Structure Determination of Drugs from Powder Diffraction Data

pstephens@sunysb.edu

http://powder.physics.sunysb.edu

This is not intended as a scholarly review or history of developments of the field.

Pharmaceuticals – some of the most costly materials in the world. Can have value >> $1000 / gram.

Enormous intellectual property issues associated with the expenses of research and bringing a product to market.

Frequently rather small organic molecules, frequently crystalline solids, frequently powders.

“Who cares about the crystal structure? The drug is in solution when it acts.”

- Crystal structure is the most decisive measure of molecular structure. (Crystallographers – do you always get the sample that was advertised?)
- Physical properties of lifetime from manufacture to use. Can be many years in the warehouse, pharmacy, user’s medicine cabinet.
- Issues of bioavailability of the drug molecule once it is ingested (or injected, inhaled, applied as ointment, …)
- Issues of intellectual property (patent) protection.

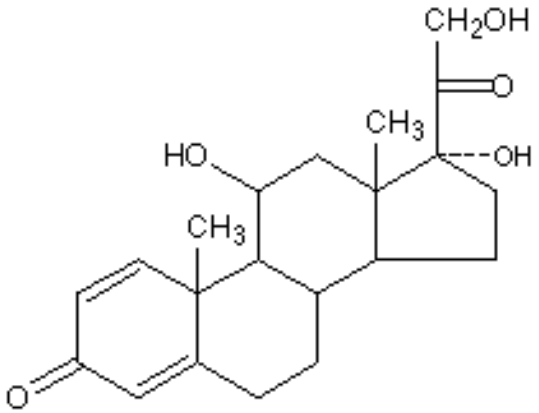
Structures of molecules:

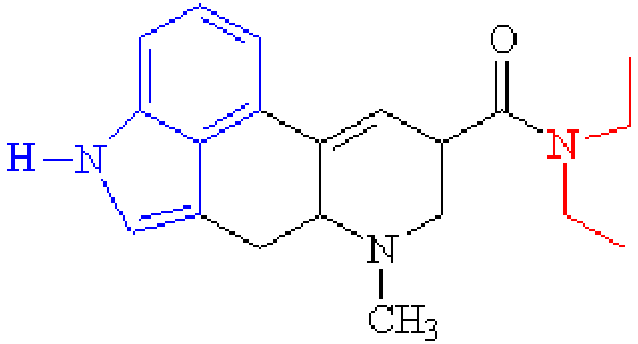
- Synthesis, design
- NMR
- IR, Raman
- Crystallographic techniques

Relevant to

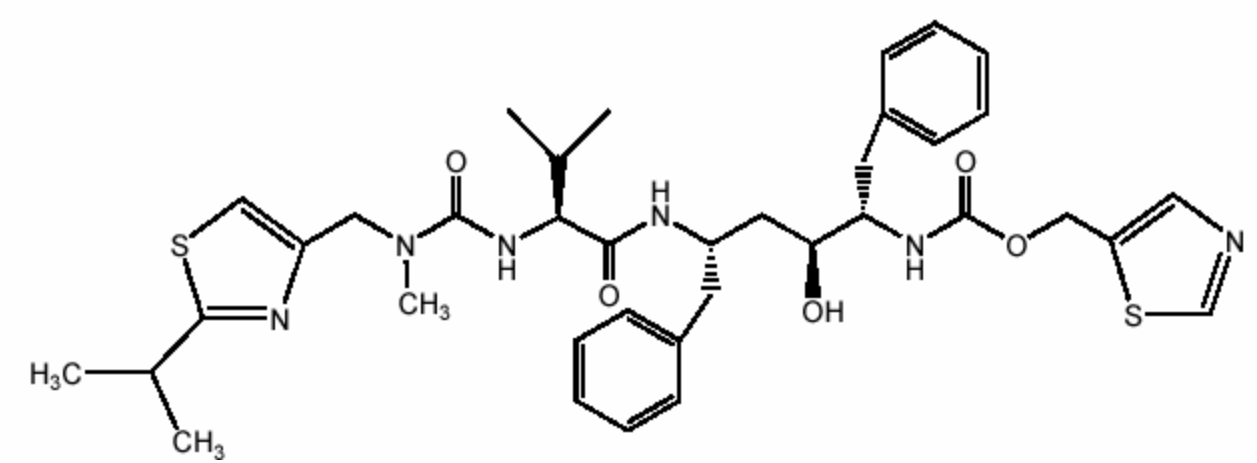
- Design of drug molecules, e.g., active sites in enzymes
- Understanding the physical chemistry of their storage, absorption into the body
- Protection of intellectual property

We are not at a state of knowledge where we can look at a molecule and judge whether it is a potent hallucinogen or if it is effective to treat inflammation.



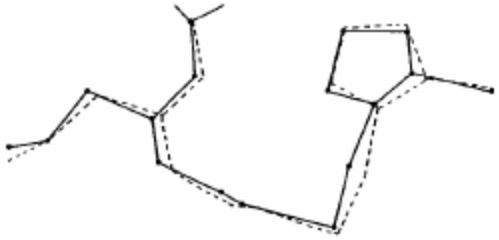


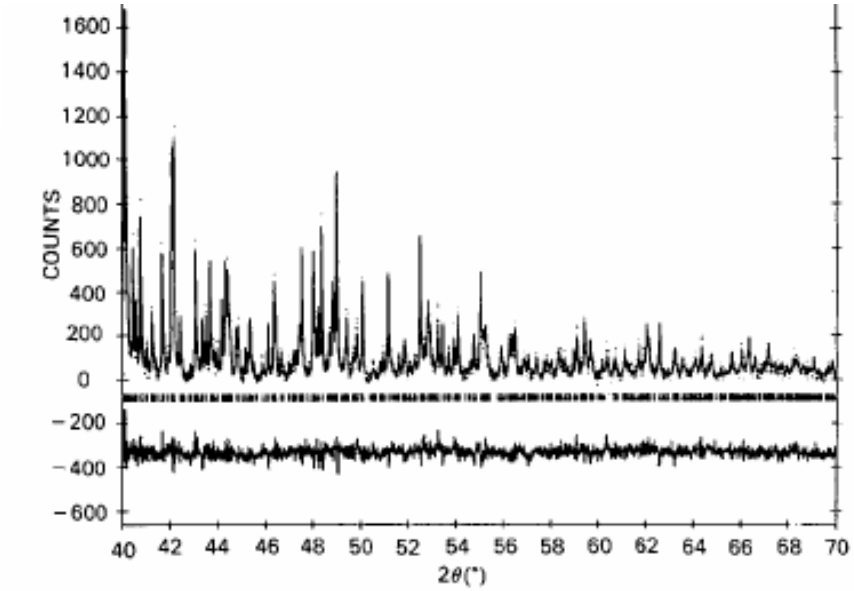
Nor are we in a state of knowledge that we can predict if a given molecule will crystallize into a form that is not biologically available, years after clinical trials are complete and it has been marketed in an amorphous form.

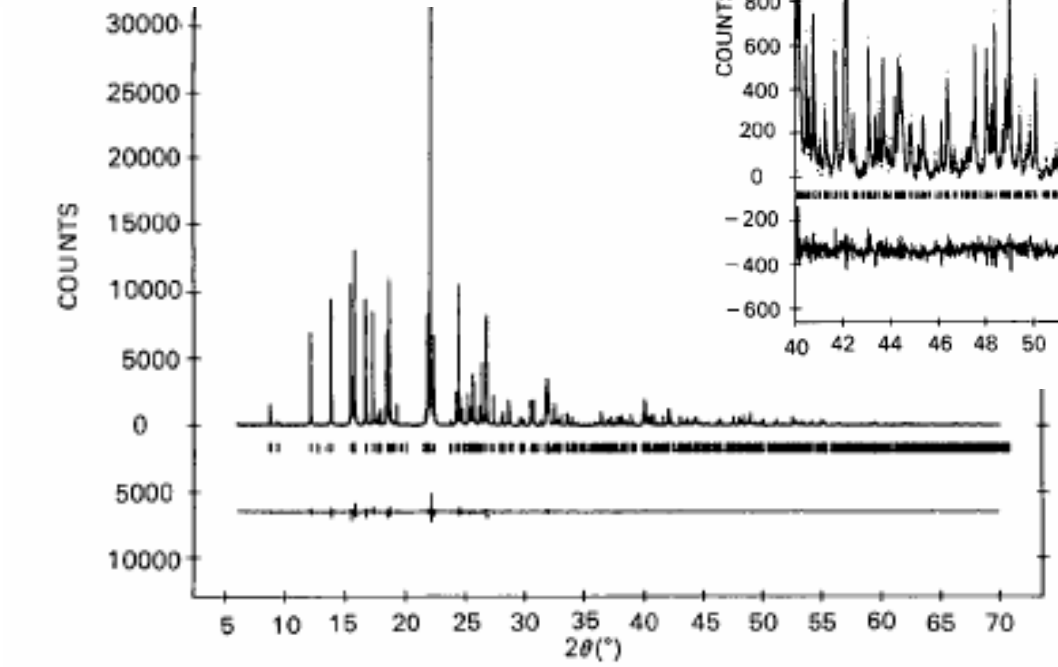


Cimetidine: histamine antagonist (stomach acid) (previously known structure)

Cernik *et al.* (1991) extracted intensities and used techniques familiar from single crystal analysis: direct methods (SIR – Burla *et al.* 1989) and repeated Fourier synthesis and Rietveld refinement.





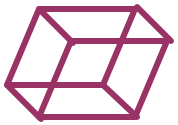


"A considerable amount of effort is necessary before this procedure can be regarded as routine."

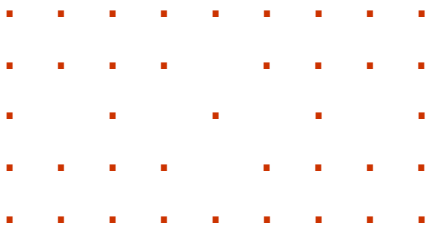
THEORY OF POWDER DIFFRACTION

(series of elementary recipes)

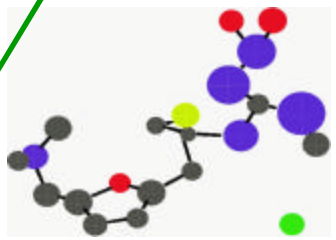
UNIT CELL



RECIPROCAL LATTICE,
SPACE GROUP



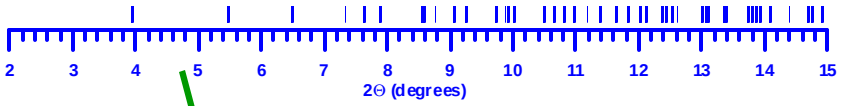
CONTENTS
OF UNIT CELL



INTENSITIES

$$I_{hkl} \sim \left| \sum f_j e^{2pi(hkl)\cdot(xyz)_j} \right|^2$$

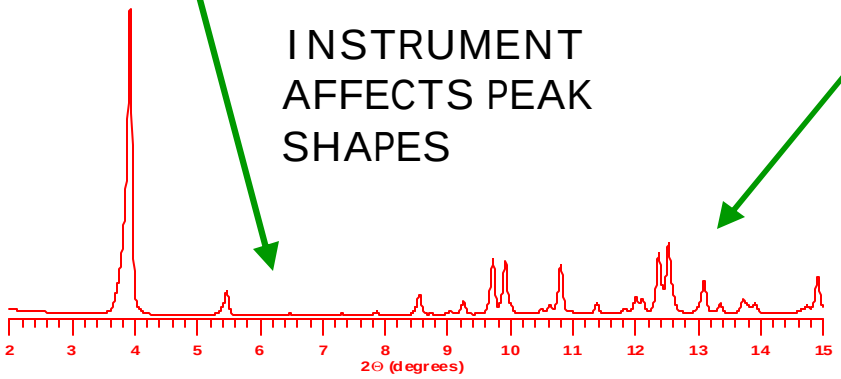
POWDER PEAK POSITIONS



PHYSICAL SAMPLE
AFFECTS LINESHAPES

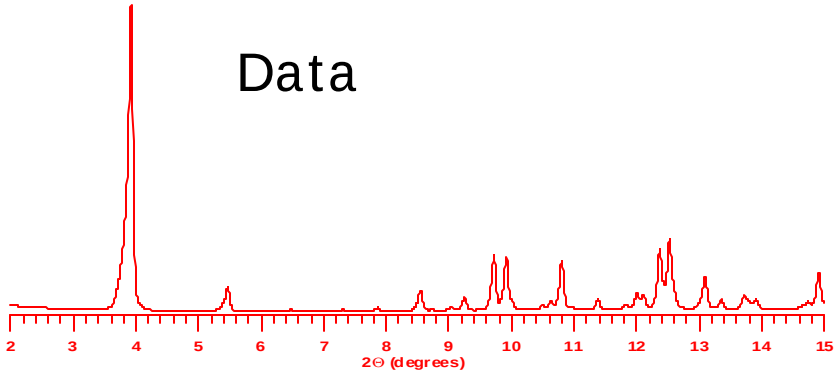
INSTRUMENT
AFFECTS PEAK
SHAPES

OTHER ARTIFACTS:
PREFERRED ORIENTATION, ETC.

