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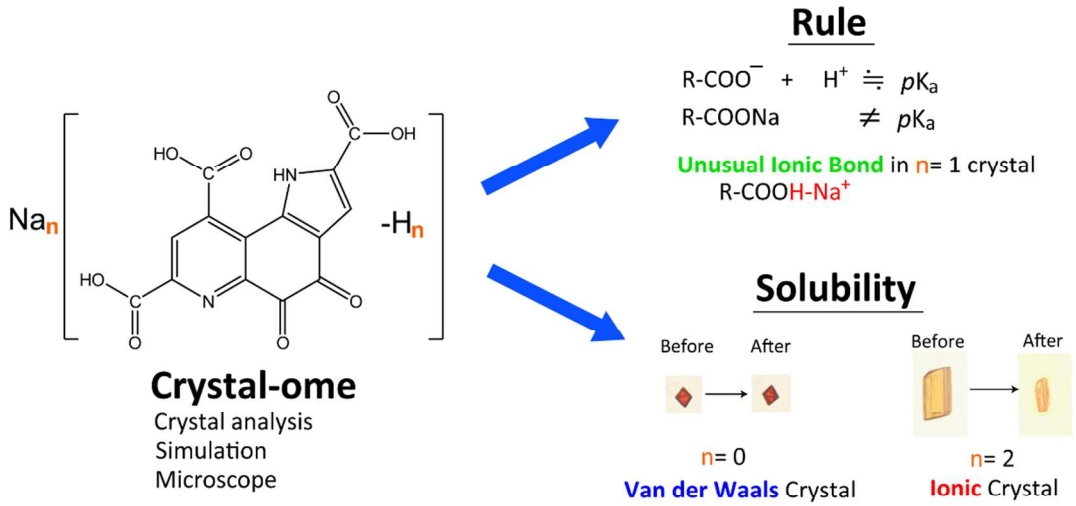
ABSTRACT: A comparative study of van der Waals and ionic crystals can provide vital information for the medical and food industries. In this work, we investigated the coenzyme pyrroloquinoline quinone (PQQ), which contains three carboxyl groups coupled to imidazole, pyridine, and quinone. Whole-crystal analysis (crystal-ome) was attempted for Na_nPQQ (n = 0–4) crystals. All deprotonation sites were found to be dependent on pK_a except for the Na sites, which cannot be explained by pK_a. The Na_1PQQ crystal exhibited an unusual ionic bond, forming COOH–Na^+ at one of the carboxyl sites in the structure. The difference in the solubility of the van der Waals and ionic crystals was also investigated, with focus on the dissolution processes of Na_0PQQ and Na_2PQQ, by combining molecular dynamics simulations with experiments that define the crystal surfaces. This study is the first step towards developing a general rule to link the different types of crystal structures with different dissolution mechanisms and rates.



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KEYWORDS PQQ, Solubility, Na, ionic bond, van der Waals

ABSTRACT

A comparative study of van der Waals and ionic crystals can provide vital information for the medical and food industries. In this work, we investigated the coenzyme pyrroloquinoline quinone (PQQ), which contains three carboxyl groups coupled to imidazole, pyridine, and quinone. Whole-crystal analysis (crystal-ome) was attempted for Na_nPQQ (n = 0–4) crystals. All deprotonation sites were found to be dependent on pK_a except for the Na sites, which cannot be explained by pK_a. The Na_1PQQ crystal exhibited an unusual ionic bond, forming COOH–Na^+ at one of the carboxyl sites in the structure. The difference in the solubility of the van der Waals and ionic crystals was also investigated, with focus on the dissolution processes of Na_0PQQ and Na_2PQQ, by combining molecular dynamics simulations with experiments that define the crystal surfaces. This study is the first step towards developing a general rule to link the different types of crystal structures with different dissolution mechanisms and rates.

INTRODUCTION

The solubility of organic crystals is a pertinent problem in the pharmaceutical and food industries. The study of crystal structures is central to better understanding, and therefore controlling this parameter. Many pharmaceutical studies have investigated the relationship between crystal structure and solubility.[1, 2] However, few have discussed the direct link between the arrangements of molecules in the crystal packing and the dissolution process.[3-5] Indeed, only a handful of studies pertaining to crystal growth discuss the dissolution process of organic crystals.[6] In light of this gap in our understanding, we investigated the difference in solubility between van der Waals and ionic crystals of the same molecular framework.

The work focused on pyrroloquinoline quinone (PQQ; 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*] quinoline-2,7,9-tricarboxylic acid) (Figure 1(a)). PQQ was originally discovered as a cofactor of bacterial dehydrogenases.[7, 8] Subsequently, several studies sought to investigate the effect of PQQ on mammalian processes such as metabolism. In 2004, it was proposed that PQQ was a new vitamin.[9] PQQ disodium is now commonly taken as a supplementary vitamin in the US and Japan owing to the reported health benefits associated with its ingestion, such as treatment of hypoglycemia, mitochondrial activation, and improvement of brain function.[10-12] More recently, it was reported that PQQ even works as an organic catalyst.[13] PQQ consists of imidazole, pyridine, and quinone groups coupled to three carboxyl groups. Each carboxyl group has a different pK_a, which implies that each carboxyl group has a different dissociation form dependent on the pH. The range of uses and structural characteristics outlined above provided the impetus behind our choice of PQQ as a model compound with which to study the solubility in van der Waals and ionic crystals. In this study, Na-stoichiometry-dependent crystals have been investigated. The structural characteristics of ionic PQQ crystals, which contain Na^+ ions in the

crystal packing structure (Na_nPQQ, n = 1, 3, 4), and van der Waals PQQ crystals (Na_0PQQ) were compared, and the different dissolution processes of these structures were analyzed.

