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A kinetic study of analyte-receptor binding and dissociation for surface plasmon resonance biosensor applications

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A fractal analysis, which takes into account, the effect of surface heterogeneity brought about by ligand immobilization on the reaction kinetics in surface plasmon resonance (SPR) biosensors is presented. The binding and dissociation of estrogen receptors, ER$\forall$ and ER$\exists$ in solution to different ligands immobilized on the SPR biosensor is analyzed within the fractal framework. The heterogeneity on the biosensor surface is made quantitative by using a single number, the fractal dimension, Df. The analysis provides physical insights into the binding of these receptors to different ligands and compounds, particularly the EDCs (endocrine disrupting compounds). These EDCs have deleterious effects on humans and on wildlife. Single- and dual-fractal models were employed to fit the ER binding data obtained from the literature. Values of the binding and dissociation rate coefficient and fractal dimensions were obtained from a regression analysis provided by Corel Quattro Pro, 8.0. Values for the affinity, KD ($= k_d/k_a$) were also calculated. This provides us with some extra flexibility in designing biomolecular assays. The analysis should provide further information on the mode of action and interaction of EDCs with the ERs. This would help in the design of agents and modulators against these EDCs.

The treatment is of a general enough nature, and should also be applicable to non biosensor applications wherein further physical insights could be obtained. It has been applied to model DNA-Hybridization, Cell-Receptor, SPR biosensor, Antigen-Antibody reactions, etc. More such studies are required to determine whether the binding and the dissociation rate coefficient are influenced by the degree of heterogeneity or roughness existing on the biosensor and other reaction surfaces. If this is correct, then experimentalists may find it worth their effort to pay a little more attention to the nature of the biosensor surface, and how it may be manipulated, for example by (i) changing the nature of the chip or the matrix, (ii) coupling homogeneous ligands or linkers, (iii) controlling immobilization density etc. to manipulate biosensor performance characteristics and to improve biosensor speed, sensitivity, response time, and robustness.

Clinical efficacy of monoclonal antibodies against SPan-1 antigen of human pancreatic carcinoma

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Introduction: Considerable progress has been made in imaging techniques over the past few years, yet this has not resulted in the ability to reach an earlier diagnosis of exocrine pancreatic cancer. The search for a noninvasive diagnostic tool capable of early diagnosis has led to the development of a series of serum tumor markers. Aim of our study is to discuss the clinical use of SPan-1 and its comparison with established markers such as CA19.9, CEA, TPA and CA242.

Methods: The markers were measured in preoperative serum samples collected from 46 patients who had undergone surgery for ductal carcinoma of the pancreas, 20 patients with chronic pancreatitis, and 23 patients with other digestive neoplasms.

Results: The sensitivity, specificity and diagnostic accuracy for pancreatic cancer were as follows:

	SPan-1	CA19.9	CEA	TPA	CA242
Sensitivity (%):	67.4	69.6	21.7	78.3	54.3
Specificity (%):	69.8	51.2	93.0	37.2	81.4
Diagnostic accuracy (%):	68.5	60.7	56.2	58.4	67.4

Conclusions: The antigenic determinant SPan-1, recognized by monoclonal antibodies, is elevated in sera of patients with exocrine pancreatic cancer.

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SPan-1 may be considered as an additional useful and reliable serum marker for the detection of this neoplasm, but it does not significantly improve the diagnostic accuracy obtained with CA 19.9.

