

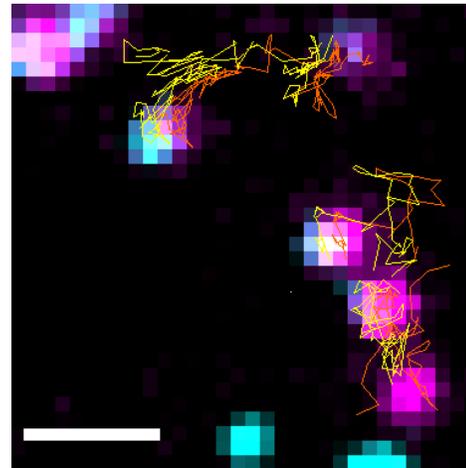
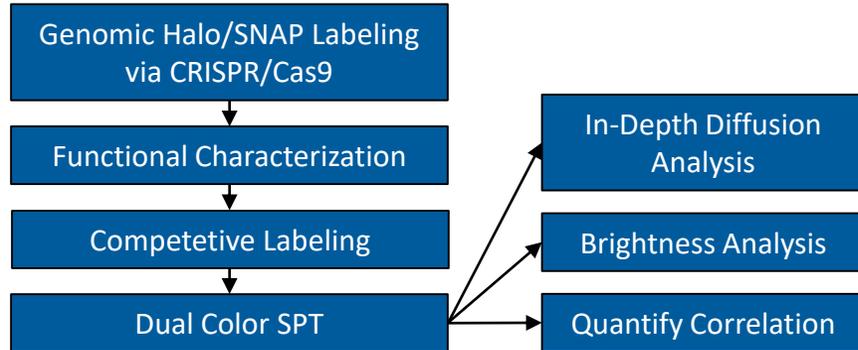
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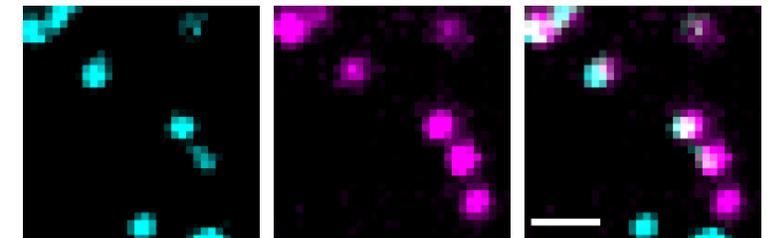
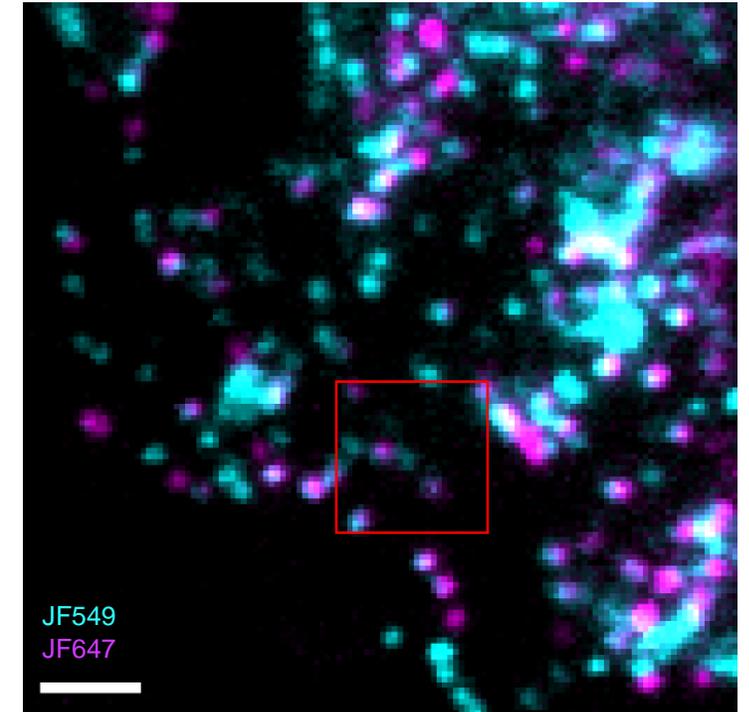
Introduction

Endoplasmic Reticulum associated degradation (ERAD) mediates degradation of misfolded proteins in the ER. A central protein complex in ERAD is the Ubiquitin E3-Ligase-complex Hrd1; a multi-protein complex formed around the name giving subunit Hrd1. During ERAD the Hrd1 complex is responsible for protein dislocation from the ER-lumen to the cytosol.