In addition to the two types of Na_2PQQ crystal structures that have already been reported, various Na_nPQQ (n = 0, 1, 3, 4) structures were crystallized and analyzed.[14, 15] The difference between the Na^+ or H^+ coordination positions within the crystal structures and molecular packing has been discussed. The dissolution processes of Na_0PQQ and Na_2PQQ in water and artificial gastric juice have also been compared. The results of these studies, reported here for the first time, provide insight into the relationship between the dissolution rate and the anisotropy of organic crystals. Thus, this work provides a crucial first step towards a general rule linking the crystal structure to dissolution.

EXPERIMENTAL SECTION

Elemental analyses were performed using a Thermo Finnigan FLASH EA1112 system. BioPQQ®, the product name of disodium pyrroloquinoline quinone trihydrate, was obtained from Mitsubishi Gas Chemical Co., Ltd. and other materials were obtained from Wako Chemicals.

Preparation and crystallization of Na_0PQQ (free form) A 200 mL aqueous solution was prepared by mixing Na_2PQQ (2.0 g) with conc. HCl (3.6 g) and stirring for 18 h. The resultant red powder (1.6 g) was filtered. The red powder (1.0 g) was mixed with water (1 L) and heated at 120 °C. Subsequently, 2 N HCl (10 mL) was added to the solution at 70 °C, which was then left to stand for 4 days while the red crystalline product precipitated.

Crystallization of Na_1PQQ-A Na_2PQQ (50 mg) was dissolved in 15 mL of aq. gastric juice analogue (15 g of NaCl and 7 mL of conc. HCl in 1 L of water). After 4 days, the red crystal was precipitated at 4 °C.

Crystallization of Na_1PQQ-B Na_2PQQ (50 mg) was mixed with same amount HCl in water (10 mL) at 70 °C. After 1 day, the red crystal was precipitated.

Crystallization of Na_3PQQ Na_3PQQ was prepared by adding NaCl to a pH 7.5 phosphate buffer solution of Na_2PQQ. An aqueous solution of Na_3PQQ 20 mg (7 mL) was mixed with 0.3 mL of a 15% NaCl/water solution. After a few days, the crystal was precipitated at room temperature.

Preparation and crystallization of Na_4PQQ An aqueous solution (0.5 mL) of Na_2PQQ (1 mg) and 0.1 mL of 25 wt% aq. NaOH were mixed. At this time, the pH was 13.4. Methanol (1 mL) was slowly added to the mixture and the solution was left to stand at room temperature for 4 days until the red crystal product was precipitated.

Quantum chemical calculations Quantum chemical calculations were performed by using density functional theory (DFT) with the B3LYP hybrid functional.[16-19] For the crystal structure analysis, the 6-31G (d,p) basis set was used. The XRD structure was used as the input geometry without any optimizations. For population analysis, we performed natural bond orbital (NBO) analysis. The DFT calculations and natural orbital analysis were performed using the Gaussian 09 program package.[20]

RESULTS AND DISCUSSION

Crystals

The previously reported Na_2PQQ crystals are Na_2PQQ trihydrate[14] and Na_2PQQ pentahydrate,[15] which contain three and five crystal waters in a unit cell, respectively. Na_2PQQ trihydrate was used as the starting material. Other Na_nPQQ crystals were prepared by replacing the Na^+ by adding HCl (Na_0PQQ), solvating Na_2PQQ in the artificial gastric juice (Na_1PQQ-A) at 4 °C, Na_1PQQ-B with HCl at 70 °C, and controlling the pH of the solution (7.5 for Na_3PQQ and 13.4 for Na_4PQQ). Na_1PQQ have two types (A, B) by temperature (4 and 70 °C).

Structural features of the carboxyl groups.

Table 1 shows a summary of the protonated forms of each carboxyl group and the number of crystal water molecules in a unit cell. Crystal structural analyses revealed that all Na_nPQQ (n = 0–4) molecules adopt the same molecular frame (Figure 1). However, upon close inspection, there are some small but crucial differences. First, the dihedral angles of the carboxyl groups vary greatly, particularly for the group labelled C13. Second, the number of crystal water molecules increases with the increasing number of Na^+ ions present in the unit cells. It is also noteworthy that each of the carboxyl groups forms either COOH (protonated), COO^- (deprotonated), or COONa (binding Na^+). The Na^+ coordinating position in each crystal was analyzed based on pK_a values, which were obtained from the literature.[21] Density functional theory (DFT) calculations were performed to assign the pK_a values for each carboxyl and pyrrole group (see Supplementary Information). These calculations revealed that the C13 (Figure 1) carboxyl group has the lowest pK_a value, followed by the carboxyl groups labelled C12 and C14 and the pyrrole group labelled N1.

The extent to which the carboxyl groups bind to a proton is dominated by the pK_a of each group. Thus, it is expected that a carboxyl group with a low pK_a value exists as COO^- or that it

will form COONa in the presence of Na^+ in a crystal structure. Indeed, the C13 carboxyl group exists in the COO^- state in all the crystal structures except in the Na_2PQQ trihydrate. Surprisingly, the most acidic carboxyl group C13 in the Na_2PQQ trihydrate crystal is present in its protonated form as COOH. The carboxyl groups C12 and C14 are coordinated to Na^+ in this crystal structure. This result may be due to the crystallization being performed in the presence of alcohols.[14]

Considering the pK_a values of each carboxyl group alone is not sufficient to explain why some groups exist as COO^- and others as COONa in these crystal structures. For example, in the Na_2PQQ pentahydrate crystal structure, all carboxyl groups exist in their deprotonated form, COO^-. Furthermore, the most acidic carboxyl group (C13) in the Na_1PQQ-A crystal structure exists in its deprotonated form (COO^-) rather than in the Na^+-coordinated form (COONa). In addition to the pK_a values of each carboxyl group, the concentration of PQQ anions and Na^+ cations present in the solution may also play a role in determining which state the carboxyl groups will be in for each structure. That is, for any one structure, the coordination of Na^+ to each carboxyl group may be affected by the crystallization conditions. This question pertaining to the position of Na^+ will be investigated in a future study.

