

Improving the design of antisense drugs using Peptides

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Chemical synthesis peptides-PMO
Cell culture assays

University of Oxford, Physiology, Anatomy and Genetics

Dr Matthew Wood and colleagues

In vivo evaluation in animal models

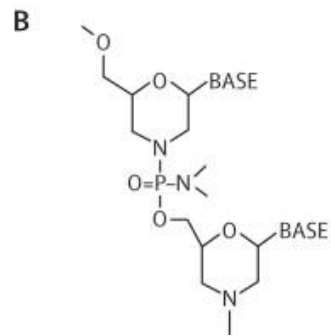
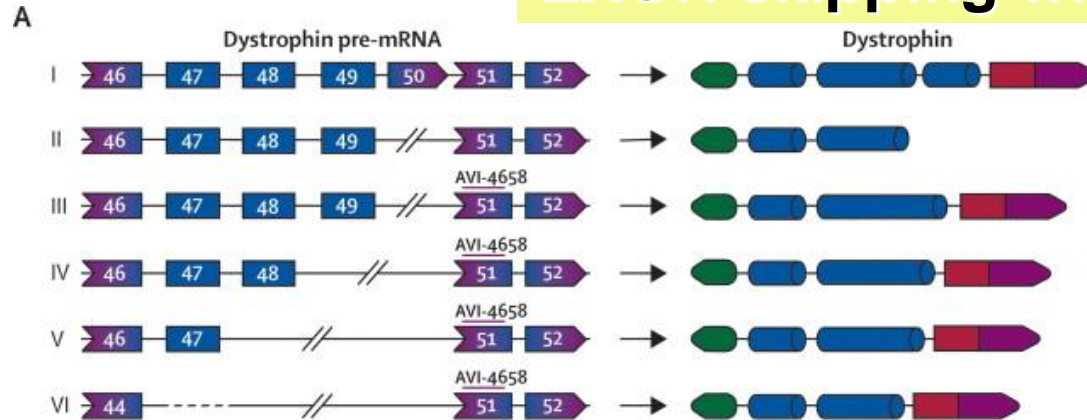
MDEX consortium partners (Muntoni, Wells, Dickson, Strauss)

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Molecular Biology

Funding from Wellcome Trust/HICF and AFM

EXON skipping with PMO



Exon skipping **Morpholino (PMO)** oligonucleotides

- bind to mutated dystrophin pre-mRNA using specific base sequence to recognise the complementary sequence by base pairing and interfering with splicing pattern
- causes exon skipping over the mutation to generate in frame mRNA
- generates shorter but active dystrophin protein

**Intravenous clinical trial of AVI 4658 (30-mer PMO targeting exon 51)
(MDEX clinical consortium and AVI Biopharma)**

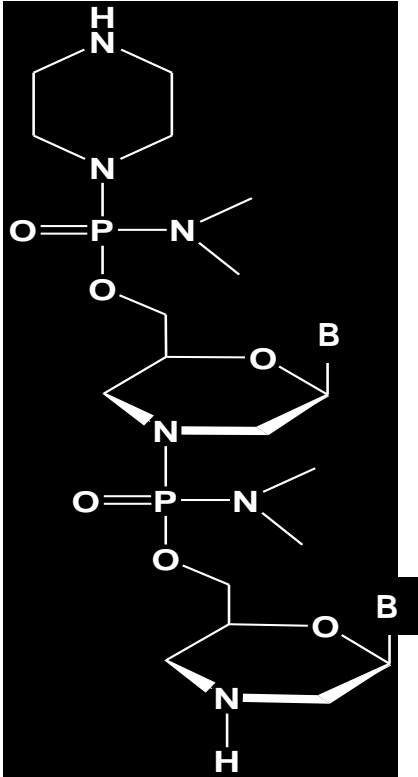
SEE

Francesco Muntoni (ICH) at 2.30 pm

Ed Kaye (AVI) at 3.00 pm

A Chemist's view of PMO: ideal antisense for exon skipping because:

