Towards an « Infinite » Number of Calcium Oxalate Structures?

C. Bonhomme*, C. Gervais, F. Babonneau

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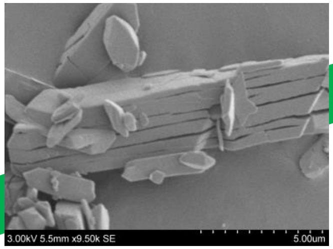
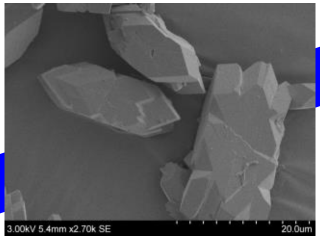


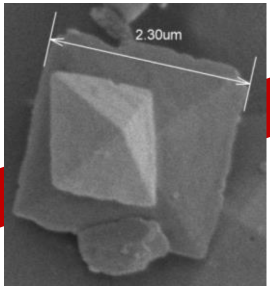
caoxite (COT)

$Ca(C_2O_4)\cdot 3H_2O$



weddellite (COD)

$Ca(C_2O_4)\cdot 2H_2O$



whewellite (COM)

$Ca(C_2O_4)\cdot H_2O$

26TH CONGRESS AND GENERAL ASSEMBLY OF THE INTERNATIONAL UNION OF CRYSTALLOGRAPHY

Commission on NMR Crystallography and Related Methods

Pathological calcifications (kidney stones, KS)

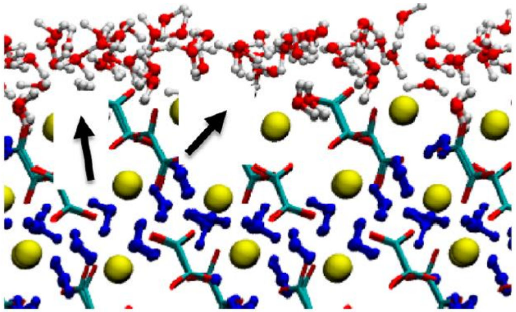
a major societal/health problem worldwide
(in France, related costs *per year* > 800 millions €)

an intrinsic structural/chemical complexity

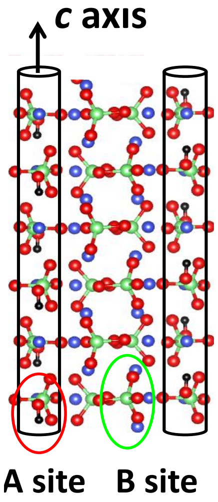
- **minerals**
- **fatty acids, triglycerides, proteins**
- **… ↔ hybrid Organic/Inorganic materials**

Ca Oxalates, CaOx (Mono-, Di-, Trihydrate)
Ca Phosphates (hydroxyapatite, HAp)

CaOx



HAp

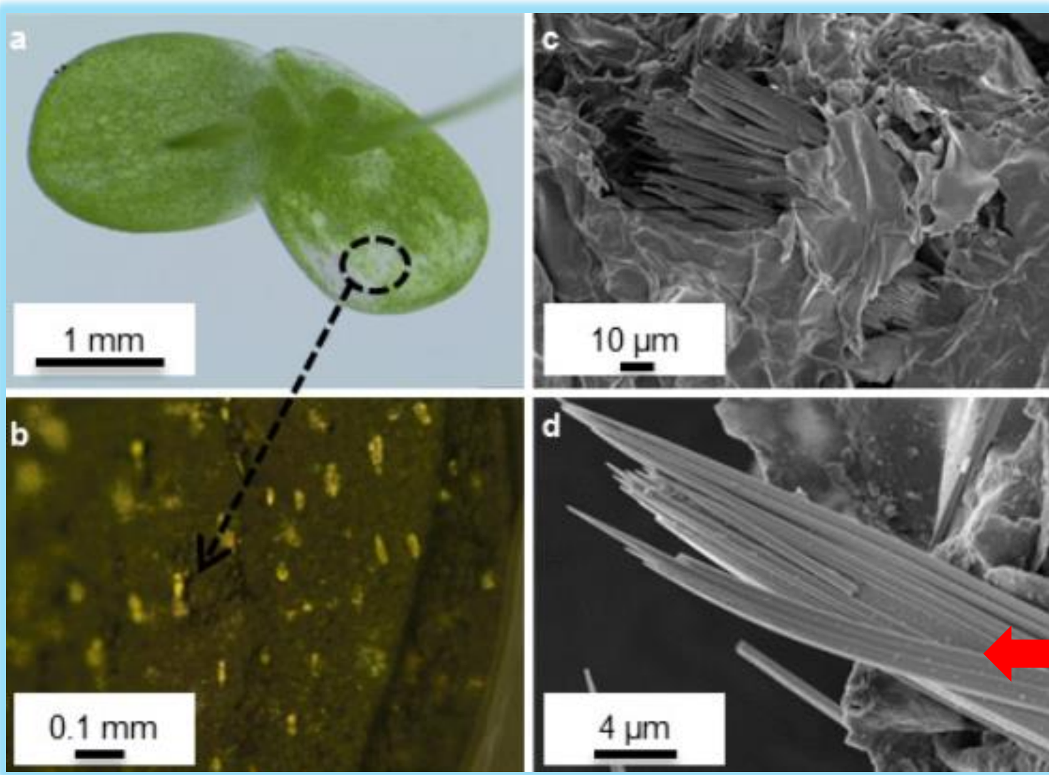


Hydrated CaOx, $Ca(C_2O_4)_2.nH_2O$, are ubiquitous

Materials Science inc. Nanomaterials & Polymers

Amorphous biogenic calcium oxalate

Eva Weber,[a, b] Andreas Verch,[b] Davide Levy,[a] Andy N. Fitch,[c] and Boaz Pokroy*[a]



whewellite (COM)
$Ca(C_2O_4)\cdot H_2O$

raphides formed by *Lemna minor* (duckweed)

Synthetic CaOx, $Ca(C_2O_4)_2.nH_2O$

ChemComm

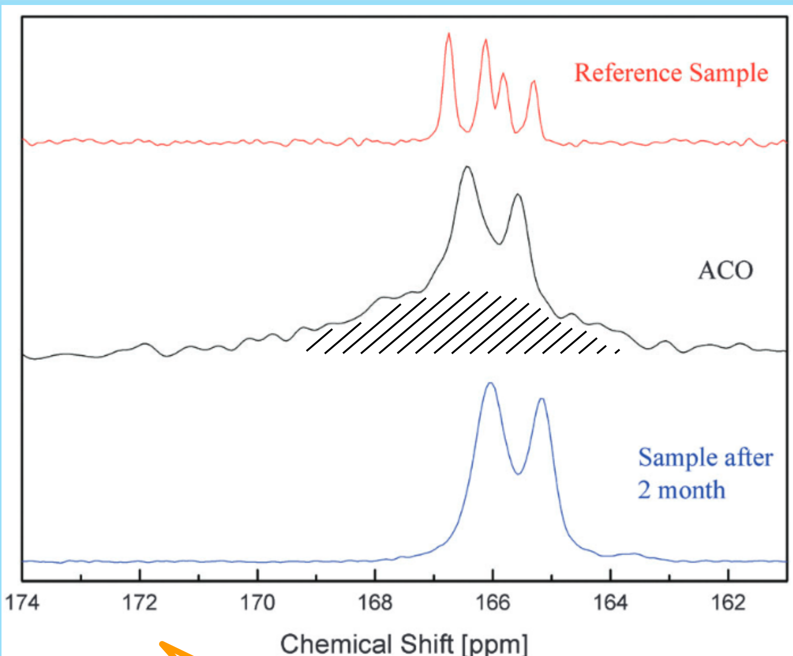
COMMUNICATION

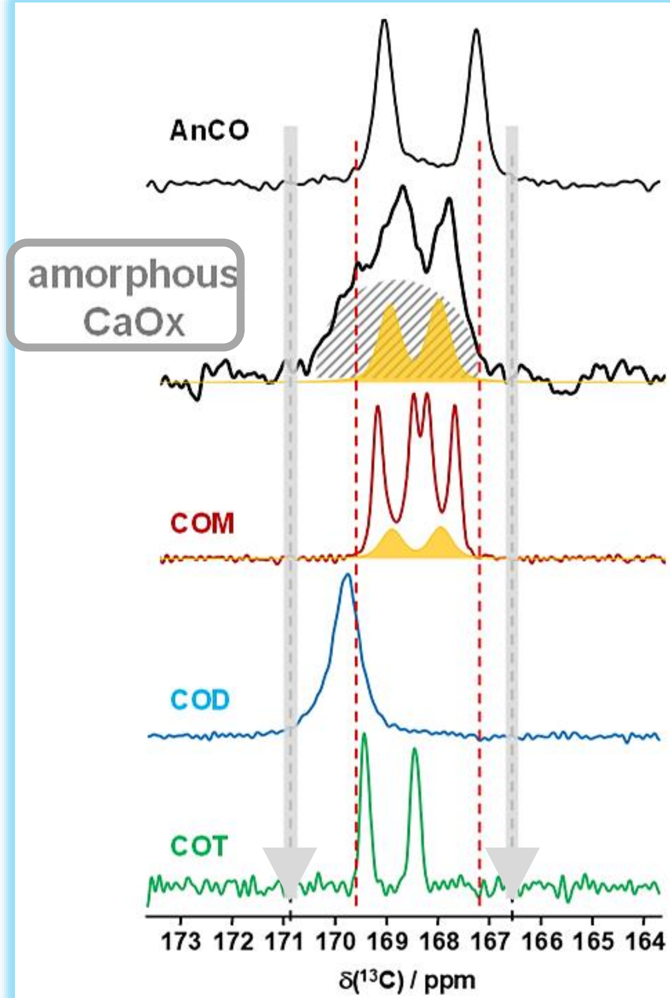
Stable amorphous calcium oxalate: synthesis and potential intermediate in biomineralization†

Myriam Hajir,[a] Robert Graf[b] and Wolfgang Tremel*[a]

Cite this: *Chem. Commun.*, 2014, 50, 6534

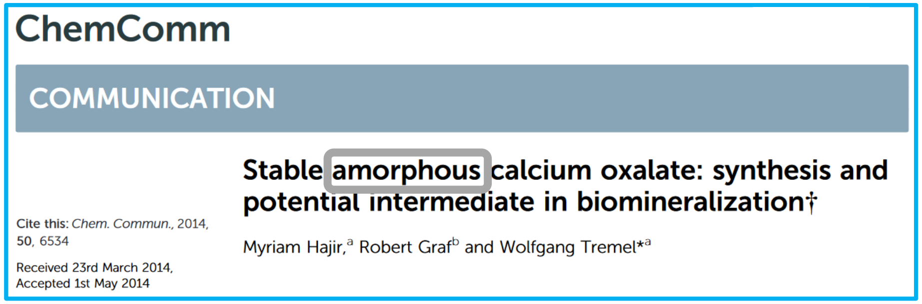
Received 23rd March 2014, Accepted 1st May 2014





13C solid state NMR

Synthetic CaOx, $Ca(C_2O_4)_2.nH_2O$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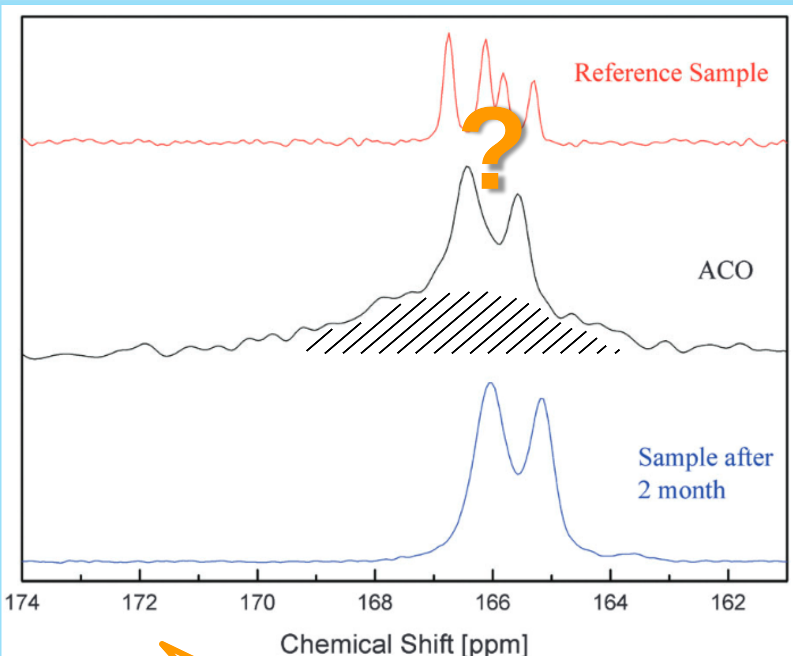
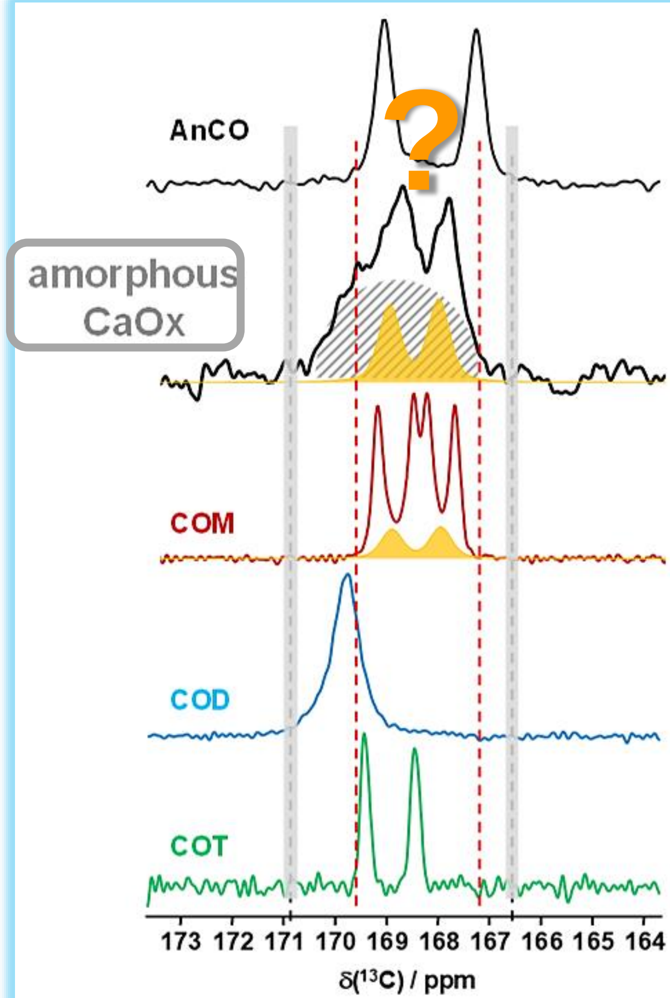




13C solid state NMR

Synthetic CaOx, $Ca(C_2O_4)_2.nH_2O$

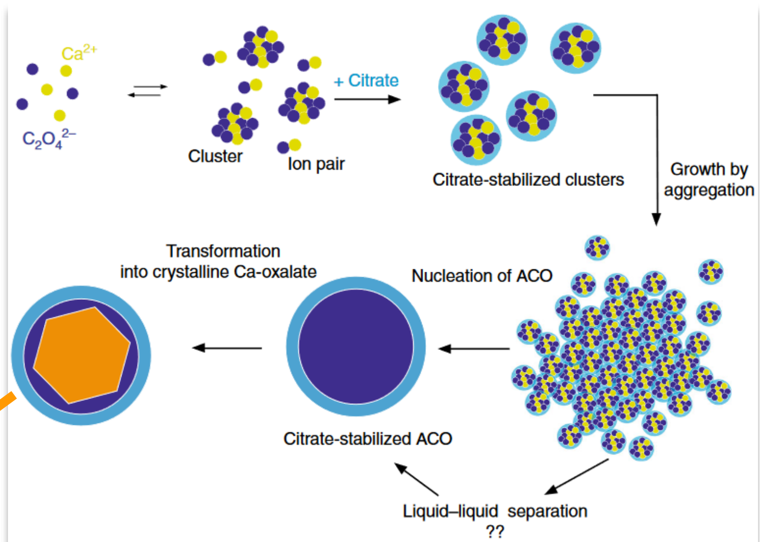
ARTICLE

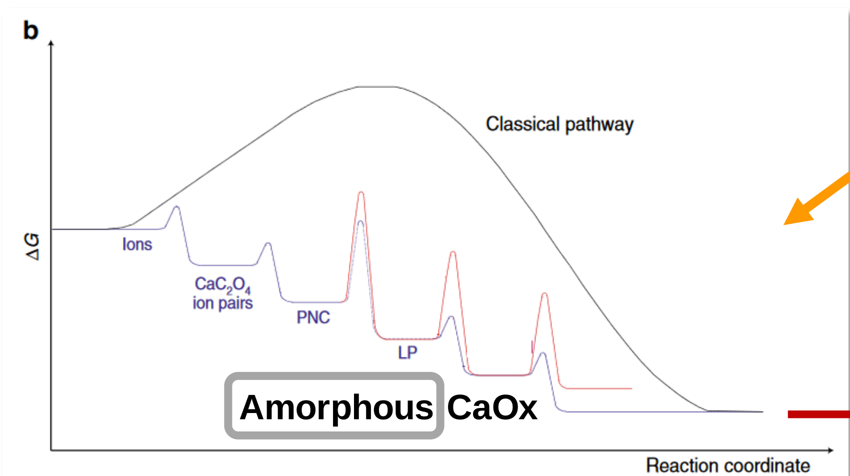
DOI: 10.1038/s41467-017-00756-5 OPEN

A non-classical view on calcium oxalate precipitation and the role of citrate

Encarnación Ruiz-Agudo[1], Alejandro Burgos-Cara[1], Cristina Ruiz-Agudo[2,3], Aurelia Ibañez-Velasco[1], Helmut Cölfen[3] & Carlos Rodriguez-Navarro[1]

► **new precipitation routes**

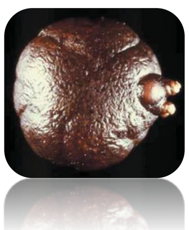




whewellite (COM)
$Ca(C_2O_4)\cdot H_2O$

Pathological calcifications (kidney stones, KS)

Tenon hospital, Paris



→ diagnosis

→ prevention

a major societal/health problem worldwide
(in France, related costs *per year* > 800 millions €)

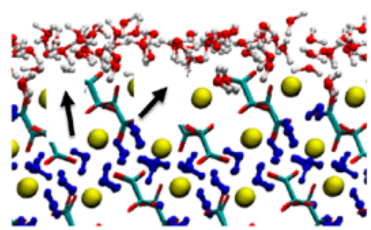
an intrinsic structural/chemical complexity

- minerals
- fatty acids, triglycerides, proteins
- ... ↔ Hybrid organic/inorganic materials

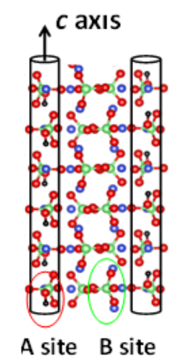
Ca Oxalates (mono-, di-, tri-hydrate)

Ca Phosphates (hydroxyapatite, HAp)

CaOx



HAp



→ NMR / DNP / crystallography

Coll.: M. Daudon, E. Letavernier, D. Bazin

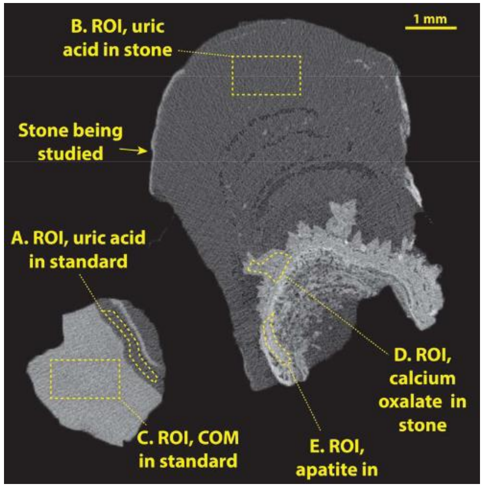
More complexity

► current lack of MR Imaging techniques:

"… Using *standard* MRI technique, stones appear as a non-specific void…"

(Brisbane, Nat. Rev. Urol., 2016)

► state of the art at hospital: μ–Computed Tomography (CT)



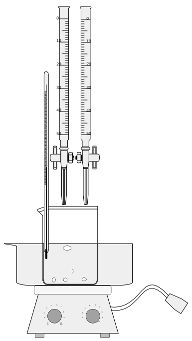
various CaOx?

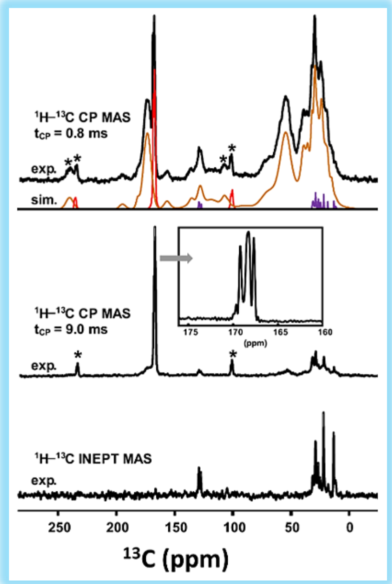
drug-induced KS, gels, non-radio opaque phases?

↓

relative lack of chemical information

- **NMR as a unique platform of characterization**
  - ► ***structure***
  - ► ***dynamics***



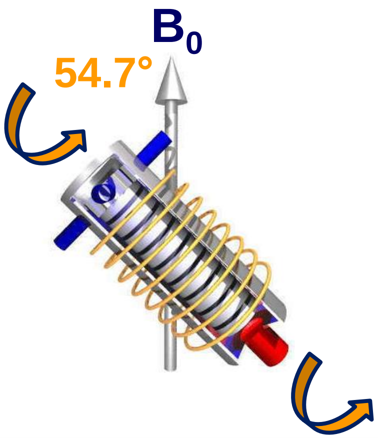


- **More sensitivity**

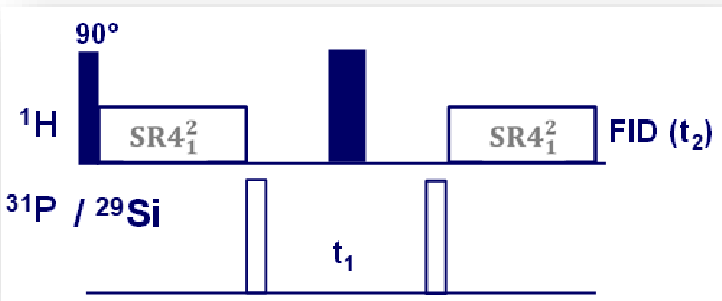
- **Dynamic Nuclear Polarization crystallography**

- **Magic Angle Spinning MRI**

The solid state NMR toolbox



★ Magic Angle Spinning (MAS) up to 150 kHz



★ ***Decoupling / Recoupling*** **of NMR interactions**

« structural local spies »

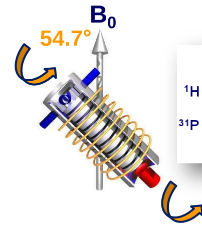
chemical shift → δ

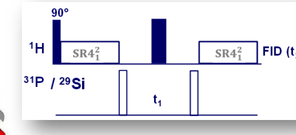
through *space* dipolar → D_{XY}

through *bond* J → J_{XY}

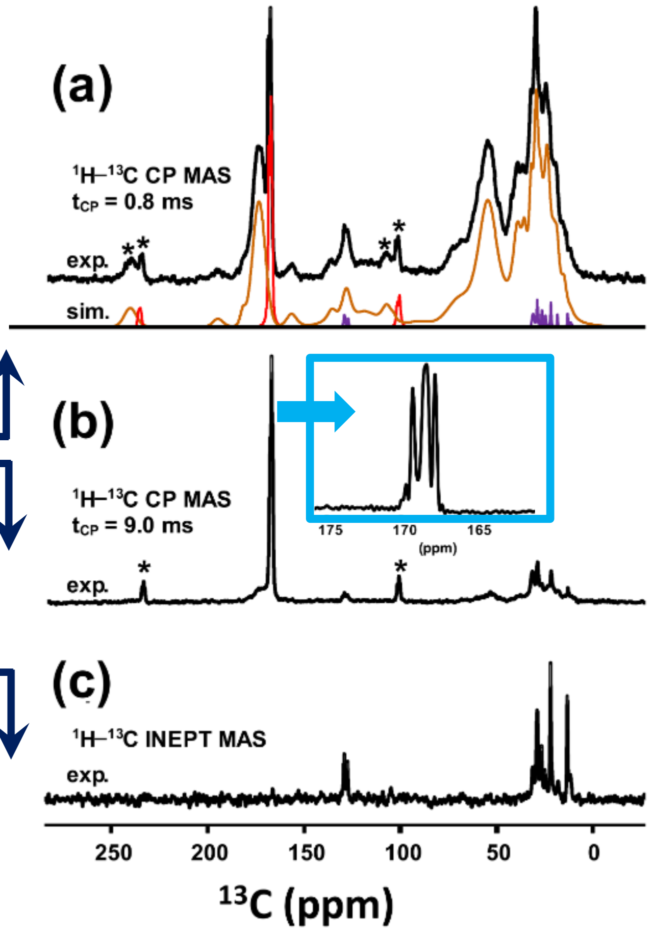
quadrupolar (I > ½ , ^2H, ^{17}O, ^{43}Ca) → Q

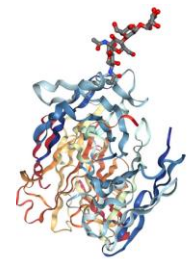
Structure, interfaces and local dynamics in KS