Na_1PQQ-A crystal structure

The crystal structure of Na_1PQQ-A is quite unique. Na_1PQQ-A forms a dimeric structure where two PQQ^- ions share two Na^+ ions (Figure 1b). The C13 group exists in its COO^- form, whereas Na^+ coordinates to the oxygen atom labelled O4 (Figure 1b) in the protonated C12 carboxyl group. Na^+ also coordinates to the nitrogen atom labelled N1 in the pyridine group and to the oxygen atom labelled O2 in the quinone group (Figure 1b). Despite the pK_a arguments

outlined above, the Na_1PQQ-A crystal structure does not support the hypothesis that Na^+ will preferentially coordinate with the most acidic carboxyl group, C13.

In order to better understand the observed crystal structures, DFT calculations were performed. $(Na_1PQQ)_2$ was used as an input structure and a population analysis was performed. Natural bonding analysis was used to determine the atomic charges of the atoms surrounding Na^+ (as described in Figure 2), all of which were found to be negative. The Na^+ environment observed is reminiscent of a crown ether.[22] Such structural features are typical for ionic bonds. Therefore, it can be concluded that the bond between Na^+ and PQQ^- is purely ionic with almost zero covalent bond character. This structure is centrosymmetric with two Na^+ ions and is held together solely by electrostatic attraction. It is remarkable that an undissociated carboxyl group can bind to a Na^+ ion in this manner. This is consistent with the reports of crystal structures in which Na^+ also coordinates to undissociated carboxyl groups, such as in sodium hydrogen maleate trihydrate.[23, 24] However, there are still very few examples in the literature of such behavior.

Solubility

Na_0PQQ and Na_2PQQ trihydrate were selected to investigate the solubility and dissolution processes of van der Waals and ionic PQQ crystals, respectively. First, the saturated solubility of each was measured. The ionic crystal Na_2PQQ showed significantly higher solubility as compared to the Na_0PQQ van der Waals crystal over a range of pH values (Table 2). This difference was maintained when the saturated solubility of the two structures was measured in a buffer solution. The saturated solubility of a structure is related to several factors such as its lattice energy, solvation energy, and ionization energy. The polarization of the PQQ may be a factor that affects the lattice and solvation energies of its structures, hence perturbing the

measured saturated solubility value. In order to further investigate the disparity in saturated solubility between the two structures of the same molecular frame, the nature of each crystal was considered. It is known that the larger the ionic radius of an ion, the smaller is its lattice energy. It follows that when a structure has a high lattice energy, it will have low solubility. Because the fully protonated PQQ and the PQQ^{2-} ions have the same molecular frame, the anion is expected to have the larger ionic radius of the two. Thus, it is expected that the Na_2PQQ crystal structure will have lattice and solvation energies that favor its dissolution more than the van der Waals structure.

Dissolution of ions having larger valences (X^{2-}, X^{3-}…) may be favorable as a result of their large solvation energies. Because a PQQ molecule has three carboxyl groups, the resulting anion can be trivalent at most. Therefore, the barrier to multivalence may explain the measured solubility difference between the Na_0PQQ and Na_2PQQ crystals. Na^+ ions coordinate to the C12 and C14 carboxyl groups of the Na_2PQQ trihydrate crystal. Thus, Na_2PQQ can easily become divalent in a solvent, irrespective of the pH. In solution, the most acidic C13 carboxyl group may deprotonate in solution to form COO^-. In contrast, all carboxyl groups present in the Na_0PQQ structure form covalent bonds with hydrogen. Therefore, one can deduce that there is a lower barrier to becoming a multivalent anion for Na_2PQQ as compared to Na_0PQQ. Thus, the ionic structure would demonstrate a higher solubility than the van der Waals structure, which is consistent with our observations.

Solvation process

The solvation process for the dissolution of the van der Waals crystal Na_0PQQ and the ionic crystal Na_2PQQ were compared. Both crystals were placed in an artificial gastric juice (HCl + NaCl) solution of pH 1.2 and the concentration of the PQQ anions liberated into solution was

monitored as a function of time. The results for Na_0PQQ and Na_2PQQ are shown in Figure 3(a) and 3(b), respectively. It is apparent from the data that Na_2PQQ has a higher solubility than Na_0PQQ. It is also particularly clear when comparing the beginning of each experiment. As both crystals have the same molecular framework, these results indicate that the molecular properties of each crystal affect the solubility. That is, the strength of crystal packing and ionicity of each structure affects the rate of dissolution.

