# Trehalose Matrices for High Temperature Dynamic Nuclear Polarization Enhanced Solid State NMR

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Supplementary Information

## 1. Materials and methods

Casein from bovine milk was purchased from Sigma Aldrich™ (CAS9000-71-9), trehalose dihydrate was purchased from Sigma Aldrich™ (CAS 6138-23-4). AMUPol and O-MbPyTol were synthesized as described previously.[1] All the samples were prepared according to literature procedure at ambient conditions using chemical compounds as received form the suppliers without any further treatment.[2]

**Synthesis of PyT radical:**

Compounds **2**, **3** and **4** were synthesized according to the reported procedures.[3] All chemicals used in synthesis were purchased from Aldrich Chemical Co. Commercially available starting materials were used without further purification. Purification of products was accomplished by flash column chromatography on silica gel (Merck silica gel 60, 230- 400mesh) or neutral alumina. NMR measurements were recorded on a Bruker AVL 300 spectrometer ($^1$H NMR 300.1 MHz and $^{13}$C NMR 75.5 MHz) using CDCl$_3$ as the solvent (internal reference). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Mass spectral analyses were carried out using a Q-STAR Elite at the Aix-Marseille Université Mass Spectrum Facility, Spectropole Saint-Jérôme Marseille. The final products were purified to ≥95% and were confirmed by HPLC-MS analysis or elemental analysis. HPLC-MS experiments were performed using an Agilent 1260 infinity system coupled with a 6120 simple quadruple. This system was equipped with a C12 column (Zorbax 1.8 μM, 3 x 50 mm) that was equilibrated with 10% vol. MeCN (containing 0.1% (v/v) formic acid) in 0.1% vol. formic acid aqueous solution at the flow rate of 0.28 or 0.40 mL/min. EPR measurements were performed on a Bruker Elexsys II spectrometer operating at 9.4 GHz (X-band) in 50 μL capillaries using the following parameters: microwave power 5 mW and modulation amplitude 0.1 G.
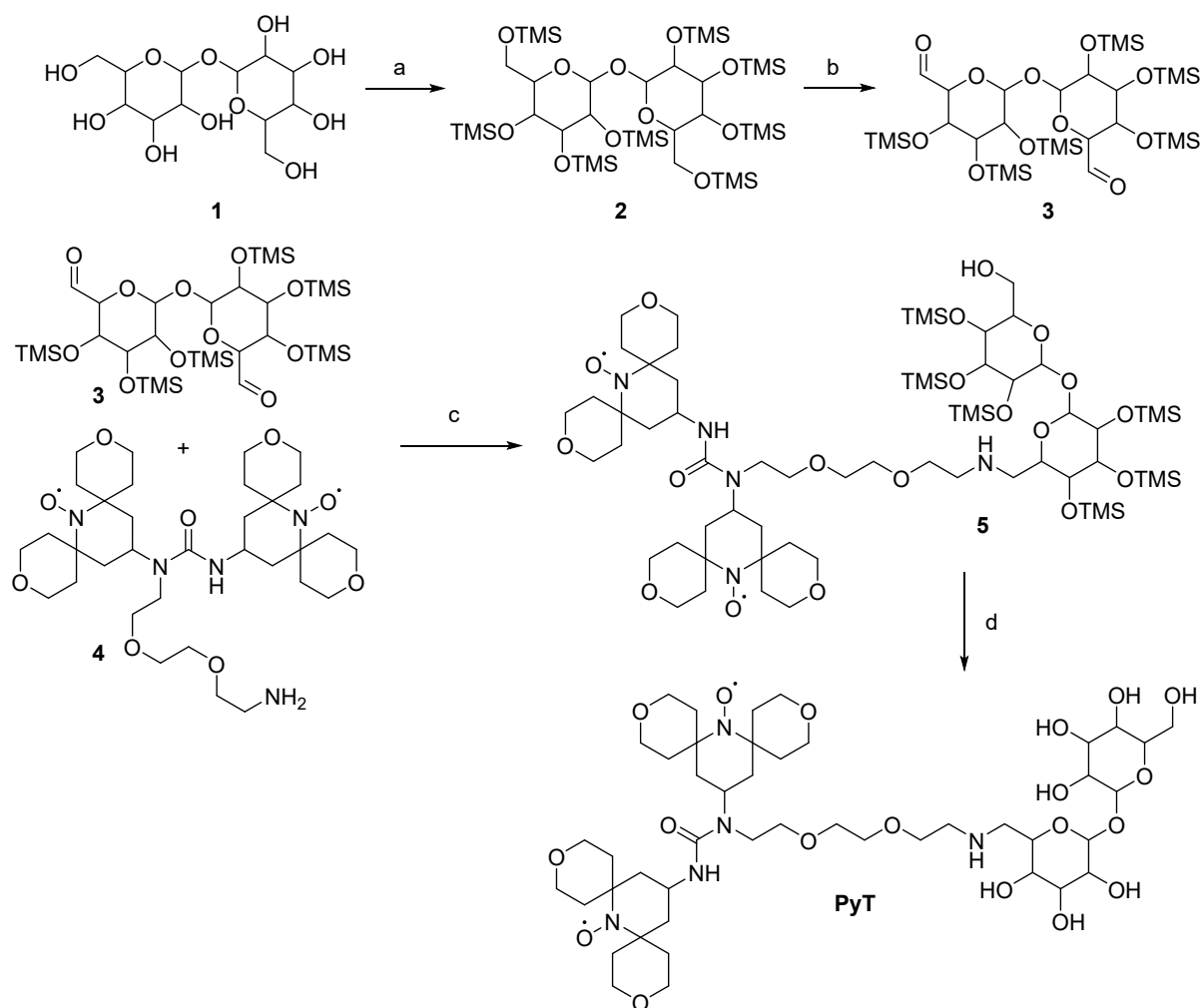
**Scheme S1:** *Reaction conditions*: (a) TMSCl, HMDS, pyridine,0°C to rt, 16h. (b) CrO₃, pyridine, 0°C to rt. (c) NaBH₃CN, CH₃CN, rt. (d) K₂CO₃, MeOH, 16h, rt.

Synthesis of compound **2**

To a solution of (D)-(+)-trehalose dihydrate in pyridine (0.04M) at 0 °C, HMDS (72 eq), and then TMSCl (60 eq) was slowly added. After 20 min at 0°C, the reaction mixture was stirred overnight at room temperature. Pyridine was removed under reduced pressure and a methanol/water mixture (90/10) was added. After 1h at 0°C, the white precipitate was filtrated and dried under high vacuum to give **2** in quantitative yield.

**¹H NMR (400 MHz, CDCl₃) δ**: 0.10 (s, 18H); 3.72; 0.12 (s, 18H); 0.14 (s, 36H); 3.38 (dd, $J$ = 6.7, 3.0, 2H); 3.62-3.74 (m, 4H); 3.43 (t, $J$ = 9.1, 2H); 3.63 – 3.71 (m, 4H)); 3.78 (m, 2H); 3.89 (t, $J$ = 9.0, 2H); 4.91 (d, $J$ = 3.0, 2H).

**¹³C NMR (75 MHz, CDCl₃) δ:** -1.1 (**C**H₃); -0.7 (**C**H₃); 0.1 (**C**H₃); 0.2 (**C**H₃); 61.3 (**C**H₂); 40.9 (**C**H₂); 72.0 (**C**H₂); 72.4 (**C**H₂); 72.8 (**C**H₂); 93.5 (**C**HO).

Synthesis of compound **3**

To a solution of DCM (200mL), add CrO₃ (5.22g, 12 eq.) then pyridine (10.5 mL, 30 eq.) freshly distillated at room temperature. Mixture was stirring for 30 min. After cooling at 0°C, compound **2** dissolved in DCM (80mL) was added slowly during 20 min. The reaction mixture was stirred for 2 hours at 0°C. Reaction evolution was monitored by TLC (DCM/EtOH: 98/2, rf= 0.3). The crude was filtrated on neutral alumina pad to remove chromium salts, the solvent were removed under reduce pressure and te compound was dried under high vacuum to yield **3** (3.13g, 93% yield) as a colorless powder.

**¹H NMR (400 MHz, CDCl₃) δ**: 0.10 (s, 18H); 0.12 (s, 18H); 0.18 (s, 18H); 3.44 – 3.50 (m, 2H); 3.54 – 3.61 (m, 2H); 3.91 – 3.97 (m, 2H); 4.46 (d, $J$ = 9.9, 1.3, 2H); 4.98 (d, $J$ = 3.1, 2H); 9.68 (s, 1H); 9.69 (s, 1H).

**¹³C NMR (75 MHz, CDCl₃) δ:** -0.8 (**C**H3); -0.1(**C**H3); 0.1 (**C**H₃); 71.5 (**C**H₂); 73.0 (**C**H₂); 76.3 (**C**H₂); 95.1 (**C**H₂); 197.3 (**C**HO).

**MS (ESI):** m/z: calc for $[M+NH_4]^+$ $C_{30}H_{70}O_{11}NSi_6$: 788.3, found 788.4

## Synthesis of compound 5

To a solution of compound **3** (4.5 mmol, 3 eq.) in $CH_3CN$ (60 mL) was added at room temperature a solution of PyPolPEGNH$_2$ (**4**) in $CH_3CN$ (5 mL). The reaction mixture was stirred during 90 min at rt, then NaBH$_3$CN (2.1 mmol, 1.4 eq.) was added. Reaction evolution was monitored by TLC (DCM/EtOH: 95/5). After 18h at r.t., solvent was removed under reduced pressure, chloroform (100 mL) was added and the mixture was washed with an aqueous solution of K$_2$CO$_3$ (35%, 100 mL). The crude product was purified by chromatography on silica gel with DCM/EtOH (100/0 to 95/5) as eluent to give a reddish powder (1.55 g, 83% yield).

