**Supporting Information**

**Structure–Activity Relationship of Antibody–Oligonucleotide Conjugates: Evaluating**

**Bioconjugation Strategies for Antibody–Phosphorodiamidate Morpholino Oligomers Conjugates**

**for Drug Development**

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**Table S1.** PMO conjugate structures.

| Conjugate | Linker | Sequence | Reagent added to reaction mixture (molar equivalent relative to mAb) | | Purity by SEC (%) | | DAR 0 (%) | Unconjugated PMO / Conjugated PMO (%) | DAR | Dose | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TCEP | PMO | Monomer | HMWS | | | | PMO (mg/kg) | mAb (mg/kg) |
| αmTfR1-MCC-pmoEx23 3' DAR3.6 (Fig 2) | MCC | GGCCAAACCTCGGCTTACCTGAAAT(3'β-Alanine) | 6 | 2.75 | 94 | 6 | 0 | 3 | 3.6 | 10.2 | 50.0 |
| | | | | | | | | | | 5.1 | 25.0 |
| | | | | | | | | | | 2.5 | 12.5 |
| αmTfR1-MCC-pmoEx23 5' DAR3.7 (Fig 2) | MCC | (5'Amine)GGCCAAACCTCGGCTTACCTGAAAT | 6 | 2.75 | 97 | 3 | 0 | 6 | 3.7 | 10.5 | 50.0 |
| | | | | | | | | | | 5.2 | 25.0 |
| | | | | | | | | | | 2.6 | 12.5 |
| αmTfR1-PEG2-pmoEx23 3' DAR3.9 (Fig 2) | PEG2 | GGCCAAACCTCGGCTTACCTGAAAT | 6 | 2.75 | 93 | 7 | 0 | 1 | 3.9 | 11.0 | 50.0 |
| | | | | | | | | | | 5.5 | 25.0 |
| | | | | | | | | | | 2.8 | 12.5 |
| αmTfR1-MCC-SCRAM DAR3.4 (Fig 2) | MCC | CGGTGTGTGTATCATTCTCTAGTGT(3'β-Alanine) | 6 | 3.5 | 93 | 7 | 0.1 | 1 | 3.4 | 9.6 | 50.0 |
| | | | | | | | | | | 4.8 | 25.0 |
| | | | | | | | | | | 2.4 | 12.5 |
| αmTfR1-MCC-pmoEx23 DAR4.1 (Fig 3) | MCC | GGCCAAACCTCGGCTTACCTGAAAT(3'β-Alanine) | 6 | 3.5 | 92 | 8 | 0 | 5 | 4.1 | 11.6 | 50.0 |
| αmTfR1-ValCit-pmoEx23 DAR4.2 (Fig 3) | ValCit | GGCCAAACCTCGGCTTACCTGAAAT(3'β-Alanine) | 6 | 3.5 | 95 | 5 | 0 | 3 | 4.2 | 11.9 | 50.0 |
| αmTfR1-BisMal-pmoEx23 DAR3.6 (Fig 3) | BisMal | GGCCAAACCTCGGCTTACCTGAAAT(3'β-Alanine) | 6 | 3.15 | 91 | 9 | 0 | 3 | 3.6 | 10.2 | 50.0 |
| αmTfR1-MCC-pmoEx23 DAR1 (Fig 4a) | MCC | GGCCAAACCTCGGCTTACCTGAAAT(3'β-Alanine) | 6 | 3.15 | 98 | 2 | 2.1 | 2 | 1.0 | | |

| Name | | Sequence | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| αmTfR1-MCC-pmoEx23 DAR2 (Fig 4) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 6 | 3.15 | 96 | 4 | 0.7 | 4 | 2.0 | 5.7<br>1.9 | 50.0<br>16.7 |
| αmTfR1-MCC-pmoEx23 DAR3 (Fig 4) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 6 | 3.15 | 98 | 2 | 0.3 | 3 | 3.0 | 8.5<br>5.7<br>2.8 | 50.0<br>33.4<br>16.7 |
| αmTfR1-MCC-pmoEx23 DAR4 (Fig 4) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 6 | 3 | 99 | 1 | 0.3 | 1 | 4.0 | 11.3<br>3.8<br>5.7 | 50.0<br>28.8<br>16.7 |
| αmTfR1-MCC-pmoEx23 DAR5.5 (Fig 4) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 6 | 2.75 | 97 | 3 | 0.8 | 1 | 5.5 | 15.6<br>5.7 | 50.0<br>18.2 |
| αmTfR1-MCC-pmoEx23 DAR3.9(Fig 4) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 6 | 3 | 98 | 2 | 0.2 | 3 | 3.9 | 11.0<br>6.4<br>3.7 | 50.0<br>28.8<br>16.7 |
| Control.mAb-MCC-PMOEx23 DAR3.9(Fig4) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 6 | 2.75 | 97 | 3 | 0 | 3 | 3.9 | 11.0 | 50.0 |
| αmTfR1-MCC-pmoEx23 DAR4 (Fig 5) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 2.2 | 5.2 | 97 | 3 | 7.6 | <1% | 3.9 | 10.0 | 45.9 |
| αmTfR1-MCC-pmoEx23 DAR7 (Fig 5) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 5 | 10 | 93 | 6 | 0.2 | <1% | 7.0 | 18.3 | 45.9 |
| αmTfR1-MCC-pmoEx23 DAR4 (Fig 6) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 2.3 | 8.2 | 95 | 5 | 8.5 | <1% | 3.9 | 10.0 | 44.2 |
| αmTfR1-MCC-pmoEx23 DAR10 (Fig 6) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 8 | 13.2 | 95 | 5 | 0 | <1% | 9.7 | 10.0 | 22.1 |
| αmTfR1-MC-pmoEx23 DAR4 (Fig 6) | MC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 2.3 | 7 | 94 | 6 | 8.5 | <1% | 3.9 | 10.0 | 44.2 |
| αmTfR1-MC-pmoEx23 DAR6 (Fig 6) | MC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 3.5 | 8.5 | 95 | 5 | 2.9 | <1% | 5.8 | 10.0 | 29.5 |

| αmTfR1-MC-pmoEx23 DAR10 (Fig 6) | MC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 8 | 11 | 92 | 8 | 1.6 | <1% | 9.7 | 10.0 | 22.1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| αmTfR1-MCC-pmoEx23 DAR4 (Fig 7/8) | MCC | GGCCAAA CCTCGGC TTACCTGA AAT(3'β-Alanine) | 2.3 | 5 | 96 | 4 | 3.7 | <1% | 4.4 | 10.0 | 44.2 |

Abbreviations: αmTfR1, anti-mouse transferrin receptor 1 antibody; BisMal, bismaleimide; DAR, drug-to-antibody ratio; mAb, monoclonal antibody; MC, maleimidocaproyl; MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; pmoEx23, PMO targeting mouse exon 23; SEC, size exclusion chromatography.

**Table S2.** Primers used for exon-skipping analysis.

| Target | Species | Primer/probe or commercial TaqMan assay ID | Sequence (5´-3´) |
|---|---|---|---|
| **Exon 23 skipped Dmd** | Mouse | Fwd primer | GCGCTATCAGGAGACAATGAG |
| | | Rev primer | GTTTTTATGTGATTCTGTAATTTCCC |
| | | Probe | CTCTCTGTACCTTATCTTAGTGTT |
| **Exon 23 non-skipped Dmd** | Mouse | Fwd primer | TGGAGGAGAGACTCGGGAAA |
| | | Rev primer | TTGAAGCCATTTTGTTGCTCTTT |
| | | Probe | ACAGGCTCTGCAAAGT |
| **Total Dmd** | Mouse | TaqMan assay ID: Mm01216925_m1 | |
| **Ppib** | Mouse | TaqMan assay ID: Mm00478295_m1 | - |

Abbreviations: Dmd, dystrophin; DMD, Duchenne muscular dystrophy; Fwd, forward; ID, identifier;
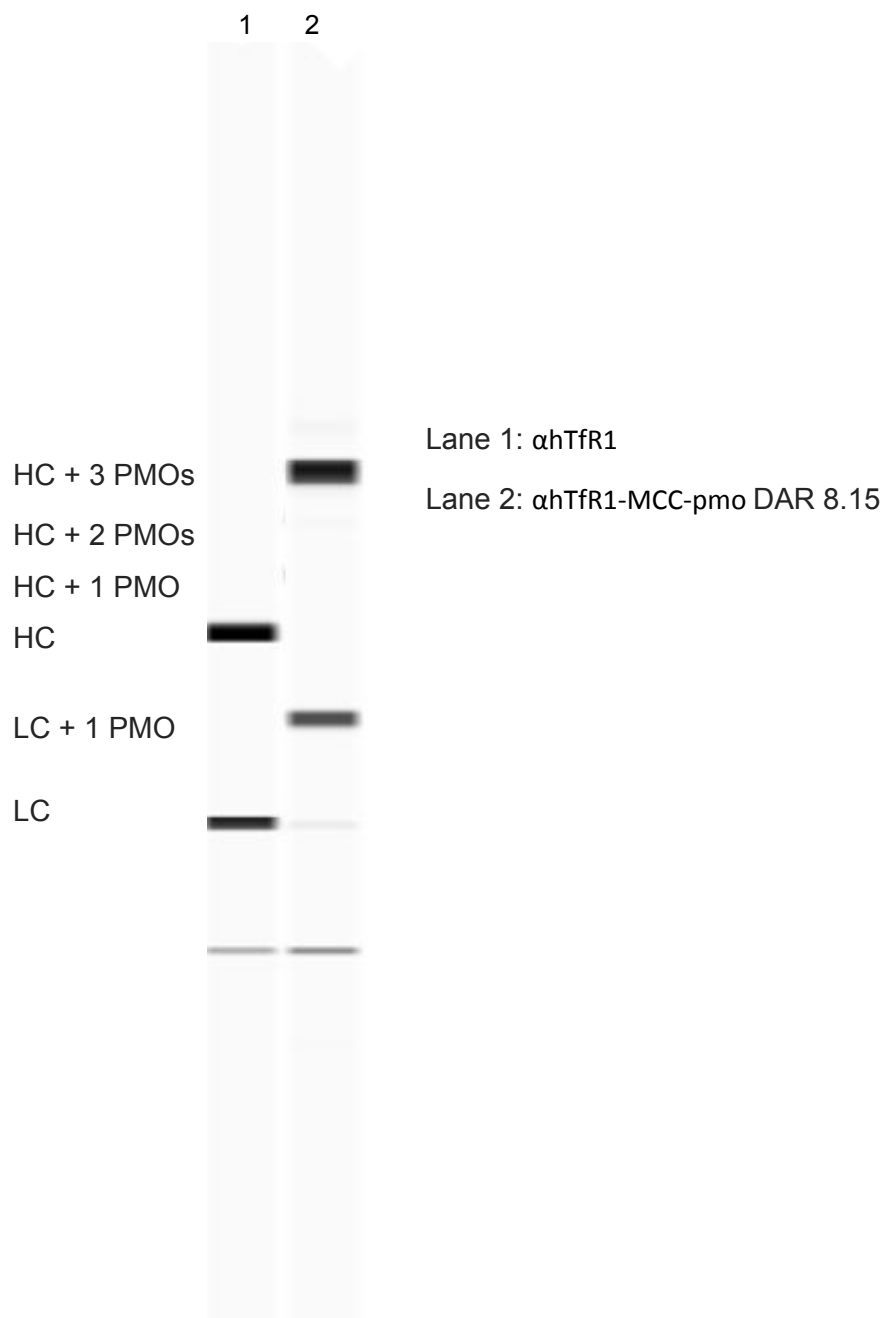
Ppib, peptidylprolyl isomerase B; Rev, reverse.

