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COMMUNICATION

Synthesis and characterization of a cell-permeable near-infrared fluorescent deoxyglucose analogue for cancer cell imaging†

Marc Vendrell,[a] Animesh Samanta,[b] Seong-Wook Yun[a] and Young-Tae Chang*[a,b]

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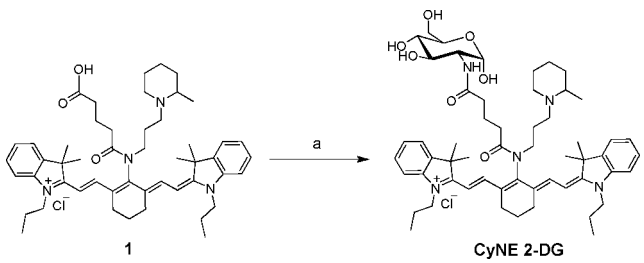
We report the synthesis and characterization of a novel NIR fluorescent deoxyglucose analogue, CyNE 2-DG. Experiments in different cell lines showed a preferential uptake of CyNE 2-DG in cancer cells and its effective competition with unlabeled D-glucose. Cell imaging experiments demonstrated the superior cell-permeability of CyNE 2-DG over the NIR standard IRDye 800CW 2-DG, and validated its application for cancer cell imaging in the NIR region.

Malignant cancer cells show an increased glycolysis rate when compared to normal cells due to the overexpression of glucose transporters (GLUTs) and the higher activity of hexokinases.[1,2] These differences in metabolism have been applied to the identification of cancer cells and tumors by optical imaging methods that rely on the preparation of reporter-containing glucose analogues. A number of fluorescent glucose derivatives have been reported. 2-[*N*-(7-Nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose (2-NBDG), the first fluorescent probe to monitor glucose uptake through GLUTs, has been widely used in cancer imaging.[3] Limitations on the photophysical properties of 2-NBDG (*e.g.* low brightness) led to the preparation of glucose analogues with improved fluorescent properties by conjugation to rhodamine, Cy3 or two-photon dyes.[4–6] The advantages of near-infrared (NIR) imaging (*e.g.* lower autofluorescence background, less photodamage) have recently raised the interest in NIR fluorescent glucose analogues.[7–10] NIR glucose derivatives have been prepared by conjugation of 2-deoxyglucose to penta- or tricarbocyanine dyes (*i.e.* Cy5.5 and IRDye 800CW respectively). Cy5.5-2DG proved to be trapped by tumor cells, but its uptake is not blocked by D-glucose, questioning its delivery through GLUTs.[8] On the other hand, **IRDye 800CW 2-DG** is uptaken in cancer cells and specifically blocked by excess of D-glucose,[10] but the highly negatively charged chemical structure may hamper its application in cell imaging due to low cell membrane permeability. Herein we prepared a NIR 2-deoxyglucose derivative (**CyNE 2-DG**) based on an amine acetylated tricarbocyanine structure. **CyNE 2-DG** showed preferential uptake in cancer cells and competition with excess D-glucose, and its suitable cell permeability asserts the potential for cancer cell imaging in the NIR region.

Our group recently developed an amine acetylated tricarbocyanine scaffold (**CyNA**) with excellent photophysical properties, low aggregation in aqueous media and fluorescence emission in the NIR region.[11] Amine acetylated tricarbocyanine dyes have been also adapted for bioconjugation purposes after incorporation of a glutaric acid linker (**1**, Scheme 1) that enables conjugation to macromolecules such as antibodies while maintaining its spectral and photostable properties.[12] The chemical structure of **1** is positively charged while many NIR fluorescent cyanine labeling dyes are highly negatively charged molecules. The minimization or absence of charges is particularly important when labeling small molecules since neutral conjugates may exhibit greater permeability properties.[13] We envisaged that the conjugation of 2-deoxyglucose to **1** would render a NIR fluorescent glucose analogue with good cell permeability. **CyNE 2-DG** was prepared by coupling 2-deoxyglucosamine to the tricarbocyanine carboxylic acid **1** using HATU as the coupling reagent (Scheme 1), and exhibited similar spectral properties to **1** with absorption and emission maxima around 790 and 815 nm respectively (Fig. 1).



