RHD

CONFERENCE POSTERS

2016 ASGCT

This is one of the most recent scientific poster that I presented at last year's American Society of Gene and Cell Therapy in Washington DC. My postdoctoral research is on bioprocess development for genetically engineered stem cells.

UNIVERSITY OF CALGARY
CUMMING SCHOOL OF MEDICINE

NON-VIRAL TRANSFECTION OF PRIMARY HUMAN FIBROBLASTS ON MICROCARRIER SUSPENSION CULTURE

Charlie Y. Hsu and Derrick E. Rancourt

Introduction

Suspension bioreactors have been employed for the large-scale production of recombinant proteins and expansion of cells for clinical applications. However, the process requires a stably-expressing cell line to begin with, which can take considerable amount of time and cost to establish. A transient-expression system not only provides a simple cost-effective platform for production in a small-medium scale, but is better suited for cellular reprogramming and tissue engineering of primary cells where only transient forced expression of factors is needed.

Here, we describe an efficient non-viral method to transfect primary human foreskin fibroblasts on suspension microcarrier using cationic reagent. This is the first step towards the development of an integrated platform to streamline the derivation and production of reprogrammed cells.

Methods

Inoculating Microcarrier

Microcarriers were first dispensed onto a polyHEMA-treated TC-dish, then single-cell suspension of fibroblasts were added to a final concentration of 100,00 cells per 10 mg of microcarriers per ml of culture media.

Growth Curve

Cell proliferation on the microcarriers were determined using the MTT assay. Each time point were expressed as a percentage over a mitomycin C-treated static fibroblasts culture

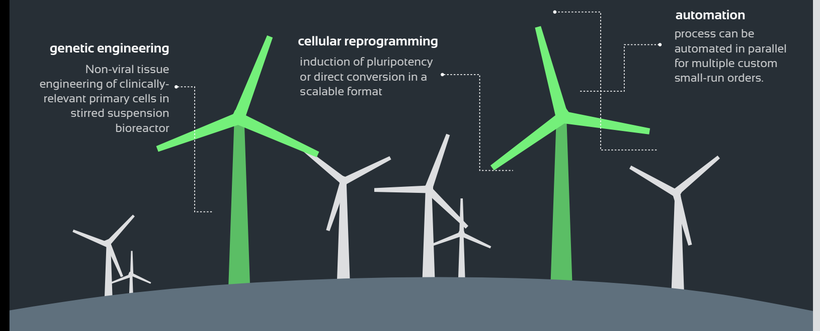
Transfection

Cells were transfected on microcarrier in static suspension using XtremeGENE-HP. Two days after transfection, cells were then dissociated by 0.25% trypsin and processed for analysis by flow cytometry.

Microcarrier	Size (µm)	Surface
Hillex II modified polystyrene	160-180	+
Glass cross linked polystyrene	125-212	+
Pronectin F cross linked polystyrene	125-212	ϟ
FACTIII cross linked polystyrene	125-212	+
Cytodex 3 cross-linked dextran	175	+
CultiSphere S modified polystyrene	130-380	/

integrated platform

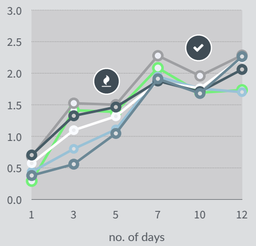
streamline the derivation and expansion of engineered cells as one vertically integrated process



Results

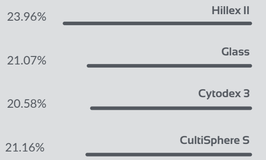
Growth Curve

A dip in the growth curve on Day 5 indicate media exhaustion. By Day 7, most of the microcarrier culture reached a plateau in cell density.



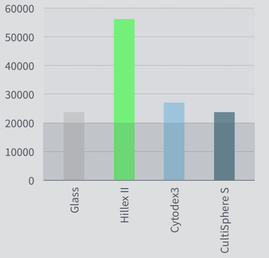
Cell Proliferation

Polymer-assisted transfection is highly dependent on the proliferation rate of cells. The optimal time frame to transfect should be around when the growth curve exhibit the sharpest positive slope.



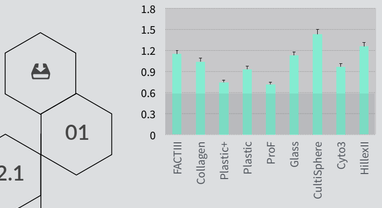
Percentage of Transfection

The percentage of cells transfected were about the same among microcarriers. Interestingly, this approximately corresponds to the percentage of cells in the mitotic phase (18-20%)



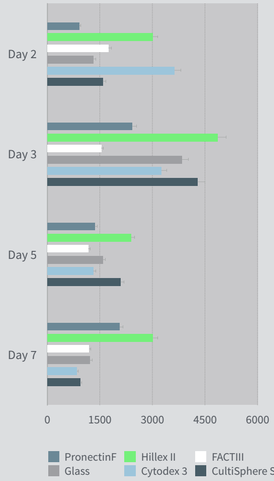
Transfection Efficiencies

Despite having similar percentages of transfected cells, the levels of transgene expression differ significantly among carriers; Hillex II was the most conducive for high transgene expression



