CC* - Linking crystallographic model and data quality

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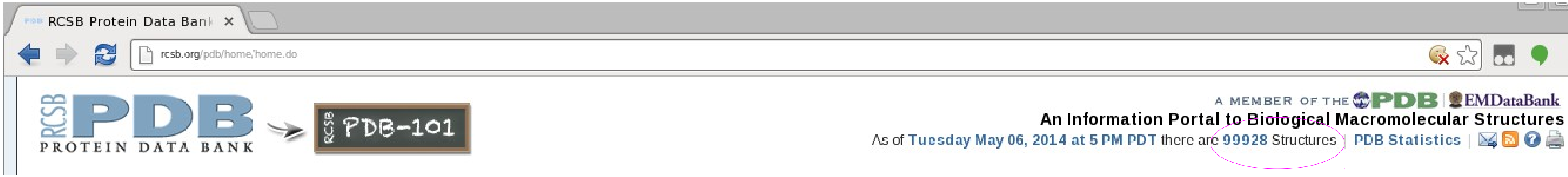
Crystallography has been highly successful

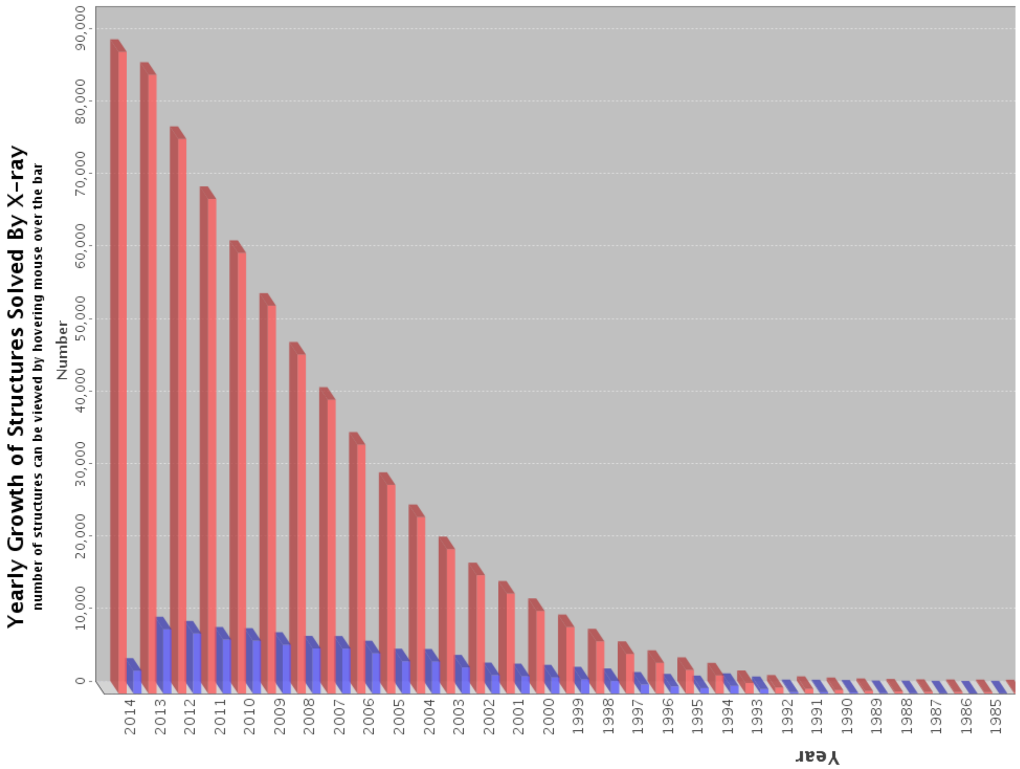


RCSB Protein Data Bank

rcsb.org/pdb/home/home.do

An Information Portal to Biological Macromolecular Structures

As of Tuesday May 06, 2014 at 5 PM PDT there are 99928 Structures | PDB Statistics |



Could it be any better?

Confusion – what do these mean?

$CC_{1/2}$

R_{merge}

R_{sym}

Mn(I/sd)

CC_{anom}

I/σ

R_{pim}

R_{meas}

R_{cum}

Topics

Signal *versus* noise

Random *versus* systematic error

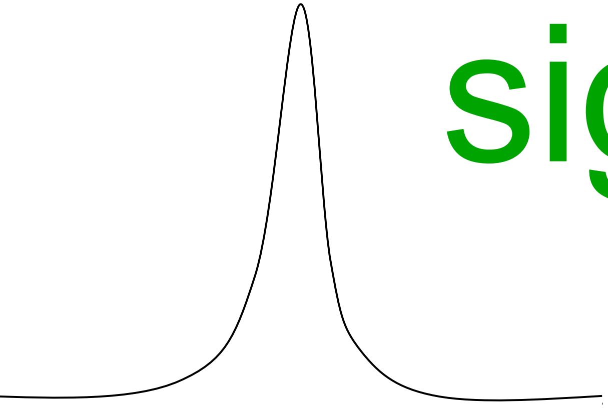
Accuracy *versus* precision

Unmerged *versus* merged data

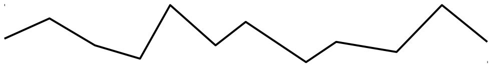
R-values *versus* correlation coefficients

