https://bl831.als.lbl.gov/~jamesh/powerpoint/CCP4_tangle_2025.pptx

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DOE-BER IDAT (Hura)

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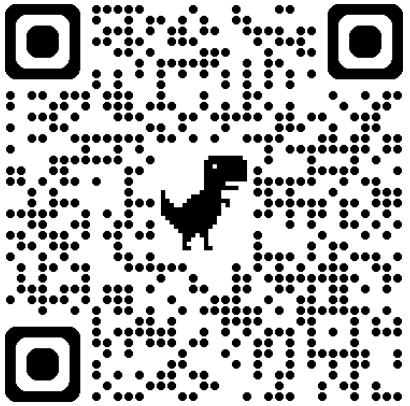
https://bl831.als.lbl.gov/~jamesh/powerpoint/CCP4_tangle_2025.pptx

Why is a beamline guy doing refinement things?



Because: the future of crystallography has alternate conformations

- ✓ Single-conf models
- ✓ poor resolution
- ✓ Low signal/noise
- ✓ Phase Problem

- ❑ Dynamics?
- ❑ Mechanism?
- ❑ Small signal?
- ❑ Overlapping states?

Map Noise:

via Parseval's Theorem

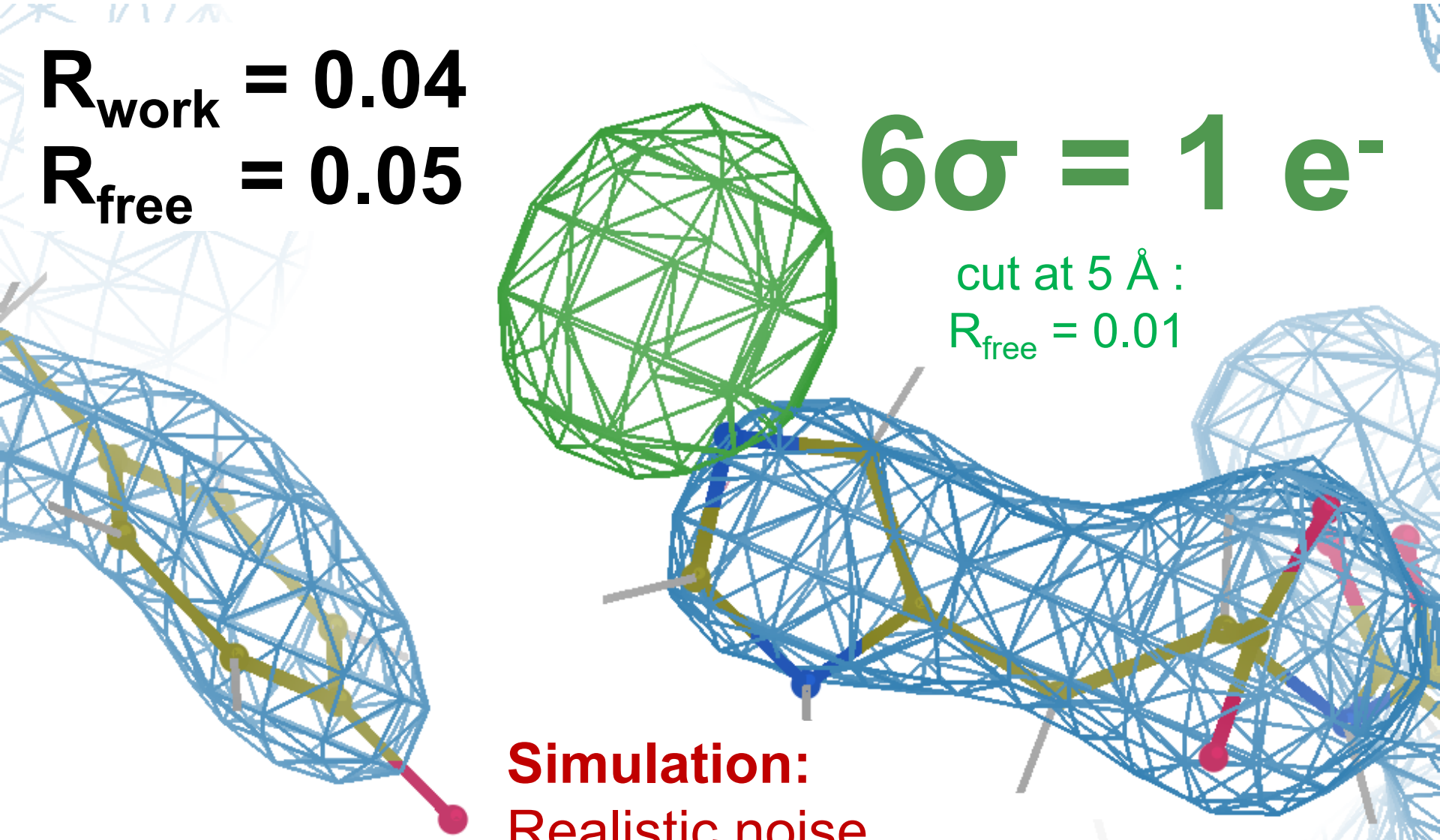
$$\frac{\rho}{\sigma(\rho)} \approx \frac{e^-}{7R}$$

e^-	number of electrons in peak
7	number of electrons in typical atom
R	R_{iso} or R_{free}
ρ	electron density peak
σ	rms deviation expected

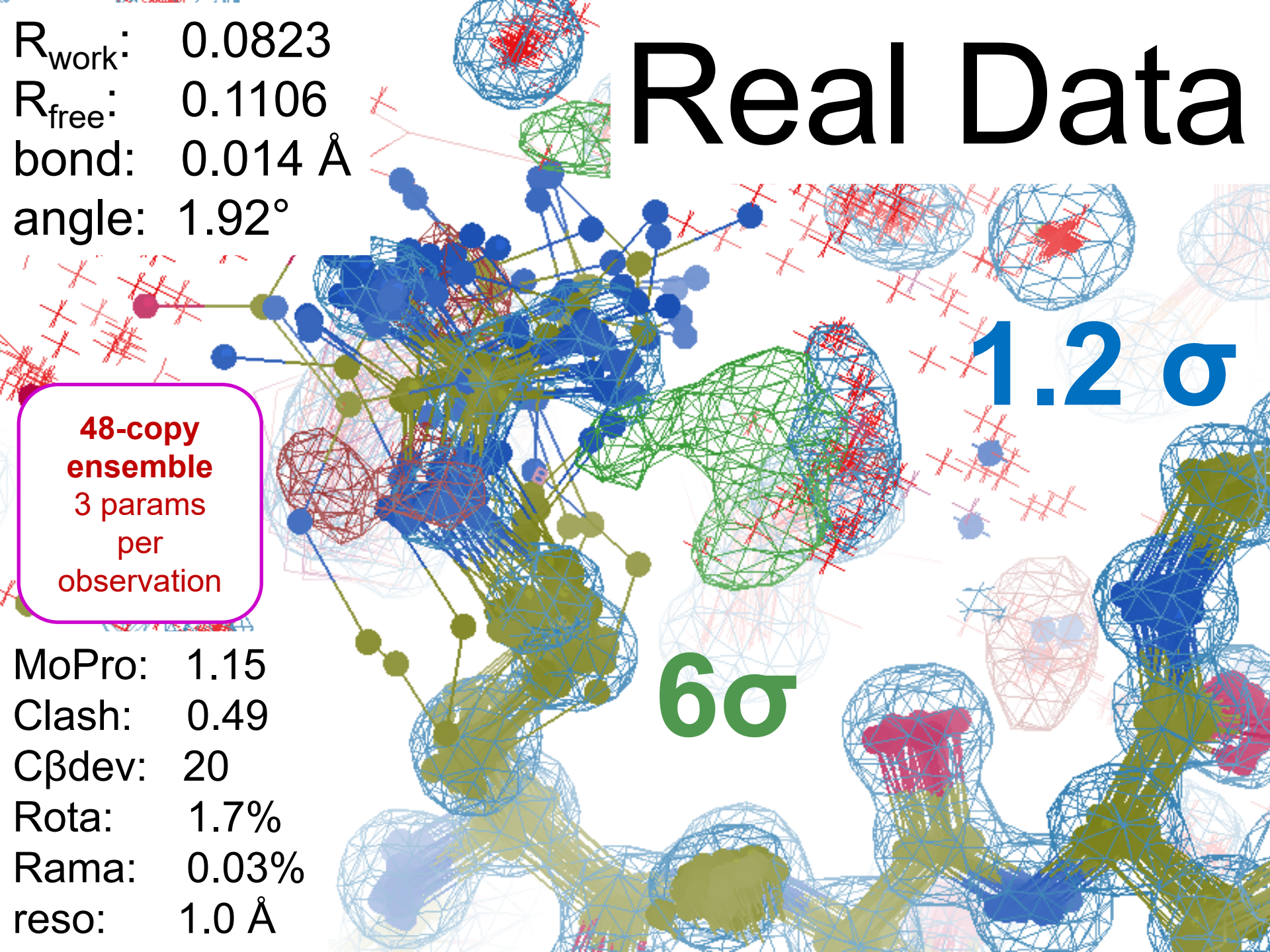
Hydrogen: visible at **3.5 Å** resolution!

