Data quality – noise, errors and their taxonomy

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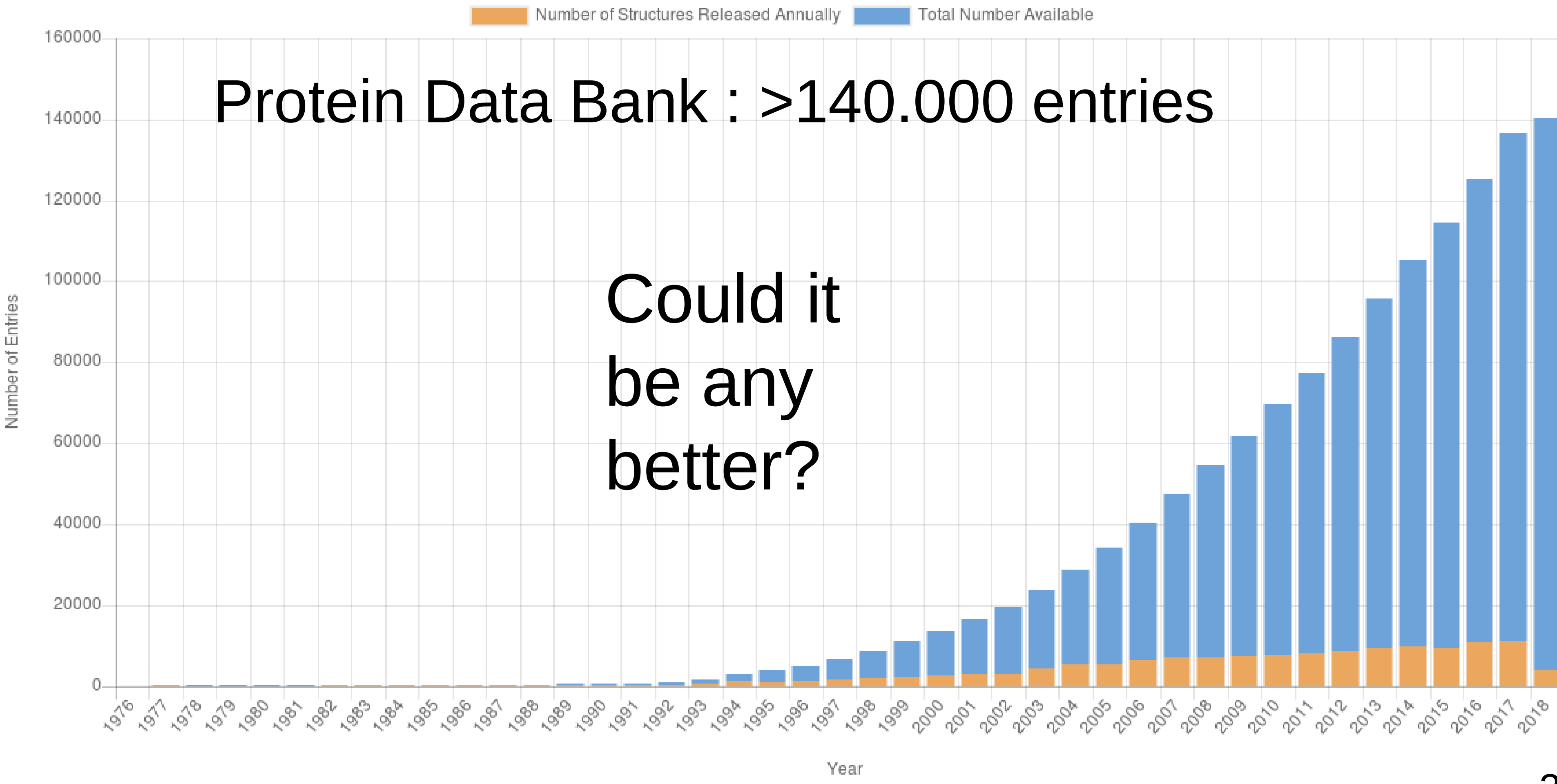
Crystallography has been extremely successful

