First Principle Electronic Structure Calculations for Molecular (and Cellular) Biology

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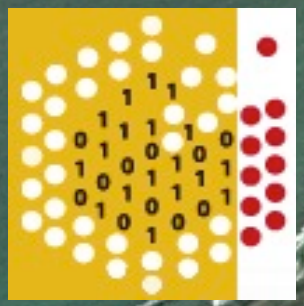
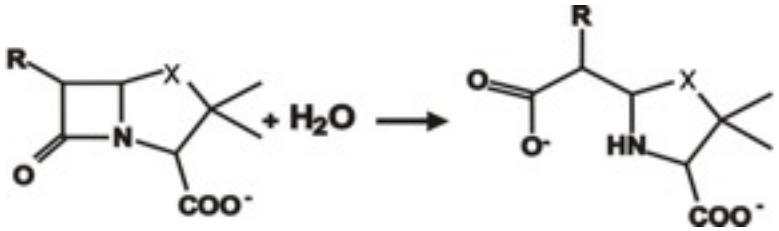
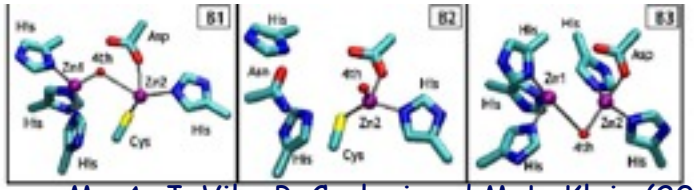


Ab initio MD

- Temperature effects are essential
- Force-field based molecular dynamics (MD) simulations predict structural dynamical and theormodynamical properties of biomolecules (submicrosecond, 10^5 particles)
- Use of effective potentials may be not appropriate in all cases, and more sophisticated and accurate approaches might be needed.
- In ab initio MD, interatomic forces are evaluated from electronic structure calculations (typically DFT) as the simulation proceeds.
- This finer level of description demands a much larger computational cost (0.01 ns, 100 atoms). However, several phenomena depend on the electronic states in such an intricate way that they cannot be modeled via effective potentials.

Bond forming and bond breaking phenomena in enzymatic reaction: drug resistance by metallo beta lactamases



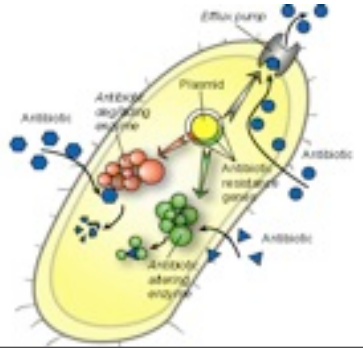


Dal Peraro, M., A. J. Vila, P. Carloni and M. L. Klein (2007). Journal of the American Chemical Society **129**(10): 2808.

Moran-Barrio, J., J. M. Gonzalez, M. N. Lisa, A. L. Costello, M. Dal Peraro, P. Carloni, B. Bennett, D. L. Tierney, A. S. Limansky, A. M. Viale and A. J. Vila (2007). Journal of Biological Chemistry **282**(25): 18286.

Dal Peraro, M., L. I. Llarrull, U. Rothlisberger, A. J. Vila and P. Carloni (2004). Journal of the American Chemical Society **126**(39): 12661.

Tomatis PE, Fabiane SM, Simona F, Carloni P, Sutton BJ, Vila AJ. Proc Natl Acad Sci U S A. 2008 105(52):20605.



Chemical shift calculations

J. Phys. Chem. B **2006**, *110*, 1437−1442

Binding of Phosphinate and Phosphonate Inhibitors to Aspartic Proteases: A First-Principles Study

Pietro Vidossich and Paolo Carloni*

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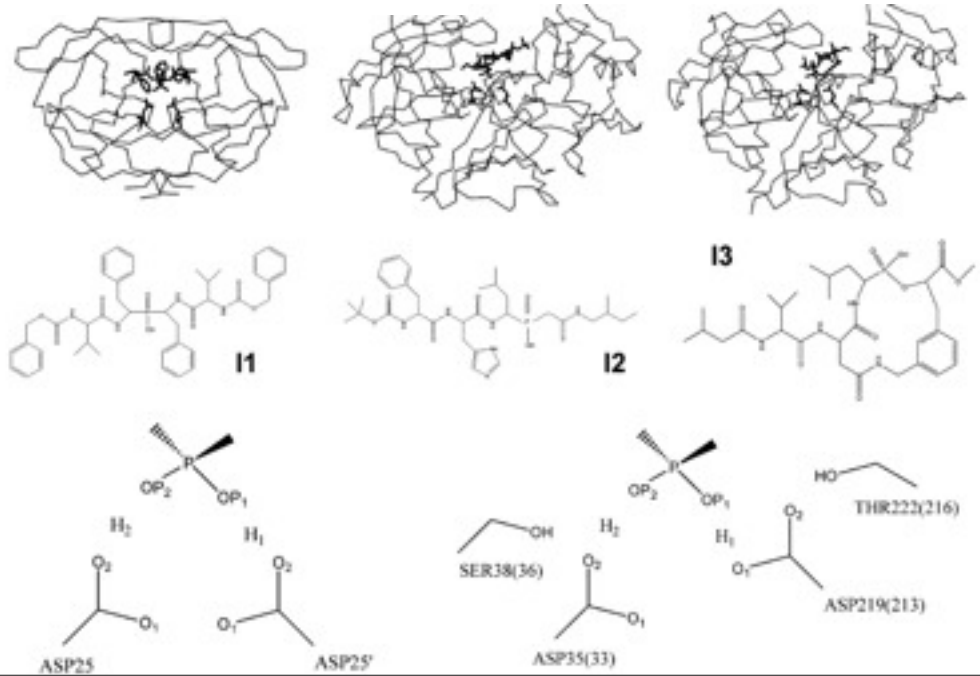
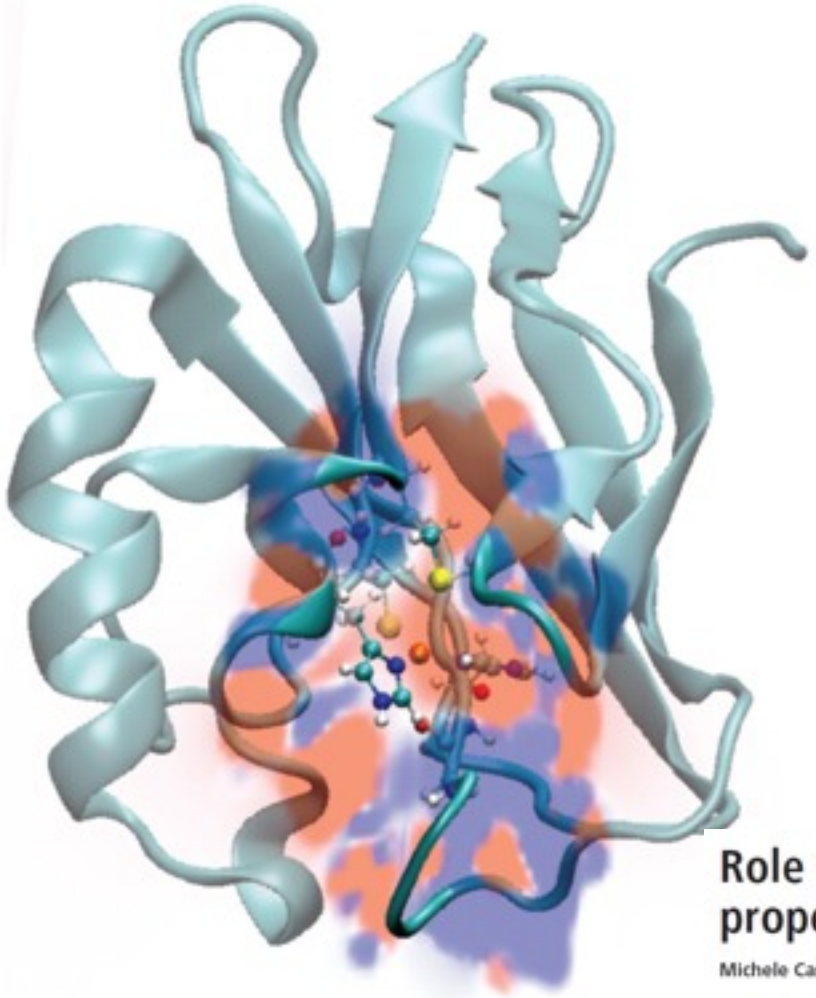
Phosphinate and phosphonate derivatives are potent inhibitors of aspartic proteases (APs). The affinity for the enzyme might be caused by the presence of low barrier hydrogen bonds between the ligand and the catalytic Asp dyad in the cleavage site. We have used density functional theory calculations along with hybrid quantum mechanics/molecular mechanics Car−Parrinello molecular dynamics simulations to investigate the hydrogen-bonding pattern at the binding site of the complexes of human immunodeficiency virus type-1 AP and the eukaryotic endothiapepsin and penicillopepsin. Our calculations are in fair agreement with the NMR data available for endothiapepsin (Coates et al. *J. Mol. Biol.* **2002**, *318*, 1405−1415) and show that the most stable active site configuration is the diprotonated, negatively charged form. In the viral complex both protons are located at the catalytic Asp dyad, while in the eukaryotic complexes the proton shared by the closest oxygen atoms is located at the phosphinic/phosphonic group.



Metallic centers in biomolecules



Open issues in force field modeling:

-Metal-ligand bond

-Ligand field may dictate geometry

-In a dynamical environment, the number of ligands and the metal oxidation state can change.

-No general consensus on functional form

Role of protein frame and solvent for the redox properties of azurin from *Pseudomonas aeruginosa*

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Molecular Dynamics -I

Molecular system of nuclear and e- coordinates ($\{\mathbf{R}_I\}, \{r_i\}$ respectively). Non-relativistic case

$$i\hbar\frac{\partial}{\partial t}\Phi(\{\mathbf{R}_I\},\{\mathbf{r}_i\},t) = \mathcal{H}_T\Phi\{\mathbf{R}_I\},\{\mathbf{r}_i\},t)$$

$$\mathcal{H}_T = -\sum_I \frac{\hbar^2}{2M_I}\nabla_I^2 - \sum_i \frac{\hbar^2}{2m_e}\nabla_i^2 + \sum_{i<j}\frac{e^2}{|\mathbf{r}_i-\mathbf{r}_j|} - \sum_{I,i}\frac{e^2 Z_I}{|\mathbf{R}_I-\mathbf{r}_i|} + \sum_{I<J}\frac{e^2 Z_I Z_J}{|\mathbf{R}_I-\mathbf{R}_J|}$$

$$\mathcal{H}_T = -\sum_I \frac{\hbar^2}{2M_I}\nabla_I^2 - \sum_i \frac{\hbar^2}{2m_e}\nabla_i^2 + \mathcal{V}_{\mathrm{n-e}} = -\sum_I \frac{\hbar^2}{2M_I}\nabla_I^2 + \mathcal{H}_{\mathrm{e}}$$

1. mean–field description of coupled nuclear–electronic quantum dynamics

$$\Phi \approx \Psi\left(\{\mathbf{r}_i\},t\right)\chi(\{\mathbf{R}_I\},t)\,\exp\left[\frac{i}{\hbar}\int_{t_0}^{t} dt' E_1(t')\right]$$

$$E_1 = \int\int d\mathbf{r}d\mathbf{R}\;\; \Psi^\star\chi^\star\,\mathcal{H}_{\mathrm{e}}\,\Psi\,\chi$$

$$i\hbar\dot{\Psi} = -\sum_i \frac{\hbar^2}{2m_e}\nabla_i^2\Psi + \left[\int d\mathbf{R}\chi^\star\mathcal{V}_{\mathrm{n-e}}\chi\right]\Psi$$

$$i\hbar\dot{\chi} = -\sum_I \frac{\hbar^2}{2M_I}\nabla_I^2\chi + \left[\int d\mathbf{r}\Psi^\star\mathcal{H}_{\mathrm{e}}\Psi\right]\chi$$

J. C. Tully, in Classical and Quantum Dynamics in Condensed Phase Simulations, Chapt. 21, p. 489, eds. B. J. Berne, G. Ciccotti, and D. F. Coker (World Scientific, Singapore, 1998).

U. Rothlisberger and P. Carloni: *Drug-Target Binding Investigated by Quantum Mechanical/Molecular Mechanical (QM/MM) Methods*, Lect. Notes Phys. 704, 449–479 (2006)
DOI 10.1007/3-540-35284-8_17

Molecular Dynamics -II

2. Classical treatment of nuclear d.o.f: Ehrenfest molecular dynamics, EMD

$$\chi(\{\mathbf{R}_I\},t) = A\, e^{(i/\hbar)S}$$

$$A(\{\mathbf{R}_I\},t), S(\{\mathbf{R}_I\},t) \in \mathbb{R}$$

In the limit $\hbar \to 0$:

$$\frac{\partial S}{\partial t} + \sum_I \frac{1}{2M_I} (\nabla_I S)^2 + \int d\mathbf{r}\, \Psi^* \mathcal{H}_e \Psi = 0$$

$$\mathbf{P}_I = \boldsymbol{\nabla}_I S$$
$$H = \sum_I \frac{1}{2M_I} (\nabla_I S)^2 + \int d\mathbf{r}\, \Psi^* \mathcal{H}_e \Psi$$

$$\frac{d\mathbf{P}_I}{dt} = -\nabla_I \int d\mathbf{r}\, \Psi^* \mathcal{H}_e \Psi$$

$$\chi^* \chi = \prod_I \delta\left[\mathbf{R}_I - \mathbf{R}_I(t)\right]$$

$$i\hbar \frac{\partial \Psi}{\partial t} = \sum_I \frac{\hbar^2}{2M_I} \nabla_I^2 \chi + \left[\int d\mathbf{r} \Psi^* \mathcal{H}_e \Psi\right] \chi = \mathcal{H}_e \Psi$$

$$M_I \ddot{\mathbf{R}}_I(t) = -\nabla_I \int d\mathbf{r}\, \Psi^* \mathcal{H}_e \Psi$$

Molecular Dynamics -III

4. Neglecting the time-dependence of electronic d.o.f: ab initio molecular dynamics

4.1 Born-Hoppenheimer MD

$E_0 = \min_{\Psi_0} \{ \int d\mathbf{r}\, \Psi_0^* \mathcal{H}_e \Psi_0 \}$

$M_I \ddot{\mathbf{R}}_I(t) = -\nabla_I E_0$

In DFT (spin unpolarized case):

$E_0 = E_{ks} + \sum_J \sum_{I<J} \frac{Z_I Z_J}{|\mathbf{R}_I - \mathbf{R}_J|}$

$E_{ks} = \sum_i \langle \phi_i(\mathbf{r}) | -\frac{1}{2}\nabla^2 | \phi_i(\mathbf{r}) \rangle + \int d\mathbf{r} \left[-\sum_I \frac{Z_I}{|\mathbf{R}_I - \mathbf{r}|} \right] \rho(\mathbf{r}) + \frac{1}{2} \int d\mathbf{r} \int d\mathbf{r}' \frac{\rho(\mathbf{r}')\rho(\mathbf{r})}{|\mathbf{r} - \mathbf{r}'|} + E_{xc}[\rho]$

$\rho(\mathbf{r}) = \sum_i |\phi_i(\mathbf{r})|^2$

Classical MD integrates out e- d.o.f. (e.g. AMBER)

$E_0 \sim E_{MM} = E_{bonded} + E_{bonded}$

$E_{bonded} = \sum_{bonds} k_b (b - b_o)^2 + \sum_{angles} k_\alpha (\alpha - \alpha_o)^2 + \sum_{torsions} k_\Theta [1 + \cos(n\Theta - \gamma)]$

$E_{nb} = \sum_{I,J} \left\{ \left[\left(\frac{A_{IJ}}{R_{IJ}} \right)^{12} - \left(\frac{B_{IJ}}{R_{IJ}} \right)^{6} \right] + \frac{Q_I Q_J}{\epsilon_o R_{IJ}} \right\}$

Molecular Dynamics -IV

4.2. Car-Parrinello MD

$$\mathcal{L}_{CP} = \sum_I \tfrac{1}{2} M_I \dot{\mathbf{R}}_I^2 + \sum_i \tfrac{1}{2}\mu|\dot{\phi}_i|^2 - E_T(\{\mathbf{R}_I\},\{\phi_i\}) + \sum_{ij}\Lambda_{ij}(\langle\phi_i|\phi_j\rangle - \delta_{ij})$$

$$M_I\ddot{\mathbf{R}}_I(t) = -\frac{\partial E_T}{\partial \mathbf{R}_I}$$

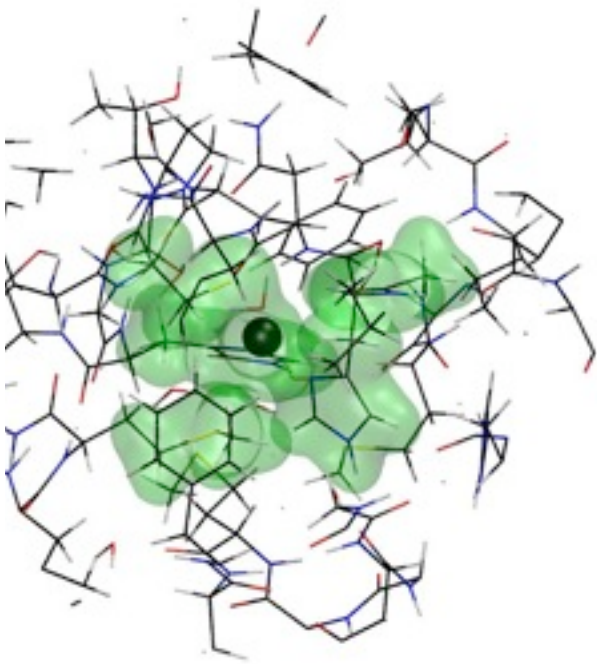
$$\mu\ddot{\phi}_i(t) = -\frac{\delta E_T}{\delta \phi_i^*} + \sum_j \Lambda_{ij}\phi_j$$

Criterion for adiabatic separation : $\sqrt{\frac{E_{gap}}{\mu}}$

- CPMD, EMD: no diagonalization except first Step
- BOMD: diagonalization
- EMD: conforms to a unitary propagation

Basis Set: Planewaves/pseudopotentials.

Hybrid CP/MM approaches



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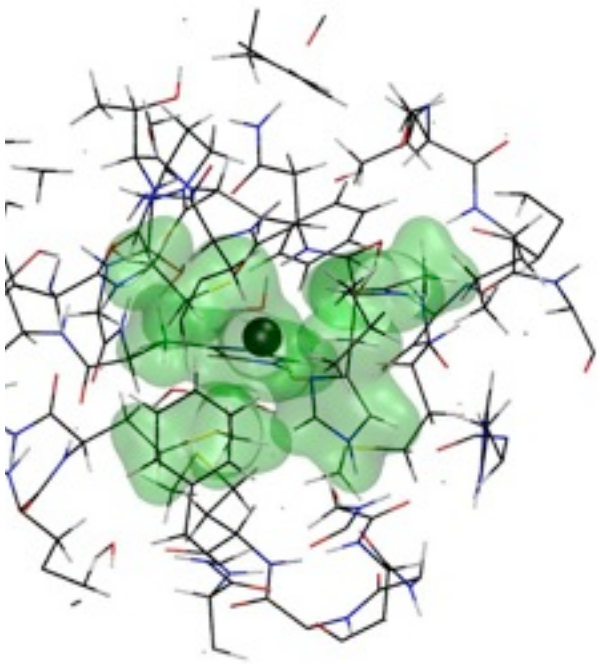

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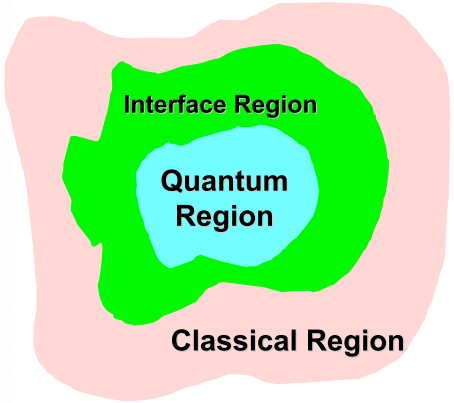

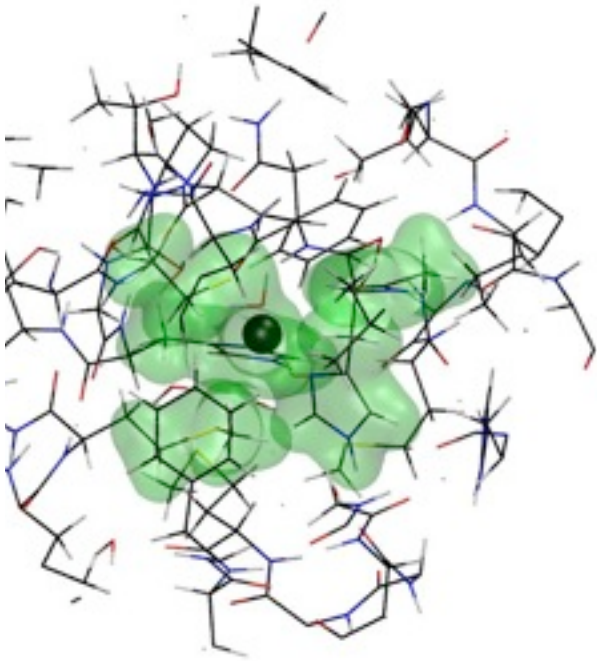

$$\mathcal{L}_{CP/MM} = \sum_I \tfrac{1}{2} M_I \dot{\mathbf{R}}_I^2 + \sum_i \tfrac{1}{2}\mu|\dot{\phi}_i|^2 - E_T(\{\mathbf{R}_I\},\{\phi_i\}) - E_{MM} - E_i + \sum_{ij}\Lambda_{ij}(\langle\phi_i|\phi_j\rangle - \delta_{ij})$$

$$E_i = \sum_{I\in MM} Q_I \int d\mathbf{r}\, \frac{\rho(\mathbf{r})}{|\mathbf{R}-\mathbf{r}|} + \sum_{\substack{I\in MM \\ J\in CP}} \left[\left(\frac{A_{IJ}}{R_{IJ}}\right)^{12} - \left(\frac{B_{IJ}}{R_{IJ}}\right)^{6}\right] + E_{bonded}$$

U. Rothlisberger and P. Carloni: *Drug-Target Binding Investigated by Quantum Mechanical/Molecular Mechanical (QM/MM) Methods*, Lect. Notes Phys. **704**, 449–479 (2006)
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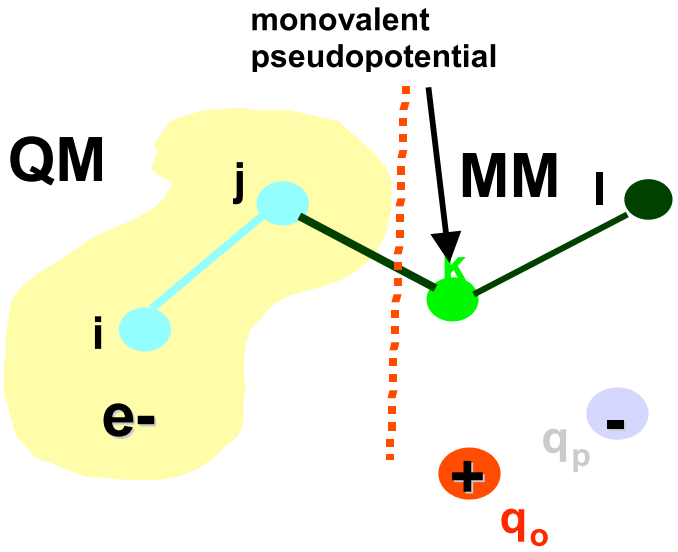
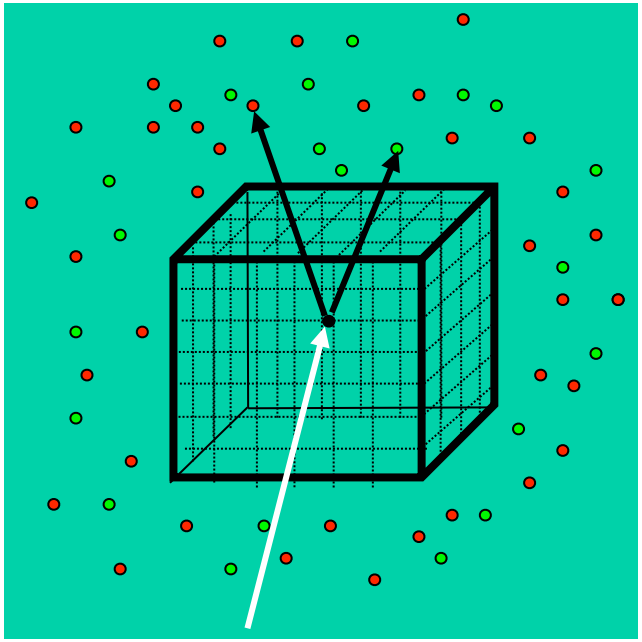
Dal Peraro et al., Curr. Op. Str. Biol. 2008

$E_{QM/MM}$:Non bonded Interactions



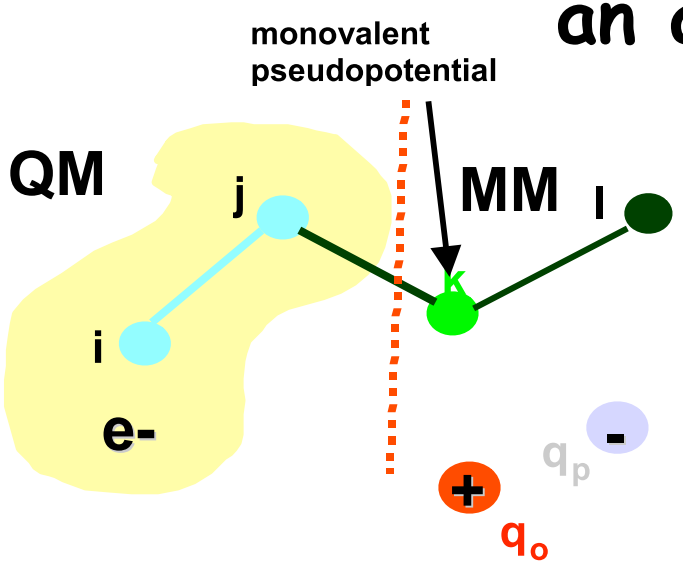


$$E^{st}_{QM/MM} = \sum_{I \in MM} q_I \int dr \rho(r) / |r - R_I| + \sum_{\substack{I \in MM \\ J \in QM}} v_{vdw}(R_{IJ})$$

1-Electron density is overpolarized at short range: electron spill-out problem

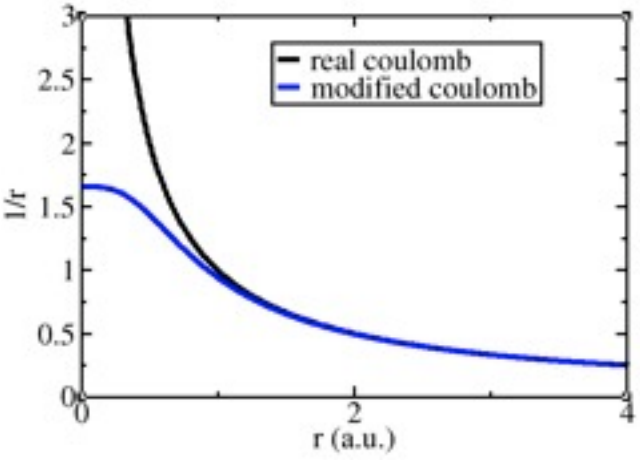
2- # operations ~N_{rsgrid} x N_{MM} ~1,000 x 10,000

1-Spill out: Replacing the Coulomb potential with an ad hoc function



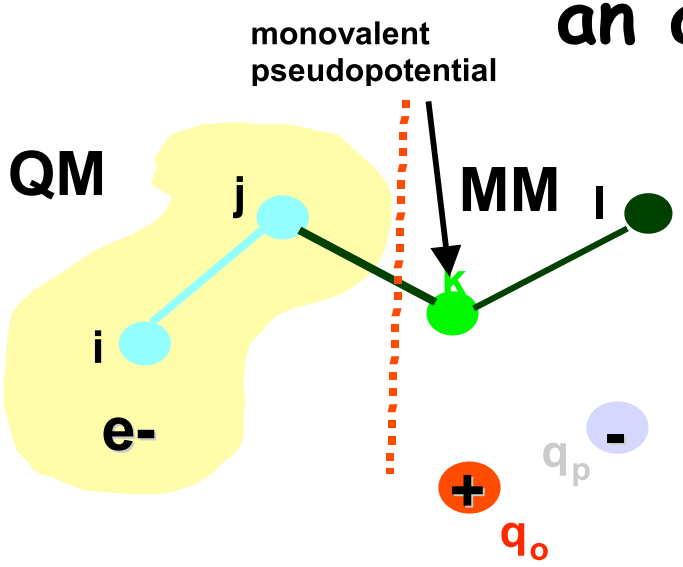
$$E^{ele}_{QM/MM} = \sum_{i \in MM} q_i \int dr \rho(r) v_i(|r - r_i|)$$

$$v_J(r) = \frac{R_{cJ}^4 - r^4}{R_{cJ}^5 - r^5}$$



R_{CJ}=cutoff radii, tested in Laio et al JCP 2002

1-Spill out: Replacing the Coulomb potential with an ad hoc function



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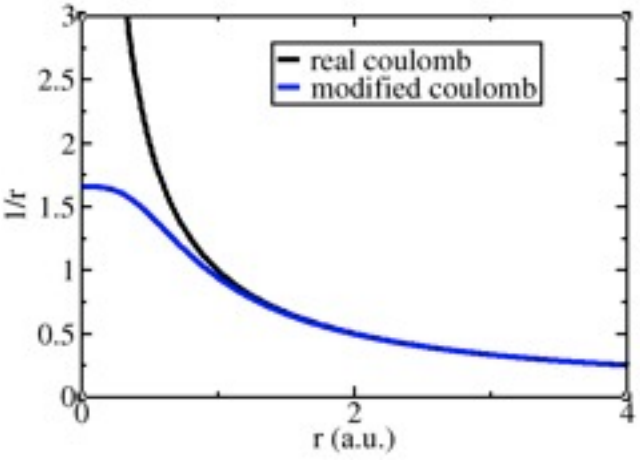

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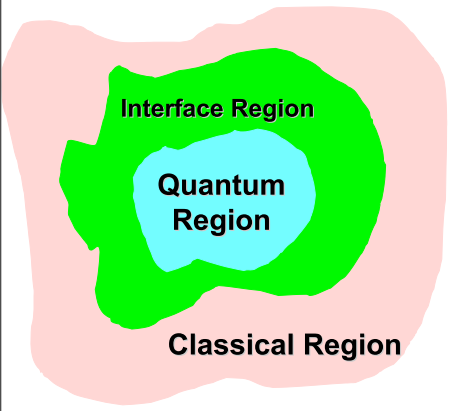
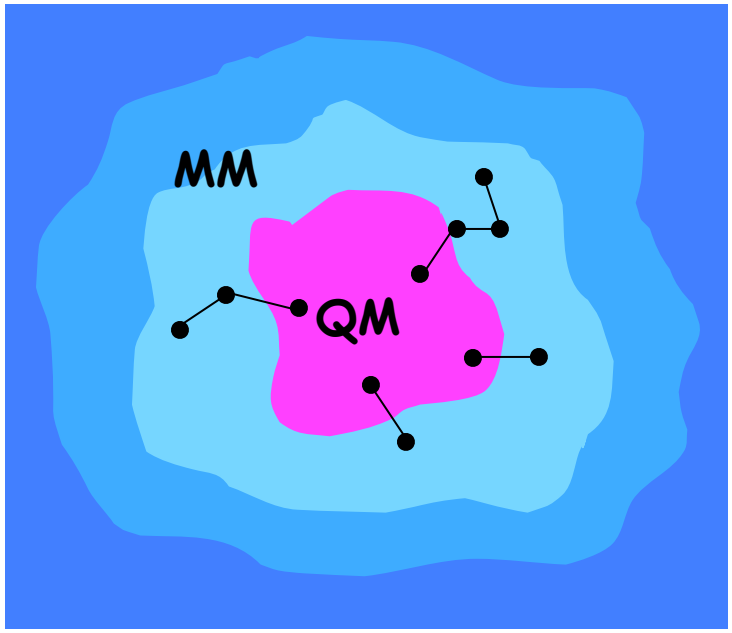
2-Computational Cost: Multiple Scheme



1-Calculate integral only
for a subset of NN atoms
2- For the MM atoms use multiple expansion

DFT/MM



- Core: QM-system
- MM-system: 3 shells
- Bonds, angles, torsionals and vdW are taken into account by the classical force field.
- Electrostatic coupling follows a hierarchical scheme:

Rothlisberger et al. J Phys. Chem. B 2000
Rothlisberger, PC, Erice Lect. Notes Phys. 2006
Dal Peraro et al. Curr. Op. Str. Biol. 2007

1. First shell: explicit coupling between QM charge density and MM point charges (within 5.3Å)

2. Second shell: D-RESP charges of QM system interact with MM charges (within 10.6 Å)

3. Third shell: Multipolar expansion of QM system interacts with MM charges

QM/MM methods

Choice of QM

- semiempirical
- DFT
- ab initio

Choice of Basis Set

- atom-centered
- plane waves

Choice of MM

- force field
- polarizable

Choice of Embedding

- mechanical coupling/ electrostatic coupling
- capping of covalent bonds

Adapted from U. Rothlisberger

Some QM/MM Methods

General QM/MM:

- A. Warshel, M. Levitt, J. Mol. Biol. 103, 227 (1976)
- U.C. Singh, P.A. Kollman, J. Comp. Chem. 7, 718 (1986) (AMBER)
- M.J. Field, P.A. Bash, M. Karplus, J. Comp. Chem. 11, 700 (1990); P.D. Lyne, M. Hodoscek, M. Karplus, J. Phys. Chem. A, 103, 3462 (1999) (CHARMM)
- review: P. Sherwood, Modern Methods and Algorithms of Quantum Chemistry, Vol. 1, John von Neumann Institute for Computing, 257 (2000) (www.fz-juelich.de/nic-series/Volume1) (CHEMSHELL)
- C.J. Cramer, D. Truhlar, ECCC10 Feature papers: QM/MM: What have we learned, where are we, and where do we go from here? (QMMM) (comp.chem.umn.edu/Truhlar/hilight/QMMMreview05.htm)
- HM Senn, W. Thiel, Angew. Chem. Int Ed Engl. 2009, 48, 1198.

