SUPPORTING INFORMATION

Cross-Fitting of Residual Dipolar Couplings

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Table S1. Chemical Shifts[(a)] of Cholesterol and 5-α-Cholestan-3-one in $CDCl_3$

	cholesterol			5-α-cholestan-3-one		
Group	**δ ^{13}C [ppm]**	**δ $^{1}H_a$[(b)] [ppm]**	**δ $^{1}H_b$[(b)] [ppm]**	**δ ^{13}C [ppm]**	**δ $^{1}H_a$[(b)] [ppm]**	**δ $^{1}H_b$[(b)] [ppm]**
C1	37.3	1.1 (α)	1.9 (β)	38.6	1.3 (α)	2.0 (β)
C2	31.6	1.5 (β)	1.8 (α)	38.1	2.3 (α)	2.4 (β)
C3	71.7	3.5	-	212.1	-	-
C4	42.3	2.2	2.3	44.7	2.1 (α)	2.2 (β)
C5	141.2	-	-	46.7	1.5	-
C6	121.6	5.4	-	29.0	1.3	1.3
C7	31.9	1.5 (α)	2.0 (β)	31.7	0.9 (α)	1.7 (β)
C8	31.9	1.5	-	35.4	1.4	-
C9	50.2	0.9	-	53.9	0.7	-
C10	36.9	-	-	36.0	-	-
C11	21.1	1.5	1.5	21.5	1.4 (β)	1.5 (α)
C12	39.8	1.2 (α)	2.0 (β)	39.9	1.1 (α)	2.0 (β)
C13	42.7	-	-	43.0	-	-
C14	56.8	1.0	-	56.3	1.0	-
C15	24.3	1.1	1.6	24.2	1.1	1.6
C16	28.3	1.3	1.8	28.3	1.2	1.8
C17	56.2	1.1	-	56.3	1.1	-
C18	11.9	0.7	-	12.1	0.7	-
C19	19.4	1.0	-	11.4	1.0	-
C20	35.8	1.4	-	35.8	1.4	-
C21	18.8	0.9	-	18.7	0.9	-
C22	36.2	1.0	1.4	36.2	1.0	1.3
C23	23.9	1.2	1.4	23.8	1.1	1.3
C24	39.6	1.1	1.2	39.5	1.1	1.1
C25	28.0	1.5	-	28.0	1.5	-
C26	22.7	0.9	-	22.8	0.9	-
C27	22.9	0.9	-	22.6	0.8	-

[(a)] Chemical shifts are referenced to the solvent signals: δ ^{1}H ($CHCl_3$) = 7.26 ppm and δ ^{13}C ($CDCl_3$) = 77.2 ppm.
[(b)] The prochiral assignment for Hα and Hβ protons was done with the help of measured RDCs (see tables (S2) and (S4)). Protons a and b have not been assigned to position α and β wherever no or inconclusive RDCs were measured.

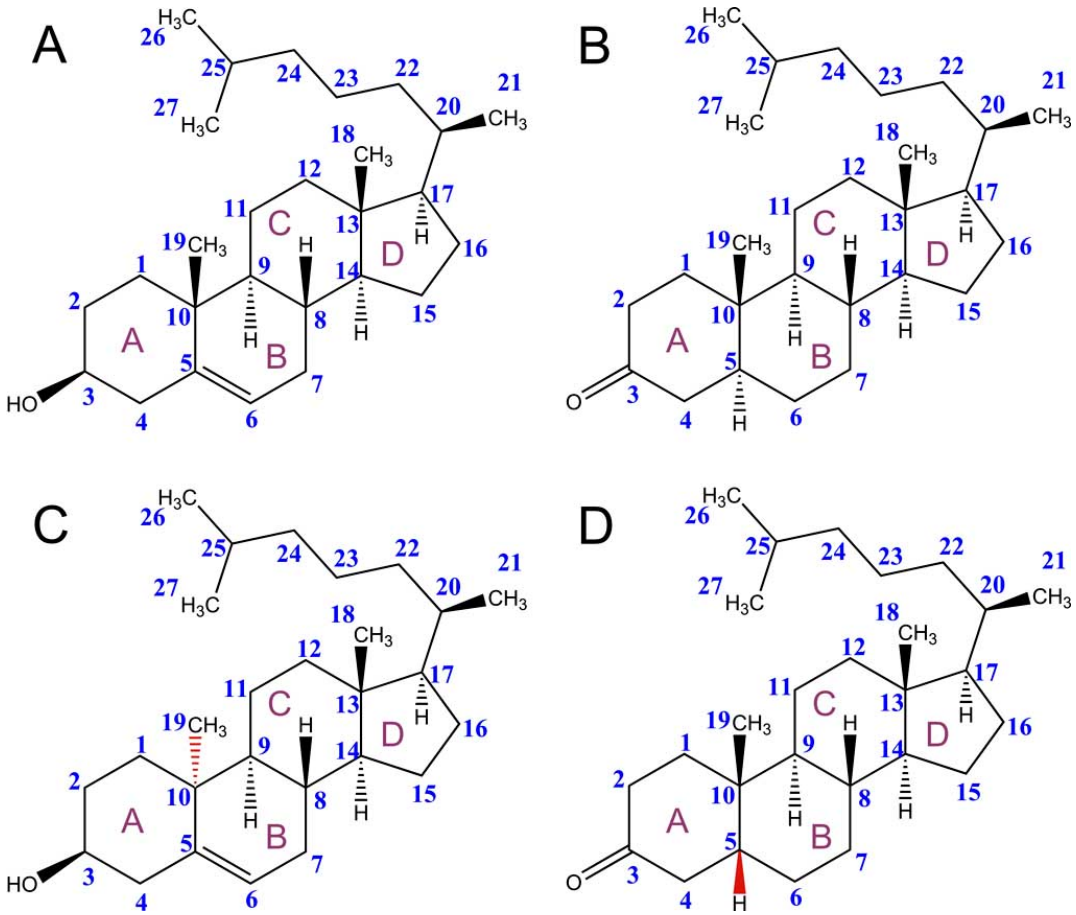

Fig. (S1). Structure and nomenclature of cholesterol (**A**), 5-α-cholestan-3-one (**B**) and their diastereomers 10-α-cholesterol (**C**) and 5-β-cholestan-3-one (**D**).

QUALITY FACTOR FOR VALIDATION OF FITS:

To compare fits of measured RDCs against different structural models, a quality factor for these fits is necessary. On the one hand it should consider how strong measured and back-calculated values differ and, on the other hand, it should take into account the experimental error of the measured values. This is described by χ^2 defined as:

$$\chi^2 = \sum^{n} \left(\frac{x_{meas.} - x_{calc.}}{\Delta x_{meas.}} \right)^2$$

with $x_{meas.}$ and $x_{calc.}$ being the measured and back-calculated values, respectively, and $\Delta x_{meas.}$ being the experimental errors of x_{meas}. As χ^2 increases with an increasing number n of measured values, we found the best measure for the quality of a fit would be the normalized n/χ^2, which should be as high as possible.

The quality factor is optimized for the comparison of different fits to a single set of experimental RDCs, including the important individual maximum error estimates for the measured values. Other quality factors are available which are adapted to other specifications. Therefore we give for comparison also the correlation coefficient, R, and the quality factor by Cornilescu *et al.*, Q,[1] in all tables of the Supporting Information.

Table S2. Couplings of Cholesterol Measured in Solution ($^1J_{CH}$) and in the Stretched PDMS Gel ($^1T_{CH}$), Corresponding RDCs ($^1D_{CH}$) and RDCs Back Calculated with the bestFit Option of PALES (SVD-Fit) [2, 3]. All Couplings are Given in Hz

Group [(a)]	$^1J_{CH}$	$^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$	$^1D_{CH}$ (exp)	$^1D_{CH}$ (calc) (SVD-fit)
C18-H18	124.3 ± 0.3	117.3 ± 0.5	-7.0 ± 0.6[(b)]	(1.9)[(b)]
C19-H19	125.6 ± 0.3	119.0 ± 0.5	-6.6 ± 0.6[(b)]	(1.8)[(b)]
C16-H16a	125.3 ± 3.0	125.6 ± 5.0	0.3 ± 5.8	-[(c)]
C16-H16b	129.7 ± 3.0	134.8 ± 5.0	5.1 ± 5.8	-[(c)]
C15-H15b	130.0 ± 3.0	144.0 ± 5.0	14.0 ± 5.8	-[(c)]
C2-H2α	129.3 ± 2.5	140.6 ± 3.0	11.3 ± 3.9	9.6
C2-H2β	125.2 ± 2.5	141.3 ± 3.0	16.1 ± 3.9	16.5
C8-H8	122.0 ± 3.0	142.7 ± 8.0	20.7 ± 8.5	22.3
C7-H7β	126.5 ± 3.0	140.7 ± 5.0	14.2 ± 5.8	11.7
C1-H1β	128.4 ± 1.0	137.4 ± 1.0	9.0 ± 1.4	9.1
C1-H1α	124.3 ± 0.8	142.5 ± 1.2	18.2 ± 1.4	19.2
C12-H12α	123.2 ± 1.0	145.6 ± 1.0	22.4 ± 1.4	21.8
C12-H12β	127.0 ± 1.0	132.1 ± 1.0	5.1 ± 1.4	4.6
C6-H6	152.7 ± 0.3	154.3 ± 3.0	1.6 ± 3.0	2.2
C9-H9	122.4 ± 0.5	146.0 ± 4.0	23.6 ± 4.0	21.7
C3-H3	142.1 ± 0.5	161.4 ± 1.5	19.3 ± 1.6	17.9
C21-H21	124.1 ± 0.3	119.2 ± 0.5	-4.9 ± 0.6	-[(d)]
C25-H25	124.8 ± 0.5	135.6 ± 0.8	10.8 ± 0.9	-[(d)]
C20-H20	123.6 ± 0.5	146.9 ± 1.0	23.3 ± 1.1	-[(d)]
C27-H27	124.1 ± 0.3	124.3 ± 0.3	-0.2 ± 0.4	-[(d)]
C26-H26	124.1 ± 0.3	123.9 ± 0.3	0.2 ± 0.4	-[(d)]
C23-H23a	124.1 ± 1.0	142.2 ± 5.0	18.1 ± 5.1	-[(d)]
C23-H23b	124.3 ± 1.0	129.5 ± 2.5	5.2 ± 2.7	-[(d)]
C22-H22a	123.0 ± 5.0	148.6 ± 4.0	25.6 ± 6.4	-[(d)]
C22-H22b	126.0 ± 10.0	134.7 ± 3.0	8.7 ± 10.4	-[(d)]
Group	$^2J_{HH}$	$^2T_{HH} = {}^2J_{HH} + {}^2D_{HH}$	$^2D_{HH}$ (exp)	$^2D_{HH}$ (calc) (SVD-fit)
H16α-H16β	-12.0 ± 2.0	5.7 ± 3.0	17.7 ± 3.6	-[(c)]
H15α-H15β	-11.0 ± 3.0	3.0 ± 3.0	14.0 ± 4.2	-[(c)]
H2α-H2β	-12.2 ± 1.0	6.7 ± 2.0	18.9 ± 2.2	20.6
H7α-H7β	-16.0 ± 3.0	1.7 ± 5.0	17.7 ± 5.8	19.8
H1α-H1β	-13.0 ± 1.0	2.0 ± 2.0	15.0 ± 2.2	15.8
H12α-H12β	-12.2 ± 1.0	2.0 ± 2.0	14.2 ± 2.2	14.1
H4α-H4β	-12.7 ± 2.0	3.0 ± 2.0	15.7 ± 2.8	16.8

