CHAPTER 1

FREE ENERGIES OF STAGING A SCENARIO AND PERPETUAL MOTION MACHINES OF THE THIRD KIND

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1.1 Abstract

It has been stated that certain chains of biological reactions can go to near completion in both directions as needed without any exterior driving force. This claim represents a thermodynamic impossibility. Yet, this impossibility is of a type not usually covered by the traditional list of impossibility devices known as perpetual motion machines. Rather, it represents a perpetual motion machine of the third kind (PM3) that becomes impossible only in finite time. At non-vanishing rate the chain of biological reactions above would constitute a finite-time perpetual motion machine. The resolution of this quandary leads directly to a notion of staging free energy: the free energy invested in choreographing all the actors of a biochemical reaction directing them to the needed places at the needed times. We conclude by noting that biological systems make ample use of invertible degrees of freedom that often operate near PM3 limits.

1.2 Introduction

It has been stated that integrative and excisive recombination of DNA through Holliday Junctions (HJ) can proceed unidirectionally in either direction as the need dictates without any free energy expenditure and at non-vanishing rates [1]. Choosing a direction is arranged with possibly different enzymes and cofactors present but either reaction can be done in a test-tube without noticeable consumption of the reagents. In fact, this reaction is used by temperate bacteriophages to integrate their genome into the host's genome or to excise it. The exact control of the directionality of this reaction, the so-called resolution of the HJ complex, is of considerable biochemical interest [1, 2, 3].

The chain of reactions in the resolution of site-specific recombination may be written in simple chemical nomenclature as

$$A \rightleftharpoons T \rightarrow E \tag{1.1}$$

for the forward reaction, and

$$A \leftarrow T \rightleftharpoons E \tag{1.2}$$

for the reverse reaction[1]. More generally

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} T \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E \tag{1.3}$$

with the rate constant $k_{-2} = 0$ for the forward reaction and $k_1 = 0$ for the reverse reaction.

Standard thermodynamics only allows spontaneous reactions if their change of free energy $\Delta G \leq 0$. For reaction (1.3) to go spontaneously in both directions would thus require $\Delta G = 0$, i.e. $G(E) = G(A)$, and the end components A and E would be in equilibrium with one another with no net reaction going either direction. If a net reaction in one direction is observed at a non-vanishing rate, ΔG must be distinctly less than zero for that reaction in order to account for internal dissipation in accordance with Onsager's flux-force relations. This dissipation represents free energy loss – free energy that must have been put into the reactants from some source.

The quandary of the HJ paradox is based on the following observations:

- Referring to reaction (3) above, the reaction can be driven to either the reactants (A) or products (E) using either Integrase alone or Integrase in combination with Excisionase. Neither of these proteins, nor the reactions themselves, require a co-factor that provides any obvious energy (e.g., hydrolysis of ATP) [1, 2].

- The energy seems to be stored in the enzymes. That is, one can isolate intermediate complexes (T) and drive them in either direction. In the case of the related

[1] For aficionados, T represents the Holliday junction (both conformers) while A and E represent the synaptic complex with intact and separate DNA duplexes.

vaccinia topoisomerase, it is even possible to isolate the complexes, store them in the freezer, and then complete the reactions months later. Again, without any obvious energy expenditures.

- While it is easy to postulate that the free energy from the initial catalysis to produce the HJ is stored in the complex, the second law requires that some energy be dissipated. So how can the reactions continue? The high energy phosphate bonds in the products and reactants are exactly the same. Lambda-like Integrases are interesting because they are directional (i.e., addition of Excisionase determines whether the products or reactants are produced) [2].

A century ago Wegscheider [4] discussed the possible consistent thermodynamic descriptions of partly irreversible reaction chains by letting the rate constant for the forbidden reaction approach zero, e.g. k_{-2} in reaction (3). A more extensive treatment of such unidirectional systems may be found in [5]. Wegscheider's conclusion was *that irreversible reactions like (1) and (2) are impossible simultaneously*. All available material would end up as either E or A, respectively, with no possibility of going back. While Wegscheider's arguments did not take into account changing the environment in which the reaction takes place, the mere presence or absence of true chemical catalysts (enzymes, cofactors) are not enough of a change to alter Wegscheider's conclusions.