**Plasma clearance of targeted versus untargeted AOC**
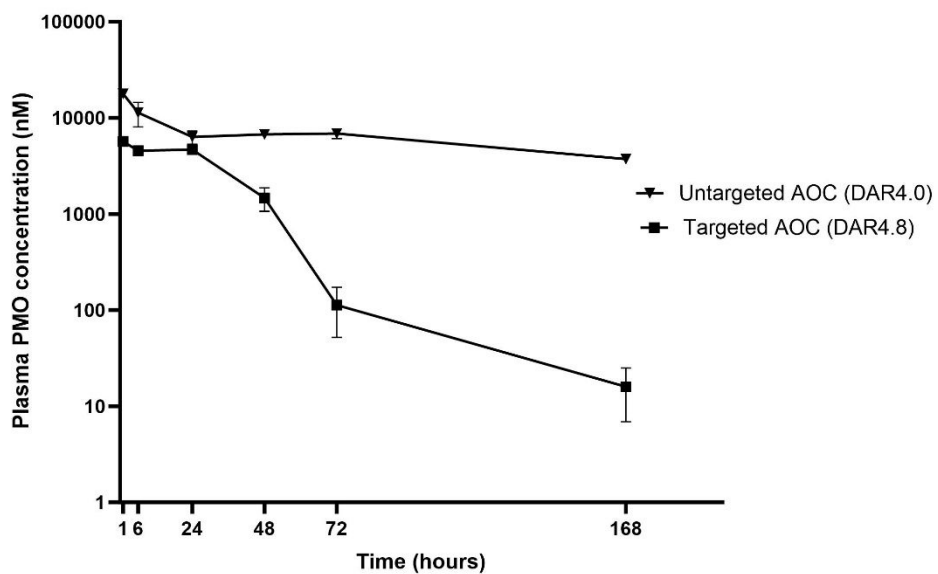
**Figure S1.** Plasma clearance of targeted versus untargeted AOCs. AOC, antibody–oligonucleotide conjugate; DAR, drug-to-antibody ratio; PMO, phosphorodiamidate morpholino oligomer.
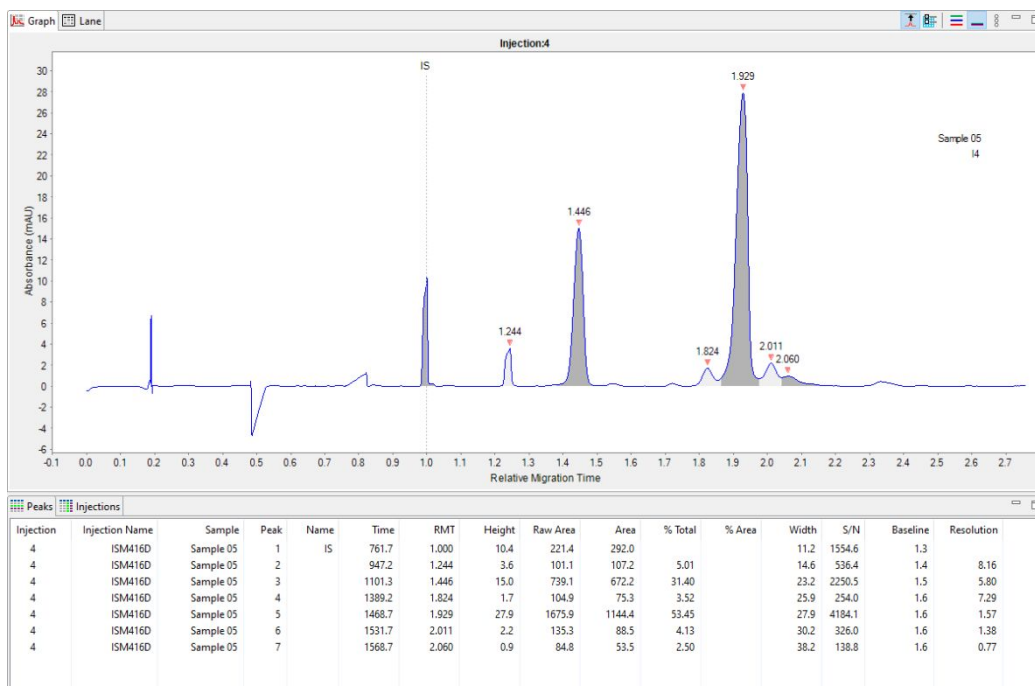
# Reduced capillary gel electrophoresis

|  | 1 | 2 |
|---|---|---|

HC + 3 PMOs

HC + 2 PMOs

HC + 1 PMO

HC

LC + 1 PMO

LC

Lane 1: αhTfR1

Lane 2: αhTfR1-MCC-pmo DAR 8.15

**b.**

# Reduced capillary gel electrophoresis



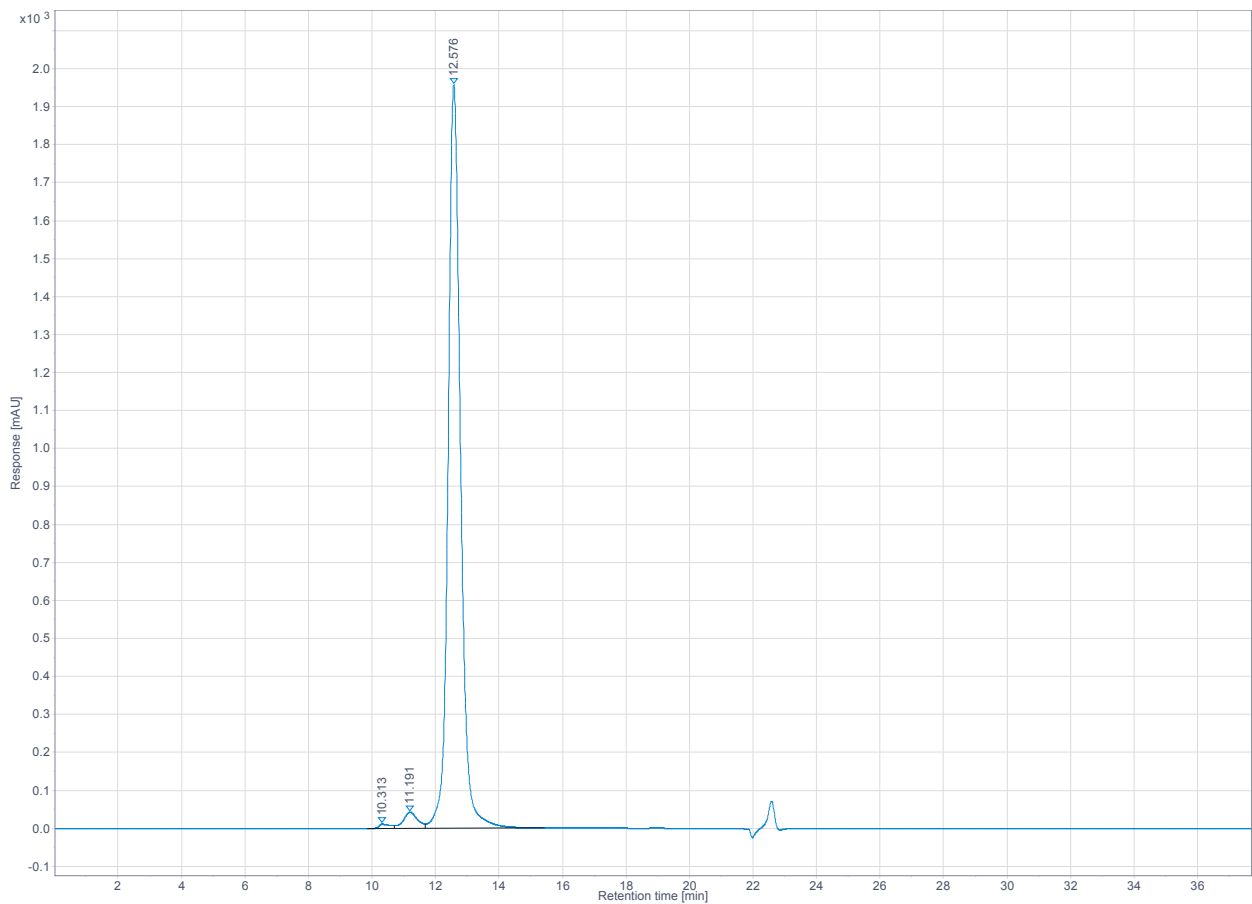| Peak | Name | | Area | Area % | Weighted Area |
|---|---|---|---|---|---|
| 1 | IS | | 292 | | |
| | | | | | |
| 2 | **LC** | | 107 | 0.14 | 0.00 |
| 3 | **LC+1** | | 672.2 | 0.86 | 0.86 |
| | | **Total LC Area** | 779.2 | | |
| | | | | | |
| | | | | | |
| 4 | **HC** | | 0 | 0.00 | 0.00 |
| 5 | **HC+1** | | 0 | 0.00 | 0.00 |
| 6 | **HC+2** | | 75.3 | 0.06 | 0.12 |
| 7 | **HC+3** | | 1144.4 | 0.87 | 2.62 |
| 8 | **HC+4** | | 88.5 | 0.07 | 0.27 |
| 8 | **HC+5** | | 53.5 | 0.04 | 0.20 |
| | | **Total HC Area** | 1308.2 | | 3.21 |
| | | | | **(LC+HC) Weighted Area** | 4.08 |
| | | | | **DAR= 2*(LC+HC) Weighted Area** | **8.15** |

