Tuning X-ray Structures:

CHEOPS Structure Preparation

**"X-ray Structures Suffer from
Inaccuracies and Inconsistencies
that Need to be Remedied
Prior to any Computation"**

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'

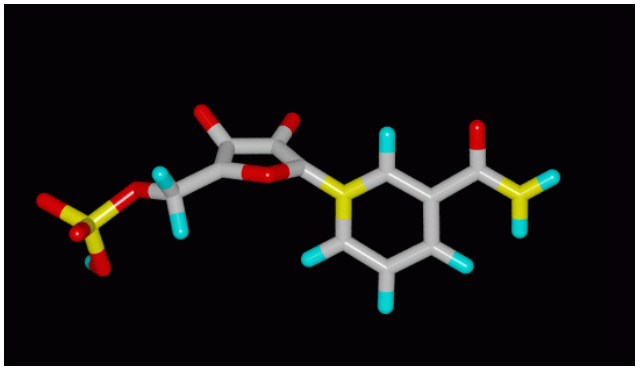
- Optimum Hydrogen Bonding Network
  - ambivalent side chain orientation
  - orientation of flexible hydrogens

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

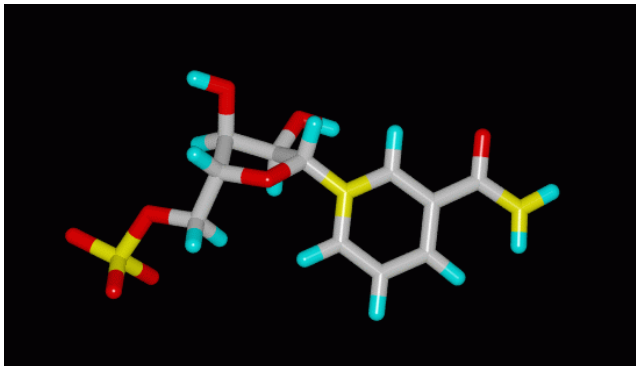
⇒ *CHEOPS* structure preparation yields *GEOMETRIES*

Hetero-Groups

- Correct for Inadequate Internal Coordinates
  Example: Nicotinamidemononucleotide



incorrect geometry=wrong center types



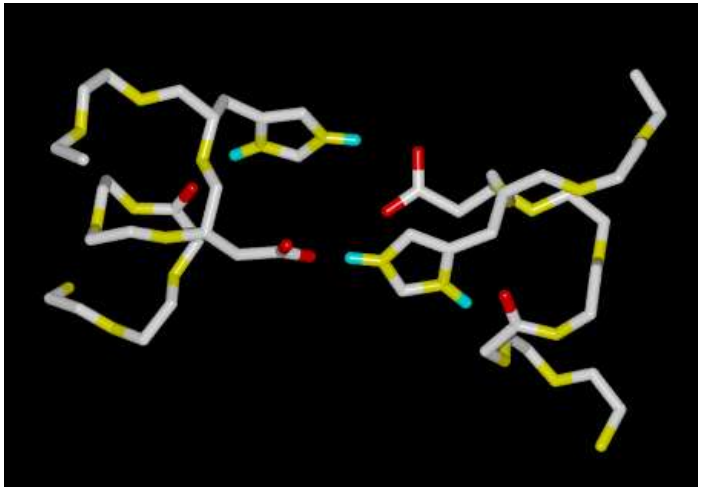
corrected center types

- Ensure Appropriate Stereochemistry
- Establish Charge Distribution
- Derive Additional Force Field Parameters (if necessary)

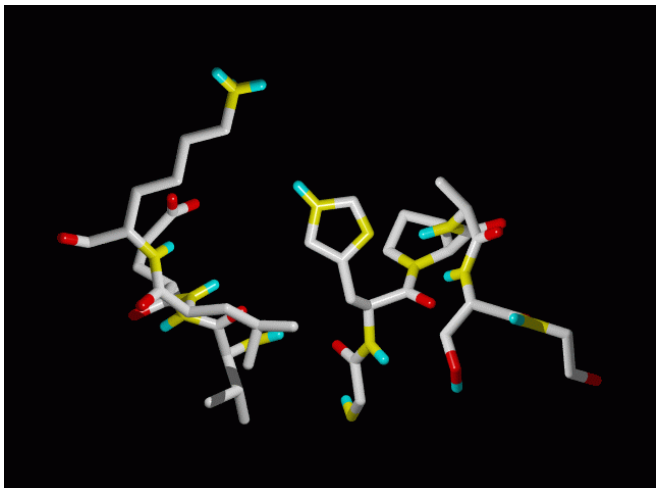
⇒ don't be surprised by the geometry of hetero-groups!

Ionizable Residues

- Actual Protein Environment Dictates State of Ionization
- Specific Environment Determines Hydrogen Positions



