Supplementary Information for

Guanine crystal formation by bacteria

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Additional file 3

Additional methods 1

Synthesis of the guanine crystalline phases using commercial guanine

For crystallization of guanine at basic pH, crystals were produced by adding 20 mg of guanine powder (Sigma−Aldrich) in 600 µL of pure water. The solid was completely dissolved after dropwise addition of a solution of NaOH 1M, adjusting the pH of the solution to a value of 10. The resulting clear solution was left open to the air at room temperature until white crystalline solids were observed. Crystalline material was then collected by centrifugation, washed several times with distilled water and finally dried under vacuum. To obtain the crystals with melanin, filtered melanized liquid culture was used to prepare the starting solution instead of water. As a result, a brown crystalline solid was obtained.

For crystallization of guanine at acid pH, crystals were produced by adding 20 mg of guanine powder (Sigma−Aldrich) in 600 µL of pure water and then, the solid was partially dissolved after dropwise addition of a solution of HCl 1M adjusting the pH of the solution to a value of 2. The resulting suspension was sonicated for 5 minutes and then heated at 90°C for an hour, to obtain a clear solution. Crystalline material was then collected by centrifugation, washed several times with distilled water and finally dried under vacuum. To obtain the crystals with melanin, filtered melanized liquid culture was used to prepare the starting solution instead of water. Due to the low pH, biogenic melanin remained partially soluble and guanine was not fully dissolved after sonication and heating. Therefore, the resulting turbid solution was filtered before crystallization. Crystalline material was then collected by centrifugation, washed several times with distilled water and finally dried under vacuum. As a result, a pale-brown crystalline solid was obtained.

Guaninium chloride dihydrate crystals were produced by adding 20 mg of guanine powder (Sigma−Aldrich) in 500 µL of pure water and then 200 µL of HCl (c) was added (pH ~ 0). The resultant suspension was warmed until complete dissolution of the guanine. The resulting clear solution was left open to the air at room temperature until white elongated needle-like crystals were observed. Crystalline material was then collected by centrifugation, washed several times with distilled water and finally dried under vacuum. To obtain the crystals with melanin, filtered melanized liquid culture was used to prepare the starting solution. Due to the low pH, biogenic

melanin remained partially soluble and guanine was not fully dissolved after sonication and heating, so the resultant turbid solution was filtered before crystallization. Crystalline material was then collected by centrifugation, washed several times with distilled water and finally dried under vacuum. As a result, pale-brown elongated needle-like crystals were obtained; this material resulted to be suitable for single crystal X-ray diffraction studies.

