

CHEMISTRY IN EXTRACELLULAR SPACE

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My view of the present state of research on exploring the chemistry–biology interface

Natural evolution has created an intricate system of coexisting molecules and selective reactions in the crowded space inside and outside of cells. This system allows the molecules of life to act, in a controlled manner, in concert. Chemical tools offer unique opportunities for interrogating how the interplay of molecules functions, for obtaining fundamental insights into the machinery of life, and for establishing methods to intervene, for example, for the purpose of disease diagnosis and therapy. Among other goals, my laboratory uses chemistry in the extracellular matrix for targeting disease states and poses the fundamental question of whether and how peptides can operate as catalysts. In the next section, I provide a brief introduction to both areas of research, along with recent contributions from my laboratory.

My recent research contributions to chemistry in the extracellular space

Targeting collagen cross-linking: As the major constituents of the extracellular matrix, collagens, the most abundant proteins in mammals, ensure the integrity of disparate tissues and organs, such as skin and bones [1]. Collagens are also involved in many signaling events that control cell differentiation, adhesion, migration, proliferation, and apoptosis [1]. Key to the properties and function of these fibrous proteins is the controlled assembly of three identical or different strands into triple helices, which then assemble into macroscopic fibrils and fibers. Whereas non-covalent interactions hold the triple helix together, covalent crosslinking plays a key role in fibril and fiber formation. Enzymes, lysyl oxidases (LOX), initiate the cross-linking by oxidizing the ϵ -amine groups of lysine (Lys) to aldehyde groups (allysine) that then undergo spontaneous aldol and related reactions to form covalent bonds between collagen strands (Figure 1) [2]. This LOX-initiated cross-linking process is essential for normal tissue growth, but prolonged and excessive LOX activity leads to excessive collagen cross-linking and impaired tissue function as observed in fibrotic and malignant diseases. Estimates associate 45% of all deaths in the industrialized world with fibrosis [3]. Tools that allow

for visualizing and targeting excessive cross-linking within the ECM, thus, present an opportunity for detecting diseased tissues.

My laboratory has interrogated the stability of collagen at the molecular level using collagen model peptides (CMPs). These studies enabled a profound understanding of the molecular principles governing collagen stability and established functional synthetic collagen triple helices. These include pH-responsive synthetic collagen [4], hyperstable lipidated triple helices [5], and self-sorting heterotrimeric collagen [6]. Building on this knowledge, we developed a chemical probe for the simultaneous imaging and targeting of LOX-generated cross-linking of collagen in the ECM (Figure 1) [7].

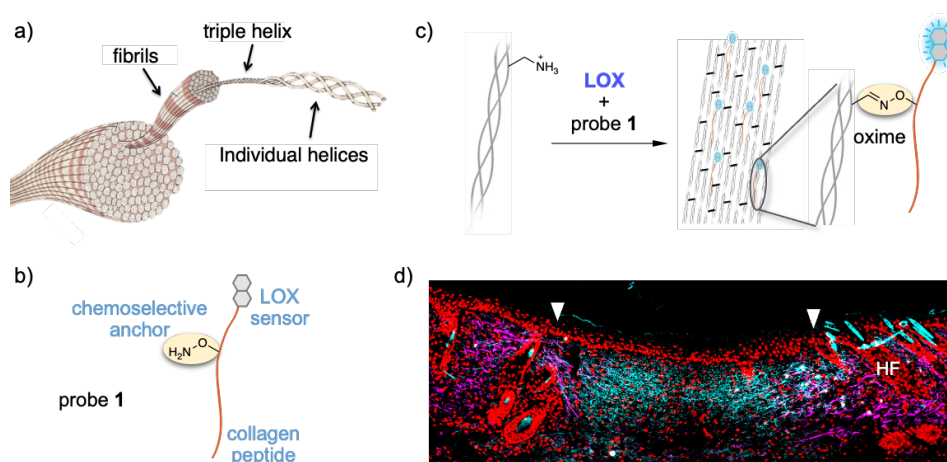


Fig. 1. Chemoselective targeting of collagen cross-linking with a chemical probe. A) Basic structure of collagen. b) Targeting probe with LOX-responsive fluorescence turn-on sensor and anchoring group. c) Targeting of collagen cross-linking sites. d) Visualization of remodeling and maturing collagen in a wound section.