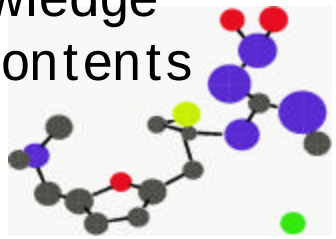


POWDER DIFFRACTION PATTERN

USE OF POWDER DIFFRACITON TO SOLVE A CRYSTAL STRUCTURE



Chemical knowledge of contents

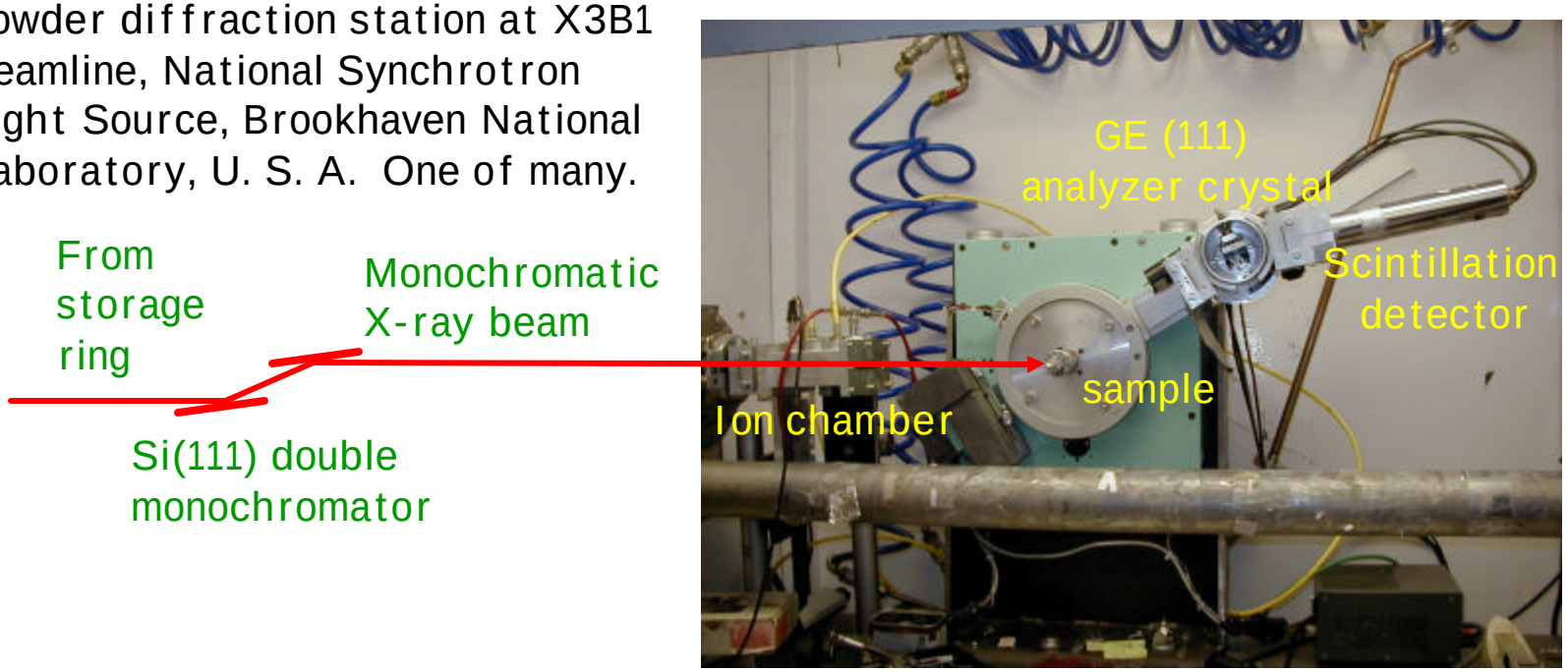


1. Start with the best data you can get (but no better).
2. Get a list of accurate diffraction peak positions.
3. Figure out a lattice that explains the peaks.
4. Guess the space group (systematic absences, # molecules).
5. Search for the best place to put the molecule(s), best conformation of the molecule.
6. Refine, refine, refine, refine, refine, …

At any stage, you can be forced to jump back to any stage.

#1, 2 This is a data-driven enterprise. Students may think that we spend all our time talking about algorithms, software, etc., but the results are no better than the data!

Powder diffraction station at X3B1 beamline, National Synchrotron Light Source, Brookhaven National Laboratory, U. S. A. One of many.



- Analyzer crystal geometry measures angles – eliminates significant aberrations of familiar Bragg-Brentano diffractometer.
- Capillary sample geometry is very helpful. Eliminates preferred orientation, peak shifts that bother flat plate

#3. Indexing

Given some values of *d* spacings, find a lattice that fits them, i.e., find {*A,B,C,D,E,F* } such that every *d* can be expressed as

$$1/d^2 = Ah^2 + Bk^2 + Cl^2 + Dkl + Ehl + Fhk$$

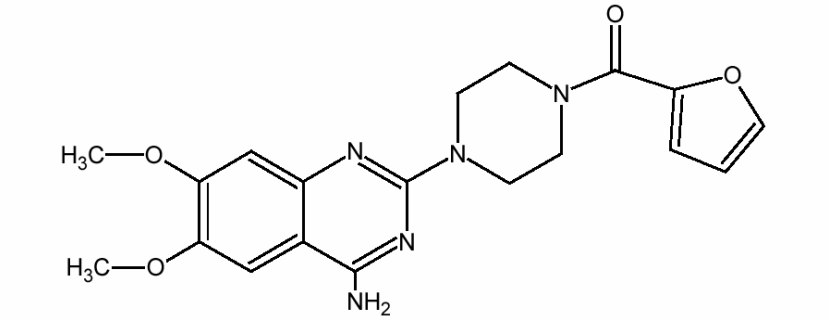
for some integers *h, k, l*.

Complicated by data of imperfect accuracy, spurious peaks from impurities.

Familiar programs, in the public domain:
TREOR, ITO, DICVOL, have their quirks, but basically they always work, given sufficiently good data. (Often possible with good lab diffractometers, nearly always with synchrotron data.) (Don't report powder data unless you can index test cases such as acetaminophen, ibuprofen.)

TOPAS (Alan Coehlo, Bruker AXS) has indexing tools that are qualitatively more powerful (in my humble opinion).

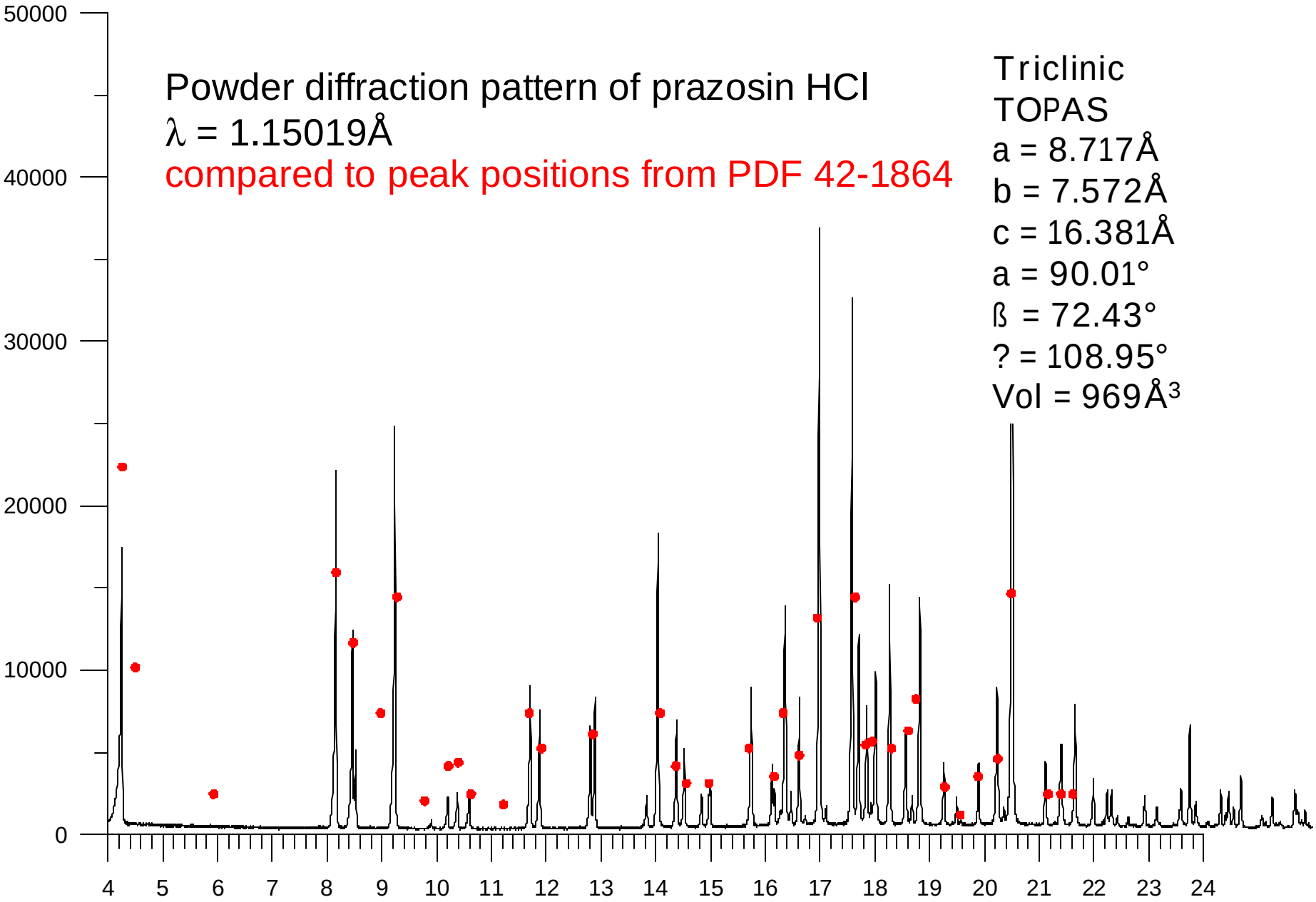
Prazosin



Designer drug – selective antagonist for a_1-adrenoceptors (blood pressure).

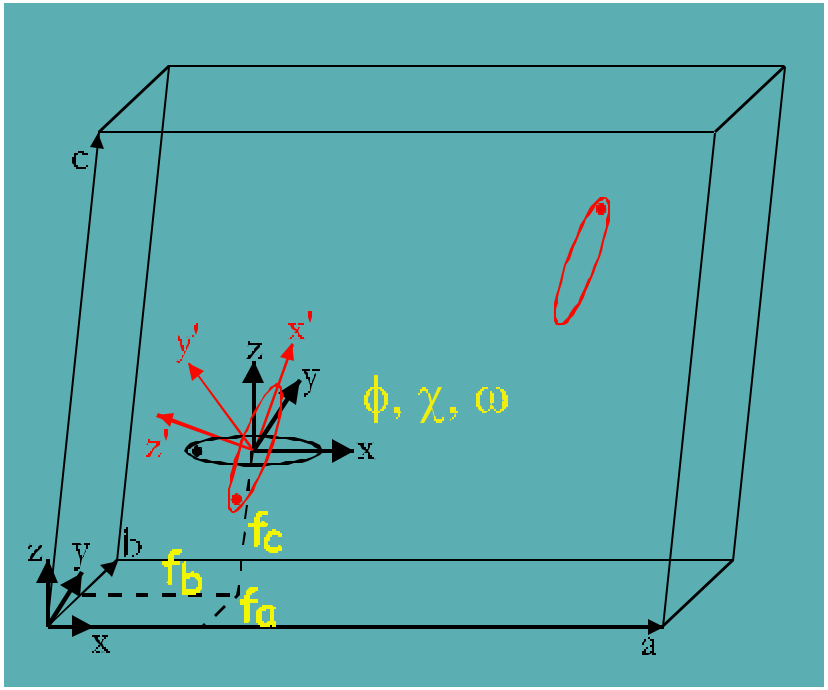
Four other polymorphs claimed in US Patents 4092315, 4739055, 4816455, and JP Patent 03206088. Department of Medicinal Chemistry, State Scientific Center of Antibiotics, Moscow, could not reproduce any of them.

Patent literature : Literature
Military intelligence : Intelligence


Powder diffraction pattern of prazosin HCl
λ = 1.15019Å
compared to peak positions from PDF 42-1864
Triclinic
TOPAS
a = 8.717Å
b = 7.572Å
c = 16.381Å
a = 90.01°
ß = 72.43°
? = 108.95°
Vol = 969Å3
50000
40000
30000
20000
10000
0
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

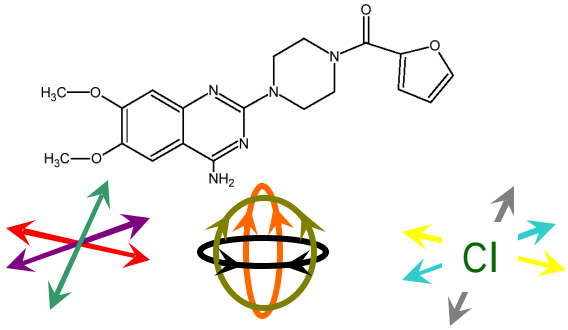
#5. Direct space methods.

Make a model of the molecule, put it into the lattice.
Move the model around seeking best agreement between calculated and observed diffraction patterns.



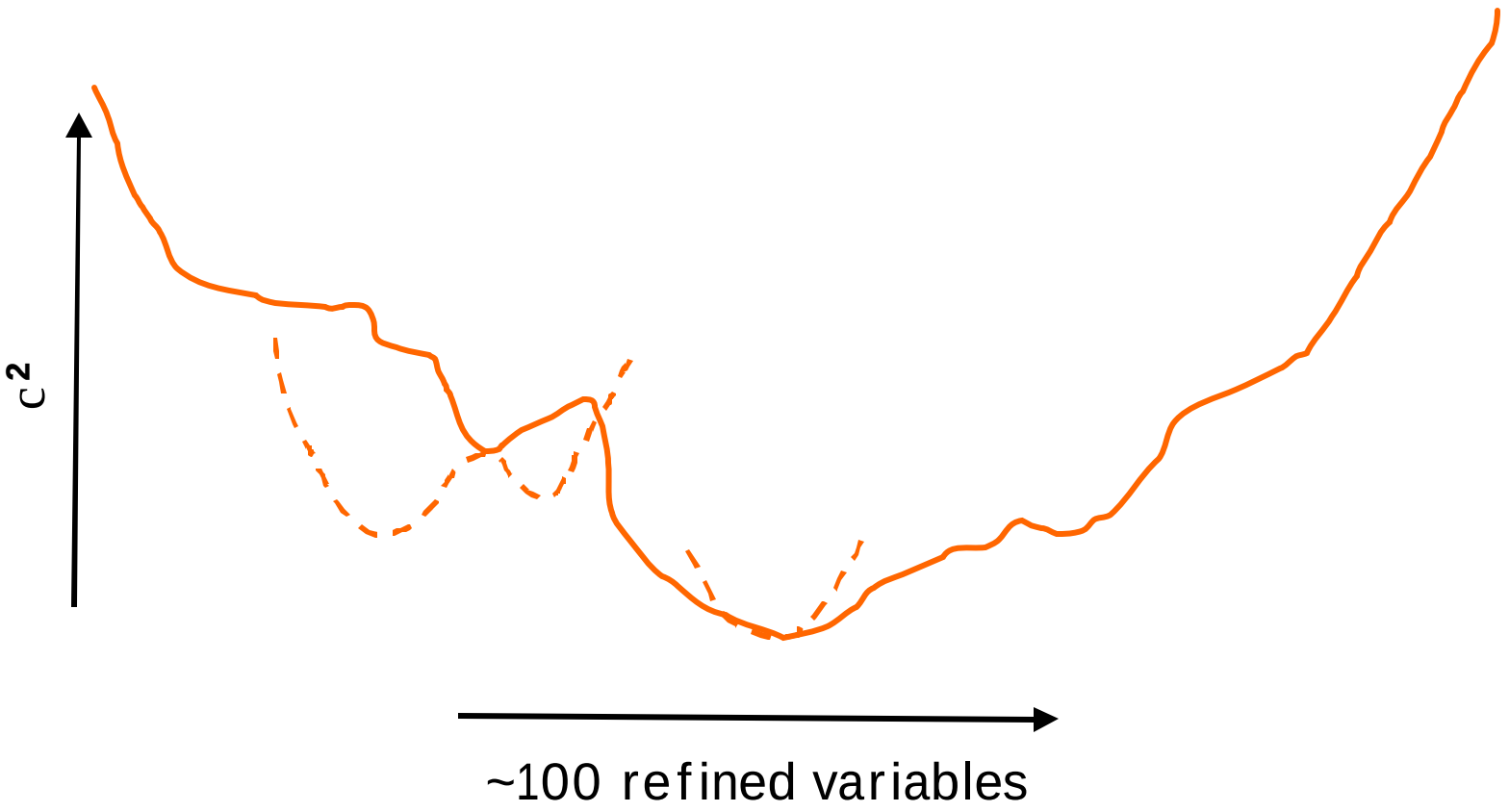
Lots of options: software DASH, PSSP, FOX, TOPAS, PowderSolve, Organa, …