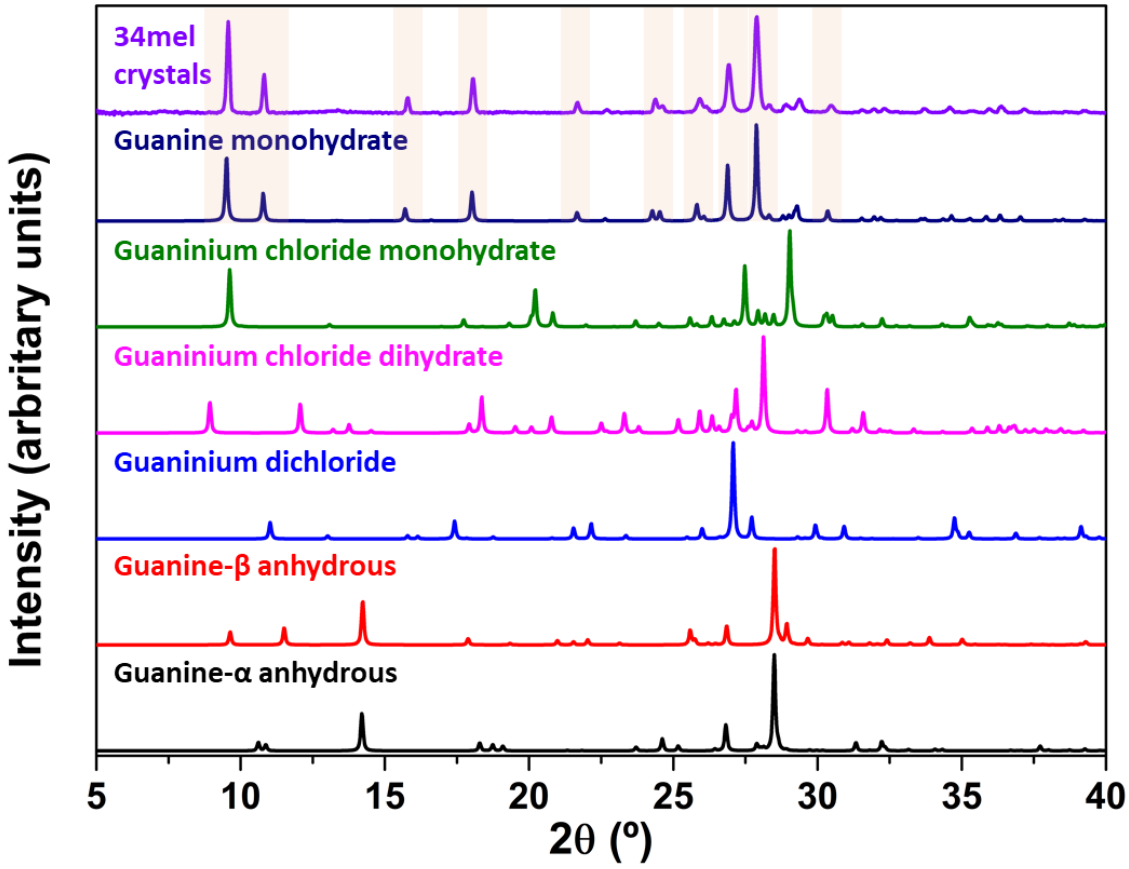

Fig. S7. X-ray diffraction studies of different crystal forms of guanine. Powder X-ray diffraction pattern of the guanine crystals produced by 34mel (violet) and the simulated powder X-ray diffraction pattern obtained from guanine monohydrate (dark blue), guaninium chloride monohydrate (green), guaninium chloride dihydrate (pink), guaninium dichloride (blue), *β* anhydrous guanine (red) and *α* anhydrous guanine (black) single-crystal X-ray data [1-3].

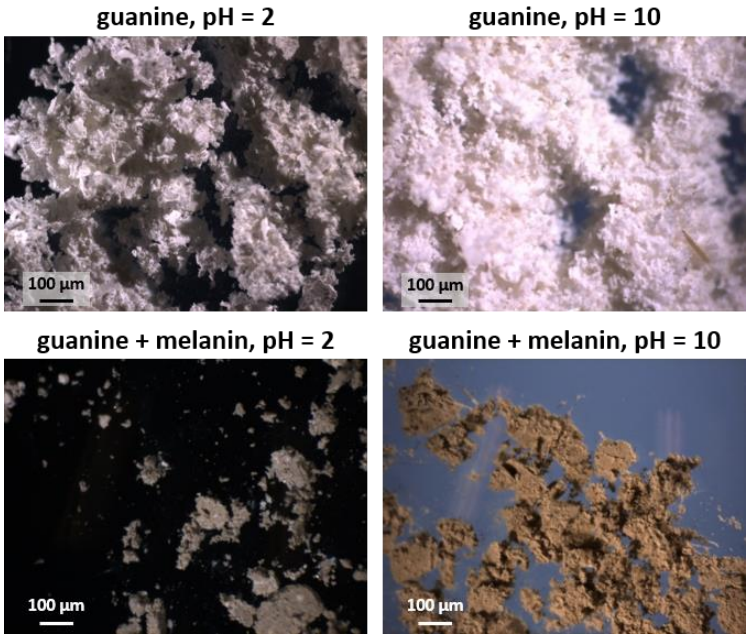

Fig. S8. Crystalline material obtained as result of crystallization experiments of commercial guanine with or without the addition of homogentisate melanin synthesized by 34mel to the solution at different pH conditions. X-ray diffraction patterns of these samples are shown in Additional file 3: Fig. S9.

