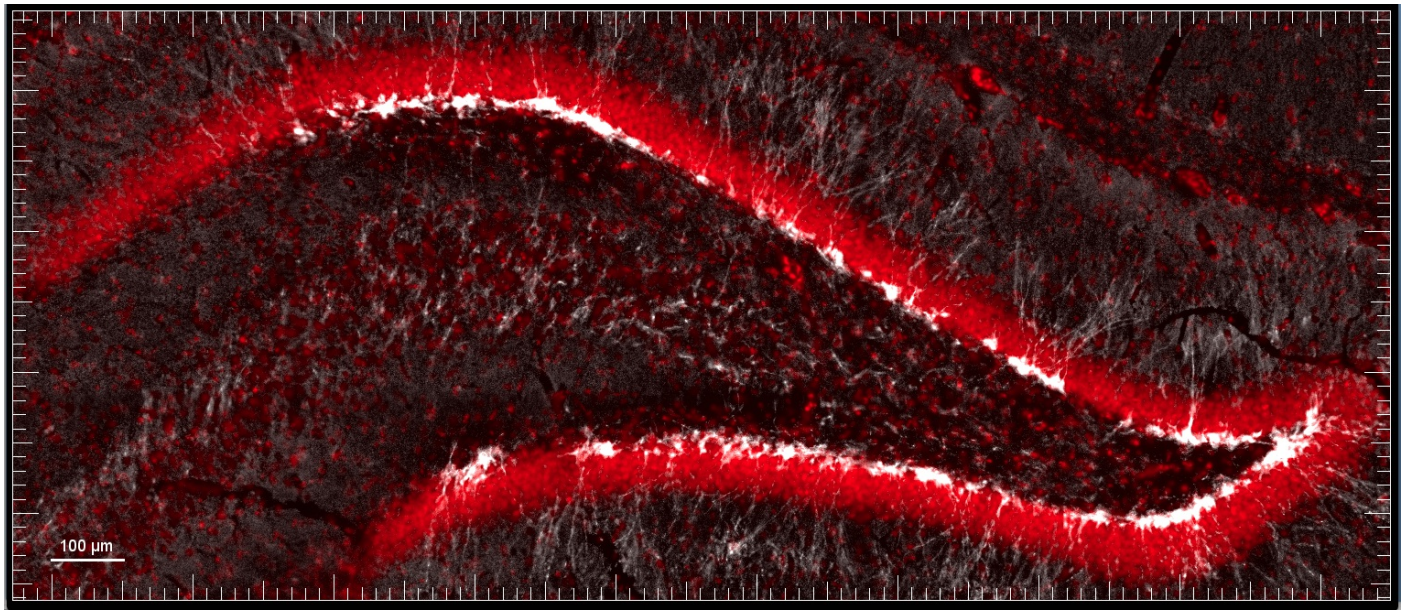
Combined test systems to advance the development of drugs for treating Alzheimer's

Which substances are suitable for treating neurodegenerative diseases such as Alzheimer's? Due to complex biochemical relationships, testing suitable drug candidates is difficult, especially in the early drug development phase. Many predictive test systems only cover individual aspects. A team from Baden-Württemberg and France is now combining different models to develop a new approach.

The search for drugs to treat Alzheimer's is going on around the globe. It is a high pressure quest that requires a great deal of manpower. Identifying potential drug candidates is one thing, testing them as early as possible in the drug discovery process for their actual suitability as a drug is a completely different challenge. Efficient predictive test systems are particularly lacking in the early drug development phase. Such systems would bring considerable benefits: the sooner the range of potential drug candidates can be narrowed down to a few with proven efficacy, the less time and resources would be wasted on substances that later turn out to be ineffective.

The EU has been supporting the A-ADAM project since June 2017 under its EUREKA-Eurostars programme. A-ADAM is a German-French research network that develops special test systems for early development phases of drugs for treating neurodegenerative diseases. The Eurostars programme promotes European cooperation in research and development projects that involve small and medium-sized enterprises (SMEs). The goal is to increase the number of innovative products and processes "Made in Europe". Two of the three A-ADAM partners come from Baden-Württemberg: the NMI Reutlingen, where Prof. Dr. Hansjürgen Volkmer is in charge of project management, and CeGaT GmbH from Tübingen. The French partner is a company called E-PHY-SCIENCE from the city of Valbonne.

Red or green light for drug development: combination tests are expected to find out



The effect of drug candidates on diseased peptide deposits in the brain can be investigated using a mouse model. The picture shows an overview of the dentate gyrus of the rat (part of the hippocampus). The nuclei of nerve cells are stained red; newly formed, immature nerve cells are shown in white.

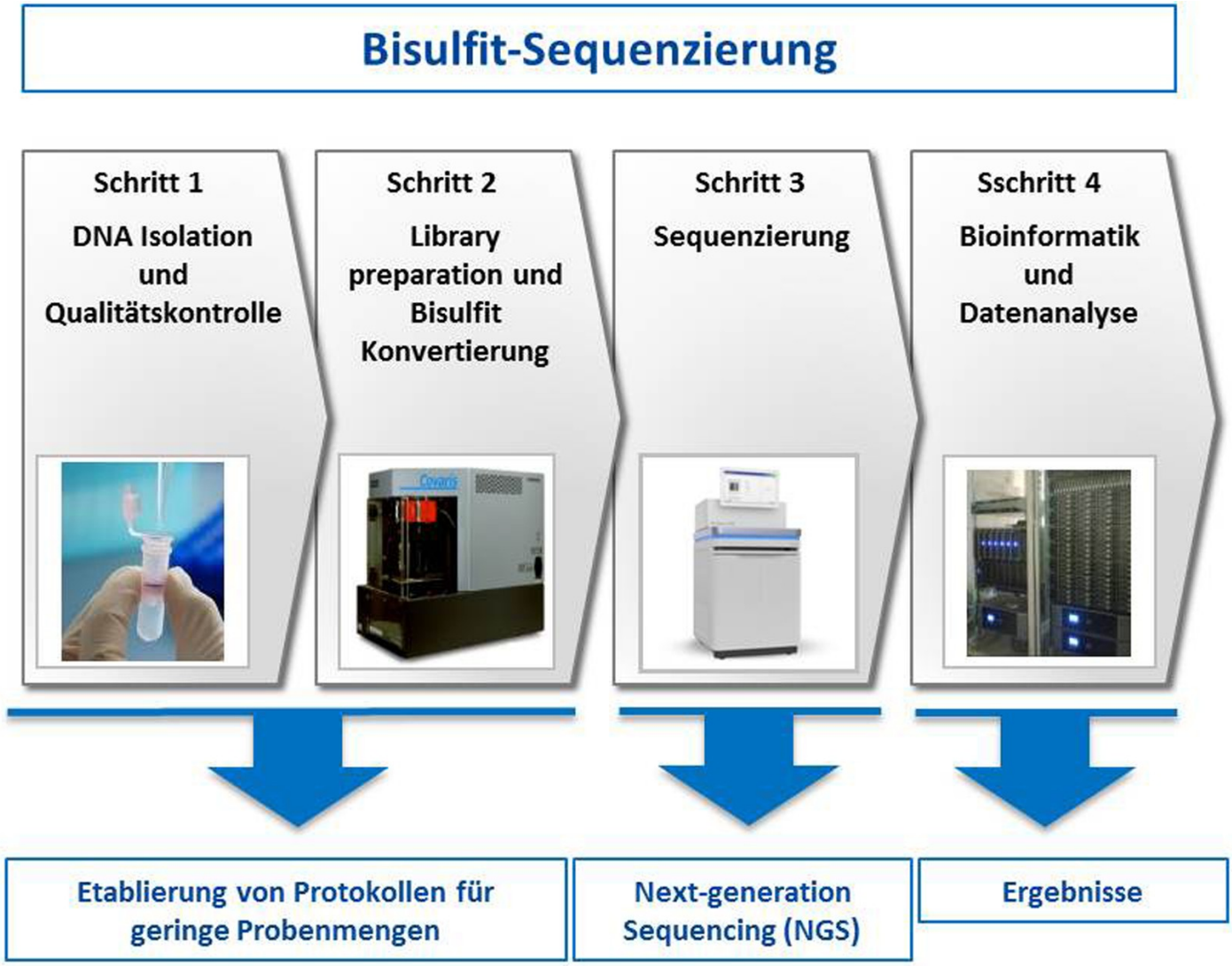
The researchers are seeking to develop a new combination of in vivo and in vitro test systems that make comprehensive early testing of drug candidates possible. Volkmer explains what this implies: "Although some disease-causing and inheritable gene mutations have already been identified for the familial form of Alzheimer's disease, the vast majority of people affected suffer from the sporadic form of Alzheimer's disease with no definable genetic influence. The major risk factor here is simply a person's age. Therefore, we are trying to visualise ageing processes of neurons in vivo and in vitro and combine them with familial risk factors." The researchers expect that the effect of potential drugs on these models will provide the basis for deciding whether or not to develop a particular drug candidate.

