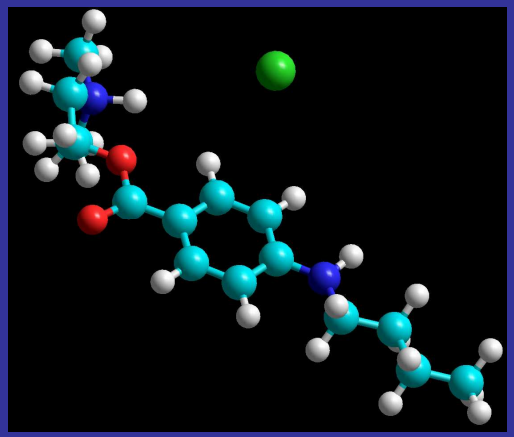
Ab initio crystal structure determination of two polymorphic modifications of a local anesthetic agent, Tetracaine Hydrochloride …



Robert J. Papoular
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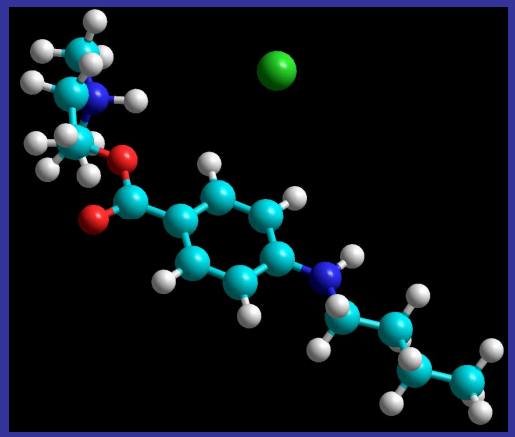
papou@llb.saclay.cea.fr



PPXRD-9 – 23 Feb' 10 – Hilton Head Island, SC, USA

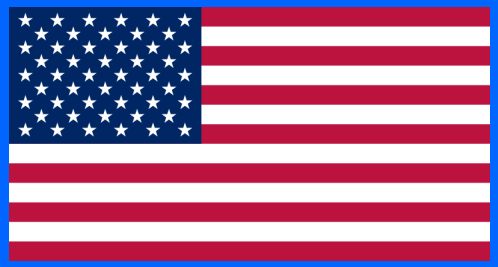
This document was presented at PPXRD - Pharmaceutical Powder X-ray Diffraction Symposium

Sponsored by The International Centre for Diffraction Data

PPXRD Website – www.icdd.com/ppxrd

ICDD Website - www.icdd.com

With :



APS / ANL

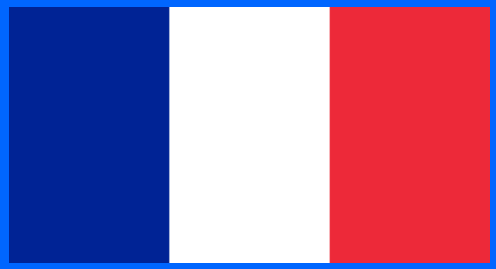
B.H. Toby

J. Doebbler

Univ. Moscow

N.B. Leonidov

A.V. Dzyabchenko



Univ. Tours

V. Agafonov

H. Allouchi

Univ. Rouen

M.-N. Petit

ENSCP, Paris

J.-M. Cense

What this talk is really about :

Ab initio structure solution

using :

High-resolution XRPD [indexing]

Molecular modeling [starting flexible molecule]

Direct space methods [Monte - Carlo]

Hydrogen bonds [chemical sense]

OVERVIEW

- Motivation
  (a) Polymorphism : induced by freeze-drying
  known tetracaine HCℓ unknown leocaine Leonidov (1995)

  (b) Anesthetics : local (eyes)
  (c) Ab Initio structure solution : a textbook example ?

- Previous work on TetraCaine HydroChloride [TCHC]
  (a) Giron *et al* (1997) (b) Nowell *et al* (2002) (c) Schmidt *et al* (2006)

- Synchrotron X-ray diffraction experiments [11-BM @ APS / ANL]

- OUR TALK : Leocaine = Modeling + Indexing + Direct Space Methods
  with a twist : Preferred Orientation included in Monte - Carlo

  --- Modeling (One molecule , PMC , four molecules)
  --- Indexing (DICVOL : ok ; N-TREOR-09 : perfect)
  --- Ab Initio Solving (FOX ! , EXPO 2009 ?)

- Inter- / Intra- molecular H- bonds

I. MOTIVATION (1) : LEOCAINE , a new effective drug

Paper ⇄ Patent ⇄ Industry

[Leocaine--a new generation of local anesthetics] [Vestn Oftalmol. 1995

PubMed Vestn. Oftalmol. 1995

U.S. National Library of Medicine
National Institutes of Health

Display Settings: Abstract

Performing your original search, ***leonidov leocaine***, in PubMed will retrieve 2 records.

Vestn Oftalmol. 1995 Oct-Dec;111(4):19-21.

[Leocaine--a new generation of local anesthetics]

[Article in Russian]

Leonidov NB, Nesterov AP, Uspenskaia SI, Knizhev VA, Fitilev SB, Avramenko NN.

Leocaine is a new crystal beta-modification of beta-dimethylaminoethyl ether of n-butylaminobenzoic
chemical formula is the same as for dicaine, but it has a number of advantages over this drug. The ar
leocaine is 2.5 times higher than that of dicaine. By the duration and depth of anesthesia 0.3% leocai
corresponds to 1% dicaine. Leocaine exerts no toxic effect on the corneal epithelium and its instillatio
cavity does not result in the reactive dilatation of corneal or episcleral vessels. Leocaine solution is st
Clinical trials of leocaine carried out on more than 2500 patients showed virtually complete absence c
Commercial manufacture of leocaine is launched at present. One of the commercial preparations rep
of the active substance in isotonic NaCl solution. Another drug contains, besides leocaine, methylcell
leocaine are recommended for practical ophthalmology instead of dicaine for local anesthesia. The d
medical use and commercial manufacture by the Ministry of Health and Medical Industry of Russia.

PMID: 8604531 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

United States Patent [19]

Leonidov 1995

[54] **CRYSTALLINE MODIFICATION OF 2-DIMETHYLAMINOETHYL-N-BUTYLAMINOBENZOATE HYDROCHLORIDE, METHOD FOR PRODUCTION THEREOF AND PHARMACEUTICAL PREPARATION FOR ANAESTHESIA OF EYES, BASED THEREON**

[76] Inventor: **Nikolai B. Leonidov**, ulitsa Zatonnaya, 12, korpus 1, kv. 158, Moscow, Russian Federation

[21] Appl. No.: **958,106**

[22] PCT Filed: **Aug. 19, 1991**

[86] PCT No.: **PCT/SU91/00168**

§ 371 Date: **Dec. 22, 1992**

§ 102(e) Date: **Dec. 22, 1992**

[87] PCT Pub. No.: **WO93/04034**

PCT Pub. Date: **Mar. 4, 1993**

[30] **Foreign Application Priority Data**

Apr. 30, 1991 [RU] Russian Federation 4927838
Apr. 30, 1991 [RU] Russian Federation 4927859

[51] **Int. Cl.**[6] **C07C 229/34**
[52] **U.S. Cl.** **560/49**
[58] **Field of Search** 560/49

[56] **References Cited**

PUBLICATIONS

Owens, J. Ass. Off. Anal. Chem. 55(6) 1171–4, (1972).

nanopharm TECHNOLOGIES 2005

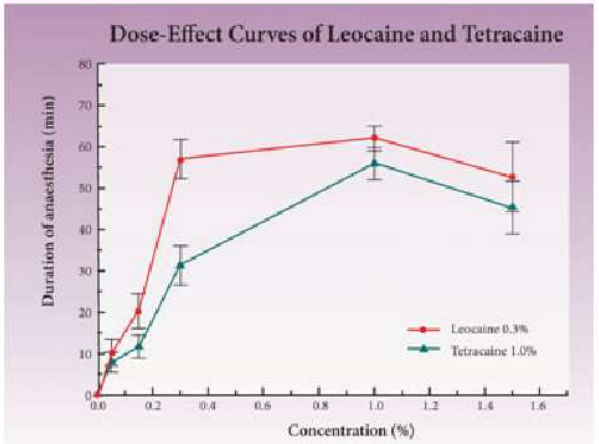
Home | About Us | Technology | Product | Activities

PRODUCT

PROJECT-LEOCAINE

- Leocaine
- Project-Leocaine
- Other Developments

Efficacy Data



... while the crystal structure remains unknown !

I. MOTIVATION (2) : Structure Solving Strategy

Preliminary modeling :

(Energy minimization)

(i) One molecular unit
- Flexible model

(Hyperchem™)

(ii) Molecular Packing (unit cell known)
- Crystal structure prediction

(PMC , Dzyabchenko)

Ab initio solving :

(i) Direct Methods
(Phase Problem)

FAILS !

(ii) Direct Space Methods

Monte-Carlo
Parallel tempering

(FOX , Favre-Nicolin)

Post modeling :

(Energy minimization)

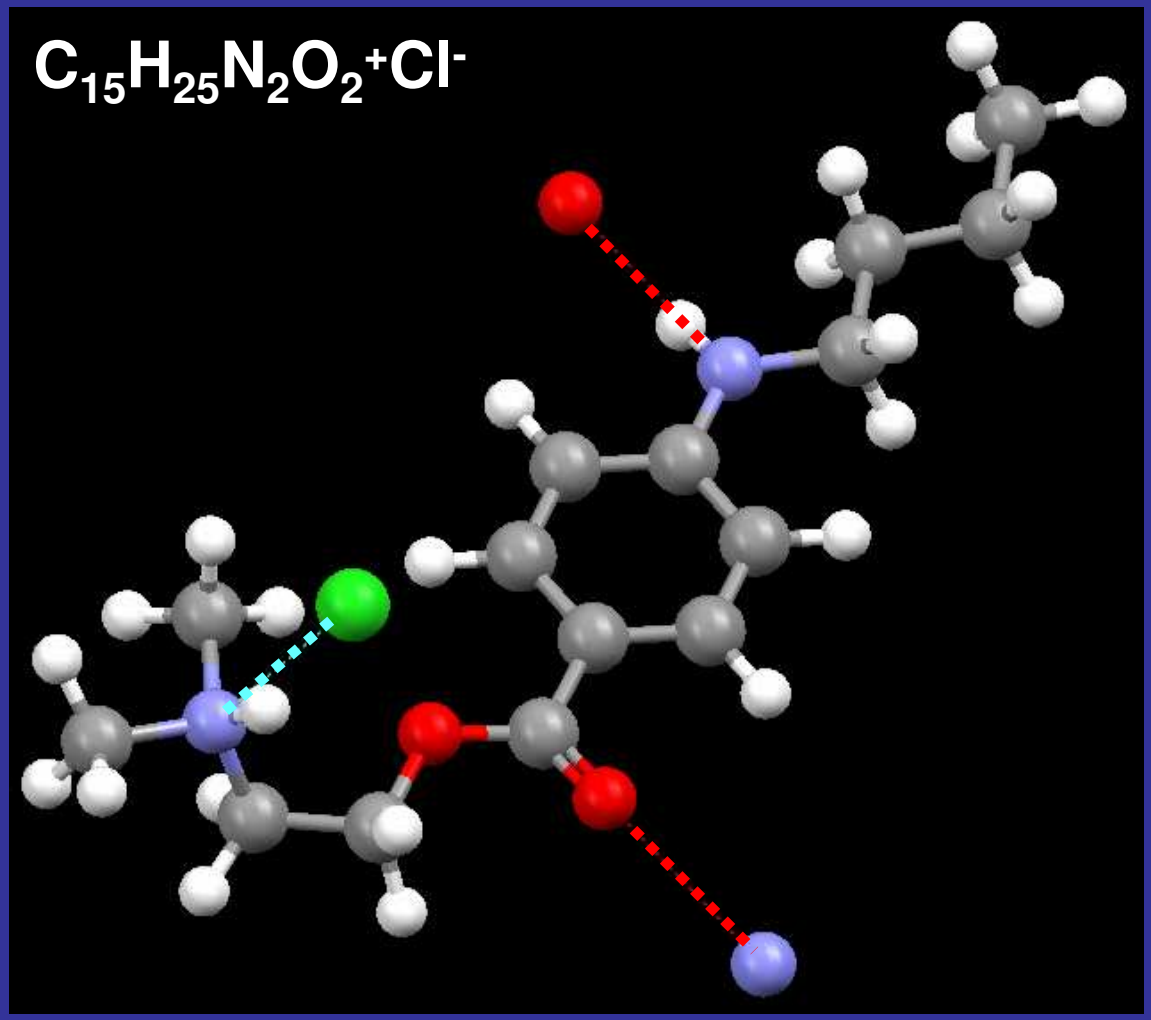
Stability check :
Crystal approximated by a few molecules

(4 for leocaine)

(Hyperchem™)

II. PREVIOUS WORK (1) : Tetracaine Hydrochloride

Inter-molecular H - bonds



Novell *et al* NJC (2002)

Triclinic
P -1

V = 822 Å^3

ab initio from XRPD

[DASH]

2 - (dimethylamino)ethyl p - butylaminobenzoate hydrochloride

II. PREVIOUS WORK (2) : Tetracaine Hydrochloride

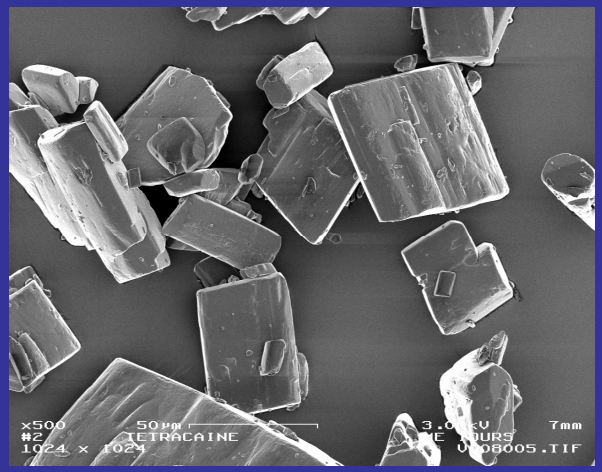
- Giron *et al*
  TCHC polymorphism (DSC , HT XRPD)
  J. Therm. Anal. (1997)
  6 anhydrous crystalline forms … 2 stable at T = 300 K.

