Protein Folding and cosmology

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Protein denaturing induced by supercooling is interpreted as a process where some or all internal symmetries of the native protein are spontaneously broken. Hence, the free-energy potential corresponding to a folding-funnel landscape becomes temperature-dependent and describes a phase transition. The idea that deformed vortices could be produced in the transition induced by temperature quenching, from native proteins to unfolded conformations is discussed in terms of the Zurek mechanism that implements the analogy between vortices, created in the laboratory at low energy, and the cosmic strings which are thought to have been left after symmetry breaking phase transitions in the early universe. An experiment is proposed to test the above idea which generalizes the cosmological analogy to also encompass biological systems and push a step ahead the view that protein folding is a biological equivalent of the big bang.

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1 Introduction

The folding of proteins can be regarded as a biological equivalent of the cosmological big bang in that its end result is evident everywhere, in every living cell, but its beginnings and main cause are shrouded in mystery. This is of course mere analogy, but connections of the problem of protein folding with basic aspects of fundamental physics are being discovered that call for a more serious interrelation. In particular, many proteins at sufficiently low temperature show a dynamical behaviour that matches that of glasses and spin glasses, while offering a lot to be earned about the physics of complexity (Frauenfelder & Wolynes, 1994).

In this paper, we push forward the above analogy in still another important respect: we look at protein unfolding in supercooled pure solvent as being a process where some internal symmetries of the native protein are spontaneously broken. On the other hand, phase transitions induced by spontaneous symmetry breaking which are thought to have occurred in the early universe could have left behind long-lived topologically stable structures such as monopoles, strings, domain walls and textures (Zel’dovich et al., 1975; Kibble, 1976; Albrecht & Turok, 1985; Vilenkin & Shellard, 1994). The possible role played by these structures in the generation of the present configuration of the observable universe has been very much discussed in recent decades (Zel’dovich, 1980; Vilenkin, 1981; Turok, 1983; Turok & Brandenberger, 1986). Therefore, the use of superfluid helium (Hendry et al., 1994; Buerle et al., 1996; Ruutu et al., 1996) or liquid crystals (Chuang et al., 1991; Bowick et al., 1994) to check in the laboratory the properties of the cosmological defects has become a matter of great interest. The possibility of performing such cosmological experiments in helium was first suggested by Zurek (1985) who noted the analogy between cosmological strings and vortex lines in the superfluid. Here, this analogy is extended to also involve vortex lines filled with folded conformations of proteins rapidly denatured by lowering the temperature.

2 Protein folding and spontaneous symmetry breaking

Globular proteins which denature in water by lowering the temperature can be described as undergoing a phase transition driven by spontaneous breakdown of the internal symmetries hidden inside the core of their folded, native structure. Proteins in aqueous medium interact with this through their hydrophobic and hydrophilic (polar) groups. At the sufficiently high temperatures for which no collective "coherent" effect can be expected to occur in the whole system, each protein molecule stabilizes by individually hiding most of its hydrophobic groups, placing them in the interior of the native, compact structure. The symmetries present in this structure can therefore be characterized by the number of contacts among its hydrophobic groups, and that number will tend to decrease as the protein unfolds (Dill & Su Chan, 1997). At lower temperatures, water can still act
like a collective field that attracts protein molecules to one another through the combined effect of the hydrophilic groups and water-to-water hydrogen bonding.

The fundamental degrees of freedom here are the molecules themselves. A way to characterize the state with the thermodynamically most stable conformations is by the number density of protein molecules in the different conformations that exist, or that have ”condensed” into this state. Since at sufficiently high temperatures the unfolded protein molecules do not attractively interact with each other (i.e. their mutual weak attractive interaction is overcome by the combined effect of repulsive interactions among water and hydrophobic groups and thermal energy), the more unfolded conformations are formed the more energy it takes. However, because protein molecules are much less rigid in their unfolded conformations, water-mediated attractive interaction among the hydrophilic groups of two or more unfolded molecules is expected to be greater than that for protein molecules in the native conformation. Therefore, when the temperature becomes low enough to allow water molecules to tend to grouping around the polar groups of the protein, so that attractive interactions start dominating, the total energy of the system begins decreasing with the number density of unfolded conformations, until the system saturates in them. At such temperatures, one would expect all or some of the internal symmetries in the core of folded proteins to be spontaneously broken, creating a more stable ensemble of unfolded protein conformations.