**HRMS (ESI):** m/z: calc for $[M+H]^+$ $C_{63}H_{126}N_5O_{19}Si_6^{2\bullet}$: 1424.7606, found: 1425.7703.

## Synthesis of PyT

Compound **5** was dissolved in 80 ml solution of K$_2$CO$_3$/MeOH (10% wt) at pH= 8. After 16 h at room temperature, the complete deprotection was performed. The solvent was removed under reduced pressure and the crude product was dissolved in $CH_3CN$, filtrated on neutral alumina pad to remove K$_2$CO$_3$ and concentrated under reduce pressure to give **PyT** as a pale reddish powder (1.02g, >99% yield).

**HRMS (ESI):** m/z: calc for $[M+H]^+$ $C_{45}H_{78}N_5O_{19}^{2\bullet}$: 992.5286, found 992.5281.
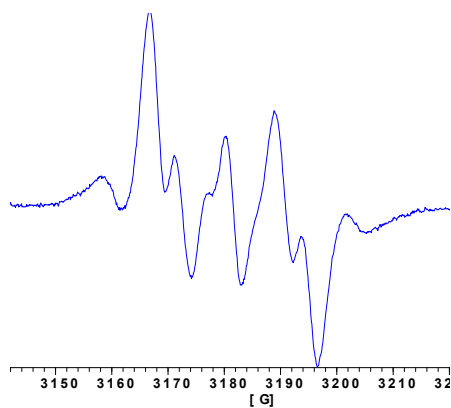


**Figure S1:** X band EPR spectrum of PyT in H$_2$O solution (2 mM) at room temperature
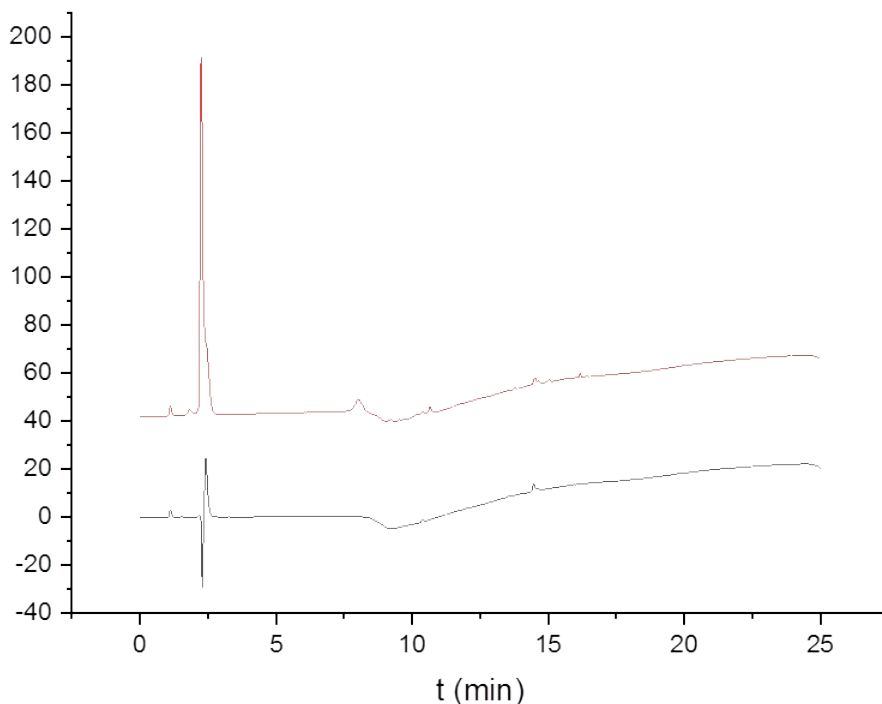
**Figure S2:** HPLC chromatograms of blank (black line) and PyT (red line) in Water/Acetonitrile/0.1% Formic Acid gradient, RP C18, UV detection at 254 nm)

**Trehalose matrix sample preparation:**

*Trehalose solution preparation.* To prepare 10 mL at 0.65 M solution: weigh 2.457 g of trehalose dihydrate in 20 mL flask and add distillated water (10 mL).

*Procedure for samples preparation (mono and binitroxides in trehalose) with the freeze-drying method.* This approach concerned the preparation of samples in 1 mL Eppendorf at any desired concentration: weigh the nitroxide (to have the desired concentration) in 1 mL trehalose solution at 0.65 M. Shake the Eppendorf (vortex) to have homogenous solution. Then put the sample in liquid nitrogen for 10 to 15 min and quickly transfer it on freeze drier for 16h at –80°C and $10^{-2}$ mbar.
Rehydration step: put the freeze-dried powder on a glass watch into hermetic box with saturated LiCl or NaCl solution (to obtain 11% or 74% rehydration respectively) for 2 days.

*Procedure for samples preparation (nitroxides and binitroxides) with the film method.* Solutions of 2, 5, 10, 20 or 50 mM nitroxide in 1:1 water/ethanol were mixed with an aqueous solution of trehalose. The solutions were placed in a thin layer on a watch glass. The solvent was evaporated between 3 and 4 h, forming a glassy film. The immobilization of nitroxide was followed by EPR. Then, the film was scraped off and transferred to the rotor.

**Protein and sugar matrix sample preparation:**

Commercially available Casein from bovine milk was dissolved in water by adjusting pH to 8.5-9 using $NH_4OH$ base (32% in water). The solution contained 2 g casein in 40 mL of solution. A stock solution of trehalose was prepared in water with 1.8 g trehalose, 0.2 g sucrose in 20 mL water. Solutions of protein, sugar, and plain water were mixed in specific ratios to form 2 mL total solution corresponding to percentage of protein and sugar. 20% casein corresponds to 20 milligrams of casein. Radical concentration of 2 to 10 mM was achieved by adding appropriate volume of a radical solution stock (432 mM) in water. Calculated weight of PyT for 2 mM, 5 mM, and 10 mM concentrations are 0.634 mg, 1.56 mg, and 3.12 mg respectively per 100 mg of casein-sugar. This mixture was treated with either freeze dry or rehydration protocol.

**Experimental parameters for the NMR experiments:**

The MAS DNP NMR experiments were performed on a Bruker Avance III 400 MHz wide bore spectrometer, equipped with triple resonance 3.2 mm and 1.3 mm low-temperature DNP MAS probes. The samples were irradiated with continuous wave high-power microwaves at a frequency of 263 GHz, with a power stability better than ±1%. The microwaves were generated by a gyrotron and delivered to the sample by a corrugated waveguide with ≈22 W of power reaching the sample. The microwave power was optimized so as to obtain maximum enhancement for each sample. Sapphire rotors were used for optimal microwave penetration. The $^1$H 1D spectra were recorded with a background suppression and phase cycled DEPTH pulse sequence using a proton 90° pulse of 2.5 ms.[4] The $^{13}$C spectra were recorded with a $^1$H-$^{13}$C cross-polarization (CP) pulse sequence, using a contact time of 1 ms. For protons a linear ramped CP was used to optimize the magnetization transfer efficiency. A proton radio frequency (RF) field of 70 kHz in the center of the ramp was applied, while the RF field on carbon-13 was adjusted for optimal sensitivity. SPINAL-64 proton decoupling[5] was applied during acquisition at a radio-frequency field of 100 kHz.

The room temperature NMR experiments reported in Figure S4 were acquired on a Bruker Avance III 500 MHz wide bore spectrometer equipped with a double resonance 4 mm probe. The $^{13}$C spectra were recorded with a $^1$H-$^{13}$C cross-polarization (CP) pulse sequence, using a contact time of 1 ms. For protons a linear ramped CP was used to optimize the magnetization transfer efficiency. A proton radio frequency (RF) field of 50 kHz in the center of the ramp was applied, while the RF field on carbon-13 was adjusted for optimal sensitivity. SPINAL-64 proton decoupling[5] was applied during acquisition at a radio-frequency field of 60 kHz.

Temperature measurements: Each sample was packed with a pellet of KBr at the bottom of the rotor. Longitudinal relaxation $T_1$ of $^{79}$Br in KBr is strongly dependent on temperature of the sample and it was recorded via saturation recovery pulse sequence to measure temperature inside the rotor.[6] Temperatures reported here are with microwave irradiation on.

The estimation of errors of the enhancements were done according to reference [7]:

$$\Delta\varepsilon = \varepsilon\left(\frac{\Delta I_{\text{on}}}{I_{\text{on}}} + \frac{\Delta I_{\text{off}}}{I_{\text{off}}}\right)$$

were $\Delta I$ is the error of the observed signal with an integral of I with and without μw, or in the case of the contribution factor, the signal intensity in a solution with or without radical.

The relative errors are higher in spectra recorded without μw. The absolute errors are higher for higher enhancement values. We estimated that the error bar was around 10 % for the measurement of the enhancement factor.

**Additional characterization:**

TGA and DSC measurements: DSC measurements were carried out using DSC Q20 (TA Instruments) under $N_2$ atmosphere with cooling/heating rate of 10 °C/min in the temperature range from −80°C to 100°C.

