YMTHE, Volume *26*

Supplemental Information

Cyclic Peptides to Improve Delivery and Exon Skipping of Antisense Oligonucleotides in a Mouse Model for Duchenne Muscular Dystrophy

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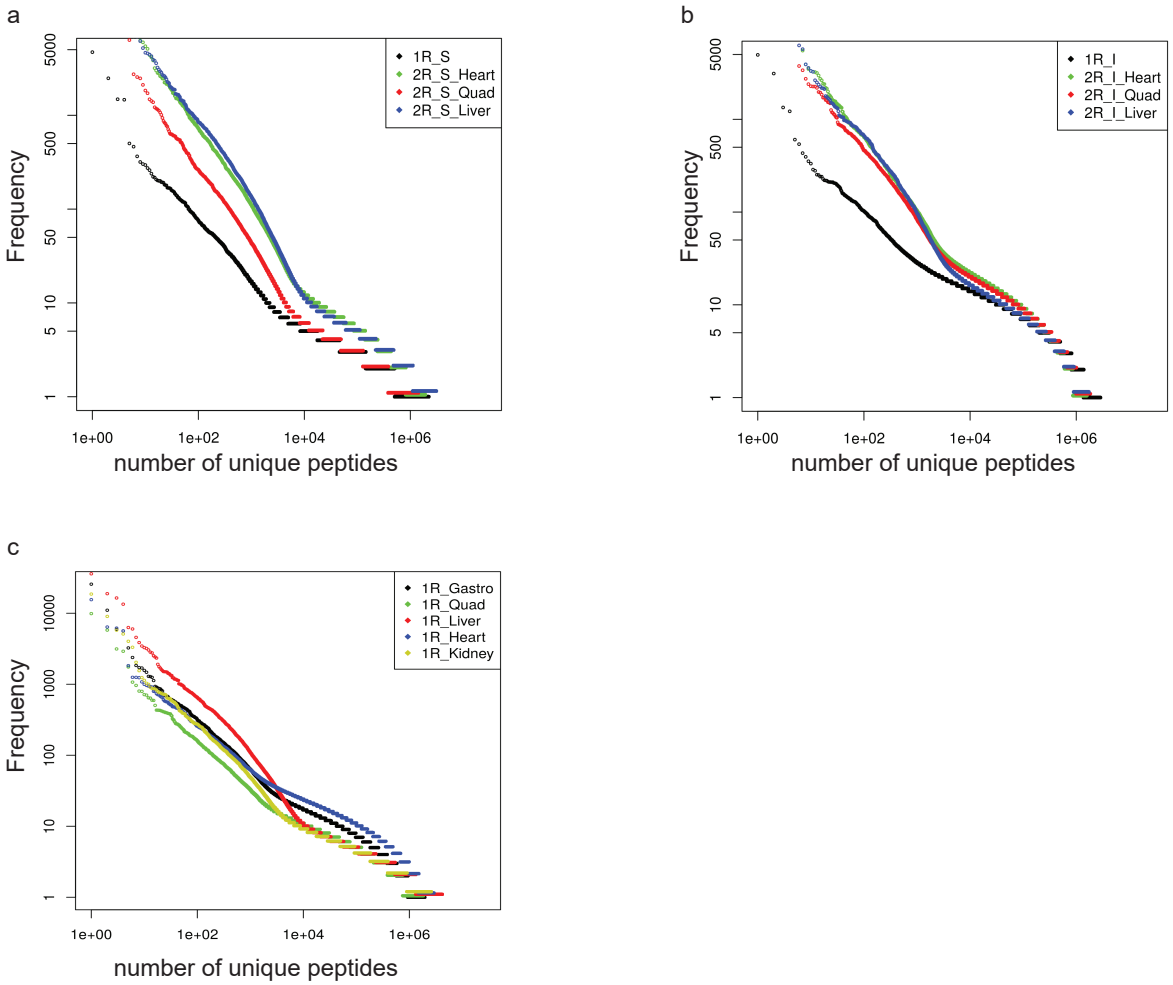
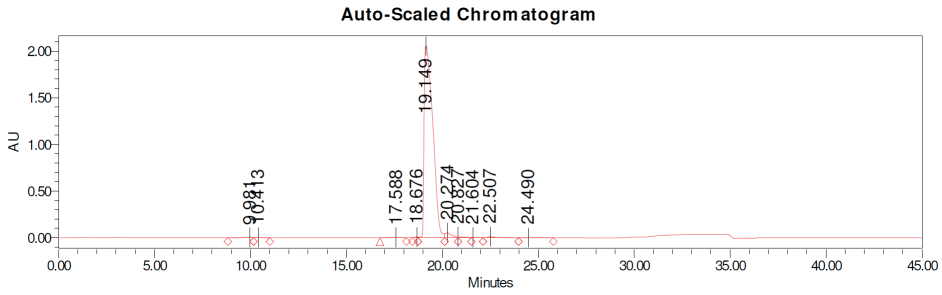
Figure S1
Next Generation Sequencing results



