Société Française d'Exobiologie, SFE, La Baule

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Proto-bioenergetics Systems and Coupled Chemical Processes

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1. Importance of phosphorus (P) in biology
2. Prebiotically plausible P-based *energy currency* molecules
3. *En route* to coupled chemical reactions

1. *Importance of phosphorus (P) in biology*

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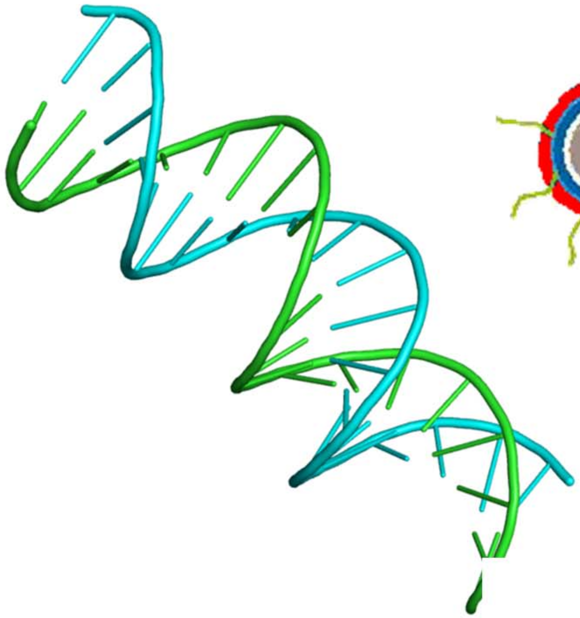
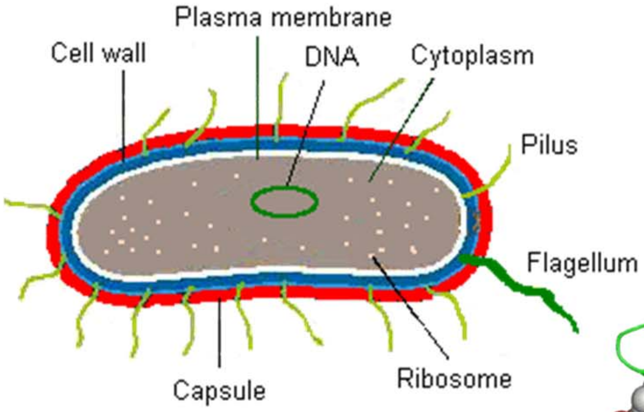
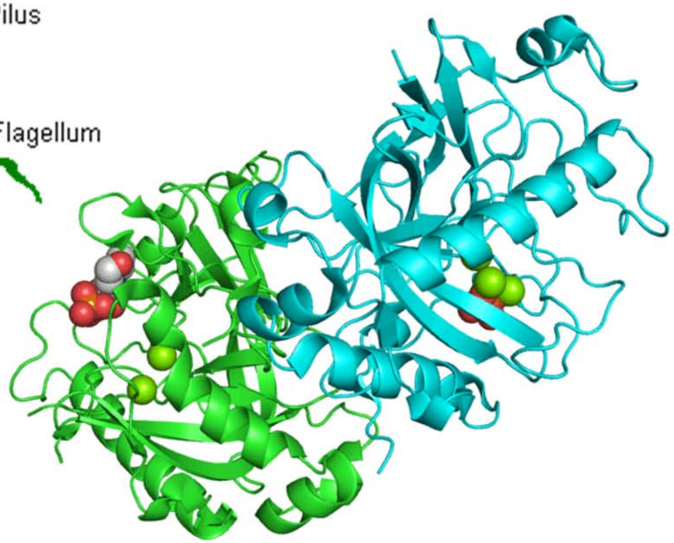
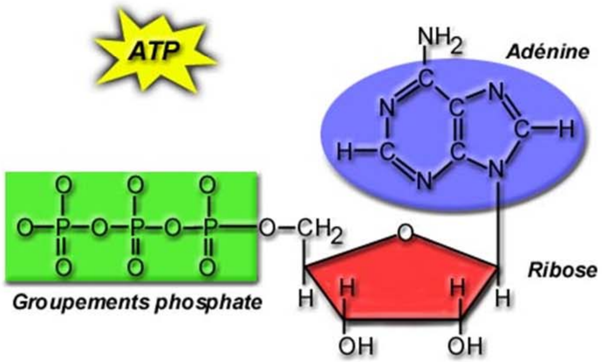








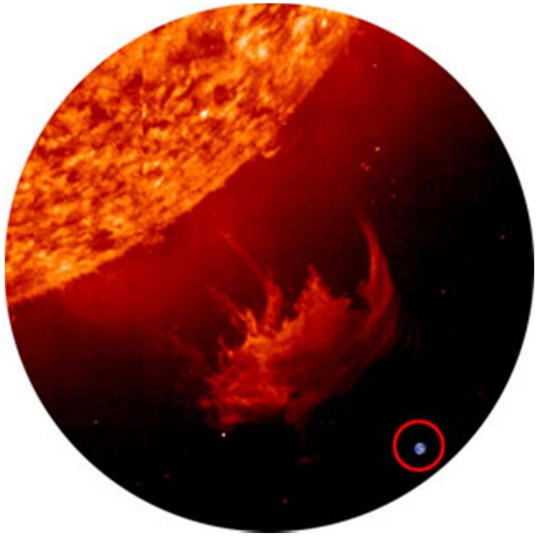
Nucleic acids

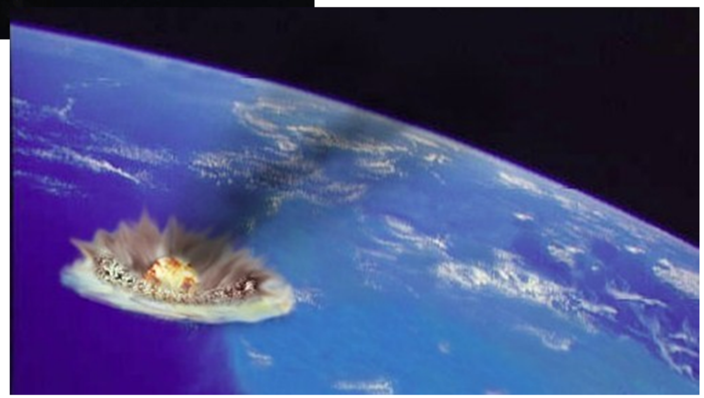
(Replication)

Proteins

(Metabolism)

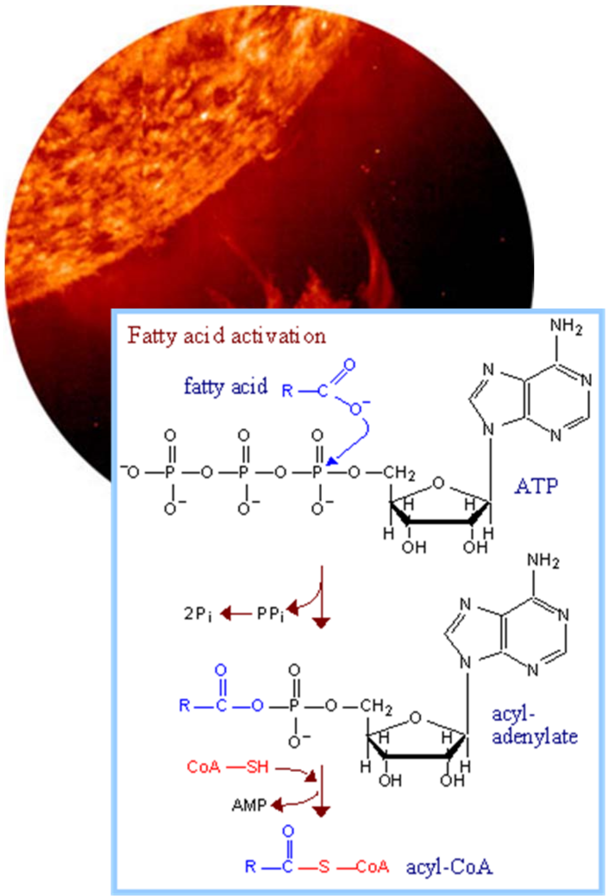
Chemical & biochemical processes require energy

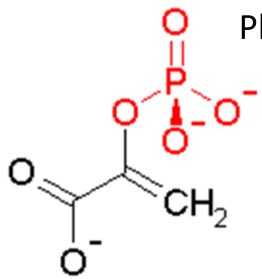




Chemical & biochemical processes require energy

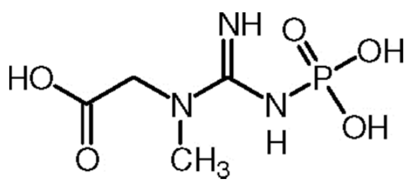
H-fusion produces *ca*. 3.8 x 10^{26} J s^{-1}
Earths surface receives *ca*. 1 kJ s^{-1} m^{-2}
Life forms store this energy in a chemically usable form

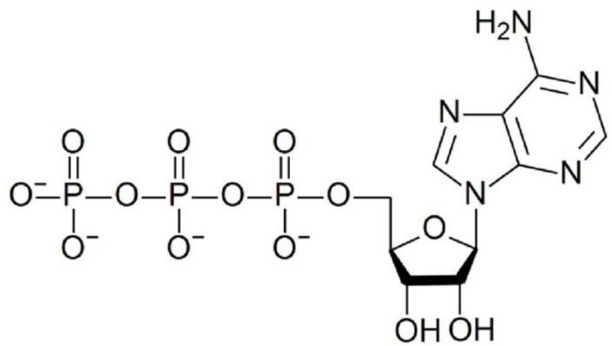




Phosphoenol pyruvate

Phosphocreatine

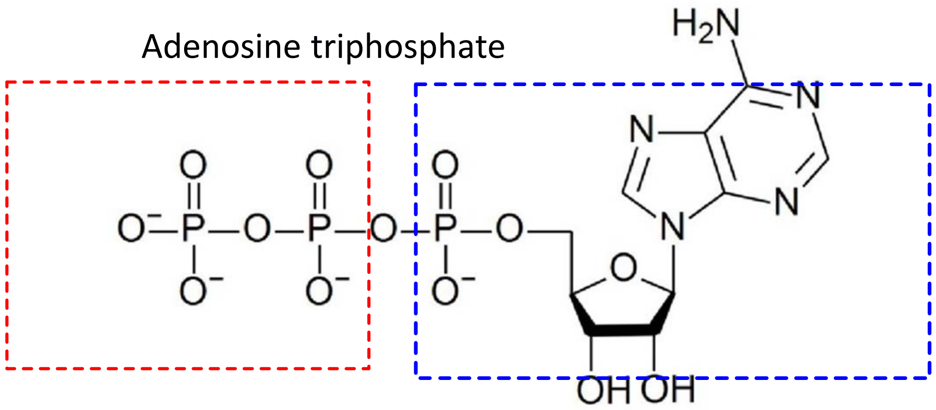


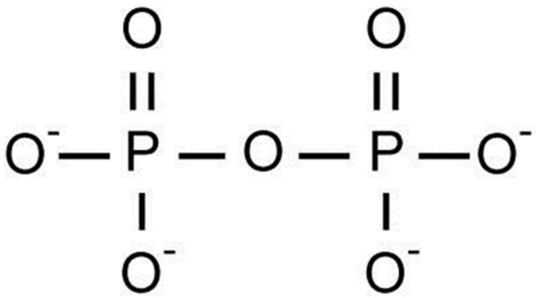


Adenosine triphosphate

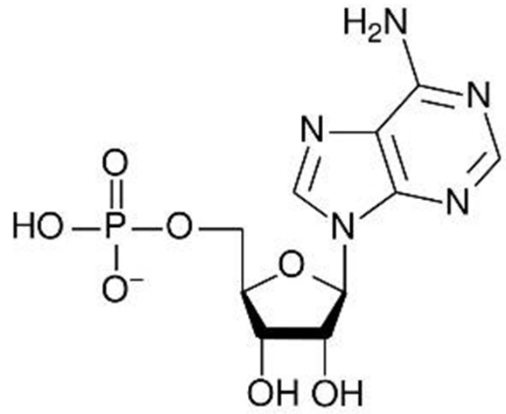
1. *Importance of phosphorus (P) in biology*

Adenosine triphosphate





Pyrophosphate



Adenosine monophosphate

1. Importance of phosphorus (P) in biology

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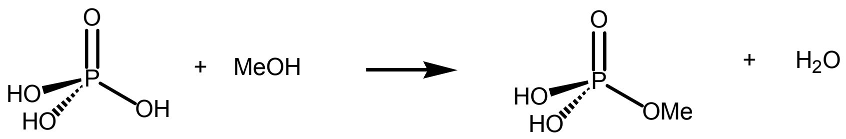
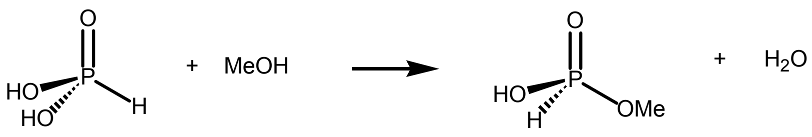
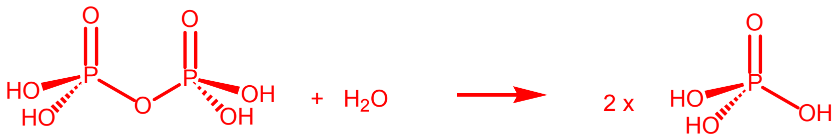
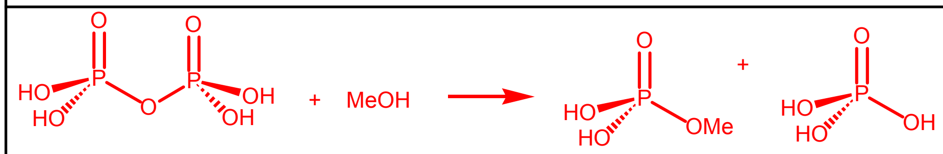
G3//g + ZPE//B3LYP/cc-pVTZ (kJ/mol)	*ΔH°* *(0K)* *(Gas)*	*ΔH°* *(298.15K)* *(Gas)*	*ΔH°* *(298.15K)* *(Gas)*	*ΔG°* *(298.15K)* *(aq.)*
	-19.0	-16.4	-12.1	+12.4
	-17.6	-15.5	-10.7	+10.9
	+10.8	+8.1	-0.1	-54.9
	-8.2	-8.3	-12.2	-42.5

The Pyrophosphate [PPi(V)] Problem

Canaphite [$CaNa_2P_2O_7 \bullet 4(H_2O)$]

- Very few geologically available pyrophosphate minerals
- Poor solublility in a salt-rich early earth ocean
- Relatively low chemical reactivity & selectivity in the absence of catalysts

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1. Importance of phosphorus (P) in biology

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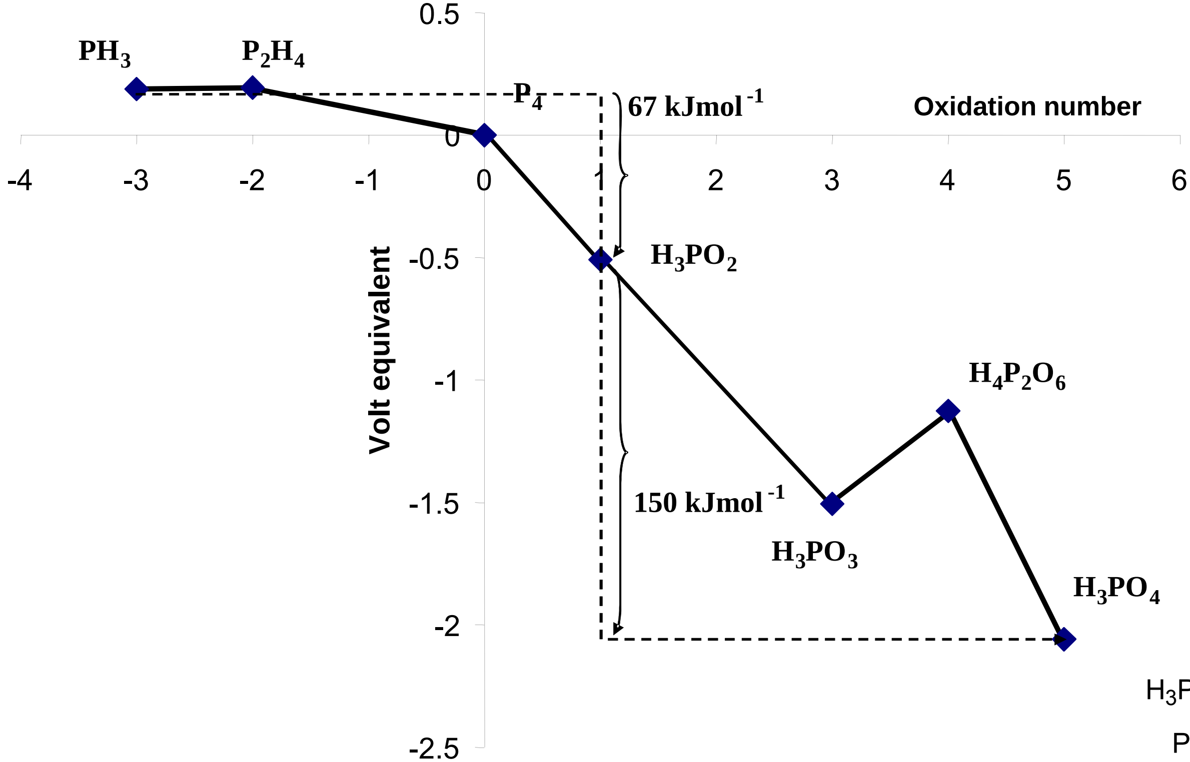
Activated P-compounds *via* redox chemistry



$$H_3PO_4 \xrightarrow{-0.276\ V} H_3PO_3 \quad \Delta G^o = +53\ kJmol^{-1}$$

$$PO_4^{3-} \xrightarrow{-1.12\ V} HPO_3^{2-} \quad \Delta G^o = +216\ kJmol^{-1}$$

$$H_3PO_4 \xrightarrow{-0.39\ V} H_3PO_2 \quad \Delta G^o = +150\ kJmol^{-1}$$

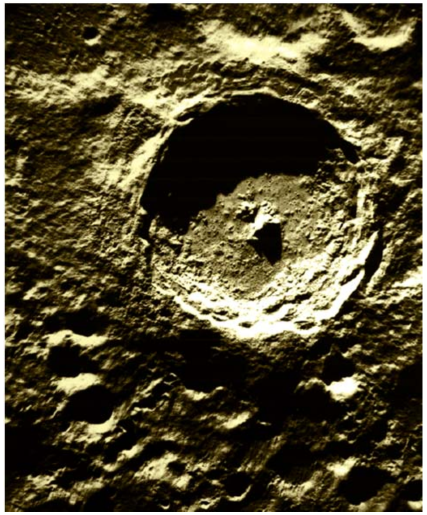
$$PO_4^{3-} \xrightarrow{-1.35\ V} H_2PO_2^{-} \quad \Delta G^o = +521\ kJmol^{-1}$$

Schreibersite or rhabdite
$(Fe, Ni)_3P$

Sikhote Alin	0.46% P
Canyon Diablo	0.26% P

(R. S. Clarke Jr., *et. al. Smithsonian Contr. Earth Sci.,* 1978, Vol 21)

Schreibersite produced *via* lightning discharge through phosphates



Impact shock reduction of phosphates

(O. I. Yakovlev,, *Geochem. Int.*, 2006, 44, 847-854)

(M. A. Pasek & K. Block, *Nature Geoscience,* 2009, 2, 529-596)

Class	**Flux (kg yr^{-1})**	**P (kg yr^{-1})**
Irons	3×10^{10}	10^{8}
CC	8×10^{7}	1000
OC	3×10^{9}	3×10^{4}
IDP	3×10^{12}	10^{8}

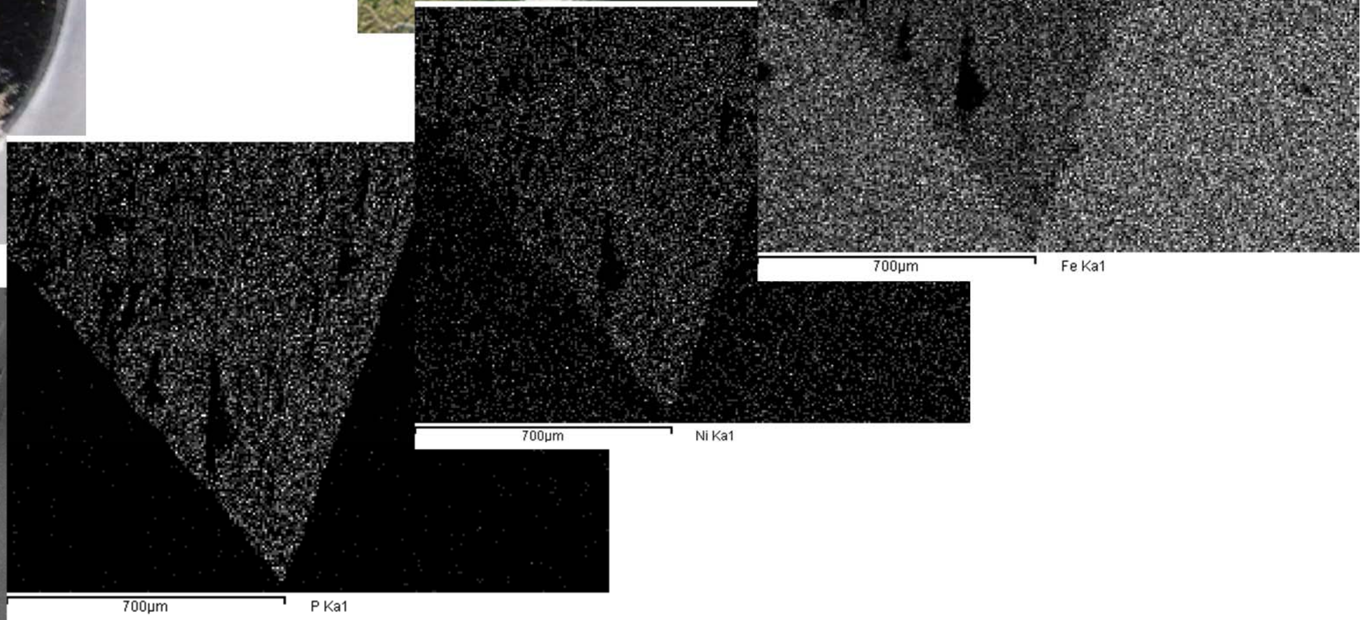
Fluxes during the Late Heavy Bombardment (LHB)
CC = carbonaceous chondrites; OC = ordinary chondrites; Irons = iron meteorites
IDPs = interplanetary dust particles. Errors ca. factor of 30.
P is mainly phosphides and phosphonic acids for CC's and IDPs, and phosphides for OC's and irons