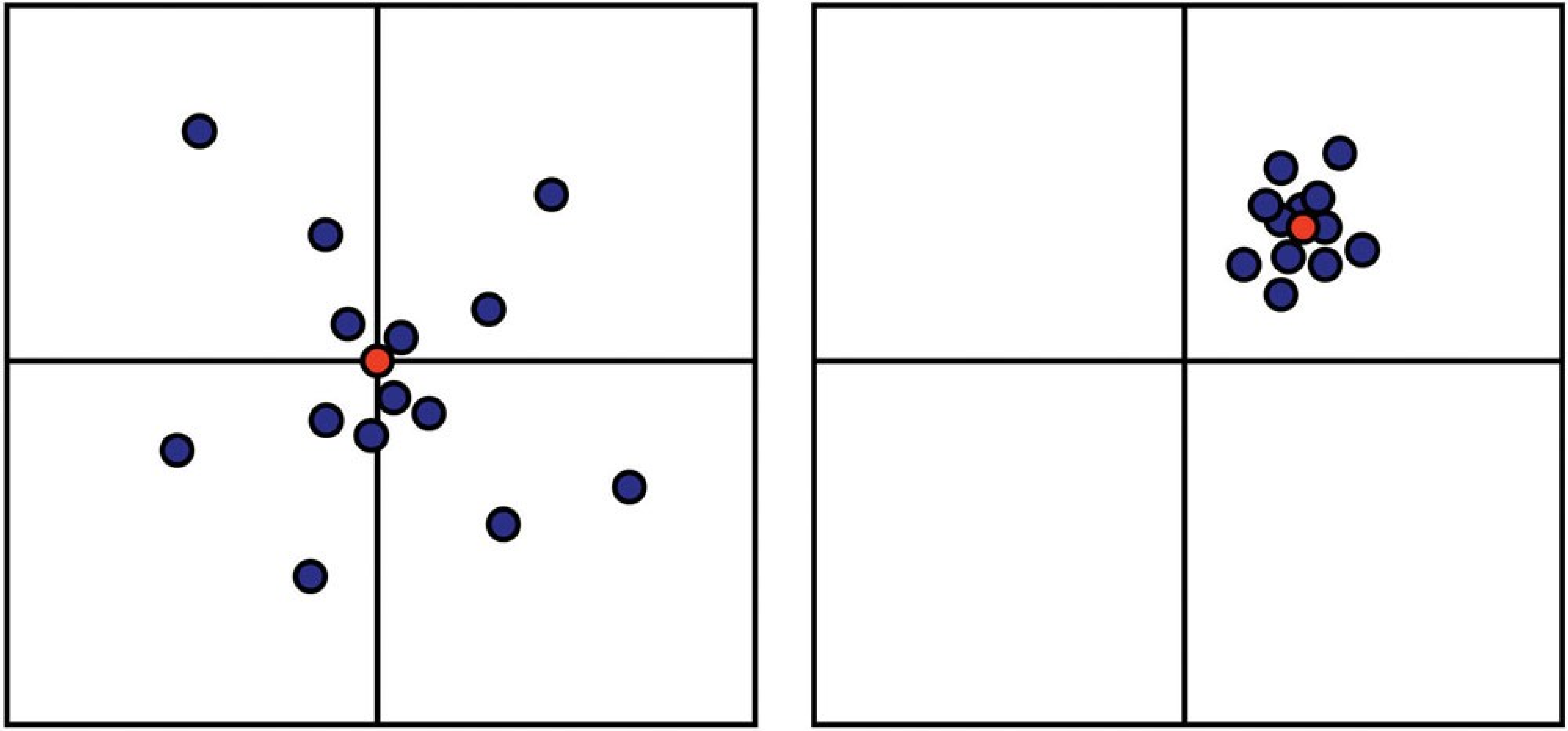


Four examples for

- *Rules* that may have been useful in the past under different circumstances, but are still commonly used today and result in wrong decisions
- *Concepts* resulting from first principles that would, if applied, deliver the information to reach the correct decision

1st example: Not understanding the difference between, and the relevance of **precision** and **accuracy**

"Quality"



B. Rupp, Bio-molecular Crystallography

Accuracy – how different from the *true value*?

Precision – how different are *measurements*?

Numerical example

Repeatedly determine π=3.14... as 3.1, 3.2, 3.0 :
observations have medium precision, medium accuracy

Precision= relative |deviation| from average value=
(0+0.1+0.1)/(3.1+3.2+3.0) = 2.2%

R_{merge} formula!

Accuracy= average relative |deviation| from true value:
=1/3*(|3.14-3.1| + |3.14-3.2| + |3.14-3.0|)/3.14 = 2.5%

Repeatedly determine π=3.14... as 2.70, 2.71, 2.72 :
observations have high precision, low accuracy.

