

Zuschriften



Die dreifarbigige Lumineszenz eines in Gegenwart von Proflavin gezüchteten Einkristalls von Kaliumhydrogenphthalat (gezüchtet, zu 200 μm dünnen Scheiben geschnitten und photographiert von J. Benedict) diente als Inspiration für das gezeigte Wasserfarbenbild von Leonel Vasquez. In ihrer Zuschrift auf den folgenden Seiten beschreiben M. Brustolon, B. Kahr, A. L. Rohl et al., wie Luminophore auf die Oberflächenladung von wachsenden Kristallflächen ansprechen.

Luminescent Probes of Crystal Growth: Surface Charge and Polar Axis Sense in Dye-Doped Potassium Hydrogen Phthalate**

Antonio Barbon, Marco Bellinazzi, Jason B. Benedict, Marina Brustolon,* Sean D. Fleming, Sei-Hum Jang, Bart Kahr,* and Andrew L. Rohl*

Luminescent labels have been a mainstay of biological chemists eager to illuminate specific noncovalent interactions.^[1] Crystal growth from solution is also controlled by specific noncovalent interactions, but luminescent probes have not been used likewise. Unlike rhodamine or green fluorescent protein covalently tethered to a protein in a cell and dangled into the cytoplasm, close packed crystals do not have the space, according to conventional wisdom, to accommodate such labels. It is now well established that crystal growth far from equilibrium can drive mixed crystal formation even when guest molecules are many times larger than those of the host.^[2] We take advantage of a luminescent probe to visualize the specificity of a guest for growing crystal faces of potassium hydrogen phthalate (commonly abbreviated as KAP for potassium acid phthalate). We have interpreted our experimental observations with calculated molecular electrostatic potential surfaces (EPS), yet another device widely used by biochemists, in this case to evaluate ligand docking sites,^[3] that has rarely^[4] found application in studies of crystal growth because of the difficulties associated with evaluating the electrostatic potential of a periodic surface. Using experiment and theory in tandem, we show

how dyes respond to the surface charges of KAP while at the same time establishing the absolute sense of its polar axis.

KAP crystals grow from aqueous solution by spontaneous nucleation at room temperature as {010} plates with the following additional forms: {111}, {11 $\bar{1}$ }, {110}, and occasionally {121}.^[5] They are built of alternating bilayers of potassium ions and polar herringbone arrays of hydrogen phthalate ions stacked along [010] in the space group *Pca*2₁ (Figure 1). The sense of the polar [001] axis, to which all (*hkl*) indices refer, has been assigned by anomalous scattering of X-rays.^[6] The Bijvoet method was similarly applied to all of the mixed crystals described herein.

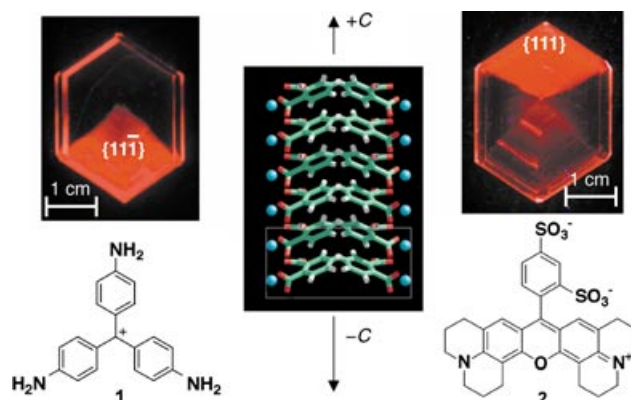


Figure 1. Images of black light (366 nm) irradiated KAP crystals grown in the presence of **1** and **2**. The crystals are similarly oriented with respect to the polar axis indicated in the view of the lattice along [100].

KAP crystals orient and overgrow more than 100 dyes in micromolar concentrations.^[7,8] Dyed crystals typically show patterns of color consistent with facets that have different affinities for the luminophores. Dyes are thus contained in polyhedral growth sectors, subvolumes of the crystals that have grown through particular faces.^[2] Two representative mixed crystals containing basic fuchsin (**1**) and sulforhodamine 101 (**2**) are shown in Figure 1. We have observed that cations such as **1** primarily recognize {11 $\bar{1}$ } growth sectors while anions such as **2** tend to recognize {111}. These findings cannot be reduced to firm rules. Several puzzling exceptions have been observed, most notably 3,6-diaminoacridine (**3**), the focus of this work, that decorates KAP to form remarkable “tricolore” crystals.

