IMéRA
Aix*Marseille Université

Call 2016/2017

Title of the project:

Cybernetic Immunology:

A systemic theory for signal transduction in lymphocytes.

Research area:

Statistical Mechanics, Complex Systems, Theoretical Immunology, Neural Nets

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Proposal summary:

As adaptive immunity crucially depends on the transfer of information among its soldiers (e.g., via cell-cell interactions, signalling molecules and signal transduction pathways), and as information theory has been largely formalised by theoretical physicists in the past, I apply the know-how of theoretical physicists on challenging problems suggested by immunologists to build a multidisciplinary project that uses techniques stemmed from statistical mechanics of disordered systems (in particular tools developed in "neural networks") to infer information processing during lymphocyte interactions. The resulting formalisation will allow to analyse the behaviour of small clusters of interacting cells from a cybernetic perspective, that is, in terms of analogies with flip-flops, operational amplifiers, inverters and (bio)-logic gates. The main goal of the present research is the design and calibration of a "translator" from immunology to cybernetics, for signal transduction pathways involved in the adaptive response (i.e. cytokine receptors, MHC-I&II receptors, BCRs and TCRs, CD** receptors), whose practical outcomes consist in deepening the reprogramming protocols for the immune system (to be possibly exploited in therapies based on monoclonal antibodies) toward new approaches for treating impaired defence systems as, for instance, in the presence of autoimmune manifestations (e.g. rheumatoid arthritis).

Key words: Biological complexity, spin glasses & neural nets, adaptive response.

(1) STATE OF THE ART

The last decade has experienced an upsurge of interest in the use of statistical mechanics for studying the adaptive immune system and, at present, various international groups are dealing with it (e.g. Kardar's group at MIT [1] or Bialek & Callan's ones at Princeton [2]). Remarkably, not only theoretical physicists, but even leading figures in the field of Medicine suggest the usage of statistical mechanics for immune system investigations. Quoting Germain (on CTL CD8+ activation), "as one dissects the immune system at finer and finer levels of resolution, there is actually a decreasing predictability in the behaviour of any particular unit of function", furthermore, "no individual cell requires two signals (...) rather, the probability that many cells will divide more often is increased by costimulation" [3]: understanding these probabilistic structures and cell's averaged behaviour is exactly the goal of statistical mechanics.

Indeed the application of this methodology to immunology has been suggested by notable scientists in the past (see e.g. the seminal work by Parisi [4]), however, only recently a renewal interest for such an approach is concretely manifested. This is because main obstacles towards a quantitative theory of lymphocyte networks (e.g., replica symmetry breaking and finite connectivity of underlying structures) have finally been overcome with the introduction of new mathematical techniques; see e.g., the seminal works by Guerra [5] and by Coolen [6], and also some recent papers that I wrote with Guerra [7,8] and with Coolen [9,10], where these techniques are applied to networks of the immune system for the first time.

In fact, in the past three years, thanks to a grant (about half a million euros), that I got as Principal Investigator from the Italian Minister of University and Research (Firb Grant "Statistical mechanics of under percolated lymphocyte networks", Nr. RBFR08EKEV), we modelled the behaviour of real lymphocyte networks in terms of "spontaneous/emergent properties" (see the selected papers at the end of the proposal, summarized in [18-22], and Fig. 1). This framework, despite constituting a minimal theory is able to reproduce several emerging phenomena (i.e. not present when analysing single cells but resulting from their interactions) of real immune systems as the low-dose tolerance, antibody cascades, "bell-shaped" response, anergy in self-directed B cells, cognitive capabilities as learning, storage and decision making, strong lymphocytosis implying transient autoimmunity and defence from several pathogens simultaneously. It is worth noticing that in this reformulation of lymphocyte network theory (that, not as a minor point, is in agreement with existing data), there is never a giant component (as postulated in the past): the 'network' where B and T clones are nodes and links are played by the chemical messages they exchange (i.e. cytokines, immunoglobulins, etc.) is split into several disconnected small clusters (see Fig. 2). This paves the way for a deeper understanding of information processing in these single cliques making up the whole immune system.

