Optimized production of reflectins and characterization of their reversible self-assembly

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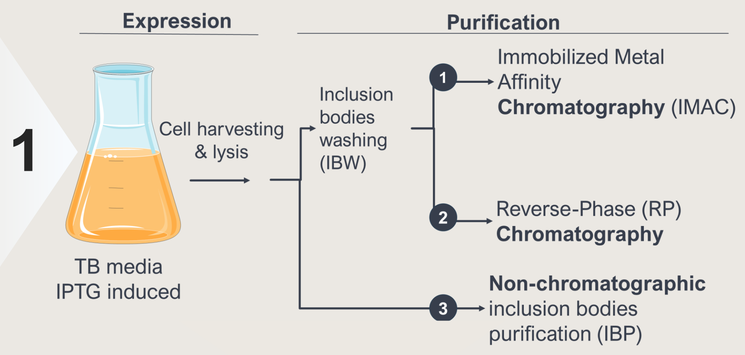
INTRODUCTION

Reflectins, crucial for cephalopod camouflage, are unique intrinsically disordered proteins within iridophores and leucophores that organize into microstructures, forming biological Bragg-reflectors in cephalopod organs [1,2]. With self-assembly triggered by a phosphorylation/dephosphorylation cascade, they possess promising hierarchical capabilities for biotechnological applications. This work aims to:

- Optimize reflectins' recombinant production & purification;
- Get insights into their charge-driven self-assembly;
- Explore the interplay between sequence and assembly reversibility;
- Identify new reflectin sequences in *Octopus vulgaris*, the most common cephalopod species along the Portuguese coast.

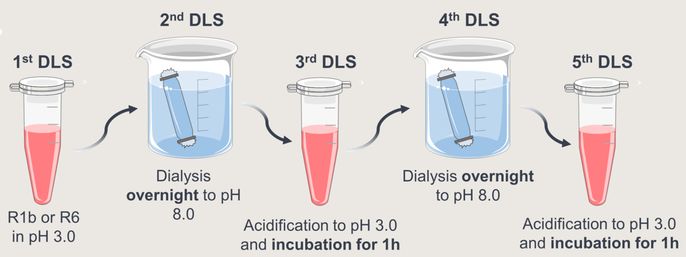
METHODS

Reflectins selection, production & purification



Characterizations & self-assembly

- Secondary structure (CD)
- Hydrodynamic diameter, stability, self-assembly reversibility (DLS & AFM)



Exploring reflectin sequences in *O. vulgaris:*

- Extract genomic DNA from octopus' epidermal layer.
- Design primers pairs based on a reflectin consensus sequence
- Apply PCR to amplify reflectin sequences.
- Use Sanger sequencing for gene sequence determination.

RESULTS

1.1 Reflectins selection

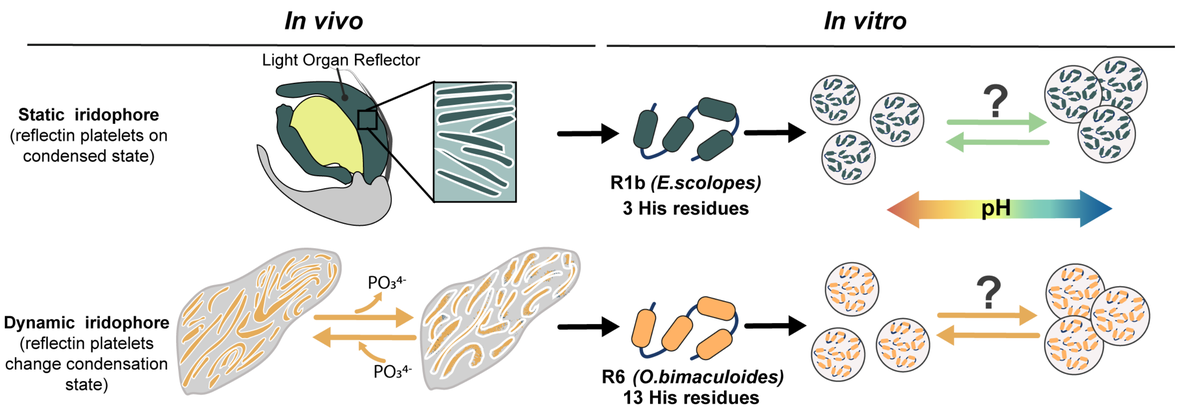


Figure 1 - Postulated *in vivo* and *in vitro* mechanisms for reflectins assembly in static and dynamic iridophores.

