

Efficient targeting of fatty-acid modified oligonucleotides to live cell membranes through step-wise assembly

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Contents

Step-wise Assembly of Membrane Anchored DNA-Duplexes For The Chemical Control of Cellular Adhesion.....	S1
Contents.....	S2
Oligonucleotide Sequences.....	S3
MALDI Data.....	S4
MALDI Traces	S5-S12
Figure S1 Labeling with Single and Double Cholesterol Anchors.....	S13
Figure S2 Cell Labeling Optimization.....	S14
Figure S3 Cell-Cell and Cell-Surface Binding.....	S15
Figure S4 Lifetime As A Function of Lipid Lipophilicity.....	S16
Figure S5 DLS Particle Size Data.....	S17
Figure S6 Labeling of Primary Cell Lines.....	S18
References.....	S19

Oligonucleotide Sequences:

Anchor Strands:

- 1) CAGT Repeat, 10 bp Duplex: Y-5' Amino-AG CTG TCA CT(T)₇₀ CAG TCA GTC AGT CAG TCA GT 3'
- 2) CAGT Repeat, 15 bp Duplex: Y-5' Amino-G ATC CAG CTG TCA CT(T)₆₅ CAG TCA GTC AGT CAG TCA GT 3'
- 3) CAGT Repeat, 20 bp Duplex: Y-5'-Amino-GTA ACG ATC CAG CTG TCA CT(T)_x CAG TCA GTC AGT CAG TCA GT 3'
- 4) ACTG Repeat, 20 bp Duplex: Y-5'-Amino-GTA ACG ATC CAG CTG TCA CT(T)₆₀ ACT GAC TGA CTG ACT GAC TG 3'
- 5) CAGT Repeat, 20 bp Duplex: 5'-Cholesterol Phosphoramidite-GTA ACG ATC CAG CTG TCA C(T)₆₀C AGT CAG TCA GTC AGT CAG T 3'

X=0, 20, 40, 60, or 80; Y= C₁₆, C₁₈, C₂₀, C₂₂, C₂₄ saturated fatty acid

Co-Anchor Strands:

- 1) 10 bp Co-Anchor: 5' AGT GAC AGC T Amino 3'-Y
 - 2) 15 bp Co-Anchor: 5' AGT GAC AGC TGG ATC Amino 3'-Y
 - 3) 20 bp Co-Anchor: 5' AGT GAC AGC TGG ATC GTT AC Amino 3'-Y
 - 4) 30 bp Co-Anchor: 5' T₁₀ AGT GAC AGC TGG ATC GTT AC Amino 3'-Y
 - 5) 40 bp Co-Anchor: 5' T₂₀ AGT GAC AGC TGG ATC GTT AC Amino 3'-Y
 - 6) 50 bp Co-Anchor: 5' T₃₀ AGT GAC AGC TGG ATC GTT AC Amino 3'-Y
- Y=C₁₆
Y=C₁₆
Y=C₁₆, C₁₈, C₂₀, C₂₂, C₂₄ saturated fatty acid, Cholesterol

Fluorescent Strands:

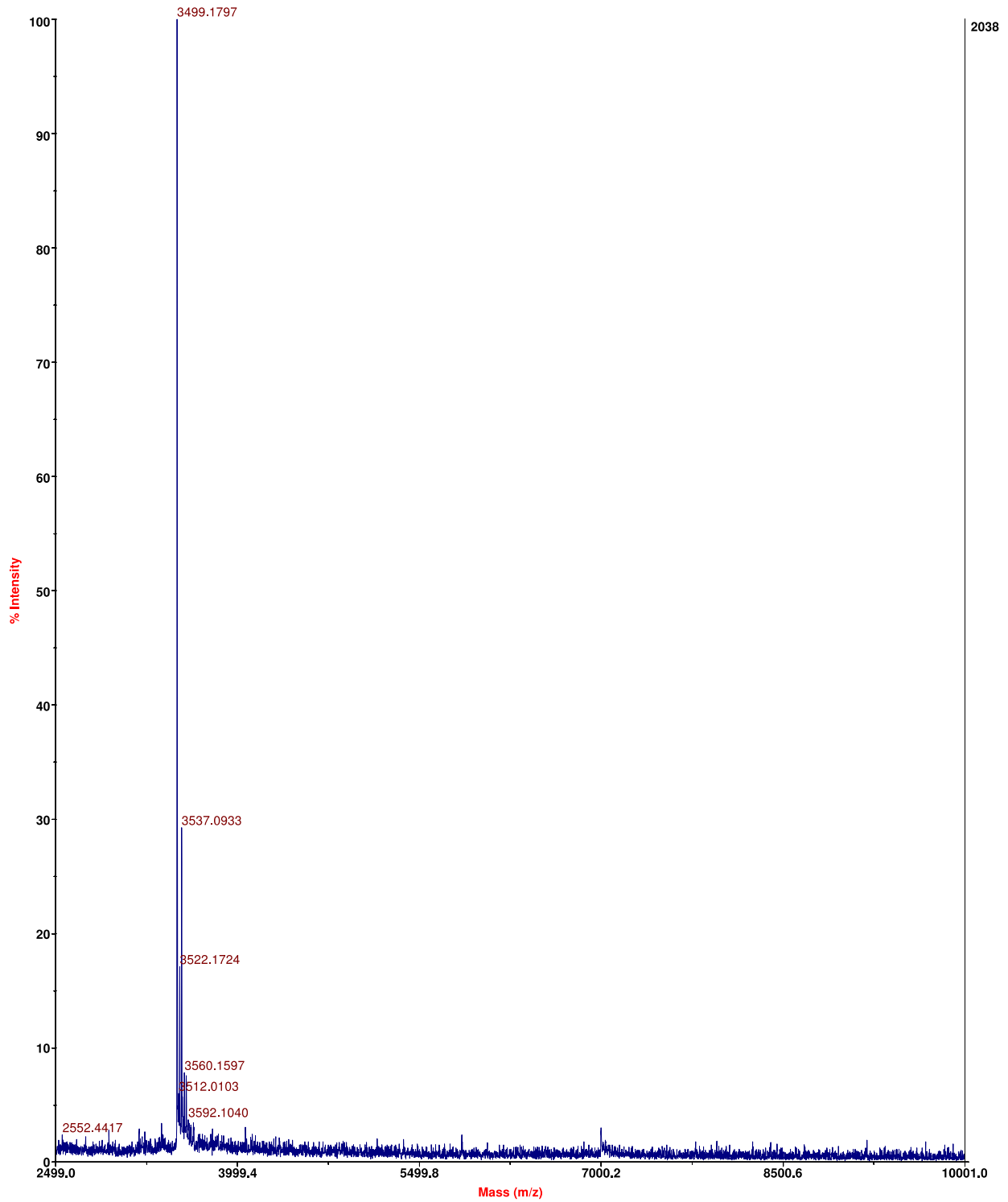
FAM-5'-(CAGT)₅-3'
FAM-5'-(ACTG)₅-3'

