

## Supplementary Information

### Solvatochromic Dye LDS 798 as Microviscosity and pH Probe

Hung Doan<sup>a</sup>, Marlius Castillo<sup>b</sup>, Micheline Bejjani<sup>c</sup>, Zhangatay Nurekeyev<sup>a</sup>, Sergei V. Dzyuba<sup>b</sup>, Ignacy Gryczynski<sup>d</sup>, Zygmunt Gryczynski<sup>a\*</sup>, Sangram Raut<sup>a, e\*</sup>

<sup>a</sup>Department of Physics and Astronomy, Texas Christian University, Fort Worth, TX, 76129

<sup>b</sup>Department of Chemistry and Biochemistry, Texas Christian University, Fort Worth, TX, 76129

<sup>c</sup>American University in Dubai, United Arab Emirates

<sup>d</sup>Institute of Molecular Medicine, UNT Health Science Center, Fort Worth, 76107

<sup>e</sup>Institute for Cardiovascular and Metabolic Disease, UNT Health Science Center, Fort Worth, 76107

#### TABLE OF CONTENTS

<b>Figure S1</b> Radiative and non-radiative rates of LDS 798 as function of viscosity of the media.....	<b>2</b>
<b>Figure S2</b> Emission spectra of LDS 798 in glycerol as function of temperature.....	<b>3</b>
<b>Figure S3</b> Fluorescence intensity decays of LDS 798 in various solvents at different temperatures...	<b>4</b>
<b>Figure S4</b> Deconvoluted absorption spectra of LDS 798.....	<b>5</b>
<b>Figure S5</b> Emission of LDS 798 in media of different pH.....	<b>6</b>
<b>Figure S6</b> LDS 798 lifetime as function of various mixtures of methanol: glycerol at 20 <sup>0</sup> C and in solvents as function of temperature.....	<b>7</b>
<b>Figure S7</b> LDS 798 lifetime as a function of media's dielectric constant (left) and E <sub>T</sub> 30-values (middle) and LDS 798 lifetime as a function of viscosity of organic solvents (right).....	<b>8</b>
<b>Figure S8</b> <sup>1</sup> H-NMR spectra of LDS 798 in acidic and neutral environments.....	<b>9</b>

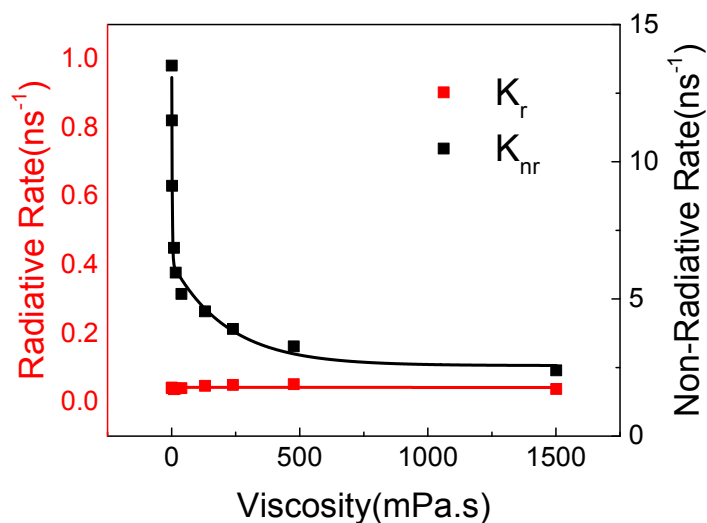
## Supplementary Information

<b>Table S1</b> Viscosity, density, moisture content and water content in methanol: glycerol mixtures.....	<b>10</b>
--	-----------

<b>Table S2</b> Viscosity of glycerol as a function of temperature and fluorescence lifetimes of LDS 798.....	<b>11</b>
---	-----------

