Photoactivatable nanoCRISPR/Cas9 system

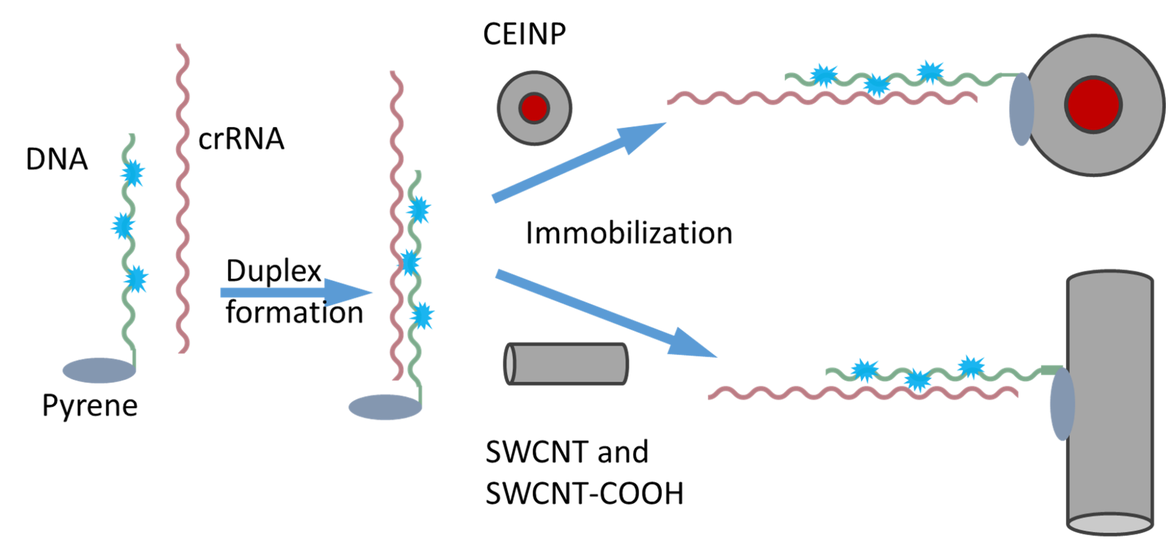
Olga Semikolenova[1,2], Lubov Sakovina[1,2], Darya Kim[1,2], Ivan Vokhtantsev[1], Sergey Novopashin[3], Alya Venyaminova[1], Darya Novopashina[1,2]

[1] Institute of Chemical Biology and Fundamental Medicine of SB RAS, Novosibirsk, Russia;
[2] Sirius University of Science and Technology, Sochi, Russia;
[3] Institute of Thermophysics of SB RAS, Novosibirsk, Russia
E-mail:danov@niboch.nsc.ru

The design of nucleic acids constructions containing photosensitive residues or linkers permits to create spatiotemporal regulated system. Photocaged oligonucleotides have been used for photoactivation of CRISPR/Cas9 gene editing systems [1,2]. Here we proposed to immobilize crRNA through the photocleavable oligodeoxyribonucleotide (PC-DNA) on carbon nanoparticles (CNP) surface for the transfection of gene editing system components into the cell with subsequent activation of nanoCRISPR/Cas9 by UV-irradiation.

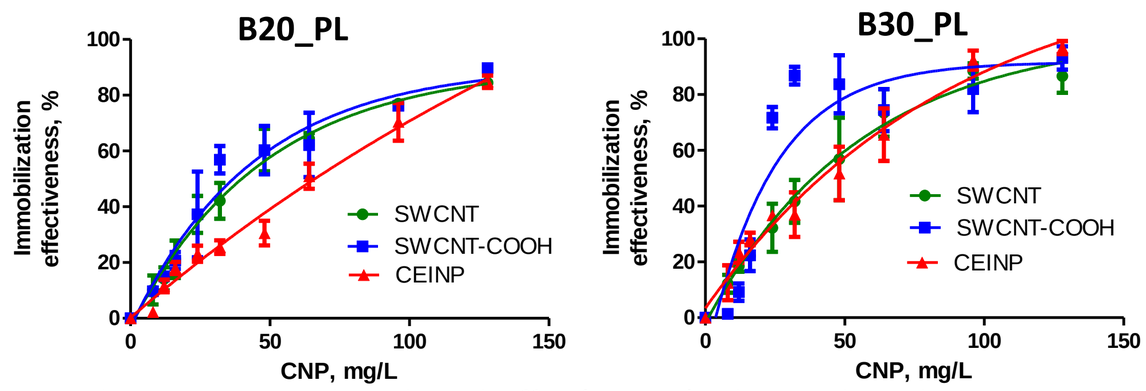
PC-DNA complementary to crRNA with two or three photocleavable linkers inside the chain and with pyrene residue at 3'-terminus have been synthesized.

