DEVELOPMENT OF A NEW BIOCHIP PLATFORM TECHNOLOGY FOR THE RECOGNITION AND VALIDATION OF PEPTIDE-PROTEIN-INTERACTIONS

A. Swistowski, K. Bialek, U. Beutling, R. Frank

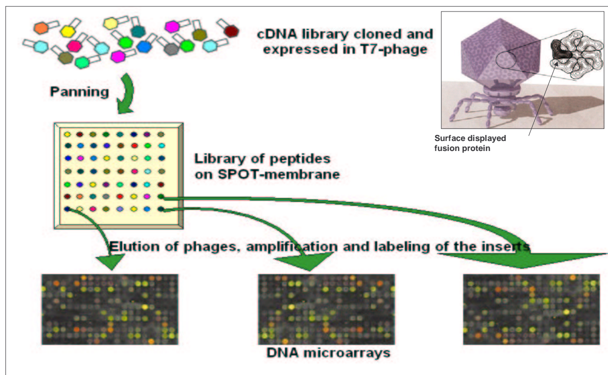
Research Group for Molecular Recognition *GBF* (German Research Centre for Biotechnology)
Mascheroder Weg 1, D-38124 Braunschweig, Germany

Introduction

Function is tantamount to interaction. Thus, global functional genome and proteome research aims at a complete description of the network of protein interactions within a cell or organ(ism) that is diagnostic for a specific cellular state such as fetal or adult, brain or liver, healthy or oncogenic/pathogenic etc. Many proteins, prominently those of regulatory function, are built from smaller domains which are stably folded structural modules still displaying their specific functional property. The catalogue of such domains that recognize linear epitopes is rapidly growing. Linear epitopes can be effectively represented by small peptide fragments that are readily available through simultaneous and parallel chemical synthesis. Peptide arrays are synthesized *in situ* by SPOT-synthesis on a planar substrate. We describe the development of a process for the genome-wide mapping of interactions between protein domains and peptide ligands entirely based on high throughput biochip technologies. A phage library displaying protein domains from a randomly fragmented and cloned cDNA library was "panned" on an array of synthetic peptide ligands. After multiplexed affinity enrichment, peptide specific phage populations were eluted, propagated, labelled and identified by hybridization to a DNA microarray. To corroborate our results, the Target Assisted Iterative Screening (Kurakin et.al. J Biomol Struct Dyn. 2002) was used. Peptide specific phage populations were lifted onto a nitrocellulose membrane and probed with biotinylated target peptide followed by streptavidine – alkaline phosphatase conjugate colour detection. Proteins interacting with the peptide ligand were identified by sequencing of the phage clone DNA and database search.

This work is funded by the German federal ministry of education and technology (BMBF) by grant No 031U102G/031U202G. The project is part of the Human Brain Proteome Project (HBPP).

The experimental concept



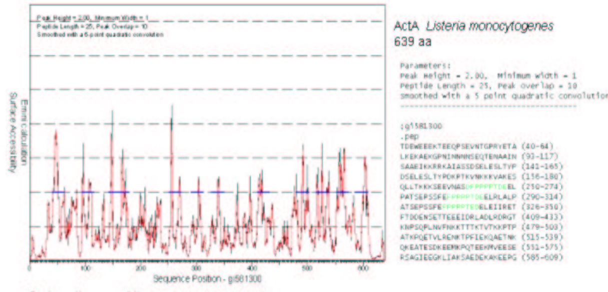
Model domains used in these studies

Characteristics of the three model protein domains selected for proof-of-principle studies.
The most important features for this study are their different affinity for the peptide ligands covering lower and higher affine interactions as well as their characteristic difference in the size of the coding DNA to allow for an easy identification by PCR and gel-electrophoretic sizing.

Code	Type of Domain	Parent protein	Peptide ligand	Kd	DNA size[a]	Domain size
E	EVH1	mMena	SFEFPPPPTDEELRL (pepE)	5 µM	467 bp	113 aa
Y	WW	hYAP	GTPPPPYTVG (pepY)	54 µM	278 bp	64 aa
F	WW	rFE65	PPPPPPPLPAPPPQP (pepF)	n.d.	228 bp	46 aa

[a] size of the PCR product with T7 Up and Down primers.

