

Automation aids peptide biomarker discovery

Researchers at JPT Peptide Technologies are using Tecan's HS 4800™ hybridization station to improve throughput and give a high level of reproducibility for patient sera screening with the Company's own PepStar™ peptide microarray technology.



Holger Wenschuh, CEO of JPT

JPT Peptide Technologies in Berlin, Germany, has developed a proprietary peptide microarray technology – PepStar – based on high throughput synthesis and immobilization of peptides onto glass slides. The Company uses these peptide arrays and Tecan's HS 4800 hybridization station to profile humoral immune responses in patient sera samples. Dr Holger Wenschuh, CEO of JPT, explained: "These profiles help in the search for diagnostic biomarkers of infections, autoimmune diseases, cancers and allergies. We receive samples from studies run by our collaborators in biotechnology companies, the pharmaceutical industry and the medical profession, searching for new peptide biomarkers which are indicative of specific stages of disease, which can be used as tools to monitor the success of a therapeutic intervention or to establish an immunological rationale for clinical findings."

Holger continued: "For each study, we optimize the assay conditions before we

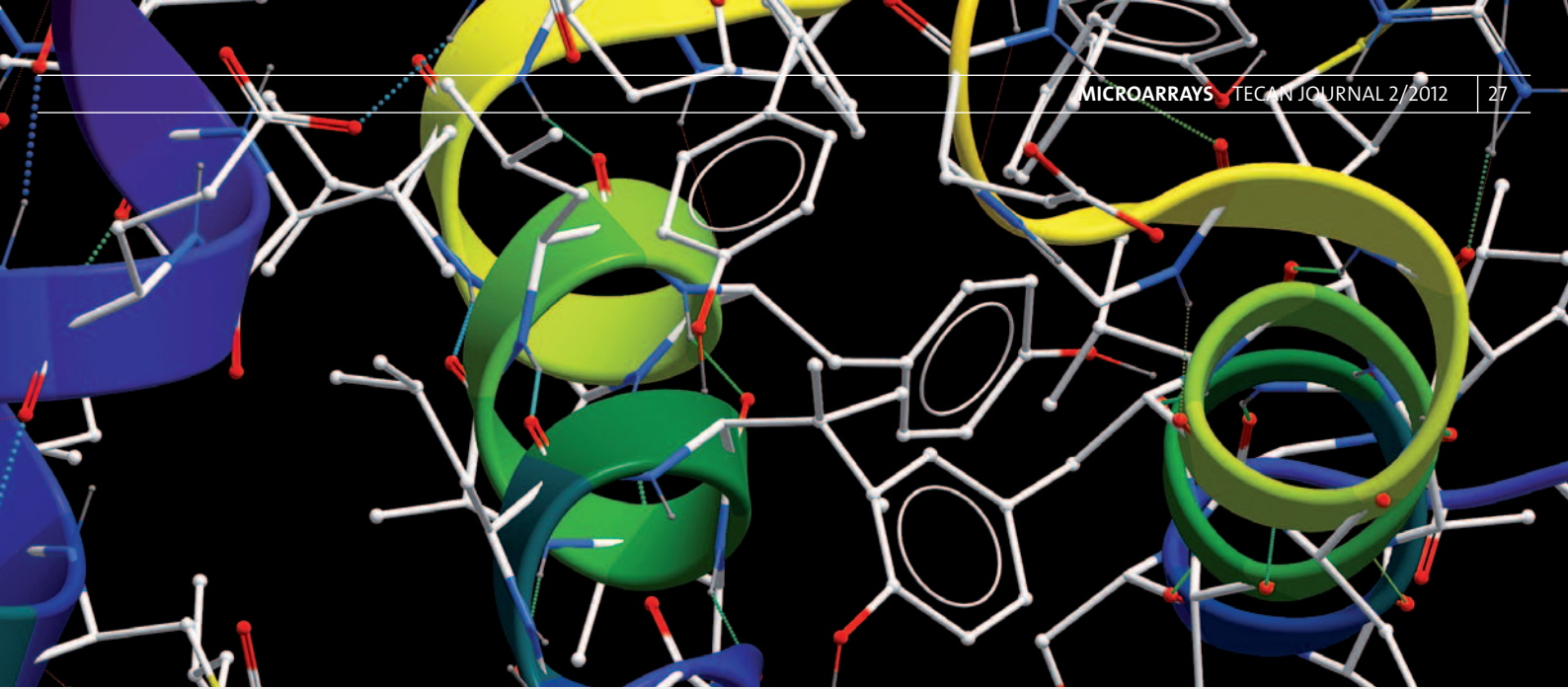
"For each study, we optimize the assay conditions before we begin testing hundreds of samples in parallel; we have to be able to prove that our protocols are reproducible and reliable."

begin testing hundreds of samples in parallel; we have to be able to prove that our protocols are reproducible and reliable. The need for profiling clinical samples under robust experimental conditions was the primary reason for automation – although throughput was also an issue – as manual hybridization introduces variances, even when standard operating procedures are followed exactly. These are significantly reduced by automated procedures and therefore, for the past three years, most of our protocols have been run on the Tecan hybridization station. After evaluating several systems, with particular regard to handling procedures and reproducibility, we concluded that the HS 4800 hybridization station was the best instrument for our purposes. Tecan was also very willing to work with us to establish instrument protocols specific for our needs."

The initial step in peptide microarray manufacture is the use of bioinformatics to generate complex, specifically tailored



Janina Seznec using JPT's hybridization station



peptide libraries that result from the human proteome or the proteomes of pathogens; these libraries typically reflect antigens related to diseases of interest. Chemical synthesis of these peptides using high throughput technology produces tens of thousands of peptides in microplates, which undergo rigorous quality control before being immobilized onto the slide surface to produce peptide microarrays. Critically, this immobilization is site-specific, yielding purified peptides with the same orientation on the slide.

The peptide microarrays are placed in the hybridization station and incubated with appropriately diluted patient samples, allowing circulating antibodies in the samples to bind to immobilized peptides with the correct linear target epitope. Any unbound antibodies are removed by a series of washes before addition of a fluorescently-labeled reporter antibody, which binds to any captured antibodies from the patient's

serum. Holger continued: "Applying peptide microarray technology in large studies – using hundreds of samples to broadly profile humoral immune responses – is quite a new area, but we were able to set up the Tecan hybridization station for this application pretty quickly, thanks to its user-friendly interface. Establishing automated protocols and standard operating procedures – either through creating new protocols or transferring existing manual ones – was achieved within two months, allowing us to quickly bring the system into routine operation. The cohort sizes vary, but a typical batch of 100 to 200 patient samples will take about two weeks to process using this set-up."

"Although most of our work focuses on the discovery of new markers and serological profiling of clinical samples, the PepStar platform is now being used in enzymatic profiling with cell lysates and a variety of other samples, which also takes advantage

of the hybridization station's reproducibility. This technique is helping us to identify appropriate substrates for enzymes with a role in pathogenesis, allowing development of high throughput screening assays for enzymatic activities indicative of diseases, a technique which is becoming increasingly useful in the field of therapeutic drug discovery."

Holger concluded: "We have been very impressed by the performance of our hybridization station, and – as a leader in the peptide microarray field – have recommended the HS Pro to several of our customers and collaborators, who are now using these instruments very successfully in their laboratories."

To find out more about Tecan's microarray solutions, visit www.tecan.com/microarray

To learn more about JPT Peptide Technologies, visit www.jpt.com



The team at JPT