Localized Basis Set QM/MM:

- F. Maseras, K. Morokuma, J. Comput. Chem. 16, 1170 (1995) (IMOMM, ONIOM@Gaussian)
- D. Bakowies, W. Thiel, J. Comp. Chem. 17, 87 (1996) (MNDO/MM)
- J. Gao, Rev. Comp. Chem. 7119 (1996) (semiemp/MM)
- P.L. Cummins, J.E. Gready, J. Comp. Chem. 18, 1496 (1997) (semiemp/MM@MOZYME)
- G. Monnard, K.M. Merz, Acc. Chem. Res. 32, 904 (1999) (semiemp/MM)
- T.K. Woo, L. Cavallo, T. Ziegler, Theor. Chem. Acc. 100, 307 (1998) (DFT/MM@ADF)
- N. Ferre, M. Olivucci, J. Am. Chem. Soc. 125, 6868 (2003) (CASPT2,CASSCF/MM)

Plane Wave Based QM/MM:

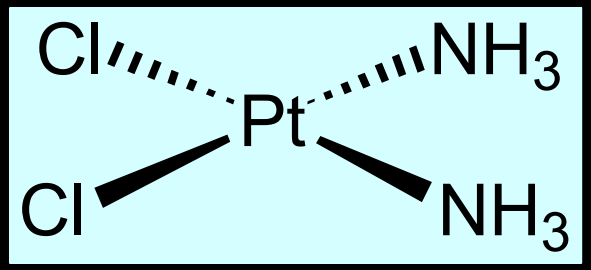
- M. Eichinger, P.Tavan, J. Hutter, M. Parrinello, J. Chem. Phys. 110, 10452 (1999) (CPMD)
- D.A. Yarne, M.E. Tuckerman, G.J. Martyna, J. Chem. Phys. 115, 3531 (2001) (PINY_MD)
- A. Laio, J. VandeVondele, U. Rothlisberger, J. Chem.Phys. 116, 6941 (2002) (CPMD)

Adapted from U. Rothlisberger

Applications in molecular medicine: An alkylating drug

Pt-based drugs targeting DNA

Cisplatin based therapies are the cornerstone of treatment of many cancers (90 % cure rate for testicular and ovarian cancer)



<u>Unfortunately, its use is severely limited by intrinsic and acquired cell resistance</u>

Resistance mechanism may include cellular uptake and efflux of the drug, increased detoxification of the drug, inhibition of apoptosis and increased DNA repair

Reedijk Proc. Natl. Acad. Sci. U.S.A. 2003, 100, 3611.
Wang, Lippard, Nat. Rev. Drug Discovery 2005, 4, 307.
Arnesano, Scintilla, Natile, Angew Chem Int Ed Engl. 2007
46(47):9062-4

Cisplatin

-In its major adduct (60%), it binds to two adjacent G's (N7), causing kink (40° -85°) towards major groove.

Kink might induce cell apoptosis and/or response of DNA repairing system

-Other adducts are formed (Hopkins, Science 96)

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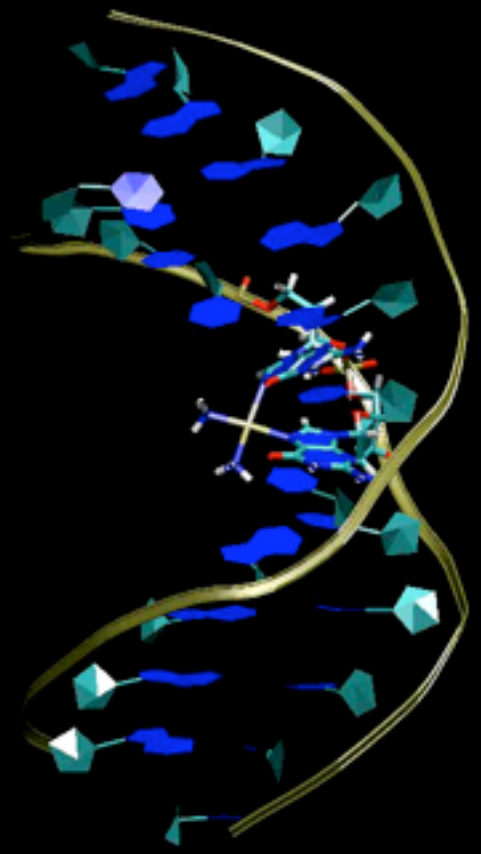

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Computer-aided design of cisplatin and other Pt-based drugs binding to DNA

Molecular simulation play a key role because of DNA flexibility , whilst there are limitations for the docking

Geometrical parameters and energetics depend on electronic structure in a rather intricate way

In Pt-drugs, QM-based methods may be a valuable alternative to parametrizations

Force field methods may require a priori geometry information: limitations for drug design

Computer-aided design of cisplatin and other Pt-based drugs binding to DNA

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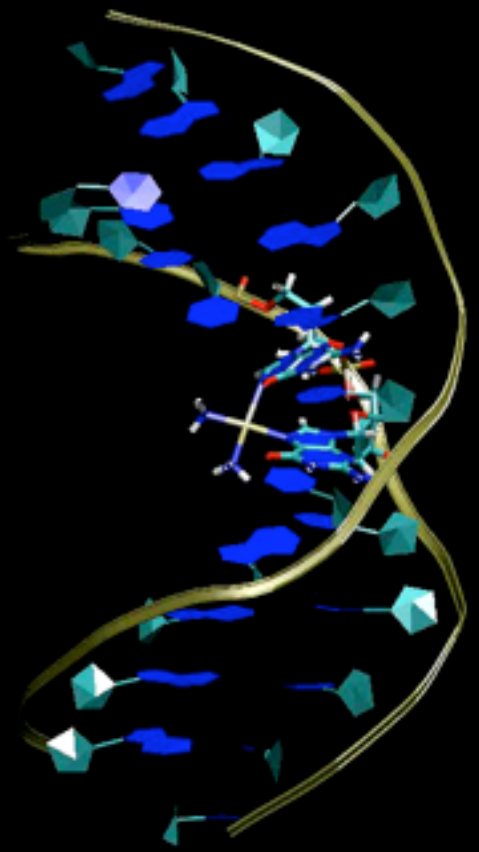
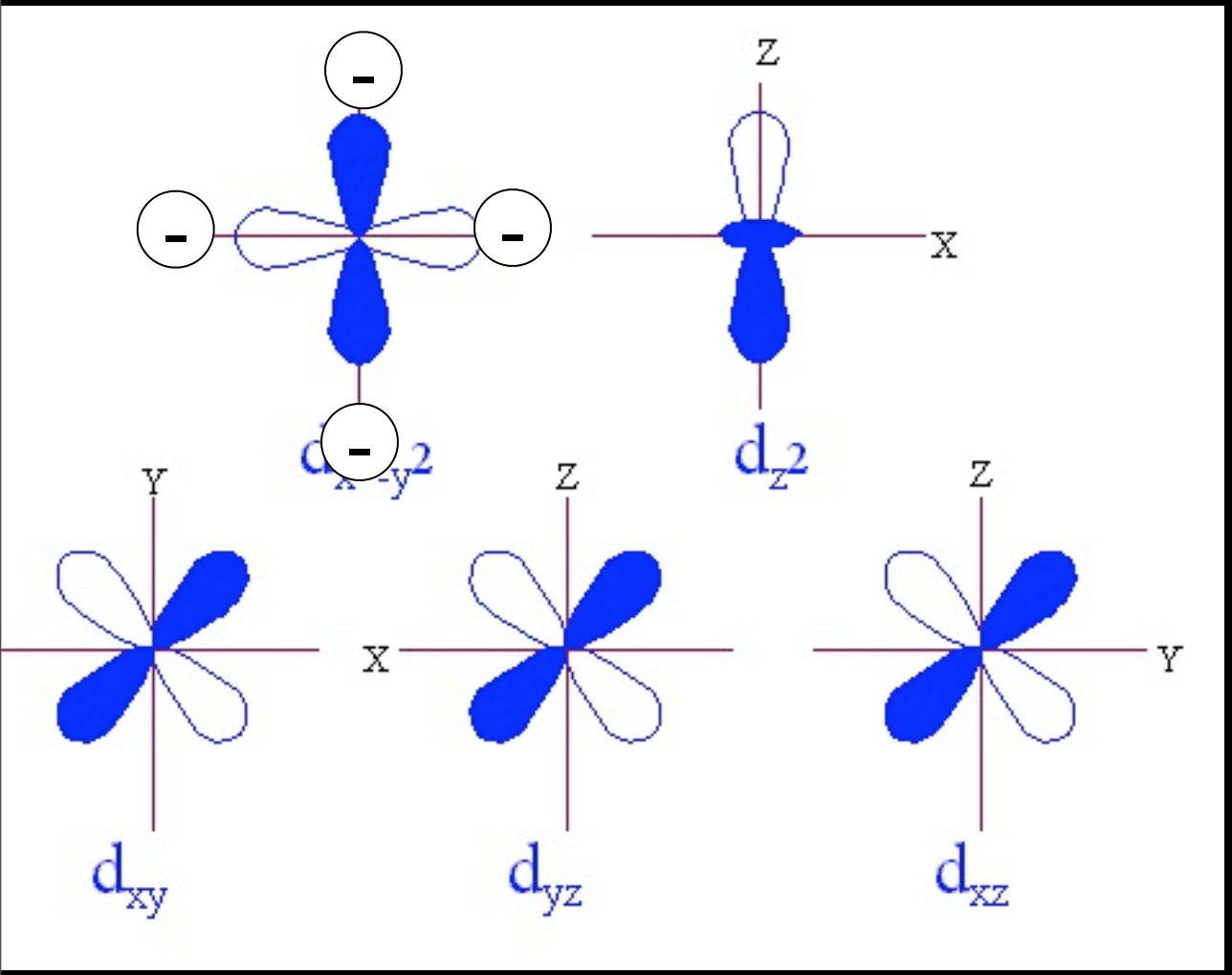
In Pt-drugs, QM-based methods may be a valuable alternative to parametrizations

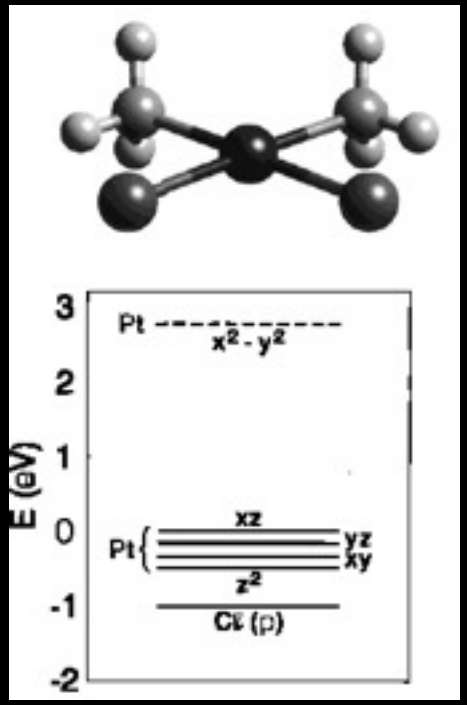
Force field methods may require a priori geometry information: limitations for drug design



Structure of lesion depends on electronic structure

PtX_4: low spin <u>diamagnetic</u>, square planar d^8 metal ion





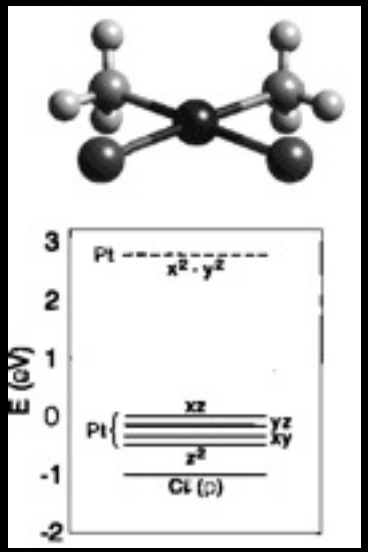
Test Calculations

Ab initio (MD)

Free drugs in the gas phase

(i) Validation of the computational approach : Pt

(ii) Characterize structure and electronic structure



Systems up to ~ 100 atoms

~ 5 ps

Test Calculations

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Free drugs in the gas phase

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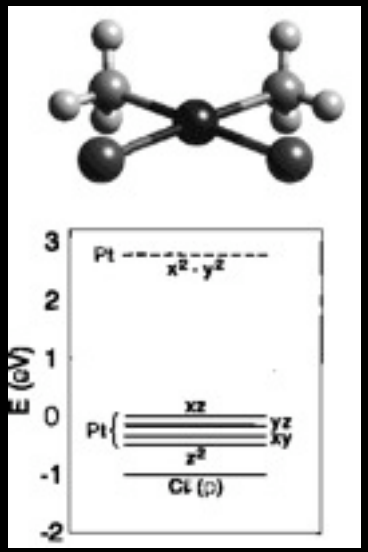

CPMD Program
DFT
BLYP, BP XC functional
Basis set: PW (70 Ry)
Martins Troullier angular momentum dependent scalar-relativistic pseudopotentials
18-electron valence shell
Δt=4-5 a.u.;μ=800 a.u.;T=300 K

Systems up to ~ 100 atoms
~ 5 ps

Test Calculations

Ab initio (MD)

Free drugs in the gas phase

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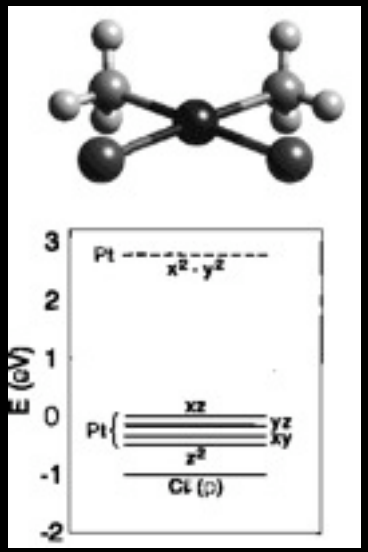
(ii) Characterize structure and electronic structure



Systems up to ~ 100 atoms

~ 5 ps

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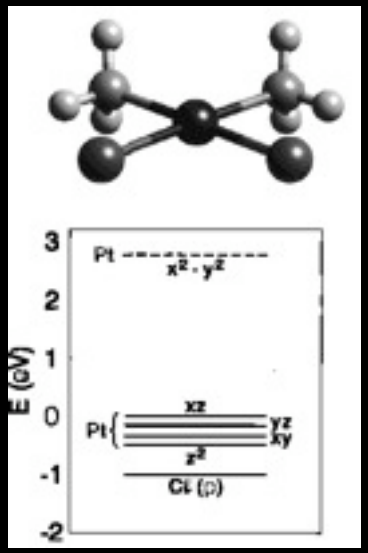


Systems up to ~ 100 atoms

~ 5 ps

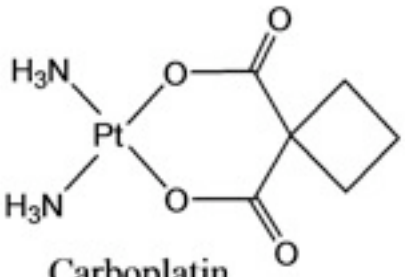
1-Structural and vibrational properties

Paolo Carloni, Wanda Andreoni, Jurg Hutter, Alessandro Curioni, Paolo Giannozzi, Michele Parrinello
Chem. Phys. Lett., 1995, 234, 50-56.

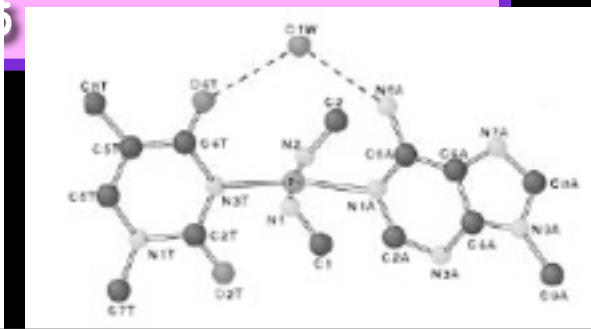
Elena Tolari, Paolo Carloni, Wanda Andreoni, Jurg Hutter, Michele Parrinello
Chem. Phys. Lett., 1995, 246, 469-474.

Platinum-Modified Nucleobase Pairs in the Solid State: A Theoretical Study
Paolo Carloni, Wanda Andreoni
J. Phys. Chem., 1996, 100, 17797 - 17800

alculations



Carboplatin



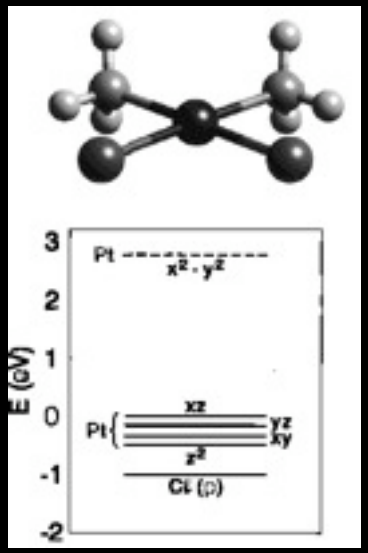
Test Calculations

Ab initio (MD)

Free drugs in the gas phase

(i) Validation of the computational approach

(ii) Characterize structure and electronic structure

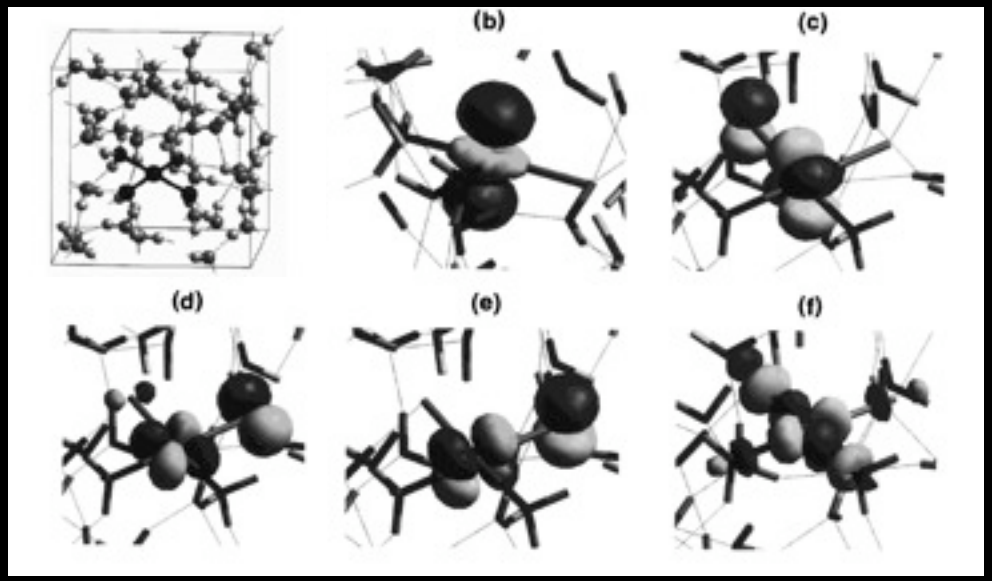


Systems up to ~ 100 atoms

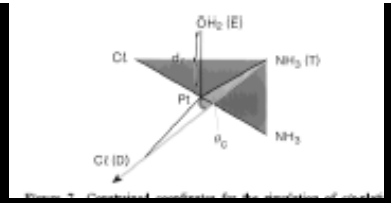
~ 5 ps

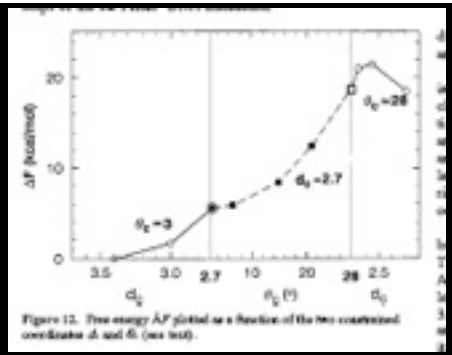
<u>3-Energetics</u> of first step of cisplatin hydrolysis by constrained ab initio MD:

$PtCl_2(NH_3)_2 + H_2O \rightarrow [PtCl(NH_3)_2 H_2O)]^+ + Cl^-$



$$\Delta F(Q) = -\int_{Q_0}^{Q} dQ' \langle \lambda \rangle_{Q'}$$





Paolo Carloni, Michiel Sprik, Wanda Andreoni

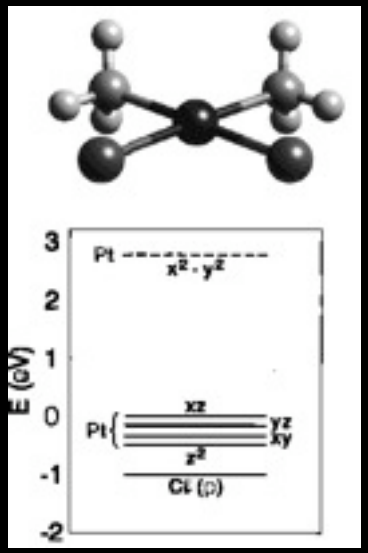
Cisplatin/DNA in water

Ab initio (MD)

Free drugs in the gas phase

(i) Validation of the computational approach

(ii) Characterize structure and electronic structure

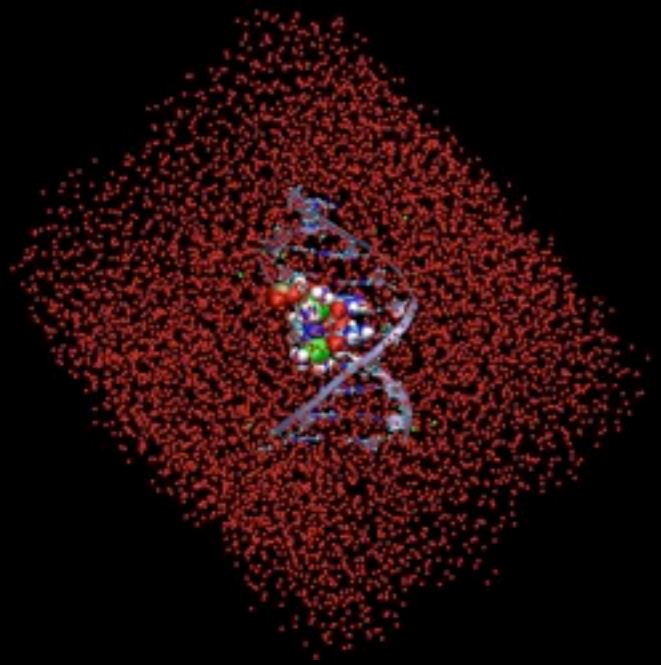


~ 100 atoms

~ 5 ps

Classical MD

To equilibrate structure



~50,000 atoms

~10-50 ns

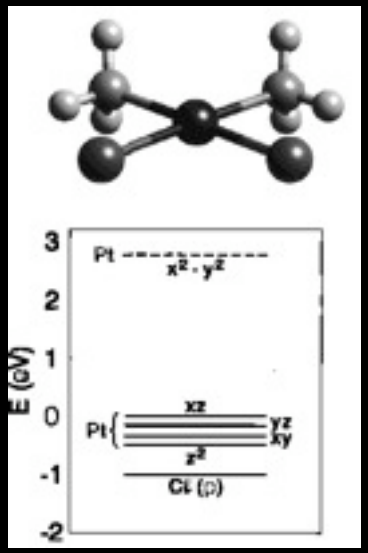
Cisplatin/DNA in water

Ab initio (MD)

Free drugs in the gas phase

(i) Validation of the computational approach

(ii) Characterize structure and electronic structure



~ 100 atoms

~ 5 ps

Classical MD

To equilibrate structure

NAMD and Amber Programs

Amber Force Field

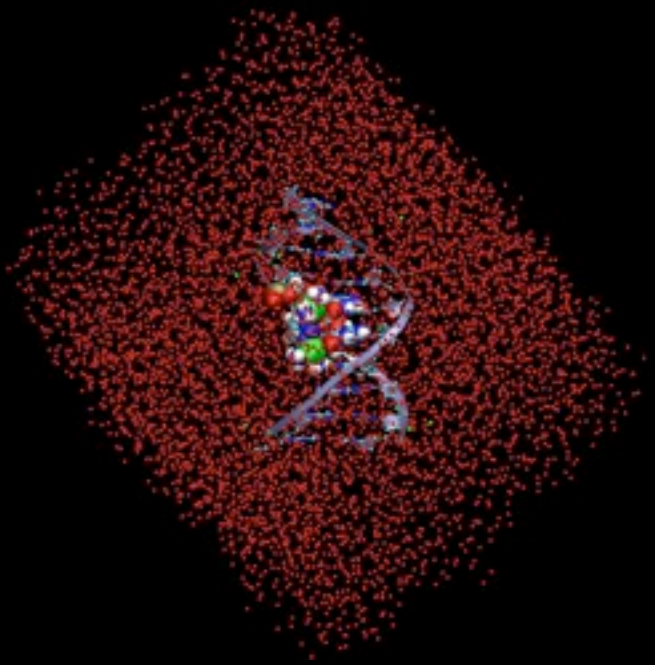
TIP3P waters

$\Delta t \sim 1.5$ fs

T=300K

P=1 atm

(Hobza and Co-workers)



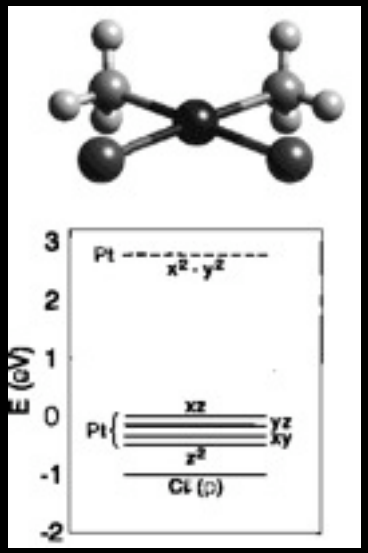
~50,000 atoms

~10-50 ns

Cisplatin/DNA in water

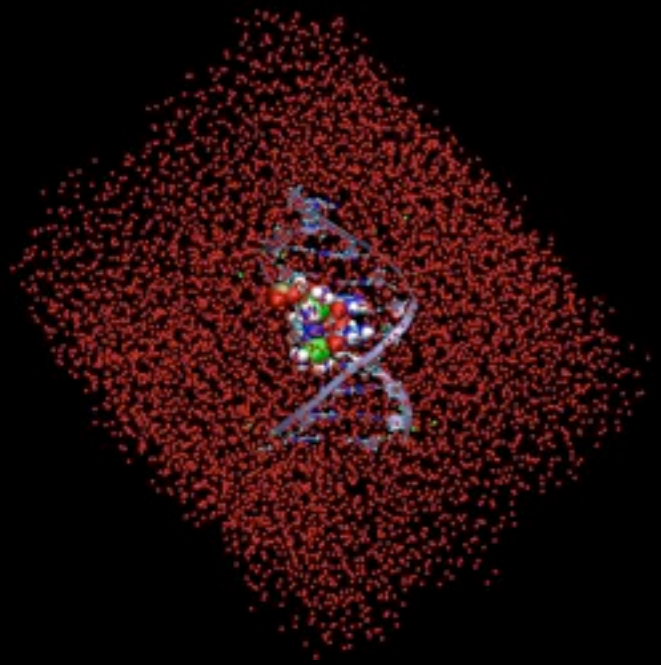
Ab initio (MD)

