TITLE PAGE

Probing the Assembly of HDL Mimetic, Drug Carrying Nanoparticles, using Intrinsic Fluorescence

Sangram Raut[a]*, Ashwini Garud[a], Bhavani Nagarajan[a], Nirupama Sabnis[a], Alan Remaley[b], Rafal Fudala[c], Ignacy Gryczynski[c], Zygmunt Gryczynski[c,d], Sergei V. Dzyuba[e], Julian Borejdo[c], Andras Lacko[a]*

a. Department of Physiology and Anatomy, UNT Health Science Center, Fort Worth, TX, USA, 76107

b. National Heart, Lung and Blood Institute, National Institute of Health, 31 Center Drive, MSC 2486, Bethesda, MD, USA, 20892

c. Department of Microbiology, Immunology and Genetics, UNT Health Science Center, Fort Worth, TX, USA, 76107

d. Department. of Phys76107ic and Astronomy, Texas Christian University, Fort Worth, TX, 76102

e. Department of Chemistry and Biochemistry, Texas Christian University, TX, 76102

Legends for Supplementary Figures

Supplementary Figure 1: CD spectra of 5A and Myr5A in PBS at different concentrations. Table below Shows percent contribution of different secondary structures color coded to match the color of the spectra.

Supplementary Figure 2: Emission spectra of tryptophan from 5A and Myr5A in EtOH and DMSO.

Supplementary Figure 3: Fluorescence intensity decays of tryptophan from 5A and Myr5A in EtOH and DMSO.

Supplementary Figure 4: Left and middle panel shows the anisotropy decays of 5A and Myr5A in EtOH and DMSO respectively. Right panel shows the anisotropy decays of N-acetyl tryptophanamide (NATA), and Myr5A for comparison.

Supplementary Figure 5: Fluorescence intensity decays of tryptophan from Myr5A with different amounts of Bis-MSB in DMSO. No change in fluorescence lifetime was noted.

Tables

Supplementary Table 1: Recovered parameters for fluorescence intensity decays of tryptophan from Myr5A with different amounts of Bis-MSB in DMSO.

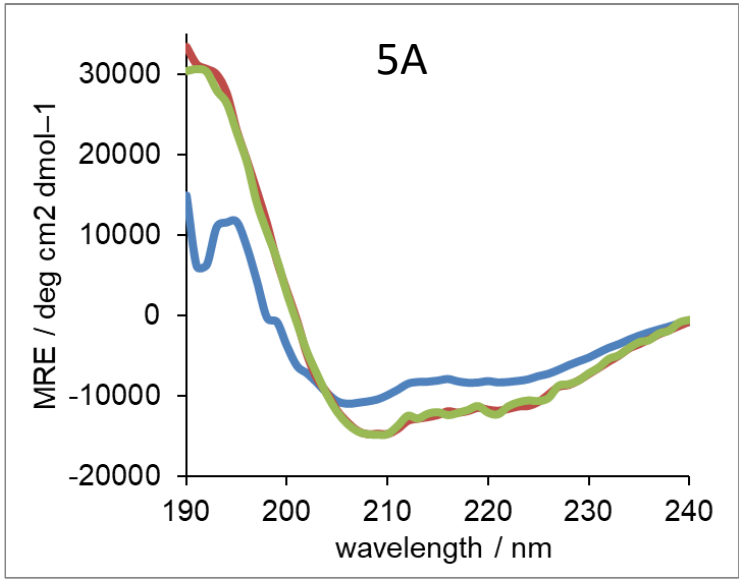
Myr5A:Bis-MSB Ratio	**Components (ns)**		**Amplitude (%)**		**Avg τ_{Int} (ns)**	**Avg τ_{Amp} (ns)**	χ^2
	τ_1	τ_2	A_1	A_2			
100:0	3.62	6.56	23.00	77.00	6.14	5.87	0.96
100:1	3.66	7.00	15.08	84.92	6.72	6.50	0.96
50:1	4.25	7.06	19.29	80.71	6.70	6.52	0.93
25:1	3.73	7.00	16.68	83.32	6.70	6.46	0.93

