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## Reprogramming Stars #14: Fast-Forwarding Cellular Reprogramming— An Interview with Dr. Mark Kotter

Mark R. Kotter<sup>1</sup> and Carlos-Filipe Pereira<sup>2</sup>

**Introduction by Dr. Carlos-Filipe Pereira  
(Editor-in-Chief, *CELLULAR REPROGRAMMING*)**

**Dr. Pereira:** Good afternoon. My name is Filipe Pereira, professor at Lund University and editor-in-chief of *Cellular Reprogramming*. I'm very happy to bring you a new episode of *Reprogramming Stars*, our flagship series capturing the findings, projects, and ideas of the leaders in cellular reprogramming.

Today, we have Mark Kotter, who is a doctor, a scientist, and a serial entrepreneur. As a neurosurgeon, he treats patients with spinal cord injuries. He is CEO and founder of bit.bio, the Cambridge, U.K.-based cell coding company democratizing access to human cells. He's also a cofounder of Meatable and clock.bio, and cofounder and trustee of myelopathy.org, the first charity dedicated to a common yet overseen condition causing slow motion spinal cord injury.

Mark was born in Canada, grew up in German- and English-speaking countries, studied medicine in Graz and obtained a PhD in stem cell biology from Cambridge. He continued his scientific career at the Max Planck Institute in Göttingen and in Cambridge and trained as a neurosurgeon at the universities of Vienna, Toronto, and Cambridge, where he currently practices.

As a scientist, Dr. Kotter is best known for discovering the importance of macrophages for brain regeneration, which inspired the first regenerative medicine trial for degenerative cervical myelopathy, and for developing opti-ox<sup>TM</sup> (optimized inducible overexpression), a gene targeting approach that enables deterministic forward reprogramming of stem cells to individual lineages and is the foundation of bit.bio. Dr. Kotter, thank you so much



**Dr. Mark R. Kotter**

**REPROGRAMMING STAR:** Dr. Mark Kotter is a stem cell biologist and neurosurgeon at the University of Cambridge and a serial entrepreneur. As a neurosurgeon, he treats patients with spinal cord injury. He is CEO and founder of bit.bio, the cell coding company generating human cells for research, drug discovery, and cell therapy, cofounder of Meatable and clock.bio and cofounder and trustee of myelopathy.org, the first charity dedicated to a

common yet often overseen condition causing a “slow motion spinal cord injury.” Mark set up bit.bio to democratize access to human cells for research, drug discovery, and cell therapies.

**for joining me today. It is a pleasure to have you featured as a *Reprogramming Star*.**

**Dr. Kotter:** Thank you so much for having me.

**Dr. Pereira:** Mark, can you tell me how you became CEO of bit.bio?

**Dr. Kotter:** This really is the result of the opti-ox technology developed in my research lab (Pawlowski et al., 2017). We discovered that by leveraging the specific properties of genomic safe harbor sites in the DNA of pluripotent stem cells, we can create a deterministic paradigm for forward programming of induced pluripotent stem cells (iPSCs) into

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somatic cell types. This was completely unexpected and nobody thought that this might even be possible. With this approach, which we called opti-ox, we can create pure cultures of somatic cells from iPSCs within days by activating transcription factors that specify cell identities. Initially, I thought someone would look at the technology and license it. But it turned out to be much too radical and novel, so the only option was to spin it out, and so I set up what's now bit.bio.

**Dr. Pereira: The possibility of creating every single human cell in a homogeneous fashion is very attractive for the field of regenerative medicine and cell therapy. What are the main aims and vision for the company?**

**Dr. Kotter:** The aim of the company is to democratize access to human cells and enable a new generation of medicines. The new paradigm of forward programming enables us to do this. There are two components to forward programming of iPSCs: the identification of transcription factor combinations that define cell types and the controlled activation of these transcription factors combinations in iPSCs. bit.bio has built an end-to-end platform to do both. We already discussed that the genomic safe harbor-driven activation of cell-type specifying transcription factor combinations results in a deterministic paradigm for manufacturing human cells that addresses three unmet needs when it comes to cell manufacturing.

First, functionality. We have demonstrated that we can program the maturity of a cell, adult versus fetal maturity, using specific sets of transcription factors. Second, we can create consistency and purity, which also has been an enormous bottleneck. And third, we can scale. We discovered that opti-ox forward programming of iPSCs is about 10 times faster than directed differentiation, and that it is also not sensitive to scale. That means it works irrespective of the volume of the tissue flask or reactor that you are using, and so, this forward programming paradigm provides a scalable solution to the fundamental problem that held back the entire stem cell field.

