EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshop Scheme

Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF Exploratory Workshop on

GPCR Signalling Systems: A New Avenue For Drug Discovery?

Paris (France), 24-25 November 2009

Convened by:
Eric Reiter[1]

SCIENTIFIC REPORT

[1] Biologie et Bioinformatique des Systèmes de Signalisation (BIOS), UMR6175, PRC, INRA/CNRS/Université de Tours, 37380 Nouzilly, FR

1. Executive summary

G-protein-coupled receptors (GPCR) account for up to 50% of currently marketed drugs. However, the rate of new drug discovery using standard approaches, based on the detection of heterotrimeric G protein-dependent activities, is slowing down despite increased investments by the pharmaceutical industry. In the mean time, an impressive amount of detailed information has been gathered over the past decade on how external *stimuli* activate plasma membrane receptors, how they translate to the activation of downstream signalling cascades and eventually affect cell fate. More specifically, an increasing number of G protein-independent transduction mechanisms have been reported for GPCRs and the notion that GPCRs are able to activate very complex signalling networks is clearly emerging. It is now widely accepted that signalling pathways are organised as coordinated communication networks in which multi-protein complexes process and integrate the signal fluxes. This complexity commands a major effort to decipher principles of GPCR-induced cellular networks organization and their functioning across several spatial and temporal scales.

Importantly, new pharmacological concepts, such as the "multidimensionality of efficacy" are being developed based on the growing complexity of GPCR-induced signalling and open avenues for the development of "functionally selective" therapeutics with less side-effects.

The workshop, supported by the European Science Foundation examined the following questions:

i) Are coordinated Systems Biology approaches combining high-throughput data generation and computational modelling adapted to unravel the complexity and dynamics of GPCRs' signalling processes?

ii) Would a system-level understanding of GPCR-mediated signalling networks be a significant asset to rationalize and speed-up the discovery of pathway-selective drugs?

The workshop was held in Paris (November 24-25, 2009) and brought together experimentalists, pharmacologists and modellers. Recents advances in GPCR structure/activity, signalling, pharmacology and investigation methods, including some emerging high-throughput methods, were reviewed and discussed. Exemples of successful systems biology approaches applied to non-GPCR signalling networks' analyses were also presented. In addition, several state of the art computational modelling methods (including topological/qualitative and dynamical/quantitative ODE-based modelling) were examined and their respective strength and limitations compared.

Participants at the meeting agreed on three general conclusions. First, the implementation of systems biology approaches was acknowledged as a necessity in order to embrace the complexity of GPCR biology. Second, the emergence of pathway selective ligands for GPCR represents a major opportunity for the development of new classes of drugs. Their discovery would greatly benefit from system level understanding of GPCR-induced signalling pathways. And third, Europe can achieve a strategic positioning in the field by fostering a powerful and concerted research initiative on systems biology of GPCR signalling.

2. Scientific content of the event

The growing complexity of GPCR-induced signalling.

The GPCR-oriented presentations made during the workshop have generally illustrated that these receptors trigger multiple signalling pathways which lead to the formation of complex signalling networks. Examples were given of GPCR coupling to multiple G protein subtypes. But everybody agreed that there is more to GPCR signalling than the classical heterotrimeric G protein recruitment and activation, followed by the generation of diffusible second messengers such as cAMP (cyclic Adenosine Mono-Phosphate), calcium or phosphoinositides. Indeed, it was reported that many GPCRs directly interact with non-G protein signalling effectors through specific protein-protein interaction domains. A lot of emphasis was placed on two protein families specifically interacting with the majority of GPCRs in their active conformation: G protein-coupled receptor kinases (GRKs) and β-arrestins. Historically, GRKs and β-arrestins have been associated with the desensitization and internalisation/recycling of most GPCRs. But, recently, β-arrestins and GRKs have been shown to act as multifunctional scaffolds and activators for a growing number of signalling pathways. These aspects were covered by several talks presenting either cell culture or transgenic mice data.

Several presentations also pointed out receptor dimerization as an important source of complexity in GPCR signalling and pharmacology. As a high point, the existence of Luteinizing hormone receptor homodimers *in vivo* by trans-complementation in transgenic mice has been reported during the workshop, nicely illustrating the physiological relevance of GPCR dimerization.

Adding to this complexity is the fact that GPCR-induced signals can be spatially and temporally encoded. This point was highlighted during the workshop with dynamic data acquired using FRET sensor assays and other real-time imaging measurements in living cells.

Pathway-selective ligands for GPCR.

