Searching for the Holy Grail; protein–protein interaction analysis and modulation

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The first EMBO workshop on 'Protein–Protein Interaction Analysis & Modulation' took place in June 2012 in Roscoff, France. It brought together researchers to discuss the growing field of protein network analysis and the modulation of protein–protein interactions, as well as outstanding related issues including the daunting challenge of integrating interactomes in systems biology and in the modelling of signalling networks.

After more than a decade spent struggling to discover what features of a protein are 'druggable', the modulation of protein–protein interactions (PPIs) has emerged from both academic and corporate research as a promising therapeutic strategy to treat human disease. As such, PPI modulators (PPIMs) are the next generation of highly innovative drugs that will reach the market. What better place to discuss this controversial newborn field—and other proteomic-related topics—than the impetuously windy region of 'Finistère'—literally 'end of the earth' in French. In the meeting's remarkably intense atmosphere, state-of-the-art presentations were often followed by quasi-philosophical discussions at the end of each session, addressing topics such as the validity of targeting 'transient' and weak protein complexes compared with 'obligate' and strong complexes. The two topics we have chosen to highlight in this meeting report—PPIs in system biology and PPI modulation—were main focuses of the workshop.

PPIs in systems biology

Discovering protein–protein interactions. At this first EMBO conference on PPIs, it was inspiring to see the evolution and implementation of innovative methodologies, as well as to appreciate the emerging dynamics of single PPIs and their networks of interaction.

Two main approaches were described to study PPIs at a systems level. The first of these is the ability to discover PPIs by

using sophisticated mass-spectrometric approaches that aim to dig deeper into the proteome by using 'data-independent' and label-free ion detection approaches. The second is the continued use of the high-throughput yeast two-hybrid system. However, a main disadvantage of the yeast two-hybrid system is that it analyses PPIs in a non-physiological context. To address this drawback, Jan Tavernier (VIB, U. Gent, Belgium) reviewed advances and artefacts associated with screens performed by using the MAPPIT system to conduct mammalian

cell two-hybrid assays, which allows the regulation of a specific PPI to be studied within the endogenous cellular environment. Further developments of this synthetic engineering approach will probably yield more physiological PPI data of potential therapeutic relevance in the future.

The actual nature of PPIs was a recurring theme throughout the workshop. Toby Gibson (EMBL, Heidelberg, Germany) highlighted the intrinsic structural disorder present in many proteins, which provides an information-rich series of linear motifs that form 'weak', dynamic, reversible but specific PPIs [1]. This developing view represents a paradigm shift in the protein science field: that many proteins are thermodynamically unstable and are composed of a large degree of intrinsic disorder. This 'additional' property of a PPI—disordered and with linear motifs [2]—might explain how so many regulatory proteins can bind to numerous proteins, and how small PPI interfaces and allosteric properties can form the basis for drug-discovery programmes. This is also an incentive to dig deeper into protein transduction network data to reveal hidden information.

Ted Hupp (Edinburgh Cancer Research UK Centre, UK) highlighted the use of phage peptide-library screens to identify new PPIs involving the MDM2 ubiquitin ligase. These screens depend on ligand-induced conformational changes that give rise to peptide motifs that reflect a 'consensus' interactome

for a target protein. The growing use of next-generation sequencing of pooled phage libraries makes it possible to develop high-throughput protein interaction screens on the basis of conformational isoforms, thus complementing yeast two-hybrid screens to reveal potentially druggable PPI interfaces.

Discovering protein–protein interaction networks. Two challenging questions emerged from the insights on the nature of PPIs presented at the meeting. First, at the mechanistic level, the question of how regulatory interactions determined by linear motifs are rewired in diseases such as cancer is challenging to answer. In this vein, Rob Russell (U. Heidelberg, Germany) discussed his lab's work to refine the use of flexible and highly specific linear domains, such as the diverse structural scaffolds present in WD40 modules, to develop new PPI interface motifs [3]. He also underlined the importance of improving open access informatics-based mining of new PPI interfaces to advance research (for example, <http://pcidb.russelllab.org> and <http://pepsite2.russelllab.org>). This insight into the role of the linear motif in driving regulatory PPIs can be extended to informatics-based systems analyses, to predict new disease-causing PPIs, by linking large genomics to protein informatics.