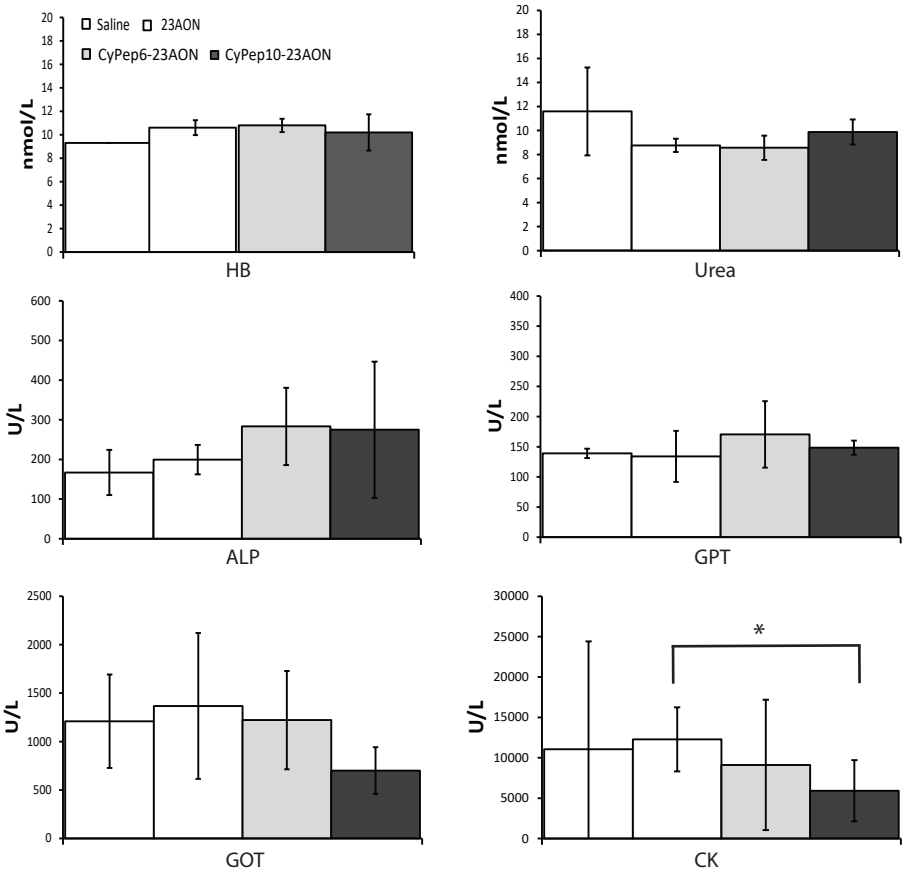
Graphical representation of the NGS output, illustrating the differences between the enriched libraries. a) Comparison of first round in vitro surface bound phages and second round in vivo with surface bound phages. b) Comparison of first round in vitro internalized phages and second round in vivo with internalized phages. c) First round in vivo with naive library.1R = frst round; 2R = second round; S = surface bound phages; I = internalized phages; Quad = Quadriceps; Gastro = Gastrocnemius

Figure S2
Chromatogram



UV chromatogram of pooled CyPep10-23AON conjugate (19.15 min, purity 96%). Method used: RP-UPLC (Waters, including a binary solvent manager, a sample manager (set at 40C) and TUV detection at 256 nm) using a C18 column (Waters OST, 1.7 µm; 2.1 x 150mm) at 850C. 5µL injection with 0.3 mL/min flow using a linear gradient from A (7.6% MeOH / 3.6% MeCN in 4 mM aq. TEA with 380 mM HFIP) to B (12.6% MeOH / 3.6% MeCN in 4 mM aq. TEA with 380 mM HFIP). System and data were controlled and analyzed by Empower III software

Figure S3
Safety evaluation



One week after the last injection of (CyPep)-23AONs or saline in mdx mice, blood was taken and evaluated for several safety markers. HB = hemoglobin, ALP = alkaline phosphatase, GPT = glutamate pyruvate transaminase, GOT = glutamic oxaloacetic transaminase, CK = creatine kinase. Bars represent mean ± SD. All markers were in normal range for mdx mice. * = significant (P=<0.05).

Table S1
Next Generation Sequencing results

Naive library, no selection	number of reads	number of unique sequences	Ratio reads/sequences
PhD C7C naive library	19,777,424	10,496,839	1.88
PhD C7C naive library amplified	21,575,275	8,904,844	2.42
First round in vitro			
surface phages	1,588,784	1,226,241	1.30
Internalized phages	3,620,792	1,911,741	1.89
Second round in vivo from surface phages			
heart	2,637,749	1,219,358	2.16
quadriceps	1,367,520	805,268	1.70
liver	3,651,870	1,895,681	1.93
Second round in vivo from internalized phages			
heart	3,265,403	1,126,767	2.90
quadriceps	3,322,155	1,292,444	2.57
liver	2,924,277	1,191,698	2.45
First round in vivo			
gastrocnemius	2,868,911	1,311,263	2.19
quadriceps	2,080,098	1,160,805	1.79
heart	4,602,680	2,006,257	2.29
liver	4,107,847	2,502,776	1.64
kidney	2,458,896	1,565,835	1.57

An overview of the coverage of the NGS output.

Table S2
Sequences of primers and TaqMan probes for ddPCR analysis of exon 23 skip in mdx mice

ddPCR assay	Target Exons	Forward Primer	Reverse Primer	Probe Sequence
non-skipped (including exon 23)	22-23	ACTGAATATGAAATAATGGAGGAGAGACT	GCCATTTTGTTGCTCTTTCAAA	AAATTACAGGCTCTGCAAAG
skipped (exon 23 skipped)	22-24	AGCAGTCAGAAAGCAAACTCTCTG	TTCAGCCATCCATTTCTGTAAGGT	TGGAGGAGAGACTCGGGAAATTACAGAATCACAT

Table S3
Molecular weight data of CyPep-AON conjugates

Conjugate	MW (calc)	MW (found)
CyPep10-h45AON	9211.9	9211.5
CyPep6-m23AON	8005.8	8006.5
CyPep10-m23AON	8189.1	8188.7