Let us consider the simplest form of a unidimensional section of the effective free-energy potential contribution satisfying the Anfinsen’s requirement (Anfinsen, 1973) that native structures be thermodynamically stable states with conformation at the global minimum of its accessible free energies when the internal symmetry is not spontaneously broken, so as the new view that proteins change tertiary structure according to a folding funnel (Dill & Sun Chan, 1997) of their accessible energy landscape. In order to mathematically describe the phase change which is associated with protein unfolding induced by supercooling the pure solvent, let us introduce the quantity $\Psi$ -the order parameter- which determines to what extend the geometrical distribution of hydrophobic groups in the unfolded phase at low temperature differs from that in the native phase, and adscribe a value $\Psi = 0$ to this (symmetric) phase and a nonzero value of $\Psi$ to the (nonsymmetric) unfolded protein phase. The order parameter $\Psi$ and the free-energy potential defined in terms of $\Psi$ will respectively be associated with a given normal mode or combination of normal modes of the protein molecule, and with the corresponding section of the folding funnel landscape.

Since the native protein is characterized by $\Psi = 0$, near the low-temperature transition point one can write the free-energy potential as a power series in $\Psi$. The number of relevant terms in this expansion should include those terms which are going to allow denaturation of the protein also at high temperature; i.e. we ought to cut off the expansion series only after the term $\Psi^8$ to allow the existence of some local minima at $\Psi \neq 0$ (visualizable at temperatures sufficiently higher than the transition temperature), while ensuring $\Psi = 0$ to be the global minimum of the symmetric phase. Thus, a simplest free-energy potential that satisfies these
requirements can be written in the form

\[ V(\Psi) = \frac{1}{2} \alpha_1 \Psi^2 + \frac{1}{4} \alpha_2 \Psi^4 - \frac{1}{6} \alpha_3 \Psi^6 + \frac{1}{8} \alpha_4 \Psi^8, \]  

(2.1)

where the \( \alpha_i \)'s are constants with the dimensions of a free energy, \( i = 1, ..., 4 \), such that \( \alpha_1 > \alpha_2 > \alpha_3 > \alpha_4 \), and, since the states with \( \Psi = 0 \) and \( \Psi \neq 0 \) are assumed to possess different symmetry, the coefficient for the linear term has been neglected. Besides, we have restricted to the most interesting case which is prepared to accommodate transitions that follow a continuous process, rather than those occurring at isolated points, and therefore all the odd-power terms have been also neglected (otherwise, the transition points would be fixed by setting to zero more than one equation for the coefficients.)

Apart of the global minimum at \( \Psi = 0 \), the potential free-energy (2.1) possesses one local minimum and one local maximum. Spontaneous symmetry breaking will occur in (2.1) when coefficient \( \alpha_1 \) is allowed to take on negative values. The potential free-energy with coefficients \( \alpha_i \) constant, resulting from changing sign of \( \alpha_1 \) in (2.1) will then show a local maximum at \( \Psi = 0 \), two local minima and one local maximum at \( \Psi > 0 \). For the position of these extrema to be at real values of \( \Psi \) in the temperature-independent approximation, the coefficients \( \alpha_i \) should moreover satisfy

\[ \alpha_1 \left[ \frac{1}{4} \alpha_1 \alpha_4^2 \pm \left( \frac{\alpha_3^2}{27} - \alpha_2 \alpha_4 \right) \right] + \frac{\alpha_2^2}{27} \left( \alpha_2 \alpha_4 - \frac{\alpha_3^2}{4} \right) \leq 0, \]  

(2.2)

where the lower signs correspond to potential (2.1) and the upper signs to the potential with negative \( \alpha_1 \). Manipulating these expressions, we obtain additional conditions on the \( \alpha_i \)'s:

\[ \alpha_2^2 > 27 \alpha_1 \alpha_3, \quad \alpha_3^2 > \frac{27}{4} \alpha_2 \alpha_4. \]  