Classical MD



~ 100 atoms

~ 5 ps



~50,000 atoms

~10-50 ns

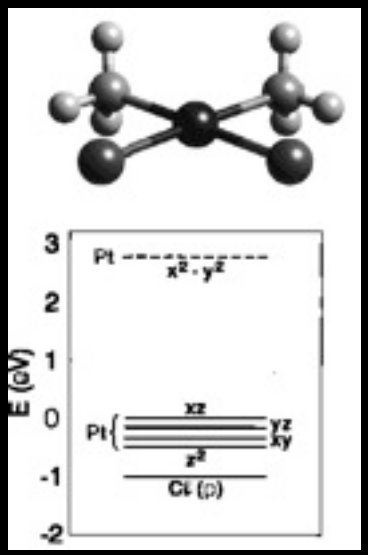
Cisplatin/DNA in water

Ab initio (MD)

Classical MD

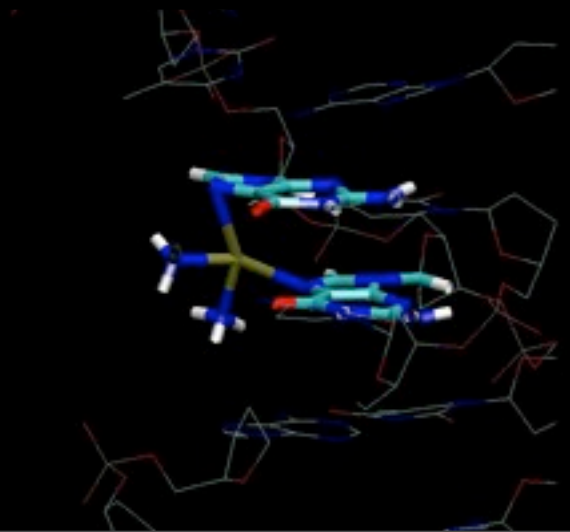
QM/MM MD

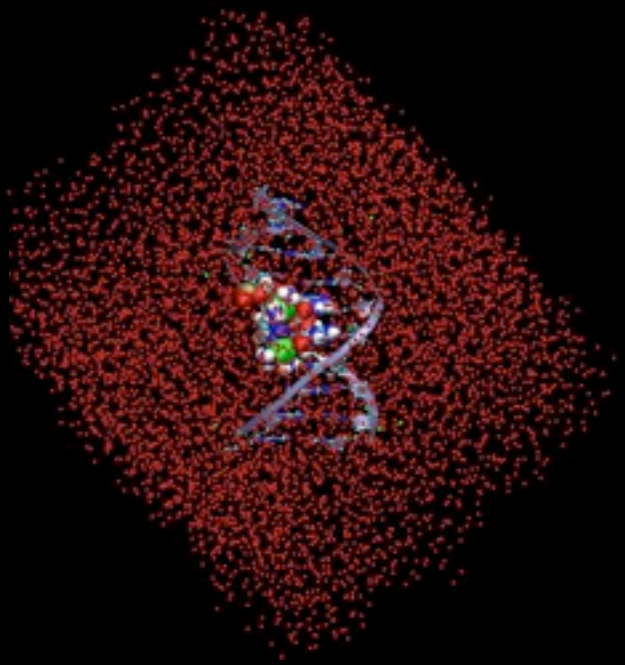
→ obtain structural ad spectroscopic information of drug -DNA complexes



~ 100 atoms

~ 20 ps





~ 50,000 atoms

~20 ns

Structural Information

Lavery, R.; Sklenar, H. Defining the structure of irregular nucleic acids. Conventions and principles. Biomol. Struct. Dyn. **1989**, 6, 655 667

pdb-entry	experiment/resolution or #struct.	sequence	specification	ax
1A2E[92]	X-ray/1.63	5′-d(CCTCG*CTCTC)- 5′-d(GAGAG*CGAGG)	interstrand	
1A84[3]	NMR/1	5′-d(CCTCTG*G*TCTCC)	intrastrand	
1AIO[1]	X-ray/2.6	5′-d(CCUCTG*G*TCTCC)- 3′	intrastrand	
1AU5[6]	NMR/1	5′-d(CCTCTG*G*TCC)	intrastrand	
1CKT[2]	X-ray/2.5	5′-d(CCUCTCTG*G*ACCTTCC)	intrastrand HMG A	
1DDP[93]	NMR/10	5′-d(GATAG*CTATG)- 5′-d(CATAG*CTATC)	interstrand	
1I1P[94]	X-ray/1.63	5′-d(CCCTCG*CTCTC)- 5′-d(GAGAG*CGAGG)	interstrand	
1KSB[19]	NMR/1	5′-d(ctccg*g*cct)	intrastrand	
5BNA[95]	X-ray/2.6	5′-d(CGCG*AATTCG*CG)- 5′-d(CGCG*AATTCGCG)	monosubstituted	

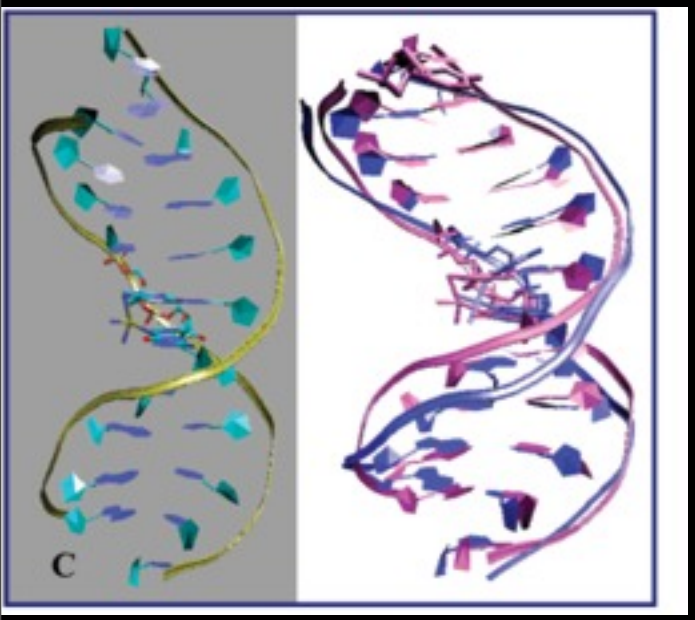
X-ray (Lippard et al., Nature 1995)
NMR (Reedijk et al., Biochemistry 1999)

Similar cisplatin-DNA X-ray and NMR adducts

Kink decreases on passing from solution to

'Ab initio' docking

cispt-d(CCTCTG*G*TCTCC)



Final QM/MM structure

initial X-ray structure

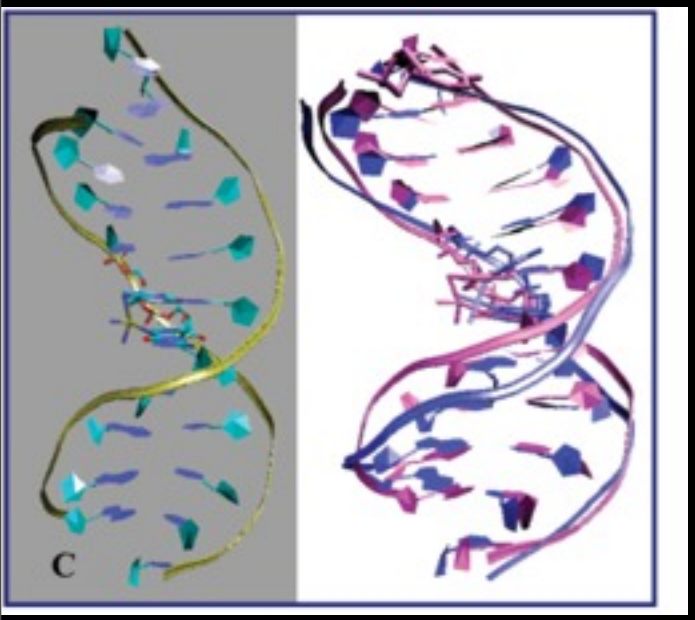
	X-ray	NMR	QM/MM
Axis bend:	40°	85°	48°

H-bond with phosphate detected in X-ray is broken in NMR and QM/MMoken

Spiegel , Carloni, Rothlisberger J. Phys. Chem. B 2004

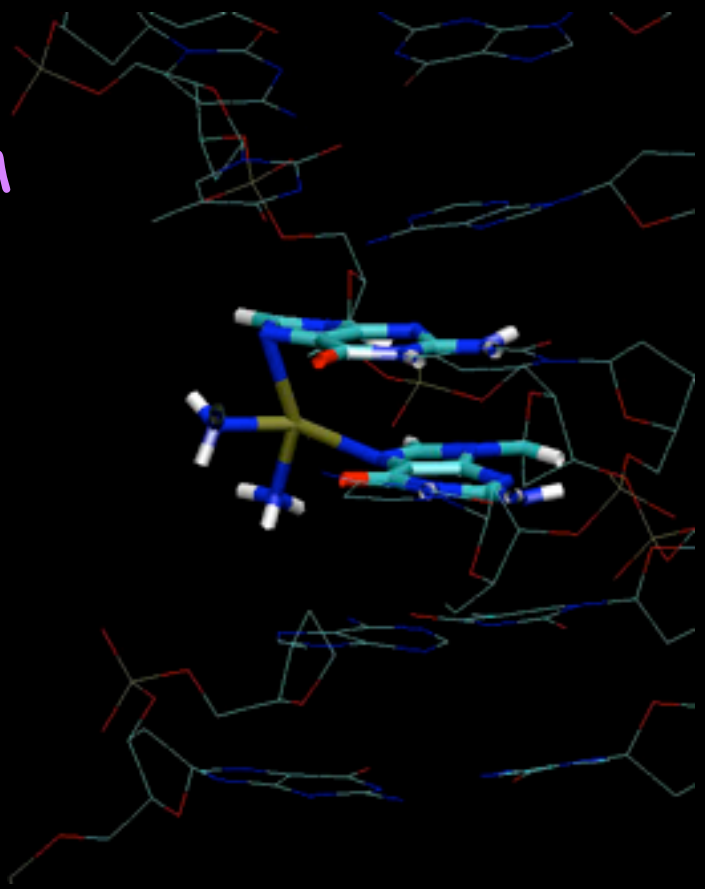
'Ab initio' docking

cispt-d(CCTCTG*G*TCTCC)



Final QM/MM structure

initial X-ray structure



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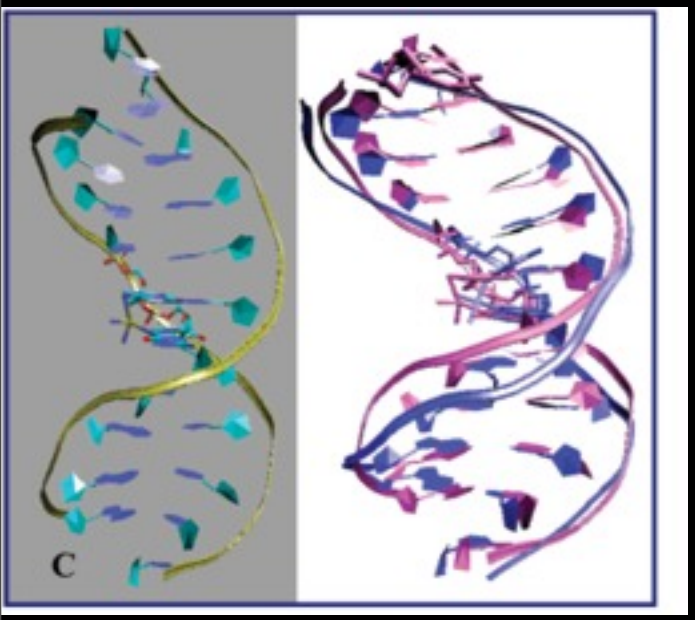

Final QM/MM structure

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Axis bend:	40°	85°	48°

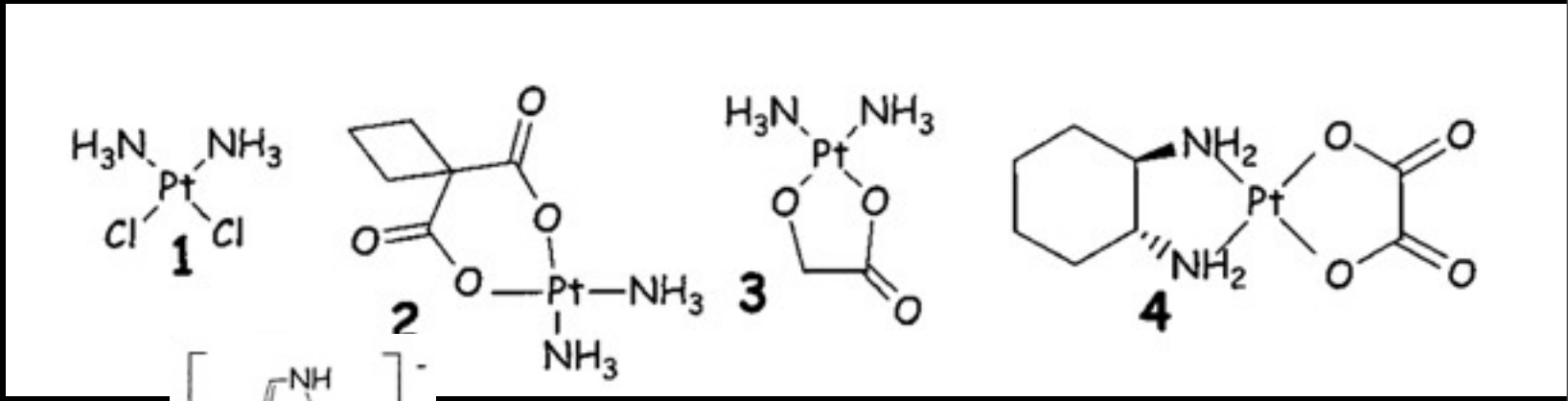
H-bond with phosphate detected in X-ray is broken in NMR and QM/MMoken

Spiegel , Carloni, Rothlisberger J. Phys. Chem. B 2004

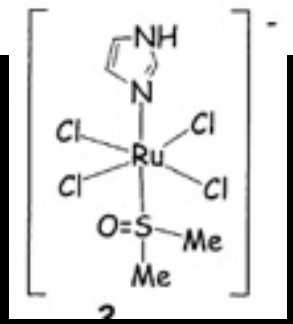
Search for novel Metal-based drugs

Repertoire of metal ions is limited:
Timescale of the ligand-exchange exchange reactions
Only Ru, Pt it has the same order of magnitude as the division of tumor cells (minutes →days)
most other metal complexes : μs-s

Pt-based clinically used drugs



Ru-based drug in clinical trials since 2000



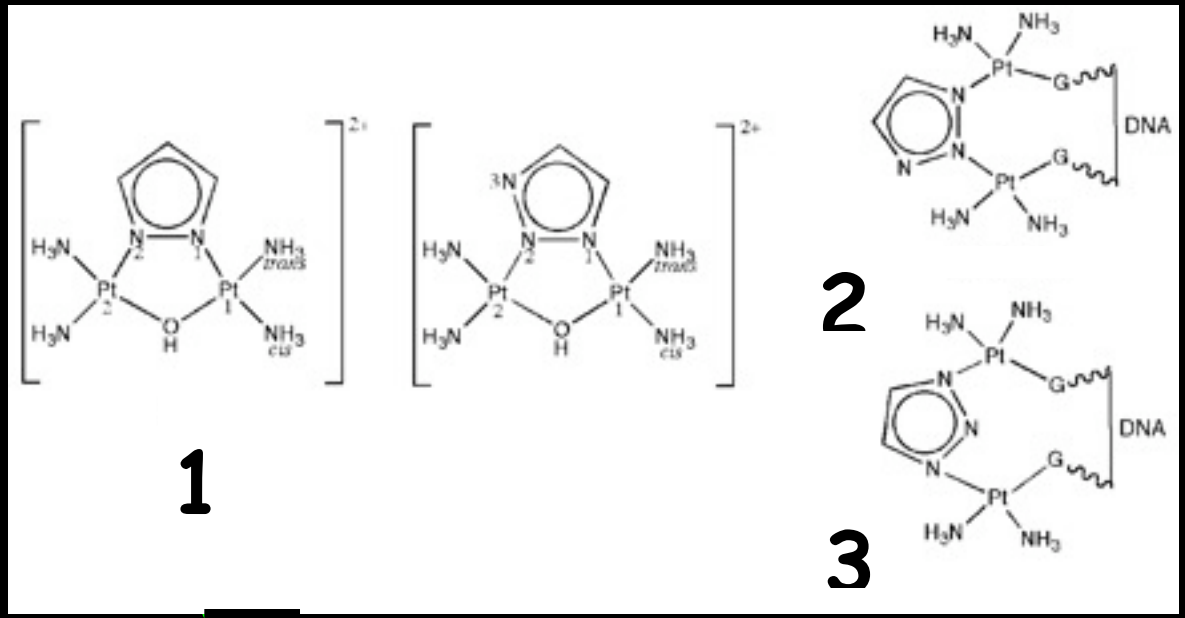
(Reedijk, PNAS 2003)

Unfortunately, mononuclear Pt drugs suffer from side effects and drug resistance, exhibiting similar binding. An increased activity of the DNA repair machinery, which is able to recognize cisplatin-induced DNA deformations, is in part responsible for resistance development

Ohndorf et al. Nature 1999, 399, 708, Brabec, Kasparkova Drug. Resist. Updates 2002

Dinuclear compounds

They may cause minor distortions to DNA by alkylating to adjacent G's, thus lowering the possibility to be recognized by DNA repairing enzymes (Reedijk PNAS 2003)

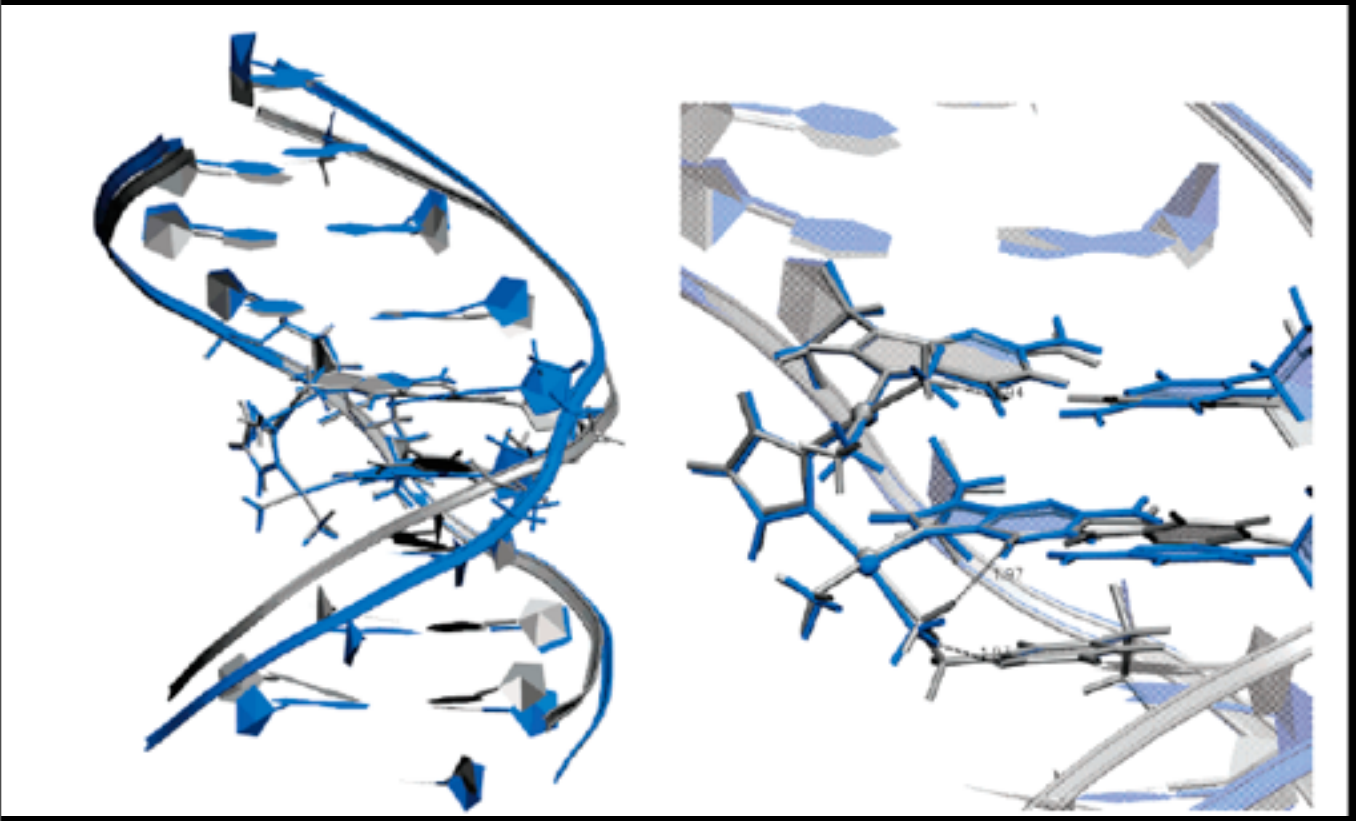


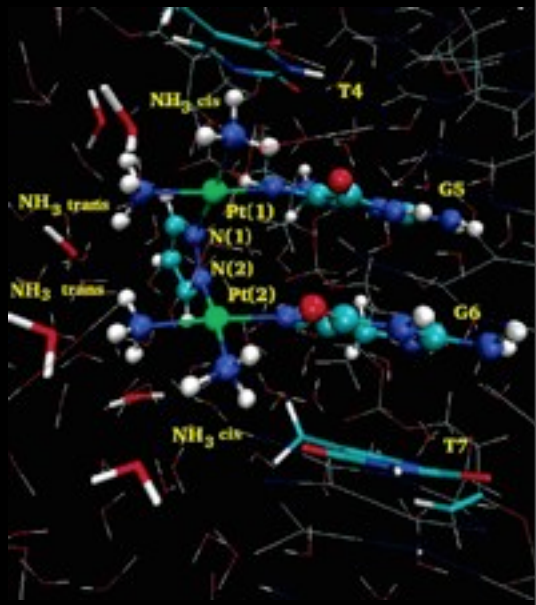
NMR structures of one complex`

1-d(CpTpCpTpG*pG*pTpCpTpCp)

Reedjik et al. J. Am. Chem. Soc. 2001, 2002

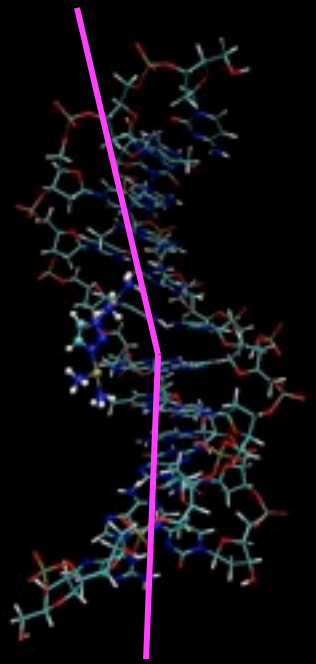
5 ps QM/MM simulation of 1-DNA: Comparison with NMR structure





RMSD between Platinated moieties in NMR and QM/MM structures: 0.6 A.

First insights in 2- and 3-DNA structures by QM/MM



Overall axis bend

51 (10) deg

19 ± 5 deg

10 ± 3 deg

8 ± 4 deg

—1-DNA

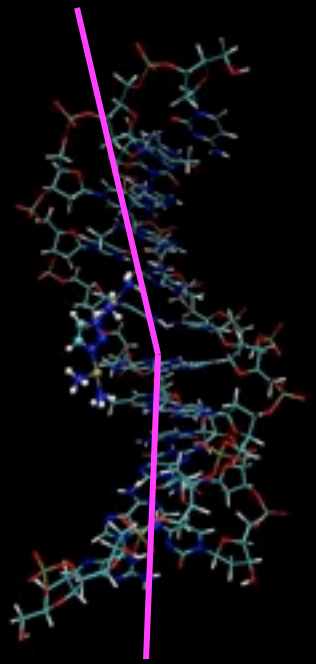
—2-DNA

—3-DNA

Magistrato et. al J. Phys. Chem. B, 2006

Dal Peraro et al. Curr. Op. Str. Biol. 2007,

First insights in 2- and 3-DNA structures by QM/MM



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Magistrato et. al J. Phys. Chem. B, 2006

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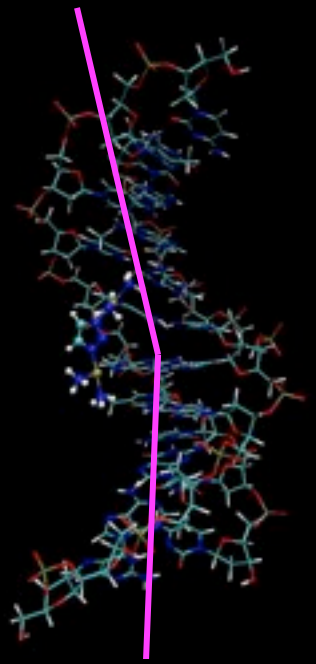

Overall axis bend

51 (10) deg
19 ± 5 deg
10 ± 3 deg
8 ± 4 deg

decrease

1-DNA
2-DNA
3-DNA

- The overall axis bend of diazole and triazole bridged decreases monotonically (minimum in 3)

- All of the three drugs do not modify largely DNA structure

Magistrato et. al J. Phys. Chem. B, 2006
Dal Peraro et al. Curr. Op. Str. Biol. 2007,

Extending the time-scale:

- QM/MM covers timescale of ~ 0.01 ns
- Because of limited time-scale, only relative comparisons are useful.
- Derive force field which reproduces structural, electrostatic mechanical properties of the QM subsystem based on QM/MM
- It takes into account biomolecular frame and temperature effects
- Bonded model versus non-bonded model: Highly directional and kinetically inert Pt-X bonds. No risk of ligand exchange.
- Calculate structural and thermodynamic averages by MD based on such force field (~ 0.01 μs)

Force Matching

- Ercolessi, Adams, Europhys. Lett. 1994, 26, 583-588
- **Fe:** Laio, A.; Bernard, S.; Chiarotti, G. L.; Scandolo, S.; Tosatti, E. Science 2000, 287,1027 1030
- **Si/SiO_2:** Csanyi, G.; Albaret, T.; Payne, M. C.; De Vita, A. Phys. Rev. Lett. 2004, 93, 175503. Lenosky, T. J.; Sadigh, B.; Alonso, E.; Bulatov, V. V.; Diaz de la Rubia, T.; Kim, J.; Voter, A. F.; Kress, J. D. Modelling Simul. Mater. Sci. Eng. 2000, 8, 825
- **Oxides**: Li, Y.; Siegel, D. J.; Adams, J. B.; Liu, X.-Y. Phys. Rev. B 2003, 67, 125101.
- Aguado, A.; Madden, P. A. Phys. Rev. B 2004, 70, 245103.
- **H_2O:** Izvekov, S.; Parrinello, M.; Burnham, C. J.; Voth, G. A. J. Chem. Phys. 2004, 120,

Force Match Approach

Force field parameters of the platinated site are obtained from QM/MM trajectories via a force matching procedure of the classical forces to ab initio forces.

Maurer et al. JCTC 2007
Spiegel et al. J. Comp. Chem 2007

Force Match Approach

Force field parameters of the platinated site are obtained from QM/MM trajectories via a force matching procedure of the classical forces to ab initio forces.

$$E = \sum_{i}^{N} \sum_{j}^{N_i^{nb}} \left(\frac{q_i q_j}{r_{ij}} + \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) + \sum_{n=1}^{N_{bon}} \frac{1}{2} k_{b_n} \left(b_n - b_{n0} \right)^2 + \sum_{n=1}^{N_{ang}} \frac{1}{2} k_{\theta_n} \left(\cos\theta_n - \cos\theta_{n0} \right)^2 +$$

$$\sum_{n=1}^{N_{imp}} \frac{1}{2} k_{\xi_n} \left(\xi_n^2 - \xi_{n0}^2 \right)^2 + \sum_{n=1}^{N_{ang}} \frac{1}{2} k_{\varphi_n} \left(1 + \cos\delta_n - \cos m_n \varphi_n \right)$$

Maurer et al. JCTC 2007

Spiegel et al. J. Comp. Chem 2007

Force Match Approach

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$$E = \sum_{i}^{N}\sum_{j}^{N_i^{nb}}\left(\frac{q_i q_j}{r_{ij}} + \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) + \sum_{n=1}^{N_{bon}} \frac{1}{2} k_{b_n}\left(b_n - b_{n0}\right)^2 + \sum_{n=1}^{N_{ang}} \frac{1}{2} k_{\theta_n}\left(\cos\theta_n - \cos\theta_{n0}\right)^2 +$$

$$\sum_{n=1}^{N_{imp}} \frac{1}{2} k_{\xi_n}\left(\xi_n^2 - \xi_{n0}^2\right)^2 + \sum_{n=1}^{N_{ang}} \frac{1}{2} k_{\varphi_n}\left(1 + \cos\delta_n - \cos m_n\varphi_n\right)$$

Van der Waals parameters are those of the force field.

Point Charges are fitted to reproduce both the electrostatic potential and electrostatic field, they are restrained to reference values, and they are averaged over different QM/MM conformations

Maurer et al. JCTC 2007

Spiegel et al. J. Comp. Chem 2007

Force Match Approach

Force field parameters of the platinated site are obtained from QM/MM trajectories via a force matching procedure of the classical forces to ab initio forces.

$$E = \sum_{i}^{N}\sum_{j}^{N_i^{nb}}\left(\frac{q_i q_j}{r_{ij}} + \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) + \sum_{n=1}^{N_{bon}}\frac{1}{2}k_{b_n}\left(b_n - b_{n0}\right)^2 + \sum_{n=1}^{N_{ang}}\frac{1}{2}k_{\theta_n}\left(\cos\theta_n - \cos\theta_{n0}\right)^2 +$$

$$\sum_{n=1}^{N_{imp}}\frac{1}{2}k_{\xi_n}\left(\xi_n^2 - \xi_{n0}^2\right)^2 + \sum_{n=1}^{N_{ang}}\frac{1}{2}k_{\varphi_n}\left(1 + \cos\delta_n - \cos m_n\varphi_n\right)$$

Van der Waals parameters are those of the force field.

