Belief theory enables detection of Caveolae in superresolution microscopy.

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We developed a novel method[1] that enables the automatic detection of CAV1 structures in STED super resolution microscopy.

Our method detects objects automatically across images with varying intensity profiles, then uses the cell level label to build a model that identifies which objects are discriminating for each cell type. Our model leverages belief theory to automatically learn a per object label, with lower and upper bound probabilities, including the uncertainty in identification of each object. We identify Caveolae and scaffolds, two types of CAV1 protein complexes, that are key to preventing membrane breakage of the cell membrane in response to stress. Our results match identification of CAV1 structures in fixed cells imaged using dSTORM[2]. Our STED based method enables the detection in live cells, where the formation dynamics can be observed and extracted.

References:

[1] Cardoen, B., Wong, T., Alan, P., Lee, S., Matsubara, J.A., Nabi, I.R. and Hamarneh, G., 2020. SPECHT: Self-tuning Plausibility Based Object Detection Enables Quantification of Conflict in Heterogeneous Multi-scale Microscopy.

[2] Khater, Ismail M., Qian Liu, Keng C. Chou, Ghassan Hamarneh, and Ivan Robert Nabi. "Super-resolution modularity analysis shows polyhedral caveolin-1 oligomers combine to form scaffolds and caveolae." *Scientific reports* 9, no. 1 (2019): 1-10.

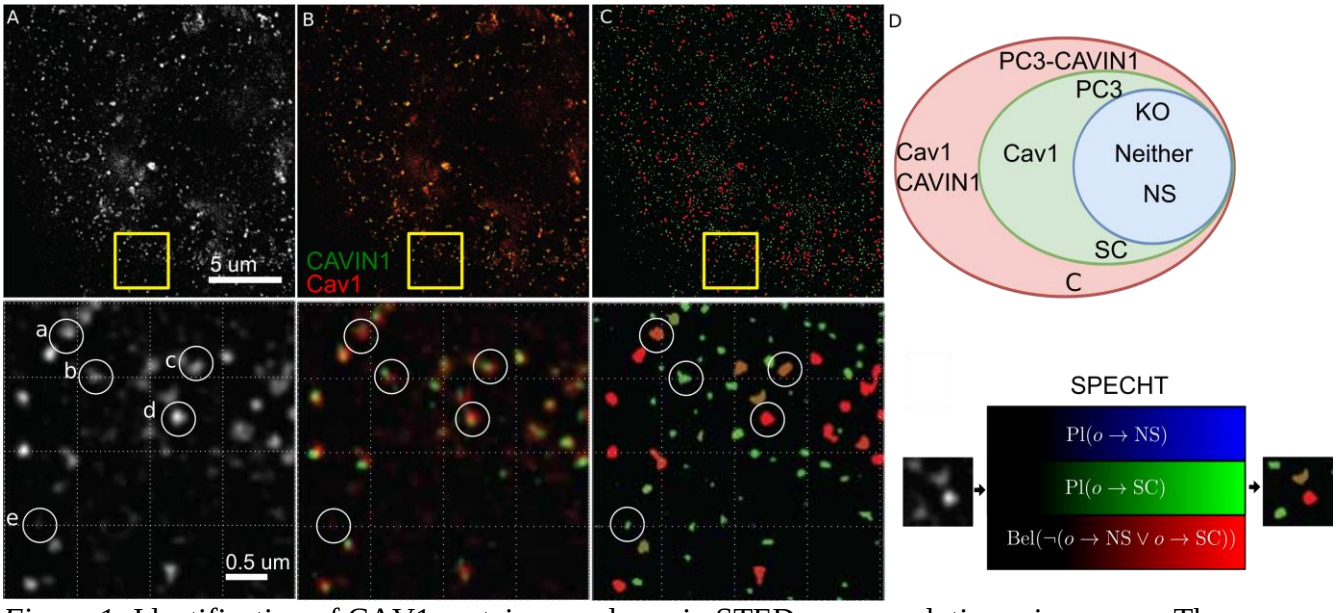

Figure 1: Identification of CAV1 protein complexes in STED superresolution microscopy. The identified Caveolae show the expected colocalization with Cavin1 (B). Our model uses 3 cell level labels to build its discriminatory capability (D). Figure based on original from [1].

SPECHT

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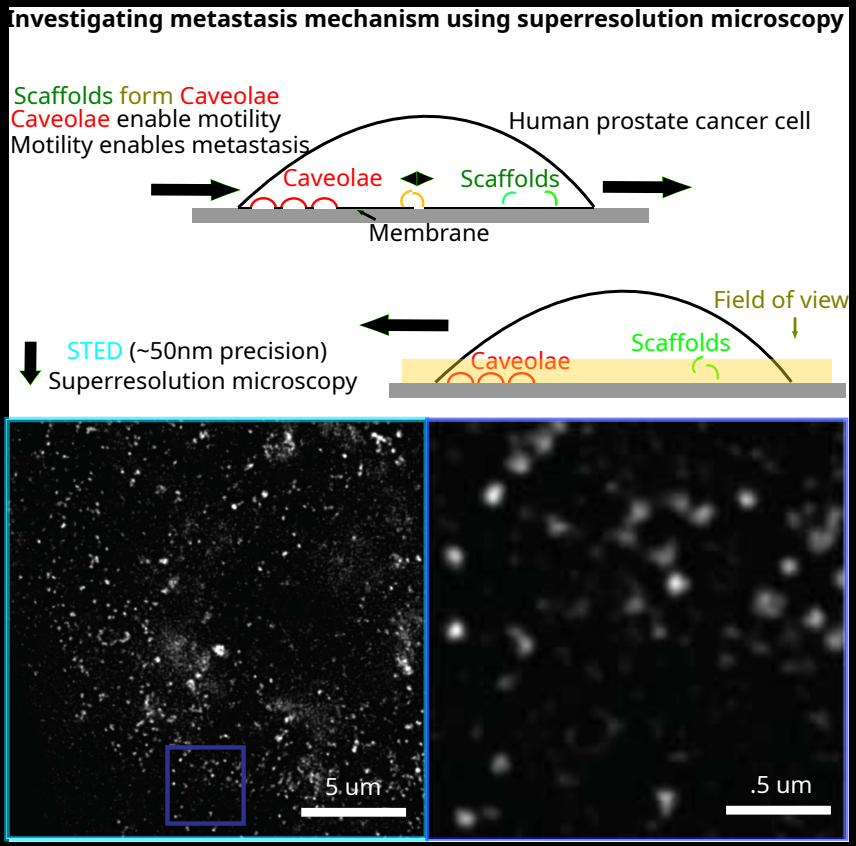
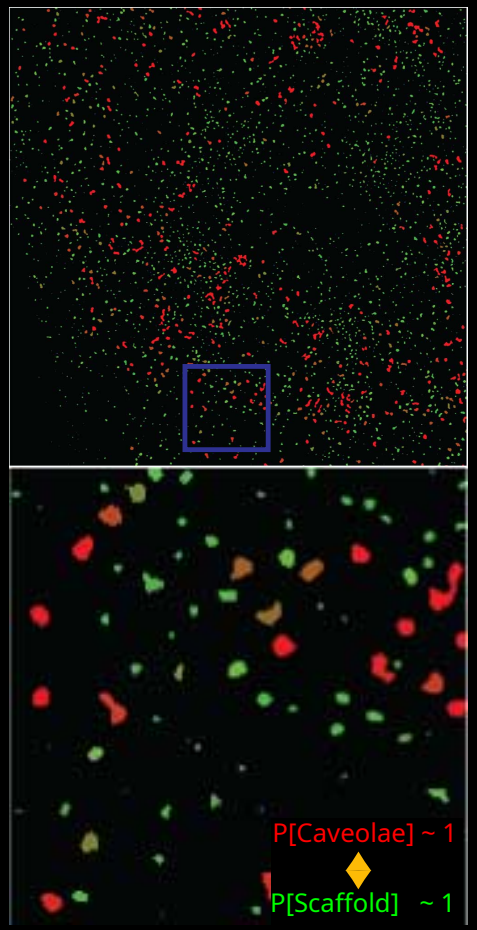
Characterizing protein complexes in 2D STED superresolution microscopy

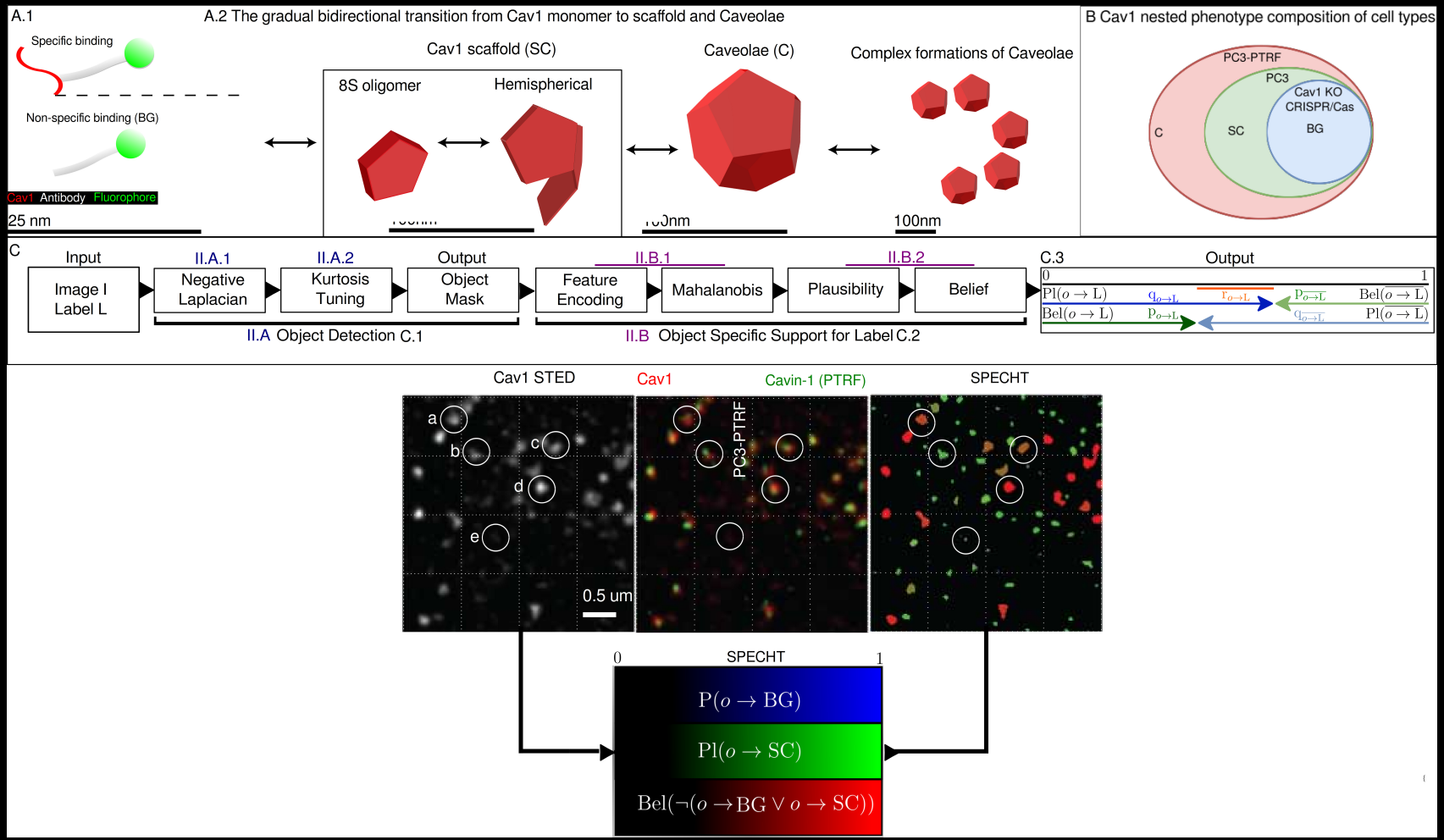


What we have : A cancer cell labelled for the protein key in forming Caveolae. Caveolae enable cell motility (metastasis) [6] and resistance to stressors.



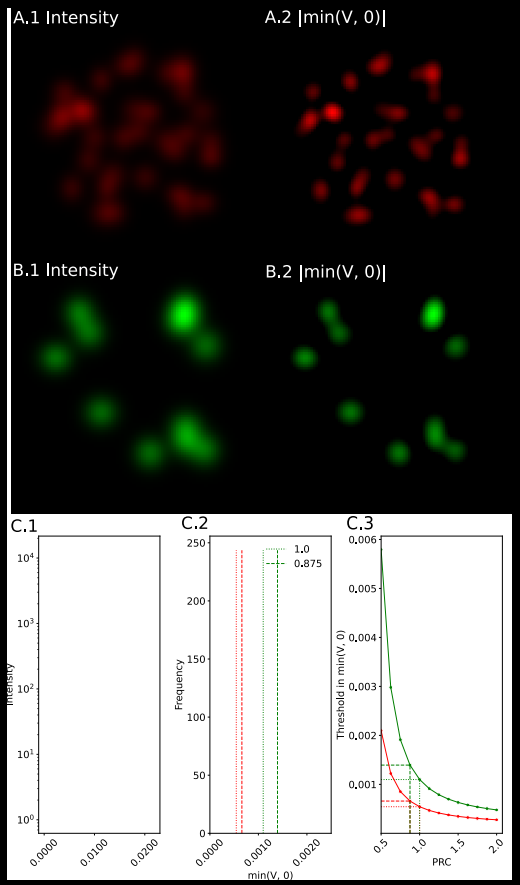
What we want: A probabilistic label that describes the type of each object, e.g. Caveolae (red), scaffold (green) and anything in between.

Method Overview



SPECHT applied to CAV1 use case. We use CRISPR to disable PTRF, essential to form Caveolae, and Cav1 to obtain images where only subsets of Cav1 structures appear.

Adaptive object detection translates user intent to algorithm specific parameters.

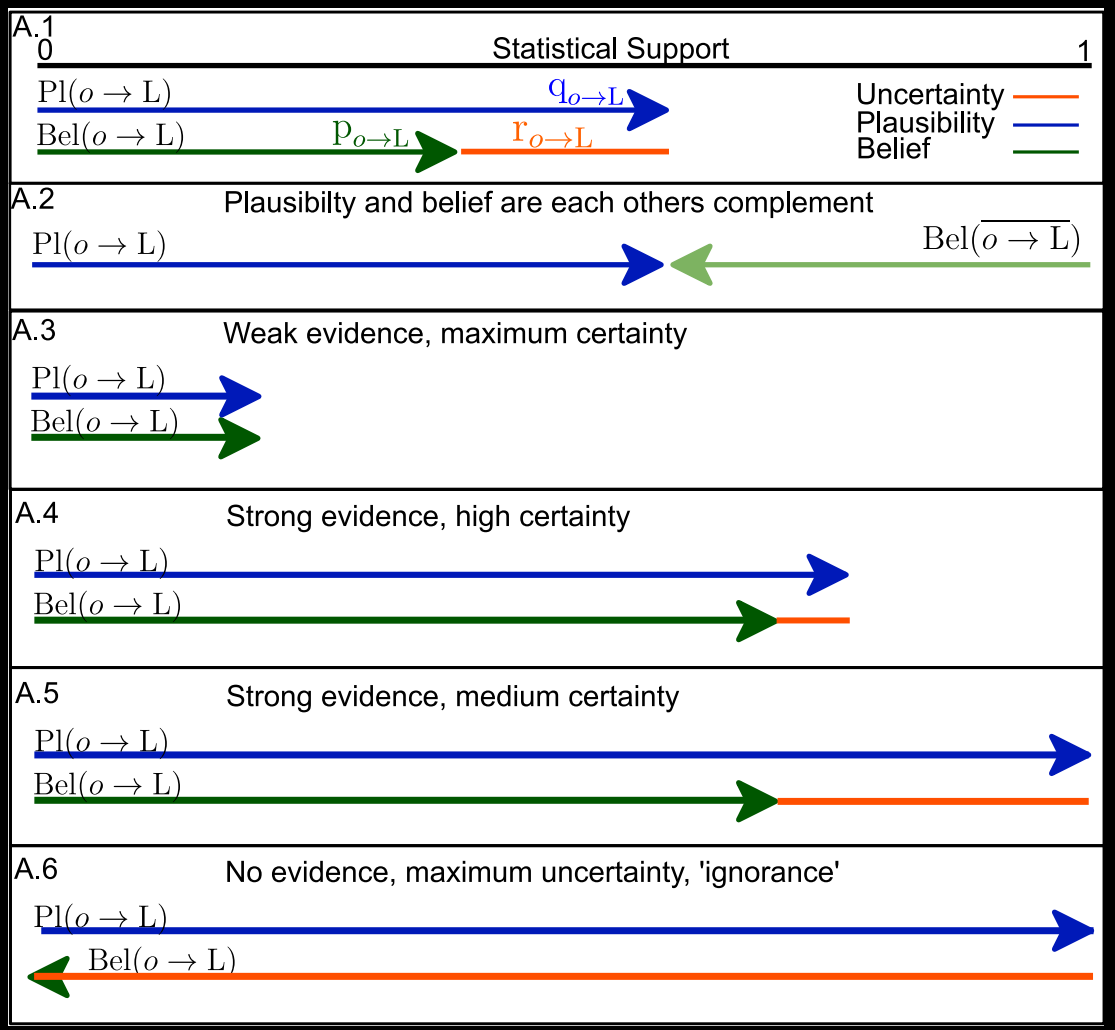


The change in distribution of bright vs dim objects is reflected in the tail of the distribution of the negative Laplacian. Reused from [2].

Use the kurtosis as a self-scaling threshold. Given an image I, with Laplacian L, find the mask M s.t.:

- $k = \text{kurtosis}(L^-)$
- $\mathbb{E}[Z_L] = T \leq k^{\frac{1}{4}}$
- $M \leftarrow I[|L^-| \geq \frac{T}{P \leftrightarrow C}]$
- $P \leftrightarrow C$: + $\rightarrow$ recall, - $\rightarrow$ favors precision. Translated in kurtosis space across image sets.

Belief theory enables robust contrastive learning.

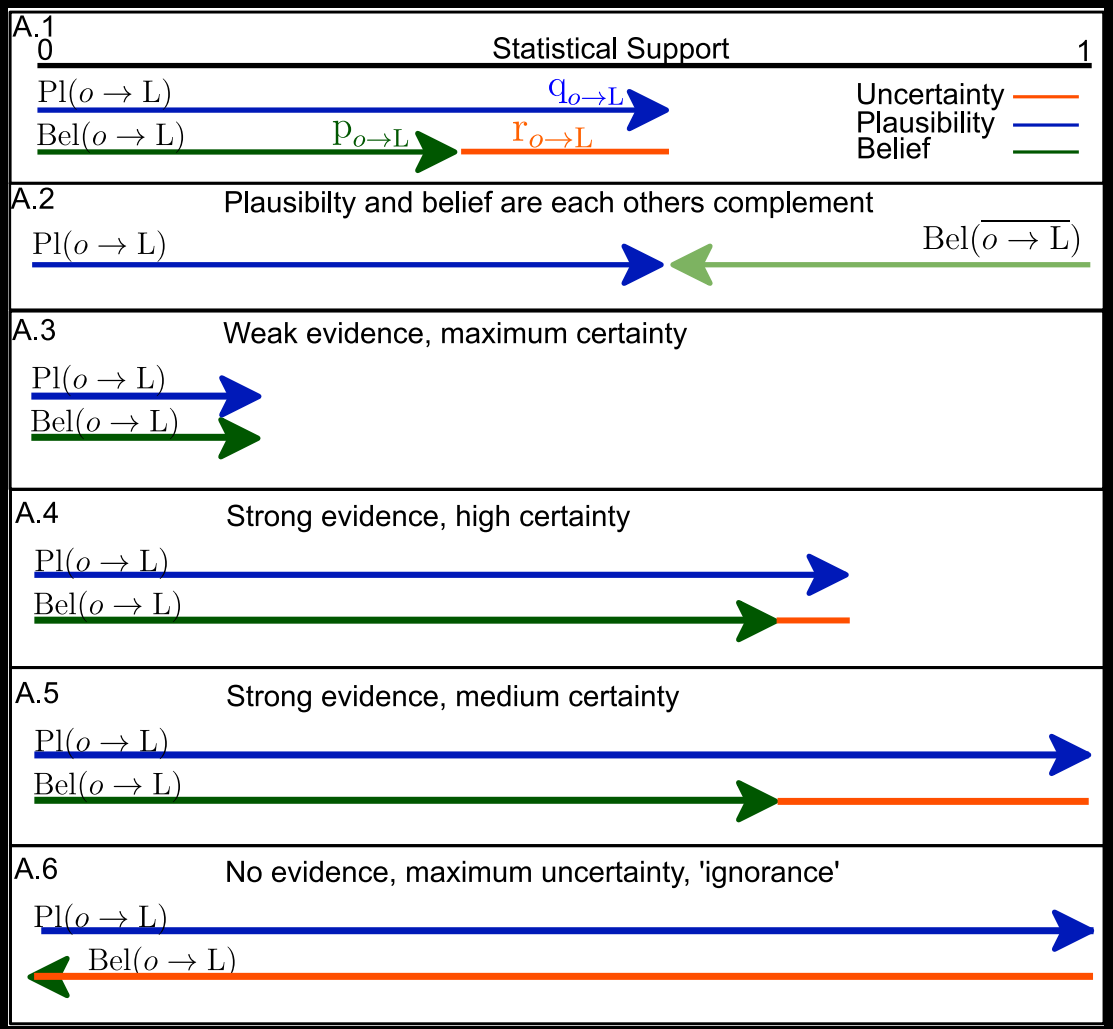


Belief Theory is a superset of standard probability theory

Proposition $o \rightarrow L$ "object o is indicative for the image label L":

- Plausibility q: **maximum** support, given the evidence.
- Belief p: the **minimum** support, given the evidence.
- Uncertainty r: The difference between q, p.

Belief theory enables robust contrastive learning.

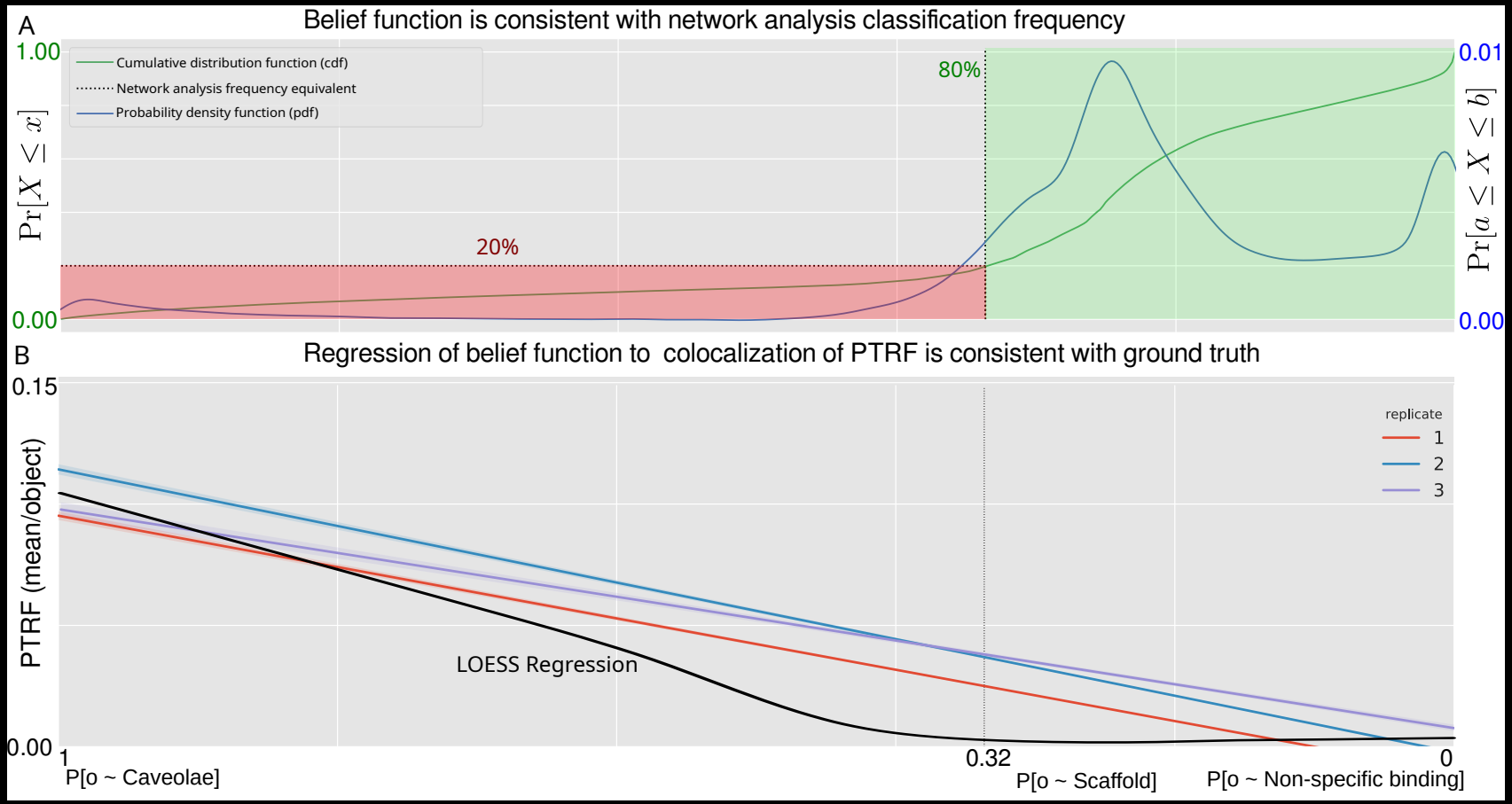


Computing Belief values [3, 4]

For each detected object o $\in I$, with image level label L we can compute:

- $d_z(o)$: normalized statistical distance in feature space to the object distribution
- Plausibility q_L [1]: $P[Z_o \geq z] \leq \frac{1}{1+z^2}$
- Using Dempster-Shafer Calculus we can derive p_L, r_L from q_L and compute the 'conflict' between different models and datasets [3].

Results are consistent with biological ground truth.



Caveolae require PTRF, and have a reported frequency of 20% [5]. We observe that the 20% frequency coincides with an elbow of the [c,p]df functions of our probability label and a marked increase of PTRF colocalization. Both linear and LOESS regressions are shown. Consistency across replicates (30 cells total) is evident.

Thanks!

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Questions?

References I

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