Interpreting Electron Densities:

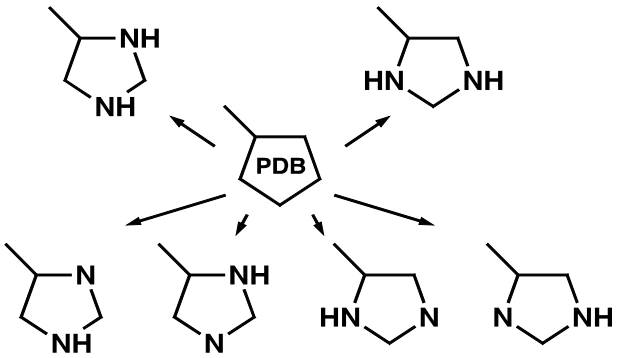
X-ray Structures

"The X-ray Structure is an Interpretation of the Electron Density and thus Susceptible to Plural Errors"

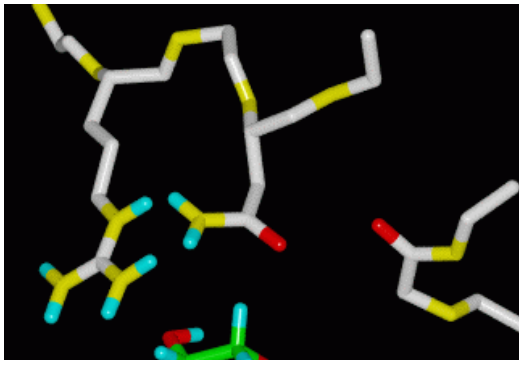
Data from X-ray Measurements

- Measured Data: diffraction pattern
- Derived Data: electron density
- Interpretation: least squares fit of density via
  - spatial coordinates
  - temperature factors

- Refined Data: coordinates with assignment of atomic numbers (C, N, O, S etc.)



Decisions: His (C/N/NH)



ASN (O/NH2 wrong!)

⇒ structure not obtained by measurement but interpretation

Widely Accepted *Sloppy Usage* of Definitions

- **Resolution:** phys. limits due to crystal imperfections
  NOT: precision of atomic positions
  e.g. bond angle deviation up to 10% in every structure
  (angle N-C_α-$C_{=O}$ often 118º)

- **B-Factors:** degree of freedom in interpretation
  NOT: vibrational motion of atomic positions
  e.g. different B-factors for symmetry related atoms
  (ortho-carbons in PHE or O/N in ASN)

- **R-Factor:** measure of global regularity
  NOT: quality of local coordinates
  e.g. lowered by well positioned water molecules

⇒ structure is a fit to experimental and theoretical restraints

Statistical Quality Checks

Reference Geometry Parameters and Standard Deviations determined from Crystal Data (e.g. PROCHECK, WHAT_IF):

- **Bond Lengths and Angles: Engh and Huber**
- **Dihedral Angles:**
  - **Ramachandran, ω**
  - **preferred conformations for χ_1, χ_2**
- **Close van der Waals Contacts**
- **Close Water Molecules**

Actual Deviations of up to
! Four Times the Standard Deviations !
in the Statistical Analyses
are Tolerated

⇒ what means 4*S-DEV? compare to force field references ...

Parameters from Crystal Data

Comparison: ENGH/HUBER-Parameters to AMBER-Force Field Reference Values

(Stat. Standard Deviation) and [Deviation of Ref. Values from Parameters]

	C-N	CA-C	CA-CB	N-CA	C-N-CA	CA-C-N	CB-CA-C	N-CA-C	N-CA-CB
Pro	1.341	-	-	1.466	122.60	116.90	-	111.80	103.00
	(0.016)			(0.015)	(5.00)	(1.50)		(2.50)	(1.10)
Amber	1.335	-	-	1.449	121.90	116.60	-	110.10	109.70
	[-0.006]			[-0.017]	[-0.70]	[-0.30]		[-1.70]	[+6.70]
Gly	-	1.516	-	1.451	120.60	116.40	-	112.50	-
		(0.018)		(0.016)	(1.70)	(2.10)		(2.90)	
Amber	-	1.522	-	1.449	121.90	116.60	-	110.30	-
		[0.006]		[-0.002]	[1.30]	[0.20]		[-2.20]	
Any	-	-	1.530	1.458	121.70	116.20	110.10	111.20	110.50
			(0.020)	(0.019)	(1.80)	(2.00)	(1.90)	(2.80)	(1.70)
Amber	-	-	1.526	1.449	121.90	116.60	111.10	110.10	109.50
			[-0.004]	[-0.009]	[0.20]	[0.40]	[1.00]	[-1.10]	[-1.00]