R_{work} = 0.04

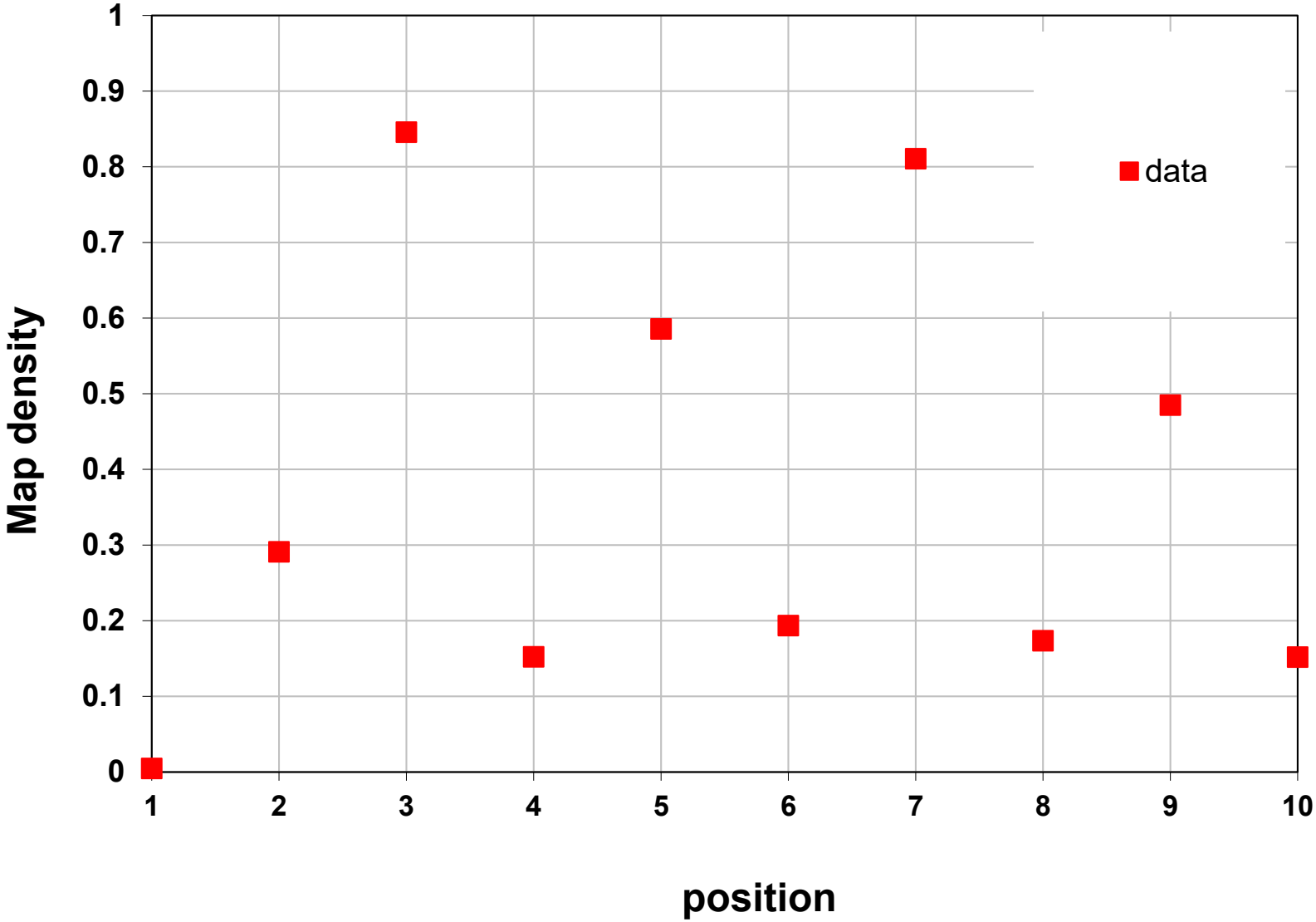
R_{free} = 0.05



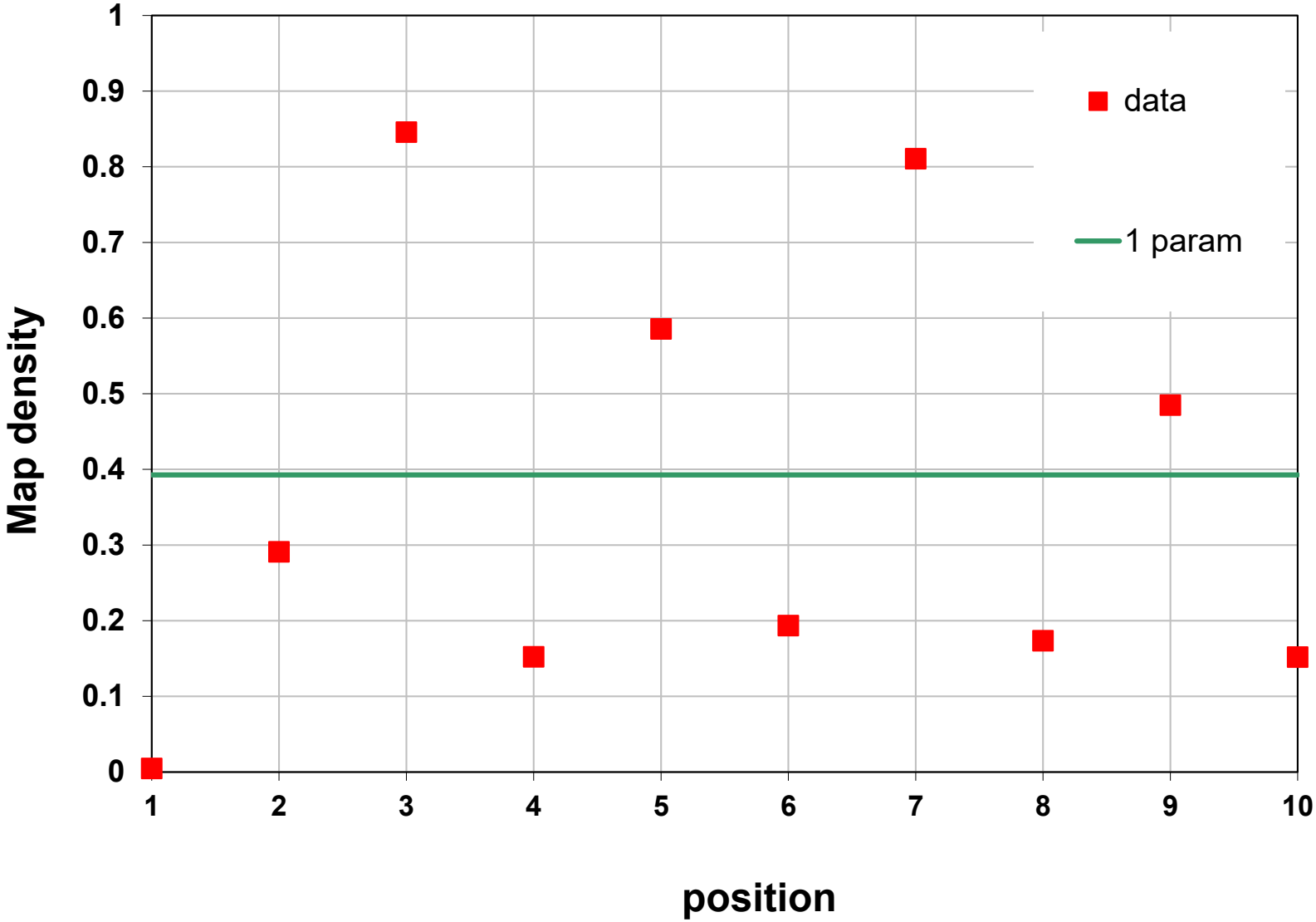
Simulation:
Realistic noise
“perfect” model


R_{work}: 0.0823
R_{free}: 0.1106
bond: 0.014 Å
angle: 1.92°
Real Data
48-copy ensemble
3 params per observation
1.2 σ
6σ
MoPro: 1.15
Clash: 0.49
Cβdev: 20
Rota: 1.7%
Rama: 0.03%
reso: 1.0 Å

