NMR vs X-ray, precision, certainty

Main methods

- X-ray crystallography and NMR

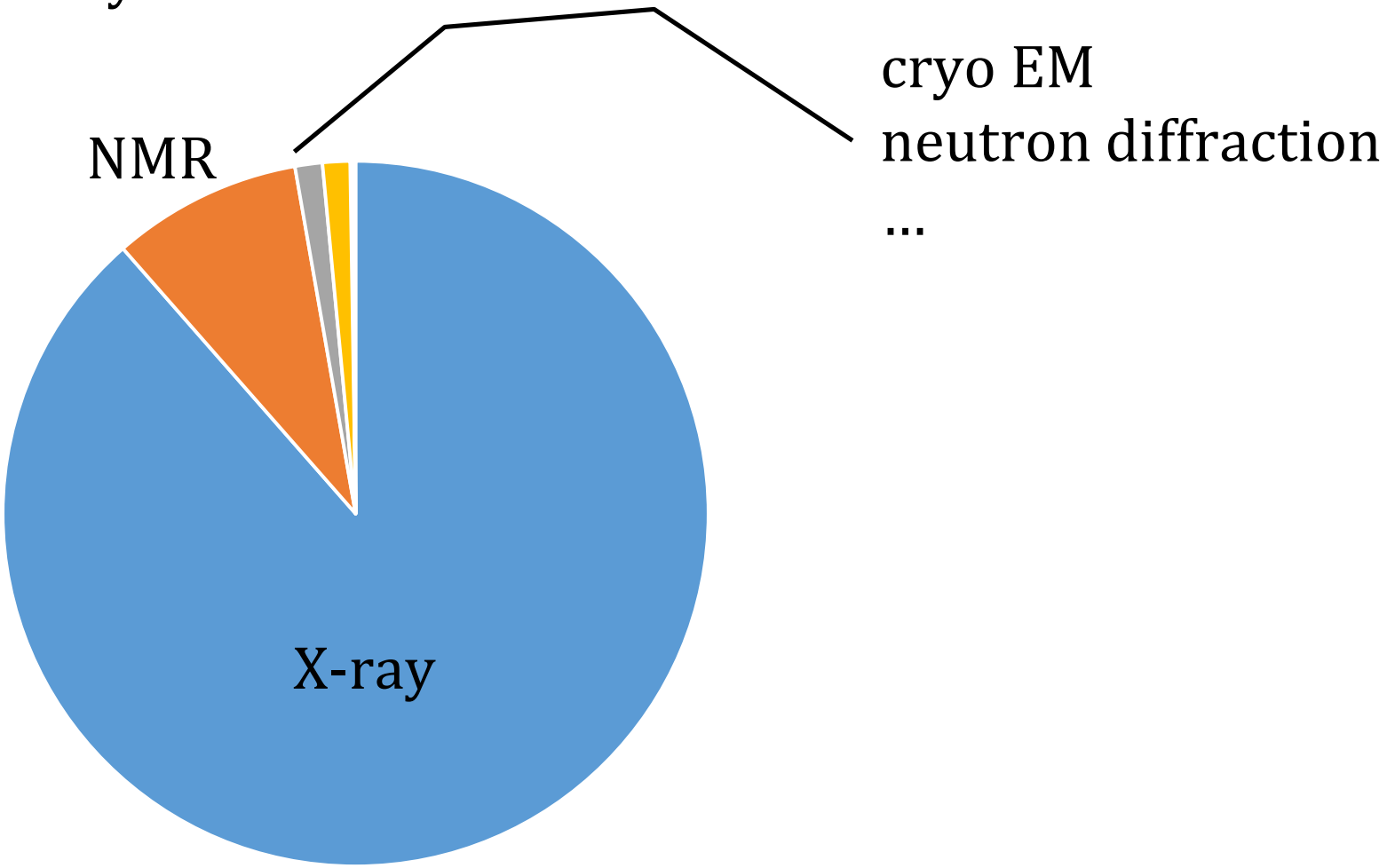
Others

- cryo-electron microscopy (cryo EM)
- small-angle X-ray scattering (SAXS)
- neutron diffraction

- Dominated by proteins, but most comments apply to nucleotides

Techniques for structures

Mostly X-ray



Structure solving techniques

X-ray	89 %	
NMR	9 %	
cryo-EM	2 %	nobel prize 2017

Can you combine methods ?

- X-ray + NMR - rare
- X-ray + cryo-EM – more common
- low and high-resolution X-ray – sometimes

Why focus on X-ray and NMR ?

- emphasis in this course on atomistic detail
- still most important

cryo-EM and SAXS

Why will I not speak about cryo-EM ?

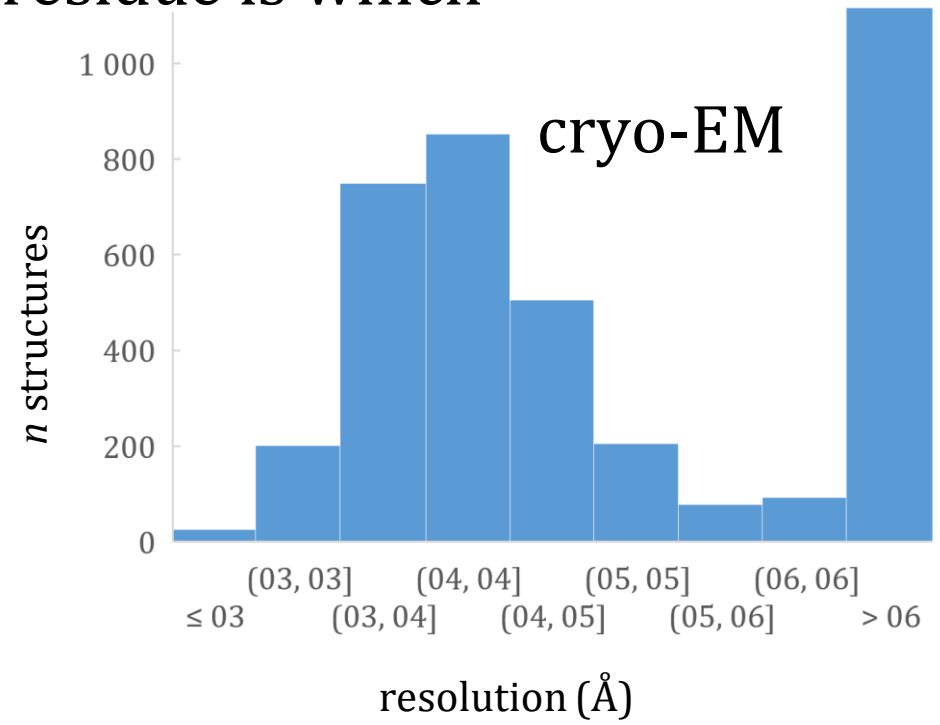
- fashionable, but look at resolution

- distance between two residues $(C_i^{\alpha}, C_{i+1}^{\alpha})$ = 3.8 Å
- cryo-EM cannot tell which residue is which

- getting better every year

- not quite atomic detail

SAXS – even less detail



SAXS = small angle X-ray scattering

Genauigkeit

Why do I care about accuracy ?

- What is a bond length ? (1.07, 1.54, 1.32 .. Å) easy
- How does the energy change as I move an atom ?
- I want to understand protein-ligand binding
  - where is my ligand ?
  - with which residues does it interact ?
  - can I predict the effect of a mutation / substitution ?

A line from the protein data bank

```
ATOM     41  N   ASP A   3      35.790  11.466  -9.466  1.00 16.15
```

$x = 35.790, y = 11.466 \;\; z = \cdots$ total fantasy (10^{-13} m)

Error definitions

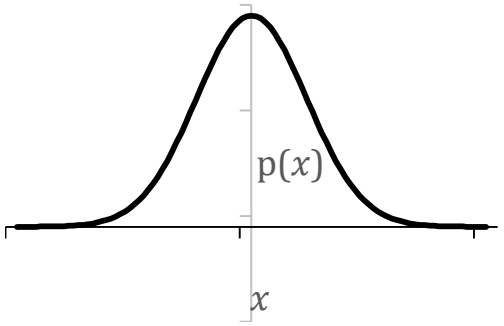
- Accuracy, precision – not good words
- Certainty / uncertainty / confidence – more in statistics
- Resolution – nice word – defined later

What do I mean by error ? $x \pm \varepsilon$

- should say 90% confidence, one σ, 75 % quartiles, ..

How do I interpret this ?

