SUPPORTING INFORMATION

Resolving Self-Assembly of Bile Acids at the Molecular Length Scale

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Absorption Spectroscopy (UV-Vis) and Circular Dichroism (CD).

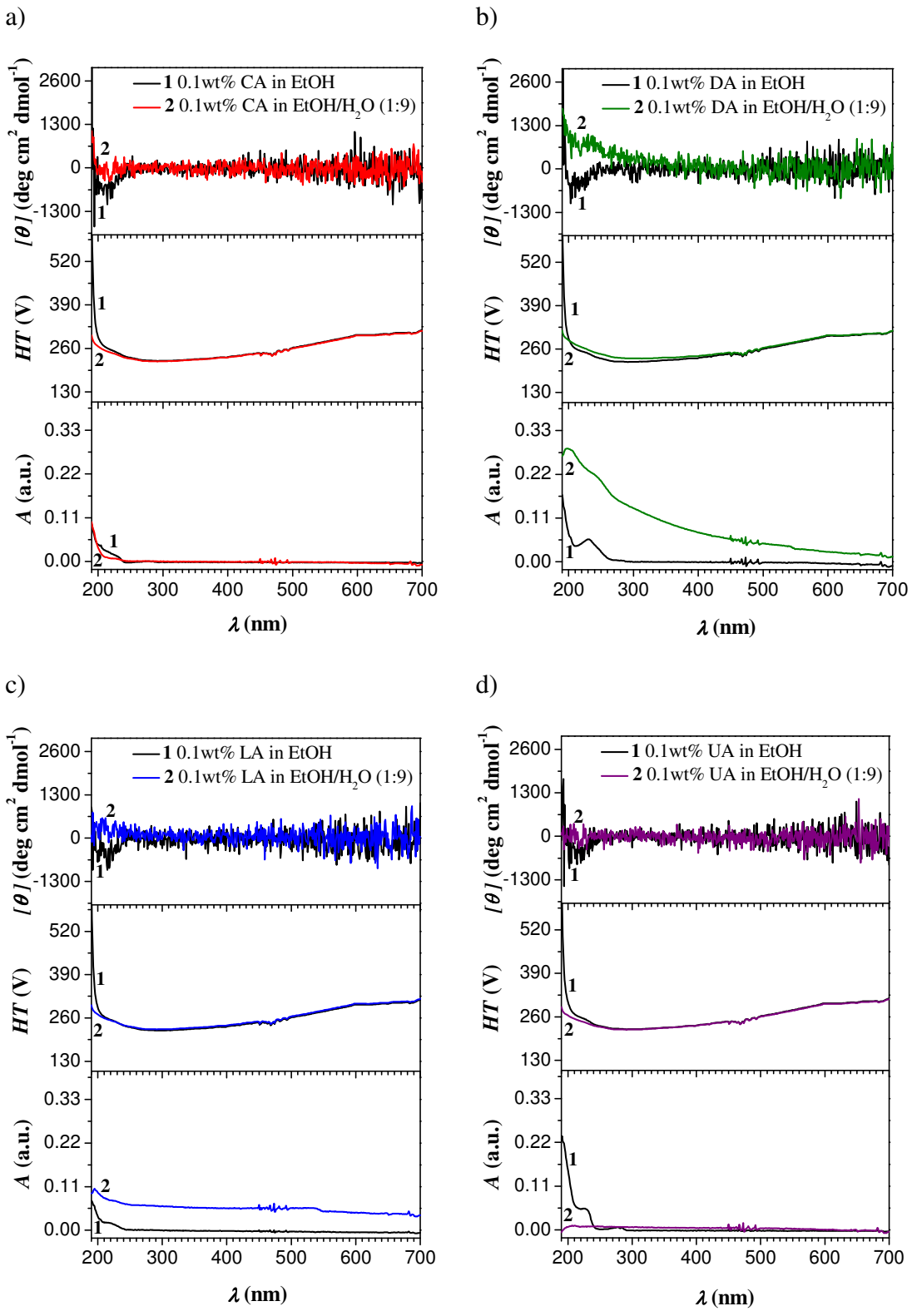


Figure SI-1. UV-Vis absorption and CD spectra of all 0.1 wt % molecular BA solutions in EtOH and in EtOH/H_2O (1:9) after the drop-wise addition of H_2O. a) Cholic acid (CA), b) deoxycholic acid (DA), c) lithocholic acid (LA), and d) ursodeoxycholic acid (UA).

Fourier-Transform Infrared Spectroscopy (FTIR).