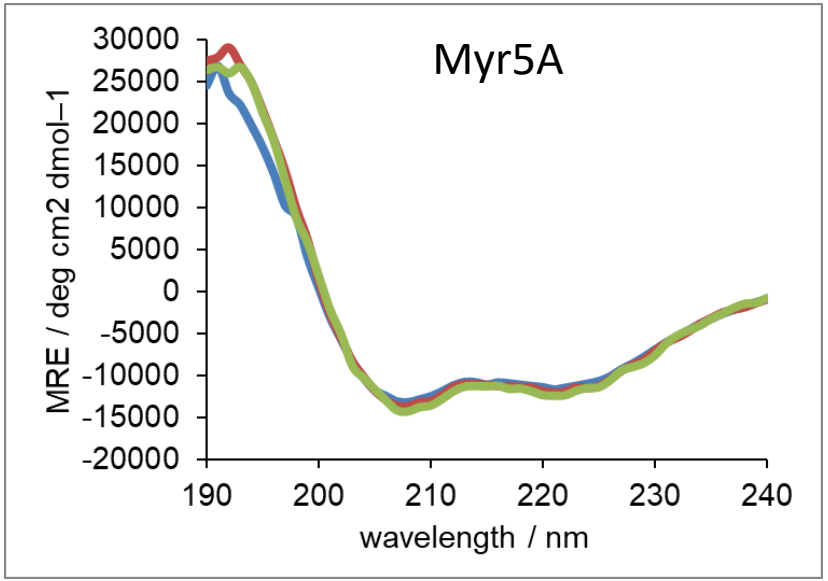
Supplementary Table 2: Recovered parameters for fluorescence intensity decays of tryptophan from Myr5A with different amounts of Bis-MSB in PBS. Significant lifetime quenching was observed.

Myr5A:Bis-MSB Ratio	**Components (ns)**			**Amplitude (%)**			**Avg τ_{Int} (ns)**	**Avg τ_{Amp} (ns)**	χ^2
	τ_1	τ_2	τ_3	A_1	A_2	A_3			
100:0	0.64	2.40	5.54	40.00	44.00	16.00	3.48	2.22	0.94
100:1	0.23	1.90	5.30	54.50	32.00	13.50	3.41	1.44	1.05
50:1	0.11	1.70	4.98	72.07	19.16	8.77	3.24	0.84	1.09
25:1	0.07	1.64	4.91	88.05	8.14	3.81	3.01	0.38	1.07

Supplementary Table 3: Stability of Valrubicin loaded Myr5A Nanoparticles over 1 month period. Particle size and zeta potential was monitored.

	Baseline	**After 1 week**			**After 1 month**		
		RT	4ºC	-20ºC	RT	4ºC	-20ºC
Average Size Number distribution (nm±SD)	113.5± 36	116.7± 33	104.9± 35	111±43	138.4± 43	105.7± 29	115.1± 40
PDI	0.101	0.059	0.102	0.124	0.145	0.054	0.126
Zeta Potential (mV±SD)	-8.68± 0.63	-7.99± 1.44	-9.84± 0.26	-9.54± 0.35	-2.54± 0.35	-5.42± 0.27	-2.16± 0.12

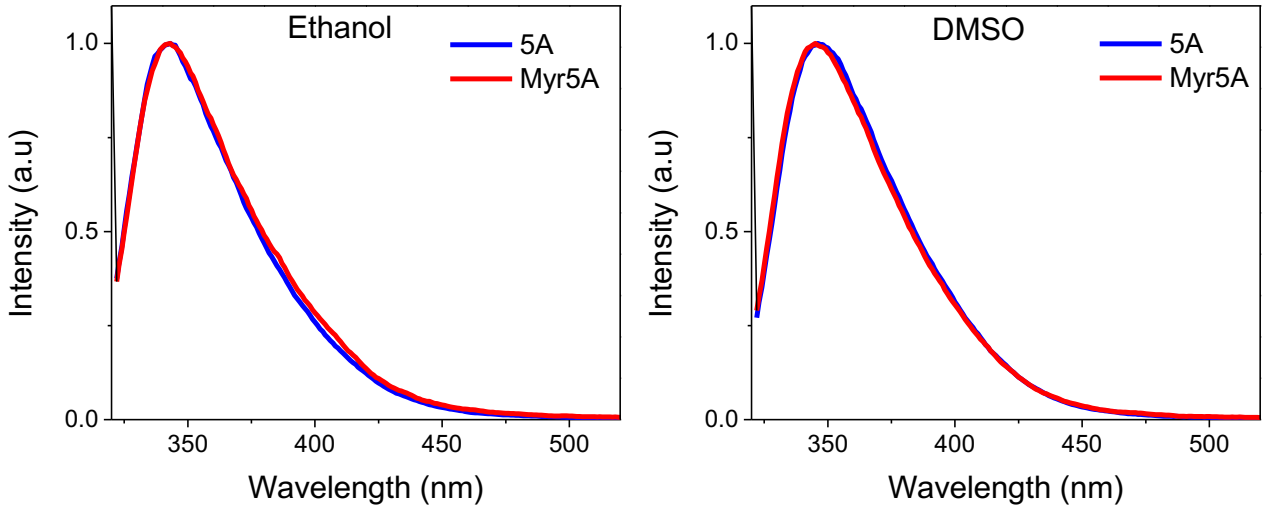




Concentration (mg/ml)	helix	strand	turn	unordered
5	40	26	8	27
2	44	17	19	20
1	43	17	18	22

