A Thermodynamic Model for Prebiotic Protein Function

Ayşe Erzan^{1,2} and Erkan Tüzel^{$\dagger 1$}

¹Department of Physics, Faculty of Sciences and Letters Istanbul Technical University, Maslak 80626, Istanbul, Turkey ²Gürsey Institute, P.O.B. 6, Çengelköy, 81220 Istanbul, Turkey (Dated: November 13, 2018)

We propose a scenario for the prebiotic co-evolution of RNA and of fast folding proteins with large entropy gaps as observed today. We show from very general principles that the folding and unfolding of the proteins synthesized by RNA can function as a heat pump. Rock surfaces can facilitate the folding of amino acid chains having polar and hydrophobic residues, with an accompanying heat loss to the surrounding rock. These chains then absorb heat from the soup as they unfold. This opens the way to the enhancement of RNA replication rates, by the enzymatic action of folded proteins present in greater numbers at reduced temperatures. This gives an evolutionary advantage to those RNA coding amino acid sequences with non-degenerate folded states which would provide the most efficient refrigeration.

I. INTRODUCTION

In order to understand the process of selection that could have lead to the observed properties of biological proteins, it is natural to look for mechanisms whereby proteins, which cannot duplicate themselves, evolved together with self-replicating molecules, "preliving" concentrations of RNA, which provide the code for protein synthesis. [1, 2, 3, 4, 5, 6, 7] The enzymatic action of proteins on the self-replication of RNA constitutes a hypercyle, which may be considered the elementary unit of evolution [1, 2].

Biological proteins fulfill their functions in unique folded states, which they are able to reach in a very short time after being synthesized. [8] Small single domain proteins fold into their secondary structures within milliseconds [9], or even faster [10]. These "native" states correspond to minimum (free) energy configurations. [11] A random sequence of amino acids, however, will typically have a degenerate ground state. [12, 13] We would like to propose a possible pathway in which those aminoacid sequences with unique ground states were selected in the course of evolution, without begging the question by referring to highly evolved biological functions. We will assume that this selection must have occurred in the prebiotic soup, where local temperature differences could have a large effect on the efficiency of RNA replication.

As a chain folds (unfolds) at constant ambient temperature, heat will be given off (absorbed) by an amount proportional to the difference in entropy between the unfolded and folded states. Since we are interested in relatively high temperatures, and our proteins do not fold into their tertiary structures, we will ignore their hydrational entropy changes. For an amino acid chain of a given length, this difference will be the largest if the folded state corresponds to a non-degenerate ground state, or several possible low-energy configurations that are well isolated from each other by very high free energy barriers. We will argue that amino acid chains which essentially behave as two-state systems [3, 14] with large entropy gaps prove to be the most effective refrigerants, if they were to be employed in a refrigeration cycle. Selection of amino-acid chains with folded states in deep free-energy wells could then be succeeded by the the evolution of more specific functions, leading to the pruning of those low-lying states so that only one, serving a highly specialized enzymatic activity, would survive. For convenience, we will henceforth use the term "proteins" to mean amino acid chains, regardless of their degree of evolution.

It has been pointed out [2] that a rudimentary form of compartition is necessary for evolutionary processes to be possible, and porous rock [6, 7] is among the likely environments to have played host to prebiotic processes, in a "soup," consisting of both organic and inorganic materials, that are assumed to be present in the prebiotic earth. For simplicity, let us concentrate on a series of compartments that contain RNA, protein molecules, amino acids and water. The surrounding rock is bathed by water at some medium temperature. The temperature range we have in mind is such that the RNA and proteins are stable, and will vary, say, between 300-360 K, (the denaturation temperatures of most proteins are in this range [9]).

In the next section, Section II, we will describe how proteins could act as a refrigerant in an adsorption refrigerator. In Section III, we discuss a toy Hamiltonian for the guided and unguided folding of chains, and describe the refrigeration cycle in the entropy-temperature plane. A discussion and pointers for further research are provided in the last section.

