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Structure and polymorphism of *trans* mono-unsaturated triacylglycerols and related fully saturated TAGs

Rene Peschar

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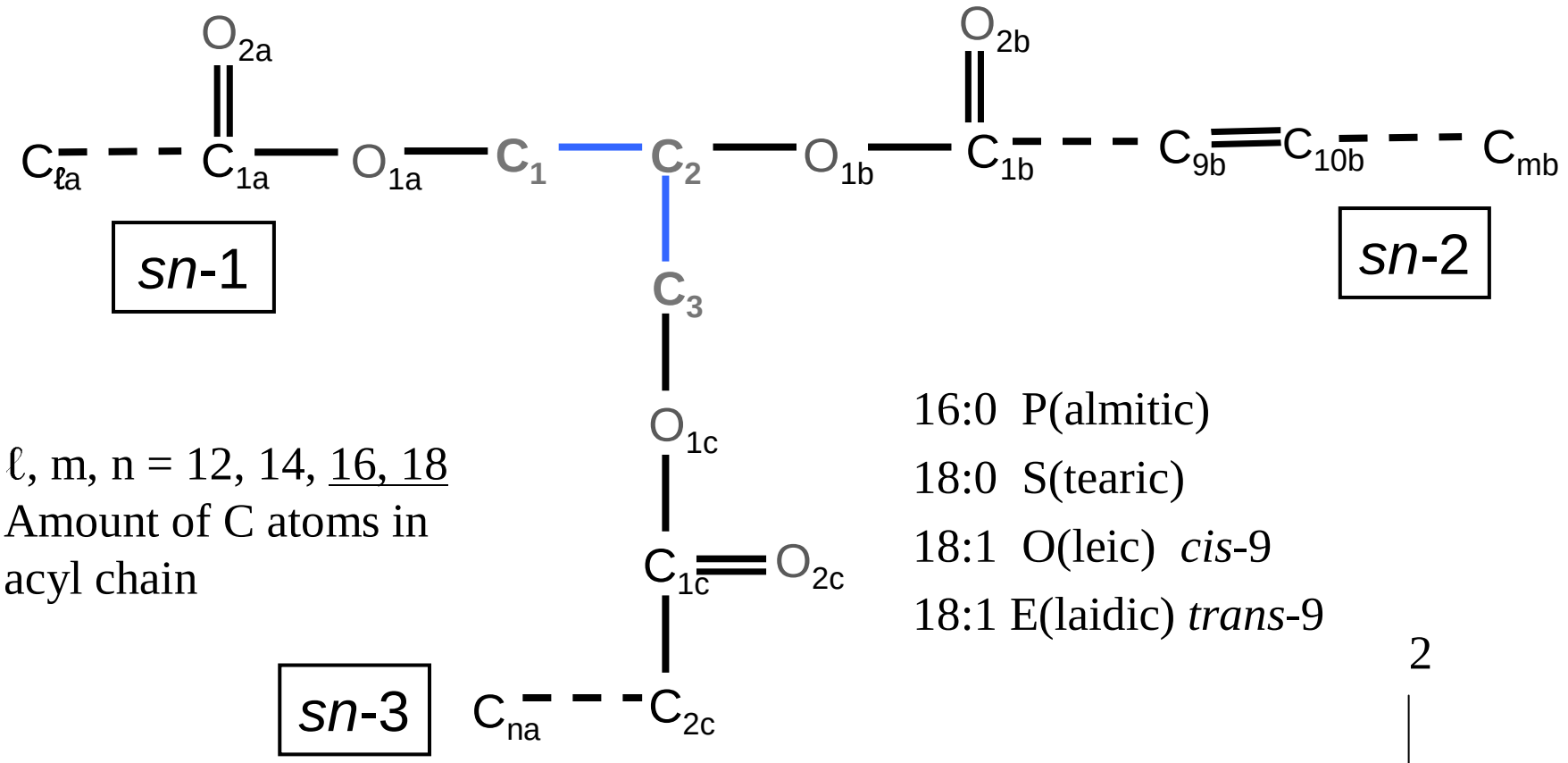
- Introduction
- Time-resolved X-ray powder diffraction (XRPD)
  -Phase transitions and stability of TAG polymorphs
- Structure determination from XRPD data
  - β -2 polymorphs
  - β'-2 polymorphs
- Conclusions

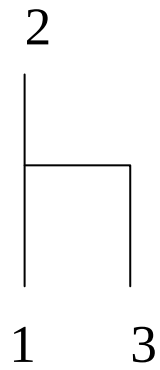
Crystallisation and Physical Properties of Fats:from Molecules to Market; Gent, june 18-19, 2008

Introduction. Triacylglycerol (TAG) conformations



ℓ, m, n = 12, 14, 16, 18
Amount of C atoms in acyl chain

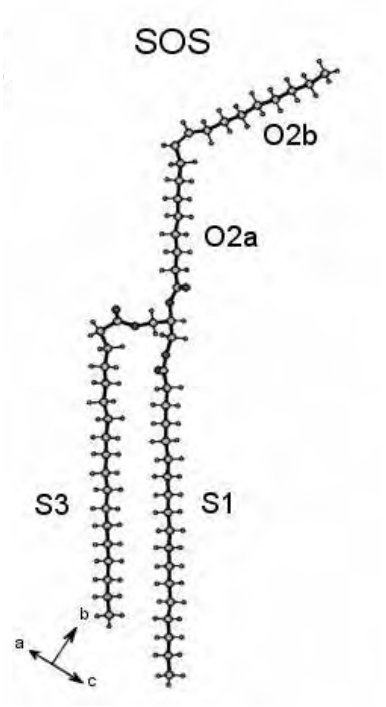
16:0 P(almitic)
18:0 S(tearic)
18:1 O(leic) *cis*-9
18:1 E(laidic) *trans*-9



e.g. [1-3] conformation sn-1 and sn-3 in the same direction

Introduction. Trans fatty acid

Hydrogenation changes cis un-saturated fatty acid chains partly into *trans* isomers



O -> S

Oleic
18:1
Cis-9

Stearic
(18:0)

E

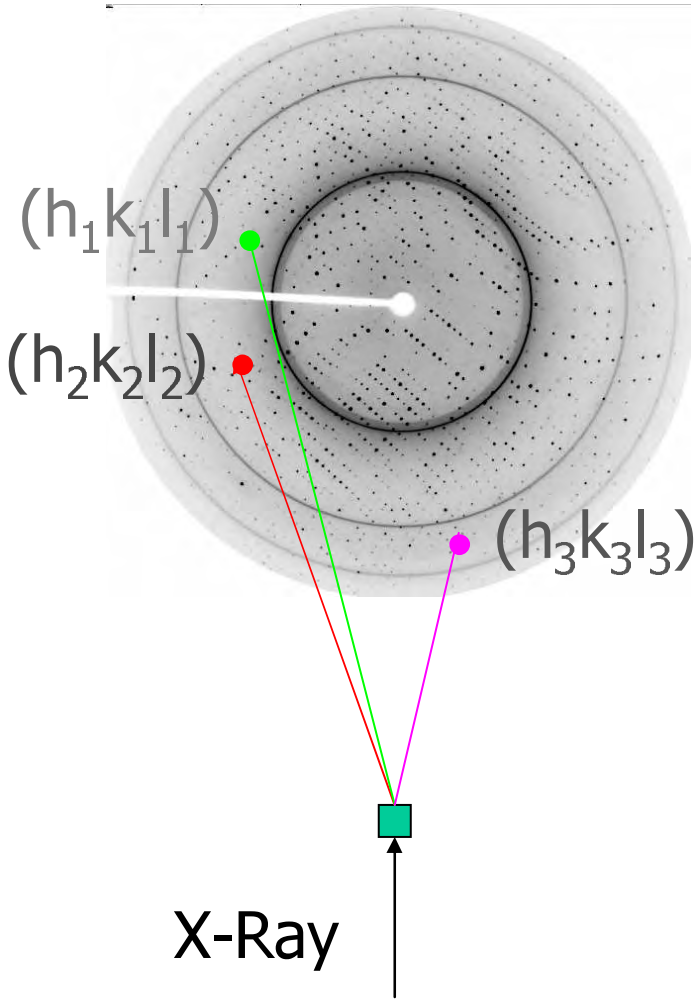
Elaidic

18:1 *trans*-9

[1-3] conformation

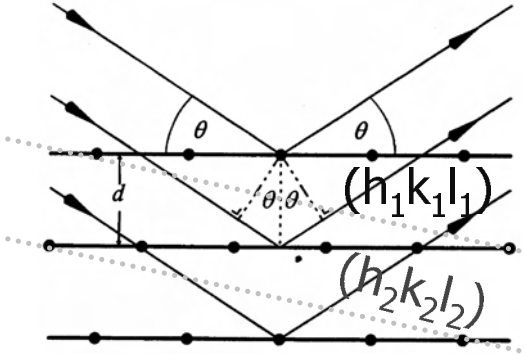
- What is the influence of the *trans* fatty acid elaidic acid on the packing and stability of TAG polymorphs ??
- To which extent can E replace an S?

Introduction. X-ray diffraction



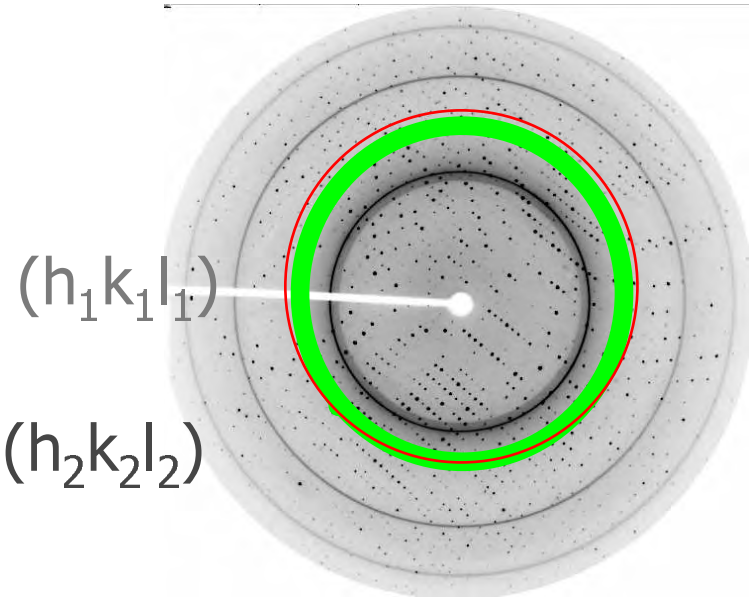
•Crystal: regular 3D stacking of identical units