Thermogravimetric analysis (TGA) measurements were carried out on a TGA 8000 thermogravimetric analyzer (Perkin Elmer). All the samples were preheated to 25 °C and kept at this temperature for 5 min then heating to 200 °C, with a heating rate of 10 °C/min in a nitrogen atmosphere.
EPR measurements: Continuous wave (cw) experiments of the NMR formulations were performed on a benchtop EMXNANO™ or on an Elexsys II spectrometer operating at 9.4 GHz.
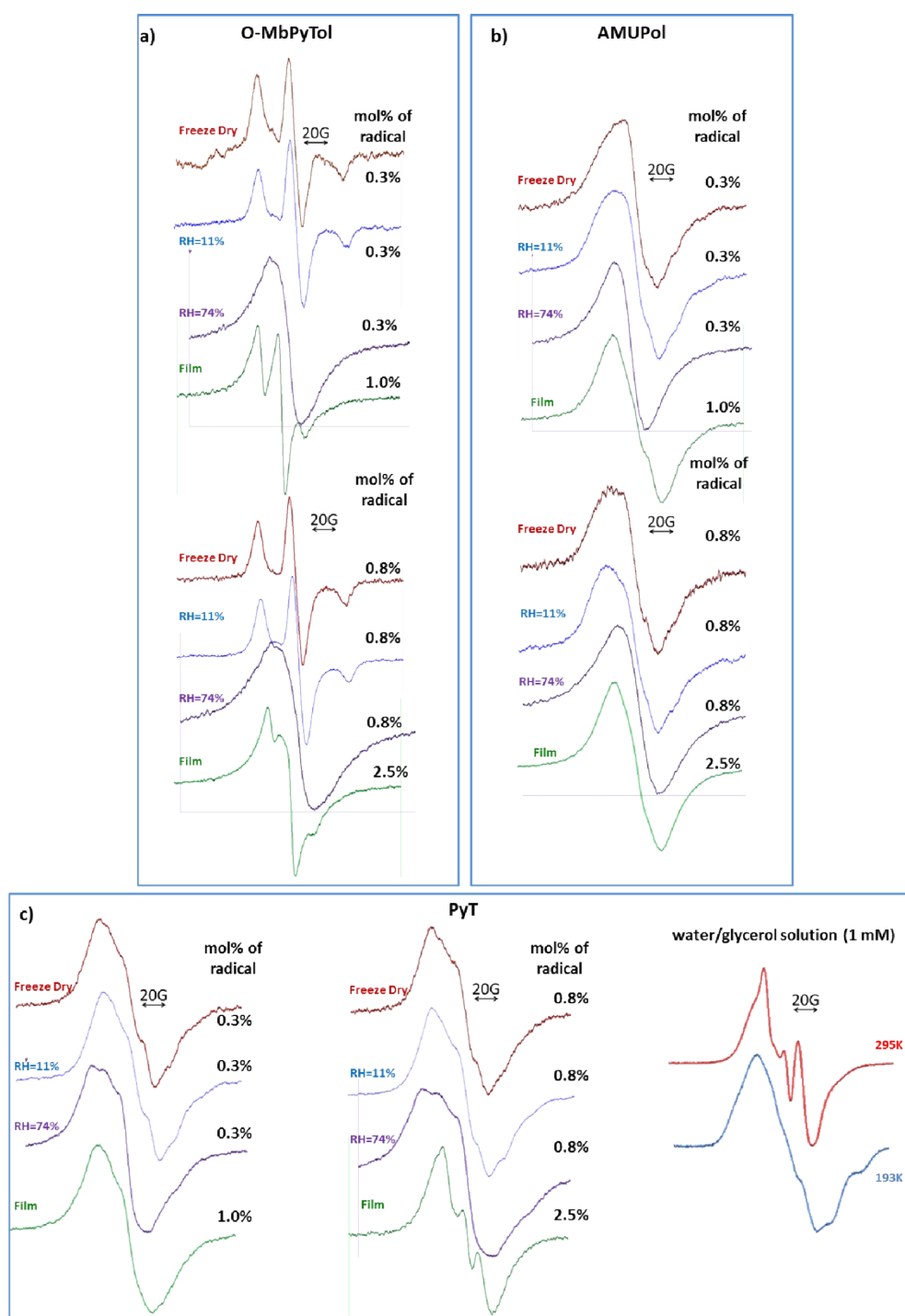
## 2. EPR data



**Figure S3.** Experimental X band EPR spectra of free radicals in trehalose matrix at room temperature. The Mol % of radicals and RH % water in matrix from TGA experiments are given on each EPR spectra. Red spectra are obtained after freeze drying. Blue spectra are obtained after freeze drying and rehydration at RH=11% with LiCl saturated solution. Green spectra are obtained after film formation. In **c)**: PyT in water/glycerol solution (1 mM) at 295 K (red spectra) and 193 K (blue spectra)
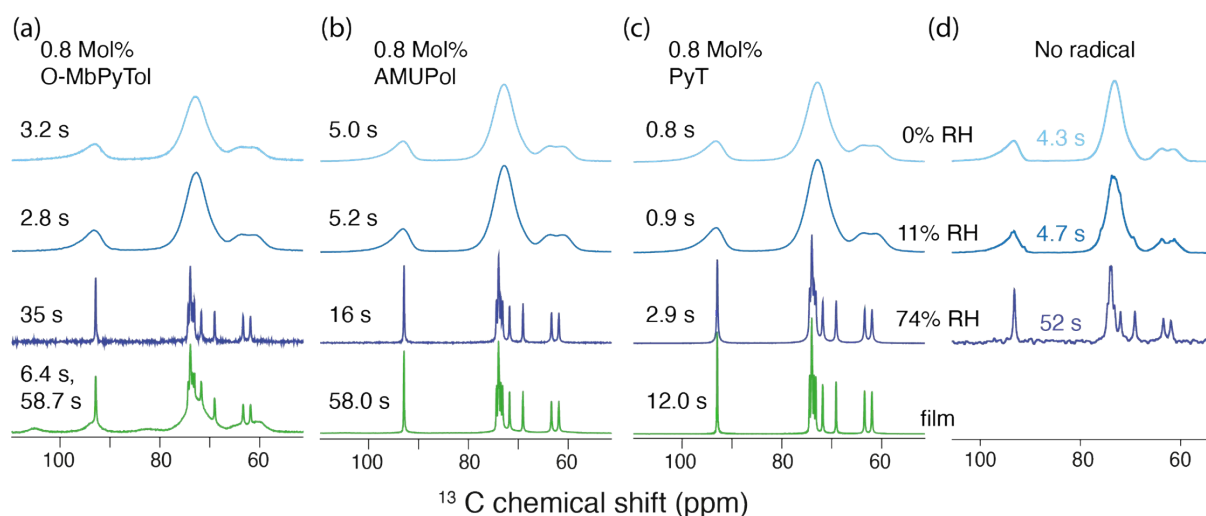
## 3. Additional DNP NMR data



**Figure S4.** (a), (b) and (c): One-dimensional DNP enhanced $^{13}$C CPMAS NMR spectra of trehalose matrices and proton polarization build-up times $T_{B,on}$ measured with a saturation recovery experiment, microwave on (the color code is the same as in Figure 2(a) in main text). The light blue, blue and dark blue spectra correspond to freeze-dried trehalose matrix containing 0.8 molar % of corresponding radical and a rehydration level of 0, 11, and 74%, respectively. (d) On the right-hand side column, the spectra in light blue, blue and dark blue are from freeze-dried trehalose matrices prepared without any radical and rehydrated at 0, 11, and 74% RH, respectively. In (a), (b) and (c), the spectra in green correspond to the film-formulation method for respective radical at the same radical concentration. Such a spectrum was not recorded for a formulation without radical. Enhancements were measured on the $^{13}$C resonance at 74 ppm of trehalose. The DNP data were at 9.4 T (on a 400 MHz DNP NMR with a gyrotron operating at a microwave frequency of 263 GHz) and of 94±2 K temperature, whereas spectra without radical were recorded at 11.75 T (on a conventional 500 MHz solid-state NMR spectrometer). A MAS frequency of 8 kHz was used for all the experiments. The proton build-up times indicated next to the spectra were measured indirectly via carbon-13 spectra, by inserting a proton saturation and recovery period just before the first proton pulse in the CP step.
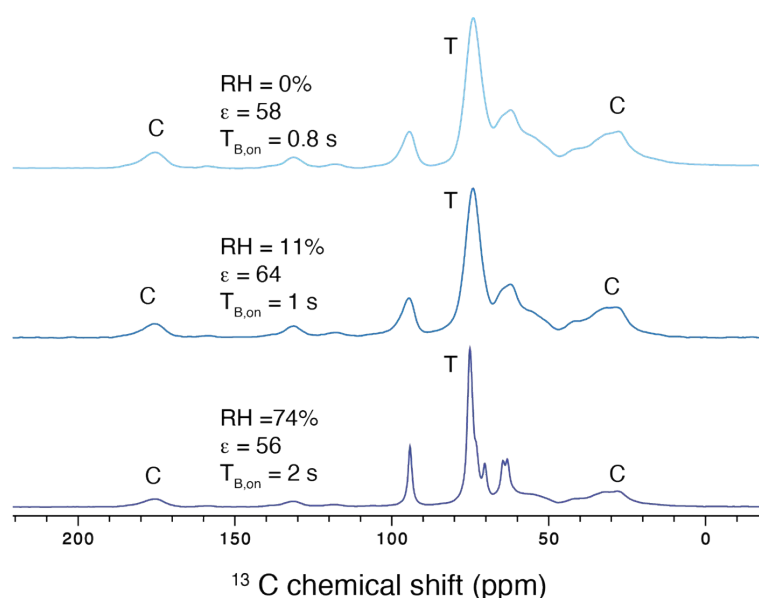


