Supplemental Information for:

In Quest of the Alanine R3 Radical: Multivariate EPR Spectral Analyses of X-Irradiated Alanine in the Solid State

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Part 1: Reconstruction of thermally annealed spectra using non-linear least squares methods

1.1 Thermal annealing

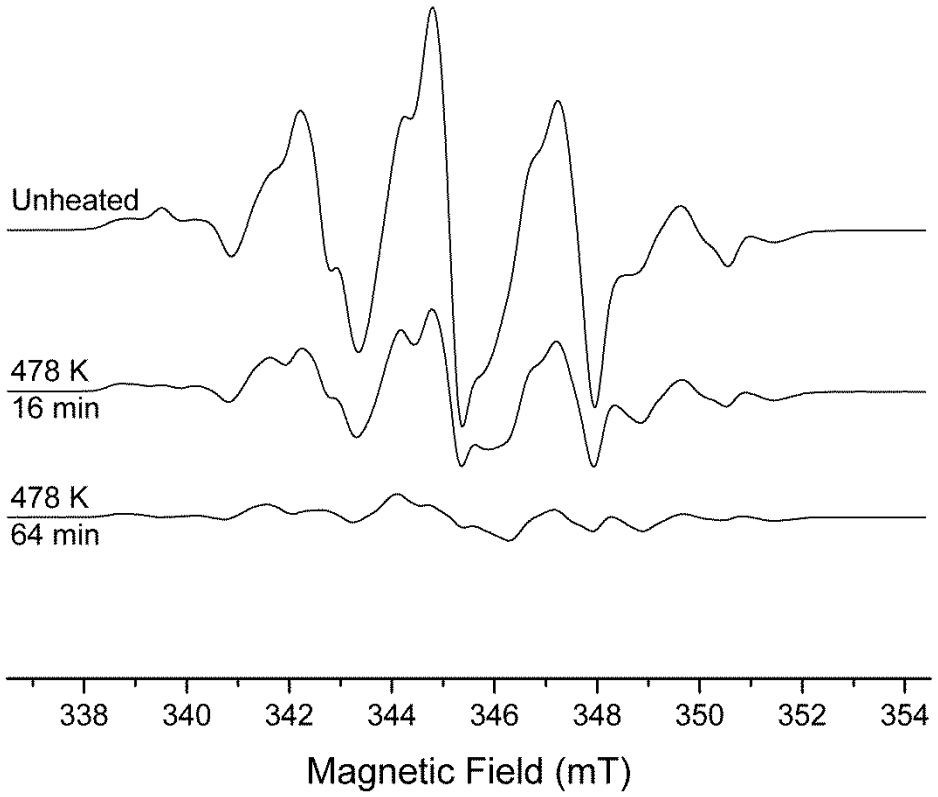

Figure S1.1: Alanine dosimeter EPR spectra as a function of annealing temperature and time. The top spectrum is recorded from one of the control pellets irradiated at 296 K and not exposed to any heat treatment. The middle and lower spectra were recorded at 296 K after heat treatment at 478 K, as indicated.

1.2 Simulated spectra

With a few minor adjustments, the previously described simulated model spectra [1-3] of the radicals **R1**, **R2** and **R3** were used for the initial component analyses. The minor adjustments, made in 2012,[4] were as follows:

- Linewidths of all three radical component spectra were increased slightly to allow for the somewhat higher microwave power used.
- Two tentative conformations of the **R3** radical were identified in the previously reported work.[5] During the work made in 2012,[4] the hyperfine coupling tensor data for the second **R3** conformation was found to be the most appropriate, whereas conformation 1 was used in references. [1-3]

The resulting component model spectra (using conformation 1) are shown in Figure S1.2.

