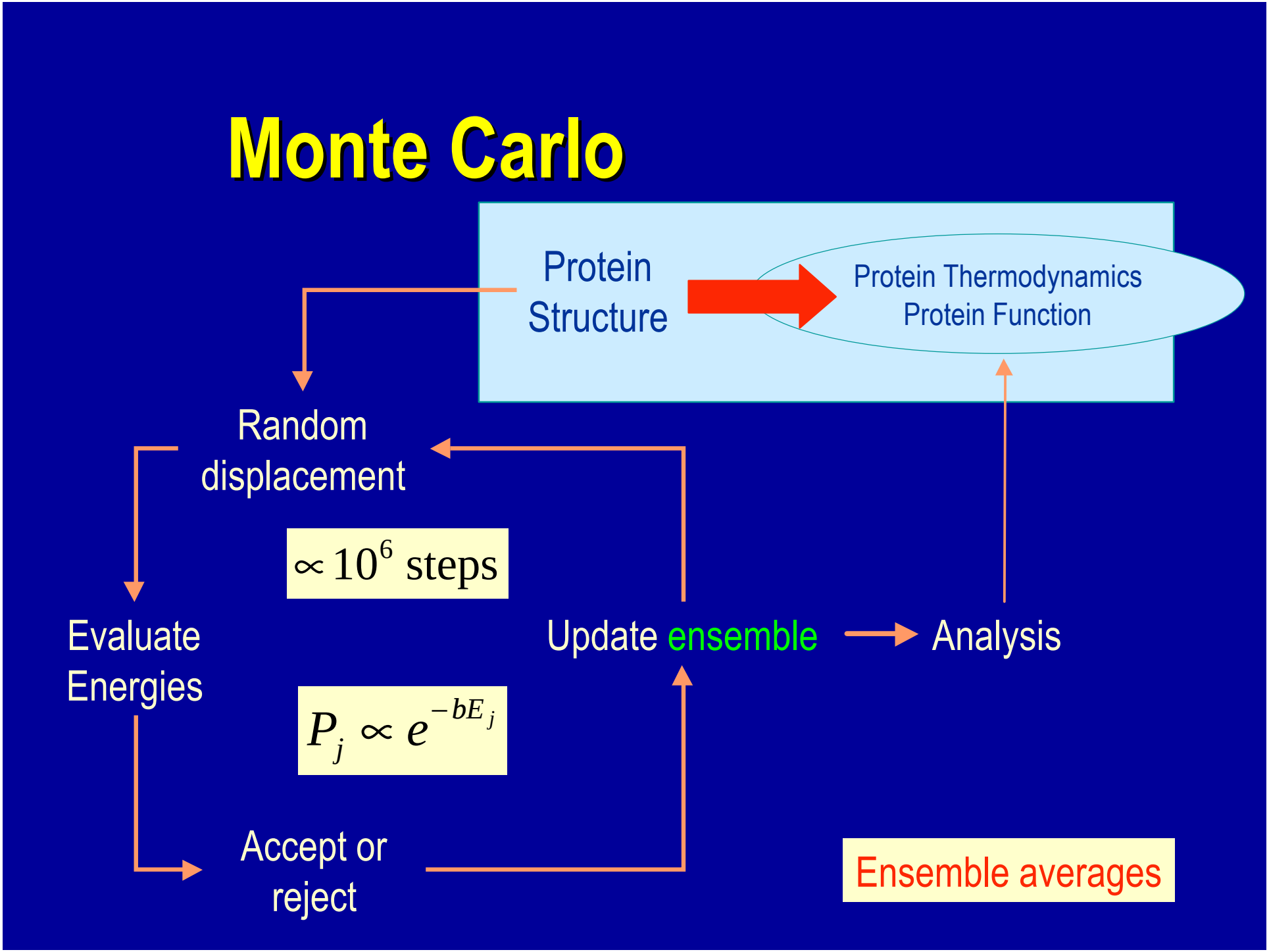
p_s : Probability of observing protonation state σ

$$p_s \propto e^{-W(\mathrm{p}K_a^{\mathrm{intr}}, \mathrm{p}H, W_{el}(s_s))/kT}$$

