Ratchets, red cells, and metastability

Frank A. Ferrone · Alexey Aprelev

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Abstract Sick cell disease is a genetic disorder in which a negatively charged glutamic acid is replaced by a hydrophobic valine on the surface of the hemoglobin molecule, leading to polymerization of the deoxygenated form, and resulting in microvascular obstruction. Because of the high volume occupancy under which polymerization occurs physiologically, this process has been an exemplar in the study of excluded volume effects on assembly. More recently, we have identified yet another type of crowding effect involving the obstruction of the ends at which the polymers grow as a consequence of the dense arrays in which these polymers form. This makes such solutions metastable, and leads to Brownian ratchet behavior in which pressure is exerted outward when the gel occupies a finite volume, as in an emulsion or red cell. Such behavior is capable of holding sickled cells in place in the microcirculation against weak pressure differentials (hundreds of Pa), but not against the typical pressures found in vivo.

Keywords Polymerization · Molecular crowding · Brownian ratchet · Sick cell disease

Introduction

The red cell is meant to function as a pliable container for hemoglobin (Hb), which typically fills 25 % of its volume. The loss of this flexibility is the central problem in sickle cell disease, a genetic disorder in which deoxygenated Hb forms

rigid polymers that distort and rigidify their erythrocytes. This can block the microcirculation, causing oxygen deprivation and attendant pain and organ damage. The polymerization reaction entails a double nucleation process (Ferrone et al. 1985a), in which the first steps are formation of unstable nuclei in solution (homogeneous nucleation), followed by polymerization, and subsequent additional nucleation (heterogeneous nucleation) onto the surface of the growing polymers. The reaction inevitably entails large corrections for molecular crowding, given the dense milieu in which it occurs. Nonetheless, the crowding corrections appear to be well described, without the need for adjustable parameters, largely by versions of scaled particle theory describing hemoglobin molecules as hard spheres, and nuclei as larger spheres of polymeric density. This aspect of the expanding field of molecular crowding has been previously reviewed (Ferrone and Rotter 2004).

However, another consequence of the density of the reaction has emerged in recent years. The ability of polymers to grow depends on the presence of available space at the polymer end. (Fig. 1) The high density at which polymers form deprives growing polymers of such space, and appears to result in a metastable state in which an outward Brownian ratchet pressure exists. In this review, we recapitulate the experimental findings that led to this observation, and the interesting physiological implications that ensue.

Experimental foundations

The discussion of growth within a dense gel begins with the growth of single fibers in the absence of the gel. The equations that govern this growth are quite simple. Polymer growth involves a monomer addition rate constant, denoted k_+ and a dissociation rate constant denoted k_- . These have units of $(\text{mM s})^{-1}$ and s^{-1} , respectively. If the concentration of monomers is given by c , and the dimensionless monomer

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F. A. Ferrone (✉) · A. Aprelev
Department of Physics, Drexel University,
Philadelphia, PA 19104, USA
e-mail: FERRONFA@drexel.edu

Fig. 1 Polymer growth rate as a function of monomer activity, measured with an optical channel method. As shown in the *inset*, polymerization is begun in the *large incubation circle at the left*. The *gray connecting channel* is then open for a period of time. Polymers that grow fast enough will cause the detection region, the *dark tip of the channel*, to polymerize by heterogeneous nucleation. Monomer activity is the product of an activity coefficient γ and c , the concentration of monomers competent to polymerize. *Empty circles* are full photolysis data for pure HbS. The *crossed circle* is 50:50 HbA/HbS mixture data. The *half-filled circle* is data obtained from partial photolysis. The linearity of the curve supports the assumption that monomer activity coefficients are the only manifestation of crowding required to describe polymer growth. The intercept at $J = 0$ gives the solubility. The value of solubility determined in sedimentation experiments is shown as the *filled square point* on the abscissa, and was not used in the best-fit straight line that is drawn through the data. The slope of the curve gives the monomer addition rate constant, k_+ , which is found to be $84 \pm 2 \text{ mM}^{-1} \text{ s}^{-1}$

activity coefficient is γ (where γ is a function of concentration), then the growth rate J is given by

$$J = k_+ \gamma c - k_- \quad (1)$$

J has units of inverse seconds as it represents the net rate of monomer addition to polymers. Only a single activity coefficient appears, viz. that of monomers. This comes from the assumed cancellation of the activity coefficient for the polymer with the activity coefficient for the activated complex.