•X-rays on crystal => diffraction
Bragg's Law : $2d_{hkl}\sin(\theta) = \lambda$

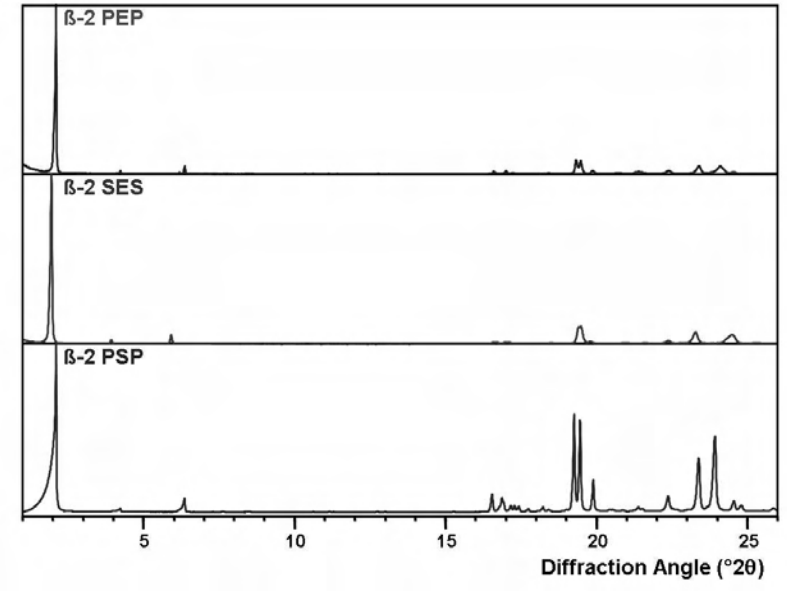


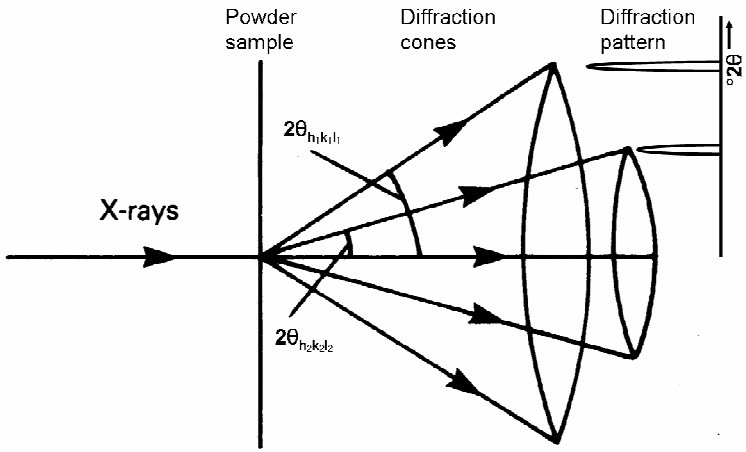
• (h k l) lattice plane or reflection

Introduction X-ray powder diffraction



Low angle Fingerprint



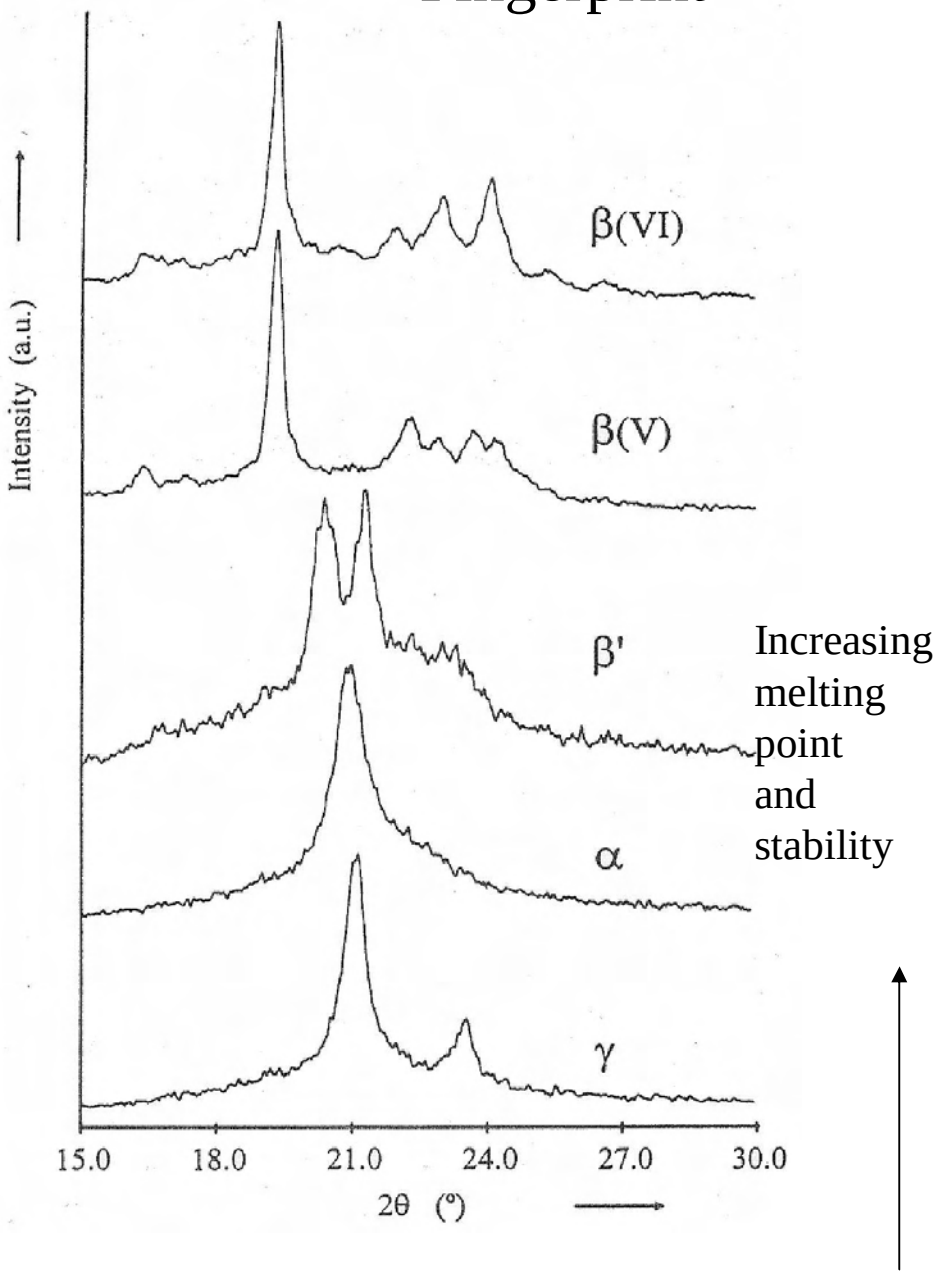


Powder = large set of small crystals
(< 10 µm)
Reflections from different crystal planes overlap

XRPD polymorph identification

Polymorph	Long spacing (Å)	Short spacings (Å)		
γ	52.4		4.14	3.69
α	48.4		4.18	
β' – 2	45.0		4.33	4.14
β-V = β_2-3	64.8	4.58	3.99	3.87 3.76 3.67
β-VI = β_1-3	64.4	4.58	4.03	3.85 3.69

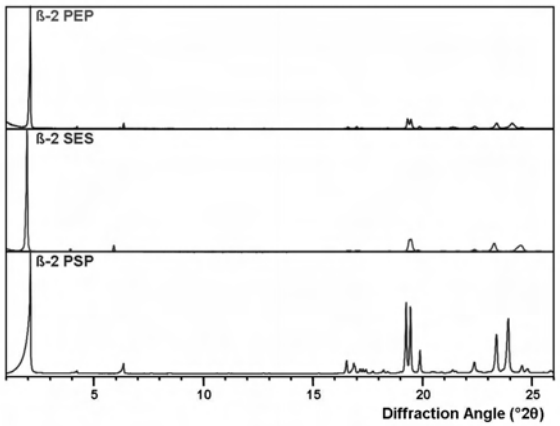
Fingerprint



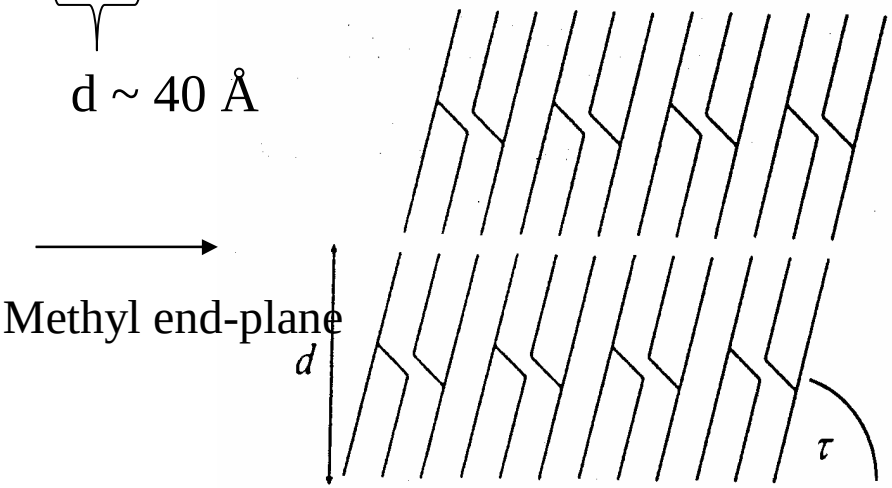
λ = 1.5406 Å

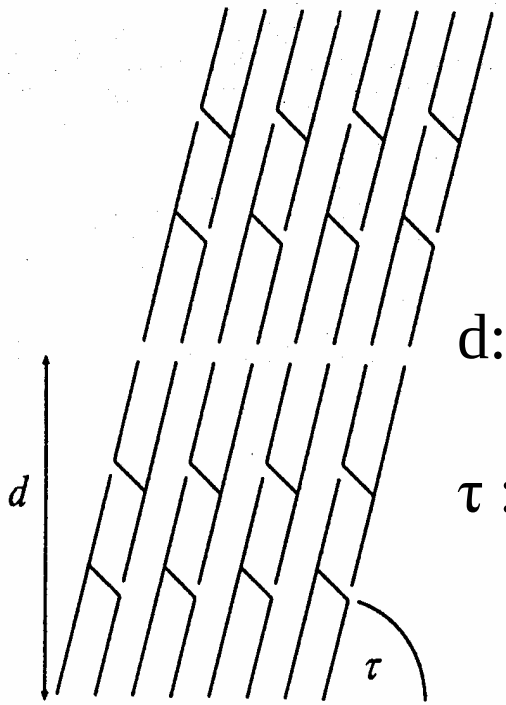
XRPD β' and β chain-length packing

Left: Double chain-length seat-facing chairs (e.g β-2)
Right: Triple chain-length packing (e.g. β-3)



