Interaction zone analysis of CAV1 - CAVIN1 in breast cancer cells

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Abstract

Formation of caveolae requires both the coat protein caveolin-1 (Cav1) and the adaptor protein CAVIN-1 (also called PTRF). The biogenesis of caveolae is defined by the interaction between Cav1 and PTRF complexes. In this work we propose and evaluate an unsupervised parameter free machine learning method that extracts from multi channel single molecule localization microscopy (SMLM) data interaction patterns in an interpretable geometric space. An interaction graph is constructed between localizations and subsequently mined for feature analysis and clustering. The modular steps of the method use self tuning parameters and are optimized to offer shorter experiment feedback loops. The developed method is validated using a realistic in-silico model based on the latest reported findings on Cav1/CAVIN1 interaction. We apply the method on SMLM imaging of MDA-MB-231 cells exposed to hypotonic shock. Supported by CIHR grants PJT-159845,PJT-156424 (IRN,GH) and NSERC Discovery (GH).

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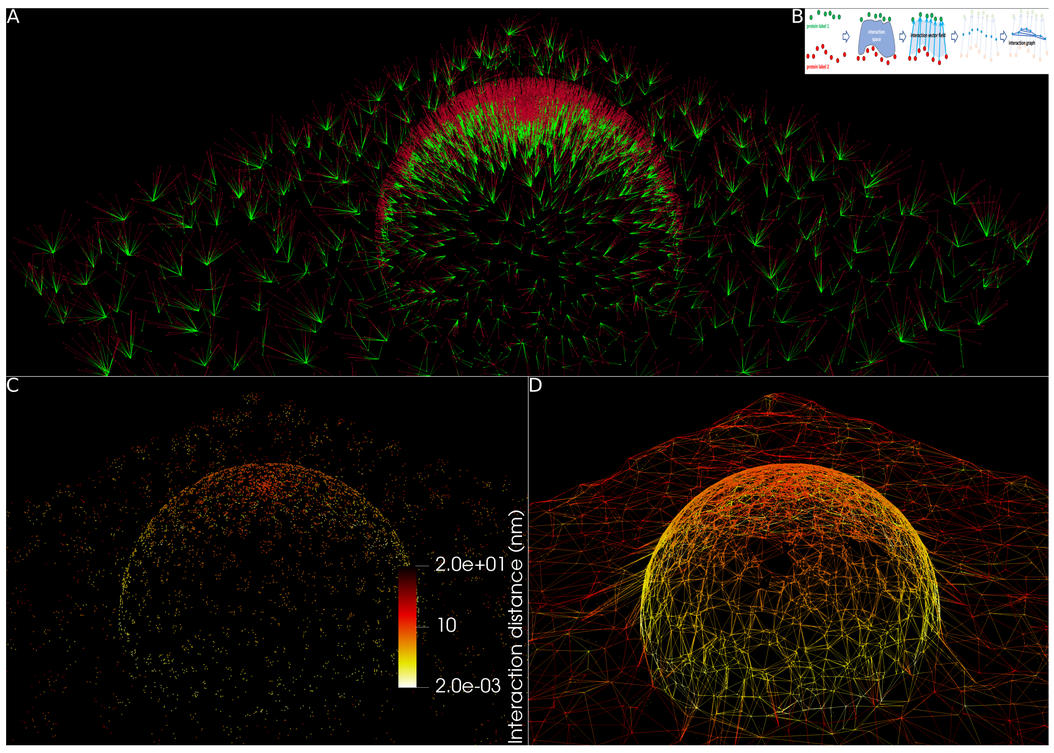
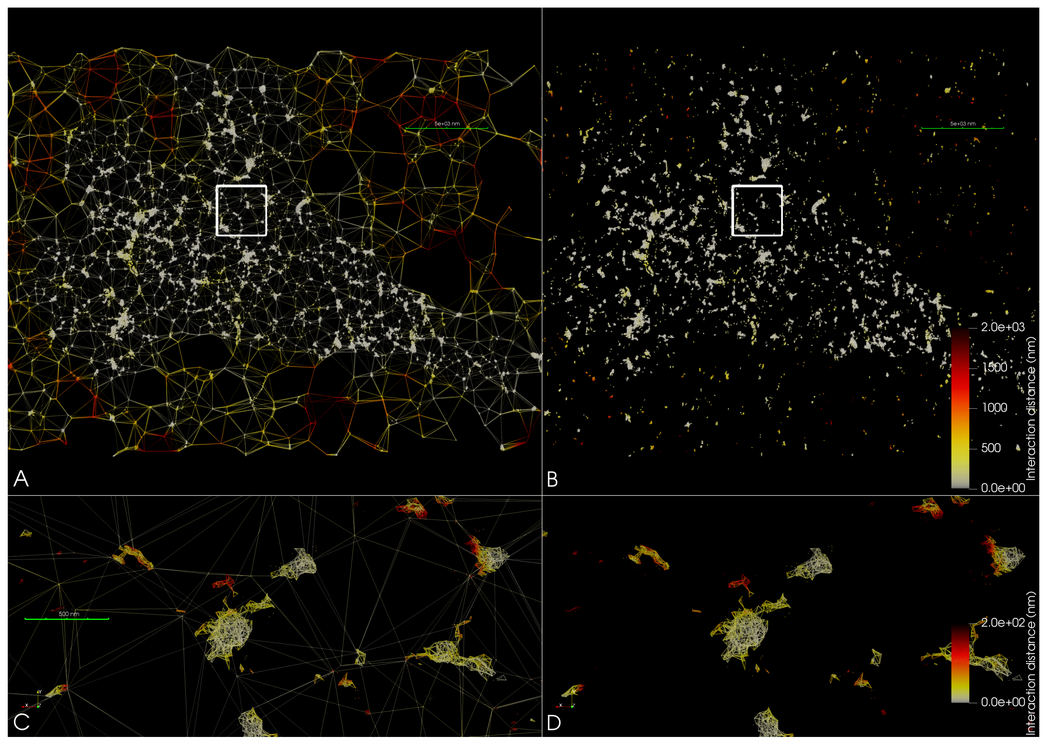
I. Visualization of interaction zone extraction



