Pre-clinical research in CRISPR

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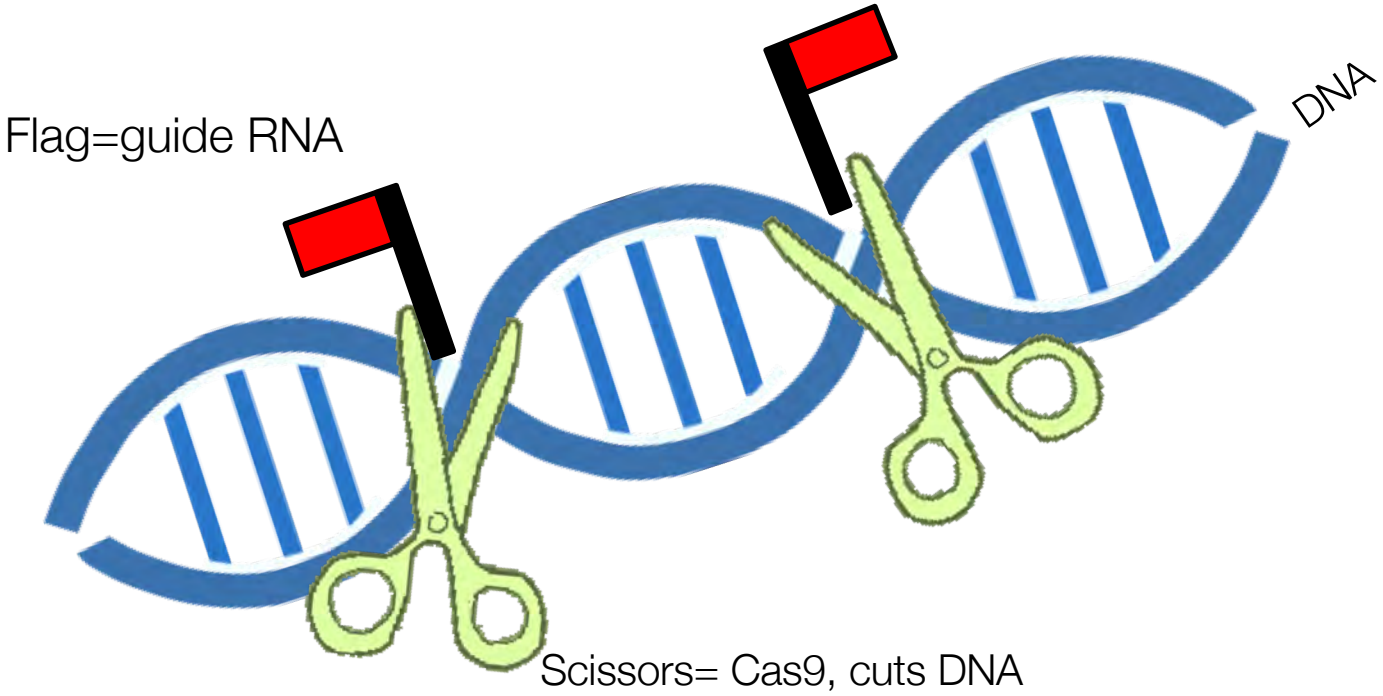
Prof. of Neurology
UCLA
06/29/19

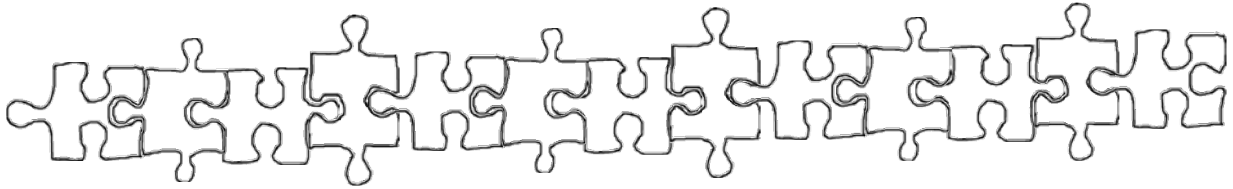
Parent Project Muscular Dystrophy
JOIN THE FIGHT. END DUCHENNE.