- 1) extremely stable metabolically
- 2) binds tightly to dystrophin pre-mRNA with good specificity
- 3) non-toxic in animal models to high doses
- 4) can be chemically synthesized readily
- 5) easy to attach other compounds such as peptides

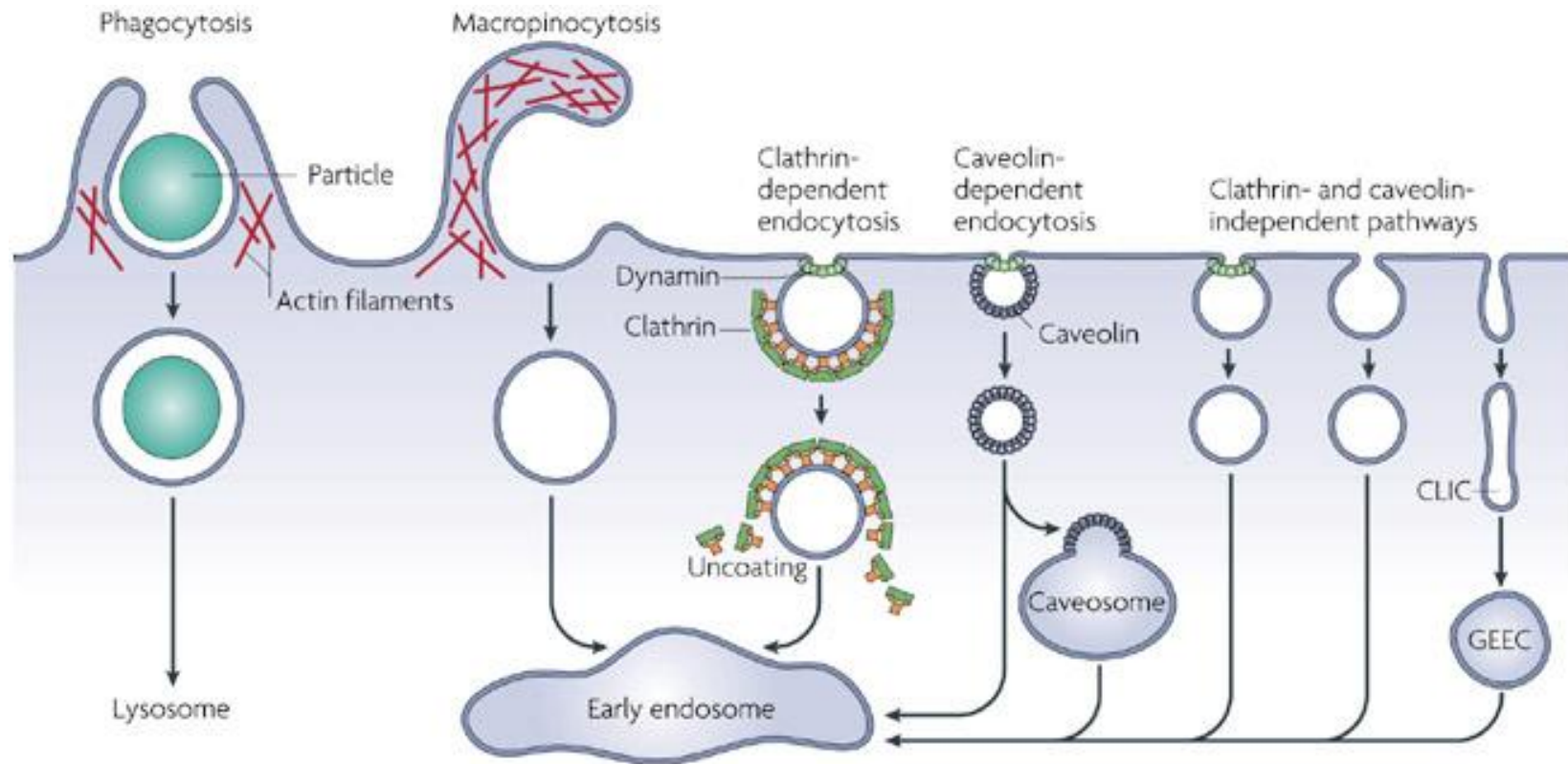


Naked PMO may need to be administered in high doses in order to generate enough dystrophin to be sufficiently effective to give clinical benefit to patients.