d ~ 40 Å





d: Long d-spacing (70 Å)
τ : Tilt angle

Polymorphs of investigated TAGs

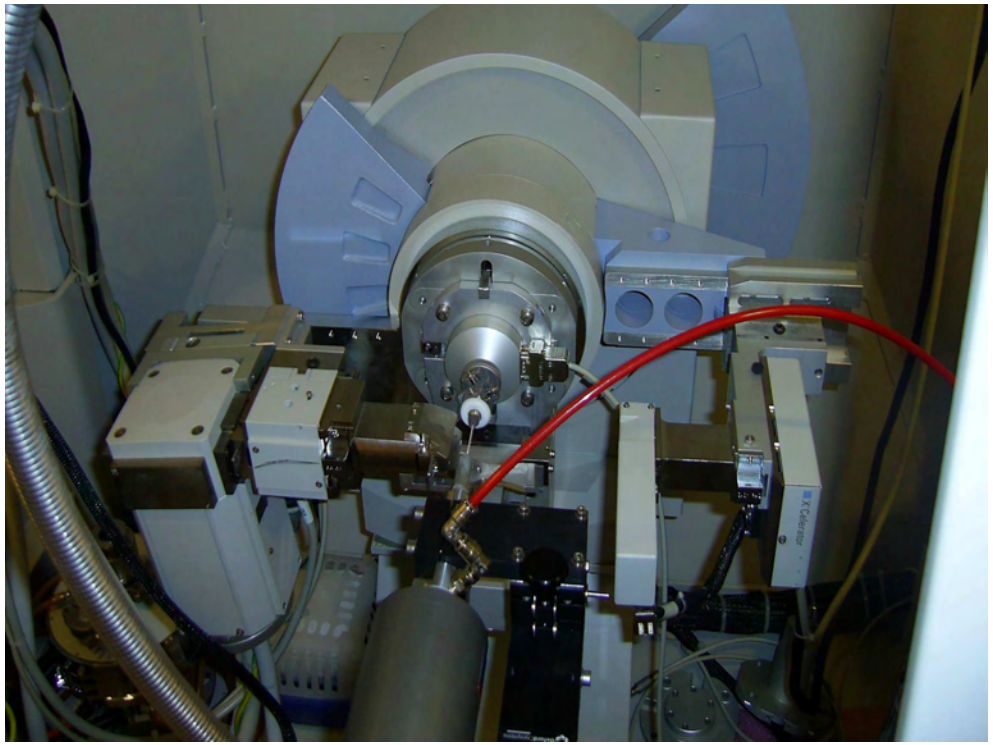
increasing stability →

TAG	α	β'$_2$-2	β'$_1$-2	β-2
PEP		MS	Stable	Exists, but lower melting!
PSP		MS	Stable	Exists, but lower melting!
PPE		MS	MS	Stable
PPS		MS	MS	Stable
PSS		MS	MS	Stable

MS: Metastable
Not reported in literature

- Are these all the possible polymorphs?
- What is the difference between β'$_2$-2 and β'$_1$-2?
- PEP and PSP are β' stable but a lower melting β exists?
- What is the packing of the polymorphs ?
- What is the influence of replacing E by S?

Time- and temperature resolved XRPD PANalytical X'pert Pro MPD



- Elliptical focusing mirror
- Soller slits (prim., sec. 0.02 rad div)
- X'celerator strip detector
- Oxford Instruments Cryostream Compact

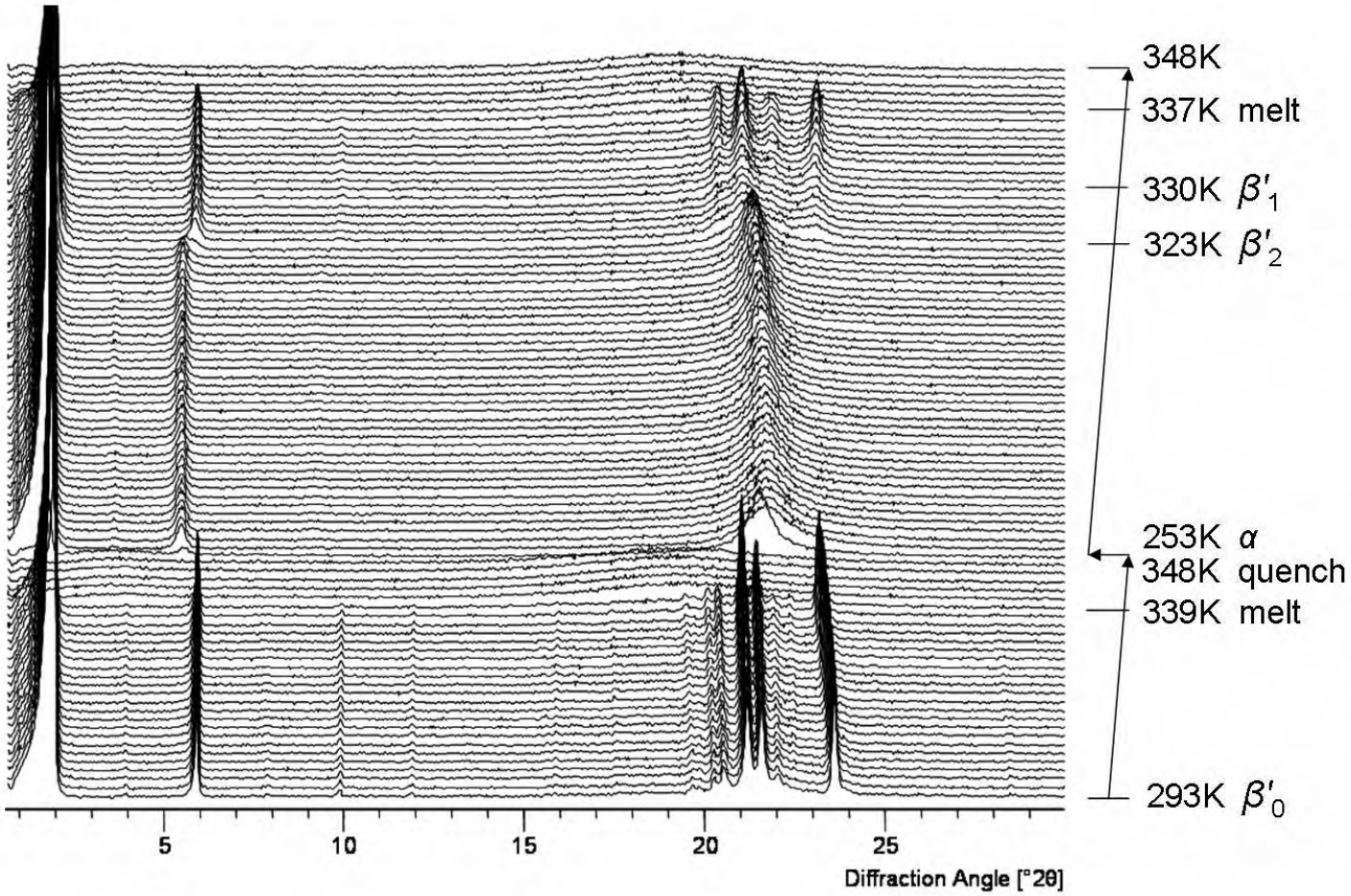
- N_2 stream parallel to capillary
- Cylindrical polymer film

Cu Kα, high flux

Typical scan settings: 1 min. 0.5-30 °2θ step 0.016 °2θ
Heating and cooling rates: 0.5 – 6/30 K min^{-1}

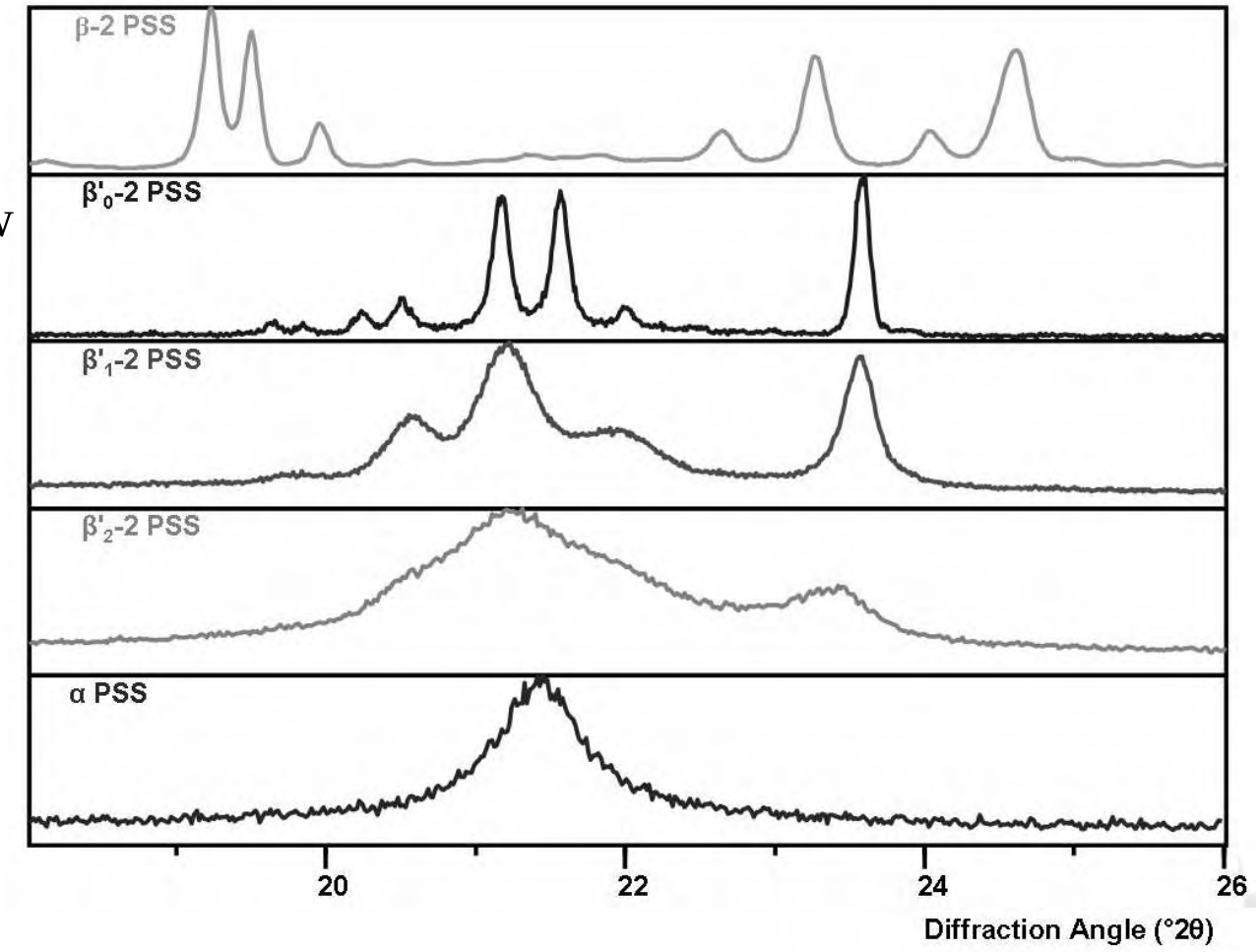
Melt and crystallization of polymorphs of PSS

quenching (30 K min^{-1})
heating (0.5 K min^{-1})



