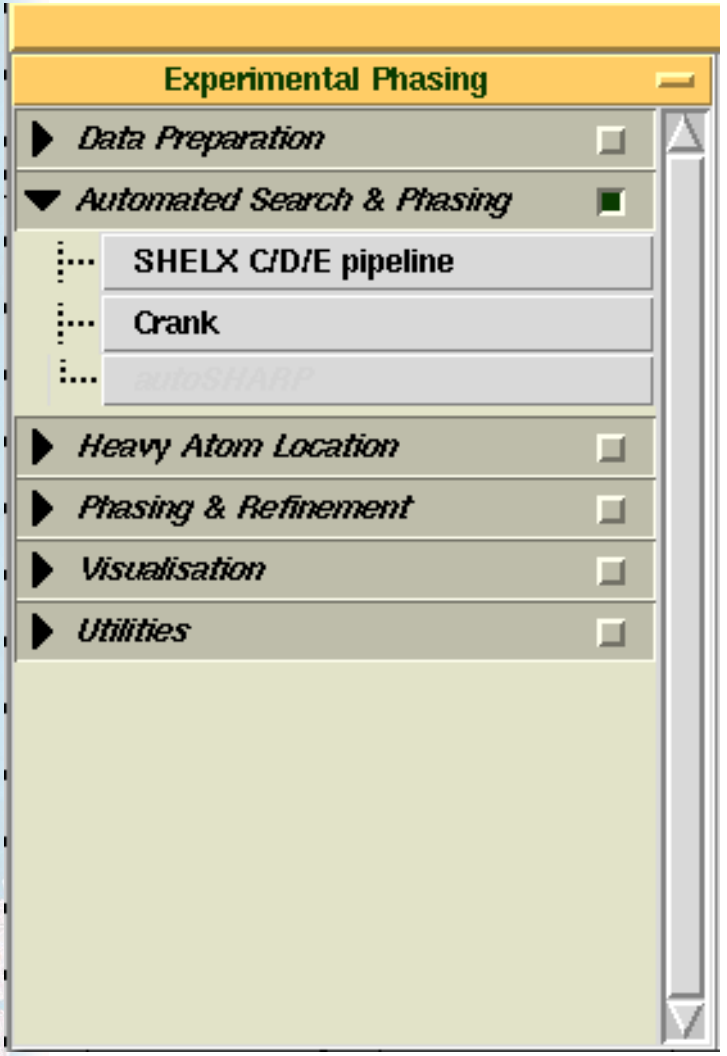
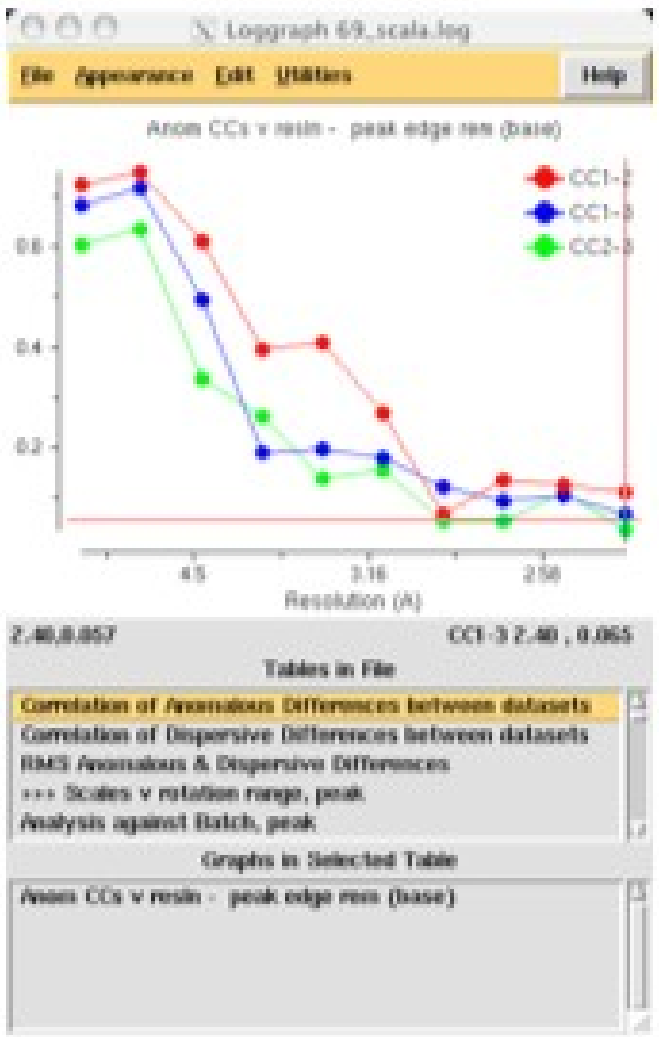
Experimental Phasing with CCP4