**c.**

# Analytical HIC (FLD)



**d.**
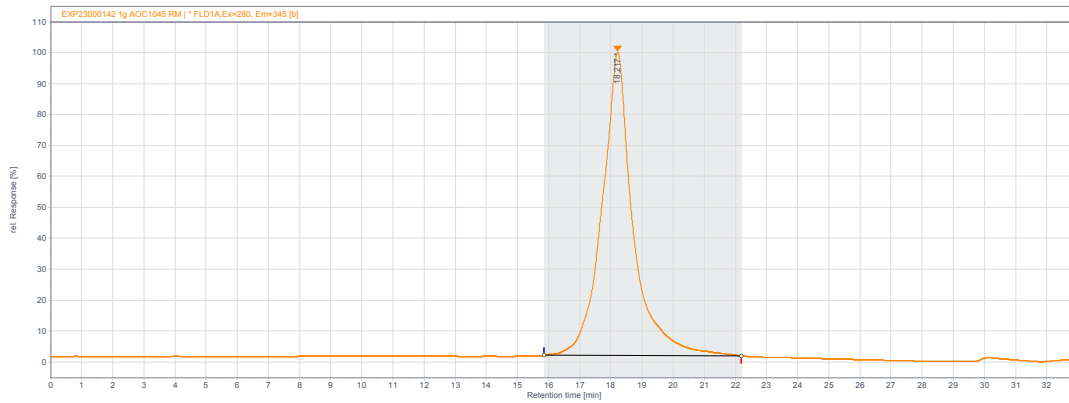
# Analytical SEC (220 nM)

2.9% HMW

**Figure S2.** Characterization of an (a) Lane view of reduced capillary gel electrophoresis and (b) electrophoretogram of reduced capillary gel electrophoresis with the weighted average calculating the average DAR to be 8.15, (c) Hydrophobic interaction chromatography showing the AOC as a single peak and (d) size exclusion chromatography for an αhTfR1-MCC-pmo DAR8 AOC (TCEP reduction at 4 eq. TCEP, PMO:mAb equivalents 9.75). AOC, antibody–oligonucleotide conjugate; DAR, drug-to-antibody ratio; eq, equivalents; FLD, fluorescence detector; HIC, hydrophobic interaction chromatography; HMW, high molecular weight species; LU, luminescence units; mAU, milli-absorbance units; MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; SEC, size exclusion chromatography; TCEP, tris(2-carboxyethyl)phosphine.
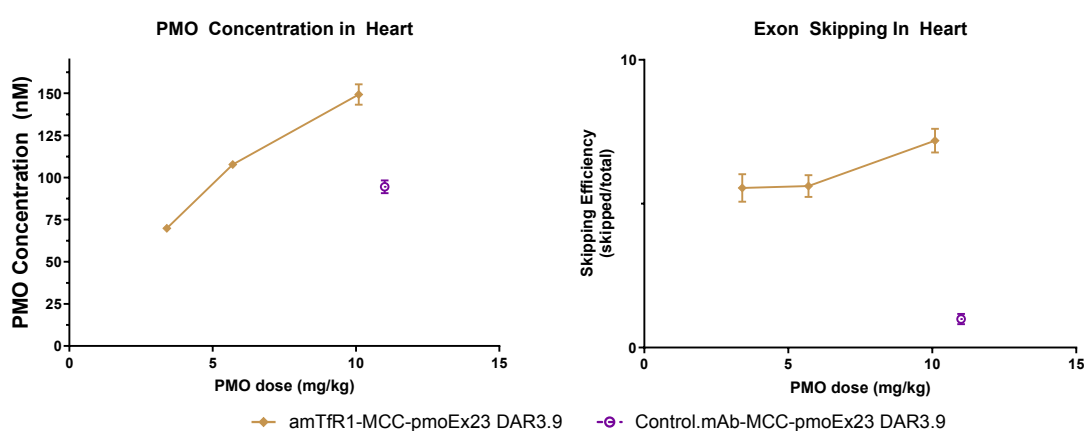


**Figure S3.** High concentrations of PMO measured in heart after treating mice with non-targeting mAb PMO-AOC however only TfR1 mediated uptake of PMO results in exon skipping.

DAR, drug-to-antibody ratio; mAb, monoclonal antibody; MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; PMO, phosphorodiamidate morpholino oligomer; pmoEx23, PMO targeting mouse exon 23.
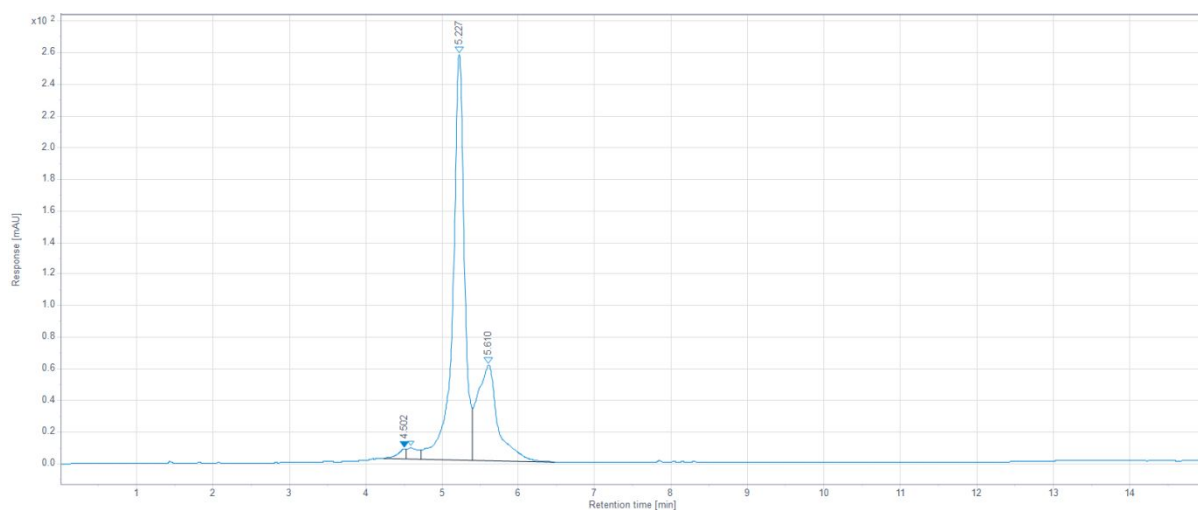
**Figure S4.** Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis to measure pmoEx23 starting material purity (69.2% main peak area). mAU, milli-absorbance units; PMO, phosphorodiamidate morpholino oligomer; pmoEx23, PMO targeting mouse exon 23.
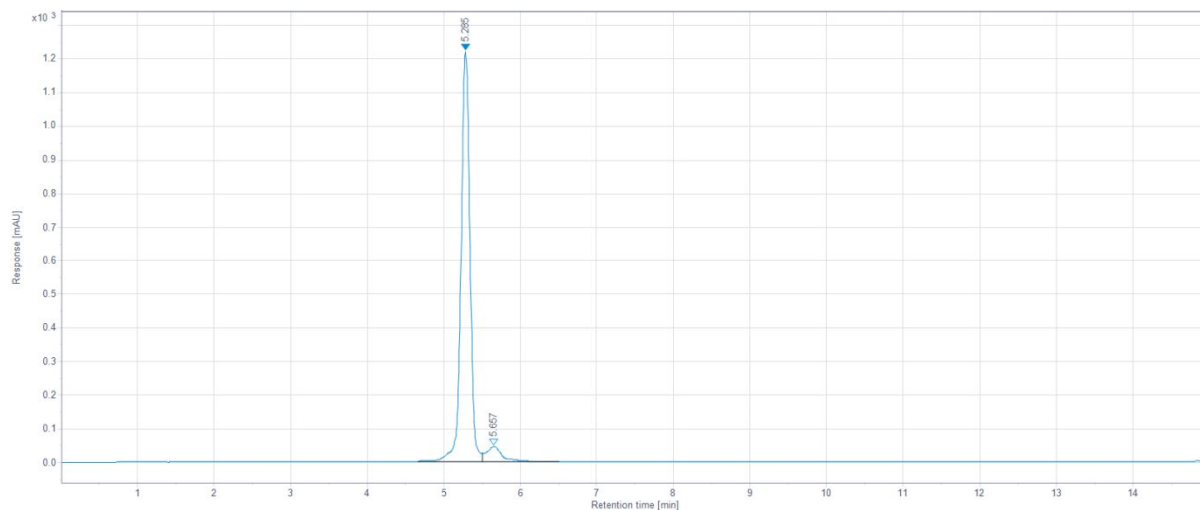
**Figure S5.** Post-strong anion exchange chromatography purification of pmoEx23: 93.7% main peak purity. mAU, milli-absorbance units; PMO, phosphorodiamidate morpholino oligomer; pmoEx23, PMO targeting mouse exon 23.

**a.**



**b.**
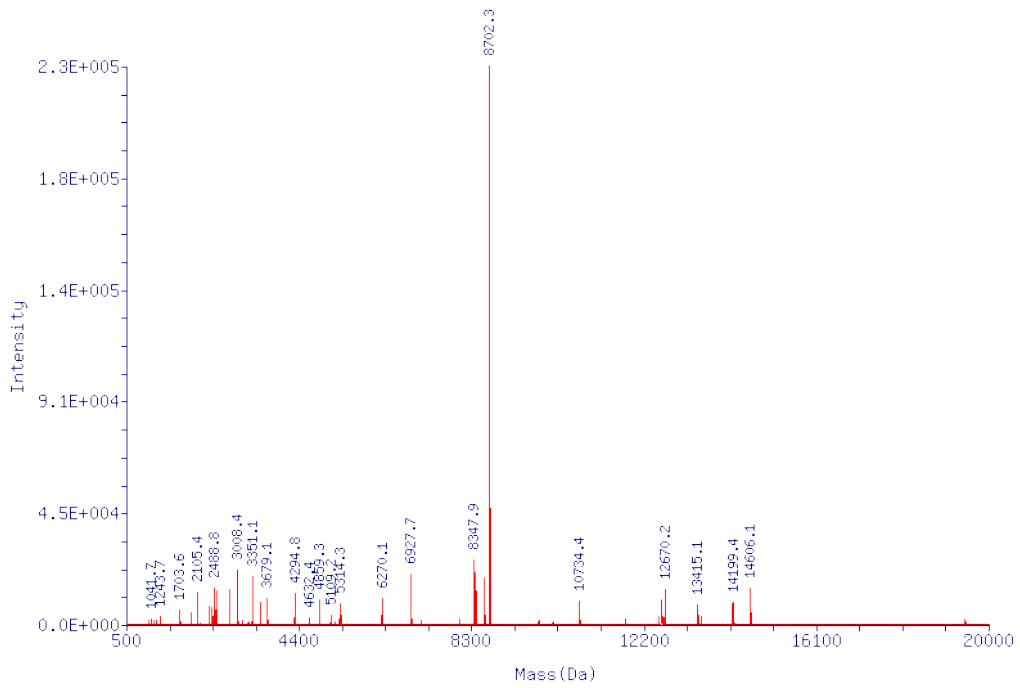
**c.**
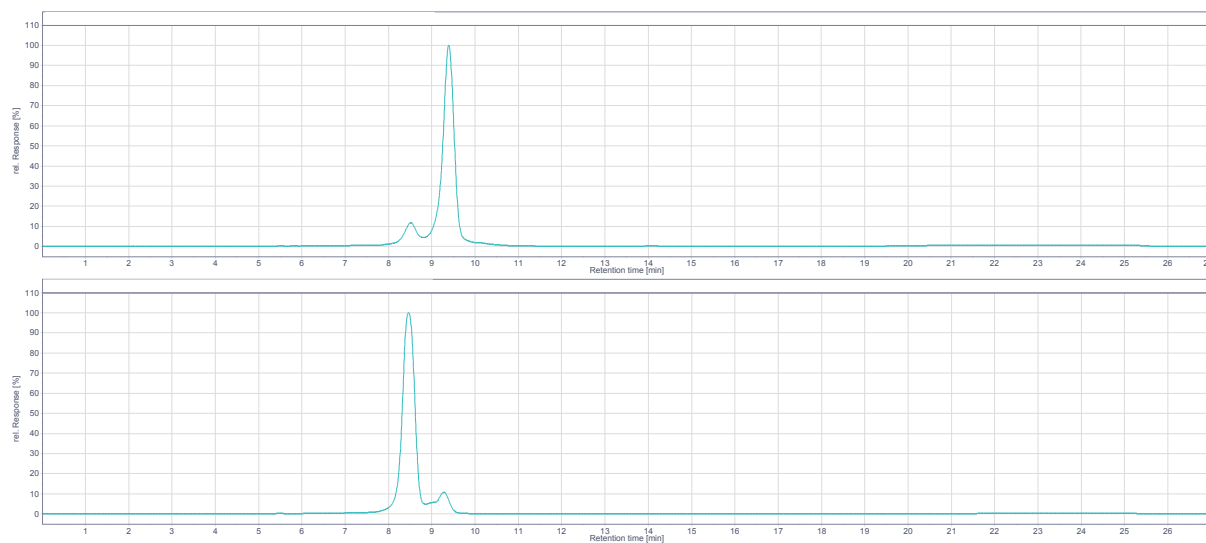


**Figure S6.** Electrospray ionization mass spectrum of pmoEx23 with 3' beta-alanine (a) before and (b) after addition of MCC linker. (c) RP-HPLC pmoEx23 with 3´ beta-alanine before (bottom) and after (top) addition of MCC linker. MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; mEx23, mouse Exon23; pmoEx23, PMO targeting mouse exon 23; rel, relative; RP-HPLC, reverse-phase high-performance liquid chromatography.

