

# THE FIRST TRANSFERRIN MADE WITHOUT A DROP OF BLOOD

The world's first recombinant transferrin offers a safer, more consistent product than the conventional transferrin derived from human or animal blood. It is being launched for use in the manufacturing of biopharmaceutical drugs through a global cooperation between Novozymes Biopharma and Millipore.

Novozymes Biopharma has developed and manufactured new CellPrime™ rTransferrin AF – the world's first microbially expressed, recombinant human transferrin made to cGMP (current good manufacturing practice) standards. Together with Novozymes' strategic alliance partner Millipore, CellPrime rTransferrin AF is being marketed for use in commercial-scale mammalian cell culture applications for production of biopharmaceuticals such as antibodies used in cancer treatment. It is specifically engineered to provide an animal-free alternative to current serum-derived human or bovine transferrin products. The problem is that these mammalian sources of transferrin are also

potential contaminants due to viruses, prions, or other diseases that could be present in the original blood.

CellPrime rTransferrin AF was first launched at the BioProcess International Conference and Exhibition on September 23–26, 2008, in Anaheim, California, and is the result of years of development work spanning many sites within the Novozymes organization (see box). Samples of the product are available from Millipore for evaluation.

### What is transferrin?

Transferrin is a protein that carries iron in the body and delivers it to cells, thereby regulating the bioavailability of iron to the cells. Iron is critical to cellular function and supports key metabolic processes including protein synthesis, DNA synthesis, and oxygen transport.

Conventional transferrin from blood serum is derived from either bovine or human sources and is a common supplement used in commercial-scale mammalian cell culture. But with increasing pressure from the regulatory authorities to reduce animal-derived components in the manufacture of biopharmaceuticals, there has been a long search for alternatives. Now Novozymes has succeeded in developing the first recombinant form of human transferrin on a scale able to supply the biopharmaceutical manufacturing market.

### Equivalent to human transferrin

The new product, CellPrime rTransferrin AF, is produced in Novozymes' proprietary expression

system using the microorganism *Saccharomyces cerevisiae*, better known as baker's yeast. This host organism has a long and successful track record in the production of protein therapeutics with GRAS (Generally Regarded As Safe) status.

The recombinant transferrin developed by Novozymes is based on nature's biological way of supplying iron to cells. Novozymes' product is a full-length transferrin so it has the whole range of functionality found in the original transferrin produced in the human body.

CellPrime rTransferrin AF has been shown to be equivalent to human transferrin in its ability to deliver iron and support the growth of a range of cell types. However, it offers a vital advantage over transferrin derived from blood. Blood-borne viruses such as HIV, as well as those causing hepatitis and TSEs (transmissible spongiform encephalopathies) are a potential risk when using human or bovine serum to extract transferrin. In comparison, recombinant transferrin produced in yeast cells is a safe, animal-free product.

### Superior to iron on its own

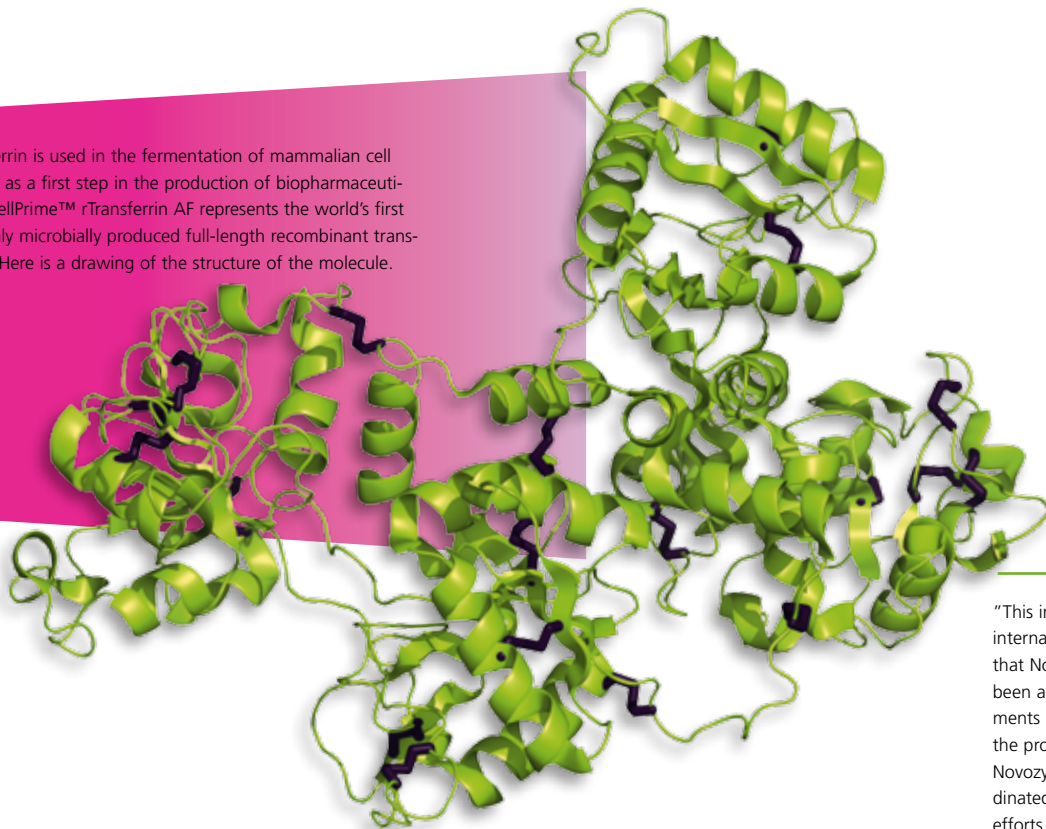
The product is loaded with iron and this explains its red color. It performs much better than simply adding iron salts to culture media because it binds specifically to transferrin receptors and facilitates the efficient uptake of iron into the cells, allowing them to perform optimally. So the cells produce a higher yield of the biopharmaceutical product.