Name	Sequences, 5’-3’
B20_PL	5’-TTTTTT-**PL**-ACAAA-**PL**-TTGAG-**PL**-TTATCC-***Pyr***
B30_PL	5’-GCTCTAAA-**PL**-ACTTTTT-**PL**-TACAAAT-**PL**-TGAGTTAT-***Pyr***
crRNA_Flu	5’-AUAACUCAAUUUGUAAAAAAGUUUUAGAGCUAUGCUGUUUUG-***Flu***
crRNA	5’-AUAACUCAAUUUGUAAAAAAGUUUUAGAGCUAUGCUGUUUUG
tracrRNA	5’-AACAGCAUAGCAAGUUAAAAUAAGGCUAGUC-CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU

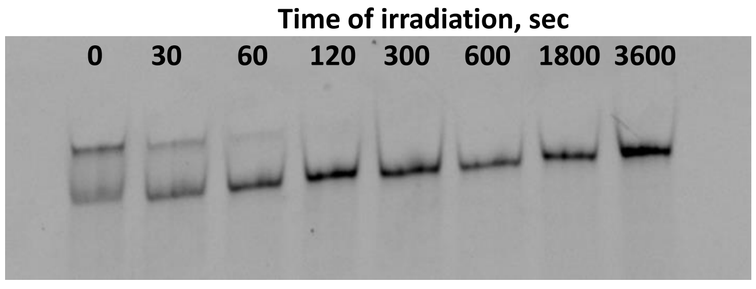


– photocleavable linker
CEINP– carbon encapsulated iron nanoparticles
SWCNT – single walled carbon nanotube
SWCNT-COOH – carboxylated SWCNT

The stability of duplexes of pyrene modified B20_PL and B30_PL with crRNA were studied by gel-shift assay (K_d n.d. and 18,5 nmole) and thermal denaturation method (28,8 and 44,7 °C). The isotherms of crRNA/PC-DNA duplexes immobilization on the surfaces of CNP were obtained using methods of pyrene fluorescence quenching [3].

