FLIM Analysis Template

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# Purpose

FLIM collects photon excitation information from cell images to examine metabolic changes within biologic samples. This information is Fourier transformed to collect two parameters: S-coordinate and G-coordinate. These parameters are used to calculate fraction bound (fB), which is the fraction of bound to unbound NADH within the cells we’re looking at. Fraction bound can tell you if the cells are using more glycolysis (more free NADH) or oxidative phosphorylation (more NADH is bound to an enzyme).

The purpose of this analysis is to compare the differences in fraction bound (fB) between STA treated and untreated samples.

# Z’ Factors for Fraction Bound and G-Coordinates

Here are the Z’ factors for fB and G-Coordinate across the 3 experiments. Raw values are calculated using all individual measurements for each sample. Mean values first average indiividual measurements within each Sample\_ID then calculates Z’ factors using the averaged values. This minimizes intra-sample variability.

Z’ Factor per Run (fB)

| Experiment | z\_prime\_factor |
| --- | --- |
| EITM-USC\_run1 | -0.1959437 |
| EITM-USC\_run2 | -19.6521376 |
| EITM-USC\_run3 | 0.0248862 |

Z’ Factor per Run (Average fB)

| Experiment | z\_prime\_factor |
| --- | --- |
| EITM-USC\_run1 | 0.5402899 |
| EITM-USC\_run2 | -2.2092288 |
| EITM-USC\_run3 | 0.8344722 |

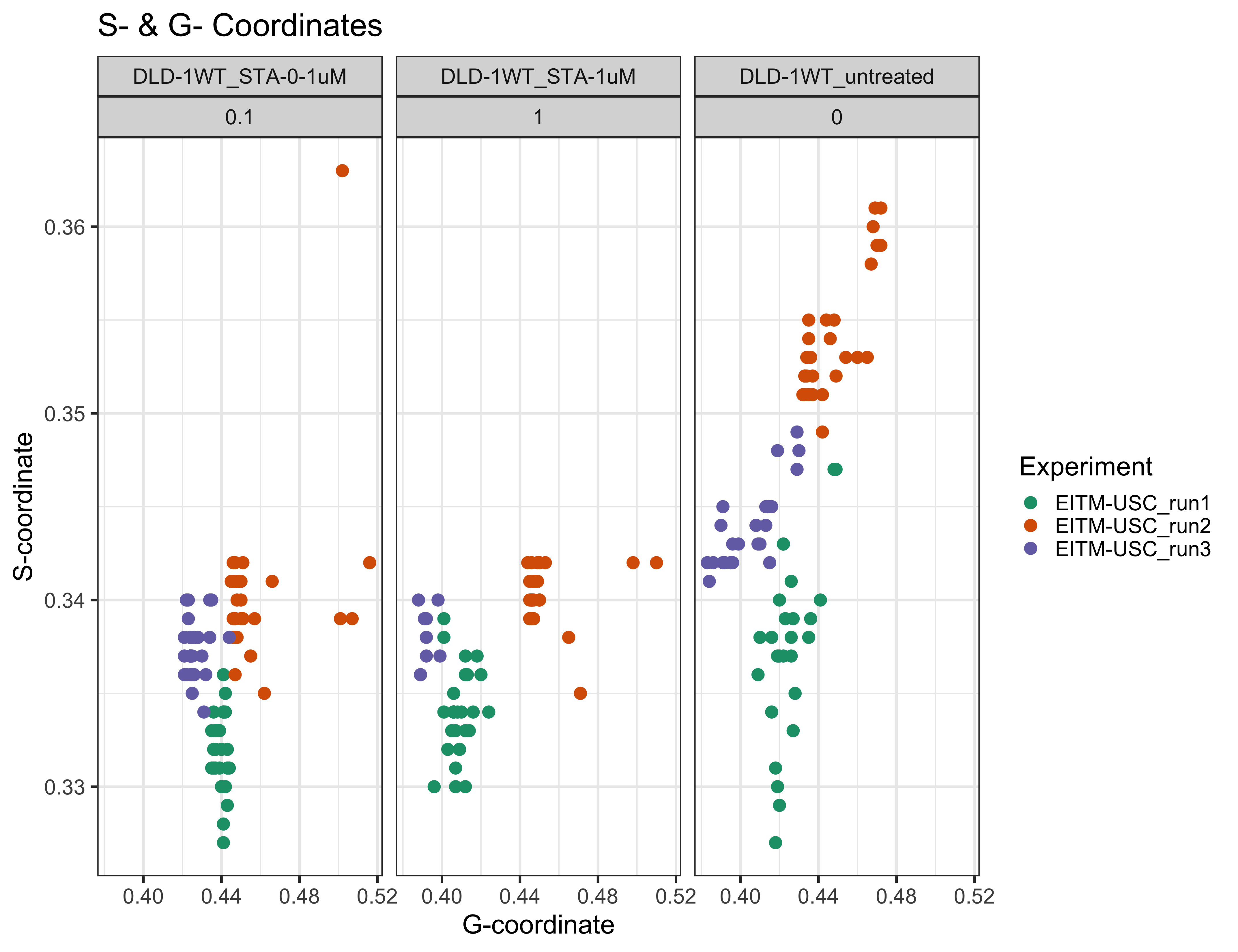
Z’ Factor per Run (G)