Monomer activity coefficients are conveniently expressed in terms of the dimensionless monomer volume fraction, $\phi = \gamma c$ where the specific volume of monomers is v . It is found that

$$\ln \gamma = 8\phi / (1 - \phi)^2 \quad (2)$$

which is virtually indistinguishable from a 7th order power series over the range of data for which almost all HbS polymerization is studied (Ferrone and Rotter 2004). This, of course, applies to a monomer in a solution where monomers are the only species that occupies significant space.

We have recently carried out measurements of the growth of single fibers using a spatially-patterned laser photolysis method (Aprelev et al. 2011). When Hb binds ligands, the quaternary structure of Hb switches, and prevents assembly. CO is a particularly useful ligand since it is released efficiently by light, and therefore permits the polymerization of HbS to be optically triggered and sustained by continuous illumination. Laser photolysis of COHbS has thus been extensively employed as an effective way to rapidly create deoxyHbS; moreover, the accompanying temperature rise from the absorption of the continuous flux of light is minimal when the dimensions are suitably small, as in all cases described here (Ferrone et al. 1985b; Galkin et al. 2007). As initially implemented, the photolysis experiments involved focusing a laser to a small spot. In the experiment used here, a larger diameter disk is photolyzed, augmented by a thinner photolyzed optical “channel” emerging as an extension from the disk. The concept of the experiment was to create an initial region in the illuminated disk where sickle hemoglobin fibers were present, with some fibers inevitably pointed normal to the disk boundary. When the thin optical channel was then illuminated, a fiber so oriented would grow along the channel. To detect the fiber, which is smaller than the wavelength of light, we monitored its ability, via heterogeneous nucleation, to generate a large mass of new fibers in a detection area (at the end of the optical channel). In this experiment, the channel between the large incubation area and smaller detection area was kept illuminated for successively longer times until a fiber successfully traversed the length of the channel and seeded large numbers of new polymers, which then could be easily viewed optically.

Figure 1 shows the results of the above experiment. As can be seen, there is excellent linearity of the growth rate as a function of γc , supporting the assumption that the activity coefficients of the polymer and activated complex have cancelled. Equally important is the intercept on the abscissa, which is the concentration at which $J = 0$, i.e. neither growth nor dissolution occurs. There, $k_- = k_+ \gamma_s c_s$ where c_s is the solubility and γ_s is the activity coefficient measured at solubility. The data point shown in the graph on the axis is the value of the solubility obtained from sedimentation experiments, and agrees well with the intercept of the line.

Finally, although slightly tangential to the main points discussed here, it is interesting to note that the linearity of growth with γc also confirms that it is monomers that add to grow the polymer, rather than some oligomeric species. Thus, the simple expression in Eq. 1 is seen to be qualitatively and quantitatively accurate for isolated individual fibers. We then turn to fibers within gels.

Two very different experimental methods have established the unexpected result that polymer growth in gels ceases before the concentration of the available monomer pool drops to the monomer concentration. The first is a

photolysis experiment on an emulsion of HbS solutions suspended in castor oil (Aprelev et al. 2007). Droplets were prepared in order that there be a finite amount of HbS in a region studied, much like a red cell. (Unlike a red cell, the sample concentration and solution ionic composition would be known precisely. This was confirmed by measuring nucleation rates in the droplets, which agreed with the equivalent solutions. Nucleation rates are highly sensitive to solution conditions.) The idea was to polymerize only a portion of a drop, and allow the remainder of the drop to serve as a reservoir for the growing gel. The concentration of hemoglobin in the reservoir would be depleted until it reached some limit, which we had expected would be the solubility. Thus, the laser beam was partially masked so as leave a part of the droplet dark, as shown in Fig. 2, in which the masked region is a square centered in an elongated drop. The original expectation was that the final concentration of monomers would be the well-documented solubility for HbS polymerization, as seen as the terminal point in the growth of individual fibers above. Instead, the polymerization terminated at a concentration well above that of the solubility, as shown in Fig. 3. Moreover, the observed terminal concentrations depended on how the result was achieved. Polymerization directly at a specific target temperature gave a different concentration than polymerization that was initiated at an intermediate temperature, followed a change to the target temperature, as shown in the figure. A true thermodynamic variable would only depend on the conditions of the final state, not of the path through which it passed. This argued that the final concentrations were not thermodynamically controlled.