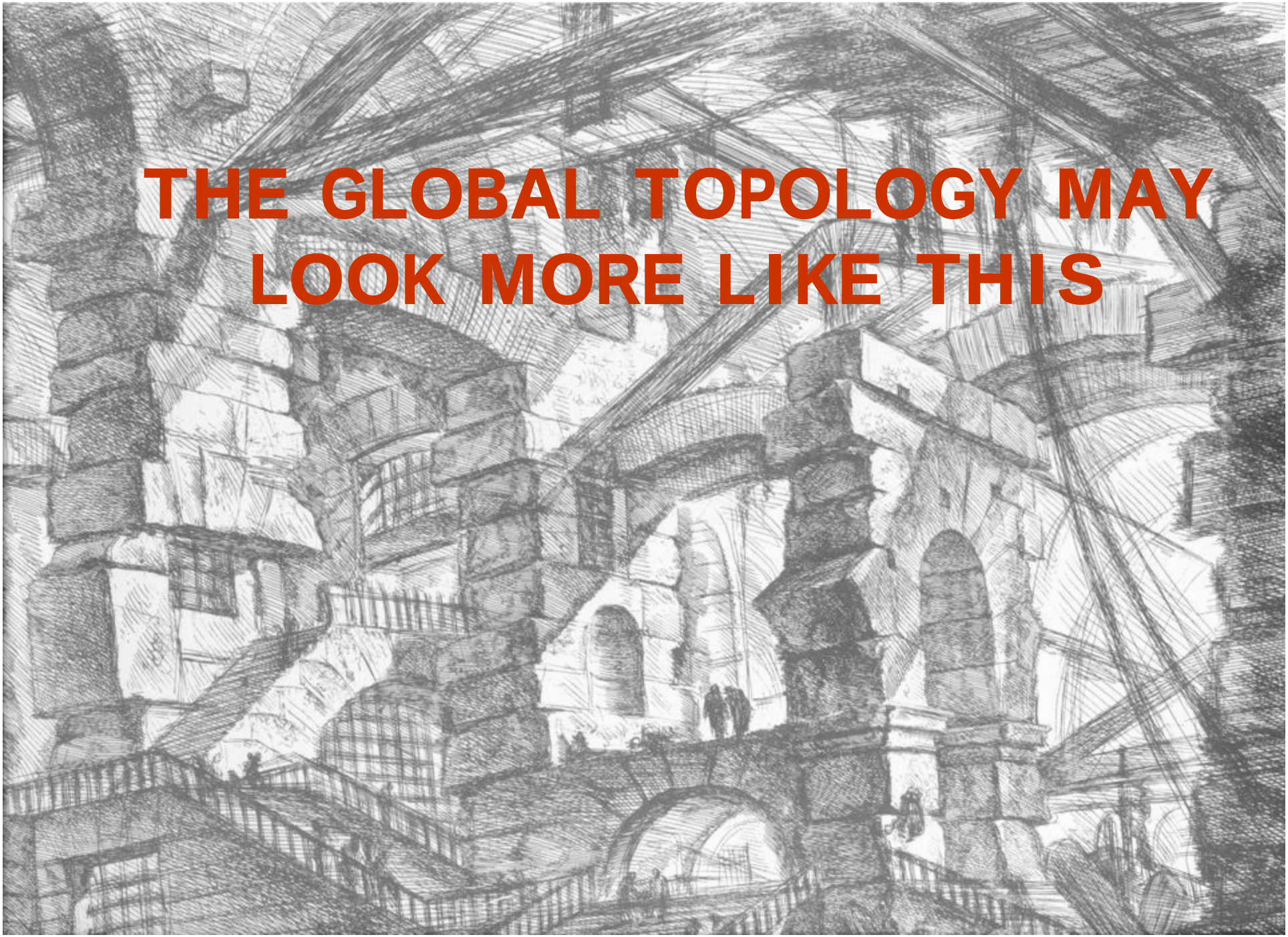
In this case, assumed $P\bar{1}$, Chernyshev searched nine parameters with software developed with H. Schenk.

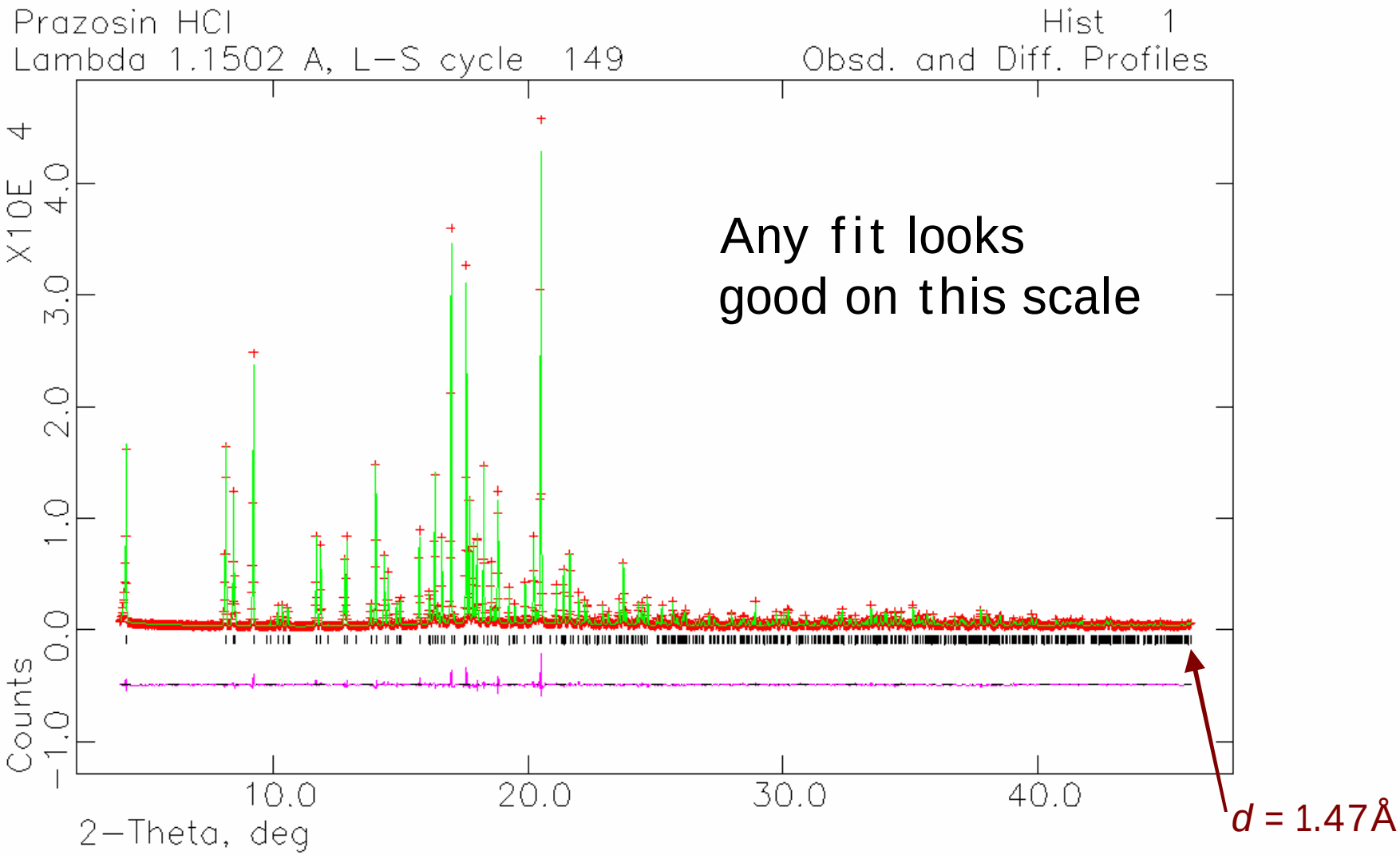


#6. Refine, refine, …

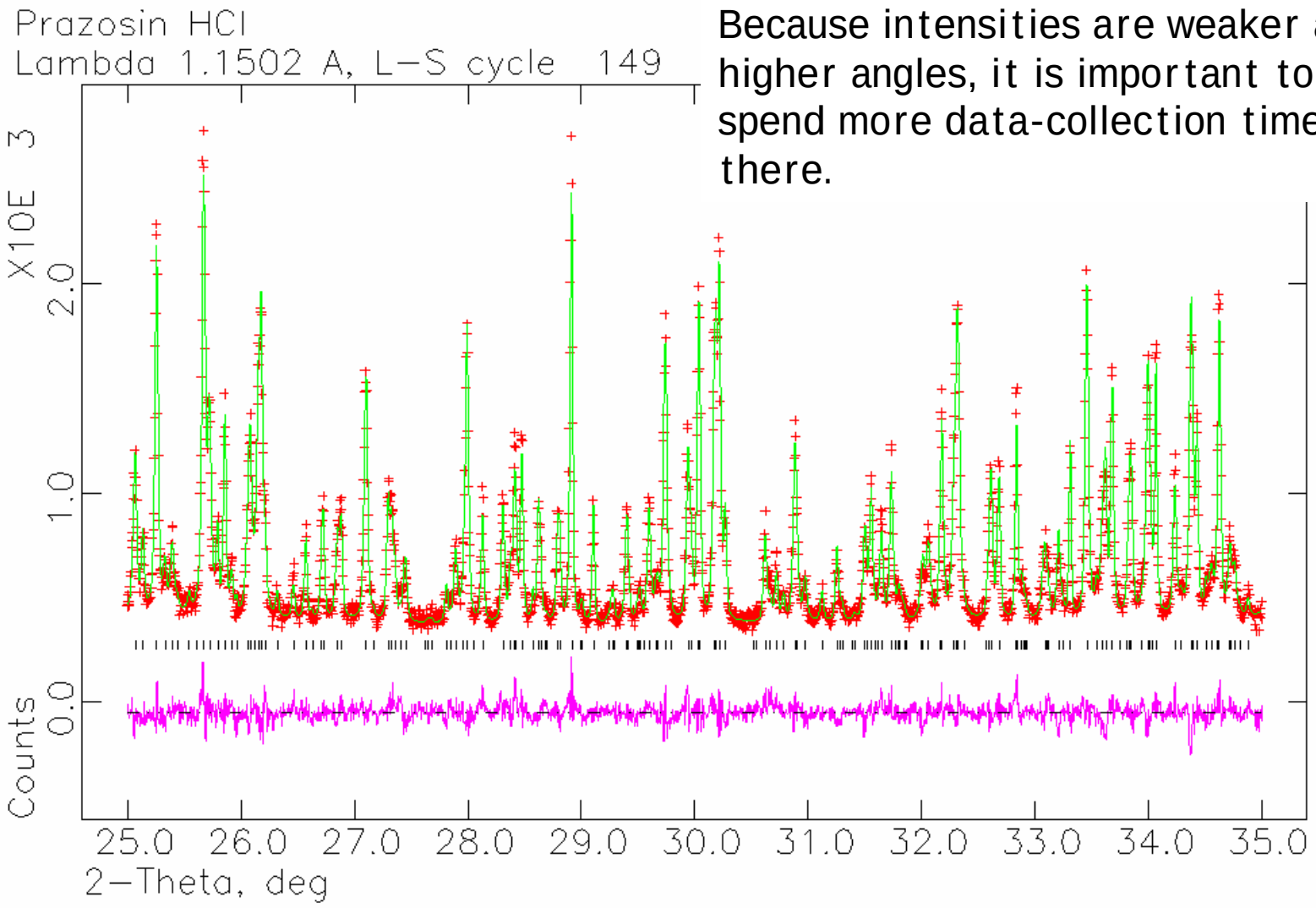
If your presumed rough solution is close enough, you can roll down hill to the correct solution, using refinement programs such as GSAS, TOPAS, FULLPROF, …




THE GLOBAL TOPOLOGY MAY
LOOK MORE LIKE THIS

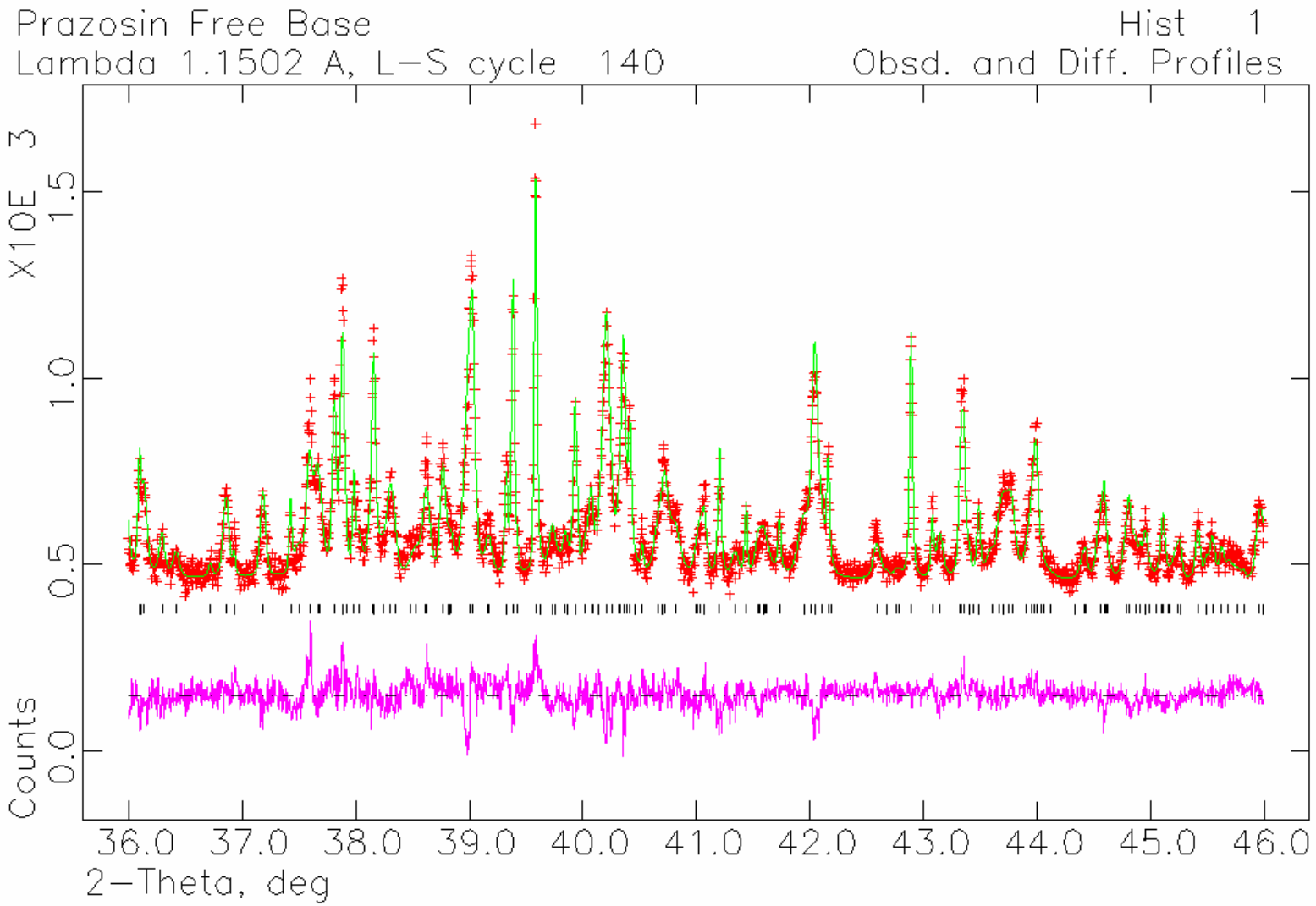


$?^2$ = 2.31, Rwp=5.92%. No restraints except for tethering all H atoms.