Point Charges are fitted to reproduce both the electrostatic potential and electrostatic field, they are restrained to reference values, and they are averaged over different QM/MM conformations

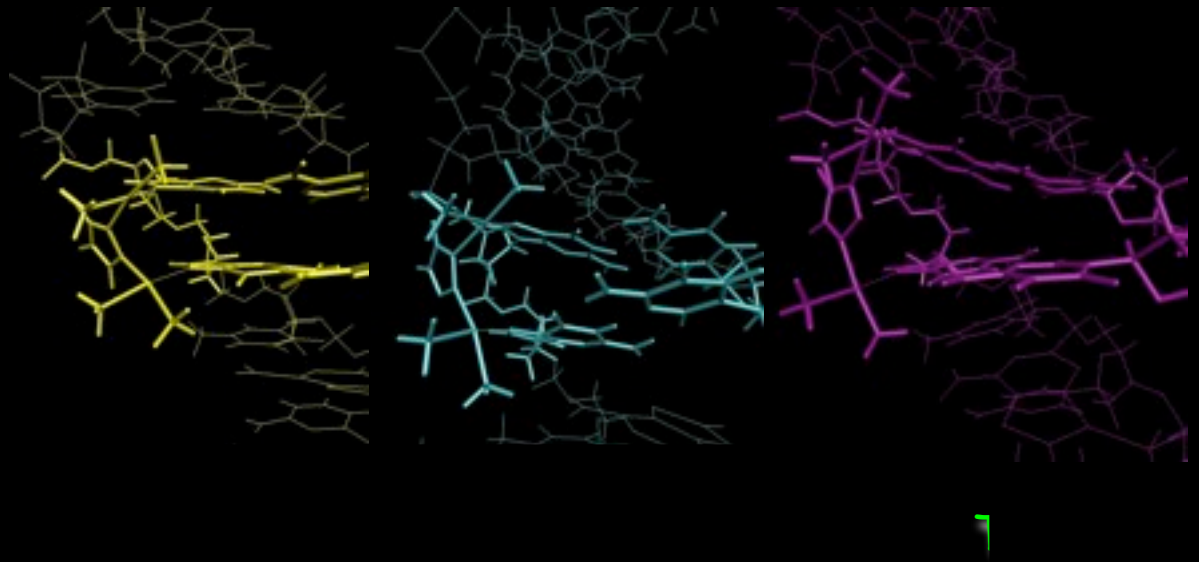
Equilibrium values for bonds, angles and torsional angles are those of the QM/MM

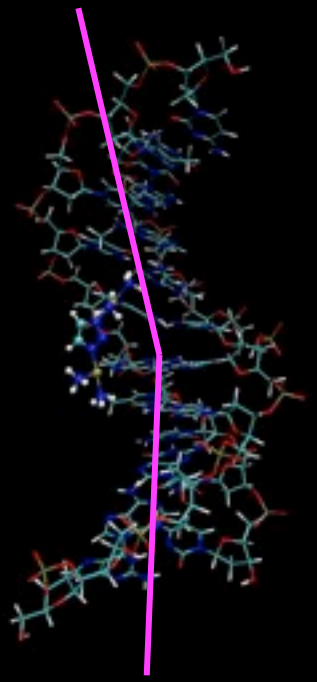
The classical bonded parameters are obtained through a least squares fit procedure to QM bonded forces.

Maurer et al. JCTC 2007

Spiegel et al. J. Comp. Chem 2007

~ 10 ns FM-MD of 1-,2-,3-DNA





axis bend 4.1 ± 0.3 Å 4.1 ± 0.5 Å

QM/MM FM

19 ± 8 deg

19 ± 5 deg 18 ± 9 deg

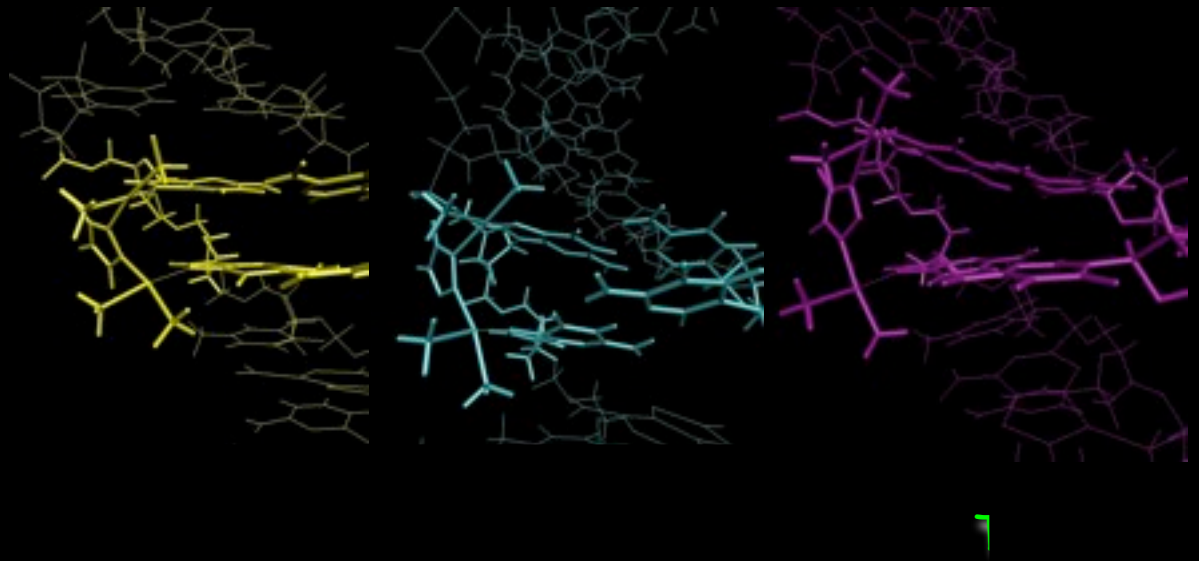
10 ± 3 deg 18 ± 8 deg

—1-DNA

—2-DNA

—3-DNA

~ 10 ns FM-MD of 1-,2-,3-DNA



axis bend 4.1 ± 0.3 Å 4.1 ± 0.5 Å
QM/MM FM

19 ± 8 deg
19 ± 5 deg 18 ± 9 deg
10 ± 3 deg 18 ± 8 deg

—1-DNA
—2-DNA
—3-DNA

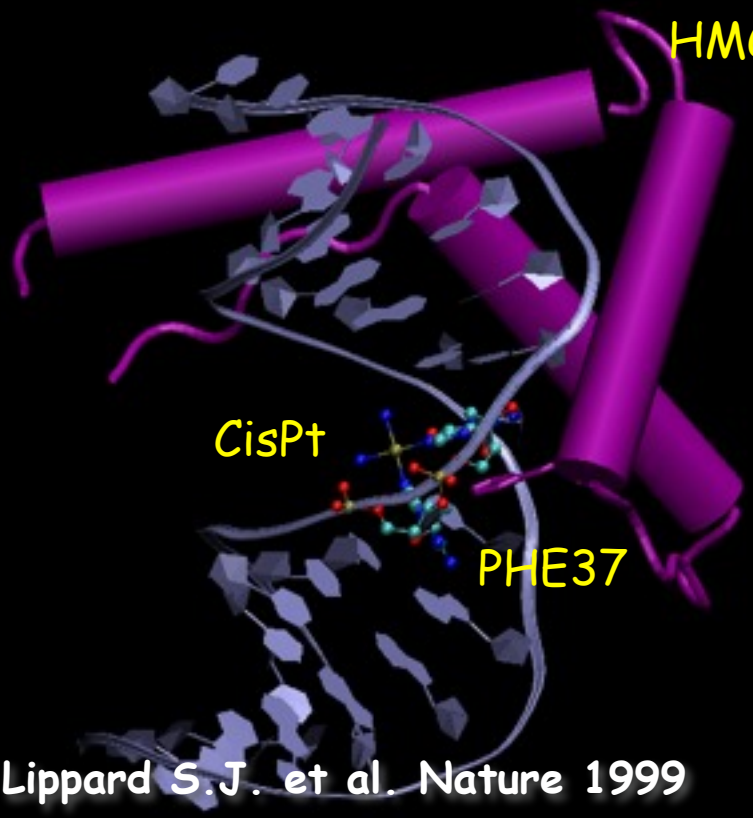
- QM/MM in qualitative agreement with FM-based MD
-1-DNA and 2-DNA almost identical
-1-DNA → 3-DNA: decrease of axis bend
- The decrease of the DNA curvature may be related with the higher cytotoxicity of the drug and to the overcome of cell resistance towards cisplatin.

Addressing resistance issues

- Search of alternative drugs

- Platinated/DNA-protein interactions

Cellular partners of platinated DNA

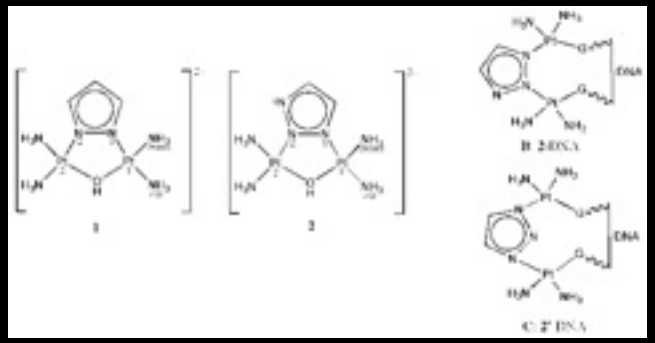


Lippard S.J. et al. Nature 1999

DNA (5'-d(CCUCTCTGGACCTTCC)-3')

HMG domain proteins bind the minor groove of cisplatin-DNA and bend the protein

Structural differences with 1-3 could affect the binding to HMG domain proteins



MD of platinated (5'-D(CCUCTCTGGACCTTCC)-3')

Complexes with DNA 16mer with the same sequence of cisPt-DNA/HMG protein complex.

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Complexes with DNA 16mer with the same sequence of cisPt-DNA/HMG protein complex.

Classical MD simulations
~ 10 ns (30,000 atoms)

MD of platinated (5'-D(CCUCTCTGGACCTTCC)-3')

Complexes with DNA 16mer with the same sequence of cisPt-DNA/HMG protein complex.

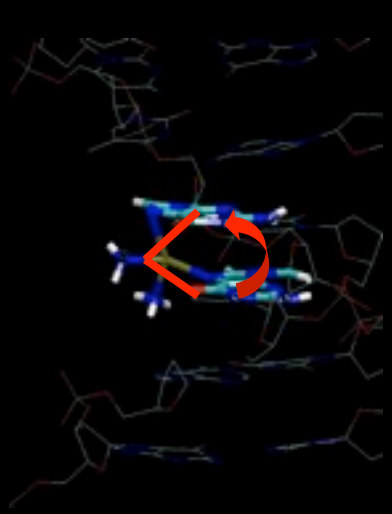
Classical MD simulations
~ 10 ns (30,000 atoms)

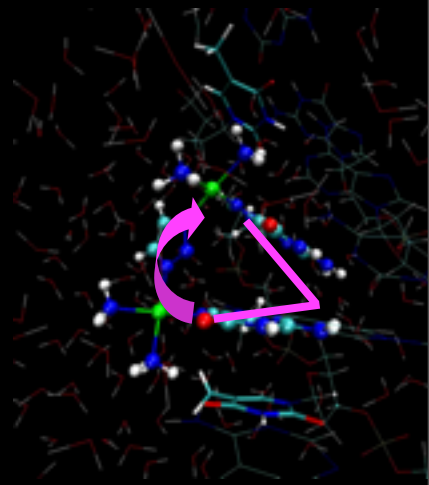
B-DNA
Rise G8-G9: 3.3 ± 0.2
Axis bend: 23 ± 11

CisPt-DNA:
Rise G8-G9: 4.6 ± 0.5
Axis bend: 49 ± 15

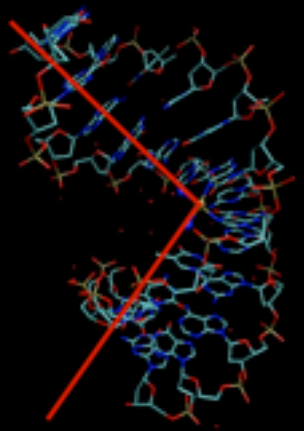
Conclusions -I

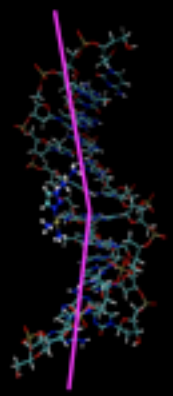
- 1- 3 cause small distortion





- Factors might be important for binding of excision repair enzymes and HGM proteins affecting cytoxicity and resistance





Spiegel, Magistrato, Carloni, Reedijk, Klein JPC B 2007

Conclusions -II

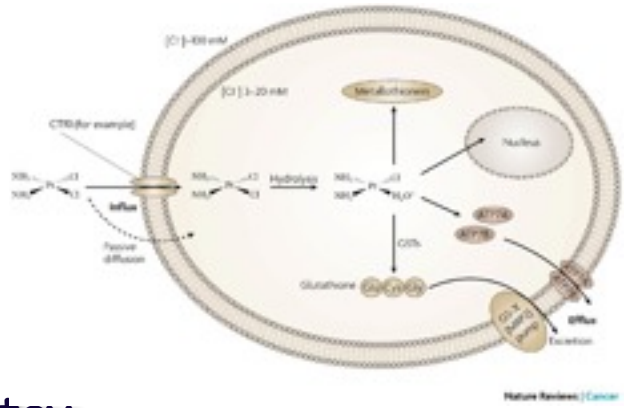
- Force matching approaches used to describe metal-based drugs and/or drugs forming covalent bonds with their targets

- Possible applications include: RNA (Flexibility, Mg^{2+} , counterions, large electric fields), metal-ions to disordered proteins

Force Matching: Pro's and Con's

- ☺ It is a largely automatic procedures
- ☺ It takes into account implicitly temperature and environment effects
- ☺ Particularly useful for compounds for which electronic structure dictates stereochemistry and/or stereochemistry is not known
- ☺ It avoids (i) unwanted solvent-ligand exchanges as non-bonded models (Teletchea at al. Chem. Eur. J. 2006); (ii) closed up' structures during optimization for RESP charges
- -) Expensive

Relevance for in vivo conditions



- Biomolecules often do not act alone:

- Cellular functions – the decisions to grow and divide, to die by programmed cell death, or to stay static – ultimately lie with macromolecules encoded by DNA.

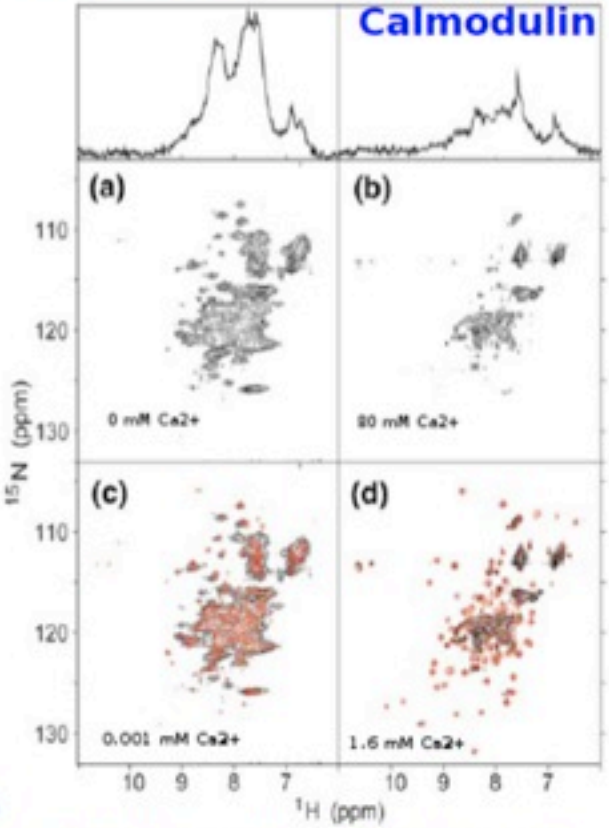
- Proteins and RNA directly control the cell through the reactions they perform, the conformations they adopt, and the interactions that they make.

- Very heterogeneous systems (cytoplasm as "molecular soup", cell membrane)

NMR, reductionism ?

Purified proteins in dilute solution

Under **physiological conditions** in cells



J. Biomol. NMR, 36(2006), 179
Biochemistry, 48(2009), 226

Black: in vitro; Red: in-cell

The cell

- Eukaryotic cell: water ~70% weight, proteins ~20%, DNA/RNA ~5% , lipids ~3%, Polysaccharides 2%

Cell Volume ≈10,000 μm^3 vs 1 μm^3

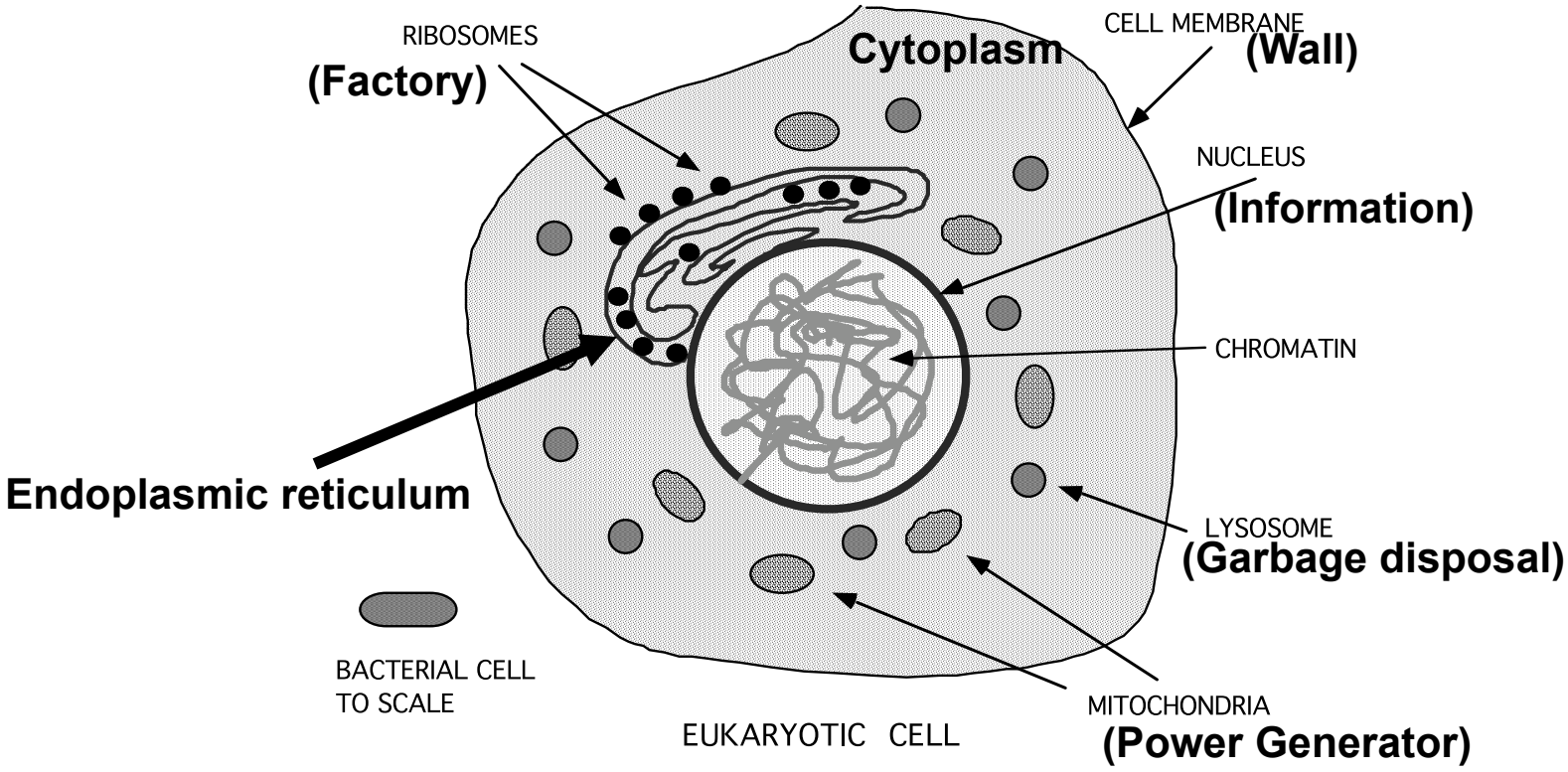
proteins ≈4 10^{10} vs 4 10^6

[proteins] ≈0.1 pM vs ≈ 1nM

Size of genome 3 10^9 bp and 30,000 genes vs 4.6 10^6 bp and 4500 genes

The medium: The cytoplasm (saline solution)

Compartmentalization: The membrane (made up by lipids)



- Cell stores its own set of instructions for carrying its functions (force or transport, metabolism, protein synthesis, control functions, production of new cells)
- Self-contained and self-maintaining. Structures potentially assemble, perform elaborate biochemical functions, vanish effortlessly when their work is done. Small molecules like drugs may hamper processes such as HIV-1 attack to the cell
- How can all of this work? (concentration of proteins less than nM)

Components of cell

·Eukaryotic cell: water ~70% weight, proteins ~20%, DNA/RNA ~5% , lipids ~3% Polysaccharides 2%

The medium: The cytoplasm (saline solution)
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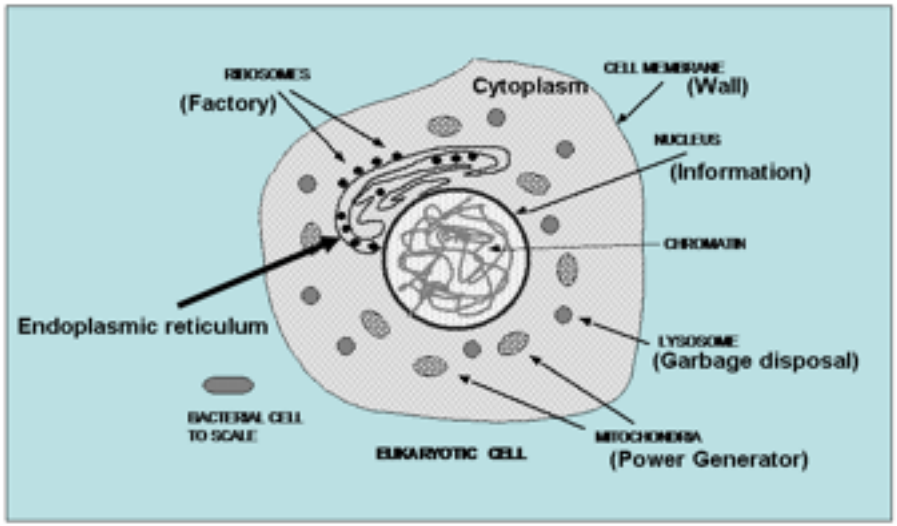
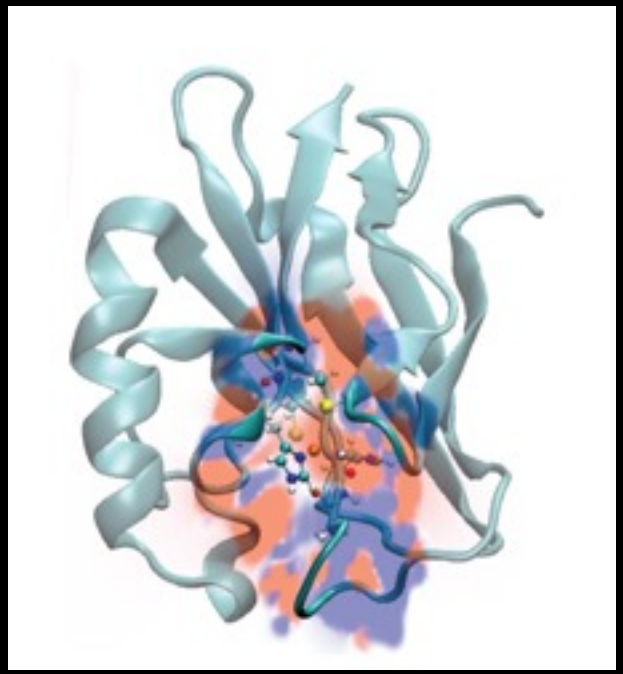


In vitro

- focus on one element…



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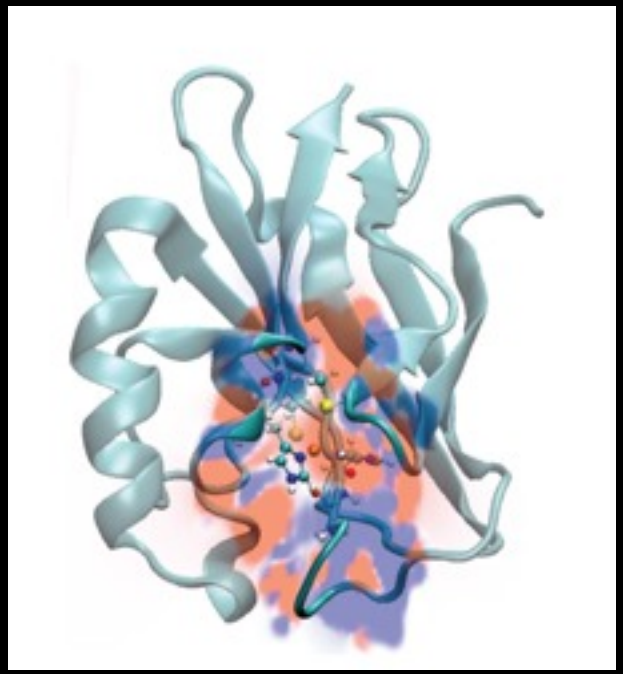


In vivo

- But processes in real life involve very complicated pathways in which **biomolecules might not act alone**

Adapted from U. Rothlisberger


SLIPPERY
WHEN WET

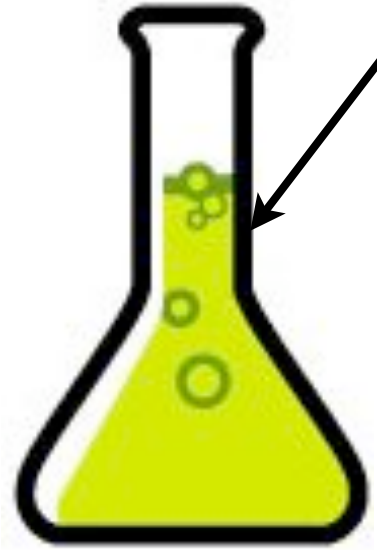

SLIPPERY
WHEN WET

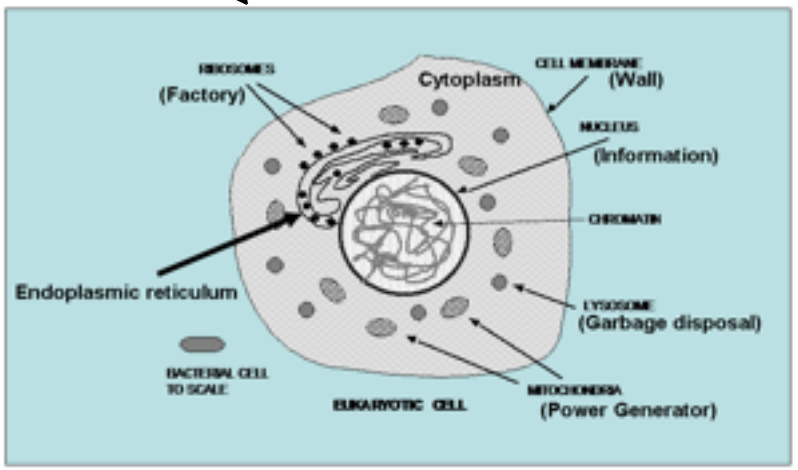
A biased biologist's view

The good, the bad (far from biology yet simple) and the ugly (but the real thing)

In silico, in vitro and in vivo

Computational methods





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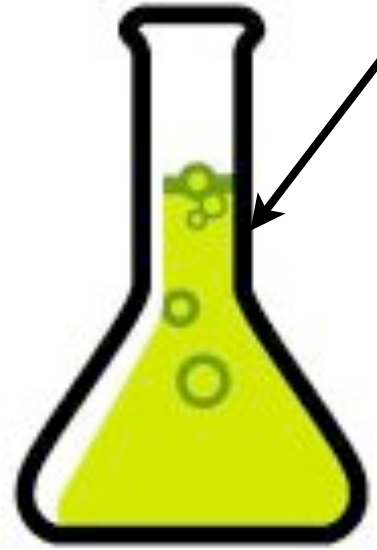
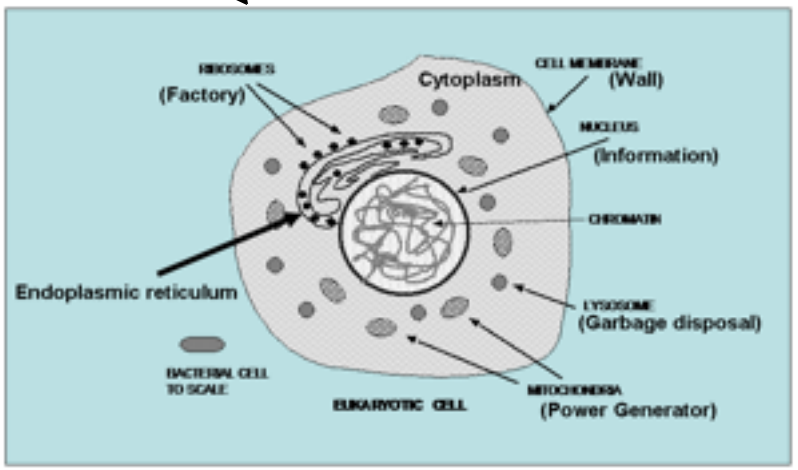




Drug with high affinity

??

