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1 **Amorphous 1-D nanowires of calcium phosphate/pyrophosphate: a demonstration**
2 of **oriented** self-growth of amorphous minerals

3

4 Chaobo Feng^{+[a]}, Bing-Qiang Lu^{+*[a]}, Yunshan Fan^[a], Haijian Ni^[a], Yunfei Zhao^[a], Shuo Tan^[a], Zhi
5 Zhou^[a], Lijia Liu^[b], Jordan A. Hachtel^[c], Demie Kepaptsoglou^[d], Baohu Wu^[e], Denis Gebauer^[f],
6 Shisheng He^{*[a]}, Feng Chen^{*[a,g]}

7

8 **Affiliations**

9 ^[a]: Center for Orthopedic Science and Translational Medicine, Department of Orthopedic, Spinal
10 Pain Research Institute, Shanghai Tenth People's Hospital, School of Medicine, Tongji University,
11 Shanghai, 200072, P. R. China.

12 ^[b]: Department of Chemistry, University of Western Ontario, London, ON, N6A5B7, Canada.

13 ^[c]: Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge,
14 Tennessee 37831, United States

15 ^[d]: SuperSTEM Laboratory, SciTech Daresbury Campus, Daresbury, WA4 4AD UK; Department
16 of Physics, University of York, York, YO10 5DD UK

17 ^[e]: Forschungszentrum Jülich GmbH, JCNS-4, JCNS at MLZ, Lichtenbergstr. 1, 85748 Garching,
18 Germany

19 ^[f]: Institute of Inorganic Chemistry, Leibniz University Hannover, Callinstr. 9, D-30167 Hanover,
20 Germany.

21 ^[g]: Shanghai Key Laboratory of Craniomaxillofacial Development and Diseases, Stomatological
22 Hospital and School of Stomatology, Fudan University, Shanghai, 200001 P. R. China.

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24 ⁺: These authors contributed equally.

25

26 **Corresponding Authors**

27 *E-mail: bqlu@tongji.edu.cn; tjhss7418@tongji.edu.cn; fchen@tongji.edu.cn.

1 **Abstract**

2 Amorphous inorganic solids are traditionally isotropic, thus, it is believed that they
3 only grow in a non-preferential way without the assistance of regulators, leading to the
4 morphologies of nanospheres or irregular aggregates of nanoparticles. However, in the
5 presence of (ortho)phosphate (Pi) and pyrophosphate ions (PPi) which have synergistic
6 roles in biomineralization, the highly elongated amorphous nanowires (denoted
7 ACPNs) form in a regulator-free aqueous solution (without templates, additives,
8 organics, etc). Based on thorough characterization and tracking of the formation process
9 (e.g., Cryo-TEM, spherical aberration correction high resolution TEM, solid state NMR,
10 high energy resolution monochromated STEM-EELS), the microstructure and its
11 preferential growth behavior are elucidated. In ACPNs, amorphous calcium
12 orthophosphate and amorphous calcium pyrophosphate are distributed at separated but
13 close sites. The ACPNs grow via either the preferential attachment of ~2 nm
14 nanoclusters in a 1-dimension way, or the transformation of bigger nanoparticles,
15 indicating an inherent driving force-governed process. We propose that the anisotropy
16 of ACPNs microstructure, which is corroborated experimentally, causes their oriented
17 growth. This study proves that, unlike the conventional view, amorphous minerals can
18 form via oriented growth without external regulation, demonstrating a novel insight
19 into the structures and growth behaviors of amorphous minerals.

20

21 **Keywords**

22 Amorphous inorganic solids; nanowires; calcium phosphate; calcium pyrophosphate;
23 oriented growth.

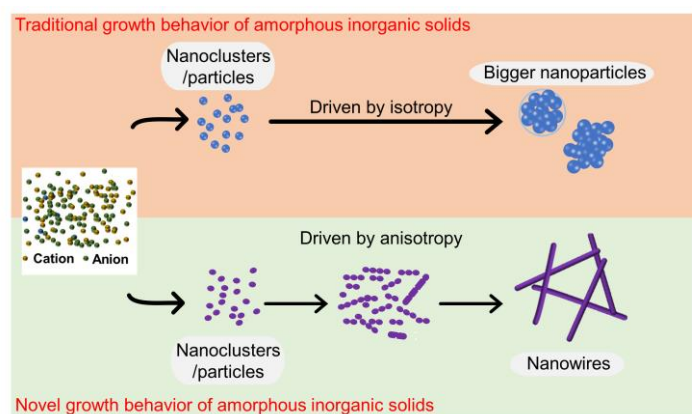
1 **1. Introduction**

2 As an important inorganic mineral, calcium phosphate is abundant in nature and
3 many organisms. For example, human bone is composed of 70% inorganic mineral
4 (mainly apatite) and 30% organic matrix (mainly collagen), while tooth enamel is 97%
5 hydroxyapatite, 1.5% proteins, and 1.5% water ¹⁻⁴. Among the diverse calcium
6 phosphates members, calcium orthophosphates (CaPi, where Pi stands for the
7 orthophosphate ion) play the major roles in hard tissue formation or biomedical
8 applications ⁵. They include the amorphous form (amorphous calcium phosphate,
9 ACaPi), and crystalline ones such as hydroxyapatite, octacalcium phosphate, brushite,
10 monetite, and (α , β) tricalcium phosphates. In comparison, calcium pyrophosphate
11 (CaPPi, where PPi stands for the pyrophosphate ion), has been much less discussed
12 regardless of its crystalline or amorphous (ACaPPi) forms. CaPPi crystals are normally
13 observed in pathological tissues, especially in cardiovascular and articular cartilage ⁶⁻⁹.
14 Moreover, these ectopic calcifications are mostly composed of both CaPPi and CaPi
15 crystals ¹⁰⁻¹⁴. In the process of bone formation, PPi, which is mainly produced by
16 hydrolyzing the phosphodiester bond in nucleotide triphosphates ¹⁵⁻¹⁷, acts as a critical
17 regulator in the formation of CaPi ¹⁸⁻²⁴, since they can effectively inhibit nucleation and
18 crystallization of CaPi by antagonizing the binding of calcium with phosphate ^{20-21, 25}.
19 It is believed that Pi and PPi synergistically control bone mineralization ^{24, 26-29}.

20 The growth behaviors of biominerals, which govern the specific construction of
21 the resulting materials (including the biomineralized tissues), have been one of the
22 central topics in **the field of** biomineralization³⁰. It has been established that substances
23 **prefer** to grow into the shapes with minimum surface free energy from the perspective
24 of thermodynamic equilibrium, which is typically described by the Gibbs–Curie–Wulff
25 Theorem ³¹⁻³³. Thus, the crystals, due to the energy differences between their different
26 facets (based on the microstructure of long-range order), **favor** the growth with certain
27 spatial preferences ^{32, 34}. This thereby leads to corresponding facets exposed on the
28 surface of crystals, forming distinct planes, angles, and edges ^{32, 35-38}. On the contrary,

1 in amorphous solids, as the atoms and ions are extremely disordered, or only ordered
2 within a very short range, they are isotropic when viewing them in the size scale of
3 nanometers or longer^{37, 39}. Therefore, there is usually no energetic driving force for
4 amorphous minerals towards a spatially preferential growth in the absence of external
5 regulators (templates, additives, electrical field, magnetic field, etc), resulting in the
6 morphologies of round nanospheres or irregular nanoparticles which have the lowest
7 specific surface area⁴⁰⁻⁴⁴. Indeed, while a few previous amorphous nanowires of
8 inorganic solids exhibited a preferential growth for their formation, the inductive
9 contributions by the applied organics, templates, and electric field can not be ruled out
10⁴⁵⁻⁵⁰. Those conclusions are particularly applicable to ACPi and ACPi⁵¹⁻⁵².

