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The manuscript presented by Ms. Joanna SOBSKA is entitled "Strategies to control biodistribution and activity of new bioactive materials and molecules".

This document is well written but its organization and the Table of content should be modified with more clearly defined paragraphs and numeration.

The introduction part is covering many fields and is well written. However, it may be too generic and some parts are not absolutely mandatory to understand the project while other parts are missing. In particular, the work is focused on the use of different nanoparticles that are not sufficiently explicated in the introduction. Just as examples, Au18 NCs need to be further explained. What does SG14 stand for? As well, the inflammation and cell response involved Ym1/2 and I was unable to find any information on that part. These are only examples and not an exhaustive list of missing parts. It is important to correct the introduction by providing the necessary information. Other parts such as the internalization process (clathrin, caveolin etc..) are on the contrary very generic and largely presented while this is not so important to understand the work presented here.
Some other minor errors need also to be corrected: EPR in page 15 and 23 seems to refer to cellular internalization which is not exact. Cyrill Richard should be changed for Cyrille Richard.

Regarding the scientific part, the project was divided into different main parts.
1/ Obtention and characterization of functional probes for the "click" reaction in vivo.
Five different BCN (bicyclo[6.1.0]nonyne) derivatives were synthesized in collaboration with the group of Pr A Wagner: BCN-PEG, BCN-benzodioxole, BCN-butanoic acid (BCN-COOH), BCN-dimethylamine (BCN-NMe2) and BCN-glucoronide.
Based on the pharmacodynamic and biodistribution profile of these molecules, in regard to that of the nanoparticles, the BCN-glucoronide probe was finally selected for its long-lasting presence in the blood before its elimination from the body through the bile and urinary tract.

2/ Characteristics of selected nanoparticles, for adapted biodistribution in vivo.
Small gold nanoclusters (Au18SG14 nanoclusters), bioluminescent nanoparticles (ZGO) and polymer nanoparticles (PLGA) were tested. The choice of nanoparticles could be discussed. In particular, why using Au18 NCs and not others such as Au25 or others possibly brighter? As well SG14 was used as ligand but others could have been selected. This is not explained and this part is really missing.
AuNCs were also tested for their immunogenic effect in microglia cells. The results showed that at low concentrations Au18 NCs display anti-inflammatory activity and in particular reduced interleukin 1-β (IL1-β) levels while higher concentrations induced expression of pro-inflammatory activity. This part of the work as well as the biodistribution of these NCs has been

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published in Nanomaterials. Unfortunately, AuNCs were not bright enough to be detected by microscopy and were not continued.
Finally, among the different type of particles tested, PLGA NP were the most satisfying ones. NP Cy5.5 PLGA PEG3000 were selected based on their biodistribution, since they circulate longer in the bloodstream and follow closely the profile of the BCN-glucoronide probe. Modified NP Cy5.5 PLGA PEG3000-N3 were thus finally chosen for further investigations. Part of this work is currently under review.

3/ Validation of click reaction in vitro and in vivo.

PLGA OH and PLGA N3 nanoparticles were then tested in the presence of the BCN library in vitro on P19 cells (this cell line would need to be described: what type of cell is it, and where do they come from?). The internalization was mainly monitored and the different BCN probes were showing different patterns of click reaction/internalization. Because the internalization pattern of PLGA N3 NPs was not affected by the presence of BCN-glucuronide and because this BCN and PLGA N3 Np had similar biodistributions, this combination was selected for in vivo tests. The distribution of the injected molecules was followed by whole body imaging as well as ex-vivo on dissected organs. The results indicate that possible modifications of the biodistribution could have been obtained. However, these results will need to be further confirmed.

Overall, the work provided is important and of good quality. The candidate was able to acquire a complete and varied training, using a number of diversified techniques.
In view of the work carried out, of the publication already accepted, and on those in preparation I give a favorable opinion to the defense of the thesis of Mrs. Joanna SOBSKA, to obtain the degree of Doctor of the University of Strasbourg and of the Wrocław University of Science and Technology.

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