# NMR vs X-ray, precision, certainty

Main methods

- X-ray crystallography and NMR

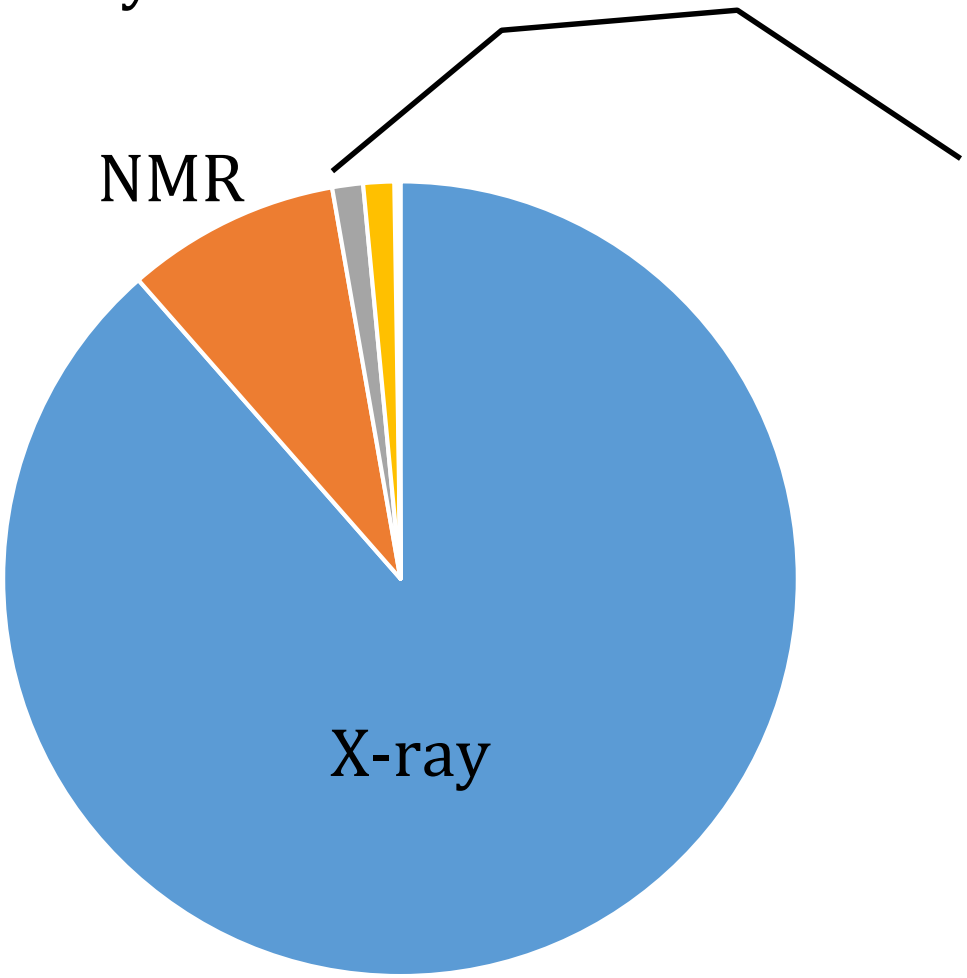
Others

- 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




cryo EM
neutron diffraction
…

# 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 ?

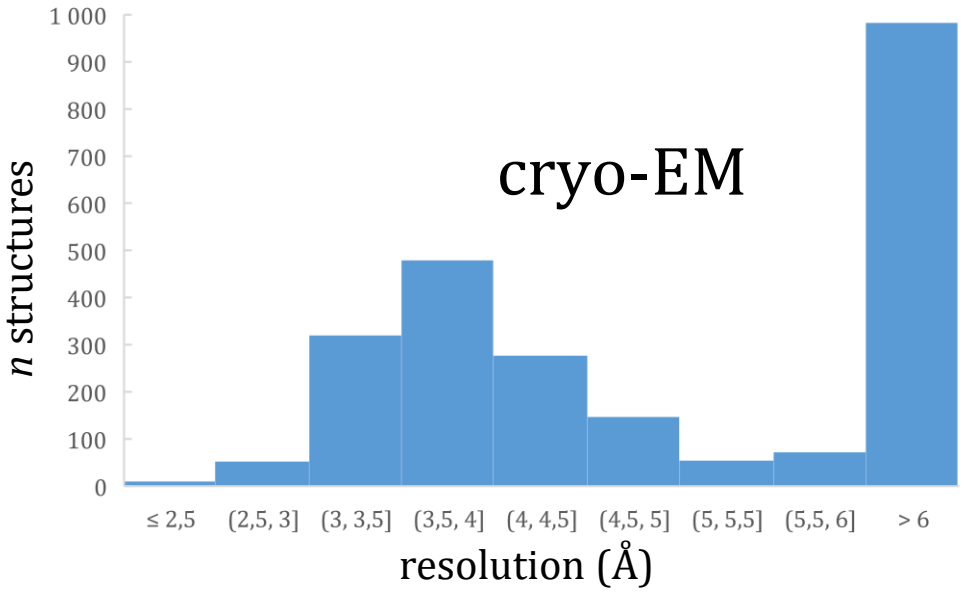
- fashionable, but look at resolution

- 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

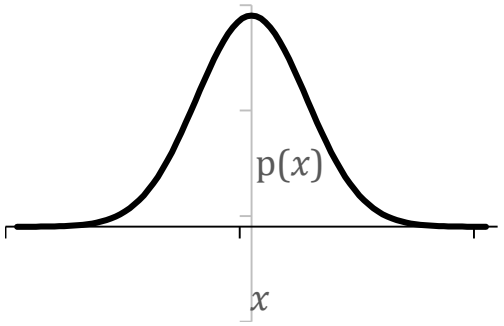
- Accuracy, precision – not good words
- Certainty / uncertainty / confidence – more in statistics
- Resolution – nice word – defined later

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

- should say 90% confidence, one $\sigma$, 75 % quartiles, ..

How do I interpret this ?

- I imagine a Gaussian (normal) distribution

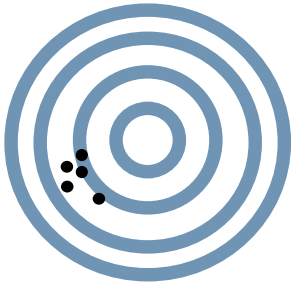


# accuracy / precision

basically bad words

- do not use Wikipedia + Übersetzung schwierig

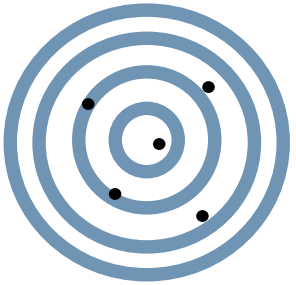
small error



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 effects – perhaps present 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
  - some methods produce more compact structures

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 probably a good starting point

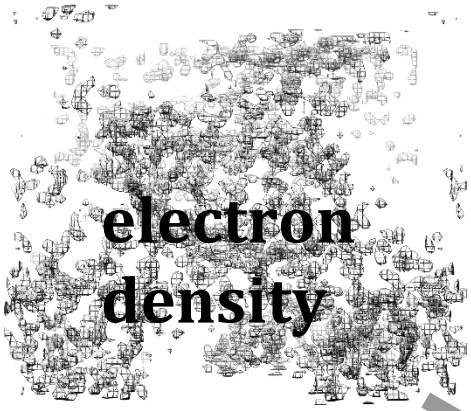
Atomic coordinates ?

