Hindawi Publishing Corporation
Journal of Thermodynamics
Volume 2012, Article ID 432143, 10 pages
doi:10.1155/2012/432143

Research Article

Nonequilibrium Thermodynamics of Cell Signaling

Enrique Hernández-Lemus

Computational Genomics Department, National Institute of Genomic Medicine, Periférico Sur 4809, Col. Arenal Tepepan, Delegación Tlalpan, 14610 Mexico City, DF, Mexico

Correspondence should be addressed to Enrique Hernández-Lemus, ehernandez@inmegen.gob.mx

Received 12 March 2012; Revised 7 June 2012; Accepted 9 June 2012

Academic Editor: Ali-Akbar Saboury

Signal transduction inside and across the cells, also called cellular signaling, is key to most biological functions and is ultimately related with both life and death of the organisms. The processes giving rise to the propagation of biosignals are complex and extremely cooperative and occur in a far-from thermodynamic equilibrium regime. They are also driven by activation kinetics strongly dependent on local energetics. For these reasons, a nonequilibrium thermodynamical description, taking into account not just the activation of second messengers, but also transport processes and dissipation is desirable. Here we present a proposal for such a formalism, that considers cells as small thermodynamical systems and incorporates the role of fluctuations as intrinsic to the dynamics in a spirit guided by mesoscopic nonequilibrium thermodynamics. We present also a minimal model for cellular signaling that includes contributions from activation, transport, and intrinsic fluctuations. We finally illustrate its feasibility by considering the case of FAS signaling which is a vital signal transduction pathway that determines either cell survival or death by apoptosis.

1. Introduction

Survival of living organisms is intimately linked to their ability to react quite efficiently to even extremely weak external signals. Common examples are the reaction of the human eye to single light photons [1, 2], the reaction of a male butterfly to a single pheromone molecule coming from a female at a distance that sometimes is in the order of kilometers [3], and so forth. Cellular receptors react to hormones, cytokines, or antigens at very low concentrations. This strong reaction to a weak impulse is attained by an amplification process which is performed by means of special pathways of free energy transduction. Mechanisms such as immune system response, thermal-shock inhibitions, and cardiovascular rearrangement in response to environmental changes are all mediated by signaling processes. Signal transduction (information flow) is, thus, equally important, if not more important, for the functioning of a living organisms than metabolism and energy flow.

Signal transduction or *cell signaling* is the generic name of the set of concatenated processes or stages in which a cell transforms a certain signal or stimulus—either intercellular or intracellular—into another signal or a specific response. Cell signaling affects the complex arrangement of biochemical reactions inside the cell that takes place by means of enzymes that are bounded to other molecules called *second messengers*. Each process takes place in fast times, with dynamic ranges between a few milliseconds in most cases, to a few seconds in the case of more complex signaling cascades. Intricate and very sensible molecular biology experiments have shown cell signaling to be rate processes, that is, kinetic-guided phenomena determined by previous systems settings [4].

The wide variety of physicochemical signals to which cells may respond may seem to imply on a wide range of signal transduction mechanisms. However, only a handful of event chains is able to generate proper response to every stimulus in different cell subtypes which points to generalistic strategies commonly beginning with the action of cell receptors. Many signal transduction processes are then usually started by the adhesion of a ligand protein to a membrane receptor that then activates either itself or other receptor (or series of

receptors) thus converting the initial stimulus into a response that once inside the cell provokes a chain of biochemical events known as a *signaling cascade* or *second messenger pathway* which results in the amplification of the signal.

The archetypal example here is that of the *epinephrine cascade*. It is known that epinephrine (adrenaline) stimulates the liver to convert glycogen to glucose in liver cells, but epinephrine alone would not convert glycogen to glucose. In an outstanding experimental *tour de force* that granted him the 1971 Nobel Prize in Physiology or Medicine, Earl Sutherland found that epinephrine had to trigger a second messenger, cyclic AMP, for the liver to convert glycogen to glucose [5]. Secondary messenger systems can be synthesized and activated by the action of enzymes, for instance, cyclases that synthesize cyclic nucleotides. Second messengers also form by opening of ion channels to allow influx of metal ions (e.g., calcium signaling). These second messengers then bind and activate protein kinases, ion channels, and other proteins continuing the signaling cascade.

The role that activation kinetics and other energy-driven dynamic processes play in cell signaling makes evident the need for a thermodynamic description. Most studies to date are based in equilibrium thermodynamics assumptions [6, 7] or, in any case, coarse-grained approaches [8]. Specific applications of nonequilibrium thermodynamics have been studied in the past [9–13] focusing on single features such as switching, sensitivity, and controllability. Thus, a nonequilibrium thermodynamics analysis of cell signaling, describing transport processes, activation kinetics and nonlocal effects is desirable.

Some particular cases of signaling dynamics have been studied, even at the nonequilibrium statistical physics level of description, for instance, by means of information theoretical approaches [14]. In such studies, a positive correlation between the *channel capacity* (i.e., the information-carrying capacity of the signaling networks) and free-energy expenditure has been observed. For phosphorylation-dephosphorylation switches, hydrolysis-free energy is in the sustained high concentration of ATP and low concentrations of ADP, that is, away from thermochemical equilibrium. This deviation from equilibrium implies, among other things, that useful hydrolysis-free energy does not come from the phosphate bond of the ATP molecule alone but from more complex-systemic mechanisms.

Nonequilibrium thermodynamic entropy and entropy production have been studied, to gain dynamic signaling information transfer, in insulin transduction [11]. In that case, entropy production rates show a broad secondary peak in time that represents a possible evidence of the decrease of the concentration of membrane GLUT4 (a so called *backflow*), thus to the reduction of insulin efficiency. Interestingly, at least in that case entropic contributions take a leading role in controlling signaling efficiency. Pathway selectivity driven by receptor-receptor interaction has also been studied by means of thermodynamic models [8]. Ligand-induced oligomerization of cell-surface receptors is driven by cooperative behavior. Oligomerization occurs due to interaction between nearest-neighbor receptors. This type of cooperativity can exhibit a first-order phase transition, corresponding to a jump in the surface density of ligand-receptor complexes. Clustering could be described by the statistical mechanics of a simple lattice Hamiltonian. Receptor-receptor interaction may lead to a first-order phase transition with a discontinuous jump in the receptor density as a function of the receptor chemical potential and/or the ligand concentration [8].

Thermodynamical studies of biomolecular switches could be quantitatively described by a simple 3-state population-shift model, in which the equilibrium between a non-binding, nonsignaling state and the binding-competent, signaling state is shifted toward the latter upon target binding. Performance of biomolecular switches can be sensibly tuned via mutations that alter their switching thermodynamics [7]. Thermodynamic conditions in the intracellular medium hence alter sensitivity, control, and effective information transfer in signaling networks [14].

Moreover, as we may see later, typical settings in signal transduction correspond with complex nonequilibrium stages [15]. In fact, even relatively simple signaling models such as the phosphorylation-dephosphorylation switches exhibit bistability due to feedback, and the related nonequilibrium steady state even presents a phase transition [16]. Such complex behavior led some researchers to propose that non-linear deterministic biochemical behavior is dynamically *trapped* between stochastic dynamics, both at the molecular signaling level and at the cellular evolution level [16]. This proposition raised from an analysis of the so-called *chemical master equation* which is founded in the tenets of non-linear nonequilibrium thermodynamics [10, 17].