**a.**
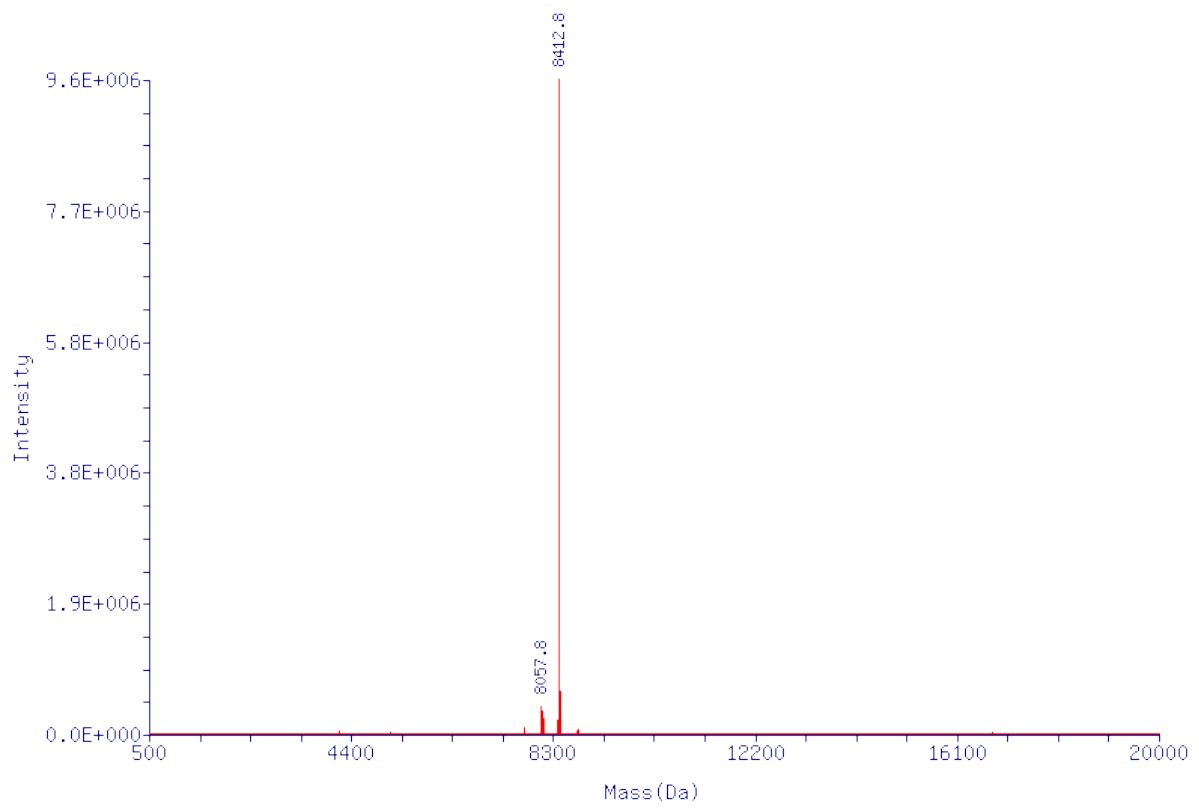
**b.**
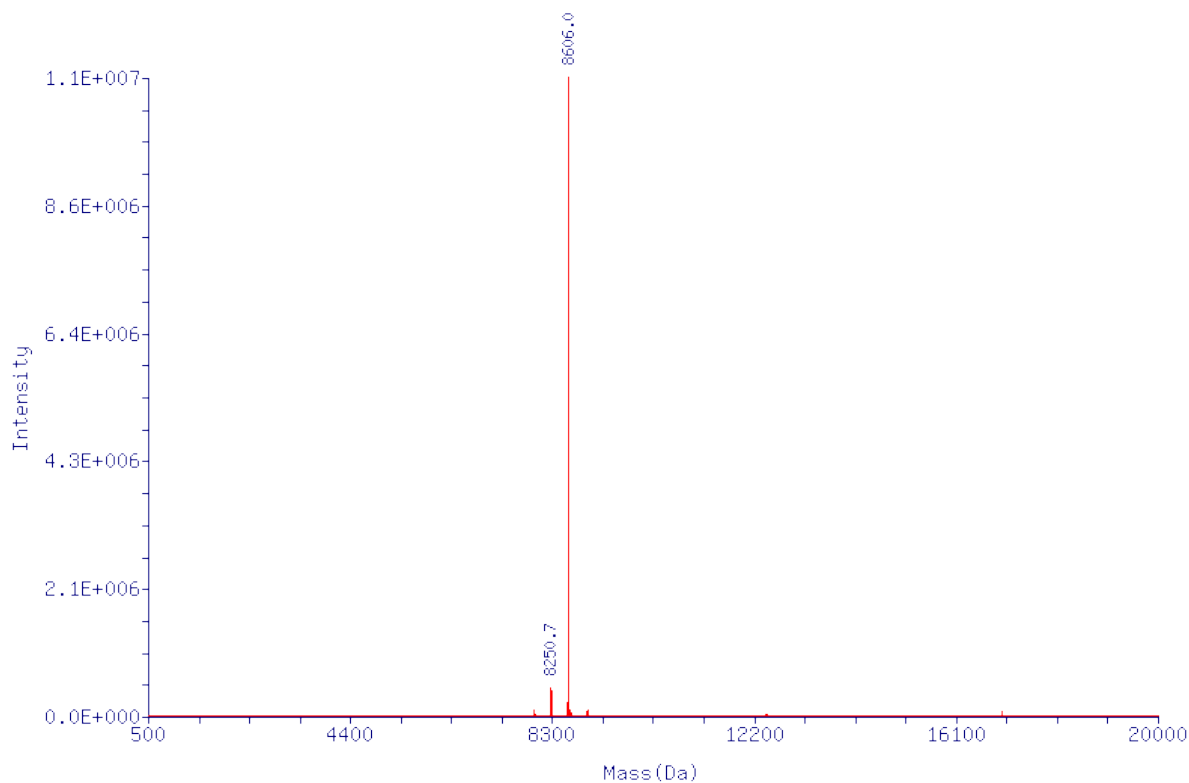


**c.**
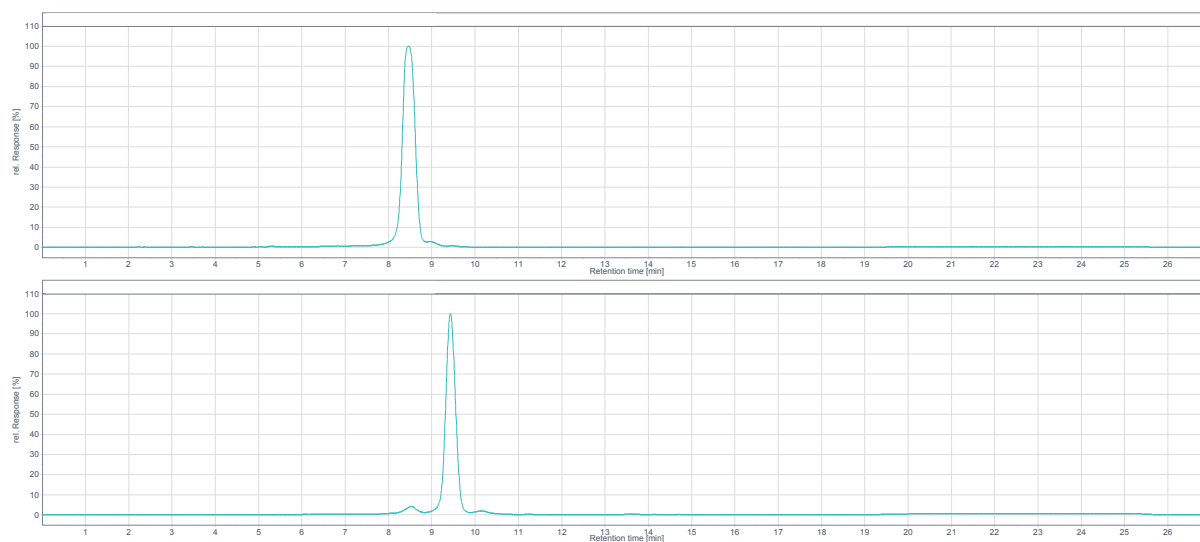


**Figure S7.** Electrospray ionization mass spectrum of pmoEx23 without 3' beta-alanine (a) before and (b) after addition of MCC linker. (c) RP-HPLC pmoEx23 before (top) and after (bottom) addition of MCC linker. ESI, electrospray ionization; MC, maleimidocaproyl; MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; PMO, phosphorodiamida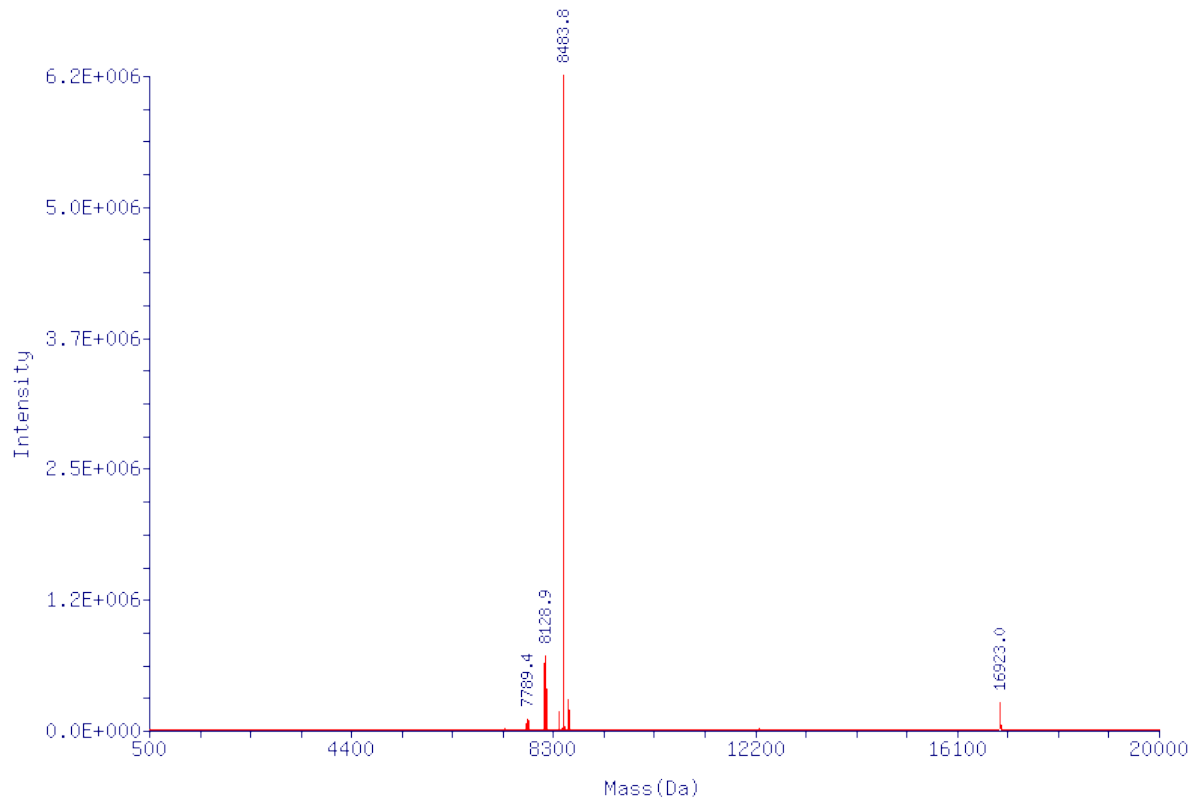te morpholino oligomer; pmoEx23, PMO targeting mouse exon 23; rel, relative; RP-HPLC, RP-HPLC, reverse-phase high-performance liquid chromatography.