- this lecture

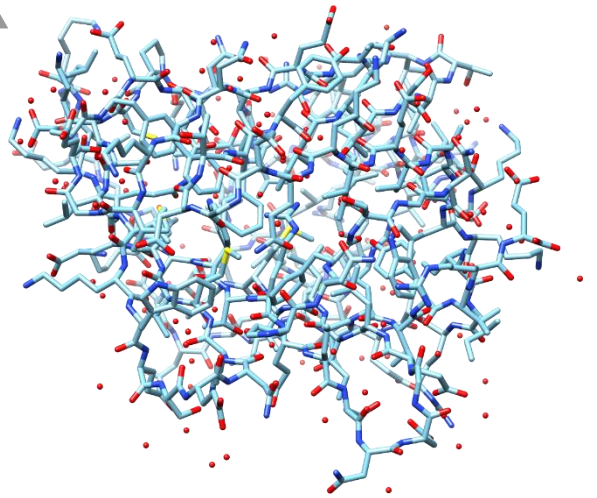
# X-ray - fitting structure to data

measured data

resolution




coordinate error and other error



# Resolution

Do we know the error in X-ray coordinates ?

- no

Do we know the resolution ?

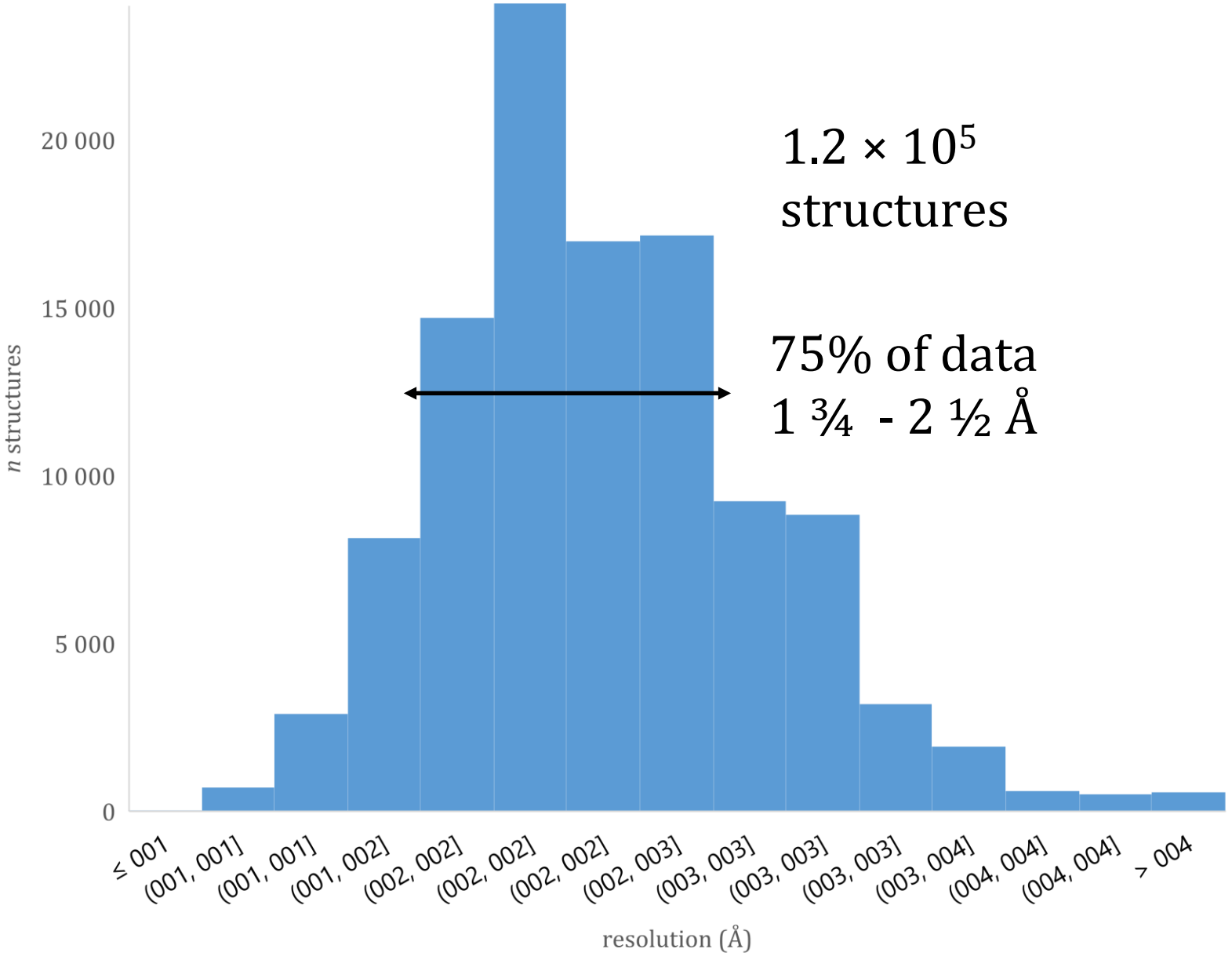
- yes
  - property of crystal and reflections one measures

What does resolution $r_{res}$ mean ?