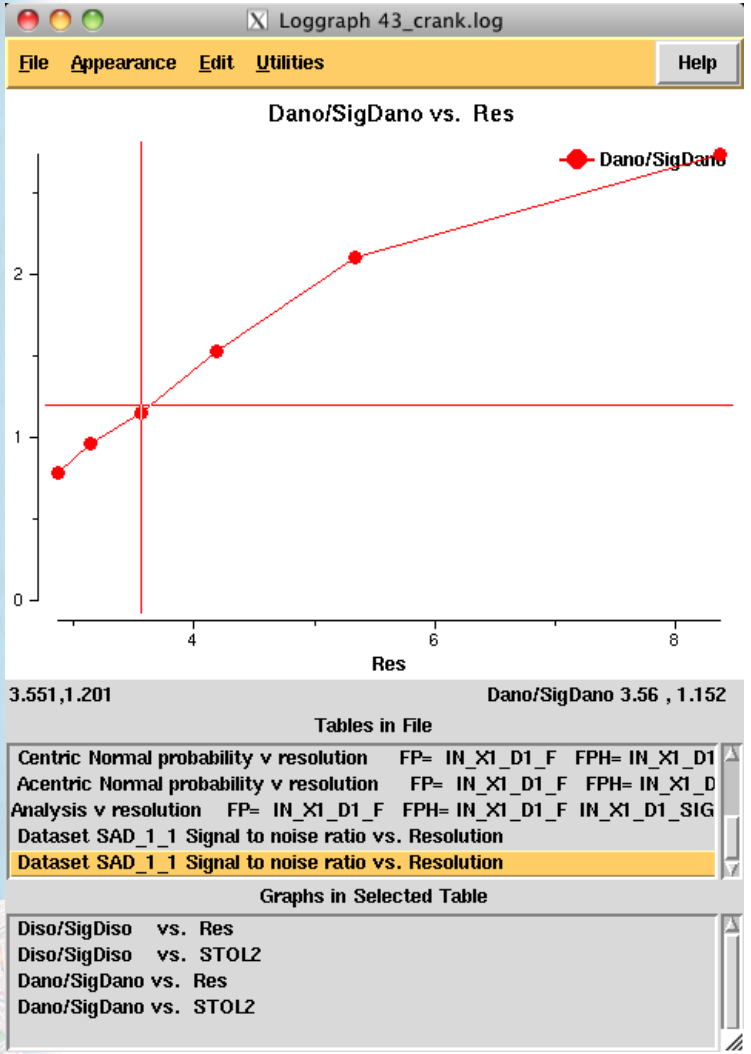
- Speaker: Charles Ballard
- Program Authors:
  - Raj Pannu, Pavol Skubak
  - Randy Read, Airlie McCoy
  - Rudolf de Graaff, Jan Abrahams
  - Kevin Cowtan
  - Garib Murshudov
  - Phil Evans
  - (George Sheldrick)
- “Every program is becoming an experimental phasing program” - Kevin Cowtan

Data and Resolution



- Spacegroup
  - Translation symmetry
  - Fixed origin
  - No special positions
  - P21 21 21 ideal
- Redundancy
  - K. Diederichs, Acta D66 (2010 733.
- Scala
  - Completeness
  - <I>/<sigI>
  - Inter dataset anomalous correlation – MAD (> 0.3)
  - Intra dataset anomalous correction - SAD