Lower AMBER-References: Strain Causes Higher Actual Value

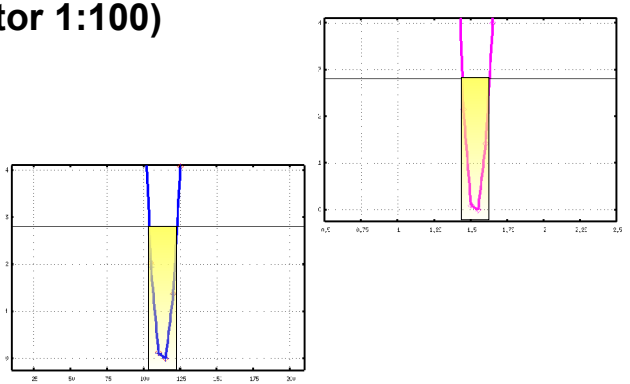
⇒ **references agree! 4*S-DEV means 0.08Å (bonds), 8° (angles)**

Energy Cost for Deviation from References

Geometry Variations with 2.8 kcal/mol Energy Penalty

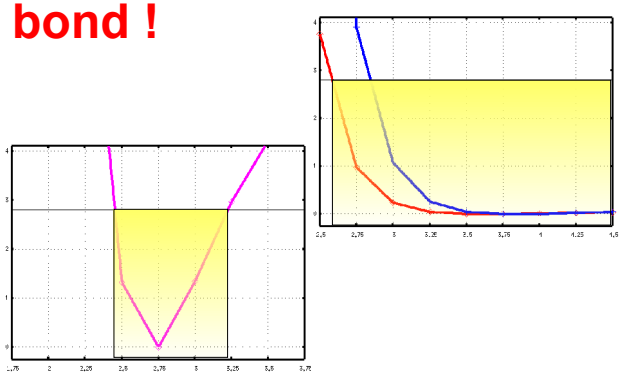
(e.g. ½ twist-chair cyclohexane, Boltzmann factor 1:100)

- **Single Bond:** +/- 0.1Å
  (C-C: 1.42 - 1.62Å)



- **Bond Angle:** +/-7.5°
  (sp^3 Carbon: 102° - 117°)

- **Dihedral Angle:** cis/trans peptide bond !



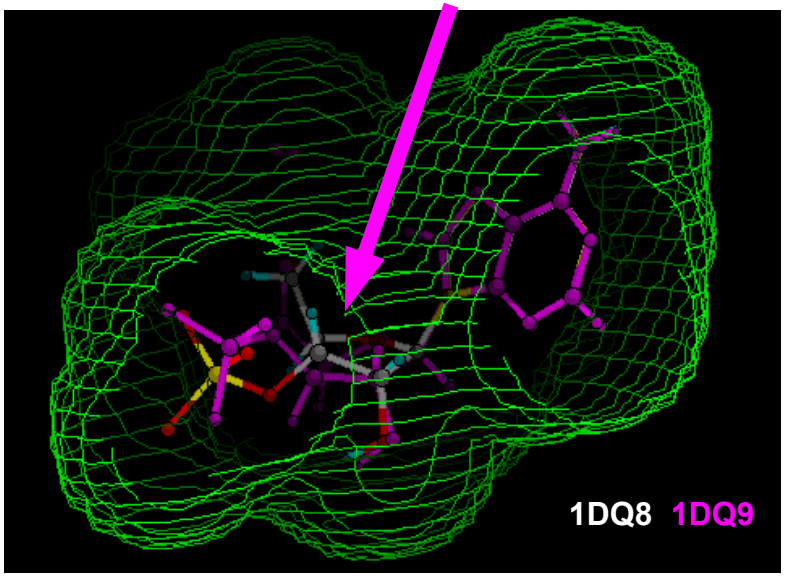
- **vdW Repulsion:** -1.0Å
  (C...C < 2.6Å)

- **HBO Repulsion:** -0.3Å +0.5Å
  (O...O: <2.5Å or > 3.3Å)

⇒ NO geometry CAN have 4*S-DEV deviations; but they do!

Other Discrepancies, Easier to Remedy?

- Conformation of Ambivalent Side Chains (e.g. ASN)
- Hetero-Groups
  (e.g. Coenzyme A: superposition of structures from 1DQ8 and 1DQ9, in 1DQ9 with wrong stereochemistry!)



Does electron density fail to show the right stereoisomer?

⇒ EVERY external information must be used, ALWAYS!

Geometry: Minimum Requirements

- Appropriate Parameters for Hetero-Groups

- Favourable Energy Contributions
  - valence terms (bonds, angles)
  - van der Waals terms (no close contacts)
  - other non valence terms (no electronic repulsion)

  ⇒ no energetical 'hot spots'

- Optimum Hydrogen Bonding Network
  - ambivalent side chain orientation
  - orientation of flexible hydrogens

Requirements *Have to be Met*
Prior to Any Theoretical Study

⇒ *CHEOPS* structure preparation takes care of this!