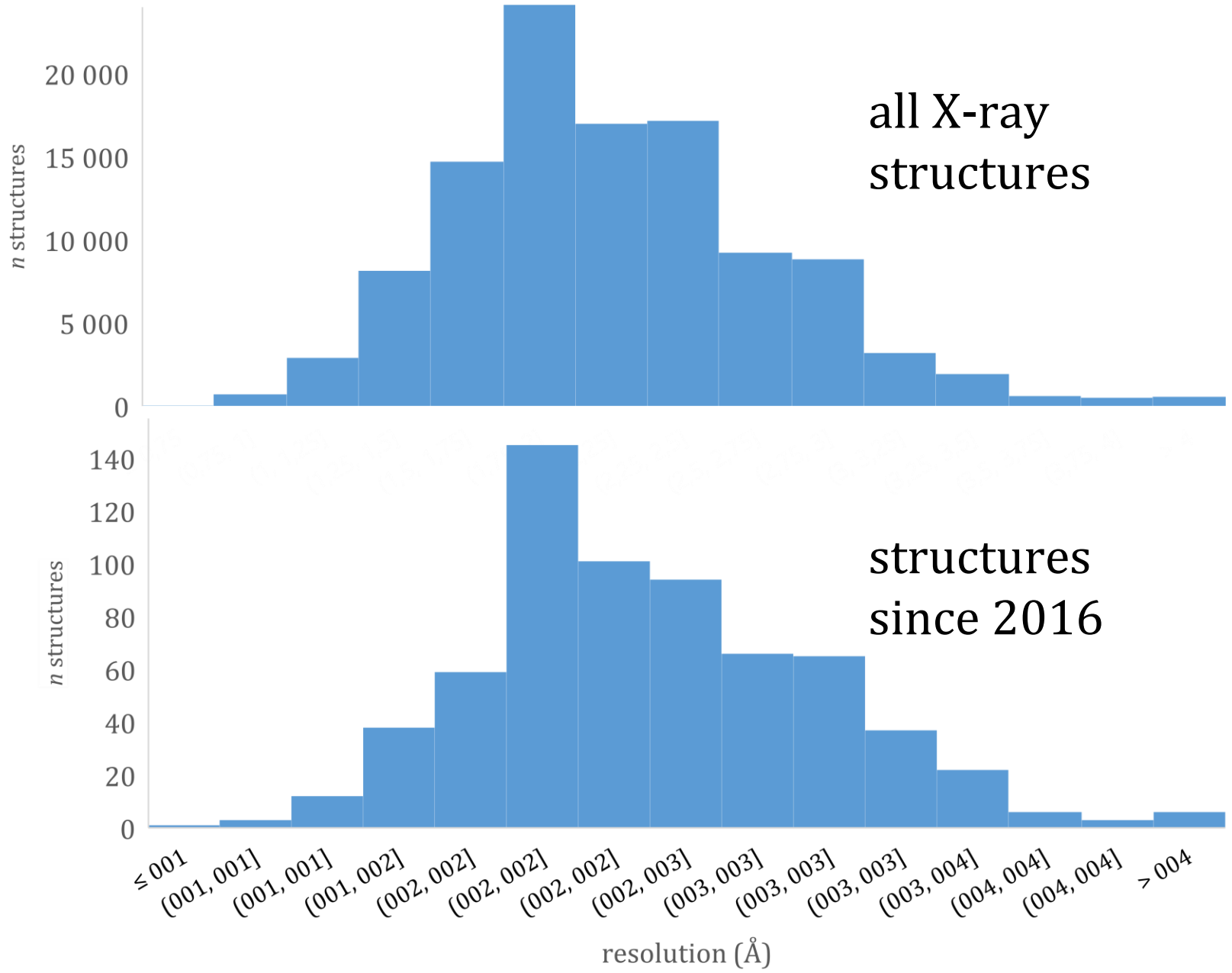
- distance $r_{ij}$ between two points $i, j$
- If $r_{ij} < r_{res}$

  I cannot resolve two points – they look like one object

# Resolution




are they getting better ?


n structures
20 000
15 000
10 000
5 000
0
all X-ray structures
n structures
140
120
100
80
60
40
20
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structures since 2016
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resolution (Å)

# X-ray resolution

Cannot say if they get better

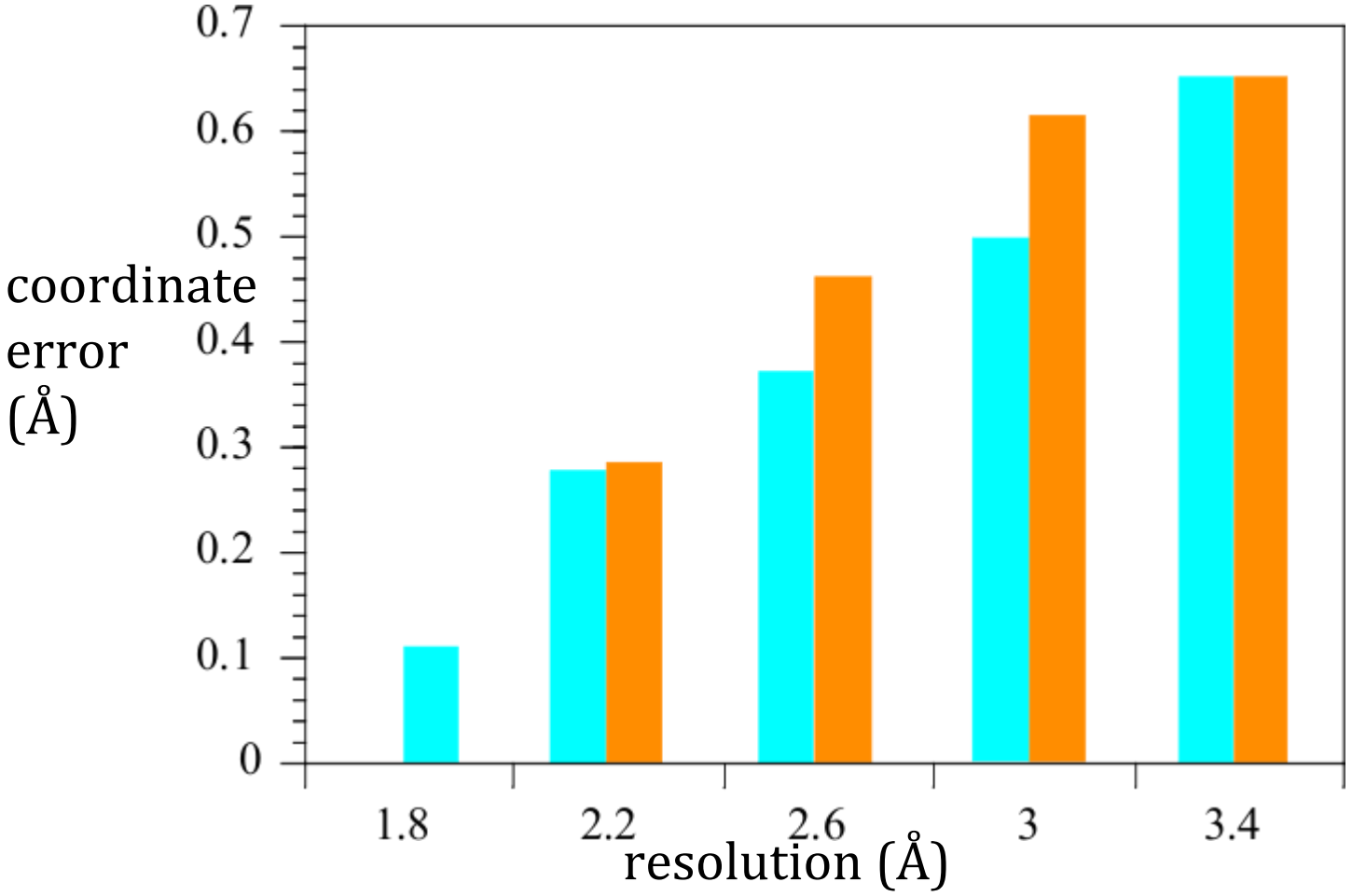
- old structures only get updated if resolution improves
- new (big) complexes are solved that could not be before (low resolution)

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) ?

