Cover Page

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1

General introduction

1.1. Molecular imaging

In biomedical and biochemical studies, it is often desirable to visualize the molecular composition of cells (*in vitro*) at the microscopic level. Such insights can help to identify different morphological structures as well as help unraveling their role in biological processes. In this *in vitro* setting, luminescence imaging is a highly desired imaging modality due to its detection sensitivity, spatial resolution, and ease of implication. Ultimately, through molecular imaging, the same type of information can be translated to the macroscopic *in vivo* scale, resulting in imaging technologies that are suitable for patients. One critical component in the realization of both *in vitro* and *in vivo* molecular imaging solutions lies in the design of imaging agents.[1, 2] Such imaging agents usually comprise of a functional moiety for targeting or activation of the molecule of interest and an imaging label that allows detection.

1.1.1. Optical imaging

Optical imaging relies on the interactions between molecules and light. Different interactions between light and molecules are possible, but perhaps the most interesting for molecular imaging is the use of luminescence emitting agents. The energy needed to generate emissions can be introduced via biochemical reactions (bioluminescence), via high energy particles (e.g. electrons, positrons), or by excitation with light of a different wavelength.[3] This last approach is used in fluorescence and phosphorescence imaging.

The primary goal of using luminescent imaging agents is to generate/enhance contrast between the (supra)molecular features of

interest and the background (the so-called signal-to-background ratio). Luminescence based methods to improve the imaging contrast can be divided into two broad categories: 1) enhancement of specificity for a particular molecular feature/structure, which helps to increase the signal to background ratio, 2) reduction of the non-specific background signal, which increases the relative visibility of the molecular feature/structure under investigation. The outcome of these two methods is illustrated in Figure 1. In this thesis, the second category was chosen as the predominant approach to improve imaging contrast.

Figure 1. Two approaches to increase contrast in molecular imaging

1.1.2. Selected methods to decrease background influence

Although most optical imaging is performed by observing the signal intensity of a luminophore (a light-emitting molecule), an alternative imaging method to distinguish between luminescence signals is based on their luminescence lifetime – the exponential decay rate of the fluorescence.[4, 5] For endogenous luminophores (e.g. DNA, NADH, proteins), responsible for the native background luminescence, the lifetime is in the order of 0.5 - 10 ns. Commonly applied organic dyes such as fluorescein and Cy5 have luminescence lifetimes in the same range as the endogenous luminophores.[4] This feature complicates lifetime-based differentiation between endogenous and exogenous signals. Obviously

separation becomes more straightforward when the differences in luminescence lifetime are more pronounced.[6] Luminescence lifetimes from 100 ns up to milliseconds can be achieved using inorganic transition metal complexes.[7] Specific localization of these luminescence signals can be the result of targeting molecular features [8] or via (enzymatic) activation processes that are specific for cellular compartments or cell types. The latter are generally referred to as “activatable” agents. Activatable imaging agents are designed so that they provide a signal after a (bio)chemical response to a (local) external factor, comparable to an ON switch.[9, 10] If a distinct alteration in (intensity or lifetime) is realized after activation, this helps to locally induce imaging contrast.

1.2. Luminescence imaging as means to study cell functionalization

As described above, cells or molecular features therein can be seen as targets for luminescence imaging. Alternatively, luminescence imaging can also be used to study the chemical functionalization of cells. At the same time luminescence imaging can be applied to monitor cellular functions such as migration and grafting. For instance in stem cell therapy, it would be groundbreaking to obtain chemical control on the migration of the stem cells to the location of choice and to monitor their activities (such as tissue re-generation *in situ*).[11-13]

1.3. Scope and outline of this thesis

The focus of the research presented in this thesis lies on the development on two subjects related to new imaging agents. Firstly, the development and use of the activatable luminescence lifetime imaging agents is described.

In **Chapter 2**, an activatable imaging agent suited for luminescence lifetime imaging was investigated. For this, the long-lifetime (100 ns) inorganic luminophore $Ir(ppy)_3$ was covalently attached to the organic luminophore Cy5 using a cleavable disulfide bond. When connected, the photophysical interactions between the two luminophores led to a quenching of the luminescence for both luminophores and a decrease of the luminescence lifetime of $Ir(ppy)_3$ to 5 ns. Chemical cleavage of the connecting disulfide bond induced luminescence of both luminophores and restored the luminescence lifetime of $Ir(ppy)_3$.

In **Chapter 3**, an MMP-2/9 enzyme-specific activatable lifetime imaging agent was generated based on the luminophores $Ir(ppy)_3$ and Cy5. By using matching ionic interactions, a peptide hairpin was created whereby both luminophores were placed within the right distance required for both intensity and lifetime quenching. *In vitro* studies revealed that in MMP-2/9 positive cell lines the peptide sequence was cleaved, resulting in the restoration of the luminescence emission (intensity and lifetime) of both luminophores.

In the second part of this thesis, luminescence imaging is used to study supramolecular modification on the cell surface. Hereby, the use of supramolecular interactions (between β-cyclodextrin and adamantane) as a means to induce cell surface modifications was explored.

In **Chapter 4**, the feasibility of supramolecular host-guest interactions on the membrane of living chemokine receptor 4 (CXCR4) expressing cells was investigated using fluorescence imaging. Live cells could be specifically decorated with multivalent cyclodextrin-polymers following the introduction of adamantane functionalized peptides that are specific for CXCR4. Not only could the surface modification be proven, it

was also possible to demonstrate how such surface functionalizations could, in the future, be used for further cell functionalization, with other molecules or with complete cells.

In **Chapter 5**, it is described how the host-guest interactions described in Chapter 4 can be used to realize layer-by-layer functionalization of a cell membrane. Hereby fluorescence imaging was used to monitor each individual step in the cell functionalization process. These functionalization steps were then related to the cell vitality and the uptake of a cytostatic drug.

Chapter 6 summarizes the main results and conclusions of every chapter.

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