The Davydov/Scott Model for Energy Storage and Transport in Proteins

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Abstract The current status of the Davydov/Scott model for energy transfer in proteins is reviewed. After a brief introduction to the theoretical framework and to the basic results, the problems of finite temperature dynamics and of the full quantum and mixed quantum-classical approximations are described, as well as recent results obtained within each of these approximations. A short survey of experimental evidence in support of the Davydov/Scott model is made and absorption spectra are calculated that show the same temperature dependence as that measured in crystalline acetanilide. Future applications of the Davydov/Scott model to protein folding and function and to misfolding diseases are outlined.

Keywords Energy transfer • Proteins • VES hypothesis • Amide I band • Temperature dependence

1 McClare's proposal

Proteins are central to the functioning of cells: they transport molecules from one region of the cell to another, catalyze reactions, pass signals, and provide basic structure. The possibility that protein folding and function involves a first step in which energy is stored in the form of vibrational excited states has recently been designated as *the VES hypothesis* [1]. A biological role for vibrational excited states was first proposed by McClare in connection with a possible crisis in bioenergetics [2] (for more information about McClare's work see the article by Luca Turin, in this issue [3]). McClare's proposal was taken up by Davydov, who wanted to explain the conformational changes responsible for muscle contraction [4, 5],

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where the trigger is the energy donating reaction of the hydrolysis of adenosinetriphosphate (ATP). Davydov's assumption was that the first event after the hydrolysis of ATP is the storing of the energy released by the chemical reaction in a vibrational mode of the peptide group, known as amide I. In the Davydov model [4, 5], the interaction of the amide I with the vibrations of the hydrogen bonds leads to a localization of the amide I excitation in a few amino acids, much as the interaction of an electron with the phonon modes of a polarizable crystal leads to a polaron. Indeed, just as the electron generates distortions (phonon modes) that in turn lead to a localized, lower energy state for the electron, in the Davydov model the amide I excitation generates distortions in the hydrogen bonds associated with it which, in turn, localize the amide I excitation. In both cases, we say that we have a self-trapped state (of the amide I or of the electron). While Davydov's studies were centered on a continuum model of the hydrogen-bonded chains that stabilize protein α -helices, the investigations by Scott and co-workers were for discrete systems that constitute more realistic representations of proteins [6]. Both Davydov's analytical results [4, 5] and Scott and co-workers numerical simulations (for a review of the work until 1992, see [6] and references therein) indicate that the state constituted by an amide I excitation and its associated hydrogen bond distortions, a self-trapped state known in the literature as the Davydov soliton, is stable at low temperatures.

The aim of this article is to review the current status of the Davydov/Scott model and the new avenues it has opened for protein research. In the next section, the Davydov/Scott model is introduced and a short survey of Davydov's and Scott's initial work, as well as of more recent studies within the same theoretical framework, is made. Section 3 describes the problems associated with finite temperature dynamics and Section 4 summarizes the recent experimental data in support of the Davydov/Scott model. The article ends with a section on future work, such as applications to protein folding, conformational changes, and misfolding diseases.

2 The Davydov/Scott model

At the conceptual level, Davydov's first main addition to McClare's proposal was to point out a specific vibrational band that is found in proteins and that is ideal for the storage and propagation of energy, namely, the amide I band. Indeed, the amide I energy is approximately eight times higher than the thermal energy at 298 K (25°C) and, at the same time, it is approximately half the energy released in the hydrolysis of ATP, i.e., the amide I vibration cannot be created thermally and is thus relatively isolated from thermal noise, but it is still sufficiently low in energy to be generated by the reaction that provides the energy needed in most biological processes. From the physical point of view, the amide I is a normal mode of vibration of the peptide group which consists essentially of the stretching of the C=O bond, and its dependence on the secondary structure of proteins has been used to determine the folds of unknown protein structures [7]. Davydov's second main contribution to the field of bioenergetics was to realize that the amide I energy depends on the strength of the hydrogen bond that may exist between the oxygen of one peptide group and the nitrogen of another, a physical ingredient that is also essential in the Davydov/Scott Hamiltonian. Thus, the Davydov/Scott Hamiltonian has three terms:

$$\hat{H} = \hat{H}_{\text{ex}} + \hat{H}_{\text{ph}} + \hat{H}_{\text{int}} \quad (1)$$

where $\hat{H}_{\text{ex}}$ is the exciton Hamiltonian, which describes the transfer of an amide I excitation between neighboring sites, $\hat{H}_{\text{ph}}$ is the phonon Hamiltonian, which describes the vibrations of the peptide groups n as a whole, and $\hat{H}_{\text{int}}$ is the interaction Hamiltonian introduced by Davydov to represent the dependence of the amide I energy of one peptide group on the strength of the associated hydrogen bond. The exciton Hamiltonian $\hat{H}_{\text{ex}}$ is:

$$\hat{H}_{\text{ex}} = \sum_{n=1}^N \epsilon \hat{a}_n^\dagger \hat{a}_n + \sum_{n>m=1}^N [V_{nm} (\hat{a}_n^\dagger \hat{a}_m + \hat{a}_m^\dagger \hat{a}_n)] \quad (2)$$

where N is the total number of sites (peptide bonds) in the system, ϵ is the energy of an isolated amide I excitation, $\hat{a}_n^\dagger (\hat{a}_n)$ is the creation (annihilation) operator for an amide I excitation at site n , and V_{nm} is the dipole–dipole interaction between the transition dipole moments of an amide I vibration at sites n and m . In most of the studies in the literature, only the nearest neighbor interactions are taken into account, and $V_{nm} = V\delta_{nm}$, V being the dipole–dipole interaction energy between neighboring amide sites.

The phonon Hamiltonian, $\hat{H}_{\text{ph}}$, is:

$$\hat{H}_{\text{ph}} = \frac{1}{2} \sum_{n=1}^N \left[\kappa (\hat{u}_n - \hat{u}_{n-1})^2 + \frac{\hat{P}_n^2}{M} \right] \quad (3)$$

where $\hat{u}_n$ is the displacement operator of peptide group n from its equilibrium position, $\hat{P}_n$ is the momentum operator of site n , M is the mass of each site, κ is the elasticity constant of the lattice, and the motions of the peptide group as a whole are assumed to be harmonic.

The interaction Hamiltonian, $\hat{H}_{\text{int}}$, is:

$$\hat{H}_{\text{int}} = \chi \sum_{n=1}^N [(\hat{u}_{n+1} - \hat{u}_n) \hat{a}_n^\dagger \hat{a}_n] \quad (4)$$

where χ is an anharmonic parameter that tells us how the amide I energy depends on the hydrogen bond length, which is measured by the displacement differences $(\hat{u}_{n+1} - \hat{u}_n)$. A positive χ means that when the hydrogen bond length decreases, the energy of the amide I vibration decreases, and vice versa. When $\chi = 0$, the amide I energy does not depend on the relative positions of the peptide groups and the amide I excitation propagates from one peptide group to the next because of the dipole–dipole interactions V . In this case, an amide I excitation that is initially located at one peptide group will spread, on a sub-picosecond timescale, to other peptide groups, and the state will quickly cease to be localized. On the other hand, when $\chi \neq 0$, an excitation initially located at one peptide group will induce a distortion of the associated hydrogen bond (a compression for positive χ and an expansion for negative χ), which, in turn, will decrease the energy of the corresponding amide I state. When the (negative) interaction energy is greater, in absolute terms, than the distortion energy, which is always positive, the state of the amide I excitation together with the distortion has an energy that is lower than the state of the amide excitation in the absence of the distortion. An example of such a lower energy state, which is known as the Davydov soliton, is depicted in the very thick curve of Fig. 1. The values of the parameters used are those estimated from a comparison of experimentally measured spectra with spectra calculated for an α -helix [8]. With these values, the Davydov soliton is broad, with a width at half maximum of 10 sites, and induces an expansion in the hydrogen bond with a maximum of 0.0011 Å.

Fig. 1 Davydov soliton (very thick line), a state at 10 K (thin line) and an average state over a statistical ensemble at 10 K (thick line) are displayed. The other parameters are: $V = -6.2 \text{ cm}^{-1}$, $\kappa = 13 \text{ N/m}$, and $\chi = -30 \text{ pN}$. The top plot shows the probability for an excitation at site n , $|\varphi_n|^2$, and the bottom plot shows the corresponding displacement differences, $u_n - u_{n-1}$, in Å. Because of their small size, for greater clarity, the displacement differences for the Davydov soliton are presented in the inset at the bottom

In (1)–(4), both the excitation and the lattice are treated quantum mechanically. Exact wave functions for the full quantum system have yet to be determined and in many studies the motion of the peptide groups as a whole is treated classically, an approximation that has been called mixed quantum-classical [9]. In the mixed quantum-classical approximation, it is possible to determine exact wavefunctions for the Davydov/Scott Hamiltonian. They are as follows:

$$|\psi(t)\rangle = \sum_{i=1}^N \varphi_i(\{u_m\}, \{p_m\}, t) a_i^\dagger |0\rangle \quad (5)$$

where the explicit dependence of the probability amplitude φ_i for an excitation at site i on the displacements and momenta of all the sites is not specified a priori. The equations of motion for the quantum variables are derived from Schrödinger's equation, while the

equations of motion for the classical variables are derived from Hamilton's equations. For a one quantum state, we obtain:

$$i\hbar \frac{d\varphi_n}{dt} = -V(\varphi_{n-1} + \varphi_{n+1}) + \chi(u_{n+1} - u_{n-1})\varphi_n, \quad (6)$$

$$M \frac{d^2 u_n}{dt^2} = \chi(|\varphi_{n+1}|^2 - |\varphi_{n-1}|^2) + \kappa(u_{n+1} + u_{n-1} - 2u_n). \quad (7)$$