- I imagine a Gaussian (normal) distribution

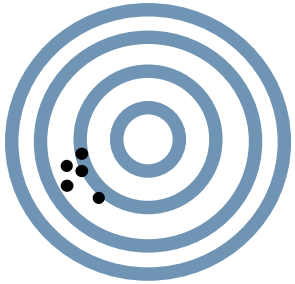


accuracy / precision

basically bad words

- do not use Wikipedia + Übersetzung schwierig

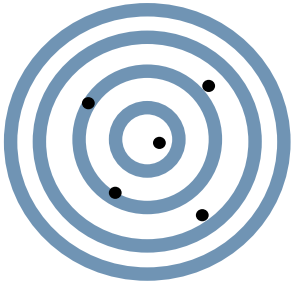
small error



systematic error

repetitions do not help

if you know it, you would correct for it



large random error

repetitions increase certainty

usually modelled with a gaussian

Systematic Errors – are they relevant ?

Hopefully not too much

- X-ray – very small – perhaps in old structures
  - not all programs use exactly the same references for bond lengths / angles
- NMR – distances
  - if you only use upper bounds are you changing the distributions ?
  - error in calibrating NOE → distance conversion
    - all distances will be too large / small
- NMR – calculation of structures
  - older structures – too compact or too expanded

Should not be much of a problem in modern data

Why is the Gaussian distribution sacred ?

Random numbers (noise, errors)

- take uniform random numbers from 0 to 1
- add a few dozen together and get the sum
- repeat many times
- the sums are normal (Gaussian) distributed around ½

If I have a process which is genuinely random

- best modelled with a Gaussian

Are errors always Gaussian ? No – more later

- Errors from your growth estimations/spectrometer ?
  - No, but good starting point

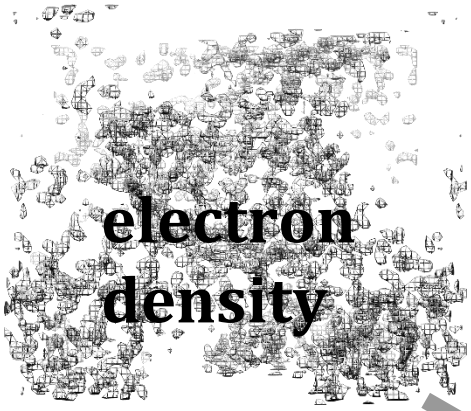
Atomic coordinates ?

- this lecture

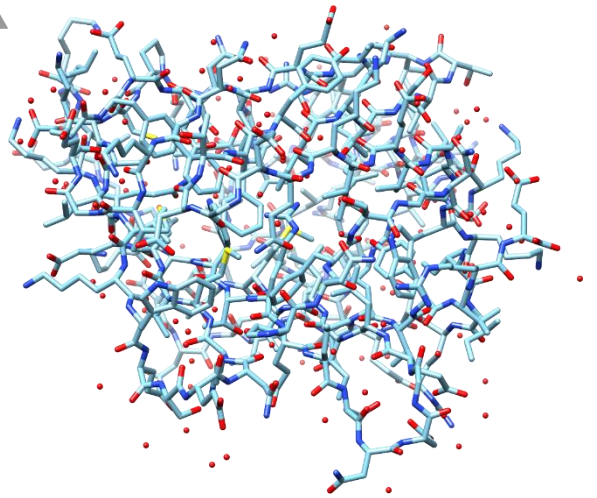
X-ray - fitting structure to data

measured data

resolution



coordinate error and other error



Resolution

Do we know the error in X-ray coordinates ?

- no

Do we know the resolution ?

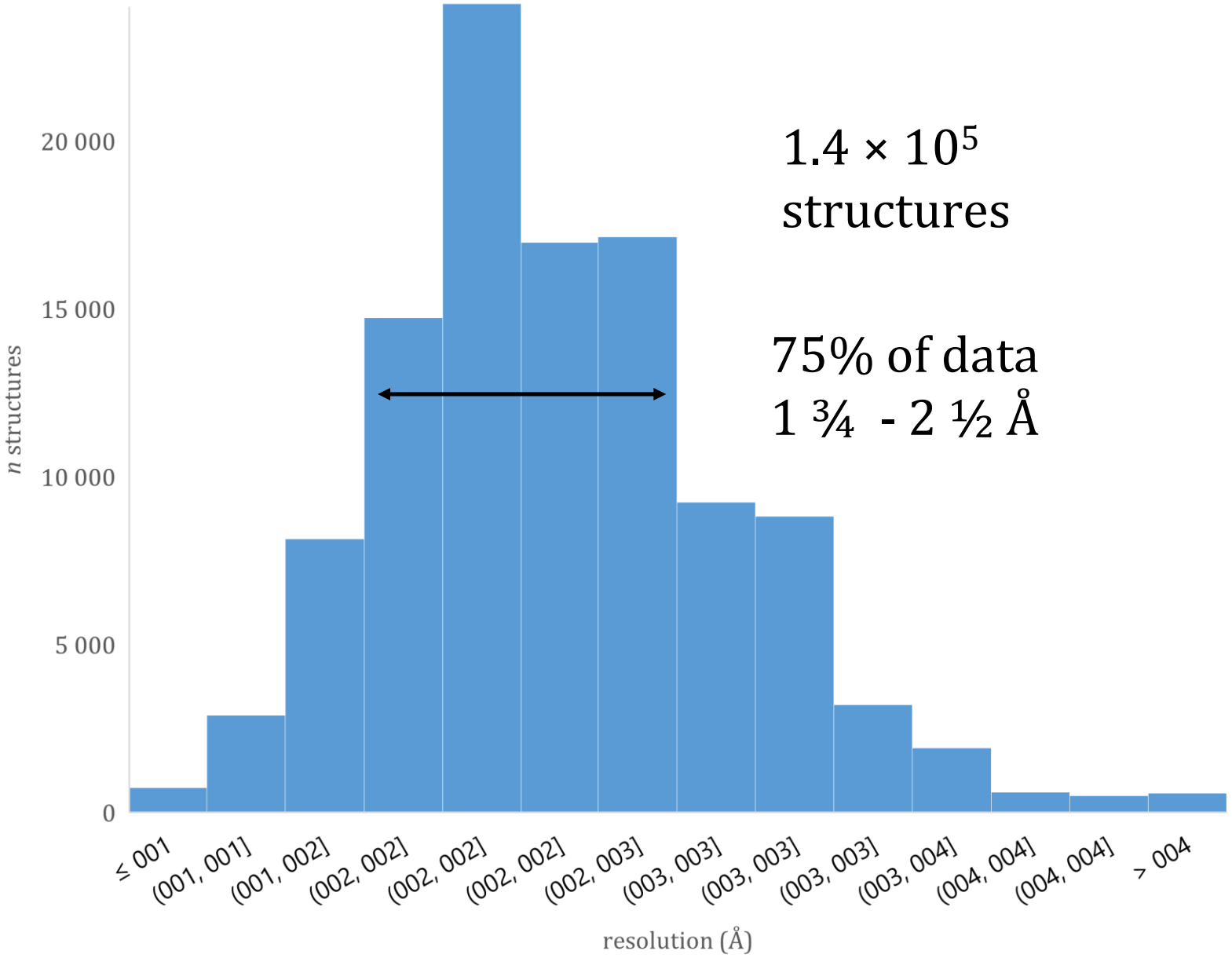
- yes
  - property of crystal and reflections one measures

What does resolution r_{res} mean ?

