Background and Motivation

The background and motivation section of your report may be written in full sentence form or in concise bullet points. Here are examples of an opening paragraph that illustrates each approach.

Paragraph form:

The intracellular signaling pathways downstream of receptor tyrosine kinases (RTKs) are highly regulated, and the aberrant activation of RTK-mediated signaling can lead to increased cell proliferation, decreased apoptosis, and metastasis – all classic hallmarks of cancer (Hanahan and Weinberg, 2012). Increased activation may be attributed to elevated RTK protein expression or dysregulation of tyrosine phosphorylation due to increased kinase activity or decreased phosphatase activity. It is possible to semi-quantitatively determine the tyrosine phosphorylation level of RTKs through biochemical methods such as Western blot, immunofluorescence, or enzyme linked immunosorbent assay (ELISA). However, these techniques measure bulk levels of tyrosine phosphorylation in cell lysate, making them end-point assays. A more informative measure of RTK activity, in real time, might facilitate drug screening and therapeutic development for cancer. Here, we developed a bioluminescence resonance energy transfer (BRET) technique to monitor the binding of several known RTK-binding SH2 domains to tyrosine-phosphorylated RTKs. Our technique can be employed in a high throughput screening platform or using live cell microscopy to monitor real-time activation of RTKs when exposed to cancer therapeutics.

Bullet point form:

Measuring RTK activation is important in cancer

- Aberrant activation of receptor tyrosine kinase (RTK) signaling can lead to cancer (Hanahan and Weinberg 2012).
- Increased RTK signaling can be due to increased protein expression or dysregulated kinase or phosphatase activity.
- Quantifying RTK activation is usually done by Western blot, immunofluorescence, or enzyme linked immunosorbent assay (ELISA).
- Classic measurement techniques are end-point assays with no real-time information.
- Real-time measurement in intact cells might be important in drug development to understand kinetics of inhibitory response.
- We developed a bioluminescence resonance energy transfer (BRET) assay that can measure RTK activation in real time.

Notice that instead of a true topic sentence, the bullet point form includes a header that informs the reader of what will be included in that section. Make sure that (in both cases) your narrative progresses logically. For both examples, the second and third paragraphs (or multi-paragraph sections) would include background information about BRET and then specific information about the experiments and hypotheses tested in the module.

Data

Below is an example of a typical data page that includes figures, a figure caption and results/discussion bullet points. A reminder: in this assignment you may add more methods information to your figure caption than you might normally due to the lack of a methods section, but do not include data interpretation.

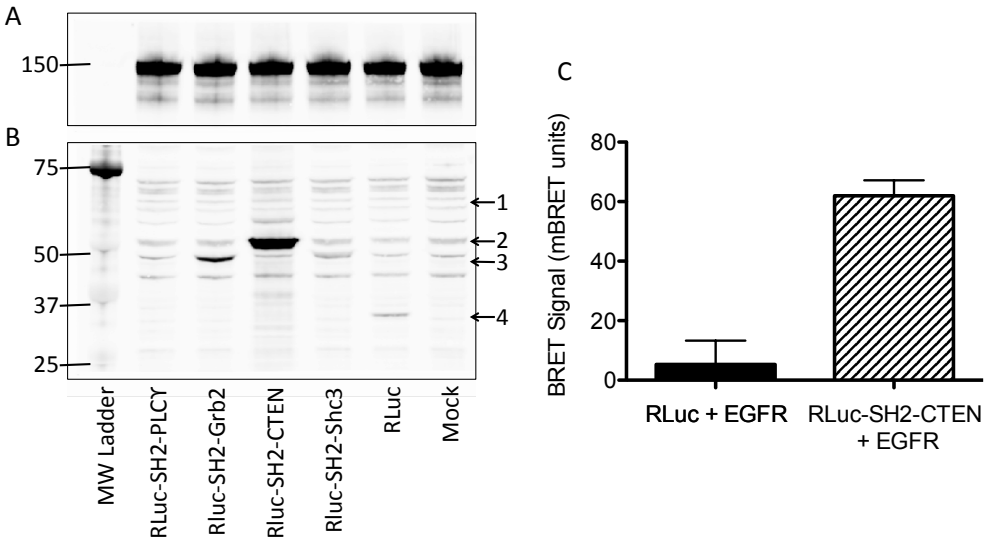

Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

Results and Discussion:

- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated by correct molecular weight band at 150 kDa (Figure 1A).
- Several protein bands are present in Mock transfection lane suggesting off-target binding of the RLuc antibody (Figure 1B).
- RLuc alone, RLuc-SH2-Grb2, and RLuc-SH2-CTEN were successfully transfected as indicated by correct molecular weight bands (Figure 1B).
- RLuc-SH2-PLCg and RLuc-SH2-Shc3 did not appear by Western blot analysis -- bands different from those in the Mock lane are not identifiable. This outcome could be due to protein expression levels below the detection limit by Western blot or to unsuccessful transfection of cDNA.

- BRET signal increased in cells transfected with Citrine-EGFR and RLuc-SH2-CTEN versus Citrine-EGFR and RLuc alone after EGF stimulation. This difference suggests that the BRET signal is specific for an SH2-EGFR interaction versus randomly localized RLuc.
- In sum, these data suggest that the RLuc-SH2 constructs can be utilized to monitor EGFR phosphorylation, as SH2 domain-EGFR association occurs only at sites of EGFR tyrosine phosphorylation. Next, we determined the dynamic range of the BRET assay.

In general, you should be able to describe more than one figure per page. Do this by combining figures in a thoughtful way into multi-panel figures. Make sure that each data page begins with an introductory statement and ends with a summary statement. The summary statement should provide a transition to the next data slide.