CRYSTAL GROWTH & DESIGN

Article

pubs.acs.org/crystal

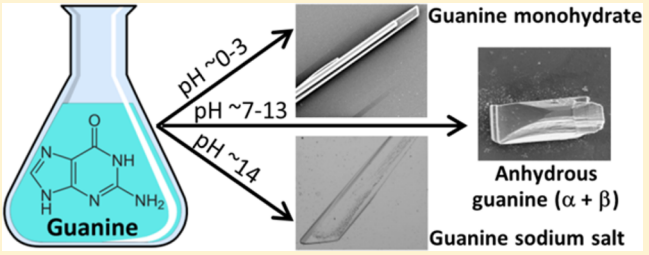
Guanine Crystallization in Aqueous Solutions Enables Control over Crystal Size and Polymorphism

Dvir Gur,[†] Maria Pierantoni,[†] Neta Elool Dov,[‡] Anna Hirsh,[⊥] Yishay Feldman,[§] Steve Weiner,[†] and Lia Addadi*[,†]

[†]Departments of Structural Biology, [‡]Organic Chemistry, [⊥]Department of Materials and Interfaces, and [§]Chemical Research Support, Weizmann Institute of Science, Rehovot, 76100, Israel

Supporting Information

ABSTRACT: Anhydrous guanine crystals are among the most widespread organic crystals used by organisms to produce structural colors. The main advantage of guanine is its exceptionally high refractive index in the reflecting direction (~1.8). For the same reason, guanine is a promising candidate material for a variety of different optical applications. Crystallization of guanine is challenging and usually involves using polar aprotic organic solvents such as dimethyl sulfoxide (DMSO). Here, we show that the crystallization of guanine from aqueous solutions is possible under conditions that provide control over crystal polymorphism and size. Using this approach we were able produce large crystals of the elusive guanine monohydrate phase. We were also able to rationalize the formation of the different phases obtained as a function of which tautomer of guanine is stable in solutions of varying pH.



1. INTRODUCTION

Some of the most brilliant colors in nature are produced by the interaction of light with structured materials, causing reflection or scattering of light. The use of these biogenic structural colors has evolved in parallel to pigmentation and many animals use a combination of the two to produce their coloration.[1−4] The use of structural colors is widespread in organisms of all the animal kingdoms and also in plants. Examples can be found in arthropods,[5−7] mollusks,[8] fish,[9−12] birds,[13,14] reptiles,[15−18] and in a variety of plants, such as the *Pollia condensata* fruit.[19] The colors are used for a variety of purposes, such as communication, thermo regulation,[20,21] vision enhancement, and camouflage.[20] There are several advantages of structural colors over pigment-based coloration for the control of brightness, hue, directionality, and polarization.[22−24] Thin guanine crystal platelets are used as highly reflective elements by many organisms.[6−8,11,15,16,25−28] The reasons why guanine crystals have evolved to fulfill this function, and how the crystals form, are not well understood.

There are three different known phases of crystalline guanine: guanine monohydrate and two polymorphs of anhydrous guanine, α and β.[29−31]Only anhydrous guanine was reported to be present in biogenic systems.[15,28,31,32] Thin plate-like crystals of β anhydrous guanine produce the broadband reflectance found in silvery fish and spiders,[25,31,33] and block-shaped β anhydrous guanine crystals scatter light, producing the white color characteristic of certain spiders.[25,31] Anhydrous β-guanine is also found underneath the cuticle of certain crustaceans, such as the sapphirinidae copepods, where it produces light induced tunable photonic crystals.[32,34] The polymorphic form of anhydrous guanine found in panther chameleons,[15] in different groups of lizards and in mollusc eyes.[8] Interestingly, in all cases where the polymorph was determined (spiders, fish and copepods), the biogenic crystal phase was only composed of the β polymorph,[31] whereas the α polymorph was obtained only in vitro.[30]

The widespread use of anhydrous guanine in nature is probably due to its exceptionally high refractive index, $n \approx 1.8$, in the reflected direction,[25,35] much higher than the refractive index of water, $n = 1.33$. This is especially significant in a multilayer reflector (1D photonic crystal consisting of an array of thin alternating layers) since the ratio of reflected to transmitted light at an interface between two materials is directly related to the difference in their refractive indices.[36] The optical properties of anhydrous guanine, together with the fact that it is nonhazardous and easy to obtain, makes it a promising candidate material to be incorporated into artificial systems. In fact, guanine is being used in a variety of products in industries, such as cosmetics, paints, and jewelry.[37−39] Guanine was recently incorporated into sophisticated systems with dynamic control over crystal orientation, providing magnetically tunable reflectivity.[40,41] However, all these systems use anhydrous guanine crystals extracted from biological specimens, namely, fish scales. The use of fish crystals limits the possibilities of varying crystal dimension, morphology, and material quantity for industrial applications. The main reasons

Received: April 13, 2016
Revised: July 6, 2016
Published: July 8, 2016

DOI: 10.1021/acs.cgd.6b00566
Cryst. Growth Des. 2016, 16, 4975−4980

impeding the use of synthetic anhydrous guanine crystals are guanine insolubility in most solvents, and the difficulty of obtaining crystals in the desired thin and wide plate morphology.