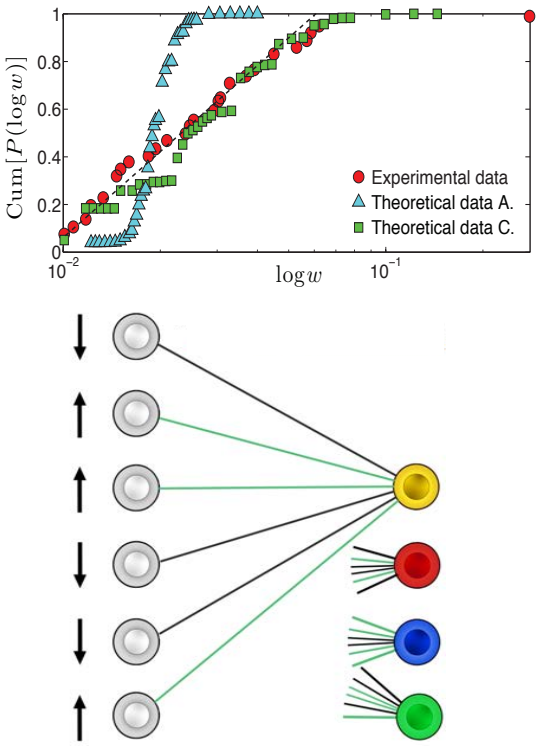


Fig. 1. Upper panel: Agreement between our "under-percolated theory" (squares) and experimental data (circles) on mice performed via ELISA technology. Note that a theory where lymphocyte networks are over-percolated (triangles) does not match the data. Lower panel: the bipartite structure underlying B (coloured circles) and T cells (white circles), interacting via both stimulating (green links) and inhibiting (black links) cytokines. See [17] for more details.

(2) RESEARCH AIMS

The scope of this project is building a robust, quantitative theory for decoding information processing in lymphocyte's dialogues (ranging from their interactions to their consequent internal signalling cascades): a very innovative theory for decoding and reprogramming adaptive responses in the immune system.
This, in turn, will provide a theoretical basis where framing the analysis of monoclonal antibody (mAbs) administrations (thought of as network's clique perturbations), toward a safe use of these novel biologics.

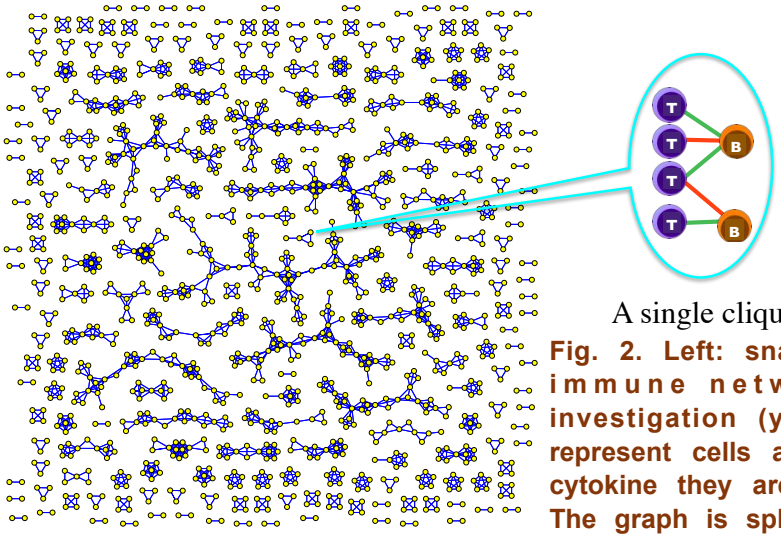
(3) DETAILED PROJECT AND METHODOLOGY

As immune networks are under-percolated (see Fig. 2), beyond the general analysis of the network as a whole, the analysis of each clique (isolated ensemble of interacting cells) is crucial. To analyse a "small loopy circuit" a convenient approach is cybernetics, that is, using Kolmogorov's definition "the science concerned with the study of systems of any nature which are capable of receiving, storing and processing information so as to use it for control".

Note that this translation into cybernetic terms (i.e. electronic equivalences encoding logical expression, that ultimately perform information processing) is quite natural for the immune system as, for instance, the so-called "two signal model" [11] is nothing but an "AND" boolean (bio)-logic gate, where the first input signal is given by antigen stimulation (upon BCR or TCR) and the "consensus to expand" -the second input- is given by an helper via diffusive stimulating cytokines and CD40 binding. If *both* inputs are "true", the outcome is "true" as well and clonal expansion starts, otherwise quiescence or even anergy are retained.

