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A naphthalimide-derived fluorogenic probe for SNAP-tag with a fast record labeling rate

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ABSTRACT

SNAP-tag is one of most popular genetically encoded protein tags that can be labeled with fluorescent molecules for visualizing a protein of interest in live cells. Fluorogenic probes keep dark until they label protein tags, significantly improving the signal-to-noise ratio to image proteins without wash-out step. However, most of reported fluorogenic probes for SNAP-tag suffered from the low or mild labeling rate comparing with non-fluorogenic ones. In this paper, we reported a 4-amino-naphthliamide derived fluorogenic probe for SNAP-tag, which exhibited the fast record labeling rate among fluorogenic probes. Finally, we applied this probe to image proteins in mitochondria and nucleus in live cells without wash-out steps.

1. Introduction

Fluorescent imaging is a powerful tool to study protein functions from the cellular to the integrative level [1]. Benefited from the small size and a broad range of emission wavelengths, organic fluorophores are attractive alternatives to fluorescent proteins to label protein [2]. But an inherent disadvantage of organic fluorophores is that they are not genetically encoded and cannot be accurately located and quantified as fluorescent proteins [3,4]. To solve this problem, chemists have developed methodologies to covalently modify proteins with artificial probes [5,6]. The represented method to site-specifically label proteins with small molecules is self-labeling protein tags, which were firstly fused in frame to a protein of target and subsequently covalently labeled by probes through highly specific enzymatic reactions [7]. In live cells imaging, there are several critical concerns to design small fluorescent probes for self-labeling protein tags [7]. These fluorescent probes should be readily cell-permeable, react rapidly with protein tags, and not binding nonspecifically to endogenous other biomolecules. Another, and perhaps most importantly, the probes should be fluorogenic after labeling with protein tags to give higher signal-to-noise ratios. Otherwise, step of washing out unreacted or nonspecifically bound probes is required before imaging, which seriously limits live cell imaging.

The most popular self-labeling protein tags are SNAP-tag and Halo-tag, which undergo an irreversible reaction with O^6-benzylguanine derivatives and primary chloroalkanes, respectively [8,9]. The typical feature of fluorogenic probes is to exhibit no fluorescence but display a selective turn-on fluorescence response when they react with the protein tags. A list of fluorogenic probes for SNAP-tag [10–21] and Halo-tag [22,23] have been reported, and some of them are already commercial available. These probes have been proved useful tools to study protein location, dynamics and interactions in live cells without washing-out steps [24–27]. A typical example reported by Johnsson et al. exhibited fluorogenicity due to the spiroring-opening reaction of silicon-rhodamine

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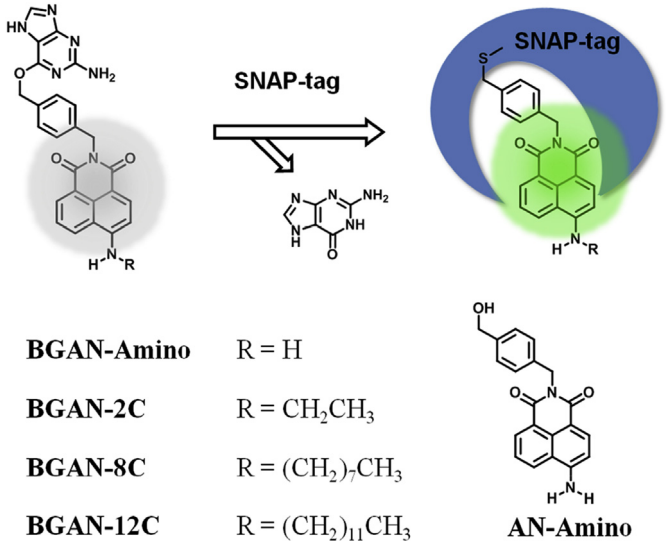
fluorophore after being labeled to SNAP-tag or Halo-tag [11]. The excellent fluorescent properties made this probe enable no-wash super-resolution imaging in live cells [25,28]. However, the labeling kinetics of most of reported fluorogenic probes were much lower than non-fluorogenic ones with corresponding protein tags. The rate constants of fluorogenic probes were usually estimated in the order of 10^3 M^{-1} S^{-1}, while non-fluorogenic ones in the order of 10^4~10^6 M^{-1} S^{-1}. Although the cause of the lower reaction rate of fluorogenic probes is not entirely clear, we can still get some reasonable information through the analysis of chemical structures. These fluorogenic probes were designed under three strategies [24], including fluorescence resonance energy transfer (FRET) quencher release, fluorophore's environmental sensitivity affected by the interactions between fluorophore and protein tag, and fluorophore generation in situ. The additional fluorescence quencher enlarged molecule size and lead to low cell permeability and long protein labeling time. Compared with non-fluorogenic probes, beside reaction site of fluorescent substrates, the additional interaction between protein tag and fluorophore reduced the speed of dynamic balance. Therefore, new fluorogenic probes with rapid labeling rates are still desirable for no-wash live cell imaging.

