Observation par RMN des hétérogénéités structurales dans les verres bioactifs $CaO-SiO_2-P_2O_5$

Franck Fayon, Cédric Duée, Ophélie Vernay, Thomas Poumeyrol, Mathieu Allix, Dominique Massiot

CEMHTI – CNRS

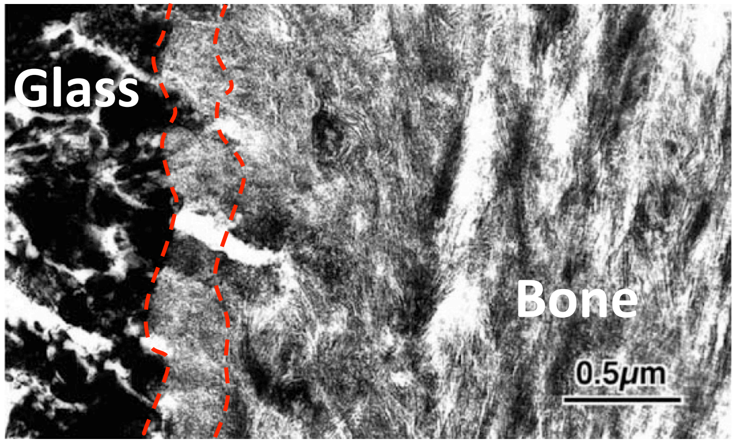
Conditions Extrêmes et Matériaux : Haute Température et Irradiation
Extreme Conditions and Materials : High Temperature and Irradiation

ORLEANS, France

Verres bioactifs

- **Calcium silicate**-based glasses doped with **phosphorus oxide**

- **Bioactive** : *Strong bonding interface*
  connecting the glass to the living tissues
  (Enable stable integration of implants)



M. Neo *et al., J. Biomed.* Mat. Res. 26, 1419 (1992).

Powder
(100-800 µm)

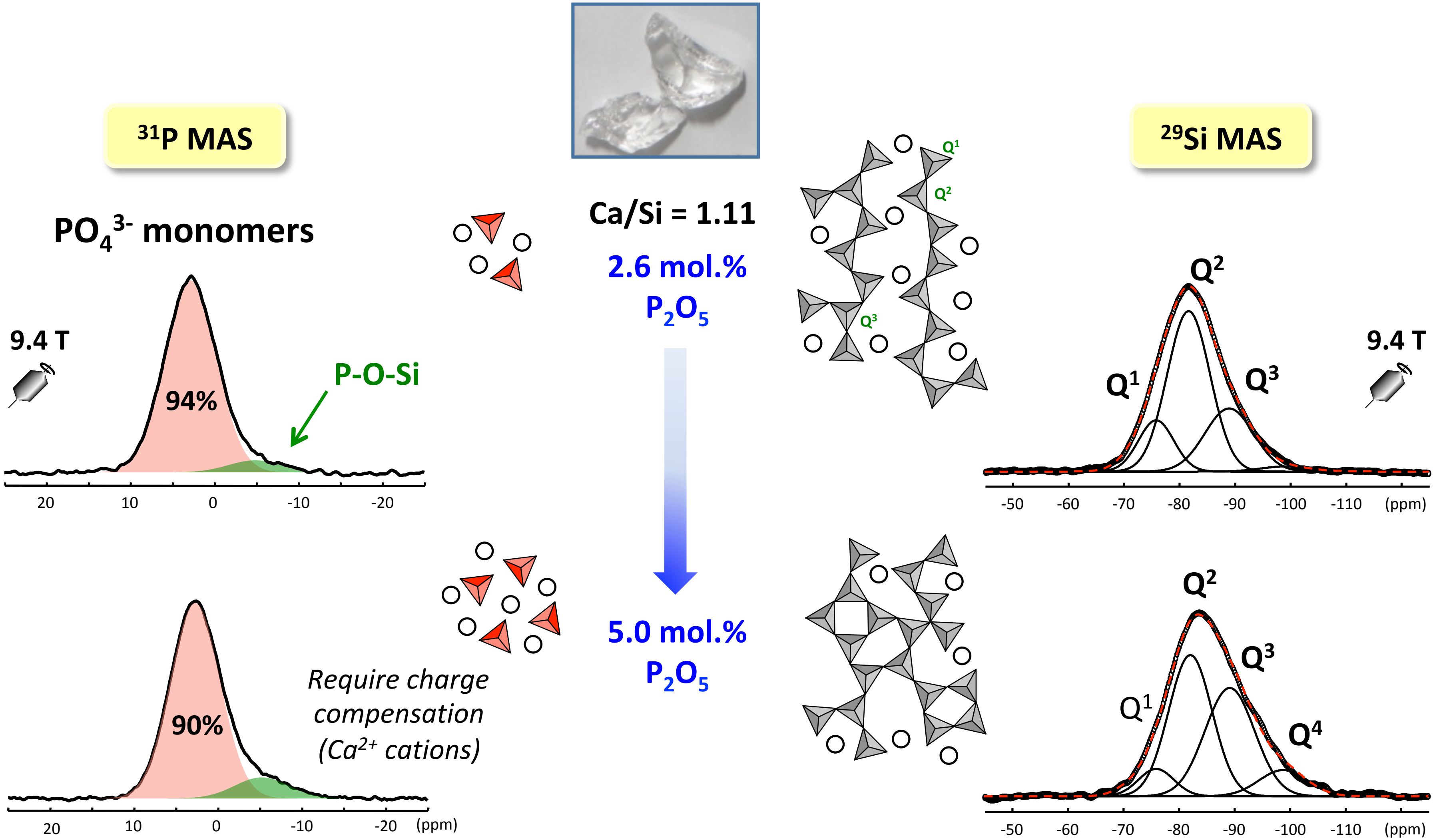
Small pieces

- Dental bone grafting material *(Perioglass®, Biogran®)*
- Orthopedic and cranofacial reconstruction (*Novabone®, Bonalive®*)
- Reconstruction of tiny bones of the middle ear

- **Bioactivity depends on glass composition and structure !**

Structure locale des verres bioactifs $CaO-SiO_2-P_2O_5$

1D MAS NMR : Nature of phosphate groups, silicate network



Repolymerization of the disordered silicate network with P addition

Liaisons P-O-Si ??

Débattue dans la littérature

J. Phys. Chem. C 2008, 112, 5522-5562

Multinuclear Solid-State NMR Studies of Ordered Mesoporous Bioactive Glasses

E. Leonova,…, M. Vallet-Regi, M. Eden

"Although the signal at ca. -7 ppm may safely be assigned to ^{31}P in *Q^1 units, it is less clear if those derive from P-O-P* or P-O-Si bonding scenarios….. **Our NMR results suggest** that the ^{31}P resonance at ca. -7 ppm derives from **P-O-Si moieties."**

Chem. Mater. 2010, 22, 5644-5652

New Insights into the Atomic Structure of 45S5 Bioglass by Means of Solid-State NMR spectroscopy and Accurate First-Principles Simulations

A. Pedone, T. Charpentier et al.

"**No Si-O-P bridges have been detected** by both ^{31}P NMR and ^{17}O MQMAS experiments, and therefore isolated orthophosphate units are able to form nanodomains that subtract sodium and calcium cations from their network modifying role into the silicate network.**"**

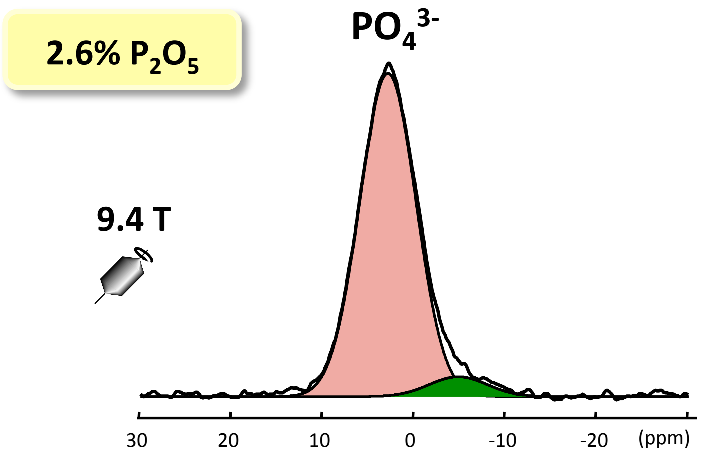
Chem. Mater. 2007, 19, 5644-5652

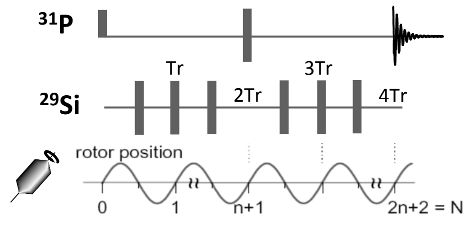
The Structure of Bioactive Silicate Glasses: New Insight from Molecular Dynamics Simulations

A. Tilocca, A. N. Cormack, et al.

"…..characterized by a marked increase in the connectivity of the silicate network and by an **increasing fraction of phosphate groups involved in P-O-Si**."

Liaisons P-O-Si !!

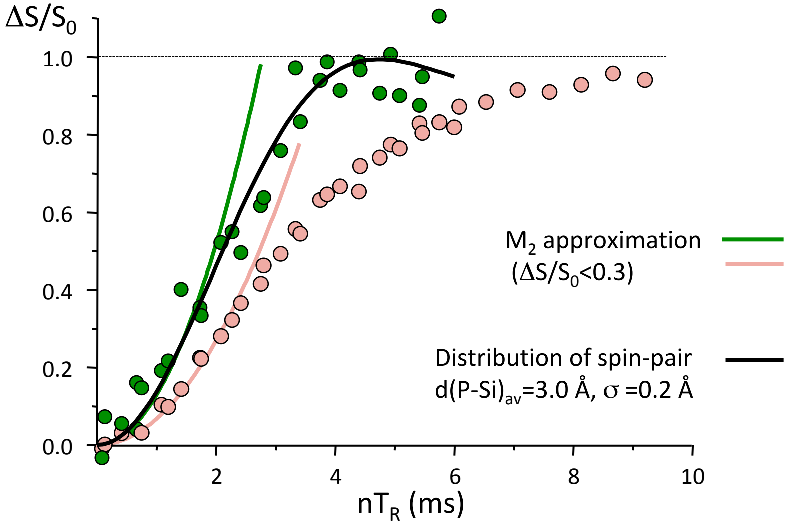




T. Gullion and J. Schaefer, JMR 1989

- Reintroduction of the heteronuclear P-Si dipolar couplings
- Dephasing of the ^{31}P echo signal

- **^{29}Si-enriched samples**



PO_4^{3-} (95-90% of the P atoms)

M_2 = 44 to 50 .10^4 rad.s^{-2}

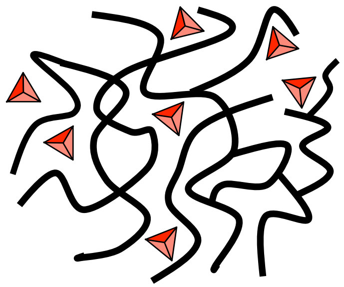
M_2 = 96 .10^4 rad.s^{-2} **d(P-Si) ≈ 3.0 Å**

→ P-O-Si bond (5-10 % of the P atoms)

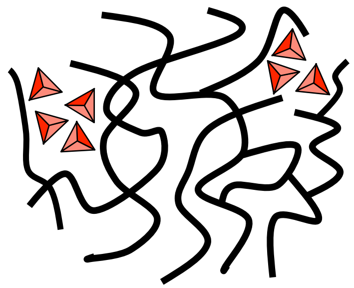
F. Fayon, C. Duée, T. Poumeyrol, M. Allix, D. Massiot, *J. Phys. Chem. C* **117**, 2283-2288 (2013)

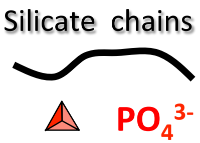
Distribution des monomères PO_4^{3-} dans le réseau Silicate

Chemical homogeneity ?