While Hrd1 has been subject to excessive biochemical and structural investigation, how it transports its cargo still remains disputed. Here we propose a dual color-single particle tracking approach that allows quantification of homo- and hetero protein-protein interactions in their native environment. We explore the architecture and the dynamics of the Hrd1-complex and directly observe its interaction with its cargo.

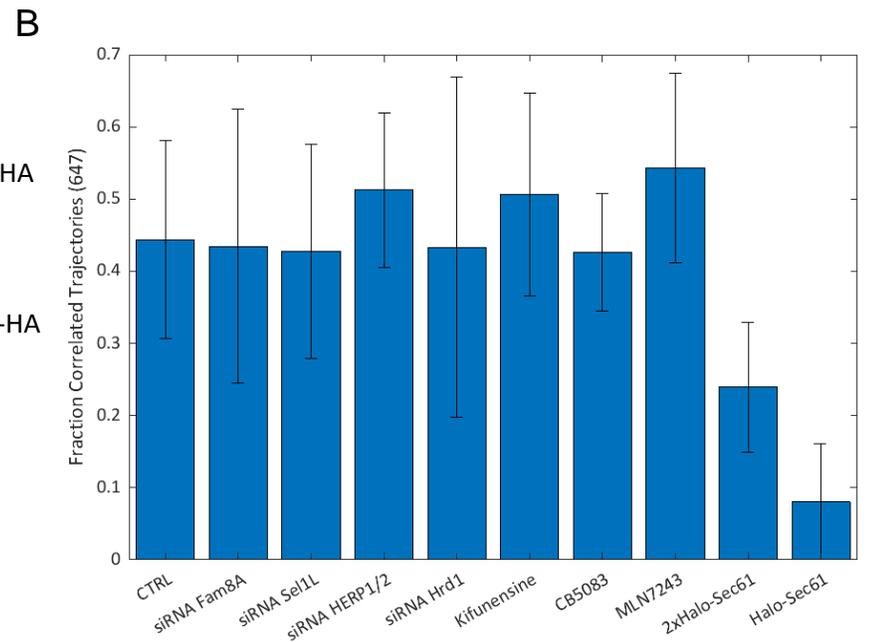
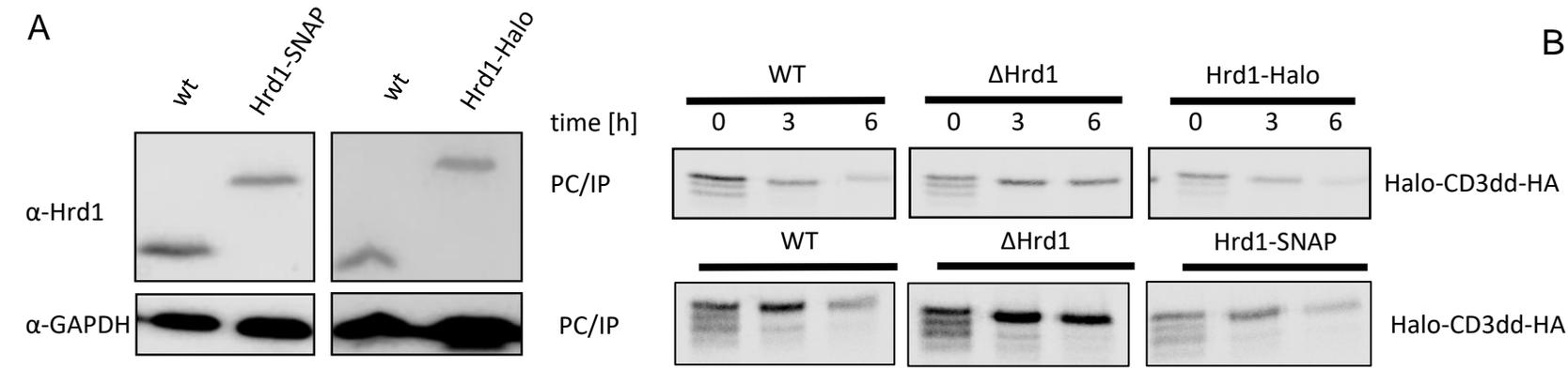
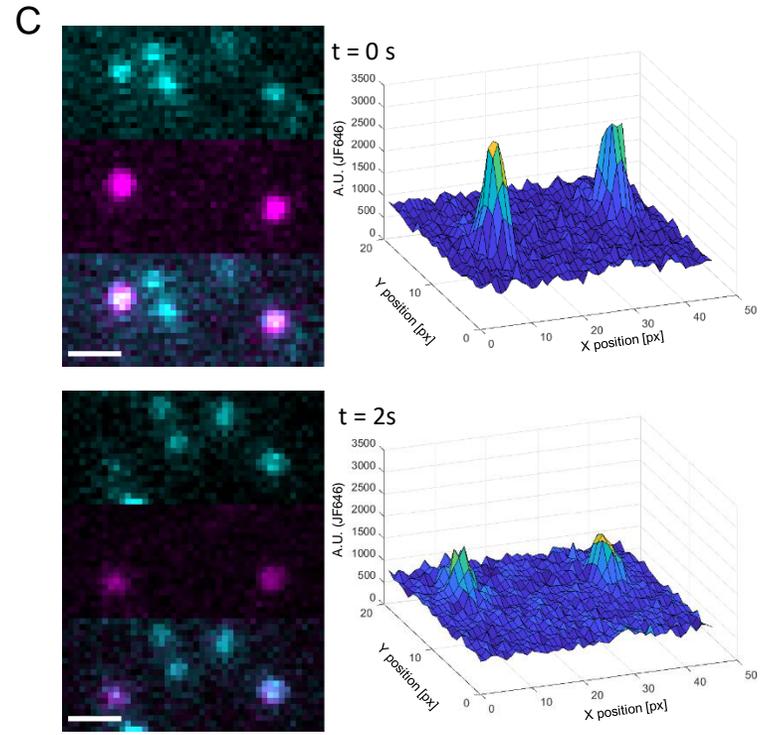