Another consequence of leveraging this paradigm is that we can bring the identification of new cell type programs encoded in combinations of transcription factors in-house. This is not possible for directed differentiation where you are metaphorically exploring Waddington's landscape with a torch, trying to find your way along multiple branch points where cells make fate choices. Because one can never be sure when one arrives at a protocol that will generate the desired cell type, the discovery of directed differentiation protocols has been left to the academic community. Over the past 25 years, there has been a lot of effort to create all sorts of different somatic cell types.

In contrast, the use of transcription factors as the drivers of cell identity developing a new protocol is a combinatorial problem. There are around 2,500 transcription factors in the human genome, and we know that between one and six define a cell identity. Therefore, with large-scale functional screens and sophisticated data processing, enhanced with machine learning, you can read out the programs that define cell identities. This is the core of bit.bio's discovery platform.

**Dr. Pereira: So the safe harbors in the genome and targeting the transcription factor combinations to these genomic locations are the key components that enable bit.bio technology?**

**Dr. Kotter:** Correct—within the overall paradigm shift of moving from directed differentiation to forward programming. I credit Harold Weintraub, Shinya Yamanaka, Thomas Südhof, and Marius Wernig for developing the idea: Weintraub discovered the first reprogramming protocol that turns fibroblasts into muscle cells using MyoD (Davis et al., 1987). Yamanaka demonstrated that a stem cell identity can be specified with four transcription factors (Takahashi et al., 2007), and Tom and Marius, with their work, explored whether this approach can be generalized. They have shown conversions from fibroblasts to neurons (Vierbuchen et al., 2010), from liver cells to neurons (Marro et al., 2011), and, finally, from pluripotent stem cells directly into neurons (Zhang et al., 2013).

However, to industrialize this new paradigm and to make it usable in a clinical setting, a second problem needed to be solved. First, there is a safety issue if you express transcription factors by randomly integrating constructs into the DNA of iPSCs. This will not comply with the regulators in a clinical setting because of the risk of off-target effects. We have seen this recently in CAR-T cells, where the random integration of CAR receptors has led to oncogenicity. So we need to be precise regarding where in the genome we insert genetic constructs to drive gene expression.

The second issue we found is that random integration events also cause stochasticity. This means not every cell will have the same load or distribution of transcription factors, which impact the efficiency and the scalability of the process and the quality of the cells.

We have also learned that non-integrating methods do not scale well. Take for example mRNA constructs. mRNA is usually turned over too fast to enable robust cell type transitions, which often require prolonged and consistent expression of transcription factors. This can be overcome with multiple dosing. However, this increases stochasticity and so, at least for the moment, it is not a great solution.

Another important challenge in the field was to create a paradigm that works for every cell type and with every genetic background. Our experience indicates that once we have a combination of transcription factors that work and we engineer it into an opti-ox cassette, we can turn any iPSC into the target cell. We have done this in 15 plus different cell types, and in 15 plus genetic backgrounds. With opti-ox, the transition from one iPSC cell line to another is no longer an issue.

If we put this all together, we arrive at our proprietary solution—leveraging the unique properties of genomic safe harbor sites. If you insert genetic code into a genomic safe harbor, you keep the cell safe as you do not disturb the physiology of the cell. And second, you keep the program safe: when you switch on the inducible cassettes, the cells do not silence the program. Both properties are essential.

**Dr. Pereira:** I'm curious about the increase in speed and the shift toward a deterministic paradigm. So what do you see as the major challenges to enable forward reprogramming toward all human cell types? Is it identifying a robust combination of transcription factors, or are there other challenges that you see linked with the complex developmental biology associated with the generation of these cells? What do you think is missing to make this process scalable for every single cell type?

**Dr. Kotter:** The first challenge and, at the same time, fantastic opportunity is the blank canvas that we have in front of us. We, as a scientific community, have not discovered many cell type programs yet. Moreover, if one thinks about the data emerging from the human cell atlas, the recurrent theme is that cell identity is much more complex than we initially thought. What we call a cell is often a subgroup of different subsets and the boundaries between cell identities and cellular states are difficult to draw.

But when we take a step back, we can imagine that any cellular state that is describable is somehow determined by a certain set of transcription factors. At bit.bio, we aim to understand how transcription factor programs relate to the transcriptomic states. When we pursue these programs, we are discovering the operating system of human cells.

