# **Supplementary Material**

# **Reagents for labeling with pH-independent fluorescein-based tags**

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#### **General Papers**





FITC 5/6-Fluorescein isothiocyanate



4'-(aminomethyl)fluorescein, hydrochloride



5-(aminoacetamido)fluorescein (fluoresceinyl glycine amide)

C

0

С

OH

0

OН



Fluorescein-5-Maleimide



Fluorescein maleimide Vector Laboratories, Inc.

H







5-iodoacetamidofluorescein



5-FAM Alkyne 5-carboxyfluorescein, propargylamide

6-fluorescein amidite (6-FAM)

Scheme S1. Chemical structures of compounds applied for introducing fluorescein moieties into organic molecules.



Figure S10. <sup>1</sup>H and <sup>13</sup>C NMR spectra of 2 in CDCl<sub>3</sub>.



Figure S11. <sup>1</sup>H and <sup>13</sup>C NMR spectra of 4 in CDCl<sub>3</sub>.



Figure S1. Absorption spectra of Olig-Flu in different buffer solutions. Concentration 1 mg/mL.



**Figure S2**. Absorption spectra of **2** (A, B) and **4** (C, D) solutions in water (pH 7) – A, C, and in 1,4-dioxane – B, D. Concentration 5  $\mu$ M.



**Figure S3**. Absorption spectra of **2** (A) and **4** (B) in different buffer solutions. Concentration 2.5  $\mu$ M for A and 10  $\mu$ M for B.



**Figure S4**. Absorption spectra of ZS-424 (A), ZS-493 (B), ZS-495 (C) in different buffer solutions. Concentrations 0.47 mg/mL for A, 0.36 mg/mL for B and 1 mg/mL for C.



**Figure S5**. Excitation spectra of fluorescein (A), Olig-Flu (B), **2** (C) and **4** (D) in different buffer solutions at emissions 523 nm . Concentrations 0.5  $\mu$ M for A and B, 2.5  $\mu$ M for C, 5  $\mu$ M for D.



**Figure S6**. Excitation spectra of ZS-424 (A) and ZS-493 (B) in different buffer solutions at emissions 523 nm. Concentrations 0.47 mg/mL for A and 0.36 mg/mL for B.



**Figure S7**. Excitation spectra of ZS-495 in different buffer solutions at emission 545 nm. Concentrations 1 mg/mL.



**Figure S8**. Emission spectra of ZS-424 (A) and ZS-493 (B) in different buffer solutions at excitation 490 nm. Concentrations 0.47 mg/mL for A and 0.36 mg/mL for B.



**Figure S9**. Emission spectra of ZS-495 in different buffer solutions at excitation 490 nm. Concentrations 1 mg/mL.