The 3^{rd} Workshop on Stochasticity in Biochemical Reaction Networks

Sotiria Lampoudi (University of California at Santa Barbara)
Brian Munsky (Los Alamos National Laboratory)
David Thorsley (Biotechnology HPC Software Applications Institute)
Aleksandra Walczak (Ecole Normale Superieure)

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Abstract

Cellular functions in biological organisms are comprised of the complex interactions of many molecular species: proteins, DNA and mRNA molecules, hormones, etc. Recent experimental developments in molecular biology have enabled researchers to characterize many of the biochemical pathways involved in these functions, and enormous amounts of data are currently available. However, despite this wealth of data, we still lack a sufficient understanding as to how cellular mechanisms combine to form the observable properties of cellular behavior. Key obstacles that restrict this understanding are the inherently complex and stochastic properties of cellular systems. Not only must we overcome the complex many-body nature of the interactions between the cellular components, but we are also we are faced with an additional difficulty, stemming from the stochastic, nonequilibrium nature of chemical reactions. The *3^{rd} Workshop on Stochasticity in Biochemical Reaction Networks* was held to discuss recent progress on the role of intrinsic stochasticity in many-body biochemical networks.

1 Overview of the Field

Cells in biological organisms are subject to vast amounts of random variation, which can cause isogenic cells to respond differently. There are many factors that may contribute to this phenotypical diversity. The simplest of these include fluctuations in environmental conditions such as nutrients, heat, radiation, etc. But even in homogenous environments, diversity can arise from the rare and discrete nature of chemical interactions within a cell. In general, molecules that are present in smaller numbers are prone to a greater extent of variable response, as single molecule events take on greater relative importance. In particular, since cells contain only one or two copies of many important genes, these cells can express vastly different behaviors when these genes become active (on) or inactive (off). Switching times from on to off and back are controlled by an uncountable number of random or chaotic events as the many cellular constituents move and interact within the cell. Effectively, genes can be (de)activated simply due to chance reactions with gene regulatory molecules. In turn, these regulatory molecules undergo their own complicated set of events, including degradation, dimerization, folding, etc. Any of these events may assist or impede a chemical's reaction with a corresponding regulatory site of a given gene.

As alluded to above, gene regulation is particularly prone to stochastic fluctuations due their extremely small population numbers. The variability in gene regulation subsequently affects the downstream regulation of other processes [30, 10, 55, 22, 41, 11, 25]. This variability is often deleterious to the organism's survival, and biology has developed many mechanisms to suppress this variability, such as negative feedback or auto-regulation [2, 9, 38], which can reduce fluctuations for a given mean expression level. As

an alternative, dynamics in one regulatory sub-network can introduce low- or band-pass filters that help diminish fluctuation frequencies coming from other sources [3, 54].

In different systems, stochastic fluctuations may be be used to the cell's advantage. When combined with nonlinear effects, stochastic behaviors can be used to amplify [43] or damp external fluctuations and tune a system's sensitivity to its environment. Fluctuations may also be used to excite and/or improve the robustness of resonant behaviors [29]. Stochastic fluctuations may also allow for the phenomena of stochastic switching, and allow organisms to express two or more very different phenotypes [1, 59, 31, 57]. For certain organisms that exist in especially hostile environments, such as parasites, this ability to switch unpredictably from one state to another ability provides an important evolutionary advantage. If the host cannot predict the response of a parasite, then it has a much harder task to devise a strategy to combat that parasite. Even in the absence of direct competition, random switching can still provide a vital role in survival within an uncertain environment [6].

Measurement of the phenotypic and/or molecular variability of single cells is a field of great progress over the recent years [45]. These techniques enable today's experimentalists to prepare cells such that the dynamics of their gene expression, protein localization, or other traits can be observed through the presence or activation of fluorescent markers. Antibodies can be attached to fluorescent dyes and then made to bind specific cellular proteins or phosphoproteins. DNA and RNA molecules can be measured with fluorescence *in situ* hybridization (FISH) techniques [42, 24, 44]. With these techniques, researchers can simultaneously measure multiple different molecule types, and even explore the spatial dynamics and colocalization of biological molecules with fluorescence (Förster) resonance energy transfer (FRET) techniques or with split fluorescent proteins [16, 4, 5]. Once tagged with fluorescent reporters, there are many techniques with which one can then measure the highlighted cellular properties. These include fluorescence microscopy, confocal microscopy, time lapse microscopy, and flow cytometry.