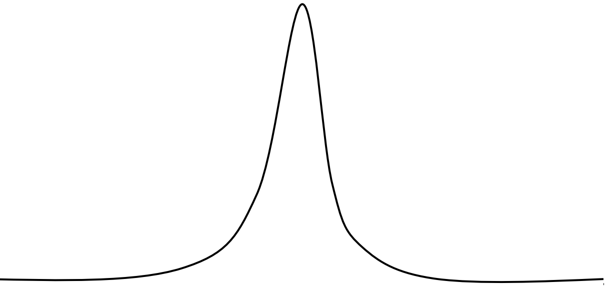
Choice of high-resolution cutoff

signal *vs* noise

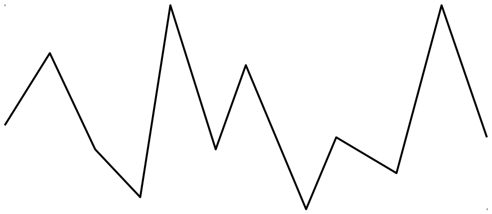


easy

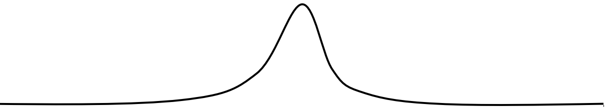




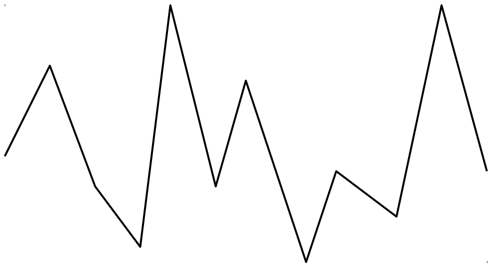
hard



threshold of “solvability”



impossible



James Holton slide

„noise“: what is noise? what kinds of errors exist?

noise = random error + systematic error

random error results from quantum effects

systematic error results from everything else: technical or other macroscopic aspects of the experiment

Random error (noise)

Statistical events:

- photon emission from xtal
- photon absorption in detector
- electron hopping in semiconductors (amplifier etc)

non-obvious

Systematic errors (noise)

- beam flicker (instability) in flux or direction
- shutter jitter
- vibration due to cryo stream
- split reflections, secondary lattice(s)
- absorption from crystal and loop
- radiation damage
- detector calibration and inhomogeneity; overload
- shadows on detector
- deadtime in shutterless mode
- imperfect assumptions about the experiment and its geometric parameters in the processing software
- ...

Adding noise

James Holton slide

Adding noise

$$1 \quad + 1 \quad = 1.4$$

James Holton slide

Adding noise

$$1 + 1 = 1.4$$

$$\sigma_1^2 + \sigma_2^2 = \sigma_{total}^2$$

James Holton slide

Adding noise

$$1^2 + 1^2 = 1.4^2$$

$$\sigma_1^2 + \sigma_2^2 = \sigma_{total}^2$$

James Holton slide

Adding noise

$$1^2 + 1^2 = 1.4^2$$

$$3^2 + 1^2 = 3.2^2$$

$$\sigma_1^2 + \sigma_2^2 = \sigma_{total}^2$$

James Holton slide

non-obvious

Adding noise

$$1^2 + 1^2 = 1.4^2$$

$$3^2 + 1^2 = 3.2^2$$

$$10^2 + 1^2 = 10.05^2$$

James Holton slide

This law is only valid if errors are independent!

non-obvious

How do random and systematic *error* depend on the *signal*?

random error obeys *Poisson statistics*
error = square root of signal

Systematic error is *proportional* to signal
error = x * signal (e.g. x=0.02 ... 0.10)

(which is why James Holton calls it „fractional error“; there are exceptions)