11 However, in this work, we find a 1-D oriented growth behavior of an amorphous
12 solid (Schematic 1). Inspired by the fact that Pi and PPI co-exist in the bodily fluids and
13 interact with each other especially during biomineralization, we studied calcium
14 phosphate formation in solutions including both of the ions. Surprisingly, we obtained
15 nanowires of amorphous phase with high aspect ratio. The nanowires are composed of
16 ACPi and ACPi and their formation involves the assembly of nanoclusters or
17 transformation of nanoparticles in a 1-D oriented fashion. The amorphous nanowires
18 (denoted amorphous calcium phosphate-pyrophosphate nanowires, ACPi) thus
19 provide a novel perspective on structures and growth behaviors of amorphous minerals.



20
21 Schematic 1. Illustration of the key finding of this work: amorphous inorganic solids
22 can grow in a 1-D oriented way without external regulators, demonstrating a novel
23 anisotropy-driven growth behavior.

1

2 2. Results and Discussion

3 2.1 Preparation of ACPNs

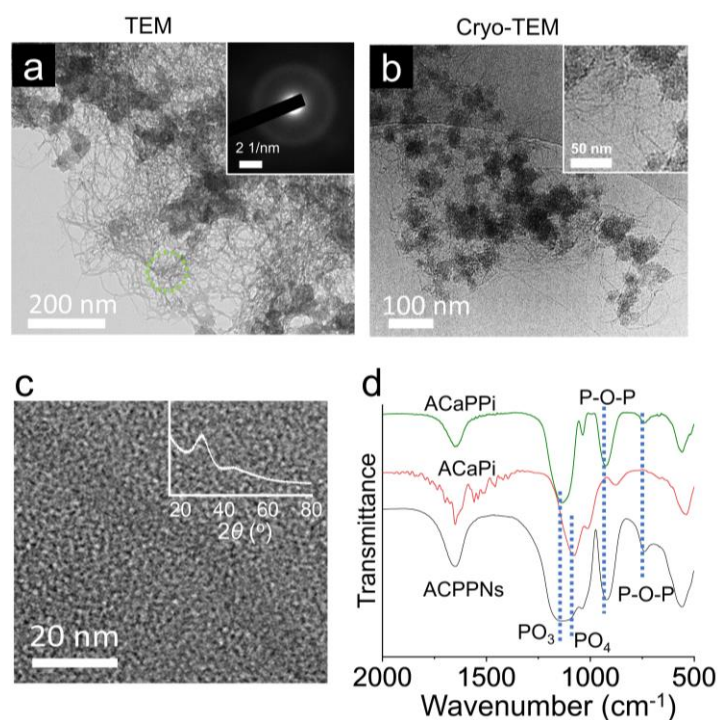
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5 **ACPNs prepared at high atomic ratio of Ca/P (>0.05) without additives**

6 ACPNs are prepared by simply mixing the solutions of calcium source (Ca^{2+})
7 and phosphorus source (Pi and PPi, denoted as P_e), where the amount of Ca^{2+} is fixed
8 at 1.00 mmol, but that of P_e is varied.

9 At first, high atomic ratio of Ca/P (>0.05 ; *i.e.*, amount of $\text{P}_e < 20$ mmol) is applied
10 and a two-step procedure is involved for ACPNs formation. Typically, for an atomic
11 ratio of Ca/P = 0.1 with 15% of P_e in the form of PPi (the remaining 85% in the form
12 of Pi), we have obtained ACPNs by adding 10.0 mmol P_e in two steps, that is, 5.0
13 mmol by 5.0 mmol sequentially. The two-step procedure facilitates **the** control over the
14 pH of the reaction solution. As shown in Fig. 1a, nanowires with average diameters of
15 2.8 nm ($n = 100$) and lengths of up to hundreds of nanometers are formed, although
16 thicker ones (e.g., diameters >10 nm) are also observed. Besides, nanoparticles with
17 irregular shapes are obtained as well. In cryogenic transmission electron microscopy
18 (Cryo-TEM) micrographs of the sample in the reaction solution, we also observe the
19 nanowires, proving that this morphology is formed in the solution, rather than during
20 the subsequent sample preparation (Fig. 1b). The electron diffraction (ED) pattern (inset
21 of Fig. 1a) of the area with nanowires (labeled by dashed circle) exhibits no distinct
22 reflections, indicating the amorphous character of the product, which is also confirmed
23 by spherical aberration correction high resolution TEM (HR-TEM, **Fig. 1c**) and X-ray
24 diffraction (XRD, inset of **Fig. 1c**). As we introduced above, nanowire morphology of
25 ACPi or ACPi has never been obtained before without external regulations such as
26 additives, templating, etc., and is not seen in other amorphous inorganic materials either,
27 to the best of our knowledge. Theoretically, when the diameter of nanowire is too thin,
28 organic additives are required to stabilize the shape especially the ones with highly

1 disordered microstructure⁵³⁻⁵⁷. In comparison, ACPNs in this work are additive-free
2 nanowires.



3
4 Fig. 1. Characterization of ACPNs prepared at Ca/P = 0.1. (a) TEM micrograph and
5 ED pattern of the area marked with a dashed green circle. (b) Cryo-TEM micrograph
6 of the sample in the reaction solution with magnified local area in the inset. (c) Spherical
7 aberration correction HR-TEM image at a temperature of -180 °C (created by liquid
8 nitrogen) and XRD pattern (inset, two broad bumps at $2\theta \approx 30$ and $\sim 45^\circ$) of the
9 ACPNs. No visible beam damage on the sample was observed after ED pattern capture.
10 (d) FTIR spectra with dashed blue line labeling characteristic bands of specific groups
11 as indicated. The assignment of the vibration modes follows the references⁵⁸⁻⁵⁹.

12

13 X-ray photoelectron Spectroscopy (XPS) (Fig. S1a) and Energy dispersive
14 spectroscopy (EDS) (Fig. S1b) prove the existence of Ca and P elements. Fourier
15 transform infrared spectroscopy (FTIR) further verify the presence of PPI ($P_2O_7^{4-}$) in
16 the ACPNs due to the asymmetric vibrational bands of P-O-P at wavenumbers of 918
17 and 739 cm^{-1} and that of PO_3 at 1146 cm^{-1} , which is consistent with $P_2O_7^{4-}$ in ACPnPi
18 (Fig. 1d). The presence of Pi (PO_4^{3-} or HPO_4^{2-}) is also confirmed by the asymmetric

1 stretching frequency of PO_4 at 1097 cm^{-1} , agreeing with the bands of $(\text{H})\text{PO}_4$ in ACaPi
2 as well (Fig. 1d). In comparison, when adding the same amount of P_e simultaneously
3 in one step (rather than in two steps as above), more nanoparticles and fewer nanowires
4 are formed, confirming that the two-step procedure is favorable for the formation of
5 nanowires (Fig. S2). Also, importantly, the ACPNs can keep the nanowire morphology
6 after being stored at 37°C even for 8 weeks without drying (Fig. S3).

7 To investigate the effect of Pi/PPi ratio on the morphology of ACPNs, we varied
8 the P_e percentages of PPi (0, 5, 10, 15, 25, 100%; other conditions are kept the same as
9 above). For 0 % PPi , *i.e.*, only Pi as the P_e , a flake-shaped product is obtained and no
10 nanowires are observed (Fig. S4a). From 5% (Fig. S4b), 10% (Fig. S4c), 15% (Fig.
11 S4d), to 25% (Fig. S4e), the proportion of nanowires increases. For 100% PPi (Fig.
12 S4f), *i.e.*, only PPi as the P_e , the TEM micrograph presents a typical irregular
13 nanosphere morphology of amorphous calcium pyrophosphate⁶⁰. Therefore, nanowires
14 only form in a certain ratio range of Pi/PPi in the P_e .