Naked PMO (and other antisense oligos) may not be able to generate sufficient dystrophin in some tissues (eg heart) to keep all muscle functions going

Activity of antisense PMO is limited by poor cell delivery and rapid excretion

One solution is to attach a Cell-Penetrating Peptide (CPP) (P-PMO)
CPPs are short strings of amino acids connected to the antisense to help it bind strongly to cells and to help it pass through the complex cell membrane system and on into the nucleus



Nature Reviews | Molecular Cell Biology

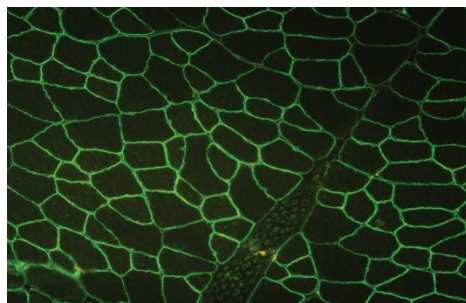
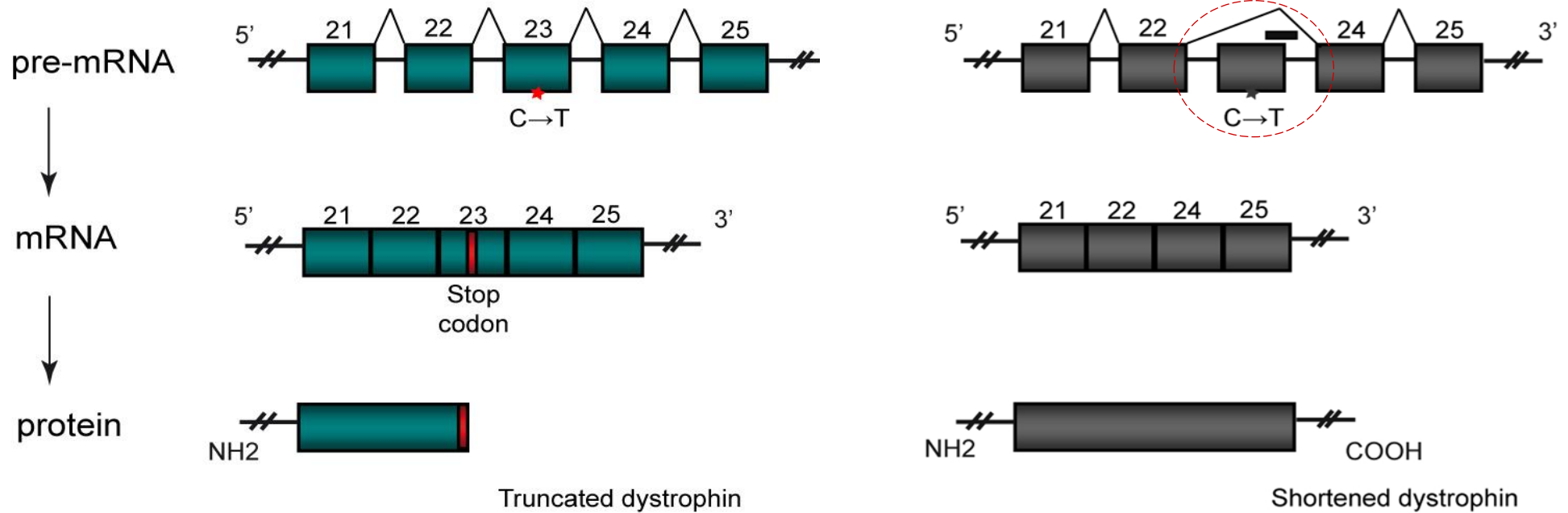
Release from endosomes into cytosol needs a cell penetrating peptide