Phosphate clusters ?





B. O. Mysen, F. J. Ryerson, D. Vigo

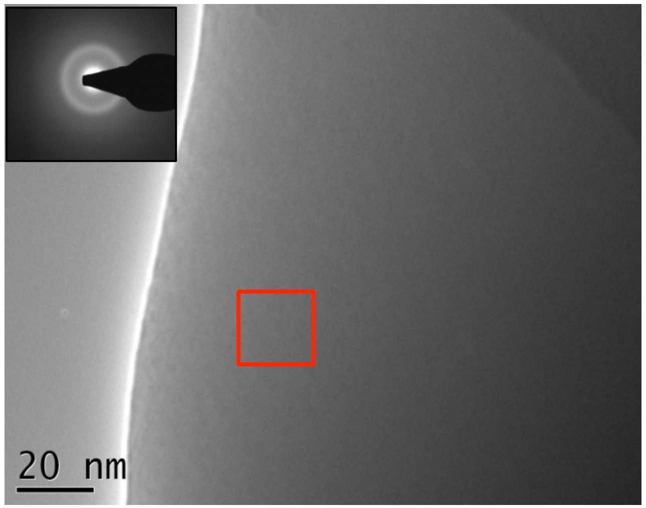
The structural role of phosphorus in silicate melts
American Mineralogist, Volume 66, p 106-117 (1981)

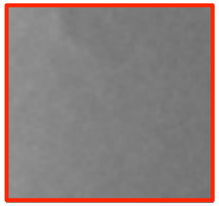
"Phosphate complexes are formed in the melts. These complexes are bonded to metal cations such as Ca^{2+},Mg^{2+}, and Na^{+}. The M-PO_4 complexes occur as ***discrete units that are larger than 20 Å in the melt***."

- **Amorphous material**
- **Very weak Z-contrast** between **Si and P**

No information with conventional techniques (SAXS, TEM, XRD)

^{31}P solid-state spin-counting multiple-quantum NMR ?

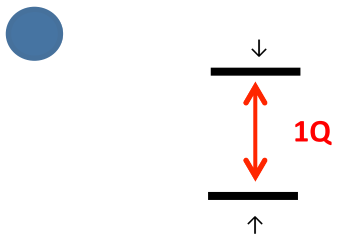




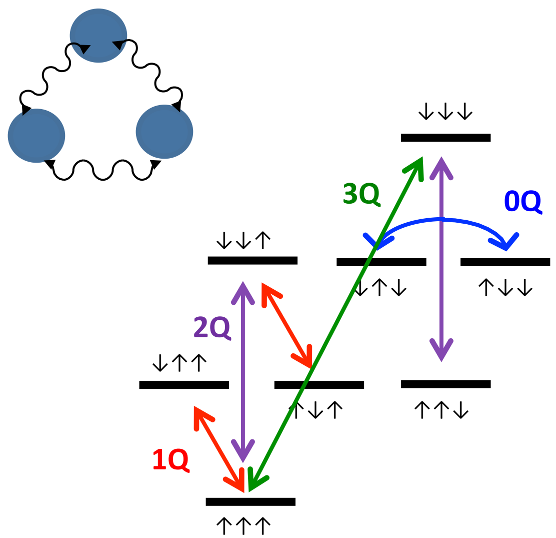
TEM (5.0 mol.% P_2O_5)

Principe de la spectroscopie RMN multi-quantum

Un spin isolé

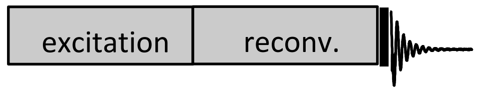


Trois spins couplés

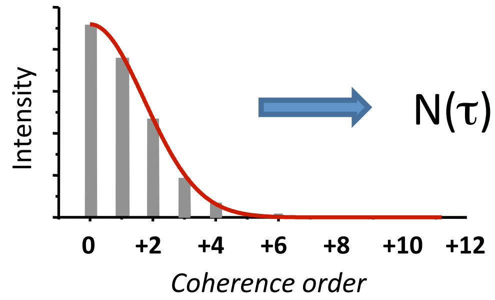


Couplages dipolaires = proximité spatiale

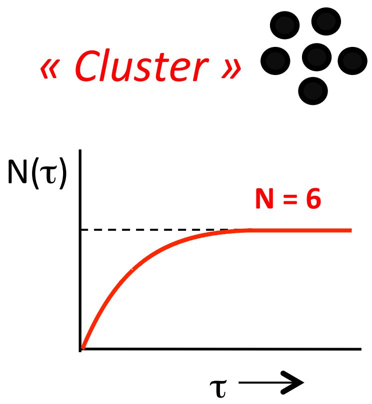
Détection indirecte des cohérences multi-quantum

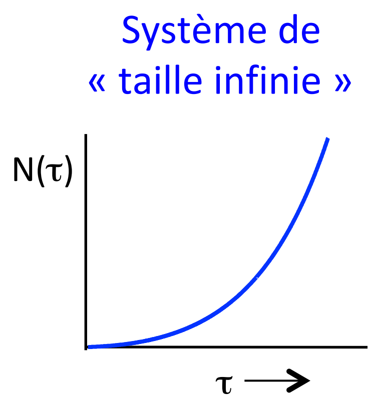


Distribution de l'intensité des cohérences multi-quantum



Deux cas limites





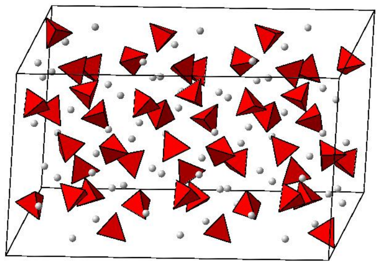
Mesure de la taille du système !!

Spectroscopie multiple-quantum ^{31}P dans un phosphate cristallin

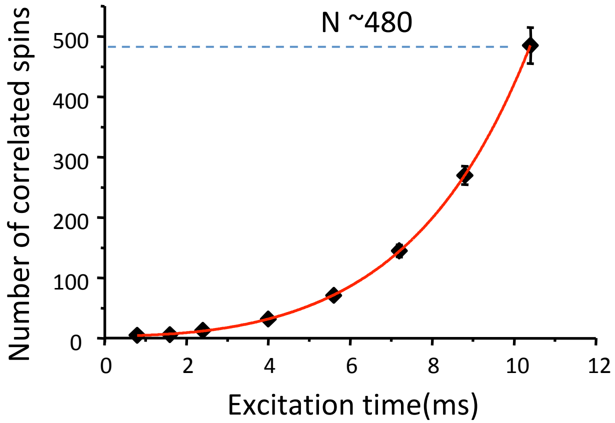
Reference compound:
Structure made of PO_4 monomers

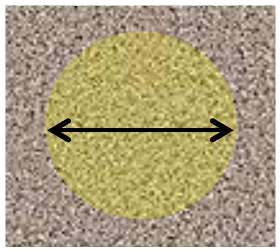
Weak homonuclear P-P dipolar couplings (D< 270 Hz)
But the excitation of Multiple Quantum Coherence is possible !!!

TriCalcium Phosphate (cristalline)



480 P atoms
(10 unit cells - 43 nm³)

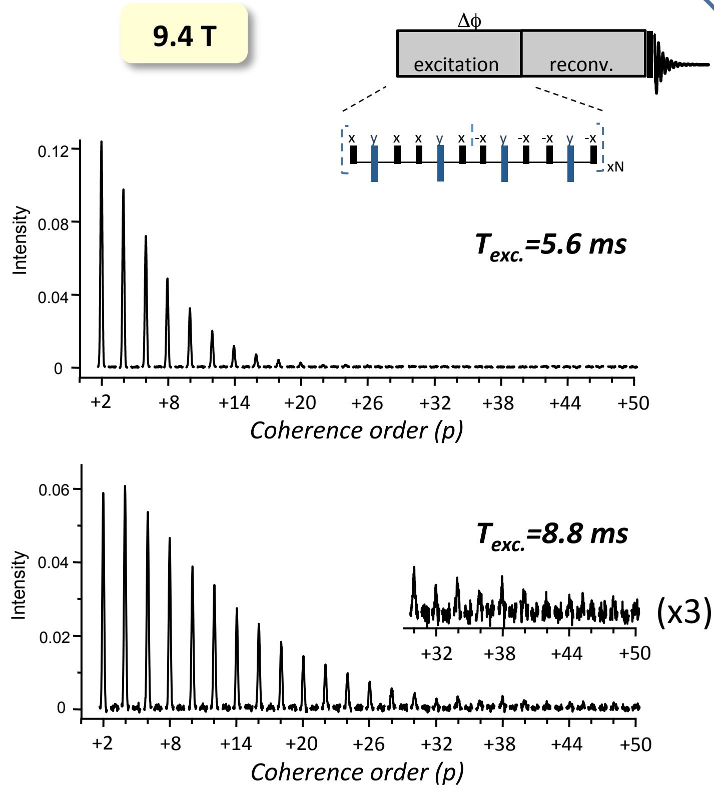




Ø ≈ 4.3 nm

Number of correlated PO_4^{3-} units (i.e. size of the system) increases continuously

9.4 T



"Infinite" (unbounded) spin system
MQC orders up to 40 !!!

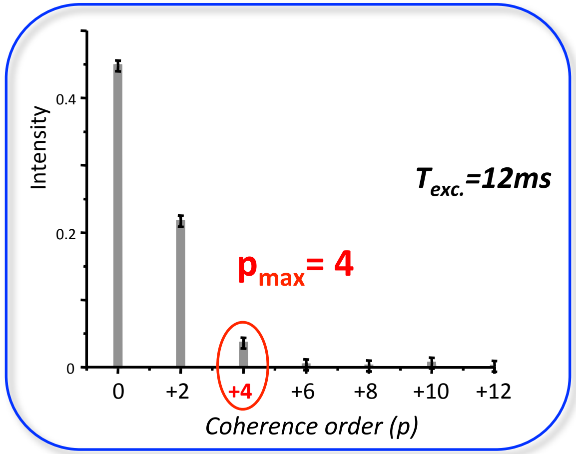
Spectroscopie multiple-quantum ^{31}P dans les bioverres

