Discovery of novel zebrafish neural tracers by organism-based screening of a rosamine library†

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Through organism based screening of a rosamine library using zebrafish larvae, novel neural tracers for live imaging were discovered with superior performance.

Diversity-oriented fluorescence library (DOFL) compounds have been shown to be a valuable source of bioimaging probes for biomarker discovery.1 These fluorescent, organic molecule sensors are generated by combinatorial synthesis, where simple building blocks can be assembled with the aim of creating diverse compounds. DOFL molecules have been widely used as sensors in a variety of analyses including those of DNA,2 RNA,3 nucleotides,4 peptides,5 proteins,6,7 and polysaccharides.8 They have also been used as living cell probes specific for subcellular organelles,9 as well as cell-status markers.10 Cell-based high throughput screening techniques have assisted in identifying new biosensors from the DOFL library. Since these probes are highly fluorescent, assays with read outs based on these properties, such as image-based assays, can facilitate the discovery procedure. However, the behavior of these compounds in a whole organism is largely unknown.

The zebrafish is a well-established model system for genetics and developmental biology. Over the past two decades work on zebrafish has resulted in the characterization of a large number of genes in vertebrate pathways, and has established the zebrafish as a model for disease and pharmaceutical research.11–13 Since zebrafish larvae (Fig. 1A) are small in size, and easy to maintain in large stocks, it becomes attractive to use them for medium-to-high throughput screening. The larvae are transparent, thus optical imaging methods are especially amenable.14 Moreover, the fluorescent nature of DOFL compounds makes it more advantageous to use the zebrafish larvae as a model system for fluorescence microscopic detection.

In this study, we applied the rosamine (RS)2 library in zebrafish larvae.† In the primary screen, zebrafish larvae were stained with RS compounds at a final concentration of 2 μM for one hour. Through fluorescence microscopy screening (ESI†) using 320 RS compounds, we found that many RS compounds label intensely the neurons located at the body surface, which include the neurons of the nose, of the internal ear and of the neuromasts. (Fig. 1A).15,16

The zebrafish nose is a paired organ located at the front end of the body, medial and anterior to the eyes (Fig. 1B and D, arrows). The internal ear is located immediate caudal to the eye (Fig. 1B, arrows with broken line). The neuromasts include the very front pair located medial to the nose (Fig. 1D, arrows with broken line) and those along the side of the body, the lateral line organ (LLO, Fig. 1B, arrowheads and Fig. 1C, arrows). The RS compounds label the neural cell bodies as well as long processes and projections (Fig. 1C and D, arrowheads).

More than 100 RS compounds label these neurons with different intensity. A semi-quantitative analysis of structure–activity relations was conducted after visually scoring the

Fig. 1 Representative RS (RS-e26, ZeN-Green and RS-c20, ZeN-Red) labeling in the 3 dpf larvae. (A) Larvae in a 96-well plate and illustration of the location of labeled neurons in a larva. (B) Structures of RS-e26 (ZeN-Green, upper panel) and RS-c20 (ZeN-Red, lower panel) and their labeling in larvae. (C) and (D) High-resolution reconstruction of confocal stacks showing that ZeN-Green (C) and ZeN-Red (D) label neuron soma (arrows) in the LLO (C) and in the nose (D), as well as their projections (arrowheads). A: anterior; P: posterior.
able to detect fluorescent neurons in all the larvae tested, however, after labeling with our neural tracers, we were not able to detect fluorescent neurons in all the larvae tested, indicating a massive destruction of these neurons during the development (Fig. 3B). To study the application of our neural tracers as indicators for acute ototoxicity induced by aminoglycosides,° 200 μM gentamicin was added together with the neural tracers into the embryo medium for one hour, a significant decrease of fluorescence was observed in the labeled neurons in inner ears in all the larvae tested, indicating dramatic neural damage even though the gross anatomy did not show detectable alteration (Fig. 3C).

Through this study, we established a mid-to-high throughput organism-based screening for DOFL in the whole organism, and discovered two novel neural tracers for live imaging of zebrafish. These novel neural tracers, ZeN-Green and ZeN-Red, can label neurons intensely and specifically, persist for a long period in live zebrafish larvae, and can be applied for environmental surveillance and as ototoxicity indicators.

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Notes and references


Fig. 2 More specific emission spectra of our compounds and longer persistence in comparison with DiA. (A) The ZeN-Green (upper) and the ZeN-Red (lower) labelled neurons only fluoresce under either the FITC filter (left) or the TRITC (right) filter sets but not both, whereas in (B) even though DiA labels the same neurons (arrows: nose, arrows with broken line: inner ear, arrowheads: LLO), the DiA labeled neurons showed fluorescence under both filter sets. (C) After labeling for an hour, larvae were raised in fresh medium, images taken after 44 h; the ZeN-Green labeled neurons are detected. However, in D, no fluorescence was detected in the nose for larvae with the same treatment using DiA. The broken line shows the outline of the larval forebrain.

Fig. 3 Application of the novel neural tracers for aquatic surveillance (B) and as acute ototoxicity indicators (C), gross development and anatomy (left pictures) do not show alteration in comparison with control (A). All larvae in the experiment were affected. Shown is the staining with ZeN-Green; staining with ZeN-Red showed similar results.

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Overall labeling intensity (Table S1†). The two best compounds were chosen as novel and superior neural tracers for live imaging because of their strong and stable fluorescence and specific emission spectra, which we dubbed ZeN (Zebrafish Neurotracer)-Green and ZeN (Zebrafish Neurotracer)-Red. Both ZeN-Green and ZeN-Red turned out to be very bright and fast accessing neural tracers which are detected after 20 min application. They were also found to be stable during routine labeling and the imaging process (data not shown). After leaving the larvae in compound solutions at 2 μM for 48 h, no obvious deterioration of the larvae was observed, and the larvae develop normally till one week, the longest period larvae were kept during the experiment. We further compared their performance with a commercially available, very widely used neural tracer, 4-Di-10-ASP (DiA). Neurons labeled with 2 μM DiA showed fluorescence with both conventional filter sets. In contrast, the emission spectra of our compounds were very specific using the conventional filter sets (Fig. 2); both of the compounds showed strong fluorescence in one channel but not in the other. Co-application of the two compounds was carried out to compare their labeling patterns in the same larva (Fig. S1†). When both compounds were applied, complete overlapping of the labeling was observed. This offers more options for labeling in combination with other dyes. In addition, our compounds persist longer than DiA of the same concentration in the stained organs (Fig. 2).

Since our compounds label the neurons of the nose, of the inner ears and of the LLO, we further explored their application in environmental surveillance and as ototoxicity indicators. Lead is a common pollutant that is known to deteriorate nervous system development.° We raised the zebrafish larvae in embryonic medium containing 100 μM lead chloride after birth; at 4 dpf, the larvae appeared normal, however, after labeling with our neural tracers, we were not able to detect fluorescent neurons in all the larvae tested,