[(a)] The prochiral assignment for all Hα and Hβ protons was determined by fitting all possible permutations with the -bestFit option of PALES [2, 3] and selecting the one with the best fitting result (in terms of highest n/χ^2 value).

[(b)] D_{CH}-couplings of methyl-groups have been converted to the corresponding D_{CC}-couplings[4]: (D_{CC}(C18-C13) = 1.9 ± 0.2 Hz; D_{CC}(C19-C10) = 1.8 ± 0.2 Hz).

[(c)] As couplings in the D-ring did not fit in the initial fittings (see main text) they were not used in further fittings.

[(d)] Couplings measured in the flexible side chain were not used for PALES fits.

Table S3. RDCs Measured on Cholesterol and RDCs Back Calculated for Cholesterol and 10-α-Cholesterol for the -bestFit Method (SVD), the Fit with Fixed Orientation Given by the Alignment Tensor of 5-α-Cholestan-3-one (Cross-fitting) and Fit with Orientation Predicted by PALES [2, 3]. Additionally Alignment Tensor Parameters and Quality Factors for the Different fits are Given: Axial and Rhombic Components (D_a, D_r) and Principal Axes of the Alignment Tensor (A_{xx}, A_{yy}, A_{zz}) with their Corresponding Eigenvectors (EV), Number of RDCs used for Fitting (n) and Quality Factors χ^2, n/χ^2, Correlation Factor (R) and Quality Factor by Cornilescu *et al.* [1]. All Couplings are Given in Hz

Group	D (exp)	D (calc) (SVD-fit)		D (calc) (cross-fitting)		D (calc) (predicted C1-C27)	
		Cholesterol	10α Cholesterol	Cholesterol	10α Cholesterol	Cholesterol	10α Cholesterol
C18-C13	1.9 ± 0.2	1.9	2.0	2.0	2.0	1.8	2.0
C19-C10	1.8 ± 0.2	1.8	1.6	2.0	0.5	1.7	1.2
C2-H2α	11.3 ± 3.9	9.6	10.9	5.0	4.6	4.9	7.8
C2-H2β	16.1 ± 3.9	16.5	16.1	20.7	22.9	15.0	18.2
C8-H8	20.7 ± 8.5	22.3	21.3	25.4	25.2	21.7	20.6
C7-H7β	14.2 ± 5.8	11.7	23.0[(a)]	7.2	23.9[(a)]	6.5	22.4[(a)]
C1-H1β	9.0 ± 1.4	9.1	6.7[(a)]	7.5	5.3	11.2	11.5[(a)]
C1-H1α	18.2 ± 1.4	19.2	17.9[(a)]	23.3	8.8	18.2	12.8[(a)]
C12-H12α	22.4 ± 1.4	21.8	20.9	24.6	24.8	21.3	20.7
C12-H12β	5.1 ± 1.4	4.6	6.0	3.5	3.7	8.7	12.5
C6-H6	1.6 ± 3.0	2.2	5.7	2.1	8.5	7.1	9.5
C9-H9	23.6 ± 4.0	21.7	18.7	24.4	24.7	21.2	19.0
C3-H3	19.3 ± 1.6	17.9	18.6	22.0	4.2	16.7	14.9
H2α-H2β	18.9 ± 2.2	20.6	23.8	21.9	18.9	19.6	20.7
H7α-H7β	17.7 ± 5.8	19.8	19.2	20.7	11.7	18.4	15.3
H1α-H1β	15.0 ± 2.2	15.8	15.6	15.1	14.8	15.3	14.8
H12α-H12β	14.2 ± 2.2	14.1	15.4	13.0	13.4	15.5	19.8
H4α-H4β	15.7 ± 2.8	16.8	13.9	16.3	2.5	15.9	14.7
	D_a	-3.94E-04	-3.56E-04	-3.97E-04		-3.72E-04	-4.06E-04
	D_r	-6.44E-05	-1.31E-04	-1.10E-04		-7.02E-05	-6.97E-05
	A_{xx}	2.98E-04	1.59E-04	2.32E-04		2.67E-04	3.02E-04
	A_{yy}	4.91E-04	5.53E-04	5.62E-04		4.77E-04	5.11E-04
	A_{zz}	-7.89E-04	-7.12E-04	-7.94E-04		-7.44E-04	-8.12E-04
	EV A_{xx}	-0.48; 0.85; -0.22	-0.36; 0.93; -0.09	-0.45; 0.87; -0.19		-0.34; 0.92; -0.17	-0.24; 0.92; -0.31
	EV A_{yy}	-0.58; -0.12; 0.80	-0.43; -0.08; 0.90	-0.65; -0.17; 0.74		-0.61; -0.08; 0.79	-0.55; 0.13; 0.83
	EV A_{zz}	0.65; 0.52; 0.55	0.83; 0.36; 0.43	0.62; 0.45; 0.64		0.71; 0.38; 0.59	0.80; 0.37; 0.47
	n	18	18	18	18	18	18
	χ^2	3.32	16.96	30.71	225.07	21.17	82.82
	n/χ^2	5.42	1.06	0.59	0.08	0.85	0.22
	R	0.984	0.911	0.940	0.679	0.900	0.800
	Q	0.082	0.195	0.214	0.435	0.198	0.275

[(a)] Hα and Hβ proton assignment was permutated compared to the assignment of cholesterol. (Of all possible permutations of the prochiral methylene groups only the fit with the best result is shown.)

