Supplementary Information

Spectroscopy on the Wing: Investigating possible differences in protein secondary structures in bird feather rachis layers using Raman spectroscopy

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Introduction

This document presents data from the Raman spectra (3200 - 100cm^{-1}) of feather rachis samples. Interpretation of the spectra gives information on the protein secondary structure in the layers and the changes between them.

Table S0.1 shows band assignments for the Amide I (1500-1700 cm^{-1}), Amide III (1200-1350 cm^{-1}) and S-S (650-400 cm^{-1}) regions.

Section 1 presents an example of a fitted Raman spectrum, and Section 2 shows examples from a parameter sweep used to decide the operational parameters to be used in obtaining experimental spectra. Section 3 presents band intensity ratios from the Amide I and Amide III regions for the outer layers of feather rachises of four birds at the Dorsal, Ventral, Leading and Trailing positions.

From these graphs and spectra presented in the main text it is clear that the errors are smaller and the fit is better in the Amide I region than in the Amide III region. Larger R^2 values were obtained for the fits in the Amide I region when compared to the Amide III and S-S regions. Typical values were 0.997, 0.987 and 0.982, respectively.

Section 4 provides band intensity ratios for a single ratio from the S-S region (510:525) and is presented as a stand alone section for convenience. Section 5 presents a preliminary IR spectrum. Section 6 presents a summary of the statistical analysis.

The fitting method used has been described in the main text. Parameters were decided after recording spectra with a range of different operational parameters. Some example conditions are shown in Figure S2.2.

Table S0.1: Band assignments used in this work, for Amide I, Amide III and S-S regions. The S-S vibrations indicate disulfide bond conformations, where G stands for Gauche and T for Trans.
*This notation refers to the CC-S-S-CC bond conformations in keratin, see the main text and the work by *Akhtar & Edwards (1997) and ES-Sendoubi et al. (2019)* which appears in the bibliography of the main text. See also Section 4 and Figure S4.0

Amide I		Amide III		S-S*	
Band (cm^{-1})	Assignment	...		...	
1553	Ring Mode (RM)	1214	Ring Mode (RM)	510	G-G-G
1585	Ring Mode (RM)	1240	β-sheet	525	G-G-T
1613	RM, Side chain	1262	Random Coil	540	T-G-T
1640	α-helix	1283	β-turn	560	S-S stretch
1668	β-sheet	1313	α-helix		and Cystine residue
1692	β-turn	1341	α-helix		

Section 1. Example of a fitted Raman Spectrum

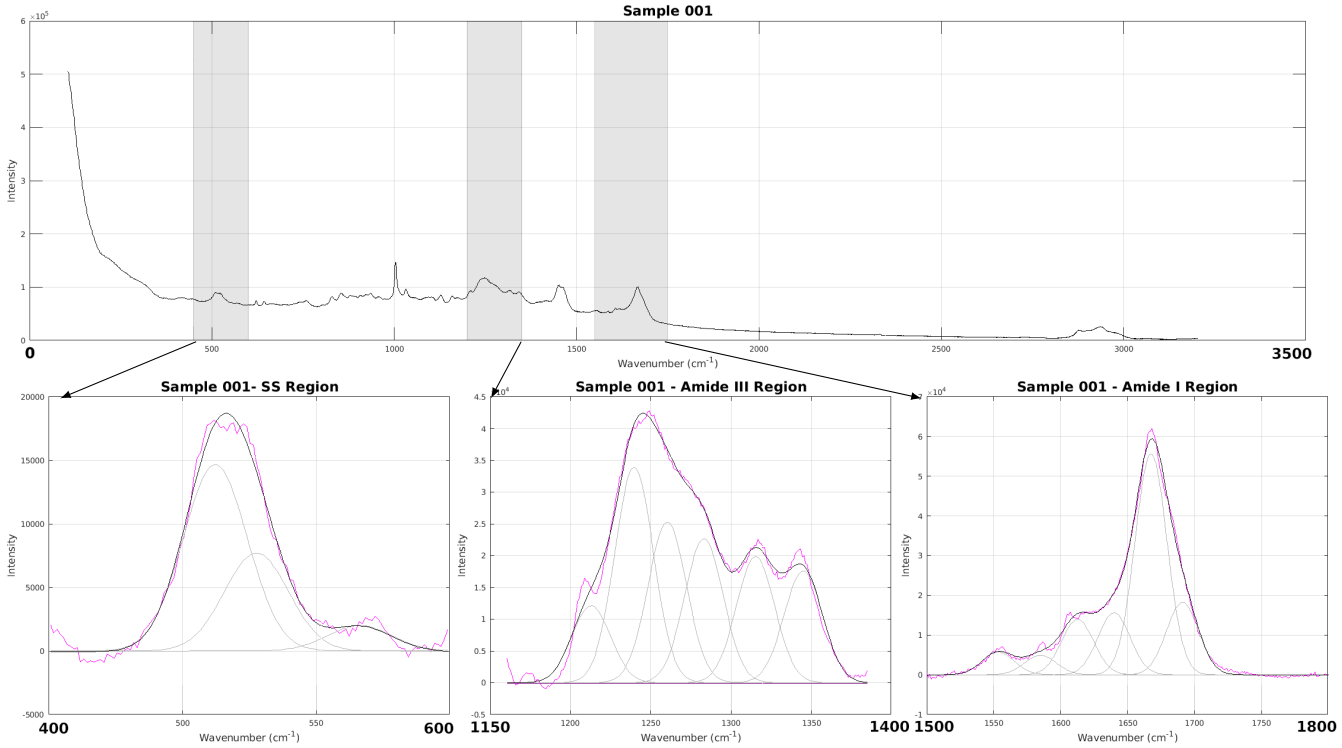


Figure S1.1: Example spectrum from the Outer layer of the Dorsal position, of a feather (P4) from left wing of a Swan. The Amide I, Amide III and S-S regions have been fitted with bands according to Table S0.1