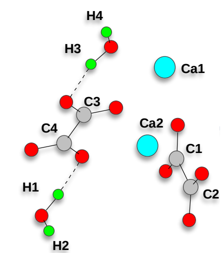




^{13}C CP MAS NMR

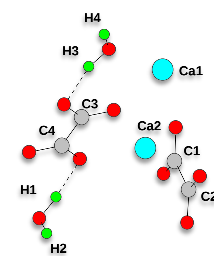






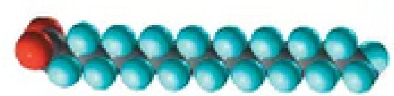
Cross Polarization

through *space*



INEPT

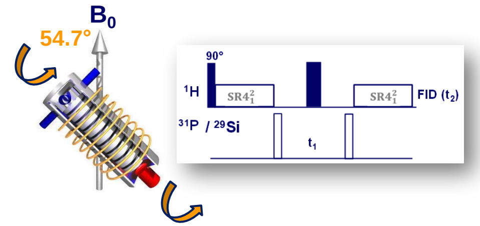
through *bond*



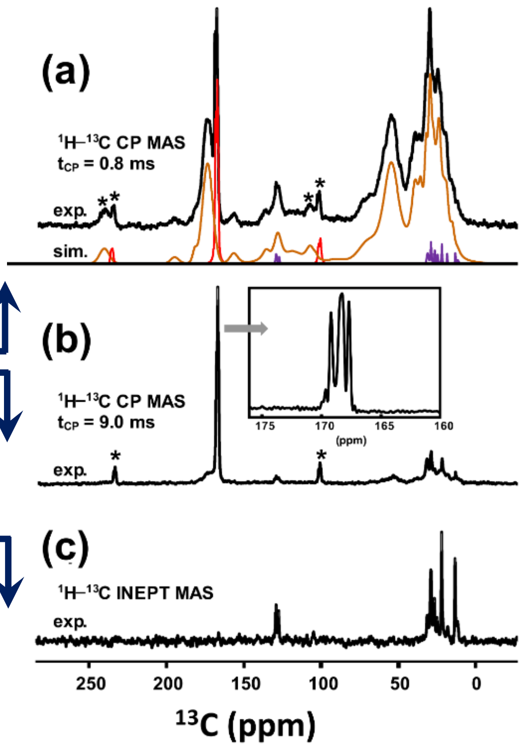
Magnetic Resonance, 2021

tribute Britannica, Sinobiol

Structure, interfaces and local dynamics in KS



^{13}C CP MAS NMR

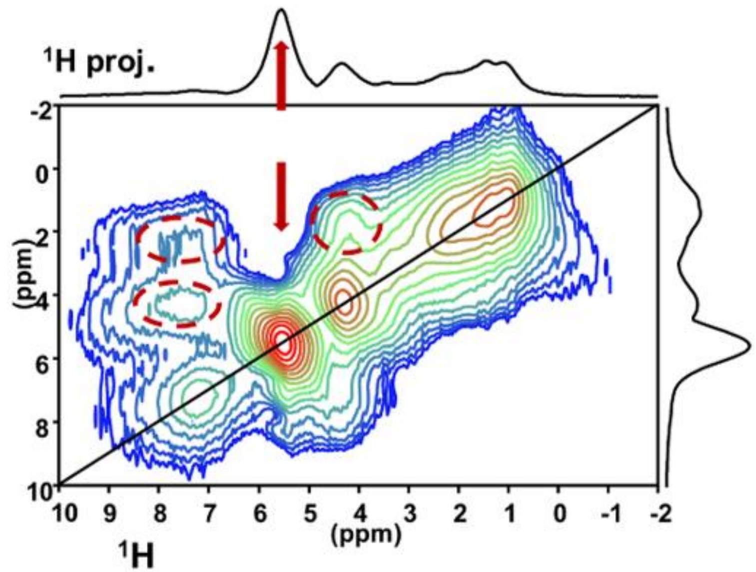


Cross Polarization

through *space*

INEPT

through *bond*



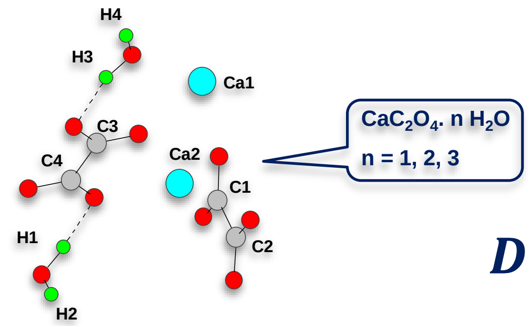
^1H DQ BABA

through space

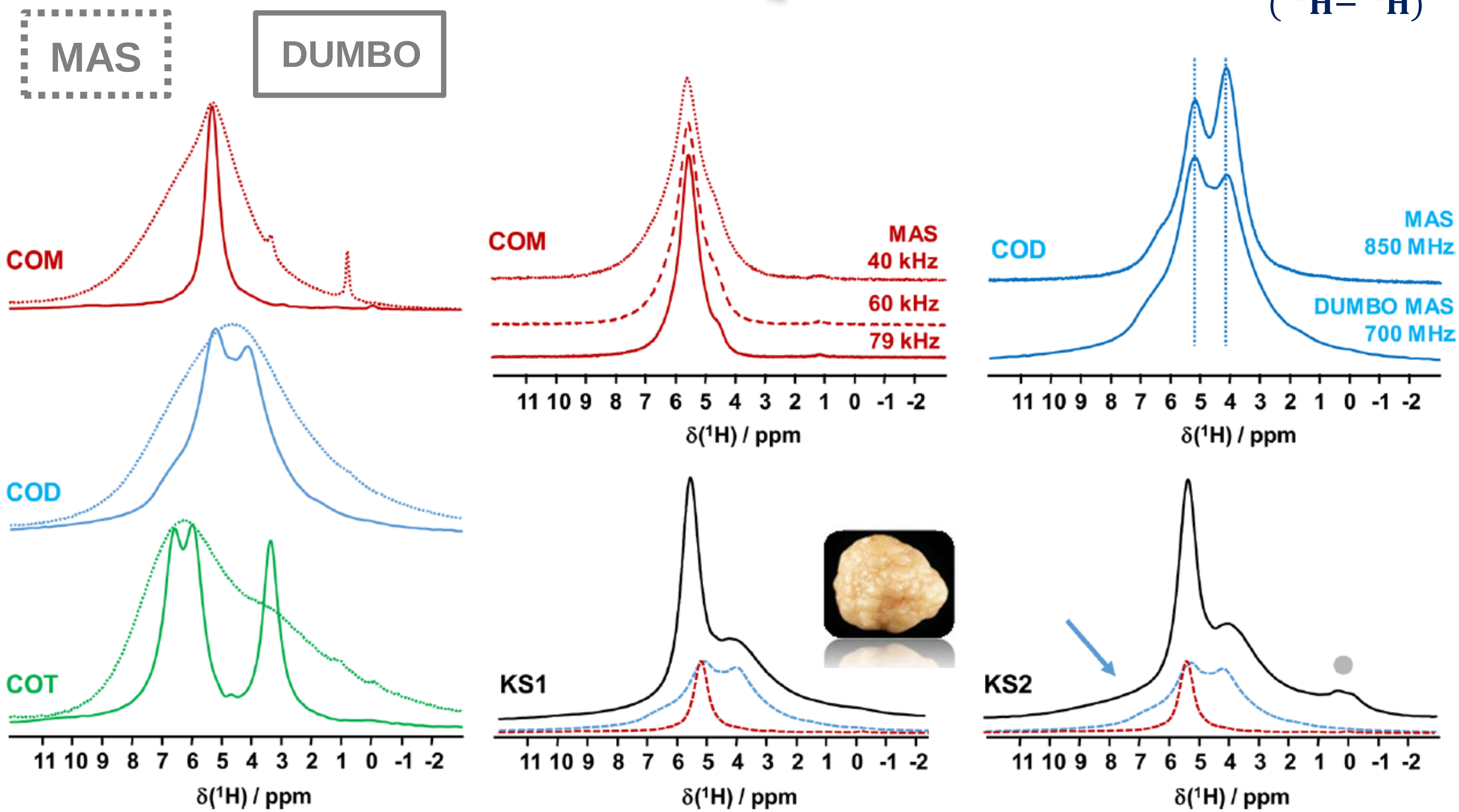
pairs of ^1H

A focus on ¹H solid state NMR

¹H fast / ultra-fast MAS *vs* DUMBO NMR



$$D \sim \frac{1}{r^3_{(\,^1\mathrm{H}-\,^1\mathrm{H})}}$$



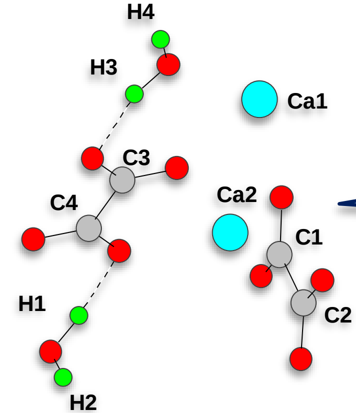
Magnetic Resonance, 2021

A focus on ^{1}H solid state NMR

neutron, XRD data

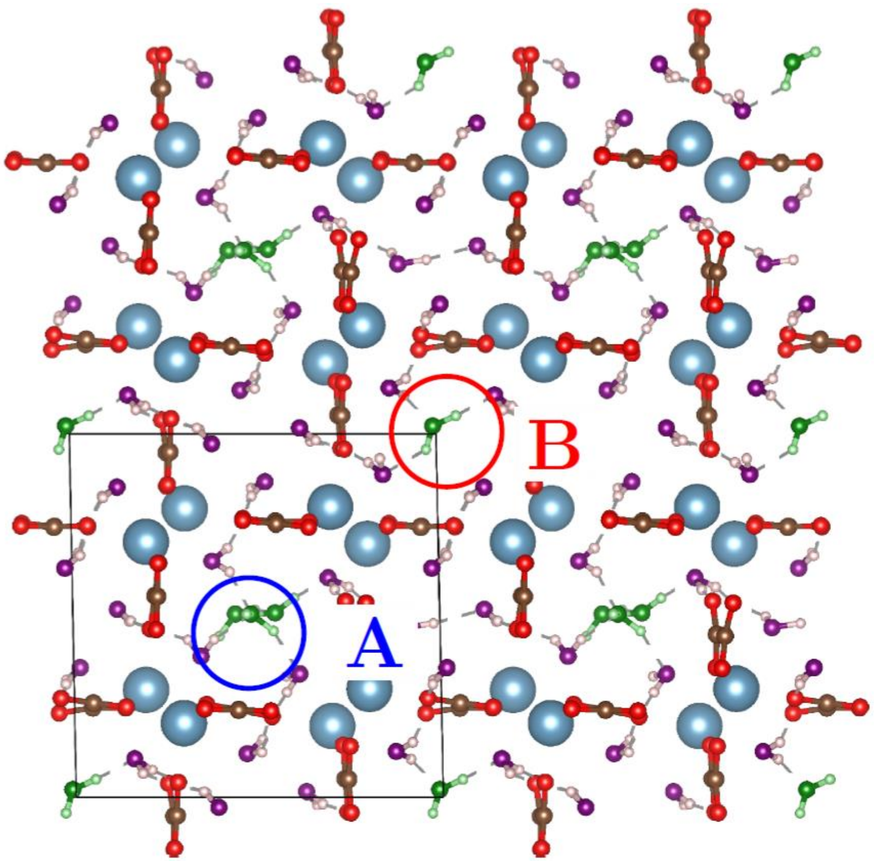
relaxation of structures at DFT level

VASP (Kresse, Hafner, Furthmüller)



$CaC_2O_4 . n\ H_2O$

n = 1, 2, 3



CaOx dihydrate (COD)

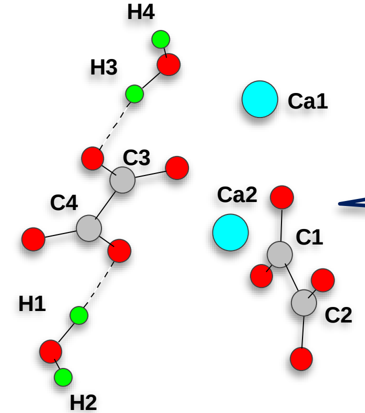
→ zeolitic structure

→ *natural* Metal Organic Framework

$Ca_8C_{16}O_{32}\,(H_2O)_{16}\,\mathit{(H_2O)_3}$

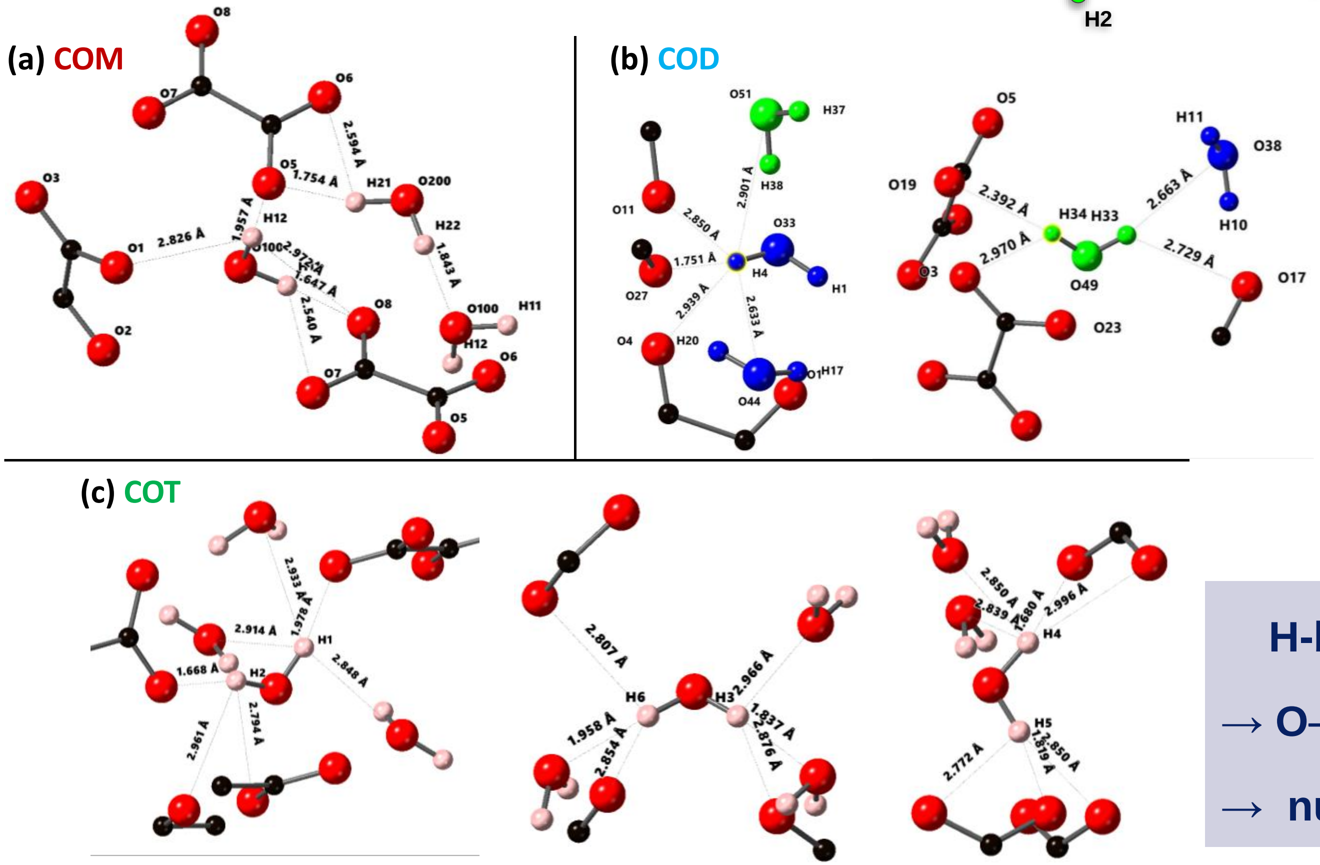
structure ***channels***

A focus on ^{1}H solid state NMR



CaC_2O_4. n H_2O

n = 1, 2, 3

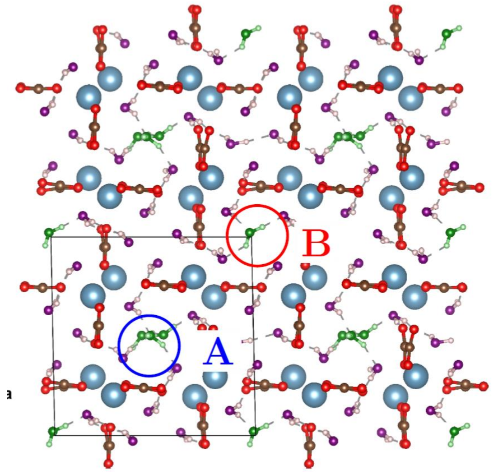


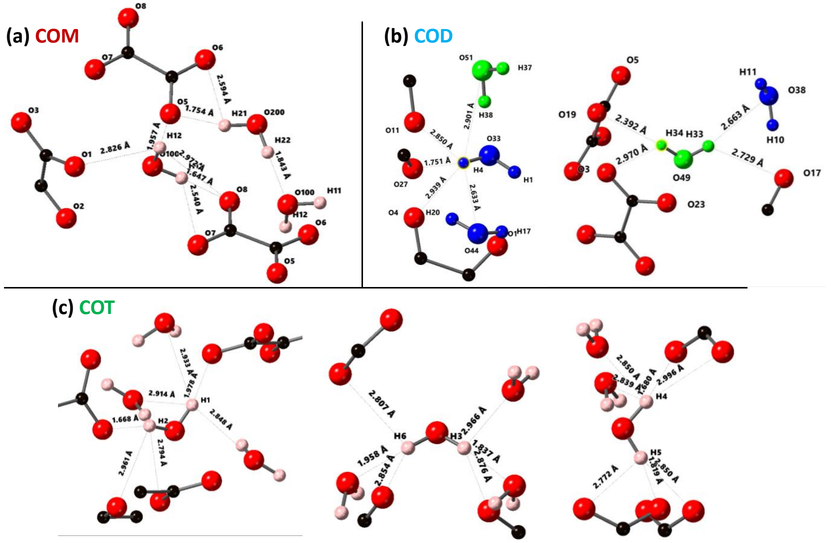
H-bond networks

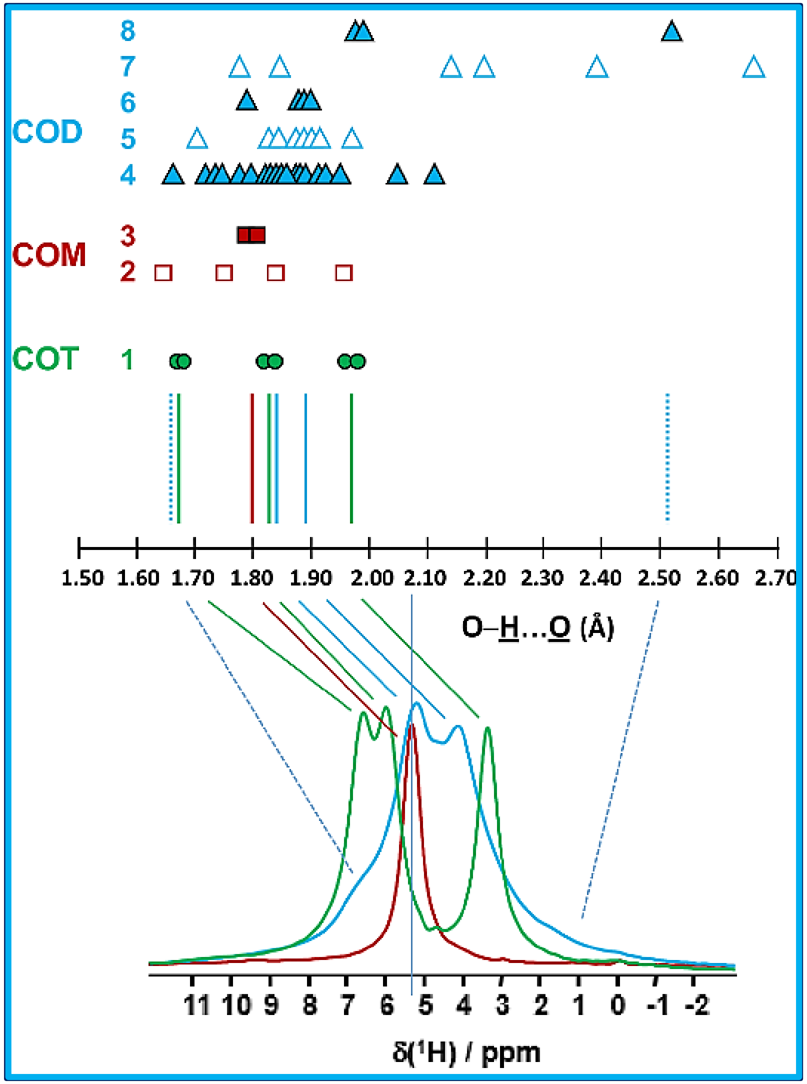
→ O–H…O (↔ δ_{iso}(^{1}H))

→ number of H…O contacts

A focus on ¹H solid state NMR







The subtle role of temperature

Hydrated Calcium Oxalates: Crystal Structures, Thermal Stability, and Phase Evolution

Alina R. Izatulina,[*,†] Vladislav V. Gurzhiy,[†] Maria G. Krzhizhanovskaya,[†] Mariya A. Kuz'mina,[†] Matteo Leoni,[‡] and Olga V. Frank-Kamenetskaya[†]

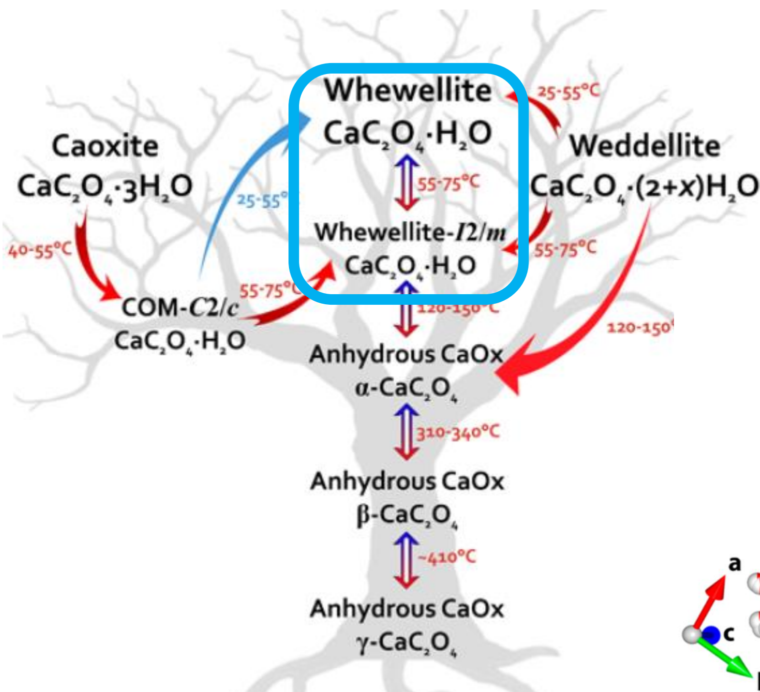
Order and Disorder in Calcium Oxalate Monohydrate: Insights from First-Principles Calculations

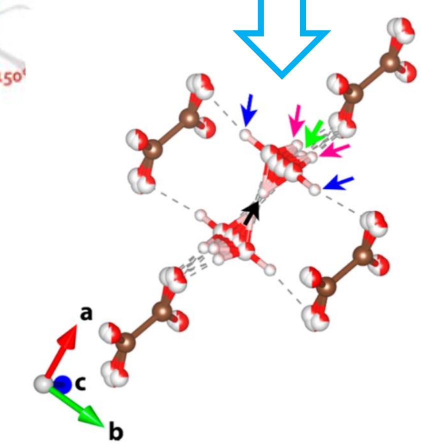
Published as part of a Crystal Growth and Design virtual special issue 'Remembering the Contributions and Life of Prof. Joel Bernstein'.