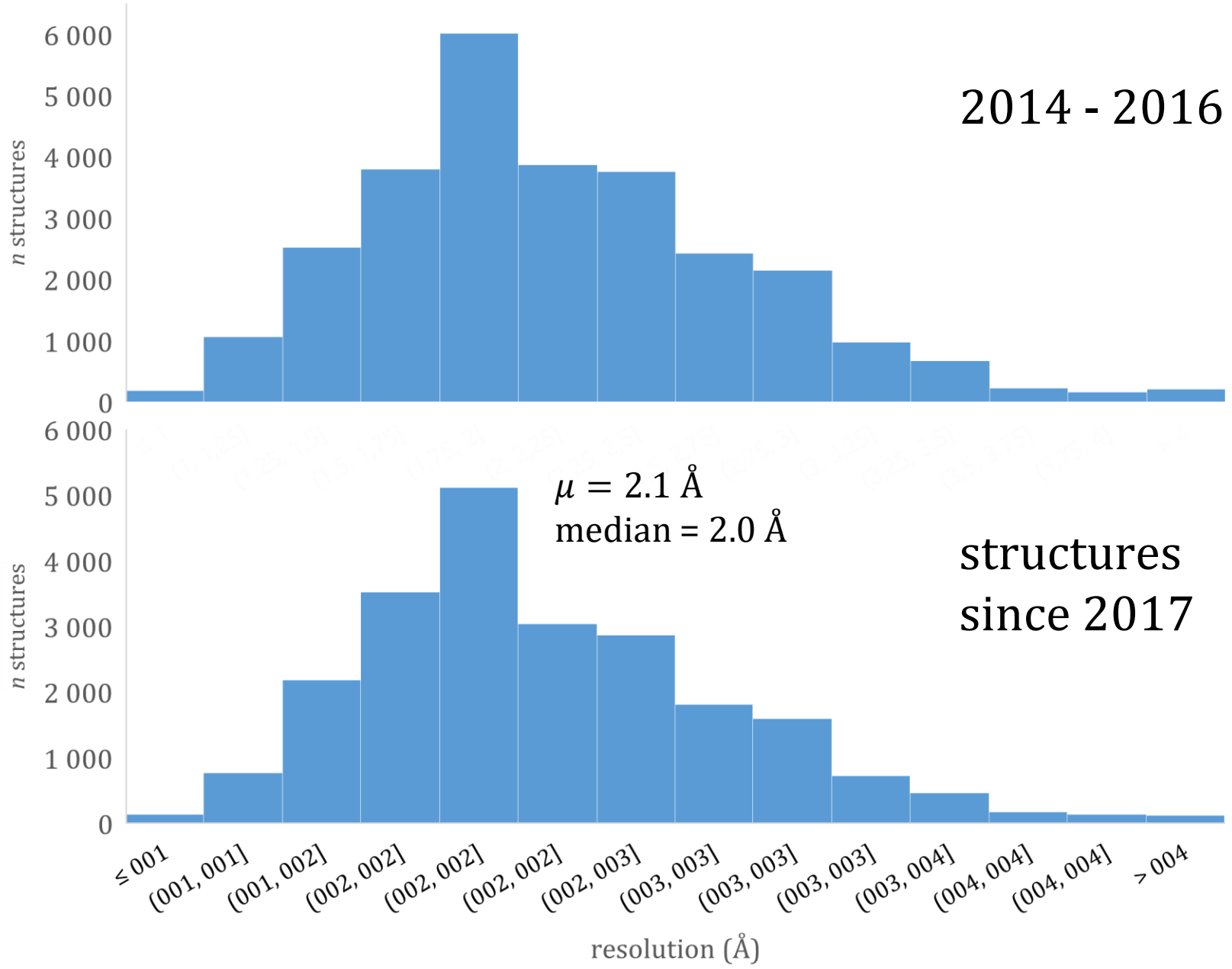
- distance r_{ij} between two points i, j
- If $r_{ij} < r_{res}$

  I cannot resolve two points – they look like one object

Resolution



are they getting better ?


2014 - 2016
structures since 2017
μ = 2.1 Å
median = 2.0 Å
n structures
6 000
5 000
4 000
3 000
2 000
1 000
0
≤ 001
(001, 001]
(001, 002]
(002, 002]
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(003, 003]
(003, 003]
(003, 004]
(004, 004]
(004, 004]
> 004
resolution (Å)

X-ray resolution

Cannot say they get better

- old structures only get updated if resolution improves
- new (big) complexes are solved that could not be before (low resolution)

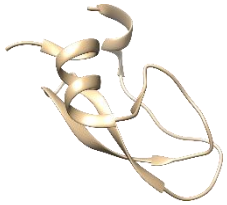
If I have 1.5 Å resolution are my coordinates only known to 1.5 Å ? No

- I have many reflections – many estimates of position
- I add much chemical information (bond lengths, angles)

What is the error really (simulated data) ?

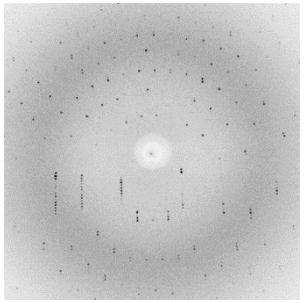
- How would you calculate it ?

