Electronic Supporting Information

Stereodifferentiation in fluorescence quenching within cholic acid aggregates

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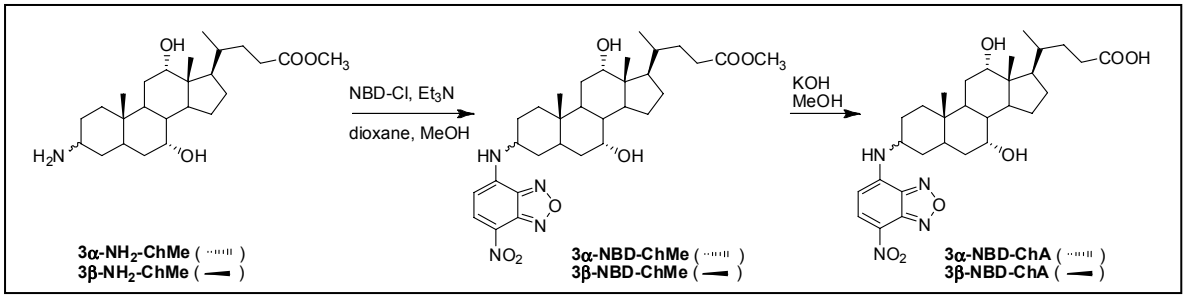
1. Synthesis of NBD-ChA derivatives

i) Amino-cholic derivatives **3α-NH_2-ChMe** and **3β-NH_2-ChMe** were prepared as previously described.[1]

ii) NBD moieties were covalently linked to these amino-derivatives as follows:



Synthesis of 3α-(7-Nitro-2,1,3-benzoxadiazol-4-yl)amino-7α,12α-dihidroxy-5β-cholan-24-oic acid (3α-NBD-ChA)

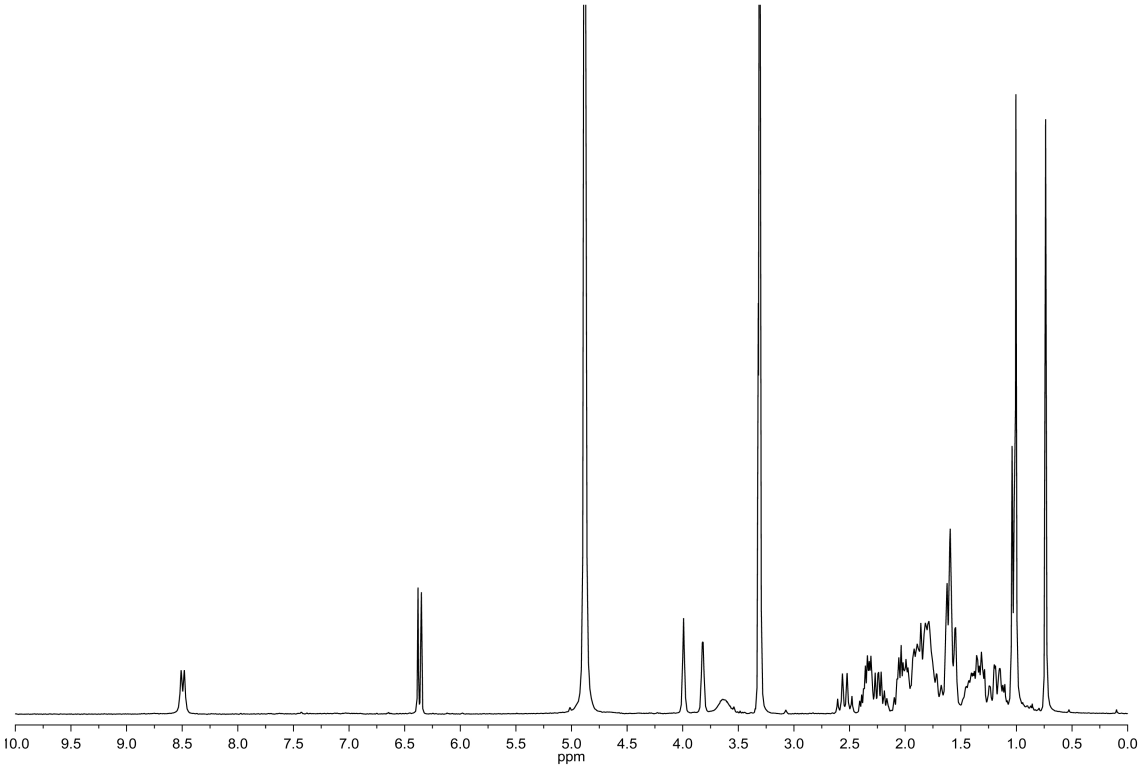
To a solution of **3α-NH_2-ChMe·HCl** (0.3 g, 0.65 mmol) in anhydrous MeOH (10 ml), Et_3N (0.27 ml, 1.96 mmol) was added, and the reaction mixture was cooled to 0 ºC. Then, a solution of NBD-Cl (170 mg, 0.85 mmol) in anhydrous 1,4-dioxane (5 ml) was added dropwise, under inert atmosphere, and the reaction mixture was stirred overnight at rt. Afterwards, the solvent was removed and the crude purified by column chromatography (SiO_2, AcOEt:*n*-hexane, 2:1) to give **3α-NBD-ChMe** as a red-brown crystalline solid (203 mg, 53%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 0.72 (s, 3H, 18-C*H*$_3$), 0.97-0.99 (m, 6H, 19-C*H*$_3$ + 21-C*H*$_3$), 3.54 (m, 1H, 3β-*H*), 3.67 (s, 3H, C*H*$_3$O), 3.90 (br s, 1H, 7β-*H*), 4.03 (br s, 1H, 12β-*H*), 6.14 (d, *J* = 8.7, 1H, C*H*(Ar)), 6.36 (br s, 1H, N*H*), 8.46 (d, *J* = 8.7, 1H, C*H*(Ar)); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 12.7 (CH_3), 17.5 (CH_3), 22.8 (CH_3), 23.3 (CH_2), 27.0 (CH), 27.1 (CH_2), 27.6 (CH_2), 28.4 (CH_2), 31.0 (CH_2), 31.2 (CH_2), 34.3 (CH_2), 35.0 (C), 35.2 (CH), 35.6 (CH_2), 35.9 (CH_2), 39.7 (CH), 42.0 (CH), 42.1 (CH), 46.7 (C), 47.5 (CH), 51.7 (CH_3O), 54.4 (3-CH), 68.4 (7-CH), 73.0 (12-CH), 104.0 (CH), 123.4 (C), 136.8 (CH), 143.3 (C), 144.1 (C), 144.5 (C), 174.8 (COO).

