nature

https://doi.org/10.1038/s41586-019-1918-4

Supplementary information

Antagonistic cooperativity between crystal growth modifiers

In the format provided by the authors and unedited

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Supplementary Information for

Antagonistic cooperativity between crystal growth modifiers

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1. The correlation between step velocity and kink blocker concentration.

The modifiers MQ and AQ inhibit step motion by adsorbing to the kinks and blocking the access of the solute molecules (Fig. 1c) [1]. We use the correlation between step velocity v and the concentrations of the two inhibitors, measured in our previous work [1], to evaluate the parameters that govern their adsorption.

To derive an expression for the correlation between the bulk concentration of a kink blocker c_B and v we rely on a fundamental tenet of crystallization that v is proportional to the kink density n_k [2-6]. We designate the fraction of kinks occupied by inhibitor molecules θ_B. Often, the fraction of kinks accessible to the adsorbing inhibitors is limited owing to spatial overlap between adsorbed inhibitor molecules, fast kink dynamics, or alternative mechanisms [1,7]. We account for this limitation by a parameter ξ, $0 < \xi < 1$. With these assumptions

$$v \propto n_{k,B} = n_{k,0}(1 - \xi\theta_B) \text{ and } \quad v = v_0(1 - \xi\theta_B)\,, \tag{1}$$

where v_0 and $n_{k,0}$ are the values of v and n_k in the absence of inhibitors and $n_{k,B}$ is the density of kinks unoccupied by kink blocker B present in the bulk solution. As $\theta_B \to 1$ v asymptotically reaches its low threshold

$$v_{sat} = v_0(1 - \xi) \text{ and } v_0\xi = v_0 - v_{sat}\,. \tag{2}$$

Combining Equations (1) and (2),

$$v = v_0 - (v_0 - v_{sat})\theta_B\,. \tag{3}$$

We model θ_B with the Langmuir adsorption isotherm, which assumes finite number of equivalent adsorption sites, no interactions between adsorbed molecules, a desorption rate

independent of the solution concentration of the inhibitor, and equilibrium between inhibitors on the surface and in the bulk,

$$\theta_B = \frac{K_{LB} c_B}{1+K_{LB} c_B}\,, \tag{4}$$

where K_{LB} is the Langmuir adsorption constant, and obtain

$$v = v_0 \left(1 - \xi \frac{K_{LB} c_B}{1+K_{LB} c_B}\right). \tag{5}$$

Equation (5) predicts that as $c_B$increases, v decreases monotonically from v_0 at c_B = 0 to its saturation value v_{sat} defined in Equation (2). Our previous work revealed that the correlations of v to the concentrations of MQ and AQ comply with this trend [1].

To evaluate $K_{LB}$and ξ from experimentally measured $v(c_B)$ correlations, we follow a similar model for the density of growth sites on crystal surfaces in the presence of inhibitors [7] and linearize Eq. (5) in coordinates $v_0(v_0 - v)^{-1}$ as a function of c_B^{-1}, sometimes referred to as "Bliznakov coordinates",

$$v_0 - v = (v_0 - v_{sat}) \frac{K_{LB} c_B}{1+K_{LB} c_B}\,, \tag{6}$$

$$\frac{v_0}{v_0 - v} = \frac{v_0}{v_0 - v_{sat}} + \frac{v_0}{v_0 - v_{sat}} \frac{1}{K_{LB} c_B}\,. \tag{7}$$

The intercept of this linear relation equals the parameter $\xi = v_0/(v_0 - v_{sat})$, according to Eq. (2). The ratio of the intercept to the slope yields the Langmuir constant K_{LB}.