Thermodynamic models of cell signaling aim to model and describe these phenomena at the basic level, and applications of thermodynamic modeling in search of therapeutic action have been recently developed [18]. Claims have been made that fast binding kinetics was advantageous for most targets with a couple of exceptions, that targeting some protein kinases could enhance rather than attenuate the pathway, and that therapeutic doses could be sensitive to the kinetic parameters of drug binding. Thermo-kinetic rates have been shown to play an important role in the dynamics of signaling and immune response. Plasmon resonance-based thermodynamics points out to slow-signaling modified second-messenger variants have similar affinities but distinctly faster dissociation rates that compared with the original messengers and that this may be behind their lower activity. Signaling deregulation could be starting not at the biochemical (recognition) but at the thermodynamical (dissociation rates) levels [19]. In fact, thermodynamic studies are now part of the drug-design tools of pharmaceutical chemistry. In fact, thermodynamic and kinetic analyses are sources of deeper insight into specificity of molecular recognition processes and signaling [20]. Such advances had led to research efforts combining statistical thermodynamic models in combination with experimental data [21]. Preliminary results of these studies are very promising.

A proposal for a nonequilibrium thermodynamics formalism including the role of fluctuations as intrinsic to the dynamics, and the role of transport processes is made in this

work. We detail a minimal model for cellular signaling that considers activation, transport, and intrinsic fluctuations.

The rest of the paper is organized as follows: in Section 2 we discuss the role that stochastic fluctuations and transport processes play in biological signal transduction, in Section 3 we develop a nonequilibrium thermodynamics formalism of cell signaling and from it we derive a minimalist model, Section 4 deals with the biology of direct FAS signaling in apoptosis that in its simplest version (here presented) is akin to our minimalist model, finally in Section 5 we discuss some potential applications, the scope and limitations of our formalism as well as some perspectives.

2. Cell Signaling and Stochasticity and Nonlocality

One source of complexity in the nonequilibrium thermodynamical characterization of cell signaling is the fact that a cell is a *small system*; that is, cellular dimensions do not permit the immediate application of the thermodynamic limit. Specifically, the role that fluctuations and stochasticity may play within such scenarios is not completely clear. A formalism to study *small systems thermodynamics* in equilibrium has been developed [22, 23], and some results were even expected to extend to local equilibrium settings within cellular sized biosystems [24]. However, one important drawback in completing such theoretical frameworks at that time was the lack of proper experimental settings to test their hypotheses. Nevertheless, with the development of modern techniques, such as microscopic manipulation by means of atomic force microscopes, optical tweezers, and cold traps, this situation has been overcome at least partially. Theories have been developed including mesoscopic thermodynamical approaches [25–29] and also studies made by means of fluctuation theorems [30–33]. Some of these theoretical results have been even experimentally tested.

Due to the low copy number of many reactants in cells, and the nonequilibrium nature of the many intracellular reactions, signal transduction may result from stochastic intracellular events. Distribution analyses of cell responses provide a means to probe the stochastic character of intracellular signaling. A goal is to determine the class of stochasticity that affects intracellular pathways [34]. Stochasticity has been measured experimentally, it has been also incorporated in molecular simulations, and it has been discovered that locality and Gaussian behavior are not always present. In fact, transient multipeak distributions have been observed in computer simulations of cell-signaling dynamics. The emergence of these complex distributions cannot be explained using either deterministic chemical kinetics or simple Gaussian noise approximation [35].

Multipeak distributions are typically transient and eventually evolve into single-peak distributions in certain cases these distributions may be stable in the limit of long times. It has also been shown that introducing positive feedback loops results in diminution of the probability distribution complexity. This effect is so strong that even stochastic resonance has been reported in signaling cascades [36] where certain *optimal reaction rates* minimize the average threshold-crossing time. A noisy signal reaches the threshold more easily when the upstream and downstream reaction time scales are related in a specific way, indicating the existence of internal resonances embedded in cellular signaling cascades [36]; that is, nonequilibrium thermodynamic couplings exist between different modes (as characterized by their corresponding relaxation times) a feature that can be accounted by certain nonequilibrium thermodynamics formalisms (see next section). This may seem to point out on how the rates of various nodes could be *collectively tuned* in protein-signaling networks in such a way that signals are optimally picked up and biological information is transmitted through the signaling cascade.

3. Irreversible Thermodynamics of Cell Signaling

As we have just pointed out, systems outside the thermodynamic limit are characterized by large fluctuations and hence stochastic effects. The classic thermodynamic theory of irreversible process (also called linear irreversible thermodynamics, LIT) [37] provides us with a *coarse-grained* description of the systems, thus ignoring the molecular nature of matter studying it as a continuum media by means of a phenomenological field theory. As such LIT is not suitable for the description of small systems because it ignores fluctuations that could become the dominant factor in the system's response. Nevertheless, in many instances, it would be desirable to have a thermodynamic theoretical framework to study small systems, most noticeably in cellular and subcellular processes like signal transduction. One way to do so is by considering the stochastic nature of the time evolution of small nonequilibrium systems. This is the approach of Mesoscopic Nonequilibrium Thermodynamics (MNETs) [26]. MNET for small systems can be understood as the extension of the equilibrium thermodynamics of small systems developed by Hill and Chamberlin [22] and Hill [23, 24].

MNET, for instance, was developed to analyze nonequilibrium small systems. Any reduction of the spatio-temporal scale description of a system implies an increase in the number of noncoarse-grained degrees of freedom. These degrees of freedom could be related with the extended variables in extended irreversible thermodynamics (EITs) [38]. In order to characterize such variables, let us say that there exist a set $\Upsilon = \{v_i\}$ of such non-equilibrated degrees of freedom. $P(\Upsilon, t)$ is the probability that the system is at a state given by Υ at time t. If one assumes [27] that the evolution of the degrees of freedom could be described as a diffusion process in Υ-space, then the corresponding Gibbs equation could be written as

$$\delta S = -\frac{1}{T}\int \mu(\Upsilon)P(\Upsilon, t)d\Upsilon. \quad (1)$$

$\mu(\Upsilon)$ is a generalized chemical potential related to the probability density, whose time-dependent expression could be explicitly be written as

$$\mu(\Upsilon, t) = k_B T \ln \frac{P(\Upsilon, t)}{P(\Upsilon)_{\text{equil}}} + \mu_{\text{equil}} \tag{2}$$

or in terms of a nonequilibrium work term ΔW as follows:

$$\mu(\Upsilon, t) = k_B T \ln P(\Upsilon, t) + \Delta W. \tag{3}$$

The time-evolution of the system could be described as a generalized diffusion process over a potential landscape in the space of mesoscopic variables Υ. This process is driven by a generalized mesoscopic-thermodynamic force $(\partial/d\Upsilon)(\mu/T)$ whose explicit stochastic origin could be tracked back by means of a Fokker-Planck-like analysis [26, 27]. MNET seems to be a good candidate theory for describing nonequilibrium thermodynamics for small systems, *provided that one has a suitable model* or microscopic means to infer the probability distribution $P(\Upsilon, t)$.

MNET and similar approaches are appropriate to deal with activated processes, like a system crossing a potential barrier. Biochemical reactions like the ones involved in signal transduction are clearly in this case. According to [27] the diffusion current in this Υ space could be written in terms of a local fugacity defined as

$$z(\Upsilon) = \exp \frac{\mu(\Upsilon)}{k_B T}, \tag{4}$$

and the expression for the associated flux it will be

$$J = -k_B L \frac{1}{z} \frac{\partial z}{\partial \Upsilon}. \tag{5}$$

L is an Onsager-like coefficient. After defining a *diffusion coefficient* D and the associated affinity $A = \mu_2 - \mu_1$, the integrated rate is given as

$$\bar{J} = J_o \left(1 - \exp \frac{A}{k_B T}\right) \tag{6}$$

with $J_o = D \exp(\mu_1/k_B T)$.