Simulating error with resolution



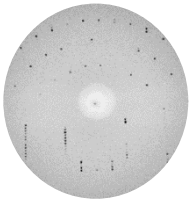
protein coordinates

calculate perfect reflections



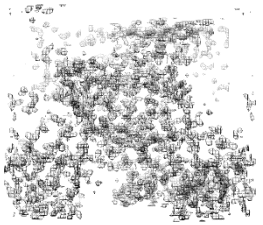
perfect (fake/ synthetic) data

throw away reflections $< x$ Å



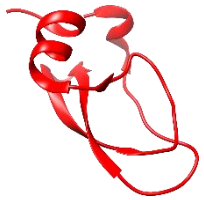
partial data

calculate density



electron density

calculate coordinates

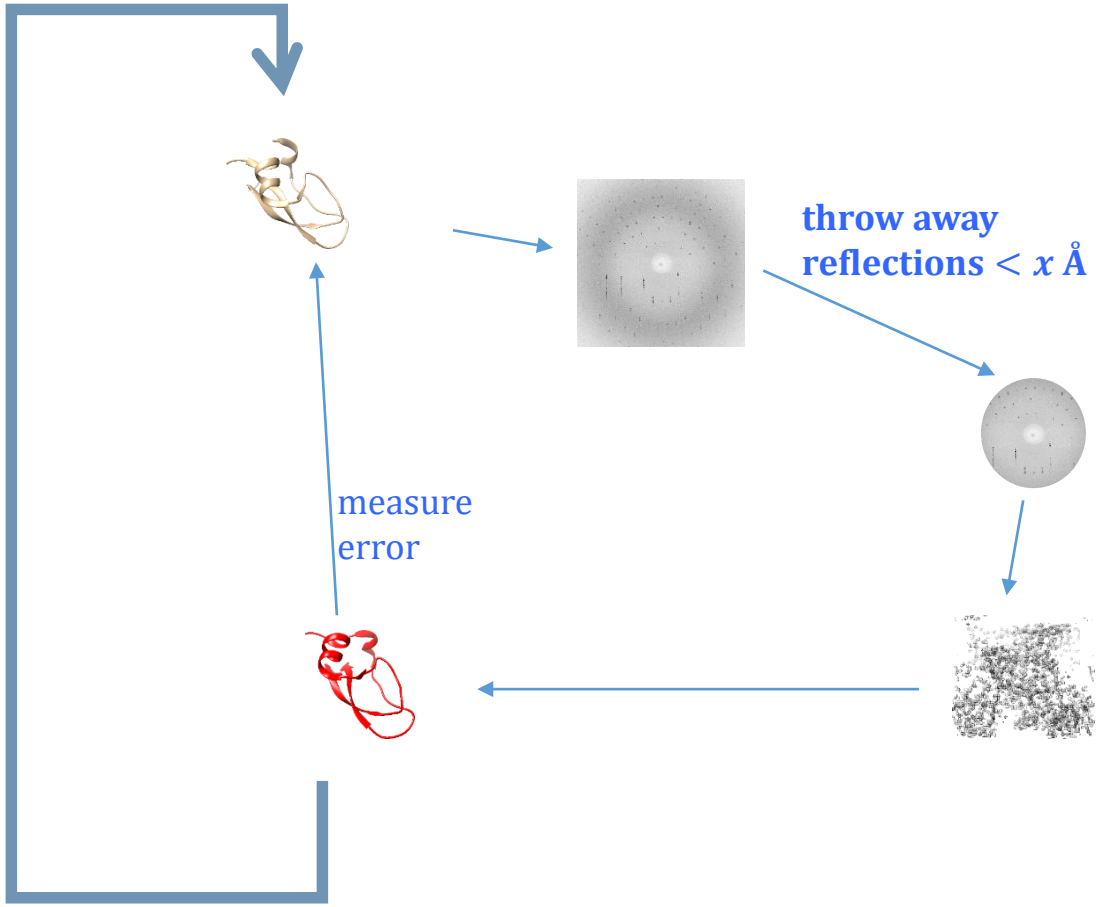


coordinates with artificial error

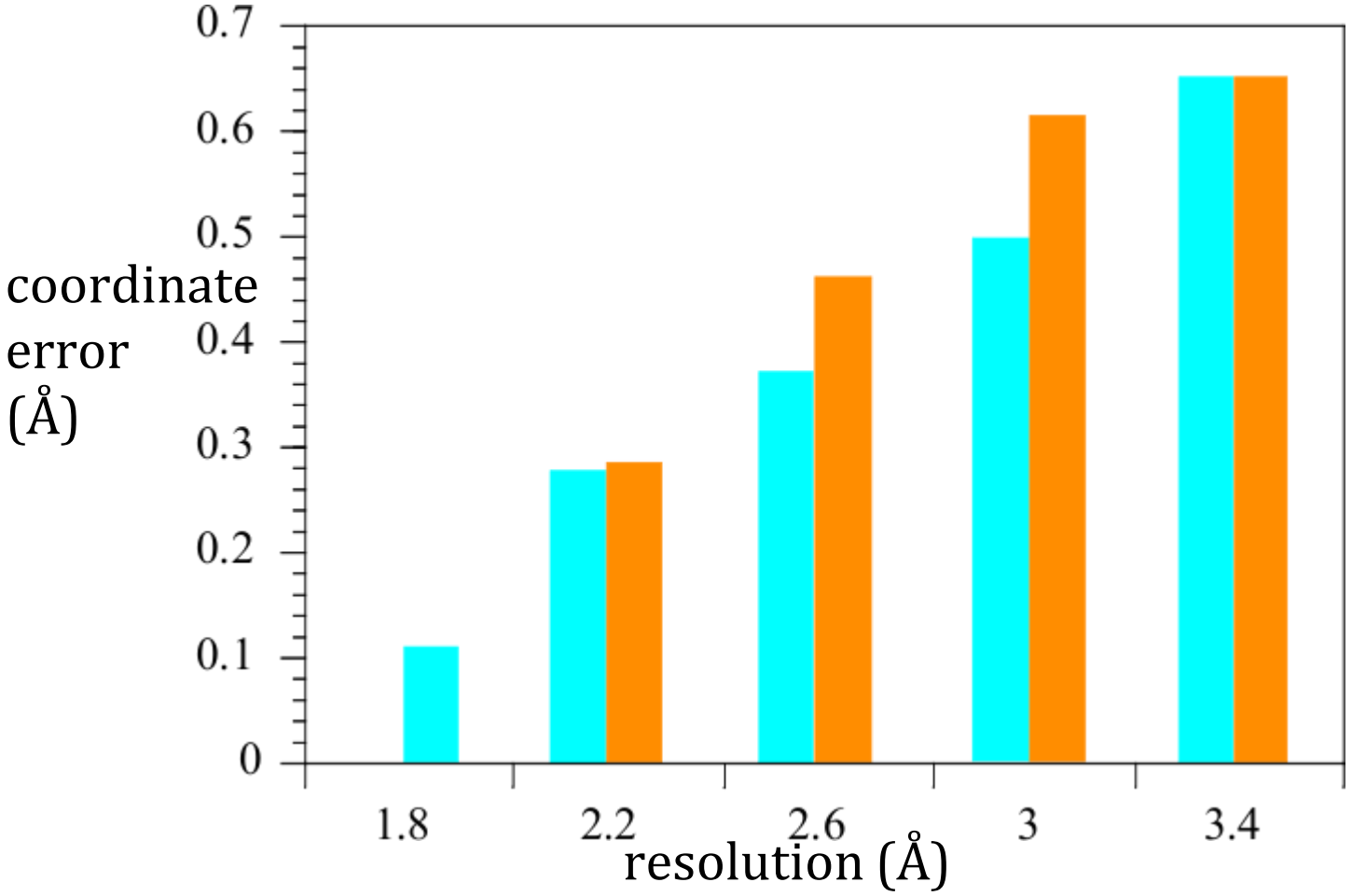
measure error

Simulating error with resolution

repeat for
values of x (1.8, 2.2, .. Å)
repeat for many proteins



X-ray coordinate error



two different estimates of coordinate error – not important for us

For resolution near 1.5 to 2.0 Å

- I have errors around 0.2 to 0.3 Å

Brünger AT, Nat Struct Biol. 1997 4, Suppl:862-865.