II. PROTEINS IN AN ADSORPTION REFRIGERATION CYCLE

The hydrophobic interactions which drive the folding of proteins into their native states [15, 16, 17] may also

[†]Present address: University of Minnesota, School of Physics and Astronomy, 116 Church St. SE, Minneapolis, MN, 55455, USA



FIG. 1: Cartoon showing the crossection of a pore, with the different steps in an adsorption–refrigeration cycle involving denatured and folded proteins.

make it favorable for non-polar residues on the chain to adsorb on nearby hydrophobic surfaces [18] lowering the free energy of the whole system by reducing the number of water molecules in interaction with the non-polar residues. If we assume that a certain fraction of the nonpolar residues have adsorbed on the surface, in a relatively stretched conformation(as shown in Fig.1), then it can slightly increase the entropy with the diffusion and aggregation of the non-polar residues on the surface, allowing the intervening sections of the chain greater freedom. The residues in the "loops" are then free to fold into "beads" or "droplets" that are the incipient building blocks of the secondary structure.

If this partially folded state is on the "correct pathway" [19, 20, 21] to a low energy folded conformation of the chain, stabilized by the specific intra-chain interactions for the given sequence of amino-acids, the rock surface can be said to act as a guide for the folding process.

We would like to propose that the relatively large heat of adsorption and denaturation which can be achieved by proteins may make them amenable to functioning as refrigerants in an adsorption refrigerator.

Unlike work-driven refrigerators, the conventional adsorption refrigerator [22, 23, 24, 25, 26] relies on the availability of a cheap heat source and has no moving parts. The refrigerant absorbes heat from the cold reservoir at T_c , as it flash evaporates, and then is led to an adsorber bed (rather than a compressor), where it adsorbes on a substrate at some medium temperature T_m , where it gives off a heat of adsorption Q_{ads} to the environment. To regenerate the refrigerant, the "low quality" heat source is used to heat the adsorber bed to a high temperature, T_h . The refrigerant that is released in this way is led to a condenser (which may be again at T_m), liquified, led through a nozzle to lower the boiling temperature, and then piped once more to the evaporation chamber. A "batch operating" system uses two "beds" (as adsorber and desorber), the cycle switching between them in turn. [27]

The "gas" and "liquid" phases correspond, in our case, to the unfolded and folded states of the protein chains. The physical environment we have in mind is a series of interconnected chambers in porous rock bathed on the outside with ambient water. Part of the system is periodically exposed to intense heat. This may, for example be due to insolation or to the heat relased from a vent in the ocean floor. [28]

The refrigeration cycle consists of the following steps.

i) The unfolded protein adsorbs on to a pore surface, where it folds.

We assume the folding to take place isothermally, at the temperature of the rock surface, which we take to be T_m . An amount of heat Q_{ads} will be given off in this process.

ii) The regenerative step involves the heating of the surface to dislodge the folded proteins from the wall. This, we propose, may be supplied by sunshine or some geothermal source. During the regenerative step, a quantity of heat $Q_{\rm des}$ will be absorbed.

The chain can now detach from the rock, and complete its folding around its hydrophobic core, with the polar residues predominantly on the "outside." [29, 30] We have shown [18], using a lattice model for the hydrophobic interactions, that above the temperature interval where the hydrophobic interactions make it favorable for a hydrophobic chain to adsorb onto a hydrophobic boundary, there is a temperature interval where the chain prefers to detach from the wall and go into a folded state. Raising the temperature further results in the unfolding of the chains.

iii) These proteins (denatured or otherwise) shall now be convected away from the hot wall. More temperate regions of the porous network, cooled by the ambient water, will act as the "condenser" in this system, and as they cool to the ambient temperature T_m once more, the proteins will equilibrate to the native, folded states. A quantity of heat $Q_{\rm con}$ is given off in the process.

iv) In order for the denaturation temperature to be lowered as the proteins enter the cool compartment at T_c , (analogously to passing them through a nozzle and lowering the pressure, in a conventional refrigerator), here use is made of the fact that the denaturation temperature depends very sensitively on the total ionization strength. The gradient of ionization strength can, in fact, be easily achieved; the RNA molecules, the amino acids and denatured proteins in the interior of the chamber will lower the unfolding temperature for the coming chains.

v) As the proteins unfold in the cool chamber due to the lowered denaturation temperature, they will absorb $Q_{\rm ev}$. This phase of the cycle corresponds to the "evaporator" of the adsorption refrigerator. Clearly, for our adsorption refrigerator to work, the adsorber "beds" must be swept clean not just of proteins, but other unreacted amino acids, or other hydrophobes. The periodic nature of the heating serves this purpose as well.