MNET then gives rise to nonlinear kinetic laws like (6). MNET has been applied successfully to biomolecular processes at the cellular level or description [28]. Non-linear kinetics are used to express, for example, RNA unfolding rates as *diffusion currents*, modeled via transition state theory, giving rise to Arrhenius-type non-linear equations. In that case the current was proportional to the chemical potential difference, so the entropy production was quadratic in that chemical potential gradient.

Signal transduction consists of a series of biochemical reactions, and many of these have unexplored chemical kinetics, due to this fact a detailed MNET analysis such as the one described above is unattainable at the present moment. On what follows, we will explore a phenomenologically based approach that takes into account similar considerations as the MNET framework already sketched but does so in a more informal, modeling-like manner. This phenomenological approach is based on the EIT assumption of enlargement of the thermodynamical variables space [39, 40].

Assuming that a generalized entropy-like function Ψ exists, we can write down a Gibbs equation which may be written in the following form [38, 41]:

$$\frac{d\Psi}{dt} = T^{-1} \left[\frac{dU}{dt} + p \frac{dv}{dt} - \sum_i \mu_i \frac{dC_i}{dt} - \sum_j \mathcal{A}_j \frac{d\xi_j}{dt} - \sum_k \mathcal{X}_k \odot \frac{d\Phi_k}{dt} \right] \tag{7}$$

or as a differential form

$$d_t \Psi = T^{-1} \left[d_t U + p d_t v - \sum_i \mu_i d_t C_i - \sum_j \mathcal{A}_j d_t \xi_j - \sum_k \mathcal{X}_k \odot d_t \Phi_k \right]. \tag{8}$$

Quantities are defined as usual, U is the internal energy per mol, T the absolute temperature, p the pressure, v the molar volume, μ_i is the chemical potential for the i species, C_i its concentration (mole fraction), $\mathcal{A}_j$ the molar chemical affinity for the reaction producing species, j (i.e., $\mathcal{A}_j = \sum_i \nu_i^j \mu_i^j$ being ν_i^j the stoichiometric coefficient for the ith species in the jth reaction and μ_i^j the corresponding chemical potential), ξ_j the reaction coordinate for the production of species, j, $\mathcal{X}_k$, and Φ_k are extended fluxes and forces for diverse k processes, and $\odot$ is the appropriate scalar product.

Here we are considering the presence of thermal processes, but also the energetics of three different contributions due to signal transduction: the effect of *bulk* chemical potentials related to concentration changes of the signaling molecules in the cellular environment (identified with the subscript i), activation kinetics (considered as generalized chemical reactions) related to the chemical affinities between ligand proteins, membrane receptors and effector proteins in the signaling cascade (identified with the subscript j), and generalized *transport* processes (including the effects of nonlocal dynamics and delays) considered as *extended* variables or *generalized fluxes and forces* (identified with the the subscript k).

3.1. Activation Kinetics. We will introduce a simple—although general—model for signal transduction including the action of ligand proteins (LPs), membrane receptors (MRs), effector proteins (EPs), and finally response proteins (RPs). In this idealized model, LPs and MRs play the role of *pulls and triggers*, then a series of m EP steps (not necessarily, but possibly sequential) constitute the core of the signaling cascade and finally, and the RPs when activated constitute the

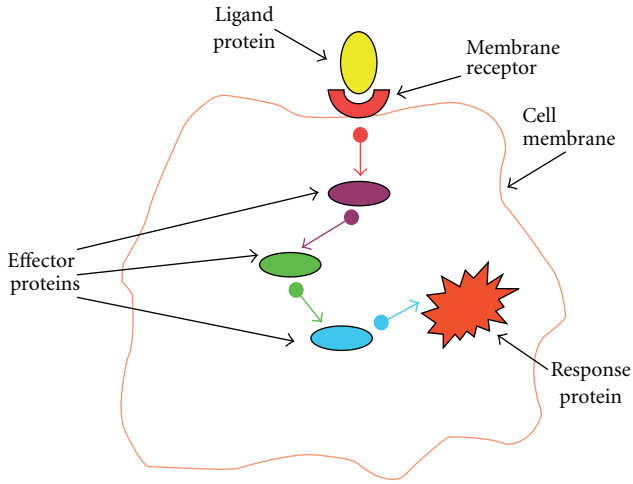

Figure 1: A toy model of cell signaling.

cell's response to the initial stimulus. The pseudo-chemical reactions could be written as follows:

$$\mathrm{LP} + \mathrm{MR} \longrightarrow \mathrm{MR}^{\star} \tag{9}$$

$$\mathrm{MR}^{\star} + \mathrm{EP}_1 \longrightarrow \mathrm{EP}_1^{\star} \tag{10}$$

$$\mathrm{EP}_1^{\star} + \mathrm{EP}_2 \longrightarrow \mathrm{EP}_2^{\star} \tag{11}$$

$$\vdots$$

$$\mathrm{EP}_i^{\star} + \mathrm{EP}_{i+1} \longrightarrow \mathrm{EP}_{i+1}^{\star} \tag{12}$$

$$\mathrm{EP}_m^{\star} + \mathrm{RP} \longrightarrow \mathrm{RP}^{\star}. \tag{13}$$

Here the superscript $\star$ refers to the *activated* form of the molecule, that is, the form which presents the corresponding biological signaling activity. For a pictorial representation, please refer to Figure 1.

3.2. Generalized Transport Processes. Let us recall (8). If we write down explicit expression for second and third terms in the r.h.s. of (8) in the context of signal transduction, we have the following generalized Gibbs form:

$$d_t G = -\Psi d_t T + \sum_i \mu_i d_t C_i + \sum_j \mathcal{A}_j d_t \xi_j + \sum_k \mathcal{X}_k \odot d_t \Phi_k. \tag{14}$$

Since signal transduction occurs within the cell, it is possible to relate an internal *work* term with the regulation process itself, being this a *far-from equilibrium* contribution. This nonlocal contribution is given by the generalized force-flux term (last term in the r.h.s. of (14)). This is so as cell signaling often does not occur *in situ* and also since is the only way to take into account (albeit indirectly) the changes in the local chemical potentials that cause the long tails in the fluctuations distributions characteristic of nonequilibrium small systems (e.g., cells). The term relating second messenger *flows* due to transduction could be written as a product of extended fluxes Φ_k and forces $\mathcal{X}_k$. Here $k = 1, \ldots m$ refers to the different second messenger species involved.

3.3. A Minimalist Model of Signal Transduction. In order to present a full detail of the different energetic contributions in (14), let us consider a *minimalist* model consisting of just four molecules under isothermal conditions: one triggering ligand protein (LP), one membrane receptor (MR), one effector protein (EP), and one response protein (RP). In such case we have the following 3 pseudoreactions:

$$\mathrm{LP} + \mathrm{MR} \longrightarrow \mathrm{MR}^{\star} \tag{15}$$

$$\mathrm{MR}^{\star} + \mathrm{EP} \longrightarrow \mathrm{EP}^{\star} \tag{16}$$

$$\mathrm{EP}^{\star} + \mathrm{RP} \longrightarrow \mathrm{RP}^{\star}. \tag{17}$$

That will give rise to the following form of (14):

$$\begin{aligned} d_t G = {} & \mu_{\mathrm{LP}} d_t C_{\mathrm{LP}} + \mu_{\mathrm{MR}} d_t C_{\mathrm{MR}} + \mu_{\mathrm{EP}} d_t C_{\mathrm{EP}} + \mu_{\mathrm{RP}} d_t C_{\mathrm{RP}} \\ & + \mathcal{A}_{\mathrm{MR}^{\star}} d_t \xi_{\mathrm{MR}^{\star}} + \mathcal{A}_{\mathrm{EP}^{\star}} d_t \xi_{\mathrm{EP}^{\star}} + \mathcal{A}_{\mathrm{RP}^{\star}} d_t \xi_{\mathrm{RP}^{\star}} \\ & + \mathcal{X}_{\mathrm{MR}^{\star}} \odot d_t \Phi_{\mathrm{MR}^{\star}} + \mathcal{X}_{\mathrm{EP}^{\star}} \odot d_t \Phi_{\mathrm{EP}^{\star}} + \mathcal{X}_{\mathrm{RP}^{\star}} \odot d_t \Phi_{\mathrm{RP}^{\star}}. \end{aligned} \tag{18}$$