General principles of CRISPR/Cas9 editing

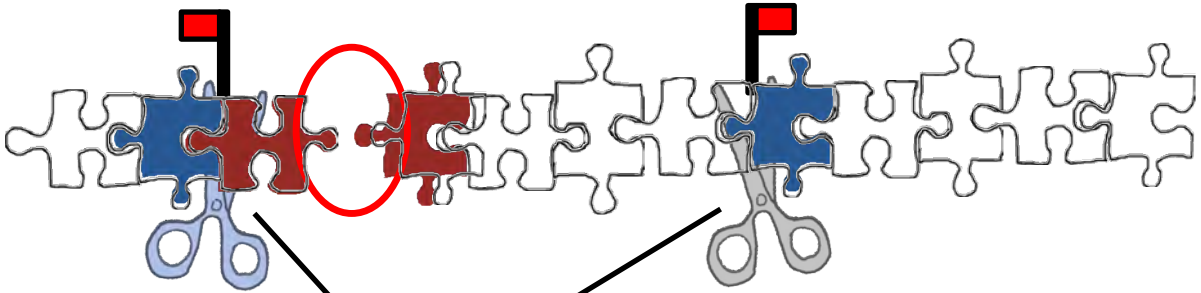


How CRISPR Works for DMD Puts the Puzzle Back Together

Non-mutated DMD gene

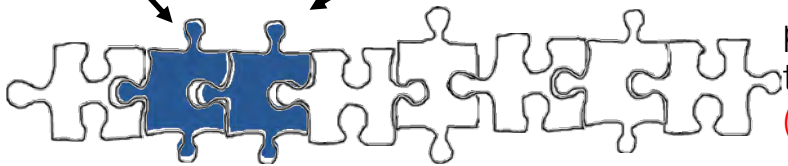


Duchenne DMD gene



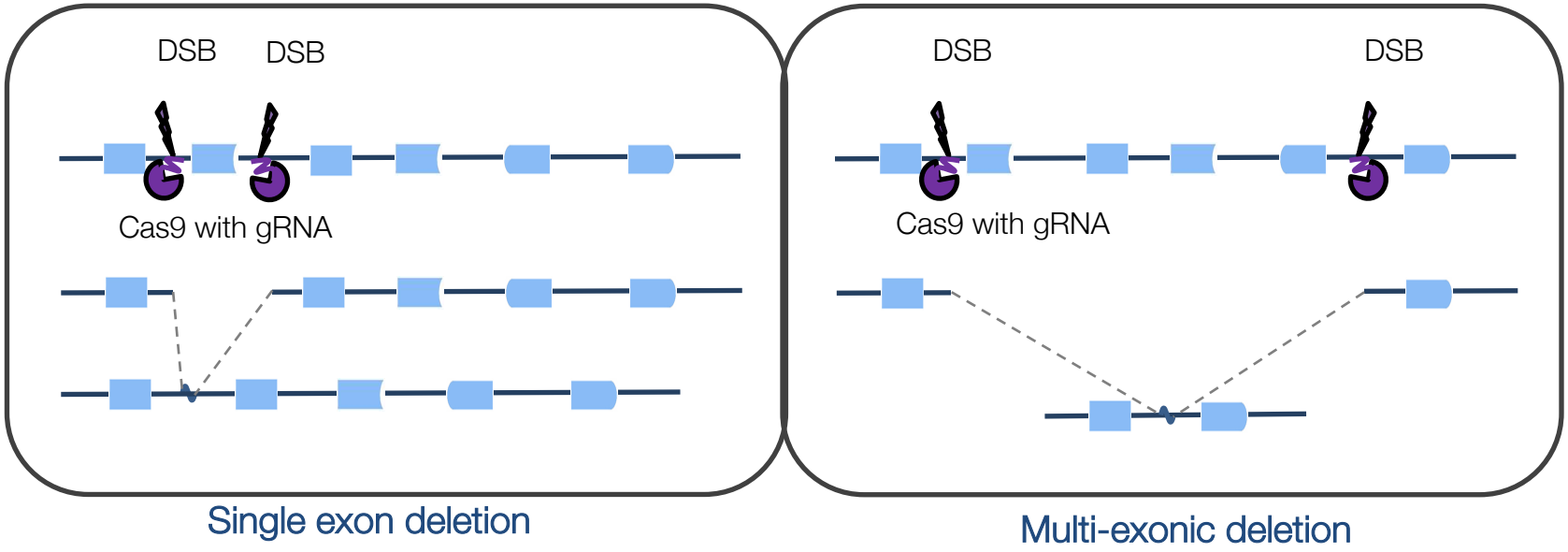
puzzle pieces do not fit together
(no dystrophin)

Treated DMD gene



puzzle pieces fit together
(dystrophin made)

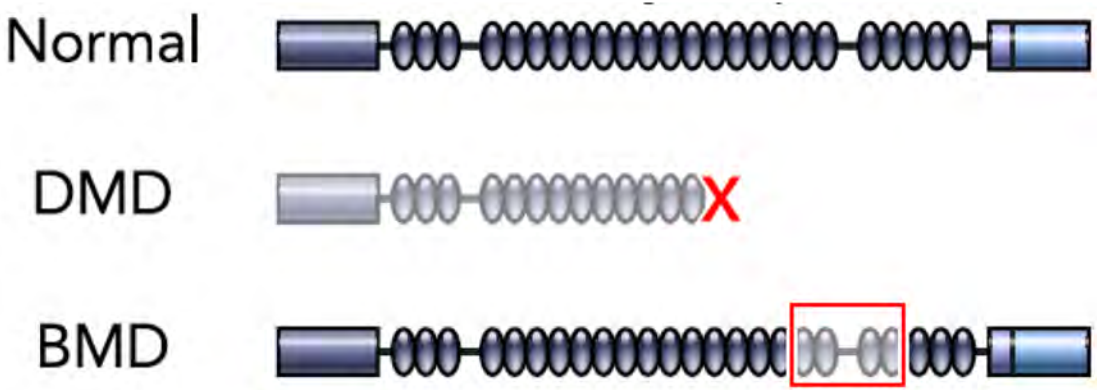
CRISPR can be used to skip one or multiple exons



SIMILAR TO EXON SKIPPING BUT CRISPR MAKES A PERMANENT CHANGE IN THE DNA

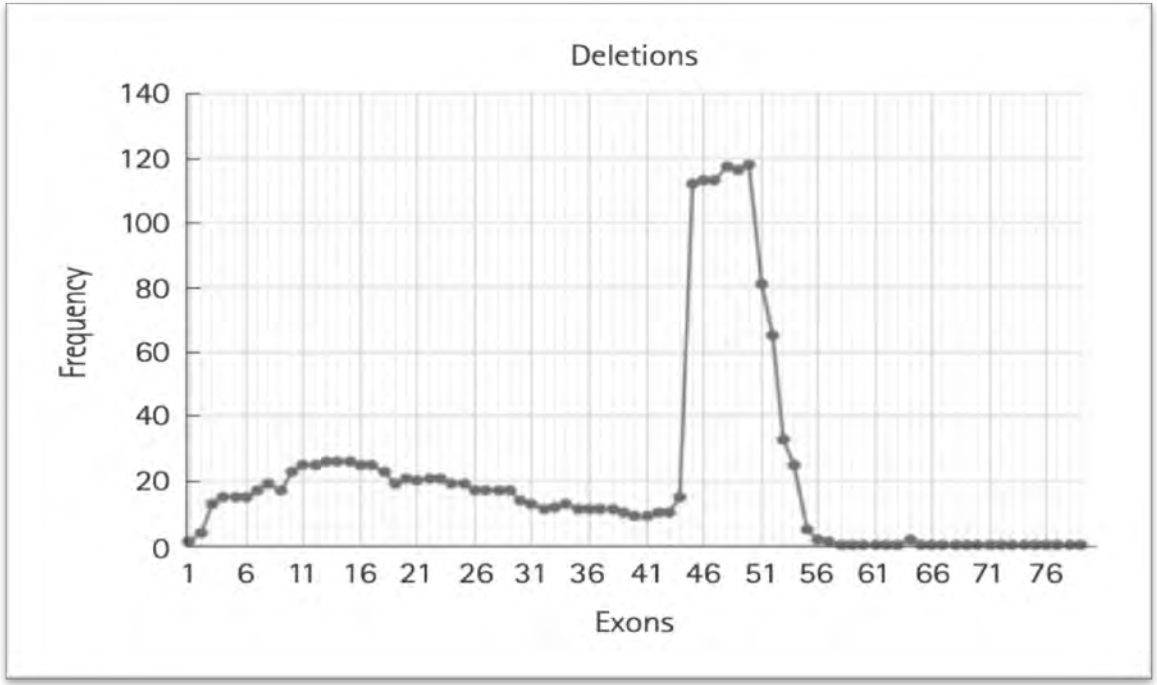
Young et al, CRISPR for Neuromuscular Disorders: Gene Editing and Beyond, Physiology in press