This refrigeration cycle is similar to magnetic cooling, if one thinks of the the action of the rock surface, facilitating the folding, as the magnetic field. Note that for the chain to fold, and to give off heat to the medium temperature reservoir, work has been done on it by the combined action of the rock surface (entropy mediated hydrophobic interactions) and intra-chain interactions. The ambient water bathing the outer surface of the rock acts as the thermal contact.

III. A HIERARCHICAL TWO-STATE MODEL FOR PROTEIN FOLDING

To investigate the refrigeration cycle in the entropytemperature plane [27], we used an exactly solvable toy system [20] to model the temperature dependence of the entropy of the backbone of the protein chain as it folds or unfolds with or without guidance. Clearly this is very schematic picture, but we believe it conveys the essential physics.

We assume that there is only one folding pathway leading to a single low-free energy folded state, to simplify the discussion. In this respect, then, the amino-acid chains under consideration are similar to modern day proteins.

The existence of a unique pathway means that an ordered sequence of binding events occur between different parts of the protein [21]; and if this particular sequence is not followed, the protein can not fold.

Slightly modifying the hierarchical zipper-like model proposed by Bakk et al. [20], let us assign a variable $\sigma_i = 1, \ldots, q$ to the different choices that can be made at each node, with only say σ_i^* leading to a correct folding move. Then, the state variable at the *i*th node on the folding pathway of N nodes [20] may be written in terms of a Kroenecker delta as,

$$\psi_i = \delta_{\sigma_i, \sigma_i^*}, \quad i = 1, \dots, N \quad . \tag{1}$$

The Hamiltonian is

$$\mathcal{H} = -\lambda \epsilon \sum_{i=1}^{N} \Psi_i - (1-\lambda) \epsilon \Psi_N \quad , \tag{2}$$

where

$$\Psi_i = \prod_1^i \psi_k \quad . \tag{3}$$

For $\lambda \neq 0$ we see that intermediate partially folded states also lower the energy, as would be the case under the guidance of chaperons [9], by an amount $\lambda \epsilon$ while the energy gap of the native state is given by ϵ . For $\lambda = 0$, this Hamiltonian allows the protein to be in two distinct states only, native and unfolded. No unfolding can occur inside an already folded part of the protein. Notice that (2) differs from the Bakk et al. model in the last term, which in our case is not multiplied by N; this allows the folding transition to shift to higher temperatures under guidence, as is experimentally observed [32].

The partition function for this model can be evaluated exactly. For a protein having a folding pathway of Nnodes, with $\beta = 1/k_BT$, k_B the Boltzmann constant, and T the temperature,

$$Z = (q-1)q^{N-1} \left(\frac{1-e^{\nu N}}{1-e^{\nu}}\right) + e^{\beta[\lambda(N-1)+1]\epsilon} \quad . \tag{4}$$

where we have defined $\nu \equiv \beta \lambda \epsilon - \ln q$. (Note that L'Hopital's rule must be used in the first term in case the denominator vanishes). From the Helmholtz free energy $F = -kT \ln Z$ one may compute the entropy $S = -\partial F/\partial T)_V$ and heat capacity $C = T\partial S/\partial T)_V$. (In this model the free energy is independent of the volume, therefore the partial derivatives above can be treated as ordinary derivatives.) The entropy is plotted in Fig.2 for different values of λ , $\lambda = 0$ corresponding to the unguided and $\lambda \neq 0$ to the guided cases. The system exhibits a sharp transition (becoming first-order in the thermodynamic limit, i.e. $\lim N \to \infty$) for the unguided case, as expected from this two- state model. The effect of guiding is to lower the entropy, smooth the transition and to shift it to higher temperatures, and is similar to the effect of turning on a field, in the case of magnetic phase transitions.