The presence of a finite boundary to the droplets suggested that polymer ends arriving at the droplet boundaries would be

thwarted in their attempts to grow, despite a clear thermodynamic drive to do so. (Heterogeneous nucleation is so highly concentration-dependent that the depletion of monomers sharply retards that process to a point where it is effectively shut down.) However, it was also apparent that there might be sufficient polymer density within the polymer mass itself to provide such end occlusion, even when far from a boundary. This led to the use of a second method, which did not require the use of finite droplets to study the final concentration in gels. In this method, a small fraction (~2 %) of the hemes were exposed to CO. Photolysis of the sample released the CO, which, when illumination was turned off, rebound at a rate controlled by the local concentration of Hb that was competent to bind CO (Liao et al. 1993, 1996). Hb bound to polymers does not compete effectively for free ligands (Shapiro et al. 1995), and so measuring the rate of CO recombination provided a second means of determining the concentration of unpolymerized HbS. Naturally, the small amount of CO meant that absorbance changes would be small in turn, and thus an averaging strategy was employed, viz., using repeated illumination. By doing this in the frequency rather than time domain, kinetics were determined by a phase measurement. This method could thus be done on thin, uniform slides. The results from this method were in excellent agreement with the reservoir method using droplets (Weng et al. 2008). Table 1 shows the comparison of modulation methods performed on droplets, so that both methods could be employed on the same samples. As can be seen, the agreement is excellent. Similar results were obtained on uniform, thin slides.

Both methods showed that about 2/3 of the amount expected actually polymerized, as seen in Fig. 4 (Weng et al.

Fig. 2 A sickle hemoglobin droplet suspended in castor oil, before and after photolysis. The size of a typical droplet was between 200 and 300 μm . A diode pumped laser (532 nm) is used as photolysis beam. A physical mask is placed along the path of laser on a conjugate image plane so that it creates an unphotolyzed mask area on the sample, which does not interfere with the probe beam for absorption measurements. The above images were taken at 432 nm, where deoxyHb

absorbs much more than HbCO, as manifested by the contrast in color when laser is turned on. The darker color inside the photolyzed area on the second image indicates the presence of polymers. As the polymerization progresses, monomers diffuse from the masked segment to the photolyzed region until a terminal concentration is reached. The terminal concentration is higher than solubility, due to the metastability of polymerization

Fig. 3 Terminal concentration as a function of temperature for droplet experiments. The *solid curve* is the solubility of HbS (Ross et al. 1977). All samples are in 200- to 300- μ m droplets, in which the masked area is 4–5 % of the total, and is placed at the center of the Hb drop. *Points connected by dashed lines* were polymerized at the higher temperature of the pair, and the temperature then was changed to the lower temperature. At the high temperatures, some symbols occlude others because the concentrations achieved were the same. All temperature-

shift experiments involved polymerization at the high temperature, followed by shifting to a lower temperature, and then finally depolymerization. Solo points (all in *darker colors*) have been polymerized directly to the final temperature. The *plus sign* shows a concentration achieved by pressing on the slide, initially gelled at 35 °C. Note that all values are above the solubility. However, the terminal value depends on the path taken, suggesting that this is not a thermodynamic measurement