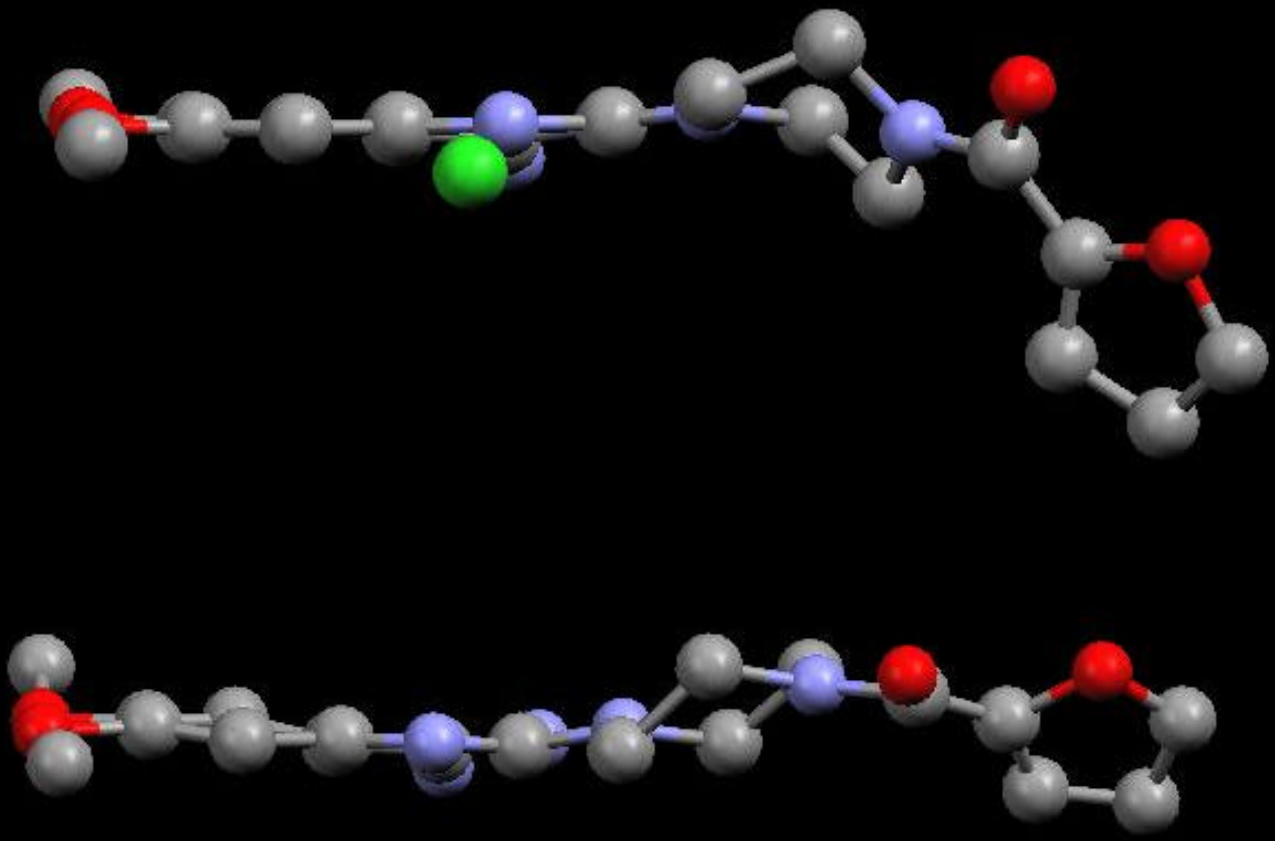
Because intensities are weaker at higher angles, it is important to spend more data-collection time there.

Same steps for prazosin free base – only 6 search coordinates



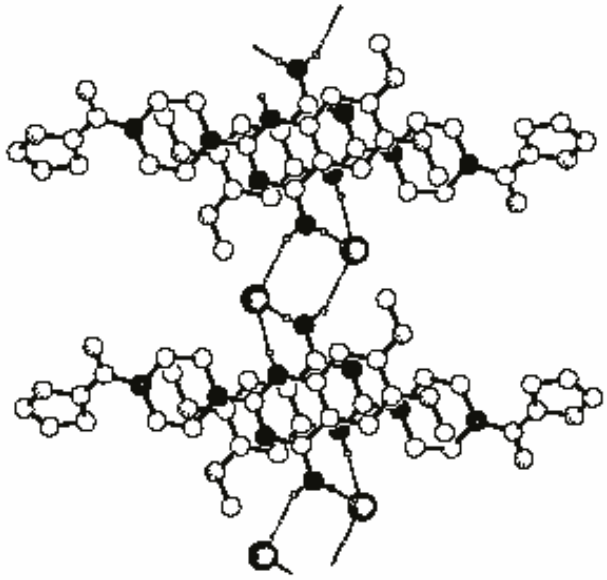
Monoclinic, *Cc*, $?^2$ = 2.78, R_{wp}=5.92%

Molecular structure comes from Rietveld refinement
all non-H atoms refined – no restraints.

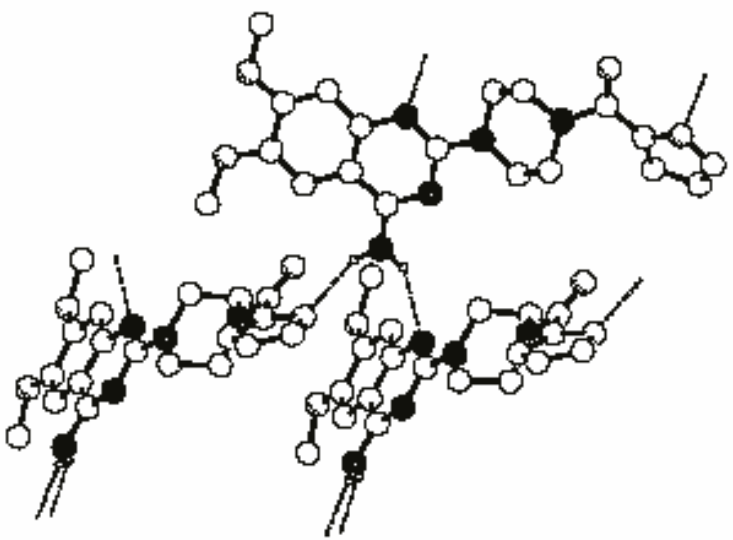


Planarity of the aromatic rings gives a measure of the degree of accuracy of the finished atomic geometry.

Hydrochloride
3 N-H…Cl

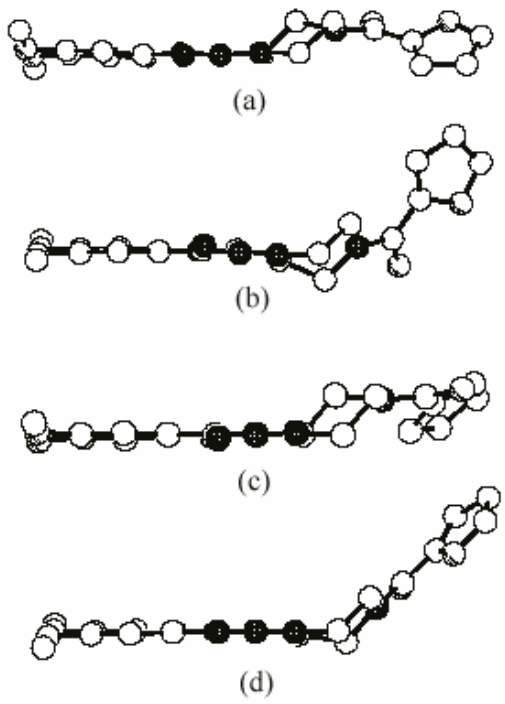


Free base
1 N-H…N 3.02Å
1 N-H…O 3.02Å



Prazosin conclusions:

- That wasn't so hard
- Of relevance to quantitative modeling of structure-activity relationships



Hydrochloride

Free base

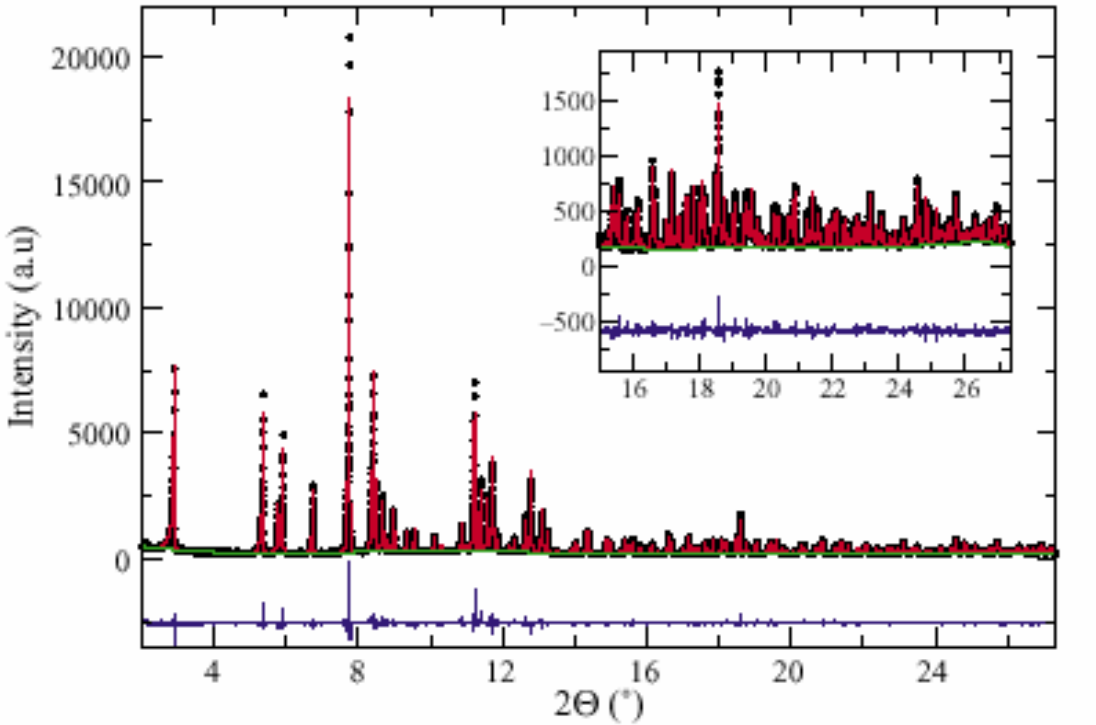
Hydrochloride methanol solvate (single xtl)

Prazosin$_2$ tetrachloro-copper(II) (single xtl)

Turkey blackhead disease – serious protozoal infection.

EC considering whether it should allow the use of Nitarsone – $C_6H_4NO_2AsO(OH)_2$ as an antibiotic feed additive.

Concerned with potential conversion to inorganic As^{V} or As^{III}. Safety margin is asserted (from old, incomplete data) but questioned.



Mail-in data collected at NSLS.

A. van der Lee, P. Richez, and C. Tapiero, *J. Molec. Struct.* (2005)

The EFSA Journal (2004) 121, 1-13

Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission related to the preliminary assessment of the safety of Nitarsone (4-nitrophenylarsonic acid), as a feed additive in accordance with Regulation (EC) N° 178/2002 and Regulation (EC) N° 1831/2003, article 15.

(Question N° EFSA-Q-2004-014)

Adopted on 28 October 2004

SUMMARY

Nitarsone (4-nitrophenylarsonic acid 4-NPA) is used to control blackhead disease, a debilitating protozoal infection in turkeys. The additive is due for evaluation to comply for a provisional authorisation for a maximum period of five years. The European Commission asked the European Food Safety Authority to make a preliminary evaluation of the safety of 4-nitrophenylarsonic acid, and its metabolites when it is used as feed additive for animal nutrition. The additive is not yet in use in the European Union.

Nitarsone (a chemically synthesized organoarsenical) at a dose level of 187.5 mg of 4-NPA kg^{-1} of feed is recommended to be administered to turkeys from 2 weeks until 12 weeks of age.

The very limited data derived from efficacy studies indicate a possible margin of safety to be four times the recommended dose (750/187.5 mg 4-NPA kg^{-1}). However, the FEEDAP Panel expressed their concern to set a preliminary margin of safety value because the studies are more than 40 years old, the number of animals per group is low, the treated animals are all infected, the duration of the experiments are short and the effects of 4-NPA to gut flora are not explored.

No data are available concerning the metabolism of 4-NPA in turkeys. The metabolic pathways of 4-NPA in the laying hen indicate that the arsanilic acid is the major metabolite excreted with no release of inorganic arsenic. However, the methods used to investigate 4-NPA metabolism are questionable in terms of specificity and sensitivity. No data are given concerning the nature of tissue residues. No data have been supplied on the metabolism of 4-NPA in laboratory animals.

