Functional Proteomics – From Protein Pathways to Drug Development

a report by
Hybrigenics SA

Genomics research – the study of the genome – has deepened the pool of potential drug targets. A major hurdle for drug development, however, is to identify rapidly what target proteins are the most appropriate to take into further development. Knowing what proteins are expressed and how – the field of proteomics – is therefore the first step to generating value from the knowledge of the human genome. Since, in the cell, proteins do not act in isolation but rather in complex pathways or cascades of protein-protein interactions, the key to drug development is the understanding of how these proteins interact with each other – the field of functional proteomics.

Moreover, inappropriate protein interactions within a cell have been shown to play an intrinsic role in disease mechanism. Elucidating how a target protein associated with a disease interacts with other proteins will give a better insight into such a mechanism. Maps showing all the relevant protein interactions in pathological cells can support decisions about the best possible targets to treat a disease. Ideally, the best target would influence the pathway of therapeutic benefit but would neither induce side effects nor toxicity through interference with other biochemical pathways.

Mapping protein-protein interactions is therefore emerging as a highly coherent method for understanding the functions of the proteins encoded in the human genome, for identifying better therapeutic targets and for generating therapeutics from the data.

What Protein-protein Interactions Maps Offer

The failure of candidate compounds in the conventional pharmaceutical approach of the drug discovery and development process is often noticed in clinical phase when millions of dollars and significant resources have already been invested. This is partly due to a lack of knowledge of the biological environment of the target proteins on which the development programmes are focused. Identifying which disease-associated targets are the most appropriate for further validation and development for therapeutics is a vital component in reducing the time and cost of drug development.

Considering that a better understanding of the protein environment is critical to achieving more precise target identification and validation, Hybrigenics has developed an original approach. The first step of this approach is to 'expand the box', i.e. to put knowledge of the target into perspective of the functional cellular pathways that the target is involved in. The outcome of expanding the box is the determination of protein interactions maps (PIMs®), in which each biochemical pathway is elucidated, thus revealing different potential targets along the pathway. With knowledge of all the targets in any given disease-related pathway therefore elucidated, it is possible to determine which would be the most appropriate to trigger a pharmaceutical effect.

The PIM® database is accessible through a powerful bioinformatic platform – PIMRider® – that optimises the display and the analysis of PIM®. It includes useful bibliographic links to the human genome, specialised databases, description of sequences and structure of proteins and represents the data in a directly exploitable format.

The information in a PIM® database can be used to identify the druggable targets and prioritise them for further evaluation. For example, a partner may have identified three potential targets that are known to be associated with a specific disease. The generation of a map will give substantially more information about these targets. One of the targets may interact with proteins in a pathway known to be involved in toxicity or side effects, therefore making an unsuitable drug target. Another may also be involved in a distinct pathway irrelevant for the disease process and its treatment so if targeted with a small molecule may induce unwanted side effects. However, the third may only interact with the desired pathway associated with the disease and play no role in any other pathway, thus making it the most suitable target for further therapeutic development.

In addition to toxicity or side-effects prediction, PIM® can show the non-linear nature of a biochemical pathway. If there is more than one route along a disease-associated pathway it is possible to

The Technology Behind the Map – Integration of Biotechnology and Bioinformatics

Hybrigenics creates its protein-protein interaction maps (PIM®) using an extremely efficient new generation of high-throughput yeast two hybrid (Y2H) screening that overcomes the drawbacks of other Y2H applications. A plasmid encoding a target of interest – the 'bait' – is inserted into one yeast cell. This cell is mated in an extremely efficient way with a series of yeast cells, each containing different plasmid fragments – the 'prey' – from a proprietary complex library of over five to ten million fragments. Mated cells are cultured in a selection medium, allowing cell growth only where the proteins, transcribed from the bait and prey, have interacted and hence showing which proteins are interacting. The preys are then selected through a stringent selection pressure that measures the affinity of interactions identified. Some of these preys can then be used as baits to iterate the process and generate new insight in the interaction cascades. This method has a high level of reproducibility.

