This script offers functions designed to determine the relative positions of proteins localization at the interface of oil droplets, with the capability to plot distance histograms for single molecule localization data, such as direct stochastic optical reconstruction microscopy (dSTORM). It also includes functions for fitting in silico model relative position distributions to experimental localization or simulation data. Additionally, the script provides peak analysis for homogeneous or heterogeneous examination of localizations at the interface.

The software functions by analyzing files containing localization data of individual droplets, which can be either simulated or segmented experimental data processed by StarDist. It evaluates the distribution of relative positions within droplet diameters and saves these positions. It then computes averages over all normalized histograms and compares the peaks of the histograms.

This PERPLE was developed by Alistair Curd of the University of Leeds in 2018, and implemented and developed for emulsion systems by Abbas Jabermoradi in 2022.

**Simulation\_of\_localizations.py**

A Python script simulates the localization of emulsifiers at the interface of oil droplets, generating an image depicting simulated droplets within a specified field of view along with the localization of each droplet.

**RPD\_per\_Droplet.py**

A Python script that calculates the relative positions between points at the interface of droplets either on fluorescence localization microscopy localizations or on simulation data. It export the distribution of the relative distances as a normalized histograms.

**Averaging\_FOV.py**

A Python script that getting relative position distribution average over all droplets present in a field of view.

**Peak\_compare.py**

This script finds the local maxima and compute the relative peak amplitude for heterogeneity analysis

**Fit.py**

A Python script that fits rotational symmetry models to relative positions among localization microscopy data. The models are generated from synthetic localization data.

**Trained\_StarDist\_model & Code\_for segmentation.ipynb**

A StarDist trained model for droplet detection and a Jupyter code for applying on the microscopy emulsion images. For further details regarding model training and the Jupyter code, please refer to the StarDist documentation (<https://github.com/stardist/stardist>).