Ca/Si = 1.11

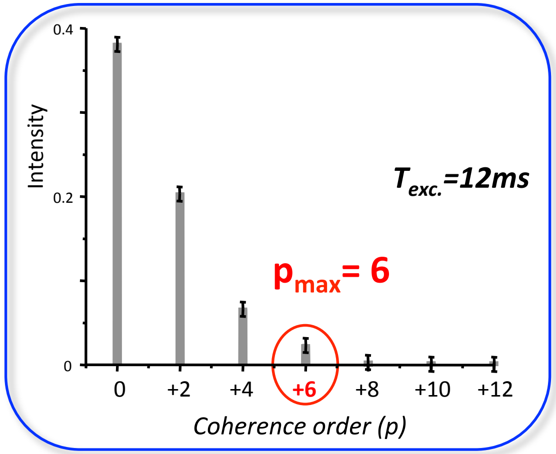
P_2O_5 content

Increasing Si network polymerization

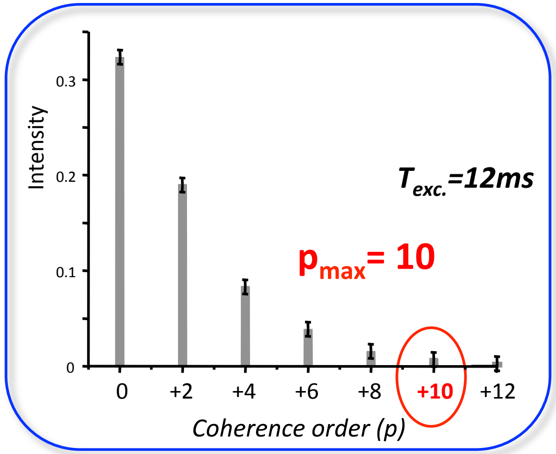
2.6 mol.% P_2O_5



3.7 mol.% P_2O_5



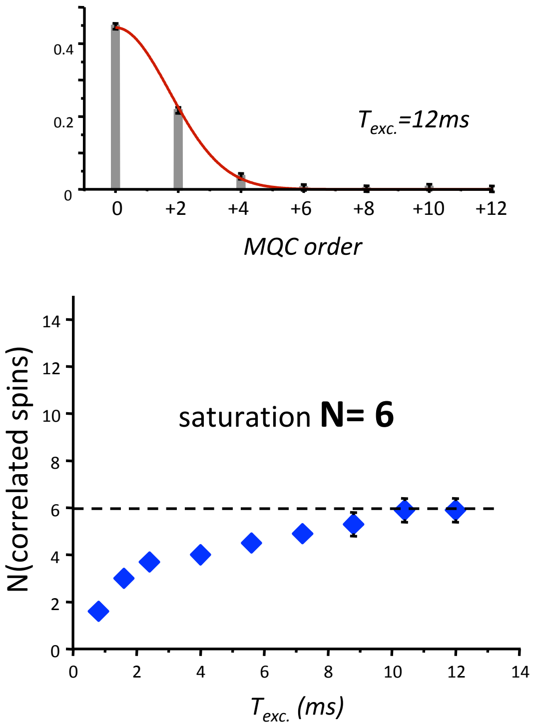
5.0 mol.% P_2O_5



Highest coherence order value (p_{max}) increases with the phosphorus content

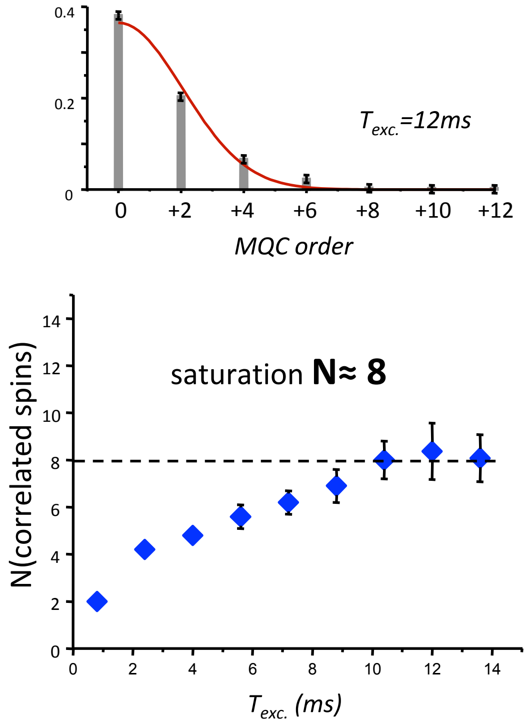
Taille des domaines phosphates dans les bioverres

2.6 mol.% P_2O_5

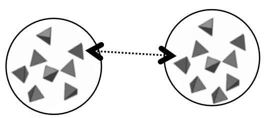


"Isolated" clusters
Average size N=6

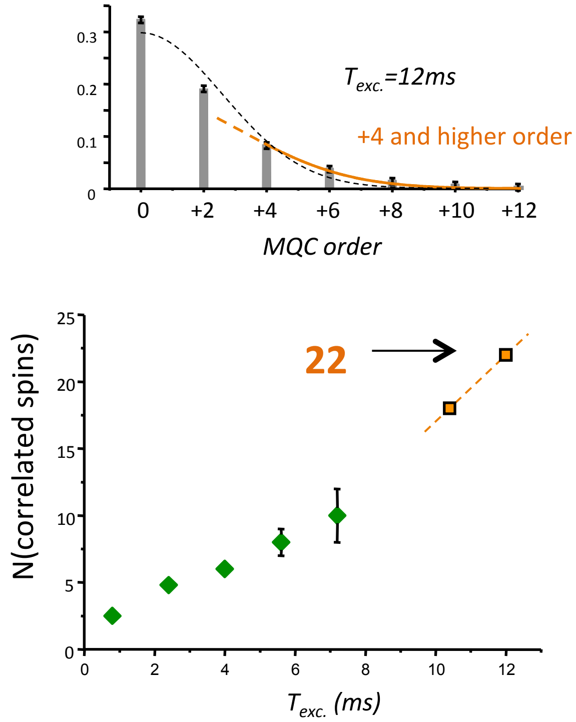
3.7 mol.% P_2O_5



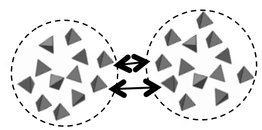
"Weak interactions" between cluster
Average size N≈8



5.0 mol.% P_2O_5



Clusters are close together
forming larger aggregates



Polymerization of the Si network

F. Fayon, C. Duée, T. Poumeyrol, M. Allix, D. Massiot, *J. Phys. Chem. C* **117**, 2283-2288 (2013)

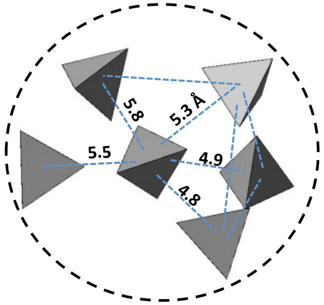
Simulations numériques / Expériences

Numerical simulations:

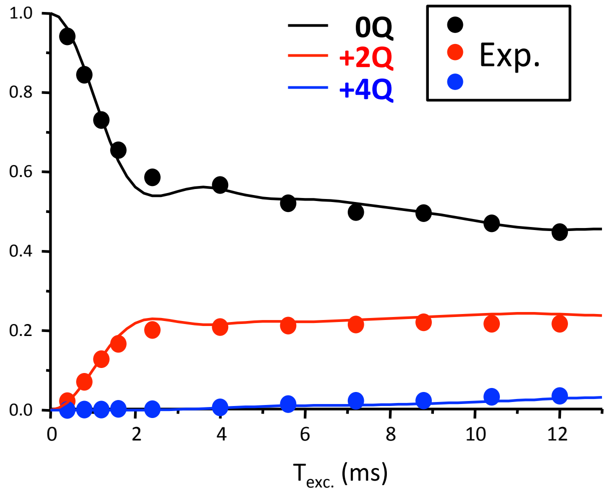
SIMPSON

Bak, Rasmussen and Nielsen, *JMR* 2000.
Vosegaard et al., *Mon. Chem* 2002.

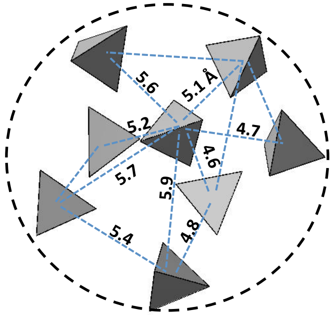
6 P cluster



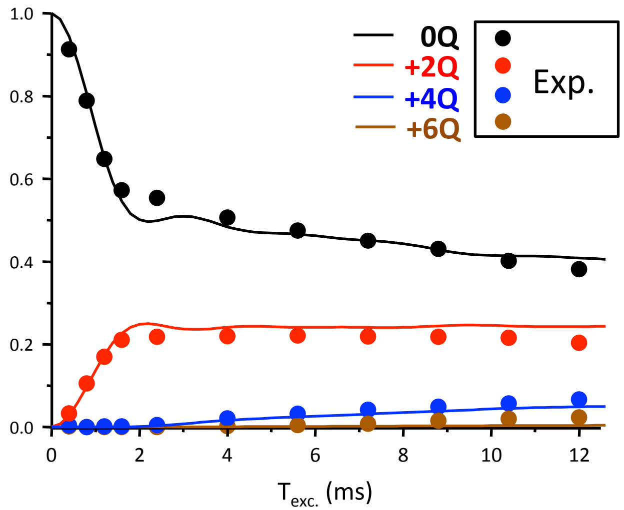
2.6 mol.%
P_2O_5 glass



8 P cluster



3.7 mol.%
P_2O_5 glass



Size of the clusters
∅ ≈ 1 nm

Conclusion

- Structure of bioactive glass shows a **non uniform** (homogeneous) **distribution of phosphate groups** and contains **chemical heterogeneities**

- **Low P_2O_5 content** (≈ < 3.7 mol.%) : Forte Bioactivité

  Small phosphate clusters, average size of **1 nm**

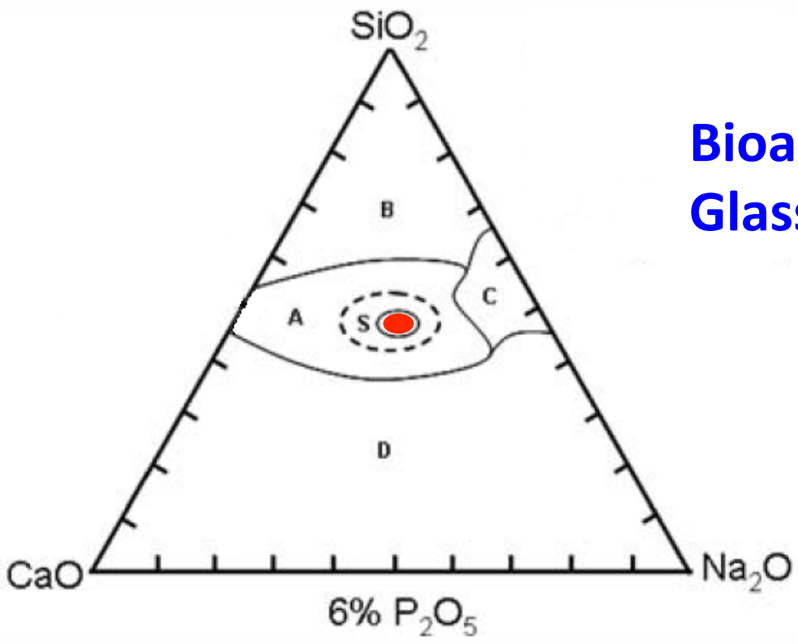
- **Higher P_2O_5 content** : Faible Bioactivité

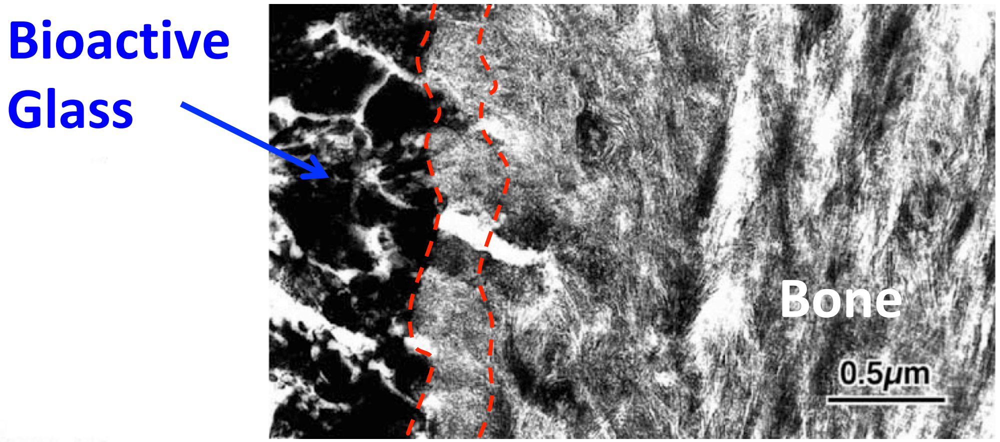
  Significant repolymerization of the silicate network
  Clusters close together forming **larger aggregate**

Bioactivité des verres

Bioglass® 45S5

46,1mol% SiO_2 – 26,9 mol% CaO – 24,4mol% Na_2O – 2,6mol% P_2O_5





M. Neo *et al., J. Biomed.* Mat. Res. 26, 1419 (1992).

Interface :
Liaison forte et
stable avec l'os

❖Caractérisation de la phase formée en surface du verre

Hench *et al.*,Journal of the American Ceramic Society, 1991, *74* (7), p.1487
Neo *et al.*, Journal of Biomedical Materials Research, 1992, *26* (11), p.1419