Correlative SPT of endogenous Hrd1-Halo in U2OS cells. Mixed-dye (JF549/JF646) labeling allows direct quantification of protein-protein interaction. Scalebar 1/2 μm .



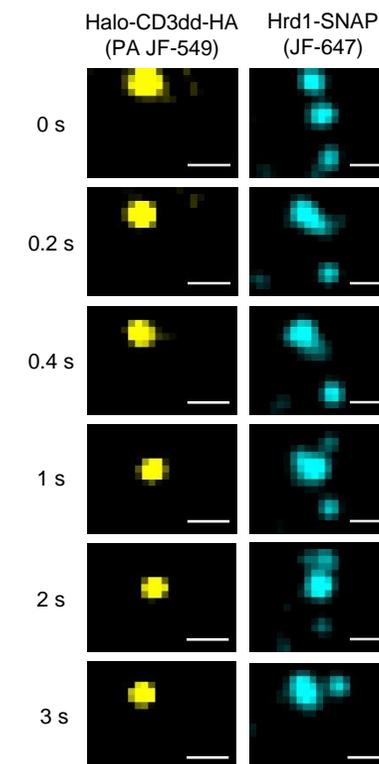
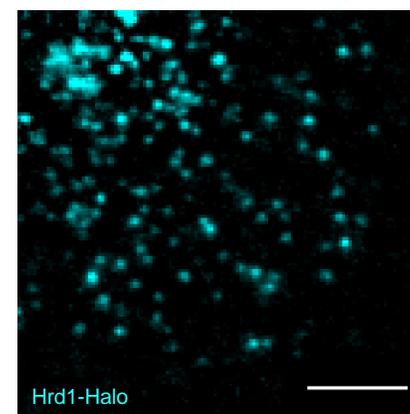
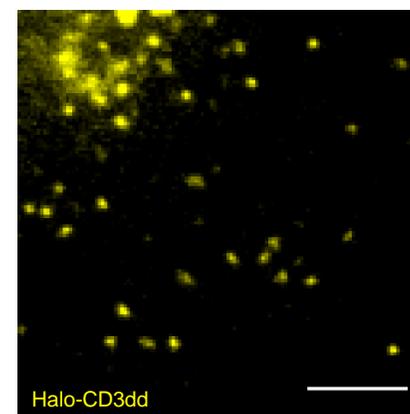
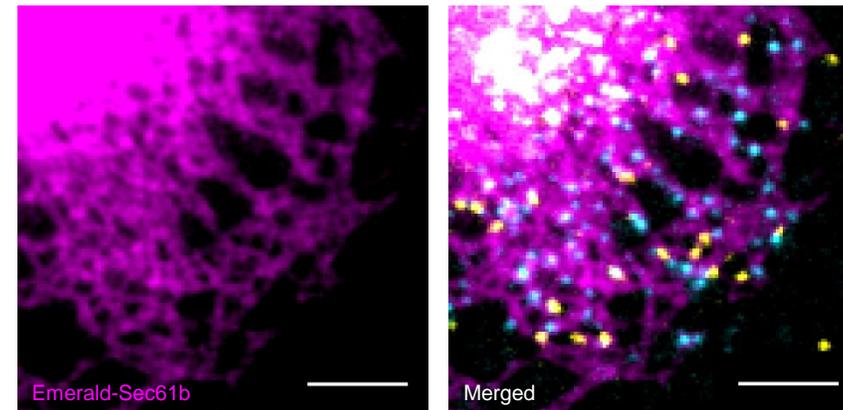
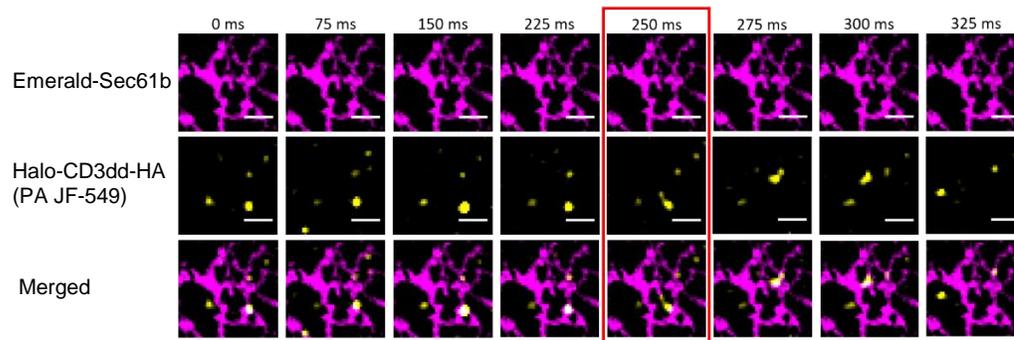
Results