Once measured, biochemical reaction networks could be modeled at many different scales. At the finest level of detail, molecular dynamics simulations are used to explore how protein movement, folding and interactions with surrounding molecules. At the other end of the scale, continuous-valued concentrations and ordinary differential equations describe large-volume chemical processes. Measurements of single-cell and single-molecule data require an approach at the *mesoscopic* scale. At this scale, each chemical species is described by an integer population, which is assumed to evolve according to Markovian dynamics. The majority of analyses at the mesoscopic scale have been conducted using kinetic Monte Carlo (MC) algorithms, such as Gillespie's Stochastic Simulation Algorithm (SSA) [17], or one of many improvements upon that approach [7, 53, 21, 51, 19, 56, 8, 46, 18]. Other analyses have been developed to approximate the solution of the chemical Master Equation, which describes the evolution of the probability densities for the Markov Process. These techniques include Finite state Projection approaches, [32, 33] and the spectral method [70, 71, 72, 73]. The participants of our workshop have also been involved in constructing and using algorithms for inferring molecular models of gene regulation [79, 78] and moment matching optimization algorithms.

1.1 A Need for Multi-Disciplinary Investigations

As discussed above, cell-to-cell variability has been studied in many different contexts and within many different disciplines, including molecular and cellular biology, physics, chemistry, computer science, optics, applied mathematics, mechanical and electrical engineering, statistics and others. The simple fact that so many different disciplines are actively researching within a relatively new topic introduces a unique set of research challenges and opportunities. One key challenge is that a strong language barrier exists that divides one discipline from another. When even physicists and engineers use unrelated sets of terminology to describe the same simple phenomena, there is little hope that mathematicians and molecular biologists could freely discuss more subtle behaviors. Faced with this concern, very few traditional journals, workshops or conferences exist to bring these myriad fields together, and established researchers have little incentive to learn the foreign terminology of another seemingly unrelated discipline. In many cases, this lack of communication leads to the duplication of efforts. On the other hand, the fact that so many different groups are focusing on similar problems has led to vast improvements in specific areas of research.

The workshop was committed to unite a broad array of young, international researchers who work on different aspects of the problem of understanding the role of stochasticity in biochemical systems. Experts in developing mathematical methods to describe cellular behavior, experimentally analyzing biochemical processes, performing advanced computations of stochastic behavior, and designing novel biological devices worked together to share the latest results in this exciting area of research, and defined new research directions for future study.

Together, the participants of this workshop formed an intellectually diverse group of researchers united

by their interest in the subject of stochasticity in biochemical reaction networks and complex biological matter; they represented the fields of biology, biophysics, engineering, chemistry, mathematics, and computer science. Each has contributed to the field of biochemical networks in either the theoretical or experimental sphere and many have contributed in both areas.

2 New Tools and Approaches to Study Stochastic Biochemical Reactions

Recent experimental techniques make it possible to measure the variation in gene expression, protein abundance, and cellular behavior. Combined with computational modeling, these techniques enable us to uncover the causes and effects of stochastic cellular dynamics. Depending on cellular function, biochemical processes may act to minimize stochastic variations or exploit them to the cell's advantage; in both cases, cellular processes have evolved to be remarkably robust to both intrinsic and extrinsic noise. By exploring this robustness in naturally occurring biological systems, we hope not only to improve our understanding of cellular biology, but also to formulate the "design principles" necessary to build similarly robust biochemical circuits and nanoscale devices.

This workshop brought together several multidisciplinary experts, who introduced the audience to many different aspects of this exciting research topic. First, we heard from experimental molecular biologists, who are continually developing and perfecting new quantitative techniques to observe single cell and single molecule dynamics. Tools such as flow cytometry and fluorescence activated cell sorting (FACS) were used by many presenters (including Lingchong You, Robert Egbert, Narendra Maheshri, Attila Becksei, and Ophelia Venturelli) measure the protein levels for millions of individual living cells in the time span of a single minute–thus conducting millions of simultaneous experiments. Time-lapse fluorescence microscopy and microfluidics studies were described by other researchers (including Rob Egbert, Nacho Molina, Gurol Suel, Gabor Balazsi) which made it possible for these researchers to measure, track and/or manipulate the behavior of single cells in carefully controlled micro-environments. Fluorescence *in situ* hybridization (FISH) techniques were presented by Gregor Neuert and Allistair Boettiger which were used to explore the spatial distributions of specific, individual RNA molecules within a cell or developing embryo. Jim Werner presented a new technique of three-dimensional single molecule tracking that could examine cellular dynamics at an even greater time and spatial resolution [85].