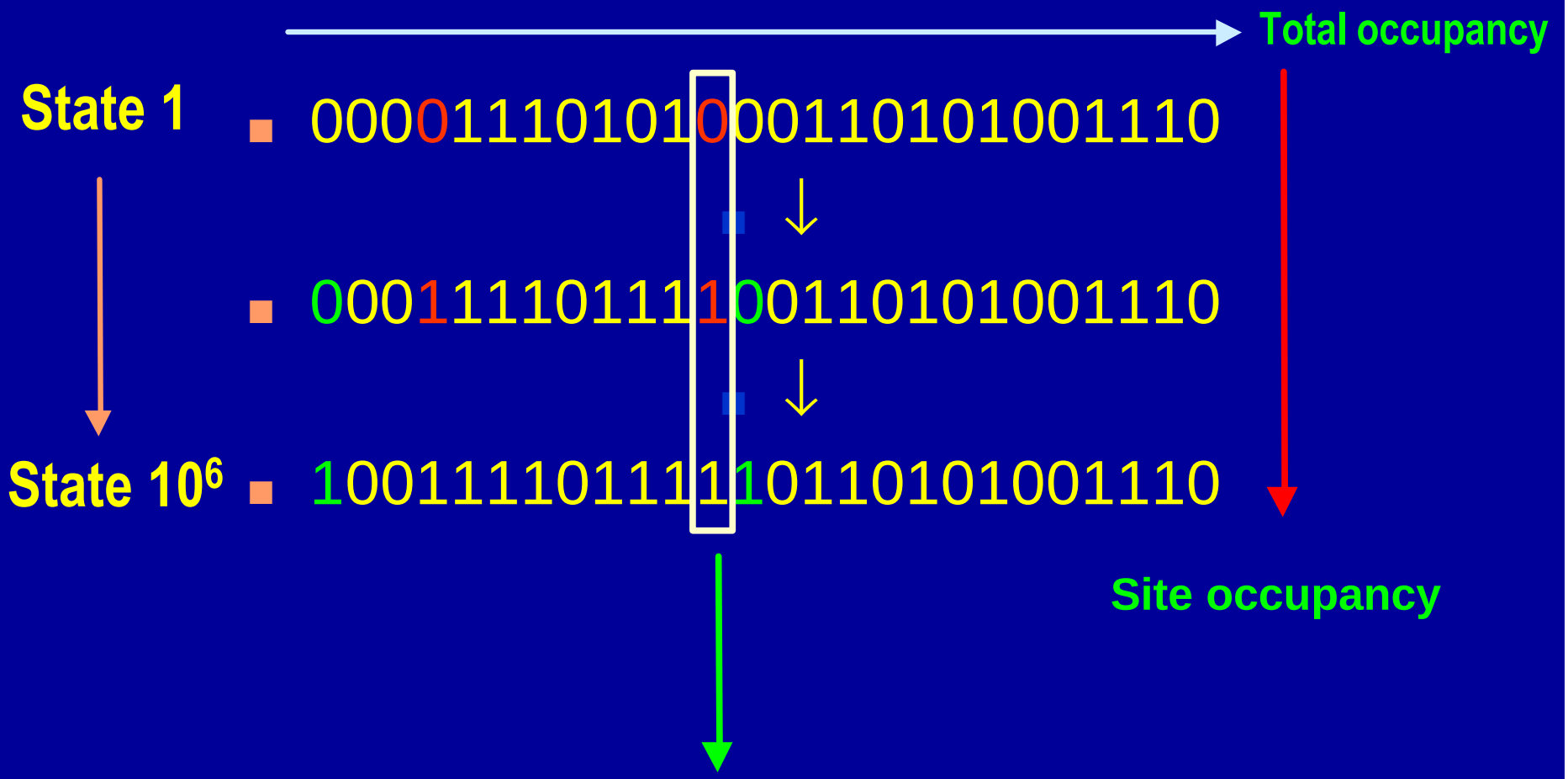
Boltzmann factor

Changes in protonation state modifies molecular charge distribution

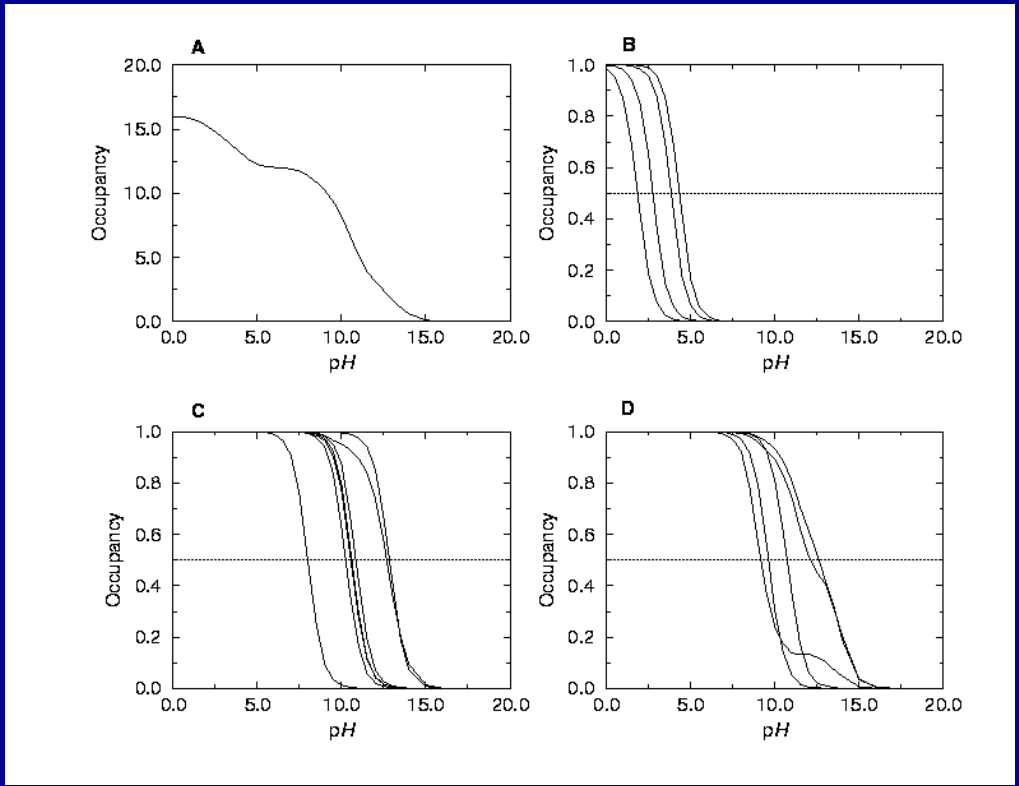
Monte Carlo


Protein Structure
Protein Thermodynamics
Protein Function
Random displacement
