**Supplementary Materials**

**Self-penetrating Oligonucleotide Derivatives: Features of Self-assembly and Interactions with Serum and Intracellular Proteins**

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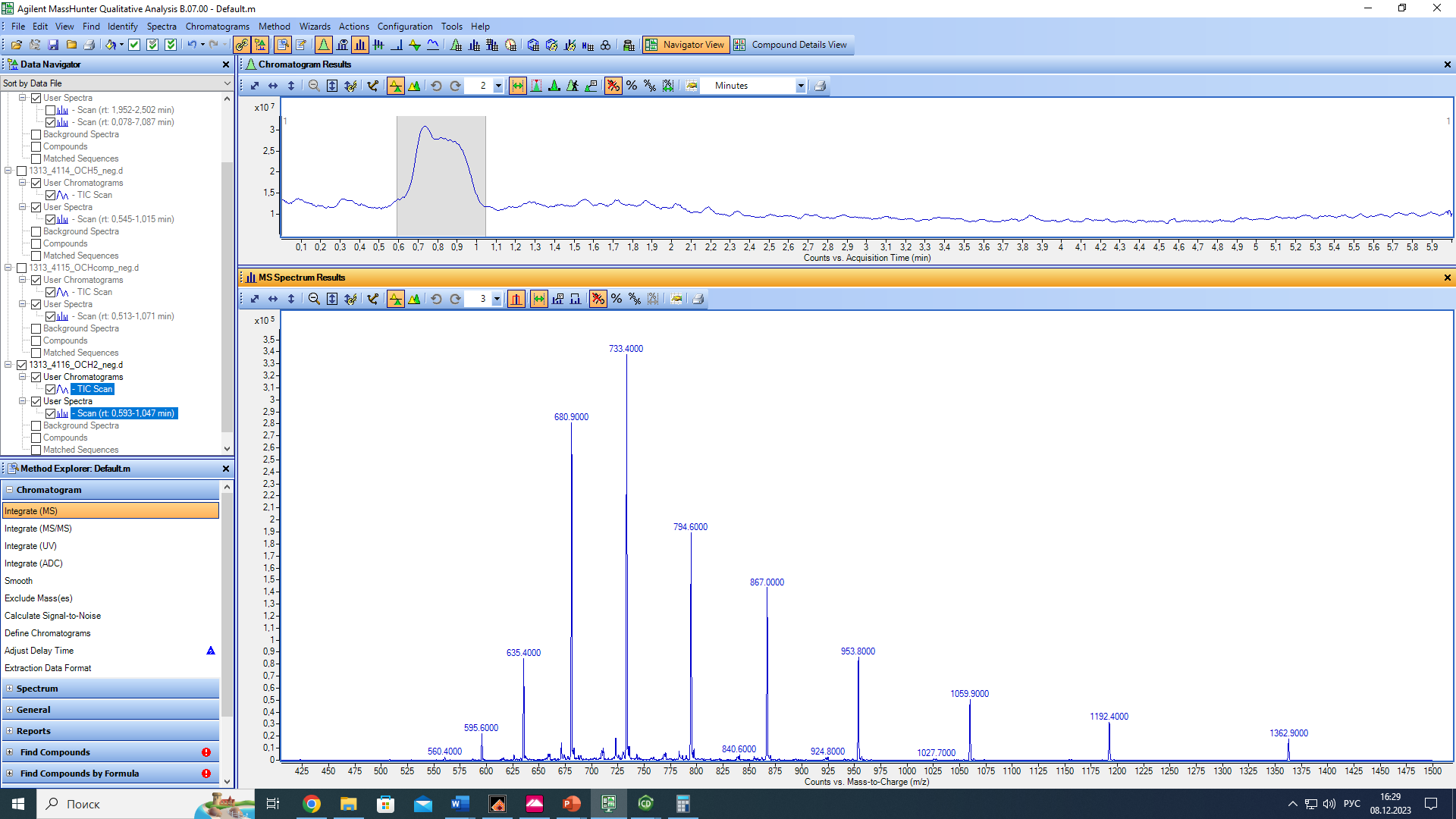
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# **S1. Result of mass-spectrometry of modified oligonucleotides**