- R1b (static iridophores) and R6 (dynamic iridophores) differ in His content, suggesting distinct pH sensitivity [1].

1.2 Recombinant production & purification [3]

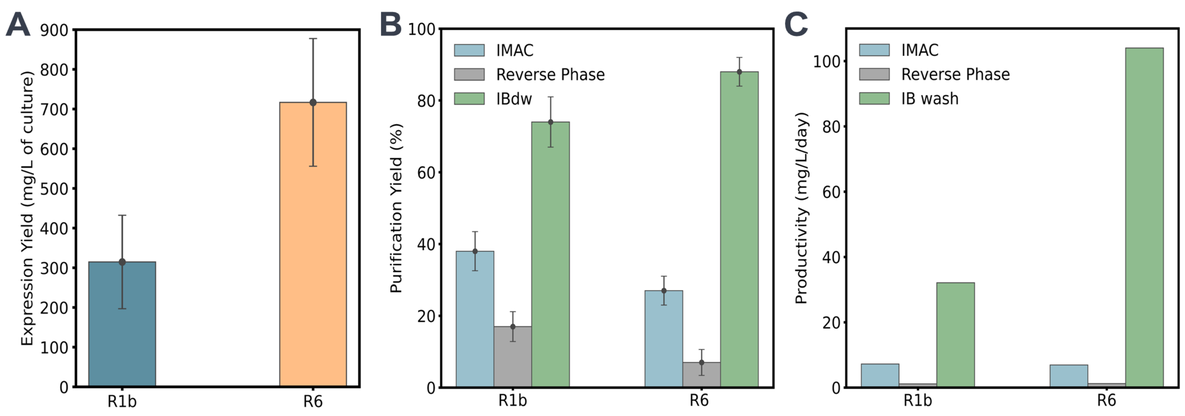


Figure 2 - **(A)** The average expression yields of reflectins (n=3). Comparison of the purification yields **(B)** and productivity **(C)** for different purification methods and both reflectins R1b and R6.

- Reflectins show high expression yields and are produced in inclusion bodies (IBs).
- Purification yields are lower with chromatographic versus non-chromatographic methods [3].

2.1 Reflectins characterization (*manuscript in preparation*)

- Both reflectins show a predominant disordered structural conformation.
- pH 3.0: R6, with a higher z-potential, forms smaller particles due to increased intra-protein electrostatic repulsion compared to R1b.
- pH 8.0: reflectins are highly unstable and nanoparticles tend to precipitate.
- pH 11.0: z-potential values indicate a high tendency for proteins to form large agglomerates.

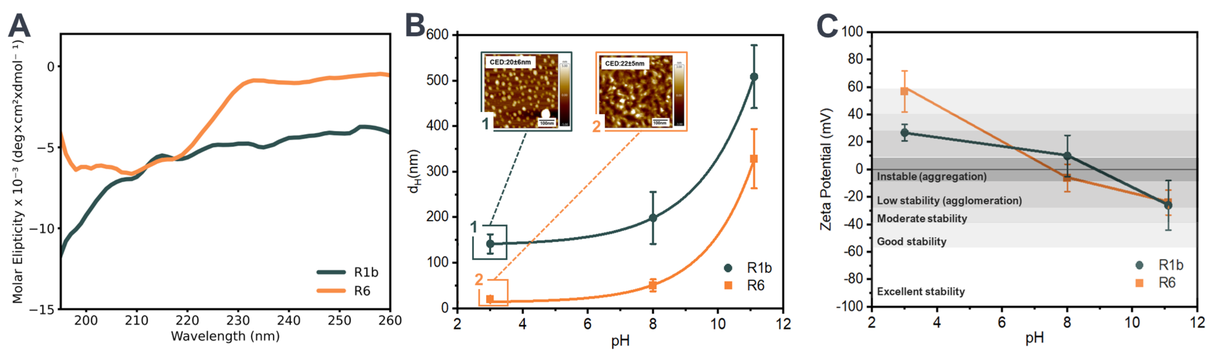


Figure 3 – **(A)** Far-UV Circular Dichroism spectra of the R1b and R6 at pH 3.0. **(B)** Average of hydrodynamic diameter obtained during DLS measurements. **(C)** Scattered plot with measured Z-potential for each protein assemblies at different pH.