As regards our HJ "paradox" described above, it is a straw man. The more reasonable voices in the HJ community acknowledge the need for some small amount of free energy supplied in some manner [1]. The fact appears to be that free energy accounting for biological systems has not yet managed to do accounting on sufficiently fine scales to track the free energy flow in the Holliday junction resolution problem.

This volume of Advances in Chemical Physics grew out of a request for open problems. The open problem this paper poses is to quantify the free energy investment required to execute a *scenario* which we define to be: a controlled sequence of biochemical reactions with a specified goal. Site specific integration and excision of bacteriophages are excellent examples of scenarios. Other examples abound and lie at the heart of living systems. Recognizing the "investment", and the associated dissipation a process must incur, represents a shift in viewpoint away from "what is possible" to "what controls are achievable". The difference is exactly the focus on the means and associated costs of control.

One important motivation for our question is the ultimate goal of understanding information flow between genomes and the environments in which they live. Much of the information regarding the control of required scenarios is embedded in an organism's genome, placed there by generations of evolution. How this information contributes to the free energy cost of staging the scenario needs much more accurate understanding of the free energy cost of the control. Some of that cost is borne by the free energy invested in controlling the process; some of it comes from the information embedded in the architecture and composition of the cell. The genome specifies the "setting" including the local architecture and chemical environment in which the control must take place. The costs of this control can be very significantly decreased by favorable settings. To separate these contributions and begin to understand the

information flow between environment and genome on evolutionary time scales, we need to establish an accounting of the entropy of staging. Such accounting is certainly very difficult and will need to include a quantification of the information used to stage (choreograph) a process.

1.3 Perpetual motion machines of the third kind

Perhaps more interesting than the resolution of the free energy paradox surrounding integrative or excisive recombination is the type of impossibility it represents. It is an impossibility that rests on the requirement of having only a finite time to perform a certain process.

Following the tradition of thermodynamics, which has put the core of the First and the Second Law of Thermodynamics into the form of the non-existence of Perpetual Motion Machines of the First and Second Kind, we propose to put the above finite-time impossibility principle into the form of the non-existence of Perpetual Motion Machines of the Third Kind (PM3), defined as follows:

Perpetual Motion Machine of the Third Kind (PM3): A real machine that continues to operate in a cycle at non-vanishing rate without input of free energy.

Perpetual motion machines of the third kind have already been introduced in the literature [6]. The fact that they become impossible only in finite time does not seem to have been previously appreciated. Here the term "real machine" characterizes the fact that all real processes involve dissipation of one sort or another, be it due to mechanical friction, ohmic resistance, or other loss mechanisms occurring when some flux is transported. Here transport represents any flow whose conjugate force in the sense of Onsager is proportional to the flow in the linear regime[2] [7, 8]. The rate of such flows in the absence of the force is zero. If it is non-zero it must incur a dissipation. This dissipation is proportional to the square of the flow rate for small flow rates and is bounded away from zero for non-zero flow rates. The important point is that all real processes involve a flow of some quantity for which the dissipation goes to zero only in the limit that the flow rate also goes to zero. Consequently, to drive an invertible mode forwards and then backwards, some free energy must enter the system and pass through moving from one chemical potential to another, some of the free energy becoming partially thermalized at each step. This much is implied by the impossibility of PM3.

Reversible heat engines, a favorite device of all thermodynamics textbooks, are examples of PM3s provided they operate at non-zero rates. On the other hand, nearly adiabatic processes such as propagation of sound or oscillation of a spring are not. These nearly adiabatic processes involve transport, in these cases between different types of energy (potential/kinetic). Such conversions are never completely lossless even when the rate of the process can approach zero. There is always some external or internal friction and thus these processes must produce entropy. For systems of at

[2]The uninitiated reader may think of this as a generalized Ohm's law or Fick's law of diffusion which results in dissipation that is proportional to the square of the current.

least mesoscopic size, such dissipation is unavoidable, and the first and second laws of thermodynamics apply immediately. For smaller systems of only a few particles, fluctuations exhibit occasional changes that actually increase free energy. On the average though, such processes do decrease free energy [9]; the first and second laws of thermodynamics do apply.