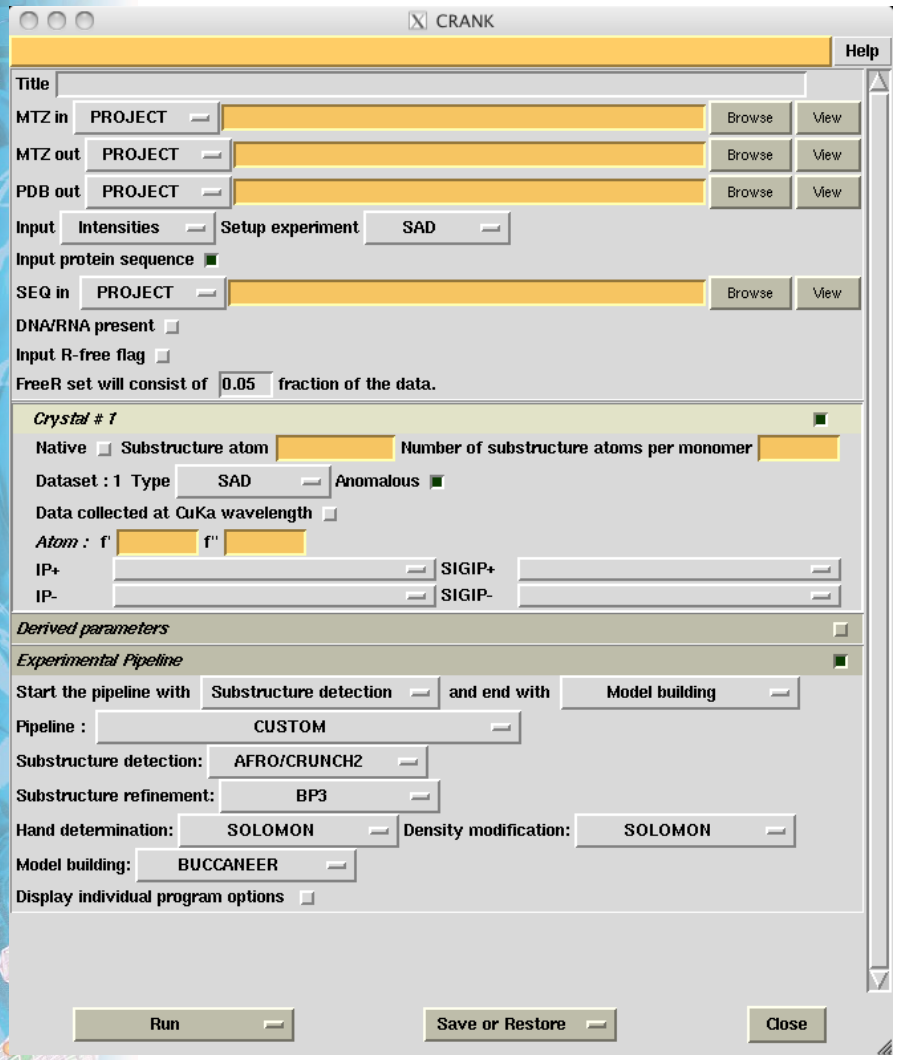
Data and Resolution II



- <Dano>/<sigDano>
  - > 1.2
  - Output by AFRO
  - Also SHELXC via crank.
- George Sheldrick's rule of thumb
  - Hi Res + 0.5
  - 3.0 A