The first determined structure of guanine, guanine monohydrate, was published in 1971.[29] The structure of guanine monohydrate is important since it may provide significant information on the interactions of water with guanine and its role in DNA mutations.[42−44] Since the structure of guanine monohydrate was reported, many theoretical studies were published on the interactions of guanine and water and on the structure and properties of guanine monohydrate;[45−50] however no experimental studies and data on guanine monohydrate crystals were reported, probably because it is very hard to produce. In fact, the conditions in which the crystals were obtained in the original publication were so harsh, involving evaporation of a dimethylamine solution, so as to be practically prohibitive.

Here we show that the crystallization of guanine from aqueous solutions is not only possible but also provides a means of controlling the crystal phase formed and the crystal size. We were also able to rationalize the formation of the different phases obtained as a function of which tautomer of guanine is stable in solutions of varying pH.

2. RESULTS AND DISCUSSION

In the structures of α and β anhydrous guanine and guanine monohydrate, neighboring guanine molecules form extended hydrogen bonded layers, held together by stacking interactions between the layers (Figure 1A and B). The α and β polymorphs of anhydrous guanine are practically identical in the hydrogen-bonded planes.[31] The difference between the two polymorphs is in the stacking of the layers that are offset one relative to the next along the *c*-axis or the *b*-axis, respectively.

In all different crystalline polymorphs guanine is in the keto-amino form. While in anhydrous guanine the hydrogens are attached to N1 and N7 (G1,7 tautomer, Figure 1C), in guanine monohydrate the hydrogens are attached to N1 and N9 (G1,9 tautomer, Figure 1C). Guanine is practically insoluble in neutral aqueous solutions. However, in aqueous acidic or basic solutions, where the molecules are ionized, guanine is much more soluble (Figure 1D).

We utilize the effect of guanine protonation and deprotonation on its solubility, in order to grow large crystals of guanine. The process involves dissolving guanine powder in either acidic or basic solutions, using HCl or NaOH respectively, and then inducing crystallization by adjusting the pH of the solution. Using this methodology we were surprised that the crystal morphologies differ significantly when carrying out the crystallization in solutions adjusted to different pH regimes. Under acidic conditions (pH 2), the crystals are elongated needles (Figure 2A and B), whereas under basic conditions (pH 10) the elongated crystals have a bulky prismatic appearance (Figure 2C and D).

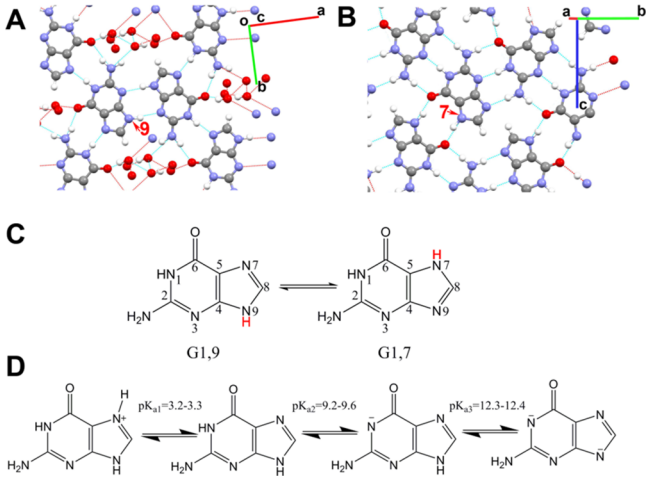


Figure 1. (A) Crystal structure of the hydrogen bonded layer in guanine monohydrate viewed down the *c* axis.[29] (B) Crystal structure of the hydrogen bonded layer in anhydrous guanine in the α or β polymorph viewed down the *a* axis.[30] (C) The two keto-amino low-energy tautomers of guanine. The designations G1,7 and G1,9 identify the nitrogen positions (1, 7, or 9) to which the hydrogen is attached. (D) The pK_a values of guanine, presented using the G1,9 tautomer. The pK_a values are given within a range, due to differences between the reported values.[52−55]