In our recent work, we reported a 4-ethylamino-Napthalimide-derived fluorogenic probe **BGAN-2C** for SNAP-tag [18]. This probe can rapidly label a SNAP-tag (k = ~2031.7 ± 63.7 M^{-1} S^{-1}) and exhibit a fluorescence increase of 36-fold due to the additive effects of environment sensitivity of fluorophores and inhibition of photo-induced electron transfer from O^6-benzylguanine to the fluorophore (Scheme 1). Notably, we found that the alkylamino group seriously affected the labeling rate. For example, the reaction rate of 4-Octylamino-naphthalimide derivative **BGAN-8C** reduced to 165.2 ± 6.0 M^{-1} s^{-1}, while the one of 4-Dodecylamino-naphthalimide derivative **BGAN-12C** was too slow to be determined. Thus we speculated that the length of alkyl chain was the main influencing factor on reaction rate. The shorter the alkyl chain, the faster the reaction rate. In this paper, we synthesized a 4-Amino-Naphthalimide derivative **BGAN-Amino** and were excited to find that this probe exhibited a record reaction rate among known fluorogenic probes for SNAP-tag and good fluorogenicity properties (Scheme 1). Finally, we used this probe to image proteins in mitochondria and nucleus in live cells without wash-out steps. Compound **AN-Amino** was synthesized as a control compound.



Scheme 1. Structure of naphthalimide-derived fluorogenic probes and the reaction with SNAP-tag. After covalently binding to the SNAP-tag protein, 1,8-naphthalimide displayed increased fluorescence due to the localization into the hydrophobic binding environment of the SNAP-tag and the release of fluorescence quencher (guanine).

2. Experimental

2.1. Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents (DMSO, DMF, tetrahydrofuran, dichloromethane, hexane, ethyl acetate, ethyl alcohol, acetone and methanol) were from Sigma-Aldrich and used without further treatment or distillation. *E. coli* strain BL21 and Trans 5α were purchased from Lucigen. Ampicillin (Amp) and isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from Sigma-Aldrich. Ni-NTA agarose and Sephadex G-50 were from GE Healthcare. All water used was from a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer, using TMS as an internal standard. Chemical shifts were given in ppm and coupling constants (J) in Hz. Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. UV-vis absorption spectra were collected on an Agilent Cary 60 UV-Vis Spectrophotometer. Fluorescence measurements were performed on an Agilent CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). The fluorescence lifetime were measured using Fluoromax-4 spectro-fluorometer equipped with a NanoLED-455 pulsed diode (excitation wavelength, 457 nm) and a DeltaHub TCSPC controller. The fluorescence lifetime of all samples was monitored at 530 nm. The quantum yields were determined via the relative determination method, with Coumarin 153 as a reference compound. The fluorescence imaging was performed by using ANDOR™ living cell laser scanning confocal microscope (Revolution WD).

2.2. Synthesis

2.2.1. Synthesis of *An-Amino*

Compound **AN-Br** and **1** were synthesized according to the previous literature [18].

Compound **AN-Br** (200 mg, 0.50 mmol) was dissolved in 10 mL DMF, and sodium azide (100 mg, 1.5 mmol) in 1 mL deionized water was added to the mixture. After stirring 6 h at 100 °C, the solution was poured into 50 mL ice water. The solid was collected and dissolved in 50 mL acetonitrile. Sodium sulfide nonahydrate (720 mg, 3.00 mmol) was added to the solution. The solvent was removed under reduced pressure after reaction at 60 °C overnight. The residue was purified through flash column chromatography (DCM:MeOH = 200:1) to give yellow powder 45 mg, yield 27%. ^{1}H NMR (400 MHz, $DMSO_{d6}$) δ 8.62 (d, J = 8.3 Hz, 1H), 8.43 (d, J = 7.2 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.49 (s, 2H), 7.28 (d, J = 7.9 Hz, 2H), 7.23 (d, J = 7.9 Hz, 2H), 6.85 (d, J = 8.4 Hz, 1H), 5.19 (s, 2H), 5.13 (t, J = 5.6 Hz, 1H), 4.43 (d, J = 5.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.27, 163.35, 153.38, 141.63, 136.83, 134.64, 131.69, 130.24, 129.96, 127.81, 126.90, 124.48, 122.11, 119.84, 108.72, 107.78, 63.14, 42.71. HRMS (ESI) calcd for $C_{20}H_{17}N_2O_3$ [MH^+] 333.1239, found 333.1237.