15 The initial Ca/P atomic ratio also has an influence on the morphology of the
16 products. When the initial Ca/P increases from 0.1 (Fig. 1) to 1.0, *i.e.*, 1.00 mmol P_e is
17 used (other parameters are not changed), nanowires are still formed in the above-
18 described two-step procedure (Fig. S5a). However, only very few nanowires are visible
19 when $\text{Ca}/\text{P}=2.0$ (Fig. S5b), and no nanowires are observed when $\text{Ca}/\text{P}=5.0$ (Fig. S5c).
20 FTIR spectra of these three samples show that Pi/PPi ratios in the products are
21 significantly higher (Fig. S5d) comparing to that prepared with $\text{Ca}/\text{P}=0.1$ (Fig. 1d). We
22 also investigated the effects of pH on the structure of the products. Based on a solution
23 of $\text{pH}=8$ as discussed above, we compared the changes of morphology at different pH
24 values. Under weakly acidic conditions ($\text{pH}=6$) (Fig. S6a), still the nanowires form, but
25 they become much fewer under weakly basic conditions at $\text{pH}=10$ (Fig. S6b).

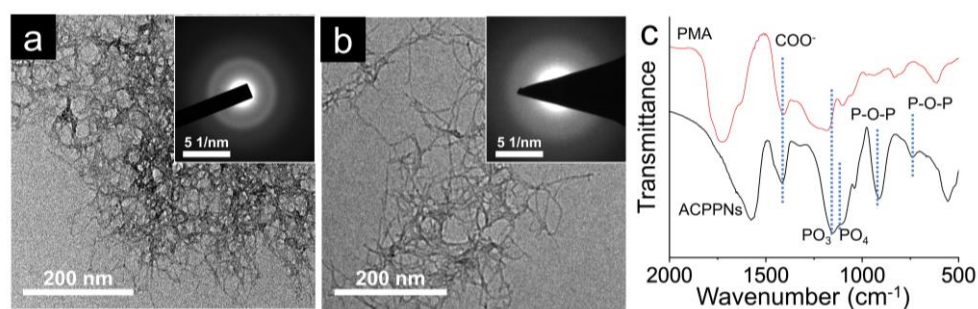
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27 ***ACPNs prepared at high atomic ratio of Ca/P (>0.05) with additives***

28 Anionic polyelectrolytes with abundant carboxylate groups, such as polymaleic

1 acid (PMA) and sodium polyacrylate (PAA), are normally used to mimic non-
2 collagenous proteins when studying biomineralization (e.g., inhibitor effects of
3 nucleation and crystallization)⁶¹, which are also typical additives for the preparation of
4 ACP nanospheres⁶². However, the introduction of PMA and PAA into the ACPPNs
5 formation system significantly increases the ratio of nanowires in the sample and even
6 results in nearly pure nanowires. As shown in Fig. 2, in the presence of either PMA (Fig.
7 2a) or PAA (Fig. 2b), nanowires with average diameters of 2.8 (n = 100, although
8 thicker ones with diameters >10 nm are also observed), and lengths of up to hundreds
9 of nanometers are prevalent in the products, while nanoparticles are hardly visible by
10 TEM observation. The predominance of nanowires in the products may be attributed to
11 an inhibitory effect of PMA and PAA against nucleation and subsequent solid growth,
12 as precipitation occurs much later than in the absence of PMA and PAA (~5 min vs. 0
13 min). We verified the amorphous phase by ED and XRD patterns (insets of Fig. 2a and
14 2b, Fig. S7), and the presence of Ca and P elements by XPS (Fig. S8). In the FTIR
15 spectra (Fig. 2c), apart from the presence of Pi and PPI corresponding to the same bands
16 as Fig. 1d, the existence of polymer is also proved by the stretching frequency of the
17 carboxy ion (COO⁻) at 1409 cm⁻¹ (Fig. 2c).

18



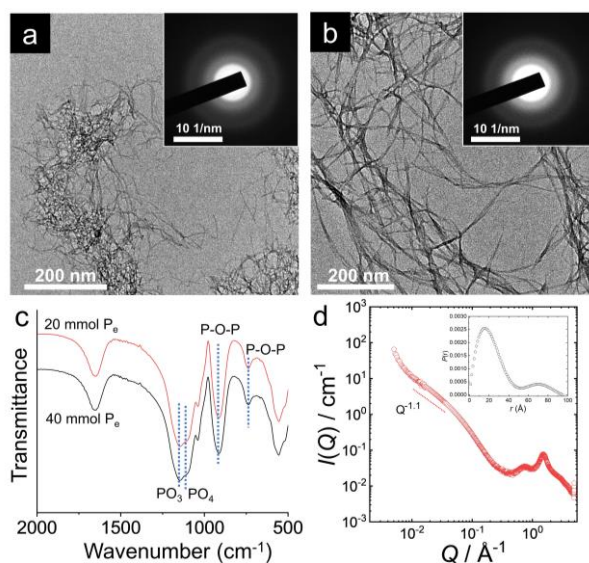
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20 Fig. 2. Characterization of ACPPNs prepared at Ca/P = 0.1 in the presence of additives.
21 (a, b) TEM images of ACPPNs prepared in the presence of PMA (a) or PAA (b). (c)
22 FTIR spectra of ACPPNs prepared with PMA.

23

24 *ACPPNs prepared at low atomic ratio of Ca/P (≤ 0.05)*

1 We further decreased the atomic ratio of Ca/P to ≤ 0.05 (amount of $P_e \geq 20$ mmol,
 2 without additives) to investigate nanowire formation in a one-step procedure. Given
 3 that the amount of P_e is much higher than that of Ca, the influence of precipitation on
 4 pH is minimal due to the buffer effect of the excess P_e , and therefore, it is not necessary
 5 to adjust the pH during the reaction as above. Hence, the one-step method can be used
 6 in this case. Here, the total P_e content is increased to 20.0 (Ca/P=0.050) and 40.0 mmol
 7 (Ca/P=0.025), while maintaining the proportion of PPI the same as above. It is found
 8 that the fraction of nanowires increases significantly (Fig. 3). Typically, when the
 9 Ca/P=0.025, nearly pure nanowires with a width of 3.0 ± 0.7 nm ($n = 100$, single
 10 nanowire or the ones at the edge of aggregates are used for measurements) and lengths
 11 of up to tens of nanometers are formed (Fig. 3b). The elemental map recorded by EDS
 12 confirms the presence of elements P and Ca (Fig. S9). With such a high P_e concentration,
 13 the stepwise addition of P_e seems to have little (or even adverse) influence on the
 14 product morphology (Fig. S10). Again, ED and XRD patterns confirm the amorphous
 15 phase (insets of Fig. 3a and b, Fig. S11). The FTIR spectra in Fig. 3c show that the
 16 intensity ratio of bands at 1146 cm^{-1} (PO_3 stretching) and 1097 cm^{-1} stretching (PO_4) is
 17 significantly higher than that in Fig. 1d, indicating the increase of PPI percentage in P_e ,
 18 which is also confirmed by nuclear magnetic resonance (NMR) measurement as
 19 discussed in the following.