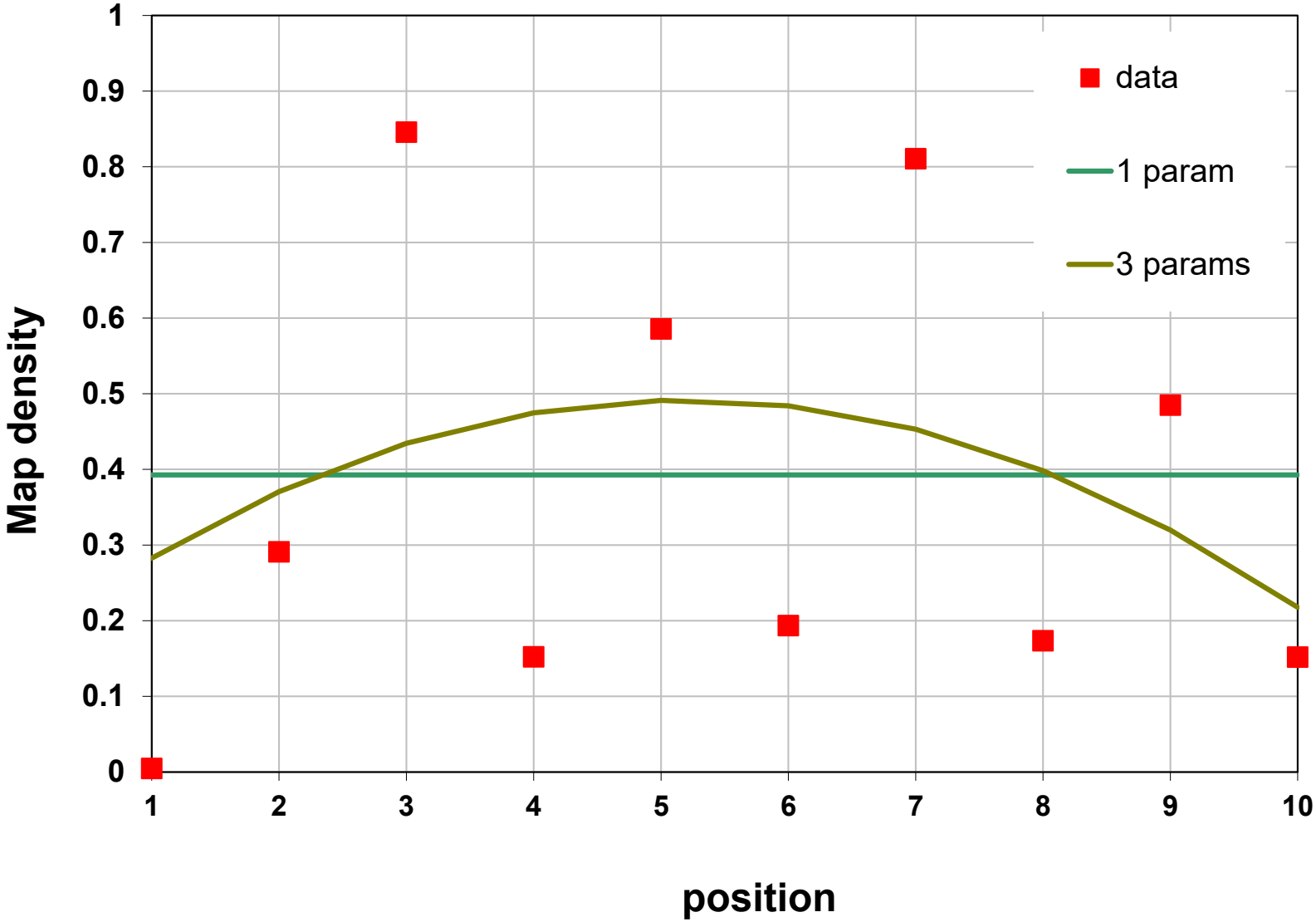
Over-Fitting data



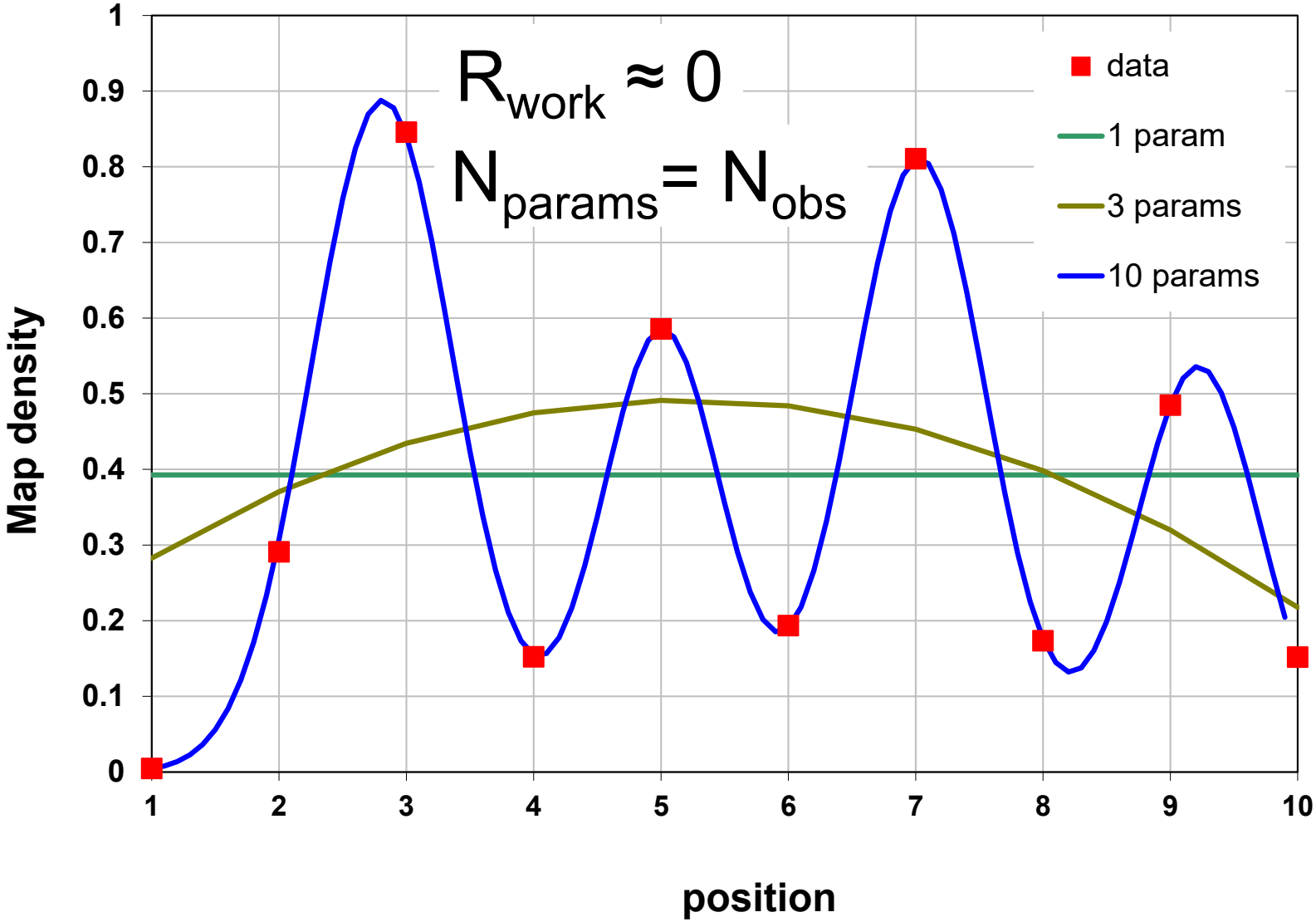
Over-Fitting data



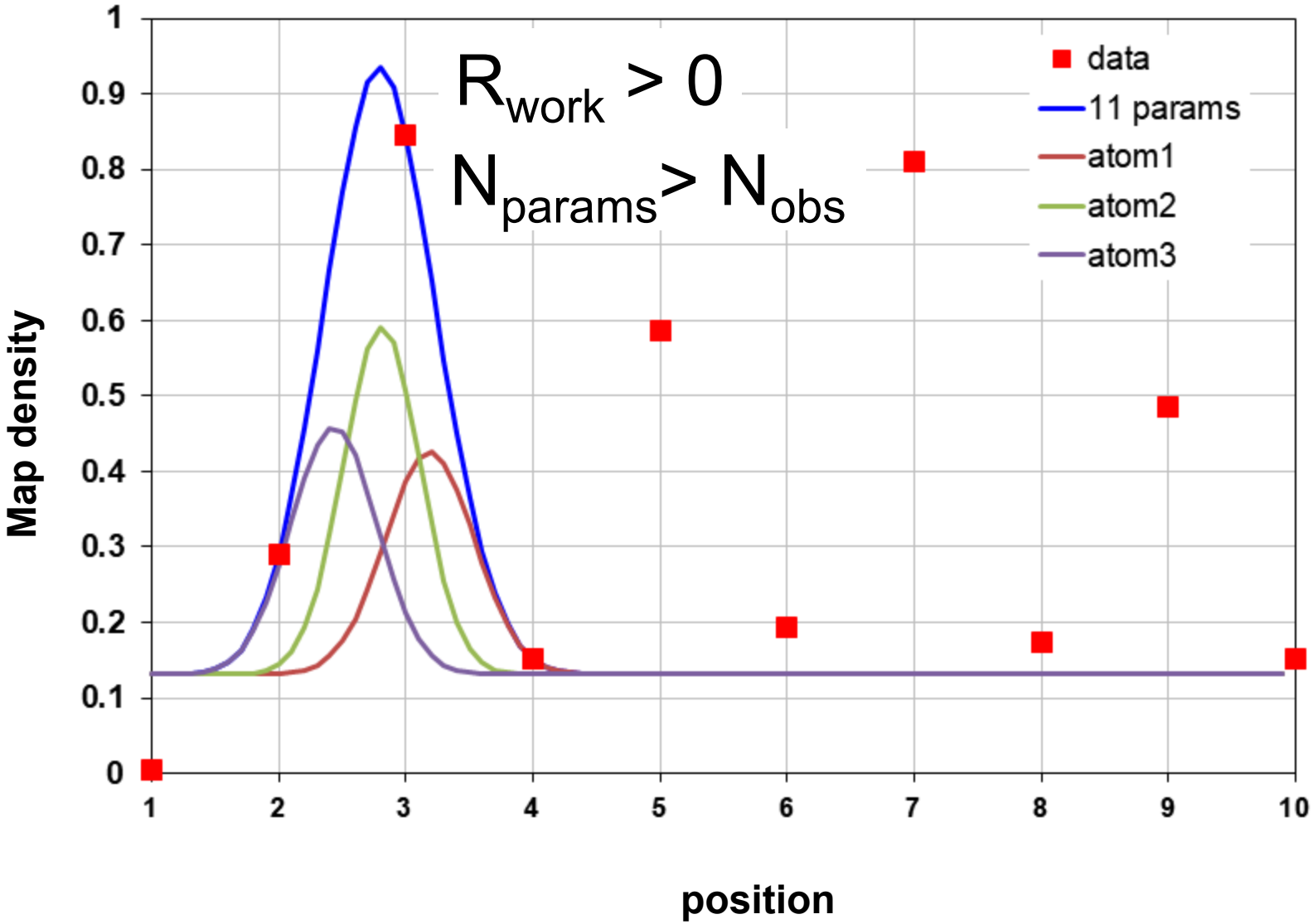
Over-Fitting data

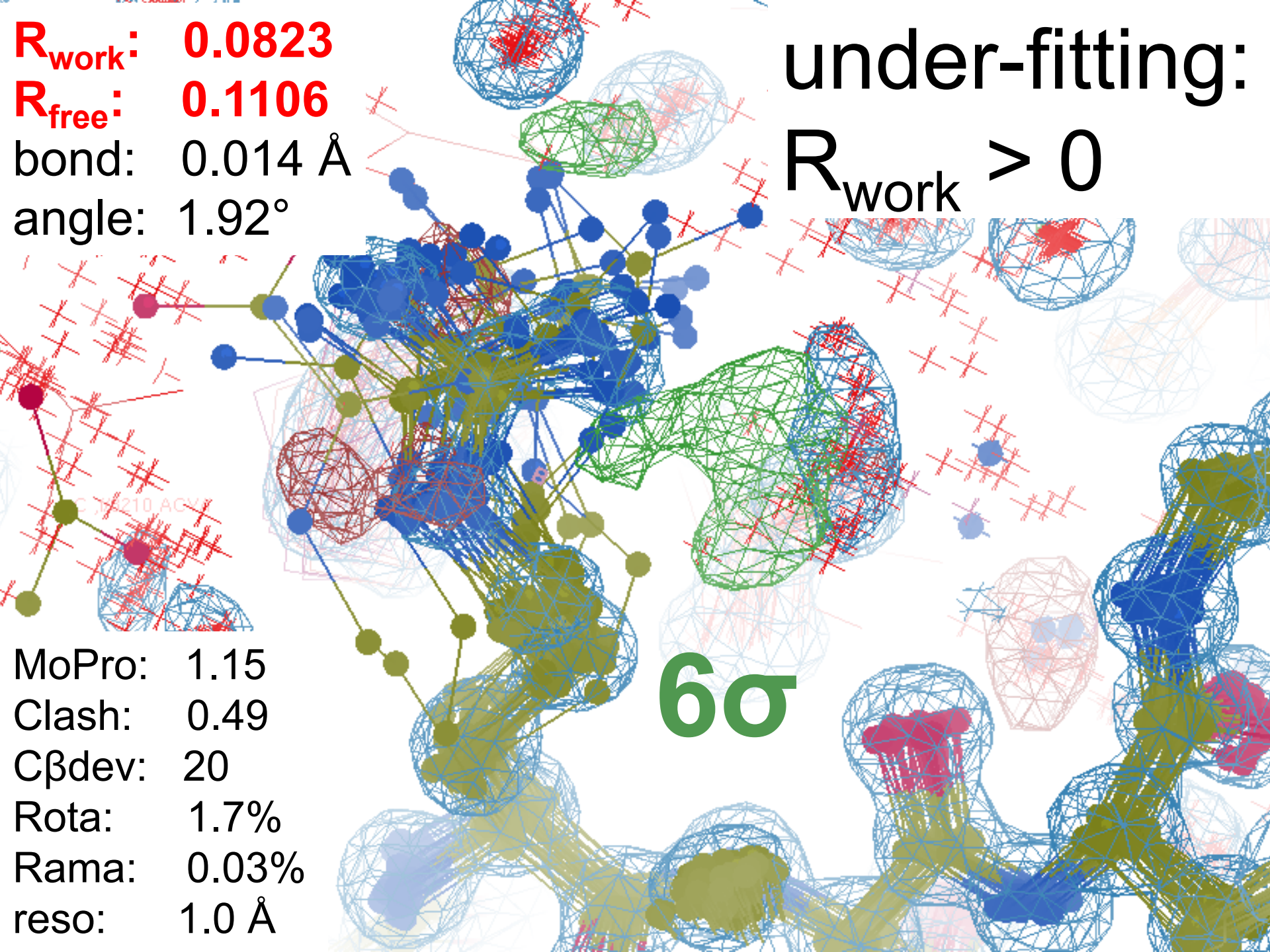


Over-Fitting data



Under-Fitting data

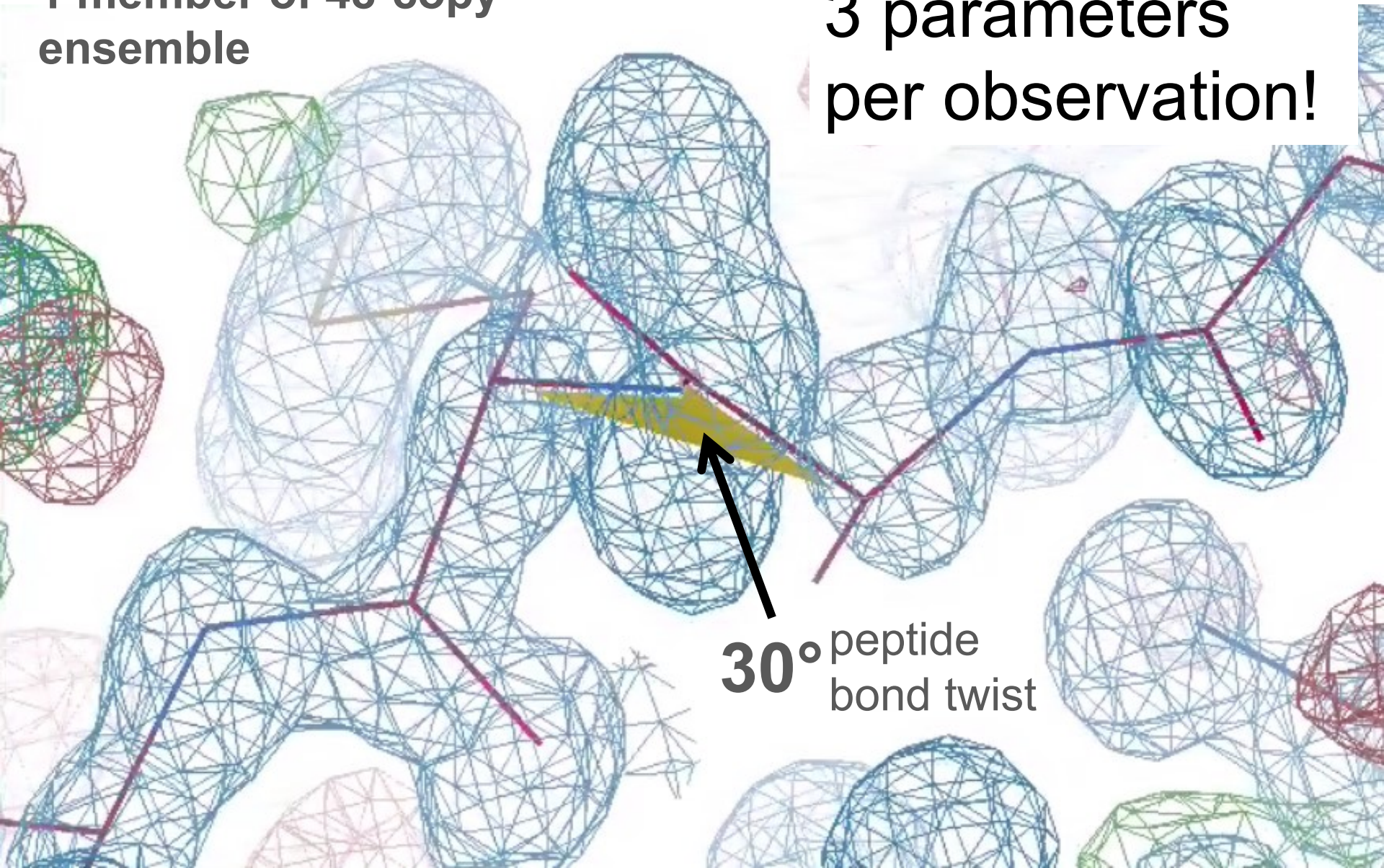



R_{work}: 0.0823
R_{free}: 0.1106
bond: 0.014 Å
angle: 1.92°
under-fitting:
R_{work} > 0
6σ
MoPro: 1.15
Clash: 0.49
Cβdev: 20
Rota: 1.7%
Rama: 0.03%
reso: 1.0 Å

What is holding it back?

1 member of 48-copy ensemble

3 parameters per observation!



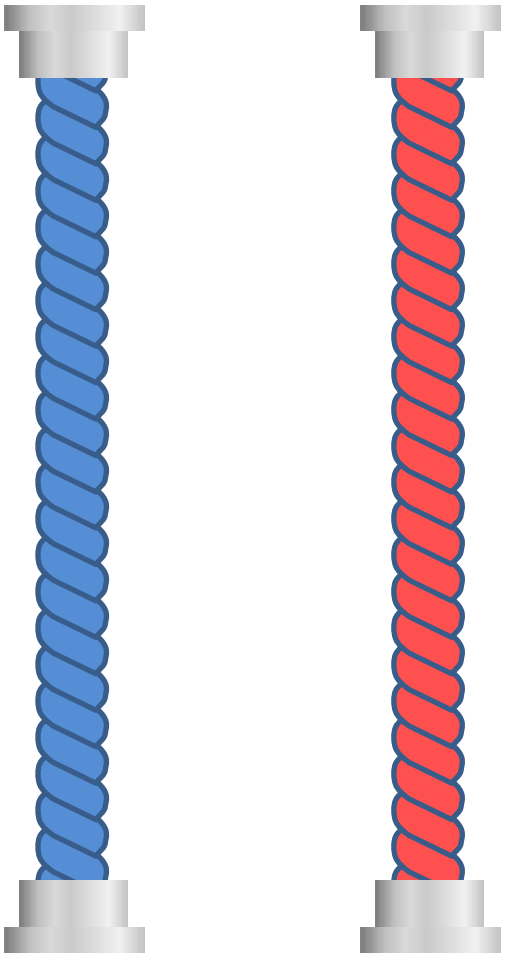
Why does ensemble refinement **suck**?

(3 parameters/observation and R_{work} still high!)

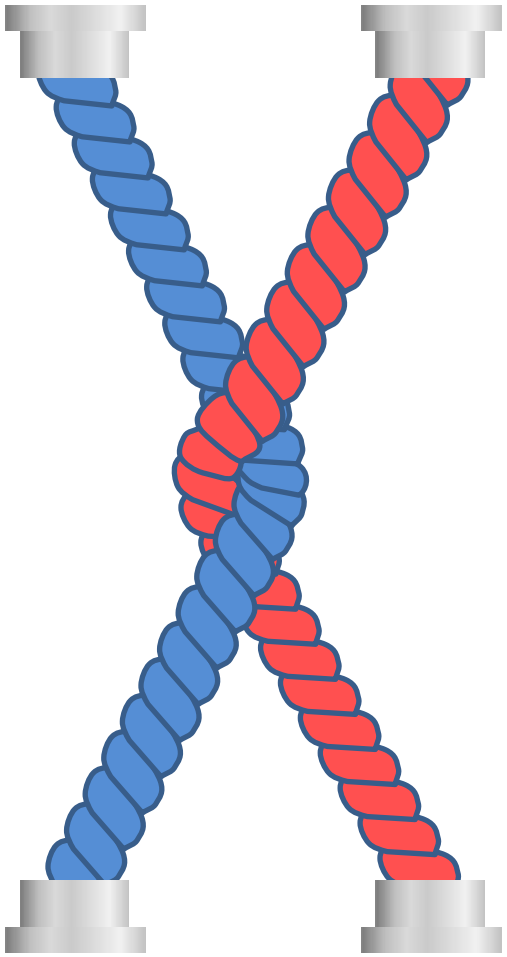
The chains are “tangled”

What do you mean, “tangled”?