MALDI Mass Spectroscopy Data

The mass of representative co-anchor samples was confirmed by MALDI-MS using a Voyager-DE Pro in reflector mode with a hydroxypicolinic acid/ammonium citrate matrix supplemented with acetone solubilized nitrocellulose². While the various anchor strands and co-anchor strands were tested for purity by analytical HPLC re-injection, only the masses of the co-anchor strands were confirmed by MALDI-MS due to the low mass resolution of MALDI for high molecular weight FA-oligonucleotides. Each measurement represents an individual spot on the MALDI plate.

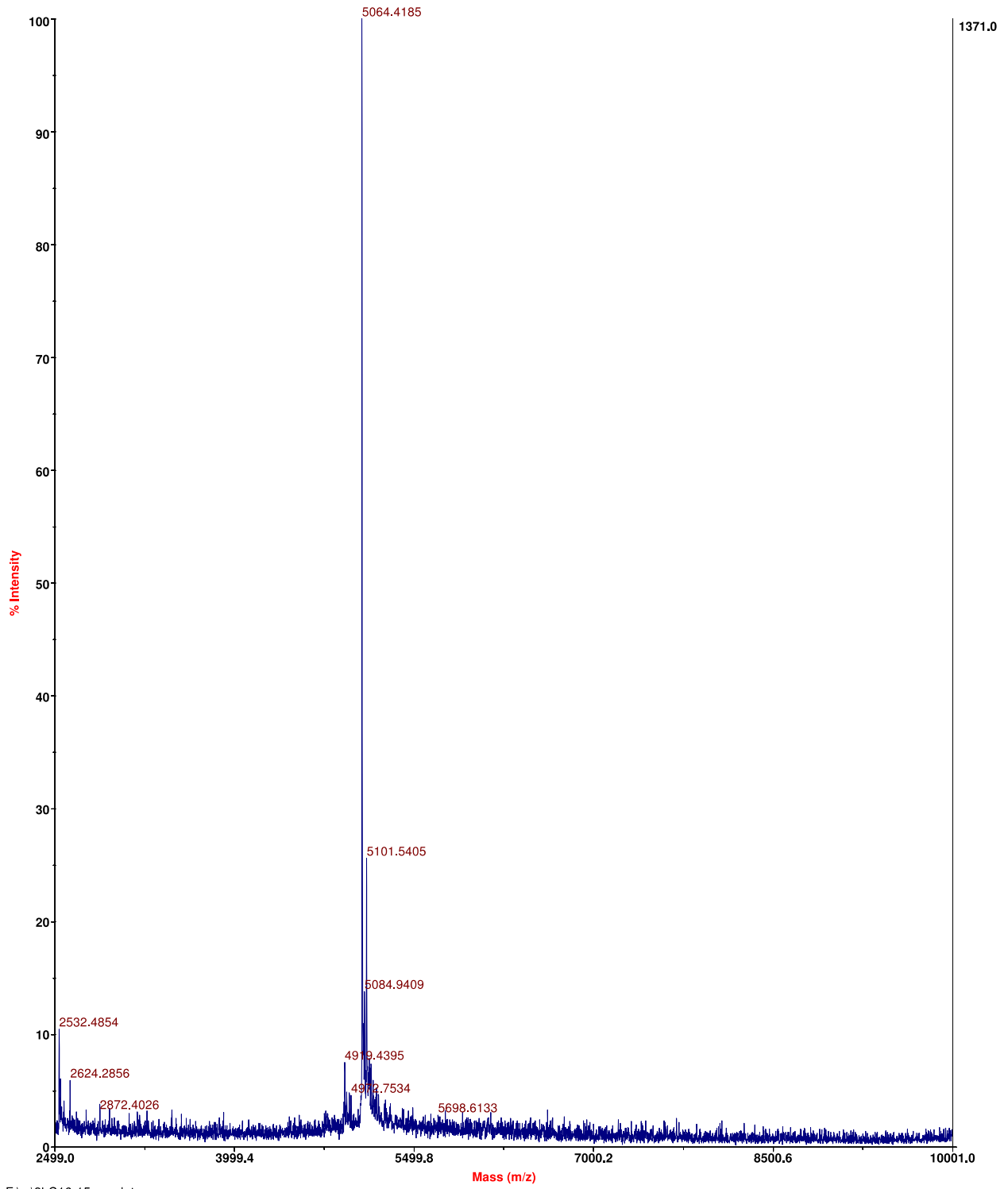
Molecule	Actual MW	Measured (M+H)					Average	StDev
		1	2	3	4	5		
10mer 3' C16	3500	349	3499	3499	3498	3499	3499	0.4
15mer 3' C16	5065	5064	5064	5064	5064	5064	5064	0.0
20mer 3' C16	6605	6603	6607	6606	6599	6598	6603	4.0
20mer 3' C18	6633	6630	6629	6630	6630	6630	6630	0.6
20mer 3' C20	6661	6656	6657	6657	6657	6657	6657	0.4
20mer 3' C22	6689	6686	6685	6685	6685	6685	6685	0.4
20mer 3' C24	6717	6717	6715	6714	6714	6713	6715	1.2
20mer 3' Cholesterol	6913	6912	6912	6912	6913	6911	6912	0.7