Fingerprint area of polymorphs of PSS

Very slow



Increasing Melting point

- β'_1-2 is a higher crystalline form of β'_2-2
- β'_0-2 is a novel polymorph

Phase transitions and stability of polymorphs

Melting points and transition temperatures in K

Heating rate 0.5 $Kmin^{-1}$

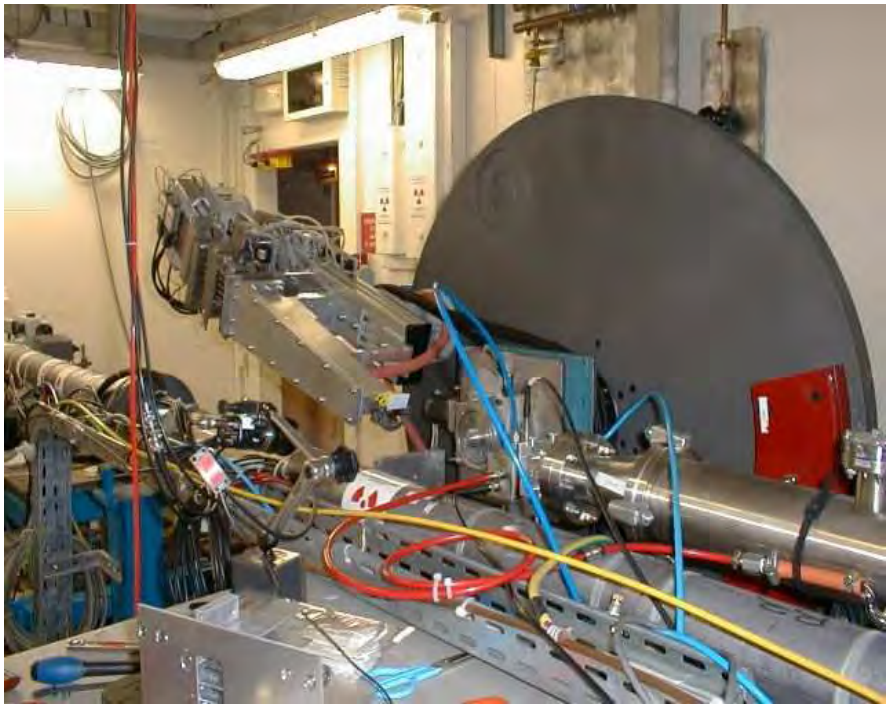
TAG	Transition α to β'_2-2	transition β'_2-2 to β'_1-2	melt β'_1-2	melt β'_0-2	melt β-2	
PEP	303	312	329	-	327	
PSP	317	319	343	-	339.5	β' stable
PPE	304	311	320	-	320	
PPS	321	323	332	-	338	β stable
PSS	323	330	337	339	339	

- Asymmetric β'_2-2 TAGs melt faster
- No significant difference E vs. S

- E stabilizes β'_2-2

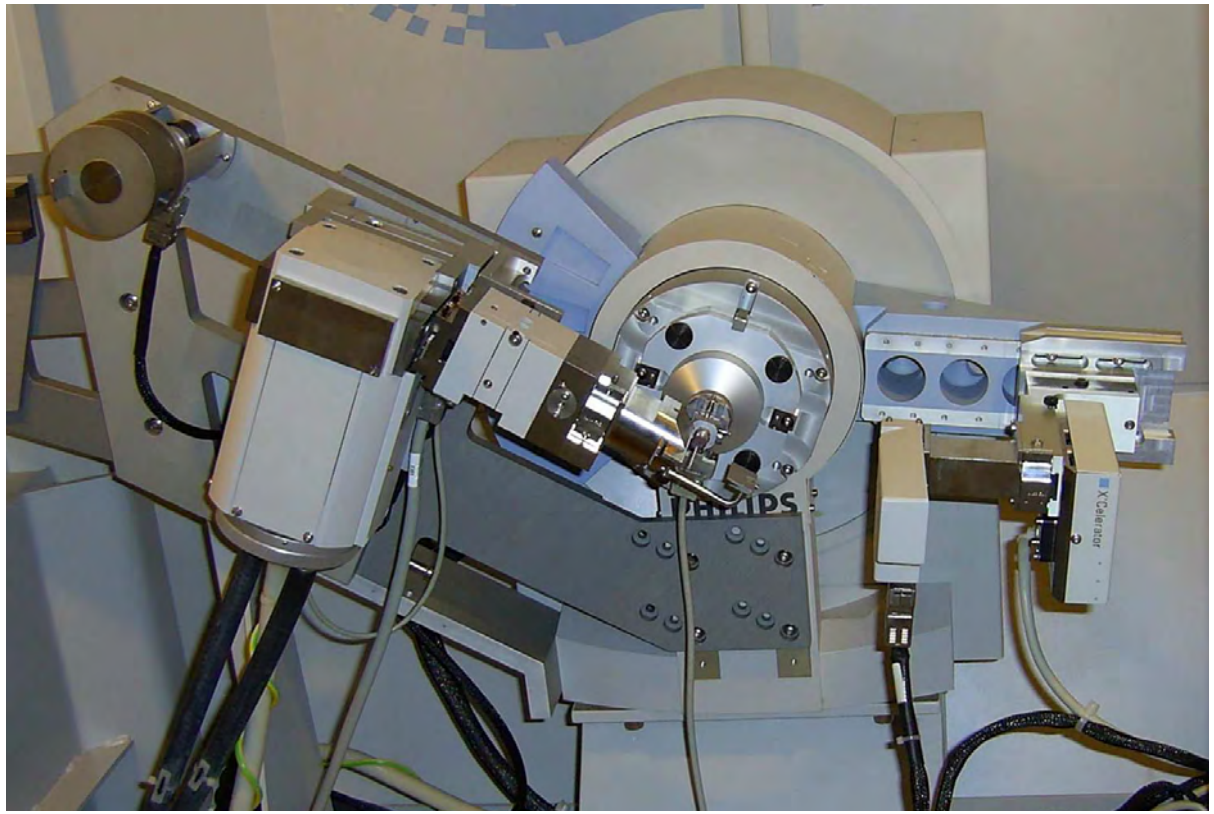
XRPD data collection for structure determination
European Synchrotron Radiation Facility (ESRF)
Grenoble France

Beamline BM01B



- 2 circle diffractometer
- 6 detectors => 6 complete patterns simultaneously
- Si 111 analyzer crystal in front of each detector => FWHM ~ 0.01 ° 2θ (λ = 1.0 Å)
- λ = 0.8 Å

XRPD data collection for structure determination
PANalytical X'pert Pro Alpha1



- Hybrid monochromator
- Soller slits (prim., sec. 0.01 rad div)
- X'celerator strip detector
- Oxford Instruments Cryostream Compact

Cu $K\alpha_1$ $\lambda = 1.5406$ Å

N_2 stream parallel to capillary
Cylindrical polymer film

Typical settings: 0.5-30 °2θ step 0.008 °2θ

Structure determination from XRPD data

1. Sample preparation (Capillary 0.7 mm)
2. Data collection
3. Find peak positions
4. Unit cell determination (indexing)
5. Direct space search
6. (Rietveld) refinement

Indexing; limit indexing search space

Make guess of volume asymmetric unit:

Use info from similar crystal structures to set limits to cell dimensions

Specific weight

Molecular weight

Volume / molecule

Z x Volume asymmetric unit = Unit cell volume

Generally only 1st 20 lines are used for indexing

- De Wolff FOM:

$$M_{20} = Q_{20} / (2 \cdot \langle Q \rangle \cdot N_{20}) \qquad M_{20}>10$$

$\langle Q \rangle$ = average $|Q_{calc}-Q_{obs}|$ N_{20} = number of calc. Q's upto Q_{20}

θ_g = selected limit $N(\theta_g)$ = number of calc. θ's upto θ_g

$\langle 2\theta \rangle$ = average discrepency N = number of observed lines upto θ_g

•Do not accept cells with ***unexplained*** non- indexed lines

Examples of indexing software

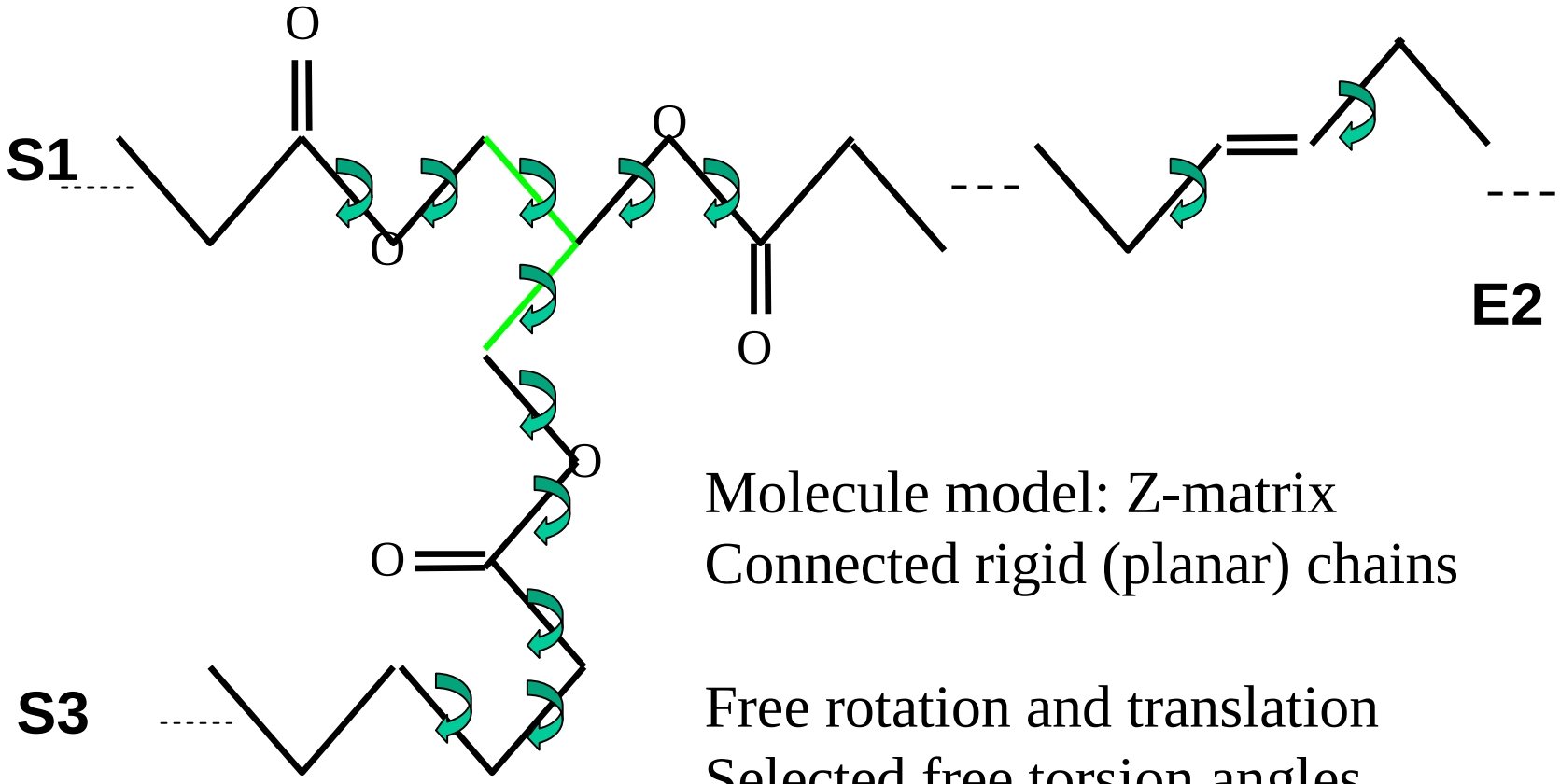
- ITO — zone finding
  needs accurate low angle data
- TREOR — trial and error + experience
  needs accurate low angle data
- DICVOL — dichotomy method
  less error sensitive
- McMaille — Monte Carlo , grid search
- Index — (slow)
- Kohl — low symmetry cells (DOS)
- Lzon — low symmetry cells (DOS)
- LSQDETC — Grid Search
- FOX — beta version

M_{20} alone is not sufficient
Check if complete pattern is covered (e.g CHEKCELL)

Structure determination from XRPD data

Direct space search

Fox, simulated annealing



Molecule model: Z-matrix
Connected rigid (planar) chains

Free rotation and translation
Selected free torsion angles

Favre-Nicolin, V. & Černý, R. (2002). *J. Appl. Cryst*. **35**, 734-743.

