phaser and phasing

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1

software for phasing

Score: find the pose that best fits the data

$$LLGI = \sum_{hkl_{info}} log\left[\frac{2E_e}{1-D_{obs}^2\sigma_A^2}exp\left(-\frac{E_e^2+D_{obs}^2\sigma_A^2E_C^2}{1-D_{obs}^2\sigma_A^2}\right)I_0\left(\frac{2E_eD_{obs}\sigma_AE_C}{1-D_{obs}^2\sigma_A^2}\right)\right]$$

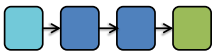
Search: strategies for exploring poses

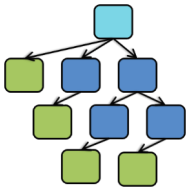
2

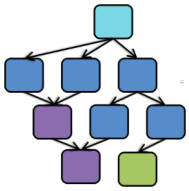
2

process flow

- path
- tree
- directed acyclic graph



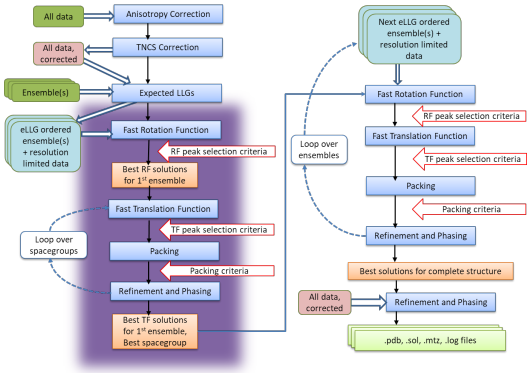




- termination condition

3

3



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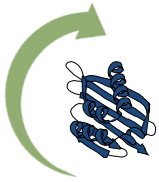
brute rotation function search

- Place model at orientations and calculate probability of each being correct



$$LLGI = \sum_{\mathbf{h}} \log\left(\frac{2E_e}{1-D_{obs}^2\sigma_A^2}\exp\left(-\frac{E_e^2+D_{obs}^2\sigma_A^2E_C^2}{1-D_{obs}^2\sigma_A^2}\right)I_0\left(\frac{2E_eD_{obs}\sigma_AE_C}{1-D_{obs}^2\sigma_A^2}\right)\right)$$

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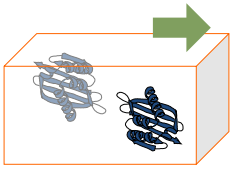
5

brute translation function search

- Place model at points in unit cell and calculate probability that it is in each position

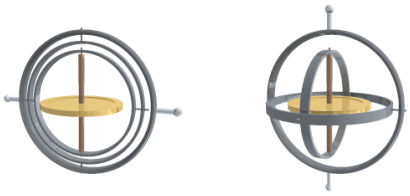


$$LLGI = \sum_{\mathbf{h}} \log\left(\frac{2E_e}{1-D_{obs}^2\sigma_A^2}\exp\left(-\frac{E_e^2+D_{obs}^2\sigma_A^2E_C^2}{1-D_{obs}^2\sigma_A^2}\right)I_0\left(\frac{2E_eD_{obs}\sigma_AE_C}{1-D_{obs}^2\sigma_A^2}\right)\right)$$

6

6

euler angles



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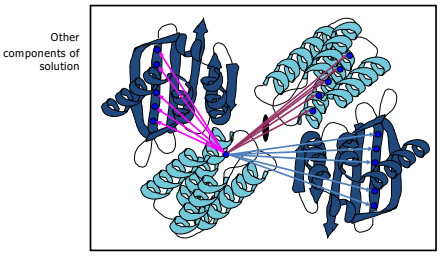
7

packing

- clash test



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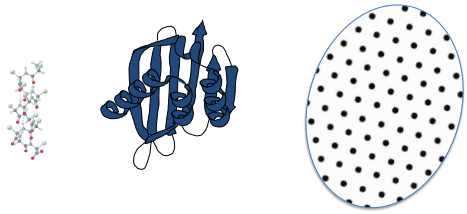
8

packing

- Small – Medium – Large
- all atoms – Cα atoms – Hexagonal Grid



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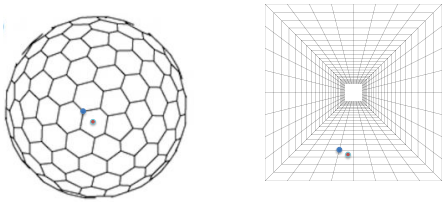
9

refinement

- Rotation and Translation searches are scored on a grid



$$LLGI = \sum_{\mathbf{h}} \log\left(\frac{2E_e}{1-D_{obs}^2\sigma_A^2}\exp\left(-\frac{E_e^2+D_{obs}^2\sigma_A^2E_C^2}{1-D_{obs}^2\sigma_A^2}\right)I_0\left(\frac{2E_eD_{obs}\sigma_AE_C}{1-D_{obs}^2\sigma_A^2}\right)\right)$$

