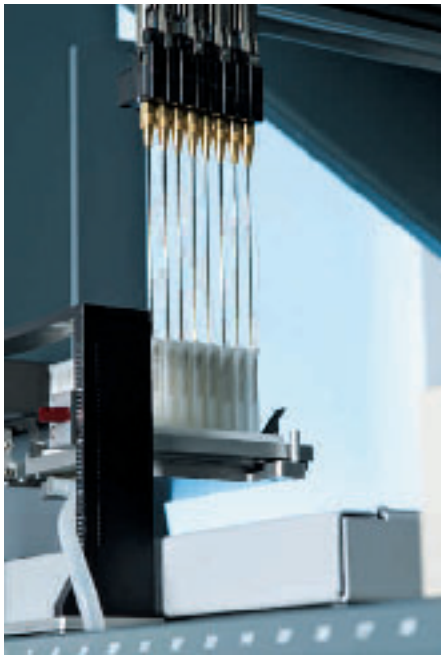
Biopharmaceuticals – manufacture in miniature

The Biomolecular Separation Engineering group at the Karlsruhe Institute of Technology, Germany, is using the Freedom EVO® liquid handling workstation, equipped with the new Te-Chrom™ module and Atoll's 96-array MediaScout® RoboColumn system, to create a fully automated chromatography platform for biopharmaceutical process development.

The Biomolecular Separation Engineering group (front row, l to r): Katrin Treier, Benjamin Maiser, Katharina Lang, Carolin Richter; (middle row, l to r): Anna Siudak, Stefan Oelmeier, Patrick Diederich; (back row, l to r): Jörg Kittelmann, Prof. Jürgen Hubbuch.

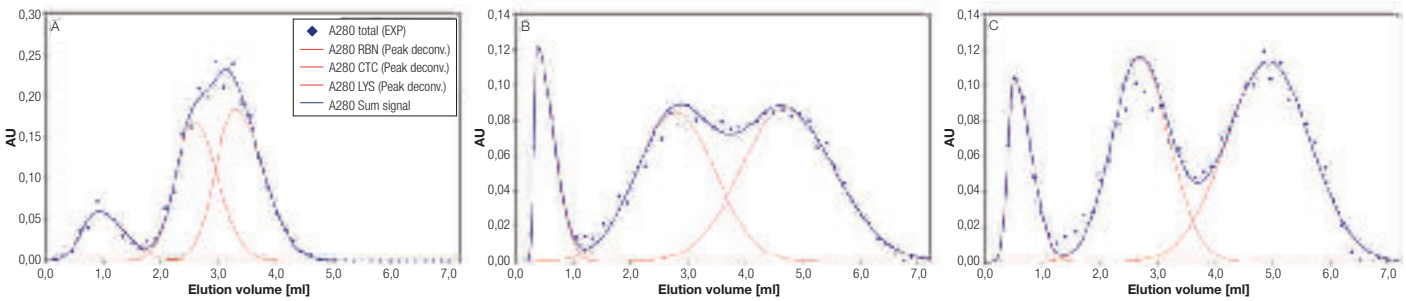


The Te-Chrom module.

The Biomolecular Separation Engineering group studies all aspects of downstream processing in the biopharmaceutical industry, focusing on rapid and optimized process development for industrial scale-up. Process development aimed at large scale production is generally highly time-consuming and expensive due to the large number of potential variables and unit operations involved. Downstream processing thus accounts currently for over 85 % of the total production costs. To tackle this problem, the group has developed high throughput techniques to mimic all relevant unit operations used in the biopharmaceutical industry. These scaled-down models allow rapid and comprehensive automated characterization of proposed processes prior to pilot scale realization.

Professor Jürgen Hubbuch explained the team's research strategy: "Originally, the group was involved in traditional experience-based sequential process development; however, it soon became clear that time pressures of the biopharmaceutical industry made it very difficult to fully optimize and understand processes prior to scale-up. Around five years ago we changed our approach, switching to automation to help us learn as much as possible about processes before process synthesis and scale-up, aiming to significantly enhance process performance and reduce production costs. When choosing an automated platform, one of our major considerations was that we wanted to be able to develop several new hardware components in-house. Therefore, we needed a flexible system that allows sufficient space to incorporate these modules and is compatible with the OEM robotics. We assessed all the automated liquid handling systems available, and found that Tecan's Freedom EVO workstations offer unrivalled flexibility and a very high level of space on the deck of the instrument."

The group is using Freedom EVO workstations in conjunction with Atoll's 96-array MediaScout RoboColumns, enabling fully automated parallel chromatography of up to eight columns. These columns are available prepacked with virtually all commercially available process separation resins, and column flow is controlled by the liquid handling arms via pressure tight inlets at the top of each column. Prof. Hubbuch continued: "As chromatographic separations are the heart of nearly all biopharmaceutical purification processes, one of the main tasks performed on our Freedom EVO workstations is assessing chromatographic separations of new biopharmaceuticals. One of the major advantages of the Freedom EVO platforms for this application is that liquid dispensing is hydraulically, rather than pneumatically, controlled. This is very important for chromatography, as pressure variations caused by compression of air in



Optimization (A-C) of a chromatographic separation of ribonuclease A, cytochrome C and lysozyme on the cation exchanger SP Sepharose FF. The separation was performed on a Tecan EVO Freedom 200 with Te-Chrom option and Atoll columns. (Blue diamonds: Experimental data from the Tecan EVO Freedom; blue line: fitted UV 280 signal of total protein content; red lines: de-convoluted signal of the individual protein species to be separated.)

the head space would affect flow rates through the columns. The combined Tecan / Atoll solution allows us to generate a large amount of data within a short time frame. The automation further allows us, for the first time, to exploit Design of Experiment (DoE) strategies combining short experimental time and low material consumption. This not only cuts the development time for process optimization significantly, but also gives a much better understanding of the process."

"We now have multiple Freedom EVO workstations, and with each successive instrument we have revised our platform design to use the latest generation of Tecan modules. We have also developed several new ideas, including the Te-Chrom module to securely position the base of the RoboColumn plates during chromatography. Our systems have been optimized for high throughput analysis, using Te-Stack™ modules to increase walkaway time and enable fraction collection during elution.

Our latest system has a Te-Stack with a Te-Chrom, LiHa and MCA™ 96 pipetting arms, a RoMa arm to maneuver plates, and both a centrifuge and Infinite® 200 microplate reader below the deck. This set-up offers a high level of flexibility and maximizes deck space for our in-house hardware, and this is particularly important for another avenue of research we are exploring – miniaturization of complete industrial processes. By creating miniaturized versions of industrial components in a modular configuration, we are able to scale down the entire process to run on the Freedom EVO workstation. This enables us to have much better insight into how changing a variable, or fluctuation in a parameter, will affect production."

"Since acquiring our first Freedom EVO workstation we have worked very closely with Tecan, and later this year we will be taking delivery of our third Freedom EVO system. This will give us some extra flexibility, but will also be used for training. We are planning on running courses on how to use the Freedom EVO system in conjunction with Tecan here at the University of Karlsruhe. Automation is now essential to our research, and the flexibility of the Freedom EVO platform has enabled us to reduce process development time drastically. The significance of our work is highlighted by the fact that some of the largest biopharmaceutical companies are following this approach, using Freedom EVO workstations for downstream process development."

You can read more about the Biomolecular Separation Engineering group at:
http://mab.ciw.uni-karlsruhe.de/

For more information on the automated chromatography platform, visit
www.tecan.com/proteinchromatography
www.gelifesciences.com/bioprocess
www.tecan.com/parallel and
www.atoll-bio.com.