In a biomimetic solution in CBSO, both MQ and AQ form complexes with hematin [1]. The predominant complexes for both MQ and AQ consist of two hematin molecules bound to one inhibitor molecule, with binding constants 14 and 510 mM^{-2}, respectively [1]. The complexation affects the rate of step propagation in two ways: First, it lowers the concentration of free hematin c_H and enforces a depression of the step velocity proportional to the decrease in hematin concentration [8]. To account for the depressed crystallization driving force, we evaluate the concentration of free hematin $[H]$ and proportionally adjust the value of v_0. Second, molecular recognition between the kinks and the inhibitor would likely eliminate one of the inhibitor forms present in the bulk solution (free inhibitor and inhibitor-hematin complex) as an active inhibitor. Evidence in the literature falls short of identifying the active inhibitor. We determine the values of $K_{LB}$and ξ assuming two alternative scenarios: 1. v is inhibited by the unliganded kink blockers and $c_B = [B]$, and 2. The active inhibitor species is the kink blocker-hematin complex and $c_B = [H_2B]$.

The values of [H], [MQ], [AQ], [H_2MQ], and [H_2AQ] at the analytical concentrations of hematin, MQ, and AQ, for which v was measured [1], are computed in Extended Data Table 3. The correlation between $v_0(v_0 - v)^{-1}$ versus c_B^{-1} are plotted in Extended Data Figure 4. The values of $K_{LB}$and ξ emerging from the analyses based on the assumptions $c_B = [B]$ and $c_B = [H_2B]$ are displayed in Extended Data Table 4.

2. Evaluation of the decrease of the surface free energy of the step edge Δγ due to adsorption of inhibitors to the kinks.

To evaluate Δγ, we assume that $n_{s,B}$, the density of kinks occupied by adsorbed kink blockers, is equal to the number of blocker molecules adsorbed at the kinks. With this assumption, $n_{s,B}$ complies with the Gibbs equation of adsorption[9]

$$\frac{n_{s,B}}{a} = -\frac{d\gamma}{d\mu_B} \tag{8}$$

where a is the step height and μ is the chemical potential of the inhibitors in the solution. Owing to the low inhibitor concentrations, the interactions between inhibitor molecules are weak and $\mu_B = \mu_{B0} + k_B T \ln c_B$, where μ_{B0} is the standard value of μ_B, k_B is the Boltzmann constant and T is temperature. The sum of occupied and free kinks $n_{s,B} + n_{k,B} = n_{k,0}$ and $n_{s,B} = \theta_B \xi n_{k,0}$.

We integrate the Gibbs equation and substitute the relations for $n_{s,B}$ and μ_B to obtain

$$-\Delta\gamma = \int_0^{c_B} \frac{n_{s,B}}{a} d\mu_B = \int_0^{c_B} \frac{\theta_B \xi n_{k,0}}{a} k_B T d \ln c_B = \int_0^{c_B} \frac{K_{LB}}{1+K_{LB}c_B} \frac{n_{k,0}\xi}{a} k_B T dc_B \tag{9}$$

and

$$-\Delta\gamma = \frac{k_B T \xi n_{k,0}}{a} \int_0^{c_B} \frac{K_{LB} dc_B}{1+K_{LB}c_B} = \frac{k_B T n_{k,0}}{a} \xi \ln(1 + K_{LB}c_B)\,. \tag{10}$$

On the {100} faces of β-hematin crystals the step height $a \cong 1.2$ nm [8]. The characteristic molecular length is 0.73 nm in the $\vec{b}$ direction and 0.80 nm in the $\vec{c}$ direction [10]. The kink density along the step is high [8] and can be approximated as the reciprocal average of these two lengths, leading to $\frac{n_{k,0}}{a} \approx 10^{18}$ m^{-2}. With this, the constant in Eq. (10) that is independent of the nature of the inhibitor is $\frac{k_B T n_{k,0}}{a} \approx 4 \times 10^{-3}$ J m^{-2}. Using the values of $K_{LB}$and ξ determined from the $v(c_B)$ correlations in the presence of MQ and AQ (Extended Data Tables 4), we obtain that the dimensionless coefficients, which account for the adsorption of inhibitors at the kinks, $\xi \ln(1 + K_{LB}c_B)$, are in the range 0.55 – 0.67. The estimates for MQ and AQ and for the two alternative inhibitor species, B and H_2B, are close. Using these values, we obtain that the predicted decrease in the surface free energy of the step edge $-\Delta\gamma$ is close to 3 mJ m^{-2}, consistent with the 3 and 5 mJ m^{-2} measured for AQ and MQ, respectively.