Section 2. Examples from a parameter sweep to obtain operational conditions

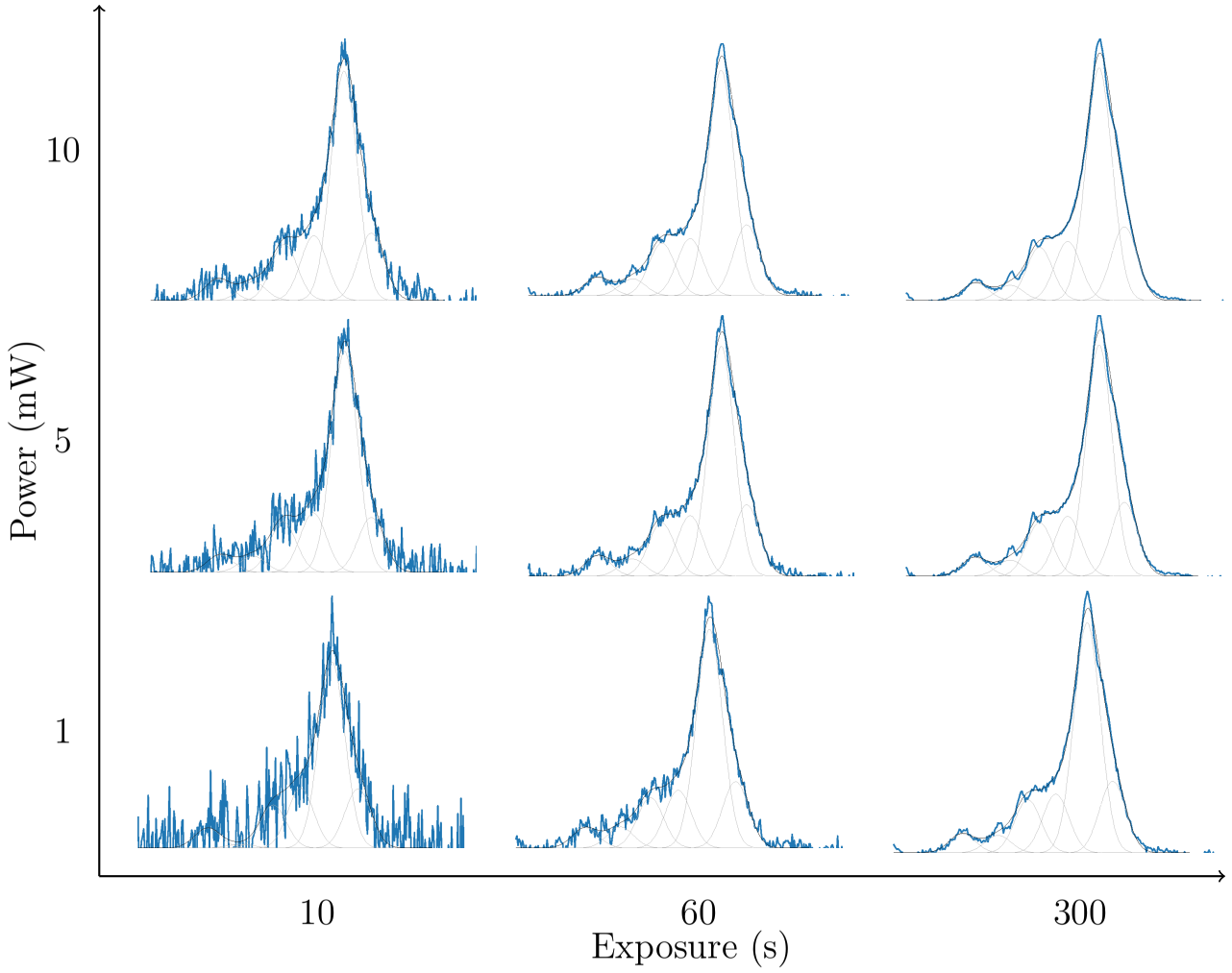


Figure S2.2: Continued on next page

Figure S2.2: A figure to show how increasing power and exposure can be used to obtain spectra with improved signal-to-noise. In these spectra
the Y axis is scaled intensity and the X axis shows wavenumbers from 1500 to 1800cm^{-1}. The blue lines are the recorded data, the black line is the sum of the six-band GauS-Sian fit, and each of those six GauS-Sians are shown with a gray line. In this work, the settings usually used to obtain spectra were 60 seconds exposure, for 8 accumulations (8 minutes total exposure) at 10mW. These settings balanced the time needed to acquire data with the risk of sample burning and returned spectra with good signal-to-noise that were normally fitted with $R^2 > 0.99$. In this figure, the R^2 values are as follows:

0.97789	0.99613	0.99701
0.95524	0.99485	0.99657
0.8676	0.98984	0.99618

The rows and columns in this table are aligned with those in the figure.

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Section Three

Band intensity ratios for the Outer Layers of feather rachises from 4 birds at the Dorsal, Ventral, Trailing and Leading positions.

All feathers are the **Right-hand**, 4th primary feather, cut at 40% length of the shaft from the base of the calamus.

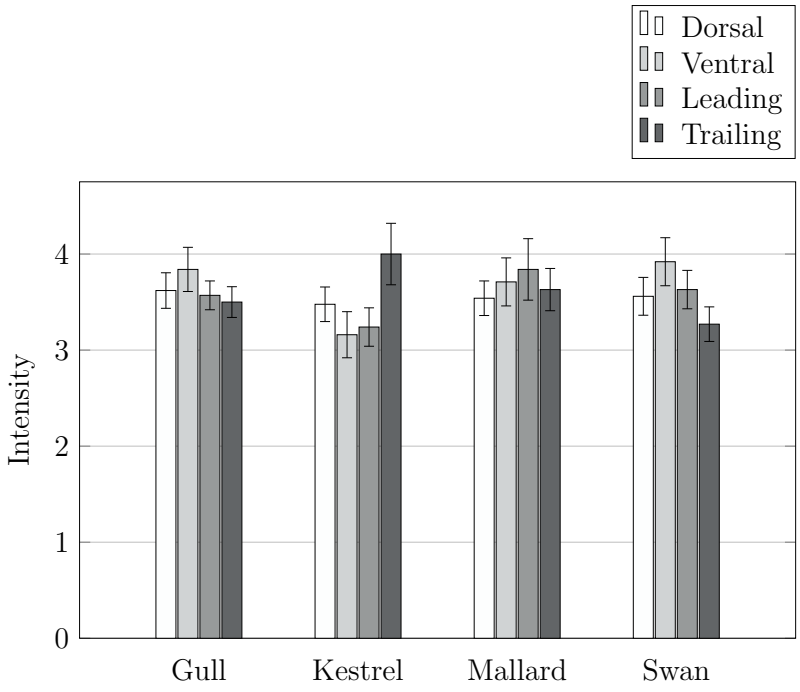


Figure 3.1: band intensity ratio 1668cm^{-1}:1640cm^{-1}.

Amide I—1668:1640;
β-sheet:α-helix

Notes

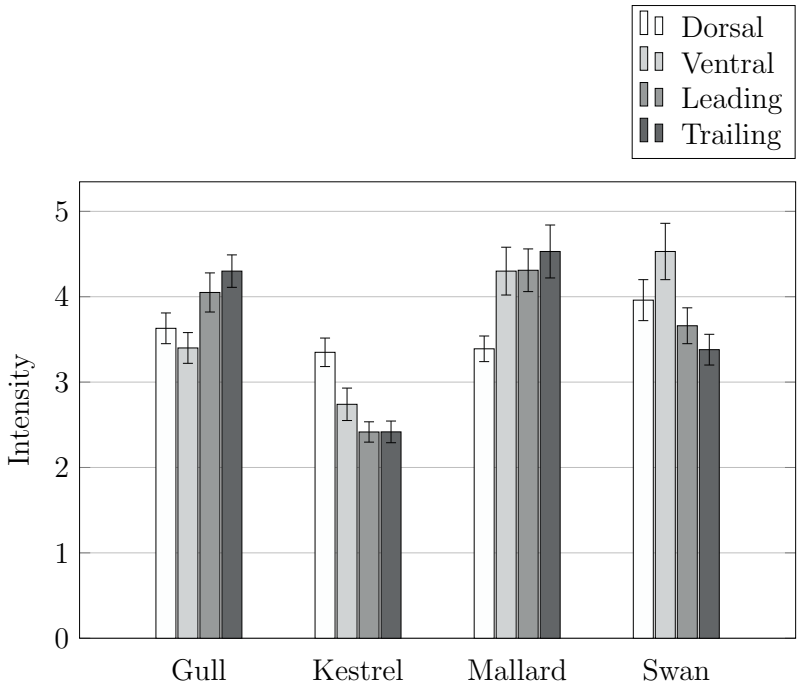


Figure 3.2: band intensity ratio 1668cm^{-1}:1613cm^{-1}.

Amide I—1668:1613;
β-sheet: Side chain

Notes

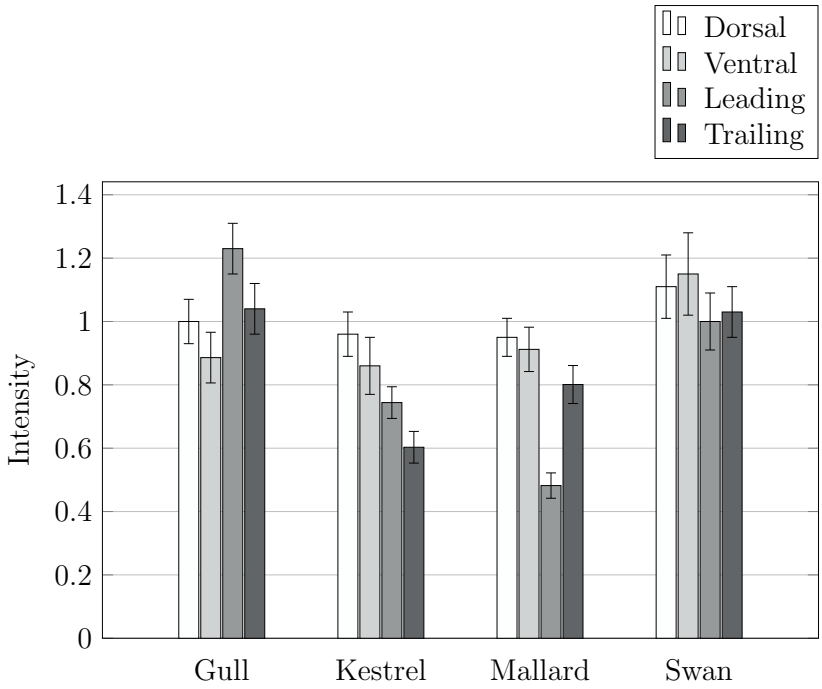


Figure 3.3: band intensity ratio 1640cm^{-1}:1613cm^{-1}.