The refrigeration cycle for the refrigerant is shown in Fig.2 in the entropy-temperature plane. The sharp unfolding transition $(1 \rightarrow 1')$ analogous to the flashevaporation in a conventional adsorption refrigerator, takes place in the cooling chamber, at T_c . The high pH conditions we take to correspond to the $\lambda = 0$ curve. The denatured proteins are convected towards the adsorbing walls (the walls could be coated by some lipids to make them more hydrophobic [28]), warming up in the process $(1' \rightarrow 2)$, and adsorb $(2 \rightarrow 3)$. Upon adsorption λ is set to unity, and the entropy of the chain drops. This happens at the (fixed) intermediate temperature T_m of the adsorbing wall, and the heat of adsorption is carried away by the water bathing the outer wall of the compartment. When the heat is turned on, as it periodically is, the wall will heat up to T_h , along the curve $3 \rightarrow 4$. In the process, Q_{des} is absorbed by the proteins. At point 4 in the cycle, they become free of the wall; and are now convected along $4 \rightarrow 4' \rightarrow 1$, back to the starting point. This happens along a curve with a nonzero value of λ , here chosen to be $\lambda = 0.13$, because, as explained in item *(ii)* of the previous section, we assume these proteins remain near the hydrophobic wall as they give off excess heat to the rock boundary washed by cooling water on the outside, and are convected back towards the cool chamber.

The various heats of evaporation, adsorption, desorption and condensation may, in principle, be computed



FIG. 2: The refrigeration cycle in the entropy-temperature plane. The curves have been drawn for $\lambda = 0, 0.13$ and 1. For this plot q = 8 and N = 10. The temperature axis is in units of ϵ/k_B .

from the entropy as a function of the temperature for the various values of λ . It is clear from the geometry of the curves in Fig. 2 that the area to the left of the curve between any two points (ij) on the curve may be obtained by doing the integral over T instead, viz.,

$$Q_{ij} = T_j S_{\lambda}^{(j)} - T_i S_{\lambda}^{(i)} - \int_{T_i}^{T_j} S_{\lambda}(T) dT \quad . \tag{5}$$

The "coefficient of performance" of the refrigerator is given by [22]

$$COP = \frac{Q_{ev}}{Q_{des} + W_g} \quad , \tag{6}$$

where W_g is a small amount of work normally needed to operate a pump. In our case, this is provided by the gravitational force driving the convection current. To maximize $Q_{\rm ev}$, one must have essentially two-state unfolding at T_c , with a maximal entropy gap. On the other hand, one must clearly have the folding-unfolding rates, $Q_{\rm con} - Q_{\rm ev} < Q_{\rm des} - Q_{\rm ads}$ to respect the first law of thermodynamics. For this, one needs the adsorption to take place at T_m values that are as low as possible, while $Q_{\rm con}$ should also be minimized. We have chosen appropriate values of λ in Fig. 2 to make this plausable.

In order to have a crude estimate of the actual pumping rates possible, one should note that the entropy of denaturation of a typical protein is about $\Delta S \sim 17 \text{J/mol-K}$ per residue. [33, 34] The folding-unfolding rates range between $k \sim 10^2/\text{s}$ to $10^5/\text{s}$ [10, 35] whereas typical protein concentrations, namely ρ , are of the order $10\mu\text{M}$ [10]. If one takes a cooling chamber of linear dimensions $r_c \sim 1$ cm, one finds that the heat pumped away, $J_{\text{ev}} = dQ_{\text{ev}}/dt$ is

$$J_{\rm ev} = \rho k r_c^3 L T_c \Delta S \sim 5 \times 10^{-1} \,\mathrm{J/s} \quad , \tag{7}$$

for typical proteins, where L, the length of a protein chain, is taken to be 100, $T_c = 300$ K and $k = 10^{-2}/s$.

On the other hand typical heat conductivities for rock are around $K_T = 2 - 6$ J/m-K-s [36, 37]. With a temperature difference between the ambient water and the inside of the cooling chamber being, say $T_m - T_c \sim 30$ K, and a surface area $\sim r_c^2$, the heat conducted into the cooling chamber from the surroundings, $J_{\rm in} = dQ_{\rm in}/dt$ will be

$$J_{\rm in} = K_T \frac{T_m - T_c}{\ell} r_c^2 \tag{8}$$

where ℓ is the thickness of the wall seperating the chamber from the ambient water. With $r_c \sim 1$ cm, and taking $K_T = 4$ J/m-K-s, one finds $J_{\rm in} \sim (1.20/\ell) {\rm J/s}$, if ℓ is expressed in cm. . The break even point where $J_{\rm in} \simeq J_{\rm ev}$ is around $\ell \sim 2.5$ cm.

