# Fluorogenic cell surface labelling using fluorescent molecular rotor-labelled peptide boronic acid conjugates

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## Abstract

Abnormally viscous mucus hydrogel layers represent the key pathological characteristic of chronic, muco-obstructive respiratory and gastrointestinal disorders such as Cystic Fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD) and Inflammatory Bowel Disease (IBD). To date, quantifying and mapping spatial nanoviscosity on living cells/tissues poses a major challenge. Our aim is to probe viscosity by anchoring fluorescent molecular rotor (FMR) dyes to cell surface glycans. For this purpose, the FMRs are attached to peptide-multiboronic acid conjugates. We show the development of fluorogenic probes that have high affinity for cell surfaces and demonstrate ratiometric imaging with an FMR and a non-FMR probe to characterize cell surface nanoviscosities.



**Developing FMR-functionalized peptide-multiboronic acid conjugates for cell surface labeling** 

**Objectives** 

A549



Hall-type probe <sup>[3]</sup>

**AeLa** 

Cy3-K-FRGDF-K 

### Zhang-type probe<sup>[4]</sup>



**boronates can facilitate cellular uptake** <sup>[5]</sup>

Internalization

**b** starting point of screening campaign of approx. 50 different probes with three different boronate receptors



Cy3-K-FDDDF-K

**Cy3-2B3D** 

A549

AMPB

New design

2 boronates (B) with 3 aspartates (D) sufficient for staining of HeLa surface, but not A549 (data not shown)

Cy3-K-FDDD-K-DDF-K AMPB AMPB AMPB

**Cy3-3B5D** 

Surface labeling

increase number to 3 boronate

receptors and 5 aspartates for

successful staining of A549 cell surface

## efficiently label the cell surface minimize unspecific cellular uptake

#### CCVJ-K-FDDD-K-DDF-K





**CCVJ** 

Wash-free surface labeling

Cy3 and CCVJ are fluorogenic probes light up under high viscosity [6] - [8]

CRC







- nanoviscosity on A549 cell
  - surface/glycocalyx
- regular fluorescence microscope without

relying on specialized FLIM setup

1.5-	****

# Fluorogenic cell surface glycan labelling with fluorescence molecular rotor dyes and nucleic acid stains <sup>[8]</sup>



wash-free A549 cell surface labelling with FMR probes

Ratiometric imaging of A549 cells upon dual covalent labeling with FMR-probe and non-FMR

- heterogenous fluorescence intensity ratio distributions on A549 cell surface (also with HeLa and CEM cell surface, data not shown)
- CCVJ/AF568 fluorescence intensity ratio 5 respond to TCEP treatment, and the same effect was observed with

**TO/AF568 fluorescence intensity ratio** 



## *Mucin bound FMR-3D-Ahx-N*<sub>2</sub>H<sub>3</sub> sensitive to changes of viscosity/crowding



Dual labeling of A549 cells with CCVJ-3D-Ahx-N<sub>2</sub>H<sub>3</sub> or TO-3D-Ahx-N<sub>2</sub>H<sub>3</sub> + AF568- $N_2H_3$  (non-FMR) mix. Cells were treated with TCEP, if added, prior to labelling.

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upon conjugation with cell surface glycans

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