Antigen-based B cell targeting technique to generate high yields of monoclonal antibodies

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Abstract: The advantage of B cell targeting technique for generating monoclonal antibodies is that the aimed B lymphocytes can be preferentially selected based on antigen-specific receptors on the immunized B lymphocytes. This step enables to select and condense B lymphocytes, even though the population of the sensitized B lymphocytes is low. Also of advantage is that the new technology drives selective fusion of the antigen-selected B lymphocyte and myeloma cell complexes by imposing an electric pulse, where the B lymphocyte and myeloma cell were brought into contact in advance by the strong and specific binding of streptavidin to the tethered biotin. We have succeeded in yielding hybridoma cells that can secrete monoclonal antibodies directed against the desired peptide sequences in presenilin 1 with high efficiency and specificity. Regardless of that the targeting technique brings about selective production of hybridoma cells secreting monoclonal antibodies against the desired antigens, several months are usually needed to obtain the aimed hybridoma cells, mainly due to the long-term immunization by *vivo*. To address it, we have employed *vitro* system to accomplish the immunization in the short term.

In this study, we focused on rapid generation of high yields of monoclonal antibodies directed against the aimed antigens using *vitro* system. Two kinds of functional molecules were selected, which are the epitope sequences in human insulin and an endocrine disrupting chemical, di-2-ethylhexyl phthalate (DEHP). The production of hybridoma cells was markedly enhanced when lipopolysaccharide (LPS) and interleukin-4 (IL-4) were included during the *vitro* immunization. Maximally, 20-40-fold increase in hybridoma production was observed. The increased number of sensitized B lymphocytes were also recognized after the immunization in vitro in the coexistence of LPS and IL-4. The addition of IL-4 played an important role for class-switching of monoclonal antibodies from IgM to IgGs, although the produced monoclonal antibodies harbored relatively broad specificities as those were obtained in the absence of IL-4. It is worth noting that B cell targeting technique enables to generate high yields of monoclonal antibodies against the functional lower antigenic molecules, even though the *vitro* system was employed for the immunization.

Here we demonstrate that adding LPS and IL-4 during the immunization by *vitro* bring about the increased number of immunized B lymphocytes, and enhance yielding of hybridoma cells secreting monoclonal antibodies. This new technique and the immunization by *vitro* could be applicable to generating high yields of human monoclonal antibodies in the short term.

Development of human monoclonal antibody tb94 and scfv fragment: Its potential in diagnosis and treatment of cancer

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TB94 is a human monoclonal antibody derived by EBV transformation of lymphocytes isolated from a patient with lung cancer. A combination of enzymatic, chemical and physical analysis indicated that the TB94 antigen (AgTB94) to be 19 kD size. Cell line specificity analysis of the hybridoma antibody indicated that TB94 recognized NCIH661, HT29 and PANC-1 and had negative reactivity with SKBR-3. Biodistribution studies in athymic mice bearing human carcinoma xenografts showed tumor targeting. For the development of recombinant TB94, antibody H+L gene was isolated from the hybridoma cells and cloned in pEE 13.4 vector from LONZA and expressed in CHOK-1 under the GS system. The expressed recombinant antibody had the same specificity as the hybridoma TB94. Single chain Fv fragment was also developed and expressed. The variable light (VL) and variable heavy (VH) chain domains are connected by a (Gly4Ser)3 linker. VL-VH version of the TB94 scfv cloned in E.coli was optimized for expression using PEL-B signal sequence, which significantly improved expression in the periplasmic space. Purification and proper re-folding of the E.coli

expressed TB94 scfv resulted in activity against target cells as seen by using a secondary anti-id mouse monoclonal antibody developed against the variable region of TB94. Using these analytical tools the potential of TB94 for its use in lung carcinoma detection, imaging and treatment is in the process of evaluation.

High affinity protein and peptide libraries

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The CRC for Diagnostics (CDx) has developed efficient libraries (primarily at CSIRO and La Trobe Uni, Melbourne) for the selection of novel, high affinity reagents against diagnostic targets. The libraries are designed to replace monoclonal antibody technology through selection of small, high-affinity, synthetic diagnostic and therapeutic molecules (CPDs) and peptides. The key feature of this technology is its rapid screening process for these peptide and protein libraries on target molecules, identifying and constructing protein and peptide reagents diagnostic of diseases and mimics of infectious pathogens for inclusion in diagnostic assays. The reagents have been shown to have high affinity against a range of commercially important target molecules (publications attached). Further, the High Affinity Reagents group has developed and continues to develop molecular evolution processes for the improvement of binding reagents (affinity and specificity), enabling extremely rapid selection of exquisitely high-affinity diagnostic reagents as biopharmaceuticals and 'immuno'-diagnostics.