Mécanismes proposés

Hydrolyse surface verre → Si-OH (silanols)

Condensation des Si-OH → **Gel de silice**

Migration Ca^{2+}, PO_4^{3-} vers surface du Gel de silice

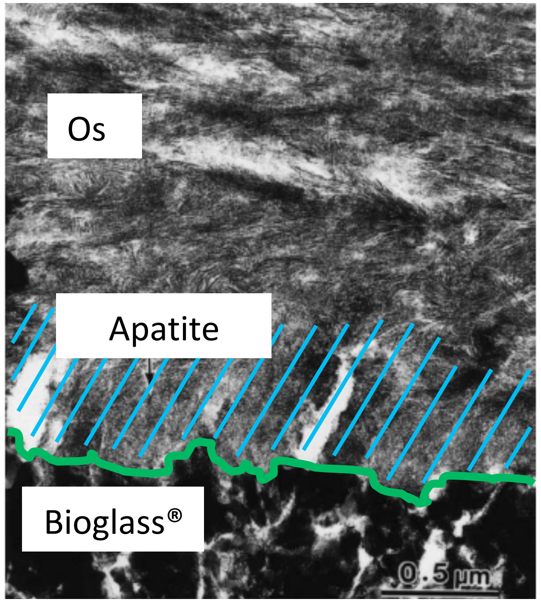
→ Formation phase **CaP amorphe**

-Migration HO^-, CO_3^{2-} vers CaP amorphe

-**Cristallisation progressive**

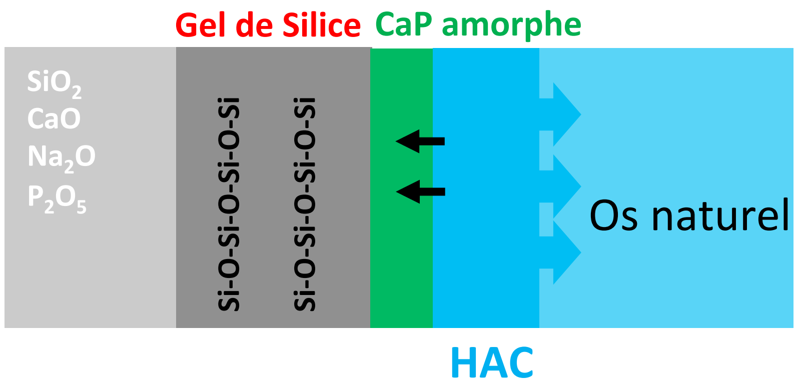
→ HydroxyApatite Carbonatée **HAC**

HAC → liaison avec l'os naturel



Interface : Liaison forte et stable avec l'os

BIOACTIVITÉ



Caractérisation des phases formées in-vitro

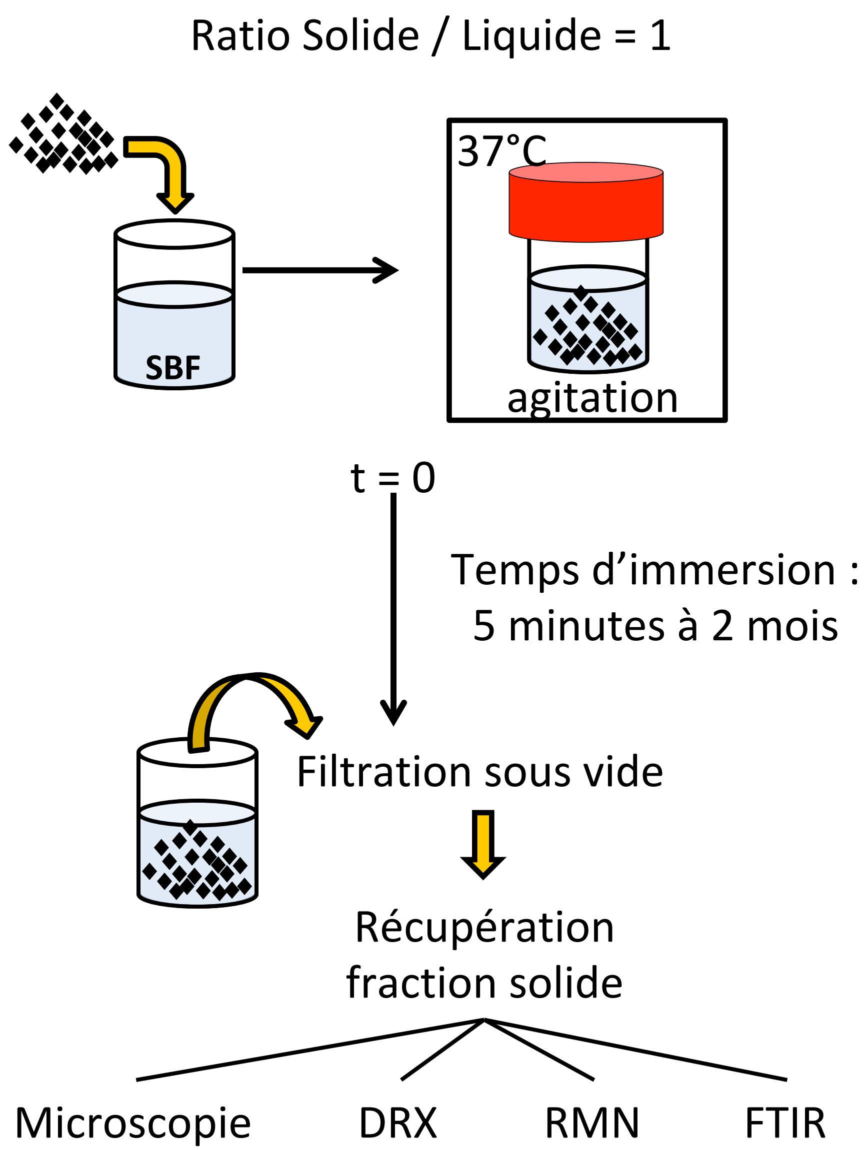
❖ Préparation SBF *(Simulated Body Fluid)*

Concentration (mMol/L)	Plasma Humain	SBF
Na^+	142,0	142,0
K^+	5,0	5,0
Mg^{2+}	1,5	1,5
Ca^{2+}	2,5	2,5
Cl^-	103,0	147,8
HCO^{3-}	4,2*	27,0
HPO_4^{2-}	1,0	1,0
SO_4^{2-}	0,5	0,5
pH	7,24-7,40	7,25-7,42

❖ Verre 45S5

Granulométrie 40-75µm

Atmosphère contrôlée

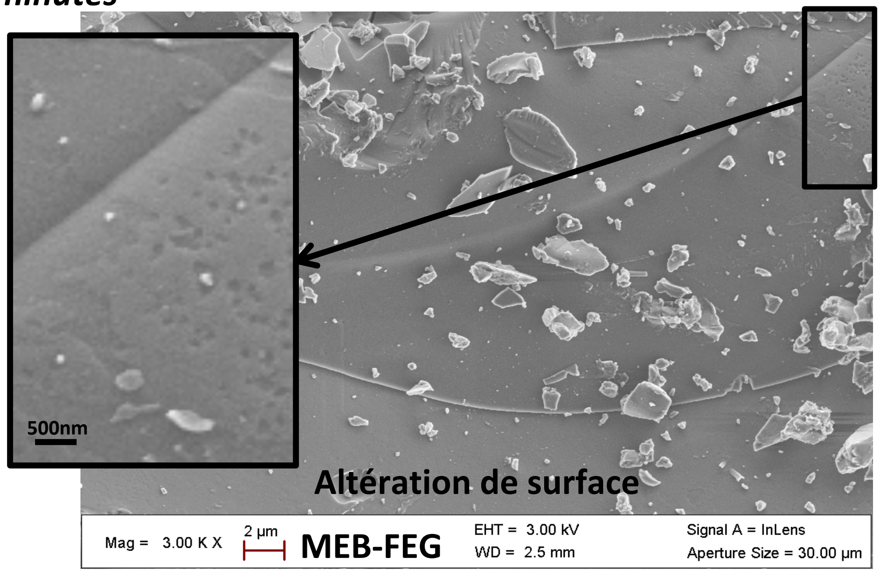


Kokubo *et al.*, Biomaterials, 2006, *27*, p.2907

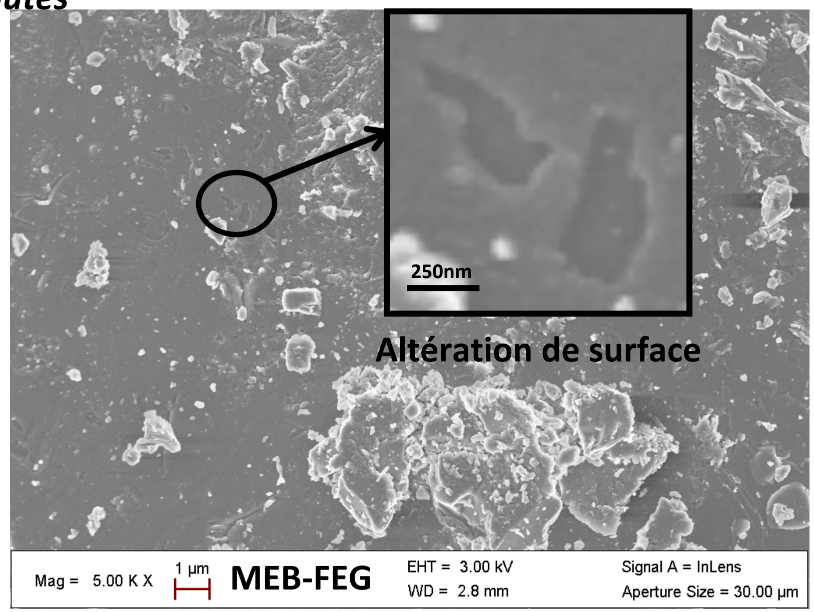
Formation d'une couche de gel de silice ?