2.2.2. Synthesis of *BGAN-Amino*

The synthetic route of **BGAN-Amino** was shown in Fig. S1. A solution of compound **AN-Amino** (30 mg, 0.09 mmol), **1** (81 mg, 0.27 mmol) and t-BuOK (70 mg, 0.54 mmol) in 5 mL dry DMF was stirred at room temperature for 10 h under N_2. The solvent was then removed under reduced pressure, and the residue was purified by

flash column chromatography (DCM:MeOH = 15:1) to give yellow powder 26 mg. Yield 62%. ^{1}H NMR (400 MHz, DMSO) δ 12.41 (s, 1H), 8.64 (d, J = 8.3 Hz, 1H), 8.45 (d, J = 6.8 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.79 (s, 1H), 7.70–7.61 (m, 1H), 7.51 (s, 2H), 7.43 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 6.86 (d, J = 8.4 Hz, 1H), 6.27 (s, 2H), 5.43 (s, 2H), 5.23 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.32, 163.37, 160.08, 153.44, 134.72, 131.79, 131.77, 130.34, 130.30, 130.12, 130.03, 129.18, 128.99, 128.01, 124.53, 122.12, 119.87, 108.74, 107.76, 66.95, 42.74. HRMS (ESI) calcd for $C_{25}H_{20}N_7O_3$ [MH^+] 466.1628, found 466.1626.

2.3. Preparation of the SNAP-tag

pSNAP$_f$ Vector (NEB) plasmid was digested by restriction enzymes Nhe I and Xho I. And then the SNAP-tag gene was cloned into pET-22b forming plasmid pET-22b-SNAP$_f$ with 6His-tag in C-terminal. The plasmid was then transformed into *E. coli* strain BL21 and cultured at 37 °C in 500 mL LB medium containing 50 mg/mL ampicillin. The protein expression was induced by the addition of 1 mM IPTG when OD_{600} reached 0.8. After an additional growing at 25 °C for 4 h, the cells were harvested by centrifugation. The protein was extracted by sonicating in 20 mM PBS buffer pH 7.4, including 1 mM PMSF and 50 mM NaCl. The supernatant liquid was loaded into Ni-NTA column running in 20 mM PBS buffer pH 7.4, and SNAP protein was eluted with 200 mM imidazole in PBS buffer. The protein was further purified by Sephadex G-50 in 20 mM PBS buffer pH = 7.4. Total protein was measured by the Bradford protein assay using BSA as a standard. Electrophoresis (SDS-PAGE) was carried out to check the purification of the protein according to the protocol of the manufacturer.

2.4. Experimental procedures

2.4.1. Fluorometric analysis in vitro

BGAN-Amino probe was dissolved in DMSO to obtain 2 mM stock solution. And 5 μM **BGAN-Amino** and 5 μM SNAP-tag protein were incubated in PBS buffer containing 1% (v/v) DMSO at 37 °C for 30 min. And then the probe before and after reacting with SNAP were measured using fluorescence spectrophotometer with excitation at 440 nm. Relative fluorescence quantum yields of the compounds were obtained via the relative determination method, with Coumarin 153 as a reference compound, which compared the area under the emission spectrum of the sample with that of Coumarin 153 in ethyl alcohol (φ = 0.53 when excited at 420 nm) for derivatives.

2.4.2. SDS-PAGE analysis

Purified SNAP-tag (10 μM) was incubated with fluorescent probes (20 μM) at 37 °C for 30 min. The reaction mixtures were heated at 95 °C for 10 min and analyzed by SDS-PAGE. The gels were then stained with Coomassie Brilliant Blue and photographed.

2.4.3. Kinetic study of the SNAP-tag labeled by BGAN-Amino

The time required for labeling half of proteins, $t_{1/2}$ was estimated by monitoring the increase in the fluorescence intensity of the probes. It was determined under the condition of 5 μM **BGAN-Amino** and 5 μM SNAP-tag protein in PBS buffer at room temperature.

To estimate the second-order-rate constant k_2, we measured the fluorescence intensity of 0.5, 1, 1.5, 2, and 2.5 μM **BGAN-Amino** reacting with 250 nM SNAP-tag, respectively. The fluorescence data were converted to labeled fractions by using the following equation:

$$F_y = (F_t - F_0) / (F_{max} - F_0)$$

where F_t, F_{max}, and F_0 represent the measured, maximum and initial fluorescence intensities.

The pseudo-first-order rate constant (k_{obs}) of different probe concentrations were obtained by fitting the plots of F_y versus time as following equation: $F_y = 1 - \exp(- k_{obs} t)$.

Then, the plots of k_{obs} versus **BGAN-Amino** concentration were obtained. By fitting the plots with the equation: $k_{obs} = k_2$[**BGAN-Amino**], the second-order-rate constant, k_2 was acquired.