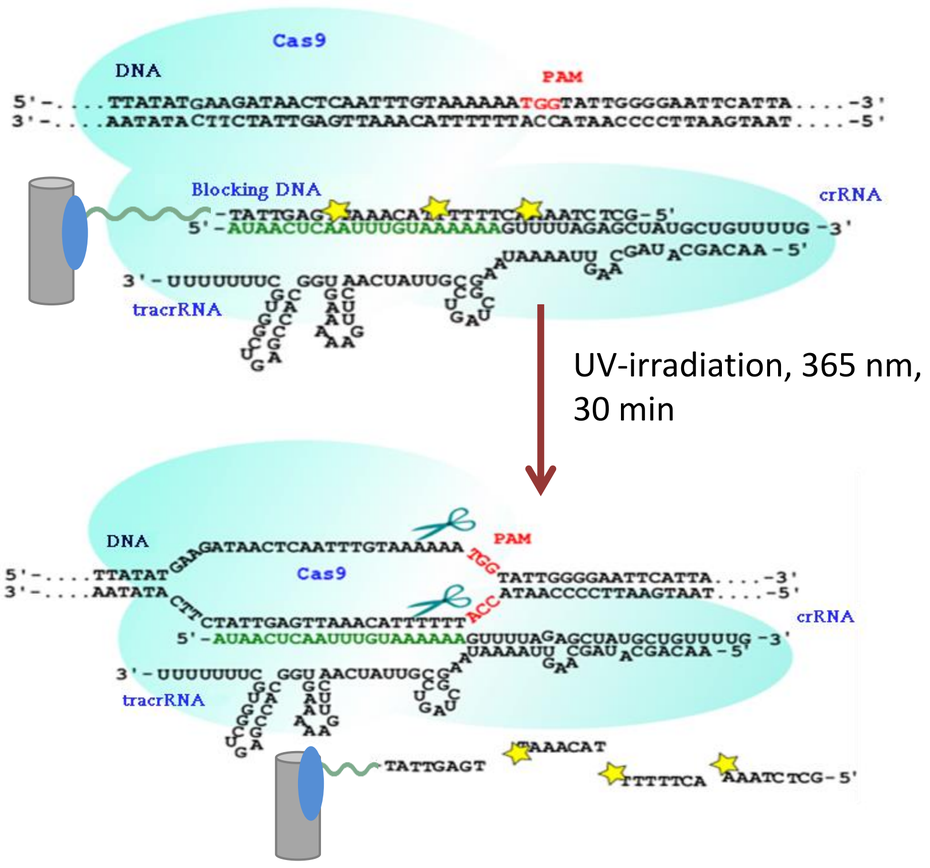


Conditions: 0,01M phosphate buffer (pH 7,5), oligonucleotide concentrations were $1 \cdot 10^{-6}$M, detection of fluorescence at 380 nm.

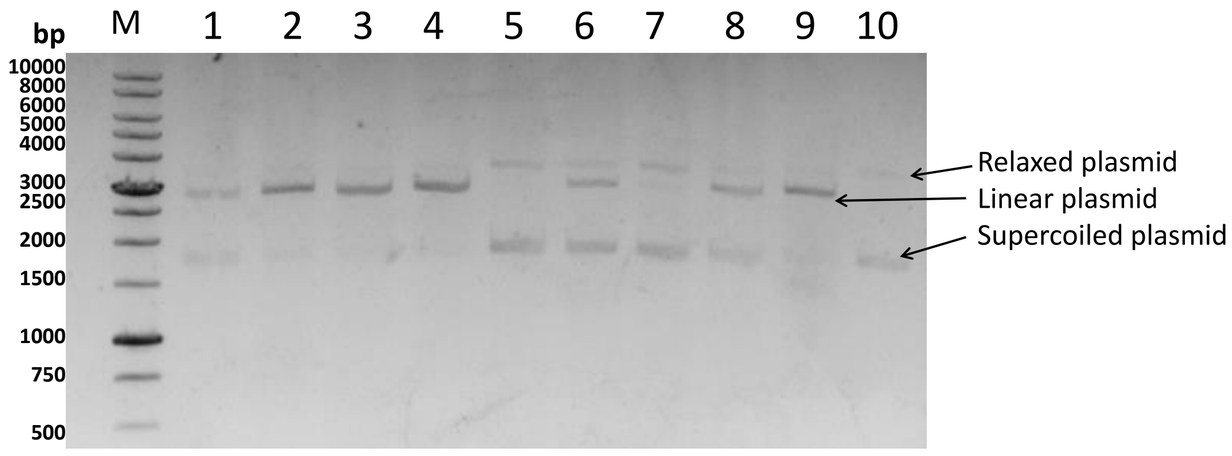


The release of fluorescent crRNA upon UV-irradiation was examined by PAGE analysis. Time of half release was about 45 sec.