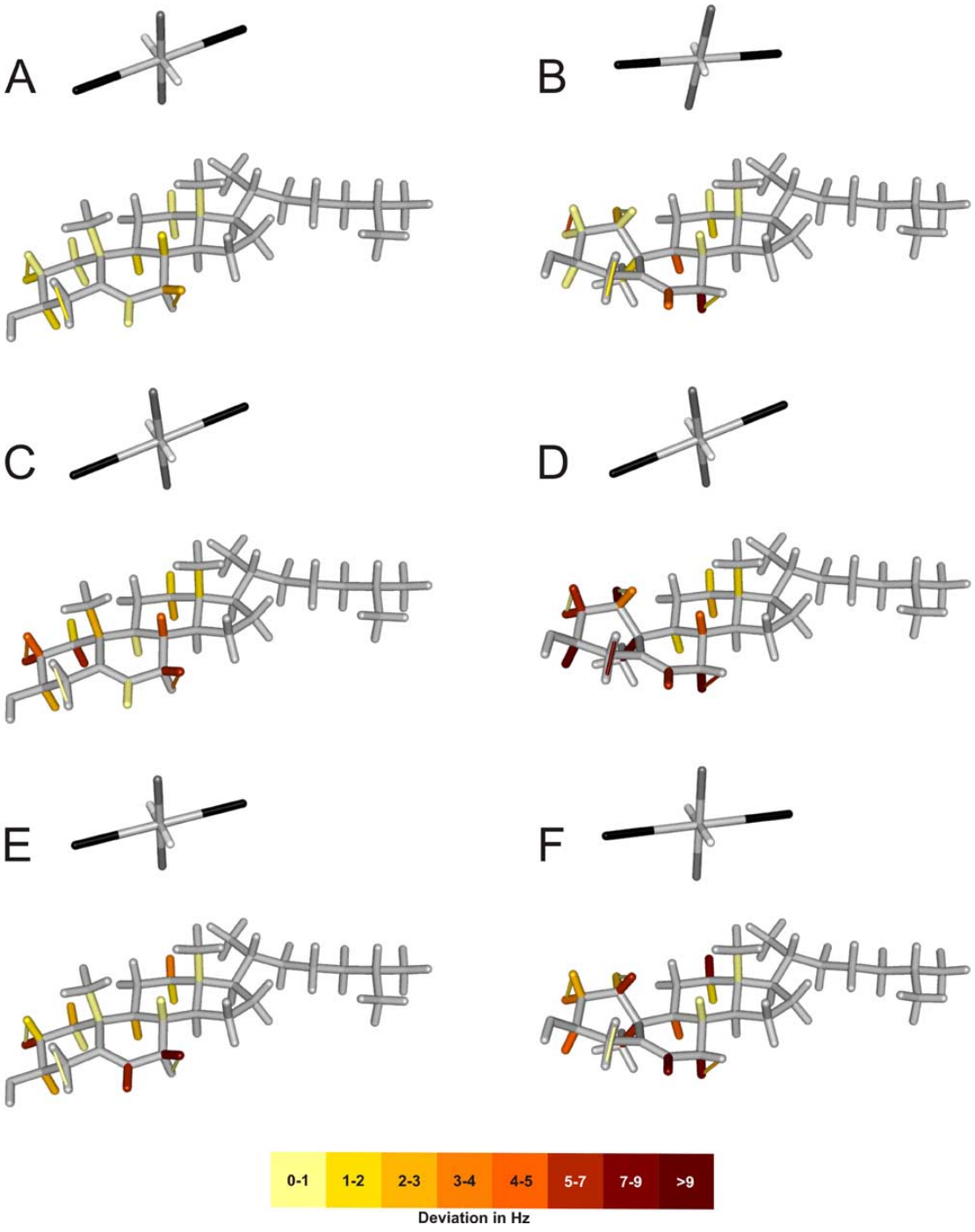

Fig. (S2). Comparison of RDCs measured on cholesterol and back-calculated for the structures of cholesterol (left: **A**, **C**, **E**) and 10-α-cholesterol (right: **B**, **D**, **F**) using the -bestFit option of PALES (SVD-fit) [2, 3] (top: **A**, **B**), the cross-fitting approach with the alignment tensor determined for 5-α-cholestan-3-one in PDMS/$CDCl_3$ (middle: **C**, **D**) and the prediction by PALES (SVD-fit) [2, 3] (bottom: **E**, **F**). The structures are shown with color-coded bonds denoting the deviation between measured and back-calculated RDCs for the different fits. The corresponding alignment tensors are visualized with their principal axis systems (black: A_{zz}; gray: A_{yy}; white: A_{xx}). For all three methods the cholesterol structure (left) gives clearly the better fit. The direct SVD-fit for 10-α-cholesterol (B) results in an alignment tensor which differs most significantly from the alignment tensors for all other fits, since it tries to match RDCs measured on cholesterol to the wrong structural model (see also Figure S6). It therefore has the least ability to distinguish the diastereomers. In contrast the fit with the fixed orientation given by the alignment tensor of 5-α-cholestan-3-one (D) shows small deviations (yellow) in regions similar to cholesterol (C-ring) and strong deviations (red) for those different to cholesterol (A- and B-ring).

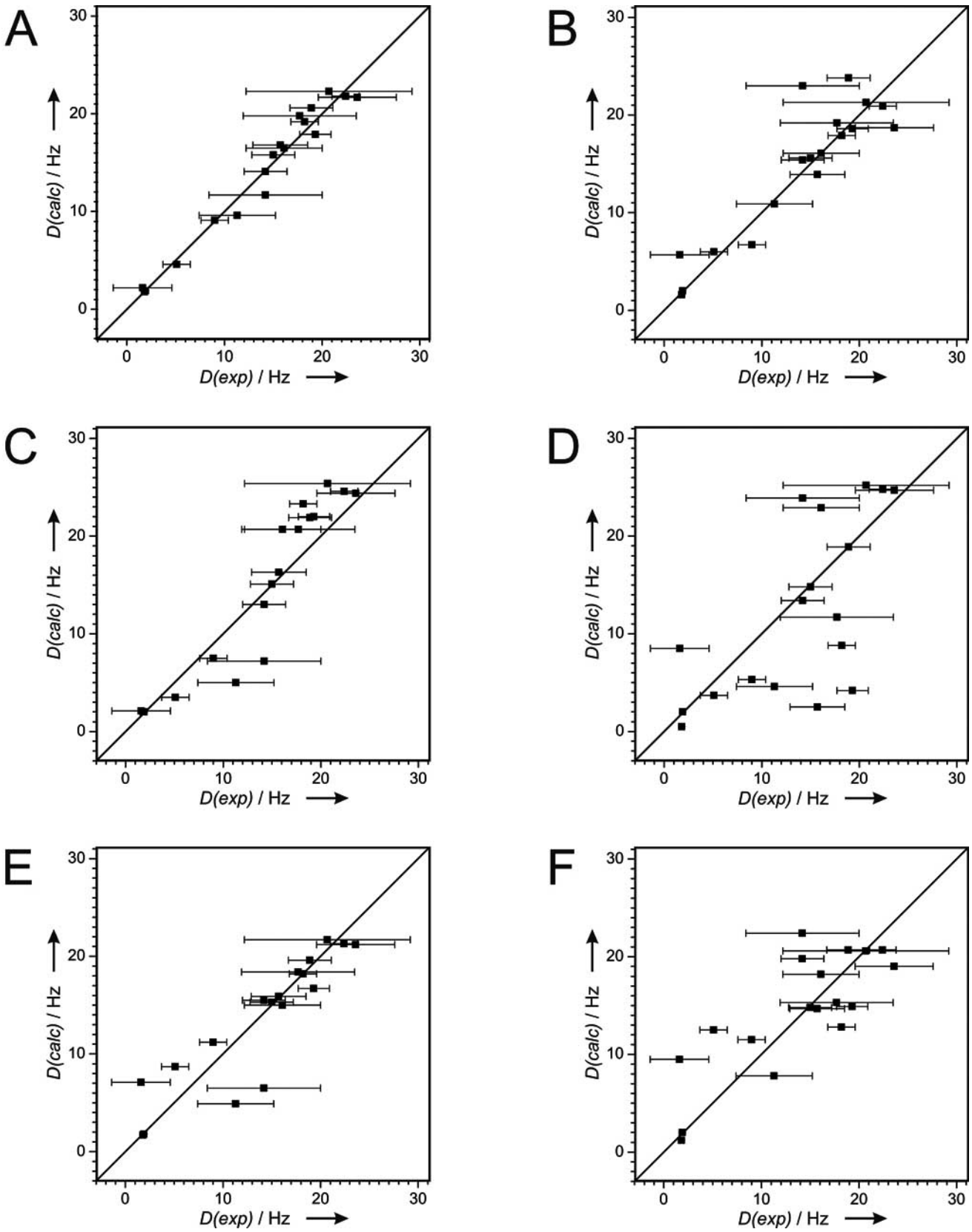

Fig. (S3). Comparison of RDCs measured on cholesterol and back-calculated for the structures of cholesterol (left: **A**, **C**, **E**) and 10-α-cholesterol (right: **B**, **D**, **F**) using the -bestFit option of PALES (SVD-fit) [2, 3] (top: **A**, **B**), the cross-fitting approach (middle: **C**, **D**) and the prediction by PALES [2, 3] (bottom: **E**, **F**). The plots show the back-calculated RDCs, D(calc), as a function of the measured RDCs, D(exp). Clearly the correct diastereomer cholesterol (left) is favored in all three methods.

Table S4. Couplings of 5-α-Cholestan-3-one Measured in Solution ($^1J_{CH}$) and in the Stretched PDMS gel ($^1T_{CH}$), Corresponding RDCs ($^1D_{CH}$) and RDCs Back Calculated with the bestFit Option of PALES (SVD-fit) [2, 3]. All Couplings are Given in Hz

Group [(a)]	$^1J_{CH}$	$^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$	$^1D_{CH}$ (exp)	$^1D_{CH}$ (calc) (SVD-fit)
C19-H19	124.4 ± 0.2	117.2 ± 0.8	-7.2 ± 0.8[(b)]	(2.1)[(b)]
C18-H18	124.1 ± 0.2	116.9 ± 0.5	-7.2 ± 0.5[(b)]	(2.0)[(b)]
C16-H16a	125.6 ± 3.0	123.7 ± 5.0	-1.9 ± 5.8	-[(c)]
C16-H16b	129.9 ± 2.0	138.4 ± 3.0	8.5 ± 5.4	-[(c)]
C15-H15a	126.6 ± 1.0	138.2 ± 5.0	11.6 ± 5.1	-[(c)]
C15-H15b	130.1 ± 1.0	144.1 ± 5.0	14.0 ± 5.1	-[(c)]
C11-H11β	122.1 ± 0.2	148.8 ± 3.0	26.7 ± 3.0	24.4
C11-H11α	125.5 ± 0.2	131.0 ± 3.0	5.5 ± 3.0	3.9
C7-H7β	127.9 ± 0.2	132.0 ± 5.0	4.1 ± 5.0	2.5
C7-H7α	122.8 ± 0.3	148.7 ± 1.5	25.9 ± 1.5	24.6
C8-H8	122.5 ± 0.3	149.7 ± 1.0	27.2 ± 1.0	25.2
C2-H2α	134.1 ± 1.0	141.0 ± 3.0	6.9 ± 3.2	6.4
C2-H2β	122.4 ± 1.0	143.5 ± 3.0	21.1 ± 3.2	20.5
C1-H1β	129.6 ± 0.7	136.9 ± 1.0	7.3 ± 1.2	7.6
C1-H1α	125.8 ± 0.7	146.5 ± 1.5	20.7 ± 1.7	23.5
C12-H12α	123.1 ± 0.5	150.5 ± 1.5	27.4 ± 1.6	24.8
C12-H12β	127.0 ± 0.5	131.3 ± 1.0	4.3 ± 1.1	3.8
C4-H4α	132.6 ± 0.7	139.3 ± 0.8	6.7 ± 1.1	6.9
C4-H4β	122.3 ± 0.7	145.0 ± 1.5	22.7 ± 1.7	23.1
C5-H5	123.3 ± 2.0	151.1 ± 3.0	27.8 ± 3.6	24.3
C9-H9	121.3 ± 0.5	145.0 ± 1.0	23.7 ± 1.1	24.7
C21-H21	124.1 ± 0.2	119.1 ± 0.7	-5.0 ± 0.7	-[(d)]
C25-H25	124.8 ± 0.2	136.2 ± 0.8	11.4 ± 0.8	-[(d)]
C20-H20	124.0 ± 0.2	147.3 ± 1.0	23.3 ± 1.0	-[(d)]
C27-H27	124.2 ± 0.2	123.7 ± 0.3	-0.5 ± 0.4	-[(d)]
C26-H26	124.0 ± 0.2	124.2 ± 0.3	0.2 ± 0.4	-[(d)]
C23-H23a	124.0 ± 1.8	144.5 ± 8.0	20.5 ± 8.2	-[(d)]
C23-H23b	123.5 ± 3.0	132.0 ± 8.0	8.5 ± 8.5	-[(d)]
C22-H22a	123.0 ± 3.0	149.7 ± 5.0	26.7 ± 5.8	-[(d)]
C22-H22b	125.8 ± 1.0	134.5 ± 5.0	8.7 ± 5.1	-[(d)]
Group	$^2J_{HH}$	$^2T_{HH} = {}^2J_{HH} + {}^2D_{HH}$	$^2D_{HH}$ (exp)	$^2D_{HH}$ (calc) (SVD-fit)
H16α-H16β	-10.1 ± 2.0	7.1 ± 3.0	17.2 ± 3.6	-[(c)]
H15α-H15β	-9.9 ± 2.0	5.8 ± 3.0	15.7 ± 3.6	-[(c)]
H11α-H11β	-13.2 ± 1.0	0.0 ± 3.0	13.2 ± 3.2	17.6
H7α-H7β	-11.8 ± 1.0	3.0 ± 3.0	14.8 ± 3.2	17.4
H2α-H2 β	-15.2 ± 1.5	3.0 ± 3.0	18.2 ± 3.4	22.6
H1α-H1 β	-12.8 ± 1.0	4.2 ± 3.0	17.0 ± 3.2	16.9
H12α-H12β	-11.8 ± 1.5	1.5 ± 3.0	13.3 ± 3.4	13.8
H4α-H4β	-14.6 ± 1.5	2.4 ± 3.0	17.0 ± 3.4	18.9