The notion that some GPCR-targeting drugs can selectively modulate a subset of the signalling events triggered by full agonists has been acknowledged by the attendees as a very promising opportunity to develop new drugs with less side effects and potentially new therapeutic indications. These ligands are often refered to as “biased”. Several examples of such biased ligands have been discussed during the course of the workshop. A conceptual framework about the structural basis for this phenomenon is emerging from the available crystal structures and has been proposed in a presentation. The idea is that many micro-conformations of an activated GPCR co-exist, some of them being preferentially stabilized by certain ligands. Each microconformation of a receptor can potentially trigger a different set of transduction mechanisms.

The added value provided by systems biology

In order to provide the attendees with concrete examples of systems biology approaches applied to cell signalling, several experts presented studies of non-GPCR mammalian signalling systems. The iteration between data generation and computational modelling has been emphasized as a necessity by most participants. Examples of model-driven predictions and validations have been given. Several modelling strategies and formalisms have been presented and compared.

As already stated above, GPCRs' complex signalling mechanisms probably lead to context-adapted cellular responses relying on emerging system-level properties that cannot be predicted from the individual components of the induced networks. In general, models could be used to better understand hypothesized mechanisms, run virtual (in silico) experiments, interpret data, suggest new drug targets, motivate experiments, and offer new explanations for observed phenomena. The consensus amongst the workshop attendees was that, indeed, a system-level grasp of GPCR-mediated signalling networks would speed-up the unravelling of GPCRs' biology and would be a significant asset to rationalize the discovery of new "pathway-selective" drugs. The goal would be to predict how an extra-cellular signal that activates a GPCR translates into a given biological or pathological response. This "global" level of analysis of GPCRs' biology is still in its infancy, since very few laboratories have invested in that direction so far. Moreover, to our knowledge, no large scale collaborative program is currently addressing this problem, not only in Europe, but also worldwide.

Challenges in high-throughput generation of signalling data.

The production of high quality biological data has become possible thanks to new experimental techniques that allow large scale accumulation of unbiased signalling data. Speeding up the production of new data and enhancing their quality is not only essential to feed model-building but also to allow testing of key model findings.

Signalling events are often propagated within the cell by post-translational modifications involving protein-protein interactions and enzymatic activities. Noteworthy, reversible protein phosphorylation is centrally involved in signal transmission within cells. The comprehensive and quantitative analysis of the protein phosphorylation patterns in different cellular backgrounds is therefore critical to reach a system level analysis of cell signalling. In particular, breakthroughs have been achieved in the isolation of phosphorylated peptides from complex samples, as well as in their analysis by mass spectrometry coupled to computational methods. Using such approaches, thousands of phosphopeptides and phosphorylation sites can now be identified in a single sample. In addition, the possibility to analyse the proteome using protein microarray-based methods has emerged. Automated spotting of concentrated and complex protein extracts permits their analysis with phosphospecific antibodies. These methods necessitate very small quantities of biological material, which allows a wide sample collection to be screened with a large panel of phospho-specific antibodies.

Approaches that use fluorescent sensors of signalling activities combine unmatched time and spatial resolution. Genetically encoded fluorescence resonance energy transfer (FRET)- or bioluminescence resonance energy transfert (BRET)-based reporters have been used in living cells to monitor the spatiotemporal patterns of diffusible second messengers, kinase activities and GPCR activation or dimerization. Protein-protein interactions can also be analysed in real time in living cells using either FRET or BRET. When used in multi-well plate format, both FRET and BRET-based methods ensure the production of huge amounts of high content dynamic data which are very well suited to feed systems biology approaches.

Challenges in system perturbation.

An important aspect when trying to decipher and model signalling networks is the ability to specifically apply perturbations and to measure the interactions of signalling pathways, considered in the recent past as isolated entities. In addition to classical approaches (e.g.: kinase inhibitors, dominant negative constructs,…), interfering RNAs offer the unique opportunity to specifically achieve gene knock-downs. A plateform performing genome-wide

siRNA screening either in multi-well liquid phase or in transfected cell array format has been presented. The participants recognized that large-scale siRNA screening may represent an extraordinary opportunity to unravel and reconstruct highly complex signalling networks.
It was also noted that, in the case of GPCRs, the use of already available pathway-selective receptor mutants and/or “biased ligands” offers great perturbation possibilities.

Challenges in data standardization.
High-throughput approaches generate huge quantities of heterogeneous data that cannot be handled with classical labbooks, or even flat files. Therefore, a necessary condition for successful modelling of GPCR signalling is to recognize the need for standards on data-collection and storage, interoperable representation as well as computational tools and standards enabling network analysis and modelling. Two important initiatives in this direction were presented during the workshop: i) ENFIN, a European Network for data integration (www.enfin.org) and ii) the Standards-based Infrastructure with Distributed Resources (SIDR) initiative (www.sidr-isb.eu).

