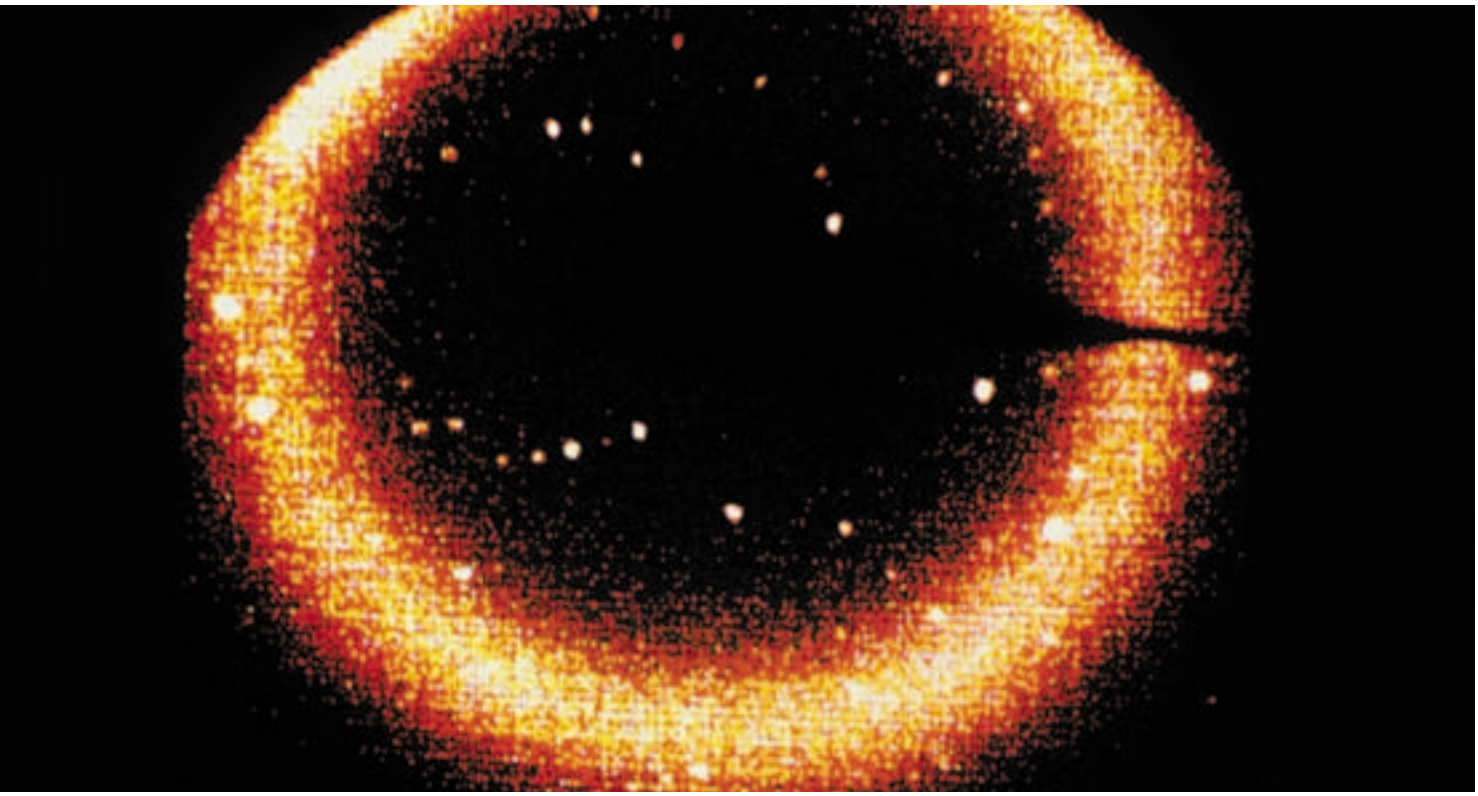
Automation greatly speeds up protein production for X-ray diffraction experiments

ACEMBL is the first fully automatable pipeline for the production of multi-protein complexes, developed by researchers at the European Molecular Biology Laboratory (EMBL) on the Freedom EVO® platform from Tecan. This system offers possibilities for experiments that were previously impossible to contemplate using manual methods. Running many simultaneous experiments in parallel has dramatically increased the likelihood of producing crystallizable molecular complexes suitable for X-ray diffraction analysis.