A BIG simplification

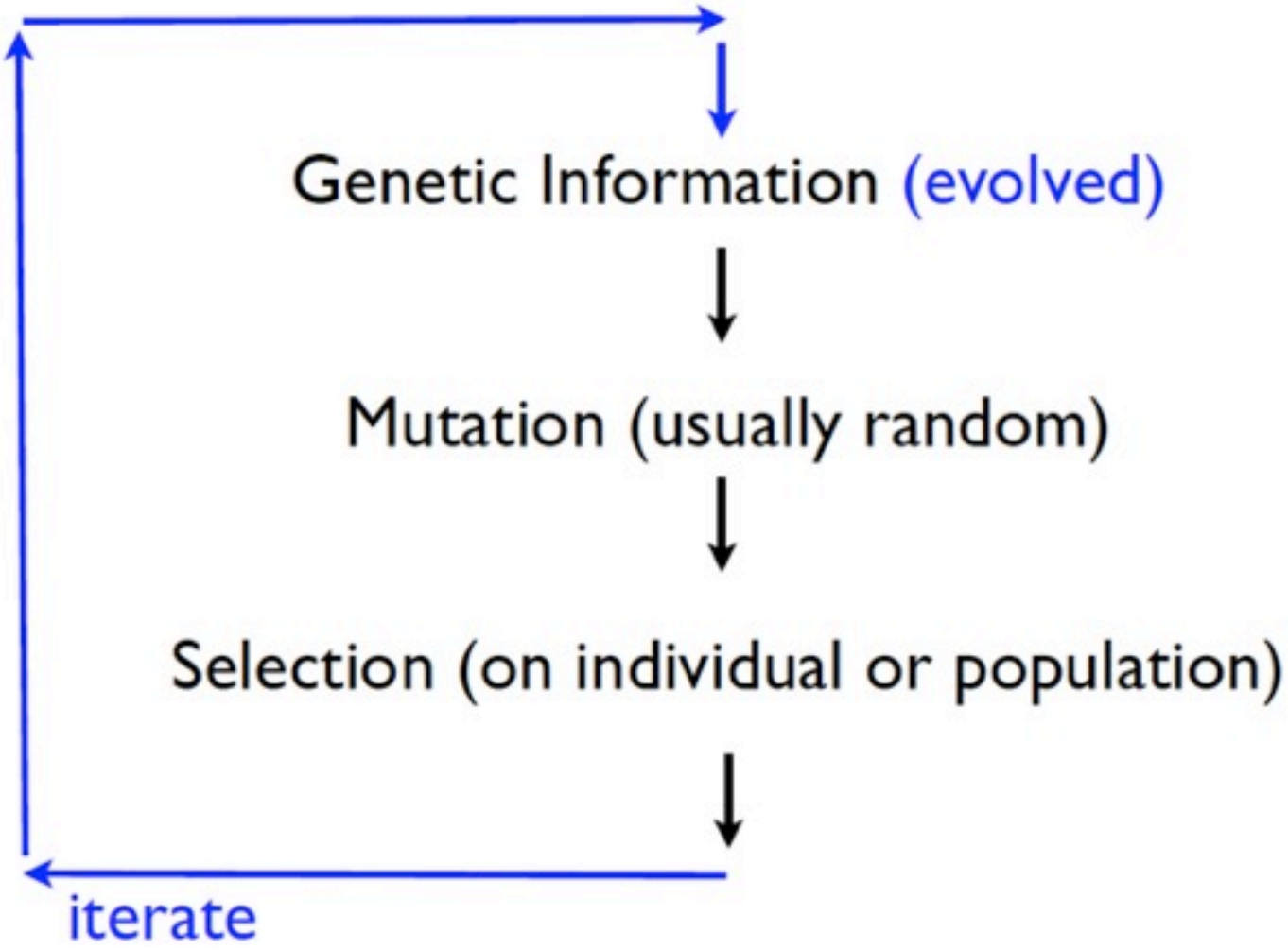
An H atom now is not different from a H atom 10^9 years ago, biological systems (~3.5×10^9 years old) are!

Darwinian Evolution consists in the survival of the fittest at each new generation of each organism. It modifies existing mechanisms rather than invent new ones. Thus biological systems are similar (e.g. plants and animal proteins share striking similarities) and they are "robust" with respect of small changes. By investigating (structural, functional, cellular) patterns, we can have clues about these very complicated systems (Bioinformatics)

…a BIG simplification: Use patterns for structural predictions and for pathways investigations

- The complexity of biological systems is determined not so much by the number of parts they use to carry out their functions, as by the number of interactions involved in the regulation of these functions.
- Thus, although eukaryotes have generally larger genomes than prokaryotes, genome sizes are not correlated with the complexity of the organism.
- Unicelullar eukaryotes have genome sizes that vary 200,000-fold, and the genome of the amoeba is about 200 times greater than that of humans.
- New protein structures reveals motifs already existing in the data banks and that have been used over and over again in related and sometimes even unrelated tasks.

The Mechanism of Evolution



Evolutionary Time Scales

Kingdom	When Evolved	Structure	Phothosynthesis
Prokaryotes:-			
Bacteria	3 to 4 billion years ago	Unicellular	Sometimes
Archaea	3 to 4 billion years ago	Unicellular	No
Eukaryotes:-			
Protista	1.5 billion years ago	Unicellular	Sometimes
Fungi	1 billion years ago	Unicellular or Multicellular	No
Animalia	700 million years ago	Multicellular	No
Plantae	500 million years ago	Multicellular	Yes

Bioinformatics

- Fundamental features of proteins are not shared by small molecules
- Proteins are the product of natural selection superimposed to random variation:Stability and Reactivity optimized for the biological environment
- Proteins evolving from a common ancestor maintained similar core 3D structures. Structural models of proteins (targets) homologous to other proteins whose 3D structure is known (templates).

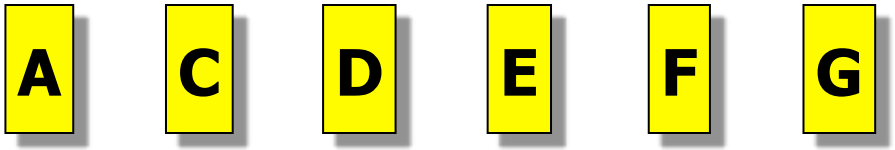
- **Exploiting the presence of patterns in biology enlarges the predictive power of computational biophysics**

Proteins that have evolved from a common ancestor may exhibit the same fold and different sequences, except for functionally important residues

From DNA...

ACTTGTAATAAATTTAGT...

...to the Protein



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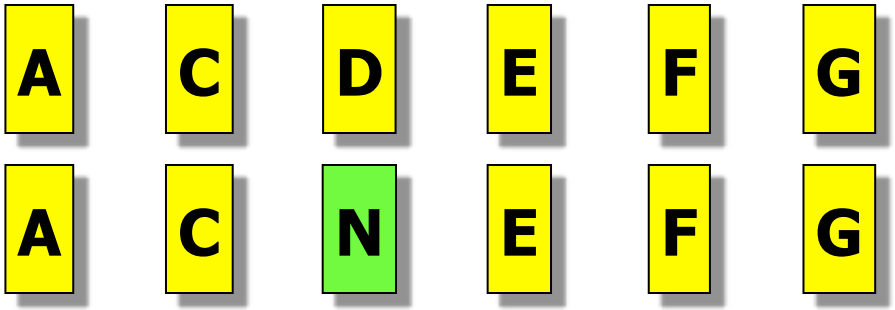

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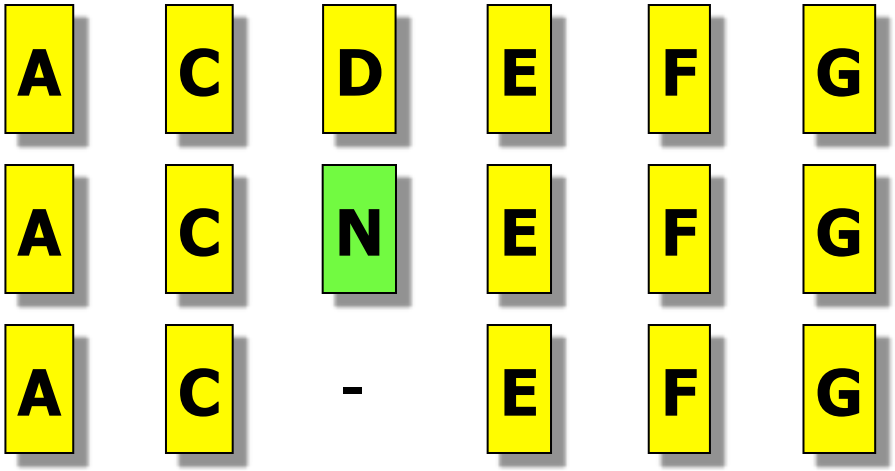

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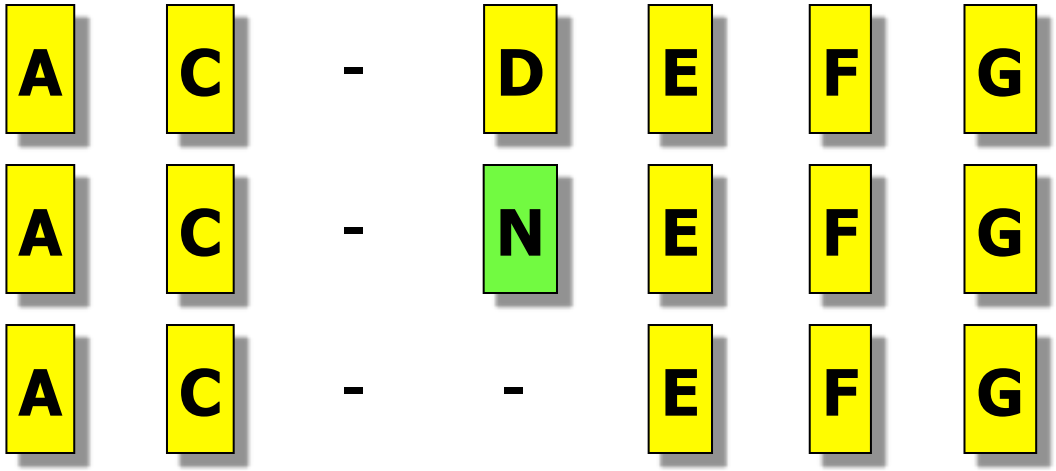

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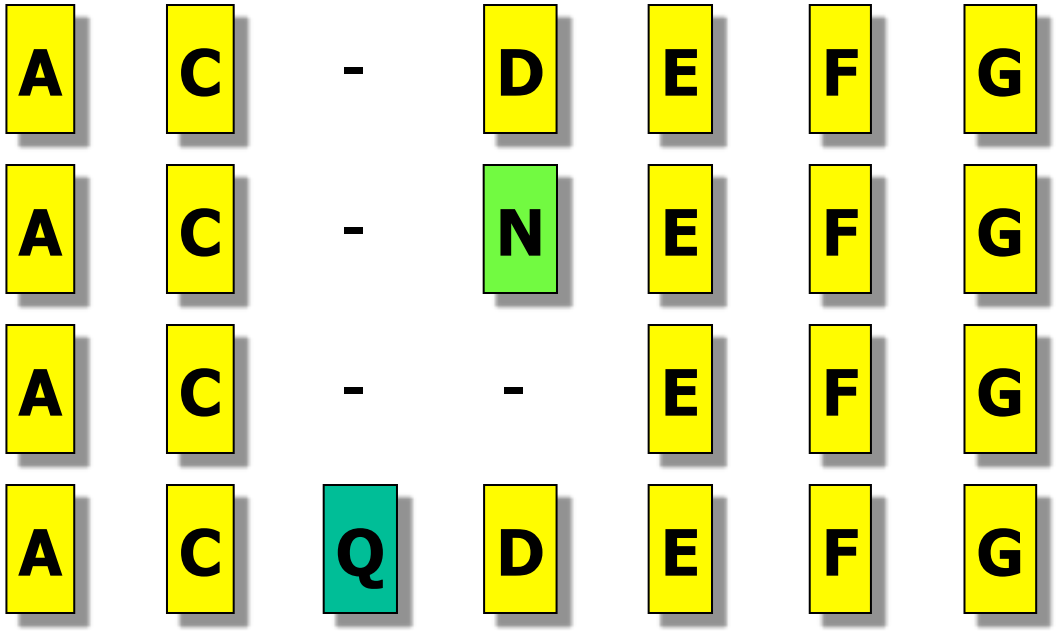

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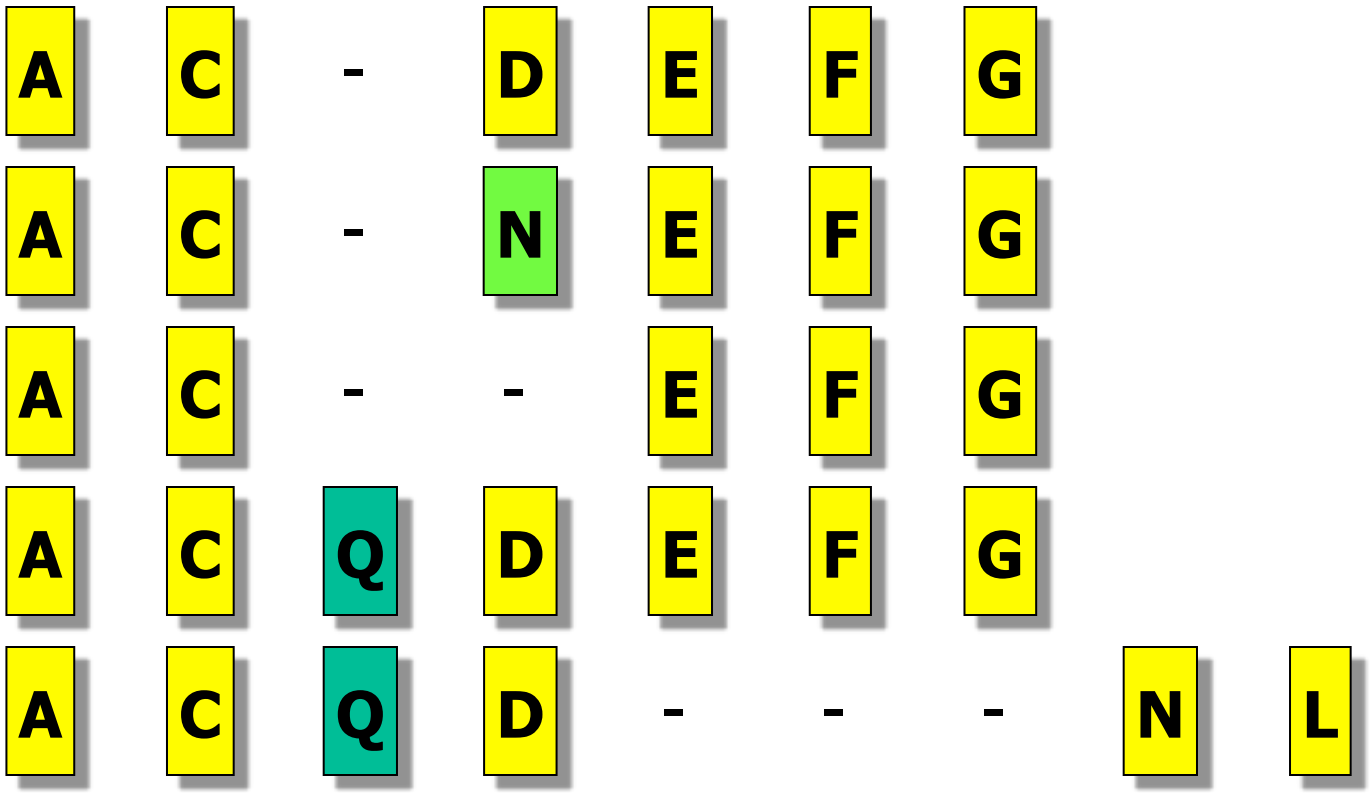

```
...PA-EEFRRRITTATA...
   ||  ||||   |||||||
...PANEEFR--ISTATA...
```

Sequence identity: 11/15 = 73%

One can assume homology if sequence identity > 30%.

The sequence space is so immense (20^{200} for a protein with 200 residues) that the probability of significant similarity between unrelated (non-hololologous) sequences is close to zero.

A striking outcome

- Understanding mechanisms. Molecular basis of e.g. enzymatic reactions or receptor signaling in the cell
- Characterizing aberrant processes (e.g. fibrillation) and eventually trying to stop them
- Delivering drugs (nano-biotechnology): e.g. delivering RNA
- Biotechnological applications: effects of mutations, gene knock-out…
- Intervening on mechanisms by improving drug affinity/ selectivity?
- Food and agriculture industry: smell, taste…

Perspectives of CPMD/MM in molecular systems biology: the case of GPCR membrane signaling

Membrane signaling confined and lower numbers of players.

GPCR's are the largest membrane-bound family of receptors (possibly selected by evolution because sensing relies on them)

Ligand binding or light induces cascade of events, in some case relatively well characterized

46 GPCR's are drug targets

The largest cluster within the family is that

Lagerstrom&Schiott, Nature Drug Disc. 2008

Olfactory signaling cascade

- Discriminate among thousands of structurally diverse odorant molecules, recognizing newly introduced odorants

  Buck, LB. (2005) Unraveling the sense of smell (Nobel lecture). Angew Chem Int Ed Engl. 44(38):6128-40

- Build artificial noses based on biological mechanisms (IIT)

- Food and parfume industry

- Localized in olfactory sensory neurons

Carloni Paolo 9/20/07
Odorant binding to an OR causes a conformational change of the receptor. The new conformation has a high affinity for the a subunit of the G protein heterotrimer in complex with GDP (Gα•GDP). As a result, the G-protein releases GDP and it binds to

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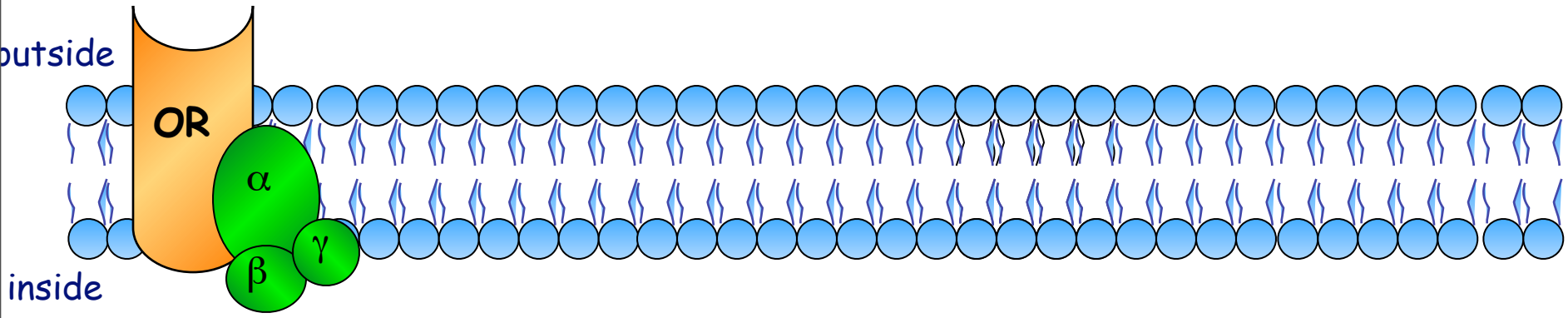
al structural information is limited



Predictions of structure and energetics

Pifferi et al., FEBS Letters 2006

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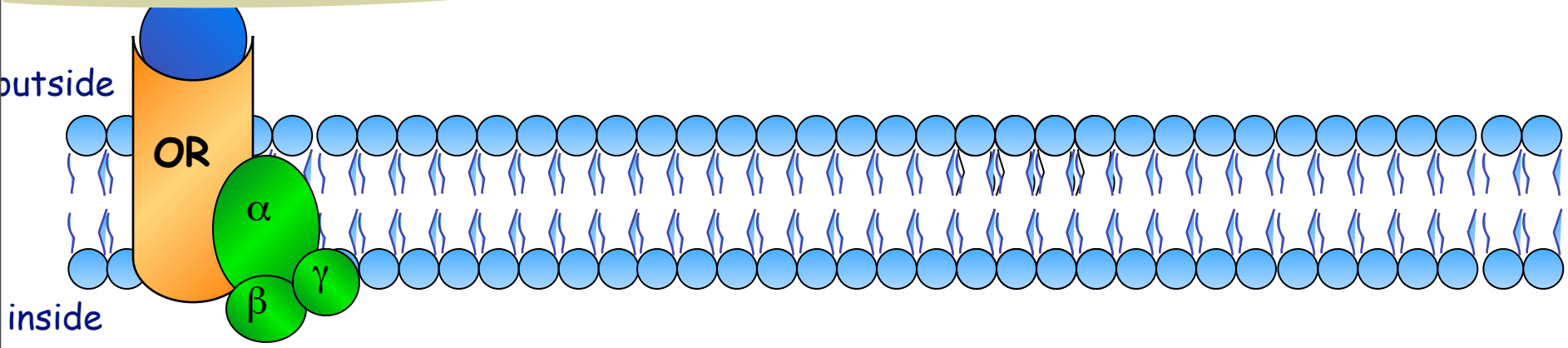

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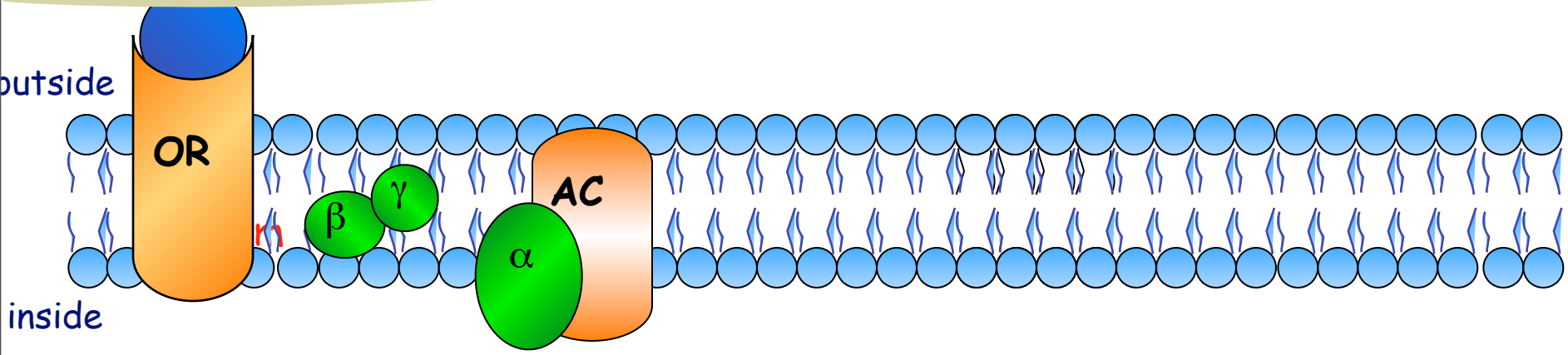

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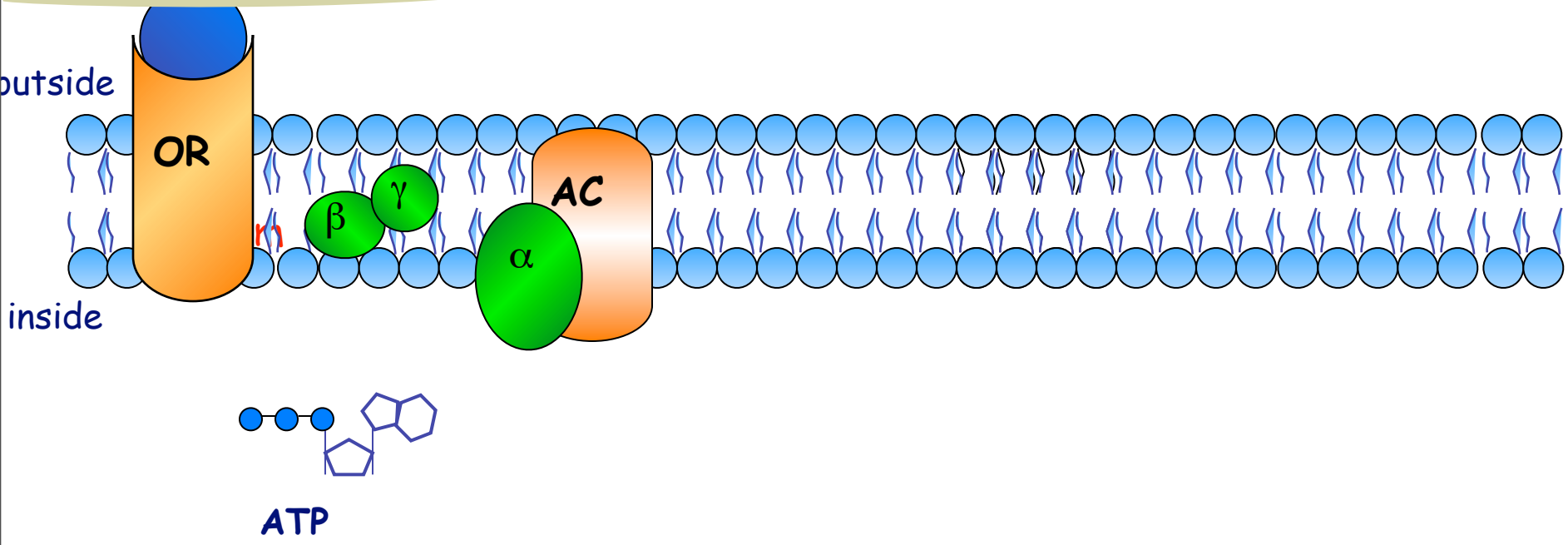

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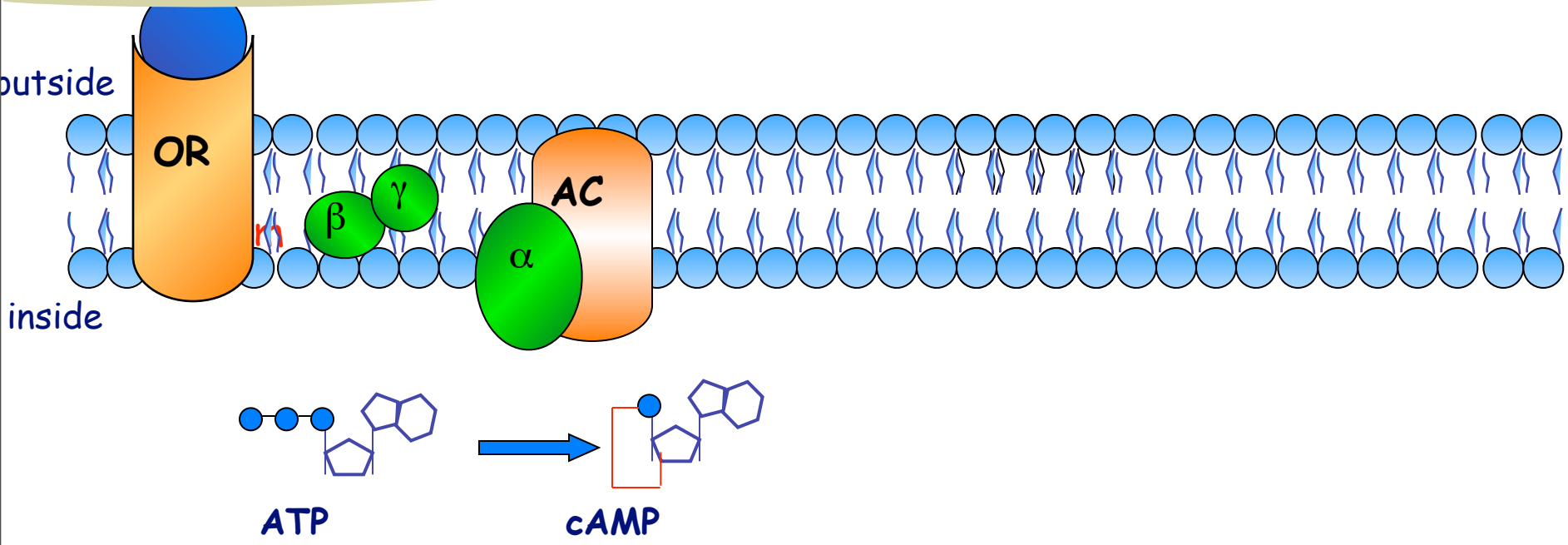

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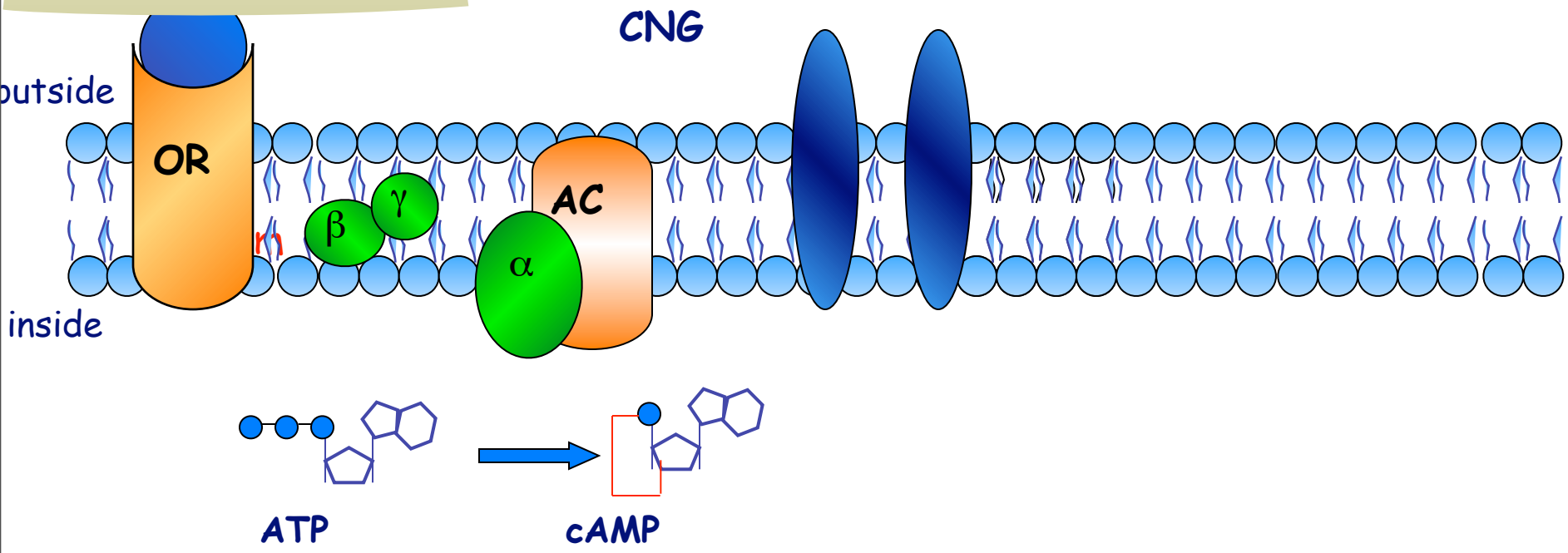

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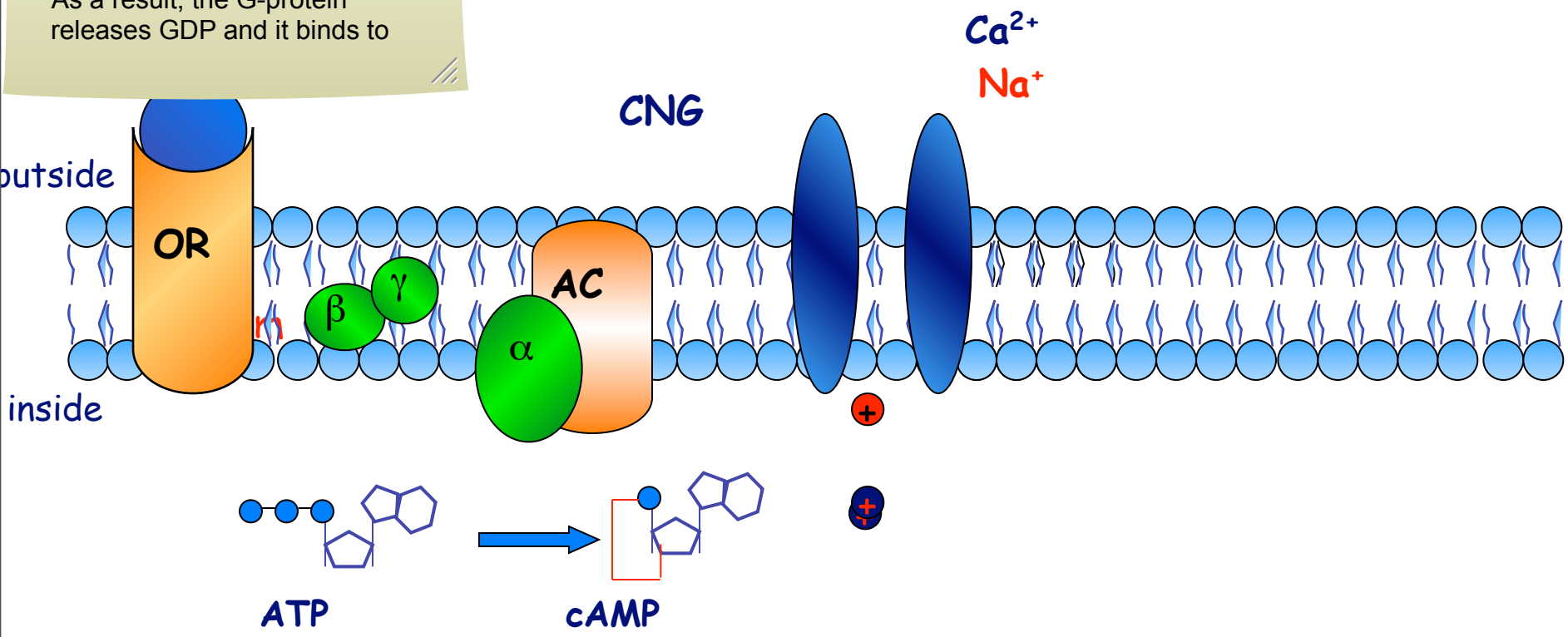

