Changes of the membrane lipid organization characterized by means of a new cholesterol-pyrene probe

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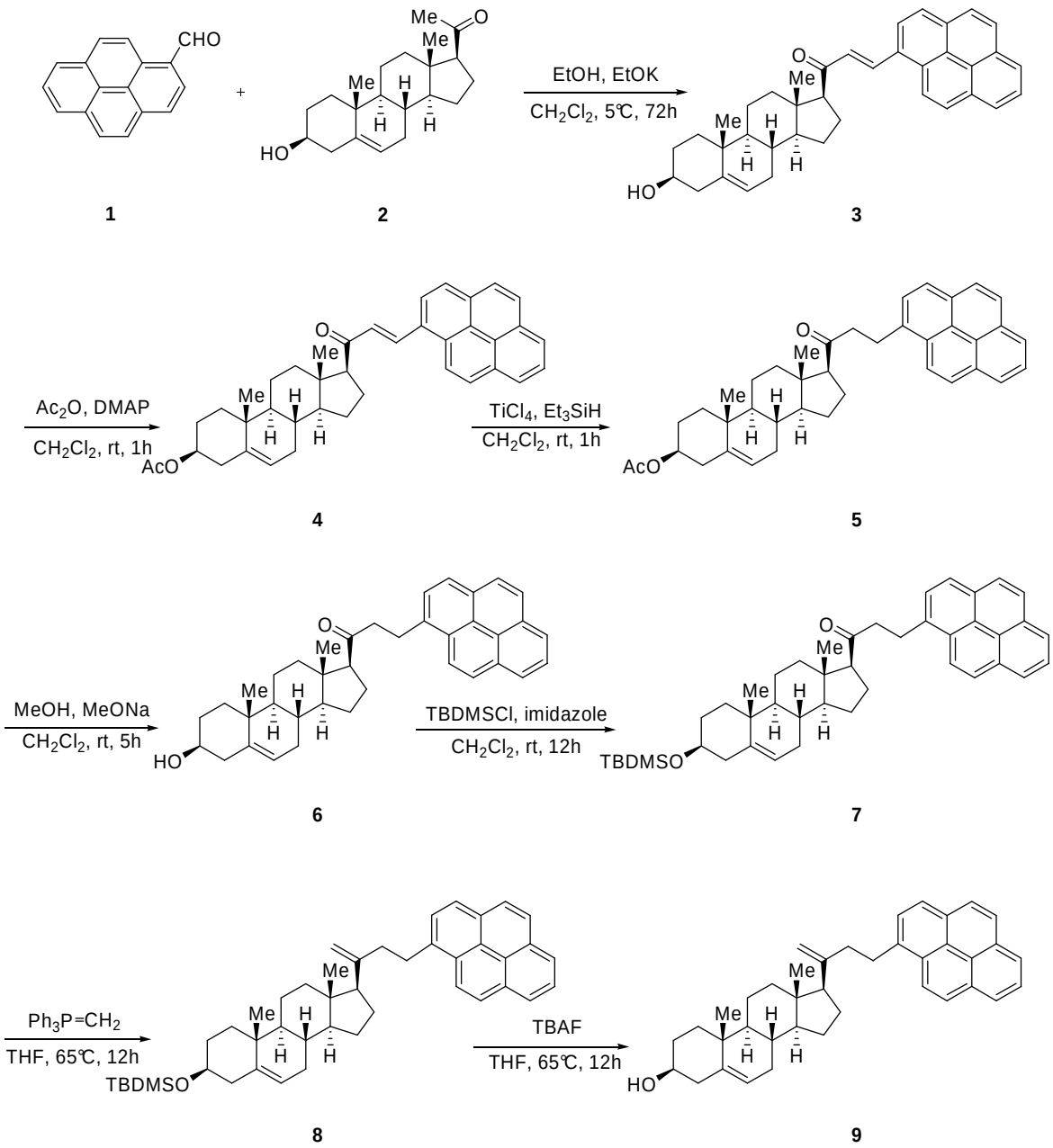
Supplementary Materials

Scheme 1. Organic synthesis of cholesterol-pyrene probe.