Voyager Spec #1[BP = 3499.2, 2038]



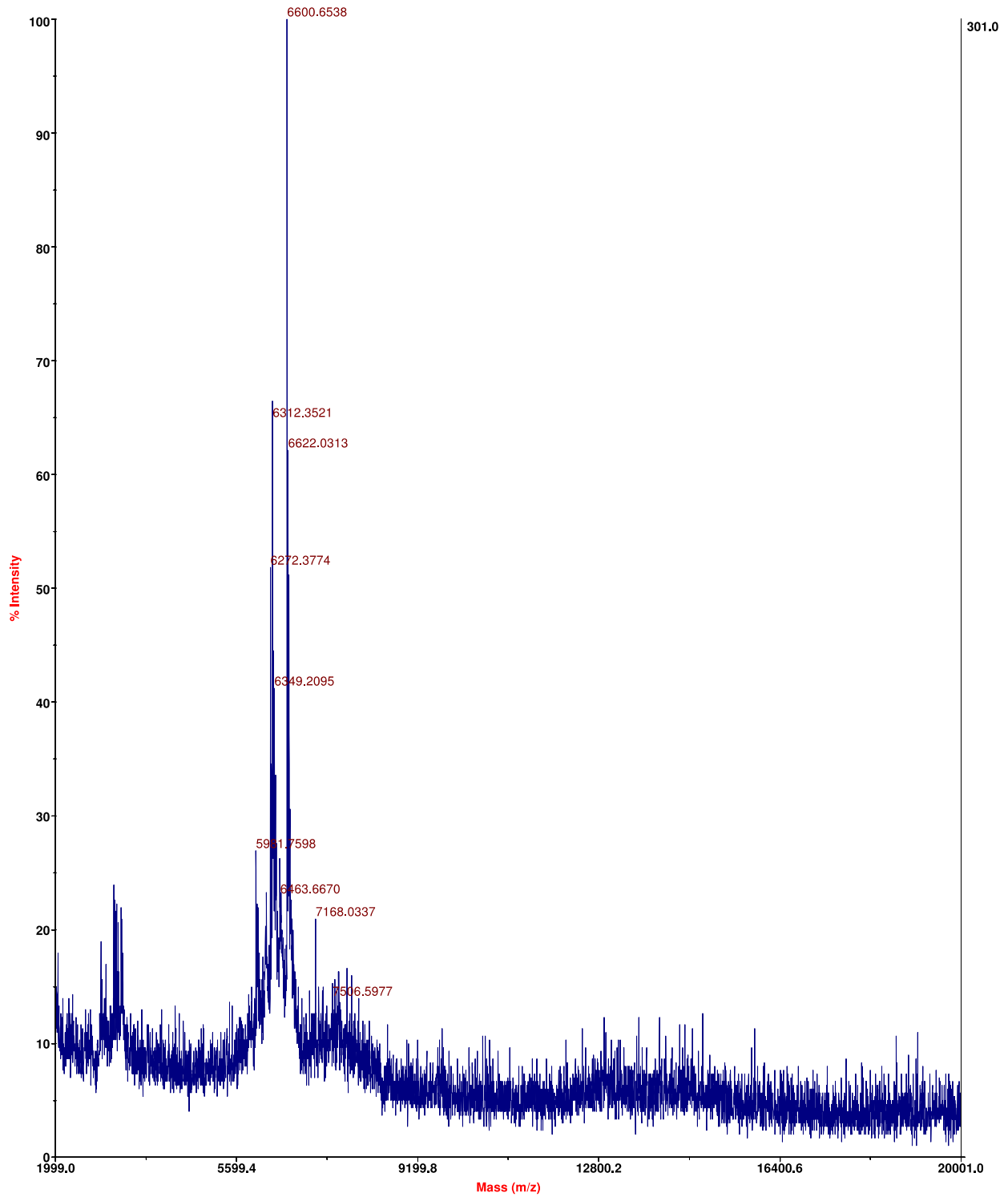
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Voyager Spec #1[BP = 5063.9, 1371]