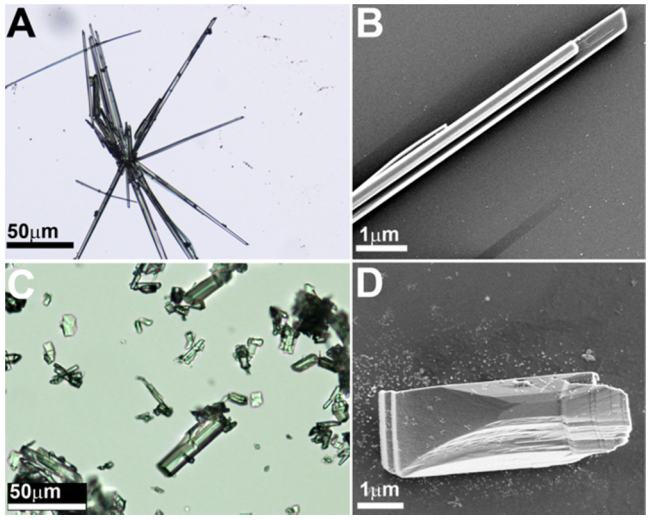


Figure 2. Morphology of guanine crystals grown in solutions with different pH. Crystallization under acidic conditions (pH < 3) results in the formation of large (1−10 mm) needle-like crystals (A and B). Crystallization under neutral and basic conditions results in the formation of prismatic crystals (C and D). A and C: Light microscope. B and D: SEM.

We used X-ray powder diffraction to characterize the crystallographic structure of the crystals with different morphologies. We discovered that whereas the crystals with prismatic bulky morphology are α and/or β polymorphs of anhydrous guanine (Figure 3C), the elongated needle-like crystals consist of the elusive guanine monohydrate phase (Figure 3A). Our previous efforts to obtain this phase of guanine using a large variety of different solvents and conditions were not successful, and to the best of our knowledge there have been no reports on the formation of guanine monohydrate since its structure was published more than 40 years ago.[29]

Experimental FTIR and Raman spectra of the monohydrate phase are not available to date. It is therefore interesting to observe that the FTIR spectrum of guanine monohydrate is distinctly different from the spectrum of anhydrous guanine (Figure 4A). Most evident differences are (i) The broad peaks at 3420 and 3200 cm^{-1} and the peak at 1596 cm^{-1}, corresponding to the water stretching modes υ1 and υ3, and

DOI: 10.1021/acs.cgd.6b00566
Cryst. Growth Des. 2016, 16, 4975−4980

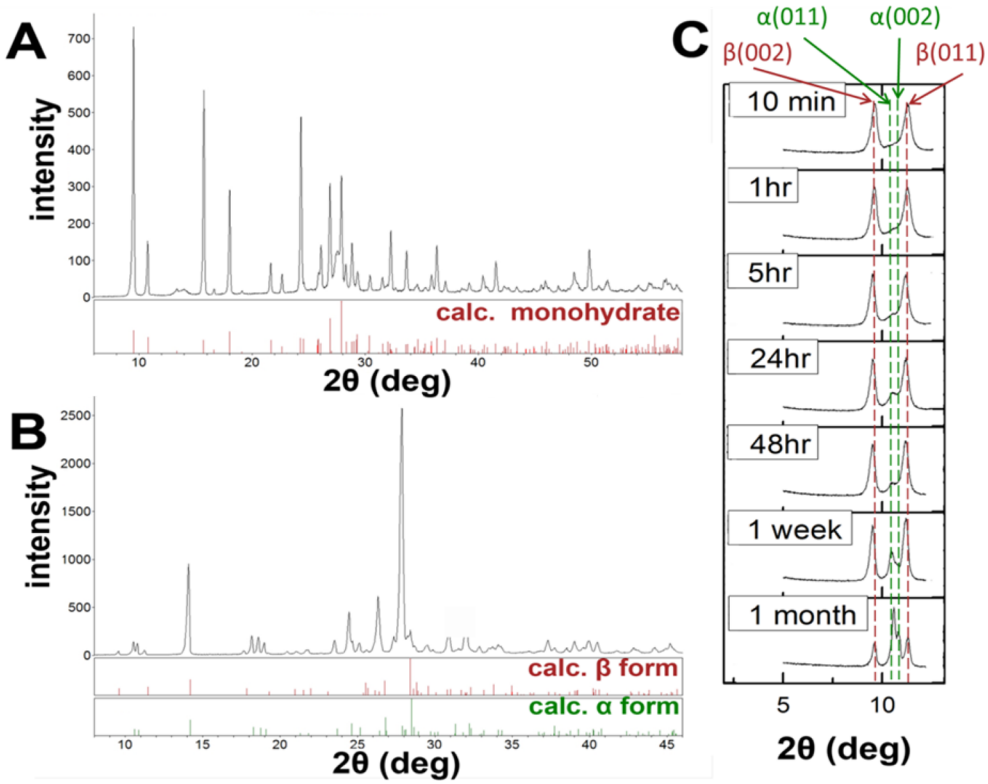


Figure 3. X-ray powder diffraction patterns obtained from crystallizations performed at pH 2 and at pH 11. (A) X-ray powder diffraction of the phase obtained at acidic pH after 24 h (black). There is a very good fit to the calculated diffraction pattern of guanine monohydrate (red). (B) X-ray powder diffraction of the phase obtained at alkaline pH (black) after 24 h. The powder pattern consists of a mixture of the two α (green) and β (red) polymorphs of anhydrous guanine. (C) Evolution in time of the anhydrous guanine polymorphs in suspension, followed through the intensity of the (011) and (002) diffraction peaks in the α polymorph (green lines) and in the β polymorph (red lines). Initially the suspension consists of the pure β form, which in time transforms into the α form.

