Structure solution with Crank2

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Crank2

- software for automated structure solution: *anomalous* data → model.
- requires minimal input, but is highly configurable.
- user friendly graphical pipelines incorporating our latest developments in substructure detection, phasing, density modification and model building & refinement as well as plugins to externally developed programs.

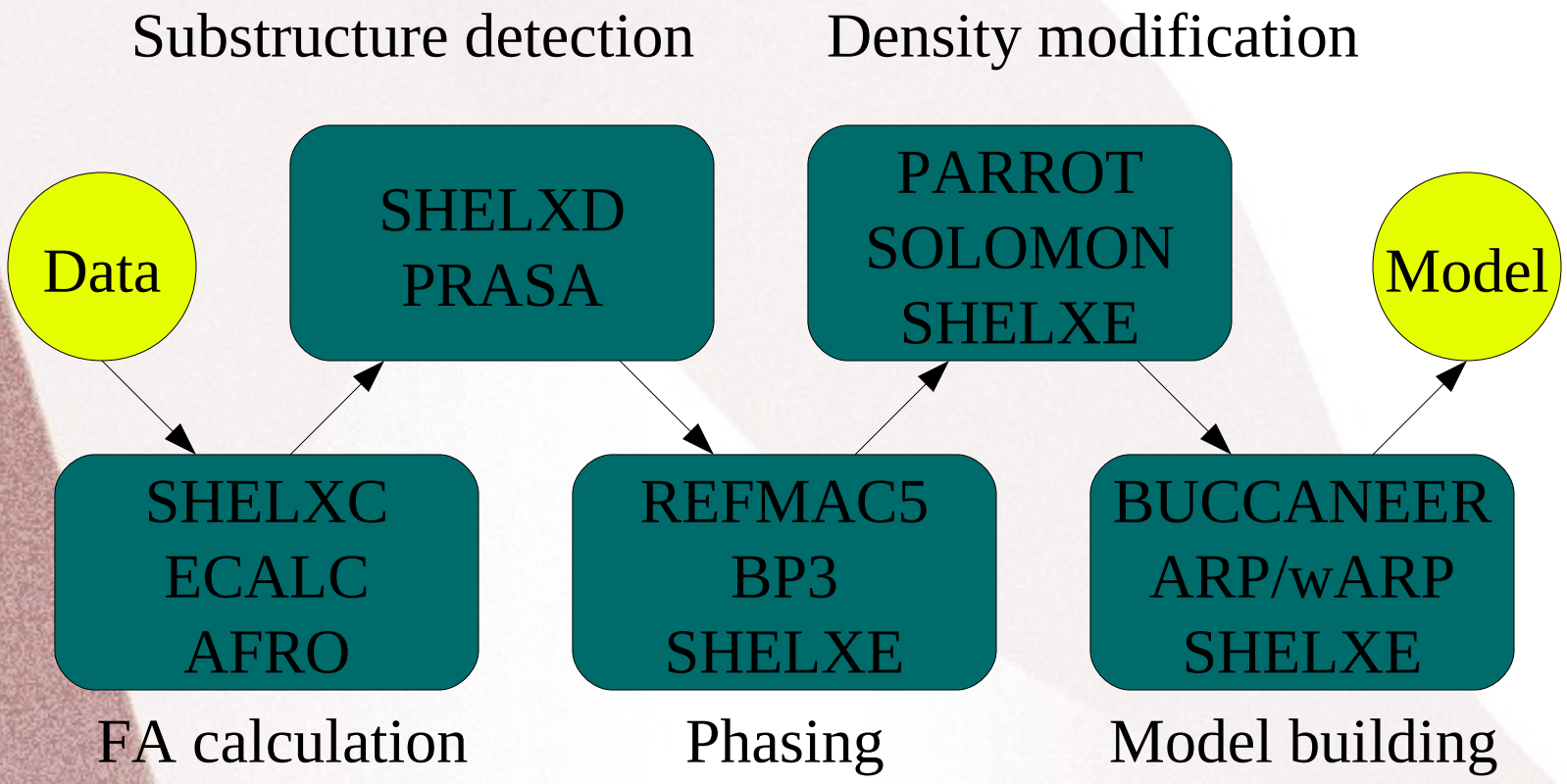
Crank2

- Several pipelines:
  - SAD pipeline using a multivariate SAD function in all steps
  - MR-SAD pipeline (for SAD after MR and for model rebuilding using anom. data)
  - SIRAS/MAD pipelines
  - SHELX pipeline via Crank2

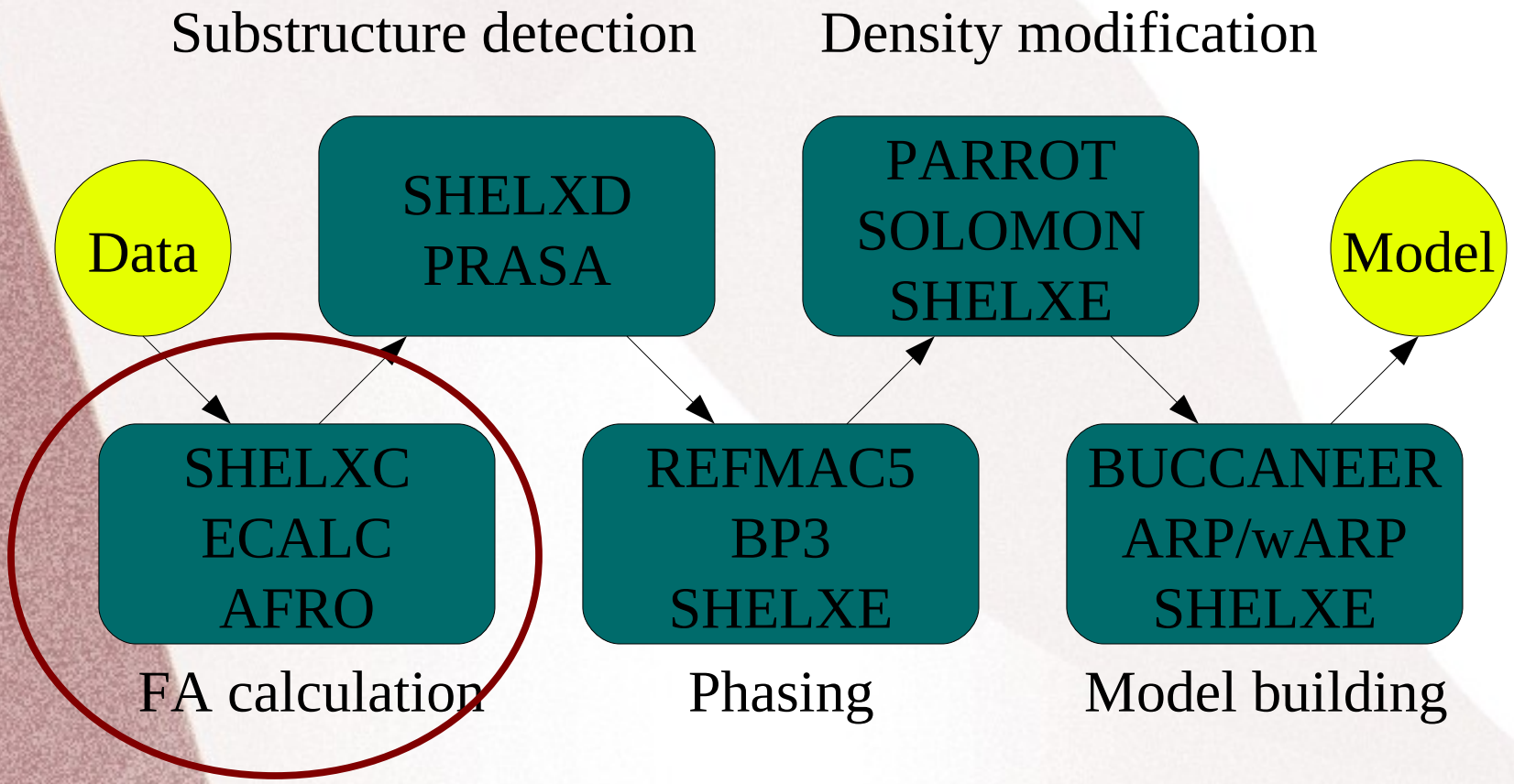
Availability

- ccp4i2 (from CCP 7.0):
  - CRANK2 for SAD,MR-SAD,SIRAS,MAD
- CCP4 Cloud (jsCoFe):
  - CRANK2 for SAD,MR-SAD,SIRAS,MAD
- CCP4 Online:
  - CRANK2 for SAD,SIRAS,MAD
- ccp4i (CCP4 7.0):
  - CRANK2 for SAD and MR-SAD
  - CRANK for SIRAS, MAD

Basic steps of structure solution from experimental phases with Crank2.



Structure solution from experimental phases with Crank2.



F_A estimation

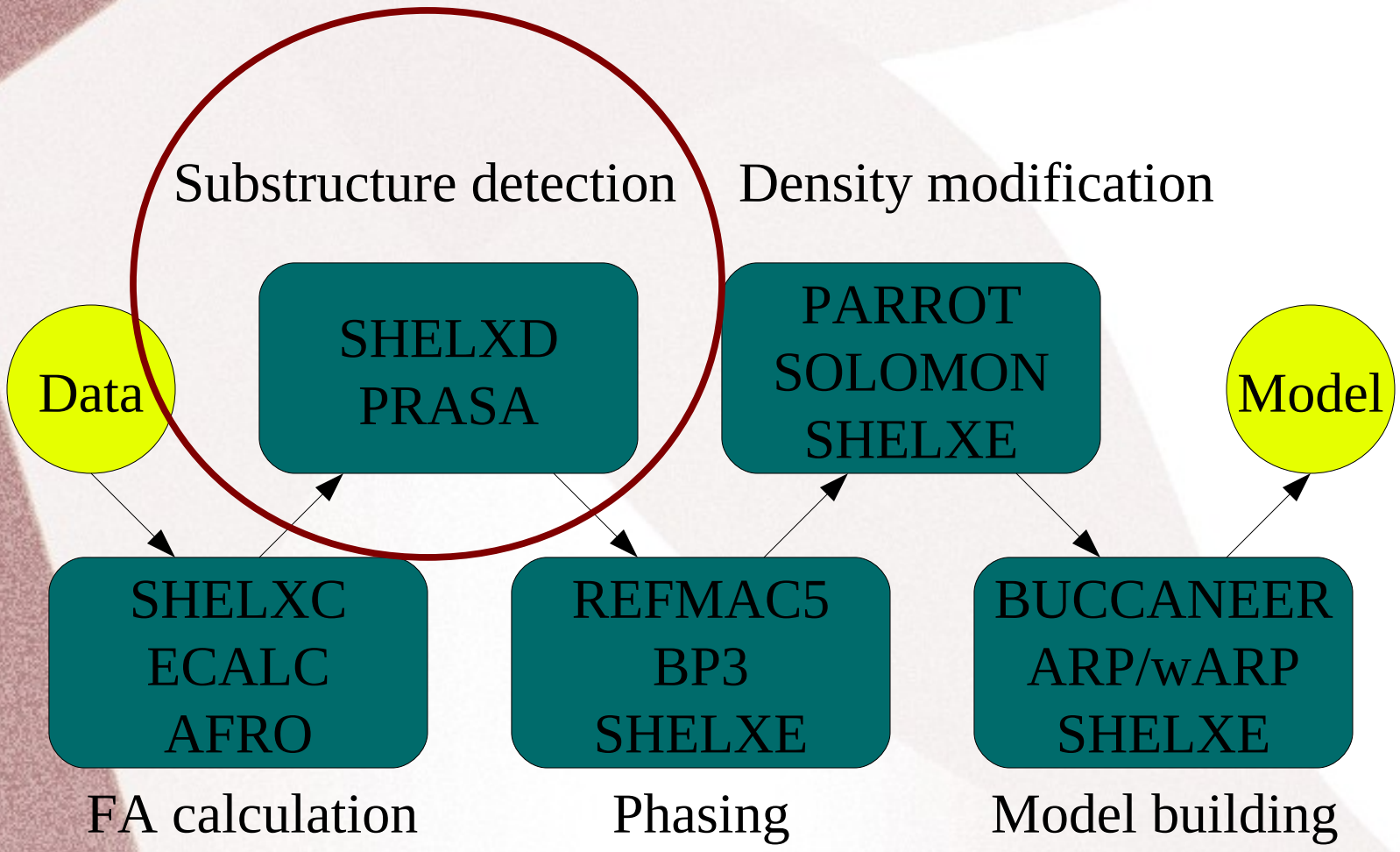
- Substructure detection methods need to first estimate "F_A" or "substructure factor amplitude"
- Improving the estimates can improve hit rates of substructure detection and solve things that could not be otherwise solved.
- The simplest estimation of F_A for SAD data is