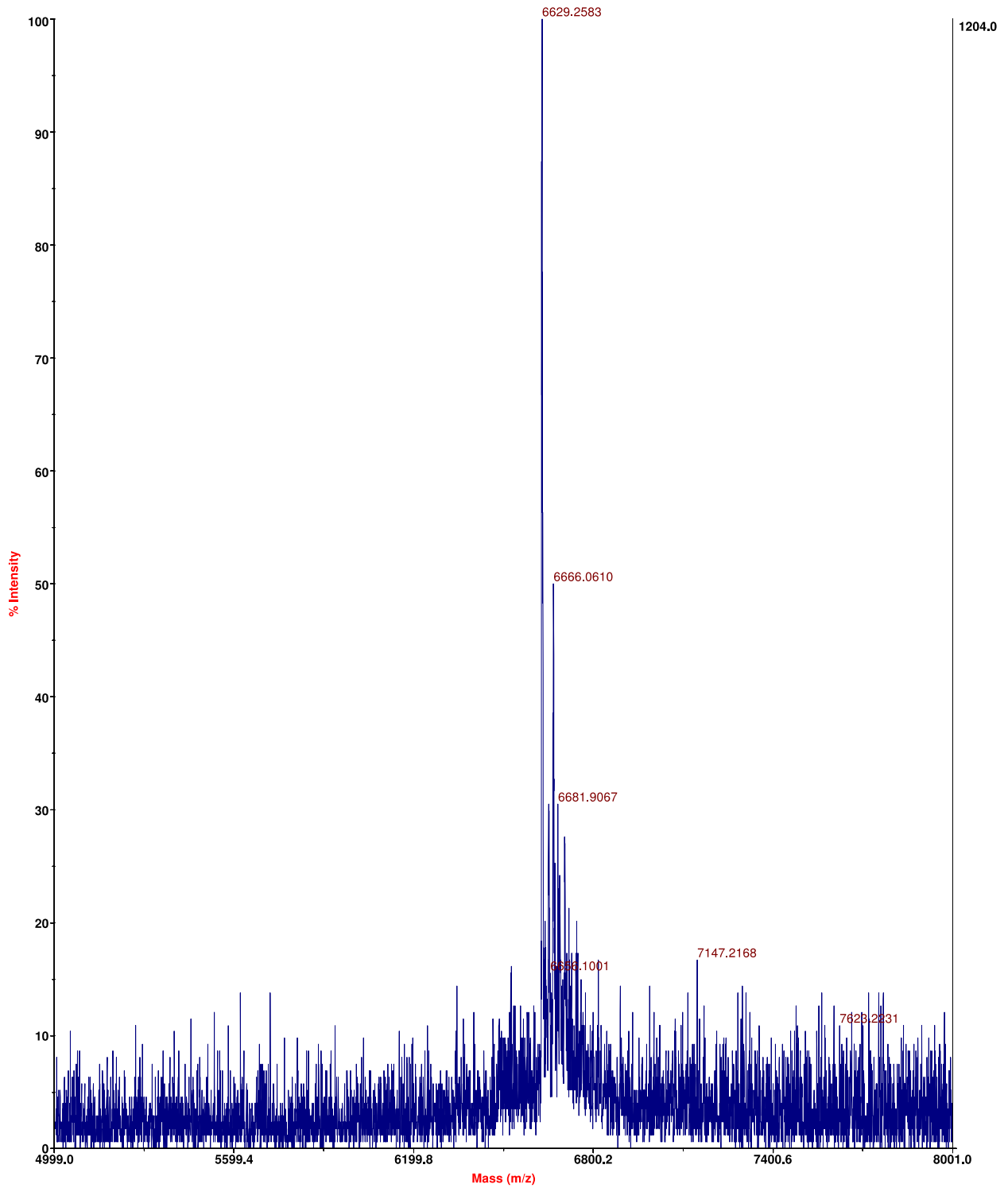
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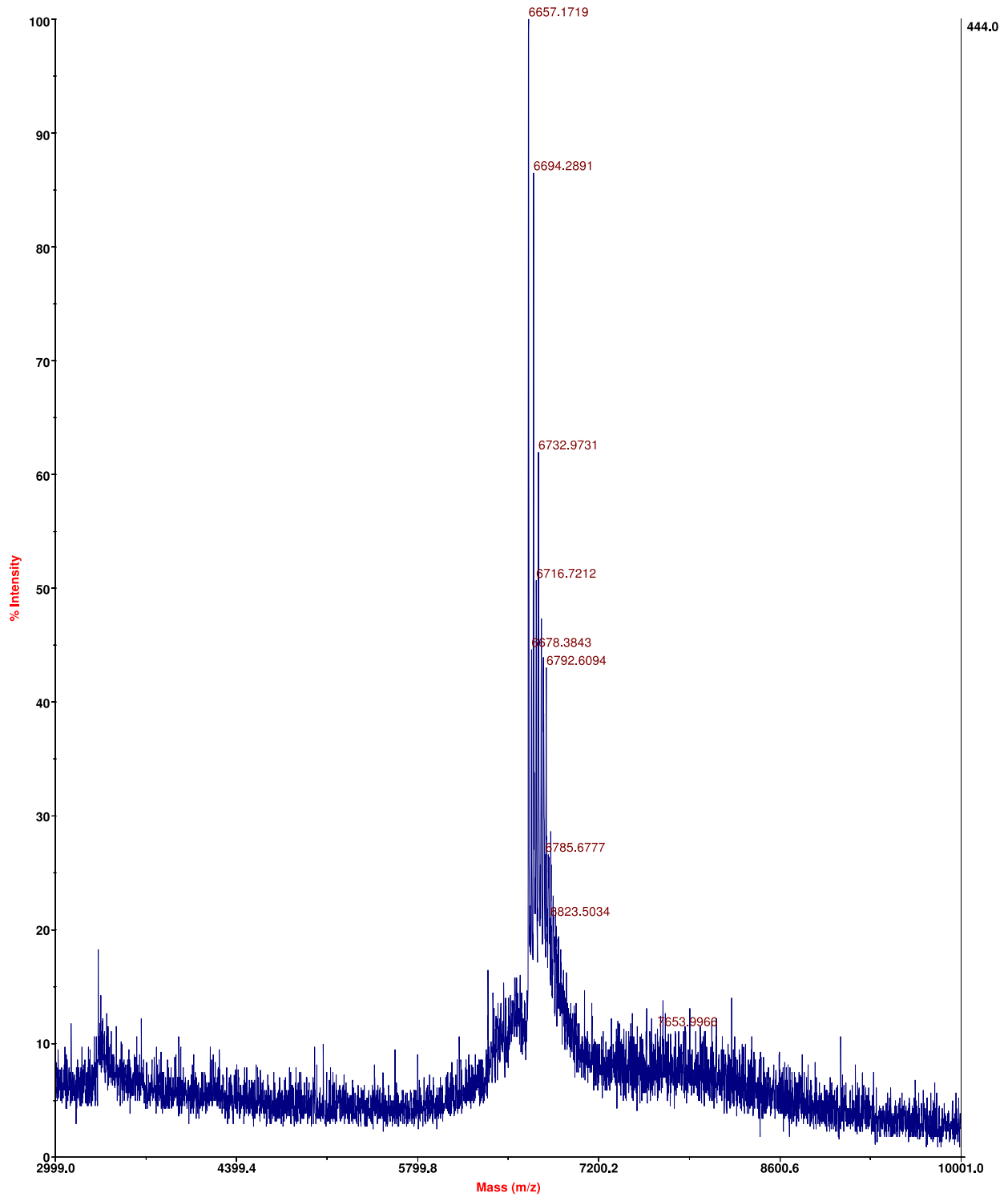
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Voyager Spec #1 [BP = 6629.1, 1204]