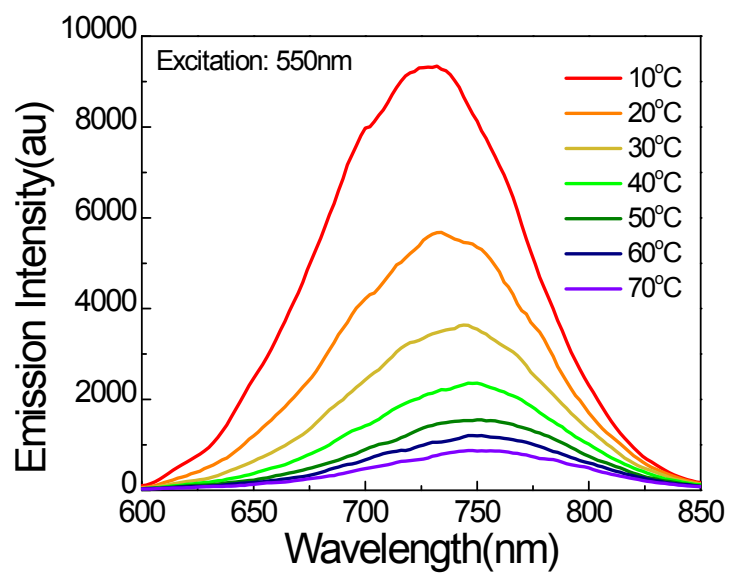
<b>Table S3</b> Viscosity of methanol as a function of temperature and fluorescence lifetimes of LDS 798.....	<b>12</b>
---	-----------

<b>Table S4</b> Viscosity of methanol: glycerol mixtures (50:50) as function of temperature and fluorescence lifetimes of LDS 798.....	<b>13</b>
--	-----------



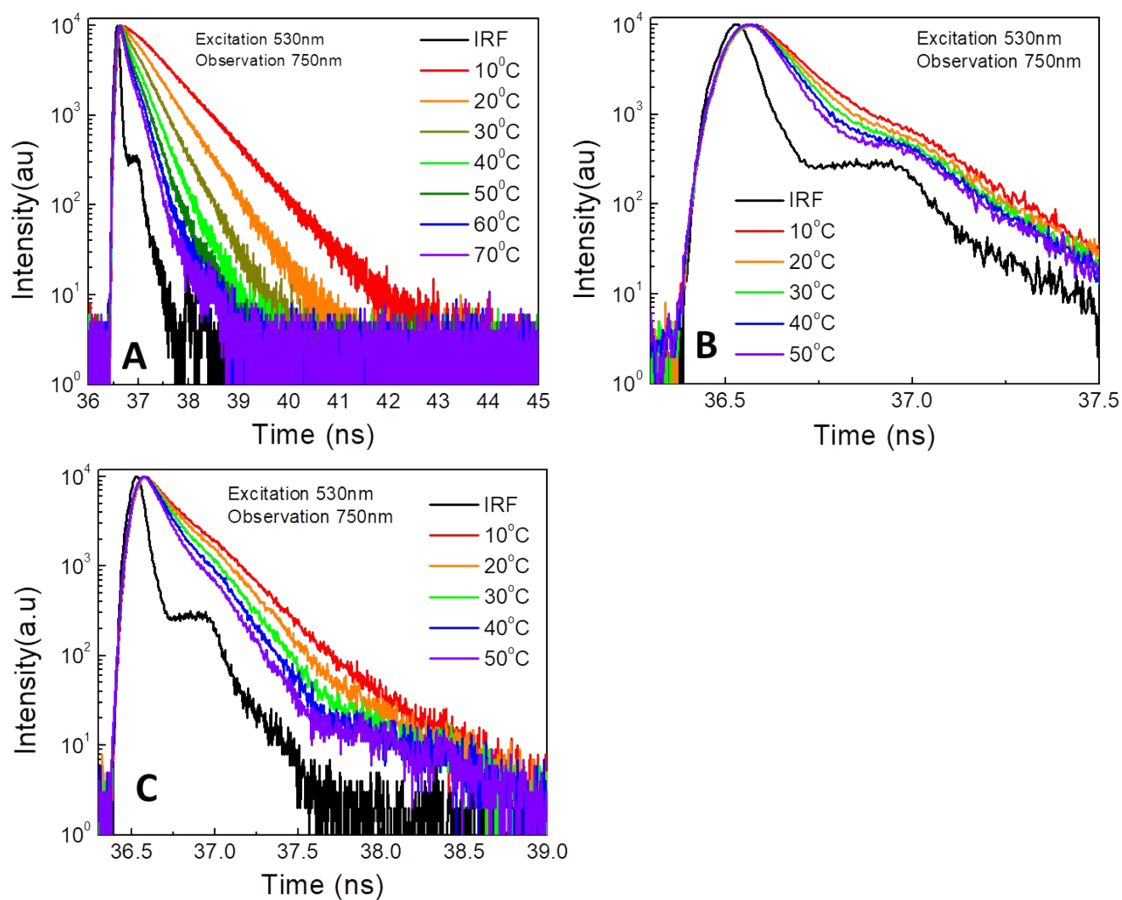
**Figure S1:** Radiative and non-radiative rates of LDS 798 as function of viscosity of the media