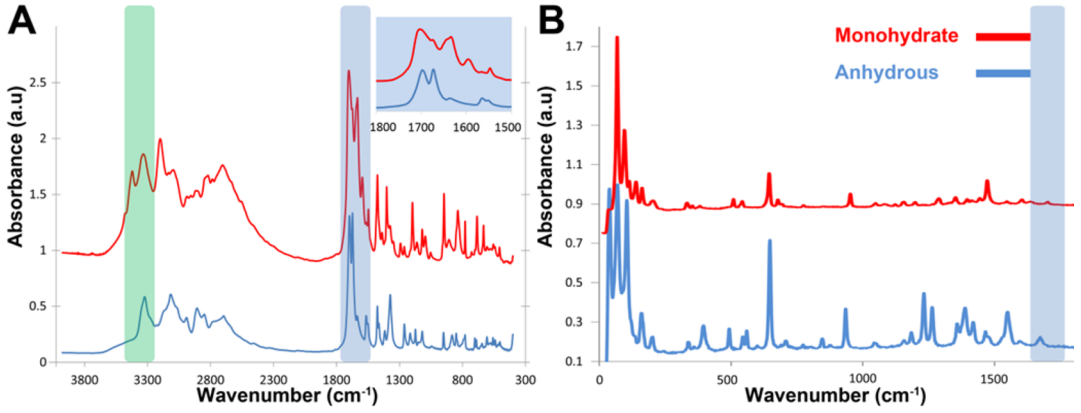


Figure 4. FTIR (A) and Raman (B) spectra of anhydrous guanine (blue line) and guanine monohydrate (red line).

the bending mode $\upsilon 2$ respectively, which are present in the monohydrate but not in the anhydrous phase; (ii) The C═O and NH_2 stretching vibrations appear as multiple peaks between 1633 and 1705 cm^{-1} in the monohydrate phase, whereas they appear as two resolved peaks at 1695 and 1672 cm^{-1} in the anhydrous phase. The Raman spectrum of guanine monohydrate is also distinctly different from the spectrum of anhydrous guanine (Figure 4B). Several vibrations are shifted: specifically, the C═O peak at 1675 cm^{-1} in the anhydrous phase shifts to 1702 cm^{-1} in the guanine monohydrate phase.

We then systematically determined which guanine phase is obtained at different pH values. To this end, we performed the crystallization at specific pH values from either acidic or basic solutions, and the crystals obtained were identified either by FTIR spectroscopy or by X-ray powder diffraction. At extremely basic solutions (~pH 14), the disodium guanine heptahydrate salt is obtained (Figure S1).[51] Guanine monohydrate was obtained from highly acidic solutions (pH ~1−3), whereas at higher pH (~4−6) a mixture of both guanine monohydrate and anhydrous guanine phases was obtained. In neutral and basic solutions (pH ~7−13) the formation of anhydrous guanine is dominant. The kinetically favored polymorph of anhydrous guanine is the β form, which in water suspension transforms with time into the α form

DOI: 10.1021/acs.cgd.6b00566
Cryst. Growth Des. 2016, 16, 4975−4980

(Figure 3C). At high pH the transformation is much slower than at neutral or acidic pH (Figure S7). The transformation requires dissolution-reprecipitation, as demonstrated by the fact that the material does not transform when kept dry.

2.1. Control of Crystal Size. One of the major challenges in guanine crystallization is producing large crystals, as both the monohydrate and anhydrous phases have a tendency to form as very small crystallites. This was also noted in the studies from which the crystal structures were determined.[29,30] Using pH induced crystallization, the interplay between the initial guanine concentration and the rate of lowering the pH allow substantial control over the crystallization process and ultimately over the crystal size. By using solutions with relatively low concentrations of guanine (0.013 M) and adjusting the pH slowly (dropwise), thus inducing fewer nucleation events, relatively large crystals were formed (Figures 2 and S2 C+D). On the other hand, using solutions with high concentrations of guanine (0.13 M) and adjusting the pH rapidly (instantly) induces the formation of very small crystals (Figure S2 A+B).

2.2. Phase Transformation. Heating a crystalline powder of guanine monohydrate results in the removal of water and subsequent phase transformation into anhydrous guanine. This was observed using TGA (Figure 5) and was confirmed by X-ray powder diffraction and FTIR spectroscopy (Figures S3 and S4). The transformation, which occurs at relatively low temperatures (~95 °C), results in the deposition of aggregates of polycrystalline anhydrous guanine with a needle-like morphology (Figure 5 inset A and B). The transformation from monohydrate to anhydrous crystals can also occur at room temperature within a few hours if the crystals are kept in suspension. However, when stored dry under ambient conditions, guanine monohydrate is stable for at least several months and no solid to solid transformation occurs (Figure S5). The transformation within the suspension probably occurs through the dissolution of guanine monohydrate and the formation of anhydrous guanine (Figure S6). In acidic conditions the crystals of guanine monohydrate grow rapidly and are the first to form in the solution, while the crystals of anhydrous guanine take much longer to form. If the suspension is not filtered and dried, anhydrous crystals continue to grow at the expense of the dissolving guanine monohydrate crystals. A suspension which initially contained almost exclusively guanine monohydrate crystals becomes a mixture of both phases within 10 min and within a few hours contains almost only anhydrous guanine.