Challenges in mathematical modelling.
Different approaches and formalisms can be used to model signalling systems. Computational models can be either static or dynamic. A first step is often to build influence graph between molecular species from the reaction/interaction graph. From the influence graph, temporal logics and model-checking algorithms have proven useful to express biological properties of complex biochemical systems and automatically verify if they are appropriate. Discrete Boolean approaches are useful to handle high-throughput data sets into very large models. Such approaches allow inferring informations about network structure. Bayesian-based methods can help evaluate the likeliness of different concurrent model structures.
However, considering the highly dynamic nature, both in time and space, of GPCR-induced signalling pathways, dynamical modelling approaches should yield more predictive power than statical approaches. Of course, a prerequisite to dynamical modelling is the generation of enough high quality dynamical data. Several dynamical modelling approaches exist, such as ordinary differential equations (ODEs, population view), Petri nets (discrete and independent mechanisms), and pi-calculus (stochastic approach). For all those methods, kinetics parameters (activation rates, probability of transition) are needed to simulate the changes in molecule concentrations over time. Frequently, many parameters are unknown. Non-linear optimization techniques are then needed to infer the unknown kinetic parameter values from the experimental data obtained under various conditions. Several techniques of parameter learning by data / property fitting can be used, such as gradient-based methods, Monte Carlo methods, and the Covariance Matrix Adaptation Evolution Strategy CMAES. Once the unknown parameters have been optimized, simulations can be performed and provide all component quantities over time. Simulations can be performed with different stimulation patterns or with in silico perturbations (modifications of total protein amounts, suppression of pathways, modulation of kinetics parameters…). Thanks to these perturbations, the robustness of the system can be appreciated.

3. Assessment of the results, contribution to the future direction of the field, outcome

The workshop gathered for the first time some of the best GPCR biologists/pharmacologists in Europe with proved leaders in systems biology and computational modelling. The general consensus, at the end of the meeting, was that indeed systems biology of GPCR signalling can have a positive impact on drug discovery and even becomes a necessity for several reasons: i) the complexity inherent to intracellular signal transmission and integration circuitry is becoming overwhelming; ii) GPCR biologists have to make sense out of exponentially increasing amounts of molecular-level data and are currently rather unprepared to meet the challenge; iii) it is now possible to selectively trigger subsets of GPCR-induced signalling pathways with so called “biased” ligands. Identifying these ligands and predicting their biological outcomes represent a major opportunity for drug discovery.

The following preliminary road-map arose from the discussion:
One major question identified: deciphering “biased” signalling and signal “channelling” processes by modelling and predicting the relationship between ligand nature, conformation of activated receptors, activation of intracellular pathways and biological outcomes. Rather than focusing on one single case study, it should be more interesting to address this question for different GPCR and in different cellular contexts, in order to identify the existing commonalities and specifities.
Data to be generated: rate constants, interacting proteins, phosphoproteome, stoechiometry of components, integrated cellular responses (eg: proliferation, differenciation, apoptosis, gene expression,…). Ideally, these data should be quantitative, dynamical, take subcellular localization into account. Control and perturbed conditions should systematically be compared. Data should be standardized (through standardized procedures or plateforms?), stored, organized (curated) and shared through common repository (it is important to share negative data as well!).
Model building: adapted modelling procedures should be applied to integrate the available data. Large qualitative models will be constructed. Smaller focused dynamical models will be built and parameterized.
Model refinement and validation: an iterative dialog between model driven prediction and experimental validation is an absolute requirement. It is also important to validate the model prediction into physiologically relevant models. Transgenic mice models offer a unique opportunity to i) specifically perturb signalling *in vivo* and ii) test “biased” ligands in disease models.

The workshop participants came to the conclusion that an efficient network needs to be established with the following objectives:

- to organize follow-up workshops/conferences on a 1 per year basis to foster the establishment of integrated research program(s) on systems biology of GPCR signalling.
- to enlarge the current core of participant to other interested European scientists involved in either GPCR biology or Systems biology.
- to prioritize objectives, scientific questions and models.
- to inform National as well as European research institutions that systems biology of GPCR signalling is a timely question with strategic implications.
- to convince pharmaceutical companies that they can also find an added value to participate in collaborative networks on this topic

These objectives will be ideally met by an ESF networking programme or a Cost action, the participants agreed to head for an application to establish a network starting in year 2011.
Other funding sources will be sought after in order to organize a conference in 2010 and keep the momentum generated by this first workshop.
The networking activities should allow us to come up with a well structured consortium for an application on systems biology area in FP7.

