



Cite this: *Org. Biomol. Chem.*, 2018, **16**, 7139

Received 24th July 2018,  
Accepted 10th September 2018

DOI: 10.1039/c8ob01773e

rsc.li/obc

## A pendant peptide endows a sunscreen with water-resistance†

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**Ultraviolet light causes skin cancer. Salicylic acid and other molecular filters absorb damaging radiation but are washed away readily. Conjugation to a collagen mimetic peptide is shown to retain salicylic acid on collagen-containing skin surrogates after repeated washing. This strategy, which is highly modular, could enhance the water-resistance of sunscreens.**

Skin is our largest organ.<sup>1</sup> Our skin not only protects underlying muscles, bones, ligaments, and internal organs, but also regulates body temperature and is the conduit for the sensations of touch, heat, and cold. Ultraviolet (UV) radiation from the sun damages skin, leading to burns, premature aging, immunosuppression, and cancer.<sup>2–13</sup> In the US, skin cancer is more prevalent than all other types of cancer combined.<sup>12,14</sup> Accordingly, UV radiation is a major public health threat. The risk can, however, be reduced by the proper use of sunscreens.<sup>2,5–9,15</sup>

UV radiation is divided into three types, based on wavelength: A, B, and C. Type C is blocked by atmospheric ozone, but UVA (320–400 nm) and UVB (290–320 nm) can penetrate human skin and damage DNA and other biomolecules.<sup>3,4,11,12</sup> Hence, sunscreens contain molecular “filters” to absorb UVA and UVB radiation.<sup>16,17</sup> Typical filters are small aromatic compounds, such as salicylates, cinnamates, benzophenones, or derivatives of *p*-aminobenzoic acid.<sup>16,18</sup>

Despite the widespread availability of sunscreens, public compliance with their use is a problem.<sup>19,20</sup> Moreover, the skin of patients with autoimmune diseases (*e.g.*, psoriasis, eczema, vitiligo, or lupus) or who take immunosuppressant drugs is highly photosensitive, and these patients have an especially

high risk of skin cancer.<sup>5–9,21</sup> Organic chemists have addressed this issue by attaching lipophilic moieties to filters. The ensuing hydrophobic interactions deter water and sweat from washing away an applied sunscreen. Unfortunately, these hydrophobic interactions are not only weak and short-lived, but also lead to undesirable greasiness that diminishes compliance.<sup>22</sup>

Collagen is the most abundant protein in the human body and the primary component of skin.<sup>23</sup> Collagen strands form triple helices that assemble into higher-order structures. Natural collagen contains loops or other interruptions in its triple helix,<sup>24–26</sup> and these regions provide binding sites for collagen mimetic peptides (CMPs<sup>27–29</sup>).<sup>30–33</sup> Damaged skin, which is more vulnerable than healthy skin to UV radiation,<sup>5–9,21</sup> is likely to contain additional binding sites. In previous work, we used CMPs to anneal pendant dyes and a cytoactive factor to collagen.<sup>32,34</sup> We reasoned that that a CMP could likewise and beneficially anchor a pendant UV-filter. Herein, we test that hypothesis and its manifestations.

For a proof-of-concept, we selected salicylic acid (Sal) because its 2-ethylhexyl ester is a common ingredient in commercial sunscreens (Fig. 1). We reasoned that the carboxyl group of salicylic acid could be tethered to a CMP *via* an amide bond. We were aware, however, that the absorbance of a UV-active molecule is sensitive to its substituents. Accordingly, an amide of salicylic acid could have a different absorbance profile than the free acid or 2-ethylhexyl ester. To search for such a perturbation, we synthesized Sal-GlyOMe, which is the glycine methyl ester of salicylic acid. We found that Sal-GlyOMe maintains absorbance of UVB radiation comparable to that of salicylic acid and its 2-ethylhexyl ester (Fig. 2).

Confident that an amide bond does not compromise the UVB absorption of salicylic acid, we synthesized a salicylic acid-CMP conjugate (Sal-LCMP) by segment condensation<sup>35</sup> on a solid support. As the collagen segment, we chose (L-Pro-L-Pro-Gly)<sub>7</sub>, which contains only L-proline and glycine residues. This peptide does not form a stable triple helix with itself but does form stable triple helices with natural collagen strands and is not toxic to dermal fibroblast cells.<sup>32,34</sup> In the conjugate, the