3. The velocity of steps in the presence of both step pinners and kink blockers

We consider the motion of steps governed by three phenomena: 1. Step curvature imposed by step pinners adsorbed on the terraces; the chemical potential of curved steps is elevated [9], enforcing lower effective supersaturation and slower step growth. 2. Kink blockers, which bar association of solute molecules to some of the kinks, and 3. Decrease of the surface free energy γ of the step edges that regulates how they respond to greater curvature.

We define the supersaturation as $\Delta\mu = k_B T \ln(c_H/c_e)$, where $\Delta\mu = \mu - \mu_e = \mu - \mu_c$, μ, μ_e, and μ_c being the chemical potentials of the solute in the growth solution, in the solution at

equilibrium with the crystal, and in the crystal, respectively; c_H is are the solute concentration; and c_e is its solubility. This definition relies on the assumption that the ratio of solute activity coefficients in the supersaturated solution and at equilibrium is close to one. For brevity we introduce σ, defined as $\sigma \equiv \Delta\mu/k_BT = \ln(c_H/c_e)$. According to transition state theory the rate of a reversible chemical reaction is proportional to $\left(\exp\frac{\Delta\mu}{k_BT} - 1\right)$[11]. Accounting for geometric factors and for the change in molecular density upon crystallization, the step velocity relates to the supersaturation as

$$v = \beta\Omega c_e\left(\exp\frac{\Delta\mu}{k_BT} - 1\right) = \beta\Omega(c_H - c_e), \tag{11}$$

where Ω is the molecular volume in the crystal, and β is the kinetic coefficient [5,12]. Linear $v(C)$ correlations have been recorded for numerous crystals growing in supersaturated solutions of solute only [13].

We assume the step pinners adsorb on the crystal surface with molecular surface concentration $n_P = \theta_P/S_0$, where θ_P is the surface coverage and S_0 is the area per adsorption site. For inhibitors with molecular size commensurate with that of the solute, S_0 is the dot product of the two lattice vectors parallel to upward facing crystal face. We evaluate the average distance between stoppers as

$$L = n_P^{-0.5} = \sqrt{S_0/\theta_P}. \tag{12}$$

To evaluate θ_P, we assume that adsorption follows the Langmuir relation between θ_P and the solution concentration of the step pinners c_P,

$$\theta_P = K_{LP}c_P/(1 + K_{LP}c_P), \tag{13}$$

where K_L is the respective Langmuir constant. We assume that pinners separated by distance L enforce step curvature with radius $R = L/2$. We obtain

$$R = \frac{1}{2}\sqrt{S_0\frac{1+K_{LP}c_P}{K_{LP}c_P}} \tag{14}$$

According to the Gibbs-Thomson relation, a step with radius of curvature R is in equilibrium with a solution of concentration c_{eR}, which is greater than the equilibrium concentration of a straight step c_e by

$$\ln\left(\frac{c_{eR}}{c_e}\right) = \frac{\Omega\gamma}{Rk_BT}, \tag{15}$$

where γ is the surface free energy of the step edge. Eq. (15) reverses the familiar application of the Gibbs-Thomson relation to define the radius R_c of a two-dimensional cluster in equilibrium with a solution with concentration c_H greater than the solubility c_e

$$R_c = \frac{\Omega\gamma}{k_BT\ln(c_H/c_e)}. \tag{16}$$

Unifying Eqs. (14) and (15), we obtain an expression for the critical supersaturation σ_d, below which a step cannot penetrate the inhibitor fence and growth ceases

$$\sigma_d = \ln\left(\frac{c_{eR}}{c_e}\right) = \frac{2\Omega\gamma}{k_B T}\sqrt{\frac{1}{S_0}\frac{K_{LP}c_P}{1+K_{LP}c_P}}. \quad (17)$$

The supersaturation range between 0 and σ_d, in which step growth is arrested, is often called "the dead zone". This expression for σ_d is identical to the one derived by Weaver and De Yoreo [14,15].