Microscopie

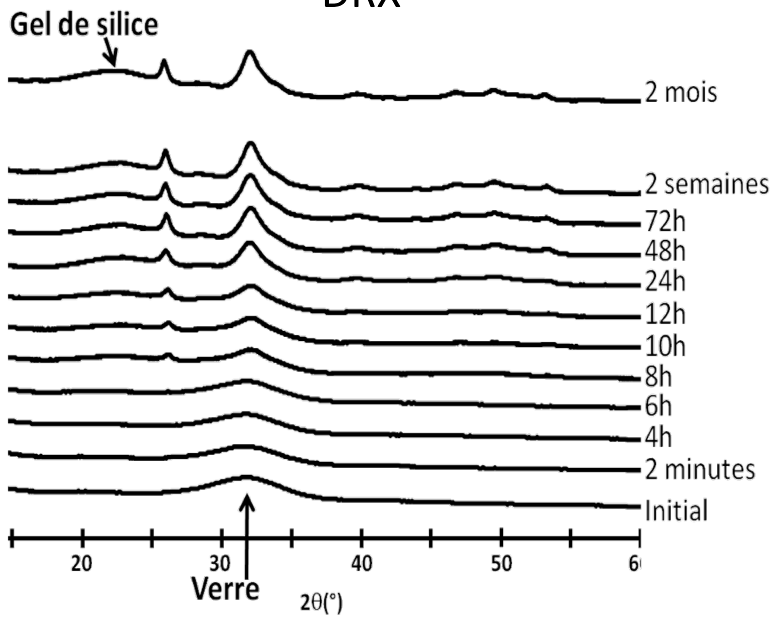
5 minutes



20 minutes

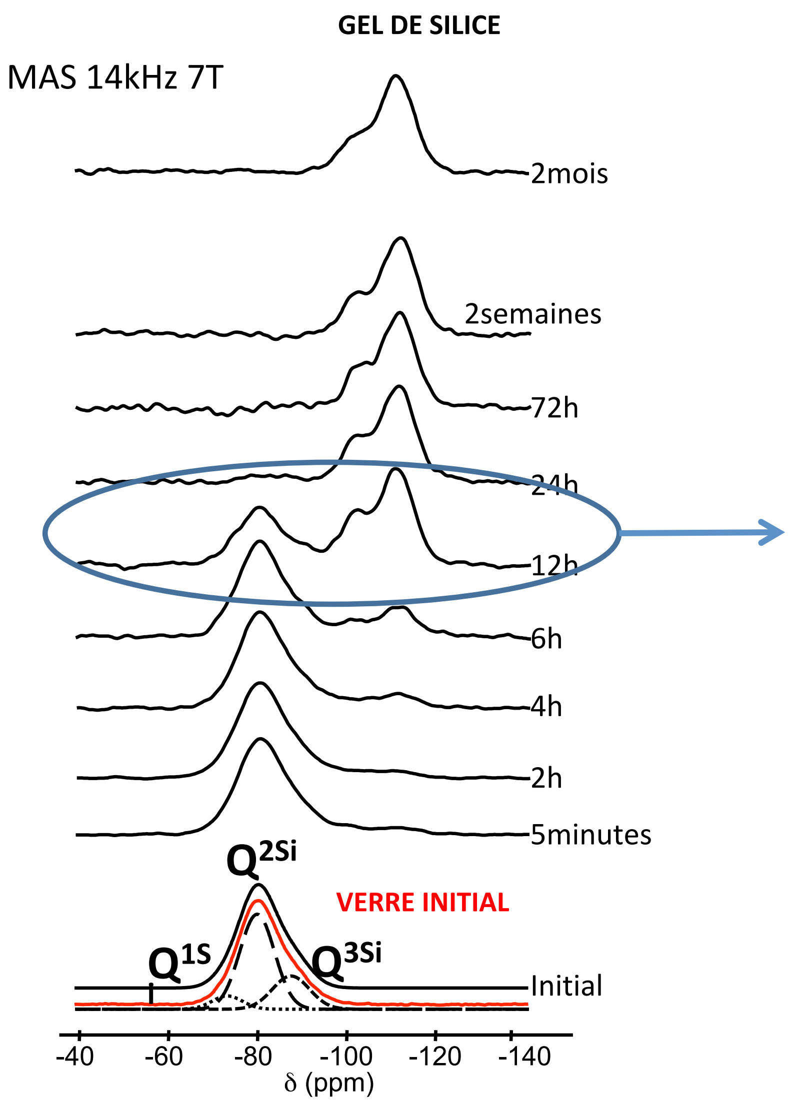


DRX



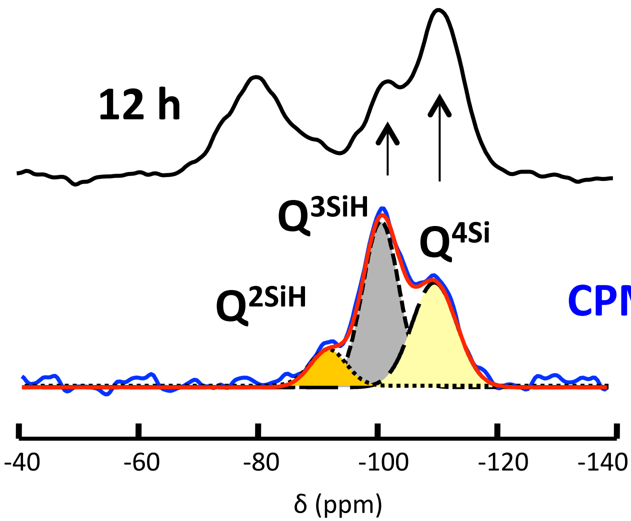
Mise en évidence difficile pour des temps de réaction courts

Formation d'une couche de gel de silice

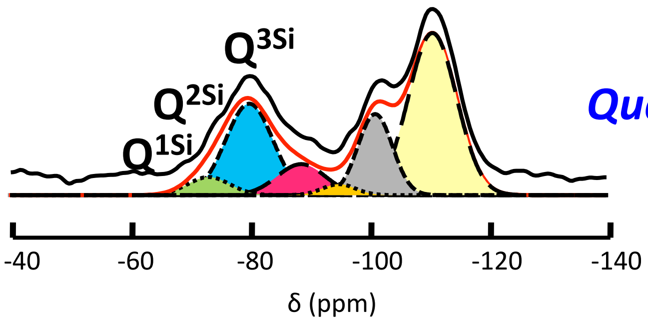


CPMAS = Cross-Polarisation MAS

Observation sélective des espèces hydratées (gel de silice)

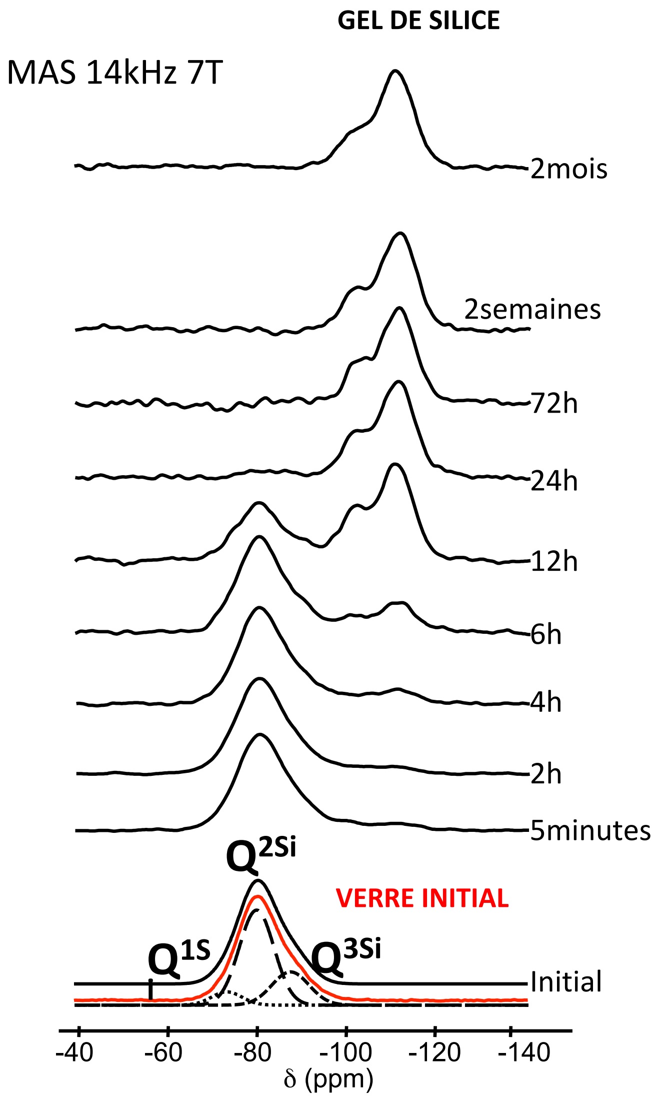


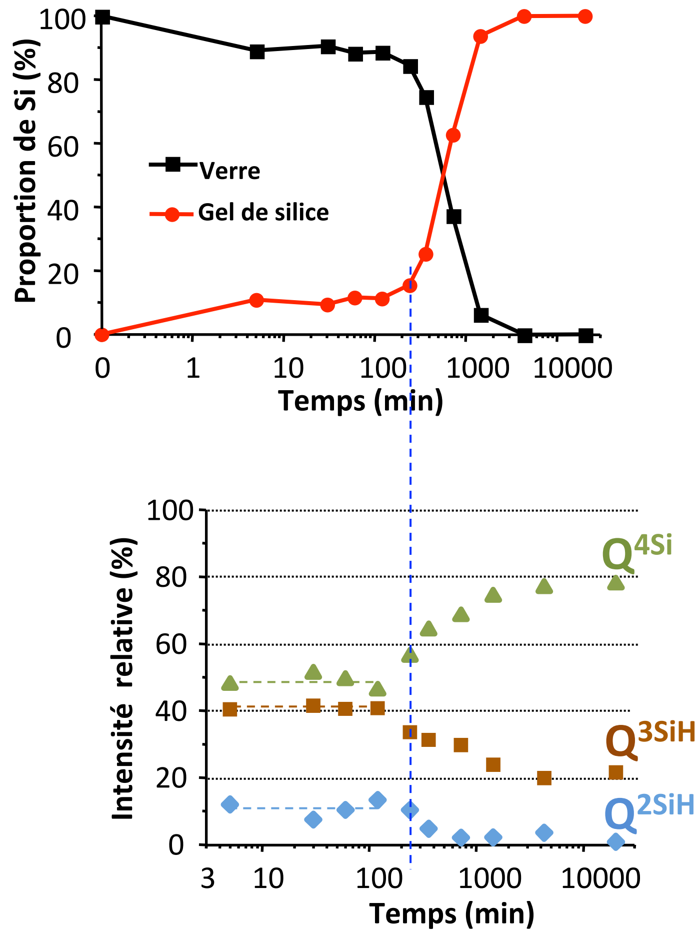
CPMAS $^{1}H \rightarrow {}^{29}Si$



Quantification

Formation d'une couche de gel de silice

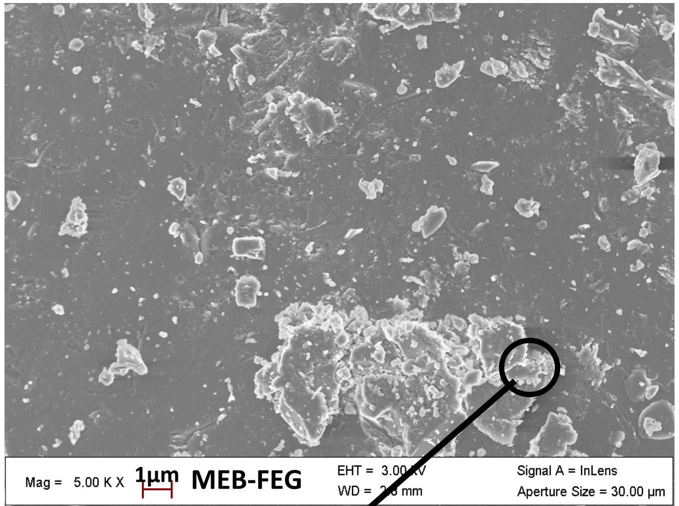




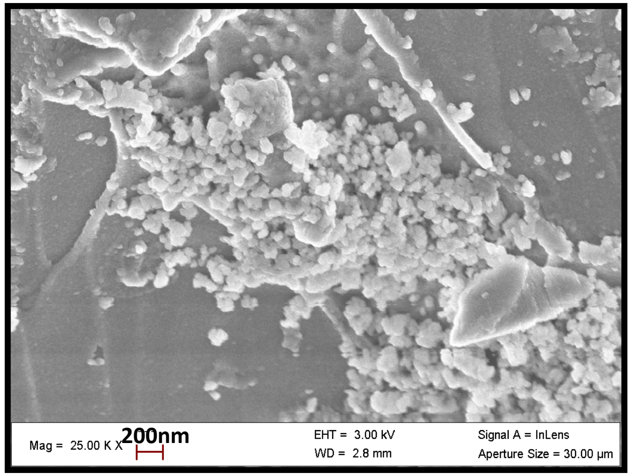
Gel de silice → majoritairement unités Q^{4Si}