BMD is associated with *DMD* deletions and a milder disease course, but with a range of severities



Del DMD exons 45-55
one of the mildest

DMD exons 45-55: hot spot for patient mutations



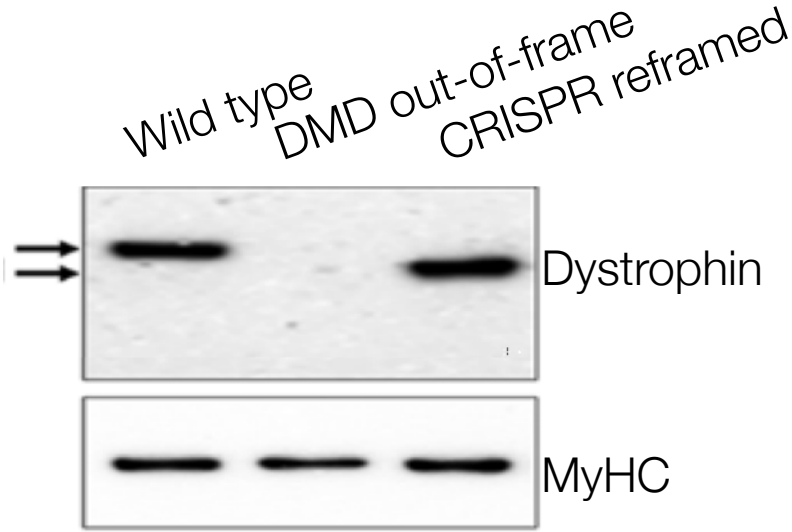
Courtney Young, Ph.D.
April Pyle, Ph.D.

Vengalli, J Clin Neurol. 2017 Jan;13(1):91-97.

Goal: mimic DMD$^{\Delta 45\text{-}55}$ using a pair of guides to accomplish CRISPR gene editing

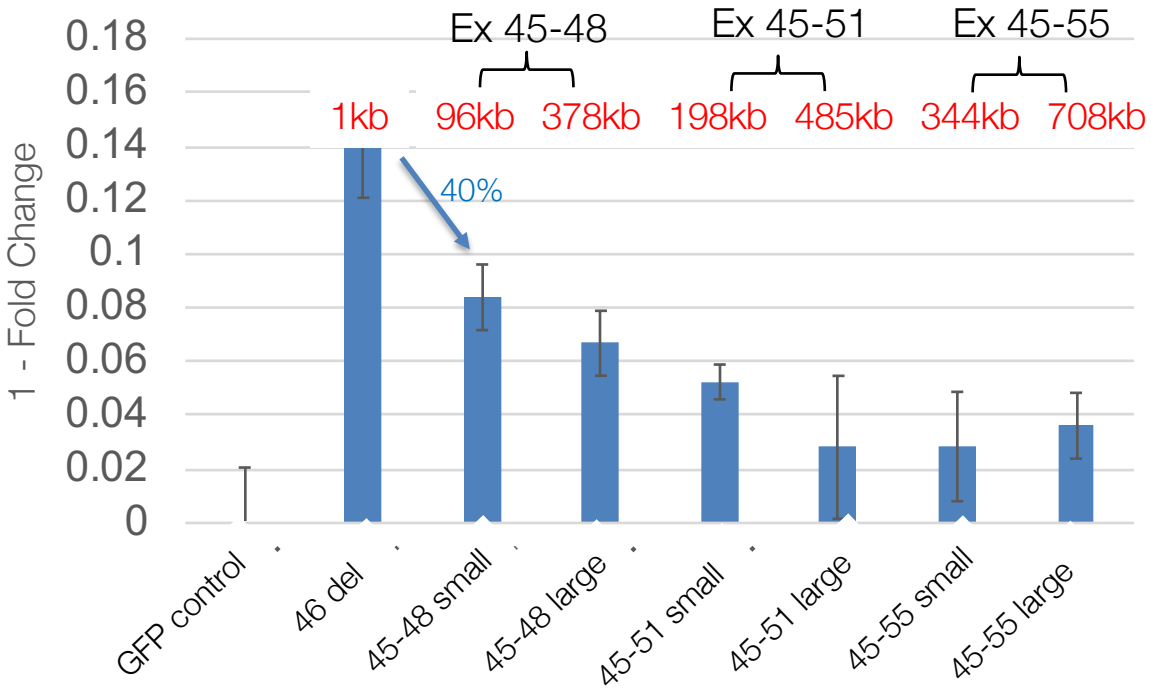
- Most mild BMD mutation
- Applicable to approximately 50% of DMD patients

CRISPR can delete exons 45-55 and generate an internally deleted but functional dystrophin protein



(66kDa shift)

Efficiency of deletion by guide distance using 2 guides



assessed by TAQman exon 46 probe

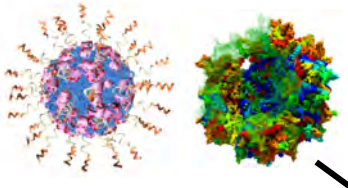
PPMD supported work

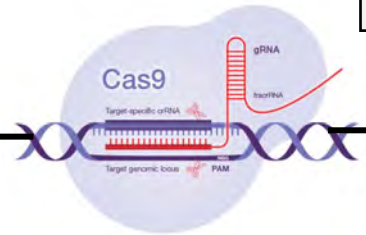
Therapeutic applications of CRISPR/Cas9 in vivo

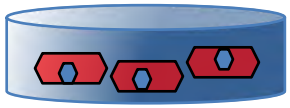
Huan Meng Ph.D.