Next, the theorists and mathematicians among us presented new quantitative methods to analyze and explain the vast amounts of statistical data gathered from such experiments. It is known that stochasticity in cells is caused in part by intrinsic noise – the variability caused by the statistical dynamics of a chemical reaction with a small number of reactants – and in part by extrinsic noise – the variability caused by random fluctuations in a cell's environment. The participants in this workshop have already developed many methods to understand and differentiate between these types of noise in experimental data. For example, Peter Swain and Andreas Hilfinger presented new theoretical approaches to discriminate between various possible sources of stochastic variation in cellular fluctuation, while Gregor Neuert, Nacho Molina [79, 78] and Gabrielle Laillacci discussed integrated experimental and computational approaches to use cell-to-cell variability to learn more about the underlying mechanisms. In addition, as experimental techniques such as FISH provide more and more information on the spatial dynamics of intracellular processes, it becomes more useful to extend these techniques to spatially heterogenous reaction dynamics, such as those discussed by Eldon Emberly and Pablo Meyer-Rojas.

Finally, these theorists and experimentalists have successfully integrated their various analyses to understand how, why and when different cellular mechanisms transmit noise in different ways, i.e. some suppress it while others amplify or exploit it. For example, control theory can help us understand feedback and feedforward regulatory motifs in cellular architectures, while an information theoretic perspective can help us to understand how cells in a developing multicellular organism can determine their exact spatial location. These analyses suggest new methods and appropriate models for mathematically demonstrating how certain motifs are useful for dealing with noise and uncertainty. Such analyses are then directly applicable to the work of more applied researchers, who can use these theories to better constructing synthetic biological circuits and devices at the nanoscale level, including biomolecular motors and DNA molecular machines. For example, Yannick Rondelez and Elisa Franco both discussed new results in the field of synthetic biology where they have designed and experimentally validated biomolecular computational devices [47, 48, 49, 12, 13]

In the following subsections, we briefly discuss a few presentations that introduced most important advances in various fields of the experimental, theoretical, and computational investigation of stochasticity

in biochemical reaction, followed by a section on how these tools have successfully been combined to gain new insight into biological behaviors.

3 Presentation Highlights

We assembled a list of presentation highlights based on the top ranked speakers from an anonymous participant survey. The vast majority of the workshop participants presented unpublished work for the first time. The interactive environment allowed them to both get feedback on their research and presentation, but also to drastically adapt the presentation of their work to suit the audience. In what follows we discuss some of the workshop's highlights. Where applicable, we have included citations to relevant past work.

3.1 Recent Discoveries of Biological Stochasticity

There is a clear shift in the experimental and theoretical parts of the field to go beyond simply characterizing noise, towards attempting to understand the possible functions of noise. This was clear in most of the experimental talks:

- Gurol Suel [6, 26, 50] talked about the role of noise in the competence circuit. He specifically contrasted the large noise present in the native circuit versus the synthetic circuits, which are less noisy. He also pointed out the importance of the correct sequential timing of events in how the cell makes its decision to sporulate or not [27, 28].
- Alistair Boettiger [65, 67, 66, 68] pointed out that the apparently redundant repetition of regulatory elements can be important in the spatial macroscopic precision of expression patterns in fly development.
- Careful characterization by means of combining dynamical theoretical models with experiments by Nacho Molina resulted in surprising gene-specific bursting characteristics in mammalian cells, that have never been observed in prokaryotes [78, 79].
- Tom Shimizu talked about linking the biochemical network that controls chemotaxis with its function in the presence of signal fluctuations [77, 60, 61, 62].
- Narendra Maheshri [58, 39, 34] and Gregor Neuert talked about linking dynamical encoding of function in gene switches.
- Gabor Balazsi [38] discussed the interplay of noise and evolution in circuits in yeast.

3.2 Cellular environment

Several speakers with a biological background pointed to the need for a more holistic view of the cell.

- One of the highlights was a thought provoking lecture by biochemist Diego Ferreiro [80, 81, 82, 83, 84] about the need for a dynamical view of regulation in the cell.
- Pablo Meyer Rojas [35, 36] discussed the importance of localization of reactions in the cell.