Amide I—1640:1613;
α-helix: Side chain

Notes

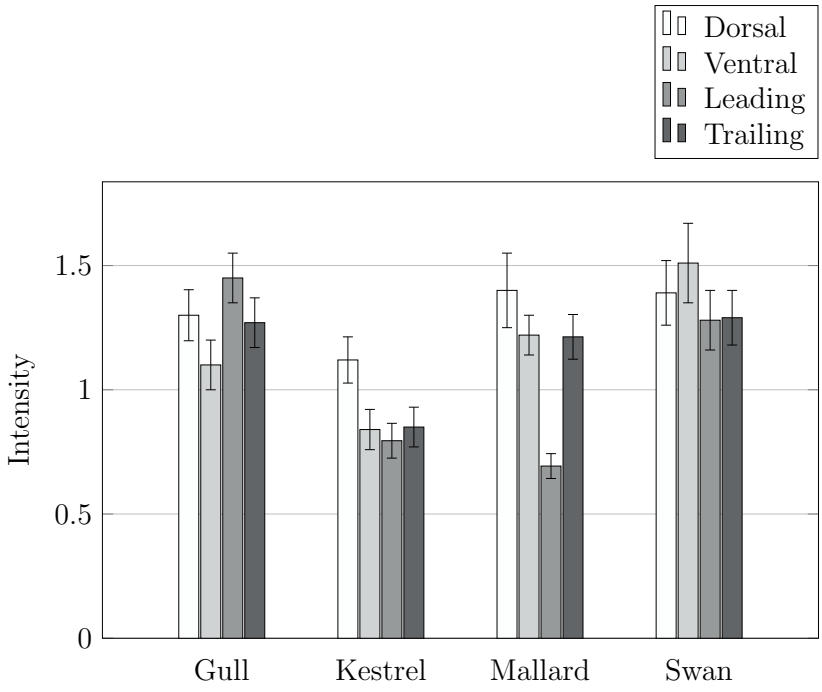


Figure 3.4: band intensity ratio 1692cm^{-1}:1613cm^{-1}.

Amide I—1692:1613;
β-turn: Side chain

Notes

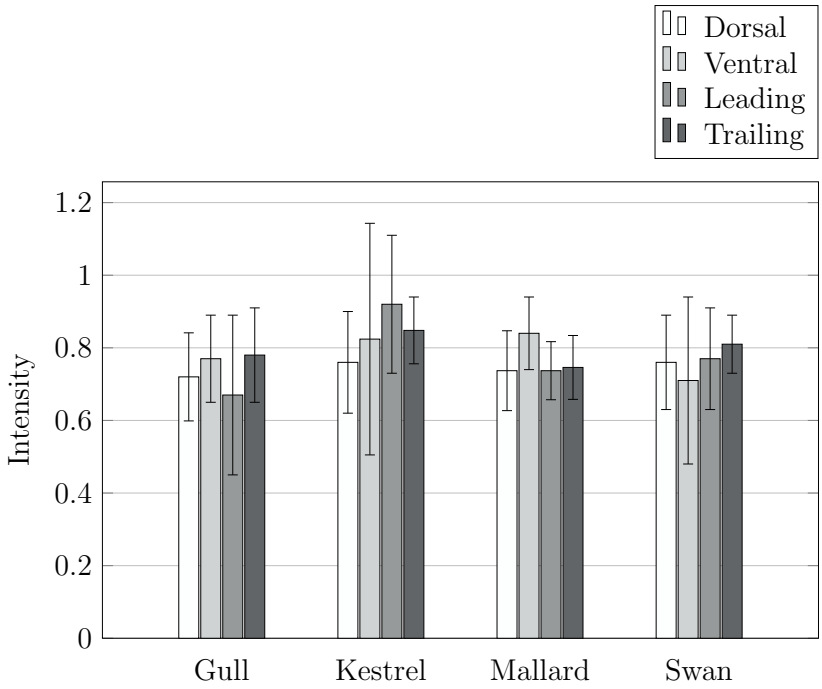


Figure 3.5: band intensity ratio 1262cm^{-1}:1240cm^{-1}.

Amide III—1262:1240;
Random coil: β-sheet

Notes

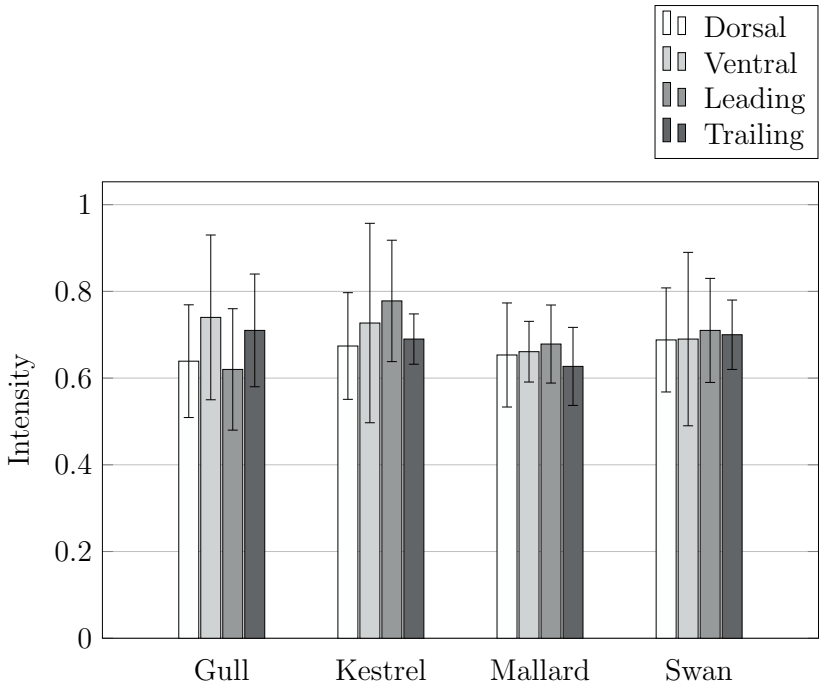


Figure 3.6: band intensity ratio 1283cm^{-1}:1240cm^{-1}

Amide III—1283:1240;
β-turn: β-sheet

Notes

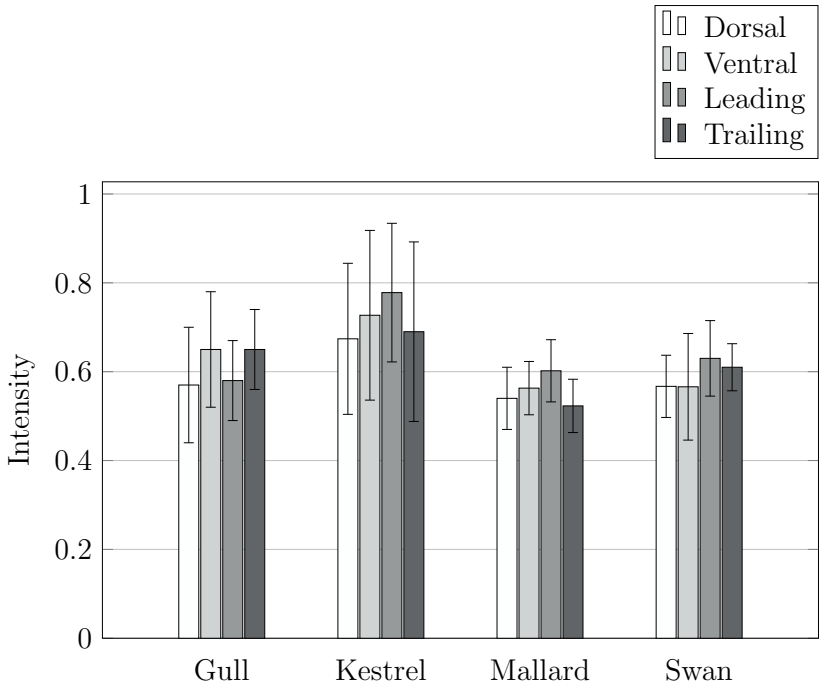


Figure 3.7: band intensity ratio 1313cm^{-1}:1240cm^{-1}.

Amide III—1313: 1240;
∝-helix: β-sheet

Notes

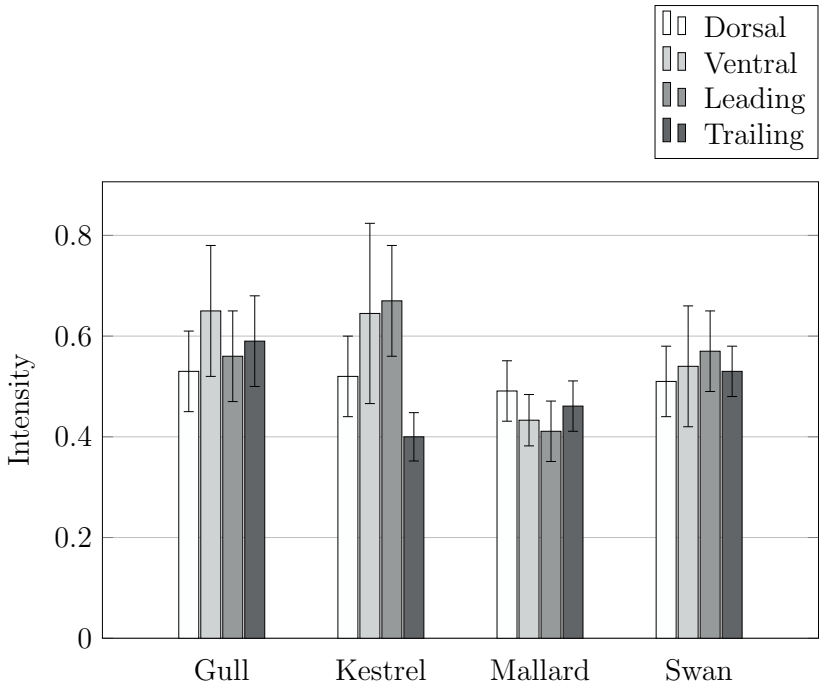


Figure 3.8: band intensity ratio 1341cm^{-1}:1240cm^{-1}.