Formation d’une phase CaP amorphe

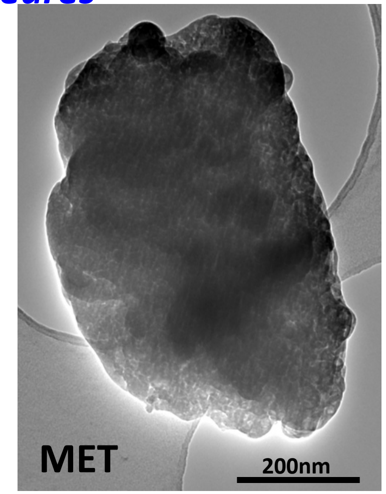
20minutes

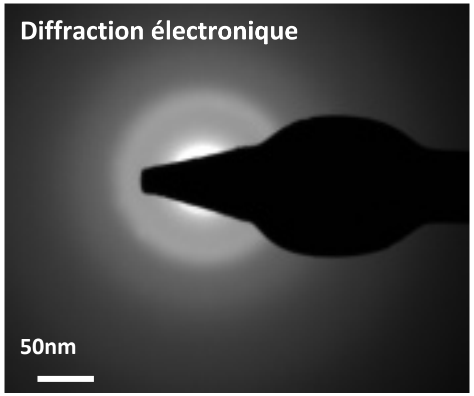


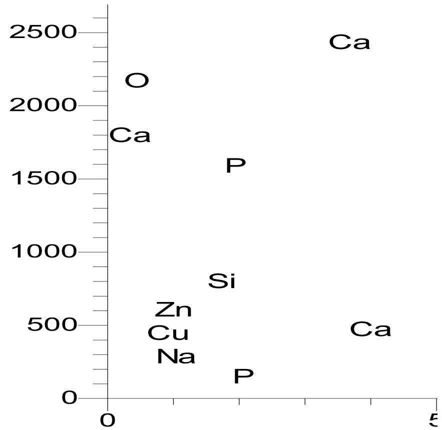
Amas de particules sphériques



2 heures



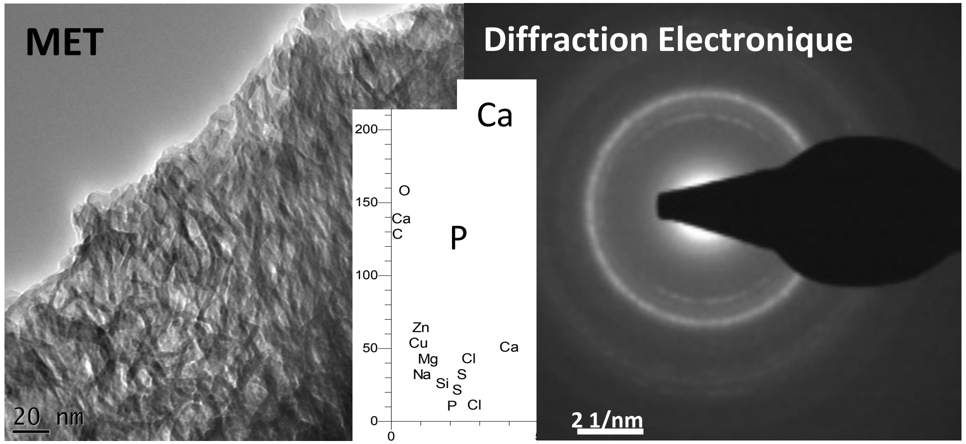




Phosphate de calcium amorphe

Cristallisation de la phase CaP amorphe

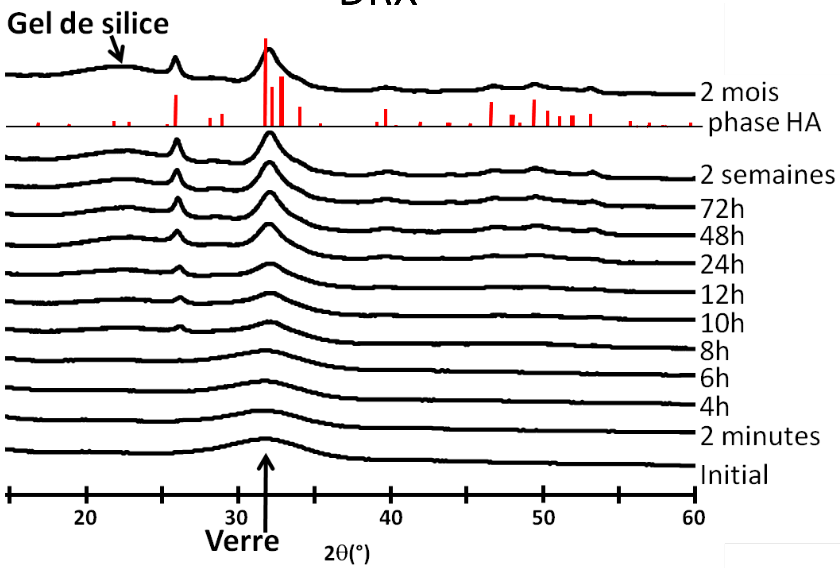
4 heures



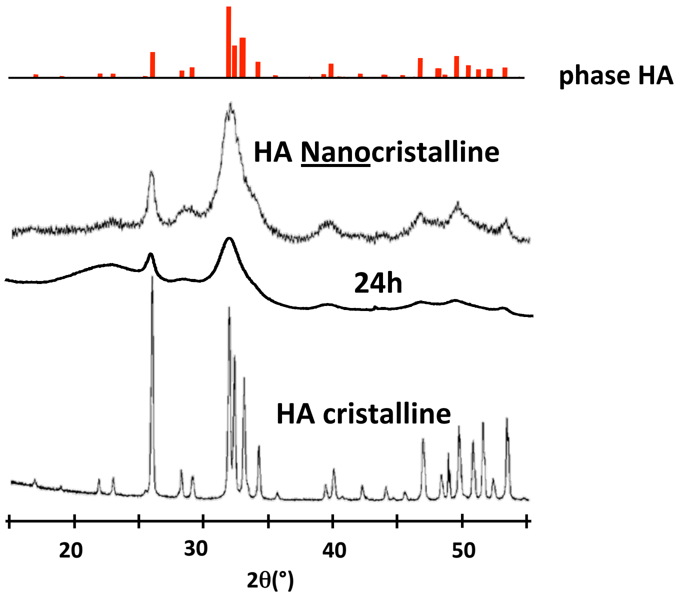
➡ Nouvelle morphologie nanocristalline

Nanocristaux d'HA

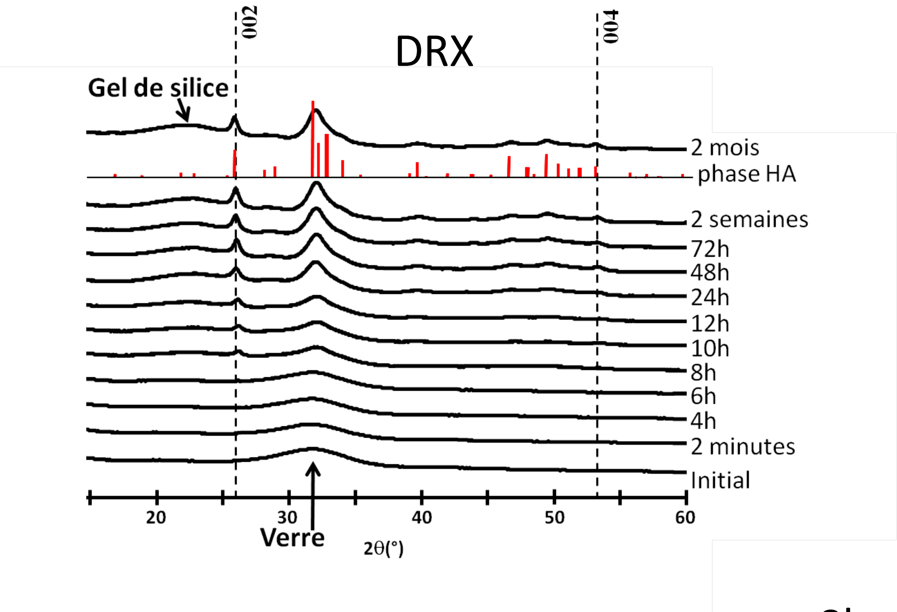
DRX



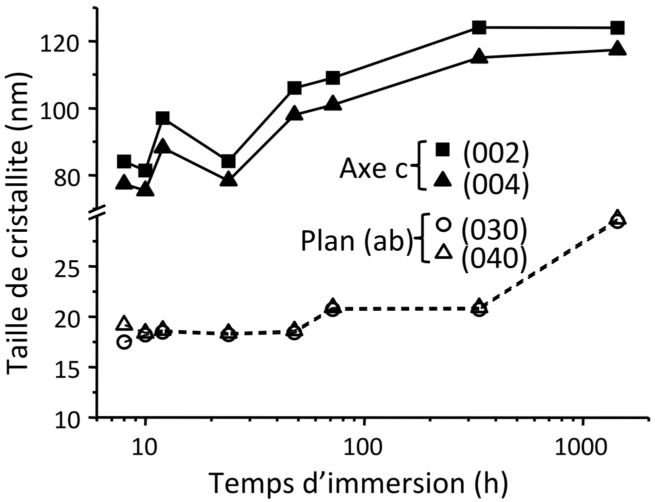
DRX



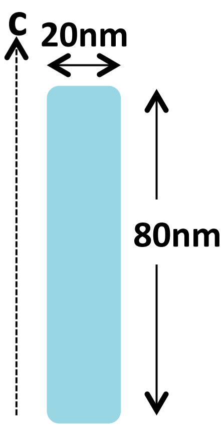
Hydroxyapatite nanocristalline



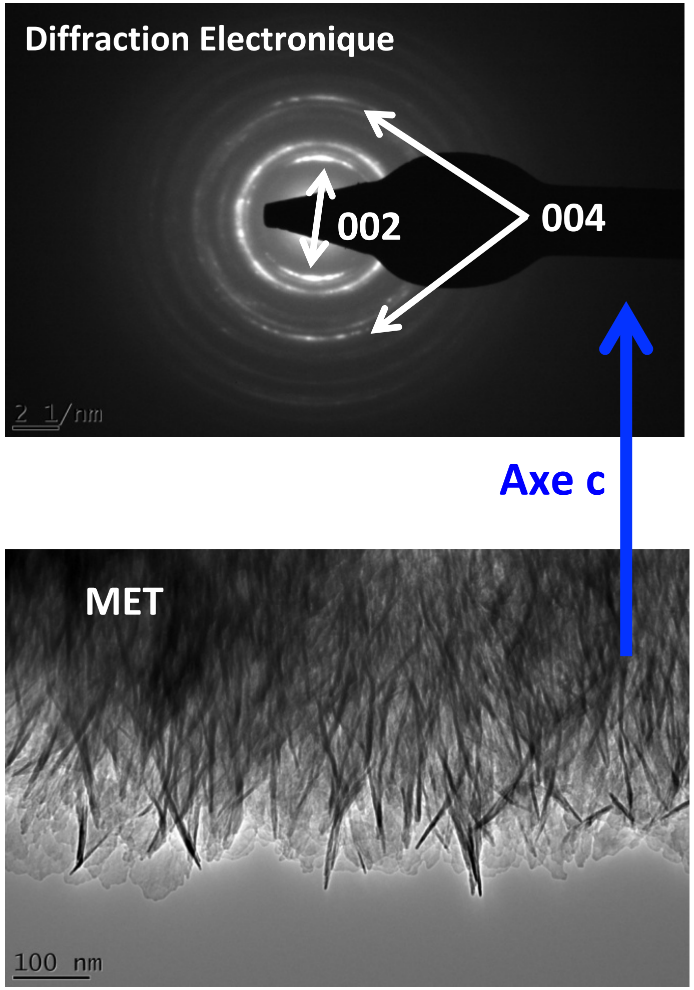
Affinements Rietveld (DRX)