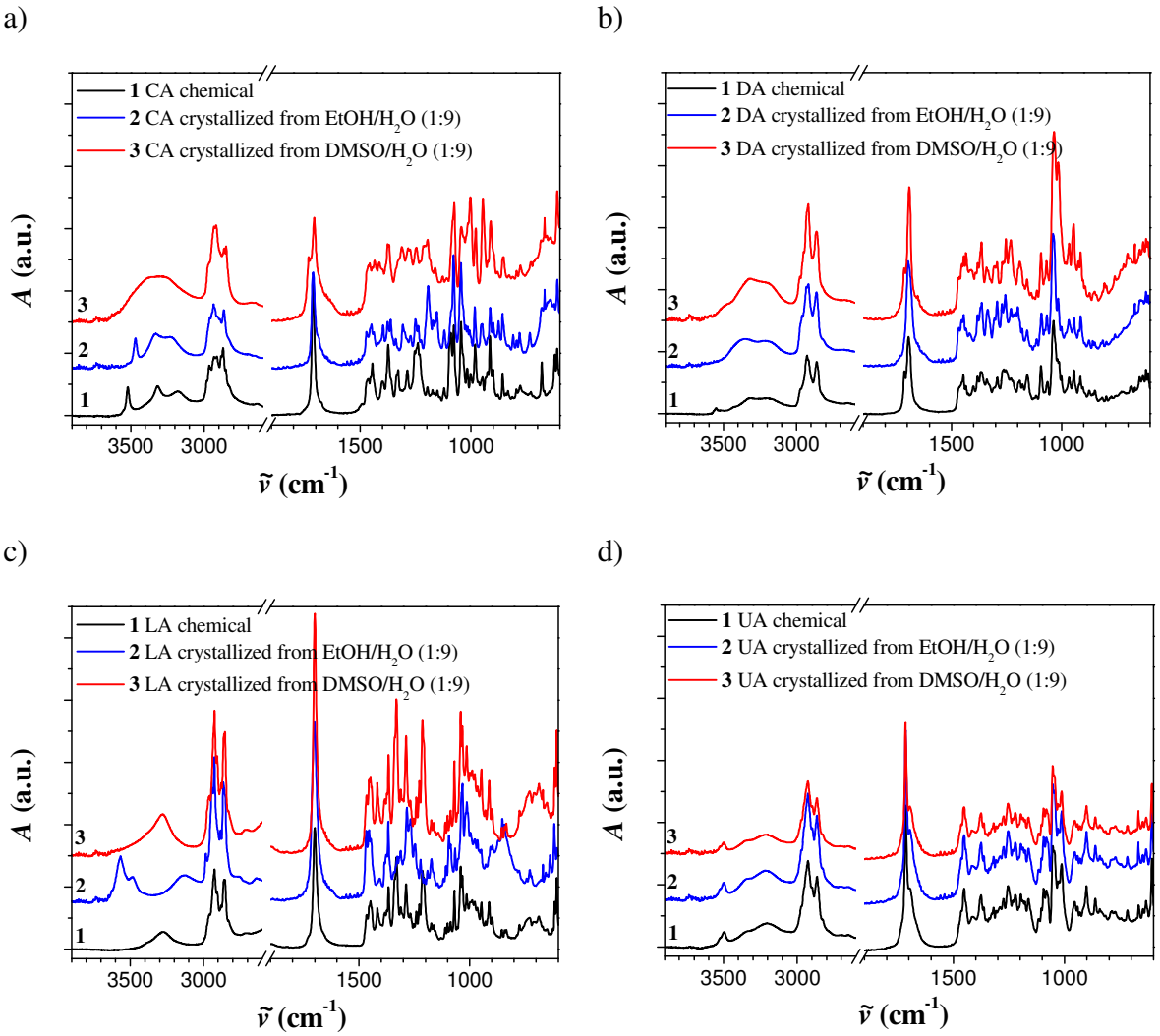


Figure SI-2. FTIR spectra of the commercial BAs and their solid-state aggregates recrystallized by slow evaporation from EtOH/H_2O (1:9) and from DMSO/H_2O (1:9) solvent mixtures. a) Cholic acid (CA), b) deoxycholic acid (DA), c) lithocholic acid (LA), and d) ursodeoxycholic acid (UA).

Wide Angle X-Ray Scattering (WAXS).