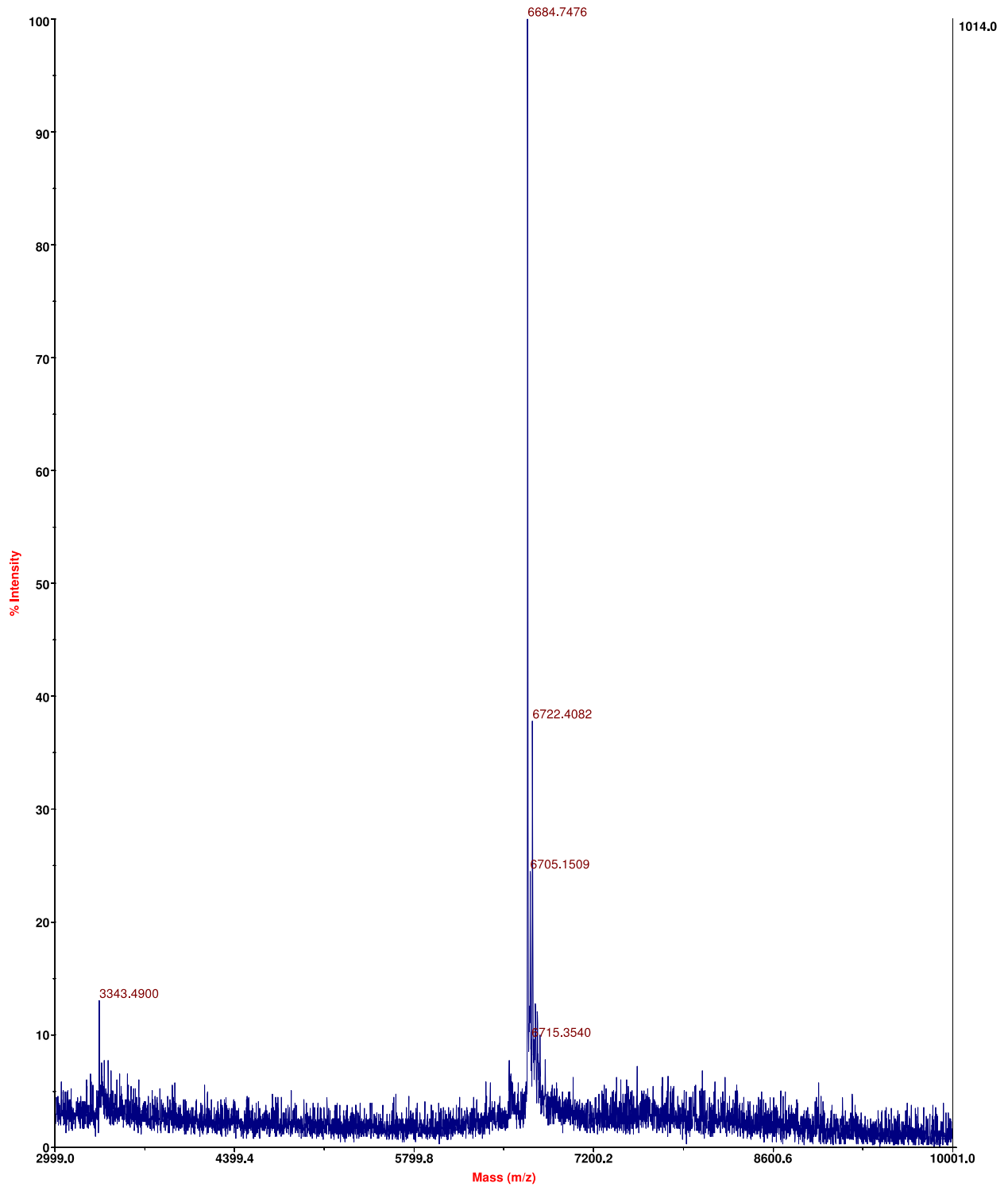
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Voyager Spec #1[BP = 6657.0, 444]