The in vivo part of the project is being carried out by E-PHY-SCIENCE, a specialist in electrophysiological test systems. The company brings to the A-ADAM project a model for familial Alzheimer's disease. The model is genetically modified mice that show pathological changes similar to those that occur in Alzheimer's disease patients. The mice accumulate pathologically altered peptides, i.e. amyloid beta, in their brains not because of old age, but because of targeted genetic intervention. "Genetic mouse models are based on rare familial mutations. These mutations accelerate the generation of pathological deposits from cleavage products of amyloid precursor protein, APP for short, or hyperphosphorylated Tau," says Volkmer. Aggregates of amyloid beta peptides are a hallmark of Alzheimer's: an excess of amyloid beta peptides leads to deposits, so-called "senile plaques", in the brain, while hyperphosphorylated Tau proteins aggregate into neurofibrils that accumulate in nerve cell projections (axons), thereby impeding their function. Both deposits disturb the transmission of signals between the neurons in the brain. E-PHY-SCIENCE is hoping to detect these signals and the effect of a potential drug on them in its model using electrophysiological measurements.

However, the model lacks the characteristics typical of ageing processes. The reduced activity of the mitochondria, i.e. the power factories of cells, has been

identified as a major factor that drives the ageing process of neurons. Reduced mitochondrial activity results in lower energy production, which ultimately leads to the age-related impairment of neuronal function, an important feature of Alzheimer's disease. Researchers at the NMI have resorted to their "molecular box of tricks" in order to recreate age-dependent mitochondrial impairments. Amongst other things, a method known as RNA interference is used to inhibit an enzyme in the respiratory chain called cytochrome C oxidase that is involved in the mitochondrial energy metabolism. The researchers at the NMI have already shown that this intervention alone reduces the number of synapses on the nerve cells. "And this, i.e. the reduced number of synapses, is characteristic of the early stages of Alzheimer's disease," says Volkmer. Together with the E-PHY-SCIENCE team, new models will now be generated by combining genetic animal models for Alzheimer's disease with an animal model for age-dependent mitochondrial impairment.

CeGaT wants expand its current business fields with epigenetic analyses



The diagram shows how bisulphite sequencing works and which devices are needed. The cytosine building blocks of the DNA are converted into uracil building blocks, which leads to a different sequencing signal. This signal allows the researchers to draw conclusions about DNA methylation, and hence epigenetic modifications of the DNA sequence.

The network of test models also addresses a third important aspect, which genetic diagnostics company CeGaT is working on. CeGaT establishes epigenetic analyses based on the brain material supplied by E-PHY-Science to substantiate the results of the drug tests. "This project takes us into the realm of epigenetic analyses, which we intend to turn into another business segment," says Britta Merz, project manager at CeGaT. Epigenetic modifications are, amongst other things, changes in the DNA methylation pattern. Epigenetic changes in the DNA of nerve cells can be a reaction to certain environmental factors that favour Alzheimer's disease. CeGaT GmbH wants to develop a test system that can be used for detecting Alzheimer-specific methylation markers that are suitable for early drug testing - but also for medical diagnostics applications. "There are studies that link changes in DNA methylation to the pathogenesis of Alzheimer's. If we were able to establish a simple blood test, we might one day be able to diagnose early disease stages," says Merz daring to look far into the future.

Each of the three partners can use the results generated in the project for their own purposes and for the further development of their own test systems. "Our technologies differ quite a bit and we use the interactions for different utilisation models," says Volkmer. The NMI itself aims to use the collaboration to develop high-throughput in vitro test systems that can be used for assessing the effectiveness of drug candidates in different neurodegenerative disease indications. "In our nerve cell cultures, for example, we want to measure calcium levels as the equivalent of electrical activity and thus make statements about neuronal function with and without the addition of a drug of interest. We are in the process of developing an experimental, high-throughput system that will later be automated. Our goal is to build a robot routine for the tests," explains Volkmer.