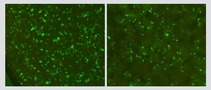
Cell Attachment

Attachment efficiencies differ among microcarriers, which translate to differences in starting cell density. This, in turn, would affect growth rate, time frame for transfection and ultimately, transfection efficiency



Transfection Time Frame

Transfection in static 2D culture is typically done at a certain cell density since cell-to-cell contact is necessary in driving the proliferation of adherent cells. Cell density on microcarrier is less straightforward to gauge, and therefore, difficult to assess culture readiness for transfection. Here, we transfected at multiple time points, bracketing around the exponential part of the growth curve.



Transfected cells on microcarrier

Representative epi-fluorescent images of HFF transfected with GFP on Cytodex 3 (left) and CultiSphere S (right).

This project is funded by the Alberta Innovates - Health Solutions Collaborative Research and Innovation Opportunities. C. Hsu is funded by a Fellowship from CIHR and a Postgraduate Fellowship from AIHS. We thank the Stem Cell Network and the NSERC CREATE Program for their generous and continued support in trainee development

RHD

CONFERENCE POSTERS

2016 ASGCT

Another poster that I presented at last year's American Society of Gene and Cell Therapy. I like to use these posters as a creative outlet to try out some new designs

I did a split panel design so I can cut it in half and put it in checked luggage instead of having to carry a giant tube as carry on.

UNIVERSITY OF CALGARY
CUMMING SCHOOL OF MEDICINE

En route to non-viral genetic engineering:

Kinetics of pDNA uptake and transgene expression following repeated transfection with multiple episomal plasmids in primary human fibroblasts

Charlie Y. Hsu and Derrick E. Rancourt

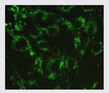
Introduction

Non-viral approach to genetic engineering of mammalian cells often involve co-transfection with multiple types of nucleic acid molecules. A significant rate-limiting step thus lies in the lack of efficient co-transfection, in which a subset of the transfected cells may be devoid of either one, two, or more of the factors required.

In this study, we used a multiplexed approach to examine the kinetics of DNA uptake and transgene expression following co-transfection with multiple fluorochrome-labeled reporter plasmids.

Methods

Non-invasive fluorescent labeling of plasmid DNA



Plasmid DNAs were labeled using the Mirus Bio Label IT® Cy3 and Cy5 Nucleic Acid Labeling Kits according to manufacturer's protocol. However, in order to measure transection efficiency from the labeled plasmid, we titrated labeling densities against uptake and transfection efficiencies. Figure A shows that the signal intensity is proportional to the amount of labeling reagent, while uptake and transfection efficiency (%Cy5 and GFP level) is inversely correlated (Figure B and C).

Transfection of human foreskin fibroblast

Primary human foreskin fibroblast were transfected with Cy5-labeled gWiz-GFP and Cy3-labeled gWiz-BFP. A 0.02 (v/w) Cy5-to-DNA labeled gWiz-BFP were mixed with a 0.1 (v/w) Cy3-to-DNA labeled gWiz-BFP at a 1:1 (w/w) DNA-to-DNA weight ratio, then transfected using XtremeGENE HP at a reagent-to DNA v/w ratio of 3.

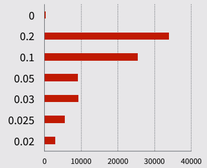


Figure A. The overall mean fluorescence (RL1) of cells transfected with plasmids labeled with decreasing ratios of Cy5-labeling reagent-to-pDNA (v/w).

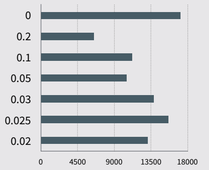


Figure B. The overall mean fluorescence (BL1) of cells transfected with gWiz-GFP labeled with decreasing ratios of Cy5-labeling reagent-to-pDNA (v/w).

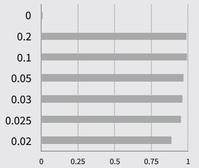


Figure C. The % of Cy5+ cells following transfection with plasmids labeled with decreasing ratios of Cy5-labeling reagent-to-pDNA (v/w).

Alberta Innovates Health Solutions
CIHR IRSC

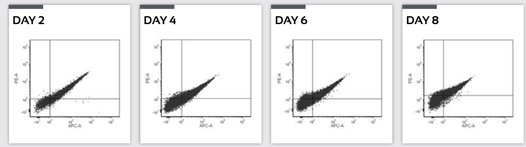
This project is funded by the Alberta Innovates – Health Solutions Collaborative Research and Innovation Opportunities. C. Hsu is funded by a Fellowship from CIHR and a Postgraduate Fellowship from AIHS. We thank the Stem Cell Network and the NSERC CREATE Program for their generous and continued support in trainee development.