The system consists of a LOX-specific turn-on fluorescent sensor, a collagen mimetic peptide, and an aminooxy group for chemoselective ligation to the aldehyde moieties created by LOX (). All three components of the synthetic reporter work in concert to visualize LOX-created cross-linking sites during the extracellular maturation of collagen. *In vivo* studies with mice and *ex vivo* studies with histological sections from, *e.g.*, tumors, wounds, fibrotic lung tissue, revealed an unprecedented level of specificity and spatial resolution for targeting newly forming and remodeling collagen, and, thus, distinguishing between healthy and disease states [7,8].

Peptide catalysis – from simple to complex environments: Peptides serve numerous functions in nature, *e.g.*, as hormones, neurotransmitters, and toxins. Yet, not a single natural catalytically active peptide is known. Instead, catalysis in nature is a hallmark of enzymes. But is this really so? During the past two decades, research by Scott Miller and my laboratory established peptides as potent catalysts for different synthetically useful

reactions [9] Many of these peptides exhibit exquisite levels of stereoselectivity. Yet, whereas enzymes catalyze reactions in aqueous media, most peptide catalysts operate in organic solvents. A further notable difference is the environment in which catalysis takes place. Enzymes work in highly complex cellular environments – reaction media that require exceptional chemoselectivity – while peptide catalysts are used in well-defined environments consisting only of substrates and products in pure solvents. Moreover, enzymes operate at significantly lower concentrations than peptide catalysts.

My laboratory has established peptides of the type H-Pro-Pro-Xaa for aldol and related addition reactions [10]. These catalysts overcome the generally low reactivity of amine-based organocatalysts, allowing for reactions in the presence of as little as 0.05 mol% of the peptide catalyst [11]. The modular structure allowed for establishing tailored catalysts for reactions with different electrophiles, including nitroolefins, dicyanoolefins, allenamides, C-substituted maleimide, and α -substituted vinyl triflones [12,13]. Our studies showed that these peptide catalysts operate for each type of reaction on a broad scope of substrates with high stereoselectivity. Furthermore, catalysis with H-Pro-Pro-Xaa type peptides is compatible with metal catalysis [14], avoids the use of protecting groups [15], can install quaternary stereogenic centers [16], and is so robust that an immobilized catalyst can be used in flow [17].

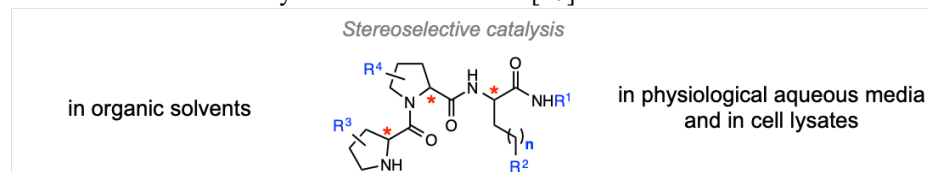


Fig. 2. Peptide catalysis in organic solvents and complex aqueous environments.

Building on these results in organic solvents, we interrogated whether a peptide catalyst can exhibit chemoselectivity reminiscent of enzymes. The lipidated peptide H-DPro-Pro-Glu-NHC₁₂H₂₅ forms conjugate addition products between aldehydes and nitroolefins with the same high stereoselectivity and comparable reactivity in water as in organic media [18]. Further studies showed that peptide catalysis can play in concert with enzymes, catalyzing cascade reactions in one pot [19]. Furthermore, our peptide catalysts proved to be sufficiently chemoselective to operate in complex mixtures, including cell lysates [18]. These results imply that peptide catalysis can take place in the complex aqueous environment out- and inside of cells and might be feasible on demand. The data also suggests that peptides have likely played a key role in the chemical evolution of enzymes

Outlook to future developments of research on exploring chemistry in the (extra)cellular space

Chemical tool will enable the precise targeting and manipulation of biological processes through stoichiometric or catalytic chemoselective reactions. Catalytic peptides

have the potential to allow for intersecting critical biosynthetic pathways. The application of tools to target, with spatial precision, the location where active collagen formation and remodeling take place opens unprecedented opportunities for probing tissue dynamics in various physiological and pathological scenarios. Chemoselective targeting probes will likely provide solutions to the diagnosis and treatment of diseases characterized by excessive tissue formation.

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