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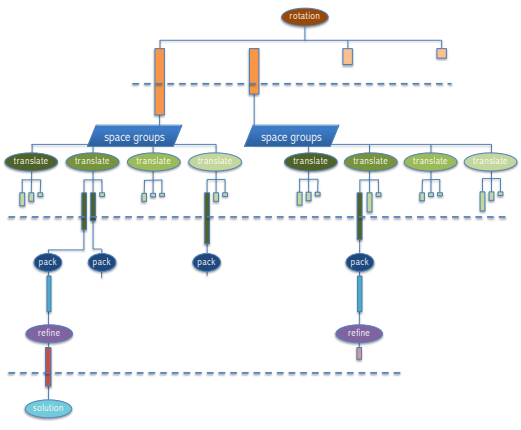
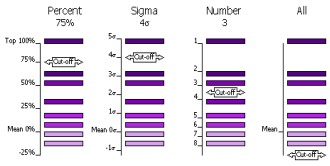


11

11

peak selection

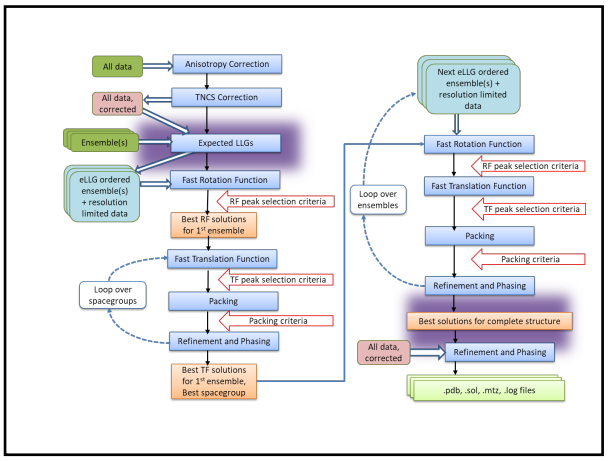
- Must chose a selection criteria to carry possible solutions through to the next step



- By default, solutions over 75% of the difference between the top peak and the mean are selected

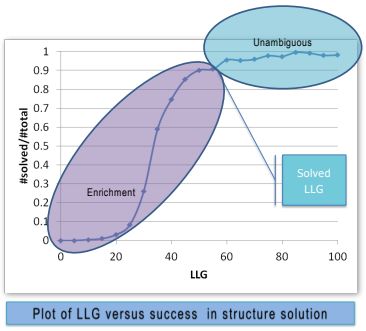
12

12



13

log-likelihood gain for solutions



Database of over 23000 MR problems

Plot of LLG versus success in structure solution

R.D. Oeffner

14

14

log-likelihood gain for solutions

TF Z-score	LLG score	Solved?
< 5	< 25	no
5 - 6	25 - 36	unlikely
6 - 7	36 - 49	possibly
7 - 8	49 - 64	probably
> 8	> 64	definitely

15

15

predicting log-likelihood gain

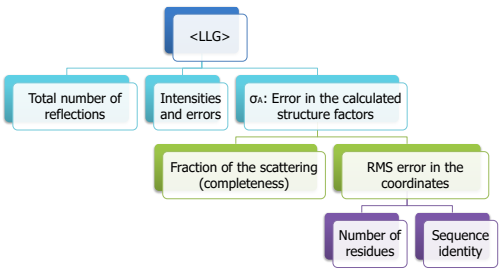
- If you can predict the log-likelihood gain you know how easy/difficult will be molecular replacement
- The expected log-likelihood gain for a model is calculated by "integrating out" the calculated structure factors from the equation
  - The error in the calculated structure factors (σ_A) is included
- We have a similar analysis for SAD phasing*
- Used to guide decision making at every step of the search strategy
  - Prioritize resources

*unpublished

16

16

expected log-likelihood gain



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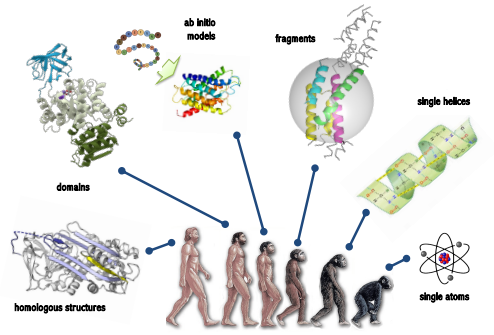