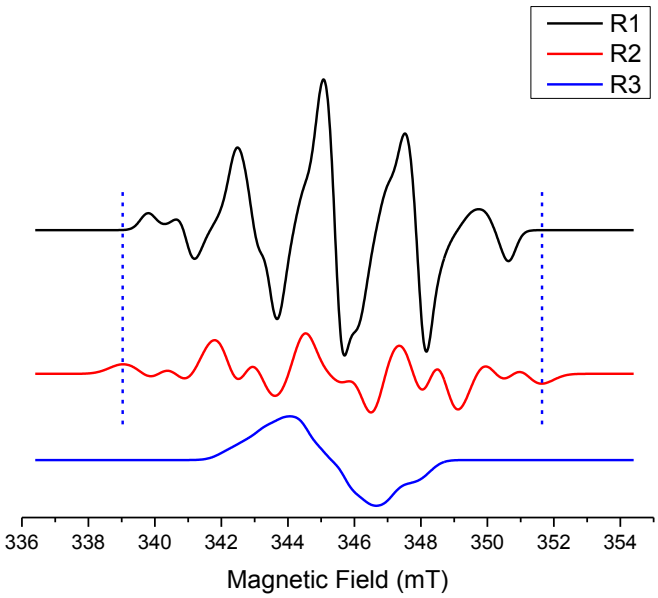

Figure S1.2: Simulated **R1**, **R2** and **R3** alanine radical model spectra used for reconstruction of the experimental alanine spectra. The area of each of these spectra is normalized to the same value. The vertical lines are visual aids.

1.3 Reconstruction of experimental data using three component spectra

The results obtained using the three model spectra in Figure S1.2 as basis for the reconstruction of all available experimental data are given in Table S1-1 and plotted in Figure S1.3

Table S1-1: Detailed results of the curve fitting shown in Fig. S1.3 using Equation S1 below[a].

	A	B	C	Correlation	A + B
R1	0.5406	-0.5295	25.6361	0.9959	0.0111
R2	0.4334	0.1774	14.0059	0.9750	0.6108
R3	0.0262	0.4038	43.3920	0.9962	0.4300
Sum	1.0002	-	-	-	1.0519

[a]The sums of **A** and of **A+B** for each radical (both being close to the expected value 1.0000) show reasonable internal consistency even if the three fits were made without any such constraints.

The curve fitting was made using the single exponential function:

$$F = A + B(1 - \exp(-t / C)) \qquad (S1)$$

where

F: Fraction of radicals (each time point normalized to 1)

t: Annealing time (minutes)

A, B, C: Fitting parameters (A is $t = 0$ value, $A+B$ is the asymptotic value ($t = \infty$) and C is the time constant (minutes) for the exponential time behavior)

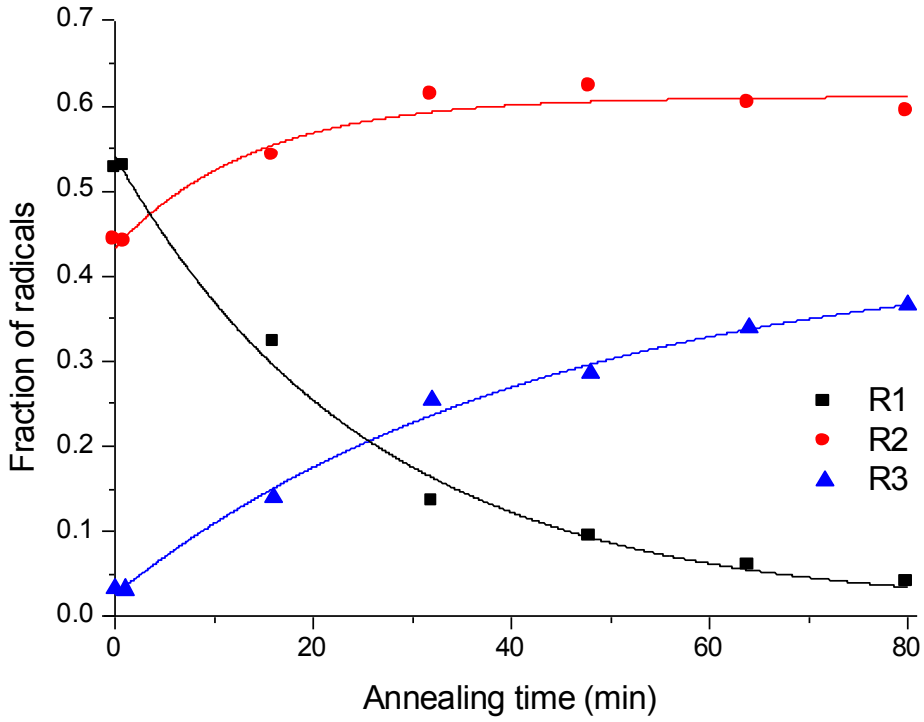

Figure S1.3: Fractions of **R1**, **R2** and **R3** radicals obtained by reconstructing the experimental EPR spectra of alanine X-irradiated to 5 kGy at room temperature. The fractional data were obtained by fitting annealing data at 478 K. Pearson's correlations for the fitting of the experimental spectra at each time point were: 1 min, 0.996; 16 min, 0.994; 32 min, 0.994; 48 min, 0.994, 64 min, 0.994; 80 min, 0.995. Microwave power: 2.0 mW. Sums of fractions are normalized at each time point. The zero time entries were obtained by reconstructing the experimental EPR spectrum from an independent control sample to a correlation of 0.996.