2 HIS cationic due to ASP counterions

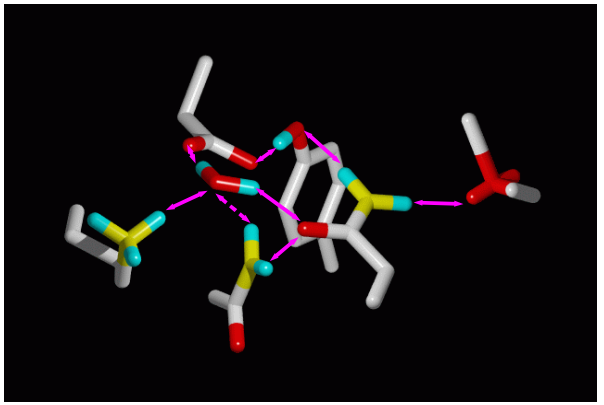


HIS neutral due to backbone donor

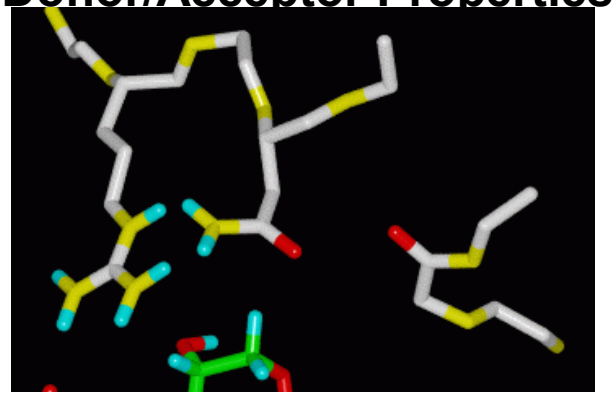
⇒ decisions based on estimate of pK_a-values

Adding Hydrogens

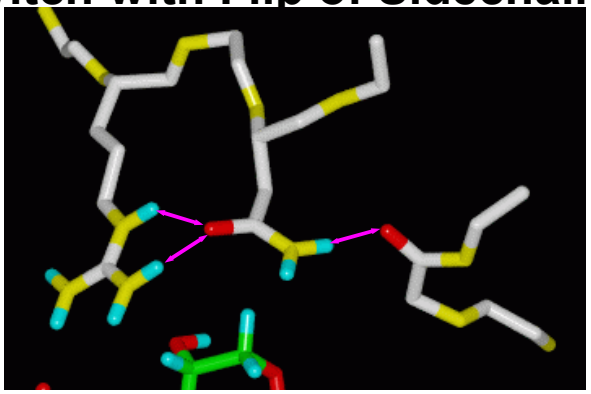
- Flexible -OH Need Orientation for Optimum HBO Network



- Donor/Acceptor Properties Switch with Flip of Sidechain



PDB assignment

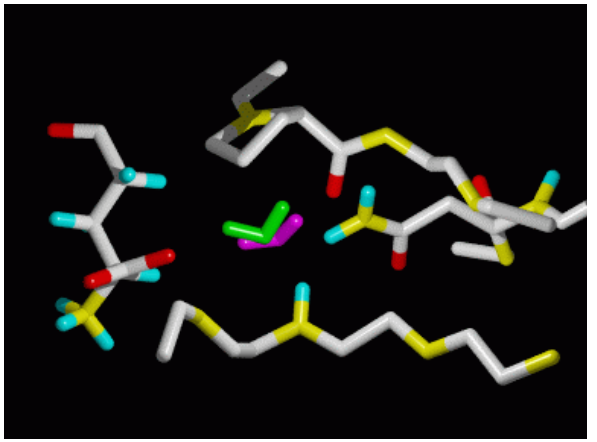


revised assignment

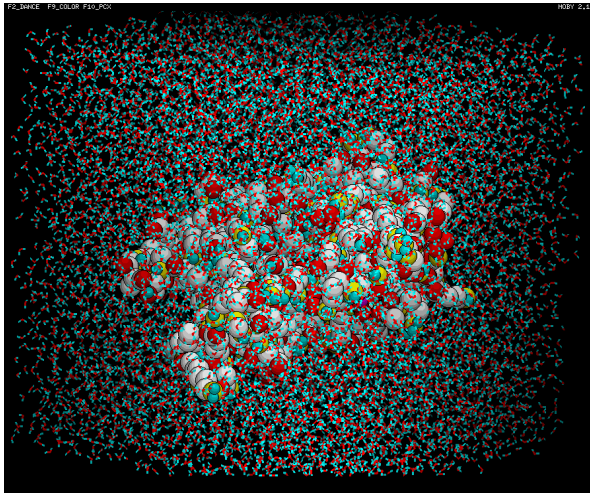
⇒ explore neighborhood for optimum HBO network

Adding Water

- Essential Water Molecules Fill Small Holes of the Protein (water added to the isolated protein finds its x-ray position)



- Final Solvation Fills the Space Starting at Hydrophilic Groups (10000 waters added to protein)



⇒ essential waters are needed to preserve the overall shape

Energy Calculation

- Detect Individual 'Hot Spots' by Energy and Gradient
  - van der Waals bumps
  - inadequate internal coordinates (bonds/angles)

- Some Simple Examples:

Structure	Energies [kcal/mol]	RMS(Gradient) [kcal/molÅ]
MET(good)	-20	10
MET(C-S=1.93Å)	-5	50
MET(N-CA-C=118°)	10	150
SER(good)	-20	10
SER(bad OH-HBO)	20	250
1NNC(old)	-13000	50
1NNC(*CHEOPS*)	-16000	10

⇒ ”small“ geometry deviations indicate further (larger) errors!

CHEOPS Structure Preparation

- **Revising Hetero-Groups:**
  center types, force field parameters, charge distribution
- **Protonation of Ionizable Residues:**
  depending on the actual surrounding
- **Positions of Protons and Hetero-Atoms:**
  optimum hydrogen bonding network
- **Water Surrounding:**
  essential water molecules and solvation
- **Valence Optimization:**
  optimization of bond lengths and bond angles

Only Well Prepared Structures are Geometries that can be Analyzed and Studied Further

⇒ **with this, computed effects are physical facts, not artefacts**