## Supplementary Information



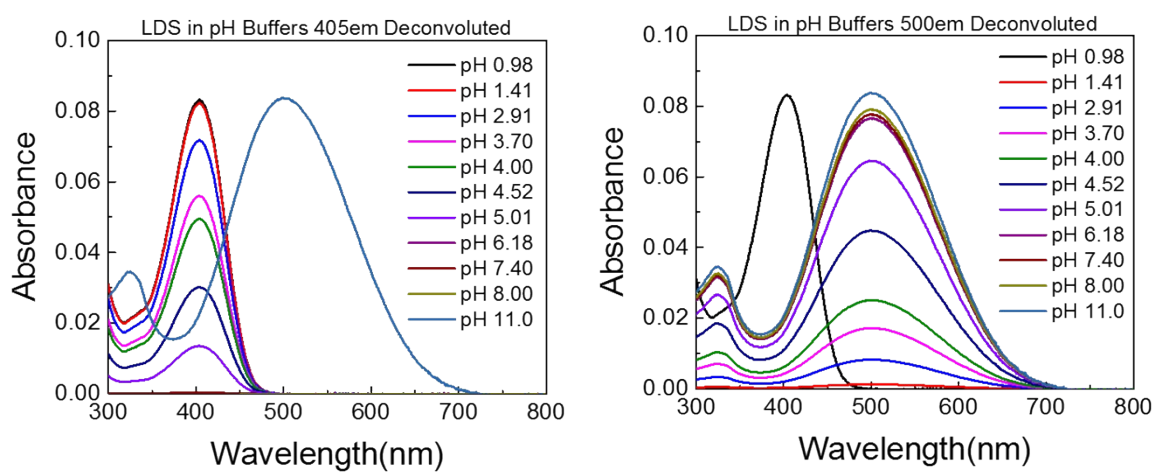
**Figure S2:** Emission spectra of LDS 798 in glycerol as function of temperature

## Supplementary Information



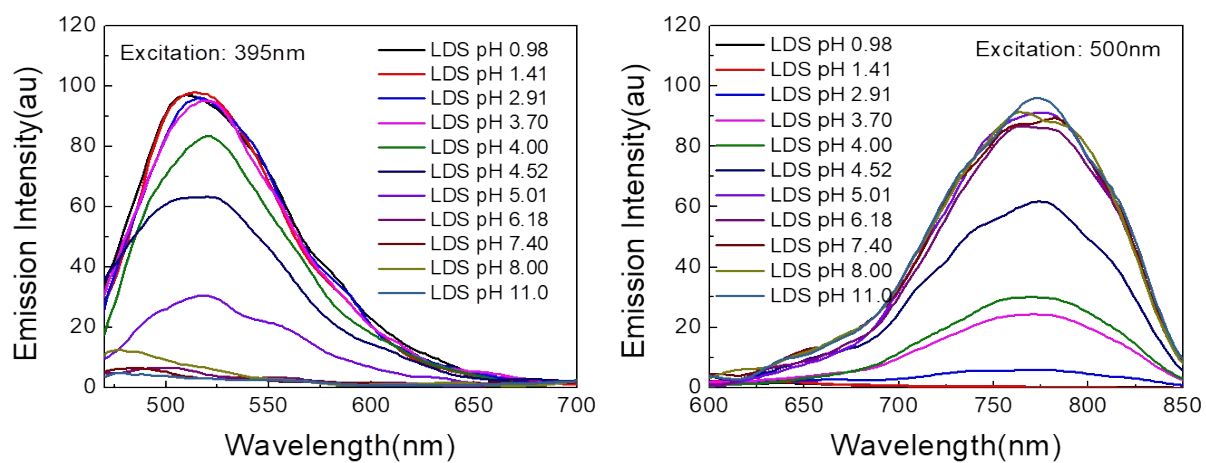
**Figure S3:** Fluorescence intensity decays of LDS 798 in various solvents at different temperatures (A) fluorescence intensity decays of LDS 798 in pure glycerol as a function of temperature. (B) Fluorescence intensity decays of LDS 798 in pure methanol as a function of temperature. (C) Fluorescence intensity decays of LDS 798 in 50:50 glycerol: methanol mixture as a function of temperature.

