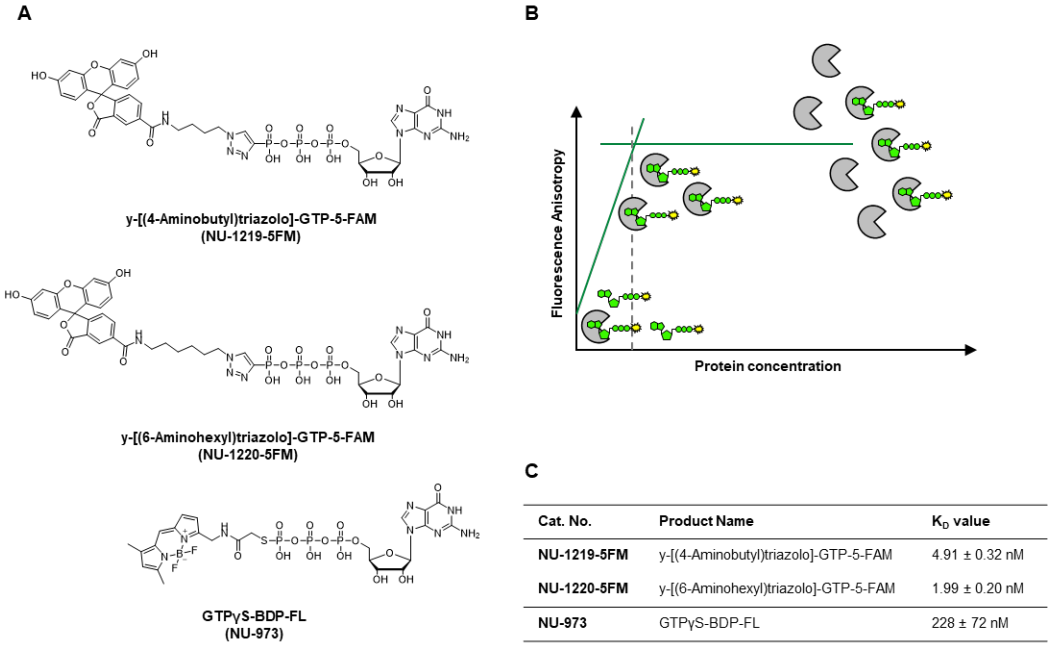
Novel fluorescent probes with improved stability and affinity for Gαi1 protein

The Gαi1 protein subunit can function as an essential molecular switches for the GPCR-mediated intracellular signaling by switching between the GDP-bound (inactive) and the GTP-bound state (active). [1] Dysregulation of the switching mechanism is associated with diverse pathophysiologies, such as heart failure, cancer etc., making Gαi proteins attractive therapeutic targets in drug research. [2] Various approaches have been described in the past using fluorescent GTP analogs targeting the Gαi1 proteins, lacking thogh both the accurate determination of the protein's guanine nucleotide binding affinity and the quantification of the active protein fraction. [3]
Pepanian *et al.* recently developed a fluorescence anisotropy (FA)-based method to determine the binding affinity at the GTP-binding site and quantify the Gαi1 protein activity. [3] Since commonly used MANT-and BODIPY- [4-6] based probes lack broad applicability for FA-based assays, a new generation of fluorescein (FAM) labeled GTP analogs was developed with beneficial properties for FA-assay including high quantum yields and relatively short fluorescence lifetimes of the excited state (3.8 ns). [3] Two high-affinity probes **y-[(4-Aminobutyl)triazolo]-GTP-5-FAM** and **y-[(6-Aminohexyl)triazolo]-GTP-5-FAM** for Gαi1 (K_D values in the one-digit nM range) have been identified that possess an improved hydrolytic stability and binding affinity compared to **MANT GTPγS** and **BODIPY FL GTPγS.** [3]



Cat. No.	Product Name	K_D value
NU-1219-5FM	y-[(4-Aminobutyl)triazolo]-GTP-5-FAM	4.91 ± 0.32 nM
NU-1220-5FM	y-[(6-Aminohexyl)triazolo]-GTP-5-FAM	1.99 ± 0.20 nM
NU-973	GTPγS-BDP-FL	228 ± 72 nM

Figure 1. 5-FAM labeled GTP analogs with improved stability and affinity for the Gαi1 protein compared to BODIPY FL GTPγS.
A) Chemical structures of y-[(4-Aminobutyl)triazolo]-GTP-5-FAM (NU-1219-5FM), y-[(6-Aminohexyl)triazolo]-GTP-5-FAM (NU-1220-5FM) and GTPγS-BDP-FL (NU-973).
B) Assay scheme for determination of binding affinity of the fluorescent GTP analogs via fluorescence anisotropy-based method. (Figure adapted from Pepanian *et al.* [3])
C) Dissociation constants (K_D) of y-[(4-Aminobutyl)triazolo]-GTP-5-FAM (NU-1219-5FM), y-[(6-Aminohexyl)triazolo]-GTP-5-FAM (NU-1220-5FM) and GTPγS-BDP-FL (NU-973).on Gαi1 protein. [3]

Table 1: Available Gαi1-specific probes developed by Pepanian *et al.* [3].

Cat. No.	Product Name	Specifications
NU-1219-5FM	γ-[(4-Aminobutyl)triazolo]-GTP-5-FAM	Purity: >/= 95 % (HPLC) Concentration: 1.0 mM – 1.1 mM pH: 7.5 ±0.5
NU-1220-5FM	γ-[(6-Aminohexyll)triazolo]-GTP-5-FAM	Purity: >/= 95 % (HPLC) Concentration: 1.0 mM – 1.1 mM pH: 7.5 ±0.5

Selected References:

[1] Nubbemeyer *et al.* (2021) Strategies towards targeting Galpha(i/s) proteins: Scanning of protein-protein interaction sites to overcome inaccessibility. *ChemMedChem.* 15: 1696-1715. DOI: 10.1002/cmdc.202100039.
[2] O'Hayre *et al.* (2013) The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. *Nat Rev Cancer.* 13:412-24.
[3] Pepanian *et al.* (2022) Fluorescence Anisotropy Assay with Guanine Nucleotides Provides Access to Functional Analysis of Gαi1 Proteins. *Anal Chem.* **94**:14410-14418.
[4] Gille *et al.* (2003) 2'(3')-O-(N-methylanthraniloyl)-substituted GTP analogs: a novel class of potent competitive adenylyl cyclase inhibitors. *J Biol Chem.* **278**:12672-9.
[5] McEwen *et al.* (2001) Fluorescent BODIPY-GTP analogs: real-time measurement of nucleotide binding to G proteins. *Anal Biochem.* **291**:109-17.
[6] Gille *et al.* (2003) Low-affinity interactions of BODIPY-FL-GTPgammaS and BODIPY-FL-GppNHp with G(i)- and G(s)-proteins. *Naunyn Schmiedebergs Arch. Pharmacol*. **368**:210.