# Review of: "3D Structure Determination of Protein Complexes using Matrix-Landing Mass Spectrometry"

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Westphall et al. describe a novel orbitrap-based mass spectrometer to separate macromolecular complexes prior to electron microscopy (EM) investigation. After ESI, ions of large protein assemblies are deposited on EM grids. Different grid preparations were investigated. It seemed that grids coated with thin film of glycerol promoted the preservation of the assemblies structures. The manuscript is interesting because it further states that EM and MS are in a very close relationship as tools to solve complicated structures of large protein complexes.

Main comments

-The research objectives are clear and interesting. Results are stimulating. However, the gas-phase purification is not fully exploited. Three large complexes have been analysed as purified assemblies. It would be extremely interesting to evaluate the results when two macromolecular complexes are mixed and separated in the gas phase.

- After ion deposition, the TEM grids are removed from vacuum and stained with uranyl acetate. I think a key point that should be evaluated is how the grid are removed. It is possible the transition from the vacuum to atmospheric pressure influences the structure preservation. Please comment about that.

-Ion beams are deposited for 60-600 seconds (For example see page 3). The time of deposition has been not systematically evaluated.

Minor comments

-Glycerol is a cryoprotective compound to preserve protein structure. However, it can reduce the resolution that can be achieved. Has it been evaluated the resolution that can be obtained in absence and presence of glycerol, when the sample is pipetted on the grid?

- Glycerol is defined as “a chemical landing matrix”. I believe that this definition is confusing. The term may be confused with Matrix-assisted laser desorption/ionization. Please suggest another definition.

-Which is the thickness of the thin film of glycerol? For example, see caption of Figure 1.

-Please use accuracy instead of precision, when appropriate.