Note that our statement about the impossibility of a perpetual motion machine of the third kind is stronger than the traditional statements of the second law. In particular this means that it does not follow from the second law and represents a genuine additional postulate regarding thermodynamic processes in finite time. Nor does our postulate follow from recent results in fluctuation theory [10, 11] which still allow $\Delta S^{\text{univ}} = 0$ as they must without restricting the time of the process. Some of the fluctuation theory results do come very close however. For example, [14] refers to a principle of dynamic irreversibility and proves a general expression for the staging dissipation of a scenario as the relative entropy of the ensemble of forward trajectories and the ensemble of reverse trajectories.

The non-existence of a Perpetual Motion Machine of the 3rd kind (PM3), in the sense defined above, is a concept which is not only of importance in biological systems. In broader terms the concept of PM3 highlights what Steve Berry and coworkers started with the field of Finite-Time Thermodynamics: processes without dissipation do not occur in nature if performed in finite time [36]. This insight has many implications, e.g. for our energy supply, for industrial processes, and even for our traffic. But as a thermodynamic principle it applies also in the realm of processes inside cells at the mesoscopic level.

PM3 formalizes the statement that site specific recombination of DNA strands cannot proceed in both directions spontaneously without some other input of free energy that is dissipated in the process. As we will see below, the real question is not whether dissipation occurs, but how to quantify it, so one gets realistic entropy production rates that give us an accurate picture of the free energy costs of finite-rate biological control.

1.4 The free energy cost of staging a scenario

We begin with some preliminaries regarding nomenclature. In what follows, we will use the term *reversible* in the thermodynamic sense to refer to processes that can go in either direction without dissipation. We adopt the term *invertible* to mean able to run forwards and backwards albeit at a free energetic cost for running at least one of the directions. What the biological and some of the chemical literature calls "reversible" should in fact be called invertible.

Recall that we adopted the term *scenario* for a controlled sequence of reactions with a specific biochemical objective. We use the term *staging cost* or *staging free energy* of the scenario to mean the free energetic investment that is required to make the scenario spontaneously reach its objective, i.e. proceed as planned in the script of the scenario with high probability. The relatively few (sometimes single!) copies of the protagonist molecule in the scenario usually require this probability to be near

one. In particular, the free energy needed to modify a reagent into its reactive form and/or an enzyme into its reactive configuration is part of what we call the *staging free energy*. Some of this activation may be recoverable at the end of the reaction, the rest will be dissipated along the reaction, becoming part of the *staging dissipation*, the net free energy loss of the process. It can be shown that this dissipated free energy equals the entropy production multiplied by the temperature[3]. We remark that similar energetic investment has been discussed previously in connection with EROEI, Energy Return on Energy Invested [13]. Staging cost generalizes this concept of energy invested to goals other than energy returned and also counts energy invested by non-human sources.

Counting the free energetic investment and dissipation associated with a general scenario is beyond our current level of understanding of biological systems. A scenario can involve a detailed choreography of which reagents need to be where at which times, and how this is arranged and paid for will take much more information than we presently possess. Our suggestion here is to focus the first efforts in this direction at invertible scenarios that living systems standardly use and to analyze the staging dissipation for running these systems forwards and backwards to make a cycle. There are some simple scenarios for which this is possible and they point out some general features of interest.

1.4.1 A trivial scenario: Protein folding

Consider the unimolecular reaction of protein folding. While some proteins need chaperone proteins to make sure they fold correctly, most proteins spontaneously fold into their native configurations at the right temperature. Now consider moving one such protein from temperature T_1 where it spontaneously folds to temperature T_2 where it unfolds, see Figure 1.1. Finally, consider cycling this process. The minimum staging dissipation required to drive this cycle at a certain rate is clear; it is the entropy production associated with the heat transport into and out of the surrounding medium at a sufficient rate to drive the cycle and is closely related to the sort of finite-time thermodynamic calculations that have been performed many times for bounding heat engine cycles [15, 16, 17, 18, 19].

Note: Wegscheider's objections do not apply. By changing the environment we make the reaction *folded* $\rightleftharpoons$ *unfolded* spontaneous forwards or backwards. We are in effect running a heat engine using the protein as the working fluid. If we connect our protein to a tether, it can do mechanical work pulling or pushing the tether as it folds and unfolds.