Libraries based on non-mammalian antibody variable domains (V_{NARs}): The IgNAR class of antibodies from sharks unusually lack the associated light chain protein found in more complicated ie murine/human antibodies. The IgNAR variable domains (VNARs) compensate by displaying a more complex loop structure, with inbuilt mechanisms to ensure loop stability. Halving the size of the active antibody-binding component means that variable domain (VNAR) fragments of these antibodies are easier to produce in mibcrobial fermentation and may prove very well suited to commercial scale-up. The loss of the light chain has an added advantage in protein stability, with VNAR domains being extremely resistant to harsh treatments that denature other antibody fragments, such as high temperature and pressure and chemical treatments. This makes them ideal candidates as the "front end" for types of environmental and biowarfare (BW) biosensors likely to be exposed to harsh conditions. Phagemid and ribosome display libraries ($>4 \times 10^8$ size) based on the VNAR domains have been developed by CDx and have been successfully selected against a range of validated clinical targets.

Libraries based on the IMM7 immunity protein from E. coli: The immunity protein IMM7 is a highly stable E. coli defensive protein which binds the DNase domain of the colicin E7 and neutralizes this toxin. Binding affinities of such colicins to their cognate immunity proteins are amongst the strongest recorded protein-protein interactions. Phagemid libraries ($>3 \times 10^8$ size) based on the Imm7 domain have been developed by modifying the exposed variable loops essential for colicin binding, while maintaining the underlying framework.

Libraries based on linear peptides: Peptides are among the smallest known protein-based binding molecules and have been shown to bind to an enormous range of targets including other proteins, carbohydrates, lipids, small organic molecules and a number of inorganic surface metals. The CDx 20-residue peptide library is diverse (in excess of 5×10^8 individual sequences), and peptide binders isolated from this library can be used directly in diagnostic and therapeutic validation strategies. Alternatively, they can be lead compounds for the identification improved peptide and non-peptide molecules that possess the desirable characteristics of high affinity and selectivity.

Multimeric Designs: CSIRO and LaTrobe have designed and constructed multimeric molecules for enhanced avidity and/or multiple specificities. These include dia/triabodies for enhanced cancer-targeting and peptide multimers as vaccines with enhanced immunogenicity.

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Monoclonal antibodies in the diagnosis of peste des petits ruminants (PPR) virus infection

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Peste des petits ruminants (PPR) is an economically important viral disease of sheep and goats, prevalent in several parts of Asia and Africa. The causative agent 'PPR virus' belongs to the genus Morbillivirus of the family Paramyxoviridae. Monoclonal antibodies are important tools for the routine diagnosis of viral diseases like PPR. With the advent of hybridoma technology, mouse monoclonal antibodies became more popular diagnostic reagents, because of the distant genetic relationship of the mouse with the target population (human beings and domesticated mammals). This genetic divergence provides an advantage to the diagnostician by avoiding cross reactivity of antibody based diagnostic assays with the target population, which adds to the specificity of the assay. Further, mouse monoclonal antibody based diagnostic tests can be used for screening of the clinical samples (antigen and antibody) originating from all species of animals except rodents. During the recent past, monoclonal antibody based competitive-ELISA (c-ELISA) has become a popular technique for the detection of antibodies to PPR virus under field conditions. Similarly, sandwich-ELISA/immunocapture-ELISA using PPR specific anti-nucleocapsid protein monoclonal antibody is a routine test for the detection of antigen in clinical specimen. Competitive-ELISA test detects the most abundant antibodies in field sera, while sandwich-ELISA detects the most abundant antigen in clinical specimens. Success in application of these tests under field conditions depends on the critical steps involved in the selection of appropriate monoclonal antibodies from the available panel. The basic criterion for the selection of such antibodies should take care of uniform presence of target antibody in field sera from wider geographic area of the world, abundant and uniform antigen in the clinical samples. The genus Morbillivirus, which include important animal and human viral pathogens viz: PPR, Rinderpest, Canine Distemper and Measles, the haemaglutinin (H) protein is most dominant antibody inducers, while nucleocapsid (N) protein remains the most abundant antigen. Successful diagnostics for Morbillivirus in field mainly target these viral proteins. Unlike the anti-measles virus monoclonal antibodies in human beings, use of monoclonal antibodies for the treatment of PPR infection is not reported, in spite of the availability of virus neutralizing monoclonal antibodies with very high antibody titres. This could be, mainly due to high cost of antibody production in relation to the cost of livestock, that to with a doubt in the success of treatment.