Scheme 1 Synthesis of **CyNE 2-DG**. Reagents and conditions: a) 2-D-deoxyglucosamine, HATU, DIEA, DMF_{anh}, r.t., 2 h.

In order to compare the uptake of **CyNE 2-DG** in different cells, serum-starved cultures of breast tumor MCF7 and MDA-MB-231 cell lines and non-tumor human foreskin fibroblasts (HFF) were incubated at 37 °C for 20 min with 20 μM **CyNE 2-DG**, and analysed by fluorescence microscopy. Mean NIR fluorescence intensity values of the different cell lines indicated that **CyNE 2-DG** was preferentially uptaken in tumor cells when compared to

[a]*Laboratory of Bioimaging Probe Development, Singapore Bioimaging Consortium, Agency for Science, Technology and Research (A*STAR), 138667, Singapore*
[b]*Department of Chemistry & MedChem Program of Life Sciences Institute, National University of Singapore, 117543, Singapore. E-mail: chmcyt@nus.edu.sg; Fax: (+) 65 6779 1691; Tel: (+) 65 6516 6774*

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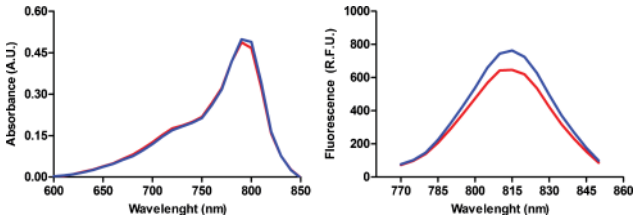

Fig. 1 Absorbance and fluorescence spectra of **1** (blue) and **CyNE 2-DG** (red) in phosphate buffer saline (PBS, pH 7.3) containing 1% DMSO. Excitation wavelength: 740 nm.

fibroblasts (Fig. 2), an observation that corresponds to the higher glycolysis rate of cancer cells.[14]

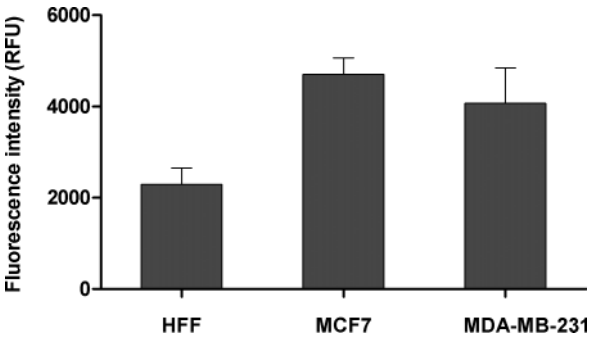

Fig. 2 NIR fluorescence intensities in human tumor cell lines and fibroblasts upon incubation with 20 μM **CyNE 2-DG**. Mean intensity values are plotted as average fluorescence intensities from 5 to 10 different regions and error bars as standard deviations.

Further experiments were aimed at determining whether the uptake of **CyNE 2-DG** was mediated by GLUTs. We performed competition experiments with increasing concentrations of D-glucose in MCF7 and MDA-MB-231 cells.[7,15] As shown in Fig. 3, the uptake of **CyNE 2-DG** was significantly reduced upon competition with D-glucose, indicating that **CyNE 2-DG** enters the cell through GLUTs. We corroborated that the uptake of **CyNE 2-DG** in MCF7 and MDA-MB-231 cells was not affected by competition with L-glucose (Figure S5 in the ESI†) demonstrating the stereoselective interaction with GLUTs. Furthermore, we confirmed the role of 2-deoxyglucose in the cellular uptake by determining higher intracellular fluorescence intensities upon incubation with **CyNE 2-DG** when compared to the carboxylic

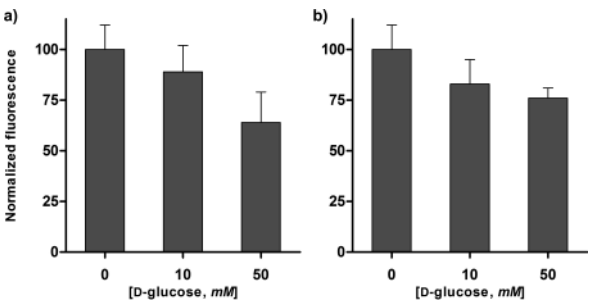

Fig. 3 Dose-dependent inhibition of the **CyNE 2-DG** uptake after competition with D-glucose in: a) MCF7 and b) MDA-MB-231 cells. Fluorescence intensity values from 5 to 10 different regions were averaged and referred to the fluorescence intensity measured in cells with no glucose competition. Error bars correspond to standard deviations.

acid **1** (Figure S6 in the ESI†). Altogether these results suggested that the uptake of **CyNE 2-DG** in tumor cell lines is specifically regulated by GLUTs rather than by passive penetration.