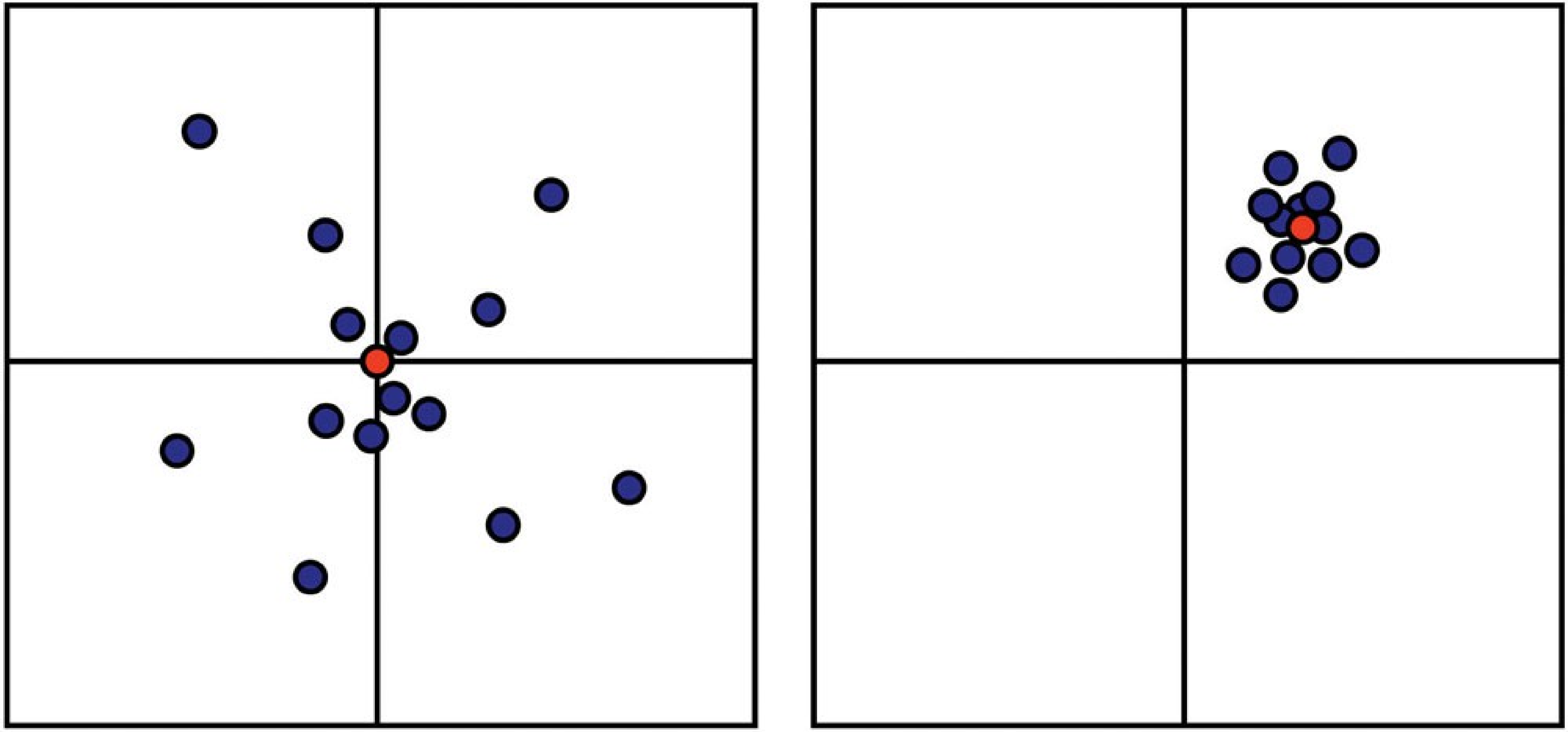
non-obvious

Consequences

- need to add both types of errors
- at high resolution, random error dominates
- at low resolution, systematic error dominates
- but: radiation damage influences both the low and the high resolution (the factor x is low at low resolution, and high at high resolution)

non-obvious

How to measure quality?



B. Rupp, Biomolecular Crystallography

Accuracy – how close to the true value?

Precision – how close are measurements?

non-obvious

What is the „true value“?

- **if only random error exists, accuracy = precision (on average)**
- if unknown systematic error exists, true value cannot be found from the data themselves
- a good model can provide an approximation to the truth
- model calculations do provide the truth
- consequence: precision can easily be calculated, but not accuracy
- **accuracy and precision differ by the unknown systematic error**

All data quality indicators estimate *precision* (only), but YOU want to know *accuracy*!

Numerical example

Repeatedly determine π=3.14159... as 2.718, 2.716, 2.720 :

high precision, low accuracy.

Precision= relative deviation from average value= (0.002+0+0.002)/(2.718+2.716+2.720) = 0.049%

Accuracy= relative deviation from true value= (3.14159-2.718) / 3.14159 = 13.5%

Repeatedly determine π=3.14159... as 3.1, 3.2, 3.0 :

low precision, high accuracy

Precision= relative deviation from average value= (0.04159+0+0.05841+0.14159)/(3.1+3.2+3.0) = 2.6%

Accuracy= relative deviation from true value: 3.14159-3.1 = 1.3%

Calculating the precision of unmerged data

Precision indicators for the unmerged (individual) observations:

$<I_i/\sigma_i>$ (σ_i from error propagation)

$$R_{merge} = \frac{\sum_{hkl} \sum_{i=1}^{n} |I_i(hkl) - \bar{I}(hkl)|}{\sum_{hkl} \sum_{i=1}^{n} I_i(hkl)}$$

$$R_{meas} = \frac{\sum_{hkl} \sqrt{\frac{n}{n-1}} \sum_{i=1}^{n} |I_i(hkl) - \bar{I}(hkl)|}{\sum_{hkl} \sum_{i=1}^{n} I_i(hkl)}$$

$R_{meas} \sim 0.8 / <I_i/\sigma_i>$

Averaging („merging“) of observations

Intensities:

$$I = \sum I_i/\sigma_i^2 \,/\, \sum 1/\sigma_i^2$$

Sigmas:

$$\sigma^2 = 1 \,/\, \sum 1/\sigma_i^2$$

(see Wikipedia: „weighted mean“)

non-obvious

Merging of observations may improve accuracy and precision

- Averaging („merging“) requires multiplicity („redundancy“)
- (Only) **if errors are unrelated, averaging with multiplicity n decreases the error of the averaged data by sqrt(n)**
- Random errors *are* unrelated by definition: averaging always decreases the random error of merged data
- Averaging *may* decrease the systematic error in the merged data. This requires sampling of its possible values - „true multiplicity“
- **If errors are related, precision improves, but not accuracy**

Calculating the precision of merged data

- using the sqrt(n) law: <I/σ(I)>

$$R_{pim}=\frac{\sum_{hkl}\sqrt{1/n-1}\sum_{i=1}^{n}\left|I_i(hkl)-\bar{I}(hkl)\right|}{\sum_{hkl}\sum_{i=1}^{n}I_i(hkl)}$$

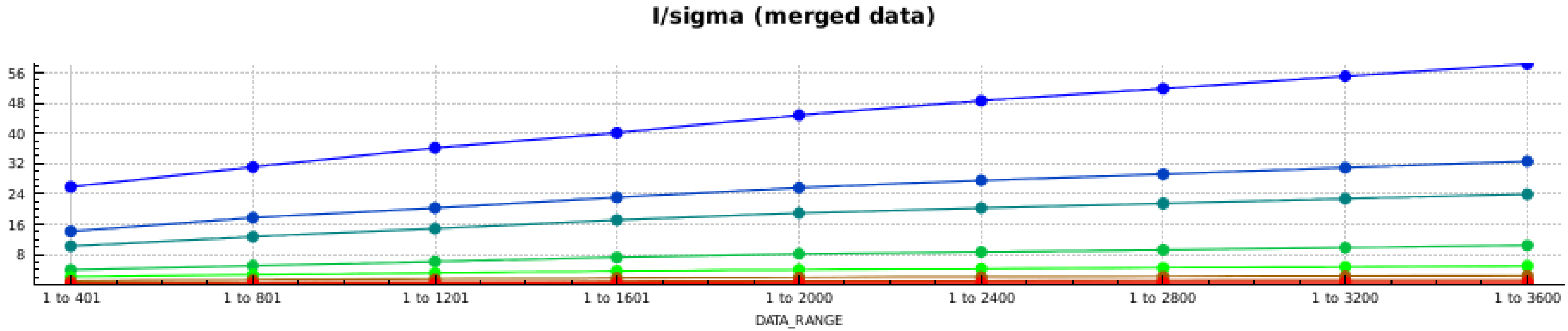
R_{pim} ~ 0.8 / <I/σ >

- by comparing averages of two randomly selected half-datasets X,Y:

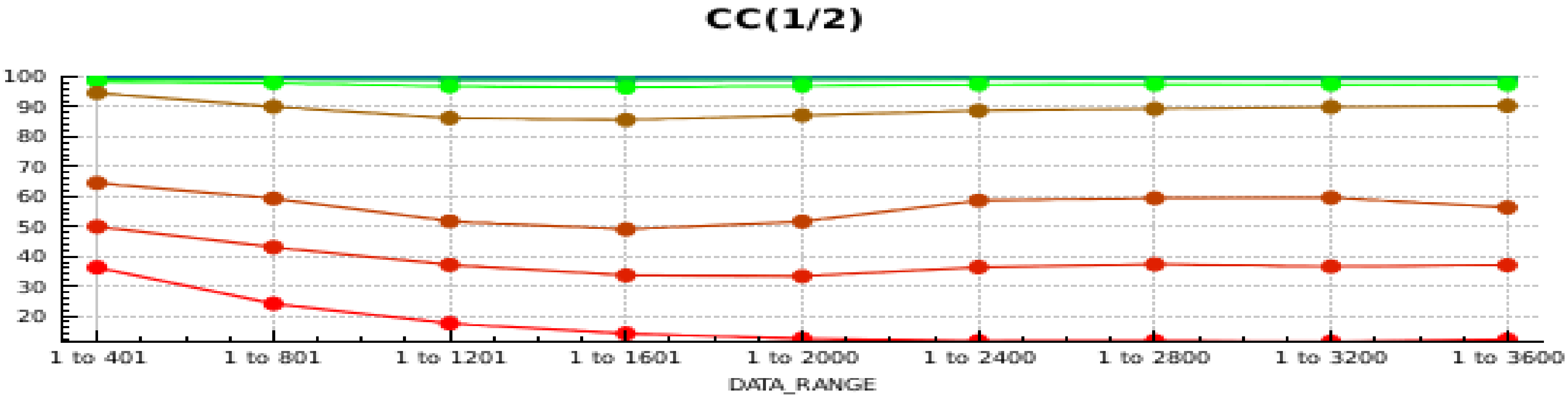
H,K,L	I_i in order of measurement	Assignment to half-dataset	Average I of	
			X	Y
1,2,3	100 110 120 90 80 100	X, X, Y, X, Y, Y	100	100
1,2,4	50 60 45 60	Y X Y X	60	47.5
1,2,5	1000 1050 1100 1200	X Y Y X	1100	1075
...				

(calculate the R-factor (D&K1997) or correlation coefficient (K&D 2012) on X, Y)

I/σ with $\sigma^2 = 1 / \sum 1/\sigma_i^2$



$$r = \frac{\sum_{i=1}^{n}\left(\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)\right)}{\sqrt{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2 \sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2}}$$



Shall I use an indicator for precision of *unmerged* data, or of *merged* data?

It is essential to understand the difference between the two types, but you don't find this in the papers / textbooks!

Indicators for precision of *unmerged* data help to e.g.
* decide between spacegroups
* calculate amount of radiation damage (see XDS tutorial)

Indicators for precision of *merged* data assess suitability
* for downstream calculations (MR, phasing, refinement)

Crystallographic statistics - which indicators are being used?

- Data R-values: $R_{pim} < R_{merge} = R_{sym} < R_{meas}$

$$R_{pim}=\frac{\sum_{hkl}\sqrt{1/n-1}\sum_{i=1}^{n}|I_i(hkl)-\bar{I}(hkl)|}{\sum_{hkl}\sum_{i=1}^{n}I_i(hkl)}$$

merged data

$$R_{merge}=\frac{\sum_{hkl}\sum_{i=1}^{n}|I_i(hkl)-\bar{I}(hkl)|}{\sum_{hkl}\sum_{i=1}^{n}I_i(hkl)}$$

unmerged data

$$R_{meas}=\frac{\sum_{hkl}\sqrt{\frac{n}{n-1}}\sum_{i=1}^{n}|I_i(hkl)-\bar{I}(hkl)|}{\sum_{hkl}\sum_{i=1}^{n}I_i(hkl)}$$

unmerged data

- Model R-values: R_{work}/R_{free}

$$R_{work/free}=\frac{\sum_{hkl}|F_{obs}(hkl)-F_{calc}(hkl)|}{\sum_{hkl}F_{obs}(hkl)}$$

merged data

- I/σ (for *unmerged* or *merged* data !)
- $CC_{1/2}$ and CC_{anom} for the *merged* data

Decisions and compromises

Which high-resolution cutoff for refinement?

Higher resolution means better accuracy and maps

But: high resolution yields high R_{work}/R_{free}!

Which datasets/frames to include into scaling?

Reject negative observations or unique reflections?