Mobility

We have uncertainty – from resolution, incomplete data

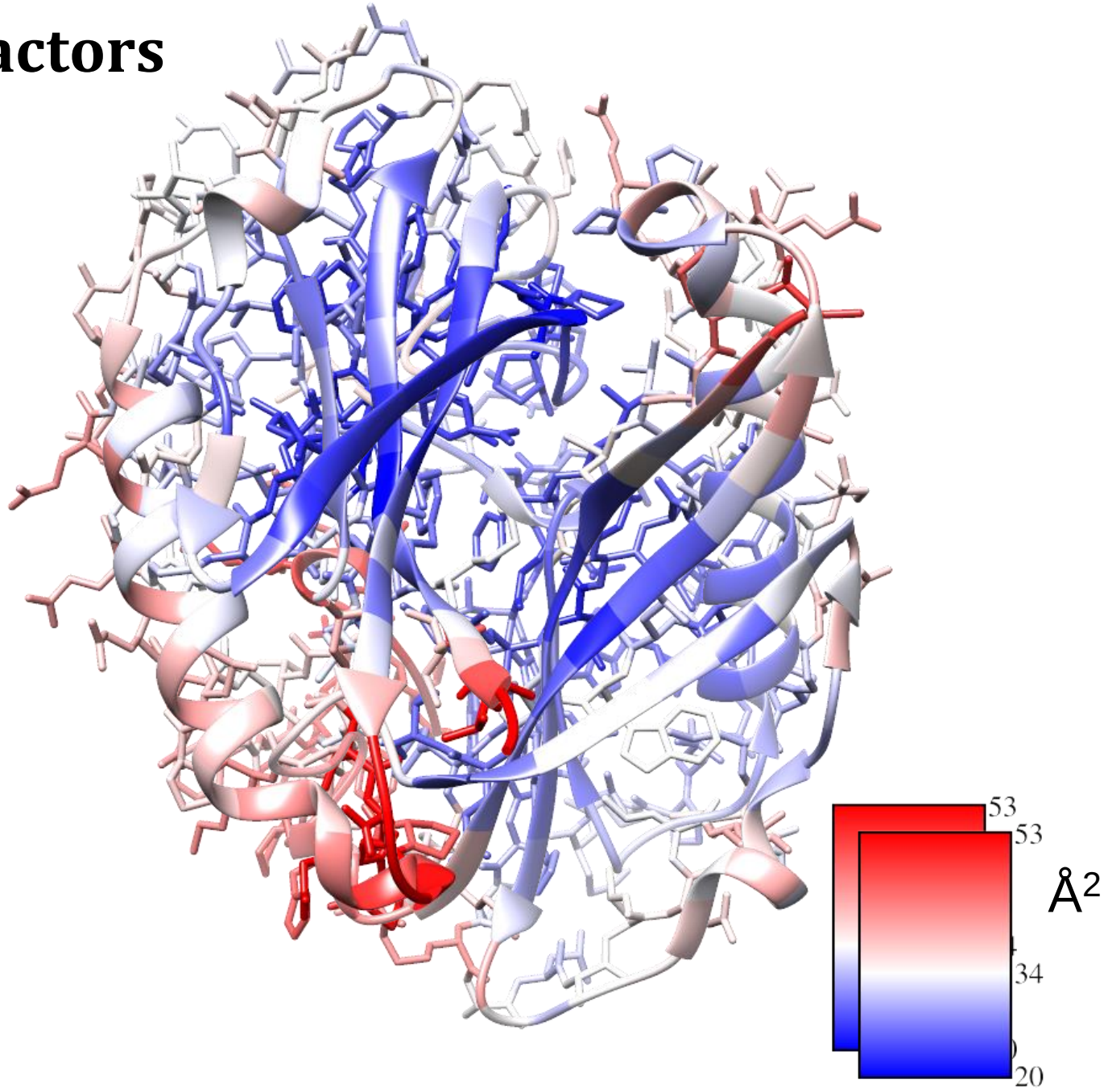
We also have mobility

- no matter how good the data is
  the positions of atoms are not fixed

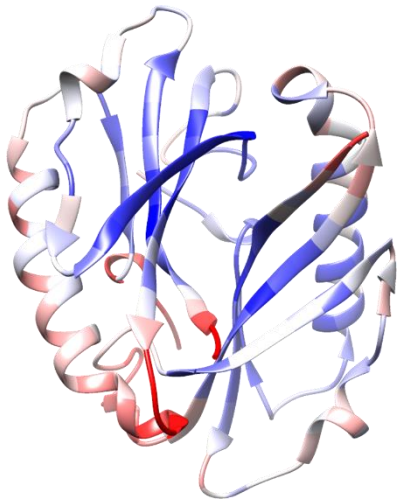
B-Factors

2ei5
all atoms

2ei5
backbone



B-factors



red – blue / mobile less mobile

- surface more mobile / core fixed

Formal meaning

$$B = 8\,\pi^2 u^2$$

say u is the average displacement

if $B = 50$ Å^2, typical displacement ≈ 0.8 Å

if $B = 20$Å^2, typical displacement ≈ 0.5 Å

units ? Å^2

- there are different kinds of B-factors

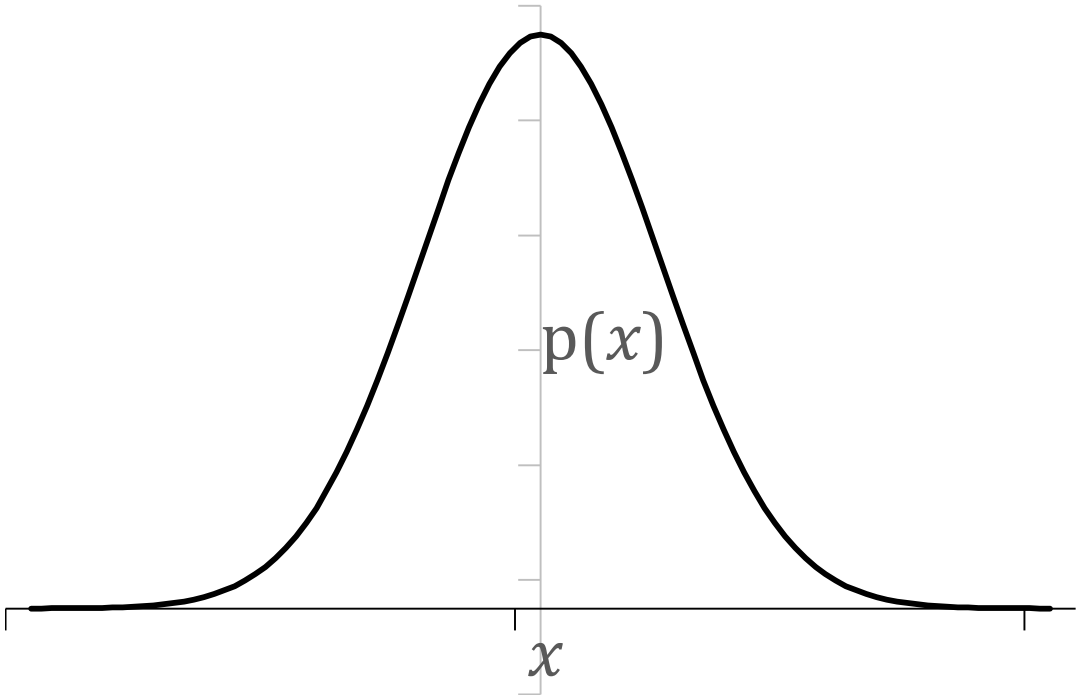
Types of *B*-factors

How reliable / meaningful ?

- the less certain the coordinates, the larger the *B*-factor (part of fitting – automatic – not done by hand)
- different programs give different values

is $8\pi^2u^2$ OK ?

- in one dimension ?

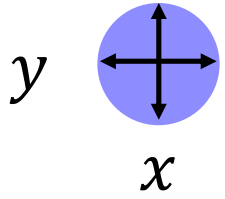


ask me where Gaussian form comes from - not for Klausur – harmonic model

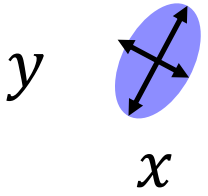
Anisotropic *B*-factors

How does an atom in a protein move ?

- the middle of a protein is not very symmetric
- we could better describe mobility with more numbers



or



one number

two numbers

Big problem

- more numbers needs better, high-resolution data rather rare

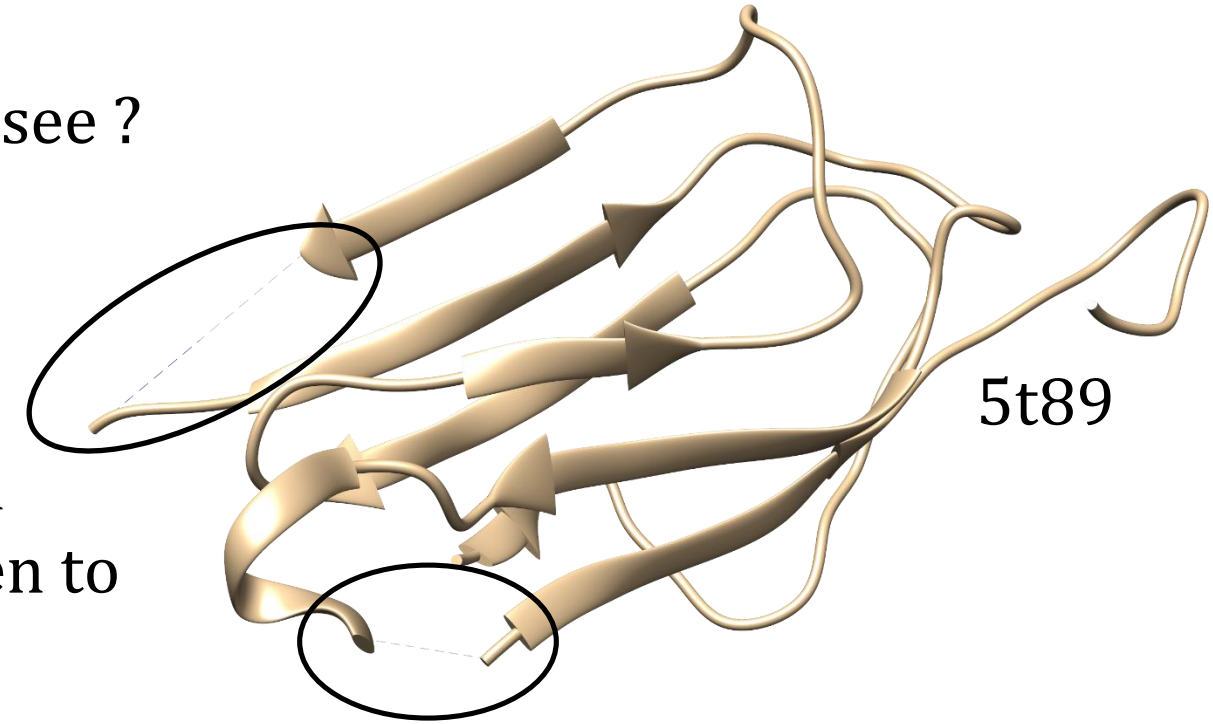
B-factors one will meet

	data necessary	number of parameters	
every atom anisotropic	lots	lots	few
every atom isotropic	normal		most common
per-residue averaged	poor data	few	older structures

very mobile atoms

- *B*–factors: physical meaning for mobility of individual atoms
  What else does one see ?

Missing atoms ?



- There is not enough electron density seen to place an atom

- Interpretation: the atoms are very mobile
- Usually only in loops, N- and C-termini

X-ray summary

- resolution is well-defined
- coordinate error is less well-defined
- resolution might be 1 ½ Å, but coordinate error is much smaller
- mobility puts a lower limit on uncertainty

How does this compare with NMR ?

NMR

How are NMR structures calculated ?

- measure NOEs between H's – convert to distances
  - maybe some angles, chemical shifts, residual dipolar couplings
- distances $\xrightarrow[\text{distance geometry}]{}$ coordinates

Distance information is

- not so accurate – often only upper bounds
- limited to short (< 5 Å) distances
- there are many sets of coordinates that fit the data

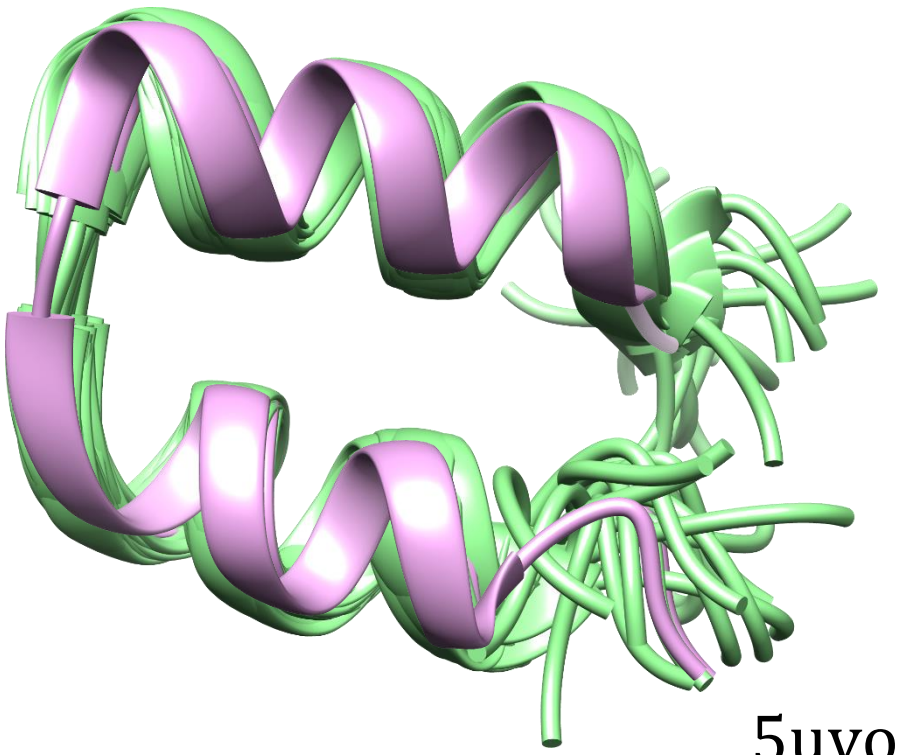
Solve the distance geometry problem 100 times