[(a)] The prochiral assignment for all Hα and Hβ protons was determined by fitting all possible permutations with the -bestFit option of PALES [2, 3] and selecting the one with the best fitting result (in term of highest n/χ^2 value).

[(b)] D_{CH}-couplings of methyl-groups have been converted to the corresponding D_{CC}-couplings[4]: (D_{CC}(C19-C10) = 1.9 ± 0.2 Hz; D_{CC}(C18-C13) = 1.9 ± 0.1 Hz)

[(c)] As couplings in the D-ring did not fit in the initial fittings (see main text) they were not used in further fittings.

[(d)] Couplings measured in the flexible side chain were not used for PALES fits.

Table S5. RDCs Measured on 5-α-Cholestan-3-one and RDCs Back Calculated for 5-α-Cholestan-3-one and 5-β-Cholestan-3-one a for the bestFit Method (SVD), the Fit with Fixed Orientation Given by the Alignment Tensor of 5-α-Cholestan-3-one (Cross-fitting) and Fit with Orientation Predicted by PALES [2, 3]. Additionally Alignment Tensor Parameters and Quality Factors for the Different Fits are Given: Axial and Rhombic Components (D_a, D_r) and Principal Axes of the Alignment Tensor (A_{xx}, A_{yy}, A_{zz}) with Their Corresponding Eigenvectors (EV), Number of RDCs used for Fitting (n) and Quality Factors χ^2, n/χ^2, Correlation Factor (R) and Quality Factor by Cornilescu *et al.* [1]. All Couplings are Given in Hz

Group	D (exp)	D (calc) (SVD-fit)		D (calc) (cross-fitting)		D (calc) (predicted C1-C27)	
	5αCholestan3one	5αCholestan3one	5βCholestan3one	5αCholestan3one	5βCholestan3one	5αCholestan3one	5βCholestan3one
C19-C10	1.9 ± 0.2	2.1	2.2	1.8	1.7	1.9	1.8
C18-C13	1.9 ± 0.1	2.0	1.7	1.9	1.9	2.0	1.9
C11-H11β	26.7 ± 3.0	24.4	22.8	21.5	21.7	23.0	22.0
C11-H11α	5.5 ± 3.0	3.9	-9.1	9.0	7.9	1.4	-0.7
C7-H7β	4.1 ± 5.0	2.5	-11.2	7.8	6.5	-0.2	-2.6
C7-H7α	25.9 ± 1.5	24.5	24.7	21.1	21.8	22.5	22.2
C8-H8	27.2 ± 1.0	25.2	25.5	22.4	22.2	23.9	22.6
C2-H2α	6.9 ± 3.2	6.4	-4.9	10.8	1.4[(a)]	4.7	2.8
C2-H2β	21.1 ± 3.2	20.5	26.6	15.7	10.4[(a)]	16.0	6.1
C1-H1β	7.3 ± 1.2	7.6	5.5	9.4	7.5[(a)]	13.0	11.5[(a)]
C1-H1α	20.7 ± 1.7	23.5	30.0	18.6	14.7[(a)]	20.0	14.5[(a)]
C12-H12α	27.4 ± 1.6	24.8	24.4	21.8	21.9	23.3	22.2
C12-H12β	4.3 ± 1.1	3.8	4.0	5.0	5.0	10.6	11.7
C4-H4α	6.7 ± 1.1	6.9	5.3[(a)]	8.6	5.1[(a)]	13.0	-10.4
C4-H4β	22.7 ± 1.7	23.1	11.0[(a)]	17.2	-15.4[(a)]	19.1	11.8
C5-H5	27.8 ± 3.6	24.3	-0.5	20.0	-23.1	21.5	-19.3
C9-H9	23.7 ± 1.1	24.7	25.3	21.5	21.5	23.0	22.0
H11α-H11β	13.2 ± 3.2	17.5	10.4	18.2	17.6	17.1	14.5
H7α-H7β	14.8 ± 3.2	17.4	9.5	18.1	16.9	16.8	13.3
H2α-H2 β	18.2 ± 3.4	22.6	26.8	20.9	17.9	21.5	19.6
H1α-H1 β	17.0 ± 3.2	16.9	16.7	16.9	13.7	17.9	12.4
H12α-H12β	13.3 ± 3.4	13.7	6.3	14.7	14.5	17.6	16.4
H4α-H4β	17.0 ± 3.4	18.8	7.7	18.2	-12.7	19.4	-2.6
	D_a	-3.97E-04	-4.29E-04	-3.94E-04		-4.32E-04	-4.04E-04
	D_r	-1.10E-04	-2.08E-04	-6.44E-05		-6.63E-05	-7.87E-05
	A_{xx}	2.32E-04	1.17E-04	2.98E-04		3.33E-04	2.86E-04
	A_{yy}	5.62E-04	7.41E-04	4.91E-04		5.32E-04	5.22E-04
	A_{zz}	-7.94E-04	-8.58E-04	-7.89E-04		-8.64E-04	-8.08E-04
	EV A_{xx}	-0.45; 0.87; -0.19	0.54; -0.84; -0.99	-0.48; 0.85; -0.22		-0.31; 0.94; -0.16	-0.36; 0.93; -0.08
	EV A_{yy}	-0.65; -0.17; 0.74	0.65; 0.49; -0.59	-0.58; -0.12; 0.80		-0.62; -0.07; 0.78	-0.62; -0.18; 0.77
	EV A_{zz}	0.62; 0.45; 0.64	0.54; 0.25; 0.80	0.65; 0.52; 0.55		0.72; 0.34; 0.61	0.70; 0.33; 0.64
	n	23	23	23	23	23	23
	χ^2	20.21	231.92	87.04	865.19	133.31	634.70
	n/χ^2	1.13	0.10	0.26	0.03	0.17	0.04
	R	0.974	0.741	0.929	0.203	0.902	0.429
	Q	0.114	0.514	0.212	0.849	0.216	0.700

[(a)] Hα and Hβ proton assignment was permutated compared to the assignment of 5-α-cholestan-3-one. (Of all possible permutations of the prochiral methylene groups only the fit with the best result is shown).