  $\Delta F = \big|\, |F^+| - |F^-| \,\big|$
- E values: normalized F_A values
- Large observation/param. ratio: data exclusion
- Available programs in Crank2: SHELXC, AFRO, ECALC

AFRO: Multivariate SAD equation for F_A estimation

- Giacovazzo previously proposed multivariate F_A estimation assuming Friedel phases are equal.
- An equation can be obtained without the equal phase assumption requiring only one numerical integration.
- The multivariate F_A calculation may improve substructures determination rate over ΔF (preliminary results)
- Currently in heavy development with Navraj S. Pannu

Structure solution from experimental phases with Crank2.



Determination of anomalous scatterers

- A crucial step in structure solution from anom. data
- Usual methods (eg SHELXD, HySS):
  - Direct methods: obtain/optimize phases using triplet relationship, tangent formula
  - Patterson methods: obtain positions of anomalous scatterers from Patterson function (or use it for seeding)
  - Space recycling: apply direct methods in reciprocal space and density modification in real space

Phase retrieval methods

- Group of algorithms attempting to solve general phase retrieval problem
- Routinely used to solve phase problem eg in astronomy
- Space recycling: iterative application of operations in the reciprocal space and the crystal space
- Do not make any use of direct / Patterson methods
- The operations in the reciprocal space alone or in the real space alone are principally NOT able to solve the phase problem

PRASA: A new program for substructure detection

- Phase Retrieval for Anomalously Scattering Atoms
- C++ program built using the CCP4 Clipper libraries, integrated in CRANK2
- charge flipping and RAAR phase retrieval algorithms
- automatic multiple high resolution cutoffs
- input of number of substructure atoms not needed (RAAR only)
- parallelized (openMP)
- beta stage, released and stable, not default yet

Charge flipping

- The simplest usable phase retrieval technique
- ***CHF***$(\rho) = R_D P_M(\rho)$
- ***CHF***$(\rho) = \begin{cases} \rho & \rho >= \delta \\ -\rho & \rho < \delta \end{cases}$
- Oszlanyi & Suto (2004, 2007, 2011)
- Palatinus (2007,2012) - program Superflip
- Dumas & van der Lee (2008)

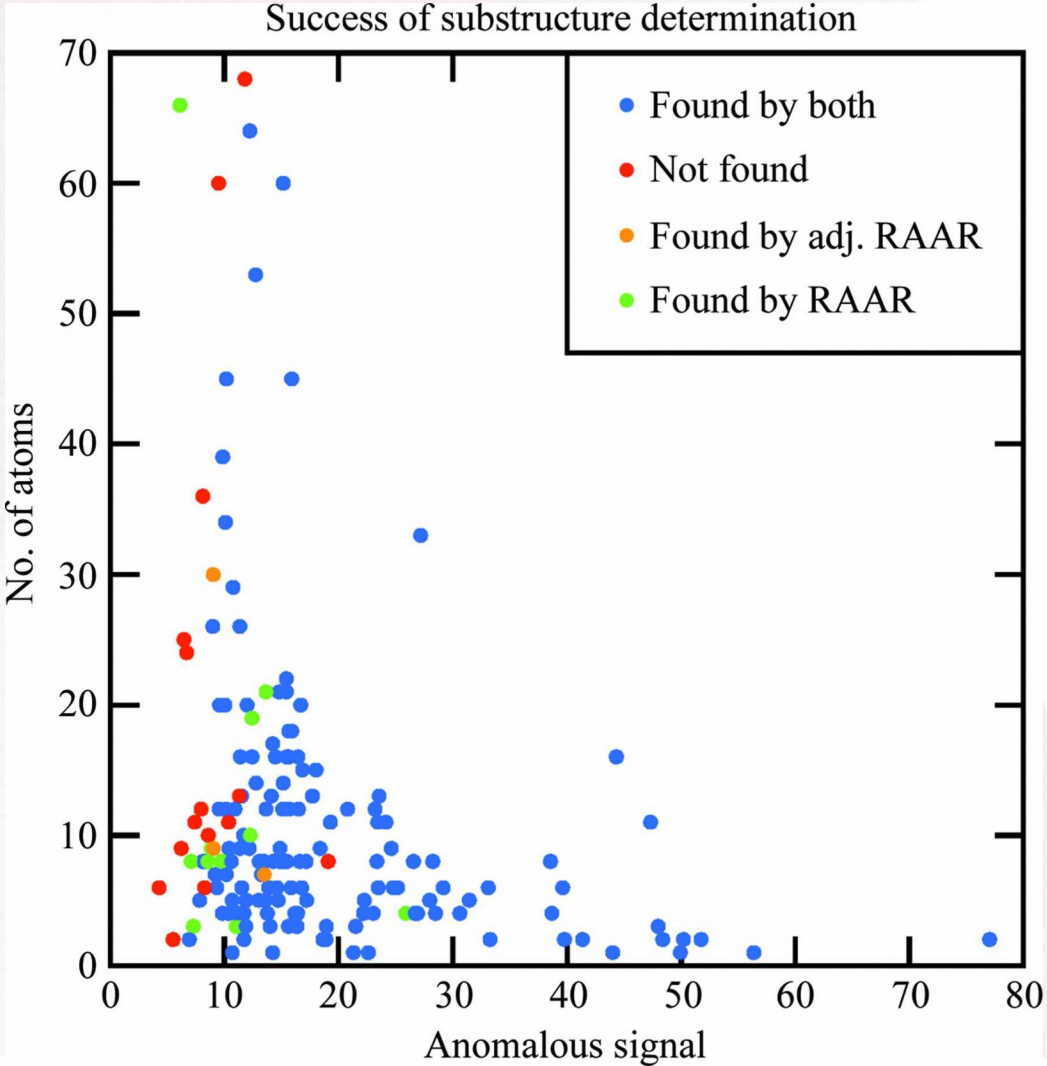
RAAR (Relaxed Averaged Alternating reflections)

- ***RAAR***$(\rho) = \frac{1}{2}\beta(R_D R_M + I)(\rho) + (1-\beta)P_M(\rho)$
- ***RAAR***$(\rho) = \rho \qquad R_M(\rho) >= \delta$

  $(1-2\beta)\rho+\beta\rho_{old} \qquad R_M(\rho) < \delta$
- $\beta = 0.8 \Rightarrow \quad -0.6\rho + 0.8\rho_{old}$

Massive testing results

- Automatic structure solution with Crank2+PRASA on ~150 SAD datasets using charge flipping and RAAR



Example: Novel SAD data solved by PRASA

- difficult SAD data from the McGill lab / Quebec, Canada by Juliana Munoz
- 3.2A dataset collected at the APS/CCP4 School workshop
- All structure solution attempts at the APS School failing
- PRASA was able to obtain a substructure solution and a highly complete protein model has been eventually built by several iterations of Crank2 combined model building (Rfree=33)

Important parameters in substructure detection

- The number of trial cycles to run.
- The number of atoms to search for (SHELXD)
  - Should be within ~20% of the actual number
  - First guess: Estimate per monomer and multiply
- The high resolution cut-off:
  - For MAD, a good guess comes from anomalous difference correlation.
  - For SAD, a first guess is high resolution limit + 0.5A; if unmerged data are available, a better guess might be derived from CCanom1/2
  - Several jobs with differing values may be needed