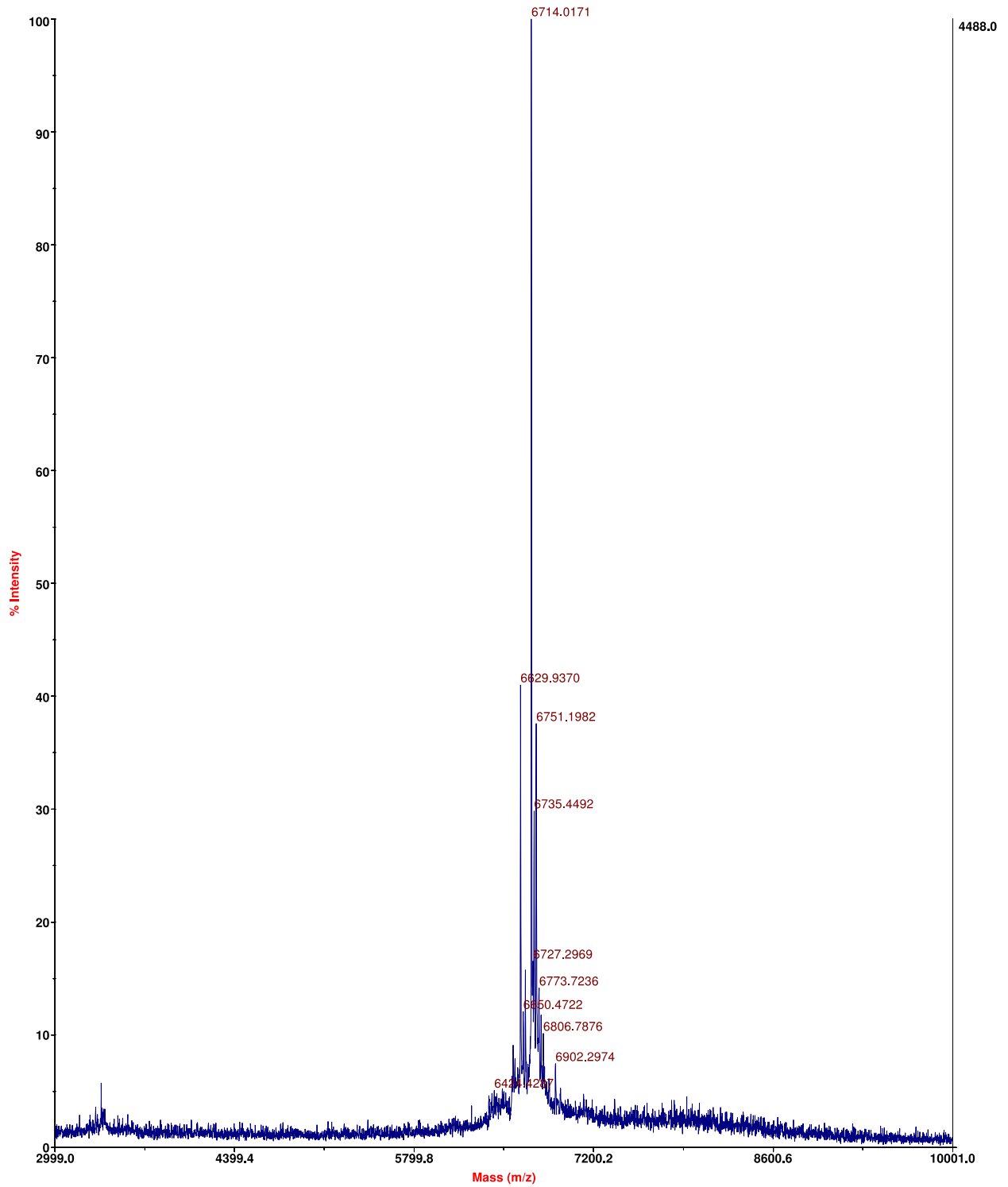
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Voyager Spec #1 [BP = 6684.8, 1014]