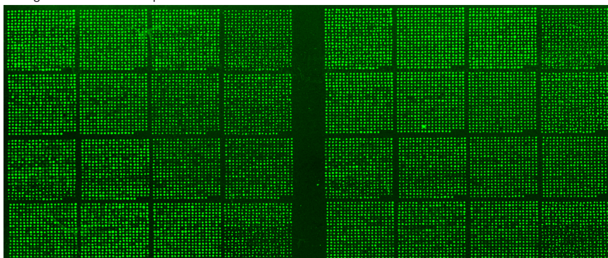
Peptide selection



A software tool was developed that imports protein sequences directly from e.g. TREMBL by it's data base accession numbers. Peptide sequences are then selected by scanning for surface exposed motifs utilizing an algorithm of Emini *et al.* (J Virol. 1985).
The peptide output file can be directly exported to the peptide spotting roboter to proceed peptide synthesis.

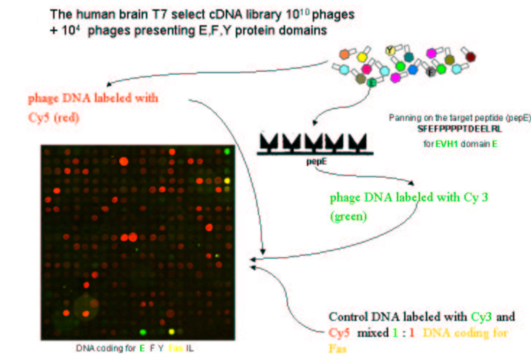
cDNA microarray fabrication

For microarray preparation 6144 full length cDNA clones derived from human brain amygdala region were PCR amplified and spotted in two replicas on the amino-activated glass substrate using the BioRobotics spotter.



The array quality was checked by hybridization with random 15 mer primers labelled with Cy3 fluorescent dye.

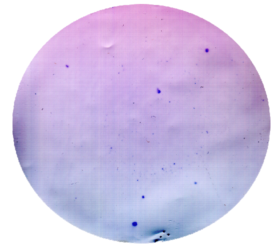
DNA microarray analysis



Simultaneous identification of peptide selective phage displayed protein domains will be achieved by DNA microarray analysis of the whole phage population enriched on a peptide spot. The figure demonstrates a successful differential hybridisation of pre- and post-panning phage library DNA to domain specific DNA probes printed onto a glass slide.

Target - Assisted Iterative Screening TAIS: mapping peptide - protein interaction

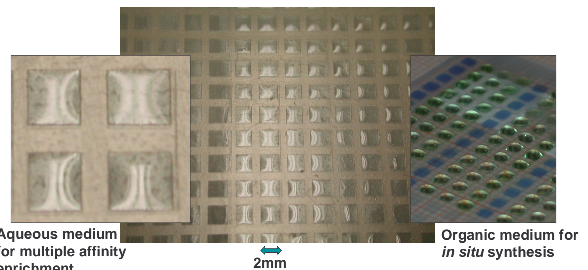
- synthesis of two copies of a peptide array: one immobilized on cellulose; second N-terminal biotinylated and cleaved into solution
- panning of the phage library over SPOT peptide
- elution and plating of specific bound phages
- phage plaque lift on the membrane
- incubation with AP conjugated peptide tetramer
- identification of positive clones by colour reaction
- sequencing and database searching



The TAIS experiment was done using target peptide sequence for WW domain of human YAP protein (GTPPPPYTVG). Two interacting proteins containing WW domains were identified. Nedd-4-like ubiquitin-protein ligase WWP1 and Itchy homolog E3 ubiquitin protein ligase

Automation

Utilizing test peptides synthesized on a cellulose membrane, after panning, individual spots have to be separated (cut apart) before elution, otherwise dissociated phages will mix together due to diffusion. We have developed a novel structurally modified plastic surface, which is suitable for a spatially addressable synthesis and elution. Hydrophobic barriers, between hydrophilic acrylamide-patches, that were graft-polymerized on a planar polypropylene sheet, prevent that phages eluted from one peptide contaminate with phages eluted from another peptide.



Aqueous medium for multiple affinity enrichment

2mm

Organic medium for *in situ* synthesis

Conclusion

Our concept of peptide array based multiplexed affinity enrichment of phage displayed protein domains was successfully applied to three model domains and their known peptide ligands supplemented to a human brain cDNA library. Now that initial results are being collected, this challenges us to advance high-throughput performance of the process in order to achieve the anticipated genome-wide mapping of peptide mediated protein-protein-interactions. This requires several further achievements related to:
- the optimised design of the peptide arrays
- the massive and cheap production of cDNA microarrays.