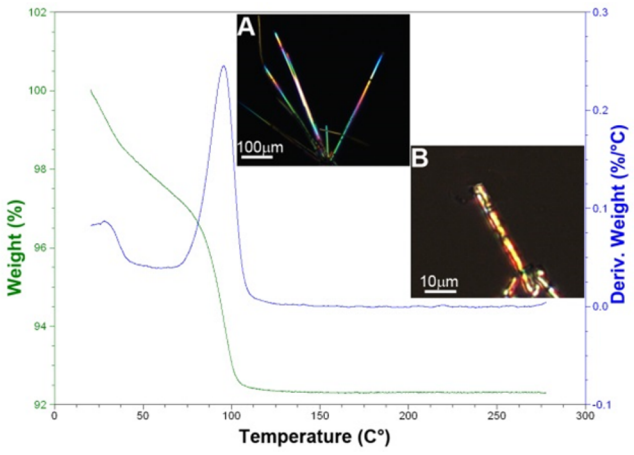


Figure 5. TGA spectra, showing the transformation of guanine monohydrate to anhydrous guanine upon heating. The phase transformation was also confirmed using FTIR spectroscopy and X-ray powder diffraction (Figure S3+S4); green line, weight (%), blue line (weight derivative (%/°C). Insets: Light microscope images through cross polarizers: (A) before heating and (B) after heating.

2.3. Discussion. Here we show that progressive pH adjustment not only enables crystallization of guanine from aqueous solution, but also permits control of the crystal phase and crystal size. These observations raise the following questions: (1) What is the chemical mechanism promoting the formation of different phases at different pH conditions? (2) Do these observations provide insight into biogenic crystal formation? (3) Can this approach replace the use of biogenic fish crystals for industrial purposes? These questions are addressed below.

2.3.1. Chemical Mechanism of Crystal Formation. Under extremely basic conditions (~pH 14), the doubly deprotonated molecular form of guanine is predominant, namely the amino−keto tautomer from which deprotonation from N1 and N7 has occurred (Figure 1D).[51] When the guanine ions reach saturation the guanine disodium heptahydrate salt crystallizes, producing large single crystals.[51] At lower pH, the neutral molecular form of guanine is formed (Figure 1D). Neutral guanine molecules have two different stable keto-amino tautomers (G1,7 and G1,9) (Figure 1C). DFT studies showed that their energies are close one to the other. While in both the gas phase and in solutions with relatively low dielectric constants the G1,7 tautomer is more stable, in solutions with relatively high dielectric constants the G1,9 tautomer is more stable.[45] Under conditions where the G1,7 tautomer is favored, anhydrous crystals should form because neighboring guanine molecules form hydrogen bonds where all hydrogen donors and acceptors are satisfied.[30,31] These hydrogen bond networks result in layers of guanine molecules held together by stacking interactions. This kind of crystal molecular arrangement is energetically stable, and therefore even small amounts of the G1,7 tautomer in the solution will eventually lead to the formation of the anhydrous crystal phase. At acidic pH, the dominant molecular form of guanine is the protonated form (Figure 1D). The crystal structure obtained under these conditions is guanine monohydrate, where the guanine molecule is neutral and the H atoms are attached to N1 and N9, rather than N1 and N7 as in the anhydrous form. In the crystal structure of the monohydrate phase the guanine molecules form hydrogen bonded layers where water molecules take part in the hydrogen bonding. The incorporation of water molecules is probably due to the impossibility to form a layer of guanine molecules where all hydrogen donors and acceptors are satisfied when using the G1, 9K tautomer (Figure S7).

Following Ostwald's rule, the crystals of the least stable guanine monohydrate grow first, while it takes a longer for the crystals of the anhydrous form to grow. Keeping the guanine monohydrate crystals in a suspension where the neutral molecular form of guanine is also present will result in the anhydrous guanine phase slowly replacing the monohydrate phase. This transformation occurs only in the presence of a suspension, via the dissolution of guanine monohydrate crystals and the precipitation of anhydrous guanine crystals. No transformation is observed when the crystals are kept dry under ambient conditions. The phase transformation from guanine monohydrate to anhydrous guanine by heating, however, results in anhydrous guanine crystals that still

DOI: 10.1021/acs.cgd.6b00566
Cryst. Growth Des. 2016, 16, 4975−4980

maintain the overall needle-like morphology of the monohydrate phase, although the individual anhydrous guanine crystals are much smaller.