**a.**

# Reduced capillary gel electrophoresis



| | 1 | 2 |
|---|---|---|
| HC + 4 PMOs | | |
| HC + 3 PMOs | | |
| HC + 2 PMOs | | Lane 1: αmTfR1 |
| HC + 1 PMO | | |
| HC | | Lane 2: αmTfR1-MCC-pmoEx23 DAR 3.9 |
| LC + 1 PMO | | |
| LC | | |
| 1 – | | – IS |

# Reduced capillary gel electrophoresis



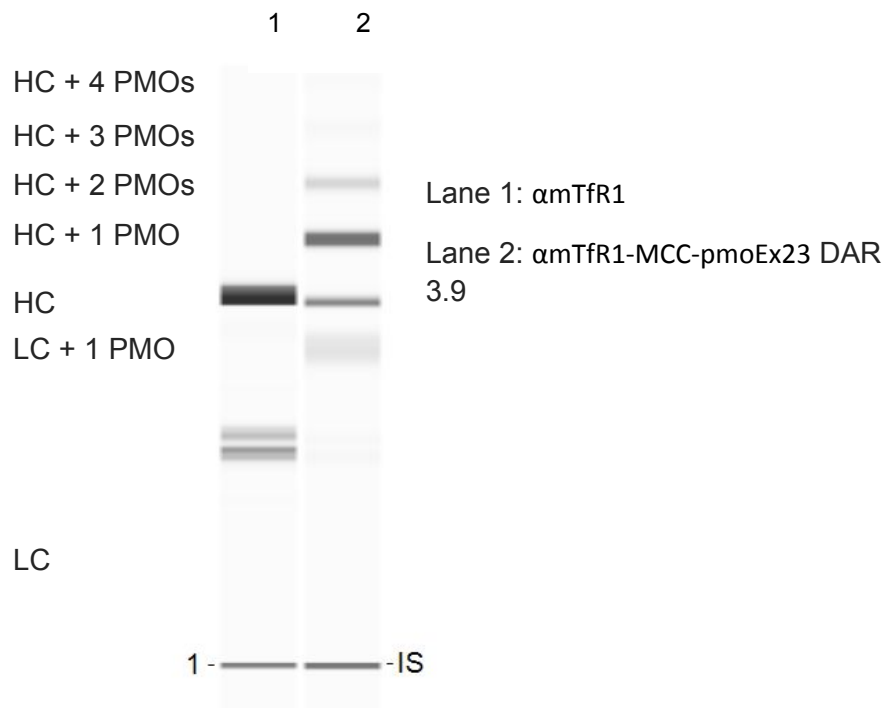| Injection | Injection Name | Sample | Peak | Name | Time | RMT | Height | Raw Area | Area | % Total | % Area | Width | S/N | Baseline | Resolution |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | DMK-028C_03 | DMK-028C | 1 | IS | 757.1 | 1.000 | 11.3 | 191.5 | 253.5 | | | 8.3 | 1212.0 | 0.9 | |
| 3 | DMK-028C_03 | DMK-028C | 2 | | 1009.8 | 1.334 | 1.5 | 109.4 | 107.4 | 8.60 | | 41.0 | 163.1 | 1.0 | 11.68 |
| 3 | DMK-028C_03 | DMK-028C | 3 | | 1139.2 | 1.505 | 3.7 | 324.7 | 284.2 | 22.76 | | 42.5 | 398.9 | 1.0 | 1.59 |
| 3 | DMK-028C_03 | DMK-028C | 4 | | 1198.7 | 1.583 | 8.5 | 251.7 | 209.6 | 16.79 | | 13.5 | 908.4 | 1.0 | 1.15 |
| 3 | DMK-028C_03 | DMK-028C | 5 | | 1275.7 | 1.685 | 12.2 | 479.6 | 375.5 | 30.07 | | 17.6 | 1302.1 | 1.0 | 2.83 |
| 3 | DMK-028C_03 | DMK-028C | 6 | | 1344.6 | 1.776 | 4.8 | 231.3 | 172.0 | 13.78 | | 20.1 | 514.5 | 1.0 | 2.09 |
| 3 | DMK-028C_03 | DMK-028C | 7 | | 1411.7 | 1.865 | 1.5 | 81.4 | 57.7 | 4.62 | | 24.0 | 157.3 | 1.0 | 1.77 |
| 3 | DMK-028C_03 | DMK-028C | 8 | | 1469.8 | 1.942 | 1.1 | 62.1 | 42.3 | 3.39 | | 25.9 | 112.6 | 1.0 | 1.37 |

| Peak | Name | | Area | Area % | Weighted Area |
|---|---|---|---|---|---|
| 1 | IS | | 253.5 | | |
| | | | | | |
| 2 | LC | | 107.4 | 0.27 | 0.00 |
| 3 | LC+1 | | 284.2 | 0.73 | 0.73 |
| | | Total LC Area | 391.6 | | |
| | | | | | |
| | | | | | |
| 4 | HC | | 209.6 | 0.24 | 0.00 |
| 5 | HC+1 | | 375.5 | 0.44 | 0.44 |
| 6 | HC+2 | | 172 | 0.20 | 0.40 |
| 7 | HC+3 | | 57.7 | 0.07 | 0.20 |
| 8 | HC+4 | | 42.3 | 0.05 | 0.20 |
| | | Total HC Area | 857.1 | | 1.24 |
| | | | | (LC+HC) Weighted Area | 1.96 |
| | | | | DAR= 2*(LC+HC) Weighted Area | 3.93 |

