DATASET BRIEF

Proteome characterization of sea star coelomocytes – The innate immune effector cells of echinoderms

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Sea star coelomic fluid is in contact with all internal organs, carrying signaling molecules and a large population of circulating cells, the coelomocytes. These cells, also known as echinoderm blood cells, are responsible for the innate immune responses and are also known to have an important role in the first stage of regeneration, i.e. wound closure, necessary to prevent disruption of the body fluid balance and to limit the invasion of pathogens. This study focuses on the proteome characterization of these multifunctional cells. The identification of 358 proteins was achieved using a combination of two techniques for protein separation (1-D SDS-PAGE followed by nanoLC and 2-D SDS-PAGE) and MALDI-TOF/TOF MS for protein identification. To our knowledge, the present report represents the first comprehensive list of sea star coelomocyte proteins, constituting an important database to validate many echinoderm-predicted proteins. Evidence for new pathways in these particular echinoderm cells are also described, and thus representing a valuable resource to stimulate future studies aiming to unravel the homology with vertebrate immune cells and particularly the origins of the immune system itself.

Keywords:
2-D SDS-PAGE / Animal proteomics / Coelomocytes / *Marthasterias glacialis* / nanoLC-MALDI-TOF/TOF MS / Sea star

Similarly to other invertebrates, echinoderms lack an acquired immune system and therefore do not express the lymphoid antibody producers' cell line responsible for the existence of immunoglobulins in vertebrates. Nevertheless, they have a very well-developed nonspecific and nonadaptive immune response that shows similarities to higher vertebrate innate immunity. This response is mediated by the circulatory cells that occupy the perivisceral coelomic cavities – coelomocytes, which are key players in clotting reactions, phagocytosis, oxygen transport, synthesis, and secretion of antibacterial and antifungal proteins [1–3] namely, hemolysins, agglutinins, and lectins [2, 4, 5].

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Abbreviation: FDR, false discovery rate

Recently, the characterization of cDNA sequences from the purple sea urchin *Strongylocentrotus purpuratus* showed evidence for the homologies in the innate immune responses within the deuterostome lineage, which include echinoderms and vertebrates [6]. Sea urchins were shown to possess proteins homologous to the vertebrate C3 and factor B complement system components, called SpC3 and SpBf, respectively [6, 7]. These two proteins act together to promote opsonization of foreign cells and particles in sea urchins and subsequent destruction by the coelomocytes [7]. The level of complexity of echinoderm immune responses has been further demonstrated by the identification of several differentially expressed proteins in the coelomic fluid of sea urchins upon bacterial challenge, namely of proteins 185/333 which seem to be tailored to produce a pathogen-specific immune response and apextrin and calreticulin that seem to be involved in the sequestration or inactivation of bacteria [8–10].

In asteroids, coelomocytes have been reported to respond to trauma stress, having an important role in wound

closure, the first stage of regeneration. This is done by a rapid and massive accumulation of coelomocytes at the wound site, which plugs and heals the wound, helping to maintain homeostasis, thus preventing the loss of body fluids and limiting the invasion of pathogens [11–13]. At the molecular level, enhanced expression of profilin transcripts in coelomocytes has demonstrated the immune response of echinoderms to minimal injury [14].

Curiously, since the sequencing of the purple sea urchin, *S. purpuratus* genome, in 2006, [15], only a minority of the genome predicted proteins were validated by a few proteomic studies on echinoderms [8, 10, 16, 17], thus strengthening the need to verify and explore this rich source of information.

In the present study, several adult specimens of both genders of the sea star *Marthasterias glacialis* (Linné, 1758) were collected at low tide on the west coast of Portugal (Estoril, Cascais). Animals were transported to ''Vasco da Gama'' Aquarium (Dafundo, Oeiras) where they were kept in open-circuit tanks with recirculating seawater at 15°C and 33‰. They were fed ad libitum with a diet of mussels collected weekly at the same site. Animals used for the experiments had similar sizes, with radius ranging from 10 to 13 cm, measured from the largest arm to the centre of the oral disc.