To a solution of **3α-NBD-ChMe** (203 mg, 0.35 mmol) in 5 ml of MeOH, a solution of KOH in MeOH (1 M, 3.5 ml) was added, and the resulting mixture was refluxed for 6 hr. The solvent was evaporated and the mixture

[1] Rohacova, J.; Marin, M. L.; Martinez-Romero, A.; O'Connor, J.-E.; Gomez-Lechon, M. J.; Donato, M. T.; Castell, J. V.; Miranda, M. A. **Synthesis of new, UV-photoactive dansyl derivatives for flow cytometric studies on bile acid uptake** *Organic & Biomolecular Chemistry* **2009**, *7*, 4973–4980.

was redissolved in H_2O, acidified with 1 M HCl, extracted twice with AcOEt and purified by column chromatography (SiO_2, AcOEt:*n*-hexane:AcOH, 100:50:1, afterwards reverse fase SiO_2-C18, MeOH:AcOEt:H_2O:AcOH, 80:10:10:1). The product **3α-NBD-ChA** was obtained as a red-orange crystalline solid (186 mg, 94%). ^{1}H-NMR (300 MHz, CD_3OD): δ (ppm) 0.72 (s, 3H, 18-C*H*$_3$), 0.99 (s, 3H, 19-C*H*$_3$), 1.02 (d, *J* = 6.3, 3H, 21-C*H*$_3$), 3.61 (m, 1 H, 3β-*H*), 3.81 (br s, 1H, 7β-*H*), 3.98 (br s, 1H, 12β-*H*), 6.33 (d, *J* = 9.3, 1H, 6'-C*H*(Ar)), 8.45 (d, *J* = 9.0, 1H, 5'-C*H*(Ar)); ^{13}C-NMR (75 MHz, CD_3OD): δ (ppm) 13.0 (CH_3), 17.6 (CH_3), 23.2 (CH_3), 24.2 (CH_2), 28.0 (CH), 28.7 (CH_2), 29.6 (CH_2), 32.0 (CH_2), 32.3 (CH_2), 35.7 (CH_2), 36.0 (C), 36.7 (CH_2), 36.8 (CH), 41.0 (CH), 43.0 (CH), 43.4 (CH), 47.5 (C), 48.0 (CH), 55.8 (3-CH), 68.8 (7-CH), 73.9 (12-CH), 99.8 (CH), 122.3 (C), 138.6 (CH), 145.5 (C), 145.7 (C), 145.8 (C), 178.2 (COOH); HRMS: obtained *m/z*: 570.3046 (calc. for $C_{30}H_{42}N_4O_7$: 570.3055).

^{1}H-NMR of **3α-NBD-ChA**:

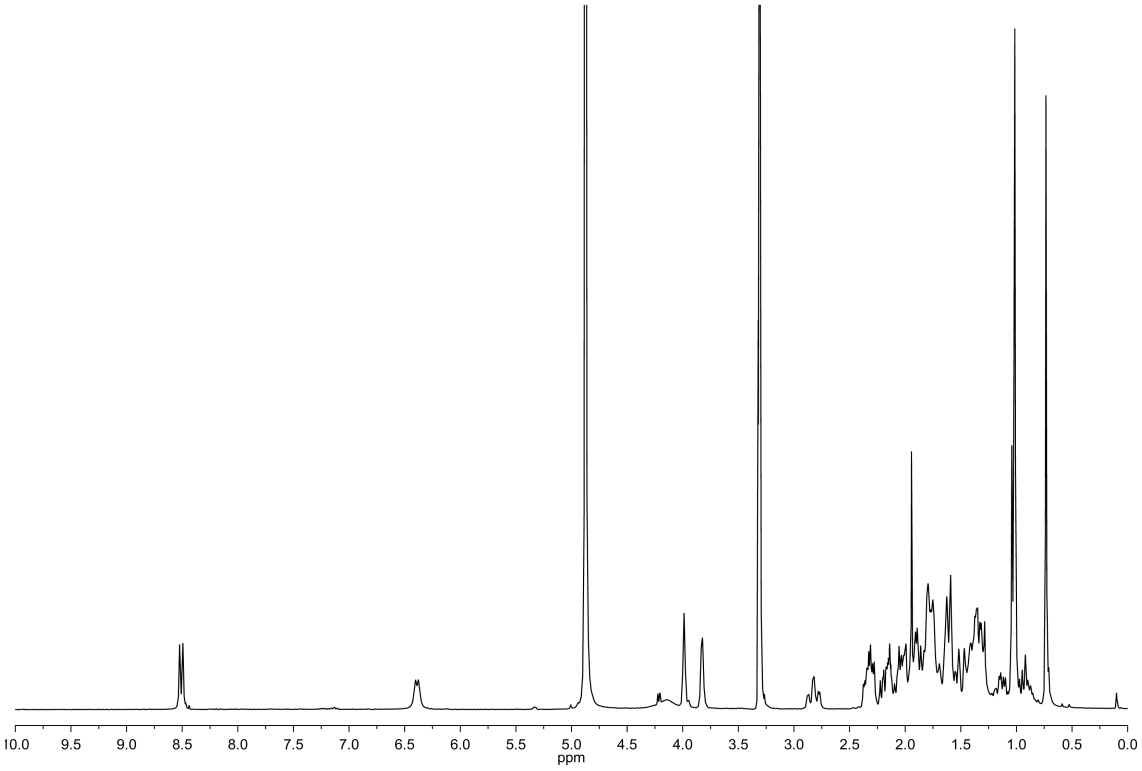


Synthesis of 3β-(7-Nitro-2,1,3-benzoxadiazol-4-yl)amino-7α,12α-dihidroxy-5β-cholan-24-oic acid (3β-NBD-ChA)

Compound **3β-NBD-ChMe** was prepared from **3β-NH_2-ChMe** following the procedure described above for **3α-NBD-ChMe**. Thus, starting from **3β-NH_2-ChMe·HCl** (0.15 g, 0.33 mmol), **3β-NBD-ChMe** (0.15 g, 80%) was obtained as a red-brown crystalline solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 0.72 (s, 3H, 18-C*H*$_3$), 0.98-1.01 (m, 6H, 19-C*H*$_3$ + 21-C*H*$_3$), 3.67 (s, 3H, C*H*$_3$O), 3.91 (br s, 1H, 7β-*H*), 4.03 (br s, 2H, 3α-*H* + 12β-*H*), 6.16 (d, *J* = 8.7, 1H, 6'-C*H*(Ar)), 6.39 (d, *J* = 7.2, 1H, N*H*), 8.48 (d, *J* = 8.7, 1H, 5'-C*H*(Ar)); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 12.7 (CH_3), 17.5 (CH_3), 23.2 (CH_3), 23.3 (CH_2), 24.0 (CH_2), 26.4 (CH), 27.6 (CH_2), 28.7 (CH_2), 31.0 (CH_2), 31.1 (CH_2), 31.2 (CH_2), 32.6 (CH_2), 34.0 (CH_2), 35.2 (CH), 35.3 (C), 37.4 (CH), 39.7 (CH), 42.1 (CH), 46.8 (C), 47.5 (CH), 49.8 (3-CH), 51.7 (CH_3O), 68.4 (7-CH), 73.0 (12-CH), 99.1 (CH), 123.8 (C), 136.8 (CH), 143.1 (C), 144.1 (C), 144.6 (C), 174.8 (COO).

Compound **3β-NBD-ChA** was prepared from **3β-NBD-ChMe** following the procedure described above for **3α-NBD-ChA**. Thus, starting from **3β-Dns-ChMe** (0.15 g), **3β-NBD-ChA** (0.13 g, 90%) was obtained as an orange crystalline solid. ^{1}H-NMR (300 MHz, CD_3OD): δ (ppm) 0.73 (s, 3H, 18-C*H*$_3$), 1.01-1.04 (m, 6H, 19-C*H*$_3$ + 21-C*H*$_3$), 3.83 (br s, 1H, 7β-*H*), 4.00 (br s, 1H, 12β-*H*), 4.15 (br s, 1H, 3α-*H*), 6.39 (d, *J* = 9.0, 1H, 6'-C*H*(Ar)), 8.51 (d, *J* = 9.0, 1H, 5'-C*H*(Ar)); ^{13}C-NMR (75 MHz, CD_3OD): δ (ppm) 13.0 (CH_3), 17.7 (CH_3), 23.4 (CH_3), 24.2 (CH_2), 27.7 (CH), 28.7 (CH_2), 29.8 (CH_2), 32.2 (CH_2), 33.0 (CH_2), 33.7 (CH_2), 35.4 (CH_2), 36.3 (C), 37.0 (CH), 38.4 (CH), 41.0 (CH), 43.0 (CH), 47.6 (CH), 48.3 (3-CH), 69.0 (7-CH), 74.0 (12-CH), 99.7 (CH), 122.0 (C), 138.2 (CH), 146.7 (C), 147.3 (C), 180.2 (COOH); HRMS: obtained *m/z*: 570.3057 (calc. for $C_{30}H_{42}N_4O_7$: 570.3055).

^{1}H-NMR of **3β-NBD-ChA**:



2. Samples preparation and photophysical measurements.

Quenching experiments

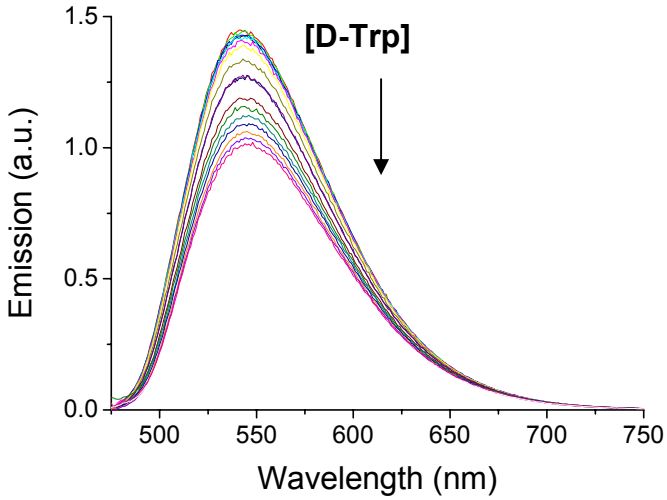
Cholic acid (ChA) solutions (4, 14 and 50 mM in aqueous 0.2 M NaCl) were prepared containing 7 μM of either 3α- and 3β- ChA-NBD. Thus absorbance of the solutions, was *ca* 0.1 at the excitation wavelength (453 nm). Then, increasing amounts of the two enantiomers of tryptophan, tryptophan methyl ester or N-acetyl-tryptophan were added to these solutions and their fluorescence spectra registered. Quenching rate constants were obtained from the slope of Stern-Volmer plots using at least 12 single points in the range from 0 to 250 μM. Every set of experiments was repeated twice. The same samples were submitted to the time-resolved fluorimeter. The kinetic traces were fitted by monoexponential decay functions using a re-convolution procedure to separate from the lamp pulse profile. A quartz cell of 1.0 cm optical path length was employed for all photophysical measurements.