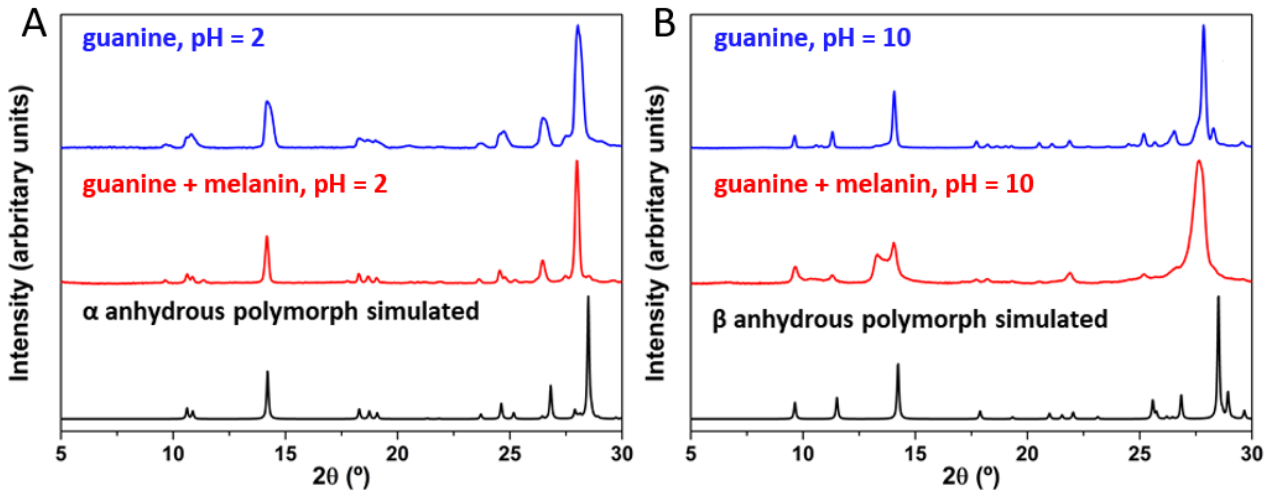

Fig. S9. Powder X-ray diffraction experiments of the crystalline material obtained under different crystallization conditions. Diffraction patterns at pH 2 (**A**) and pH 10 (**B**) for solutions containing commercial guanine with or without melanin produced by 34mel.

Additional methods 2

Single Crystal X-ray diffraction (XRD)

Crystals of guaninium chloride dihydrate crystallized from a solution containing biogenic melanin were studied by single crystal XRD using an Oxford Diffraction Gemini E lab diffractometer with Mo Kα (λ = 0.71 Å) radiation (Additional file 3: Fig. S10). The full data collection was planned using the CrysAlis Pro strategy tool and data was reduced using CrysAlis Pro software (Agilent Technologies, Oxfordshire, UK). A Gaussian method implemented in WinGX [4], or a numerical model [5] was used for the absorption correction. Using Olex2 [6] the structures were solved by Intrinsic phasing employing ShelXL [7] and refined with the ShelXL [8] package using Least Squares minimization. Non-hydrogen atoms were anisotropically refined. Hydrogen atoms were mostly included at geometrically calculated positions with thermal parameters derived from the parent atoms. Hydrogen atoms attached to the water molecules were located on Fourier maps, fixed, and given isotropic displacement parameters depending on the parent atoms. Chloride anions disorder was modeled over two sets of sites with occupancies of 0.26 and 0.74 (Additional file 3: Table S1). Disorder was not modeled for solvent molecules and is represented through large ellipsoids. Additional Crystallographic information is shown in table S1. Crystallographic data for guaninium chloride dihydrate - melanin have been deposited into the Cambridge Structural Database under the deposition number CCDC 2156488.These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures

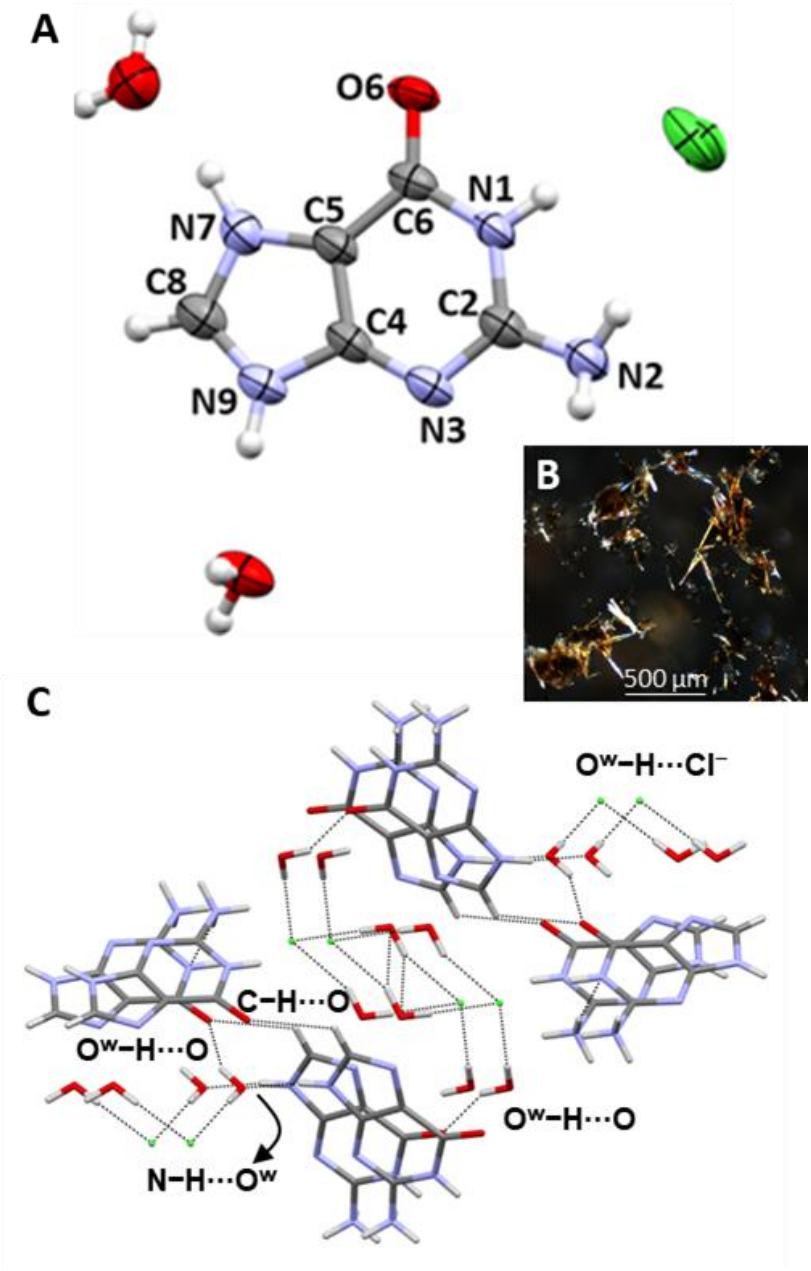

Fig. S10. Structure of guaninium chloride dihydrate crystallized in presence of melanin produced by 34mel determined by single crystal X-ray diffraction. **A** ORTEP plot of the asymmetric unit with displacement ellipsoids drawn at 50% probability level, with numbering. **B** Image of the crystals seen through a polarized light microscope. **C** H-bonded supramolecular network.

Table S1. Crystal data and structure refinement for guaninium chloride dihydrate crystallized in presence of melanin produced by 34mel.

Crystal data and structure refinement for Guaninium chloride dihydrate - melanin	
Empirical formula	$C_5H_{10}ClN_5O_3$
Formula weight	223.63
Temperature/K	298
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	4.8276(10)
b/Å	13.417(2)
c/Å	14.6730(14)
α/°	90
β/°	94.127(14)
γ/°	90
Volume/Å^3	947.9(3)
Z	4
ρ_{calc} g/cm^3	1.567
μ/mm^{-1}	0.395
F(000)	464
Crystal size/mm^3	0.1 × 0.05 × 0.05
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	8.242 to 57.544
Index ranges	-5 ≤ h ≤ 6, -14 ≤ k ≤ 18, -19 ≤ l ≤ 18
Reflections collected	6247
Independent reflections	2201 [R_{int} = 0.1620, R_{sigma} = 0.1534]
Data/restraints/parameters	2201/0/148
Goodness-of-fit on F^2	0.992
Final R indexes [I ≧ 2σ (I)]	R_1 = 0.0907, wR_2 = 0.2024
Final R indexes [all data]	R_1 = 0.1606, wR_2 = 0.2638
Largest diff. peak/hole / e Å^{-3}	0.43/-0.49

Bond lengths for Guaninium chloride dihydrate - melanin		
Atom	**Atom**	**Length(Å)**
O1	C2	1.231(5)
N1	C1	1.370(5)
N1	C2	1.389(6)
N2	C1	1.336(5)
N2	C3	1.334(6)
N3	C4	1.373(6)
N3	C5	1.309(5)
N4	C3	1.382(5)
N4	C5	1.328(6)
N5	C1	1.321(6)
C2	C4	1.420(5)
C3	C4	1.381(6)

Atomic occupancy for Guaninium chloride dihydrate - melanin	
Atom	***Occupancy***
Cl1	0.78(5)
Cl2	0.22(5)

Hydrogen bonds for Guaninium chloride dihydrate-melanin

D	**H**	**A**	**d(D-A) (Å)**	**D-H-A (°)**
O2	H2A	O11	2.786(5)	151.9
O3	H3B	Cl22	2.95(4)	153.3
N4	H4	O2	2.687(5)	170.8
N3	H3	O3	2.645(6)	169(4)

1 + x, 3/2 - y,-½ + z; -1+x, 3/2 - y ,-½ + z

Table S2. Comparative analysis of different guaninium chloride dihydrate single crystal X-ray diffraction data

	a	b	c	α	β	γ	Space Group	Ref.
Guaninium chloride dihydrate (GUANCD01)	4.8708(7)	13.237(3)	14.638(2)	90	93.906	90	$P2_1/c$	[9]
Guaninium chloride dihydrate (GUANCD02)	4.8587(11)	13.228(3)	14.612(3)	90	93.862(4)	90	$P2_1/c$	[10]
Guaninium chloride dihydrate-melanin	4.8276(10)	13.417(2)	14.6730(14)	90	94.127(14)	90	$P2_1/c$	this work

Supplementary References

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