1 Biosensor for blood testing at home

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Just eat what tastes good? This is absolutely taboo for many metabolic diseases. A strict diet and regular laboratory tests are part of everyday life – a psychological and social burden. Our latest research results could soon bring great relief to these and many other patients: "protein engineering" enables the design of artificial biosensors for rapid and autonomous blood value checks.

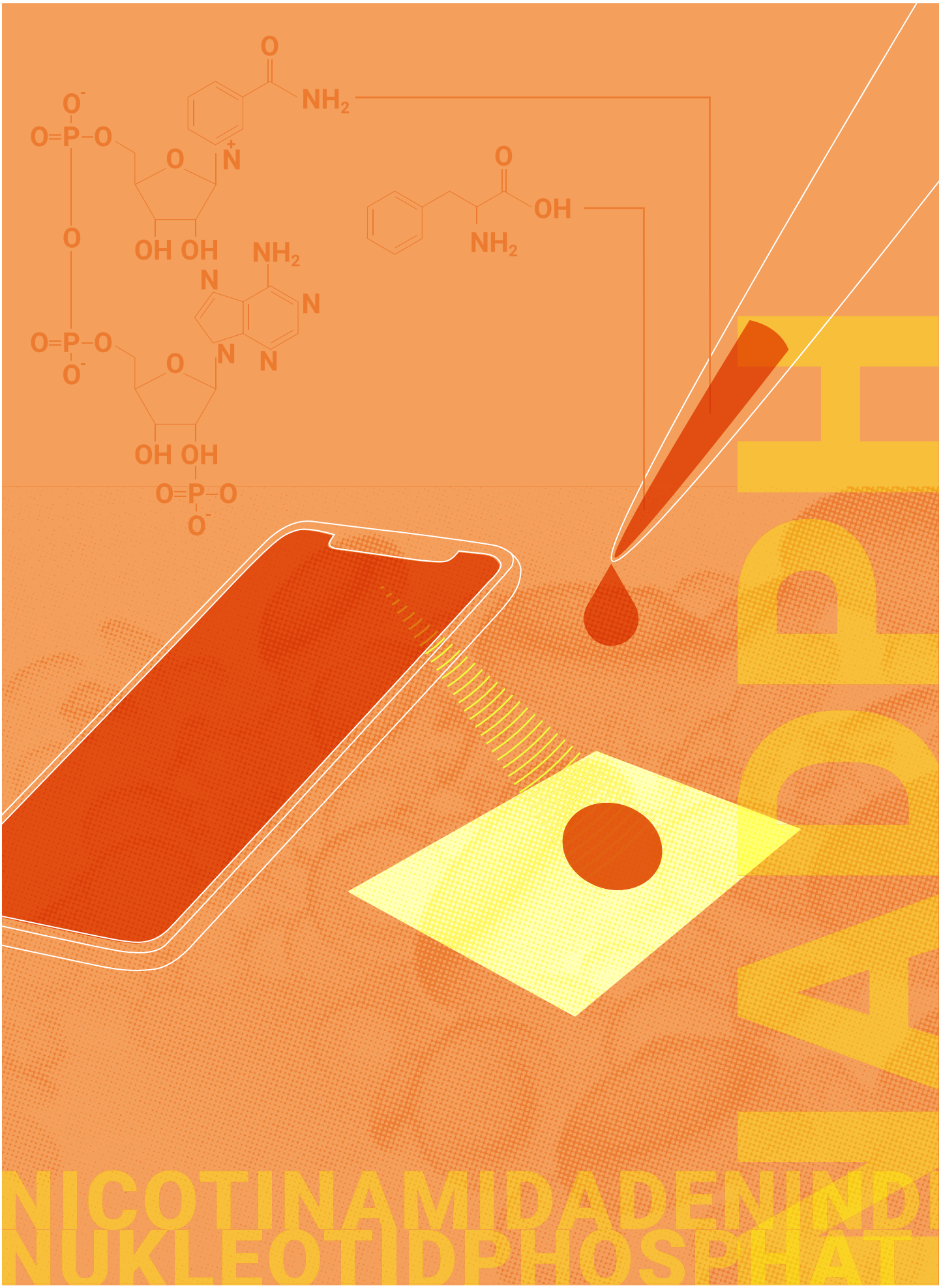
Doctors usually diagnose the metabolic disease phenylketonuria within the first few days of a baby's life. Research into this hereditary disease, which affects one in every 10,000 children, has been ongoing since the 1970s. The amino acid phenylalanine accumulates in the blood due to a non- or only partially functional phenylalanine hydroxylase enzyme and can damage the growing brain. In everyday life, the disease means not eating most natural foods. Instead, those affected have to take special protein substitutes, because the amino acid tyrosine, which the body produces from phenylalanine, is vital for us.