However, the idea of reading with cybernetic glasses small ensembles of interacting lymphocytes has a much broader interest, as I explain hereafter. "Signal transduction" occurs whenever an extracellular signalling molecule (e.g. an antigen brought to the BCR by an APC or seen by a TCR via an interaction with MHC) activates a specific receptor located on the cell surface –oligomeric BCR or TCR in these examples. Thus, all the immune dynamics are based on signal transduction pathways and lymphocyte's membranes are dense of receptors for this scope (i.e. antigen receptors –namely BCRs and TCRs- antigen presenting molecules –that is MHC class I and class II- the families of co-receptors CD4, CD8, CD19, etc.). In turn, these receptors trigger a biochemical chain of events inside the cell, involving well studied signalling molecules (see Fig. 3 and Tab. 1), ultimately creating a response: this is a marvellous input-output relation on which an arsenal of cybernetic methods for extracting information it conveys has already been developed in theoretical physics (e.g. Wiener-Kintchin relations, Convolution Theorem, Signal and Cable Theories, etc. [12]) but never applied on the present subject. Indeed while extensive data storage on these biochemical phenomena has been achieved, their full understanding seems still lacking: quoting Cohen, "now the question is how to turn information into comprehension" [13].

In this context, the present project would not require further experiments but rather a careful and patient work of data mining and elaboration [14] (see the "equivalence example" in Fig. 3). To make things clear, at this point, I introduce the basic phenomenological equivalences between biological processing and information processing in silico (that I recently developed [15, 18]): more complex cascades (as those of interest sketched in Fig. 3) will then be analysed "piece by piece" reducing the general problem of signal transduction (and more generally of lymphocyte's interaction) into a time-ordered ensemble of intermediary steps, for each of which the equivalences shown in Figs. 4 and 5 apply. In fact, on the one hand in biochemical kinetics (that we use to quantify immunological dialogues) we deal with concentration of substrate and ligands and they are related by the saturation curve (whatever the type, e.g. Michaelis-Menten, Hill, Adair, MWC or Koshland like); on the other hand, in electronics, we compare input voltage versus output voltage and they are related by the transfer function (whatever the operator, e.g. amplifier, inverter, flash, flip-flops, etc.). Remarkably, the two phenomenologies can finally be analogously formalised (due to [15]) in a unique



The whole network

A single clique

Fig. 2. Left: snapshot of the immune network under investigation (yellow circles represent cells and blue links cytokine they are exchanging). The graph is split into several disconnected clusters. Right: a magnification of a single cluster.

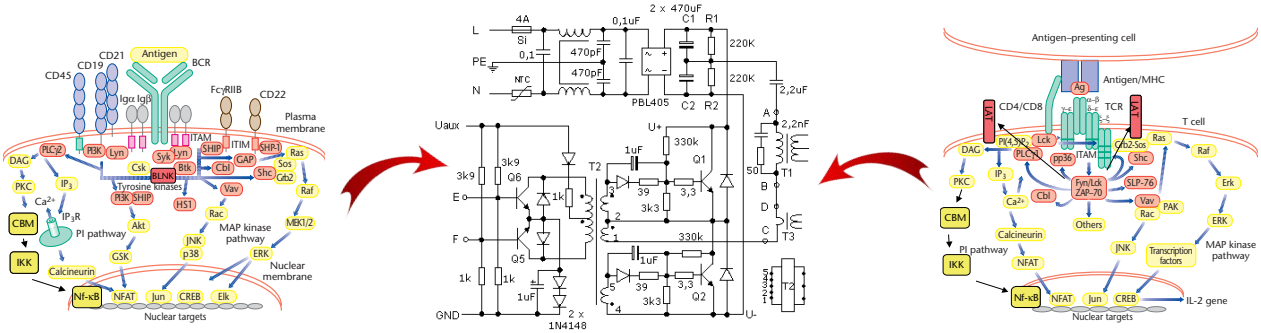


Fig. 3. The signalling pathways following BCR (leftmost panel) and TCR (rightmost panel) stimulation [16]. Example of electronic circuit (middle panel).

frame via statistical mechanics where we deal with order parameters and external fields and they are linked in the self-consistency (see Fig. 4). This bridge allows switching from a representation to another, namely to "translate" biochemical pathways into electronic circuits (whose comprehension is handily). Finally, the mirror between reaction kinetics and information processing, can be further extended at the level of logical gates (for instance, in Fig. 5, I show the YES, NOT, OR, NOR, AND, NAND gates only for simplicity).
Therefore, we now have very sophisticated tools to extract, from the biochemical processes underlying the lymphocyte responses, the information flux exchanged by cell themselves: the reward lies in a quantitative scaffold that will pave the strand for a comprehension of information processing during lymphocyte interactions. The final stage of the project will be devoted to the calibration of the theory by exploiting preliminary results on mAbs technology.