Cycling the temperature of the system to switch from one T to another is not as artificial as it might seem. Heat shock proteins (or stress proteins) which prepare the cell for stressful situations are widespread. Cycling the pH or the ionic strength of the solution will do in place of the temperature for many proteins. The cost of cycling these is also straightforward to quantify assuming access to reservoirs of

[3]This is known as the Guoy-Stodola theorem [12].

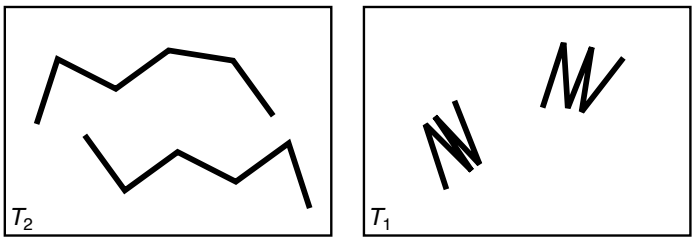

Figure 1.1 Proteins unfolded at temperature T_2 (left) and folded at T_1 (right). Changing the temperature requires the flow of entropy to or from the location of the proteins, thus producing additional entropy due to the degradation of the free energy from higher to lower temperatures.

ions. The cost of such access (maintaining the reservoirs) may however itself be more significant than transporting ions to and from the stage of the scenario.

1.4.2 Staging free energy

Even for the simple example above, determining the staging free energy of folding (or unfolding) is not straightforward. How much of the cooling should we count? Starting from what "normal" temperature? How is the cooling achieved and what is the cost of doing that? For this process, as used by real cells, the temperature shift comes from the environment. The staging cost is thus borne by agents external to the cell. How are we to count it? The situation becomes clearer if we also include the backwards process. The staging dissipation for the *cycle* driven at a specified rate is better defined and is also open to experimental measurement.

One huge complication in living systems is less than complete knowledge of the exact initial and final states of various actors in a scenario. There are many other simultaneous scenarios running and separating their effects is not easy. Biological systems are typically very complicated with a very large number of molecules present in the reaction compartment, all interacting in many different ways. In such surroundings it is difficult to exactly carve out what are reactants, products, and the "environment" of a given scenario. One resolution would be to measure the change in free energy of the whole system initially and at as many time points during the scenario as possible. In principle this would allow us to "follow the money", i.e. the free energy flow and loss through the system.

One common cost of staging a scenario is making sure all the actors are present. In our context, this means sufficient concentration of certain key enzymes, a standard control needed for most scenarios. Our HJ resolution example needs significant concentrations of several proteins that catalyze the reaction. The cost of producing and maintaining such enzymes is considerable and omnipresent. How much of this cost should be "charged" to the cost of staging the scenario is not clear. One complication is that many enzymes participate in more than one reaction. Another is the question of how many times the enzyme is used before needing maintenance.

Despite daunting difficulty, determining the staging free energy for some scenarios is possible. The staging investment of many scenarios (pathways) is detailed down to an integer number of ATP molecules [30]. The staging dissipation is less well understood. As we write this manuscript, systems biology [20] is assembling the huge databases needed to calculate staging investment and dissipation for many more scenarios.

1.5 Near PM3 processes

Many biological processes seem to operate near the PM3 limit. In many cases the location where the free energy dissipation occurs is not even clear. Thus claims of the reversibility of such processes are not surprising. Below we present a number of processes for which the energy dissipation is surprisingly low.

- The molecular motor ATP synthase operates very nearly reversibly [21, 22].
- Myriad crista shapes of the inner mitochondrial membrane are isoergic and interconvert freely [23].
- Lipid composition of *E. coli* adjusts to ambient temperature so the sol-gel phase transition temperature is just below ambient [24]. This brings the sol-gel transition within reach of many local fluctuations, e.g. in pressure or charge.
- Twisting and untwisting DNA mediated by DNA-binding proteins that perform extensive DNA remodeling or distortion are frequent processes. The isothermal enthalpy / entropy compensation that keeps these reactions nearly isoergic is well documented [25, 26].