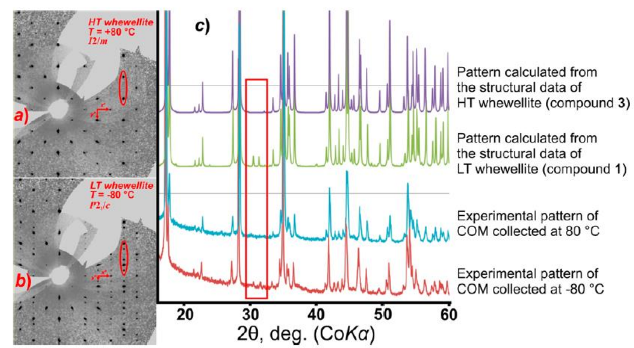
Margarita Shepelenko,[†] Yishay Feldman,[‡] Leslie Leiserowitz,[*,†] and Leeor Kronik[*,†]

CRYSTAL GROWTH & DESIGN

CRYSTAL GROWTH & DESIGN



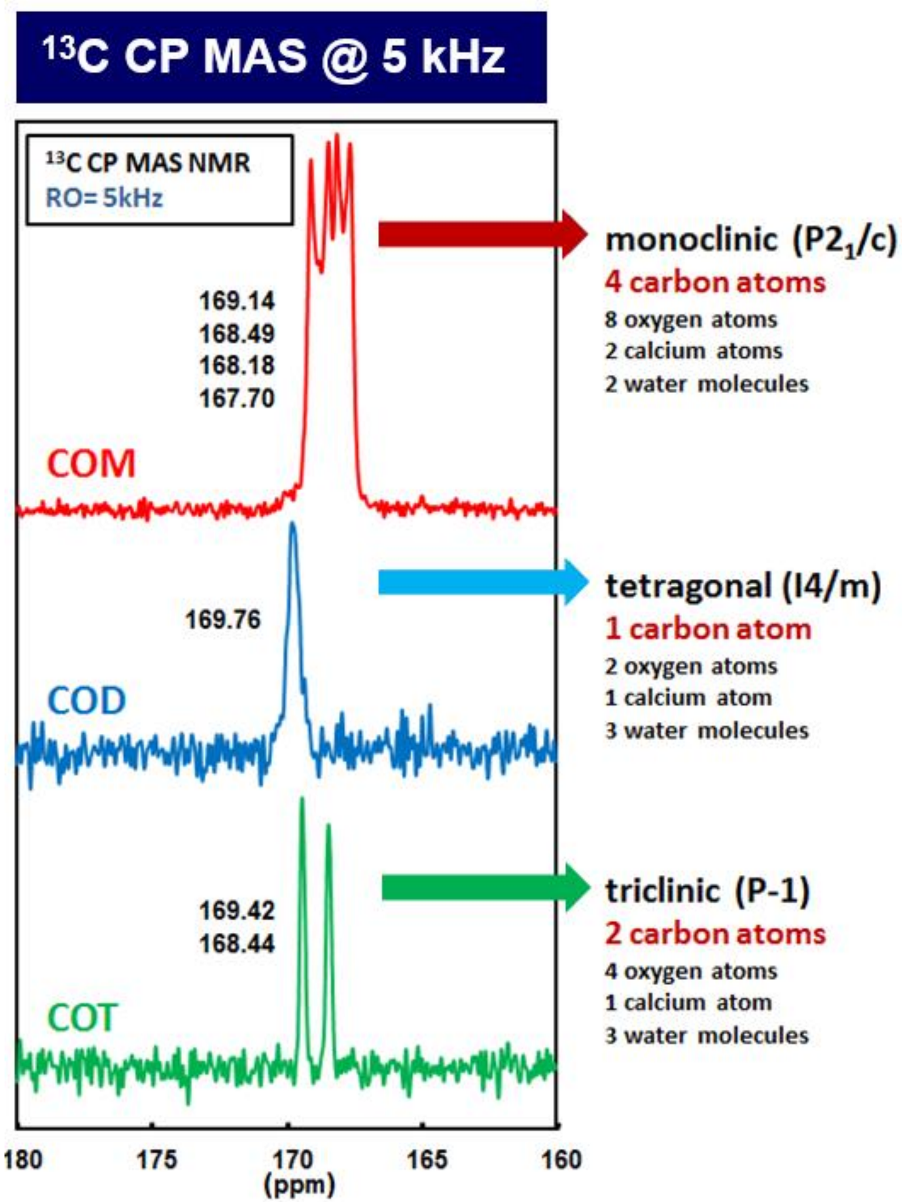




XRD experiments

DFT calculations

In situ transformations: modifying the temperature of the sample by MAS



Cross Polarization

^{1}H–^{13}C dipolar interaction

through *space*

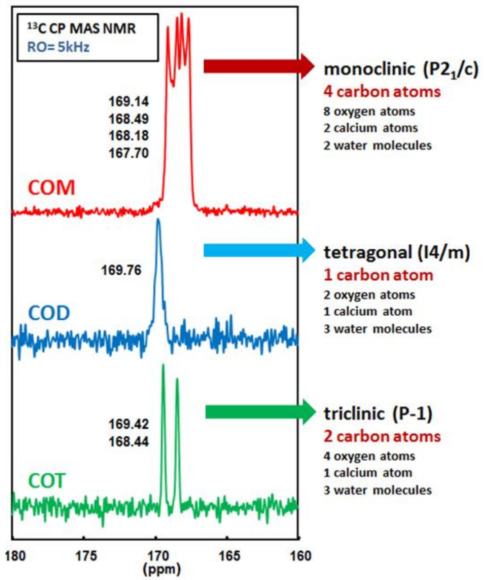
$$D \sim \frac{1}{r^{3}_{(^{1}H-^{13}C)}}$$

In situ transformations

... To : COM

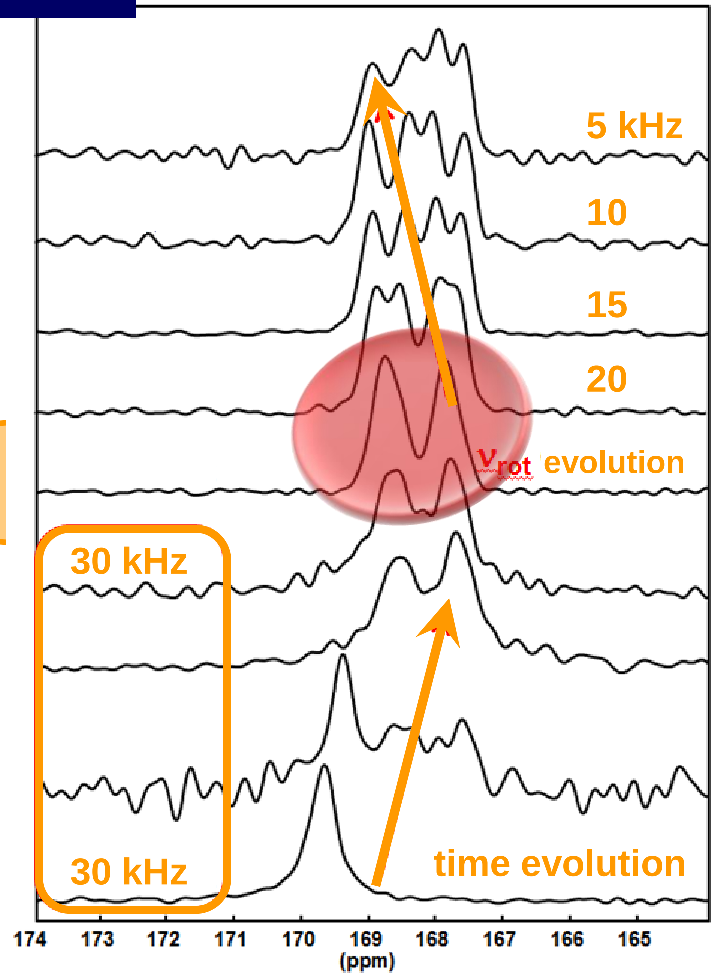
^{13}C CP MAS @ 30 kHz

^{13}C CP MAS @ 5 kHz



intermediate phase

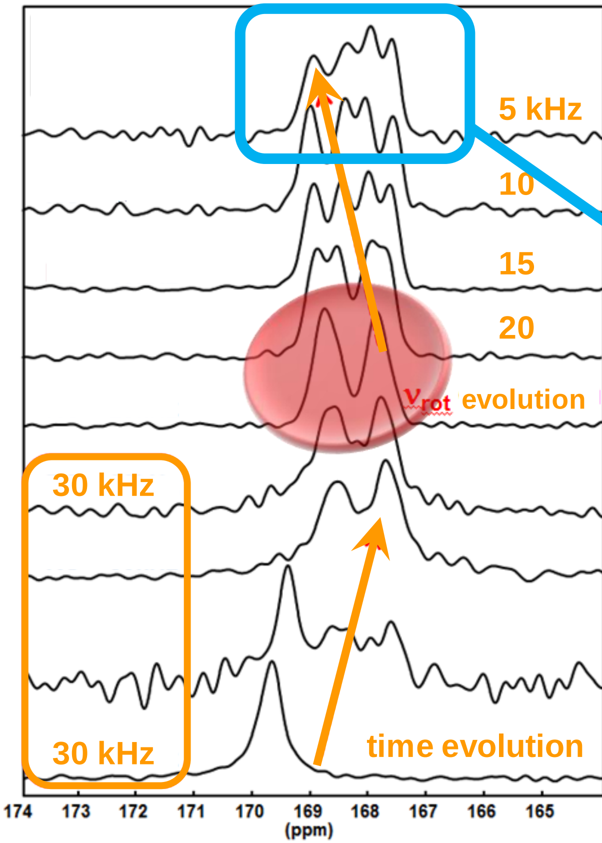
?



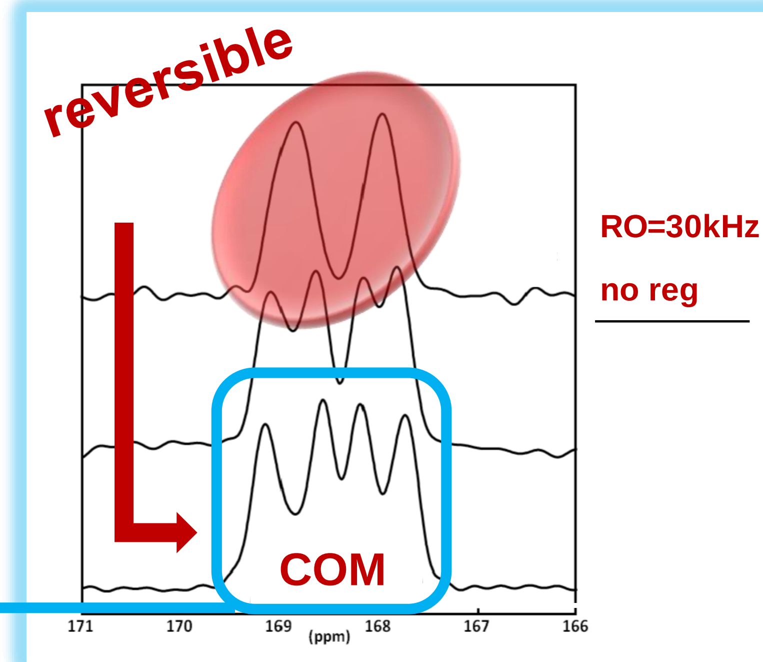
From: COD ...

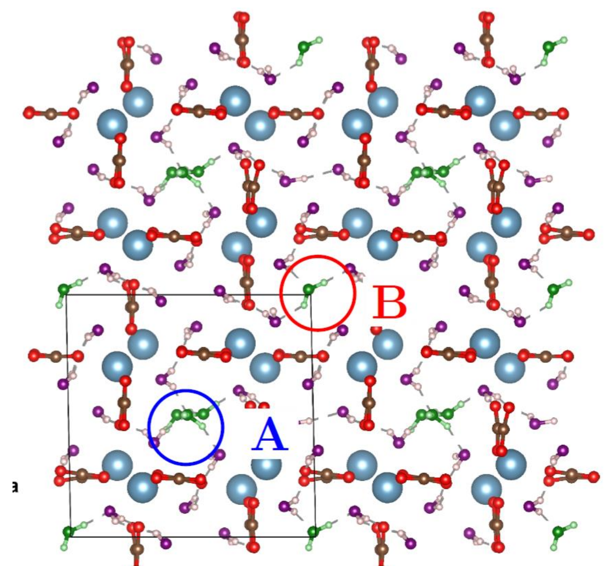
An intermediate phase

… To : COM

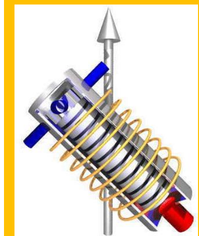


From: COD …





In situ dehydration: ^{79}Br MAS NMR

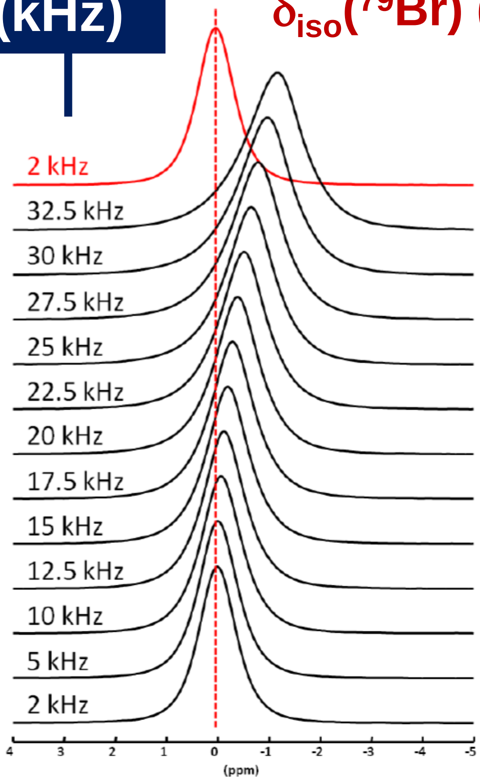


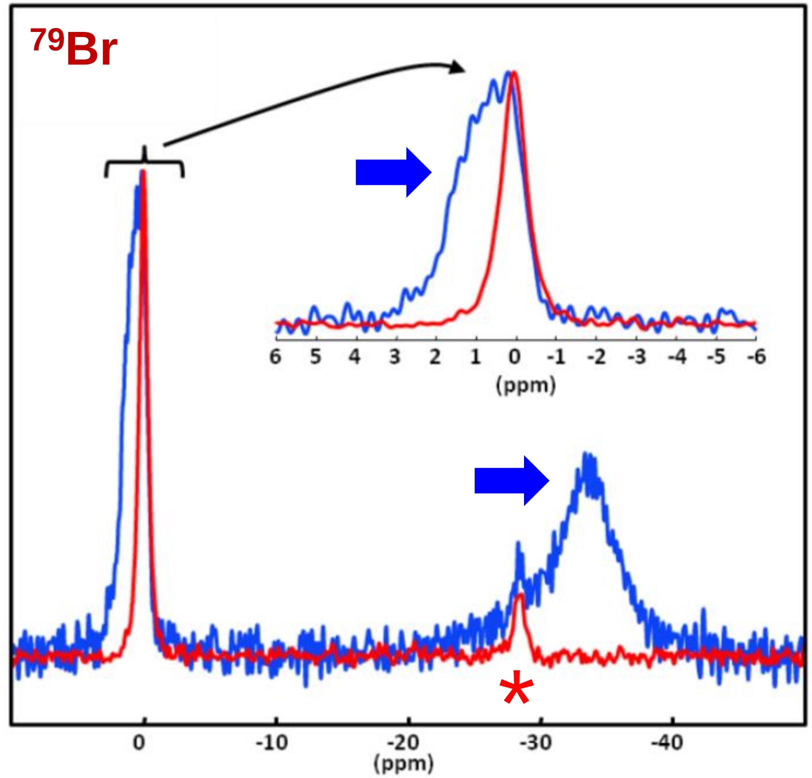
30 kHz

{COD + KBr}

ν_{rot} (kHz)

$\delta_{iso}(^{79}Br)$ ($T_1(^{79}Br)$)





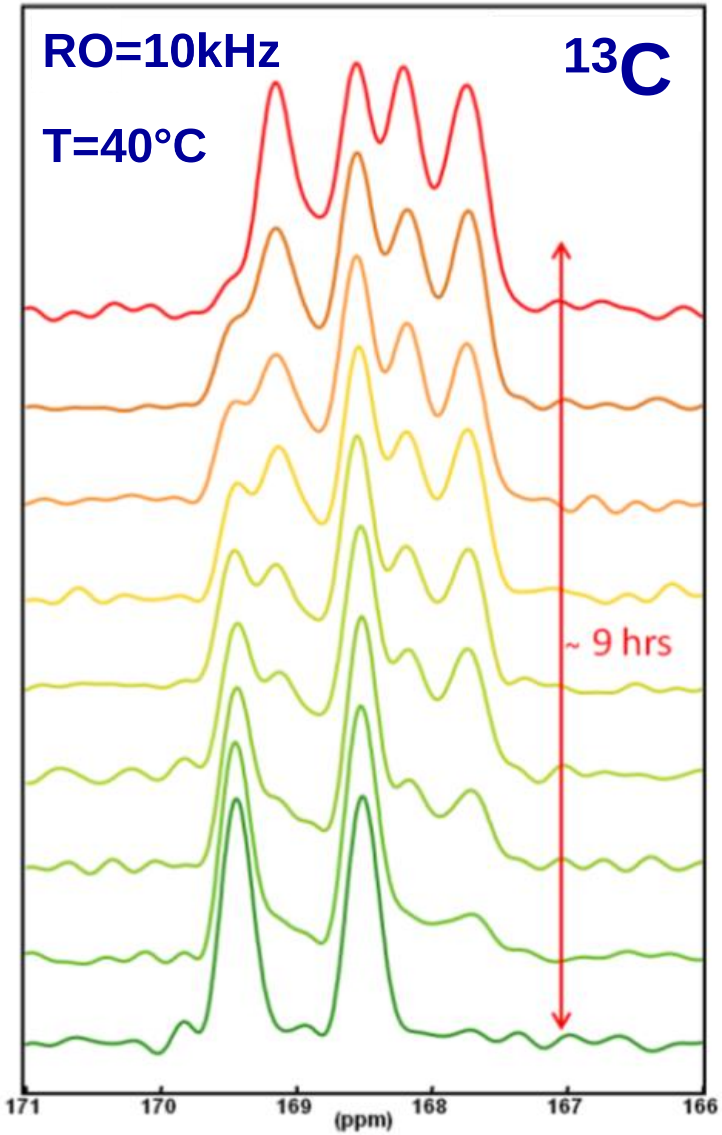
► **dry KBr**

► **{COD + KBr}**

***i.e.* KBr + H_2O (droplet)**

K. Thurber *et al.*, 2009

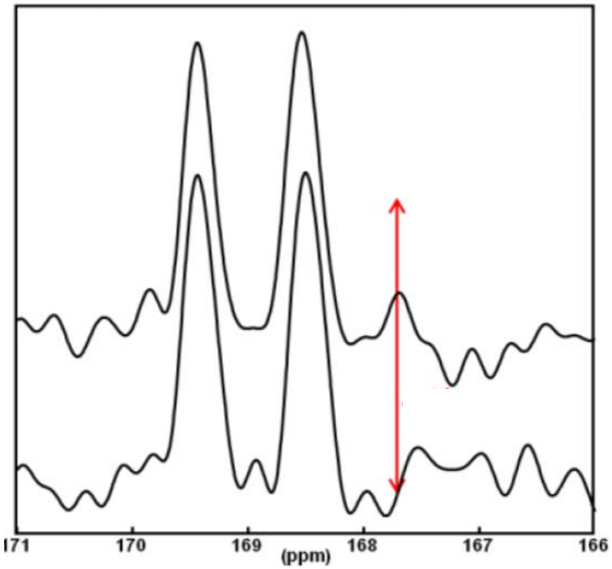
From COT to COM



… To : COM

clinical observation:

no occurrence of COT in pathological calcifications

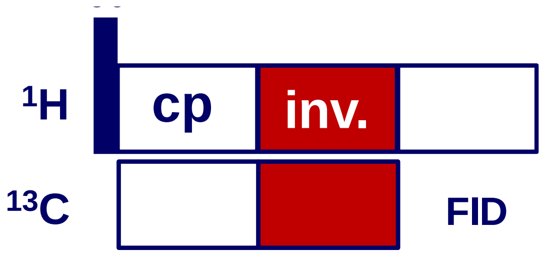


RO=30kHz

T=25°C

From: COT …

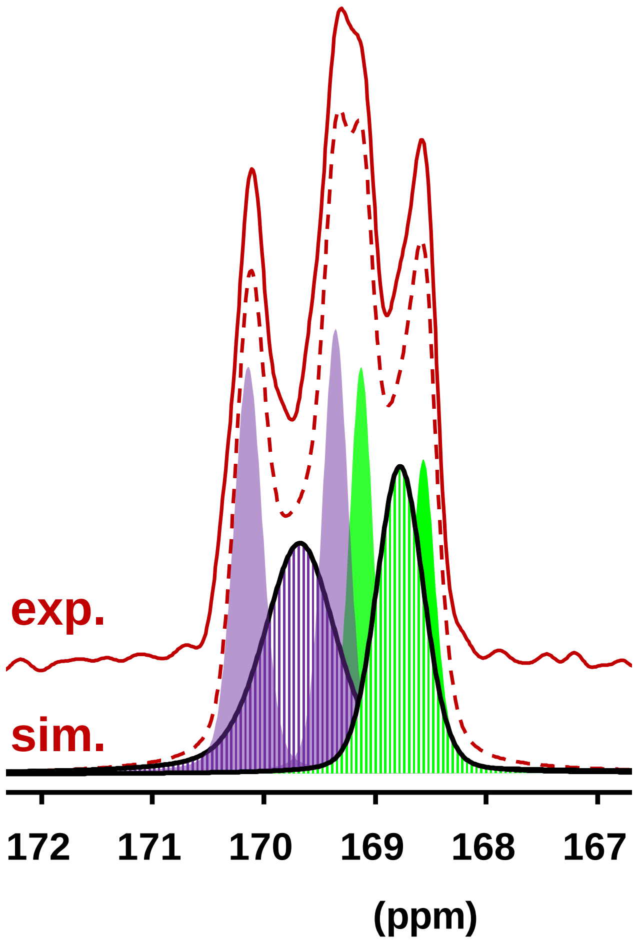
^{1}H–^{13}C SLF (Separated Local Field) by inversion of polarization



Zilm *et al*., Tekely *et al*…

CaOx monohydrate

COM



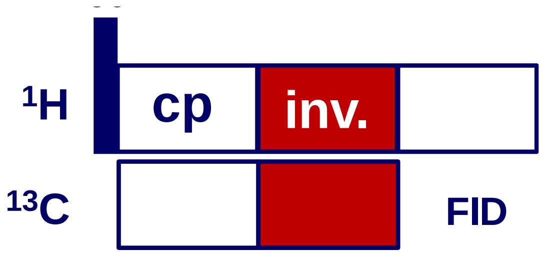
Cross Polarization

^{1}H–^{13}C dipolar interaction

through *space*

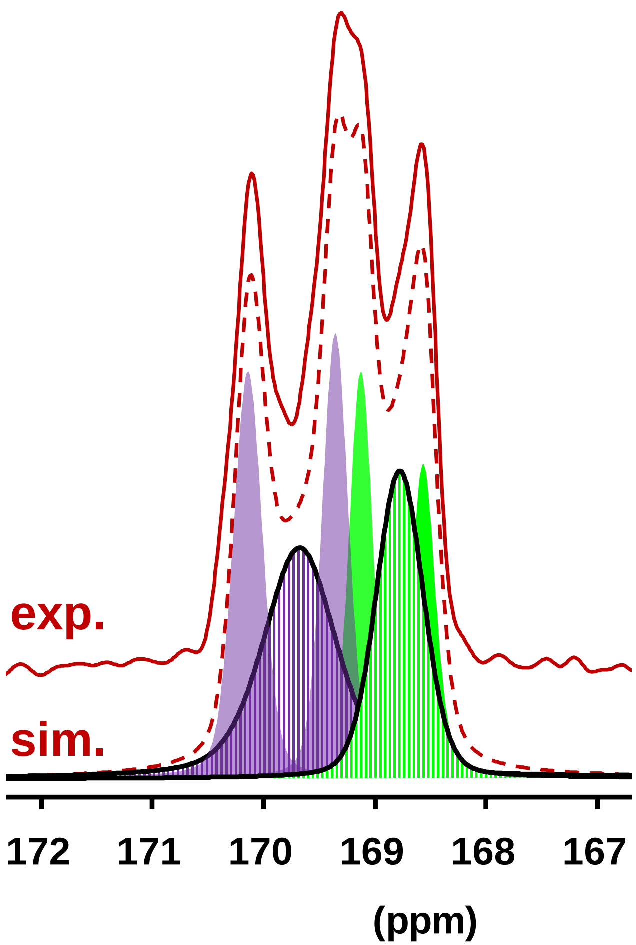
« … the stronger the interaction, the faster the inversion… »