**Figure S5.** One-dimensional $^{13}$C CPMAS NMR spectra of 50/50 casein/trehalose formulations containing 2 weight% PyT and exposed to 0, 11, and 74% relative humidity (RH) respectively. Proton polarization build-up times measured on respective proton spectra using saturation recovery experiment, microwave on. The enhancement factors were measured on the protein bulk resonances and were similar for the trehalose peaks.
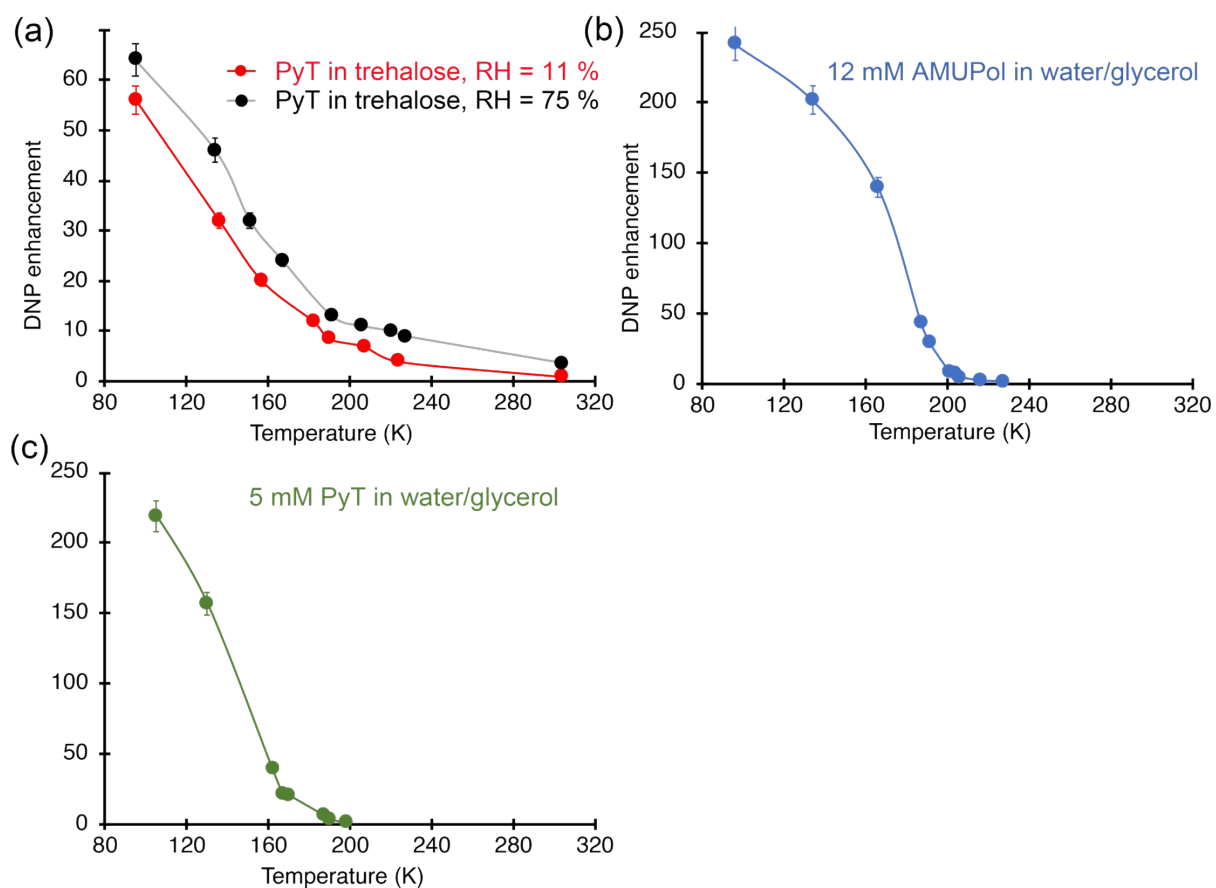
**Figure S6.** DNP enhancement factors as a function of sample temperature (measured inside the rotor). (a) The DNP enhancements shown here are measured for the 50/50 casein/trehalose sample prepared with the freeze-dried method and rehydrated at 11% RH (in red) or 75% (in black). I(b) The DNP enhancements shown here correspond to 12 mM AMUPol in a 60:30:10 glycerol-$d_8$:$D_2O$:$H_2O$ solution containing 0.2 M proline. (c) The DNP enhancements shown here correspond a 5 mM PyT in a 60:30:10 glycerol-$d_8$:$D_2O$:$H_2O$ solution.
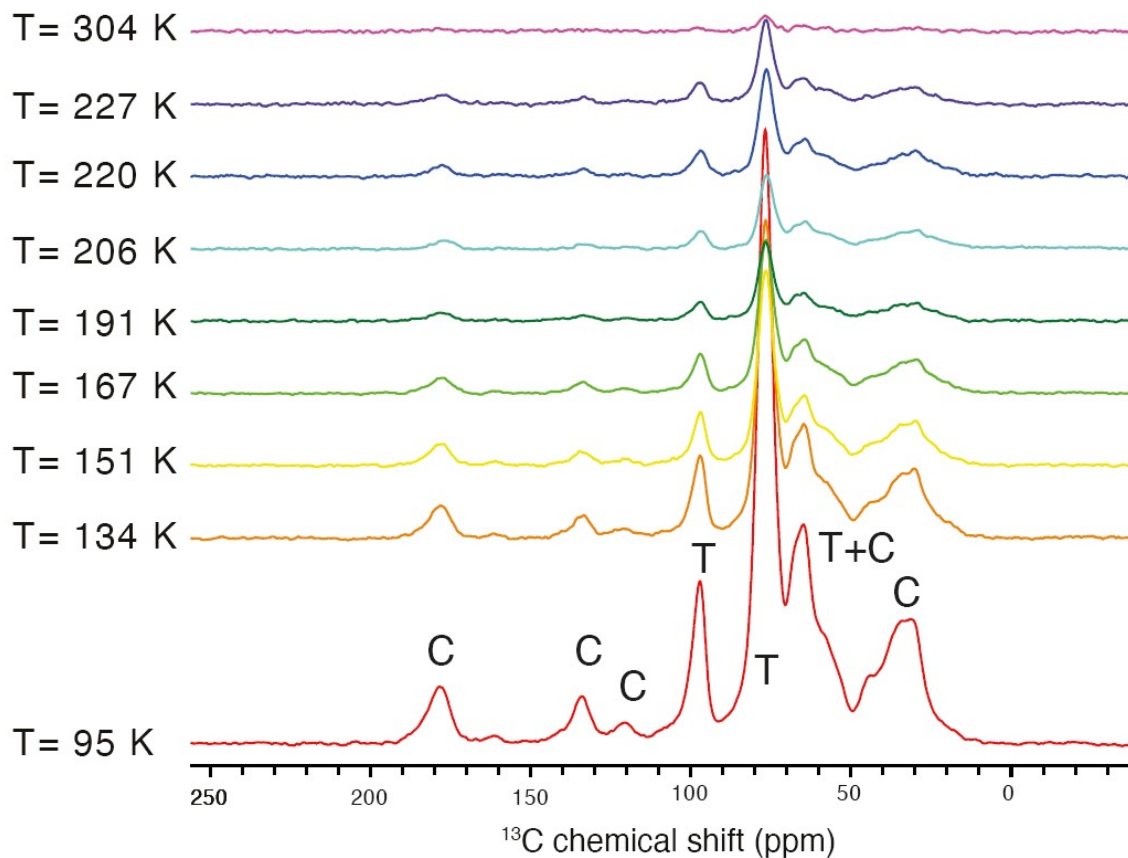
**Figure S7..** Series of carbon-13 spectra recorded as a function of sample temperature, corresponding to the data shown in Figure 4. The sample corresponds to a freeze-dried and rehydrated formulation containing 50 wt % of casein and 2 wt% in PyT (11% RH). The data were recorded in a 1.3 mm DNP MAS probe at 9.4 T (263 GHz microwave frequency). A MAS frequency of 20 kHz was used. The resonances of the Casein (C) and of the trehalyose (T) are indicated. An exponential line broadening of 100 Hz was applied to the processed data.

## 4. Details on the experimental results

A series of samples were prepared with the freeze-drying method at a higher radical concentration with no rehydration, namely 3.2 molar %. Both M-ObPyTol and PyT radicals underperform at such a high concentration. In particular, the best performing radical PyT gave an $\varepsilon$ of 10 in contrast to 44 obtained with 0.3 molar %. Additionally, at 3.2 molar% concentration, a proton build-up time of less than 1 s was measured and the NMR resonances were significantly broadened. Therefore, only 0.3 molar % and 0.8 molar % radical concentrations were further investigated.