Transfer to another compartment needs just a few particular amino acids

Targeting pre-mRNA in the nucleus to redirect required for exon skipping

Targeting microRNAs (fine tuners of gene expression) involved in muscle function that are over expressed in DMD

The *mdx* mouse, nonsense mutation in exon 23 of dystrophin



Muscle tissue antibody detection of dystrophin

Normal mouse muscle



Mdx mouse

Peptide-conjugated PMO delivery

PMO

Cell Penetrating Peptide

GGCCAAACCTCGGCTTACCTGAAAT-**B peptide**

Name	Sequence	Abbreviation	Length
M23D	5'-GGCCAAACCTCGGCTTACCTGAAAT-3'	PMO	25
B peptide	N- RXRRBRRXRRBRXB -C	B	14

Natural amino acid arginine (R)
Spacers aminohexanoyl (X)
and beta-alanine (β)
Developed by AVI Biopharma

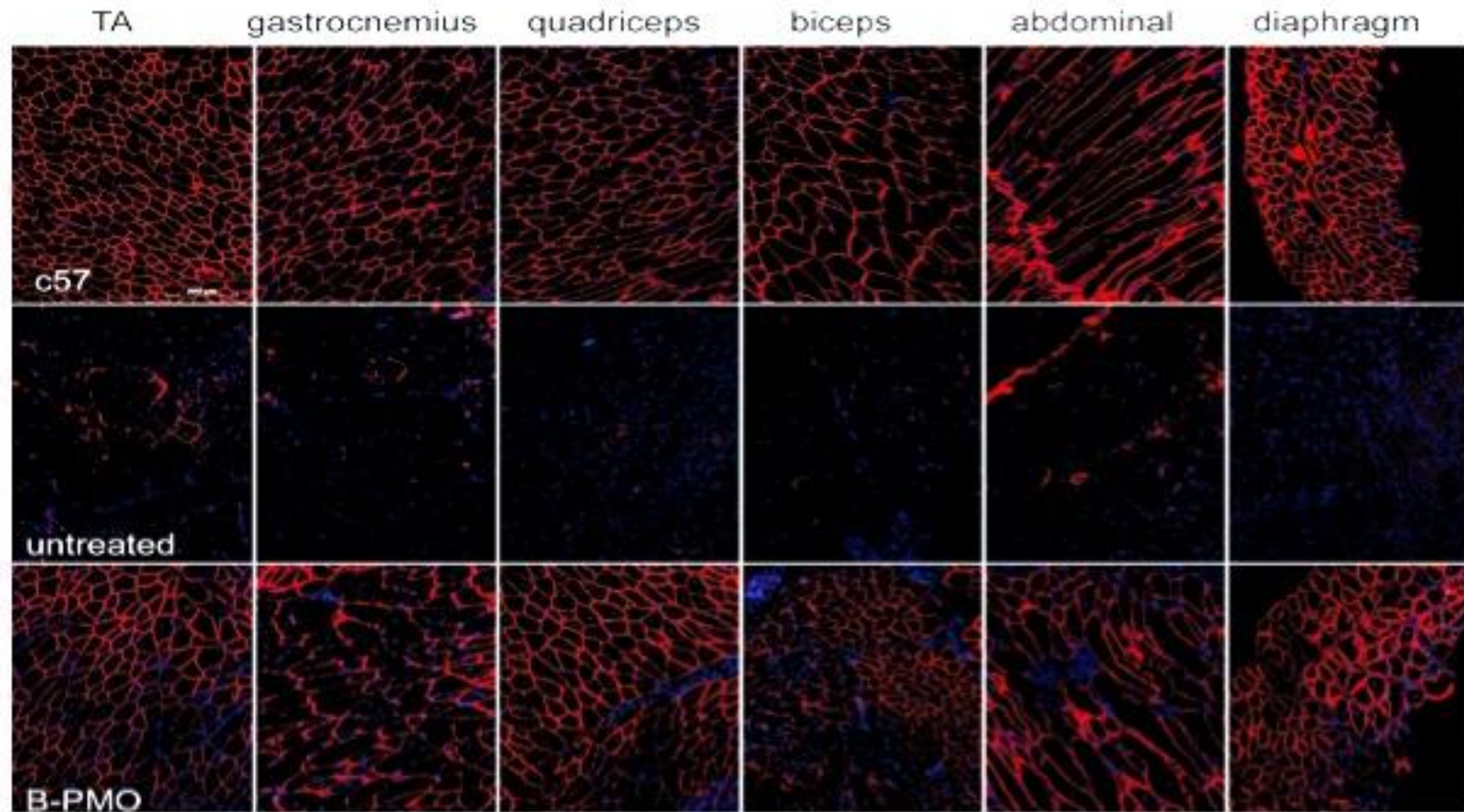
B-PMO shows substantially enhanced dystrophin production and exon skipping in **mdx mouse model of DMD**