**Dr. Pereira:** From your experience at bit.bio, do you see differences in speeds across different lineages? For instance, the lineages that emerge in the embryo first would also be generated faster with forward programming, rather than the ones that come later in development?

**Dr. Kotter:** We and others have looked at time-course series of what happens during forward reprogramming and found that it is not entirely synthetic. The transition from pluripotent stem cells to somatic cell types seems to follow a developmental trajectory, but greatly accelerated.

In our hands, the range of time it takes to program iPSCs into a *bona fide* cell type is between 4 and 10 days. We see differences between different cell types, but there is no clear relationship between cells that take longer in human development, and those that require longer reprogramming trajectories. However, there are some cells, for example, oligodendrocyte precursor cells, that come very late during development, and that are difficult to program. But I wouldn't say there is a clear correlation between the two.

**Dr. Pereira:** What was the most surprising finding from running these 15 different programs to generate these 15 different cell types by forward reprogramming?

**Dr. Kotter:** What is really amazing is the consistency of opti-ox reprogramming. Last year at International Society for Stem Cell Research (ISSCR), we released data showing that there are no differences between batches of our research grade glutamatergic neurons. We used bulk mRNA-Seq techniques and revealed that there was not one differentially expressed gene between batches of cells from three different manufacturing runs. And looking at the representation of the cells on single cell RNA-Seq and ATAC-Seq UMAP plots,

these are identical. I thought that we were doing well when it came to reproducibility, but to show that bit.bio can produce with such extreme biological consistency is not something that I expected. We do not have a good scientific explanation for it yet, but it is real, and we are working on the why.

**Dr. Pereira:** Relating to the current stage of development of the company, what is bit.bio doing now? I know that you announced a cell therapy to use hepatocytes for liver failure. Can you tell us more about the current operations and projects?

**Dr. Kotter:** The company is now nearly 5 years old, and we have about 200 employees. We have raised significant capital—almost \$200 million—and we were able to industrialize both the creation of new cell types via the identification of new cell type defining transcription factor combinations, and our manufacturing paradigm. Our aim is to democratize access to human cells. There are two key opportunities, two big unmet needs that we are addressing: the development of cell and regenerative medicine therapies, and the use of human cells as tools to help bridge the translation gap. For various reasons mainly related to the novelty of the technology, we started with the creation of research-grade cell products. A few years later, the feedback that we are getting is that bit.bio's cells are best-in-class.

Over time, our confidence has grown, and hence, we decided to make that next leap with the announcement of a clinical pipeline late last year. I am very excited about our lead candidate based on hepatocytes for the treatment of acute liver diseases, a very serious group of diseases with high mortality rates and unmet needs. Previous studies used donor-derived hepatocytes and, while small in study size, completely changed the outcome for patients. Specifically, from an 85% to 90% chance of mortality without transplant, these have indicated that you can reverse the odds of survival back to a 90% chance of a good outcome. This is truly transformational. We will replicate these studies and use cells created by bit.bio instead of donor-derived cells. In this indication, the cells are only required transiently, so they can be encapsulated and ectopically implanted. This reduces the complexity on our way in the clinic.

**Dr. Pereira:** Is it autologous or allogeneic?

**Dr. Kotter:** bit.bio focuses on allogeneic treatments because we need to make cell therapies accessible. Currently, cell therapies can cost up to \$600,000 per dose to manufacture, which means these transformational therapies are reserved for a minority. It really restricts the application of cell therapies. Hence, at the moment, we can only treat late-stage cancers, for example, with CAR-T cells. But would you not want a single-curative treatment as soon as your cancer has been detected and avoid conventional chemotherapy and radiation? It is not possible, at the moment, because these therapies are too expensive.

Consequently, the U.S. and U.K. governments stated the need to fundamentally change the economics of cell therapies. The aim of the U.S. Executive Order, released last year, was to reduce the cost of goods for cell therapies by

one order of magnitude. At bit.bio, we believe that we can reduce the manufacturing costs of cell therapies by two orders of magnitude. This is truly transformative as it means that you can produce therapies for the many, not the few, and that these can be applied to earlier stages of disease.

**Dr. Pereira: I am aware of the manufacturing problems in cell therapy and the several start-ups using very creative manufacturing approaches for generating more cells, like lasers, and so on. But it is an important challenge that the field has yet to overcome for cell therapy to become a blockbuster.**

**Dr. Kotter:** I think the potential of these therapies is incredible. So we need to ask how do we get these therapies to every patient that might benefit?

