Public summary of PhD-thesis of Anniek den Hamer
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Engineering molecular modular components to direct signalling within cells

Biological cells are hugely complex chemical systems, with fabulous capacities, like self-repair, self-rejuvenation, cooperation, battling diseases, etc. Profound understanding of complex molecular systems in cells will one day enable us to design and make synthetic cells for purposes like diagnosis and medical treatment. Science has unraveled most individual components in a cell, but relatively little is known yet about the complex cooperation between them.

Cells are continuously registering, processing and producing signals to work properly. Therefore insight into the functioning of and cooperation between the molecular components required in these signalling processes is fundamental. I have developed new signalling tools to mimic and manipulate biological signalling networks. The goal is to apply them in future synthetic cells or other complex molecular applications.

The basic component of the new tools I developed is the natural 14-3-3 protein, which is a scaffold protein. Scaffold proteins are pivotal in signalling pathways, as they bring together several signalling proteins simultaneously. I developed a molecular system in which the addition of a small-molecule (fusicoccin) leads to enhanced activity of the caspase enzyme due to binding on the 14-3-3 protein scaffold. As caspases are crucial in cell death, the new tool may eventually play a role as a 'kill switch'. Such a switch is required in genetically engineered cells, to be able to switch them off after performing their duty.

Another system I developed used phosphorylation (a chemical process that is part of many cellular processes) as input, instead of a small-molecule, to bring proteins together on the 14-3-3 scaffold. The phosphorylation input led to reassembly on the scaffold of a light-emitting enzyme (luciferase) that was split in two. Upon reassembly the enzyme produces light. This system may be used to assemble other types of enzymes that play a role in cell signalling, using several types of input.

Finally I engineered a module that can be used to detect the activity of the caspase enzyme. It consists of a luciferase and the fluorescent protein mNeonGreen, with a linker in between them that can only be cut by active caspase. As long as the luciferase and mNeonGreen are linked, the ensemble emits green light. mNeonGreen absorbs the energy of the luciferase blue light emission in a process called Bioluminescence Resonance Energy Transfer. If active caspases are present, the linker is cut which results in reduced energy transfer, leading to increased blue light emission of the luciferase. The shift in color allows for the detection of active caspases.

Title of PhD-thesis: Modules for the synthetic supramolecular signalling toolbox.

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