The disadvantage of providing free iron on its own in the form of iron salts is that the iron can induce the formation of free radicals in the



Pharmaceutical companies want to go animal free in order to avoid risks. The AF in the product name stands for "animal free."

Transferrin is used in the fermentation of mammalian cell culture as a first step in the production of biopharmaceuticals. CellPrime™ rTransferrin AF represents the world's first and only microbially produced full-length recombinant transferrin. Here is a drawing of the structure of the molecule.



"This is one of the most international projects that Novozymes has ever been a part of," comments Dorrit Aaslyng, the project director at Novozymes who coordinated all the different efforts around the world.



#### TRANSFERRIN PROJECT TRULY GLOBAL

The development of the new product has involved:

- Novozymes' center of excellence in yeast expression in Nottingham, UK, where the project started
- Cell Culture R&D facility in Adelaide, Australia
- Biopharma Production and Development in Lund, Sweden
- ProMan – Novozymes' Corporate Project Management
- The Enzyme Business pilot plant in Copenhagen, Denmark
- Novozymes Biopharma cGMP production in Nottingham, UK
- Novozymes Biopharma Sales and Marketing, UK and USA
- Millipore, headquartered in Billerica, MA, USA

fermentation broth. This creates oxidative stress which causes the cells to die giving lower yields of the desired biopharmaceutical product.

"Transferrin is the optimal way to deliver iron to the cell," comments Caspar Foghsgaard, Strategic Product Director for Novozymes Biopharma's upstream products. "CellPrime rTransferrin AF behaves exactly like transferrin was designed to do by nature. We have a unique product that is new to the world and highly innovative. No one else has been able to do anything like this and make recombinant transferrin economically viable. We sell the product at a price that is attractive to the pharmaceutical industry."

#### Need for consistency

"The drug development process takes a long time, and the pharmaceutical industry wants to know if a particular supplement will still be avail-

## "Consistency determines success in biopharmaceuticals"

Caspar Foghsgaard, Strategic Product Director for Novozymes Biopharma's upstream products

able in 10–15 years. We can upscale our production of CellPrime rTransferrin AF as much as we need to meet demand. You won't find the same consistency in products derived from blood. For example, the quality of transferrin or albumin from cows varies depending on the time of year. The price can also vary due to limited supply. Our recombinant products are the same batch after batch," assures Caspar Foghsgaard. "In biopharmaceuticals, the process defines the product. If the inputs for the process are different from

batch to batch, then you are likely to see some differences in the final product. Our consistent transferrin product fits the requirements of the pharmaceutical industry."

#### CellPrime Albumin AF-G is next

"CellPrime is the brand name for Millipore's and Novozymes' product portfolio of high-quality, animal-free alternatives for use in cell culture and other upstream biopharmaceutical applications.

"The joint launch of CellPrime rTransferrin AF was a major milestone for the CellPrime alliance. It's our first product to become commercially available, and Millipore has the exclusive rights to market it worldwide for mammalian cell culture applications," says Caspar Foghsgaard.

The next challenge in the alliance is the joint launch of CellPrime rAlbumin AF-G, which will take place in April 2009. Like the new recombinant transferrin, it is also derived from a genetically modified microorganism, but in this case of the *Aspergillus* family. Albumin AF-G is a supplement aimed at large-scale mammalian cell culture applications for use in biopharmaceutical production. This new recombinant albumin is a powdered product, produced under ISO certification, that will complement Novozymes other recombinant albumin products called CellPrime rAlbumin AF-S (for use in stem cell culture) and Recombumin® used, for example, in vaccines.

#### Know-how for customers

Customer support is available from Millipore and from Novozymes' Cell Culture R&D facility in Adelaide, Australia. The team of scientists in Adelaide, headed by Tony Simula, is responsible for demonstrating how the products work under practical conditions, such as bioreactors. They also test such attributes as the stability of Novozymes'

products in media, how they should be handled, filtered, and even measured in the final medium formulation.

"We determine the best way to use our products so the customer is able to achieve the highest yields possible in their processes," says Tony Simula, R&D Director at Novozymes Biopharma AU Ltd in Australia. "A considerable number of companies have moved away from the use of serum-derived transferrin in the last 20 years due to the level of risk. Our new, safe, animal-free version will bring them back into the market for transferrin." ■

CellPrime™ is a trademark owned by Novozymes and Millipore. CellPrime rTransferrin AF is sold and distributed exclusively by Millipore.

FOR MORE INFORMATION  
Caspar Foghsgaard  
cfog@novozymes.com