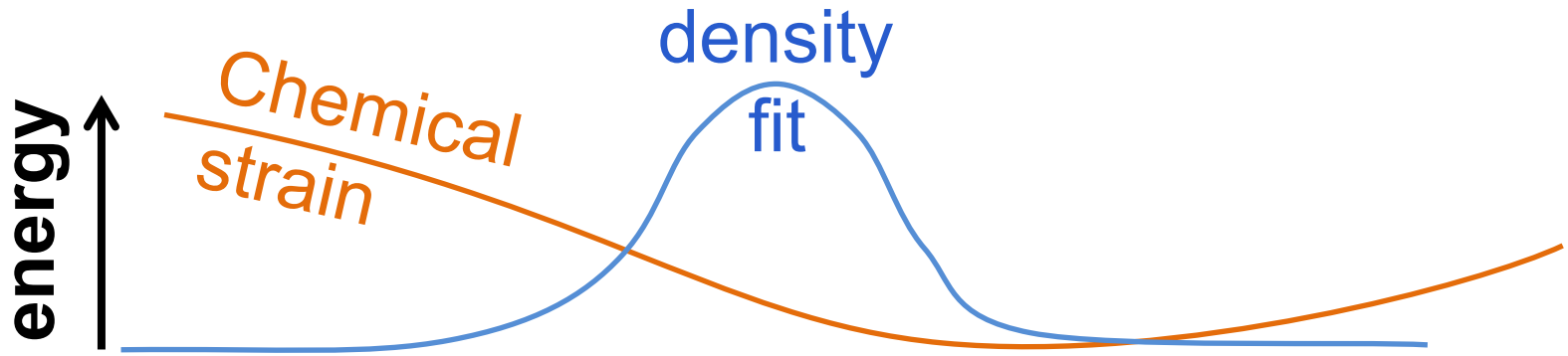
Ground Truth

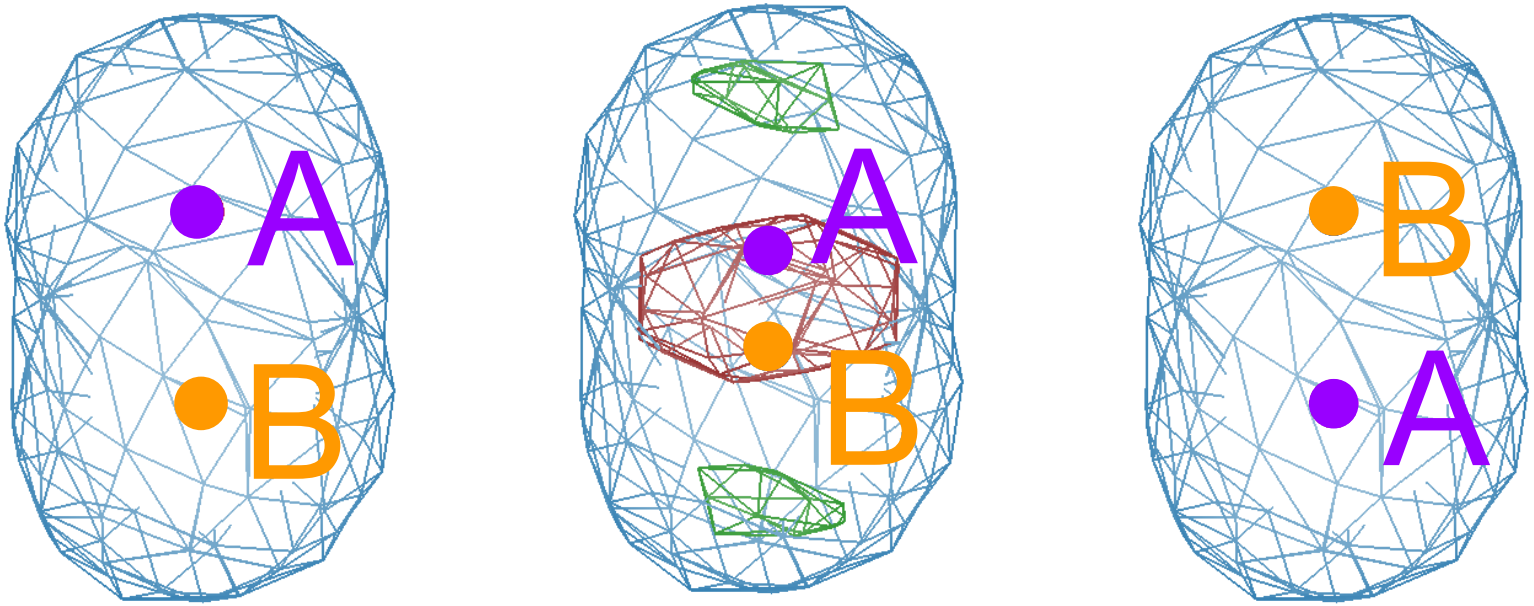


Local Minimum



It takes two to tangle



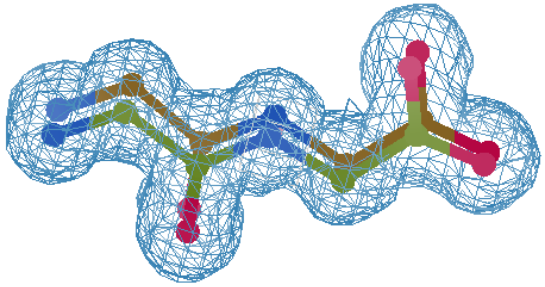


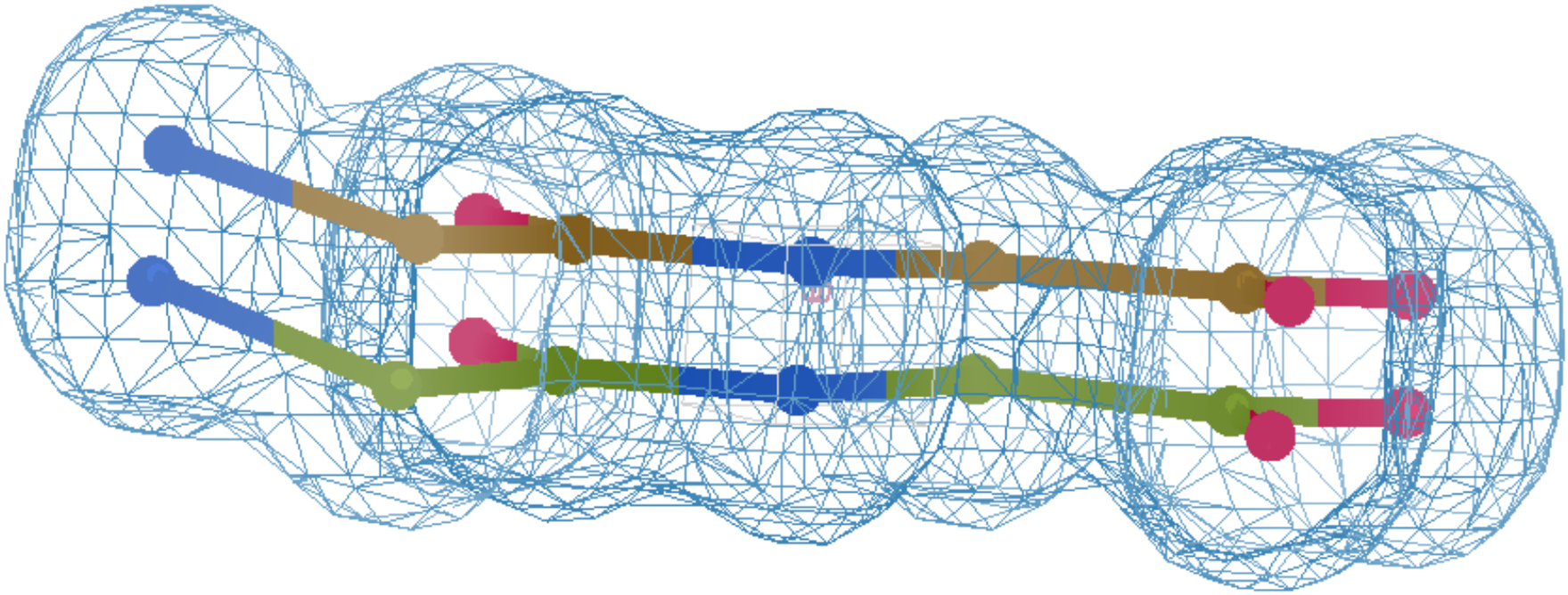
tangled barrier untangled

Refine from right answer

R = 0.8%

rms bond = 0.0002

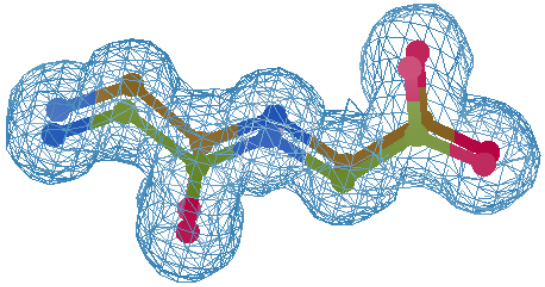


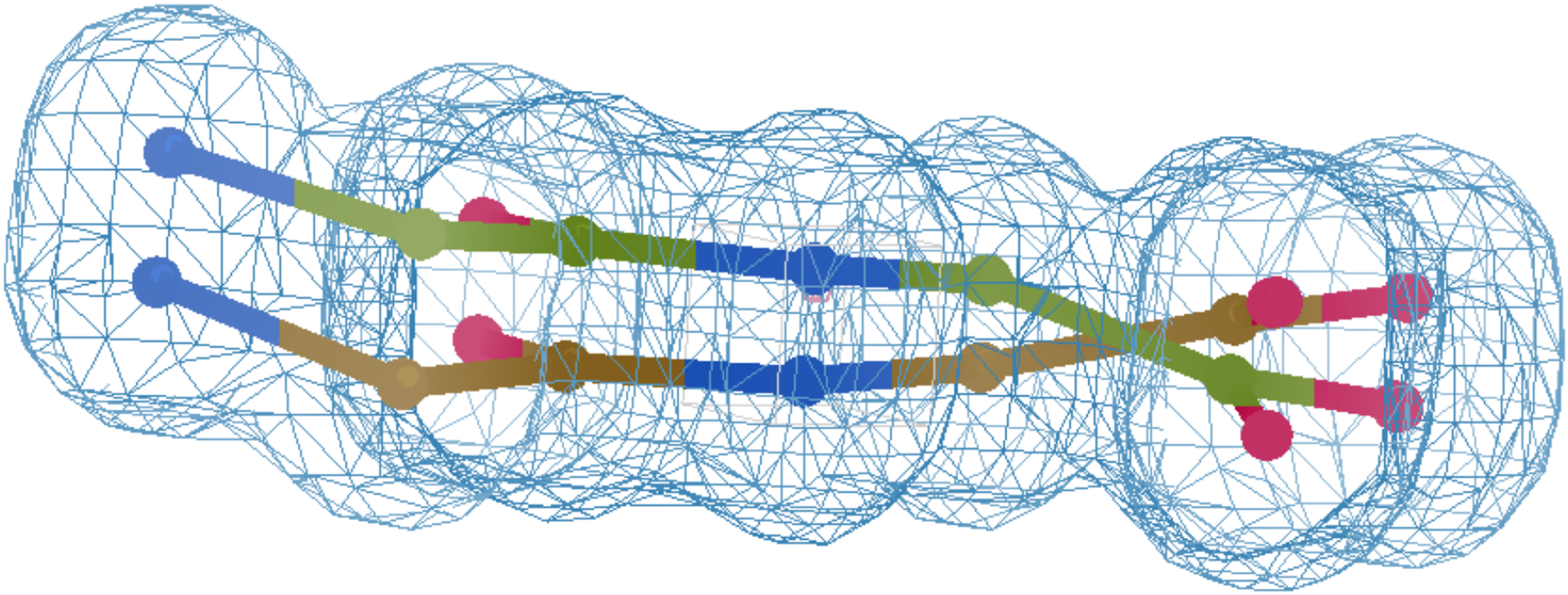


Refine from wrong starting point

R = 7.07%

rms bond = 0.001





UNTANGLE: sim-data Ground Truth

- As simple as possible:
  - 2 conformers
  - 50:50 occupancy
  - flat bulk solvent
  - no false outliers
- Accessible
  - 64 residues
  - 78 waters
  - phenix, refmac, shelxl
  - clear score
- Every chance of success
  - 1.0 Å reso
  - “one thing wrong”
  - Experimental error only
  - perfect phases

The UNTANGLE Challenge

`best.pdb`

`refme.mtz`

11) Build better than the best:

as-good or better wE, R_{free} as best.pdb

non-equivalent ensemble

10) Recover best.pdb from refme.mtz

true phases, auto-building allowed

no cheating (don't use best.pdb as guide)

Prove: true ensemble is unique
and can be recovered

https://github.com/jmholton/UnTangle

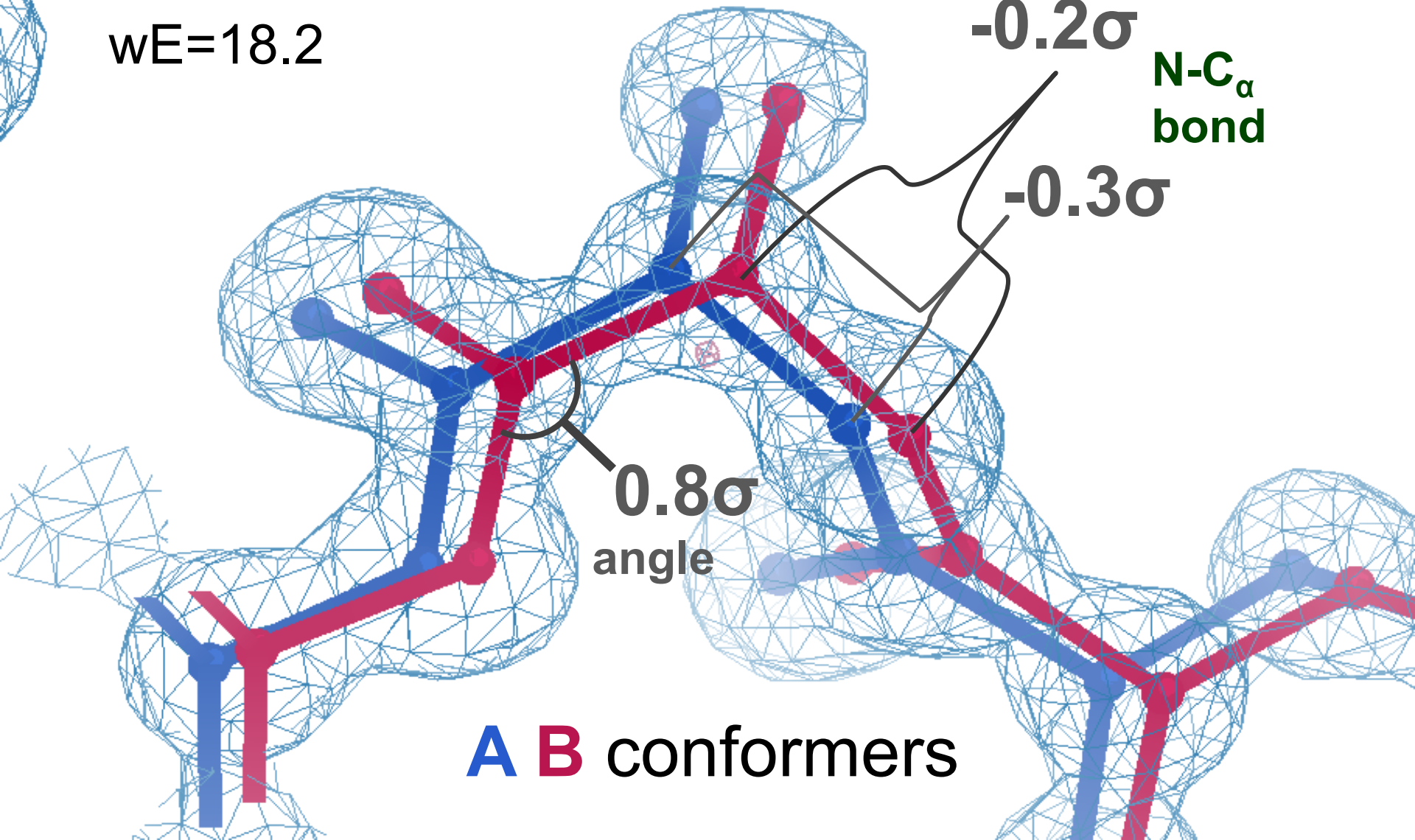
verified Score sheet

wE	R_{work}	R_{free}	method
185	20.6	21.4	Alphafold2
109.6	8.87	9.44	Phenix.ensemble_refine
97.3	8.84	9.27	qFit best
91.7	3.64	4.27	manual
80.5	4.31	4.75	lotswrong
65.2	6.35	6.93	Amber24
54.6	5.87	6.57	single conf w aniso B
33.5	2.94	3.32	One thing wrong: Ala39
23.5	2.95	3.31	One thing wrong: Val1
21.4	3.12	3.48	Rectified Simulated annealing
18.1	17.9	18.4	Phenix autobuild
18.2	2.79	3.14	best (ground truth)