1.4 Fitting of experimental data using two (R1, R2) spectral components

Table S1-3: The least squares fitting of the experimental EPR spectra using only two radical components, **R1** and **R2**, and their simulated spectra given in Figure S1.2[a].

Temperature [^{o}C]	Time [min]	R1	R2	LOF	Correlation
Control 1	0	0.60	0.40	0.14	0.99
Control 2	0	0.60	0.40	0.14	0.99
470	1	0.62	0.38	0.15	0.99
470	30	0.24	0.76	0.26	0.96
470	60	0.24	0.76	0.26	0.97
470	90	0.12	0.88	0.37	0.93
470	120	0.11	0.89	0.38	0.92
470	150	0.12	0.88	0.37	0.93
478	1	0.60	0.40	0.14	0.99
478	16	0.36	0.64	0.20	0.98
478	32	0.16	0.84	0.33	0.94
478	48	0.11	0.89	0.38	0.93
478	64	0.07	0.93	0.45	0.89
478	80	0.05	0.95	0.49	0.87
486	1	0.61	0.39	0.15	0.99
486	10	0.29	0.71	0.23	0.97
486	20	0.11	0.89	0.38	0.92
486	30	0.06	0.94	0.47	0.89
486	40	0.05	0.95	0.48	0.88
486	50	0.02	0.98	0.58	0.81

[a]The contribution fractions of each radical to the fit is shown. The lack of fit (LOF) and the Pearson correlation between the fitted and the experimental spectra are given. The arrows are visual aids, showing the increase in LOF and the decrease in correlation with heating time.

Part 2: PCA, ICA and Factor Analysis results

Table S2-1. The Pearson correlations between the simulated spectra **R1**, **R2**, **R3**, and the component spectra estimated using PCA (PC1-3), MLCFA (F1-3) and ICA (IC1-3)[a].

Method		R1	R2	R3	LOF	Correlation
PCA						
Mean-centered	PC1	0.99	-0.54	0.11		
	PC2	-0.06	0.75	0.49	0.012	0.999
	PC3	-0.03	0.06	0.09		
Mean-centered heated samples	PC1	-0.82	-0.85	-0.36		
	PC2	0.52	-0.35	-0.57	0.037	0.997
	PC3	0.03	0.04	-0.06		
Normalized & mean-centered	PC1	0.55	-0.31	-0.58		
	PC2	-0.34	-0.71	0.28	0.053	0.997
	PC3	-0.05	-0.04	0.00		
MLCFA						
All samples	F1	0.20	0.80	0.74		
	F2	0.97	0.35	-0.08	0.048	0.999
	F3	-0.06	0.35	-0.58		
Heated samples	F1	-0.11	0.53	0.85		
	F2	0.92	0.70	0.03	0.050	0.999
ICA						
All samples	IC1	-0.06	-0.10	0.07		
	IC2	0.15	0.81	0.69	0.014	0.998
	IC3	0.98	0.37	-0.03		
Heated samples	IC1	0.41	0.15	-0.68		
	IC2	-0.15	-0.81	-0.52	0.019	0.999
	IC3	0.86	0.41	0.26		

[a]The lack of fit (LOF) and the correlation between the original data matrix **X** and the reconstructed data matrix **X*** are also shown. All experimental spectra or a subset of only spectra of samples heated at 478 K or 486 K for > 20 min were used in the analyses. Spectra were normalized to unit area prior to decomposition, except for two PCA models, where only mean-centering was used.

