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# Targeting and mimicking collagens via triple helical peptide assembly

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As the major structural component of the extracellular matrix, collagen plays a crucial role in tissue development and regeneration. Since structural and metabolic abnormalities of collagen are associated with numerous debilitating diseases and pathologic conditions, the ability to target collagens of diseased tissues could lead to new diagnostics and therapeutics. Collagen is also a natural biomaterial widely used in drug delivery and tissue engineering, and construction of synthetic collagen-like materials is gaining interests in the biomaterials community. The unique triple helical structure of collagen has been explored for targeting collagen strands, and for engineering collagen-like functional assemblies and conjugates. This review focuses on the forefront of research activities in the use of the collagen mimetic peptide for both targeting and mimicking collagens via its triple helix mediated strand hybridization and higher order assembly.

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## Introduction

Collagen, the most abundant protein in mammals, plays a critical role in tissue development and regeneration. It is a major structural component of the extracellular matrix (ECM), where cells proliferate and differentiate. While fibrous collagens (e.g., type I, II) provide mechanical strength to connective tissues, network-like collagens (e.g., type IV) form the basic scaffold of the basement membrane where cells attach and grow into organized tissues. Abnormal collagen remodeling activities are typically seen during wound healing response and in chronic pathological conditions such as cancer, osteoporosis, arthritis, and fibrosis. Therefore, the ability to target remodeling collagens could help understand the progression of such diseases, as well as provide new diagnostic and therapeutic opportunities. Collagen is one of the most widely used natural biomaterials for medical applications in biocompatible coatings, drug delivery and tissue engineering. However, in order to overcome the structural and compositional complexity of animal-derived collagen, researchers are constructing artificial collagen-like scaffolds with tunable physico-chemical properties and unique biological functions. Collagen mimetic peptide (CMP) is a family of small synthetic peptides that mimic natural collagens: they share the collagen's hallmark structural motif — the triple helix, as well as the Gly-Xaa-Yaa triplet repeat sequence, where Xaa and Yaa are largely populated by proline and 4(*R*)-hydroxylproline, respectively [1]. These peptides were traditionally used as synthetic models to study the structure and folding behaviors of collagens [1]. In this review, we will discuss recent progress in two distinct research areas in the biomedical application of CMPs: firstly targeting collagens in pathological tissues [2••,3,4,5•], and secondly creating self-assembled collagen-like biomaterials and molecular constructs [6,7,8••], both of which are based on the CMP's unique triple helical structure. With our eyes set on identifying applications in bioimaging, drug delivery, and tissue engineering, we will review recent progress in CMP-based collagen/gelatin targeting in the context of other collagen-targeting molecules, as well as highlight various CMP derivatives, and collagen mimetic assemblies and conjugates that are inspired by the structure and function of natural collagen.

## Collagen-targeting molecules

Among many collagen binding molecules, only a few have been explored in the context of collagen targeting. One example is CNA35, a 35 kDa collagen-binding domain in the adhesin protein found on the surface of bacterium *Staphylococcus aureus*, which hugs the triple helical collagen molecule with its two subdomains through hydrophobic interactions [9]. Merkx group and others developed fluorescently labeled recombinant CNA35 and CNA35-functionalized micelles for visualization of collagens in various biological samples including cell culture (e.g., collagen-producing myofibroblasts), engineered tissue constructs, and animal tissues (e.g., blood vessels and kidney) [10–12]. They found out that fluorescent CNA35 administered *in vivo* shows high uptake in atherosclerotic arteries, particularly in the areas of atherosclerotic plaque that are rich in collagen networks [13]. Using the phage display method, Caravan and coworkers developed a type I collagen-binding cyclic peptide which was used as MRI contrast agent for myocardial scar [14]. During the phage display efforts to find cartilage binding peptides,