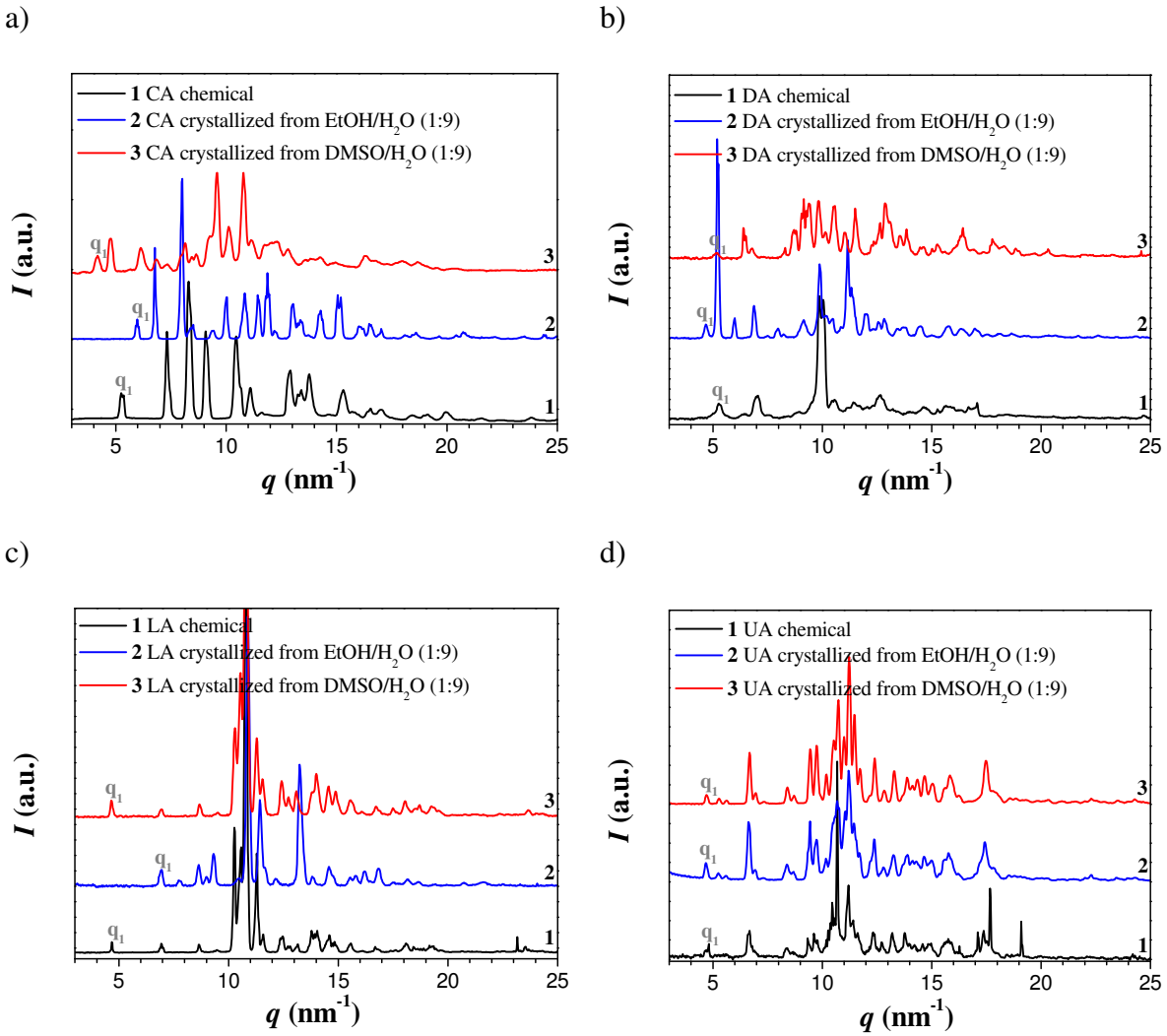


Figure SI-3. WAXS scattering patterns of solid-state aggregates recrystallized from EtOH/H_2O (1:9), from DMSO/H_2O (1:9), and with the corresponding commercial compounds. a) Cholic acid (CA), b) deoxycholic acid (DA), c) lithocholic acid (LA), and d) ursodeoxycholic acid (UA).

Bile Acid Molecules.

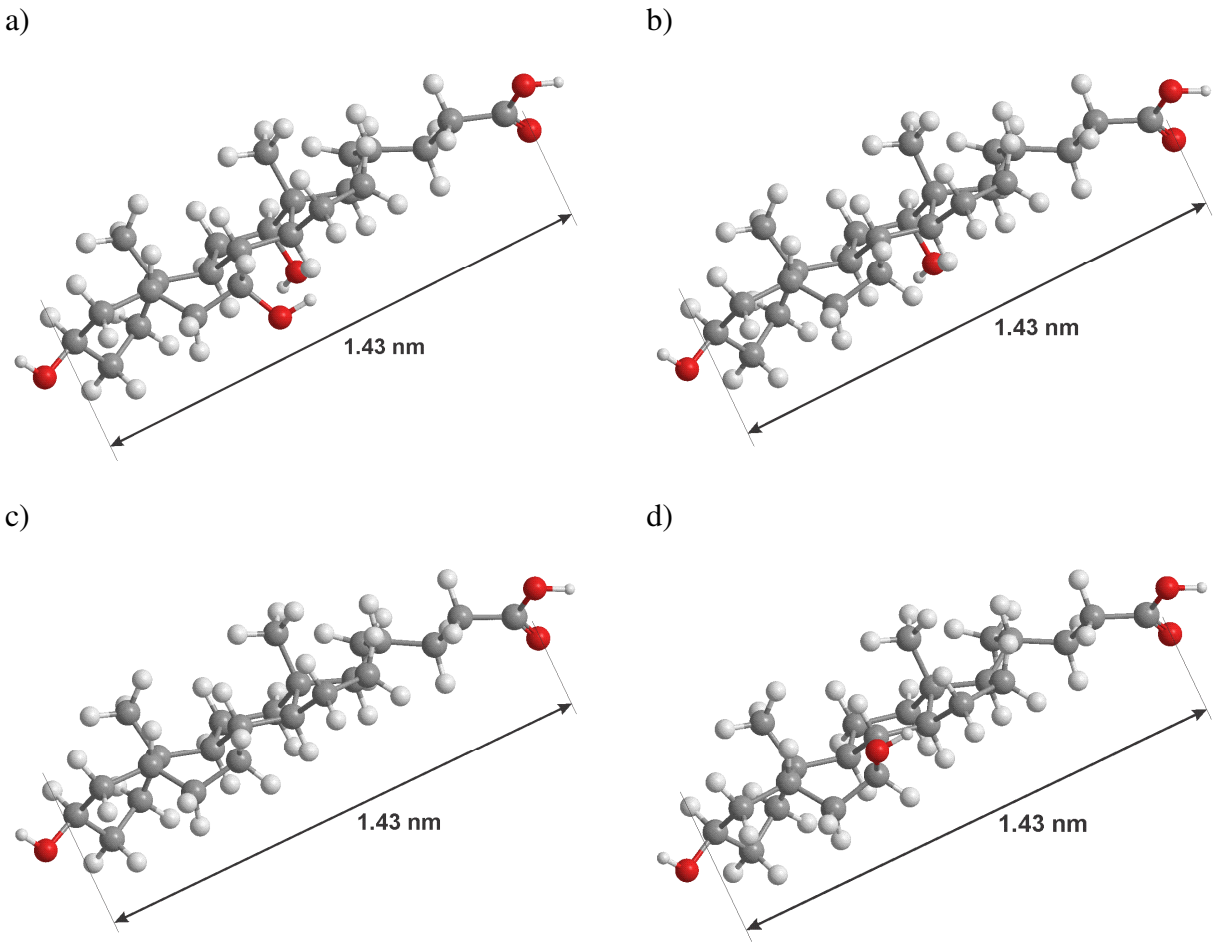
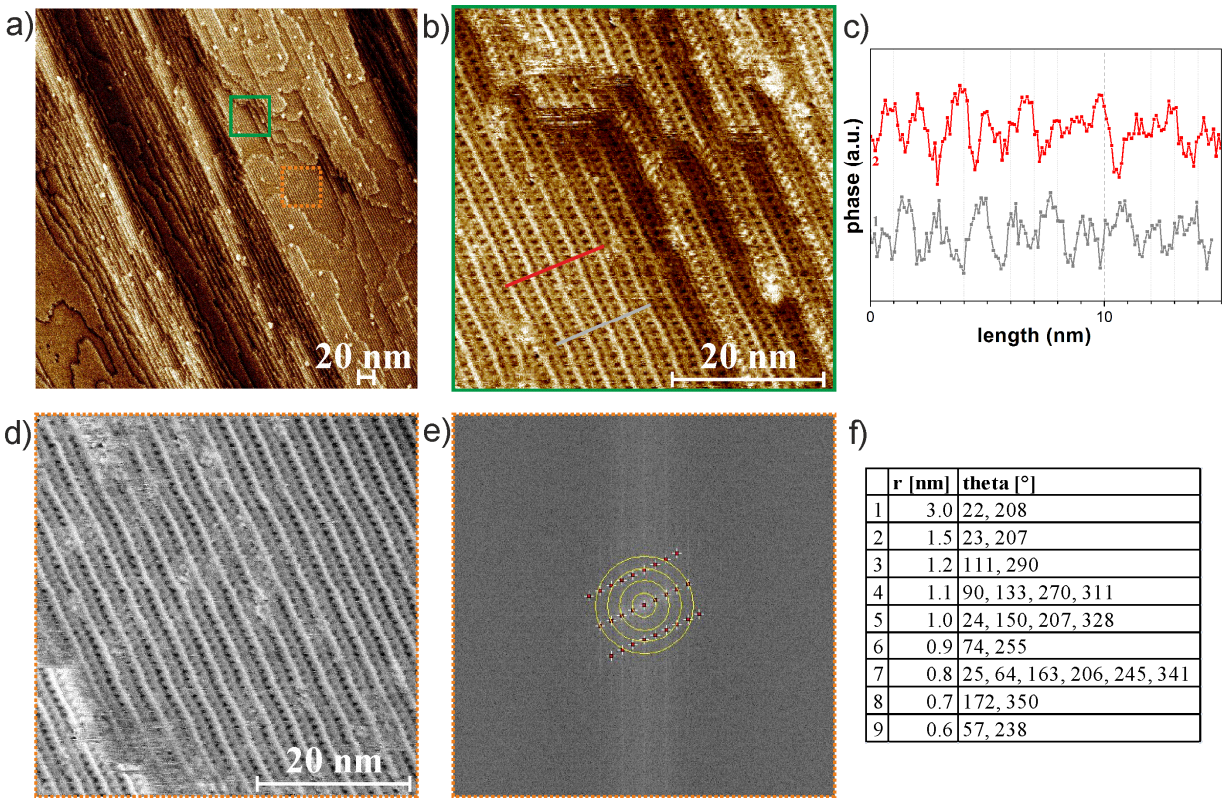