RESULTS

Kinetics of co-pDNA uptake

Figure 1. Fluorescent intensities of Cy5 and Cy3 over a course of 8 days

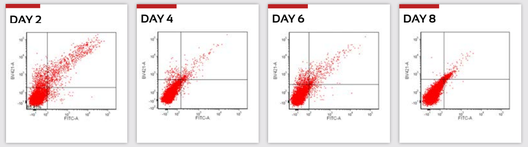
More than 80% of the cells with pDNA uptake were positive for both Cy3 and Cy5, suggesting both plasmid DNAs were complexed and efficiently co-delivered together.



Kinetics of co-transfection

Figure 2. Fluorescent intensities of GFP vs BFP over the course of 8 days.

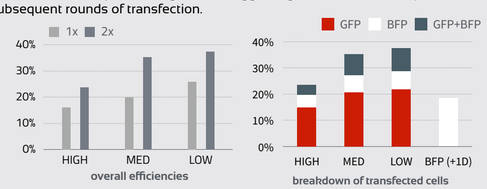
While majority of the transfected cells co-express both BFP and GFP, small proportions were singly transfected with either BFP or GFP. Further, the relative levels of transgene expression were not equivalent; some cells had more BFP than GFP, and vice versa.



Repeat Transfection

Figure 3. Transfection efficiencies following sequential transfection

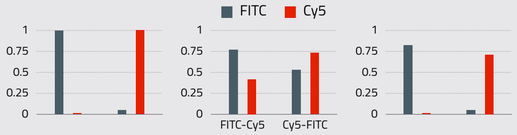
We then looked at the effects of repeated transfection by first transfecting with BFP then with GFP. To our surprise, the increase in overall transfection efficiency came from new events, not pre-existing events, suggesting transfected cell may be refractory to subsequent rounds of transfection.



Sequential Transfection

Figure 4. DNA uptake following sequential transfection

We then looked at whether cells were refractory to even DNA uptake. By transfecting cells sequentially with two different fluorochrome-labeled plasmid DNA, we saw that there is indeed a reduction in the capacity to take up DNA.