4. Final programme

Tuesday 24 November 2009

13.00-14.00	**Arrival of the attendees**
14.00-14.15	**Welcome by Convenor** **Eric Reiter** (INRA/CNRS/Université de Tours, Nouzilly, France)
14.15-14.30	**Presentation of the European Science Foundation (ESF)** **Pilar Perez** (ESF Standing Committee for Life, Earth and Environmental Sciences, LESC)
14.30-16.30	**Session 1: Systems biology of cell signaling (I).**
14.30-14.45	**Presentation 1 "Spatiotemporal dynamics and signalling specificity of RTK networks"** **Boris Kholodenko** (University College Dublin, Dublin, Ireland)
14.45-15.00	**Presentation 2 "Modelling approach to the spatio-temporal organization of Ca2+ signalling"** **Geneviève Dupont** (Université Libre de Bruxelles, Brussels, Belgium)
15.00-15.15	**Presentation 3 "Systems Biology of the ERK signalling network"** **Walter Kolch** (University College Dublin, Dublin, Ireland)
15.15-15.30	**Presentation 4 "Modelling the molecular mechanism of circadian clocks and related disorders of the sleep-wake cycle"** **Jean-Christophe Leloup** (Université Libre de Bruxelles, Brussels, Belgium)
15.30-15.45	**Presentation 5 "Mathematical modeling and systems theoretic analysis of cell signaling"** **Frank Allgower** (University of Stuttgart, Stuttgart, Germany)
15.45-16.00	**Presentation 6 " Methods for in silico exploration of the cell interactome "** **Anne Poupon** (INRA/CNRS/Université de Tours, Nouzilly, France)
16.00-16.15	**Presentation 7 "High-Content RNAi Screening"** **Holger Erfle** (Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany)
16.15-17.15	**Discussion**
17.15-17.45	*Coffee / Tea Break*
17.45-20.30	**Session 2: Emerging concepts and approaches in GPCR signalling (I).**
17.45-18.00	**Presentation 1 "Our embarrassing lack of understanding structural basis for affinity not to mention efficacy in 7TM receptors"** **Thue Schwartz** (University of Copenhagen, Copenhagen, Denmark)
18.00-18.15	**Presentation 2 "Increasing complexity of GPCR complexes and signaling networks"** **Marc Parmentier** (Université Libre de Bruxelles, Brussels, Belgium)
18.15-18.30	**Presentation 3 "GPCR crosstalk: is it all about dimerization?"** **Rob Leurs** (Vrije Universiteit Amsterdam, Amsterdam, The Netherlands)
18.30-18.45	**Presentation 4 "Receptors as moving targets"** **Michaël Freissmuth** (Medical University of Vienna, Vienna, Austria)
18.45-19.00	**Presentation 5 "Evidence for in vivo function of GPCR homodimers"** **Ilpo Huhtaniemi** (Imperial College London, London, United Kingdom)
19.00-19.15	**Presentation 6 "Understanding the complexity of dopamine receptor regulation and signaling in vivo"** **Raul Gainetdinov** (Italian Institute of Technology, Genova, Italy)

19.15-19.30 **Presentation 7 "Activation of oncogenic signaling networks by (viral) GPCRs"**
Martine Smit (Vrije Universiteit Amsterdam, Amsterdam, The Netherlands)

19.30-20.30 **Discussion**

20.30 *Dinner*

Wednesday 25 November 2009

08.30-10.10 Session 3: Emerging concepts and approaches in GPCR signalling (II).

08.30-08.45 **Presentation 1 "Temporal and spatial analysis of signaling steps of GPCRs"**
Martin Lohse (University of Würzburg, Würzburg, Germany)

08.45-09.00 **Presentation 2 "G-protein coupled receptors (GPCRs): a complex signalling world"**
Joël Bockaert (CNRS, Montpellier, France)

09.00-09.15 **Presentation 3 "GRKs: interactome and novel cellular functions"**
Federico Mayor (Universidad Autónoma Madrid, Madrid, Spain)

09.15-09.30 **Presentation 4 "Assymetric signalling of dimeric Class C GPCRs"**
Jaroslav Blahos (Charles University in Prague, Hradec Kralove, Czech Republic)

09.30-09.45 **Presentation 5 "Functional interactions of GPCR dimers and oligomers"**
László Hunyady (Semmelweis University, Budapest, Hungary)

09.45-10.10 **Discussion**

10.10-10.40 *Coffee / Tea Break*

10.40-12.20 Session 4: Systems biology of cell signaling (II).