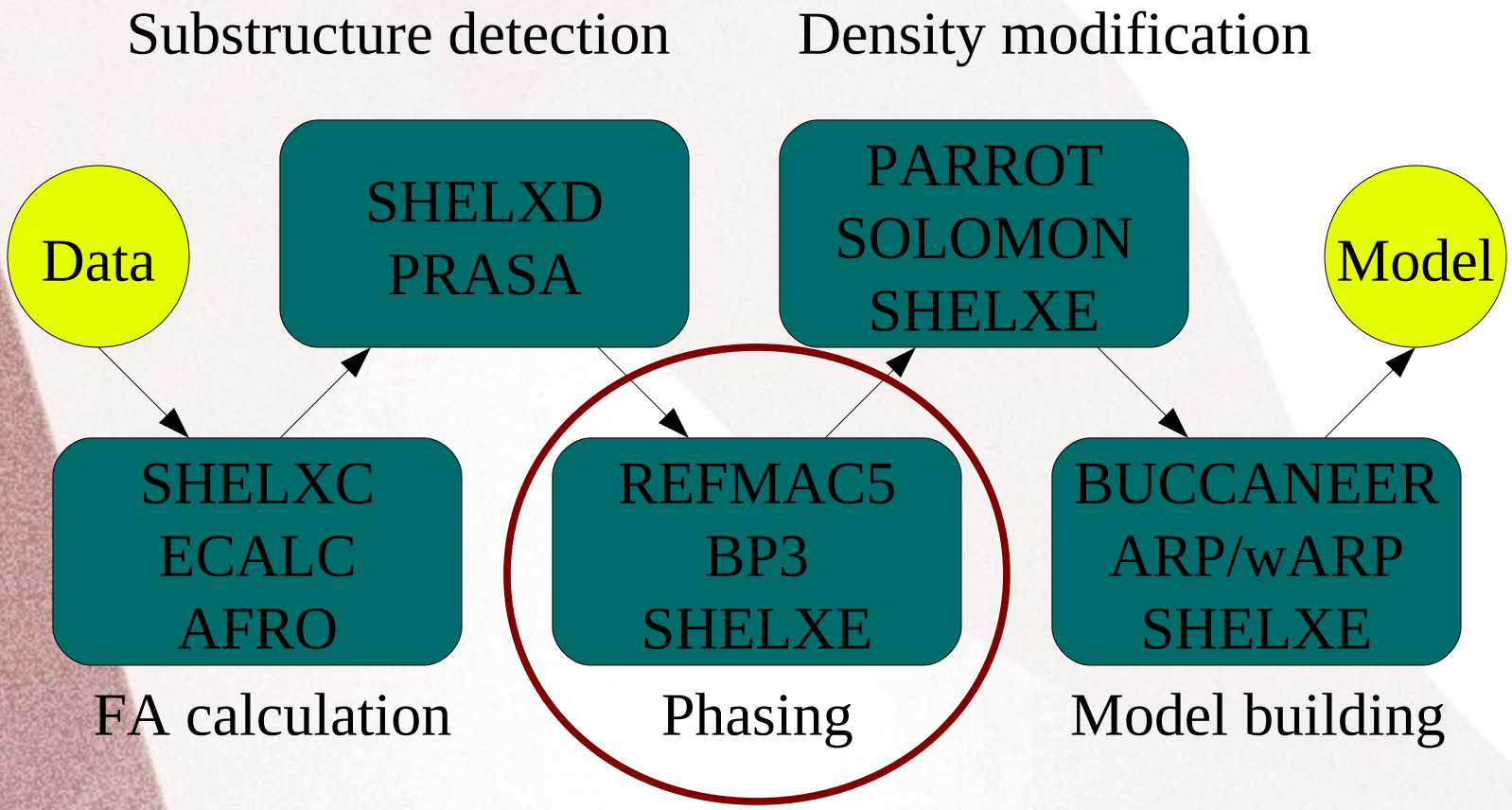
Substructure determination validation

- Usually either a highly complete solution is found or the solution is incorrect
- Indicators of a correct solution:
  - CFOM > 75 for SHELXD (120 for MAD)
  - CLD*FOM*CC > 35 for PRASA

(conservative criteria for a solution -

half or even less may be still a solution)

Structure solution from experimental phases with Crank/Crank2.



Substructure refinement and phasing

- Refinement of the substructure parameters and error parameters
- The refined parameters are used for initial estimation of phases
- Available programs in Crank2: REFMAC5, BP3

Substructure refinement and phasing by BP3 and Refmac

- Can be used for SAD, SIRAS, MAD (BP3 only)
- Using multivariate SAD/SIRAS phasing function
- Outputs the estimates of “best” initial phases PHIB and their reliability FOM and four Hendrickson-Lattman (HL) coefficients “encoding” the phase distribution (all in the output MTZ file)
- REFMAC is usually faster except if there are just a few substructure atoms (FFT vs direct summation)

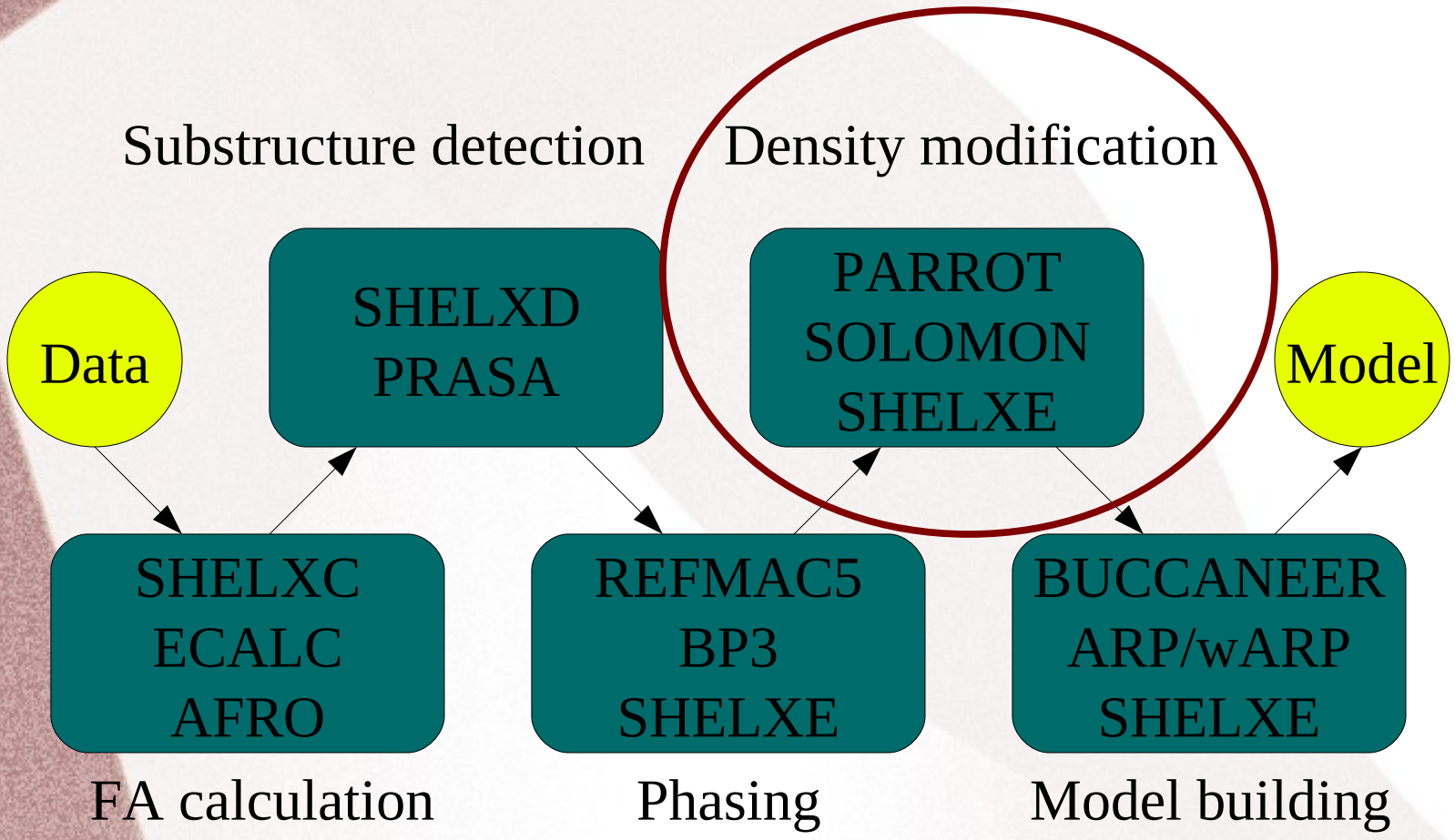
Multivariate distribution for a SAD experiment

- Include effect of model and measurement errors and correlation between observed and calculated Friedel pairs.
- Required multivariate joint probability distribution

$$P_{ph}\left(F_o^+, F_o^- \middle| F_H^+, \varphi_H^+, F_H^-, \varphi_H^-\right)$$

- The function can be further extended for phase combination and refinement in density modification and model building stages

Structure solution from experimental phases with Crank/Crank2.



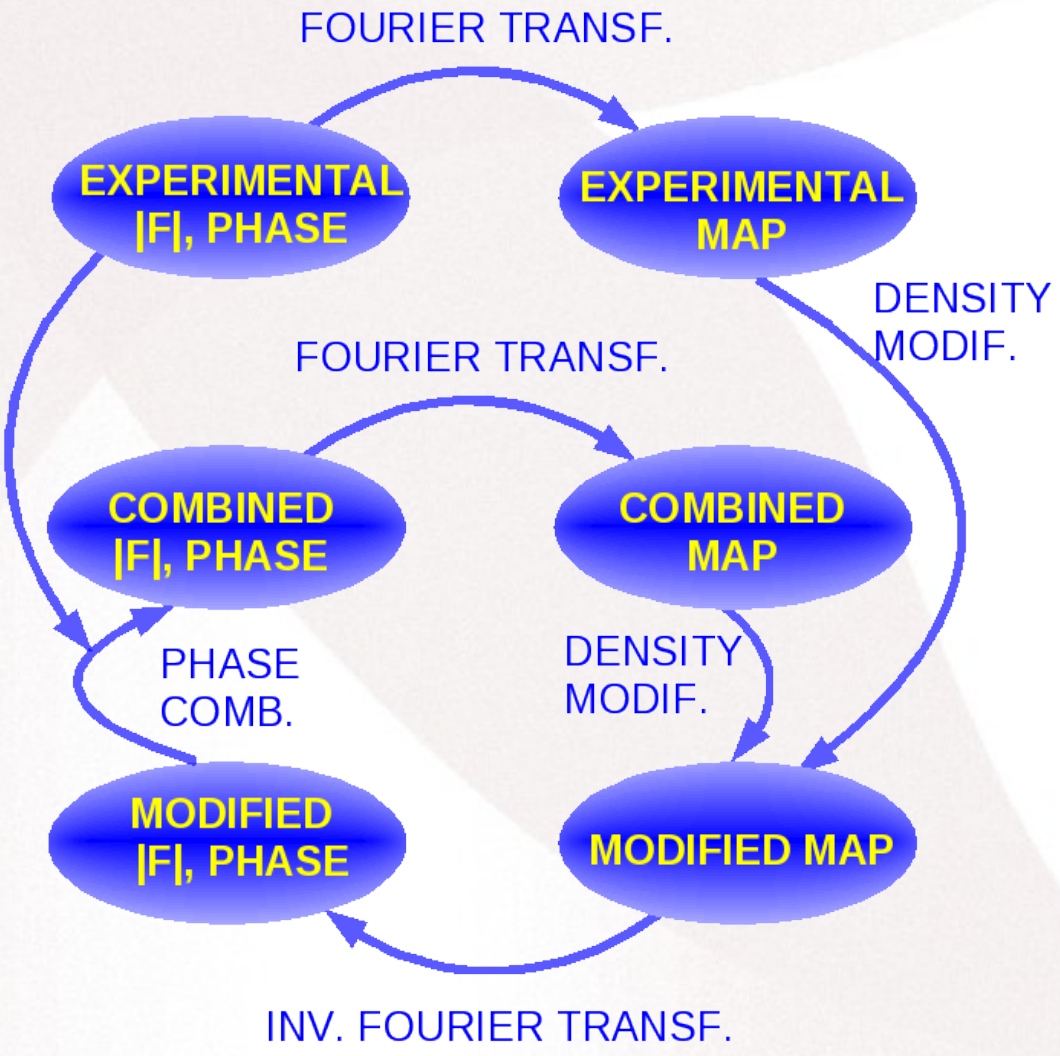
After we have initial phases: Density modification