$\propto 10^6$ steps
Evaluate Energies
$P_j \propto e^{-bE_j}$
Update ensemble
Analysis
Accept or reject
Ensemble averages

Monte Carlo simulation



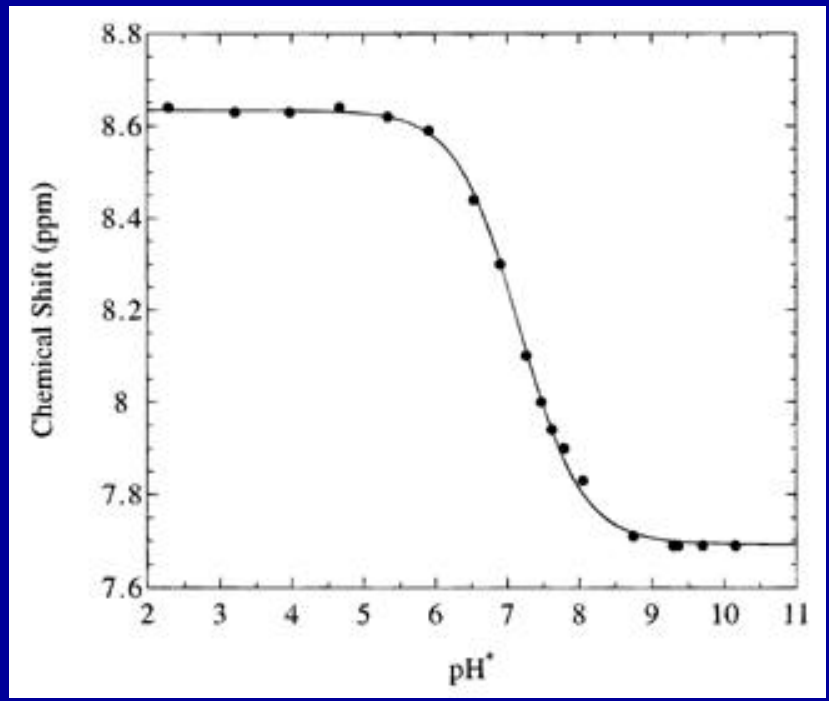
Outcome of computation



Prediction

Biochem. Cell. Biol., 76,198-209 (1998).

