**Development of a New Biochip Platform Technology for the Recognition and Validation of Peptide-Protein-Interactions**

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**Introduction**

Function is tantamount to interaction. Thus, global functional genome and proteome research aims to at a complete description of the network of protein interactions within a cell or organism 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 synthetic 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 “pinned” on an array of synthetic peptide ligands. After multiplexed affinity enrichment, peptide specific phage populations were eluted, propagated, labeled and identified by hybridization to a DNA microarray. To corrobore our results, the Target Assisted Iterative Screening (Kurakin et al. J Biol Mol 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.

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**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 affinity 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.

<table>
<thead>
<tr>
<th>Code</th>
<th>Enzyme</th>
<th>Peptide ligand</th>
<th>Full DNA size</th>
<th>Domain size</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>E1vH1</td>
<td>Y W W H Y A P</td>
<td>5 µM</td>
<td>54 aa</td>
</tr>
<tr>
<td>Y</td>
<td>E V E</td>
<td>G T P P Y T V V</td>
<td>278 bp</td>
<td>66 aa</td>
</tr>
<tr>
<td>F</td>
<td>W W D</td>
<td>P P P P P P Y</td>
<td>224 bp</td>
<td>66 aa</td>
</tr>
</tbody>
</table>

* view of the PCR product with T7 Up and Devan primers.

**Peptide Selection**

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.

The TAINS experiment was done using target peptide sequence for WW domain of human YAP protein (GTPPPPYYTVG). Two interacting proteins containing WW domains were identified. Nedd4-like ubiquitin-protein ligase WW1 and fihy homing E3 ubiquitin protein ligase.

**DNA Microarray Analysis**

- 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

**Conclusion**

The novel 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.