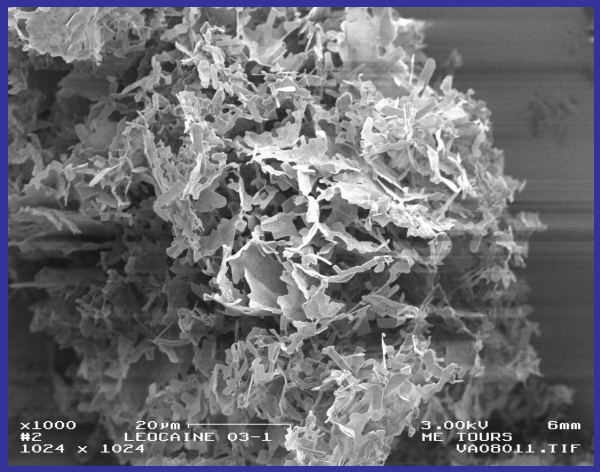
- Schmidt *et al*
  TCHC mod.4 (SXD)
  Pharmaceutical Research (2005)
  Polymorphism caused by conformal flexibility of the molecules …

- Hamaed *et al*
  TCHC (SXD, ^{35}Cl NMR)
  JACS (2008)

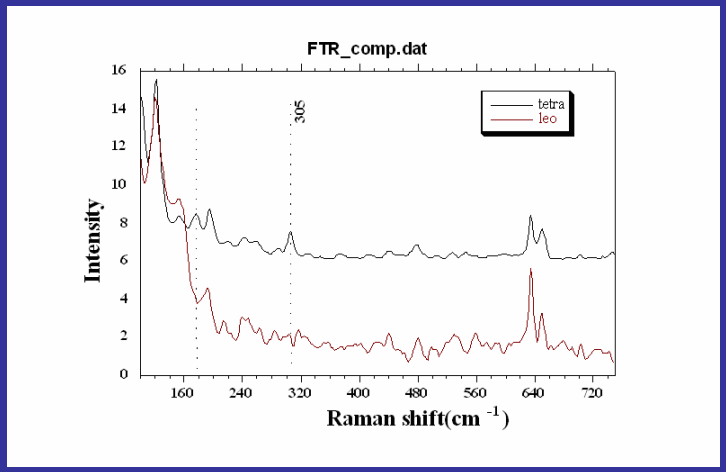
II. Our Experiments (1) : Sample characterization

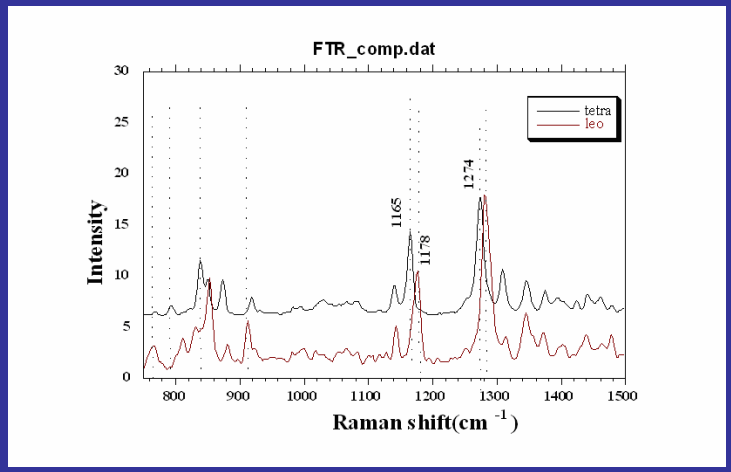
- Electron microscopy (SEM)





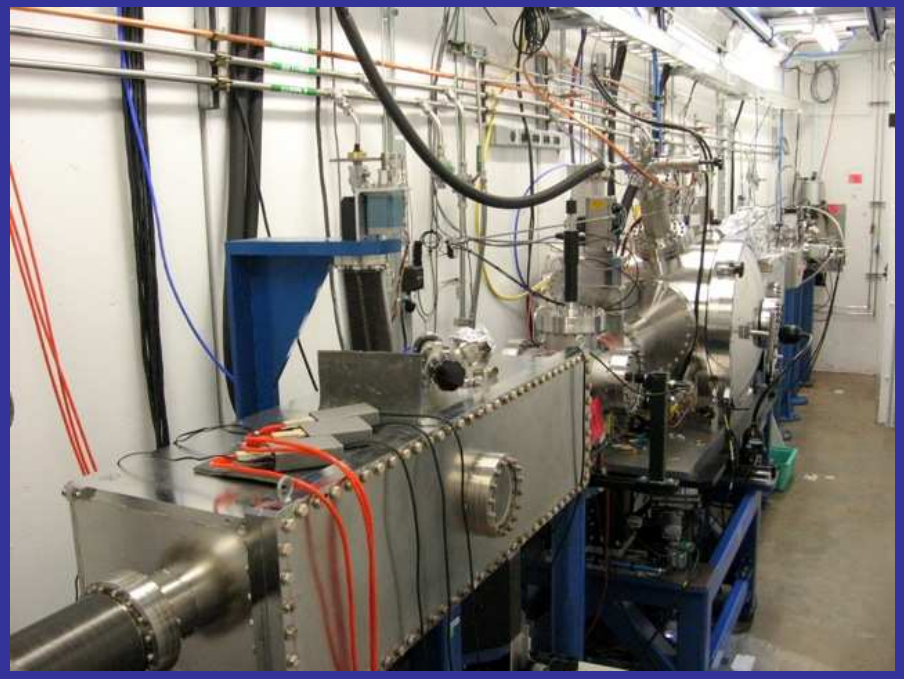
- Raman spectroscopy (FTR)

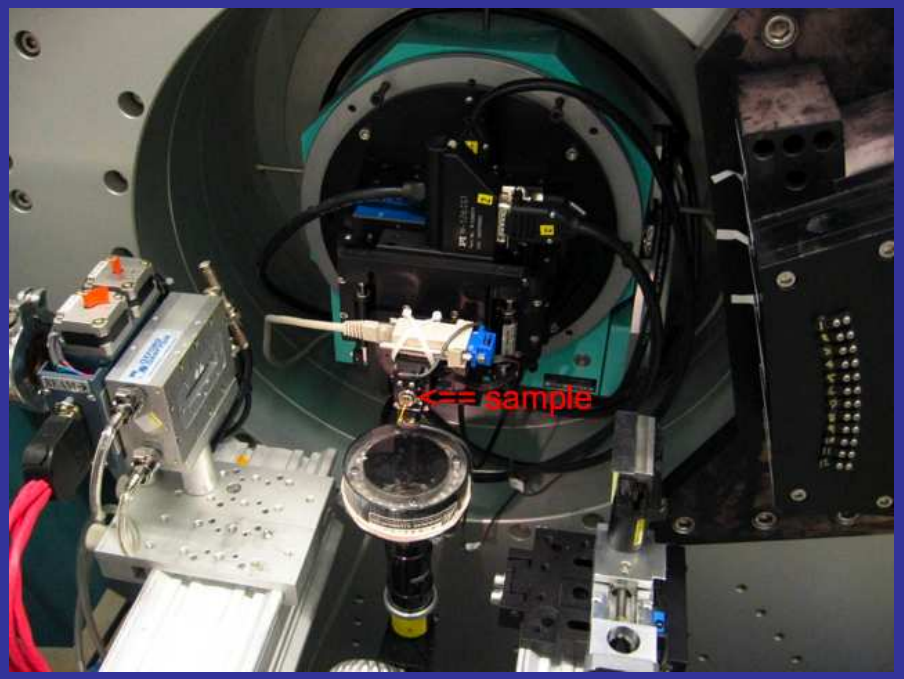




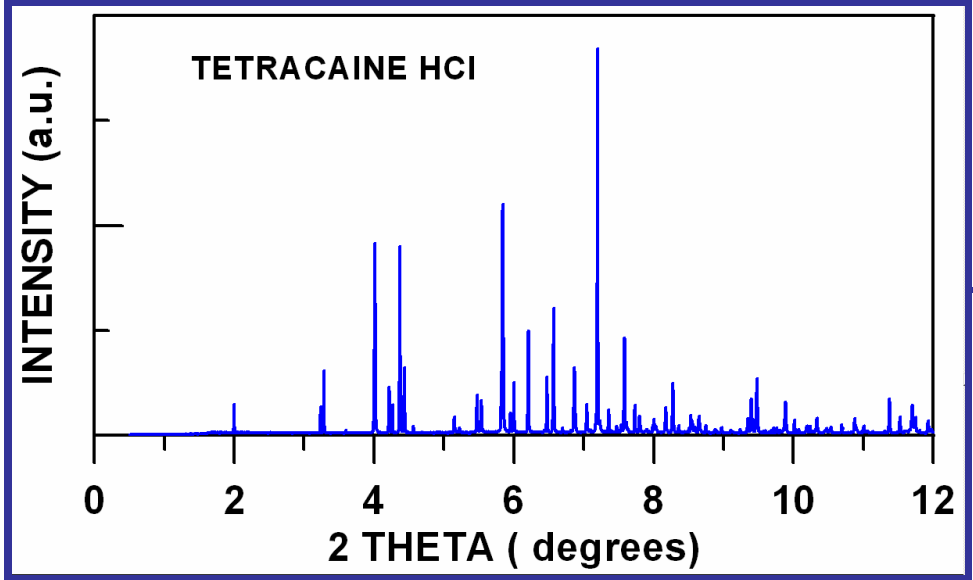
II. Our Experiments (2) : High-resolution Synchrotron XRPD

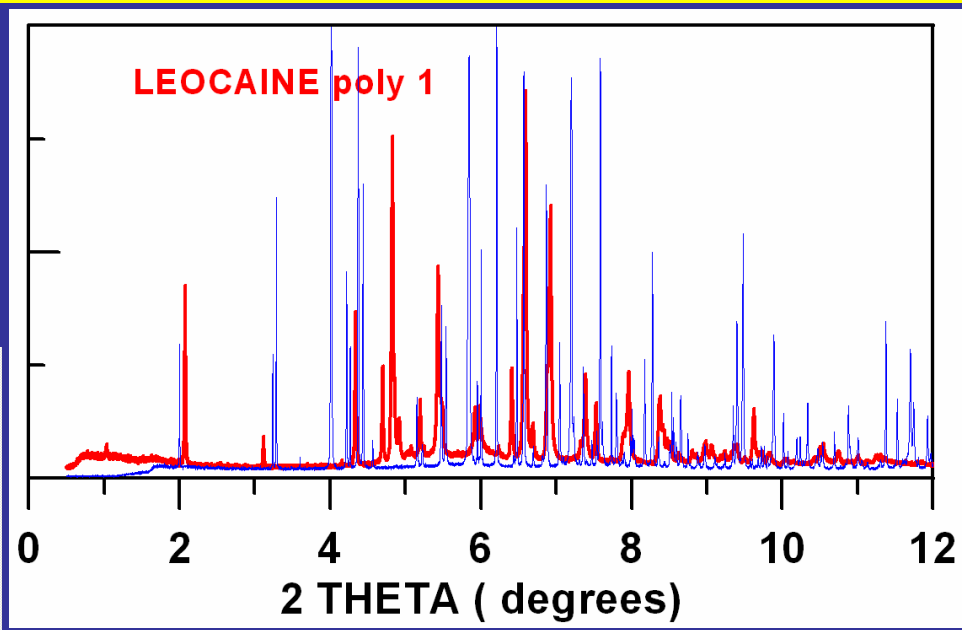
11-BM beamline @ APS / ANL (Argonne , USA)