2008). This result included different temperatures as well as different initial concentrations. Sedimentation experiments, which established the solubility and showed it to have thermodynamic properties such as path independence (Ross et al. 1977), were able to avoid the problem of the metastable state by breaking up the polymers as the gel was centrifuged. This explains why the sedimentation-measured solubility agrees well with single fiber measurements, as described above. On the other hand, in experiments that do not have the disruptive forces of centrifugation, including intracellular polymerization, the reaction will terminate at the higher concentrations as we have shown. Recognition of this difference also had the salutary effect of rectifying a discrepancy that had long existed between calorimetry measurements and van't Hoff analysis of solubility (Eaton and Hofrichter 1990). The calorimetry measurements entailed less polymerized hemoglobin than had been calculated, and thus the comparison with sedimentation

measurements led to apparent disagreement. When corrected for the lower amount of polymerized hemoglobin, the agreement was excellent (Weng et al. 2008).

Theoretical framework

A theoretical description of these phenomena begins with the polymer growth equation (Eq. 1). When species other than monomers are present, the simple monomer activity coefficient should be modified to account for them. Various scaled particle theories have been effective treatments for multiple species with the ability to crowd solutions. However, in the case at hand, the volume occupancy is substantial; moreover, the treatments are for assemblies of convex particles. What we have done instead is to employ an expression derived by Ogston for the permeation of

Table 1 Similarity of the terminal concentrations (in g/dl) using modulation and reservoir methods, from Weng et al. (2008)

Temperature (°C)	Initial c	Terminal c (Modulation)	Terminal c (reservoir)	c_s^b
25.4	29.1	23.9 $\pm$ 0.7	24.1 $\pm$ 0.2	17.5
24.8	23.1	20.3 $\pm$ 0.8 ^a	20.1 $\pm$ 1.0	17.7
24.6	28.2	24.2 $\pm$ 0.8	24.2 $\pm$ 0.4	17.7

^a Uniform sample (all others are droplets)

^b Taken from Ross et al. (1977)

Fig. 4 Dependence of the amount of polymer formed on expected amount of polymer. The amount of polymer is computed by subtracting the terminal concentration from the initial concentration. The amount of polymer expected is the initial concentration minus the solubility. Measurements were made at 35 °C (*filled symbols*) and 25 °C (*open squares*). Data measured by modulation is denoted by circles and data determined on droplets by the reservoir method is denoted by triangles and squares. The line was constrained to go through the origin, and has a best-fit slope of 0.67. The *inset* shows our understanding of the process. When a polymer end encounters another polymer, even though there is a thermodynamic driving force to grow, the obstruction halts the growth and leaves the system in a metastable state

spaces (Ogston 1970). Ogston described the volume fraction of a gel available to a spherical solute of radius r . Let this (dimensionless) fraction be designated as Φ . Then, Eq. 1 is rewritten as

$$J = \Phi k_+ \gamma c - k_- = k_+ (\Phi \gamma c - \gamma_s c_s) \quad (3)$$

This is energetically equivalent to making two nonideality contributions additive, viz.

$$\ln \gamma_{\text{eff}} = \ln \Phi + \ln \gamma \quad (4)$$

The metastable state occurs when J in equation 4 is zero, so that

$$\Phi \gamma' c' = \gamma_s c_s \quad (5)$$

where c' is the metastable concentration.

The fibers that make up the gel are assumed to be long enough that their length is irrelevant, and have a radius a . The volume fraction occupied by the hemoglobin fibers is given by ϕ_p . Monomers are not included in this term, since their contribution has already been taken into account. Then, the available volume fraction is given by

$$\Phi = \exp \left[(1 + r/a)^2 \phi_p \right] \quad (6)$$

The volume fraction of polymer, ϕ_p , is related to the final concentration of monomers c' as well as the initial monomer

concentration c_o . If the specific volume of the polymer is v_p , which is approximately 1/(69 g/dl) (Sunshine et al. 1979), then

$$\phi_p = v_p (c_o - c') \quad (7)$$

We can combine Eqs. 5, 6 and 7 and solve numerically for r/a , the size of the pores relative to the fiber radius, for given values of c_o and c' . Naturally, we expect $r/a > 1$, since penetration of a fiber through the voids in a gel would require a hole radius r at least the size of the polymer radius a . When this theory is applied to the data, a consistent representation arises for $r/a = 1.4$ to 1.5.