3.3 Synthetic Circuits

Another series of experimental talks presented ideas about how our increased knowledge of molecular biology, chemistry and noise can be used in the field of synthetic biology to build more precisely designed switches and networks:

- Rob Egbert described how design of regulatory sequences can lead to precise tuning of observed gene regulatory circuit dynamcs.
- Yannick Rondelez [47, 48, 49] described a toolbox for building predictable switches using DNA elements.
- Elisa Franco [12, 13] directed our attention to interactions between well-functioning modules and biochemical switches.

3.4 Theoretical Techniques

On the theoretical side, there was a strong emphasis on understanding the environment in which biochemical networks function: the temporally changing signals and resources. Similarly to experimental and computational approaches the theoretically-focused speakers discussed the time varying response of gene expression.

- Pointing to the fact that gene expression occurs in a cellular environment, Rosalind Allen talked about the effects of mRNAs competing for ribosomes [20].
- The precision of temporal signaling in the context receptors was discussed by Thierry Mora.
- Peter Swain [40] and Andreas Hilfinger [23] both talked about novel frameworks for distinguishing between intrinsic and extrinsic fluctuations in dynamical systems.
- Paul Francois discussed the evolution of function in the context of morphogenic gradients [14, 15].
- Eldon Emberly presented a computational model based on simple physical ideas of spatial patterning of proteins in bacteria [52].

3.5 Computational Techniques

Important computational advances were discussed in the context of both analyzing experimental data and developing novel methods for model selection. Once again the topic of understanding the dynamics of gene regulation was prominent.

- Gregor Neuert and Nacho Molina [78, 79] discussed computational approaches that allow for the efficient analysis of gene expression data.
- Gabrielle Lillacci talked about how to increase the reliability of model selection algorithms while reducing computational costs.

4 Scientific Progress Made

A few new topics emerged as a result of the workshop. Many of the discussions aimed to point out that biochemical networks function in a specific context: they are part of the cellular environment, they rely on nutrients, they evolve, they interpret signals, they fulfill a function. Biochemical networks involve proteins, which are dynamical entities. As a result, a dynamical picture of both gene regulation and signaling is needed, to understand the functioning of cells. The importance of the dynamics of molecular interactions was addressed both theoretically (e.g. Peter Swain, Nils Becker) and experimentally (Atilla Becksei, Tom Shimizu). However, it clearly emerged as an important future direction.

On a similar note, it became clear that more attention must be directed to understanding the ability of biochemical circuits to react in a wide range of environments and conditions.

Discussions also pointed towards the abundance and spatial availability of proteins, enzymes and molecular machinery in the cell. This direction emerged as a worthwhile direction to study both theoretically and experimentally.

As noted before, many of the discussion centered around function and evolution of biological circuits. These topics were present in the talks (see section 3), but also in private discussions.

During the specially allocated discussion time, groups of varying size met to brainstorm about new ideas. One fruitful interdisciplinary interaction concerned protein and DNA interactions, and involved discussions between molecular biologists, physicists, chemists and control theorists. Specifically the group considered the analogies and differences between the chemical circuits used in bio-engineering (“molecular devices”) and the existing biochemical networks in cells. The workgroup led to experimental ideas that the participants will try to verify.

An exchange between participants interested in creating DNA switches in cells and theorists working on describing real noisy biochemical networks resulted in a discussion about how feedback can be encoded in molecular circuits. The portraying of this seemingly simple questions in the light of DNA switches led the participants to re-visit some basic assumptions made in molecular modeling. As a result, the experimentalist had new ideas that could be tested. This exchange also resulted in sharing existing literature among the theorists.

In the field of DNA switches, it became clear that many of the initial technical problems have now been solved. However to obtain well functioning molecular devices, it is now necessary to consider many

different ways of implementing feedback. The interactions between participants working on DNA switches from a chemical and control interaction background led them to establish international collaborations.

Similarly the workshop put in touch people interested in the relevance of spatial positioning of biochemical networks in pathways.

In the anonymous survey, the participants clearly claimed that the workshop led them to start new collaborations, discover new fields and point their research in novel directions. Since the survey was anonymous, we do not know the names of these participants, but we are excited to learn about these projects in a future meeting.