2.3.2. Insight into Biogenic Crystal Formation. The formation of guanine crystals in biological systems is enigmatic. Huge amounts of insoluble guanine molecules must be transported from the cell nuclei (where they are formed) to the location where crystallization takes place.[56] Taking into account that biological systems set up and control biomineralization using strategies that are very different from those used in vitro, we can still cautiously draw some insight into biological guanine mineralization from the information obtained in vitro. It is unlikely that guanine molecules are soluble in the aqueous cytoplasmic fluids of the cell, because of the very low solubility of guanine in close to neutral pH aqueous solutions. Solubilizing guanine using either acidic or alkaline environments in confined areas is a conceivable in vivo scenario. In fact similar mechanisms exist in shell-forming organisms such as the foraminifera[57] and in silicon deposition of diatoms.[58]

The fact that only the β phase of anhydrous guanine phase was found so far in biological samples, indicates that organisms stabilize the polymorph that is kinetically favored but thermodynamically marginally less stable in vitro[31](Figure 3C). How this is achieved is not known, although preventing a dissolution-reprecipitation process from occurring is sufficient to stabilize the β form indefinitely. We note that both morphology and optical properties are expected to be the same for the two polymorphs, because they share the same fundamental structural units of extended hydrogen bonded layers coupled with stacking interactions. There is thus no obvious advantage to one polymorph relative to the other. The specific guanine polymorph obtained in vivo could also be influenced by the presence of an amorphous precursor that was reported for certain fish[12].

The fact that using this methodology guanine crystallization occurs in an aqueous environment also permits studies on processes/systems which could not have been studied using organic solvents such as DMSO or dimethyl amine (DMA). For example, studying guanine crystallization within liposomes or in the presence of proteins could contribute to the understanding of biological processes that are relevant to biogenic guanine crystal formation.

2.3.3. Replacing Biogenic Fish Crystals for Industrial Use. The use of aqueous solutions provides a more cost-effective and environmentally friendly approach that has the potential to replace the current methodologies for guanine formation, and specifically the use of biogenic anhydrous guanine crystals extracted from fish. Fish guanine crystals are used in a variety of industries including cosmetics and paints.[37−39] While for some of the industrial uses fish guanine is suitable, for others, controlling the size and morphology of the guanine crystals could prove to be highly beneficial. Earlier studies on guanine crystals have shown that control over crystal morphology is possible.[33] A similar approach could be taken using the pH dependent crystallization reported here (a patent was recently filed[59]). Recent studies where biogenic guanine is incorporated into sophisticated tunable devices show intriguing capabilities,[40,41] as in such systems larger and/or thicker crystals of guanine could allow the production of more efficient synthetic light reflectors. Furthermore, the methodology presented here could be used for achieving crystallization of other purines and pteridines, for which the crystal structures have yet to be determined. For example the crystallization of xanthine and isoxhantopterine is possible using this methodology and studies to determine their structure are currently taking place.

3. CONCLUSIONS

In conclusion, herein we present a methodology that enables control over guanine crystal phases, polymorphism and crystal size, by varying the pH of the crystallization solutions. The different phases obtained are a function of the guanine tautomer stable in solutions of varying pH.

4. EXPERIMENTAL SECTION

4.1. Guanine Crystal Formation. Guanine crystals were produced by dissolving 20−200 mg of guanine powder (Sigma−Aldrich), in 10 mL solution of either 1 M HCl or 1 M NaOH. The solutions were then filtered using a PVDF filter (22 μm, Millipore) and 0.1 mL of 1 M NaOH or 1 M HCl, respectively, were added to the solutions to ensure that all of the guanine was dissolved. Guanine crystallization was induced by adjusting the pH of the solution using NaOH or HCl respectively in a drop by drop manner. The pH of the solution was followed using a pH meter and the solution was adjusted to different pH values (1−14). The obtained suspensions were collected and filtered.

4.2. X-ray Powder Diffraction Measurements. The XRD data were collected using a Rigaku Ultima III (with Cu Kα radiation). All XRD data analyses were made with the Jade software package (Materials Data Inc.).

4.3. FT-IR and Micro-Raman Measurements. *FTIR:* A few milligrams of sample were ground in an agate mortar and pestle. About 0.2 mg were left in the mortar, mixed with about 20 mg of KBr, and pressed into a 3 mm pellet using a hand press (Pike). Infrared spectra were obtained at 4 cm^{-1} resolution for 32 scans using a Nicolet iS5 (Thermo).

Micro-Raman: Measurements were made in air at room temperature using LabRAM HR Evolution Microscope. The laser used for these measurements was 633 nm, with an average power of 20 mW. The scattered light was measured using a 300 gr/mm grating, acquiring the Raman spectrum for 10 s and averaging 5 times. The signal was measured using the Andor EMCCD detector (1600 × 200 pixel front illuminated EMCCD camera cooled to −60C), and collected with an Olympus UIS2 50x long working distance objective LMPlanFL N NA 0.5.

