Raising antibodies against protein complexes

Proteins are vital for the inner workings of cells. Complex networks of interactions form between protein molecules, and because these associations drive cellular activity, an accurate knowledge of them is vital for understanding cell biology and biochemistry. One way of gaining an insight into the protein interaction networks forming within a specific cellular population is through immunoprecipitation – using the molecules of the immune system to bind proteins and draw them out of solution so they can be studied.

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Immunoprecipitation is a molecular technique capable of providing an accurate picture of protein associations. The technique involves breaking open living cells to access their contents, thus releasing a complex mixture of proteins, then using antibodies to attach to a specific target protein, thereby capturing it and permitting it to be pulled away from the mixture. Importantly, the antibody must be able to bind to its target in the context of the physical associations the target forms with other proteins in the cell, and with minimum off-target binding. When done properly, immunoprecipitation permits groups of interacting proteins to be collectively purified from cells. Co-purifying proteins are said to be guilty by association. That is, if the biological function of one or more of the proteins in the purified group is known, the rest often also prove to be implicated in the same or related biological functions. With antibodies against every human protein, researchers could map the vast networks of protein associations responsible for life. When protein associations go wrong, the resulting altered interactions may lead to disease. The study of these changes therefore has high clinical value.

A Census of Protein Associations
LaCava’s work is important because, despite significant advances in genome characterisation and protein identification, the global networks of protein interactions that occur within cells (rubbed together) remain poorly characterised. It is estimated that 10% of human protein interactions, or fewer, are currently mapped – and this figure does not include the disease-specific interactions which are arguably of most interest. As part of their collaboration with CDI Laboratories Inc., Dr LaCava’s group is currently focused on identifying interactions involving transcription factors, proteins which are master regulators of gene expression and commonly implicated in cancer progression. Changes in these proteins are often responsible for the unregulated proliferation of tumours, so understanding their associations and activities in both the natural and disease states will assist with identifying potential targets for therapy.

NOTHING WORTHWHILE IS EVER EASY; DISCOVER, OPTIMISE, REPEAT
This task is made more challenging because of the now widely recognised problem that many antibodies are not capable of reliably capturing their target protein and its associated interaction partners. Moreover, even otherwise reliable antibodies may not perform well under all experimental conditions, and protein associations existing in cells are not all equally stable and analytically tractable once they are released from cells and subjected to immunoprecipitation. Therefore, each antibody and immunoprecipitation experiment must be subjected to procedural optimisations, a labour-intensive and often time-consuming process. Dr LaCava and his collaborators at CDI have therefore set about generating and evaluating a suite of new antibody candidates, as well as developing robust processes to use them in optimal conditions.

The process is not entirely straightforward. Protein interactions within cells (in vivo) exist in a highly specific set of naturally occurring conditions. These conditions are inevitably altered during immunoprecipitation, which requires the cells’ contents to be transferred into artificial conditions within test tubes (in vitro) in order to mix them with antibodies used for protein capture. An undesirable yet common side-effect of transferring proteins out of cells into an artificial environment is that interacting groups of proteins sensitive to the change will rapidly dissociate from one another (off-target, false negatives). Dissociation can also occur because interactions are context-dependent and may form, wrongly implicating these spurious interactions in biological processes linked to the target of the immunoprecipitation (false positives). Hence, different components of the interactome require different conditions for in vivo and in vitro protein complexes formed with transcription factors within established cell lines. Understanding these differences is key to being able to capture a range of complexes for the next stage of the programme.

It is thought that as little as 10% of human protein interactions are currently mapped.

A NEW TOOLBOX FOR BIOMEDICAL RESEARCHERS
Using the building blocks of their screening techniques, specific antibodies, and identified optimal conditions, the team hope to be able to capture a range of complexes for the next stage of the programme. Presently, antibodies are typically generated on a case-by-case basis. In such a workflow, a protein of interest (such as a recombinant human transcription factor) is, for example, injected into a mouse, provoking an immune response. Antibody producing B-cells are then harvested from the mice and cultured in the lab to provide a renewable source of those antibodies. In the hands of Dr LaCava and CDI, these antibodies are tested for their efficacy in immunoprecipitation, as described above. CDI has made a major advance in the field developing a proprietary monoclonal antibody production reduction pipeline, named Pasteur’s. Overall, however, this remains an expensive, labour-intensive and time-consuming process.
What if this process could be sped up? In order to meet the ultimate goal of mapping the entire human interactome in health and disease, good antibodies against every human protein and variant are needed. To solve this problem, Dr LaCava and CDI have recently turned to the interactome for new protein associations – generating a virtuous cycle. Dr LaCava hopes to exponentially expand the portfolio of antibodies useful for interactome studies and, likewise, rapidly increase the coverage of bona fide human protein-protein associations.

This approach also has added value. When using intact, endogenously assembled protein complexes as immunogens, the antibodies generated may recognise variables that are part of the gamut of naturally occurring protein processing. These may include alternative isoforms and truncations, post-translational modifications, and interfaces formed only when proteins are associated together (referred to as the quaternary structure). Creating reagents that can distinguish bound and unbound proteins, and capture only those protein complexes in a given state of protein production, can then be easily genetically manipulated on a genome-wide scale, leaving more disease relevant clinical samples off the table.

Taken together, the drive for this research is to provide tools for expanding biomedical research capabilities and findings while also improving reproducibility.

**UNIQUE APPROACH TO AN OLD PROBLEM**

Dr LaCava’s approach is therefore providing novel solutions to long-standing, under-articulated problems in protein biochemistry and affinity proteomic research. Leveraging CDI’s proprietary mononuclear antibody technology, Dr LaCava’s team is determined to further expand the current possibilities of immunoprecipitation techniques and bring this to the commercial marketplace themselves.

The ultimate goal is of course to improve patient outcomes and develop new drugs capable of combating cancer. Indeed, the team believe that the intact, purified protein complexes they obtain will provide an unparalleled opportunity to test drug candidates for their ability to modulate proteins as they are found within cells, and in doing so, treat disease. Hence, the benefits of this research are likely to be recognised across both diagnostics and therapeutics, as it becomes increasingly possible to quickly characterise the biochemical profiles of tumours and develop weapons against their aberrant activity.

**How high is the market for these sorts of biomarkers?**

The global antibody market is expected to grow from $80 billion and continues to expand each year. The antibody market comprises three major sectors, therapeutic applications, diagnostics tests and research use. Scientific research institutes use immunoprecipitation technology for protein-target discovery and characterisation. The re-use only antibody market generates between $2.2 billion and $2.7 billion per year and growing (2015). Our market research indicates that the proteomics global economy is projected to be valued at over 20 Billion USD by 2021.

**What makes you think that this technique would succeed where others have failed?**

Firstly, others have not had the ability to screen antibodies for success in immunoprecipitation in such a comprehensive way. Secondly, others have lacked the array-based pre-screen of CDI to select for antibodies likely to be specific to begin with. Finally, to our knowledge, others have not been able to readily purify enough endogenous complexes from human cells to routinely inject them in mice for antibody production – a recent preparative ‘trick’, coupled with our already highly effective protocols helped us make the leap.

**How will this research impact on cancer diagnosis and therapy?**

When aberrant molecular interactions are identified, they may prove to be diagnostic of cancer sub-types or prognostic of outcomes, and rational approaches to intervene may be effectively employed as therapies. A therapeutic approach may seek to reverse the aberration by e.g. stabilising a stable disease interaction, or by destabilising a stable diseased interaction, or otherwise modulate disease with the potential to grow.