Repurposing keratin waste as a support for biocatalysts via keratin binding fusion modules

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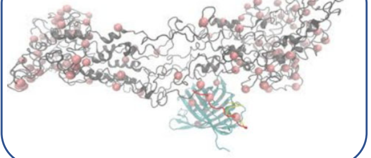
INTRODUCTION

Keratin is the main structural protein in nature, presented in various forms, including wool, feathers and hair. Millions of tons of keratin waste are produced every year, mostly disposed of in landfills or incinerated. Repurposing keratin waste as a support for biocatalysts is of industrial interest, adding value and reducing waste. A previously characterised keratin-based peptide reportedly binds to keratin through a cysteine-mediated disulfide-bridge (1-4). The phenomenon can be observed in **Fig. 1**, where the cysteine residue of the keratin peptide binds to cuticular keratin (1).

Fig. 1 – Peptide binding to keratin.



These peptides were fused to a chromoprotein and successfully used to dye overbleached Asian hair, resisting shampoo washes (**Fig. 2**) (1).

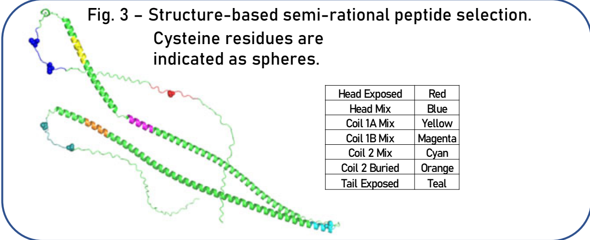
Fig. 2 – Chromoprotein fusion with keratin-based peptide dying hair.

The objective of this study is to investigate the binding capacity of keratin-based peptides from various regions of the keratin structure with and without cysteine-residues in textile waste.

MATERIAL AND METHODS

8 peptides with 13 amino acids were designed based on the keratin type II sequence. The selection considered various regions of the keratin structure, including head, coil and tail (**Fig. 3**). The peptides were fused to the fluorescent protein mVenus-Q69M (**Fig. 4**), expressed in *E. coli* BL21(DE3) with a pET28a vector. Proteins were purified with a HisTrap HP column using ÄKTA pure (**Fig. 5**), analysed and dialysed. Keratin binding assays were performed using multiple substrates, including cattle hair, 100 % wool, feather meal, and wool blends (n = 9).

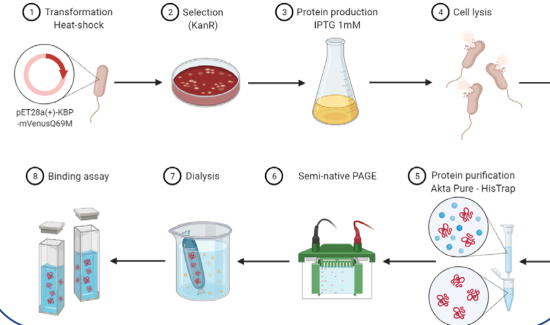
Fig. 3 – Structure-based semi-rational peptide selection. Cysteine residues are indicated as spheres.



Head Exposed	Red
Head Mix	Blue
Coil 1A Mix	Yellow
Coil 1B Mix	Magenta
Coil 2 Mix	Cyan
Coil 2 Buried	Orange
Tail Exposed	Teal

Fig. 4 – Fusion protein design – linearised plasmid

Fig. 5 – Protein purification rationale.



RESULTS

The expression of fusion proteins was successful (**Fig. 6A**). Fluorescence microscopy overlay showed that peptides prefer to bind at the ends of fibres (**Fig 6B**). Fusions did not bind to feather meal (**Fig. 7**). Though, binding was observed in wool blends and pure wool ($p < 0.0001$). Head and tail-based peptides presented binding capacity comparable to the literature peptide (**Fig. 7A**) (40-60%), whereas coil peptides did not show efficient binding (**Fig. 7B**) (0-20%), possibly due to the fewer binding sites available in the coiled coils, compared to head and tail. Low non-specific binding was observed with fluorescent protein without a peptide (**Fig. 7C**) .

Fig. 6A – Peptides did not affect protein fluorescence or expression levels.

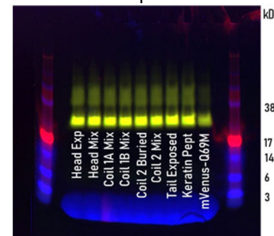
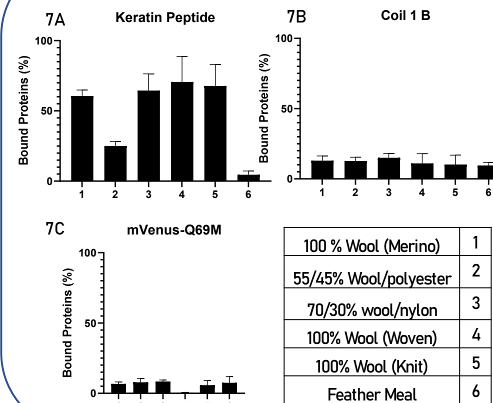


Fig. 6B– Fluorescence overlay showed that peptides bind at the ends of fibres.

Fig. 7 – Keratin binding assays



100 % Wool (Merino)	1
55/45% Wool/polyester	2
70/30% wool/nylon	3
100% Wool (Woven)	4
100% Wool (Knit)	5
Feather Meal	6

CONCLUSIONS AND PERSPECTIVES

Keratin-based peptides from the head and tail tend to bind better than coil peptides, possibly due to the keratin coiled-coil structure and absence of cysteine residues.

Fluorescence microscopy showed that these peptides prefer to bind to the ends of wool fibres.

Future work will involve developing new constructs, fusing the best candidates to enzymes and immobilising in a keratin-based column.

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