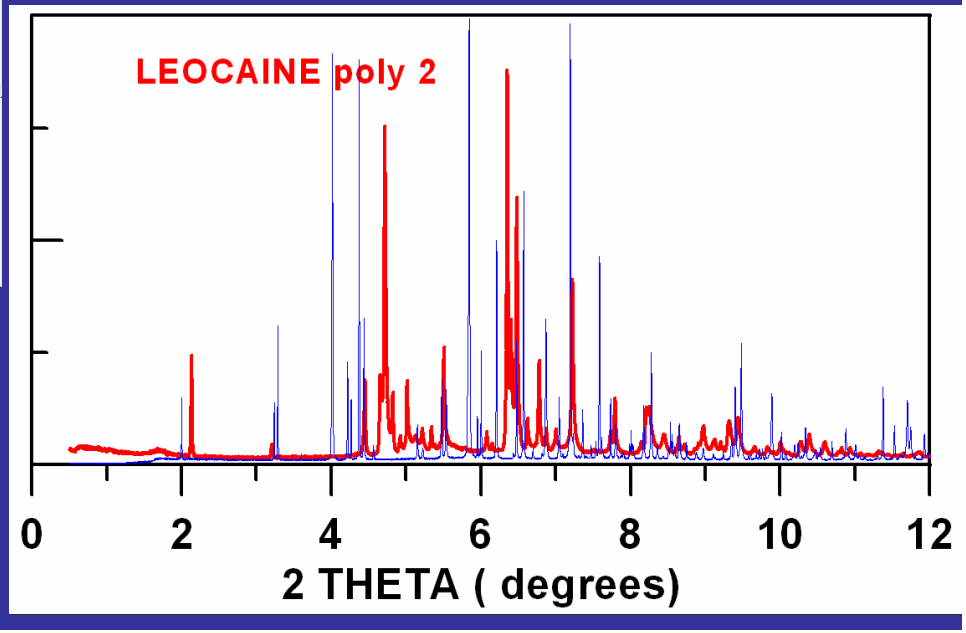




II. Our Experiments (3) : High-resolution Synchrotron data







III. Our Structure Solutions (1) : INDEXING with N-TREOR-09

Tetracaine (TCHC)

Total number of plausible solutions = 3

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	31	13.7376	8.5802	7.4115	98.6903	90.8451	106.1796	0.0020	0	828.
2	3	24.1806	6.8277	18.8280	90.0000	102.1412	90.0000	0.0000	2	3039.
3	3	24.1969	6.8476	18.8098	90.0000	102.0099	90.0000	-0.0020	2	3048.

NIX is the number of unindexed lines.

Leocaine poly # 1

Total number of plausible solutions = 4

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	22	25.4800	6.2351	10.9343	90.0000	96.8923	90.0000	-0.0040	0	1725.
2	2	12.4406	12.4406	77.8686	90.0000	90.0000	120.0000	-0.0020	6	10437.
3	2	12.6063	12.6063	76.9181	90.0000	90.0000	120.0000	0.0000	1	10586.
4	1	NaN	6.2364	NaN	90.0000	90.0000	90.0000	-0.0060	1	NaN

NIX is the number of unindexed lines.

Leocaine poly # 2

Total number of plausible solutions = 2

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	28	25.0079	6.0831	11.3201	90.0000	100.0335	90.0000	0.0000	0	1696.
2	6	24.7547	12.1383	5.6728	90.0000	92.6190	90.0000	0.0000	1	1703.

NIX is the number of unindexed lines.

III. Our Structure Solutions (1) : INDEXING with N-TREOR-09

Tetracaine (TCHC)

Total number of plausible solutions = 3

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	31	13.7376	8.5802	7.4115	98.6903	90.8451	106.1796	0.0020	0	828.
2	3	24.1806	6.8277	18.8280	90.0000	102.1412	90.0000	0.0000	2	3039.
3	3	24.1969	6.8476	18.8098	90.0000	102.0099	90.0000	-0.0020	2	3048.
		13.6923	8.5669	7.4002	98.778	90.853	106.211	Novell *et al* (2002)		

NIX is the number of unindexed lines.

Leocaine poly # 1

Total number of plausible solutions = 4

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	22	25.4800	6.2351	10.9343	90.0000	96.8923	90.0000	-0.0040	0	1725.
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3	2	12.6063	12.6063	76.9181	90.0000	90.0000	120.0000	0.0000	1	10586.
4	1	NaN	6.2364	NaN	90.0000	90.0000	90.0000	-0.0060	1	NaN

NIX is the number of unindexed lines.

Leocaine poly # 2

Total number of plausible solutions = 2

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	28	25.0079	6.0831	11.3201	90.0000	100.0335	90.0000	0.0000	0	1696.
2	6	24.7547	12.1383	5.6728	90.0000	92.6190	90.0000	0.0000	1	1703.

NIX is the number of unindexed lines.

III. Our Structure Solutions (1) : INDEXING with N-TREOR-09

Tetracaine

(TCHC)

P -1

Total number of plausible solutions = 3

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	31	13.7376	8.5802	7.4115	98.6903	90.8451	106.1796	0.0020	0	828.
2	3	24.1806	6.8277	18.8280	90.0000	102.1412	90.0000	0.0000	2	3039.
3	3	24.1969	6.8476	18.8098	90.0000	102.0099	90.0000	-0.0020	2	3048.
		13.6923	8.5669	7.4002	98.778	90.853	106.211	Novell *et al* (2002)		

NIX is the number of unindexed lines.

Leocaine

poly # 1

P 2_1 / c

Total number of plausible solutions = 4

Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	22	25.4800	6.2351	10.9343	90.0000	96.8923	90.0000	-0.0040	0	1725.
2	2	12.4406	12.4406	77.8686	90.0000	90.0000	120.0000	-0.0020	6	10437.
3	2	12.6063	12.6063	76.9181	90.0000	90.0000	120.0000	0.0000	1	10586.
4	1	NaN	6.2364	NaN	90.0000	90.0000	90.0000	-0.0060	1	NaN

NIX is the number of unindexed lines.

Leocaine

poly # 2

P 2_1 / c

Total number of plausible solutions = 2

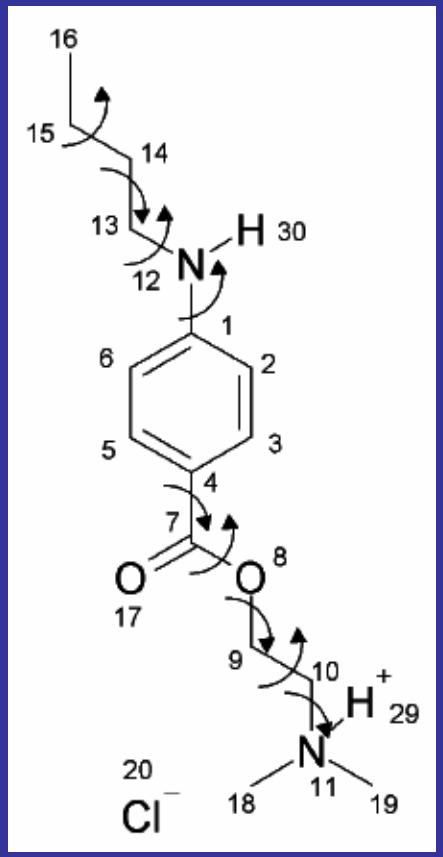
Sol.	M20	a	b	c	alpha	beta	gamma	2Theta-shift	NIX	Vol.
1	28	25.0079	6.0831	11.3201	90.0000	100.0335	90.0000	0.0000	0	1696.
2	6	24.7547	12.1383	5.6728	90.0000	92.6190	90.0000	0.0000	1	1703.

NIX is the number of unindexed lines.

III. Our Structure Solutions (2): Flexible molecular model

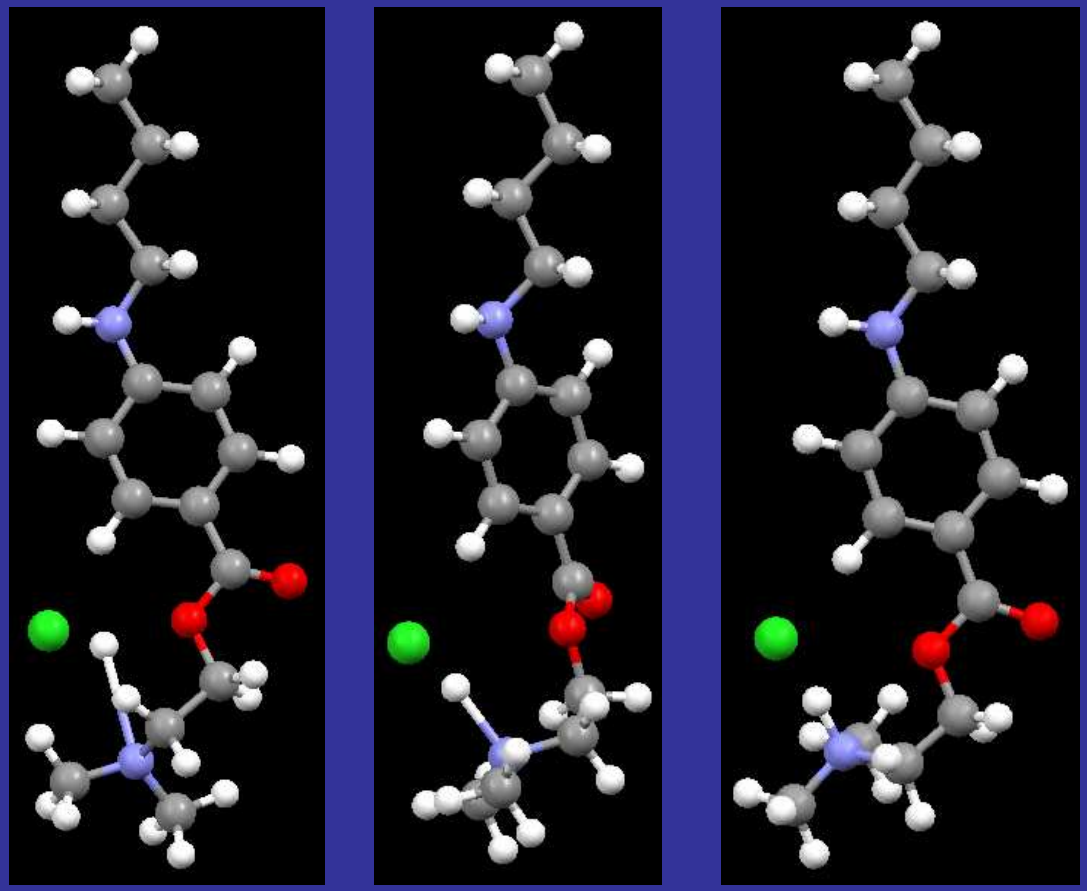
for Tetracaine

(Novell , NJC, 2002)



Quantum-Mechanical Semi-Empirical Methods :

AM1 PM3 ZNDO (best)

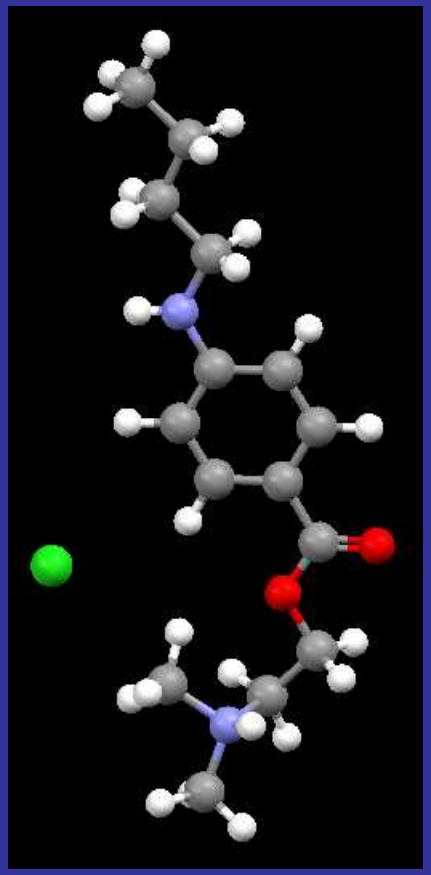


III. Our Structure Solutions (3): Flexible molecular model

for Leocaine

2 more experimental models

Tetracaine (TCHC) XRPD (Novell,2002)