Sim challenge data

Ala39

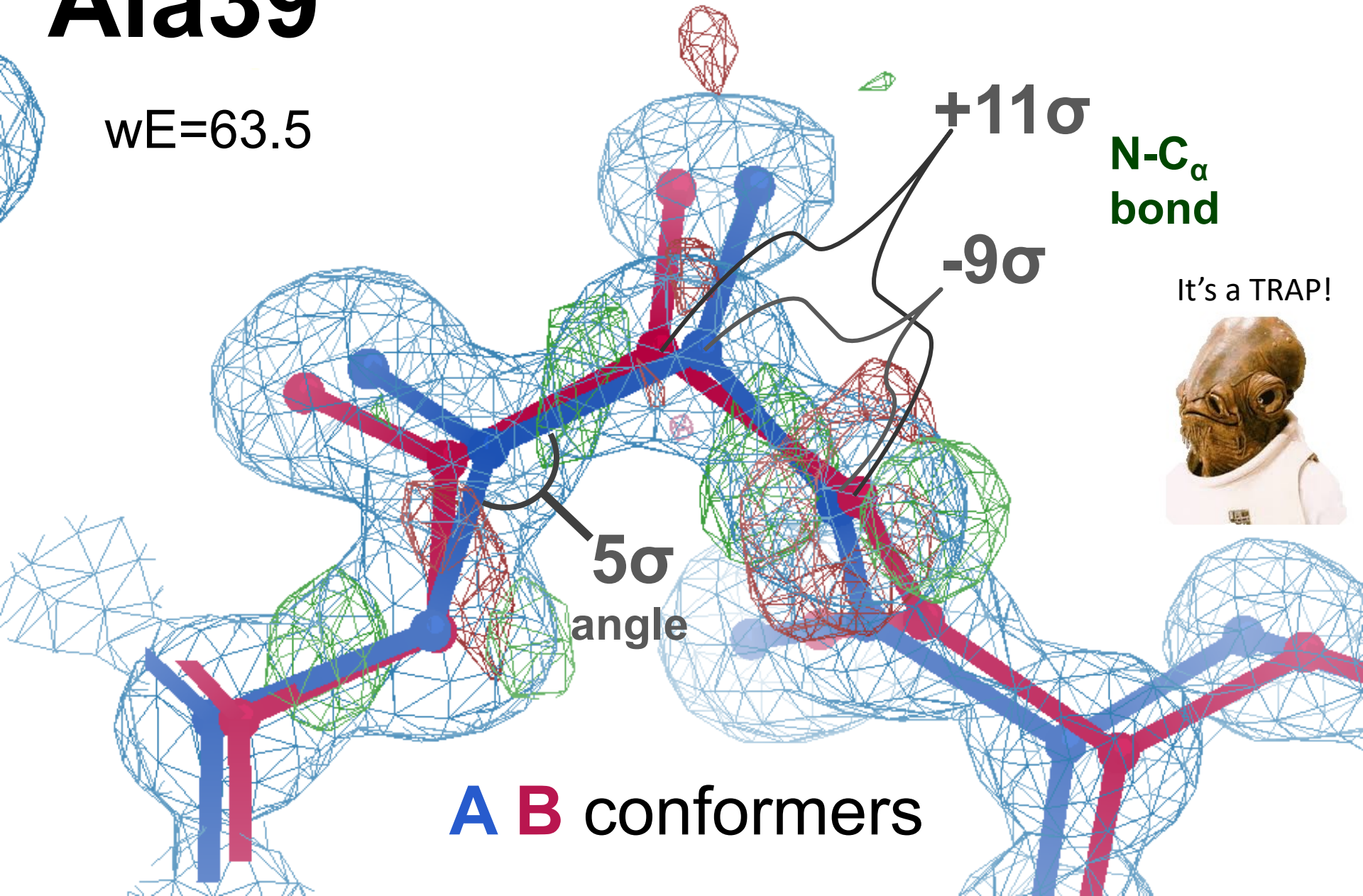
wE=18.2



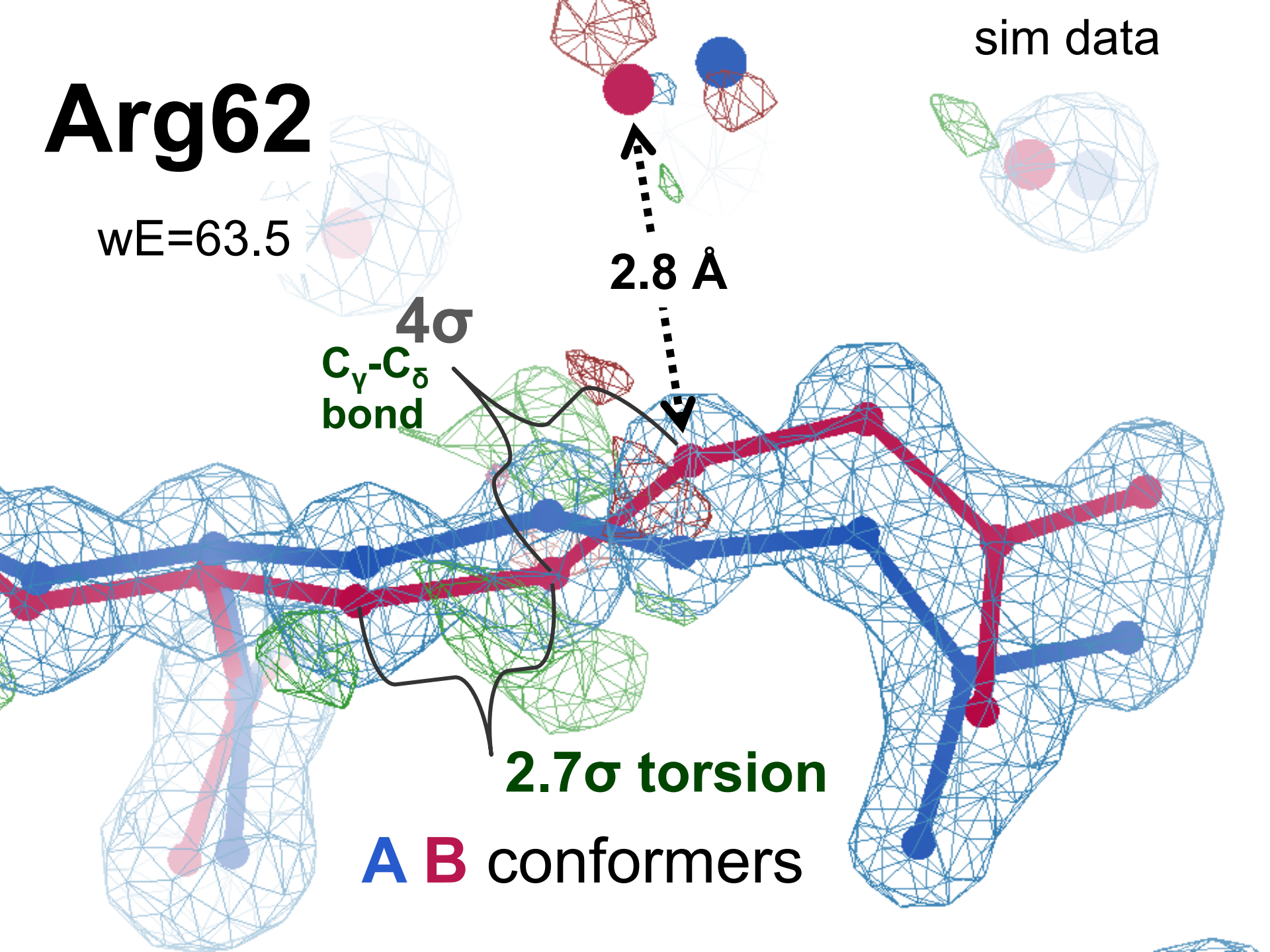
Sim challenge data: “otw39”