Studies within this mixed quantum-classical approximation include those of Davydov and co-workers in the continuum and adiabatic limit of (6) and (7) (see [5] and references therein), and those of Scott and co-workers for the more realistic discrete system (see [6] and references therein). While Davydov obtained a nonlinear Schrödinger equation for the probability amplitudes, which leads to exact soliton solutions [4], the numerical integration of (6) and (7), and of extensions of these equations by Scott and co-workers [6, 10], which can represent the three interacting chains that are present in an α -helix, showed that also in the discrete system soliton solutions can form and travel in the chains, confirming Davydov's analytical studies. While in the continuum limit a nonzero value, no matter how small, of the nonlinearity parameter χ leads to localized amide I excitations, in the discrete chains localized excitations will only form above a given threshold [11], which tends to zero as the number N of peptide groups increases [12].

More recent studies within the mixed-quantum classical approximation include those by Brizhik and co-workers who have studied (1) the influence of electromagnetic radiation (EMR) on soliton solutions and have identified two EMR frequencies, one which induces soliton decay and another which leads to emission of sound waves [13, 14] and (2) the influence of helical symmetry on low temperature solutions, finding that solitons that preserve helical symmetry are dynamically unstable, while those that break translational and helical symmetries have lower energies and can propagate [15, 16]. On the other hand, full quantum mechanical studies of the Davydov/Scott model have also been pursued (see [6] and references therein, and [17–22] for more recent results). A question that remains open, in spite of many investigations, is the degree of accuracy of the mixed quantum-classical approach. When comparing the latter approximation with full quantum versions, however, it is important to make sure that the variables estimated do indeed represent the same physical quantities in both regimes. For example, it should be noted that the mixed-quantum classical probability $|\varphi_n|^2$ (see (5)) cannot be directly compared with the full quantum probability. This is because the mixed quantum-classical probability is already a correlation, i.e., it represents the probability that an amide I excitation is site n when the lattice conformation is that specified by the displacements u_n . While the Davydov/Scott Hamiltonian (1–4) is translation invariant, and its full quantum wave function leads to equal probability for the amide I excitation to be at all sites, the mixed quantum-classical wave function represents a situation in which the translation invariance has already been broken. A second problem with the assessment of the validity of the mixed quantum-classical approach is that in fact both regimes include approximations whose validity is hard to evaluate but without which it is not possible to arrive at quantitative results.

3 Thermal stability of localized states

While the low temperature results mentioned above indicate the existence of soliton, or self-trapped, states, an important question is whether these states are stable at biological

temperatures and, if not, how long they last. While Davydov studied this problem using a thermally averaged Hamiltonian [23], dynamical coupling to a thermal bath was first modeled by extending the equations of motion into Langevin equations [24], that is, by adding stochastic forces $F(n)$ and damping terms $-\Gamma \frac{du_n}{dt}$ to the right hand side of (7), so that these terms obey the fluctuation–dissipation relations: $\langle F_n(t) F_m(t') \rangle = 2M\Gamma k_B T \delta_{nm} \delta(t - t')$, k_B being the Boltzmann constant and T the temperature. The result of such simulations is that soliton solutions disperse in a few picoseconds at biological temperatures [24]. However, these results clashed with exact quantum Monte Carlo (QMC) simulations [25] which showed that the distortion in the hydrogen bonds induced by the excitation becomes more localized and, in absolute term, *increases* as the temperature increases. This conflict was resolved by showing that the coupling of a classical bath to a mixed quantum-classical system leads to a classical behavior, not only of the lattice, but also of the quantum amide I excitation [9, 26]. While the states of a classical amide I excitation at finite temperature that are sampled by the Langevin simulations are predominantly delocalized, the states of a quantum amide I excitation in the same thermodynamic conditions are predominantly localized. One example of the localized states sampled by the amide I excitation at finite temperature is that given by the thin line in Fig. 1, which should be contrasted with the Davydov soliton given by the thick line in that figure.

A set of dynamic equations for finite temperature simulations of the mixed quantum-classical system was proposed [26, 27] which, for sufficiently long runs, leads to the same equilibrium averages for the lattice distortion as the QMC simulations. According to the latter set of equations, instead of coherent propagation along the chain, as is expected from solitons, at biological temperatures, localized amide I excitations jump stochastically from one peptide group to another peptide group, changing their shape and their velocity as they travel [9]. Although from a mathematical and physical perspective this stochastic amide I propagation may seem less elegant than the coherent soliton propagation, from a biological point of view, it is more appealing because it is a much more robust manner to transfer energy and information, capable of surviving many mutations, as what happens with protein structure and function.

