**Project:** ROS-specific Huntingtin Interactions

**Experiment:** Measuring huntingtin chromatin recruitment dynamics by fluorescence recovery after photobleaching (FRAP) of the YFP-tagged huntingtin-specific intrabody, nucHCB2

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**Purpose:** To test whether veliparib affects endogenous huntingtin chromatin recruitment dynamics in response to oxidative stress.

Experiment 1

*2019-03-18*

Seeded 3 x 35-mm glass-bottom dishes with 1 mL/10 mL from an 80% confluent 10-cm plate of RPE1s (p5).

*2019-03-19*

Next day, cells are 100% confluent, look healthy.

Transfection

* Replaced with fresh media
* Transfection mixture: 300 uL SFM + 3 ug nucHCB2 + 6 uL Turbofect, vortex, 15 min incubation
* 100 uL transfection mixture per plate
* Replaced with fresh media after 2 hours

*2019-03-21*

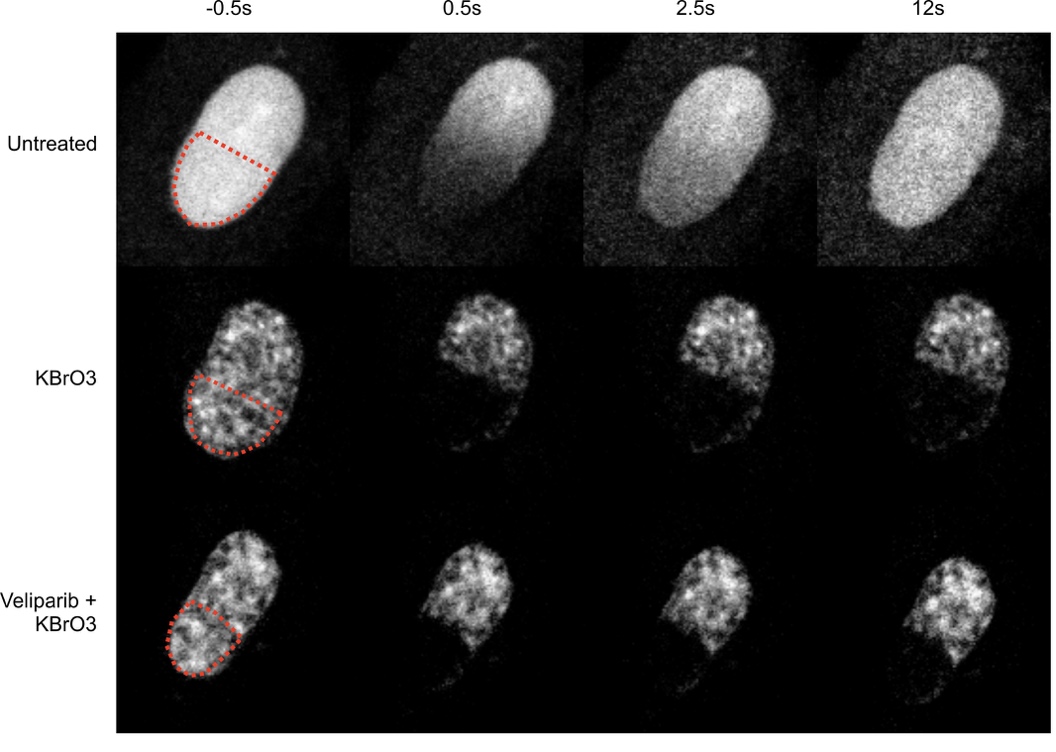
Next day, cells are healthy at dense 100% confluence with relatively low transfection efficiency.

Treatment and FRAP

* Replaced media with HBSS on untreated plate, performed FRAP experiment which took 15-20 min per plate to complete
* 100 mM KBrO3 in HBSS treatment was for 20 min plus imaging time
* 10 uM veliparib pre-treatment was 1 hour, then veliparib + KBrO3 treatment was for 20 min plus imaging time
* Performed FRAP on the Nikon A1 confocal. In 10 cells for each condition, an ROI covering half the nucleus was drawn. 5 pre-bleach images were acquired, then the region was photobleached using 50% laser power on the 489.6 nm laser with 1/2 frame speed, and images were acquired every 0.5 seconds for 20 seconds. Had to reduce resolution to 256 x 256 in order to scan fast enough
* In ImageJ, measured the intensity within the ROI for each image and subtracted the background
* For each cell, normalized the background-subtracted intensity of the ROI for each frame to the average pre-bleach intensity (fractional recovery) and plotted against time

Results

* KBrO3 really slows down the intrabody, which remains diffuse in the nuclei of untreated cells but displays a different (punctate? Nuclear speckles?) pattern in treated cells
* Unexpectedly, veliparib DECREASED huntingtin mobility. Is this related to the CO-IP result in which veliparib increased the amount of PARylated proteins pulled down by huntingtin despite decreasing overall levels of PAR?