- Improving phases of the initial map by incorporation of prior information about protein map features into it
- Space recycling with phase combination
- Available programs in Crank2:
  - Parrot
  - Solomon
  - SHELXE
- In case of SAD or SIRAS, Parrot and Solomon can use external multivariate combination with bias reduction by MULTICOMB or REFMAC

Density modification principles

- Idea: apply prior information about protein electron density maps to the experimental map
- Solvent flatness prior: the density in solvent regions is close to constant → solvent flattening
- Histogram similarity prior: the histograms of protein density maps are similar → histogram matching
- NCS prior: the density in NCS related regions should be very similar → NCS averaging

Density modification procedure



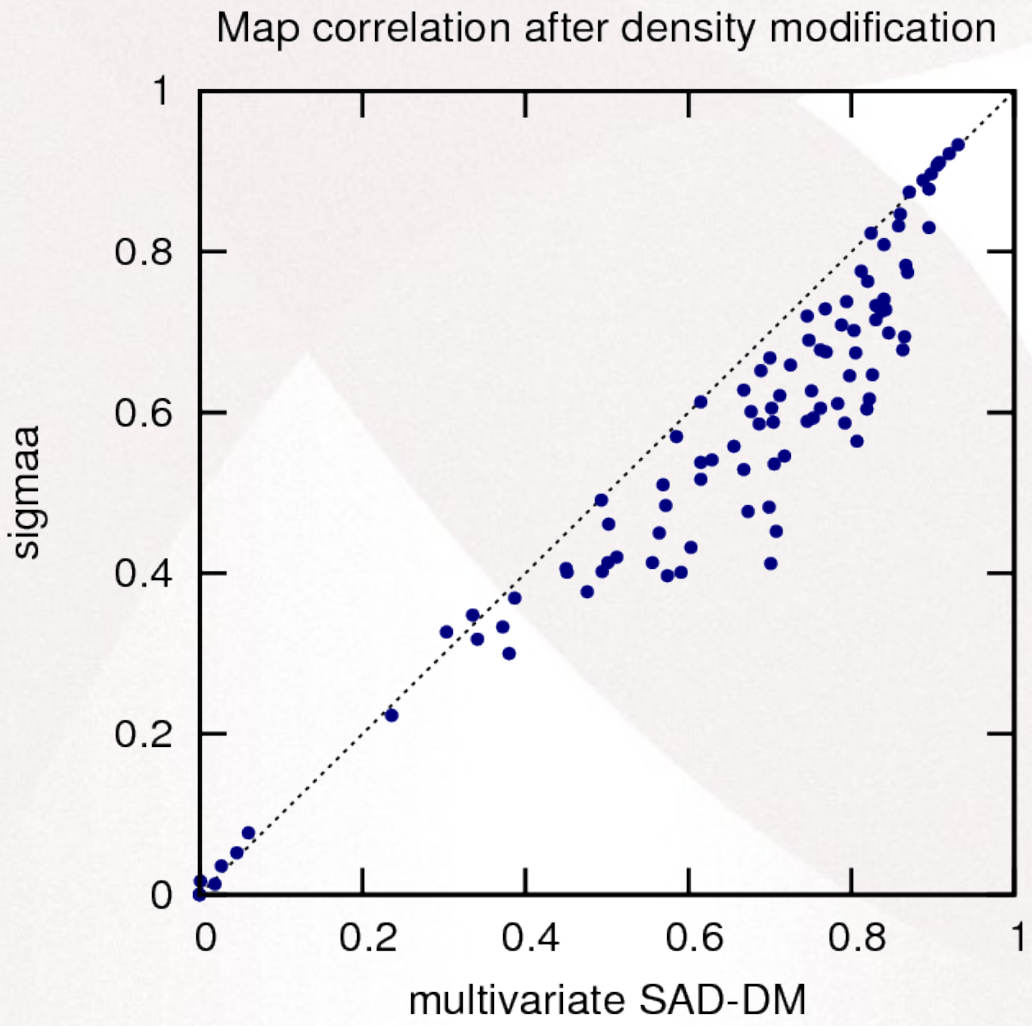
Multivariate phase combination for density modification

- Density modification procedures usually neglect the correlation between the original map and the density modified map
- Our approach: multivariate SAD-DM probability distribution

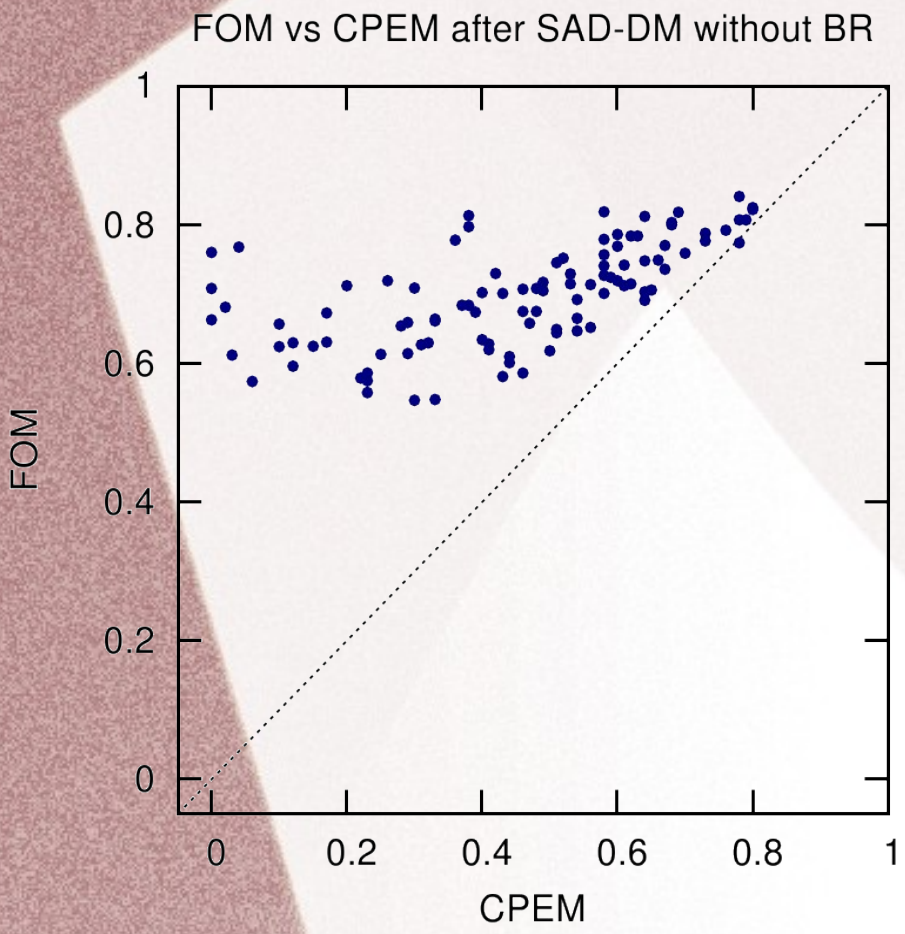
$$P_{DM}\left(F_o^+, F_o^- \middle| F_H^+, \varphi_H^+, F_H^-, \varphi_H^-, F_{DM}, \varphi_{DM}\right)$$

- Advantages: no independence assumption, dynamic construction of phase probabilities rather than static HL
- Implementation: MULTICOMB, REFMAC