Synthesis procedures, spectral and analytical characterizations of cholesterol probe in Scheme 1:

(21E)-3β-hydroxy-pregna-5,21-diene-22-(1-pyrenyl)-20-one (3). Fifteen mL of dry ethanol and then 25 mL of a 24 wt. % solution of potassium ethanolate in ethanol were added to a solution of 1-pyrenecarboxaldehyde (**1**) (3.2 g, 14 mmol) and pregnenolone (**2**) (4 g, 12.64 mmol) in 30 mL of dry CH_2Cl_2. The resulting solution was stirred for 72 h at +5°C, 200 mL of water was added and the mixture was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with saline solution, dried over sodium sulfate, filtered and concentrated on a rotatory evaporator. The crude product was purified by flash chromatography on silica G60 (0.040-0.063 mm), eluting with ethyl acetate in CH_2Cl_2 (2/98) then with ethyl acetate in CH_2Cl_2 (15/85) to give 6.12g (91%) of **3** as a bright yellow solid; mp: 153°C; TLC (CH_2Cl_2/AcOEt 15:85) R_f 0.5; IR (KBr, cm^{-1}) 3300m, 2932s, 1676m, 1590s, 1436w, 1370w, 1352w, 1315w, 1234w, 1192w, 1102m, 1052m, 973w, 848s, 709w; ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H), 0.87 (s, 3H), 0.70-2.38 (m, 22H), 2.73 (t, J = 8.7 Hz, 1H), 3.42 (sept, J = 4.8 Hz, 1H) 3.55 (t, J = 9 Hz, 2H), 5.24 (d, J = 4.8 Hz, 1H), 6.87 (d, J = 15.6 Hz, 1H), 7.87-8.17 (m, 8H), 8.36 (d, J = 9.3 Hz, 1H), 8.58 (d, J = 15.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 200.2, 140.8, 138.0, 132.7, 131.3, 130.7, 130.2, 128.7, 128.6, 128.5, 127.3, 126.3, 126.0, 125.8, 125.0, 124.9, 124.6, 124.0, 122.5, 121.4, 71.7, 62.5, 57.1, 53.5, 50.0, 45.1, 42.3, 39.2, 37.3, 36.5, 32.0, 31.8, 31.6, 24.7, 22.8, 21.1, 19.4, 13.5; MS (CI, NH_3) m/z 529 (100, $[M + H]^+$); optical rotation $[\eta]^{20}_D$ +34.0 (c 1.1, $CHCl_3$).

(21E)- 3β-acetoxy-pregna-5,21-diene-22-(1-pyrenyl)-20-one (4). Acetic anhydride (1 mL, 5.28 mmol) 4-(dimethylamino)pyridine (30 mg, 246 µmol) and triethylamine (343 µL, 2.46 mmol) were successively added to a solution of **3** (1.3 g, 2.46 mmol) in 20 mL of CH_2Cl_2. The resulting solution was stirred for 3h at RT then 150 mL water was added and the organic products were extracted with CH_2Cl_2 (3 x 80 mL). The combined extracts were dried over sodium sulfate, concentrated on a rotatory evaporator and subjected to column chromatography (CH_2Cl_2/AcOEt 98/2) to give 1.16 g (83%) of **4** as a bright yellow solid; mp 99°C; TLC (AcOEt/CH_2Cl_2 2:98) R_f 0.9; IR (KBr, cm^{-1}) 2938s, 1728s, 1676w, 1590s, 1371m, 1242s, 1102m, 1126m, 845s; ^{1}H NMR ($CDCl_3$) δ 0.58 (s, 3H), 0.87 (s, 3H), 1.95 (s, 3H), 0.70-2.36 (m, 21H), 2.71 (t, J = 9 Hz, 1H), 4.52 (m, 1H), 5.24 (d, J = 4.8 Hz, 1H), 6.85 (d, J = 15.6 Hz, 1H), 7.87-8.17 (m, 8H), 8.36 (d, J = 9.6 Hz, 1H), 8.56 (d, J = 15.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 200.1, 170.6, 139.6, 137.9, 132.7, 131.3, 130.7, 130.2, 128.7, 128.6, 128.5, 127.3, 126.3, 126.0, 125.8, 125.0, 124.9, 124.6, 124.0,

122.5, 122.4, 73.9, 62.4, 57.0, 49.9, 45.0, 39.1, 38.1, 37.0, 36.6, 31.9, 31.8, 27.8, 24.7, 22.8, 21.5, 21.1, 19.3, 13.5 (only 39 of the 40 expected resonances were observed); MS (CI, NH_3) m/z 571 (100, $[M + H]^+$); optical rotation $[\eta]^{20}_D$ +63.6 (c 0.86, $CHCl_3$).