4.4. Thermogravimetric Analysis (TGA). Few milligrams of sample was placed in a platinum pan and heated to 300 °C together with an empty reference pan. The heating was performed under nitrogen atmosphere, with a heating rate of 2.5 °C/min using a Shimadzu DTG-50 instrument equipped with a microbalance. The baseline spectrum, obtained under the same conditions with two empty platinum pans, was subtracted from the measurement spectrum.

4.5. Optical and SEM microscopy. Optical images were taken using a Nikon microscope (Eclipse E600 Pol), with or without polarizers in cross position. SEM images were taken using LEO Supra 55 and Ultra 55 (Zeiss).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.cgd.6b00566.

Heptahydrate disodium guanine salt, control of crystal size, X-Ray powder diffraction spectra, FT-IR spectra, solid to solid transformation, and transformation in solution (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: lia.addadi@weizmann.ac.il.

DOI: 10.1021/acs.cgd.6b00566
Cryst. Growth Des. 2016, 16, 4975−4980

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Prof. Leslie Leiserowitz and Prof. Leeor Kronik for helpful discussions and Dr. Iddo Pinkas for micro-Raman data collection. This work was supported by the Israel Science Foundation (Grant 2012\224330*). L.A. and S.W. are the incumbents of the Dorothy and Patrick Gorman Professorial Chair of Biological Ultrastructure and the Dr. Trude Burchardt Professorial Chair of Structural Biology, respectively.