20
 21 Fig. 3. Characterization of ACPPNs prepared at the atomic ratio of Ca/P ≤ 0.050 via a

1 one-step procedure. (a, b) TEM images of the ACPNs prepared when the Ca/P is at
2 0.050 (P_e content 20.0 mmol) (a) and 0.025 (P_e content 40.0 mmol) (b). (c) FTIR spectra
3 of the ACPNs with dashed blue line labeling characteristic bands of specific groups
4 as indicated. (d) One-dimensional integrated SAXS/WAXS profiles of the ACPNs
5 (Ca/P=0.025) dispersed in ethanol. The inset of (d) shows the pair distribution function
6 $P(r)$ obtained from the SAXS scattering curve. Q : scattering vector.

7
8 The morphology and phase of the nanowires are also confirmed with small-angle
9 X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) (denoted as
10 SAXS/WAXS). As shown in Fig. 3d, within the scattering vector (Q) range spanning
11 from 0.009 \AA^{-1} to 0.5 \AA^{-1} , the scattering patterns predominantly stem from nanowires,
12 which is consistent with the TEM observation. The scattering curves exhibit Q^{-1} type
13 scattering at lower Q values, indicating that the scatterers are largely constituted of
14 flexible nanowires. Within the inset of Fig. 3d, the pair distribution function ($p(r)$)
15 illustrates two distinct maxima within 0-50 \AA and 50-100 \AA , where the initial maxima
16 is associated with the nanowire's diameter, and the latter one corresponds to their
17 aggregations. Regarding the WAXS segment ($Q > 0.5 \text{ \AA}^{-1}$), the findings indicate that
18 the nanowires dispersed in ethanol do not exhibit any crystal formation.

19 20 **ACPNs prepared with Pi and PPi added in separate steps**

21 The observations above beg the question whether the pre-synthesized ACPi and
22 ACPi can transform into ACPNs? In order to explore this further, we added Pi and
23 PPi sequentially in two steps with a time interval of 3 s (Ca/P=0.1, P_e is 10.0 mmol, in
24 which Pi and PPi are 8.50 and 0.75 mmol), by which ACPi or ACPi was initially
25 formed by the first added Pi or PPi, then further reacted with the next added PPi or Pi.
26 Like above, nanowires are generated in the presence of both Pi and PPi although they
27 are added in separate steps (Fig. S12a-b). Moreover, there are many more nanowires
28 (Fig. S12a) when the PPi is added in the first step and Pi in the second (denoted as PPi-

1 and-Pi), than in the case of the reverse sequence of P_e addition (Pi-and-PPi) (Fig. S12b).
2 It should be noted that when PPI or Pi is first added, pure ACPi or ACPi forms
3 primarily, and the next addition of Pi or PPI can only partially substitute PPI or Pi in the
4 products. Furthermore, we extended the time interval between PPI and Pi addition and
5 removed the excess P_e source during the interval by washing the precipitates at the first
6 addition with a large amount of H_2O to avoid effects of the first-added excessive P_e .
7 Again, nanowires form with PPI added in the first step (PPI-and-Pi, Fig. S12c), but they
8 are nearly invisible with P_e added in reverse sequence (only crystalline CaPi, Fig. S12d).
9 All of the above reveals that ACPi or ACPi can transform into ACPNs although
10 their performance varies under different conditions. In essence, as discussed above, the
11 nanowire mainly consists of ACPi rather than ACPi, but only the addition sequence
12 of PPI-and-Pi yields nearly pure nanowires (Fig. S12c). This further confirms that, (1)
13 in addition to PPI, Pi is indeed included in nanowires as well; (2) excludes the
14 possibility that PPI acts as the surfactant to induce the nanowire formation because PPI
15 has been consumed in the first step of addition in the PPI-and-Pi sequence.

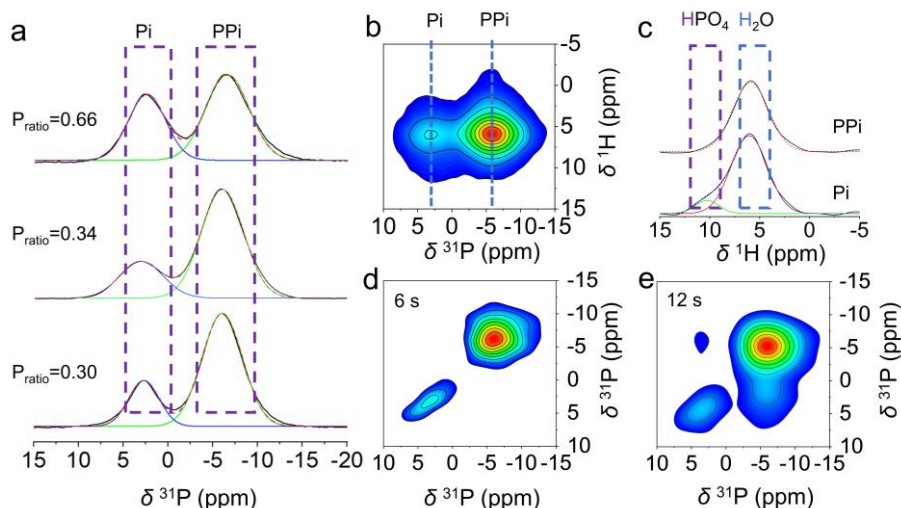
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17 2.2 Microstructure of ACPNs

18 We analyzed the typical products by solid state NMR spectroscopy (Fig. 4). The
19 single pulse ^{31}P MAS NMR spectra show two resonances with chemical shifts of ~ 2.6
20 ppm and ~ 6.5 ppm, which can be assigned to Pi (PO_4^{3-} or HPO_4^{2-}) and PPI ($P_2O_7^{4-}$)
21 groups, respectively (Fig. 4a)⁶³. The molar ratio (P_{ratio}) of P_e in Pi and PPI for the
22 samples is calculated by integrating the corresponding Gaussian peaks after
23 deconvolution as shown in Fig. 4a. Based on the P_{ratio} value and TEM results, it can be
24 inferred that the increase of the fraction of nanowires is associated with the higher PPI
25 content in the products, indicating that the formed nanoparticles consist of more Pi
26 while the nanowires contain more PPI. In the case of uniform nanowires, the P_{ratio}
27 remains $\sim 1:3$ regardless of different synthesis procedures (one using PMA, and the
28 other one at $Ca/P=0.025$ without additives).