3β-acetoxy-pregn-5-ene-21-(1-methylpyrenyl)-20-one (5). To an iced cooled solution of **4** (1 g, 1.75 mmol) in 20 mL of CH_2Cl_2, 4.2 mL of a 1M solution of $TiCl_4$ in CH_2Cl_2 was added and the resulting deep brown solution was stirred for 10 min. Et_3SiH (419 µL, 2.63 mmol) was added all at once and the solution was warmed to room temperature and stirred for 45 min. The solution was poured into 150 mL of a saturated solution of $NaHCO_3$ and the organic products were extracted with CH_2Cl_2 (3 x 80 mL). The combined extracts were dried over sodium sulfate, concentrated on a rotatory evaporator and subjected to column chromatography (CH_2Cl_2/AcOEt 98:2) to give 601 mg (60%) of **5** as a pale yellow solid; mp 91°C; TLC (CH_2Cl_2 /AcOEt 98:2) R_f 0.9; IR (KBr, cm^{-1}) 2938s, 2902s, 1729s, 1701s, 1456w, 1436w, 1362m, 1246s, 1095w, 1031m, 906w, 845m, 731m; ^{1}H NMR ($CDCl_3$) δ 0.49 (s, 3H), 0.86 (s, 3H), 1.94 (s, 3H), 0.6-2.4 (m, 20H), 2.77 (t, J = 8.1 Hz, 2H), 3.51 (t, J = 7.5 Hz, 2H), 4.49 (m, 1H), 5.23 (d, J = 4.2 Hz, 1H), 7.76-8.14 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 210.5, 170.5, 139.6, 135.6, 131.4, 130.9, 130.0, 128.5, 127.6, 127.5, 127.3, 126.7, 125.9, 125.1, 125.0, 124.9, 124.8, 123.0, 122.3, 73.8, 63.1, 56.7, 49.7, 46.1, 44.2, 38.8, 38.1, 36.9, 36.5, 31.7, 31.6, 27.7, 27.4, 24.5, 23.0, 21.4, 20.9, 19.2, 13.4 (only 39 of the 40 expected resonances were observed); MS (CI, NH_3) m/z 590 (100, $[M + NH_4]^+$), 573 (98, $[M + H]^+$); optical rotation $[\eta]^{20}_D$ -9.0 (c 1.37, $CHCl_3$).

3β-hydroxy-pregn-5-ene-21-(1-methylpyrenyl)-20-one (6). We successively added 5 mL of dry methanol and 5 mL of a 30 wt. % solution of sodium methanolate in methanol to a solution of **5** (600 mg, 1 mmol) in 10 mL of CH_2Cl_2 and stirred the solution for 4 h at room temperature. We added 200ml water and extracted the organic products with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with saline solution, dried over sodium sulfate, filtered and concentrated on a rotatory evaporator. Purification by flash chromatography on silica G60 (0.040-0.063 mm), eluting with CH_2Cl_2 in AcOEt (85/15) gave 302 mg (95%) of **6** as an off-white solid; mp 199°C (dec.); TLC (CH_2Cl_2/AcOEt 85:15) R_f 0.5; IR (KBr, cm^{-1}) 3428m, 2930s, 1700s, 1458w, 1436w, 1357w, 1108w, 1054m, 845s, 755s; ^{1}H NMR ($CDCl_3$) δ 0.55 (s, 3H), 0.89 (s, 3H), 0.70-2.24 (m, 19H), 2.40 (t, J = 9 Hz, 1H), 2.84 (t, J = 6 Hz, 2H), 3.42 (m, 1H) 3.55 (t, J = 9 Hz, 2H), 5.24 (d, J = 4.8 Hz, 1H), 7.80-8.18 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 210.5, 140.7, 135.7, 131.4, 130.9, 130.0, 128.5, 127.6, 127.5, 127.3, 126.8, 125.9, 125.1, 125.0, 124.9, 124.8, 123.0, 121.4, 71.7, 63.2, 56.9, 49.9, 46.1, 44.3, 42.2, 38.9, 37.2, 36.5, 31.8, 31.7, 31.6, 27.4, 24.5,

23.0, 21.0, 19.3, 13.5 (only 37 of the 38 expected resonances were observed); MS (CI, NH_3) m/z 548 (100, $[M + NH_4]^+$), 531 (17, $[M + H]^+$); optical rotation $[\eta]^{20}_D$ -12.1 (c 0.74, $CHCl_3$).