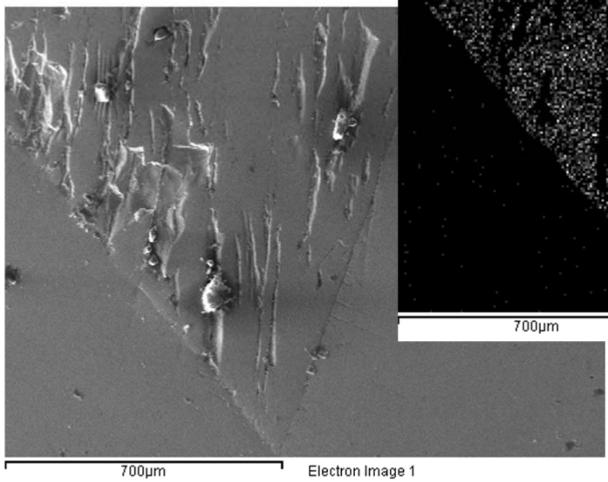
- Oceanic in-fall could have resulted in dispersion to [P] *ca.* 10^{-6} to 10^{-8} M ([P] *ca*. 10^{-3} M in cell cytoplasm)
- Consider a large iron impactor (10^{10} kg) affording a 5 km diameter crater of *ca*. 16 km^2
- Assuming ~5% of P survives Earth collision as schreibersite; ≈**100,000 kg of reactive P per km^2**
- This local flux is ≈ 1 million year's worth of reactive P from IDPs

(M. A. Pasek & D. Lauretta, *OLEB,* 2008, 38, 5-21)

2. *Prebiotically plausible P-based* energy currency *molecules*

Sikhote-Alin (eastern Russia)
Fell 1947; *ca.* 70 tonnes survived





2. *Prebiotically plausible P-based* energy currency *molecules*

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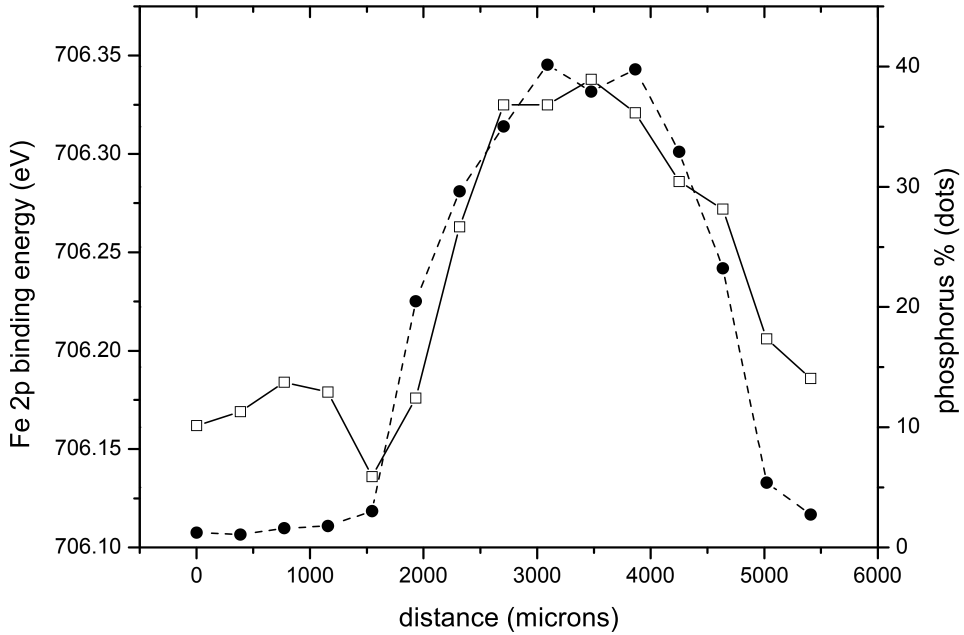
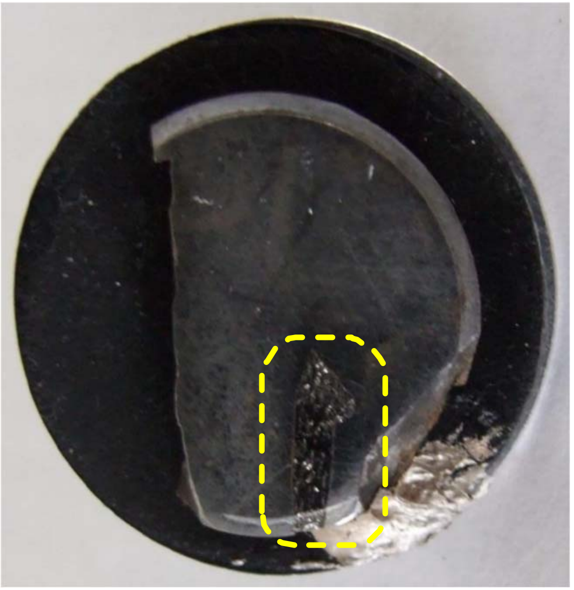
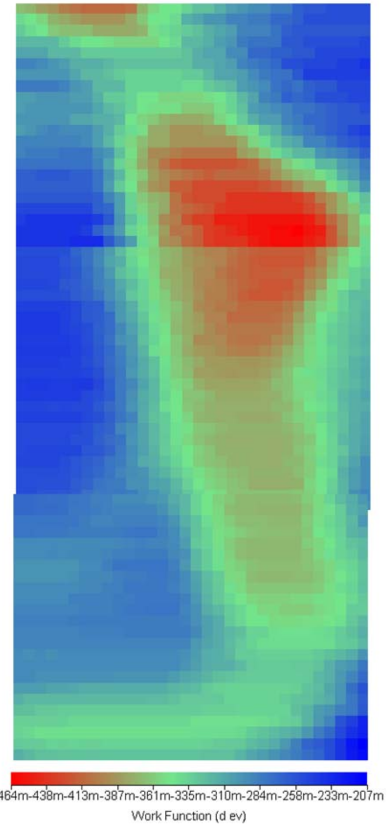
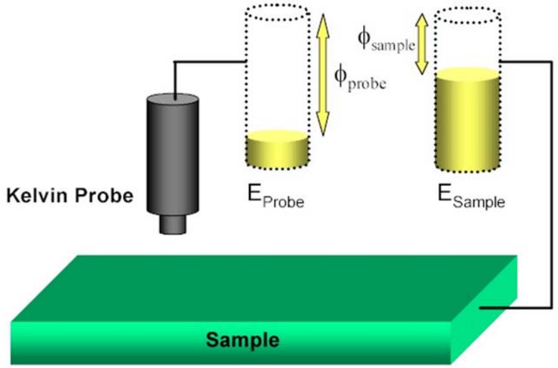


- Fe-2$p_{3/2}$ XPS line scan analysis traversing matrix-schreibersite-matrix region of sectioned Sikhote Alin.
- P-composition locates the inclusion region
- Increasing Fe-2$p_{3/2}$ binding energy within inclusion links to greater Ni-content

(D. E. Bryant et al, GCA, 2013, <u>109</u>, 90-112)





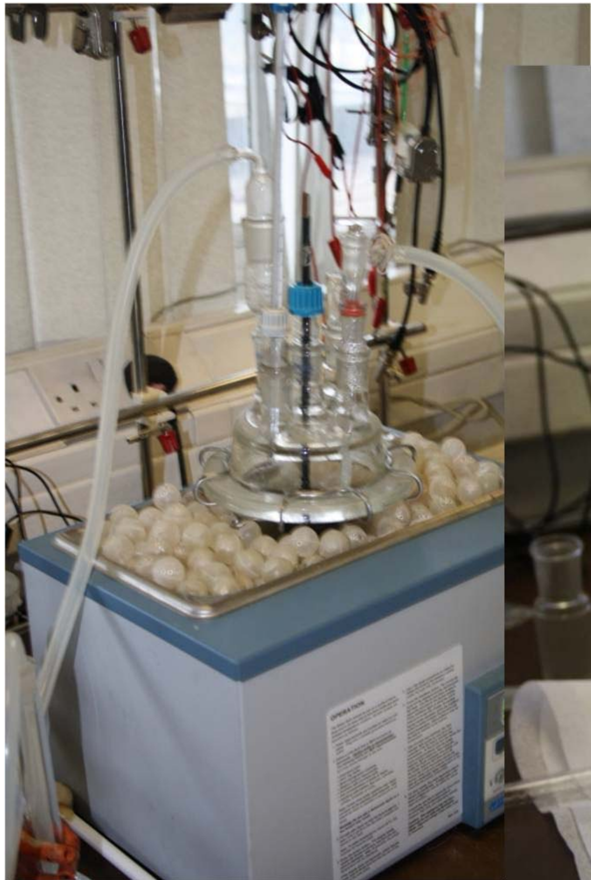


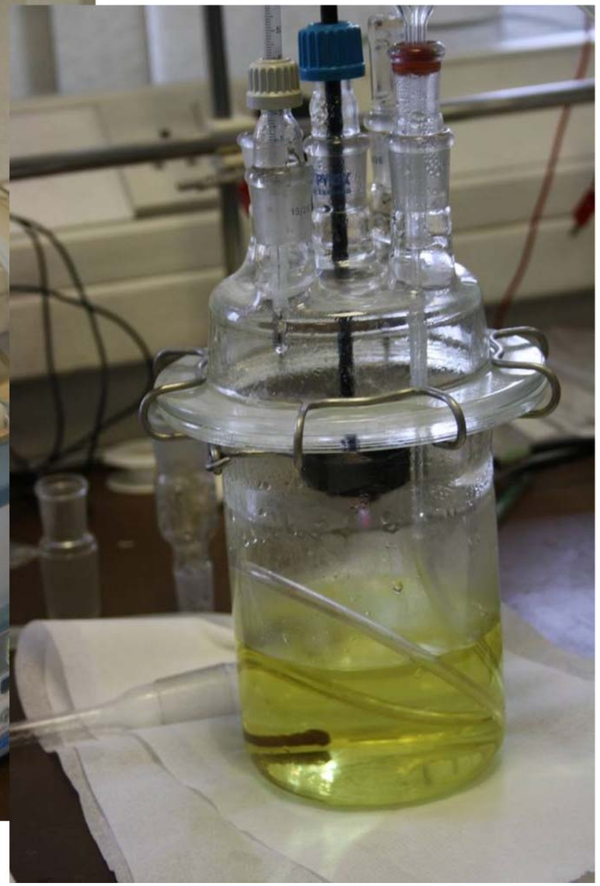
- ➢ ΔE between the inclusion and the substrate *ca*. 550 mV.
- ➢ ΔE acts as driving force for a galvanic current between inclusion and matrix.
- ➢ Result is to polarise the matrix **anodically** making the matrix more susceptible towards oxidation than inclusion.

(D. E. Bryant et al, GCA, 2013, <u>109</u>, 90-112)

2. *Prebiotically plausible P-based* energy currency *molecules*

Anaerobic, acid-mediated digestion of Sikhote Alin





500 cm^3, 10% aqueous HCl; N_2; 5 days 50^oC

2. *Prebiotically plausible P-based* energy currency *molecules*

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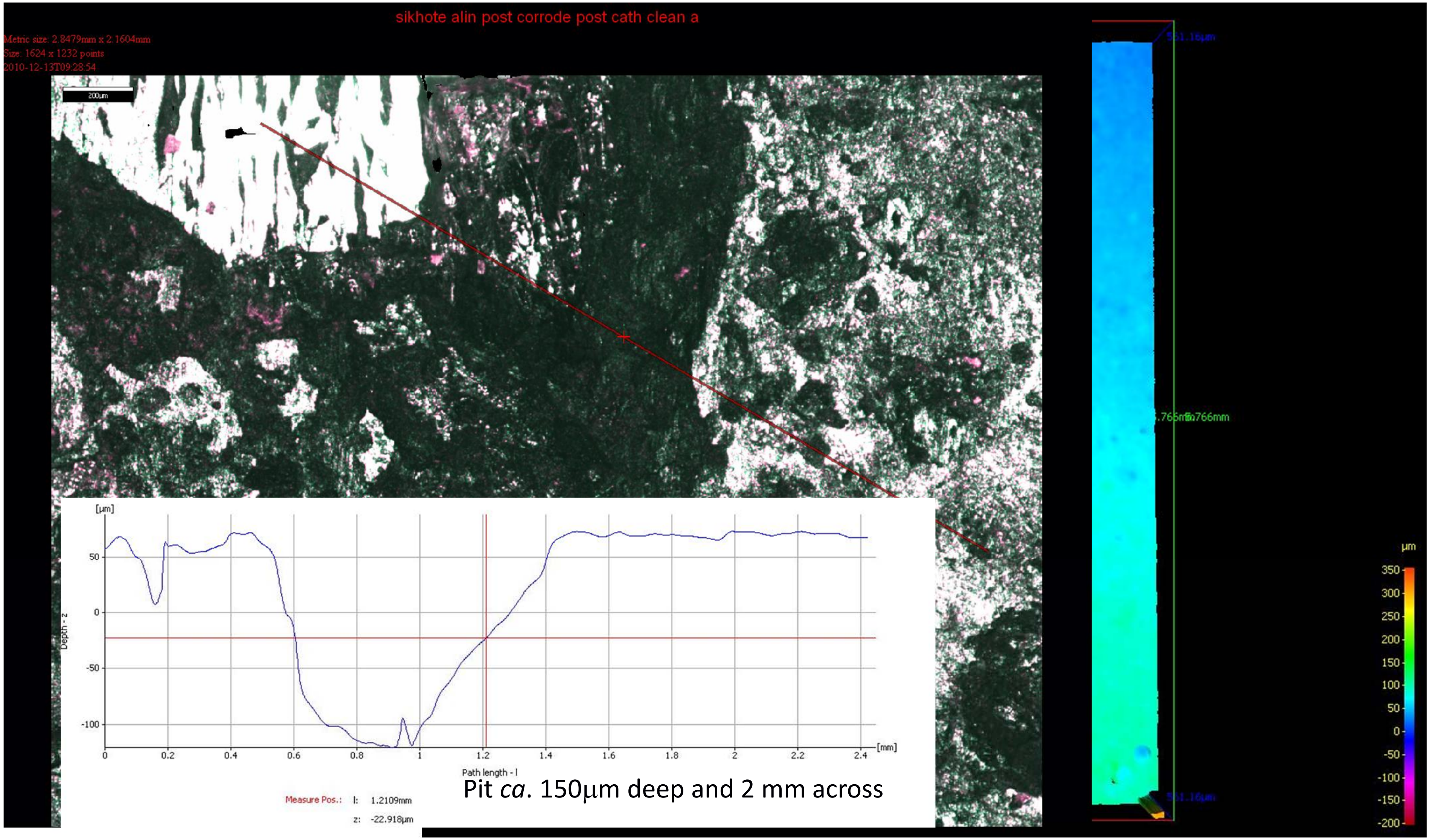

Pit *ca*. 150μm deep and 2 mm across

2. *Prebiotically plausible P-based* energy currency *molecules*

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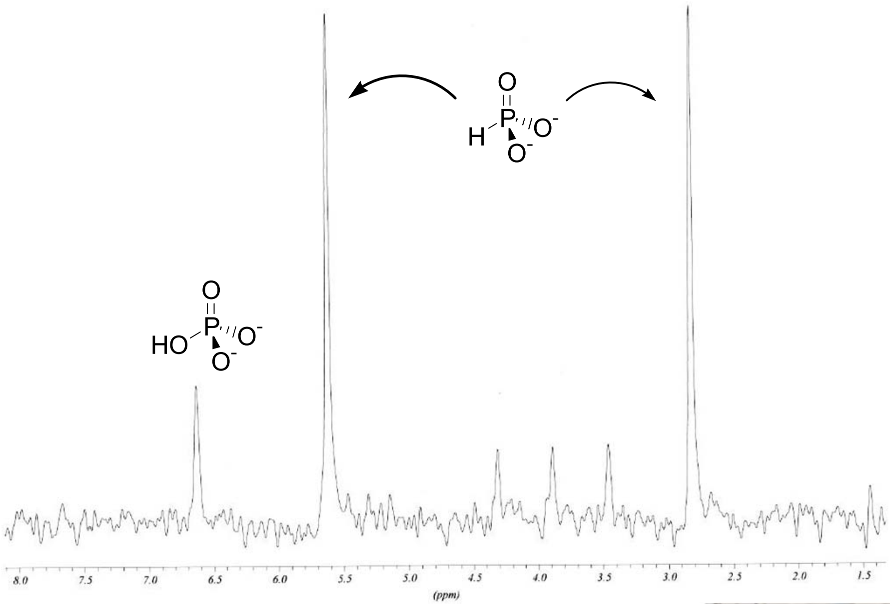
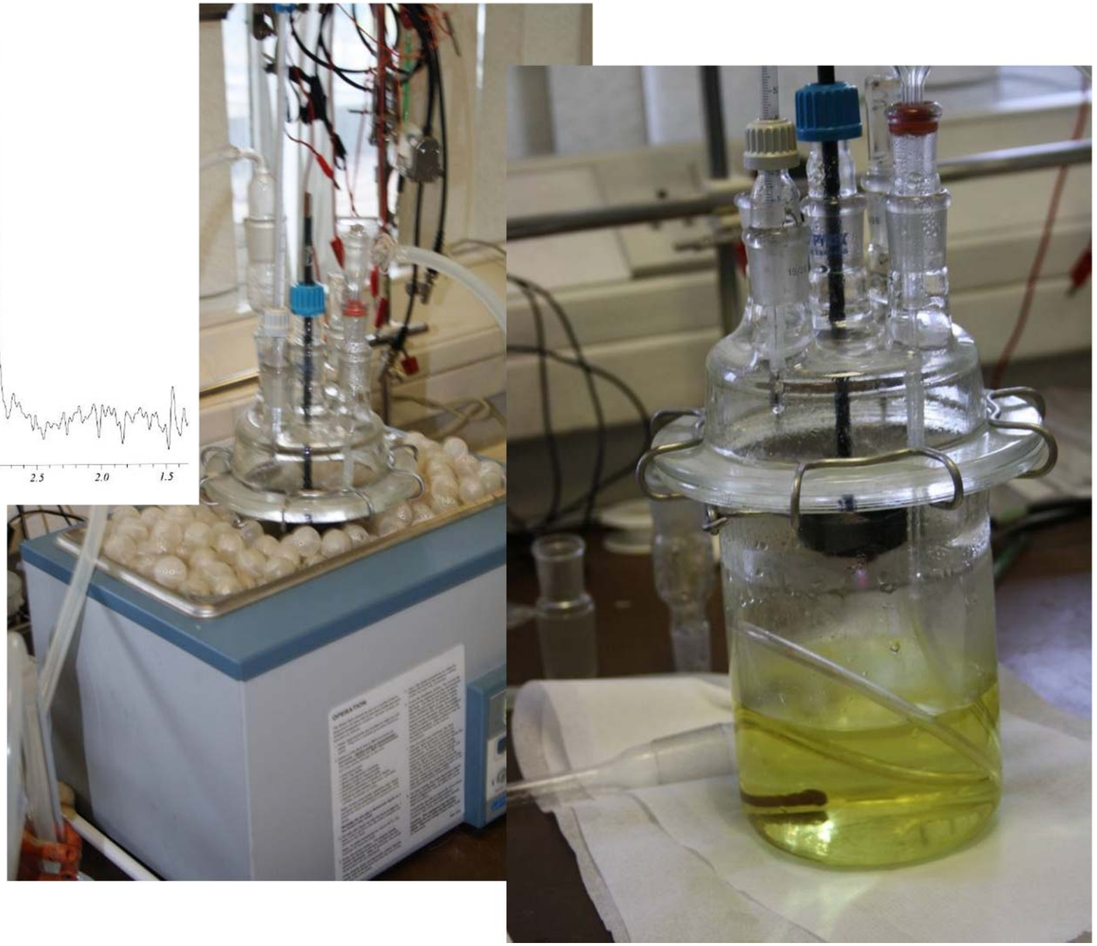


^{31}P-NMR spectrum (202.456 MHz; D_2O)
$[HPO_4]^{2-}$ δ6.63ppm.
$[HPO_3]^{2-}$ δ4.22ppm; $^1J_{PH}$ = 566.9Hz
$[DPO_3]^{2-}$ δ3.89; $^1J_{PD}$ = 85Hz

500 cm^3, 10% aqueous HCl; N_2; 5 days 50^{o}C
440 ppm Fe
20 ppm Ni
0.7 ppm P

Anaerobic, acid-mediated digestion of Sikhote Alin



2. *Prebiotically plausible P-based* energy currency *molecules*

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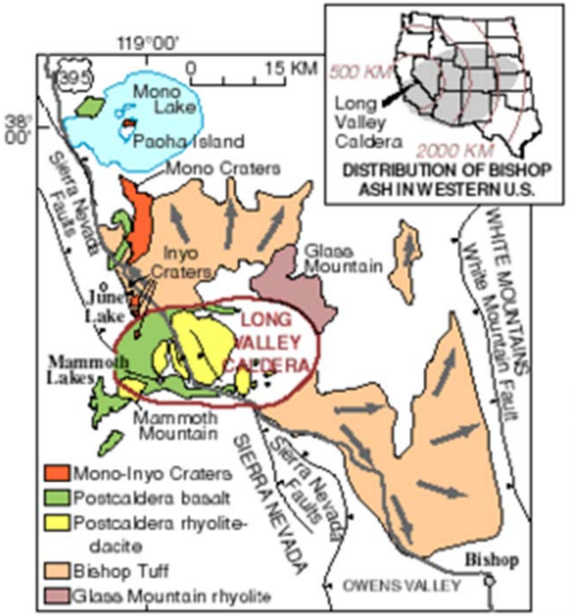
Redcued phosphorus, specifically **H-phosphite**, $\mathbf{H_2PO_3^-}$ has recently been detected at 0.06 ± 0.02 µM levels in geothermal pools linked to volcanic activity.