Day 1
Transfected Once on Day 1 Only

Sequential
Transfected Twice, Sequentially

Day 2
Transfected Once on Day 2 Only

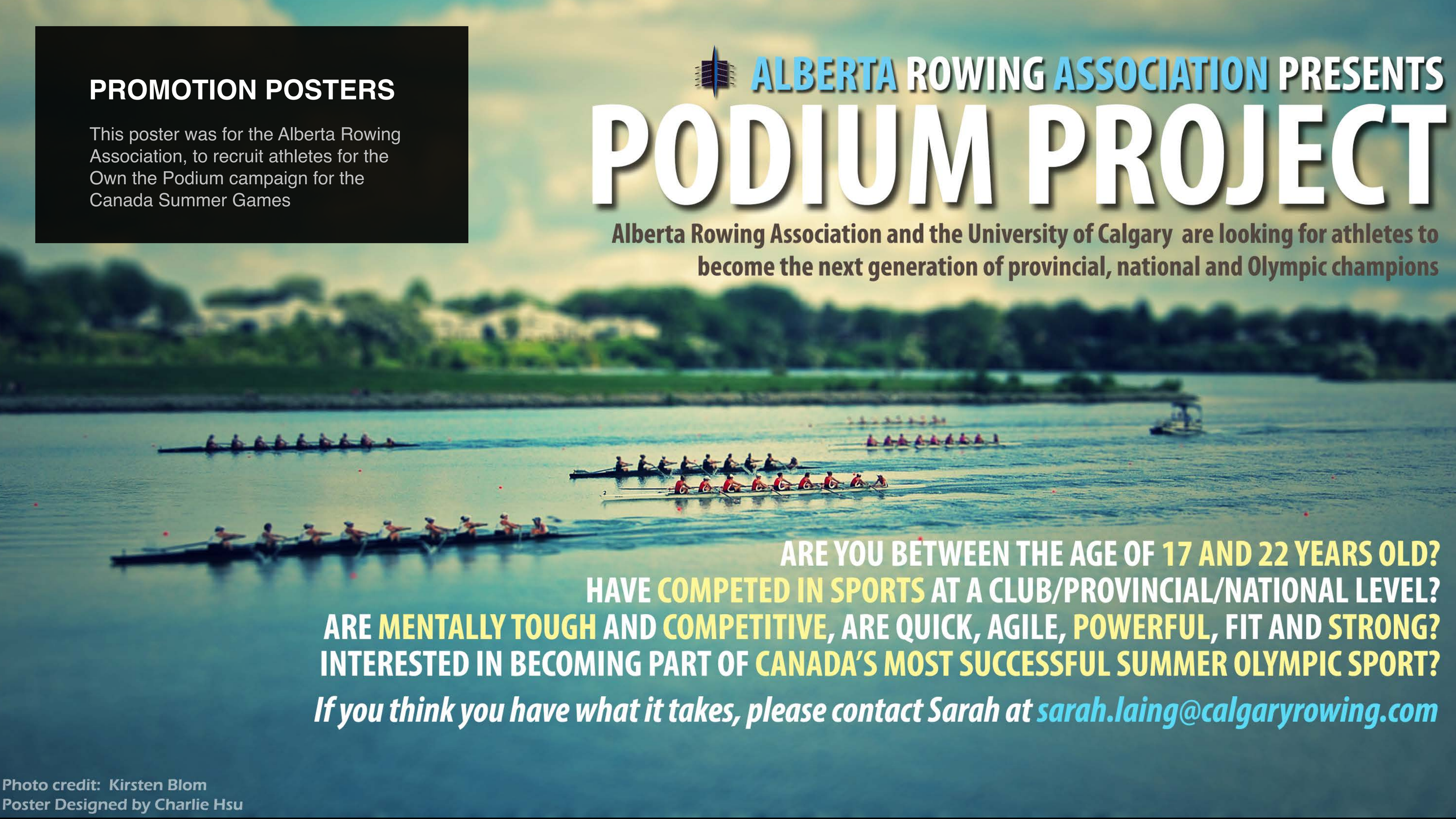
Summary

Co-transfection with multiple plasmid DNAs invariably results in asymmetry in the levels of transgene expression. Over time, the differences in relative expression levels widens, in which the stoichiometric ratios of the expressed factors deviates significantly from the intended input weight ratios.

In the context of cellular reprogramming where pluripotency-associated transcription factors are delivered separately by multiple episomes, this presents a major rate-limiting step. This may in part, explain why some iPSC-like colonies appear to be partially reprogrammed while only a few commit to full pluripotency.

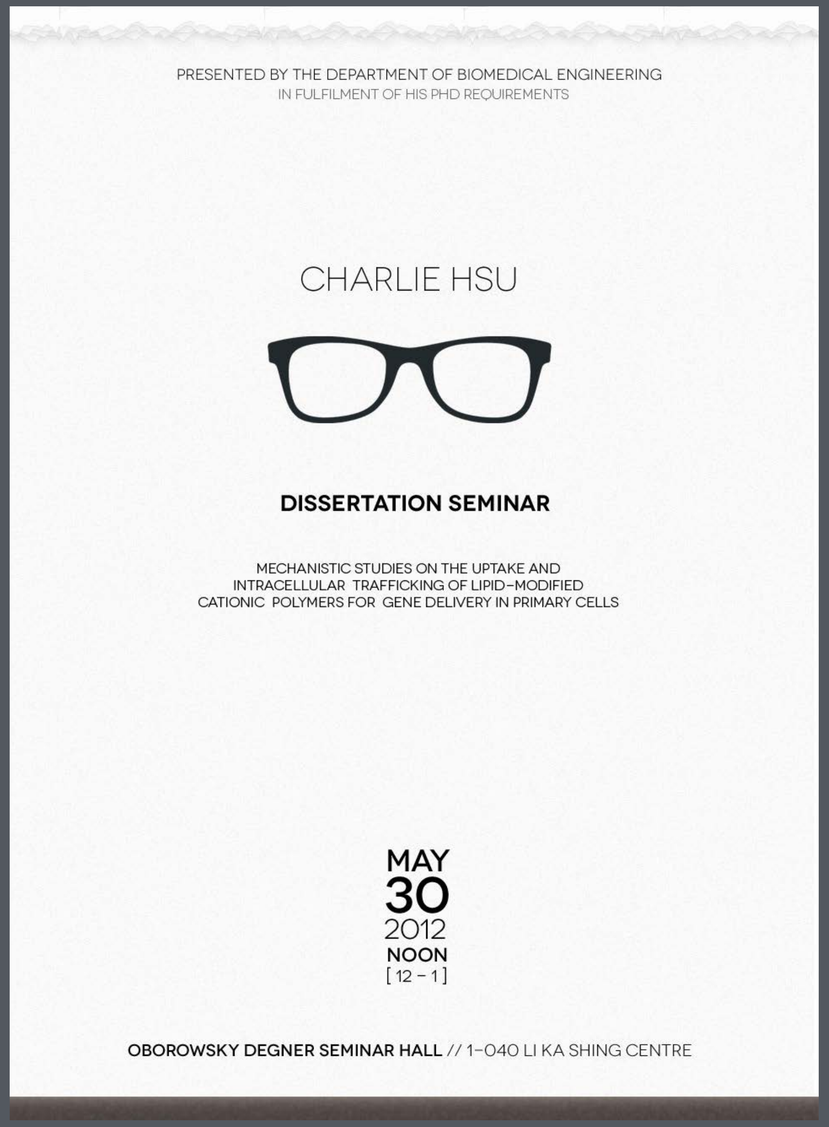
PROMOTION POSTERS

This poster was for the Alberta Rowing Association, to recruit athletes for the Own the Podium campaign for the Canada Summer Games