3β-tert-butyldimethylsilyloxy-pregn-5-ene-21-(1-methylpyrenyl)-20-one (7). We added via a syringe, a solution of tert-butyldimethylsilyl chloride (68 mg, 452 mmol) in 2 mL of CH_2Cl_2 to a solution of **6** (200 mg, 377 µmol) and imidazole (51 mg, 754 mmol) in 5 mL of CH_2Cl_2. After 12h stirring at RT, 50 mL water was added and the organic products were extracted with dichloromethane (3 x 50 mL). The combined organic extracts were washed with saline solution, dried over sodium sulfate, filtered and concentrated on a rotatory evaporator. Purification by flash chromatography on silica G60 (0.040-0.063 mm), eluting with dichloromethane, gave 231 mg (95%) of **7** as an off-white solid; mp 100°C; TLC (CH_2Cl_2) R_f 0.9; IR (KBr, cm^{-1}) 2929s, 2852s, 1703s, 1461m, 1381m, 1359m, 1250m, 1183w, 1091s, 843s, 773m; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6H), 0.47 (s, 3H), 0.60-2.22 (m, 32H), 2.72 (t, J = 7.5 Hz, 2H), 3.39 (m, 1H) 3.49 (t, J = 7.8 Hz, 2H), 5.17 (d, J = 3.9 Hz, 1H), 7.73-8.12 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 210.4, 141.4, 135.7, 131.4, 130.9, 130.0, 128.5, 127.6, 127.5, 127.3, 126.8, 125.9, 125.1, 125.0, 124.9, 124.8, 123.1, 120.9, 72.6, 63.0, 56.7, 49.9, 46.0, 44.2, 42.8, 38.8, 37.3, 36.5, 32.1, 31.8, 31.7, 27.4, 26.2, 26.0, 25.8, 24.5, 23.0, 21.0, 19.4, 18.3, 13.4, -4.5 (only 42 of the 44 expected resonances were observed); MS (CI, CH_4) m/z 645 (100, $[M + H]^+$); optical rotation $[\eta]^{20}_D$ -4.3 (c 0.84, $CHCl_3$).

3β-tert-butyldimethylsilyloxy-pregn-5-ene-21-(1-methylpyrenyl)-20-methylidene (8). NaH (48 mg, 2 mmol) was added to 3 mL of dry DMSO and the suspension was stirred and warmed to 65°C until all the sodium hydride dissolved. The solution was cooled at RT and methyl triphenylphosphonium bromide (715 mg, 2 mmol) was added in aliquots over a period of 5 min. The reaction was slightly exothermic and the solution was stirred for an additional 45 min. This solution was mixed with a solution of **7** (645 mg, 1 mmol) in 5 mL of THF and the resulting solution was stirred and warmed at 70°C for 12h. After cooling the mixture, we added 50 mL water and the organic products were extracted with dichloromethane (3 x 50 mL). The combined organic extracts were washed with saline solution, dried over sodium sulfate, filtered and concentrated on a rotatory evaporator. Purification by flash chromatography on silica G60 (0.040-0.063 mm), eluting with pentane in CH_2Cl_2 (75/25), gave 514 mg (80%) of **8** as an off-white solid; mp 63°C; TLC (pentane/CH_2Cl_2 75:25) R_f 0.85; IR (KBr, cm^{-1}) 2928s, 2851s, 1636w, 1460m, 1434m, 1380w, 1249m, 1088s, 888m, 842s, 773m; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6H), 0.54 (s, 3H), 0.83 (s, 9H), 0.91 (s, 3H), 0.75-2.30 (m, 32H), 2.47 (m, 2H), 3.40 (m, 3H), 4.85 (s, 1H), 5.01 (s, 1H), 5.24 (d, J = 6.0 Hz, 1H), 7.77-8.20 (m, 9H); ^{13}C NMR ($CDCl_3$) δ