(2.3)

If we consider the dependence of the free-energy potential on the thermodynamic quantities \( P, T \) of the protein, then the corresponding thermodynamic potential should be represented as a function \( V(P, T, \Psi) \), where \( \Psi \) must be determined from the thermal equilibrium condition for which \( V \) is a global minimum. Restricting to processes taking place at constant pressure, it follows that \( \alpha_1(T) \) must vanish at the transition point because this term must be larger than zero in the symmetric phase (to have a global minimum at \( \Psi = 0 \)) and smaller than zero in the phase with broken symmetry to account for the existence of a global minimum at \( \Psi \neq 0 \). Besides, for the transition point at which \( \alpha_1 \) vanishes to be a stable state, it is necessary that the last term \( \alpha_4 \) be positive and constant. However, one can expect that the terms \( \alpha_2(T) \) and \( \alpha_3(T) \) still show a singularity where they change sign as function of \( T \). These singularities would correspond to new transitions occurring at temperatures which must differ from the main transition temperature. Thus, for a given \( P = \text{const.} \), one may write \( \alpha_1 \propto (T - T_{c1}) \), \( \alpha_2 \propto (T - T_{c2}) \) and \( \alpha_3 \propto (T - T_{c3}) \), and, given the shape of the potential, \( T_{c1} < T_{c2} < T_{c3} \).
Once the temperature-independent potential (2.1) is fixed, the above results can also be obtained by using the following procedure. If we were dealing with a field theory in which coordinate $\Psi$ was taken to be the matter field, then we had a precise prescription to investigate the symmetry behaviour of the effective theory with negative $\alpha_1$ at nonzero temperature (Linde, 1979). However, what we, instead, have is a phenomenological theory with a potential linear in free energy which is not but a generalization of that of Ginzburg and Landau for superconductivity (Ginzburg & Landau, 1950). Field theory, in turn, should be expected to be nothing but a covariant generalization of the Ginzburg-Landau theory for a potential given in terms of energy densities rather than thermodynamic potentials (free energies). To obtain a temperature-dependent potential in our phenomenological theory using the field-theoretical prescription (Linde, 1979), we note that the potential of the Ginzburg-Landau theory can be formally reproduced by regarding the order parameter as a field, and using then the field-theoretical prescription as applied to a "fermionic-like" field, rather than the usual boson case. The ultimate reason to follow this formal procedure resides in the fact that the Ginzburg-Landau potential, or potential (2.1), is linear in free energy, rather than energy-density.

Starting with the temperature-independent potential (2.1), we shall extend this trick to attain the corresponding temperature-dependent effective potential for our unidimensional symmetric slice of a protein folding funnel landscape. The Lagrange equation for the "field" coordinate $\Psi$ in the broken symmetry phase would be given by

$$\left( \Box + \alpha_1 - \alpha_2 \Psi^2 + \alpha_3 \Psi^4 - \alpha_4 \Psi^6 \right) \Psi = 0. \tag{2.4}$$

We then tentatively interpret the global minimum in (2.1) as defining a "conformational vacuum". Therefore, in Eqn. (2.4) we should first insert a shift $\Psi \rightarrow \bar{\Psi} = \Psi + \sigma(T)$ to recover the picture where we can define usual creation and annihilation operators with vanishing conformational vacuum expectation for the "field" $\Psi$, and then take the Gibbs average (Landau & Lifshitz, 1975)

$$\langle ... \rangle = \frac{\text{Sp}[\exp(-H/T)]}{\text{Sp}[\exp(-\frac{H}{T})]} ,$$

with $H$ the Hamiltonian and $T$ the temperature. We then obtain

$$\Box \sigma(T) + \alpha_1 \sigma(T) - \alpha_2 \left( \sigma(T)^3 + 3 \sigma(T) \langle \Psi^2 \rangle \right)
+ \alpha_3 \left( \sigma(T)^5 + 10 \sigma(T)^3 \langle \Psi^2 \rangle \right)
- \alpha_4 \left( \sigma(T)^7 + 21 \sigma(T)^5 \langle \Psi^2 \rangle \right) = 0, \tag{2.5}$$

