To Whom It May Concern,

the diploma thesis of Bc. Anastasia Rakhimbekova (Structure-assisted development of a continuous carboxypeptidase assay) is written in almost perfect English and divided somewhat non-classically in three chapters: Introduction, Aims, and the Experimental part which is further divided into Materials, Methods, Results, Discussion and Conclusion. The introduction chapter provides good overview of the GCP-II all a lot of details and also contains more than 180 citations.

The Aims are clear, the main aim was to develop a continuous fluorescence-based GCPII activity assay with several specific aims including a structural analysis of the complex enzyme:substrate by X-ray crystallography.

The Result section is impressive, it contains the biophysical characterization of the fluorescent substrates and their complexes with the GCPII enzyme including kinetic analysis and X-ray crystal structure of a representative substrate with the GCPII enzyme.

All together this is an excellent diploma thesis and deserves the grade excellent (výborně). However, I do have few questions:

What are the SI units of fluorescence polarization and fluorescence anisotropy? How are those related to the mP unit used in this work?

How was the resolution cut-off determined? That is not very clear from Table 15.

How clear was the electron density map for the ligands? Could you show an omit map?

Could the assay developed be directly used on the cells? Would there be any advantage over the *in vitro* system?

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