

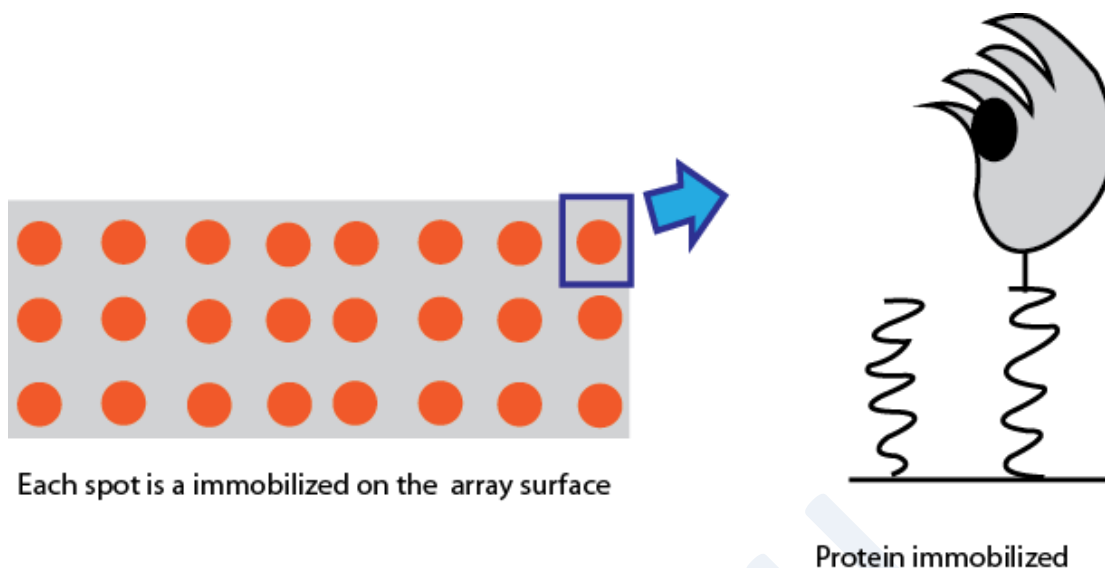
Lecture 24. Protein Chip Technology

Protein chip/arrays are solid-phase ligand binding assay systems using immobilised proteins on surfaces which include glass, membranes, microtiter wells, mass spectrometer plates, and beads or other particles. The assays are highly multiplexed and are often miniaturised (microarrays, protein chips). On one array plate several hundred proteins are spotted (immobilized). Thus, in a single experiment data about several hundred proteins is generated. The process is rapid and automatable. Recent advances in detection techniques provide very high sensitivity. The data handling and data comparison analysis requires sophisticated software.