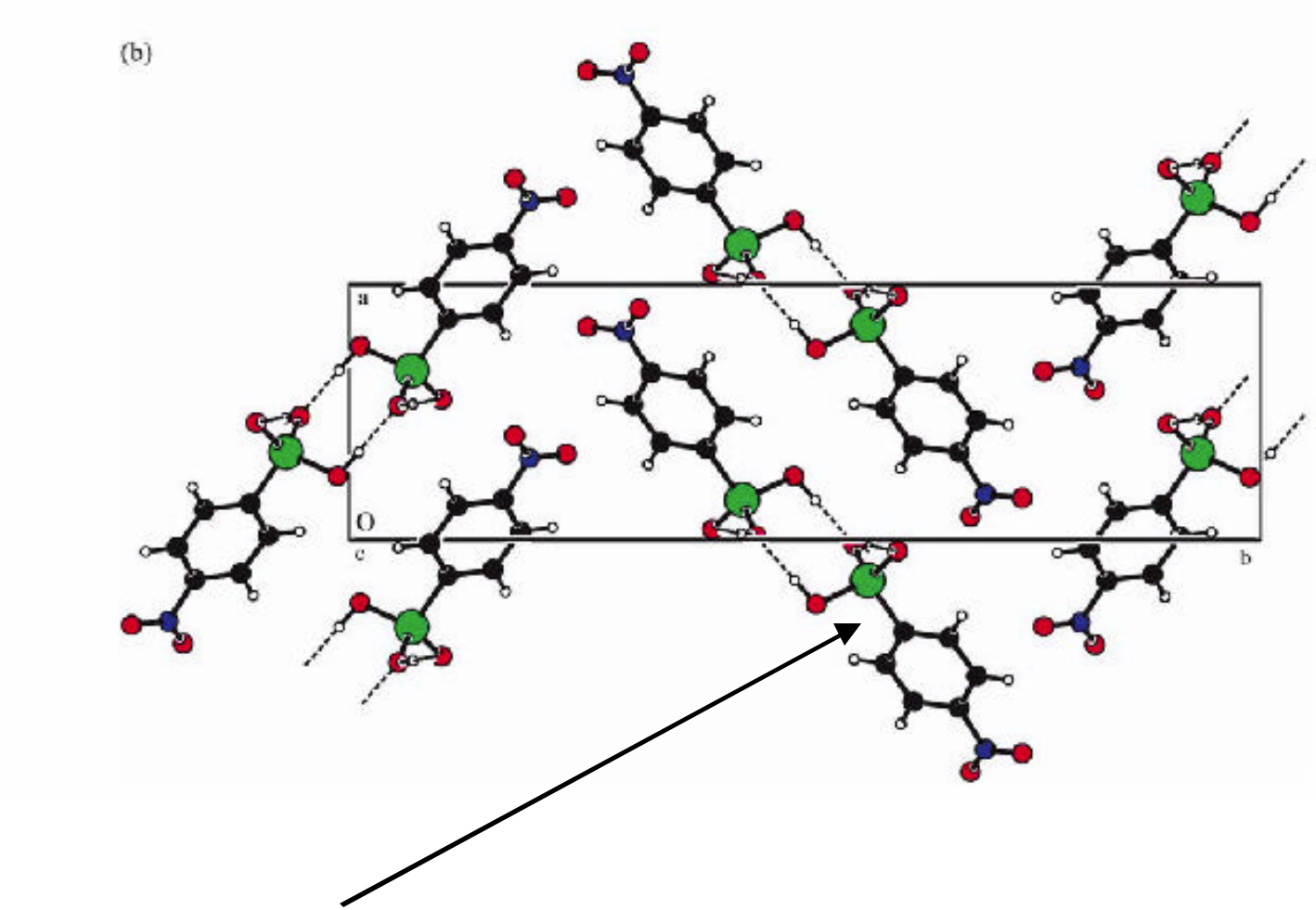
Residue studies of total arsenic in turkey tissues indicate that arsenic concentrations increase following exposure of the animals to 4-NPA but decline to control levels after a 9-day withdrawal period. However, no data are given concerning the kinetics of 4-NPA residues in turkey tissues.

The limited data available give no indication of genotoxicity or carcinogenicity but no data at all are available on developmental and reproductive effects. A NOEL can not be firmly established but it would be approximately 2 mg 4-NPA kg^{-1} bw day^{-1}, based on the chronic toxicity study in rats. However, there is evidence that dogs are maybe more sensitive to 4-NPA and a properly conducted study in dogs may give a lower NOEL. Additional studies would be needed in order to further refine the assessment of the consumer safety.

Since a NOEL cannot be confidently established from the available toxicity data an acceptable daily intake (ADI) value cannot be determined. The data available does not allow the FEEDAP Panel to propose maximum residue limit (MRL) values.

No validated control methods are supplied for 4-NPA in premixes and feedstuffs. Validated control methods would be required for any marker residue when this can be established.

Previous efficacy and safety studies are 40 yrs old, subject to substantial criticism.



C-As distance of 1.86 Å shows partial double bond character, clarifying why the molecule does not degrade to release free As *in vivo*.

Zopiclone (Zimovane® hypnotic)

(N. Shankland *et al.*, 2001)

Manufacturer had substantial batch-to-batch variation in physical form in commercial manufacture.

Monoclinic dihydrate – racemic (I)

Monoclinic anhydrous – racemic (II)

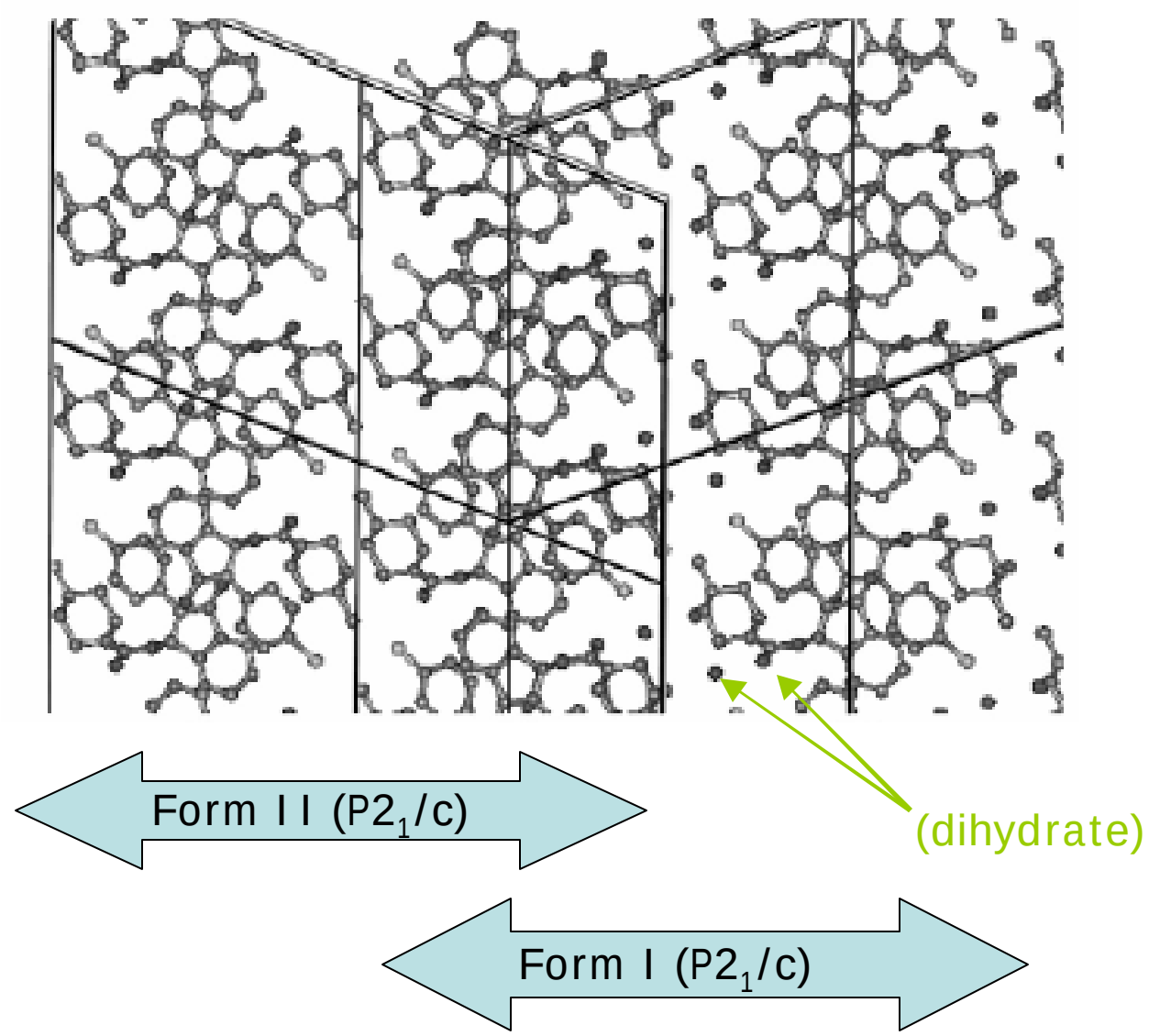
Reversible

Irreversible

Orthorhombic anhydrous (resolved) (III)

New anhydrous monoclinic form (II) provides a kinetic pathway to anhydrate. Processed anhydrate may consist of either (II) or (III).

Reversible transformation between anhydrate and dihydrate.



Polymorphism – multiple crystal structures for the same chemical entity. Metastable or truly concomitant.

A crystal structure can be patented as long as it is not obvious, not in prior art.

Patented polymorphs have been at the focus of several lawsuits pertaining to generic drug companies gaining access to markets > $1G/yr. (Ranitidine - Zantac®, Paroxetine - Paxil®, …)

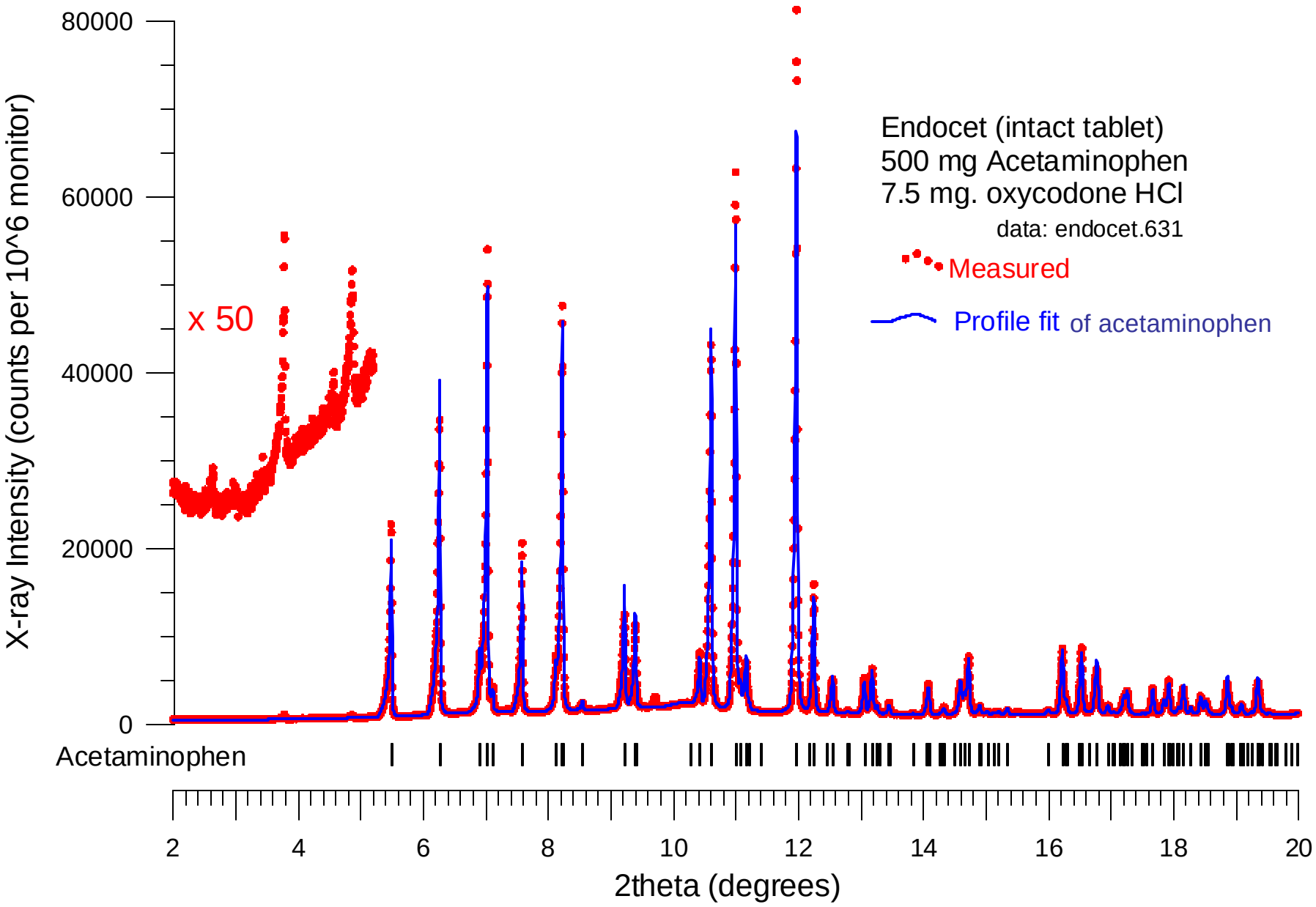
The most direct probe is diffraction (structure). In many polymorphic systems, single crystals are not available of all of the forms.

- Brief diversion – use of powder diffraction to detect polymorphs
- Study (in progress) of a polymorphic system in which different polymorphs have very different bio-availabilities.

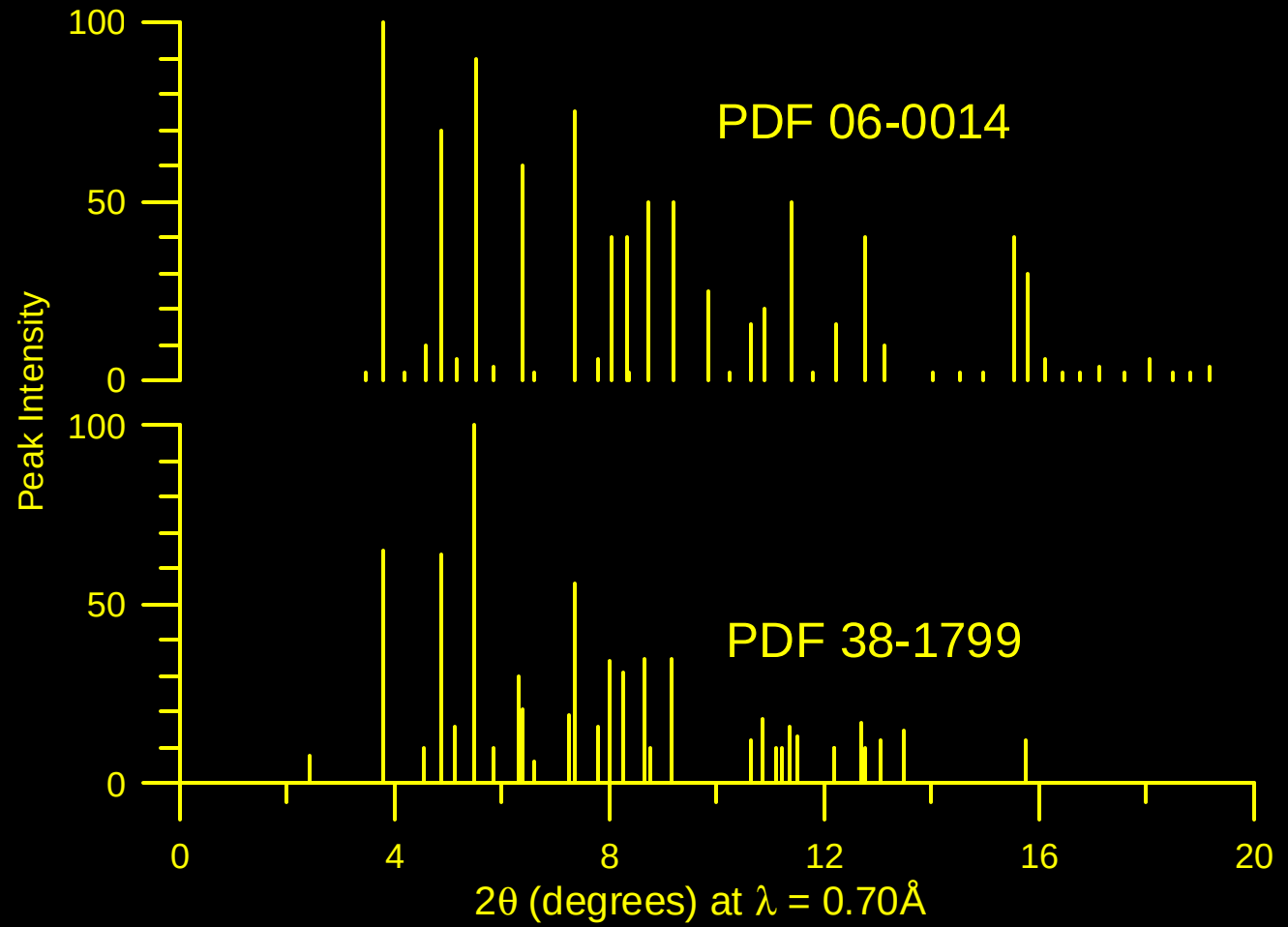
Real business problem:
_____ has a patented polymorph of _____ , and suspects that _____ is selling material that infringes. It is desired to examine the commercial tablets and determine the polymorph of the API for potential litigation.

