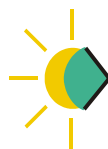


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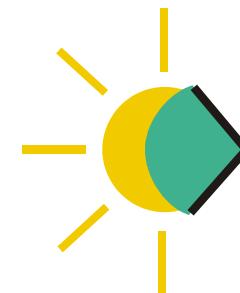
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High Affinity Reagents



Versatile reagents for
any diagnostic
sensing platform

The CRC for Diagnostics

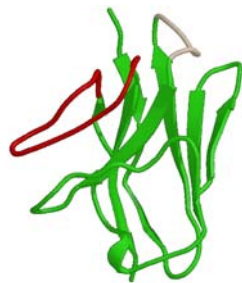
An Australian Co-operative Research Centre,
with world class researchers and end users
in a strong alliance of research & commercialisation expertise.

High Affinity Reagents

Inspiration

Monoclonal antibodies are used widely in diagnostic assays. Recombinant gene libraries may be 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.



Shark NAR

Cooperation

The CRC for Diagnostics (CDx) has developed efficient libraries at CSIRO Parkville and La Trobe University, for the selection of novel, high affinity reagents against diagnostic targets. The project involves research staff from CHRI, CSIRO, LTU and QML.

Investigation

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 ($> 5 \times 10^8$ individual sequences) and peptide binders isolated from this library can be used directly in diagnostic & 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.

Libraries based on non-mammalian antibody variable domains (V_{NAR})

The IgNAR class of antibodies from sharks unusually lack the associated light chain protein found in murine & human antibodies. The IgNAR variable domains (V_{NAR}) 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 (V_{NAR}) fragments of these antibodies are easier to produce in microbial fermentation and may prove very well suited to commercial scale-up.

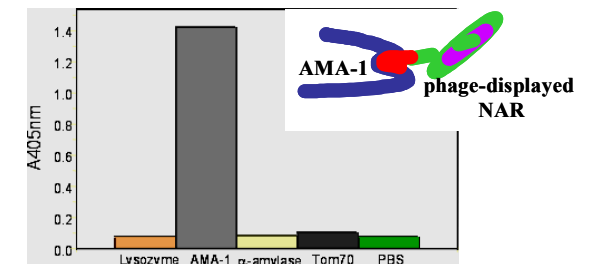
The loss of the light chain has an added advantage in protein stability, with V_{NAR} domains being extremely resistant to harsh treatments that denature other antibody fragments, such as high temperature and pressure and chemical treatments. This makes them ideal candidates as the “front end” for types of environmental and biowarfare (BW) biosensors exposed to harsh conditions.

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.

Validation

Phagemid libraries ($>4 \times 10^8$ size) based on the V_{NAR} domains have been developed by CDx and have been successfully selected against a range of validated clinical targets.



Application

Reagents developed in this project will have sufficient versatility to be integrated into virtually any diagnostic sensing platform. We are continuing to develop molecular evolution processes for the improvement of binding reagents (affinity and specificity), enabling extremely rapid selection of exquisitely high-affinity diagnostic reagents, which may also have therapeutic potential.