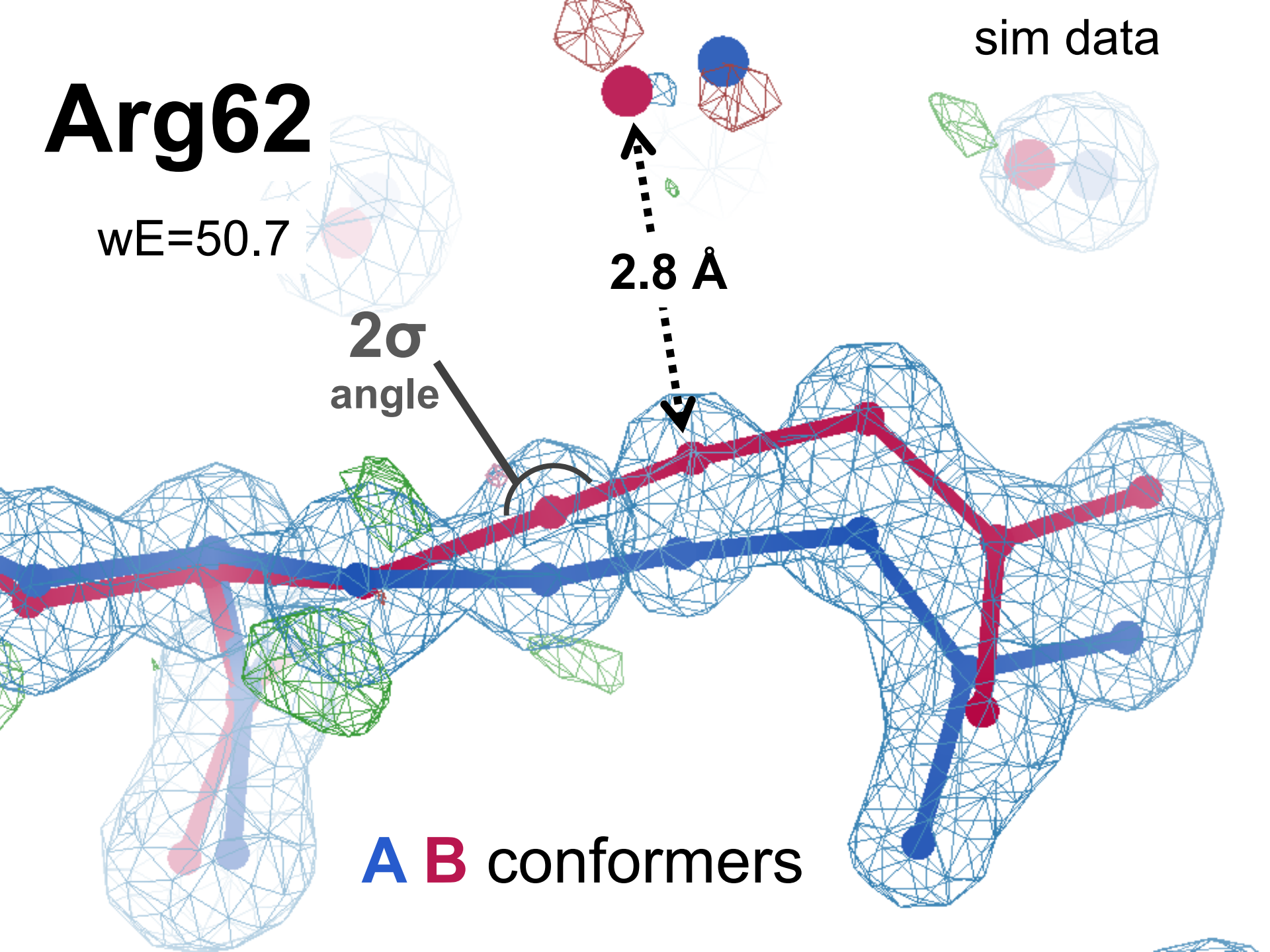
Ala39

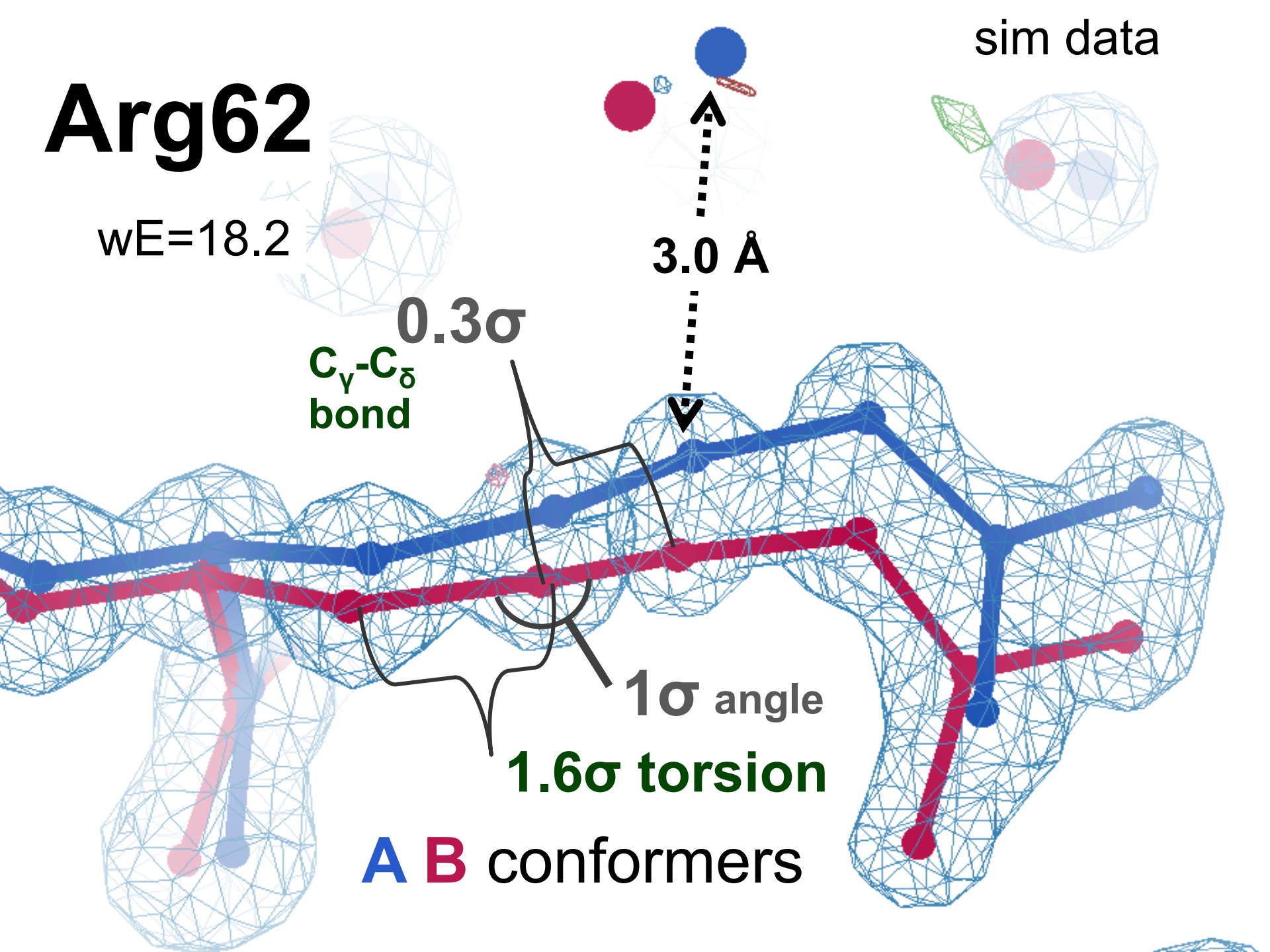
wE=63.5



It’s a TRAP!

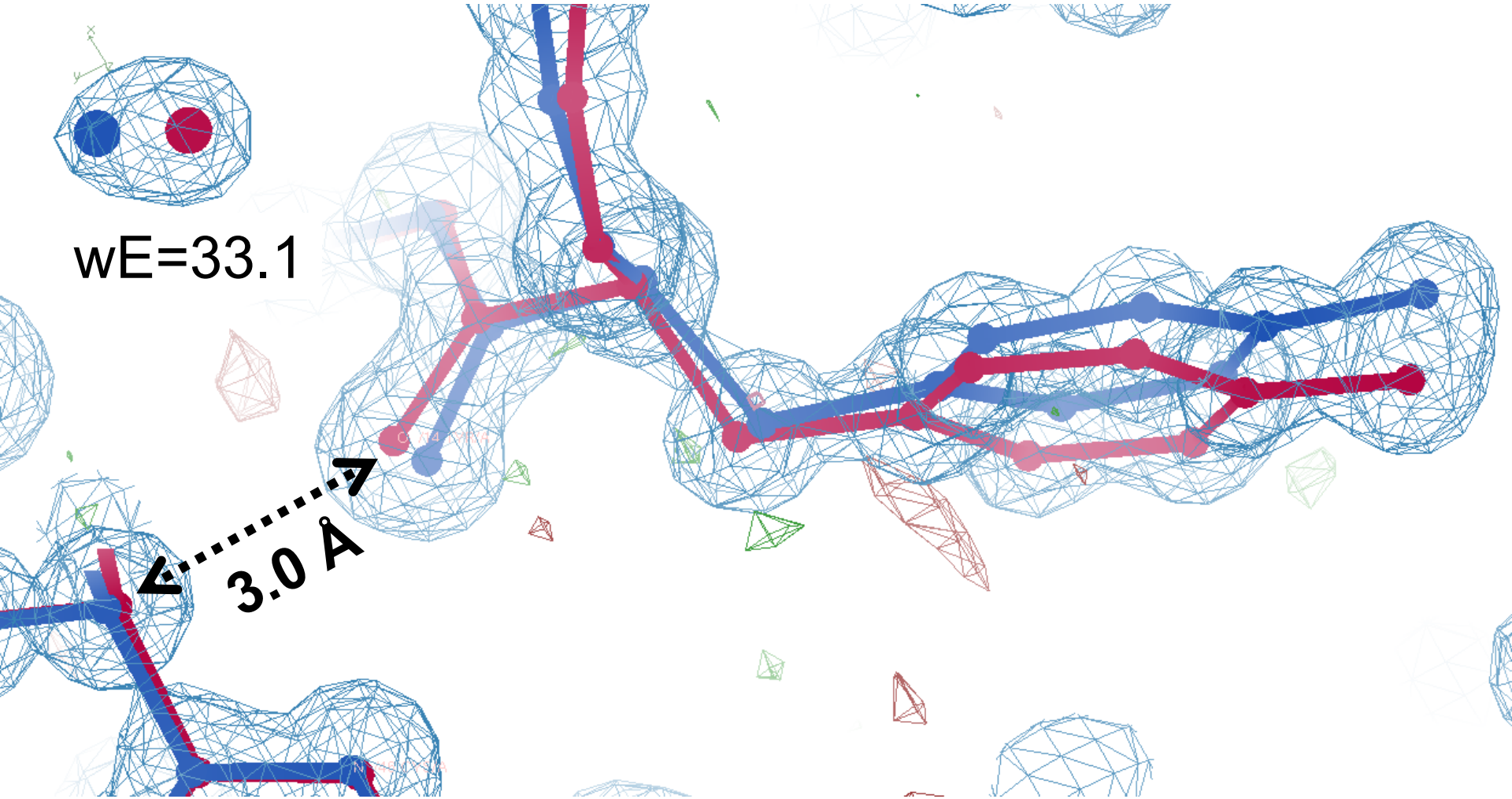

Arg62
wE=63.5
sim data
2.8 Å
4σ
C_γ-C_δ
bond
2.7σ torsion
A B conformers


Arg62
wE=50.7
sim data
2.8 Å
2σ
angle
A B conformers


Arg62
wE=18.2
sim data
3.0 Å
0.3σ
C_γ-C_δ bond
1σ angle
1.6σ torsion
A B conformers