The large number of fragments in Hybrigenics's library is key to alleviating false negatives. As a given protein is represented in the library numerous times, following mating of the yeast cells, the bait protein will interact with many prey protein fragments. This confirms and reconfirms the interaction, reducing the probability of a false negative.

Hybrigenics's powerful bioinformatics enables the computation of the precise domain of a target that is interacting with other proteins. Again, this is because the relevant 'active' domain of a target is represented in the library as numerous, different-sized plasmid fragments. The SID® fragment is the part shared by all the prey fragments found for an interaction.

To reduce the number of false positives, Hybrigenics has generated a unique confidence index – PBS® – that reveals how genuine the interactions are. This is a scale of relevance, not affinity, that grades the interactions from A (not random, high biological relevance) to E (false positive, 'sticky').

Hybrigenics has developed a unique powerful proprietary bioinformatics platform called PIMBuilder® that integrates, stores and organises data obtained in screening experiments, interfaces with robotics and ensures strict quality control. Data from other sources is also included, such as information on sequence, structure and family groupings of all the proteins. These results are available in a fully annotated database delivering complete information in an exploitable format that is accessed through PIMRider®, a software interface that allows complete navigation inside the map. PIMRider® for *Helicobacter pylori*, Hepatitis C virus, and HIV host cell (data from literature) is currently available for subscription.

determine a chosen target, even if it is known to have an affiliation to a disease, as it will be bypassed and antagonised when modulated with a drug. This may explain the discrepancy between pharmacological effect induced by the drug at the level of the target and the overall therapeutic benefit of this drug.

Beyond Protein-protein Interaction Mapping – Dissecting Biological Pathway, Validating Targets and Selecting Drug Candidates

Once the pathways are mapped, these need to be analysed and validated functionally in a biological model. In order to determine the validity of these specific targets, Hybrigenics takes advantage of a unique feature resulting from its screening technology, i.e. the specific interacting domain (SID®) computed for each protein-protein interaction. Hybrigenics exploits the ability of these domains to block protein-protein interactions and use them as a 'molecular scalpel' for dissecting pathways.

Pathways of interest can be disrupted at various positions and respective phenotypes can be observed. In a cellular assay, the SID® fragment acts as a competitive inhibitor, blocking the selected target and therefore preventing it from interacting with its real protein partner. If the selected target no longer interacts with its protein partner and the pathway cascade is terminated then a phenotypic change of the cell will be observed.

Beyond drug target validation, it should also be possible to use SID® in the chemical compound selection. In this case, SID® would be used as a control in chemical compounds screening assays. Compounds inducing an identical phenotypic change than that observed when SID® is used to disrupt pathways could be considered as potentially active on one or several proteins in the targeted pathway.

Advantages and Benefits

Hybrigenics has developed a technology platform for protein-protein interactions screening that provides a better understanding of the genome-wide biology. The reliability and comprehensiveness of the screening enables access to novel information that is difficult to observe with other technologies.

Hybrigenics has also developed efficient tools that provides users with quantifiable decision criteria for target prioritisation. For instance, predicted biological score (PBS®) that allows the ranking of interactions found according to their biological relevance and SID® fragments that enable functional cellular validation of selected targets.

The information arising from this validation provides a prediction of the potential therapeutic effects of the targets that can help pharmaceutical partners make the choice of the more appropriate targets to study further.

The key benefits of Hybrigenics's technologies for a biopharmaceutical partner are twofold. First, a significantly improved target identification and validation decision support that turns into risk reduction, time shortening and money sparing and, second, an innovative way to build, reinforce or defend intellectual property position.

