Researchers in Germany's Rhine-Neckar region are connecting to make scientific breakthroughs in health and bioscience.

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HEALTH AND BIOSCIENCE BRILLIANCE: FROM IMAGING BRAIN TUMOUR NETWORKS TO BUILDING CELLS FROM SCRATCH

HOW SUPPORTIVE INFRASTRUCTURE AND KNOWLEDGE SHARING is boosting biomedical science in Germany

"There has not been much progress in brain tumour treatment in recent decades," says Varun Venkataramani, physician-scientist at the Heidelberg University and University Hospital Heidelberg. Initially, research conducted on other cancers was repurposed, in the hope that these ideas would also work for brain tumours. "But this has failed," says Venkataramani. "The truth is we don't fully understand the biology of brain cancers yet."

Venkataramani's research focuses on treatment-resistant gliomas — aggressive tumours that infiltrate and colonize the brain. After recent advances in imaging technologies and collaboration with other research groups in the Heidelberg region, Venkataramani's team were able to track the development of single brain tumour cells. They discovered that these cells form intricate multi-cellular networks, both between themselves and with other brain cell types.

"This opens a new field within brain cancer research, right at the edge of image analytics, oncology and neuroscience," says Venkataramani. "The Health + Life Science Alliance is a vital source of collaboration and infrastructure for our work."

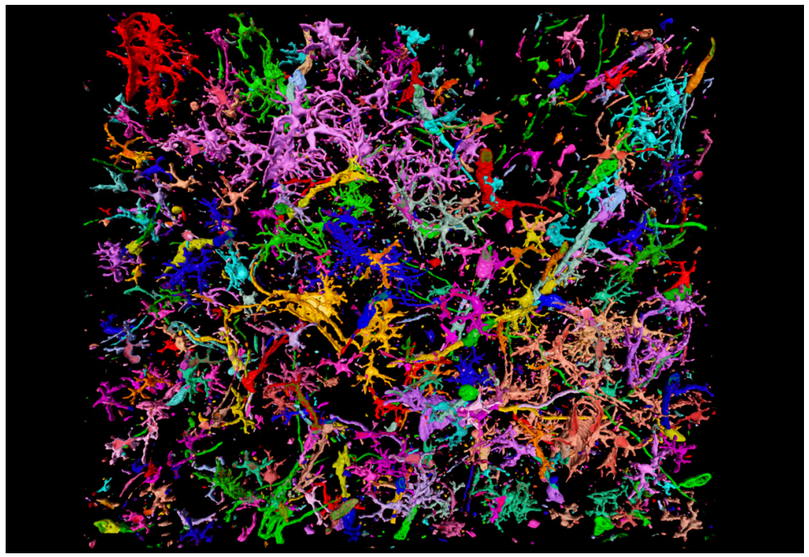
UNDERPINNING COLLABORATIVE BIOMEDICINE

The Health + Life Science Alliance, founded in 2022, is an inter-institutional public benefit company that supports cross-disciplinary research across biomedical sciences in Germany's Rhine-Neckar region. The Alliance comprises seven leading academic university and non-university institutions in Heidelberg and Mannheim, providing infrastructure and funding to enable scientists to pursue their research goals. The Alliance's aim is to become the leading health and life science campus in Germany — deepening the strong links between hospitals, academic institutions and industry to ensure that research is translated and applied effectively.

The Alliance can bring together leading minds, like Venkataramani and Anna Kreshuk, a computer scientist at the European Molecular Biology Laboratory in Heidelberg. Their team are creating novel analytical tools for imaging the intricate networks created by brain tumour cells.

"All scientists are driven by curiosity and we flourish when continually learning from each other," says Kreshuk. "The Alliance channels this wish to connect and collaborate."

"The Alliance facilitates interactions and then actively supports those newly formed relationships," says Venkataramani. "I was delighted that Kreshuk and her team were keen to help us further understand brain tumour networks."



▲ Novel analytical tools for brain tumour imaging show that these cells form intricate multi-cellular networks, both between themselves and with other brain cell types.

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THE BRAINS BEHIND TUMOUR IMAGING

"As an image analyst and technology developer, working with brains is very exciting; you don't often get more complex and dynamic data than this," says Kreshuk. Neurons are already extremely challenging to image because they are thin, long and difficult to segment, even more so when they are imaged *in vivo*. Tumour networks add another step within this puzzle because they move continuously — the cells create irregular shapes, extending and morphing to make new connections, Kreshuk adds. "This process is a fascinating challenge to capture."

As well as answering questions about key biological processes, the team hopes that their imaging techniques will help identify potential treatment targets. By automating image analysis using machine learning, the researchers can identify patterns more quickly than ever before.

"For example, we recently worked together to automatically identify white matter tracts and blood vessels in brain images," says Venkataramani.

Kreshuk and her colleagues relish the challenge of optimizing algorithms that are not trained on vast datasets. "No-one has done this kind of research before, so we don't have data to use from hundreds of experiments," she says. "It's more than just doing the same things as humans do, but faster. AI is now finding patterns that humans can't easily distinguish."

The Alliance supports the use of state-of-the-art technologies by sharing resources and tools between institutions. The Alliance also hosts regular lectures, seminars and training sessions across the region, enabling people from different disciplines to meet. Kreshuk and Venkataramani will launch a proof-of-concept trial (EuDRACT 2023-503938-52-00) designed by Frank Winkler, a leading neuro-oncologist at Heidelberg University and the German Cancer Research Center.