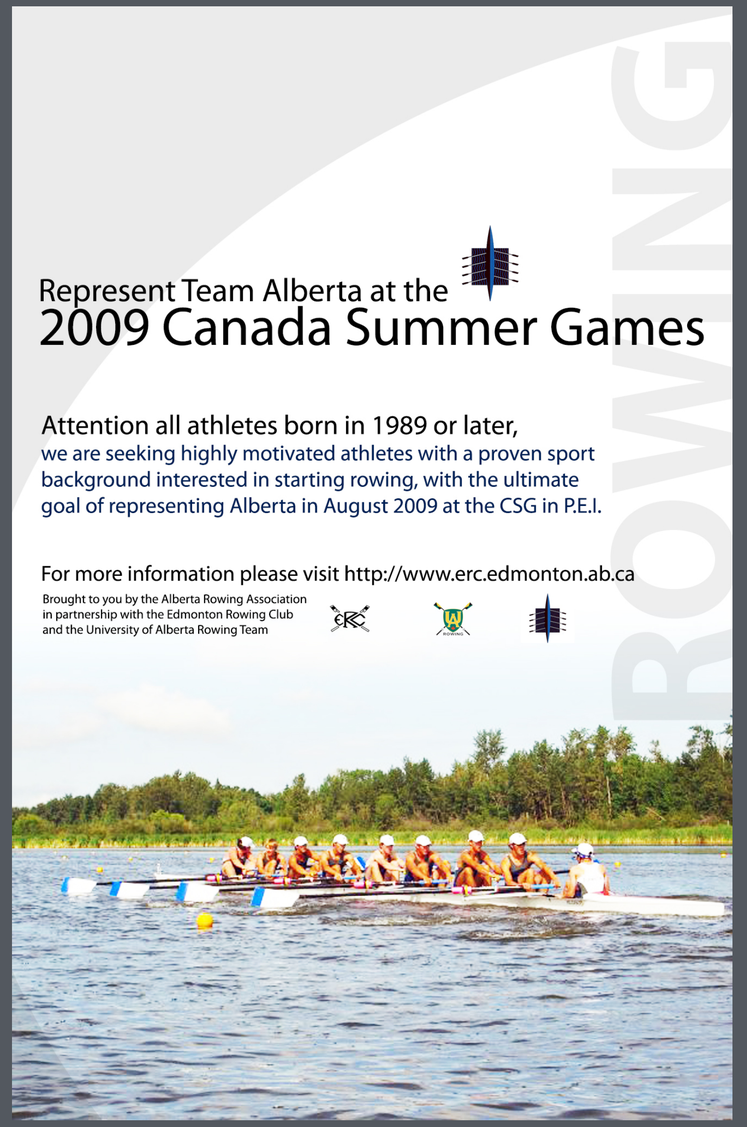
PROMOTION POSTERS

Another poster I did for the Alberta Rowing Association's Podium Project.