Crystal structures of polymorphs solved

Melting points and transition temperatures in K

	Transition **α to β'$_2$-2**	transition **β'$_2$-2 to β'$_1$-2**	melt **β'$_1$-2**	melt **β'$_0$-2**	melt **β-2**
• PEP	303	312	329	-	327
• PSP	317	319	343	-	339.5
• PPE	304	311	320	-	320
• PPS	321	323	332	-	338
• PSS	323	330	337	339	339

Crystal structure determined

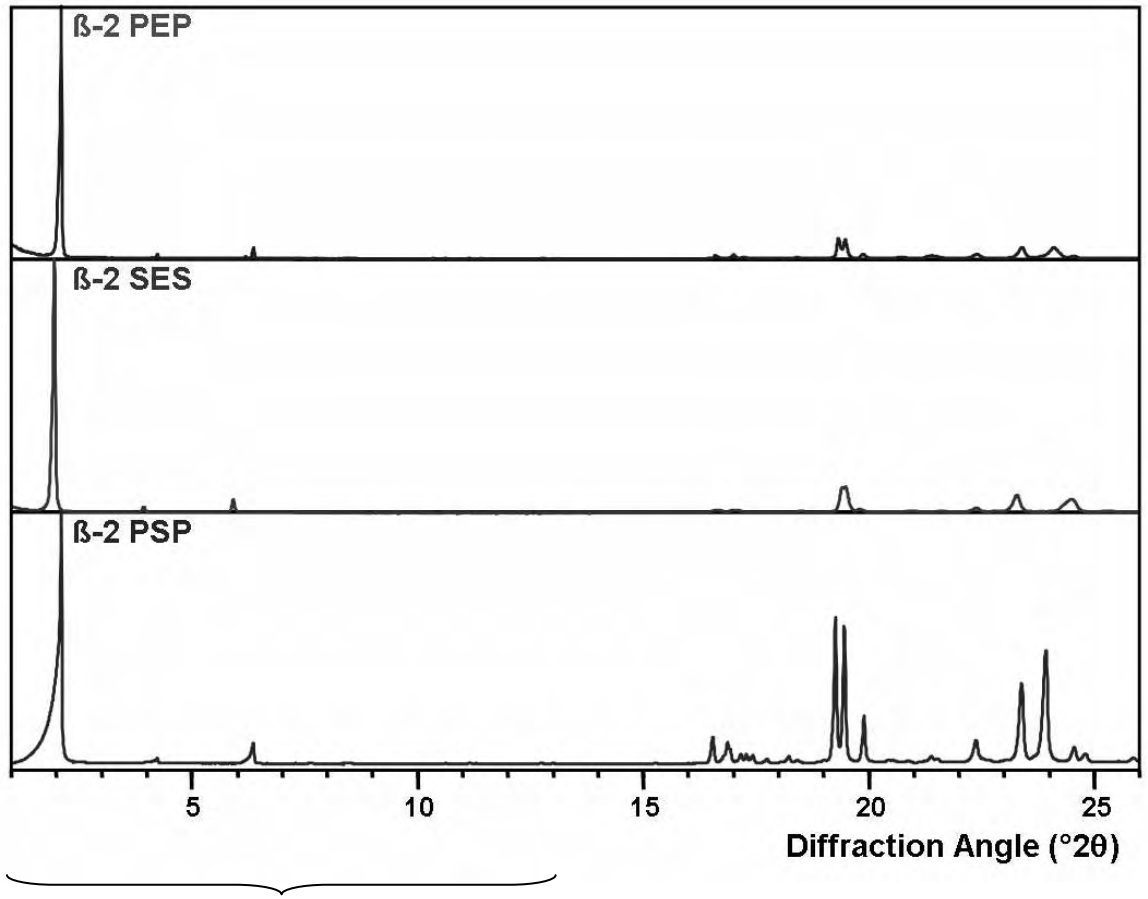
Novel polymorph, crystal structure determined

XRPD patterns of β-2 polymorphs of symmetric TAGs

a, b ,c in Å
α, β, γ in °

5.417 12.13 41.54
88.3 92.09 99.95

BM1b



5.425 11.95 44.64
92.01 88.76 100.18

BM1b

5.439 12.18 41.60
88.73 93.10 99.97

BM16

Space group P-1

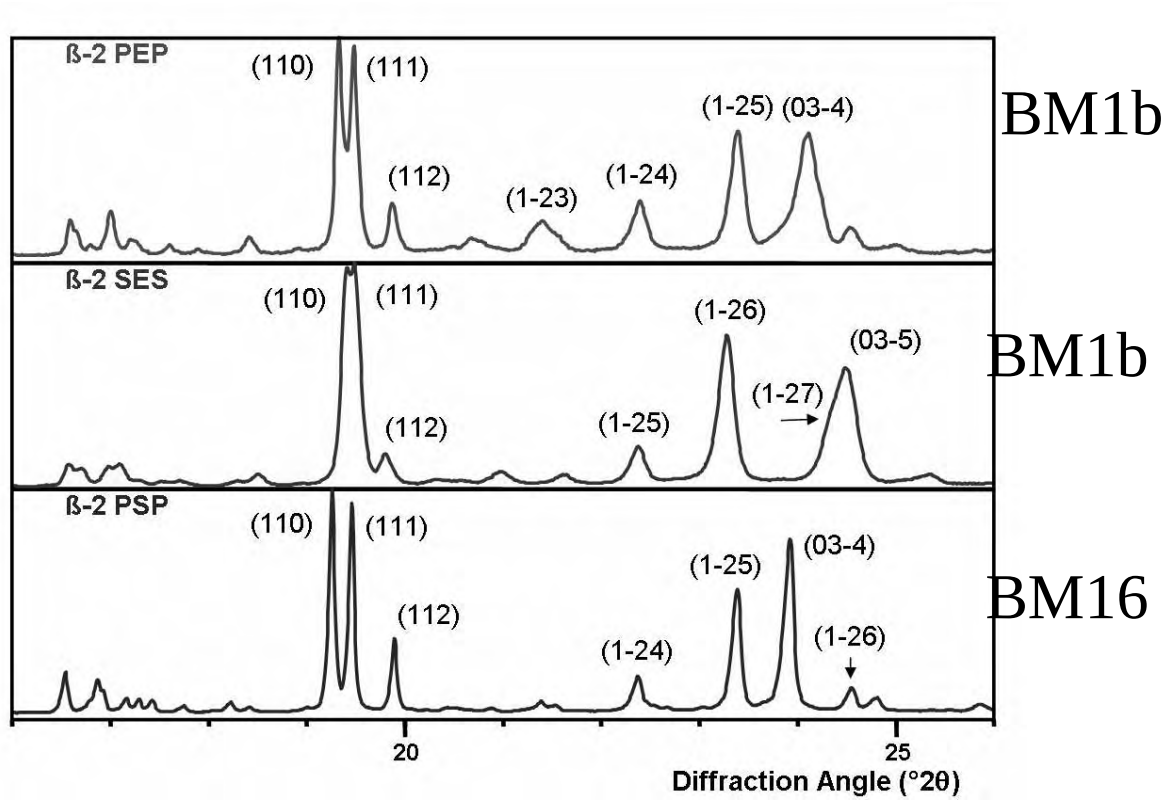
(0 0 ℓ)

All data scaled to CuKα1

•Van Mechelen et al. (2008) Acta Cryst B64, 240-248.

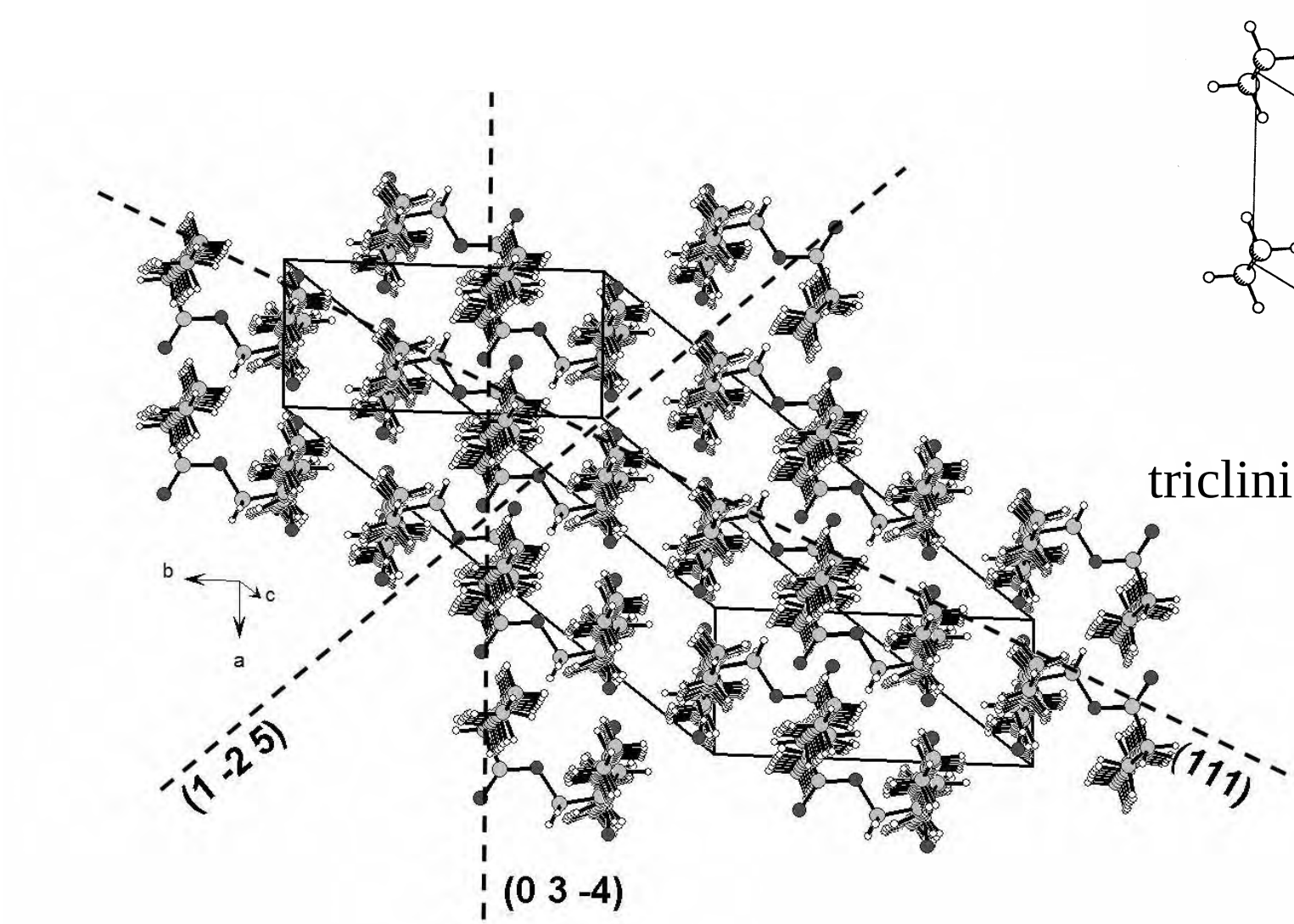
XRPD fingerprint area of β-2 polymorphs of symmetric TAGs

