Novel non-toxic, cell permeable, pH-dependent Fluorescent Dye for Live Imaging

Unique Features of Ageladine A

- Wide range (pH 4 to pH 8). pH-dependent fluorescent dye in the blue-green range upon excitation with UV light. Stronger under acidic conditions and barely detectable in alkaline solutions.
- Non-toxic, highly cell/membrane permeable dye. No AM-esters or esterases involved. Trapped within the cells and acidic organelles through hydrophobic interactions with the inner side of the membranes.
- Barely metabolized and exerts long-term stability.
- Allows long term pH monitoring in acidic organelles, vesicles, cells, tissue and small animals over several days without side effects.

LITERATURE:

- Incorporated nematocysts in Aeolidiella stephanieae (Gastropoda, Opisthobranchia, Aeolidoidea) mature by acidification shown by the pH sensitive fluorescing alkaloid Ageladine A: D. Obermann, et al.; Toxicon 60, 1108 (2012)

APPLICATIONS:

Fluorescence microscopic monitoring of acidic organelles like lysosomes and endosomes, whole animals like plathelmintms, cnidaria, larvae and eggs from different species • Screenings • Viability tests • Flow cytometry • Fluorescence lifetime imaging microscopy (FLIM)


FIGURE: CFLSM scan of M. lignano stained with Ageladine A (fluorescence is shown in false color). Ageladine A shows a pH-dependent fluorescence. Highly fluorescent areas (white) are more acidic than dark areas (dark red).
**Imaging Specifications**

**Loading times:**
- Cells in culture: ~10-30 minutes. Small animals (e.g. larvae, platelhelmintes): ~30-120 minutes. Serum, salt content and ion assembly of the culture medium plays no role. All common buffers and culture media buffered up to pH 7.5 can be used.

**Spectral properties:**
- Excitation between 325 & 415nm; max. at 370nm
- Emission from 415nm to >500nm; max. at 415nm.

Experiments can be done with common filter settings (like for FURA-2). The fluorescence rises with lower pH in a linear range between pH 4 and pH 8. Quantitative results are achieved with ratiometric methods or FLIM.

**Photobleaching** occurs, but plays no role for monitoring at low excitation intensities and exposition times under usual laboratory conditions (even for several days).

**Leakage** was not observed after several days of incubation.

**Toxic effects** were not observed at concentrations up to 30µM. Patch clamp experiments with PC12-cells showed weak changes of the membrane potential at concentrations >10µM.

**Recommended concentrations:**
- Between 1µM (cells) and 30µM (whole animals).

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**Imaging Examples**

PC12-cells staining with ageladine A during UV excitation and the transmission image of the cells.

Nematocysts in different regions of Aiptasia sp. stained with Ageladine A: left: in acontia; right: in tentacles. Monitored by a confocal laser scanning microscope. Fluorescence is displayed in red (false colour).

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**Antiangiogenic Agent**

Ageladine A is a unique brominated pyrrole-imidazole alkaloid, first isolated from the marine sponge Agelas nakamurai. It exhibits *in vitro* and *in vivo* antiangiogenic activity, which was initially considered to be associated with its moderate inhibition of various subtypes of matrix metalloproteinases (MMPs), but subsequently confirmed to result from its selective inhibition of kinases including dual specificity tyrosine-phosphorylation-regulated kinase (DYRK)1A, DYRK2, tyrosine kinase 2 (TYK2) and yeast Sps1/Ste20-related kinase 4 (YSK4). It is a highly selective angiogenesis inhibitor with no cytotoxicity against a panel of human cancer cell lines.

**LITERATURE:**