OUTCASST

Out-of-cell CRISPR Activated Sequence-specific Signal Transducer

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Ultra-sensitive sequence-specific detection of nucleic acids

The major drawback of existing nucleic acid detection methods is their need for high quantities of the DNA/RNA for accurate detection. This quantity can be reached through amplification but these amplification steps also enhance the chance of producing artefacts, especially, if the starting amount of the target sequence is diluted in a pool of similar sequences.

We introduce a mammalian cell-based sequence-specific nucleic acid sensor, composed of two membrane protein chains with two different catalytically inactive Cas-like proteins (dCas) attached to the extracellular part of the chains (e.g. Cas9 and Cpf1). The Cas-like proteins serve as sequence-specific nucleic acid sensors that induce dimerization of the membrane protein chains upon nucleic acid binding. Dimerization of the receptors causes the intracellular protease domain of chain 1 to cleave off the effector molecule of chain 2 which is then released into the cytosol. The effector molecule could be a transcription factor (TF), signalling molecule, enzyme, fluorescent protein and many more. The advantages of our approach are simplicity, modularity and potentially very high sensitivity. Here we overcome the need for DNA amplification prior to detection which simplifies the methodology while at the same time maintaining high sensitivity by making use of established cellular amplification mechanisms (e.g. via TF release).

Pathogens

During most infections, small amounts of pathogen DNA/RNA are present in a patient's blood. Sequence-specific detection of these could improve diagnosis of infectious diseases.

Tumor detection

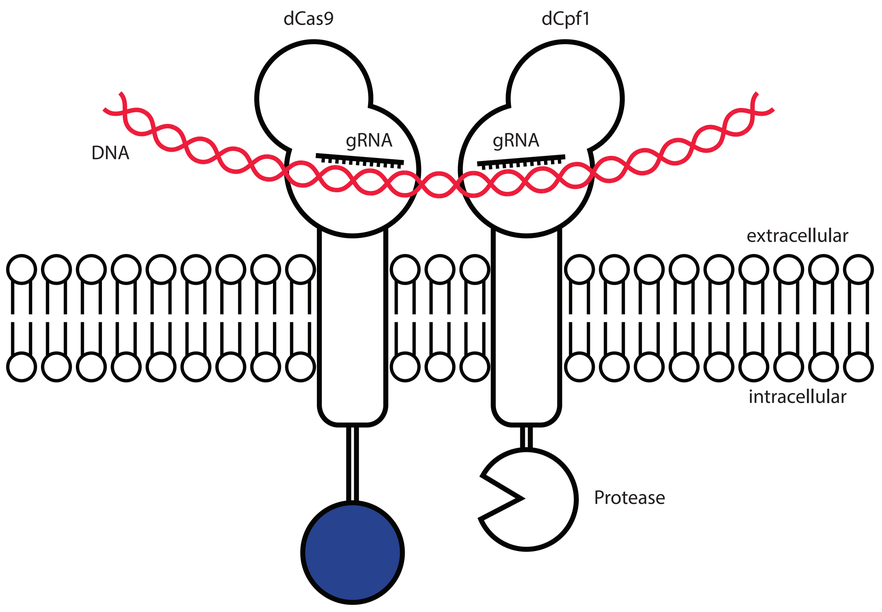
Detection of trace amounts of DNA in blood or tissue samples could help diagnosing cancer at a very early stage.

Synthetic DNA

DNA-Libraries, mRNA-Display and other high-throughput techniques often require the fishing for very specific targets in a large pool of sequences.

Environmental samples

Sequence-specific DNA detection has countless applications in environmental biology and public health. The low concentration of DNA is a constant issue when working with environmental samples.



input

output

input

output

Transcription factors

By activation of reporter genes, transcription factors can serve as an amplifier module of the sensor and make trace amounts of extracellular DNA detectable.

Enzymes

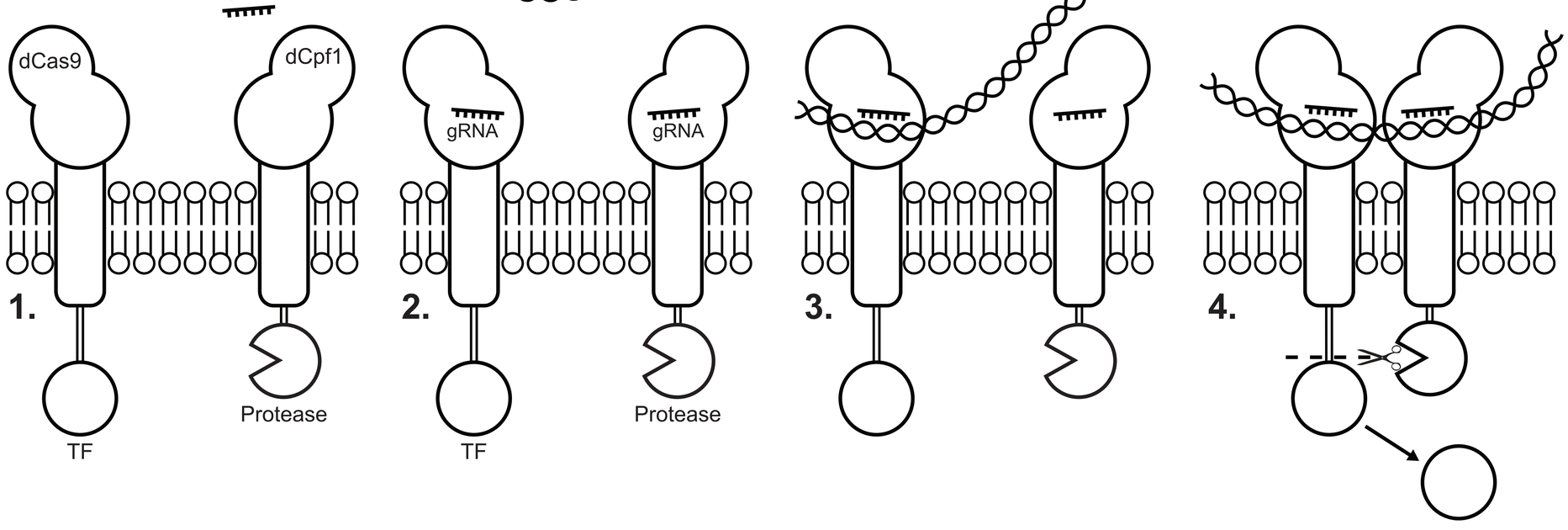
The release of enzymes into the cytosol can cause an immediate reaction without any transcription-translation delay.

Fluorescent proteins

Split-proteins that dimerize in the cytosol after their release can serve as an immediate visual read-out upon DNA detection.

Signalling molecules

Coupling of our detection system with existing mammalian signalling pathways could be achieved by release of kinases or secondary messenger molecules.



In the first step, the two guide-RNAs (gRNA) are added to the system. Only the gRNA determines which DNA will be detected, thus the system is very easy to use and versatile.

The incorporation of the gRNA into the Cas-like proteins happens spontaneously and with high affinity. Once the gRNA is bound, the sample containing the DNA can be applied.

The two gRNAs target two distinct stretches on the DNA molecule by complementary base-pairing. The "scaffold" region of the gRNA determines the Cas-likes protein it will bind to.

Once a DNA molecule has bound the two membrane protein chains, the intracellular protease domain can release the effector molecule from the other chain.

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Patent pending