The power needed for this system can be found from $J_{\rm des} = dQ_{\rm des}/dt$. The insolation at the earth surface is of the order of $W_{\rm sol} = 200 \text{ J/m}^2$ -s. Requiring

$$J_s \equiv W_{\rm sol} r_{\rm b}^2 > J_{\rm des} = \frac{J_{\rm ev}}{\rm COP} \tag{9}$$

where $r_{\rm b}$ is the radius of the adsorption bed receiving the solar power and inserting numbers, one finds $r_{\rm b}$ should be of the order of a few tens of cm, for COP as low as 0.1.

IV. DISCUSSION

We have shown above that a protein soup within a porous rock could function as a self-regulatory refrigeration cycle and lower the temperature of the soup within the pore, for realistic ranges of the physical and chemical parameters. The efficiency of the cycle strongly depends on the size of the entropy gaps.

It is well known that the presence of protein molecules acting as enzymes may effect the RNA replication rates (which we have not considered) by factors of up to 10^4 [1, 38]. Catalytic activity is a function of the spatial structure [1] and therefore requires the proteins to be in a unique folded state, whose stability also depends on the temperature and is optimized only for a definite temperature interval [9, 35]. Moreover, RNA replication rates depend non-monotonically on the temperature [38] and drop off outside a definite temperatre range. We have shown that proteins with large entropy gaps are able to achive temperatures in a small compartment that are lowered relative to the ambient temperature. This vindicates our initial assumption that this criterion could concievably have played a role in their selection, in a environments that are too warm for the optimal self-replication of RNA.

There is experimental evidence that the folding transition is like a two-state system for many single-domain proteins [9, 14, 31]. Although the proteins fold via a twostate folding pathway, especially at higher temperatures, the presence of some intermediate states might be necessary for the folding process to find the native state. It has been experimentally observed [32] that the concentration of chaperons in E. coli rises as the temperature increases, indicating that at high temperatures E. coli needs help in order to fold its proteins.

The efficiency of protein folding can be adversely affected if partially folded proteins aggregate in order to reduce exposed hydrophobic residues. Molecular chaperons bind reversibly to these partially folded chains preventing their aggregation and promoting their passage down the folding pathway. Therefore, one might speculate that these chaperons have taken the role of rock surfaces in the course of higher evolution.

It is interesting to note that there exist so called heatshock proteins which are synthesized in large numbers when the temperature of the environment is suddenly raised above (or dropped below) those temperature above and below which proteins in the cells would normally denature, and chaperone the correct folding of other proteins in the cell. [39] These proteins observed today under

- [1] M. Eigen, Naturwissenschaften, **10**, 58 (1971).
- M. Eigen, Steps towards Life, a Perspective on Evolution (Oxford University Press, Oxford, 1992); also see M. Eigen and P. Schuster, The Hypercycle (Springer, Berlin, 1979).
- [3] R. F. Gesteland and J. F. Atkins, *The RNA World* (Cold Spring Harbor Laboratory Press, New York, 1993).
- [4] R. Dawkins, *The Selfish Gene* (Oxford University Press, Oxford, 1990).
- [5] J. M. Smith and E. Szathmáry, *The Major Transitions in Evolution* (Oxford University Press, Oxford, 1995).
- [6] J. M. Smith and E. Szathmáry, *The Origins of Life* (Oxford University Press, Oxford, 1999).
- [7] S.A. Kauffmann, The Origins of Order: Self-Organization and Selection in Evolution (Oxford University Press, Oxford, 1999).
- [8] H. Frauenfelder, S.G. Sligar and P.G. Wolynes, Science 254, 1598 (1991).
- [9] A. Fersht, Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding (W. H. Freeman, New York, 1998).
- [10] U. Mayor, M. C. Johnson, V. Daggett and A. R. Fersht, Proc. Natl. Acad. Sci. USA, 97, 13518 (2000).
- [11] P.G. Wolynes, J.N. Onuchic and D. Thirumalai, Science 276, 1619 (1995).
- [12] E.I. Shakhnovich and A.M. Gutin, Proc. Natl. Acad. Sci., USA, 90, 7195 (1993).
- [13] A. Sali, E.I. Shakhnovich, M.J. Karplus, Nature **369**, 248 1994).
- [14] H. Kaya and H. S. Chan, Proteins: Struct., Funct., Genet., 40, 637-661 (2000).
- [15] B.T. Nall and K.A. Dill, eds., Conformations and Forces in Protein Folding,..
- [16] B. Erman and Dill, J. Chem. Phys. 112, 1050 (2000).
- [17] G.T. Barkema and B. Widom, J. Chem. Phys, in press; G.M. Schütz, I. Ispolatov, G.T. Barkema, and B. Widom,

