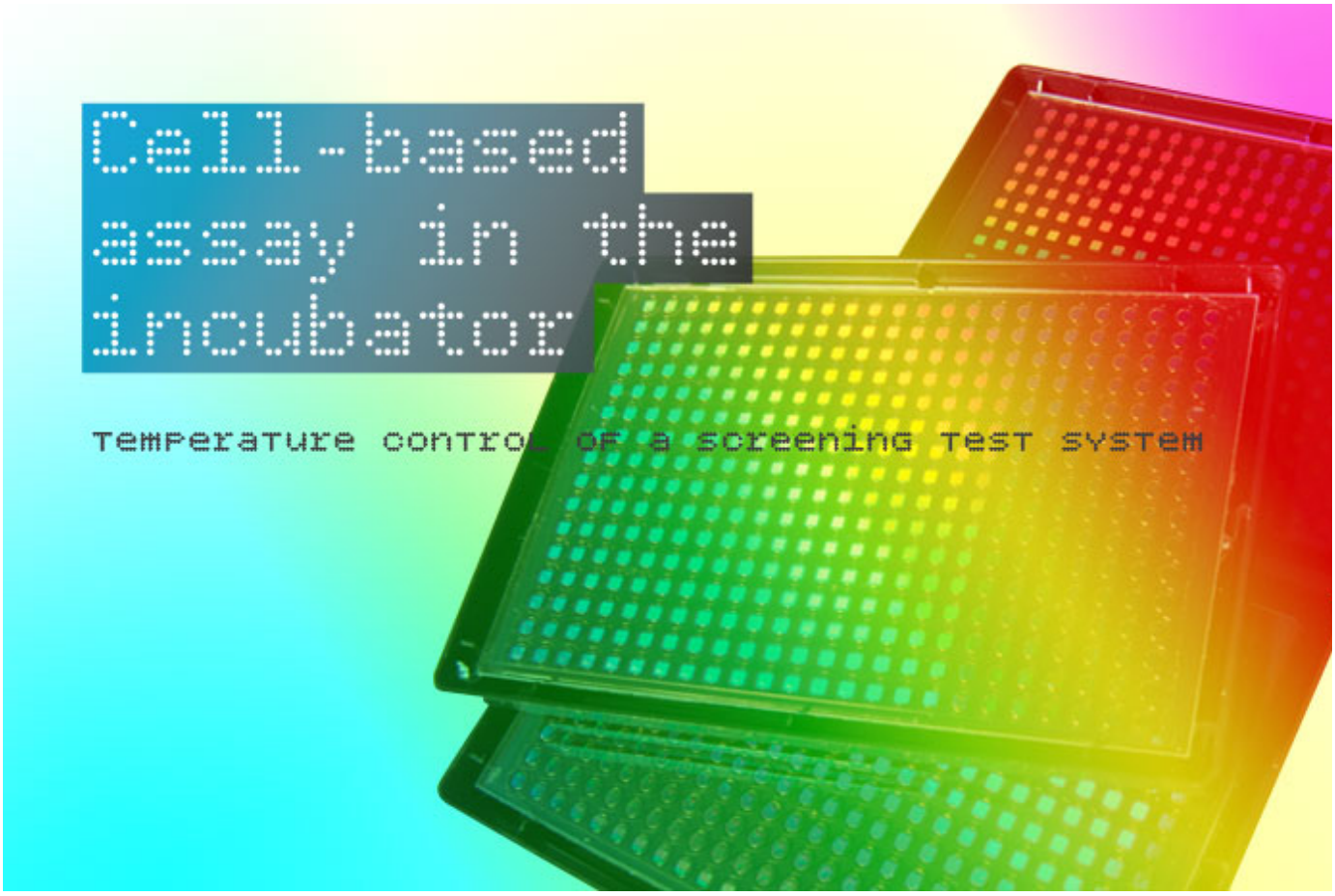
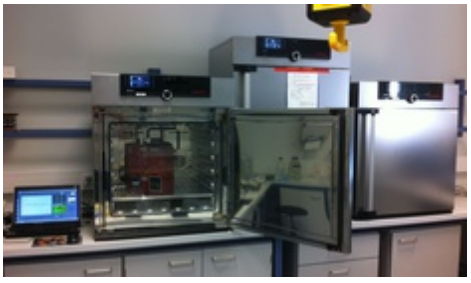
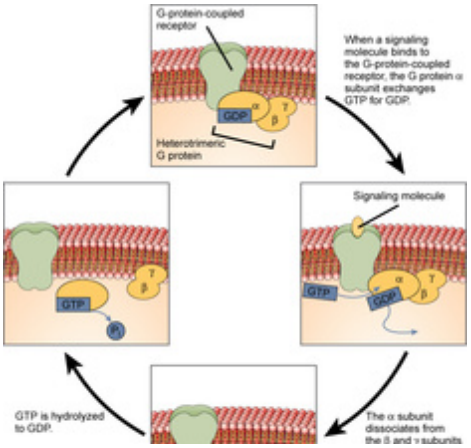