With **1** and **2**, only one end of each KAP mixed crystal is luminescent (Figure 1), which reveals the presence of a polar axis. Other chemical methods for assigning the sense of a polar axis include reactions with gases,^[9] the Kundt powder test in pyroelectric crystals,^[10] and changes in crystal habit, surface topography, and symmetry with so-called “tailor-made additives” of known activity.^[11] Given the sensitivity of luminescence, mixed crystal formation with fluorescent probes is an attractive alternative.

To understand the selectivity of **1** and **2** for KAP, we first determined the energetically stable surfaces that correspond to the (111), (11 $\bar{1}$), and (110) faces. For any given set of indices (*hkl*), there can be a number of discrete surfaces, especially

[*] Dr. A. Barbon, M. Bellinazzi, Prof. M. Brustolon
Dipartimento di Chimica Fisica
Università di Padova
Via Loredan 2, 35131 Padova (Italy)
Fax: (+39) 049-827-5135
E-mail: m.brustolon@chfi.unipd.it

J. B. Benedict, Dr. S.-H. Jang, Prof. B. Kahr
Department of Chemistry
University of Washington
Box 351700, Seattle, WA 98195-1700 (USA)
Fax: (+1) 206-685-8665
E-mail: kahr@chem.washington.edu

Dr. S. D. Fleming, Assoc. Prof. A. L. Rohl
Nanochemistry Research Institute
Curtin University of Technology
PO Box U 1987, Perth, 6845 (Australia)
Fax: (+61) 8-9266-4699
E-mail: andrew@power.curtin.edu.au

[**] This work was supported by the US National Science Foundation, the Petroleum Research Fund of the American Chemical Society, the Italian Ministry of Universities and Research by the FIRB program (Nano-organizzazione di molecole ibride inorganiche/organiche con proprietà magnetiche ed ottiche), the Government of Western Australia via the Premier's Research Fellowship Program, and the Australian Research Council by the International Researcher Exchange (IREX) scheme.

for multicomponent crystals. It is well established for simple salts that the electrostatic contribution to the lattice energy is dominant; others have used only the electrostatic contribution to study surface energetics.^[12] This approximation facilitates the quick evaluation of stable surfaces and their electrostatic potentials for any salt without going through the intensive process of deriving a force field. With the program GULP,^[13] we calculated the attachment energies of all surface terminations of the (111), (11 $\bar{1}$), and (110) faces by using the partial charges from the CVFF force field.^[14] We then calculated the EPS for the stable faces by using our GDIS open source code.^[15,16] The basis for the preponderance of anions recognizing (111) and cations recognizing (11 $\bar{1}$) is immediately evident in the EPS (Figure 2); the former is exclusively positive whereas the latter is exclusively negative. The (110) is comparatively neutral; symmetry requires no net charge but the molecular surface is slightly negative.

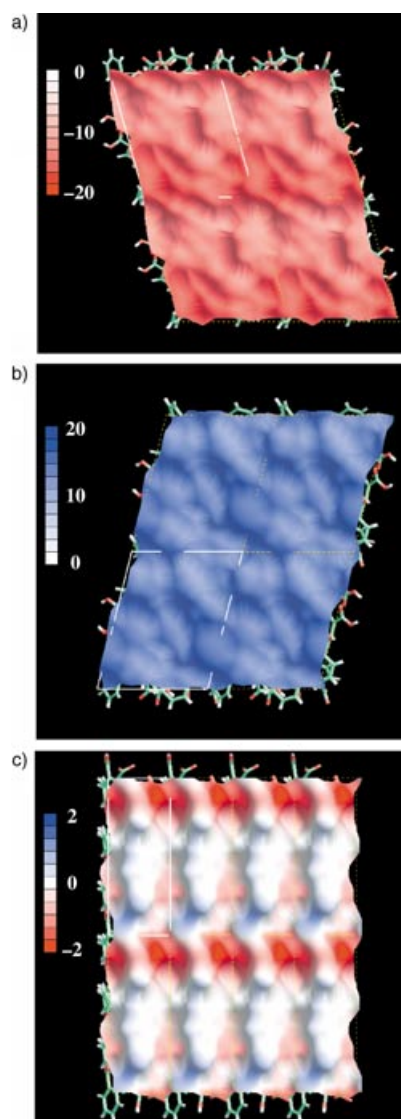


Figure 2. EPS for the stable terminations of a) (11 $\bar{1}$), b) (111), and c) (110) surfaces of KAP. Red regions are areas of negative potential and blue regions positive potential in units of electron volts. Note that the maximum values for (c) are ten times smaller than that of (a) and (b).

