

Supporting Information for

Theoretical Simulation of Red Cell Sickling Upon Deoxygenation Based on the

Physical Chemistry of Sick Hemoglobin Fiber Formation

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SI-1: Solubility of mixtures of HbS with other Hb variants as a function of fractional saturation of hemoglobin with oxygen

The following description follows in spirit that given by Cellmer, et al. (in preparation), except that the treatment here is not restricted to a specific type of mixture. The procedure described here is actually designed to compute the solubility as a function of the partial pressure of oxygen; some relationship between partial pressure and fractional saturation, such as that provided by a model description of the Hb oxygen binding curve, is required to establish the functional relationship between fractional saturation and solubility.

The fundamental thermodynamic linkage between solubility and oxygen binding in the two-phase system (solution/polymer) satisfies a Gibbs-Duhem relation for the three-component system of hemoglobin, oxygen and solvent. This relation ultimately takes the form¹

$$\ln \left(\frac{\gamma_s c_s}{\gamma_s^0 c_s^0} \right) = 4 \int_{-\infty}^{\ln(p)} \frac{y_s(p') - y_p(p')}{1 - (1/c_p - v)/(1/c_s - v)} d \ln(p') \quad (\text{SI-1.1})$$

where y_s and y_p are the fractional saturations as a function of oxygen partial pressure (p) of the free tetramers and fibers, respectively. $a_s = \gamma_s c_s$ is the activity of the free tetramers at partial pressure p , where γ_s is the activity coefficient at concentration c_s , and the corresponding quantities with zero superscripts refer to their values at zero pressure; these activities will be discussed in more detail below. c_p is the concentration of hemoglobin in the polymer phase ($= 0.69$ g/cc), and v is the partial specific volume of hemoglobin ($= 0.75$ cc/g). We note here that, with prescriptions for $y_s(p)$ and $y_p(p)$ and for the dependence of the activity coefficient γ_s on the concentration c_s (see below), (SI-1.1) may be transformed into an ordinary differential equation for c_s as a function of $\ln(p)$, greatly facilitating its solution for c_s as a function of oxygen partial pressure.

We focus on situations where tetrameric Hb exists in solution as a mixture of HbS with one or more other Hb variants (HbA, HbF, etc.). We formulate the model for copolymerization of these species, in a manner ultimately equivalent to (SI-1.1), as follows: We begin with a simple expression of overall mass conservation encompassing the Hb tetramers existing in the solution and fiber phases:

$$c_s v_s + c_p v_p = c_0 \quad (\text{SI-1.2})$$

where v_s and v_p are the volume fractions of the solution and fiber phases, respectively, c_s and c_p are the Hb concentrations in the two phases, and c_0 is the total Hb concentration. Noting that $v_s = 1 - v_p$, we have

$$v_p = \frac{c_0 - c_s}{c_p - c_s} \quad (\text{SI-1.3})$$

We consider the general case of q distinct additional variants of Hb which have some probability of copolymerizing with HbS in the fiber; any variants assumed to have the same copolymerization probability may be merged for the purpose of the following computations. We define for each variant the mole fraction x_i in solution, the mole fraction f_i in the fiber phase, and the copolymerization probability ε_i , all pressure-dependent; we also assign an overall mole fraction X_i to each variant. Mass conservation for variant i may be written

$$x_i c_s (1 - v_p) + f_i c_p v_p = X_i c_0 \quad (\text{SI-1.4})$$

which from (SI-1.3) yields

$$x_i c_s (c_p - c_0) + f_i c_p (c_0 - c_s) - X_i c_0 (c_p - c_s) = 0 \quad (\text{SI-1.5})$$

In these equations, the pressure dependence of the unknown quantities c_s , x_i and f_i has been suppressed to simplify the notation.

The fundamental copolymerization equations of Minton² for mixtures of HbS with other Hb variants, incorporating non-ideality and the water activity term required by the Gibbs-Duhem relation, may be written

$$\frac{\gamma_s c_s}{\gamma_s^0 c_s^0} \left(\frac{a_{H_2O}}{a_{H_2O}^0} \right)^n = \frac{1}{\sum_{i=1}^q x_i \varepsilon_i} = \Gamma \frac{c_s}{c_s^0} \quad (\text{SI-1.6})$$

$$f_i = \frac{x_i \varepsilon_i}{\sum_{i=1}^q x_i \varepsilon_i}$$

where γ_s is the activity coefficient and a_{H_2O} is the water activity; as in (SI-1.1), these symbols with superscript 0 refer to the values at zero pressure, and those without superscript refer to quantities at the pressure of interest. n is the total number of water molecules involved in the copolymerization reaction for a single Hb tetramer.

