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Conference on 2nd Drug Development for the Third World: From Computational Molecular Biology to Experimental Approaches

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Forces and Energies

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Forces and Energies

Lecture notes for Trieste

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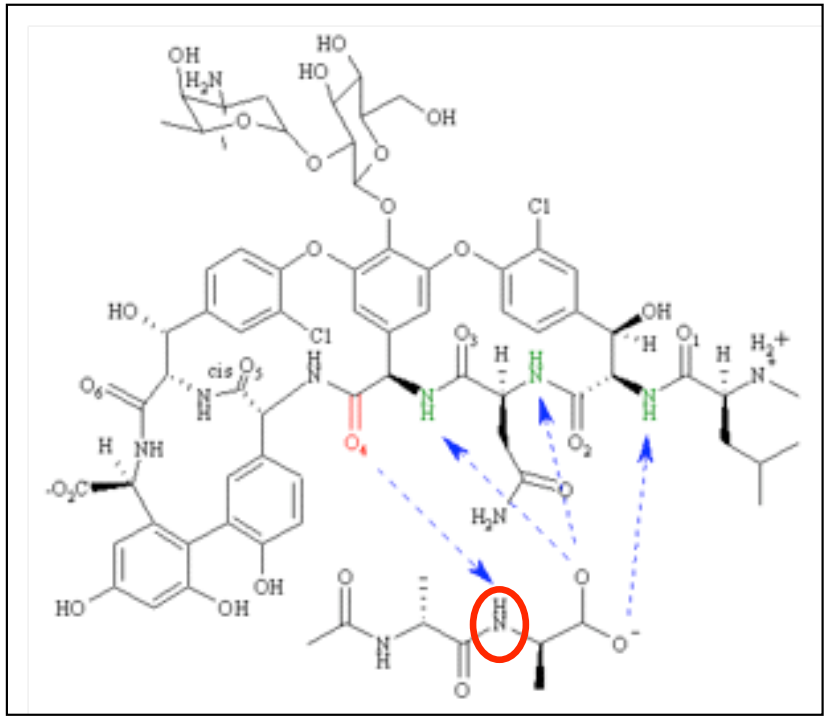
Contents

1. Introduction: typical drug design questions (1)

- Bacterial resistance to vancomycin:
  lactate instead of D-Ala to build the cell wall

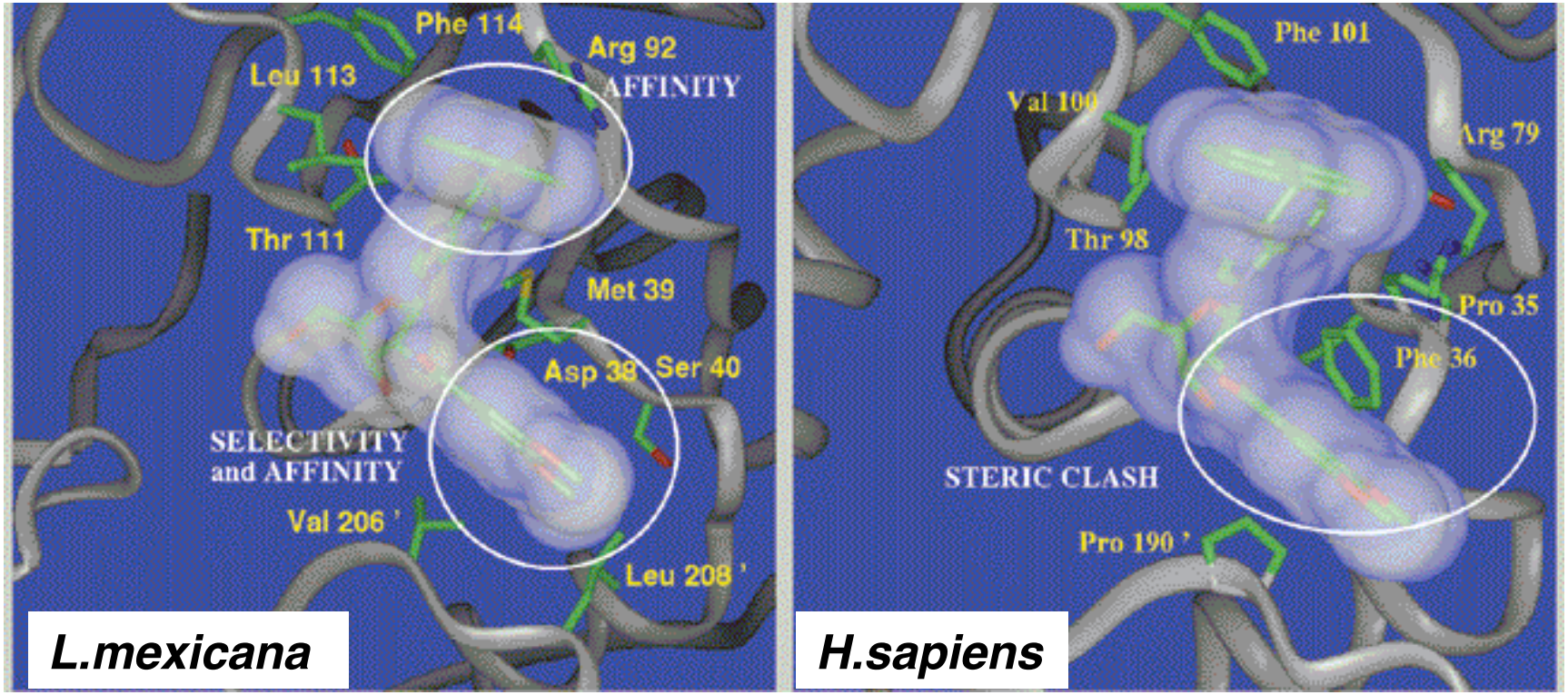


Predicted drop in affinity? (answer: 1,000 x)

Angew. Chem. Int. Ed. 42: 730-65

Introduction: typical drug design questions (2)

- Design a potent and selective inhibitor of *L.mexicana* GAPDH starting from adenosine