5 Outcome of the Meeting

The workshop emphasized recent improvements in the theoretical, computational, and experimental investigation of stochasticity at the cellular and nanoscale levels. Each of the participants at the meeting contributed to this progress in at least one, and in many cases two or three, of these advances. The workshop promoted cross-disciplinary communication and collaboration between researchers in mathematical fields such as stochastic processes, Markov models, stochastic simulation and information theory, engineering fields such as control theory, computer science, and circuit design, and scientific fields such as computational biology, nucleic acid research, biophysics, biochemistry, and nanotechnology. The event was highly successful in encouraging the development of a research community uniquely qualified to investigate the phenomenon of stochasticity in biochemical reaction networks.

In addition to presenting significant progress on the topics of stochasticity in biochemical reactions, the workshop also highlighted the persisting need for continued improvements in the analysis of such reactions. For example, combining new techniques for measuring spatial variability in cellular components with spatially non-homogenous analyses may yield new insights into cell regulatory behaviors. Similarly, the expanding usage of experimental techniques such as flow cytometry, time lapse fluorescence microscopy, and other techniques involving the use of fluorescent proteins leads to a demand of a much more quantitative characterization of these important proteins. Finally, with researchers from many diverse disciplines exploring stochasticity in the fields of synthetic and computational biology, a real need is arising for an improved and standardized toolkit for researchers to describe and computationally analyze cellular variability. These and other discussion topics that arose during the meeting will be revisited in the next workshop on stochasticity in biochemical reaction networks.

5.1 Open and Emerging Questions

Recent advances in experimental molecular biology have revolutionized the way people conduct biological research. Techniques such as flow cytometry, fluorescence activated cell sorting, time-lapse fluorescence microscopy, and microfluidics have made it possible for researchers to measure and manipulate the behavior of single cells and even single molecules within them. These experiments have shown that cellular dynamics are intrinsically noisy and that individual cells may both regulate and exploit this noise. To further understand the mechanisms of organism development, evolution, cancer, disease and drug efficacy, we must improve our understanding of the effects of noise on the corresponding biochemical reaction processes. Such explanations require the close integration of new mathematical models, techniques and theories with these emerging experimental techniques. An improved understanding of these systems will help explain newly observed phenomena and may suggest methods by which new behaviors can be engineered.

The main goal of the workshop was to suggest new research directions and new synergies between researchers in complementary fields within the main field of systems biology. As becomes clear from participant testimonies, the workshop fulfilled this goal. Many new directions that emerged from discussions have been summarized in sections 4 and 3. Here we present them from the point of view of the questions we asked before the workshop. The workshop was organized around a sequence of questions that begins and ends with experimental evidence:

- **What new experiments are possible and what can they tell us?** In the last few years, many of our participants have devised new experimental techniques to measure intracellular dynamics. Even in their infancy, the tools previously presented at BIRS in September 2009 have already improved our understanding of intrinsic and extrinsic noise biochemical reaction networks. During this workshop it became clear that there has been a shift from studying prokaryotic cells to developing tools to study eukaryotic cells, including mammalian cells. The experimental talks on biological cells can be divided into those that talked about techniques to describe noise in eukaryotic cells (e.g. Neuert,

Molina) and those that looked at the level of the whole organism (e.g. Boettiger, Shimizu, Meyer-Rojas). We note that many of the experimental results were presented with advanced statistical or theoretical analysis (e. g. Molina, Neuert, Shimizu), marking a significant shift in the field. The experimental advances presented at the workshop, including new, unpublished techniques offered the starting point of the workshop.