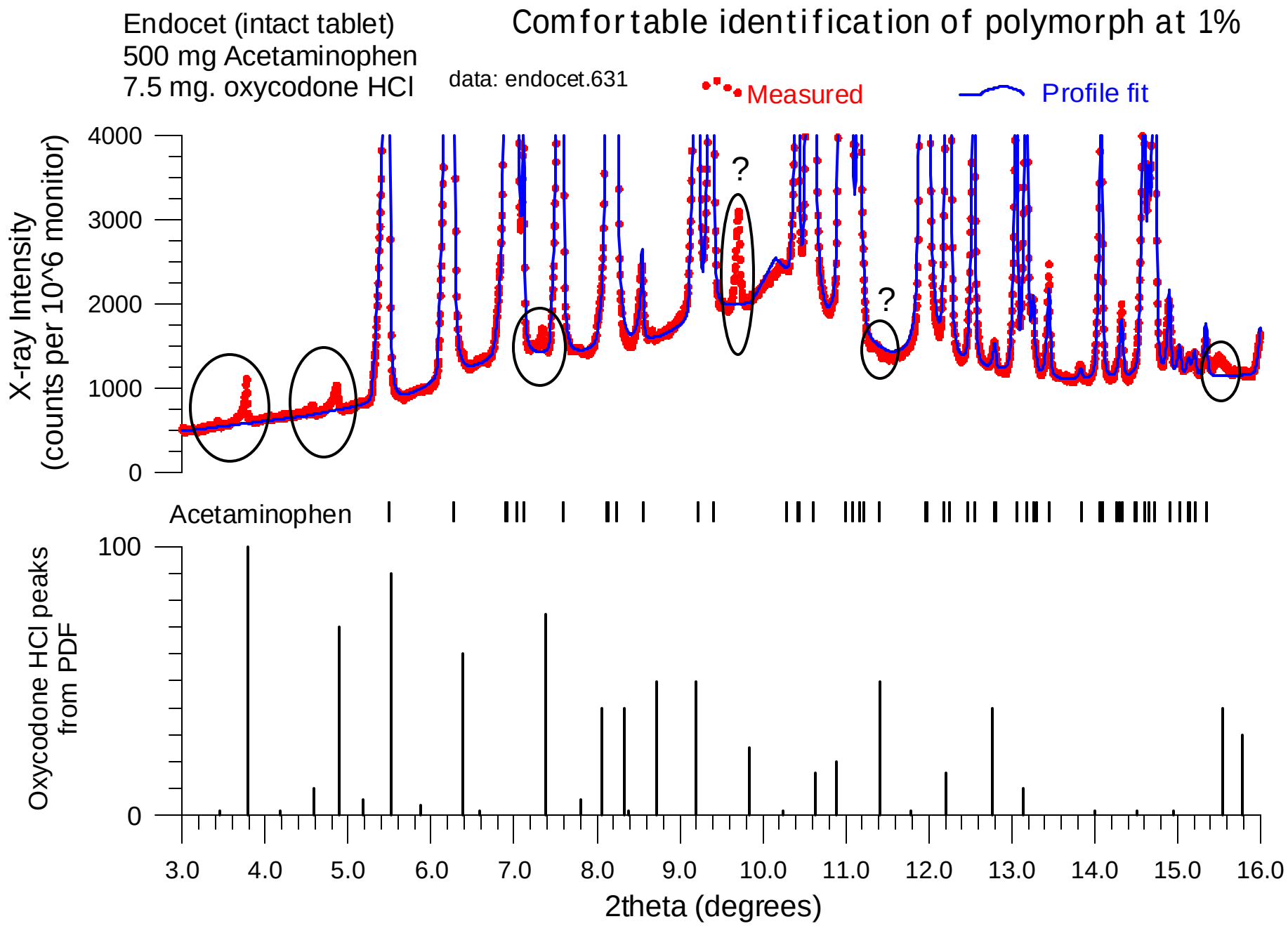
Proxy:
Examine commercial tablet of Endocet 500/7.5
Gross tablet 607 mg
Acetaminophen 500 mg – known lattice & structure
Oxycodone (as HCl) 7.5 mg – pattern in PDF but lattice unknown,*

* In general, I'd like to get better info into the PDF database. Please get in touch if you can help.


Endocet (intact tablet)
500 mg Acetaminophen
7.5 mg. oxycodone HCl
data: endocet.631
Measured
Profile fit of acetaminophen
x 50
X-ray Intensity (counts per 10^6 monitor)
80000
60000
40000
20000
0
Acetaminophen
2
4
6
8
10
12
14
16
18
20
2theta (degrees)

Powder patterns of oxycodone hydrochloride from ICDD Powder Diffraction File.
Strucutures and lattices are not known.




Endocet (intact tablet)
500 mg Acetaminophen
7.5 mg. oxycodone HCl
Comfortable identification of polymorph at 1%
data: endocet.631
Measured
Profile fit
X-ray Intensity
(counts per 10^6 monitor)
4000
3000
2000
1000
0
?
?
Acetaminophen
Oxycodone HCl peaks
from PDF
100
0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
2theta (degrees)

Chloramphenicol palmitate.

Has 3 forms α, β, ? (B, A, C). Only β structure is known from single crystal.

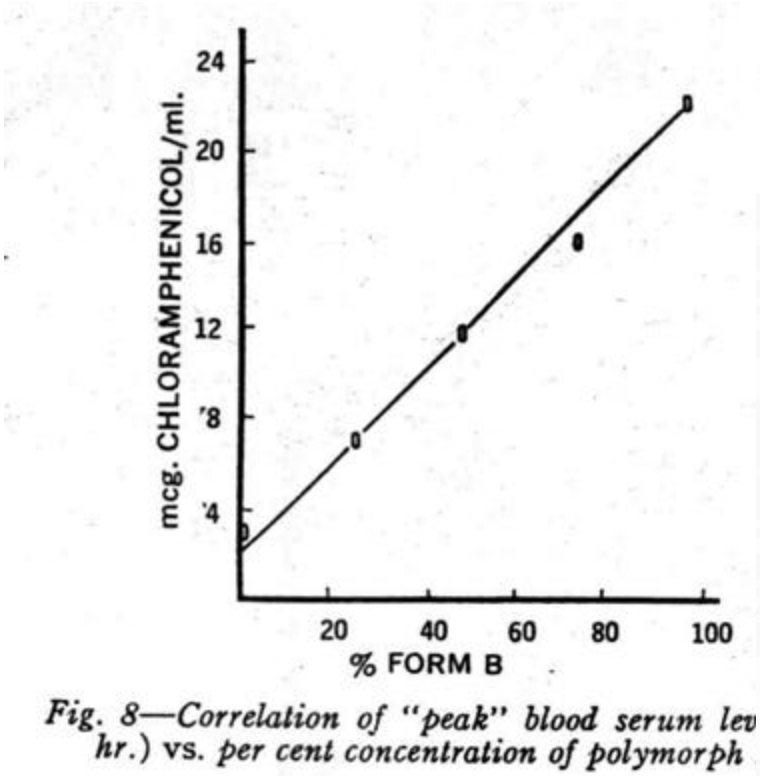
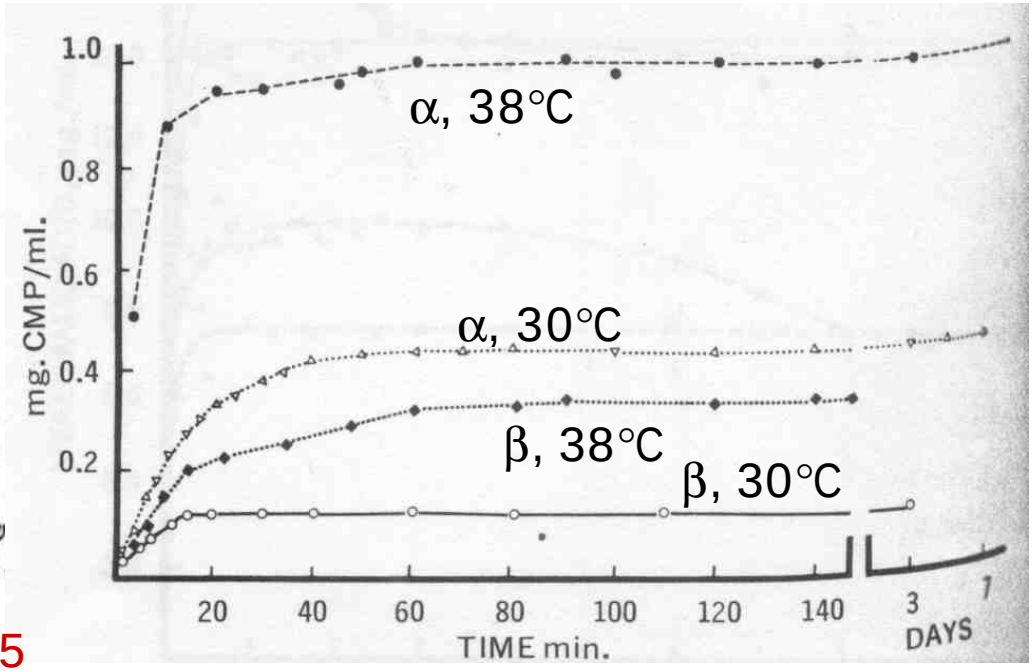


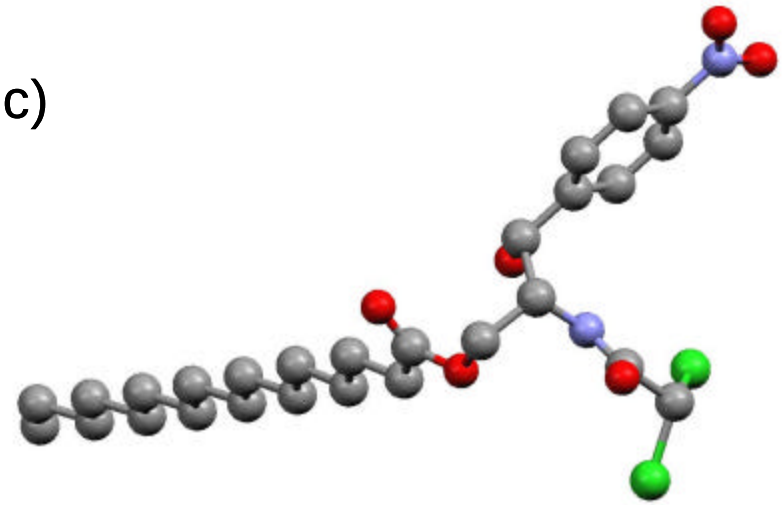
Fig. 8—Correlation of "peak" blood serum lev
hr.) vs. per cent concentration of polymorph

Aguiar *et al.* (1967). Oral dose 1.5 gm. Peak (2 hr) blood serum level.



Aguiar & Zelmer (1969). Dissolution curves in 35% tertiary butanol & water.

Chloramphenicol palmitate (antibiotic)



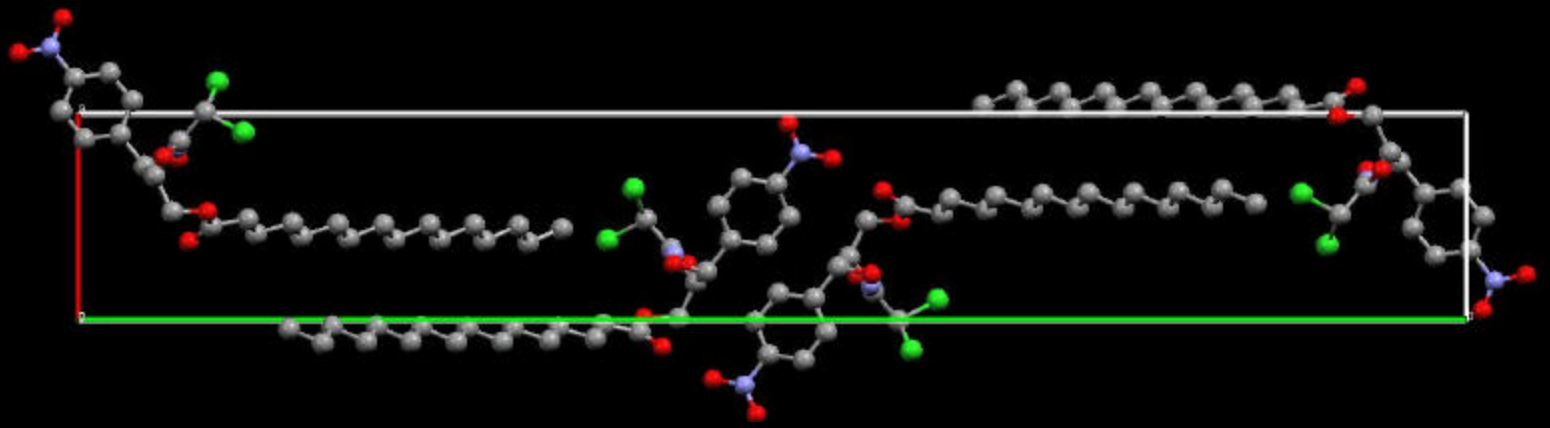
ß (A) Single crystal structure known, least bioavailable, highest melting point. $P2_12_12_1$, 7.805Å x 52.503Å x 7.414Å, Z'=1

α (B) Readily available in solution. C2, 34.110Å x 4.897Å x 39.45Å, β=110.17°, Z'=2.