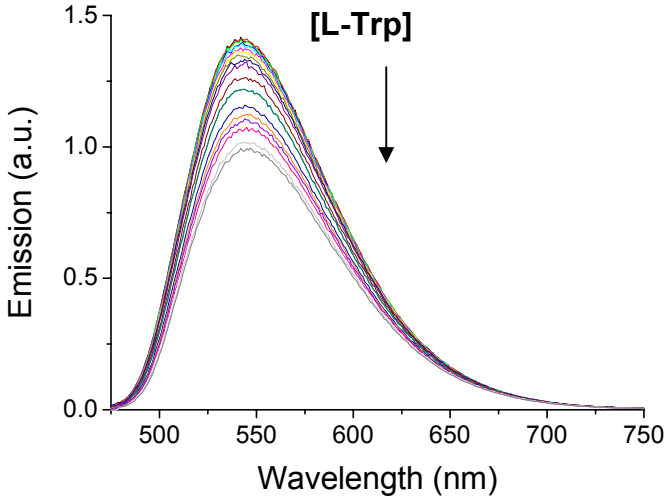
Job's plot

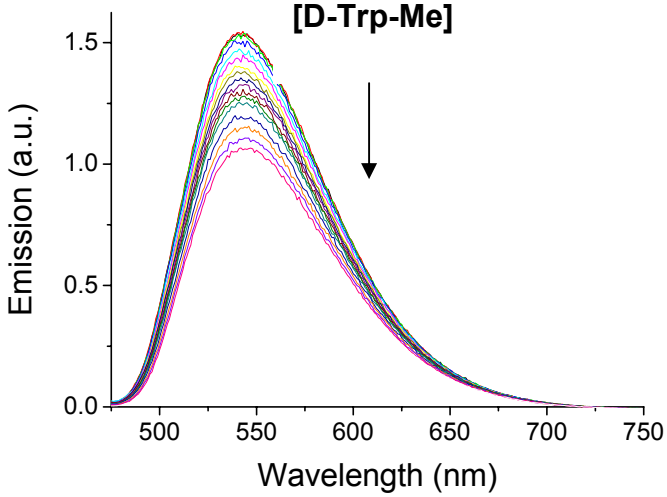
To determine the stoichiometry of the NBD-ChA/tryptophans complexes, absorbance changes were measured and plotted against NBD-ChA molar fraction, keeping the total (NBD-ChA + tryptophan) concentration constant. The obtained Job plots indicate that a 1:1 complex is formed for all the derivatives. Analogous experiments based on fluorescence measurements showed similar results.

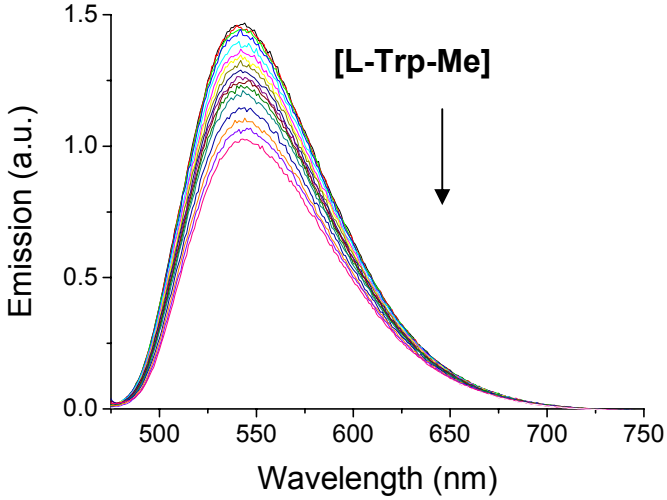
3.Original fluorescence quenching data

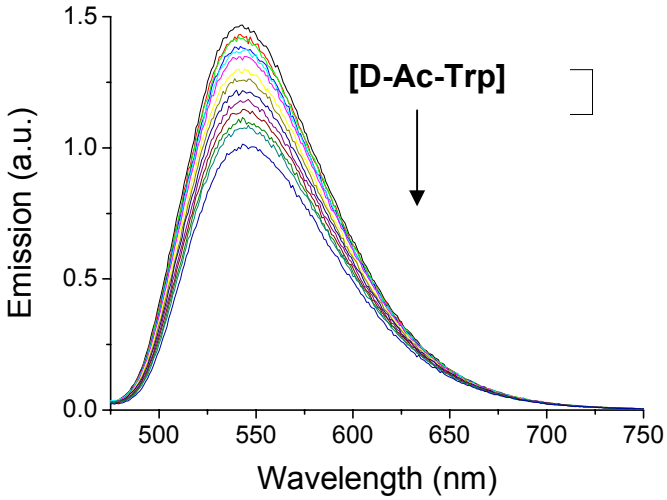
3α-NBD-ChA in primary aggregates (A-I)