# 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

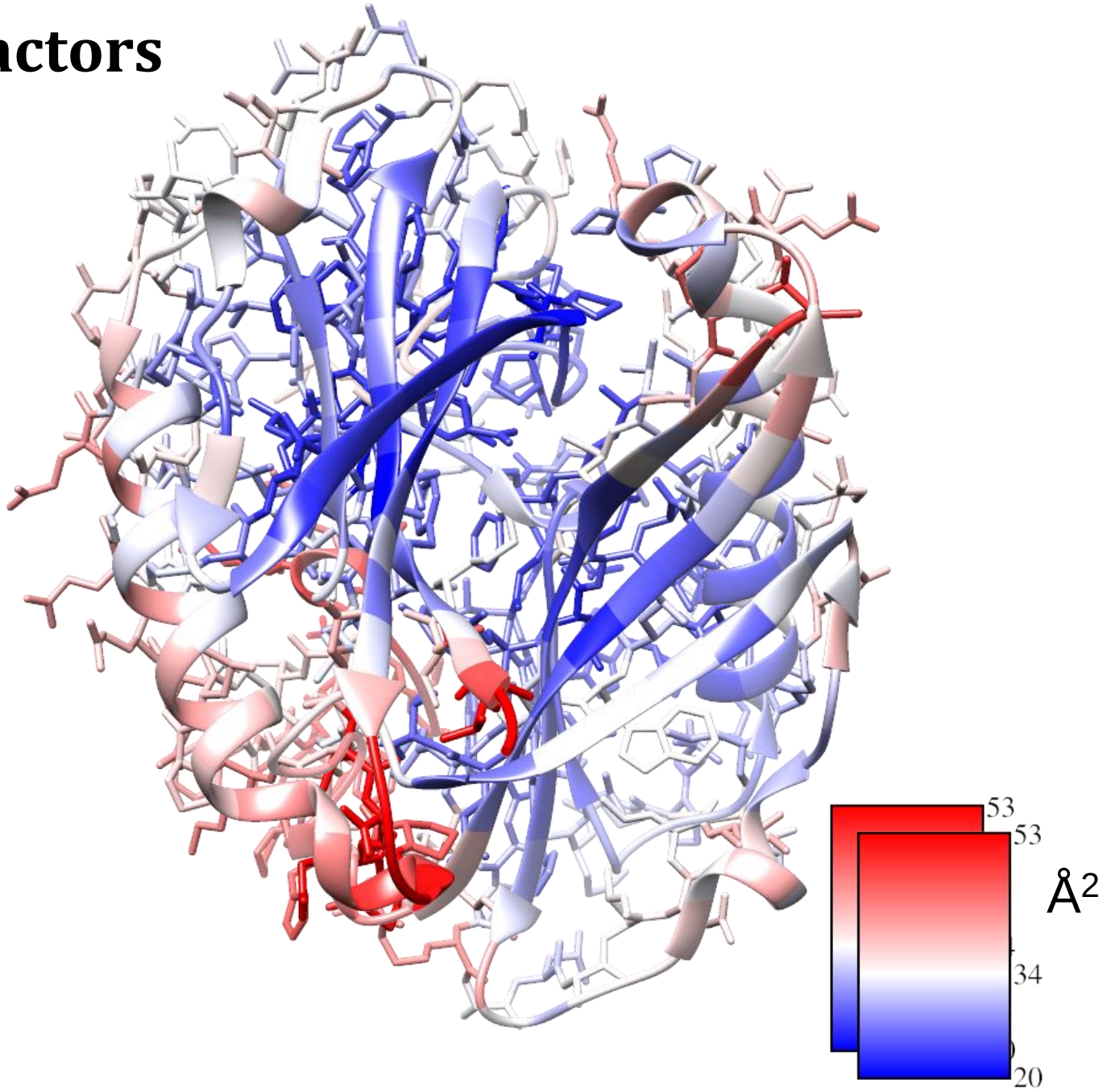
- We have uncertainty – from resolution, incomplete data
- we also have mobility

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

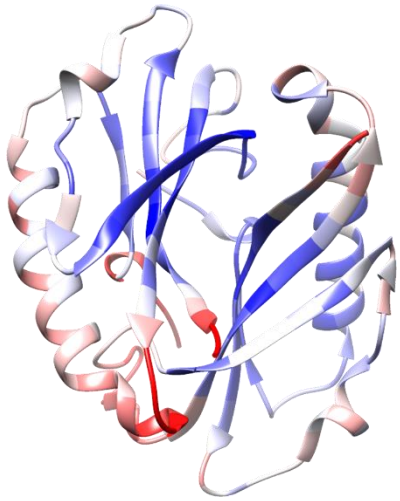
# B-Factors

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 $\approx 0.8$ Å

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

units ? Å$^2$

- there are different kinds of $B$-factors

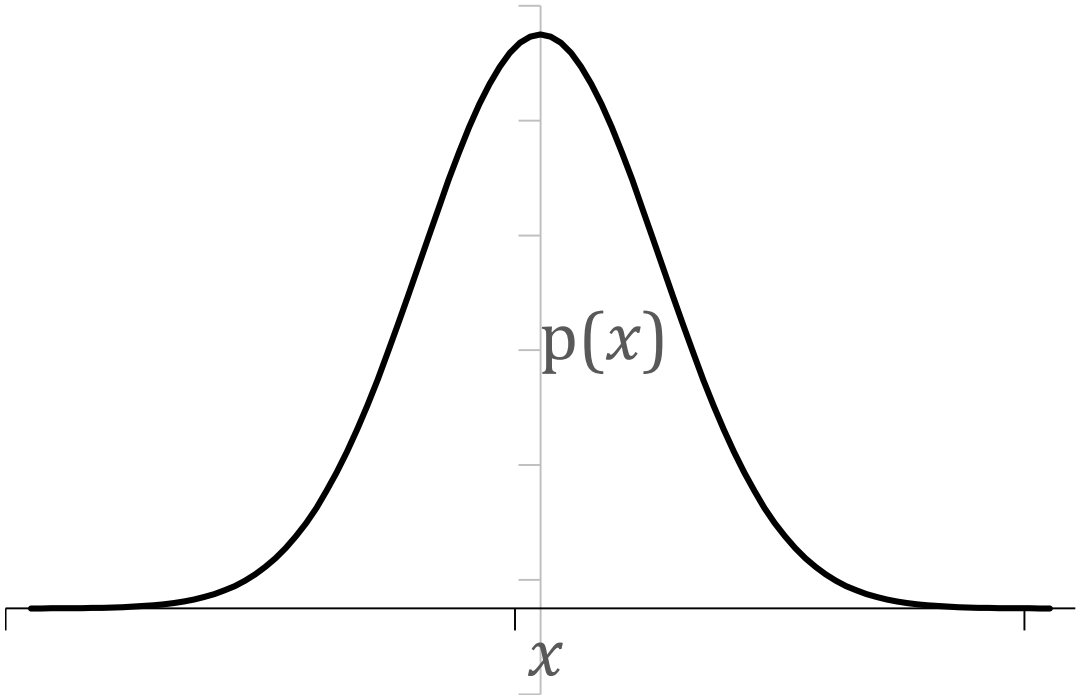
# Types of *B*-factors

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 ?