Amide III—1341:1240;
α-helix: β-sheet

Notes

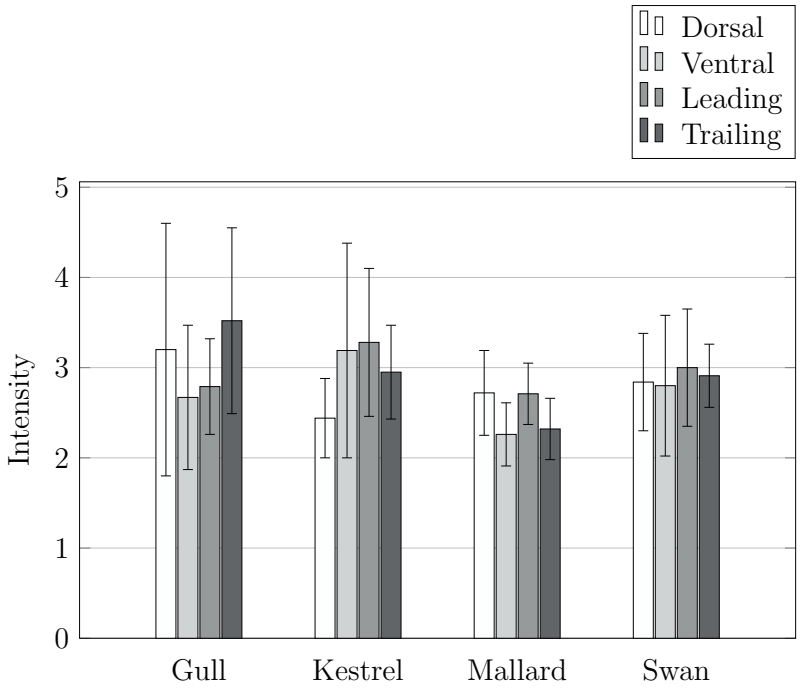


Figure 3.9: Band intensity ratio 1240cm^{-1}:1214cm^{-1}.

Amide III—1240:1214;
β-sheet: Ring mode

Notes

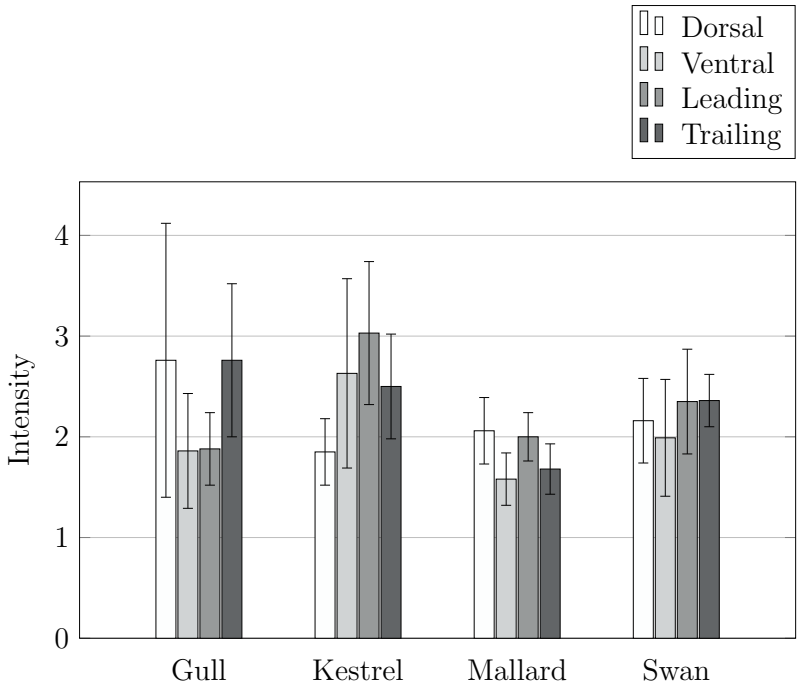


Figure 3.10: Band intensity ratio 1262cm^{-1}:1214cm^{-1}.

Amide III—1262:1214;
Random coil: Ring mode

Notes

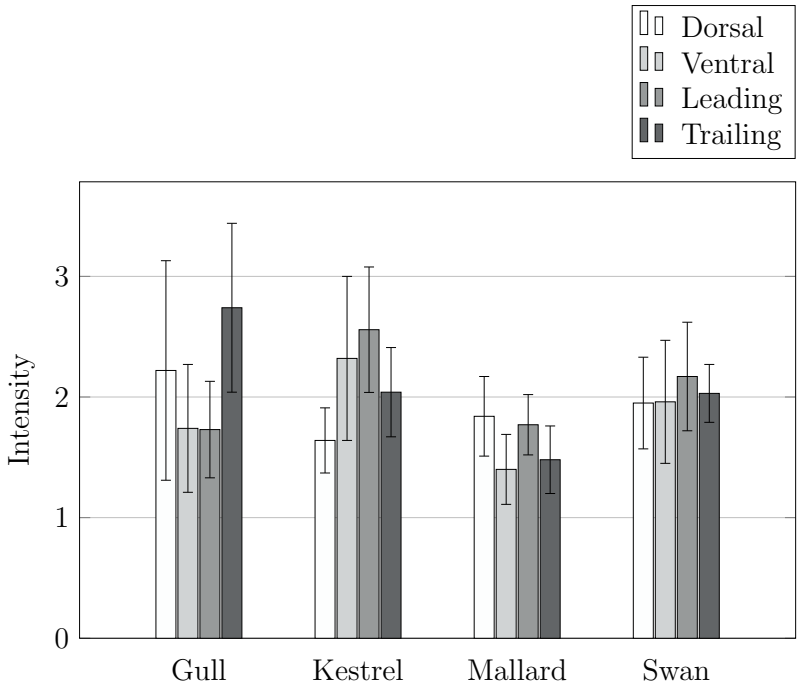


Figure 3.11: Band intensity ratio 1283cm^{-1}:1214cm^{-1}.

Amide III—1283:1214;
β-turn: Ring mode

Notes

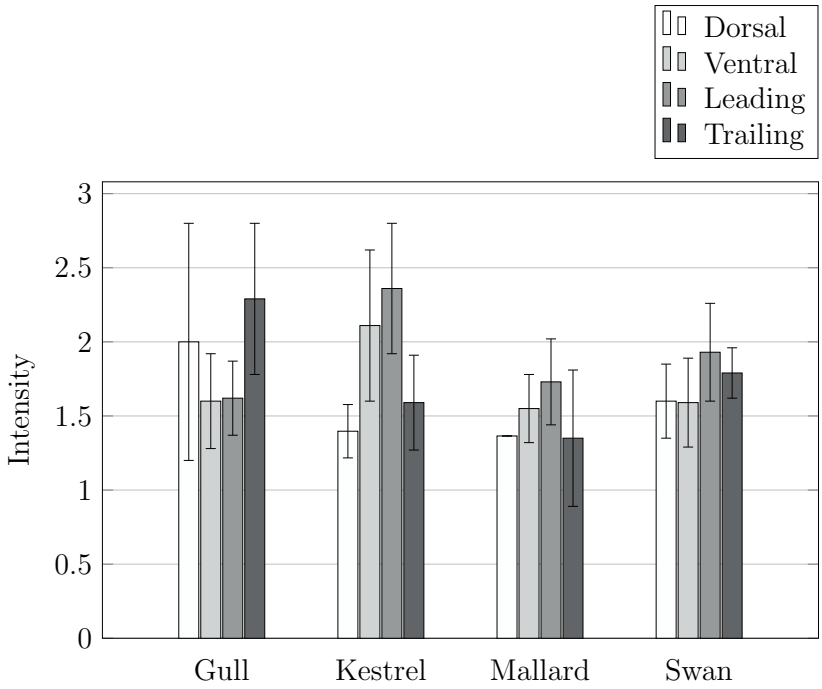


Figure 3.12: Band intensity ratio 1313cm^{-1}:1214cm^{-1}.