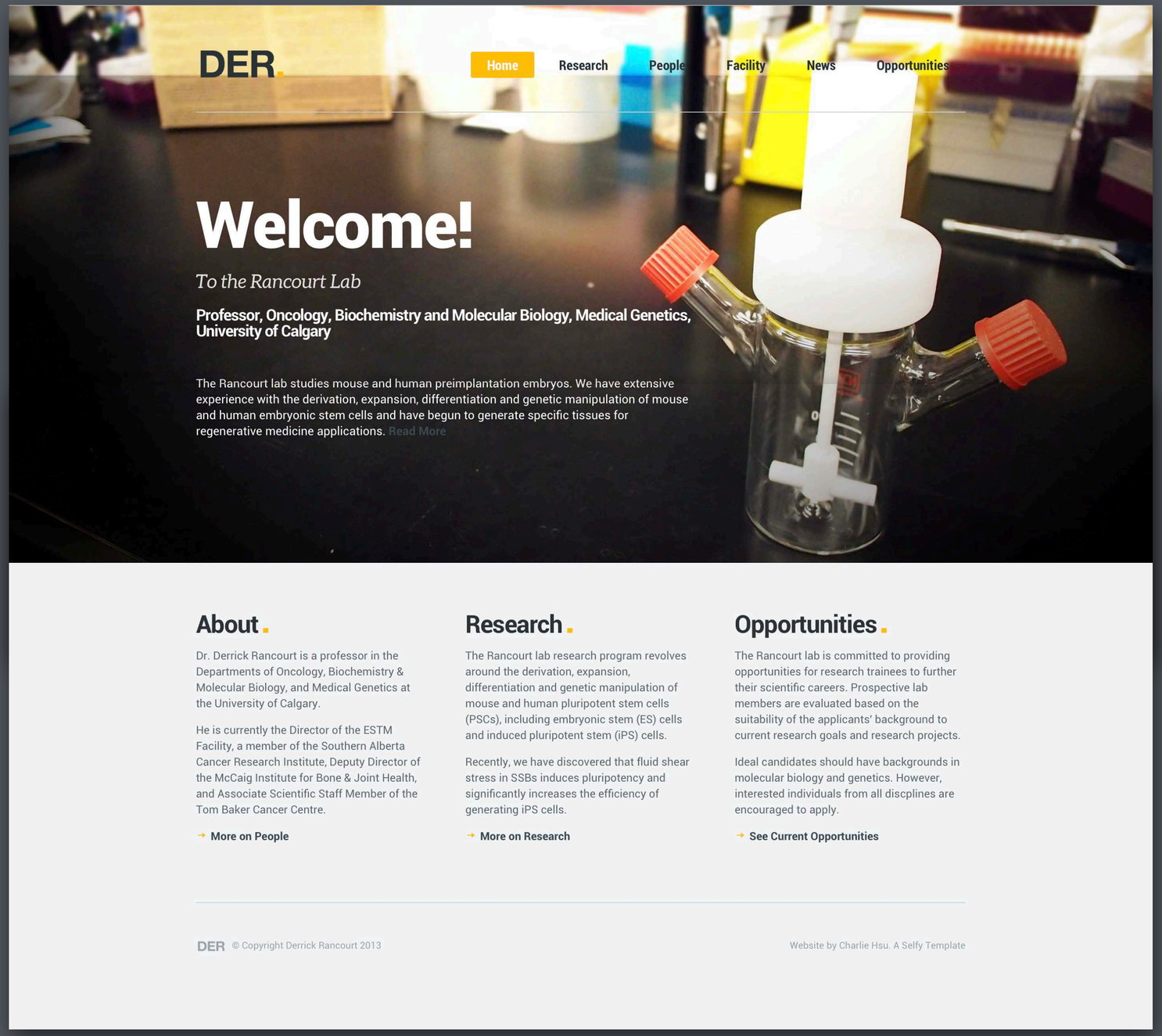
THESIS SEMINAR

I designed a poster for my thesis defence public seminar, but the department insisted on using their own Microsoft 3D text poster instead.



CANADA SUMMER GAMES

Another poster for the Alberta Rowing Association, for the 2009 Canada Summer Games.



RANCOURT GROUP

LAB WEBSITE

2013-Present

I re-did the website for my postdoctoral research supervisor. I used a template to save time on hard coding everything then customized the layout and graphics afterwards.

RESPONSIVE CONTENT

RANCOURT LAB

DYNAMIC VIEWING

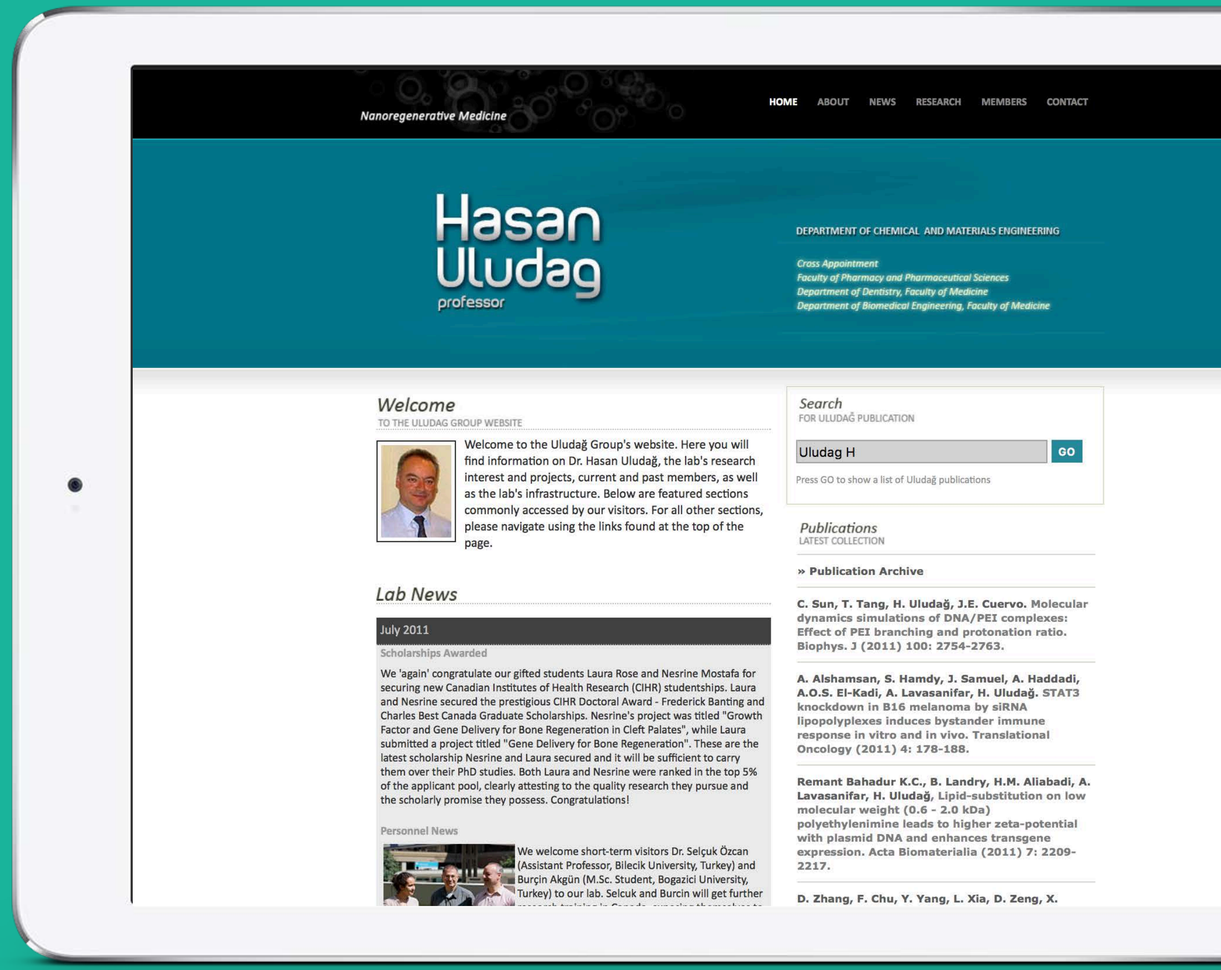
The HTML/CSS template has built in responsive content functionality, which means viewing is optimized for both web and mobile devices

