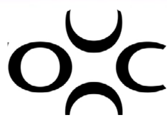

# human protein function arrays
# applied to the characterisation of wild type and variant p53

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## introduction

Many protein arrays measure protein expression, but few protein arrays measure protein function [1]. Here we present a novel human protein function array. Central to the technology is the use of a proprietary tag that ensures arrayed proteins are folded and therefore functional. The arrays produced are compatible with standard microarray equipment. The array described here contains wild type p53 and 49 variants. p53 is inactivated in the majority of cancers, mostly through missense mutations that cause single residue changes in the DNA binding core domain of the protein. We applied several assays to this array in order to determine the effects of SNP and other mutations on DNA binding and conformation of the core domain. The data presented illustrate the potential of studying the function of many proteins in parallel in a single controllable environment. A wide range of functional assays is possible in order to identify and characterise many different protein properties, including interaction with other proteins, protein modifications and their effects, DNA-binding and small molecule interactions.

## methods

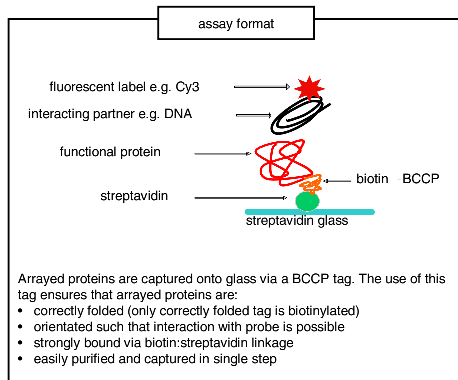
Cloning and expression: wild type and variant full length p53 genes were cloned, sequence-verified, tagged to BCCP and expressed. Western blotting of cell lysates was used to confirm that all of the p53 proteins were biotinylated and therefore correctly folded.

Microarray production: cell lysates containing wild type and mutant p53 were spotted onto streptavidin coated slides using a commercially available robotic system. Slides were blocked, washed and then stored at -20°C prior to assay.

DNA binding assay: to study protein function an assay was devised which measured the ability of arrayed proteins to bind Cy3-GADD45 promoter DNA. After binding, the intensity of fluorescent signals for mutant p53 was compared with that for wild type p53 so that any effect of mutation on the ability of p53 to bind DNA could be determined. The data were presented as a bar chart comprising a DNA binding profile.

Antibody binding assay: to study protein structure, an assay was devised to measure the ability of the arrayed proteins to bind a conformation-specific antibody. The antibody was applied to the array and detection was via a Cy-labelled secondary antibody. As a control (so that any loss of binding could be attributed to the effect of mutation rather than absence of protein), the assay was repeated with an conformation-independent antibody, and the results compared.

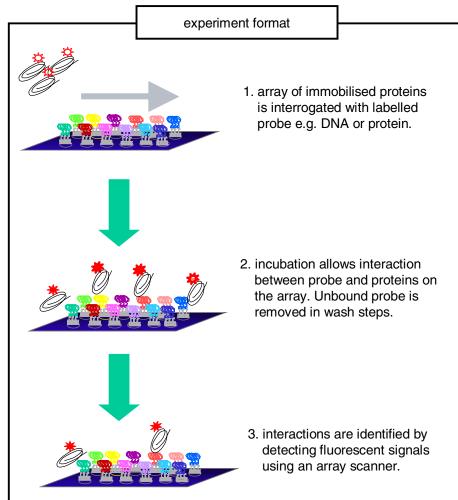
### assay format



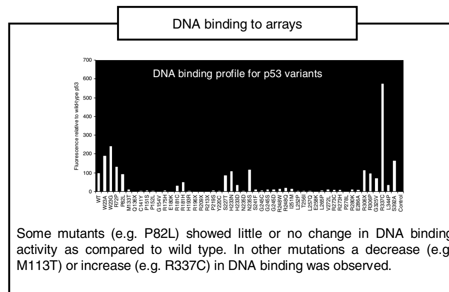

Arrayed proteins are captured onto glass via a BCCP tag. The use of this tag ensures that arrayed proteins are:

- correctly folded (only correctly folded tag is biotinylated)
- orientated such that interaction with probe is possible
- strongly bound via biotin:streptavidin linkage
- easily purified and captured in single step

### experiment format

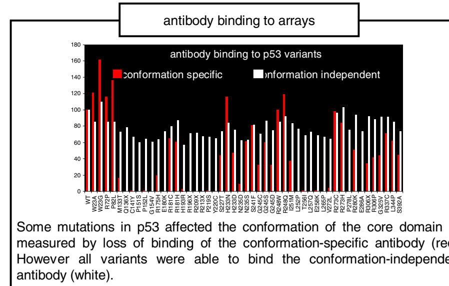



### DNA binding to arrays



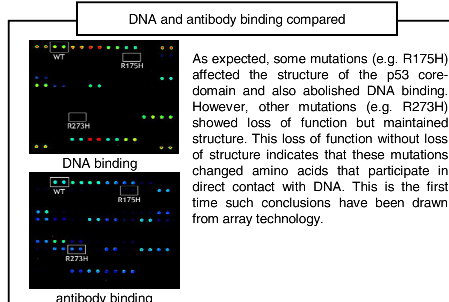

Some mutants (e.g. P82L) showed little or no change in DNA binding activity as compared to wild type. In other mutations a decrease (e.g. M113T) or increase (e.g. R337C) in DNA binding was observed.

### antibody binding to arrays




Some mutations in p53 affected the conformation of the core domain as measured by loss of binding of the conformation-specific antibody (red). However all variants were able to bind the conformation-independent antibody (white).

### DNA and antibody binding compared




As expected, some mutations (e.g. R175H) affected the structure of the p53 core-domain and also abolished DNA binding. However, other mutations (e.g. R273H) showed loss of function but maintained structure. This loss of function without loss of structure indicates that these mutations changed amino acids that participate in direct contact with DNA. This is the first time such conclusions have been drawn from array technology.

## discussion

The use of a BCCP tag is effective in ensuring that arrayed proteins are correctly folded, strongly bound to the slide and orientated such that interaction with probe is possible. This means that proteins on the array retain their biological activity so that reliable and reproducible data for protein function can be obtained.

In this study, it was observed that some p53 variants had reduced or abolished ability to bind DNA. The results were in agreement with what is already known about p53 variants and mirror observations that p53 mutations are a feature of most cancers.

A wide range of other experiments with this array is possible. Indeed, these arrays have already been used to demonstrate a variety of other p53 characteristics, including interaction with MDM2 (a regulatory oncoprotein), the pattern and effects of on-chip phosphorylation of p53 by Casein Kinase II and the determination of $B_{max}$ and $K_d$ binding kinetics between p53 variants and DNA [2].

The versatile nature of this technology will enable many different questions to be asked of proteins, such as how they interact with other proteins or DNA, the effects of post translational modifications (e.g. phosphorlyation or glycosylation) on these interactions, and which of these interactions are blocked by small molecules.

By providing a technology in which these and other questions about protein function can be addressed in parallel, this generic platform will be a valuable tool for functional genomics.

References:

[1] Cutler P (2003). Proteomics 3, 3-18.
[2] Boutell JM, Hart DJ, Godber BLJ, Kozlowski RZ and Blackburn JM (2004). Proteomics 4, 1950-1958.

## CONCLUSIONS

Proteins captured on these arrays are correctly folded and orientated so their interaction with probes, such as other proteins or DNA, can be studied effectively.

In this study, p53 variants were characterised in terms of their structure (via antibody binding) and function (via DNA binding). A comparison of the two data sets enabled novel conclusions to be drawn concerning the regions of p53 that are required for contact with DNA.

This technology provides a powerful multiplex tool for characterising protein function. The p53 array will enable many more questions to be addressed concerning how mutations in this protein modulate its oncogenic properties.

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