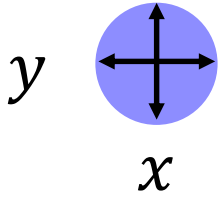



ask me where Gaussian form comes from - not for Klausur – harmonic model

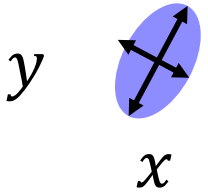
# Anisotropic *B*-factors

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

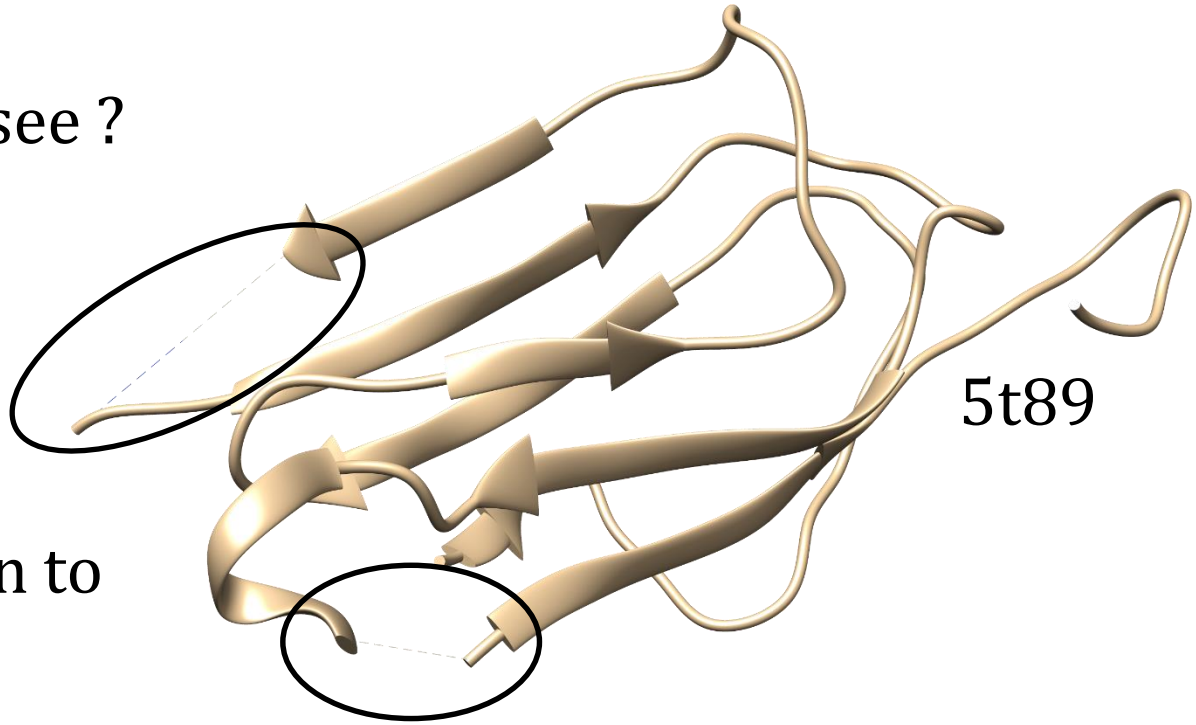
# *B*-factors one will meet

| | data necessary | number of parameters | |
|---|---|---|---|
| every atom anisotropic | lots | lots | few |
| every atom | 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

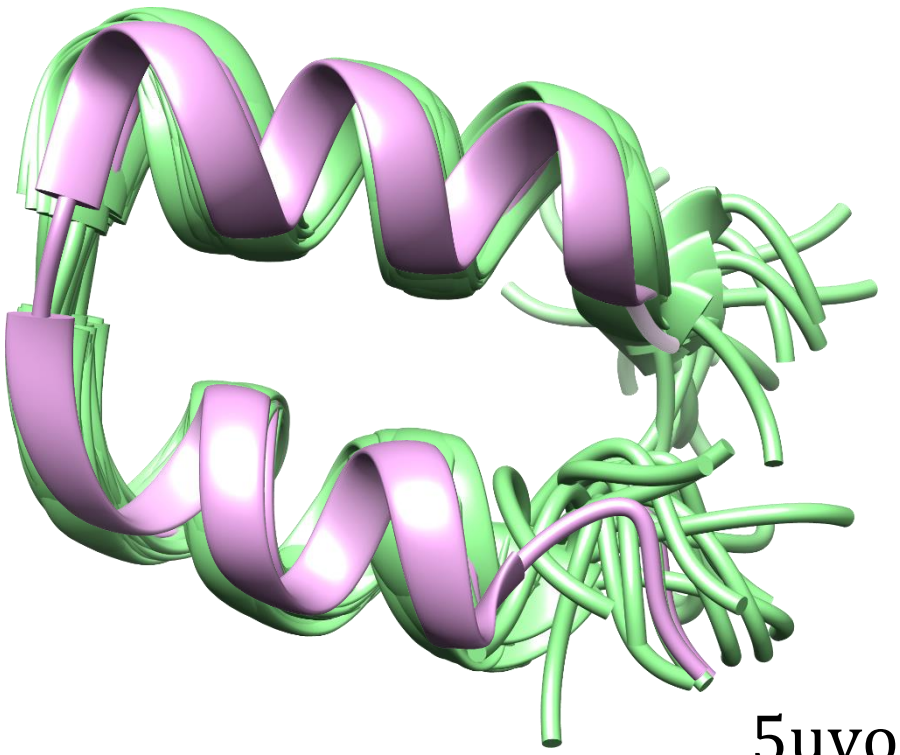
Solve the distance geometry problem 100 times

- send the best 20 or 30 structures to data bank

# NMR coordinate error

- purple – what you see when you open the file

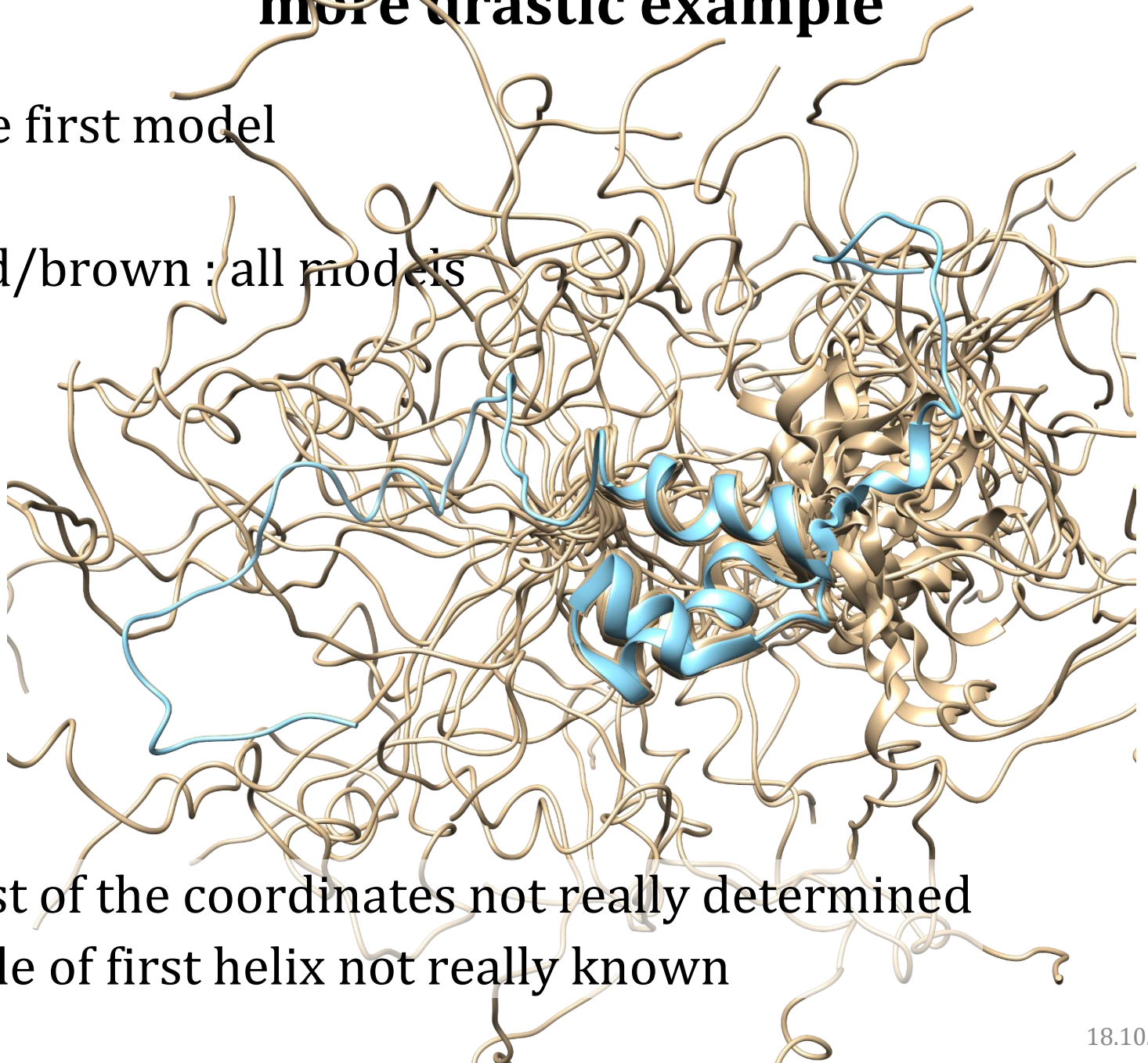
- green – 20 more “models”