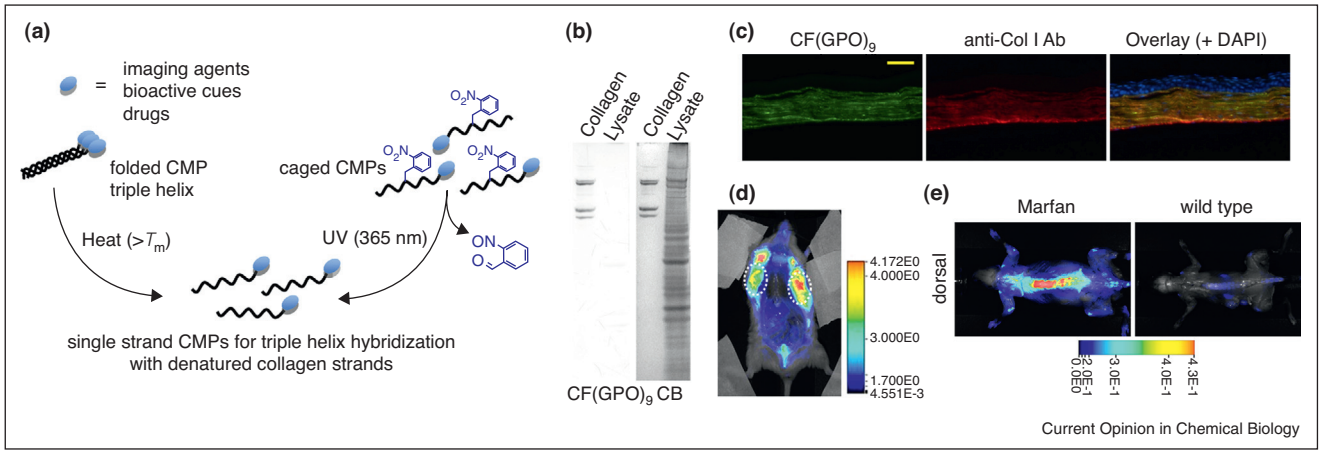
Hubbell's research group identified a type II collagen binding peptide of sequence WYRGRL. This peptide supported local delivery and immobilization of polymeric nanoparticles in knee cartilages after intra-articular injection [15]. A peptide mimetic of glycoprotein VI, the main platelet receptor of type I and III collagens, was used for *in vivo* radioactive imaging of lung fibrosis and scars in healed myocardial infarction [16]. A peptide derived from the collagen-binding proteoglycan, decorin, was also used to target collagens to modulate collagen fibrillogenesis [17], and to reduce collagen degradation and dermal scarring [18].

Since collagen is present ubiquitously in the body, collagen-binding molecules discussed above face the challenge of distinguishing collagens in the diseased tissue from those in the healthy tissue, if they are to be used for targeted drug delivery and molecular imaging [13]. Some strategies have been taken to design or select collagen targeting molecules that are more specific to collagens in diseased tissues. Recombinant CNA35 constructs whose collagen-binding is activated by matrix metalloproteinases (MMPs) showed improved selectivity for collagens undergoing MMP-mediated remodeling [19,20]. Library approaches have been used to identify monoclonal antibody [21,22] and peptides [23] that specifically bind to cryptic sites in collagen strands that become exposed after denaturation. For example, humanized monoclonal antibody (D93) that recognizes the GPO repeating sequence in denatured collagen strands was developed by Baeuerle and coworkers [22,24]. Studies showed that D93 specifically adheres to vascular basement membrane in tumors but not to blood vessels in normal tissues [22]. The peptide sequence, TLTYTWS, which was selected by phage display for binding to MMP2-modified type IV collagen was found to accumulate in tumors and inhibit angiogenesis *in vivo* [23].

## CMP-collagen hybridization

The triple helix, which is the hallmark structure of collagen, provides a unique mechanism for targeting denatured collagen strands. The triple helical tertiary structure is nearly exclusively seen in collagens except as small sub-domains in a few non-collagen proteins [1]. During tissue remodeling, the collagen molecules within the collagen fibers and networks are degraded by proteases (e.g., MMPs or cathepsin) and become denatured at body temperature. We recently discovered that the collagen mimetic peptide [sequence: $(GPO)_n$, $n$ = 6–10] with its strong triple helix folding propensity can specifically bind to such denatured collagen strands both *in vitro* and *in vivo* (Figure 1) [2••,3,25]. This binding is primarily driven by the triple helix hybridization between monomeric CMPs and the denatured collagen strands, which is similar to DNA fragments binding to complimentary DNA strands. Because homotrimeric CMPs have little driving force for collagen hybridization, CMPs had to be thermally dissociated to the monomeric state before binding to collagen substrates (Figure 1a, left panel). Such thermally induced CMP hybridization allowed us

**Figure 1**




**(a)** Schematic illustration of two approaches (heat and UV activation) to generate single strand CMPs that hybridize with denatured collagen strands. **(b)** Fluorescent images of SDS-PAGE gel loaded with type I collagen and endothelial cell lysate stained by carboxyfluorescein-labeled CMP [$CF(GPO)_9$] (left) in comparison to the same gel stained by coomassie blue (CB) (right) showing high specificity of CMP-collagen hybridization (adapted from Ref. [3]). **(c)** Fluorescent micrographs of fixed mouse cornea sections stained by photo-triggered caged carboxyfluorescein-labeled CMP (green), and co-stained with anti-collagen I antibody (red) and DAPI (blue). The CMP staining clearly reveals the parallel organization of collagen fibrils in the corneal stroma (adapted from Ref. [3]). **(d)** Near infrared (NIR) fluorescence image of a mouse bearing PC-3 prostate tumors at forward flanks (circled) administered with UV-activated caged and NIR fluorophore labeled CMPs, indicating stable and tumor specific CMP uptake (adapted from Ref. [2••]). **(e)** Comparative NIR fluorescence images of mouse model with Marfan syndrome showing high CMP uptake in the skeleton of the diseased mouse (adapted from Ref. [2••]).