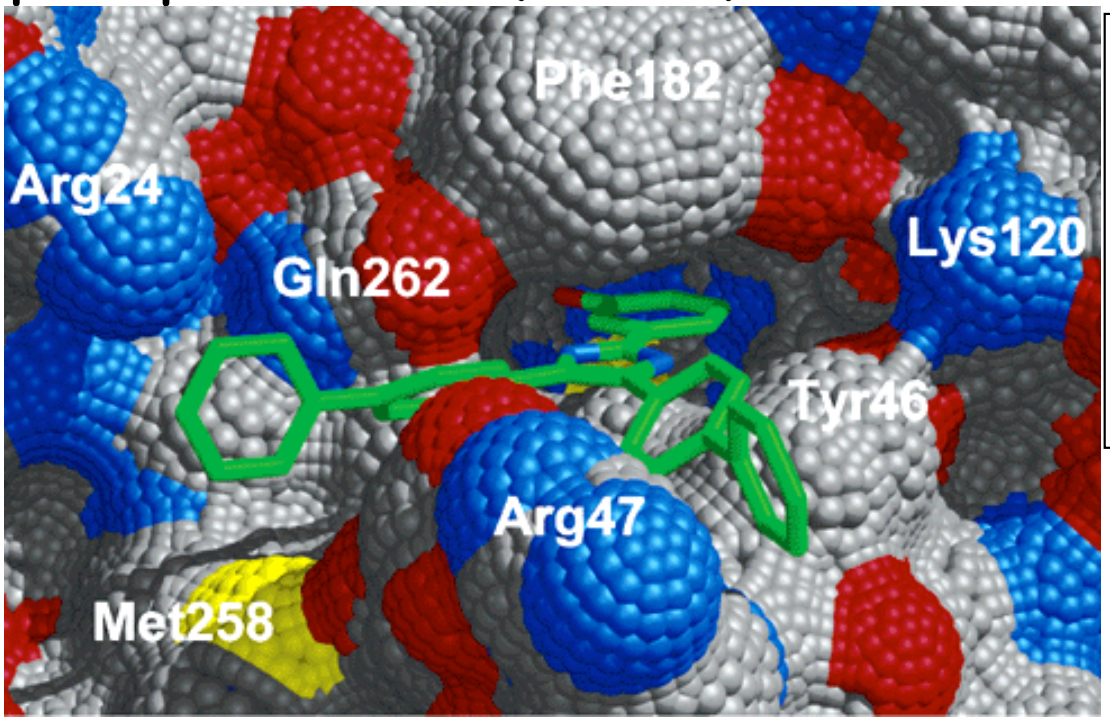


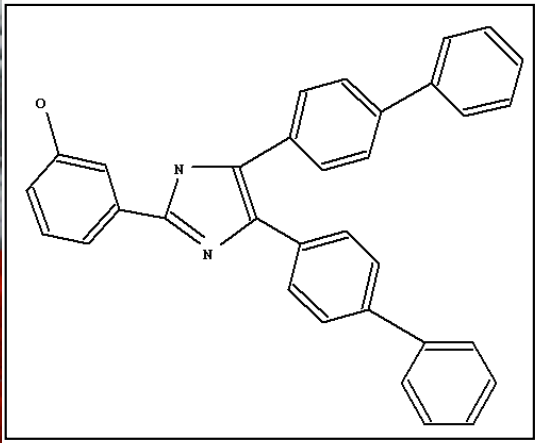
Answer: add naphtalenemethyl to N6 and 3-methoxybenzamido to C2' . Affinity x 10^5

PNAS USA 96: 4273-4278

Introduction: typical drug design questions (3)

- Dock 400,000 available molecules to protein phosphatase B (PTP-B) to find new inhibitors.





Result: 10 μM inhibitor

J.Med.Chem. 45: 2213-2221

Introduction: typical drug design questions (4)

To address the previous questions we need to examine the following relationships:

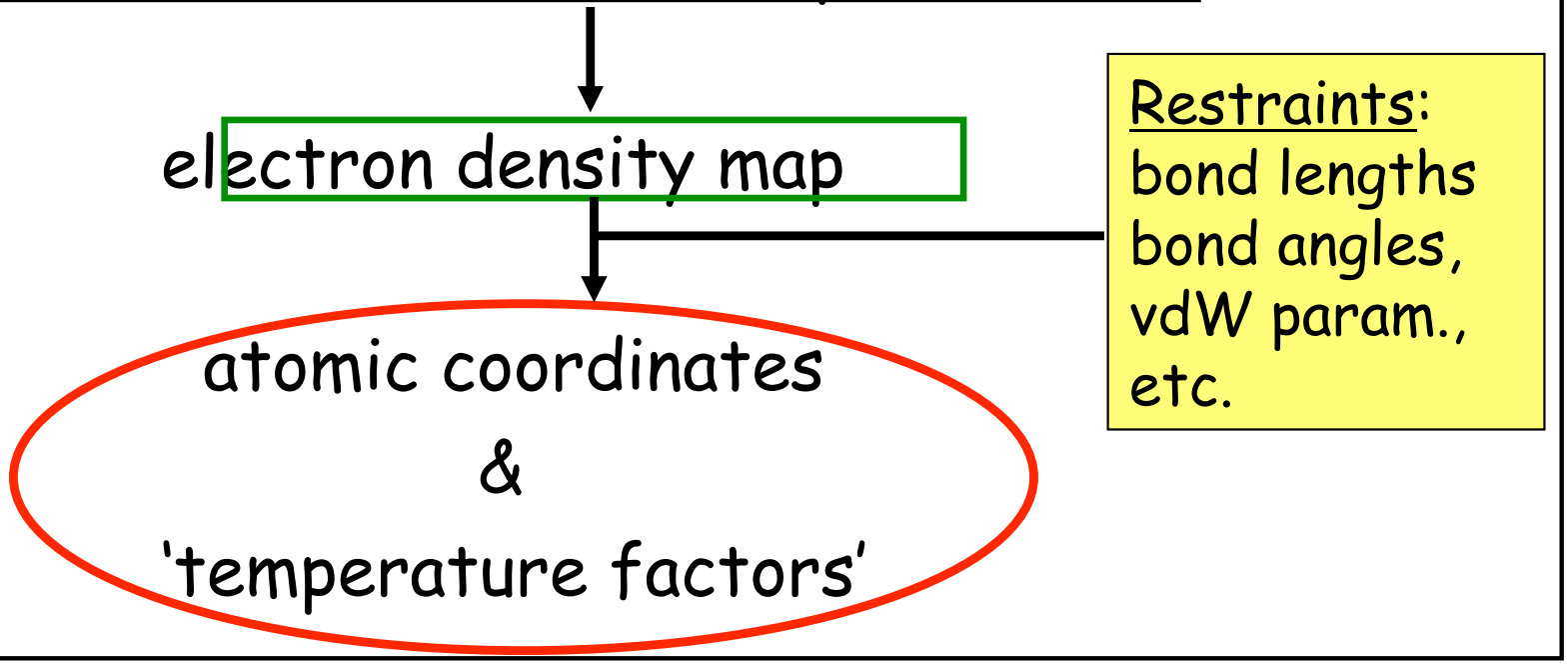
structure	↔ forces ↔	energy	↔ affinity
x,y,z		ΔG $\Delta H, \Delta S$	K_d

2. Structure

Three techniques produce structures for drug design: Crystallography, NMR, and Comparative modeling.

2.1 X-ray crystallography

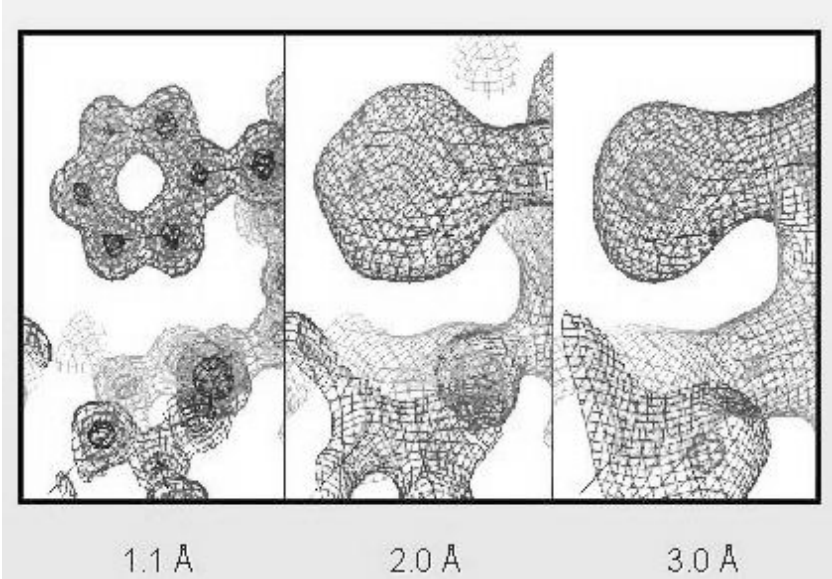
Principle: diffraction intensities + phase info



X-ray crystallography (cont.)

Issues:

- Resolution: e.g. Phe at 3 levels



- *R*-factor (R_{work}): $R = \sum \left| |F_o| - |F_c| \right| / \sum |F_o|$

  ok if $R \leq$ resolution/10 ('law of Drenth')
- Free *R*-factor: calculated from F's left out

  ok if $R_{free} \leq R + 0.05$

X-ray crystallography (cont.)