Yin H, et al. (2008) *Hum Mol Genet* 15:17(24):3909-3918

Jearawiriyapaisarn N, et al. (2008) *Mol Ther* 16(9):1624-1629

Wu, B, et al. (2008) *Proc Natl Acad Sci U S A* **105**: 14814-14819

PMO restores dystrophin expression



Single intravenous
injection 25mg/kg

Yin et al. Human Molecular Genetics (2008)

Novel peptides for improved AO delivery

CPP domain

AO domain

B peptide

-GGCCAAACCTCGGCTTACCTGAAAT

Improved cell penetrating peptides (CPPs)
Chimeric tissue specific peptides for targeting to muscle



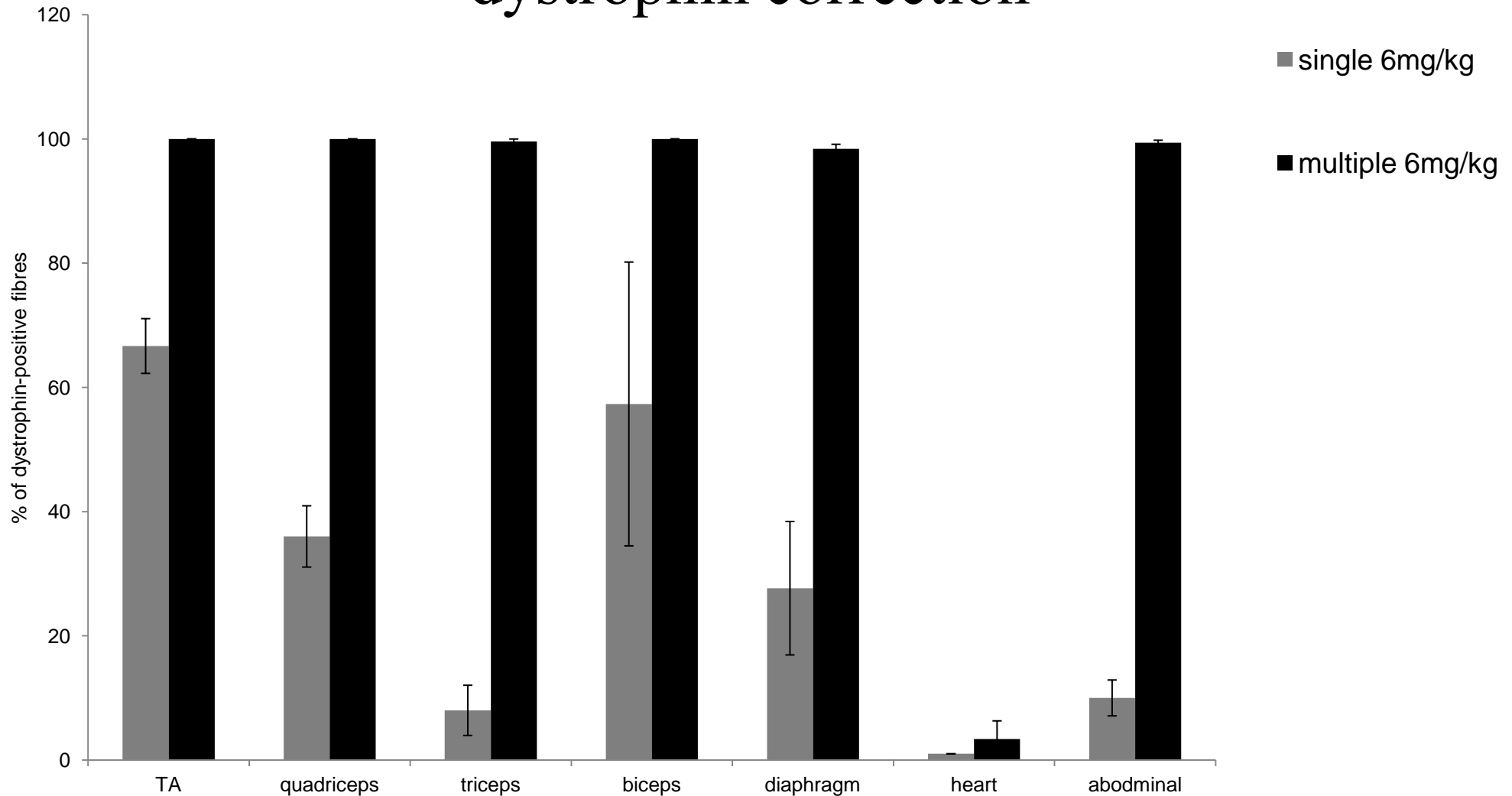
B peptide

MSP

-GGCCAAACCTCGGCTTACCTGAAAT

- Muscle-specific peptide (MSP) – ASSLNIA
- 7-mer with 50-100-fold increased skeletal myocyte binding affinity