- send the best 20 or 30 structures to data bank

NMR coordinate error

- purple – what you see when you open the file

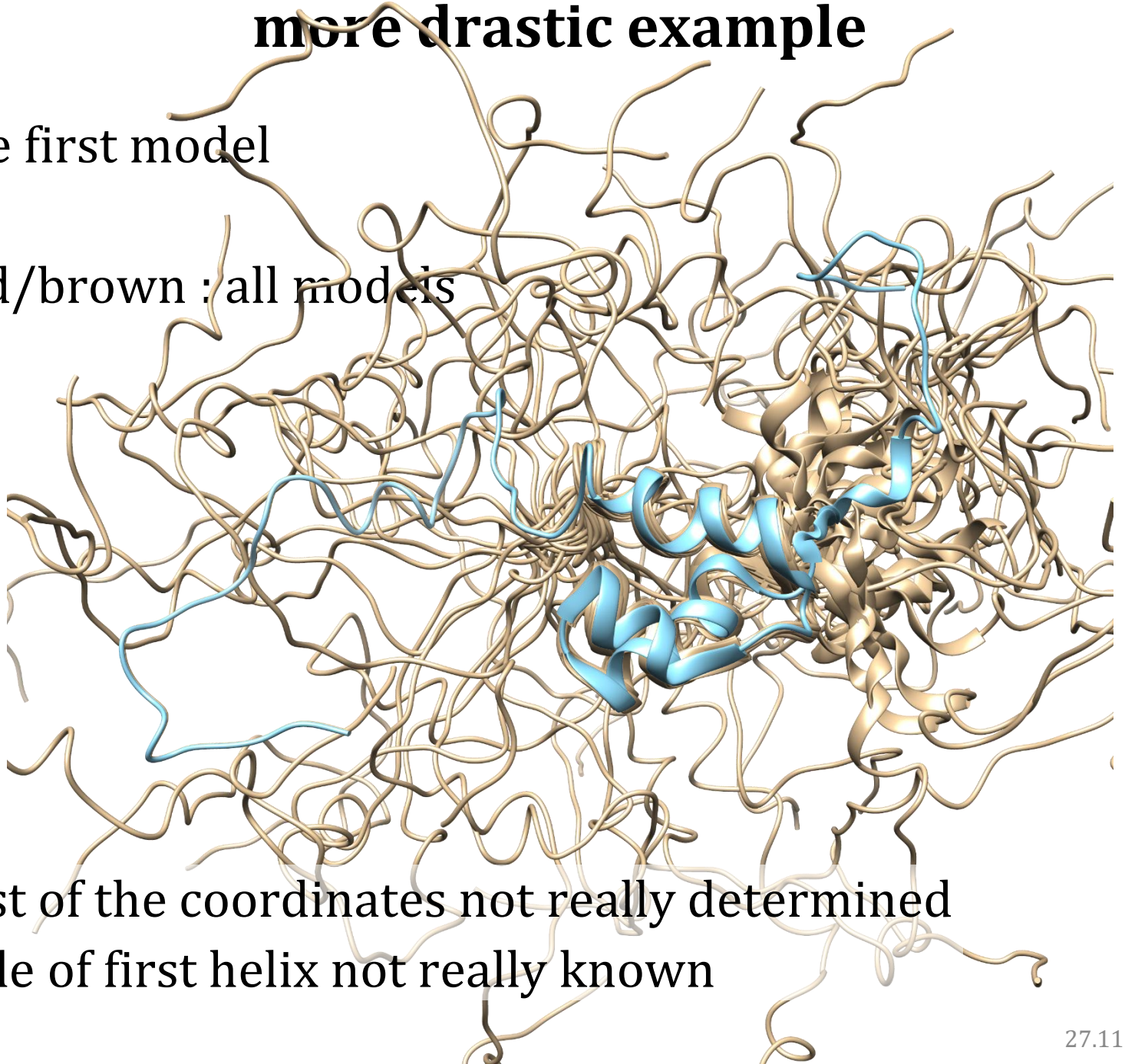
- green – 20 more “models”



5uyo

more drastic example

- blue first model
- gold/brown : all models



5nr6

- most of the coordinates not really determined
- angle of first helix not really known

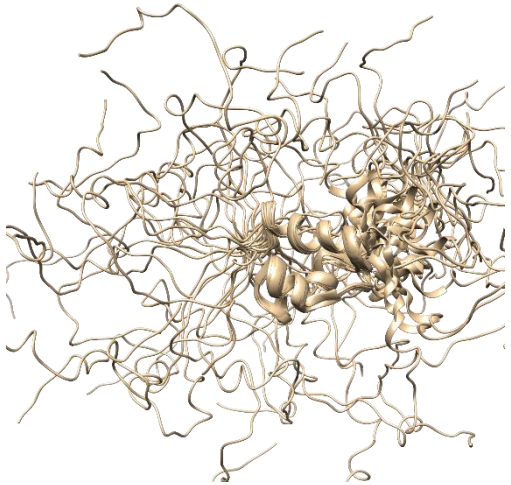
Meaning of models

Interpretation

- Each of the models in the data file agrees with the experimental information
- All of the models are reasonable solutions

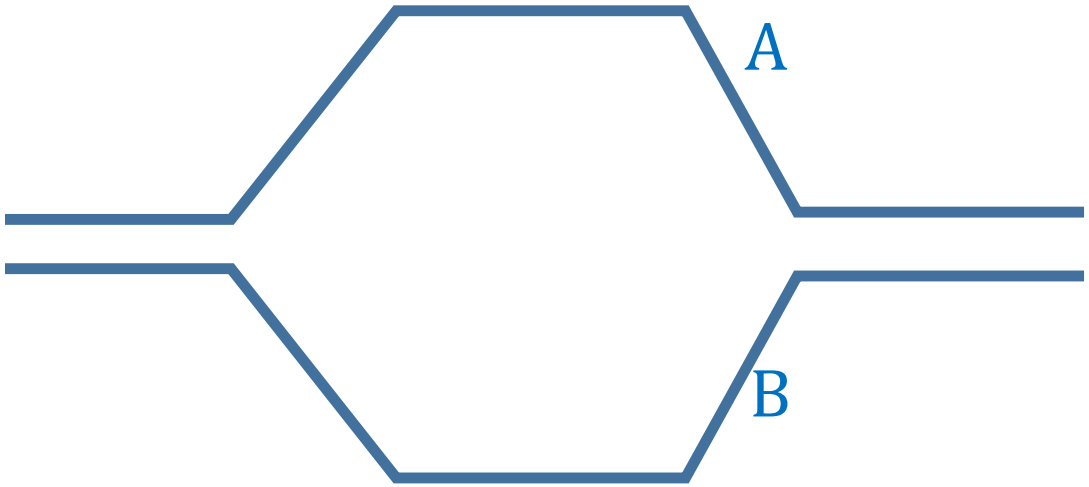
Can we take the average ?

- what would the average look like ?



What do average coordinates generally look like ?

You cannot average coordinates

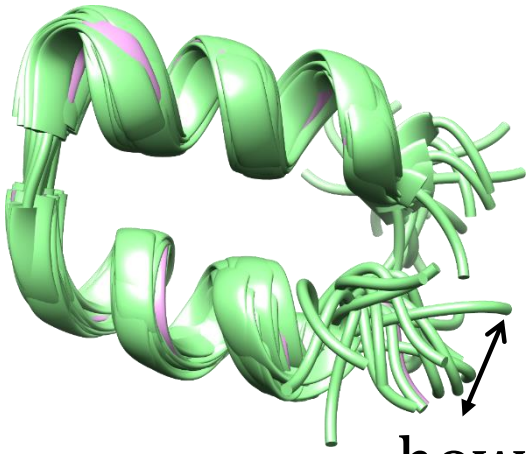


coordinates with normal bond lengths / angles

averaged (A, B) coordinates silly bonds, angles

Using NMR coordinates

- average may have little meaning
- pick a model of your choice ?
  - if the models are good – OK
  - if the models are very different you have a problem