Amide III—1313:1214;
∝-helix: Ring mode

Notes

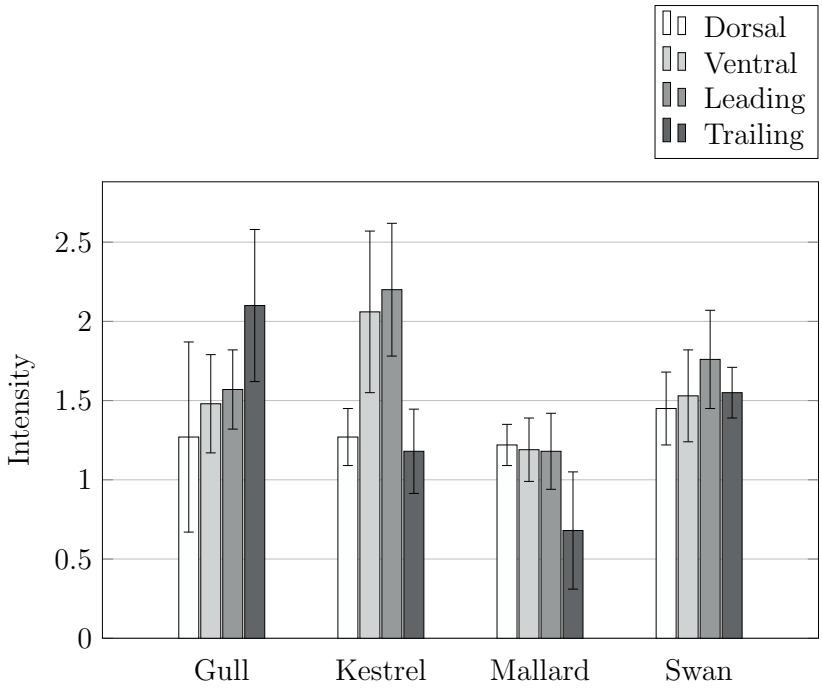


Figure 3.13: Band intensity ratio 1341cm^{-1}:1214cm^{-1}.

Amide III—1341:1214;
∝-helixRing mode

Notes

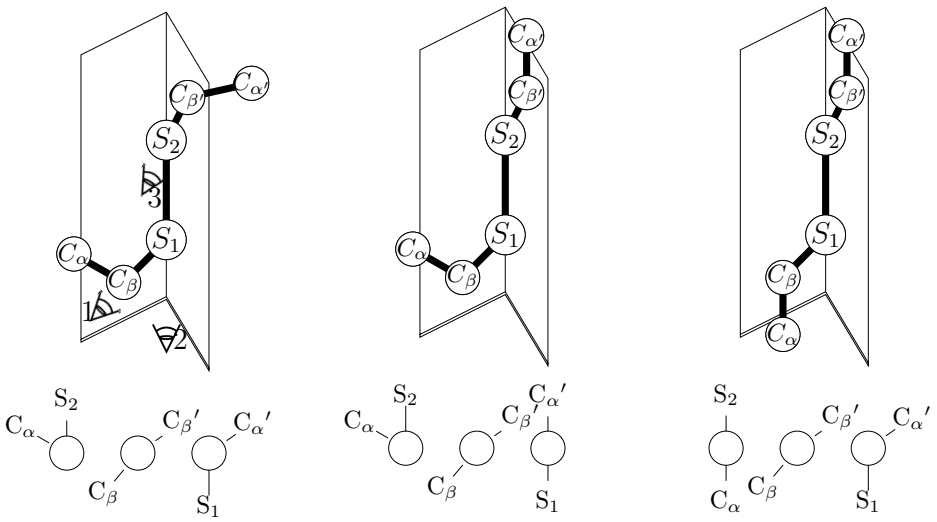
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Section 4

Band intensity ratios for the S-S region (400–600 cm^{-1})

The band intensity ratio $\bar{\omega} = 510\ cm^{-1}$: $\bar{\omega} = 525\ cm^{-1}$, in the S-S region is presented for 4 birds at the dorsal position of the fourth primary feather, which had been cut at 40% from the end of the calamus and measured at the inner and outer layers. Values for feathers from the left (L) and right (R) wings of the same bird are shown, and the order is Inner L, then Inner R; Outer L, then Outer R. This is indicated for one set in each graph by the characters 'L' and 'R'. Also, for the fitted spectra, the band intensity ratios have large errors which make it difficult to discern clear trends on going from the Inner layer to the Outer layer.

The ratio of the G-G-G conformation to the total disulphide bonds is considered a criterion for the stability of disulphide bonds in keratin *(Choe et al. 2017; Essendoubi et al. 2019)*, see Figure S4.0. This may indicate that the intensity ratio 510:525 cm^{-1} will decrease on going from the Inner layer to the Outer layer. The 510 cm^{-1} band is assigned to the G-G-G conformer and the 525 cm^{-1} band is assigned to the G-G-T conformer. See also Table S5.1.



(a) G-G-G **(b)** G-G-T **(c)** T-G-T

Figure S4.0: Reproduced from *Akhtar & Edwards (1997)*: Diagrammatic representation of CC-S-S-CC bond conformations of the disulphide bond in keratins (a) *gauche-gauche-gauche*, (b) *gauche-gauche-trans* and (c) *trans-gauche-trans*. Three bond angles are shown beneath each subfigure, which correspond to the viewpoints 1, 2 and 3, illustrated in (a).

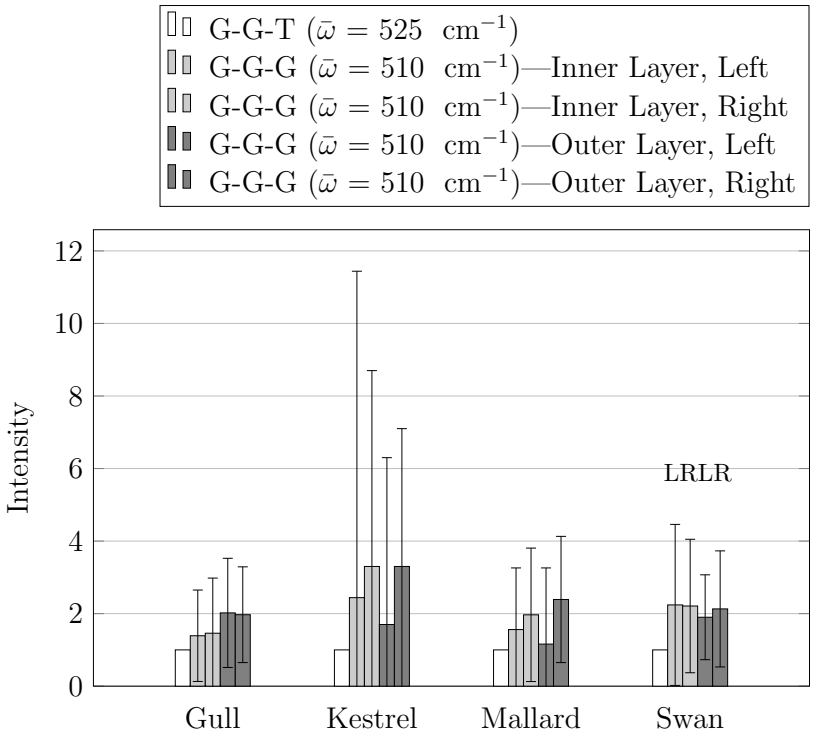


Figure S4.1: Band intensity ratios in feathers from the Gull, Kestrel, Mallard and Swan. The contribution from the Gauche-Gauche-Trans conformation is set to one.

SS—$\frac{510}{525}$;$\frac{G-G-G}{G-G-T}$ where G is Gauche and T is trans.

Notes

- Expectation: A decrease on going from Inner to Outer
- Expected trend not observed, although errors are large.