where we have used the condition $\langle \Psi \rangle = 0$ for the new "conformational vacuum", and restricted to work in the lowest order in $\alpha_2$, $\alpha_3$ and $\alpha_4$, where the quantities $\langle \Psi^3 \rangle$, $\langle \Psi^5 \rangle$ and $\langle \Psi^7 \rangle$ vanish, and the contribution from $\langle \Psi^4 \rangle$ can be discarded.

Since in the harmonic approximation at constant $\sigma$, $\alpha_1 \langle \Psi^2 \rangle$ is linear in the free-energy, this quantity can now be calculated according to the fermionic rules as

$$\alpha_1 \langle \Psi^2 \rangle = \frac{1}{(2\pi)^3} \int \frac{d^3p}{2\omega_p} (2n_p - 1), \tag{2.6}$$
in which $\omega_p$ is the energy of the particles with momentum $p$ and mass $m$, and $n_p$ is the occupation number. Taking $n_p = \left( \exp \frac{\omega_p}{T} + 1 \right)^{-1}$, discarding the temperature-independent term $-\int d^3p/2\omega_p^2$, which can be eliminated by mass renormalization at $T = 0$, and recalling (Linde, 1979) that all interesting effects should take place at $T \gg m$, expression (2.6) becomes

$$\alpha_1 \langle \Psi^2 \rangle = \frac{1}{2\pi^2} \int_0^\infty \frac{dp}{e^{\frac{p}{T}} + 1} = \frac{T \ln 2}{2\pi^2},$$

so that we would finally have a Lagrange equation that again corresponds to an effective potential free-energy given by

$$V = -\frac{1}{2} \alpha_1 \epsilon_1 \Psi^2 + \frac{1}{4} \alpha_2 \epsilon_2 \Psi^4 - \frac{1}{6} \alpha_3 \epsilon_3 \Psi^6 + \frac{1}{8} \alpha_4 \Psi^8,$$

where the relative temperature $\epsilon_j$ are defined as:

$$\epsilon_j = \frac{T_{cj} - T}{T_{cj}}, \quad j = 1, 2, 3$$

with the critical temperatures

$$T_{c1} = f_1 \frac{\alpha_1^2}{\alpha_2}, \quad T_{c2} = f_2 \frac{\alpha_1 \alpha_2}{\alpha_3} > T_{c1}, \quad T_{c3} = f_3 \frac{\alpha_1 \alpha_3}{\alpha_4} > T_{c2},$$

in which conditions (2.3) have been used and the dimensionless numerical coefficients $f_1$, $f_2$ and $f_3$ are all of order unity. The same approximate result would also be obtained if we took into account the discarded terms proportional to $\langle \Psi^4 \rangle$ in (2.5), since such terms, which can be calculated by the above procedure, would contribute $T_{c1}$ and $T_{c2}$ with a factor of order unity because of conditions (2.3). These results can readily be generalized to the more realistic case of a potential with an arbitrary number of higher-order potential terms, the highest of which being even and positive, and terms with odd power of $\Psi$ which break invariance under $\Psi \rightarrow -\Psi$. For the purposes of this work, however, it will suffice working with the simplest potential slice (2.8).

Note that (2.8) reduces in fact to the typical thermodynamical potential of the Ginzburg-Landau theory (Ginzburg & Landau, 1950) in the limit $\alpha_3, \alpha_4 \rightarrow 0$, with $\alpha_4$ going to zero more rapidly than $\alpha_3$. The considered phase transition for protein folding can therefore be regarded as a generalization from the second-order phase transitions taking place in the phenomenological theory of superconductivity. At $T_{c1}$ there will be a second-order phase transition from a potential with a shape as in (2.1) to the corresponding potential with the broken internal symmetries. According to (2.8), other transitions can also occur at the generally higher critical temperatures $T_{c2}$ and $T_{c3}$, these taking place between states with the folded protein at the global minimum.
3 Cosmology in a protein