Comparison of univariate vs. multivariate (SAD-DM) function



Density modification bias

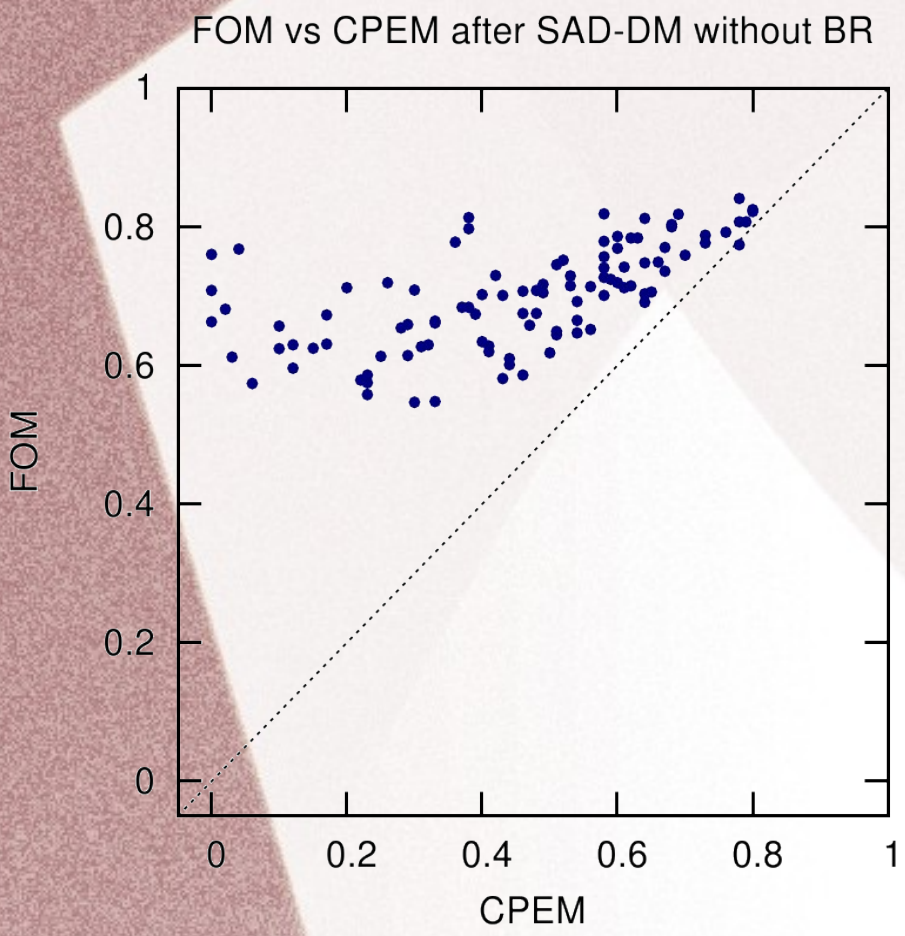


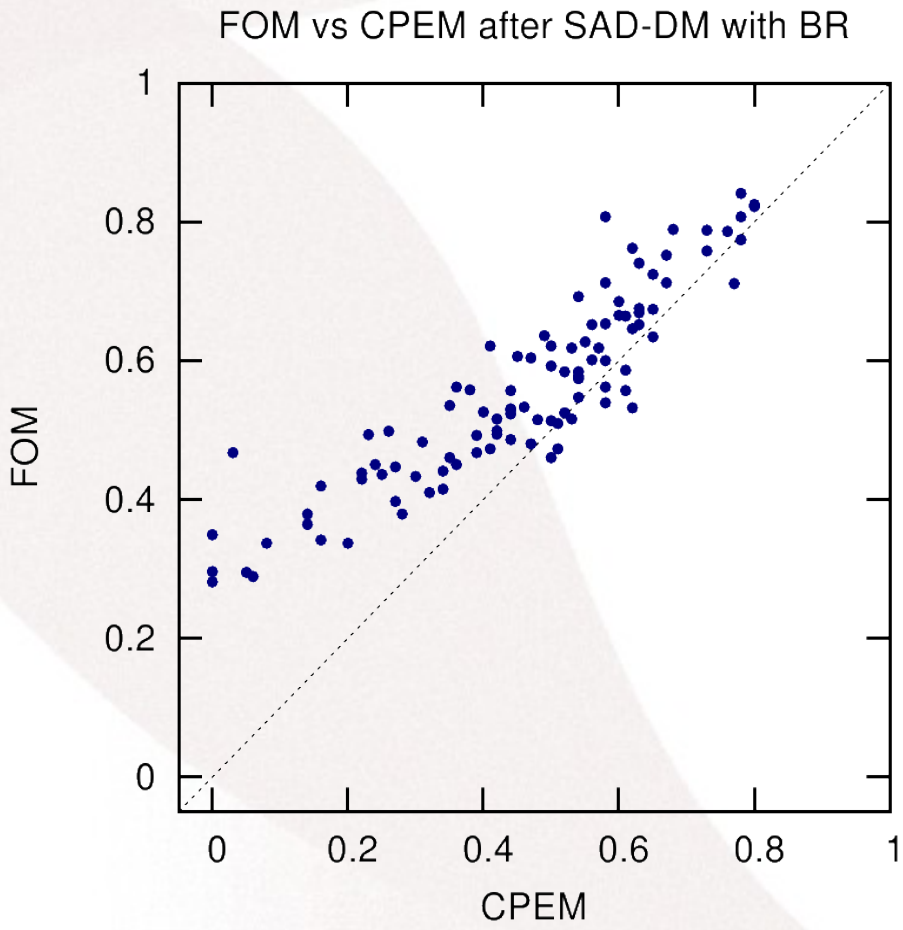
- Estimated <FOM> is much larger than <cos(ph.error)>
- Reason: the phase quality is estimated from fit between the observations and the model - which was constructed from the observations

Solution for SAD-DM: β-correction

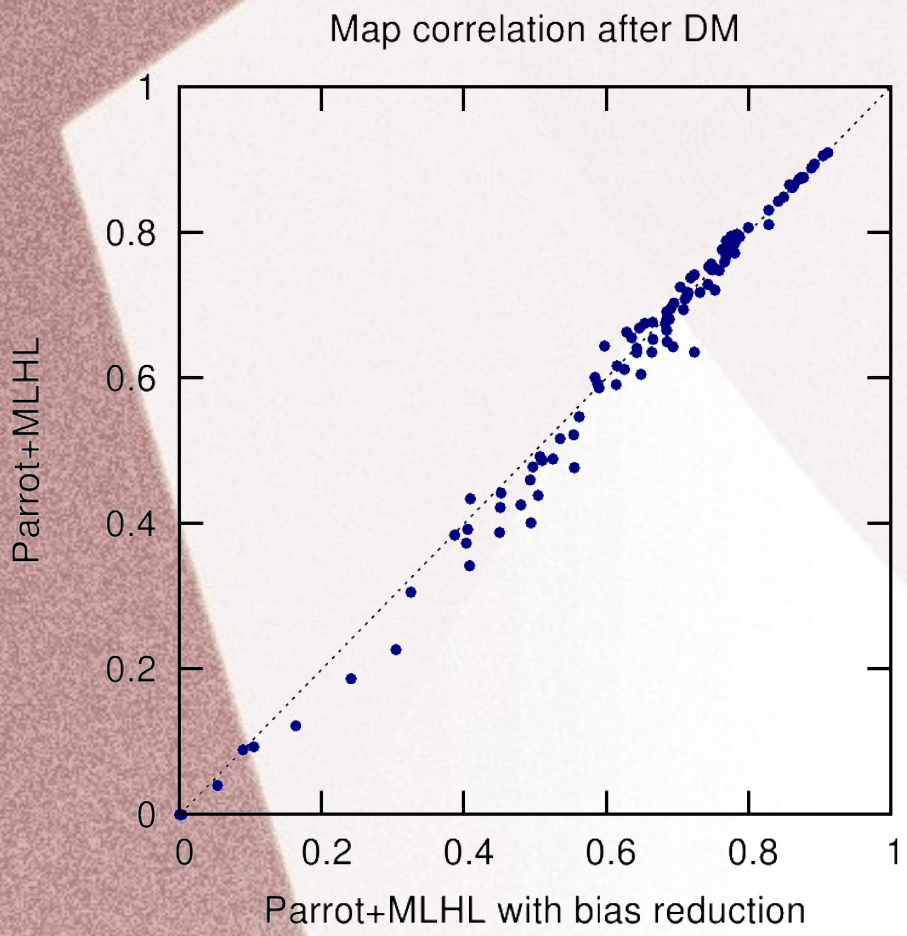
- Estimate overall bias parameter β:
  - run 4-5 DM cycles with 'free' set of reflections set aside and comparing the correlation between F_o and F_c in free and working set
- Run standard DM using β (and all reflections):
  - β is applied to the correlation term betwen F_o and F_c in the covariance matrix
- β is ~1 in case of no bias and can reach 0.2-0.3 in case of very strong bias

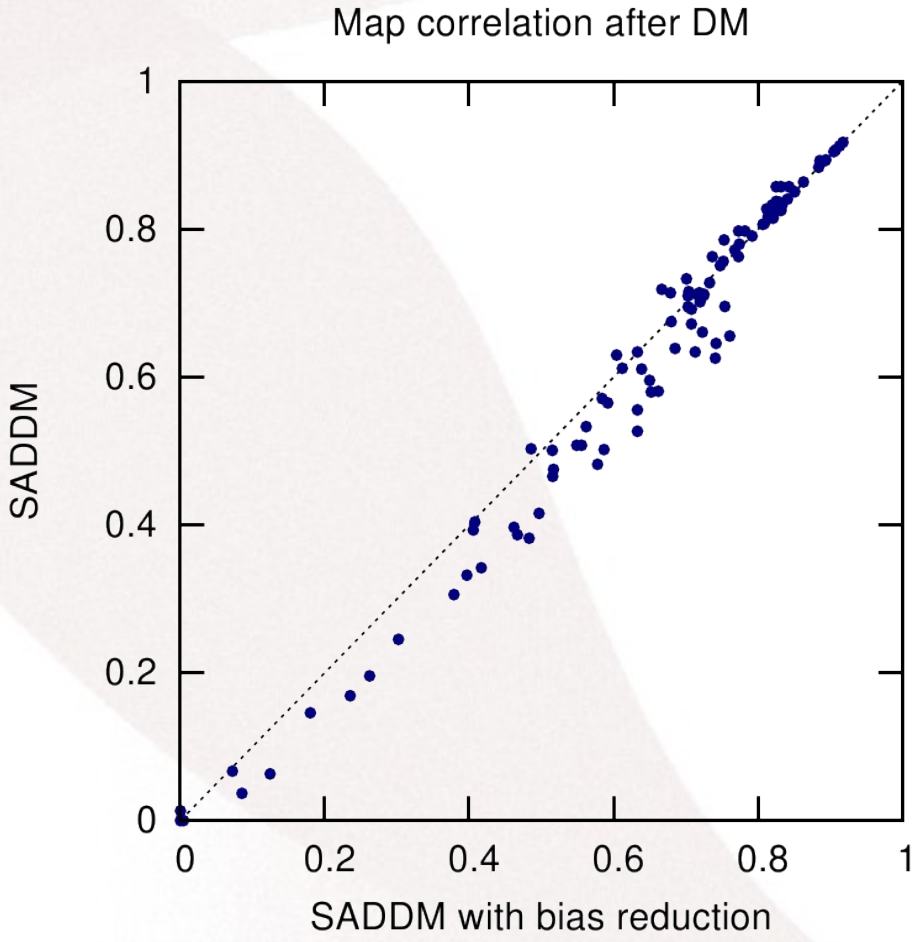
FOM and phase error after DM with/ without bias reduction





Map correlation after DM with/without bias reduction





Automatic hand determination

- The hand is not known - either the found substructure or its inverse is correct
- Safe (albeit slower) approach: try building with both hands
- Crank2 chooses the hand before building, assuming that a correctly handed substructure provides a better map
- Criteria used: combination of CLD of the map after phasing with FOM after “quick” density modification
- The latest version of Crank2 continues with both hands if distinction between the scores of hands is small

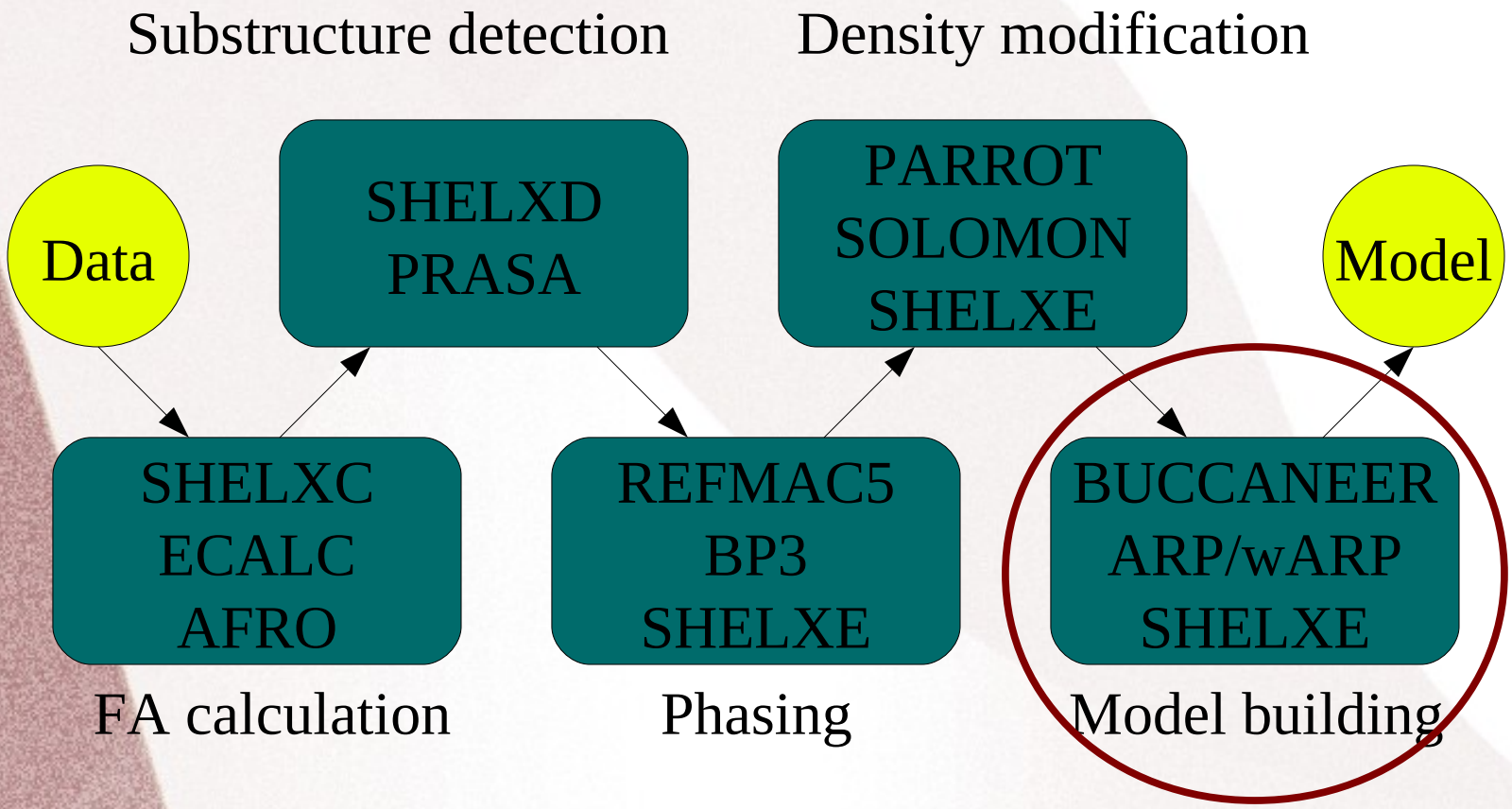
How are we doing?