Equation (18) considers the energies of formation for four molecules (as given by the μ's), the energies of activation of three species (as given by the chemical affinities $\mathcal{A}$'s) as well as the energies related with transport of the active species, given by their respective thermodynamic forces ($\mathcal{X}$'s). If we now refer to (6) for the definition of *signaling fluxes* [25, 28], we can write down expressions for the Φ's, namely,

$$\Phi_{\mathrm{MR}^{\star}} = D_{\mathrm{MR}^{\star}} \exp^{\mu_{\mathrm{MR}}/K_B T} \times \left(1 - \exp^{\mathcal{A}_{\mathrm{MR}^{\star}}/K_B T}\right) \tag{19}$$

$$\Phi_{\mathrm{EP}^{\star}} = D_{\mathrm{EP}^{\star}} \exp^{\mu_{\mathrm{EP}}/K_B T} \times \left(1 - \exp^{\mathcal{A}_{\mathrm{EP}^{\star}}/K_B T}\right) \tag{20}$$

$$\Phi_{\mathrm{RP}^{\star}} = D_{\mathrm{RP}^{\star}} \exp^{\mu_{\mathrm{RP}}/K_B T} \times \left(1 - \exp^{\mathcal{A}_{\mathrm{RP}^{\star}}/K_B T}\right). \tag{21}$$

Hence their temporal derivatives are given by:

$$\begin{aligned} d_t \Phi_{\mathrm{MR}^{\star}} = {} & \frac{D_{\mathrm{MR}^{\star}}}{K_B T} \exp^{\mu_{\mathrm{MR}}/K_B T} \\ & \times \left[\left(1 - \exp^{\mathcal{A}_{\mathrm{MR}^{\star}}/K_B T}\right) d_t \mu_{\mathrm{MR}} - \exp^{\mathcal{A}_{\mathrm{MR}^{\star}}/K_B T} d_t \mathcal{A}_{\mathrm{MR}^{\star}}\right] \end{aligned} \tag{22}$$

$$\begin{aligned} d_t \Phi_{\mathrm{EP}^{\star}} = {} & \frac{D_{\mathrm{EP}^{*}}}{K_B T} \exp^{\mu_{\mathrm{EP}}/K_B T} \\ & \times \left[\left(1 - \exp^{\mathcal{A}_{\mathrm{EP}^{*}}/K_B T}\right) d_t \mu_{\mathrm{EP}} - \exp^{\mathcal{A}_{\mathrm{EP}^{\star}}/K_B T} d_t \mathcal{A}_{\mathrm{EP}^{\star}}\right] \end{aligned} \tag{23}$$

$$\begin{aligned} d_t \Phi_{\mathrm{RP}^{\star}} = {} & \frac{D_{\mathrm{RP}^{\star}}}{K_B T} \exp^{\mu_{\mathrm{RP}}/K_B T} \\ & \times \left[\left(1 - \exp^{\mathcal{A}_{\mathrm{RP}^{\star}}/K_B T}\right) d_t \mu_{\mathrm{RP}} - \exp^{\mathcal{A}_{\mathrm{RP}^{\star}}/K_B T} d_t \mathcal{A}_{\mathrm{RP}^{\star}}\right]. \end{aligned} \tag{24}$$

If we consider, as it is often done in irreversible thermodynamics, that the thermodynamic forces $\mathcal{X}$'s are proportional to the fluxes Φ's, with proportionality constant $\mathcal{R}$, we have

$$\mathcal{X}_{\mathrm{MR}^\star} = \mathcal{R}_{\mathrm{MR}^\star} D_{\mathrm{MR}^\star} \exp^{\mu_{\mathrm{MR}}/K_BT} \times \left(1 - \exp^{\mathcal{A}_{\mathrm{MR}^\star}/K_BT}\right) \quad (25)$$

$$\mathcal{X}_{\mathrm{EP}^\star} = \mathcal{R}_{\mathrm{EP}^\star} D_{\mathrm{EP}^\star} \exp^{\mu_{\mathrm{EP}}/K_BT} \times \left(1 - \exp^{\mathcal{A}_{\mathrm{EP}^\star}/K_BT}\right) \quad (26)$$

$$\mathcal{X}_{\mathrm{RP}^\star} = \mathcal{R}_{\mathrm{RP}^\star} D_{\mathrm{RP}^\star} \exp^{\mu_{\mathrm{RP}}/K_BT} \times \left(1 - \exp^{\mathcal{A}_{\mathrm{RP}^\star}/K_BT}\right). \quad (27)$$

We now define the following generalized transport coefficients:

$$\Theta_{\mathrm{MR}^\star} = \frac{\mathcal{R}_{\mathrm{MR}^\star} D^2_{\mathrm{MR}^\star}}{K_BT};$$

$$\Theta_{\mathrm{EP}^\star} = \frac{\mathcal{R}_{\mathrm{EP}^\star} D^2_{\mathrm{EP}^\star}}{K_BT}; \quad (28)$$

$$\Theta_{\mathrm{RP}^\star} = \frac{\mathcal{R}_{\mathrm{RP}^\star} D^2_{\mathrm{RP}^\star}}{K_BT}.$$

By substitution of (22) to (28) in (18) we have

$$\begin{aligned} d_tG = {} & \mu_{\mathrm{LP}} d_t C_{\mathrm{LP}} + \mu_{\mathrm{MR}} d_t C_{\mathrm{MR}} + \mu_{\mathrm{EP}} d_t C_{\mathrm{EP}} + \mu_{\mathrm{RP}} d_t C_{\mathrm{RP}} \\ & + \mathcal{A}_{\mathrm{MR}^\star} d_t \xi_{\mathrm{MR}^\star} + \mathcal{A}_{\mathrm{EP}^\star} d_t \xi_{\mathrm{EP}^\star} + \mathcal{A}_{\mathrm{RP}^\star} d_t \xi_{\mathrm{RP}^\star} \\ & + \Theta_{\mathrm{MR}^\star} \exp^{2\mu_{\mathrm{MR}}/K_BT} \left[1 - \exp^{\mathcal{A}_{\mathrm{MR}^\star}/K_BT}\right]^2 d_t \mu_{\mathrm{MR}} \\ & - \Theta_{\mathrm{MR}^\star} \exp^{2\mu_{\mathrm{MR}}/K_BT} \left[\exp^{\mathcal{A}_{\mathrm{MR}^\star}/K_BT} - \exp^{2\mathcal{A}_{\mathrm{MR}^\star}/K_BT}\right] d_t \mathcal{A}_{\mathrm{MR}^\star} \\ & + \Theta_{\mathrm{EP}^\star} \exp^{2\mu_{\mathrm{EP}}/K_BT} \left[1 - \exp^{\mathcal{A}_{\mathrm{EP}^\star}/K_BT}\right]^2 d_t \mu_{\mathrm{EP}} \\ & - \Theta_{\mathrm{EP}^\star} \exp^{2\mu_{\mathrm{EP}}/K_BT} \left[\exp^{\mathcal{A}_{\mathrm{EP}^\star}/K_BT} - \exp^{2\mathcal{A}_{\mathrm{EP}^\star}/K_BT}\right] d_t \mathcal{A}_{\mathrm{EP}^\star} \\ & + \Theta_{\mathrm{RP}^\star} \exp^{2\mu_{\mathrm{RP}}/K_BT} \left[1 - \exp^{\mathcal{A}_{\mathrm{RP}^\star}/K_BT}\right]^2 d_t \mu_{\mathrm{RP}} \\ & - \Theta_{\mathrm{RP}^\star} \exp^{2\mu_{\mathrm{RP}}/K_BT} \left[\exp^{\mathcal{A}_{\mathrm{RP}^\star}/K_BT} - \exp^{2\mathcal{A}_{\mathrm{RP}^\star}/K_BT}\right] d_t \mathcal{A}_{\mathrm{RP}^\star}. \end{aligned} \quad (29)$$

Equation (29) gives a complete irreversible thermodynamical description of the minimal model given by (15) to (17). The model is then to be supplemented with the appropriate constitutive relations; in this case, the time evolution for the concentrations, chemical potentials, and chemical affinities as given by biochemical kinetics.