Like in superconductors and superfluids (Tilley & Tilley, 1986), or field theories (Nielsen & Olesen, 1973), in the present phenomenological model strings filled with a protein in its symmetric, native phase will be able to form when the order parameter $\Psi$ is allowed to be complex

$$\Psi = |\Psi|e^{i\theta}, \quad (3.1)$$

where $\theta$ is a fixed phase. The possibility of formation of vortex lines in the considered phase transition in proteins required therefore having a two-dimensional section of the inverted folding funnel (Dill & Su Chan, 1997) that corresponded to the breakdown of a complete given symmetry. Folded proteins contain a lot of internal symmetries which can be visualized as contacts between hydrophobic groups in the protein core (Dill, 1995; Dill & Su Chan, 1997). Each of these symmetries can be independently broken by variation of one, two or possibly more normal coordinates. In the first case, a domain wall could be formed; when variation of two normal coordinates were required to break down the hydrophobic contacts defining one of such internal symmetries, then one would expect the formation of stringy topological defects, a case which we assume to hold hereafter.

Generally, strings can be formed because in the broken-symmetry phase where $T < T_{c1}$, the first term in (2.1) is negative and, since the last term of this potential is always positive, $V$ has the shape of a wrinkled mexican sombrero; then, the manifold of the most stable conformations (conformational “vacuum” manifold) is not simply connected and, therefore, possesses nontrivial loops. By the first homotopy group (Kibble, 1980; Vilenkin & Shellard, 1994) it follows that axisymmetric topological defects (vortex lines) should then be expected to form along the phase transition at temperature $T_{c1}$. This can be most clearly understood by looking at the broken-symmetry phase with inverted folding funnel potential as a quantum system.

Below the critical temperature $T_{c1}$, a temperature-dependent fraction of protein macromolecules become trapped in their various unfolded conformations and, together with the solvent molecules, will form a phase which can be described by a wave function $\Psi(r)$. The properties of the protein-solvent complex in the broken-symmetry phase can be understood assuming that this wave function satisfies a wave equation of the form

$$i\hbar \frac{\partial \Psi}{\partial t} = -\frac{\hbar^2}{2m}\nabla^2 \Psi + \mu \Psi, \quad (3.2)$$

where $m$ is taken to be the mass of the complex formed by a protein macromolecule and its hydration shell, and $\mu$ is the chemical potential, i.e. the energy gained by the system when one molecule of protein is unfolded at constant volume and entropy.

Let us assume that the free energy can be expanded in powers of $|\Psi|^2$ (which
would now play the role of the order parameter) and has the form:

\[ F(r) = \alpha_1(T)|\Psi|^2 + \frac{1}{2}\alpha_2(T)|\Psi|^4 - \frac{1}{3}\alpha_3(T)|\Psi|^6 + \frac{1}{4}\alpha_4|\Psi|^8, \]  

\( \text{(3.3)} \)

where \( \alpha_j(T) = \epsilon_j \alpha_j \).

The chemical potential \( \mu \) can be evaluated by using (3.3) so that the Schrödinger equation becomes

\[ i\hbar \frac{\partial \Psi}{\partial t} = -\frac{\hbar^2}{2m}\nabla^2 \Psi + \alpha_1(T)\Psi + \alpha_2(T)|\Psi|^2\Psi - \alpha_3(T)|\Psi|^4\Psi + \alpha_4|\Psi|^6\Psi. \]  

\( \text{(3.4)} \)

Eq. (3.4) can now be re-scaled to assure a dimensionless form, which is given by

\[ i\dot{\eta} = -\nabla^2 \eta + \left(-1 + |\eta|^2 - |\eta|^4 + |\eta|^6\right)\eta, \]  

\( \text{(3.5)} \)

by introducing the quantities

\[ \tau_1 = \frac{\hbar}{|\alpha_1(T)|} = \frac{\tau_{01}}{\epsilon_1}, \quad \tau_{01} = \frac{\hbar}{\alpha_1}; \]  