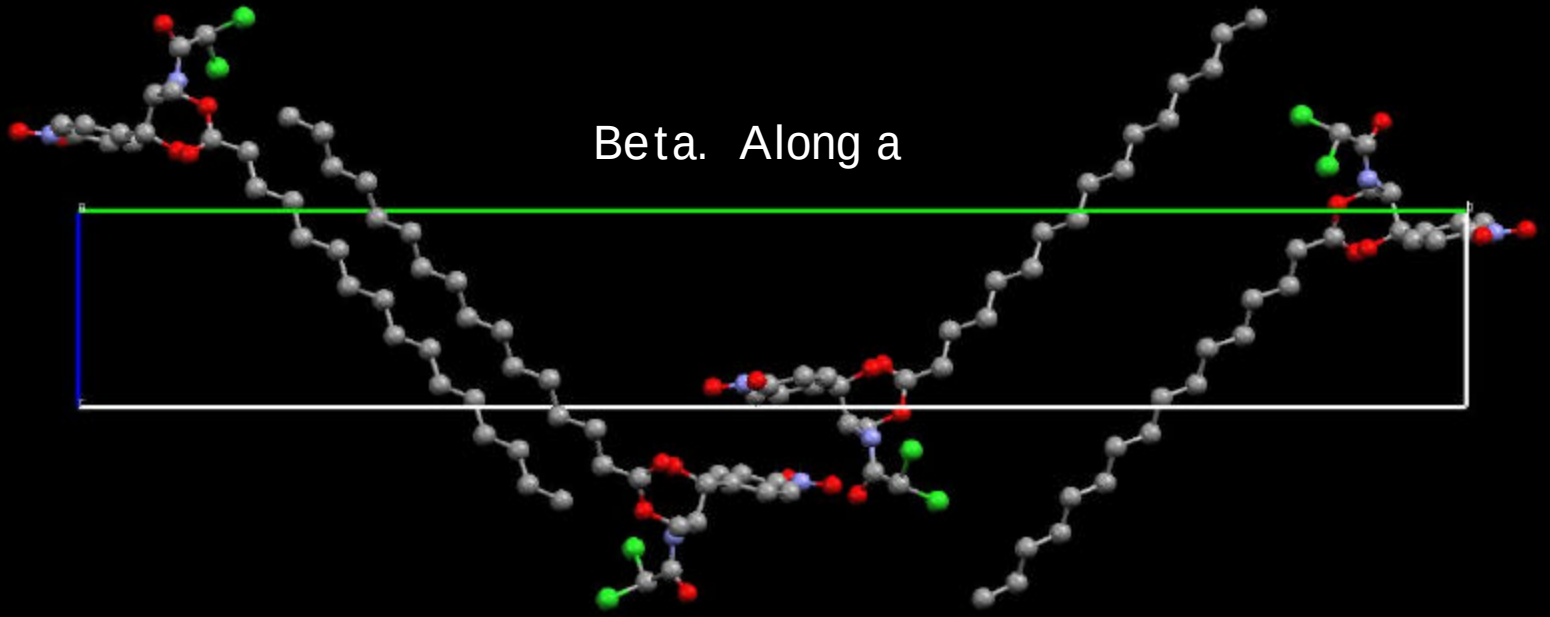
? (C) Intermediate solubility. $P2_1$, 35.53Å x 16.45Å x 5.185, β=90.15°, Z'=2.

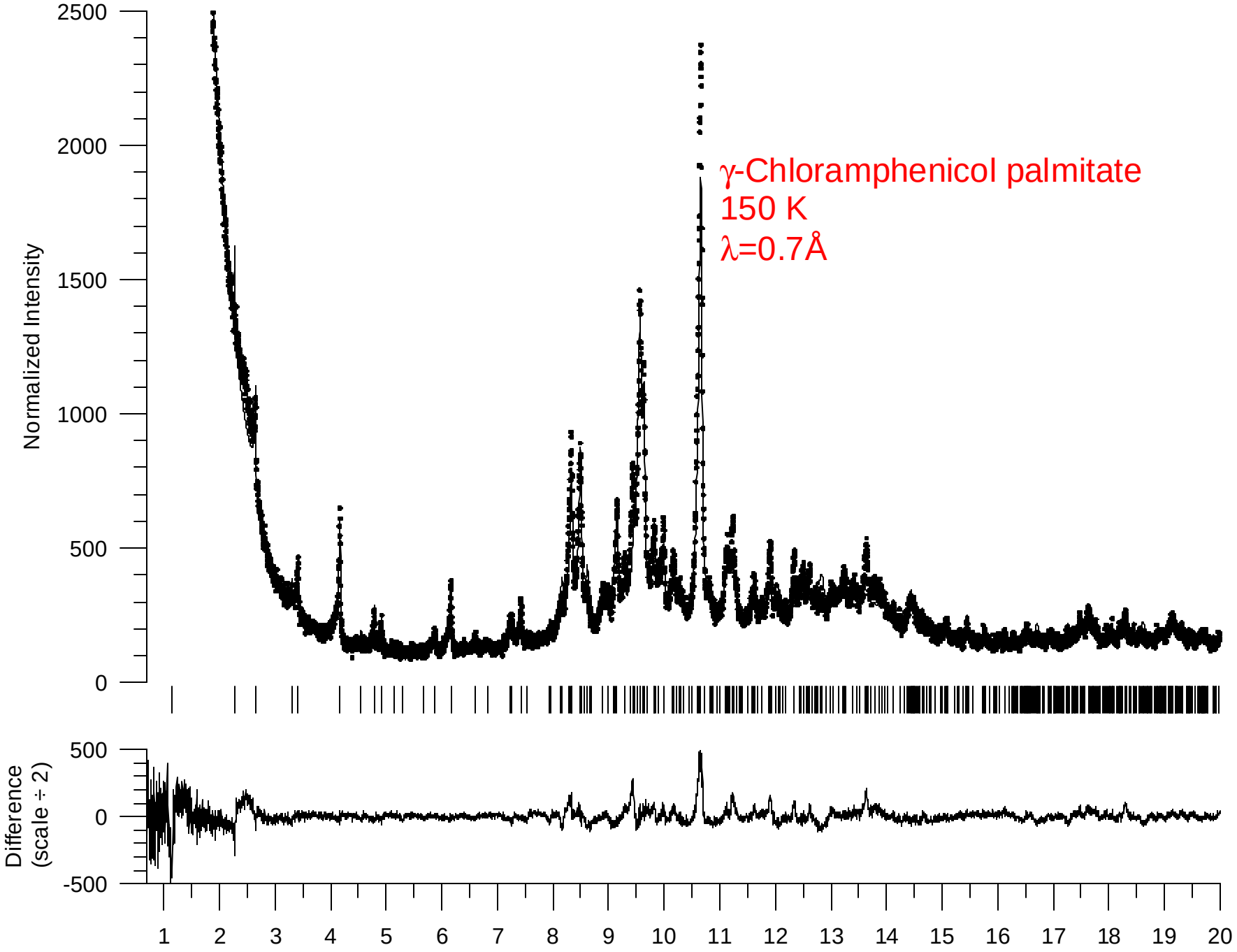
α and ? have been described in literature, lattice parameters given, but no structure solutions.

