Improving low-resolution refinement of nucleic acids in *Phenix*

Oleg V. Sobolev[a], Pavel V. Afonine[a], Alexandre Urzhumtsev[b,c], Nigel W. Moriarty[a] and Paul D. Adams[a,d]

[a]*Lawrence Berkeley National Laboratory, Berkeley, CA*
[b]Department of Integrated Structural Biology, IGBMC, Illkirch, France
[c]Physical Department, Université de Lorraine, France
[b]*Department of Bioengineering, University of California at Berkeley, Berkeley, CA*

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Restraints and data resolution

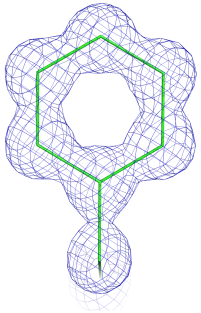
- **Refinement target** - a weighted sum of experimental data (E_{data}) and *a priori* chemical knowledge terms (restraints; $E_{restraints}$): $E_{total} = w * E_{data} + E_{restraints}$
- **Choice of restraints** depends on data quality (resolution):

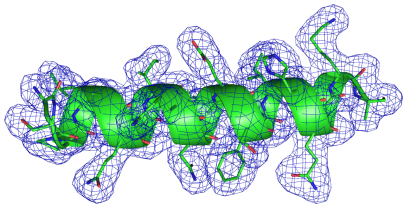
<1Å: unrestrained refinement

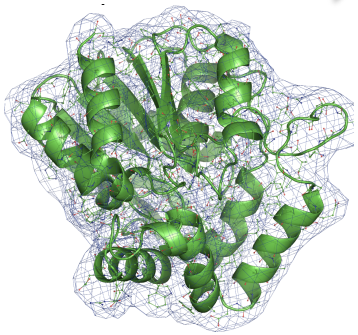
>3Å: more restraints needed

$E_{restraints} = \ldots + E_{Ramachandran} + E_{NCS} + E_{ReferenceModel} + E_{SecondaryStructure} + \ldots$

High Resolution **Low**





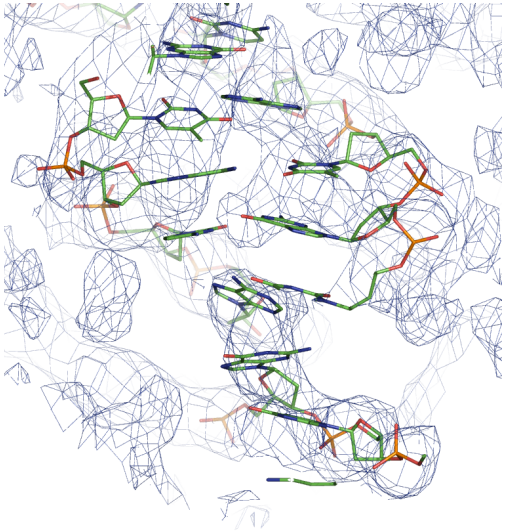


1-3Å: *standard* restraints are necessary

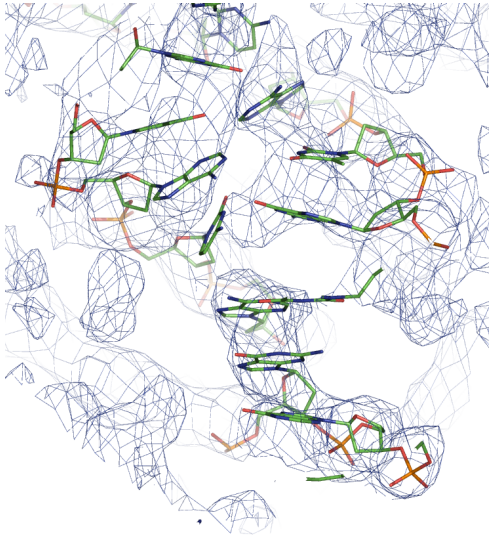
$E_{restraints} = E_{bond} + E_{angle} + E_{dihedral} + E_{nonbonded} + E_{planarity} + E_{chirality}$

Insufficiency of standard restraints at low resolution

- Example: real-space refinement with simulated annealing of 3gbi against 4Å 2mFo-DFc map with *phenix.real_space_refine*
- Refinement with standard restraints fits model into map well, but geometry is poor (no correct basepairing and stacking interactions)