^1H–^{13}C SLF (Separated Local Field) by inversion of polarization



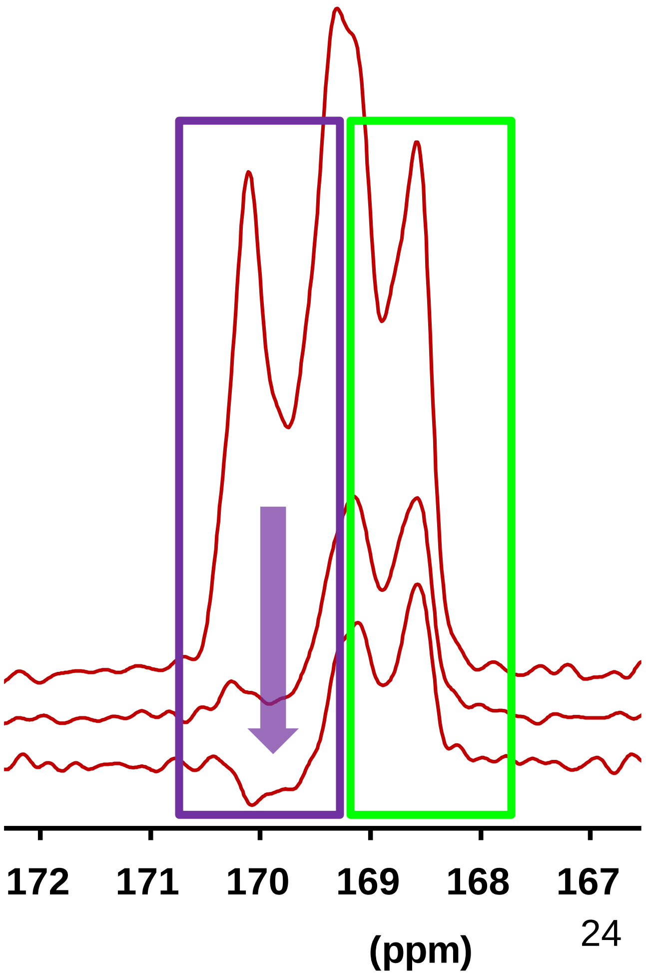
Zilm *et al*., Tekely *et al*…

CaOx monohydrate
COM

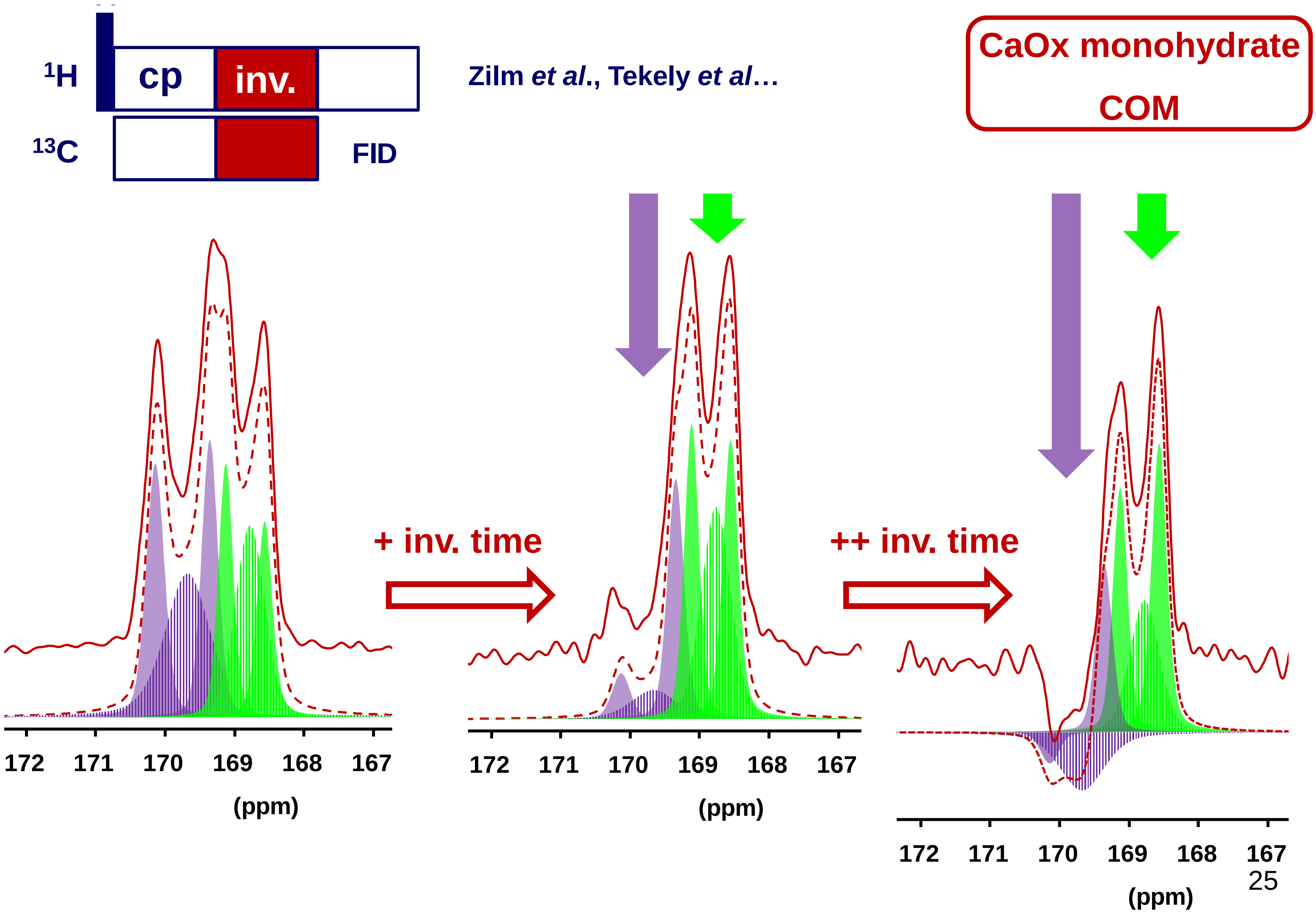


$t_{inv.}$ ~ 0 μs

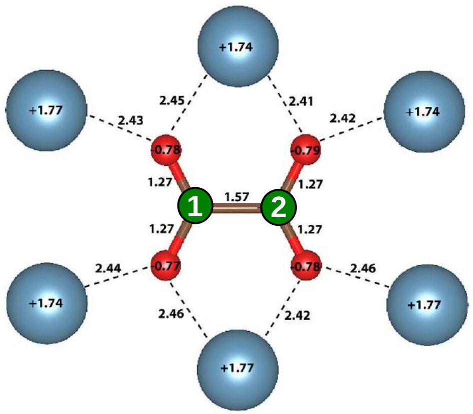
1000 μs

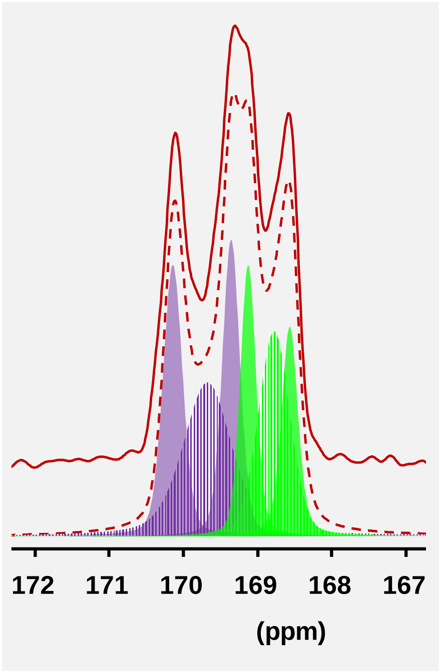


^{1}H–^{13}C (SLF) Separated Local Field by inversion of polarization

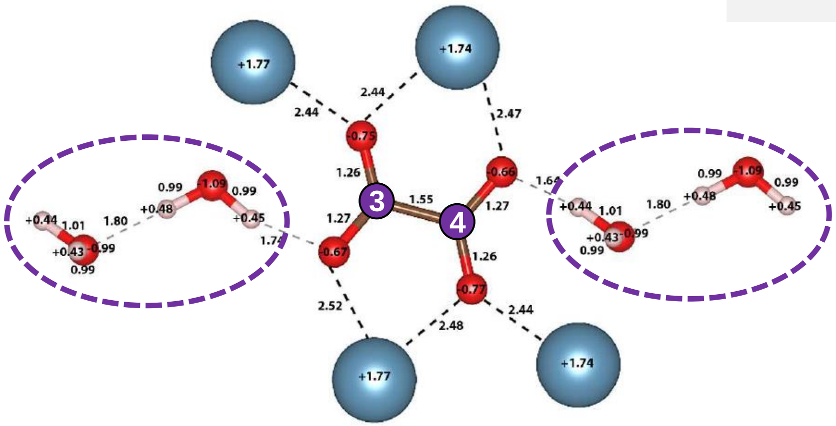


Full interpretation of the ^{13}C CP MAS NMR spectra of COM



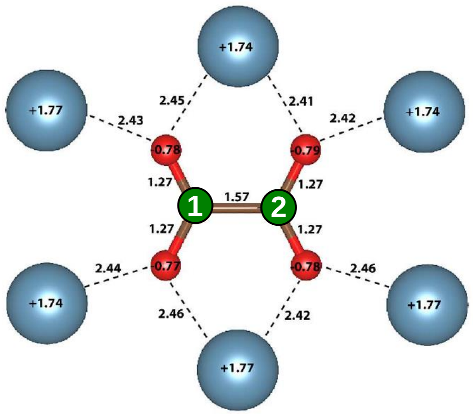


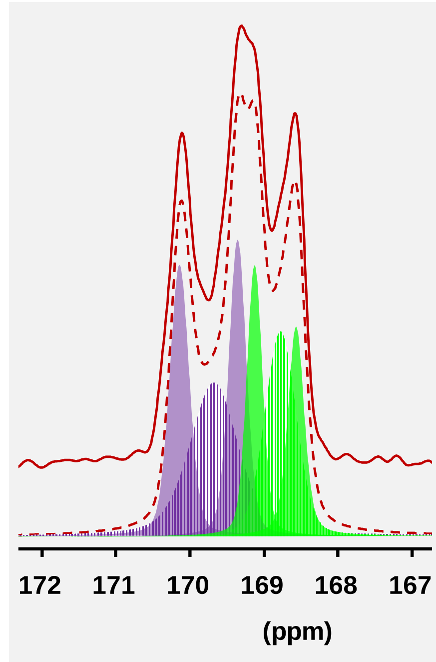
P2$_1$/c: **C$_1$, C$_2$, C$_3$, C$_4$**

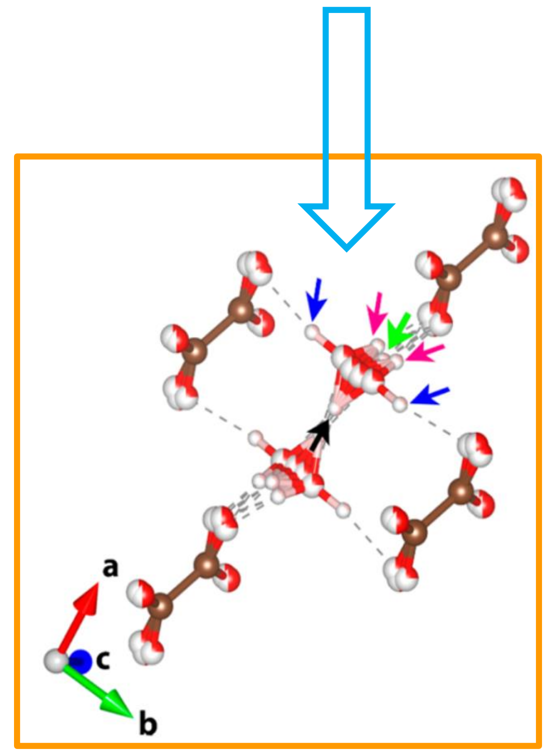


√ **COM phase: *P2$_1$/c* space group**

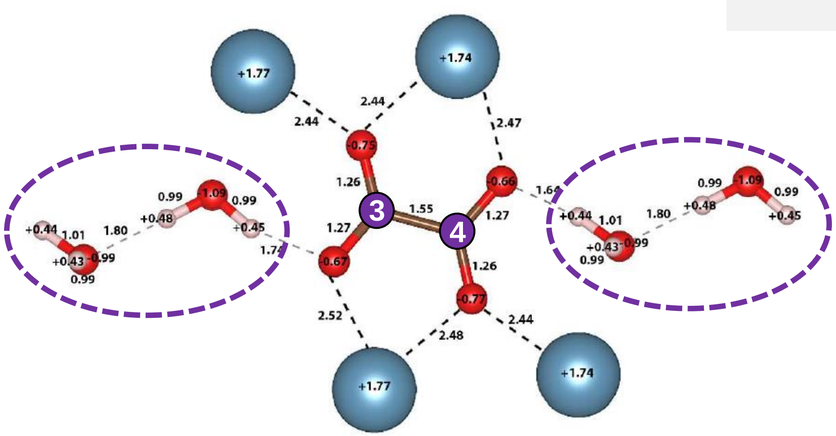
Full interpretation of the ^{13}C CP MAS NMR spectra







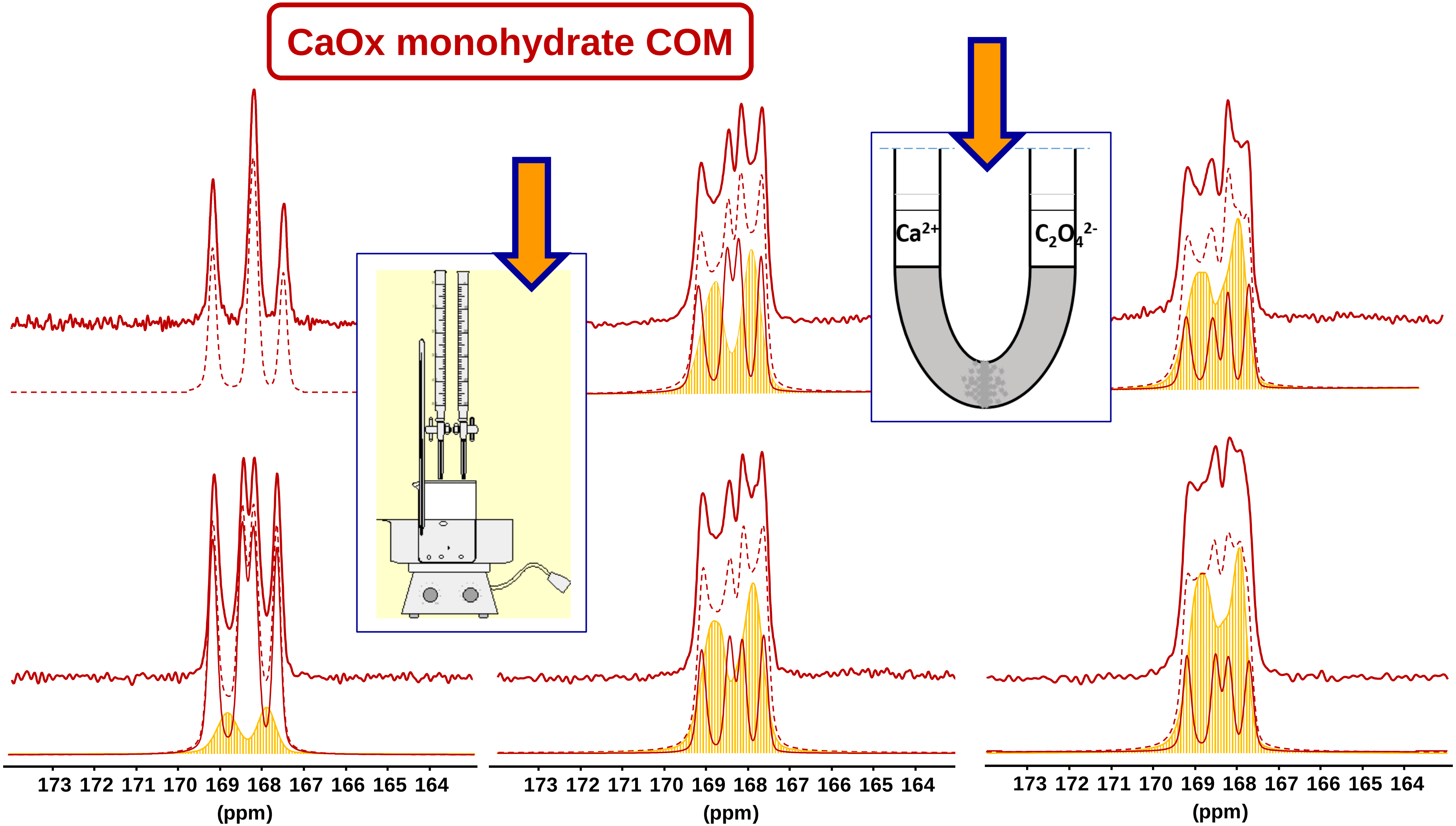
***$P2_1/c$*: C_1, C_2, C_3, C_4**



√ **COM phase: *$P2_1/c$* space group**

√ **disordered COM phase: statistical *I2/m* space group (Shepelenko *et al*., 2020)**

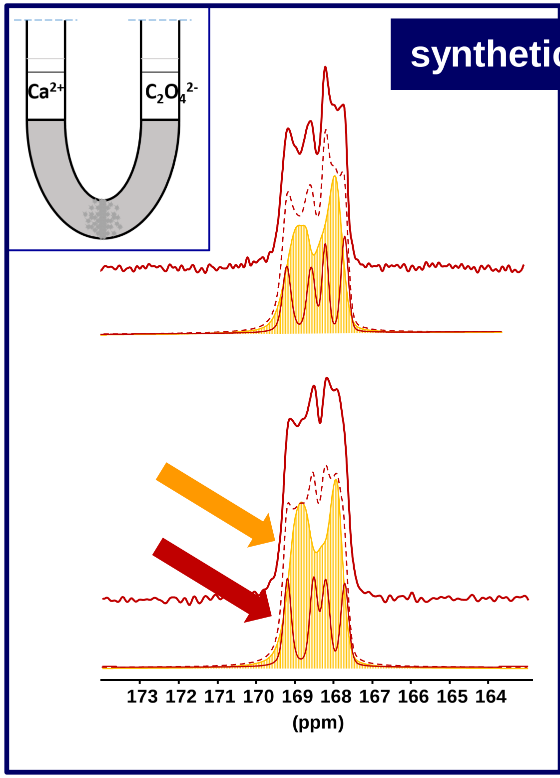
The new phase (from NMR…) is ubiquitous in COM syntheses

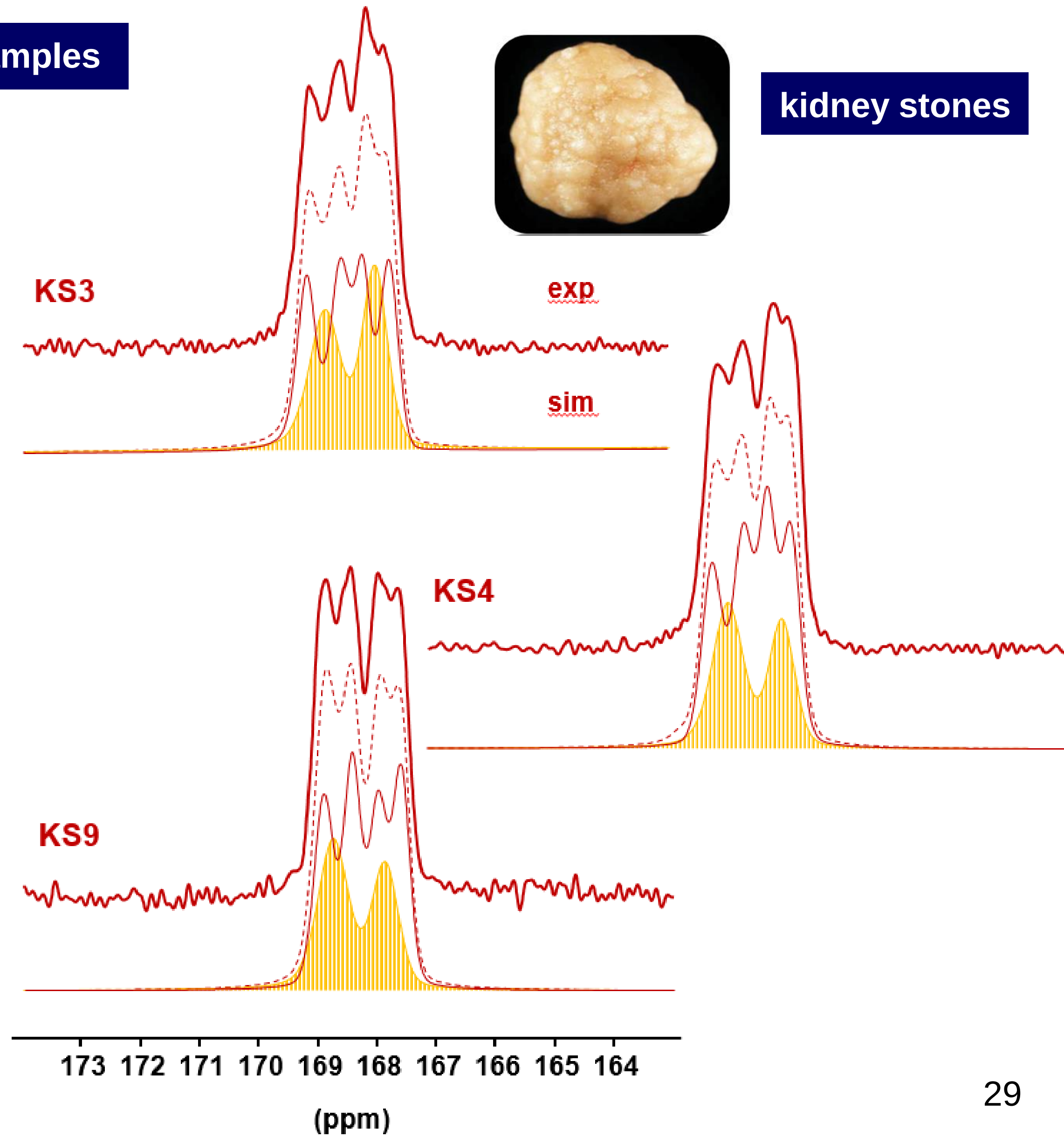


√ **COM phase: *P*2$_1$/*c* space group**

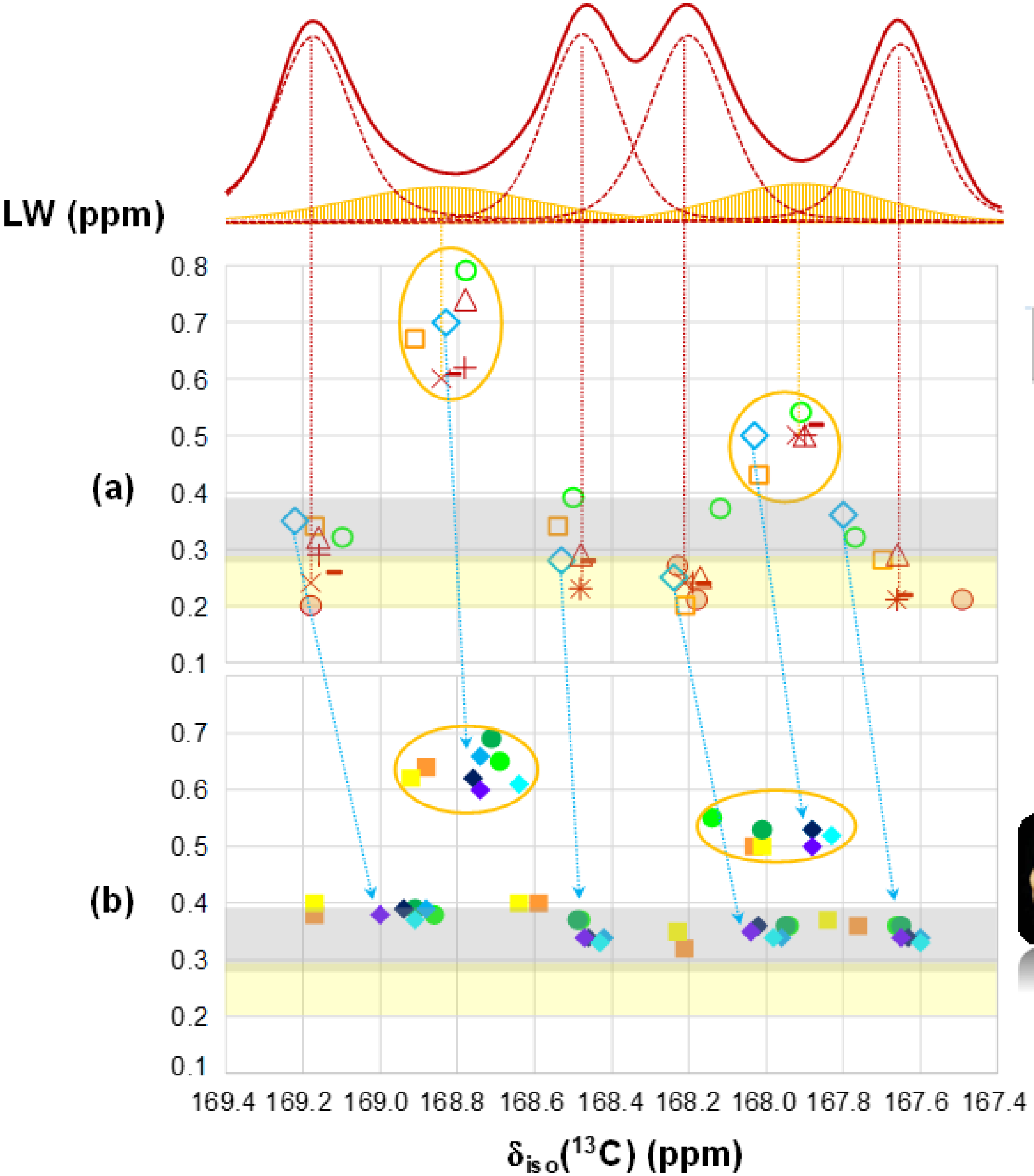
√ **disordered COM phase: statistical *I*2/*m* space group (Shepelenko *et al*., 2020)**

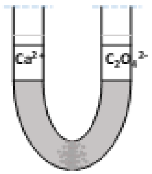
Towards *artificial* kidney stones

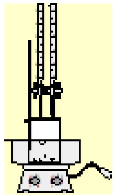




The new phase (from NMR) is ubiquitous in KS





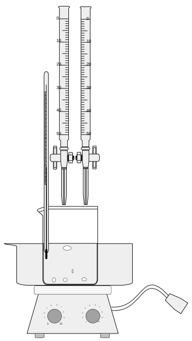


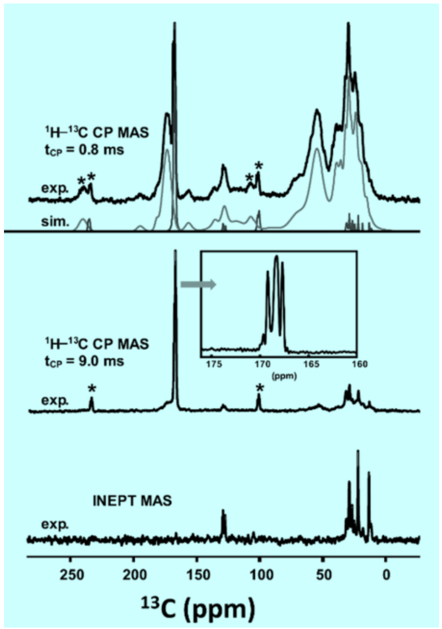
→ diagnosis

■ NMR as a unique platform of characterization

▶ *structure*

▶ ***dynamics***





■ More sensitivity

■ Dynamic Nuclear Polarization crystallography

■ Magic Angle Spinning MRI

Dynamics of water molecules in COM and calcium Pyrophosphates (CaPP)

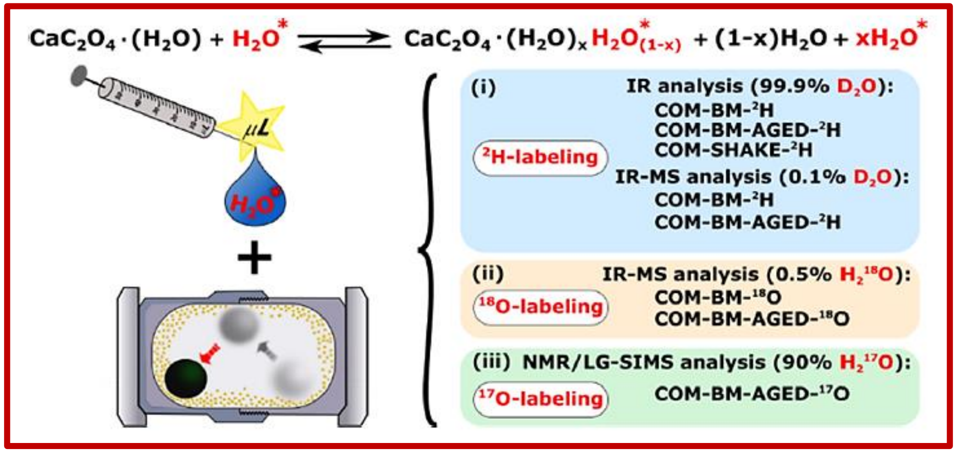
synthesis of labeled samples (^{17}O) by mechanochemistry


Angewandte Chemie International Edition
GDCh A Journal of the German Chemical Society