DIRECT DELIVERY AND EDITING IN VIVO

EX VIVO EDITING AND ENGRAFTMENT







Non viral or viral Direct Delivery

Ex vivo editing + engraftment- Stem cell therapy

April Pyle, Ph.D.

collaboration with April Pyle and Huan Meng labs

Issues to consider for AAV mediated delivery of CRISPR/Cas9

Immune response to virus prevents re-administration of the same serotype
Approximately 60-70% of adults have pre-existing immunity;

Sa and SpCas9 are also **immunogenic,** thus, long term expression of Cas9 will likely lead to toxicity;

approximately 96% estimated to have pre-existing immunity

AAV can **integrate** into the cut site (Chamberlain, Gersbach, etc.)

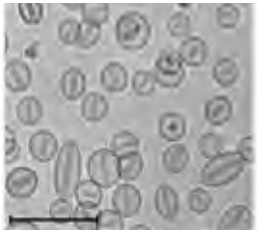
Benefits of on-viral carriers, such as nanoparticles,

- Can be modified to increase functionality
- Biodegradable
- Largely non-immunogenic: can be re-administered

Challenge: Identifying a nanomaterial able to transport a large plasmid or mRNA?

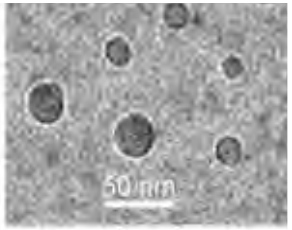
Nanoparticles can be generated from a variety of materials

Organic



Liposomes

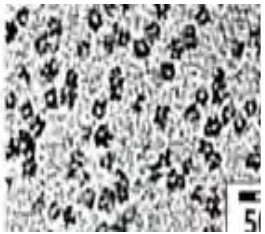
Protein based



Polymer

Inorganic

Iron-Oxide



Silica

Gold

>50 approved nanomedicines

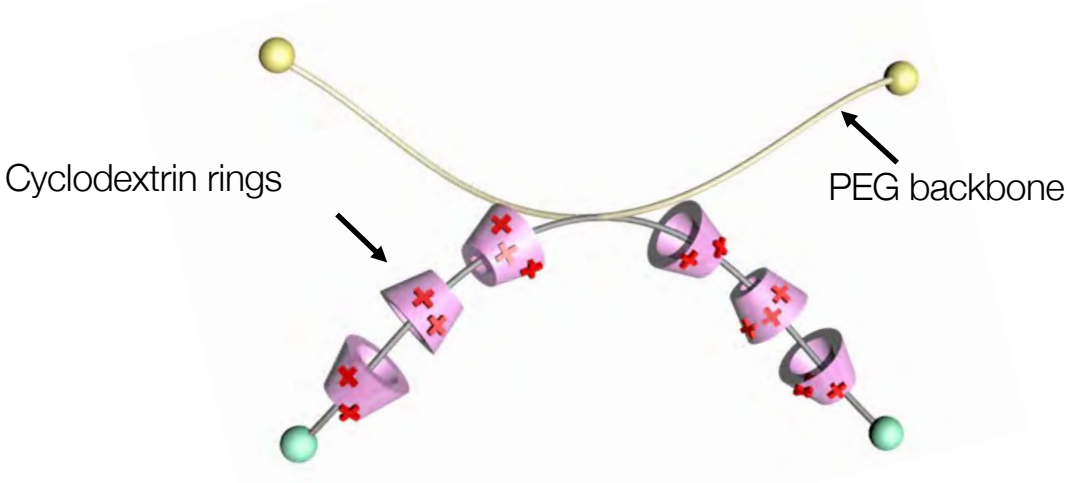
>75 in clinical trials

David Geffen School of Medicine

(Bobo et al 2016)

CDMD Center for Duchenne Muscular Dystrophy

Polyrotaxane (PRX) Nanocarriers



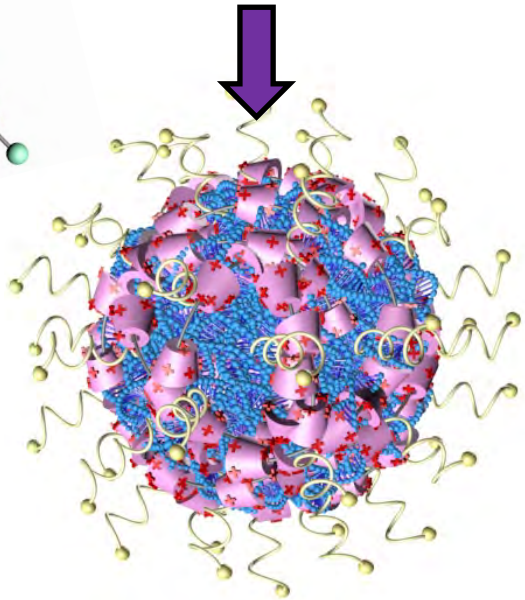
Tunable
- # CD rings
- Charge (# amines per ring)
- ratio of PEG
- Functionalize with peptides or linkers
- Ratio of particle to nucleic acid

