DOCTORAL THESIS

Series of porphyrin-ru conjugates as two-photon induced bifunctional therapeutic vectors: synthese, characterization, photophysis, cell imaging and photodynamic therapy

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Series of Porphyrin-Ru Conjugates as Two-Photon Induced Bifunctional Therapeutic Vectors: Synthesis, Characterization, Photophysis, Cell Imaging and Photodynamic Therapy

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Abstract

This thesis focuses on the development of biocompatible porphyrin derivatives as two-photon **PDT** agents to use in a so-called "see and treat" method, i.e. dual-model imaging and local photodynamic cell killing. In addition, we proposed a novel **PDT** strategy of specific targeting to the cyclin A protein for gastric cancer treatment through the coupling of the porphyrin-Ru complex and the cyclin A-targeting antibody.

Firstly, we focus on the improvement of two-photon absorption cross section in the porphyrin-Ru systems. In such sense, we prepared and characterized the homometallic conjugates **Ru-1**, **Ru-2** and heterobimetallic conjugates **Ru-Zn-1**, **Ru-Zn-2** through modifying the conjugated connections between the β position of tetraphenylporphyrin and Ruthenium polypyridyl complex. The detailed photo-physical properties were studied by UV-Vis absorption spectrum, fluorescence spectrum including solvent-dependent emissions, singlet oxygen phosphorescence and two-photon induced fluorescence and absorption. The two-photon cross sections and singlet oxygen quantum yields were found to be improved efficiently in these conjugated systems. These Ruthenium compounds show high cellular uptake in the cytoplasm without specific localization, low dark-cytotoxicity, high photo-cytotoxicity (*via* $^1O_2$) on HK-1 or HeLa cells. **Ru-1**, in particular, has adequate fluorescence and can be excited and emitted in the "biological window" *in-vitro* which make them potent potential candidates as bifunctional

two-photon **PDT** agents in the coming generation–to be able to generate singlet oxygen and has *in-vitro* near-infrared emission with excitation at around 800 ~ 900 nm.

Secondly we focus on the distinct cellular behavior resulted from modifying the different connections between the *meso* position of the porphyrin and the Ruthenium polypyridyls complex. We synthesized and characterized three homometallicporphyrin-Ru compounds **Ru-3**, **Ru-4** and **Ru-5** and three heterobimetallicporphyrin-Zn-Ru compounds **Ru-Zn-3**, **Ru-Zn-4** and **Ru-Zn-5** with different linkages such as an amide bond, a triple bond or a PEG chain between two moieties. **Ru-3**, **Ru-4** and **Ru-5** showed different emission sensitivities to pH variations. Interestingly, **Ru-3** and **Ru-4** are specifically localized at the mitochondria and lysosome organelles of the HeLa cell respectively, and **Ru-5** is localized in the cytoplasm without specific distribution. Furthermore, the flow cytometric analysis demonstrated that **Ru-3** and **Ru-4** have a faster uptake by the HeLa cells than **Ru-5**. The MTT assay and confocal imaging results proved that mito-tracker **Ru-4** showed the highest photocytotoxic on HeLa cell, followed by lyso-tracker **Ru-3** then the nonspecific **Ru-5**, regardless of excitation *via* one photon or two-photon process. These comparative studies make **Ru-4** the most suitable two-photon induced **PDT** dual probe – imaging and local photodynamic cancer cell killing.

Finally, we propose a novel strategy of targeting cyclin A protein, which is frequently found to be overexpressing in gastric cancer patients. Following

this concept, we synthesized and characterized two porphyrin precursors which bear a primary amine group on one side of porphyrin macrocycle and are ready to couple with the cyclin A-specific antibody.

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