



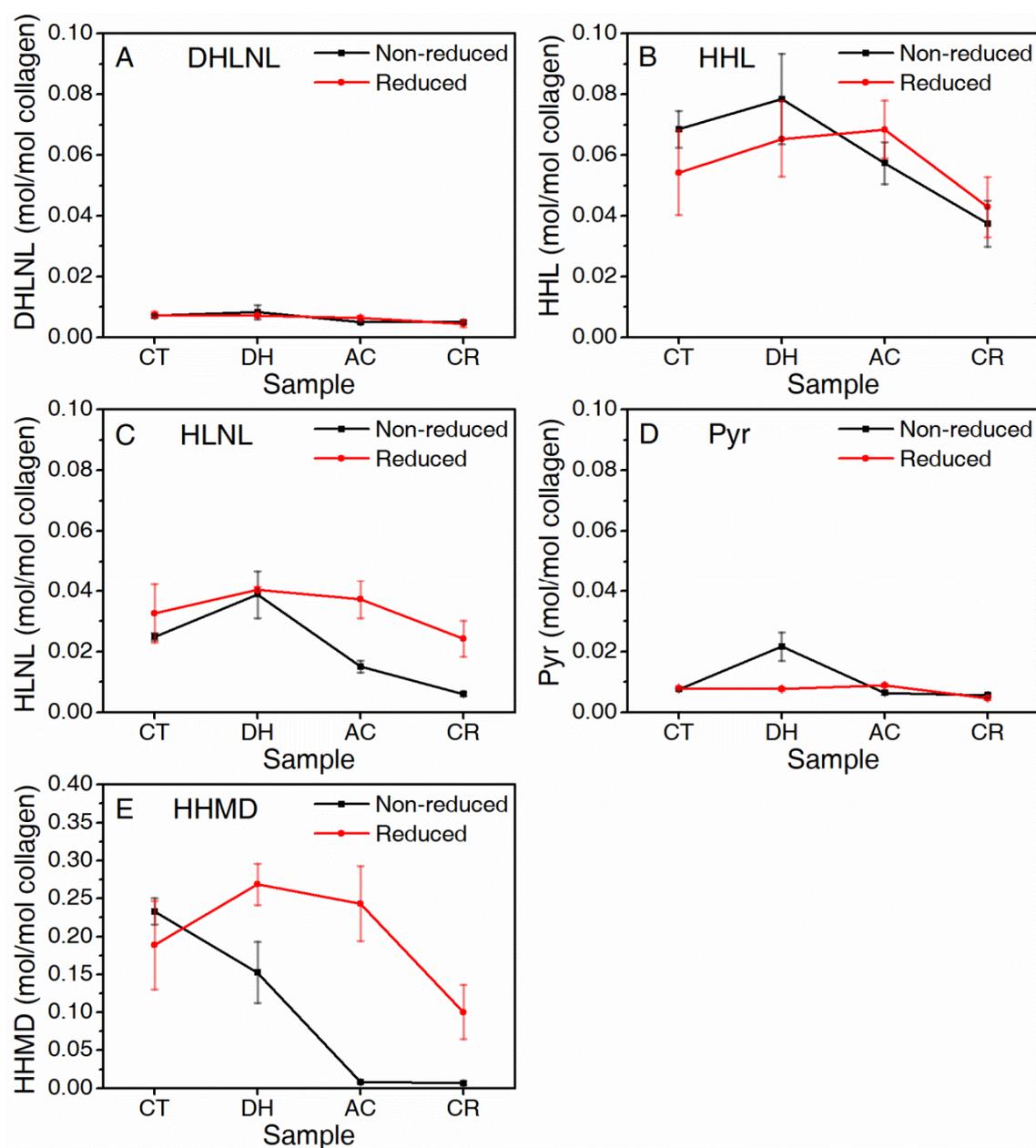
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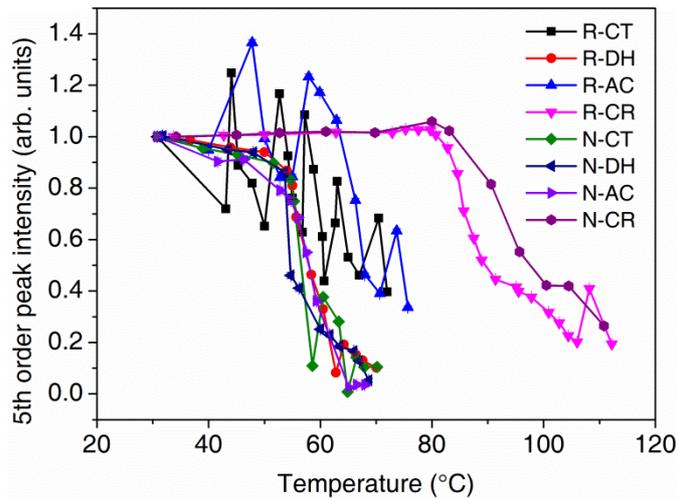
**Supporting information for article:**