The very thick line in Fig. 1 shows the distortion induced in the hydrogen bond lengths by the amide I excitation and the localized state that arises because of that distortion. On the other hand, the thick line is the result of an average over four million amide I states from an equilibrium ensemble at 10 K and reveals that, when the thermal noise is averaged out, a localized lattice expansion, correlated with the position of the amide I excitation, becomes visible. However, it should be noted that the existence of a correlation between the distortion and the amide I excitation does not necessarily mean that self-trapped states are involved. Comparing the distortions induced by thermal agitation (thin line) with those induced by the amide I excitation alone (very thick line), we see that the former are 50 times larger than the latter. While, for the Davydov soliton (very thick line), the localization of the amide I vibration is due to self-trapping, the much greater localization of the amide I excitation at 10 K (thick and thin lines) is due to the lattice disorder, i.e., it is due to Anderson localization. We have two regimes: one, at sufficiently low temperature, in which the distortion in the hydrogen bonds induced by thermal fluctuations is smaller than that induced by the amide I excitation, and in which there is a correlation between the lattice distortions and the amide I localization because of self-trapping, and a second regime, at higher temperatures, when the distortion induced by thermal fluctuations is larger and in which such a correlation arises because of Anderson localization. The lattice distortion associated with the self-trapped state *decreases* with temperature, but the distortion of

the Anderson localized states *increases* with temperature (see Fig. 1), which explains the results obtained in the QMC calculations [25]. This prediction of self-trapped states at low temperature and Anderson localization at biological temperatures [9] is also what is found experimentally, as is detailed in Section 4.

4 Experimental support

The first evidence for a Davydov-soliton-like state was obtained in the crystal of acetanilide (ACN) [6] which possesses hydrogen-bonded chains identical to those that stabilize the secondary structure of proteins and thus constitutes a possible model system in which to test the Davydov/Scott model. Careri and co-workers found an anomalous line, visible at low temperatures and 15 cm^{-1} below the amide I excitation in that crystal, which was interpreted as a self-trapped, soliton-like state, as described in Al Scott's review article [6]. This interpretation has been confirmed by Hamm and co-workers who have applied nonlinear spectroscopic methods to the study of both the amide I and NH stretch excitations in ACN and have identified two phonon modes that mediate the self-trapping [28–31] (see also Hamm's article in this issue [32]). Furthermore, Hamm and co-workers also find evidence for self-trapped states in the organic crystal of *N*-methylacetamide (NMA) [30, 31] and even in a small polypeptide [33] and speculate that such states may arise in all hydrogen-bonded systems [31]. Their experimental data [29] also confirms the results obtained in computer simulations [9], according to which at low temperature vibrational excitations are self-trapped, while at biological temperatures they are localized because of static and dynamical disorder (Anderson localization).

An important question in a biological context is whether the anharmonic interactions present in the Davydov/Scott Hamiltonian exist in proteins. The experimental evidence already available strongly supports the existence of such anharmonicity [33–36]. This being so, another important issue is how long the vibrational states last. Indeed, an early objection to a biological role of vibrational excited states in proteins was their short lifetimes, thought to be in the subpicosecond range [2] and Davydov's argument was that the soliton state, having a lower energy than the delocalized, free exciton, states would last longer [4, 5]. The experimental data so far indicate that the lifetime of the amide I modes is system- and temperature-dependent and can vary from 30 to 35 ps at around 50 K to 5 ps at 280 K in myoglobin [34, 36]. Computer simulations in real proteins show that in a few picoseconds the amide I energy can travel from the active site, where the energy is generated, to any other region in the protein where the energy is needed for work [1, 9]. Finally, experiments in the group of Hochstrasser [37] show that amide I vibrations can be delocalized in two different helices, as occurs in computer simulations [1], and as must happen in real proteins if the VES hypothesis is valid.

In short, the experimental evidence for the kind of interactions first postulated by Davydov for vibrational excitations in proteins is now strong, both for amide I and NH stretch vibrations. The lifetimes of these excitations vary from system to system, but they are comparable to the time needed for an excitation to go from a protein's active site to another region where the energy is needed for work, according to computer simulations. The conclusion is that both the theoretical results so far and the available experimental evidence support the viability of VES hypothesis. It is now important to make predictions about variables that can be measured experimentally. To this end, in Fig. 2, the temperature dependence of the absorption spectrum of a one dimensional Davydov/Scott chain with