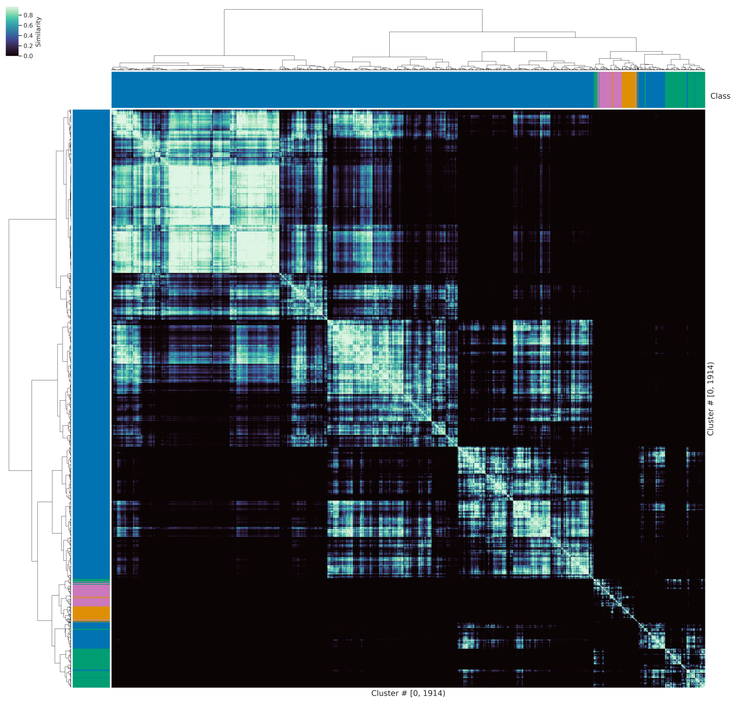
Nanometer scale interaction between 2 protein localizations of a half sphere embedded in a plane with a 10-1 (red to green) stochiometry. A vector field is created (A,B) between both channels from red to green. The vector representation (C) induces a graph (D) that captures the interaction zone (IZ) features.

