Fluorescent Polarization in Microgravity: Validation of Microplate Reader Aboard the ISS

Siobhan Malany and Stefan Vasile
Sanford-Burnham Medical Research Institute, Orlando, Florida, United States of America

The Opportunity

As recipient of The International Space Station Research Competition (ISSRC) sponsored by **Space Florida** and **NanoRacks LLC,** Team Micro-gRx had the opportunity to access the Molecular Devices M5 microplate reader on the ISS and gain payload space aboard SpaceX CRS-4 launched from Cape Canaveral on 21 September 2014. The objective of the payload was to:

- Validate fluorescence intensity (FI) and fluorescent polarization (FP) modes of the M5 reader
- Perform 384-well FP bio-affinity assays and compare results in gravity (g) vs microgravity (µg)
- Validate the UV-Visible mode in 384-well using three different absorbance wavelengths

The Florescence Polarization Payload and Protocol

Biotin (B-vitamin) binding to its antibody, α-biotin, is a stable, well established bio-affinity assay. Concentrations of mouse α-biotin (Life Tech) were added to 20nM FITC labeled biotin in buffer (PBS, 0.005% tween, pH 7.0). *For competition assays:* 200nM α-biotin was added to concentrations of D-biotin in buffer containing 20nM FITC-biotin. Total volume was 140µl in 384-well black plates (Greiner). Plates were heat sealed at 140°C with optical seals (Agilent).

- Payload included Four 384-well microtiter plates and 7 USB drives
- Duplicate sets of black and clear plates incl. for fluorescence and abs readings, respectively
- Astronaut Reid Weisman removed each plate from payload (4°C), incubated @RT for 1h
- Protocol was uploaded to M5 (Softmax Pro v5) from USB drive coded for plate and λ
- Data was downloaded from ISS Command Window to NanoRacks
- Data was compared to ground controls read on a M5 reader at Sanford Burnham

Principal of Florescence Polarization: Fluorescein (FITC) is excited with polarized light. Before emission, the FITC-molecule rotates and the polarization of emitted light differs from the excitation plane. Polarization response is measured using emission filters parallel (S) and perpendicular (P) to the excitation filter and given as mP (milli-Polarization) obtained from equation:

$$\text{Polarization (mP)} = 1000 * (S-G*P)/(S+G*P)$$

Where **S** and **P** are fluorescence counts and **G** (grating) is an instrument dependent factor and set to 1.1.

FITC-molecules are small and rotate rapidly in solution (low mP); when bound to increasing concentrations of a larger molecule, rotation slows proportionality (high mP).

The Results: ISS vs. Ground Studies

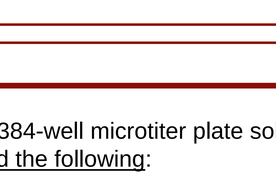
Fluorescein and rhodamine linear regression analysis (**Fig.1**)

- R^2 values 0.96-0.99 obtained in **µg** and **g** for both fluorophores
- Relative fluorescence units (RFU) comparable for both fluorophores tested in **µg**

Fluorescein milli-polarization (mP) values *(**Fig 2**).*

- Decrease trend in mP values with increasing concentrations
- Concentrations ≥ 10nM display saturating mP values.
- Average mP values comparable in **µg**

Figure 2: Fluorescence Polarization of Fluorescein



mP values for concentrations of fluorescein determined in **g** at L-84h (green) and **µg** at L+13,14d (Averages in red). A plate copy was tested in **g** at L-84h and L+14d (Averages in black). n=6 ± std. dev.

Alpha-biotin binding to FITC-biotin (Fig 3)

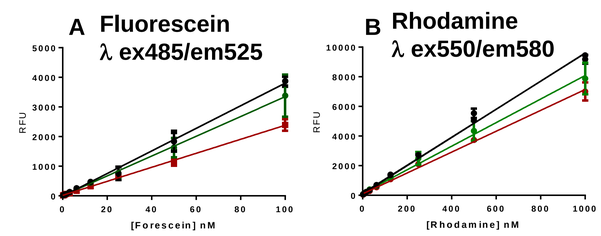
- EC_{50} = 106±7nM (ΔmP =137) and 82±13nM (ΔmP =135) in **g** pre-launch (C) and in **µg** (D), respectively
- EC_{50} = 99.5±6nM (ΔmP =125) and 86.3±5nM (ΔmP =117) for plate copy tested in **g** pre- (A) and post-launch (B), respectively.