**c.**

# Analytical HIC (FLD)
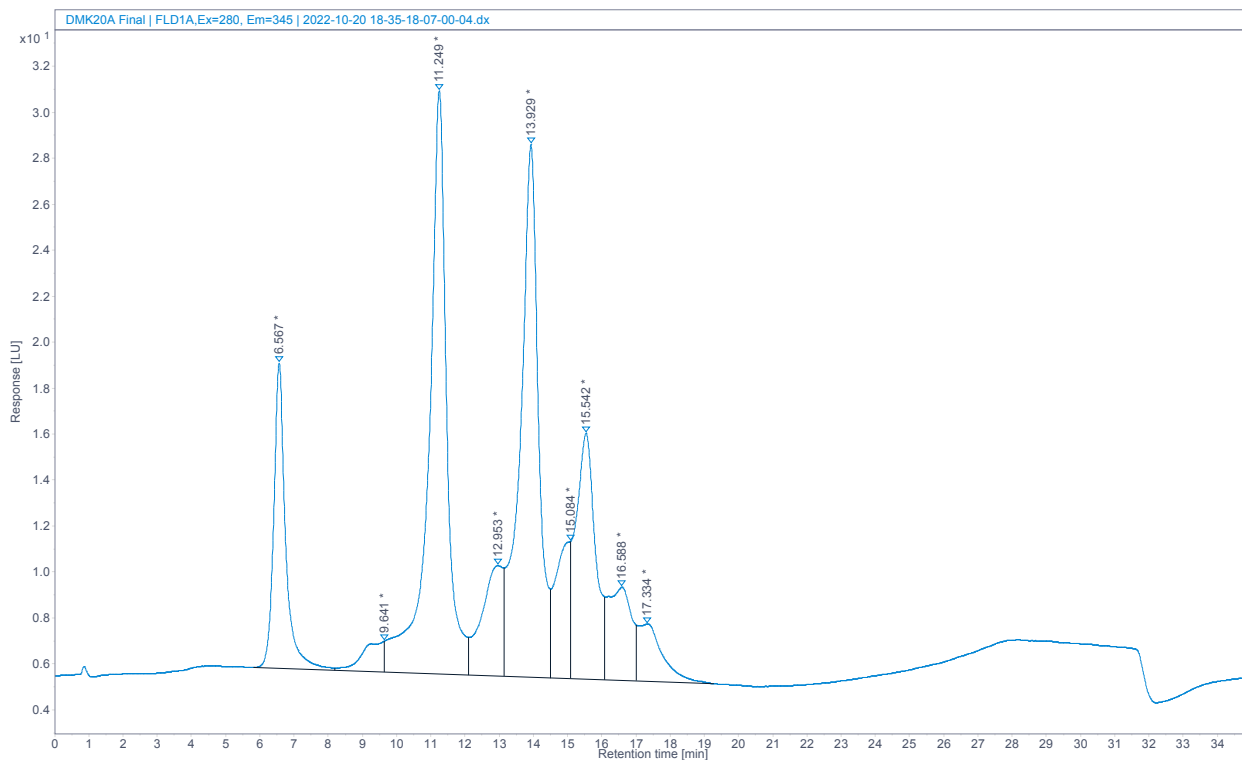


DMK20A Final | FLD1A,Ex=280, Em=345 | 2022-10-20 18-35-18-07-00-04.dx

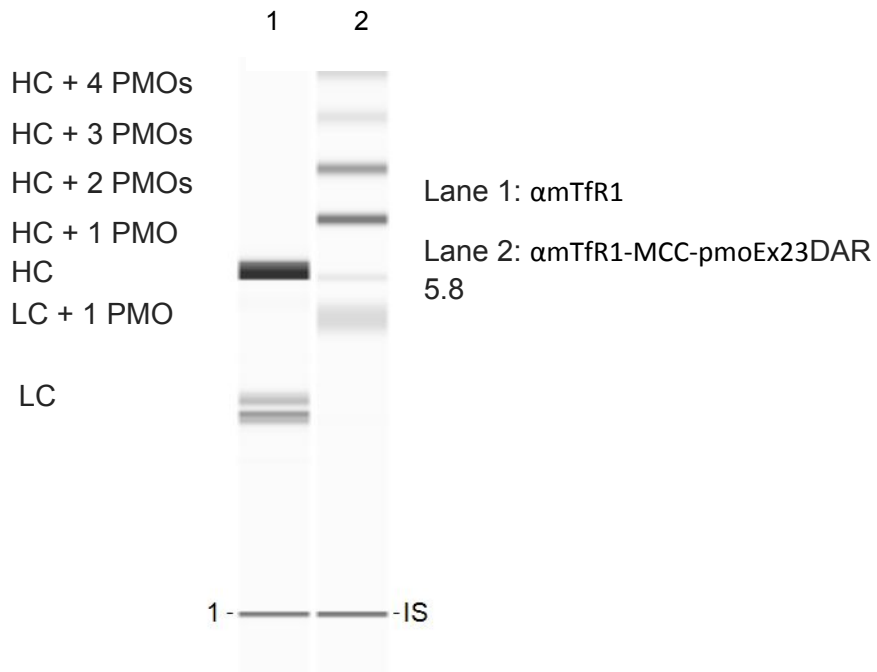| # | DAR | Signal description | RT (min) | Area (LU·s) | Area% | Height (LU) | Height% | Start time (min) | End time (min) | Weighted Area (Area% *0.01*DAR) |
|---|-----|--------------------|----------|-------------|-------|-------------|---------|------------------|----------------|----------------------------------|
| 1 | 0 | FLD1A,Ex=280, Em=345 | 6.567 | 319.036 | 9.633 | 13.288 | 14.57 | 5.828 | 8.2 | 0 |
| 2 | 1 | FLD1A,Ex=280, Em=345 | 9.641 | 58.233 | 1.758 | 1.347 | 1.48 | 8.2 | 9.646 | 0.01758 |
| 3 | 2 | FLD1A,Ex=280, Em=345 | 11.249 | 934.442 | 28.214 | 25.385 | 27.82 | 9.646 | 12.108 | 0.56428 |
| 4 | 3 | FLD1A,Ex=280, Em=345 | 12.953 | 210.097 | 6.343 | 4.801 | 5.26 | 12.108 | 13.147 | 0.19029 |
| 5 | 4 | FLD1A,Ex=280, Em=345 | 13.929 | 863.113 | 26.06 | 23.187 | 25.42 | 13.147 | 14.503 | 1.0424 |
| 6 | 5 | FLD1A,Ex=280, Em=345 | 15.084 | 179.591 | 5.422 | 5.975 | 6.55 | 14.503 | 15.09 | 0.2711 |
| 7 | 6 | FLD1A,Ex=280, Em=345 | 15.542 | 426.818 | 12.887 | 10.706 | 11.73 | 15.09 | 16.084 | 0.77322 |
| 8 | 8 | FLD1A,Ex=280, Em=345 | 16.588 | 194.97 | 5.887 | 4.055 | 4.44 | 16.084 | 17.01 | 0.47096 |
| 9 | 10 | FLD1A,Ex=280, Em=345 | 17.334 | 125.723 | 3.796 | 2.487 | 2.73 | 17.01 | 19.247 | 0.3796 |
| | | | | | | | | | DAR=Sum of Weighted % Area | 3.71 |

d.

# Analytical SEC (220 nM)
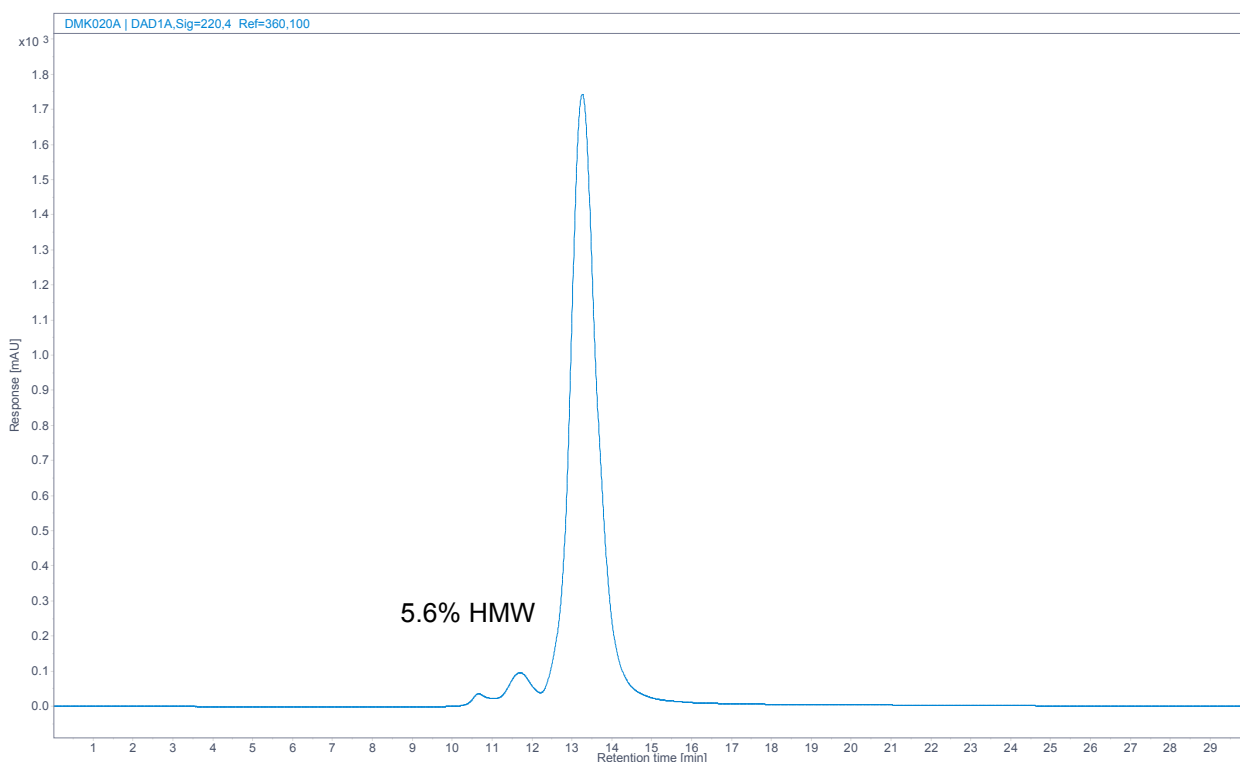


**Figure S8.** Characterization of αmTfR1-MCC-pmoEx23 DAR4 AOC. (a) Lane view of reduced capillary gel electrophoresis and (b) electrophoretogram of reduced capillary gel electrophoresis with the weighted average calculating the average DAR to be 3.93 (c) weighted average calculations of the integrated AUC from Hydrophobic interaction chromatography showed the DAR to be 3.7 and (d) size exclusion chromatography for a αmTfR1-MCC-pmoEx23 DAR4 AOC (partial TCEP reduction at 2.3 eq. TCEP, PMO:mAb equivalents 7). AOC, antibody–oligonucleotide conjugate; DAR, drug-to-antibody ratio; eq, equivalents; FLD, fluorescence detector; HIC, hydrophobic interaction chromatography; HMW, high molecular weight species; LU, luminescence units; MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate**;** mAU, milli-absorbance units; PMO, phosphorodiamidate morpholino oligomer; pmoEx23, PMO targeting mouse exon 23; SEC, size exclusion chromatography; TCEP, tris(2-carboxyethyl)phosphine.
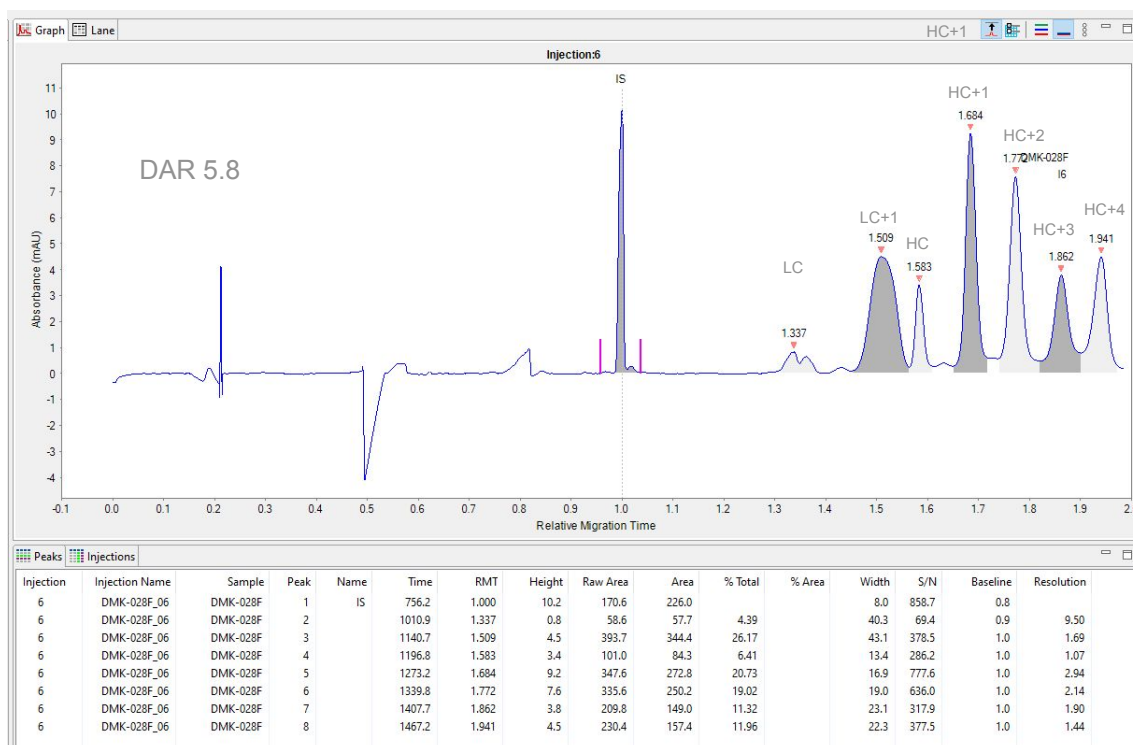
# Reduced capillary gel electrophoresis

1      2

HC + 4 PMOs

HC + 3 PMOs

HC + 2 PMOs

HC + 1 PMO

HC

LC + 1 PMO

LC

1 -

-IS

Lane 1: αmTfR1

Lane 2: αmTfR1-MCC-pmoEx23DAR 5.8

**b.**

# Reduced capillary gel electrophoresis



| Peak | Name | | Area | Area % | Weighted Area |
|------|------|------|------|--------|---------------|
| 1 | IS | | 226 | | |
| | | | | | |
| 2 | LC | | 57.7 | 0.14 | 0.00 |
| 3 | LC+1 | | 344.4 | 0.86 | 0.86 |
| | | Total LC Area | 402.1 | | |
| | | | | | |
| | | | | | |
| 4 | HC | | 84.3 | 0.09 | 0.00 |
| 5 | HC+1 | | 272.8 | 0.30 | 0.30 |
| 6 | HC+2 | | 250.2 | 0.27 | 0.55 |
| 7 | HC+3 | | 149 | 0.16 | 0.49 |
| 8 | HC+4 | | 157.4 | 0.17 | 0.69 |
| | | Total HC Area | 913.7 | | 2.02 |
| | | | | (LC+HC) Weighted Area | 2.88 |
| | | | | DAR= 2*(LC+HC) Weighted Area | **5.76** |