2.4.4. SNAP-tag labeling competition experiments

The commercial available probe **SNAP-surface-549** (structure shown in supporting information) had a similar labeling rate compared with **BGAN-Amino**. To evaluate the labeling properties of **BGAN-Amino**, the solution of 2 μM **BGAN-Amino** was mixed with that of 2 μM **SNAP-surface-549** to react with SNAP-tag at the same time. **BGAN-Amino** and **SNAP-surface-549** can be excited by 420 nm and 550 nm separately, and the fluorescence emissions centered at 532 nm and 570 nm were collected, respectively. The final percentage of labeling SNAP-tag with different probes can be calculated by the following equation.

$$A = (F - F_0) / (F' - F_0) * 100\%$$

A: The percent of labeled SNAP-tag.
F_0: The fluorescence intensity of 2 μM probe.
F': The fluorescence intensity of 2 μM probe treated with 1 μM SNAP-tag.
F: The fluorescence intensity of 2 μM BGAN-Amino and 2 μM **SNAP-surface-549** treated with 1 μM SNAP-tag at corresponding fluorescence emissions center.

2.4.5. Cell toxicity assay

The cytotoxicity of the fluorescence probe was determined using the MTT cell proliferation and Cytotoxicity Detection Kit (KeyGEN BioYECH) The HEK 293T cells were seeded into 96-well plate at a density of 1×10^4 cells/well in 200 μL DMEM (10% FBS) and then treated with fluorescence probe **BGAN-Amino** (at a final concentration of 0, 1, 2, 5, and 10 μM) 12 h under 5% CO_2, 37 °C. After incubation, the cell supernatant was removed and cells were supplemented with fresh 150 μL medium and 50 μL 1x MTT per well and incubated for another 4 h (5% CO_2, 37 °C). Then the cell supernatant was removed and the cells were resuspended with 150 μL DMSO. The absorption was recorded at 490 and 570 nm using a UV-Vis microplate reader. The cell viability was determined by comparing the probe treated cells and the untreated control.

2.4.6. Fluorescence imaging of HEK 293T cells

We transfected HEK 293T Cells (1000 cells/well) seeded in plates with plasmids pSNAP$_f$-Cox8A (NEB) and pSNAP$_f$-H2B (a gift from Prof. Yi Xiao, Dalian University of Technology) by using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol. After the incubation of the cells at 5% CO_2 37 °C for 48 h, the cells were incubated with 2 μM **BGAN-Amino** at 37 °C for 5 min and then imaged without washout process. The cell mitochondria were co-stained with 1 μM Mitotracker Red, and the cell nucleus were co-stained with 3 μM Hoechst 33342. Microscopic images of the cells were recorded using live-cell confocal laser scanning microscope.

3. Results and discussion

3.1. Fluorogenicity of **BGAN-Amino** to label SNAP-tag

3.1.1. Optical properties of **BGAN-Amino** and **AN-Amino** in different solvents

We have demonstrated that the fluorogenicity of **BGAN-2C** for SNAP-tag resulted from the additive effects of environment sensitivity of naphthalimide and inhibition of photo-induced electron transfer from O^6-benzylguanine to naphthalimide after labeling with SNAP-tag to release the fluorescence quencher of guanine group. To examine the fluorogenicity of **BGAN-Amino**, the fluorescence and absorption properties of **BGAN-Amino** and control compound **AN-Amino** were firstly studied to evaluate if the fluorescence of 4-amino-naphthalimide was also high polarity-sensitive and could be quenched by guanine group. As shown in Fig. 1 and Table 1, with decreasing solvent polarity, significant blue-shifts in emissions (from 549 nm to 503 nm; 541 nm−504 nm; $H_2O/CHCl_3$) and fluorescence increases (~29 fold; ~5 fold) were observed for **BGAN-Amino** and **AN-Amino**, respectively. The absorption spectra of these two compounds displayed about 30 nm blue-shifts in non-polar solvents. These data demonstrated that **BGAN-Amino** displayed typical solvatochromic properties. The different fluorescence enhancements of **BGAN-Amino** and **AN-Amino** in non-polar solvents demonstrated that the guanine group did act as a quencher in **BGAN-Amino.**

3.1.2. SANP-tag labeling in vitro

Then, **BGAN-Amino** was examined to label SNAP-tag. Compounds **BGAN-Amino** showed weak fluorescence in PBS buffer solutions. After labeling with SNAP-tag, a large fluorescence enhancement (12 fold) was observed (Fig. 2a, Fig. S2). This fluorescence enhancement was a result of both the inhibition of guanine quenching and 1,8-naphthalimide's environmental sensitivity. The binding characteristics of **BGAN-Amino** with SNAP-tag were further investigated by SDS-PAGE (Fig. 2b). Purified SNAP-tag was incubated with **BGAN-Amino** and analyzed. One strong fluorescence band in accordance with **BGAN-Amino** was observed, which was consist with that in solutions. The result demonstrated that **BGAN-Amino** reacted with SNAP-tag covalently.