The free-energy coupling given by the corresponding generalized Maxwell relations (since d_tG is an exact differential form, integrability conditions imply the existence of Maxwell-like relations [41]), as well as Gibbs-Duhem constrains (not all the concentrations and chemical potentials are independent) once explicit kinetics are given, constitutes the energetic core behind the complex processes of signal transduction. This is possibly the key contribution of this work, the explicit derivation from a nonequilibrium thermodynamics formalism showing that cell signaling *control* is, indeed, an energy-driven process. Of course, free-energy transduction has been known to be responsible for *the initiation* of signaling cascades. However, our model has shown that *every step* of the process is controlled and *locked* via the local chemical potentials even in the presence of stochasticity and fluctuations, provided that the assumptions of MNET hold.

4. Case Study: FAS Signaling in Apoptosis

An important family of signal transduction pathways is related with the onset and regulation of *programmed cellular death* or, apoptosis. Any functional disruption in the balance that apoptotic cells encounter may affect death signaling thus leading to diseases ranging from cancer in the case of subnormal apoptosis to degenerative disorders in supernormal apoptosis. Hence the control of the process as given by signal transduction pathways is of foremost relevance. One of the simplest example of such pathways is apoptosis regulation by FAS signaling. FAS is a cell-surface receptor protein that when triggered by an stimulus induces apoptosis in FAS-expressing cells. This process is highly linked with immune response, as the ligand for FAS, FAS-L, is mostly present on cytotoxic T cells and TH1 cells central players in innate immunity. FAS is composed of an extracellular region, one transmembrane domain, and an intracellular region. FAS activity is governed by interaction with its ligand (FAS-L). Activation of FAS through binding to its ligand or FAS antibody induces apoptosis, which has been confirmed by many experiments [42].

In order for signal transduction to occur, cross-linking of FAS with its ligand must occur. FAS trimerizes to properly bind to its ligand, which exists as a trimer. This creates a clustering of FAS that is necessary for signaling. In its intracellular region, FAS contains a conserved sequence deemed as *death domain*. An adaptor protein, FADD, interacts with the death domain on the FAS receptor. Subsequent binding to another region of FADD by procaspase 8 promotes grouping of pro-caspase 8 molecules bound to each of the clustered FADD proteins. This entire cluster is sometimes called a *death-inducing signaling complex*, or DISC [43]. Pro-caspase 8 transactivates itself once grouped, cleaving and releasing active caspase 8 molecules intracellularly.

As is clear from Figure 2, there is a correspondence between the model given by (15) to (17) and direct FAS signaling (Figure 2). In this case the ligand protein (LP) is the FAS-L molecule, the membrane receptor (MR) is FAS-R that when activated ($\mathrm{MR}^\star$) becomes FAS and then interacts with the effector protein (EP), in this case FADD, that carries the biosignal activating procaspase-8 (a response protein) that when activated ($\mathrm{RP}^\star$) becomes caspase-8, the molecule responsible for the no-return apoptotic response leading to cellular death.

FAS signaling is a well-characterized process [45], some thermodynamic parameters may be thus obtained by experiments [46, 47] or by means of molecular simulations [48]. This is the case of activation energies—especially when activation occurs by means of ATP produced by oxidative

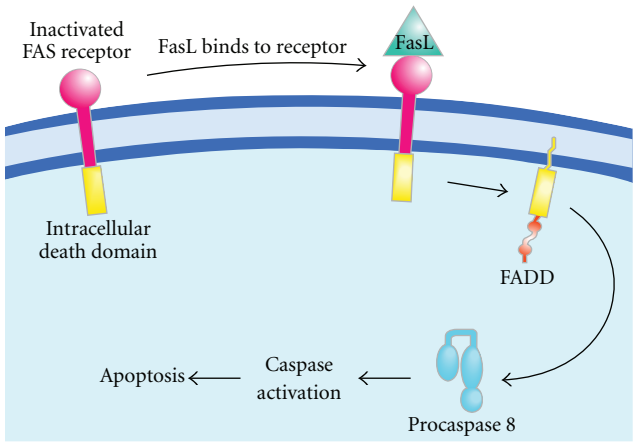

FIGURE 2: A *real-life* toy model of cell signaling: FAS signaling in apoptosis [44].

phosphorylation—and free energies of formation. However, transport processes have not been measured accurately (and in most cases have not been even measured at all). Being signaling pathways so important for the understanding of cell function, and in many instances for their biomedical importance as pharmacological targets; we hope that this situation soon will change. At the present moment, some insight on particular signaling pathways may be obtained by molecular dynamics simulations [49–54].

Experimental techniques have been refined that allow thermomolecular characterization of signaling processes. The technical challenges are, however, gigantic. Cell-signaling thermodynamic parameters must be experimentally measured by combining many different methodologies involving different scales of description: protein-protein electrostatic interactions, the electrohydrodynamic effect of the medium, cleavage and protein structure, free energies of folding/unfolding, transport processes, and so forth. Nonetheless, progress is being made in the actual realization of such experimental challenges, by using a clever combination of surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), and ultracentrifugation (UC) of the thermodynamics of T-cell signaling in the MHC pathway have been unveiled [55].

SPR is extensively used to study receptor/ligand binding both qualitatively and quantitatively. However, results are commonly ambiguous, and every conclusion needs to be independently verified. SPR provides both kinetic and equilibrium data; data acquisition is fast, comparative studies are easily performed, and low affinities can be detected with relatively low amounts of protein. The accuracy of the SPR-derived kinetic constants depends crucially on other various parameters such as mass transport, sensor chip capacity, and flow rate. In conclusion, SPR experiments are useful but partial and sometimes even dubious. In contrast, equilibrium methods, such as ITC, often result in more reliable, specially when used in conjunction with SPR and analytical techniques as ultracentrifugation in which sedimentation velocity, and equilibrium experiments provide insight into the hydrodynamic and thermodynamic properties of the sample, thus enabling the inference of transport parameters and free energies via association constants; on the other hand, SPR experiments could shed some light on biochemical kinetics and their associated relaxation times [55]. ITC has also used in the experimental analysis of the interaction between TRAF and tumor necrosis factor receptors [56].

FAS signaling proceeds by typical physicochemical mechanisms. Being this the case, common ranges for the parameters in cell signaling may be used as proxy values instead. For instance, the characteristic concentration of signaling protein molecules ranges from 0.01 to 1 μ-molar, with molecule counts between 120,000 and 20,000,000 depending on molecular weights and type of cell [57]. RAS concentration in HeLa cells has been measured to be 0.4 μ-molar [58]. Thresholding signal duration times for whole processes range between 2 minutes and 24 hours. NF-κB signaling (which is related with FAS signaling) takes about 320 minutes in epithelial cells [59, 60].