**TZ30** 5'- TCC-TGA-CAT-ACT-TGA-TAC-TTA-GAC-ATT-CT**\***T -3'

Мr [calculated] / Мr [found] = 9546.0 / 9547.3

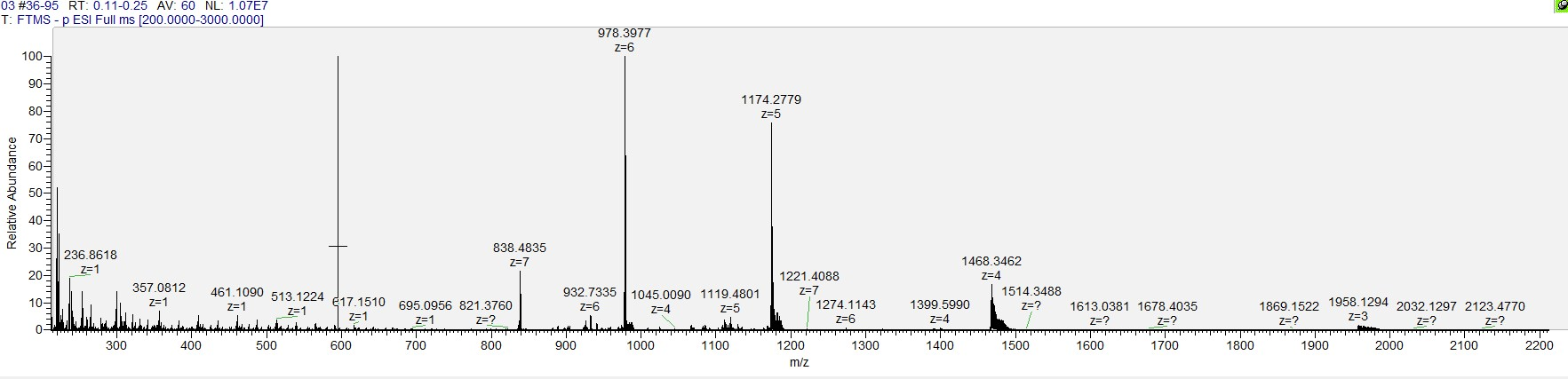
|  |  |  |
| --- | --- | --- |
| **Ion charge** | **Calculated** | **Found** |
| -7 | 1362.71 | 1362.9 |
| -8 | 1192.25 | 1192.4 |
| -9 | 1059.67 | 1059.9 |
| -10 | 953.60 | 953.8 |
| -11 | 866.82 | 867.0 |
| -12 | 794.50 | 794.6 |
| -13 | 733.31 | 733.4 |
| -14 | 680.86 | 680.9 |
| -15 | 635.40 | 635.4 |



**FAM-TZ16** 5′-[FAM]CTGACTATGAAGTAT**\***T-3′

Мr [calculated] / Мr [found] = 5877.5 / 5876.5

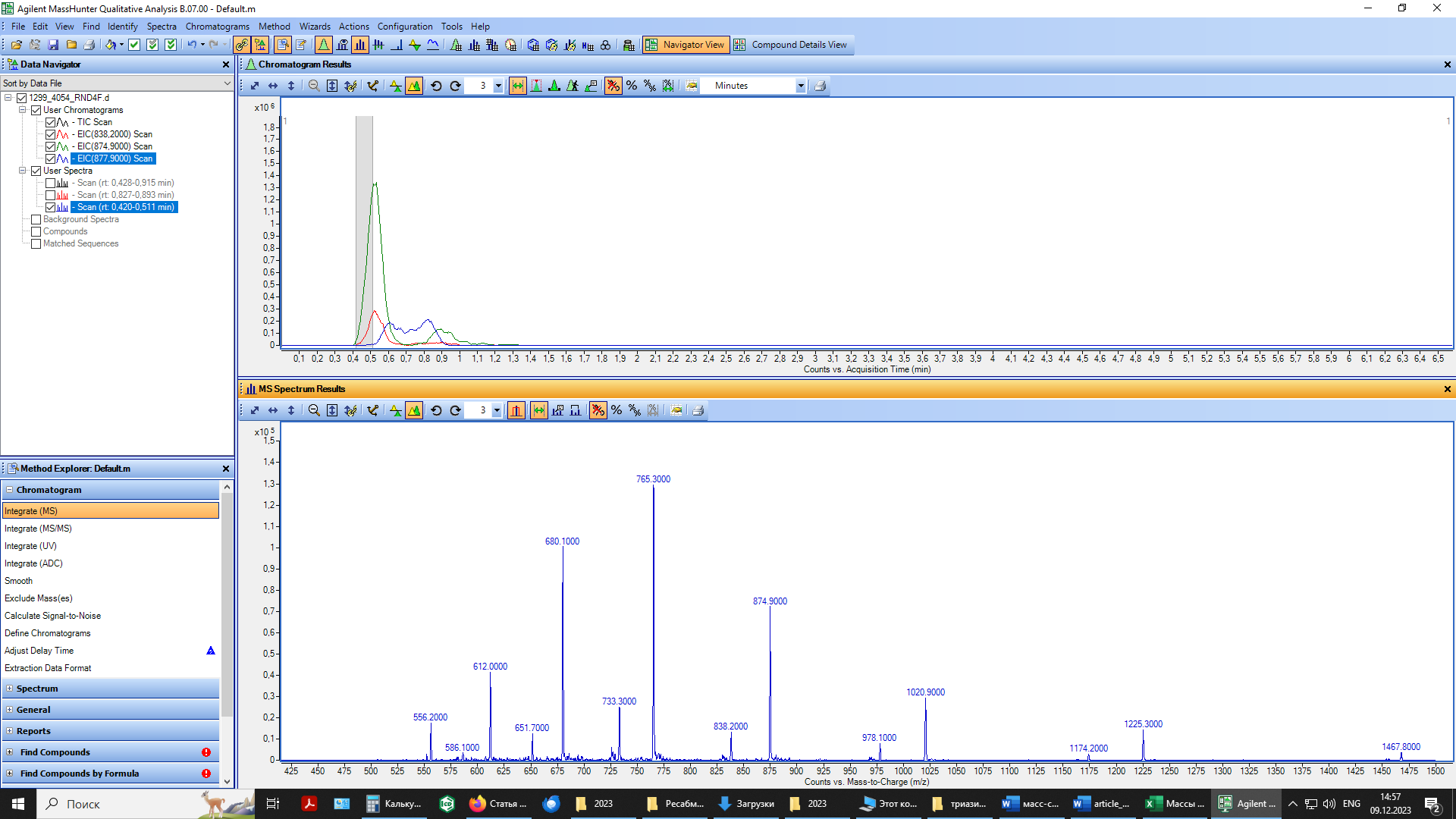
|  |  |  |
| --- | --- | --- |
| **Ion charge** | **Calculated** | **Found** |
| -4 | 1468.38 | 1468.35 |
| -5 | 1174.50 | 1174.28 |
| -6 | 978.58 | 978.35 |
| -7 | 838.64 | 838.35 |