NMR experiment



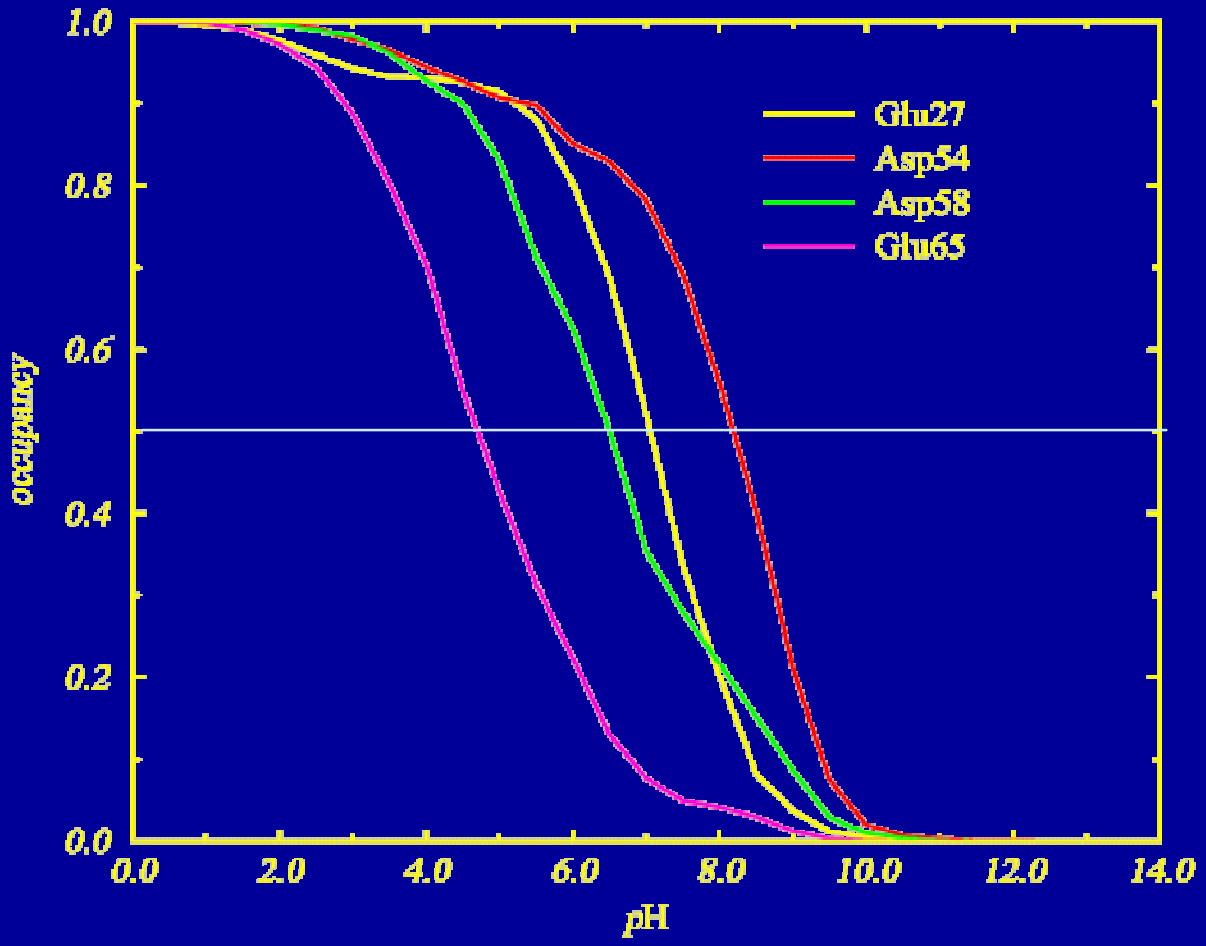
Predictions are useful

- Molecule level explanation of titration curves
- Details on electrostatic network: protein structure
- Statements about enzyme activity: protein function (J. Biol. Chem., **275**, 25633-25640, 2000)

Problems

- Correlation between protein dynamics and protonation state is commonly ignored.
- At low or high pH, proteins become unstable or denaturate.
- New models properly should sample both conformation and protonation states simultaneously:
  - Coarse grained model.