The activity coefficients are themselves functions of the pressure-dependent solution concentrations (solubilities) and reflect excluded volume effects in concentrated protein solutions present in the red blood cell. They are computed from the virial expansion for hard spheres with specific volume V ($=0.79 \text{ cc/g}$) as³

$$\ln(\gamma(c)) = \sum_{k=1}^6 B_{k+1} c^k$$

$$B_2 = 8 V$$

$$B_3 = 15 V^2$$

$$B_4 = 24.48 V^3$$

$$B_5 = 35.3 V^4$$

$$B_6 = 47.4 V^5$$

$$B_7 = 65.9 V^6 \quad (\text{SI-1.7})$$

Using this expression, and a Gibbs-Duhem relation coupling Hb and water, an expression for the water activity may be derived¹

$$\left(a_{H_2O}(c)\right)^n = \exp\left(-\int_0^c \frac{1/c_p - v}{1/c' - v} \left(1/c' + \sum_{k=1}^6 k B_{k+1} c'^{k-1}\right) dc'\right) \quad (\text{SI-1.8})$$

which allows us to write

$$\left(\frac{a_{H_2O}}{a_{H_2O}^0}\right)^n = \exp\left(-\int_{c_s^0}^{c_s} \frac{1/c_p - v}{1/c - v} \left(1/c + \sum_{k=1}^6 k B_{k+1} c^{k-1}\right) dc\right) \quad (\text{SI-1.9})$$

where c_s^0 is the solubility at zero oxygen pressure.

For notational simplicity, we have in (SI-1.6) defined

$$\Gamma \equiv \frac{\gamma_s}{\gamma_s^0} \left(\frac{a_{H_2O}}{a_{H_2O}^0}\right)^n \quad (\text{SI-1.10})$$

which is itself a function of the concentration c_s at the pressure of interest. This allows us to rewrite (SI-1.5) as

$$x_i c_s (c_p - c_0) + \Gamma \frac{c_s}{c_s^0} x_i \varepsilon_i c_p (c_0 - c_s) - X_i c_0 (c_p - c_s) = 0 \quad (\text{SI-1.11})$$

There is one such equation for each of the q non-HbS variants. We can create an additional condition for HbS by invoking overall conservation of mass:

$$\begin{aligned}
 x_S c_s (c_p - c_0) + \Gamma \frac{c_s}{c_0} x_S \varepsilon_S c_p (c_0 - c_s) - X_S c_0 (c_p - c_s) &= 0 \\
 x_S &= 1 - \sum_{i=1}^q x_i \\
 X_S &= 1 - \sum_{i=1}^q X_i
 \end{aligned} \tag{SI-1.12}$$

The combination of (SI-1.11) and (SI-1.12) provides a system of $q+1$ nonlinear equations in $q+1$ unknowns (x_1, \dots, x_q, c_s) , to be solved at each pressure of interest. It is possible to algebraically reduce this system to a single nonlinear equation in c_s , as follows: First, (SI-1.11) is used to express each individual x_i in terms of the single unknown c_s .

$$x_i = \frac{X_i c_0 (c_p - c_s)}{c_s (c_p - c_0) + \Gamma \frac{c_s}{c_0} \varepsilon_i c_p (c_0 - c_s)} \tag{SI-1.13}$$

Then the mass conservation expression in (SI-1.12) is used to likewise express x_S in this fashion. The resulting expression, now itself a function of the single unknown c_s , both explicitly and through the quantity Γ defined in (SI-1.10), is then substituted in the first equation (SI-1.12), yielding a single nonlinear equation in c_s . This algebraic reduction procedure is essentially equivalent to that followed in Cellmer, et al. for a restricted special case. It may be performed explicitly for sufficiently simple specific cases, or implicitly by performing the sequence of operations described above numerically, and

solving the resulting single nonlinear equation for c_s ; alternatively, the equivalent solution may be pursued by directly solving the system of simultaneous equations (SI-1.11) and (SI-1.12).

Within this model for the copolymerization, the system of equations (SI-1.11) and (SI-1.12) provides a general and exact setting (equivalent to (SI-1.1)) for determining the solubility c_s of the mixture as a function of pressure. The crux of its practical application is the specification of the pressure-dependent copolymerization probability ε_i for each variant, which requires model representations and various assumptions. For example, structural considerations suggest that only Hb tetramers in the T quaternary structure are able to be incorporated significantly into the fiber, so the copolymerization probability is tied to the probability of incorporation of a tetramer in the T structure. If we assume that the variation of this probability with pressure is the same in all variants, we can write the pressure-dependent copolymerization probability ε_i for each variant as this single pressure-dependent probability $Z^T(p)$ weighted by some intrinsic overall probability e_i of incorporation of that variant into the fiber, that is

$$\varepsilon_i(p) = e_i Z^T(p) \quad (\text{SI-1.14})$$

We can further assume that the incorporation probability $Z^T(p)$ varies with ligation state or tertiary configuration of subunits, which allows us to write

$$Z^T(p) = \sum_{k=0}^s x_{T_k}(p) \pi_k \quad (\text{SI-1.15})$$

where $s + 1$ is the number of internal states (ligation state or tertiary configuration), $x_{T_k}(p)$ is the pressure-dependent probability that the tetramer is in the T quaternary structure in internal state k , and π_k is the intrinsic probability of incorporating this state of the tetramer into the fiber.