18

fragments

- Fragments are just models with low completeness
  - Low σ_A
- Success of fragment based MR relies on other contributions to the <LLG> being favourable
  - Lots of reflections
  - Low RMS

Success does NOT depend on
- The resolution (strictly)
- The type of fragment

19

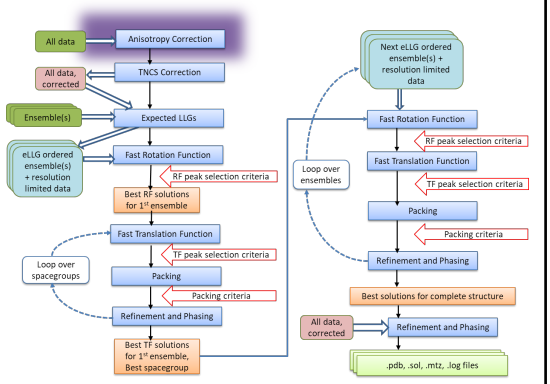
19

What chance of success?

- *Better than 40% identity: usually easy, unless large conformational changes are involved.*
- *30-40%: MR possible, but sometimes more difficult.*
- *20-30%: MR difficult, careful model preparation required.*
- *Less than 20%: MR unlikely to work in most cases.*
- *In terms of RMSD, above 2.5A is very unlikely to work, while 1.5A or less is preferable.*

20

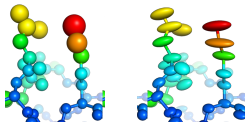
20



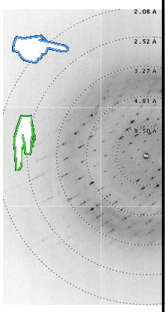
21

Anisotropy

- Systematically weak and strong diffraction directions
- Caused by underlying anisotropic movement of atoms



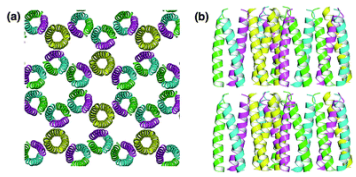
Visualization of individually refined isotropic (left) versus anisotropic (right) B-factors for a high-resolution structure



22

22

Anisotropy in coiled coils



(a) Honeycomb-like P6 crystal lattice of a computationally-designed coiled-coil protein building block. (b) The layer structures were stacked by hydrogen bond interactions between layers. 4DAC

23

23

Anisotropy

Before

- Easy phasing cases and/or mild anisotropy, can ignore
- In difficult cases, must corrected before structure solution
- Global Anisotropic B-factors calculated with reference to the Wilson distribution

After

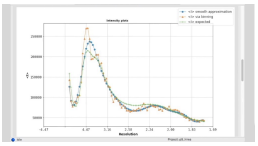
- Corrections will be better after structure solution when you have a model
- Global Anisotropic B-factor calculated with reference to calculated structure factors
  - TLS
  - Atomic anisotropic B-factors

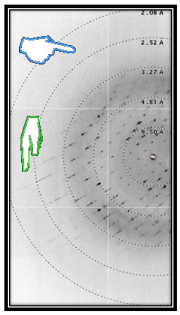
24

24

Wilson Plot

- Correct data with reference to the Wilson plot
- Make intensity distribution the same in all directions by refining parameters of the anisotropy tensor





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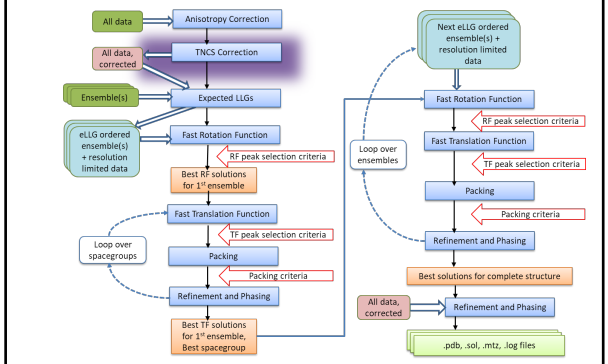
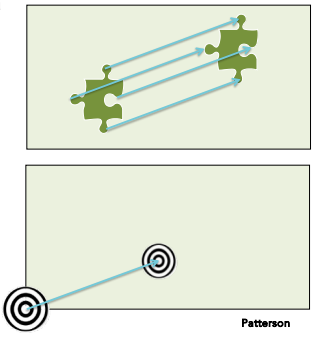