***In situ* structural studies during denaturation of natural and synthetically crosslinked collagen using synchrotron SAXS**

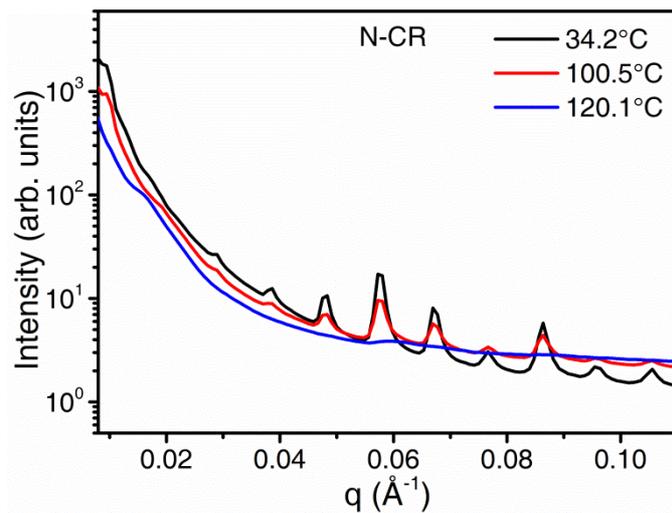
**Yi Zhang, Jenna Buchanan, Rafea Naffa, Bradley Mansel, Catherine Maidment, Geoff Holmes and Sujay Prabakar**



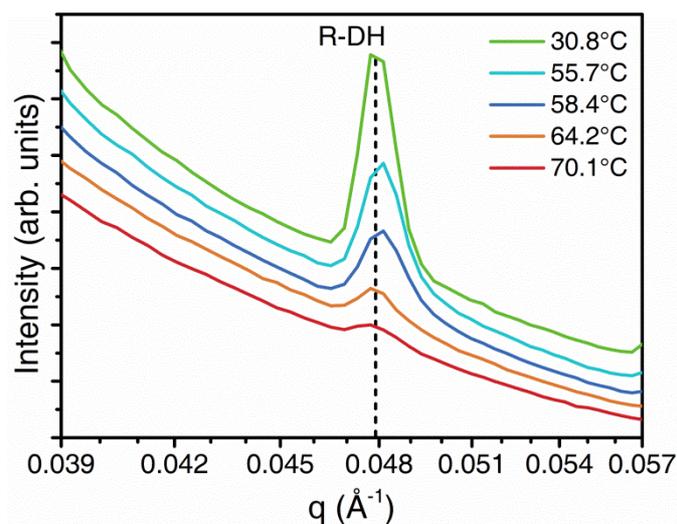
**Figure S1** Quantity of individual natural crosslink, i.e., dihydroxylysinoxorleucine (DHLNL), Hydroxylysinoxorleucine (HLNL), histidinohydroxymerodesmosine (HHMD), histidinohydroxylysinoxorleucine (HHL) and pyridinoline (Pyr), in reduced and non-reduced bovine skin samples CT = control (pH = 8), DH = dehaired (pH = 8), AC = acidified (pH = 3), CR = crosslinked (pH = 4)). Concentrations were normalized to collagen content in each sample.



