Challenges For Structure Determination At Low Resolution

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PUBLIC ACCESS TO X-RAY DIFFRACTION DATA

To the Editors:

The undersigned have a long standing concern with the problem of public access to the results of single crystal X-ray diffraction studies on biological macromolecules. The actual data from such research are the measured X-ray intensities, and the primary results are the lists of atomic coordinates derived from those data. While many papers on various proteins and nucleic acids have been published describing and interpreting the results of such a structure analysis, the actual data and results are often not made easily accessible, if at all. We are requesting that the journals which play a major role in the publication of such structural studies adopt and enforce rules for documentation similar to those which apply in all other areas of scientific research. Studies where the structural information has not been made available must be considered incomplete, as would any other piece of research where the data were not provided in published or deposited form.

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A list of the signers of this letter follows. The original signatures are on file in the office of the undersigned.

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Corresponding cosignatory on behalf of the following individuals:

ca. 1990

The Low Resolution Problem

How can one obtain a "good" crystal structure at low resolution?

There are generally more independent reflections than torsion angles at 5 Å resolution

Problems:

- poor observable to parameter ratio
- electron density maps may be difficult to interpret
- potential model bias

Need for powerful reciprocal and real space methods

Outline

- Overview of DEN-refinement
- MR for a new structure at 3 Å (high B-factors)
- Refinement at 7.4 Å resolution

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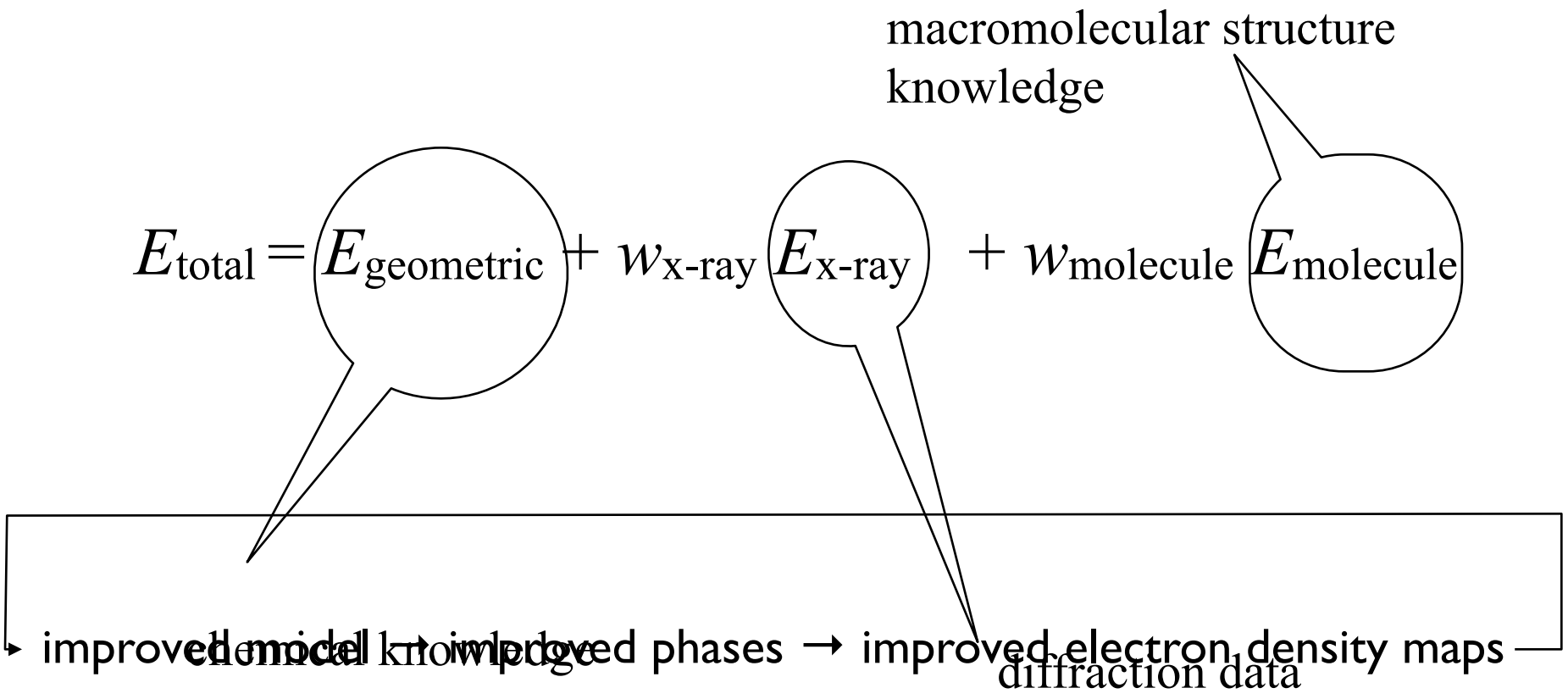
Target Function For Macromolecular Refinement



DEN (Deformable Elastic Network) Restraints

$$E_{\text{total}} = E_{\text{geometric}} + w_{\text{x-ray}} E_{\text{x-ray}} + w_{\text{DEN}} E_{\text{DEN}}$$

$$E_{\text{DEN}}(n) = \sum_{N\ pairs\ i,j} \left(d_{ij} - d_{ij}^{0}(n)\right)^{p}$$

Reference model ➔ selected inter-atom distances ➔sparse distance network $d^0_{ij}(n)$

- randomly selected atom pairs within a specified distance range, and separated by a specified interval in the primary sequence
- typically ~ 1 distance restraint per atom

***Schröder**, Levitt, Brunger, Nature 2010*

Implementation Of The DEN Method

Several (typically 10) macrocycles consisting of:

- torsion angle refinement (slow-cooling molecular dynamics)
- default option: restrained grouped *B*-factor refinement
- default option: last two cycles are without DEN restraints

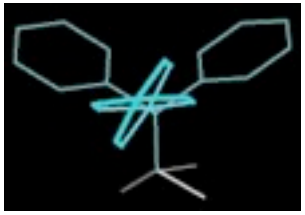
Target function:

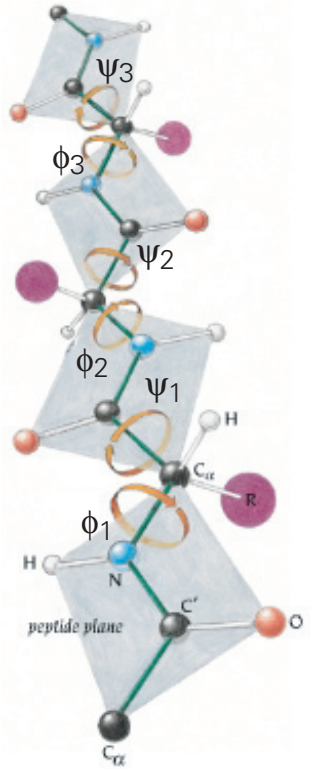
$$E_{total} = E_{geometric} + w_a E_{ML} + w_{DEN} E_{DEN} \qquad E_{DEN}(n) = \sum_{N\ pairs\ i,j} \left(d_{ij} - d_{ij}^0(n)\right)^p$$

During slow-cooling cycles, periodic updates of the DEN equilibrium distances are performed

$$d_{ij}^0(n+1) = (1-\kappa) d_{ij}^0(n) + \kappa\left[\gamma d_{ij} + (1-\gamma) d_{ij}^{ref}\right]$$

γ: *deformation factor* (adjustable)
w_{DEN}: *weight* for DEN restraints (adjustable)
κ: damping factor (usually set to 0.1)
d^{ref}_{ij}: distances from reference model
$d^{o}_{ij}(n)$: current DEN restraint distances
d_{ij}: current distances of refinement model





Multiple trials for each (γ, w_{DEN}) parameter pair and temperature
Trial with the best R_{free} is used for subsequent steps

How Does It Work?