To further characterize the copolymerization probability (SI-1.14), we must first adopt a model to describe the internal state probabilities $x_{T_k}(p)$. For example, the MWC model⁴ identifies the relevant internal states with the ligation state of the tetramer (i.e., $s = 4$) and describes ligand-binding and quaternary equilibria in terms of three parameters: the equilibrium constant L between the T and R quaternary structures in their respective zero-liganded states and the equilibrium constants K_T and K_R for oxygen binding to the T and R states, respectively. The MWC partition function provides the following for the probabilities of the distinct ligation states of the T structure:

$$x_{T_k}(p) = \frac{4!}{k!(4-k)!} \frac{L(K_T p)^k}{L(1+K_T p)^4 + (1+K_R p)^4} \quad (\text{SI-1.16})$$

To determine the intrinsic ligation-state-dependent probability π_k , we note that Hb in the fiber is known to bind oxygen non-cooperatively, with an apparent affinity $K_p < K_T$. This suggests a progressive destabilization of the incorporated tetramer with increasing ligation state, i.e.,

$$\pi_k = \left(\frac{K_p}{K_T} \right)^k \quad (\text{SI-1.17})$$

Combining (SI-1.16) and (SI-1.17) we have the MWC form

$$Z^T(p) = \frac{L(1+K_p p)^4}{L(1+K_T p)^4 + (1+K_R p)^4} \quad (\text{SI-1.18})$$

If we adopt instead a TTS model description (see main text), the “natural” identification of internal states of the tetramer is in terms of tertiary configuration of subunits, for example the number of subunits in the r or t tertiary structure. An expression analogous to (SI-1.16) may then be derived from the TTS partition function; combining this with some estimate of the intrinsic incorporation probabilities of different tertiary configurations leads to an expression analogous to (SI-1.18).

The mutation in HbS is a single point mutation in the β subunit, with the α subunit the same as in HbA; reflecting this difference, we write the full subunit configurations of HbA as $\alpha_2\beta_2^A$ and of HbS as $\alpha_2\beta_2^S$. It is well known that Hb in solution has a tendency to dissociate into $\alpha\beta$ dimers, which in the case of more than one Hb variant will create some population of hybrid tetramers containing dimers from distinct variants. For example, a HbS/HbA mixture will produce some population of hybrid $\alpha_2\beta^A\beta^S$ tetramers in solution. To model the copolymerization in such a mixture, we assume that the HbS homotetramer $\alpha_2\beta_2^S$ incorporates into the polymer with an intrinsic overall probability e_i of 1, the HbA homotetramer $\alpha_2\beta_2^A$ incorporates with zero probability (i.e., $e_i = 0$), and the hybrid tetramer $\alpha_2\beta^A\beta^S$ incorporates with some intermediate probability, estimated to be $e_i = 0.4$.¹ The presence of an additional possible variant HbA₂, where considered (see main text), has different consequences from that of HbA; specifically, in contrast with HbA, the hybrid tetramer $\alpha_2\beta^{A2}\beta^S$ is assumed not to incorporate into the polymer (i.e., $e_i = 0$).¹

SI-2. Theoretical treatment of the desaturation of red blood cells

The desaturation of red blood cells at the bottom of a well is observed to take place on a time scale of many minutes, at least a couple of orders of magnitude slower than the time scale for binding and dissociation reactions of Hb with oxygen. The time scale for desaturation is also much slower than the expected time scale for exchange of oxygen between the cell interior and the surrounding solution. These expectations lend some confidence to the simplifying assumption that, on the time scale of our experiments, the saturation of Hb in the red blood cell is always in equilibrium with the instantaneous concentration of free oxygen in the solution at the bottom of the well.

The initial intracellular reservoir of oxygen, largely that bound to the Hb in the red blood cell, is quite a bit higher than the concentration outside the cell because of the very high intracellular Hb concentration (~ 20 mM in heme). However, the fraction of the solution volume occupied by the red blood cells in these experiments is very small ($\ll .01$), so this reservoir represents only a small perturbation of the oxygen content of the external solution, which has been equilibrated with the atmospheric concentration of oxygen. Therefore, the release of intracellular oxygen during desaturation, while it may result in a transient elevation of the solution concentration of oxygen in the vicinity of individual cells, is expected to have a very small effect on the overall time course of the dissipation of oxygen in the sample.

These considerations allow a theoretical description of the time-dependent desaturation of red blood cells to be formulated in which the progressive depletion of the oxygen in the solution, via escape through the interface between the solution and the oxygen-free atmosphere outside the well, is

effectively decoupled from the oxygen binding and dissociation reactions with the Hb in the cell. The model treatment of this depletion then becomes a relatively simple problem of free diffusion of oxygen through the well solution, and the instantaneous saturation of Hb in the cells at any time may be calculated using some model for the equilibrium binding of oxygen to Hb.