We repeated the dissolution experiments described above in water and observed the change in the shape of each crystal through an optical microscope, the results of which are shown in Figure 3(c) and 3(d) for the van der Waals and ionic crystals, respectively. The volume and shape of the van der Waals crystal, Na_0PQQ, did not change after soaking. Conversely, a large section of the Na_2PQQ crystal dissolved within 5 min of soaking. Notably, the length of the longest side of the crystal did not change relative to the other sides, which indicates that the surfaces of the Na_2PQQ structure have varying solubility. In order to further understand this observation, the molecular orientation in the unit cells of each crystal was determined and the surface indices were measured. The results are shown in Figure 3(e) and 3(f) for Na_0PQQ and Na_2PQQ, respectively. The longest side of the ionic crystal observed through the optical microscope was assigned to the *a*-axis in the Na_2PQQ unit cell. As PQQ^{2-} anions stack along the *a*-axis in the Na_2PQQ crystal structure, the results indicate that exfoliation along the *a*-axis is difficult as compared to the other axes of the crystal. Conversely, when observing the dissolution of Na_0PQQ in water, it was found that the crystal dissolved slowly and in an isotropic manner. This may be attributed to the staggered structure of the crystal and the covalent bonds between the carboxyl groups and protons. The surfaces of the van der Waals crystal are hydrophobic, which may explain the slow, isotropic dissolution of this crystal in water. Thus, one can infer that the dissolution process

depends on the polarization of the crystal (i.e., ionic or van der Waals), surface orientation, and packing.

Molecular dynamics (MD) simulations were employed to further investigate the dissolution processes described above and to better understand our observations. The possible mechanism of dissolution can be studied by monitoring the simulated movement of ions, which cannot be observed via an experimental microscope. The MD simulations were performed on systems that contained each crystal lattice in the TIP3P water box (see Supporting Information for further details). The 250 ns MD simulation showed that the ionic Na_2PQQ crystal underwent anisotropic dissociation. While dissolution along the *a*-axis was not observed, it instead took place along the axis normal to the *a*-axis. The calculated results from the 250 ns MD simulation matched those observed experimentally through the optical microscope. However, Na_0PQQ dissolution was not observed in the time frame of the 250 ns MD simulation. When comparing MD simulations to experimental results, the vastly different time and space scales of the two techniques must always be considered. Thus, further studies are required to definitively understand the observed dissolution behavior. Nevertheless, the MD simulations indicate that the two types of crystal, Na_0PQQ and Na_2PQQ, undergo distinctly different dissolution processes. The similarity in the calculated dissolution of the ionic crystal compared to what has been observed is stark.

The simulations and experiments discussed in this paper provide insights into the different dissolution processes of a van der Waals crystal and an ionic crystal of the same molecular framework. Combining macroscopic observations with MD simulations has provided an explanation for the enhanced dissolution process of Na_2PQQ relative to Na_0PQQ, as shown in Figure 4. The results presented in this paper indicate that

(i) Na^+ ions located near the corner of a crystal structure dissociate first. If the dissociating Na^+ is a bridge between the PQQ^- ions, then repulsion within the structure increases.

(ii) PQQ^- ions then begin to dissociate from the structure.

Here, we present an explanation for the link between crystal properties and dissolution. This study provides insight into how crystal structures can be designed to manipulate their dissolution behavior. Further surface measurement studies are being performed in our group.

CONCLUSIONS

A new concept in crystallography has been suggested. Whole crystal analysis (crystal-ome) of Na_nPQQ (n = 0–4) arrived at two important results. First, the deprotonation of almost all sites depends on the pK_a. However, Na sites cannot be explained by pK_a. In fact, the Na_1PQQ-A crystal exhibited an unusual ionic bond forming COOH-Na^+ at one of the carboxyl sites in the structure. Second, the study and comparison of the solubility of van der Waals and ionic crystals is vital information for medical and food industries. The difference in the solubility between the van der Waals and ionic crystals was investigated. The dissolution processes of Na_0PQQ and Na_2PQQ were also studied by combining molecular dynamics simulations with experiments that define the crystal surfaces. This study is the first step towards developing a general rule to link types of crystal structure with different dissolution mechanisms and rates.