Tetracaine (TCHC) SXD (2010)

```
_diffrn_radiation_monochromator   graphite
_diffrn_radiation_source          'fine-focus
sealed tube'
_diffrn_radiation_type            MoK\a
_diffrn_radiation_wavelength      0.71073
_diffrn_reflns_av_R_equivalents   0.0513
_diffrn_reflns_av_sigmaI/netI     0.0884
_diffrn_reflns_limit_h_max        9
_diffrn_reflns_limit_h_min        -9
_diffrn_reflns_limit_k_max        11
_diffrn_reflns_limit_k_min        -9
_diffrn_reflns_limit_l_max        17
_diffrn_reflns_limit_l_min        -17
_diffrn_reflns_number             5899
_diffrn_reflns_theta_full         27.44
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```
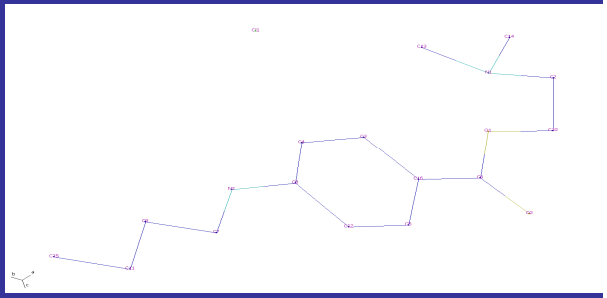



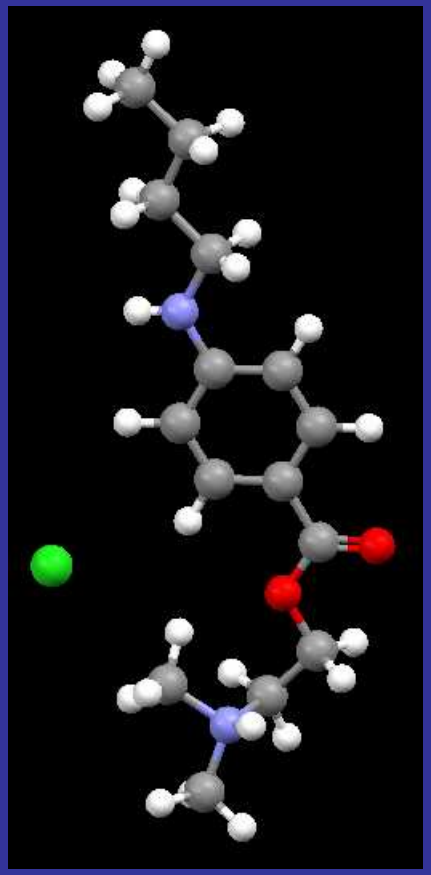
[IL MILIONE]

III. Our Structure Solutions (3): Flexible molecular model

for Leocaine

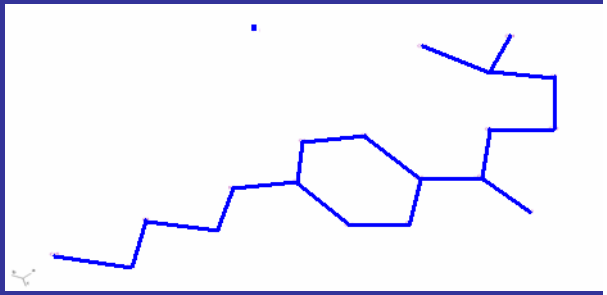
2 more experimental models

Tetracaine (TCHC) XRPD (Novell,2002)



Tetracaine (TCHC) SXD (2010)