Emami et al, Adv. Therapeutics in press

Polyrotaxane Nanocarriers self assemble with nucleic acid to form the particle

4-arm PRX
positively charged

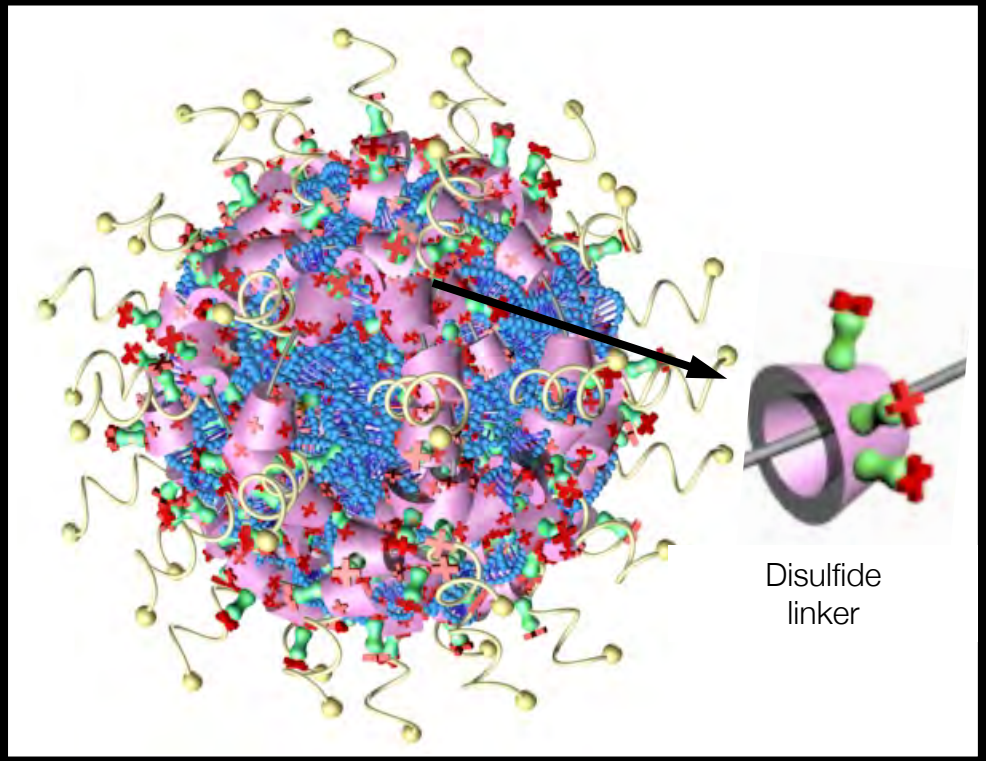
+



pDNA or RNA
negatively charged

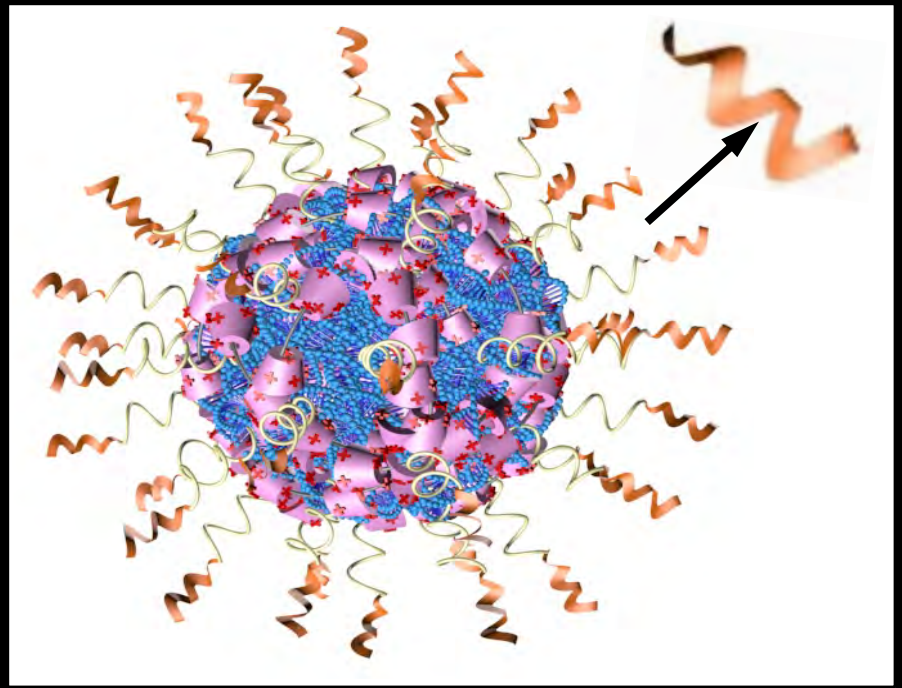
Emami et al, Adv. Therapeutics in press

Addition of disulfide linker facilitates plasmid release



Emami et al, Adv. Therapeutics in press

Addition of peptide facilitates nanoparticle uptake and cellular trafficking

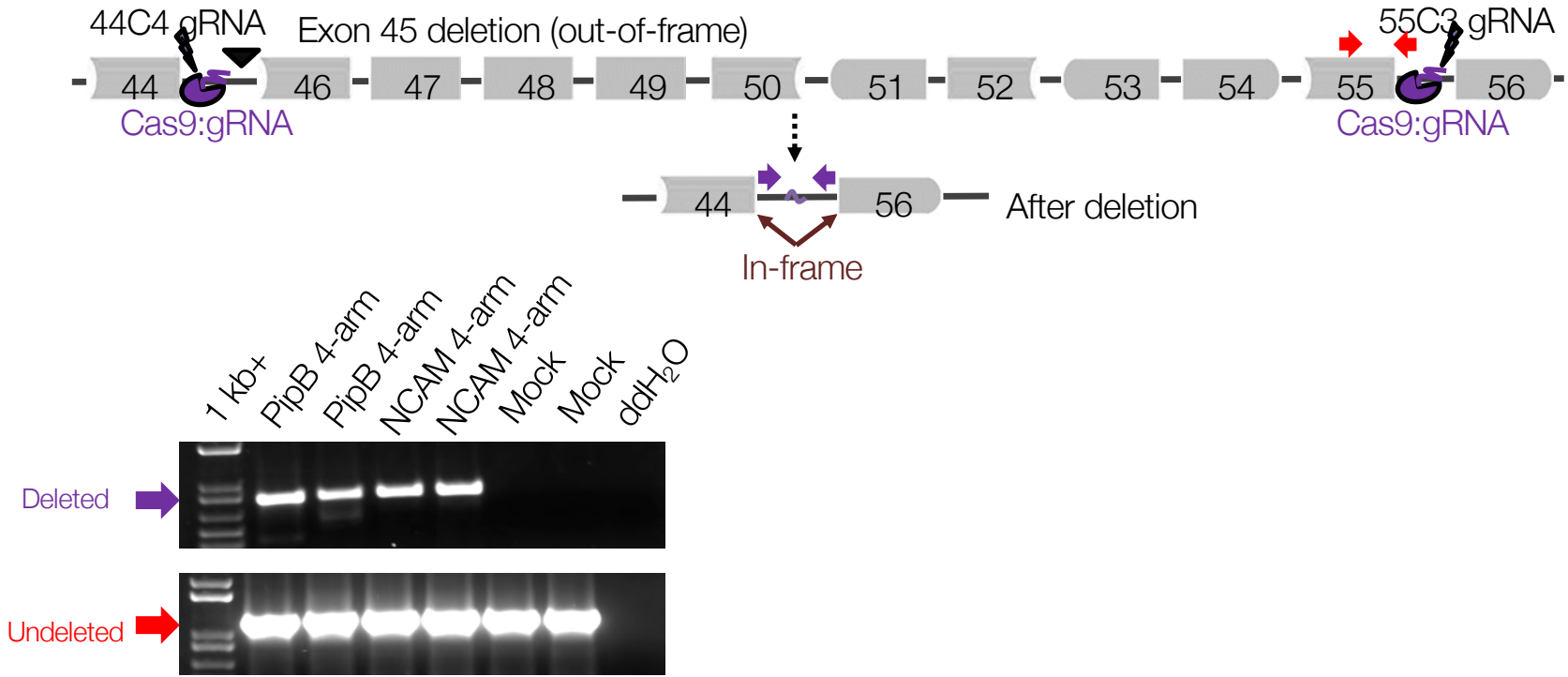


PipB-cell penetrating peptide

NCAM peptide
-Satellite cell targeting

Emami et al, Adv. Therapeutics in press

CRISPR gene editing in hDMD cells after PRX



Emami et al, Adv. Therapeutics in press

Systemically delivered PRX nanoparticles biodistributed to skeletal muscle in mdx mice

