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GLUT1 and GLUT9 as major contributors to glucose influx in HepG2 cells identified by a high sensitivity intramolecular FRET glucose sensor

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Abstract

Genetically encoded FRET glucose nanosensors have proven to be useful for imaging glucose flux in HepG2 cells. However, the dynamic range of the original sensor was limited and thus it did not appear optimal for high throughput screening of siRNA populations for identifying proteins involved in regulation of sugar flux. Here we describe a hybrid approach that combines linker-shortening with fluorophore-insertion to decrease the degrees of freedom for fluorophore positioning leading to improved nanosensor dynamics. We were able to develop a novel highly sensitive FRET

nanosensor that shows a 10-fold higher ratio change and dynamic range (0.05â€“11Â mM) in vivo, permitting analyses in the physiologically relevant range. As a proof of concept that this sensor can be used to screen for proteins playing a role in sugar flux and its control, we used siRNA inhibition of GLUT family members and show that GLUT1 is the major glucose transporter in HepG2 cells and that GLUT9 contributes as well, however to a lower extent. GFP fusions suggest that GLUT1 and 9 are preferentially localized to the plasma membrane and thus can account for the transport activity. The improved sensitivity of the novel glucose nanosensor increases the reliability of in vivo glucose flux analyses, and provides a new means for the screening of siRNA collections as well as drugs using high-content screens.

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Abbreviations

FLIP, fluorescent indicator protein; FLIIP, fluorescent intramolecular indicator protein; FRET, fluorescence resonance energy transfer; Gluc, glucose; CYT, cytosol

Keywords

GLUT; Transporter; Liver; Blood; Homeostasis

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