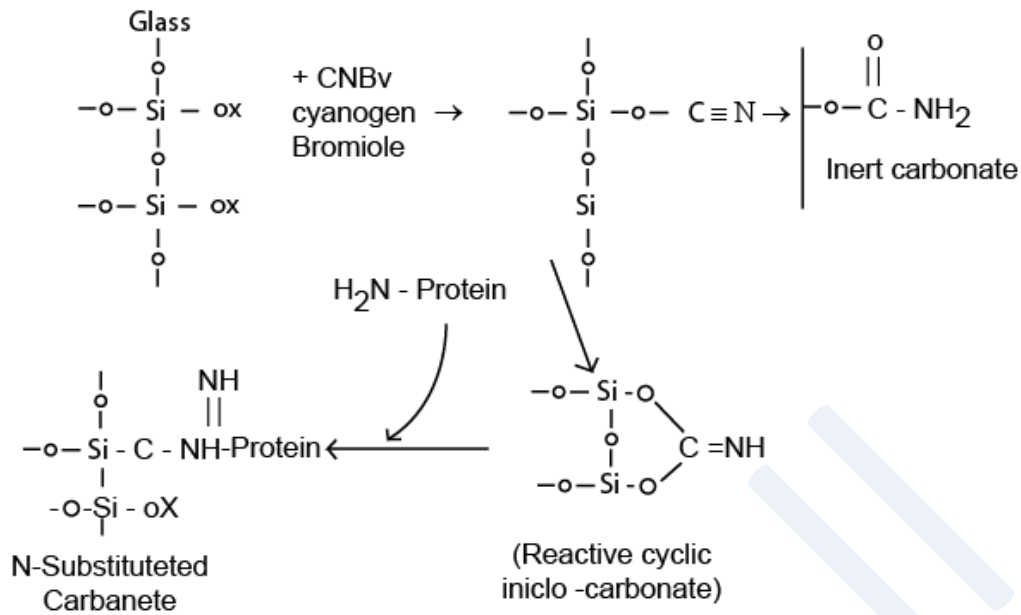
Manufacture of protein chips

It is important that the chip retains proteins preferably in their active state and several proteins can be spotted on a chip (high density chip). Many substrates like polystyrene, poly vinylidene flouride (PVDF), and nitrocellulose membranes, which have been used to attach proteins in traditional biochemical analysis are used as protein chip support material on which proteins are spotted (immobilized).

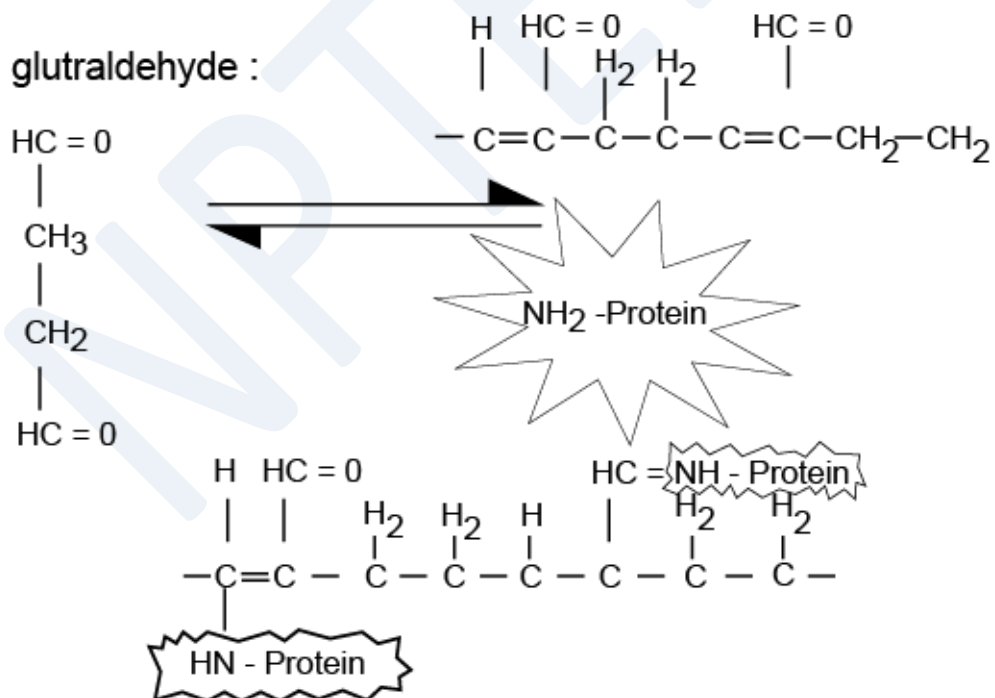


A very convenient method is to cover the glass surface with nitrocellulose membrane or poly-L-lysine such that protein can be adsorbed. Protein stays on the surface with random orientation and can be washed in stringent conditions. To increase the strength of binding of protein to the surface, protein can be cross-linked to the surface with cross-linkers which has functional group that react with hydroxyl group on the glass surface and functional group on protein. Many substrates like cyanogen bromide, ethyl chloroformate, carbodimide, glutaraldehyde, polystyrene, nitrocellulose membranes etc, which have been used to attach protein in traditional biochemical analyse are used as protein chip support material on which proteins are spotted (immobilized);