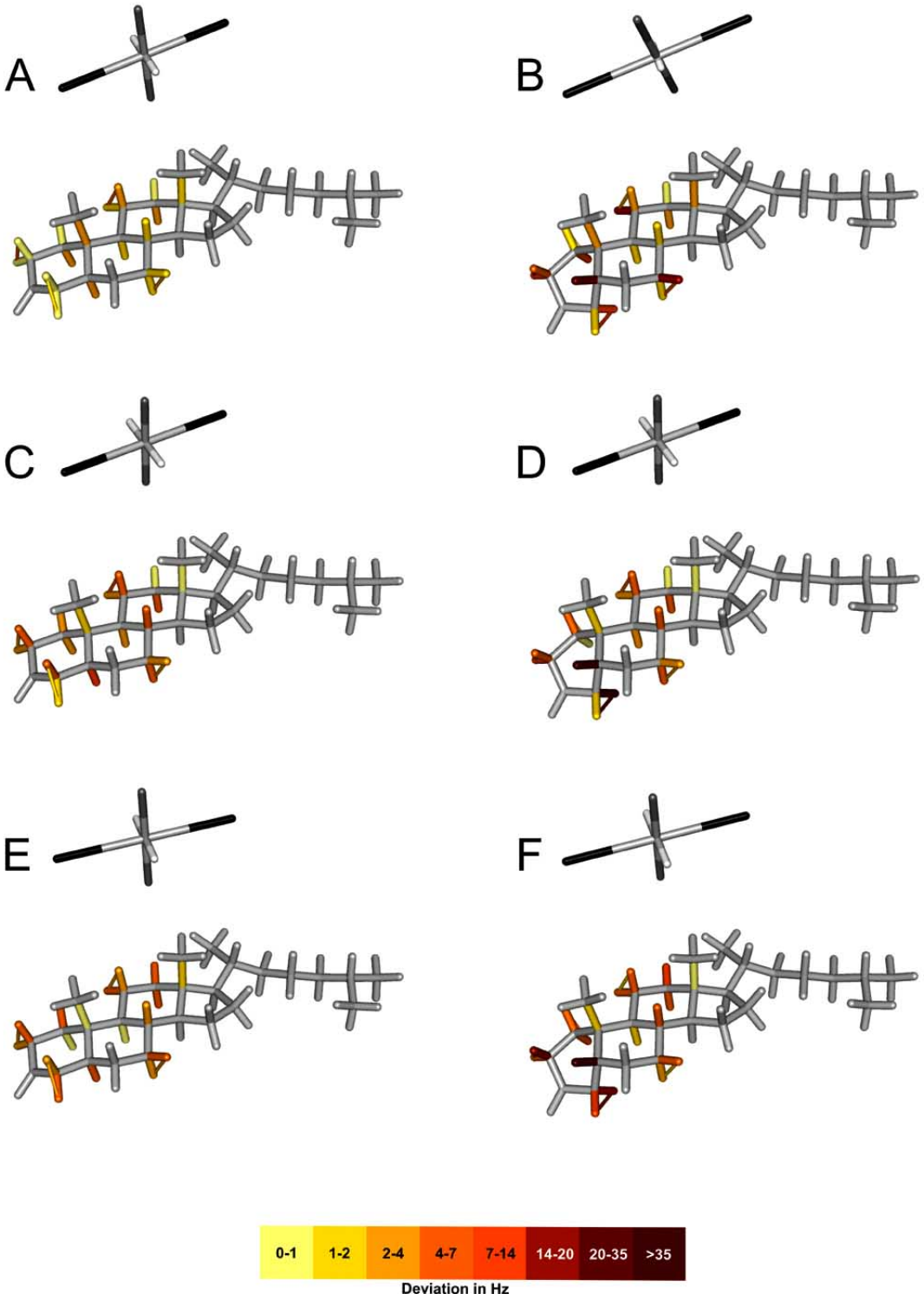

Fig. (S4). Comparison of RDCs measured on 5-α-cholestan-3-one and back-calculated for the structures of 5-α-cholestan-3-one (left: **A**, **C**, **E**) and 5-β-cholestan-3-one (right: **B**, **D**, **F**) using the -bestFit option PALES [2, 3] (top: **A**, **B**), the cross-fitting approach with the alignment tensor determined for cholesterol in PDMS/$CDCl_3$ (middle: **C**, **D**) and the prediction by PALES [2, 3] (bottom: **E**, **F**). The structures are shown with color-coded bonds denoting the deviation between measured and back-calculated RDCs for the different fits. The corresponding alignment tensors are visualized with their principal axis systems (black: A_{zz}; gray: A_{yy}; white: A_{xx}). For all three methods the 5-α-cholestan-3-one structure (left) gives clearly the better fit.

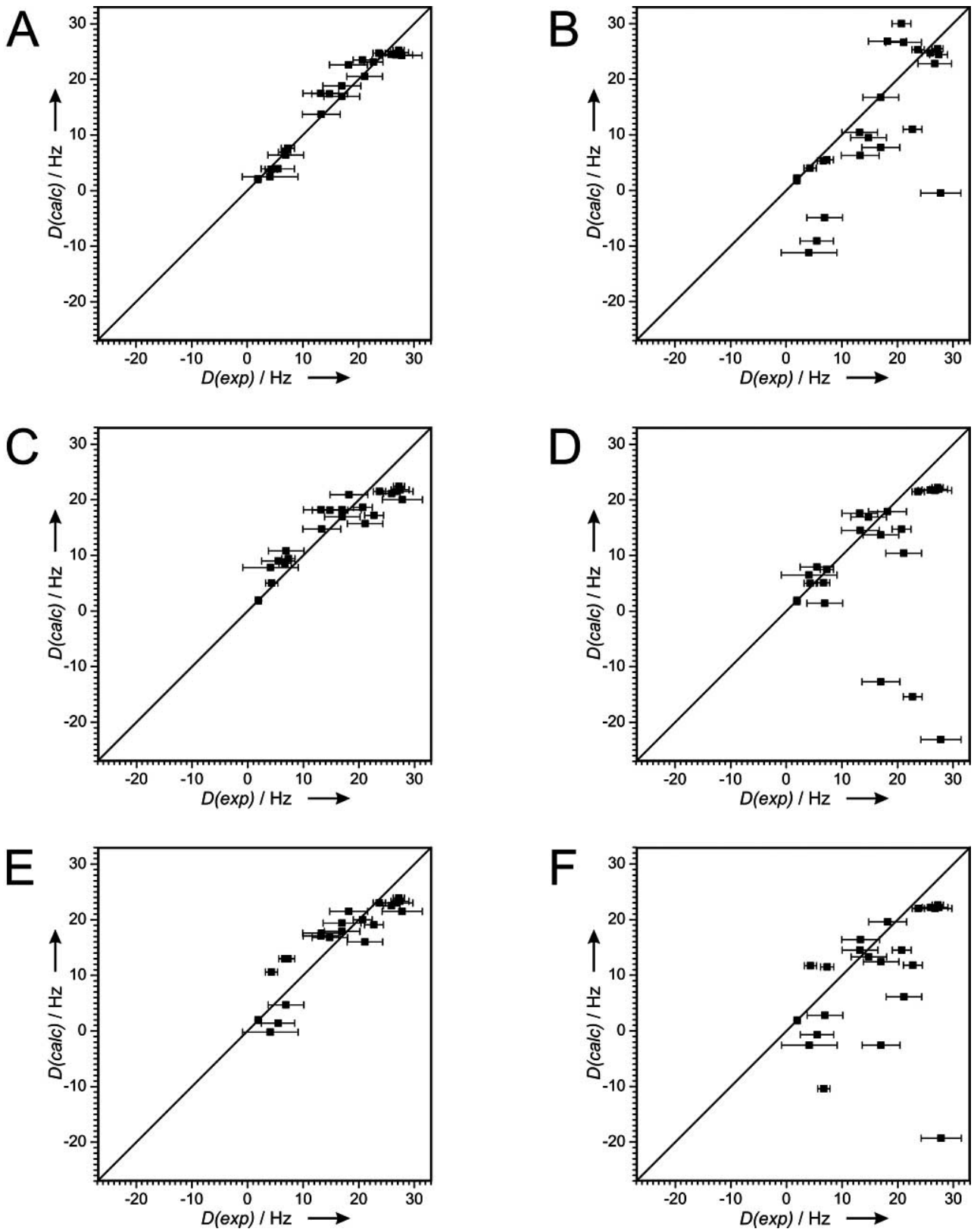

Fig. (S5). Comparison of RDCs measured on 5-α-cholestan-3-one and back-calculated for the structures of 5-α-cholestan-3-one (left: **A**, **C**, **E**) and 5-β-cholestan-3-one (right: **B**, **D**, **F**) using the -bestFit option of PALES [2, 3] (top: **A**, **B**), the cross-fitting approach (middle: **C**, **D**) and the prediction by PALES [2, 3] (bottom: **E**, **F**).The plots show the back-calculated RDCs, D(calc), as a function of the measured RDCs, D(exp). Clearly the correct diastereomer 5-α-cholestan-3-one (left) is favored in all three methods.

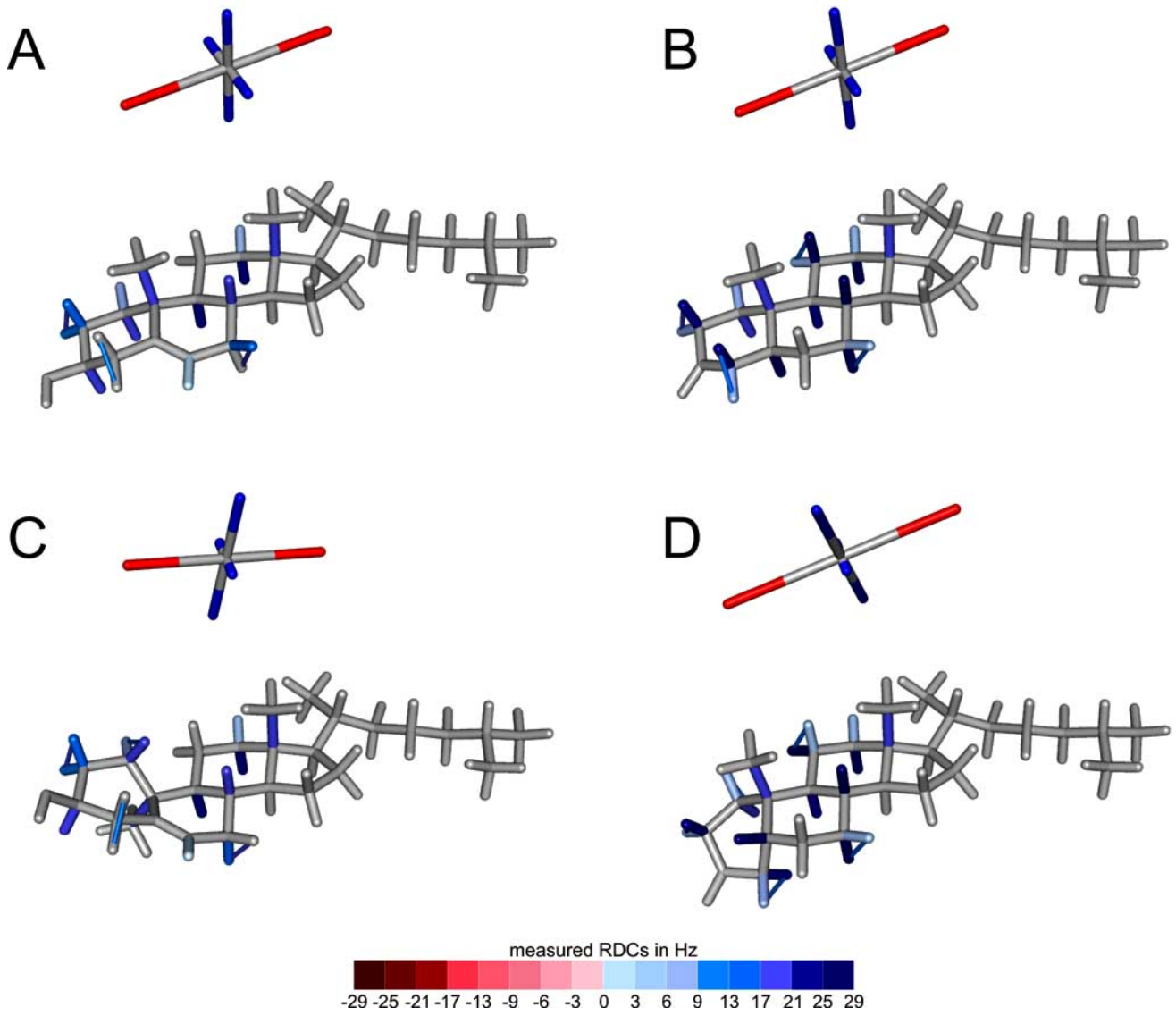

Fig. (S6). Visualization of the alignment tensor of different steroids as obtained with the SVD method. RDCs measured on cholesterol in a stretched PDMS/$CDCl_3$ gel have been fitted with the SVD method (-bestFit option in PALES) to the structural models of cholesterol (**A**) and its diastereomer 10-α-cholesterol (**C**) and RDCs measured on 5-α-cholestan-3-one in a stretched PDMS/$CDCl_3$ gel have been fitted with the SVD method (-bestFit option in PALES) to the structural models of 5-α-cholestan-3-one (**B**) and its diastereomer 5-β-cholestan-3-one (**D**). All structures are shown with color-coded bonds representing negative (red) and positive (blue) RDCs and the principle axes of the corresponding alignment tensors are drawn next to it.