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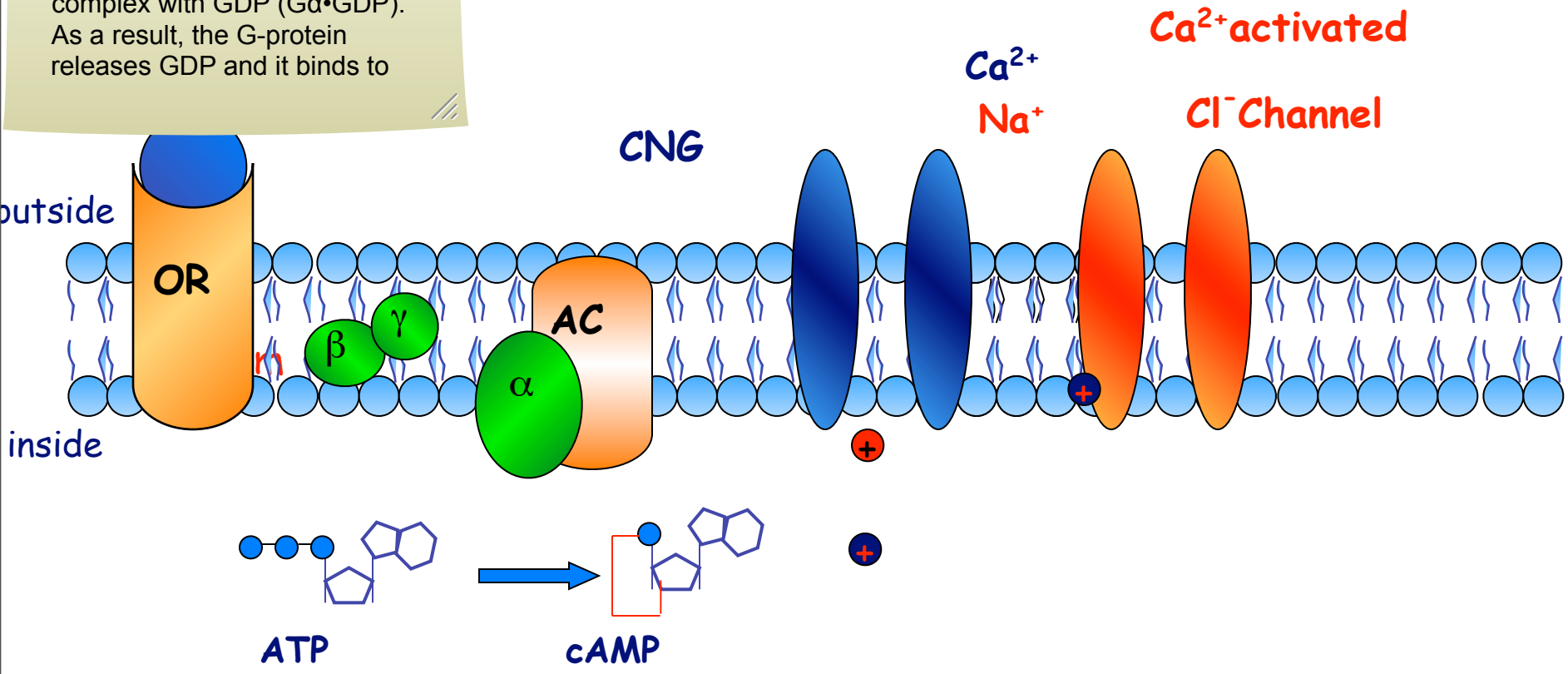

Predictions of structure and energetics

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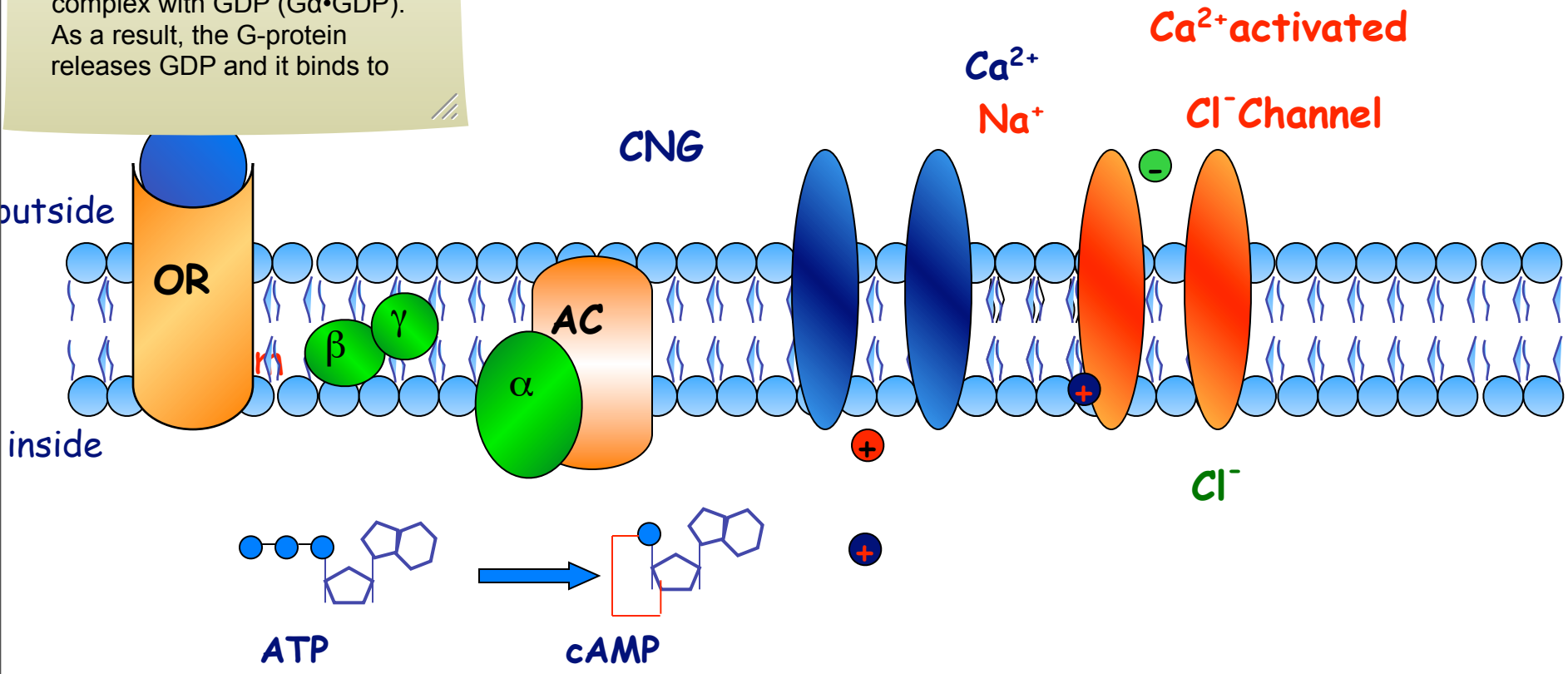

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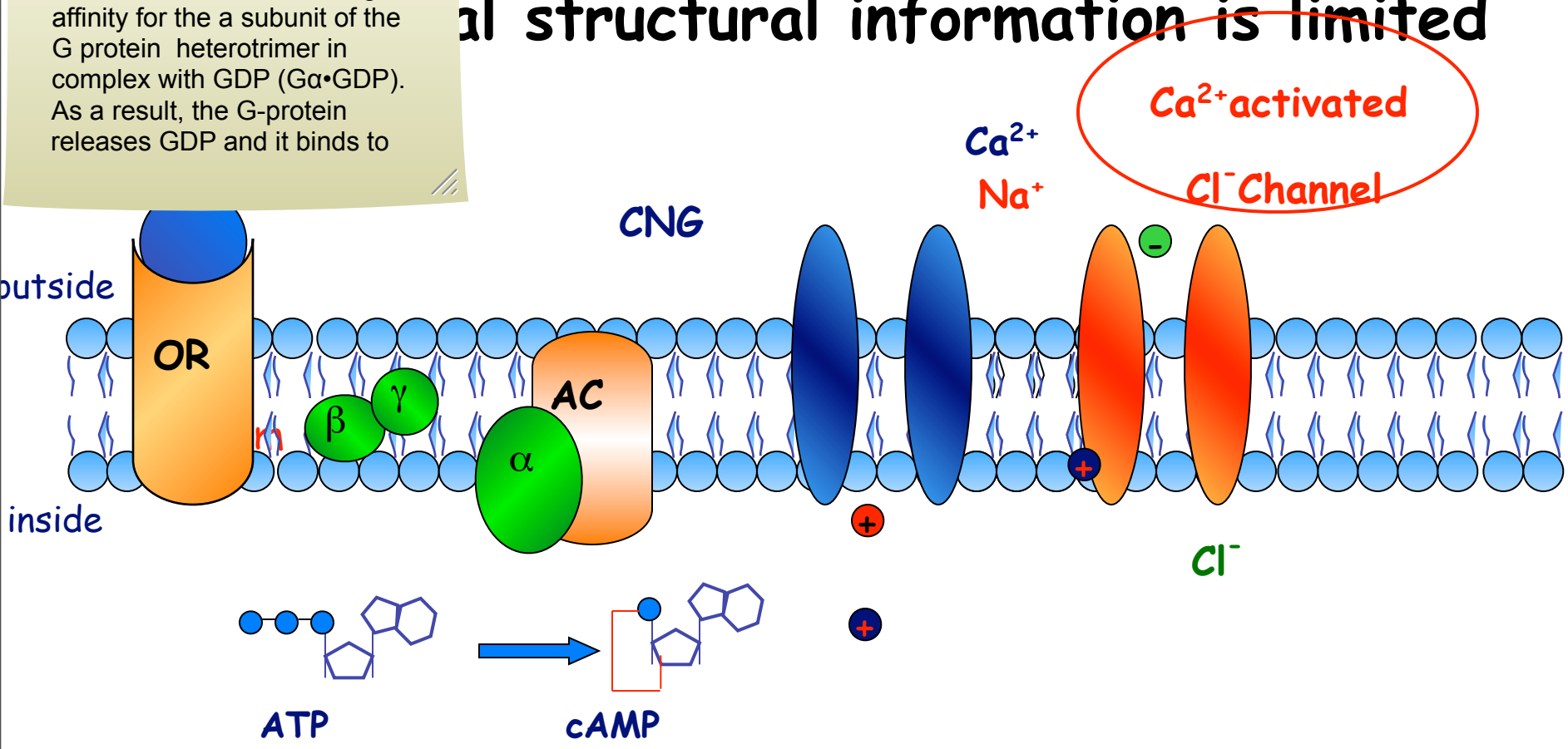

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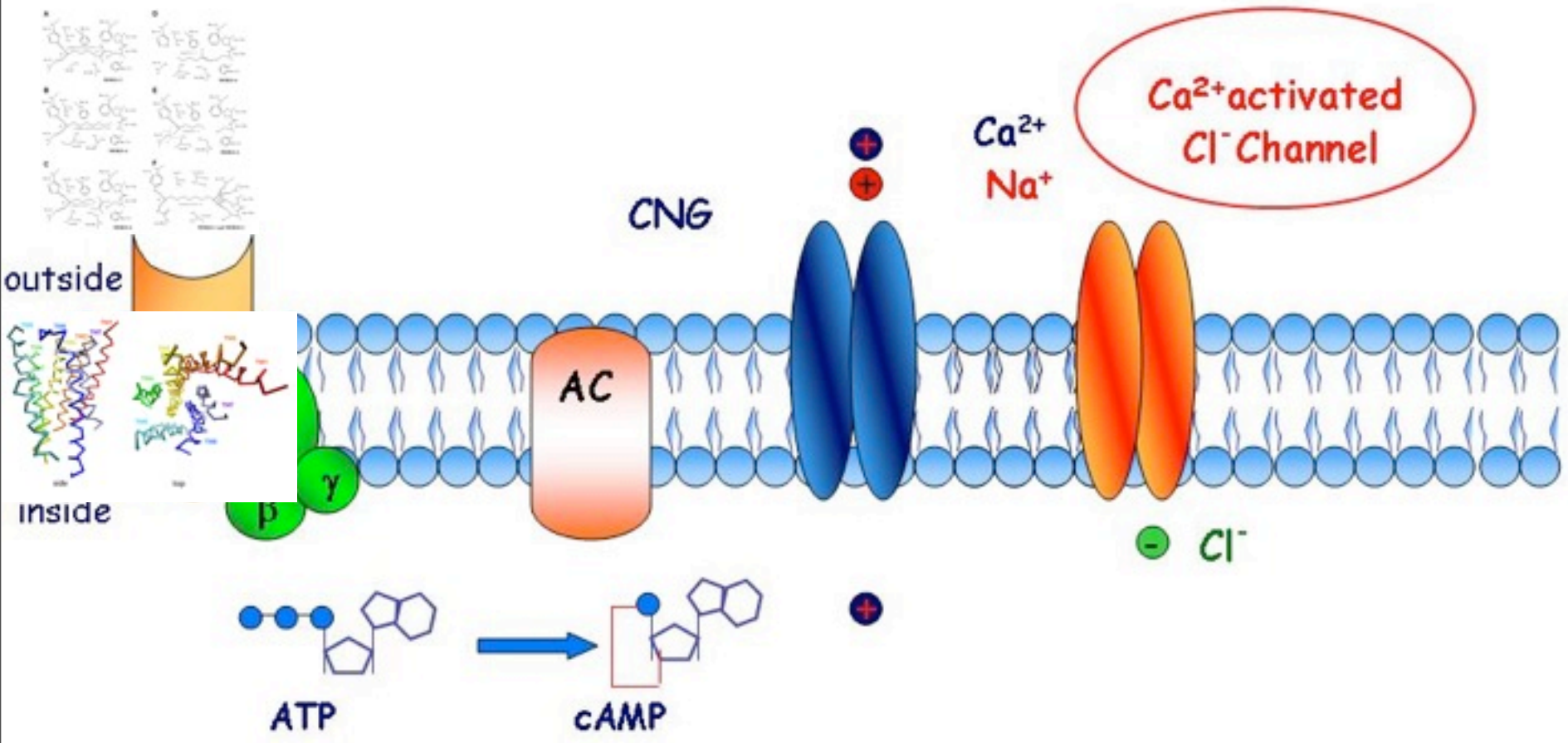
Signal transduction in the olfactory sensory neurons



Lupieri et al. HFSP Journal, in press

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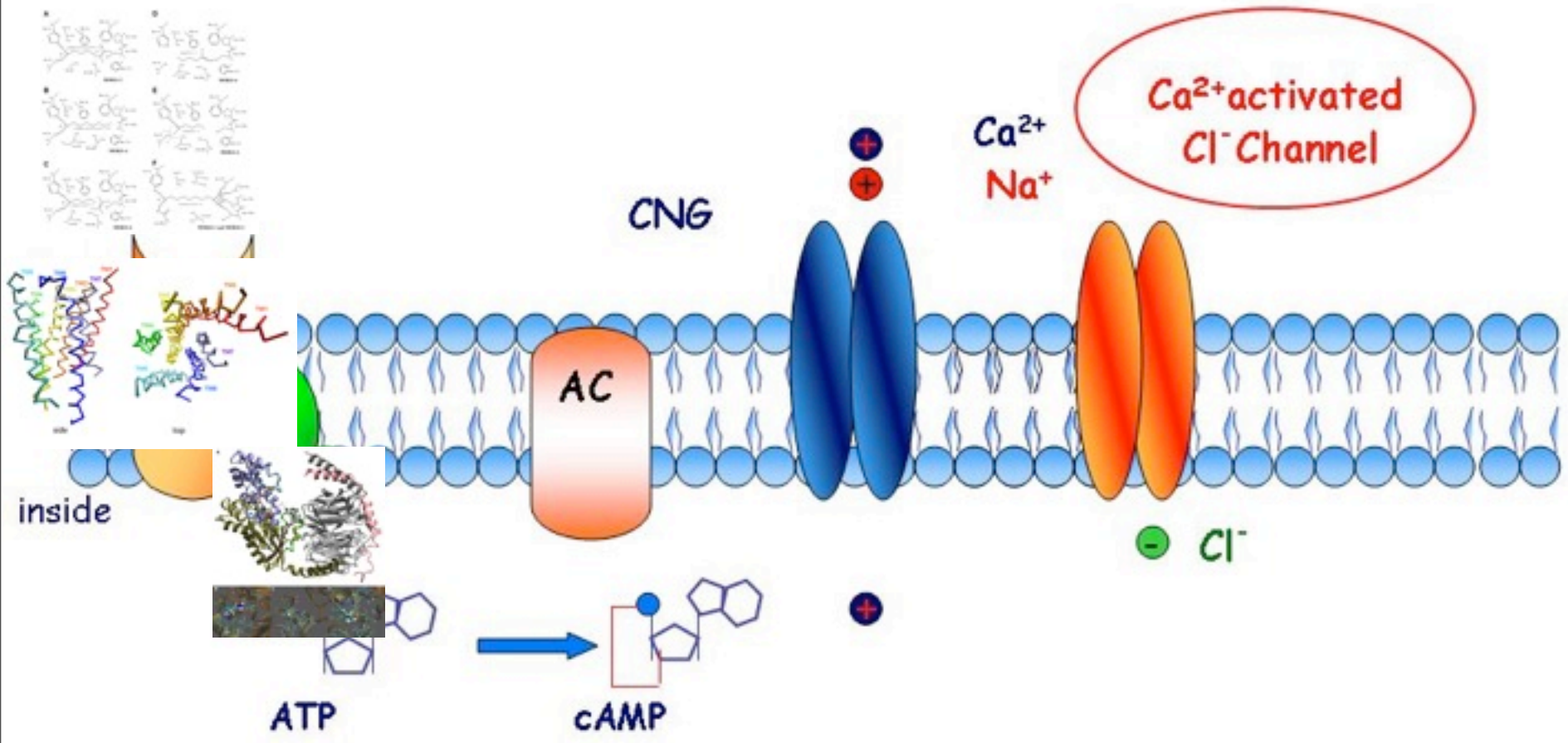

Khavizov et al. Proteins, 2008

Pifferi et al., FEBS Letters 2006

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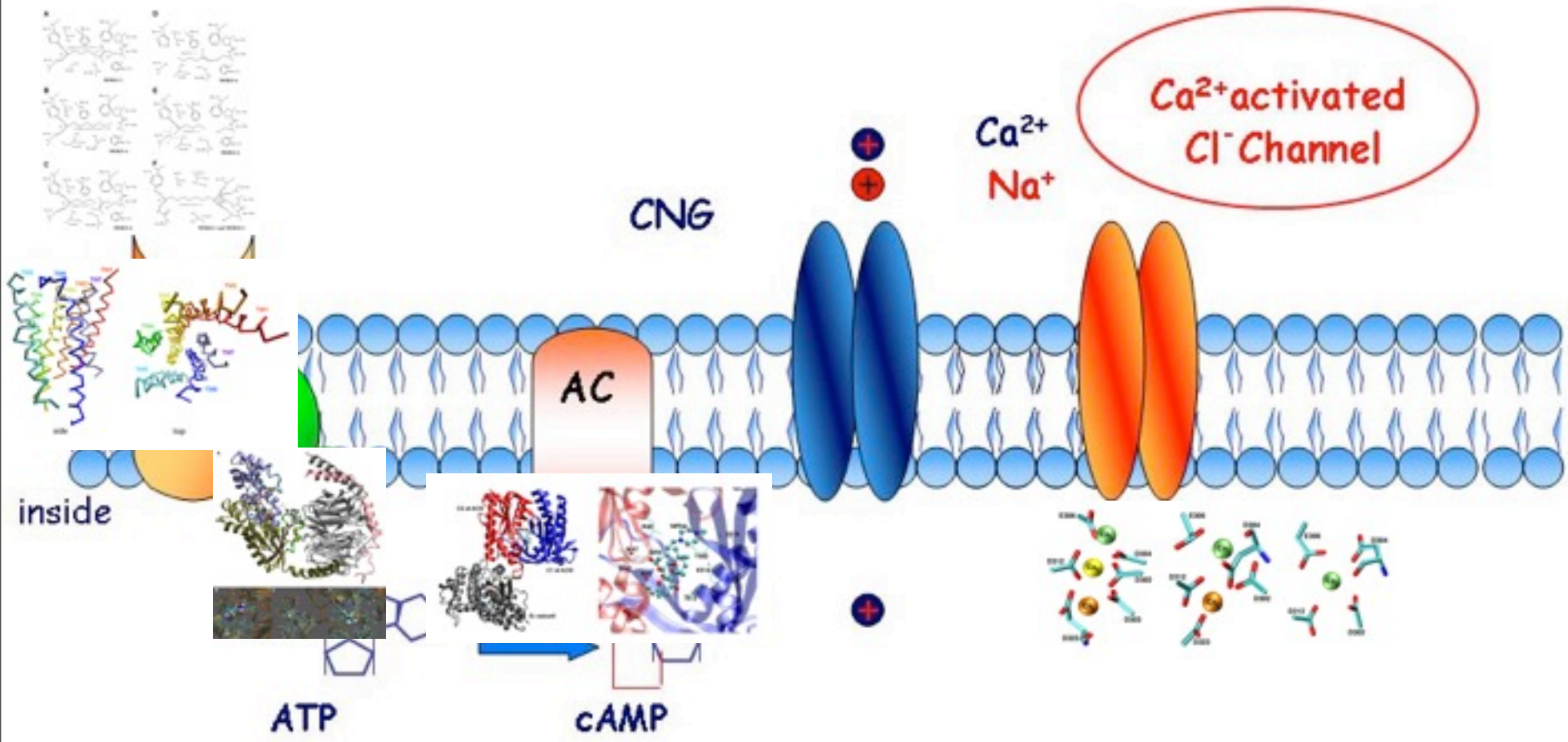

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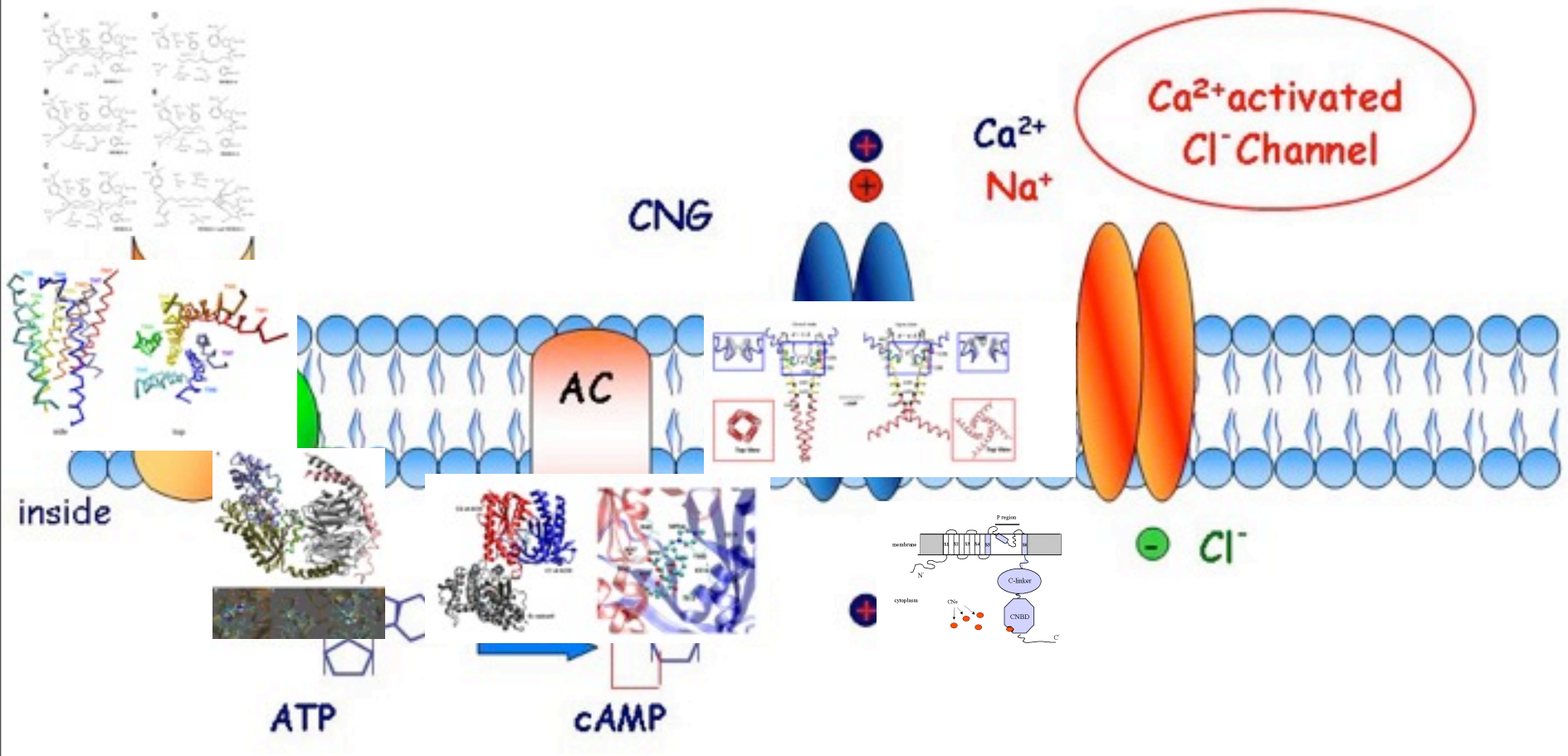

Giorgetti et al. Biophys. J., 2005.
Anselmi et al,, Proteins, 2007
Giorgetti et al., FEBS letter 2005

Berrera, Pantano, PC , Biophys J. 2006

Pifferi et al., FEBS Letters 2006

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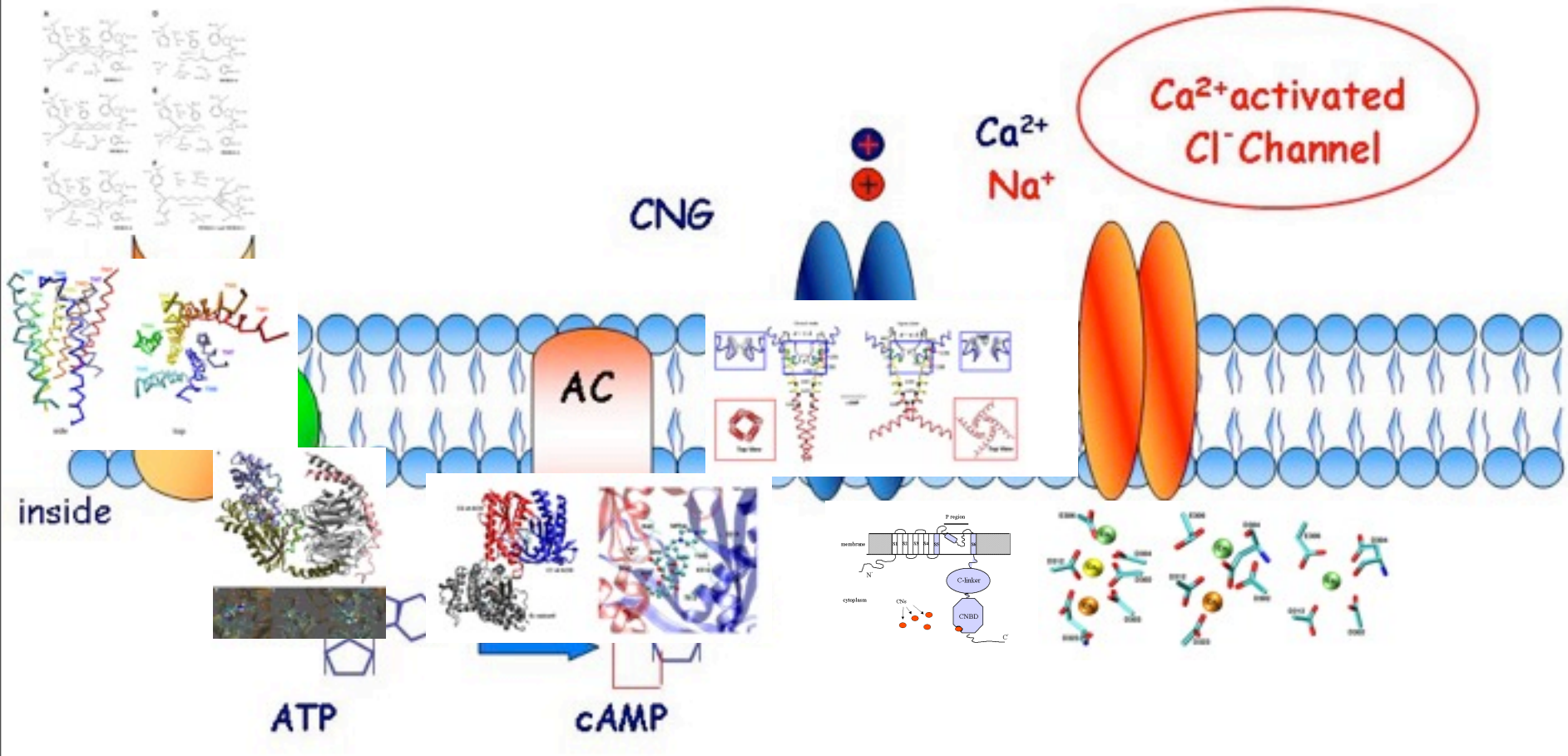
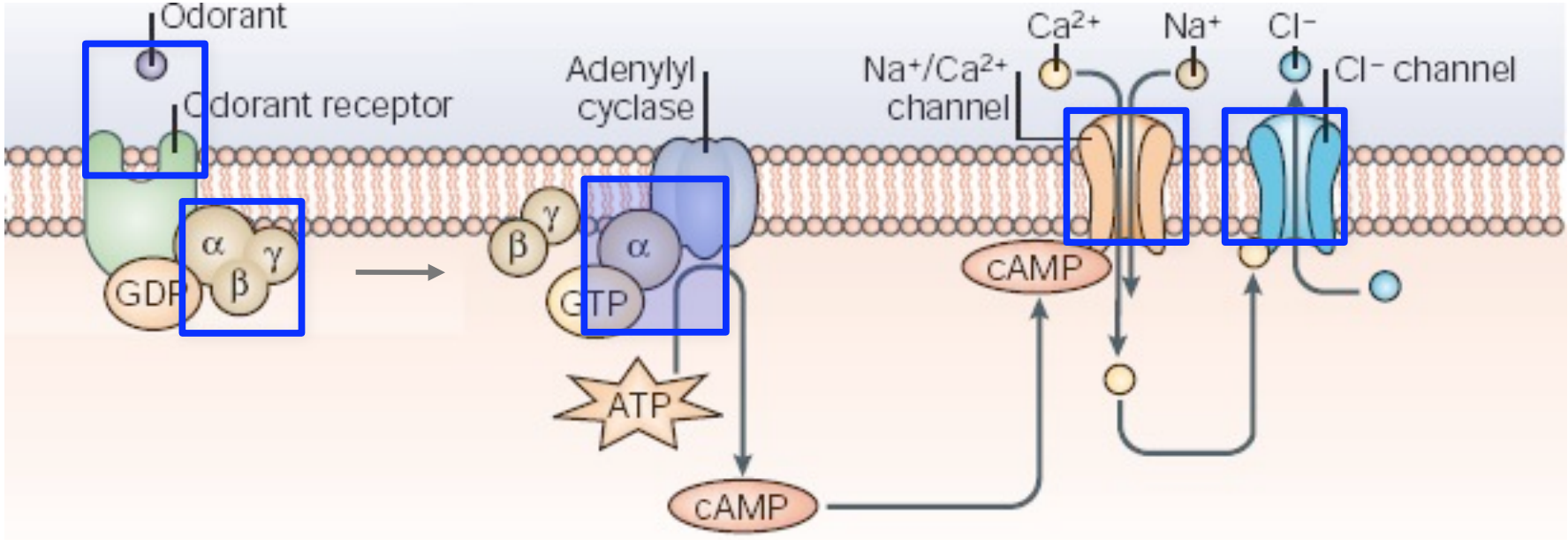


Kranjic et al. PLOS one, in press.