Production and characterization of monoclonal antibodies against human IgG in Balb/c mouse

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IgG is principal immunoglobulin in natural human serum, and It constitutes 70 to 75 percent of all immunoglobulins. IgG is the main antibody of second immune responses and It is the only class that has antitoxic effect. Monoclonal antibodies have many applications in diagnostic, treatment and purification. Conjugated monoclonal antibodies against human IgG are used in most diagnostic kits. For production of monoclonal antibody against IgG, five female Balb/c mice 6 to 8 weeks old were immunized against human IgG. Then the most immune mouse was selected for fusion. The fusion of Mouse's spleen immune cells with sp2/0 cells (myeloma cells) were done in presence of Poly Ethylen Glycol (PEG). Supernatant of hybridoma cells were screened for detection of antibody by ELISA. The suitable clones were selected for limiting dilution (L.D). Then supernatant of suitable mono clones were assessed for cross reactivity with IgM & IgA by ELISA and confirmed by immunobloting. The subclasses of the selected monoclonal antibodies were determined and the clones freezed and kept in liquid nitrogen. Finally, suitable mono clone was injected intraperitonealy to mouse that had been primed with Pristane. In this study, 127 clones were obtained that 15 clones had absorbance more than 1 which two of them with absorbance about 1.5 were selected for limiting dilution. The yield of limiting dilution was 6 clones with absorbance about 1.8 that didn't show cross reactivity with IgM & IgA. The titer of mab produced in acitic fluid was 100000. Therefore, this clone with IgG1 subclass and high affinity and without any cross reactivity with IgM and IgA certainly can be used in diagnostic kits of infectious diseases.

Bioprocessing of human mutated J-chain in *Escherichia coli* yielded a soluble protein

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Abstract: The immunoglobulin J (joining) chain plays an important role in the assembly of polymeric immunoglobulin (dimeric IgA and pentameric IgM) and in the selective transport of these molecules across epithelial cell layers. The amino acid sequences of human J-chain, as well as the gene sequencers are known. Based on these sequences data, disulfide bond (2 bonds) assignment secondary structure predictions, and chemical properties, a model for J-chain folding has been proposed. While the real crystal structure of the J-chain is still far from assembled, because the J-chain expression and its protein downstream has a permanent aggregation problems, due to its two free thiol groups. Our attempt to mutate the two cysteine to serine has been yielded a soluble (6.5 mg/l) J-chain protein migrate (SDS-PAGE) at 15–17 KDa. We were used pET20b expression vector and E.coli BL21 (DE3) to producing the j-chain protein. The strategy of batch culture in CSTR bioreactor was developed for maximization of the production yield of J-chain foreign protein. Here we described the scaling-up production in term of kinetic behavior to the recombinant E.coli and optimization of cultivation parameters in 3-L bench-top bioreactor. The results showed an obvious increasing in biomass by 5.98 g/L after 11.5 h.