**FAM-TZ17** 5′-[FAM]AGTCTCGACTTGCTAT\*T-3′

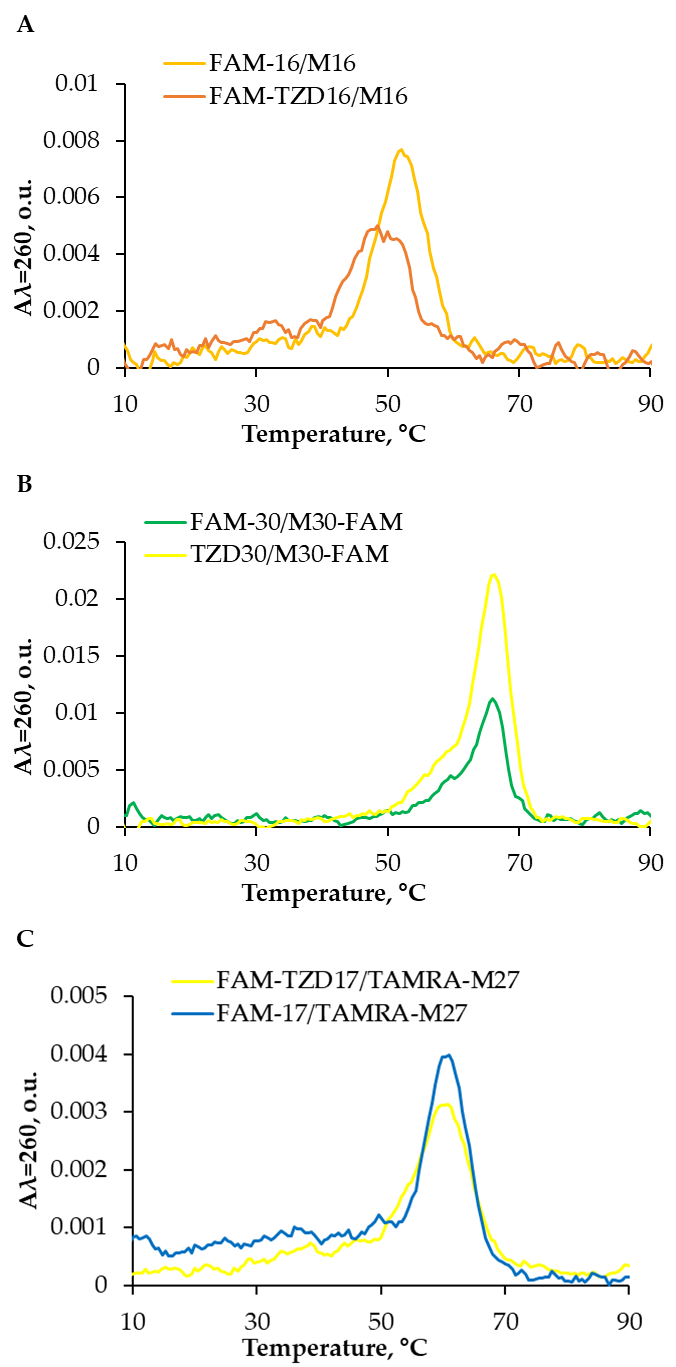
Мr [calculated] / Мr [found] = 6130.4 / 6132.0

|  |  |  |
| --- | --- | --- |
| **Ion charge** | **Calculated** | **Found** |
| -5 | 1225.08 | 1225.3 |
| -6 | 1020.73 | 1020.9 |
| -7 | 874.77 | 874.9 |
| -8 | 765.30 | 765.3 |
| -9 | 680.16 | 680.1 |
| -10 | 612.04 | 612.0 |
| -11 | 556.31 | 556.2 |