The reason why it is difficult to answer “R-value questions” is that no proper mathematical theory exists that uses absolute differences; concerning the use of R-values, Crystallography is disconnected from mainstream Statistics

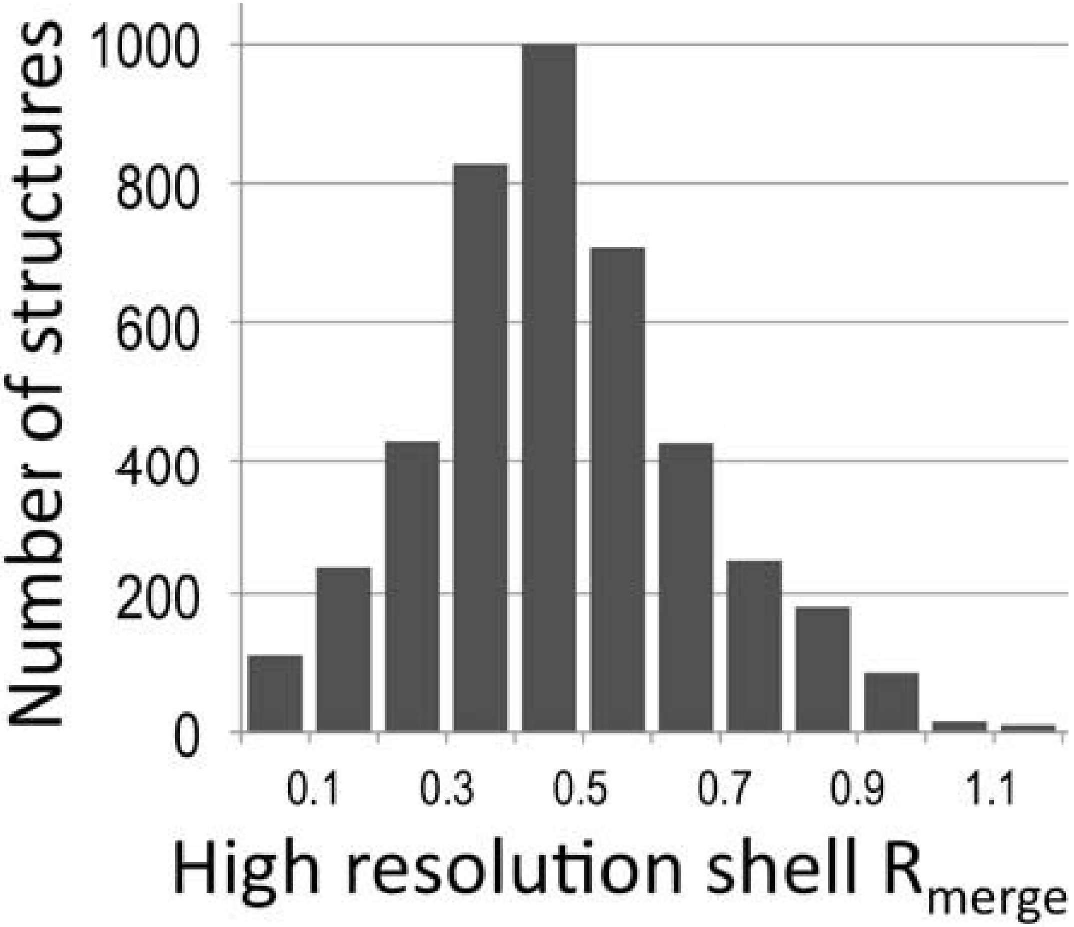
Conflicting views

„An appropriate choice of resolution cutoff is difficult and sometimes seems to be performed mainly to satisfy referees ... Ideally, we would determine the point at which adding the next shell of data is not adding any statistically significant information ... R_{merge} is not a very useful criterion.“
P. Evans (2011) An introduction to data reduction: space-group determination, scaling and intensity statistics. *Acta Cryst.* **D67**, 282

"At the highest resolution shell, the R_{merge} can be allowed to reach 30–40% for low-symmetry crystals and up to 60% for high-symmetry crystals, since in the latter case the redundancy is usually higher."
A. Wlodawer, W. Minor, Z. Dauter and M. Jaskolski (2008) Protein crystallography for non-crystallographers, or how to get the best (but not more) from published macromolecular structures. *FEBS J.* **275**, 1

“... the accepted resolution limit is where the I/sigI falls below about 2.0. R_{merge} may then reach 20-40%, depending on the symmetry and redundancy.“ Z. Dauter (1999) Data-collection strategies. *Acta Cryst* **D55**, 1703