PRX/Cy3-labeled mCherry plasmid, mdx mice, 24 h post i.v. injection

Quad EDL TA Gast Tri Dia Hrt

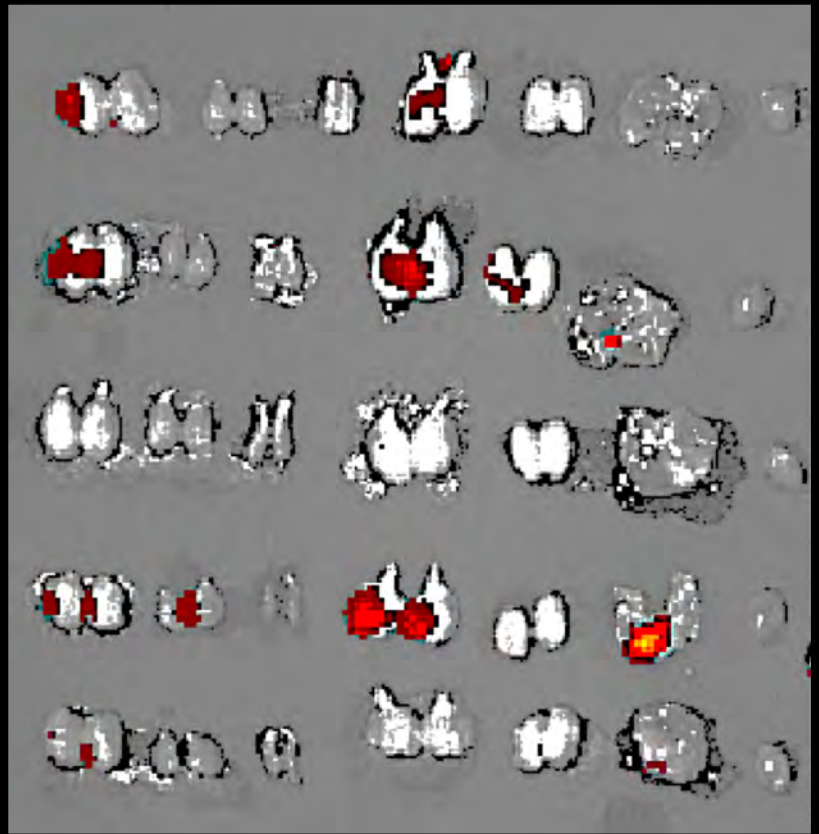
No linker unconjugated

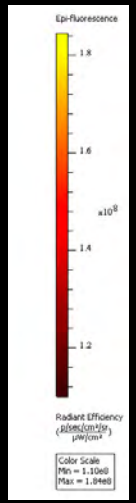
No linker CPP

No linker NCAM

→ Unconjugated + linker

Untreated

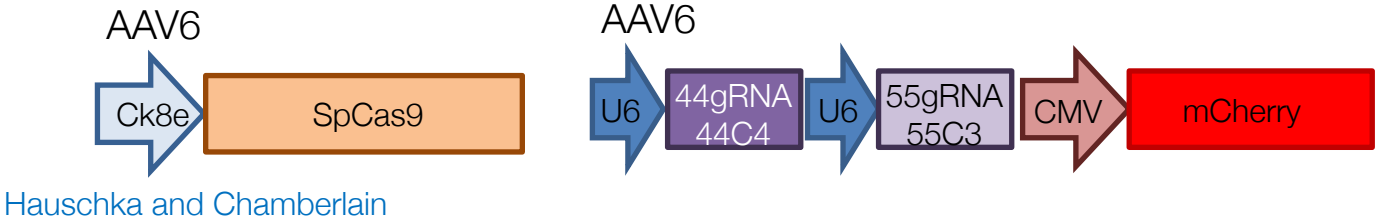




How well does DMD$^{D45\text{-}55}$ perform with AAV delivery?

Other groups previously showed efficacy using AAV to deliver CRISPR in mouse models of DMD on the mouse gene

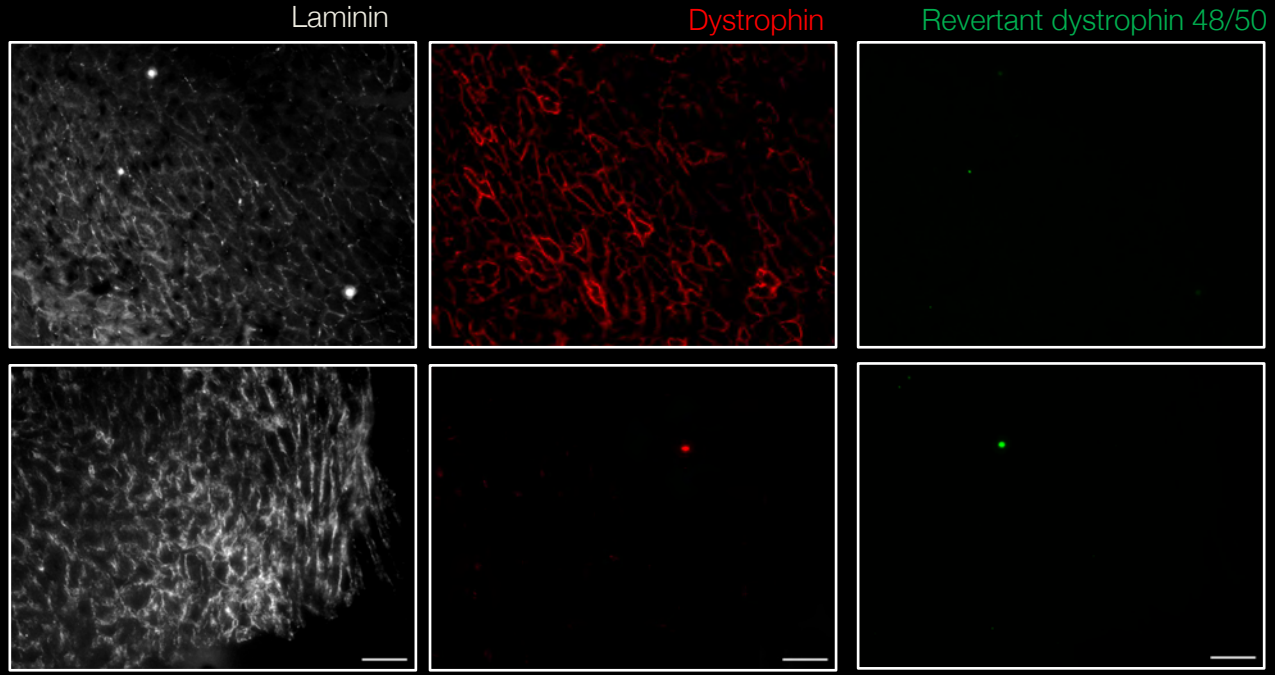
(Long et al. 2015, Nelson et al. 2015 and 2019, Tabebordbar et al. 2015, Bengtsson et al. 2017, Hakim, 2019)



