

## K KINIEXUS Kinetworks<sup>™</sup> and Kinex<sup>™</sup> proprietary analyses uses over 1800 of the world's best antibodies cherry-picked from over 26 vendors and validated in-house Targets low abundance cell signalling proteins Quantifies protein expression and phosphorylation ◆ Kinetworks<sup>™</sup> multi-immunoblotting is more accurate and guantitative than competing methods Kinex™antibody microarray is more comprehensive and sensitive than other methods with unfractionated cell/tissue lysates 2

Kinexus Bioinformatics Corporation is seeking to map and monitor the molecular communications networks of living cells to support biomedical research into the diagnosis, prognosis and treatment of human diseases. In particular, we track enzymes called protein kinases that control other proteins by carrying out their phosphorylation at regulatory sites known as phosphosites. These sites of reversible covalent modification act as on/off switches for proteins. Their malfunction has been linked to more than 1000 human diseases, including cancer, cardiovascular disease, diabetes and inflammation.

Currently, Kinexus measures the presence and activation states of over 650 different protein kinases and other regulatory proteins and many of their phosphosites in cell and tissue specimens through our integrated platform of cell culture, protein microarray, immunoblotting, mass spectrometry and immunohistochemistry services. This is supported by the use of over 900 pan- and phosphosite-specific antibodies that have been cherry-picked from over 6000 from over 26 leading vendors and tested in-house at Kinexus. In addition, Kinexus has recently developed its own line of over 900 additional antibodies for protein kinases, protein phosphatases and other signal transduction proteins for use in our services.

These innovative proprietary services utilize unique and stringent screening methods to facilitate the discovery and validation of biomarkers, drug targets and the rapid exclusion of inappropriate drug leads in the early stages of the drug discovery process. This is further augmented by our suite of open source, online bioinformatics services. Our bioinformatics studies are helping us to create new tools for biomedical research that include enzymes, antibodies and peptides.



The Kinexus integrated proteomics platform enables our clients to undertake antibody-driven biomarker discovery for disease diagnosis or tracking the actions of therapeutic drugs and toxins. To examine clinical specimens, we recommend initially separately pooling the disease and control samples to identify leads with our Kinex<sup>™</sup> KAM Antibody Microarrays. This reduces costs and permits the most consistently affected proteins to emerge from the analyses. With our custom Kinetworks<sup>™</sup> multi-antibody Immunoblotting (KCPS) service, those antibodies that demonstrate the strongest protein signals and most robust changes are then subjected to validation of their ability to be specific for their target proteins in these type of specimens. If cross-reactive proteins turnout to show marked changes, then they can be identified with our Protein Identification by Mass Spectrometry (PIMS) services. To examine biological variation in patient specimens, hundreds of samples can be spotted on to our custom lysate microarrays or nitrocellulose macroarrays and probed with promising biomarker antibodies. Since our immunoblotting services establish the specificity of biomarker antibodies, these can be used in follow up immunohistochemistry studies also available from Kinexus. Query of our SigNET KnowledgeBase can provide further insights into how potential biomarkers might be affected by other types of perturbations in others organs, tissues and cells. Our In Silico services permit predictions of protein phosphorylation and protein interactions, and facilitates the development of probes (e.g. antibodies and substrate peptides) to track drug targets and biomarkers for disease diagnosis and personalized medicine.



Our unique business model permits the growth of Kinexus through revenues from provision of our specialized services and products, access to thousands of experimental animal and human specimens, and the development of working relationships with thousands of researchers. Since our inception, we have reinvested all of the proceeds from our services back into R&D. For near 18 years, Kinexus has provided our unique services to over 2000 laboratories in 40 countries. We view our clients as our research partners and treat them accordingly.

Our proteomics and bioinformatics services are designed to assist academic and industrial clients at all stages of the research discovery and development continuum. In principle, our clients do not actually have to possess a wet laboratory of their own. Our clients can solicit Kinexus' services at different stages and obtain data that enables them to continue to follow up on the research with Kinexus or independently in their own laboratory, often with reagents that are also available from our company. Kinexus can even assist in the preparation of presentation and publication ready figures and tables with the data resulting from our services. Over 450 scientific publications have appeared with data generated in part by Kinexus.



The following series of slides provide an overview of the various services that we presently offer to our clients. We also sell products to further enable our clients in their research endeavors.

Our In Vivo services permit our clients to have their cell lines of interest or a panel of well characterized human cancer cell lines sourced from Kinexus cultured and treated according to their specifications. Kinexus can prepare lysates from these cells that are suitable for follow up with our diverse proteomics services. Clients can also ship frozen cell pellets and tissues to Kinexus that we can homogenize and subject to subcellular fractionation. Many of our cell and tissue lysate preparations are also available for sale upon enquiry.