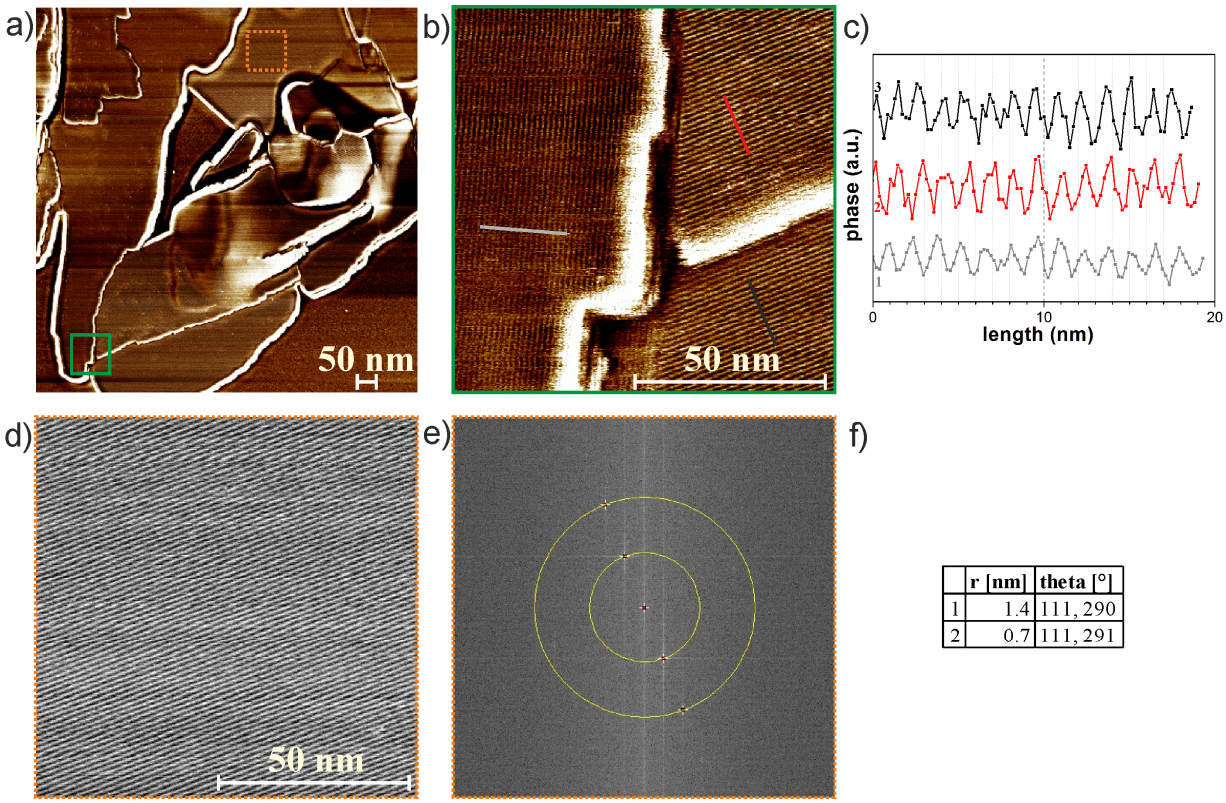
Figure SI-4. 3D Chemical structures and long axis molecular lengths for a) cholic acid (CA), b) deoxycholic acid (DA), c) lithocholic acid (LA), and d) ursodeoxycholic acid (UA).

Atomic Force Microscopy (AFM).