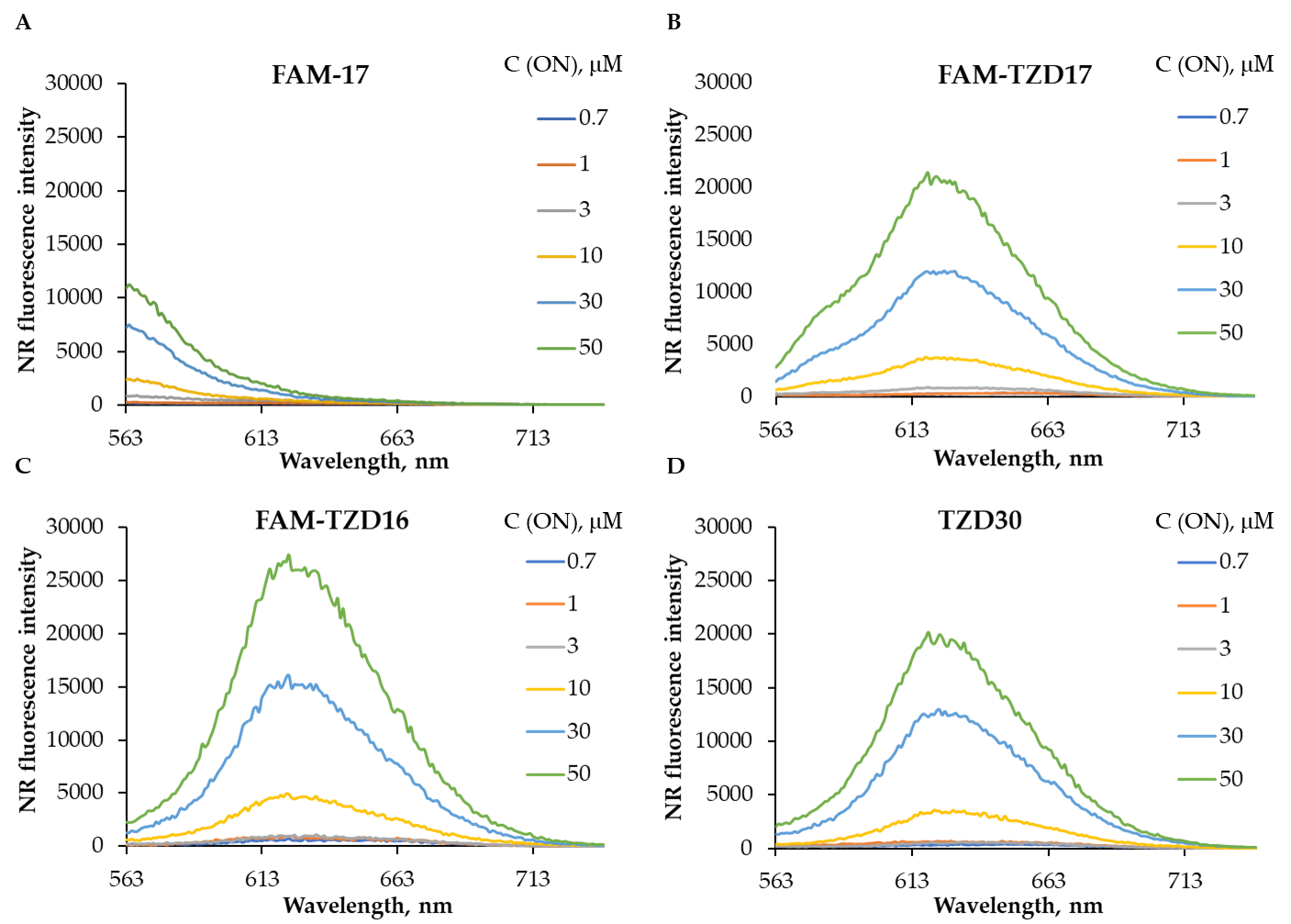
**Figure S1.** Result of mass-spectrometry of modified oligonucleotides TZ30, FAM-TZ16, FAM-TZ17

# **S2. Duplex thermal denaturation experiments**

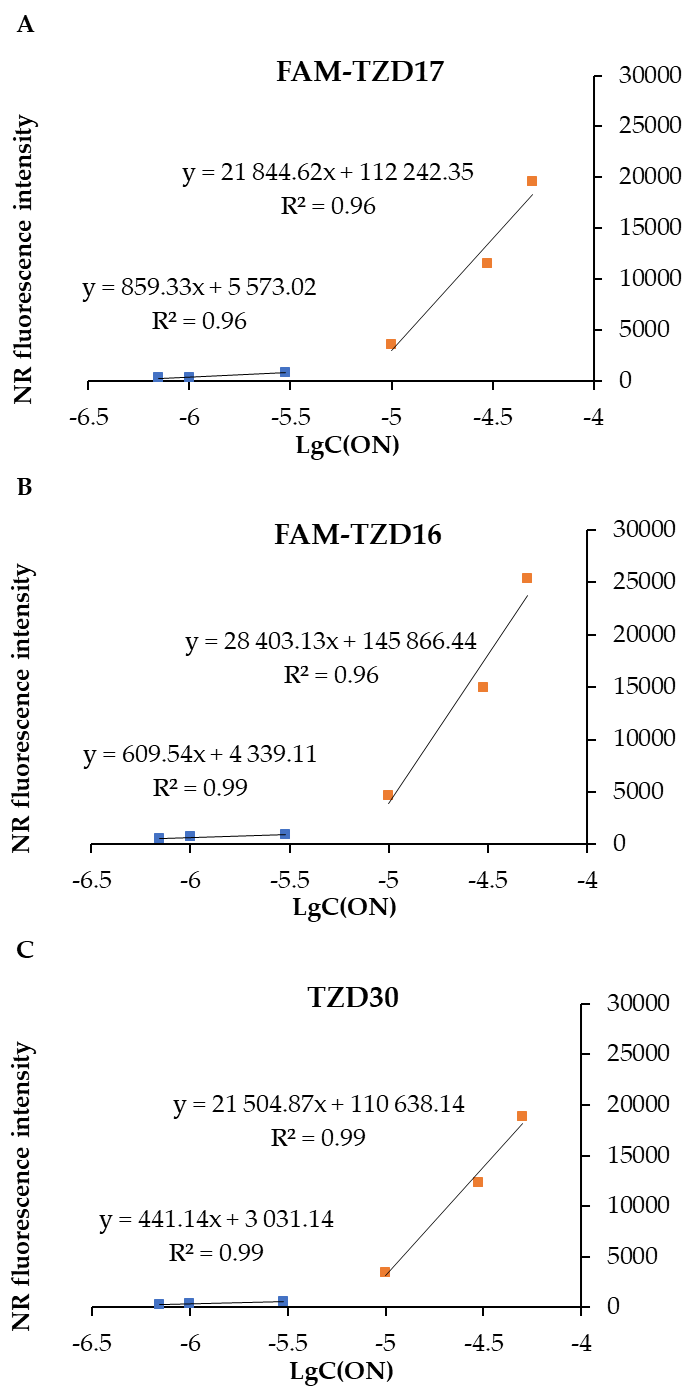


**Figure S2.** Differential melting curves of the duplexes under study; each sample contained 5 µM of a complementary strand in 10 mM NaCac, pH 7.0, and 100 mM NaCl.

