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Monitoring penetratin interactions with lipid membranes and cell internalization using a new hydration-sensitive fluorescent probe

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Table 1. Spectroscopic properties of the 7AF label in solvents.

	Solvent	$E_T(30)$	ε	λ_{Abs}, nm	λ_{N*}, nm	λ_{T*}, nm	I_{N*}/I_{T*}	QY, %
1	toluene	33.9	2.4	402	445	583	0.004	31.6
2	dioxane	36	2.2	394	441	586	0.011	25.9
3	THF	37.4	7.4	397	451	586	0.007	25.7
4	acetone	42.2	20.5	399	469	589	0.013	23.6
5	DMF	43.2	37.2	404	478	596	0.029	21.9
6	octanol	48.3	9.9	409	491	571	0.23	43.6
7	MF	54.1	180	409	506	590	0.38	29.8
8	EtOH	51.9	24.9	408	505	572	0.32	36.4
9	MeOH	55.4	32.6	410	511	572	0.57	38.1
10	Water*	63.1	78.4	411	534	593	13	11.2

$E_T(30)$ – empiric polarity index [20]. ε– dielectric constant, λ_{ABS} – position of absorption maxima, λ_{N*} and λ_{T*} - position of fluorescence maxima of N* and T* forms, respectively. I_{N*}/I_{T*} - ratio of the intensities of the two emission bands at their peak maxima (errors are ± 2%). QY, fluorescence quantum yield (errors are ± 5%), measured using 3-hydroxy-4'-diethylaminoflavone in EtOH (QY = 51% [48]) as a reference. Abbreviations: THF – tetrahydrofuran, DMF – *N,N*-dimethylformamide, MF – *N*-methylformamide. *Fluorescence parameters were obtained by mathematical deconvolution of spectrum. 20 mM phosphate buffer, 150 mM NaCl, pH = 7.4 was used. Excitation wavelength was 408 nm.

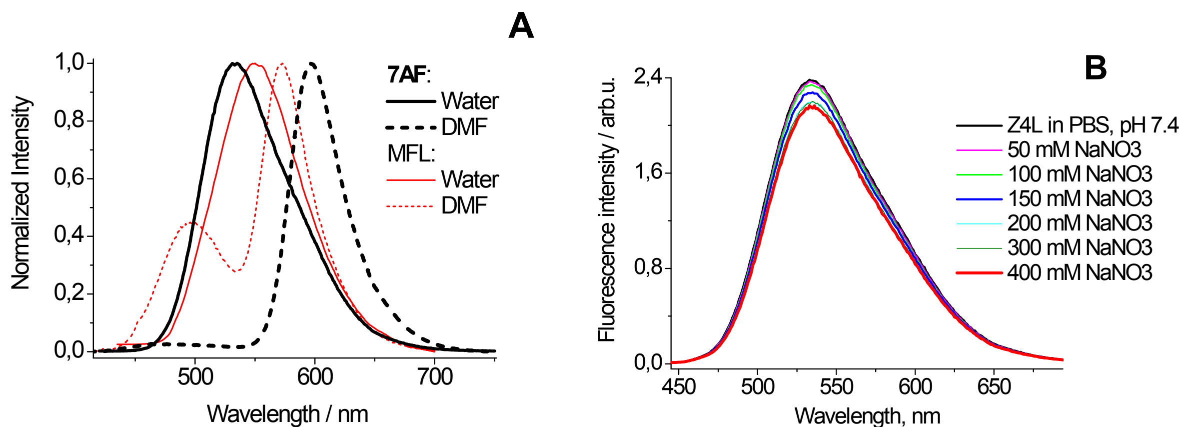


Fig. S1. Normalized fluorescence spectra of MFL [24] and 7AF labels in DMF and water (A). Changes in fluorescence spectra of 7AF label in PBS buffer versus the concentration of sodium nitrate (B). Excitation wavelength is 400 nm (for organic solutions) and 415 nm (for water ones).

