

## Automated Glycan Assembly Using the Glyconeer 2.1 Synthesizer

PNAS 2017, 114, E3385–E3389

According to Professor Peter H. Seeberger from the Max Planck Institute of Colloids and Interfaces (Potsdam, Germany), synthesizing oligosaccharides has always been one of the great challenges of organic synthesis. “Manipulating monosaccharide protecting groups and designing a strategy for the preparation of oligosaccharides was as much a form of art as it was science,” said Professor Seeberger. “Access to these very important molecules was limited because the preparation of oligosaccharides was very challenging and time-consuming, as it requires many purification steps and depends on many variables, which are not trivial to control.”

The project of automating the synthesis of oligosaccharides was pioneered by Seeberger and co-workers in 1998, when he started as an assistant professor at MIT (USA). “The idea behind automating the synthesis of oligosaccharides came from the immense success of automated DNA and peptide synthesis,” explained Professor Seeberger. “Creating an accessible and reproducible process, while minimizing the number of purification steps, was the goal of the development of the Glyconeer.” Professor Seeberger revealed that during the construction of several oligosaccharide synthesizer prototypes – over almost two decades – it became clear that a new synthesizer would not only facilitate the access to oligosaccharides but would also provide online feedback to achieve reproducible results.

Professor Seeberger emphasized: “Many new functionalities have been integrated so that the Glyconeer will meet these requirements. The system was designed to have multiple building block vials to ensure the stability of monosaccharides during synthesis and to allow the synthesis of large oligosaccharides. Multiple solvent and reagent containers were introduced to minimize manipulation of the synthesizer during and in between runs. Furthermore, cooled compartments were integrated in order to guarantee stability of the activating reagents throughout the entire synthesis.”

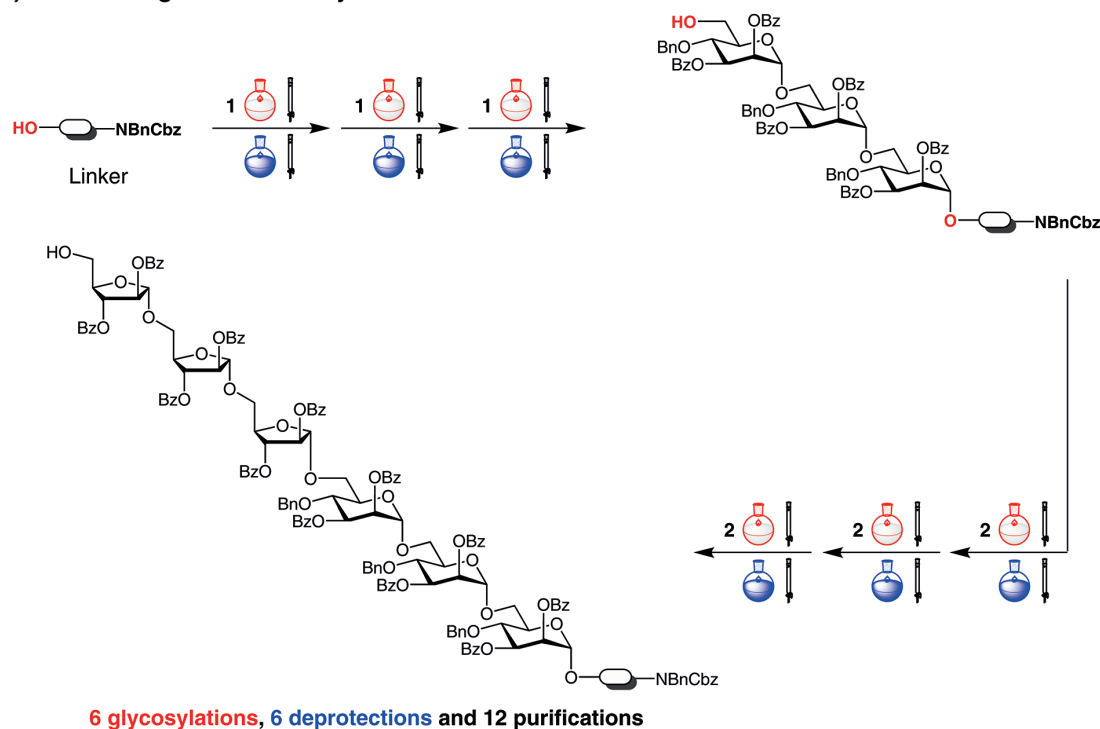
Professor Seeberger said: “In collaboration with engineers and software experts, we designed software that has the capability of controlling all reaction parameters in order to adjust conditions that may vary for each glycosylation. In addition, we added a feedback process that relies on an online detector during the synthesis and set of analytical steps used after synthesis to guarantee the purity of the oligosaccharide.”