The detailed kinetic analysis of the protein labeling was carried out by monitoring the fluorescence intensity of the probe, in which the concentration of probes and the protein were 5 μM (Fig. 2c). The time required for 50% labeling of SNAP-tag was about 11 s for **BGAN-Amino**. In further kinetic analysis, the second-order rate constant (k_2) for reaction between **BGAN-Amino** and SNAP-tag was determined to be 14436 ± 1189 M^{-1} s^{-1}. The labeling rate of **BGAN-Amino** is much faster than quencher-based SNAP-tag fluorogenic probes **DRBG-488** (396 ± 32 M^{-1} s^{-1}) [16] and **CBG-549-QSY7** (1027 ± 457 M^{-1} s^{-1}) [10], environment sensitive probe **BGSBD** (7200 ± 1600 M^{-1} s^{-1}) [12]. In our previous paper, we have demonstrated that the longer the hydrophobic carbon chains of 4-Amino-substituted naphthalimide, the slower the reaction rate with SNAP-tag. The reason may be that most of hydrophobic cavity of proteins was easy to be occupied by hydrophobic alkyl chains which restrained the covalent binding between the SNAP-tag and the BG ligand. Then we concluded that the fast labeling rate of **BGAN-Amino** was due to its small size without the interference to interact with SNAP-tag. Notably, as we know, **BGAN-Amino** displayed the fast record labeling rate with SNAP-tag among reported fluorogenic probes, even as faster as commercial available probe **SNAP-Surface-549**, which had a similar labeling rate (k = ~11138 M^{-1} s^{-1}) compared with **BGAN-Amino**, but can only label membrane proteins. To further evaluate the labeling kinetics of **BGAN-Amino**, the solution of 2 μM **BGAN-Amino** was mixed with that of 2 μM **SNAP-surface-549** to react with SNAP-tag at the same time (Fig. S3). The results indicated that **BGAN-Amino** had the equal ability with SNAP-Surface-549 to label SNAP-tag, but higher fluorescence signal-to-noise ratio, since **SNAP-Surface-549** displayed only 1 fold fluorescence enhancement after labeling with SNAP-tag.

3.1.3. BGAN-Amino enables no-wash imaging in live cells

Since **BGAN-Amino** displayed rapid and special labeling to

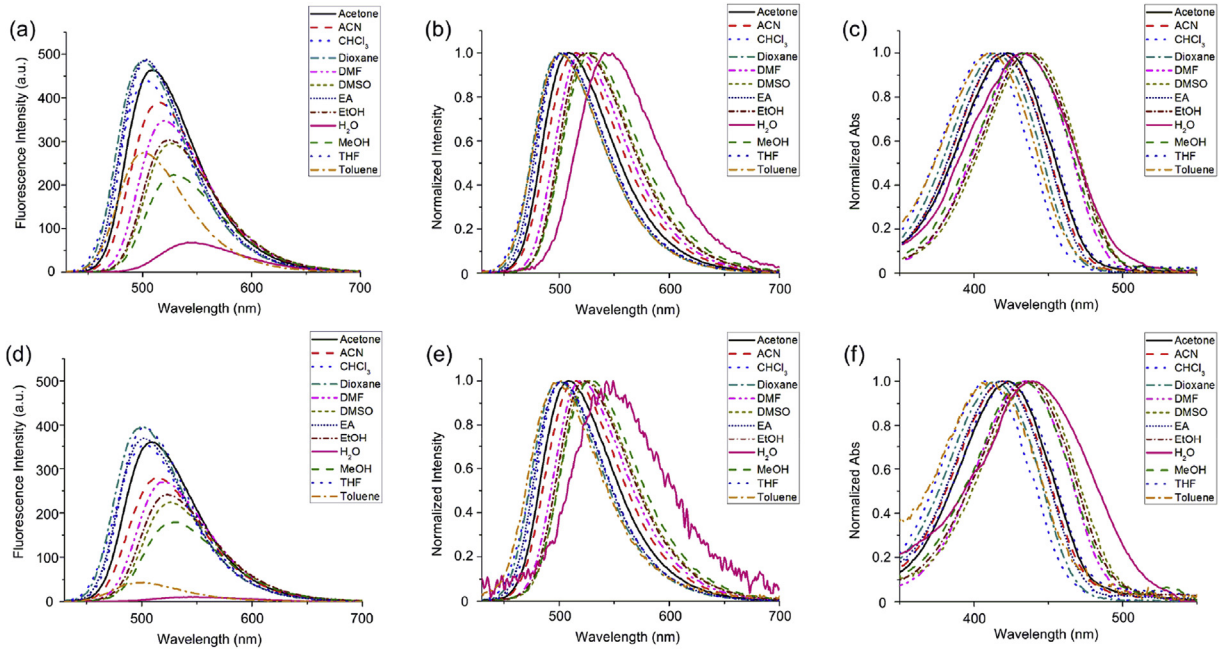


Fig. 1. (a) Fluorescence, (b) normalized and (c) absorption spectra of 10 μM **AN-Amino** in different solvents. (d) Fluorescence, (e) normalized and (f) absorption spectra of 10 μM **BGAN-Amino** in different solvents.

