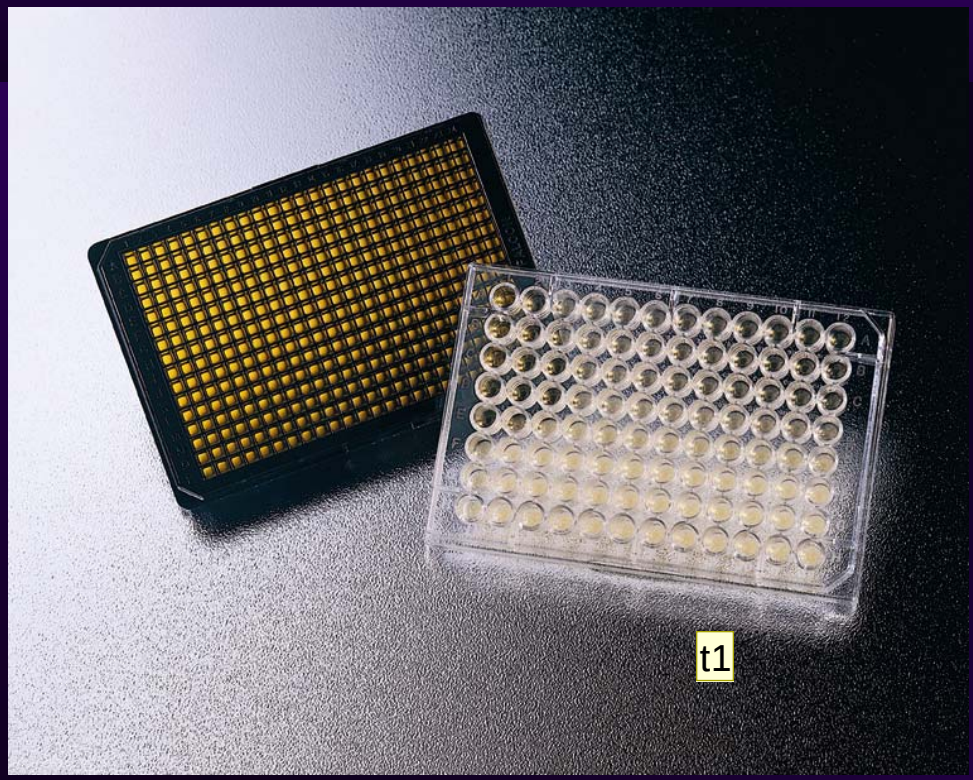


OCR Index As A Rapid Potency Metric of Isolated Islets of Langerhans

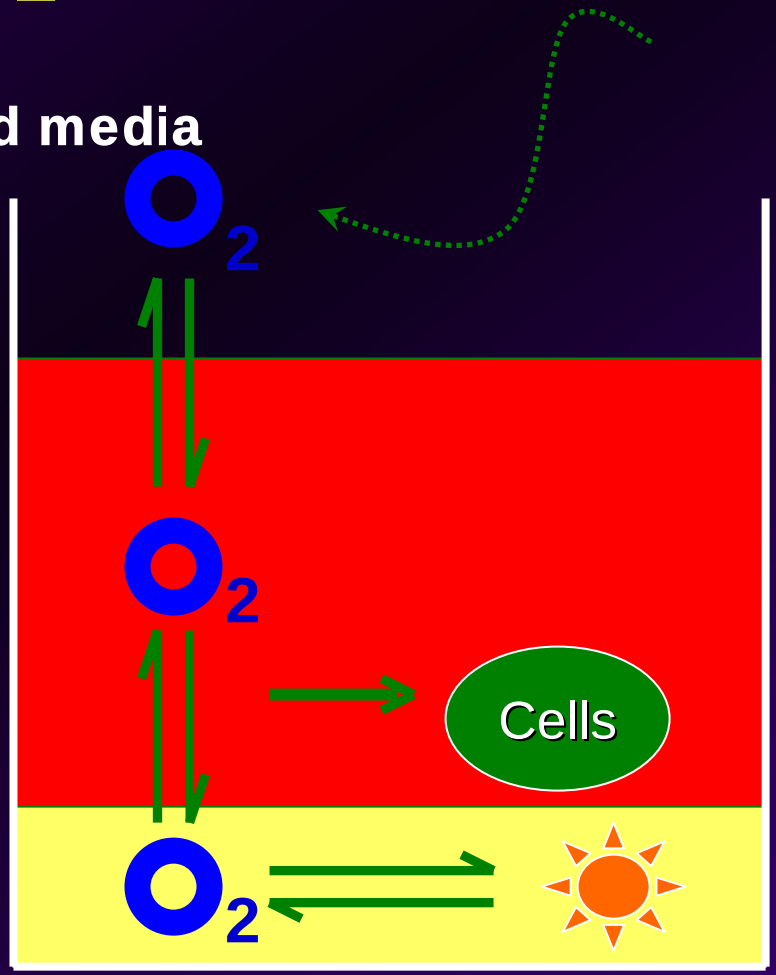
By Chris Fraker

University of Miami School of Medicine

OBS: How it works

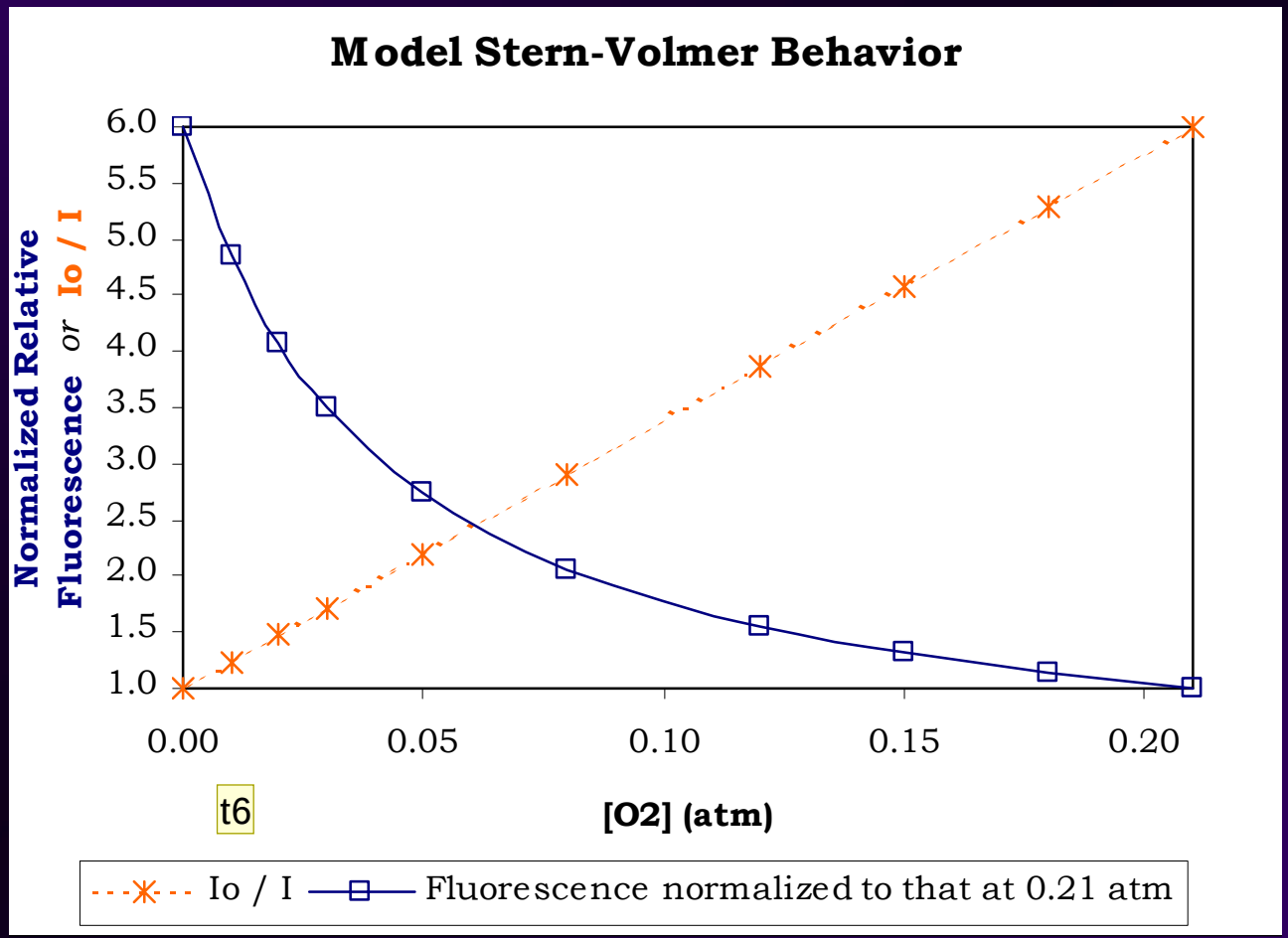
An oxygen sensitive Ruthenium-based fluorophore is adsorbed to silica particles and embedded in silicone rubber matrix

- Diffusion from air into well and media
- Consumption by cells
- Diffusion across matrix
- O_2 - quenched $Ru(DPP)_3Cl_2$



METHODS:

Oxygen concentration is calculated using modified equationsof Stern-Volmer Theory



$$I_0 / I_A = 1 + K_{SV} [O_2]_A$$

METHODS:

Oxygen concentration is calculated using modified equations of Stern-Volmer Theory

- I_0 / I_A is the dynamic range of the plate fluorescence signal, DR
- K_{SV} is the Stern Volmer constant defined as $(DR-1)/ [O_2]_A$
- Fluorescence values for each well were normalized (by division) to their initial value and then to the average value of no-cell controls at each time point (NRF)
- Ratio of $DR/NRF = I_0 / I_{NRF}$
- $DR/NRF = 1 + K_{SV} * [O_2]_{NRF}$
- So, $NRF = DR/ (1 + K_{SV} * [O_2]_{NRF})$
- Fluorescence values so-normalized were used to compute oxygen concentrations per Equation [1].