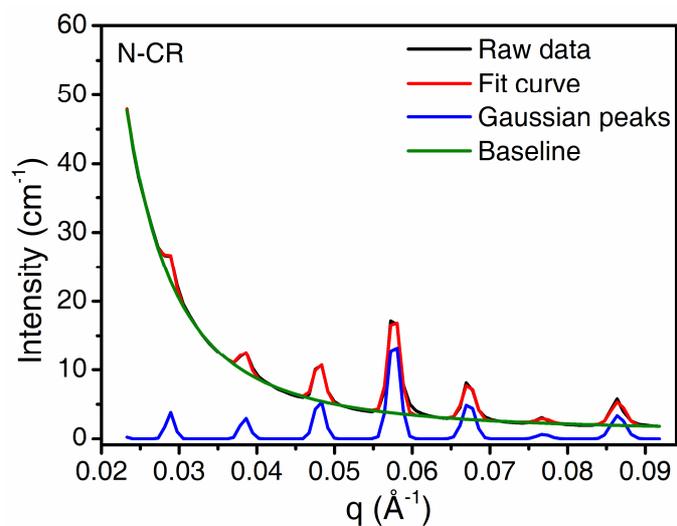
**Figure S2** Changes in the 5<sup>th</sup> order peak intensity (normalised to sample at room temperature) obtained from *in situ* SAXS data analysis during the heat denaturation of the collagen in bovine skins (N- = non-reduced, R- = reduced, CT = control (pH = 8), DH = dehaired (pH = 8), AC = acidified (pH = 3), CR = crosslinked (pH = 4)).



**Figure S3** 1D SAXS profile of non-reduced crosslinked sample (N-CR) at different temperatures during the *in situ* SAXS experiment (in correspondence to the 2D SAXS patterns in Figure 2A).



**Figure S4** Shifts in position and changes in width of the 5<sup>th</sup> order peak at different temperatures during the *in situ* SAXS experiment (in correspondence to Figure 2K). Black dashed line highlights the peak centre at 30.8°C. Peak shifts to higher  $q$  after the onset temperature (55.7°C) then revert back to the original centre at 70.1°C.



**Figure S5** 1D SAXS profile of non-reduced crosslinked sample (N-CR) at 34.2°C and the fitting of diffraction peaks using Gaussian function.

**Table S1** Peak modelling results including  $D$ -period (based on the position of the peaks of different orders ranging from the 3<sup>rd</sup> to the 9<sup>th</sup>), and the parameters for peak width ( $a$  and  $b$ ) at different temperatures from the *in situ* SAXS experiment (in correspondence to Figure 2F to 2M). Fitting uncertainties are included. The parameter ‘ $a$ ’ allows for individual deviation of the peak width while ‘ $b$ ’ permits linear peak width changes with  $q$ . The parameters allow for effects such as sample inhomogeneity where peak broadening occurs due to polydispersity in the  $D$ -period.

Sample	Temperature (°C)	$D$ -period (nm)	Fitting uncertainty (nm)	$a$ ( $10^{-3} \times \text{Å}^{-1}$ )	Fitting uncertainty ( $10^{-3} \times \text{Å}^{-1}$ )	$b$ ( $10^{-3}$ )	Fitting uncertainty ( $10^{-3}$ )
N-CT	30.8	65.594	0.001	0.982	0.002	0.244	0.044
	39.0	65.658	0.001	0.976	0.002	0.357	0.049
	45.2	65.684	0.001	0.968	0.002	0.508	0.051
	51.6	65.699	0.001	0.950	0.002	0.784	0.053
	54.7	65.690	0.001	0.986	0.003	0.923	0.061
	55.3	65.654	0.001	1.016	0.003	1.383	0.071
	58.6	65.671	0.005	1.048	0.020	4.287	0.617
	60.5	65.642	0.002	1.116	0.006	2.946	0.172
	63.3	65.702	0.002	1.101	0.008	2.849	0.206
	64.9	65.681	0.057	1.166	0.207	0.656	6.190
	66.5	65.707	0.004	1.113	0.013	3.071	0.359
	67.9	65.684	0.005	1.121	0.017	3.290	0.496
	70.2	65.684	0.005	1.120	0.017	3.316	0.496
N-DH	30.8	65.473	0.003	0.892	0.009	4.903	0.232
	31.8	65.476	0.003	0.890	0.009	5.061	0.237
	43.5	65.552	0.003	0.914	0.009	4.293	0.217
	48.0	65.553	0.003	0.895	0.009	4.355	0.217
	53.6	65.464	0.004	0.906	0.010	4.678	0.265
	54.7	65.396	0.010	1.248	0.031	4.803	0.826
	56.2	65.334	0.021	1.221	0.035	5.171	0.914
	60.0	65.327	0.021	1.149	0.070	9.659	1.967
	61.7	65.367	0.019	1.151	0.061	6.431	1.679