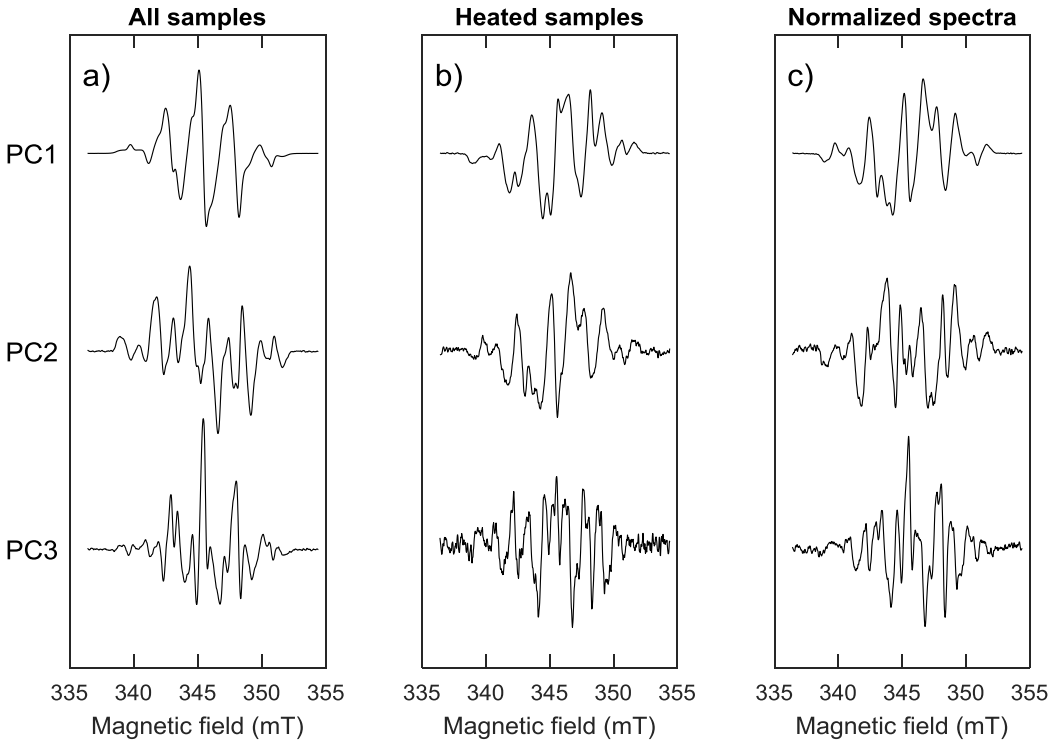

Figure S2.1. The PCA principal component loadings for PC1 (top), PC2 (middle), PC3 (bottom), as a function of magnet field for (a) all EPR sample spectra, (b) a subset of spectra of samples heated for more than 20 min at 478 K and 486 K, and (c) normalized spectra.