- DEN is a *general* refinement method that guides torsion angle molecular dynamics by restricting it to reasonable conformational changes (e.g., differences between homologous models or motions)
- DEN introduces information that is specific for the particular starting structure (e.g., homology model)
- Degree of deformation is determined by γ
  - $\gamma=0$: no deformations of reference distances allowed
  - $\gamma=1$: deformations track refined model (akin "jelly body")
  - $0<\gamma<1$: deformations allowed according to some interpolation between current and reference distances
- Grid search for best (γ, w_{DEN}) parameters and annealing temperature

Some Notes And Considerations

- General modes
  - New refinement: initial model = reference model
  - Re-refinement: initial model ≠ reference model
- Special options
  - DEN-restraints active throughout
  - w_{DEN}=0 ↔ torsion angle simulated annealing (without DEN)
- Implementation in CNS 1.3 and Phenix.refine (development)
- Resource for grid search: SBGrid Science Portal (www.sbgrid.org)

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Structure Of Cgl1109 (JCSG HP3342)
(A Putative Succinyl-Diaminopimelate Desuccinylase (dapE) From *C. Glutamicum*)

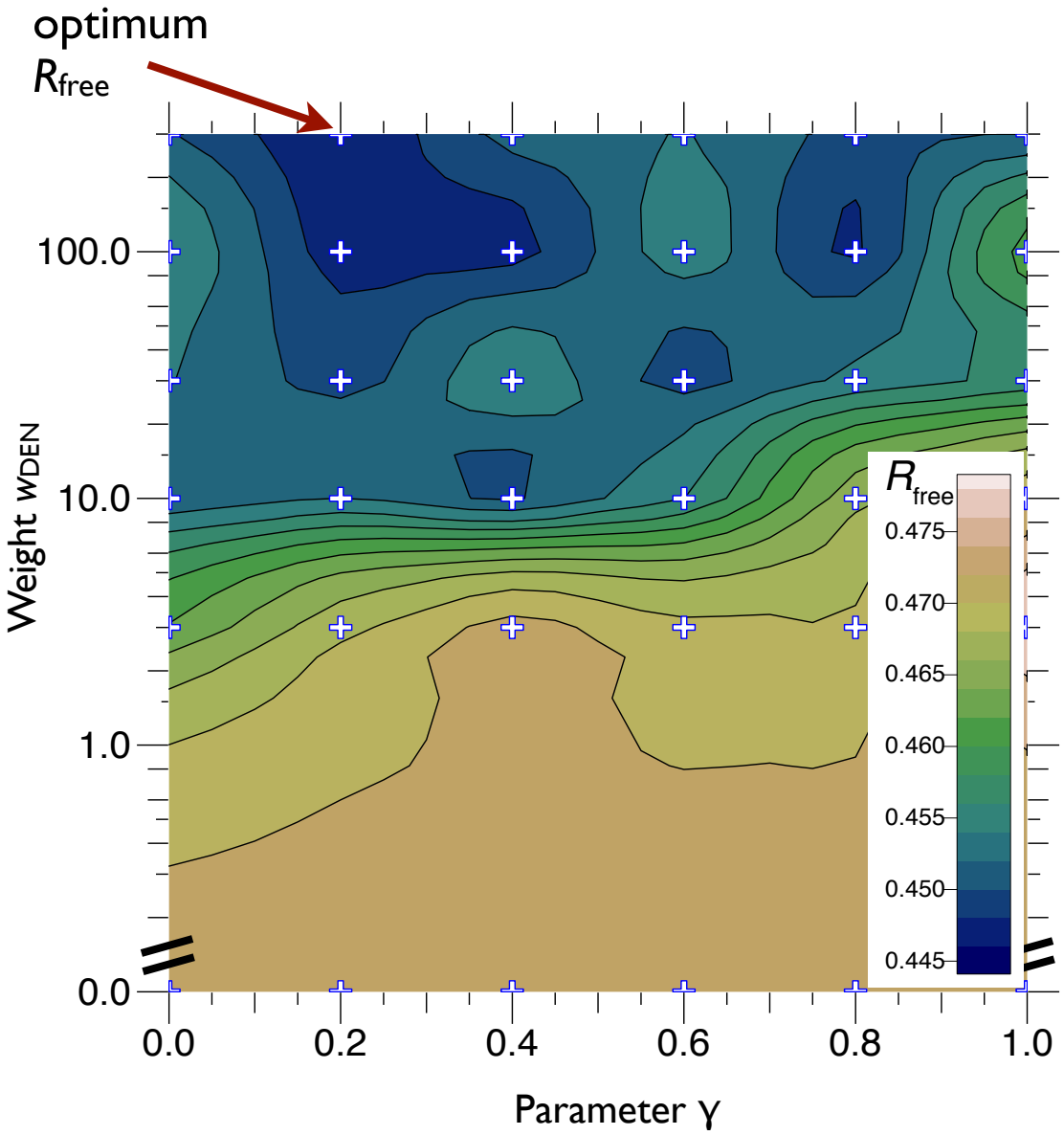
Collaboration with D. Das, A.Deacon, J.Grant, T. Terwilliger, R. Read, P.Adams, M. Levitt, G. Schröder

- Anisotropic diffraction data to ~ 3 Å resolution
- MAD data available, but experimental map was not easily interpretable
- Weak homology to known structures (1vgy with 25% identity)
- One of the cases used by DiMaio et al. (2011)
- MR solution for both 1vgy and Modeller model of Cgl1109 (Phaser: RFZ=3.2, TFZ=9.9, LLG=75, R_{cryst}=0.65)

DEN Refinement
+ Automated Model Building With AutoBuild

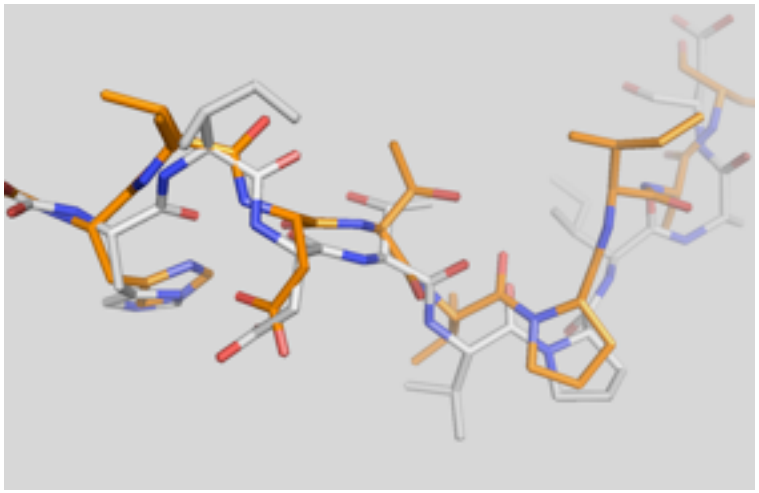
- Reference model = initial model = MR solution (search model: Modeller homology model)
- Standard DEN protocol: but restrained individual *B*-factor refinement
- AutoBuild with "morphing" and "rebuild in place"