- Coordinate uncertainty: ~ 0.2 Å
- B-factors: spherical / ellipsoidal Gaussian model for harmonic motion

$$B = 8\pi^2 \langle u^2 \rangle$$

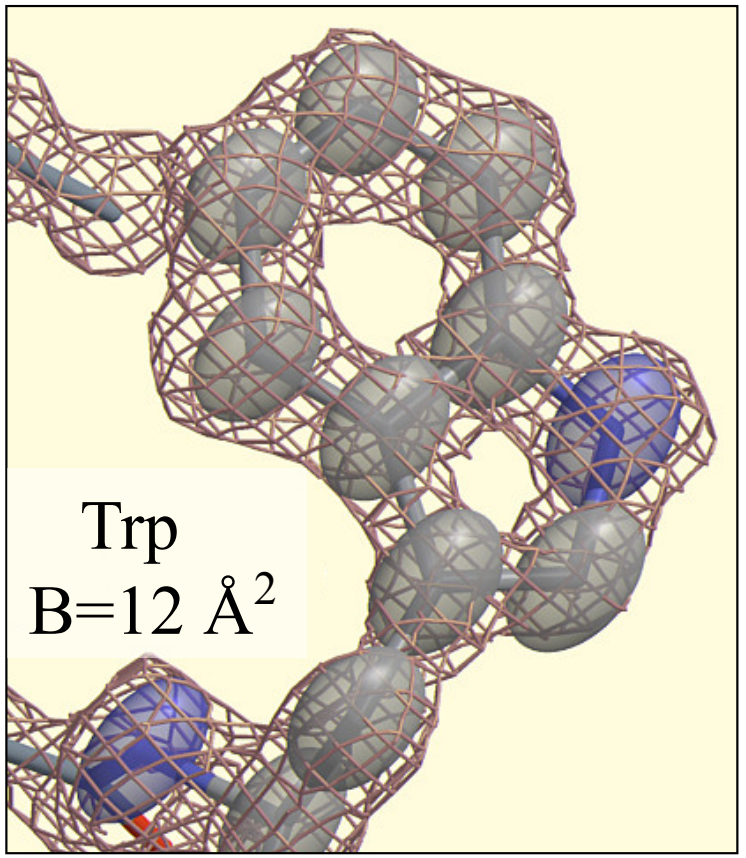
u is atomic displacement from rest position

e.g. if B = 10 Å^2,

then u = 0.35 Å

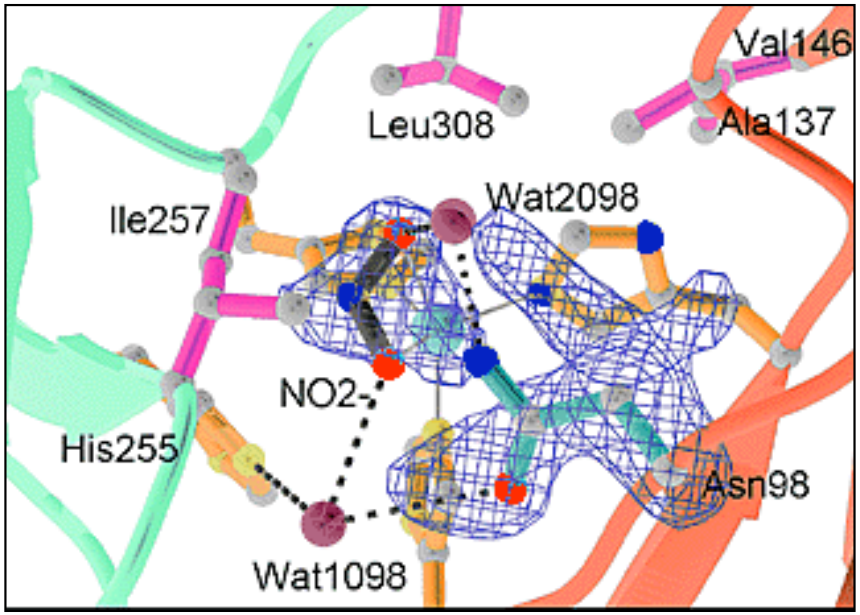
if B = 80 Å^2,

then u = 1.00 Å



X-ray crystallography (cont.)

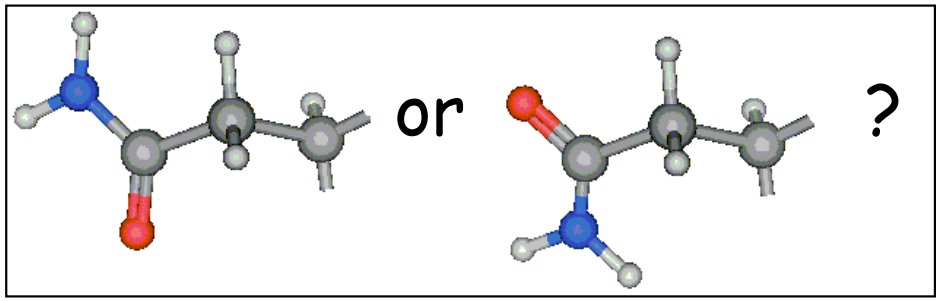
- Alternative conformations



Nitrite reductase at 1.0 A resolution: evidence for second Asn conformation

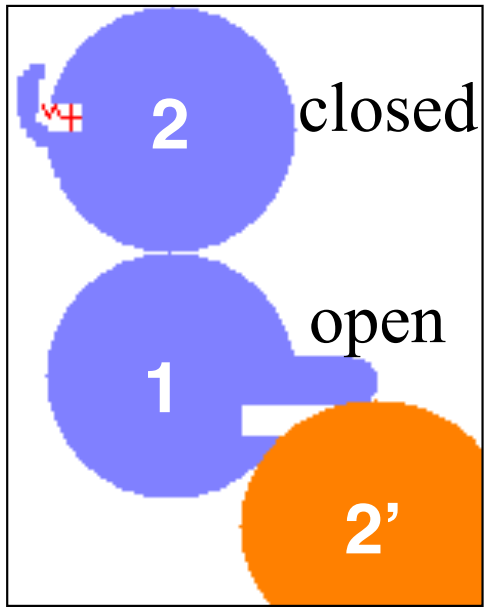
Biochem. 40: 9132-9141

- 'Flipped' amide or imidazole conformations may be needed: Asn (15%), Gln (15%), His (9%)



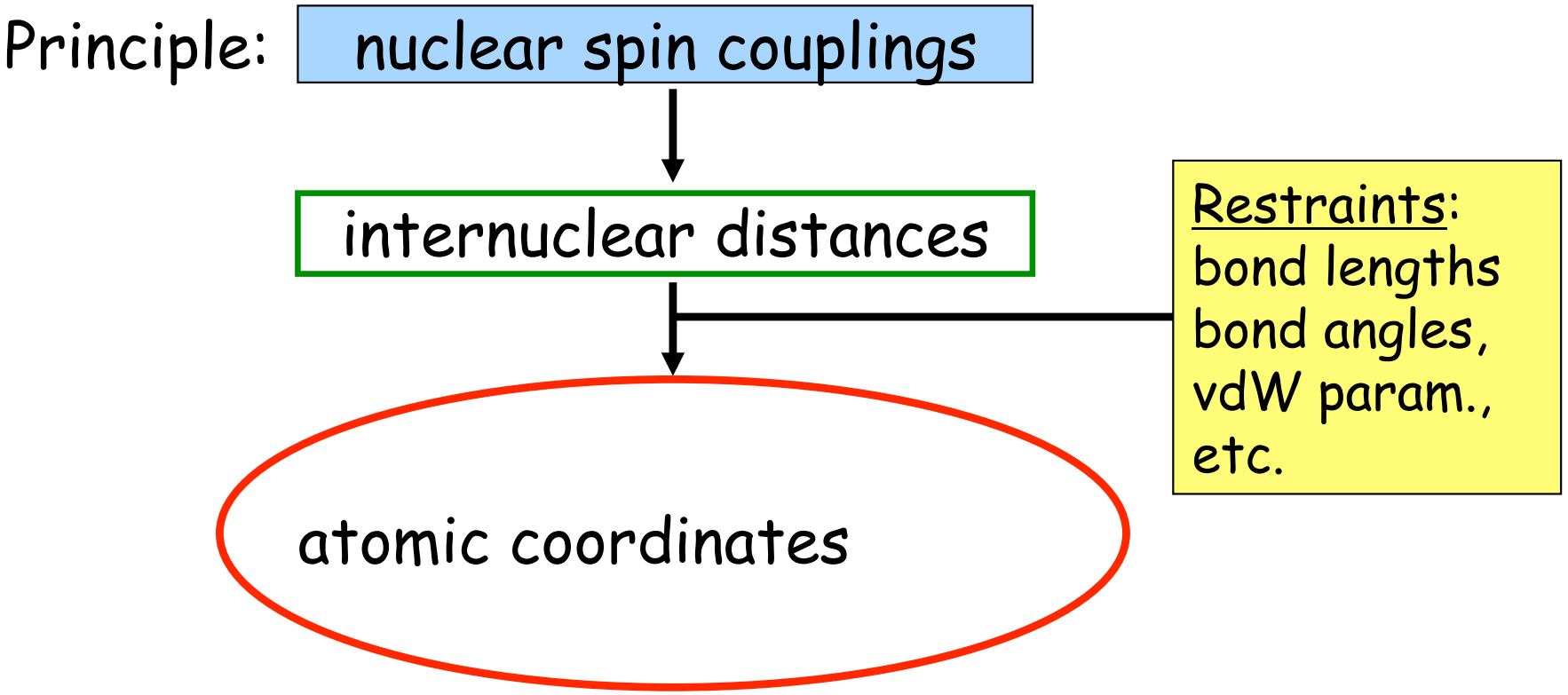
X-ray crystallography (cont.)