THE POWER OF RATIONAL DESIGN FOR GENE THERAPY

Kerstin Göpfrich is a biophysicist at the Center for Molecular Biology of Heidelberg University (ZMBH) and the Max Planck Institute for Medical Research. Her career goal is to build a fully functional synthetic cell. The cell will consist of a lipid vesicle operated by engineered molecular machinery, and the functional components will be built using DNA and RNA origami.

"The beauty of our research is that researchers can use the tools we're creating now, long before we have a functioning synthetic cell in our lab," says Göpfrich. "Through the people we're meeting within the Alliance, we're harnessing the full potential of every element of our progress."

Göpfrich's origami tools came to the attention of Dirk Grimm, an expert in viral vector technology and gene therapy at the Medical Faculty of Heidelberg University. The pair now work together to optimize the targeted delivery of gene therapies using adeno-associated viral vectors (AAVs). AAVs are used as carriers to take new genetic material, such as the code to repair a mutated gene, to the correct place in the body. AAVs have been used in gene therapies for diseases including Alzheimer's Disease, spinal muscular atrophy and cancers.

AAVs consist of a protein shell — or capsid — which holds a single DNA strand. The viral DNA strand is small and very simple, which makes it easy to engineer to carry therapeutic genetic material. However, there are limitations associated with current AAVs. For example, if there is any deficit in the DNA packaging process, then the AAVs releases a potentially

inactive cargo into patient cells. Moreover, even intact particles often require very high doses to effectively reach a target organ or cell in the body and to express the encoded transgenes at therapeutic levels. This flood of foreign particles into the body can be toxic and may trigger over-active immune responses.

"We've witnessed some serious side effects with AAVs recently, so there is pressure to improve the quality of these products," says Grimm. "For several decades, we've worked almost exclusively 'top down' in AAV development. We've made gigantic libraries of billions of viral variants and screened them extensively," he adds. "But none of this is rational design."

"Engineering purpose-fit AAVs from scratch in a 'bottom up' manner should allow us to block these potentially harmful effects," says Göpfrich.

Synthetic cells and bottom-up synthetic immunology have the potential to transform gene therapies and many other areas. The newly established faculty for engineering sciences at Heidelberg University will explore these techniques: enhancing knowledge of the basic biology of adeno-associated viruses, helping to overcome packaging issues, safety concerns and more.

FOLDING IN THERAPEUTIC GENETIC COMPONENTS

Another limitation to AAVs, which Grimm hopes the origami techniques can tackle, is that the virus capsid is rigid and cannot expand infinitely.

"If we want to package a gene larger than 5000 bases, it will not fit. This applies to many relevant therapeutic genes," says Grimm.

One solution is to split the therapeutic genome into pieces and deliver it in two or three capsids. However, all capsids must enter the same cell simultaneously and work together to reassemble the full-length therapeutic gene. This is technically very challenging, especially when applied in patients. But DNA and RNA origami techniques could prove invaluable here.

"DNA origami is the art of folding DNA," says Göpfrich. "It exploits complementary base pairing for architecture purposes."

The technique typically starts with single-stranded genomic DNA from a bacteriophage which is folded into the desired shape with about 200 short synthetic DNA pieces. These short DNA 'staples' are designed computationally. Each position on the DNA origami thus is a unique sequence and is therefore addressable by chemical functionalization. In this way, it is possible to link AAVs with DNA origami.

"We can thus deliver larger AAV-packaged genomic cargos by linking individual AAVs with a DNA origami," says Göpfrich. An RNA origami is slightly different: it is produced by transcription from a gene template. "The beauty is that DNA and RNA origami are programmable. We can position components precisely how we want them and determine their function."

The team could feasibly fold the DNA of larger therapeutic genes into AAVs — opening the technology to treat numerous diseases. Grimm also hopes to incorporate guide RNAs in combination with Cas9 protein.

"With most types of gene modification, you want a hit and run mechanism: a short boost, and then it should disappear — not expressed in the body long-term," says Grimm. Current vectors activate once they're inside the cell, and there is typically no way to turn them on and off, or to regulate activity. "This leaves us with very little control," he adds. This problem could be alleviated by delivering at least part of the gene editing machinery, such as the Cas9 nuclease, only transiently as an origami-coupled protein rather than a constitutively expressed gene.

▲ **To optimize gene therapy delivery, Kerstin Göpfrich uses DNA origami to package large genes in small viral vectors.**

BUILDING ON A VIBRANT REGIONAL ECOSYSTEM

The Rhine-Neckar region already has a high number of start-up companies based on the scientific research conducted there. This ecosystem is being enhanced through the inter-institutional collaborations enabled by the Alliance, which is encouraging researchers at all levels in academia, together with experts in industry and entrepreneurs, to consider joining this flourishing community.

According to the QS World University Rankings by Subject 2023, Heidelberg University took first place in life sciences and medicine in Germany. The Alliance continues to support the development of the region as a premier location for health and life science in Europe.

"The Alliance has opened many doors in my own research that I never dreamed I would be working on," says Göpfrich. It's a wonderful environment for students and early-career researchers, because they can see highly specialized technologies being developed step-by-step, and then witness how collaborators in other fields use the tools directly, she adds.

"If you come with an open mind, and a readiness to share data and results," Kreshuk says, "then this is an excellent, nurturing place to be." ■

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