Optimization Of DEN Parameters

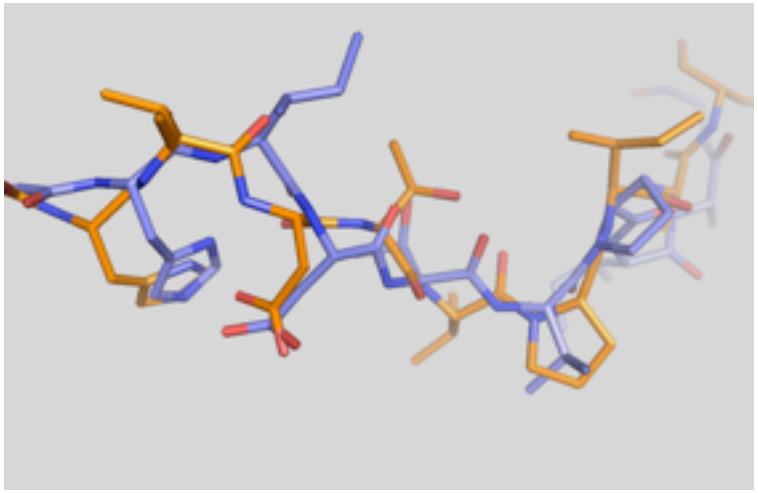


Larger Radius Of Convergence For DEN Vs. Standard Refinement

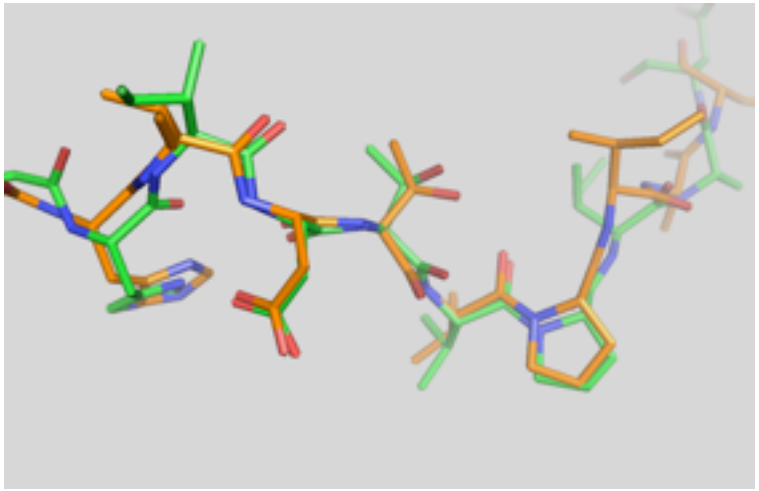
Standard refinement (R_{free}=0.52) vs. final (orange)



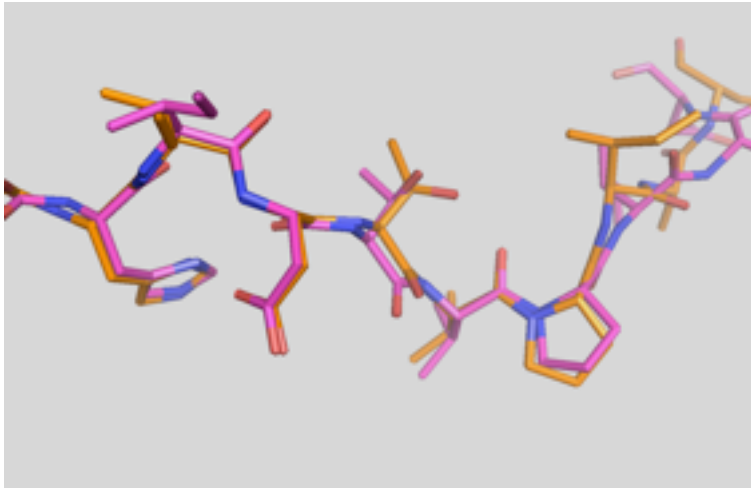
Standard ref. + AutoBuild (R_{free}=0.48) vs. final (orange)



DEN (R_{free}=0.44) vs. final (orange)

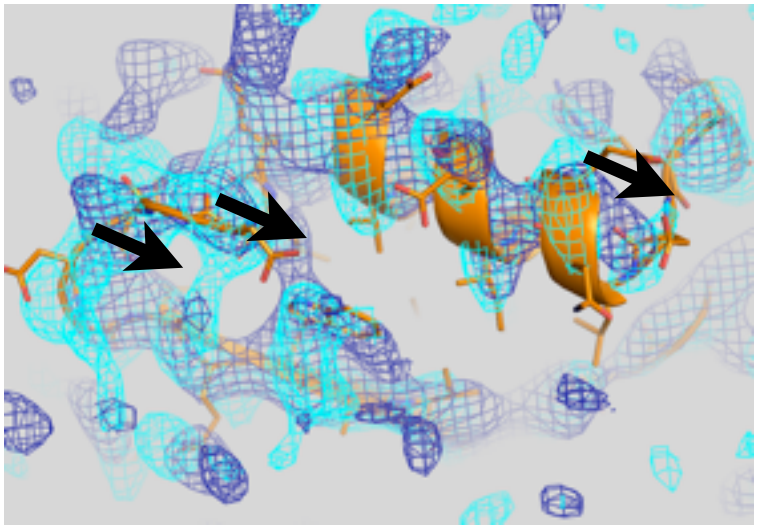


DEN + AutoBuild (R_{free}=0.42) vs. final (orange)

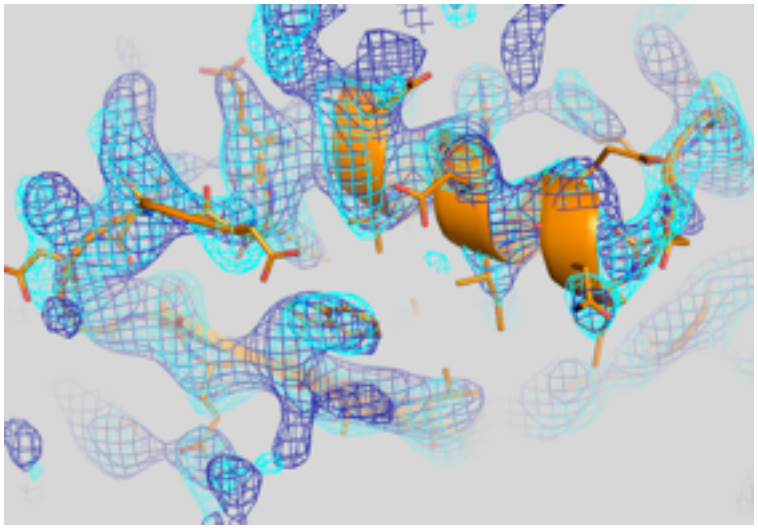


DEN-Refinement Produced Better $2mF_o$-DF_c Maps Than Standard Refinement

Standard ref. (blue) + AutoBuild (cyan)