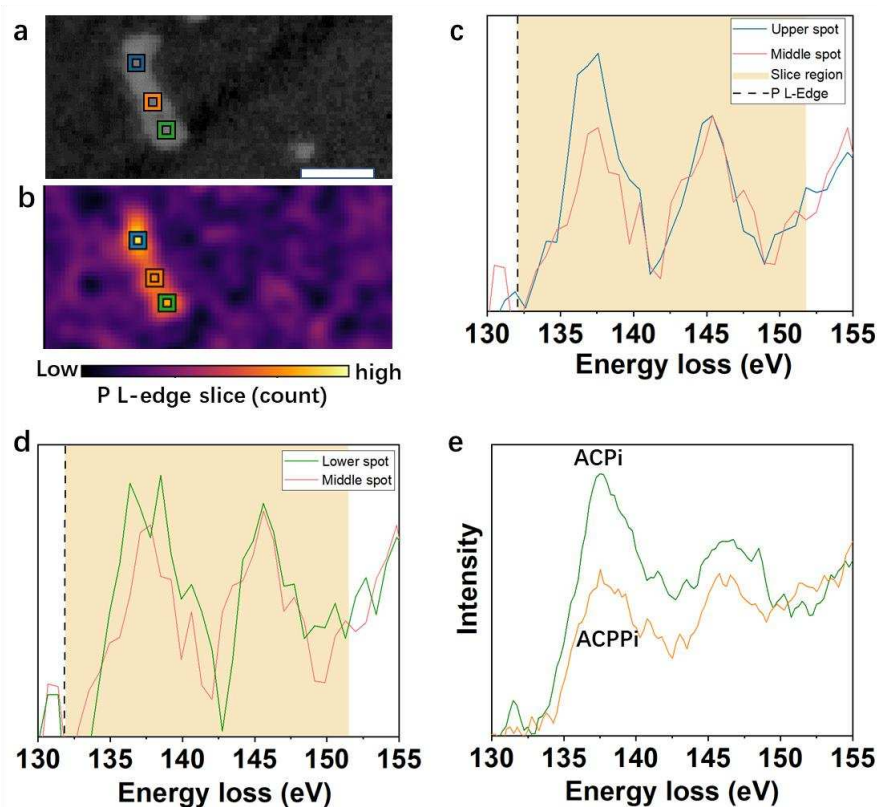
1 Subsequently, we measured the ACPNs prepared at Ca/P=0.025 via 2D solid
2 state nuclear magnetic resonance (Fig. 4b-e). In the 2D ^1H - ^{31}P HETCOR CPMAS NMR
3 spectra (Fig. 4b), both Pi and PPi are strongly correlated with H. We further extracted
4 the cross-section spectra at the ^{31}P chemical shift of Pi and PPi as indicated in Fig. 4c.
5 They show that the PPi peak correlates with the resonance of H from
6 adsorbed/structural H_2O while that of the Pi peak correlates with H of both H_2O and
7 HPO_4^{2-} . This reveals that a certain fraction of phosphates is protonated to yield HPO_4^{2-} ,
8 but pyrophosphates are not. As the Ca/P molar ratio determined by ICP-OES analysis
9 is 0.98, *i.e.*, very close to 1, we conclude that nearly all of the Pi are protonated in this
10 sample. So, with these results and TG measurement (Fig. S13), the chemical formula
11 of the nanowire is estimated to be $\text{CaHPO}_4 \cdot (\text{Ca}_2\text{P}_2\text{O}_7)_{1.5} \cdot x\text{H}_2\text{O}$ (the content of H_2O is
12 flexible depending on the drying conditions).

13 Further insight into the relationship of Pi and PPi is obtained by ^{31}P - ^{31}P NOESY
14 CPMAS NMR spectra (Fig. 4d, e). At a mixing time of 6 s, no exchanges between the
15 P_e from Pi and PPi are observed (Fig. 4d). However, when extending the mixing time
16 to 12 s, which is still very short, exchanges (positive) peaks are clearly observed (Fig.
17 4e). This result shows that Pi and PPi are separately distributed at independent sites, but
18 stay very close to each other. This anisotropy can be corroborated by STEM-EELS as
19 well: data obtained on a short rod (Fig. 5, S14) indicates fundamental changes in the
20 coordination of P_e within inner and outer spaces of ACPNs, which is consistent with
21 the spectra of ACPi and ACPi, and thus agree with the anisotropic distribution of Pi
22 and PPi. Therefore, we propose the structural model of ACPNs as Fig. S15 where
23 ACPi is in inner space and is coated by ACPi on the surface.



1

2 Fig. 4. Solid state nuclear magnetic resonance analysis of ACPPNs. (a) ^{31}P MAS NMR
 3 spectra. The measured nanowires are prepared at Ca/P=0.1 (top, same as the sample in
 4 Fig.1), at Ca/P=0.1 with PMA (middle, same as the sample in Fig.2a), and at
 5 Ca/P=0.025 (bottom, same as the sample in Fig.3b), respectively. Black solid curve:
 6 original spectrum; blue and green curve: Gaussians from the deconvolution of the
 7 original spectrum; red dashed curve: the sum of the Gaussians. The P_{ratio} , ratio of P_e in
 8 Pi and PPi, is shown for the corresponding spectrum of each sample. Dashed rectangles
 9 indicate the chemical shifts attributed to Pi and PPi as indicated. (b) 2D ^1H - ^{31}P
 10 HETCOR CPMAS NMR spectra of ACPPNs prepared at Ca/P=0.025. (c) Extracted
 11 cross-section ^1H spectra at the ^{31}P chemical shifts of Pi and PPi as indicated in the
 12 dashed line of (b). Dashed rectangles show the resonance of H from H_2O and HPO_4^{2-} ,
 13 respectively. (d, e) ^{31}P - ^{31}P NOESY CPMAS NMR spectra (same sample as **b**) at mixing
 14 times of 6s (d) and 12s (e).



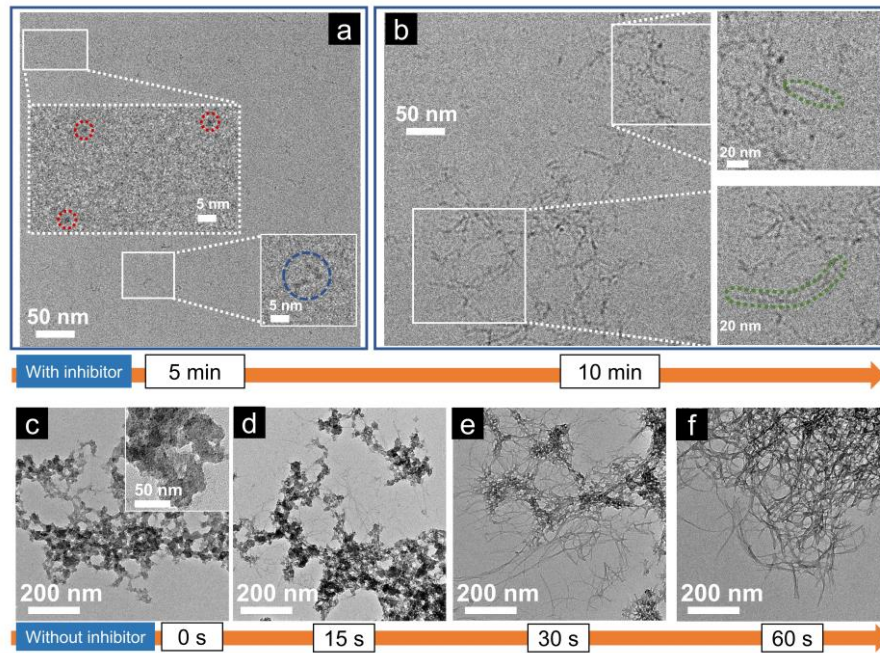
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2 Fig. 5. STEM-EELS analysis on ACPNs. (a) HAADF image (upper) of a short-rod-
 3 like structure used to acquire P EELS. Due to the low signal intensity of thin nanowires,
 4 a thicker rod was selected for measurements (thus, the structural differences between
 5 thick and thin nanowires can not be ruled out). Scale bar: 50 nm. (b) Background-
 6 subtracted, smoothed, spectrum image (SI) slice of the P L-Edge on the ACPN rod. (c,
 7 d) Representative EEL spectra from the regions marked in (a) and (b). The relative
 8 intensities of the peaks at 138 eV and 146 eV, corresponding to the L₂ and L₃ peaks,
 9 vary between the spots. The significant changes of the L₂:L₃ ratio, e.g., upper spot VS
 10 middle spot (c) and lower spot VS middle spot (d), indicate fundamental changes in the
 11 P bonding of the inner and outer spaces of ACPNs. (e) EELS of ACPi and ACPPi. The
 12 difference of the L₂:L₃ ratio between the two specimens is consistent with that of (b)
 13 and (c), which thus corroborates the anisotropic distribution of Pi and PPi. We
 14 convolved the spectrum image with a gaussian of about 15 pixel diameter to retain the
 15 spatial information but obtain a better signal-to-noise ratio.

16 2.3 Formation mechanism of ACPNs—oriented growth

1 *The formation process of ACPNs*

2 We investigated the formation process of ACPNs by tracking the evolution of the
3 formed species in the reaction solutions. As the process takes about 5 minutes in the
4 presence of PMA (Ca/P=0.1) to form visible precipitation after adding the P_e source
5 due to its inhibitory effect on the nucleation, which is a much slower reaction than that
6 without polymers (immediate precipitation), it becomes possible to characterize
7 intermediate species of the reaction. Therefore, we first studied the formation process
8 involving inhibitor PMA by cryo-TEM. It shows that, 5 min after P_e addition,
9 nanoclusters with a size of ~1-2 nm are obtained, and some of them begin to aggregate
10 or assemble into short nanorods (Fig. 6a); 10 min after P_e addition (Fig. 6b), nanowires
11 with a diameter of ~2 nm, which is similar to that of the former nanoclusters, are
12 predominant in the micrographs. Moreover, in some nanowires, which should be the
13 early formed ones, distinct boundaries in between the nanoclusters are observed. The
14 FTIR spectra show that the ratio of P_i and PP_i does not change very much during the
15 ACPNs formation according to the relative intensities of corresponding bands, which
16 also reveals that P_i and PP_i simultaneously react with Ca^{2+} in the solution instead one
17 by one. (Fig. S16a). Therefore, for the formation process, nanoclusters with a size of 1-
18 2 nm are formed in the solution first, after adding P_e ; then, they aggregate into short
19 rods; finally, the rods grow into nanowires by attaching more nanoclusters (Fig. 7).



1

2 Fig. 6. Study of the ACPNs formation mechanism. (a, b) Cryo-TEM images of the
 3 formed species in the solution at 5 min (a) and 10 min (b) during the PMA (Ca/P=0.1)
 4 involved process. The magnified area indicated by dashed circles highlight single
 5 nanocluster⁶⁴, short nanorods (blue), and long nanowires (green) formed by oriented
 6 aggregation in 1-D. (c-f) TEM images of the formed species in the solution at different
 7 times during the process of Ca/P=0.025 as indicated.

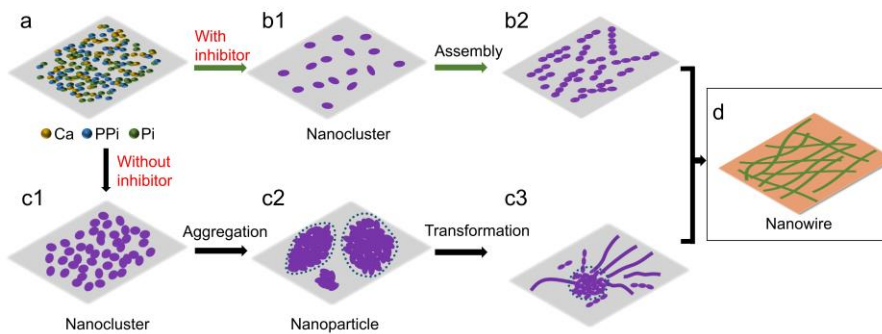
8

9 The formation of ACPNs without polymer inhibitors progresses so rapidly that
 10 precipitation occurs as soon as P_e is added. Thus, the question whether nanowires are
 11 formed immediately or after a certain lag time spurs us to investigate the formation
 12 progress at Ca/P=0.025. Fig. 6c shows that only nanoparticles, which seem to be built
 13 up by nanocluster aggregation, are observed in the sample at 0 s. Over time, nanowires
 14 appear at 15 s, with their fraction increasing afterwards, and dominating at 60 s (Fig.
 15 6d-f). This indicates that the nanowires are formed at the expense of nanoparticles.
 16 Again, FTIR spectra of the samples at different time points do not vary significantly
 17 over time indicating that the chemical compositions remain largely unchanged (Fig.
 18 S16b). Therefore, in this system, nanoclusters are formed first, but instead of
 19 subsequent aggregating in 1-D (like in the presence of PMA), they then quickly

1 aggregate into nanoparticles. However, the nanoparticles finally transform into
 2 nanowires by rearrangement or a dissolution-reprecipitation process (Fig. 7). It is
 3 reasonable to understand the aggregation of early formed nanoclusters: without
 4 nucleation inhibitor and stabilizer like PMA, a large number of nanoclusters are formed
 5 in a very short time, which tend to first randomly aggregate due to the high specific
 6 surface area and high surface free energy.

7 Besides, when tracking the transformation process with P_e addition in the sequence
 8 of PPi -and- Pi by Cryo-TEM, we find that nanowires are formed via the transformation
 9 of nanoparticles as well after adding Pi (Fig. S17).

10 The transformation of nanoparticles into nanowires at later stages indicates that
 11 nanowire formation should be controlled by thermodynamics rather than kinetics, that
 12 is, the nanowires seem to be more thermodynamically favored than nanoparticles in the
 13 long term, although we cannot categorically rule out that also kinetic aspects play a role.



14
 15 Fig. 7. Schematic illustration of possible ACPNs formation pathways with nucleation
 16 inhibitor (PMA) and without nucleation inhibitor. In the presence of nucleation inhibitor,
 17 ions (a) react to form nanoclusters with a size of 1-2 nm (b1) firstly after adding the P_e
 18 source; then, they aggregate into short rods (b2); and finally grow into nanowires (d).
 19 However, for ACPNs preparation without nucleation inhibitor, nanoclusters are
 20 formed first (c1), then quickly aggregate into nanoparticles (c2), but finally transform
 21 into nanowires (c3) by rearrangement or a dissolution-reprecipitation process.

22 ***The cause for the oriented growth of ACPNs***

23 It has been established that, in crystals, the energy differences between different

1 facets, drives the preferential growth according to Wulff's rule ⁶⁵, leading to certain
2 facets exposed on the surface of crystals, forming distinct planes, angles, and edges.
3 However, it is surprising that ACPNs, with an amorphous phase confirmed by multiple
4 characterizations, grow into this 1-dimensional structure instead of nanospheres or
5 irregular aggregates of nanoparticles without any external regulation. We also exclude
6 the effect of mechanical shear stress of stirring, by obtaining nanowires without stirring
7 after P_e addition (Fig. S18). Generally, the amorphous solids exhibit **the** order in their
8 microstructure only within a very short range ⁵², where the atoms and ions are, on
9 average of nanometer scale and **shorter**, uniformly distributed in the solids ^{37, 51}. So,
10 when the amorphous solids grow by either attaching nanosized particles or atoms/ions,
11 there are no energy differences between each spatial direction, that is, all directions are
12 equally favored by the atoms and ions during attachment. This should, like in the other
13 amorphous solids, lead to the morphologies of nanospheres or irregular aggregates of
14 nanoparticles. Thus, the fact that we obtain nanowires with such high aspect ratio
15 expands this conventional view.

16 Considering that various preparation conditions (high or low Ca/P ratio, one-step,
17 two-step, with or without polymers, P_e addition of PPi-and-Pi), and different
18 intermediate states (nanoclusters and nanoparticles with different compositions) all
19 result in ACPNs formation, the growth of the nanowire should be mainly driven by its
20 inherent properties. Therefore, it is reasonable to propose that the anisotropy of the
21 ACPNs microstructure causes the oriented growth. As discussed above, the
22 anisotropic distribution of Pi and PPi within the nanowires has been corroborated by
23 ³¹P MAS NMR spectra (Fig. 4) and STEM-EELS (Fig. 5, **Fig S14**), and is consistent
24 with the fact that the specific addition sequence of PPi-and-Pi is important for yielding
25 nearly exclusively nanowires (Fig. S12). Thus, during the growth, the building units
26 (ions or nanoclusters) attach to the nanowire in a preferential rather than random way,
27 depending on the energy differences as shown in Fig. S19. A similar mechanism is also
28 seen in an organic system (but not in amorphous inorganic ones): the core-shell

1 structured cylindrical micelles, where the inner core and outer shell consist of distinct
2 components and drive the 1-D oriented growth⁶⁶.

