

## Separation of chemically modified oligonucleotides using YMC-Triart Bio C4

U190829AE

Antisense DNA and siRNA have been widely used for gene silencing in basic research and in medicinal applications. Effective delivery of the oligonucleotides into cells is important for clinical applications. Previous methods took several hours or more to deliver oligonucleotides to cytoplasm. However, oligonucleotides modified with low molecular weight disulfide units at their terminuses reached the cytoplasm 10 minutes after administration to cell culture<sup>1)</sup>.

In RP-HPLC using C18 columns, such disulfide modified oligonucleotides show poor peak shape due to too strong interaction between highly hydrophobic their disulfide units and C18 phase. On the other hand, YMC-Triart Bio C4 show a good peak shape because of its short alkyl chains and low hydrophobic interaction.

### Separation of disulfide-modified oligonucleotides

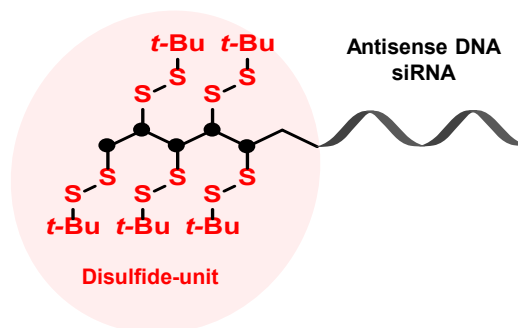
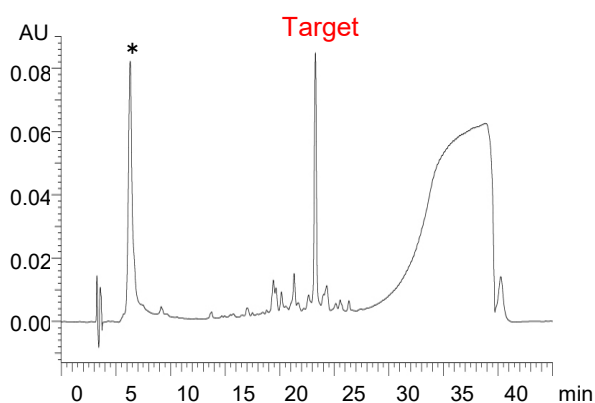
#### 【Cell membrane permeable oligonucleotides】

Oligonucleotides are negatively charged polymers, resulting in low cell membrane permeable efficiency. Various strategies are being pursued including chemical modification of oligonucleotides itself. Recently, it is reported that the disulfide-modified oligonucleotides are efficiently internalized into cytoplasm through disulfide exchange reactions with thiol groups on cell surface.<sup>1)</sup>

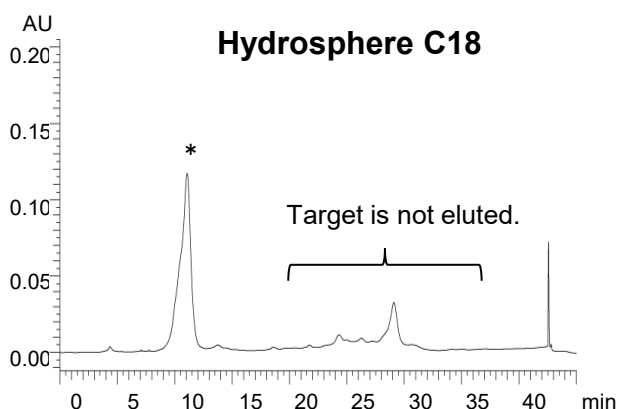
#### 【Separation of highly hydrophobic oligonucleotides】

The chromatograms show the separation of phosphorothioate oligonucleotides modified with highly hydrophobic disulfide units. The target disulfide-modified oligonucleotides are not completely eluted from the C18 column due to the strong hydrophobic interaction. On the other hand, a good peak shape is obtained using YMC-Triart Bio C4, which has short alkyl chains, by the moderate interaction.

#### YMC-Triart Bio C4



Disulfide-modified oligonucleotides



Column	: 5 μm, 250 X 4.6 mm I.D.
Eluent	: A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5) B) acetonitrile 5-95%B (0-30 min), 95%B (30-35 min), 95-5%B (35-35.1 min), 5%B (35.1-45 min)
Flow rate	: 1 mL/min
Temperature	: 50°C
Detection	: UV at 260 nm
Sample	: crude reaction mixture

\*triethylammonium acetate

\* phosphorothioate oligonucleotides without disulfide-unit

Reference 1)

Zhaome Shu et al. (2019) Disulfide-Unit Conjugation Enables Ultrafast Cytosolic Internalization of Antisense DNA and siRNA. *Angew. Chem.*, 131, 6683-6687

Courtesy of Saki Kawaguchi, Chemistry Department, Nagoya University, Japan