5uyo

# more drastic example

- blue first model
- gold/brown : all models



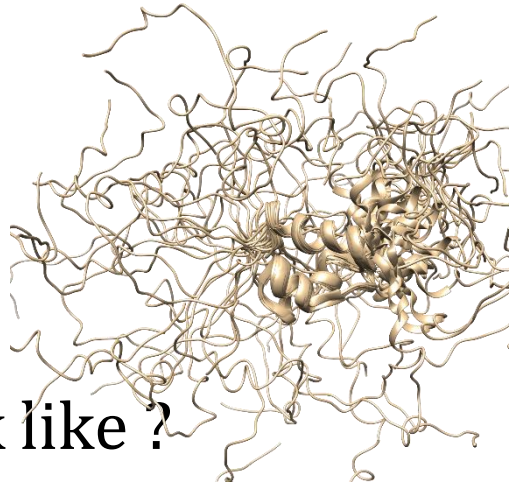
5nr6

- 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

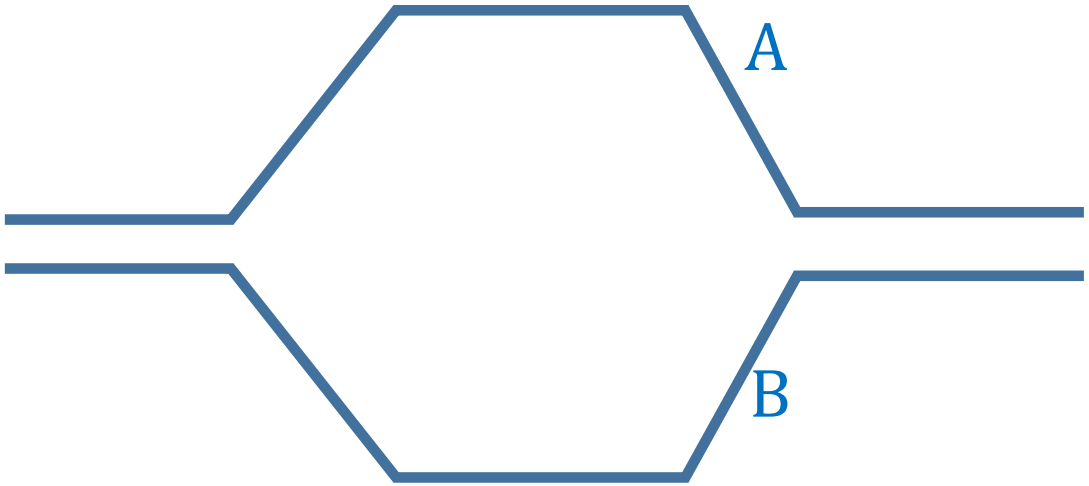


Can we take the average ?

- what would the average look like ?

What do average coordinates generally look like ?