**Table S1.** Summary of $^1H$ enhancements obtained at higher molar % of radical in the trehalose matrix. Measurements were done at 100 K (temperature sense by auxiliary thermocouple sensor in the probe) using a 3.2 mm probe on a 400 MHz DNP spectrometer.

| Radical | Mol% (radical) | $^1H$ enhancement |
|---|---|---|
| M-ObPyTol | 3.2% | 5.6 |
| M-ObPyTol and 5 mM Casein | 3.2% | 5.4 |
| PyT and 5 mM Casein | 3.2% | 10 |

**Table S2.** Summary of data shown in Figure 2 of main text and in Figure S4. Enhancements and $T_{B,ON}$ were measured for the freeze-dried samples and film samples. To estimate the sensitivity enhancement per unit of time, E= $\varepsilon$ /sqrt($T_{1B,on}$) was calculated in each case. The error on the enhancement factor is estimated to be 10% as described above. The experimental points of the inversion-recovery curve were fitted using a monoexponential function. The biggest error of the fit was found to be ± 7 %. In some cases, a bi-exponential function was considered.

| RH | ObPyTol | | | | | | AMUPOL | | | | | | PyT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.3 molar% | | | 0.8 molar% | | | 0.3 molar% | | | 0.8 molar% | | | 0.3 molar% | | | 0.8 molar% | | |
| | $\varepsilon$ | $T_{B,on}$ | E | $\varepsilon$ | $T_{B,on}$ | E | $\varepsilon$ | $T_{B,on}$ | E | $\varepsilon$ | $T_{B,on}$ | E | $\varepsilon$ | $T_{B,on}$ | E | $\varepsilon$ | $T_{B,on}$ | E |
| 0 % | 11 | 9.1 | 3.6 | 11 | 3.2 | 6.1 | 32 | 9 | 10.7 | 24 | 5.0 | 10.7 | 52 | 3 | 30.0 | 29 | 0.8 | 32.4 |
| 11 % | 13 | 8.5 | 4.5 | 12 | 2.8 | 7.2 | 32 | 8.4 | 11.1 | 24 | 5.2 | 10.5 | 65 | 3.7 | 33.8 | 31 | 0.9 | 32.7 |
| 74 % | 1.7 | 57 | 0.2 | 1.5 | 35 | 0.3 | 12 | 15 | 3.1 | 10 | 16 | 2.5 | 42 | 6.1 | 17 | 24 | 2.9 | 6.1 |
| film | | | | 11 | 58.7 6.4 | 1.4 4.3 | 55 | 28 | 10.4 | 30 | 58 | 3.9 | 52 | 8.1 | 18.27 | 30 | 12 | 9.1 |

**Table S3.** Summary of the data presented in Figure 3 of the main text. Proton build-up times and enhancements were measured for each formulation. The signal to noise ratio per unit of time and unit of mass was calculated in the protein or trehalose region. For samples at higher protein ratio a biexponential $^1$H $T_{B, on}$ is observed and we report here the two components.
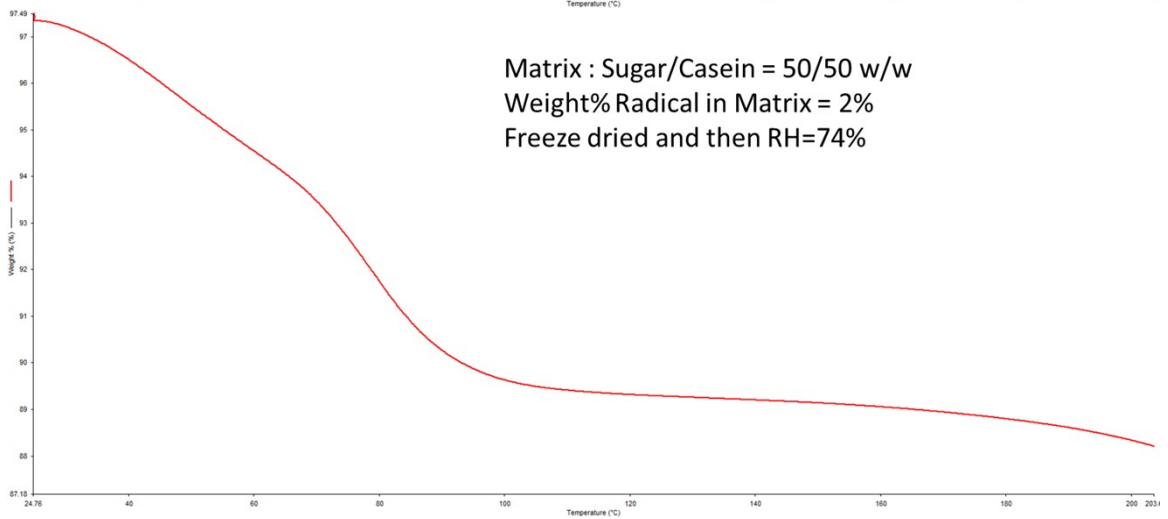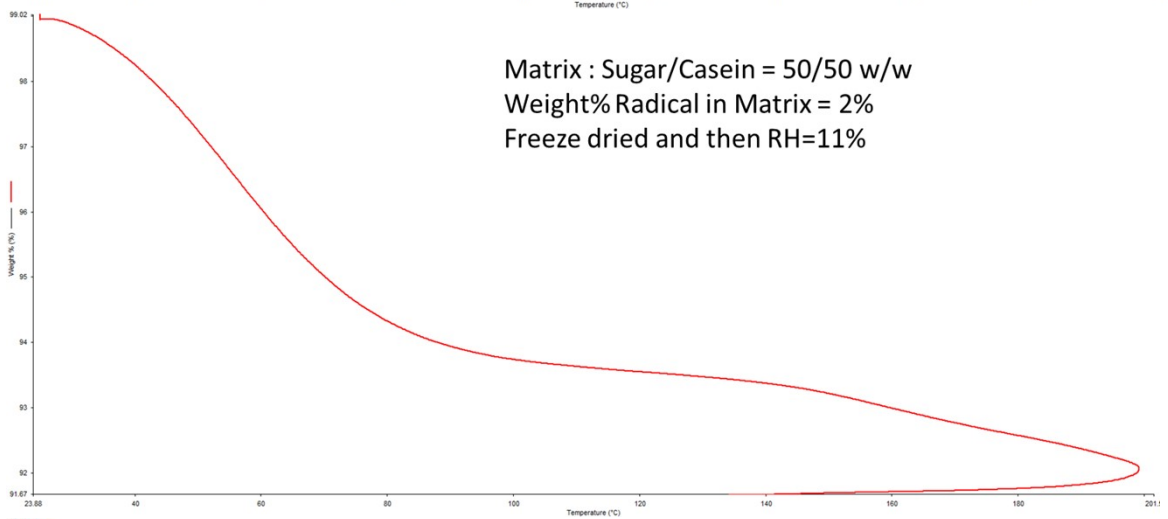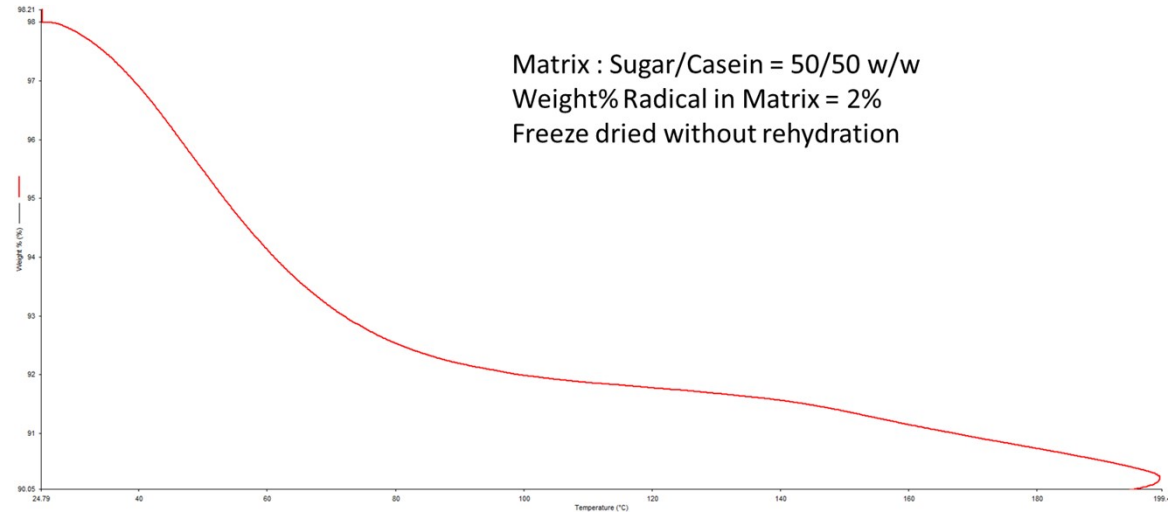
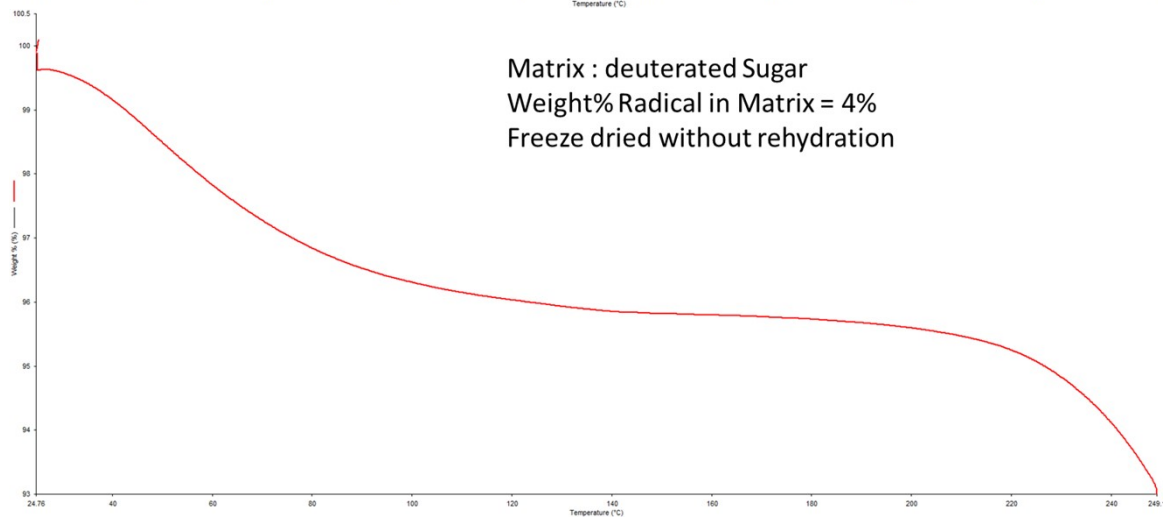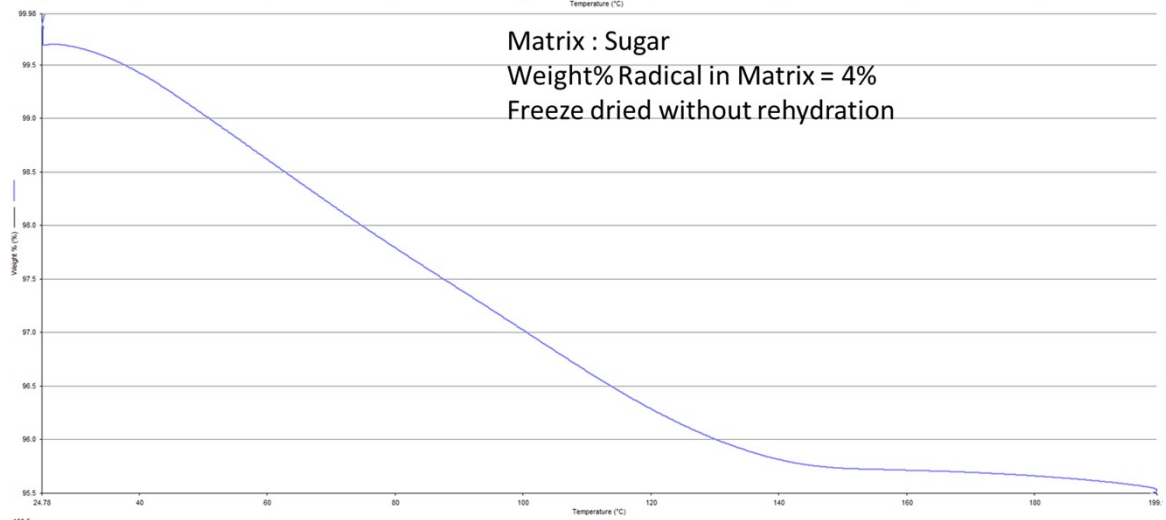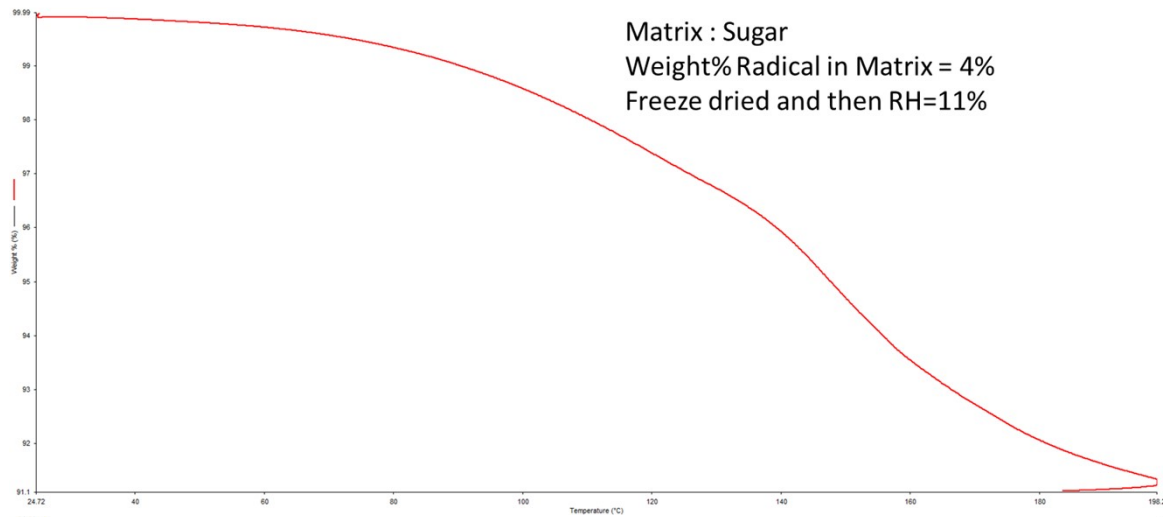| Ratio of casein/sugar | PyT weight% in matrix | $\varepsilon(^1H)$ DNP | $\varepsilon(^{13}C\ CP)$ casein | $\varepsilon(^{13}C\ CP)$ trehalose | $^1H\ T_{B,on}$ (s) | Weight (mg) | Sensitivity per unit time and mass on protein region | Sensitivity per unit time and mass on trehalose region |
|---|---|---|---|---|---|---|---|---|
| 20/80 | 0.8 | 51 | 48 | 48 | 2 | 2.8 | 7.8 | 128.7 |
|  | 2 | 40 | 40 | 40 | 0.9 | 2.8 | 14.7 | 56 |
| 50/50 | 0.8 | 48 | 42 | 44 | 2 | 2.5 | 35.4 | 139.3 |
|  | 2 | 54 | 58 | 60 | 0.8 | 2.3 | 51.6 | 64 |
| 80/20 | 0.8 | 31 | 29 | 32 | 1.5 | 2.5 | 40.2 | 38.9 |
|  | 2 | 38 | 35 | 38 | 0.6, 9.5 | 2.3 | 54.5 | 54 |
|  | 4 | 26 | 26 | 26 | 0.3, 17 | 2.6 | 17.1 | 14.6 |

**Table S4.** Enhancement vs temperature for the 50/50 casein/sugar formulation prepared with a 1.55 mM concentration of PyT at 11% and 75% RH. The data are compared with a frozen solution PyT in 60:30:10 glycerol-$d_8$:$D_2O$:$H_2O$ (5 mM radical concentration), and with a frozen solution of AMUPol in 60:30:10 glycerol-d8:D2O:H2O containing 0.2 M proline (12 mM radical concentration). The enhancements were measured on the carbon-13 signals of the protein resonances.

| Trehalose matrix 11 % RH PyT radical | | Trehalose matrix 75 % RH PyT radical | | DNP juice PyT radical | | DNP juice AMUPol radical | |
|---|---|---|---|---|---|---|---|
| Temp. (K) | $\varepsilon_{C\ CP}$ | Temp. (K) | $\varepsilon_{C\ CP}$ | Temp (K) | $\varepsilon_{C\ CP}$ | Temp. (K) | $\varepsilon_{C\ CP}$ |
| 95 | 64 | 95 | 56 | 105 | 210 | 99 | 274 |
| 134 | 46 | 136 | 32 | 105 | 219 | 105.5 | 246 |
| 151 | 32 | 157 | 20 | 130 | 157 | 117 | 236 |
| 167 | 24 | 182 | 12 | 162 | 39 | 127 | 216 |
| 191 | 13 | 190 | 8.5 | 167 | 22 | 147 | 195 |
| 206 | 11 | 207 | 7 | 170 | 21 | 168 | 82 |
| 220 | 10 | 224 | 4 | 187 | 7 | 193 | 38 |
| 227 | 9 | 304 | 1 | 190 | 4 | 198 | 11 |
| 304 | 3.6 |  |  | 198 | 1 |  |  |

## 5. TGA data

Thermogravimetric analysis (TGA) measurements were carried out on a TGA 8000 thermogravimetric analyzer (Perkin Elmer). All the samples were preheated to 25°C and kept at this temperature for 5 min then heating to 200°C, with a heating rate of 10°C/min in a nitrogen atmosphere.