Apparently the alignment tensors of cholesterol and 5-α-cholestan-3-one in a stretched PDMS/$CDCl_3$ gel are very similar but not fully identical. In contrast, the alignment tensors obtained by fitting experimental RDCs against the structure of a wrong diastereomer (**C**, **D**) differ significantly from those for the correct diastereomers (**A**, **B**).

Out of the 18 measured RDCs of cholesterol, various subsets of 15, 12, 9, 8, 7 and 6 RDCs were generated by random selection of RDC-combinations. As with a decreasing number of RDCs within a subset the influence of the actual composition of the subset increases, we created the more subsets the less RDCs are contained within the subsets.

Table S6. Composition of RDCs used in Each Subset with 18, 15, 12, 9 or 8 RDCs

Group	D [Hz]	Name of Subset																										
		18A	**15A**	**15B**	**15C**	**12A**	**12B**	**12C**	**12D**	**12E**	**9A**	**9B**	**9C**	**9D**	**9E**	**9F**	**9G**	**9H**	**8A**	**8B**	**8C**	**8D**	**8E**	**8F**	**8G**	**8H**	**8I**	**8J**
C18-C13	1.9 ± 0.2	x	/	x	x	/	x	x	/	x	x	/	x	/	x	/	x	/	x	/	/	x	/	/	/	x	/	x
C19-C10	1.8 ± 0.2	x	x	x	x	/	x	x	/	x	/	x	x	x	/	x	x	/	/	x	x	x	/	x	/	x	x	/
C2-H2α	11.3 ± 3.9	x	x	/	x	x	x	x	x	/	x	/	/	x	x	x	x	/	x	/	x	/	x	x	/	x	/	/
C2-H2β	16.1 ± 3.9	x	x	x	x	x	x	x	x	/	/	x	/	/	x	x	x	/	/	x	/	/	x	x	x	/	/	x
C8-H8	20.7 ± 8.5	x	x	x	/	/	x	x	/	x	x	/	x	/	/	/	x	/	/	/	x	x	/	/	x	/	x	/
C7-H7β	14.2 ± 5.8	x	/	x	x	x	x	x	x	/	/	x	x	x	/	/	x	/	x	/	/	/	x	/	x	/	/	/
C1-H1β	9.0 ± 1.4	x	x	x	x	x	x	x	/	x	x	/	/	x	x	/	x	/	/	x	/	/	/	/	x	/	x	/
C1-H1α	18.2 ± 1.4	x	x	x	x	x	/	/	x	x	/	x	/	/	x	x	x	/	x	/	x	x	/	/	/	x	/	x
C12-H12α	22.4 ± 1.4	x	/	x	x	x	/	/	x	x	x	/	x	/	/	x	x	/	/	x	/	x	x	x	/	x	/	/
C12-H12β	5.1 ± 1.4	x	x	x	x	/	x	/	x	x	/	x	x	x	/	x	/	x	/	x	x	/	x	x	x	/	x	x
C6-H6	1.6 ± 3.0	x	x	/	x	x	x	/	/	x	x	/	/	x	x	/	/	x	x	/	x	/	/	/	x	/	/	x
C9-H9	23.6 ± 4.0	x	x	x	/	x	/	x	x	x	/	x	/	/	x	/	/	x	/	x	/	x	/	/	x	/	x	/
C3-H3	19.3 ± 1.6	x	x	x	x	x	/	x	x	/	x	/	x	/	/	/	/	x	x	/	/	/	x	/	x	/	/	x
H2α-H2β	18.9 ± 2.2	x	x	x	x	x	x	/	x	/	/	x	x	x	/	x	/	x	/	x	/	/	/	x	/	/	x	/
H7α-H7β	17.7 ± 5.8	x	x	/	x	x	/	x	x	x	x	/	/	x	x	x	/	x	/	/	x	x	/	x	/	/	x	/
H1α-H1β	15.0 ± 2.2	x	x	x	x	x	/	x	/	x	/	x	/	/	x	x	/	x	x	/	/	x	/	x	/	x	/	x
H12α-H12β	14.2 ± 2.2	x	x	x	/	/	x	/	x	x	x	/	x	/	/	/	/	x	/	x	/	/	x	/	/	x	x	/
H4α-H4β	15.7 ± 2.8	x	x	x	x	/	x	x	x	/	/	x	/	x	/	/	/	x	x	/	x	/	x	/	/	x	/	x

x = RDC used in this subset, / = RDC not used in this subset.

Table S7. Composition of RDCs used in Each Subset with 7 or 6 RDCs

Group	D [Hz]			Name of Subset																								
				7A	**7B**	**7C**	**7D**	**7E**	**7F**	**7G**	**7H**	**7I**	**7J**	**7K**	**7L**	**6A**	**6B**	**6C**	**6D**	**6E**	**6F**	**6G**	**6H**	**6I**	**6J**	**6K**	**6L**	**6M**	**6N**	**6O**
C18-C13	1.9	±	0.2	x	/	x	/	/	x	/	/	/	/	x	/	x	/	/	x	/	/	x	/	/	/	x	/	/	x	/
C19-C10	1.8	±	0.2	/	x	/	x	x	x	/	/	x	/	/	x	/	x	/	x	/	/	x	/	/	x	/	/	x	/	/
C2-H2α	11.3	±	3.9	/	/	x	/	x	x	x	/	x	/	/	/	/	/	x	/	x	/	x	/	/	/	/	x	/	x	/
C2-H2β	16.1	±	3.9	x	x	/	/	/	/	x	/	/	x	/	x	x	/	/	/	x	/	x	/	/	/	x	/	/	/	x
C8-H8	20.7	±	8.5	x	/	/	x	/	/	x	/	x	x	/	x	/	x	/	/	/	x	x	/	/	x	/	/	x	/	/
C7-H7β	14.2	±	5.8	/	/	x	/	x	/	/	x	/	/	x	/	/	/	x	/	/	x	x	/	/	/	/	x	/	x	/
C1-H1β	9.0	±	1.4	/	x	/	/	x	/	/	x	/	/	/	/	x	/	/	/	/	x	/	x	/	x	x	/	/	/	/
C1-H1α	18.2	±	1.4	x	/	/	/	/	/	/	/	x	/	/	x	/	x	/	x	/	/	/	x	/	/	/	x	/	x	x
C12-H12α	22.4	±	1.4	/	/	x	/	/	x	/	x	x	/	x	x	/	/	x	x	/	/	/	x	/	/	x	/	x	/	/
C12-H12β	5.1	±	1.4	/	x	/	x	/	/	/	x	/	x	/	/	x	/	/	/	x	/	/	x	/	x	/	/	/	/	x
C6-H6	1.6	±	3.0	x	/	/	x	/	/	x	/	/	x	/	/	/	x	/	/	x	/	/	x	/	/	/	/	x	/	/
C9-H9	23.6	±	4.0	/	/	x	/	x	/	x	/	x	/	x	/	/	/	x	/	/	x	/	x	/	/	x	/	/	/	x
C3-H3	19.3	±	1.6	/	x	/	/	/	/	x	/	/	/	x	x	x	/	/	/	/	x	/	/	x	x	/	/	/	x	/
H2α-H2β	18.9	±	2.2	x	/	/	x	/	/	/	x	/	x	/	/	/	x	/	/	/	x	/	/	x	/	/	x	/	/	x
H7α-H7β	17.7	±	5.8	/	/	x	/	x	/	/	x	/	x	/	/	/	/	x	x	/	/	/	/	x	/	x	/	x	/	/
H1α-H1β	15.0	±	2.2	/	x	/	/	x	x	/	/	x	/	x	x	x	/	/	x	/	/	/	/	x	/	/	/	/	x	/
H12α-H12β	14.2	±	2.2	/	/	x	x	/	x	x	/	/	/	x	/	/	x	/	/	x	/	/	/	x	x	/	x	/	/	x
H4α-H4β	15.7	±	2.8	x	x	/	x	/	x	/	x	/	x	/	/	/	/	x	/	x	/	/	/	x	/	/	x	x	/	/

x = RDC used in this subset, / = RDC not used in this subset.

To investigate the influence of the flexible side chain on the orientation predicted by PALES [2, 3] we created several pdb-files of both steroids with decreasing length of the side-chain. The alkyl chain has been shortened stepwise and the resulting fragments are named after the containing carbon atoms (e.g. C1-C24 is the fragment with carbon atoms 1 to 24 and all adjacent oxygen and hydrogen atoms. Accordingly C1-C27 is the whole steroid molecule). With all steroid fragments prediction of alignment and back-calculation of the measured RDCs were performed with PALES (-stPales mode) assuming a rod-shaped alignment medium (-pf1 flag) and including all hydrogen atoms (-H flag) [2, 3]. The concentration of the alignment medium (-wv flag) [2, 3] which only scales the resulting RDCs linearly, was varied in steps of 0.001 to give the best result (best n/χ^2 value).