D-biotin binding to EC_{70} α-biotin and FITC-biotin (Fig 3)

- IC_{50} = 20±7µM and 29±12µM in **g** (C) and **µg** (D), respectively.
- IC_{50} = 20±10µM and 30±10µM in g copy pre- (A) and post-launch (B), respectively.

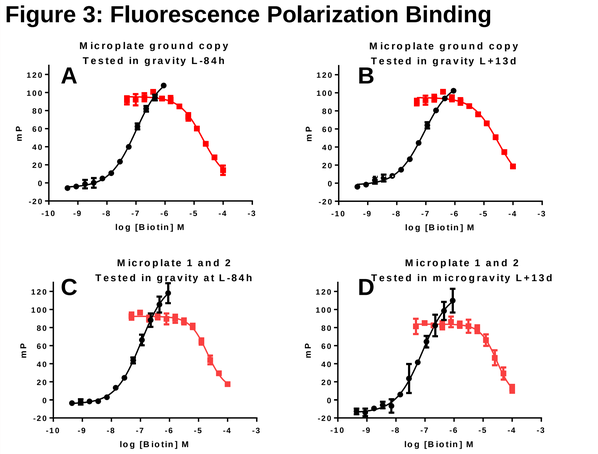
Absorbance readings performed within spec (data not shown)

Figure 1: Fluorescence Intensity



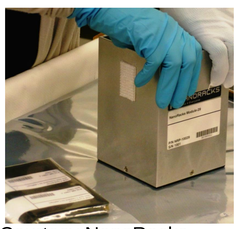
Linear-regression analysis of fluorescein (A) and rhodamine (B) concentrations determined in **g** at L-84h (green) and **µg** at L+13,14d (Averages in red). A plate copy was tested in **g** at L-84h and L+14d (Averages in black). n=6 ± std. dev.

Figure 3: Fluorescence Polarization Binding

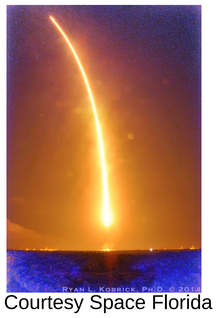


FITC-biotin binding to various concentrations of mouse α-biotin (black curve fit) and to 200nM α-biotin in the presence of various competing concentrations of D-biotin (red curve fit). n=6 ± std. dev.

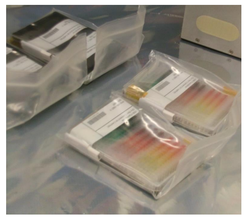
The Payload Timeline


Courtesy NanoRacks


Courtesy SpaceX


Courtesy Space Florida


Courtesy NASA


Courtesy NanoRacks

Ground Prep L-84h → NanoRacks Handoff L-80h → Hardware to NASA L-72h → Launch scrubbed → **Launch** Sept 21 1:52AM L0 → Dragon Berth L+2d → Mod29 Dragon to ISS L+3d → **Run 1 and 2** Microplate 1 L+13d → **Run 3 and 4** Microplate 2 L+14d → **Run 5 and 6** Microplate 3,4 L+38d → Data downloaded to NanoRacks

Our Sponsors

Summary

Team Micro-gRx's 384-well microtiter plate solution based experiments were a first of a kind tested on the ISS. Our payload proved the following:

- Fluorescence intensity, fluorescence polarization and absorbance λ measurements taken on the M5 reader are reproducible in microgravity.
- Binding of molecules detected by fluorescence polarization behave similarly in microgravity and rotational speed of a molecule is not dependent on microgravity.
- Provided a workflow for the M5 plate reader and established a baseline for microgravity spectroscopic analysis and use.

Fluorescence polarization 384-well assays are homogeneous, high throughput assays common in the Drug Discovery field. By transferring advanced technologies such as these to the ISS, researchers may explore biochemical and cellular pathways in multi-well format to understand how molecules and drugs may interact on a molecular level in microgravity.

http://micro-grx.com/