```
_diffrn_radiation_monochromator   graphite
_diffrn_radiation_source          'fine-focus
sealed tube'
_diffrn_radiation_type            MoK\a
_diffrn_radiation_wavelength      0.71073
_diffrn_reflns_av_R_equivalents   0.0513
_diffrn_reflns_av_sigmaI/netI     0.0884
_diffrn_reflns_limit_h_max        9
_diffrn_reflns_limit_h_min        -9
_diffrn_reflns_limit_k_max        11
_diffrn_reflns_limit_k_min        -9
_diffrn_reflns_limit_l_max        17
_diffrn_reflns_limit_l_min        -17
_diffrn_reflns_number             5899
_diffrn_reflns_theta_full         27.44
_diffrn_reflns_theta_max          27.44
_diffrn_reflns_theta_min          3.39
```



[IL MILIONE]

III. Our Structure Solutions (4) : Theoretical Crystal Model

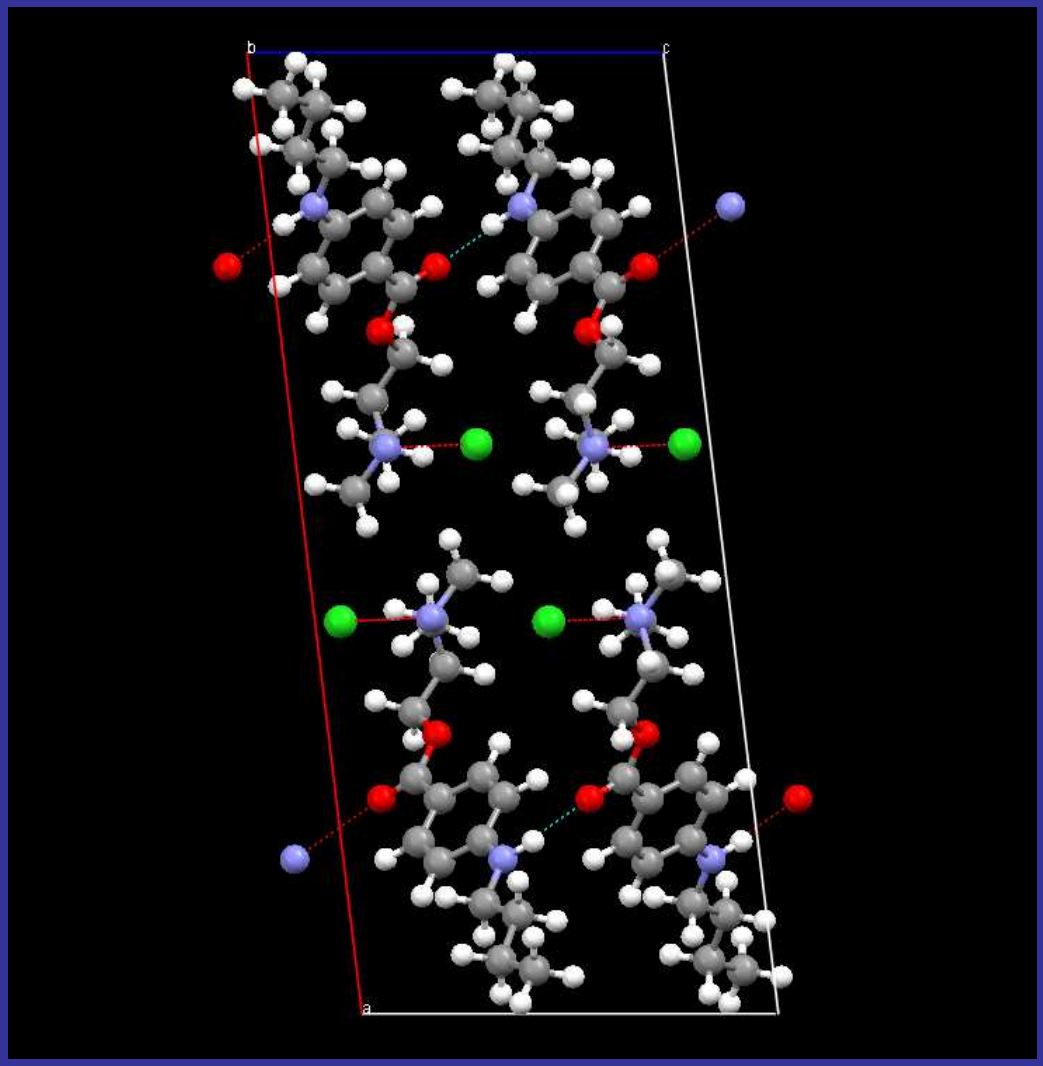
PMC :

Packing of
Molecules
in **C**rystals

(Dzyabchenko , 2008)
Russ. J. Phys. Chem.

- minimizes the energy of the lattice

- requires :
  - the unit cell
  - a rigid molecular model



III. Our Structure Solutions (5) : SOLVING THE STRUCTURE !

OUR STRATEGY :

(i) Fine-tune the solving procedure on a known case :

(Tetracaine Hydrochloride)

(ii) Transpose it to cases of similar complexity :

The two new polymorphs (Leocaine)

III. Our Structure Solutions (6) : SOLVING THE STRUCTURE !

EXPO-2009 ?

(i) DIRECT METHODS [Phase problem] FAIL

WHY : Not enough triplets / quartets …
… in the Lebail- extracted F(hkl)'s

(ii) SIMULATED ANNEALING FAILS (for now …)

WHY : Preferred Orientation not yet included …
… in the Monte-Carlo search

III. Our Structure Solutions (7) : SOLVING THE STRUCTURE !

FOX ! (Favre-Nicolin , Cerny)
J. Appl. Cryst. 2002

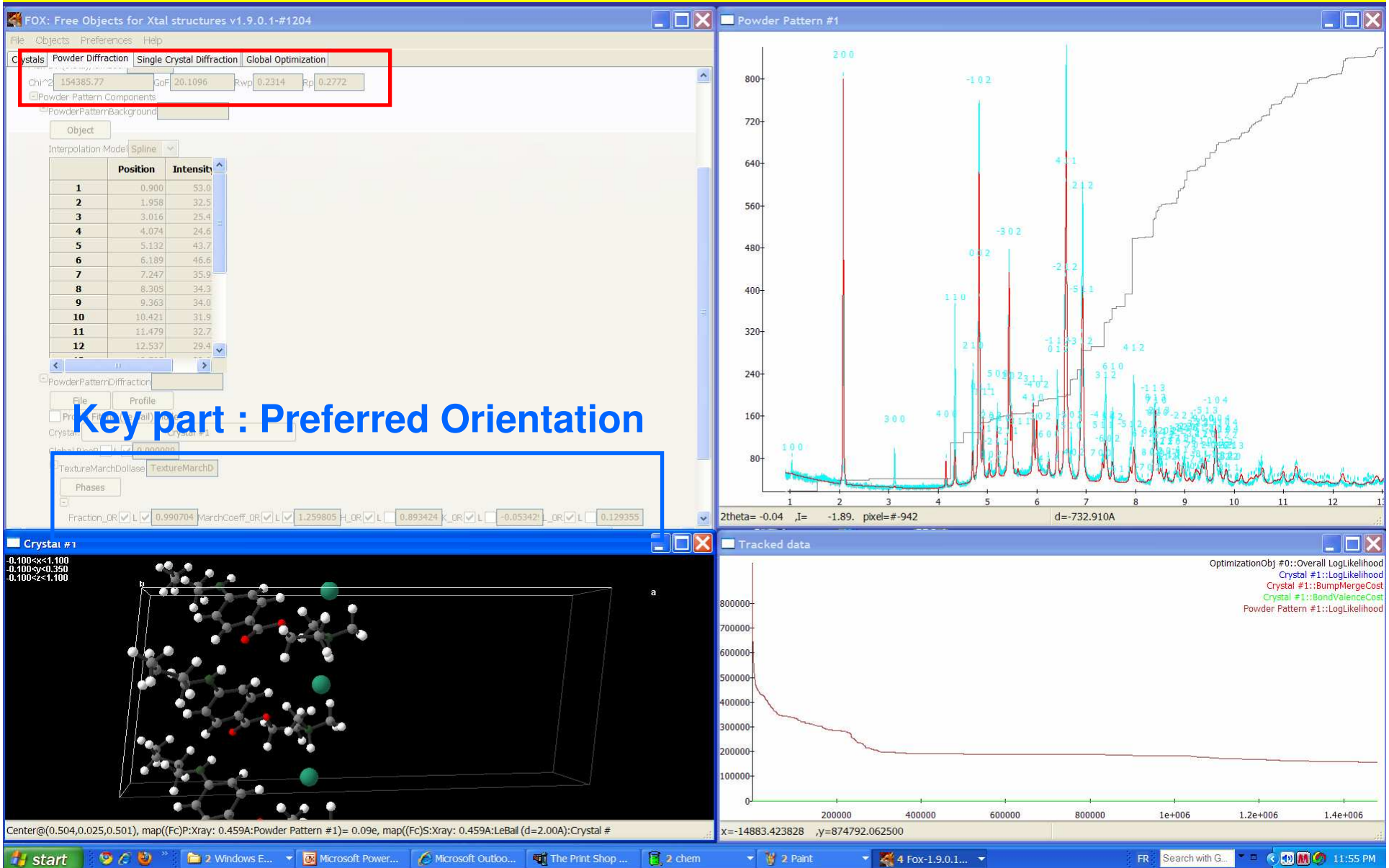
WHAT : DIRECT SPACE METHODS

HOW : Monte-Carlo Parallel Tempering
+
Relaxed Flexible Molecular Model

WHY : Preferred Orientation IS included …
… in the Monte-Carlo search

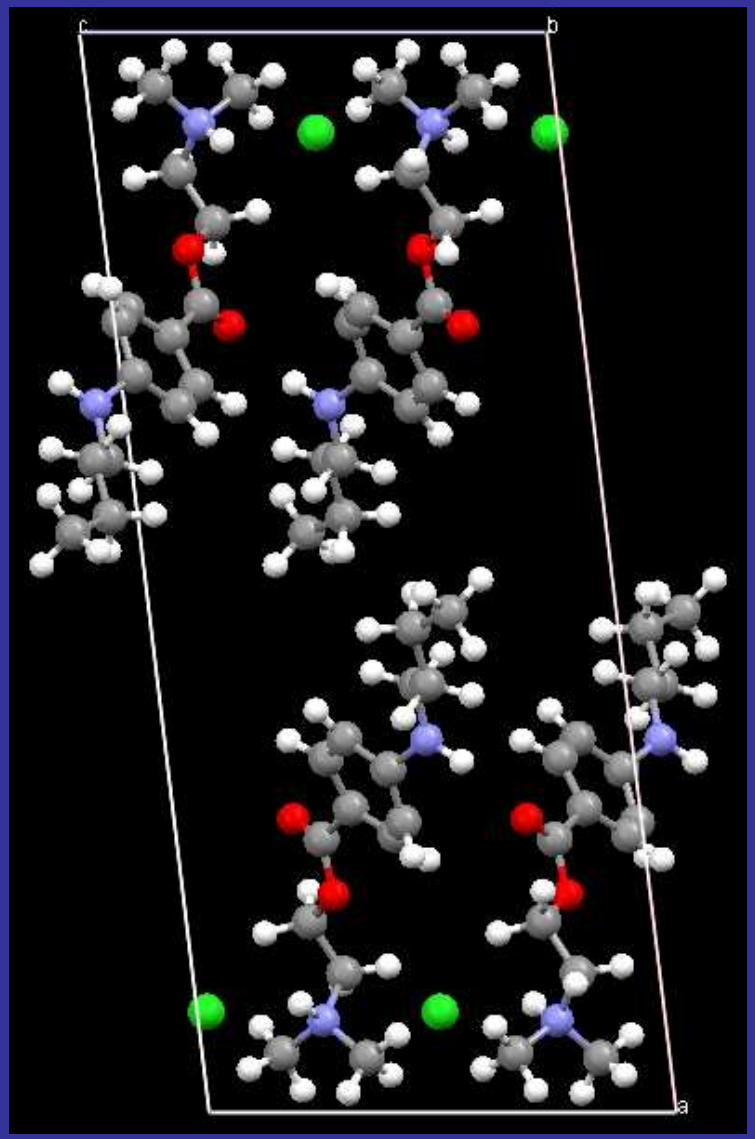
WORKS WITH TETRACAINE ⇙ WE TRY IT ON LEOCAINE

III. Our Structure Solutions (8) : A TYPICAL FOX SCREEN

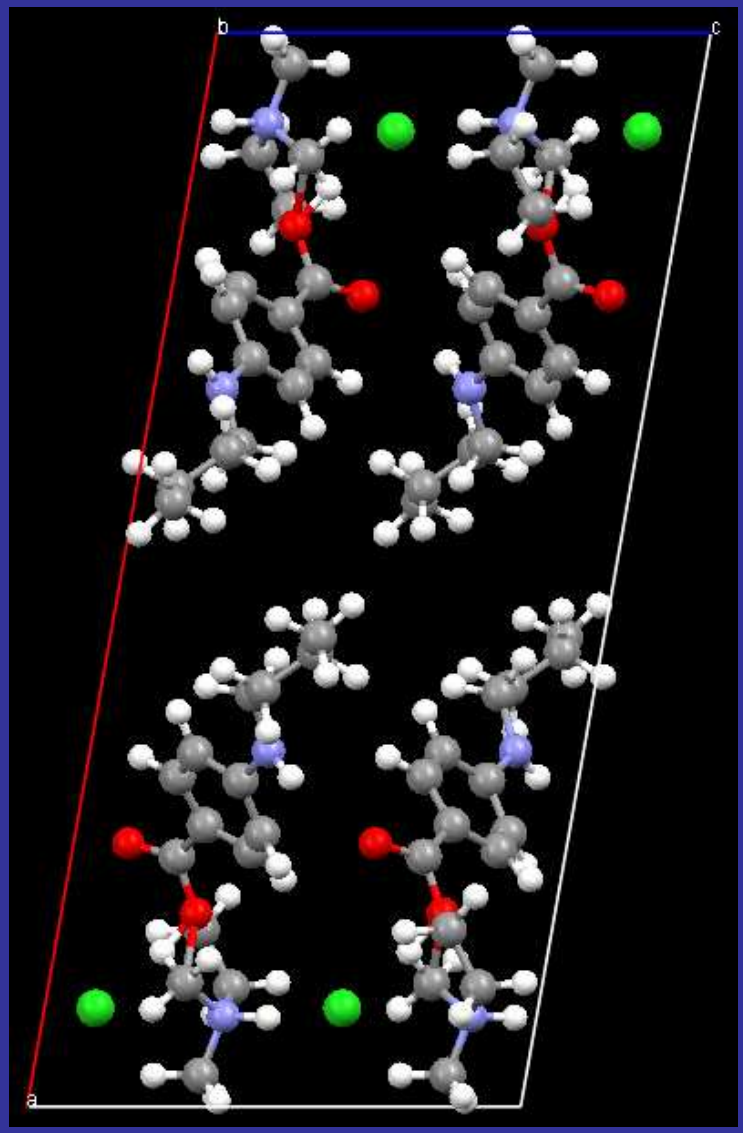


III. Our Structure Solutions (9) : FOX solutions for LEO

Polymorph # 1