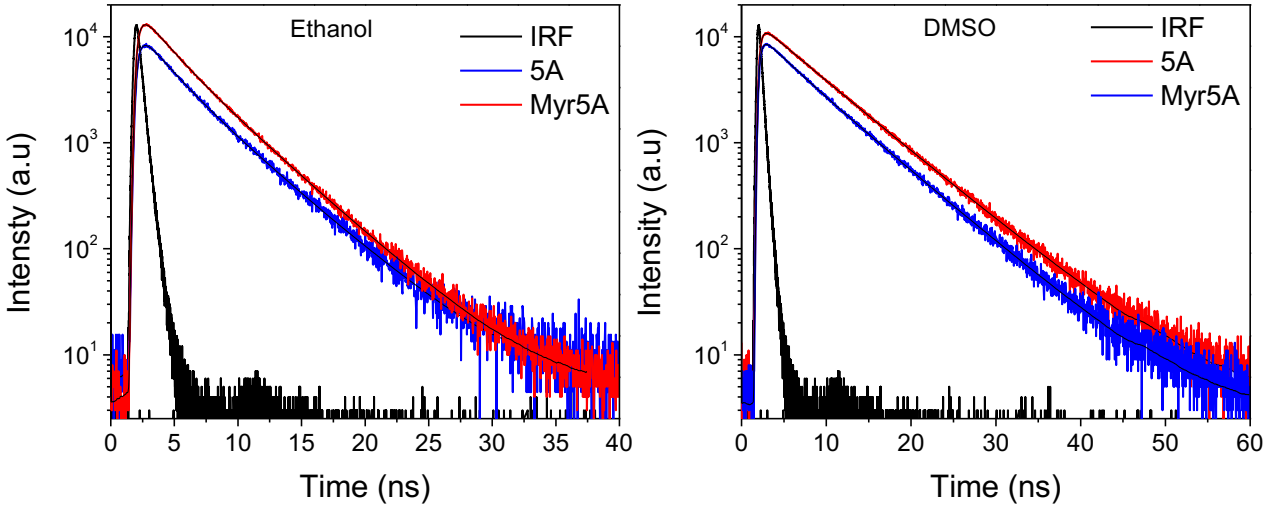
concentration (mg/ml)	helix	strand	turn	unordered
5	38	13	17	29
2	43	12	19	25
1	43	12	18	27

S1


Ethanol
5A
Myr5A
Intensity (a.u)
1.0
0.5
0.0
350
400
450
500
Wavelength (nm)
DMSO
5A
Myr5A
Intensity (a.u)
1.0
0.5
0.0
350
400
450
500
Wavelength (nm)