26

Translational NCS

- Molecules related by a vector translation
- Patterson shows a single vector
  - and symmetry copies



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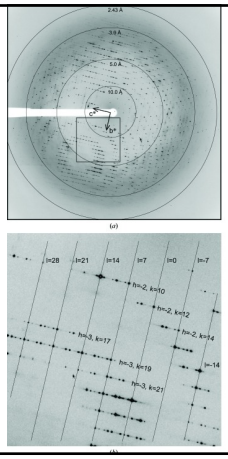
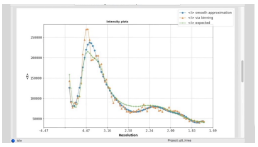


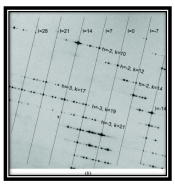
Hyp-1 (PR-10)
(a) A sample full image, with resolution rings and reciprocal-lattice directions indicated,
(b) Enlarged fragment on which layer lines of constant l = 7n are marked and annotated
(c) A histogram of intensity distribution in layers of l.

28

Wilson Plot

- Correct data with reference to the Wilson plot
- Make intensity distribution in tncs modulation directions the same by refining parameters of the tncs





29

29

TNCS

Before

- In difficult cases, must corrected before structure solution
- TNCS parameters calculated with reference to the Wilson distribution
- TNCS parameters generate epsilon factors for each reflection, to make them closer to the Wilson distribution

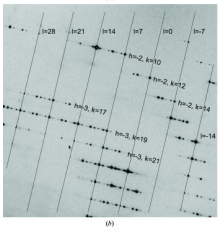
After

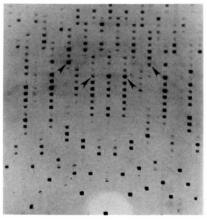
- Corrections will be better after structure solution when you have a model
- TNCS parameters calculated with reference to the oriented and placed models in the asymmetric unit

30

TNCS and Twinning

- TNCS – broadens intensity distribution
- Twinning – narrows intensity distribution





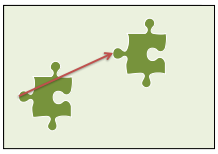
31

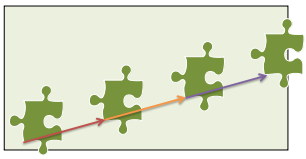
31

PHASER: Two classes of tNCS

Pairs of molecules related by one vector

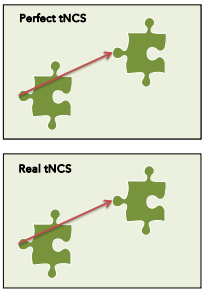
Molecules related by multiples of one vector





32

Translational NCS: Pairs

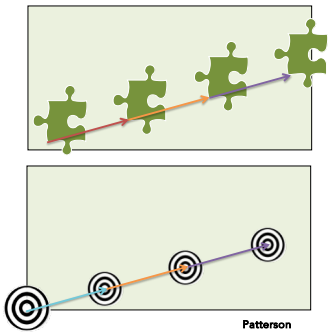


- Three TNCS parameters refined from data alone
  1. Rotation
  2. Translation
  3. RMSD between copies

33

33

Translational NCS: Multiples



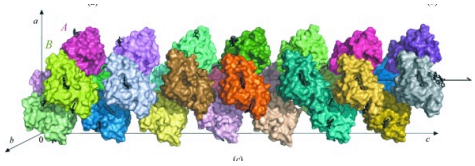
- Two TNCS parameters refined from data alone
  1. Translation
  2. RMSD between copies

34

34

Hyp-1 (PR-10)

- Repeats 7/2 along c* (sevenfold translational NCS)



Acta Cryst D STRUCTURAL BIOLOGY

Likelihood-based molecular-replacement solution for a highly pathological crystal with tetartohedral twinning and sevenfold tNCS Sliwiak J, Jaskolski M, Dauter Z, McCoy AJ, Read RJ. Acta Cryst D70, 471 (2014)