## Supplementary Information



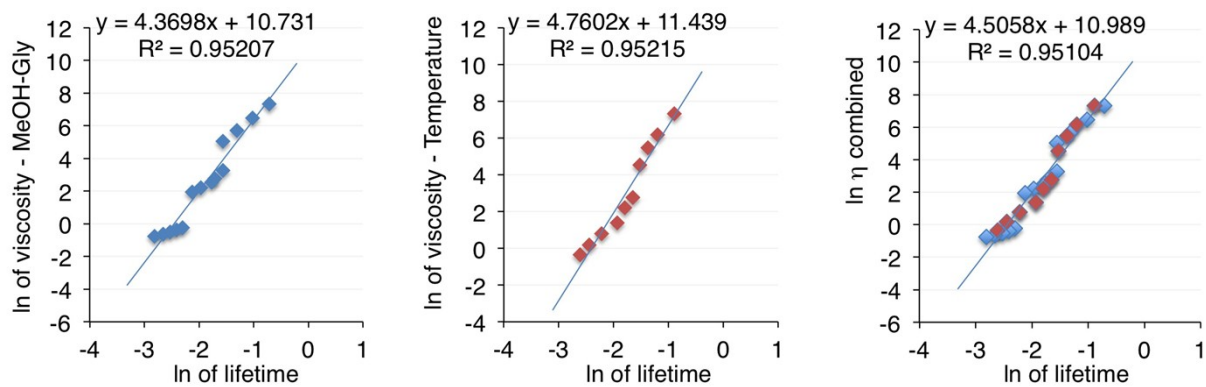
**Figure S4:** Deconvoluted absorption spectra of LDS 798. 2 distinct species were assumed.

## Supplementary Information



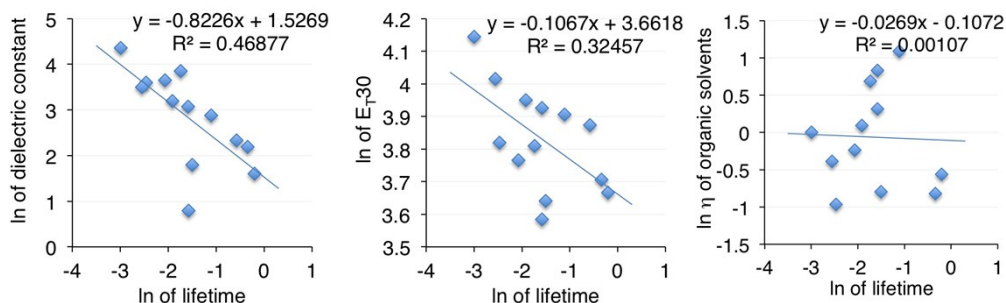
**Figure S5:** Emission of LDS 798 in media of different pH.

## Supplementary Information



**Figure S6:** LDS 798 lifetimes: as a function of viscosity of methanol : glycerol mixtures at 20°C (left), as a function of viscosity of methanol, glycerol and 50:50 mixture of methanol : glycerol at various temperature (middle), and as a function of viscosity of mixtures, individual solvent at various temperature, i.e., overlaid data set (right).