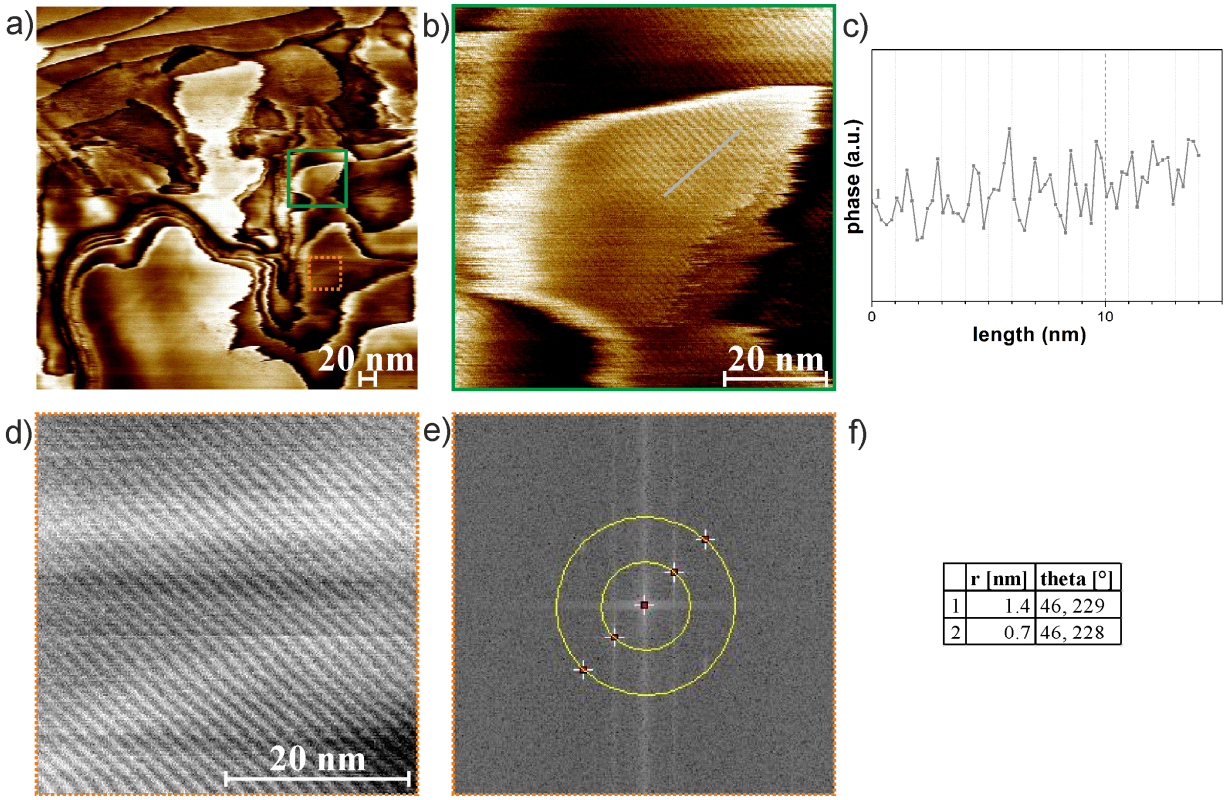
	r [nm]	theta [°]
1	3.0	22, 208
2	1.5	23, 207
3	1.2	111, 290
4	1.1	90, 133, 270, 311
5	1.0	24, 150, 207, 328
6	0.9	74, 255
7	0.8	25, 64, 163, 206, 245, 341
8	0.7	172, 350
9	0.6	57, 238

Figure SI-5. a) AFM phase image of the self-assembled ursodeoxycholic acid (UA) from DMSO/H_2O (1:9), showing crystalline aggregates in arranged multilayers. b) Zoom-in from the green square in a. c) Phase intensity sections along the colored lines in b, showing a lattice spacing of 1.5 nm. d) Zoom-in from the orange dotted square in a. e) FTT image from d. f) FFT analysis from e, showing two major reflections at 3.0 and 1.5 nm.



	r [nm]	theta [°]
1	1.4	111, 290
2	0.7	111, 291

Figure SI-6. a) AFM phase image of the self-assembled deoxycholic acid (DA) from DMSO/H_2O (1:9), showing crystalline aggregates in arranged multilayers. b) Zoom-in from the green square in a. c) Phase intensity sections along the colored lines in b, showing a lattice spacing of 1.4 nm. d) Zoom-in from the orange dotted square in a. e) FTT image from d. f) FFT analysis from e, showing two major reflections at 1.4 and 0.7 nm.



	r [nm]	theta [°]
1	1.4	46, 229
2	0.7	46, 228

Figure SI-7. a) AFM phase image of the self-assembled deoxycholic acid (DA) from DMSO/H_2O (1:9), showing crystalline aggregates in arranged multilayers. b) Zoom-in from the green square in a. c) Phase intensity section along the colored line in b, showing a lattice spacing of 1.4 nm. d) Zoom-in from the orange dotted square in a. e) FTT image from d. f) FFT analysis from e, showing the major reflection at 1.4 nm.