- Crystal waters: 30 – 70% of crystal is water
- Packing
  e.g. triosephosphate isomerase
  active site loop open / closed



- pH
- Hydrogen atoms: only visible at very high resolution (examine the 1.1 Å electron density map on page 8).

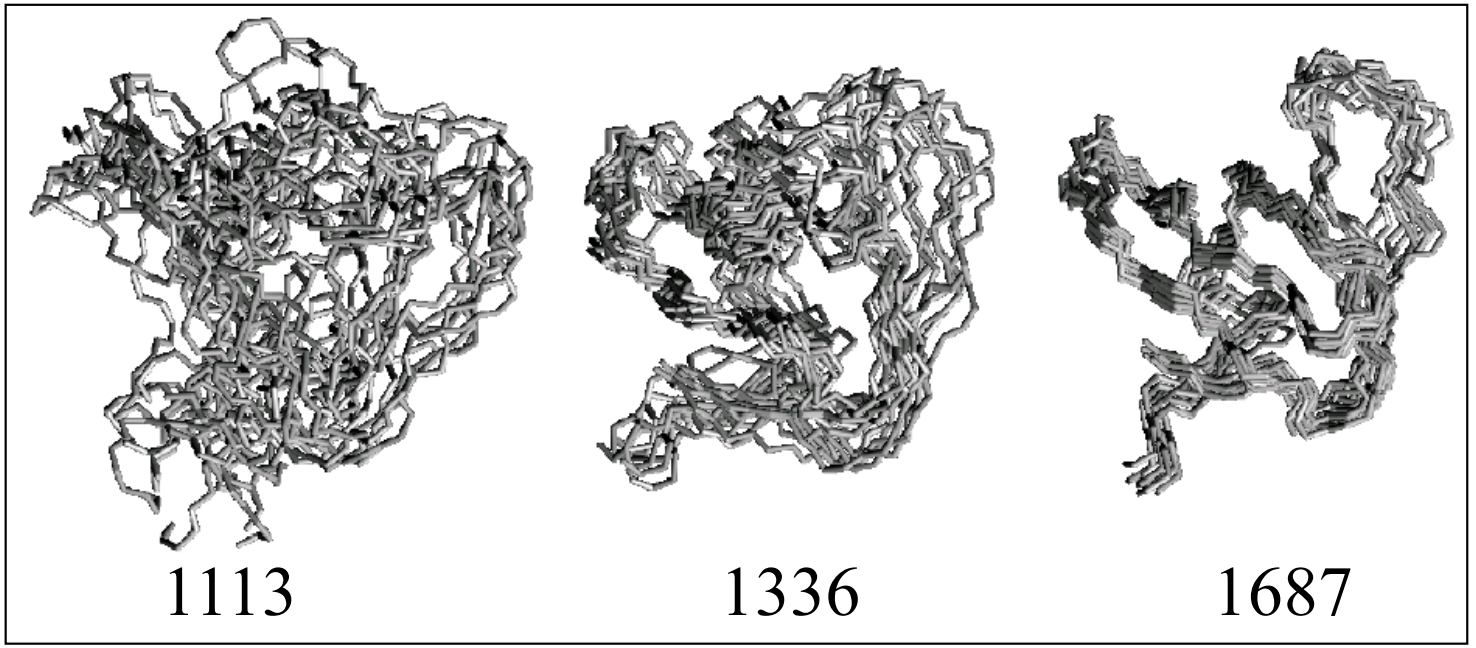
2.2 NMR


Principle:
nuclear spin couplings
internuclear distances
atomic coordinates
Restraints:
bond lengths
bond angles,
vdW param.,
etc.

NMR (cont.)

Issues:

- Number of restraints

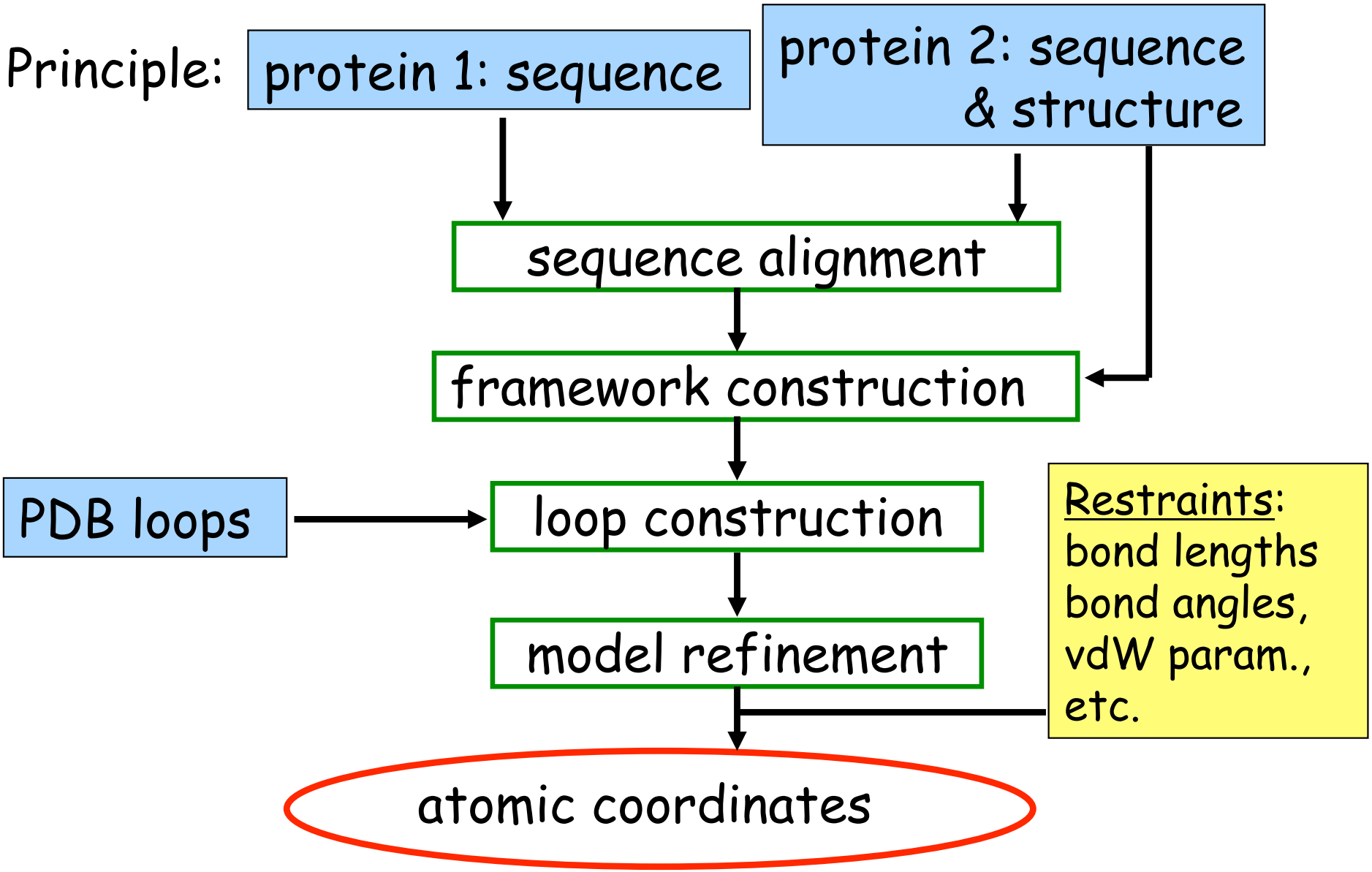


NMR ensemble: SH3 domain of Lck Tyr kinase

Biotechniques 29: 1278-1294

- All information is local

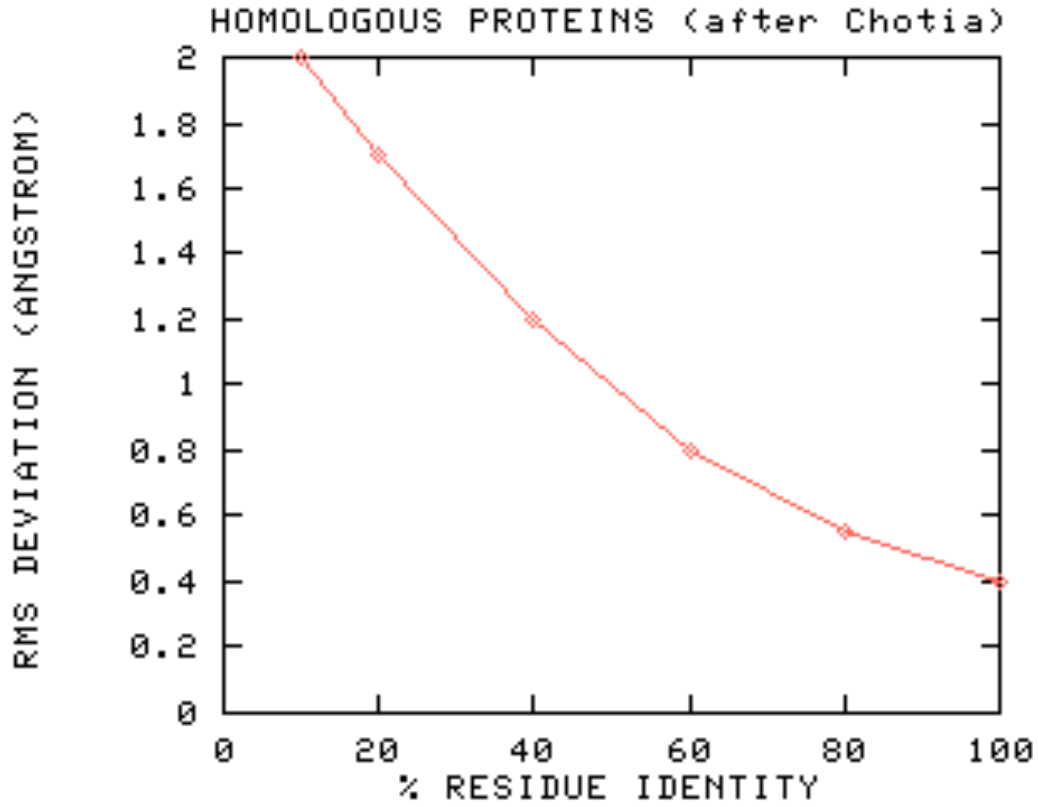
2.3 Comparative modeling



Comparative modeling (cont.)

Issues:

- sequence identity of template



EMBO J. 5: 823-826

- loops: mostly incorrect if more than 5 residues (CASP5 competition, 2002)

3. Forces between molecules: electromagnetic

Fundamental forces of nature:

Force	*Relative strength*	*range*
strong	1	10^{-15} m
electromagnetic	$7\ 10^{-3}$	∞
weak	10^{-5}	10^{-17} m
gravitation	$6\ 10^{-39}$	∞

Molecular contacts occur at the Ångström level (10^{-10} m). Hence, only the electromagnetic force is relevant.

Forces between molecules (cont.)

Literature abounds with interactions:

- Ionic
- Coulombic
- Charge transfer
- Dipole
- Quadrupole
- Salt bridges
- Hydrogen bonds
- Dispersion interactions
- Van der Waals
- …

All are manifestations of the electromagnetic force !

The electromagnetic force is described by Maxwell's equations:

1. The electric flux out of a closed surface is proportional to the enclosed charge:

$$\oint E \cdot dA = q/\varepsilon_o = 4\pi k q \quad or \quad \nabla \cdot E = \rho/\varepsilon_o = 4\pi k \rho$$

2. There is no net magnetic flux out of a closed surface:

$$\oint B \cdot dA = 0 \quad or \quad \nabla \cdot B = 0$$

3. The line integral of the electric field around a closed loop equals the negative of the rate of change of the magnetic flux through the area enclosed by the loop:

$$\oint E \cdot ds = -\frac{d\Phi_B}{dt} \quad or \quad -\nabla \times E = -\frac{\partial B}{\partial t}$$

4. The line integral of the magnetic field around a closed loop is proportional to the electric current flowing through the loop:

$$\oint B \cdot ds = \mu_o i + \frac{1}{c^2}\frac{\partial}{\partial t}\int E \cdot dA \quad or \quad \nabla \times B = \frac{4\pi k}{c^2} J + \frac{1}{c^2}\frac{\partial E}{\partial t}$$

Maxwell's equations (cont.)

Symbols:

E = electric field	B= magnetic field
ρ = charge density	ε_0 = permittivity
J = current density	μ_0 = permeability
i = electric current	c = speed of light

4. From quantum to molecular mechanics

Electromagnetism at the atomic level: Schrödinger equation

For any system of electrons (…,i, j, …) and nuclei (…A,B,…):

$$H\Psi(\ldots i, j, \ldots, A, B, \ldots) = E\Psi(\ldots i, j, \ldots A, B, \ldots)$$

$$H = \frac{-h^2}{8\pi^2}\sum_A \frac{1}{m_A}\nabla_A^2 + \frac{-h^2}{8\pi^2}\sum_i \frac{1}{m_i}\nabla_i^2 - \sum_i\sum_A \frac{Z_A e^2}{r_{iA}} + \sum_i\sum_{<j} \frac{e^2}{r_{ij}} + \sum_A\sum_{<B} \frac{Z_A Z_B e^2}{r_{AB}}$$

kinetic energy nuclei	kinetic energy electrons	pot. E nuclei-electr.	pot. E electrons	pot. E nuclei

4. From quantum to molecular mechanics

Simplification: Born-Oppenheimer approximation

Nuclei are much more massive than electrons. Therefore, nuclei are nearly fixed with respect to electron motion.

Consequence:

$$H = \frac{-h^2}{8\pi^2}\sum_A \frac{1}{m_A}\nabla_A^2 + \frac{-h^2}{8\pi^2}\sum_i \frac{1}{m_i}\nabla_i^2 - \sum_i\sum_A \frac{Z_A e^2}{r_{iA}} + \sum_i\sum_{<j} \frac{e^2}{r_{ij}} + \sum_A\sum_{<B} \frac{Z_A Z_B e^2}{r_{AB}}$$

0

constant

From this simplified wave function the electron density can be calculated.