Table 1
Spectroscopic data of **AN-Amino** and **BGAN-Amino** in different solvents.

solvent		toluene	$CHCl_3$	acetone	THF	EA	dioxane	CH_3CN	EtOH	DMF	MeOH	DMSO	H_2O
λ_{abs}(nm)	**AN-Amino**	409	409	423	423	419	415	419	436	434	435	439	432
	BGAN-Amino	409	407	423	423	418	415	419	436	434	434	439	439
λ_{em}(nm)	**An-Amino**	501	504	508	502	504	500	515	524	521	529	526	541
	BGAN-Amino	502	503	509	502	501	500	516	523	520	529	526	549
ε ($M^{-1}cm^{-1}$)	**An-Amino**	8076	11658	12517	12172	12247	11412	11291	12932	12138	11988	12456	11739
	BGAN-Amino	1255	10218	10487	10547	9466	9764	8592	10668	9845	10063	10137	7436
$\Delta\lambda$ (nm)	**An-Amino**	92	95	85	79	85	85	96	88	87	94	87	109
	BGAN-Amino	93	96	86	79	83	85	97	87	86	95	87	110
φ	**An-Amino**	0.83	0.67	0.72	0.80	0.77	0.90	0.67	0.53	0.68	0.40	0.66	0.13
	BGAN-Amino	0.84	0.84	0.67	0.76	0.75	0.80	0.64	0.51	0.66	0.39	0.61	0.04
τ (ns)	**An-Amino**	9.1	10.3	10.2	10.0	10.0	10.8	10.4	8.77	10.1	7.42	10.0	3.21
	BGAN-Amino	4.32 (16%)	3.77 (7%)	9.86	9.72	9.74	10.4	10.2	9.90	9.90	7.45	9.87	2.66 (71%)
		9.15 (84%)	10.3 (93%)										6.23 (29%)
CHISQ	**An-Amino**	1.20	1.12	1.15	1.21	1.18	1.21	1.14	1.09	1.20	1.16	1.14	1.20
	BGAN-Amino	1.08	1.12	1.31	1.36	1.57	1.60	1.42	1.19	1.25	1.31	1.18	1.41