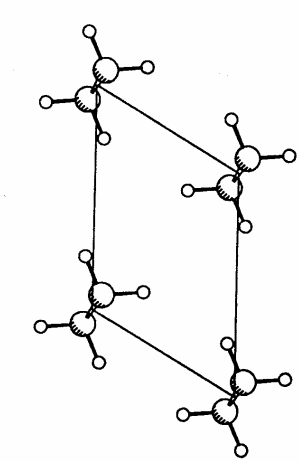
Polymorph	Short spacings (Å)		
γ		4.14	3.69
α		4.18	
β' – 2		4.33	4.14
β_2-3	4.58	3.99	3.87 3.76 3.67
β_1-3	4.58	4.03	3.85 3.69



Dominant reflections: (111) and (0 -3 4)

β-2 polymorphs dominant reflections



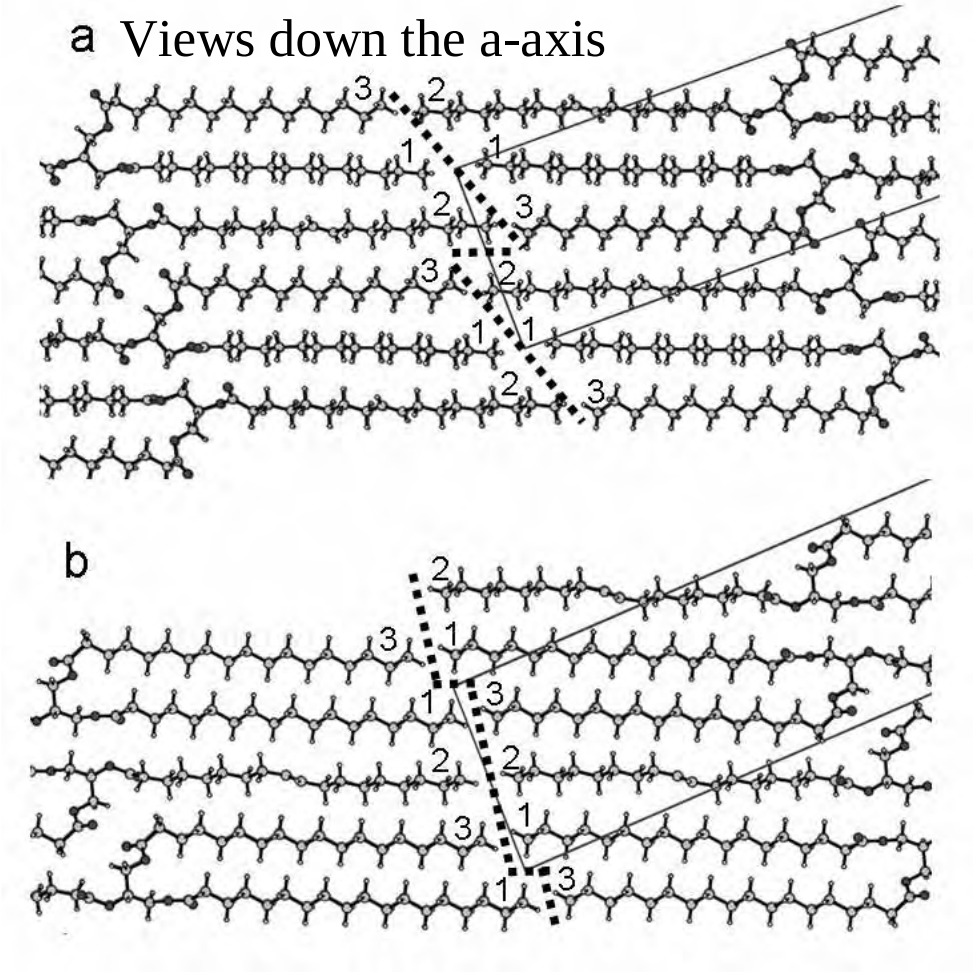


triclinic subcell $T_{//}$

PSP

Packing characteristics of the β-2 polymorphs

- Chair-shaped [1-3] conformation, seat-facing (inversion centres) (same as in e.g. β-2 SSS)
- Pairs of chairs form 'two-packs' : layer with double-chain thickness
- Two-packs face each other (shifted over a/4) at the methyl end-plane



PEP

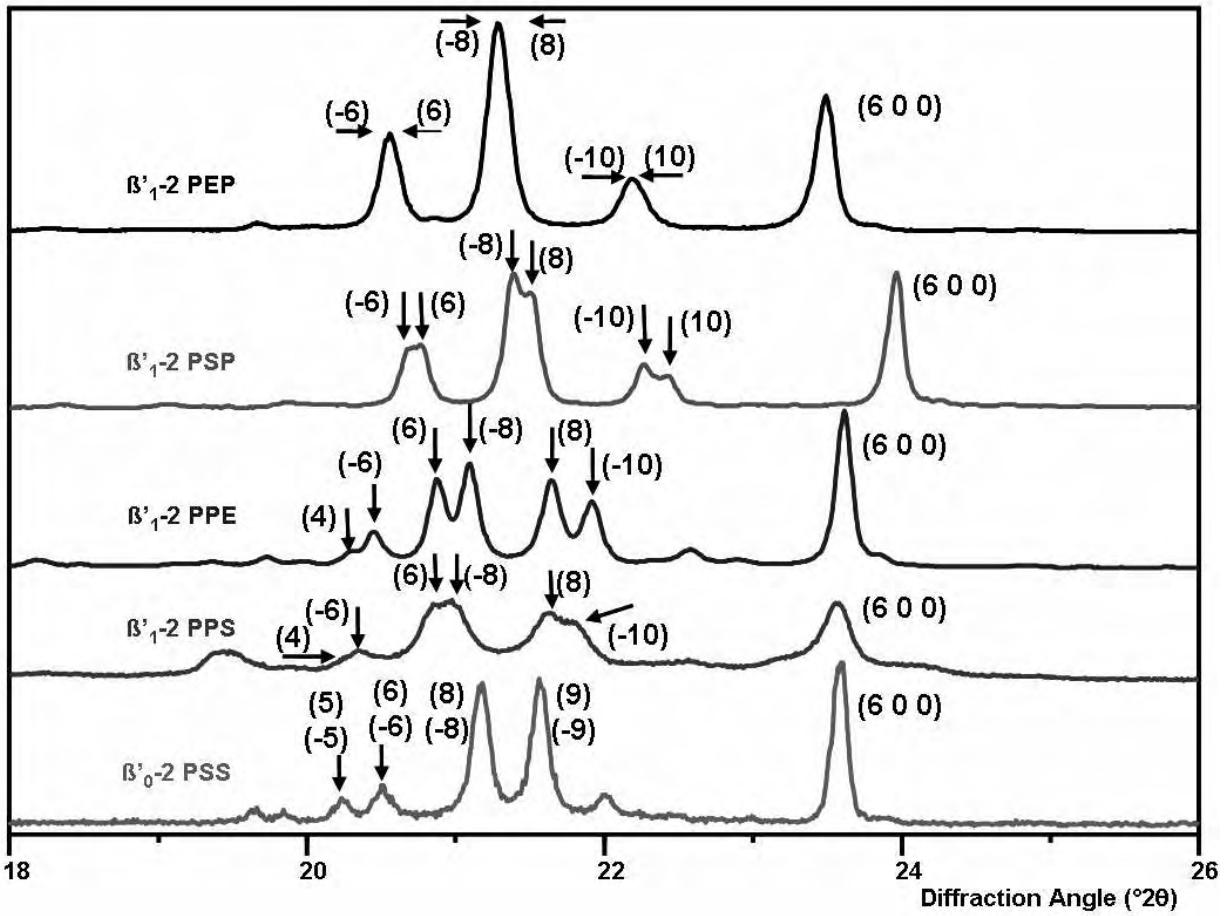
- Chain directions (1-1) aligned
- Two-packs tilted
- Small angle between step plane (- - -) and chain direction (1-1)

SES

- Chain directions (2-2) aligned
- Two-packs not tilted
- Large angle between step plane (- - -) and chain directions (2-2)

No significant difference in packing for E vs S !