ULUDAG GROUP

LAB WEBSITE

2009-2011

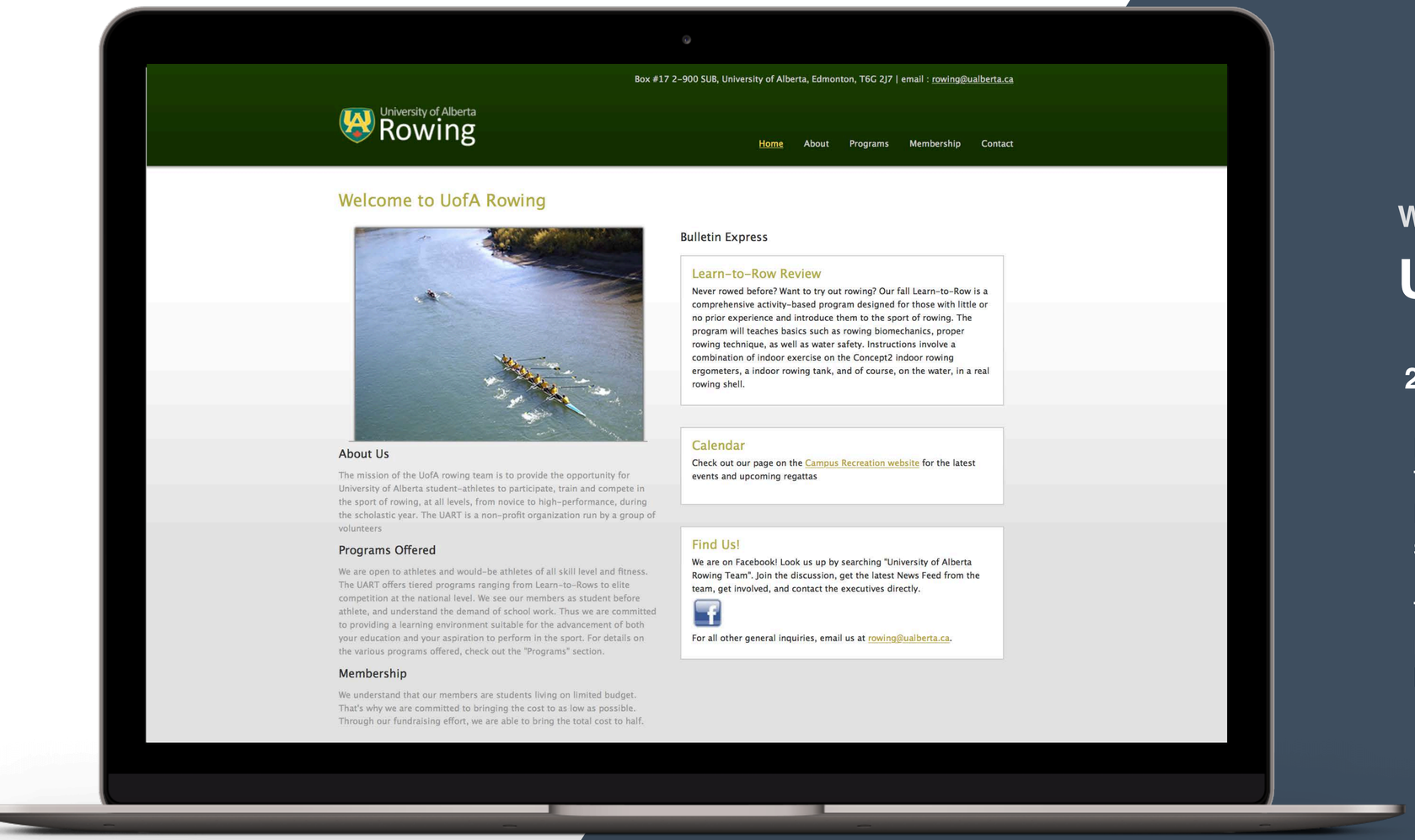
I also did the lab website for my doctoral thesis supervisor. This is from 2009-2011, pre-responsive content. I start migrating towards a Web 2.0 interface at this point. But this is still coding HTML/CSS by hand, which is very time consuming.