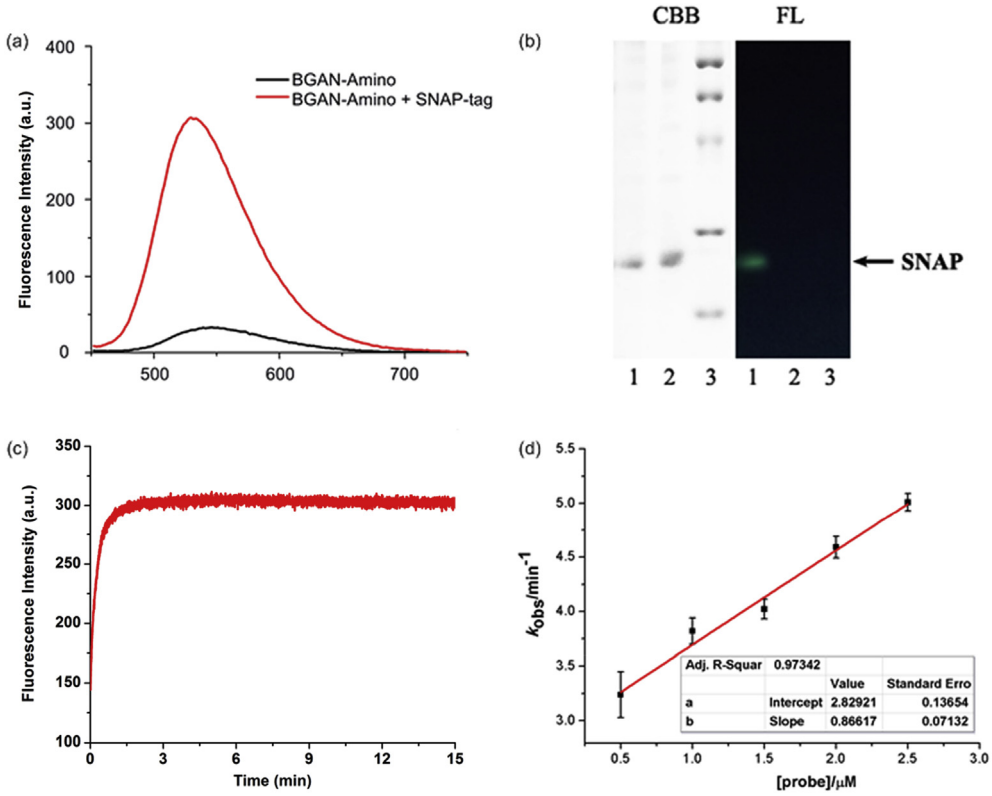


Fig. 2. Fluorescence spectra, SDS-PAGE, and kinetic analysis of the labeling reaction between SNAP-tag and **BGAN-Amino**. (a) Fluorescence spectra of 5 μM **BGAN-Amino** in the absence and presence of 5 μM SNAP-tag in PBS buffer (1% DMSO). (b) SDS-PAGE analysis of purified SNAP-tag (lane 2) and the labeling reaction of SNAP-tag with **BGAN-Amino** (lane 1). The molecule weight of protein marker (lane 3) are 97.2, 66.4, 44.3, 29.0, and 20.1 KDa, respectively. (c) Time course of fluorescence intensity of 5 μM **BGAN-Amino** in the presence of 5 μM SNAP-tag. (d) Plots of the pseudo-first-order rate constant (k_{obs}) versus **BGAN-Amino** concentrations. k_2 was the slope of the fitting line.

SNAP-tag *in vitro*, we next sought to apply **BGAN-Amino** for labeling intracellular proteins in live cells. First, toxicity test indicated that the probe is nontoxic to the HEK 293T cells (Fig. S4). HEK 293T cells which expressed SNAP-tag fusion proteins in mitochondria, and nucleus were investigated (Fig. 3). Commercialized plasmids $pSNAP_f$-Cox8A and $pSNAP_f$-H2B (a gift from Prof. Yi Xiao), which fused SNAP-tag to cytochrome *c* oxidase subunit 8 (Cox8A) and human histone H2B, respectively, were transiently transfected to HEK 293T cells. After 5 min incubation with 2 μM **BGAN-Amino**, the cells were observed wash-free with the probe. And clear green fluorescence was observed without obviously background fluorescence (Fig. 3a). Co-staining of the probe with red emission from Mitotracker Red confirmed that the probe localized in mitochondrial area (Fig. 3b–c). Meanwhile, specific labeling of SNAP-H2B fusion proteins in the nucleus was also observed (Fig. 3d–f). These results demonstrated that **BGAN-Amino** is high cell-permeable. More importantly, its fluorogenicity made it a good probe to visualize a target proteins at a specific location with no-wash procedure required.

In the further studies, competition experiments in live cells with **BG-TMR**, which was reported a SNAP-tag substrate but had no fluorogenicity [11], were carried out to evaluate the intercellular