After validating **CyNE 2-DG** as a NIR fluorescent glucose analogue, we compared its application for cell imaging studies to **IRDye 800CW 2-DG**, a tumor targeting NIR fluorescent agent that is uptaken by cancer cells through GLUT family proteins.[10] **CyNE 2-DG** and **IRDye 800CW 2-DG** have similar fluorescence emission maxima (794 nm for **IRDye 800CW 2-DG**, 815 nm for **CyNE 2-DG**), but the negatively charged structure of **IRDye 800CW 2-DG** entails a lower permeability that can hinder its application in cell imaging experiments. We compared the NIR fluorescence images of MCF7 cells upon incubation with **CyNE 2-DG** and **IRDye 800CW 2-DG** (both at 20 μM) at 37 °C for 20 min. Images taken under the same acquisition conditions proved that the cytoplasmatic fluorescence intensities of the cells incubated with **CyNE 2-DG** were significantly higher than those treated with **IRDye 800CW 2-DG** (Fig. 4). These results demonstrated the superior uptake of **CyNE 2-DG** in cancer cells and validated the good cell permeability properties of **CyNE 2-DG**.

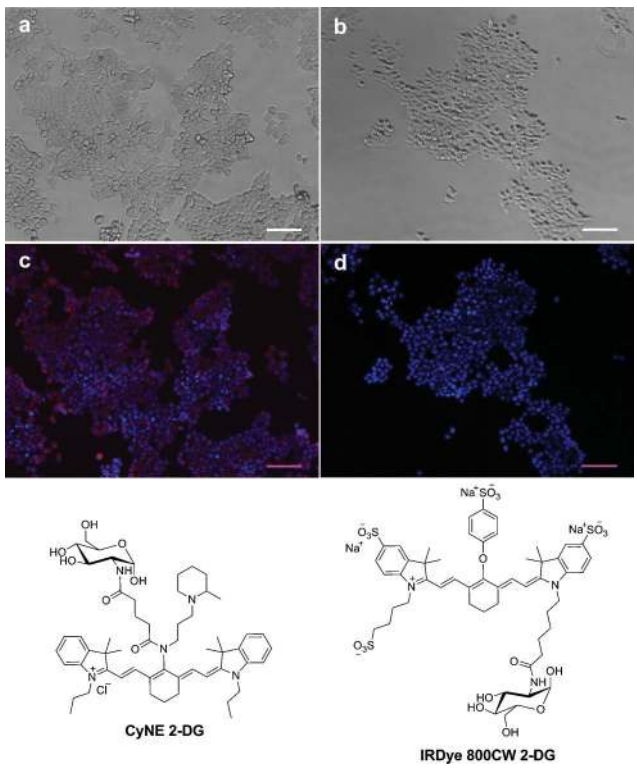

Fig. 4 Brightfield and merged fluorescence images of MCF7 cells upon incubation with **CyNE 2-DG** (a and c) and **IRDye 800CW 2-DG** (b and d). Cells were incubated with 20 μM **CyNE 2-DG** or **IRDye 800CW 2-DG** at 37 °C for 20 min followed by nuclear staining with Hoechst, and fluorescence images were taken under the same acquisition conditions. NIR fluorescence is shown in red color and Hoechst staining in blue color. Scale bar: 100 μm.

In conclusion, we synthesized and characterized **CyNE 2-DG** as a novel NIR fluorescent glucose derivative. **CyNE 2-DG** showed a higher uptake in cancer cell lines than in primary fibroblasts, and its competition with unlabeled D-glucose implied that its

uptake into the cells may be mediated by GLUTs. **CyNE 2-DG** exhibited a brighter staining of cancer cells when compared to the commercially available **IRDye 800CW 2-DG**, which suggests its good cell permeability properties and its applicability to cancer cell imaging in the NIR region.

Acknowledgements

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