Fig. 2 Absorption spectrum for $T = 80$ K (*very thick line*), $T = 40$ K (*thick line*) and $T = 20$ K (*thin line*) for $V = -6.2$ cm^{-1} (*top*) and $V = +9.1$ cm^{-1} (*bottom*). Other values of the parameters are: $\kappa = 13$ N/m and $\chi = -30$ pN. The energy is in cm^{-1} and the zero corresponds to the isolated amide I energy ϵ

nearest neighbor amide I dipole–dipole interaction, V , is presented. Protein alpha-helices are stabilized by three hydrogen-bonded chains and, for an internal dielectric constant of one, the distribution of V_{nm} of the dipole–dipole interactions between all pairs of amide sites has a peak centered at the negative value of $V = -6.2$ cm^{-1} which comes from amide I dipole–dipole interactions within the same chain and another peak centered at the positive value of $V = +9.1$ cm^{-1} which comes from amide I dipole–dipole interactions of amide sites that belong to different chains [8]. In Fig. 2, both these values have been considered. The figure shows that for negative values of the dipole–dipole interaction V , an increase in temperature leads essentially to a broadening of the amide I line. On the other hand, when V is positive, at low temperatures, the amide I band is split into two peaks, of which only the high energy peak survives at higher temperatures. These qualitative features resemble what is found for the amide I band of ACN [6, 29, 31], although a quantitative fit has not been obtained. The qualitative reason for the spectra in the bottom panel of Fig. 2 is that when $V > 0$, the oscillator strength of the higher energy states is larger than that of the lower energy states, which compensates for the reverse trend in the density of states. At low energies, even small populations of high energy states lead to absorption (and hence two peaks), and as temperature increases and the higher energy states become more populated,

the lower energy peak becomes weaker. A more detailed explanation of this phenomenon is presented elsewhere [38].

5 Future perspectives

In spite of decades of research, the way proteins work remains obscure. However, it is known that to perform their functions, proteins must, first of all, acquire a well-defined average structure, known as the native structure. A fundamental question for biology, medicine, and the pharmaceutical and biotechnology industries, known as the protein folding problem, is how a given sequence of amino acids, in cells, most of the time assumes the native structure [39, 40]. Since Anfinsen's renaturing experiments, the answer to the protein folding problem has been the thermodynamics hypothesis, that is, the idea that the native structure of a protein is that which minimizes its free energy [40]. More recent theories of protein folding further assume that the free energy landscape of proteins is funnel-shaped [41–43] and that the native structure is that which is found at the bottom of the funnel. In this view, the folding of a given amino acid sequence into a protein native structure is simply driven by thermal agitation, and computationally, it can be reached by free energy minimization. This is the current paradigm according to which the protein folding problem is essentially solved [44] since finding a protein structure from its sequence on a computer depends only on having a sufficiently accurate potential to describe the interactions of its atoms with one another and on the availability of enough computer power. However, in spite of more than two decades of work, and millions of CPU hours [45], de novo folding of a protein from its amino acid sequence is still elusive.

On the other hand, the first experimental evidence for that the native structure of proteins is a kinetic trap was reported in 1968 by Levinthal who found two forms of an alkaline phosphatase at 317 K, one active and the other inactive, synthesized at different temperatures, in mutants of *E. coli* [39]. More recently, other cases have been found of proteins that can assume more than one structure in the same thermodynamical conditions [46–50]. All this latter experimental evidence points to a multi-funnel free energy landscape. Of course, in a multi-funnel free energy landscape, the crucial step for the definition of the structure of a protein is that in which a specific funnel is selected. One way to achieve this selection is by a kinetic mechanism, for which there is *direct* experimental evidence in a few cases [48–50] and which was already proposed by Levinthal, who suggested that there are specific pathways for folding [39]. In spite of the statements about the reconciliation between Levinthal's and Anfinsen's visions for folding [51], the fact is that the possibility that the native structure is that which minimizes the free energy, and that this structure can be accessed by many pathways, is fundamentally different from the possibility that the native structure is one among many thermodynamically equivalent kinetic traps and must be accessed by a specific pathway. Considering that a pathway for folding can be characterized by the intermediates that form along that pathway, in spite of the theoretical prevalence of Anfinsen's thermodynamic hypothesis and of the funnel models, one can say that the experimental evidence for a kinetic mechanism is indeed substantial [52, 53] and may even include proteins that apparently follow a two-state process [53, 54].

Thus, from the experimental point of view, there is actually at least some evidence for a multi-funnel free energy landscape and a kinetic mechanism for protein folding. On the other hand, the idea of a free energy bias to the native state is based on the fact that chemically denatured proteins refold to the native structure when the native conditions are

restored. Indeed, it is thought by many that, in denatured states, protein structures are essentially random, so that protein refolding starts from these random coil conformations. This idea is mainly based on the fact that random coil models can predict the experimentally determined dimensions of denatured proteins. However, it has been demonstrated that an ensemble of protein structures in which some 92% of the structure is fixed in the native conformation can exhibit the same statistical properties as random coils, as far as end-to-end distances and radii of gyration are concerned [55]. Therefore, it may well be that the starting point for the refolding experiments is not a random coil, as is usually assumed, but an opened up and distorted native structure, that is, a structure that is either still within, or not far from, the native basin and that naturally returns to it when the conditions go back to normal.

