

# **Pepscan Technology for Fighting Cancer: From Vaccine/Antibody Development to Screening Assays for SUMO Modified Peptides**

*Klaus Schwamborn*

The idea that the immune system can recognize and respond to tumors was formed in the late 19<sup>th</sup> century when William Coley, a surgeon at the Memorial Sloan-Kettering Cancer Center in New York noted that rare events of spontaneous tumor regression were often preceded by infectious episodes. However, the modern era of tumor immunology using vaccine and antibody technology began several decades later.

Pepscan, a worldwide recognized Dutch immunotherapy company in the field of vaccine and monoclonal antibody development, applies these two major strategies for fighting cancer and other man threatening diseases. Our key technology platform is the peptide synthesis on solid support combined with CLIPS<sup>TM</sup> chemistry:

Mimotopes which bind specifically to selected broadly neutralizing monoclonal antibodies are constructed by synthetic chemistry. In different research programs at Pepscan we use peptide libraries or random peptide libraries to map the antibody binding site of broadly neutralizing antibodies. After finding the energetic core of the epitope we improve the binding by searching for the possible missing parts of the epitope by a synthesizing and screening combinations of the energetic core of the epitope with all other peptides. Binding peptides will be combined into peptide constructs to create a mimotope. As pioneered by Pepscan many groups try to mimic protein function using flexible, linear peptides. However to generate functional mimics of an epitope, out of necessity conformational constraints have to be introduced at certain stages. To this end Pepscan Systems has developed new technology to make mimotopes work as vaccines. This technology, CLIPS<sup>TM</sup> (Chemically linked Peptides on Scaffolds), has been developed to make synthetic peptides, representing binding sites, biologically functional. Using small, chemical "Scaffolds" one or more peptides can be organized in a systematic fashion into a single constrained, spatially defined molecule. These mimotopes much better resemble the native structure of a protein domain compared to linear peptides as shown by their biological activity.

CLIPS<sup>TM</sup> Peptides have shown to be exquisitely suited to map and reconstruct complex interaction sites on protein targets. The compounds serve as excellent immunogens in vaccines. When structural data of the antibody-antigen complexes become available within this program, we will use these data to build mimotopes with optimal structural resemblance to the epitope of interest. In addition to the CLIPS<sup>TM</sup> technology we will also use structural mimics of an epitope based on small natural peptides or small proteins. Such naturally occurring rigidly folded peptides with structural homology to the epitope will be used as a natural scaffold on which the sidechains of the epitope of interest will be transplanted. The modified natural scaffold will mimick the epitope surface and can be used as an antigen.

The mimotopes will be coupled to carrier proteins and will be used in a prime-boost strategy that will specifically generate antibodies against these neutralization sites which are otherwise rarely recognized by the immune system. The results from the immunization studies will be used to optimise the mimotopes in an iterative process.

In an effort to expand our portfolio to fight cancer we applied the well established peptide synthesis and CLIPS technology platform on another area of biology: The SUMO research field.

Posttranslational modification by small ubiquitin-like modifiers (SUMO) is an important mechanism regulating a diversity of cellular functions, including subcellular distribution, gene transcription and protein stability. Sumoylation has also been shown to be linked with the pathogenesis of various disorders, including Alzheimer's and Huntington's disease, highlighting the importance of this protein modification. More recently it became also clear that proteins involved in cancer development are also targets for SUMO modification, indicating the link between cancer and SUMO.

The identification and validation of new SUMO substrates and deciphering of the SUMO acceptor site in a given protein are of considerable interest. We established a sumoylation assay with peptide array on solid support, mainly applicable for consensus SUMO target sites. The high-throughput SUMO assay format with peptide array technology might also be useful for drug discovery and diagnosis.

For non-classical sumoylation sites we developed a specific research program:

It has been suggested that some non-consensus sumoylation sites depend for the recognition not on the typical motif ( $\psi$ ,xKE/D) but rather on the helical structural context. Therefore, we wanted to reconstruct minimal helical peptides with non-consensus sites to investigate if just a helical structure is enough for sumoylation of such sites. Furthermore, we want to do a systematic analysis of such helical peptides to study both the dependency on helicity and on the rest of the site, to come up with a consensus site for non-consensus helical SUMO sites.

Recently, we have shown that some helical templates with non-consensus sites could be SUMOylated at a low level. The throughput was still low because we were not able to do the assay on the solid support of the pepsan card and because we were not able to reconstruct relatively large and complex helical structures on the surface of a pepsan card.

Pepsan synthesis is limited by the length of the peptide (30 residues max.) and this length is normally too short to reconstruct folded protein domains with a fixed secondary structure. The simplest helical peptides that can be made are typical dimeric Leucin-zipper based peptides (40 residues). The advantage of a helical zipper is that one part (20 residues) of the dimer can be synthesized on the pepsan card, and the other biotin-labeled monomeric part

(the other half) of the dimer can be added later to fold it into a stable dimer. We have tested this concept for a heterodimeric idealized helix dimer of 2 x 20 residues and it was proven very successfully. So, even though we can not synthesize long peptides that fold into stable structures on the cards, we can accomplish this when we assemble it from 'prefab' components. Next we have tried to perform it with a non-ideal, longer homodimeric helix similar to GCN4 (app. 60 residues). The advantage of this system is that it can be used as a cassette in which all residues of the helical template can be changed except for the Leu – zipper. This system is a bit more complicated because premature dimerization occurs before the addition of the second soluble monomeric 'half' to solid phase monomeric half. Binding of the tagged soluble monomer to the solid phase monomer in this homodimeric system appeared to be much less sensitive. We have obtained evidence that it is indeed caused by the fact that the solid phase peptides attached to the hydrogel of the pepsan card are already prematurely folded into a very stable helical dimer. Therefore, we have the technology ready to use the system of the helical peptide domains (of about 60 residues long!) for screening helical SUMO target sites.

The here described assay format could also provide a snapshot of SUMO targets in cancer cells. The role of modified SUMO substrates in cancer development could be investigated by testing cell lysates from cancer and non cancer cells in this assay. The sumoylation pattern obtained would indicate which substrates were the most likely to be involved in the studied cancers. The high-throughput assay format is particularly suitable for the screening of chemical compounds potentially affecting the modification of therapeutic SUMO targets.

In summary, Pepsan is using its key technologies, i.e. peptides synthesis on solid support and CLIPS chemistry, and applies it to fight cancer with different therapeutically approaches: Vaccine/Antibody Development and Screening Assays for SUMO Modified Peptides.