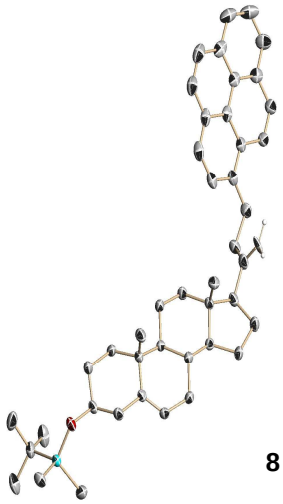
149.1, 141.6, 136.8, 133.9, 133.6, 131.5, 131.0, 129.8, 128.7, 128.6, 128.5, 128.4, 127.6, 127.3, 127.2, 126.6, 125.6, 125.1, 124.9, 124.7, 123.4, 121.1, 110.1, 72.7, 56.7, 56.3, 50.3, 43.3, 42.9, 39.9, 38.8, 37.4, 36.7, 32.9, 32.3, 32.1, 31.9, 26.0, 24.3, 21.2, 19.5, 18.3, 12.9, -4.5 (only 44 of the 45 expected resonances were observed); MS (CI, NH_3) m/z 660 (10, $[M + NH_4]^+$), 511 (100); optical rotation $[\eta]^{20}_D$ -35.0 (c 0.76, $CHCl_3$).

3β-hydroxy-pregn-5-ene-21-(1-methylpyrenyl)-20-methylidene (9). We added TBAF (2.5 mL of a 1M solution in THF) to a solution of **8** (300 mg, 466 µmol) in THF (5 mL), and the solution was stirred and warmed at 65°C for 12h. After cooling, 100 mL of water was added and the organic products were extracted with dichloromethane (3 x 50 mL). The combined organic extracts were washed with saline solution, dried over sodium sulfate, filtered and concentrated on a rotatory evaporator. Purification by flash chromatography on silica G60 (0.040-0.063 mm), eluting with CH_2Cl_2 in AcOEt (98/2), gave 209 mg (85%) of **9** as an off-white solid; mp 84°C; TLC (CH_2Cl_2/AcOEt 98:2) R_f 0.4; IR (KBr, cm^{-1}) 3364m, 2929s, 1636w, 1458w, 1434w, 1375w, 1182w, 1056m, 888m, 843s, 712w; ^{1}H NMR ($CDCl_3$) δ 0.52 (s, 3H), 0.65-2.25 (m, 32H), 2.3-2.6 (m, H), 3.27-3.44 (m, H), 4.83 (s, 1H), 4.99 (s, 1H), 5.24 (d, J = 4.5 Hz, 1H), 7.75-8.18 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 149.1, 140.8, 136.8, 131.5, 130.9, 129.8, 128.6, 127.5, 127.3, 127.2, 126.6, 125.8, 125.1, 124.9, 124.7, 123.4, 121.6, 110.1, 71.8, 56.6, 56.3, 50.2, 43.2, 42.3, 39.9, 38.8, 37.3, 36.6, 32.9, 32.3, 31.8, 31.7, 29.7, 26.0, 24.3, 21.1, 19.4, 12.9 (only 38 of the 39 expected resonances were observed); MS (CI, NH_3) m/z 546 (38, $[M + NH_4]^+$), 529 (89, $[M + H]^+$), 217 (100); optical rotation $[\eta]^{20}_D$ -46.8 (c 1.23, $CHCl_3$).

X-ray Crystallography.

Structural data presented was collected at low temperature using an oil-coated shock-cooled crystal on a Bruker-AXS CCD 1000 diffractometer with MoKα radiation (λ = 0.71073 Å). The structure was solved by direct methods (1) and all non-hydrogen atoms were refined anisotropically using the least-squares method (2) on F^2.

Figure S1: Molecular structures of **8** with thermal ellipsoids at the 50% probability level.



Crystal data. $C_{45}H_{58}OSi$, M = 643.00, monoclinic, $P2_1$, a = 13.273(1) Å, b = 7.966(1) Å, c = 17.590(1) Å, β = 101.502(1)°, V = 1822.5(3) $Å^3$, Z = 2, T = 133(2) K. 10706 reflections (5635 independent, R_{int} = 0.0265) were collected. Largest electron density residue: 0.309 e $Å^{-3}$, R_1 (for I>2(I)) = 0.0412 and wR_2 = 0.1028 (all data).

1. Sheldrick, G. M. 1990. Phase annealing in SHELX-90 : direct methods for larger structures. Acta crystallographica, Section A, Foundations of crystallography 46:467-473.
2. Sheldrick, G. M. 1997. Program for Crystal Structure Refinement.