# **S3. Critical aggregation concentration determination by Nile red encapsulation assay**

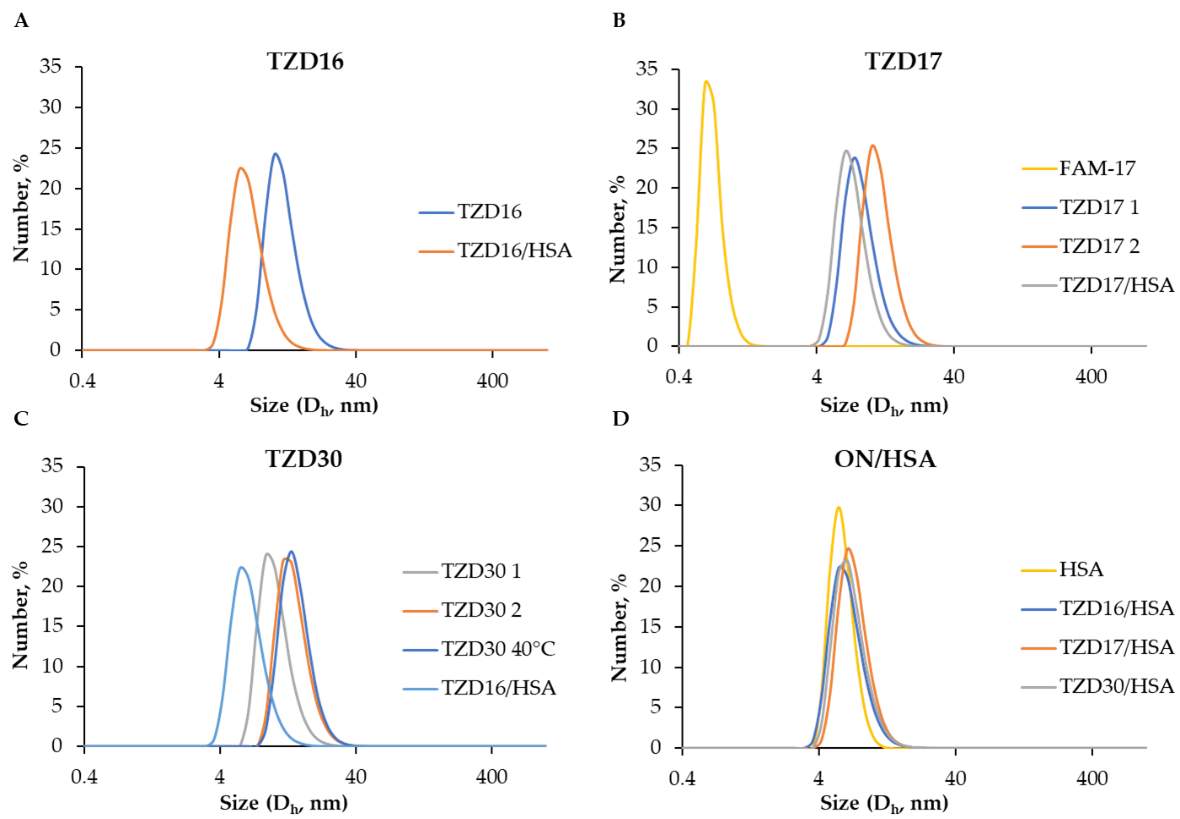


**Figure S3.** Nile Red fluorescence emission spectra in solutions (TAM buffer) of indicated concentrations of oligonucleotides under study.



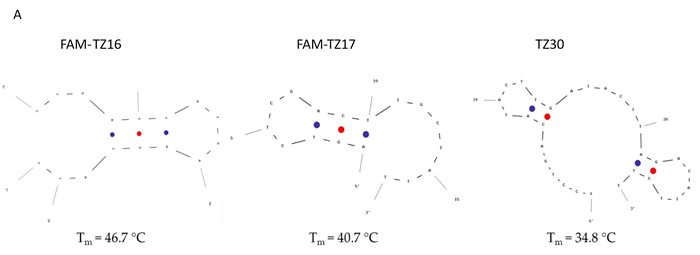
**Figure S4.** Dependence of the Nile Red emission intensity at 630 nm on the logarithm of oligonucleotide concentration (M).

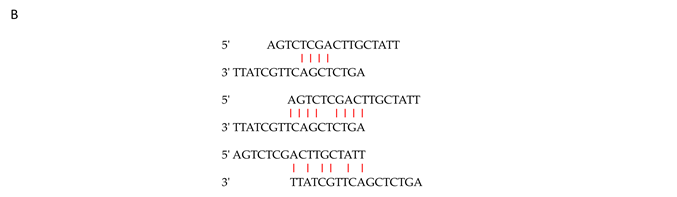
# **S5. Dynamic light scattering measurements**



**Figure S5.** Size distribution by Number of oligonucleotide particles and HSA-oligonucleotide associates after 12 h incubation in TAM as measured using DLS; oligonucleotide samples contained 10 µM oligomer; ON/HSA ratio in the respective samples 1:1.