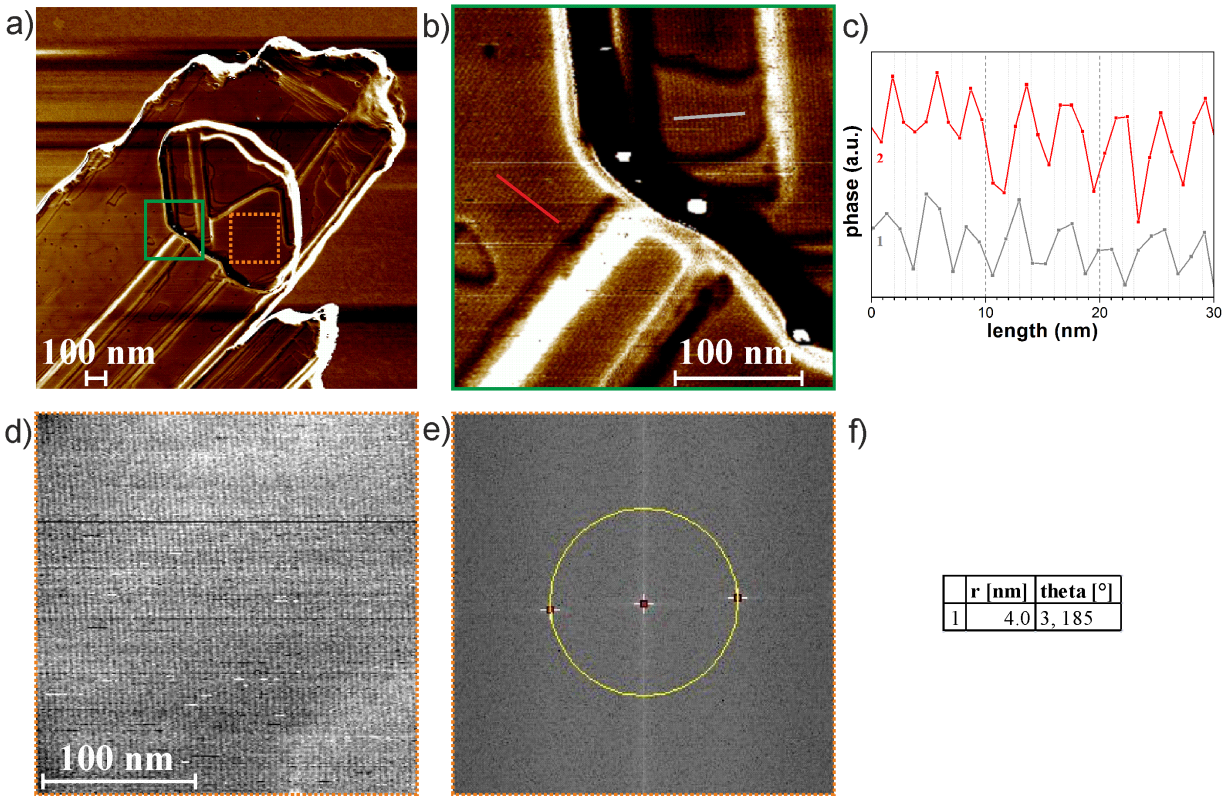
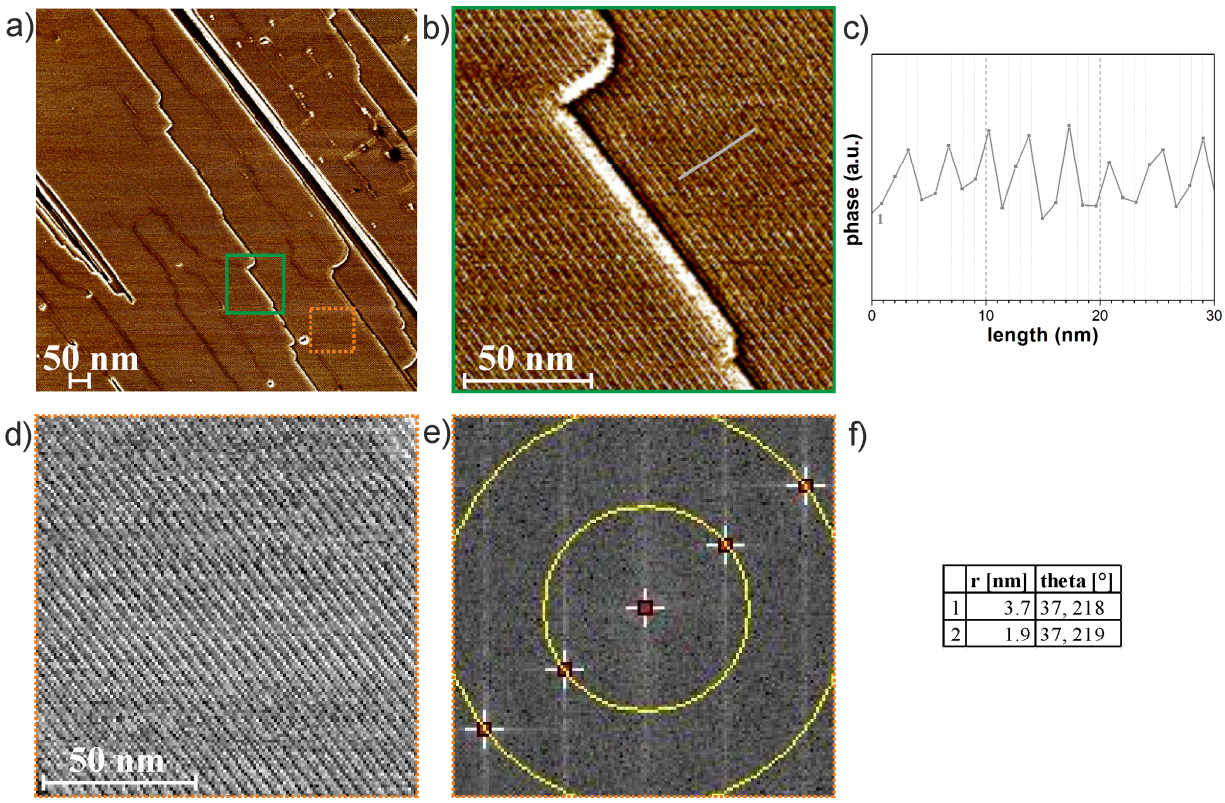
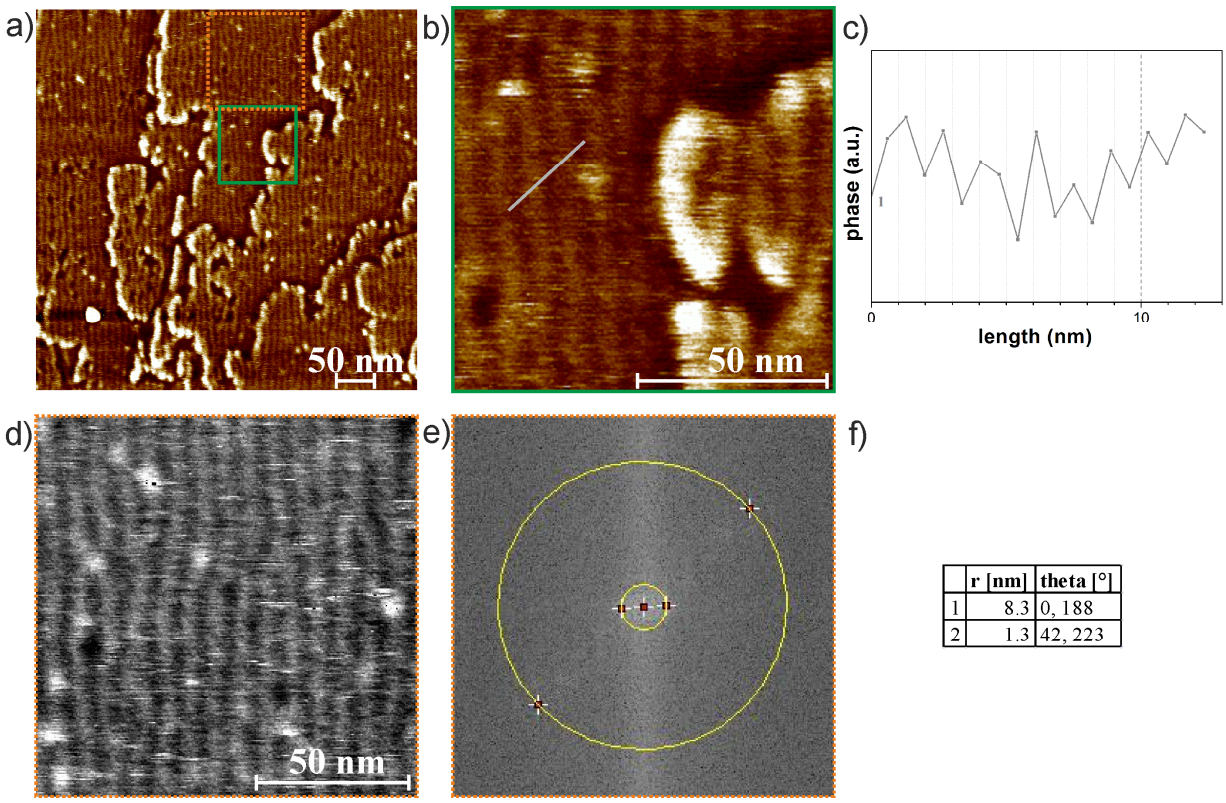