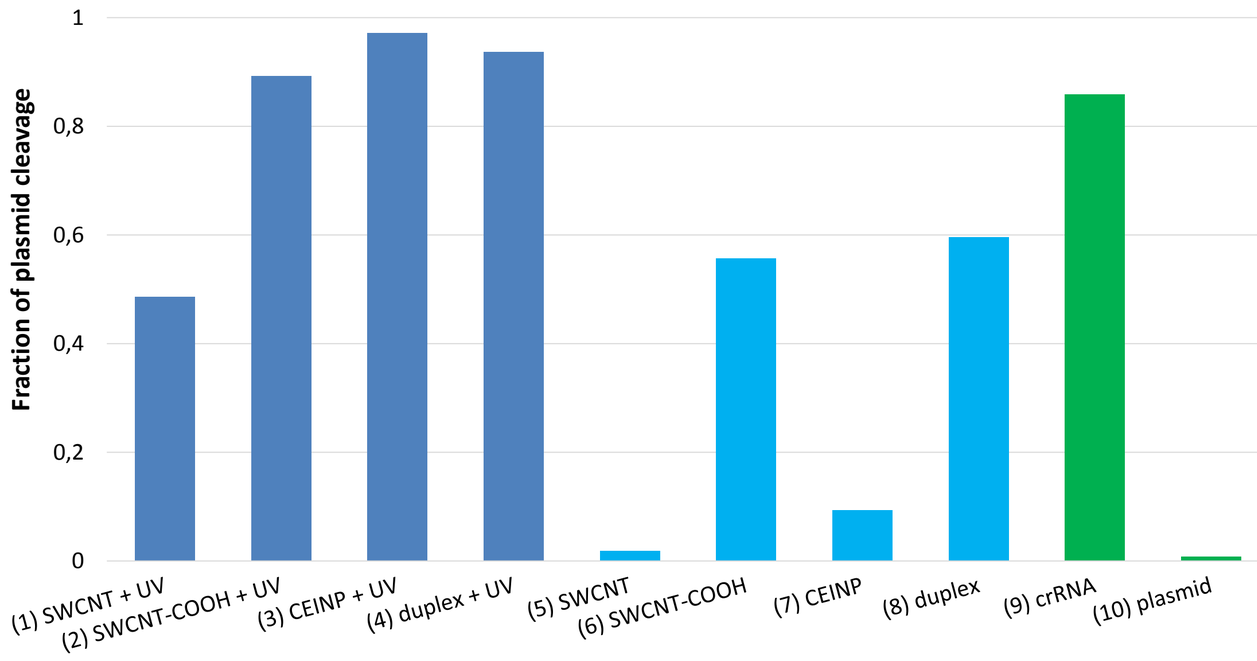
15% denaturating PAGE, oligonucleotide concentration B30_PL was $1 \cdot 10^{-6}$ M, temperature was 25°C, wavelength of irradiation was 365 nm.



The immobilized crRNA were used in vitro as component of nanoCRISPR/Cas9 system. The possibility of nanoCRISPR/Cas9 system activation by UV-irradiation and effectiveness dsDNA cleavage was observed.



Conditions: 20 mM HEPES (pH 7,5), 100 mM KCl, 1 mM DTT, 0,5 mM Na_2EDTA, 2 mM $MgCl_2$, 5% glycerol. Cleavage of pBS2SKM by nanoCRISPR/Cas9 systems were performed at 37 °C during 1 h. Reaction mixture were analyzed in agarose gel-electrophoresis.



Effectiveness of dsDNA cleavage by nanoCRISPR/Cas9 system with activation by UV-radiation was better than that by system without activation. nanoCRISPR/Cas9 system immobilized on CEINP showed the best result with low plasmid cleavage before UV-radiation and high plasmid cleavage after UV-radiation.

Cell penetration crRNA/PC-DNA/CNP complex in presence of lipofectamine was demonstrated by cytofluorometry. The possibility of PC-DNA photodegradation and crRNA released from CNP surface as well as nanoCRISPR/Cas9 system activation by UV-irradiation was demonstrated.

The proposed approach for the design of CNP-immobilized photoactivatable crRNA for cell transfection and UV-activation of CRISPR/Cas9 system is prospective for spatiotemporal gene editing.

References:
1. Brown W. et al. ChemBioChem. 2021 V.22. N.1. N.63-72. doi: 10.1002/cbic.202000423.
2. Akmetova E.A. et al.Russ. J. Bioorg. Chem. 2021 V.47. N.2. P.495-503. doi: 10.1134/S1068162021020023
3. Apartsin E.K. et al. ACS Appl Mater Interfaces. 2014. V.6. N.3. 1454-1461. doi:10.1021/am4034729

The reported study was funded by RFBR, project number 19-34-51026.

COST ACTION CA 17140
NANO2CLINIC
CANCER NANOMEDICINE - FROM THE BENCH TO THE BEDSIDE