3 As for the possible anisotropy in the nanoclusters, we can hardly determine it by
4 experimental characterizations due to their transient state in the solution and very small
5 size. Actually, it is believed that some amorphous solids are composed of clusters with
6 specific structures whose sizes are comparable to the short order range, for example,
7 the so-called Posner's cluster or CHPC cluster⁶⁷. Also, anisotropy of a special ACPi
8 building block stabilized by a small molecule was indicated by Tang et al.⁶⁸⁻⁶⁹. In their
9 work, linear ion oligomers of amorphous calcium phosphate were prepared, where the
10 chemical environment of ends and middle segments are obviously different. However,
11 oligomer attachment did not occur in 1-dimension during the growth, and nanowires
12 were not observed. As for ACPi, its microstructure has been much less studied than
13 that of ACPi. Although an order range of 8 Å was indicated by the pair distribution
14 function pattern¹⁷, potential anisotropy was not studied further. These all indicate that,
15 within the size of short and medium range order, anisotropic nanoclusters could be
16 formed. In the nanoclusters-involved ACPNs growth, beside the anisotropic
17 nanowires, Ca, Pi, PPI, and H₂O should also have formed anisotropic building blocks,
18 *i.e.*, the nanoclusters, with a heterogeneous distribution of components. In this sense,
19 our observations may again resemble the formation of core-shell structured cylindrical
20 micelles⁶⁶. That is, the nanoclusters can be regarded as inorganic analogues of
21 amphiphilic organic diblock copolymers, or surfactants, which subsequently self-
22 assemble. Please note that the formation of ACPNs is not dependent on the presence
23 of organic amphiphiles, but an analogous, purely amorphous inorganic phenomenon.

24 Moreover, the rather slow assembly velocity of building blocks seems necessary
25 for the nanowire formation. Otherwise, the building blocks do not have sufficient time
26 for direction selection to form nanowires. In the case of PMA and PAA involved
27 reaction, it takes more than 5 minutes to initiate nucleation and further 10 minutes to
28 finish the assembly; in the case without polymers, 1 min is needed to transform

1 nanoparticles into nanowires. This also inspires us that, a slow transformation from one
2 amorphous solid to another amorphous one, may proceed via oriented growth as well
3 in other materials.

4

5 **3. Conclusion**

6 The amorphous calcium phosphate-pyrophosphate nanowires (ACPPNs) are
7 formed via an oriented growth in the aqueous regulator-free solution containing Ca^{2+} ,
8 Pi , and PPi . It is composed of amorphous calcium phosphate and amorphous calcium
9 pyrophosphate and displays the morphology of highly elongated nanowires with an
10 average diameter of $\sim 2\text{-}3$ nm, and lengths of up to hundreds of nanometers. Their
11 amorphous phase are confirmed in multiple ways. Both Pi and PPi are indispensable
12 for the formation of nanowires, in which the ACaPi and ACaPPi are distributed at
13 separated sites but stay close to each other. Further studies show that the ACPPNs form
14 via either the preferential attachment of ~ 2 nm nanoclusters in a 1-dimension fashion
15 (in the presence of nucleation inhibitor), or the transformation of bigger nanoparticles
16 (without nucleation inhibitor). An anisotropy of the ACPPNs microstructure should
17 cause their preferential growth, which is rarely seen in inorganic amorphous solids. This
18 proves that, unlike the conventional view, amorphous solids can form via oriented
19 growth, expanding the conventional view on the structure and growth behavior of
20 amorphous minerals. This finding may also provide a new perspective for biomineral
21 growth in vivo, which will be studied in the future. On the other hand, given the
22 flectional morphology in the TEM images and the possible micelle-like growth
23 behavior of ACPPNs, they display the characters of organic polymers. This work, along
24 with previous reports such as amorphous inorganic oligomers⁶⁹ and sub-one-nanometer
25 inorganic materials⁷⁰, inspires us to further explore the organics-like behaviors in
26 inorganic substances.

27 Due to technical limitations, characterizing the microstructure of amorphous solids
28 remains a great challenge. Therefore, the specific arrangements of the ions in ACPPNs

1 were not determined in this study. We also would like to remind that, during this study,
2 we found significant content of impurity PPI in the chemical Na_2HPO_4 (analytical grade)
3 from different commercial suppliers, which may have impacted or will further affect
4 the results of researches.

5 **4. Experimental Procedures**

6 **4.1 Materials**

7 Sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$, PPI source) was purchased from Aladdin
8 Biochemical Technology Co., China. Sodium phosphate dibasic (Na_2HPO_4 , Pi source)
9 was purchased from Sigma-Aldrich. Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was
10 purchased from Sinopharm Chemical Reagent Co., China. Glycerin, sodium
11 polyacrylate (PAA, 50% in H_2O , Mw 3000-5000), and polymaleic acid (PMA, 50% in
12 H_2O , Mw 400-800) were purchased from Macklin Biochemical Co., China. All the
13 chemicals were used without further processing.

14 **4.2 Preparation of calcium phosphate-pyrophosphate nanowires (ACPPNs)**

15 ACPPNs are prepared by simply mixing the solutions of calcium source (Ca^{2+})
16 and phosphorus source (Pi and PPI, denoted as P_e), where the amount of Ca^{2+} is fixed
17 at 1.00 mmol, but that of P_e is varied.

18 ***Preparation of ACPPNs at high atomic ratio of Ca/P (>0.05) by a two-step procedure***

19 Typically, three solutions were prepared as follows: 1.00 mmol of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
20 was dissolved in 100 ml of deionized water (solution A, 0.01 M); 5.00 mmol P_e source
21 with 15% in the form of PPI (0.375 mmol $\text{Na}_4\text{P}_2\text{O}_7$) and 85% in the form of Pi (4.25
22 mmol Na_2HPO_4) was dissolved in 50 ml of deionized water (solution B, 0.1M), and
23 another 5.00 mmol P_e source with same Pi/PPI ratio in 5 ml deionized water (solution
24 C, 1M), respectively. All the pH of the solutions was adjusted to 8.00 using 1M HCl
25 and NaOH solutions. Under vigorous stirring, mix solutions A and B and adjust the pH
26 to 8.00, then add solution C and keep the pH at 8.00 during the reaction. The two-step
27 procedure can facilitate the control over the pH of the reaction solution. After the

1 reaction, the solution was centrifuged at 7000 rpm for 1 min, and the precipitation was
2 washed with deionized water for 5 times and absolute ethanol for twice. All procedures
3 were conducted at ambient temperature.

4 The ratio of P_e forms (Pi and PPI) was varied (P_e of PPI: 0, 5, 10, 15, 25, 100%)
5 while other conditions kept the same as above to investigate the effects on the
6 morphology of ACPNs. For 100% PPI (only PPI as the P_e), as the solubility of PPI
7 source, sodium pyrophosphate, in H_2O is too low to prepare solution C
8 (<https://cameochemicals.noaa.gov/chemical/25074>), so the saturated PPI solution was
9 used.

10 For the PAA involved preparation, 0.40 ml PAA (50% in H_2O , Mw 3000-5000)
11 was added into solution A, and other conditions were the same as above. For the PMA
12 (50% in H_2O , Mw 400-800) involved in the preparation, 1.20 ml PMA was added into
13 solution A, and the solvent in solution A was replaced by a mixed one of 80 mL water
14 + 20 mL glycerol. Other conditions were the same as above.