35

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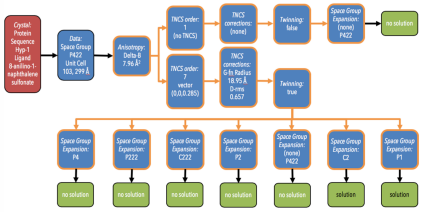
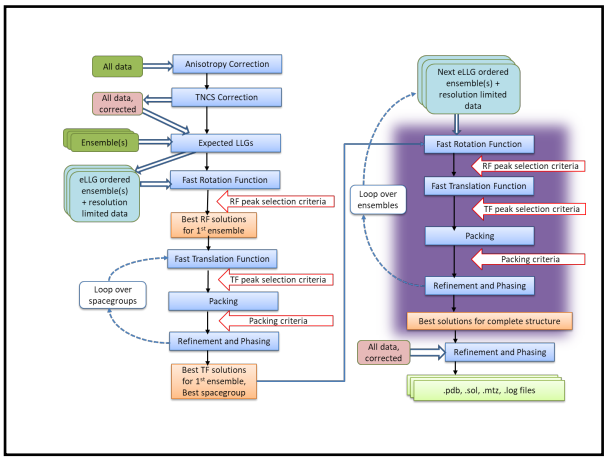


36



37

Matthew's coefficient

- First calculated by Brian Matthews in 1968 (over 3500 citations)
- Most crystals are 50% protein by volume
- Can be used to estimate the contents of the asymmetric unit
- *Self Rotation Function*
- *TNCS Order*

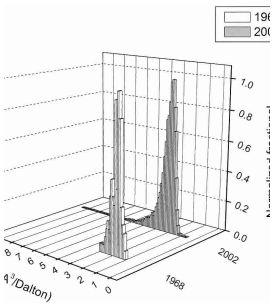
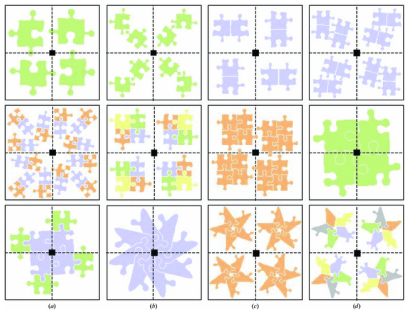


Figure 1: Kantardjieff and Rupp (2003)

38

38

contents of the asymmetric unit



39

39

using partial structure

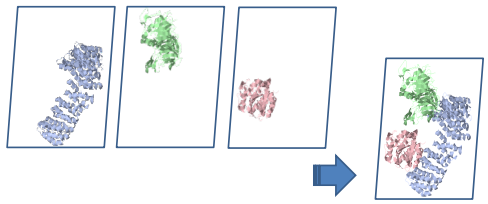
- The maximum likelihood rotation and translation functions can use models that have already been placed in the asymmetric unit
- Patterson Rotation Function cannot account for placed models
  - The Patterson Correlation Coefficient Translation Function can account for known positions (but not their errors)
  - Most of the difficulty in molecular replacement is the rotation function, because the signal to noise ratio is much lower

40

40

searches for multiple components

- It may not be possible to find each component in a separate search

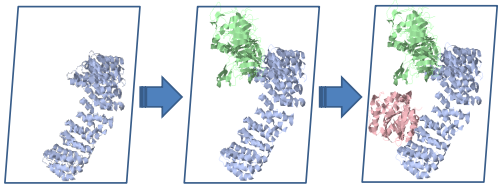


41

41

searches for multiple components

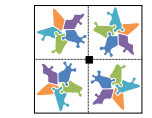
- maximum likelihood functions include partial structure information from previous placements
- structure builds up by addition



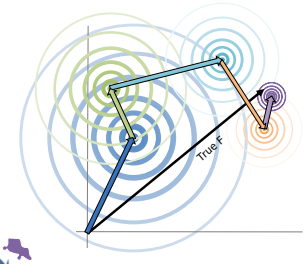
42

42

σ_A and fragments



Structure builds by addition



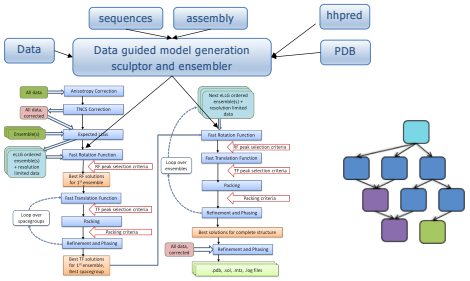
Whenever a component is placed, the variances are reduced, thus increasing the signal-to-noise of the search for the next component

43

43

phaser.voyager

- Data guided, statistical model generation
- DAG tracking and control



44

44

Acknowledgements

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Tristan Croll
Claudia Millán

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Isabel Usón
Iracema Caballero

BERKELEY LAB

Billy Poon
Pavel Afonine

Phenix
CCP4

45

45