Electronic Supplementary Information (ESI)

Spectral properties of ionic benzotristhiazole based donor-acceptor NLO-phores in polymer matrices and their one- and two-photon cellular imaging ability

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Table S1: Absorption maximum of BTT NLO-phores 1-3a, 1b, 2b and 1c in methanol (MeOH) and chloroform $(CHCl_3)$.

Sample ^a	MeOH ^b	CHCl ₃ λ_A	
	λ_{A}		
	nm	nm	
1a	540	566	
2a	580	626	
3a	598	643	
1b	528	565	
2b	550	598	
1c	586	605	

a) Structure of the NLO-phores according to Figure 1; ^{b)} data obtained from Ref. 24;



Figure 1: Absorption spectra of BTT chromophore 2*a* in solvents of different polarity (methanol and chloroform).

Light absorption characteristics

As typical for increased π -conjugation length in push-pull systems, the long-wavelength band shifts bathochromically with increasing length of the conjugated bridge in both polymer matrices (Table S2; Figures 2 and 3 in the main manuscript). Weaker ICT character in diphenylamino derivatives **1b** and **2b** due to resonance delocalization (-M effect) and thus weaker electron density at $-NPh_2$ nitrogen manifests itself in hypsochromically shifted λ_A values compared to NLO-phores **1a-3a** with $-NMe_2$ donor. Compared to NLO-phores **1a** and **1b** with the same length of conjugated bridge, compound **1c** with the julolidine donor exhibits the largest red-shift of absorption maximum ($\lambda_A = 609$ and 623 nm in PVC and PSS, respectively). Contrary to $-NMe_2$ and $-NPh_2$ donors in **1a** and **1b**, nitrogen in julolidine substituent is rigidized and rehybridized from pyramidal to planar configuration and thus enables better conjugation and more effective charge transfer. Extinction coefficient (ε) of NLO-phores in polymer matrices follows the trend observed in methanol (Table S2).

Decrease in ε for most of the studied chromophores with the exception of **1a** in polar anionically charged PSS matrix indicates specific chromophore interaction with the polymer chain. Partially structuralized absorption spectra in PSS, mainly for **1a-3a** and **1c** derivative, confirm this assumption. Absorption maxima of all studied derivatives are hypsochromically shifted compared to less polar PVC (Table 1) and thus exhibit "negative solvatochromic-like" behaviour also in the polymer matrix. In all cases, the chromophore band shoulder position in PSS (Figure 3A in the main manuscript) more or less correlates with chromophore λ_A in PVC. As shown in previous study [24], the quaternization of BTT nitrogen has an expressive influence on the high withdrawing character of the benzotristhiazolium acceptor unit when compared to corresponding neutral benzotristhiazoles. On the other side, the electron density of HOMO orbital is dominantly localized on the donor part of the molecule [24]. It is also commonly known that the PPS salt dissolution in solvent leads to adsorption of positively charged adsorbates by the negatively charged side group [82]. Therefore, interaction of PSS sulfonate anion with cationic BTT moiety results in additional BTT NLO-phore ground state stabilization and thus to HOMO-LUMO energy gap increase (Figure S2). However, also chromophore-polymer $\pi(\delta)$ - $\pi(\delta+)$ interactions cannot be excluded (Figure S3). Consequently, the λ_A position in PSS is blue-shifted compared to PVC and almost approaches the λ_A value in polar MeOH. As discussed further, fluorescence measurements exclude the formation of both H- and J-aggregates.

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peel a perjor mea in methanol, 1 + C ana 1 55 polymer maintees.						
Sample ^a	Methanol ^b		PVC		PSS	
	λ_{A}	3	$\lambda_{\rm A}$	3	λ_{A}	3
	nm	mol ⁻¹ Lcm ⁻¹	nm	mol ⁻¹ Lcm ⁻¹	nm	mol ⁻¹ Lcm ⁻¹
1a	540	63 800	573	52 860	549; 580sh	49 920
2a	580	76 300	634	64 300	588; 655sh	51 420
3 a	598	54 300	648	44 070	613	18 600*
1b	528	53 900	564	46 660	543	31 130
2b	550	52 600	586	42 790	555	35 160
1c	586	98 000	609	103 320	590sh; 622	71 040

 Table S2: Spectral parameters of BTT NLO-phores 1-3a, 1b, 2b and 1c according to absorption

 spectra performed in methanol, PVC and PSS polymer matrices.

^{a)} Structure of the NLO-phores according to Figure 1; b) data obtained from Ref. 24; λ_A - Absorption band maximum (band shoulder); ε - molar extinction coefficient; * - not good film forming properties in PSS.



Figure S2: Proposed interaction of studied benzotristhiazolium NLO-phores with PSS polymer matrix.



Figures S3: Possible chromophore-polymer $\pi(\delta)$ - $\pi(\delta+)$ interactions of studied BTT NLO-phores with PSS polymer matrix.