In order to figure out the order of magnitude of kinetic parameters, let us consider the case of the values of fluxes and dissociation constants in the Wnt-signaling pathway [61]. Dissociation constants for several second messenger molecules range around 10–1200 nM, with protein concentrations in the 15 to 100 nM regime. The degradation flux of β-catenin via the proteasome is 25 nM/h. The characteristic time of the associated phosphorylation-dephosphorylation switch is 2.5 minutes for APC. Relaxation times for GSk3-β association/dissociation is 1 minute, and that of Axin degradation is 6 minutes [61]. Decay rates (half-life times) $t^{1/2}$, for signaling molecules in the MAPK pathway and the STAT pathway, are valued between 0.75 and 24 h [57]. More closely related with FAS signaling, duration times on switch of apoptosis and duration of apoptotic death in HeLa cells exposed to different levels of TRAIL are between 19 and 27 minutes for switching and between 140 and 660 minutes for cell death [62]. Rate constants for diffusion-limited enzymes may vary around 10^8 and 10^{10} M^{-1} s^{-1} [63] although there are other kinetic mechanisms for second messengers that in some cases seem to be cell-type specific [64], these figures may serve as reference to infer chemical kinetic behavior since the general behavior seems to be quite common [65]. Once the rate constants are measured, one can infer activation energies from them, following a kinetic model [66].

In relation to energetics, it is known that ATP hydrolysis releases between 28 and 33.5 kJ/mol [67] depending on cell type and condition whereas for other energy-rich compounds involved in substrate level phosphorylation range around 23.44 and 88 kJ/mol [68]. Free energy profiles may also help us to understand the role that protein-coupling plays in cell signaling. In Ras signaling, for instance, binding free energy determines the fate of Ras/Raf dynamics [69]. Diffusion coefficients measured inside the cell differ according to cell medium and molecule size. Inside the cell nucleus typical diffusion constants vary between 10 and 100 $\mu m^2/s$ [70]. In reference with signaling proteins, this is usually also the case even in cytoplasm and/or aqueous solution. The diffusion rate of phosphoglycerate

kinase (around 45 kDa) has been measured as $63.8\,\mu m^2/s$ [71], while heavier molecules as 62 kDa Dextran move slowly, around $39\,\mu m^2/s$ [72]. Smaller second messenger molecules like insulin (5.808 kDa) can diffuse much faster, $D = 150\,\mu m^2/s$ [73]. The combination of different experimental/computational modeling techniques and estimated parameters just sketched may allow to construct quantitative thermodynamic models for cell signaling, following the lines of the present work, in the near future.

5. Discussion

Signaling transduction is a quite complex yet extremely important physicochemical phenomenon in cell biology. As we have seen cellular signaling is characterized by a combination of stochastic effects, activation biochemical kinetics, and multiple transport processes all setup in a far-from thermodynamic equilibrium setting. Is is known that free-energy transduction plays a key role in the process of signaling cascades. For this reason a nonequilibrium thermodynamics description at the mesoscopic level is desirable. In the present work we have presented such a formalism in the context of MNET [26].

The role of stochasticity is taken into account (albeit in an indirect way) by means of incorporating the probability distribution for the nonequilibrated degrees of freedom into a generalized chemical potential as is described in (2) to (6). In this scheme, the thermodynamic forces (equations (25) to (27))—that reflect in a coarse grained way the effect of stochasticity—are identified as the gradients in the space of mesoscopic variables of the logarithm of the ratio of the probability density to its equilibrium value. The main idea is to generalize the definition of the chemical potential to account for these additional mesoscopic variables. Thus it is possible to assume that the evolution of these degrees of freedom is described by a diffusion process and formulate the corresponding Gibbs equation.

The effect of generalized transport processes related with the distribution of relaxation times of the kinetics (it takes some (in general different) time for every biochemical reaction to activate the corresponding signaling molecule) and nonlocalities in the molecular processes (i.e., a second messenger has to travel some distance, say by diffusion, until it reaches its target molecule) is given by the last term at the r.h.s. of (14). In particular, relaxation times for the coarse-grained processes are given in terms of generalized transport coefficients (28). Our formalism is written in such a way that we can distinguish between local equilibrium effects (corresponding to the energetics of non-activated molecules at the top of the signaling cascade, as given by the first 4 terms at the r.h.s. of (29)), deterministic activation kinetics depending exclusively on the rate equations for the chemical reactions (corresponding to the 5th to 7th terms at the r.h.s. of (29)), and far-from equilibrium effects, involving both stochasticity and transport processes (terms 8th–13th at the r.h.s. of (29)) involving the dynamics of the evolution of nonconserved variables. We could think of this structure as a hierarchy in which *trains of signals* are coupled with each other via their relative relaxation times (as given by the corresponding generalized transport coefficients, (28)).

The potential application of such a formalism is wide, in particular with respect to the detailed study of the dynamics for important biological pathways. Consider, for instance, the extremely important scenario of calcium signaling. Phenomena like *calcium waves* [74, 75] and *calcium-induced calcium* release [76] could be understood more clearly (and even modeled and simulated) in the light of a nonequilibrium thermodynamic description like the one presented above. For instance, the role of energy releasing pathways in the dynamics and control of cell signaling under a system biology-like philosophy becomes almost crystal clear. In turn these free-energy triggers may be appropriate candidates for pharmacological targets for drug-therapy in cases of diseases associated with abnormal signaling. This may be the case of cardiac arrhythmias, neurological disorders [75, 77], and metabolic diseases [76].

Of course, such general modeling strategy has some drawbacks. On the one hand being a fully thermodynamical description, this framework depends entirely on experimental data for the activation kinetics and other constitutive relations or in any case in a good set of molecular simulations. On the other hand, ultrafast kinetics may be accompanied by noncompensated stochasticity that could not be handled entirely under the MNET paradigm. In conclusion, much work has still to be done in order to establish the validity and feasibility of these physical models into biological and biomedical research.

Acknowledgments

The author gratefully acknowledges support by Grant PIUTE10-92 (ICyT-DF) (Contract 281-2010) as well as federal funding from the National Institute of Genomic Medicine (México). He also acknowledges suggestions of the anonymous reviewers that, no doubt, contributed greatly to improve this work.

References

[1] D. A. Baylor, T. D. Lamb, and K. W. Yau, "Responses of retinal rods to single photons," *Journal of Physiology*, vol. 288, pp. 613–634, 1979.

[2] S. Hecht, S. Schlaer, and M. H. Pirenne, "Energy, quanta and vision," *Journal of the Optical Society of America A*, vol. 38, pp. 196–208, 1942.

[3] J. Andersson, A. K. Borg-Karlson, N. Vongvanich, and C. Wiklund, "Male sex pheromone release and female mate choice in a butterfly," *Journal of Experimental Biology*, vol. 210, no. 6, pp. 964–970, 2007.

[4] F. H. Johnson, H. Eyring, and B. J. Stover, *The Theory of Rate Processes in Biology and Medicine*, John Wiley & Sons, New York, NY, USA, 1974.

[5] T. N. Raju, "The Nobel chronicles. 1971: Earl Wilbur Sutherland, Jr. (1915–74)," *The Lancet*, vol. 354, no. 9182, article 961, 1999.

[6] E. Mertz, J. B. Beil, and S. C. Zimmerman, "Kinetics and thermodynamics of amine and diamine signaling by a

trifluoroacetyl azobenzene reporter group," *Organic Letters*, vol. 5, no. 17, pp. 3127–3130, 2003.

[7] A. Vallée-Bélisle, F. Ricci, and K. W. Plaxco, "Thermodynamic basis for the optimization of binding-induced biomolecular switches and structure-switching biosensors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 13802–13807, 2009.