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In addition to studying the fundamental role of linear domains in rewiring or shifting the equilibrium of PPIs in disease, a second question emerges: at the therapeutic level, which of the many PPIs in large disease-causing networks are actually 'druggable'? One answer might come from a previous pioneering 'systems biology' approach [4] that used mass spectrometry to map the breadth of the ATM/ATR kinase interactome. The study highlighted that this signalling hub has more than 700 interacting proteins, and that genetic depletion of many of them can lead to defects in the checkpoint response. This suggests that targeting either the ATM or its effectors would have the same radio-sensitizing phenotype and, if true, opens up the potential to drug many more PPIs at the edges ('edgetics'). Indeed, Luis Serrano (CRG, Barcelona,

Spain) described heroic efforts to construct a systems biology level quantification of large numbers of regulatory proteins. He stressed that small changes in adaptor protein concentrations can have a significant impact on cell signalling and feedback loops. His work forms a blueprint for future studies aimed at trying to annotate dominant druggable PPIs.

Matthias Gstaiger (ETH Zurich, Switzerland) described systematic approaches to define CMGC kinase superfamily interactomics and defined the range of inter-laboratory reproducibility, a major limiting factor of high-throughput 'omic' technologies. One of the main insights from these studies is that CMGC kinases cluster with other kinase family members, suggesting that kinase hubs could partly explain the unanswered problem of how the multi-site phosphorylation of a given protein spatially occurs in a cell. The exploration of allosteric sites as new PPI modulators represents an exciting new landscape in kinase signalling. The intrinsic interdependence of proteins in networks suggests that pharmacological exploitation can be achieved at any point surrounding a hub.

PPI modulation

Target selection and druggability. To improve the quality of target selection, more needs to be discovered about PPI networks and their weak points. Gene products do not function in isolation, but operate within highly interconnected 'interactome' networks, modelled as graphs of nodes and edges representing macromolecules and the interactions between them, respectively. The ability to modulate these 'edgetics' in a particular signalling pathway is the key to innovative drug discovery for patients. Anne-Claude Gavin (EMBL, Heidelberg, Germany) illustrated this idea by presenting an unpublished screen of protein–lipid interactions in yeast by using lipid arrays, which provided an excellent resource to enhance our understanding of lipid signalling in eukaryotic systems, and allowed us to decipher the molecular mechanisms behind cellular processes. To illustrate the biological value of this data set, she further studied several new interactions involving sphingolipids, a class of conserved bioactive lipids with an elusive mode of action. She presented the integration of outstanding live-cell imaging, which suggested new cellular targets for these molecules, including several with pleckstrin homology (PH) domains.

Pascale Zimmerman (Catholic U. Leuven, The Netherlands & Centre de Recherche en Cancérologie de Marseille, INSERM, France) presented a screen of the human proteome for phosphoinositide (PtdInsP)-interacting PDZ domains that used a combination of *in vivo* cell-localization studies, *in vitro* dot blot and surface plasmon resonance experiments involving synthetic lipids and recombinant proteins. Her screen substantially expands the set of PtdInsP-interacting PDZ domains, and demonstrates that a full understanding of the biology of PDZ proteins requires comprehensive insight into the intricate relationships between PDZ domains and their peptide and PtdInsP ligands.