Refinement with standard restraints

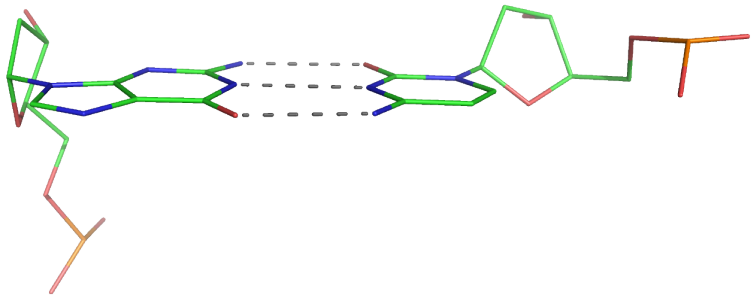


Starting model

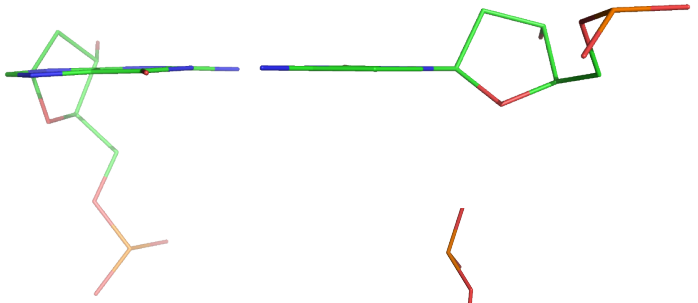
Basepairs are not parallel, poor hydrogen bonding

Geometry restraints for DNA/RNA in Phenix

1. **Hydrogen bonds** between base pairs:
   - Bond length restraints
   - Bond angles restraints



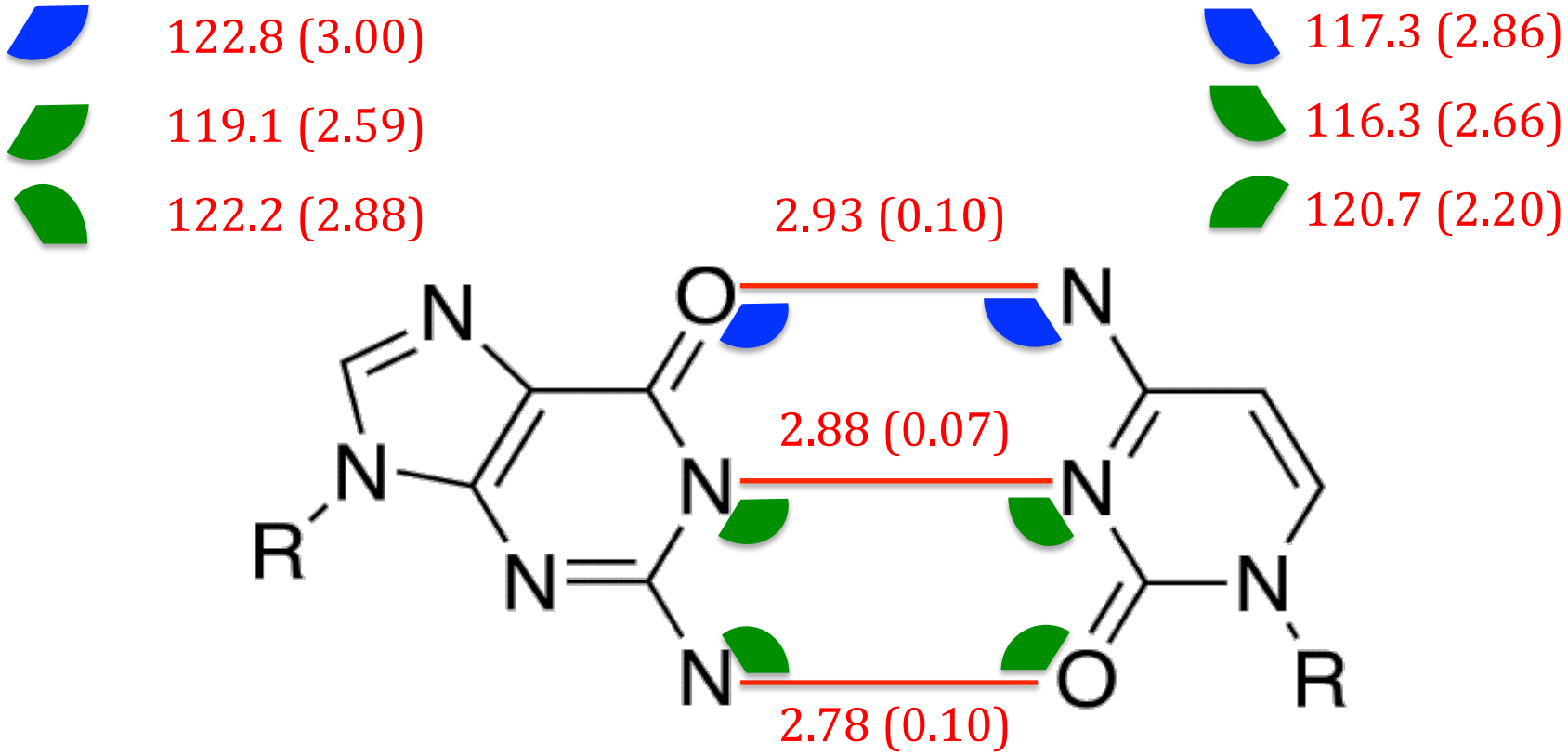
2. **Planarity** of base-pairs:
   - Planarity restraint
   - Parallelity restraints



3. **Parallelity** of stacking nucleobases:
   - Parallelity restraints

Hydrogen bond restraints

The values for hydrogen bond lengths differ for different basepairing type and participating atoms