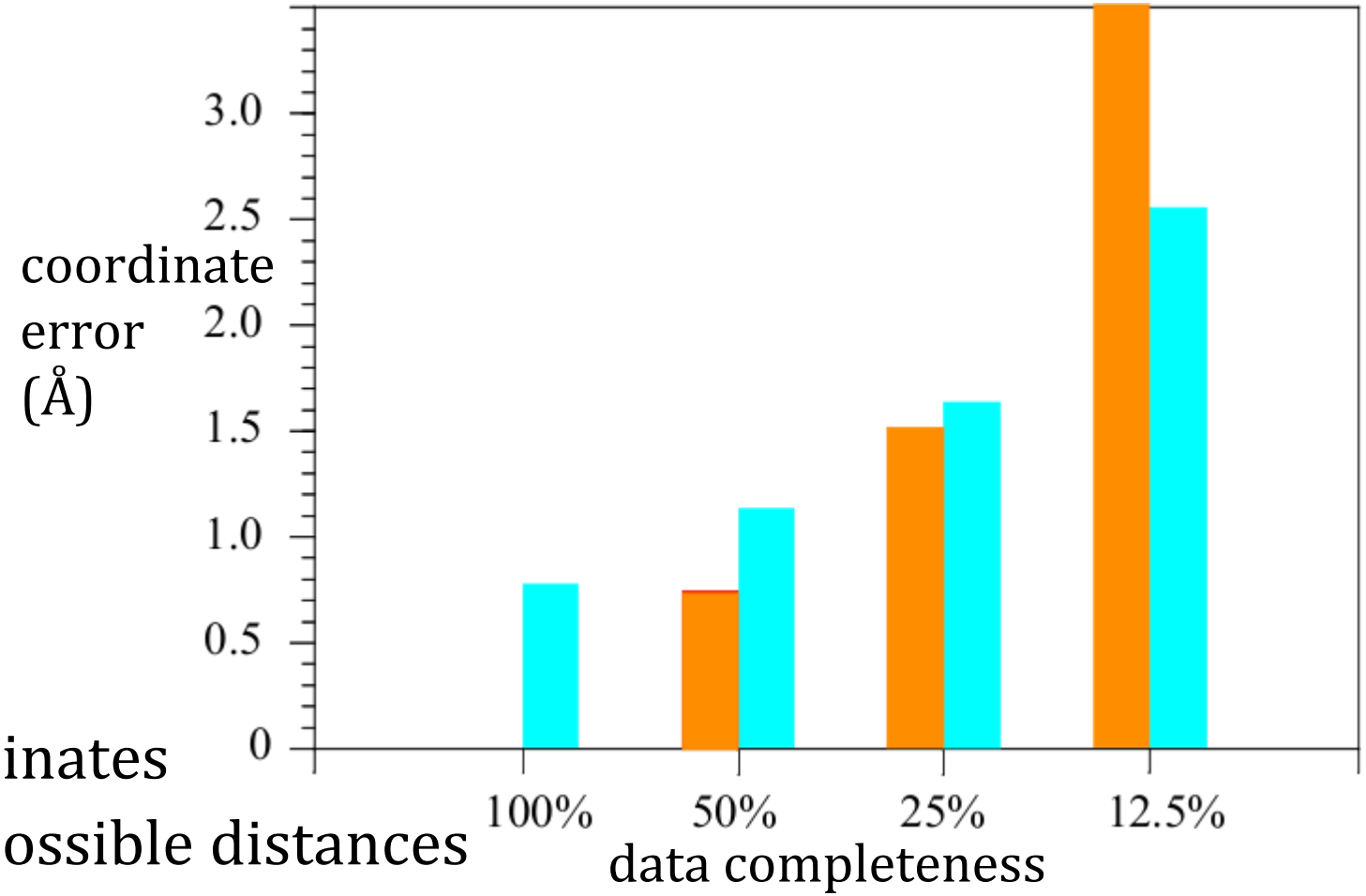
how big is this ?

Can one talk about accuracy/certainty ?

- If you think the models cover the allowed space
- what is the average distance compared to average coordinates ? (root mean square)

- What does one expect ?

NMR



- take coordinates
- generate possible distances
- delete randomly
- calculate structures / compare to known coordinates

Brünger AT, Nat Struct Biol. 1997 4, Suppl:862-865.

Certainty with NMR

- take set of solutions (20 to 50)
- fit to each other or average
- for each site (maybe C^{α}) calculate root means square difference

- gives estimate at each site of spread

- maybe average over all sites – gives very rough idea of certainty

- Gaussian distribution assumption ? Weak

- compare some features of NMR and X-ray..

Is NMR terrible ?

Uncertainty is

- bigger than with X-ray
- less well estimated

There are problems with crystallography

- many proteins never crystallise
- some are difficult to phase
- a synchrotron is much more expensive than an NMR spectrometer

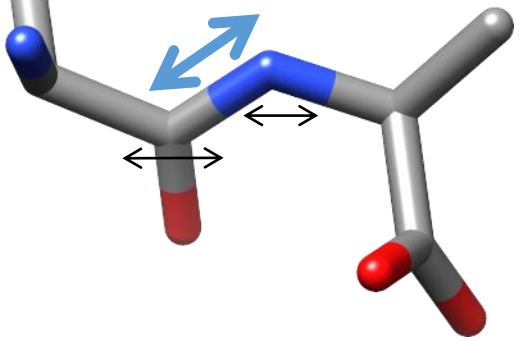
Distribution of errors

I say I have 2 Å resolution or 2 Å difference between structures or 0.2 Å uncertainty – what does it mean ?

Can I use simple / classic error analysis ?

Say $y = x_1 - x_2$

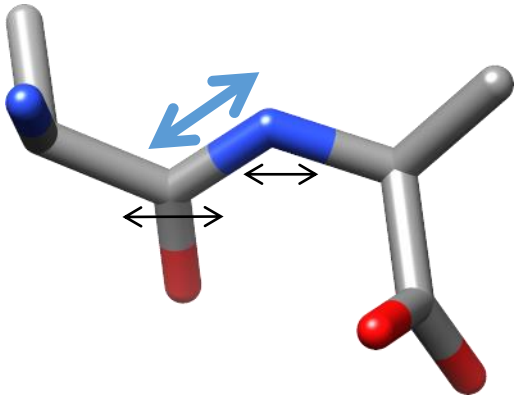
- then with errors $y = (x_1 \pm \epsilon_1) - (x_2 \pm \epsilon_2)$
  - final error is $\epsilon = \left((\epsilon_1)^2 + (\epsilon_2)^2\right)^{\frac{1}{2}}$



Distribution of errors

Can we apply the formula here ?

- imagine $^1/_4$ Å error on C and N
- final error on d_{CN}



$$\epsilon_{d_{CN}} = \left(\left(\frac{1}{4}\right)^2 + \left(\frac{1}{4}\right)^2\right)^{\frac{1}{2}} = \left(\frac{1}{8}\right)^{\frac{1}{2}} \approx 0.35\text{Å}$$

Silly. I know that CN bond length is 1.32 Å

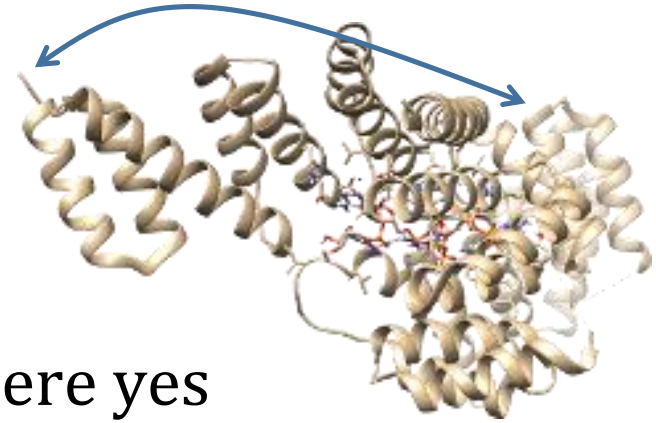
What have I done wrong ?

Intuitive – some distances are known and fixed

Formally

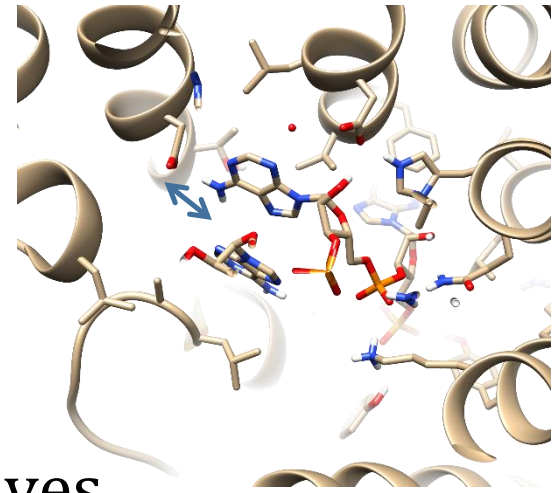
- error analysis only works with independent errors
- bonded C and N coordinates are highly correlated

Does simple error analysis ever apply ?