- Statistics from substructure phasing:
  - FOM (>0.3 usually a solution, <0.15 usually not)
  - CLD (or skewness) of the map - outputted by MAPRO utility (difference>0.02 usually a solution)
- Statistics from density modification:
  - FOM (>0.5 usually a solution, <0.35 usually not - only useful if bias reduction was employed!)
- Statistics from hand determination:
  - Distinction in score between the hands
- Does it look like a protein? (visualization)

Further improving the map

- Adjusting solvent content can improve the map after density modification. (Since the number of monomers is generally not known beforehand, neither is the solvent content.)
- Try to determine NCS – sometimes the automatic NCS detection in Parrot does not succeed
- Try to find additional anom. scatterers or remove likely wrong atoms (the automatic peak picking and removal thresholds in Crank2 are conservative)

Structure solution from experimental phases with Crank/Crank2.

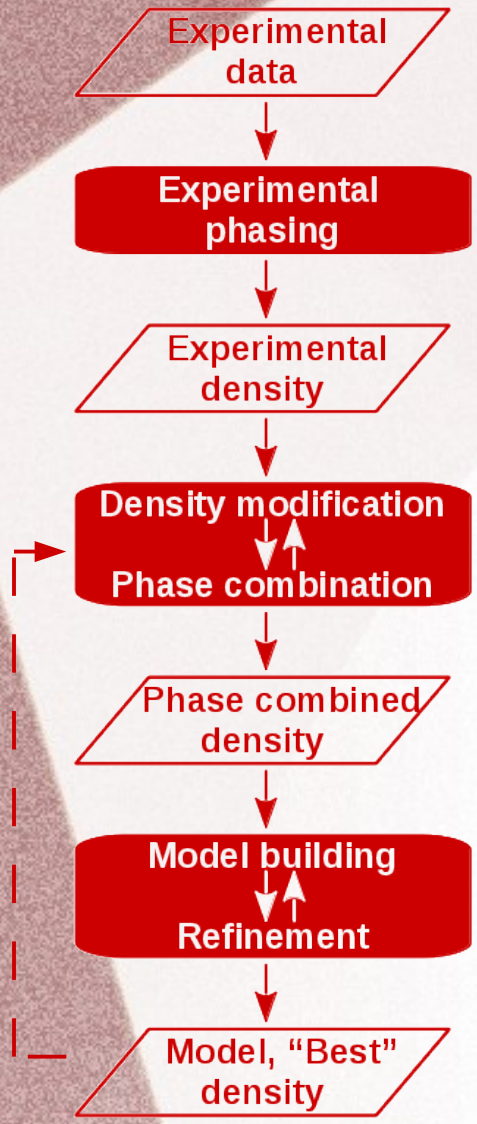


Automatic model building

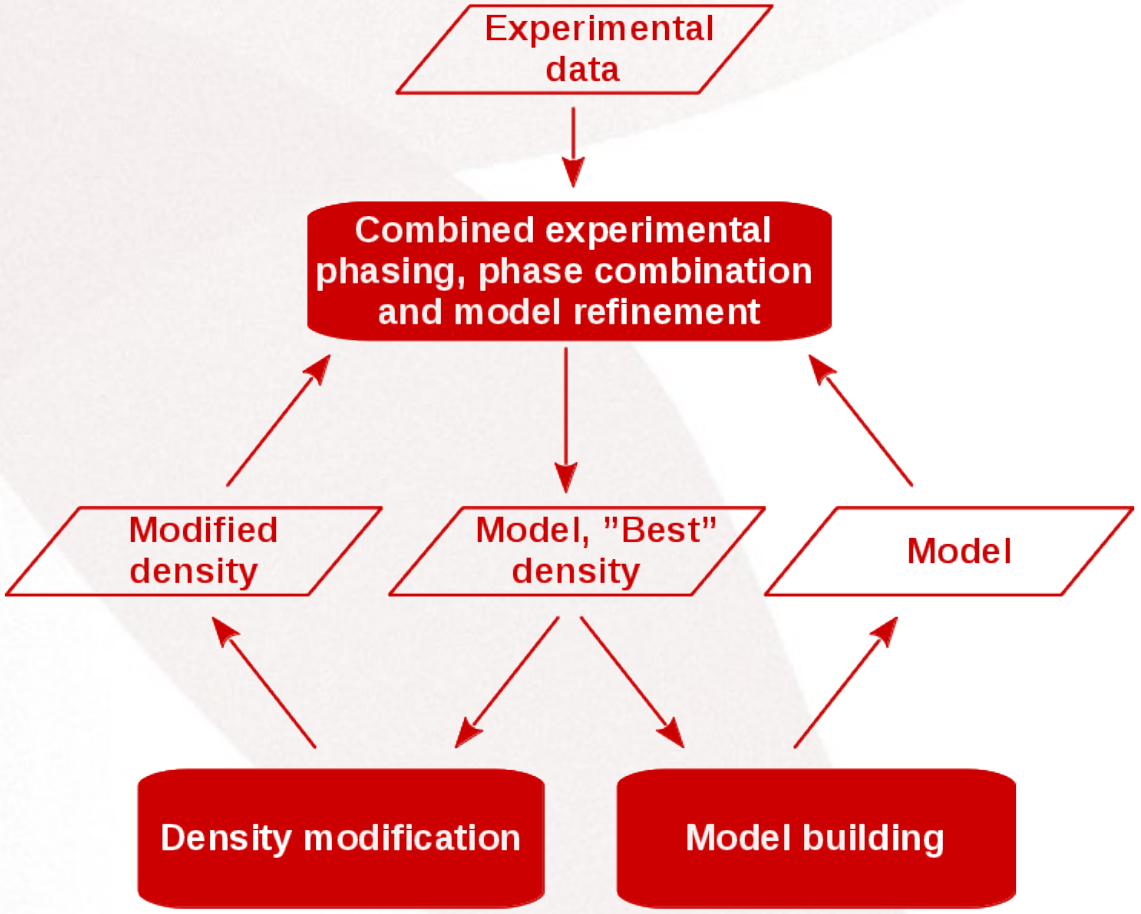
- Available programs in Crank2:
  - Buccaneer
  - ARP/wARP
  - SHELXE
- in the Crank2 pipelines, model building is either iterated with model refinement by Refmac or more complicated algorithms are used

Combined algorithm: Flowchart

step-wise



Combined (SAD only)



(Skubak&Pannu, Nature Comm., 2013)

Multivariate probability distributions

- Phasing (2003):

$$P_{ph}\left(F_o^+, F_o^- \mid F_H^+, \varphi_H^+, F_H^-, \varphi_H^-\right)$$

- Phase combination (2010):

$$P_{DM}\left(F_o^+, F_o^- \mid F_H^+, \varphi_H^+, F_H^-, \varphi_H^-, F_{DM}, \varphi_{DM}\right)$$

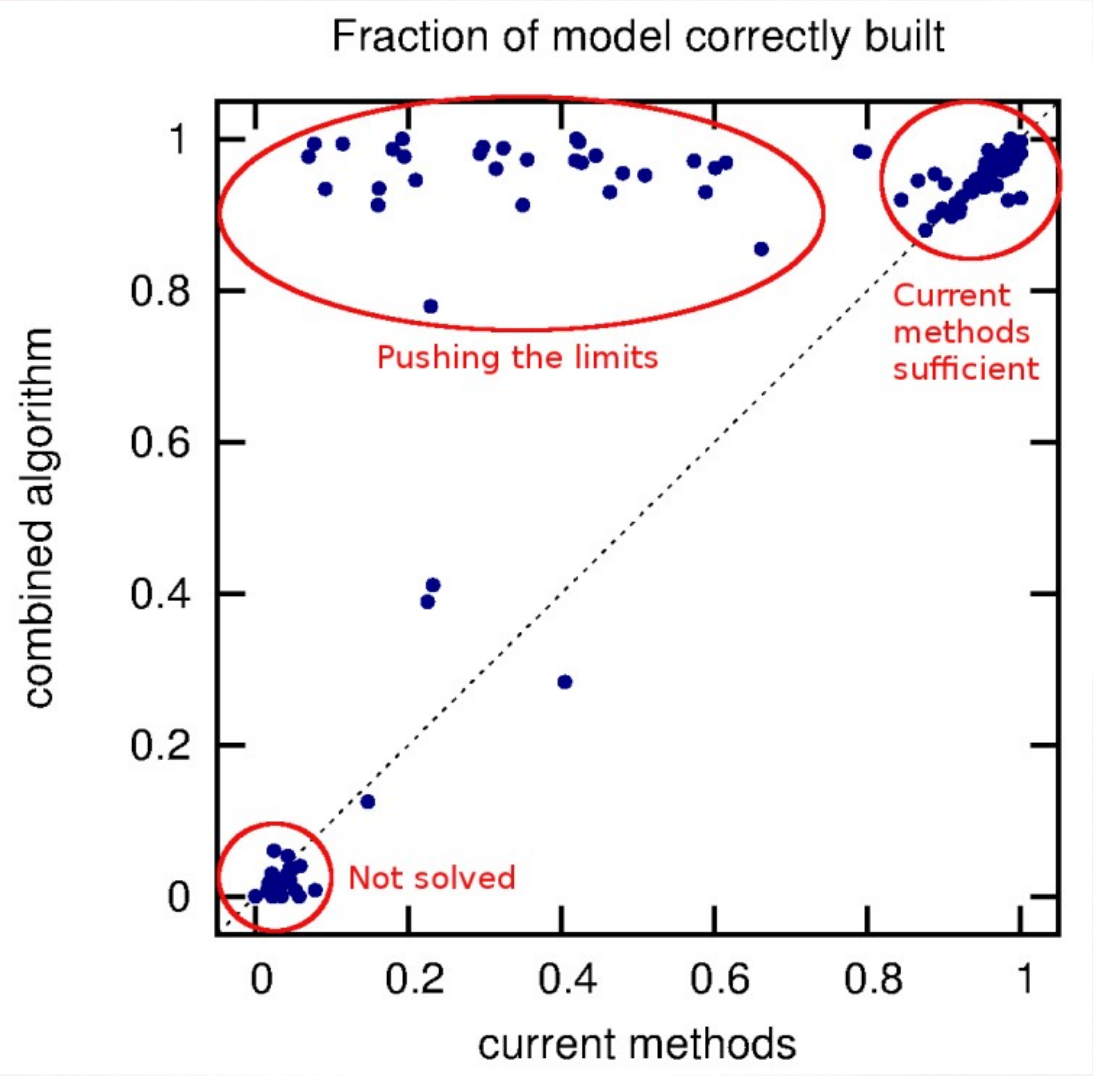
- Refinement (2005):