Repeat dose B-MSP-PMO provides complete muscle dystrophin correction



Yin et al (2009) *Human Mol. Gen.*

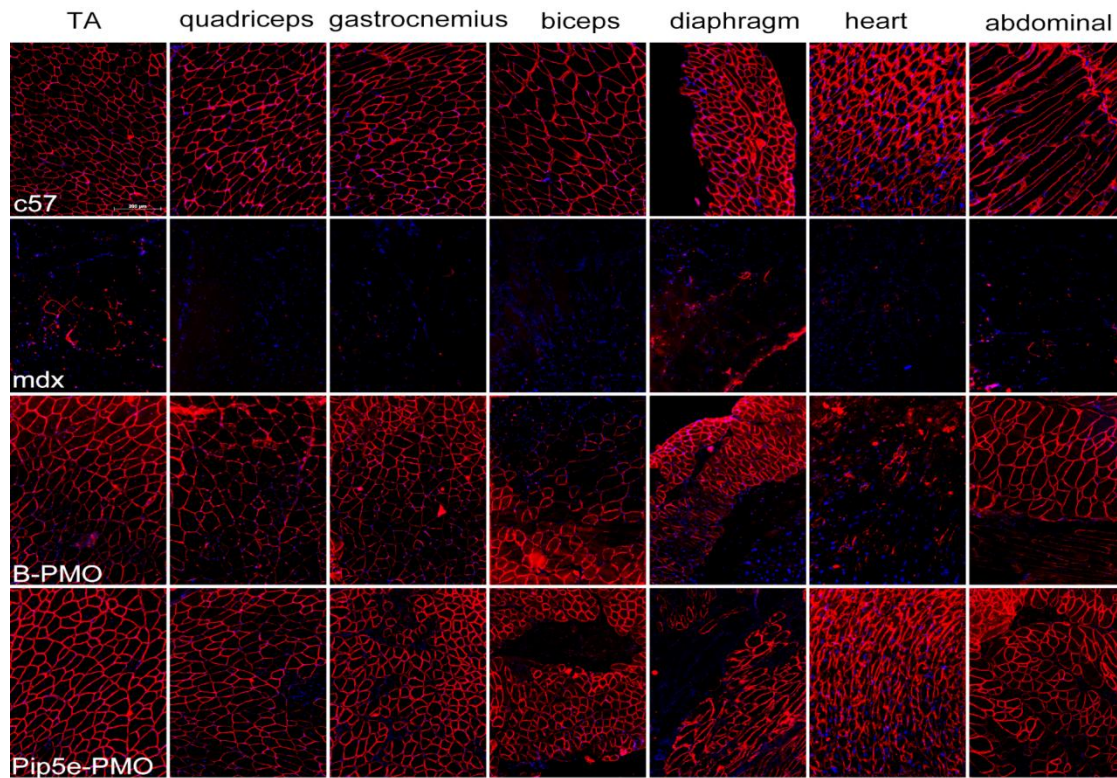
Yin et al (2010) *Mol. Ther.*

Second class of peptides developed in Cambridge