Simple arguments confirm the reasonableness of such values. Elsewhere we have presented an elementary lattice model for a gel (Zakharov et al. 2010). It can easily be shown that the size of the lattice for 2 mM of gel (typical of that studied in the results quoted here) is about 41 nm. Given that the “connecting rods” of the lattice are not infinitesimally thin lines, but polymers of thick (20 nm) diameters, the remaining available central lattice space to accommodate a growing polymer would be a lattice square, diminished on each side by 10 nm, with an available central area of 441 nm². Since the cross-sectional area of a fiber is 314 nm², the area ratio is 1.4 compared with a ratio of 2 expected from the Ogston theory. This is reasonable agreement, given the extreme simplicity of the lattice picture, and supports the notion that fiber permeation through the gel is substantially restricted. We next turn to the consequences of such restriction.

Cellular implications

The flexibility of the red cell is a physiological necessity because the average capillary diameter is narrower than the average red cell. It was long thought that capillary occlusion was central to the pathophysiology of the disease (Eaton and Hofrichter 1987). Animal model studies using tissues perfused with sickle blood challenged that assumption, showing blockages further downstream, in the somewhat larger venules, occurring at the site of adherent red or white cells. Such cells diminished the vessel lumen without fully obstructing the flow (Kaul et al. 1989).

To understand what happens in cells sickling in capillaries, we used microfluidic methods to mimic the microcirculation by constructing channels in transparent polydimethylsiloxane (PDMS), having a cross-section that is smaller than the resting diameter of red cells (Aprelev et al. 2012). These permit only one cell at a time to pass through them. We again used laser photolysis to remove CO, and cause the cells to sickle.

The experiment began by parking a cell inside such a channel; channel and cell were both saturated with CO. Since the channel thickness was known, it was possible to determine the intracellular hemoglobin concentration by measuring its optical absorption spectrum. The cell was then

Fig. 5 Pressure to dislodge a cell sickled in a microchannel, as a function of intracellular concentration. The *inset* illustrates the experiment. A cell enters a narrow microchannel, and is parked at the center. Laser photolysis (using the 488 nm line of an Argon ion laser) then causes deoxygenation followed by polymerization. While the cell is polymerized, the pressure is increased until the cell breaks free. Note that typical intracellular concentrations are around 32 g/dL. The line shows the Brownian ratchet theory described in the text. The coefficient of friction (0.036) is within the observed range, and is the only parameter varied. The pressures seen here are much less than found in the circulation across typical capillaries

illuminated by laser intensity sufficient to remove all CO and allow polymerization. During the laser illumination, hydrostatic pressure was applied until the cell broke free. Figure 5 shows the result of that experiment. The magnitude of the dislodging pressure increased with increasing intracellular Hb concentration, implying that an increased mass of polymerized hemoglobin is more difficult to dislodge. A clear concentration threshold for capture was seen. The threshold observed is significantly higher than the solubility, which was 18.5 g/dL here. (Note that the thermodynamic solubility is the relevant

threshold variable here, not the higher terminal concentrations discussed above. The metastability driven concentration is a *final* concentration, while what is considered here is the minimum concentration below which polymers could not form.)

The metastability described above nicely explains these phenomena both qualitatively and quantitatively. Metastability gives rise to Brownian ratchet forces which Briehl and coworkers have shown leads to dramatic fiber buckling and cell distortion when individual fibers are isolated in sickle cells as shown in Fig. 6 (Wang, Kwong and Briehl, personal communication.) The force exerted by the ratchet has been shown to be (Peskin et al. 1993; Daniels et al. 2006)

$$f = (kT/\delta) \ln S(c) \quad (8)$$

where k is Boltzmann's constant, T the absolute temperature, δ the net spatial elongation from addition of a single monomer, and S is the dimensionless supersaturation of the solution when the metastable limit is reached, at monomer concentration c . S is defined as

$$S \equiv \gamma c / \gamma_s c_s \quad (9)$$

In this calculation, c is taken as the terminal concentration, computed from the above finding that in this metastable system the amount of polymerized hemoglobin is 2/3 that expected.