2010 PDB depositions



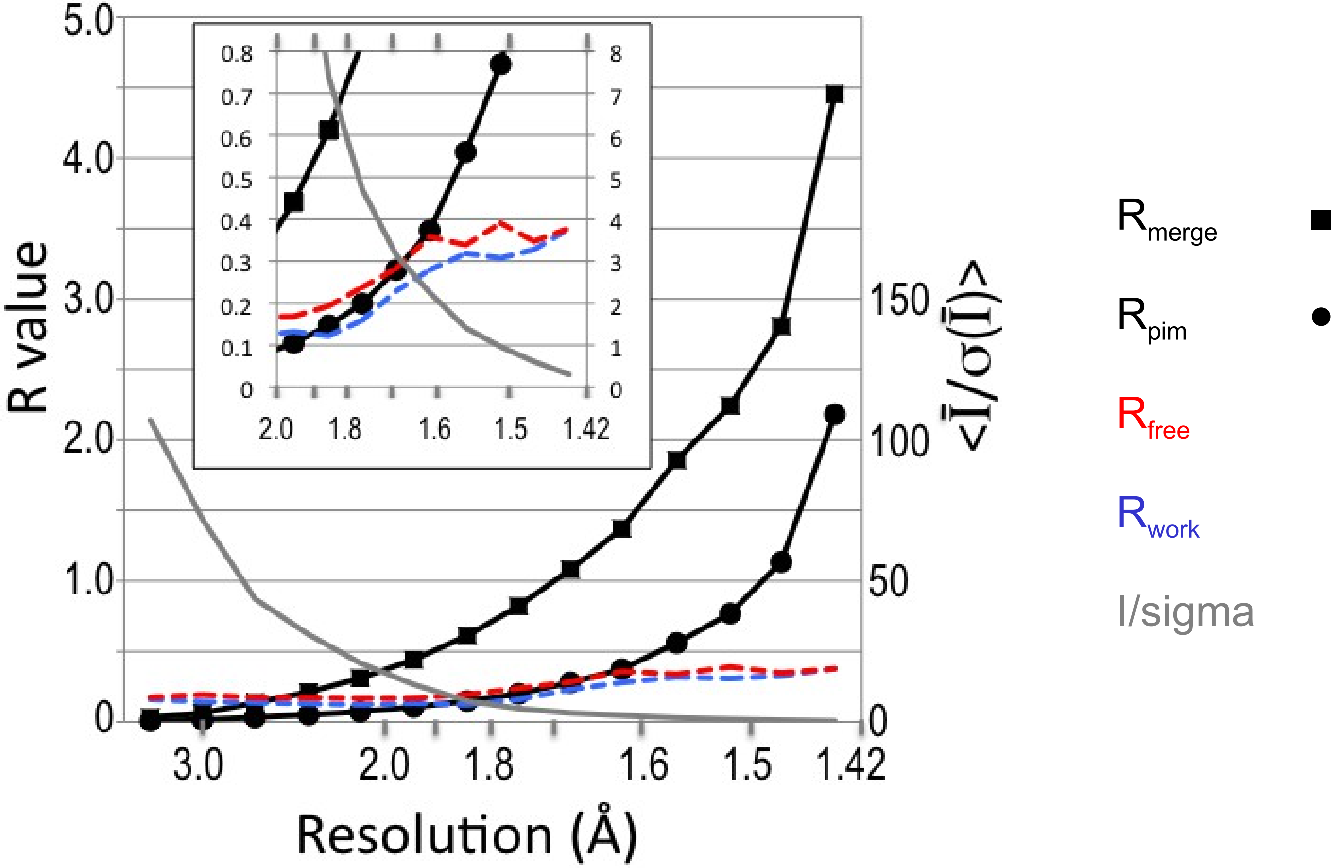
Improper crystallographic reasoning

- typical example: data to 2.0 Å resolution
- using all data: R_{work}=19%, R_{free}=24% (overall)
- cut at 2.2 Å resolution: R_{work}=17%, R_{free}=23%
- „cutting at 2.2 Å is better because it gives lower R-values“

Proper crystallographic reasoning

1. Better data allow to obtain a better model
2. A better model has a lower R_{free}, and a lower R_{free}-R_{work} gap
3. *Comparison* of model R-values is only *meaningful* when using the *same* data
4. Taken together, this leads to the *„paired refinement technique“*: compare models in terms of their R-values against the *same* data.

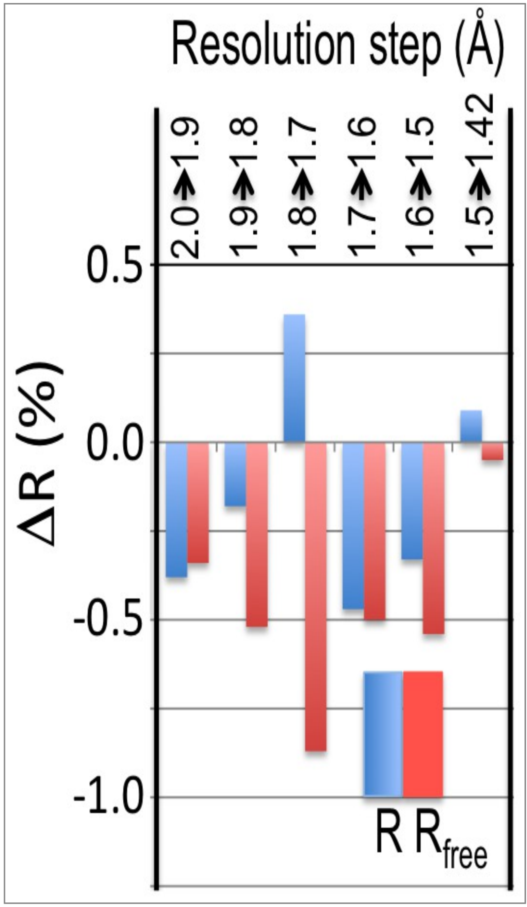
Example: Cysteine DiOxygenase (CDO; PDB 3ELN) re-refined against 15-fold weaker data

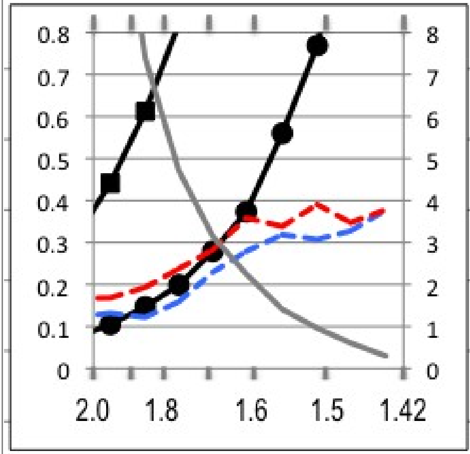


Is there information beyond the conservative hi-res cutoff?