t = 8h



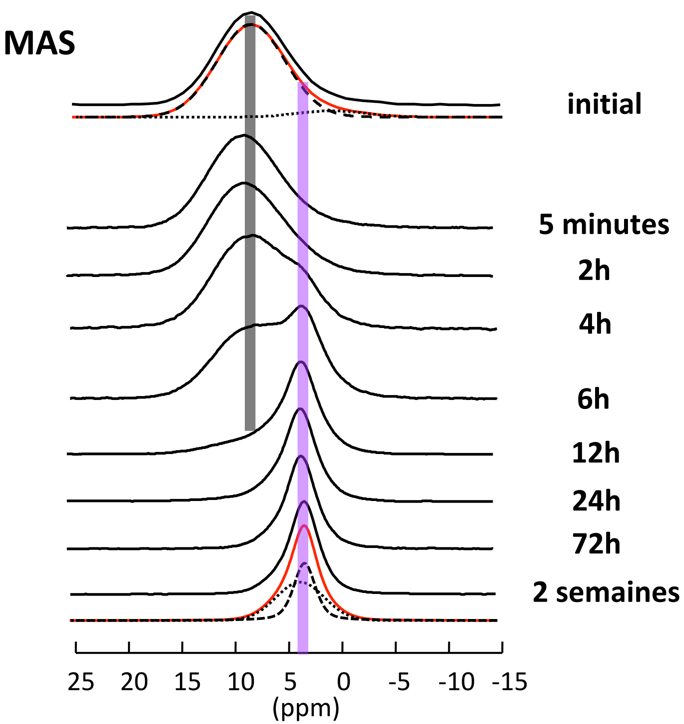
2semaines

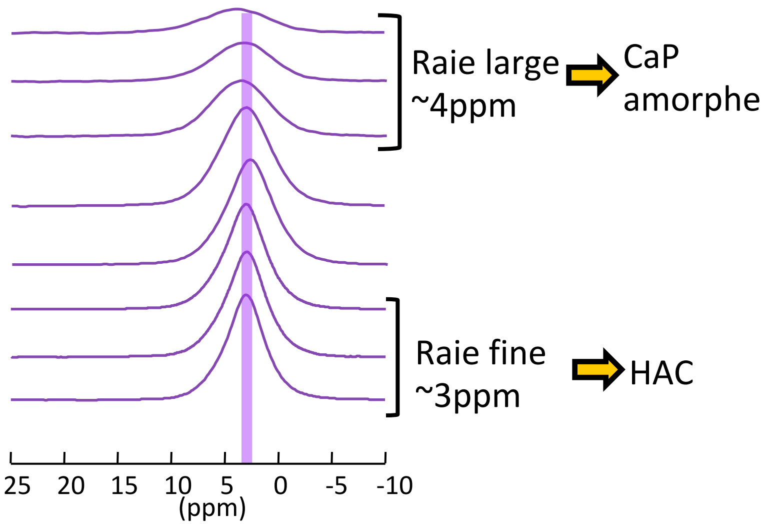


Nanocristaux = batonnets

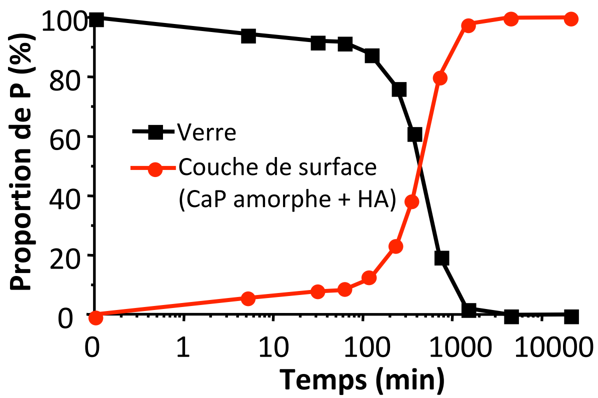
Cristallisation de la phase CaP amorphe

CPMAS = Cross-Polarisation MAS
Observation sélective de la phase amorphe et de l'hydroxyapatite





Quantification

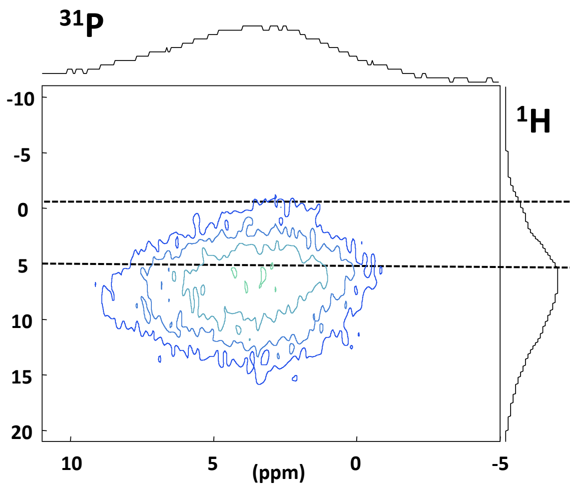


Structures des phases phosphocalciques

Corrélation hétéronucléaire RMN ^{31}P / ^{1}H

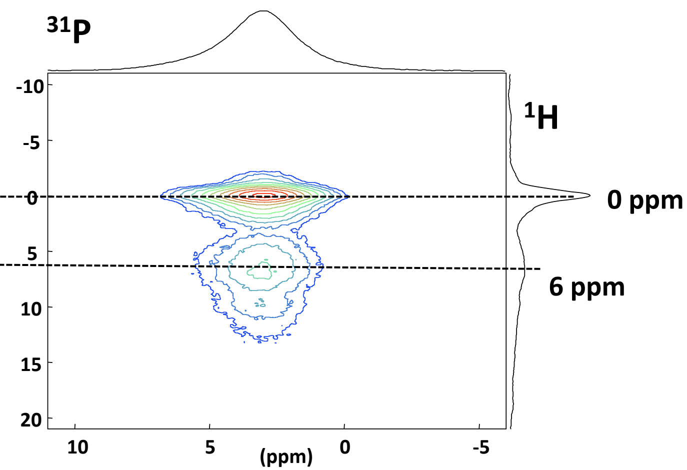
Verre immergé 2 heures

CaP amorphe



Verre immergé 2 mois

HAC nanocristalline



Absence d'HO^-
Riche en ions HPO_4^{2-} et H_2O

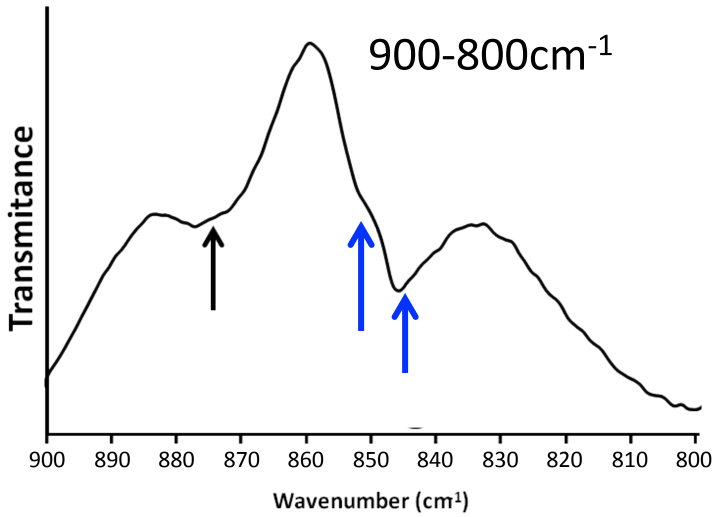
cristallisation →

Cœur HAC
Pauvre en ions HPO_4^{2-} et H_2O
Riche en ions HO^-

Surface
Riche en ions HPO_4^{2-} et H_2O
Pauvre en ions HO^-

Structures des phases phosphocalciques

Spectroscopie IR



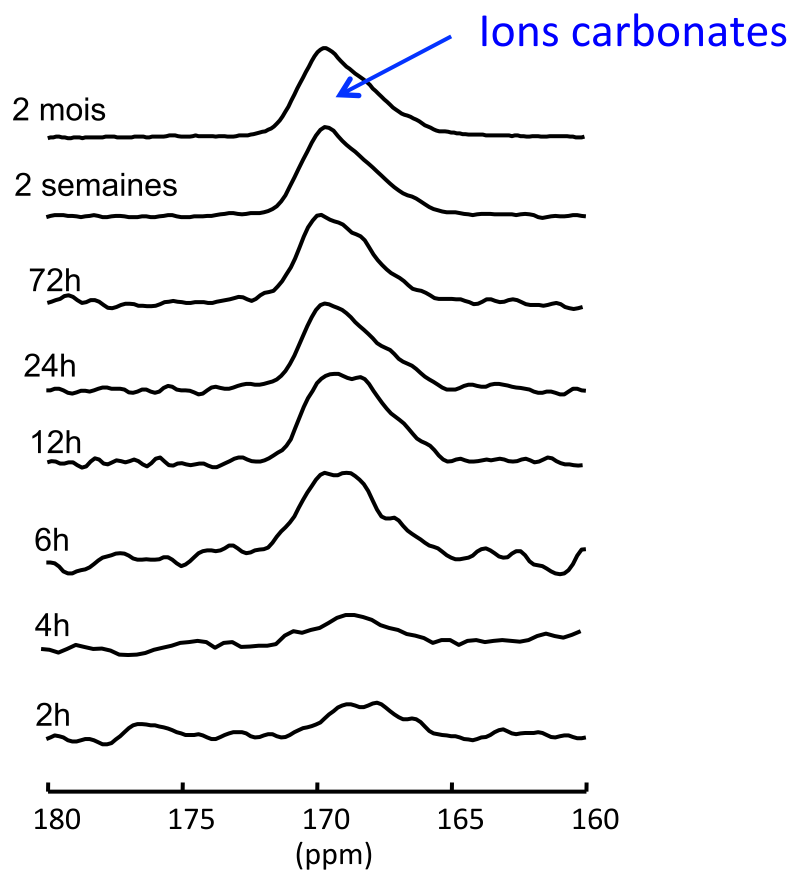
Enrichissement en ^{13}C du SBF :
distinction des bandes des CO_3^{2-} et des HPO_4^{2-}

852cm^{-1} ➔ CO_3^{2-} type A

846cm^{-1} ➔ CO_3^{2-} type B

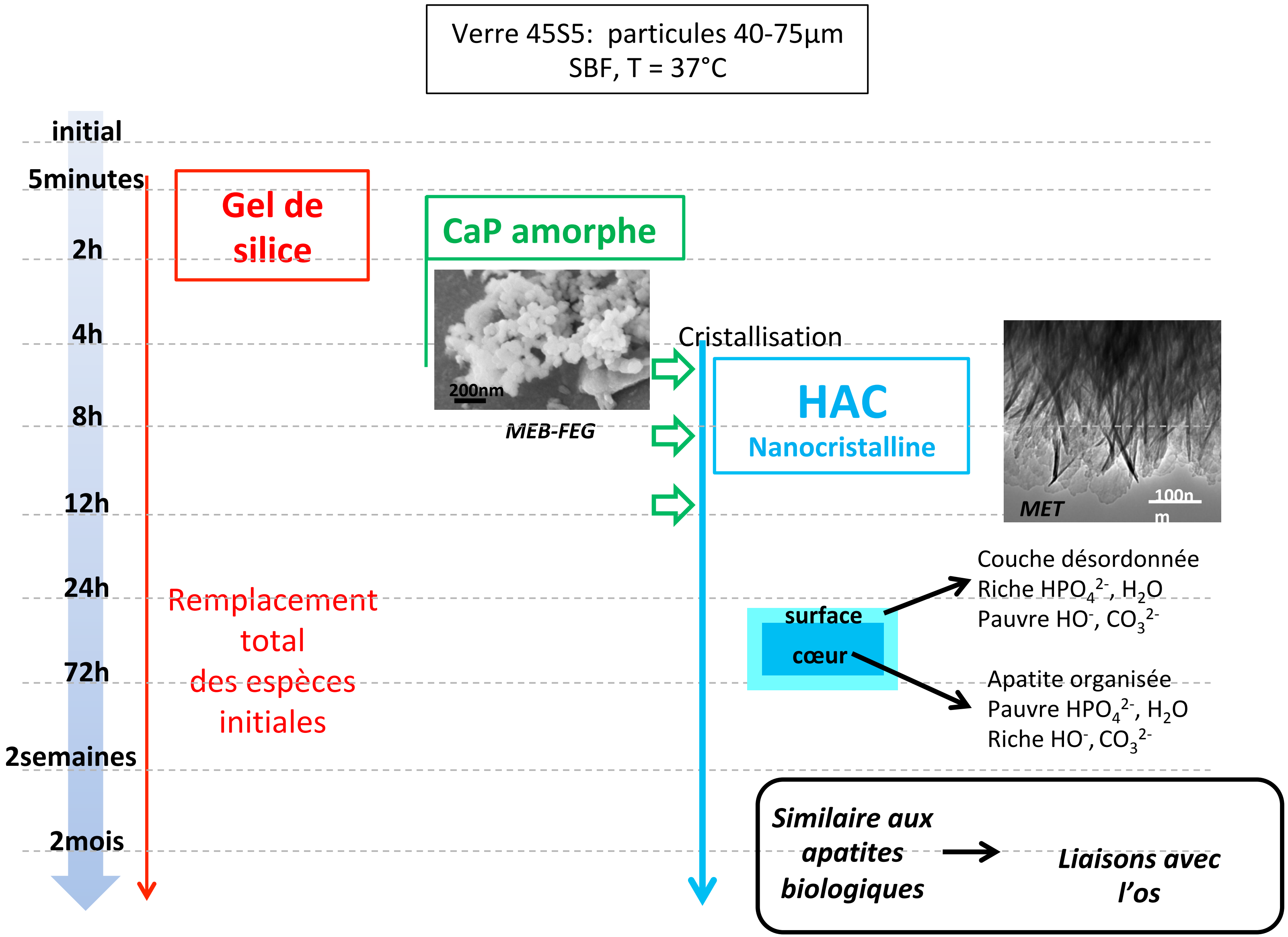
>870cm^{-1} ➔ HPO_4^{2-} en phase désordonnée