Tyr14

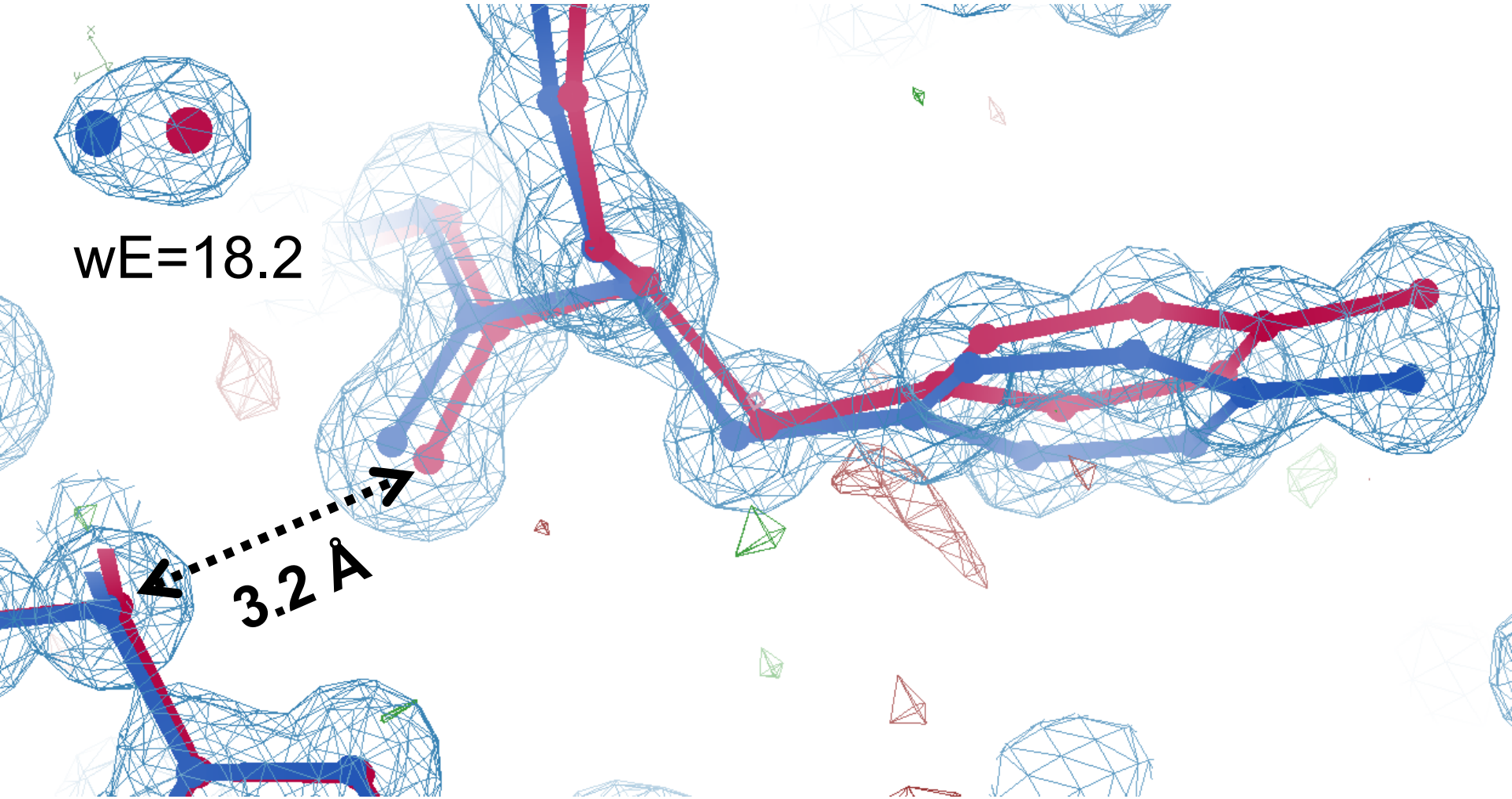
sim data



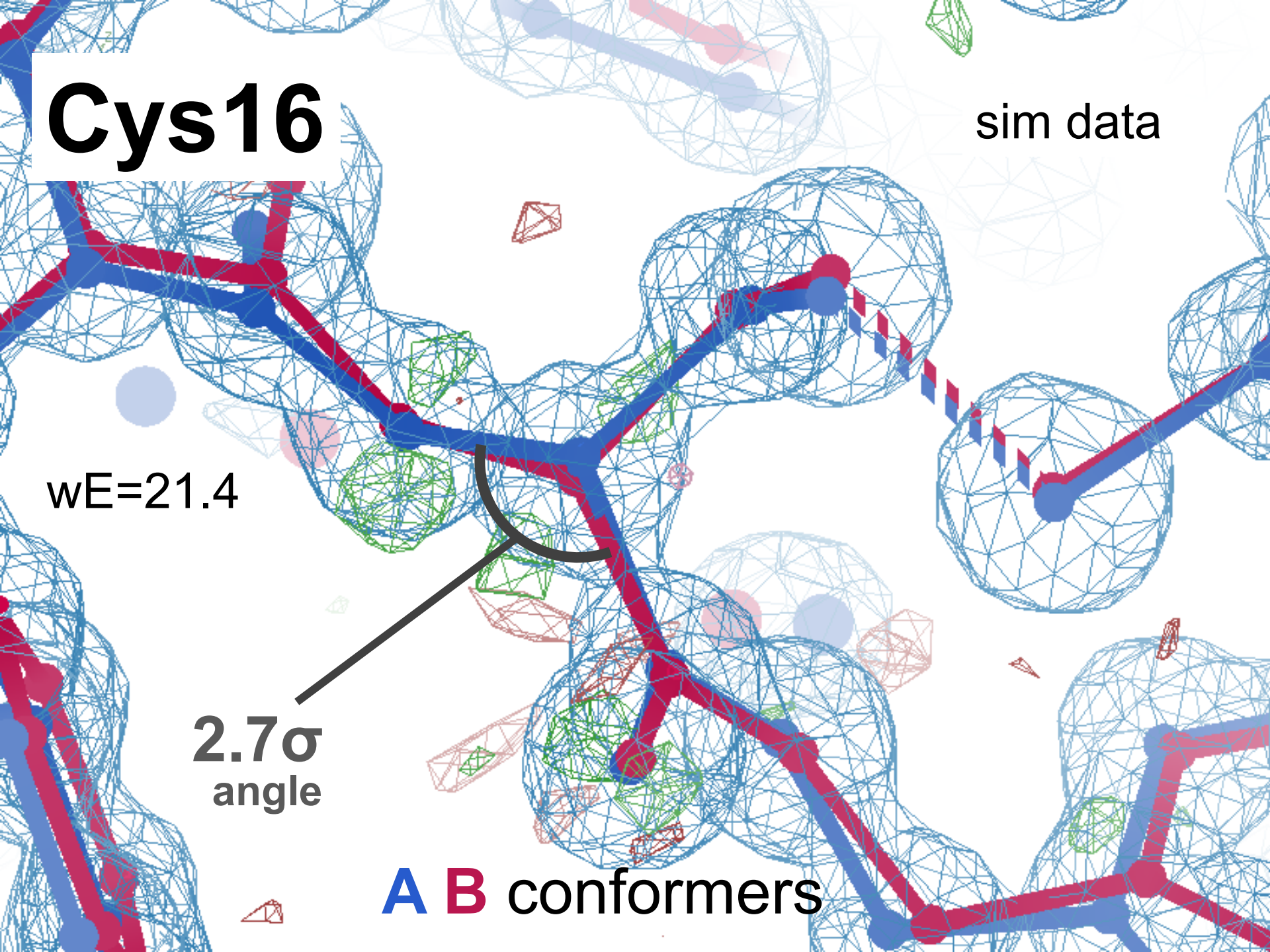
A B conformers

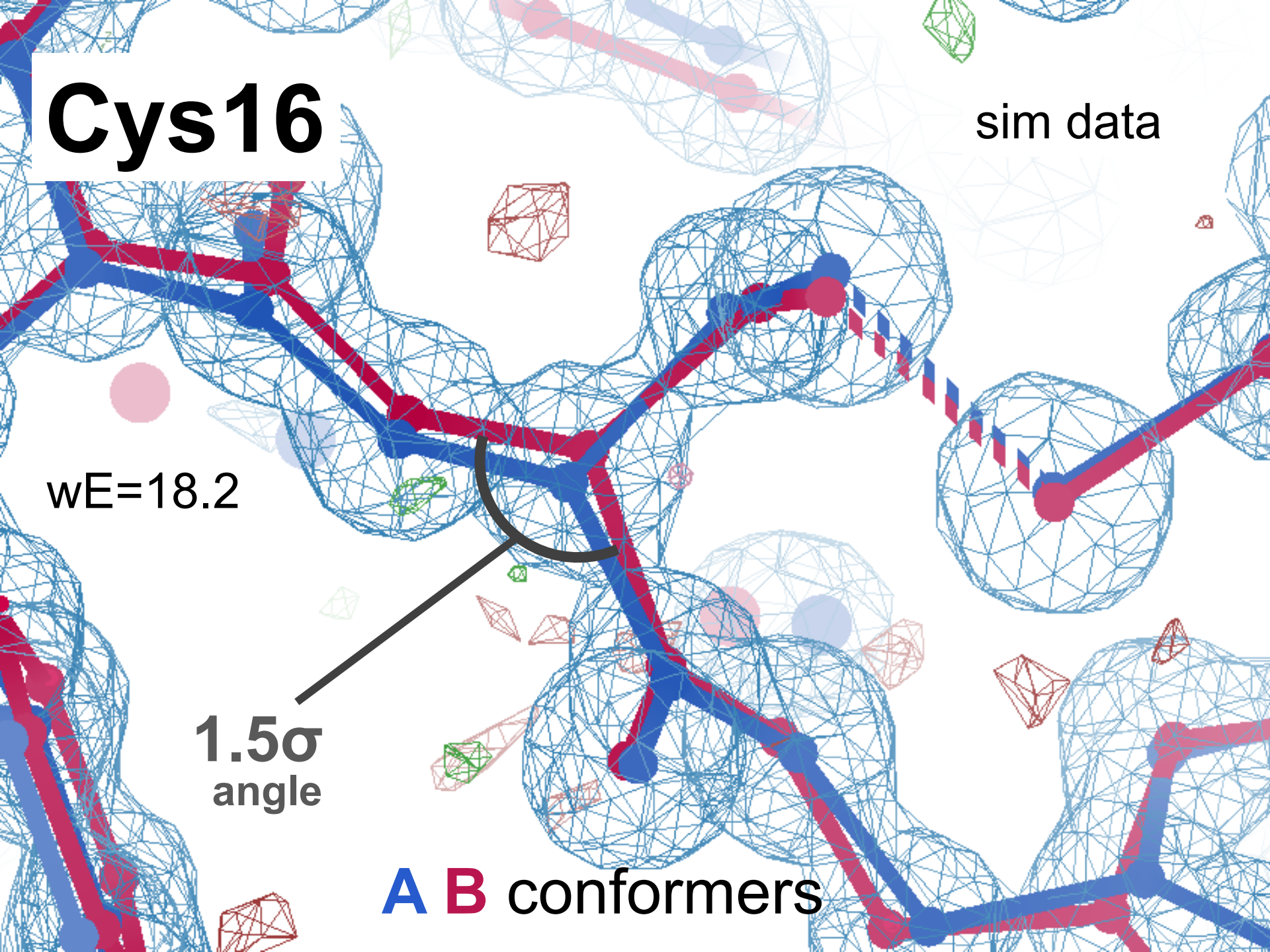
Tyr14

sim data



A B conformers


Cys16
sim data
wE=21.4
2.7σ
angle
A B conformers


Cys16
sim data
wE=18.2
1.5σ
angle
A B conformers

wE score: key concepts

- Energy $\overset{\text{sqrt()}}{\leftrightarrow}$ dev/sigma $\overset{\text{erf()}}{\leftrightarrow}$ Probability
- Probability outlier is not noise
  - "clip" outlier energy at "10"
  - Not so sharp transition to outlier
- Tighter Non-bond (Lennard-Jones)

Statistical Potential

$$E = \left(\frac{v - v_0}{\sigma}\right)^2$$

E	"energy"
v	model value
v_0	ideal value
σ	rms deviation expected

Outlier problem: $1 \text{ x } 6\sigma \leftarrow 40\text{x } 1\sigma$

solution: weight by
"probability its not noise"

Glu32

real data

Genuine outlier
in ground truth

-80°

Torsion outlier

New Tools!

Tom Terwilliger

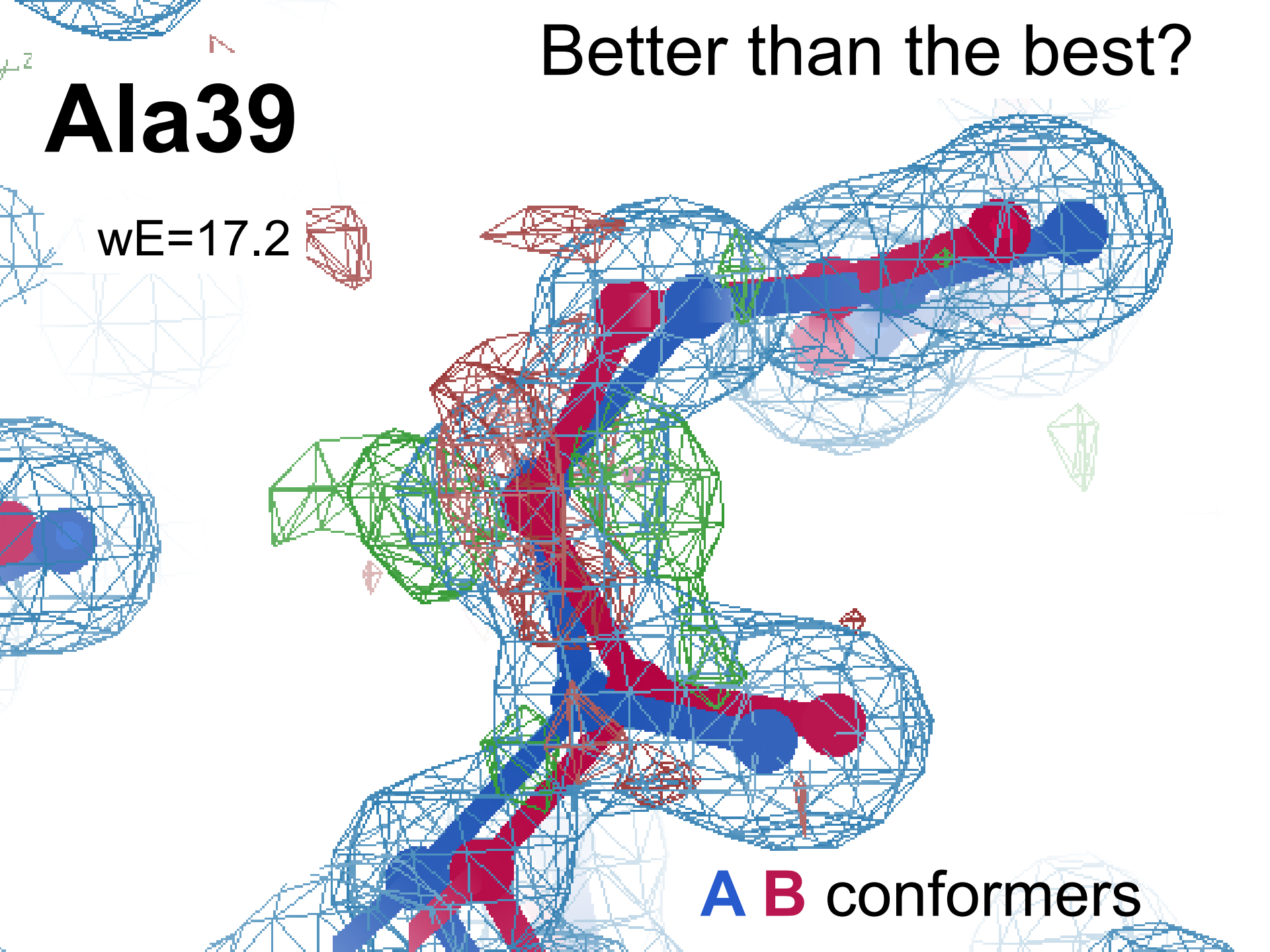
```
phenix.holton_geometry_validation test.pdb

phenix.create_alt_conf phenix_autobuild.pdb
refme.mtz \
nproc=48

phenix.refine \
model.pdb refme.mtz \
main.number_of_mac=10 \
fit_altlocs_method=masking \
ordered_solvent=true \
include_altlocs=true \
ordered_solvent.mode=every_macro_cycle_after_first \
refine_oat=true
```

Pavel Afonine

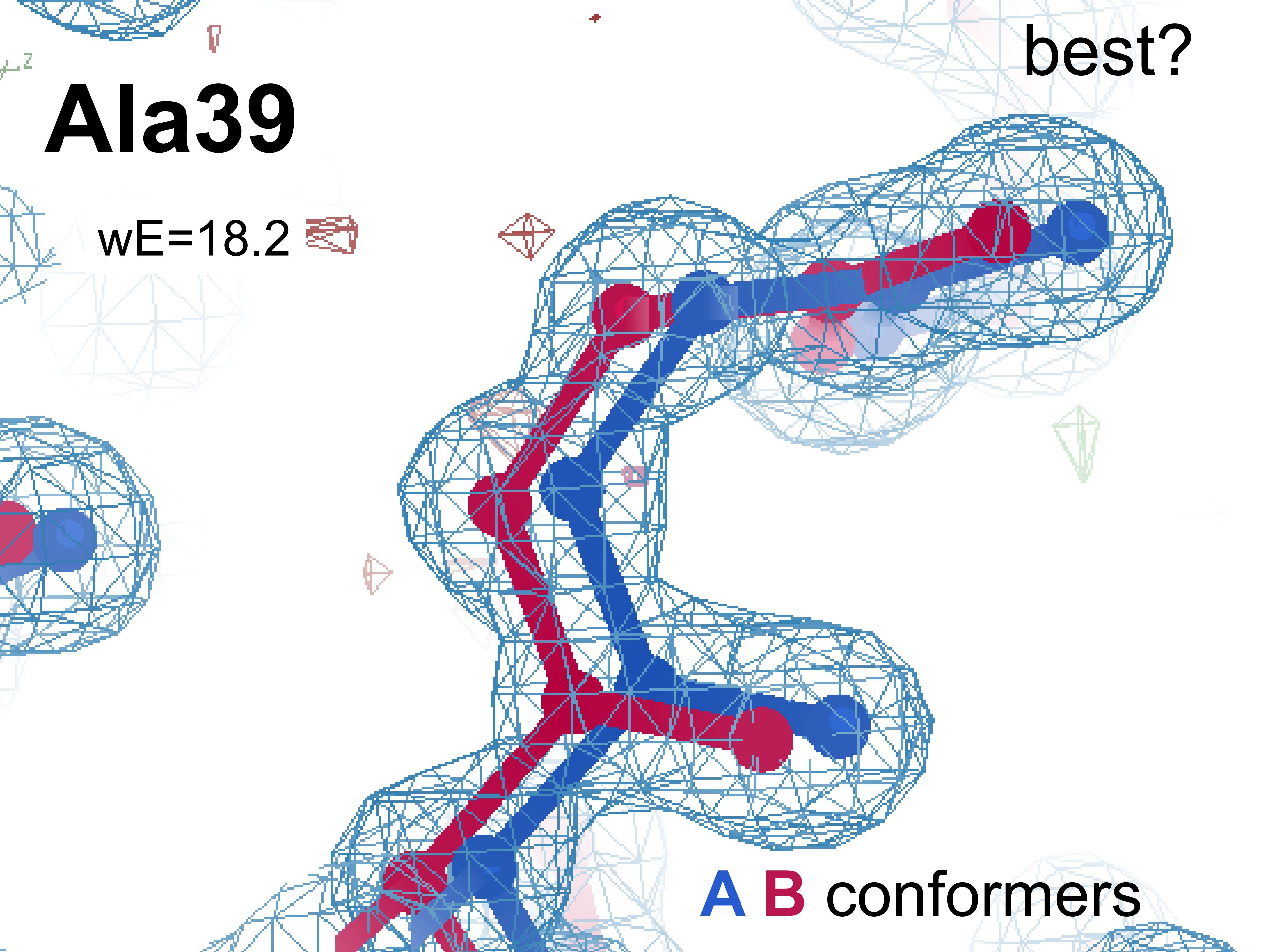
Ala39

wE=17.2

Better than the best?