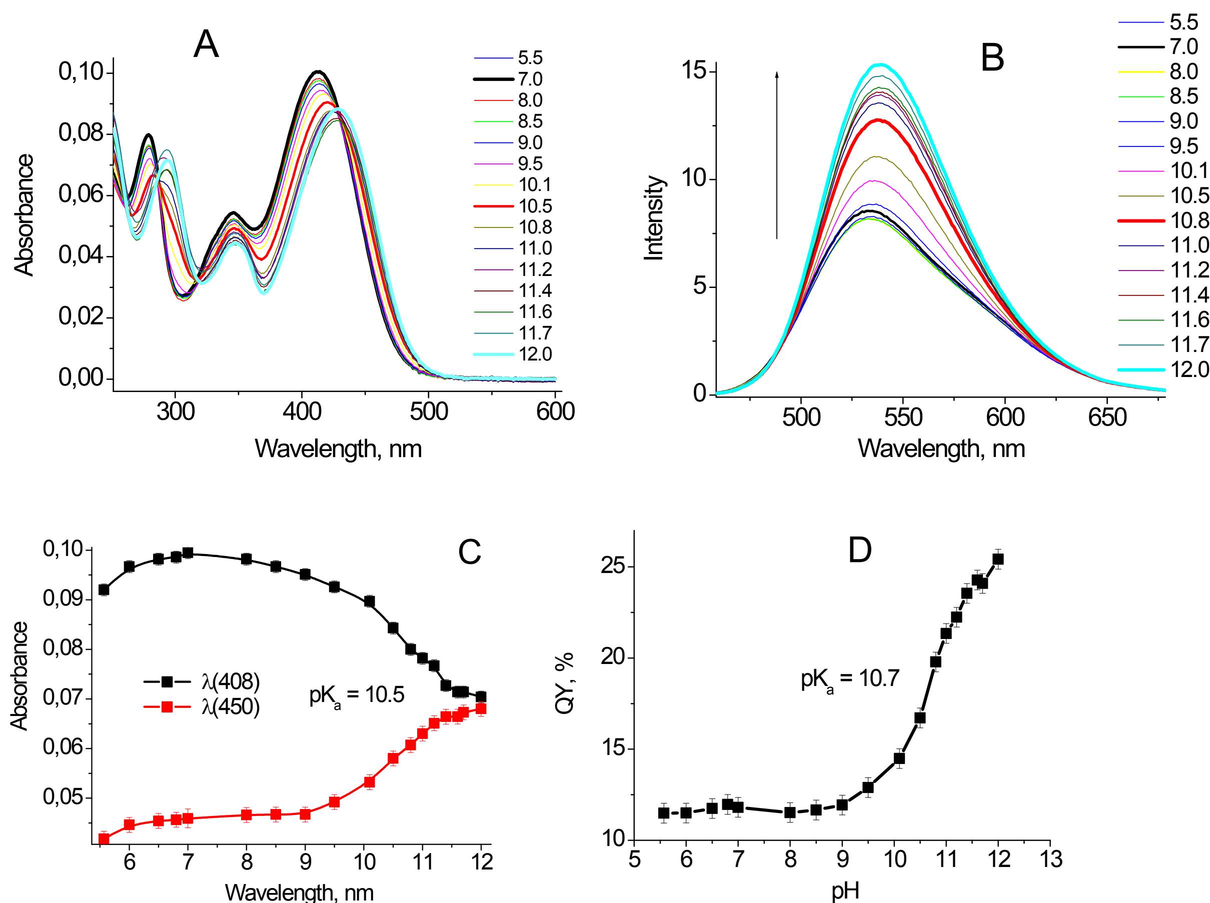
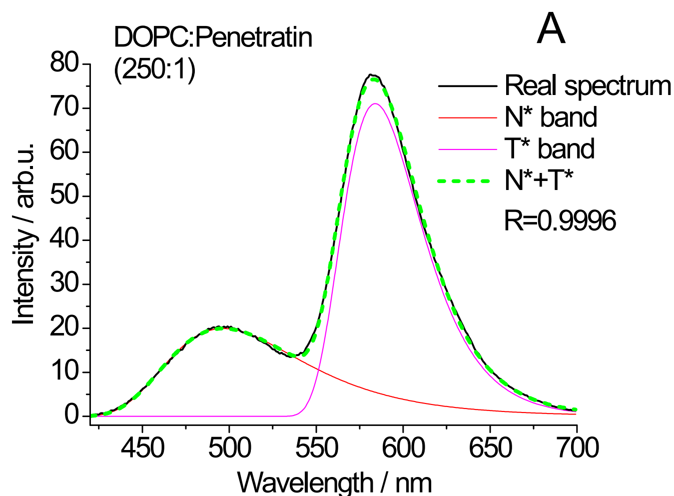


Fig. S2. Absorption (A), fluorescence (B) spectra of 7AF label in PBS buffer at the changes of pH from 5.5 to 12.0. Changes of absorbance (C) and fluorescence intensity (D) versus pH. Excitation wavelength is 415 nm.

Deconvolution of spectra.