From Eq. (15), the solution concentration at equilibrium of a curved step with radius R is

$$c_{eR} = c_e \exp\frac{\Omega\gamma}{Rk_B T} \quad (18)$$

At a concentration $c > c_{eR}$, a step with radius of curvature R grows with velocity

$$v_R = \beta\Omega(c_H - c_{eR}) = \beta\Omega\left(c_H - c_e \exp\frac{\Omega\gamma}{Rk_B T}\right) = v_\infty \frac{c_H - c_e \exp\frac{\Omega\gamma}{Rk_B T}}{c_H - c_e}, \quad (19)$$

where v_∞ is the velocity of a straight step, defined in Eq. (11). An equivalent form of this relation, derived by Weaver, *et al.* [14], is

$$v_R = v_\infty\left(1 - \frac{e^{\sigma_d}-1}{e^{\sigma}-1}\right). \quad (20)$$

Introducing the step pinning mechanism of inhibitor action, Cabrera and Vermilyea [16] evaluated the effective step velocity as the geometric mean of the maximum velocity, corresponding to uninhibited step propagation in the gaps between the stoppers and following the kinetic law in Eq. (11) and, and the minimum v due to stopper inhibition according to Eq. (19). In the resulting step motion law, this mode of averaging invoked an exponent of ½ on the expression in parenthesis in Eq. (19). Instead of using the geometric mean, subsequent models assume that the measured step velocity corresponds to an arithmetic mean [17] or just to the minimum velocity expressed by Eq. (19) [18]. The impact of additional factors—kink blocking by the pinners, step anisotropy, variable step curvature [19], stopper mobility, slow stopper adsorption, and others—on the correlation between the step velocity and the surface distribution of step pinners is summarized in Lee-Thorp, *et al.* [20]. Even the most elaborate models fail to predict quantitatively experimentally measured $v(c_P)$ correlations [20]. Importantly, recent solid-on-solid kMC results reveal that uniformly distributed pinners inhibit moving steps to the same degree as randomly positioned pinners of the same surface concentration, i.e., the surface distribution of step pinners has no effect on the step velocity [21]. To avoid the ambiguity associated with averaging inhibited, partially inhibited, and uninhibited step velocities, below we only model the effects of kink blockers on the velocity of steps with greatest curvature enforced by step pinners, v_R. The evaluated v_R, contained by step pinners at concentration c_P would correspond to the measured step velocity if the approximation introduced De Yoreo [15] and Weaver, *et al.* [14 18], that $v = v_R$ and follows Eqs. (19) and (20), is valid.

Combining Eq. (19) with Eq. (14) on the relation between the step radius of curvature R, dictated by the surface concentration of step pinners, and the bulk concentration of step pinners c_P engenders a relation between the v_R and c_P

$$v_R = \frac{v_\infty}{c-c_e}\left(c_H - c_e \exp\frac{2\Omega\gamma}{k_BT}\sqrt{\frac{K_{LP}c_P}{S_0(1+K_{LP}c_P)}}\right). \quad (21)$$

According to Eq. (21), the velocity $v_R$of a step with curvature R enforced by step pinners with solution concentration c_P decreases monotonically from v_∞ at $c_P = 0$ and $R = \infty$ to zero as c_P increases.