Hot Creek Gorge, Mammoth Lakes, California



(H. Pech *et al Environ Sci Technol*. 2009, 43, 7671)

2. *Prebiotically plausible P-based* energy currency *molecules*

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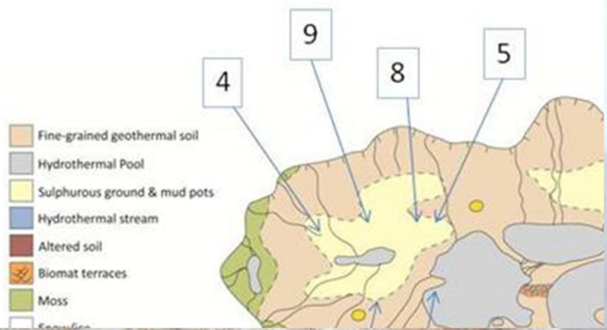

2. *Prebiotically plausible P-based* energy currency *molecules*

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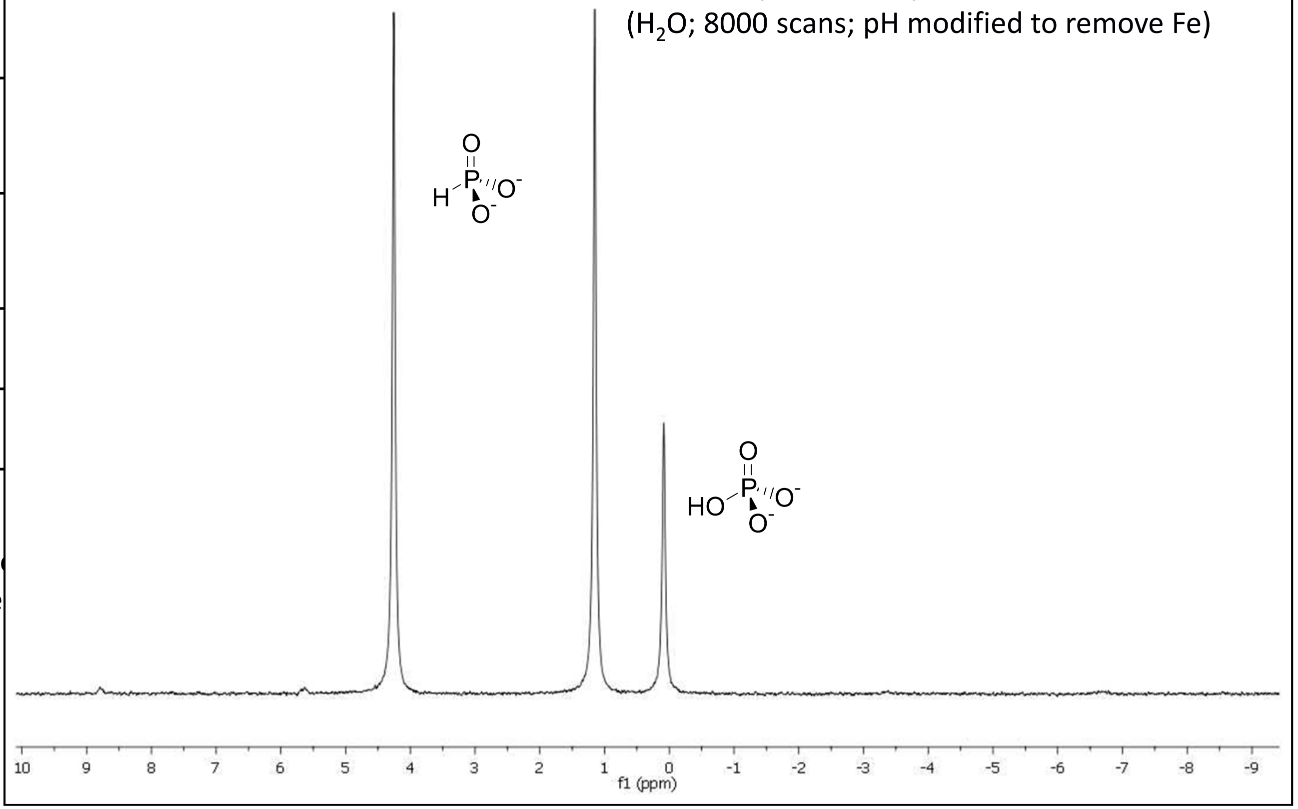
Sample	Fe	Ni	P	Ca	Mg	S	F	Cl	pH	T (°C)
Blank	-0.07	-0.04	**-0.04**	-2.51	-0.50	0.10	0.28	0.92	-	-
Blank	-0.07	-0.04	**-0.05**							
Blank	-0.07	-0.04	**-0.02**							
Sikhote Alin 1	7.82	0.02	**16.78**							
Sikhote Alin 2	0.35	0.67	**0.17**							
Sikhote Alin 4	68.01	7.52	**-0.01**							
Sikhote Alin 5	11.97	1.88	**0.06**							

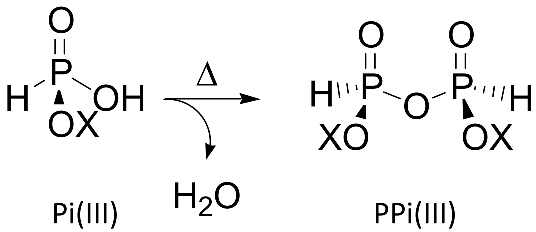
Dissolved cation (ICP-AES) and ani
on Sikhote Alin samples incubate



[31]P-NMR spectrum of post-incubation SA1 fluid (H_2O; 8000 scans; pH modified to remove Fe)

(D. E. Bryant et al, GCA, 2013, <u>109</u>, 90-112)

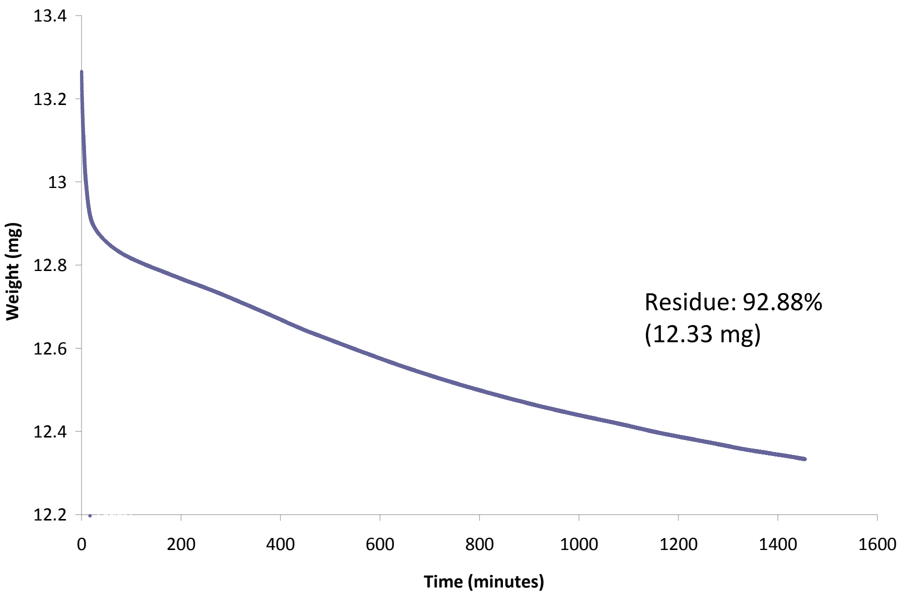
2. *Prebiotically plausible P-based* energy currency *molecules*

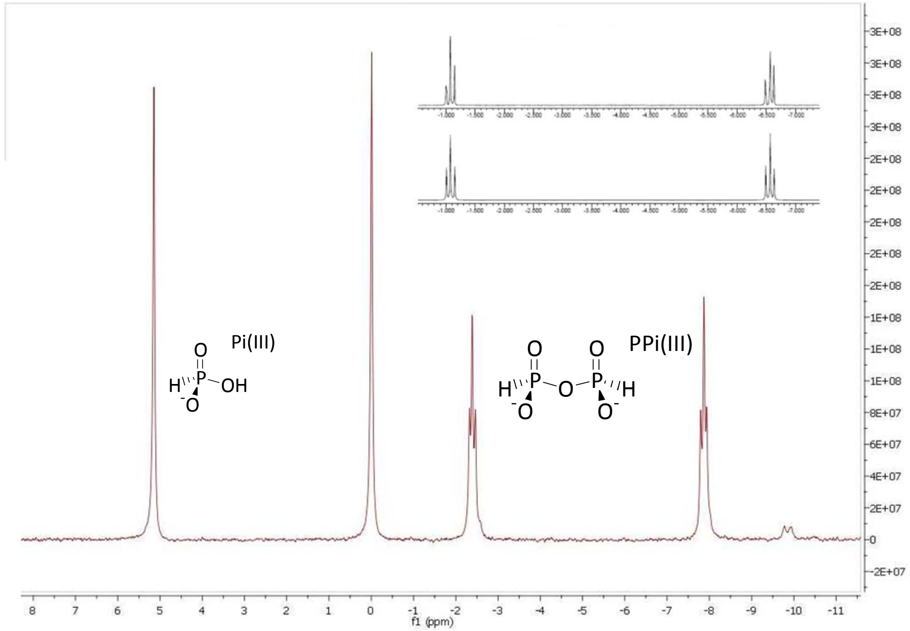


X = Na^{+}, Mg^{2+}, Ca^{2+}, Zn^{2+}

AA'XX' simulation (gNMR):
δH 6.745; δP -3.818; $^{1}J_{PH}$ 665.88; 0.65; $^{2}J_{PP}$ 17.33

Isothermal TGA for NaH_2PO_3 Pi(III) (**90ºC**; N_2 gas flow 60 mLmin^{-1})





^{31}P-NMR (H_2O; pH *ca*. 4) post-TGA. *ca*. 53:47 Pi(III):PPi(III) ratio

2. *Prebiotically plausible P-based* energy currency *molecules*

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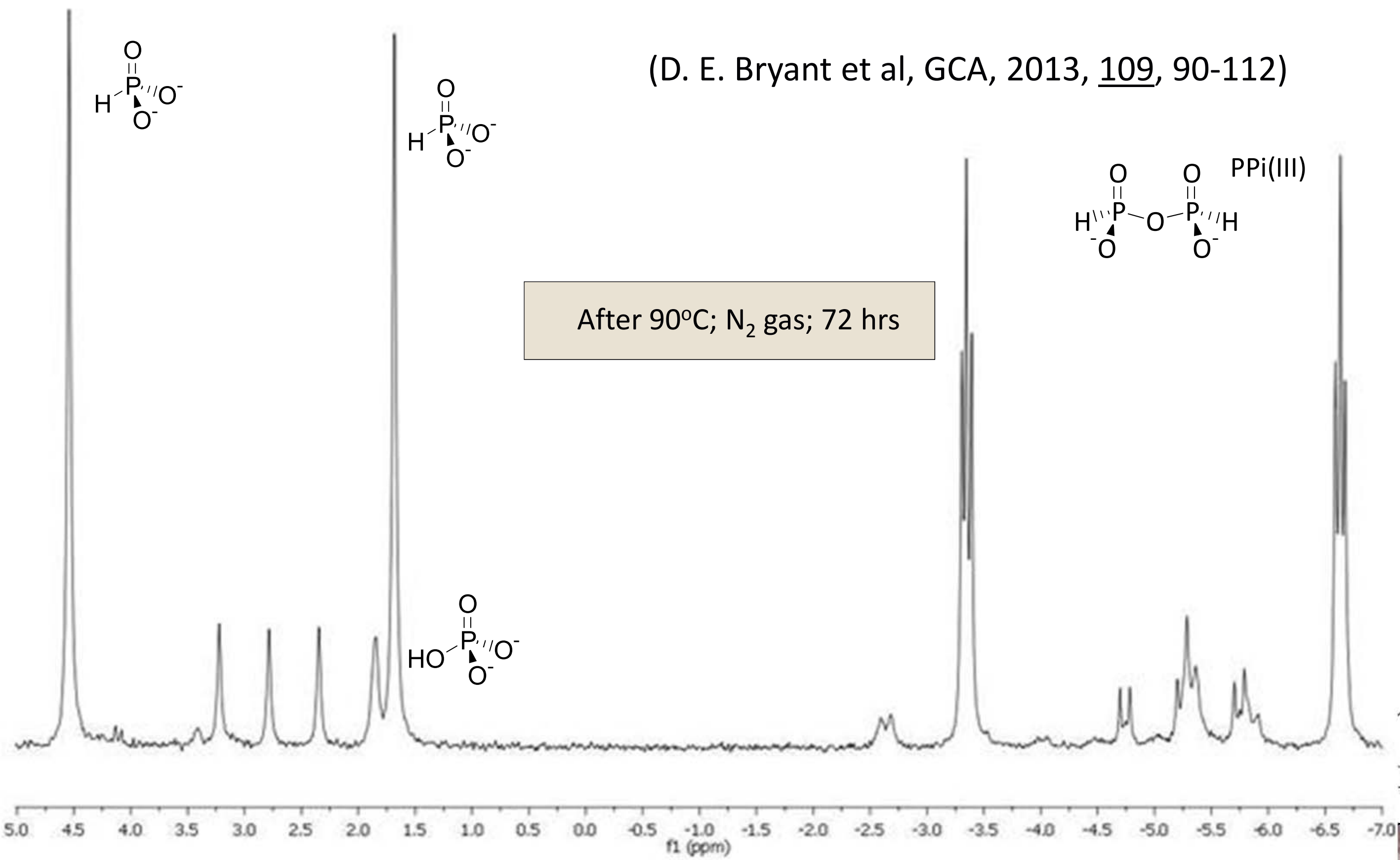
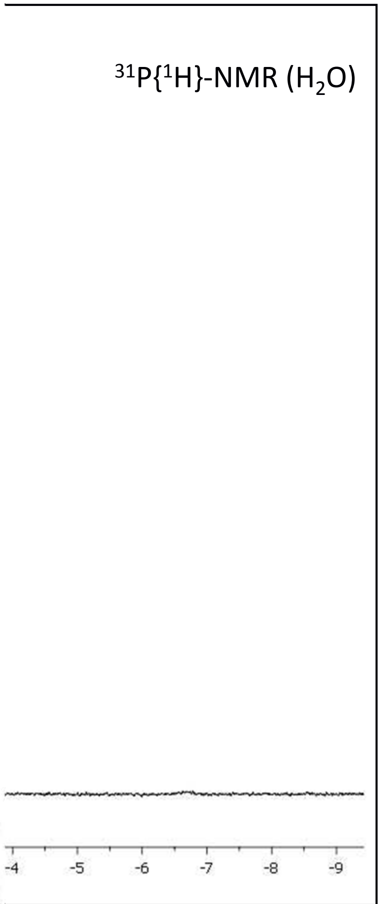
$^{31}P\{^1H\}$-NMR (D_2O)

(D. E. Bryant et al, GCA, 2013, <u>109</u>, 90-112)

After 90°C; N_2 gas; 72 hrs





(3 weeks ambient)

2. *Prebiotically plausible P-based* energy currency *molecules*

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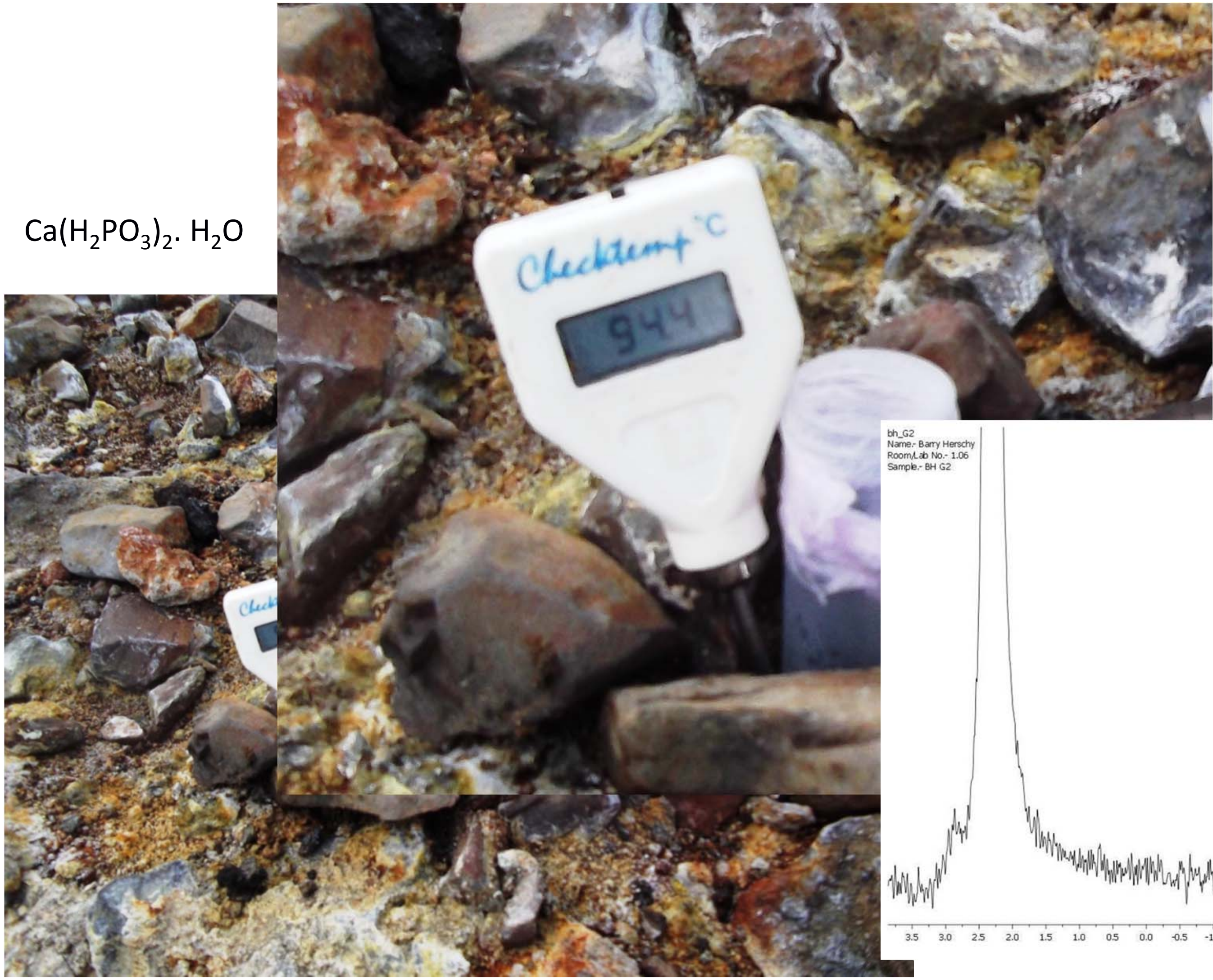
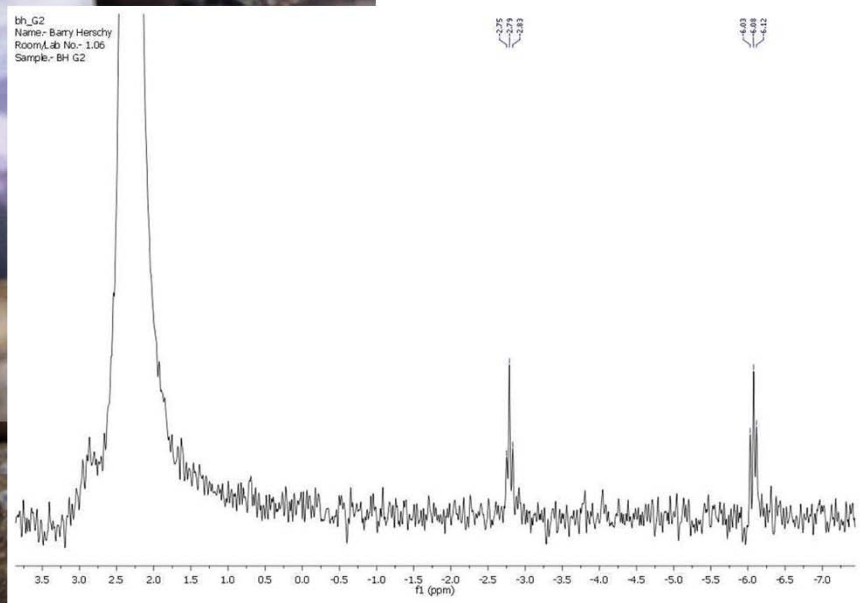
$Ca(H_2PO_3)_2 . H_2O$





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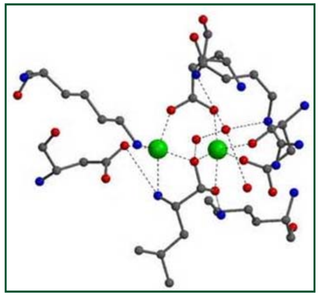
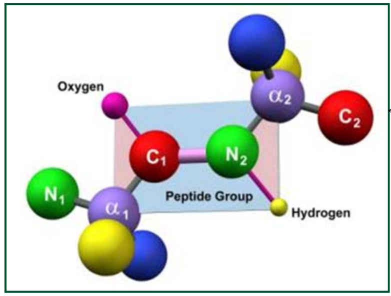
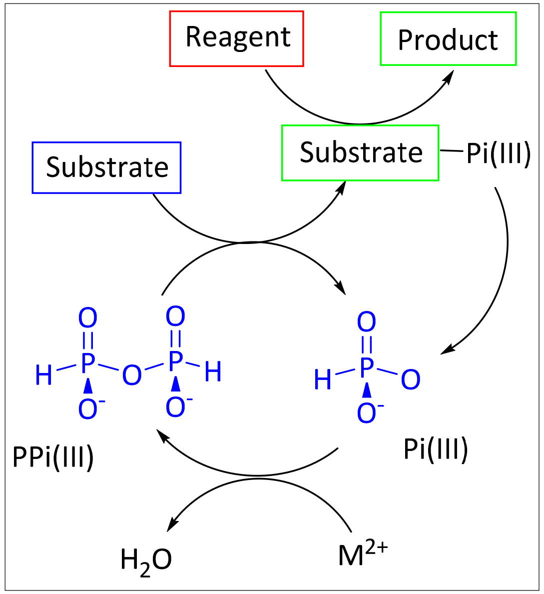
3. *En route* to coupled chemical reactions

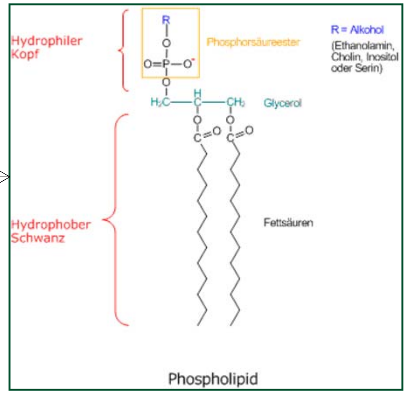
3. En route *to coupled chemical reactions*

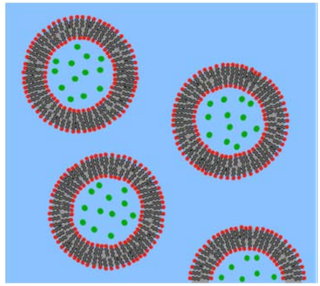


Function
(catalysis)

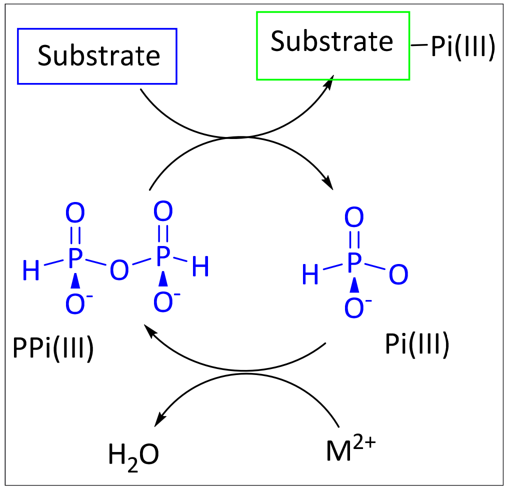








Function
(self-assembly)



3. En route *to coupled chemical reactions*

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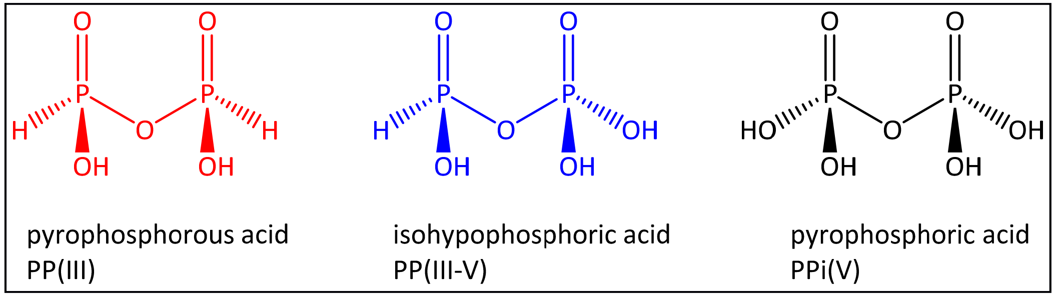

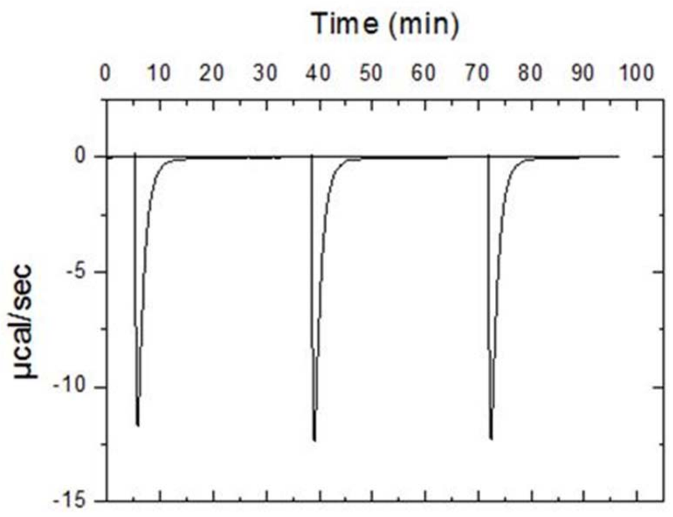

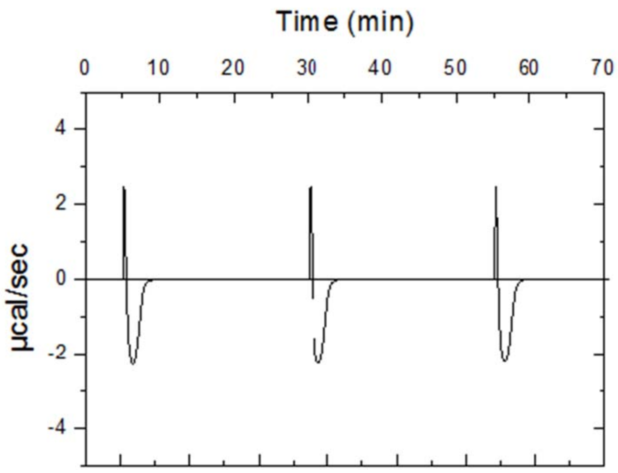

10.05mM aq. PPi(III) at pH 7, 1M ionic strength added to cell; pH 13, 1M ionic strength.

Exotherm of **-113 kJmol^{-1}** at pH 13

10mM aq. PPi(V) added to cell; 3mM $MgCl_2$, 20mM HEPES buffer pH 8.4, 0.1M ionic strength & 1x10^{-6} mM *E.coli* pyrophosphatase.

Exotherm of **-31 kJmol^{-1}** at pH 8.4 (enzyme-induced)

3. En route *to coupled chemical reactions - peptides*

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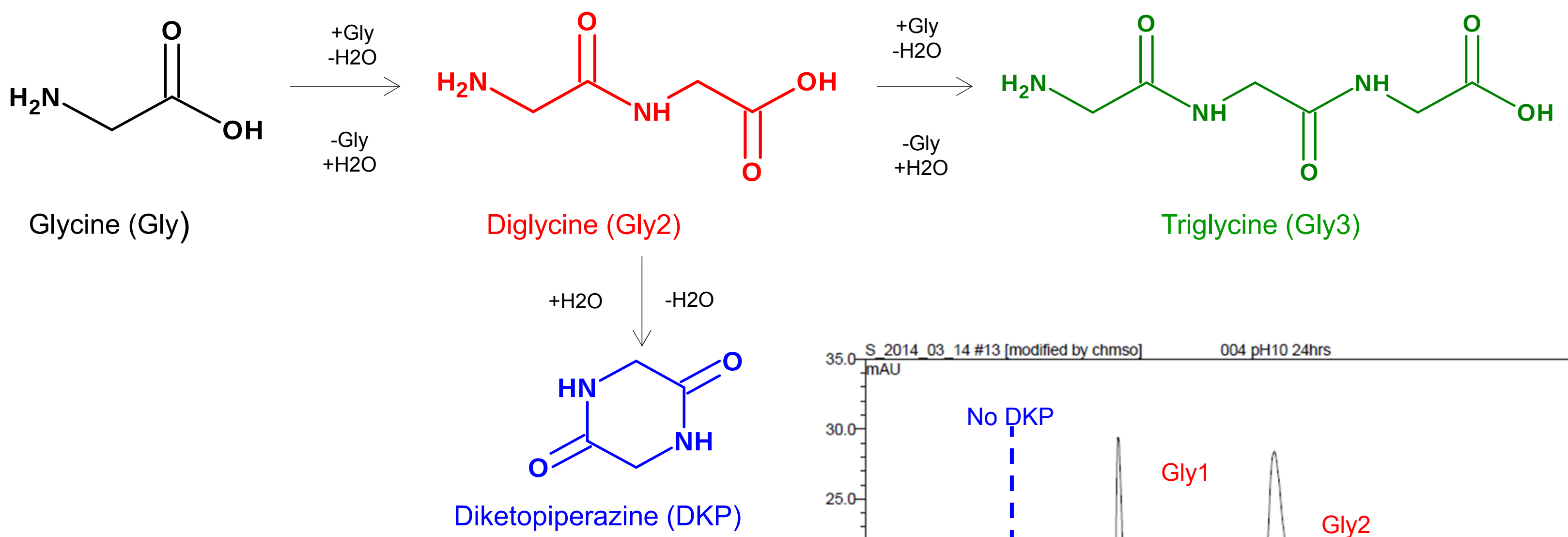
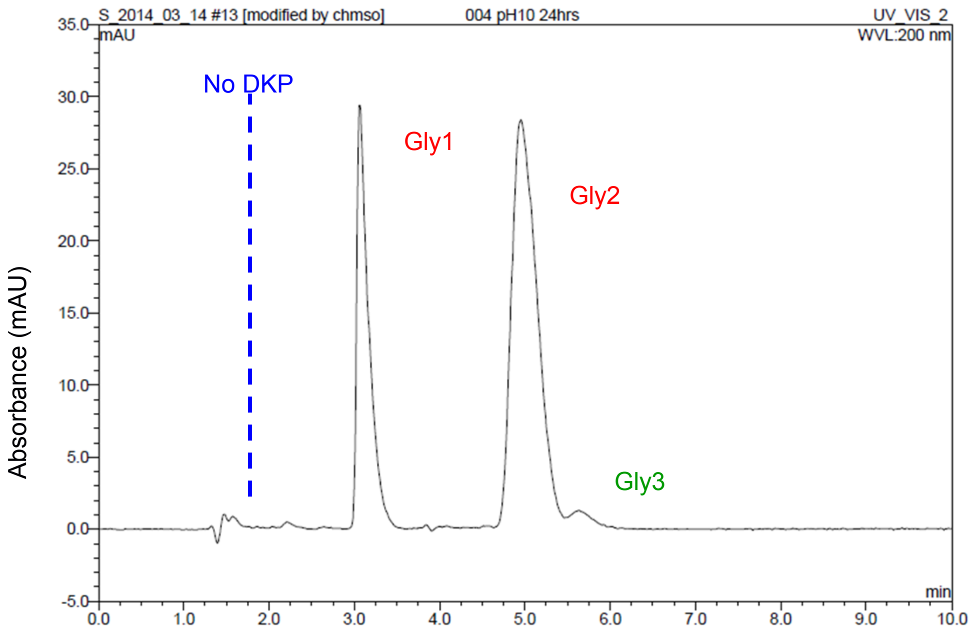
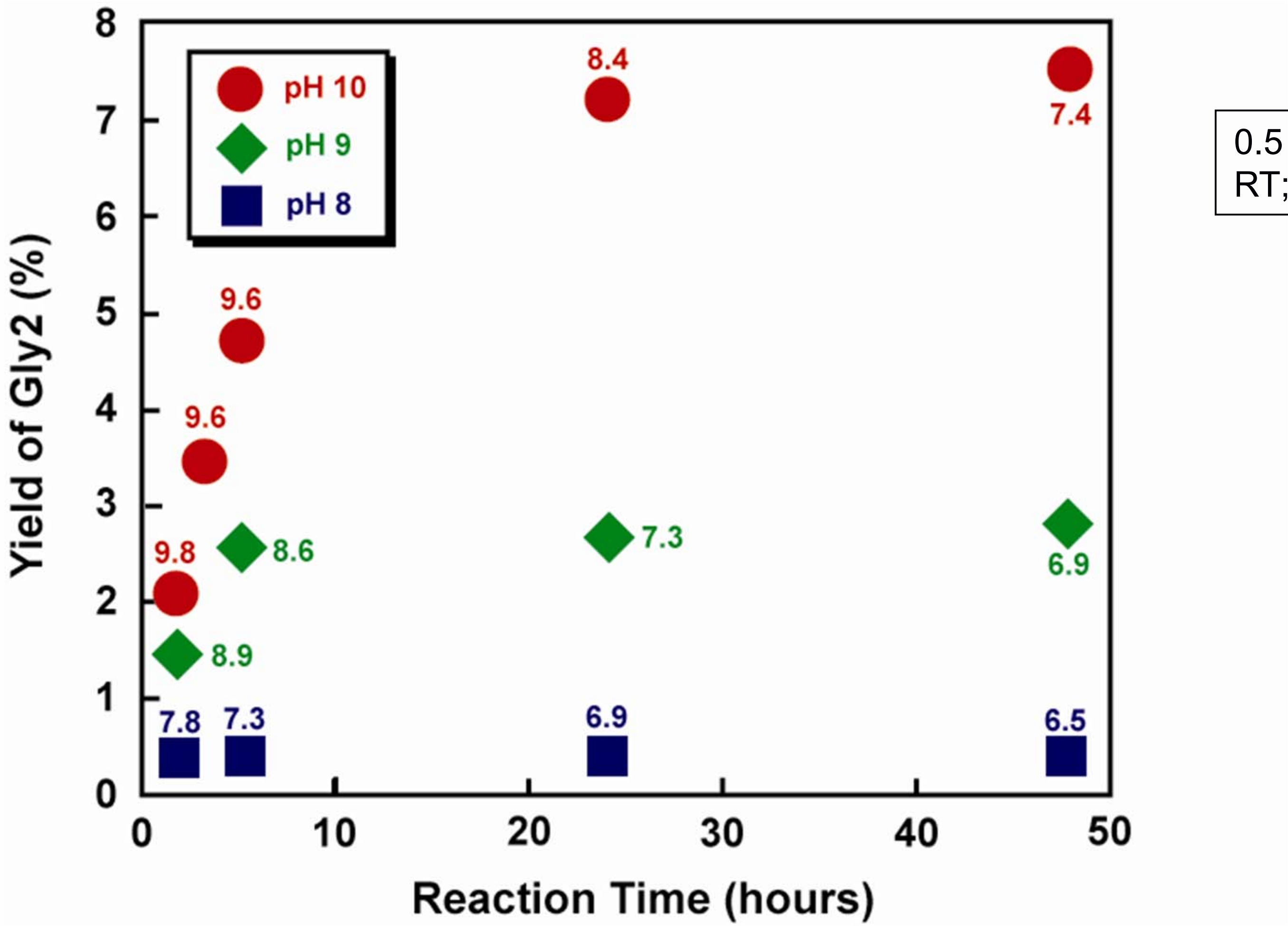




HPLC chromatogram: 0.5 M Gly &1.0 M PPi(III); RT; pH 10; 24 h



0.5 M Gly &1.0 M PPi(III); RT; pH 10; 24 h

3. En route *to coupled chemical reactions - peptides*

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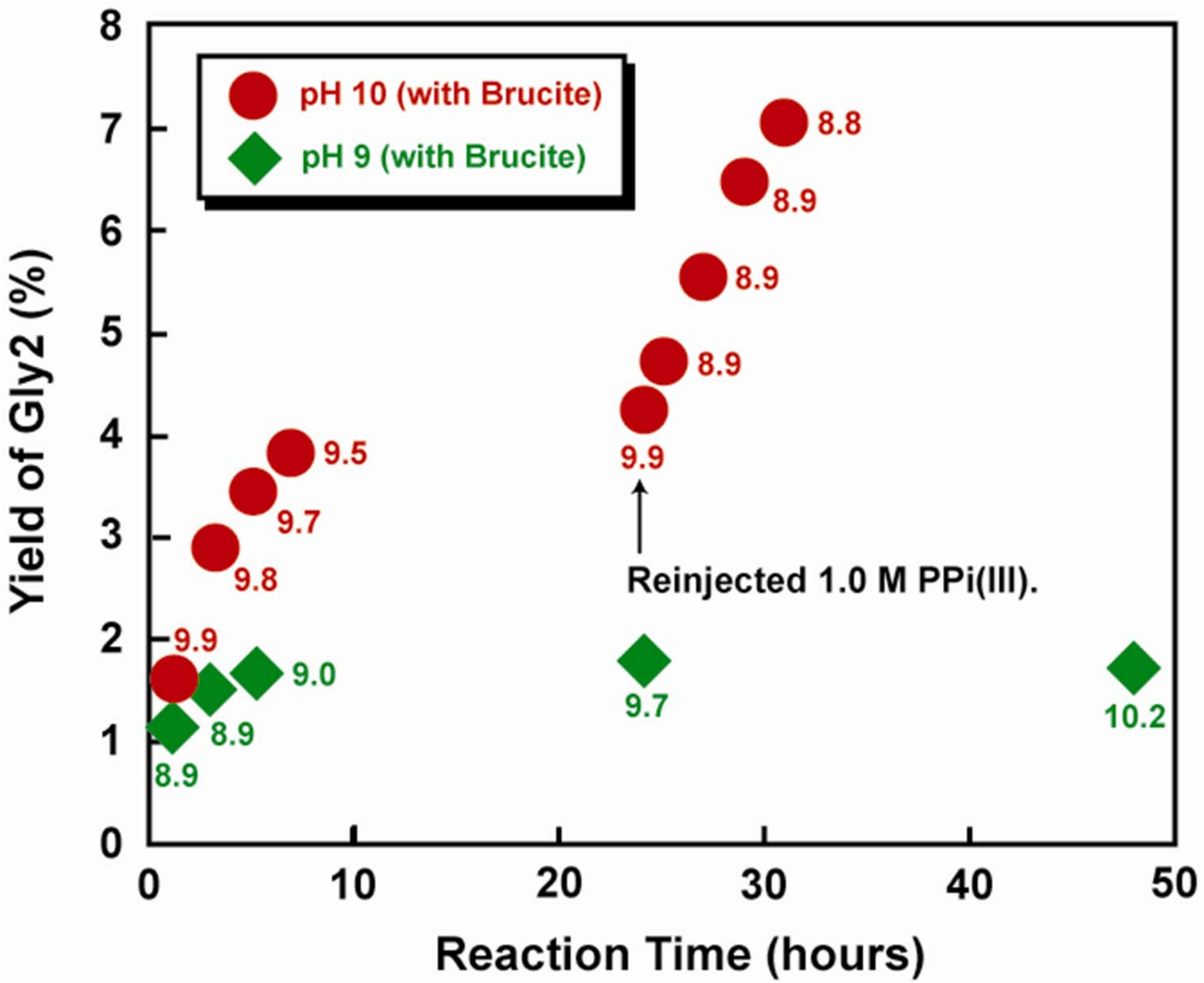
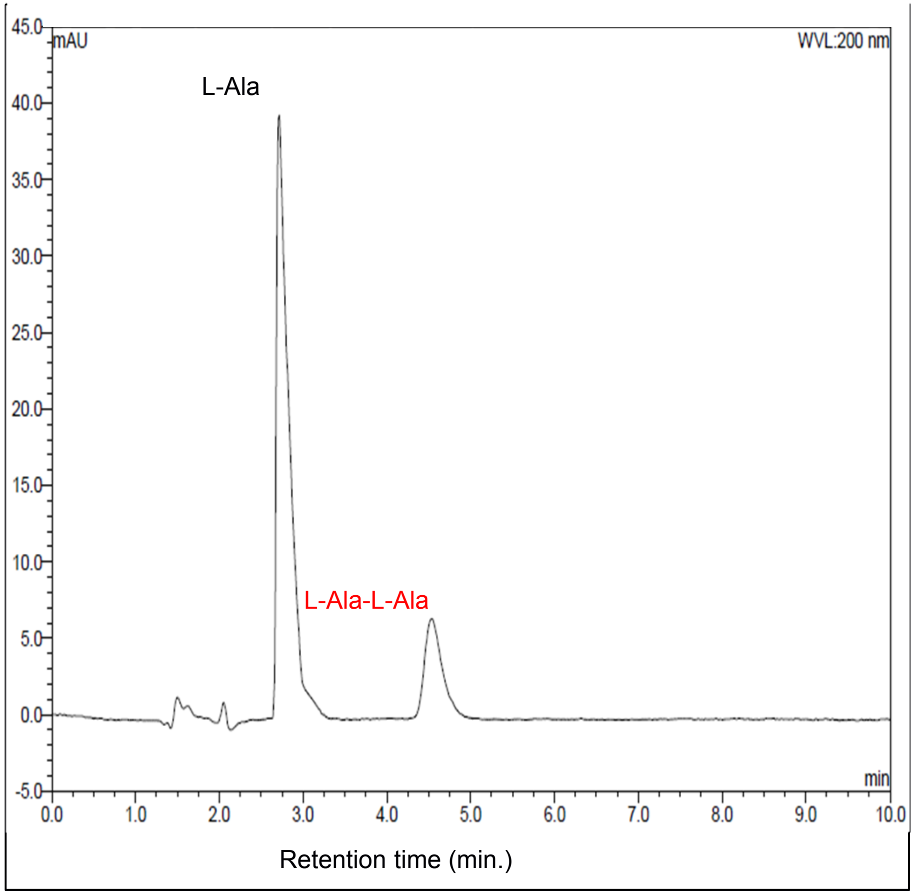
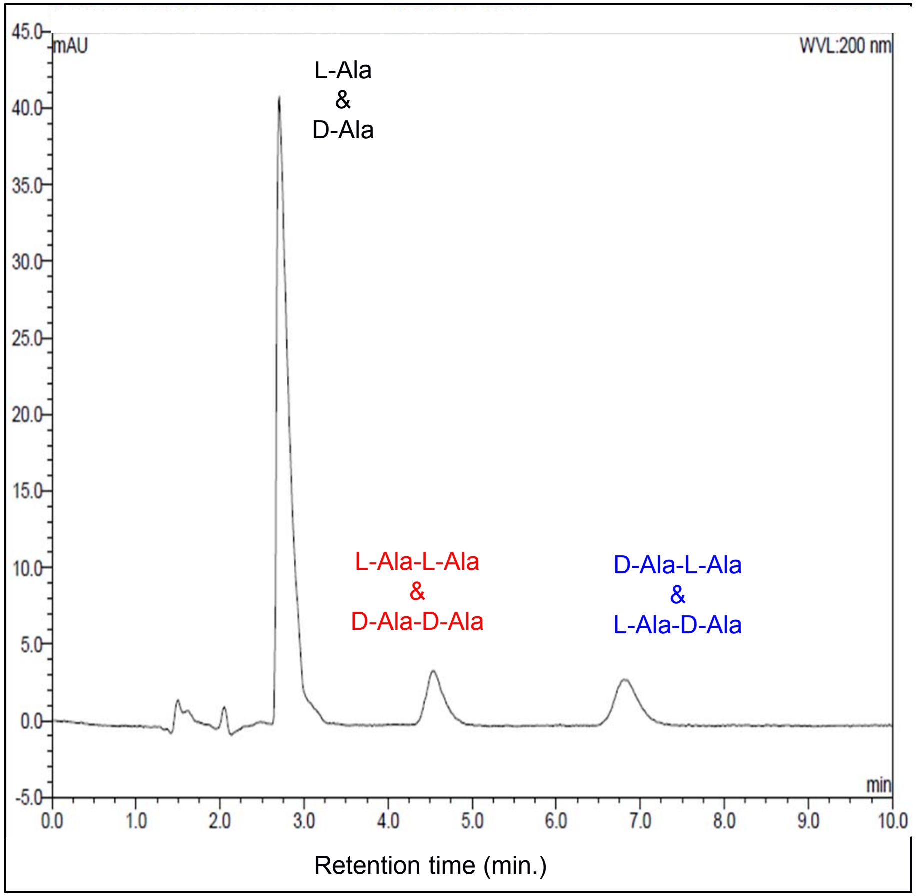


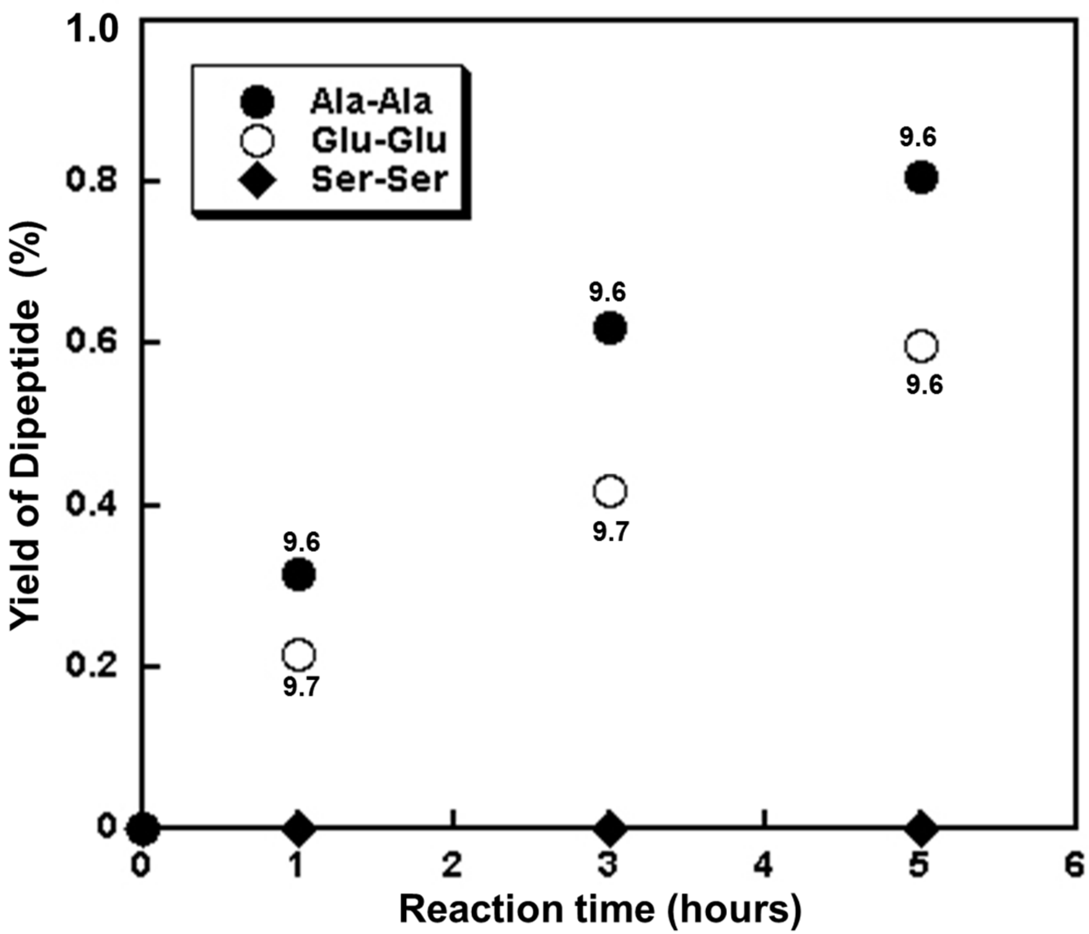
0.5 M Gly &1.0 M PPi(III); RT; pH 10; 24 h

Buffered by brucite [$Mg(OH)_2$]

HPLC chromatograms of the solutions of 1.0 M PPi(III) and:
(A) 0.5 M L-Ala
(B) 0.5 M DL-Ala
reacted at RT and pH 10 for 24 hours







Dipeptides from 1.0 M PPi(III) with:

(A) 0.5 M L-Alanine,
(B) 0.5 M L-Glutamate
(C) 0.5 M L-Serine

reacted at RT; pH 10; 1-5 h

3. En route *to coupled chemical reactions - peptides*

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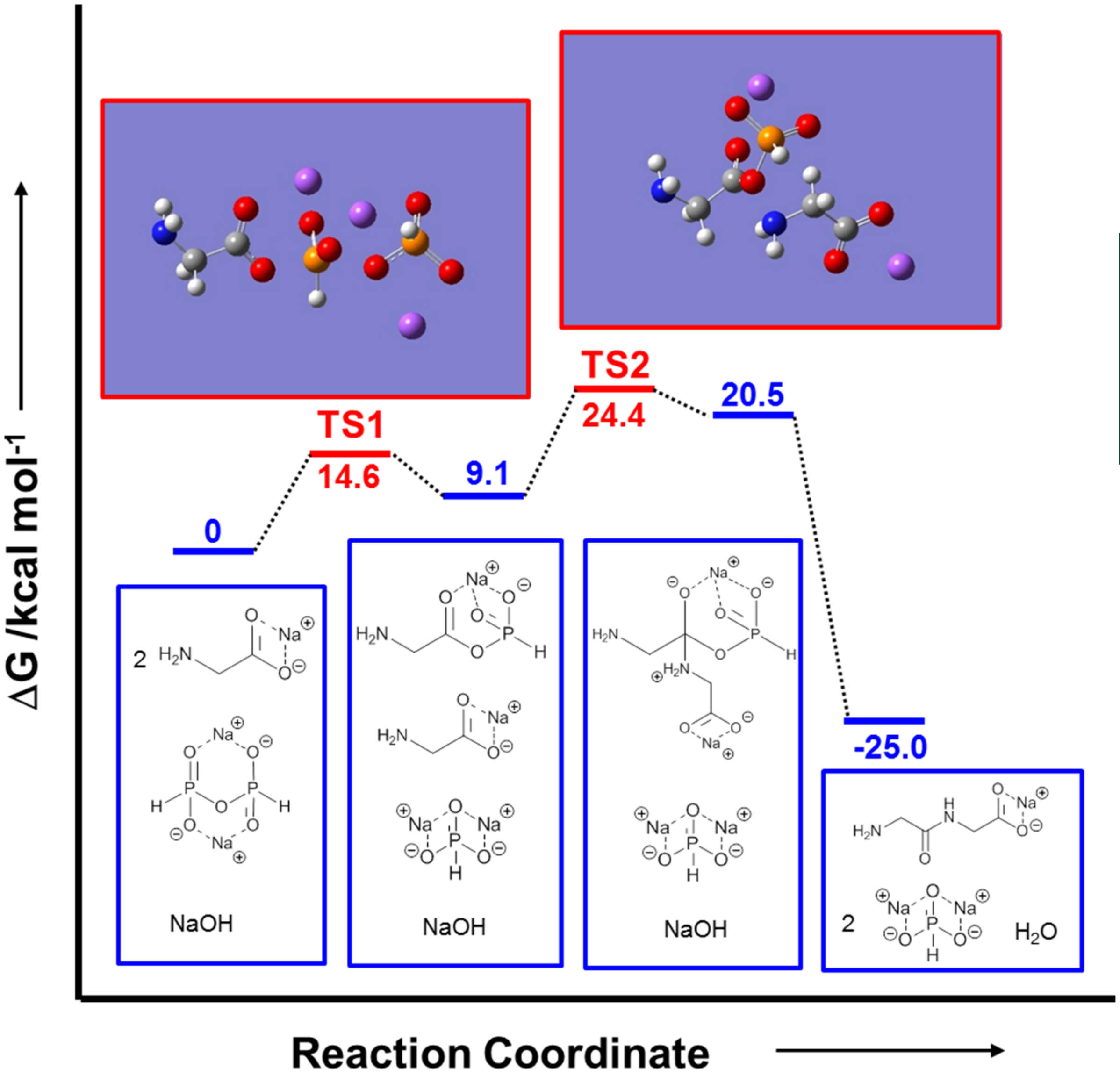
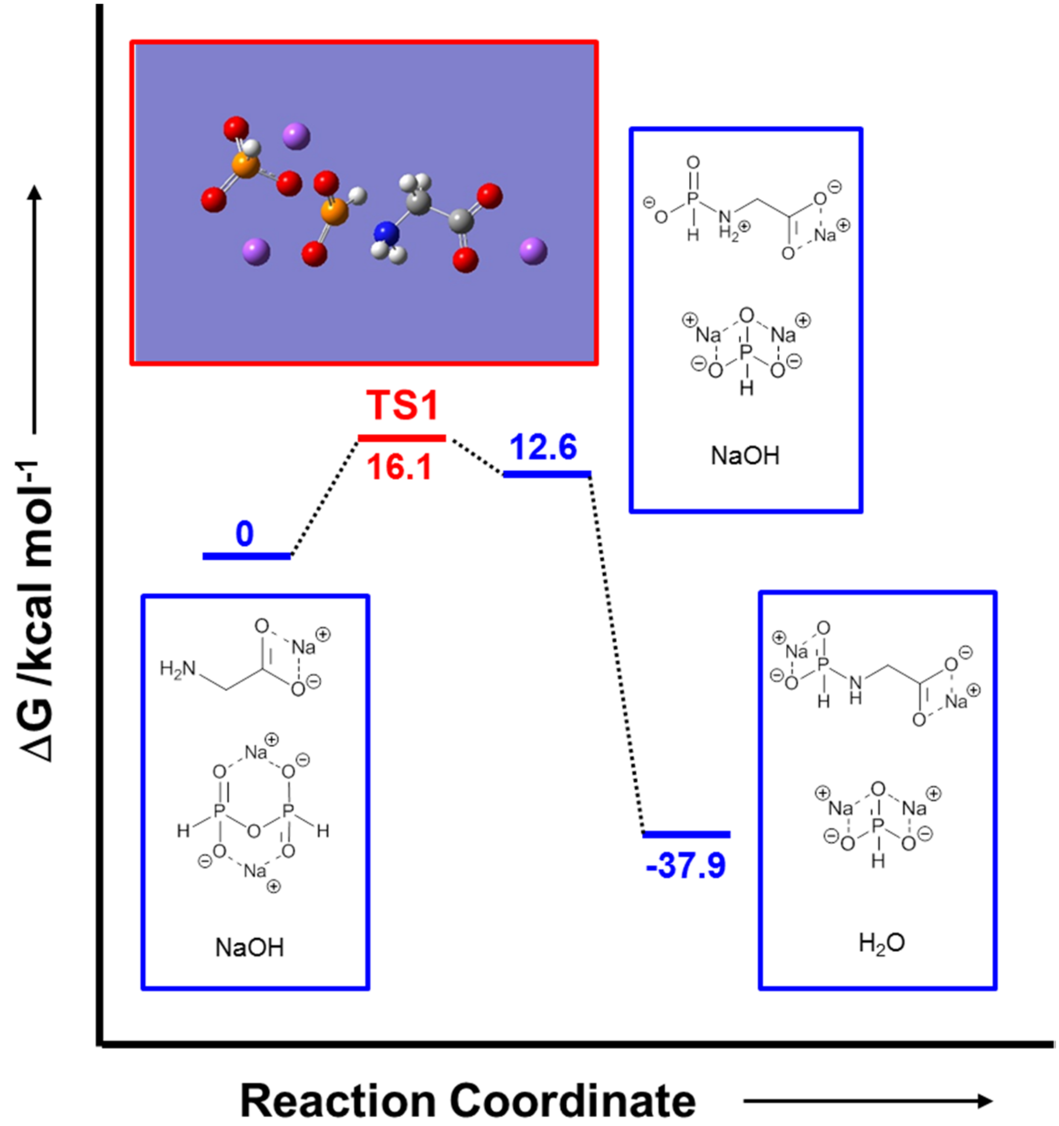


DFT calculations M06-2X df 6-311++G(d,p) basis set.

Solvation modelled via (SMD) polarized continuum method



DFT calculations M06-2X df 6-311++G(d,p) basis set.

Solvation modelled via (SMD) polarized continuum method

3. En route *to coupled chemical reactions - peptides*

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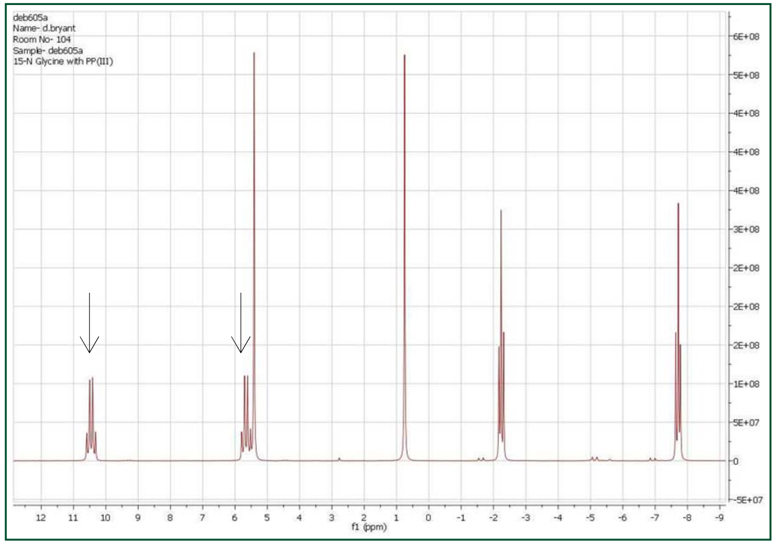
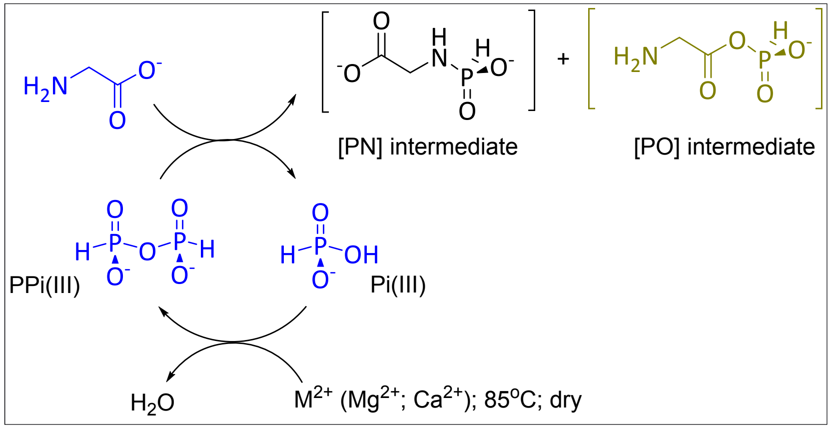
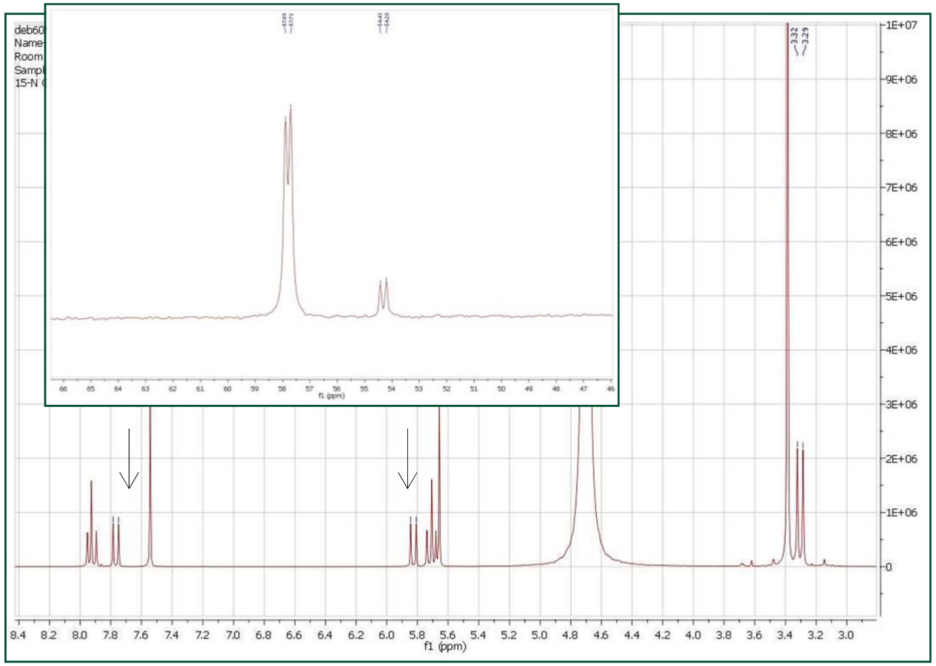


^{15}N-glycine (1.0M); PP(III) (0.5M) pH 10.8-11.0.
^{31}PNMR: δ 8.1ppm, dq, $^{1}J_{PH}$ 580Hz, ^{3}J 11Hz ($^{1}J_{PN}$ = $^{3}J_{PH}$ = 11Hz; 23% total P
^{1}H-NMR: δ 6.77ppm, dd, 1H $^{1}J_{PH}$ 580Hz, $^{2}J_{NH}$ = 11Hz; δ3.3ppm, 2H, $^{2}J_{NH}$ = 11Hz ($^{1}J_{PN}$ = $^{3}J_{PH}$ = $^{2}J_{NH}$ = 11Hz)
^{15}N-NMR: δ54.4ppm, d, $^{1}J_{PN}$ = 11Hz, δ 57.8ppm, d, $^{1}J_{PN}$ = 10Hz





3. En route *to coupled chemical reactions*

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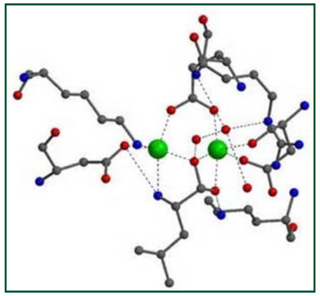
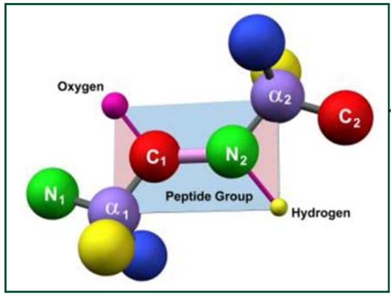
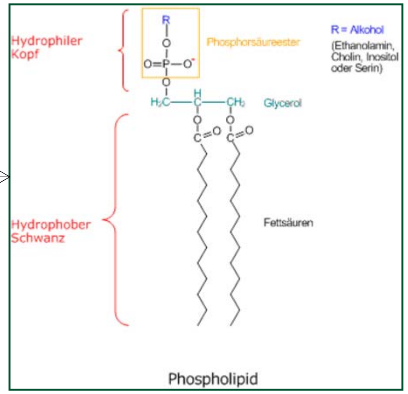
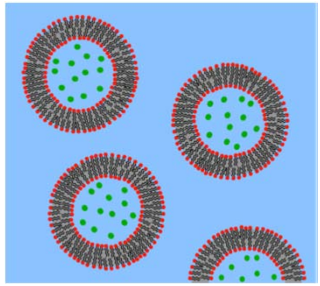


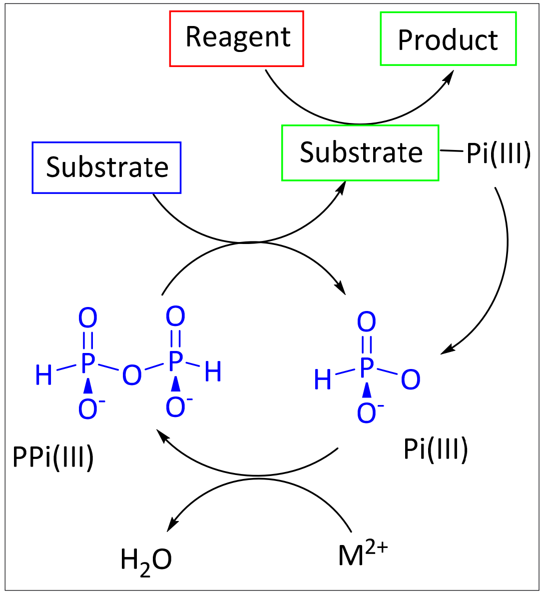
Function
(catalysis)

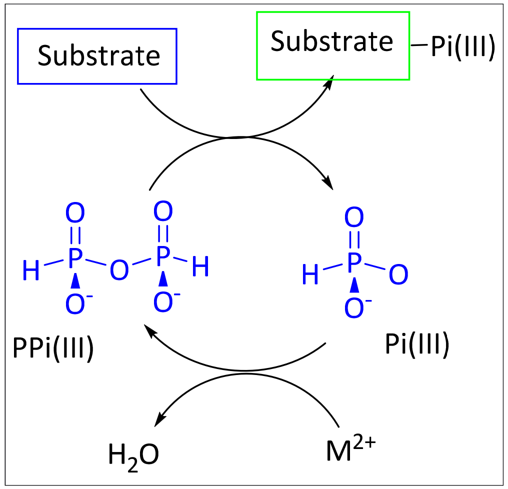




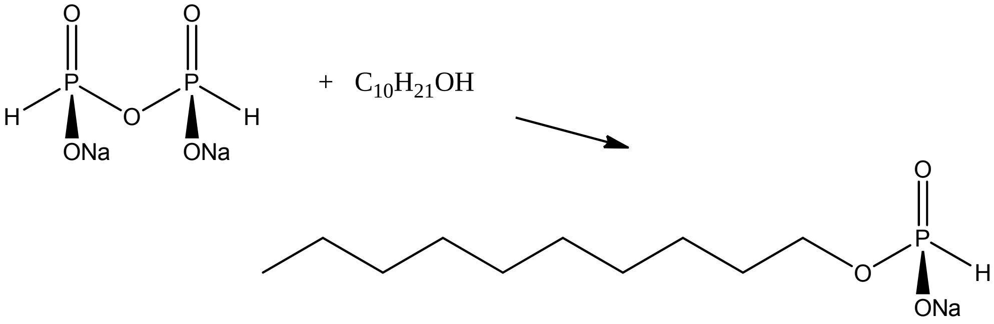


Function
(self-assembly)

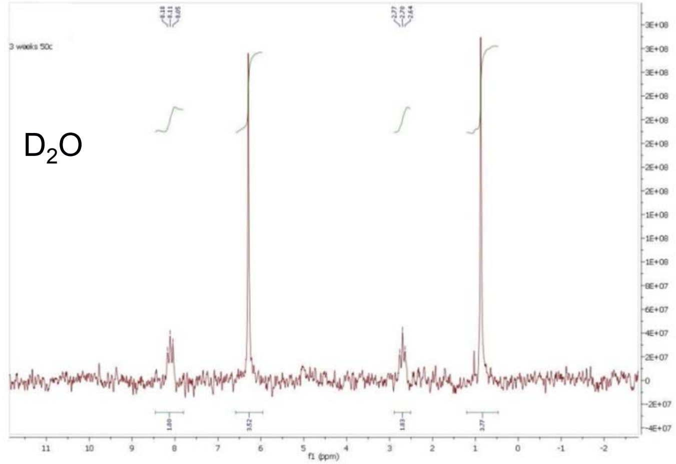




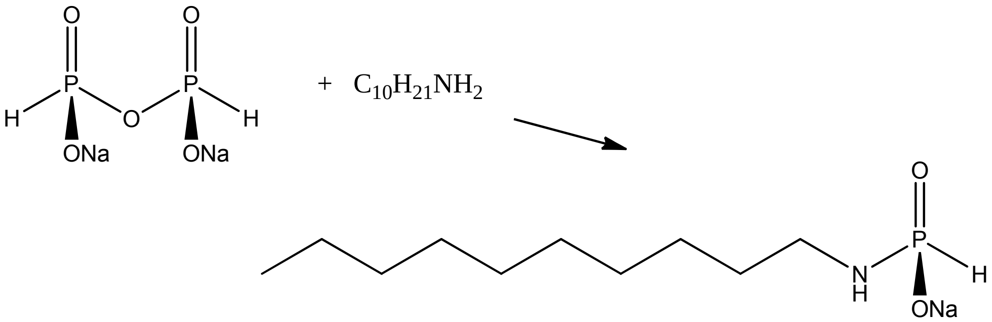
3. En route *to coupled chemical reactions - compartments*

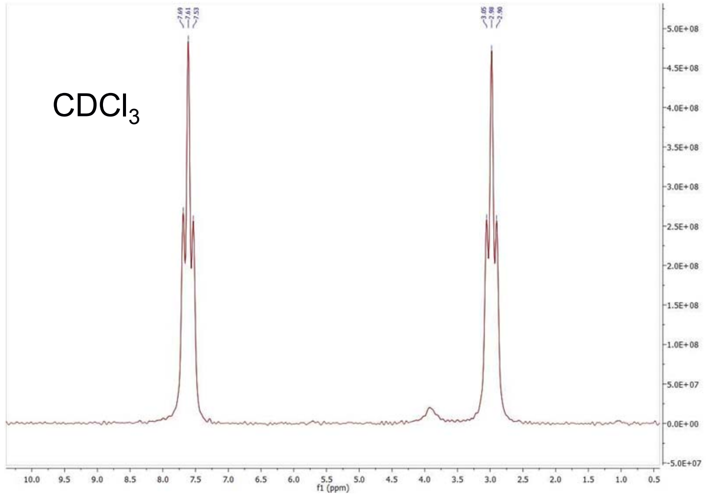


3 weeks at 50°C. Slow



^{31}P NMR

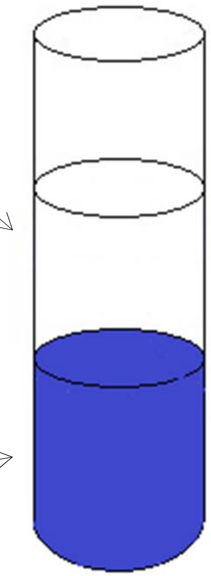




Amines are better nucleophiles.
Faster reaction, minutes at ambient

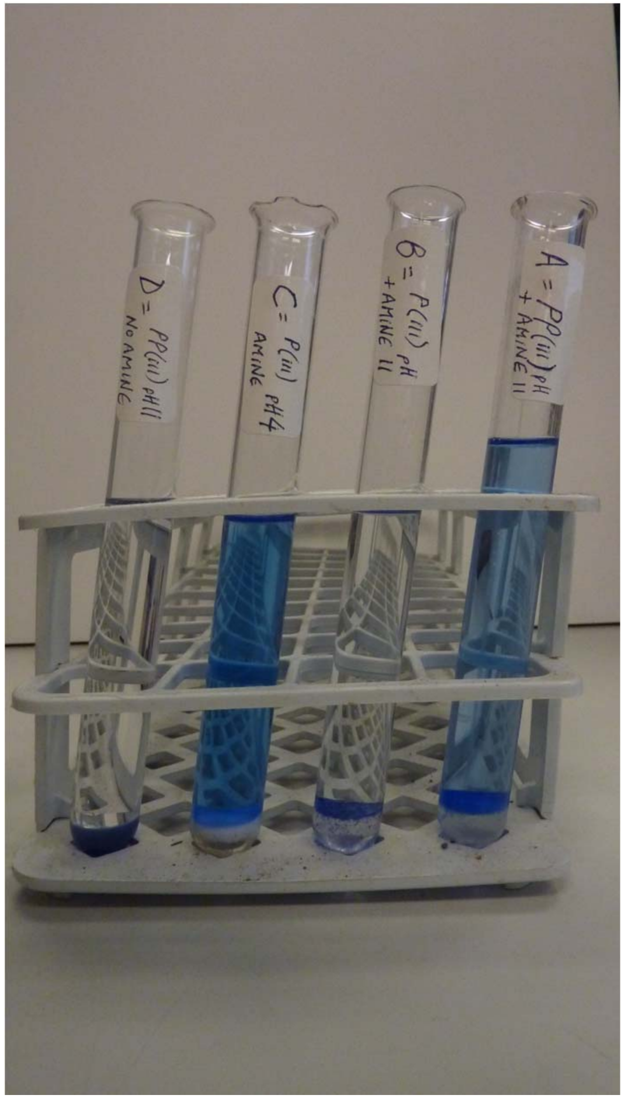
3. En route *to coupled chemical reactions - compartments*

Organic layer (toluene) Containing amine



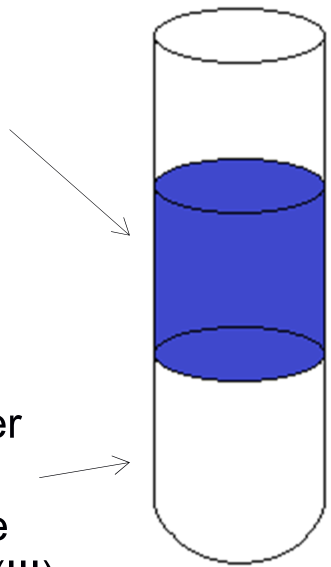
Aqueous layer containing Water soluble blue dye, PP(III) pH 11

Sample (Right, A) and blanks
B = P(III) pH11
C = P(III) pH4
D = PP(III) no amine



3. En route *to coupled chemical reactions - compartments*

Organic
layer
(toluene)
Containing
amine



Aqueous layer
containing
Water soluble
blue dye, PP(III)
pH 11

Sample (Right, A)
and blanks
B = P(III) pH11
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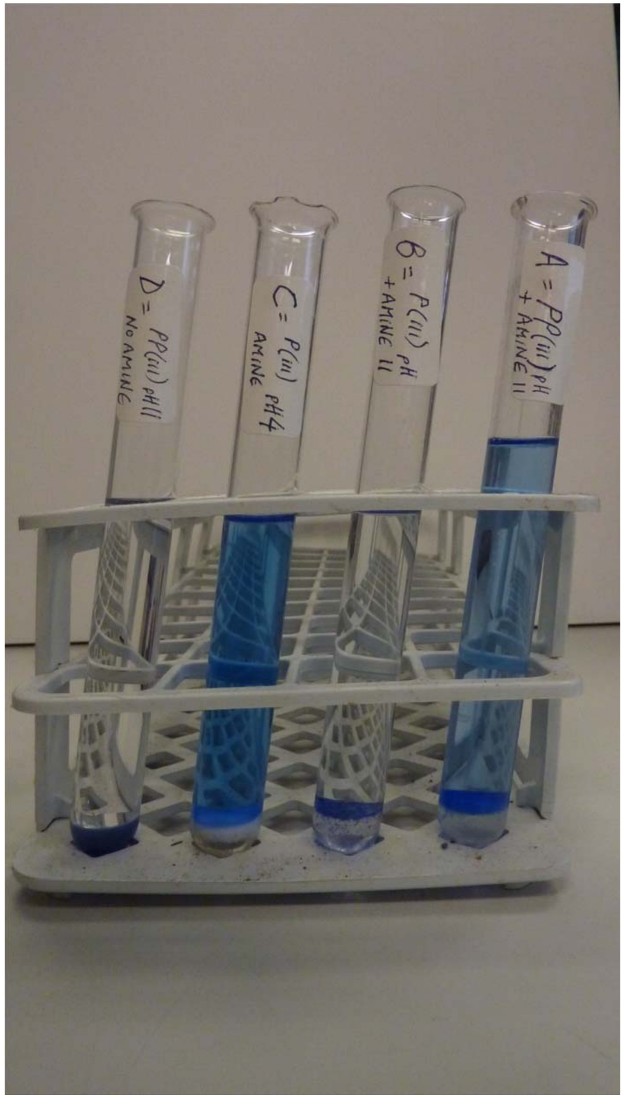
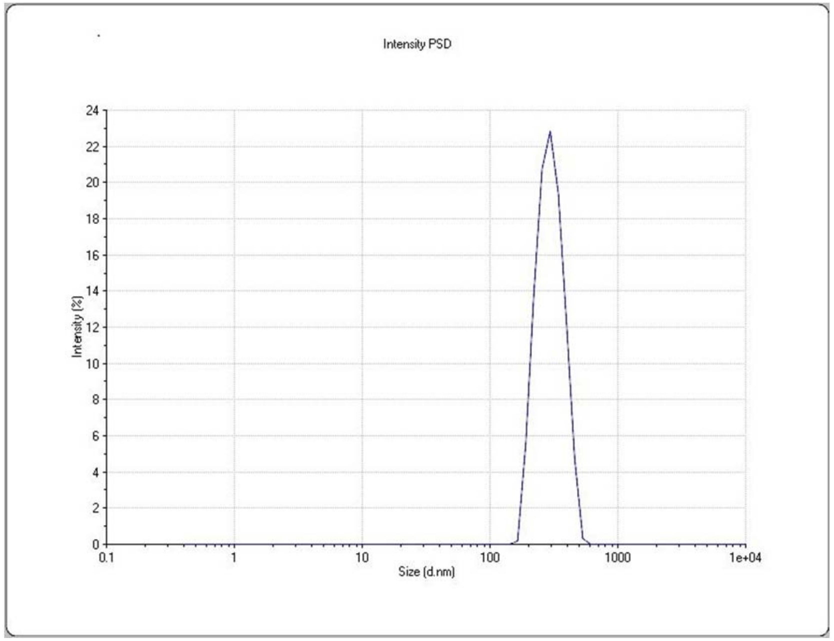
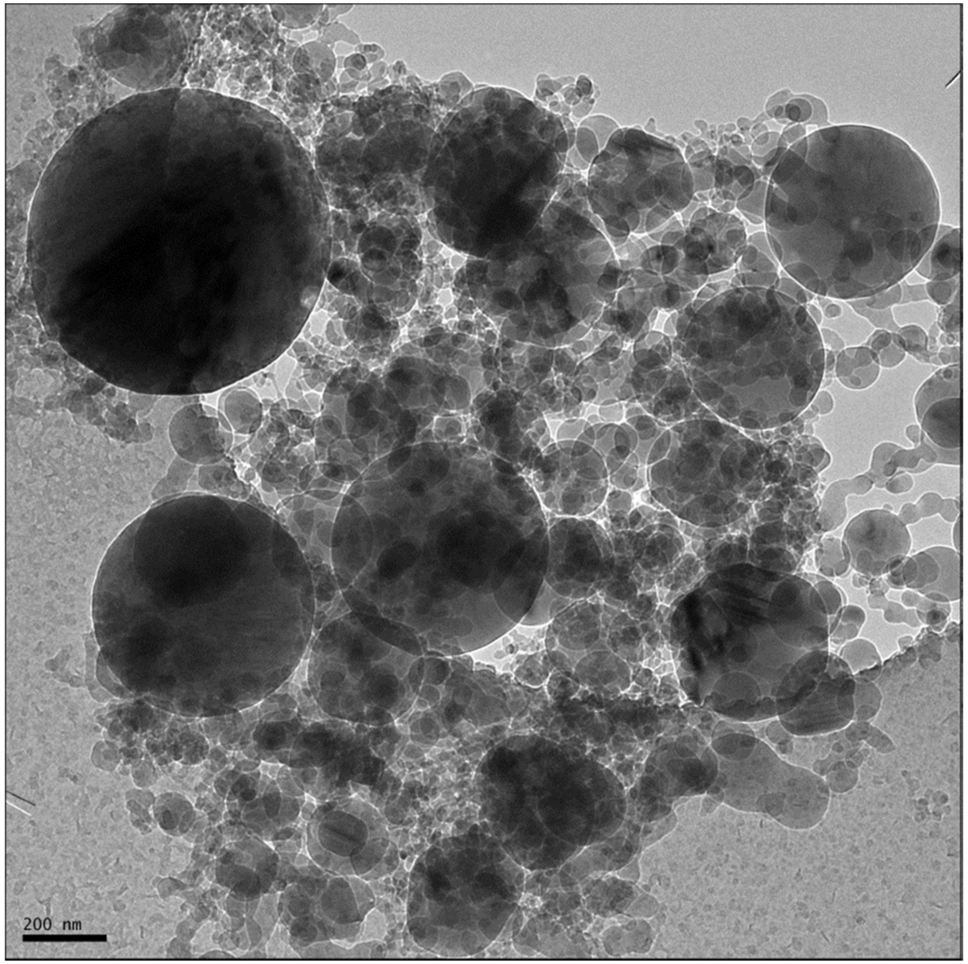


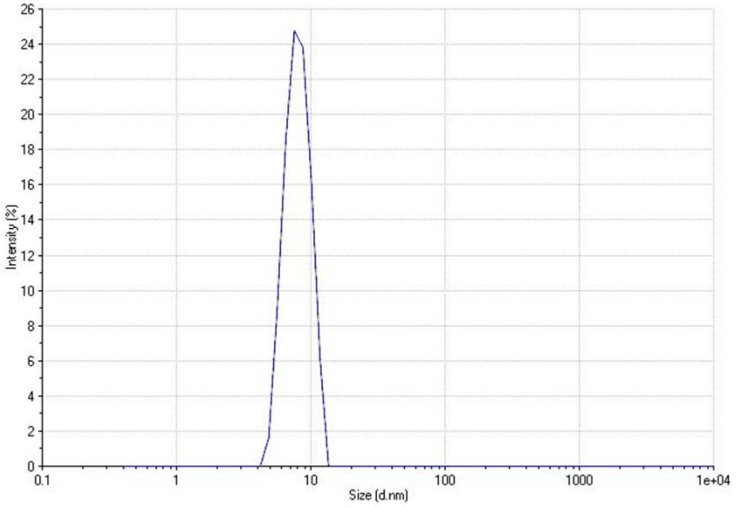


Dynamic light Scattering analysis
Showing population at ~300nm diameter

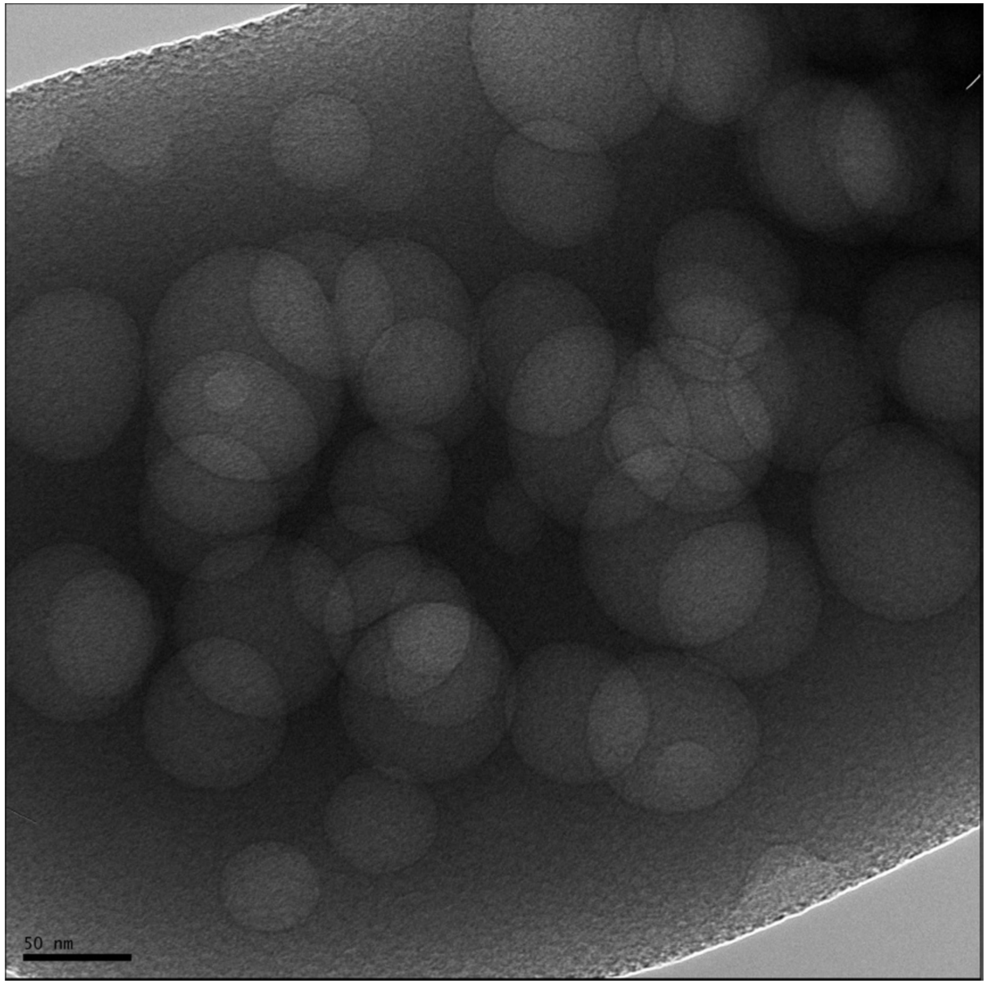


Long chain amine = decylamine ($C_{10}H_{21}NH_2$)