\( \text{(3.6)} \)

\[ \xi_1 = \frac{\hbar}{\sqrt{2m|\alpha_1(T)|}} = \frac{\xi_0}{\sqrt{\epsilon_1}}, \quad \xi_0 = \sqrt{\frac{\hbar \tau_{01}}{2m}}; \]  

\( \text{(3.7)} \)

\[ \sigma_j^2 = -\frac{\alpha_1(T)}{\alpha_j \neq 1(T)} = -\frac{\alpha_1 \epsilon_1}{\alpha_j \neq 1(T)}; \]  

\( \text{(3.8)} \)

where \( \tau_1 \) is to be interpreted as the relaxation time, \( \xi_1 \) as the correlation length, and the \( \sigma_j^2 \)'s as equilibrium linear densities characterizing the broken-symmetry phase for the unfolded protein. In these definitions, \( \tau_{01} \) is a time parameter that also characterizes the phase with broken symmetry.

When we restrict ourselves to deal with the time-independent solutions to Eq. (3.5) in cylindrical coordinates \((\rho, \phi, z)\), i.e.

\[ \frac{\partial \eta}{\partial \rho} + \frac{\partial^2 \eta}{\partial \rho^2} + \frac{\partial^2 \eta}{\rho \partial \phi^2} = \left(|\eta|^2 - |\eta|^4 + |\eta|^6 - 1\right)\eta, \]  

\( \text{(3.9)} \)

one has the trivial solution \( |\eta|^2 = 1 \) and the axisymmetric solution

\[ \eta = f(\rho) \exp(in\phi), \]  

\( \text{(3.10)} \)

with \( n \) a whole number. The radial part of (3.10) is regular for \( \rho \ll 1 \), where \( f(\rho) \simeq \rho^n \), and approaches the equilibrium value of the trivial solution at \( \rho \gg 1 \), for which \( f^2(\rho) \simeq 1 - n^2/2\rho^2 \). Thus, solution (3.10) represents a stringy topological defect which, for \( n = 1 \), is still of the kind first considered by Ginzburg and Pitaevskii in the context of the phenomenological theory of superfluidity (Ginzburg & Pitaevskii, 1958). Around the axis of symmetry, there will be a flow caused by the phase gradient, with velocity \( v = \hbar \nabla \theta/m = \hbar/mr \). The resulting vortex will be filled with protein molecules keeping the native structure,
or some partially folded conformations, and will be surrounded by a distribution of distinct unfolded configurations. It appears that a certain nonzero density of these vortex lines would be formed if the second-order phase transition is induced by a sufficiently rapid temperature quenching.

However, the isotropy assumption for the order parameter, by which variation of the accessible free energy with respect to normal coordinates $\Psi_1$ and $\Psi_2$ are regarded to be equal, is obviously not but an approximation. Strictly speaking, the cylindric coordinates cannot be circular, but only closed on the $z$-constant sections. This would lead to stringy solutions corresponding to a cylinder deformed on its $z$-constant sections and closed flow streamlines no longer perfectly circular. These deformed solutions would satisfy (3.10) only approximately.

We shall estimate the density of vortex lines formed during the phase transition of a protein induced on a dynamical time scale by a rapid decrease of temperature. In the vicinity of $T_{c1}$, the relaxation time scale $\tau_1$ (i.e. the time scale on which the order parameter can adjust to the new thermodynamic parameters) will become quite longer than the time on which the quench proceeds. Then, the order parameter $\Psi$ will be practically frozen on that time scale and correlated over large domains where $\xi_1 \to \infty$ (impulse regime). Sufficiently far from $T_{c1}$, $\tau_1$ will be much smaller than the quench time and $\Psi$ will become at an equilibrium configuration with $\xi_1$ determined by the instantaneous value of $\epsilon_1$ (adiabatic regime) (Zurek, 1996).

If the transition is made fast enough, then the growing regions of the new phase met and coalesced, making it impossible to avoid the creation of topological defects. That will happen on the boundary between the impulse and adiabatic regimes, at the freeze out time $\hat{t}$ (Zurek, 1996), if

$$ R_b > \hat{\xi}_1, \quad (3.11) $$

where $R_b$ is the effective radius of the region with the starting symmetric phase, and $\hat{\xi}_1$ is the characteristic correlation length at the freeze out time $\hat{t}$.