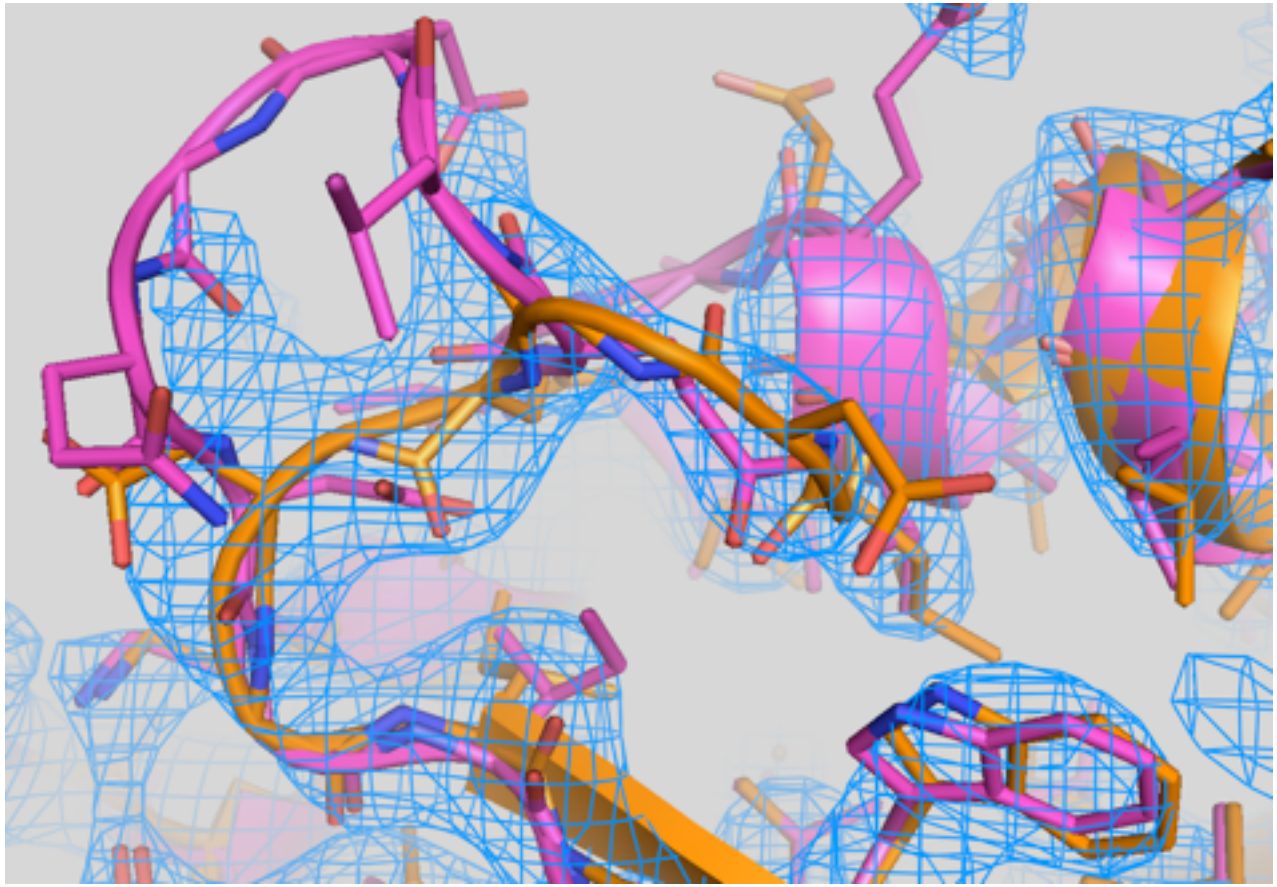


DEN (blue) + AutoBuild (cyan)



Orange: final model

DEN+Autobuild Map Showed How To Correct The Model

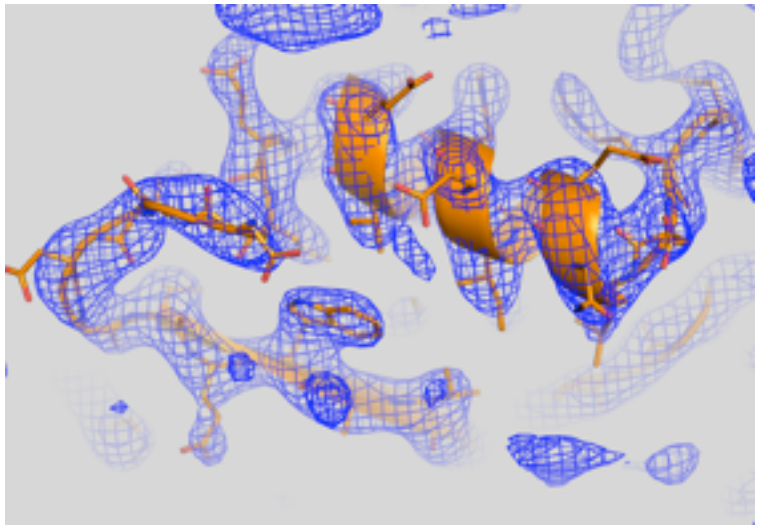


Magenta: model after first round of DEN+AutoBuild
Cyan: corresponding DEN+AutoBuild $2mF_o$-DF_c map
Orange: final model

Final Model

R_{free}	0.258
R_{cryst}	0.234
Ramachandran favored	92.7%
Ramachandran outliers	0.8%
Molprobity score	2.41 (96th percentile)

Final $2mF_o$-DF_c



Results For The Refinement Of Cgl1109

- Synergism between DEN-refinement and AutoBuild
- Improved model ➔ improved phases ➔ better starting point for AutoBuild
- Semi-automated completion of the structure with AutoBuild

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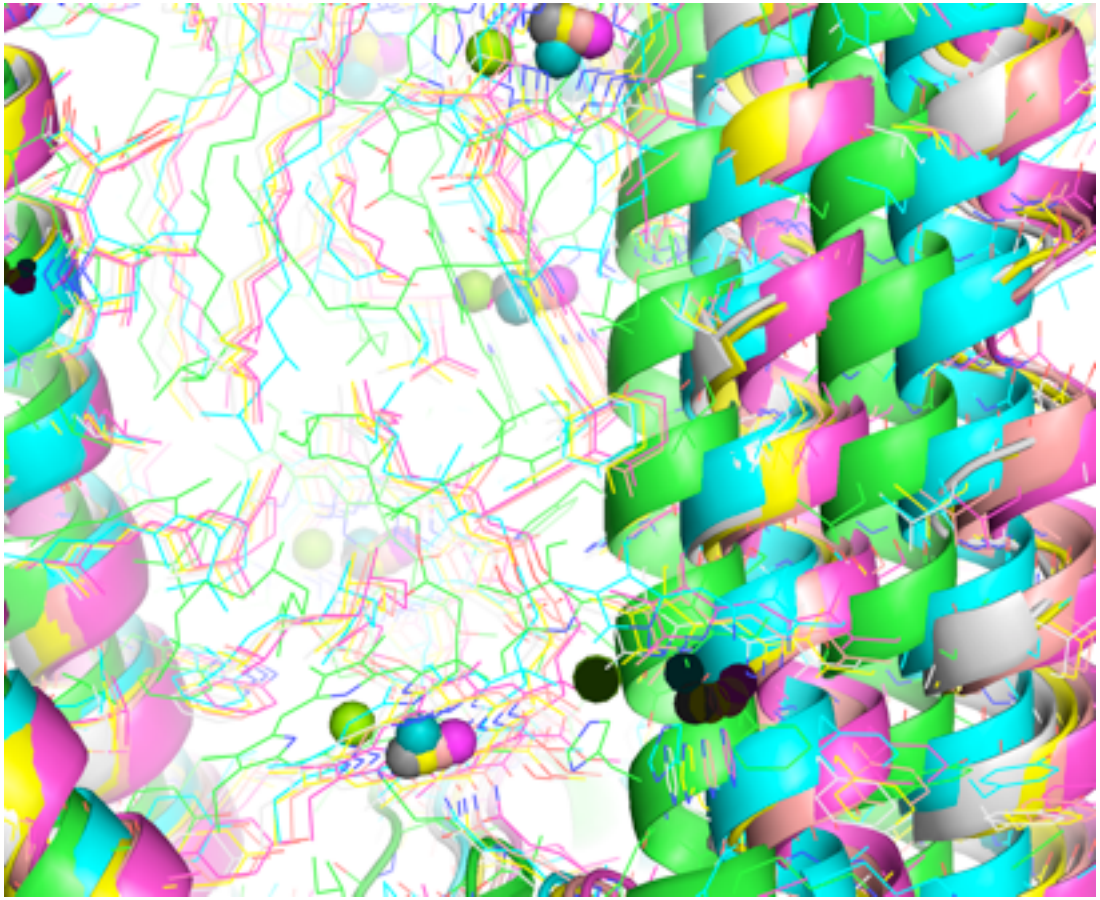
Refinement Of Photosystem I (PSI) At 7.4 Å Resolution