Kink blockers present in the solution at concentration c_B, which adsorb to the kinks with Langmuir constant K_{LB}, impact step motion in two ways: 1. they diminish the number of kinks available for solute association, and 2. they lower the surface free energy of the step edge. These two consequences can be quantified by Eqs. (5) and (10), respectively, leading to

$$v_R = \beta\Omega\left(1 - \xi\frac{K_{LB}c_B}{1+K_{LB}c_B}\right)\left(c_H - c_e \exp\left(\frac{2\Omega}{k_BT}\left(\gamma_0 - \frac{k_BTn_{k,0}}{a}\xi\ln(1+K_{LB}c_B)\right)\sqrt{\frac{K_{LP}c_P}{S_0(1+K_{LP}c_P)}}\right)\right), \quad (22)$$

where $v_0 = \beta\Omega(c_H - c_e)$ is the step velocity in the absence of both step pinners and kink blockers and γ_0 is the surface free energy of the step edge in the absence of kink blockers.

4. Cooperativity between step pinners and kink blockers in suppressing step motion.

According to a recently proposed rationale, the cooperativity between two inhibitors, designated 1 and 2, of any process can be classified as synergistic, additive, or antagonistic [22] using the value of a combination index, *CI*, defined as

$$CI = \frac{C_{1,combination}}{C_{1,alone}} + \frac{C_{2,combination}}{C_{2,alone}}, \quad (23)$$

where C_i are the concentrations (or doses, for pharmaceuticals [23,24]) of the respective inhibitor applied to attain a certain degree of inhibition in combination with the other inhibitor or acting alone. If CI= 1, the cooperativity between the two inhibitors is considered additive; CI values lower than one are viewed as indications of synergistic cooperativity, and CI > 1 designates antagonism. The definition of CI in Eq. (23) implies that only functional relations of a response V (process rate, disease spread, parasite survival, etc.) to C_1 and C_2, which contain linear combinations of these two variables, could result in additive cooperativity. Such dependencies are of the type $V(C_1, C_2) = V(A_1C_1 + A_2C_2)$ and suggest that both inhibitors employ the same mechanism of action, in which inhibition is a linear function of the inhibitor concentration. For instance, if $V(C_1, C_2)$ is written as $V = V_0/(1 + A_1C_1 + A_2C_2)$, then 50% inhibition by each of the inhibitors acting alone, for which $V_0/(1 + A_1C_i) = 0.5V_0$, is driven by $C_1 = 1/A_1$ and $C_2 = 1/A_2$. If the two inhibitors are combined, the equality $\frac{V_0}{1+A_1C_1+A_2C_2} = 0.5V_0$ is satisfied by $C_1 = 1/2A_1$ and $C_2 = 1/2A_2$. Substituting the four concentration in Eq. (23) yields *CI* = 1. This evaluation yields the same result for *CI* for any other inhibition fraction. An example of a crystal growth inhibition mechanism that complies with the requirements for additive synergy is suppression of step

motion by kink blockers, discussed by Sangwal [26,27] and others [28,29]. Functional dependencies that contain the product of C_1 and C_2 can lead to both behaviors. For example, if $V(C_1, C_2) = V(A_1C_1 + A_2C_2 + BC_1C_2)$, then using $A_1C_{1,alone} = A_2C_{2,alone} = A_1C_{1,combination} + A_2C_{2,combination} + BC_{1,combination}C_{2,combination}$ gives $A_1C_{1,alone} = A_2C_{2,alone} = C_{1,combinatino} + C_{2,combination} + AC_{1,combination}C_{2,combination}CI = 1 - \frac{BC_{1,combination}C_{2,combination}}{A_1C_{1,combination}+A_2C_{2,combination}+AC_{1,combination}C_{2,combination}}$ which is always less than 1 for positive B (i.e., the cooperativity is synergistic) and greater than one for negative B (corresponding to antagonistic cooperativity).

Attempts to correlate mechanisms of action of more than one inhibitor of crystal growth to the strict definition of cooperativity in Eq. (23) have been rare and mostly focus on cooperativity between crystal growth inhibitors in bulk crystal production. These investigations concluded that the two inhibitors enhance each other's activity if they suppress adjacent crystal faces [25,26].