Cryo-TEM image of structures formed



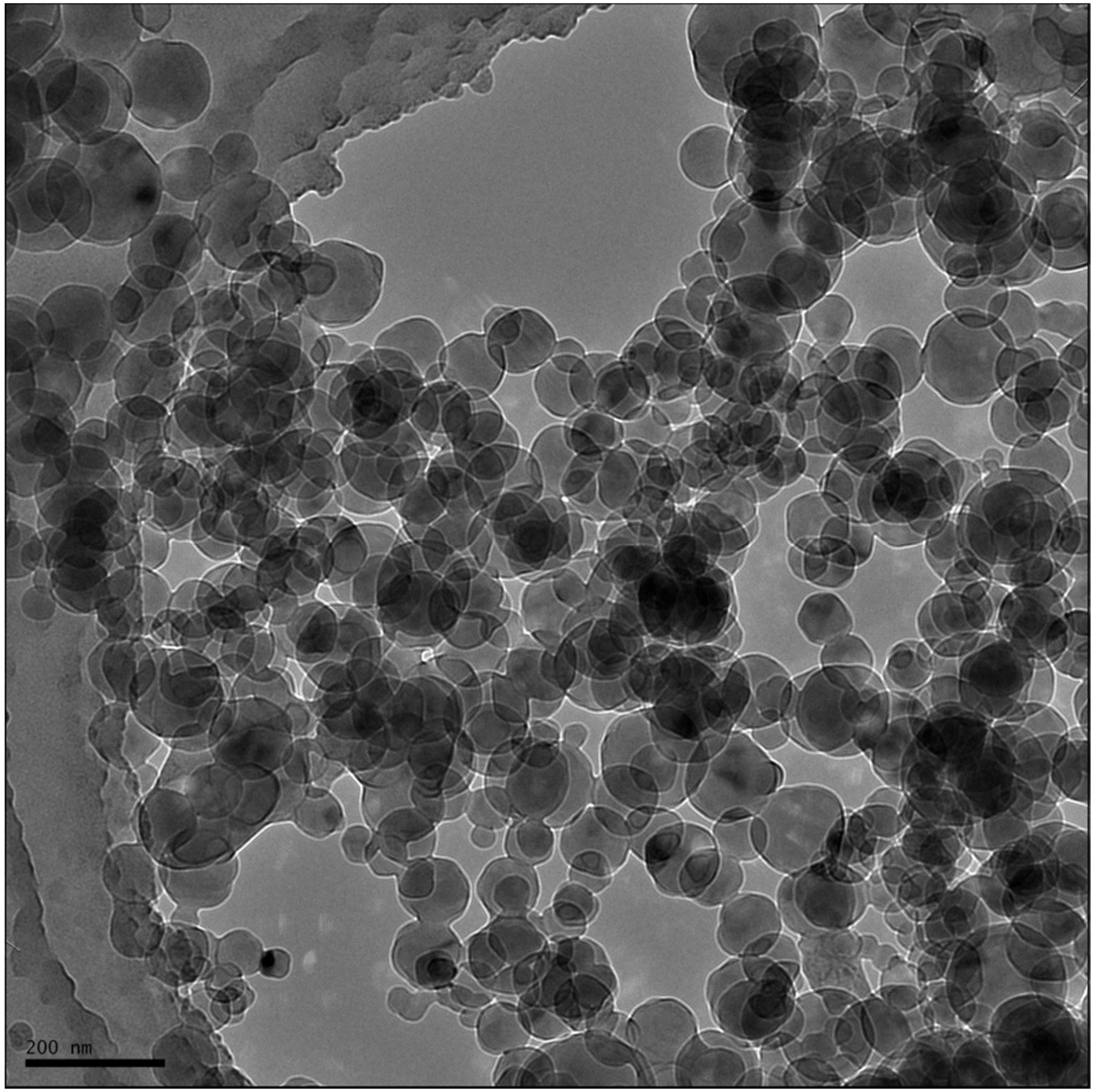
Dynamic light Scattering analysis
Showing population at ~10nm diameter



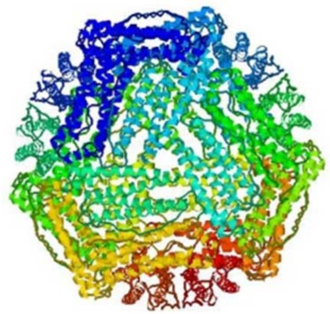
Cryo-TEM image of structures formed

Long chain amine = oleylamine ($C_{18}H_{34}NH_2$)

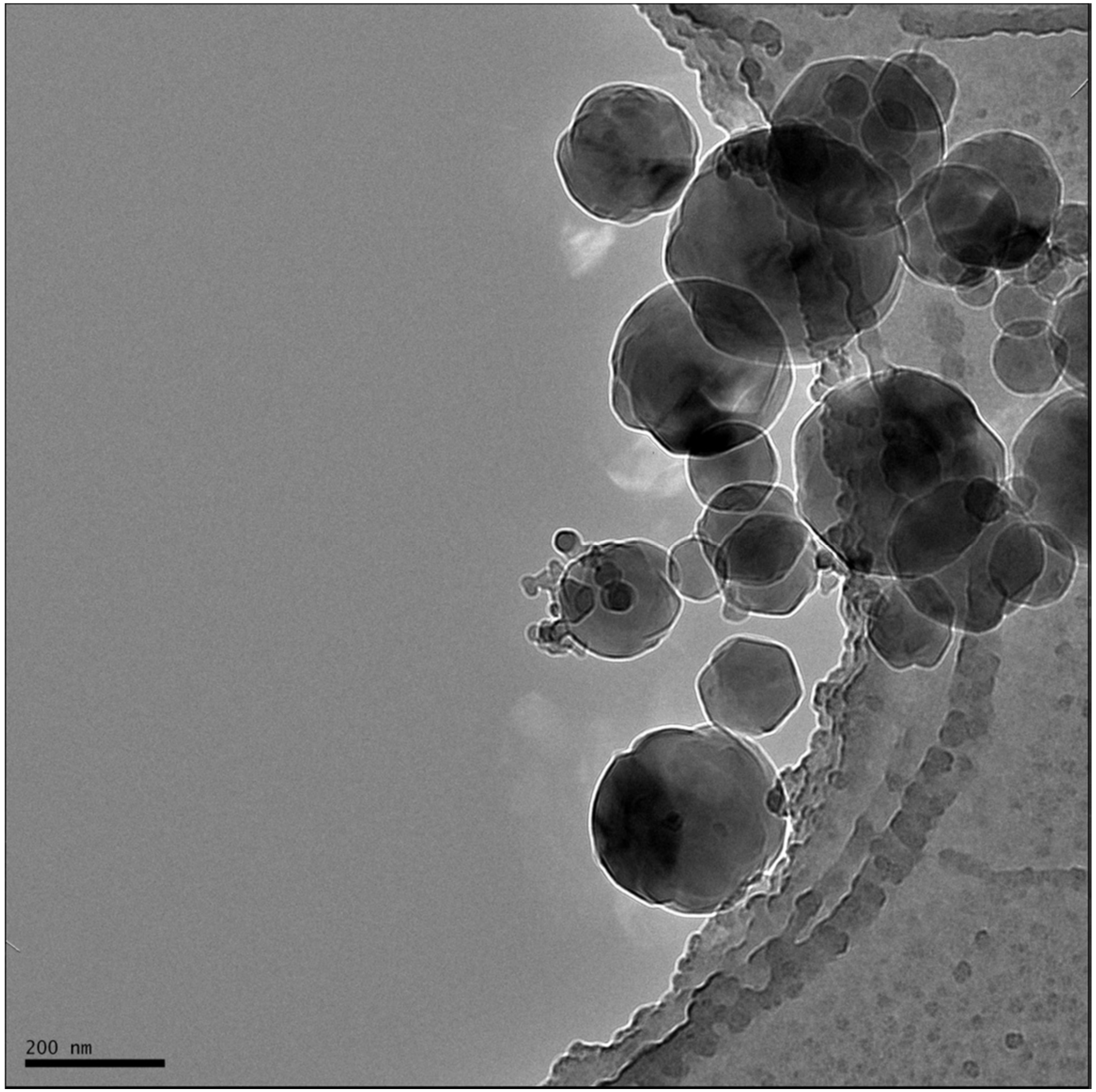
3. En route *to coupled chemical reactions - compartments*



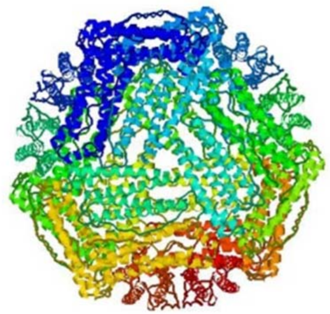
Ferritin: iron containing protein



Water soluble contrast agent shows internal spaces are water



Ferritin: iron containing protein



Water soluble contrast agent
(av. Diam. 12 nm)
shows internal spaces contain
water

3. En route *to coupled chemical reactions - peptides*

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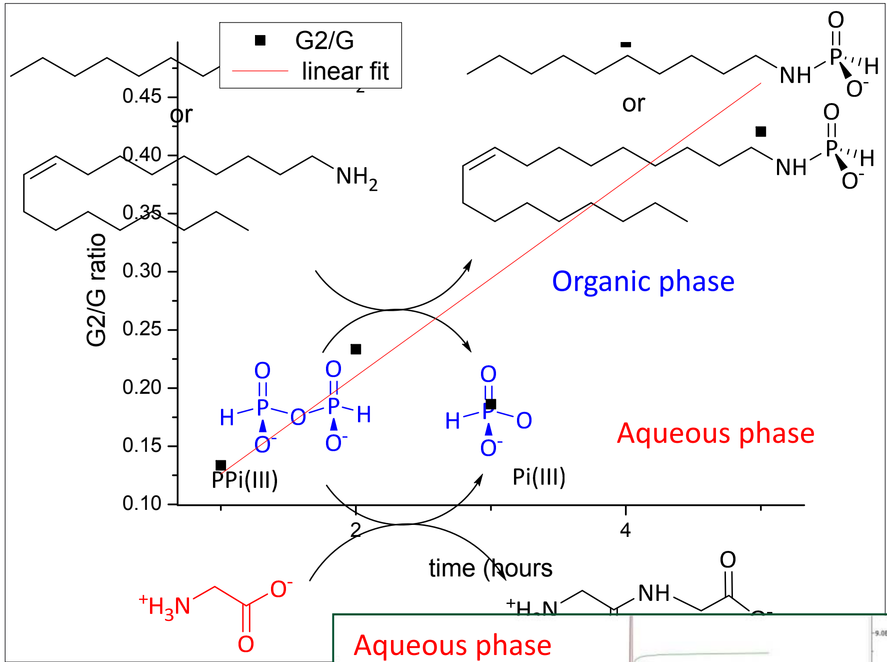
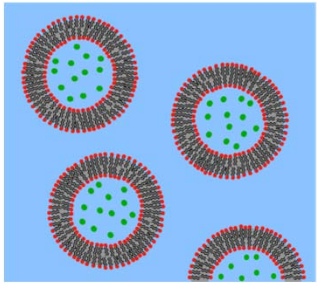
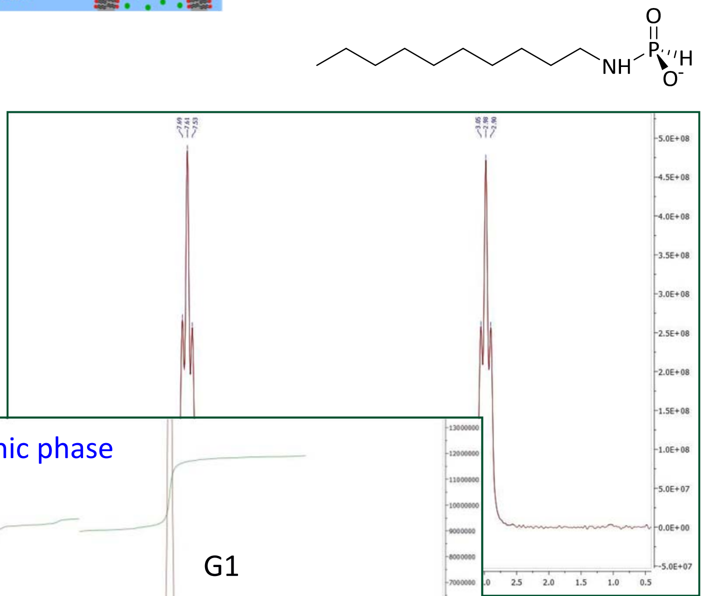
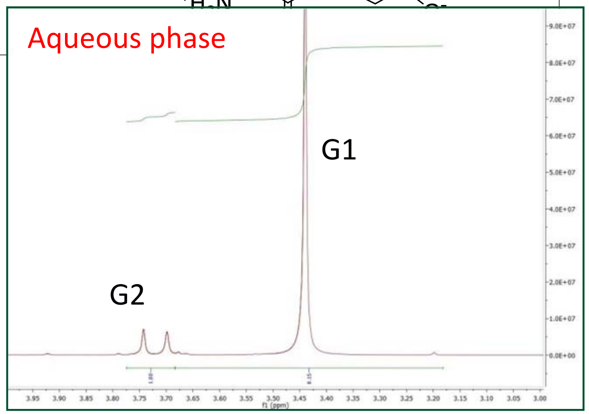
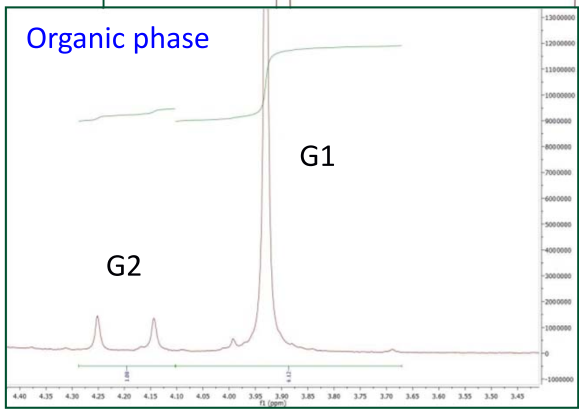




Amphiphile formation followed
by self-assembly in the organic phase
To form [PN] compound







Aqueous Phase (5 mL)
PPi(III) (1.0 M)
Anthraquinone blue dye
pH 11
Glycine (1.0 M)

3. En route *to coupled chemical reactions - compartments*

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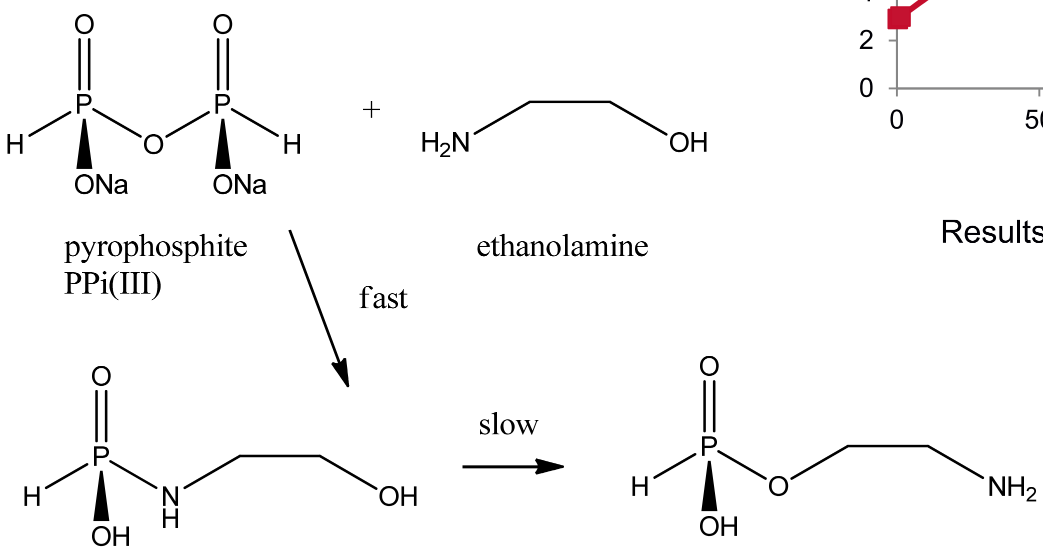
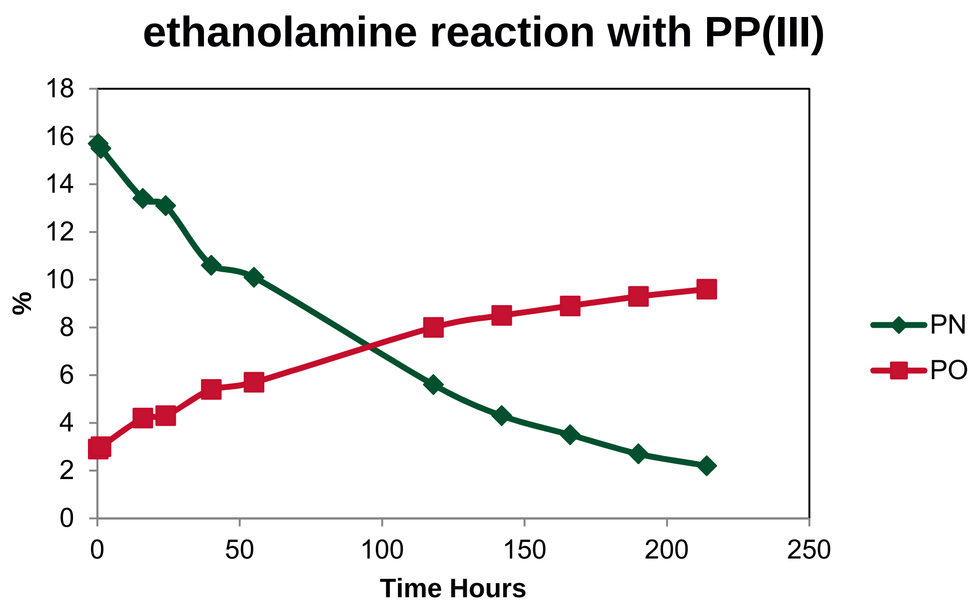
Reaction of PPi(III) with ethanolamine