**Dr. Pereira: When is bit.bio becoming a clinical stage company?**

**Dr. Kotter:** The earliest we can go into the clinic is next year. This is not that far off...

**Dr. Pereira: It's amazing that bit.bio is so close to the clinic. For the drug screening approaches, was this done mainly in collaboration with others or will you develop your own molecules?**

**Dr. Kotter:** We are a product business, and our products are cells. Whether they are clinical, with clinical packages, or whether they are research grade. On the research side, we enable large-scale drug screening campaigns, which usually require billions of cells. Conventional directed differentiation struggles to produce the volume and, of course, the consistency of cells. Because of the opti-ox technology, we can do this without blinking an eye. On the cell therapy side, we are also enabling partners to use our cells. For example, we recently announced collaboration with BlueRock Therapeutics, which is an independent subsidiary within Bayer AG.

**Dr. Pereira: I've been in the field of cellular reprogramming for 20 years, and it is really striking to see how the landscape has changed in the last 6 years or so. From an exclusive academic pursuit to a field where companies emerge and are working hand-in-hand with academics.**

**Dr. Kotter:** I agree. Six years ago, we were at the absolute fringe and the then director of my institute told me I should not be a part of the Stem Cell Institute because cell reprogramming is not stem cell biology. Now, when you go to the ISSCR conference, all the main labs seem to be moving into the cell programming space because they recognize that it is the only option to industrialize the manufacture of cells—the only way to get real precision is to use a programming paradigm.

**Dr. Pereira: What do you think changed and motivated investment in cell reprogramming companies like yours and others?**

**Dr. Kotter:** If you think about stem cells or stem cell-derived cells as a new modality, you expect it to take 15,

maybe 18 years to get to an approved therapy. In the case of CRISPR, we saw from invention to approved drug, a period of only 12 years, which are extraordinary. In the case of stem cell-derived cells, it has now been nearly 26 years and nothing has been approved. We have had a few early-stage clinical trials that look promising, but there is still some way to go until an approval. So I think the sentiment among investors was that conventional differentiation alone is probably not going to work. They were looking for an alternative, and I really do think that cell reprogramming can offer a solution.

**Dr. Pereira: I agree. Can you please highlight the main challenges for someone thinking about starting a company in this space? What are the main challenges to overcome right now?**

**Dr. Kotter:** For a platform company starting out today, it may already be a bit late. The market conditions are not very favorable at the moment. It is more likely that investors will request you to focus on a pipeline of assets, rather than building a company that has the ambition to recreate any human cell type, like bit.bio, because that requires substantial amounts of capital. What we have built will, I believe, be very hard to replicate because of the capital needs and the expertise that you have to bring together.

The second thing I would say is that with focus, there is a huge opportunity because we are at the beginning of developing this new modality. Take, for example, the many companies that exist in the mRNA space. Before mRNA, antibody technologies were a major driver creating an entire industry with many large and small players. So, overall, the opportunity in front of us is huge, and the more people join and develop this new modality, the better for patients.

**Dr. Pereira: So your suggestion is to have a certain focus. I am curious... what does it take to close a \$150 million series B round of investment in Europe?**

**Dr. Kotter:** On the one hand, you need a unique technology that enables certain breakthroughs and is protected by a very strong IP position. On the other hand, you need a rockstar team. I was very lucky that people involved in bit.bio from the beginning had deep experience building companies, deep experience creating and leading commercial organizations, and developing cell therapies. The combination of these factors was really what helped to close out a financing round like that.

**Dr. Pereira: Do you have any advice for medical doctors or scientists considering becoming a CEO?**

**Dr. Kotter:** When you make the move from academia to industry, you learn a few things. Academia is essentially a single-player game, it is very individualistic and competitive. When you make the transition to industry, you must leave this behind; you need to bring people together to work on the big program or question that you have formulated. So you must substantially change your context.

You have also got to be willing to learn new things that you have never heard of before, whether it is on the finance side,

on the human resource (HR) side, or commercial, and you need to figure out how these pieces fit together. This is a problem set that we do not face in academia. The growth from a small, early-stage company to a mid- or late-stage is also incredibly demanding. The entire company culture, which I think is very important, has to adapt and change, and you need to find the right talent for the right stage. To be a CEO, you need to be willing to lean in and accept all these challenges. It is hard but also very exciting.

**Dr. Pereira: I totally agree, culture is key. What's your approach to define culture and to refine the culture while the team is growing?**

**Dr. Kotter:** Culture is foundationally important, so we were very conscious about creating a framework and defining a culture at bit.bio at a very early stage. When we were just three to four people, we agreed on a set of values.