Pifferi et al., FEBS Letters 2006

Assembling the pieces of the puzzle



Peter Mombaerts, Nature Reviews Neuroscience 5, 263-278 (2004)

-Coarse-grained models, protein/protein docking,energetics of binding
-Physiological conditions for structure, function and regulation
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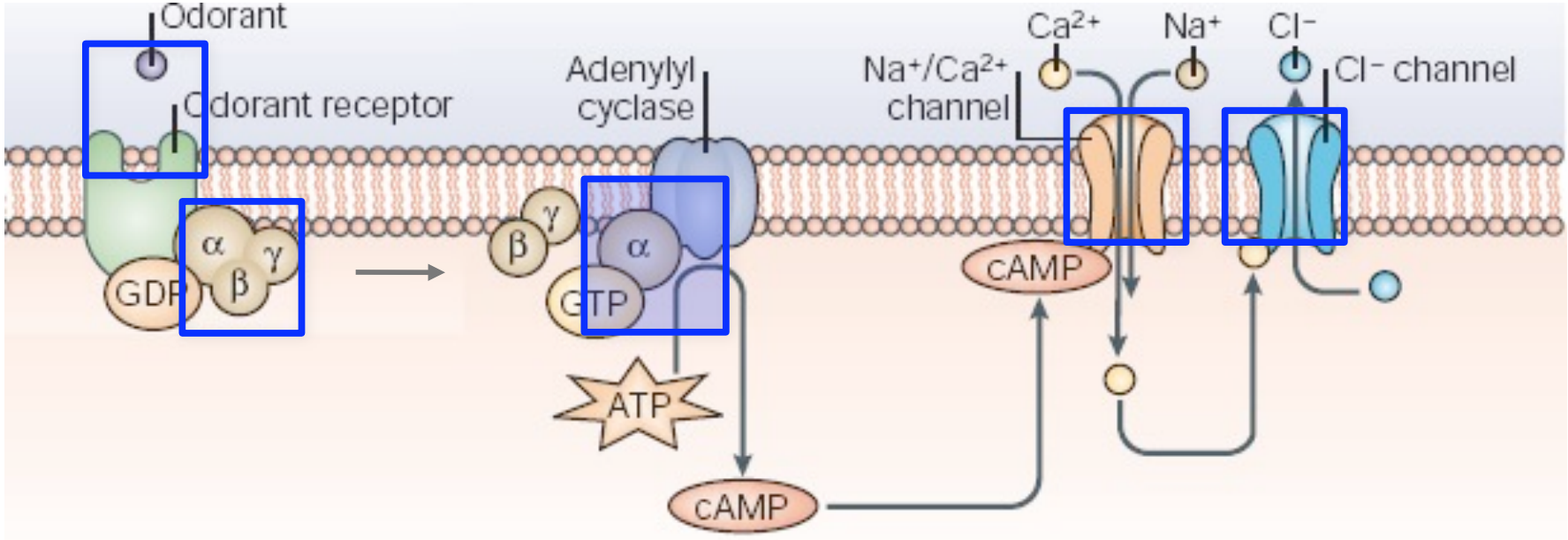

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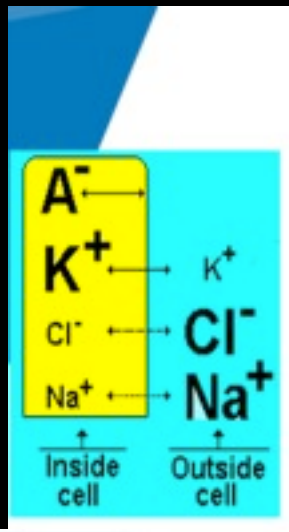
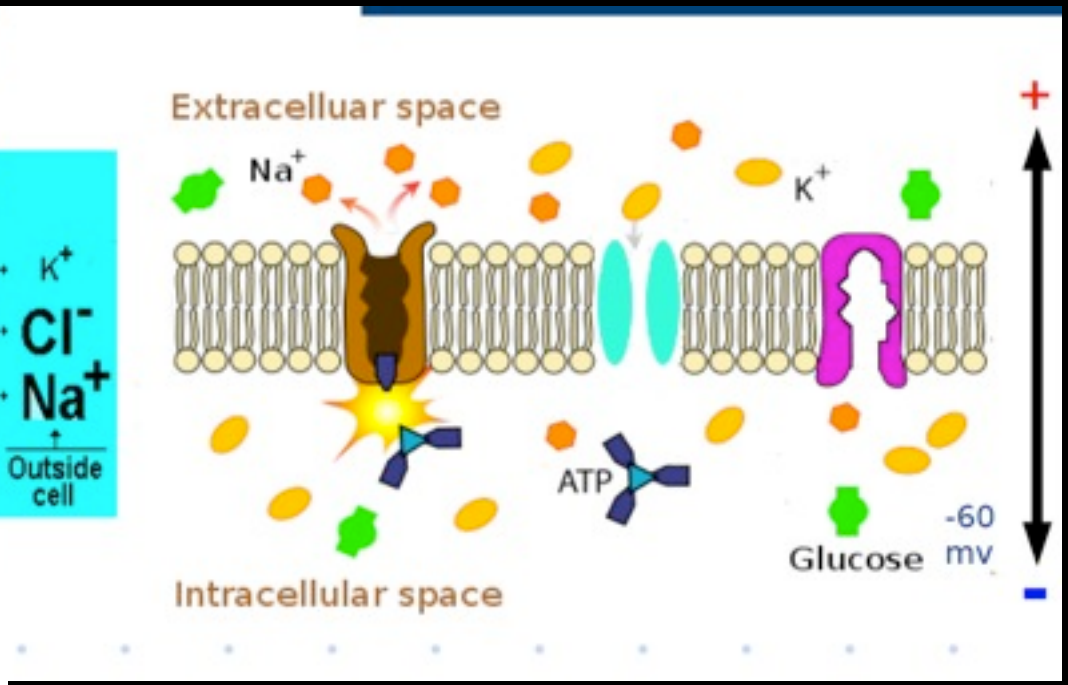
Towards a more realistic representation of the cytoplasm

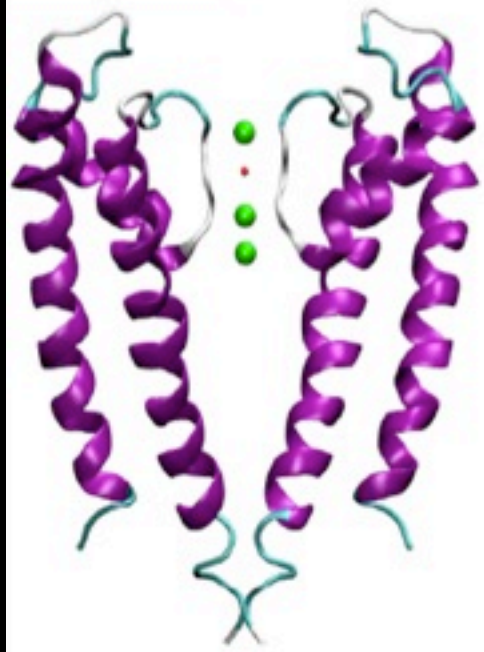


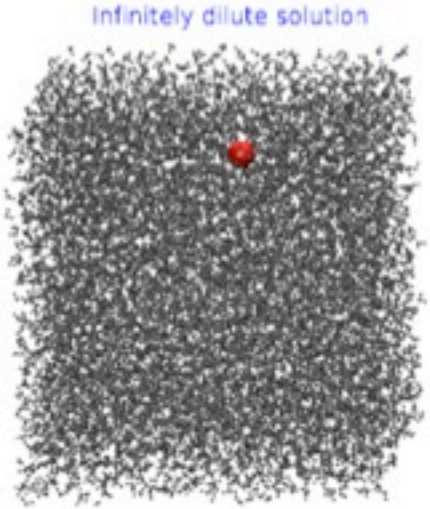


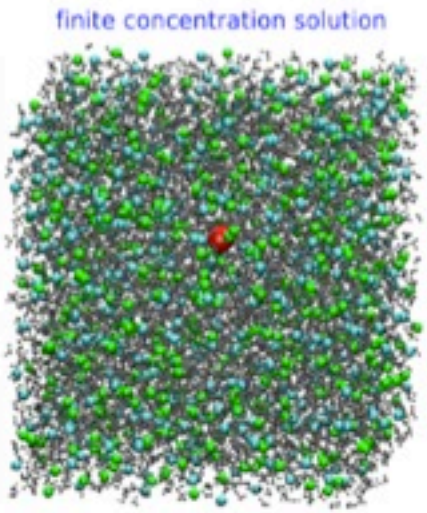
	Concentration, mM		
Ion	Plasma	Cytosol	Seawater
Na^+	135~146	25~35	480
K^+	3.5~5.2	130~145	10.4
Mg^{2+}	0.8~1.4	4~20	54
Ca^{2+}	2.1~2.7	<0.01	10.6
Cl^-	98~108	50~60	559
HCO_3^-	23~31	4~12	54
PO_4^{2-}	0.7~1.4	90~110	<0.1

Physiol. Rev. 86(2006), 1049

The missing factor





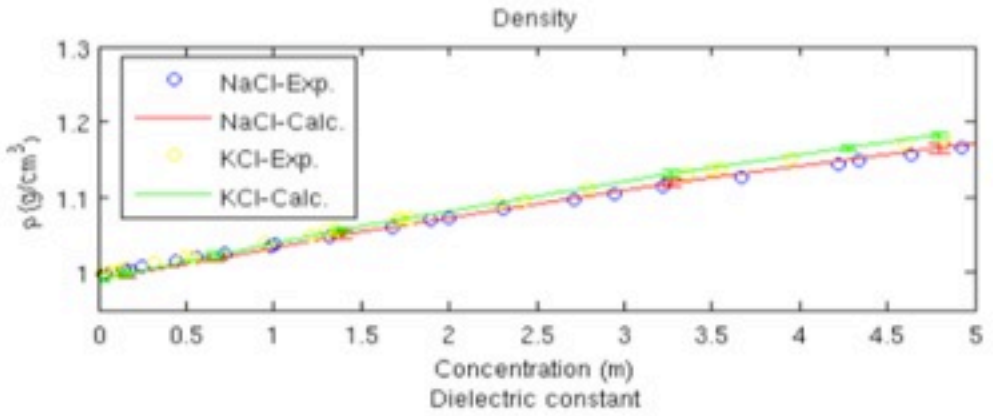


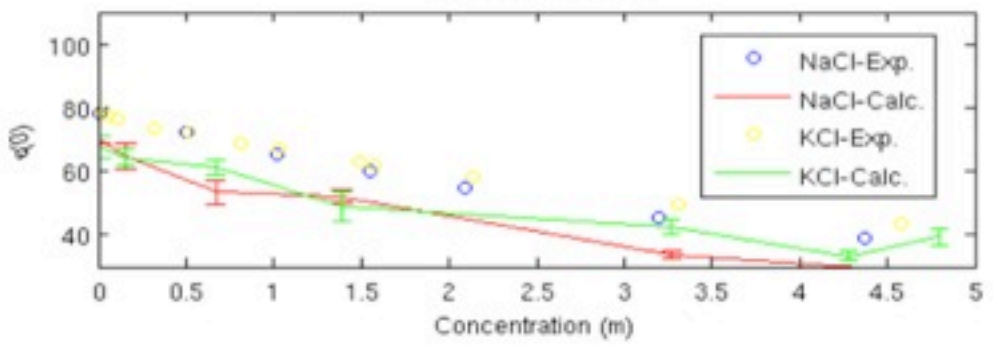
We have tested **AMBER**, CHARMM, OPLS, Dang95 parameter se
with TIP3P and **SPC/E** model. (~500ns, 20000 atoms)

+/-

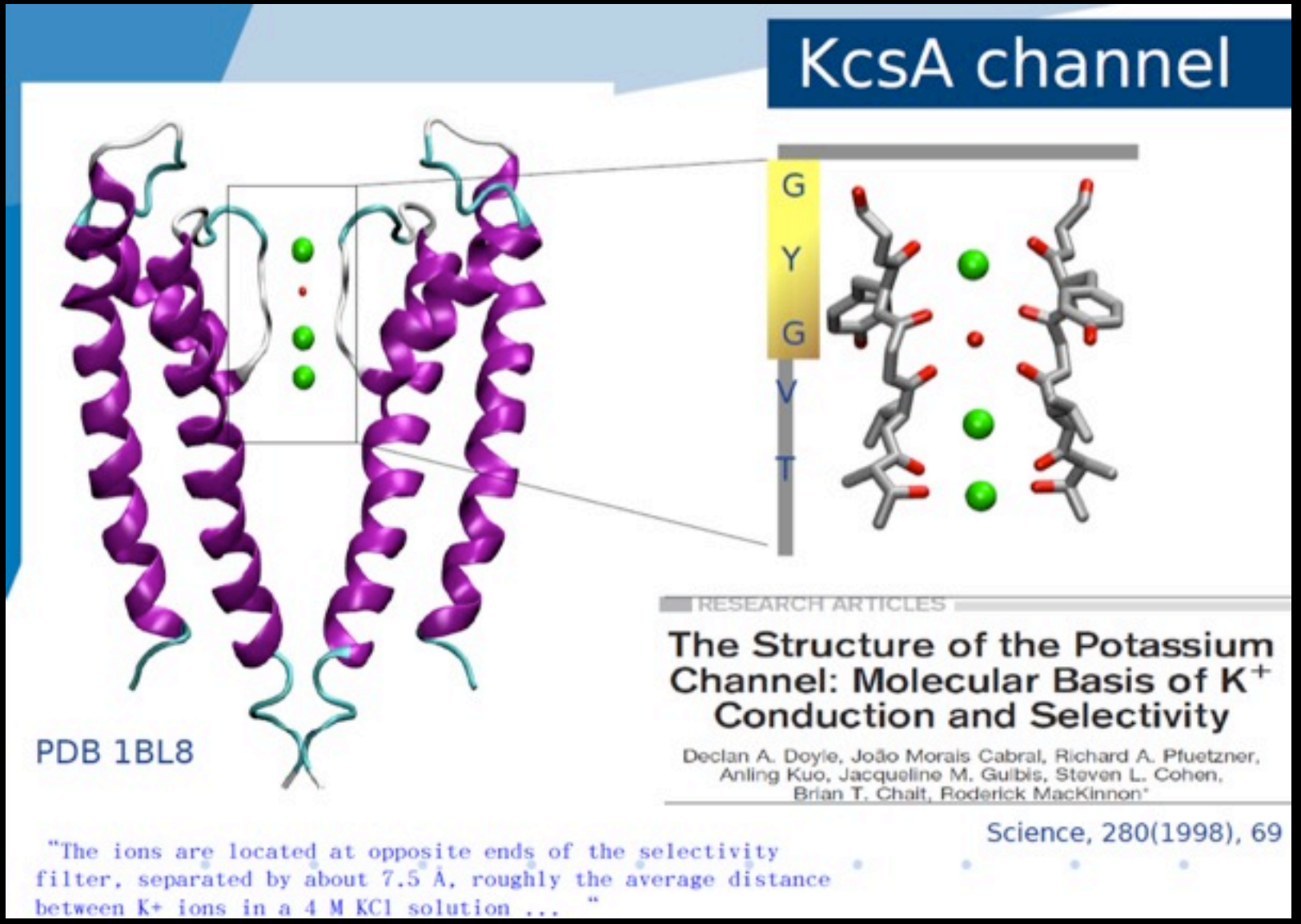
$$U_{ij} = 4\epsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] + \frac{q_i q_j}{r_{ij}}$$

Bulk properties





What about permeation?
Ionic strength



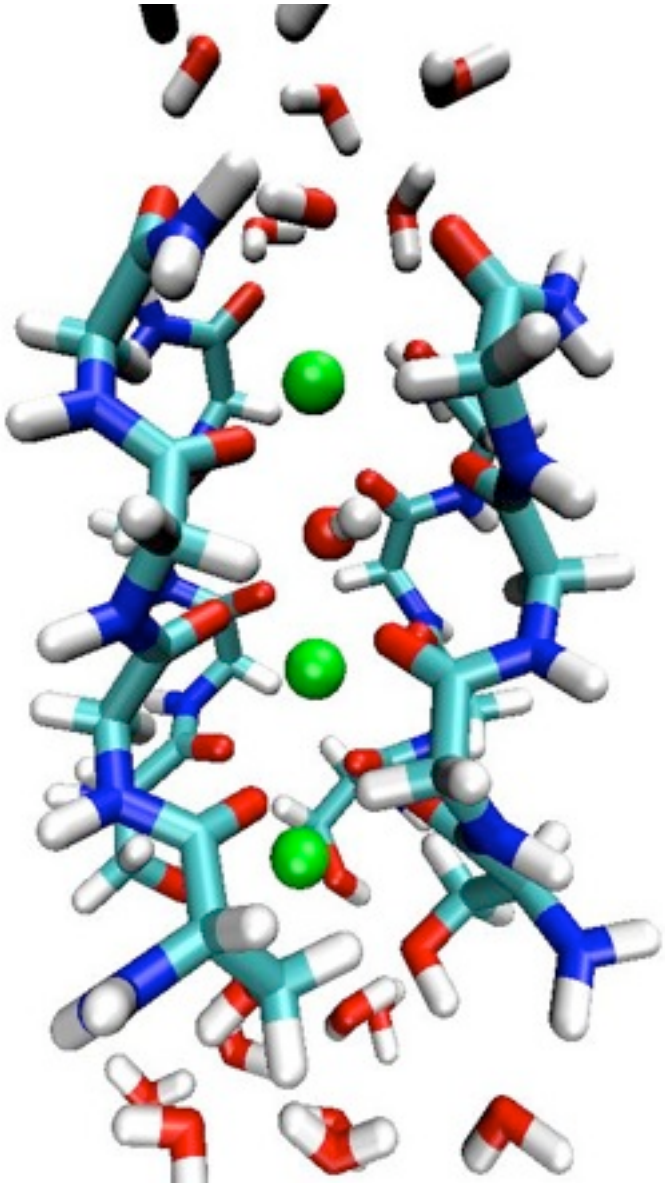
Charges averaged over 17 QM/MM conformations

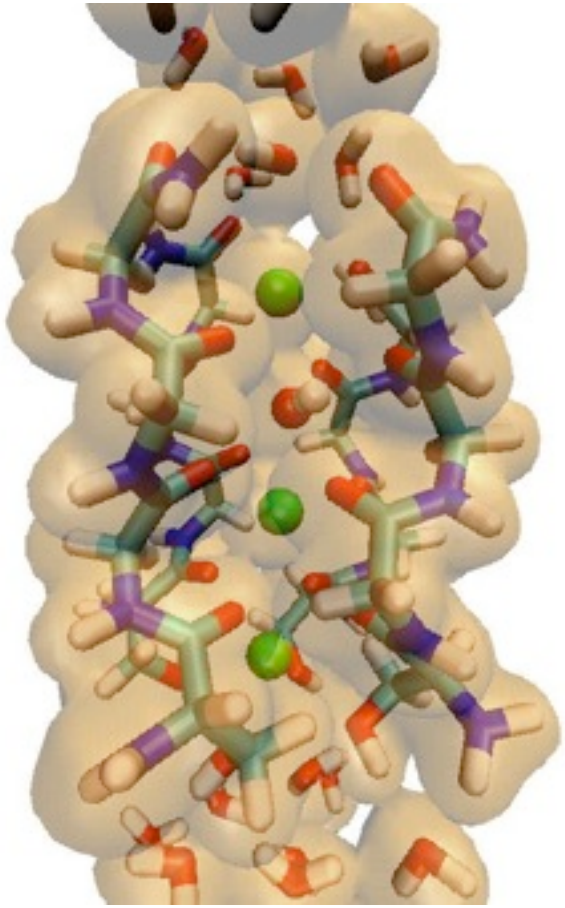
K1=0.91

Wat=0.03

K2=0.87

K3=0.90





Bucher D et al. Biophys. Chem. 2006

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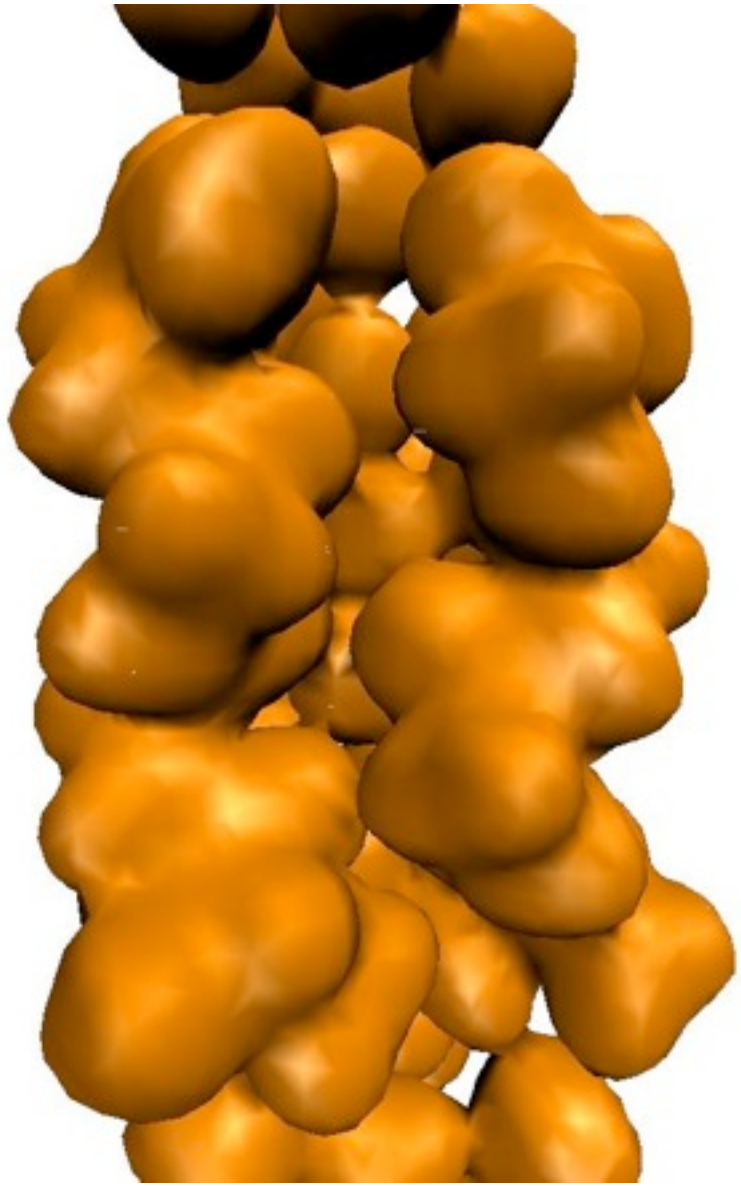
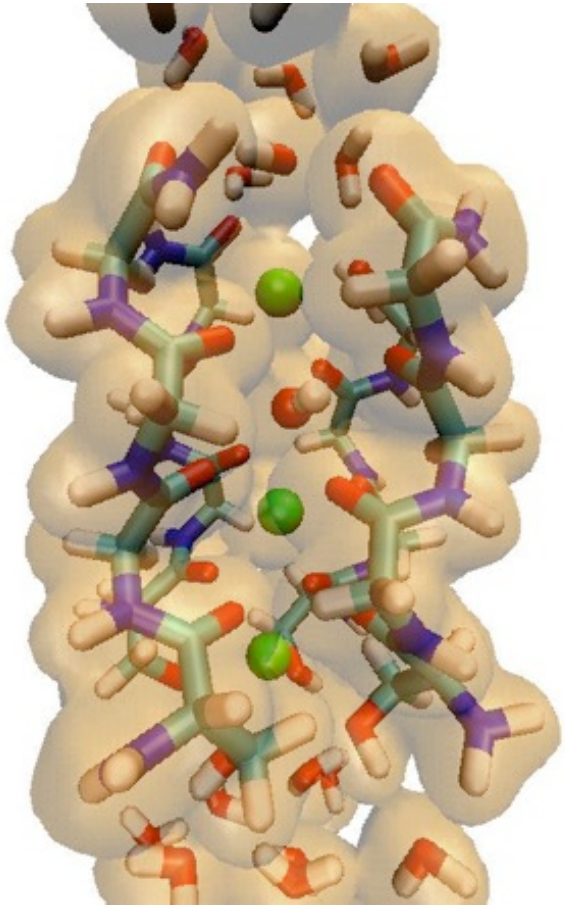
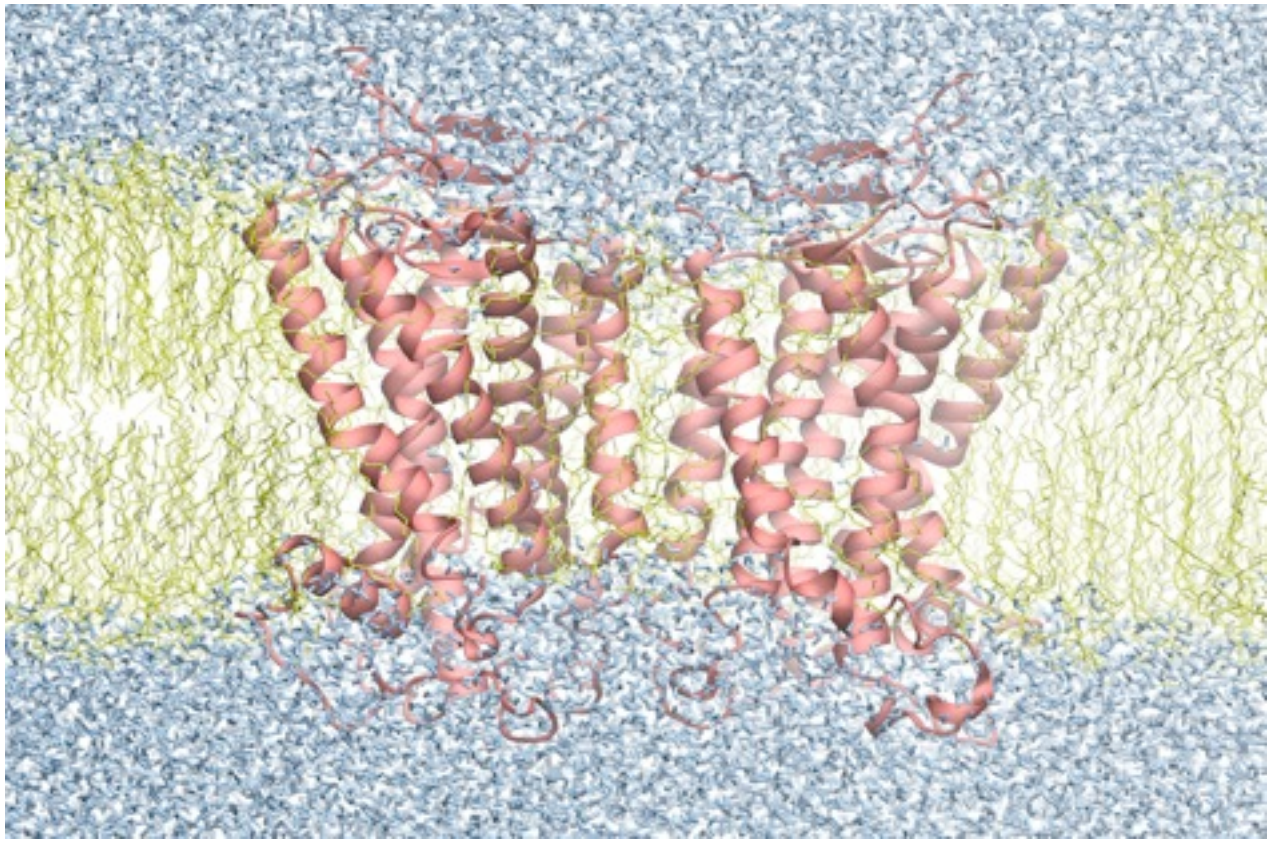




Bucher D et al. Biophys. Chem. 2006

What is the effect of voltage on entire system?

