17th International Echinoderm Conference &
2nd International Hemichordate Meeting

15 – 19 July 2024
Puerto de la Cruz, Tenerife

Interspecific Analysis of the Glycans Present in Sea Urchin Adhesive Organs

Ventura, I.[1], Eeckhaut, I.[2] , Dupont, S.[3], Byrne, M.[4], Flammang, P.[2] and Santos, R.[1]*

[1] MARE – Marine and Environmental Sciences Centre / ARNET – Aquatic Research Network, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Portugal

[2] Biology of Marine Organisms and Biomimetics Laboratory, Research Institute for Biosciences, University of Mons, Belgium

[3] Department for Biological and Environmental Sciences, University of Gothenburg, Sweden

[4] School of Life and Environmental Sciences, University of Sydney, NSW, Australia

Sea urchins possess specialized adhesive organs, the tube feet, that produce adhesive and de-adhesive secretions to attach and detach repeatedly from the substrate. Given the biotechnological potential of their strong reversible adhesive, the protein and glycan molecules that compose these secretions have been the object of investigation.

Although initially research focused on a single species, *Paracentrotus lividus* (Simão et al. 2020), a follow-up study was performed using three other species, *Diadema africanum*, *Arbacia lixula* and *Sphaerechinus granularis* (Gaspar et al., 2021). The results indicated a high interspecific variability in the glycans involved in sea urchin adhesion, but there appears to be greater conservation among taxonomically related species such as *P. lividus* and *S. granularis*. However, while a broad range of lectins (molecules that bind specifically to glycans) was used for *P. lividus*, only five lectins were tested on the tube feet of the other three species, thus limiting the screening only to the detection of Nacetylglucosamine and N-acetylgalactosamine.

To further confirm these results, there is a need to expand to more species and lectins, to find out if there are common key elements in their adhesives and whether these similarities are related with their geographical distribution, habitat, or taxonomy.

In the present work we carried out a broader comparative study including 15 species with contrasting taxonomy (four orders, seven families and 11 genera) and habitat (in terms of depth, substrate, and hydrodynamics) and used a battery of 22 lectins and two complementary techniques (lectin histochemistry and lectin blotting).

Our aim was to evaluate whether there is a taxonomy-related conservation of the glycans which are conjugated with the adhesive proteins, thus contributing to a better understanding of the evolution of adhesion mechanisms in echinoids thereby allowing the identification of key elements that explain their temporary adhesion and as potential for replication as biomimetic adhesives.

P1

Transcriptome analysis of the tube feet in the sea urchin *Sphaerechinus granularis*

Ventura, I.[1,2], Delroisse, J.[3,4], Henriques, B.J.[2], Flammang, P.[3] and Santos, R.[1]*

[1] MARE – Marine and Environmental Sciences Centre / ARNET – Aquatic Research Network, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Portugal

[2] BioISI – Biosystems and Integrative Sciences Institute, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

[3] Biology of Marine Organisms and Biomimetics Unit, Research Institute for Biosciences, University of Mons, Belgium

[4] Cellular and Molecular Immunology, GIGA-R, University of Liège, Belgium

Sea urchins possess specialized adhesive organs, the tube feet, which are able to attach to various substrates through the secretion of a strong adhesive, and detach from it within seconds. Given its potential biotechnological applications, our lab has been focusing on the molecular characterization of the sea urchin biological adhesive.

Although there are still gaps in our understanding of temporary adhesion, some common features have been identified as a direct result of the increasing number of transcriptomic and proteomic studies on the adhesive organs and/or secretions of several aquatic invertebrates. For sea urchins, molecular information is only available for the species *Paracentrotus lividus* (Lebesgue et al. 2016; Pjeta et al. 2020). Tube foot transcriptome sequencing combined with footprint proteome, together with tube foot differential gene and protein expression analyses (adhesive disc versus non-adhesive stem) and in situ hybridization (ISH), allowed to identify 16 adhesion-related proteins potentially involved in tube foot temporary adhesion. Of these, 6 shared significant similarity with orthologue adhesion-related proteins from the sea star *Asterias rubens* (Lengerer et al. 2019), indicating functional sequence conservation among echinoderms.

To provide new insights regarding the key elements responsible for the adhesion of the sea urchin species *Sphaerechinus granularis*, we first produced the tube foot transcriptome. Using differential gene expression analysis comparing whole tube feet to non-adhesive stem tissues, adhesive protein candidates have been identified and retrieved. Comparing them with the adhesive proteins of *P. lividus* makes it possible to determine whether sea urchin species with contrasted taxonomy and habitats share common adhesion- related proteins or if there are species-specific molecules.

P44

Sea urchin-inspired biological adhesives – from protein production to characterization

Santos, M.R.[1], Ventura, I.[1,2], Gomes, C.M.[1], Santos, R.[2]* and Henriques, B.J.[1]

[1] BioISI – Biosystems and Integrative Sciences Institute, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

[2] MARE – Marine and Environmental Sciences Centre / ARNET – Aquatic Research Network, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Portugal

Nowadays there is a great need in biomedicine and biotechnology for biological adhesives that are non-cytotoxic and effective in wet/humid environments. It is already known that marine invertebrates can attach to several substrates in the presence of seawater (high dielectric and ionic strength similar to physiological fluids) through the production of protein-based adhesive secretions with remarkable adhesive properties. This knowledge has inspired the development of a few biomimetic adhesives, nonetheless, there is still a need for new ones with novel capabilities. To contribute to the development of these new materials, our group has been studying sea urchins' adhesion, aiming to uncover the adhesive proteins responsible for their attaching abilities.

In the last decade, studies on *Paracentrotus lividus* identified Nectin as an important adhesive protein, present in both its adhesive organs and secretions. Nectin is a relatively large protein with ~110 kDa, presenting six galactose-binding discoidin-like (DS) domains which are thought to be important for its adhesive function. Here, our main goal is to discover one or more combinations of DS domains that will simultaneously confer adhesive properties and adequate stability comparable to the full-length protein, as well as optimized protocols for expression and purification of the identified targets.

We have analyzed recombinant protein expression in *E. coli* of several constructs, and the top targets were purified using a combination of chromatographic methodologies. The ones produced in significant amounts were structurally and conformationally characterized using spectroscopic techniques like circular dichroism (CD), fluorescence spectroscopy and differential scanning fluorometry (DSF). Briefly, we have managed to successfully express four different Nectin constructs, and one was purified with a 90-95% purity grade. This construct demonstrated to be fairly thermal stable and presented a folded conformation with a typical α/β structure.

We believe that this project contributes to increase the current knowledge on *P. lividus* adhesion mechanisms and opens new avenues for the development of sea urchin inspired bioadhesives for biomedical/biotechnological applications.