Can EPS explain the activities of probes such as **3** (Figure 3), which has distinct properties after having associated with each of the principal faces? Compound **3** recognizes both ends of the KAP polar axis and the (110) face in different

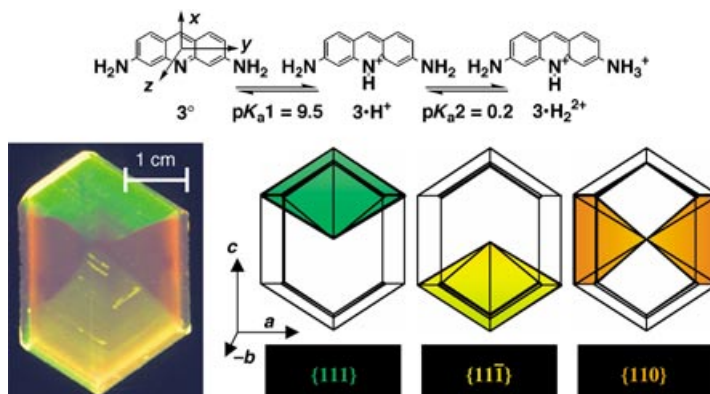


Figure 3. Top: protonation scheme for **3**. Bottom: fluorescence image of a KAP crystal grown in the presence of **3** with idealized representations of the event indicating the growth sectors and their respective colors of luminescence.

states that are easily distinguished by the colors of light the growth sectors emit (Figure 3). Presumably **3** was overgrown in different states of protonation including the commonly observed forms: neutral (**3**⁰), monocation (**3**·H⁺), and dication, (**3**·H₂²⁺).

The visible λ_{max} for the {110} and {111} sectors are 481 and 460 nm, respectively (Figure 4).^[17–19] The corresponding spec-

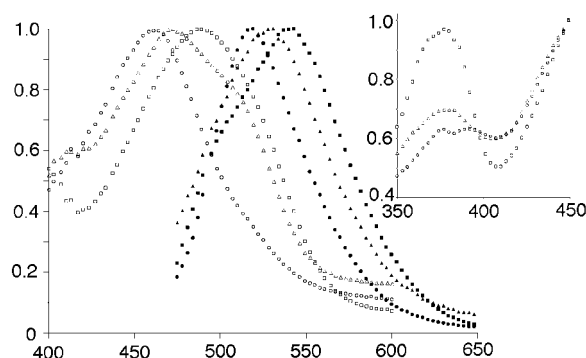


Figure 4. Absorption (open) and luminescence (solid) spectra for the individual {111} (○), {11 $\bar{1}$ } (△), and {110} (□) sectors of KAP with **3**.

trum for the {11 $\bar{1}$ } sector is a superposition of the two bands characteristic of {110} and {111}. The peak at 460 nm is characteristic of **3**·H⁺, the predominant solution species at neutral pH, while the species absorbing at 481 nm has been assigned to **3**·H₂²⁺. A characteristic companion peak in the near UV only found in the {11 $\bar{1}$ } and {110} further supports assignment of the latter.^[18] The corresponding photoluminescence energies were 519, 531, and 542 nm for {111}, {11 $\bar{1}$ }, and {110}, respectively.^[17,20]

How can we use the electrostatic potentials to rationalize the photophysics of the mixed crystals? Sensibly, we can say that the strongly negative (11 $\bar{1}$) surface adsorbs any cation,

$3\cdot\text{H}^+$ or $3\cdot\text{H}_2^{2+}$. The slightly negative (110) attracts only the dication; for an electrostatic interaction to be substantive either the field or the corresponding charge must be large. Furthermore, the (110) face contains areas of negative potential separated by 6.5 Å, which closely matches the two primary sites of positive charge on $3\cdot\text{H}_2^{2+}$. Why then does the strongly electropositive surface adsorb $3\cdot\text{H}^+$ at all?