Analysis of the time-dependent depletion of oxygen in the well. The basic model picture treats the solution in a single well as a roughly cylindrical structure, with a lower base and walls which are impermeable to oxygen, and a liquid surface through which oxygen may exchange with the surrounding atmosphere. The design of the current experiments is such that the solution is initially equilibrated with the oxygen concentration in air (~ 150 torr), and the concentration of oxygen in the surrounding atmosphere is held at zero by the nitrogen purge.

The evolution of the time- and position-dependent oxygen concentration $\phi(\vec{r}, t)$ within the well is governed by the diffusion equation

$$\nabla \cdot (D \nabla \phi) = \frac{\partial \phi}{\partial t} \quad (\text{SI-2.1})$$

where D is the diffusion coefficient, which is itself in principle time- and position-dependent; for our purposes, this coefficient will always be a simple constant, in which case the equation simplifies to

$$D \nabla^2 \phi = \frac{\partial \phi}{\partial t} \quad (\text{SI-2.2})$$

The solution of this equation requires 1) the specification of region Ω , which we assume to be bounded, over which the solution is defined, 2) conditions to be satisfied by the solution on the boundary $\partial\Omega$ of this region, and 3) initial values of the solution throughout the region. It is assumed that all derivatives of the solution appearing in the equation are defined within and on the boundary of the region. For a limited set of problems, an analytical solution in terms of elementary and special functions is possible; for a much broader set of problems, numerical methods are available to obtain approximate solutions to a specified accuracy.

Analytical solution of the diffusion equation in an idealized geometry. We provide here a fairly detailed recipe for solving the diffusion equation in an idealized cylindrical geometry. If we require the well to be a perfect cylinder, cylindrical coordinates offer the “natural” coordinate system in which to frame the problem. In this system, we specify an origin, a single axis passing through the origin, a base plane passing through the origin perpendicular to the axis, and a reference direction within the base plane. A three-dimensional point is then identified using three coordinates: the distance ρ from the axis, the signed perpendicular distance z from the base plane, and the azimuthal angle φ with respect to the reference direction of the projection of the point into the base plane.

Using the Laplacian in cylindrical coordinates, the diffusion equation (SI-2.2) for the density $\phi(\rho, z, \varphi, t)$ may be written as

$$\frac{1}{\rho} \frac{\partial}{\partial \rho} \left(\rho \frac{\partial \phi}{\partial \rho} \right) + \frac{1}{\rho^2} \frac{\partial^2 \phi}{\partial \varphi^2} + \frac{\partial^2 \phi}{\partial z^2} = \frac{1}{D} \frac{\partial \phi}{\partial t} \quad (\text{SI-2.3})$$

We will only consider here solutions which are axially symmetric, which allows us to suppress the dependence on the angle ϕ , and consider only functions of the form $\phi(\rho, z, t)$. We can then rewrite the equation as

$$\frac{\partial^2 \phi}{\partial \rho^2} + \frac{1}{\rho} \frac{\partial \phi}{\partial \rho} + \frac{\partial^2 \phi}{\partial z^2} = \frac{1}{D} \frac{\partial \phi}{\partial t} \quad (\text{SI-2.4})$$

Methods for solving this equation over simple regions are covered in many applied mathematics textbooks.

We stipulate that, for a solution to represent a physically meaningful concentration, it must be non-negative everywhere within and on the boundary of the region, for all times. Before we discuss the solution, we first define more precisely the region within which diffusion takes place and sketch the applicable boundary conditions on possible solutions of the equation. The well encloses the medium in a cylinder with radius r and axis coinciding with the z direction of the coordinate system. The lower boundary, the base of the cylinder, lies in the $z = 0$ plane. The surface of the medium through which oxygen may exchange with the surrounding atmosphere is assumed to be planar, with constant $z = L > 0$.

The walls and lower base of the cylindrical well are assumed to be impermeable to oxygen. This zero-flux condition translates to a requirement that the component of the gradient of the concentration normal to these surfaces be zero. That is

$$\hat{\mathbf{n}} \cdot \nabla \phi = 0 \quad (\text{SI-2.5})$$

where $\hat{\mathbf{n}}$ is the unit vector normal to the surface. (Such boundary conditions are referred to as Neumann boundary conditions.)

In our experiments it is assumed that the oxygen concentration in the surrounding atmosphere is zero. We wish to impose conditions which enforce the transition of the solution to this value across the interface between the medium and the surrounding atmosphere. The simplest way to accomplish this is by explicitly setting the value of the solution to zero everywhere on the boundary (a so-called Dirichlet boundary condition), that is

$$\phi(z=L) = 0 \quad (\text{SI-2.6})$$

With this condition, an exact solution of the problem at any time $t > 0$ will approach zero as z approaches L from below.