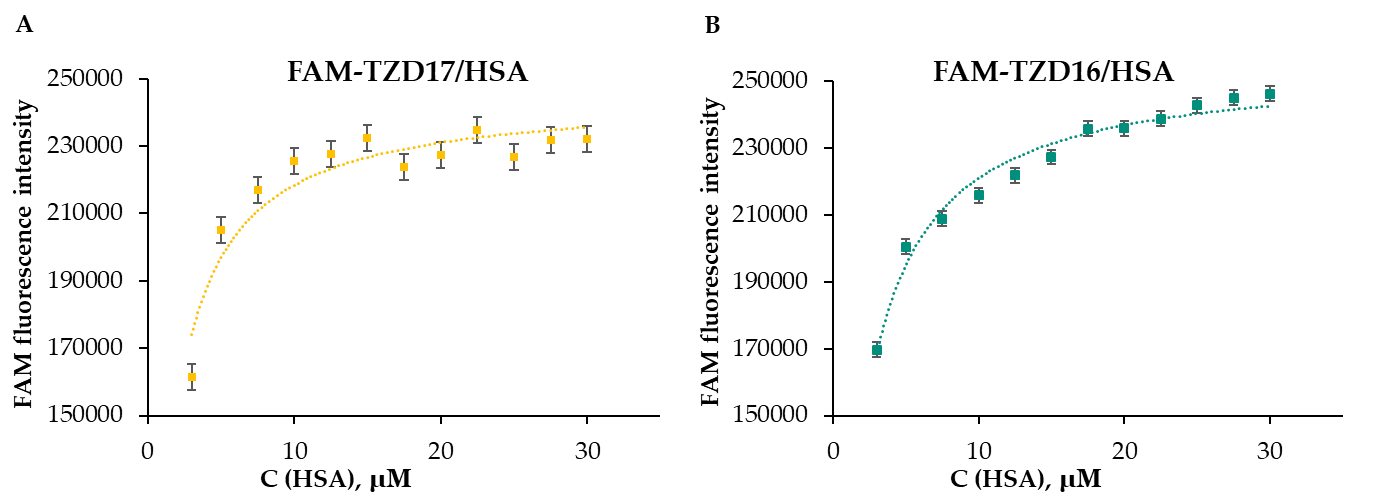
# **S6. Possible secondary structures formed by oligonucleotides under study**

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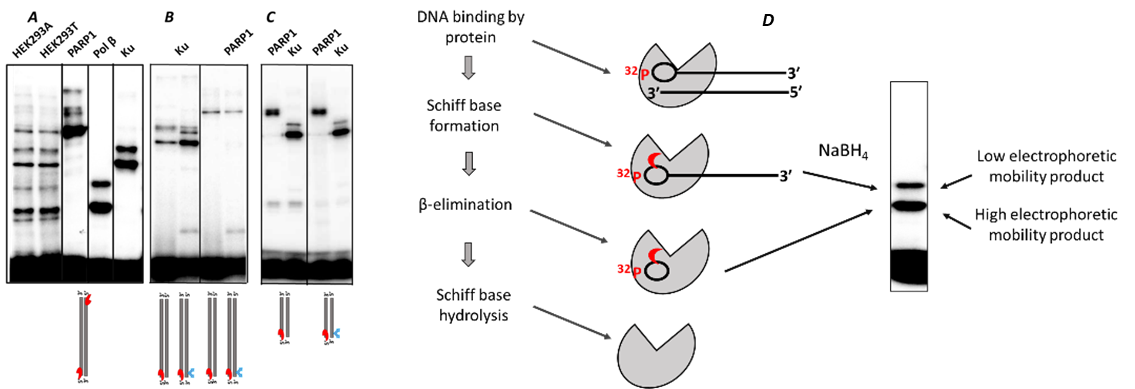
**Figure S6.** Secondary structures and calculated melting temperatures were predicted using OligoAnalyzer software lacking the triazinyl phosphoramidate modification (A) and possible structures of FAM-TZ17 self-dimers (B).

# **S7. Kd determination by fluorescence titration experiment**



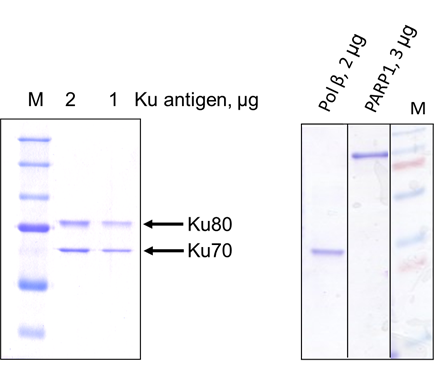
**Figure S7.** Binding curves for the complexes FAM-TZ17/HSA and FAM-TZ16/HSA after 30 min incubation in PBS buffer, at 37 °C; each sample contained 10 µM oligomer.