The dependence of the step velocity on the concentrations of step pinners and kink blockers $v_R(c_P, c_B)$ in Eq. (22) suggests a definition of cooperativity based on the molecular mechanisms of action of the individual inhibitors. The cooperativity between a step pinner at concentration c_P and a kink blocker at concentration c_B would be additive if $v_R(c_P, c_B)$ belongs to a straight line anchored at $v_R(c_P, 0)$ and $v_R(0, c_B)$. Values of $v_R(c_P, c_B)$ that are lower or greater that the value belonging to the straight line between $v_R(c_P, 0)$ and $v_R(0, c_B)$ define, respectively, synergistic or antagonistic cooperativity. Unfortunately, the dependence $v_R(c_P, c_B)$ in Eq. (22) is more complex than the three functional forms discussed above. Neither c_P nor c_B can be expressed as analytical functions of v_R at a certain fraction inhibition. Consequently, cooperativity analysis in terms of Eq. (23) of the combined action of step pinners and kink blockers on step growth can only be carried out numerically. Plots of $v_R(c_P, c_B)$ using independently determined governing parameters (Extended Data Fig. 5) reveal that for low degrees of inhibition, $v_R/v_0 > 0.7$, the $v_R(c_P, c_B)$ is concave, i.e., a combination of a the two inhibitors achieves a certain degree of inhibition at concentrations c_p and c_B that are lower than those at the straight line connecting $v_R(c_P, 0)$ and $v_R(0, c_B)$, an indication of synergistic cooperativity. In contrast, for $v_R/v_0 < 0.7$ the $v_R(c_P, c_B)$ surface is convex indicating that the concentrations of the two inhibitors required to reach a certain degree of inhibition at higher than those along the line connecting the anchor points at $v_R(c_P, 0)$ and $v_R(0, c_B)$, a signature of antagonistic cooperativity. This partition between synergistic and antagonistic cooperativities conforms with data on the inhibition of hematin step velocity v by the joint action of CQ/AQ, CQ/MQ, QN/MQ, and QN/AQ in Fig. 2 c, e and the respective isobolograms in Extended Data Fig. 2b.

Eq. (22) affords an alternative approach to rationalize the cooperativity between step pinners and kink blockers. We distinguish between synergistic and antagonistic cooperativities using the signs of the derivatives $\left(\frac{\partial v_R}{\partial c_P}\right)_{c_H, c_B}$ and $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H, c_P}$. If both derivatives are negative in a certain range of c_P and c_B, the cooperativity between step pinners and kink blockers would be synergistic. Transition from negative to positive values of one of these derivatives would mark a crossover to antagonistic cooperativity.

The derivative $\left(\frac{\partial v_R}{\partial c_P}\right)_{c_H,c_B}$ characterizes the response of the step velocity to increasing concentration of step pinners c_P at constant concentrations of solute $c_H > c_e$ and kink blockers c_B,

$$\left(\frac{\partial v_R}{\partial c_P}\right)_{c_H,c_B} = \left(\frac{\partial v_R}{\partial R}\right)_{c_H,c_B}\left(\frac{\partial R}{\partial c_P}\right)_{c_H,c_B}. \tag{24}$$

Here R is the radius of step curvature governed by the separation between step pinners adsorbed on the surface, quantified by Eq. (14), so that

$$\left(\frac{\partial R}{\partial c_P}\right)_{c_H,c_B} = -\frac{S_0}{8RK_{LP}c_P^2}.$$

From Eq. (19),

$$\left(\frac{\partial v_R}{\partial R}\right)_{c_H,c_B} = \frac{v_B}{c_H-c_e}\frac{\Omega\gamma_B}{R^2k_BT}c_e\exp\frac{\Omega\gamma_B}{Rk_BT}.$$

