# Targeting specific DNA sequences with I-125-labelled Triplex-forming-oligonucleotides

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Purpose: Triplex-forming-oligonucleotides (TFOs) are able to bind DNA in a sequence specific manner and are a promising tool to manipulate genes or gene regulatory units in a cellular environment. TFOs have also therapeutic potential e.g. as a carrier for Auger-Electron-Emitter (AEE) to target DNA of tumour cells. We established a method for the effective labelling of TFOs with I-125 and studied the DNA binding capabilities of labelled TFOs *in vitro*. Furthermore we examined the intracellular biokinetic of TFOs with the focus on the transfer from the cytoplasm into the cell nucleus.
Methods: TFOs specific for the genes *cdkn2a*, *bcl2*, *brca1*, *chk2*, *cdk4* were designed using TFO Target Sequence Search (Univ. of Texas). TFO labelling with I-125 was performed with the primer extension method. Formation of DNA triplexes was visualized with MS Imaging Plates on a FLA-5000 Imaging System (Fujifilm, Düsseldorf) and electrophoretic-mobility-shift-assay (EMSA). For biokinetic studies SCL-II cells were transfected by electroporation with Alexa488-labelled TFOs. Transfected cells were subsequently cultured for 1, 6, 12, 18, 24, 30, 48 and 72 h and TFO signal intensity was determined in single cells and in isolated cell nuclei by flow cytometry (FACS-Canto II, BD).
Results: The desired Triplex-DNA-formation could be confirmed for 53 % of all tested TFOs by EMSA. Triplex-formation of I-125-labelled TFOs was confirmed for 10 % by autoradiographic analysis. The biokinetic studies showed that TFO-Alexa488-positive cells were detectable as soon as 1 h after transfection and the signal intensity remained constant for at least 30 h. 72 h after transfection the signal was less intense but still detectable. A substantial loss of TFO-Alexa488-labelled positive cell nuclei was observed within the first 6 h post-transfection followed by a significant increase up to 18 h post-transfection.
Conclusions: Labelling of TFOs with I-125 has a strong influence on their binding capacities. TFOs initially located in the cytoplasm are re-located to the cell nucleus within 12 h after delivery of the TFOs probably during cell division.