2.2 Self-assembly reversibility

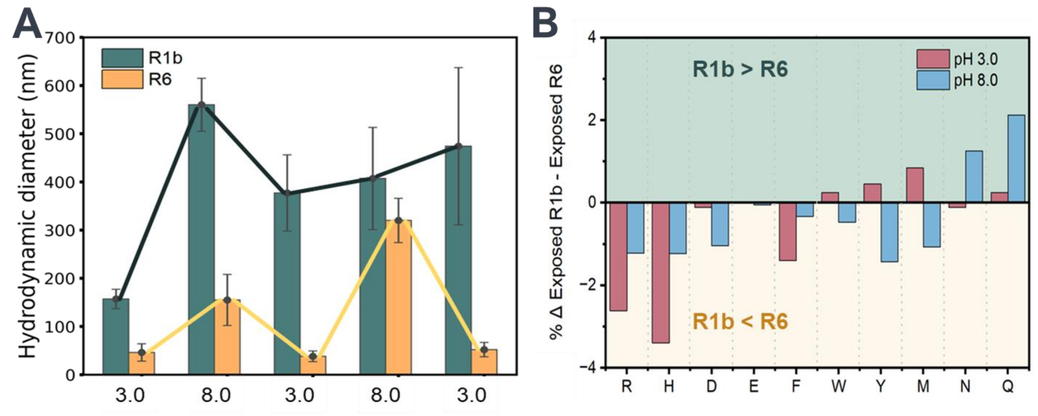


Figure 4 – (A) Variation of the dH measured by DLS. (B) Variation of % of exposed amino acid residue between R1b and R6 at different pH.

- Both proteins experience significant dH changes with the initial pH shift from 3.0 to 8.0. While R1b remains consistently stable in size, R6 shows noticeable dH variations, consistently recovering its size after each pH change.
- R6 has a higher percentage of exposed charged residues than R1b at both pH levels.

3. Exploring new reflectin sequences in *Octopus vulgaris*

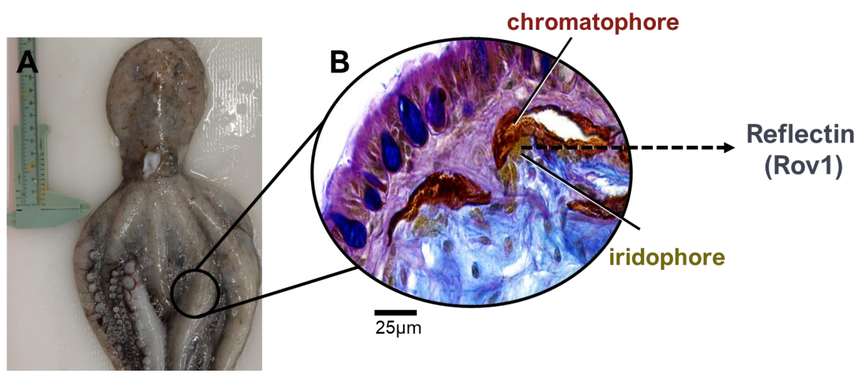


Figure 5 – **(A)** *Octopus vulgaris* specimen collected in Costa da Caparica, Portugal. **(B)** Paraffin section of *Octopus vulgaris* skin with Tetrachromic staining.

- Rov1 (344 residues and MW of 44.5 kDa) exhibits a characteristic reflectin sequence structure and composition. It shares 57% sequence identity with R1b and 80% with R6.

CONCLUSIONS

- Non-chromatographic purification (IBs purification) is highly efficient, involving IB resuspension and recovery through centrifugation, eliminating the need for a stationary phase.
- Reflectins' disordered conformation enables structural flexibility, adapting to pH variations and facilitating hierarchical self-assembly through protein-protein interactions.
- pH-induced reversible self-assembly relies on the composition of charged residues, their exposure, and the protein's dynamic capacity for interactions.
- Rov1 is the 1st described reflectin sequence in *O. vulgaris*; however, further studies are needed to confirm if it is the single reflectin in this species.

REFERENCES

[1] DeMartini D. *et al.*, *Jorn. Biol. Chem.*, vol.290, 2015; **[2]** Hanlon R.T. *et al.*, *Bioinspir. Biomim. Chem.*, vol.20, 2018 **[3]** Lychko I. *et al., Sep. Purif. Technol*., vol.315, 2023

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