II. Interaction graph construction

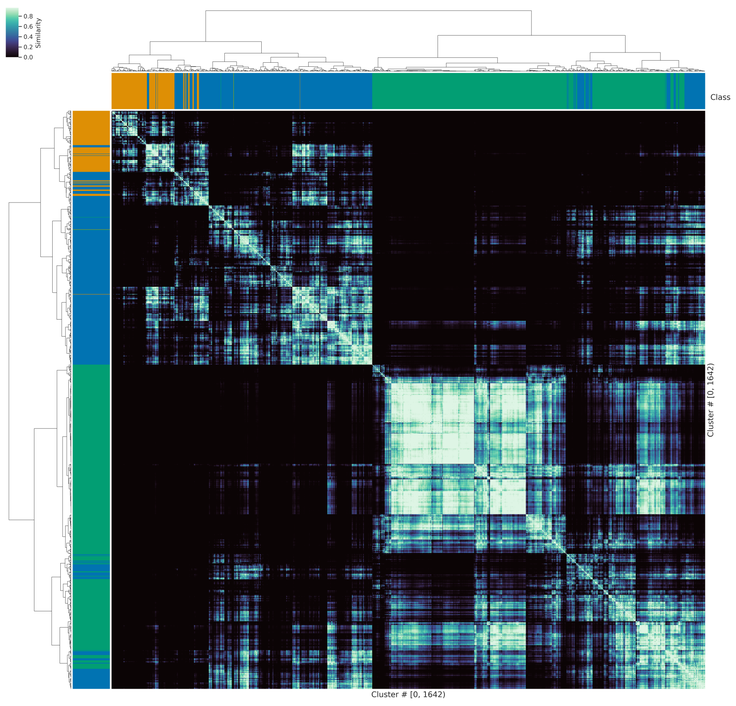


The interaction graph construction of CAV1 to CAVIN1 in a MDA-MB-231 breast cancer cell imaged with a superresolution microscope (ground state depletion, Leica). The sparse graph representation (A) of the interaction vector field is decomposed into zones (B) where each zone represents a local interaction vector field (inset, C-D) between CAV1 and CAVIN1 clusters at nanometer scale (resolution $\sim$ 10-50 nm). Points are localizations of fluorophore labelled CAV1 and CAVIN1 with double antibody chains.

III. Hierarchical spectral clustering finds 3 distinct interaction zone classes

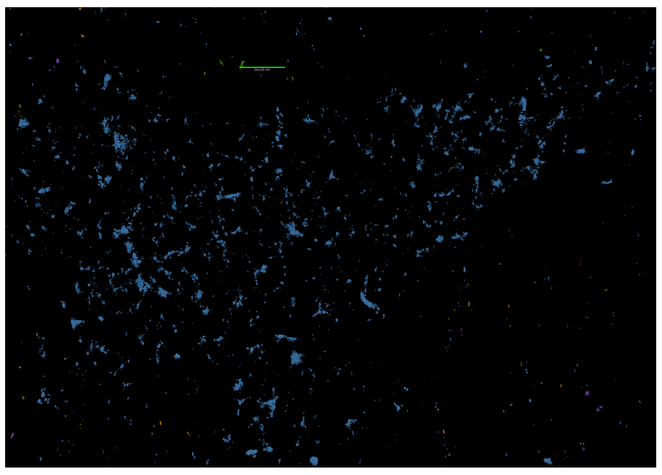


First stage of classification identifies a single self similar class (blue) representing the majority (85+%) of zones with 5 smaller classes. The affinity matrix holds entries at row i, column j describing the similarity between zone i and zone j with a value of 1 for identity.

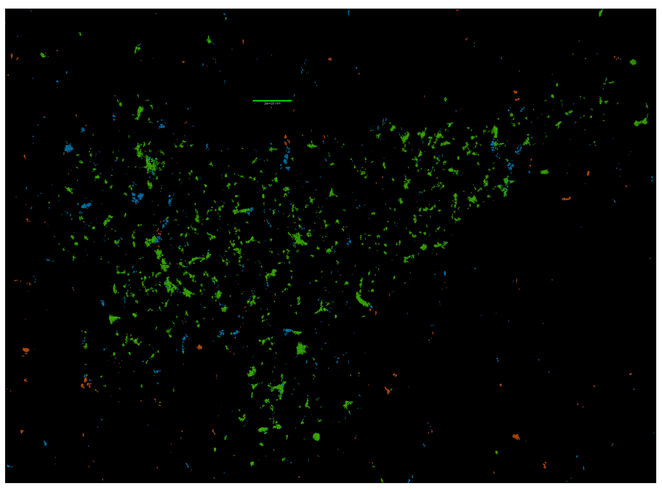


Classifying the large central interaction zone results in 3 distinct classes (colored bar). The square similarity patterns are indicative of the clear classification boundaries between the 3 classes.