extremely hot conditions, in archeabacteria are relatively small and have very fast folding rates. [2, 9, 10] These examples seem to point also in the direction of a direct correlation between thermal properties of the environment and protein functions, which may have evolved from the rudimentary function proposed here.

Other studies also have demonstrated that rock surfaces may have played a selection role in prebiotic conditions, c.f., the selection of right handed amino acids binding to optically active surfaces of calcite crystals with same chirality [40].

Clearly we are making no claims that the present proteins are identical to the end products of one particular selection mechanism. Once the temperature was sufficiently lowered, other, more complex evolutionary pressures would come into play. However, our calculations show that the proposed refrigeration cycle could very well have played an important role in the co-evolution of fast folding proteins with large entropy gaps and the RNA molecules which code them.

"Symmetry effects and equivalences in lattice models of hydrophobic interaction," preprint.

- [18] P. Önder, A. Erzan, Eur. Phys. J. E 9, 467 (2002).
- [19] C. Levinthal, J. Chem. Phys. 65, 44 (1968).
- [20] A. Bakk, J. S. Hoye, A. Hansen, K. Sneppen and M. H. Jensen, Biophysics Journal, 79, 2722 (2000).
- [21] A. Hansen, M. H. Jensen, K. Sneppen and G. Zocchi, Europhys. J. B., 6, 157-161 (1998).
- [22] Y.A. Çengel and M.A. Boles, *Thermodynamics: an Engineering Approach* (McGraw Hill, N.Y., 1996; in Turkish translation by Literatür Yayınevi, Istanbul, 1996) p 549.
- [23] J.S. Hsieh, Solar Energy Engineering (Prentice Hall, Englewood Cliffs, N.J., 1986) p.303.
- [24] Y. Teng, R.Z. Wang, asnd J.Y. Wu, App. Therm. Eng., 17, 327 (1997)
- [25] F. Meunier, F. Poyelle and M.D. LeVan, App. Therm. Eng. 17, 43 (1997).
- [26] M. Pons, App. Therm. Eng. 17, 615 (1997
- [27] H.T. Chua, K.C. Ng, A. Malek, and N.M. Ono, J. Appl. Phys. 89, 5151 (2001).
- [28] W. Martin, M.J. Russell, Phil Trans. Royal Soc. B (Biol. Sci.) (London), **358**, 59 (2003)
- [29] E. Tüzel and A. Erzan, Phys. Rev. E 61, R1040 (2000).
- [30] E. Tüzel and A. Erzan, J. Stat. Phys. **100**, 405 (2000).
- [31] P. L. Privalov and N. N. Khechinashvili, J. Mol. Biol., 86, 665 (1974).
- [32] S. L. Herendeen, R. A. VanBogelen and F. C. Neidhardt, J. Bacteriol., 139, 185 (1979).
- [33] P.L. Privalov, Adv. Protein Chem., 33 167 1988.
- [34] M. Daune, Molecular Biophysics: Stuctures in Motion (Oxford University Press, Oxford 1999).
- [35] M. Oliveberg, Y. Tan and A. R. Fersht, Proc. Natl. Acad. Sci. USA, 92, 8926 (1995).
- [36] R.C. Weast, ed., CRC Handbook of Chemistry and Physics (CRC Press. Inc., Florida, 1989).
- [37] E.G. Dwight, ed., American Institute of Physics Hand-

book (McGraw-Hill, New York, 1972).

- [38] N.A. Campbell, Biology 3rd ed. (The Benjamin/Cummins Publ. Co. Inc., New York, NY 1993).
- [39] K.B. Arnvig, S. Pedersen, and K. Sneppen, Phys. Rev.

Lett., 84, 3005 (2000).

[40] R. M. Hazen, T. R. Filley and G. A. Goodfriend, Proc. Natl. Acad. Sci. USA, 98, 5487 (2001).