Collaboration with Henry Chapman, Thomas White (CFELS, DESY), Petra Fromme, Raimund Fromme (Univ. of Arizona)

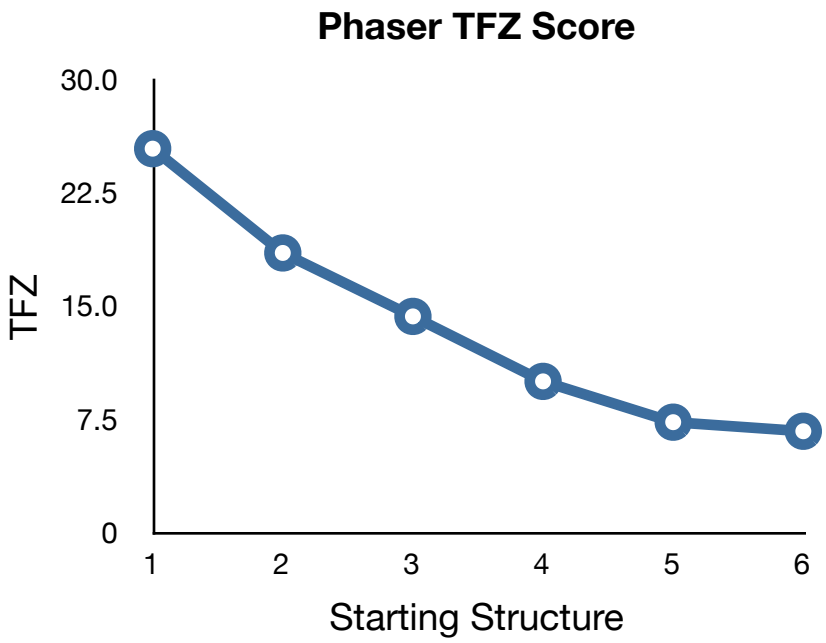
- DEN-refinements using synchrotron (ALS) data of a PS1 crystal in harvesting buffer used for nano-crystal generation (Chapman et al., Nature, 2011), d_{min} ~ 5 Å
- Data truncated to 7.4 Å to make it comparable to FEL (LCLS) data of PS1
- Increasingly scrambled starting models (Cα rmsd to target 1JB0: 0, 2.3,...,4.4 Å)
- Omitted three Fe-S clusters (for validation)
- Reference model = starting model
- Standard DEN protocol, except DEN active throughout, sparse random selection between all atoms, and only overall *B*-factor refinement

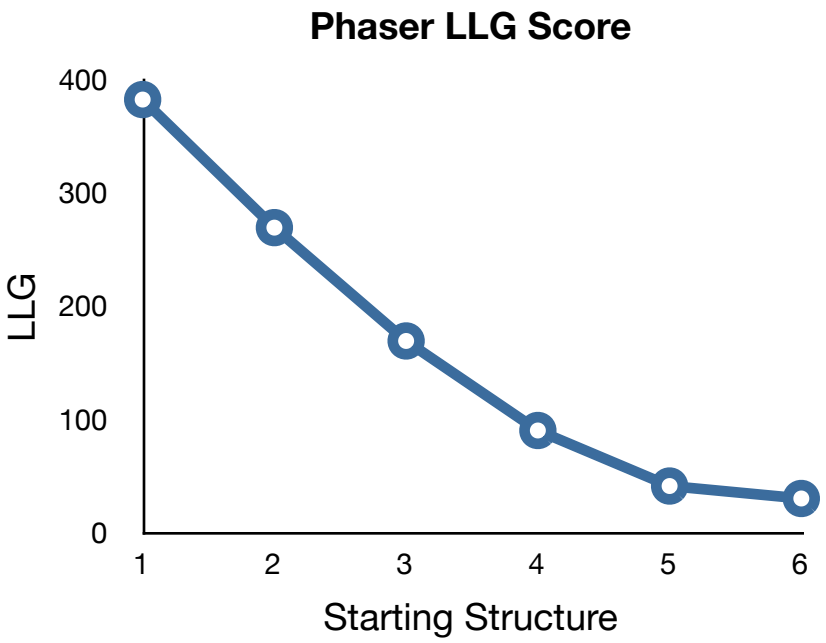
Increasingly Scrambled Starting Structures



Green: PS1 (PDB ID1JB0)
Magenta: maximum scrambled structure
(Cα rmsd: 4.4 Å, right most helix displaced by 5.4 Å)

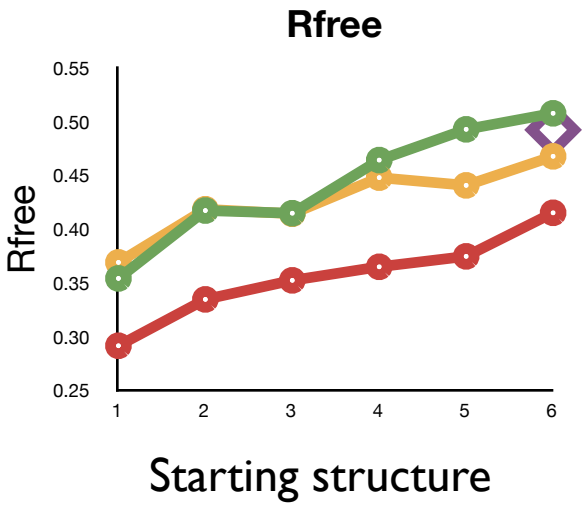
Molecular Replacement (7.4 Å Resolution)