From quantum to molecular mechanics (cont.)

Forces on nuclei: Hellmann-Feynman theorem

The force on any nucleus, considered fixed, in any system on nuclei and electrons is just the classical electrostatic interaction exerted on the nucleus by the other nuclei and by the electron charge density distribution for all electrons.

One step further: empirism

Year 2000: Very high quality quantum chemical calculations, CCSD(T):

- Feasible for ~ 15 atoms
- Relative energy error for conformations: ~ 0.2 kcal/mol

But, SBDD deals with thousands of atoms …

Empirical methods are used: molecular mechanics.

5. Molecular mechanics: assumptions

1. Born-Oppenheimer approximation.
2. Hellman-Feynman theorem.
3. Electron density around nuclei can be collapsed with the nuclear charge, yielding partial atomic charges.

   Issues:

   - Potentials are needed to replace the electron density 'glue'. E.g. bond length potentials.
   - Atomic 'ownership' of electron density in any part of space.

     Partial atomic charge is no physical property !

Molecular mechanics: assumptions (cont.)

4. Quantum model can be replaced by classical force-field that reproduces molecular properties.

   Issues:

   - What properties? Geometry, relative conformational energy, heat of formation, absorption frequencies, …
   - Partitioning:

     $E = E_{bonds} + E_{angles} + E_{dihedrals} + E_{coulomb} + E_{vdW} + \ldots$

     Complete? (e.g. cross-terms needed? polarization?)
     There is no 'correct' way of partitioning: different in all ff. Consequence: swapping parameters between ff. is dicey.

Molecular mechanics: assumptions (cont.)

5. Transferability:

   i.e. the validity of force-field parameters for molecules that were not used for parameterization.

   Issue:

   - How many atom types needed ?

6. Additivity:

   i.e. $E(A,B,C) = E(A,B) + E(A,C) + E(B,C)$

   Issues:

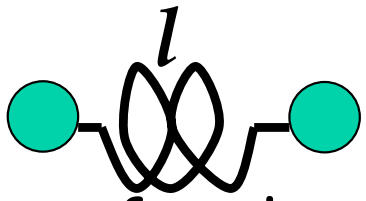
   - Polarization effects are non-additive.

     e.g. with pairwise vdW potentials the dispersion interactions of crystalline Ar are underestimated by 10%.

   - Entropy effects are non-additive.

6. Force-fields

Bond lengths



Bonds between similar atoms in a wide variety of molecules have similar lengths. Hence the idea of a reference bond length l_0. Deviations from the reference cost energy:

$$E(l) = E(l_0) + \Delta l \partial E \frac{\partial E}{\partial l} + \frac{(\Delta l)^2}{2} \frac{\partial^2 E}{\partial l^2} + \cdots (Taylor\ series)$$

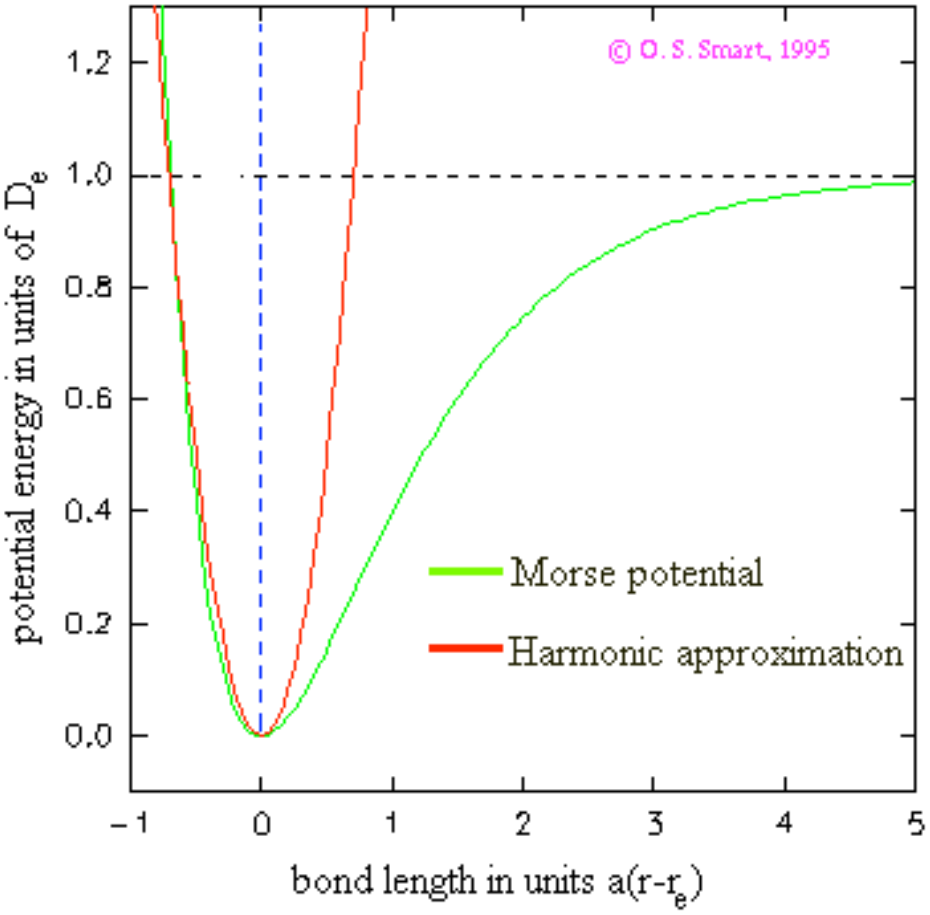
set = 0 near l_0 = 0 significant

$$E(l) = \frac{k_{bond}}{2}(l - l_0)^2$$

harmonic approximation

Bond lengths (cont.)

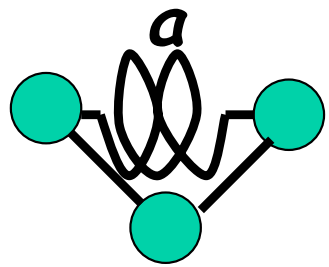
- Harmonic approximation: ok near equilibrium.
- Morse potential: closer to reality

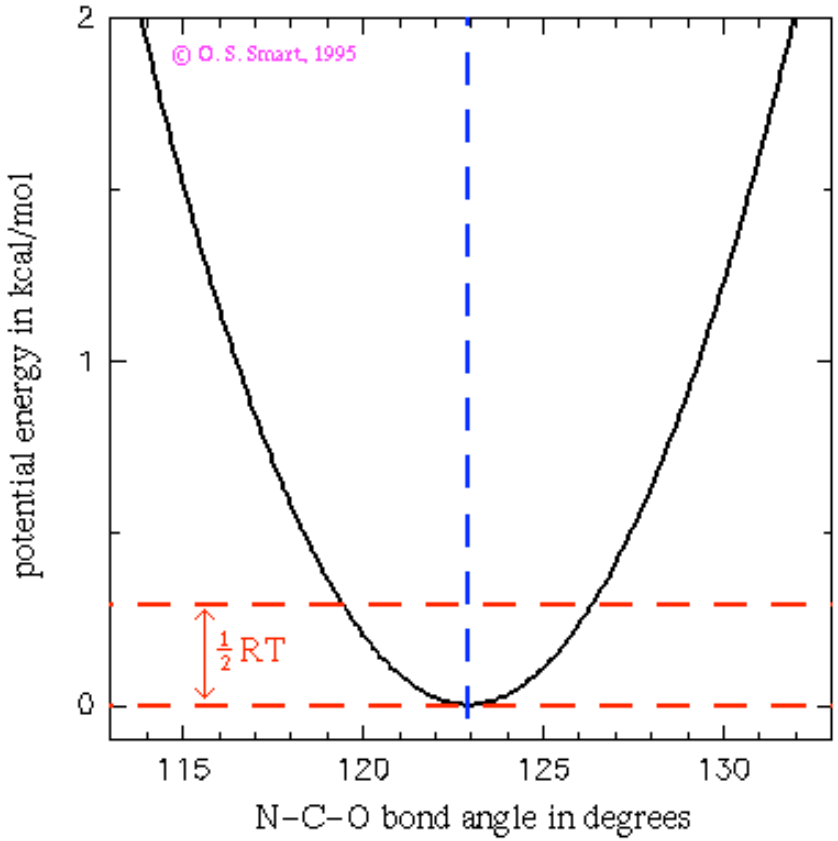


- Typical k_{bond}: $C(sp^3)$-$C(sp^3)$ 300 kcal/(Å^2 mol)