To refine our understanding of the orientation of **3** in KAP, we turned to a time-resolved electron paramagnetic resonance (TREPR) analysis of its photo-excited triplet states.^[21] EPR lines are typically narrow relative to the overall spectral dispersion, thus enabling the identification of subpopulations not detected in electronic spectroscopy.^[22]

The {110} sector that contained only the dication was EPR silent. For the {111} and $\{11\bar{1}\}$ sectors, more than a dozen distinct triplet orientations were detected, though in each case a single orientation was predominant, which was five times more intense than any other. The elements of the zero-field splitting (ZFS) tensors that represent the dipolar couplings of the unpaired electrons in the triplet states could be extracted by a first-order fit of the angular dependencies of the spectral splittings in the principal planes. The presence of magnetically inequivalent sites in the crystal structure gives rise to a doubling of curves that become isochronous when the field is along a crystallographic axis. Each curve can be matched with two different curves in another plane giving rise to ZFS tensors, whose off diagonal elements differ in sign. This ambiguity was resolved by comparing the two sets of principal values with those obtained from the spectra of glasses and powders of individual sectors. The eigenvalues for one of the two magnetically inequivalent sites for {111} and $\{11\bar{1}\}$ are reported in Table 1.

Table 1: Eigenvalues (D_i) in Gauss (± 10 G) of ZFS tensors.

	X	Y	Z
{111}	−43	−361	402
$\{11\bar{1}\}$	−28	−390	418
aprotic	−140	−334	474
pH 12	−133	−344	477
pH 5	−71	−412	483
pH 2	−55	−365	410

The principal directions *X*, *Y*, and *Z* can be assigned to the molecular axes, as shown in Figure 3, on the basis of experimental and calculated results for similar triplet systems.^[23] The eigenvectors (Figure 5) indicate distinct orientations within KAP. In $\{11\bar{1}\}$ the mean plane of **3** is almost parallel (20°) to one of the symmetry related faces, whereas in {111} the molecular plane is nearly perpendicular (87°) to its respective face. Edge approach in the latter case minimizes repulsive electrostatic interactions, thus enabling adsorption of a cation to a formally positive surface.^[24] TREPR spectra in disordered frozen matrices of 1:1 propionitrile/butyronitrile (PBCN) and 1:4 glycerol/water were obtained at various pHs (Table 1). The crystal data most closely resemble those from low pH glasses, which suggests that the signals in {111} and $\{11\bar{1}\}$ are from $3\cdot\text{H}_2^{2+}$, the predominant triplet species at pH < 4.^[17] These data seem to contradict the absorbance that

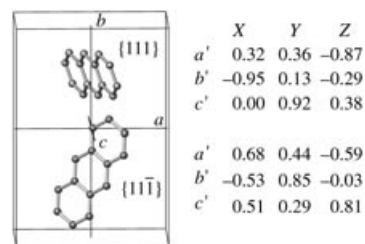


Figure 5: The orientation of the acridine nucleus of **3** in the KAP unit cell derived from the eigenvectors (right) of TREPR data. The prime symbol on the axes in the eigenvector tables indicates a normalized unit cell length.

indicates that $3\cdot\text{H}^+$ is the predominant species in {111}; however, the $3\cdot\text{H}_2^{2+}$ triplet signal arises as a result of excited-state protonation.^[25] Excitation and subsequent protonation of the $3\cdot\text{H}^+$ ground state leads to the formation of a long-lived triplet, presumably a consequence of an energy barrier for deprotonation, thus allowing the molecule to return to the $3\cdot\text{H}^+$ ground state. After excitation, molecules initially in the $3\cdot\text{H}_2^{2+}$ ground state return to their starting configuration at rates faster than excited-state proton transfer or intersystem crossing. Hence, the {110} sectors that contain only $3\cdot\text{H}_2^{2+}$ remain EPR silent.

Given recent advances in, and adoption of, sensitive light detectors,^[26] there is every reason to expect that luminescent probes of noncovalent chemistry during crystal growth from solution can be as revealing as they are in illuminating associations that underlie biological chemistry. First, we must learn to translate the language in which the probes are broadcasting their information.

Received: January 23, 2004

Revised: June 9, 2004 [Z53839]

Keywords: crystal growth · electrostatic interactions · EPR spectroscopy · luminescence · luminescent probes

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