# **S8. Affinity modification of Ku antigen, PARP1 and DNA polymerase β by [32P]5′-dRp-DNAs**



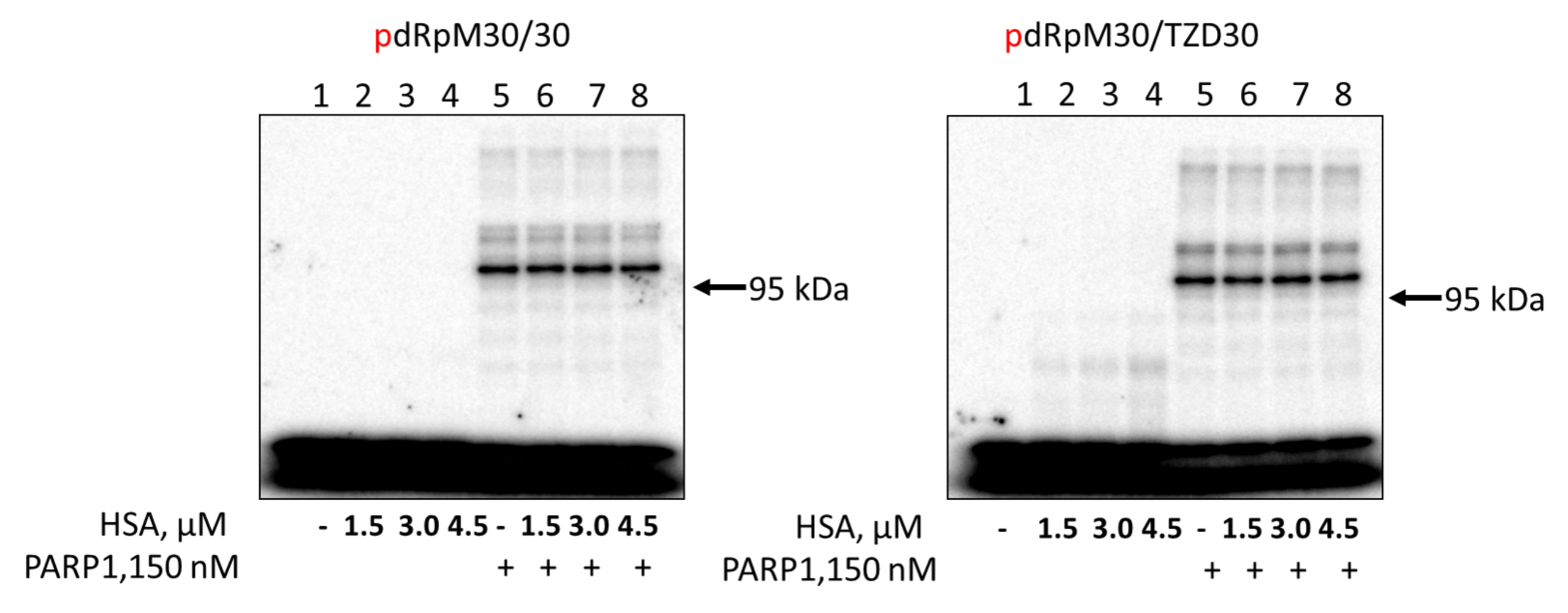
**Figure S8.** Affinity modification of Ku antigen, PARP1, and DNA polymerase β by [32P]5′-dRp-DNAs. A – modification of proteins by DNA-duplex composed from two 30-mer oligonucleotides bearing [P32]5′-dRp group at each chain. All reaction mixtures contained 100 nM 5′-dRp-DNA and proteins of whole-cell extracts, 1 mg/ml, or 30 nM Ku antigen, or 150 nM PARP1, or 50 nM DNA polymerase β. The reaction mixtures were incubated at 37 °C for 5 min followed by borohydride treatment for 30 min at 0 °C. The products of DNA-protein cross-linking were separated in 7.5% PAAG according to [85]. B - modification of 20 nM Ku antigen, 20 nM PARP1 by 30-mer DNA duplexes. The reaction mixtures were incubated at 37 °C for 10 min followed by borohydride treatment for 30 min at 0 °C. The products of DNA-protein cross-linking were separated in 10% PAAG according to [85]. C - modification of 50 nM Ku antigen, 50 nM PARP1 by 16-mer DNA duplexes containing [P32]5′-dRp-oligonucleotide and non-modified or TZD-modified oligonucleotides. The reaction mixtures were incubated at 37 ⁰C for 10 min followed by borohydride treatment for 30 min at 0 °C. The products of DNA-protein cross-linking were separated in 12.5% PAAG. The gels were dried and exposed to a phosphor imaging screen. Positions of [32P]5′-dRp group and TZD-modification are shown by red and blue symbols, respectively. D - Scheme of [32P]5′-dRp DNA cross-linking to protein and release from covalent complex with protein. The products to be analyzed by SDS-PAAGE were obtained by sodium borohydride treatment of the reaction products.

