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Supervisor's Opinion on the Doctoral Thesis by

Aneta Závodná

titled

"Synthesis of Rotaxane Structures with Multitopic Ligands"

Aneta Závodná's thesis represents a classic journey through the landscape of fundamental research, where initial expectations and aspirations ultimately lead to completely different, yet far more intriguing, discoveries than we had dared to hope for at the beginning.

The original aim of the thesis was to prepare multivalent ligands that would allow the experimental observation and, potentially, quantification of both attractive and repulsive interactions between various macrocycles based on cyclodextrins and cucurbit[*n*]urils. However, the idea of using central linkers of varying lengths proved problematic, as non-optimal linker lengths did not permit the preparation of sufficiently pure rotaxanes, and the purification of mixtures was initially beyond Aneta's abilities. Here, I would like to make a brief digression: it might be worth revisiting these compounds, as by the end of her work, Aneta developed a method for the purification of rotaxanes, but she did not return to these original systems.

After the first concept proved unsuccessful, Aneta shifted her focus to exploring the possibilities of structural modification at the site where the CB6 macrocycle enters (and exits) the ligand. However, it became apparent that the use of a *tert*-butyl end group completely inhibited the formation of rotaxanes, even though the *tert*-butyl residue was shown to slowly bind within the CB6 cavity.

The final stage of this research evolution involved the structural variation of a high-affinity binding site based on adamantane scaffold. Through a subsequent investigation of six rotaxane derivatives using ITC, MS, and, most notably, extensive NRM titration experiments, Aneta discovered that the CB6 macrocycle could be displaced from its rotaxane position through repulsive interactions with the CB7 portal, which was tightly bound to an allosteric regulatory adamantane site. Subtle structural modifications of this regulatory site enabled control over the rate of this process.

The outcome of this work was a supramolecular switch that responds to a molecular signal—a practical implementation of a concept that, while previously known, had been conspicuously absent in the field of cucurbituril rotaxane chemistry. The significance of this discovery is reflected in the publication of the results in the prestigious journal *Chemical Science*.

In addition to the main topic of her thesis, Aneta also contributed to other projects, such as spiroheptane ligands, where she made a significant contribution to the publication of results in *The Journal of Organic Chemistry*. During her mandatory internship at Universidad de Huelva in the group of Professor Pischel, she focused on the supramolecular chemistry of DASA photoswitches.

The submitted thesis is written in English and exceeds the usual standard in both scope and quality of execution.

In conclusion, I find that the submitted thesis unquestionably contains novel and original findings and fulfils all formal requirements for a doctoral dissertation. This work represents a significant advancement in the field of cucurbituril rotaxane chemistry and has already initiated several follow-up projects within our group aimed at utilising the discovered switching mechanism.

For the reasons outlined above, I consider the thesis to be exceptionally well-executed and recommend it as a basis for awarding the degree of *Philosophiae Doctor*.

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