The quest for function in systems with two dynamic covalent bonds

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The Quest

for Function in Systems with Two Dynamic Covalent Bonds:

Supramolecular Self-Assembly, Self-Replication and Hydrogels for Biomedical Applications

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The front cover art puts in focus the main protagonists of each research chapter (Chapters 3-6): the peptide building block (a drop), assemblies (a wave), hydrogels (a sea), and finally, cells cultured on hydrogels (a ship sailing over the sea).

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PhD thesis

to obtain the degree of PhD at the
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on the authority of the
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and in accordance with
the decision by the College of Deans.

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by

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Mojim bakama i dedama.

(To my grandmothers and grandfathers.)

Preface

The Aim and Outline of this Dissertation

The work on which my research initiated was based and proposed in earlier publications[1-3] of our group. Although the long-term goals remained constant, the topics investigated went beyond the original plan: new ideas were developed, fueled by the scientific surroundings, fruitful conversations and conferences, current state of the art, but also my growing understanding. All of the above gave a fresh direction to the research presented on the following pages.

I aimed to discover and introduce new functions into molecular systems with orthogonal dynamic covalent bonds. The focus on this adventurous chemistry allowed me to learn about, and address questions relevant to fundamental science, particularly the process of self-replication in the context of how the machinery of life might come to be. Contemporaneously, applicational aspects were also explored concerning bioinspired functional biomaterials.

As beautifully outlined in the review article by Matile *et al.*,[4] functional molecular networks that operate with one or more types of dynamic covalent bonds often deal with highly challenging, interesting, and original concepts. The diverse collection of functions investigated thus far nicely outlines the potential of such systems. With this perspective, embarking on a journey to new behavior and processes appeared prosperous and exciting. We envisioned to expand on the idea of previously described self-replicators[5] by making them a part of increasingly complex systems through an ingredients-approach, to observe additional functions. Thus, the objective was to extend from their monofunctionality[6] towards multifunctionality with multiple reversible covalent bonds at work, simultaneously or independently.

This dissertation begins with two separate introductory sections to acquaint the reader with the essential concepts, ideas, and goals studied in the research chapters.

In **Chapter 1** the main tools to construct dynamic reaction networks and functional molecular and supramolecular systems are presented. A few relevant applications have been selected and discussed out of the remarkably rich set of functions attained using (multiple) dynamic covalent bonds. This overview ends with the section about the post-assembly modification of systems that are not necessarily based on dynamic covalent reactions but showcases this approach's utility for late-stage derivatization of self-assembled complexes.

Chapter 2 deals with recent advances in the field of biomimetic materials. Several types of naturally and synthetically derived biomaterials for cell-culture are discussed, emphasizing design principles. The complexity and dynamic character of extracellular matrix (ECM) were also considered, as it has motivated the development of many simplified, yet functional hydrogels for biomedical application.

The work described in **Chapters 3 and 4**, firmly rooted in concepts of dynamic combinatorial chemistry, slowly translates into a prospective application in **Chapter 5**. It finally reaches its (full, in years to come) realization in **Chapter 6**, which requires the broader perspective given by the summary of proceedings in the field of biomaterials.
Inspired by the elegant ways of nature to expand the functional repertoire of proteins by chemical alterations, **Chapter 3** focuses on developing a self-assembling system that can undergo constitutional post-modification. We describe a building block which uses two dynamic covalent bonds to form a supramolecular polymer and engages in further functionalization. Our results show that both post-modification of and exchange within the supramolecular structure can be accomplished. The new research lines that are directed towards modification for obtaining particular functions are proposed to illustrate the potential of such a system.

The work in **Chapter 4** expands further on the molecule design discussed in Chapter 3, by introducing new hydrazide-functionalized peptide building blocks and investigating their behavior. Contradicting our initial hypotheses (and expectations), these molecules yielded self-replicating species, thus we devoted some time to examine whether the system can be directed towards the foldamer formation. This led to new findings about the relationship between self-replication and folding processes in fully synthetic systems.

In **Chapter 5**, the supramolecular polymer synthesized and studied in Chapter 3 is taken as a component for soft material fabrication. Here, the idea of post-modification for a specific outcome was exploited to achieve hydrogel formation by (reversible) covalent reaction of hydrazide-moieties of fibrous assemblies and carbonyl groups of cross-linkers. Hydrogels are formed at remarkably low critical gelation concentrations (CGCs) and exhibit short gelation times (2 – 10 min). These materials were then characterized by cryo-EM, SEM and rheological measurements.

In **Chapter 6**, the material explored in Chapter 5 is further investigated as a substrate for cell growth. We show that our material is biocompatible but can also incorporate biologically relevant ligands, highlighting the value of our approach towards tailor-made

and on-demand biomimetic soft surfaces. Surprisingly, materials “decorated” with cell adhesion motives (RGD and LDV) did not exhibit improvement when it comes to cell morphology, as the visualization of cells revealed that cells in the original substrate’s presence already display extended morphologies, characteristic for cell spreading and adhesion.

Finally, in **Chapter 7** the main achievements of preceding chapters are put in a broader perspective, emphasizing the importance of research along these directions.

References and notes

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6 By monofunctionality, the ability of the system to only self-replicate is meant. Recently, an example of a self-replicator that can catalyze the retro-aldol reaction has been reported: J. Ottelé, A. S. Hussain, C. Mayer, S. Otto, *Nat. Catal.* **2020**, *3*, 547-553.