## c. Analytical HIC (FLD)
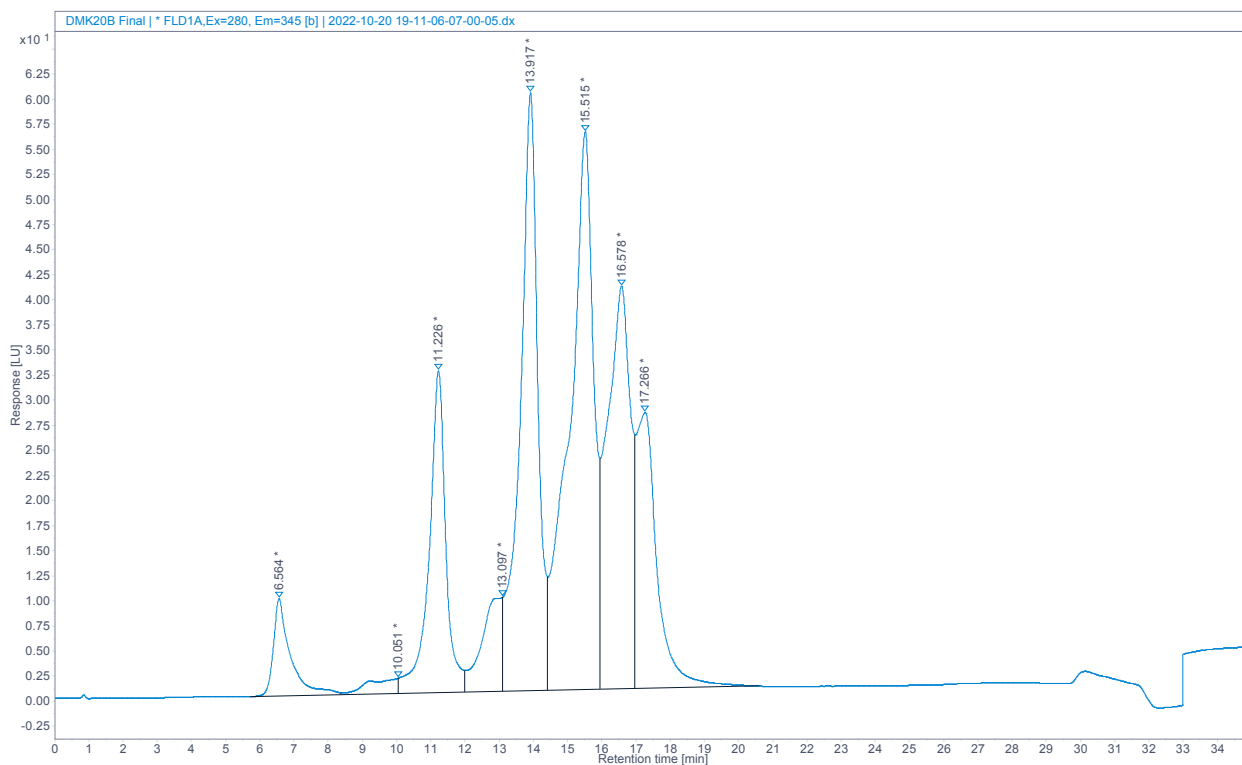


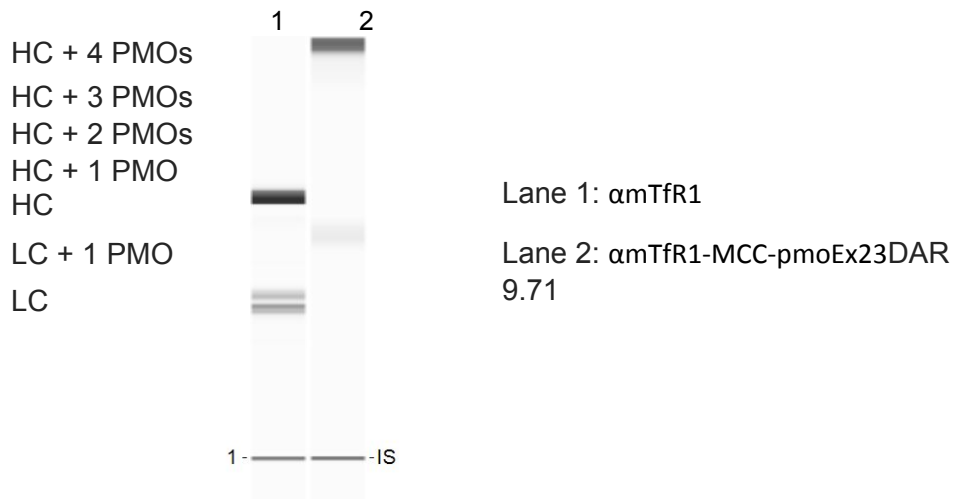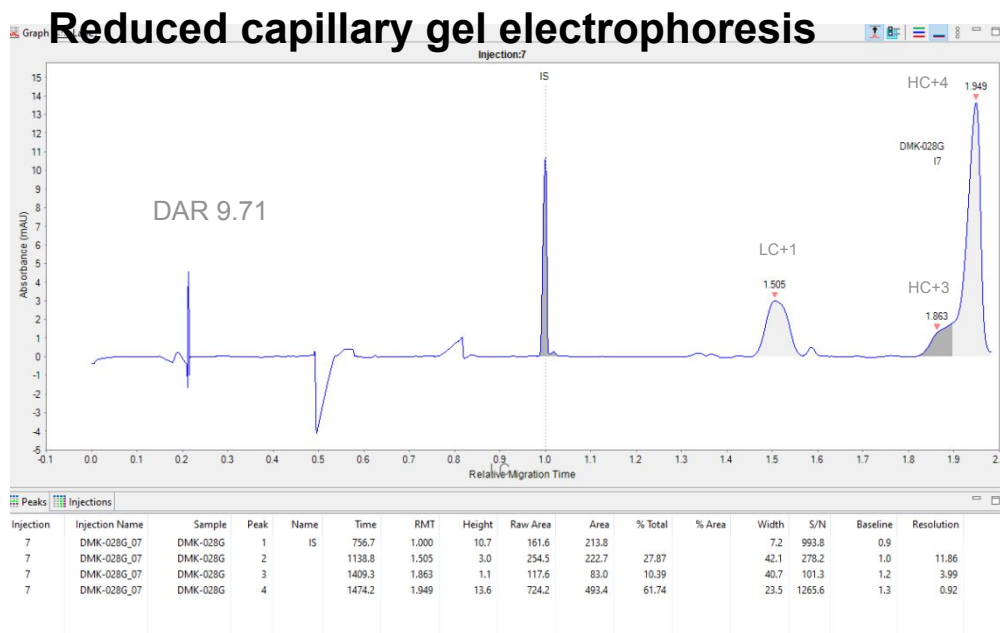| # | DAR | Signal description | RT (min) | Area (LU·s) | Area% | Height (LU) | Height% | Start time (min) | End time (min) | Weighted Area (Area% *0.01*DAR) |
|---|-----|-------------------|----------|-------------|-------|-------------|---------|------------------|----------------|----------------------------------|
| 8 | 10 | * FLD1A,Ex=280, Em=345 [b] | 17.266 | 1172.512 | 11.866 | 27.482 | 11.67 | 16.965 | 20.58 | 1.1866 |
| 7 | 8 | * FLD1A,Ex=280, Em=345 [b] | 16.578 | 1889.871 | 19.126 | 40.13 | 17.04 | 15.949 | 16.965 | 1.53008 |
| 6 | 6 | * FLD1A,Ex=280, Em=345 [b] | 15.515 | 2746.694 | 27.797 | 55.596 | 23.61 | 14.413 | 15.949 | 1.66782 |
| 5 | 4 | * FLD1A,Ex=280, Em=345 [b] | 13.917 | 2160.3 | 21.862 | 59.603 | 25.31 | 13.102 | 14.413 | 0.87448 |
| 4 | 3 | * FLD1A,Ex=280, Em=345 [b] | 13.097 | 389.065 | 3.937 | 9.374 | 3.98 | 11.995 | 13.102 | 0.11811 |
| 3 | 2 | * FLD1A,Ex=280, Em=345 [b] | 11.226 | 1095.516 | 11.087 | 32.062 | 13.62 | 10.053 | 11.995 | 0.22174 |
| 2 | 1 | * FLD1A,Ex=280, Em=345 [b] | 10.051 | 93.226 | 0.943 | 1.526 | 0.65 | 8.381 | 10.053 | 0.00943 |
| 1 | 0 | * FLD1A,Ex=280, Em=345 [b] | 6.564 | 334.197 | 3.382 | 9.673 | 4.11 | 5.738 | 8.381 | 0 |
| | | | | | | | | | DAR=Sum of Weighted % Area | 5.61 |