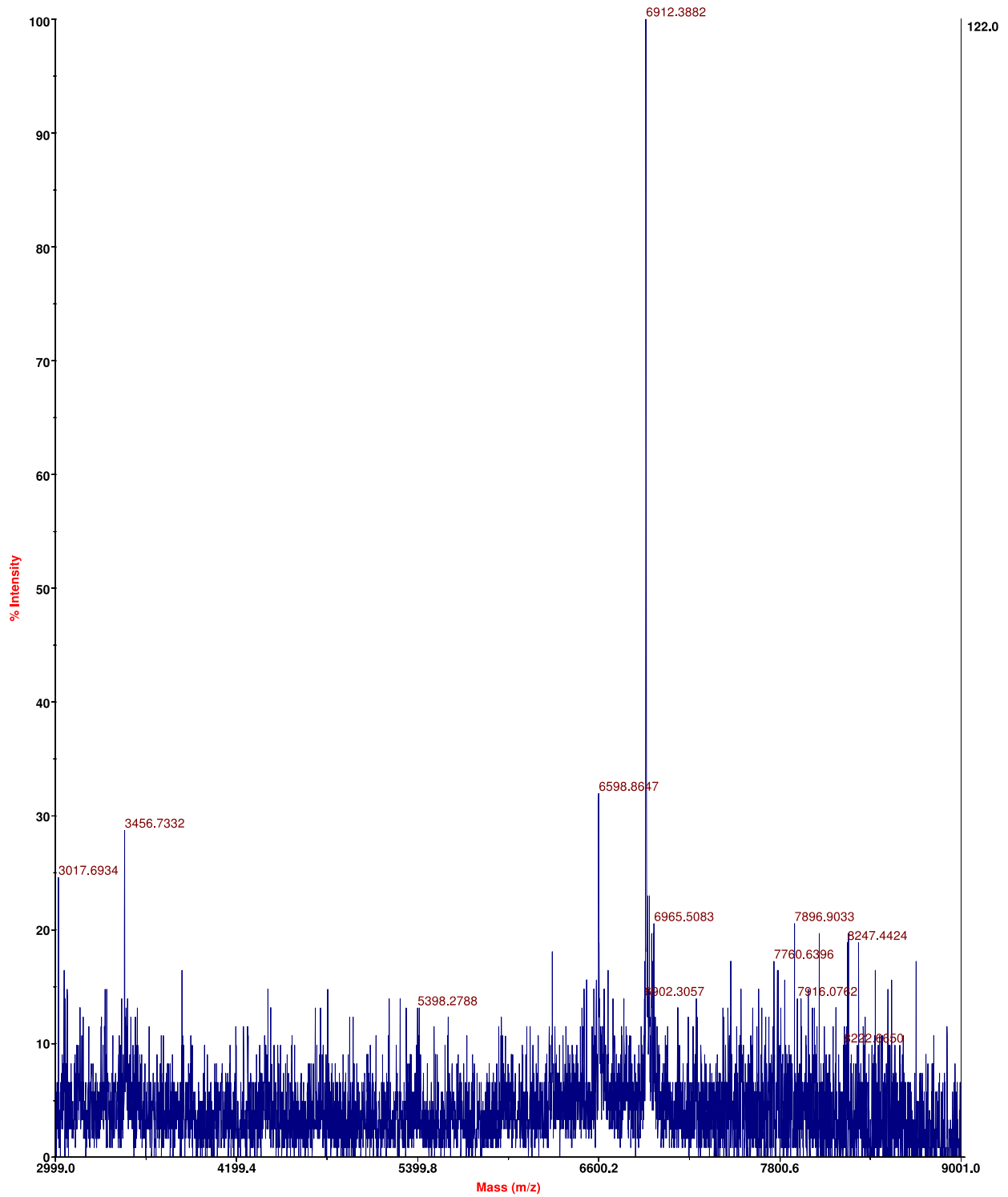
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Voyager Spec #1 [BP = 6713.9, 4488]



F:\...13-C24-mer.dat
Acquired:

Voyager Spec #1[BP = 6912.5, 122]



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Acquired:

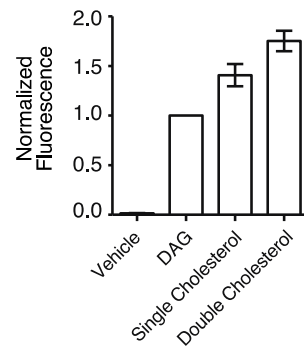


Figure S1. Labeling of Jurkats with single and double cholesterol anchors.

Single cholesterol indicates cells a single anchor strand linked to cholesterol (Anch-Chol) alone. Double cholesterol indicates cells labeled with Anch-Chol and a co-anchor modified with cholesterol (cA-Chol) with a 20 bp duplex. Fluorescence was normalized to DAG. Error bars are standard deviations of at least 3 independent measurements.

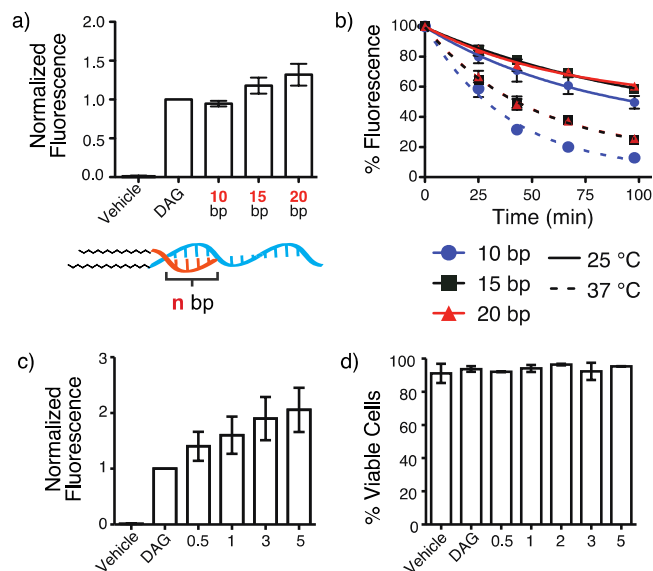


Figure S2: Cell labeling Optimization.

The combination of FAs used for this experiment was 5'-Anch₁₀₀-C₁₈ and 3'-cA-C₁₆. a) Dependence of initial cell labeling on the length of duplex formed by the anchor and co-anchor. b) Stability of cell labeling overtime at 25 and 37 °C as a function of duplex length. c) Initial cell labeling as a function of FA-DNA concentration. d) Effect of FA-DNA concentration on cell viability. Error bars are standard deviations of at least 3 independent measurements.