Matrix : Sugar/Casein = 50/50 w/w
Weight% Radical in Matrix = 2%
Freeze dried without rehydration

Matrix : Sugar/Casein = 50/50 w/w
Weight% Radical in Matrix = 2%
Freeze dried and then RH=11%

Matrix : Sugar/Casein = 50/50 w/w
Weight% Radical in Matrix = 2%
Freeze dried and then RH=74%

Matrix : Sugar
Weight% Radical in Matrix = 4%
Freeze dried and then RH=11%

Matrix : Sugar
Weight% Radical in Matrix = 4%
Freeze dried without rehydration

Matrix : deuterated Sugar
Weight% Radical in Matrix = 4%
Freeze dried without rehydration
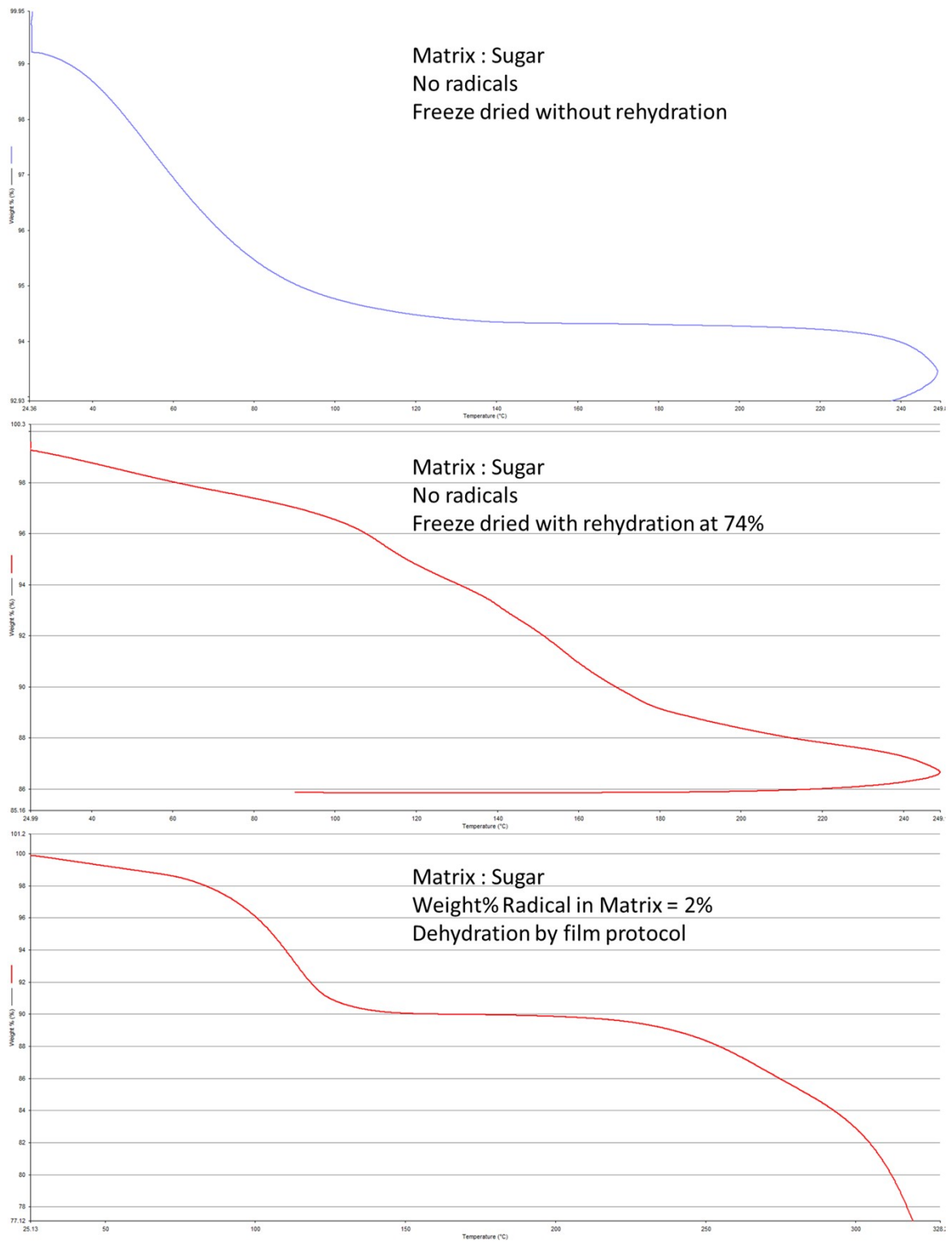
**Figure S8.** TGA experiments for the determination of the water content in trehalose matrixes.

|  | Weight % of water | | |
| --- | --- | --- | --- |
|  | **PyT 5% wgt** | **Casein 50/50 w/w PyT 2% wgt** | **Trehalose** |
| Freeze dry | 4% | 6.3% | 4.8% |
| Freeze dry and RH 11% | 3% | 5.3% | 3.1% |
| Freeze dry and RH 74% | 10.5% | 11% | 12% |

**Table S5:** Weight % of water in different samples.

## 6. DSC data

DSC measurements were carried out using DSC Q20 (TA Instruments) under $N_2$ atmosphere with cooling/heating rate of 10°C/min in the temperature range from −90°C to 150°C.
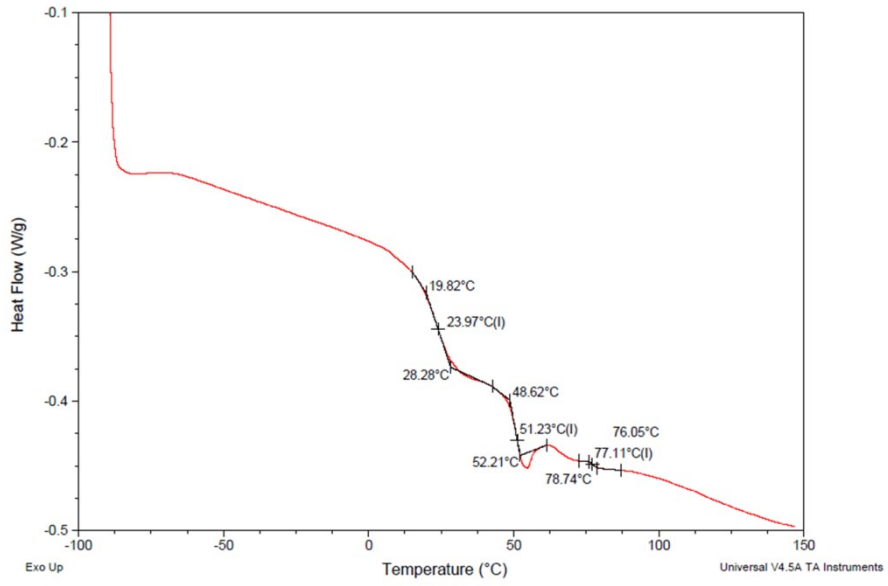
**a)**