Alternatively, we can couple the flux through the boundary and the value of the concentration at the boundary, such that the outward flux is large when the concentration is large, and diminishes to zero as the concentration approaches the assumed zero concentration outside the boundary. At the boundary, the flux is simply the gradient, with appropriate sign, of the concentration with respect to the coordinate normal to the boundary. The coupling is accomplished by a so-called mixed boundary condition, the simplest of which is linear, of the form

$$\left. \frac{\partial \phi}{\partial z} \right|_{z=L} = c \phi(z=L) \quad (\text{SI-2.7})$$

for some constant $c < 0$. For $\phi > 0$, this has the net effect of imposing a roll-off of ϕ with increasing z in the vicinity of the boundary. In one view, this mathematical condition represents a “permeability” of the interface between the two media to the passage of oxygen, as reflected in the value of the constant c . As $c \rightarrow 0$ this condition approaches a zero-flux condition through the interface, and as $c \rightarrow -\infty$ the behavior of the solution approaches that imposed by the simple condition (SI-2.6); the latter may reasonably be viewed as representing an interface through which oxygen passes without resistance.

The usual approach to the solution of (SI-2.4) uses the method of separation of variables, in which we write the function $\phi(\rho, z, t)$ as the product of functions of the individual independent variables, that is

$$\phi(\rho, z, t) = R(\rho)Z(z)T(t) \quad (\text{SI-2.8})$$

Substituting into (SI-2.4), after some manipulations we arrive at

$$\left[\frac{1}{R} \left(R'' + \frac{1}{\rho} R' \right) \right] + \left[\frac{Z''}{Z} \right] = \left[\frac{1}{D} \frac{T'}{T} \right] \quad (\text{SI-2.9})$$

where primes indicate derivatives of each function with respect to its single independent variable. This equation contains three distinct (bracketed) terms, each of which is by assumption the function of a single variable, ρ , z and t , respectively. For this equation to hold, each of these terms must individually be equal to a constant.

For the term dependent on t , we write this constant as $-\lambda^2$, where the real parameter λ is otherwise undetermined for the moment. This specification leads to

$$\frac{1}{D} \frac{T'}{T} = -\lambda^2 \quad (\text{SI-2.10})$$

$$T(t) \propto \exp(-D\lambda^2 t)$$

By constraining the constant $-\lambda^2$ to be non-positive, we exclude solutions exhibiting exponential growth. Substituting this into (SI-2.9) yields

$$\left[\frac{1}{R} \left(R'' + \frac{1}{\rho} R' \right) \right] + \left[\frac{Z''}{Z} \right] = -\lambda^2 \quad (\text{SI-2.11})$$

Each of the bracketed terms on the left is individually equal to a constant. We temporarily write the constant for the first term as $-\nu^2$, which allows us to write the equation for the z -dependent term as

$$\frac{Z'}{Z} = \nu^2 - \lambda^2 \quad (\text{SI-2.12})$$

This has solutions of the general form

$$Z = A \exp(i\mu z) + B \exp(-i\mu z) \quad (\text{SI-2.13})$$

$$\mu = \sqrt{\lambda^2 - \nu^2}$$

We require μ to be real (i.e., $\nu \leq \lambda$) to insure that Z remains bounded as $z \rightarrow \pm\infty$.

In a bounded region, the boundary conditions result in a discrete spectrum of allowed eigenvalues μ and associated eigenfunctions. The first boundary condition in z is the zero-flux condition (SI-2.5) applied to the lower ($z = 0$) boundary of the cylinder, which yields

$$\begin{aligned} Z'(0) &= 0 \\ \mu(A-B) &= 0 \end{aligned} \tag{SI-2.14}$$

The two methods described above for treating the boundary condition at the upper surface of the medium lead to different sets of eigenvalues and eigenfunctions. Both boundary conditions, when combined with (SI-2.14), lead to the functional form

$$Z(z) \sim \cos(\mu z) \tag{SI-2.15}$$

The sets of real eigenvalues in the two cases, while different, both appear as sets of $(-\mu_i, \mu_i)$ pairs. Because of the symmetry of (SI-2.15) with respect to this sign inversion, we need only retain the positive values.

If we apply the boundary condition (SI-2.6), we arrive at

$$\begin{aligned} \cos(\mu L) &= 0 \\ \mu &= \frac{\left(n + \frac{1}{2}\right)\pi}{L} \end{aligned} \tag{SI-2.16}$$

for non-negative integer values of n .

If we instead apply the boundary condition (SI-2.7), after some calculation we arrive at the constraint on allowable eigenvalues

$$\mu \sin(\mu L) + c \cos(\mu L) = 0 \quad (\text{SI-2.17})$$

For $c < 0$ this admits an infinite set of discrete real, positive roots μ , which must be computed numerically. (The case $c > 0$, which produces a positive concentration gradient across the boundary layer, admits an additional single imaginary root, which is responsible for the expected nonphysical exponential growth of concentration across the boundary layer; this case is not considered further here.)