Deconvolution of the fluorescence spectra into N* and T* bands was performed using the Siano software kindly provided by Prof. A.O. Doroshenko (Kharkiv, Ukraine), as previously described [35]. The program is based on an iterative nonlinear least-squares method, where the individual emission bands were approximated by a log-normal function accounting for several parameters: maximal amplitude, I_{max}, spectral maximum position, ν_{max}, and position of half-maximum amplitudes, ν_1 and ν_2, for the blue and red parts of the band, respectively. These parameters determine the shape parameters of the log-normal function, namely the full width at the half-maximum, FWHM=$\nu_1-\nu_2$, and the band asymmetry, P=$(\nu_1-\nu_{max})/(\nu_{max}-\nu_2)$. All parameters were allowed to vary in the iteration process, due to that a sum of two separated bands was in good correlation with an initial spectrum ($R\geq0.998$ for the experiments with lipids, $R\geq 0.9998$ for solvent mixtures). In the case of spectra from neat water T*-band FWHM parameter was determined by averaging the data from binary solvent mixtures and then fixed upon the procedure of deconvolution. The resulting integral fluorescence intensities of the separated N* and T* bands (I_{N*} and I_{T*}) were used for calculation of the N/T ratio.

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C:\_PVG\CHEM_PRO\SDL\OPC250.DTX
23800  14280
R=0.99963
  νmax(cm-1) HW   (Band asymmetry data)      Peak intensity          Integral intensity
1  20143  3741  0.777  1635  2106     199628.516 *   3402.62 =  6.7926E+0008
2  17125  1573  0.740   669   904     710406.438 *   1434.68 =  1.0192E+0009
Fluo
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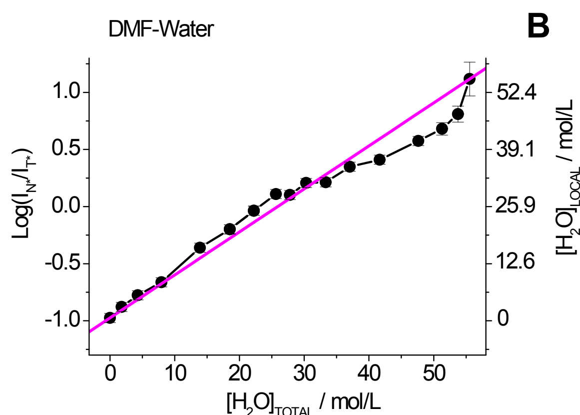

Figure S3. (A) Example of deconvolution of spectra of 7AF labeled penetratin in DOPC LUVs at L/P ratio= 250 (presented in λ scale) and numerical data of the deconvolution. (B) Logarithm of N* and T* bands intensity ratio Log(N/T) of 7AF probe versus the total concentration of water in the mixture. Approximation: *y=0,0377x-0,975* (R= 0.987, SD=0.0956).

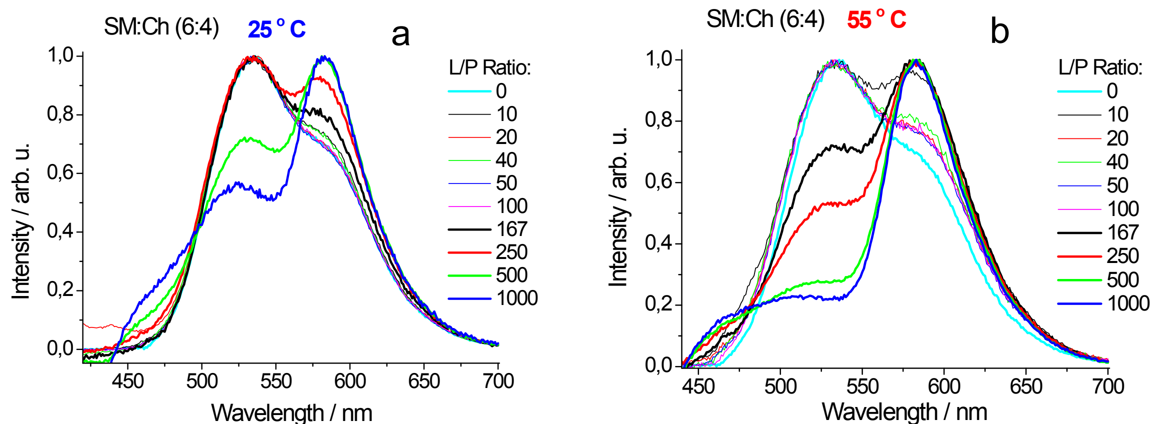

Fig. S4. Fluorescence spectra of 7AF- labeled penetratin in SM-cholesterol (6:4) LUVs at 25ºC (A) and 55ºC (B) at different lipid-peptid ratio. Excitation wavelength is 400 nm.