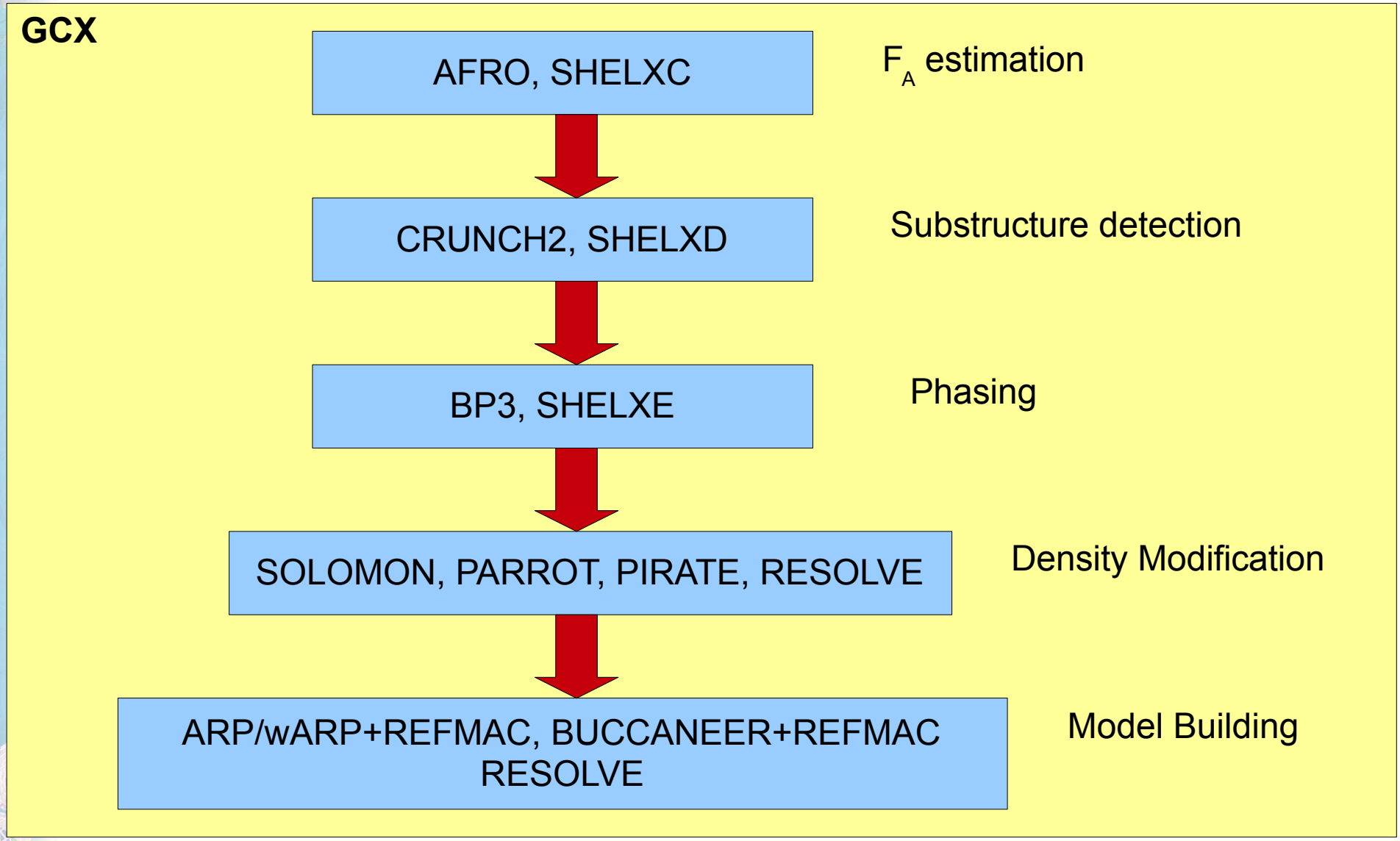
Running CRANK

http://www.bfsc.leidenuniv.nl/software/crank/



- Takes Merged data
- Experiment type
- Atom types plus f' and f''
- Customize pipeline
- Heart of CRANK
  - AFRO
  - CRUNCH2
  - BP3

Flow inside CRANK



AFRO: Multivariate SAD equation for F_A estimation

- $E(|F_A|;|F^+|,|F^-|) =$

$$\frac{\iiint |F_A| P(|F_A|,\alpha_A,|F^+|,\alpha^+,|F^-|,\alpha^-)\, d|F_A|\, d\alpha_A\, d\alpha^+\, d\alpha^-}{\iiint P(|F_A|,\alpha_A,|F^+|,\alpha^+,|F^-|,\alpha^-)\, d|F_A|\, d\alpha_A\, d\alpha^+\, d\alpha^-}$$

- Direct methods very sensitive to FA values
- The multivariate FA estimation leads to more substructures being determined than $\Delta F = ||F+|-|F-||$
-