t6

t3

$$[O2]_{NRF} = \frac{DR}{NRF-1} \times \frac{1}{K_{sv}} \qquad [1]$$

Transient State dO_2 Slope and Equilibrium OCR Calculations

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Short-term (< ~1 hr)

- Quantitate based on rate of change of signal
- Transient dO_2 Slope ~ Slope of 1-1/NRF vs. time
- Ideal for Islets

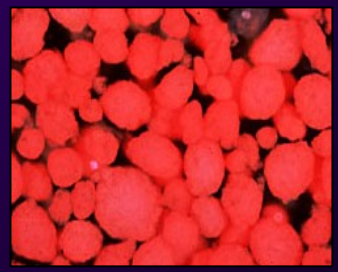
Steady State

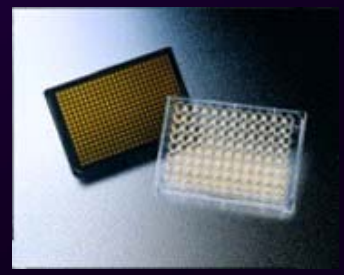
- Quantitated based on equilibrium OBS signal
- **OCR = D S L Δp / h**
  t4
- OCR ~ Actual 1-1/NRF ~ DdO_2
- Additional comparitive metric of islet potency. "True OCR"
- Time to equilibrium depends on volume of medium used.

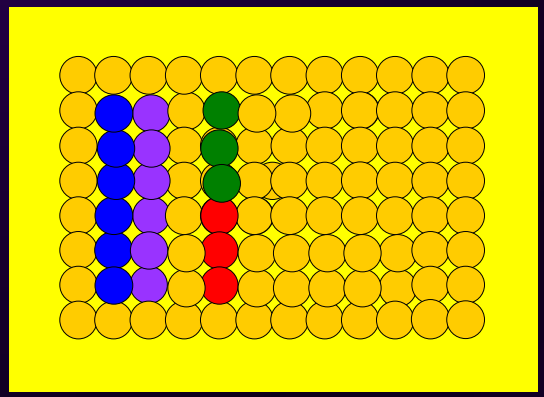
  $T = h^2/D$

D = 3.3 x 10-5 cm^2/s
S = 0.31 cm2 for a 96-well plate
h = 0.65 cm for 200 µL
L = 6.0 x 107 L/cm3 * fmol/µmol * s/min
Δp= in µM.

METHODS:
Triplicate wells of IEQs in Low (2.2mM) and High (22mM)Glucose Supplemented Krebs Buffer







Islets Low glucose

Islets High glucose

Media CNT

Sulfite CNT

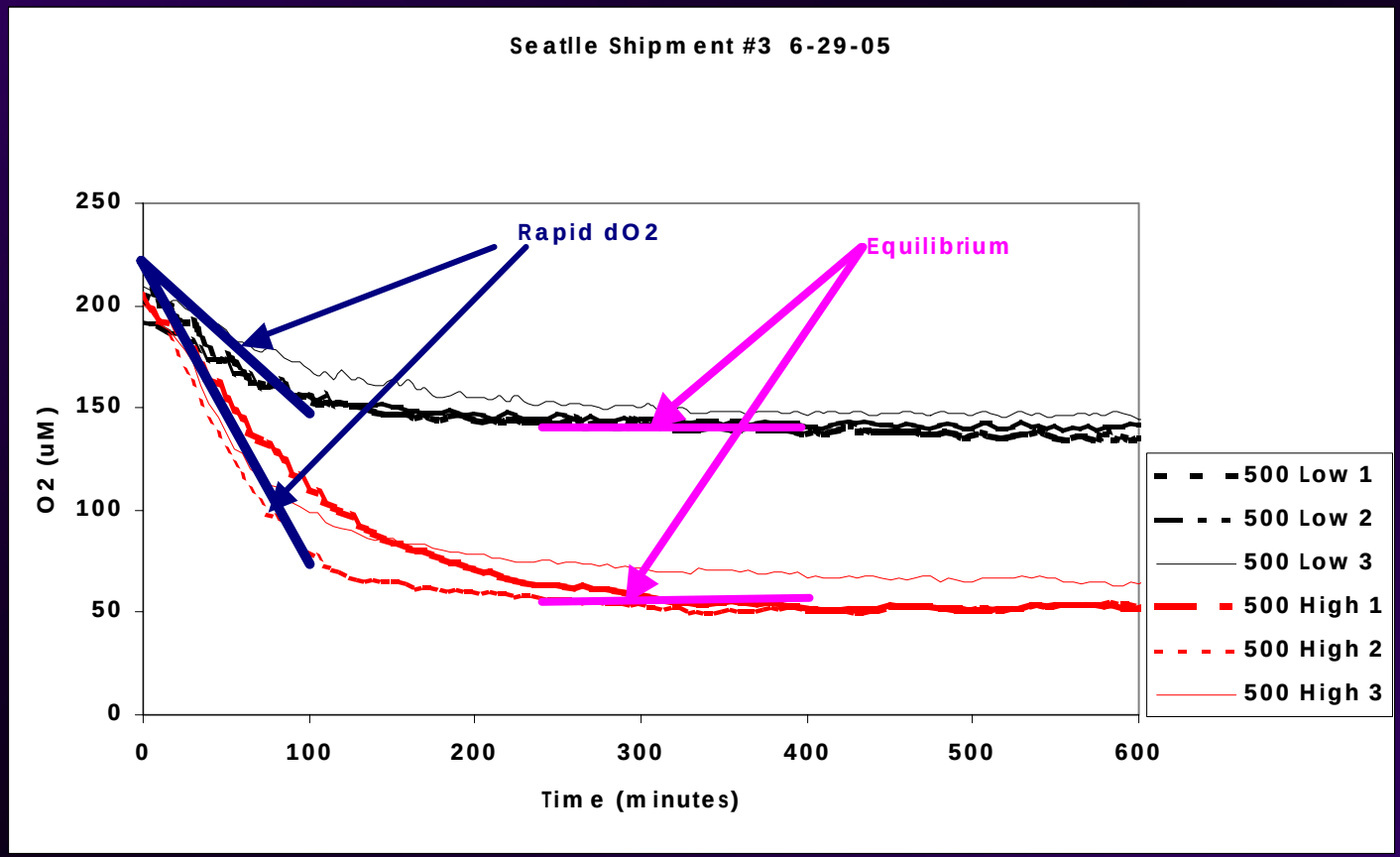
- **Measure fluorescence change for 200 cycles 5 minute intervals**