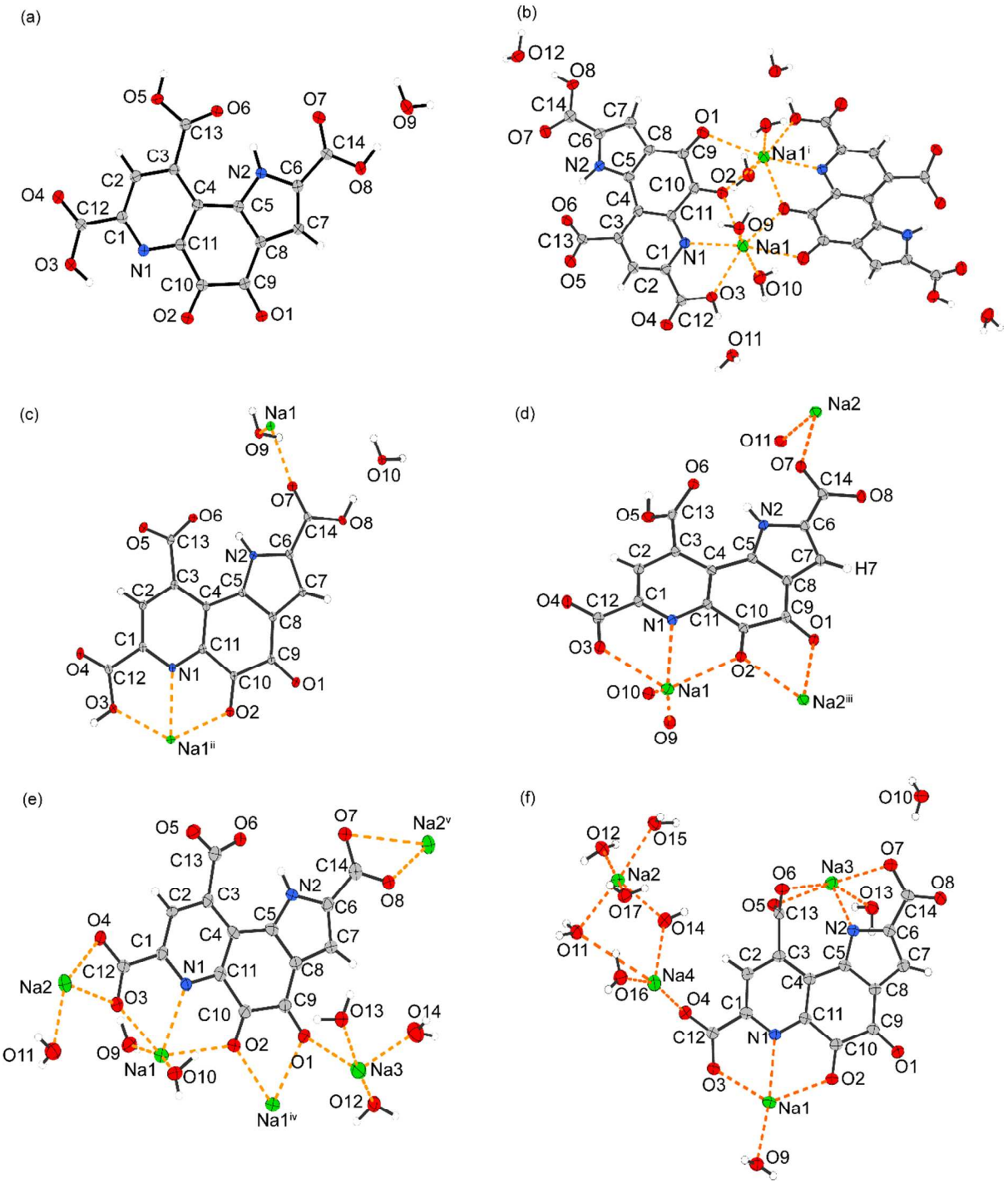


Figure 1 Molecular structures of the Na_nPQQ (n = 0–4) crystals. Atoms colored in green, red, blue, grey, and white are sodium, oxygen, nitrogen, carbon, and hydrogen respectively. Displacement ellipsoids are drawn at the 50% probability level for non-H atoms.

(a) Na_0PQQ, (b) Na_1PQQ-A, (c) Na_1PQQ-B, (d) Na_2PQQ,[12] (e) Na_3PQQ, (f) Na_4PQQ [Symmetry code: (i) –x, y, 1/2−z, (ii) −1+x, −1+y, z, (iii) x, y, 1+z, (iv) –x, 2−y, 1−z, (v) 1/2−x, 2−y, 1/2+z.]

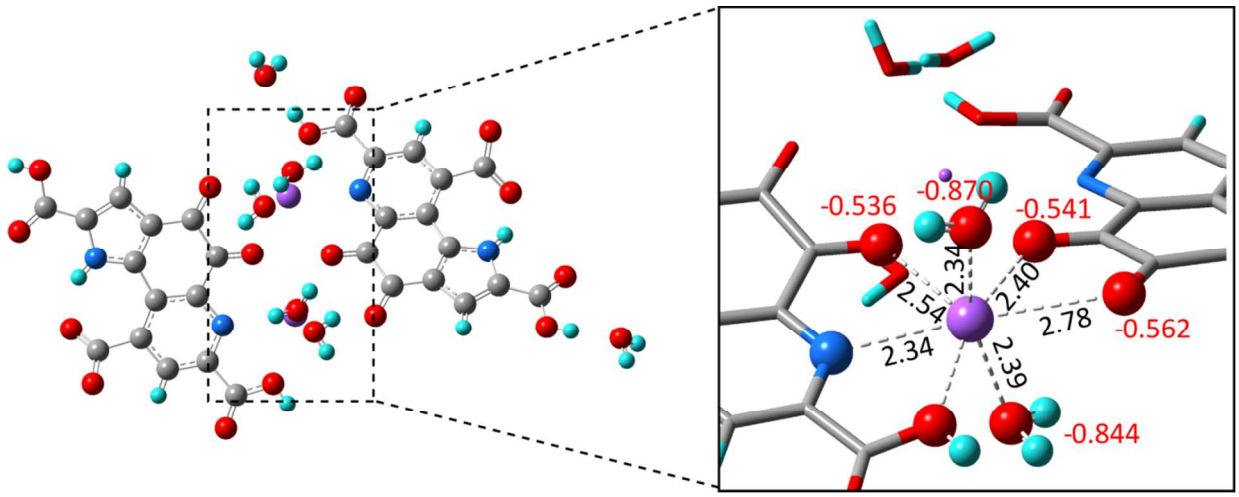


Figure 2 Environment around Na^+ in the Na_1PQQ-A crystal structure. The values in red color are the atomic charges obtained from natural orbital analysis. The values in black color are the distances between Na^+ and each of the surrounding atoms.