Bond angles

$$E(l) = \frac{k_{angle}}{2}(a - a_0)^2$$

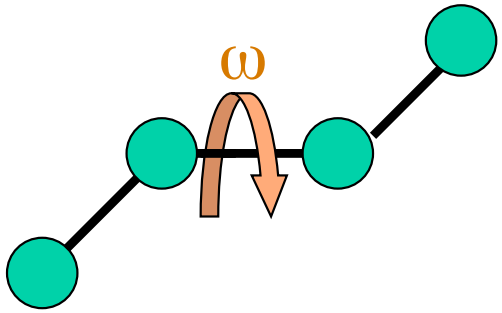




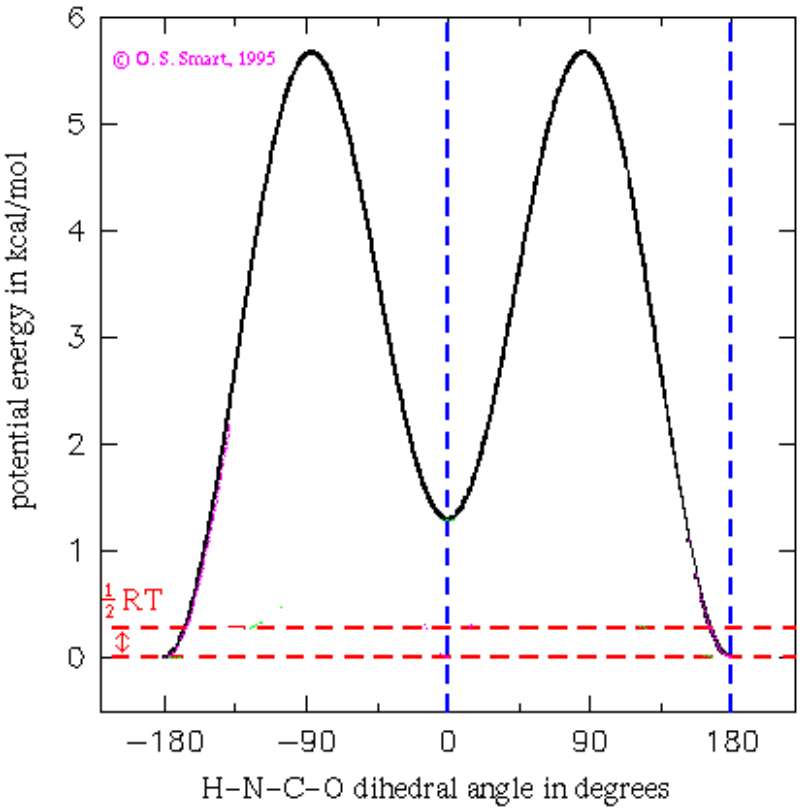
- Typical k_{angle}: $C(sp^3)$-$C(sp^3)$-$C(sp^3)$ 0.01 kcal/($^{\circ 2}$ mol)
- Central atom usually participates in several angles: not independent (6 angles for C (sp^3)).

Torsion angles (aka dihedral angles)

Non-bonded interactions (Coulomb and vdW) are insufficient to reproduce energies of conformers and torsion barriers.



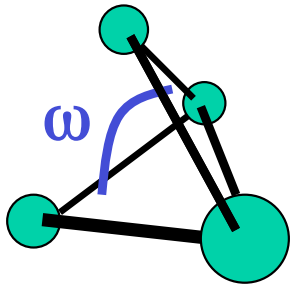
Why? Does not include hyperconjugation, etc.



$$E_{torsion} = \sum_{n=0}^{N} \frac{V_n}{2}\left[1 + \cos(n\omega - \omega_0)\right]$$

- n: multiplicity, the number of minima
- ω_0: phase factor, the angular shift of the cos function

Improper torsions

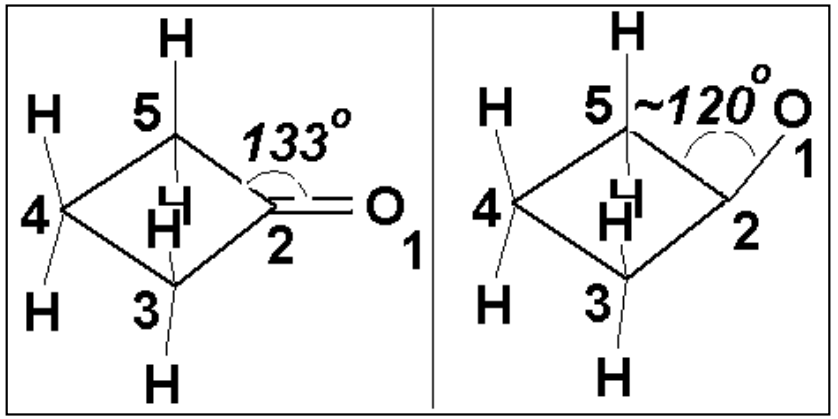


Often correct hybridization geometry is not maintained, especially in MD simulations:

- planarity
- chirality

Example: cyclobutanone

- ⟨CCO = 133 °, but a_0=120 °
- the slightest non-planarity will lead to one of the ⟨CCO → 120 °
- solution: impose improper dihedral ω 1-5-3-3-2 = 0



$$E_{impr} = k_{impr}(1 - \cos 2\omega)$$

Electrostatics

Maxwell's law for the electrostatic field: $\oint E \cdot dA = q/\varepsilon$

- In vacuum: $\varepsilon = \varepsilon_0$
- In a homogeneous dielectric: $\varepsilon = \varepsilon_0 \varepsilon_r$

  ε_r is called the dielectric constant or relative permittivity
- Special case: two interacting point charges separated by distance r_{12}

$$E_{ES} = \frac{q_1 q_2}{4\pi\varepsilon r_{12}}$$ aka Coulomb's law

if q_1, q_2 in units of $|e^-|$, then $E_{ES} = -332 \frac{q_1 q_2}{\varepsilon r_{12}} kcal/mol$

- Problems:
  - biology occurs in non-homogeneous dielectric media !
  - partial atomic charges

Electrostatics (cont.)

Electrostatics in non-homogeneous dielectric media will be discussed in a later lecture on solvation.

Various schemes to derive partial atomic charges are in use. In order of increasing quality:

- Gasteiger-Marsili charges: based on electronegativity equilibration.
- Mulliken population analysis: squaring of the wave function.
- Bader charges: volume integration of the squared wave function.
- CHELP, CHELPG, RESP: charges fitted to reproduce the electrostatic potential derived from the wave function.

the only acceptable charges

van der Waals

- Repulsion

  At short distances atoms repel each other. Why?

  - Pauli exclusion principle: no two electrons can have the same quantum numbers.
  - Consequence: electrons get excited to higher levels.
  - Result: electron density between nuclei decreases, leading to nuclear repulsion.

$$E_{rep} = C_{rep} / r^{12}$$

There is no theoretical justification for the r^{-12} form of this potential.

van der Waals (cont.)