In each of these examples a degree of freedom is kept near equilibrium thereby lowering the associated dissipation needed in changing that degree of freedom. Moving along such neutral degrees of freedom is a nice trick for any control minimizing dissipation. As illustrated in Figure 1.2, to get the rolling pin from one end of the table to the other, we need only lift one end of the table a bit as the table is flat. General bounds on dissipation in finite time relate such dissipation to the thermodynamic distance traversed [27]. This distance is zero along exactly such equilibrium modes [28]! Our list above serves as a partial argument that living systems sometimes exploit these degrees of freedom to achieve their control of the scenarios needed for life. How the various scenarios needed to keep a living system alive are controlled, and how the dissipation of running them is paid for are the general questions explored here.

1.6 Energy sources for staging

Despite our near PM3 examples above, most scenarios do need considerable staging free energy. The textbook example of counting staging costs is for reactions driven

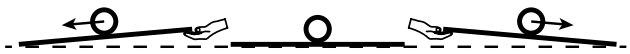

Figure 1.2 An illustration of the energetics along a neutral degree of freedom: the horizontal table. Note that only a very small elevation suffices to make the process go in one direction or the other.

by coupling to the ATP⇌ADP reaction. Often this proceeds via the standard protocol of up-front paying for the scenario to take place by phosphorylating a protein involved in the reaction. It is relatively easy to use "follow the money" approaches to tracing the flow and degradation of free energy and this has been done for scenarios that use ATP as "fuel". The number of ATPs needed to stage many cellular reactions are known and tabulated [29, 30]. Note that these are the investment costs; the dissipation costs have not been as thoroughly studied.

The other well-known and tracked example of currency to pay the investment cost of control is via the transport of an ion that is maintained at a concentration difference across a membrane. Examples of this include the electrical polarization maintained by H^+ ions responsible for the electrical gradient across a mitochondrial membrane or the Na^+ gradient maintained across the plasma membrane. Coupling to the passage of such ions across the gradient is another frequent power source.

Our present interest is taking this free energy accounting beyond the rough scale that an integer number of ATP molecules allows and considering more general situations than forced proximity to membranes allows. Our example of HJ resolution shows such accounting to be needed[4]. The cell must have some subtle forms of control which use far less than one ATP (or GTP) worth of free energy and yet follow a careful script. Are there other possible yet general purpose ways to carry activation, i.e. packets of free energy available for easy coupling to a variety of reactions? One possible free energy source that has been suggested [2] for the HJ resolution reaction, is via the torque exerted due to the supercoiling state of the bacterial DNA. This wound-up state can in general act as a spring capable of powering otherwise non-spontaneous reactions. While this may be the power source for the HJ reaction, we suspect that there exist other general purpose currencies. One possibility would be enzymes folded so as to leave several hydrophobic arms exposed to the aqueous environment, in effect creating the protein in a slightly activated state. This would also account for aging effects and concomitant loss of enzyme activity. Such loss of activity is indeed observed for the enzymes in the HJ reaction.

Our final example is not exactly a free energy source for driving reactions. Rather it is a device whereby the free energy one would expect to be needed for a conversion step comes without perceptible cost in the right staging environment. It concerns a choreographic device for controlling the direction of an invertible reaction via the concentration dependence of the free energy. Consider a device facilitating the reac-

[4]We have in mind something like the exergy accounting standardly performed for chemical plants that charts the transfer and degradation of exergy, a generalized free energy [31].

tion $A \overset{\text{device}}{\rightleftharpoons} E$. When A is plentiful but there is no E and the *reference* free energies of A and E are comparable, A will convert to E. Vice versa, if E is present but A is not, E will convert to A. The hidden free energy input here could happen through the removal of E and the introduction of A. This mechanism is likely to be important especially when the scenario requires a long sequence of steps. The choreography required for having the reactant/enzyme that will whisk the previous step's product on to the next step is interesting. One example of this is the location of the cytochromes for the electron transport scenario. Note that this scenario shows that much of the information required for the choreography is carried in the genetic map of the cell and expressed through its architecture.

1.7 Conclusions

In this paper we used established facts about site-specific recombination to set up a straw man: a biochemical reaction that can proceed in either direction without input of free energy. Closer examination reveals however that a reaction that goes forwards and then backwards is not really a violation of the second law. It is however a violation of a strengthened second law which precludes such processes *in finite time*. While the second law requires only that total entropy not decrease

$$\Delta S^{\text{univ}} \geq 0, \tag{1.4}$$

the finite-time second law in the sense introduced above takes this to be a strict inequality

$$\Delta S^{\text{univ}} > 0 \tag{1.5}$$

for any real, finite-rate process. In particular, this implies an impossibility principle for perpetual motion machines of the third kind – ones which keep running at perceptible rates without input of free energy.