| Experiment | z\_prime\_factor |
| --- | --- |
| EITM-USC\_run1 | 0.1179575 |
| EITM-USC\_run2 | -19.7009225 |
| EITM-USC\_run3 | 0.1939339 |

Z’ Factor per Run (Average G)

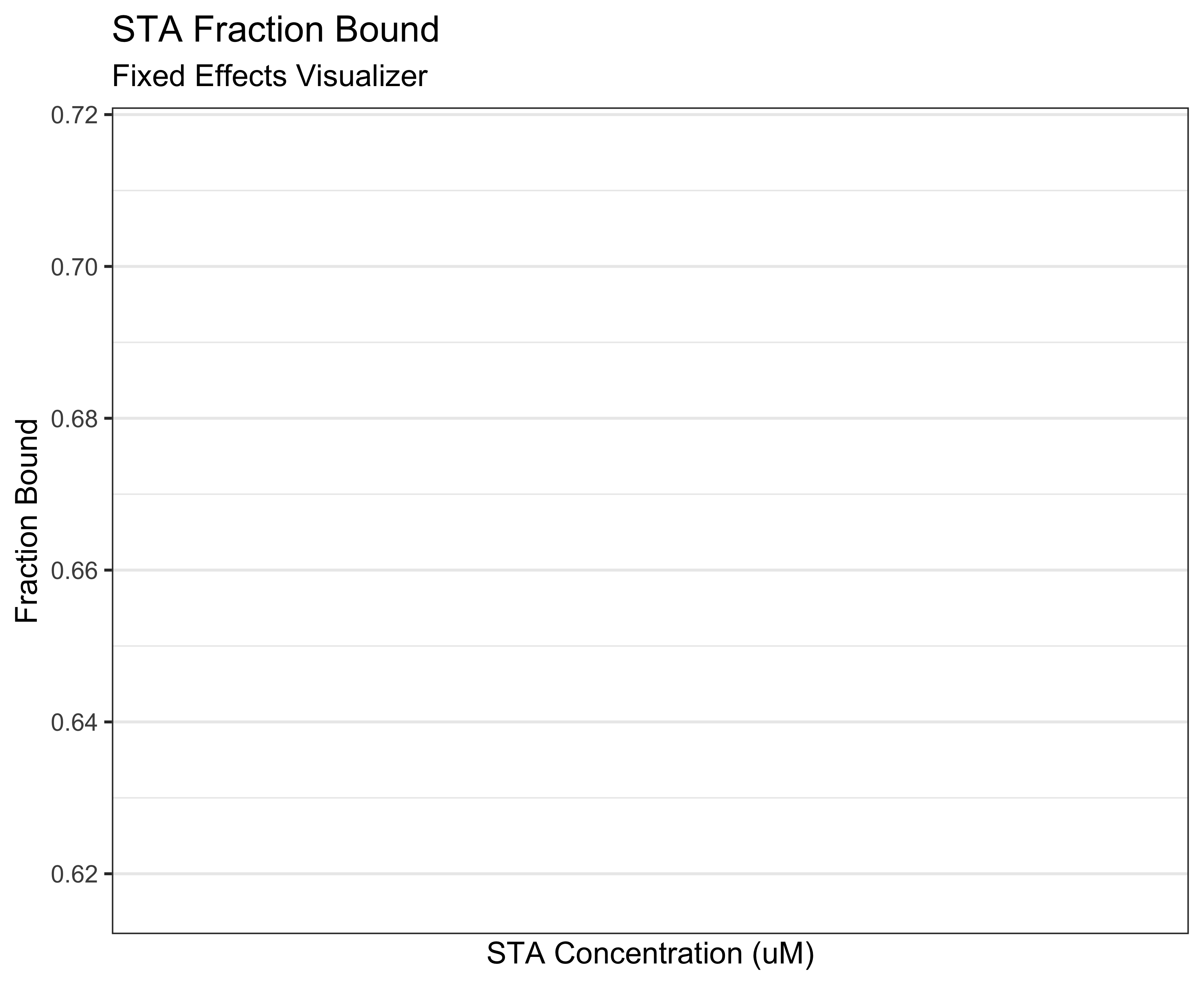
| Experiment | z\_prime\_factor |
| --- | --- |
| EITM-USC\_run1 | 0.6898841 |
| EITM-USC\_run2 | -4.4975397 |
| EITM-USC\_run3 | 0.8750364 |