- **Calculate transient stat(t6)dO2 and equilibrium OCR and normalize for DNA content.**

t3

- **Index = Mean High glucose values/ Mean Low glucose values**

t10 Example Assessment: Islet Shipment From UPS/Seattle 6-29-05



Transient Period Index: 1.51

Equilibrium Index: 1.56

Transient period dO2 slope and Equilibrium OCR scale kinetically with cell number

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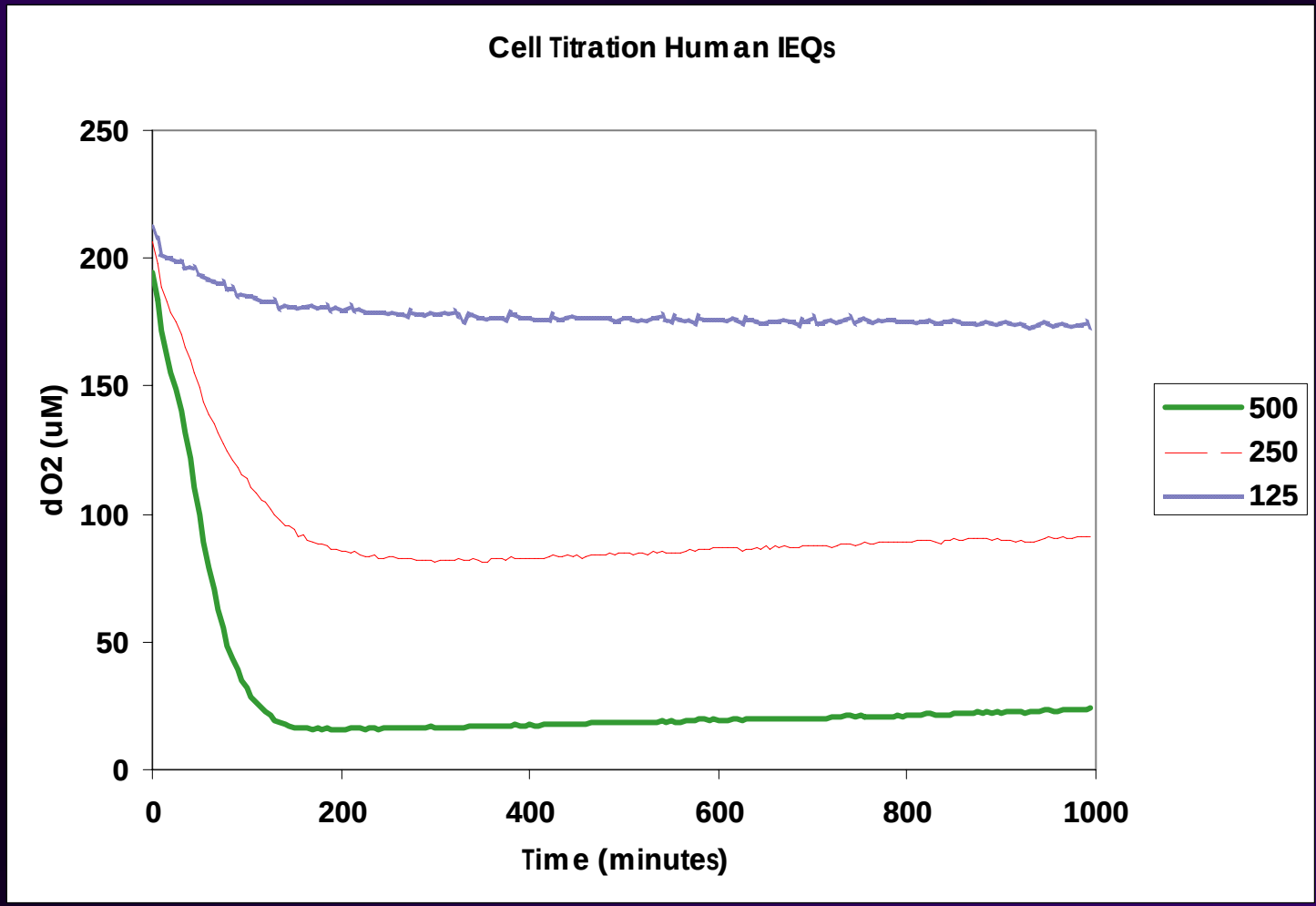
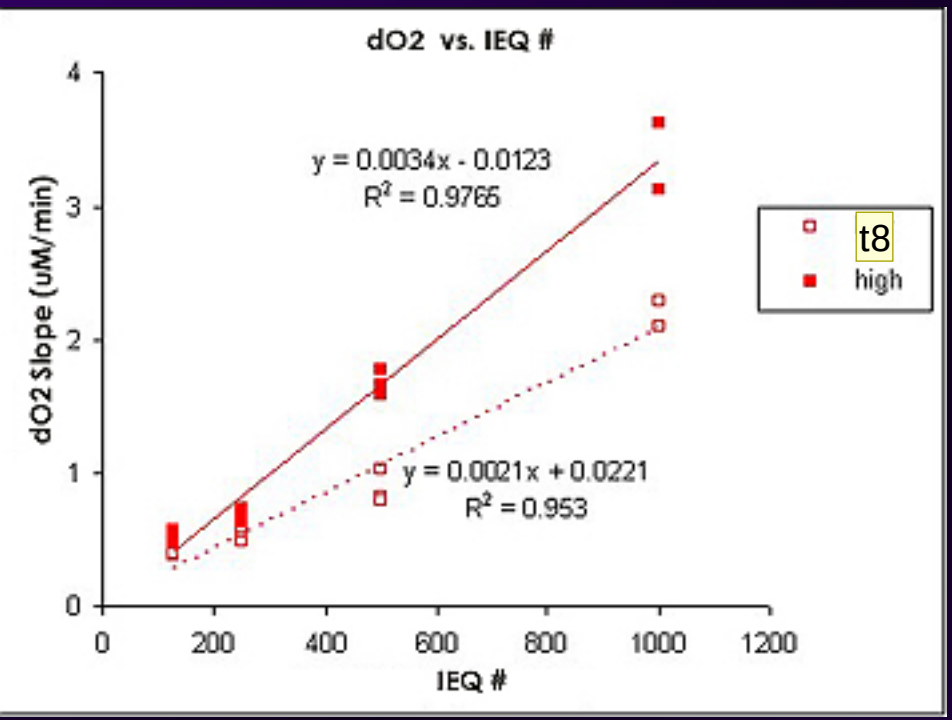
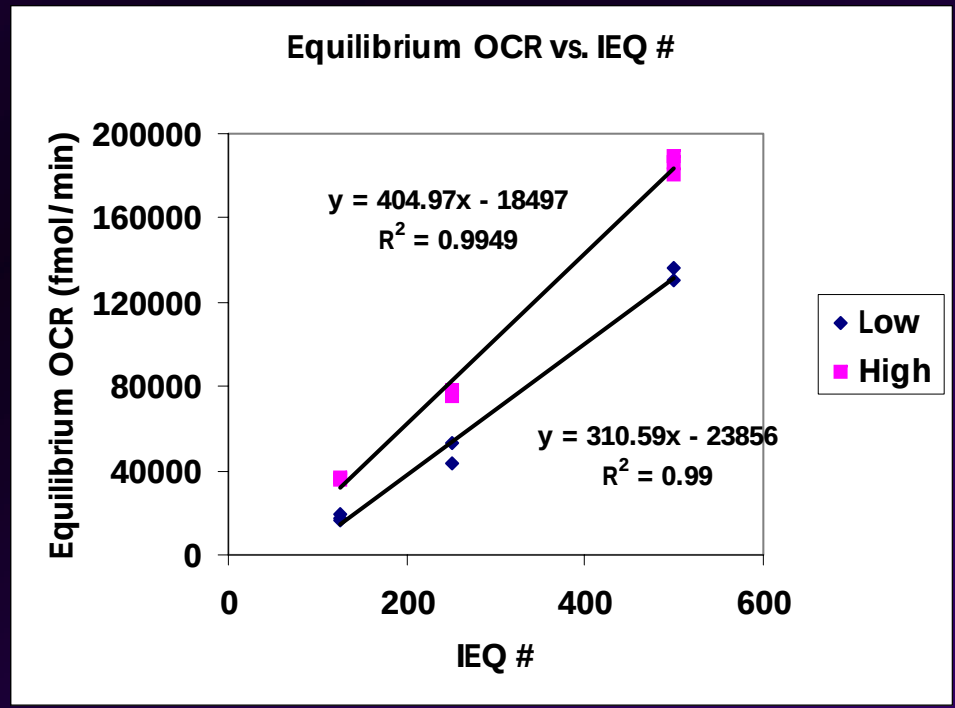