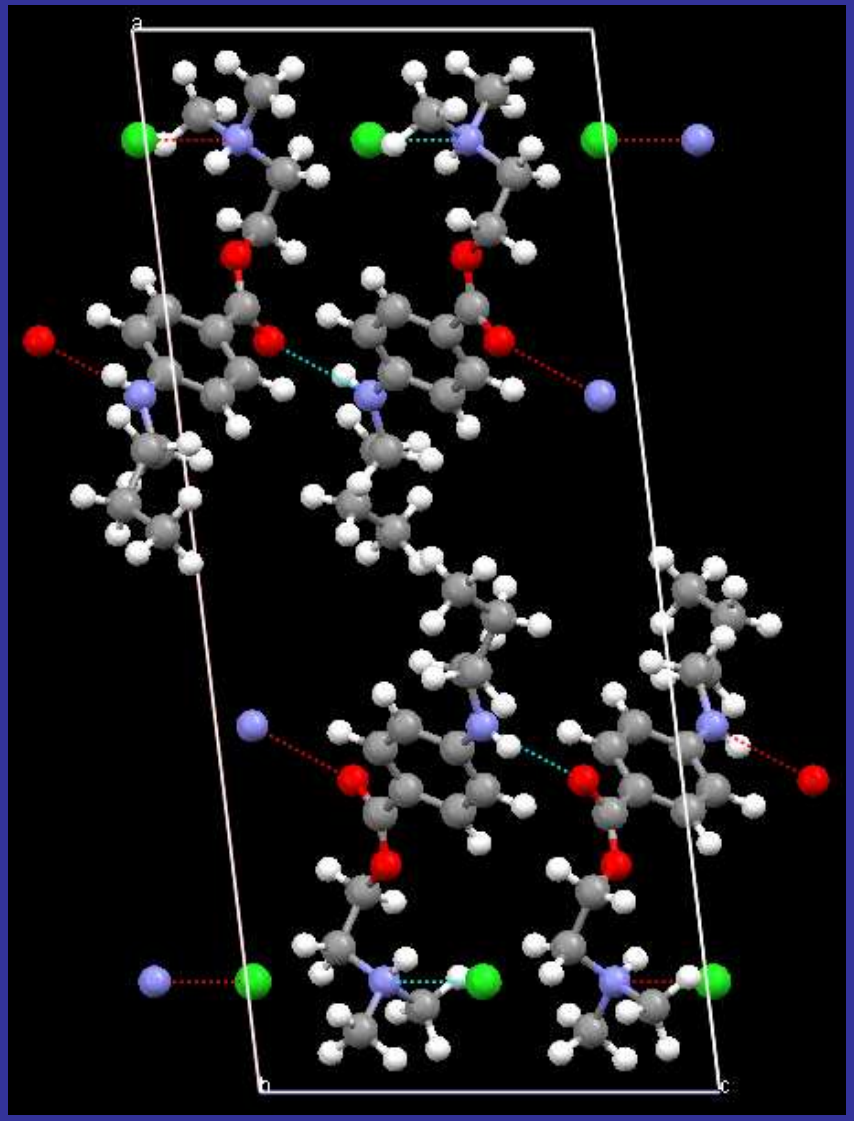
Polymorph # 2



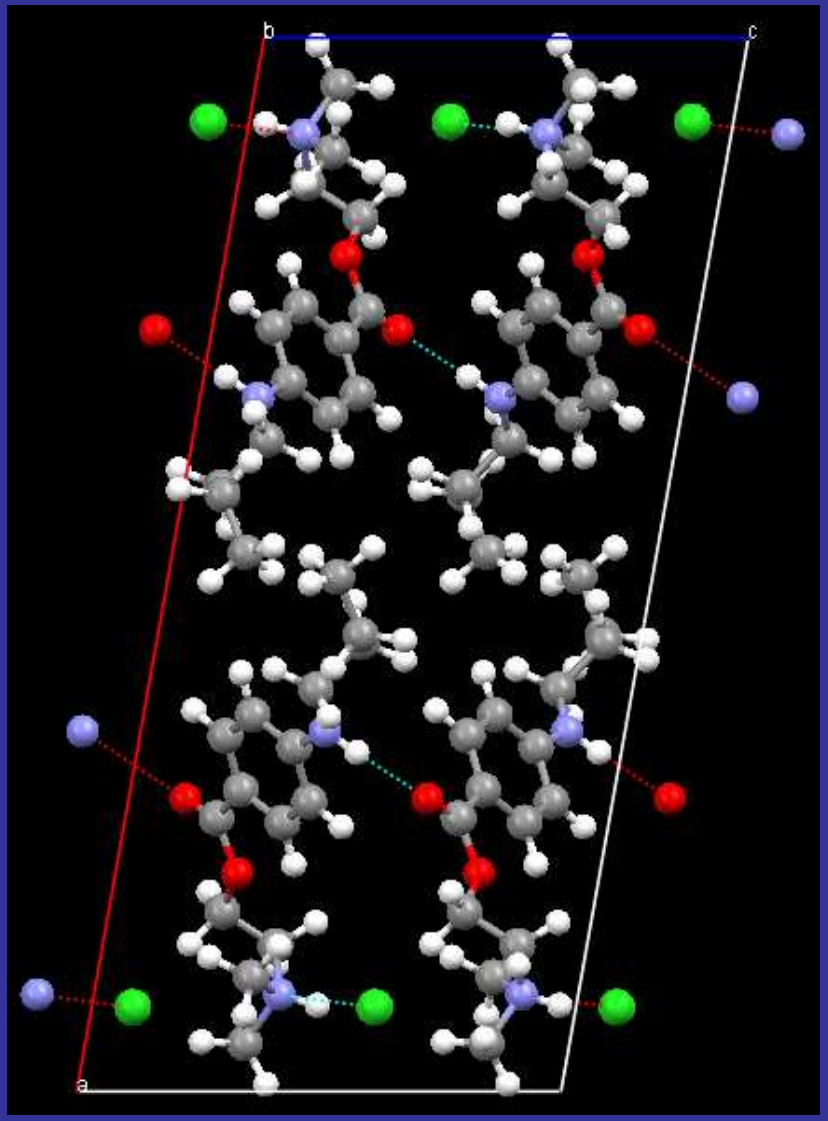
… after GSAS refinement

III. Our Structure Solutions (10) : FOX solutions for LEO

Polymorph # 1

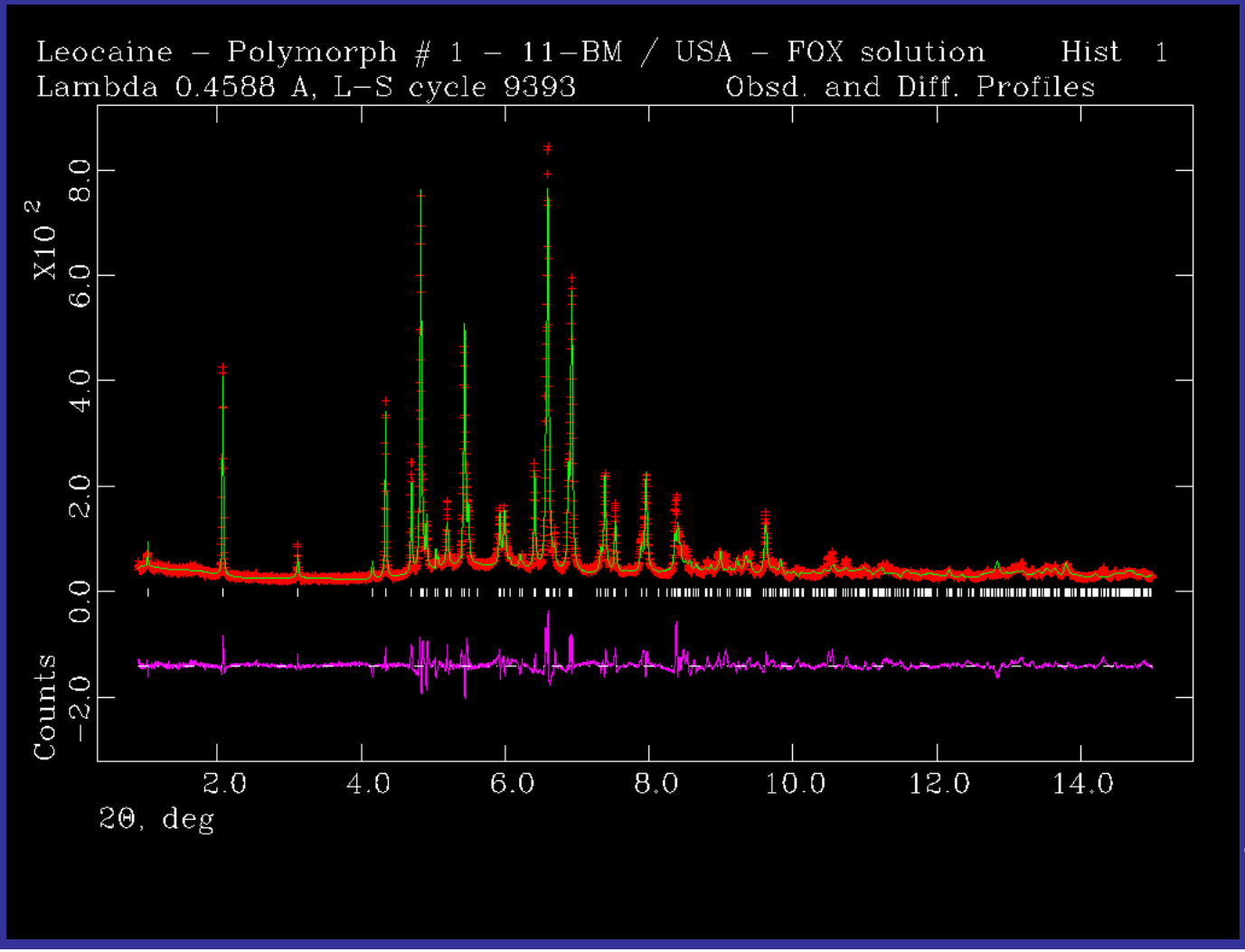


Polymorph # 2



III. Our Structure Solutions (11) : FOX solutions for LEO

Polymorph # 1 : GSAS-fit diffraction data



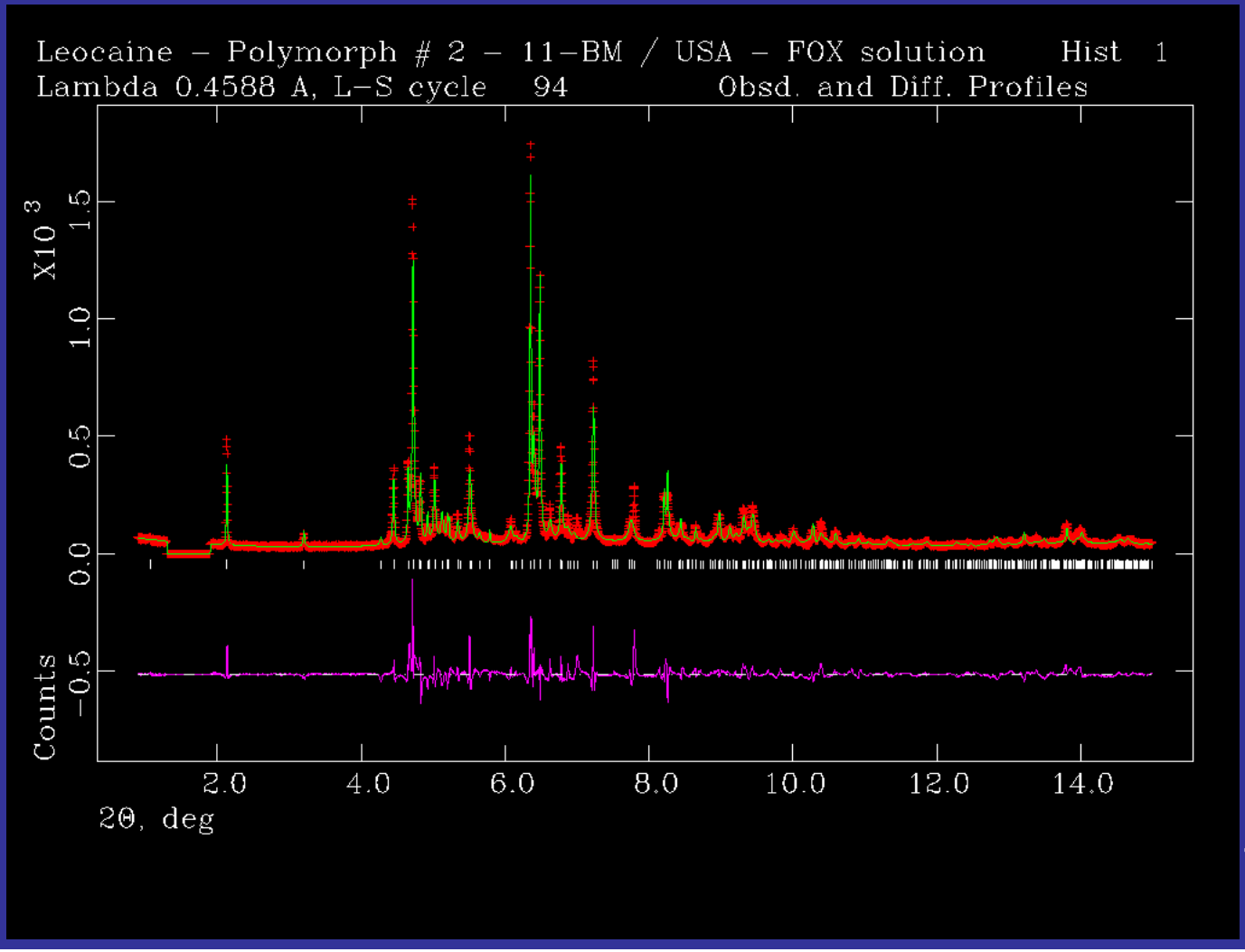
R_{wp} = 0.139

R_p = 0.104

156 variables

III. Our Structure Solutions (12) : FOX solutions for LEO

Polymorph # 2 : GSAS-fit diffraction data



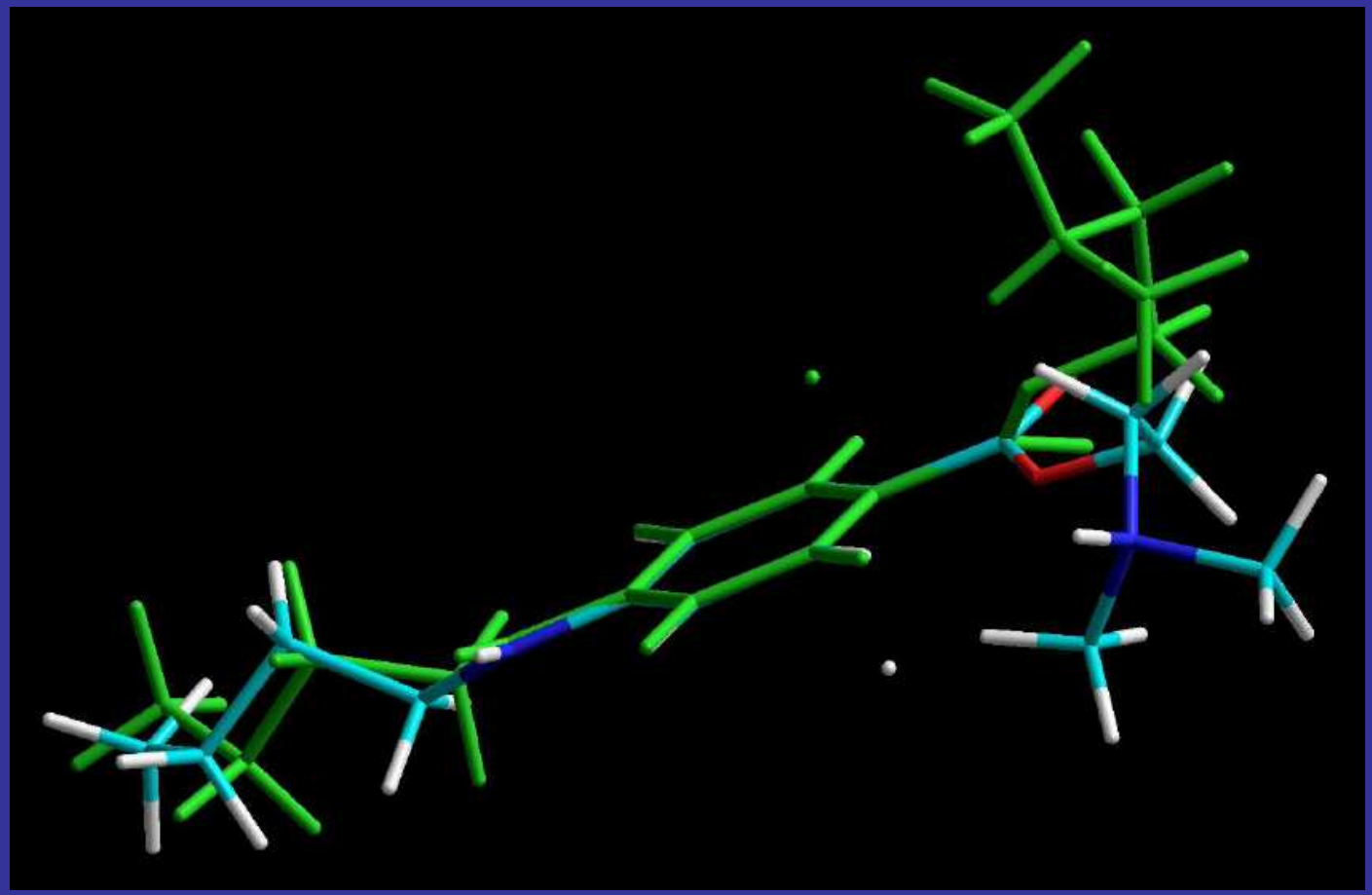
R_{wp} = 0.168

R_p = 0.131

144 variables

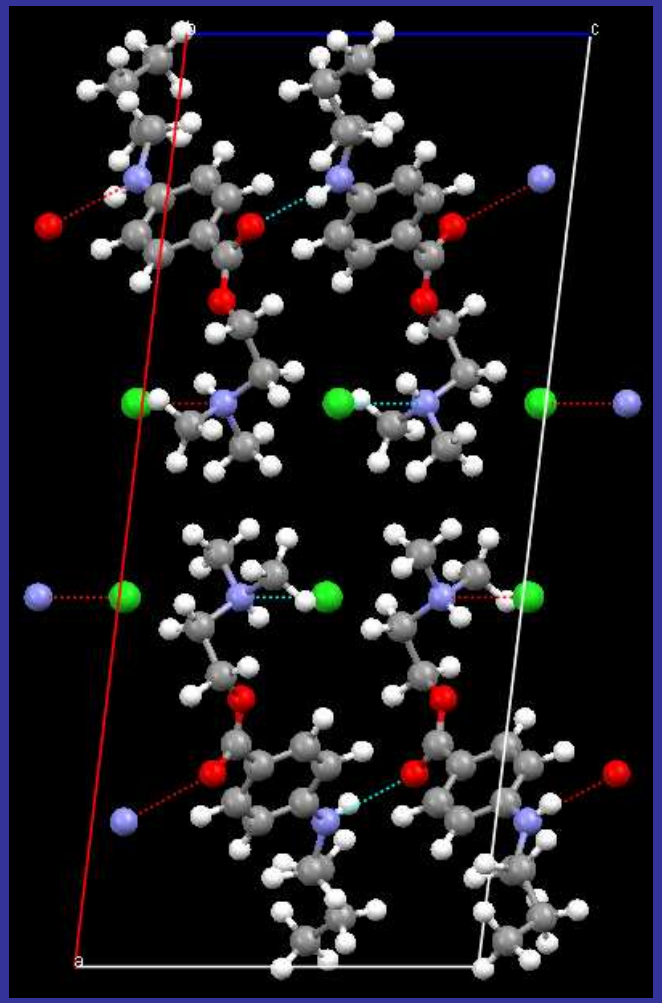
III. Our Structure Solutions (13) : FOX solutions for LEO

Polymorph # 1 versus Polymorph # 2

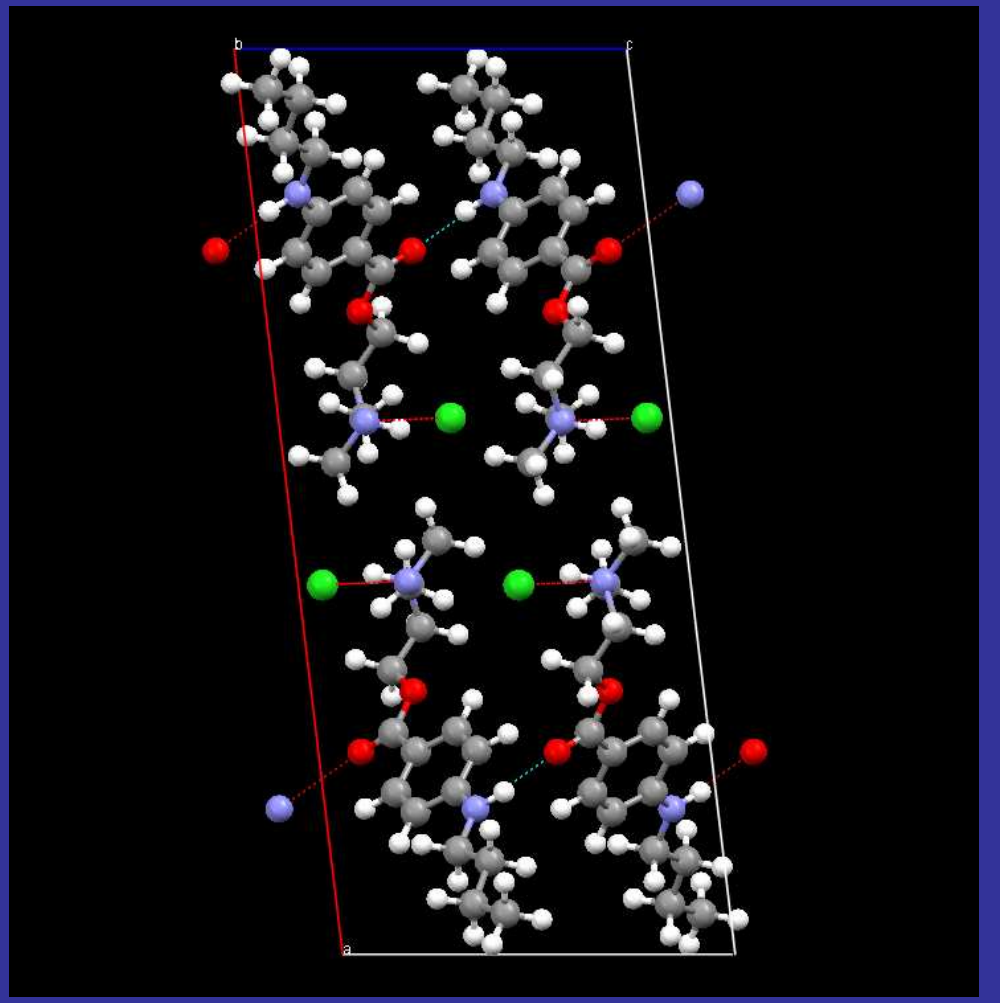


III. Our Structure Solutions (14) : Do they make sense ?

Polymorph # 1



PMC / THEORY



III. Our Structure Solutions (15) : Do they make sense ?

TETRACAINE

Fragment	Classification	No. of hits	Query value	Mean	Std. dev.	| z-score |	| x - mean |	Minimum	Maximum	Median	| d(min) |	Local de
C19 N11 C18	Not unusual (enough hits)	793	105.593	110.526	2.520	**1.958**	4.932	83.958	132.651	110.609	0.000	
C13 N12 C1	Not unusual (enough hits)	52	123.544	123.352	2.410	**0.080**	0.192	116.725	126.836	123.738	0.006	
C14 C13 N12	Not unusual (enough hits)	140	108.445	111.333	2.828	**1.021**	2.888	102.176	127.860	111.120	0.004	
C15 C14 C13	Not unusual (enough hits)	375	111.361	114.206	6.035	**0.472**	2.845	99.956	157.142	113.236	0.002	
C16 C15 C14	Not unusual (enough hits)	10000	110.364	113.643	8.886	**0.369**	3.279	28.671	180.000	113.132	0.001	
O8 C7 C4	Unusual (enough hits)	1592	117.284	112.266	1.707	**2.940**	5.017	96.366	128.246	112.168	0.001	
C9 C10 N11	Unusual (enough hits)	33	107.973	113.243	2.482	**2.123**	5.270	107.969	119.756	113.426	0.004	
C2 C1 N12 C13	Not unusual (enough hits)	371	-2.818								0.001	**0.353**
C6 C1 N12 C13	Not unusual (enough hits)	371	176.112								0.000	**0.388**
O8 C7 C4 C3	Not unusual (enough hits)	10000	179.083								0.002	**0.377**
O17 C7 C4 C3	Not unusual (enough hits)	10000	6.007								0.002	**0.434**