	63.5	65.438	0.017	1.003	0.051	6.413	1.418
	66.0	65.489	0.020	0.830	0.058	7.609	1.632
	67.0	65.494	0.026	0.934	0.076	6.088	2.017
	68.7	65.532	0.051	0.789	0.145	9.191	3.846
N-AC	30.8	65.749	0.003	1.114	0.012	3.470	0.344
	41.6	65.802	0.003	1.069	0.011	2.029	0.298
	46.3	65.803	0.003	1.111	0.013	3.468	0.349
	52.8	65.814	0.003	1.119	0.013	2.859	0.352
	54.7	65.782	0.004	1.145	0.013	2.453	0.371
	56.0	65.761	0.004	1.094	0.014	3.602	0.407
	57.5	65.713	0.004	1.111	0.016	2.920	0.480
	59.3	65.655	0.006	1.096	0.027	4.168	0.842
	65.1	65.674	0.058	1.286	0.210	-0.582	6.099
	66.5	65.702	0.124	1.047	0.687	22.544	22.027
	68.0	65.674	0.123	1.037	0.693	22.675	22.163
N-CR	34.2	65.385	0.001	0.749	0.008	12.648	0.137
	45.0	65.421	0.001	0.770	0.008	12.400	0.136
	52.7	65.446	0.001	0.762	0.008	12.521	0.136
	61.0	65.465	0.001	0.751	0.008	12.727	0.135
	69.8	65.476	0.001	0.788	0.008	12.150	0.134
	80.0	65.438	0.001	0.743	0.008	12.978	0.126
	83.1	65.351	0.001	0.792	0.008	12.320	0.124
	90.6	65.213	0.001	0.881	0.010	12.962	0.161
	95.7	65.214	0.001	1.060	0.018	13.712	0.290
	100.5	65.330	0.002	0.961	0.023	13.775	0.369
	104.5	65.364	0.002	1.252	0.022	9.526	0.353
	110.8	65.227	0.002	1.227	0.025	9.600	0.402
R-CT	30.8	65.491	0.001	1.079	0.003	-0.943	0.059
	43.1	65.591	0.002	1.151	0.005	-0.669	0.113