Kinetic product if P-N
Thermodynamic product is P-O

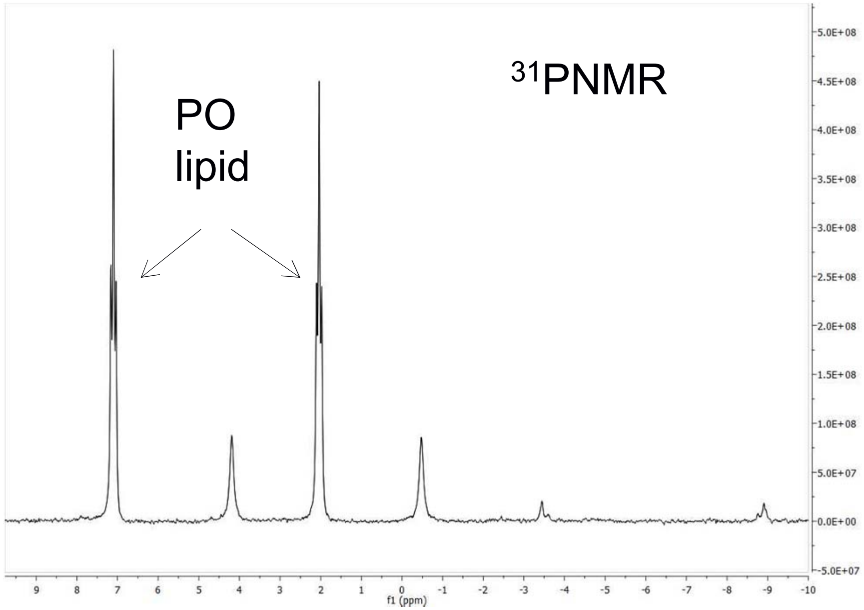


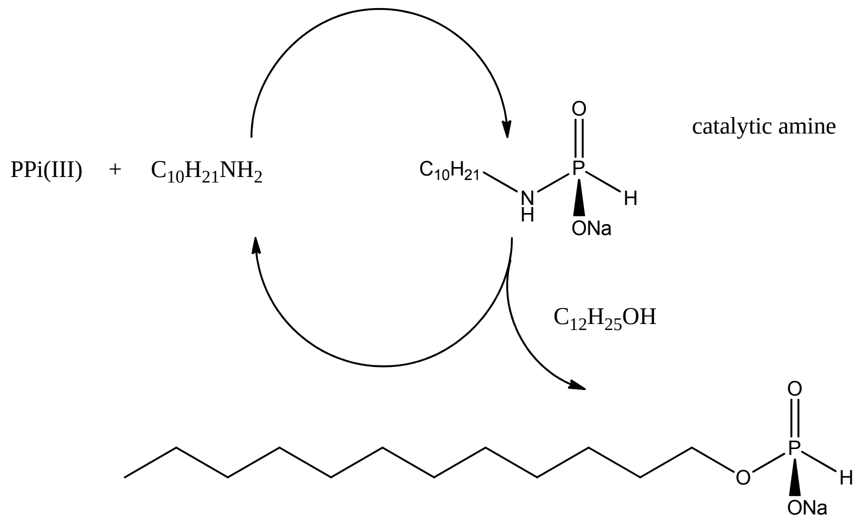
PN → PO

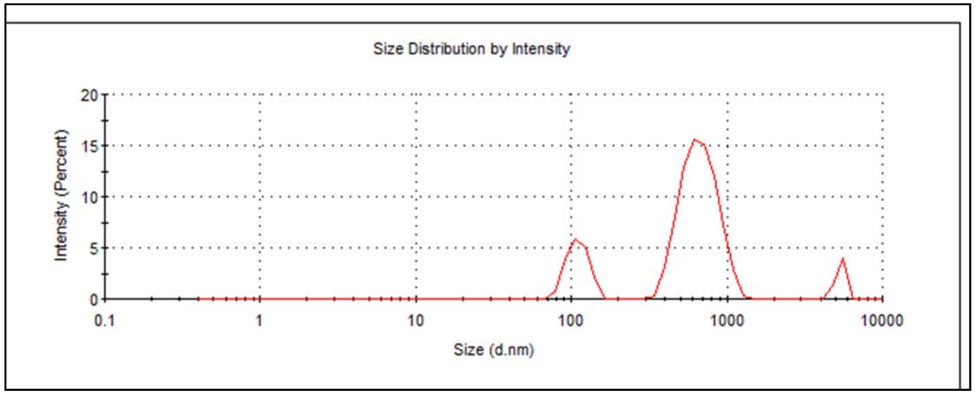


Results based on the integration of ^{31}P NMR spectra

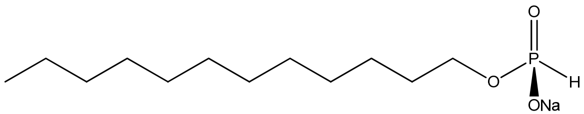
P-N formation can facilitate P-O formation

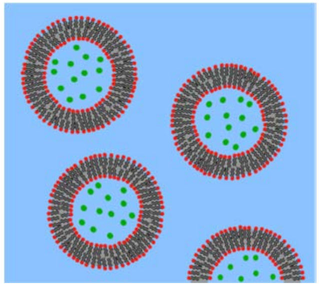


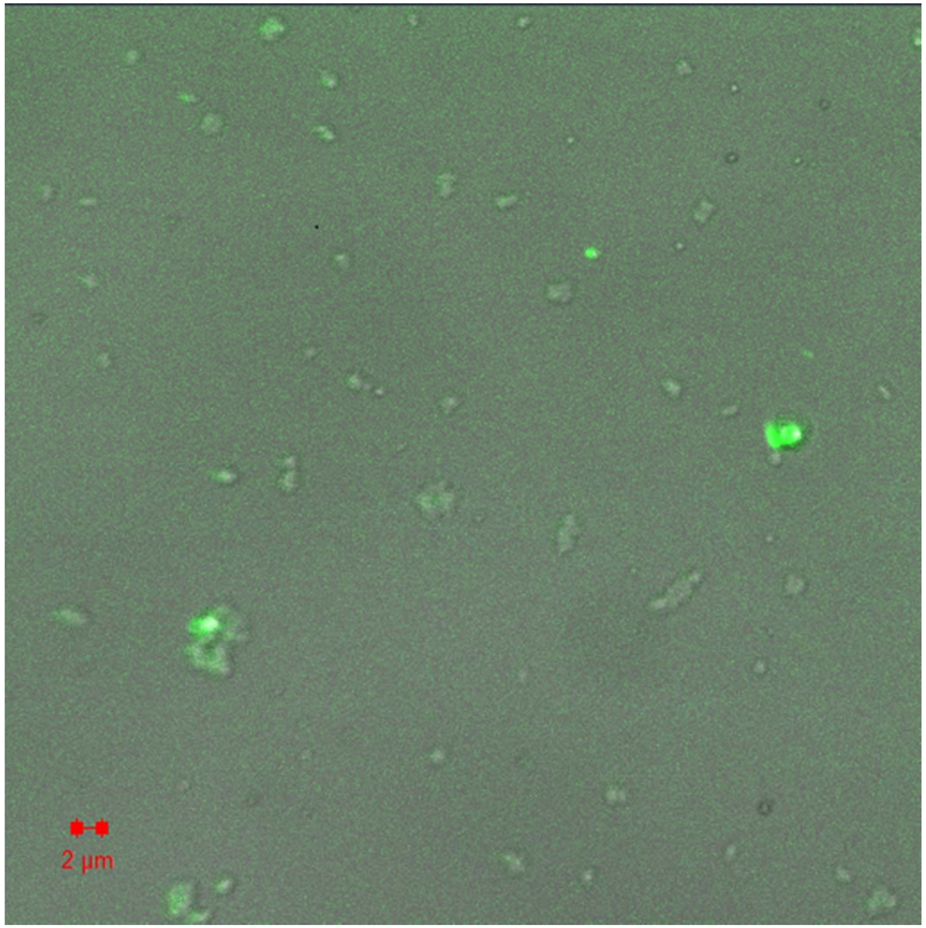




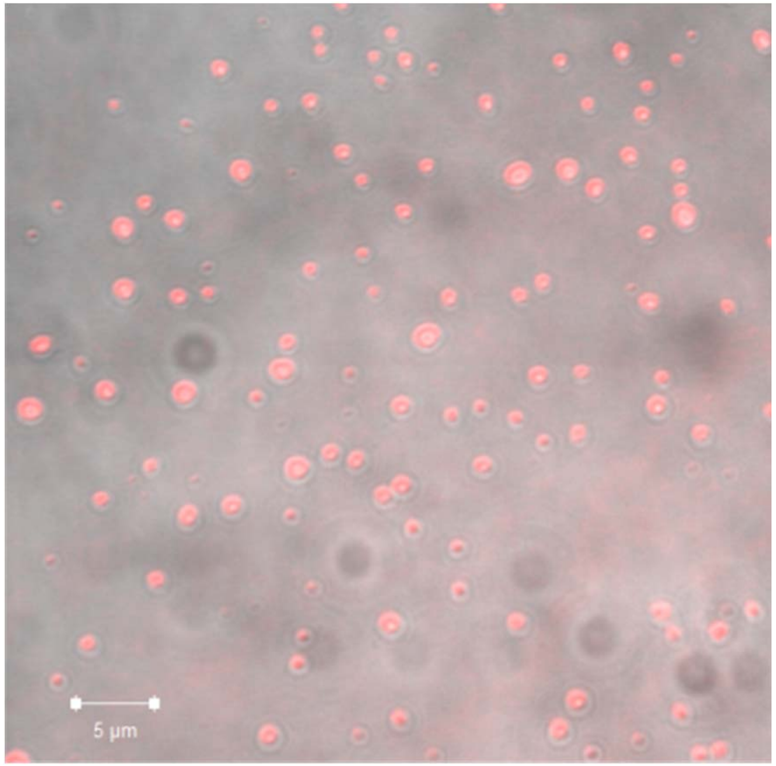
DLS analysis of aqueous vesicles from PPi(III), dodecanol & decylamine showing some agglomeration

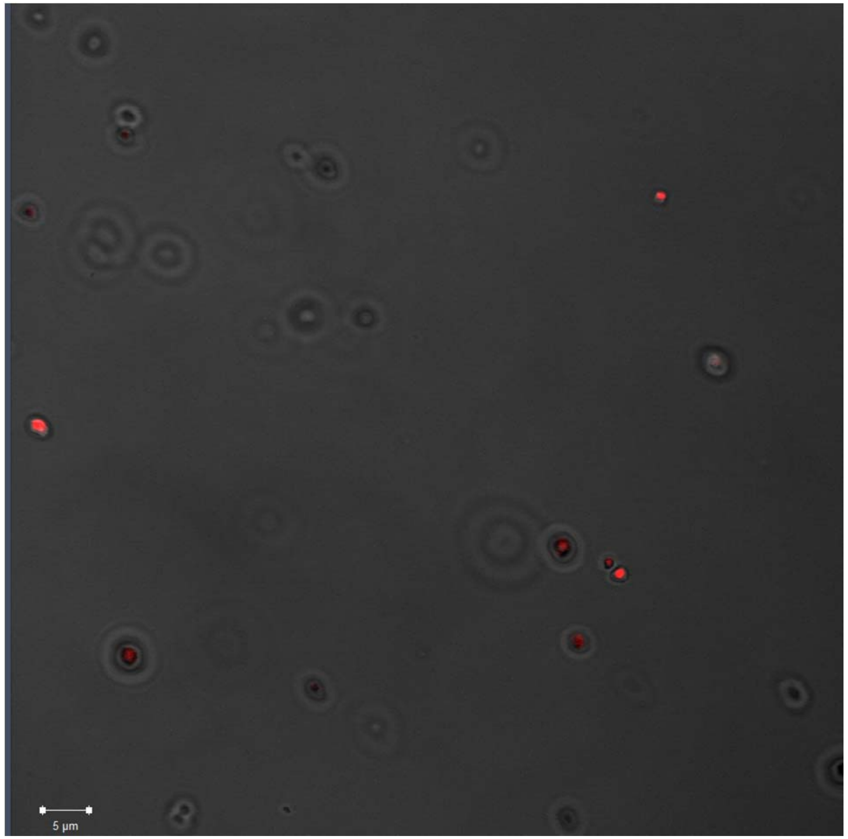






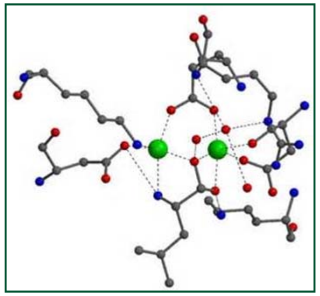
Fluorescence microscope image of aqueous vesicles containing water soluble fluorescein dye



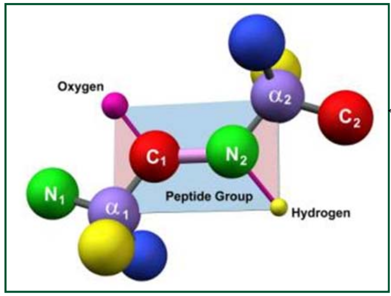


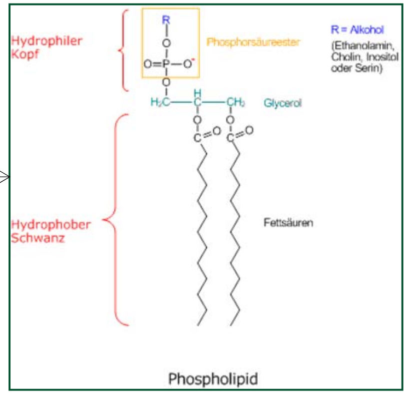
Phosphonolipid bilayers visualised by lyophilic Rhodamine 6G fluorescent indicator in the bilayer

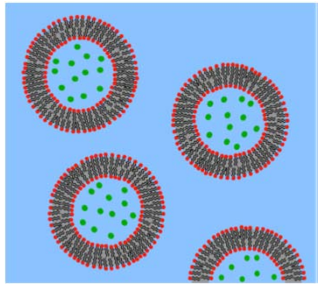
3. En route *to coupled chemical reactions*



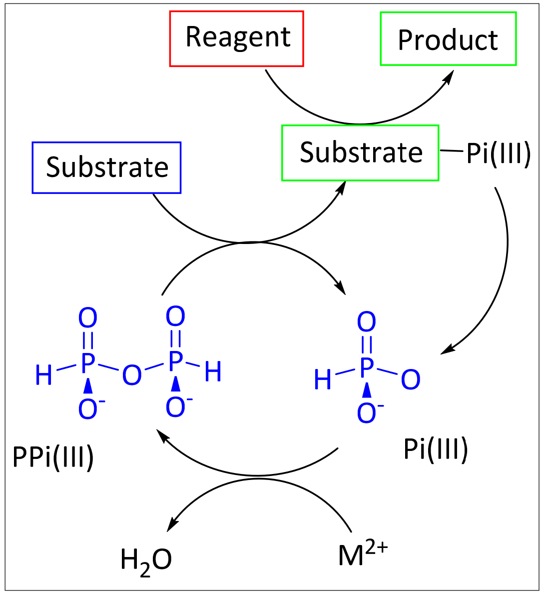
Function
(catalysis)

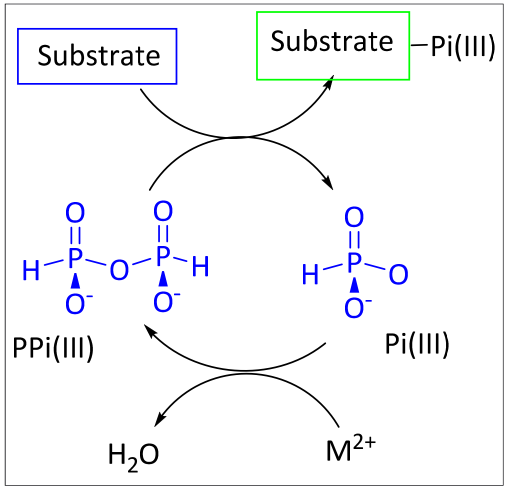






Function
(self-assembly)





3. En route *to coupled chemical reactions*

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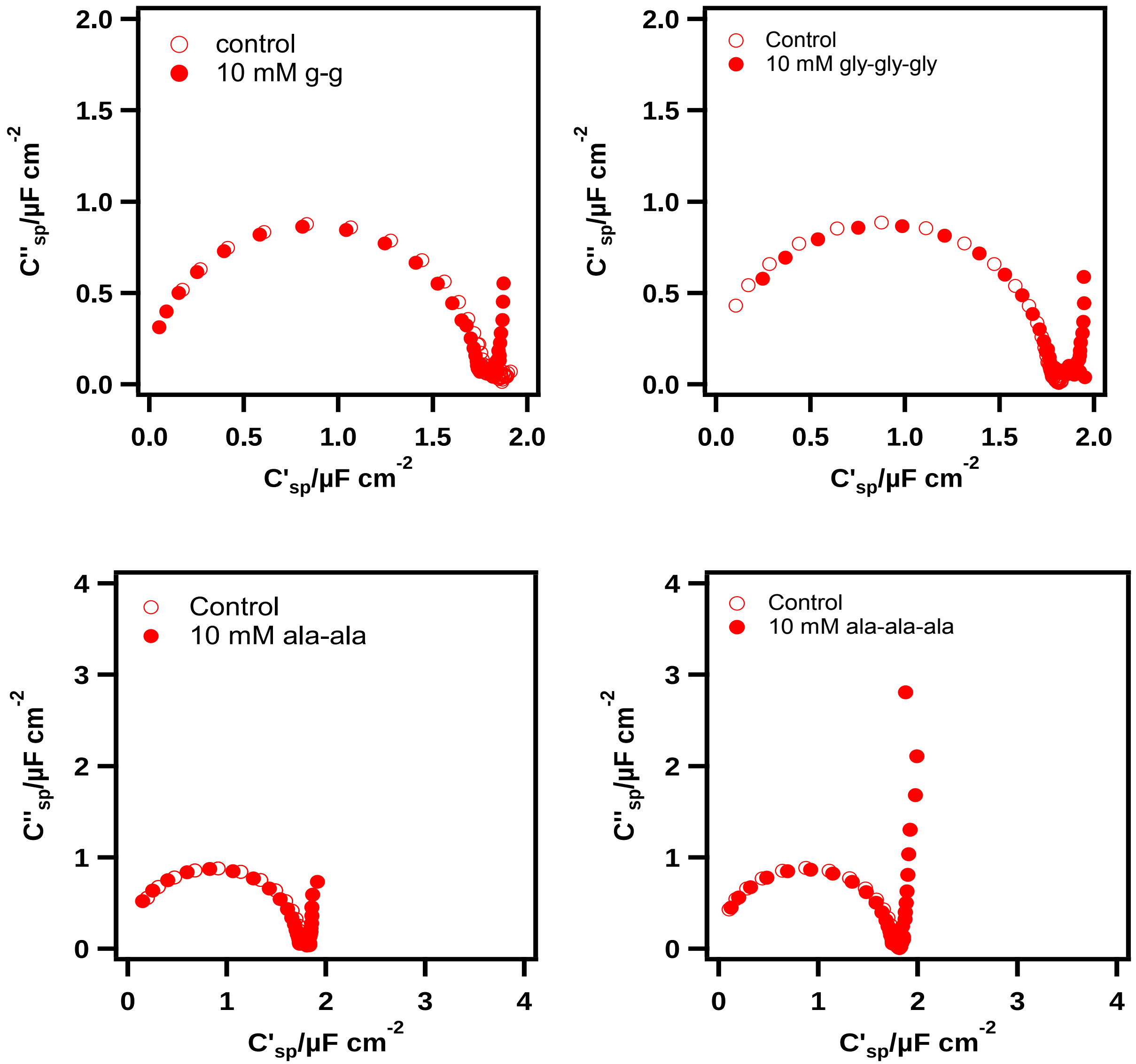
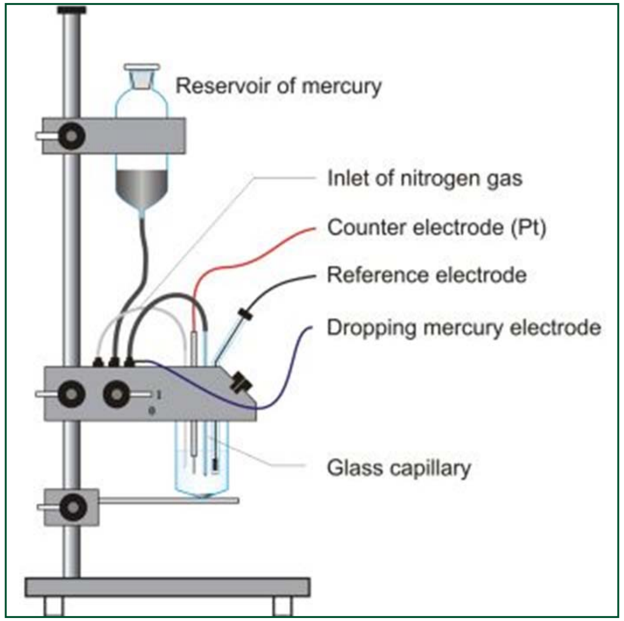


Complex capacitance impedance data of DOPC coated Hg chip electrode in 0.1 mol dm-3 KCl with 0.001 mol dm-3 phosphate bufferr
10 mmol dm-3 of gly-gly (a), gly-gly-gly (b), ala-ala (c) and ala-ala-ala (d)
acquired using EIS at -0.4 V.



1. Importance of phosphorus (P) in biology

2. Prebiotically plausible P-based *energy currency* molecules

3. En route to coupled chemical reactions

Electrochemistry & IFM
Dr D. Greenfield (*SHU*)
Prof. A. Nelson (UoL)

SEM, EDX, XPS
Dr J. Harrington (*UL*)

Meteoritics & minerals
Dr C. Smith (*NHM*)
Dr M. A. Pasek (*NAI; USF*)

DLS
A. Kazlauciunas (UoL)

IT calorimetry
Prof.M. Page (UoH)

Cryo-TEM
Dr P. Wang (UoL)

PEM fuel cell (CCFD)
Dr Mohammed Ismail
Prof. Mohamed Pourkashanian

Dr David Bryant
Dr Shohei Ohara
Dr Ian Gorrell
Joshua Hampton
Kieran Conville

Dr Barry Herschey (UCL)
Dr Claire Cousins (UCL/Birkbeck)
Dr Ian Crawford (UCL/Birkbeck)
Icelandic Glaciological Society
Dr Laurie Barge (JPL-Caltech)

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