RMN de ^{13}C



Carbonates en substitution de type B dans le cœur des nanoparticucles d'HAp

Conclusion


Verre 45S5: particules 40-75µm
SBF, T = 37°C
initial
5minutes
2h
4h
8h
12h
24h
72h
2semaines
2mois
Gel de silice
Remplacement total des espèces initiales
CaP amorphe
200nm
MEB-FEG
Cristallisation
HAC
Nanocristalline
MET
surface
cœur
Couche désordonnée
Riche HPO_4^{2-}, H_2O
Pauvre HO^-, CO_3^{2-}
Apatite organisée
Pauvre HPO_4^{2-}, H_2O
Riche HO^-, CO_3^{2-}
Similaire aux apatites biologiques
Liaisons avec l'os

Remerciements

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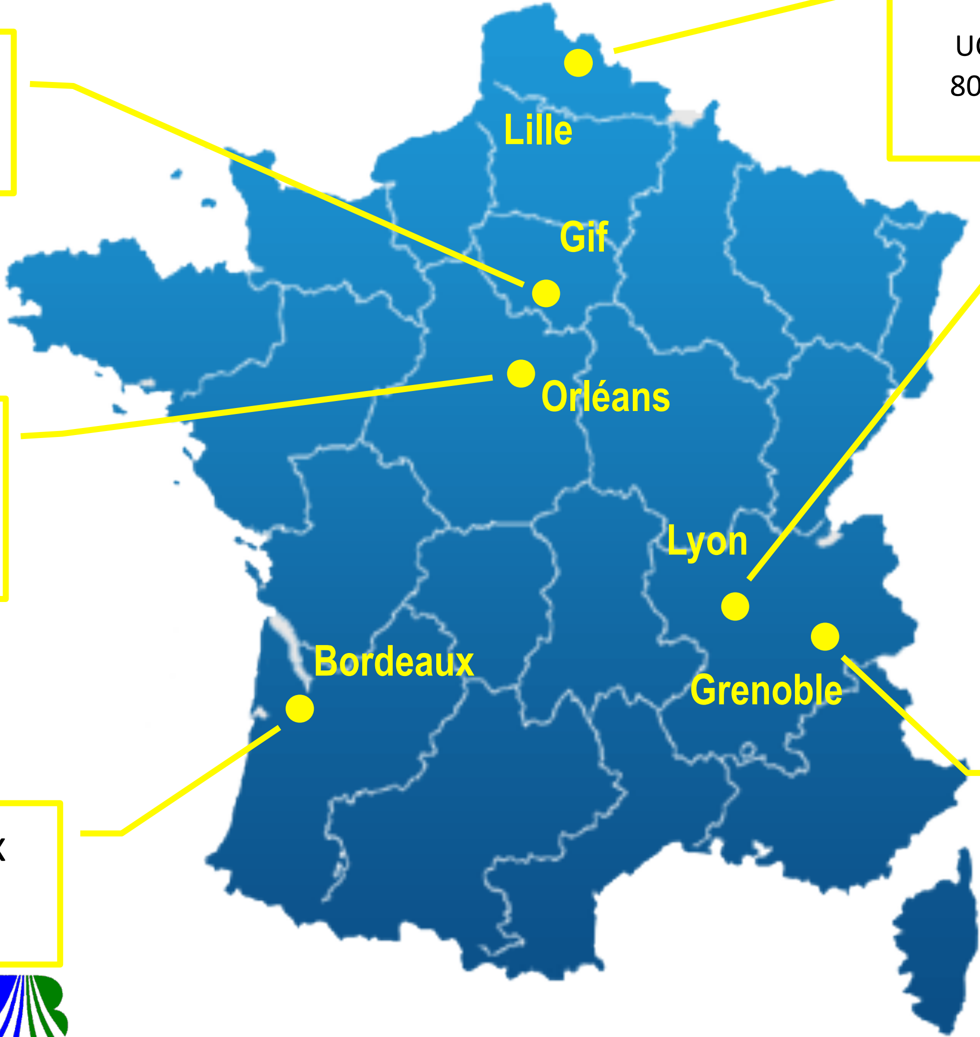
Emmanuel Véron, Domingos De Sousa Meneses, Dominique Massiot

TGIR RMN

http://www.tgir-rmn.org/



Gif
ICSN
950 SB SB liq.

INC

Orléans
CEMHTI
750 WB sol/HT
850 WB sol/HT

INC

Bordeaux
CBMN IECB
800 SB

INC/INSB

Lille
UCCS / UGSF
800 SB sol/liq
900 SB

INC

Lyon
CRMN
*800 SB sol/liq**
800WB DNP
1000 SB

INC

EU-NMR

Grenoble
IBS
950 SB liq

INSB

Nov. 2013

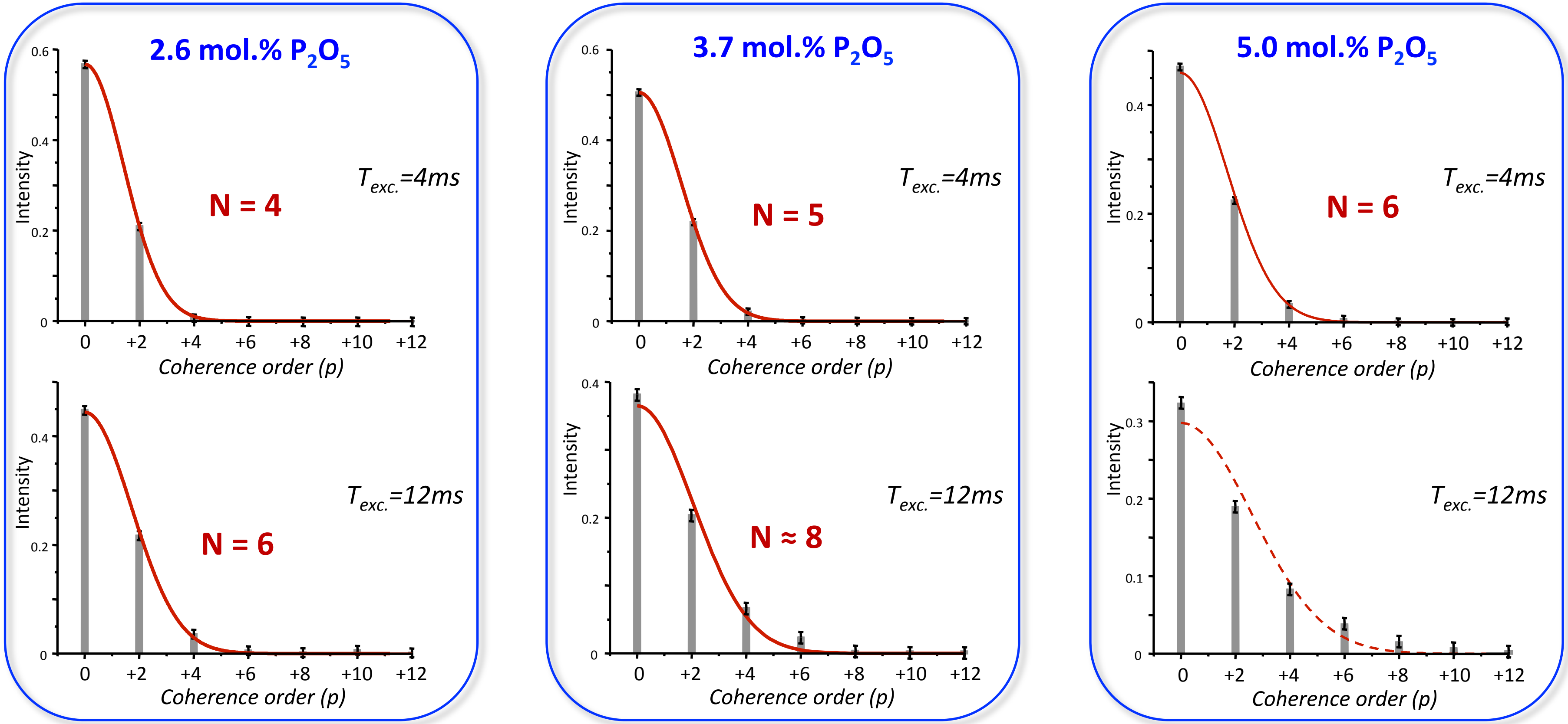
^{31}P multiple-quantum experiments in bioglasses

Statistical model :
(Pines *et al.*)

Number of correlated spins is time dependant N(τ)
with a binomial distribution of MQC intensities

Baum, Munovitz, Garoway and Pines, J Chem Phys 1985.
Munovitz, Pines and Mehring, J Chem Phys 1987.

$$\frac{(2N)!}{(N-p)!\,(N+p)!} \approx \mathbf{exp}\left(\frac{-\boldsymbol{p}^2}{\boldsymbol{N}}\right)$$



Local structure of bioactive Glasses

Ca/Si = 1.11

2.6 mol.% P_2O_5

Ca/Si = 1.11

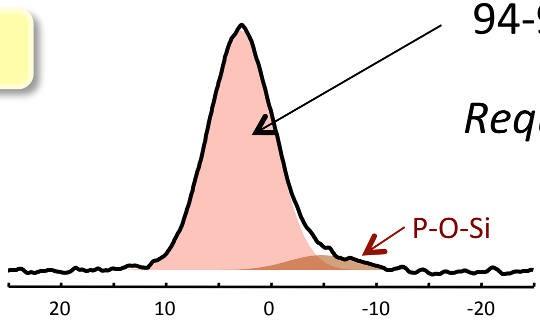
5.0 mol.% P_2O_5

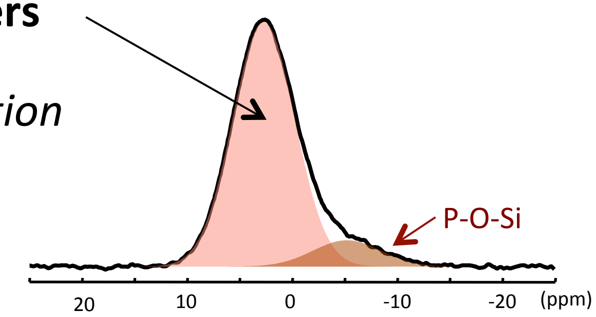
^{31}P MAS

94-90 % of **PO_4^{3-} monomers**

Require charge compensation

(Ca^{2+} cations)





^{29}Si MAS

Repolymerisation of the silicate network with P addition