Figure SI-8. a) AFM phase image of the self-assembled lithocholic acid (LA) from EtOH/H_2O (1:9), showing crystalline aggregates in arranged multilayers. b) Zoom-in from the green square in a. c) Phase intensity sections along the colored lines in b, showing a lattice spacing of 4.0 nm. d) Zoom-in from the orange dotted square in a. e) FTT image from d. f) FFT analysis from e, showing the major reflection at 4.0 nm.



	r [nm]	theta [°]
1	3.7	37, 218
2	1.9	37, 219

Figure SI-9. a) AFM phase image of the self-assembled lithocholic acid (LA) from DMSO/H_2O (1:9), showing crystalline aggregates in arranged multilayers. b) Zoom-in from the green square in a. c) Phase intensity section along the colored line in b, showing a lattice spacing of 3-4 nm. d) Zoom-in from the orange dotted square in a. e) FTT image from d. f) FFT analysis from e, showing the major reflection at 3.7 nm.



	r [nm]	theta [°]
1	8.3	0, 188
2	1.3	42, 223

Figure SI-10. a) AFM phase image of the self-assembled lithocholic acid (LA) from DMSO/H_2O (1:9), showing crystalline aggregates in arranged multilayers. b) Zoom-in from the green square in a. c) Phase intensity section along the colored line in b, showing a lattice spacing of 1.4 nm. d) Zoom-in from the orange dotted square in a. e) FTT image from d. f) FFT analysis from e, showing the two major reflections at 8.3 and 1.3 nm.