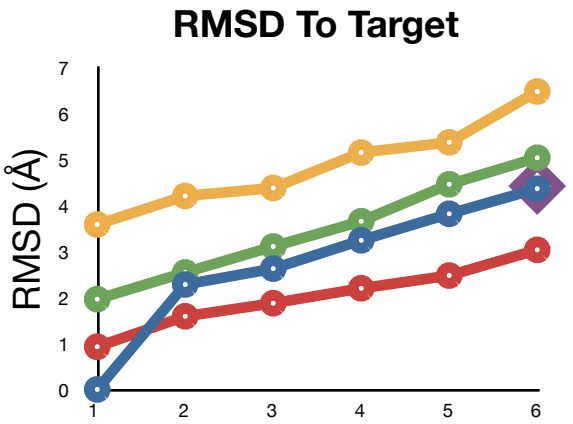




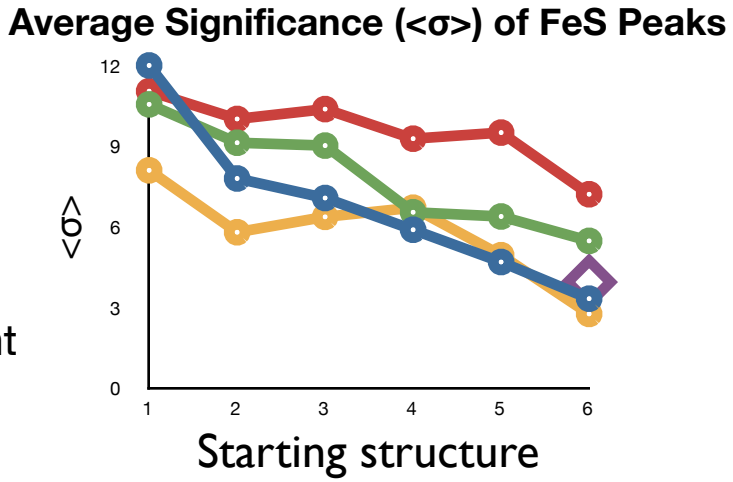
Solutions were found for all starting structures

Comparison Of Refinement Protocols



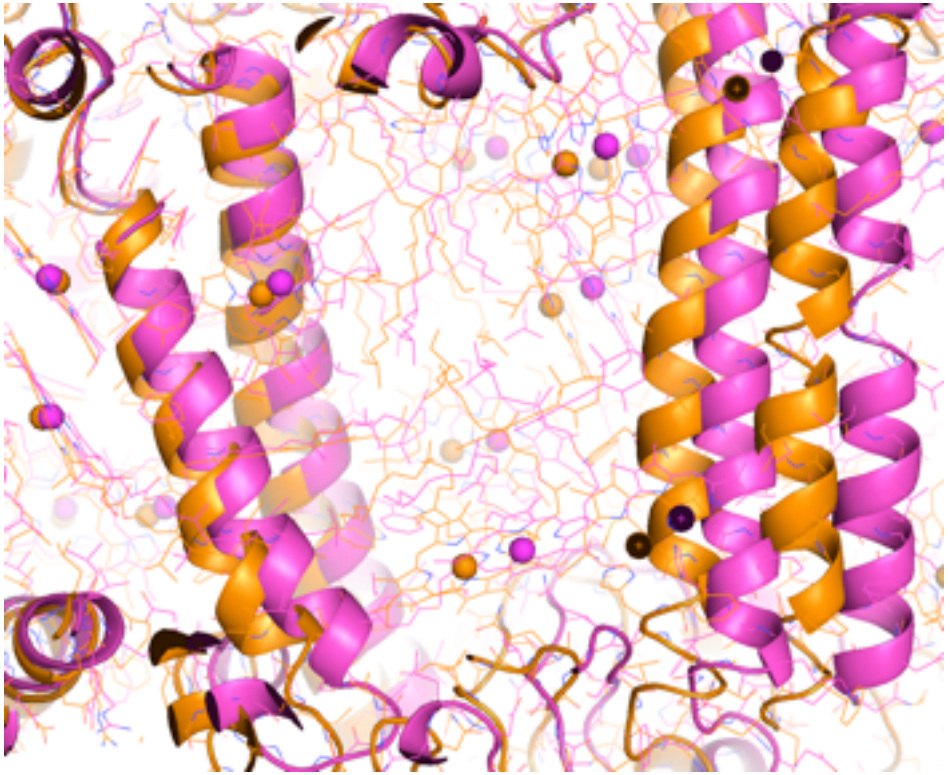


- overall rigid body refinement
- standard refinement
- torsion simulated annealing refinement
- DEN-refinement
- secondary structure restrained refinement

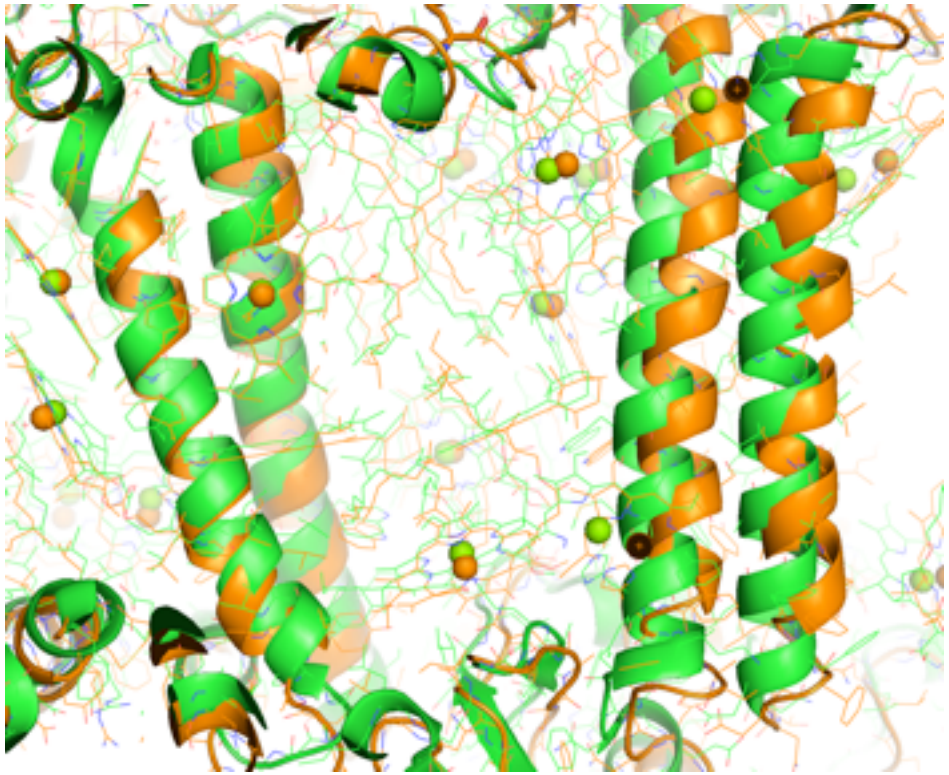


Results For Maximally Scrambled Starting Structure

Mg ions are shown as spheres



DEN (orange) vs. starting (magenta)