To calculate the net force, the total number of fibers must be known. The concentration of polymers $[p(t)]$, measured by counting the concentration of polymer *ends*, initially grows exponentially, described by

$$[p(t)] = (AB/2J) \exp(Bt) \quad (10)$$

where A and B are parameters related to nucleation, and J is the polymer elongation rate, as described (Bishop and Ferrone 1984). A is measured in mM, and B in s^{-1} . Because A and B are both extremely concentration-dependent, they

Fig. 6 Distortion of a red cell by a polymer that crosses the cell (Wang, Kwong and Briehl, personal communication.) By repeated polymerization/depolymerization experiments, an individual fiber (or bundle) is isolated, and allowed to grow. The fiber and cell are viewed in DIC optics. The *left panel* shows the cell before the fiber presses

against the walls. The *right panel* shows the pressing fiber distorting the cytoskeleton as well as causing the fiber to buckle. This is dramatic evidence of the Brownian ratchet force in operation, which is responsible for the outward forces that hold the cell in place in Fig. 5

will drop dramatically once a significant number of monomers begin to add to polymers, thereby diminishing the remaining monomer concentration. Given the extreme concentration dependence of the reaction, this rapidly shuts off further polymerization at approximately the tenth time (the time when the reaction has reached 1/10 of its maximum). Thus, the $[p(t_{1/10})] \approx [p(\infty)]$. Moreover, at one-tenth of the reaction, the time-dependent concentration of monomers $\Delta(t)$, measured in mM, is

$$\Delta(t_{1/10}) = \frac{1}{2} A \exp(Bt_{1/10}) = \Delta(\infty)/10, \quad (11)$$

and thus

$$[p(\infty)] = (B/J)(\Delta(\infty)/10) = (B/J)(c_o - c_s)/15 \quad (12)$$

To convert the polymer concentration to the number of fibers, the volume of the cell was taken as $90 \mu\text{m}^3$. This calculation shows, as expected, that the number of polymers in the cell is highly concentration-dependent, thanks to B , and very few fibers are produced at concentrations just above solubility, although the number grows sharply as concentration rises. This is the main contribution to the threshold in holding force shown by the data.

Knowing the force per fiber, and the total number of fibers, the net force against the wall can be computed. With the addition of a coefficient of friction, this then allows a determination of the force that a trapped cell can withstand. Dividing the force by the cross-sectional area across which the force is applied yields a prediction of the dislodging pressure, which can then be compared to the data.

Using this result with two further small corrections (Aprelev et al. 2012), the results of the theory are compared with the data in Fig. 5. The coefficient of friction has been taken to be 0.036, well within the established values of 0.03–0.06 (Dunn et al. 2007). Thus, the metastability, arising from the crowded solution conditions, has given rise to an outward force that can hold a red cell in place against moderate pressures.

Typical arterio-venous pressure differentials are, however, larger than this, ranging from 0.7 kPa (in hamster skin) (Cabral et al. 2004) to 7.9 kPa (in rat mesentery) (Pries et al. 1994). This argues that cells sickling in capillaries will not get stuck there, and that the observations seen in the animal model studies are essential results. This provides a satisfying connection from an unusual feature of crowded solutions and the important biomedical issue of understanding sickle cell disease.

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Conflict of Interest The authors declare no conflicts of interest.