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Expanding on our ability to define 'interactomes', Vincent Lotteau (Inserm U851, Lyon, France) presented the first comparison of five virus–host interactomes (the Infection Mapping Project, IMAP and the VINLANDdb—unpublished data) by using yeast two-hybrid screens. His laboratory has robust data demonstrating that viruses have a core set of targets (three times more protein partners on average compared with cellular proteins)—the 'virosthome'—that take part in the cellular response to infection, which they use to hijack the cell machinery for their own survival strategy. Common cellular targets and pathways could thus be used to identify broad-spectrum antiviral drugs. Overall, the ability to target virus–host interactions would considerably broaden the landscape of drugs that could be developed in the near future.

Success stories. Over the past decade, the modulation of PPIs has emerged as a new way to control the activity of proteins. Global research efforts have driven pharmaceutical successes, which have led to the embrace of PPIs as drug targets by the scientific community [5, and references therein]. PPIMs can be subdivided into two main classes: orthosteric and allosteric. Orthosteric PPIMs interfere directly with the interface of the protein complex, whereas allosteric PPIMs bind away from the interface and cause or prevent conformational changes that preclude formation of the complex.

The dynamic view of allostery as an unexplored approach to drug discovery was discussed by Mike Tyers (U. Edinburgh, UK), who described a stunning example of the use of complex phenotypic cell-based screens to identify new chemical inhibitors of E2 ubiquitin ligases. This class of proteins has been considered undruggable because its members do not contain the 'classical' pockets necessary to develop specific drugs. Tyers presented crystallographic studies of an E2-inhibitor complex revealing that the inhibitor forms a binding site through allosteric changes in E2 that were not obvious from the apo-E2 structure [6]. The possibility to allosterically drug the E2 superfamily would be as revolutionary as was the realization that protein kinases can be targeted by exploiting their ATP pocket.

PPIs and chemical space. The success of experimental screening techniques used to identify innovative molecules depends on both the specificity of the input—that is, the quality of target selection—and the chemical libraries that are used to find the 'nugget'. The latter have clearly demonstrated their limitations for discovering PPIs and their improvement represents an exciting challenge for the chemoinformatics community during the next decade.

John P. Overington (European Bioinformatics Institute, Hinxton, UK) emphasized this challenge, as he has already partly addressed it through the development of the ChEMBL database (<https://www.ebi.ac.uk/chembl/>), which contains around 1.3 million compound structures and their associated bioactivity and interaction data, 12,000 actual clinical candidates and 1,500 approved drugs. This database also indicates

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all drug approvals with new codes tagged by a symbol system—for example, cyclization, truncation, disulphide, unnatural or natural, and natural orthologues.

Such database developments coupled with original algorithms, for example, the '2P2IHUNTER' support vector machine (<http://2p2idb.cnrs-mrs.fr>) presented by Xavier Morelli (Cancer Research Center of Marseille, CNRS UMR7258, France)—a new generation of PPI-focused libraries—increase the likelihood of finding efficient compounds within reasonably sized libraries. Morelli's team highlighted the properties of PPIMs for a subset of identified inhibitors with binding modes validated by three-dimensional structure and presented the 'rule of 4' (Ro4) as a guideline for PPI inhibitor development.

The future

In conclusion, the conference gave a good account of the massive amounts of data that are or will soon be available to the scientific community to better discover, understand, validate and target protein–protein interactions related to human health and diseases. Pierre Colas (INSERM, Station Biologique Roscoff, France) summarized the fundamental questions in the PPI field including: (i) what is the nature of a PPI; (ii) when do we consider a PPI network to be 'complete'; (iii) how can we integrate the different disciplines—by using combinatorial synthetic protein modules, computational science,

structural biology and the concept of allostery, 'disorder' and 'fuzziness'—to improve our simplistic view of how networks produce phenotype; (iv) as PPIs are emerging as compelling drug targets and are increasing the chemical space available for pharmacological intervention, how do we decide if a PPI or a key PPI within a hub is 'druggable'? These challenges will drive innovation forward to further exploit PPIs as a rich source of information for the future, and will certainly be discussed in a second 'PPI and Modulation' meeting to be held in 2014.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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