Experiment 2

*2019-03-25*

Seeded 3 x 35-mm glass-bottom dishes with 1 mL/10 mL from a 95% confluent 10-cm plate of RPE1s (p8).

*2019-03-26*

Next day, cells are 95-100% confluent, look healthy. Transfected as above.

*2019-03-27*

Next day, cells are 100%, look healthy with better transfection efficiency than previous experiment (cell passage? Density?).

Treatment and FRAP

As in experiment 1.

Results

* Once again, KBrO3 reduced huntingtin mobility, although the effect was not as strong (higher intrabody expression levels due to better transfection efficiency? Cell passage? Density?)
* Once again, veliparib reduced huntingtin mobility even further than KBrO3 alone, however the effect was not as strong as in experiment 1

Experiment 3

*2019-03-26*

Seeded 3 x 35-mm glass-bottom dishes with 2 mL/10 mL from a 50% confluent 10-cm plate of RPE1s (p8).

*2019-03-27*

Next day, cells are 90-100% confluent, look healthy. Transfected as above.

*2019-03-28*

Next day, cells are 100%, look healthy with even better transfection efficiency than expt 2.

Treatment and FRAP

As in experiment 1.

Results

* This time, KBrO3 effect was even less than expt 2 (therefore KBrO3 effect variability not due to intrabody expression levels), but veliparib effect was stronger than expt 2

# Experiment 4

*2019-04-01*

Seeded 3 x 35-mm glass-bottom dishes with 1 mL/10 mL from a 95% confluent 10-cm plate of RPE1s (p11).

*2019-04-02*

Next day, cells are 90-100% confluent, look healthy. Transfected as above.

*2019-04-03*

Next day, cells are 100%, look healthy with good transfection efficiency. NucHCB2 localization seems a bit more cytoplasmic than usual (not as many cells have strictly nuclear localization).

## Treatment and FRAP

## As in experiment 1.

## Results

## This time, KBrO3 effect was stronger than experiments 1 and 2, but veliparib had very little effect at all. Cell passage too high? NucHCB2 localization was more cytoplasmic than usual, maybe having less nuclear huntingtin lessens the effect of veliparib?

# Experiment 5

*2019-04-02*

Seeded 3 x 35-mm glass-bottom dishes with 1 mL/10 mL from a 95% confluent 10-cm plate of RPE1s (p12).

*2019-04-02*

Next day, cells are 90-100% confluent, look healthy. Transfected as above.

*2019-04-03*

Next day, cells are 100%, look healthy with good transfection efficiency. Once again, nucHCB2 localization seems more cytoplasmic than usual.

## Treatment and FRAP

## As in experiment 1.

## Results

## This time, KBrO3 effect was not very strong and veliparib effect was negligible

## Conclusions

* KBrO3 reduces huntingtin mobility as would be expected, however the effect is highly variable from experiment to experiment. This is not surprising as KBrO3 shows high degrees of variability in other experiments as well (effect on PAR levels, etc)
* Veliparib treatment was expected to increase huntingtin mobility if huntingtin uses PAR binding to recruit to chromatin. Unexpectedly, veliparib treatment decreased huntingtin mobility. This could be related to the increased binding of huntingtin to PARylated proteins upon veliparib treatment as measured by CO-IP (see Zenodo entry <https://zenodo.org/record/2586924#.XS4P3pNKh-U>)
* The veliparib effect appeared to be lost in experiments 4 and 5. The cells were high passage and the intrabody displayed a more cytoplasmic pattern than usual

Normalized values from 5 experiments

The effect of KBrO3 on huntingtin mobility was variable from experiment to experiment (relative to the untreated condition). Combining the values from 5 experiments would cause the KBrO3 and veliparib + KBrO3 values to appear to overlap. It is therefore useful to normalize the values from each experiment to the KBrO3 condition to visualize the effect of veliparib. All values were normalized to the KBrO3 t = 20s value for each experiment. Error bars = SEM.