Chloramphenicol palmitate. Beta. $P2_12_12_1$. View along c

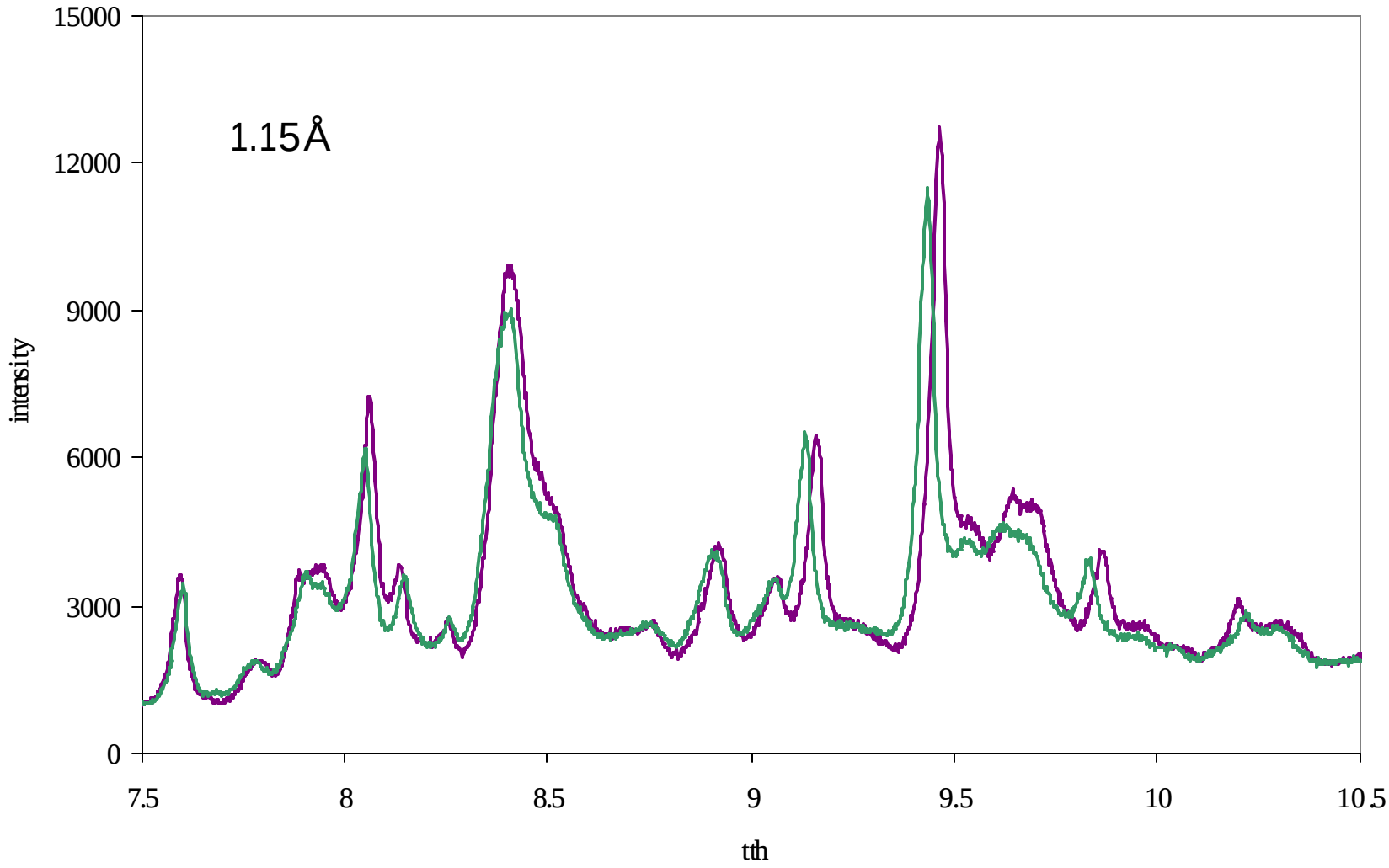


Beta. Along a

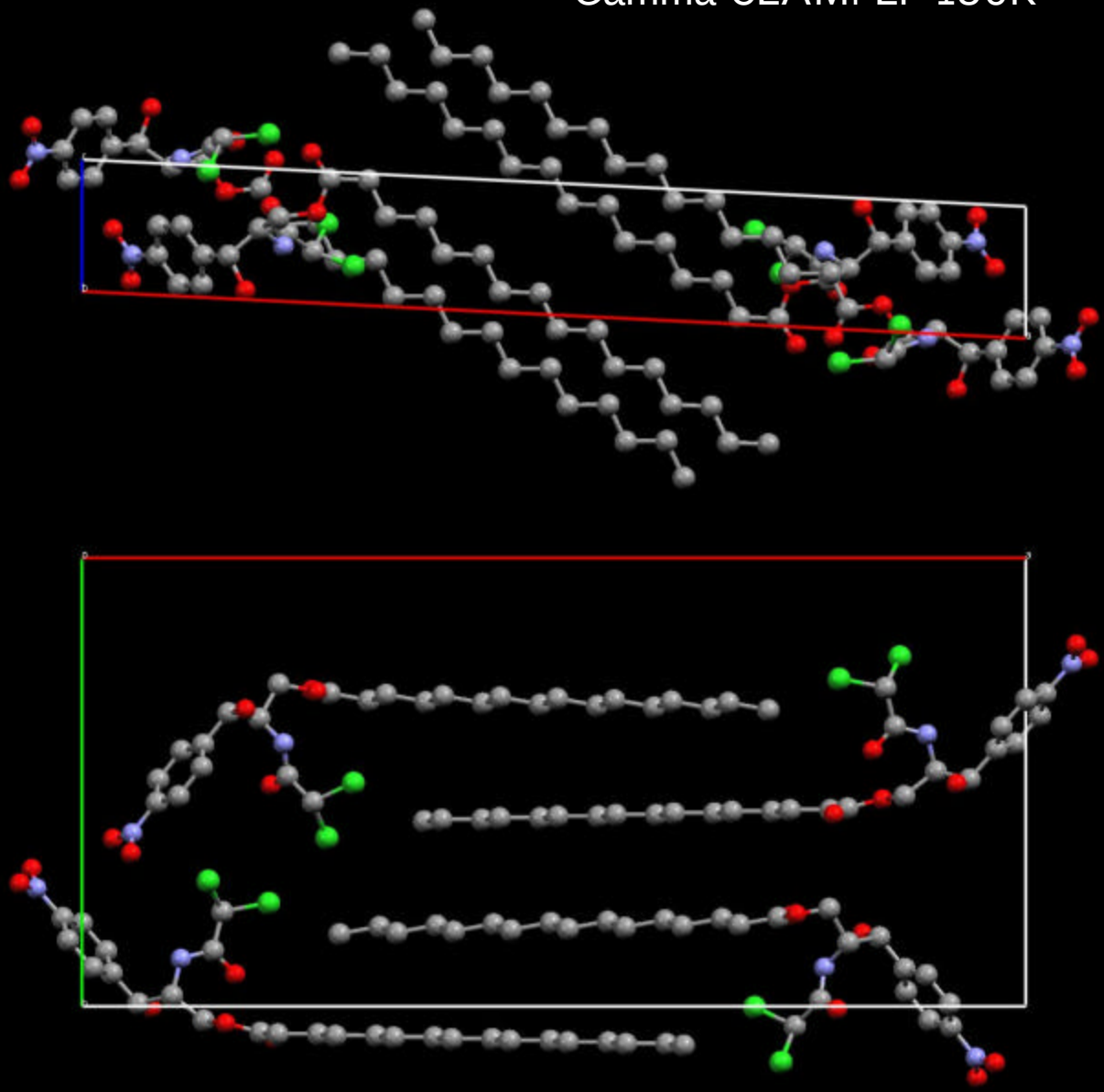


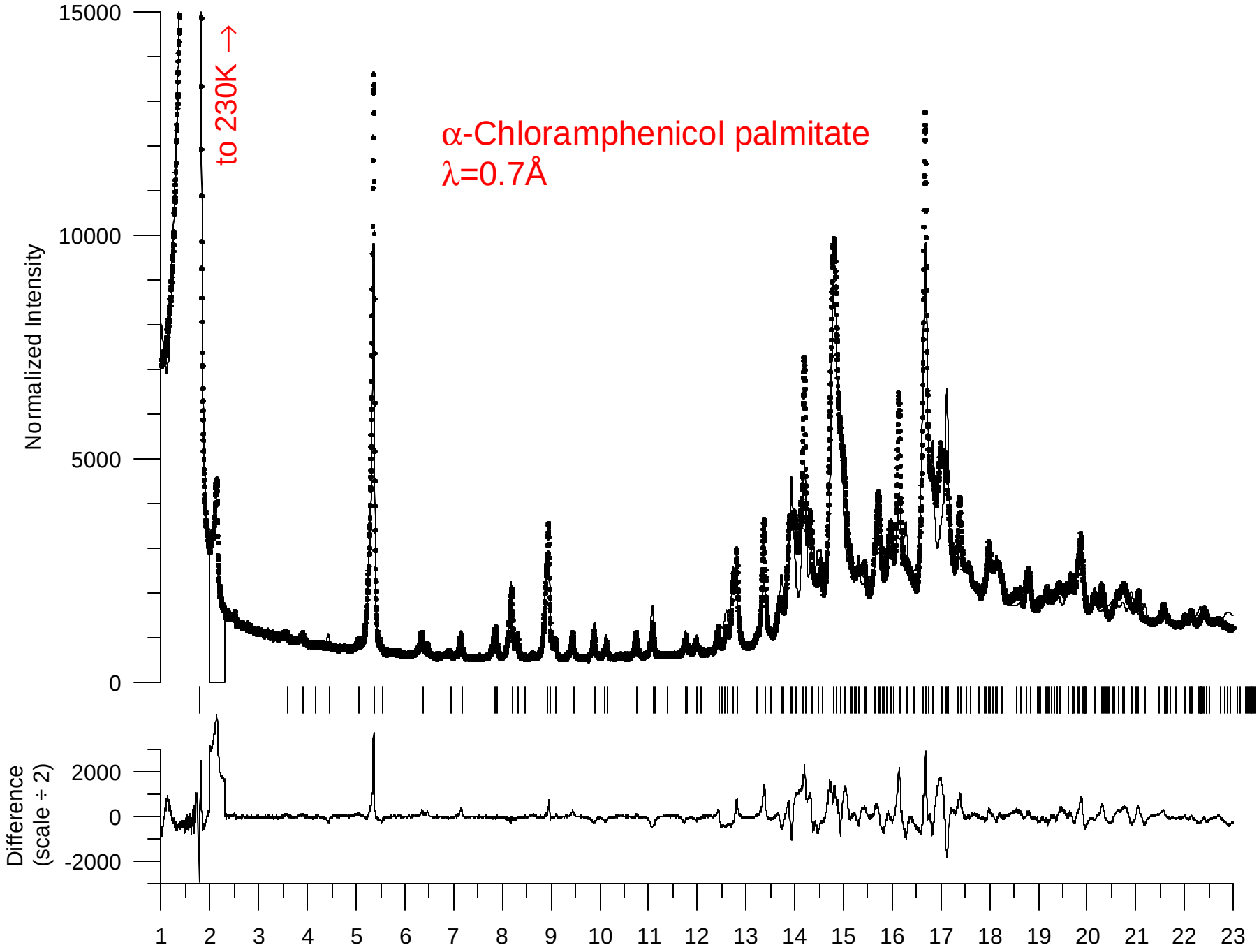

γ-Chloramphenicol palmitate
150 K
λ=0.7Å
Normalized Intensity
2500
2000
1500
1000
500
0
Difference
(scale ÷ 2)
500
0
-500
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

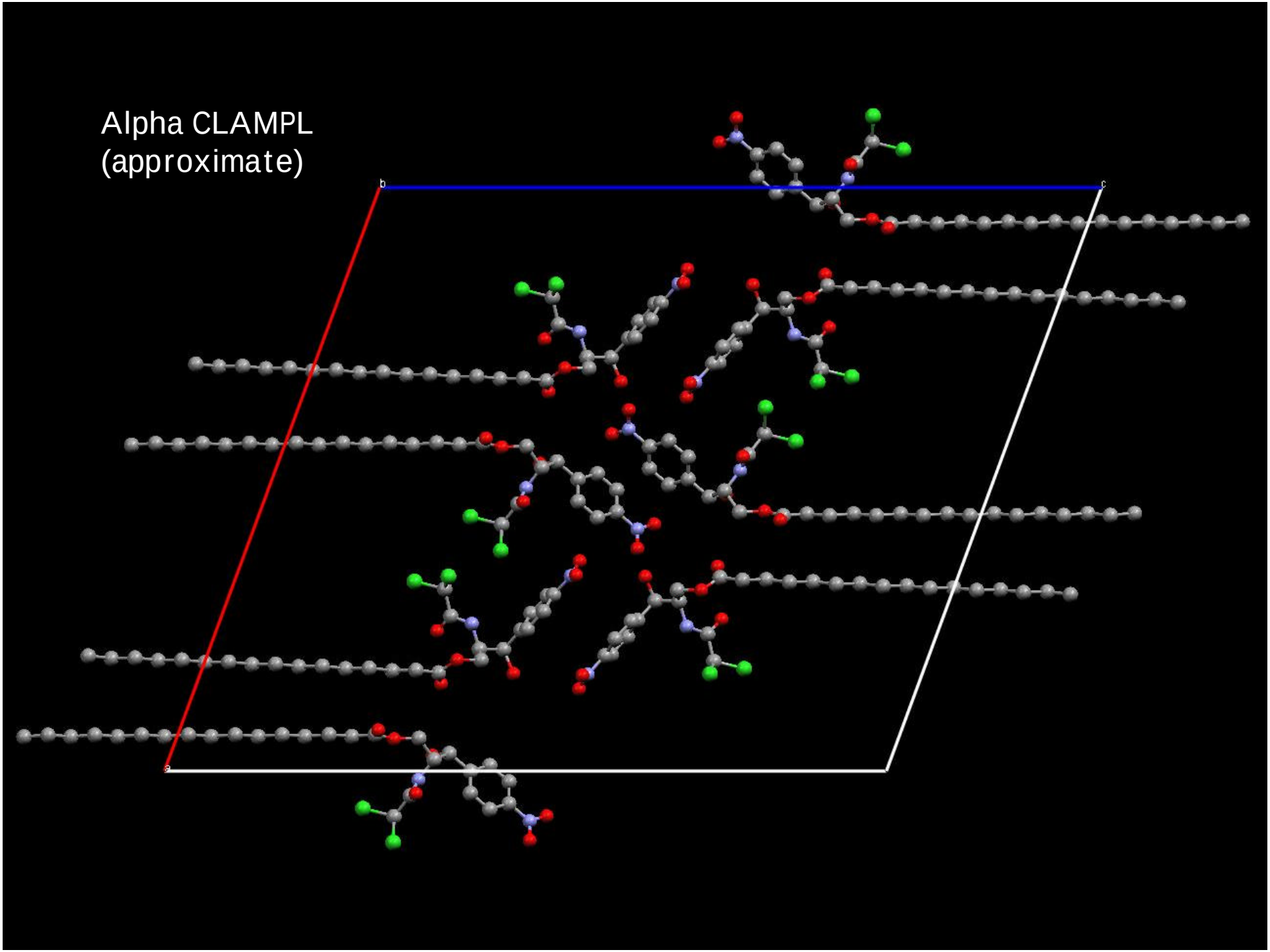
Sample is quite radiation sensitive – expands along chain direction.

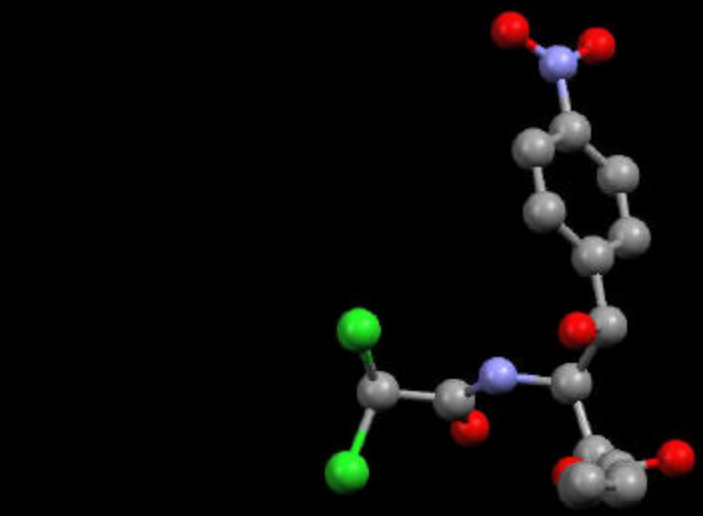


Gamma CLAMPL. 150K

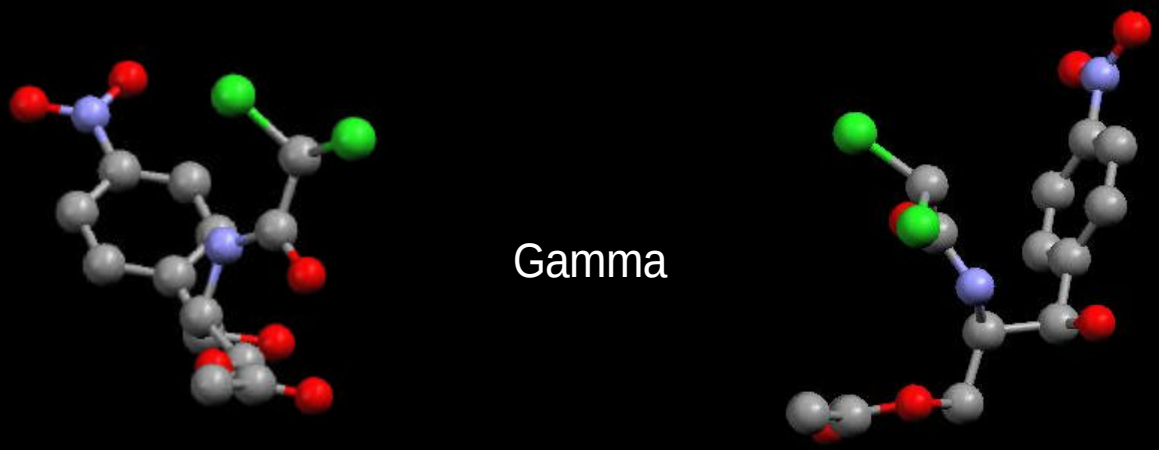



15000
10000
5000
0
Normalized Intensity
to 230K →
α-Chloramphenicol palmitate
λ=0.7Å
2000
0
-2000
Difference
(scale ÷ 2)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23


Alpha CLAMPL
(approximate)
b
c

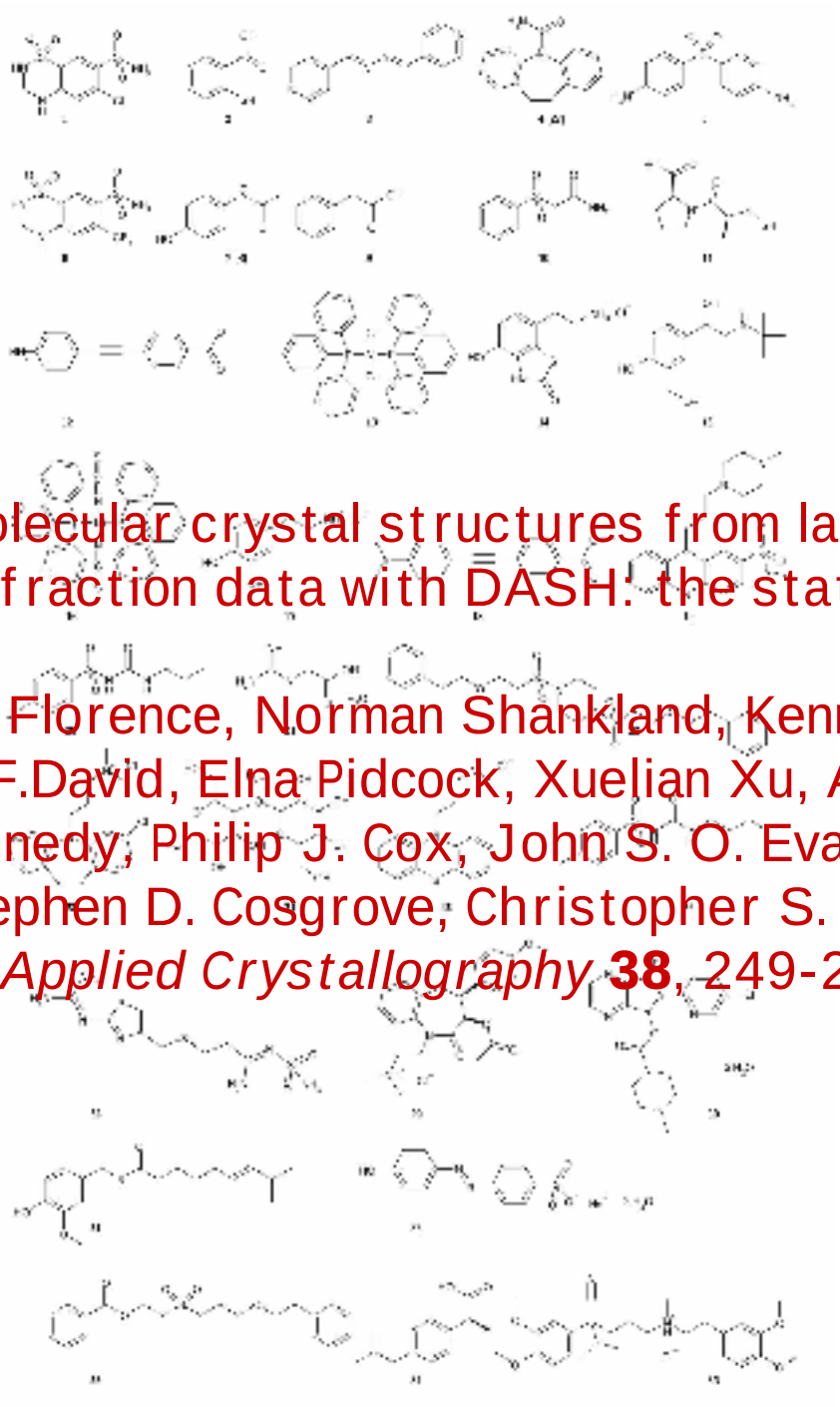


Beta



Gamma

Still to come – analysis of stability based on structures



"Solving molecular crystal structures from laboratory X-ray powder diffraction data with DASH: the state of the art and challenges"

Alastair J. Florence, Norman Shankland, Kenneth Shankland, William I. F.David, Elna Pidcock, Xuelian Xu, Andrea Johnston, Alan R. Kennedy, Philip J. Cox, John S. O. Evans, Gerald Steele, Stephen D. Cosgrove, Christopher S. Frampton

Journal of Applied Crystallography **38**, 249-259 (2005)

Acknowledgments:

Specific collaborations discussed here:
Vladimir Chernyshev, A.V. Yatsenko, O.B. Ryabova, V.A. Makarov, Cristian Botez, Jae-Hyuk Her, Ken and Norm Shankland.

Other close collaborators: Silvina Pagola and Ashfia Huq

People who have led the way, especially W.I.F. David and Ken Shankland.

Lots of other people who have written software: Alan Coelho, Bob Von Dreele, Jan Visser, Juan Rodriguez-Carvajal, Giacovazzo group, …