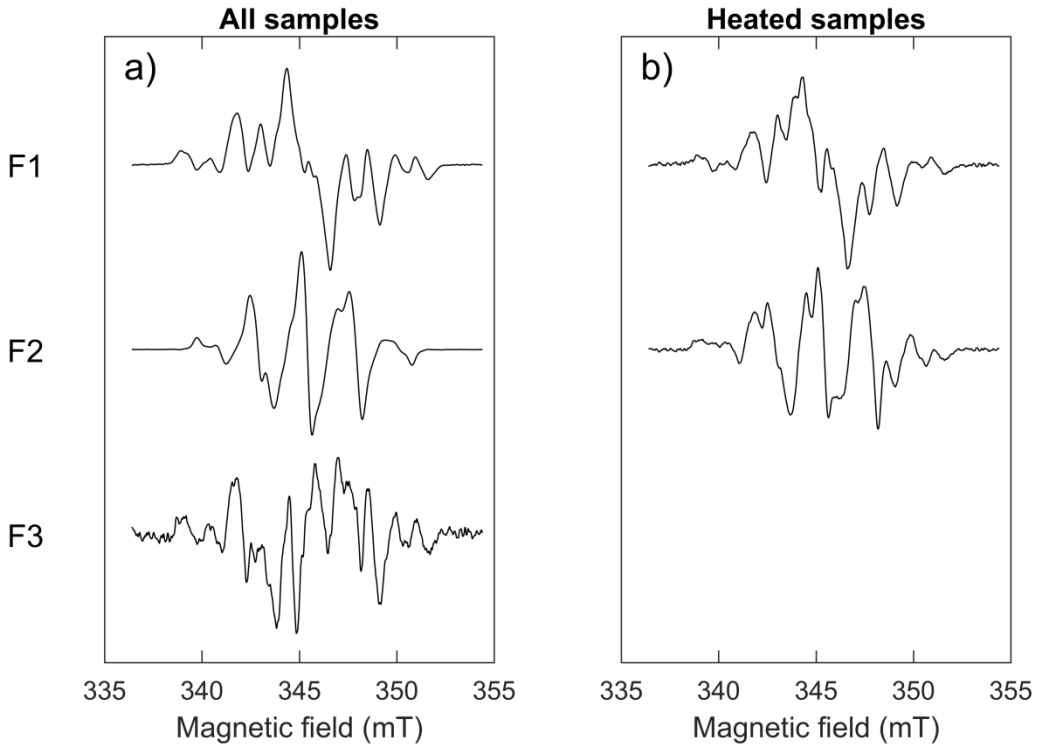

Figure S2.2. The common factors, F1 (top), F2 (middle), F3 (bottom), estimated by MLCFA using (a) all EPR sample spectra and (b) a subset of spectra of samples heated for > 20 min at 478 K and 486 K.

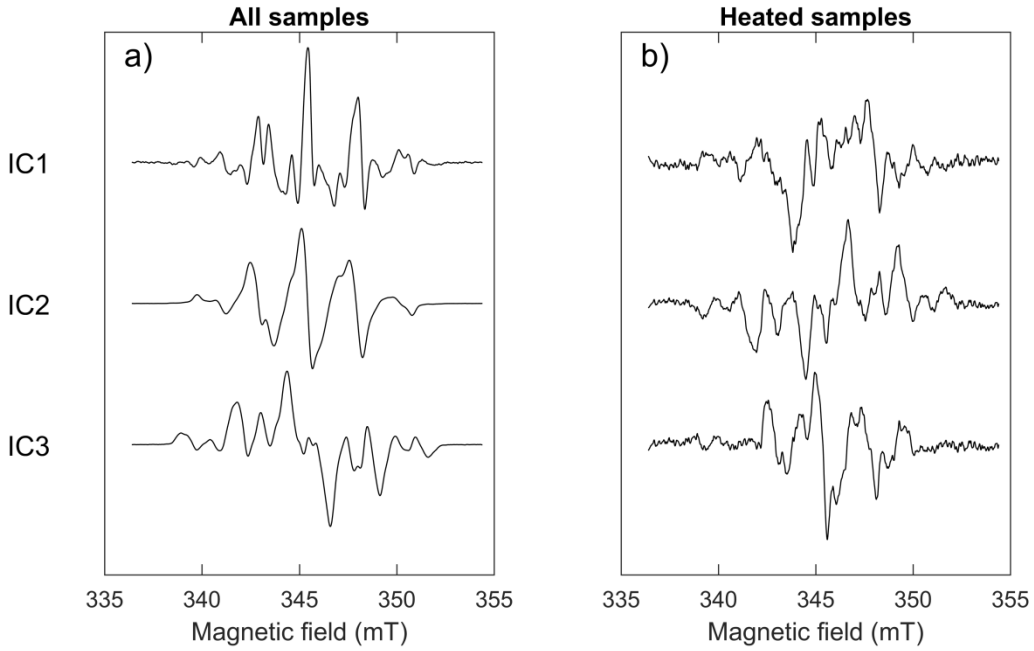

Figure S2.3. The independent components, IC1 (top), IC2 (middle), IC3 (bottom), estimated by ICA using (a) all EPR sample spectra and (b) a subset of spectra of samples heated for > 20 min at 478 K and 486 K.

References (Supplemental Information only)

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(2) Malinen, E.; Heydari, M. Z.; Sagstuen, E.; Hole, E. O. Alanine radicals, part 3: properties of the components contributing to the EPR spectrum of x-irradiated alanine dosimeters. *Radiat. Res.* **2003,** *159*, 23-32.

(3) Malinen, E.; Hult, E. A.; Hole, E. O.; Sagstuen, E. Alanine radicals, part 4: relative amounts of radical species in alanine dosimeters after exposure to 6-19 MeV electrons and 10 kV-15 MV photons. *Radiat. Res.* **2003,** *159*, 149-153.

(4) Krivokapic, A.; Knudtsen, I. S.; Malinen, E. LET effects of heavy ion irradiations of alanine. *Unpublished data* **2012**.

(5) Sagstuen, E.; Hole, E. O.; Haugedal, S. R.; Nelson, W. H. Alanine radicals: structure determination by EPR and ENDOR of single crystals x-irradiated at 295 K. *J. Phys. Chem.* **1997,** *A101*, 9763-9772.