O8 C7 C4 C5	Not unusual (enough hits)	10000	-1.982								0.001	**0.388**
O17 C7 C4 C5	Not unusual (enough hits)	10000	-175.058								0.002	**0.418**
C9 O8 C7 C4	Not unusual (enough hits)	2181	176.771								0.000	**0.969**
C9 O8 C7 O17	Not unusual (enough hits)	2405	-10.361								0.005	**0.887**
C7 O8 C9 C10	Not unusual (enough hits)	191	-171.505								0.000	**0.492**
O8 C9 C10 N11	Not unusual (enough hits)	590	-73.342								0.001	**0.205**
C14 C13 N12 C1	Not unusual (enough hits)	112	175.351								0.001	**0.518**
C15 C14 C13 N12	Not unusual (enough hits)	1309	178.668								0.003	**0.517**
C13 C14 C15 C16	Not unusual (enough hits)	10000	-177.304								0.001	**0.668**
C9 C10 N11 C18	Unusual (enough hits)	92	94.607								0.001	**0.033**
C9 C10 N11 C19	Unusual (enough hits)	92	-149.398								0.003	**0.043**

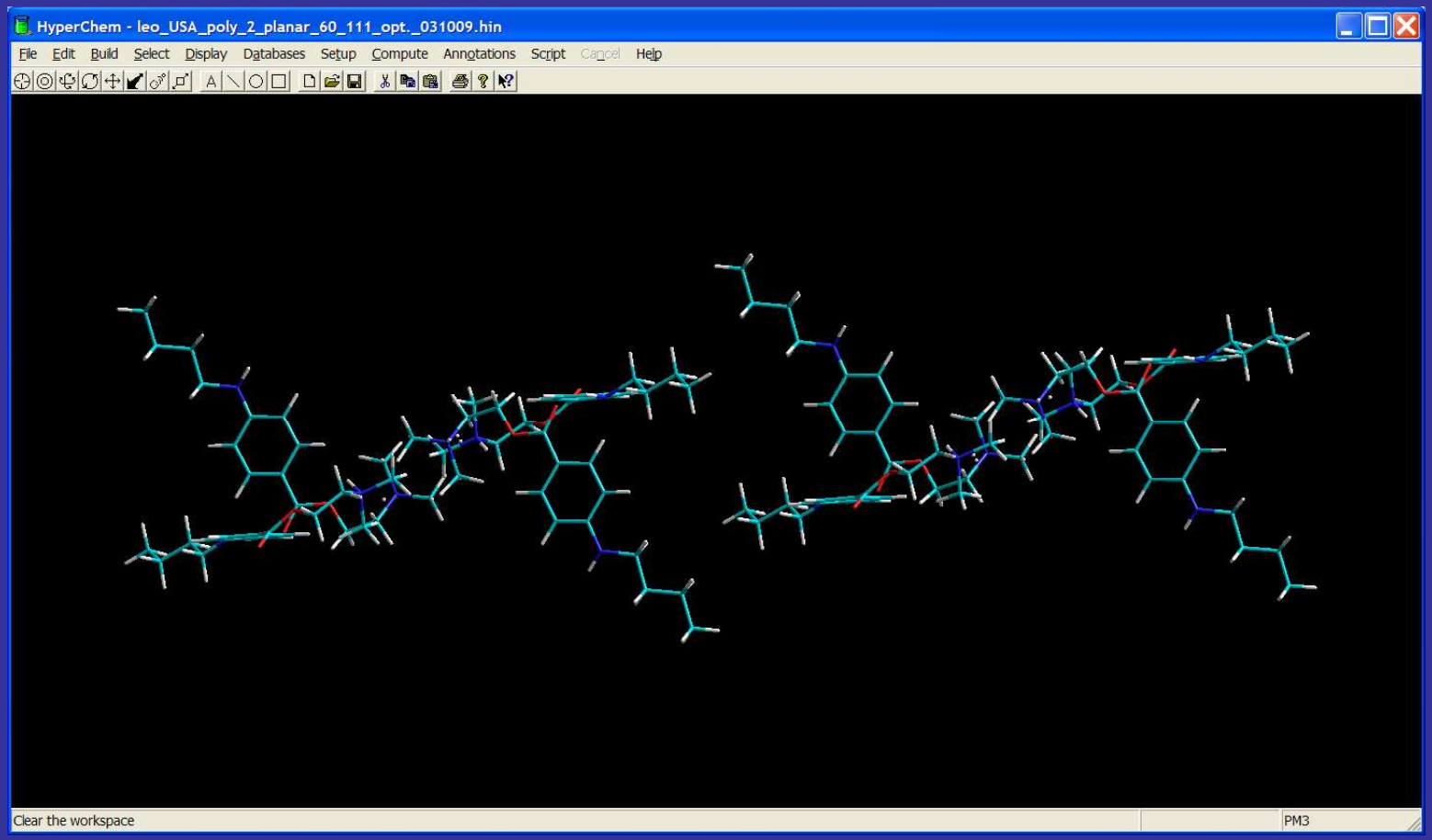
MOGUL
[CCDC]

LEO Polymorph # 1

Type	Molecule	Fragment	Classification	No. of hits	Query value	Mean	Std. dev.	| z-score |	| x - mean |	Minimum	Maximum	Median	| d(min) |	Local de
		O1 C7 C4	Unusual (enough hits)	1592	119.430	112.266	1.707	**4.197**	7.164	96.366	128.246	112.168	0.034	
		C8 O1 C7	Unusual (enough hits)	626	123.960	116.351	2.206	**3.449**	7.608	97.347	127.366	116.375	0.202	
		C8 C9 N1	Unusual (enough hits)	33	108.056	113.243	2.482	**2.090**	5.187	107.969	119.756	113.426	0.087	
⊟ torsion														
	⊟ 0001_1													
		C8 O1 C7 C4	Not unusual (enough hits)	2181	179.760								0.001	**0.939**
		C8 O1 C7 O2	Not unusual (enough hits)	2405	0.761								0.001	**0.938**
		O1 C8 C9 N1	Not unusual (enough hits)	590	58.275								0.041	**0.615**
		C8 C9 N1 C14	Not unusual (enough hits)	92	-165.363								0.153	**0.348**
		C8 C9 N1 C15	Not unusual (enough hits)	92	77.700								0.310	**0.120**
		C2 C1 N2 C10	Not unusual (enough hits)	371	20.482								0.847	**0.156**
		C6 C1 N2 C10	Not unusual (enough hits)	371	-159.503								0.158	**0.154**
		C11 C10 N2 C1	Not unusual (enough hits)	112	-150.084								1.061	**0.098**
		O1 C7 C4 C3	Unusual (enough hits)	10000	144.315								0.058	**0.006**