A **B** conformers


best?
Ala39
wE=18.2
A B conformers

https://bl831.als.lbl.gov/~jamesh/powerpoint/CCP4_tangle_2025.pptx

Summary

- Rfree < 3% → single e^-
  - Not over-fitting
- Main barrier: tangled ensemble
- Sensible scoring function
  - Probability its not noise
  - some outliers are real
- Recover underlying ensemble
  - Might be required!
- Cooperative motions

verified Score sheet

wE	R_{work}	R_{free}	method
185	20.6	21.4	Alphafold2
109.6	8.87	9.44	Phenix.ensemble_refine
97.3	8.84	9.27	qFit best
91.7	3.64	4.27	manual
80.5	4.31	4.75	lotswrong
65.2	6.35	6.93	Amber24
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wE score: key concepts

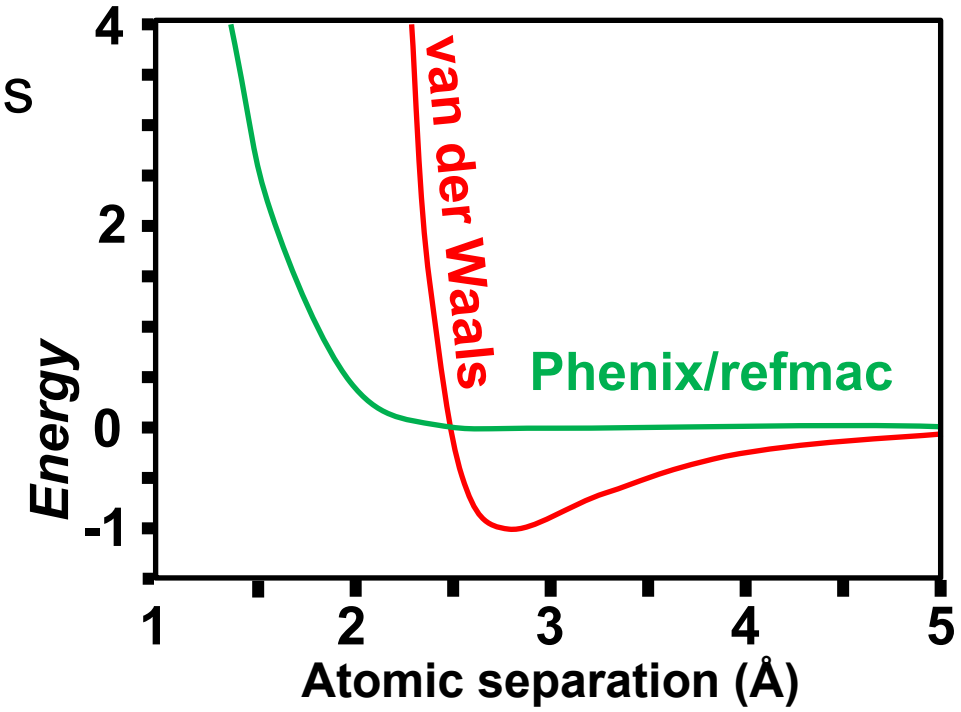
- Probability outlier is not noise
  - "clip" outlier energy at "10"
  - Not so sharp transition to outlier
- Tighter Non-bond (Lennard-Jones)

Sensible scoring function

- Statistical potential
  - bonds, angles, planes, torsions, chiral
- Lennard-Jones vdW
  - non-bond and clashes
- Probability -> σ dev
  - Rota, rama
- Peptide bond

  $\sigma \rightarrow 5°$
- $C_\beta$dev

  $\sigma = 0.05$ Å



Probability that it's not noise

$$P_{nn} = \mathrm{erf}\left(\left|\frac{v - v_0}{\sigma\sqrt{2}}\right|\right)^{N_{things}}$$

P_{nn} probability
v-v_0 deviation from ideal
σ rms deviation expected
erf() integral of a Gaussian
N_{things} number of trials

$\mathrm{erf}(1.0/\sqrt{2})$ = 68%

$\mathrm{erf}(1.0/\sqrt{2})^{10}$ = 2%

$\mathrm{erf}(2.0/\sqrt{2})$ = 95%

$\mathrm{erf}(2.0/\sqrt{2})^{10}$ = 63%

$\mathrm{erf}(4.0/\sqrt{2})^{10,000}$ = 53%

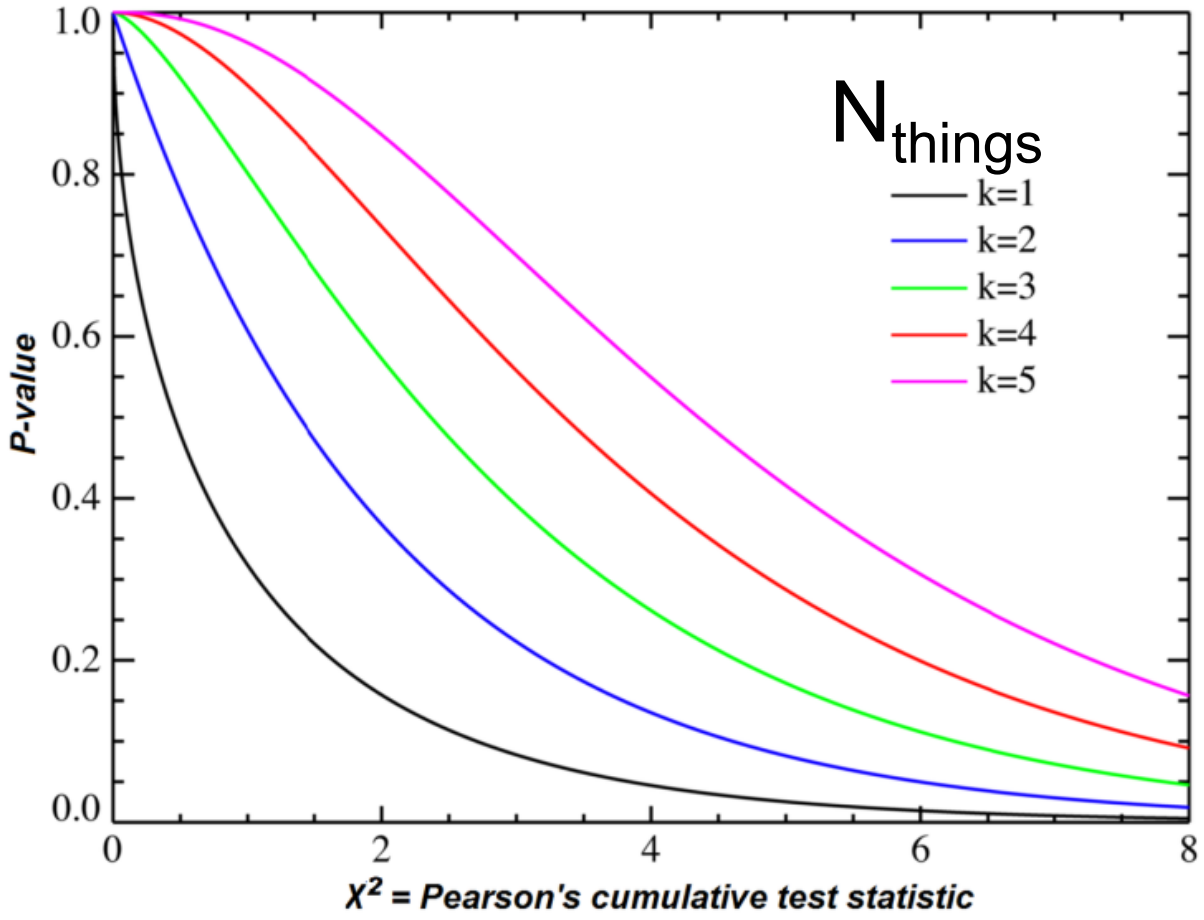
Probability that it's not noise

average/sum of N_{things}

χ^2 test

Probability
That its
Gaussian

tie breaker



“clip” for tolerating true outliers

$$\text{clip}(E) = \begin{cases} E, & if\ E < 10 \\ 10 + \log(E) - \log(10) \end{cases}$$

Statistical Energy

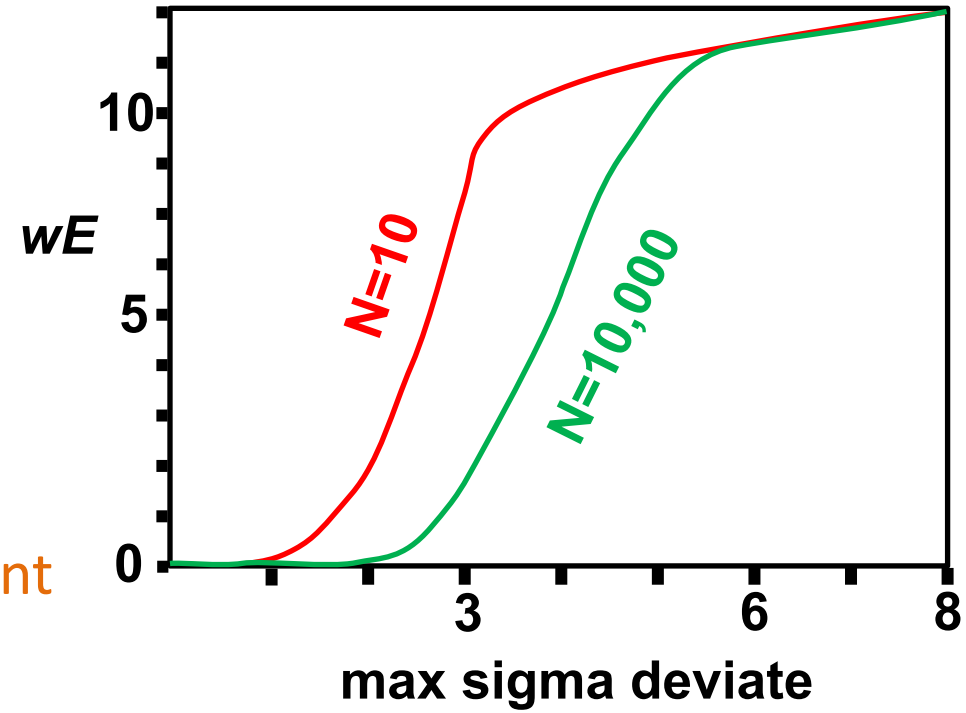
$$E = \left(\frac{v - v_0}{\sigma}\right)^2$$

Colloquially:

OK! OK! Its an outlier!

Let’s not agonize about it

10σ vs 10.1σ **not** more important than 3.3σ vs 3.0σ



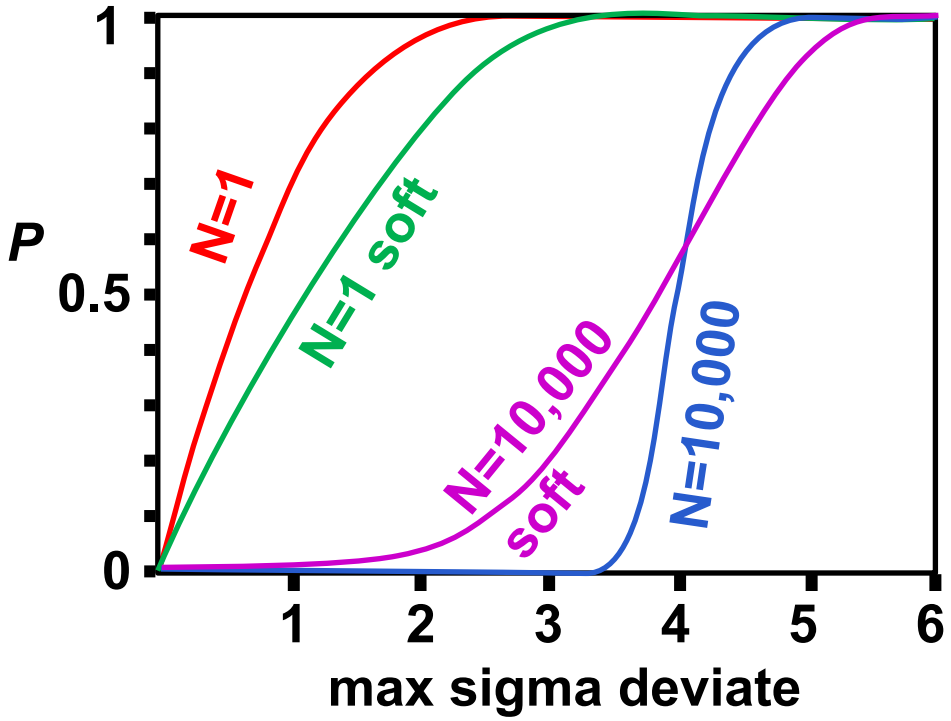
Probability that it's not noise

softer

$$P_{nn}^{soft} = 1 - 2^{\left(\left(\frac{|v - v_0|}{\sigma_{P50}}\right)^5\right)}$$

P_{nn} probability
v-v_0 deviation from ideal
σ rms deviation expected
inverf() inverse erf()
N_{things} number of trials

$$\sigma_{P50} = \sqrt{2}\,\mathrm{inverf}\left(0.5^{1/N_{things}}\right)$$



weighted Energy scoring function

$$wE = \sum_{\text{validation types}} w_{avg}\langle E\rangle + w_{max}\max\big(\text{clip}(E)\big)$$

phenix.holton_geometry_validation

$$w_{max} = \mathrm{P}_{\mathrm{nn}}\max\big(\text{clip}(E)\big)$$

$$w_{avg} = \chi^2\left(\sum_{\mathrm{N_{bonds}}} E\right)$$

$$E = \left(\frac{v - v_0}{\sigma}\right)^2$$

max() = worst outlier

$$\text{clip}(E) = \begin{cases} E, & if\ E < 10 \\ 10 + \log(E) - \log(10) \end{cases}$$