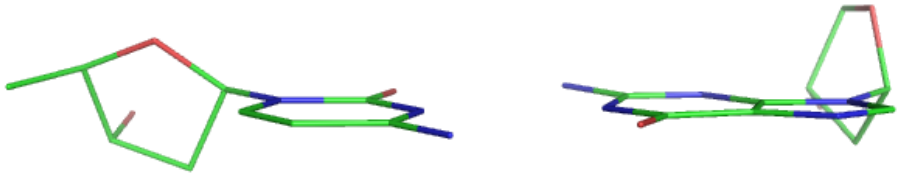


The values for hydrogen bond lengths of the same type are essentially the same for DNA and RNA

Stacking and basepairing restrains

Basepairing
RMSDs from 0:

- parallelity: 14.87°
- planarity: 0.188Å

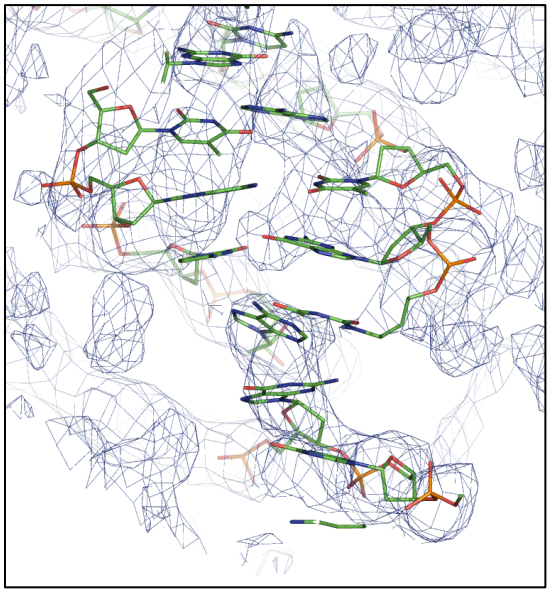


Stacking parallelity
RMSD from 0°: 11.54°

DNA/RNA: example of low-resolution refinement

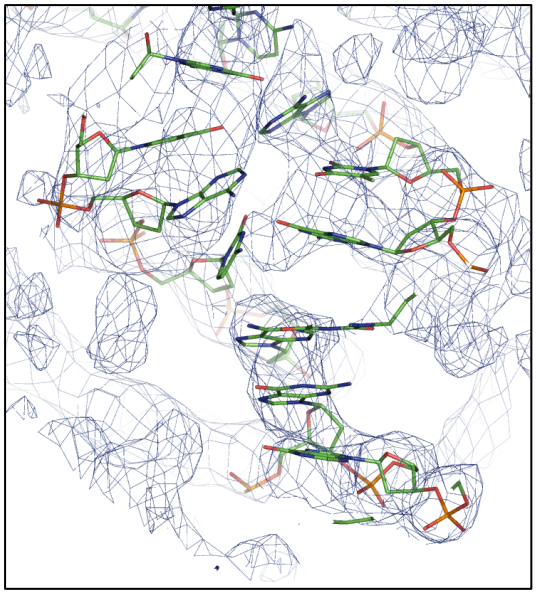
- Real-space refinements with simulated annealing against 4Å 2mFo-DFc map with *phenix.real_space_refine*

Starting model



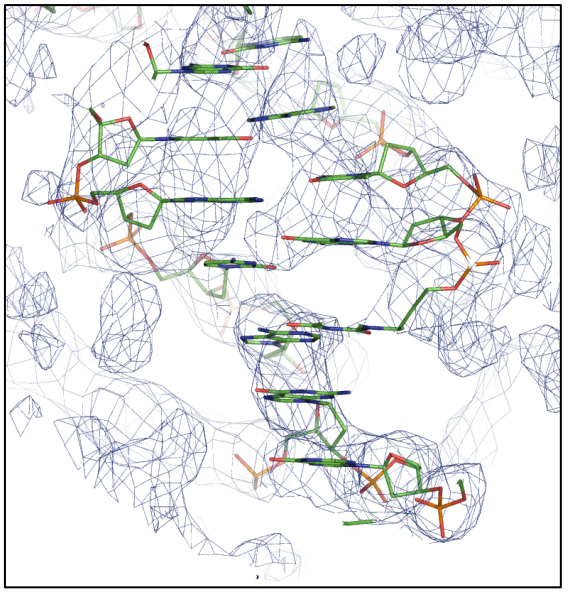
Nucleobases somewhat parallel with a number of outliers

Standard restraints



Geometry becomes worse: nucleobases are not parallel, poor H-bonding

Standard + stacking + basepair + H_{bond} restraints



Nucleobases are parallel, correct H-bonding

Implementation in Phenix

- Available in all relevant tools (phenix.refine, phenix.real_space_refine, phenix.geometry_minimization, phenix.dynamics) via secondary_structure.nucleic_acid scope

- Turn on restraints: secondary_structure.enabled=True

- Generate phil file with NA definitions: phenix.secondary_structure_restraints <model.pdb>