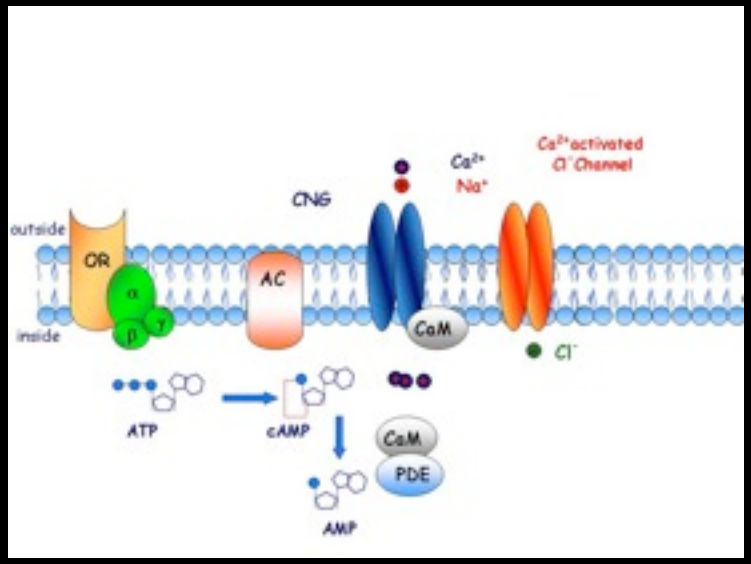


Shutting down the signal: calmodulin

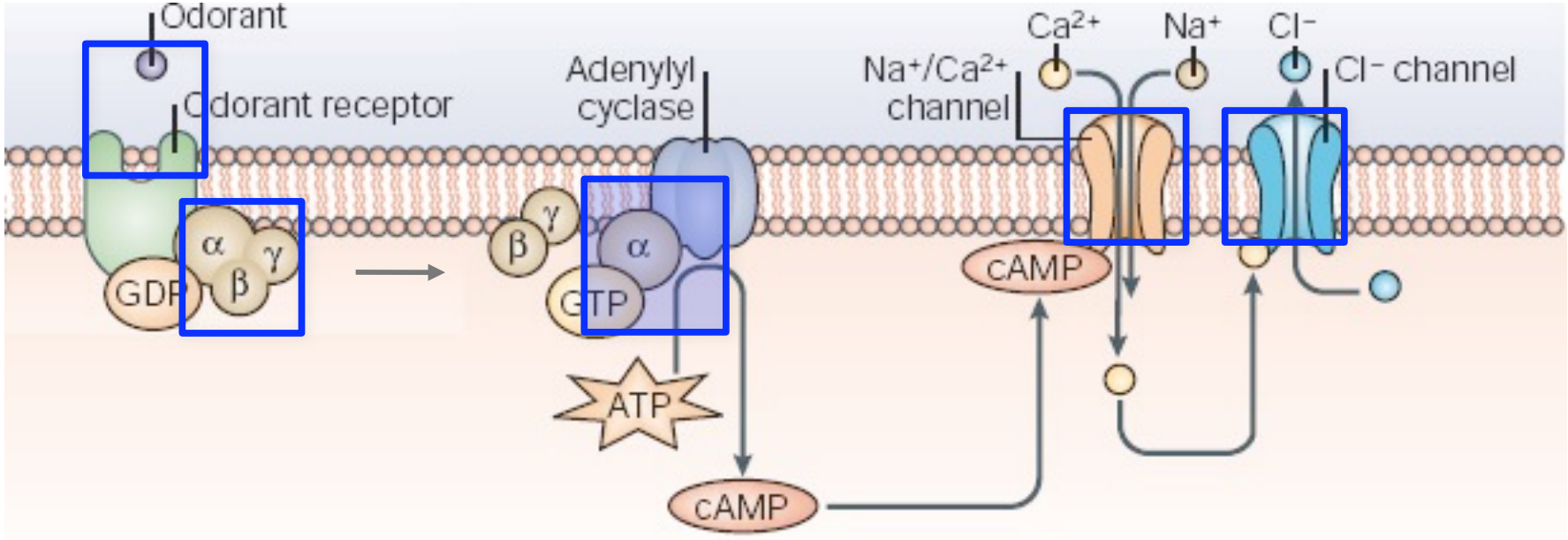
Increase of cytoplasmatic [Ca^{2+}]: excitatory and inhibitory effect by calmodulin (CaM) binding:

(i) Decrease open probability of CNG channels by binding to their N-term (Contessa et al., J. Biom. NMR 2005)

(ii) Cause cAMP → AMP by binding and activating CaM-dependent



Assembling the pieces of the puzzle



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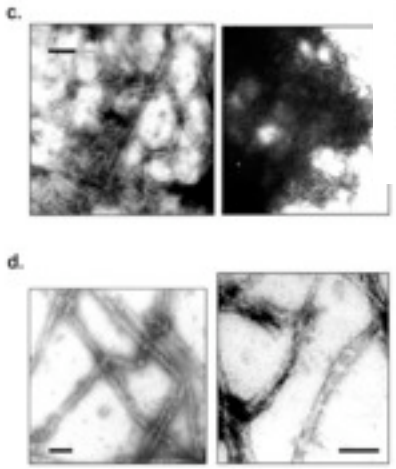
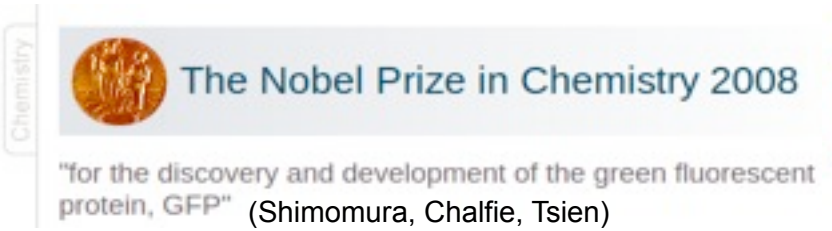
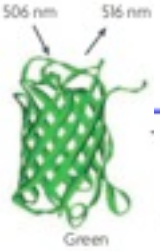
FLUORESCENT PROBES

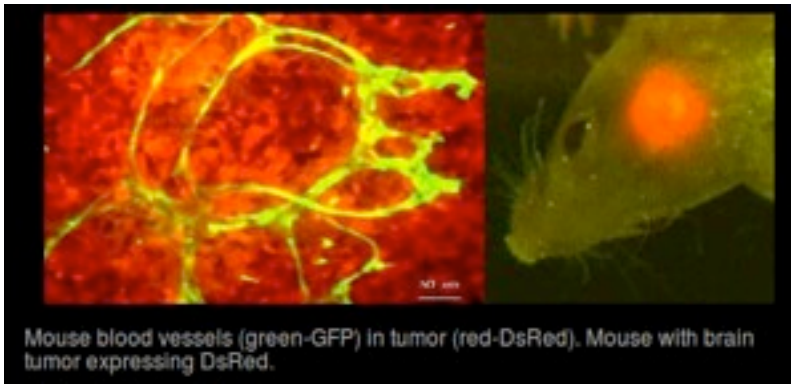
FOR CELL BIOLOGY AND STRUCTURAL BIOLOGY



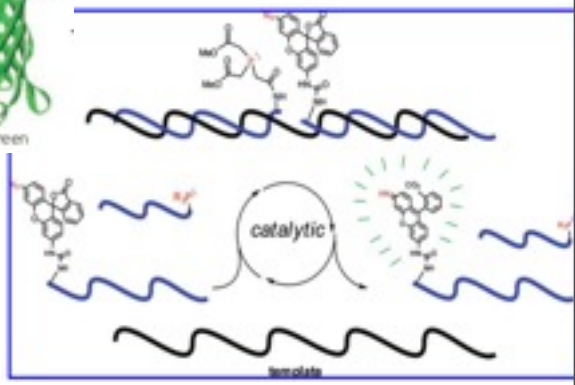
^ Fibrils formation from prion protein (Baskakov et al, J Biol Chem, 2002)



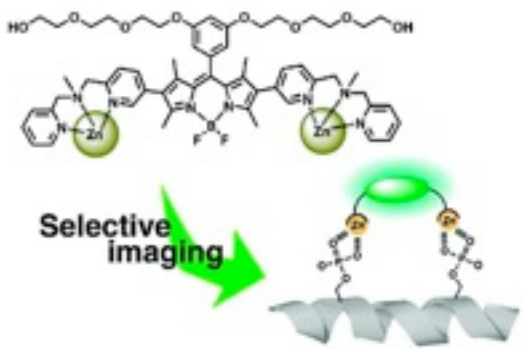


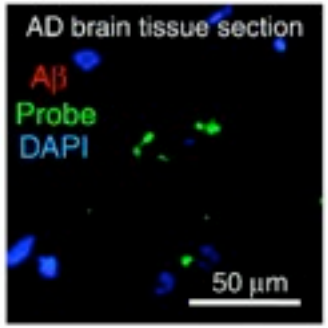


source: http://www.conncoll.edu/ccacad/zimmer/GFP-ww/GFP-1.htm



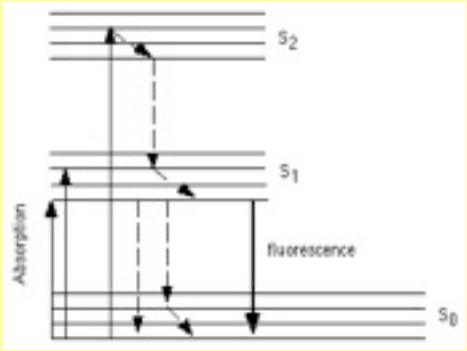
^ Imaging of mRNA in live cells via nucleic acid reduction of a rhodamine probe (Pianowski et al, JACS 2009)



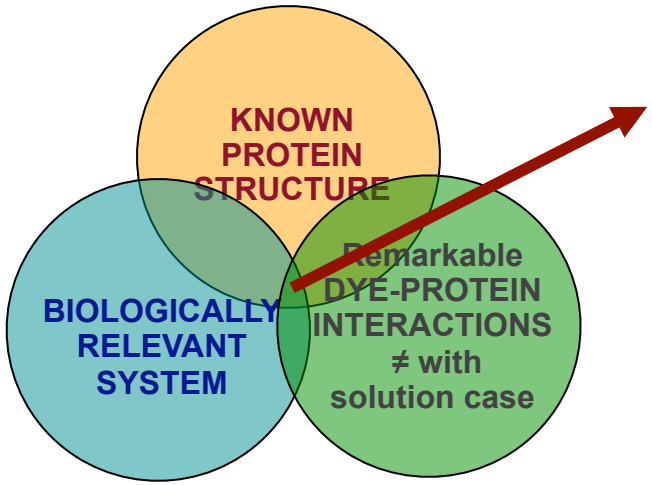


< Fluorescent BODIPY-based Zn^{2+} complex to detect neurofibrillary tangles in Alzheimer-affected brains (Ojida et al, JACS 2009)

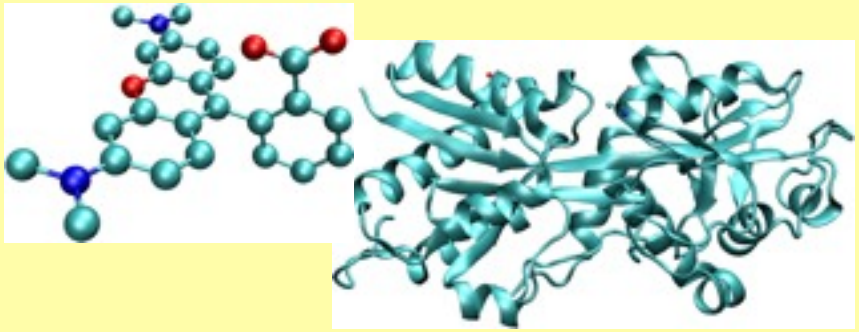
FLUORESCENCE IN A NUTSHELL:



Test system

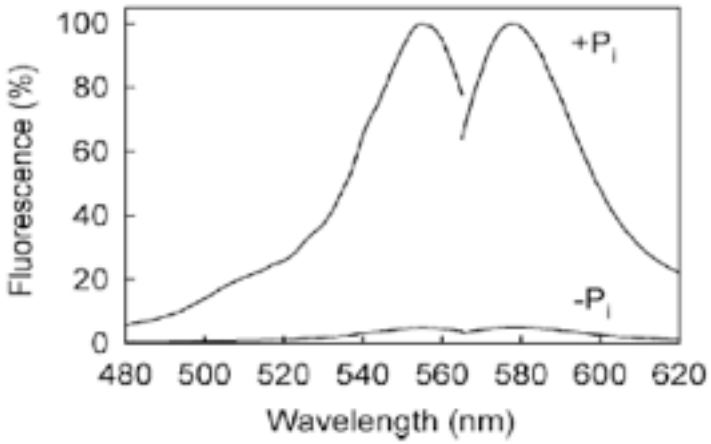


(A17C, A197C) PBP from *E. coli* labeled with 2 rhodamines



- Good probe for Pi:
  - K_d = 70 nM
  - 18-fold change of fluo intensity upon Pi binding
  - Linear increase of emission with increasing of Pi concentration
- System suitable for simulation
  - Protein coordinates known
  - ~ 5,000 atoms
  - Known spectral properties
- Experimental support:
  - Webb's group will do mutations designed by myself and perform NMR
- Biologically interesting
  - Pi release is a major assay target (ATPases, GTPase, phosphatases, ...)

Okoh et al., Biochemistry (2006)



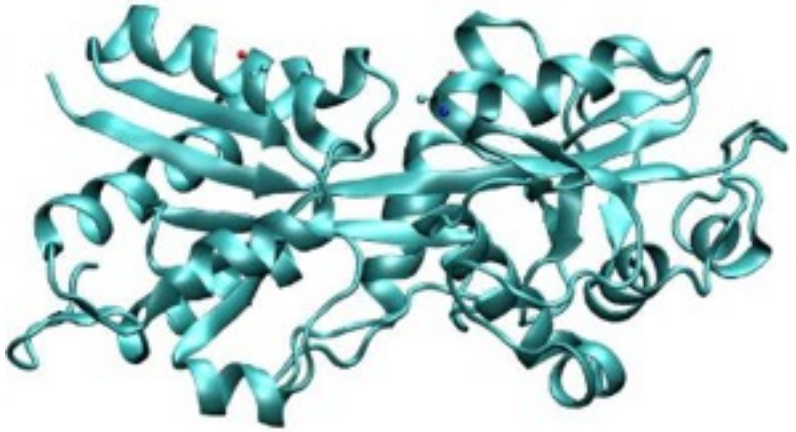
Paola Lupieri

The phosphate binding protein (PBP) as a sensor for phosphate (Pi)

PBP from *E. coli:*

- FUNCTION: scavenges the periplasm for Pi, transfers it to a membrane protein for transport into the cytoplasm.
- ACTIVATED and brought to periplasm in Pi-starvation conditions
- CONFORMATION CHANGE upon Pi binding

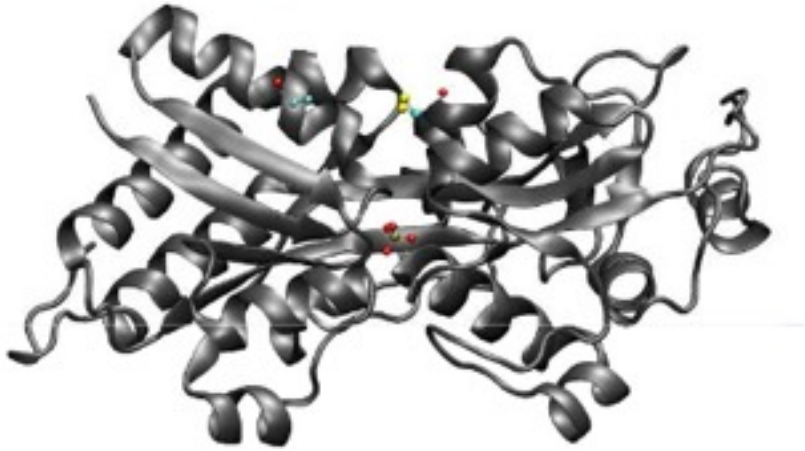
Luecke and Quiocho, Nature (1990);
Brune et al., Biochemistry (1998)



As a probe for Pi :

- MONITOR Pi RELEASE
- Fluorescent labeling exploiting conformation changes: **SWITCH**
- INNOVATIVE STRATEGY: **2 DYES**

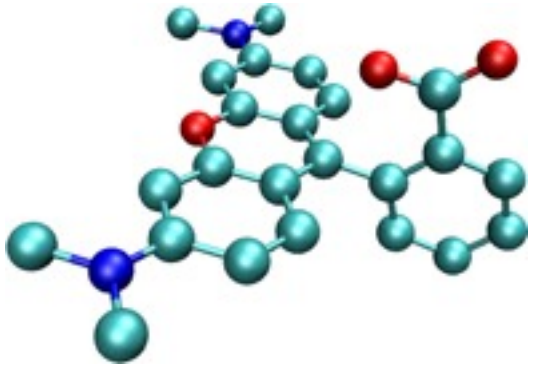
Okoh et al., Biochemistry (2006)



Rhodamines stacking & fluorescence

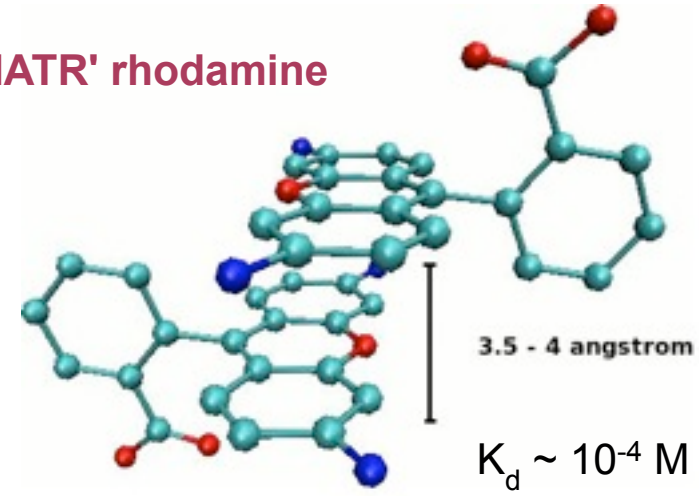
Structural data about rhodamines from X-ray
(Abrams et al, Inorg Chem (1986))
and NMR (Ilich et al, Spectrochimica Acta 1994):

2 x



≈

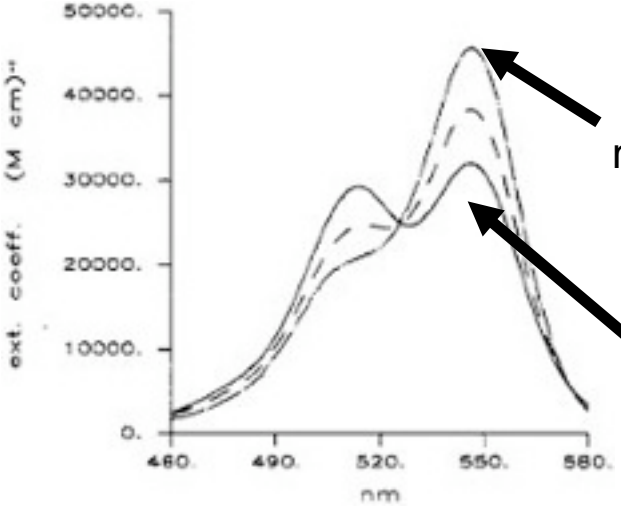
6-IATR' rhodamine



In WATER SOLUTION

$K_d \sim 10^{-4}$ M

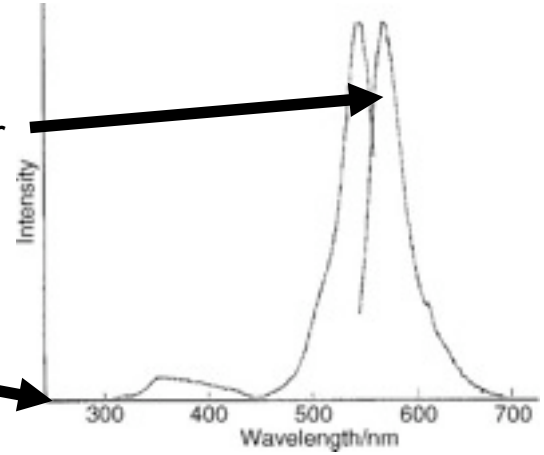
Absorption spectrum:



monomer

dimer

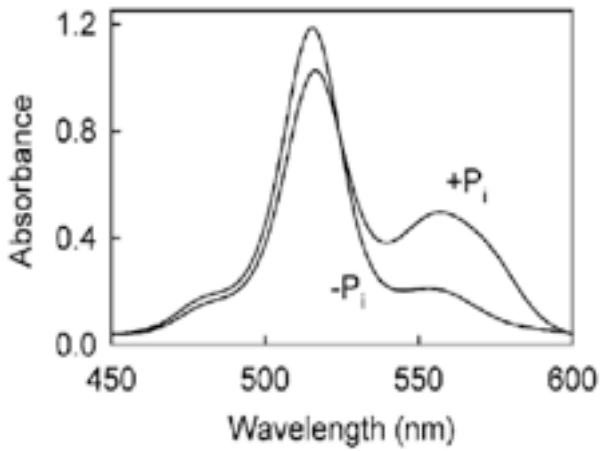
Emission spectrum:

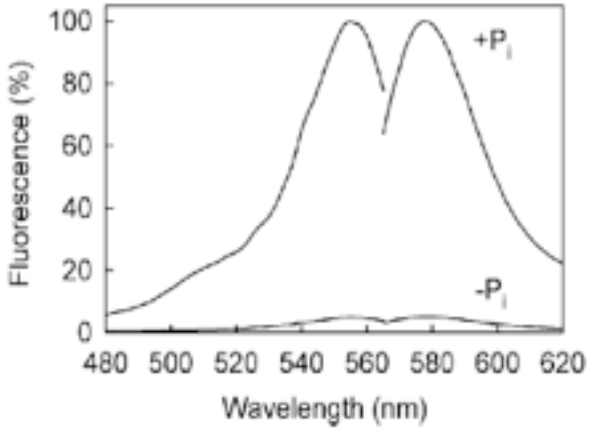


DIMER is NON-FLUORESCENT and has blue-shifted ABSORPTION w.r.to the monomer

Ajtai et al, Biochemistry (1992), Van Zandvoort et al, PCCP (1999)

In the PROTEIN SCAFFOLD

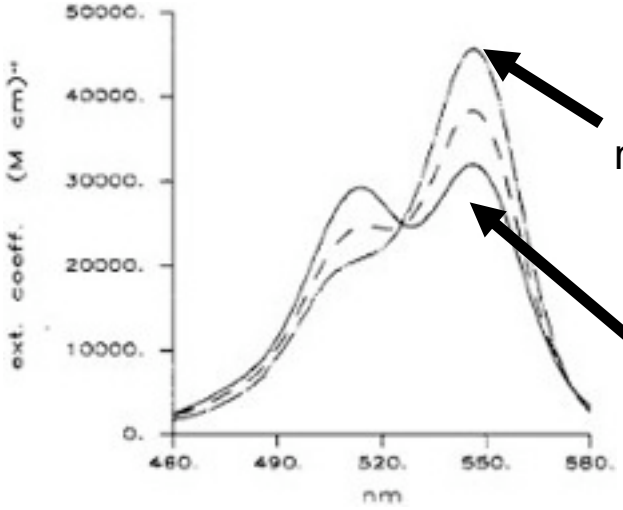




- The -Pi complex conformation CAN HAVE a dimeric-like conformation of rhodamines, DISRUPTED in the +Pi conformation
- The disrupted dimer MUST keep some interaction btw the rhodamines: +Pi and -Pi structures are likely to have dimer character but spectra more complex than "solution" spectra

In WATER SOLUTION

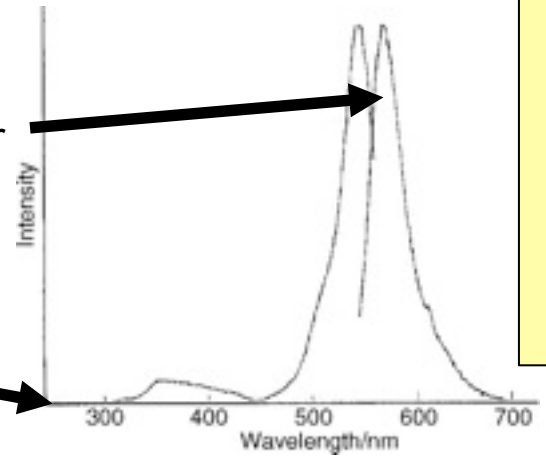
Absorption spectrum:



monomer

dimer

Emission spectrum:



NO STRUCTURE OF PBP/2-RHOs COMPLEXES: NEED FOR MOLECULAR SIMULATION

Ajtai et al, Biochemistry (1992), Van Zandvoort et al, PCCP (1999)

Validation of protocol

Dock the RHOs on the mutated structures of PBP:
monomeric/dimeric-like geometry

Classical MD of the obtained complexes
in a simulation box of water, NPT conditions

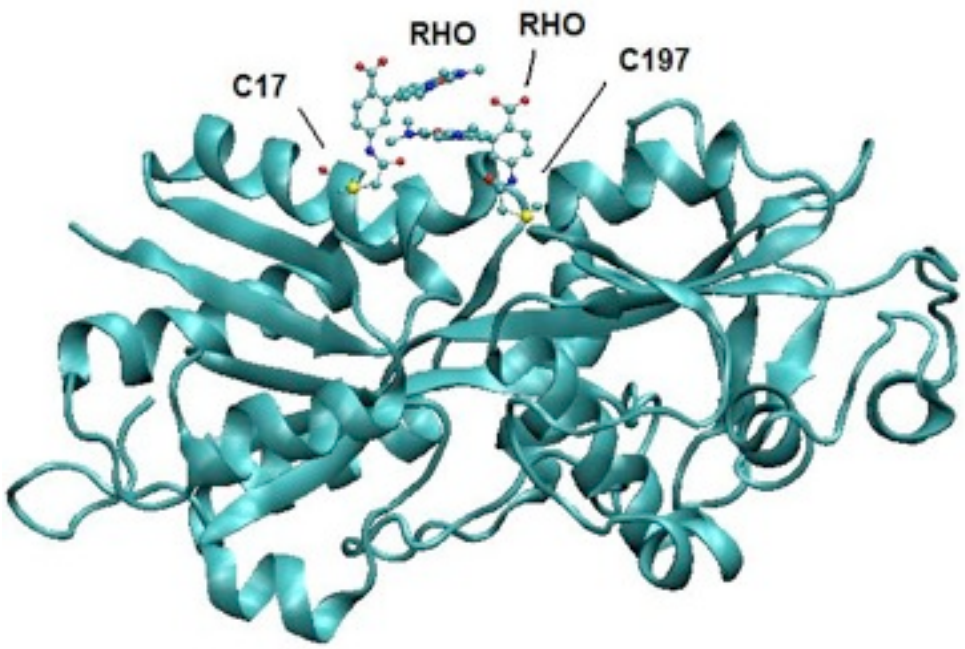
Extract snapshots clustering structures

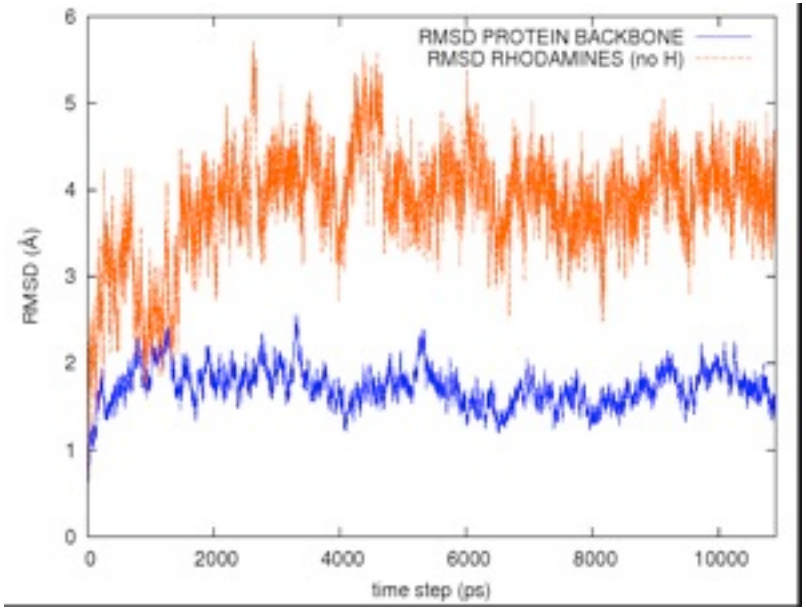
QM/MM dynamics

Calculation of optical properties
with TD-DFT, GW-BSE, ...

COMPARISON WITH EXPERIMENTAL DATA FOR VALIDATION
spectra in water and on the protein, structure in water, ...

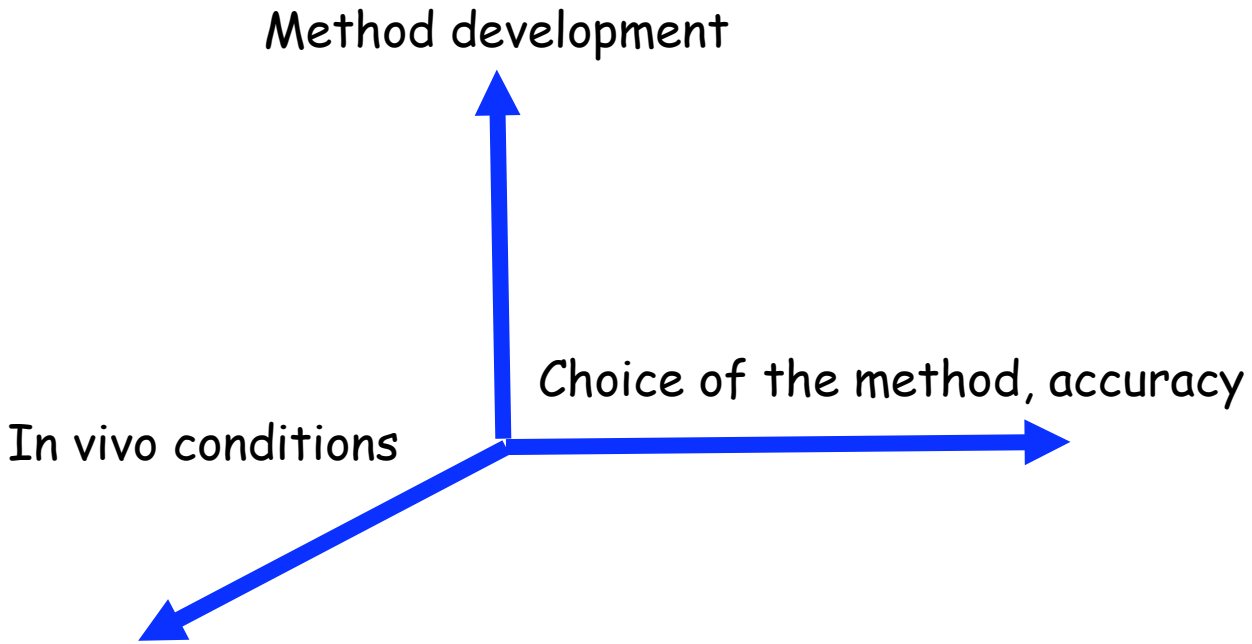
- **Stable trajectory (~10ns) from classical MD of PBP/2-RHO without Pi from a NON-CONVENTIONAL dimer conformation**





- The same conformation in solution DID NOT produce a STABLE trajectory: **PROTEIN STABILIZATION OF RHODAMINES' CONFORMATION**

Expanding the scope of Biomolecular Simulation



Acknowledgments

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