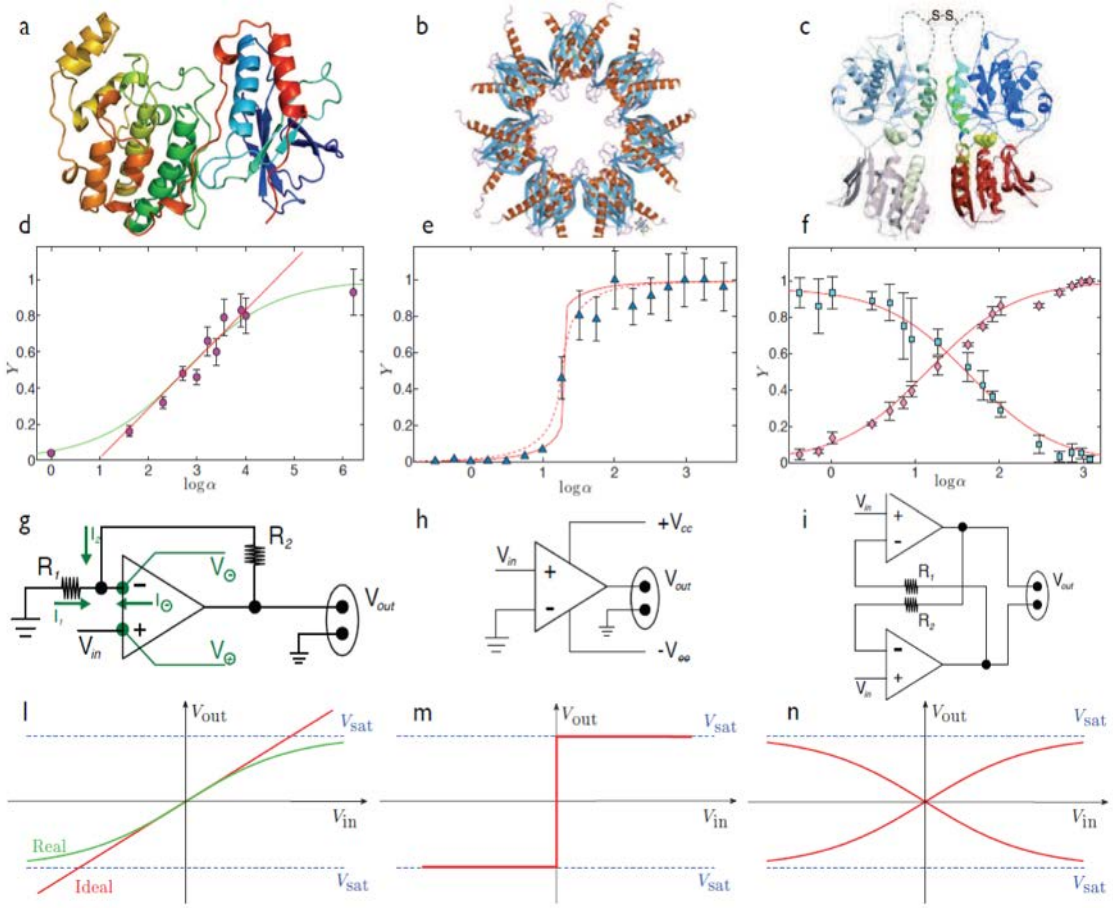


Fig. 4. The first row presents pictures of three molecules exhibiting cooperativity [positive (a), ultra-sensitive (b), and negative (c)]. The related saturation curves (binding isotherms) are shown in the second row (panels d, e and f, respectively), where symbols stand for experimental data and lines are best fits performed through the analytical expression I obtained from statistical mechanics (see [15] for details). In the third row we sketch the cybernetic counterparts, [operational amplifier (g), analog-to-digital converter (h), and flip-flop panel (i)]. The (theoretical) transfer functions corresponding to the circuits are shown in the fourth row (panels l, m and n, respectively).