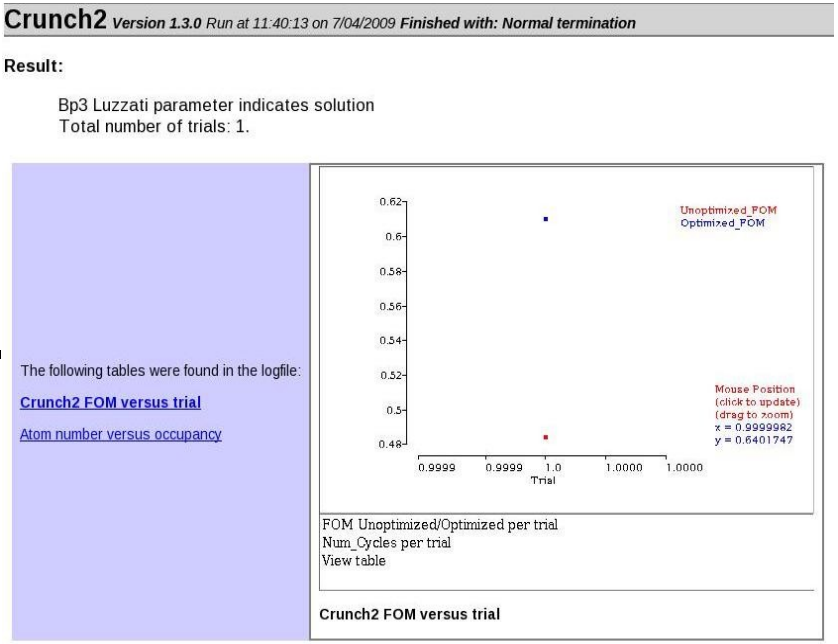
Crunch2: substructure detection

- Algebraic approach based on rank reduction of Karle/Hauptman matrices
- Includes higher order collections of reflections, not just triplets
- de Graff et al. (2001) Acta Cryst. D57, 1857-1862.
- Standalone via crank interface

Scoring the substucture solution

- **CRUNCH2**: FOM > 1.0
- **SHELXD**: CC_{weak} > 30%
- Both conservative criteria.

  Solutions may be missed



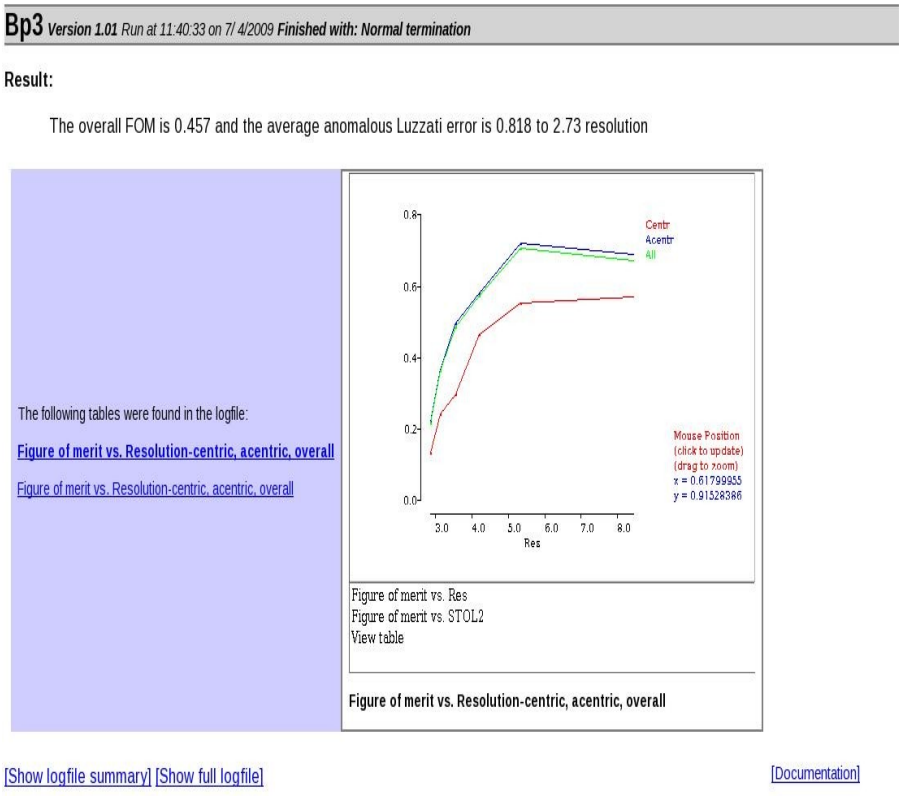
- BP3 can be run in “PHAS” mode to verify