		O2 C7 C4 C3	Unusual (enough hits)	10000	-36.649								0.147	**0.011**
		O1 C7 C4 C5	Unusual (enough hits)	10000	-36.344								0.026	**0.007**
		O2 C7 C4 C5	Unusual (enough hits)	10000	142.692								0.036	**0.011**
		C7 O1 C8 C9	Unusual (enough hits)	191	137.257								0.725	**0.026**
		C12 C11 C10 N2	Unusual (enough hits)	1309	-140.048								5.325	**0.002**
		C10 C11 C12 C13	Unusual (enough hits)	10000	-120.937								0.056	**0.010**

III. Our Structure Solutions (16) : Are they stable ?

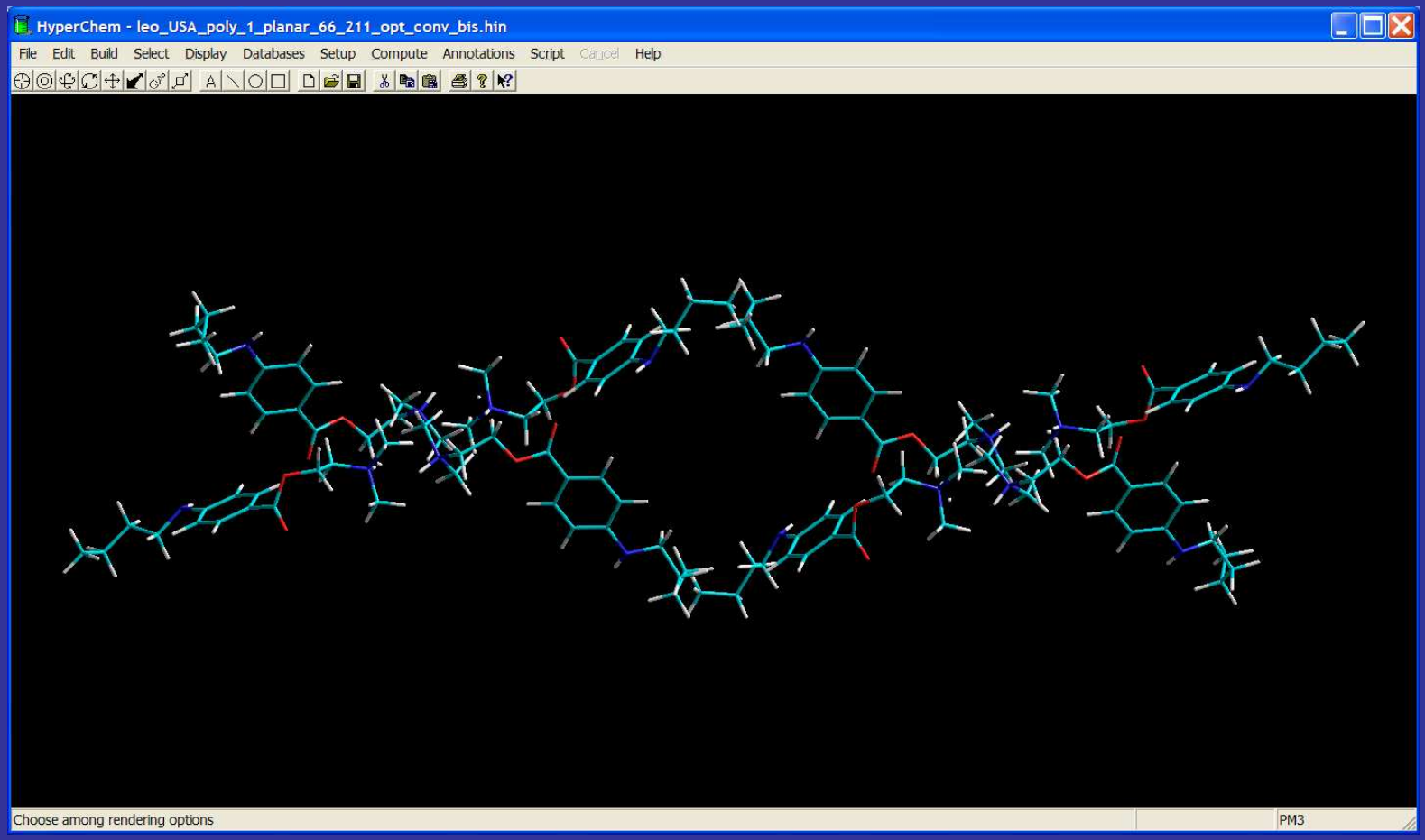
LEOCAINE polymorph # 1



Hyperchem / PM3

III. Our Structure Solutions (17) : Are they stable ?

LEOCAINE polymorph # 2



Hyperchem / PM3

III. Our Structure Solutions (18): Intramolecular H-bonds ?

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(2000)

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Gregory P. Shields[b] and
Judith A. K. Howard[a]*

Intramolecular hydrogen bonds: common motifs, probabilities of formation and implications for supramolecular organization

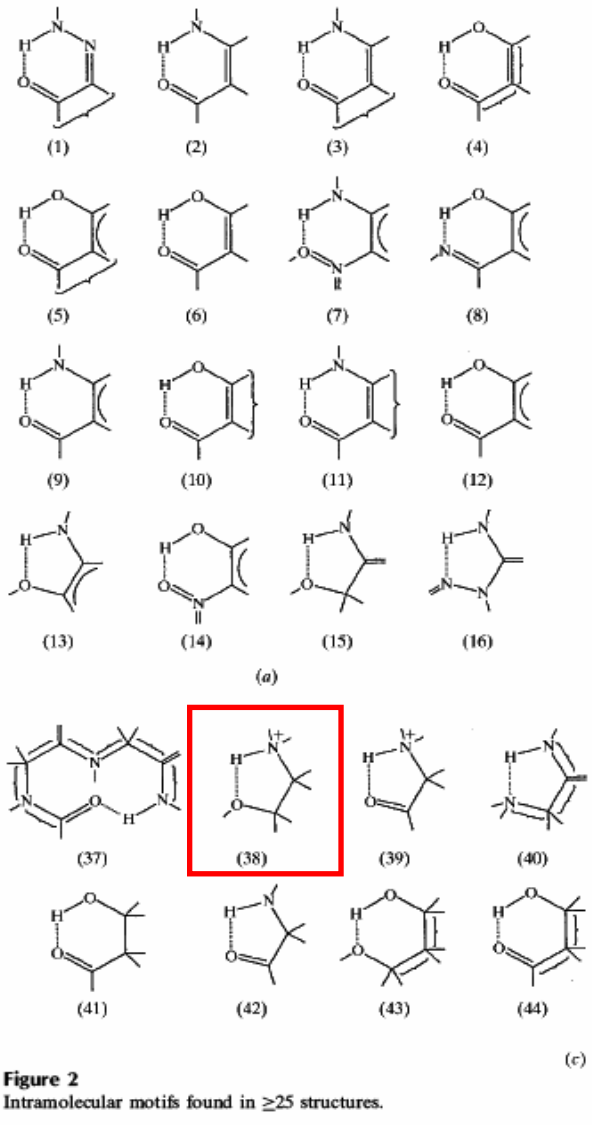
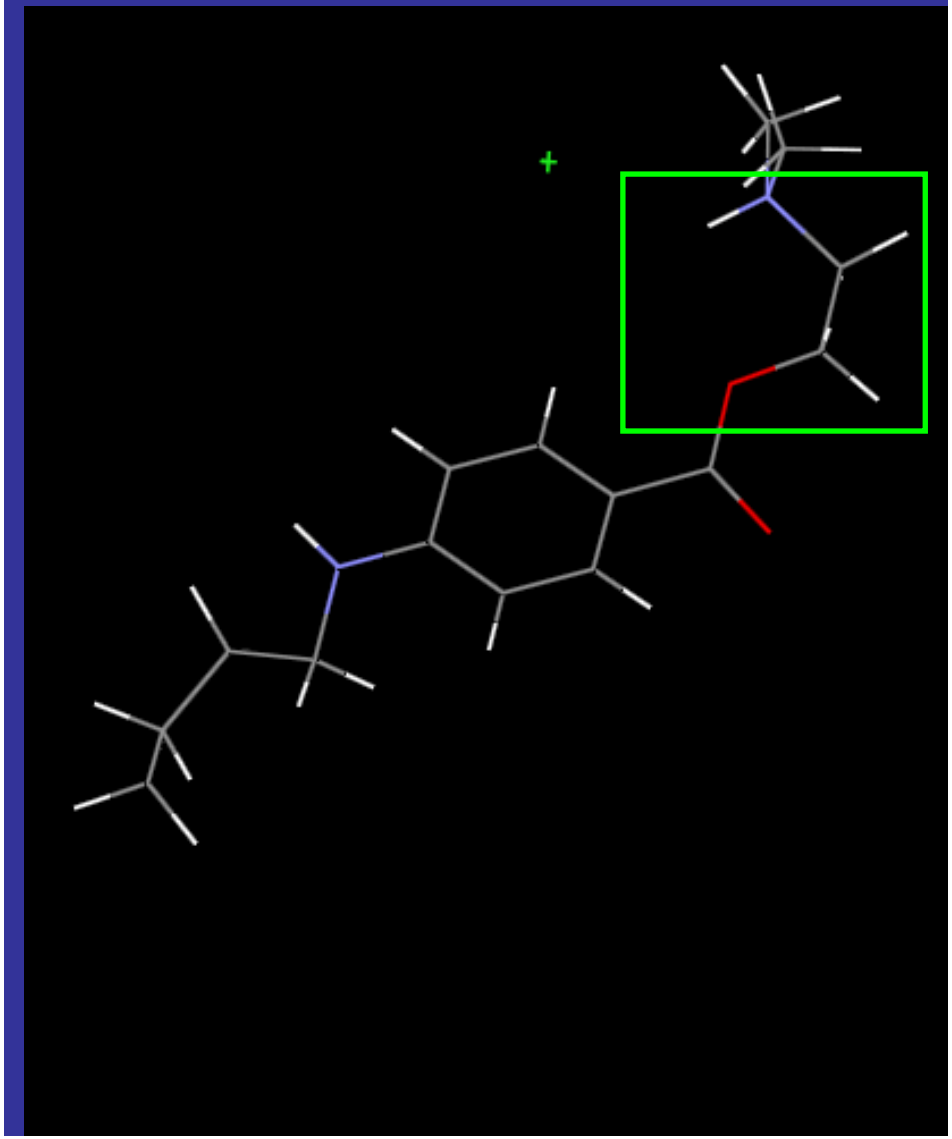


Figure 2
Intramolecular motifs found in ≥25 structures.

Leocaine : poly # 1



CONCLUSION

- **Freeze-drying** TCHC generates a new unknow polymorph:
  Leocaine #1

- **Leocaine #1** exposure to **intense X-ray beams** [synchrotron , 27 keV]
  produces a second new polymorph: **Leocaine #2**

- **Structure solution** with :
  Index [**N-TREOR-09** / **EXPO-2009**] ; Solve [**FOX**] ; Refine [**GSAS**]

- **FOX** requires a **flexible molecular model**:
  (i) Molecular Modeling **(ii)** XRPD [TCHC , 2002] **(iii)** SXD [TCHC]

- Why **FOX** works better than **EXPO-2009** on Leocaine …
  … **Preferred Orientation** is included in the Monte-Carlo search

- **Next : observe** Leocaine #1 ⇄ Leocaine #2 **transition in situ**

THANK YOU :

- **Prof. Carmelo GIACOVAZZO** : IC-CNR, U. Bari, Italy
  - N-TREOR-09
  - EXPO-2009
  - IL MILIONE

- **Free Web resources** : GSAS [R. Von Dreele]
  - FOX [V. Favre-Nicolin]
  - MERCURY [CCDC]

Last but not least :

ICDD staff : Newton Square, PA, USA
my participation …