Hauschka and Chamberlain

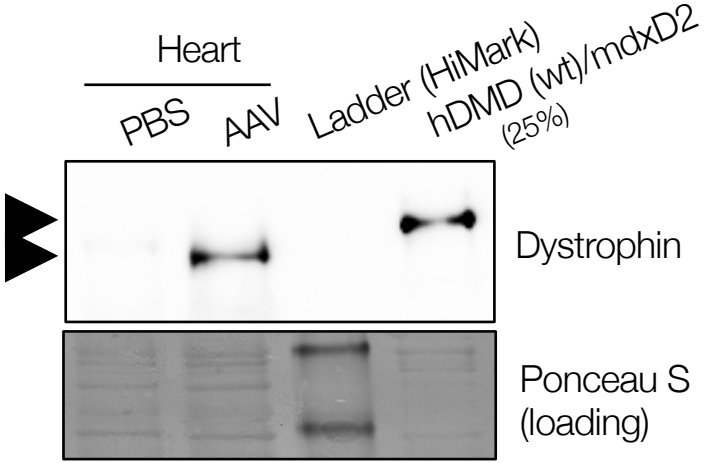
Jeffrey Chamberlain and Nic Bengtsson, (U Washington)

AAV6-CRISPR mediated dystrophin expression in heart after systemic injection



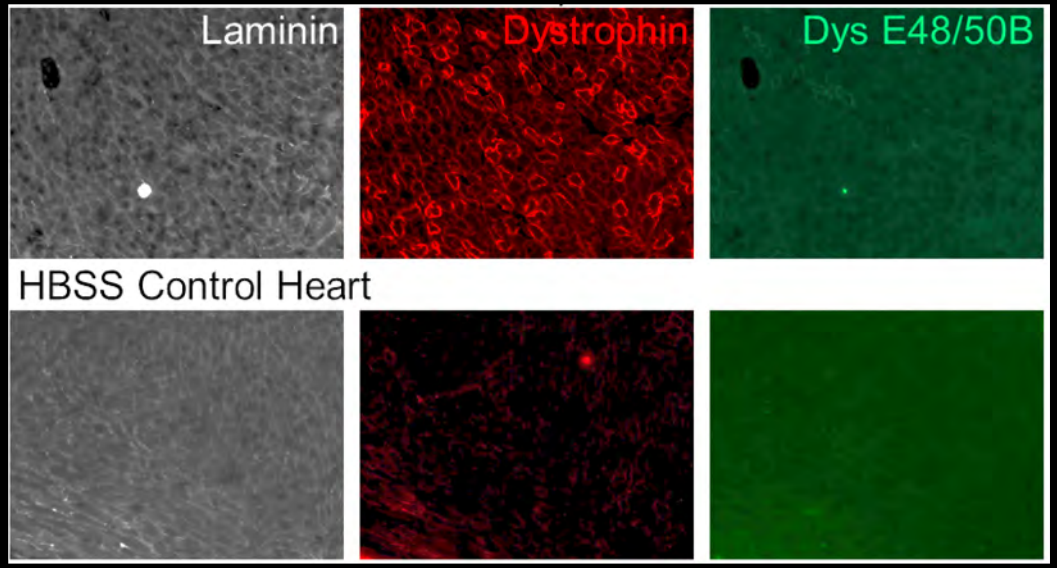
2.85x10^{12} v.g. of each vector @ 5 wks of age