BP3: substructure refinement

- Multivariate substructure refinement
  - Includes effect of model and measurement errors
- SAD, MAD, SIR(AS) and MIR(AS)
- Refines atomic and error parameters
- Outputs H-L coeffs, FOM and PHIB
- Normal and fast phasing (PHAS)

Solution validation

- FOM
- SAD - Anomalous Luzatti Parameter
  - > 0.7 strong solution
  - 0.3 unlikely
- Build



Density Modification

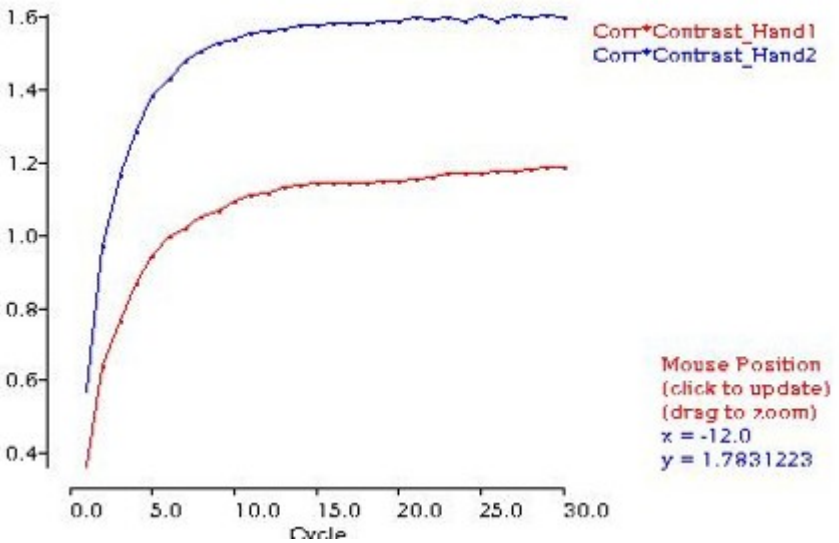
- Map improvement
  - SOLOMON, (SHELXE)
  - PIRATE – statistical in recipriocol space
  - PARROT – MLHL target function, pairwise NCS
- SAD Hand selection
  - **SOLOMON/SHELXE**:

    contract local map density

    - higher better
  - **PARROT**:

    sigmaa value – higher better

    (DM:Real Space Free R, lower better)
  - Tolerences about 0.05

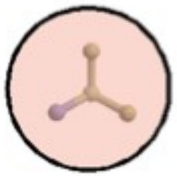


Refinement - Refmac

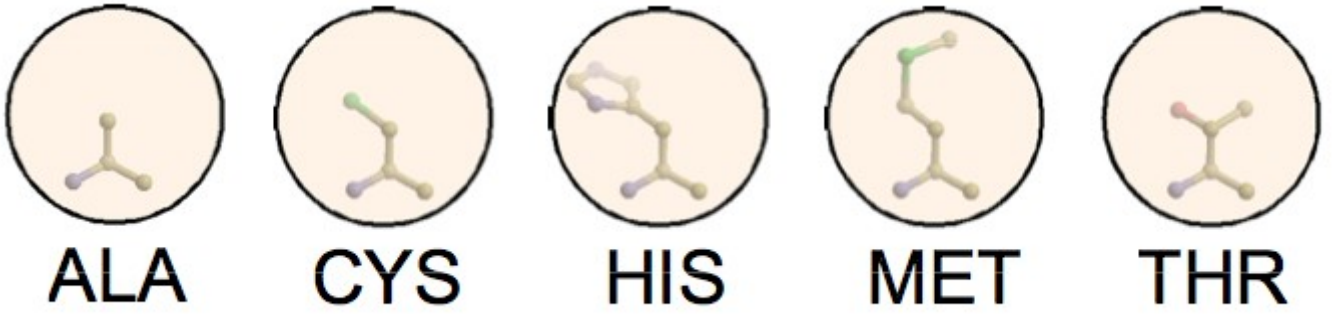
- Relevant refinement targets
  - SAD (and SIRAS coming)
    - Greatly improves maps
    - Skubak et al (2004) Acta D
  - MLHL
    - Prior phase information used indirectly in the form of H-L coefficients
    - Assume prior phase information is independent of refined phases
    - Generally applicable
    - Pannu et al (1998) Acta D