The hypothesis that underlies this author's work on energy transfer in proteins is that free energy landscape of proteins is multi-funnel-shaped and that protein folding and function involve two steps [56]: a first, kinetic, step in which a specific funnel is selected (most of the times that funnel being the native funnel) and a second step in which the structure relaxes as its free energy is minimized within the funnel selected, as first proposed by Anfinsen [40] and as was later incorporated in the funnel models [41–44]. Within this two-step picture of folding, proteins denature reversibly as long as the process of denaturation does not make them diffuse away from the native funnel and denature irreversibly otherwise. One advantage of this picture is that while protein misfolding in the absence of mutations is difficult to explain in a funnel model, in a multi-funnel, it can be easily rationalized as a case in which a funnel different from the native funnel was selected. On the other hand, within a multi-funnel picture, the difficulty in determining the native structure from a given amino acid sequence using a free energy bias is not just due to the size of the conformational space and the lack of computer power, but, more essentially, to the fact that the native structure is not a well-defined global free energy minimum, and even the most accurate potential energy functions will not be able to get around that problem.

According to the VES hypothesis [1], the drivers of protein conformational changes (which mediate protein function and also the selection of a specific funnel in protein folding) are vibrational excited states. Thus, according to the VES hypothesis, protein folding and function are not driven by thermal agitation, as happens according to Anfinsen's thermodynamic hypothesis, and, instead, the drivers of protein folding and function are the transient forces that arise from the nonradiative decay of vibrational excited states. These ideas have already been explored in a number of studies [1, 57–60]. For example, nonconservative Davydov/Scott models in which the amide I excitation decays so that its energy is transferred to other degrees of freedom have been explored in connection with protein conformational changes [57–59], and the amide I energy absorbed by a protein from the bending mode of water molecules has been found to be larger in proteins involved in misfolding diseases, something that, according to the VES hypothesis, can explain their structural instability [1, 60]. The ultimate aim of these studies is to open up a new avenue for the understanding of protein folding and function, in a multi-funnel-shaped free energy landscape. The general idea is that the classical atomistic interactions, described by potentials, such as AMBER [61], do not describe all the behavior of proteins and that what is missing is the effect of vibrational excited states (the VES hypothesis). From this perspective, in order to understand protein folding and function, we need to include the forces that arise from VES.