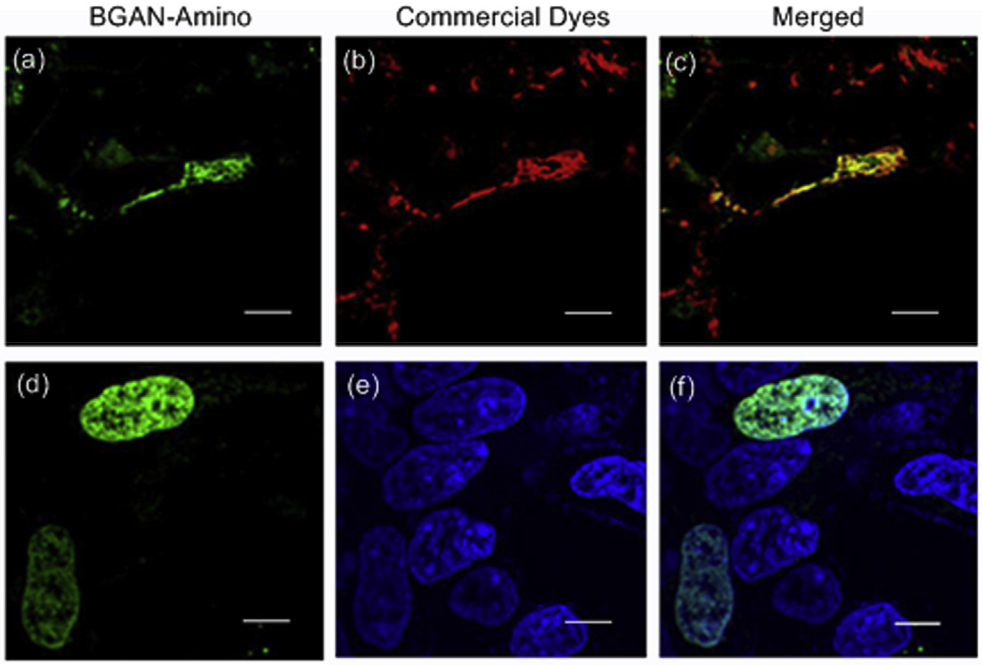


Fig. 3. No-wash HEK 293T live cell imaging of SNAP-tagged proteins labeled with **BGAN-Amino**. Cells were incubated with 2 μM BGAN-Amino at 37 °C for 5 min (a)–(c) and (d)–(f) present HEK 293T cells transiently expressing $pSNAP_f$-Cox 8A and $pSNAP_f$-H2B, respectively. (a) and (d) was treated with **BGAN-Amino** (Ex: 488 nm; Slit: 500–550 nm), (b) with Mitotracker Red (Ex: 561 nm; Slit: 580–653 nm), (e) with Hoechst 33342 (Ex: 405 nm; Slit: 417–477 nm). (c) and (f): overlay images of **BGAN-Amino** and commercial dyes. Scale bar = 10 μm.

labeling properties of **BGAN-Amino**. HEK 293T cells expressing SNAP-tag fused H2B was observed when incubated with the mixture of 1 μM **BGAN-Amino** and 1 μM **BG-TMR** without washing step. Fluorescence images (Fig. 4a) demonstrated the strong fluorescence exclusively in nucleus in 20 min with low background. It also revealed that **BGAN-Amino** can pass through the cell membranes and label nucleus specifically in 5 min. While, the cells incubated with **BG-TMR** showed high background and lower labeling speed (Fig. 4b).

4. Conclusion

In summary, we have developed a 4-amino-naphthalimide-derived fluorogenic probe **BGAN-Amino** for SNAP-tag. The typical feature of this probe is its very fast labeling rate with SNAP-tag, which would be much helpful for cell imaging. To our best knowledge, **BGAN-Amino** may display the fast record labeling rate with SNAP-tag among known fluorogenic probes. This characteristics allowed **BGAN-Amino** to have the ability to compete with commercial available probes to label proteins. Finally, we applied

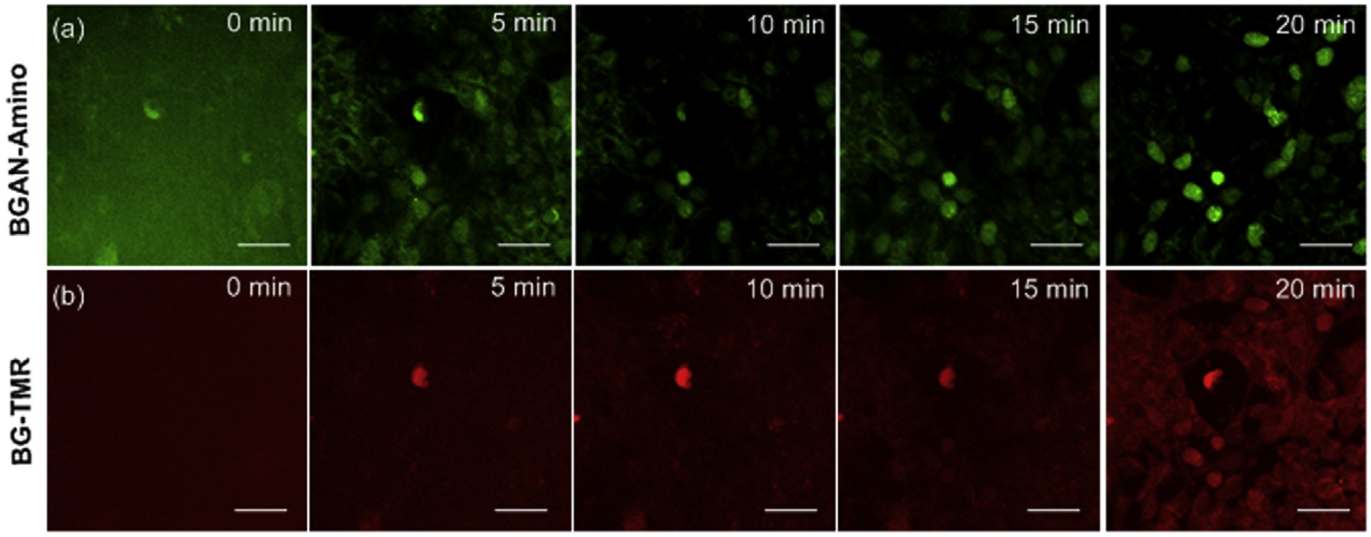


Fig. 4. No-wash HEK 293T live-cell imaging of nucleus labeled with 1 μM **BGAN-Amino** (Ex: 488 nm; Slit: 500–550 nm) and 1 μM **BG-TMR** (Ex: 561 nm; Slit: 580–653 nm), simultaneously. The confocal imagings were recorded at different points in time. Scale bar = 40 μm.

this probe to image proteins in mitochondria and nucleus in live cells without wash-out step.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2017.08.032.

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