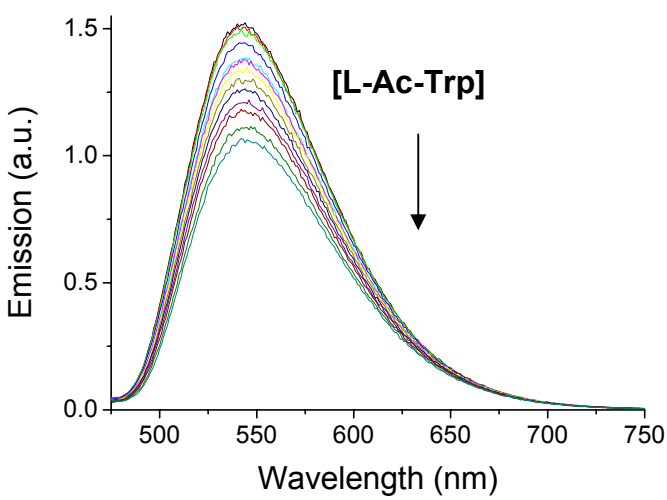




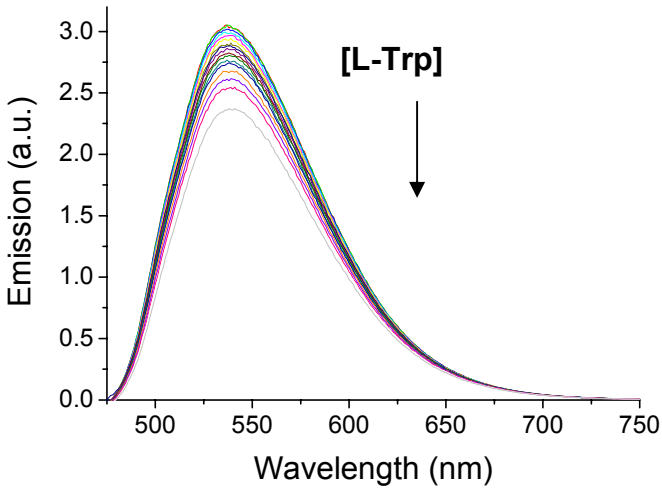


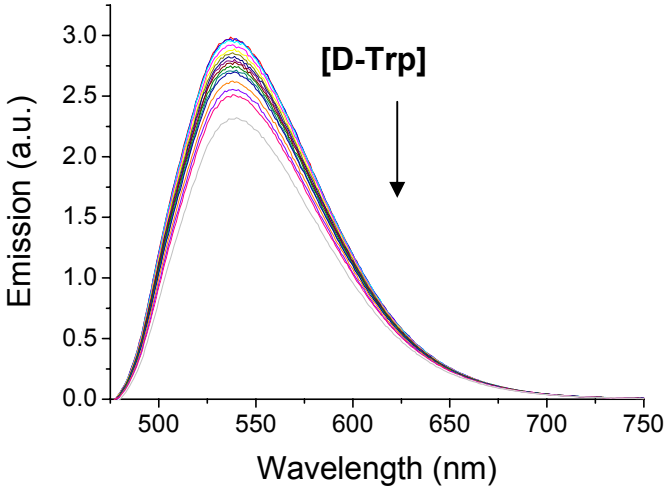


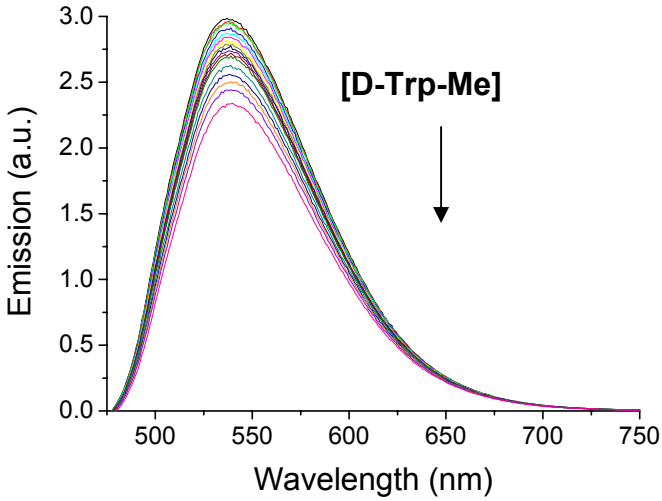


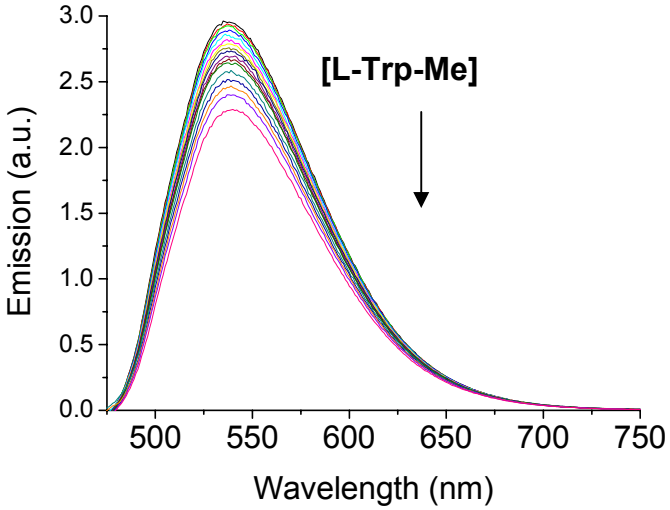


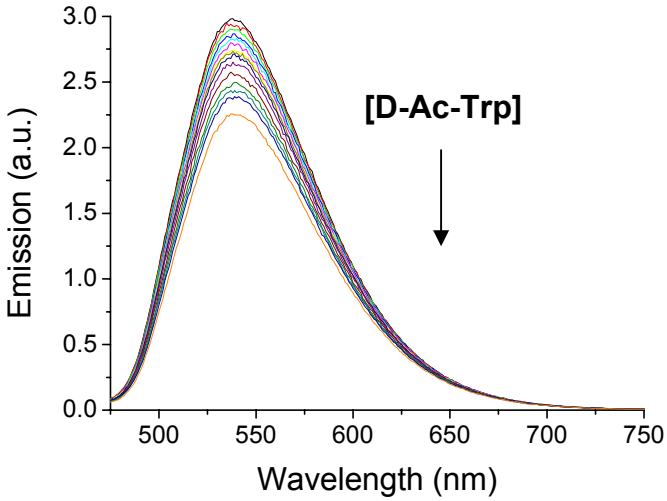
3α-NBD-ChA in secondary aggregates (A-II)

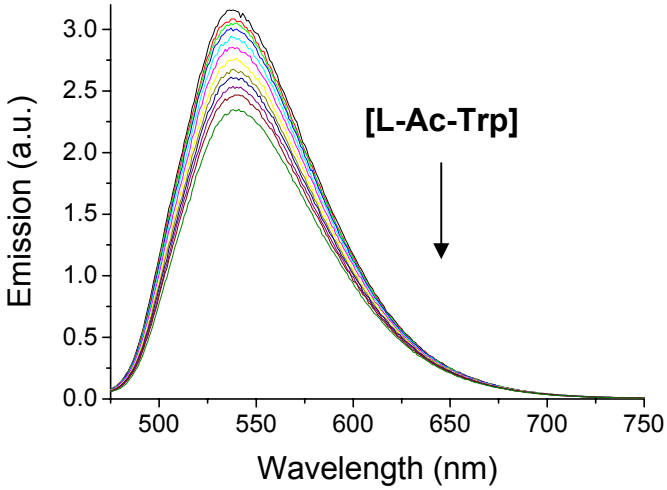




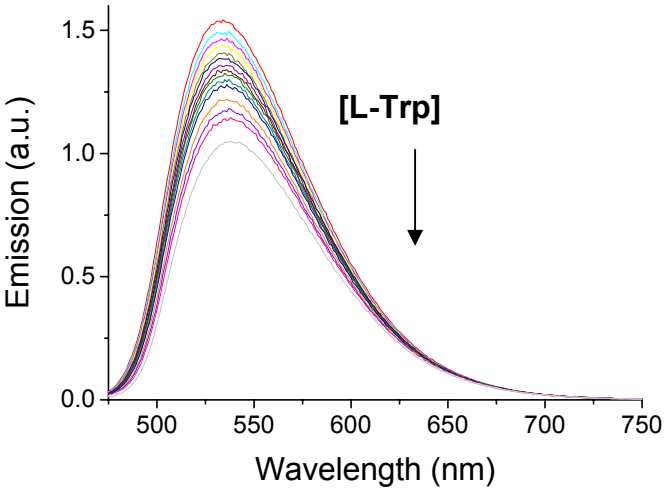


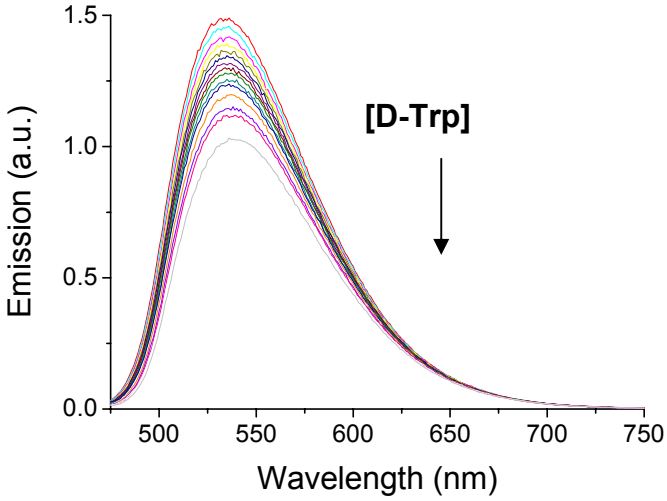


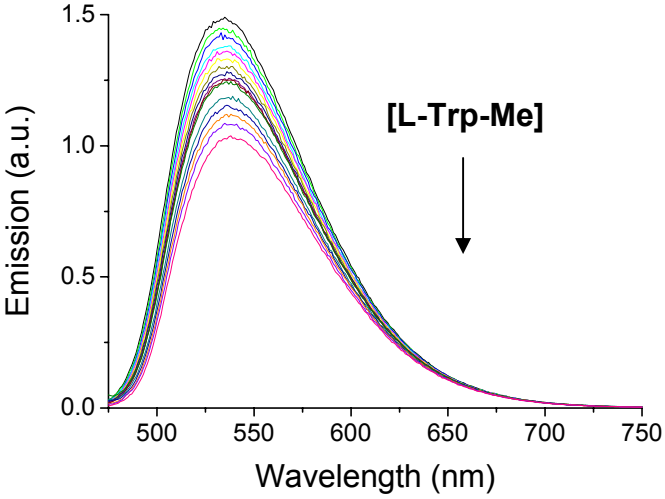


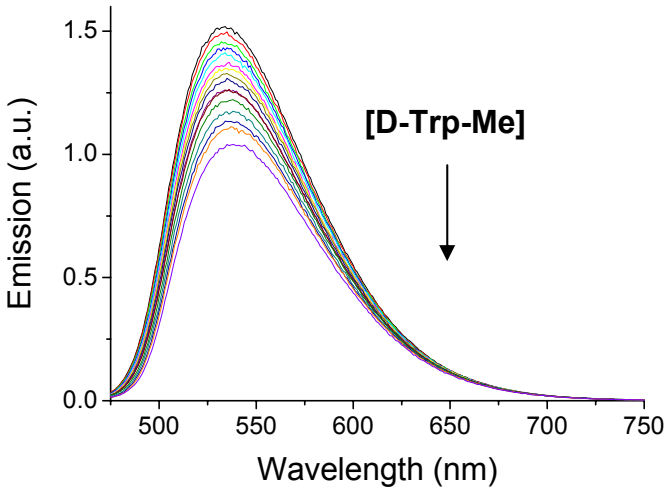


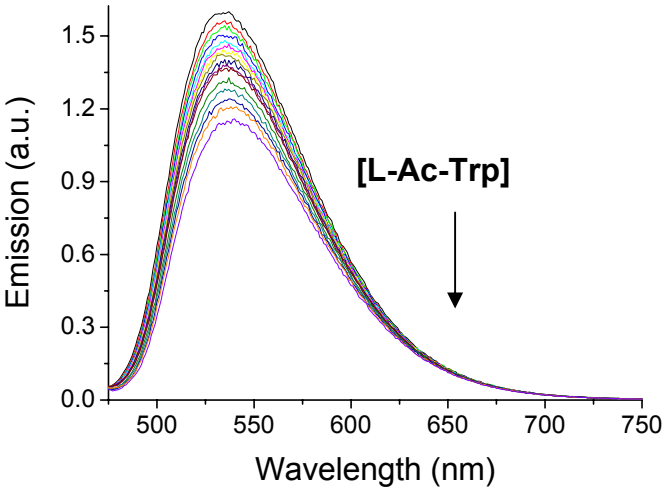
3β-NBD-ChA in primary aggregates (A-l)