Dystrophin westerns after systemic delivery of AAV6-CRISPR DMD$^{del45-55}$



31% dystrophin + fibers
10% WT dystrophin

AAV9-CRISPR mediated dystrophin expression in heart after systemic injection

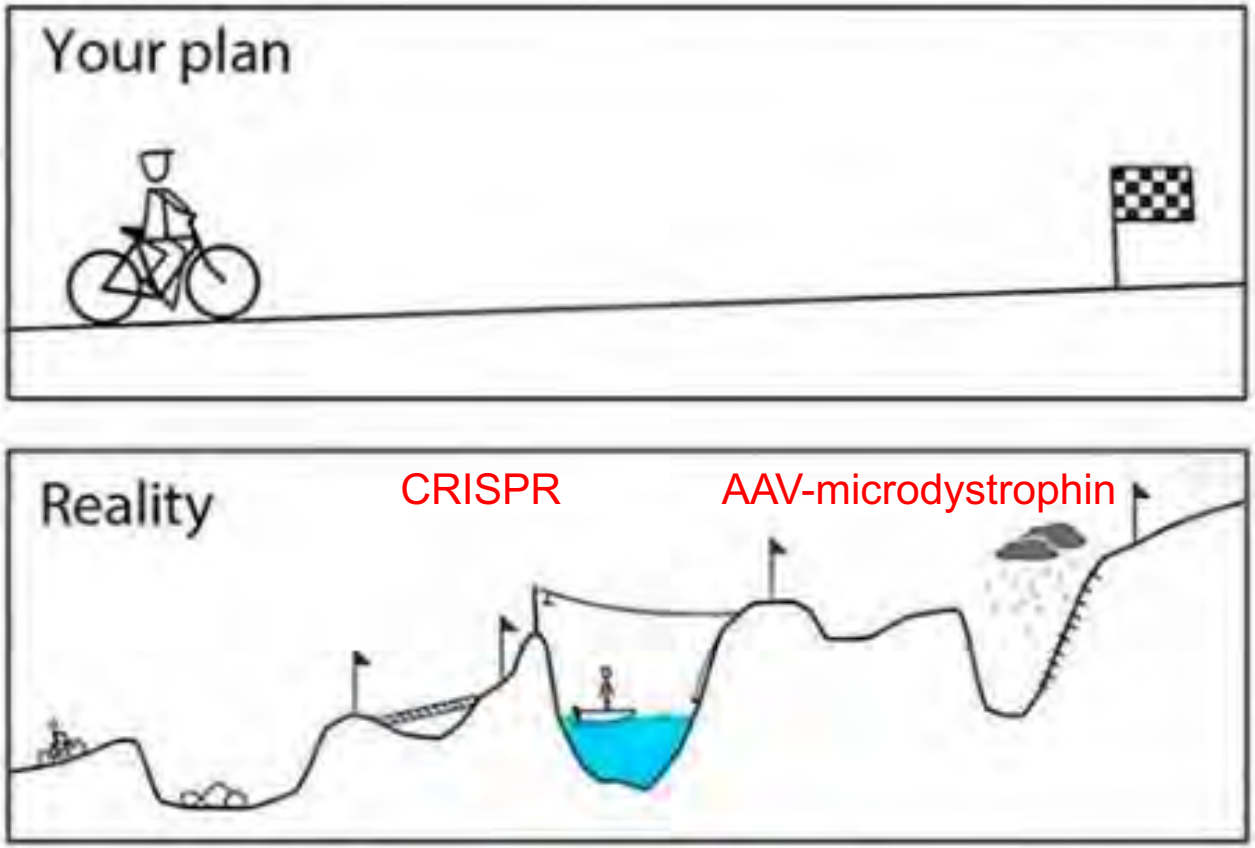


5E11vg AAV9-SPY-DYS$^{45\text{-}55}$
day 6

hDMD del45 mdx mice

Summary

1. CRISPR platform targets 50% of patients; delivery by viral and non-viral delivery methods
2. CRISPR efficiency is higher over short distances, but with distances greater than 200kb, size has less effect on efficiency.
3. Currently optimizing platform and delivery methods.
    1. Efficiency is too low to use single AAV injection;
    2. Need to ”kill” Cas9;
    3. Need to establish off target effects;
    4. Testing high fidelity nucleases



Slide from Dr. Barry Byrne

Acknowledgements

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- Devin Gibbs
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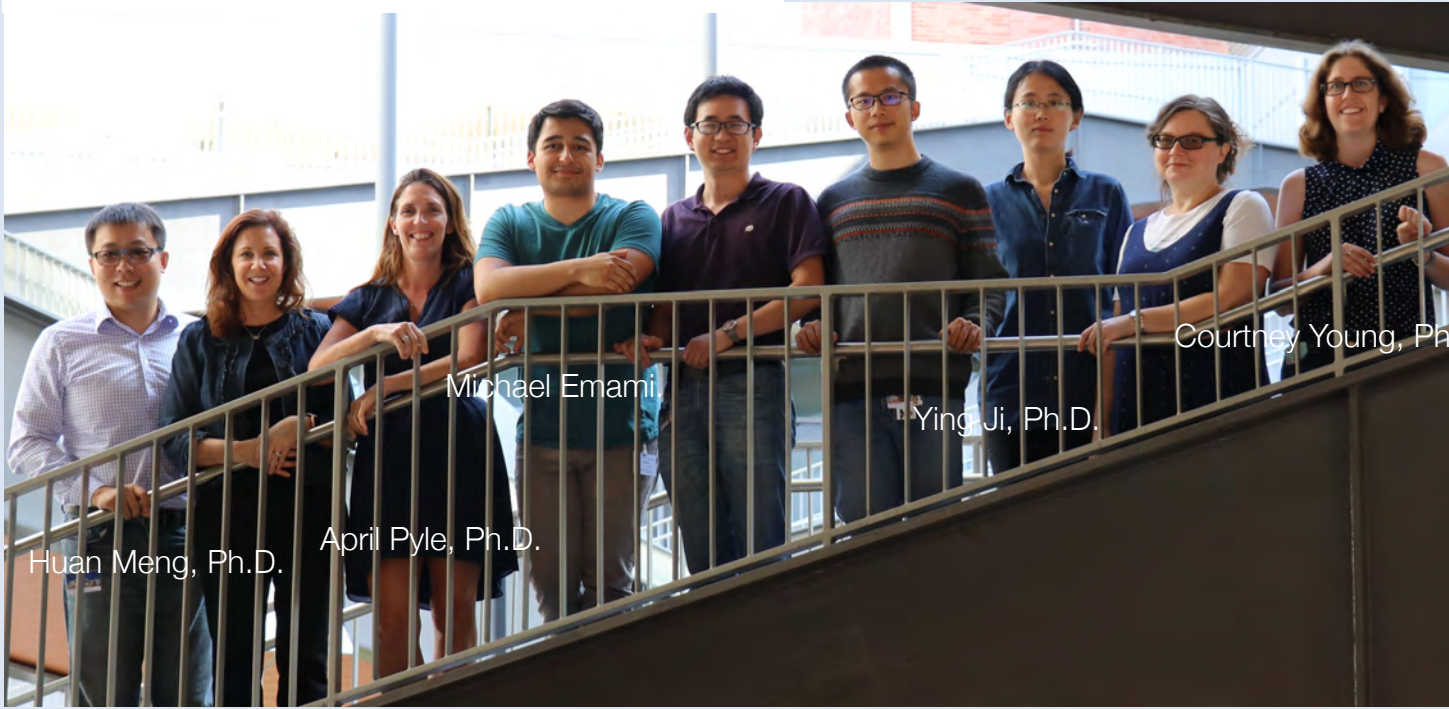
Stanley Nelson
Carrie Miceli
Stephen Cannon



- UCLA BSCRC
- Jesse's Journey

Thank you!