- CRISPR/Cas9 mediated “Knock-In” of either the SNAP- or the Halo-Tag results in a functional variant of Hrd1 (Figure A).
- Simultaneous labeling with two different Halo-ligands enables dual-color single particle tracking of Hrd1 (Slide 1). The low endogenous expression gives SM resolution without tricks.
- Correlated particle movement is robustly identified by direct correlation of Hrd1-trajectories, which allows quantification of Hrd1-oligomerization (Figure B).
- Comparing the degree of correlation of Hrd1 with a dimeric reference construct hints towards more than two Hrd1 copies per complex. This is further supported by the occurrence of bleaching steps in correlated trajectories (Figure C).



(A) Hrd1-Halo/SNAP cell lines generated by CRISPR/Cas9 mediated HDR. Degradation of an Halo-tagged model-substrate is followed via radio-active pulse-chase assays and confirms the functionality of the fusion constructs. (B) Fraction of correlated Hrd1-trajectories in dependence on siRNA mediated Knock-downs or ERAD Inhibitors. Hrd1 alone is sufficient for homo-oligomerization and correlates stronger than a 2xHalo tandem. (C) Two correlated trajectories show a bleaching step indicative of at least 3 Hrd1-copies. Shown is the raw data as well as a 3D intensity plot. Scalebar 1 μ m.

(Left) Using PALM, single molecules can be observed dissociating from the ER. Scalebar 1 μ m. (Right) Dual-color imaging allows direct visualization of Hrd1-Substrate interaction. Scalebar 1/5 μ m.



Results

- Using Dual color SPT we directly observe the interaction between the Hrd1-dependent ERAD-substrate Halo-CD3dd and Hrd1-SNAP. Unlike previously described, the interaction occurs throughout the peripheral ER
- Single Molecules can be observed dissociating from the ER

Future Perspective

- Observe Halo-CD3dd ER dissociation after correlated movement with Hrd1
- Combine correlation analysis with step-wise bleaching analysis
- Test approach on a dynamically assembled ER-resident protein complex (IRE1)
- Expand set of calibration constructs
- Estimate labeling-efficiency using Halo-GFP fusion constructs
- Evaluate the correlation algorithm using simulated data