Apo form of Calbindin



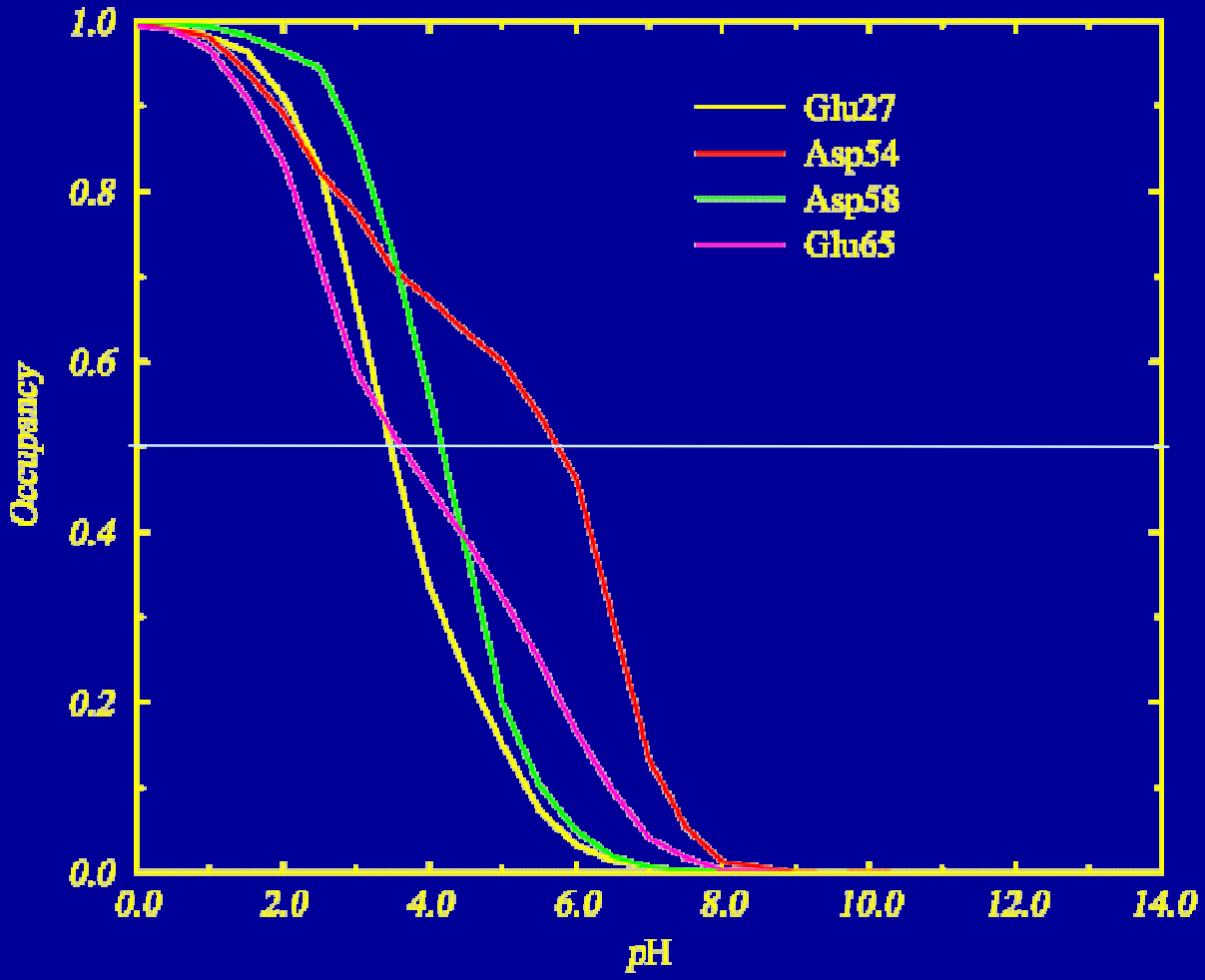
Calbindin
Titration curves of ion ligating groups.

No structure relaxation upon ion release.