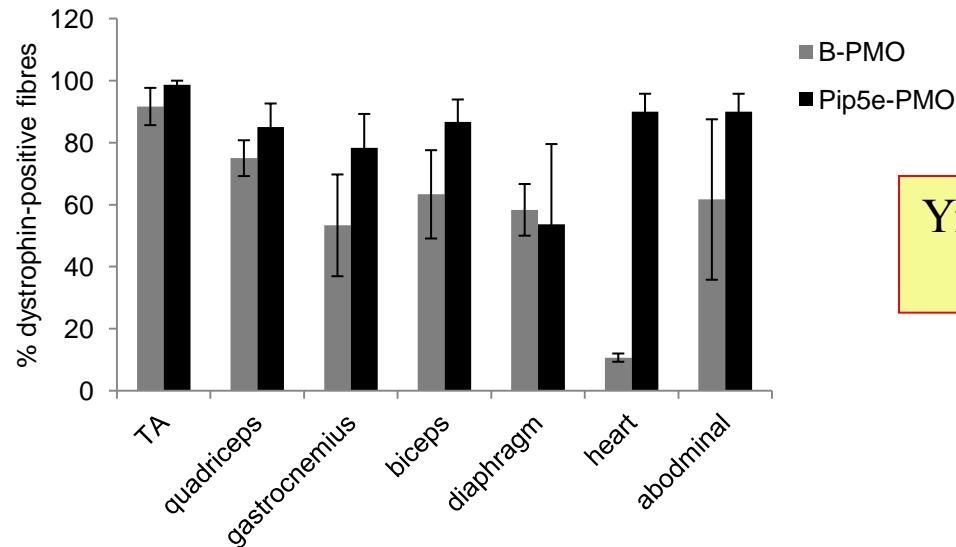
PMO Internalization Peptides (Pip)



Outer sections composed of
Arginine R, Aminohexanoyl X and beta alanine B,
Inner section: 5 natural amino acid hydrophobic core