15

16 ***Preparation of ACPNs at low atomic ratio of Ca/P (≤ 0.05) by a one-step procedure***

17 Based on the procedure above, all of the P_e was dissolved only in solution B, and
18 the addition of P_e was finished in one step (instead of two). Other conditions remained
19 the same as above.

20 As the amount of P_e is much higher than Ca, the effect of chemical reaction on the
21 pH is negligible, and it is not very necessary to adjust pH during the reaction. Therefore,
22 one-step method was used when adding high (≥ 20 mmol) amount of P_e ($Ca/P \leq 0.05$).

23 To investigate the formation progress, at a specific time point, 0.5 mL reaction
24 solution was quenched with 5.0 mL ethylene glycol, then immediately centrifuged at
25 12000 rpm for 1 min, washed with ethylene glycol for 3 times, and absolute ethanol for
26 3 times. The samples were characterized for TEM or FTIR (after drying in vacuum).

27 As the controls for characterizations, ACPi with the P_e in the form of HPO_4^- (same
28 as this work) was prepared following the previous protocol³⁸: Na_2HPO_4 (0.95 mmol)

1 and $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ (0.05 mmol) was dissolved in 3 mL deionized water, then quickly
2 added into 2 mL CaCl_2 (1.67 mmol) aqueous solution. After 3 s, 80 mL methanol was
3 added to quench the reaction; ACPi was prepared via the protocol same as that of Fig.
4 4F.

6 *ACPPNs formation when adding Pi and PPi in separate steps*

7 The protocol follows that of a two-step procedure with modifications. Three
8 solutions were prepared as follows: 1.00 mmol of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 100 ml
9 of deionized water (Ca solution, 0.01M); 0.75 mmol PPi was dissolved in 10 ml
10 deionized water (PPi solution, 0.075M); 8.50 mmol Pi was dissolved in 50 ml deionized
11 water (Pi solution, 0.17M). In the Ca solution, Pi and PPi in separate steps: add PPi and
12 Pi solution successively (PPi-and-Pi) with 3 s interval; add Pi and PPi solution
13 successively (Pi-and-PPi) with 3 s interval; add PPi solution, separate the precipitation
14 by centrifugation, wash it with H_2O , then add Pi solution (PPi-and-Pi); add Pi solution,
15 separate the precipitation by centrifuge, wash it with H_2O , then add PPi solution. All of
16 the resulting precipitates were separated by centrifugation and washed with deionized
17 water and absolute ethanol.

18

19 **4.3 Solid state nuclear magnetic resonance spectroscopy**

20 Solid state nuclear magnetic resonance characterization, including 1D ^{31}P and ^1H
21 magic angle spinning (MAS), 2D ^1H - ^{31}P Heteronuclear Correlation (HETCOR)
22 CPMAS NMR, 2D ^{31}P - ^{31}P Nuclear Overhauser exchange (NOESY) CPMAS NMR
23 were conducted using a Bruker AVANCE-III 400MHz widebore spectrometer. The
24 frequency of magic angle spinning was 25 kHz. $\text{NH}_4\text{H}_2\text{PO}_4$ and adamantane were used
25 as references for ^{31}P and ^1H MAS NMR, respectively. Fine powdered samples were
26 filled in the rotor with a diameter of 1.9 mm, and then directly measured. The NMR
27 peaks were integrated after deconvolution for quantitative calculations.

28 **4.4 Transmission electron microscopy (TEM)**

1 The samples were dispersed in absolute ethanol, then dropped on a carbon coated
2 copper grids, and finally measured with the Transmission electron microscope (JEM-
3 2100F and FEI Tecnai G2 F20).

4 **4.5 Cryogenic -transmission electron microscopy (Cryo-TEM)**

5 2 μ L aqueous samples solution were taken for cryo-electron microscopy. After
6 dropping on a plasma treated grid, it was quickly vitrified using automated vitrification
7 robot (Vitrobot Mark IV/Leica EM GP2) with blot time of 5 s, wait time of 1 s, and
8 drain time of 0s. The sample was characterized using Thermo-Fisher Transmission
9 Electron Microscope (Talos L120C G2) for observation.

10 **4.6 Electron Energy-Loss Spectroscopy (EELS)**

11 The data EELS was acquired on a Nion High Energy Resolution Monochromated
12 STEM-EELS operating at 60 kV equipped with a Dectris ELA direct electron detector.
13 Samples were cooled to a temperature of 100 °K using a Gatan Elsa single tilt holder
14 to mitigate beam damage. Light monochromation was used to reduce the fill-width at
15 half-maximum of the electron beam to ~ 100 meV, which resulted in a beam current of
16 ~ 30 pA. A convergence semiangle of 30 mrad and a collection semiangle of 25 mrad
17 was used for the experiment. Spectra were background fitted with a powerlaw from two
18 background fit regions (125-130 eV before P L-Edge, and 270-280 eV before C K-
19 Edge). Spectra were also filtered with a Gaussian-averaging-kernel with a sigma of 15
20 pixels to improve signal to noise.

21 **4.7 X-ray powder diffraction (XRD)**

22 The XRD patterns were acquired by Rigaku Ultimate IV powder X-ray Cu Ka
23 radiation diffractometer ($\lambda=1.5418\text{\AA}$) with voltage of 40 kV, current of 40 mA, scanning
24 angle of $2\theta = 5-80^\circ$, and scanning speed of $10^\circ/\text{min}$.

25 **4.8 Fourier transform infrared spectroscopy (FTIR)**

26 FTIR was obtained using a spectrometer (Nicolet IS50, Thermo Scientific;
27 IRSpirit, SHIMADZU) by following the KBr technique. KBr and the sample mixture
28 at a ratio of 100:2 were pressed into plates for measurement.

1 **4.9 Thermogravimetric analysis (TGA)**

2 TGA measurements were performed by the analyzer (NETZSCH STA 409 PC/PG)
3 in the air atmosphere and the heating rate was 10 °C/min. 10-20 mg of powder samples
4 were used for testing.

5 **4.10 X-Ray Photoelectron Spectroscopy (XPS)**

6 XPS was collected by a spectrometer (Thermo Scientific K-Alpha) with Al K α X-
7 rays ($h\nu = 1486.6$ eV) at 12 kV and 6 mA. All high-resolution spectra were recorded at
8 a pass energy of 1 eV. Sample charging was corrected by setting the lowest BE
9 component of the C1s spectral envelope to 284.80 eV.

10 **4.11 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)**

11 The powder samples were dissolved in 1% HNO₃ to determine their elemental
12 composition using an Inductively Coupled Plasma Optical Emission Spectrometry
13 (Agilent 5100).

14 **4.12 Small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS)**

15 The SAXS/WAXS experiments were conducted at the JCNS MLZ using a
16 laboratory-based SAXS-WAXS beamline, KWS-X (XENOCSS XUESS 3.0 XL). The
17 sample prepared Ca/P =0.025 (P_e=40 mmol) via a one-step procedure, and dispersed
18 ethanol for measurements. A MetalJet X-ray source (Excillum D2+) with a liquid metal
19 anode operated at 70 kV and 3.57 mA, emitting Ga-K α radiation with a wavelength (λ)
20 of 1.314 Å. Solution samples were measured in a sealed glass capillary (2 mm diameter
21 and 0.05mm thickness) at the capillary. The sample-to-detector distances ranged from
22 0.1 m to 1.70 m, covering the scattering vector Q range from 0.003 to 4.5 Å⁻¹ (Q is the
23 scattering vector, $Q=(4\pi/\lambda)\sin(\theta)$, where 2θ represents the scattering angle). The SAXS
24 patterns were normalized to an absolute scale, and azimuthally averaged to obtain the
25 intensity profiles. Ethanol as background was subtracted.

26

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