10.40-10.55 **Presentation 1 "Systems Biology of ERBB signaling and miRNAs for targeted breast cancer therapy"**
Özgür Sahin (German Cancer Research Center, Heidelberg, Germany)

10.55-11.10 **Presentation 2 "Metadata Generation, annotation and Management: the SIDR Initiative"**
Magali Roux (CNRS, Nancy, France)

11.10-11.25 **Presentation 3 "ENFIN - a European Network for data integration"**
Pascal Kalhem (European Bioinformatics Institute, Hinxton, United Kingdom)

11.25-11.40 **Presentation 4 "A computational method based on temporal logic for parameter search and robustness analysis of dynamical models of biological processes"**
François Fages (INRIA, Paris-Rocquencourt, France)

11.40-11.55 **Presentation 5 "Current methods for the modelling and inference of signalling pathways"**
Achim Tresch (Ludwig Maximilians University, Munich, Germany)

11.55-12.20 **Discussion**

12.20-14.00 *Lunch*

14.00-16.30 Round table

14.00-15.00 **The following questions will be discussed:**

Can systems biology have an impact drug discovery? What GPCRs, what cells, what models, what read outs, what can we predict? How can we best share data and resources?

15.00-16.30 **Discussion on follow-up activities/networking/collaborations**

16.30 *End of Workshop and departure*

5. Final list of participants

Convenor:

1. **Eric REITER**
   INRA/CNRS/Université de Tours,
   Nouzilly, France

ESF Representative:

2. **Pilar PEREZ**
   Consejo Superior de Investigaciones Científicas (CSIC),
   Universidad de Salamanca Edificio Departamental,
   Salamanca, Spain

Participants:

3. **Frank ALLGOWER**
   University of Stuttgart,
   Stuttgart, Germany

4. **Jaroslav BLAHOS**
   Charles University in Prague, Hradec Kralove, Czech Republic

5. **Joël BOCKAERT**
   CNRS UMR 5203,
   Institut de Génomique Fonctionnelle,
   Montpellier, France

6. **Geneviève DUPONT,**
   Université Libre de Bruxelles,
   Brussels, Belgium

7. **Holger ERFLE**
   Ruprecht-Karls-Universität Heidelberg,
   Heidelberg, Germany

8. **François FAGES**
   INRIA Paris-Rocquencourt,
   Le Chesnay, France

9. **Michael FREISSMUTH**
   Medical University of Vienna,
   Vienna, Austria

10. **Raul GAINETDINOV**
    Italian Institute of Technology,
    Genova, Italy

11. **Ilpo HUHTANIEMI**
    Imperial College London,
    London, United Kingdom

12. **László HUNYADY**
    Semmelweis University,
    Budapest, Hungary

13. **Pascal KALHEM**
    European Bioinformatics Institute,
    Cambridge, United Kingdom

14. **Boris N KHOLODENKO**
    University College Dublin,
    Dublin, Ireland

15. **Walter KOLCH**
    University College Dublin,
    Dublin, Ireland

16. **Jean-Christophe LELOUP,**
    Université Libre de Bruxelles,
    Brussels, Belgium

17. **Rob LEURS**
    Vrije Universiteit Amsterdam,
    Amsterdam, The Netherlands

18. **Martin J LOHSE**
    University of Würzburg,
    Würzburg, Germany

19. **Federico MAYOR**
    CSIC-Universidad Autónoma Madrid,
    Madrid, Spain

20. **Marc PARMENTIER**
    Université Libre de Bruxelles,
    Brussels, Belgium

21. **Anne POUPON**
    INRA/CNRS/Université de Tours,
    Nouzilly, France

22. **Magali ROUX**
    INIST-CNRS,
    Vandoeuvre-lès-Nancy Cedex, France

23. **Thue SCHWARTZ**
    University of Copenhagen,
    Copenhagen, Denmark

24. **Özgür SAHIN**
    German Cancer Research Center,
    Heidelberg, Germany

25. **Martine J SMIT**
    Vrije Universiteit Amsterdam,
    Amsterdam, The Netherlands

michael.freissmuth@meduniwien.ac.at

26. **Achim TRESCH**
    Ludwig Maximilians University,
    Munich, Germany

6. Statistical information on participants

Total of 26 attendees (including the ESF representative)

Age structure:

30-39: 3
40-49: 10
50-59: 9
> 60: 3

Gender repartition:

Female: 5
Male: 21

Countries of origin:

Germany: 5
France: 5
Belgium: 3
United Kingdom: 2
The Neterlands: 2
Ireland: 2
Spain: 2
Austria: 1
Czech Republic: 1
Denmark: 1
Hungary: 1
Italy: 1

Scientific background:

Experimental biologist: 16
Mathematics/informatics: 7
Both: 3