Precision= relative |deviation| from average value=
(0.01+0+0.01)/(2.70+2.71+2.72) = 0.24%

R_{merge} formula!

Accuracy= average relative |deviation| from true value=
1/3*(3.14-2.70 + 3.14-2.71 + 3.14-2.72)/3.14 = 13.7%

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
- 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 (should) want to know *accuracy*!

- ***Rules***: "The data processing statistics tells me (and the reviewers!) how good my data are.
  To satisfy reviewers, the indicators must be good."

- *Suboptimal result*: these rules encourage
  - overexposure of crystal to lower R_{merge}
  - data collection "strategy" with low multiplicity
  - statistics massaging: throw away potentially useful data

- ***Concepts***:
  - Data processing logfiles report the *precision* (consistency) of the data, *not* their *accuracy* (agreement with truth).
  - averaging increases accuracy *unless* the data repeat systematic errors
  - outliers may be correctly ("true positive") or incorrectly ("false positive") identified. Rejections always *increase* precision, but may *decrease* accuracy!

2nd example: confusion by multitude and properties of crystallographic indicators

Confusion – what do these mean?

R_{merge}

$CC_{1/2}$

R_{sym}

Mn(I/sd)

CC_{anom}

I/σ

R_{pim}

R_{meas}

R_{cum}

Calculating the precision of unmerged (individual) observations

$\langle I_i/\sigma_i \rangle$ (σ_i from error propagation, i=individual measurement)

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

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

$R_{meas} \sim 0.8 / \langle I_i/\sigma_i \rangle$

Calculating the precision of merged data

using the √n law of error propagation (Wikipedia “weighted arithmetic mean”):

$\langle I/\sigma(I)\rangle$

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

$R_{pim} \sim 0.8 / \langle I/\sigma \rangle$

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	Average I of 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 $CC_{1/2}$ (K&D 2012) on X, Y)

Measuring the precision of merged data with a correlation coefficient

- Correlation coefficient $cc_{xy}=\frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2\sum(y_i-\bar{y})^2}}$ 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
- Using "random half-datasets" of crystallographic intensity data: → $CC_{1/2}$
- From $CC_{1/2}$, we can analytically estimate **CC of the merged dataset against the true** (usually unmeasurable) **intensities** using

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

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

- ***Rule***: “the quality of the data that I use for refinement can be assessed by R_{merge}/R_{meas} . Data with R_{merge}/R_{meas} > e.g. 60% are useless.”

- Suboptimal result: Wrong indicator. Wrong high-resolution cutoff. Wrong data-collection strategy.

Concept: - use an indicator for the precision of the *merged* data if you are interested in the suitability of the data for MR, phasing and refinement.

- Use <I/σ> or <I>/<σ> (but how to calculate σ; and which cutoff??)

- Use $CC^* = \sqrt{\frac{2CC_{1/2}}{1+CC_{1/2}}}$ if you want to know how high (numerically) CC_{work} , CC_{free} in refinement can become (i.e. how *data quality limits model quality*):
CC_{work} larger than CC* implies overfitting, because in that case the model agrees better with the experimental data than the true signal does.

This does not work with R-values because data R-values and model R-values have different definitions!

3rd example: *improper* crystallographic reasoning

situation: 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%

- ***Rule***: “The lower the R-value, the better.”

„cutting at 2.2 Å is better because it gives lower R-values“

- (Potentially) suboptimal result: throwing away data.

- ***Concept***: indicators may only be compared if they refer to the *same* reflections.

Proper crystallographic reasoning

…. requires three concepts:

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

Taking these together, this leads us to the *„paired refinement technique“*: compare models in terms of their R-values against the *same* data.

P.A. Karplus and K. Diederichs (2012) Linking Crystallographic Data with Model Quality. *Science* **336**, 1030-1033.

Highly controversial?

4^{th} ex.: Resolution of the data

Rules:

1. Worst: cutoff based on R_{merge}/R_{meas} (which value?)
2. Better: cutoff based on $\langle I/\sigma(I)\rangle$ (which value?) merged data
3. Even better, but not good: cutoff based on $CC_{1/2}$ (which value?)