■ REFERENCES

(1) Land, M. *Prog. Biophys. Mol. Biol.* **1972**, *24*, 75.
(2) Parker, A. R. *J. Opt. A: Pure Appl. Opt.* **2000**, *2*, R15.
(3) Fox, D. L. *Animal Biochromes and Structural Colours: Physical, Chemical, Distributional & Physiological Features of Coloured Bodies in the Animal World*; University Press, 1953.
(4) Prum, R. O. *Bird Coloration* **2006**, *1*, 295.
(5) Umbers, K. D. L.; Fabricant, S. A.; Gawryszewski, F. M.; Seago, A. E.; Herberstein, M. E. *Biol. Rev.* **2014**, *89*, 820.
(6) Chae, J.; Nishida, S. *Mar. Biol.* **1994**, *119*, 205.
(7) Mueller, K. P.; Labhart, T. *J. Comp. Physiol., A* **2010**, *196*, 335.
(8) Land, M. *J. Exp. Biol.* **1966**, *45*, 433.
(9) Denton, E. J.; Nicol, J. A. C. *J. Mar. Biol. Assoc. U. K.* **1965**, *45*, 683.
(10) Denton, E. J.; Land, M. F. *Proc. R. Soc. London, Ser. B* **1971**, *178*, 43.
(11) Denton, E. J. *Philos. Trans. R. Soc., B* **1970**, *258*, 285.
(12) Gur, D.; Politi, Y.; Sivan, B.; Fratzl, P.; Weiner, S.; Addadi, L. *Angew. Chem., Int. Ed.* **2013**, *52*, 388.
(13) Hill, G. E.; McGraw, K. J. *Bird Coloration*; Harvard University Press, 2006.
(14) Mason, C. W. *J. Phys. Chem.* **1922**, *27*, 201.
(15) Teyssier, J.; Saenko, S. V.; van der Marel, D.; Milinkovitch, M. C. *Nat. Commun.* **2015**, *6*, 6368.
(16) Rohrlich, S. T.; Rubin, R. W. *J. Cell Biol.* **1975**, *66*, 635.
(17) Rohrlich, S. T. *J. Cell Biol.* **1974**, *62*, 295.
(18) Taylor, J. D.; Hadley, M. E. *Cell Tissue Res.* **1970**, *104*, 282.
(19) Vignolini, S.; Rudall, P. J.; Rowland, A. V.; Reed, A.; Moyroud, E.; Faden, R. B.; Baumberg, J. J.; Glover, B. J.; Steiner, U. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 15712.
(20) Doucet, S. M.; Meadows, M. G. *J. R. Soc., Interface* **2009**, *6*, S115.
(21) Stuart-Fox, D.; Moussalli, A. *Philos. Trans. R. Soc., B* **2009**, *364*, 463.
(22) Kinoshita, S.; Ghiradella, H.; Björn, L. In *Photobiology*; Björn, L. O., Ed.; Springer: New York, 2015.
(23) Kinoshita, S.; Yoshioka, S. *Structural Colors in Biological Systems—Principles and Applications*; Osaka University, Osaka, Japan, 2005.
(24) Srinivasarao, M. *Chem. Rev.* **1999**, *99*, 1935.
(25) Levy-Lior, A.; Shimoni, E.; Schwartz, O.; Gavish-Regev, E.; Oron, D.; Oxford, G.; Weiner, S.; Addadi, L. *Adv. Funct. Mater.* **2010**, *20*, 320.
(26) Lythgoe, J. N.; Shand, J. *J. Physiol.* **1982**, *325*, 23.
(27) Gur, D.; Palmer, B. A.; Leshem, B.; Oron, D.; Fratzl, P.; Weiner, S.; Addadi, L. *Angew. Chem., Int. Ed.* **2015**, *54*, 12426.
(28) Jordan, T. M.; Partridge, J. C.; Roberts, N. W. *Nat. Photonics* **2012**, *6*, 759.
(29) Thewalt, U.; Bugg, C. E.; Marsh, R. E. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1971**, *27*, 2358.
(30) Guille, K.; Clegg, W. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **2006**, *62*, o515.
(31) Hirsch, A.; Gur, D.; Polishchuk, I.; Levy, D.; Pokroy, B.; Cruz-Cabeza, A. J.; Addadi, L.; Kronik, L.; Leiserowitz, L. *Chem. Mater.* **2015**, *27*, 8289.
(32) Gur, D.; Leshem, B.; Pierantoni, M.; Farstey, V.; Oron, D.; Weiner, S.; Addadi, L. *J. Am. Chem. Soc.* **2015**, *137*, 8408.
(33) Levy-Lior, A.; Pokroy, B.; Levavi-Sivan, B.; Leiserowitz, L.; Weiner, S.; Addadi, L. *Cryst. Growth Des.* **2008**, *8*, 507.
(34) Gur, D.; Leshem, B.; Farstey, V.; Oron, D.; Addadi, L.; Weiner, S. *Adv. Funct. Mater.* **2016**, *26*, 1393.
(35) Hinrichs, K.; Silaghi, S. D.; Cobet, C.; Esser, N.; Zahn, D. R. T. *Phys. Status Solidi B* **2005**, *242*, 2681.
(36) Kinoshita, S.; Yoshioka, S.; Miyazaki, J. *Rep. Prog. Phys.* **2008**, *71*, 076401.
(37) Draelos, Z. *Arch. Dermatol.* **2002**, *138*, 1262.
(38) Delattre, N.; Thevenet, L. Int. Patent Application WO2014097134A1, 2014.
(39) Panush, S.; Gelmini, J. M., European Patent Application EP0439112A1, 1991.
(40) Mizukawa, Y.; Iwasaka, M. *J. Appl. Phys.* **2015**, *117*, 17B730.
(41) Iwasaka, M.; Miyashita, Y.; Mizukawa, Y.; Suzuki, K.; Toyota, T.; Sugawara, T. *Appl. Phys. Express* **2013**, *6*, 037002.
(42) Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. *FASEB J.* **2003**, *17*, 1195.
(43) Kasai, H. *Mutat. Res., Rev. Mutat. Res.* **1997**, *387*, 147.
(44) Chworos, A.; Coppel, Y.; Dubey, I.; Pratviel, G.; Meunier, B. *J. Am. Chem. Soc.* **2001**, *123*, 5867.
(45) Yu, L. J.; Pang, R.; Tao, S.; Yang, H. T.; Wu, D. Y.; Tian, Z. Q. *J. Phys. Chem. A* **2013**, *117*, 4286.
(46) Lopes, R. P.; Marques, M. P. M.; Valero, R.; Tomkinson, J.; de Carvalho, L. A. E. B. *Spectroscopy* **2012**, *27*, 273.
(47) Shukla, M. K.; Leszczynski, J. *Int. J. Quantum Chem.* **2010**, *110*, 3027.
(48) Monajjemi, M.; Honarparvar, B.; Nasseri, S. M.; Khaleghian, M. *J. Struct. Chem.* **2009**, *50*, 67.
(49) Gorb, L.; Leszczynski, J. *J. Am. Chem. Soc.* **1998**, *120*, 5024.
(50) Parac, M.; Doerr, M.; Marian, C. M.; Thiel, W. *J. Comput. Chem.* **2010**, *31*, 90.
(51) Gur, D.; Shimon, L. J. W. *Acta Cryst. E* **2015**, *71*, 281.
(52) Jang, Y. H.; Goddard, W. A.; Noyes, K. T.; Sowers, L. C.; Hwang, S.; Chung, D. S. *J. Phys. Chem. B* **2003**, *107*, 344.
(53) Verdolino, V.; Cammi, R.; Munk, B. H.; Schlegel, H. B. *J. Phys. Chem. B* **2008**, *112*, 16860.
(54) Podolyan, Y.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2000**, *104*, 7346.
(55) Greco, F.; Liguori, A.; Sindona, G.; Uccella, N. *J. Am. Chem. Soc.* **1990**, *112*, 9092.
(56) Higdon, C. W.; Mitra, R. D.; Johnson, S. L. *PLoS One* **2013**, *8*, e67801.
(57) Bentov, S.; Brownlee, C.; Erez, J. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 21500.
(58) Vrieling, E. G.; Gieskes, W. W. C.; Beelen, T. P. M. *J. Phycol.* **1999**, *35*, 548.
(59) Gur, D.; Weiner, S.; Addadi, L. Israeli patent application IL 246428, June, 2016.

DOI: 10.1021/acs.cgd.6b00566
Cryst. Growth Des. 2016, 16, 4975−4980