For either choice of boundary conditions, we produce a set of ordered positive eigenvalues $\{\mu_i, i=0, 1, 2, \dots\}$ and corresponding eigenfunctions $Z_i(z) = \cos(\mu_i z)$. For the set of eigenvalues obtained from boundary condition (SI-2.6), these functions satisfy the orthogonality conditions

$$\int_0^L \cos(\mu_k z) \cos(\mu_l z) dz = \frac{1}{2} L \delta_{kl} \quad (\text{SI-2.18})$$

For the set of eigenvalues obtained from the boundary condition (SI-2.7), the orthogonality condition takes the form

$$\int_0^L \cos(\mu_k z) \cos(\mu_l z) dz = \left(\frac{1}{2} L + \frac{1}{4 \mu_k} \sin(2 \mu_k L) \right) \delta_{kl} \quad (\text{SI-2.19})$$

Finally, recall that we temporarily assigned the first bracketed term in (SI-2.11) to the constant $-v^2$. This leads to the equation

$$\rho^2 R'' + \rho R' + v^2 \rho^2 R = 0 \quad (\text{SI-2.20})$$

Setting $v = 0$ results in the trivial solution $R = \text{constant}$. For nonzero v , the substitution $\bar{\rho} = v\rho$ transforms this to an equation of Bessel type, for which the solutions are the Bessel and Neumann functions $J_0(v\rho)$ and $Y_0(v\rho)$, respectively. We retain only the former, because the latter is not finite at $\rho = 0$.

Once again, applying boundary conditions leads to a discrete spectrum of eigenvalues v . Specifically, imposing the zero-flux condition (SI-2.5) on the curved cylindrical boundary ($\rho = r$) leads to the requirement

$$J_0'(vr) = 0 \quad (\text{SI-2.21})$$

Using the relation

$$J_0'(x) = -J_1(x) \quad (\text{SI-2.22})$$

we can find the values of v which satisfy (SI-2.21) from the zeroes of $J_1(x)$.

Major mathematical libraries provide functions for computing sequences of zeroes of Bessel functions of specified order, which for a specified value of r leads to the corresponding eigenvalues v . We incorporate the $v = 0$ case considered above by defining $v_0 = 0$, with corresponding function $R_0(\rho) = \text{constant}$. To merge the two cases we identify this function with $J_0(0)$, thus producing a complete sequence of ordered non-negative eigenvalues $v_j, j=0, 1, 2, \dots$ and corresponding eigenfunctions $R_j(\rho)=J_0(v_j\rho)$. It may be shown that these eigenfunctions satisfy the orthogonality relation

$$\int_0^r \rho J_0(v_k \rho) J_0(v_l \rho) d\rho = \frac{r^2}{2} (J_0(v_k r))^2 \delta_{kl} \quad (\text{SI-2.23})$$

For specific choices of the eigenvalues μ_i and v_j we can use (SI-2.13) to compute the corresponding value of the constant λ^2 in the time-dependent factor (SI-2.10). This allows us to construct a single “component” of the solution associated with the indices i and j as

$$\phi_{ij}(\rho, z, t) \sim J_0(v_j \rho) \cos(\mu_i z) \exp\left(-D(\mu_i^2 + v_j^2)t\right) \quad (\text{SI-2.24})$$

where no attempt has been made to normalize the individual components.

The full solution is then a linear combination of all of these components, that is

$$\phi(\rho, z, t) = \sum_{i=0}^{\infty} \sum_{j=0}^{\infty} c_{ij} \cos(\mu_i z) J_0(v_j \rho) \exp\left(-D(\mu_i^2 + v_j^2)t\right) \quad (\text{SI-2.25})$$

The coefficients c_{ij} are computed from the specified initial conditions $\phi(\rho, z, 0)$, by exploiting the orthogonality relations (SI-2.18) or (SI-2.19) and (SI-2.23), with the result

$$c_{ij} = \frac{\int_0^r \rho J_0(v_j \rho) \int_0^L \phi(\rho, z, 0) \cos(\mu_i z) dz d\rho}{\int_0^r \rho (J_0(v_j \rho))^2 d\rho \int_0^L \cos^2(\mu_i z) dz} \quad (\text{SI-2.26})$$

where the integrals in the denominator are taken from the orthogonality relations for the appropriate boundary conditions.

In practice the infinite sums in (SI-2.25) are truncated to a finite number of terms sufficient to produce acceptable fidelity to the initial conditions; the dependence of the exponential decay rates in the individual terms on the squares of the eigenvalues insures that the error incurred in this truncation will itself decay exponentially in time at quite a high rate.

