

## 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 key in all phases of tumor progression. Hence, in this work we are using this receptor family as a model to develop a subtomogram averaging image processing workflow, to solve its structure in a close to native environment. Vesicles from SKBR3 cancer cells, enriched with HER2 membrane receptors (~170 kDa) were used to acquire cryo-electron tomography (cryo-ET) data. The heterogeneity of the vesicle membrane protein content together with the small size of the target receptor, 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 PySeg software [1] to perform a vectorial picking on CTF-corrected tomograms [2]. A combination of exhaustive 2D procedures using PySeg, and 3D classification and alignments implemented in RELION4 [3]; were used to try to identify HER2 protein on the membrane surface. The implementation of this new workflow into Scipion3 [4], 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:

- [1] Martínez-Sánchez A, *et al. Nat Methods.* 2020;17:209-216.
- [2] Xiong Q, *et al. J Struct Biol.* 2009;168(3):378-387.
- [3] Zivanov J, *et al. BioRxiv* 2022
- [4] Jiménez de la Morena J, *et al. Struct Biol.* 2022;214:107872.

<sup>1</sup> Agirrezabala X *et al.* (2005) *EMBO J.* **24** :3820-9.