Evaluation of the PhD thesis
"Titanium Dioxide – Phosphonate Assemblies as Medical Nanoprobes"
by Ivan Řehoř, Charles University, Prague 2011

This excellent dissertation describes the results of an impressive study on the design of a new class of multimodality theranostics. A titanium dioxide nanoparticulate carrier is covered with a mixture of Gd-DOTA chelates endowed with a phosphonate containing pendant arm and a phosphonate conjugate of rhodamine B. The resulting assemblies have potential in both medical diagnostics and therapy. Thanks to the Gd^{3+}, they are efficient MRI contrast agents, whereas the rhodamine B can be used in optical imaging. On top of that, the titanium dioxide core induces production of OH· radicals with tumor cell killing properties, upon irradiation with UV light. The study is set up very systematically: first the possibility to anchor phosphonates to titanium dioxide is demonstrated, then the surface of the particles is physico-chemically characterized, and finally the applicability of the assemblies is tested in a set of experiments on stem cells, HeLa cells, leukocytes, and pancreatic islets. The obtained results are very promising. The thorough treatment of such very different aspects of drug design in a single thesis is a remarkable achievement.

The work is of high scientific quality; the thesis is well-written and leads to sound conclusions. Therefore, I find that the dissertation is worthy of being defended and that its quality fulfils the criteria for obtaining the PhD degree by the candidate.

Some questions that may be discussed during the defence of this thesis:

1. What is the opinion of the candidate on the future of OI-MRI probes? The low penetration depth of OI puts a limit on the combined OI-MRI image as well. The resolution of both techniques is high, so what is the added value of the combination?
2. In Appendix 2, p. 3, left column, l. 3, it is stated that the amount of TiO_2 decreases during processing. Where does it go? Has the amount of Ti in the dialysate been determined? If it is true that amorphous TiO_2 dissolves, it may be expected that Gd-chelate is lost at the same time. Are there any indications for that? Has the presence of amorphous TiO_2 in the starting material been demonstrated with an XRD spectrum?
3. The adsorption curves in Appendix 2, Figure 2 seem to be almost perfect Langmuir type. Why has been decided for a qualitative interpretation of the data rather than for a more

quantitative one using the Langmuir equation (*Cf.* T. Vitha *et al.*, Langmuir 24 (2008) 1952).

4. How many layers of the bisphosphonate would be present on the TiO_2 particles? Are they formed stepwise? What is the estimated thickness of the layers? If the core diameter does not change upon coating, why is the average diameter of the whole particles as determined with DLS not changing upon coating?
5. How is the photocatalytic activity of the multimodal probe compared to that of bare TiO_2? Is the TiO_2 oxide surface in the probe still accessible for water or plays the Ti in the covering layers a dominant role? Is the selectivity of the tumor killing effect (Appendex 3, Figure 6) high enough for applicability?
6. PVA with a molecular weight above the cutoff of the dialysis membrane has been applied for stabilization of the colloid. Where does the PVA end up? Does it play a role in the relaxivity, which is unexpectedly high?

Delft, May 9, 2011,

Dr. J.A. Peters