Fingerprint area of β'_1-2 PEP, PSP, PPE, PPS and β'_0-2 PSS



	Cell parameters	Spacegroup
	22.715 5.656 85.110 90.2	I2
	22.253 5.634 85.263 90.8	I2
	22.988 5.641 86.265 93.52	I2
	22.7515 5.650 86.746 93.97	I2
	22.651 5.653 89.462 90.01	C2/c

Z = 8

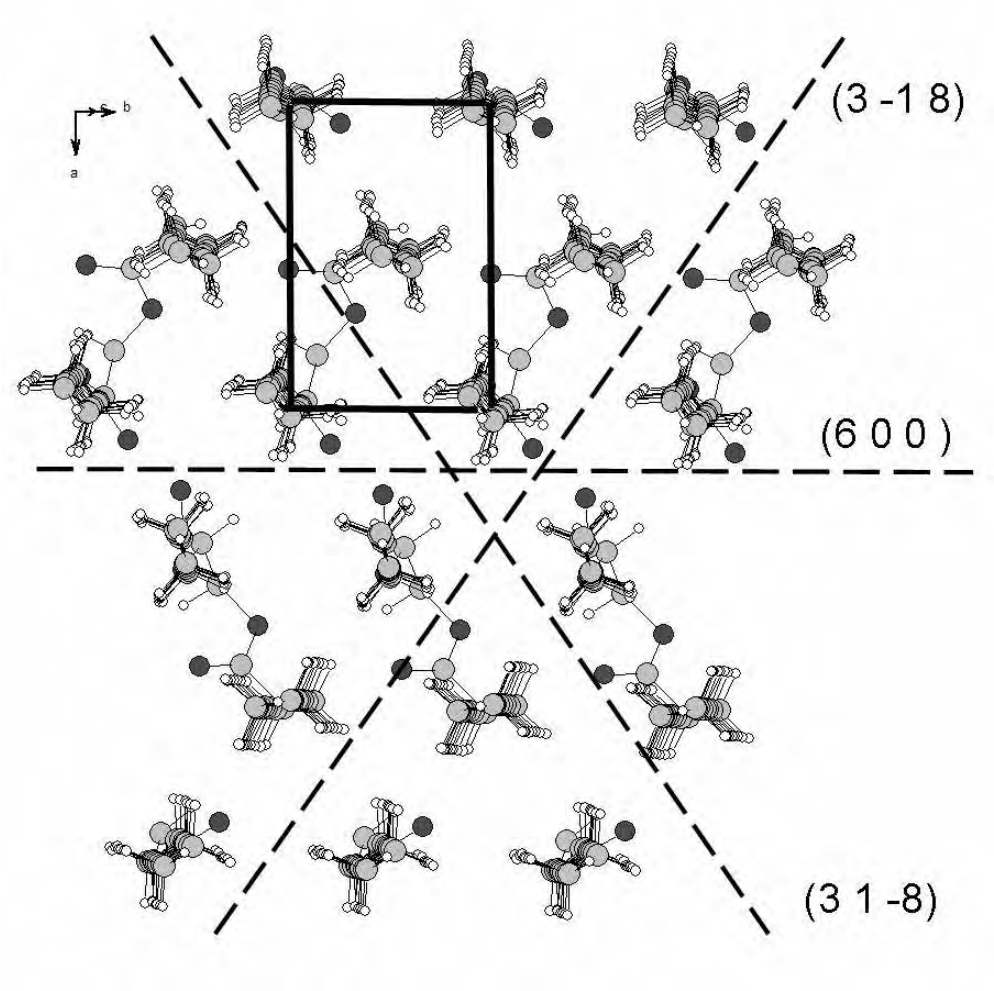
Only the ℓ of the (31ℓ) is shown.

PSP (synchrotron data) rescaled to Cu $K\alpha_1$

(31 8) (3 1 -8) and (600) dominant

Van Mechelen et al. (2008) Acta Cryst B64, 249-259.

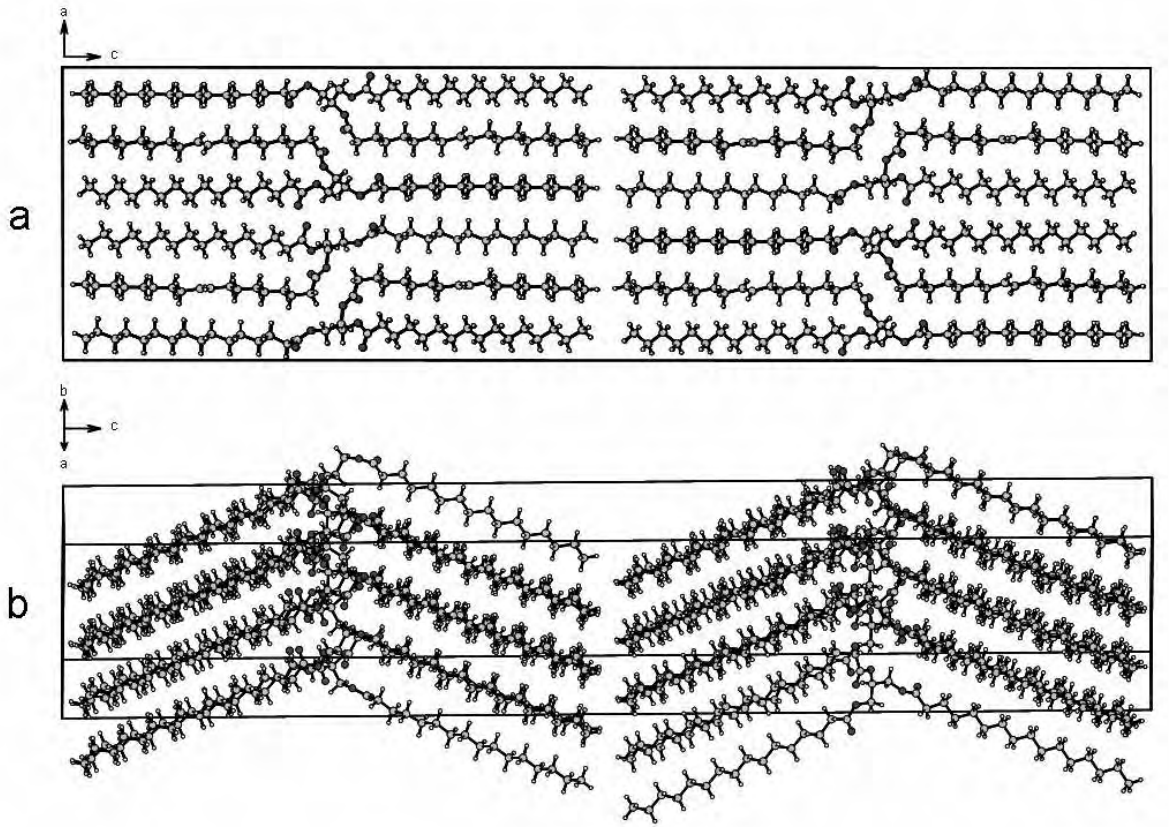
Dominant reflections in β'_1-2 polymorphs (PSP)



Orthorhombic O⊥ subcell

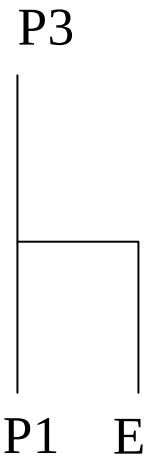
Packing characteristics of the β'_1-2 polymorphs

- Two pack layers
- Chair-shaped molecules, seat-facing, seat of chair is S or E
- β’ bend (~ 130°) between the back and back leg of the chair
- Symmetric TAGs (PEP, PSP) have a [1-2] conformation



123

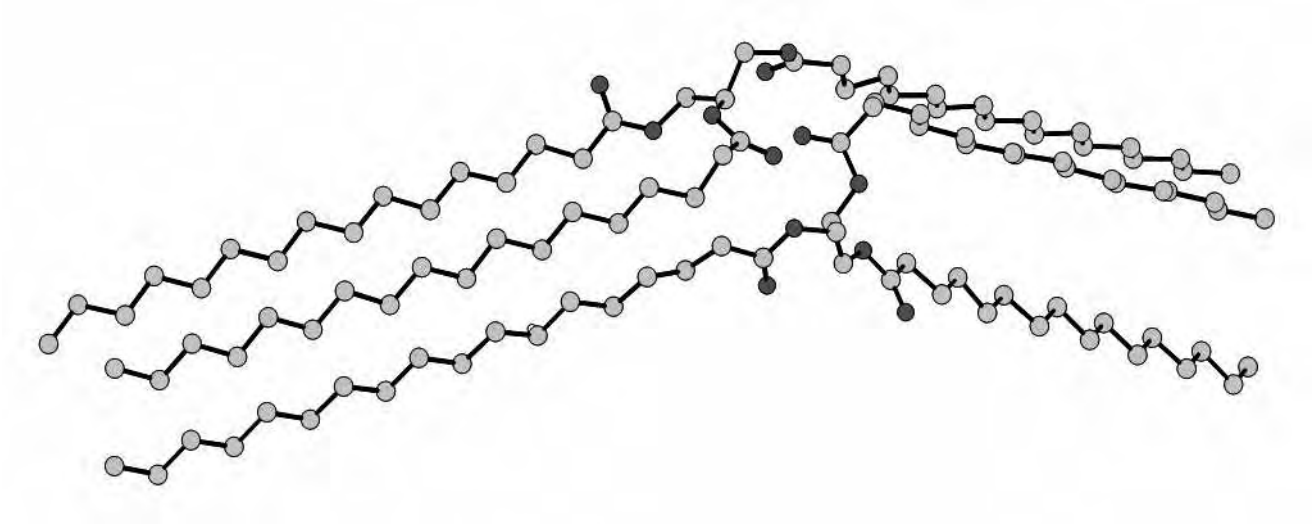
[1-2] β'_1-2 PEP



Bends in molecules point in the same direction

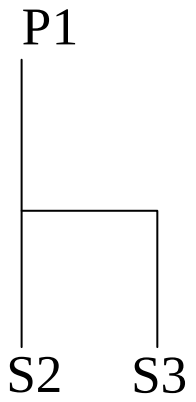
Packing characteristics of the β'_1-2 polymorphs

- Two pack layers
- Chair-shaped molecules, seat-facing, seat of chair is S or E
- β’ bend (~ 130°) between the back and back leg of the chair
- Asymmetric TAGs (PPE, PPS) have a [2-3] conformation