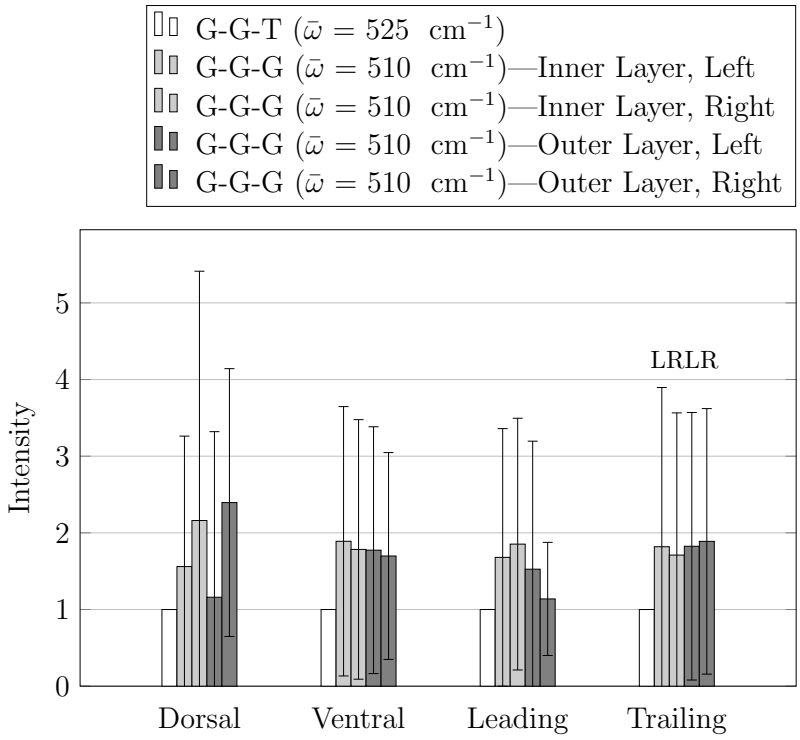


Figure S4.2M: Mallard band intensity ratios. The contribution from the Gauche-Gauche-Trans conformation is set to one.

SS—$\frac{510}{525}$;$\frac{G-G-G}{G-G-T}$

Notes

- Expectation: a decrease on going from Inner to Outer
- Expected trend not clearly observed, although errors are large.

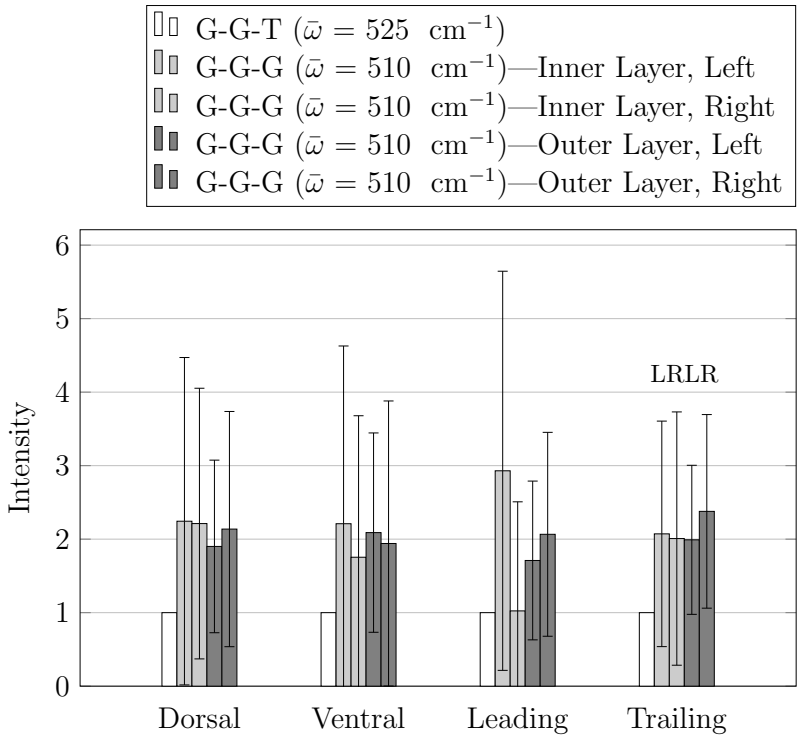


Figure S4.3Sw: Swan band intensity ratios. The contribution from the Gauche-Gauche-Trans conformation is set to one.

SS—$\frac{510}{525}$;$\frac{G-G-G}{G-G-T}$

Notes

- Expectation: a decrease on going from Inner to Outer
- Expected trend not clearly observed, although errors are large.

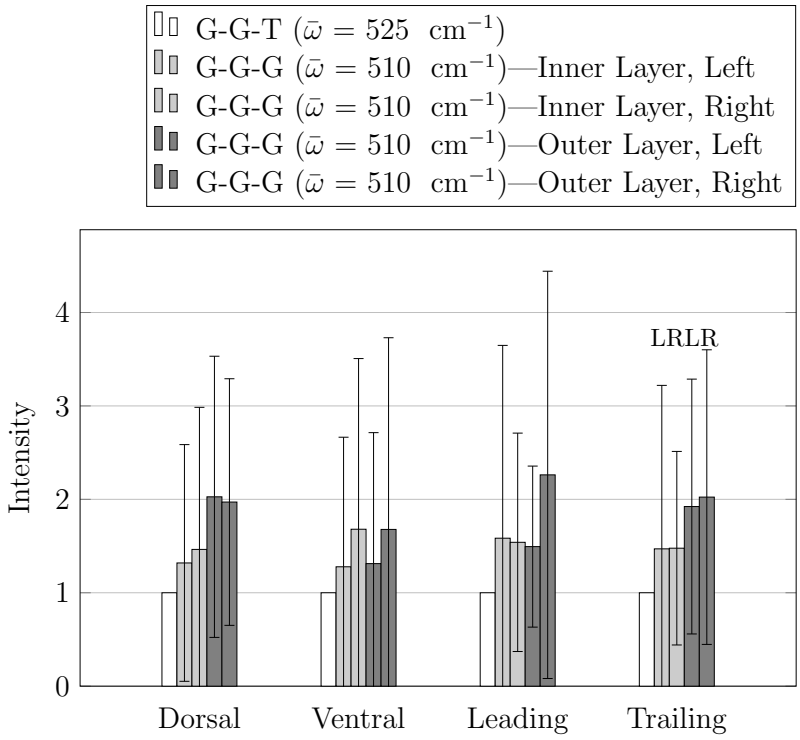


Figure S4.4SG: Gull band intensity ratios. The contribution from the Gauche-Gauche-Trans conformation is set to one.

SS—$\frac{510}{525}$;$\frac{G-G-G}{G-G-T}$

Notes

- Expectation: a decrease on going from Inner to Outer
- Expected trend not clearly observed, although errors are large.

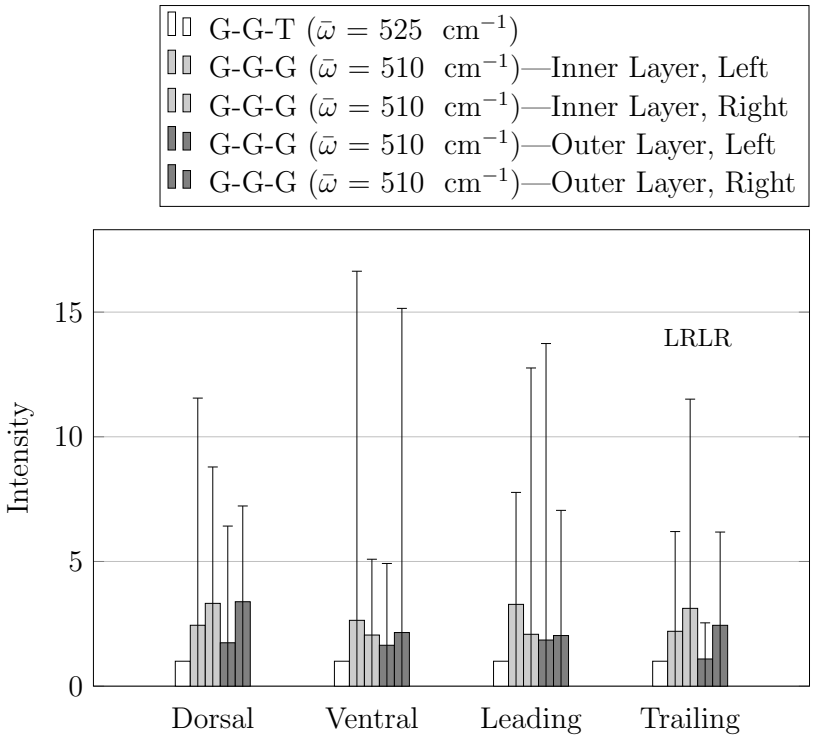


Figure S4.5K: Kestrel band intensity ratios. The contribution from the Gauche-Gauche-Trans conformation is set to one.

SS—$\frac{510}{525}$;$\frac{G-G-G}{G-G-T}$

Notes

- Expectation: a decrease on going from Inner to Outer
- Expected trend observed, although errors are large.