- **What are the available computational tools? How good do they need to be? What new mathematical approaches may be developed to meet these requirements?** The addition of stochasticity to gene regulatory network models severely complicates numerical analyses. Several of the workshop participants have pioneered new techniques for the analysis, reduction, and solution of stochastic processes in the context of gene regulatory networks and many are extending these results to treat spatially heterogeneous systems. Many efficient computational methods were presented in the context of the analysis of experimental data (e. g. Molina, Neuert). Others (Becker, Lilacci) pioneered new techniques to target spatially and temporally fluctuating environments.
- **How does noise affect cellular mechanisms? How do cellular mechanisms affect noise?** The signaling network in the cell is vast and only approximately known. Many of our participants have developed new ways to examine control, stability, robustness, adaptability, computation and information transfer under this highly uncertain setting. As noted above a key shift has been to try and link the observed stochasticity to function (e.g. Suel, Balazsi) or understand the dynamics of the response in fluctuating environments (e.g. Maheshri). Once again it is worth emphasizing that our participants discussed the role of precision of expression at the multicellular level, by means of both theoretical (Francois) and experimental (Boettiger) techniques. An interesting novel topic was considering the fluctuations in resources (Allen) and the spatial precision of events (Meyer-Rojas).
- **What sorts of synthetic biochemical processes can be designed and constructed?** As our computational and theoretical understanding of cell regulation improves, we can obtain more detailed quantitative characterizations of biochemical building blocks. Many of our workshop participants in synthetic biology used these "design principles" to build new organic constructs to perform specific biological and micro-mechanical tasks, both in gene regulatory (Egbert) and DNA-switch circuits (Franco, Rondelez).
- **What new experiments should we do?** Measurements at the single cell level are difficult, expensive and sometimes even disappointingly uninformative. One of the main objectives of this workshop was to suggest new approaches and collaborations to integrate stochastic modeling and experimental studies. This goal has been realized in a much wider range of interactions than we anticipated. Not only did experimentalists and computational researchers interact to build better data analysis software, but experimentalists and theorists discussed the prospect of tackling the newly emerging questions, such as the dynamics and spatial organization in cells.

5.2 Comments on the Workshop Organization and Logistics

Although other meetings had previously been organized to explore stochasticity in biochemical reaction networks, this workshop was unlike any other in the field. Upon conclusion of the workshop, it seems beneficial to discuss the particular items that made this a success. The key ingredients that set this workshop apart were (i) a multi-disciplinary and international organizing committee and participant list, (ii) an emphasis on young researchers and new ideas rather than tenured professors and established techniques, (iii) a flexible schedule with ample discussion time, and (iv) a specific focus on the integration of experimental and theoretical/computational investigations. Even though we adopted our title and location from two previous *Workshops on Stochasticity in Biochemical Reaction Networks*, these ingredients represented a significant and very successful shift in focus and organization. The original workshop was organized and attended almost exclusively by researchers connected to the control engineering community in the USA, whereas this workshop brought together a multi-disciplinary group of international researchers–including not only control engineers, but also physicists, chemists, mathematicians and biologists (including new organizers). Whereas the original workshops considered mostly theoretical and computational studies of small networks, this workshop emphasized the systematic integration of computational, theoretical and experimental techniques to investigate the interactions of cellular components at myriad length and time scales. Moreover, the five-day length of the workshop allowed much more time for discussion and collaboration than the breakneck pace of the previous two-day workshops.

As discussed above, one of the main goals of this workshop was to encourage collaboration between researchers from diverse fields, who often might not be aware of each others' research. The diverse participant list helped us to achieve this goal. The workshop participants included representatives of diverse

fields: chemists, engineers, control theorists, molecular biologists, physicists, mathematicians and computer scientists. These researchers also represented a diverse set of locations including US (19), Canada (4), Europe (12), Asia (1) and South America (1). Most of the participants were young: 14 are pre-tenure faculty members; 7 will have just started their faculty positions this year; and 6 are currently post-doctoral researchers. 7 women attended the workshop: five invited attendees and two organizers, all of them junior faculty (4), post-docs (1) or graduate students (2). This is a high ratio for research disciplines which include such male dominated fields as physics, computer science, engineering and theoretical chemistry.

We note that the ability to bring together such a young internationally diverse group of people was made possible by the award we received from International Complex Adaptive Matter Institute (ICAM). This award allowed us to subsidize the rather high travel costs of young faculty and post-docs, allowing many of them to attend. The Director of ICAM, Professor Daniel Cox, attended our workshop as a participant and acknowledged our success in achieving the goals of the institute to foster the exchange of scientific ideas.

5.2.1 Meeting Researchers From Diverse Communities

One of the major positive outcomes of the workshop was successfully bringing together people from extremely different backgrounds. Although many workshops invite people from different communities and create the potential for the exchange of ideas, our workshop was truly unique compared to other meetings we have attended. Unlike in other events, the participants exchanged ideas and points of view and engaged in discussion both during the sessions and during the plentifully designated discussion time. Due to the variety of backgrounds, not a lot of "basal" knowledge was shared by participants. We strongly encouraged our participants to ask questions, and *every single talk at the workshop elicited several questions and discussions*. This suggestion on one hand resulted in incredibly lively discussions. On the other hand it allowed for a bridging of the gap between the different backgrounds of participants. These prolonged introductions often led to the emergence of interesting questions (see section 4 for details). Frankly, the level of interactions, exchange of ideas and mixing of fields greatly surpassed the expectations of the organizers.