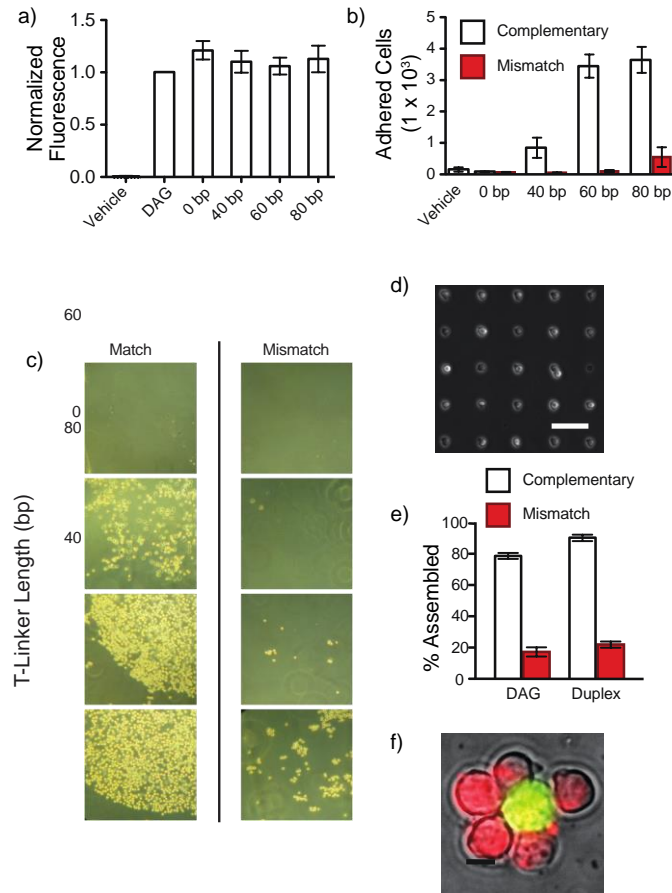


Figure S3: Binding of cells bearing Anch and cA ssDNA duplexes to cells and surfaces bearing complementary oligonucleotide sequences. a) Labeling efficiency of various poly-T linker lengths on the anch strand. b,c) Model cell line, Jurkats, labeled with DNA complexes bearing poly-T linker lengths of increasing length were incubated with aldehyde glass modified with spots of complementary or non-complementary ssDNA (scale bar = 100 μm) and the number of adhered cells was quantified. d) An array of Jurkat cells prepared from printed DNA spots approximately 1 cell in diameter (scale bar is 100 μm). e) Assembly of Jurkats into small clusters when labeled with complementary and non-complementary DNA strands. f) Representative image of one such cluster. Error bars are standard deviations of at least 3 independent measurements.

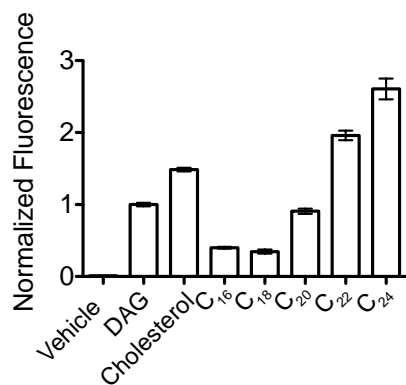


Figure S4. The effect of FA-Anch length on cell surface DNA retention after 90 minutes at 37 °C.

The Anch strand was linked to saturated fatty acids of increasing length and fluorescence was measured after a 90 minute time course and normalized to DAG which was arbitrarily set to 1. The cA strand was linked to 3'-CA₂₀-C₁₆ fatty acid for all samples. Error bars are standard deviations of at least 3 independent measurements.

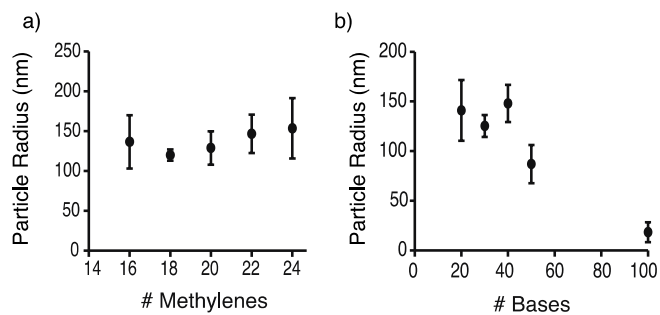


Figure S5. Particle size by DLS of 3'-cA strands of with various hydrophobicities and charges.

a) Particle size gradually increases as the hydrophobicity of the linked fatty acid increases for 3'-cA₂₀. Particle size drops for 3'-cA-C₂₄ as more nucleotides are added thus increasing the charge. Error bars are standard deviations of at least 3 independent measurements.

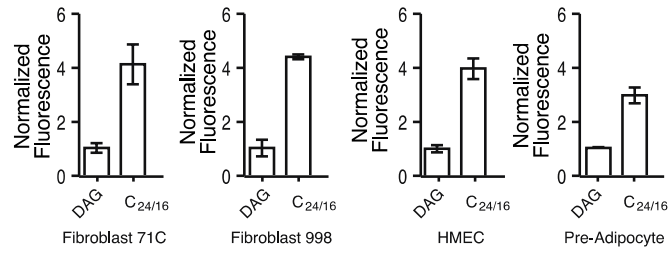


Figure S6. Cell Labeling of Several Primary Cell Lines

Cells were labeled with either DAG or a combination of 5¹-Anch₁₀₀-C₂₄/3¹-cA₅₀-C₂₄ and the relative fluorescence was plotted. Error bars are standard deviations of at least 3 independent measurements.

References

1. Research, G. 5'-MMT-Amino-Modifiers. *Technical Note* 1–2 (2013).
2. Selden, N. S. *et al.* Chemically Programmed Cell Adhesion with Membrane-Anchored Oligonucleotides. *J. Am. Chem. Soc.* **134**, 765–768 (2012).
3. Debnath, J., Muthuswamy, S. K. & Brugge, J. S. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods* **30**, 256–268 (2003).
4. Limberg, R., Rumlung, R. & Resch, M. An optimised sample preparation for the MALDI- ToF-MS of oligonucleotides. *Technical Note* 1–4 (1998).