dO_2 and Equilibrium OCR Scale Linearly with IEQ





Both metrics of OCR scale with IEQ and each other

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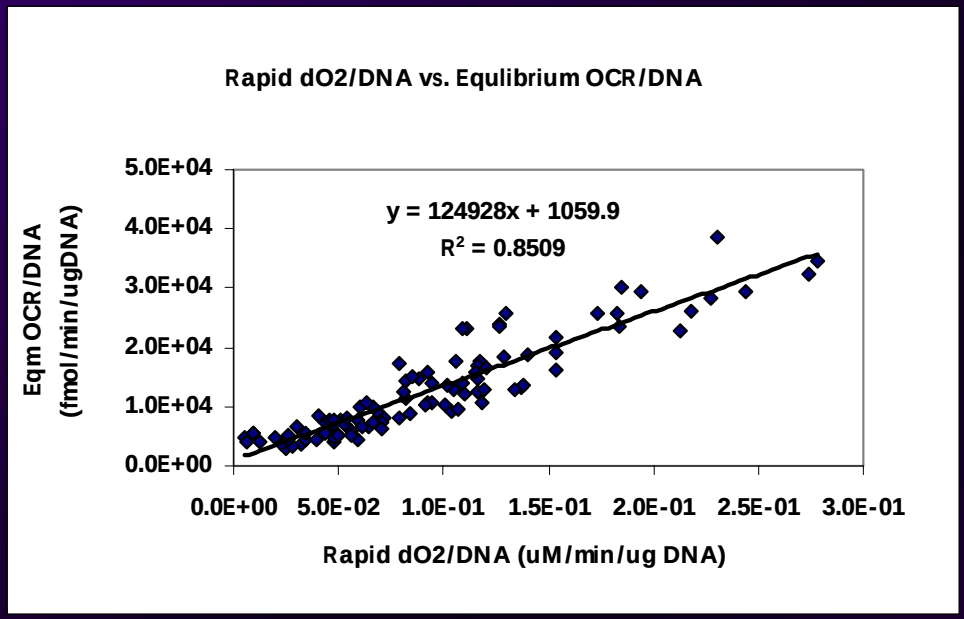
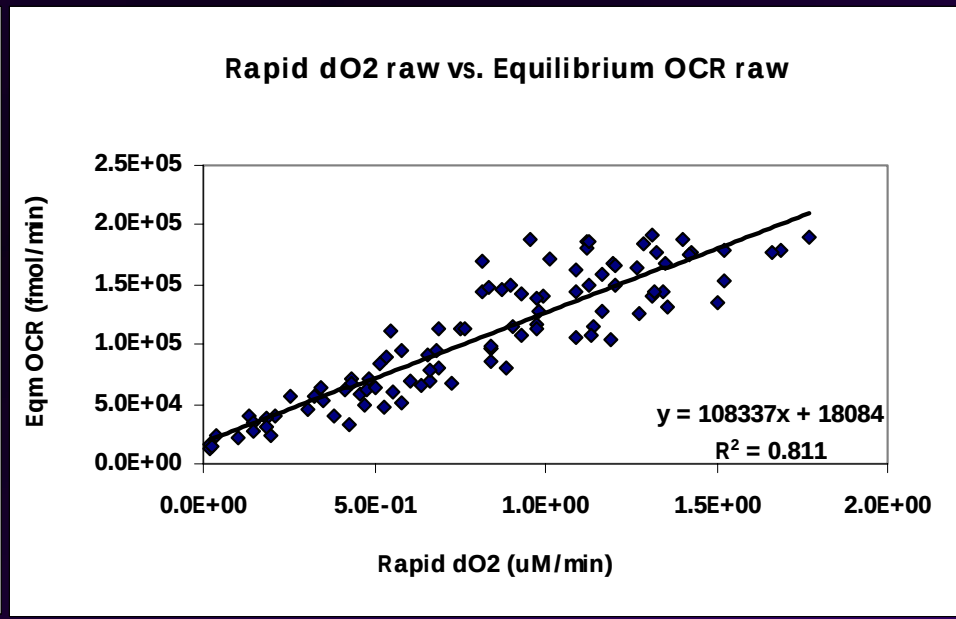
t9