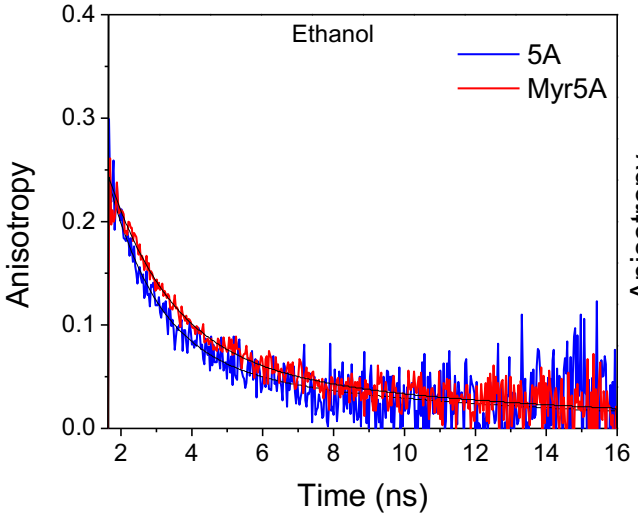
S2

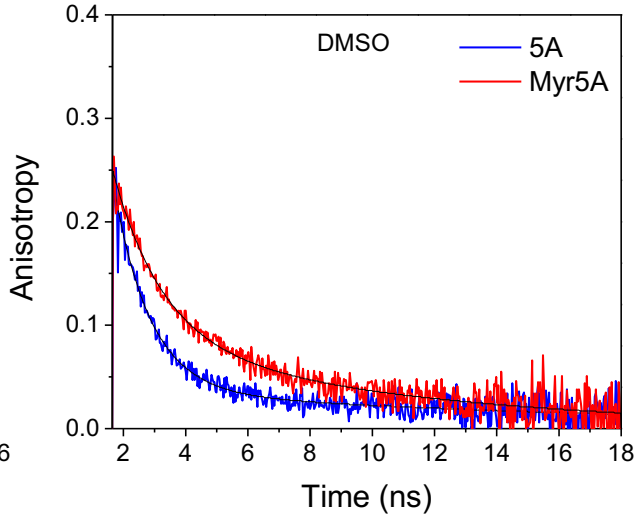
Lifetime Different Solvents

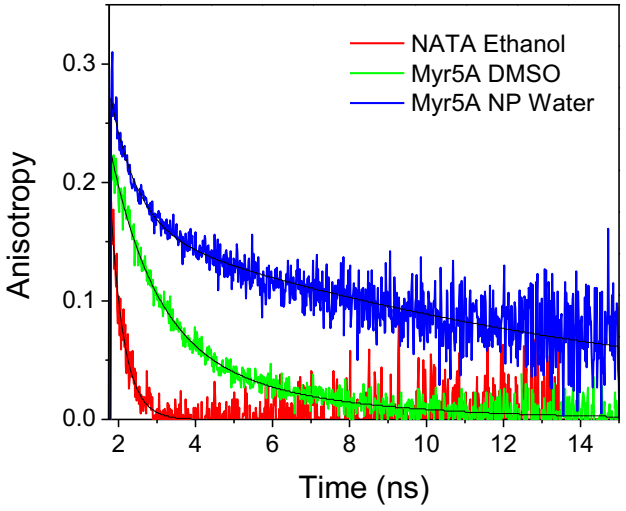


S3:

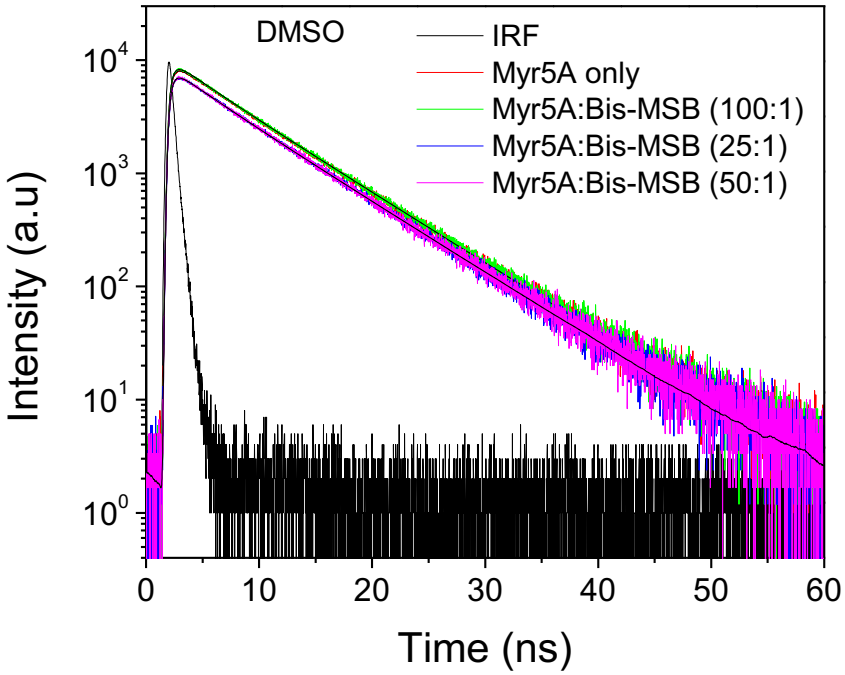
Anisotropy Different Solvents







S4


DMSO
IRF
Myr5A only
Myr5A:Bis-MSB (100:1)
Myr5A:Bis-MSB (25:1)
Myr5A:Bis-MSB (50:1)
Intensity (a.u)
10^0
10^1
10^2
10^3
10^4
0
10
20
30
40
50
60
Time (ns)