“**Paired refinement technique**“:

- refine at (e.g.) 2.0Å and at 1.9Å using the *same* starting model and refinement parameters
- since it is *meaningless* to compare R-values at *different* resolutions, calculate the overall *R-values of the 1.9Å model at 2.0Å* (`main.number_of_macro_cycles=1 strategy=None fix_rotamers=False ordered_solvent=False`)
- $\Delta R = R_{1.9}(2.0) - R_{2.0}(2.0)$





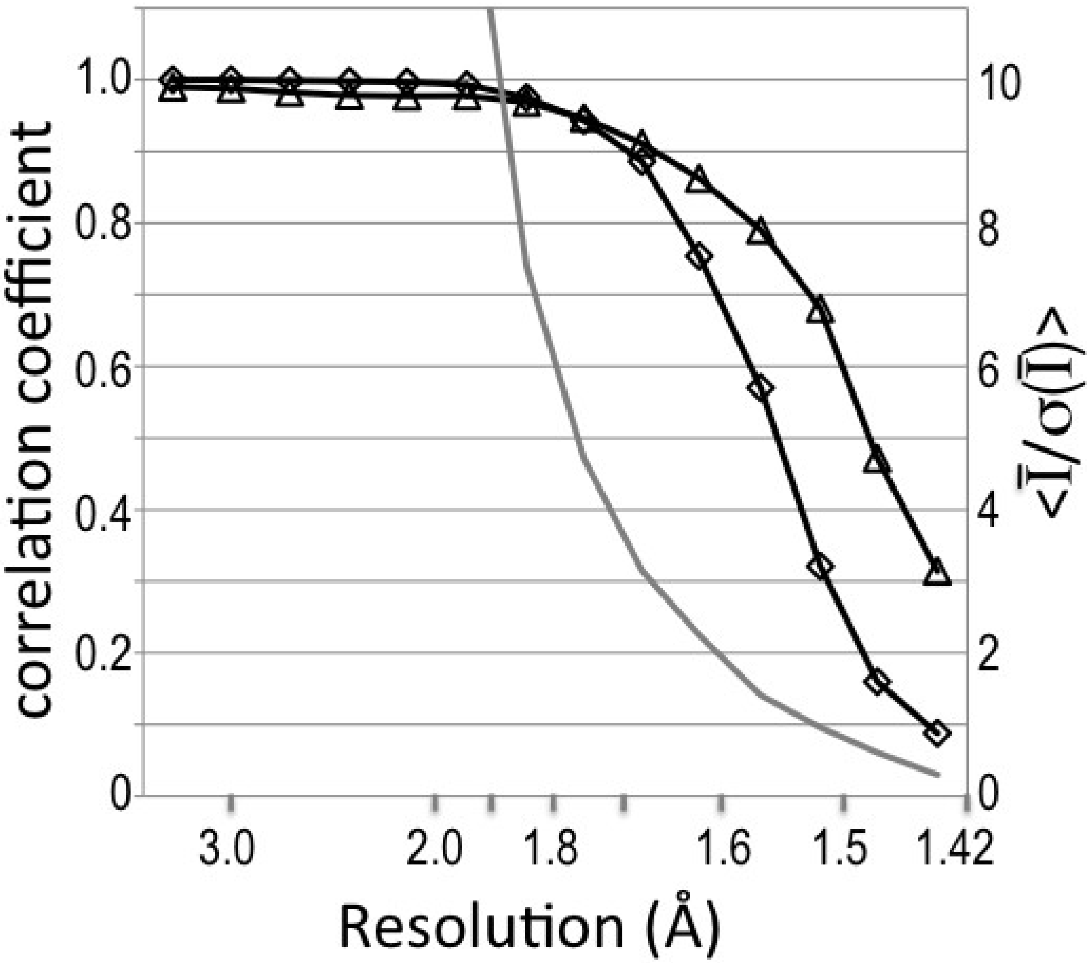
Measuring the precision of merged data with a correlation coefficient

- Correlation coefficient has clear meaning and well-known statistical properties
- Significance of its value can be assessed by Student's t-test
  (e.g. CC>0.3 is significant at p=0.01 for n>100; CC>0.08 is significant at p=0.01 for n>1000)
- Apply this idea to crystallographic intensity data: use "random half-datasets" → $CC_{1/2}$ (called CC_Imean by SCALA/aimless, now $CC_{1/2}$)
- From $CC_{1/2}$, we can analytically estimate **CC of the merged dataset against the true** (usually unmeasurable) **intensities** using

$$CC^* = \sqrt{\frac{2\,CC_{1/2}}{1+CC_{1/2}}}$$

- (Karplus and Diederichs (2012) *Science* **336**, 1030)