Existing Applications and Perspective

Hybrigenics currently has several drug target identification and validation programmes in areas of therapeutic importance, such as infectious diseases (bacterial and viral), cancer and various metabolic diseases (obesity and diabetes). Some of these programmes are partnered with biopharmaceutical companies or leading scientific teams of renowned academic institutes.

Anti-infectious Programmes

Hybrigenics is conducting programmes on several pathogens, such as *Helicobacter pylori*, gram-positive bacteria and viruses. The company has a strategic alliance with Institut Pasteur (Paris, France) to strengthen scientific expertise in the field of infectious diseases.

As recently highlighted in *Nature*[1], the company mapped the protein-protein interactions of *Helicobacter pylori*. This was the first published bacterial PIM® and it clearly validated the technology. The corresponding PIM® database is currently available through PIMRider® for a free subscription to academic scientists.

Other on-going projects are focused on the identification and the validation of potential drug target candidates in *Staphylococcus aureus* and *Streptococcus pneumoniae*.

As far as viruses are concerned, Hybrigenics also mapped the interactions occurring between the Hepatitis C virus (HCV) polypeptides. The corresponding PIM® database is available through subscription licence. The company is now mapping the interactions between virus and host cells. Studying such interactions is a novel approach and should lead to new insights in antiviral drug discovery. Other activities in the field of HCV include a partnership with XTL Biopharmaceuticals Ltd (Rehovot, Israel), aimed at selecting and developing small molecules therapeutic compound candidates.

Concerning Human Immunodeficiency Virus (HIV) projects, Hybrigenics has compiled and integrated all the protein interaction information known and available in the scientific literature about the HIV and its host cell. This PIM® database is also available for subscription. The company is now building the map of the interactions occurring in HIV as well as the ones between HIV and its host cell. Part of this programme was supported by l'Agence Nationale de Recherches sur le SIDA (ANRS) (French AIDS agency). Studying such interactions is a novel approach and should lead to new insights in antiviral drug discovery.

Metabolism Programmes

In the field of metabolic diseases, Hybrigenics is conducting a project of target identification and validation in obesity. Part of this programme is conducted in collaboration with Lynx Therapeutics, Inc., Hayward, California, that recently led to the discovery of a first drug target candidate involved in adipogenesis – a key specific mechanism in the development of obesity.

Cancer Programmes

In the field of cancer, Hybrigenics has several on-going projects, focusing on pathways of major interest such as cell cycle related pathways for discovery of novel anticancer therapeutics. One of these projects, which targets an undisclosed pathway, is sponsored by the pharmaceutical company Servier (Paris, France). Hybrigenics has also entered into a

1. *J C Rain, L Selig, H De Reuse, V Battaglia, C Reverdy, S Simon, G Lenzen, F Petel, J Wojcik, V Schächter, Y Chemama, A Labigne and P Legrain (2001), "The protein-protein interaction map of Helicobacter pylori",* Nature *,409, pp. 211–215.*

strategic collaboration with Institut Curie (Paris, France) to strengthen scientific expertise in the field of cancer.

Partnering Opportunities at Hybrigenics

The future application of Hybrigenics's technology platform is vast and offers various opportunities for partnership in drug target discovery and validation to the biopharmaceutical industry. It is possible to map, analyse and validate the biological relevance of any drug target related cellular pathway in any disease area. Partnerships can be related either to technology platform-oriented programmes or driven by in-house programmes.

In technology platform-oriented programmes, there is no restriction in terms of disease area. Basically, Hybrigenics will expand the box around the partners' selected targets and dissect the network of protein interactions that they are involved in. A specific case of interest is to apply Hybrigenics's technology to revitalise programmes shelved because of severe side effects and toxicity. In that case, PIM® may allow the identification of safer target candidates in the pathway of interest.

The other opportunity for pharmaceutical companies to benefit from Hybrigenics's unique technology is to join and collaborate with in-house projects in Hybrigenics's key areas of expertise, i.e. infectious diseases, cancer and various metabolic diseases. ■