To characterize the proteome of *M. glacialis* coelomocytes, the coelomic fluid of five sea stars was obtained by puncturing the animal epidermis at the arm tip with a needle and collecting the fluid by gravity into separate ice cold recipients, containing a protease inhibitor cocktail to prevent endogenous proteolysis. Then, low-speed centrifugation ($800 \times g$; 10 min; 4°C) was used to separate the circulating cells from the coelomic fluid.

The number of coelomocytes present in *M. glacialis* coelomic fluid was determined using a syringe with an anticoagulant to avoid clotting (1.2 mL paediatric syringes, S-Mono Vet Sarsted and 20Gx 1(1/2)' hypodermic needle, S-Mono Vet needle, Starsted). Cells were then counted using a Burker chamber, being comprised between 1 and 2×10^6 cell/mL which is in the range of the values reported for the sea star *Asterias rubens* [11].

For the proteomic experiments, pelleted coelomocytes were flash frozen in liquid N_2 and stored at −80°C until further processing. The deep frozen coelomocytes were then mechanically disrupted as described previously [18] and the resulting powder was resuspended in 1-D sample buffer [19] or 2-D solubilization buffer [20] (see Supporting Information 1 for a detailed description of all the procedures used). After cellular debris removal by a low-speed centrifugation ($1000 \times g$; 20 min, 4°C), the total protein concentration was determined using the 2D Quant Kit™ (GE Healthcare).

For 1-DE protein separation, 12.5% w/v acrylamide gels were loaded with 25 μg total protein per lane and stained with Colloidal Coomassie [21]. Then, gel lanes were sliced for in-gel digestion as shown in Fig. 1A.

Coelomocyte protein extracts were also subjected to 2-DE separation using an IPGphor system (GE Healthcare), 11 cm Immobiline pH 3–11 nonlinear DryStrip loaded with 400 μg total protein and 1% v/v of ampholytes (GE Healthcare) in the rehydration buffer. IEF was carried out with a minor adaptation due to high salt content of sea star coelomocytes. After a two-step equilibration of the strips for reduction and alkylation, the second dimension was performed in an Ettan DaltSix (GE Healthcare). The 24 cm gels containing two IEF strips were stained with Colloidal Coomassie [21] (Fig. 1B), scanned with LabScan (GE Healthcare), and analyzed with Progenesis SameSpots v.3.3 (Nonlinear Dynamics, Newcastle Upon Tyne, UK). To characterize the proteome of *M. glacialis* coelomocytes, five 2-D gels were run, each with the coelomocytes protein extract from one sea star. The spots were only selected for protein identification if they were present consistently in all analyzed 2-D gels. The excised 2-D gel spots and 1-D gel bands were digested as described previously [20] and the tryptic peptides were resuspended in 5% v/v formic acid.

Peptides from the 1-D gel digested bands were further separated with Proxeon Easy-nano-LC (Proxeon Biosystems, Odense, Denmark) using a C18 reversed-phase EASY-Column (10 cm, 75 μm id; Proxeon Biosystems). The obtained fractions were mixed with a solution of 5 mg/mL CHCA in 50% v/v ACN, 2.5% v/v formic acid, and deposited

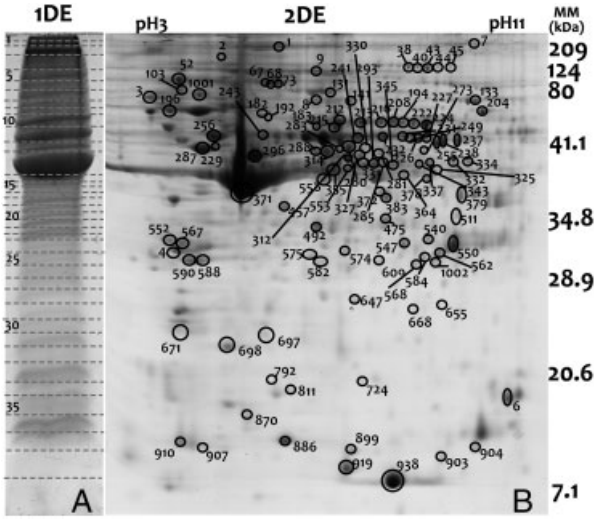

Figure 1. Gel separation of protein extract of the sea star *M. glacialis* coelomocytes by 1-D (A) and 2-D (B) electrophoresis. (A) Horizontal lines in the 1-D protein separation indicate the sections excised for in-gel tryptic digestion. See Supporting Information Material for the proteins identified in each band (Supporting Information Tables ST1 and ST3). (B) Black circles indicate spots with protein identification compliant with the specified criteria. The identified 2-D spots are annotated with numbers to facilitate a comprehensive reading of the Supporting Information material (Supporting Information Tables ST1 and ST2).

on a MALDI target plate using an online SunCollect automatic spotting system (SunChrom, Friedrichsdorf).