[8] C. Guo and H. Levine, "A thermodynamic model for receptor clustering," *Biophysical Journal*, vol. 77, no. 5, pp. 2358–2365, 1999.

[9] H. Qian, "Thermodynamic and kinetic analysis of sensitivity amplification in biological signal transduction," *Biophysical Chemistry*, vol. 105, no. 2-3, pp. 585–593, 2003.

[10] H. Qian and T. C. Reluga, "Nonequilibrium thermodynamics and nonlinear kinetics in a cellular signaling switch," *Physical Review Letters*, vol. 94, no. 2, Article ID 028101, 4 pages, 2005.

[11] E. Liu and J. M. Yuan, "Dynamic sensitivity and control analyses of metabolic insulin signalling pathways," *IET Systems Biology*, vol. 4, no. 1, pp. 64–81, 2010.

[12] D. Hu and J. M. Yuan, "Time-dependent sensitivity analysis of biological networks: coupled MAPK and PI3K signal transduction pathways," *Journal of Physical Chemistry A*, vol. 110, no. 16, pp. 5361–5370, 2006.

[13] N. R. Nené, J. Garca-Ojalvo, and A. Zaikin, "Speed-dependent cellular decision making in nonequilibrium genetic circuits," *PLoS ONE*, vol. 7, no. 3, Article ID e32779, 2012.

[14] H. Qian and S. Roy, "An information theoretical analysis of Kinase activated phosphorylation dephosphorylation cycle," *IEEE Transactions on NanoBioscience*, vol. 99, no. 1, pp. 1–17, 2012.

[15] M. Kurzynski, *The Thermodynamic Machinery of Life*, Springer, Berlin, Germany, 2006.

[16] H. Ge and H. Qian, "Non-equilibrium phase transition in mesoscopic biochemical systems: from stochastic to nonlinear dynamics and beyond," *Journal of the Royal Society Interface*, vol. 8, no. 54, pp. 107–116, 2011.

[17] T. G. Kurtz, "The relationship between stochastic and deterministic models for chemical reactions," *Journal of Chemical Physics*, vol. 57, no. 7, pp. 2976–2978, 1972.

[18] M. Goyal, M. Rizzo, F. Schumacher, and C. F. Wong, "Beyond thermodynamics: drug binding kinetics could influence epidermal growth factor signaling," *Journal of Medicinal Chemistry*, vol. 52, no. 18, pp. 5582–5585, 2009.

[19] K. Matsui, J. J. Boniface, P. Steffner, P. A. Reay, and M. M. Davis, "Kinetics of T-cell receptor binding to peptide/I-E(k) complexes: correlation of the dissociation rate with T-cell responsiveness," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 26, pp. 12862–12866, 1994.

[20] N. J. De Mol, F. J. Dekker, I. Broutin, M. J. E. Fischer, and R. M. J. Liskamp, "Surface plasmon resonance thermodynamic and kinetic analysis as a strategic tool in drug design. Distinct ways for phosphopeptides to plug into Src- and Grb2 SH2 domains," *Journal of Medicinal Chemistry*, vol. 48, no. 3, pp. 753–763, 2005.

[21] C. C. Mello and D. Barrick, "An experimentally determined energy landscape for protein folding," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, pp. 169–178, 2004.

[22] T. L. Hill and R. V. Chamberlin, "Extension of the thermodynamics of small systems to open metastable states: an example," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 22, pp. 12779–12782, 1998.

[23] T. L. Hill, *Thermodynamics of Small Systems*, Dover, New York, NY, USA, 2002.

[24] T. L. Hill, *Free Energy Transduction and Biochemical Cycle Kinetics*, Dover, New York, NY, USA, 2004.

[25] J. M. Rubí, "Non-equilibrium thermodynamics of small-scale systems," *Energy*, vol. 32, no. 4, pp. 297–300, 2007.

[26] D. Reguera, J. M. Rubí, and J. M. G. Vilar, "The mesoscopic dynamics of thermodynamic systems," *Journal of Physical Chemistry B*, vol. 109, no. 46, pp. 21502–21515, 2005.

[27] J. M. Rubí, "Mesoscopic non-equilibrium thermodynamics, atti dell'accademia peloritana dei pericolanti, classes di scienze fisiche," *Matematiche E Naturali*, vol. 86, C1S081020, supplement 1, Article ID 081020, 2008.

[28] J. M. Rubí, D. Bedeaux, and S. Kjelstrup, "Unifying thermodynamic and kinetic descriptions of single-molecule processes: RNA unfolding under tension," *Journal of Physical Chemistry B*, vol. 111, no. 32, pp. 9598–9602, 2007.

[29] F. Ritort, "The nonequilibrium thermodynamics of small systems," *Comptes Rendus Physique*, vol. 8, no. 5-6, pp. 528–539, 2007.

[30] D. J. Evans and D. J. Searles, "Fluctuation theorem for stochastic systems," *Physical Review E*, vol. 50, no. 2, pp. 1645—1648, 1994.

[31] G. Gallavotti and E. G. D. Cohen, "Dynamical ensembles in nonequilibrium statistical mechanics," *Physical Review Letters*, vol. 74, no. 14, pp. 2694–2697, 1995.

[32] C. Jarzynski, "Nonequilibrium equality for free energy differences," *Physical Review Letters*, vol. 78, no. 14, pp. 2690–2693, 1997.

[33] G. E. Crooks, "Nonequilibrium measurements of free energy differences for microscopically reversible Markovian systems," *Journal of Statistical Physics*, vol. 90, no. 5-6, pp. 1481–1487, 1998.

[34] M. N. Artyomov, J. Das, M. Kardar, and A. K. Chakraborty, "Purely stochastic binary decisions in cell signaling models without underlying deterministic bistabilities," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 48, pp. 18958–18963, 2007.

[35] Y. Lan and G. A. Papoian, "Evolution of complex probability distributions in enzyme cascades," *Journal of Theoretical Biology*, vol. 248, no. 3, pp. 537–545, 2007.

[36] Y. Lan and G. A. Papoian, "Stochastic resonant signaling in enzyme cascades," *Physical Review Letters*, vol. 98, no. 22, Article ID 228301, 4 pages, 2007.

[37] S. R. de Groot and P. Mazur, *Non-Equilibrium Thermodynamics*, Dover, New York, NY, USA, 1984.

[38] D. Jou, C. Pérez-García, L. S. García-Colín, M. Lapez De Haro, and R. F. Rodríguez, "Generalized hydrodynamics and extended irreversible thermodynamics," *Physical Review A*, vol. 31, no. 4, pp. 2502–2508, 1985.

[39] D. Jou, J. Casas-Vazquez, and G. Lebon, *Extended Irreversible Thermodynamics*, Springer, New York, NY, USA, 1998.

[40] D. Jou, J. Casas-Vazquez, and G. Lebon, "Extended irreversible thermodynamics," *Reports on Progress in Physics*, vol. 51, no. 8, article 02, pp. 1105–1179, 1988.

[41] M. Chen and B. C. Eu, "On the integrability of differential forms related to nonequilibrium entropy and irreversible thermodynamics," *Journal of Mathematical Physics*, vol. 34, no. 7, pp. 3012–3029, 1993.

[42] N. Itoh, S. Yonehara, A. Ishii et al., "The polypeptide encoded by the cDNA for human cell surface antigen fas can mediate apoptosis," *Cell*, vol. 66, no. 2, pp. 233–243, 1991.

[43] A. O. Hueber, M. Zörnig, D. Lyon, T. Suda, S. Nagata, and G. I. Evan, "Requirement for the CD95 receptor-ligand pathway

in c-myc-induced apoptosis," *Science*, vol. 278, no. 5341, pp. 1305–1309, 1997.

[44] http://upload.wikimedia.org/wikipedia/commons/f/f5/Fas-signalling.png.