Kinexus has an in-house inventory of lysates prepared from 16 human cancer cell lines that have been selected to represent a diversity of tissues, gender and age of the originator. These cell lines have been treated with different stimuli that affect diverse cell signalling pathways. Lysates are also available from a wide range of tissues prepared from monkey, rat and mouse for use in our proteomics services to permit characterization of proteins or antibodies for our clients.



Over 1800 different commercial antibodies sourced from over 25 vendors or developed by Kinexus in-house are printed on the Kinex<sup>™</sup> Antibody Microarray chips. These antibodies have been tested in-house at Kinexus and shown to perform on Western blots and proto-type microarrays to bind to their intended target proteins. Our latest KAM-900P chip features about 265 pan-specific antibodies and about 613 phosphosite-specific antibodies that are intended primarily for tracking protein kinases and other cell signalling proteins. Our next KAM-1100E chip utilizes over 1100 pan-specific antibodies, which encompass a wide range of cancer and neurobiology target proteins. These antibodies were selected from about 7000 that have been tested in-house at Kinexus. Nearly half the antibodies on our antibody microarrays were developed by Kinexus and available directly from our company. The Kinex<sup>™</sup> Antibody Microarray approach is the most effective strategy for quantification of alterations in global protein expression or phosphorylation with a broad panel of antibodies starting with as little as 25 µg of crude lysate protein.

With the Kinex<sup>™</sup> Antibody Microarray services, two lysate samples are analyzed in duplicate with a single dye, non-competitive method. This results in higher signal to noise ratios and less dye-related artefacts than obtained with the two dye, competitive method that is adopted by our competitors. We have also developed a chemical cleavage step for use with the KAM-900P antibody microarray chips that significantly reduces the contribution of signals from proteins that may be physically associated with target proteins and normalizes differences in dye-related signals due to vast differences in target protein size.



As antibody microarrays typically use non-denatured, native proteins in lysates for analyses, there is a high risk for detection of antibody cross-reactivity and changes in protein-protein interactions. Kinexus provides an economical custom Kinetworks<sup>™</sup> multi-immunoblotting follow-up service in which any 18 antibody leads from a Kinex<sup>™</sup> antibody microarray can be validated for showing alterations in the expression or phosphorylation of intended target proteins. Our quantitative Kinex<sup>™</sup> antibody microarray reports also provide over 220 custom pathway analyses using our Kinections Pathway Maps. Moreover, with our KiNetscape Mapping services, we can produce signalling pathway maps that can connect the lead target proteins from our antibody microarrays that show the greatest changes with diverse treatments of cells and animals. Representations of Kinections maps and KiNetscape maps are shown in subsequent slides.



Kinexus has developed protocols that better preserves the phosphorylation status of proteins prepared from cells and tissues, lowers backgrounds and provides more sensitive detection than competing strategies. The KAM-900P antibody microarray permits detection of phosphorylated target proteins with as little as 25  $\mu$ g of crude lysate protein with a dynamic range of greater than 10,000-fold and a median % error in duplicate measurements of 12-14%.



The slide shows a close-up image of a scanned KAM-900P analyses of lysates from overnight serum-starved human A431 cell cervical epidermoid cells treated with and without 100 nM EGF for 5 minutes before harvesting and homogenization. Strongest signals appear red and the weakest are blue if detectable.



With the KAM-1100 antibody microarray, the antibody spots are printed with ink-jet spray technology on epoxy slides, and the top 8 fields are printed in duplicate below. With the probing of captured lysate protein on the chip, we use dye-labelled rabbit polyclonal PYK generic phosphotyrosine antibody (Cat. AB-PG001) that was developed by Kinexus. This antibody displays higher sensitivity, specificity and selectivity for physiological tyrosine phosphorylation sites than any of the mouse monoclonal phosphotyrosine-specific antibodies that we have tested from other vendors.



As part of its Kinex<sup>™</sup> KAM-900P Antibody Microarray Reports, Kinexus offers its panel of over 220 signalling pathway maps with mini-tables with the generated data from each analyses for the proteins featured on the maps. These static maps prepared in MS-PowerPoint are also downloadable for free from the open-access PhosphoNET and KinaseNET websites.