The tryptic digests from the 2-D gel spots were desalted and concentrated on chromatographic microcolumns using GELoader tips (Eppendorf) packed with POROS R2 (bead size, 20 μm) and directly eluted onto the MALDI plate using 0.5 μL of 5 mg/mL α-CHCA in 50% v/v ACN with 2.5% v/v formic acid.

Tandem MS was performed using a MALDI-TOF/TOF 4800 plus mass spectrometer (AB Sciex, USA). Raw data were generated by the 4000 Series Explorer Software v3.0 RC1 (AB Sciex) and contaminant *m/z* peaks resulting from trypsin autodigestion were excluded when generating the peptide mass list used for database search.

For the 2-D coelomocyte proteome characterization, in order to overcome the lack of a complete sea star genome information, which impairs the success of protein identification, two different protein identification algorithms were used: PARAGON® provided with the ProteinPilot software (version 3.0, revision 114732; AB Sciex) and MOWSE® from MASCOT (version 2.2; Matrix Science, Boston, MA, USA) and three different protein sequence databases were used for protein identification (Fig. 2). Protein identification files derived from MASCOT were converted to mzML files using PRIDE Converter tool [22] and are available in the PRIDE database [23] under accessions numbers 15332/15334.

For the nanoLC experiments regarding the tryptic digests from 1-D separation, peptides were identified using the algorithm PARAGON® provided with ProteinPilot software and the false discovery rate (FDR) was determined individually for each gel band using PSPEP algorithm supplied with this search engine, using the reversed and original database joined together. Identified proteins were selected if a FDR < 1%.

Protein clustering and parsimony analysis was performed using the MassSieve software [24] in order to reduce protein name redundancy. Protein isoforms were considered only if the unique peptide(s) were identified, and the lists of the identified proteins in both 1-D and 2-D experiments have all been compiled into the data set (Supporting Information Table ST1).

Sea star coelomocyte proteins identified as uncharacterized/unknown or homologous to *S. purpuratus* proteins were further submitted to protein–protein BLAST searches against Swiss-Prot database using the Basic Local Alignment Search tool available at NCBI website (http://blast.ncbi.nlm.nih.gov/). STRAP software [25] was used to fully annotate the identified proteins using the UniProt gene ontology information. Three independent sets of ontology were used in the annotation: the biological function in which the protein participates (Fig. 3A), their subcellular location (Fig. 3B) and their molecular functions (Fig. 3C). However, since cellular pathways focus on physical and functional interactions between proteins rather than merely taking the gene-centric view of GO-based analyses, a pathway analysis using DAVID functional annotation tools

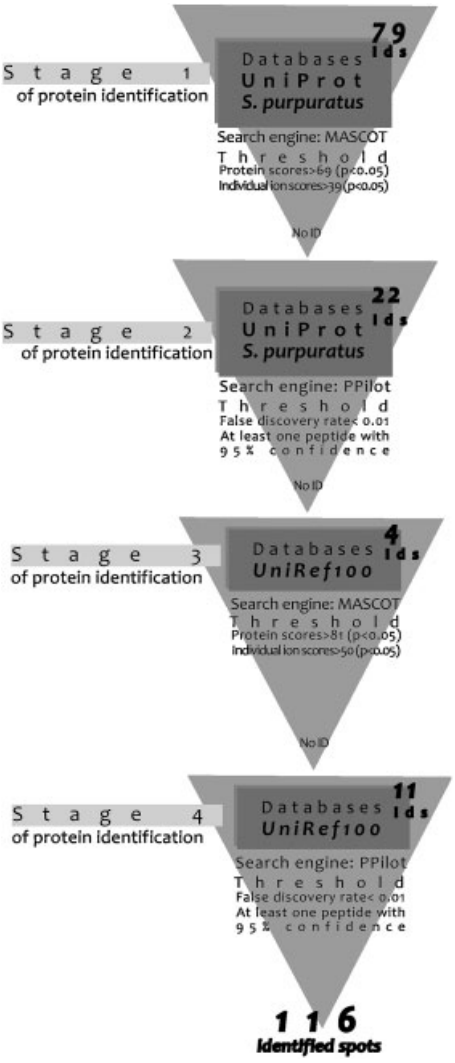

Figure 2. Coelomocytes 2-DE protein identification workflow. Schematic representation of the protein identification workflow using different search engines (MASCOT®, ProteinPilot®) and several protein databases : UniProt/Swiss-Prot (release 2010_04) joined together with the purple sea urchin *S. purpuratus* predicted database (42 420 entries; December 2006) and the nonredundant database Uniref100 (release 2010_06; 10 246 365 entries).

(http://david.abcc.ncifcrf.gov/home.jsp) [26] was also performed and a more comprehensive overview of the relevant functions enrolled by these cells is also presented (Supporting Information Table ST1).

The excised spots from the coelomocytes 2-D gels were processed for protein identification by MS and altogether, more than 85% of the selected spots were successfully identified (Supporting Information Table ST2) which was only possible using a protein identification workflow involving different databases and search algorithms. The coelomocytes reference 2-D map with the corresponding identified spots is shown in Fig. 1B, complemented by a

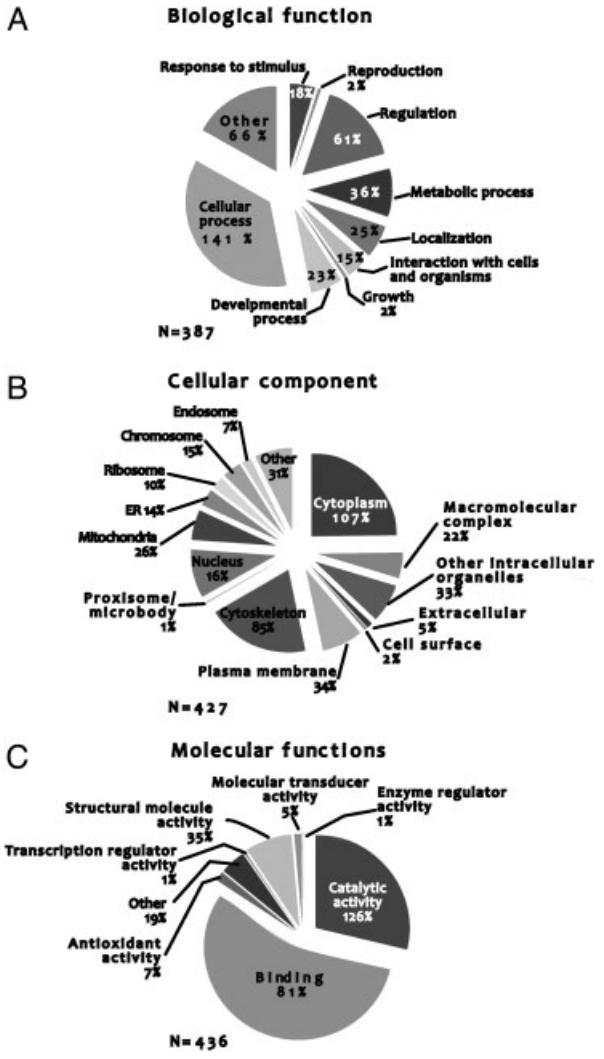

Figure 3. Gene ontology annotations of the identified sea star *M. glacialis* coelomocytes proteins. Biological functions (A) cellular component (B) and molecular functions (C).

detailed information on the number of identified proteins in each step of the described protein identification workflow shown in Fig. 2.

As in each 1-D band (Fig. 1A) there is a high probability of protein co-migration, an extra separation at the peptide level was also performed. This was achieved by injecting each band digest in a nano-flow HPLC coupled to a MALDI plate spotter. The peptides for each 1-D band were separated in one chromatographic run and the obtained 72 fractions per gel band were applied onto the MALDI sample plate. This approach, followed by database search using independent data for each band, allowed the identification of approximately six proteins per band from which were derived a total of 242 proteins with an estimated FDR of 1% (Supporting Information Table ST3).

The combination of two techniques for protein separation (1-D SDS-PAGE coupled with nanoLC and 2-D SDS-PAGE) followed by MALDI-TOF/TOF MS allowed the identification of 358 proteins, many constituting, to our knowledge, new assignments for echinoderm coelomocytes. Also, some of the identified proteins were present in more than one 2-D spot, indicating the presence of possible post-translational modifications or different protein isoforms that should be further investigated in order to obtain a more complete characterization of the coelomocytes proteome. Since only a few sea star proteins are deposited on the available protein sequence databases (1438 results for Asteroidea in UniProt of which only 58 are curated sequences), the present study is a homology-driven proteomic characterization. As expected, a high number of identified proteins were homologous with other echinoderm proteins deposited on the searched protein sequences databases (30%). However, several of the identified proteins from the sea star coelomocytes shared homology with proteins from other organisms (i.e. Chordata 34%; Nematode and Annelida 9%; Arthropoda 6%; Bacteria 6%), in some of the cases with only one identified peptide. This suggests the presence of novel forms of the proteins predicted in the sea urchin genome, which need to be further validated, and/or simply highlights the urgent need to increase the available information on genomes/proteomes of other echinoderm species.

A functional overview of the identified proteins in *M. glacialis* coelomocytes clearly highlights the multiple roles of these cells in the biology of echinoderms. The newly identified proteins provide preliminary evidence for several molecular pathways that have never been reported in coelomocytes of which some examples are described below:

Cytoskeleton regulation and cellular adhesion-related proteins: The phagocytic cell population (a dendritic-like coelomocyte phenotype) in *A. rubens* can perform a rapid morphological transition from petaloid to filipoidal shape [11]. In order for a cell to move and change shape, its cytoskeleton must undergo rearrangements that involve breaking down and reforming filaments. Two major pathways involved in these events are here revealed through several identified proteins. The first is the integrin signaling pathway, which is triggered when integrins in the cell membrane bind to extracellular matrix components causing downstream events such as actin reorganization and activation of MAPK and other signaling cascades [27]. The second pathway involves regulation by Rho GTPase, a family of key regulatory molecules that link surface receptors to the organization of the actin cytoskeleton. Also, several proteins which play a role in the regulation of cell adhesion and cytoskeleton organization were found: profilin, (already reported as being associated with changes in cell shape in the sea urchin coelomocytes [14]), ezrin; α-parvin, filamin A and C, several actin-binding and -capping proteins, clathrin-associated proteins and linker proteins.

Signaling, cellular regulation, and proliferation-related proteins: As coelomocytes secrete a number of regulating

factors into the coelomic fluid, the pathways that lie at the base of important biological events, like vesicular protein secretion mediated by G-protein receptor-activated pathways, were also represented through several identified proteins namely: clathrin heavy chain, AP-1 complex subunit μ, AP-2 complex subunit sigma, and ras-related protein Rab-11A. Moreover, several Ca^{2+}-binding proteins, such as calmodulin, calpain, calreticulin, and gelsolin were identified, indicating that like in other immune cell, calcium intracellular concentration is also an important second messenger in the signaling events [28] of echinoderm coelomocytes. Other regulatory proteins such as, cell division cycle and apoptosis regulator protein 1 LIM, senescent cell antigen-like-containing domain protein 2 and Rho-related GTP-binding protein RhoB were also found. Cell proliferation is tightly regulated by exposure to serum, growth factors, survival factors, and other cues from the cellular environment. This was shown to be the case also for coelomic fluid coelomocytes [1, 29, 30]. Several RAS family proteins and growth factors were also identified in this study, such as Ras-related proteins (e.g. Rab-10, Rab-6A, and Rab-7A) and the growth factor receptor-bound protein 2-B; LIM and senescent cell antigen-like-containing protein and the pre-B-cell colony-enhancing factor.

Regeneration-related proteins: Coelomocytes are involved in the very early stages of regeneration namely in the wound-healing phase [31], and the *wnt* genes have already been described as being involved in the formation of the thickened wound epithelia that is vital for regeneration in the echinoderm *Amphiura filiformis* [32]. Several proteins belonging to this pathway were also identified in the present study, namely, cAMP-dependent histone kinase and guanine nucleotide-binding protein subunit β-1.

Contrary to our expectations, no homologous proteins of the vertebrate complement system were identified. This suggests that to extend this proteomic characterization, new methodologies for the preparation of coelomocyte subcellular fractions, and the enrichment or depletion of low or abundant proteins will need to be developed. Nevertheless, the present study constitutes the first high-throughput proteomic characterization of echinoderm coelomic fluid-circulating cells, the coelomocytes. The newly identified coelomocyte proteins provide evidence for several unreported signaling pathways, eventually responsible for the diverse functions enrolled by these cells. Characterization of coelomocyte proteins post-translation modifications will further elucidate how the described pathways are being regulated. This comprehensive list of coelomocyte proteins is of extreme importance as a ground-work that will lead to future studies, which might clarify the homology with vertebrate immune cells or discover the pathways responsible for the coelomocytes functions during sea star regeneration events.

Protein identification files derived from MASCOT are available in the PRIDE database [23] under accession numbers 15332/15334.

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The authors have declared no conflict of interest.

References

[1] Cavey, M. J., Märkel, K. In: Harrison, F. W., Chia, F. S. (Eds.), *Microscopic Anatomy of Invertebrates. Echinodermata, vol. 14*, Wiley-Liss, New York 1994, pp. 169–245.

[2] Gross, P. S., Al-Sharif, W. Z., Clow, L. A., Smith, L. C., Echinoderm immunity and the evolution of the complement system. *Dev. Comp. Immunol.* 1999, *23*, 429–442.

[3] Haug, C. L. T., Stensvag, K., Antimicrobial peptides in echinoderms. *Invert. Surviv. J.* 2010, *7*, 132–140.

[4] Cervello, M., Arizza, V., Cammarata, M., Matranga, V., Parrinello, N., Properties of sea urchin coelomocytes agglutinins. *Italian J. Zool.* 1996, *63*, 353–356.

[5] Tahseen, Q., Coelomocytes: biology and possible immune functions in invertebrates with special remarkes on nematodes. *Int. J. Zool.* 2009, *2009*, 1–13.

[6] Al-Sharif, W. Z., Sunyer, J. O., Lambris, J. D., Smith, L. C., Sea urchin coelomocytes specifically express a homologue of the complement component C3. *J. Immunol.* 1998, *160*, 2983–2997.

[7] Smith, C. L., Shih, C. S., Dachenhausen, G., Coelomocytes express SpBf, a homologue of factor B, the second component in the sea urchin complement system. *J. Immunol.* 1998, *161*, 6784–6793.

[8] Dheilly, N. M., Nair, S. V., Smith, L. C., Raftos, D. A., Highly variable immune-response proteins (185/333) from the Sea Urchin, *Strongylocentrotus purpuratus*: proteomic analysis identifies diversity within and between individuals. *J. Immunol.* 2009, *182*, 2203–2212.

[9] Nair, S. V., Valle, H. D., Gross, P. S., Terwilliger, D. P., Smith, L. C., Macroarray analysis of coelomocyte gene expression in response to LPS in the sea urchin. Identification of unexpected immune diversity in an invertebrate. *Physiol. Genomics* 2005, *22*, 33–47.

[10] Dheilly, N. M., Haynes, P. A., Bove, U., Nair, S. V., Raftos, D. A., Comparative proteomic analysis of a sea urchin (*Heliocidaris erythrogramma*) antibacterial response revealed the involvement of apextrin and calreticulin. *J. Invert. Pathol.* 2011, *106*, 223–229.

[11] Pinsino, A., Thorndyke, M., Matranga, V., Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*. *Cell Stress Chaperones* 2007, *12*, 331–341.

[12] Carnevali, C., Bonasoro, F., Microscopic overview of crinoid regeneration. *Microsc. Res. Tecnhiq.* 2001, *55*, 403–426.

[13] Holm, K., Dupont, S., Sköld, H., Stenius, A., Thorndyke, M., Hernroth, B., Induced cell proliferation in putative hematopietic tissues of the sea star, *Asterias rubens*. *J. Exp. Biol.* 2008, *211*, 2551–2558.

[14] Smith, L. C., Britten, R. J., Davidson, E. H., SpCoel1, a sea urchin profilin gene expressed specifically in coelomocytes in response to injury. *Mol. Biol. Cell.* 1992, *3*, 403–414.

[15] Consortium, Sea Urchin Sequencing. The genome of the sea urchin *Strongylocentrutus purpuratus*. *Science* 2006, *10*, 941–952.

[16] Mann, K., Poutka, A. J., Mann, M., The sea urchin (*Strongylocentrotus purpuratus*) test and spine proteomes. *Proteome Sci.* 2008, *6*, 22.

[17] Sewell, M. A., Eriksen, S., Middleditch, M. J., Identification of protein components from the mature ovary of the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). *Proteomics* 2008, *8*, 2531–2542.

[18] Butt, R. H., Coorssen, J. R., Pre-extraction sample handling by automated frozen disruption significantly improves subsequent proteomic analyses. *J. Proteome Res.* 2006, *5*, 437–448.

[19] Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, *227*, 680–685.

[20] Franco, C., Santos, R., Coelho, V., Exploring the proteome of an echinoderm nervous system: 2-DE of the sea star radial nerve cord and the synaptosomal membranes subproteome. *Proteomics* 2011, *11*, 1359–1364.

[21] Neuhoff, V., Harold, N., Ehrhardt, W., Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis* 1988, *9*, 255–262.

[22] Barsnes, H., Vizcaino, J. A., Eidhammer, I., Martens, L., PRIDE converter: making proteomics data-sharing easy. *Nat. Biotechnol.* 2009, *27*, 598–599.

[23] Vizcaíno, J. A., Côté, R., Reisinger, F., Foster, J. M., et al., A guide to the Proteomics Identifications Database proteomics data repository. *Proteomics* 2009, *9*, 4276–4283.

[24] Slotta, D. J., McFarland, M. A., Markey, S. P., MassSieve: panning MS/MS peptide data for proteins. *Proteomics* 2010, *10*, 3035–3039.

[25] Bhatia, V. N., Perlman, D. H., Costello, C. E., McComb, M. E., Software tool for researching annotations of proteins: open-source protein annotation software with data visualization. *Anal. Chem.* 2009, *81*, 9819–9823.

[26] Da Wei Huang, D. W., Sherman, B. T., Lempicki, R. A., Systematic and integrative analysis of large genelists using DAVID bioinformatics resources. *Nat. Protoc.* 2009, *4*, 44–57.

[27] Yoo, Y., Guan, J.-L., Integrin signaling through focal adhesion kinase, in: LaFlamme, S. E., Kowalczyk, A. P. (Eds.), *Cell Junctions: Adhesion, Development, and Disease*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2008.

[28] Vig, M., Kinet, J. P., Calcium signaling in immune cells. *Nat. Immunol.* 2009, *10*, 21–27.

[29] Patruno, M., Thorndyke, M. C., Candia Carnevali, M. D., Bonasoro, F., Beesley, P. W., Growth factors, heat-shock proteins and regeneration in echinoderms. *J. Exp. Biol.* 2001, *204*, 843–848.

[30] Carnevali, C., Bonasoro, F., Welsch, U., Thorndyke, M. C., *Arm Regeneration and Growth Factors in Crinoids*, Mooi and Telford, San Francisco 1998, pp. 145–150.

[31] Coteur, G., DeBecker, G., Warnau, M., Jangoux, M., Dubois, P., Differentiation of immune cells challenged by bacteria in the common European starfish, *Asterias rubens* (Echinodermata). *Eur. J. Cell Biol.* 2002, *81*, 413–418.

[32] Dupont, S., Thorndyke, M., Bridging the regeneration gap: insights from echinoderm models. *Nat. Rev. Genetics* 2007, *8*, 1–4.