It is important for the parents of sick children to know their children's phenylalanine levels, as this is the only way for them to assess the leeway for a normal life. Did the kid "sin" on the school trip? In the morning, a muesli bar was eaten in the kindergarten without permission: could that have consequences? How much restriction must there be after that? However, getting a phenylalanine blood value means visiting the doctor for a blood test, then waiting two to three days for the result, by which time the school trip and muesli bar are long forgotten.

Blood value for dosage of drugs

Drugs that can improve tolerance to phenylalanine in about 30 percent of patients have been approved for adults. In this context too, the laboratory test result is an important tool for setting the correct dosage and checking the efficacy of the medication. And for pregnant women who suffer from phenylketonuria, the strict avoidance of phenylalanine is particularly important to avoid placing the unborn child at risk.

Being able to monitor the blood concentrations of phenylalanine in a timely and patient-friendly manner would significantly improve the everyday life and quality of life of patients. But for such a project, we researchers have to dig deep into our biochemical bag of tricks: the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technology we used to bring such an analysis within reach is known as "molecular engineering".

Blood values from the lab

Currently, metabolic products or other biomarkers in blood samples are mostly detected using conventional laboratory methods. Most attempts to achieve a prompt diagnosis at the patient's bedside or in the patient's home environment aim to reduce the size of existing analysis technology.

Our method, on the other hand, is based on a fundamentally different approach. It is based on a novel molecular tool, a so-called semisynthetic biosensor, which consists of a multifunctional protein and an artificial dye that is covalently linked to the protein.
The protein itself consists of two components: a luciferase – an enzyme that emits blue light, and is responsible, for example, for the glow of fireflies; and a receptor for the dye. The special trick: this receptor can only bind the dye in the presence of the co-factor nicotinamide adenine dinucleotide (short: NADPH). If the dye now binds to the receptor and thus comes close to the luciferase, the colour of the light emitted by the luciferase changes from blue to red. This colour change is even visible to the naked eye.

Molecular engineering thus produces a luminous biosensor that changes its colour in the presence of the NADPH cofactor. One molecule of NADPH is formed for each molecule of phenylalanine in an enzymatic reaction. This couples the NADPH concentration to the colour of the biosensor and the amount of phenylalanine. The amount of NADPH produced is therefore directly proportional to the phenylalanine concentration. The colour change from blue to red can be measured with a simple camera and the phenylalanine concentration can be calculated from this value.

However, the same biosensor can also be used for the detection of other metabolites by forming an NADPH molecule for each molecule of the metabolites in a specific enzymatic reaction. We have already shown that our sensor can not only be used in the laboratory for determining the blood concentrations of phenylalanine but also of glutamate and glucose – and there are many other possible applications.

How is the test done? First, a small drop of blood is obtained with a simple lancet of the type familiar to diabetics from the sugar test. A small portion of this sample is then diluted with enzymes that convert the phenylalanine contained in the sample into the corresponding amount of NADPH. The sample is then applied to a test strip with the dried biosensor. The biosensor begins to emit light, which is recorded by a digital camera or smartphone. The ratio of blue to red, i.e. the colour of the light, directly reflects the phenylalanine concentration. The entire procedure takes only about ten minutes and could be carried out directly at the patient's home using simple equipment. The accuracy of the results is comparable to the accuracy of modern standard methods in clinical laboratories.

Successful tests on patients

Our new analytical method has already been successfully tested on patients in collaboration with scientists from the University Hospitals of Heidelberg and Lausanne. Currently, these initial promising results are waiting to be confirmed in further studies in Heidelberg. The fast and easy handling as well as the accuracy of the method predestines it for future applications in diagnostics at the bedside or at home. Of course, it will have to be further simplified, automated and tested for this.

The prospect of providing affected patients with an innovative tool for a more autonomous life is a particular motivation for us to master these challenges. From a scientific point of view, our work is also an example of how the synergy between synthetic chemistry and protein engineering can be used to produce semisynthetic biomolecules with completely new properties. ○