## Supplementary Information

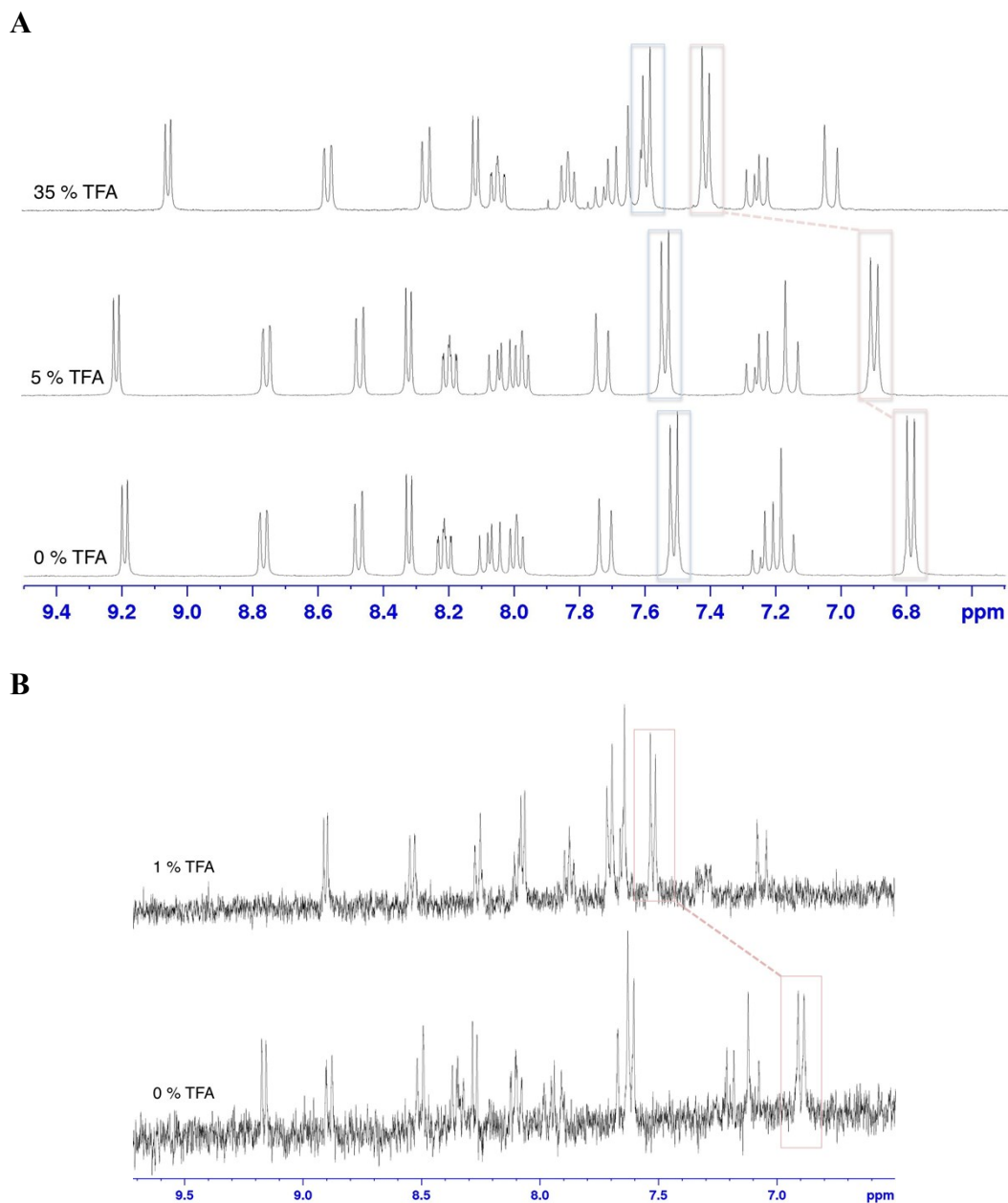


Solvents	Dielectric Constants	$E_{T30}$ Value	Viscosity (mPa.S)	Lifetime (ns)
Dioxane	2.21	36.0	1.37	0.206
Ethyl acetate	6.02	38.1	0.45	0.223
Acetonitrile	36.6	45.6	0.38	0.085
DMF	38.3	43.2	0.79	0.126
DMSO	47.2	45.1	1.99	0.176
1-octanol	10.3	48.1	7.36	0.562
1-butanol	17.85	49.7	2.98	0.330
1-propanol	21.65	50.7	2.30	0.205
Ethanol	24.3	51.9	1.10	0.147
Methanol	33.1	55.4	0.68	0.078
Chloroform	4.98	39.1	0.57	0.815
Dichloromethane	8.93	40.7	0.44	0.712
Water	78.3	63.1	1.00	0.050
Glycerol	42.5	57.0	1501	0.410

**Figure S7:** LDS 798 lifetime as a function of media's dielectric constant (left) and  $E_{T30}$ -values (middle) and LDS 798 lifetime as a function of viscosity of organic solvents (right). Table below shows the organic solvents their dielectric constants,  $E_{T30}$  values, and viscosity along with fluorescence lifetimes of LDS 798 in respective solvents derived from a previous publication; Biophys. Chemistry 153.1 (2010): 61-69.



## Supplementary Information



**Figure S8:** **A:** Effect of TFA on the aromatic region of LDS 798 in DMSO- $d_6$  as a function of various amounts of TFA. The chemical shift changes of ortho-protons (light red) and meta-protons (light blue) are highlighted as the most prominent protons to be affected by the changes in acidity. **B:** Effect of TFA on the aromatic region of LDS 798 in  $D_2O$ . The chemical shift changes of ortho-protons (light red) are highlighted as the most prominent protons to be affected by the changes in acidity.

## Supplementary Information

**Table S1.** Viscosity, and density in methanol : glycerol mixtures.<sup>a</sup>

Glycerol content (%)	Viscosity (mPa•s)	Density (g/mL)