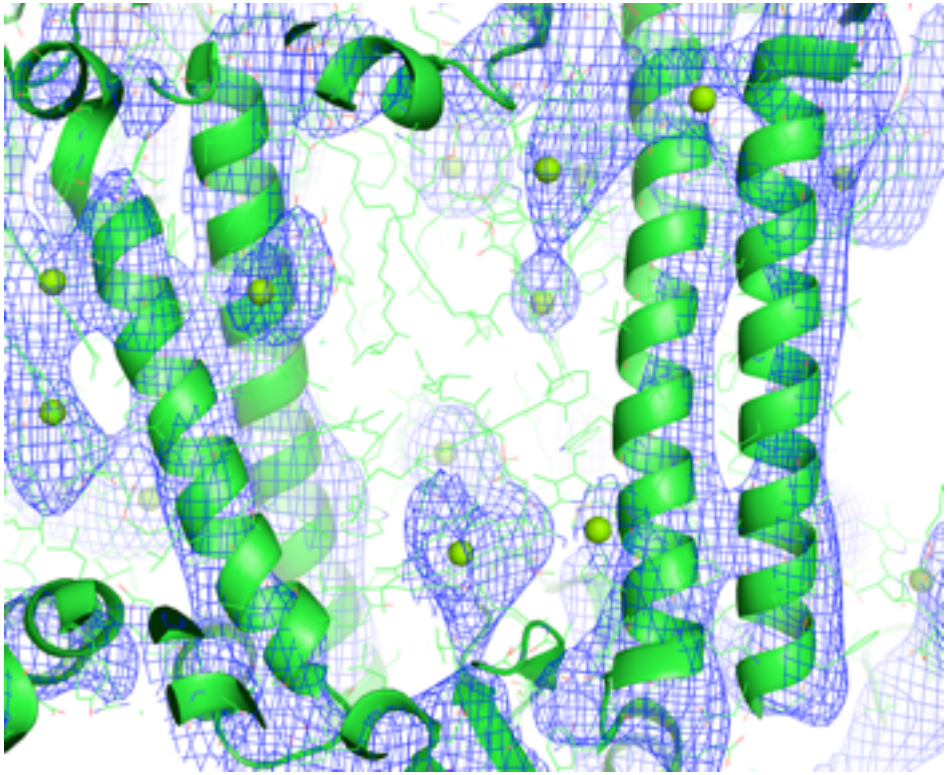


DEN (orange) vs. target 1JB0 (green)

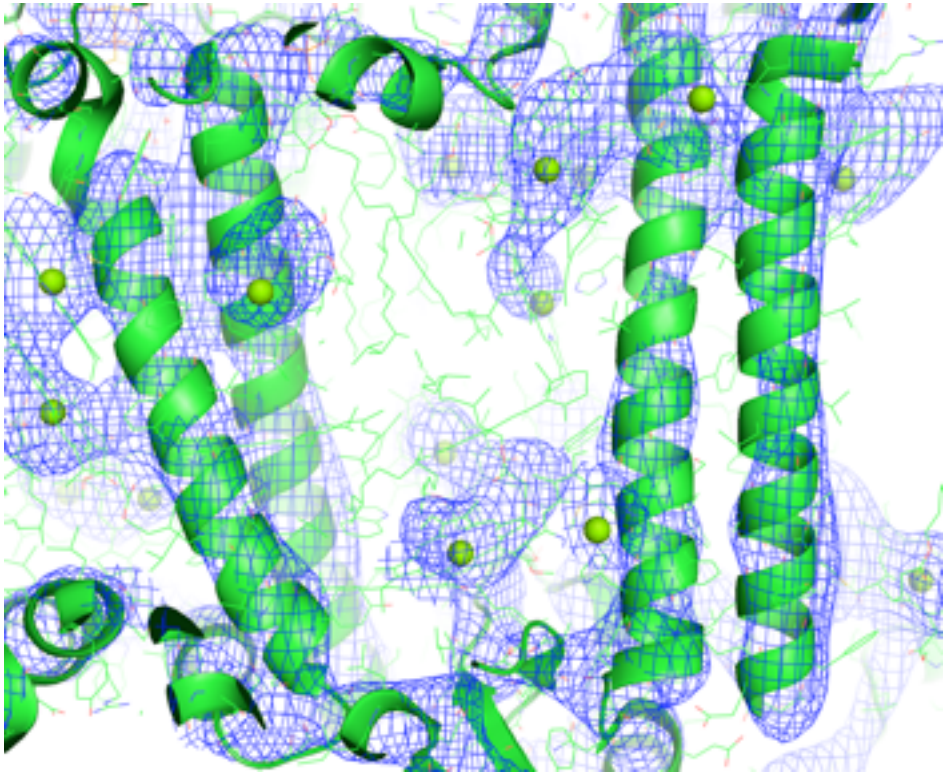
DEN-refinement shifted helices by 5.5 Å

Results For The Maximally Scrambled Starting Structure

Mg ions are shown as spheres



DEN $2mF_o$-DF_c map (blue)
vs. target 1JB0 (green)

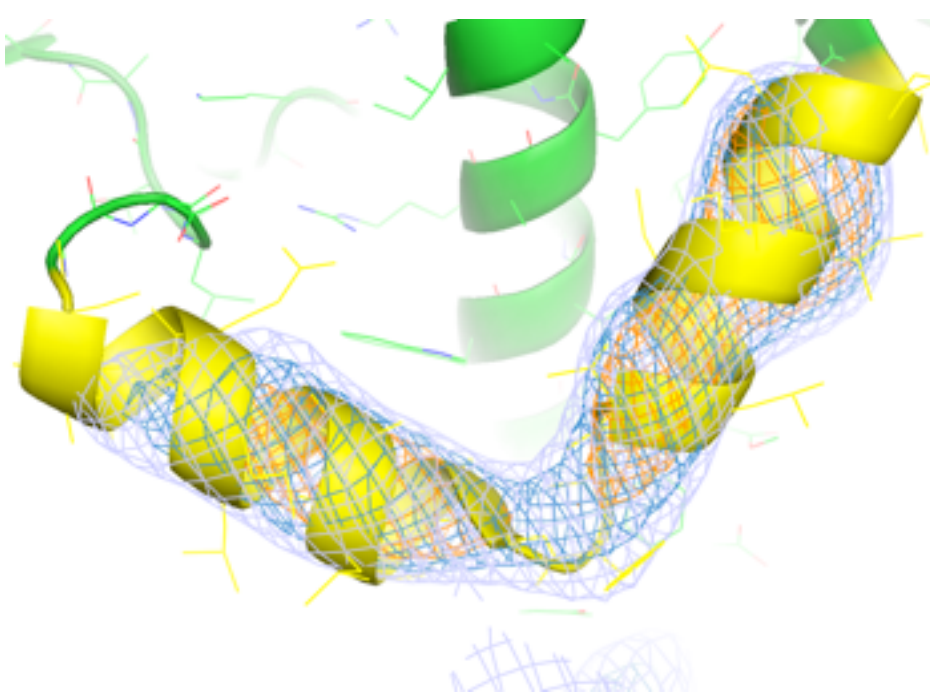


target $2mF_o$-DF_c map (blue)
vs. target 1JB0 (green)

1.5 σ contour level

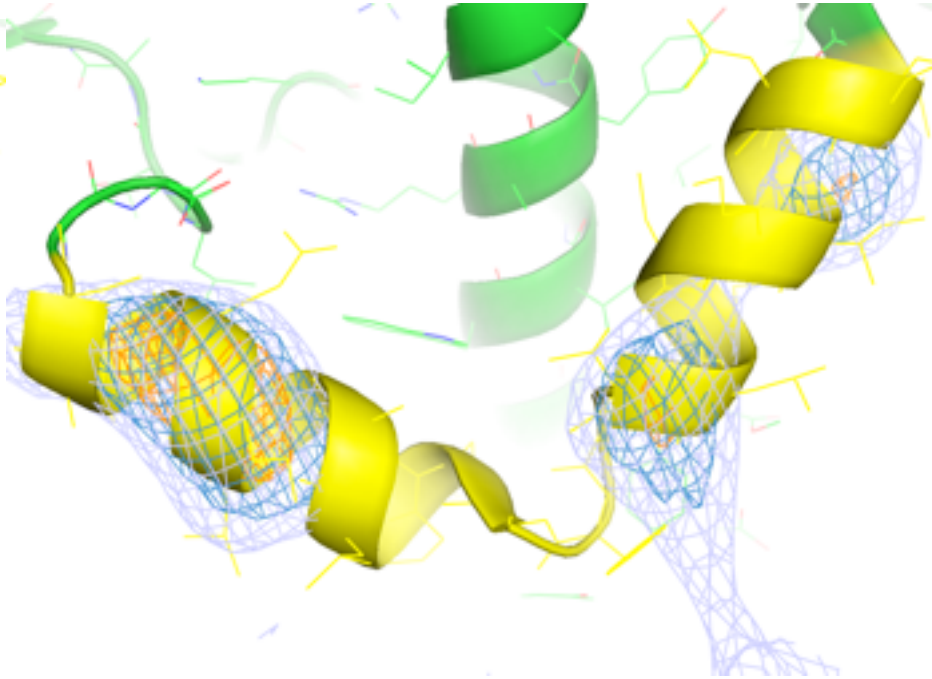
DEN-Refinements With Two Helices Omitted (Chain F, Residues 103:126 - Yellow)