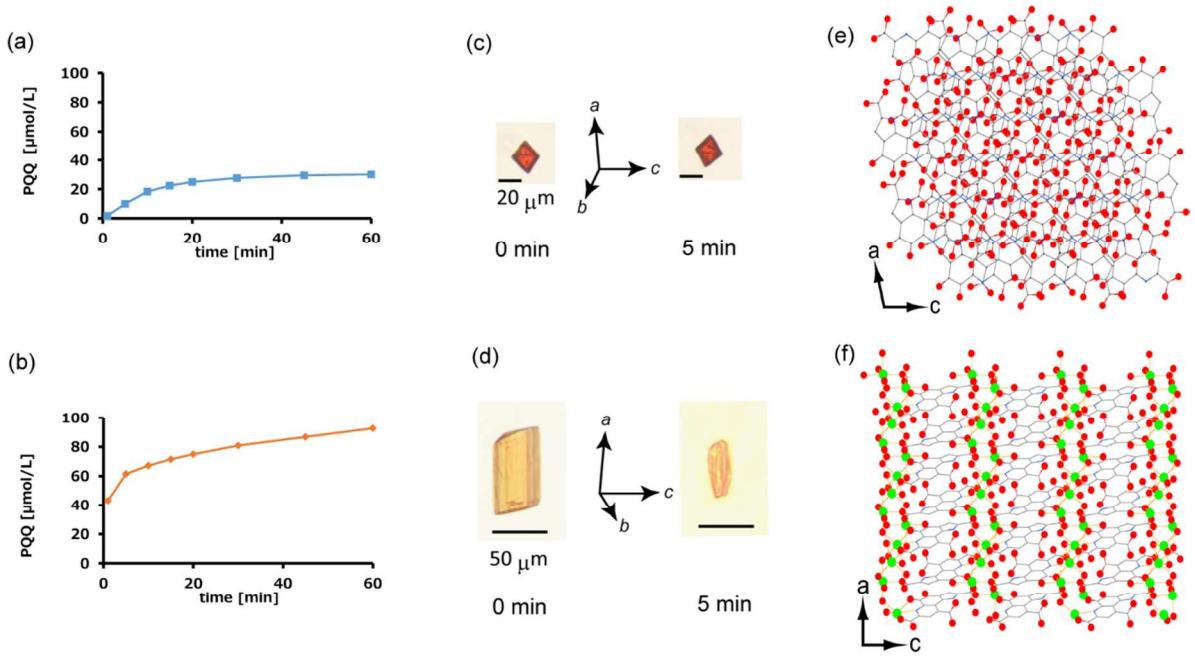


Figure 3 The solubility of (a) Na_0PQQ and (b) Na_2PQQ in gastric juice as a function of time. Microscopic observations of dissolution of (c) Na_0PQQ and (d) Na_2PQQ crystals. Molecular orientation viewed along the *b*-axis of (e) Na_0PQQ and (f) Na_2PQQ.

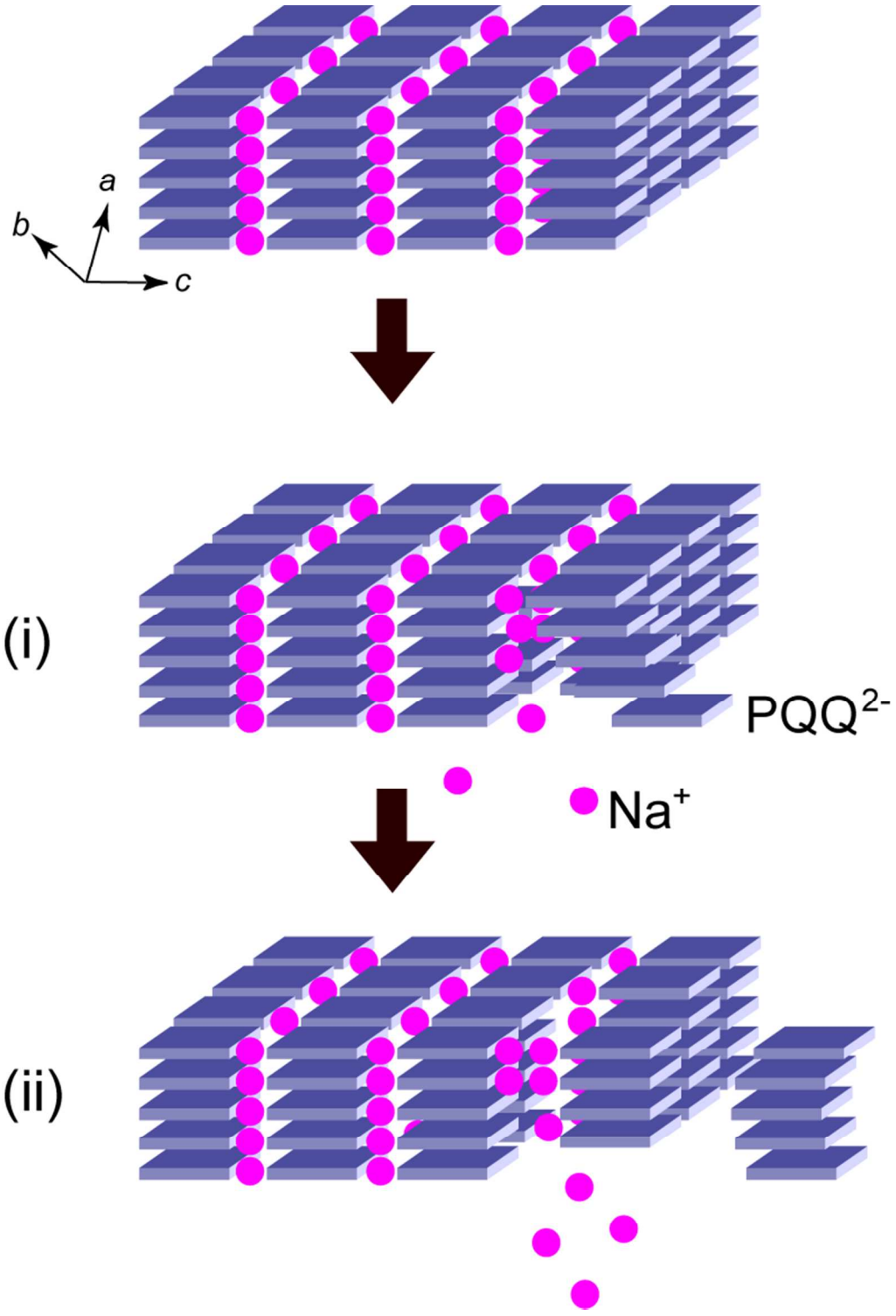
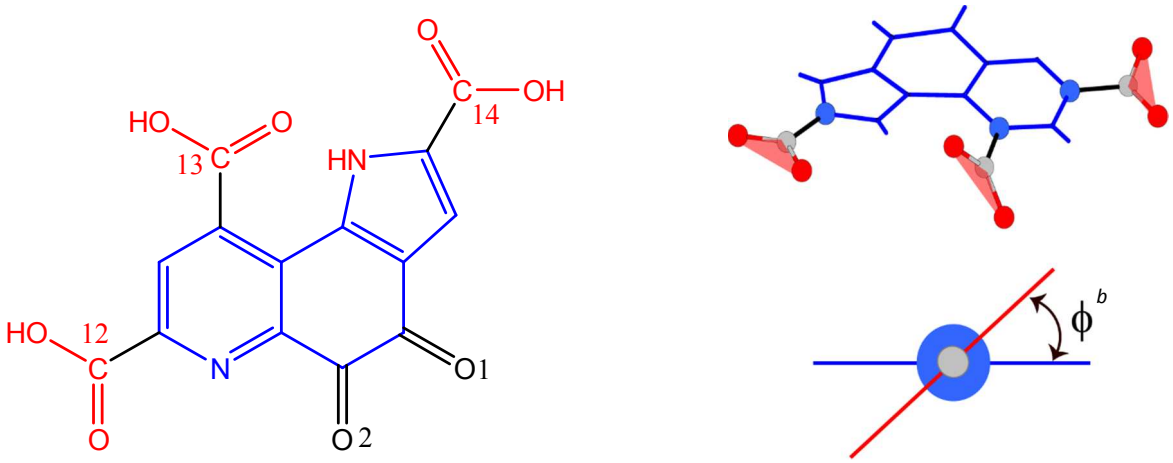


