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Measuring the pH levels inside lysosomes

The pH level varies in different organelles inside a cell and there is a diversity of function carried out within. Any deviations of pH from normal levels could signal cellular stress or dysfunction and may indicate serious diseases such as cancer or Alzheimer's. Lysosomes are very important acidic organelles (pH range 4.5 to 5.5) and are degradation centers involved in the recycling of macromolecules such as proteins, carbohydrates, and lipids. Lysosomes also play a role in cell signaling, immunologic response, and energy metabolism. In cases of diseases like cancer, the microenvironment of tumors shows an increase in extracellular acidity, which impacts lysosomal pH as well as its autophagy functions. The question we address is how to determine lysosomal pH values within a cell as abnormal levels can lead to lysosomal disorders. We accomplish this by synthesizing lysosome specific molecular probes that change color depending on pH levels. The color change is accomplished by a molecular switch mechanism which responds to the pH at certain levels. We use a spirolactam switch which is present in a ring format at high pH levels and then upon lowering the pH, the ring opens causing a change in the fluorescence properties of the molecule.

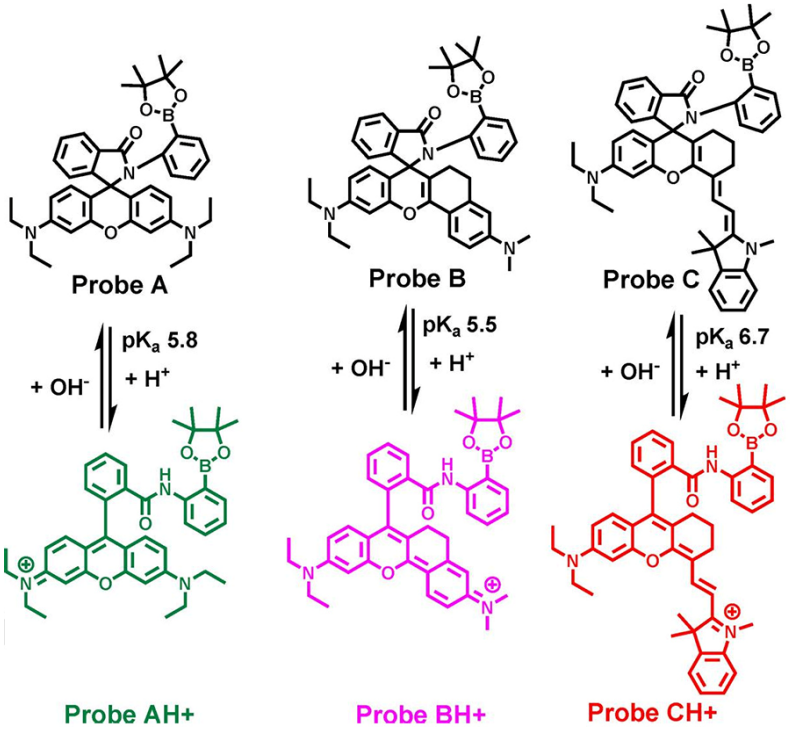

Fig. 1.

Our probes have a desirable attribute over other rhodamine probes in that they are fluorescent under acidic, neutral, and basic conditions. We have surmounted the non-fluorescent limitation of these fluorescent probes with closed spirolactam switches under basic pH conditions to take advantage of the outstanding photophysical properties of rhodamine and its derivatives. Our article features the

design and syntheses of three fluorescent probes (**A**–**C**) bearing closed spirolactam ring configurations with high pK_a values for lysosomal pH detection in living cells by introducing significantly bulky 2-aminophenylboronic acid pinacol ester sections to traditional rhodamine B and its near-infrared derivative, and near-infrared hemicyanine dyes, respectively, to improve the spectroscopic properties of the dyes. We found that probes **B** and **C** based on near-infrared rhodamine and hemicyanine dyes, with pKa values of 5.45 and 6.97, display significant fluorescence peaks at 644 nm and 744 nm under basic pH level of 8.8, respectively. The higher pKa value in probe **C** compared to that for probe **B** may be due to increased bulkiness.

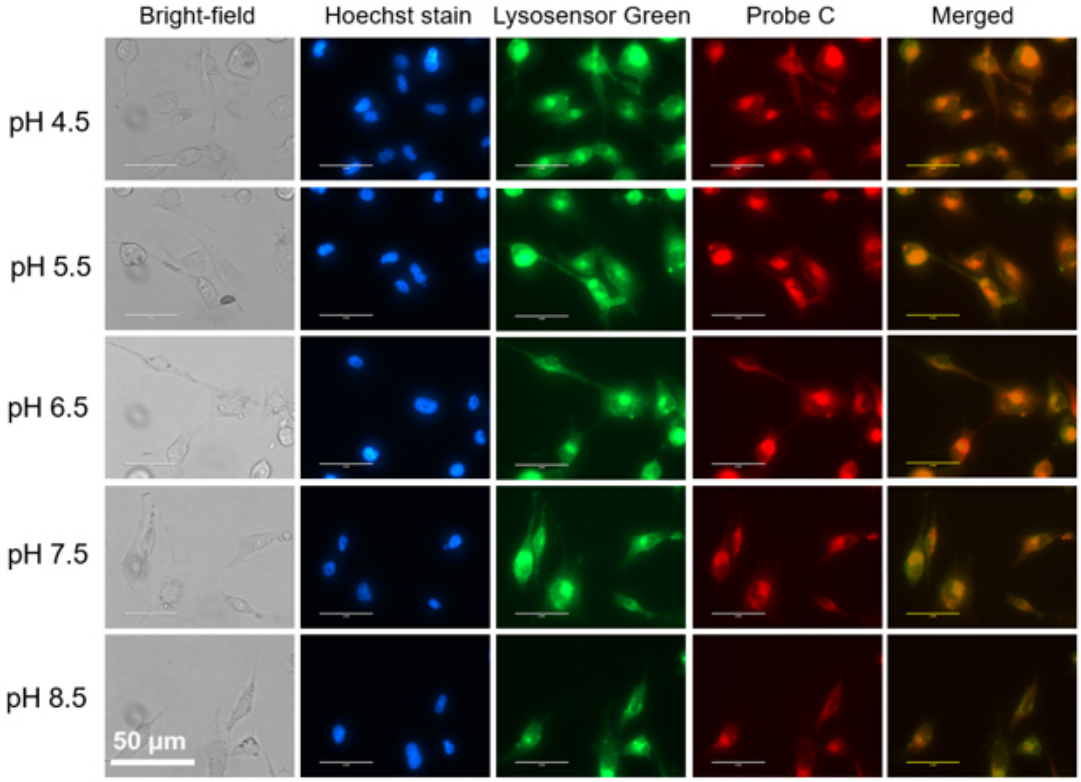

Fig. 2.

Probe **A** similar to fluorescent probes based on traditional rhodamine dyes is non-fluorescent with a pKa value of 5.81 under basic pH conditions. Probes **B** and **C** display activated fluorescent responses to both acidic and basic intracellular pH ranges and are capable of monitoring acidic pH variations in lysosomes. The ability of probe **C** to function in buffers with different pH levels is shown in Figure 2 and illustrates the capability of the probe to show fluorescence variance compared to Lysosensor Green which is insensitive to intracellular pH changes.

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Publication

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