First, purpose. You need to be purpose-bound, and your purpose needs to match the purpose of the company. For bit.bio, this is to democratize access to human cells and enable a new paradigm in medicine. This creates an umbrella. If you have a scientific interest in reprogramming, bit.bio is the right company for you. If you want to treat patients, it is the right company for you. If you are interested in disease models, it is the right company. If you are interested in finance and want to have an impact on people's lives, it is the right company. So be very specific with your purpose.

The second value that we formulated is ambition, and, of course, there are two sides to ambition: selfish ambition, or ambition toward the purpose. We focused on people that are ambitious toward their purpose and try to eliminate anyone with selfish ambitions.

The third is trust and collaboration. These go together because trust is the basis for collaboration. Again, I think there are two kinds of trust. One of them is: I have known you for 10 years, we've always been on the same side therefore I trust you. But there is another kind of trust where you think, "Hey, I don't know you, but I trust you and if you do something wrong, I'll let you know and we can change it—and vice versa." I call this trusting forward—it is very powerful but very hard to embed into a company.

And then, finally, we also landed on being empirical and data-driven as an important positive value. Like most companies, we are not quite there, not quite living our values 100% every day. It is very hard and you have to work at it every day. But when other people visit bit.bio, they do get a sense that this company is different. I think of bit.bio more as a movement than a traditional company.

**Dr. Pereira: In one of the meetings you organized in London, I saw the deck of cards produced by bit.bio, each card with a single cell. I thought it was a brilliant idea to create a strong sense of purpose in the team to generate all those cells.**

**I would like to end the interview with some questions that are not strictly related to science, so the audience of**

***Cellular Reprogramming* knows you better. The first one is, if you could answer any scientific question regardless of your expertise or chosen field, what would it be?**

**Dr. Kotter:** I made the transition from physics to medicine relatively early in my life. I started off studying maths, and the reason why I changed to medicine was because, somewhat naively, I was not able to see how I could have positive impact. This seemed much clearer in medicine and the change helped me to get out of what felt like an existential crisis. Now I am in a very privileged position where I am asking the two questions I am most passionate about.

The first question is: what is cell identity? What defines a cell? This is a foundational question in biology, and bit.bio is really the engine that asks that question every day. What we know already is that it is a transcriptional state that is driven by transcription factors. Now, we want to understand this more deeply, including using and building AI models that help us decode and deconvolute the relationship between transcriptional networks and transcription factors. To me this is absolutely fascinating.

I also started another company recently called clock.bio, which is a reprogramming company focusing on rejuvenation. And, in that context, I would say people often ask the question, "What is aging?" I fundamentally think it is the wrong question. The question should be: "What is youth and how can we prolong or reenter a youthful state?" So that is the second foundational question in biology. I am in an incredible situation where I am able to pursue research in two fundamental areas of cell biology.

**Dr. Pereira: I agree. Many of us are interested in these two questions! What is the biggest misconception about science-based start-ups that you would like to resolve or to see resolved?**

**Dr. Kotter:** I would say, as an academic, I tended to believe that the science in companies might be less sophisticated. I would say that is fundamentally wrong. The science that we now do in bit.bio is incomparable to what we were able to do in the academic lab. Within bit.bio, there are 50 or 60 people working on cell type discovery at the moment—a combination of synthetic biologists, cell type biologists, stem cell biologists, bioinformaticians, and AI experts. You cannot build this in academia. So that is probably one assumption in the heads of academics that needs to change.

The second misconception is the notion that you cannot ask deep scientific questions within a company context because you always have to create a "product." For bit.bio, this is not correct because we ask the most fundamental question: what is cell identity? How is a cell defined? Are there any generalizable patterns? And the moment we can answer this question for a particular cell type, we have a new product. We are incredibly privileged because we ask the most foundational scientific questions—and once we have the answer it translates into something tangible.

**Dr. Pereira:** As an entrepreneur myself, I was also shocked with the level of scrutiny and scientific rigor in every interactions I had with venture capitalists (VCs).

**Dr. Kotter:** Exactly. Think about the suggestion that 50% to 60% of research findings published cannot be reproduced. You cannot afford this in a company setting. You must find something that works reliably and consistently and not only in a specific lab for a specific set of experiments.

**Dr. Pereira:** Dr. Kotter, thank you so much for joining me today. It was really a pleasure to know more about bit.bio and yourself. So thank you so much for your insight.

**Dr. Kotter:** Thanks so much for having me again. The pleasure was mine.

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