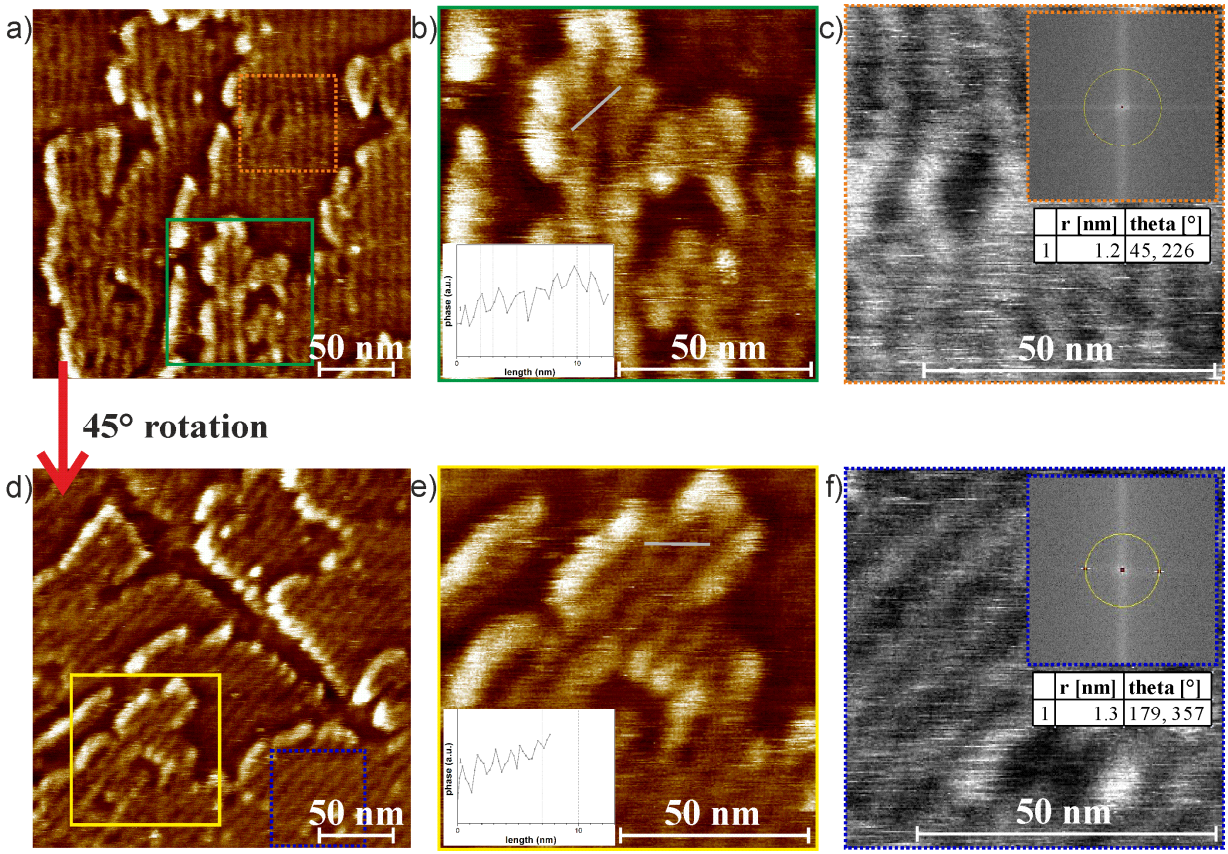


Figure SI-11. a) AFM phase image of the self-assembled lithocholic acid (LA) from DMSO/H_2O (1:9), showing crystalline aggregates in arranged multilayers. b) Zoom-in from the green square in a, showing a spacing of about 1.3 nm. c) Zoom-in from the orange dotted square in a with the corresponding FTT image (inset), showing a major reflection at 1.2 nm. d) AFM phase image of the self-assembled lithocholic acid (LA) in DMSO/H_2O (1:9), after rotating the sample by 45°. e) Zoom-in from the yellow square in d, showing a spacing of about 1.3 nm. c) Zoom-in from the blue dotted square in a with the corresponding FTT image (inset), showing a major reflection at 1.3 nm.

Table SI-1. The first ten scattering vector q values from the WAXS scattering patterns of commercial BAs and the corresponding solid-state crystalline aggregates from EtOH/H_2O (1:9) and from DMSO/H_2O (1:9). FFT peak signals from the corresponding AFM images of the crystalline aggregates.

Sample	Source	Technique	q [nm^{-1}]									
Cholic acid	commercial	WAXS	5.3	7.3	8.3	9.1	10.5	11.1	11.6	12.9	13.4	13.8
	EtOH/H_2O	WAXS	6.0	6.8	8.0	8.5	9.4	10.0	10.8	11.4	11.9	12.2
		FFT	-	-	-	-	-	-	-	-	-	-
	DMSO/H_2O	WAXS	4.2	4.8	6.1	6.8	7.4	8.2	8.4	8.6	9.3	9.6
		FFT	-	-	-	-	-	-	-	-	-	-
Deoxycholic acid	commercial	WAXS	5.3	6.4	7.1	9.0	9.9	10.0	10.6	11.4	12.7	14.7
	EtOH/H_2O	WAXS	4.7	5.2	6.0	6.9	8.0	8.2	9.2	9.9	10.1	10.5
		FFT	-	-	-	-	-	-	-	-	-	-
	DMSO/H_2O	WAXS	5.2	6.4	6.8	8.3	8.7	9.2	9.4	9.8	10.1	10.6
		FFT	4.5	9.0	-	-	-	-	-	-	-	-
Lithocholic acid	commercial	WAXS	4.7	6.9	8.6	9.5	10.3	10.6	10.8	11.3	11.6	12.5
	EtOH/H_2O	WAXS	7.0	7.7	8.6	9.0	9.3	10.9	11.4	12.1	13.3	13.8
		FFT	1.6	-	-	-	-	-	-	-	-	-
	DMSO/H_2O	WAXS	4.7	6.9	8.7	9.5	10.3	10.6	10.8	11.3	11.5	12.4
		FFT	0.8	4.8	-	-	-	-	-	-	-	-
Ursodeoxycholic acid	commercial	WAXS	4.8	6.7	8.4	9.3	9.6	10.5	10.7	11.2	11.4	11.6
	EtOH/H_2O	WAXS	4.7	5.3	5.6	6.6	6.9	8.4	8.7	9.4	9.7	10.2
		FFT	-	-	-	-	-	-	-	-	-	-
	DMSO/H_2O	WAXS	4.7	5.3	5.6	6.7	7.0	7.3	8.4	8.7	9.4	9.7
		FFT	2.1	4.2	5.2	5.7	6.3	7.0	7.9	9.0	10.5	-

Table SI-2. Overview of experimental WAXS and AFM results of the four BA aggregates obtained from EtOH/H_2O (1:9) and from DMSO/H_2O (1:9). WAXS presents real space distances d calculated from lowest scattering vectors q_1; AFM summarizes the smallest real space and FFT analysis lattice spacing.

Sample	Good solvent	d_{WAXS} [nm]	d_{AFM} [nm]	d_{FFT} [nm]
Cholic acid	EtOH	1.05	-	-
	DMSO	1.50	-	-
Deoxycholic acid	EtOH	1.34	-	-
	DMSO	1.22	1.3 – 1.4 [a]	1.4 [a]
Lithocholic acid	EtOH	0.90	4.0 [b]	4.0 [b]
	DMSO	1.35	1.0 – 1.4 [a]	1.2 – 1.3 [a]
Ursodeoxycholic acid	EtOH	1.34	-	-
	DMSO	1.34	2.9 – 3.3 / 1.6 – 1.7 [a]	3.0 / 1.5 [a]

[a] High resolution AFM scanning parameters [b] Low resolution AFM scanning parameters