## d. Analytical SEC (220 nM)
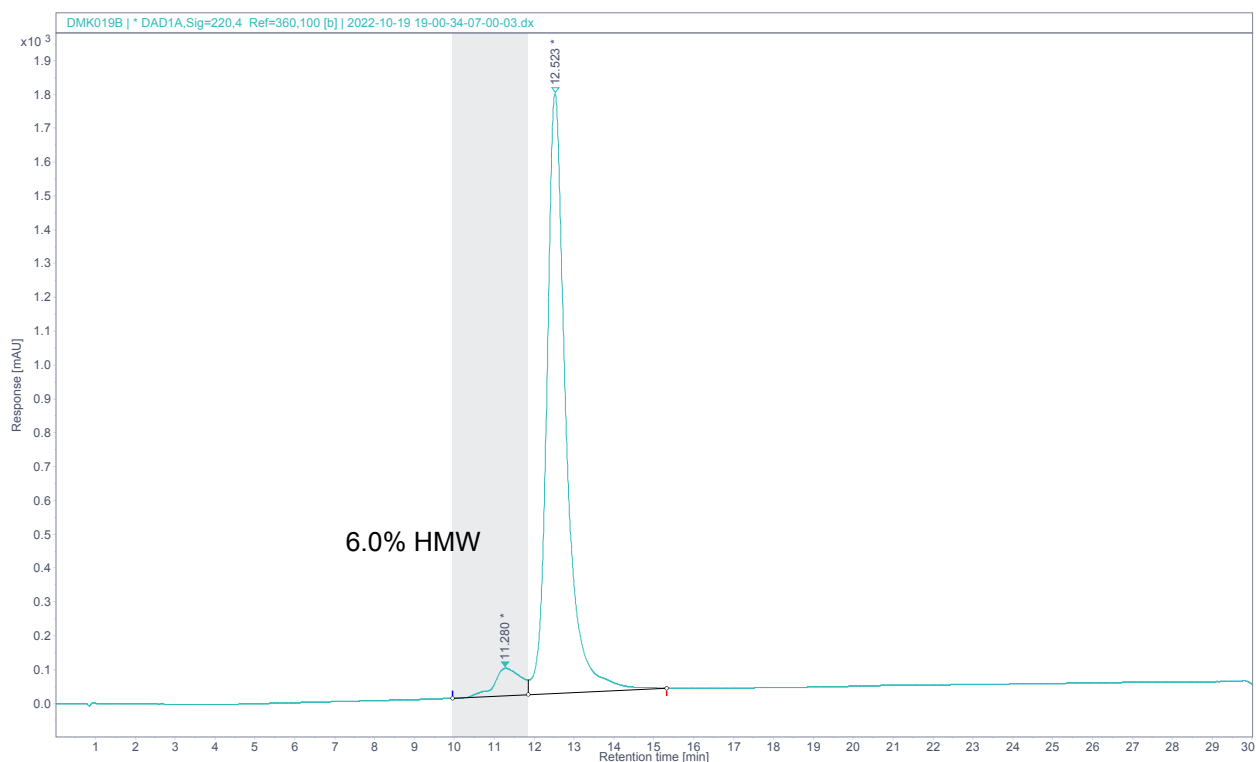


**Figure S9.** Characterization of αmTfR1-MCC-pmoEx23 DAR6 AOC. (a) Lane view of reduced capillary gel electrophoresis and (b) electrophoretogram of reduced capillary gel electrophoresis with the weighted average calculating the average DAR to be 5.8 (c) weighted average calculations of the integrated AUC from Hydrophobic interaction chromatography showed the DAR to be 5.6 and (d) size exclusion chromatography for a αmTfR1-MCC-pmoEx23 DAR6 AOC (partial TCEP reduction at 3.5 eq. TCEP, PMO:mAb equivalents 8.5). AOC, antibody–oligonucleotide conjugate; DAR, drug-to-antibody ratio; eq, equivalents; FLD, fluorescence detector; HIC, hydrophobic interaction chromatography; HMW, high molecular weight species; LU, luminescence units; MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; mAU, milli-absorbance units; pmoEx23, PMO targeting mouse exon 23; SEC, size exclusion chromatography; TCEP, tris(2-carboxyethyl)phosphine.
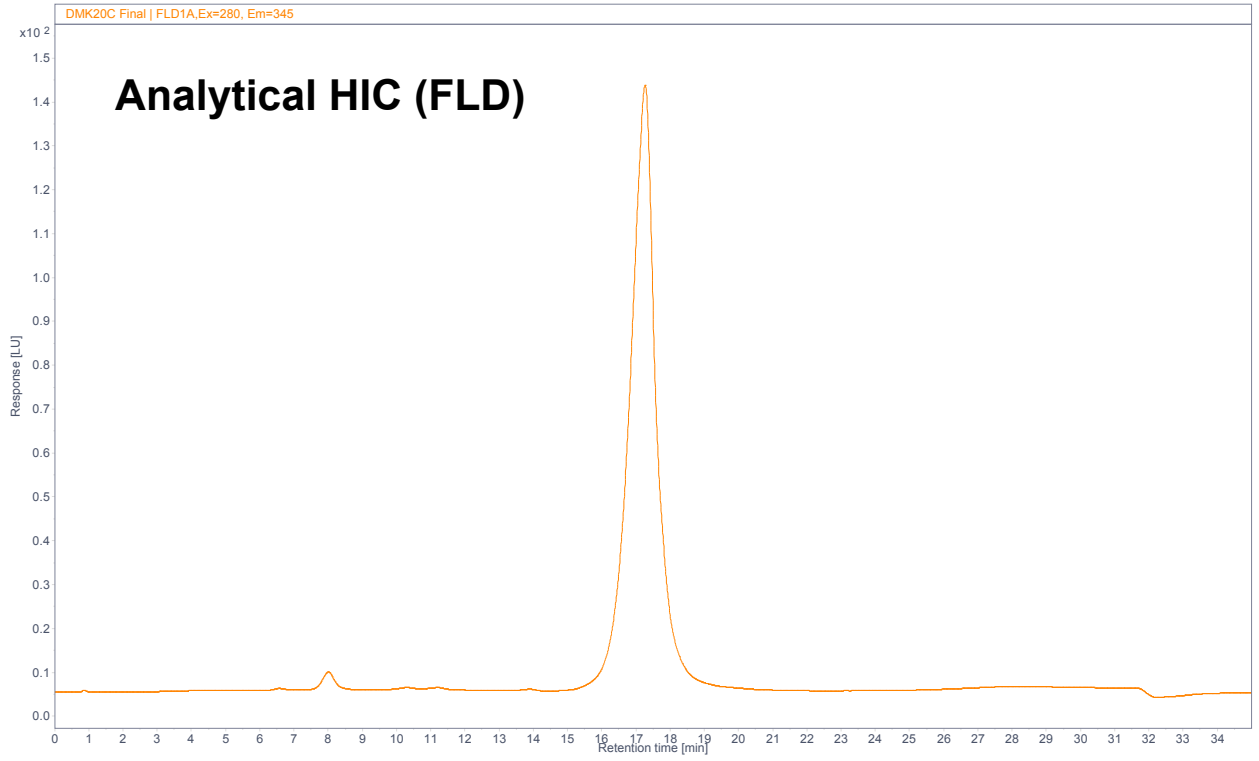
**a.**

# Reduced capillary gel electrophoresis



HC + 4 PMOs
HC + 3 PMOs
HC + 2 PMOs
HC + 1 PMO
HC

LC + 1 PMO

LC

Lane 1: αmTfR1

Lane 2: αmTfR1-MCC-pmoEx23DAR 9.71

1 -          - IS

**b.**

# Reduced capillary gel electrophoresis



| Injection | Injection Name | Sample | Peak | Name | Time | RMT | Height | Raw Area | Area | % Total | % Area | Width | S/N | Baseline | Resolution |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | DMK-028G_07 | DMK-028G | 1 | IS | 756.7 | 1.000 | 10.7 | 161.6 | 213.8 | | | 7.2 | 993.8 | 0.9 | |
| 7 | DMK-028G_07 | DMK-028G | 2 | | 1138.8 | 1.505 | 3.0 | 254.5 | 222.7 | 27.87 | | 42.1 | 278.2 | 1.0 | 11.86 |
| 7 | DMK-028G_07 | DMK-028G | 3 | | 1409.3 | 1.863 | 1.1 | 117.6 | 83.0 | 10.39 | | 40.7 | 101.3 | 1.2 | 3.99 |
| 7 | DMK-028G_07 | DMK-028G | 4 | | 1474.2 | 1.949 | 13.6 | 724.2 | 493.4 | 61.74 | | 23.5 | 1265.6 | 1.3 | 0.92 |

| Peak | Name | | Area | Area % | Weighted Area |
|---|---|---|---|---|---|
| 1 | IS | | 213.8 | | |
| | | | | | |
| 2 | LC | | 0 | 0.00 | 0.00 |
| 3 | LC+1 | | 222.7 | 1.00 | 1.00 |
| | | Total LC Area | 222.7 | | |
| | | | | | |
| | | | | | |
| 4 | HC | | 0 | 0.00 | 0.00 |
| 5 | HC+1 | | 0 | 0.00 | 0.00 |
| 6 | HC+2 | | 0 | 0.00 | 0.00 |
| 7 | HC+3 | | 83 | 0.14 | 0.43 |
| 8 | HC+4 | | 493.4 | 0.86 | 3.42 |
| | | Total HC Area | 576.4 | | 3.86 |
| | | | | (LC+HC) Weighted Area | 4.86 |
| | | | | DAR= 2*(LC+HC) Weighted Area | **9.71** |

**c.**



**Analytical HIC (FLD)**

**d.**

**Analytical SEC (220 nM)**



7.8% HMW
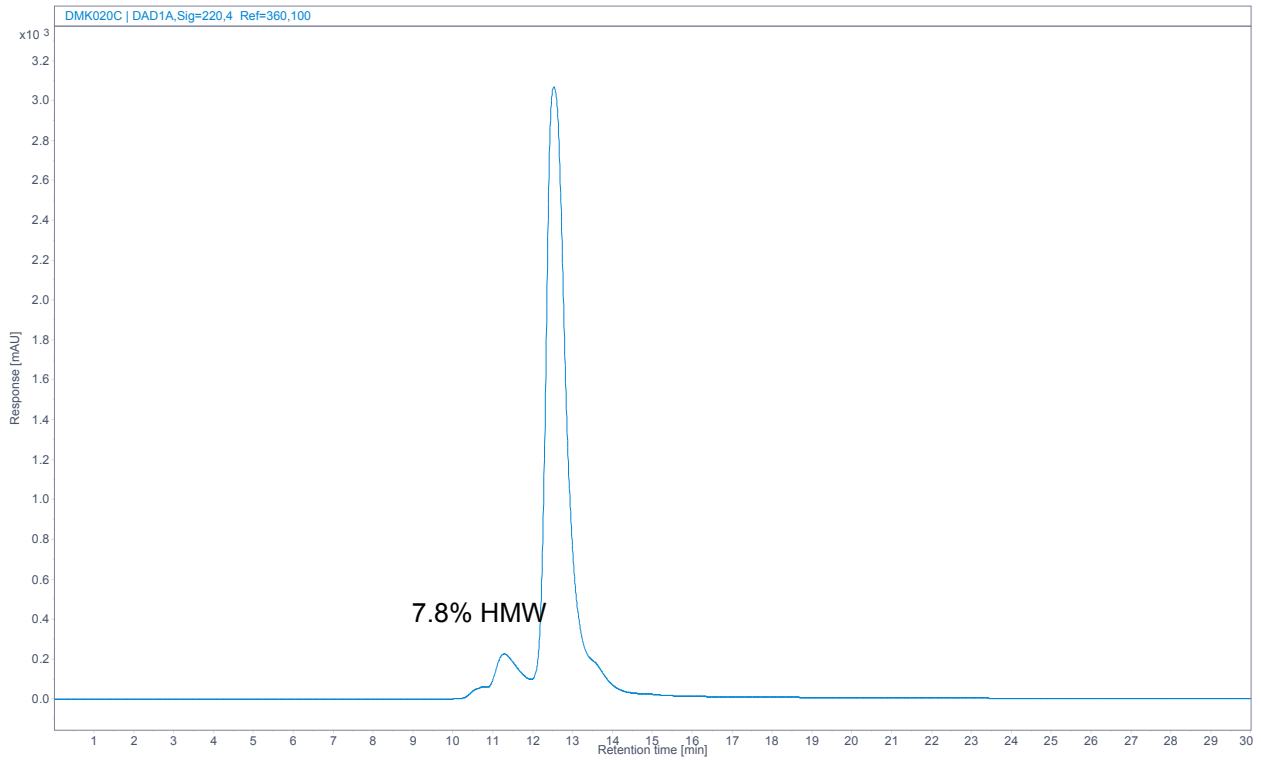
**Figure S10.** Characterization of αmTfR1-MCC-pmoEx23 DAR10 AOC. (a) Lane view of reduced capillary gel electrophoresis and (b) electrophoretogram of reduced capillary gel electrophoresis with the weighted average calculating the average DAR to be 9.74 (c) Hydrophobic interaction chromatography showing the high DAR AOC appears as a single peak and (d) size exclusion chromatography for a αmTfR1-MCC-pmoEx23 DAR10 AOC (partial TCEP reduction at 8 eq. TCEP, PMO:mAb equivalents 13.2). AOC, antibody–oligonucleotide conjugate; DAR, drug-to-antibody ratio; eq, equivalents; FLD, fluorescence detector; HIC, hydrophobic interaction chromatography; HMW, high molecular weight species; Ig, immunoglobulin; LU, luminescence units; MCC, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; mAU, milli-absorbance units; pmoEx23, PMO targeting mouse exon 23; TCEP, tris(2-carboxyethyl)phosphine.