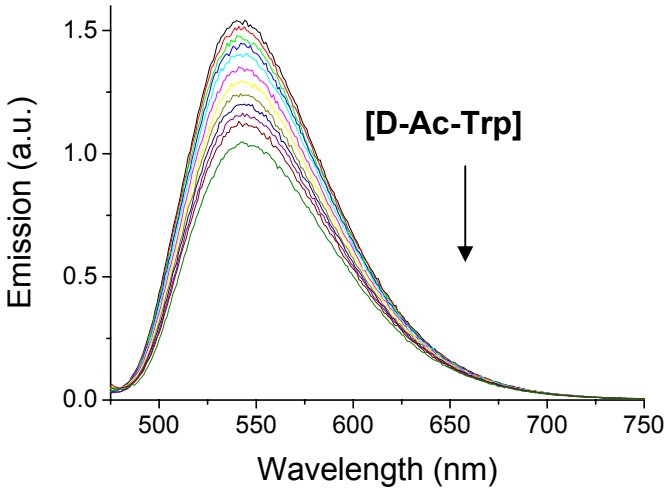




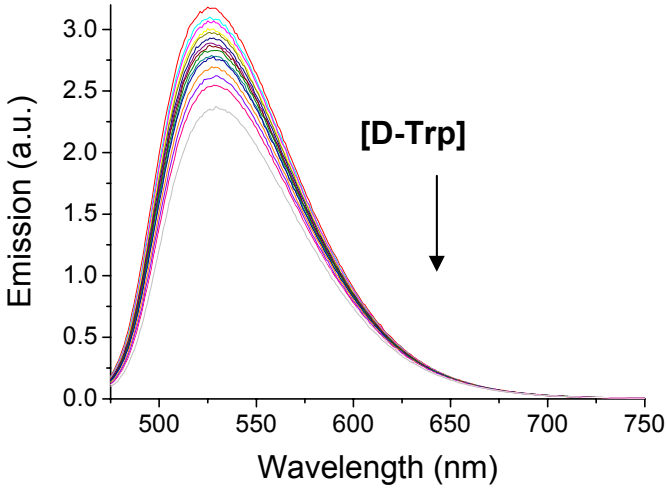


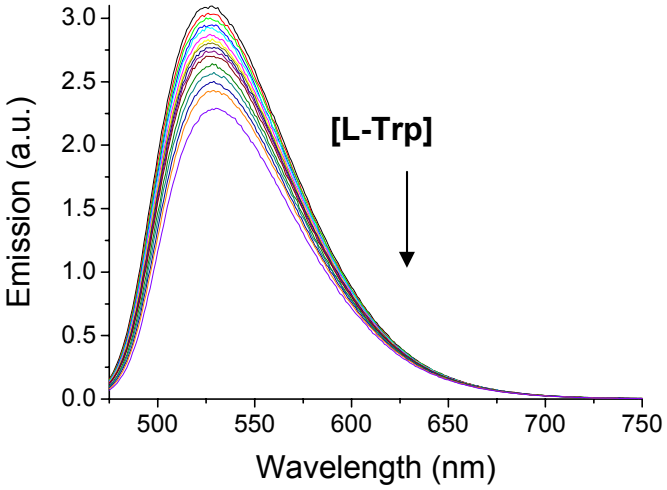


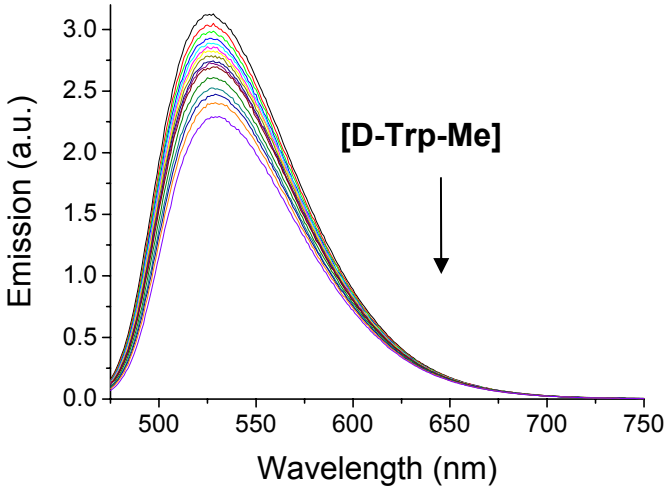


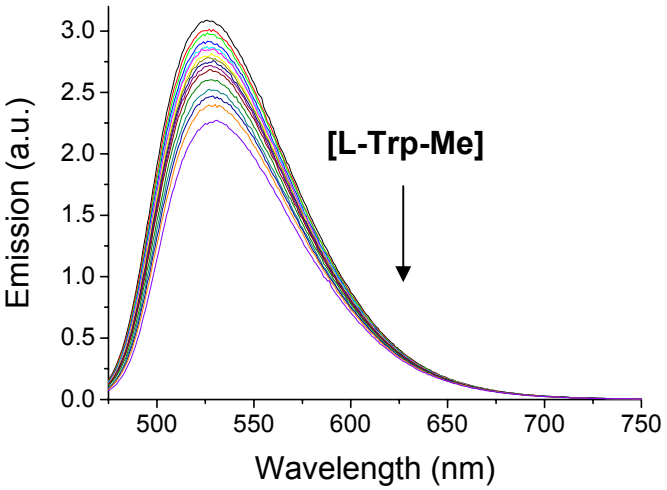


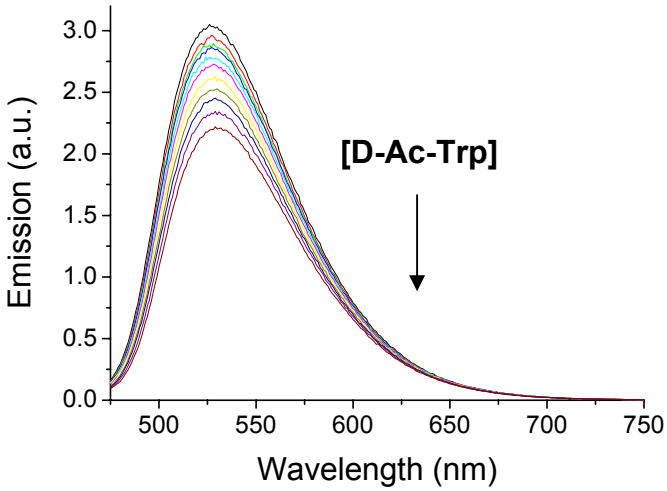