(a) cyanogen bromide

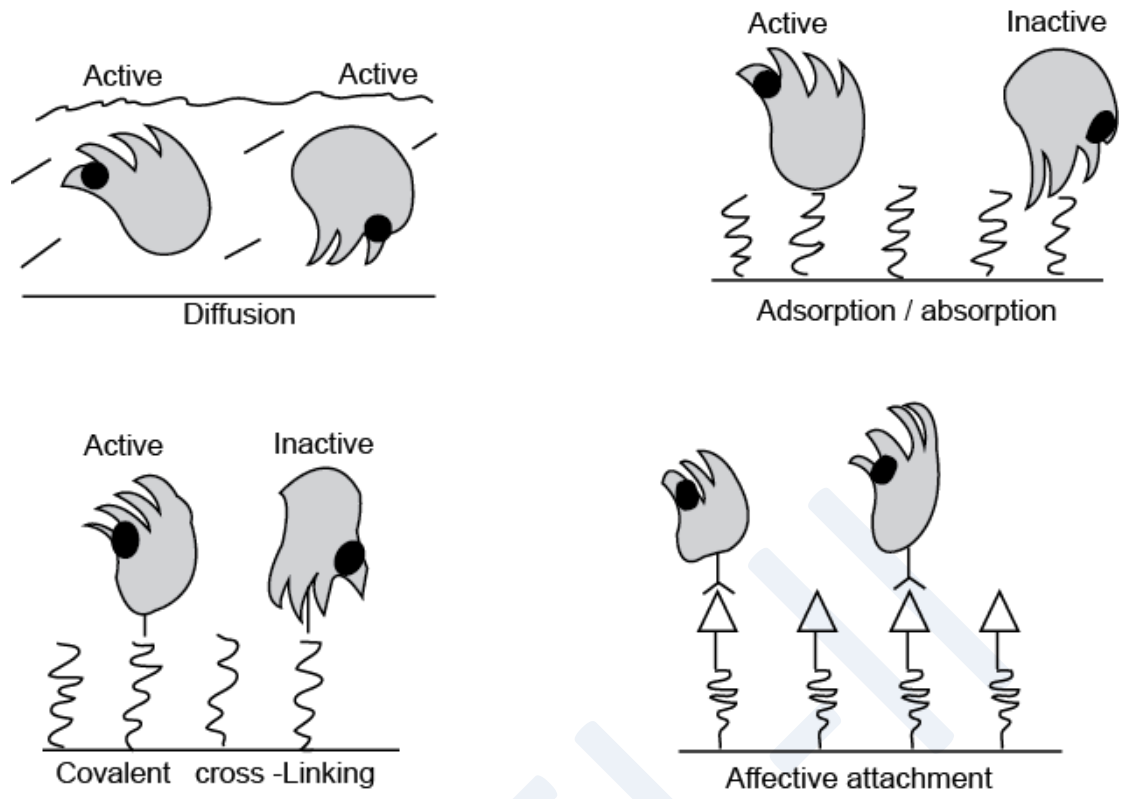


(b) Glutaldehyde



Use of cyanogen bromide and glutaraldehyde for immobilization and cross-linking of protein