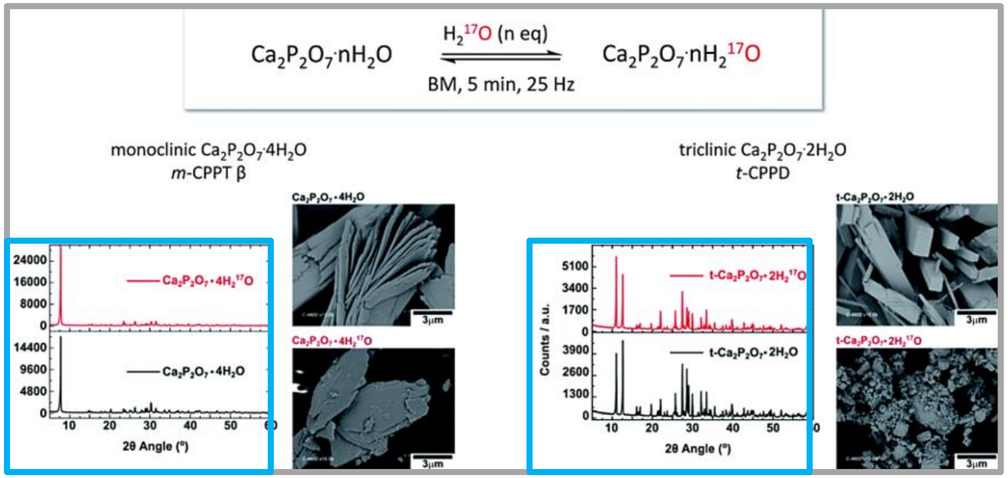
Communication

Unleashing the Potential of ^{17}O NMR Spectroscopy Using Mechanochemistry

Dr. Thomas-Xavier Métro, Prof. Christel Gervais, Anthony Martinez, Prof. Christian Bonhomme, Dr. Danielle Laurencin



COM

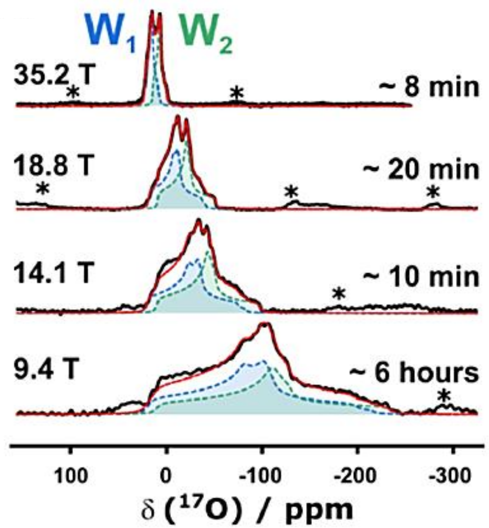


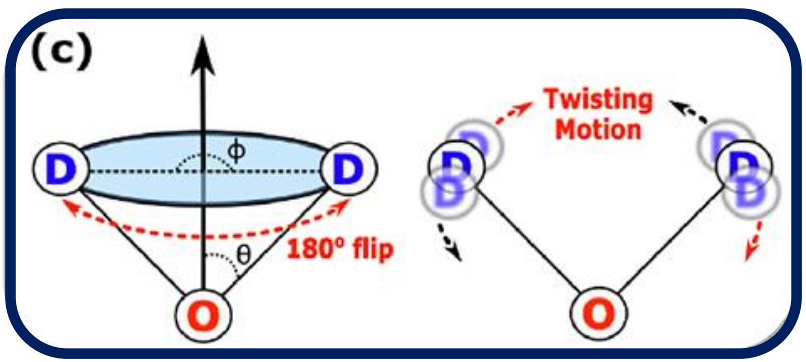
CaPP

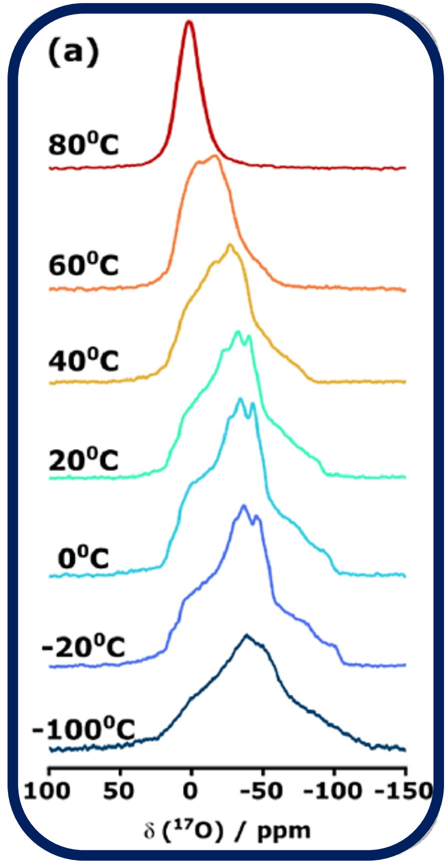
Coll.: D. Laurencin, Montpellier (France) **JPC C , 2022; Faraday Discuss., 2023**

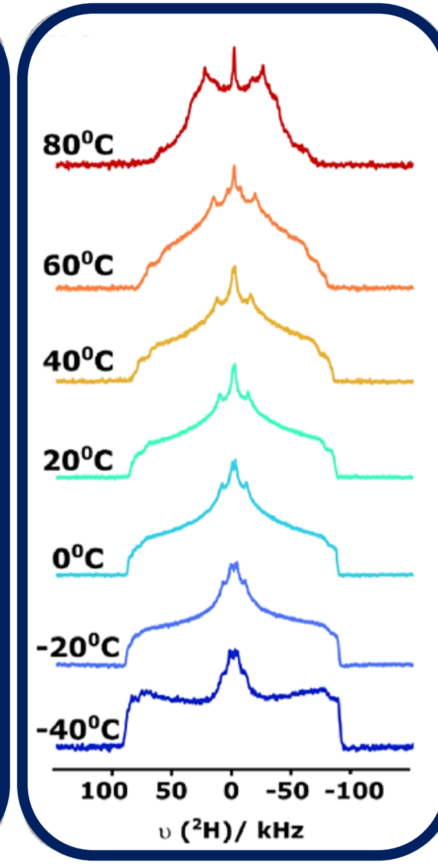
Dynamics of water molecules in COM

^{17}O, I = 5/2, Q









^{17}O

^{2}H, I = 1, Q

JPC C , 2022

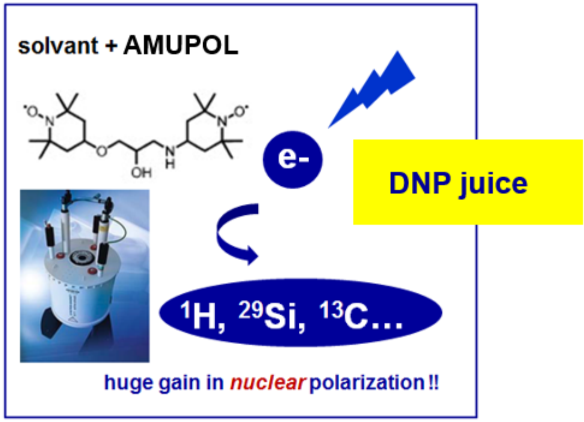
Outline

■ NMR as a unique platform of characterization

► *structure*

► *dynamics*

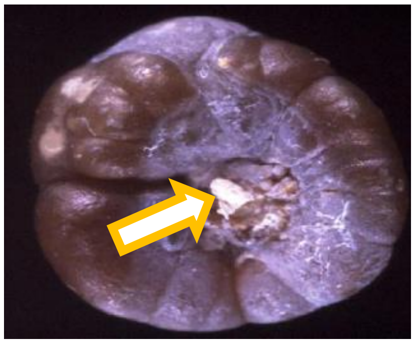
■ **More sensitivity**



■ Dynamic Nuclear Polarization crystallography

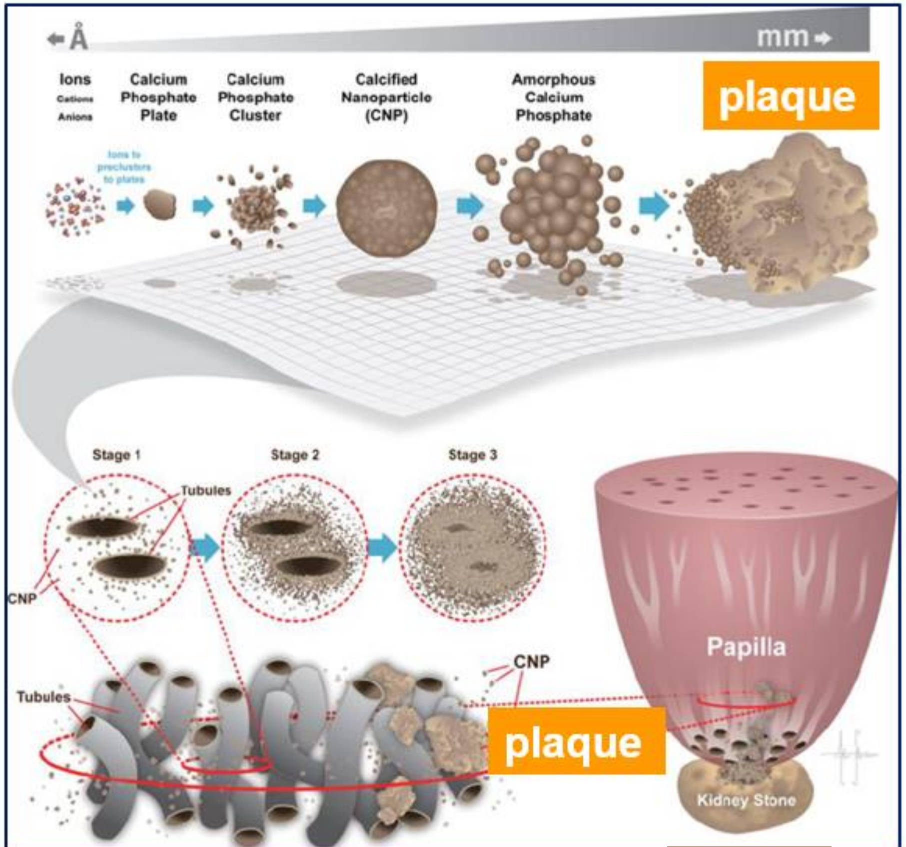
■ Magic Angle Spinning MRI

The Randall's plaque: a calcium phosphate (hydroxyapatite, HAp)



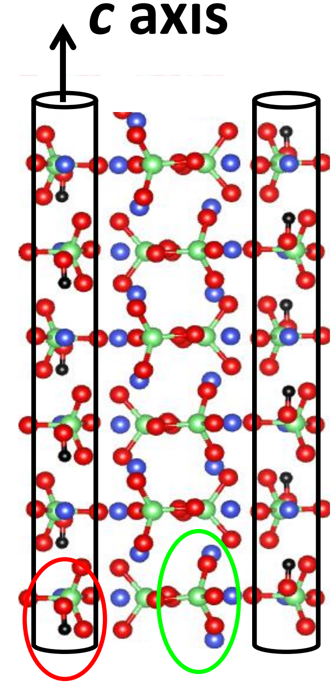
d < 1 mm

m < 100 μg



KS (COM)

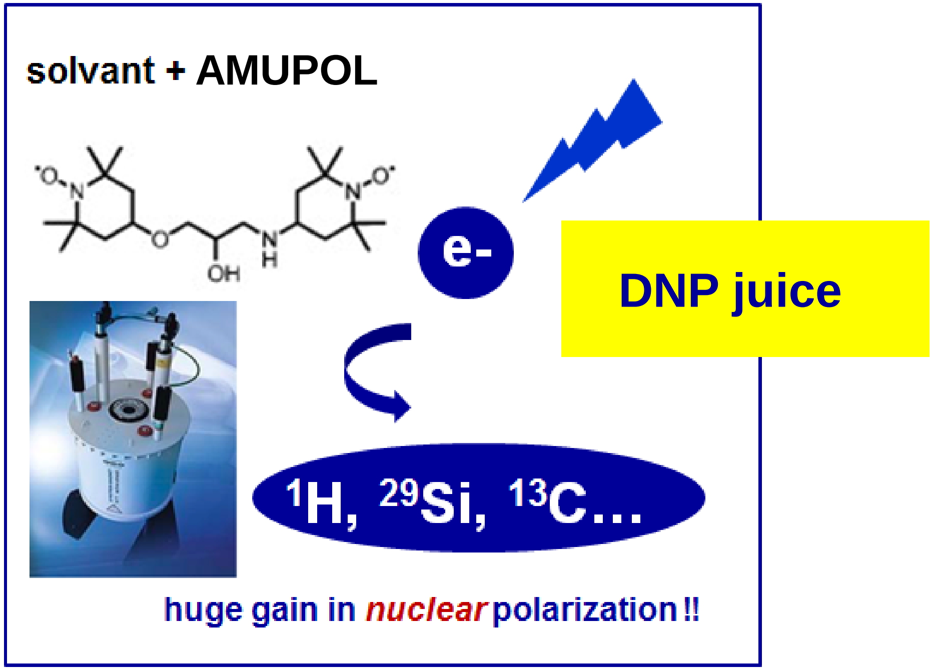
HAp

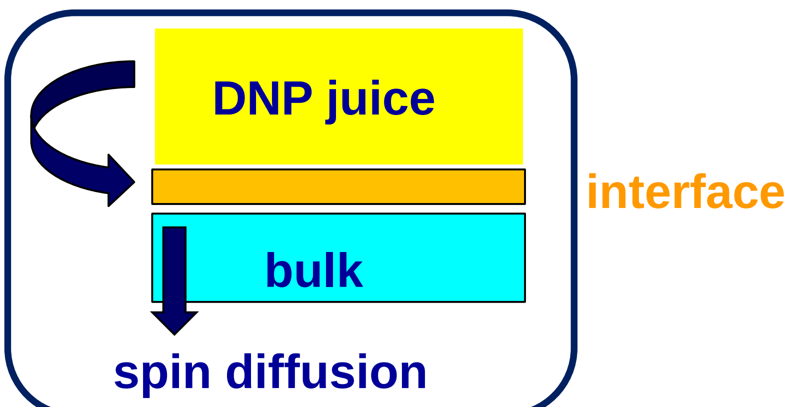


$Ca_{10}(PO_4)_6(OH)_2$

Sherer, Acta Biomater., 2018

Dynamic Nuclear Polarization (DNP) MAS





- **SENSITIVITY**

"impossible experiments"

- **LOW TEMPERATURE & MAS**

(~ 100 K or lower…)

depending on the sample…

- **enhanced spin locking during CP**
- **better homonuclear decoupling**
- **…**

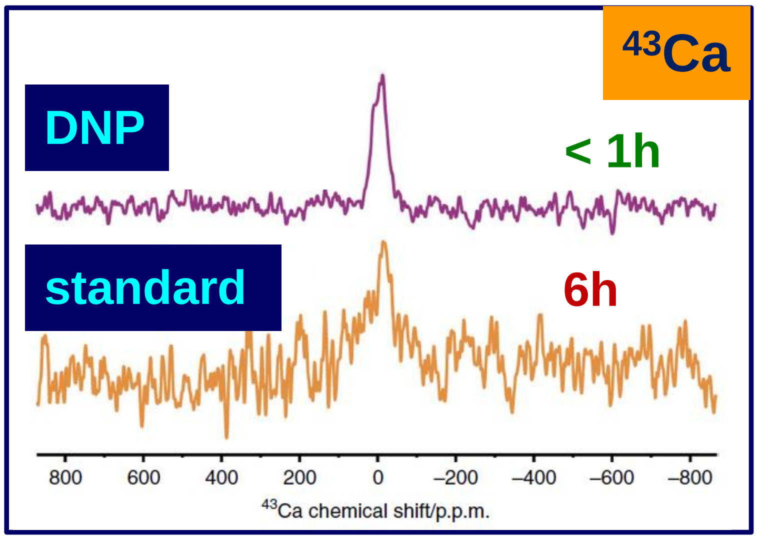
Griffin, Bodenhausen, Emsley…

Natural abundance ^{43}Ca DNP spectroscopy (N.A. 0.14%, low γ, $I = 7/2$)

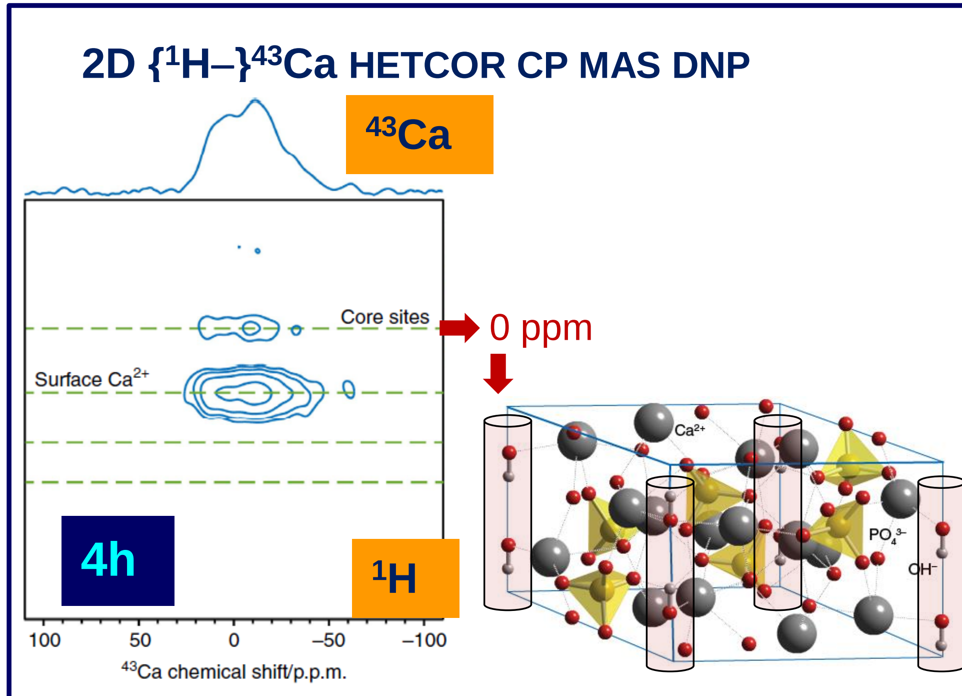
$\nu_0(^{43}Ca)$ = 26,94 MHz, 100 K, DNP juice: glycerol-d_8/D_2O/H_2O (60/30/10; v/v/v) + AMUPol,

sample: ~ 20 mg

1D NMR



nat. abund. 2D NMR



Nature Commun., 2017

Coll.: D. Lee, G. De Paëpe, Grenoble, France

Some perspectives in the study of Randall's plaque

m < 100 μg

^{13}C DNP CP MAS approach (400 MHz & 100 K)

saphire rotor

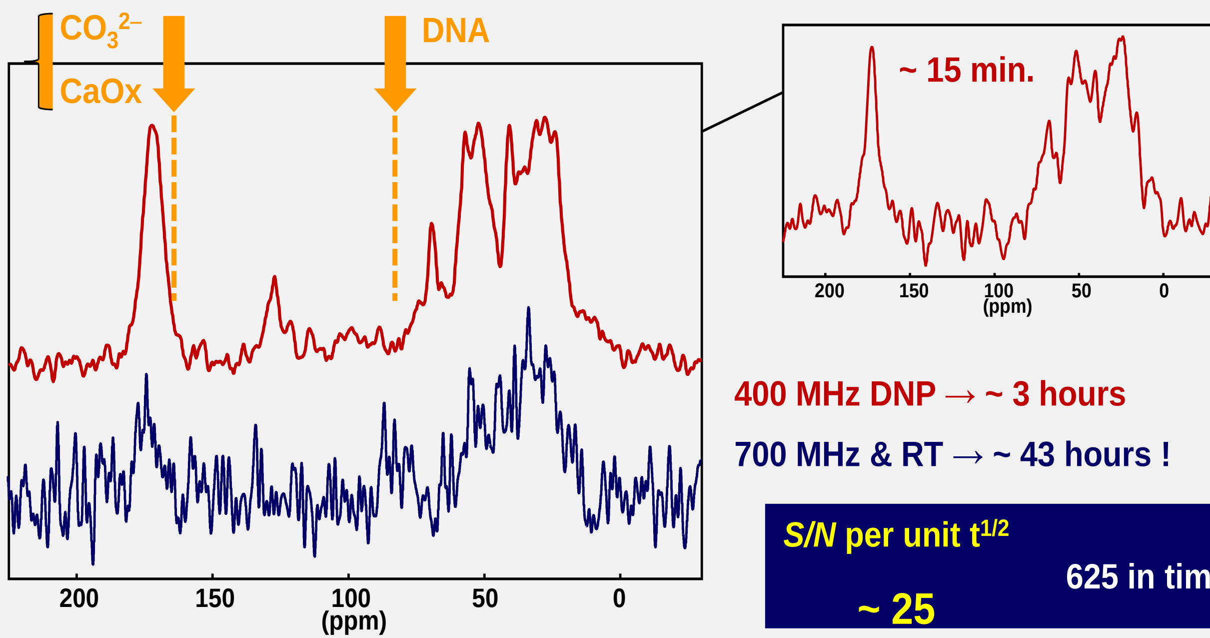
unique R plaque

+ {AMUpol in D_2O/H_2O (9:1)}

Duer *et al.* (*J. Urol*., 2011)

"Unfortunately, it is challenging to collect sufficient Randall's plaque material in the mg to tens of mg quantities necessary for $^{13}C\{^{31}P\}$ REDOR".

^{13}C



400 MHz DNP → ~ 3 hours

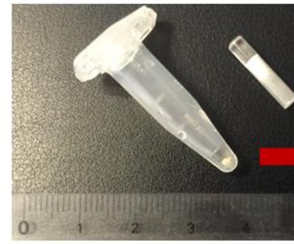
700 MHz & RT → ~ 43 hours !

S/N per unit $t^{1/2}$ ~ 25

625 in time

Some perspectives in the study of pathological calcifications

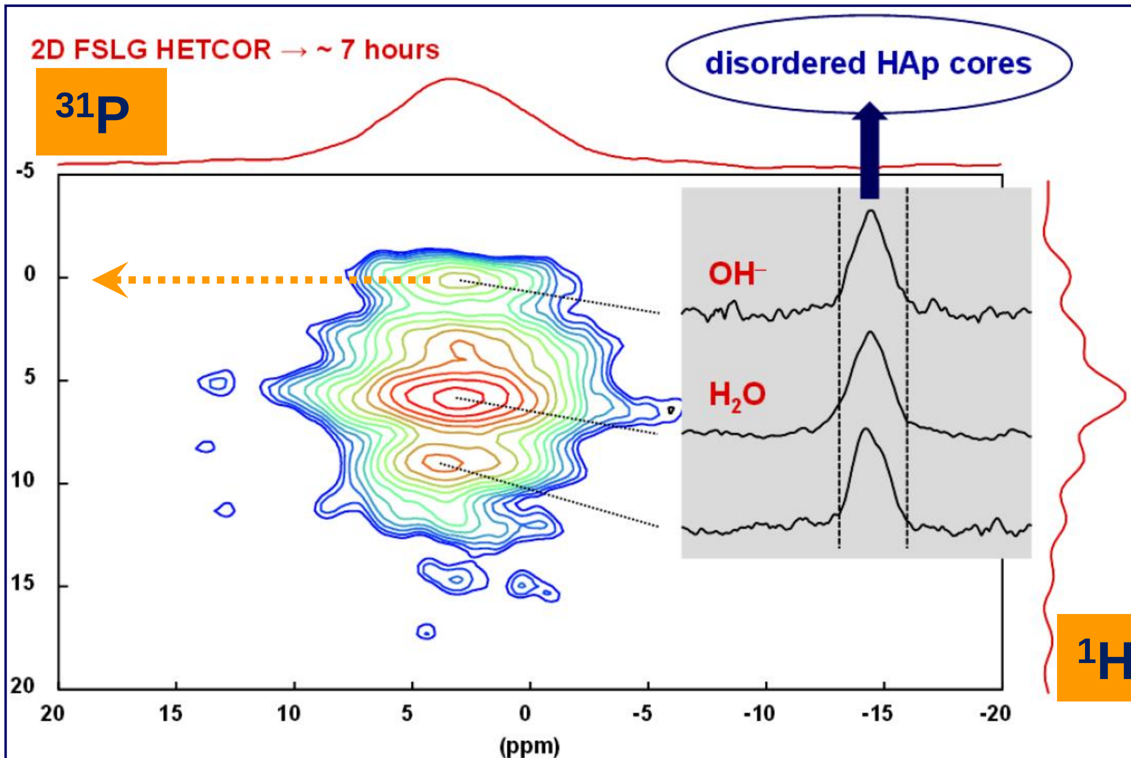
low mass samples



saphire rotor

unique R plaque

+ {AMUpol in D_2O/H_2O (9:1)}



Outline

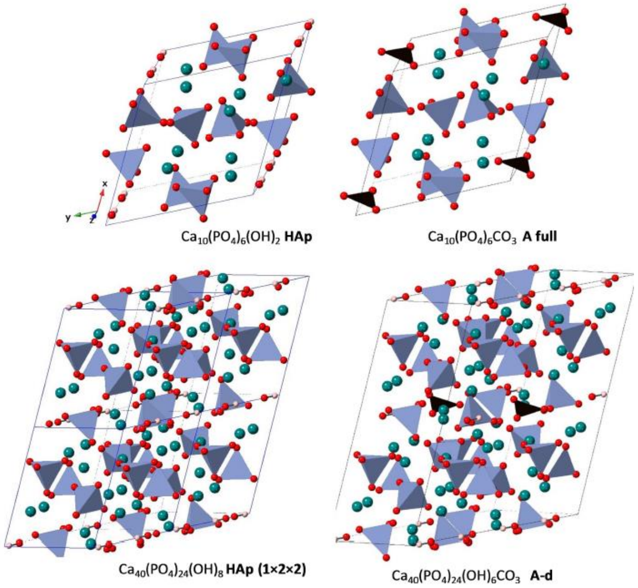
■ NMR as a unique platform of characterization

► *structure*

► *dynamics*

■ More sensitivity

■ **DNP crystallography**



■ Magic Angle Spinning MRI

GIPAW calculations

Pickard, Mauri, *Phys. Rev. B* (2001)

DFT

periodic systems

all-electron hamiltonians

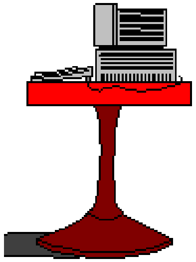
GIPAW

evaluation of $j^{(1)}(r')$ using pseudopotentials

$$B_{in}^{(1)}(r) = 1/c \int d^3r' \, j^{(1)}(r') \times \frac{r-r'}{|r-r'|^3}$$

CSA

EFG **J**

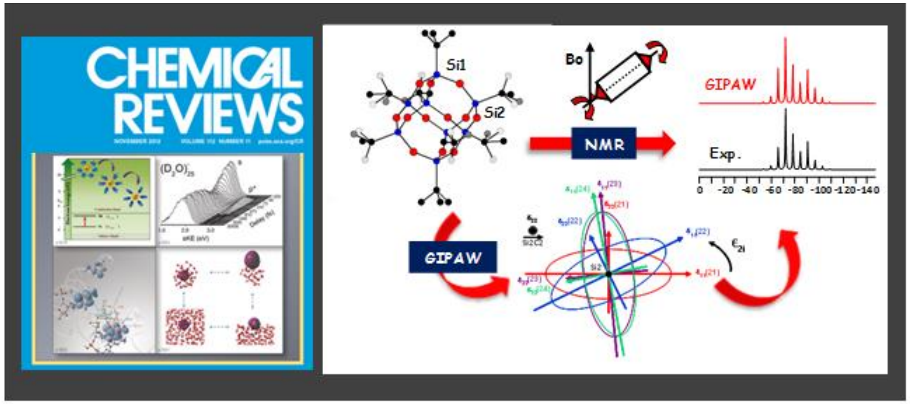


IDRIS

- structure / assignment of spectra
- dynamics
- amorphous slabs
- distributions

δ, C_Q, J

Coll.: J. Yates, Oxford (UK)