Model Building - Buccaneer

- Statistical model building. Likelihood function based on conserved density
  - Requires phase probabilities
- **Finding Growing**: Cα environment

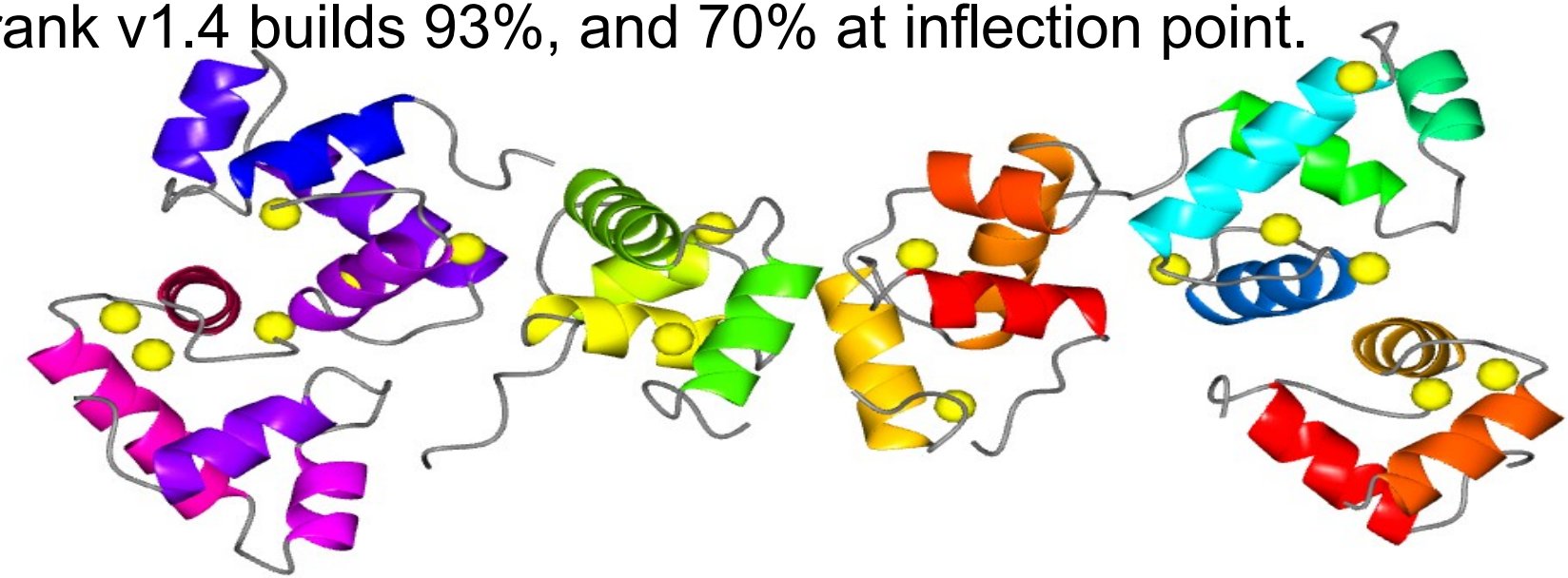


- **Sequencing**: Cβ environment



GerE SAD – a challenging example solved by default

- Example data with CCP4. Originally MAD+native
- 2.7Å SAD peak data with 12 Se (F`-3.6, F” 6.0)
- Early Crank version failed
- Crank v1.3 builds 70% by default
- Crank v1.4 builds 93%, and 70% at inflection point.