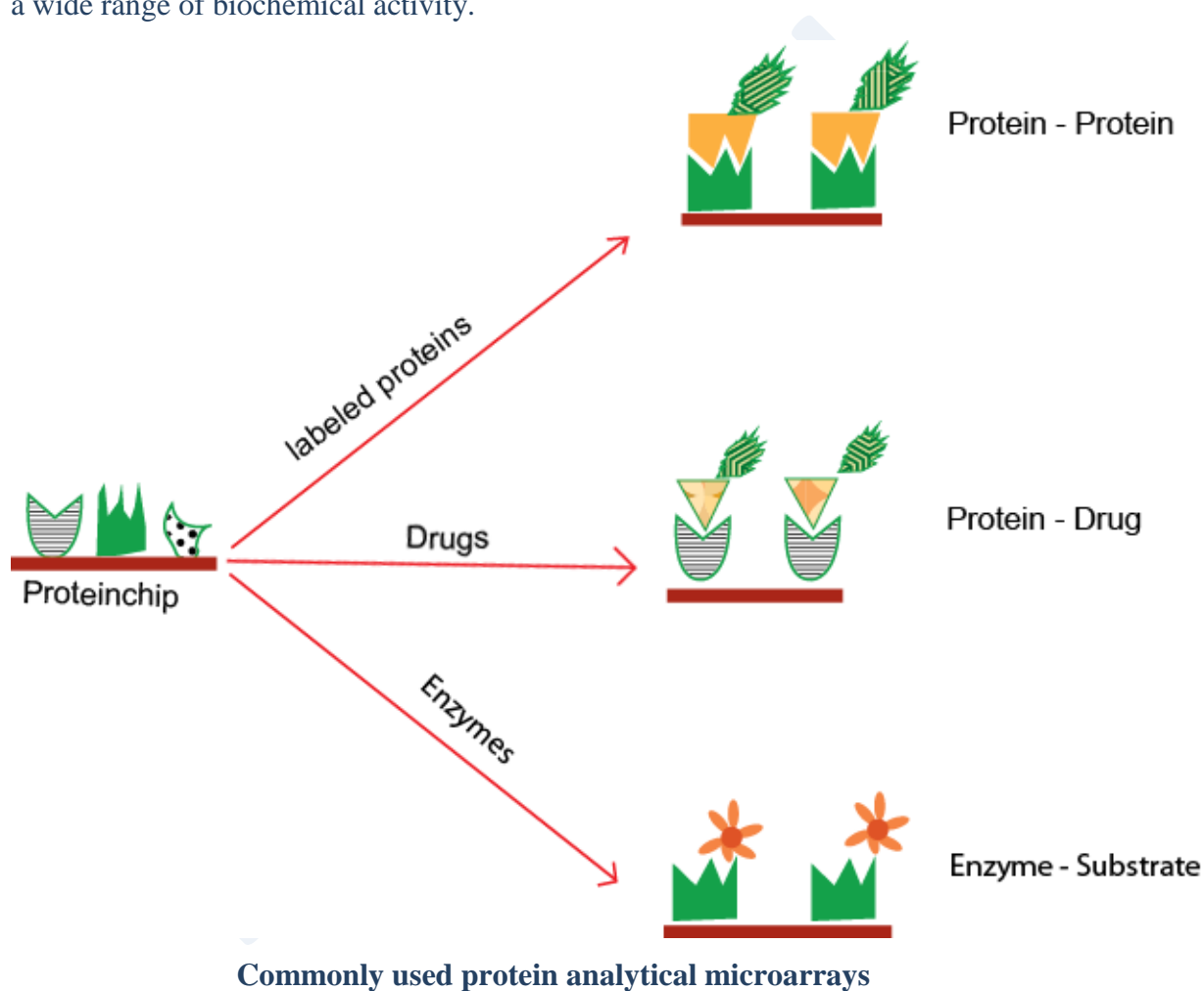
Protein can also be attached via diffusion, adsorption and absorption. In all the methods except affinity attachment, proteins are usually laid on the surface in a random manner, which may alter native conformation of proteins.



Comparison of different protein attachment method

Two functional classes of protein microarray:

There are two general type of protein chips/microarray. Firstly, **the analytical microarray** in which antibodies, antibody mimics (molecules which are structurally similar to antigen binding domain of antibody but do not actually functions as antibody) or other proteins are immobilized and used to measure the presence and concentration of protein in a complex mixtures. Secondly, functional protein microarray in which sets of protein or an entire proteome are prepared and arrayed for a wide range of biochemical activity.



Functional protein chips/ microarray are constructed by immobilising large number of purified protein on a solid surface. Unlike the analytical array, which are mainly developed for diagnostics, functional protein chips have enormous potential in basic research as well as drug target identification. For example: Functional protein chips have been used to conduct enzymatic assays to identify downstream targets of kinases; the substrates are covalently immobilised to individual nanowells and then

individual kinases (which are radiolabelled with ATP) were added. After washing and removing kinases, phosphorylations of substrates were analysed. It was found that 1/4th of yeast kinases phosphorylates at tyrosine residue however they are a members of Ser-Thr family of protein kinases.

Recommended article for further reading (Current Opinion in Chemical Biology, 2003, 7, 55-63.

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Protein chip technology

Heng Zhu* and Michael Snyder*†

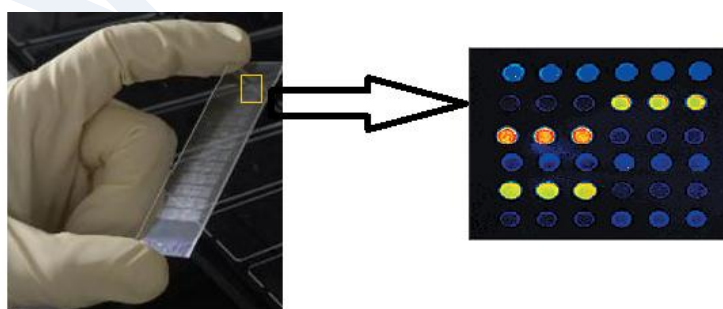
Microarray technology has become a crucial tool for large-scale and high-throughput biology. It allows fast, easy and parallel detection of thousands of addressable elements in a single experiment. In the past few years, protein microarray technology has shown its great potential in basic research, diagnostics and drug discovery. It has been applied to analyse antibody-antigen, protein-protein, protein-nucleic-acid, protein-lipid and protein-small-molecule interactions, as well as enzyme-substrate interactions. Recent progress in the field of protein chips includes surface chemistry, capture molecule attachment, protein labeling and detection methods, high-throughput protein/antibody production, and applications to analyse entire proteomes.

approaches [7,8]. In a microarray format, capture molecules are immobilized in a very small area, and probed for various biochemical activities. High signal intensities and optimal signal-to-noise ratios can be achieved under ambient analyte conditions [3]. The microarray format has become the leading technology that enables fast, easy and parallel detection of thousands of addressable elements and side-by-side measurements.