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	44.1	65.611	0.001	1.110	0.003	-0.609	0.063
	45.3	65.622	0.001	1.086	0.004	-0.152	0.085
	47.8	65.641	0.001	1.089	0.004	-0.465	0.091
	50.0	65.623	0.002	1.157	0.006	-1.051	0.122
	52.7	65.646	0.001	1.096	0.003	-0.264	0.069
	54.2	65.657	0.001	1.059	0.004	0.057	0.087
	55.0	65.656	0.001	1.057	0.005	0.147	0.098
	56.8	65.618	0.002	1.139	0.006	-0.703	0.126
	57.2	65.628	0.001	1.088	0.003	-0.050	0.071
	58.8	65.623	0.001	1.038	0.004	0.815	0.091
	60.4	65.604	0.002	1.036	0.006	2.195	0.142
	60.7	65.557	0.003	1.060	0.010	3.540	0.240
	62.7	65.597	0.002	1.128	0.008	1.953	0.176
	63.0	65.643	0.001	0.998	0.005	2.304	0.109
	65.0	65.566	0.003	1.210	0.009	2.013	0.215
	67.0	65.638	0.003	1.179	0.009	2.282	0.208
	70.5	65.622	0.002	1.121	0.006	2.639	0.141
	72.0	65.613	0.003	1.103	0.009	3.097	0.230
R-DH	30.8	65.504	0.001	1.088	0.004	0.603	0.085
	36.7	65.541	0.001	1.081	0.004	0.741	0.083
	43.9	65.571	0.001	1.069	0.004	0.853	0.084
	50.0	65.568	0.001	1.070	0.004	0.787	0.084
	54.0	65.515	0.001	1.086	0.004	0.865	0.091
	55.0	65.423	0.002	1.097	0.005	1.966	0.115
	55.7	65.296	0.002	1.065	0.007	5.688	0.178
	58.4	65.212	0.004	1.098	0.011	8.287	0.306
	60.5	65.208	0.005	1.209	0.017	7.840	0.469
	62.8	65.363	0.019	1.143	0.061	6.746	1.657
	64.2	65.405	0.008	1.352	0.025	3.242	0.673

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	66.4	65.424	0.009	1.139	0.030	6.444	0.813
	67.6	65.440	0.011	1.139	0.035	6.686	0.955
	70.1	65.454	0.015	1.205	0.047	6.676	1.294
R-AC	31.7	65.891	0.002	1.055	0.008	3.117	0.165
	40.0	65.926	0.002	1.059	0.007	2.877	0.152
	47.8	65.954	0.001	1.006	0.005	3.322	0.099
	50.0	65.967	0.002	1.050	0.007	3.605	0.154
	52.8	65.972	0.002	1.112	0.008	1.879	0.160
	55.0	65.976	0.002	1.065	0.007	2.531	0.151
	57.9	65.982	0.001	0.992	0.005	3.474	0.101
	59.9	65.973	0.001	0.979	0.005	3.389	0.102
	62.9	65.902	0.001	0.973	0.005	3.803	0.113
	66.3	65.813	0.002	0.952	0.006	5.059	0.147
	68.0	65.837	0.003	1.009	0.010	4.787	0.250
	70.7	65.915	0.003	1.043	0.011	4.083	0.278
	73.7	65.953	0.002	1.115	0.008	2.167	0.183
	75.7	65.912	0.003	0.999	0.011	4.072	0.290
R-CR	33.7	65.447	0.001	0.821	0.005	8.211	0.072
	42.7	65.490	0.001	1.109	0.004	3.325	0.068
	50.0	65.514	0.001	1.160	0.004	2.511	0.067
	62.8	65.552	0.001	1.180	0.004	2.157	0.065
	72.9	65.557	0.001	1.206	0.004	1.772	0.064
	75.3	65.526	0.001	1.181	0.004	2.330	0.065
	77.6	65.478	0.001	1.101	0.004	3.765	0.068
	79.7	65.408	0.001	0.999	0.004	5.799	0.069
	80.7	65.293	0.001	0.860	0.004	8.375	0.066
	82.8	65.189	0.001	0.929	0.005	8.635	0.073
	84.6	65.125	0.001	0.928	0.006	11.053	0.097
	85.7	65.093	0.001	0.924	0.008	13.035	0.127

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87.5	65.080	0.001	0.988	0.010	13.663	0.159
88.9	65.084	0.001	1.090	0.012	13.793	0.198
91.4	65.187	0.001	1.207	0.014	11.637	0.221
95.1	65.325	0.001	1.238	0.013	8.645	0.210
95.5	65.326	0.001	1.226	0.014	8.862	0.220
97.8	65.307	0.001	1.270	0.015	8.615	0.246
100.9	65.307	0.002	1.285	0.018	8.577	0.286
102.8	65.278	0.002	1.307	0.021	8.685	0.339
104.4	65.248	0.002	1.293	0.026	9.520	0.414
106.0	65.252	0.002	1.176	0.025	9.631	0.411
108.2	65.315	0.001	0.757	0.010	11.311	0.165
112.2	64.899	0.002	0.789	0.027	17.853	0.445

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