Fig. S5. Changes in shape of fluorescence spectra of 7AF label alone (a) and 7AF-labeled penetratin (b,c) in DPPC LUVs at heating (a) and cooling (b,c). In (c) and (d) spectra are normalized at N* or T* maximum for better representation. Excitation wavelength is 400 nm.

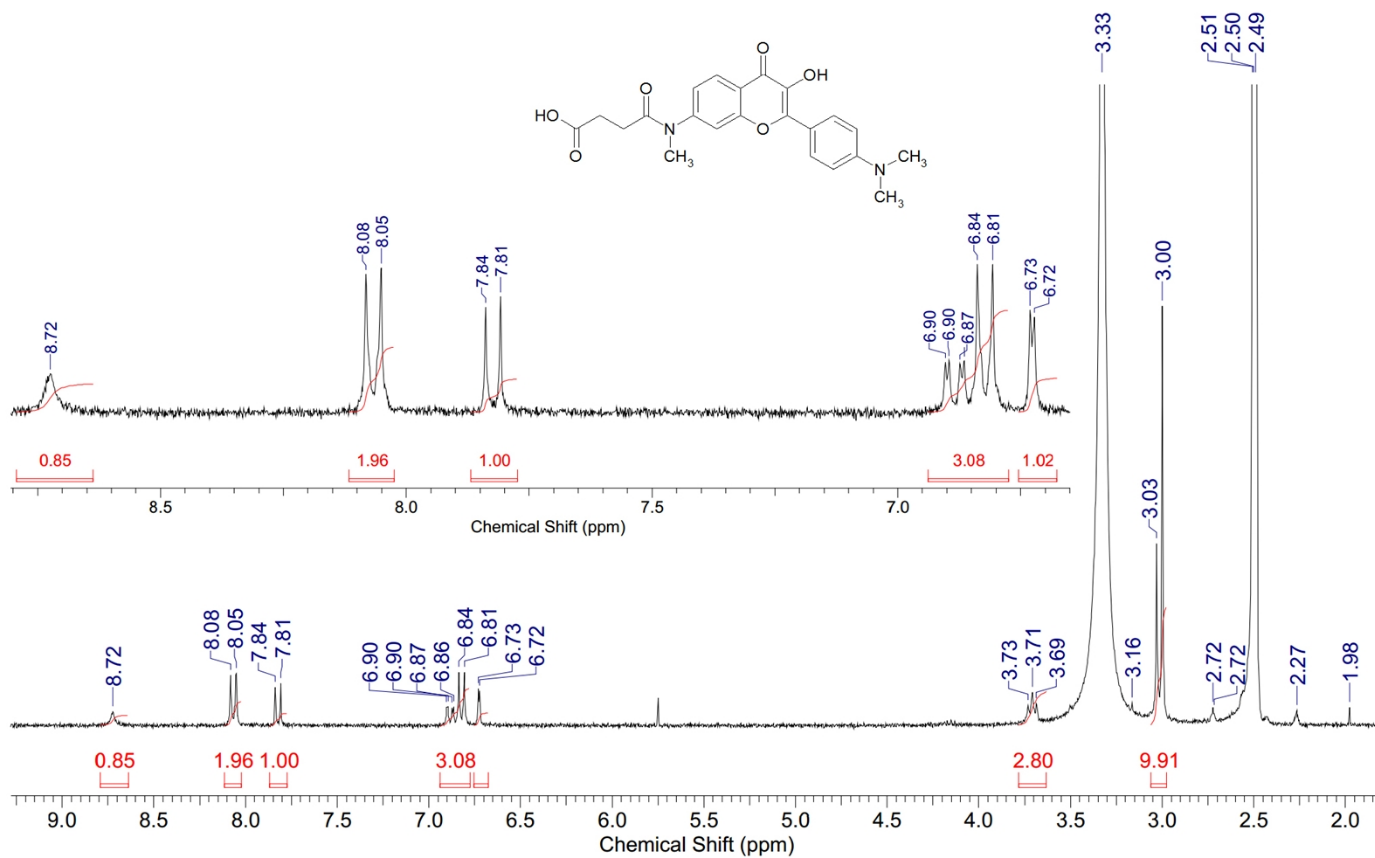

Figure S6. *1H*-NMR spectrum of 7AF label in DMSO-d_6.