Prof. Evi Kostenis is the head of a research group at the Institute for Pharmaceutical Biology of the University of Bonn that validated a label-free screening test system for pharmaceutically active substances based on optic biosensors and now uses it for numerous research projects with much success. They use a Peltier-cooled incubator IPP and an incubator I, both by Memmert, to ensure precise temperature control during the assays.



The setup consists of a Peltier-cooled incubator IPP110, an incubator IN160 and a Corning Epic® BT system for label-free assays.

With about 1000 members, the G-protein-coupled receptors (GPCRs) constitutes the largest protein family there is. Research on this family of super-proteins was honoured with two Nobel prizes, one in 1994[I] and one in 2012[II] , reflecting how important it is for intercellular communication. The exact way many physiological processes work in animal and vegetable organisms was a well-kept secret for many years and to this day, biologists, chemists and



pharmacologists are still working on reproducing these molecular mechanisms as precisely as possible.

Inter-/intracellular signal transducers: G-protein-coupled receptors

Many messenger substances such as hormones, neurotransmitters and pheromones do not enter the cells. Instead, they latch onto highly specialized receptors on the outside of the cell membrane. In the human organism, only the about 1000 different G-protein-coupled receptors[III] transmit the signals into the inside of the cells, triggering further events. Therefore, they are among the most researched target structures in pharmacology. Many pharmaceuticals such as beta blockers, antihistamines or neuroleptics are activated with GPCRs and researching further effect mechanisms opens sheer endless therapeutic possibilities. "GPCRs and their associated G-proteins are involved in many physiological effects, e.g. blood pressure regulation, airway muscle tone, cell movement, metabolism and cell proliferation." [IV]

Increasing relevance of cell-based assays

The research group led by Prof. Kostenis at the Institute for Pharmacological Biology of the University of Bonn mainly investigates G-protein-coupled receptors and intracellular signalling pathways and therefore also focuses on determining pharmaceutically active substances that have an effect on GPCRs and the family of heterotrimeric G-proteins.

To analyse such substances, which end up both in the development of pharmaceutical products and as important tools to decode complex signalling processes, the intracellular signal transduction processes have to recorded first. This can be done by recording individual events within the cells, often using molecules died with chemical contrasting agents (e.g. with fluorescent substances) and lysing the receptor-bearing cells. However, there is always the risk that the died molecules themselves influence the interactions that are to be recorded.

For this reason, the Kostenis research group also worked

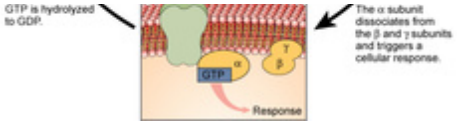


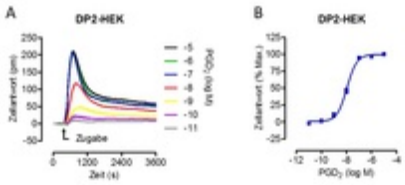
Diagram of the activation cycle of G-proteins with G-protein-coupled receptors, by OpenStax, Licence: CC BY 4.0

with contrast agent-free analysis methods, known as label-free assays, such as dynamic mass redistribution (DMR) recording. This method records minimal changes in the optical density of the cells, as they occur when the GPCRs are activated (Schröder et.al. 2010, 2011). The DMR method is a holistic alternative to record complex signalling processes in intact living cells. What makes this method special is that it can record all four main GPCR signalling pathways, which would otherwise require an individual assay platform each.

