

Implementation of a workflow for the structural characterization of HER2 by subtomogram averaging.

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Members of the Epithelial Growth Factors Receptor family (EGFRs) influence cell growth and proliferation, and are pivotal in all phases of tumor progression. We will use this receptor family as an example with which to develop an image processing workflow towards high resolution, in a native environment. Here, we present the structural analysis of HER2 membrane receptors (~170 kDa) present in SKBR3 breast cancer vesicles cells by applying cryo-electron tomography (cryo-ET). The heterogeneity of the vesicle membrane together with the small size of the protein, settles this image processing on the limit of the nowadays tomography tools, making it mandatory to push the different existing methods to their maximal capabilities. We used the software PySeg [1] to perform a vectorial picking on CTF-corrected tomograms [2]. A combination of exhaustive 2D and 3D classification procedures using PySeg and RELION [3] was used to identify HER2 protein on the membrane surface, allowing to obtain a preliminary model at 30Å resolution. The implementation of this new workflow into Scipion3, will facilitate the use of the different softwares in a single platform, allowing in a near future to push image processing to a higher resolution.

References:

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[3] Bharat TA, Scheres SH. Resolving macromolecular structures from electron cryo-tomography data using subtomogram averaging in RELION. *Nat Protoc*. 2016;11(11):2054-2065.