**b)**
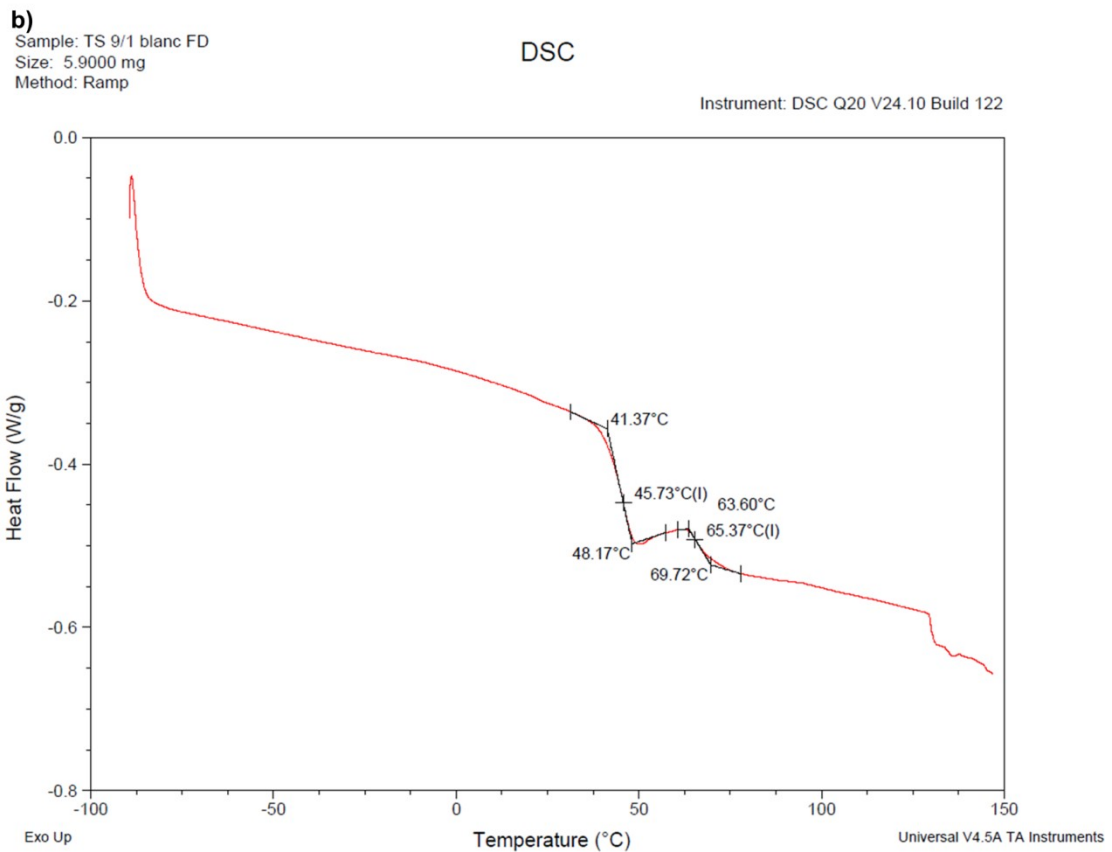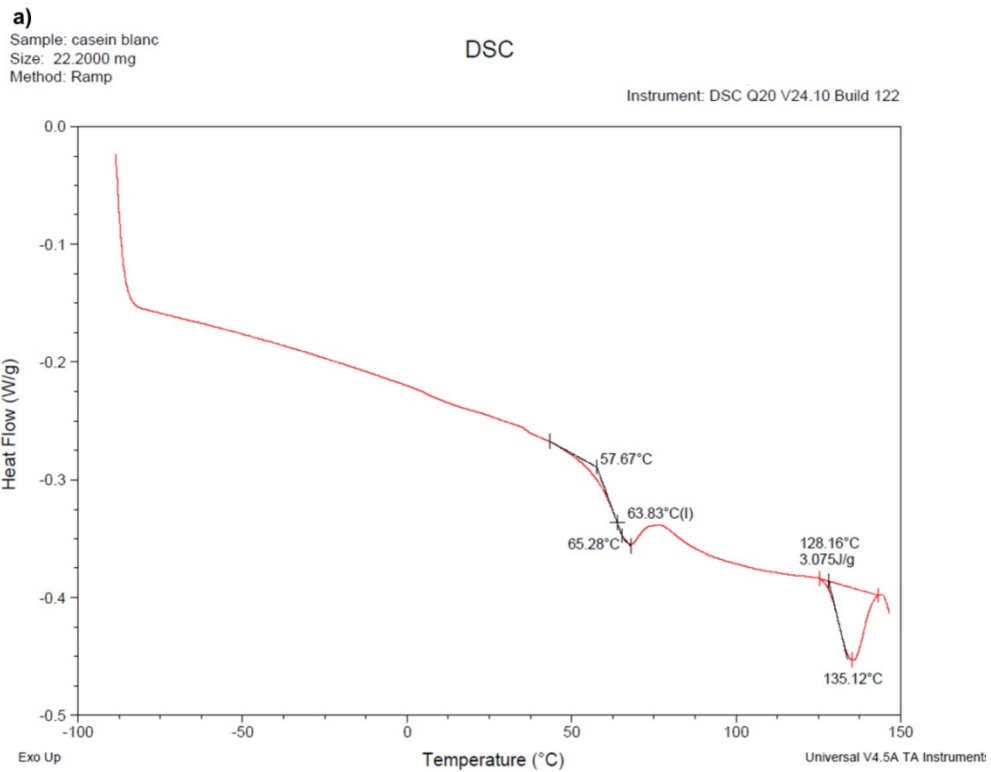
**c)**

Sample: TS.casein 100.0 pyt.mat1.20 FD
Size: 6.2000 mg
Method: Ramp

DSC

Instrument: DSC Q20 V24.10 Build 122



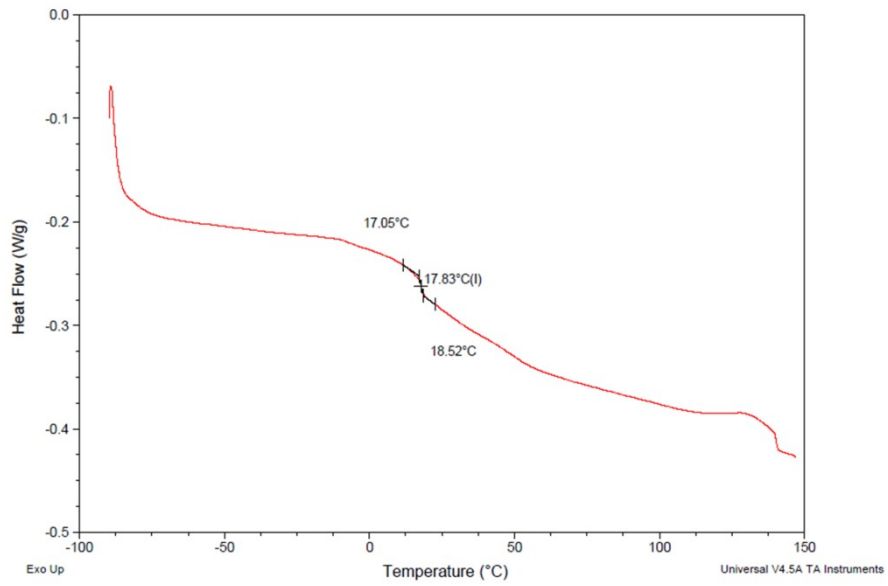Exo Up                    Temperature (°C)                    Universal V4.5A TA Instruments

**d)**

Sample: TS.casein 50.50 pyt.mat1.20 11rh
Size: 6.2000 mg
Method: Ramp

DSC

Instrument: DSC Q20 V24.10 Build 122



Exo Up                    Temperature (°C)                    Universal V4.5A TA Instruments

**e)**



Sample: TS.casein 50.50 pyt.mat1.20 74rh
Size: 7.7000 mg
Method: Ramp

DSC

Instrument: DSC Q20 V24.10 Build 122

15.76°C
16.96°C(I)
17.00°C

Heat Flow (W/g)
Temperature (°C)
Exo Up
Universal V4.5A TA Instruments

**f)**



Sample: TS.casein 50.50 pyt.matrix1.20 F
Size: 3.3000 mg
Method: Ramp

DSC

Instrument: DSC Q20 V24.10 Build 122

16.27°C
16.69°C(I)
17.90°C
31.05°C
34.36°C(I)
37.01°C

Heat Flow (W/g)
Temperature (°C)
Exo Up
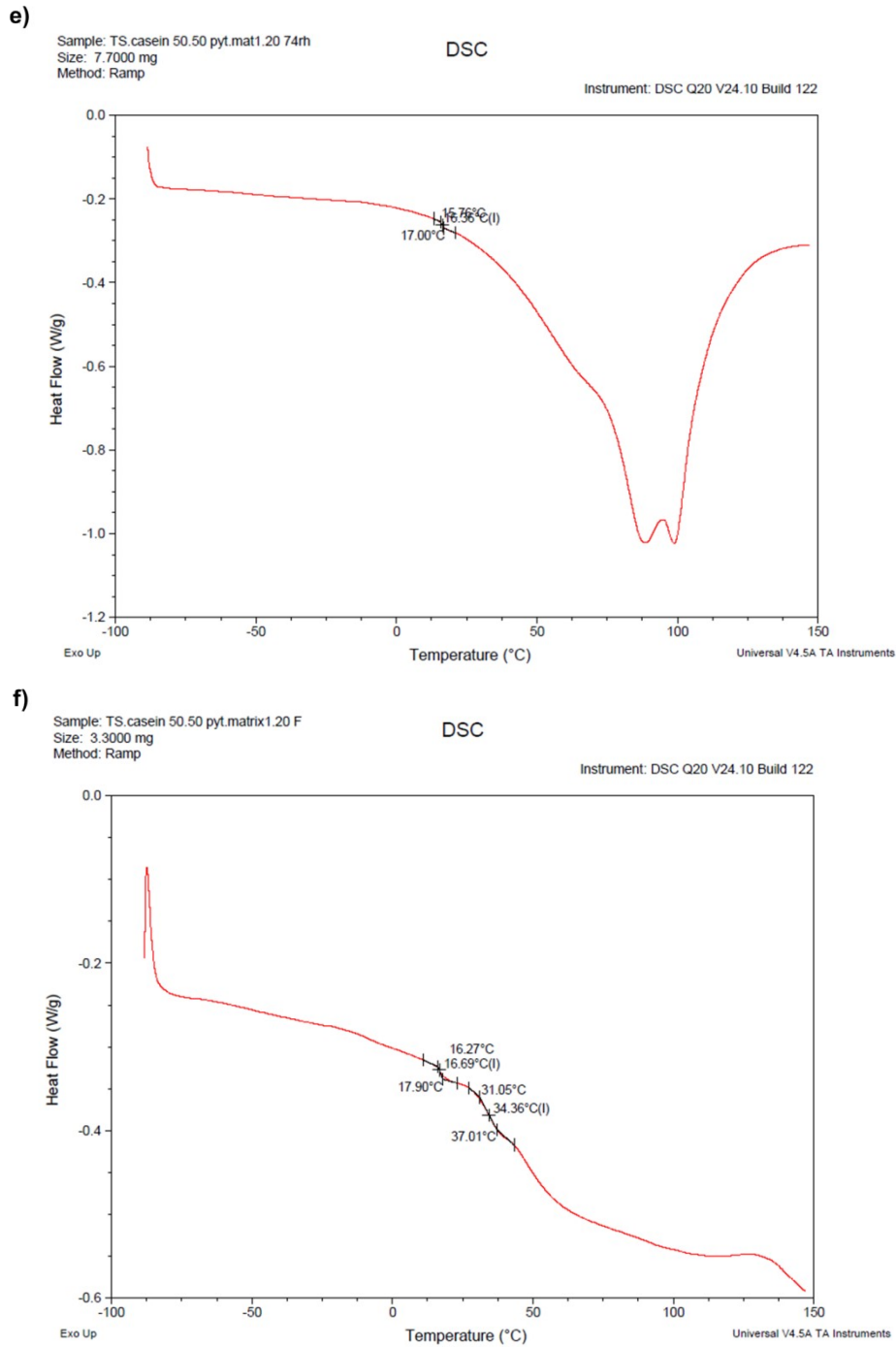Universal V4.5A TA Instruments

**Figure S9.** DSC experiments of **a)** Casein alone; **b)** Freeze dried trehalose matrix; **c)** Freeze-dried trehalose matrix and PyT 5 wt% **d)** Freeze dried trehalose matrix and casein 50 wt% with PyT 5 wt%, RH=11 %; **e)** Freeze dried trehalose matrix and casein 50 wt% with PyT 5 wt%, RH=74 %; **f)** Freeze dried trehalose matrix and casein 50 wt% with PyT 5 wt%, RH=0 %

## 7. References

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