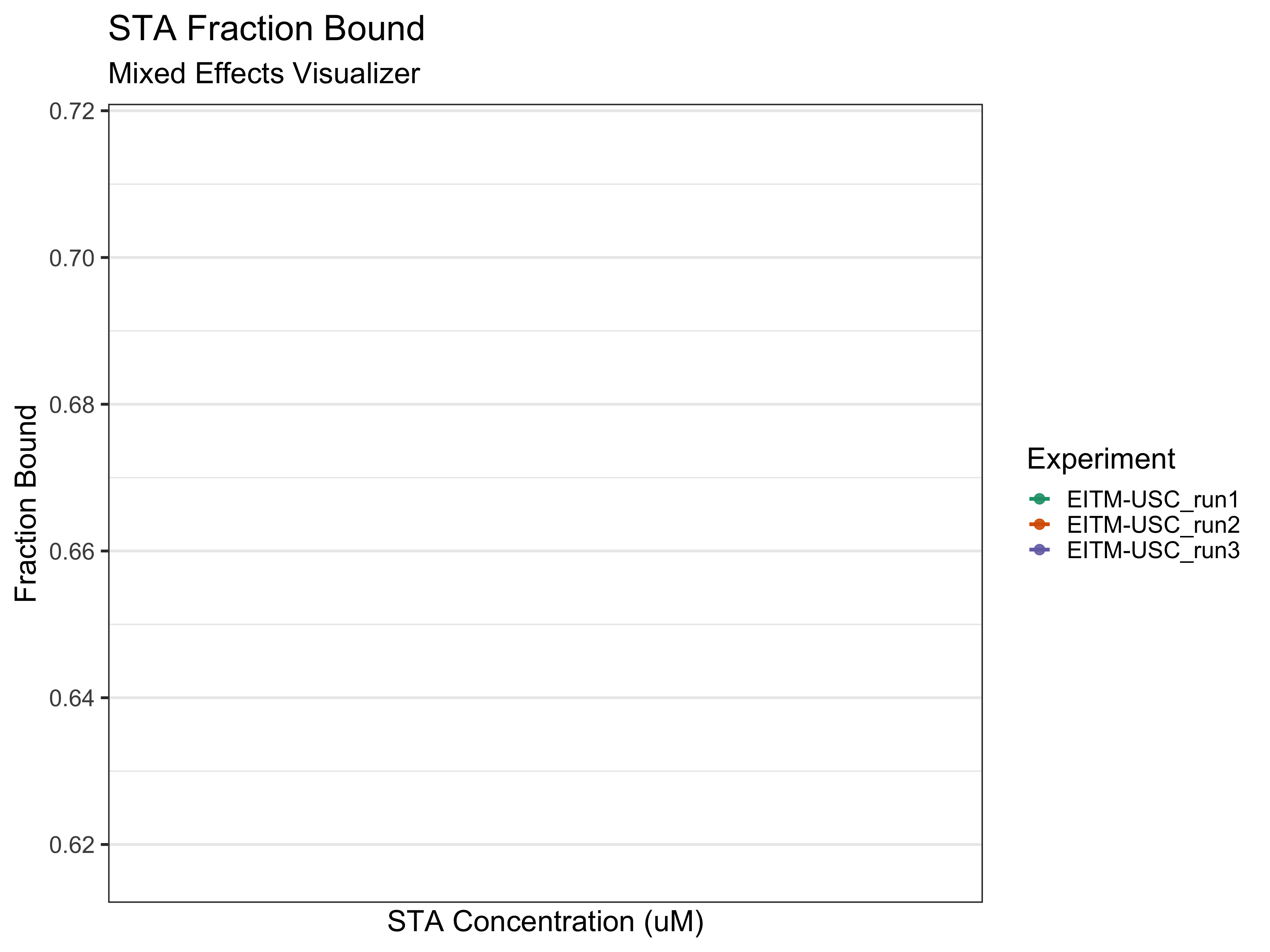
# Scatterplot of S- & G- Coordinates of STA Treatments



# Mixed Effects Models (Fraction Bound)







The mixed effect model examines the effects of STA Concentration on fraction bound, with experiment as a random variable to assess differences between experiment runs.