At a sufficiently rapid pace, on a quench time scale $\tau_Q$ (controlled by the rate at which the temperature is lowered), one can assume (Zurek, 1996) that $\epsilon_1$ is proportional to time, $\epsilon_1 = \frac{t}{\tau_Q}$, in the vicinity of $T_{c1}$. Thus, at $\hat{t}$ there will be a freeze out temperature $\hat{\epsilon}_1 = \hat{t}/\tau_Q$, so that using (3.6), we obtain $\hat{t} = \sqrt{\tau_{01}\tau_Q}$. The transition between the adiabatic and impulse regimes will therefore take place at the relative temperature $\hat{\epsilon}_1 = \epsilon(\hat{t}) = \sqrt{\tau_{01}/\tau_Q}$, corresponding to a freeze out correlation length which, using (3.7), can be expressed as

$$ \hat{\xi}_1 = \frac{\xi_0}{\sqrt{\hat{\epsilon}_1}} = \sqrt{\frac{h}{2m\alpha_1}}(\tau_{01}\tau_Q)^{\frac{1}{2}} = \left(\frac{h^3\tau_Q}{4m^2\alpha_1}\right)^{\frac{1}{2}}. \quad (3.12) $$

Since $\alpha_1$ corresponds to an free energy which may be associated with a large number of the protein internal motions, such as unfolding of $\alpha$-helices (Creighton, 1992) and hindered rotations, each with energies typically of the order $10^{-13}$ erg., for most proteins $\alpha_1$ will take on values within the interval $(10^{-10} - 10^{-13})$ erg.,
and hence $\hat{\xi}_1$ will have values in the interval $(10^{-6} - 10^{-7})\frac{1}{\tau_Q}$ cm. For sufficiently rapid, but still not very fast temperature quench, we then obtain $\hat{\xi}_1 \ll 1$ cm, and therefore there will be a copious initial production of vortex lines with density $\hat{\xi}_1^{-2}$ (Zurek, 1996).

We propose finally an experiment to check the possible formation of vortex lines and other topological defects during the phase transition leading from folded to unfolded conformations in apomyoglobin (aMb). The transition will be induced by rapid supercooling, following excitation by focused pulses of a tunable infrared laser. The experiment is a variant of that recently carried out by Ballew et al. (1996) in order to follow the main fastest events of the aMb folding. It would consist of first denaturing aMb by supercooling it in water or D$_2$O at around -10 C in a perfectly insulated cell equipped with a high precision device to monitor variations of energy locally. This could be e.g. made by measuring the transmission of a calibrated low-power diode laser focused at the given region. The sample would be held in a cuvette with optical path length of the order 1 mm, cooled by thermoelectric devices keeping the temperature constant at around -10 C, or inducing a fast recovery of that temperature, in the region previously heated by the infrared laser, with a thermistor feedback loop. Two counterpropagating beams from an infrared laser would here coherently and uniformly heat a cylindric region with section $\sim 1$ mm$^2$, of the previously cooled sample, inducing the folding of aMb in the resulting irradiated volume. The pulsed infrared laser delivered short pulses ($\sim 1$ ns) at wavelengths able to excite a near-infrared mode of water only (Ballew et al., 1996). After absorbing the infrared photons, water will almost instantly transform all absorbed energy into heat that locally induced naturation of aMb.

After the laser excitation, the created interphase would immediately start traveling inward the irradiated volume, quenching it to the denatured conformations in a time $\tau_Q$ short enough to satisfy (3.11), so creating a tangle of topological defects filled with folded or partly folded aMb conformations. The presence of these defects would finally be detected by measuring the deficit in energy released as, in the irradiated volume, aMb molecules in the symmetric phase pass back into the low-temperature unfolded phase. By ascribing this deficit to the formation of vortices and other topological defects (Buerle et al., 1996), one could infer the resulting defect density.

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