We obtain

$$\left(\frac{\partial v_R}{\partial c_P}\right)_{c_H,c_B} = -\frac{v_B}{c_H-c_e}\frac{S_0}{8R^3K_{LP}c_P^2}\frac{\Omega\gamma_B}{k_BT}c_e\exp\frac{\Omega\gamma_B}{Rk_BT}. \tag{25}$$

where $v_B = v_0\left(1-\xi\frac{K_{LB}c_B}{1+K_{LB}c_B}\right)$ is the step velocity in the presence of kink blockers only, Eq. (5), and $\gamma_B = \gamma_0 - \Delta\gamma = \gamma_0 - \frac{k_BTn_{k,0}}{a}\xi\ln(1+K_{LB}c_B)$, Eq. (10), is the surface free energy of the step edge reduced by the adsorption of kink blockers; both v_B and γ_B are positive. With this, $\left(\frac{\partial v_R}{\partial c_P}\right)_{c_H,c_B}$ is always negative, i.e., the addition of step pinners lowers the step velocity at any concentration of kink blockers.

The derivative $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P}$ predicts how step motion reacts to the presence of kink blockers. Since kink blockers affect both the density of kinks available for solute incorporation and the step edge surface free energy, the expression for this derivative contains two summands,

$$\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P} = \left(\frac{\partial v_R}{\partial v_B}\right)_{c_H,c_P}\left(\frac{\partial v_B}{\partial c_B}\right)_{c_H,c_P} + \left(\frac{\partial v_R}{\partial \gamma_B}\right)_{c_H,c_P}\left(\frac{\partial \gamma_B}{\partial c_B}\right)_{c_H,c_P}. \tag{26}$$

From Eqs. (19), (5) and (10),

$$\left(\frac{\partial v_R}{\partial v_B}\right)_{c_H,c_P} = \frac{1}{c_H-c_e}\left(c_H - c_e\exp\frac{\Omega\gamma_B}{Rk_BT}\right),$$

$$\left(\frac{\partial v_B}{\partial c_B}\right)_{c_H,c_P} = -v_0\frac{\xi K_{LB}}{(1+K_{LB}c_B)^2}, \tag{27}$$

$$\left(\frac{\partial v_R}{\partial \gamma_B}\right)_{c_H,c_P} = -\frac{v_B}{c_H - c_e}\frac{\Omega}{Rk_BT}c_e \exp\frac{\Omega\gamma_B}{Rk_BT}, \text{ and}$$

$$\left(\frac{\partial \gamma_B}{\partial c_B}\right)_{c_H,c_P} = -\frac{\xi k_BT n_{k,0}}{a}\frac{K_{LB}}{(1+K_{LB}c_B)}.$$

Substituting these expressions into Eq. (26),

$$\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P} = -\frac{v_0}{c_H - c_e}\frac{\xi K_{LB}}{(1+K_{LB}c_B)^2}\left(c_H - c_e \exp\frac{\Omega\gamma_B}{Rk_BT}\right) + \frac{v_B}{c_H - c_e}\frac{\Omega n_{k,0}}{Ra}\frac{\xi K_{LB}}{(1+K_{LB}c_B)}c_e \exp\frac{\Omega\gamma_B}{Rk_BT}. \quad (28)$$

The first summand in this expression describes the reduction of the number of kinks available for solute incorporation and it is, accordingly, always negative. The second summand accounts for the reduction of surface free energy due to the adsorption of kink blockers to the step edge and it is positive. The difference between the magnitudes of the two summands determines the sign of $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P}$. Since $\left(\frac{\partial v_R}{\partial c_P}\right)_{c_H,c_B}$ is always negative, the sign of $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P}$ determines the type of cooperativity between step pinners and kink blockers. At low values of c_P, the enforced step curvature radius R is high and the correction to c_e, which reduces the magnitude of the first summand of Eq. (28), is small. This negative second summand dominates the expression on the left-hand side of Eq. (28) and determines the sign of $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P}$, leading to synergistic cooperativity of the kink blockers with the step pinners, as observed in Fig. 2 c and e in the same c_P range. Elevated concentrations of step pinners lead to high density of adsorbed pinners on the crystal surface and low R. The correction to c_e increases, which minimizes the driving force for step growth, $\left(c_H - c_e \exp\frac{\Omega\gamma_B}{Rk_BT}\right)$ in the first summand of Eq. (28). The dominance of the second positive summand enforces a positive sign of $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P}$, indicating that higher concentrations of step pinners lead to faster v_R. Remarkably, positive values of $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P}$ that correspond to the counterintuitive faster step growth at higher kink blocker concentrations are only possible in the presence of step pinners. If c_P = o, $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P} = \left(\frac{\partial v_B}{\partial c_B}\right)_{c_H}$ and, according to Eq. (27), is always negative.