# **S9. Electrophoretic analysis of Ku antigen, PARP1, and DNA polymerase β**



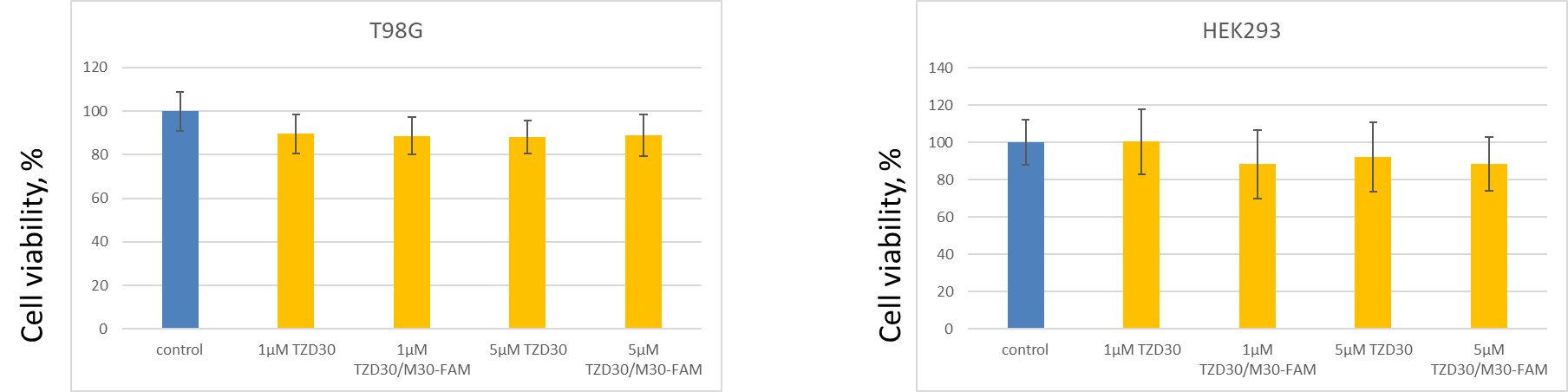
**Figure S9.** Electrophoretic analysis of used proteins. Purified proteins were analyzed by SDS-PAGE according to the Laemmli method. The gels were 7.5% for Ku antigen and 12.5% for other proteins. The gels were stained with Coomassie R250.

# **S10. Cross-linking of [32P]-dRp-containing DNAs to PARP1 and HSA**



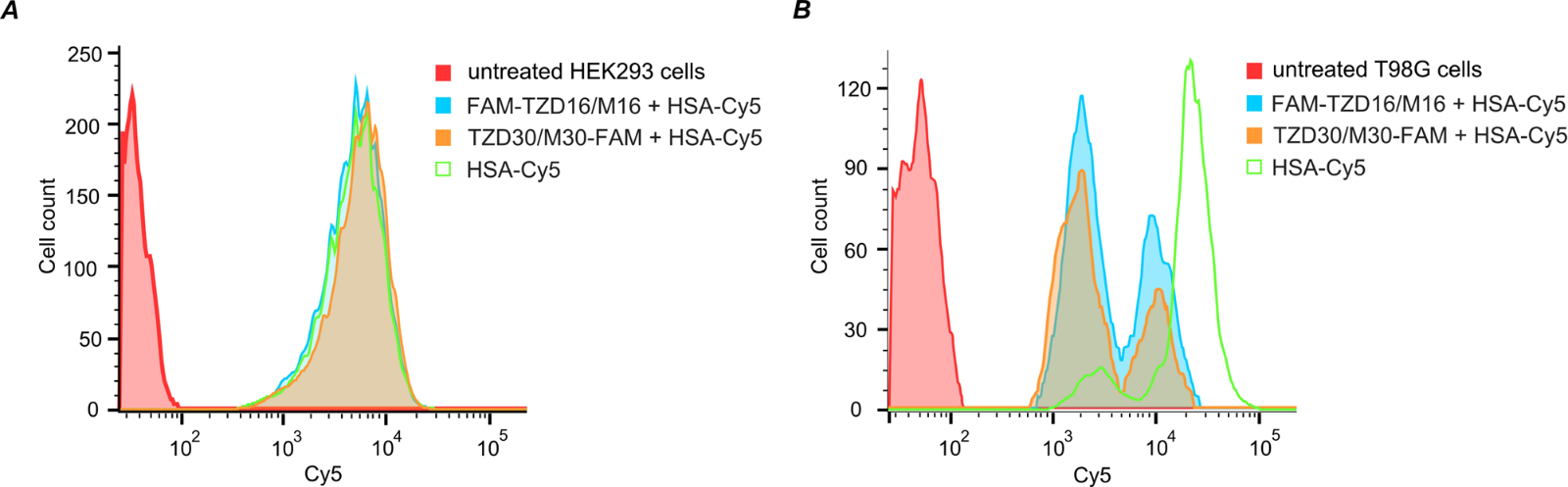
**Figure S10**. Cross-linking of [32P]-dRp-containing DNAs to PARP1 and HSA. 100 nM [32P]-dRp-containing DNAs were incubated at 37⁰C for 10 min with 1.5 μM HSA (lanes 2), 3.0 μM HSA (lanes 3), 4.5 μM HSA (lanes 4), 150 nM PARP1 (lanes 5), 150 nM PARP1+ 1.5 μM HSA (lanes 6), 150 nM PARP1+ 3.0 μM HSA (lanes 7), and 150 nM PARP1+ 4.5 μM HSA (lanes 8). Lanes 1 – DNA, control without protein(s). After incubation, the reaction mixtures were supplemented with 20 mM sodium borohydride to reduce the Schiff base for 30 min at 0 °C. The products were resolved in 7.5% SDS-PAAG according to the Laemmli method. The gels were dried and exposed to a phosphor imaging screen. The type of DNA is indicated above the autoradiograph.

# **S11. Oligonucleotide cytotoxicity studies**

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**Figure S11.** MTT test results for TZD30 and TZD30/M30-FAM on HEK293 and T98G cell lines.

# **S12. Penetration of HSA and its complexes with TZD16 and TZD30 oligomers into cells**



**Figure S12.** Penetration of HSA and its complexes with TZD16 and TZD30 oligomers into cells. HEK293 (A) and T98G (B) cells were incubated for 4 hours with complexes containing 5 µM HSA and 5 µM oligomers.