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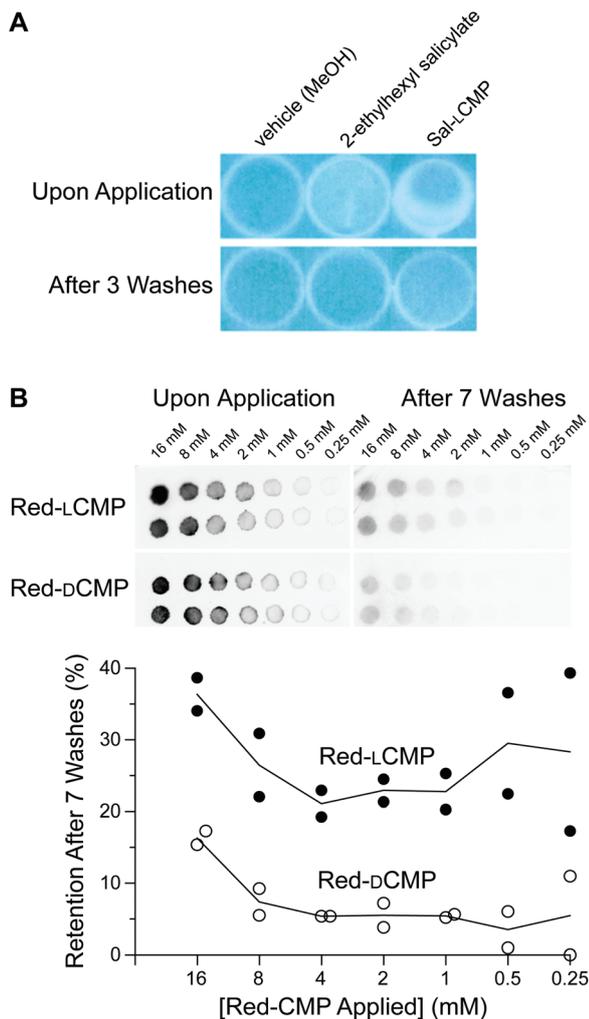
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† Electronic supplementary information (ESI) available: Synthetic and analytical procedures. See DOI: 10.1039/c8ob01773e

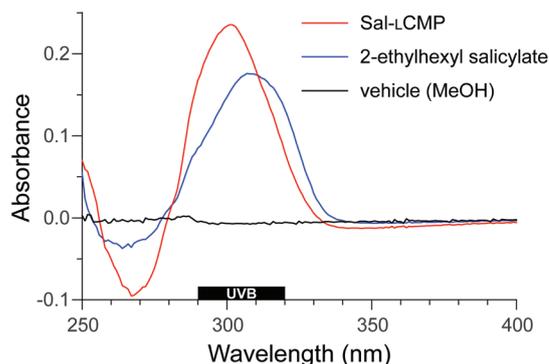




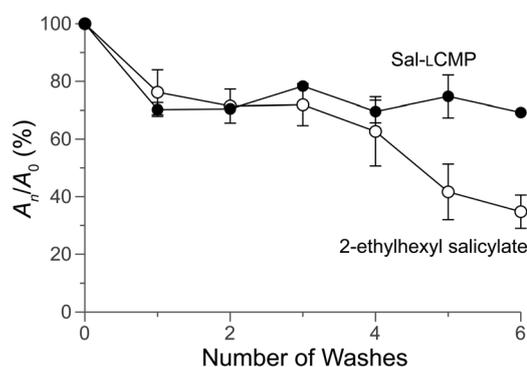


**Fig. 4** Images showing the adherence of CMP conjugates and related analytes to collagen surfaces, before and after washing. (A) Photographs of cyanotype paper after being covered by collagen-coated wells that had been treated with Sal-LCMP or a related analyte. (B) Top, inverse-fluorescence images of Vitro-skin® spotted with 5  $\mu$ L of an aqueous solution of Red-LCMP or Red-DCMP (16  $\rightarrow$  0.25 mM). Bottom, Graph of the data as quantified with ImageJ software.<sup>37</sup> For experimental details, see: ESI, sections IV–VI.†

Towards that end, we spread Sal-LCMP and 2-ethylhexyl salicylate on the surface of Vitro-skin®. Then, we obtained the absorption spectra of the surfaces with a solid-state UV-vis spectrometer. Again, we observed comparable UV-vis spectra, showing greatest absorbance in the UVB range (Fig. 5). Next, we tested the longevity of the two sunscreens through a series of washes. The monitoring at 300 nm shows that Sal-LCMP and 2-ethylhexyl salicylate diminishes by 30% after the first wash (Fig. 6). With further washes, Sal-LCMP maintains UV absorption whereas that of 2-ethylhexyl salicylate continues to diminish. This result also indicates that the Sal-LCMP conjugate not only protects against UVB radiation, but also enhances the longevity of that protection compared to 2-ethylhexyl salicylate.



**Fig. 5** Solid-state UV spectra of Vitro-skin® treated with a methanolic solution of Sal-LCMP and 2-ethylhexyl salicylate at 0.14  $\mu$ mol  $\text{cm}^{-2}$ .



**Fig. 6** Graph showing the adherence of Sal-LCMP and 2-ethylhexyl salicylate to Vitro-skin®, before and after washing. Absorbance was measured at 300 nm initially ( $A_0$ ) and after a wash ( $A_n$ ), and values are the mean  $\pm$  SD from triplicate measurements. For experimental details, see: ESI, section VII.†

## Conclusions

We conclude that CMPs can anchor a pendant UV-filter on a collagen surface through multiple washes. There, the filter is able to absorb UV light. Hence, the use of a CMP tether merits consideration as a means to endow sunscreens with water resistance. This approach could be especially beneficial to patients with photosensitive skin. In addition, anchoring to collagen could diminish any systemic cytotoxicity of a sunscreen, expediting approval from regulatory agencies. Future applications could benefit from the multimeric display of UV-filters on a single CMP as well as from the use of state-of-the-art UV-filters<sup>18,41</sup> and CMPs containing L-fluoroproline residues, which can anneal extremely strongly to natural collagen.<sup>32</sup>

## Conflicts of interest

There are no conflicts to declare.



## Acknowledgements

We are grateful to Dr M. M. Vestling for advice and assistance. A. J. E. was supported by Chemistry–Biology Interface Training Grant T32 GM008505 (NIH). This work was supported by Grant R01 AR044276 (NIH).

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