# You cannot average coordinates

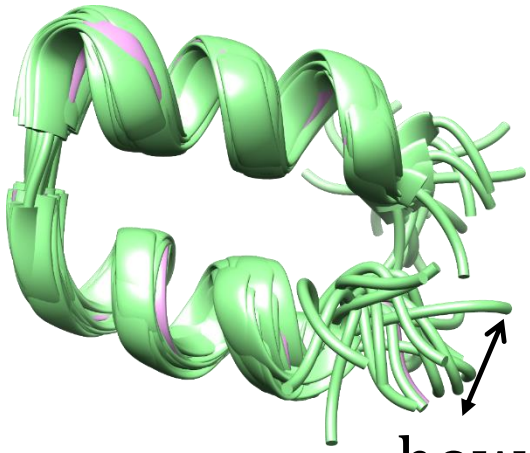



coordinates with normal bond lengths / angles

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



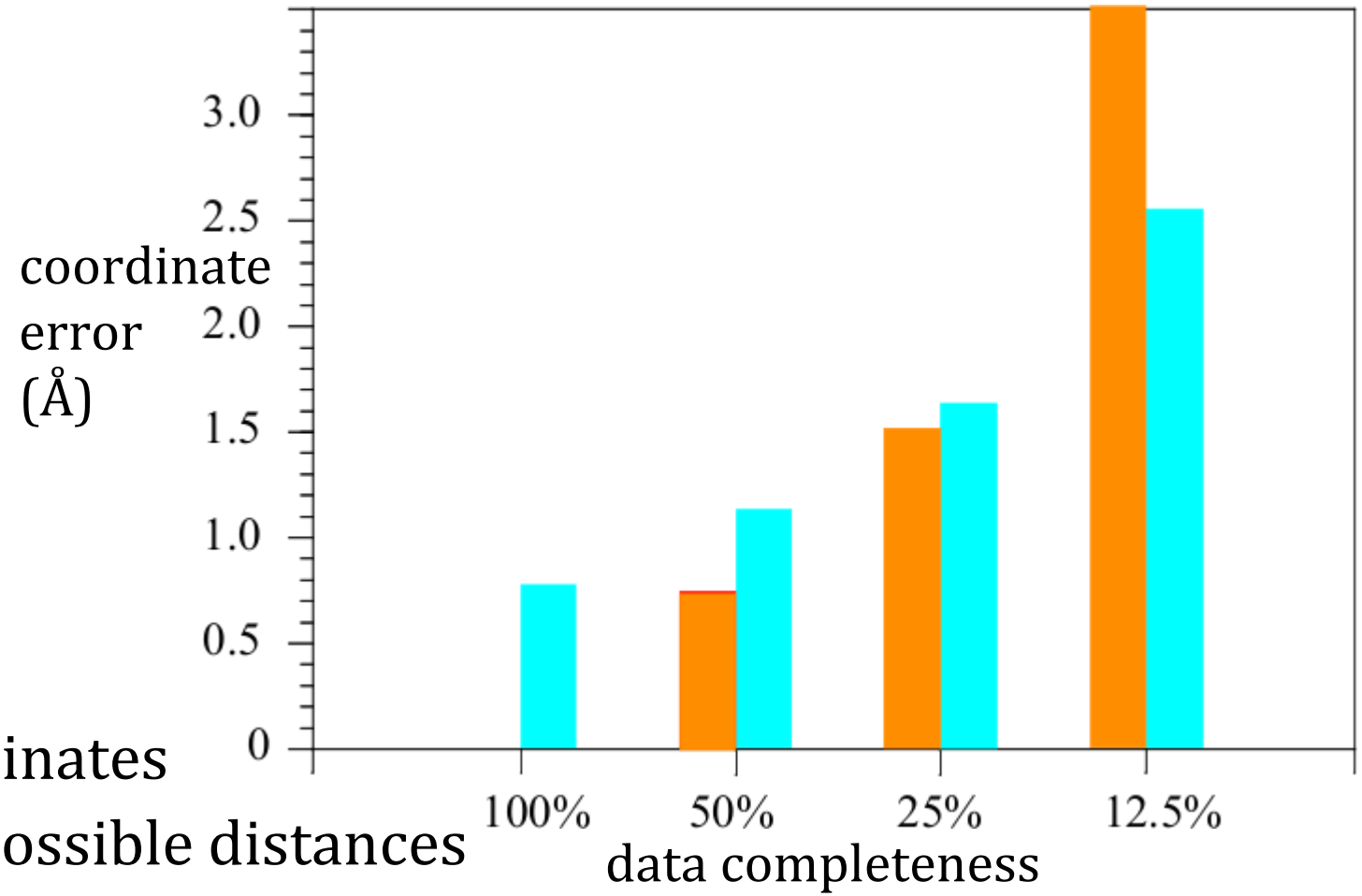
how big is this ?

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 $C^{\alpha}$ ) 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

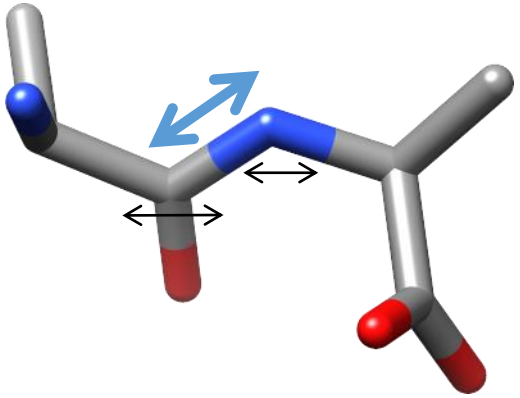
- bigger than with X-ray
- less well estimated

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 ?
- simple / classic error analysis
- if $y = x_1 - x_2$ with errors $y = (x_1 \pm \epsilon_1) - (x_2 \pm \epsilon_2)$
  - final error is $\epsilon = \left((\epsilon_1)^2 + (\epsilon_2)^2\right)^{\frac{1}{2}}$
- imagine $^1/_4$ Å error on C and N
- final error on $d_{CN}$

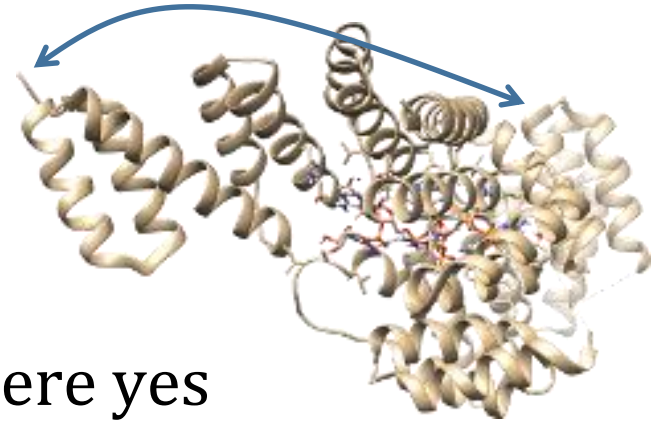


$$\epsilon_{d_{CN}} = \left(\left(\frac{1}{4}\right)^2 + \left(\frac{1}{4}\right)^2\right)^{\frac{1}{2}} = \left(\frac{1}{8}\right)^{\frac{1}{2}} \approx 0.35\text{Å}$$

silly. I know that CN bond length is 1.32 Å

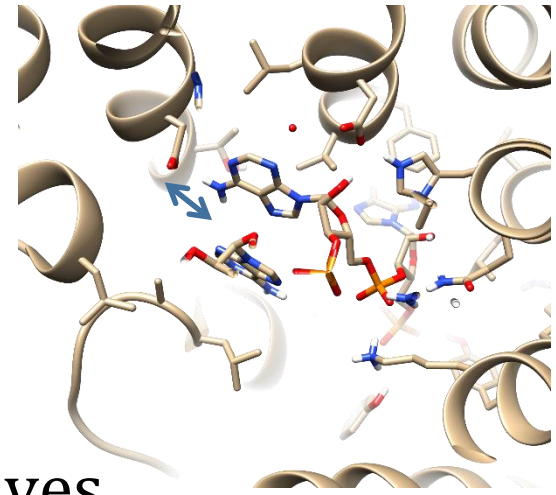
What have I done wrong ?

- Intuitive – some distances are known and fixed
- Formal statistical – rule only applies to independent errors
  - bonded C and N coordinates are highly correlated
- Does simple error analysis ever apply ?