$$P_{ref}\left(F_o^+, F_o^- \mid F_P^+, \varphi_P^+, F_P^-, \varphi_P^-\right)$$

- Combined (2013):

$$P_{comb}\left(F_o^+, F_o^- \mid F_P^+, \varphi_P^+, F_P^-, \varphi_P^-, F_{DM}, \varphi_{DM}\right)$$

Model building results on almost 150 real SAD data sets

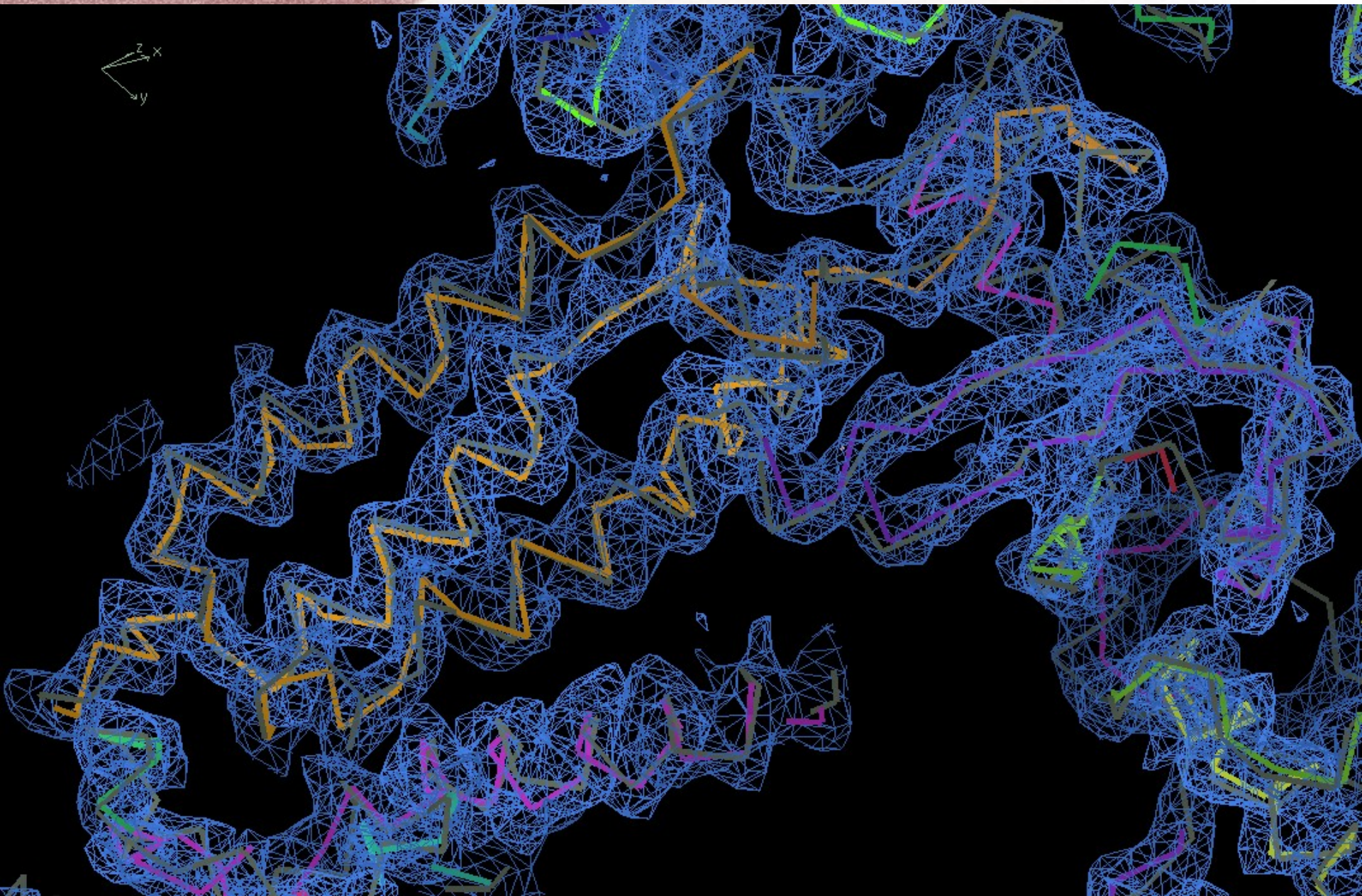


12-subunit RNA polymerase II

- 3.8A resolution dataset with anomalous signal from eight intrinsic zinc ions

  (Meyer P.A. et al., *J.Biol.Chem.*, 2009)
- ~4000 residues in the asym. unit; no NCS
- Originally solved by a partial model MR followed by multi-crystal MAD phasing, MR-MAD phase combination and manual iterative model building
- The combined approach in CRANK2 can build ~70% of the protein backbone automatically from a single SAD dataset only, with R-free of 37.5

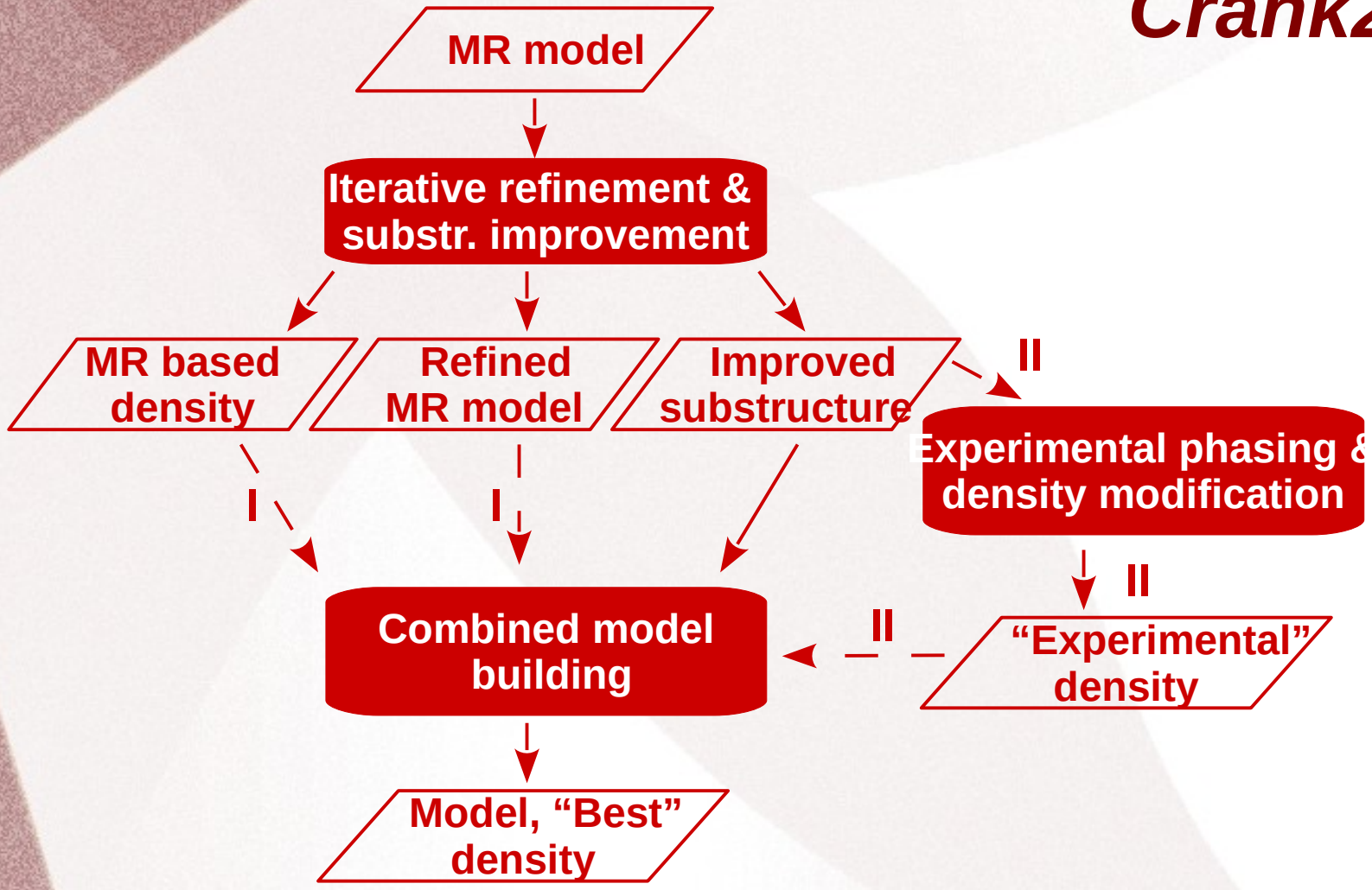
12-subunit RNA polymerase II



SAD after MR (MR-SAD)

- If initial phases are from MR but also significant anomalous information is available, it can improve the model building
- Basic steps:
  - Anomalous substructure completion
  - Model building using the “combined” algorithm
- Two approaches:
  - Rebuild from the MR phases and combine with SAD phases (MR-SAD rebuilding)
  - Rebuild from the SAD map obtained from the (completed) MR substructure

