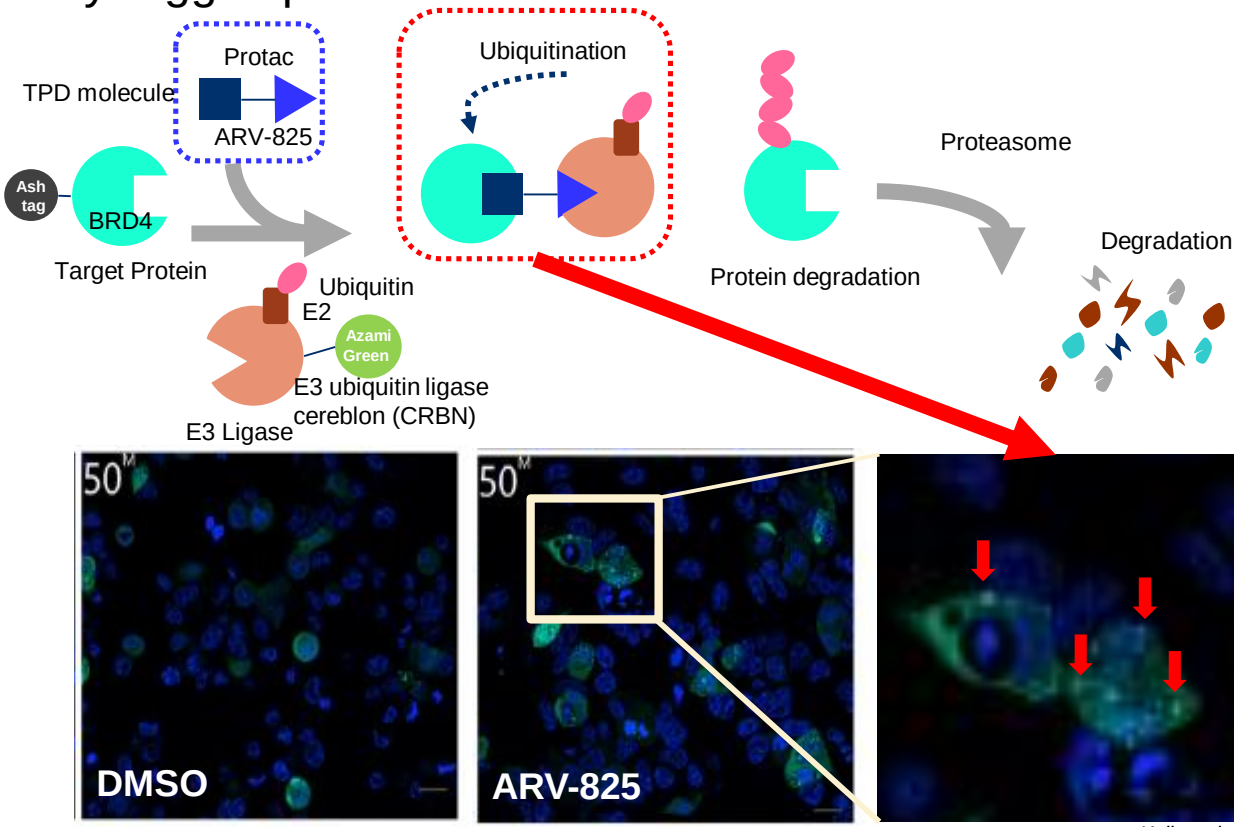
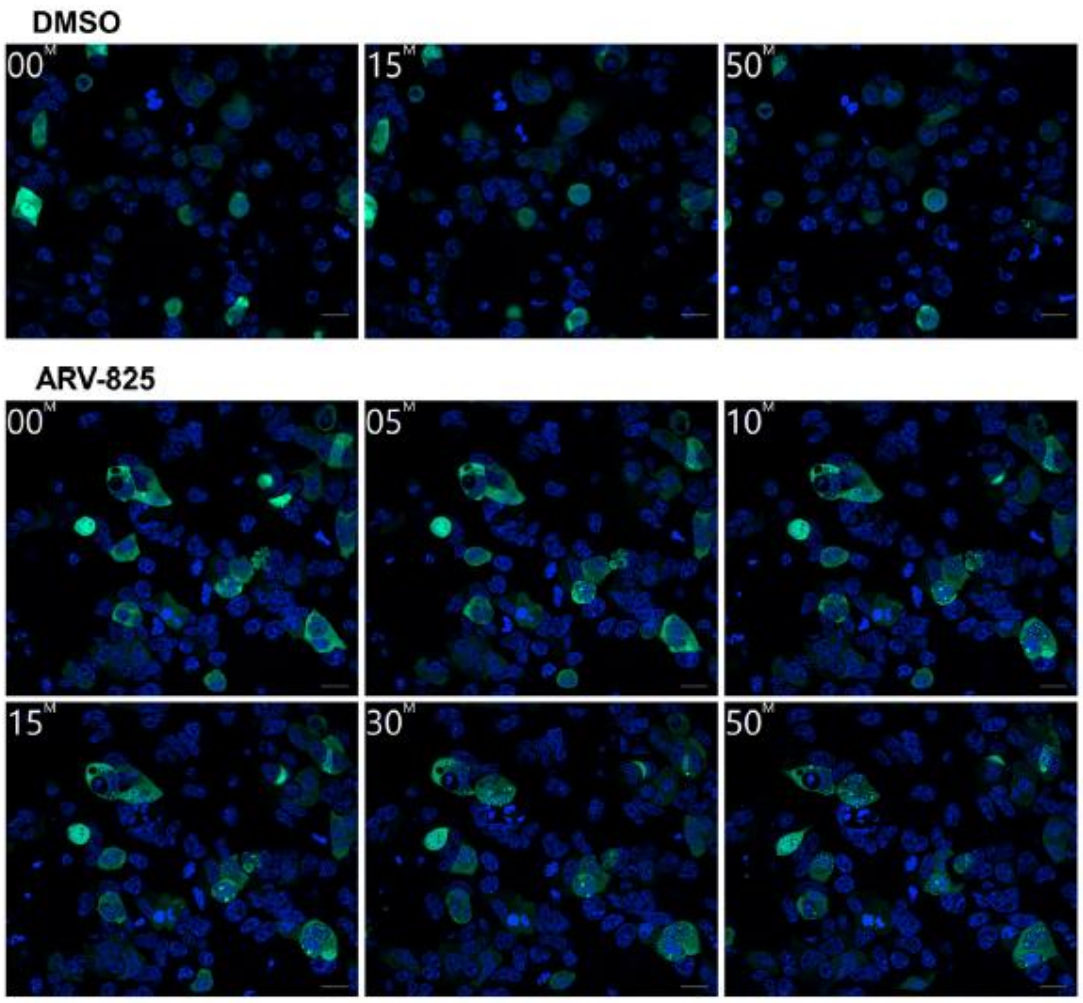
Real time imaging of TPD foci formation in live cells

