Assessing data quality – noise, errors and mistakes

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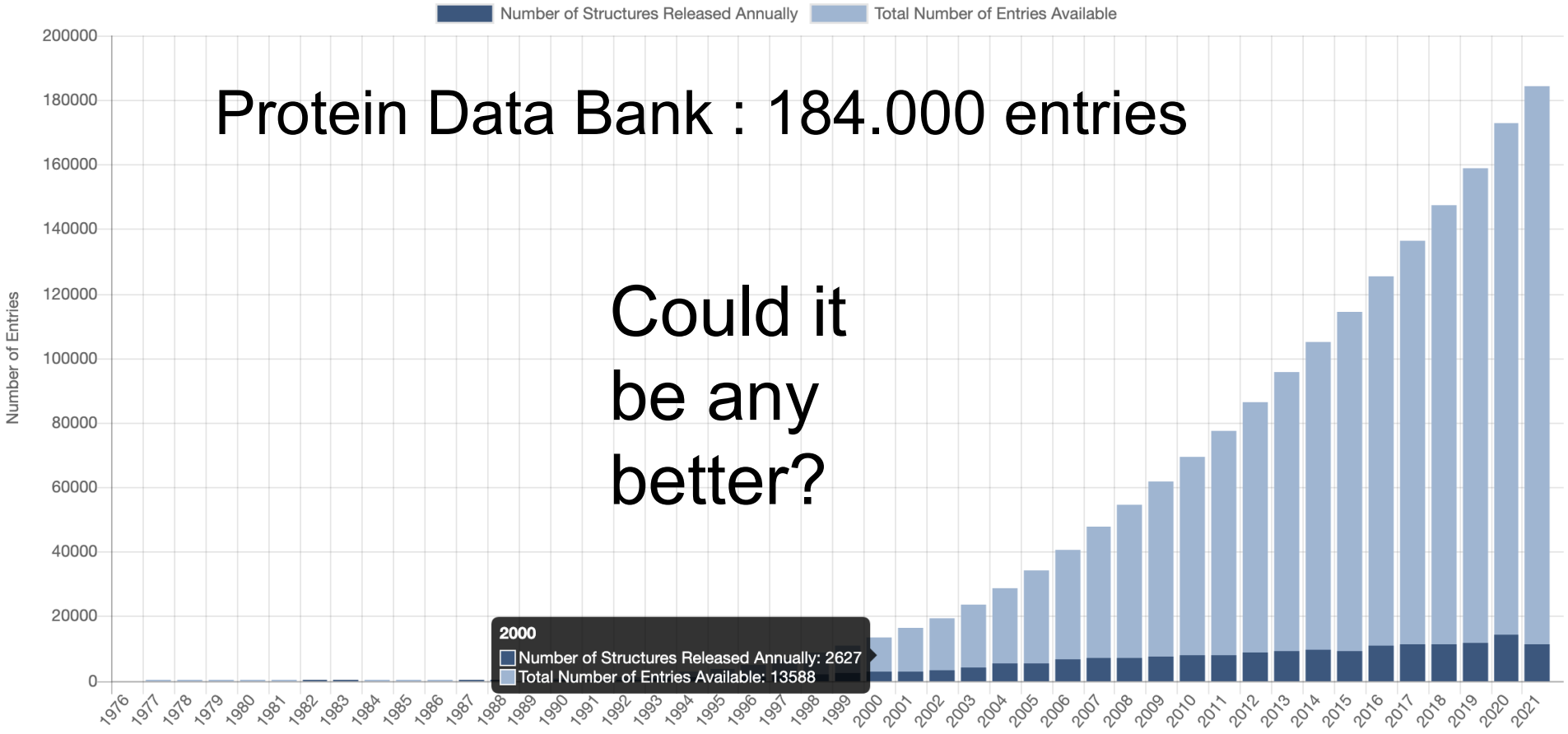
Crystallography has been extremely successful