For certain classes of initial conditions $\phi(\rho, z, 0)$, considerable simplifications in (SI-2.25) and (SI-2.26) are possible. For example, if this function is independent of ρ , that is $\phi(\rho, z, 0) = \phi(z)$, then the sum over j collapses to the single term $j = 0$, with the single eigenvalue $v_0 = 0$ and the solution remains independent of ρ for all subsequent times. The problem has been reduced essentially to a one-dimensional description of diffusion along the z direction, and with the zero-flux boundary condition along directions orthogonal to this direction becomes mathematically equivalent to the simple one-dimensional problem with a zero-flux boundary condition at $z = 0$, and condition (SI-2.6) or (SI-2.7) in effect at $z = L$.

With this simplification we can write the practical truncation of (SI-2.25) to $n+1$ terms as

$$\phi(z, t) = \sum_{i=0}^n c_{i0} \cos(\mu_i z) \exp(-D\mu_i^2 t) \quad (\text{SI-2.27})$$

One way to estimate the oxygen concentration at the bottom of the well, from which the time-dependent saturation of Hb in the red blood cells is computed, is to simply evaluate this expression at $z = 0$. In practice, we exploit the simplicity of (SI-2.27) to compute instead the time-dependent average concentration over a thin region of height ϵ at the bottom of the well, that is

$$\begin{aligned} \bar{\phi}(t) &= \frac{1}{\epsilon} \sum_{i=0}^n c_{i0} \left(\int_0^{\epsilon} \cos(\mu_i z) dz \right) \exp(-D\mu_i^2 t) \\ &= \frac{1}{\epsilon} \sum_{i=0}^n c_{i0} \frac{\sin(\mu_i \epsilon)}{\mu_i} \exp(-D\mu_i^2 t) \end{aligned} \quad (\text{SI-2.28})$$

Numerical solution of the diffusion equation in a realistic geometry. Physical problems formulated as partial differential equations for which an analytical solution is possible comprise a small fraction of the problems of interest in physics and engineering. Several classes of methods are available for the numerical solution of problems not amenable to analytical approaches. One powerful such class falls under the general heading of finite-element methods.

An in-depth discussion of such methods is outside the scope of this article. Broadly, the method exploits the consequences of requiring the original equation to hold only under integration against a set of test functions over the region of interest, leading to a so-called “weak form” of the problem. As a simple example, we consider the time-independent problem of solving Poisson's equation over some bounded region Ω

$$\nabla^2 \phi(\mathbf{r}) = -\rho(\mathbf{r}) \quad (\text{SI-2.29})$$

with some conditions on the boundary of the region $\partial\Omega$. We start by multiplying by a test function $u_i(\mathbf{r})$ from some space of such functions and integrating over the region

$$\int_{\Omega} u_i \nabla^2 \phi d\Omega = \int_{\Omega} u_i \nabla \cdot \nabla \phi d\Omega = - \int_{\Omega} u_i \rho d\Omega \quad (\text{SI-2.30})$$

Using Green's identity (i.e., multidimensional integration by parts) on the left side yields

$$\oint_{\partial\Omega} u_i (\mathbf{n} \cdot \nabla \phi) dS - \int_{\Omega} (\nabla u_i) \cdot (\nabla \phi) d\Omega = - \int_{\Omega} u_i \rho d\Omega \quad (\text{SI-2.31})$$

where the first integral is over the boundary of the region, \mathbf{n} is the outward-facing normal of the surface and dS is the oriented surface area element. If we then expand our unknown function as a linear combination of test function from the chosen space

$$\phi(\mathbf{r}) = \sum_{j=1}^N U_j u_j(\mathbf{r}) \quad (\text{SI-2.32})$$

we have transformed the original equation to a linear algebra problem in the vector of coefficients $\mathbf{U} = \{U_j\}$

$$(\mathbf{S} - \mathbf{A})\mathbf{U} = \mathbf{F} \quad (\text{SI-2.33})$$

where the matrices \mathbf{S} and \mathbf{A} and vector \mathbf{F} are defined by

$$\begin{aligned} S_{ij} &= \oint_{\partial\Omega} u_i (\mathbf{n} \cdot \nabla u_j) dS \\ A_{ij} &= \int_{\Omega} (\nabla u_i) \cdot (\nabla u_j) d\Omega \\ F_i &= - \int_{\Omega} u_i \rho d\Omega \end{aligned} \quad (\text{SI-2.34})$$

In the special case of zero-gradient (homogeneous Neumann) boundary conditions, the first term in (SI-2.31), and therefore the matrix \mathbf{S} , are identically zero.

The solution of the problem generally proceeds by first prescribing a mesh which covers the region and selecting the set of functions u_i to have finite support centered on various positions within this mesh; these functions are the elements which give the method its name. The accuracy of the solution will depend on the fineness of the mesh (and therefore the size of the function space $\{u_i\}$), as well as the properties of the functions themselves. The finite-support requirement results in the matrices \mathbf{S} and \mathbf{A} being sparse, which allows for efficient solution on even quite large sets of elements.