“We put the Glyconeer to the test by synthesizing oligosaccharides of different complexity to prove that the platform

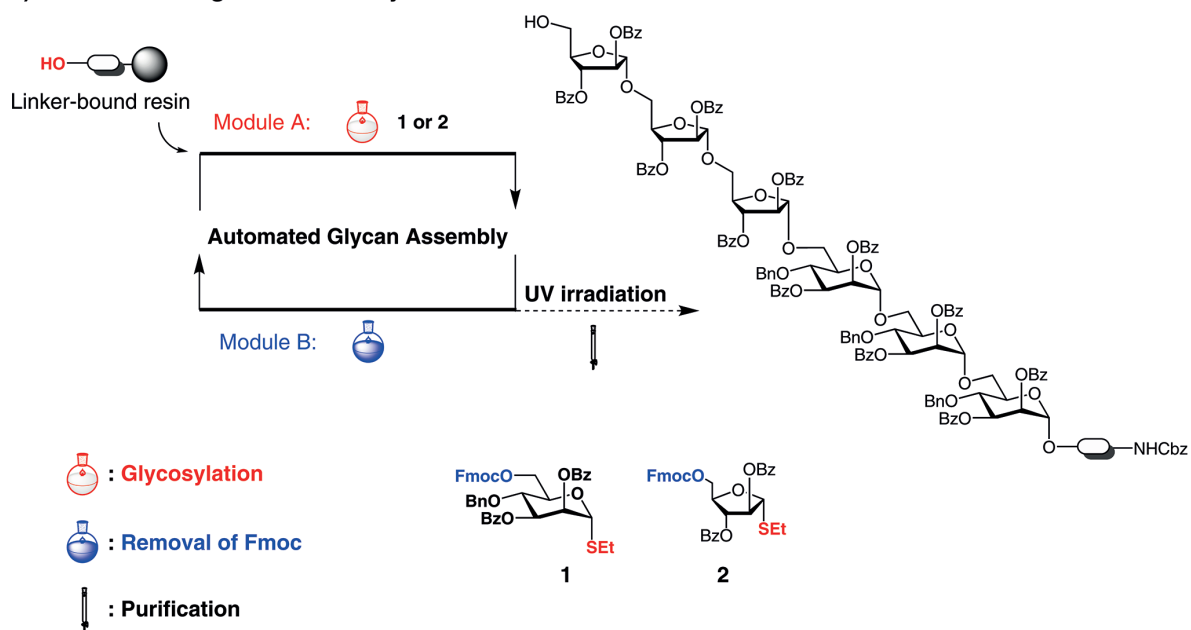
is reliable, reproducible and also flexible,” said Professor Seeberger, who concluded: “We are certain that this benchtop synthesizer can find a wide audience in the field of carbohydrate research since it is a straightforward way to synthesize oligosaccharides.”



