Bio 200C Assignment Bhumil Patel

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Review: Optimized FRET pairs and quantification approaches to detect the activation of Aurora kinase A at mitosis.

In this manuscript, Bertolin et al. improve on their original Aurora Kinase A biosensor to produce a second generation that would help follow AURKA activation in regions where it is extremely low in concentration and undetectable with the original AURKA biosensor. The authors develop two independent strategies to improve on their previous work. First, they develop a single-color AURKA biosensor for multiplex FRET and second, a method to observe and quantify FRET efficiency in areas with very low AURKA abundance. The authors show that dark acceptors ShadowG and ShadowY allow for single-color FRET/FLIM measurements while first generation tandem GFP isn't suitable due to low concentration of AURKA. They also show the inability of the original construct to measure FRET by 2c-FCCS and thus develop a novel method by replacing the donor-acceptor pair with a mTurquoise2 and novel superYFP. The experiments allowed the authors to develop guidelines when making new FRET biosensors such as characterizing the nature of the protein and making sure the conformational changes of the protein fall within the Forster's radius of the donor-acceptor pair.

The improvements to AURKA biosensors represent a novel way for studying the function of this kinase. While fluorescence anisotropy has been used in the past to study FRET in different kinases such as PKA, ERK, and cAMP, it has not been known to work with AURKA due to the nature of the protein and it's function. Also, given the fact that levels of AURKA is regulated throughout the cell cycle, the ability to detect it at low levels will help understand it's function in diverse contexts.

The authors provide good explanations with regards to the anomalies seen in their data and point out any results that deviate from their expected hypothesis. However, experiments with regards to characterizing the effects of inserting a novel superYFP on the cell and AURKA's function need to be seen. The author's also fail to provide clear explanations for discrepancies between the inactivated kinases in Fig. 1B and 1C. The author's work is systematic, giving context when constructing new strains, and provides clear explanations when talking about new methods of quantifying FRET. One thing that I did have a hard time understanding was the use of anisotropy to measure FRET, and I think the authors could have done a better job introducing the concept.

In terms of experiments that need to be done in order to further validate the results. As mentioned previously, the differences observed in Δlifetime for inactivated ShG-AURKA-mTurq2 and ShY-AURKA-mTurq2 need to be investigated or explained better. Similarly, effects of inserting flanking donor-acceptor pairs on the function of the kinase need to be quantified. It would be relevant to see how insertion of the flanking pairs affect AURKA localization to the spindle poles and morphology of the cell compared to wildtype. It would also be interesting to see if normal, non-arrested cells can function properly for multiple generations with the inserted constructs.

There are minor spelling mistakes that can be attributed to continental differences. But for the most part, the article is easy to read and well written, however, explaining the thresholds in Fig. 1 and 2 will help

the readers. As someone who is not familiar with analyzing fluorescence data, I did have a tough time understanding Fig. 3 and 4C, but the data and the author's interpretation are clear and convincing.