Kinexus also offers its KiNetscape mapping service to allow integration of leads that are generated from the Kinex<sup>™</sup> KAM-900P analyses into protein phosphorylation networks. In the map shown here, a qualitative representation is provided for the key EGF-induced changes in protein expression or phosphorylation from a Kinex™ KAM-900P antibody microarray analyses of the lysates from serumstarved A431 cells that were treated without or with 100 ng/ml EGF for 5 minutes. Lysates were prepared by directly homogenizing the cells into CCC buffer and subsequently biotinylated. Relevant kinase-substrate relationships were imported into the Cytoscape 3.4 program (The Cytoscape Consortium). With this style of protein signalling map, protein kinases are represented with circular icons and other proteins with rounded box icons (nodes). Activating phosphorylation events are shown with green dotted lines and arrows, inhibitory phosphorylations with red dotted lines and phosphorylations with undefined effects with grey dotted lines (edges). Proteins that showed increased expression changes greater than 45% are coloured orange, but appear blue if there was decreased expression greater than 45%. Protein expression changes less than 45% are not identified and these protein icons are coloured purple. If the phosphorylation of a site on a protein was induced more than 45%, then the text for this phosphosite is coloured orange. If its phosphorylation was reduced more than 45% in response to EGF, the text is colored blue. Changes in phosphorylation less than 45% are not indicated and the text for these phosphosites appears grey. The appearance of a positive or negative sign in front of the phosphorylation site text shows if the site is known to be stimulatory or inhibitory, respectively. A portion of the full map is shown.



Kinexus offers our Standard Kinex<sup>™</sup> Reverse Lysate Microarray (KRLM) services with a chip that has been printed with individual spots of 230 diverse animal tissue lysates and lysates from human cells treated at different times and dose with several hormones and drugs. This service is ideal for further characterization of a protein for which an antibody is available for its detection. Such a protein might be uncovered from our Kinex<sup>™</sup> Antibody Microarray services. Each cell/tissue lysate is printed at 4 dilutions in triplicate to ensure linearity in antigen detection on the chip. Each chip is normally probed with a separate antibody.

Kinexus can print custom Kinex<sup>™</sup> Reverse Lysate Microarrays with lysates that are provided by our clients. A minimum of 20 chips must be ordered by our clients to qualify for this service. The custom KRLM chips are the most economical method to explore the biological variation in potential biomarkers that are uncovered with our Kinex<sup>™</sup> Antibody Microarray and Kinetworks<sup>™</sup> Multi-immunoblotting services. Kinexus can also print macroarrays with cell lysates or antibodies on nitrocellulose membranes and glass slides with up to 300 spots.



Kinexus offers microarrays with diverse substrate peptides to explore the specificities of protein kinases and protein phosphatases or for antibody epitope mapping. Kinexus can also print custom macroarrays with peptides and phosphopeptides on nitrocellulose membranes and glass slides with up to 300 spots. Over 1200 peptides and phosphopeptides are already available from KInexus, and we can perform custom peptide synthesis to meet our client's needs.

Kinexus' bioinformatics research program has cracked the protein kinase specificity code for 492 human protein kinase catalytic domains such that their optimal substrate recognition sequences can be predicted solely from the amino acid sequences of their catalytic domains. The Kinex<sup>™</sup> Kinase Substrate Microarray (KKSM) chip features about 450 peptides that were predicted by our algorithms to be optimally phosphorylated by all of these kinases. The deduced amino acid specificity preferences for each of these human protein kinases is available with our In Silico Kinase Specificity Prediction (IKSP) Services. This In Silico service has proven to be effective for prediction of kinase substrate peptide specificities for species as diverse as yeast.

Each KKSM chip permits the experimental testing of the specificity of up to three different protein kinases along with a blank control. Alignment of the amino acid sequences of those substrate peptides on the KKSM chip that reveal the strongest phosphorylation by an added protein kinase in the presence of ATP permits deduction of the optimum consensus sequences for recognition by that kinase. This information is useful to define optimal peptide substrates for high throughput drug screening with target kinases and defining potential phosphosites in physiological substrates that could serve as biomarkers for these kinases.



Immunoblotting remains the gold standard for specific phosphoprotein detection and quantification. Kinexus offers four standard Kinetworks<sup>™</sup> Multi-immunoblots (KPSS) Screens that utilize 35 to 40 well tested commercial antibodies to track alterations in protein phosphorylation. Kinexus has performed over 10,000 Kinetworks<sup>™</sup> analyses over the last 18 years. With our Custom Graphics services, we are pleased to produce annotated figures of the immunoblots that we generate for presentation and publication purposes for our clients.



Our Custom Kinetworks<sup>™</sup> Multi-immunoblotting services permit flexibility for our clients to choose from any of over 800 antibodies used in our proteomics services to investigate specific proteins of interest in their biological specimens of cells and tissues. The KCPS screen is ideal for checking by immunoblotting the specificity or potency of antibody leads from our Kinex<sup>™</sup> Antibody Microarray services. Our KCSS screen permits the testing of 8 different cell/tissue lysate samples on the same immunoblot with up to three different antibodies. This can generate data for publication ready figures with replicates for statistical analyses. However, if large numbers of samples need to be analyzed against a multitude of antibodies, our Kinex<sup>™</sup> Reverse Lysate Microarray analyses are the most economical approach and represent the best value proposition.