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Section Five

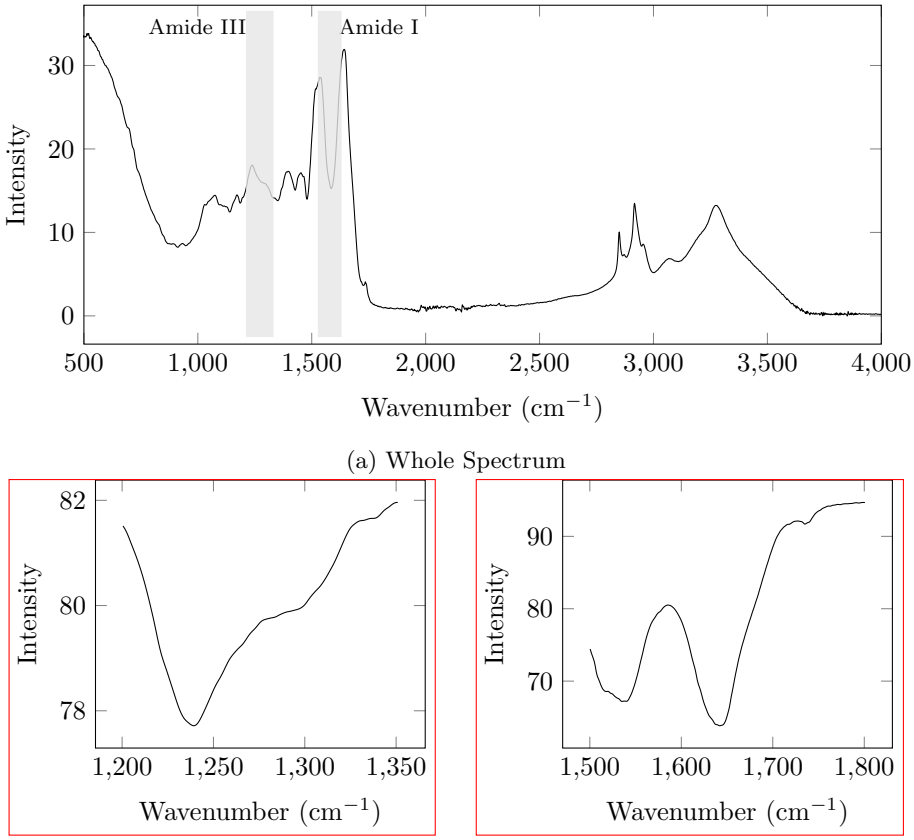
A preliminary IR spectrum

For the sake of completeness, an IR spectrum of a feather rachis sample for a Swan is included here and fitted in the same way as the samples in the sections above and the main text. The infra-red spot-size on the sample was ≈ 1 mm and no spatial sampling of the type carried out in the Raman work was possible. As this was only a preliminary spectrum, the sample was investigated 'as received' and spectra were taken from the side of the rachis *i.e* with the infrared beam incident on the side of the rachis not 'end-on' as in the Raman work.

The spectrum was taken using a Thermo ScientificNicole iS5 FTIR Microplate Spectrometer, equipped with iD7 ATR optics and a DTGS detector. The spectrum (see Figure S5.1) was recorded with 16 scans in the range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}.

Bands were fitted using the same model used in previous sections (See Figure S5.2), though a seven-band fit was used in the Amide I region The fitted band positions are presented in Table S5.1.

5 IR spectra

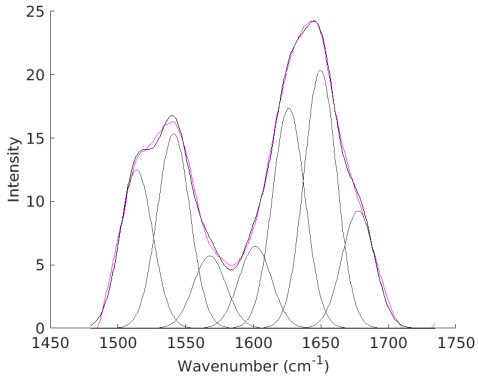


(a) Whole Spectrum

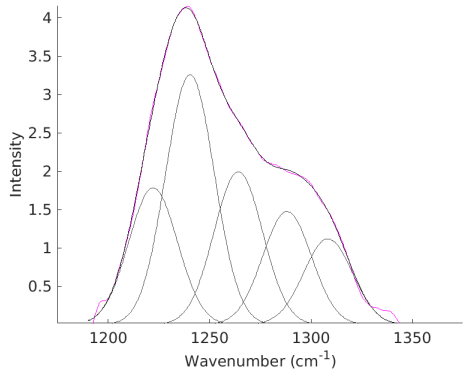
(b) Amide III

(c) Amide I

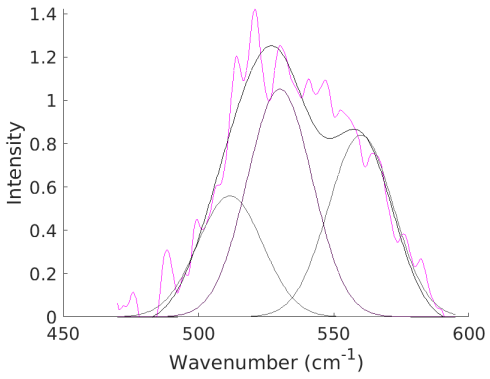
Figure S5.1: An IR absorption spectrum of a Swan rachis sample shows less detail in the Amide I region than a Raman spectrum. The Amide III region is better resolved than the Amide I region and shows similar features to the spectra obtained using Raman spectroscopy. Bands were fitted using the same model used in previous sections.



(a) Amide I



(b) Amide III



(c) S-S

Figure S5.2: An example of the fits used for a preliminary IR spectrum obtained of an 'as received' rachis sample of a Swan . The same method used to fit a Raman spectrum was used to fit an IR spectrum.

Band	Amide 1			Amide 3			SS		
	Raman	IR	Assignment	Raman	IR	Assignment	Raman	IR	Assignment*
1	-	1515	-	1214	1217	Ring Mode	510	518	G-G-G
2	1553	1542	Ring mode	1240	1237	β-sheet	525	535	G-G-T
3	1585	1570	Ring mode	1262	1261	Random coil	560	558	S-S stretch and
4	1613	1604	Side chain, Ring mode	1283	1284	β-turn			cystine residue absorption
5	1640	1627	α-helix	1313	1306	α-helix			
6	1668	1650	β-sheet	1343	1336	α-helix			
7	1692	1678	β-turn						

Table S5.1: Band positions from Raman and IR data. For (G=Cauche, T=Trans).
Gaussian bands were used in the fit with a half-width of 28.2 cm^{-1}.
* These vibrational assignments refer to S-S vibrations of the CC-S-S-CC bond conformations between two cysteine residues (the dimer is formed on oxidation and is called cystine) in keratin, see *Akhtar & Edwards(1997)*.

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Section 6— Statistical results

List of Tables

S6.1 Tukey tests compare four band intensity ratios for the inner and outer layers in feather from all four birds. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 3

S6.2 Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the swan. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 4

S6.3 Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the kestrel. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 4

S6.4 Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the gull. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 5

S6.5 Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the mallard. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 5

S6.6 ANOVA results between band intensity ratios 1668:1613cm^{-1}(Ratio A) taken from the inner layer only of cross section taken from swan rachises at different lengthwise (%) positions. 6

S6.7 Post-Hoc Tukey tests between band intensity ratios 1668:1613cm^{-1}(Ratio A) taken at different lengthwise positions on rachis samples. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 6

S6.8 ANOVA results between band intensity ratios 16681640cm^{-1}(Ratio B) taken from the inner layer of different lengthwise rachis sections. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 7

S6.9 Post-Hoc Tukey tests between band intensity ratios 1668:1640cm^{-1}(Ratio B) taken at different lengthwise positions on rachis samples. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 7

S6.10 ANOVA results between band intensity ratios 1640:1613cm^{-1}(Ratio C) taken from the inner layer of different lengthwise rachis sections. 8

S6.11 Post-Hoc Tukey tests between band intensity ratios 1640:1613cm^{-1}(Ratio C) taken at different lengthwise positions on rachis samples. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 8

S6.12 ANOVA results between band intensity ratios 1692:1613cm^{-1}(Ratio D) taken from the inner layer of different lengthwise rachis sections. 9

S6.13 Post-Hoc Tukey tests between band intensity ratios 1692:1613cm^{-1}(Ratio D) taken at different lengthwise positions on rachis samples. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference. 9

Table S6.1: Tukey tests compare four band intensity ratios for the inner and outer layers in feather from all four birds. *n.b.* $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	Ratio	mean(In)	s.d.	mean(Out)	s.d.	diff	se	tail	T	p-Tukey	hedges	Result
A	1668:1613	4.241	0.649436	3.450	0.696084	0.791	0.168	two-sided	4.702	0.001000	1.161	**
B	1668:1640	3.923	0.175181	3.653	0.269490	0.269	0.057	two-sided	4.721	0.001000	1.166	**
C	1640:1613	1.081	0.159542	0.944	0.174344	0.136	0.042	two-sided	3.257	0.001126	0.804	*
D	1692:1613	1.330	0.210333	1.157	0.240685	0.173	0.056	two-sided	3.072	0.002129	0.759	*

