Chloride Sensor: measuring sub-cellular chloride in live cells

A nucleic acid based chloride sensor *Clensor* images and quantifies spatiotemporal chloride transport associated with endosomal maturation in the living cell.

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Quantitative molecular imaging is a rapidly growing technology for quantification of chemicals and biological molecules and for monitoring phenomena in the living cell. Chloride is one of the target chemicals for intracellular sensing, which functions for pH regulation, cell volume regulation, cell migration, and cell proliferation. In the cytosol and lumen of organelles, chloride concentration is stringently regulated by the chloride transporters and channels.[1,2] Despite the importance of monitoring chloride in the organelles, the real time quantification in a specific organelle using a fluorescent probe is still challenging because it requires the ability to monitor a wide range of chloride concentration and should also be insensitive to pH changes. Writing in *Nature Nanotechnology*, Yamuna Krishnan and colleagues report a nucleic acid based chloride sensor called "*Clensor*", which monitors spatiotemporal chloride concentration in the organelles in the living cell. The problem of the present protein-based chloride sensors is their pH sensitivity and their restriction to specific, narrow ranges of Cl^- concentration.[3]

Krishnan and colleagues overcome these limitations using functionalized nucleotides that contain sensing, normalizing, and targeting modules (Figure 1a). Nucleic acid-based sensors are widely investigated for investigating various phenomena in living cells especially by fluorescence imaging.[4,5] They employed a fluorescent and chloride-sensitive molecule, 10,10′-Bis[3- carboxypropyl]-9,9′-biacridinium dinitrate (BAC), which was used for sensing

chloride in subcellular compartments.[6,7] The chloride insensitive Alexa 647 fluorophore was used for normalizing the fluorescence intensity in a ratiometric fashion. By simply assembling these modules, the quantitative chloride reporting nanodevice *Clensor* was realised (Figure 1a). The R/G ratio of *Clensor*, where the fluorescence intensity of Alexa 647 (R) is divided by that of BAC (G), shows a linear dependence on Cl^- concentration ranging from 5 mM to 200 mM and is also insensitive to solution pH (Figure 1b). *Clensor* shows excellent Cl^- sensing performance *in vitro* experiments, as well as quantitatively monitors intracellular Cl^- concentration (Figure 1b).

Krishnan and colleagues applied *Clensor* to determine the Cl^- concentration in specific endo-lysosomal compartments including early endosome (EE), late endosome (EE), lysosome (LY), and recycling endosome (RE). *Clensor* is internalized by endocytosis via anionic ligand binding receptor (ALBR) pathway. Then endosomal/lysosomal maturation was monitored by R/G ratio imaging with *Clensor* having confirmed organelle localization using specific endocytic markers. Remarkably, *Clensor* can acquire organelle specific targeting ability by just replacing the targeting module of the nanodevice. Conjugating an RNA aptamer targeting a transferrin receptor, *Clensor* can be internalized via transferrin pathway (*Clensor^{Tf}*), which was efficiently uptaken by transferrin receptor expressing cells.

Intracellular functionality of *Clensor* was examined by R/G ratio by comparing experiments *in vitro* and in the endosome of *Drosophila* hemocytes by changing Cl^- concentrations. In both experiments, R/G ratios show the good agreements of the quantitative performance of *Clensor* in a range of 5-120 mM (Figure 1c).

Next, Krishnan and colleagues demonstrated the quantification of Cl^- concentrations during the endosome-lysosome maturation. Cl^- concentrations in endosome and lysosome were directly measured by R/G ratio with *Clensor*. Compartments are acidified along the endocytic pathway from pH 6.0 in early endosomes to pH ~5.0 in lysosomes, accompanying the increase of Cl^-

concentration. From the distribution of R/G ratios, Cl^- concentration increased during the endosome-lysosome maturation. Importantly, Cl^- concentration in lysosomes was first measured using this sensor, whose values are consistent with the theoretical model.

Finally, they demonstrate characterization of the functions of the putative CLC family proteins, DmClC-b and DmClC-c, during the endosome-lysosome maturation. To investigate the function of DmClC-b and DmClC-c, the expression of these proteins was suppressed by the corresponding RNAi and the R/G ratios were measured in each endocytic organelle and also correlated to lumenal pH. They found that DmClC-c accumulates Cl^- in the early endosome with acidification. On the other hand, DmClC-c worked in late endosome and lysosome to accumulate Cl^- , especially in the lysosome, without acidification. *Clensor* clearly revealed the different roles of these proteins in endolysosomal maturation.

