

Automation gives scientists time for science

In Yokohama, Japan, RIKEN (The Institute of Physical and Chemical Research) has just completed a structural genomics and proteomics project to determine numerous protein structures, for which the researchers chose Tecan's liquid handling workstations to prepare the protein samples for NMR spectroscopy and X-ray crystallography.



RIKEN Yokohama Institute in Yokohama (photo courtesy of RIKEN)

RIKEN was organized 90 years ago as Japan's first comprehensive research institute for natural sciences, carrying out high level basic and applied research in a wide range of fields including physics, engineering, chemistry and biology. RIKEN scientists enjoy a unique environment, as researchers in diverse fields work alongside each other and are exposed to stimulation that they would not normally experience at other research institutes.

Since 1998, RIKEN's Genomic Sciences Center (GSC) has carried out comprehensive genomic studies, creating the term 'omic space' to describe the levels of biological phenomena from genome, through transcriptome, proteome, metabolome to phenome. Currently, six groups within the GSC carry out research at each of these levels. In response to the rapidly increasing availability of genome sequences and cDNA resources, the RIKEN Structural Genomics/Proteomics Initiative (RSGI) was formed in 2001 to promote structural genomics research by coordinating the

efforts of these and other research groups in RIKEN. Since FY 2002 the RSGI has been involved in the Protein 3000 Project (the National Project on Protein Structural and Functional Analyses) organized by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, to propel research in protein structure and function and obtain deeper insight into biological networks.

"Although structural genomics research is now being carried out throughout the world, we, in Japan, commenced the Protein 3000 Project in the early days of structural genomics," explained Dr Masaaki Aoki, senior scientist in the NMR Pipeline Methodology Research Team of the Protein Research Group at the GSC. "For five years, our group has been responsible for a program for comprehensive studies within the Protein 3000 Project, and has carried out NMR analysis of approximately 300 protein structures a year. Our set-up is probably quite unusual – we have integrated various systems at the world's leading

edge into our pipeline at the NMR Facility Yokohama Institute, including Tecan's workstations."

"In the Protein Research Group we prepare protein samples for three-dimensional structural analysis by NMR spectroscopy or X-ray crystallography. At the beginning of the project, this was performed manually but, to handle the increasing sample numbers and to obtain experimental results that endure scrutiny, robotic systems were introduced to automate each process in the pipeline. Data reproducibility and consistency are the main advantages of process automation and, to achieve this, it is essential that the instruments perform stably and reliably."

Dr Aoki continued: "Tecan's Genesis RWS™ 150 and Genesis RWS 200 systems perform the rearranging of cDNA clones and PCR primers into 96- and 384-well plates. The Genesis 150 uses plate hotels and handles 45 plates, while the Genesis 200 uses a carousel and can handle 189



(Left) The Genomic Sciences Center NMR complex (photo courtesy of RIKEN)

(Below) The Protein Research Group's Freedom EVO workstation set-up, including the custom-made tilting carrier which is tilted to the right (as shown) when reaction solutions are collected from flat-bottom wells



prepare linear templates that can be introduced directly into the cell-free system for protein synthesis. Because cloning is not required, this system is extremely quick. The protein synthesis, from cDNA to protein product, can be finished in a day or two and there is no need to worry about biological obstacles like toxic protein products that may kill living cells. It is also much easier to automate the cell-free reaction compared to protein expression and purification from living cells. All steps from synthesis to purification are automated on the Freedom EVO platform yielding, on average, 2 mg of protein."

"The three-dimensional coordinate data were one of the final output of the Protein 3000 Project, and have been deposited in the Protein Data Bank (PDB), a database containing structural data of proteins that is freely accessible on the internet by everyone. In addition, from August 2007, supported by MEXT, our structural analysis pipeline is on general release as 'The program for creating innovation by sharing advanced research facilities: The strategic industrial use' (<http://ynmr.riken.jp/>, only in Japanese). We at RIKEN set up this facility for the Protein 3000 Project and, with this project now successfully completed, we would like others, for example people from pharmaceutical enterprises or university researchers, to be able to use it for their own protein analyses."



The Protein Research Group members (from l to r): Hitomi Saito, Dr Masaaki Aoki, Takeshi Nagira, Kazuharu Hanada, Masaomi Ikari and Yasuko Tomo

"We first screen for proteins that yield suitable quality and quantity of product, because we need proteins that can be prepared in milligram amounts in soluble form for analysis using current technologies like NMR or X-ray crystallography. We then study and optimize the synthesis and purification parameters of each protein. Manually finding these conditions for hundreds of proteins is hard work, but the process itself is essentially a monotonous task so we automate this procedure, screening for the best conditions using the Freedom EVO workstation."

plates in one run. The Freedom EVO® 200 platform carries out the automated synthesis and affinity purification of proteins, and can process 96 samples per run, operating up to four times a week. A tilting carrier has been installed to allow efficient collection of reaction solutions from flat-bottom wells with dialysis membranes, and Tecan's MIO™ shaking incubators have been integrated, as well as an automated centrifuge underneath the platform."

"We have developed several unique technologies for protein preparation during this project, including two-step PCR and cell-free protein synthesis, because cloning all the genes of interest, expressing them in living cells and harvesting the cells to extract the proteins are extremely labor-intensive and time-consuming processes. We developed the two-step PCR to complement the cell-free reaction, to

"Although automation has increased our throughput, for us it is not simply chasing the numbers, it is the benefit we derive. Rather than go home at night exhausted from the monotony, we let the Freedom EVO carry out the monotonous work overnight to yield data in a report for us to analyze in the morning. We study the results, decide what to do next, and we go home at night exhausted by the brain work instead – this is how we work," concluded Dr Aoki.