Table S8. RDCs Measured on Cholesterol and RDCs Back Calculated for Various Cholesterol Fragments as Result of the Orientation Predicted by PALES [2, 3]. Alignment Tensor Parameters and Quality Factors for the Different Fits are Given: Axial and Rhombic Components (D_a, D_r) and Principal Axes of the Alignment Tensor (A_{xx}, A_{yy}, A_{zz}) with Their Corresponding Eigenvectors (EV), Number of RDCs used for Fitting (n) and Quality Factors χ^2, n/χ^2, Correlation Factor (R) and Quality Factor by Cornilescu *et al.* [1] (Q). Additionally the Concentration (-wv) used for the Best Prediction is Given. All Couplings are Given in Hz

Group	D (exp)	D (calc) according to prediction by PALES for the fragment of cholesterol							
		C1-C27	C1-C26	C1-C25	C1-C24	C1-C23	C1-C22	C1-C21	C1-C20
C18-C13	1.9 ± 0.2	1.8	1.8	1.8	1.9	1.8	1.8	1.8	1.7
C19-C10	1.8 ± 0.2	1.7	1.7	1.8	1.8	1.8	1.8	1.8	1.8
C2-H2α	11.3 ± 3.9	4.9	5.7	4.5	10.5	7.1	10.9	8.6	6.2
C2-H2β	16.1 ± 3.9	15.0	14.5	16.7	15.8	18.4	17.8	19.2	18.8
C8-H8	20.7 ± 8.5	21.7	21.2	21.1	22.3	21.8	22.0	21.6	22.3
C7-H7β	14.2 ± 5.8	6.5	7.9	7.2	12.8	9.4	12.7	10.0	9.4
C1-H1β	9.0 ± 1.4	11.2	12.8	11.5	10.4	7.3	5.4	2.2	8.0
C1-H1α	18.2 ± 1.4	18.2	17.7	19.1	18.7	20.3	19.9	20.7	20.7
C12-H12α	22.4 ± 1.4	21.3	20.8	20.8	21.7	21.5	21.7	21.3	21.4
C12-H12β	5.1 ± 1.4	8.7	9.8	7.9	5.7	2.8	0.2	-2.7	3.1
C6-H6	1.6 ± 3.0	7.1	8.2	6.1	3.4	0.4	-2.7	-5.3	1.8
C9-H9	23.6 ± 4.0	21.2	20.7	20.8	21.6	21.5	21.6	21.2	21.3
C3-H3	19.3 ± 1.6	16.7	16.2	17.9	17.3	19.4	18.9	19.9	19.7
H2α-H2β	18.9 ± 2.2	19.6	19.5	18.4	21.0	19.0	20.1	18.9	19.4
H7α-H7β	17.7 ± 5.8	18.4	18.6	17.3	20.4	17.9	19.2	17.6	19.0
H1α-H1β	15.0 ± 2.2	15.3	15.9	16.5	16.1	15.8	15.2	13.5	14.1
H12α-H12β	14.2 ± 2.2	15.5	15.6	14.9	14.5	13.1	12.4	10.4	10.9
H4α-H4β	15.7 ± 2.8	15.9	16.4	17.3	16.9	17.0	16.5	15.1	15.4
	D_a	-3.72E-04	-3.89E-04	-3.81E-04	-4.10E-04	-3.73E-04	-3.84E-04	-3.48E-04	-3.88E-04
	D_r	-7.02E-05	-5.27E-05	-5.41E-05	-5.74E-05	-7.02E-05	-6.62E-05	-8.58E-05	-7.29E-05
	A_{xx}	2.67E-04	3.10E-04	2.99E-04	3.24E-04	2.68E-04	2.85E-04	2.19E-04	2.78E-04
	A_{yy}	4.77E-04	4.68E-04	4.62E-04	4.96E-04	4.78E-04	4.83E-04	4.76E-04	4.97E-04
	A_{zz}	-7.44E-04	-7.79E-04	-7.61E-04	-8.20E-04	-7.46E-04	-7.68E-04	-6.95E-04	-7.76E-04
	EV A_{xx}	-0.34; 0.92; -0.17	-0.39; 0.91; -0.14	-0.34; 0.90; -0.28	-0.52; 0.84; -0.15	0.40; -0.84; -0.37	0.50; -0.80; 0.34	0.47; -0.78; 0.43	-0.52; 0.83; -0.18
	EV A_{yy}	-0.61; -0.08; 0.79	-0.59; -0.12; 0.80	-0.67; -0.02; 0.74	-0.53; -0.19; 0.82	-0.69; -0.01; 0.73	-0.63; -0.06; 0.78	0.70; 0.03; -0.71	-0.62; -0.23; 0.75
	EV A_{zz}	0.71; 0.38; 0.59	0.71; 0.39; 0.59	0.66; 0.43; 0.61	0.67; 0.51; 0.55	0.61; 0.54; 0.58	0.60; 0.60; 0.53	0.54; 0.63; 0.55	0.58; 0.50; 0.64
	n	18	18	18	18	18	18	18	18
	χ^2	21.17	33.57	18.26	5.48	10.35	24.45	69.94	12.57
	n/χ^2	0.85	0.54	0.99	3.29	1.74	0.74	0.26	1.43
	R	0.900	0.890	0.911	0.981	0.970	0.976	0.952	0.959
	Q	0.198	0.205	0.187	0.09	0.128	0.135	0.228	0.145
	-wv	0.049	0.050	0.057	0.064	0.068	0.073	0.074	0.088

Table S9. RDCs Measured on 5-α-Cholestan-3-one and RDCs Back Calculated for Various 5-α-Cholestan-3-one Fragments as Result of the Orientation Predicted by PALES [2, 3]. Alignment Tensor Parameters and Quality Factors for the Different Fits are Given: Axial and Rhombic Components (D_a, D_r) and Principal Axes of the Alignment Tensor (A_{xx}, A_{yy}, A_{zz}) with Their Corresponding Eigenvectors (EV), Number of RDCs used for Fitting (n) and Quality Factors χ^2, n/χ^2, Correlation Factor (R) and Quality Factor by Cornilescu *et al.* [1] (Q). Additionally the Concentration (-wv) used for the Best Prediction is Given. All Couplings are Given in Hz

Group	D (exp)	D (calc) according to prediction by PALES for the fragment of 5-α-cholestan-3-one							
		C1-C27	C1-C26	C1-C25	C1-C24	C1-C23	C1-C22	C1-C21	C1-C20
C19-C10	1.9 ± 0.2	1.9	1.8	2.0	1.9	2.1	2.1	2.2	2.2
C18-C13	1.9 ± 0.1	2.0	2.0	2.0	2.1	2.0	2.1	2.0	1.9
C11-H11β	26.7 ± 3.0	23.0	22.3	23.6	23.5	24.8	24.9	24.9	24.3
C11-H11α	5.5 ± 3.0	1.4	2.7	2.0	8.4	4.6	8.7	6.2	3.0
C7-H7β	4.1 ± 5.0	-0.2	1.2	0.6	7.0	3.4	7.6	5.2	1.6
C7-H7α	25.9 ± 1.5	22.5	21.8	23.2	23.0	24.6	24.6	24.9	24.9
C8-H8	27.2 ± 1.0	23.9	23.1	23.7	24.5	24.8	25.3	24.8	24.8
C2-H2α	6.9 ± 3.2	4.7	5.5	4.1	10.6	6.4	10.5	7.8	4.7
C2-H2β	21.1 ± 3.2	16.0	15.4	18.5	16.9	21.1	20.4	22.6	22.3
C1-H1β	7.3 ± 1.2	13.0	14.5	13.5	12.0	8.7	6.7	3.2	8.9
C1-H1α	20.7 ± 1.7	20.0	19.2	21.3	20.4	23.2	22.6	24.1	24.8
C12-H12α	27.4 ± 1.6	23.3	22.5	23.4	23.8	24.6	24.8	24.6	24.6
C12-H12β	4.3 ± 1.1	10.6	11.7	10.0	7.6	4.1	1.3	-2.1	4.7
C4-H4α	6.7 ± 1.1	13.0	14.0	12.9	11.1	8.2	6.1	2.8	7.4
C4-H4β	22.7 ± 1.7	19.1	18.3	20.3	19.1	21.9	21.1	22.8	24.7
C5-H5	27.8 ± 3.6	21.5	20.7	22.4	21.9	24.0	23.7	24.6	25.1
C9-H9	23.7 ± 1.1	23.0	22.2	23.3	23.5	24.6	24.7	24.8	24.7
H11α-H11β	13.2 ± 3.2	17.1	16.9	15.4	19.6	16.5	18.9	16.7	15.7
H7α-H7β	14.8 ± 3.2	16.8	16.7	15.3	19.4	16.3	18.6	16.4	15.9
H2α-H2 β	18.2 ± 3.4	21.5	21.0	20.4	22.8	21.2	22.4	21.1	21.9
H1α-H1 β	17.0 ± 3.2	17.9	18.1	19.5	18.8	19.3	18.8	17.6	17.2
H12α-H12β	13.3 ± 3.4	17.6	17.6	17.5	16.7	15.6	15.0	12.8	12.8
H4α-H4β	17.0 ± 3.4	19.4	19.3	20.7	20.1	20.9	20.6	19.7	18.9
	D_a	-4.04E-04	-3.89E-04	-4.18E-04	-4.18E-04	-4.39E-04	-4.04E-04	-4.12E-04	-3.79E-04
	D_r	-7.87E-05	-5.27E-05	-5.84E-05	-6.78E-05	-6.61E-05	-9.59E-05	-9.29E-05	-1.16E-04
	A_{xx}	2.86E-04	3.10E-04	3.30E-04	3.16E-04	3.40E-04	2.60E-04	2.73E-04	2.05E-04
	A_{yy}	5.22E-04	4.68E-04	5.06E-04	5.19E-04	5.38E-04	5.47E-04	5.51E-04	5.53E-04
	A_{zz}	-8.08E-04	-7.79E-04	-8.36E-04	-8.35E-04	-8.77E-04	-8.07E-04	-8.24E-04	-7.58E-04
	EV A_{xx}	-0.31; 0.94; -0.16	-0.33; 0.93; -0.15	-0.28; 0.92; -0.28	-0.46; 0.87; -0.19	0.35; -0.87; -0.36	0.44; -0.82; 0.37	0.41; -0.80; 0.44	-0.47; 0.87; -0.17
	EV A_{yy}	-0.62; -0.07; 0.78	-0.62; -0.09; 0.78	-0.69; 0.01; 0.72	-0.58; -0.14; 0.80	0.71; 0.00; -0.70	0.67; 0.03; -0.74	0.74; 0.01; -0.68	-0.67; -0.23; 0.71
	EV A_{zz}	0.72; 0.34; 0.61	0.71; 0.36; 0.60	0.66; 0.40; 0.64	0.67; 0.48; 0.57	0.61; 0.50; 0.62	0.60; 0.57; 0.56	0.54; 0.60; 0.59	0.58; 0.45; 0.69
	n	23	23	23	23	23	23	23	23
	χ^2	133.31	183.64	121.17	81.52	24.91	35.16	78.96	26.57
	n/χ^2	0.17	0.13	0.19	0.28	0.92	0.65	0.29	0.87
	R	0.902	0.882	0.920	0.931	0.974	0.956	0.962	0.974
	Q	0.216	0.238	0.195	0.201	0.114	0.152	0.143	0.115
	-wv	0.056	0.057	0.067	0.075	0.081	0.088	0.093	0.109