(some people say 50%, others 30-50%; EM "gold standard" is 14.3%) merged data, no σ

Concepts:

1. "ideally, we would determine the point at which adding the next shell of data is not adding any statistically significant information" (P. Evans)
2. paired refinement method proper comparison
3. only a good model can extract information from weak data external
4. R_{work}/R_{free} of model against *noise* is ~43% (G. Murshudov) validation

Advice: be generous at the data processing stage, and
decide only at the very end of refinement
Deposit the data up to the resolution where $CC_{1/2}$ becomes insignificant!

Highly controversial?

Resolution of the model

Rule:

the resolution of the *model* is the resolution of the data it was refined against

Concepts:

1. the notion “resolution of a model” is misguided – it answers the wrong question!

2. *resolution of a map* (Urzhumtsev *et al*) is well-defined: how far are features apart that we can distinguish? depends on Wilson-B

3. better to ask about precision and accuracy of the model
   - precision: reproducibility of coordinates
   - accuracy: which errors are present? much more important!

Understand your data!
(and your problems)

The signal and the noise: random and systematic errors

Random error

True randomness occurs due to the quantum nature of matter.

- counting photons
- electronic noise (detector, electronics)

Random error is proportional to square root of measured value

Systematic error

- crystal: conditions, composition, conformation, damage due to experiment, ...
- apparatus: shadows, absorption, vibrations, photon/electron flux ...
- processing software: inaccurate or incomplete modelling of experiment

Systematic error is proportional to measured value (often 1..10% but sometimes much more e.g. in case of shadows and overloads)

XDS: Level of systematic error is given by ISa = asymptotic signal/noise

Two types of systematic errors

- Some kinds of systematic errors (e.g. beam intensity fluctuation, crystal vibration) “average out” with high multiplicity: they behave like random error upon averaging. We can consider them as “isomorphous systematic errors”.

- Other systematic errors (better: differences) lead to “non-isomorphism” (other fields call this “heterogeneity”)

- Indexing ambiguity (potential in about every third project)
- Cell parameters (may not be determined precisely anyway)
- Radiation damage (within or between data sets)
- Relative humidity (fishing, handling, ...)
- Composition of crystal (ligands, HA, Se, …)
- Conformation of molecules / loops / sidechains

These do not “average out” !

Taxonomy of errors, and how to understand and deal with them

Type of error	Reason	Diagnostics	Treatment	Model accuracy and fit to data
Random	counting photons electronic noise	weak pattern bad R_{merge} good ISa	high multiplicity	good CC_{free} approaches CC*
Systematic, isomorphous	vibrations absorption ...	bad R_{merge} bad ISa CC analysis*: small angle	high multiplicity use kappa gonio	good CC_{free} approaches CC*
Systematic, non-isomorphous	radiation damage overloads / shadows wrong geometry wrong space group twinning ...	bad R_{merge} bad ISa CC analysis*: wide angle	cut later frames mask pixels reprocess try lower symmetry analyze data	bad CC_{free} is below CC*

CCP4BB 28.9.18: “R-merge is too high !!”: good pattern, bad R_{merge}, good model R/R_{work} - this means high systematic but isomorphous errors. https://www.jiscmail.ac.uk/cgi-bin/webadmin?A2=ccp4bb;89f7c3ce.1809

CC analysis*: Diederichs, K. (2017) Dissecting random and systematic differences between noisy composite data sets. *Acta Cryst.* **D73**, 286-293

Summary

- Crystallographic decisions are often based on *rules* of (if anything) only historical interest. These rules frequently lead to *improper shortcuts* being taken
- “make everything as simple as possible, but not simpler” (attributed to A. Einstein)
- Rules may be needed in expert systems; however, humans should rather learn, apply and further develop the underlying *concepts*
- Taxonomy of errors explains “weird” findings

Thank you for your attention!

References:

Karplus, P.A. and Diederichs, K. (2015) Assessing and maximizing data quality in macromolecular crystallography. *Current Opinion in Struct.Biol.* **34**, 60-68.

Diederichs, K. (2015) Crystallographic data and model quality. in: Nucleic Acids Crystallography (Ed. E. Ennifar), *Methods in Molecular Biology* **1320**, 147-173.

Diederichs, K. (2017) Dissecting random and systematic differences between noisy composite data sets. *Acta Cryst.* **D73**, 286-293.

(PDFs at http://cms.uni-konstanz.de/strucbio/diederichs-group/publications)