(Ashbrook, Gervais, CB, 2012)

ROYAL SOCIETY OF CHEMISTRY

D. Bryce

"Modern NMR Crystallography: Concepts and Applications" (2024)

Disordered materials **(Ashbrook, Gervais, CB)**

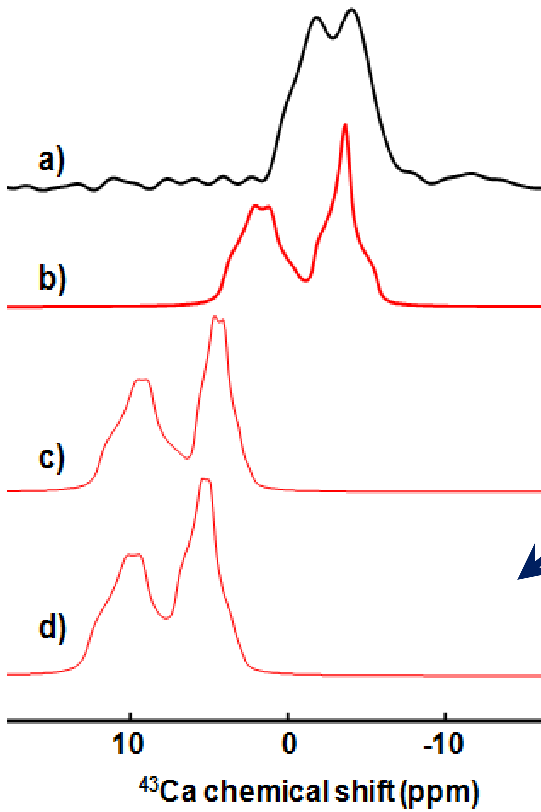
^{43}Ca GIPAW calculations

NATIONAL HIGH MAGNETIC FIELD LABORATORY

^{43}Ca, I = 7/2, Q

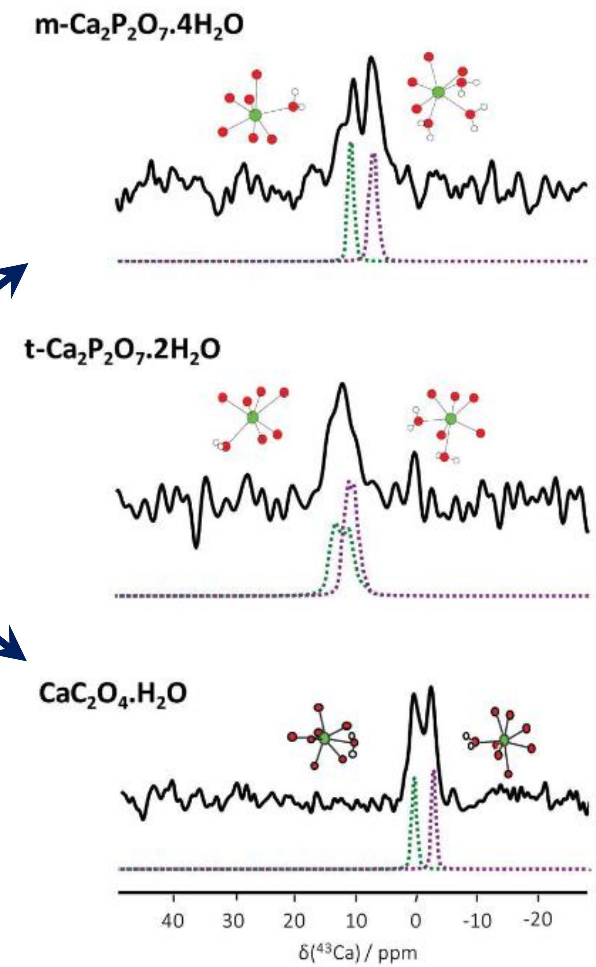
exp.

calc.



Ca PyroP

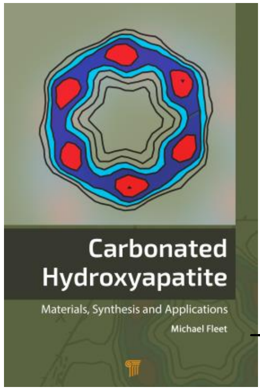
COM



CrystEngComm., 2013

Chem. Commun., 2018

Carbonate substituted hydroxyapatite (CHAp) ➡ low wt % in C



- ▶ **hundreds of publications (except NMR)**
- ▶ **common *qualitative* observations**

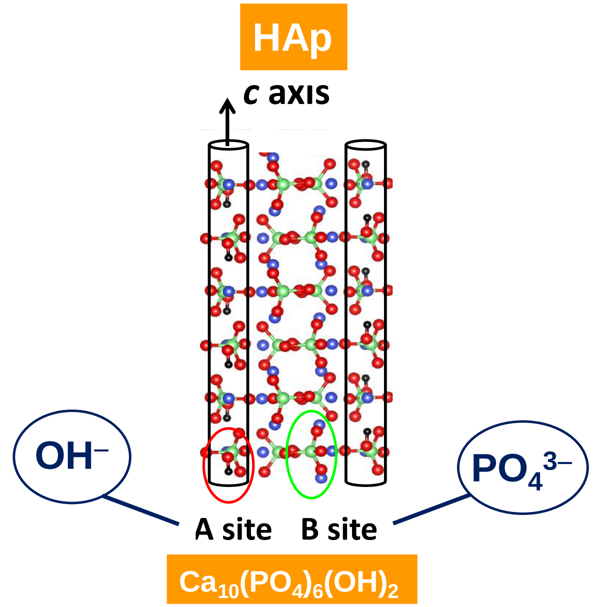
→ **LW (^{31}P) × 3 ; < δ_{iso}(^{31}P) > ↗**

→ **LW (^{1}H) ↗**

→ **multiplicity / overlap of ^{13}C resonances:**

A, B, A/B, B/B… *substitutions*

- ▶ **challenges of *quantitative* nature**



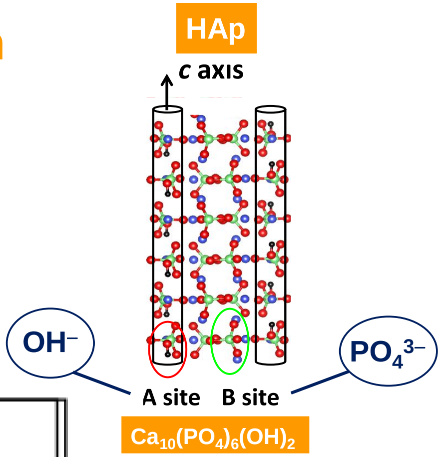
DNP… + DFT modeling … see: Peroos, de Leeuw, Ugliengo, Astala, Marisa… among others!

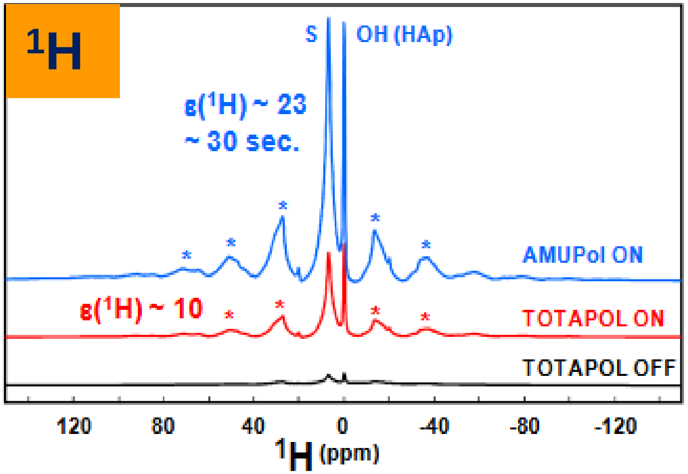
Coll.: F. Babonneau, C. Gervais

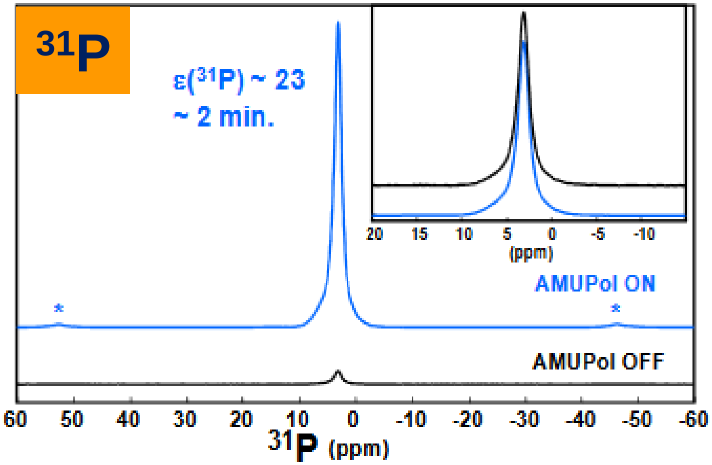
optimization of geometry at DFT level
PBE, van der Waals Grimme D3
VASP, QUANTUM-ESPRESSO, GIPAW

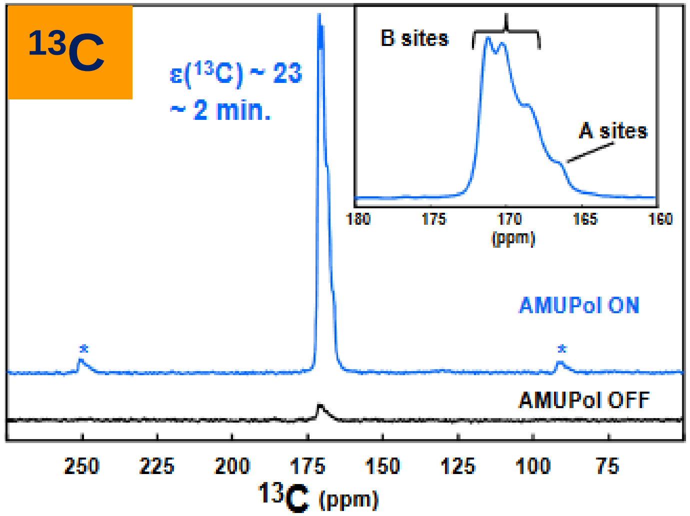
Synthetic carbonated nanosized HAp: DNP characterization

- ▶ synthetic HAp, ~ 1 wt % in C, labeled in ^{13}C
- ▶ 1D, 2D, double– and triple resonance CP, SQ-DQ experiments





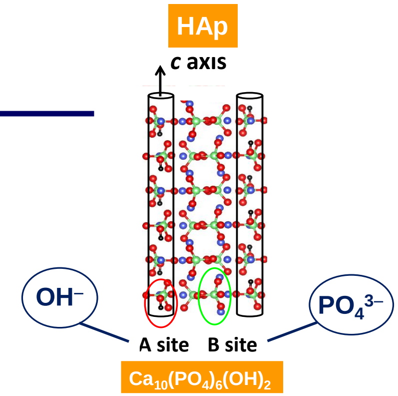




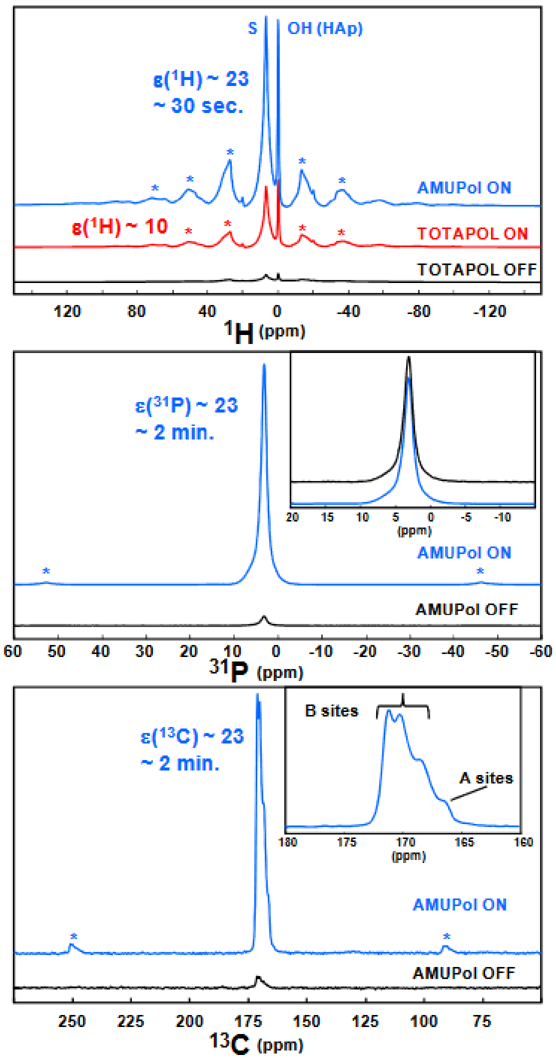
ON

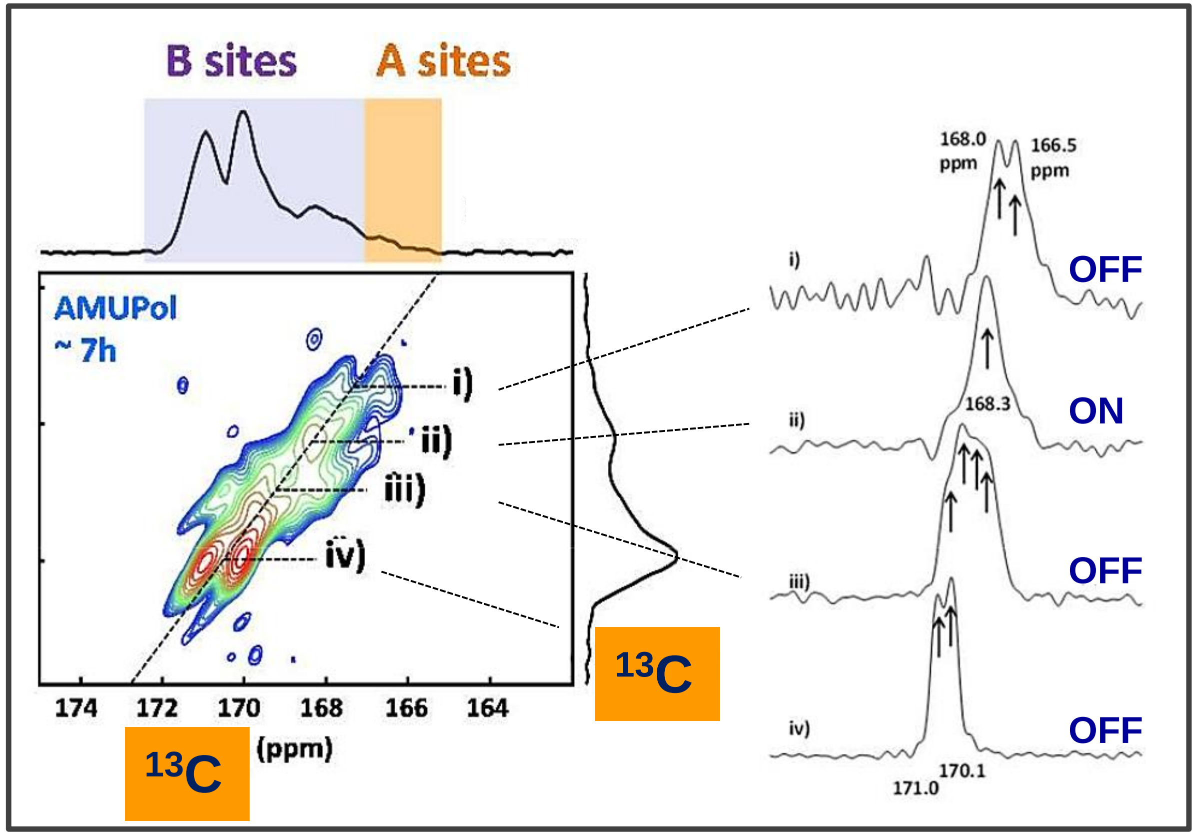
OFF

Synthetic carbonated nanosized HAp: DNP characterization



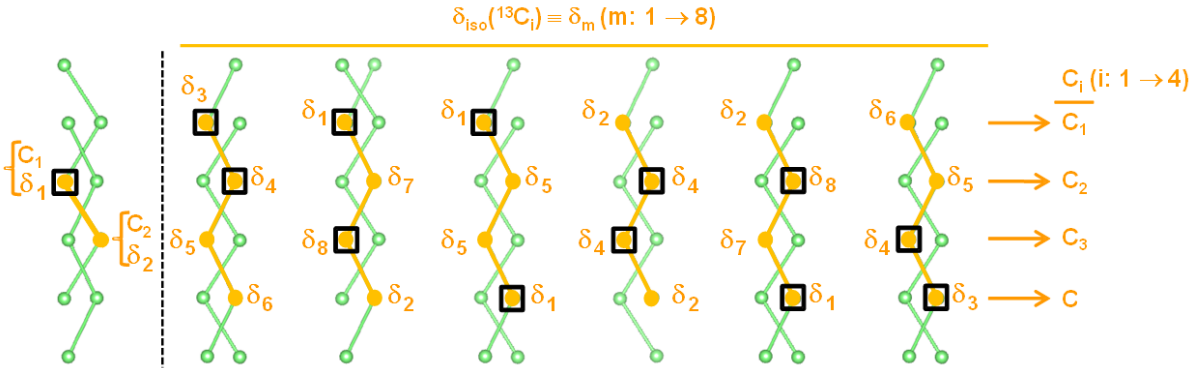
- synthetic HAp, ~ 1 wt % in C, labeled in ^{13}C
- 1D, 2D, double– and triple resonance CP, SQ-DQ experiments