ULUDAG GROUP WEBSITE

2006-2009

The first version of the lab website that I did back in 2006. There was no advanced CSS functionalist, so limited to Flash-based menu with simple animation



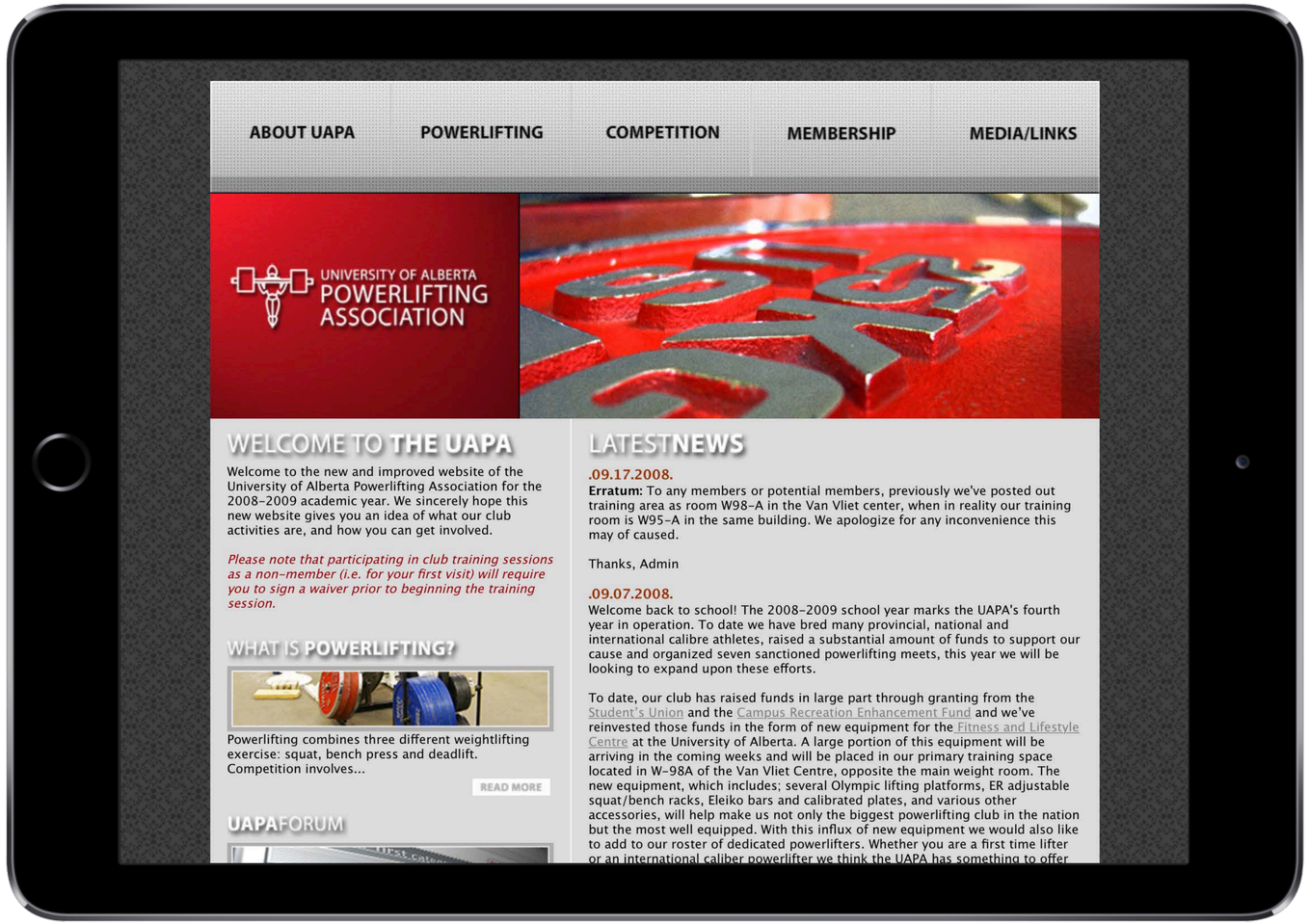
WEB DESIGN

UNIVERSITY ROWING

2006-2008

I was with the university rowing club from 2004-2010. This is the third website I did for the University of Alberta Rowing Team before migrating to a content management system.

The other two were done between 2004-2006, which did not have CSS or Web 2.0 features and were purely just HTML hardcode.



POWERLIFTING CLUB

2007-2009

I was also part of the University of Alberta Powerlifting Association from 2007-2012. This was the first website I did for them before they went under the auspices of Alberta Powerlifting Union and adopted Facebook Page exclusively.

EDMONTON ROWING CLUB

I was with the Edmonton Rowing Club from 2004-2013. I did a number of websites for them. This is the longest running one from 2005-2008 before I implemented a Drupal-based content management system with a customer relation database