Supramolecular host-guest chemistry for applications in theranostics

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CHAPTER 1

Introduction and Outline

In the last few decades our knowledge in the fields of molecular biology and medicine has vastly increased. Serious illnesses are being tackled with the help of a large selection of pharmaceuticals and clinical procedures. However, with the increase in therapeutic know-how, the insight has grown in the fact that current therapies are far from perfect and that sometimes the side-effects of the cure are worse than the disease itself. To reduce collateral damage and side effects, increasing effort is being spent on the development of personalized medicine and the integration of diagnostics and therapy into one treatment plan, giving rise to the field of theranostics.

With the idea of personalized medicine, the demand for the availability of a generic chemical toolbox that supports refinement of therapeutic strategies has risen. Such a chemical toolbox could, for example, include interchangeable targeting groups and/or therapeutic entities allowing the target (e.g. specificity) and doses of the therapy to be adjusted to the patient's needs. Furthermore, theranostic approaches often demand for new drug/tracer designs, where both imaging labels (fluorescence and/or radio-label) and therapeutic agents are either combined in one tracer, or their location and action can be linked.

There has already been a lot of research towards combining different functional features into one targeting tracer in the field of nanomedicine.[2,3] One prime example is the combined integration of a radioactive and fluorescent signature in the clinically applied multimodal nanoparticle Indocyanine green-^{99m}Tc-nanocolloid.[4] Unfortunately, controlling the amount and number of labels on the nanoparticles remains challenging.

Nature's way to introduce complex functionalizations is presented by supramolecular interactions.[5,6] For example: the folding and replication of DNA, the formation of protein capsids (e.g. ferritin, viruses), and the specific binding of proteins to cellular receptors. When analyzing the basis of these systems, many are based on multivalent host-guest interactions. Zooming in, these interactions occur with the help of hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces and/or electrostatic effects.[7] On their own these chemical interactions are relatively weak, their strength however, lies in their numbers. Through multiple interactions strong but still revisable binding is accomplished leading to highly specific interactions.

A well-described supramolecular system is the host-guest interaction between adamantane (Ad) and β-cyclodextrin (β-CD)(hydrophobic forces[7]). One CD-Ad interaction has a relative low binding strength ($K_D = 5{\cdot}10^4\ M^{-1}$),[8,9] but three or more of such interactions increases the binding strength significantly ($K_D = 1{\cdot}10^7$–$1{\cdot}10^{10}\ M^{-1}$).[10,11] Hence in the field of supramolecular chemistry/nanotechnology, CD-Ad interactions are utilized as driving

forces for compound aggregation.[12,13]

In this thesis supramolecular host-guest interactions and self-assembly processes are investigated in relation to the development of theranostic and diagnostic drugs/tracers.

Chapter 2 explores the possibility of utilizing the supramolecular host-guest chemistry between β-CD and Ad to drive cell functionalization and cell-cell interaction in an *in vitro* environment. To this end cellular surfaces are functionalized in a two-step pre-targeting set-up and the effects were monitored using fluorescence imaging.

After exploring the CD-Ad interaction *in vitro*, the supramolecular host-guest interaction was further tested *in vivo*, as described in **chapter 3**. Here a pre-targeting setup for liver radioembolization is discussed, again exploring the interaction between the β-CD and Ad-functionalized protein microparticles. Radiolabeling with ^{99m}Tc supported *in vivo* SPECT imaging and quantitative biodistribution studies (%ID/g; 2h post injection). In **chapter 4** the pre-targeting principle for radioembolization is further explored by using the two radioisotopes: ^{99m}Tc and ^{111}In. Via dual-isotope multiplexing and by monitoring the individual components via dual/-isotope SPECT and %ID/g analysis more light is shed on the *in vivo* co-localization of β-cyclodextrin polymers and Ad-functionalized protein microparticles.

In **chapter 5**, use of a self-assembled protein for drug/tracer development is described. The supramolecular self-assembly interaction of (apo)ferritin was utilized to obtain control over multi-functionalization of these bionanoparticles. Re-assembly of stochiometric mixtures of functionalized (apo)ferritin subunits, was combined with tests that underline the preservation of ferritins natural iron mineralization properties.

As fluorescence plays an important part in the analysis technologies used in this thesis, and can provide clinical theranostic value in the form of fluorescence guided surgery, **chapter 6** was devoted to the photophysical properties of different fluorophores. Through systematic alteration of the Cy5-dye structure more insight was obtained in the structure/chemical- and photo-physical-property relationships.

In **chapter 7** the future of supramolecular interactions in theranostic procedures is discussed and a summary of the thesis is provided in **chapter 8**.

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