Here yes
but probably not
so interesting

5W5I

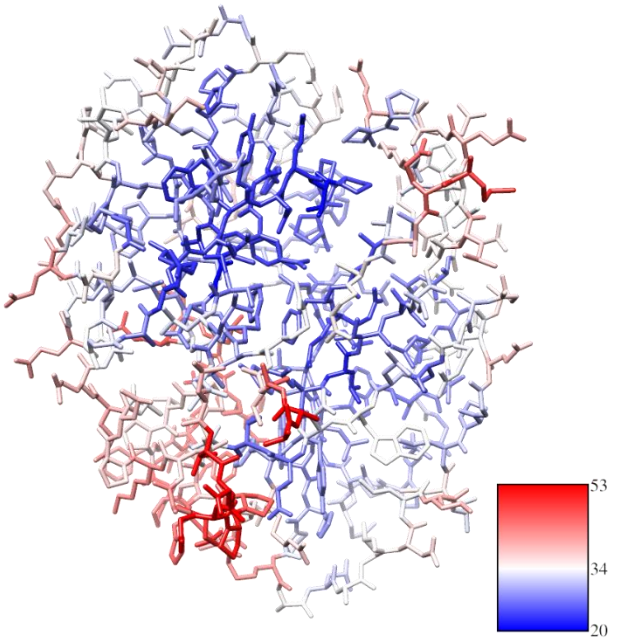


Here yes
and probably
important

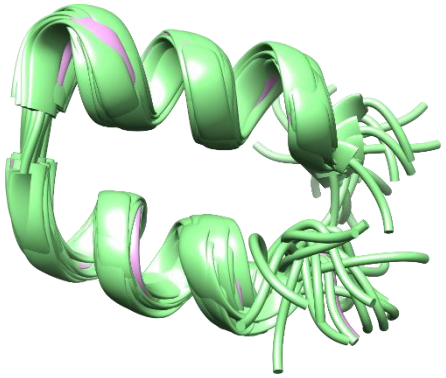
# uncertainty is more complicated

Mobility is not evenly distributed

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



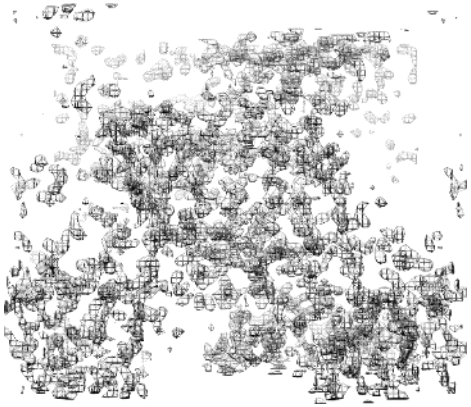

- NMR
  - uncertainty also reflects mobility

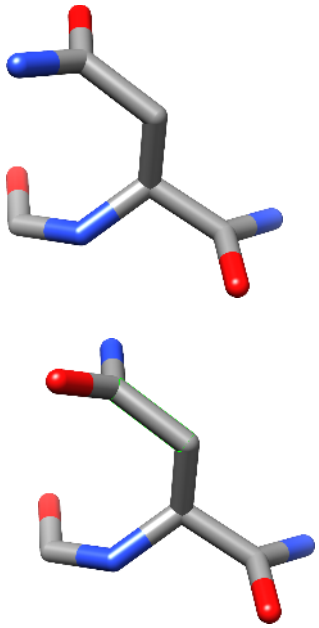


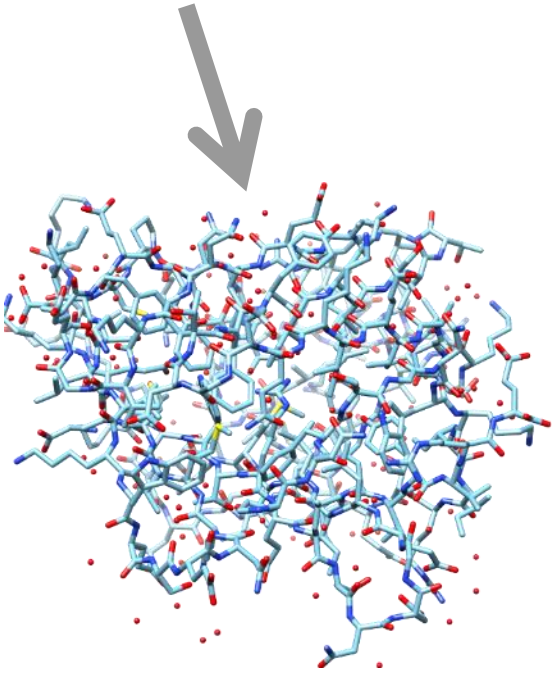
# Mistakes –not random, not systematic

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

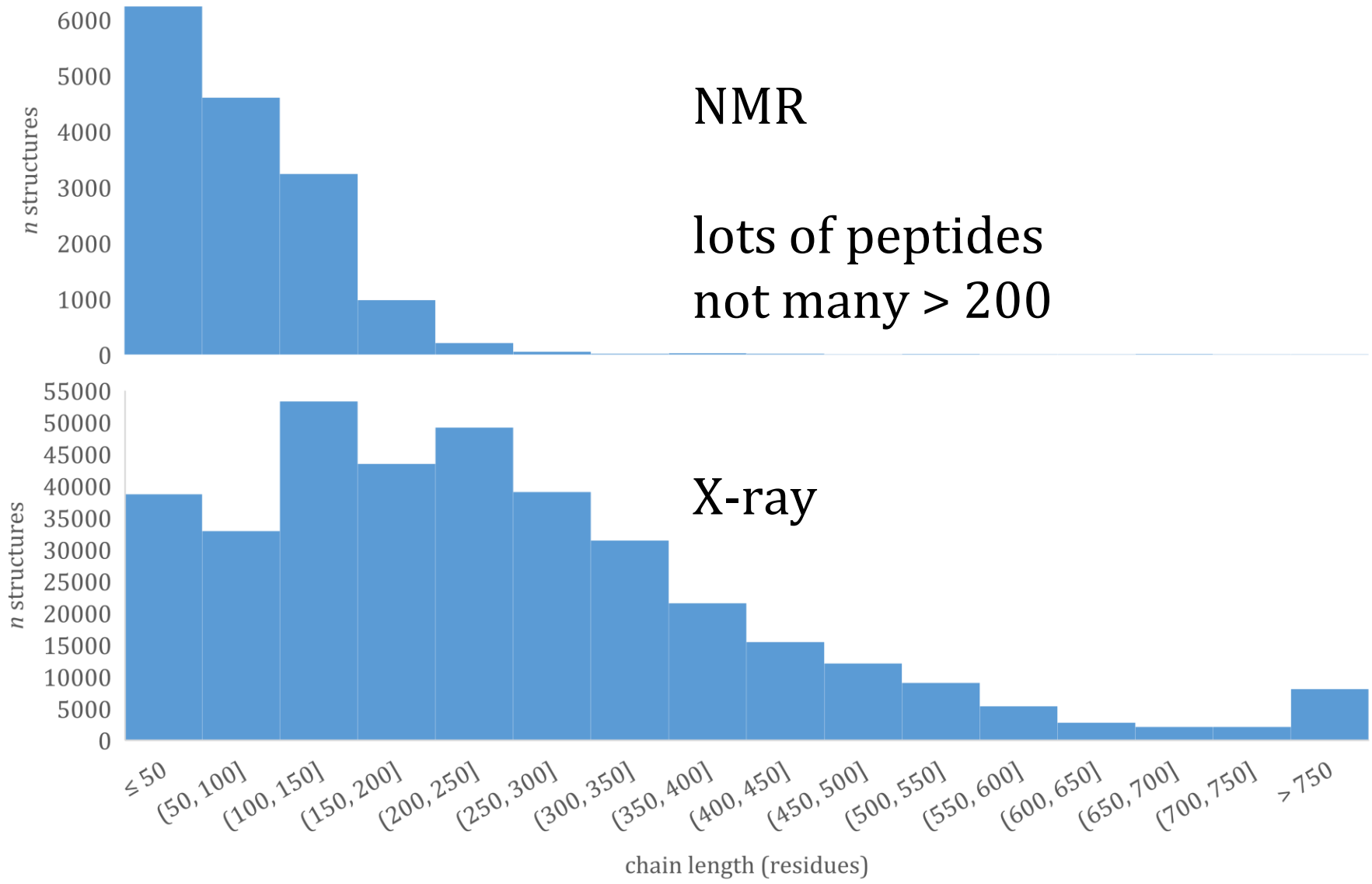
- finding errors ?

# 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 | | |