Molecular architecture of clathrin plaques and pits defined by super-resolution network analysis (SuperResNET)

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SuperResNET is an integrated software that performs a set of processing operations to quantify and visualize 3D point clouds generated by single-molecule localization microscopy (SMLM) data. SuperResNET is equipped with computational modules to correct for multiple-blinking of single fluorophores, filter-out noisy events, segment clusters of localizations, extract various cluster features, and apply machine learning-based grouping to identify the biological clusters. SuperResNET was previously applied to caveolin-1 labeled cells, identifying the structure of caveolae and three distinct non-caveolar scaffolds. Here, application of SuperResNET to clathrin labeled HeLa cells identifies three groups: Class I: larger plaques; Class II: intermediate sized hollow pits and vesicles; and Class III: smaller oligomers. Cos7 cells present reduced number of plaques and treatment with the clathrin endocytosis inhibitor pitstop decreases plaque number in HeLa cells. Consistently, large clathrin plaques are not observed by platinum replica electron microscopy in pitstop-treated cells. A second grouping of Class II pits based on shape features distinguishes larger, spherical and more hollow structures from smaller, elongated and more compact structures that accumulate upon pitstop treatment. Association of clathrin pits with plaques together with loss of plaques upon pitstop inhibition of clathrin endocytosis supports clathrin plaques as a reservoir for clathrin-dependent endocytosis. SuperResNET 2D and 3D visualization tools help detail the structural transition of clathrin budding from flat lattices into curved pits representing a powerful tool for analysis of the molecular architecture of subcellular, macromolecular organelles and structures.
Molecular architecture of clathrin plaques and pits defined by super-resolution network analysis (SuperResNET)

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Abstract

SuperResNET is an integrated software that performs a set of operations processes to quantify and visualize 3D point clouds generated by single-molecule localization microscopy (SMLM). SuperResNET is equipped with computational modules to correct multiple-blinking of single fluorophores, filter-out noisy events, segment clusters of localizations, extract various cluster features, and apply machine learning-based grouping to identify the biological clusters. SuperResNET was previously applied to caveolin-1 labeled cells, identifying the structure of caveolae and three distinct non-caveolar scaffolds. Here, application of SuperResNET to clathrin labeled HeLa cells identifies three groups: Class I: larger plaques; Class II: intermediate sized hollow pits and vesicles; and Class III: smaller oligomers. Cos7 cells present reduced number of plaques and treatment with the clathrin endocytosis inhibitor pitstop decreases plaque number in HeLa cells. Consistently, large clathrin plaques are not observed by platinum replica electron microscopy in pitstop-treated cells. A second grouping of Class II pits based on shape features distinguishes larger, spherical and more hollow structures from smaller, elongated and more compact structures that accumulate upon pitstop treatment. Association of clathrin pits with plaques together with loss of plaques upon pitstop inhibition of clathrin endocytosis supports clathrin plaques as a reservoir for clathrin-dependent endocytosis. SuperResNET 2D and 3D visualization tools help detail the structural transition of clathrin budding from flat lamellae into curved pits representing a powerful tool for analysis of the molecular architecture of subcellular, macromolecular organelles and structures.

Background

Unlike standard microscopy imaging modalities, single molecule localization microscopy (SMLM) generates big data of millions of 2D or 3D point cloud localizations. As SMLM is a relatively new imaging technique, winning the 2014 Nobel Prize in Chemistry, methods for quantification, visualization and interpretation of SMLM data have not kept up with the development of SMLM imaging. Many methods that are based on second-order statistics are sensitive to noise and imaging artifacts, have not been applied to 3D data, do not leverage machine-learning formulations, and are not scalable for big data analysis. SuperResNET is an integrated software for the analysis of SMLM point-cloud data capable of running large datasets. SuperResNet has previously been applied to study Caveolin-1 (Khater, 2018. Sci Rep) and detect molecular changes due to mutations in Caveolin-1 (Wong et al. 2021. Sci Rep). Here we apply SuperResNet to study the structure of clathrin pits, vesicles and plaques.

Conclusions

Application of SuperResNET, an integrated software for the analysis of 3D SMLM point cloud data, to clathrin heavy chain labelled SMLM data: 1) Classifies clusters into Clathrin plaques, pits/vesicles and oligomers 2) Detects reduction of plaques in Cos7 3) Identifies two classes of clathrin pits/vesicles based on shape features 4) Shows that Pitstop2 reduces the abundance of plaques and the size of both groups of pits/vesicles