[45] Y.-Y. Mo and W. T. Beck, "DNA damage signals induction of Fas ligand in tumor cells," *Molecular Pharmacology*, vol. 55, no. 2, pp. 216–222, 1999.

[46] J. Albanese, S. Meterissian, M. Kontogiannea et al., "Biologically active fas antigen and its cognate ligand are expressed on plasma membrane-derived extracellular vesicles," *Blood*, vol. 91, no. 10, pp. 3862–3874, 1998.

[47] T. Nguyen and J. Russell, "The regulation of FasL expression during activation-induced cell death (AICD)," *Immunology*, vol. 103, no. 4, pp. 426–434, 2001.

[48] J. D. Suever, Y. Chen, J. M. McDonald, and Y. Song, "Conformation and free energy analyses of the complex of calcium-bound calmodulin and the Fas death domain," *Biophysical Journal*, vol. 95, no. 12, pp. 5913–5921, 2008.

[49] N. Ota and D. A. Agard, "Intramolecular signaling pathways revealed by modeling anisotropic thermal diffusion," *Journal of Molecular Biology*, vol. 351, no. 2, pp. 345–354, 2005.

[50] H. Park, W. Im, and C. Seok, "Transmembrane signaling of chemotaxis receptor tar: insights from molecular dynamics simulation studies," *Biophysical Journal*, vol. 100, no. 12, pp. 2955–2963, 2011.

[51] J. E. Pessin and A. L. Frattali, "Molecular dynamics of insulin/IGF-I receptor transmembrane signaling," *Molecular Reproduction and Development*, vol. 35, no. 4, pp. 339–345, 1993.

[52] K. Watanabe, K. Saito, M. Kinjo et al., "Molecular dynamics of STAT3 on IL-6 signaling pathway in living cells," *Biochemical and Biophysical Research Communications*, vol. 324, no. 4, pp. 1264–1273, 2004.

[53] Y. Kong and M. Karplus, "Signaling pathways of PDZ2 domain: a molecular dynamics interaction correlation analysis," *Proteins*, vol. 74, no. 1, pp. 145–154, 2009.

[54] E. P. G. Arêas, P. G. Pascutti, S. Schreier, K. C. Mundim, and P. M. Bisch, "Molecular dynamics simulations of signal sequences at a membrane/water interface," *Journal of Physical Chemistry*, vol. 99, no. 40, pp. 14885–14892, 1995.

[55] M. G. Rudolph, J. G. Luz, and I. A. Wilson, "Structural and thermodynamic correlates of T cell signalling," *Annual Review of Biophysics and Biomolecular Structure*, vol. 31, pp. 121–149, 2002.

[56] H. Ye and H. Wu, "Thermodynamic characterization of the interaction between TRAF2 and tumor necrosis factor receptor peptides by isothermal titration calorimetry," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 16, pp. 8961–8966, 2000.

[57] S. Legewie, H. Herzel, H. V. Westerhoff, and N. Blüthgen, "Recurrent design patterns in the feedback regulation of the mammalian signalling network," *Molecular Systems Biology*, vol. 4, article 190, 2008.

[58] A. Fujioka, K. Terai, R. E. Itoh et al., "Dynamics of the Ras/ERK MAPK cascade as monitored by fluorescent probes," *The Journal of Biological Chemistry*, vol. 281, no. 13, pp. 8917–8926, 2006.

[59] D. D. Hershko, B. W. Robb, C. J. Wray, G. J. Luo, and P. O. Hasselgren, "Superinduction of IL-6 by cycloheximide is associated with mRNA stabilization and sustained activation of p38 map kinase and NF-κB in cultured Caco-2 cells," *Journal of Cellular Biochemistry*, vol. 91, no. 5, pp. 951–961, 2004.

[60] A. Hoffmann, A. Levchenko, M. L. Scott, and D. Baltimore, "The IκB-NF-κB signaling module: temporal control and selective gene activation," *Science*, vol. 298, no. 5596, pp. 1241–1245, 2002.

[61] E. Lee, A. Salic, R. Krüger, R. Heinrich, and M. W. Kirschner, "The roles of APC and axin derived from experimental and theoretical analysis of the Wnt pathway," *PLoS Biology*, vol. 1, no. 1, article e10, 2003.

[62] J. G. Albeck, J. M. Burke, S. L. Spencer, D. A. Lauffenburger, and P. K. Sorger, "Modeling a snap-action, variable-delay switch controlling extrinsic cell death," *PLoS Biology*, vol. 6, no. 12, article e299, pp. 2831–2852, 2008.

[63] M. E. Stroppolo, M. Falconi, A. M. Caccuri, and A. Desideri, "Superefficient enzymes," *Cellular and Molecular Life Sciences*, vol. 58, no. 10, pp. 1451–1460, 2001.

[64] C. Kiel and L. Serrano, "Cell type-specific importance of Ras-c-Raf complex association rate constants for mapk signaling," *Science Signaling*, vol. 2, no. 81, article ra38, 2009.

[65] C. Kiel, D. Aydin, and L. Serrano, "Association rate constants of ras-effector interactions are evolutionarily conserved," *PLoS Computational Biology*, vol. 4, no. 12, Article ID e1000245, 2008.

[66] C. Kiel and L. Serrano, "Affinity can have many faces: thermodynamic and kinetic properties of Ras effector complex formation," *Current Chemical Biology*, vol. 1, pp. 215–225, 2007.

[67] J. Rosing and E. C. Slater, "The value of G degrees for the hydrolysis of ATP," *Biochim Biophys Acta*, vol. 267, no. 2, pp. 275–290, 1972.

[68] R. K. Thauer, K. Jungermann, and K. Decker, "Energy conservation in chemotrophic anaerobic bacteria," *Bacteriological Reviews*, vol. 41, no. 1, pp. 100–180, 1977.

[69] H. Gohlke, C. Kiel, and D. A. Case, "Insights into protein-protein binding by binding free energy calculation and free energy decomposition for the Ras-Raf and Ras-RalGDS complexes," *Journal of Molecular Biology*, vol. 330, no. 4, pp. 891–913, 2003.

[70] T. Misteli, "Physiological importance of RNA and protein mobility in the cell nucleus," *Histochemistry and Cell Biology*, vol. 129, no. 1, pp. 5–11, 2008.

[71] P. G. Squire and M. E. Himmel, "Hydrodynamics and protein hydration," *Biochem Biophys*, vol. 6, no. 1, pp. 165–177, 1979.

[72] R. Peters, "Nucleo-cytoplasmic flux and intracellular mobility in single hepatocytes measured by fluorescence microphotolysis," *The EMBO Journal*, vol. 3, no. 8, pp. 1831–1836, 1984.

[73] S. Vogel, *Life's Devices: The Physical World of Animals and Plants*, Princeton University Press, Princeton, NJ, USA, 1988.

[74] J. Amundson and D. Clapham, "Calcium waves," *Current Opinion in Neurobiology*, vol. 3, no. 3, pp. 375–382, 1993.

[75] W. N. Ross, "Understanding calcium waves and sparks in central neurons," *Nature Reviews Neuroscience*, vol. 13, no. 3, pp. 157–168, 2012.

[76] M. Shahidul Islam, P. Rorsman, and P. O. Berggren, "Ca^{2+}-induced Ca^{2+} release in insulin-secreting cells," *FEBS Letters*, vol. 296, no. 3, pp. 287–291, 1992.

[77] C. Trueta, S. Sánchez-Armass, M. A. Morales, and F. F. De-Miguel, "Calcium-induced calcium release contributes to somatic secretion of serotonin in leech Retzius neurons," *Journal of Neurobiology*, vol. 61, no. 3, pp. 309–316, 2004.