Proteins, 41, 554-567, 2000

Xray holo structure with Ca^{2+} removed

Apo form of Calbindin

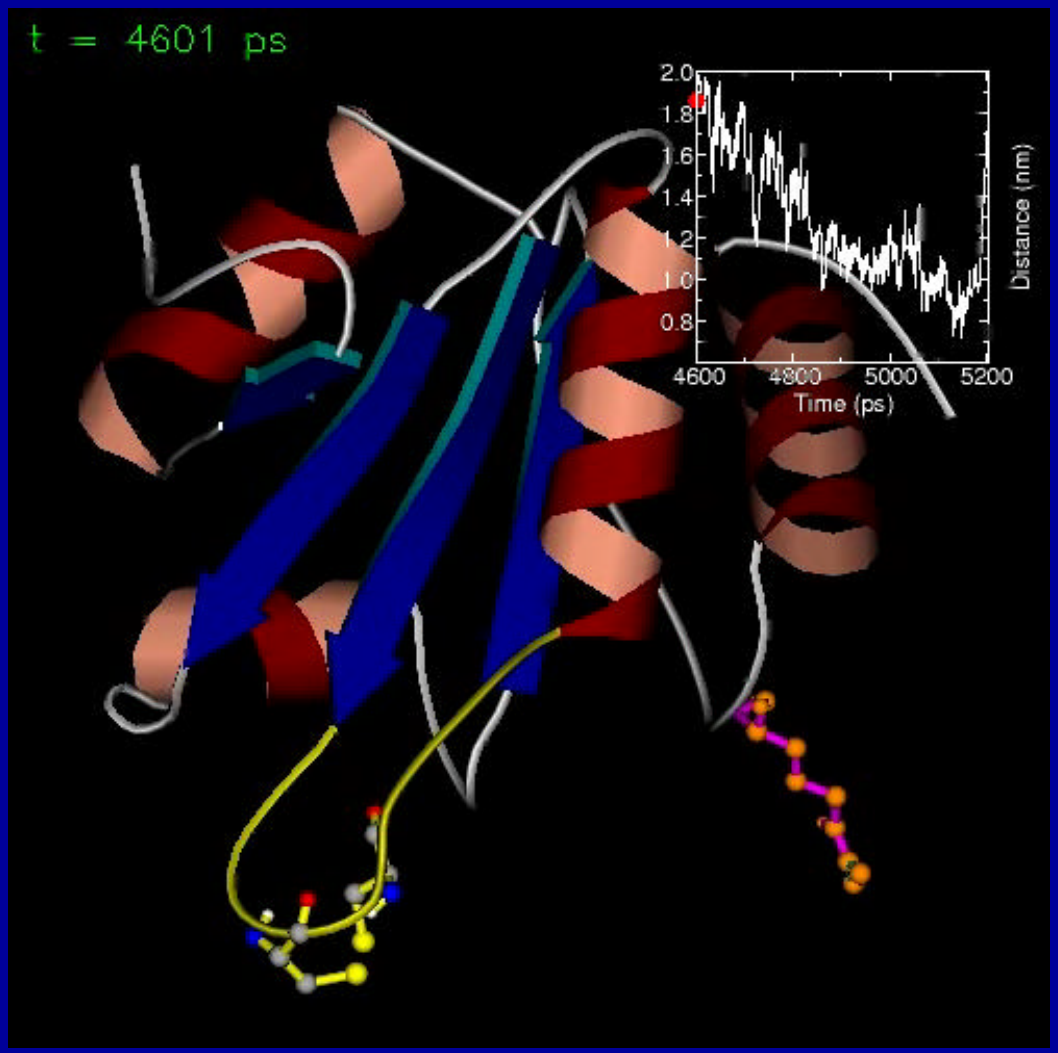


Calbindin
Titration curves of ion ligating groups.

With structure relaxation upon ion release.

NMR apo structure

The Arg in the a-domain of PDI



Structure	Cys53	Cys56
NMR Model 1	8.1	12.8
NMR Model 1 Neutral Arg 120	8.0	12.4
NMR model 19	3.8	3.5
NMR model 19 Neutral Arg 120	4.6	7.6
Native, MD simulation 15,834 ps	8.8	6.1
Native, MD simulation 15.834 ps	9.3	8.4

J. Mol. Biol., 335, 283-295, 2004