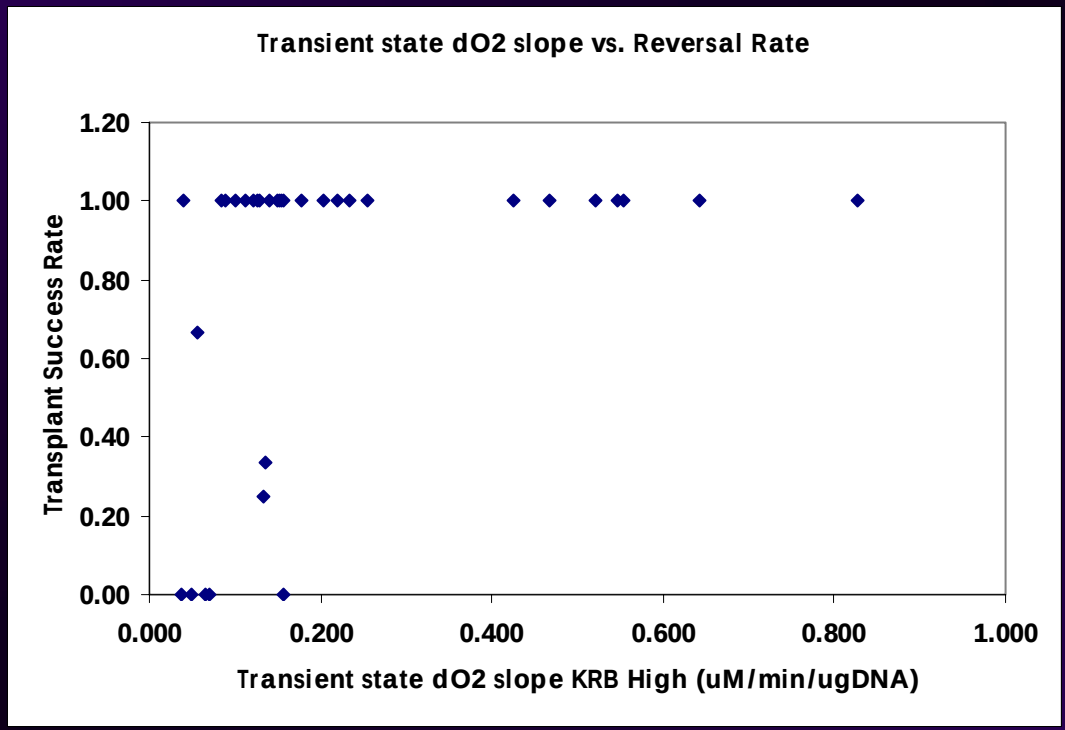


Diabetic Mouse Model for Potency

- Chemically Induced Diabetic Mice
- Islets Transplanted into Kidney Capsule
- Recipients chosen based on body weight and blood glucose profile similarities.
- 2000 IEQ per mouse
- Monitor blood glucose daily
- Cure is defined as two consecutive days < 200 mg/dl fasting blood glucose
- Days to cure used as comparative measure of potency.

OCR/DNA shows some correlation to reversal…

t13



...but OCR Index is much more predictive

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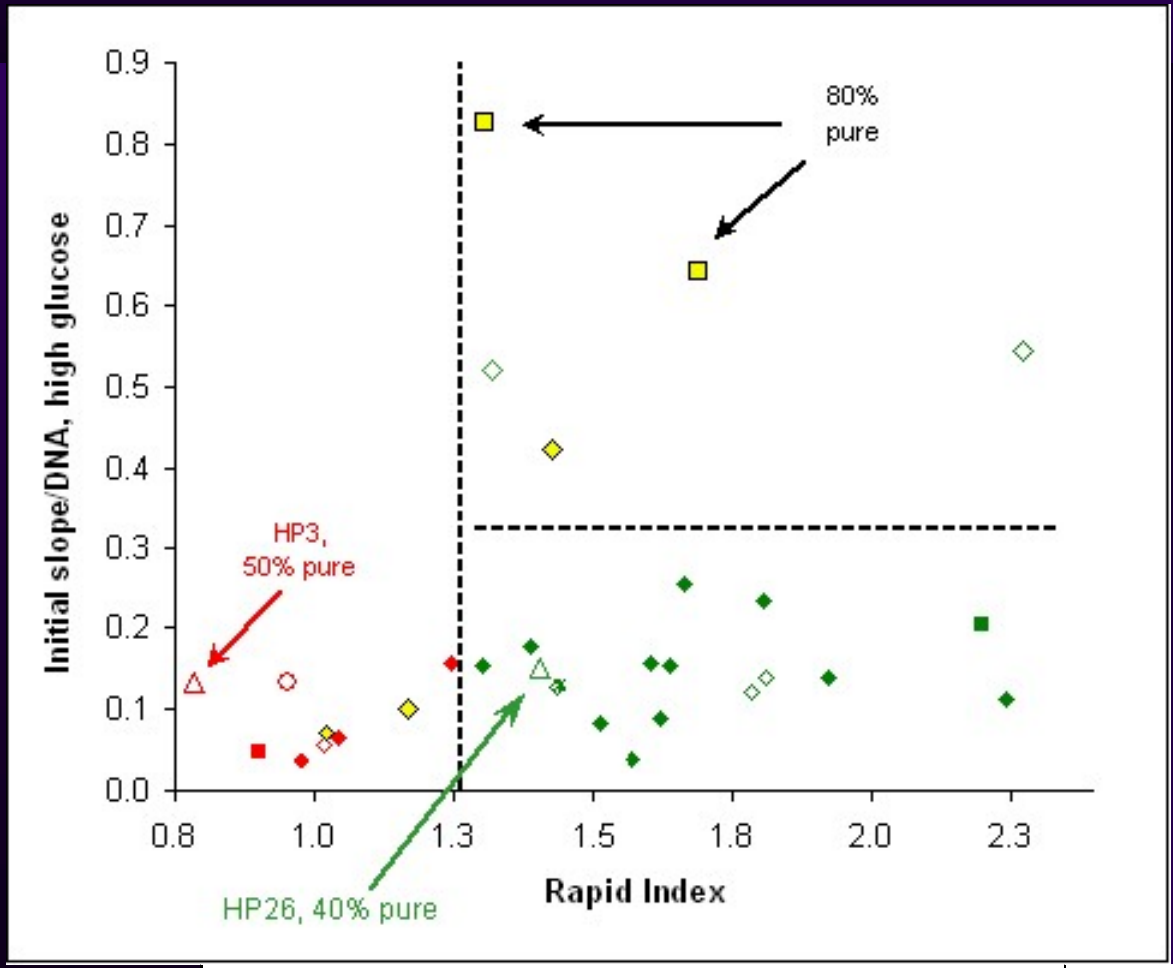
t13