As a last remark, let us focus in more details on the underlying dinamical aspects of these reaction kinetics: so far we discussed mainly "static quantities" as, once fixed the quantity of substrate given to a reaction, the resulting saturation value is its "thermodynamic value". However, as mentioned earlier, completely novel information can be inferred during off-equilibrium dynamics. Accessing to dynamical quantities is of prohibitive difficulty in vivo -and quite hard also in vitro (depending on the reaction)- mainly for the fine tuning on the external field necessary in order to have well detectable responses, but, once the formal bridge has been developed, they are easily accessible in silico (i.e. via numerics) as we briefly explain hereafter.
We related 1:1 equilibrium saturation plots with self-consistencies in Statistical Mechanics and transfer function in Electronics; remarkably their (simplest) dynamical extension (that can be obtained easily via Langevin and/or Fokker-Planck stochastic equations in Statistical Mechanics) also do extend properly to tackle the dynamics of these biochemical and electronic counterparts, resulting respectively into the so called "chemical master equations" for the former and into stochastic ODEs that generalize classical Kirchhoff laws for the latter (these are not-standard RLC filters due to the crucial presence of active elements, i.e. transistors). Once these equations will be made available on computing machines, there are two main routes to pave to extract information from them: one dealing with standard operational approaches (Fourier and Laplace frequency analyses) -that will give us information of the involved timescales and energy spectra- and one dealing with entropy production and energy loss that can be performed by investigating the resulting hysteresis areas that generalize the self-consistencies in off-equilibrium regimes. Their investigation will reveal dynamical properties that have never been analyzed so far, possibly significantly extending our know-how on these biochemical reaction patterns.

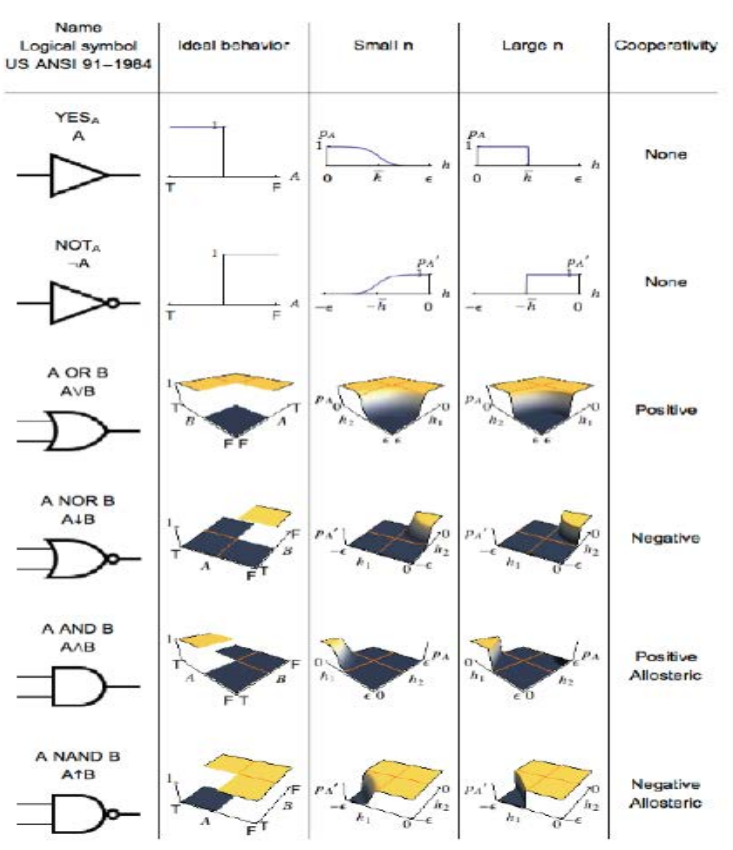
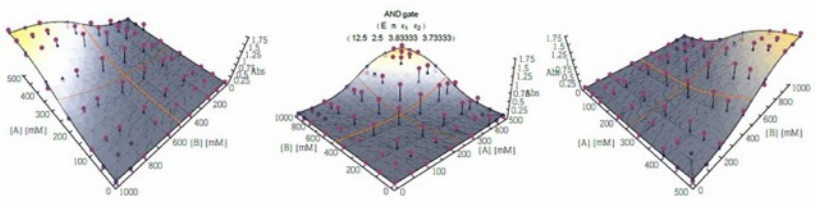


Fig. 5. This table recapitulates the analogy between allosteric kinetics and information processing: the first column collects the logical gates, the second one shows their ideal behaviour, while the third and the fourth columns display the activity probability for the pertinent allosteric reactions, while the last column clearly states if, in terms of statistical mechanics, we have cooperativity and, for positive answers, if the latter is real cooperativity or indirect regulation only. It is to remar that the four logical operators we have shown here form a logic bases hence, from first principles, they could be arranged (i.e. in chain-cascades) to perform complex operations as desired.

Fig. 6: This picture shows a real biological stochastic AND gate realized by us in [18] where the statistical mechanical formulation of stochastic calculus via these gates has been developed.



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