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References

1. Cruzeiro, L.: Why are proteins with glutamine- and asparagine-rich regions associated with protein misfolding diseases? *J. Phys.: Condens. Matter* **17**, 7833–7844 (2005)
2. McClare, C.W.F.: Resonance in bioenergetics. *Ann. N. Y. Acad. Sci.* **227**, 74–97 (1974)
3. Turin, L.: Colin McClare (1937–1977): a tribute. *J. Biol. Phys.* doi:[10.1007/s10867-009-9131-6](https://doi.org/10.1007/s10867-009-9131-6)
4. Davydov, A.S.: The theory of contraction of proteins under their excitation. *J. Theor. Biol.* **38**, 559–569 (1973)
5. Davydov, A.S.: *Solitons in Molecular Systems*, 2nd edn. Kluwer Academic, Dordrecht (1991)
6. Scott, A.: The Davydov soliton revisited. *Phys. Rep.* **217**, 1–67 (1992)
7. Krimm, S., Bandekar, J.: Vibrational Spectroscopy and conformation of peptides, polypeptides and proteins. *Adv. Protein Chem.* **38**, 181–364 (1986)
8. Cruzeiro, L.: Influence of the nonlinearity and dipole strength on the amide I band of protein α -helices. *J. Chem. Phys.* **123**(23), 234909 (2005)
9. Cruzeiro-Hansson, L., Takeno, S.: Davydov model: the quantum, mixed quantum-classical and full classical systems. *Phys. Rev. E* **56**, 894–906 (1997)
10. Scott, A.C.: Dynamics of Davydov solitons. *Phys. Rev. A* **26**, 578–595 (1982)
11. Hyman, J.M., McLaughlin, D.W., Scott, A.C.: On Davydov's α -helix solitons. *Physica D* **3**, 23–44 (1981)
12. Cruzeiro-Hansson, L., Kenkre, V.M.: Localized versus delocalized ground states of the semiclassical Holstein Hamiltonian. *Phys. Lett. A* **190**, 59–64 (1994)
13. Brizhik, L., Cruzeiro-Hansson, L., Eremko, A.: Influence of electromagnetic radiation on molecular solitons. *J. Biol. Phys.* **24**, 19–39 (1998)
14. Brizhik, L., Cruzeiro-Hansson, L., Eremko, A.: Electromagnetic radiation influence on nonlinear charge and energy transfer in biosystems. *J. Biol. Phys.* **24**, 223–232 (1999)
15. Brizhik, L., Eremko, A., Piette, B., Zakrzewski, W.: Solitons in α -helical proteins. *Phys. Rev. E* **70**(3), 031914, 1–16 (2004)
16. Brizhik, L., Eremko, A., Piette, B., Zakrzewski, W.: Charge and energy transfer by solitons in low-dimensional nanosystems with helical structure. *Chem. Phys.* **324**, 259–266 (2006)
17. Pouthier, V.: Two-vibron bound states in α -helix proteins: the interplay between the intramolecular anharmonicity and the strong vibron-phonon coupling. *Phys. Rev. E* **68**(2), 021909 (2003)
18. Pouthier, V., Falvo, C.: Relaxation channels of two-vibron bound states in α -helix proteins. *Phys. Rev. E* **69**(4), 041906 (2004)
19. Falvo, C., Pouthier, V.: Vibron–polaron in α -helices. I. Single-vibron states. *J. Chem. Phys.* **123**(18), 184709 (2005)
20. Falvo, C., Pouthier, V.: Vibron–polaron in α -helices. I. Two-vibron states. *J. Chem. Phys.* **123**(18), 184710, 1–12 (2005)
21. Kenkre, V.M., Giuggioli, L.: Study of some approximation schemes in the spin-boson problem. *Chem. Phys.* **296**, 135–148 (2004)
22. Cuevas, J., Silva, P.A.S., Romero, F.R., Cruzeiro, L.: Dynamics of the Davydov–Scott monomer in a thermal bath: comparison of the full quantum and semiclassical approaches. *Phys. Rev. E* **76**(1), 011907, 1–8 (2007)
23. Davydov, A.S.: Soliton motion in a one dimensional molecular lattice with account taken of thermal oscillations. *Sov. Phys. JETP* **51**, 397–400 (1980)
24. Lomdahl, P.L., Kerr, W.C.: Do Davydov Solitons exist at 300 K? *Phys. Rev. Lett.* **55**, 1235–1238 (1985)
25. Wang, X., Brown, D.W., Lindenberg, K.: Quantum Monte Carlo simulation of the Davydov model. *Phys. Rev. Lett.* **62**, 1796–1799 (1989)
26. Cruzeiro-Hansson, L.: Dynamics of a mixed quantum-classical system at finite temperature. *Europhys. Lett.* **33**, 655–659 (1996)