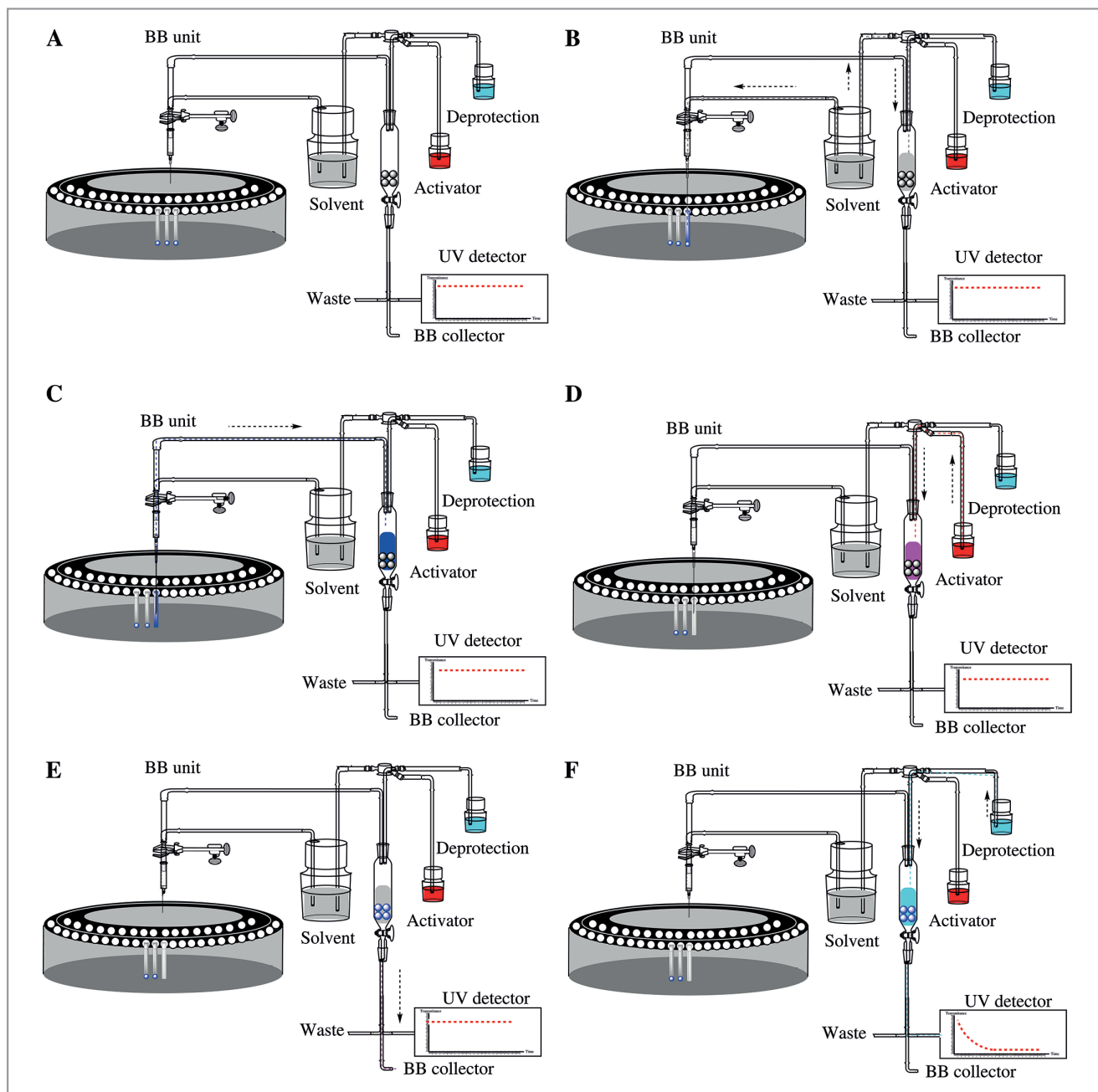
## I) Manual Oligosaccharide Synthesis



## II) Automated Oligosaccharide Synthesis

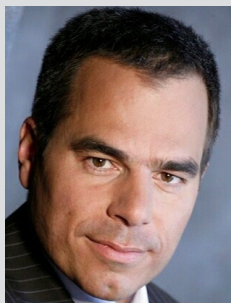


**Figure 1** The main differences between I) manual oligosaccharide synthesis, which requires multiple purification steps and is time-consuming, and II) automated oligosaccharide synthesis, which requires only setting up the synthesizer and applying two modules in an iterative manner



**Figure 2** A complete cycle for the elongation and deprotection performed on the synthesizer: **A)** The system in standby. **B)** Solvent is added to dissolve the monosaccharide building blocks as the solid support in the reaction vessel (RV) is being washed. **C)** RV is cooled and the dissolved building blocks (BB) are delivered to the RV. **D)** Activator is added to the RV to initiate the reaction. **E)** Glycosylation solution is discarded. **F)** Deprotection solution is added to RV and washed out after incubation through UV detector to monitor the reaction progress

## About the authors



F. Schuhmacher

**Frank Schuhmacher** was born in Frankfurt (Germany). He was trained as a physics laboratory assistant at Hoechst AG (Germany) and later graduated from his studies in physical technology in Wiesbaden (Germany). After this, he worked as application engineer at Additive in Friedberg (Germany), where he later established a training and education department. In 2000, Frank founded Elexon AG, an independent IT development and training company in Frankfurt (Germany). In 2010, he joined the Max Planck Institute of Colloids and Interfaces in Professor Peter Seeberger's group to complete his Ph.D. studies, which focused on developing automated platforms for oligosaccharide synthesis.



Dr. S. Eller

**Steffen Eller** was born in Lichtenfels (Germany). He received his undergraduate education at the University of Bayreuth (Germany) and his Ph.D. in chemistry from the same university. He was a postdoctoral fellow at Max Planck Institute of Colloids and Interfaces in Berlin (Germany) in the Biomolecular Systems group of Professor Peter Seeberger. He joined Chemspeed Technologies, a leading provider of high-throughput and high-output research & development workflow solutions in 2012 as an Automation Chemist. From March to October 2015, he worked as a Workflow Architect before he became Head Workflow/Chemistry/Support and a member of the extended management board in November 2015.