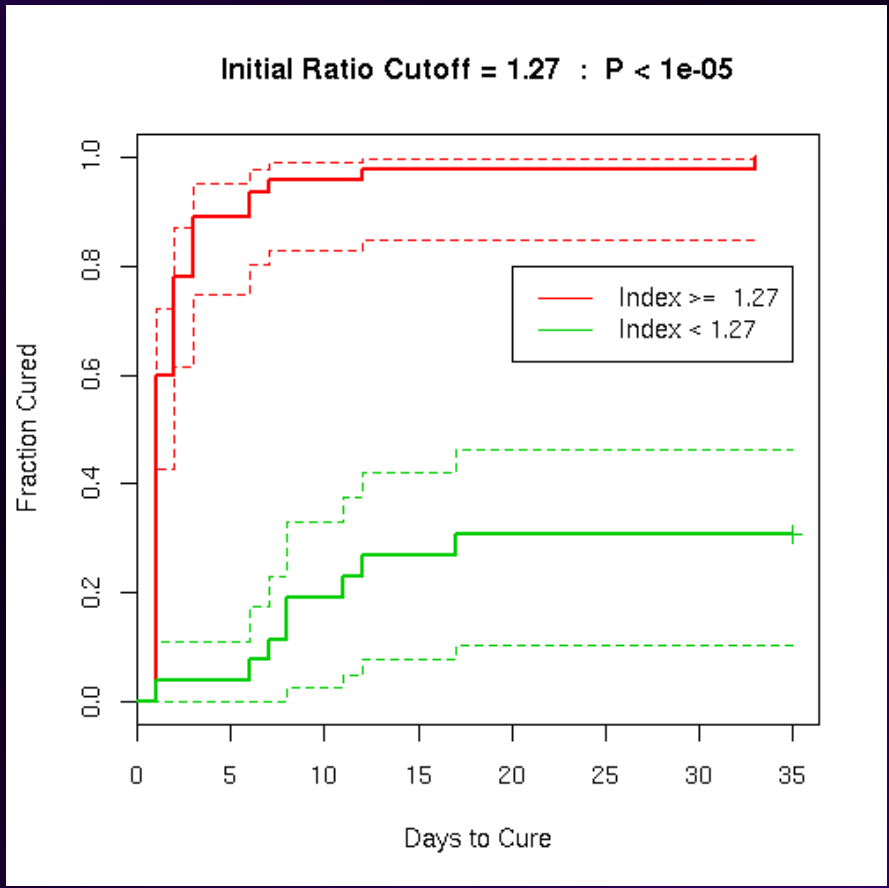


- ◆ solid red = pelleted --> all failed
- ◇ open red = not pelleted, but failed at least some replicates
- ◆ yellow = 100% reversal, but not all by day 3
- ◆ green = 100% reversal by day 3
- ◇ open green: n=1
- * asterisk = PFC

Kaplan-Meier type survival analysis.

t15

- P-values calculated using a log-rank test. P < .00001
- Top curve "cure" curve for mice with ratios > 1.27
- Bottom curve, mice with ratios < 1.27.
- Dashed lines 95% confidence intervals for the fraction cured.



In 31 human islet preparations analyzed, statistical analysis bears out that index is by far the best predictor

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t14

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