The chief strength of mass spectrometry approaches for proteomics studies is the breadth of proteins that can be analyzed without the need for specific detection reagents such as selective antibodies. Our suite of mass spectrometry services permit our clients to identify proteins phosphosites that are regulated in vivo and in vitro. Cross-reactive proteins with antibodies used in our Kinex<sup>™</sup> Antibody Kinetworks™ Multi-immunoblotting services can Microarray and be identified following immunoprecipitation with our Protein Identification by Mass Spectrometry (PIMS) services. Our phosphoproteome-wide scan (MSPP) services allow the tracking of hundreds to thousands of phosphosites in cells that are subjected to perturbations under tissue culture conditions. However, if the need is to identify the phosphosites that are affected in a specific protein of interest, then our custom Mass Spectrometry Phosphosite Identification (MSPS) services are most appropriate. Finally, Kinexus offers our unique Mass Spectrometry Kinase Substrate (MSKS) service for identification of physiological substrates in lysates from cultured cells. Clients should contact Kinexus if they are interested in having rabbit polyclonal antibodies developed to recognize novel proteins identified from our mass spectrometry analyses.



Kinexus has developed a powerful algorithm that predicts the frequency of occurrence of amino acids that border protein phosphorylation sites that are targeted by about 500 different human protein kinase catalytic domains. These kinase specificity matrices, which identify the optimal phosphosite amino acid sequences for substrate recognition, are available with our In Silico Kinase Specificity Prediction (IKSP) services. As long as a typical protein kinase from any eukaryotic species is identified with a Uniprot number, we can provide a matrix for substrate specificity prediction. Using the human substrate specificity prediction matrices that we have developed, we can identify the top 100 protein kinases that are likely to phosphorylate any 15 amino acid peptide that contains a phosphorylated amino acid at the 8th amino acid position in the peptide with our In Silico Kinase Match Prediction (IKMP) services. Our In Silico Phosphoprotein Match Prediction (IPMP) services permit identification of the top 100 protein kinases that target all of the known and predicted phosphosites in a protein of interest. The IPMP service also provide for identification of the top 5,000 out of 650,000 human phosphosites that are likely to be targeted by a specific protein kinase. Most of the predicted protein kinase phosphorylations of peptides can also be confirmed experimentally by Kinexus for clients with our In Vitro Kinase and Phosphopeptide testing (IKPT) services. Kinexus can also produce recombinant kinase substrate proteins for testing with the IKPT services. Identification of the specific sites of phosphorylation of in vitro phosphorylated recombinant proteins can be determined with our custom Mass Spectrometry Phosphosite Identification (MSPS) services.



Kinexus offers a wide range of services for production of recombinant proteins including active protein kinases, and synthetic peptides with diverse tags and modifications. These peptides can serve as substrates for kinases and phosphatases, or to elicit antibody production in animals. Our custom peptide arrays are useful for defining epitopes for antibody recognition and structural determinants for kinase and protease recognition. These peptide arrays can be generated by SPOT synthesis on cellulose membranes or robotically spotted on to nitrocellulose membranes following the synthesis and purification of peptides in solution. Clients that might be interested in the production of antibodies and custom peptide microarrays should contact our Technical Services representatives.







Kinexus has created the KiNET-AM DataBase so that clients using our Kinex<sup>™</sup> KAM antibody microarrays have a large database of protein measurements with which to compare their results. However, it should be appreciated that antibody microarray data can be confounded with issues of cross-reactivities, protein-protein interactions and blocked epitopes.











Multiple mapping initiatives are currently underway at Kinexus. One of these is the KinATLAS website, which generates maps of known and predicted kinase-substrate, kinase-drug and protein-protein interactions. KinATLAS maps of protein-protein interactions and kinase-substrate interactions can be generated in a cell- and tissue-specific manner. These maps permit direct linkages to other SigNET Knowledgebases and other useful bioinformatics websites.



Hovering a mouse cursor above an interacting protein generates a pop up box with direct links to more information available about that protein on other websites. Clicking on an interacting protein results in the generation of a new map with that protein as its hub. KinATLAS is highly customizable and provides more depth about protein interactions that available from any other source in a cell/tissue-specific manner.



The KinATLAS Kinase-Drug module permits queries starting with over 496 human protein kinases or over 850 different kinase inhibitory compounds. The Kinase-Substrate module is currently under development, but will feature over 12,000 kinase-substrate relationships when it is on-line.



We look forward to assisting you in your research and development programs. Contact our friendly service representatives to learn how we maximize your results with the most cost-effective strategies available.