H. S. Hahm

**Heung Sik Hahm** was born in Sokcho (South Korea). After obtaining a B.S. in chemistry in 2002, and an M.S. in chemistry in 2004 at the Sogang University (South Korea), he was exposed to stem-cell biology using small molecules under the supervision of Professor Sheng Ding at The Scripps Research Institute (TSRI), La Jolla (USA) between 2004 and 2008. After quitting a doctoral program of chemistry and biochemistry at the University of

Colorado, Boulder (USA), he moved to Berlin (Germany) to study for his Ph.D. under the supervision of Professor Peter Seeberger at the Max-Planck-Institute of Colloids and Interfaces (2009 to present). While there, he worked on glycan synthesis using the Automated Glycan Synthesizer and showcased the synthesis of dozens of oligosaccharides including  $\alpha$ -glucan-containing multiple 1,2-*cis*-glycosidic linkages, glycosaminoglycans, and a 50mer polymannoside. Furthermore, he participated in the development of the Glyconeer 2.1 Synthesizer as the first commercial glycan synthesizer. At the end of 2015, he joined the laboratory of Professor Ku-Lung (Ken) Hsu as a research scientist at the University of Virginia (USA) to pursue his interests in the area of chemoproteomics and metabolomics using chemical probes.



J. Hofmann

**Johanna Hofmann** was born in Berlin (Germany). She studied chemistry at the Humboldt-Universität zu Berlin (Germany) and received her diploma in 2012. For her thesis, she investigated a cisplatin-specific antibody and its antibody-antigen complexes by native mass spectrometry. In 2012, she started her Ph.D. at the Fritz Haber Institute of the Max Planck Society and the Free University Berlin (Germany) under the supervision of Professor Kevin Pagel. Her research focus is the investigation of glycans using ion mobility-mass spectrometry.



Prof. K. Pagel

**Kevin Pagel** was born in Werdau (Germany). He studied chemistry at the University of Leipzig (Germany), where he received his diploma in 2003. Afterwards he moved to the Free University Berlin (Germany) to work on his Ph.D. under the supervision of Professor Beate Koksche. The focus of his thesis, which he successfully defended in 2007, was the design and development of amyloidogenic model peptides. After a brief postdoctoral stay at the Free University Berlin, he joined the group of Professor Dame Carol V. Robinson as a fellow of the German Academia of Science Leopoldina, first at the University of Cambridge (UK, 2008–2009) and later at the University of Oxford (UK, 2010). In early 2011, he returned to Berlin to establish his own research. From 2011 until 2014 he worked under the

supervision of Professor Gerard Meijer as a research associate in the Molecular Physics department of the Fritz Haber Institute of the Max Planck Society. In 2014, he was appointed as Assistant Professor at the Free University Berlin, where he was promoted to Associate Professor in early 2017. In addition, he is affiliated to the Fritz Haber Institute of the Max Planck Society as a guest scientist. His current research is focused on the analysis of biomolecules using ion mobility-mass spectrometry and gas-phase IR spectroscopy, with a special focus on oligosaccharides and amyloidogenic peptides.



*Dr. M. Schlegel*

**Mark Schlegel**, born in Pennsylvania (USA), received his undergraduate education at Moravian College (USA) and subsequently graduated from the University of Pennsylvania (USA), receiving his Ph.D. in the group of Professor Eric Meggers in 2009. After postdoctoral research at the Max Planck Institute of Colloids and Interfaces with Professor Peter Seeberger (Germany), he joined Glycouniverse GmbH & CO KGaA (Germany) in 2013

where he worked on the design, development, and implementation of the first commercial Glyconeer in 2014. Currently he is a Senior Scientist at Alnylam Pharmaceuticals (USA) with a focus on developing siRNA-based therapeutics.



*Dr. M. Hurevich*

**Mattan Hurevich** was born in Jerusalem (Israel). He received his undergraduate education at the Hebrew University of Jerusalem (Israel), where he continued for an M.Sc. and a Ph.D. in the group of Professor Chaim Gilon. Mattan joined the oligosaccharide automation team in the group of Professor Peter Seeberger at the Max Planck Institute of Colloids and Interfaces (Germany), where he focused on the development and implementation

of several automated platforms. Mattan recently rejoined the Hebrew University as a Senior Lecturer where he continues his research in automated oligosaccharide synthesis.



*Prof. P. H. Seeberger*

**Peter H. Seeberger** studied chemistry in Erlangen (Germany) and completed his Ph.D. in biochemistry in Boulder, Colorado (USA). After performing research at the Sloan-Kettering Cancer Center in New York (USA), he built an independent research program at MIT (USA) where he was promoted to Firmenich Associate Professor with tenure after just four years. After six years as Professor at ETH Zurich (Switzerland), he assumed

positions as Director at the Max-Planck Institute in Potsdam and Professor at the Free University Berlin (Germany). His research covers the glycosciences from chemistry to immunology as well as flow chemistry.