3β-NBD-ChA in secondary aggregates (A-II)

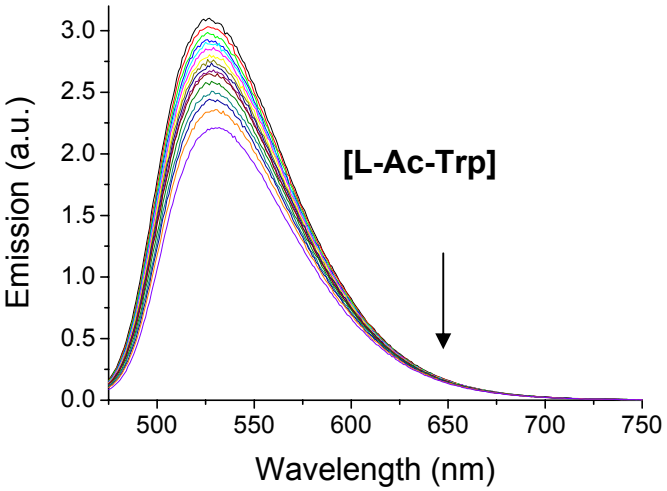






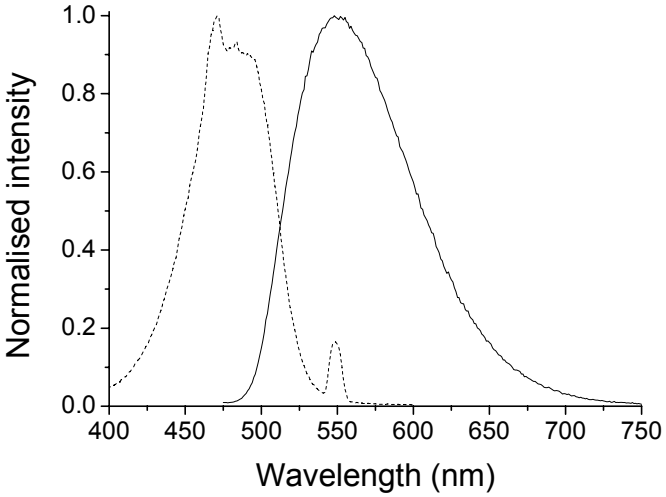




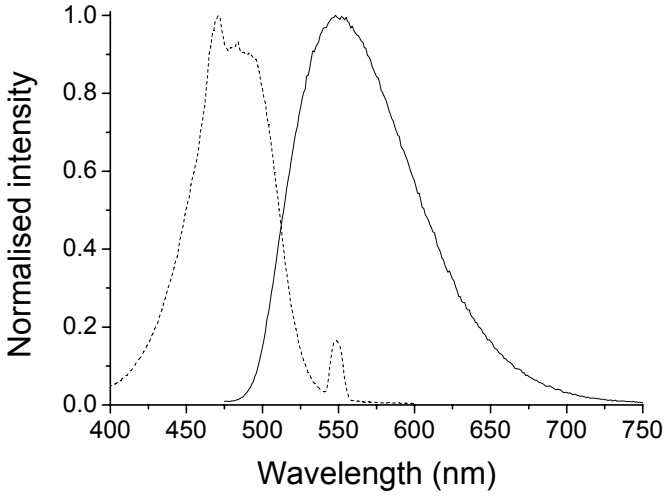


4. Determination of NBD-ChA singlet energy in the aggregates

No aggregates



A-I



A-II