Figure 4 Proposed dissolution scheme of Na_2PQQ crystal. (i) Na^+ ions located at the corner of the structure dissociate first. (ii) PQQ^{2-} ions begin to dissociate from the cluster.

Table 1. Structural summary of Na_nPQQ crystals. [a]



		Acidic site							*Quinone*		*Py*
		C12		C13		C14		N			
		(pK_a 2.2)		(pK_a 1.6)		(pK_a 3.3)		(pK_a10.4)	O1	O2	N
n	**m**	ϕ		ϕ		ϕ					
0	1	1.3(2)	COOH	8.9(2)	COOH	6.9(2)	COOH	NH	-	-	-
1-A	4	10.2(2)	COOH-Na	28.9(2)	COO^-	9.6(2)	COOH	NH	O-Na	O-Na	N-Na
1-B	2	1.69(2)	COOH-Na	28.0(2)	COO-Na	10.3(2)	COOH-Na	NH	O-Na	-	N-Na
2[c]	3	8.6(2)	COO-Na	51.7(2)	COOH	5.9(2)	COO-Na	NH	O-Na	O-Na	N-Na
2[d,e]	5	8.59(6)	COO-Na	34.4(2)	COO-Na	3.79(5)	COOH-Na	NH	O-Na	O-Na	N-Na
		7.90(4)	COO-Na	30.7(2)	COO-Na	8.75(4)	COOH-Na	NH	-	O-Na	N-Na
3	6	2.1(5)	COO-Na	24.0(2)	COO^-	6.1(3)	COO-Na	NH	O-Na	O-Na	N-Na
4	8.5	3.12(3)	COO-Na	87.49(5)	COO-Na	6.35(3)	COO-Na	N-Na	-	O-Na	N-Na

[a] Dihedral angles and the coordination states of the functional groups in $Na_nPQQ \cdot m(H_2O)$. The pK_a for each carboxyl group has been noted.[19] [b] ϕ indicates dihedral angles of two least-squares planes defined by N1,N2/C1-C11 and COO^-. [c] Ref. 14. [d] Ref. 15. [e] In this crystal, PQQ^{2-} ions exist in two conformations.

Table 2 The solubility of ionic Van der Waals crystal (Na_0PQQ) and crystal (Na_2PQQ) in several solvents.

	Na_0PQQ (10^{-3} mol L^{-1})	Na_2PQQ (10^{-3} mol L^{-1})
Water	0.93	9.69
Artificial gastric juice (pH = 1.2)	0.03	0.24
Artificial intestinal juice (pH = 6.8)	1.85	22.84

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

Experimental details, CIF data.

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Author Contributions

K.I.: Chemical experiments, R.T. and N. M.: crystallography, Y. S., K.O. and S. N.: Physical chemistry (computer)

Notes

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REFERENCES

(1) Tawashi, R., *Science* **1968,** 160, 76.

(2) Watanabe, A.; Yamaoka, Y.; Takada, K., *Chem. Pharm. Bull.* **1982,** 30, 2958-2963.

(3) Tamura, R.; Fujimoto, D.; Lepp, Z.; Misaki, K.; Miura, H.; Takahashi, H.; Ushio, T.; Nakai, T.; Hirotsu, K., *J. Am. Chem. Soc.* **2002,** 124, 13139-13153.