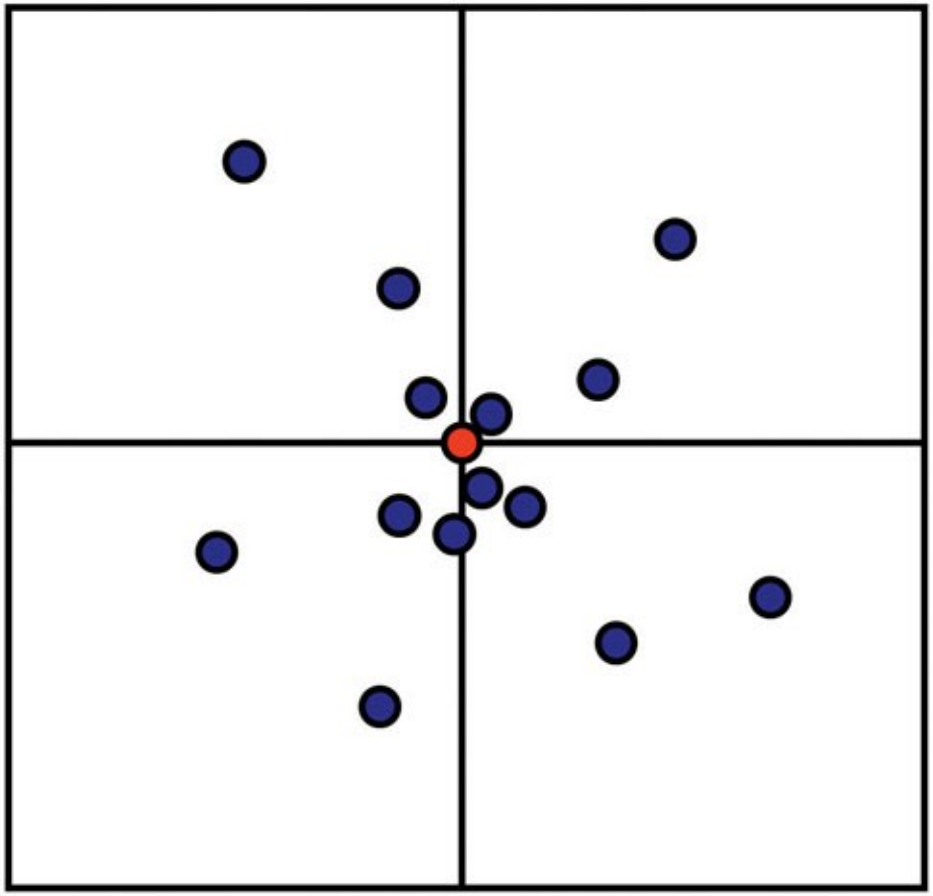


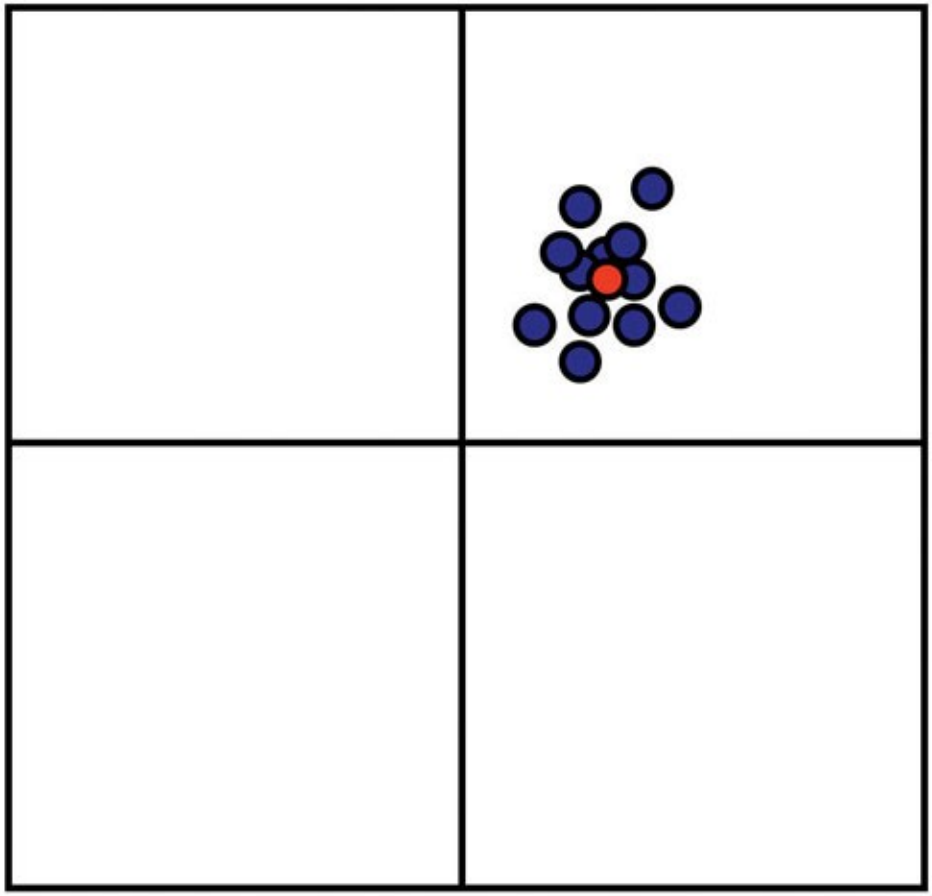
Four examples for

- *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"





Accuracy – how different from the *true value*?

Precision – how different are *measurements from each other*?

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%

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%

R_{merge} formula!

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

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%

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%

R_{merge} formula!

What about the “true value“?

- if only **random error** exists, <accuracy> ≈ <precision> . But how can we be sure?
- if unknown **systematic error** exists, true value cannot be found from the data themselves.
- <accuracy> and <precision> differ by the unknown systematic error
- <precision> can easily be calculated, but not <accuracy>
- <precision> overestimates <accuracy>; <accuracy> is always worse than <precision>

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
-

➔ ***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)

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

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

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

Calculating the precision of merged data

a) using the √n law of error propagation (Wikipedia "weighted arithmetic mean"):

<I/σ(I)>

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

R_{pim} ~ 0.8 / <I/σ>

b) by comparing averages of 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
...				

Then calculate **Pearson correlation coefficient: $CC_{1/2}$ 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