- TPD (targeted protein degradation) molecules form ternary complex with target proteins and E3 ligase, inducing the ubiquitin-dependent target degradation. TPD technology is attracting considerable attentions as a new therapeutic strategy.
- Fluoppi system visualizes the formation of protein complex as local foci of fluorescently-tagged proteins.

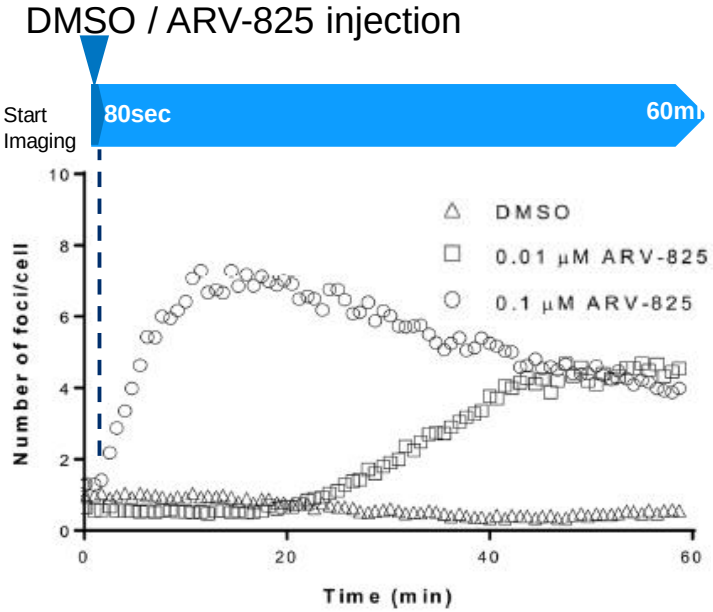


Kaji et al., Sci Rep.10, Article number: 3088 (2020)

Real time imaging of TPD foci formation in live cells



Blue; Hoechst (nucleus)
Green; AzamiGreen (CRBN)



Early foci formation changes according to time course.

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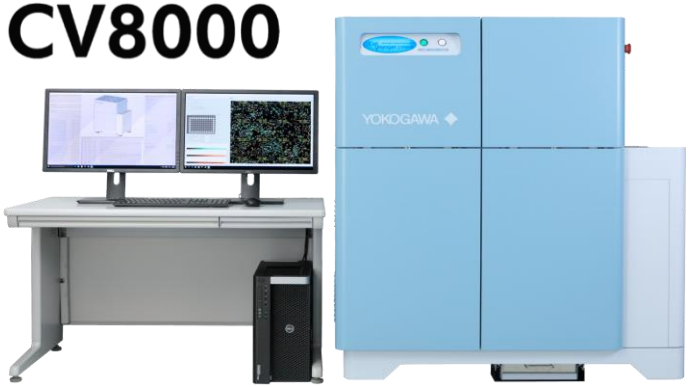
Real time imaging of TPD foci formation in live cells

High Content Screening System

CellVoyager CV8000



Experiment

Materials

- Cell Line: 293A cells
- TPD Molecule: ARV-825
- Nucleolus marker: Hoechst 33342
- Fluoppi system: AzamiGreen-tagged CRBN and Ash (Assembly Helper)-tagged BRD4

Imaging and analysis

- Magnification: 60x Water immersion lens
- Excitation wave length: 405nm, 488nm
- Field of View: 3 field of view per well
- Time lapse: every 45 sec for 1 hour
- TPD molecule injection: built in robot pipetter
- Analysis: count the TPD foci every time point by CellPathfinder.

Reference : . Tomohiro Kaji, Hiroshi Koga, Mutsumi Kuroha, Toshihiko Akimoto & Kenji Hayata . *Characterization of cereblondependent targeted protein degrader by visualizing the spatiotemporal ternary complex formation in cells*. Scientific Reports volume 10, Article number: 3088 (2020)

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