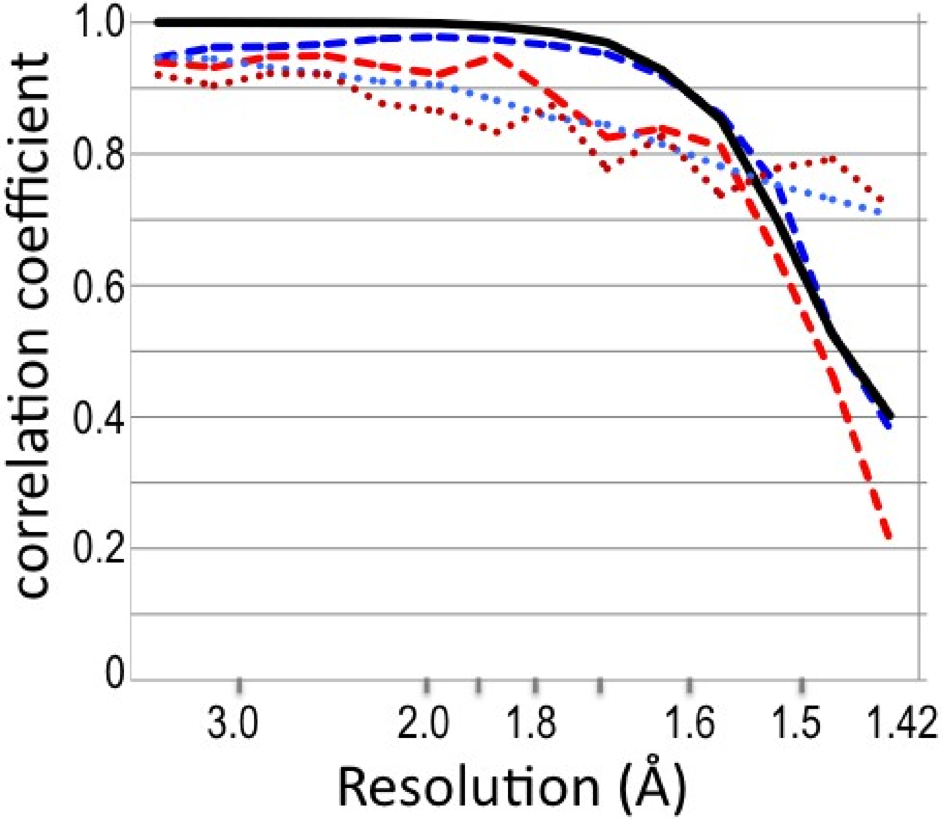
Data CCs



$CC_{1/2}$	◊
CC*	Δ
I/sigma	

Model CCs

- We can define CC_{work}, CC_{free} as CCs calculated on F_{calc}^2 of the working and free set, against the experimental data
- CC_{work} and CC_{free} can be directly compared with CC*



— CC*

Dashes: CC_{work} , CC_{free} against weak exp‘tl data

Dots: $CC‘_{work}$, $CC‘_{free}$ against strong 3ELN data

Four new concepts for improving crystallographic procedures

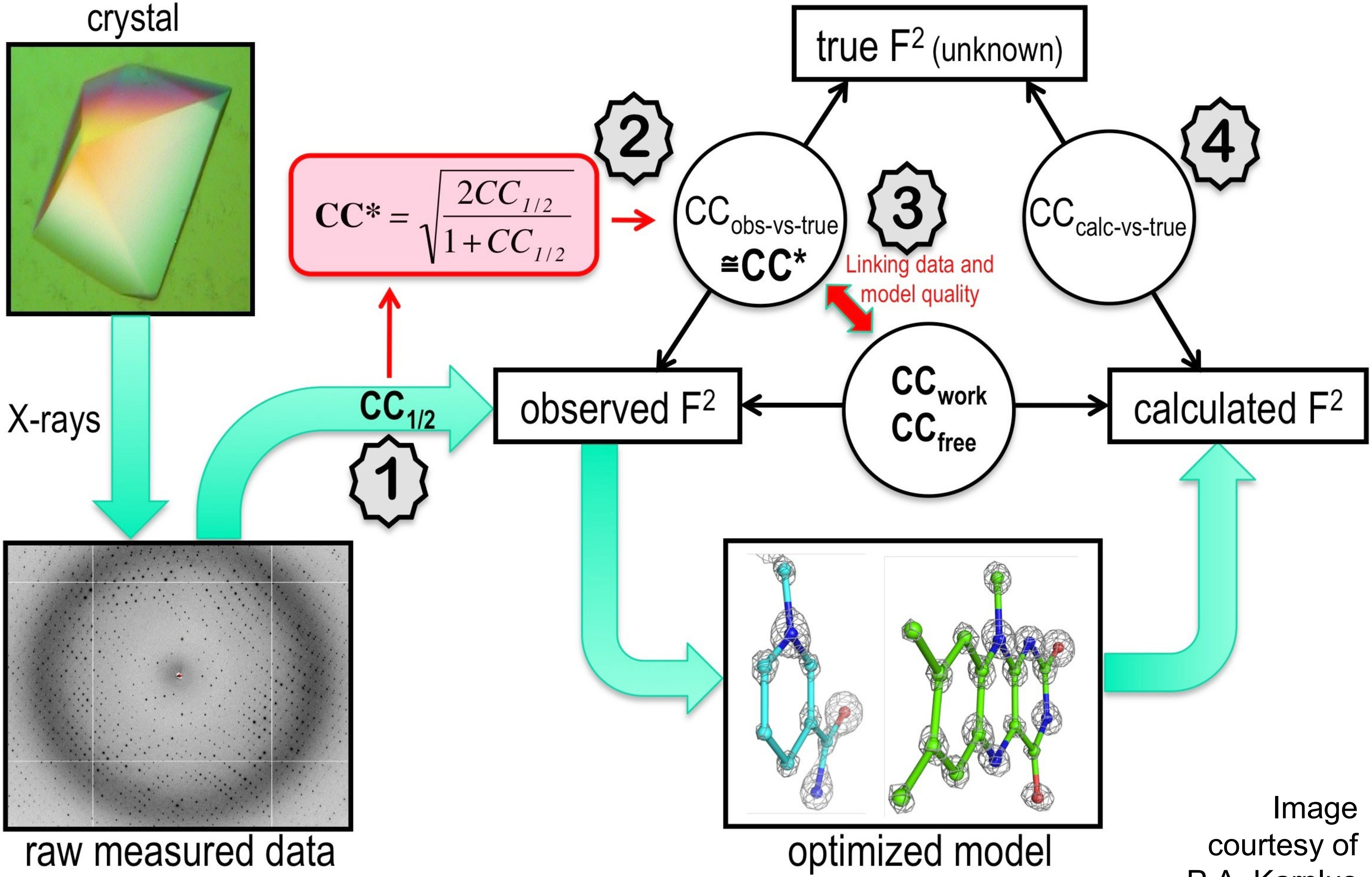


Image courtesy of P.A. Karplus

Summary

- To predict suitability of data for downstream calculations (phasing, MR, refinement), we should use indicators of merged data precision
- R_{merge} should no longer be considered as useful for deciding e.g. on a high-resolution cutoff, or on which datasets to merge, or how large total rotation
- I/σ has two drawbacks: programs do not agree on σ, and its value can only rise with multiplicity
- $CC_{1/2}$ is well understood, reproducible, and directly links to model quality indicators

References

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