MR-SAD pipelines in Crank2



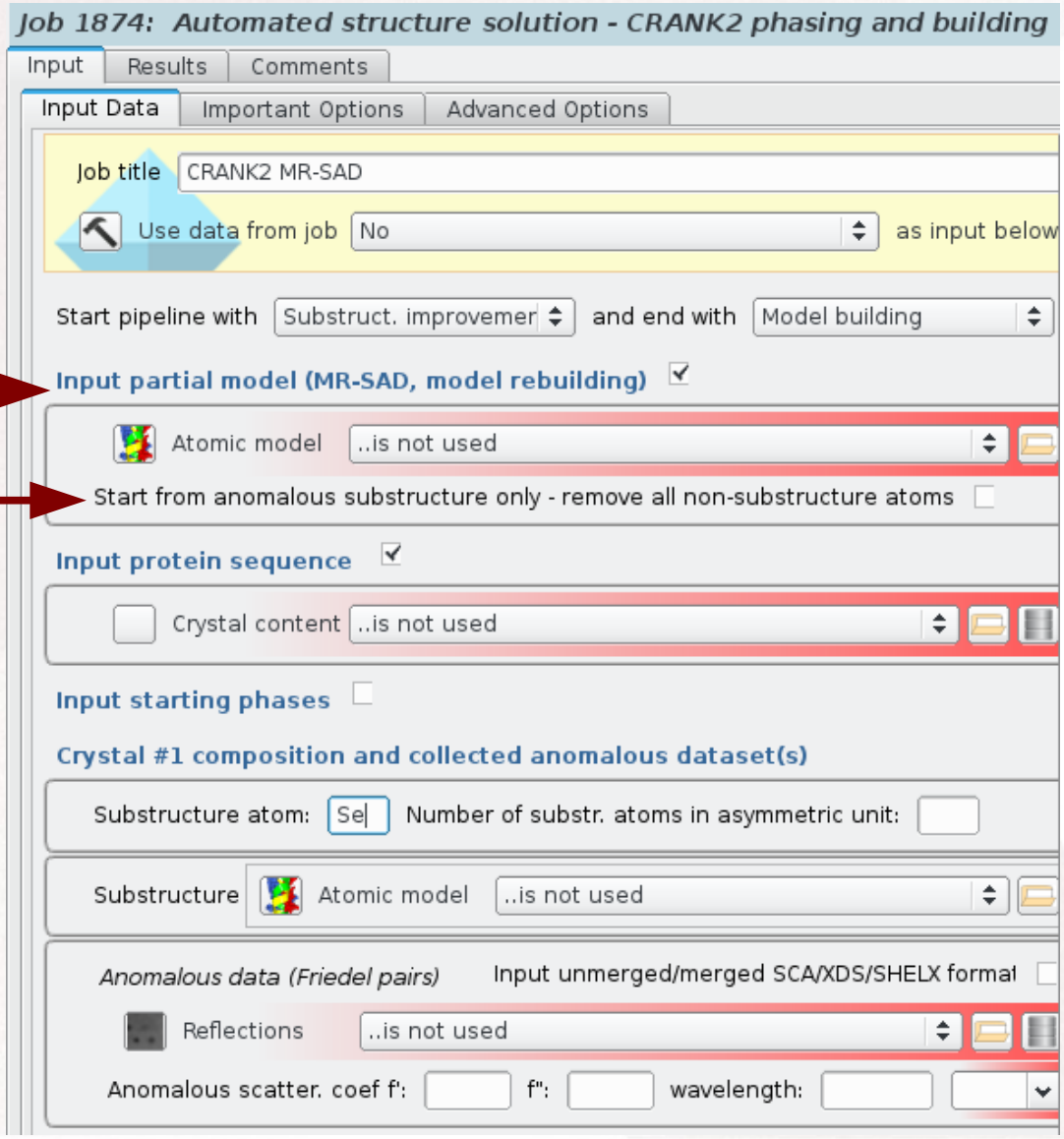
I = MR-SAD rebuilding pipeline
II = SAD-only pipeline (from the MR anom. substructure)

Low resolution MR-SAD examples

	resol. [A]	Rfree init.MR ref.	SAD-only	MR-SAD
• unpubl.1	3.6	51.2	32.6	29.8
• unpubl.2	3.2	53.7	51.4	35.8
• 5kvm	3.0	48.6	39.1	38.4
• 4d80	3.6	47.5	39.0	40.9
• 3din	4.5	51.8	39.9	39.6
• 3u5z	3.5	56.8	40.8	39.8

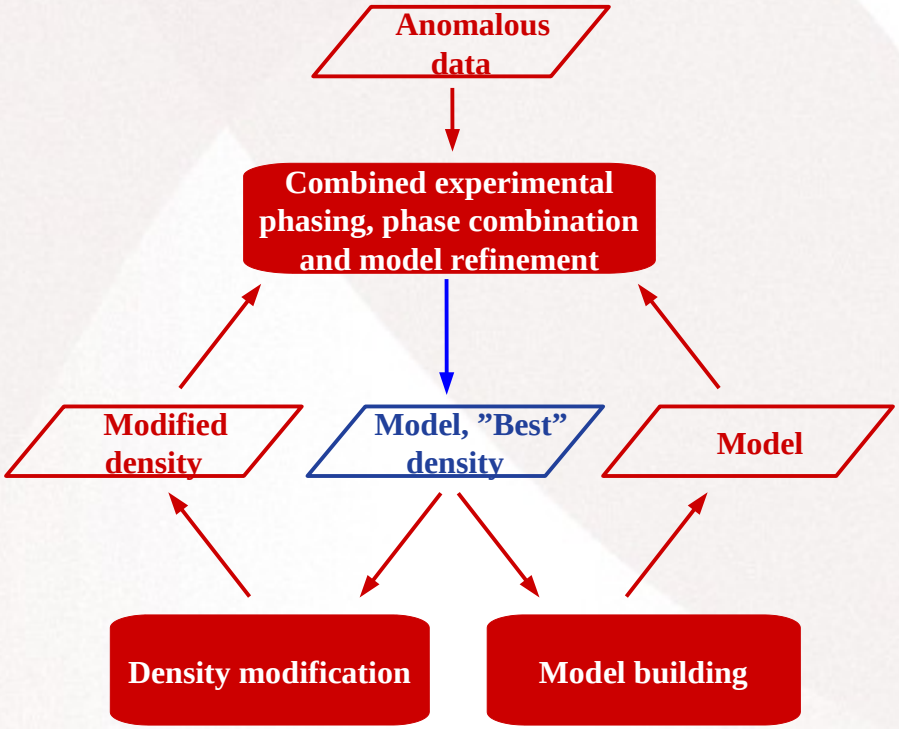
(Skubak et al, IUCrJ, 2018)

MR-SAD / SAD-only in ccp4i2



Parallel combined model building

Idea: iteratively run several processes in parallel with differing parametrization, and in each iteration pick the best result(s) or merge (denoted by the blue color)



Implemented in Crank2 in 2019, available as an option in i2

Parallel model building example:

- Difficult 3.2A SAD data from the McGill lab / Quebec, Canada by Juliana Munoz, collected at the APS/CCP4 School workshop
- Eventually, the structure was solved by manually running a large number of Crank2 jobs (~30) in 6 steps, where each step consisted of ~5 different jobs run in parallel:

  Rfree 51% →49% →47% →40% →37% →33%
- The current implementation of parallel parameter perturbation model building can provide an Rfree of 36-41% within a single job (depending on the number of parallel processes)

Parallel model building output

▾ Combined model building

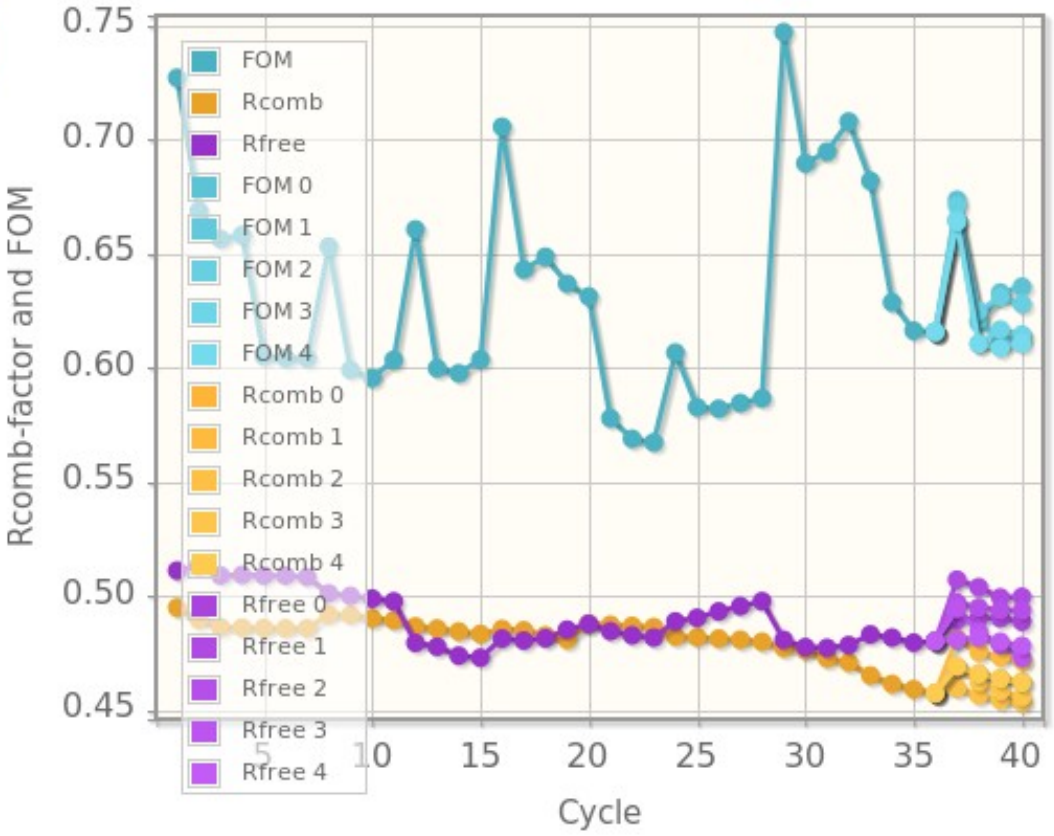
Programs used:

Parrot, REFMAC5, Buccaneer

Graph Data

- ▾ Refinement statistics
  - Rcomb and FOM vs refinement cycle
- ▸ Model building statistics

Print



Conclusions, remarks

- CRANK2 aims to:
  - push the anomalous signal and resolution limits for automatic structure solution
  - provide as good models as possible - by default, better rather than fastest methods

- If structure is not automatically built (or only partially built), first try to determine which step has failed (or can be improved): CRANK2 attempts to make re-running steps easy.

Acknowledgements

- All dataset contributors (JCSG, SSGCID, Z. Dauter, M.Weiss, A.Sharma, ...)

- Garib Murshudov, Kevin Cowtan, George Sheldrick, Andrea Thorn, Jon Agirre, Victor Lamzin, Charles Ballard, Andrey Lebedev, Ville Uski, Eugene Krissinel, Ronald v. Brempt, Francois Remacle, Peter Briggs, Norman Stein, Martyn Winn