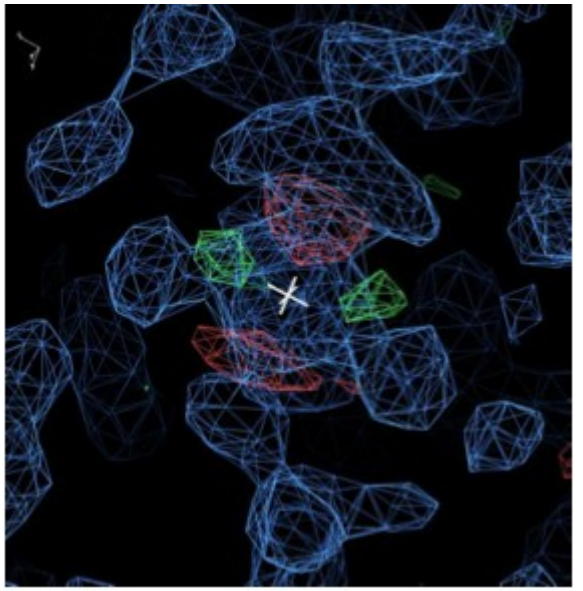
Phaser

- Multivariate SAD target
- Refines coordinates, temperature, occupancy
- Iterative atom location
  - Improves substructure and sensitivity
- Maps
  - Electron density
  - Log likelihood gradient (by atom type)

CCP4

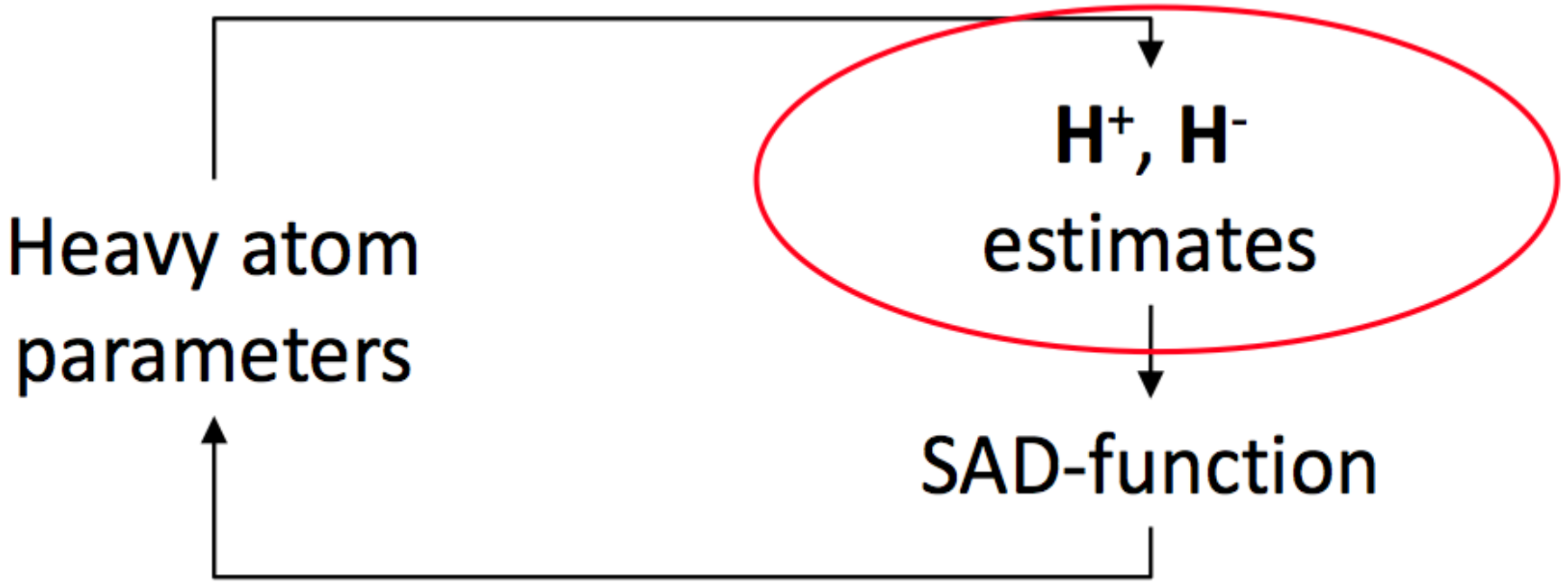
Phaser – log likelihood gradient

- Peak height dependent on f'/f''' ration
  - May distinguish atom types
  - Can be fooled if normal scatter present at site
- Very sensitive to detail
  - Image shows anisotropic



MR-SAD

- Input: partial structure/MR solution
- Possible atom types



- Better maps

Other Programs

- Acorn
  - Direct method based density modification
  - Free lunch
- OASIS
  - SAD ambuguity breaking
- Venerable
  - RANTAN, MLphare
- External
  - SHELX, HKL2MAP
  - Autosharp