We believe that the unusually successful and creative atmosphere during our workshop is a combination of a number of factors:

- The wonderfully intimate facilities at BIRS, that forced people who did not know each other to interact (for example: eat together and engage in discussions).;
- The schedule of the meeting that left plenty of time for discussions and collaborations. For this reason people were not tempted to skip sessions to work, they paid attention during the sessions and could continue discussions privately after the sessions;
- Most of our participants were young faculty members - experienced researchers who are very open minded and curious about other fields.
- The strong emphasis and encouragement to ask questions.

To second the fact that this is not only our subjective impression, we quote samples of post-workshop surveys that specifically say this was the best conference people have ever attended.

To emphasize one of the main successes of the workshop we will cite the comment of one participant who met and started to collaborate with another participant - Rosalind Allen. After the week, this participant decided to spend his or her sabbatical visiting the newly found collaborator whom they previously did not know existed:

"Because of the workshop, I realized Rosalind Allen's research interests overlap with my own. Consequently, I am arranging to spend a sabbatical in Edinburgh next summer. That connection certainly would not have been made if not for the fortuitous arrangement of speakers."

Some additional quotes from participants:

"Through the workshop I've developed one potential collaboration with someone whose research I was unaware of before coming to Banff. ..."

"... I was able to establish collaborations with two different scientists during this event, which would have been extremely hard to achieve without such an intimate environment."

"The amply allocated discussion time allowed for deep communition with people whom I'm unlikely to have interacted with otherwise, and has led to three (or possibly more) concrete leads for future collaborations. This would not have occurred in a typical conference setting ..."

"I really enjoyed the feeling that we were on the cutting edge of defining the future course of this very new field of study."

"This workshop allowed work on a collaboration that includes two people from different European countries and one from the US."

"Time is the most valuable thing for people, and this workshop did a good job in allowing people to have just the right amount of time to meet and talk with each other, by organizing properly informal discussions slot (including meals)."

"This was the BEST workshop I ever attended. I learned deeply from topic that i believed to be out of my specific field but turned out to be very close. I feel this will mark a before/after point in my career."

"Very much like the emphasis on promoting the field in a collaborative way - this is exemplary!"

5.2.2 The Continued Demand for Such a Meeting

The views cited above (and the ones we did not have room to quote) clearly show that the most important outcome of the workshop was for people to meet, exchange ideas and start new collaborations, often with people whose research they were previously unfamiliar with. A large number of participants noted the importance of fostering a collaborative environment, the time and space to exchange ideas, and meeting other young researchers. Practically all participants emphasized the non-standard nature of this meeting (the large allocation of time to questions and discussions) as opposed to the many talks + posters format present elsewhere.

To illustrate the demand for such a workshop in the community, we can recall the large number of participants who were eager to apply to organize the subsequent meeting from this series. Finally four participants were chosen, from four different communities (molecular biology, physics, control theory and bioengineering), and we fully expect that future meetings will retain the kind of scientific diversity and energy that made this meeting a strong success.

6 Summary

We organized a workshop that aimed to bring together researchers studying different aspects of the emergent behavior of cellular networks. Despite extreme progress over the last decade, our understanding of how many cellular components, which interact in an intrinsically stochastic manner, come together and result in reliable outcomes of cell behavior is still in its infancy. The organization of our workshop allowed for free discussion between scientists who have been studying similar problems with very different tools. Although the large scale goal of understanding the complexity of cells (the space of possible output states, their relation to biological phenotypes and genotypes, the stability of these states, and their connectivity) is clear, the intermediate problems the community needs to solve are, in general, not obvious. This workshop made precise some missing links in our understanding of how cells function. By studying eukaryotic cells, we saw that many ideas the community considered to be resolved in prokaryotes are not at all simple in eukaryotic cells and at the multi-cellular level. The resulting main idea of the workshop is to call for a more holistic view of cells. At this stage of the development of the field it is essential to keep bringing together diverse young scientist to attempt to propose novel approaches to these problems. Our goal was to provide such a venue, introduce scientists from different fields to each other, and encourage informal discussions and strong, long-lasting collaborations. We feel we have been very successful in providing this platform for the exchange of ideas.

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