Maxime Chaillet (left), engineer, and Imre Berger (right), Group leader, with the Freedom EVO at EMBL

Metabolic processes in eukaryotic cells depend on large multi-subunit assemblies where proteins combine with one another and with other molecules. Transcription factor complexes, the subject of gene expression studies by Dr Imre Berger and his group at EMBL in Grenoble, France, are good examples of such assemblies. Imre explained: "X-ray diffraction analysis is the only method that is capable of producing the high resolution necessary to elucidate how these very large protein complexes operate during transcription and gene expression processes, including binding to DNA and chromatin, and recruiting RNA polymerase II. For X-ray diffraction analysis, the multi-subunit complexes have to be crystallized first; to ensure that we have sufficient quantity and quality for this, we produce the proteins by a recombinant over-expression method based on baculovirus-infected insect cell cultures that we developed, called MultiBac-EMBL. However, producing and purifying protein complexes does not guarantee successful crystallization, so creating many variants of each of the complex's subunits is essential in obtaining complexes that will crystallize. As an example, a complex with four protein subunits, each with five genetic variants, would produce over 600 different combinatorial permutations. This is impossible to handle manually as I realized



X-ray diffraction involves the interpretation of complex diffraction patterns to elucidate protein structures

some years ago, due to the essentially sequential nature of experimentation by hand. This is when we had the idea to harness the power of automation by using robotics for our purposes. Automation is the essential solution for protein complex expression and production, when many fold variations in the individual proteins is required for experimental success. With a robot, many experiments can be done simultaneously and in parallel, with a precision that is unattainable in manual mode."

ACEMBL is an automatable multi-gene combination and expression system developed by Imre and his colleagues, to generate multi-gene baculoviral expression vectors by tandem recombination procedures. Firstly, one or several genes encoding proteins of interest are introduced into small plasmids; these so-called 'donor' and 'acceptor' plasmids recombine *in vitro* at specific sites to produce larger multi-gene vectors. These vectors, in turn, are combined into the baculovirus genome by transposition. The resultant recombinant baculoviruses, containing fluorescent reporter genes to track virus amplification and protein production, are used to infect insect cell cultures where the proteins of interest are produced. ACEMBL has great potential for revealing currently unknown molecular mechanisms of health and disease that could be vital in drug discovery.

"Before moving to the EMBL in 2007, I researched at Zurich, and visited the Paul Scherrer Institut (PSI), Villigen, Switzerland, where I familiarized myself with the Tecan systems in Michel O Steinmetz's Biomolecular Research Group," Imre continued: "We designed and developed modules for the Freedom EVO platform at the PSI to automate protein-complex expression. We are still closely collaborating on multi-protein complex production technology, producing joint publications. Our Tecan system in Grenoble consists of a Freedom EVO 200 platform with three identical liquid handling modules for, respectively, gene integration into small plasmids, multi-gene plasmid fusion and integration of plasmids into baculoviral genomes. This creates a pipeline with all the modules executing routine and easily scripted plasmid manipulation and transformation steps simultaneously. Additionally, the platform has a sterile, covered compartment for growing cell cultures, which allows eukaryotic cell cultures to be distributed into deep well plates, before they are transformed with the recombinant baculovirus."

"The two main advantages of automation are diversification and throughput. We are introducing diversity through parallel processing into many genes, coding for variants of the same subunit of the protein complex we are studying. This vastly increases the chances of a successful outcome compared to manual procedures. Instead of one candidate that may or may not crystallize, we obtain, in a few days, 96 variants of one protein complex that will probably include at least one or two that will crystallize. Our research and optimization of the procedures are ongoing, with the aim of producing a routine and robust high throughput application in the foreseeable future, with several automated platforms running in parallel, repeating the ACEMBL procedure many thousands of times with up to 95 % efficiency."

"We have an excellent working relationship with Tecan, which is a collaboration of mutual benefit. We are continuously in contact, and often have discussions for method development. From our experience, it is essential to have a dedicated and well-trained technician to operate the automated systems, to get the best out of them. Investing a little extra time and effort from the beginning really has paid dividends later on," Imre concluded.

ACEMBL has recently been described in an article published in Nature Methods, vol 6 (June 2009), pg 447-450.