Engineered antibodies are high-value Biopharmaceutical and Diagnostic reagents and also validate novel proteomic biomarkers.

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Introduction:
Engineered antibodies are the fastest growing field in biotechnology (1-3), and form a new wave of high-value clinical products (diagnostics and therapeutics). Applications include cancer therapeutics, anti-inflammatory drugs, novel Neuro-active therapeutics and rapid response reagents to emerging diseases. By 2008, engineered antibodies will account for >30% of all revenues in the biotechnology market, many as high-affinity targeting for nanostructured delivery/signalling devices.

Methods and Innovative aspects:
Engineered antibodies (diabodies, triabodies, minibodies and single-domain antibodies) offer unique and superior properties for a range of diagnostic and therapeutic applications, and particularly to enhance the efficacy of therapeutic payloads (radionuclides, toxins, enzymes, liposomes and viruses). Recently, single antibody domains have been designed and selected as targeting reagents against hitherto immunosilent cavities in enzymes, receptors and infectious agents, thus expanding the repertoire of antibody-based reagents against the vast range of novel biomarkers being discovered through proteomics. The challenge remains to design and validate these antibody fragments either as robust in vitro reagents for nanobiosensors, or as non-immunogenic in vivo biopharmaceuticals that possess ideal biodistribution and blood clearance properties. Further challenges include the development of targeted delivery systems for active contrast reagents for image based diagnosis and innovative therapeutic agents for delivery across physiological barriers.

Results:
For the biological targeting component; protein and antibody libraries are now widely used for selection of high-affinity targeting reagents (1-3) and these molecules have been engineered as the front-end sensor in a variety of *in vitro* and *in vivo* Nano-devices. We have constructed display libraries of novel proteins to complement and extend the current range of natural and synthetic antibody repertoires (1-6) and selected against Malaria, SARS, and various biowarfare targets (eg anthrax). These libraries comprise efficient domain scaffolds for the presentation and selection of constrained polypeptides displayed as long surface loop structures. Affinities of both antibodies (scFv) and domains have been improved; a) by nanoscale design of point mutations at the antigen binding surface and b) by rapid mutation and selection strategies to explore larger sequence diversity.

Avidity (functional affinity) has been enhanced by multimerisation, for example by careful antibody (scFv) linker design to direct the formation of diabodies, triabodies or tetrabodies (1,8). Importantly, the improved tumor penetration and fast blood clearance of scFv multimers (~60-100kDa in size) makes them valuable for cancer targeting and other *in vivo* applications. Importantly, the construction of radionuclide conjugates as nanogenerators is a rapidly expanding field of nuclear medicine. Our latest nano-structures of two cancer targeting diabodies and the latest pre-clinical data using murine xenografts show excellent tumour:blood ratios (1).

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