DEN-refinement starting from 1JB0



DEN mF_o-DF_c map (mesh)
vs. target 1JB0 (green and yellow)

DEN-refinement starting from max. scrambled

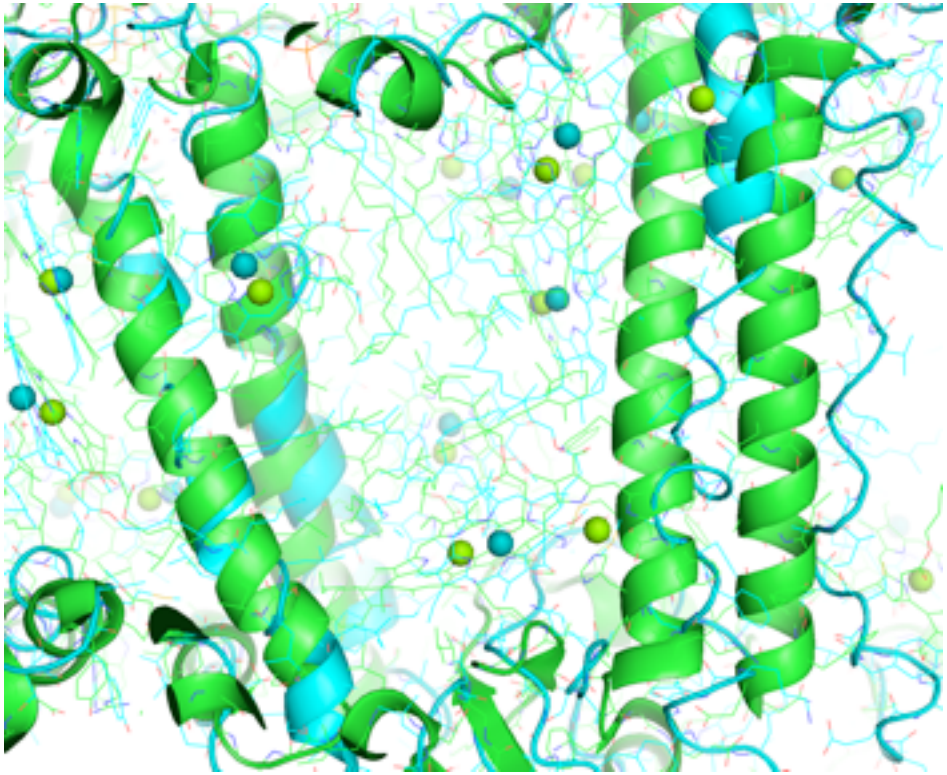


DEN mF_o-DF_c map (mesh)
vs. target 1JB0 (green and yellow)

3 (orange), 2.5 (blue), 2 σ (light blue) contour levels

Results For The Maximally Scrambled Starting Structure: Standard Refinement

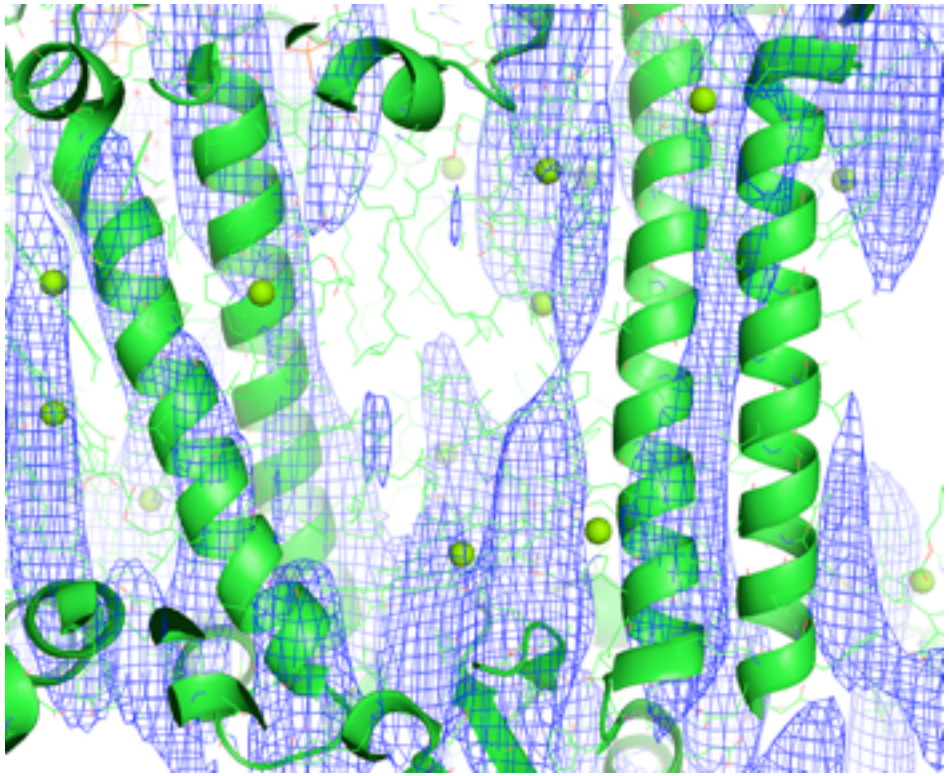
Mg ions are shown as spheres



standard refinement (teal)
vs. target 1JB0 (green)

Results For Maximally Scrambled Starting Structure: Standard Refinement

Mg ions are shown as spheres



standard refinement $2mF_o$-DF_c map (blue)
vs. target 1JB0 (green)

1.5 σ contour level

DEN-Refinement At 7.4 Å Resolution Is Beneficial

Compared to overall rigid body, standard, or simulated annealing refinement, DEN-refinement

- moves closer to the true structure
- recovers information not included in the refined model - more significant features in difference maps

Overall Conclusions For DEN-Refinement

- DEN-refinement is a general method that can produce better models than standard refinement or simulated annealing protocols
- DEN-refined model phases are a better starting point for (automated) model building
- Most benefit is for starting structures that are far from the true structure, low-resolution, or high B-factor cases

Acknowledgments

DEN refinement

Gunnar Schröder, Michael Levitt

Cgl1109/HP3342 structure determination

Debanu Das, Tom Terwilliger, Randy Read, Paul Adams, et al.

DEN-refinement of photosystem 1

Henry Chapman, Thomas White, Petra Fromme, Raimund Fromme, et al.

SBGrid Science Portal

Dan O'Donovan, Piotr Sliz