Table S6.2: Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the swan.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	Ratio	mean(In)	s.d.	mean(Out)	s.d.	diff	se	tail	T	p-Tukey	hedges	Result
A	1668:1613	4.704	0.405509	3.866	0.374382	0.838	0.195	two-sided	4.297	0.001000	2.031	**
B	1668:1640	3.854	0.069941	3.600	0.196256	0.255	0.074	two-sided	3.433	0.001000	1.623	**
C	1640:1613	1.221	0.109430	1.072	0.056322	0.148	0.045	two-sided	3.321	0.001000	1.570	**
D	1692:1613	1.472	0.117282	1.330	0.114307	0.142	0.057	two-sided	2.483	0.013013	1.174	*

Table S6.3: Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the kestrel.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	Ratio	mean(In)	s.d.	mean(Out)	s.d.	diff	se	tail	T	p-Tukey	hedges	Result
A	1668:1613	3.730	0.978664	2.971	0.679487	0.759	0.421	two-sided	1.801	0.071690	0.851	*
B	1668:1640	3.976	0.283493	3.587	0.463798	0.389	0.192	two-sided	2.022	0.043138	0.956	*
C	1640:1613	0.932	0.213804	0.829	0.153922	0.103	0.094	two-sided	1.098	0.272172	0.519	-
D	1692:1613	1.181	0.274473	0.985	0.244577	0.196	0.130	two-sided	1.501	0.133387	0.710	-

Table S6.4: Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the gull.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	Ratio	mean(In)	s.d.	mean(Out)	s.d.	diff	se	tail	T	p-Tukey	hedges	Result
A	1668:1613	4.049	0.286016	3.881	0.354912	0.168	0.161	two-sided	1.041	0.297930	0.492	-
B	1668:1640	3.854	0.153406	3.743	0.188239	0.111	0.085	two-sided	1.305	0.191860	0.617	-
C	1640:1613	1.050	0.055712	1.038	0.091410	0.012	0.039	two-sided	0.322	0.757015	0.152	-
D	1692:1613	1.293	0.105228	1.302	0.133185	-0.009	0.059	two-sided	-0.147	0.884715	-0.069	-

Table S6.5: Tukey tests compare four band intensity ratios for the inner and outer layers in feathers from the mallard.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	Ratio	mean(In)	s.d.	mean(Out)	s.d.	diff	se	tail	T	p-Tukey	hedges	Result
A	1668:1613	4.483	0.104119	3.083	0.768655	1.400	0.274	two-sided	5.104	0.001	2.413	**
B	1668:1640	4.006	0.071622	3.684	0.118600	0.322	0.050	two-sided	6.438	0.001	3.043	**
C	1640:1613	1.120	0.037858	0.838	0.206512	0.281	0.074	two-sided	3.794	0.001	1.793	**
D	1692:1613	1.374	0.208866	1.009	0.221739	0.365	0.107	two-sided	3.404	0.001	1.609	**

Table S6.6: ANOVA results between band intensity ratios 1668:1613cm^{-1}(Ratio A) taken from the inner layer only of cross section taken from swan rachises at different lengthwise (%) positions.

Source	ddof1	ddof2	F	p-unc	np^2
length	3	65	3.68	0.016384	0.145

Table S6.7: Post-Hoc Tukey tests between band intensity ratios 1668:1613cm^{-1}(Ratio A) taken at different lengthwise positions on rachis samples.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	A	B	$\bar{x}_A$	s.d.	$\bar{x}_B$	s.d.	diff	se	tail	T	p-Tukey	hedges	Results
0	20	40	4.533	0.380835	4.349	0.496233	0.184	0.174	two-sided	1.055	0.691719	0.332	-
1	20	60	4.533	0.380835	4.153	0.681043	0.380	0.180	two-sided	2.111	0.152803	0.685	-
2	20	80	4.533	0.380835	3.947	0.623127	0.586	0.187	two-sided	3.134	0.010301	1.057	*
3	40	60	4.349	0.496233	4.153	0.681043	0.196	0.186	two-sided	1.052	0.693524	0.353	-
4	40	80	4.349	0.496233	3.947	0.623127	0.402	0.193	two-sided	2.083	0.161822	0.724	-
5	60	80	4.153	0.681043	3.947	0.623127	0.206	0.198	two-sided	1.041	0.699894	0.371	-

Table S6.8: ANOVA results between band intensity ratios 16681640cm^{-1}(Ratio B) taken from the inner layer of different lengthwise rachis sections.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

Source	ddof1	ddof2	F	p-unc	np^2
length	3	65	0.957	0.4183	0.042

Table S6.9: Post-Hoc Tukey tests between band intensity ratios 1668:1640cm^{-1}(Ratio B) taken at different lengthwise positions on rachis samples.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	A	B	mean(A)	s.d.	mean(B)	s.d.	diff	se	tail	T	p-tukey	hedges	Result
0	20	40	3.848	0.437033	3.690	0.195957	0.159	0.109	two-sided	1.450	0.469184	0.456	-
1	20	60	3.848	0.437033	3.698	0.434827	0.150	0.113	two-sided	1.328	0.539125	0.431	-
2	20	80	3.848	0.437033	3.798	0.135463	0.051	0.118	two-sided	0.432	0.900000	0.146	-
3	40	60	3.690	0.195957	3.698	0.434827	-0.009	0.117	two-sided	-0.073	0.900000	-0.024	-
4	40	80	3.690	0.195957	3.798	0.135463	-0.108	0.121	two-sided	-0.889	0.784667	-0.309	-
5	60	80	3.698	0.434827	3.798	0.135463	-0.099	0.125	two-sided	-0.797	0.835852	-0.284	-

Table S6.10: ANOVA results between band intensity ratios 1640:1613cm^{-1}(Ratio C) taken from the inner layer of different lengthwise rachis sections.

Source	ddof1	ddof2	F	p-unc	np^2
length	3	65	5.366	0.002309	0.198

Table S6.11: Post-Hoc Tukey tests between band intensity ratios 1640:1613cm^{-1}(Ratio C) taken at different lengthwise positions on rachis samples.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	A	B	mean(A)	s.d.	mean(B)	s.d.	diff	se	tail	T	p-tukey	hedges	Result
0	20	40	1.184	0.069453	1.178	0.114239	0.006	0.038	two-sided	0.165	0.900000	0.052	-
1	20	60	1.184	0.069453	1.127	0.140175	0.058	0.039	two-sided	1.473	0.455676	0.478	-
2	20	80	1.184	0.069453	1.037	0.143584	0.147	0.041	two-sided	3.604	0.002066	1.215	*
3	40	60	1.178	0.114239	1.127	0.140175	0.052	0.041	two-sided	1.268	0.572731	0.425	-
4	40	80	1.178	0.114239	1.037	0.143584	0.141	0.042	two-sided	3.341	0.005217	1.161	*
5	60	80	1.127	0.140175	1.037	0.143584	0.089	0.043	two-sided	2.062	0.168777	0.734	-

Table S6.12: ANOVA results between band intensity ratios 1692:1613cm^{-1}(Ratio D) taken from the inner layer of different lengthwise rachis sections.

Source	ddof1	ddof2	F	p-unc	np^2
length	3	65	6.393	0.000729	0.228

Table S6.13: Post-Hoc Tukey tests between band intensity ratios 1692:1613cm^{-1}(Ratio D) taken at different lengthwise positions on rachis samples.
n.b. $*p \leq 0.1, **p \leq 0.001, ***p \leq 0.0001, -$ no significant difference.

	A	B	mean(A)	s.d.	mean(B)	s.d.	diff	se	tail	T	p-tukey	hedges	Result
0	20	40	1.426	0.062296	1.394	0.107388	0.032	0.038	two-sided	0.847	0.808241	0.266	-
1	20	60	1.426	0.062296	1.356	0.154847	0.070	0.039	two-sided	1.793	0.279182	0.582	-
2	20	80	1.426	0.062296	1.256	0.138969	0.170	0.041	two-sided	4.167	0.001000	1.405	**
3	40	60	1.394	0.107388	1.356	0.154847	0.038	0.041	two-sided	0.940	0.756196	0.315	-
4	40	80	1.394	0.107388	1.256	0.138969	0.138	0.042	two-sided	3.271	0.006590	1.136	*
5	60	80	1.356	0.154847	1.256	0.138969	0.100	0.043	two-sided	2.303	0.100423	0.820	*

Ratio D