Table S10. RDCs Measured on Sodium Cholate and RDCs Back Calculated for Sodium Cholate with the SVD Method, the Fit with Fixed Orientation Given by the Alignment Tensor of 5-α-Cholestan-3-one (Cross-fitting) and Fit with Orientation Predicted by PALES [2, 3]. Additionally Alignment Tensor Parameters and Quality Factors for the Different Fits are Given: Axial and Rhombic Components (D_a, D_r) and Principal Axes of the Alignment Tensor (A_{xx}, A_{yy}, A_{zz}) with their Corresponding Eigenvectors (EV), Number of RDCs used for Fitting (n) and Quality Factors χ^2, n/χ^2, Correlation Factor (R) and Quality Factor by Cornilescu *et al.* [1] (Q). All Couplings are Given in Hz

Group	$^1D_{CH}$ (exp) [(a)]	$^1D_{CH}$ (calc) (SVD-fit)	$^1D_{CH}$ (calc) (cross-fitting)	$^1D_{CH}$ (calc) (predicted)
C3-H3	1.5 ± 1.0	1.9	-22.7	0.0553
C5-H5	0.9 ± 1.0	0.5	-16.8	0.0530
C7-H7	2.5 ± 1.0	2.7	1.3	-0.0024
C8-H8	-7.8 ± 1.0	-7.2	25.3	-0.0570
C9-H9	-7.5 ± 1.0	-7.0	24.8	-0.0560
C12-H12	1.6 ± 1.0	1.4	3.7	-0.0307
C14-H14	-6.6 ± 1.0	-6.9	24.2	-0.0546
C17-H17	-6.0 ± 1.0	-6.8	23.8	-0.0533
	D_a	-8.047E-05	-3.97E-04	1.16E-06
	D_r	-3.11E-05	-1.10E-04	8.66E-08
	A_{xx}	3.38E-05	2.32E-04	-1.03E-06
	A_{yy}	1.27E-04	5.62E-04	-1.29E-06
	A_{zz}	-1.61E-04	-7.94E-04	2.31E-06
	EV A_{xx}	-0.08; 0.98; 0.16	-0.45; 0.87; -0.19	-0.54; 0.84; 0.04
	EV A_{yy}	0.74; -0.05; 0.68	-0.65; -0.17; 0.74	-0.53; -0.38; 0.76
	EV A_{zz}	-0.67; -0.17; 0.72	0.62; 0.45; 0.64	0.65; 0.39; 0.65
	n	8	8	8
	χ^2	4.35	4881.82	205.30
	n/χ^2	5.42	0.002	0.04
	R	0.994	-0.867	0.782
	Q	0.094	4.839	0.992

(a) Experimental data from Mangoni *et al*. [5]. As no experimental errors are given in by Mangoni *et al.* [5] they were set to 1Hz for the fitting with PALES.

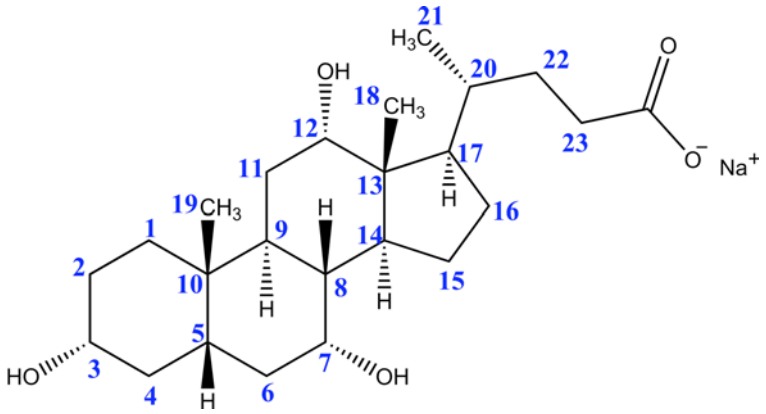

Fig. (S7). Structure and nomenclature of sodium cholate.

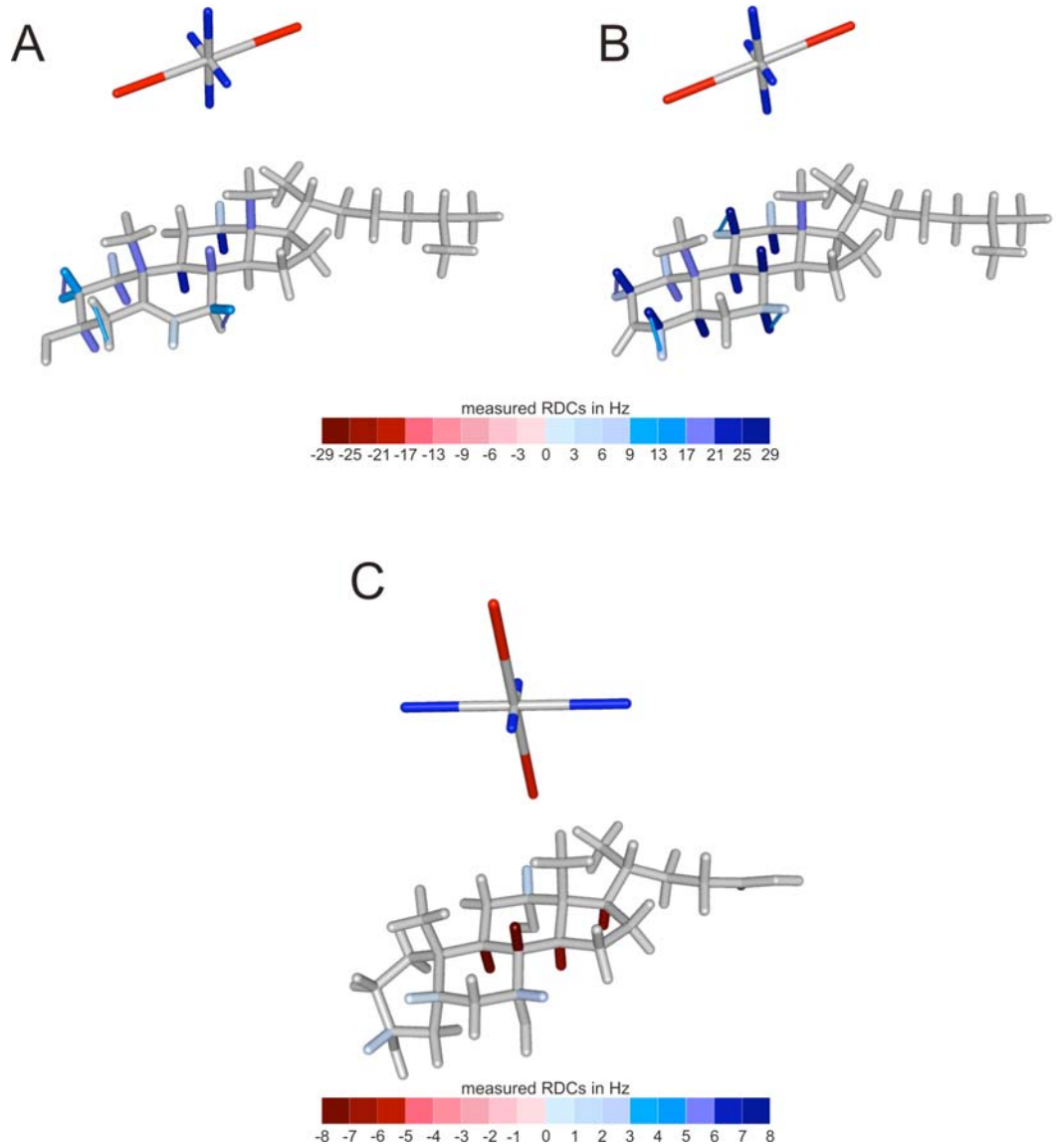

Fig. (S8). Comparison of alignment tensors for steroids in different alignment media. RDCs have been measured for cholesterol (**A**) and 5-α-cholestan-3-one (**B**) in stretched PDMS/$CDCl_3$ gels and for sodium cholate (**C**) in a compressed PAA/D_2O gel. The structures are shown with color-coded bonds representing negative (red) and positive (blue) RDCs and the axes of the corresponding alignment tensors (as obtained with the -bestFit option of PALES [2, 3]) next to it.

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