The extension of this approach to the time-dependent diffusion problem over the more realistic well geometry was implemented using the excellent deal.II general-purpose finite-element library⁵, which provides extensive facilities for setting up meshes and corresponding element spaces over a region, as well as support for linear-algebra and other methods required to address the weak-form analyses (e.g. (SI-2.33)) of a wide range of problems.

SI-3. A heuristic discussion in support of the use of eq. 2 in the main text.

This work relies on the calculation of overall sickling delay times from time-dependent delay times by solving the integral equation (2) of the main text. This simple equation is derived by Cellmer, et al. (in preparation) by exploiting the linearization of the equations describing the heterogeneous nucleation mechanism for polymer formation.⁶ The purpose of this brief discussion is to provide a somewhat broader context in support of the validity of this equation.

We start with a single generic differential equation describing the evolution of a population p :

$$\frac{dp}{dt} = \phi(p, t) \quad (\text{SI-3.1})$$

We consider two specific cases, one involving evolution by an exponential decay process, and the other involving a power-law growth in population. In the former case, we introduce a decay rate $k(t)$ which may itself be a function of time:

$$\begin{aligned} \phi(p, t) &= -k(t) p \\ \frac{dp}{dt} &= -k(t) p \\ p(t) &= p_0 \exp\left(-\int_0^t k(t') dt'\right) \end{aligned} \quad (\text{SI-3.2})$$

The time-dependent rate k may be associated with a time-dependent characteristic time

$$k(t) = \frac{1}{\tau(t)} \quad (\text{SI-3.3})$$

and the overall characteristic time τ_c of the relaxation process, at which the decay has reached $1/e$ of the initial value, may be computed from

$$\int_0^{\tau_c} \frac{1}{\tau(t')} dt' = 1 \quad (\text{SI-3.4})$$

Now we consider the case of power-law growth, with a control constant κ :

$$\begin{aligned} \phi(p) &= \kappa p^n \quad n > 1 \\ \frac{dp}{dt} &= \kappa p^n \\ \frac{dp}{p^n} &= \kappa dt \\ -\frac{1}{n-1} (p^{-(n-1)} - p_0^{-(n-1)}) &= \kappa t \\ p(t) &= p_0 \left(1 - (n-1) p_0^{n-1} \kappa t \right)^{-\frac{1}{n-1}} \end{aligned} \quad (\text{SI-3.5})$$

This solution will experience an “auto-catalytic explosion” at the time t given by

$$(n-1) p_0^{n-1} \kappa t = 1 \quad (\text{SI-3.6})$$

or we can alternatively speak of a generalized “upward-turn” time t at which

$$(n-1)p_0^{n-1}\kappa t = c \leq 1 \quad (\text{SI-3.7})$$

We therefore define a characteristic time τ_c associated with the generalized upward turn by

$$\tau_c = \frac{c}{(n-1)p_0^{n-1}\kappa} \quad (\text{SI-3.8})$$

We now introduce a time dependence into the control constant κ , leading to the modified differential equation and solution:

$$\begin{aligned} \frac{dp}{dt} &= \kappa(t)p^n \\ p(t) &= p_0 \left(1 - (n-1)p_0^{n-1} \int_0^t \kappa(t') dt' \right)^{-\frac{1}{n-1}} \end{aligned} \quad (\text{SI-3.9})$$

for which the overall upward-turn time τ_c is defined by

$$(n-1)p_0^{n-1} \int_0^{\tau_c} \kappa(t') dt' = c \leq 1 \quad (\text{SI-3.10})$$

We now use (SI-3.8) to rewrite the time-dependent control parameter $\kappa(t)$ in terms of a corresponding time-dependent upward-turn time $\tau_c(t)$ as

$$\kappa(t) = \frac{c}{(n-1)p_0^{(n-1)}\tau_c(t)} \quad (\text{SI-3.11})$$

This allows us to arrive at a prescription for the overall upward-turn time τ_c in terms of the upward-turn times corresponding to the time-dependent control parameter:

$$\begin{aligned} (n-1) p_0^{(n-1)} \int_0^{\tau_c} \left(\frac{c}{(n-1) p_0^{(n-1)} \tau_c(t')} \right) dt' &= c \\ \int_0^{\tau_c} \frac{1}{\tau_c(t')} dt' &= 1 \end{aligned} \quad (\text{SI-3.12})$$

In the two cases being considered here, the time-dependent characteristic times have very different natures, in the former case a $1/e$ decay time, in the latter an upward-turn time characterizing an auto-catalytic explosion. Nevertheless, in both cases the relationship between the overall characteristic times and the underlying time-dependent characteristic times assumes the same simple form

$$\int_0^{\tau_c} \frac{dt'}{\tau_c(t')} = 1 \quad (\text{SI-3.13})$$

In both cases, the control of the time dependence of the underlying process is through a simple pre-factor, in one case an exponential relaxation rate and in the other a control parameter for power-law evolution. This common linearity in the time-dependent control of the process, exploited in the derivation by Cellmer, et al., no doubt contributes to the generic nature of (SI-3.13).

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