a) 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

b) From $CC_{1/2}$, we can analytically estimate **CC of the merged dataset against the true** (unknown) **intensities** using $CC^*=\sqrt{\frac{2CC_{1/2}}{1+CC_{1/2}}}$, assuming absence of systematic error.

CC* = upper limit of CC_{work}/CC_{free} in refinement (*data quality limits model quality*): $CC_{work}>CC^*$ implies overfitting = model agrees better with data than the true signal does

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

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

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

- Like R_{merge}/R_{meas}, $\mathbf{R_{pim}}$ goes to infinity for weak data, whereas R_{work}/R_{free} approach a constant: R_{pim} cannot predict model agreement with data
- $\langle I/\sigma \rangle$ or $\langle I \rangle / \langle \sigma \rangle$ - but how to calculate σ; and which cutoff??
- $CC_{1/2}$, CC* - no need for σ; normalized; predicts agreement of data with optimal model

Assmann, G., Wang, M., and Diederichs, K. (2020), Acta Cryst D76, 636: **validation of $CC_{1/2}$ as optimization target**

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?

4th 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!

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*

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.

Assmann, G., Wang, M., and Diederichs, K. (2020) Making a difference in multi-data-set crystallography: simple and deterministic data-scaling/selection methods. *Acta Cryst.* **D76**, 636-652

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