Despite the success of DNA microarrays in gene expression profiling and mutation mapping, it is the activity of encoded proteins that directly manifest gene function. Thus, one would expect protein microarrays, in which proteins are prepared, arrayed and analysed at high spatial

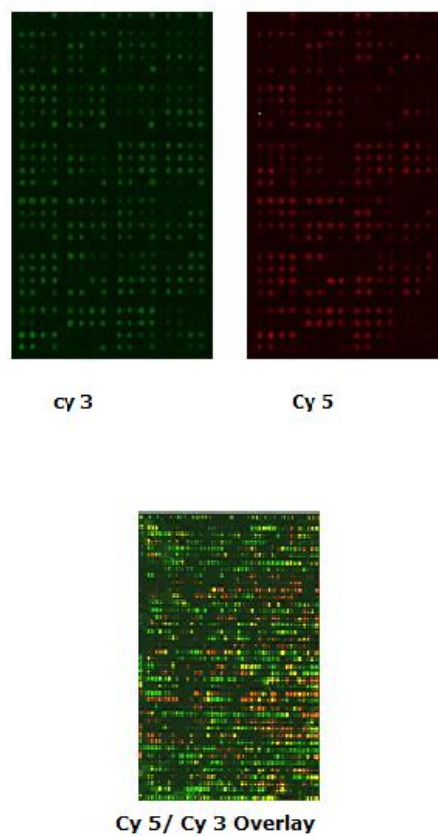
Detection system:

Various proteins are immobilized as individual spot on a microarray and commercially available. Let us take an example of Clontech Microarrays (Spotted Antibodies). This contains 500 antibodies spotted (immobilized) on the microarray chip.



Clontech Microarrays (Spotted Antibodies) may be used to find differences in protein expression of antigens against antibody in biological samples (for example differences in expression in healthy Vs diseased person. Recall our discussion during Lecture 13 about detection of difference in expression using Cy-2, Cy-3 and Cy-5 dye in two