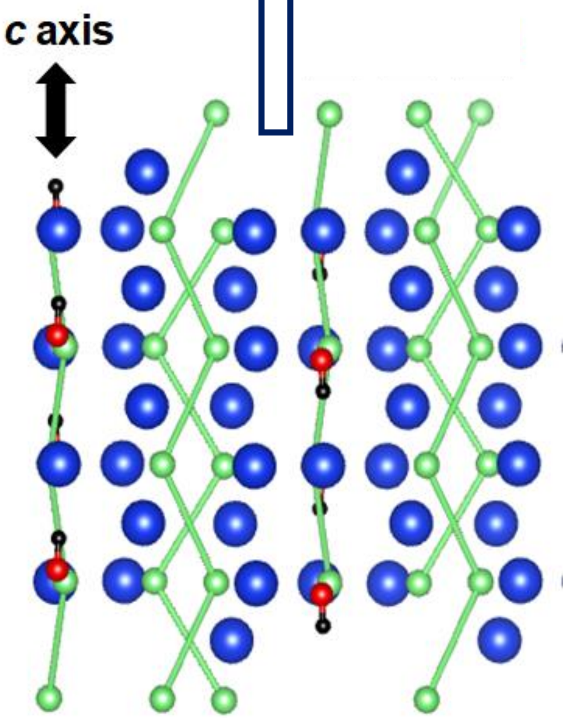


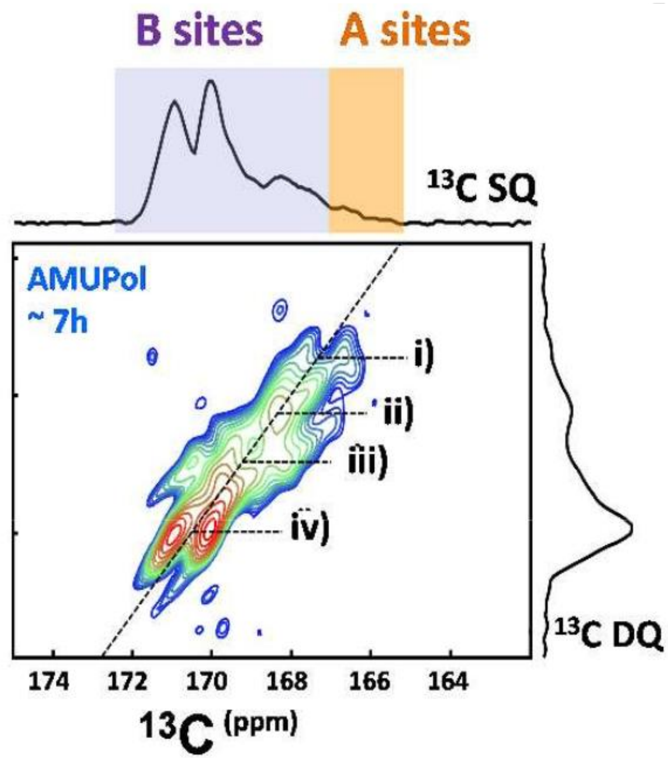


Analytical Chem., 2017

Towards structural models

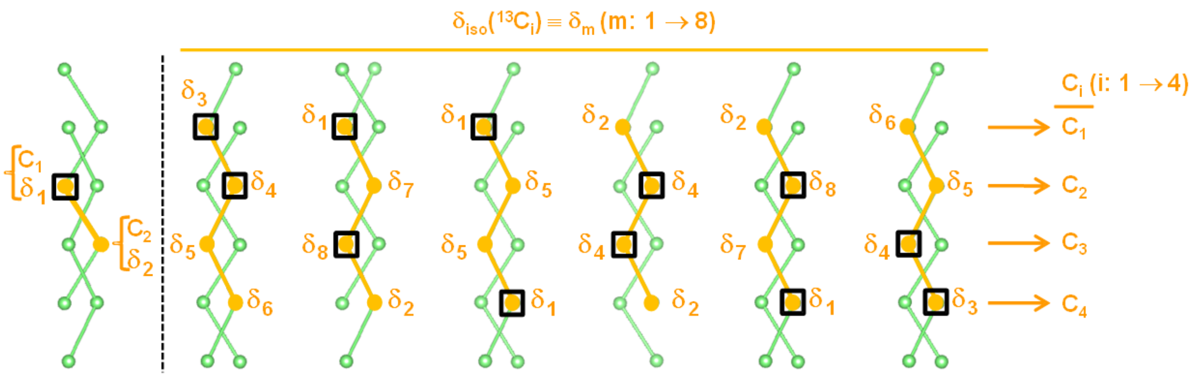


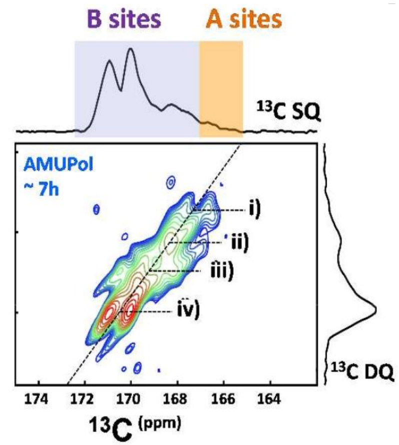


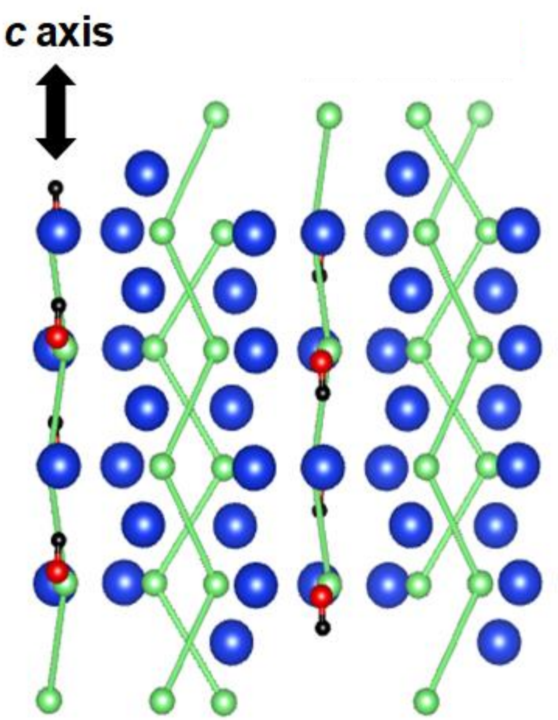


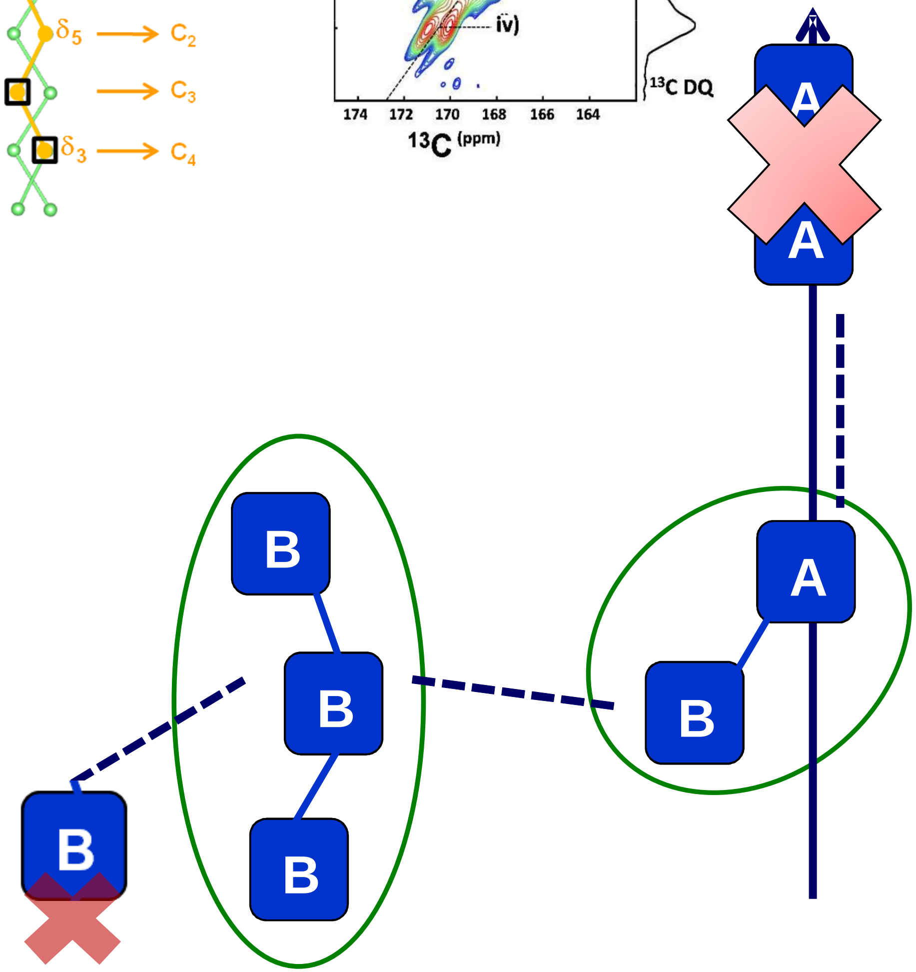
Analytical Chem., 2017

Towards structural models





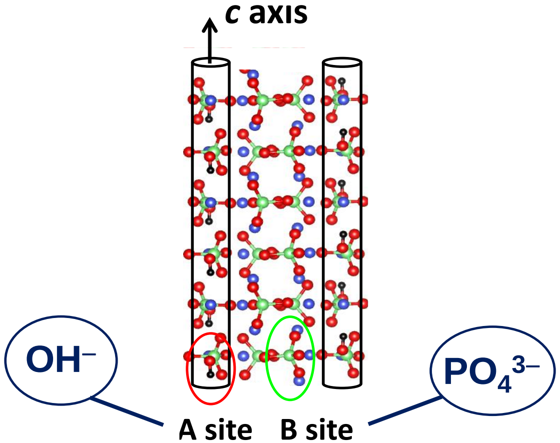




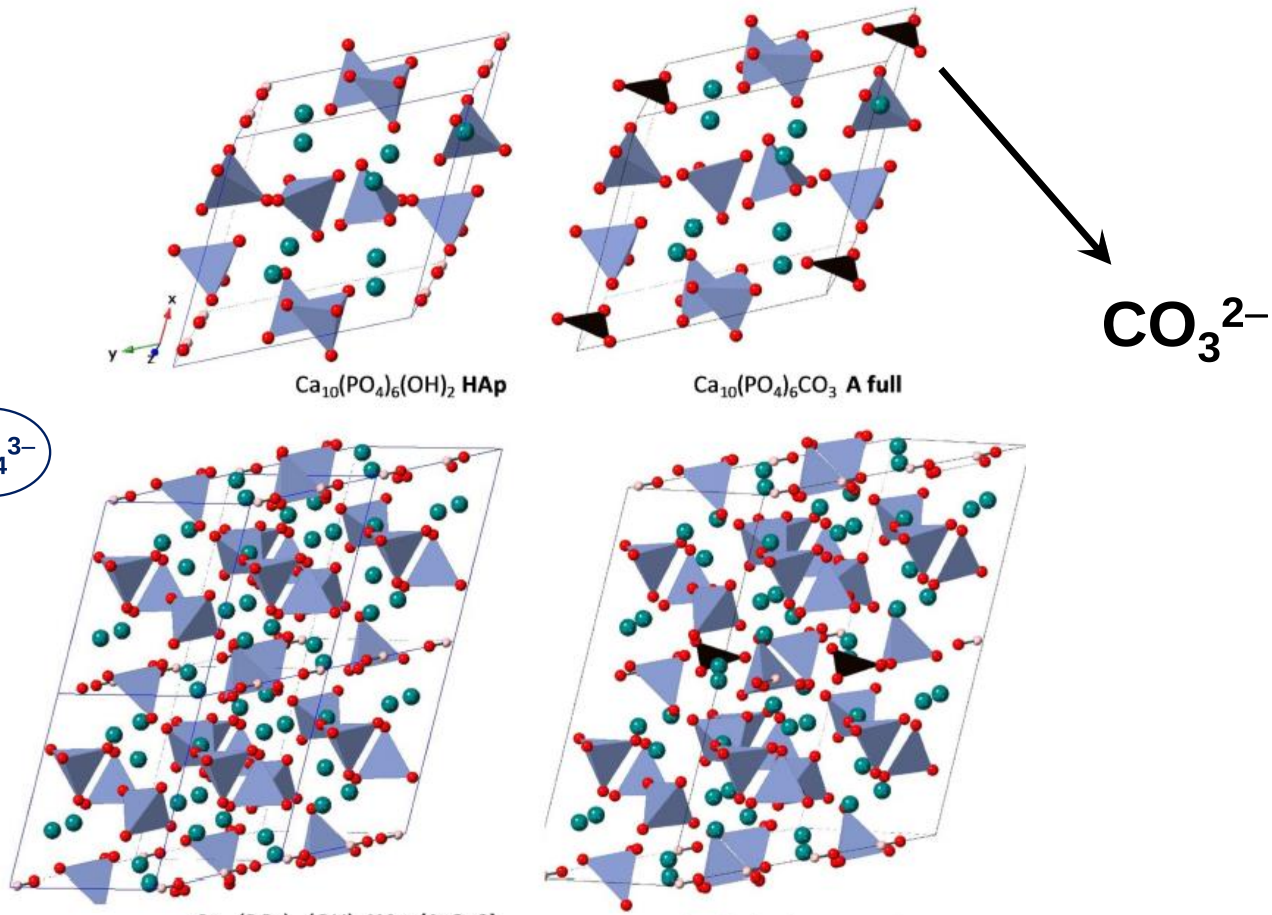
Analytical Chem., 2017

Carbonate substituted hydroxyapatite (HAp)

A, B, A/A, B/B … + charge compensation mechanisms → structural models



$Ca_{10}(PO_4)_6(OH)_2$



$Ca_{10}(PO_4)_6(OH)_2$ **HAp**

$Ca_{10}(PO_4)_6CO_3$ **A full**

CO_3^{2-}

$Ca_{40}(PO_4)_{24}(OH)_8$ **HAp (1×2×2)**

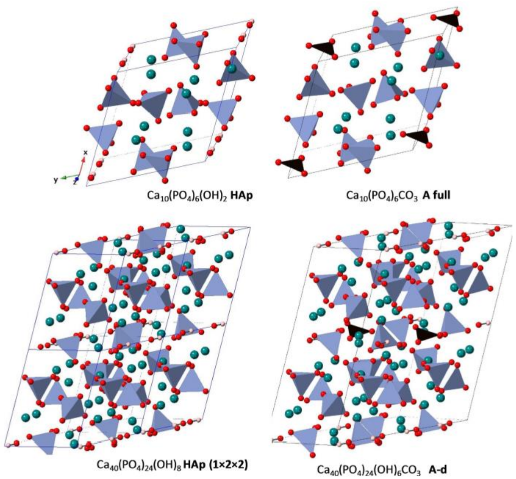
$Ca_{40}(PO_4)_{24}(OH)_6CO_3$ **A-d**

A type (full, diluted)

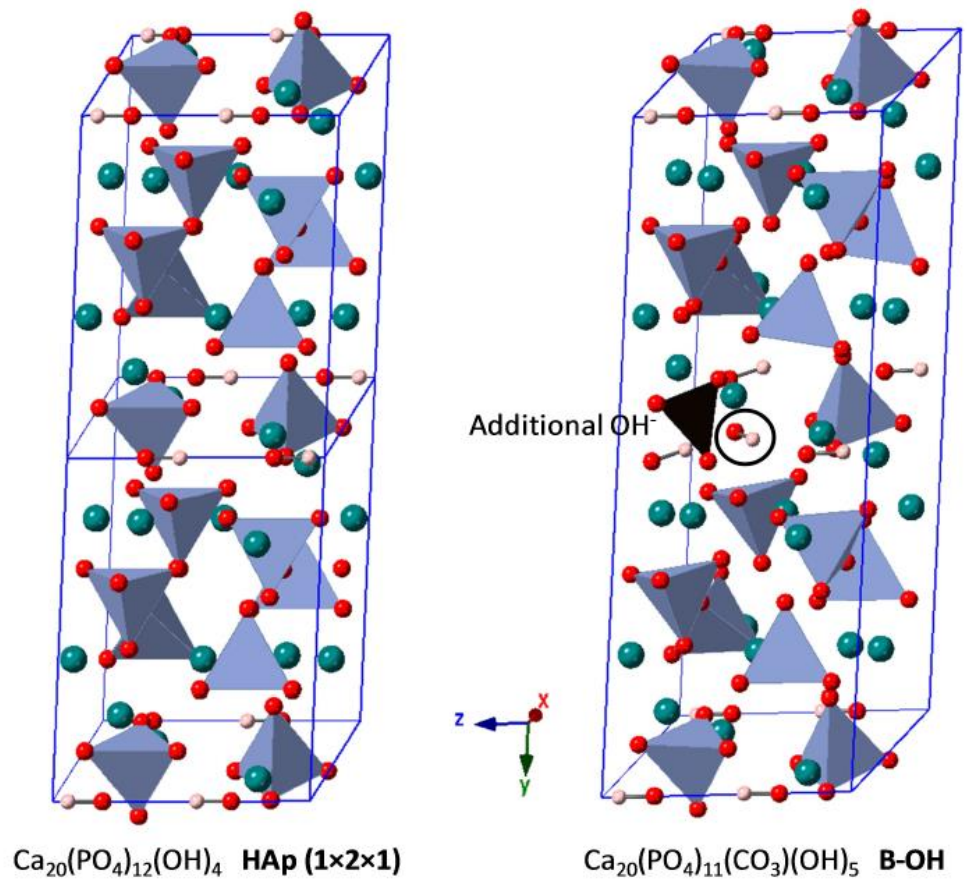
Chemistry–Methods, 2023

Carbonate substituted hydroxyapatite (HAp)

A, B, A/A, B/B … + charge compensation mechanisms → structural models



A type (full, diluted)

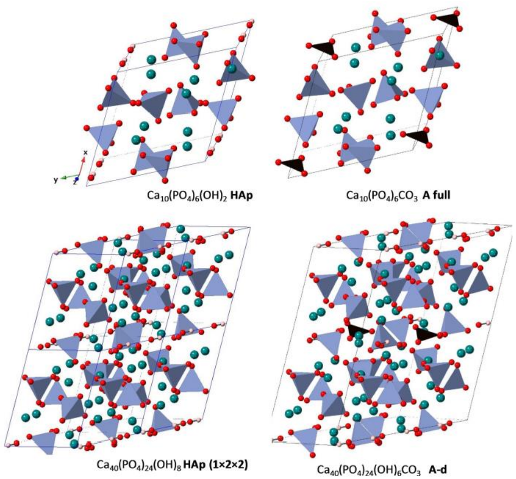


B type (with OH^- or M = Na^+, K^+…)

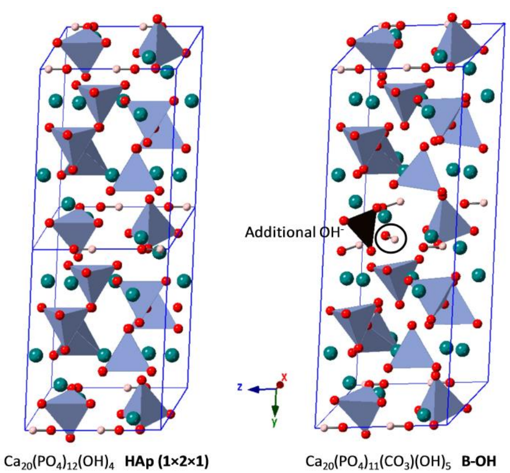
Chemistry–Methods, 2023

Carbonate substituted hydroxyapatite (HAp)

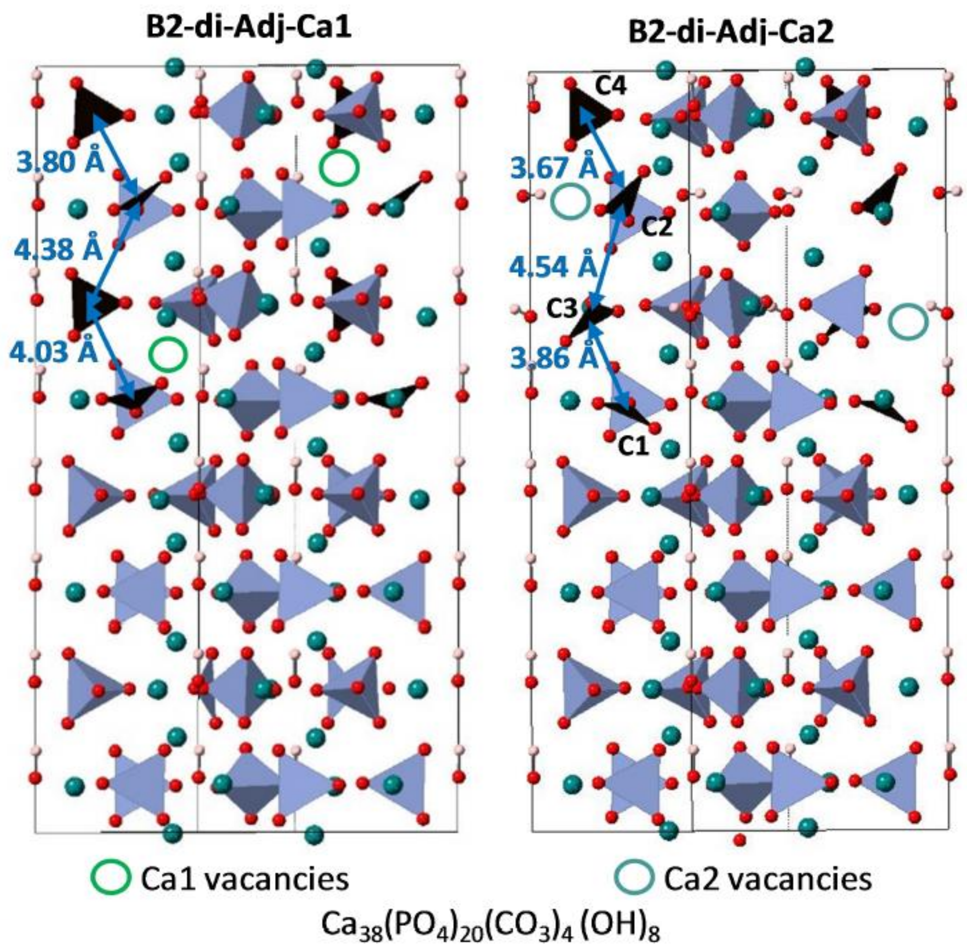
A, B, A/A, B/B … + charge compensation mechanisms → structural models



A type (full, diluted)

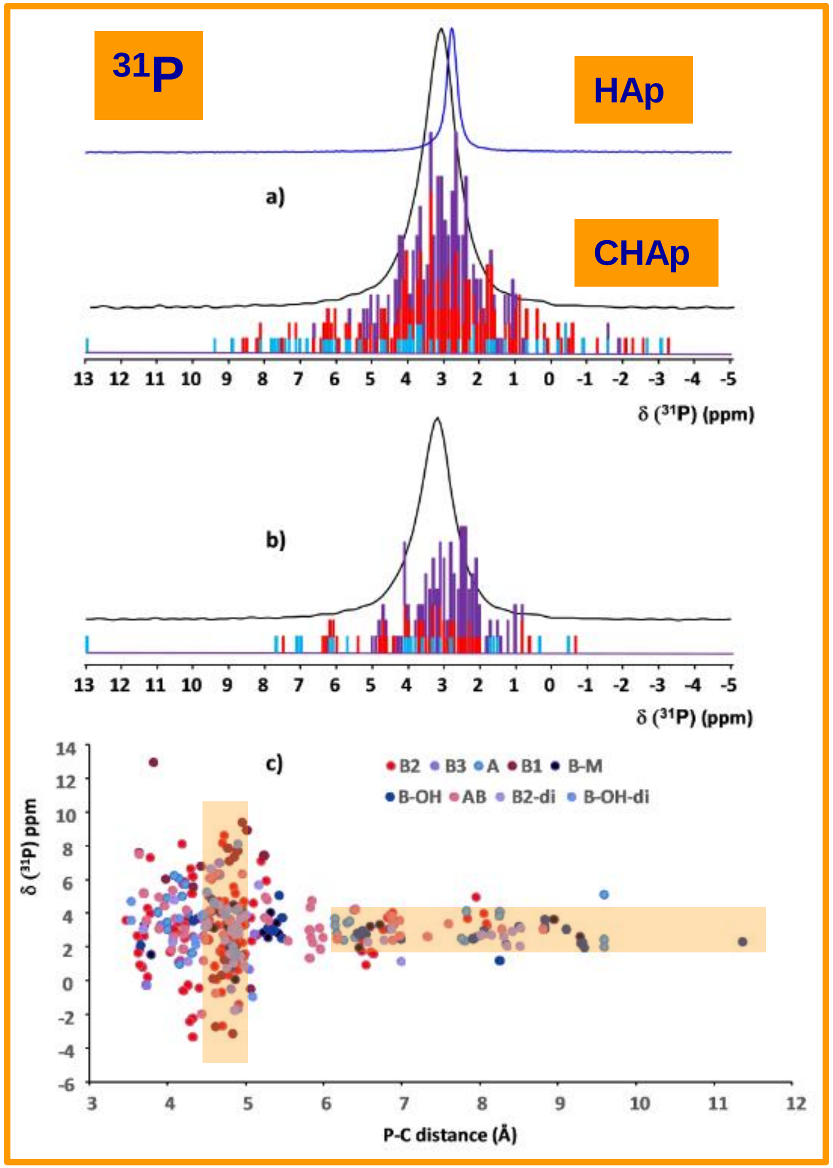


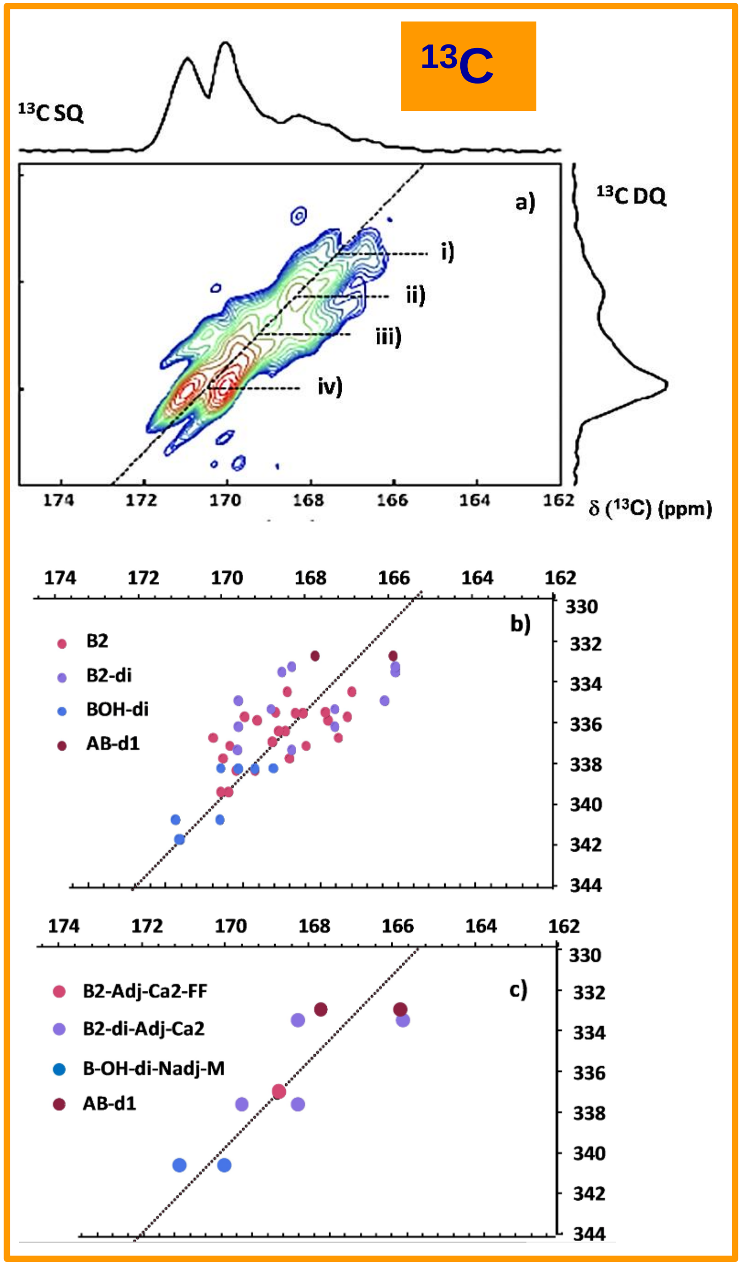
B type (with OH^- or M = Na^+, K^+…)



B /B associations

Towards a global understanding of CHAp related NMR data

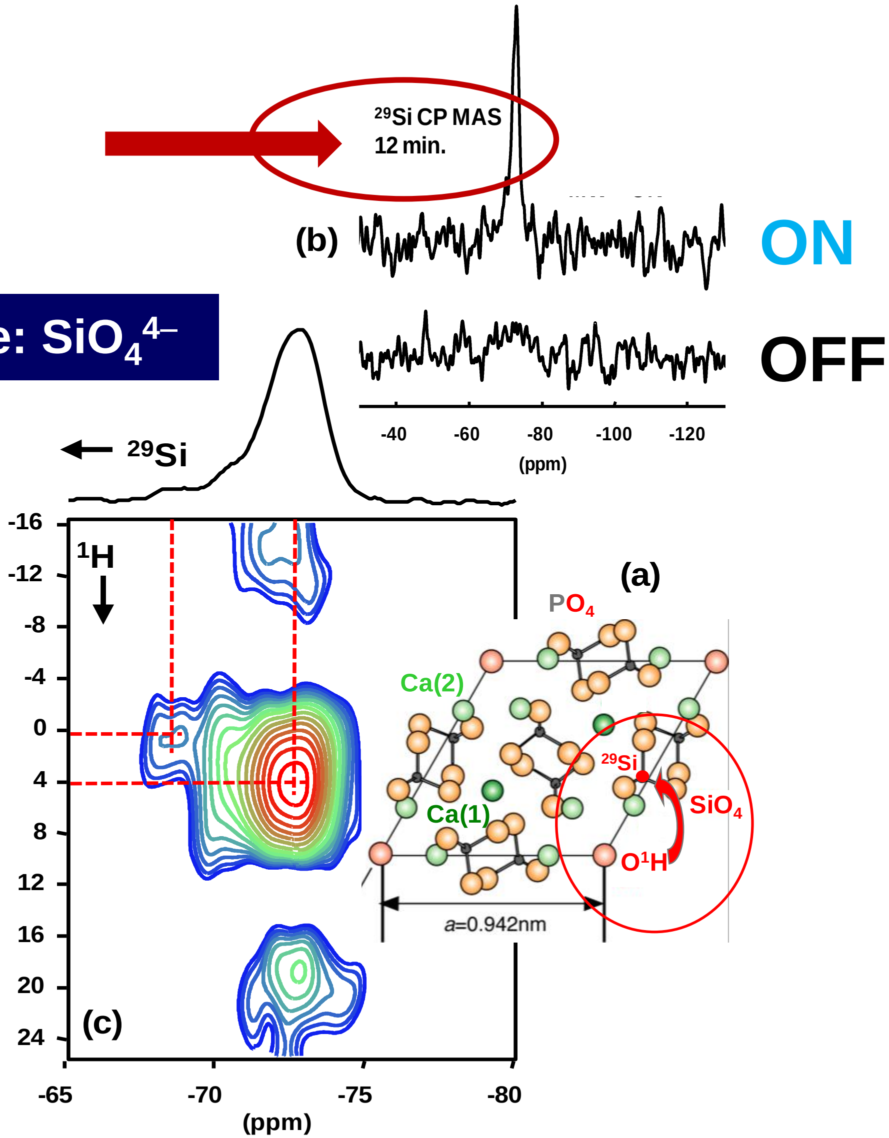




Silicate substituted HAp nanoparticles

Coll.: D. Marchat, Saint-Etienne, France

B type: SiO_4^{4-}

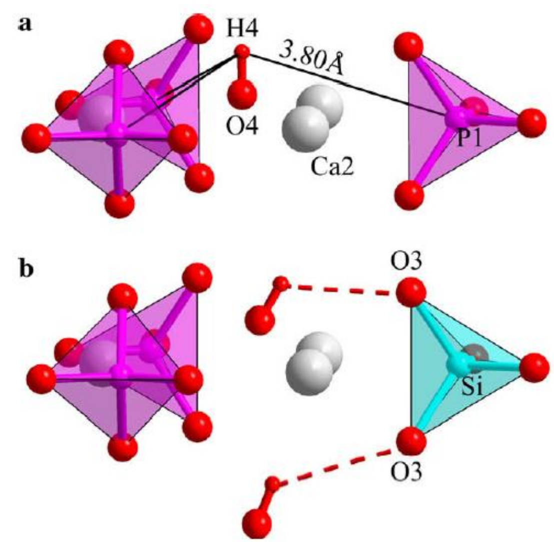


Magn. Reson. Chem., 2008

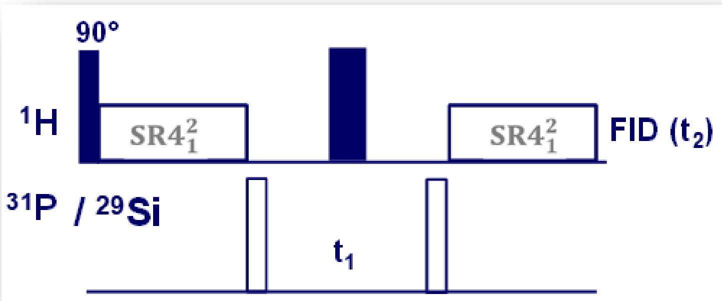
Acta Biomat., 2010

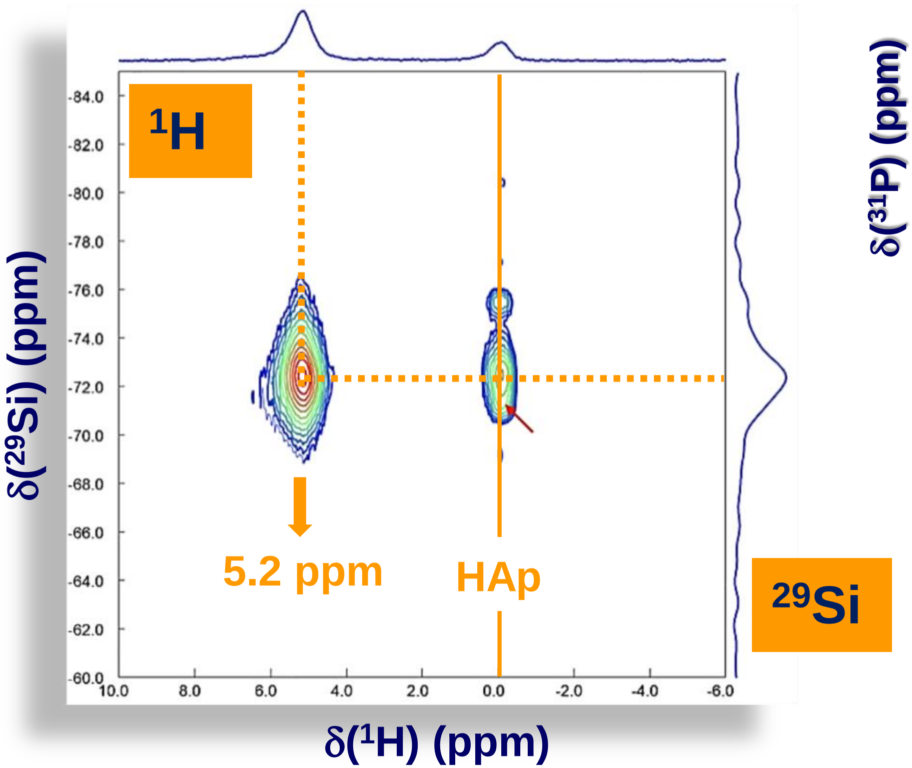
Nuclear Magnetic Resonance as a Tool for the Investigation of Interfaces and Textures in Nanostructured Hybrid Materials, (2017) Wiley