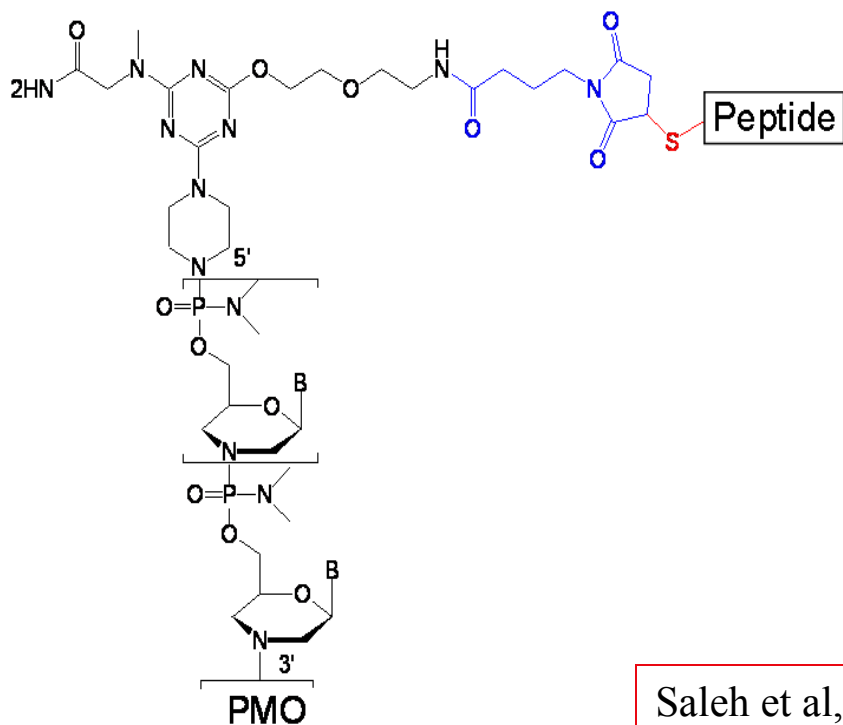
Single 25 mg/kg **i.v. injection** of **Pip5e-PMO** into mdx mouse shows much higher exon skipping and dystrophin production in **heart muscle** than B-PMO



Yin et al. (2011) *Molecular Therapy*
19, 1295–1303

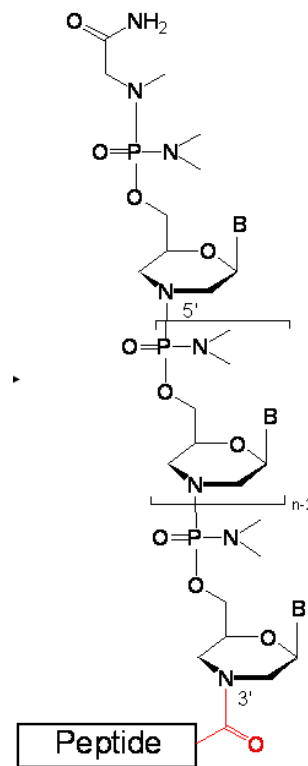
**It does not matter which end the peptide is added
PMO equally active in both cases**

PMO-Pip5e



Saleh et al, *Coll. Symp. Series* (2011)
12, 292-296

Pip5e-PMO



Why does the central core of Pip5e give rise to higher dystrophin production in heart muscle?

Pip-5e Arg-rich Hydrophobic Arg-rich
RXRRBRRXR ILFQY RXRBRXR

WORK IN PROGRESS

Pip 6 series: The central core acts like a hydrophobic spacer

1. Altering the order of amino acids sequence specificity does not affect heart activity
2. Shortening the core is detrimental to the heart activity

Pip 7-9 series: Development of a therapeutic Peptide-PMO candidate

Funding from Wellcome/HICF

Aim to take a compound to the clinic within 2-3 years for DMD treatment that has enhanced dystrophin production in mouse models compared to naked PMO whilst providing sufficiently low toxicity in animal models.

LMB 2010



ACKNOWLEDGEMENTS

Conjugate synthesis
Cell Studies and assays

Amer Saleh, Thibault Coursindel
Andrey Arzumanov

COLLABORATIONS

Hai-Fang Yin, Matthew Wood and colleagues (**University of Oxford**)

New Building 2012



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Laboratory of
Molecular Biology