Pair of PSS molecules with facing seats

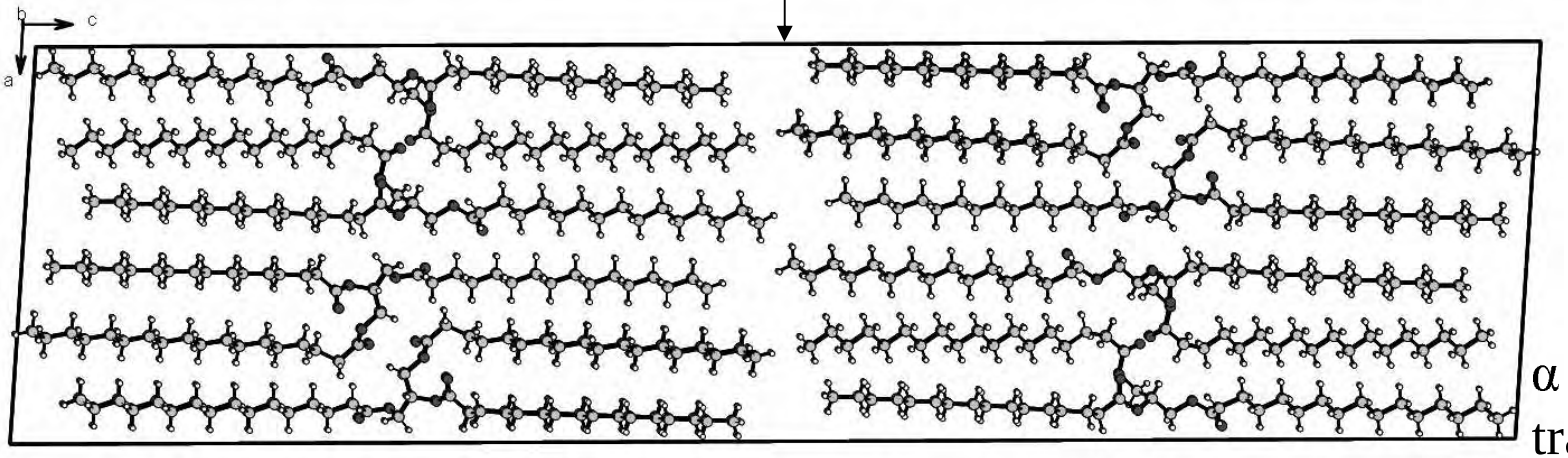
123
[2-3] β'_1-2 PSS



Packing characteristics of the β'_1-2 polymorphs
Symmetric vs asymmetric TAGs

Methyl-end plane

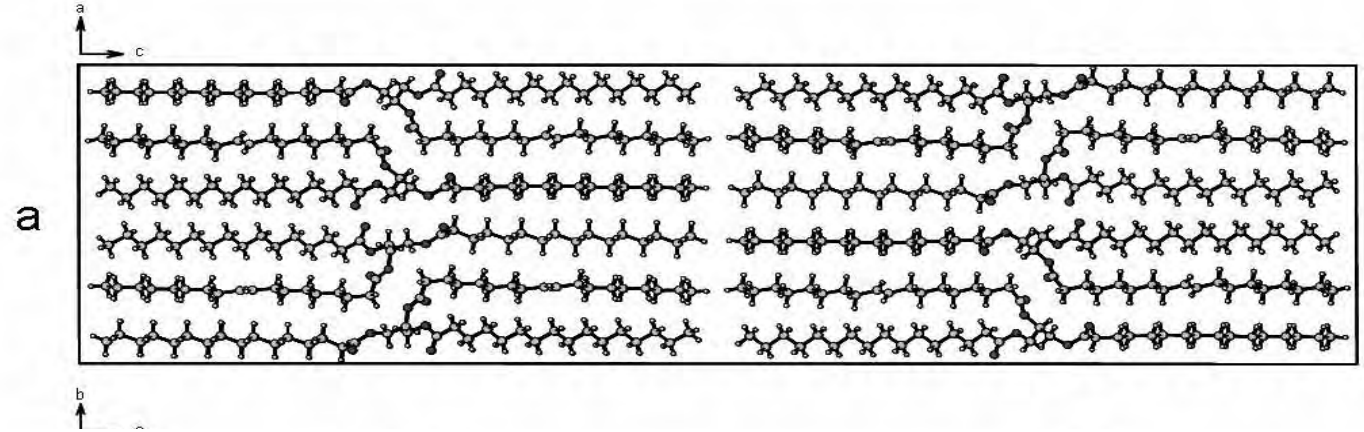
View parallel to b axis



Not aligned

[2-3] β'_1-2 PSS

α melt in α -> β'_2 transition

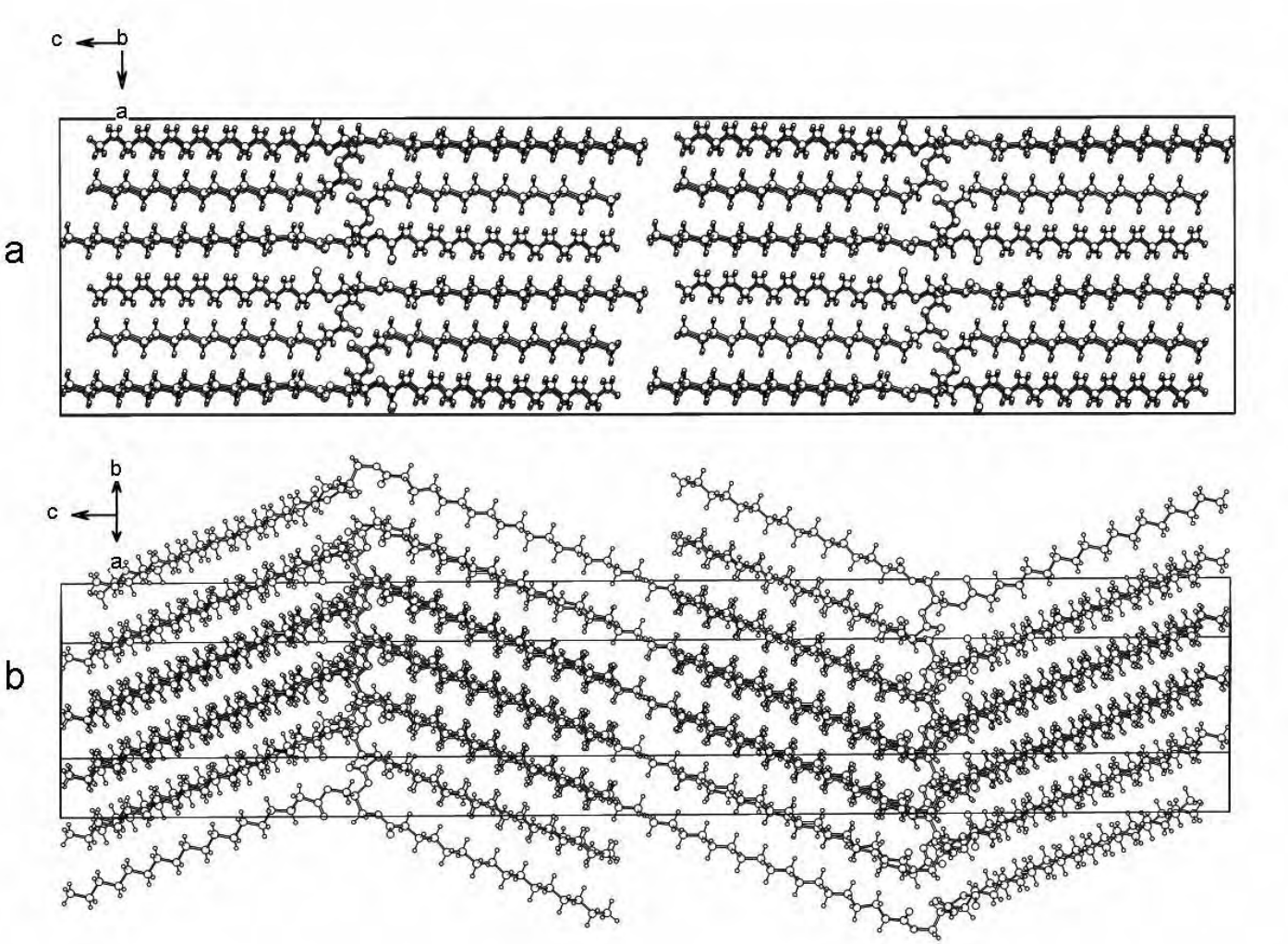


Aligned

[1-2] β'_1-2 PEP

No α melt in α -> β'_2 transition

Packing characteristics of the novel β'_0-2 PSS polymorph



[1-2] conformation

Two packs related by inversion centres

Stepped methyl end-plane

T_m (in K) and conformations of molecules in TAG polymorphs

Space group	I2	C2/c	P-1
TAG	**β'_1-2**	**β'_0-2**	**β-2**
PEP	329 [1-2]	-	327 [1-3]
PSP	343 [1-2]	-	339.5 [1-3]
PPE	320 [2-3]	-	320 [1-3]
PPS	332 [2-3]	-	338 [1-3]
PSS	337 [2-3]?	339 [1-2]	339 [1-3]

β' stable

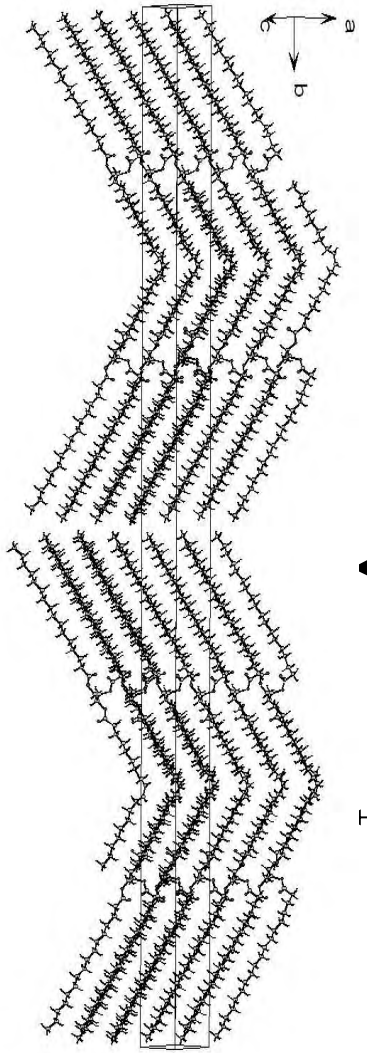
[1-2] => [1-3] not observed for symmetric TAGs

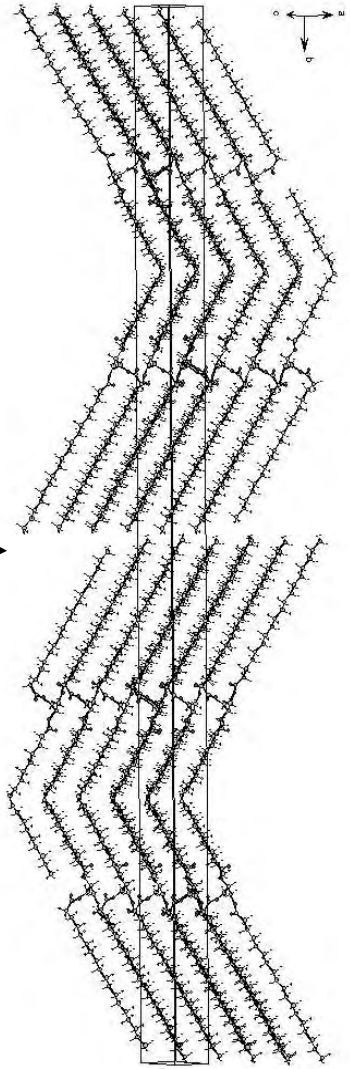
β stable

β'_1-2 [2-3] => β'_0-2 [1-2] would require an orientation inversion of every other two pack (similar as in β_2-3 SOS => β_1-3 SOS)

Three-pack conversion in SOS

Van Mechelen et al. (2006). *Acta*.*Cryst*. B**62**, 1131-1138.





methyl end plane

Double bonds lined up

2 x 3-pack layer

β_2-packing (Cc)

β_1-packing ($P2_1/n$)

Conclusions

Time-resolved XRPD

- No definite influence of E vs. S except that E stabilizes β'_2-2
- The β'_2-2 is a less crystalline form of the β'_1-2

Structure determination from XRPD

- Symmetric and asymmetric β-2 polymorphs are in the [1-3] conformation. No definite influence of E vs S.
- Symmetric β'_1-2 (PSP, PEP) are in a [1-2] conformation,
- Asymmetric β'_1-2 (PPS, PPE) in a [2-3] conformation.
- The β'_0-2 PSS is in the [1-2] conformation

Solid state β' to β-2 phase transition unlikely because of the different conformations

Low resolution data

d > 3.0 Å

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