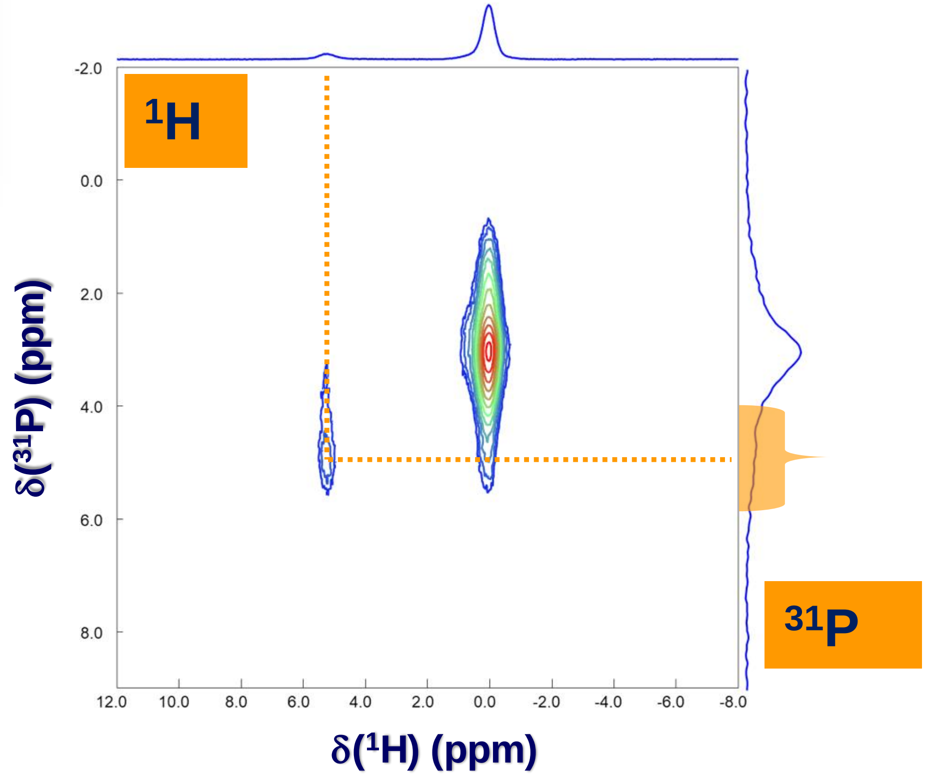
Solid-State NMR Characterization of Sol–Gel Materials: Recent Advances, The Sol-Gel Handbook: Synthesis, Characterization, and Applications, (2015) Wiley



Silicate substituted HAp nanoparticles

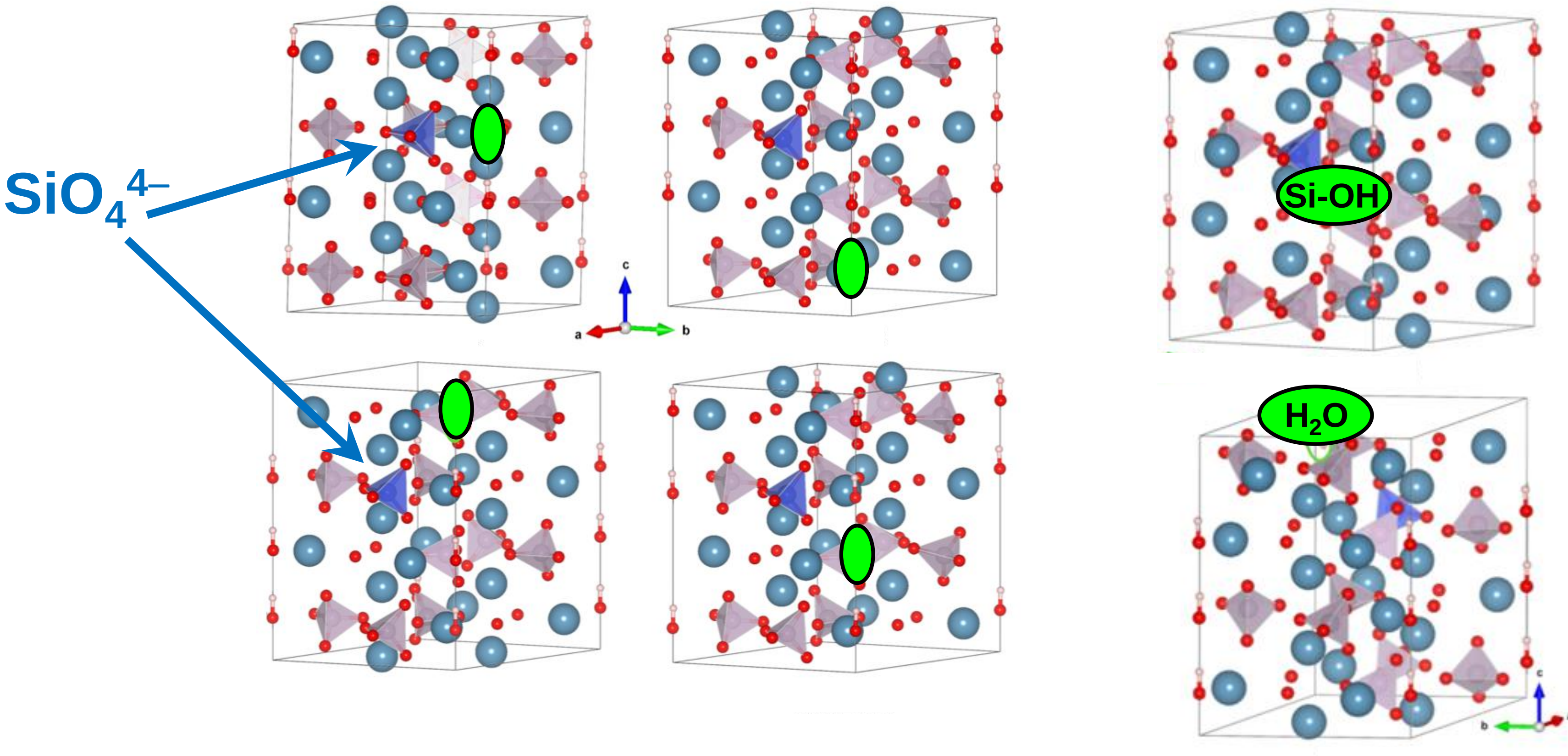






Silicate substituted HAp nanoparticles

B type: SiO_4^{4-}, $SiO_3(OH)^{3-}$, H_2O, HPO_4^{2-}... + charge compensation (V_{OH^-})



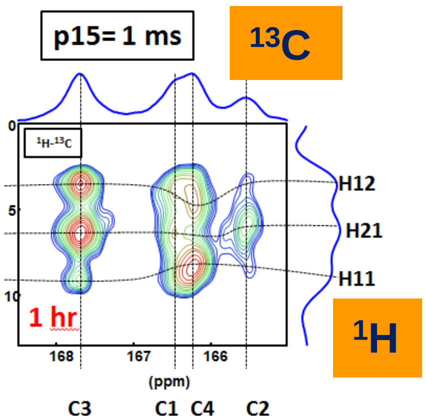
$\delta(^1H)$ = 5.2 ppm ↔ protonated silicate

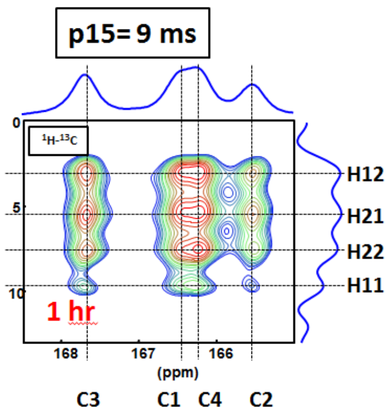
A. Froment, PhD, October 2023

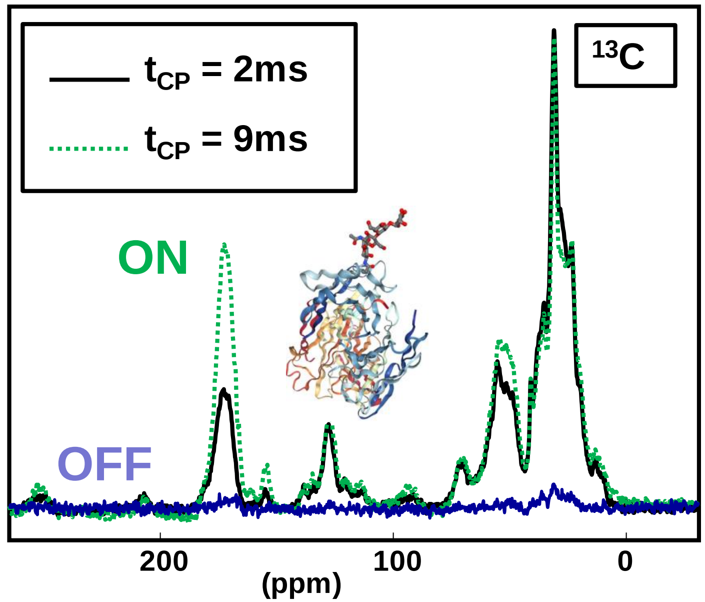
Back to KS: DNP crystallography

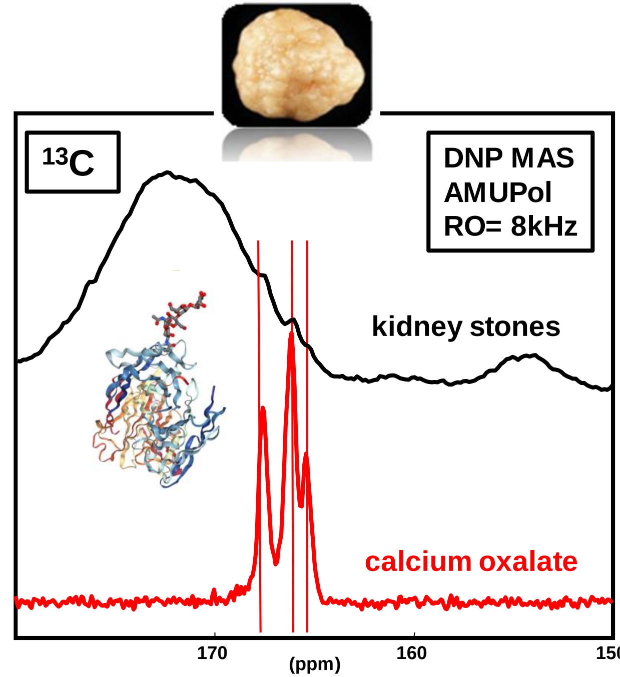
TEKpol in d-TCE/TCE (9:1)

^{1}H-^{13}C FSLG HETCOR

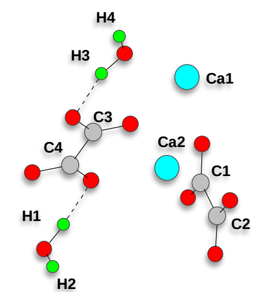








- **heterogeneity**
- **choice of the solvent / radical**

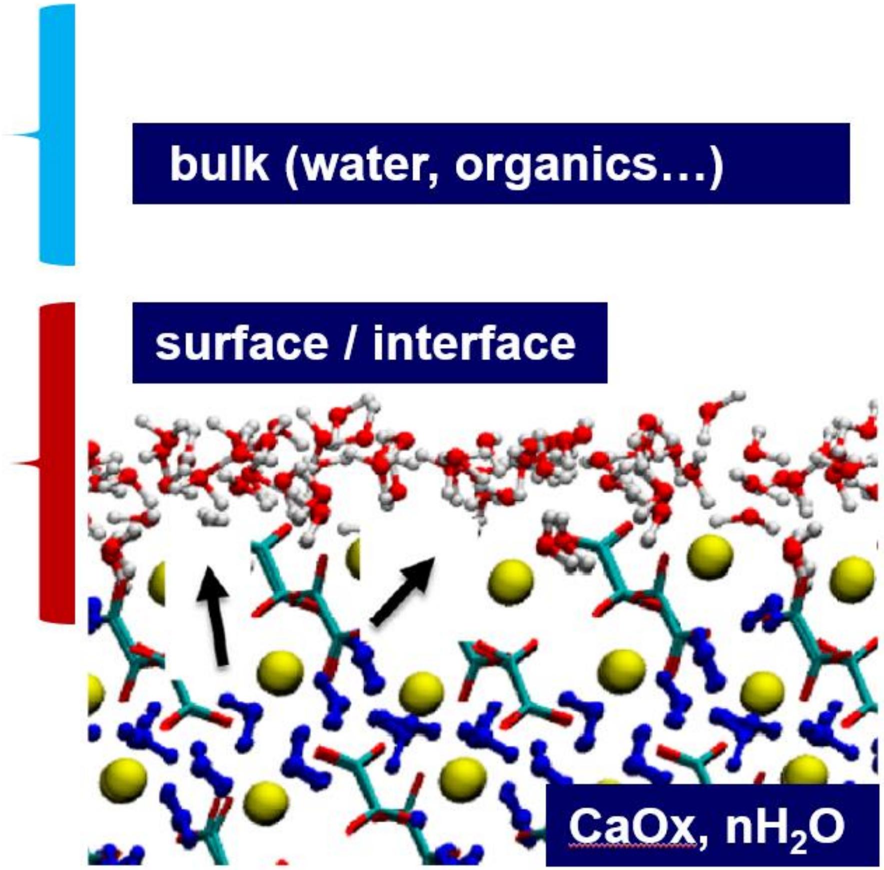


→ assignments for synthetic COM, COD, COT

→ towards interfaces

→ GIPAW (slabs…)

First principles calculations of realistic CaOx structures



CP2K/quickstep DFT

Gaussian plane wave hybrids

PBE / D3 Grimme / OptPBE-vdW

BO-MD

GROMACS, Gromos force field 54a7

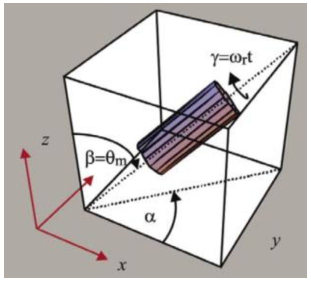
⬇

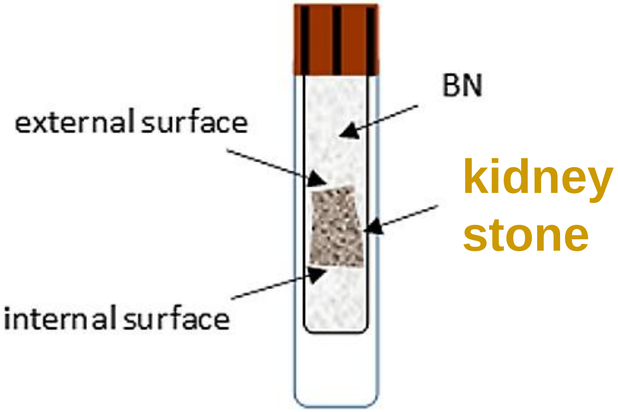
role of water, layers of solvation at DFT level…

Coll.: F. Tielens, Brussels (Belgium)

Crystal Growth & Design, 2020

- NMR as a unique platform of characterization
    - *structure*
    - *dynamics*
- More sensitivity
- DNP crystallography
- **Magic Angle Spinning MRI**





First MAS MR Imaging of kidney stones

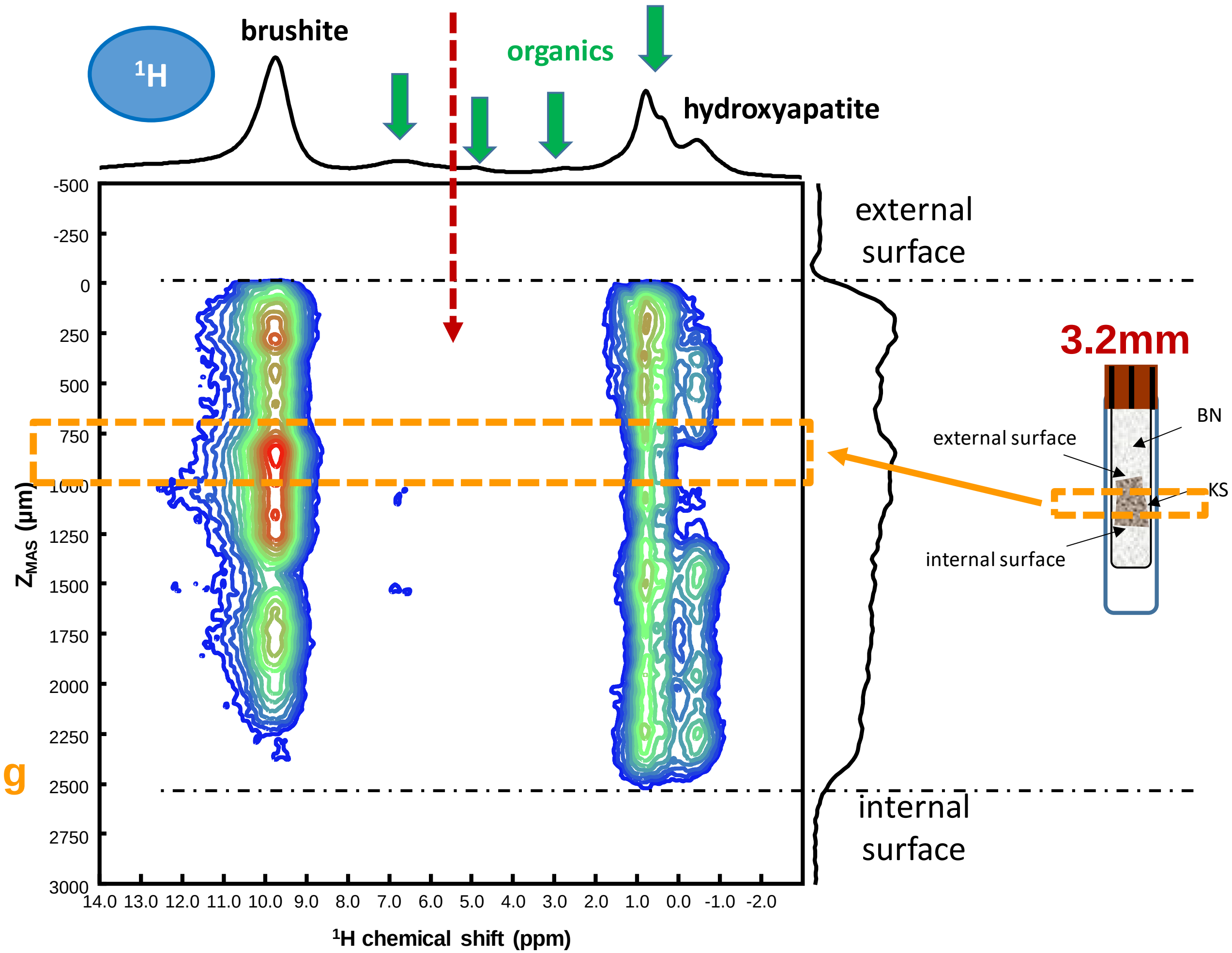
"… Using standard MRI sequences, stones appear as a *non-specific void*"
(Brisbane *et al*., Nat. Rev. Urol., 2016)

brushite +++

organics +

HAp no…

Chemical Shift Imaging

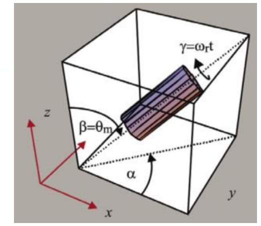


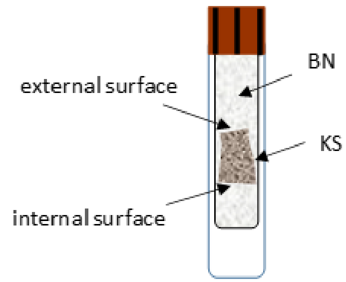
Coll.: V. Sarou-Kanian, F. Fayon, Orléans , France

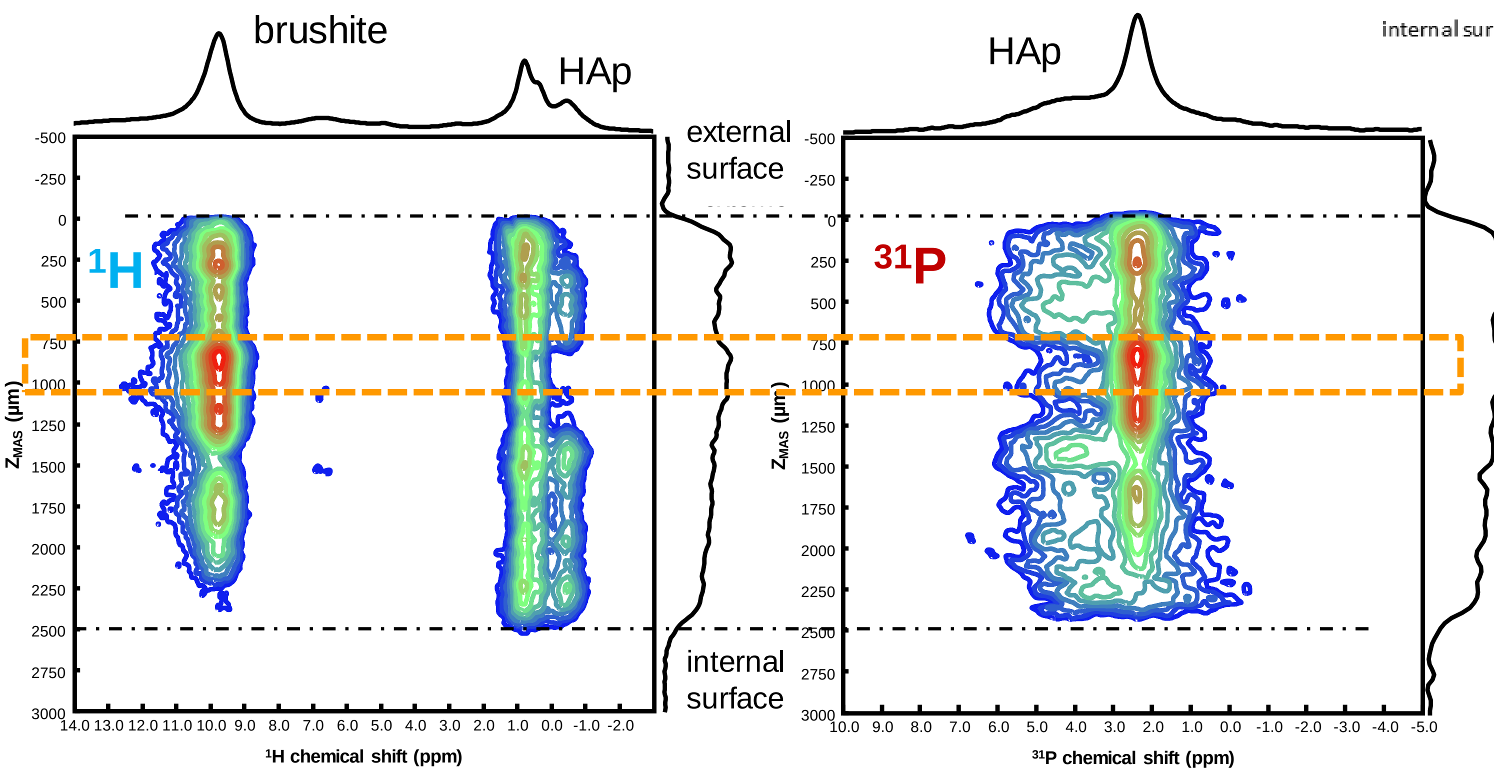
First MAS images of kidney stones

WB 750 MHz AVANCE III HD, 17.6 T. Bruker *Micro 2.5*. 2.5 G.cm^{-1}A^{-1} (60 A per axis). 3.2mm Bruker probe (up to 24 kHz). FOV ~ 3.5mm. Res. ~ 31 μm, 61μm.

see: Pampel, 2006, Sarou-Kanian, 2015, Maudsley *et al*., 1982
***spectral* dim.: direct**
***spatial* dim.: indirect**







tribute Pampel

Conclusions and acknowledgments

- ^{1}H and ^{13}C nuclei as pertinent targets for diagnosis at hospitals
- *in situ* monitored phase transformations
- DNP + crystallography
- MAS MRI

W.-C. Teh, A. Froment, I. Goldberga, C. Leroy, T. Debroise, Y. Petit

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Fondation pour la Recherche Médicale

anr