100	1501	1.26057
90	477	1.21838
80	239	1.18405
70	131	1.15154
60	39	1.09840
50	16	1.04791
40	9.0	1.00512
30	4.0	0.95428
20	2.2	0.90394
10	1.2	0.84710
0	0.7	0.79183

<sup>a</sup> – viscosity and density measurements were performed at 20 °C

## Supplementary Information

**Table S2:** Viscosity of glycerol as a function of temperature and fluorescence lifetimes of LDS 798.<sup>a</sup>

Temperature (°C)	Viscosity (mPa•s)	Lifetime (ns) <sup>a</sup>	
		$\tau_{\text{int}}$	$\tau_{\text{amp}}$
10	ND <sup>b</sup>	0.70	0.70
20	1501	0.49	0.49
30	634	0.36	0.34
40	300	0.27	0.25
50	154	0.21	0.19

<sup>a</sup> – 530 nm excitation / 570 nm observations;

<sup>b</sup> – ND not determined due to the melting point of glycerol

## Supplementary Information

**Table S3:** Viscosity of methanol as a function of temperature and fluorescence lifetimes of LDS 798.<sup>a</sup>

Temperature (°C)	Viscosity (mPa•s)	Lifetime (ns) <sup>a</sup>	
		$\tau_{\text{int}}$	$\tau_{\text{amp}}$
10	0.79	0.10	0.10
20	0.68	0.09	0.09
30	0.60	0.08	0.08
40	0.52	0.07	0.07
50	0.47	0.06	0.06

<sup>a</sup>– 530 nm excitation / 570 nm observation

## Supplementary Information

**Table S4:** Viscosity of methanol: glycerol mixtures (50:50) as function of temperature and fluorescence lifetimes of LDS 798.

Temperature (°C)	Viscosity (mPa•s)	Lifetime (ns) <sup>a</sup>	
		$\tau_{\text{int}}$	$\tau_{\text{amp}}$
10	26.0	0.21	0.19
20	16.0	0.18	0.17
30	12.5	0.17	0.14
40	9.0	0.14	0.12
50	7.0	0.12	0.11

<sup>a</sup> – 530 nm excitation / 570 nm observation