References

- Aprelev A, Weng W, Zakharov M, Rotter M, Yosmanovich D, Kwong S, Briehl RW, Ferrone FA (2007) Metastable polymerization of sickle hemoglobin in droplets. *J Mol Biol* 369(5):1170–1174
- Aprelev A, Liu Z, Ferrone FA (2011) The growth of sickle hemoglobin polymers. *Biophys J* 101(4):885–891. doi:10.1016/j.bpj.2011.05.064
- Aprelev A, Stephenson W, Noh HM, Meier M, Ferrone FA (2012) The physical foundation of vasoocclusion in sickle cell disease. *Biophys J* 103(8):L38–L40. doi:10.1016/j.bpj.2012.09.003
- Bishop MF, Ferrone FA (1984) Kinetics of nucleation controlled polymerization: a perturbation treatment for use with a secondary pathway. *Biophys J* 46:631–644
- Cabral P, Tsai AG, Intaglietta M (2004) Microvascular pressure and functional capillary density in extreme hemodilution with low- and high-viscosity dextran and a low-viscosity Hb-based O₂ carrier. *Am J Physiol Heart Circ Physiol* 287(1):H363–H373. doi:10.1152/ajpheart.01039.2003
- Daniels DR, Wang JC, Briehl RW, Turner MS (2006) Deforming biological membranes: how the cytoskeleton affects a polymerizing fiber. *J Chem Phys* 124(2):024903
- Dunn AC, Zaveri TD, Keselowsky BG, Sawyer WG (2007) Macroscopic friction coefficient measurements on living endothelial cells. *Tribol Lett* 27:233–238
- Eaton WA, Hofrichter J (1987) Hemoglobin S gelation and sickle cell disease. *Blood* 70:1245–1266
- Eaton WA, Hofrichter J (1990) Sickle cell hemoglobin polymerization. *Adv Protein Chem* 40:63–280
- Ferrone FA, Rotter MA (2004) Crowding and the polymerization of sickle hemoglobin. *J Mol Recognit* 17(5):497–504
- Ferrone FA, Hofrichter J, Eaton WA (1985a) Kinetics of sickle hemoglobin polymerization II: a double nucleation mechanism. *J Mol Biol* 183:611–631
- Ferrone FA, Hofrichter J, Eaton WA (1985b) Kinetics of sickle hemoglobin polymerization I: studies using temperature-jump and laser photolysis techniques. *J Mol Biol* 183:591–610
- Galkin O, Nagel RL, Vekilov PG (2007) The kinetics of nucleation and growth of sickle cell hemoglobin fibers. *J Mol Biol* 365(2):425–439
- Kaul DK, Fabry ME, Nagel RL (1989) Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: pathophysiological implications. *Proc Natl Acad Sci USA* 86(9):3356–3360
- Liao D, Jiang J, Zhao M, Ferrone FA (1993) Modulated excitation of singly ligated carboxyhemoglobin. *Biophys J* 65:2059–2067
- Liao D, Martin de Llano JJ, Himanen J-P, Manning JM, Ferrone FA (1996) Solubility of sickle hemoglobin measured by a kinetic micromethod. *Biophys J* 70:2442–2447
- Ogston AG (1970) On the interaction of solute molecules with porous networks. *J Phys Chem* 74:668–669
- Peskin CS, Odell GM, Oster GF (1993) Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys J* 65(1):316–324. doi:10.1016/S0006-3495(93)81035-X
- Pries AR, Secomb TW, Gessner T, Sperandio MB, Gross JF, Gaetgens P (1994) Resistance to blood flow in microvessels in vivo. *Circ Res* 75(5):904–915
- Ross PD, Hofrichter J, Eaton WA (1977) Thermodynamics of gelation of sickle cell deoxyhemoglobin. *J Mol Biol* 115:111–134

- Shapiro DB, Esquerra RM, Goldbeck RA, Ballas SK, Mohandas N, Kliger D (1995) Carbon monoxide religation kinetics to hemoglobin S polymers following ligand photolysis. *J Biol Chem* 270(44):26078–26085
- Sunshine HR, Hofrichter J, Eaton WA (1979) Gelation of sickle cell hemoglobin in mixtures with normal adult and fetal hemoglobins. *J Mol Biol* 133:435–467
- Weng W, Aprelev A, Briehl RW, Ferrone FA (2008) Universal metastability of sickle hemoglobin polymerization. *J Mol Biol* 377(4):1228–1235. doi:[10.1016/j.jmb.2008.01.083](https://doi.org/10.1016/j.jmb.2008.01.083)
- Zakharov MN, Aprelev A, Turner MS, Ferrone FA (2010) The microrheology of sickle hemoglobin gels. *Biophys J* 99(4):1149–1156. doi:[10.1016/j.bpj.2010.04.079](https://doi.org/10.1016/j.bpj.2010.04.079)