Within this line of argument, the line defined by the relation

$$\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P} = 0 \quad (29)$$

may be viewed as another approximate separator of the areas of synergistic and antagonistic cooperativities in the (c_P, c_B) plane. Extended Data Fig. 6 reveals that synergistic cooperativity, defined using the derivate $\left(\frac{\partial v_R}{\partial c_B}\right)_{c_H,c_P}$, is confined to low concentrations of the two inhibitors; by contrast, elevated concentrations of c_P and c_B lead to antagonistic cooperativity.

5. Cooperativity between step pinners and kink blockers in suppressing the generation of new crystal layers by 2D nucleation.

On the (100) surface of β-hematin crystals, new layers are generated by two-dimensional nucleation. The rate of generation of new layers J_{2D} is governed by the supersaturation according to the relation [8,27]

$$J_{2D} = J_0 \exp\left(-\frac{\pi\Omega\gamma^2 h}{\Delta\mu k_B T}\right). \quad (30)$$

In Eq. (30), J_0 accounts for the association of molecules to newly nucleated islands. It is often assumed that J_0 relates to the solute concentration c_H in a manner similar to that of v [27]. Whereas this may be true for relatively large 2D nuclei that form at low supersaturations, the 2D critical radii on hematin crystal surfaces range from 8 to 16 nm (see Fig. 3). At these sizes, the curvature of the nucleated islands may enforce kink structures and densities that diverge significantly from those on straight steps. Thus, the association of kink blockers to the edges of newly nucleated islands may deviate from the mechanism that underlies Eqs. (1) – (7) above. Even though deriving explicit relations on the effects of kink blockers on J_0 is a challenge, it is likely that the association of kink blockers to the edges of the island nuclei will lower J_0.

Both step pinners and kink blockers may affect the ratio in the exponent in Eq. (30). Step pinners adsorbed on crystal surfaces enforce additional curvature on nucleating 2D islands. The excess curvature would effectively increase the solubility. A possible way to account for the solubility increase is through the Gibbs Thomson relation, Eq. (18), which leads to

$$\frac{\Delta\mu_R}{k_B T} = \ln\frac{c_H}{c_e} - \frac{\Omega\gamma}{R k_B T} = \frac{\Delta\mu_0}{k_B T} - \frac{\Omega\gamma}{R k_B T}, \quad (31)$$

where R is the average distance between adsorbed stoppers, defined by Eq. (14), and $\Delta\mu_0$ is the supersaturation. Kink blockers adsorbing on the island edge lower its free surface energy γ, as defined in Eq. (10). Quantitative predictions of the degree of inhibition induced by these mechanisms would be inaccurate owing to the poorly understood structure of the island edge.

Still, these qualitative considerations suggest that step pinners inhibit the 2D nucleation rate by cutting down the effective supersaturation that drives nucleation. Kink blockers inhibit 2D nucleation by suppressing J_0 synergistically with the step pinners, but may weaken the effect of step pinners by lowering γ. Since γ impacts more strongly the nucleation rate J_{2D} than the velocity of propagation of layers v, this effect on γ indicates that the kink blockers constrain the inhibition of J_{2D} by step pinners to a greater extent than that of v. This prediction is borne for three of the four tested pairs of inhibitors (CQ/MQ, CQ/AQ, and QN/AQ) by the isobolograms in Extended Data Fig. 2 and the Combination Index values in Extended Data Table 1, which reveal stronger antagonism between the two classes of inhibitors in suppressing J_{2D} than in impeding v.

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