Here yes
but probably not
so interesting

5W5I

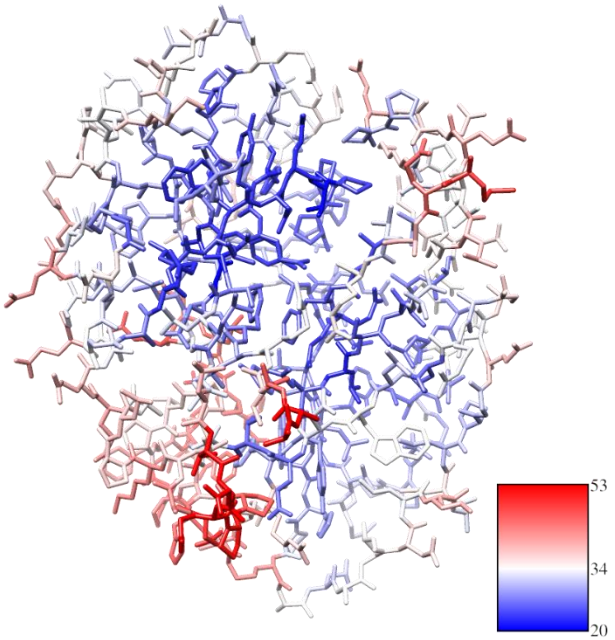


Here yes
and probably
important

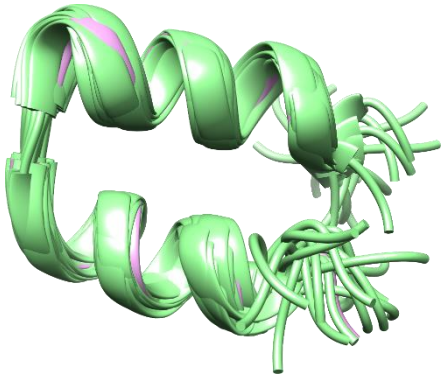
uncertainty is more complicated

Mobility is not evenly distributed

- X-ray B-factors
  - very uneven
  - surface is most mobile
  - long sidechains are very mobile



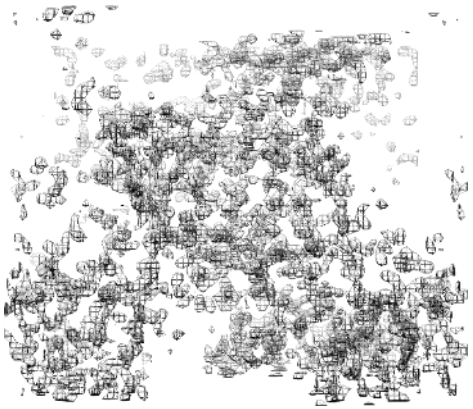
- NMR
  - uncertainty also reflects mobility

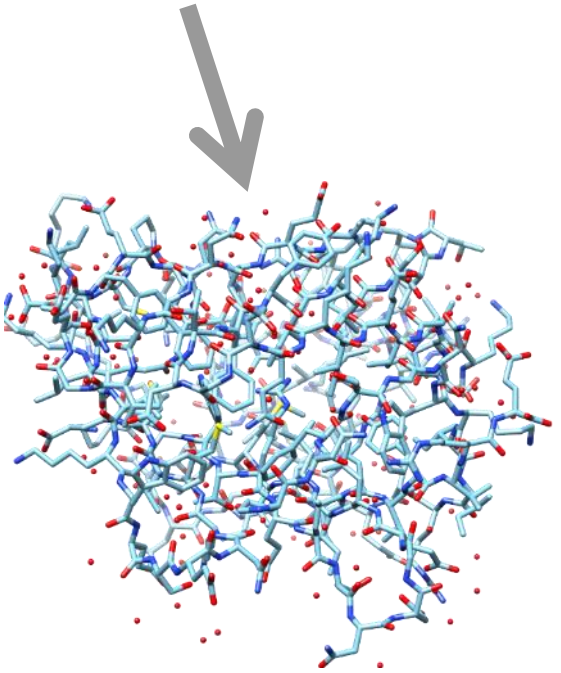


Mistakes – not random, not systematic

X-ray

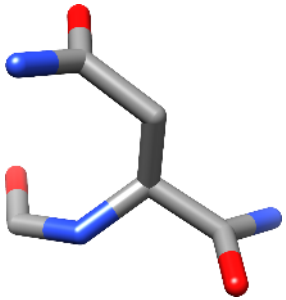
- usually in fitting atoms into density
- trace chain backwards
- asn and gln – N and O have the same electron density

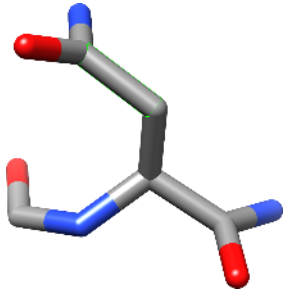




NMR

- misassignment of peaks





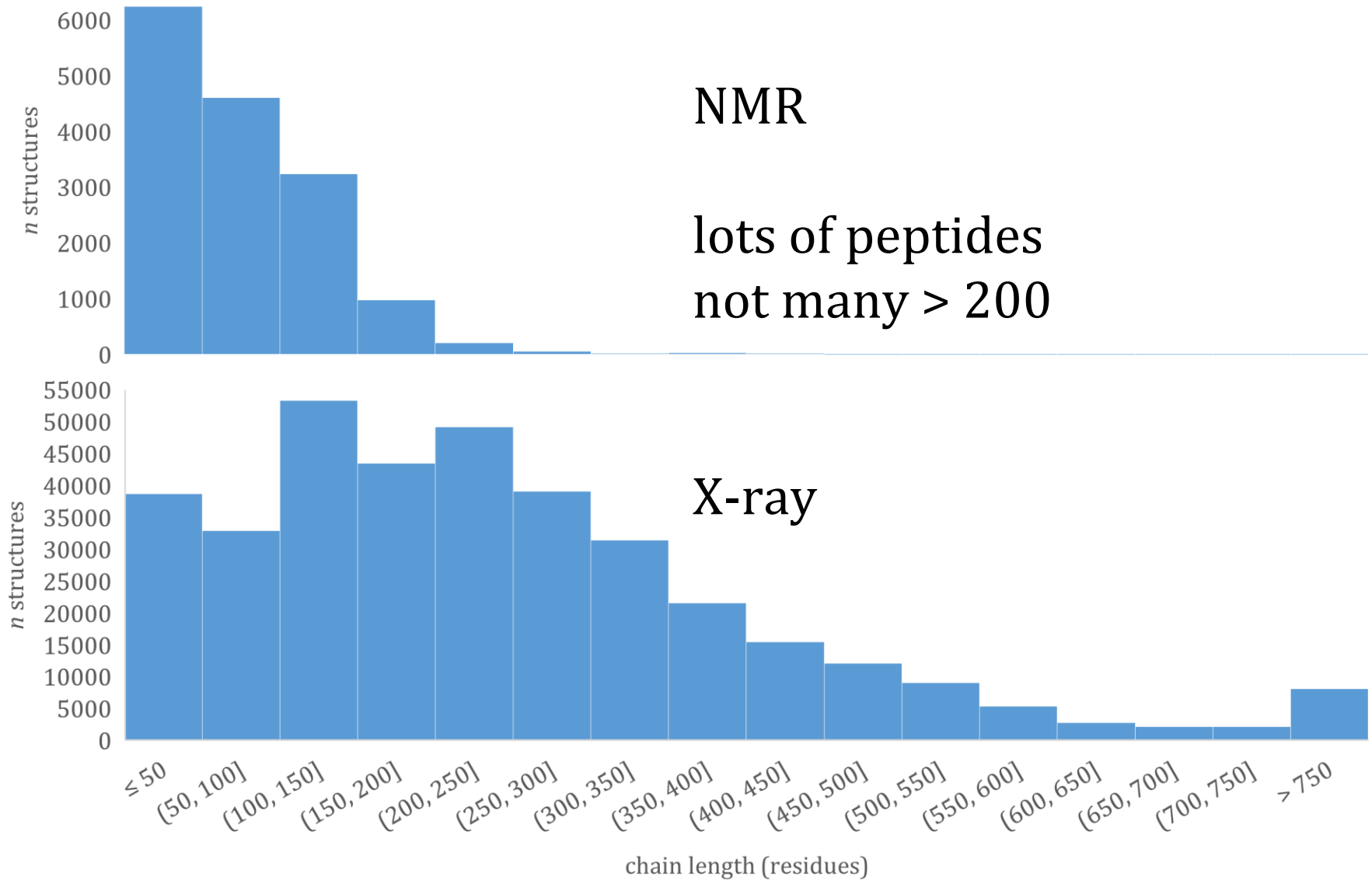
- finding errors ?

finding errors

- a structure is solved again and looks different
- a structure is solved under slightly different conditions
- a very homologous structure is solved

- properties of structures
- are all bond lengths / angles OK ?

sizes of chains



	X-ray	NMR	cryo-EM	SAXS
resolution	1½ - 2½ Å	n/a		
certainty	< 1 Å	from < Å to bad	> few Å	blobs
cost	$$$$	$$$	$$$	like for X-ray
you have protein.. how difficult is structure ?	easier if similar to known structure	less reliance on known structure		