(4) Coquerel, G., In *Advances in Organic Crystal Chemistry*, Tamura, R.; Miyata, M., Eds. Springer Japan: Japan, 2015; chapter 20, pp 393-420.

(5) Uchida, Y.; Iwama, S.; Coquerel, G.; Tamura, R., *Chem. Eur. J.* **2016,** 22, 11660-11666.

(6) Piana, S.; Reyhani, M.; Gale, J. D., *Nature* **2005,** 438, 70-73.

(7) Duine, J.; Van Zeeland, J., *FEBS Lett.* **1979,** 108, 443-446.

(8) Salisbury, S.; Forrest, H.; Cruse, W.; Kennard, O., *Nature* **1979,** 280, 843-844.

(9) Kasahara, T.; Kato, T., *Nature* **2003,** 422, 832-832.

(10) Akagawa, M.; Minematsu, K.; Shibata, T.; Kondo, T.; Ishii, T.; Uchida, K., *Sci. Rep.* **2016,** 6.

(11) Akagawa, M.; Nakano, M.; Ikemoto, K., *Bioscience, biotechnology, and biochemistry* **2016,** 80, 13-22.

(12) Takada, M.; Sumi, M.; Maeda, A.; Watanabe, F.; Kamiya, T.; Ishii, T.; Nakano, M.; Akagawa, M., *Biochem. Biophys. Res. Commun.* **2012,** 428, 315-320.

(13) Uchida, W.; Wakabayashi, M.; Ikemoto, K.; Nakano, M.; Ohtani, H.; Nakamura, S., *Chem. Phys. Lett.* **2015,** 620, 13-18.

(14) Ikemoto, K.; Sakamoto, H.; Nakano, M., *Chem. Cent. J* **2012,** 6, 57.

(15) Ishida, T.; Doi, M.; Tomita, K.; Hayashi, H.; Inoue, M.; Urakami, T., *J. Am. Chem. Soc.* **1989,** 111, 6822-6828.

(16) Becke, A. D., *Phys. Rev. A* **1988,** 38, 3098-3100.

(17) Becke, A. D., *J. Chem. Phys* **1993,** 98, 5648-5652.

(18) Hohenberg, P.; Kohn, W., *Phys. Rev. B* **1964,** 136, B864-B871.

(19) Lee, C.; Yang, W.; Parr, R., *Phys. Rev. B* **1988,** 37, 785-789.

(20) Frisch, M.; Trucks, G.; Schlegel, H.; Scuseria, G.; Robb, M.; Cheeseman, J.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G., Gaussian 09, revision D. 01. In ed.; Gaussian, Inc., Wallingford CT: 2009.

(21) Zhang, Z.; Tillekeratne, L.; Kirchhoff, J. R.; Hudson, R. A., *Biochem. Biophys. Res. Commun.* **1995,** 212, 41-47.

(22) Pedersen, C. J., *J. Am. Chem. Soc.* **1967,** 89, 7017-7036.

(23) Guo, M.-L., *Acta Crystallogr. Sect. Sect. E: Struct. Rep. Online* **2004,** 60, m1684-m1685.

(24) Olovsson, G.; Olovsson, I.; Lehmann, M., *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.* **1984,** 40, 1521-1526.

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Unusual ionic bond and solubility mechanism of Na_nPQQ (n = 0–4) crystals

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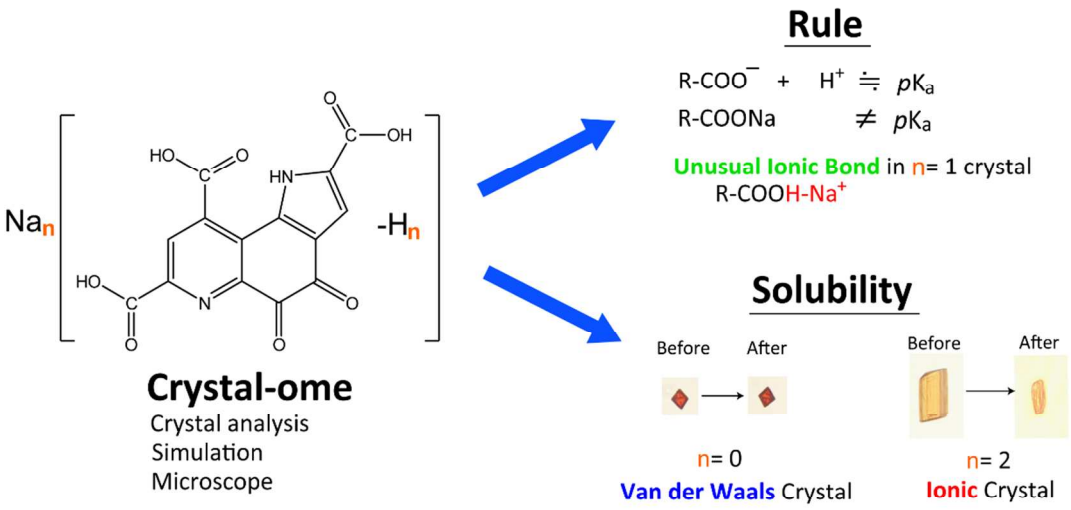

Whole-crystal analysis (crystal-ome) is applied to Na_nPQQ (n = 0–4). While the deprotonation sites are dependent on the p*K*a value, the location of Na cannot be explained by p*K*a. Na_1PQQ exhibited an unusual ionic bond COOH–Na^+. The difference in the solubility of the van der Waals (Na_0PQQ) and ionic (Na_2PQQ) crystals is explained by a combination of molecular dynamics simulations and experiments that define the crystal surfaces.