Therefore, it is possible to obtain complex, cell-based and real-time data already at an early stage of the pharmacological research process. This is the method that comes closest to in-vivo test series, potentially contributing to reducing the need for animal testing. Moreover, the DMR method is also suitable for high-throughput screening (HTS) and therefore of great interest for the pharmaceutical industry when it comes to testing libraries consisting of hundreds of thousands of substances for selected target structures.

Cell-based assays and label-free detection

The possibility of measuring physiological cell processes without labels and in real time - that is, without taking the detour over the reaction of luminescent or radioactive substances - and holistic recording are the main advantages modern research sees in these new screening technologies. The Institute for Pharmaceutical Biology uses an Epic® BT[V] system by Corning for label-free detection on G-proteins. The measuring system consists of a broadband light source, an optical biosensor integrated in a 384-well microplate and a detector. When polarized broadband light enters the biosensor, the light is reflected in a certain wave length. If a substance is added and the signalling pathways are activated, the distribution of the cellular mass changes, changing the wavelength of the reflected light. The measurement results show the picometre wavelength change of the reflected light, mirroring the change in optical density near the biosensor. This would typically be used to, for instance, quantify the effect of different concentrations of



Signals typical for DMR after activating the receptors, here: (A) After adding the agonist prostaglandin D2 (PGD2) to human embryonic kidney cells (HEK) that were genetically modified to carry D-prostanoid receptor type 2 (DP2) (DP2-HEK), concentration-dependent signals are detected over time. (B) Quantifying the maximum signals results in a sigmoid concentration/effect curve, a

certain substances with concentration/effect curves (see figure).

concentration/effect curve, a typical indication of active receptors. (Data: R. Schröder, E. Kostenis, University of Bonn)

Regulating the temperature of the Corning Epic® BT in the incubator

The measurement setup of the institute includes two Memmert incubators. A small robot, which is brought to temperature in the Memmert incubator IN160, pipets the wells. DMR measurements are temperature-sensitive, therefore the temperature of the Corning Epic® BT system is brought to exactly 28 °C or 37 °C in a Peliter-cooled incubator IPP110. The incubator's cooling ability makes it possible to choose temperatures below the current room temperature on the one hand, and on the other hand allows for a faster transition between different subsequent measurements at different temperatures.

The cooled incubator IPP is especially silent and almost vibration-free, which according to Dr. Ralf Schröder and the University of Bonn was the main factor of success for the validation of the testing system. The measurements are carried out at constant temperature for about 60 to 90 minutes. Once the basal signal level has been measured, the substances used to measure the reactions have to be added - and therefore the incubator door has to be opened for about 5 seconds. In addition to the temperature stability ensured by this device, the researchers of the institute also praised the fast recovery times after opening and closing the door. To prevent radical changes in temperature, a glass inner door and a smaller special door (door in door) was installed (approx. 120 x 160 mm) that can be opened individually, keeping temperature fluctuations to a minimum.

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Overview of the main topics

- University of Bonn
- Institute for Pharmaceutical Biology
- Pharmacology
- Dynamic Mass Redistribution (DMR)
- cell-based assays
- Screening
- Protein analysis
- Biosensors
- label-free detection
- G-protein-coupled receptors
- Corning, Epic® System
- Memmert incubator, Peltier-cooled incubator

Laboratory equipment for incubation

Incubator I
Cooled incubator ICP
Peltier-cooled incubator IPP
CO_2 incubator ICO
CO_2 incubator INCOmed
Cooled storage incubator IPS

Sources and further reading

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[II]
https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2012/lefkowitz-lecture.html

[III]
http://www.nature.com/scitable/topicpage/gpcr-14047471

[IV]
https://www.uni-bonn.de/forschung/startseite-forschung/DFG-Verbundforschungsprojekte/for-2209

[V]
https://www.corning.com/media/worldwide/cls/documents/CLS-ES-038%20REV4%20DL.pdf

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Autor: