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demonstrated a rapid shift in bioluminescent dynamics (Figure 2). Within 8 hours of infection bioluminescence had significantly decreased compared to uninfected control cells and by 9 hours it had been completely eliminated. While the magnitude of the initial bioluminescent dynamics differed between infection with virulent and non-virulent strains of *E. coli* O175:H7, the cessation of bioluminescence resulting from infection of either strain occurred in a similar fashion and across similar timescales. These results demonstrate that the use of autonomous bioluminescence presents a facile method for tracking cellular changes in a remote, automated fashion, without the need for investigator intervention. This makes *lux*-based imaging of cell cultures ideal for rapid, high throughput detection of changes in cellular growth and metabolic dynamics while reducing the screening cost compared to traditional substrate-dependent luciferase systems.

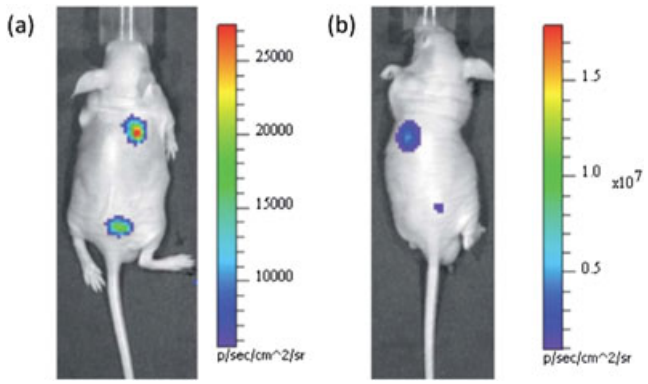

Figure 1. The autonomous bioluminescent signal from a) human optimized lux genes is similar in its pseudocolor detection to that of b) human optimized luc gene expression following treatment with its luciferin compound.

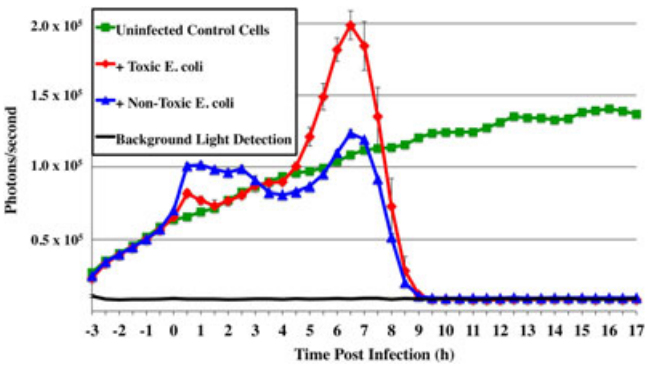

Figure 2. Human kidney cells expressing human optimized lux genes respond rapidly to infection with E. coli O157:H7.

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Chemiluminescence Functionalized Nanoprobes for Bioassays

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Recently, nanomaterials as biological labels have received increasingly considerable attention in chemiluminescence (CL) and electrochemiluminescence (ECL) bioassays due to their excellent chemical reactivity, catalytic property, surface property, biocompatibility and ease of self-assembly. Various CL/ECL functionalized nanoprobes have been exploited for bioassays. In these cases, one analytical probe can carry a number of signal reporters so that CL/ECL signals can be greatly amplified. From structural point of view, these CL/ECL functionalized nanoprobes can be divided into two types. One protocol involves the CL reagents indirect capping on the surface of nanomaterials by virtue of bridge molecules. Another protocol is to dope the CL/ECL reagents into nanomaterials. Although these protocols can achieve very high sensitivity for bioassays, there are some drawbacks. For example, analytical process is complicated and time-consuming; the doped reagents are readily to leak; the labeling procedure is also complicated. These problems limit their practical applications. Thus, it is highly desired to exploit new CL/ECL functionalized nanoprobes with high CL/ECL efficiency, stability and biocompatibility for bioassays.

Herein, we report current progress on CL/ECL functionalized nanoprobes for bioassays in our research group. In our group, a direct synthesis strategy was proposed for the preparation of CL functionalized nanoprobes. It was found that CL/ECL reagents, including luminol, isoluminol, N-(aminobutyl)-N-(ethylisoluminol), could directly reduce $HAuCl_4$ or $AgNO_3$ in aqueous solution to form CL/ECL functionalized gold or silver nanoparticles (NPs) [1–4]. These CL/ECL functionalized NPs are synthesized via such a simple method and a great number of CL/ECL molecules as stabilizers are coated on the surface of the AuNPs or AgNPs, which exhibited good CL and ECL activities. Subsequently, the CL/ECL functionalized NPs were used as CL/ECL labels to build bio-probes and ultrasensitive CL/ECL sensors were developed for immunoassays, DNA assays and the detection of small molecules. These bioassays show extremely high sensitivity. Moreover, they are also simple, stable, specific, and time-saving. Additionally, the labeling procedure is also superior to that of other reported CL/ECL functionalized nanoprobes in simplicity, stability, labeling property and practical applicability. They are of great application potential in the fields of public health, food safety, environmental science and so on.

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Between emission and perception: do luminous brittle-stars perceive their own light?

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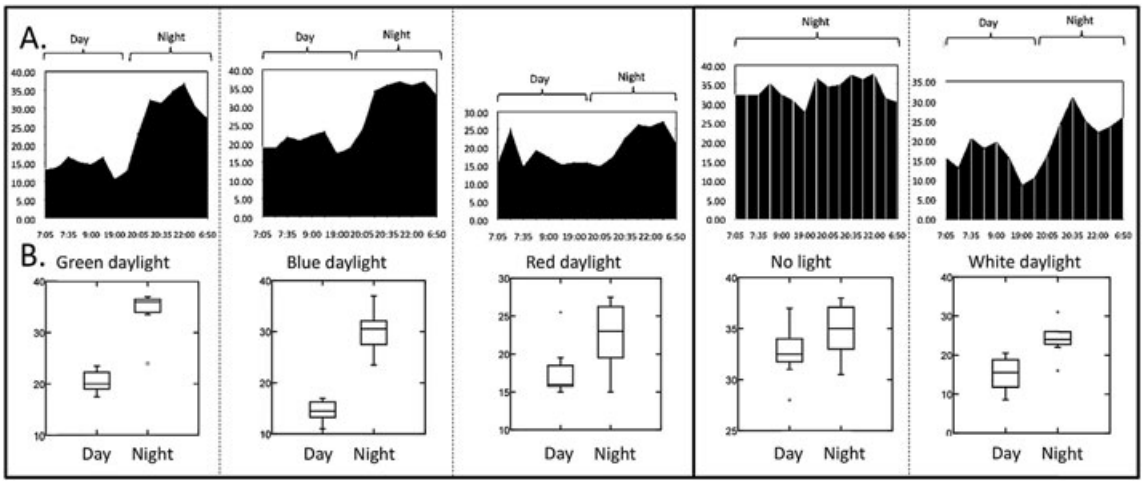

Figure 1. A. Histograms shows average number of "active arms" during the day and night (averaged over 3 days) for five different treatments (green daylight, blue daylight, red daylight, no light, white light. The number of arms is presented on y axis and the time on thex axis. B. Box-plots oth the data distribution for each treatment during the day and night. Significantly differences are present between the day and the night for the green and the blue daylight treatment.

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Since life appeared on earth, light has been one of the most important selective evolutionary forces for living organisms (1). In the marine environment, two predominant phenomena are directly related to light: photoreception and bioluminescence. Bioluminescence is present in at least thirteen phyla and in more than seven hundred identified genera (2). Its implications in the biology of living organisms are multiple (reproduction, nutrition, defense and communication. . .) (3). In echinoderms, luminescent species predominantly occur in the class Ophiuroidea, the brittle-stars, which comprises at least 66 species able to emit light (on 175 tested, (2)). In these organisms, luminescence, which is always intrinsic, stems from specialized cells, called photocytes, mainly located along the arms.

Recently, molecular markers of photoreception (opsins, arrestin, rhodopsin kinase,. . .) have been identified in the photophores of the sepiolid squid *Euprymna scolopes* (4). Bioluminescence in this species is produced by a bacterium - *Vibrio fisheri* - present in the photophore (extrinsic bioluminescence). Mutant bacteria in which the lux gene is non-expressed (inducing the lack of bioluminescence) do not persist in this organ (5). These observations suggest that squid photophores would be able to control their own bacterial population though extraocular photosensitivity. Could such a mechanism be present in organisms with intrinsic bioluminescence, such as ophiuroids? One can indeed think that extraocular perception by such organism would constitute an adequate control of photogenesis. The presence of extraocular photosensitivity in a light emitting organ poses some fascinating questions, which have been left unanswered until now. Are luminescent brittle-stars able to perceive their own light production? Does light detection differ in bioluminescent and non-bioluminescent species? Do bioluminescent species perceive light in a more efficient way than non-bioluminescent species, or conversely? These are the questions addressed in this study.

A behavioral approach, conducted in aquaria, permitted to highlight the photoreception capabilities of different bioluminescent (blue or green emitters) and non-bioluminescent brittle-star species. Depending on the ecology of the targeted brittle-stars, two different experiments are used. For brittle-stars considered as relatively photoreactive (*Ophiocomina nigra, Ophiopsila aranea*. . .), a high-intensity illumination is used and the escape behavior is analyzed. For less photoreactive brittle-stars as for example the borrowing species (*Amphiura filiformis, Amphiura chiajei*. . .), a modification of the photoperiod (ambient light) by color restriction is used. *A. filiformis* is mainly active during the night and is known to use photoreception to perceive the nycthemeral cycles (6). For photoperiod manipulation experiments, light intensity is calibrated using data collected in the field to match natural conditions encountered by the studied species. Specific monochromatic color lighting (red, blue, green, yellow. . .) are used to target the range of wavelengths these organisms can detect. Different colored LEDs are used for the experiments and their spectra are first evaluated with a microspectrophotometer. The results provide us with new information about the ecology of the luminous brittle-stars and the putative interaction between the processes of bioluminescence and photoreception.

Experiments on the species *A. filiformis*, a blue light emitter, revealed a spectral sensibility mainly to green light and also to blue light (fig. 1). The photosensibility seems to depend mostly on the ambient light present in the environment (fjord waters at a depth of 30 m where green light is the predominant wavelength), more than on bioluminescence. Work is in progress regarding the non-burrowing species, the green emitters and the non-bioluminescent brittle-stars species.

Acknowledgements

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Bioluminescent measurement of innate immunity bactericidal factors

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Innate immunity comprises molecular and cellular bactericidal mechanisms that protect the host from pathogens in a non-specific manner. That's why the activity of innate immunity bactericidal factors has important diagnostic and prognostic value, but routine methods of this detection are labour-consuming and of low technology.

The goal of this study is the development of the novel bioluminescent methods for molecular and cellular innate immunity measurement with inherent simplicity, sensitivity, and selectivity.

The first group of methods is based on bacteria bioluminescence inhibition that displays a loss of their viability in contact with bactericidal factors. Developed luminescent assay uses recombinant luminescent *Escherichia coli* and *Bacillus subtilis* strains with cloned *luxCD(AB)E* genes of *Photobacterium leiognathi* and gives the possibility for differential quantitative determination of blood serum molecular bactericidal systems presented by complement or platelet cationic proteins (PCP). The important step of this procedure is preliminary removal of antibodies from blood serum that excludes influence of specific (adaptive) immunity on the measurement result.

The similar principle is used at determination of phagocytosis completeness with neutrophils and macrophages separated after density gradient centrifugation. The luminescent *Escherichia coli* strain opsonized only by normal human immunoglobulin is used as phagocytosis particles that exclude preliminary loss of its viability in contact with others blood serum components. Developed simultaneous analysis of bacterial destruction and oxygen-dependent phagocyte system activation also uses chemiluminescent agent luminol and has two variants of realization. The first one is carried out in two separate tests: (i) leukocytes + luminescent bacteria for bioluminescence measurement, (ii) leukocytes + luminol + bacteria with thermoinactivated luminescent system for chemiluminescence measurement. Another variant is carried out in one probe consist of leukocytes, luminol, and luminescent bacteria by means of differentiated measurement of a bioluminescence at ≥ 540 nm and chemiluminescence at ≤ 420 nm.

Alternative bioluminescence methods for the differential determination of reactive oxygen species (ROS), including superoxide anion, and hydrogen peroxide, which are formed during phagocytes «oxidative burst» use *Escherichia coli* strains *soxS'::lux* and *katG '::lux* carrying fusions between promoters of oxidative stress genes and structural *luxCDABE* genes. The presence of *soxS'::lux* fusion led to specific bioluminescence induction of bacterial cells treated with *N*,*N*'-dimethyl-4, 4'-bipyridinium dichloride (paraquat) and *katG '::lux* to similar reaction with hydrogen peroxide. Carrying out of same experiments in phagocytosis system led to a primary induction of *soxS'::lux* fusion at contact with macrophages and *katG '::lux* with neutrophils, that can be defined by distinctions in generated ROS spectrum. In addition a luminescence induction of phagocytised bacteria with *recA '::lux* fusions it is revealed as SOS-reaction on DNA damage by ROS and most probably by hydroxyl radical.

The developed principles and experimental protocols of bioluminescent analysis of innate immunity bactericidal factors are based on the spectra of bioluminescent viability and gene expression tests and realized on a universal technological platform (figure 1).

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Fast kinetics of bioluminescent emitting species

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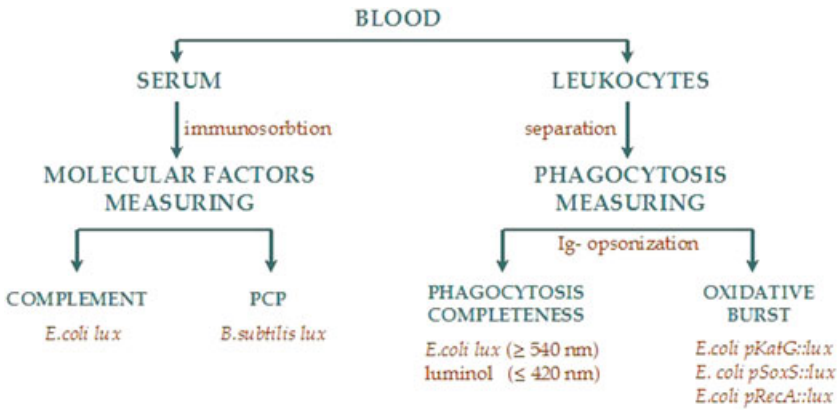

Figure 1. Principles of bioluminescence innate immunity measurement.