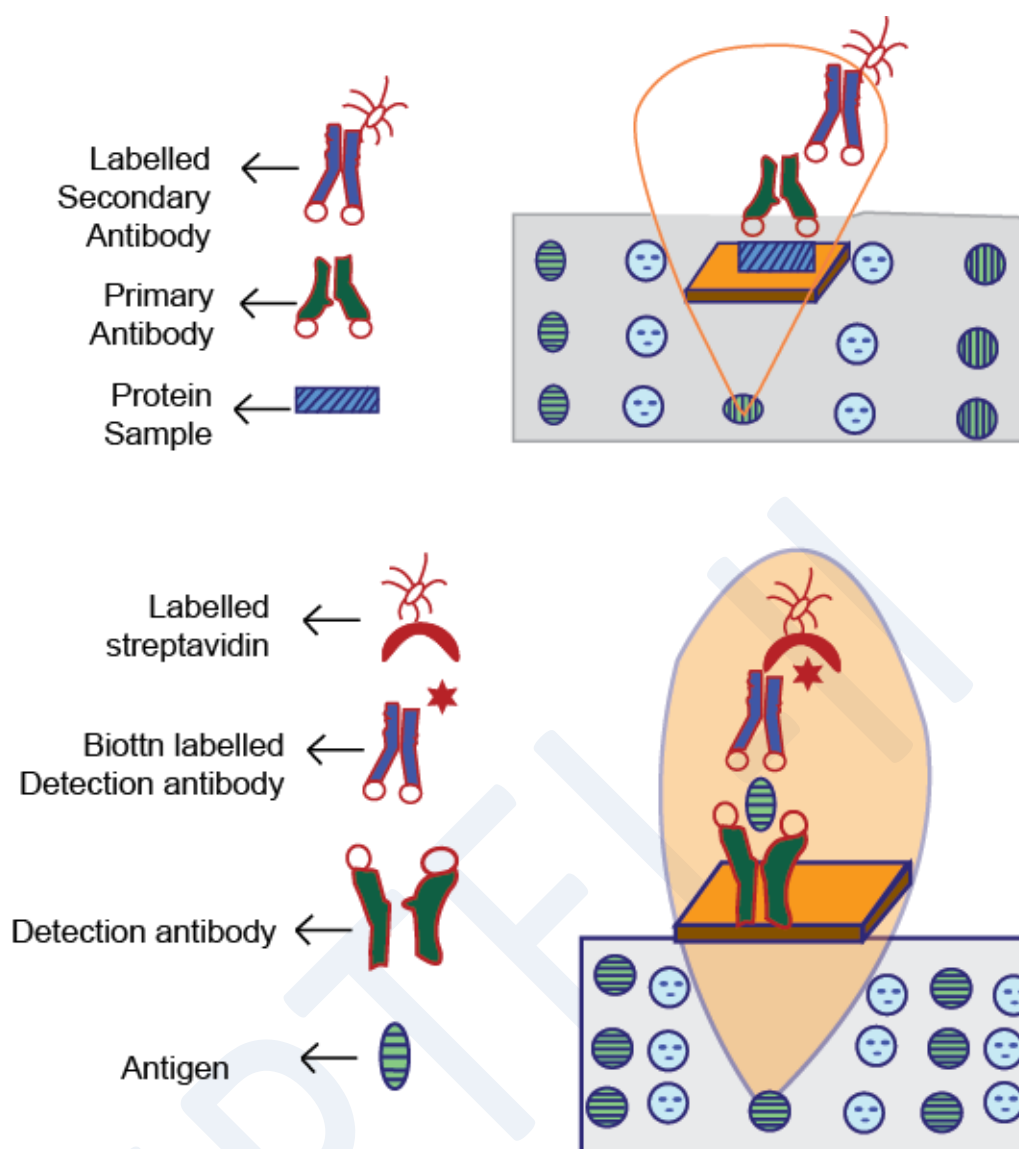
dimensional gel electrophoresis [Please read Lecture 13 to understand Cy Dyes concepts)



Other examples of microarray chips are **Sigma Panorama Microarrays** (Spotted Antibodies)

- Panorama AbMicroarray -Cell Signaling -224 Cell signaling related proteins
- Panorama AbMicroarray -Gene regulation -112 gene regulation related proteins.
- Panorama AbMicroarray -MAPK & PKC Pathways-84 MAPK & PKC related proteins.
- Panorama AbMicroarray -Antibody Array -p53 Pathways -112 p53 related antibodies

Other detection system includes simple immunological detection as shown in the figure



Application of protein chip technology

The analytical protein arrays are used to detect target molecules in mixtures such as plasma or tissue extracts. Typically a complex mixture would be applied to arrays of possibly thousands of specific binders, and the individual bound analytes detected in parallel by appropriate labelling and scanning. In diagnostics, it can be used to carry out multiple immunoassays in parallel, both testing for several analytes in individual sera for example and (by segmenting the array) testing many serum samples simultaneously. In proteomics it is used to quantitate and compare the levels of proteins in different samples in health and disease, i.e. protein expression profiling. Functional protein arrays are used in for *in vitro* functional interaction screens and particularly to detect antibodies in individual patient or animal sera, during disease or to monitor immune responses. The capture reagents themselves will need to be selected and screened for cross-reactivity against many proteins, which can also be

done in a multiplex array format against multiple protein targets. Protein chip technology allows thousands of samples to be analysed simultaneously on the same platform, greatly increasing throughput and simplifying quantitative analysis between samples. Furthermore, an exceedingly small amount of sample is required for printing the arrays, thus permitting the analysis of rare and valuable patient samples. Thus, broadly speaking, there are at least four major areas where protein arrays are being applied, each of which requires appropriate formats and readout methods:

- **Diagnostics:** detection of antigens and antibodies in blood samples; profiling of sera to discover new disease markers; environment and food monitoring. Applications in autoimmunity, allergy and cancer are listed in the tables below.
- **Proteomics:** Protein expression profiling.
- **Protein function analysis:** protein-protein interactions; ligand-binding properties of receptors; enzyme activities.
- **Antibody characterisation:** cross reactivity and specificity, epitope mapping.