Figures S4 and S5: Normalized absorption, excitation and emission spectra of investigated BTT chromophores 1a and 2a in PSS matrix at concentration of 2×10^{-4} mol kg⁻¹.



Figures S6 and S7: Normalized absorption, excitation and emission spectra of investigated BTT chromophores 1b and 1c in PSS matrix at concentration of 2×10^{-4} mol kg⁻¹.



Figure S8: Fluorescent quantum yield of studied BTT chromophores 1a and 1b in methanol/glycerol mixtures of different viscosity.

Table S3: Fluorescent quantum yields of BTT NLO-phores 1b and 1c in solvents of different polarity.

Solvent	Dielectric constant	$\mathbf{\Phi}_{\mathrm{F}}$		
		1b ^a	1c ^a	
1,4-dioxane	2.25	0.03	_*	
toluene	2.39	_*	_**	
EA	6.07	_**	_**	
THF	7.58	0.02	0.008	
CH_2Cl_2	8.93	0.01	0.03	
MeOH	33.6	0.002 ^b	0.007 ^b	

^{a)} Structure of the NLO-phores according to Figure 1; ^{b)} data obtained from Ref. 24; EA – ethyl acetate; THF – tetrahydrofuran; CH₂Cl₂ – dichloromethane; MeOH – methanol; Φ_F – quantum yield of fluorescence determined using integration sphere (excitation wavelength = λ_A); * – low solubility; ** – nonfluorescent.



Figure S9: Bathochromic shift of absorption maximum of BTT chromophores **1b** and **1c** in dichloromethane (CH_2Cl_2) compared to absorption maximum in other medium polar solvents (EA - ethylacetate and THF - tetrahydrofuran).

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Sample ^a	au ^b				
	ns				
	Glycerol/MeOH		Glycerol/MeOH		
	3:7 (v/v)		9:1 (v/v)		
1b	$\tau_1 = 0.1$	$(\alpha_1 = 0.485)$	$\tau_1 = 0.3$	$(\alpha_1 = 0.744)$	
	$\tau_2 = 1.4$	$(\alpha_2 = 0.267)$	$\tau_2 = 1.9$	$(\alpha_2 = 0.072)$	
	$\tau_3 = 9.5$	$(\alpha_3 = 0.004)$	$\tau_3 = 15.7$	$(\alpha_3 = 0.002)$	
	$\overline{\tau} = 1.9$		$\overline{\tau} = 2.2$		
1c	$\tau_1 = 0.1$	$(\alpha_1 = 1.342)$	$\tau_1 = 0.9$	$(\alpha_1 = 0.430)$	
	$\tau_2 = 1.6$	$(\alpha_2 = 0.041)$	$\tau_2 = 2.5$	$(\alpha_2 = 0.017)$	
	$\tau_3 = 10.0$	$(\alpha_3 = 0.006)$	$\tau_3 = 15.0$	$(\alpha_3 = 0.002)$	
	$\overline{\tau} = 2.4$		$\bar{\tau} =$	= 2.0	

Table S4: Fluorescence lifetime of BTT NLO-phores *1b* and *1c* in MeOH/glycerol solvent mixtures of different viscosity.

^{a)} Structure of the NLO-phores according to Figure 1; ^{b)} τ – Fluorescence lifetime (Excited state lifetime); α – pre-exponential factors; $\overline{\tau}$ – calculated average lifetime; Quality of fluorescence lifetime fitting did not exceed χ^2 = 1.200



Figure S10: The average intensity of *1c* fluorescence signal during the time lapse experiment. The sample was continuously scanned with 561 nm excitation at 15 μ W intensity at the sample.



Figure S11: Normalized emission spectra of investigated BTT chromophore *1c* under *1P* (561 nm) and *2P* (*1120 nm*) excitation (with emission maximum around 640 nm) in *1c* stained cells.



Figure S12: Excited state lifetime decays of 1c in mitochondria of live HeLa cells grown on glass coverslip in every 10^{th} second during 100s FLIM time series acquisition. Individual decays are labelled with different colors, as indicated on this figure, and overlap perfectly.



Figure S13: Normalized emission spectra of investigated BTT chromophore 1b under 1P (561 nm) and 2P (1120 nm) excitation (with emission maximum around 640 nm) in 1b stained cells.



Figure S14: Normalized 1P excitation spectra of investigated BTT chromophores 1b and 1c in BTT chromophore stained cells.



Figure S15: HeLa cells stained with 100 nM **1b** ($M = mol \ dm^{-3}$). The frame sequential images were acquired with one photon 561 nm (Image A) and 2P excitation at 1120 nm (Image B). The colour is proportional to the real emission spectra. The white bar indicates 10 μ m.



Figure S16: Fluorescence decay (normalized) of investigated BTT chromophores 1b and 1c in BTT chromophore stained cells.