In an attempt to generalize the straw-man example, we are led to introduce and explore the free energetic costs of staging a scenario, i.e. controlling a sequence of biochemical reactions having a specific goal. Examples of scenarios include (1) producing ATP by transporting electrons along the cytochrome chain, (2) photon capture and conversion in chlorophyll, or even (3) mitosis and (4) meiosis. The scenario we are describing might well involve only a single copy of a molecule that is to undergo a long sequence of events. The fidelity of the control exercised must be high in order to arrive at the desired goal. At each step there must be enough affinity to assure that there are no bottlenecks while at the same time keeping each reaction close to equilibrium in order to minimize dissipation.

The free energy costs of staging a scenario are of at least two forms: (1) The staging free energy is the free energy investment of arranging the local environment so the desired reaction becomes spontaneous. (2) The staging dissipation is the free energy degraded to heat as a result of the scenario. We noted that equilibrium modes offer a living system near PM3 performance for some important invertible scenarios

and explored some possible means of powering scenarios by means other than direct coupling to ATP degradation.

Deciding whether or not to stage a scenario often needs information regarding the environment of the cell. The excision scenario by λ-phage is triggered by an environmental sensor that measures [cAMP] which in turn controls the expression of Integrase and Excisionase. The dissipation cost of measuring an environmental concentration has been calculated by Mehta and Schwab [32]. Their model couples the activity of a detector on the surface of the cell to the activation rate of a certain internal protein, thereby enabling the cell to sense the concentration of an external substrate. In our terminology, setting reliability of the measurement is part of staging this measurement scenario. The reliability of the measurement is inversely related to the variance in concentration of the internal moiety and directly related to the dissipation in the process. The model quantifies the tradeoff between the two.

This line of reasoning also points to a very different way to think about our simple scenario of folding or unfolding a protein. Building and maintaining such proteins is a way for the cell to collect information regarding its environment. It bears a cost similar to Mehta and Schwab's: maintaining the heat-shock protein intact and functional in the presence of a background of catabolic processes[5]. With our new perspective, the goal of the protein folding scenario should not have been to fold or unfold the protein but rather to sense the temperature change in the environment. Note that this change of goals has a large effect on both the staging investment and the staging dissipation required to make it happen.

Following John Roth's lead in his definition of microbial species as a *business plan* [33], we can define an *ideal cell* as an agent that runs a particular blend of scenarios. This blend will depend on environmental conditions and constitutes Roth's business plan in a more concrete fashion that should enable one to make the definition operational once enough biochemical information becomes available to assemble all the scenarios used by a species. This has been done for some bacteria, albeit without our perspective, in an approach called energy and flux balance analysis [34, 35].

As regards apparent PM3 processes in biological systems, the culprit is certainly an incomplete description of the initial and final states. Some free energy must be dissipated each way, and at least one of the directions needs investment of free energy. The origin of any perceived perpetual motion (PM3) must be due to an incomplete description of the reaction sequence. In particular in biological/biochemical systems there is plenty of room for incomplete description. Some contributions may be energetic (e.g. charge interaction or twisting of a molecule), others may be entropic in the sense that the molecules involved must attain a particular shape or be in a particular position relative to one another.

We conclude by noting that the problem described above fits squarely into the original program for finite-time thermodynamics laid out by Steve Berry and two of the authors thirty some years ago (BA and PS) [34]. The staging cost of a scenario is an instance of the finite-time thermodynamics problem: what is the minimum cost of

[5]Some of this maintenance is done by chaperone proteins that couple ATP hydrolysis to folding (and refolding) certain proteins.

achieving a desired net physico-chemical effect in a finite time? What is new is the realization that reversible processes require infinite time. Finite rate processes need $\Delta S > 0$. The equality case is important but only as a limit with which to calculate.

The accompanying note by Hoffmann et al. [28] in this volume describes some tools from finite-time thermodynamics and possible suggestions for how such tools may be applied to the staging costs problem.

1.8 Acknowledgements

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