27. Cruzeiro-Hansson, L.: The Davydov Hamiltonian leads to stochastic energy transfer in proteins. *Phys. Lett. A* **223**, 383–388 (1996)
28. Edler, J., Hamm, P., Scott, A.C.: Femtosecond study of self-trapped vibrational excitons in crystalline acetanilide. *Phys. Rev. Lett.* **88**, 067403.1–067403.4 (2002)
29. Edler, J., Hamm, P.: Self-trapping of the amide I band in a peptide model crystal. *J. Chem. Phys.* **117**, 2415–2424 (2002)
30. Edler, J., Hamm, P.: Two-dimensional vibrational spectroscopy of the amide I band of crystalline acetanilide: Fermi resonance, conformational substates, or vibrational self-trapping? *J. Chem. Phys.* **119**, 2709–2715 (2003)
31. Edler, J., Hamm, P.: Spectral response of crystalline acetanilide and N-methylacetamide: vibrational self-trapping in hydrogen-bonded crystals. *Phys. Rev. B* **69**, 214301 (2004)
32. Hamm, P.: Femtosecond IR pump-probe spectroscopy of energy localization in protein models and model proteins. *J. Biol. Phys.* doi:[10.1007/s10867-009-9126-3](https://doi.org/10.1007/s10867-009-9126-3)
33. Edler, J., Pfister, R., Pouthier, V., Falvo, C., Hamm, P.: Direct observation of self-trapped vibrational states in α -helices. *Phys. Rev. Lett.* **93**(10), 106405, 1–4 (2004)
34. Xie, A., van der Meer, L., Hoff, W., Austin, R.H.: Long-lived amide I vibrational modes in Myoglobin. *Phys. Rev. Lett.* **84**, 5435–5438 (2000)
35. Xie, A., van der Meer, A.F.G., Austin, R.H.: Excited-state lifetimes of far-infrared collective modes in proteins. *Phys. Rev. Lett.* **28**, 147–154 (2002)
36. Austin, R.H., Xie, A., van der Meer, L., Shinn, M., Neil, G.: Self-trapped states in proteins. *Nucl. Instrum. Methods Phys. Res.* **507**, 561–563 (2003)
37. Fang, C., Senes, A., Cristian, L., DeGrado, W.F., Hochstrasser, R.M.: Amide vibrations are delocalized across the hydrophobic interface of a transmembrane helix dimer. *Proc. Natl. Acad. Sci. U. S. A.* **103**(45), 16740–16745 (2006)
38. Cruzeiro, L.: Influence of the sign of the coupling on the temperature dependence of optical properties of one dimensional exciton models. *J. Phys. B: At. Mol. Opt. Phys.* **41**(19), 195401 (2008)
39. Levinthal, C.: Are there pathways for protein folding? *J. Chim. Phys.* **65**, 44–45 (1968)
40. Anfinsen, C.B.: Principles that govern the folding of protein chains. *Science* **181**, 223–233 (1973)
41. Bryngelson, J.D., Onuchic, J.N., Socci, N.D., Wolynes, P.G.: Funnels, pathways, and the energy landscape of protein folding: a synthesis. *Proteins* **21**, 167–195 (1995)
42. Onuchic, J.N., Luthey-Schulten, Z., Wolynes, P.G.: Theory of protein folding: the energy landscape perspective. *Annu. Rev. Phys. Chem.* **48**, 545–600 (1997)
43. Wolynes, P.G.: Recent successes of the energy landscape theory of protein folding and function. *Q. Rev. Biophys.* **38**, 405–410 (2005)
44. Karplus, M., Kuriyan, J.: Molecular dynamics and protein function. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 6679–6685 (2005)
45. Callaway, E.: The shape of protein structures to come. *Nature* **449**, 765 (2007)
46. Prusiner, S.B.: Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144 (1982)
47. Prusiner, S.B., McCarty, M.: Discovering DNA encodes heredity and prions are infectious proteins. *Annu. Rev. Genet.* **40**, 25–45 (2006)
48. Baker, D., Sohl, J.L., Agard, D.A.: A protein-folding reaction under kinetic control. *Nature* **356**, 263–265 (1992)
49. Sohl, J.L., Jaswal, S.S., Agard, D.A.: Unfolded conformations of alpha-lytic protease are more stable than its native state. *Nature* **395**, 817–819 (1998)
50. Tsutsui, Y., Liu, L., Gershenson, A., Wintrobe, P.L.: The conformational dynamics of a metastable serpin studied by hydrogen exchange and mass spectroscopy. *Biochemistry* **45**, 6561–6569 (2006)
51. Lazaridis, T., Karplus, M.: “New view” of protein folding reconciled with the old through multiple unfolding simulations. *Science* **278**, 1928–1931 (1997)
52. Evglander, S.W.: Protein folding intermediates and pathways studied by hydrogen exchange. *Annu. Revs. Biophys. Biomol. Struct.* **29**, 213–238 (2000)
53. Brockwell, D.J., Radford, S.E.: Intermediates: ubiquitous species on folding energy landscapes? *Curr. Opin. Struct. Biol.* **17**, 30–37 (2007)
54. Roder, H., Colón, W.: Kinetic role of early intermediates in protein folding. *Curr. Opin. Struct. Biol.* **7**, 15–28 (1997)
55. Fitzkee, N.C., Rose, G.D.: Reassessing random-coil statistics in unfolded proteins. *Proc. Natl. Acad. Sci. U. S. A.* **101**(34), 12497–12502 (2004)
56. Cruzeiro-Hansson, L., Silva, P.A.S.: Protein folding: thermodynamic versus kinetic control. *J. Biol. Phys.* **27**, S6–S9 (2001)
57. Silva, P.A.S., Cruzeiro-Hansson, L.: A reduced set of exact equations of motion for a non-number-conserving Hamiltonian. *Phys. Lett. A* **315/6**, 447–451 (2003)

58. Silva, P.A.S., Cruzeiro, L.: Dynamics of a nonconserving Davydov monomer. *Phys. Rev. E* **74**(2), 021920, 1–13 (2006)
59. Pouthier, V.: Energy relaxation of the amide-I mode in hydrogen-bonded peptide units: a route to conformational change. *J. Chem. Phys.* **128**(6), 065101 (2008)
60. Cruzeiro, L.: Protein's multi-funnel energy landscape and misfolding diseases. *J. Phys. Org. Chem.* **21**, 549–554 (2008)
61. Case, D.A., Pearlman, D.A., Caldwell, J.W., Cheatham, T.E. III, Ross, W.S., Simmerling, C.L., Darden, T.A., Merz, K.M., Stanton, R.V., Cheng, A.L., Vincent, J.J., Crowley, M., Tsui, V., Radmer, R.J., Duan, Y., Pitera, J., Massova, I., Seibel, G.L., Singh, U.C., Weiner, P.K., Kollman, P.A.: AMBER 6. University of California, San Francisco (1999)