IV. Zone classification visualized

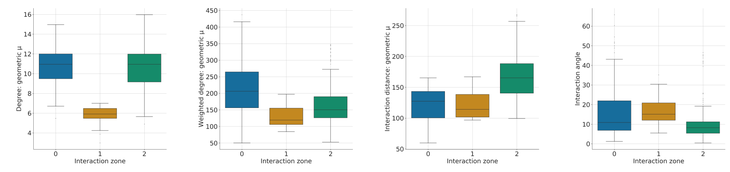


The first stage of classification identifies a single large class (blue) of interaction zones with the remainder of the classes localized at the edges of the acquisition frame.

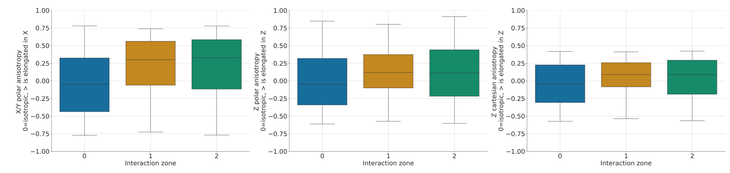


The second stage decomposes the single large class of interaction zones into smaller distinct subclasses with a frequency: IZ0 (blue): 40%, IZ1 (orange): 11%, IZ2 (green): 39%.

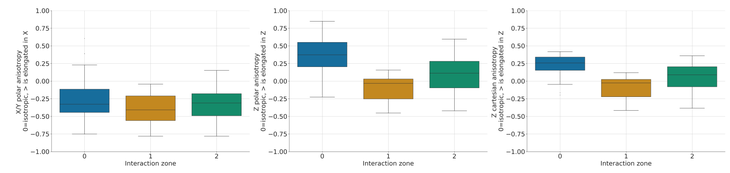
V.1 Zone and vector field analysis describes representative zones



IZ0 and IZ2 display dense interaction zones (weighted degree) whereas IZ1 is clearly sparse. IZ2 has a higher interaction distance with IZ0 most proximate. The interaction angle differentiates in its variance with the angular distribution of IZ2 evidence in support of a planar zone.



IZ0 is an isotropic zone (ratio between axis) in XY whereas 1 and 2 are elongated in X. All three are isotropic in Z with high variance.



The anisotropy of the interaction vector field in Z distinguishes the three classes: IZ0 is characterized by a large Z-elongation indicative of upward oriented interaction with IZ1 isotropic and IZ2 mildly anisotropic.

V.2 Correlate zones with CAV1 classes

Ours	IZ0	IZ1	IZ2
Khater et al.	Caveolae	S2	S1B

For each interaction zone classes we identify the most representative (feature class centroid) zone and identify the corresponding CAV1 class [2]. S2 and S1B are non-caveolar clusters ('scaffolds').

VI. Conclusion

- We demonstrate a novel interpretable interaction zone analysis method [1] for double-channel superresolution microscopy.
- We identify 3 classes of interaction zones between CAV1 and CAVIN1 in a MDA-MB-231 breast cancer cell:
  - **IZ0** ($\sim$ Caveolae) displays a dense proximate interaction vector field that elongates in Z.
  - **IZ1** ($\sim$ S2) displays a sparse isotropic interaction vector field
  - **IZ2** ($\sim$ S1B) displays a dense Z-anisotropic vector field with remote interaction and planar angular distribution

Acknowledgement

We thank CIHR, CFI and NSERC-CREATE for funding.

References

[1] Ben Cardoen et al. "Data driven interaction zone analysis for multi-channel superresolution microscopy". In: *In preparation* ().

[2] Ismail M Khater et al. "Super resolution network analysis defines the molecular architecture of caveolae and caveolin-1 scaffolds". In: *Scientific reports* 8.1 (2018), p. 9009.