Krishnan and colleagues successfully created a structurally simple nucleic acid based nanodevice by combining the functionalized modules to integrate the sensing and targeting functions. These modules can be easily replaced in a "mix-and-match" format for the desired sensing and targeting purposes. *Clensor* works in subcellular compartments and the sensing ability was completely preserved. This simple nucleic acid-based nanodevice can be a general tool for imaging complicated intracellular phenomena and real time quantification of chloride in the living cell for diverse biological problems.

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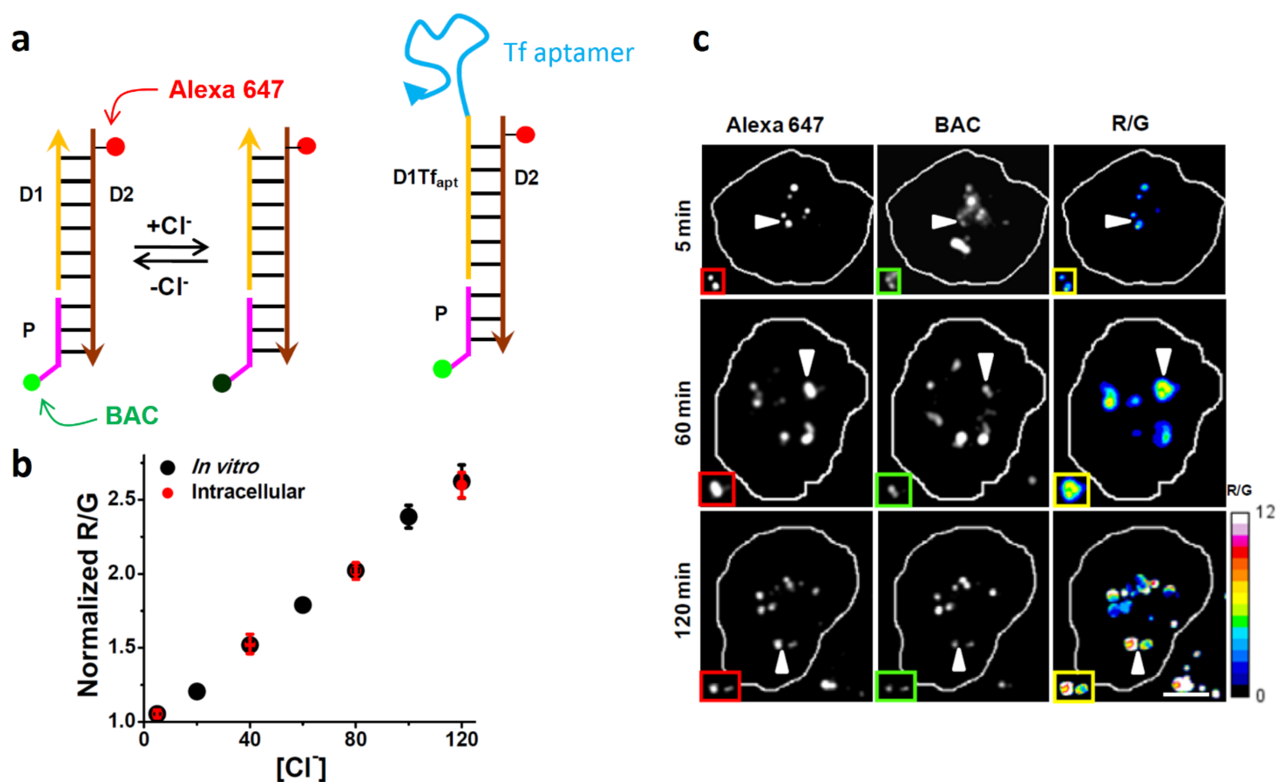
Figure 1: ***Clensor* functions in the subcellular components.**



(a) Cl^--sensitive fluorophore BAC (G) and reference Alexa 647 (R) modules are assembled using DNA and analogs to construct *Clensor*. Fluorescence of BAC is quenched by increasing of Cl^- concentration. (b) Quantitative performance of *Clensor*. Linearity of R/G ratios vs. Cl^- concentration *in vitro* and in cell. (c) Alexa 647, BAC, and pseudocolour R/G map of live *Drosophila* hemocytes labeled with *Clensor* in EE (5 min), in LE (60 min), and LY (120 min). Scale bar: 10 µm.