- Attraction
  - Between all atoms and molecules there is an attractive force due to temporarily induced changes in electron density (dipole-dipole, dipole-quadrupole,quadrupole-quadrupole, …).

$$E_{London} = -C_{disp}/r^6 - C_{disp}/r^8 - C_{disp}/r^{10} - \ldots$$

Higher order terms are usually absorbed in the 1st term.

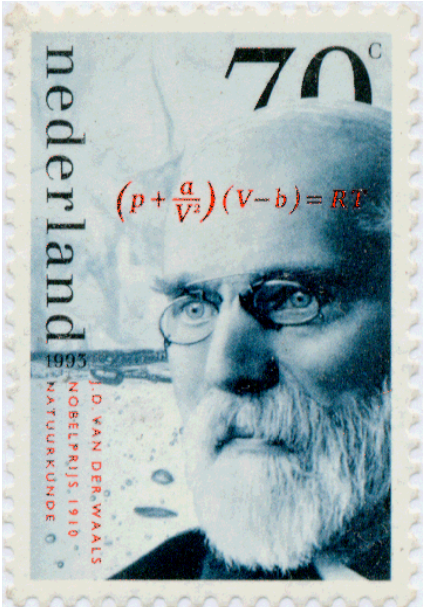
  - When polar molecules approach each other their permanent electrostatic field induces dipoles.

$$E_{pol,ind} = -C_{pol,ind}/r^6$$

  - Rotational polarization involves orienting dipoles.

$$E_{pol,ori} = -C_{pol,ori}/r^6$$

van der Waals (cont.)



- Sum: Lennard-Jones potential

$$E_{LJ} = C_{rep}/r^{12} - C_{attr}/r^{6}$$

Often recast in terms of:

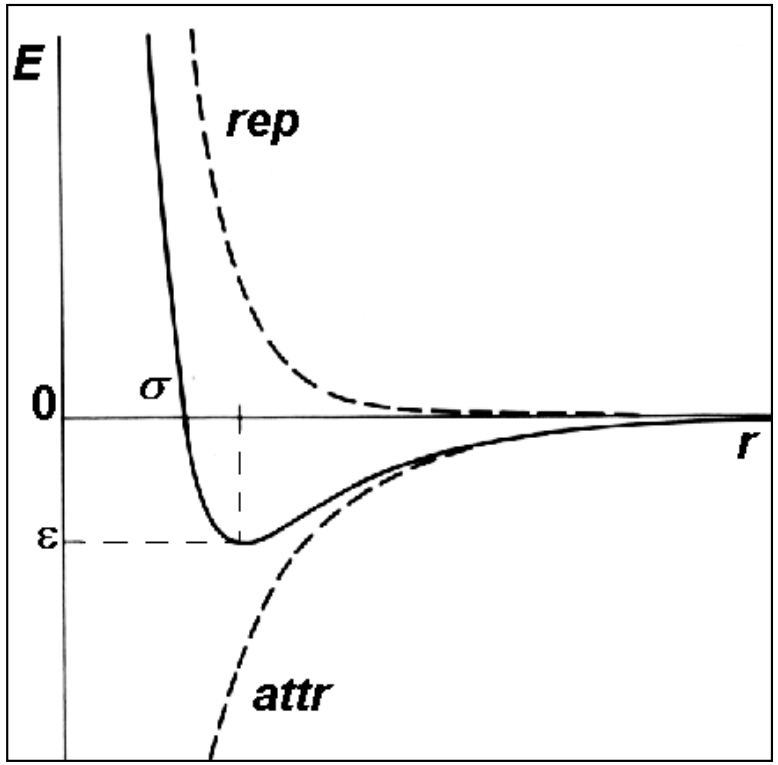
- R, distance where E is a minimum
- $\in$, the E of the minimum

$$E_{LJ} = \in\left[\left(\frac{R}{r}\right)^{12} - 2\left(\frac{R}{r}\right)^{6}\right]$$

Or in terms or σ, the collision diameter, where E is zero:

$$E_{LJ} = 4\in\left[\left(\frac{\sigma}{r}\right)^{12} - 2\left(\frac{\sigma}{r}\right)^{6}\right]$$

van der Waals (cont.)



Common mixing rules (Lorentz-Berthelot):

$$\sigma_{AB} = \tfrac{1}{2}\left(\sigma_{AA} + \sigma_{BB}\right)$$

$$\in_{AB} = \left(\in_{AA} \in_{BB}\right)^{0.5}$$

van der Waals (cont.)

- In most force-fields 1-3 vdW interactions are ignored.
- In many force-fields 1-4 vdW are scaled down.
- Several force-fields replace the E_{LJ} in case of hydrogen bonds with:

$$E_{LJ} = \left[C_{rep} / r^{12} - C_{attr} / r^{10} \right] \cos^4 \theta$$

where θ is the donor-H…acceptor angle.

7. Suggested readings

General:

- Andrew R. Leach. "Molecular modelling: Principles and applications". Addison-Wesley Longman, Harlow, 1996.
- Jacob N. Israelachvili. "Intermolecular and surface forces". 2nd ed., Acad. Press., London, San Diego, 1991.

Examples from introduction:

- Vancomycin: Hubbard B.K., Walsh C.T.(2003). Vancomycin assembly: nature's way. Angew. Chem. Int. Ed. 42: 730-65.
- GAPDH: Aronov A.M., Buckner F.S., Van Voorhis W.C., Verlinde C.L.M.J., Opperdoes F.R., Hol W.G.J., Gelb M.H. (1999). Structure-based design of sub-micromolar, biologically active inhibitors of trypanosomatid glyceraldehyde- 3-phosphate dehydrogenase. *PNAS USA* 96: 4273-4278.
- PTP-B: Doman T.N., McGovern S.L., Witherbee B.J., Kasten T.P., Kurumbail R., Stallings W.C., Connolly D.T., Shoichet B.K. (2002). Molecular docking and high-throughput screening for novel inhibitors of protein tyrosine phosphatase-1B. *J.Med.Chem.* 45: 2213-2221.

Suggested readings (cont.)

Structures:

- X-ray crystallography:
  - Davis A.M, Teague S.J., Kleywegt G.J.(2003). Application and Limitations of X-ray Crystallographic Data in Structure-Based Ligand and Drug Design. *Angew. Chem. Int. Ed.* 42: 2718-2736.
  - Kleywegt G.J., Henrick K., Dodson E.J., van Aalten D.M. (2003). Pound-wise but penny-foolish: How well do micromolecules fare in macro-molecular refinement? *Structure* 11: 1051-1059.
  - McDonald I.K. and Thornton J.M. (1995) The application of hydrogen bonding analysis in X-ray crystallography to help orientate asparagine, glutamine and histidine side chains. *Protein Eng.* 8: 217–224.
- NMR:
  - Wider G. (2000). Structure Determination of Biological Macro-molecules in Solution Using NMR spectroscopy BioTechniques 29: 1278-1294.

Suggested readings (cont.)

Structures (cont.):

- Comparative modeling:
  - Ring, C.S., Cohen F.E. (1993). Modeling protein structures: construction and their applications. *FASEB J.* 7: 783-790.
  - Chothia C, Lesk AM. (1986). The relation between the divergence of sequence and structure in proteins. EMBO J. 5: 823-826.
  - Tramontano A, Morea V. (2003). Assessment of homology-based predictions in CASP5. Proteins 53 Suppl 6: 352-368.

Quantum chemistry:

- Barden C.J., Schaeffer H.F.(2000). Quantum chemistry in the 21st century. Pure and Applied Chem.72: 1405-1423.
- Pople J.A. (1999). Quantum chemical models (Noble lecture). Angew. Chem. Int. Ed. 38: 1894-1902.

Suggested readings (cont.)

Additivity

- Dill, K.A. (1997). Additivity principles in biochemistry. *J. Biol. Chem.* 272: 701-714.

Force-fields

- See: Leach, Chapter 3 "Empirical force-field models".