**Supporting Information**
**(10 pages, 3 Tables, 6 Figures)**

# Facilitating nitrification inhibition through green, mechanochemical synthesis of a novel nitrapyrin complex

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**Single Crystal X-ray diffraction**

**Table S1.** Crystal data and details of measurement for nitrapyrin (NP).

| | **NP** |
|---|---|
| **Formula** | $C_6 H_3 Cl_4 N$ |
| **Mr (g mol$^{-1}$)** | 230.89 |
| **Temperature (K)** | 293 |
| **Morphology, colour** | Prism, colourless |
| **Crystal System** | Monoclinic |
| **Space group** | $P2_1/m$ |
| **Z** | 4 |
| **a (Å)** | 9.0327(4) |
| **b (Å)** | 7.2075(6) |
| **c (Å)** | 13.7485(6) |
| **α (deg)** | 90 |
| **β (deg)** | 102.079(4) |
| **γ (deg)** | 90 |
| **V (Å$^3$)** | 875.25(9) |
| **d (g/cm$^3$)** | 1.752 |
| **μ (mm$^{-1}$)** | 1.281 |
| **θ-range (deg)** | 3.645 – 26.301 |
| **Refls collected/unique** | 3920/2139 |
| **GoF** | 1.0354 |
| **Threshold expression** | $Fo > 4\sigma(Fo)$ |
| **R1(obs)** | 0.0570 |
| **wR2 (all)** | 0.1798 |

**Powder X-ray diffraction.**

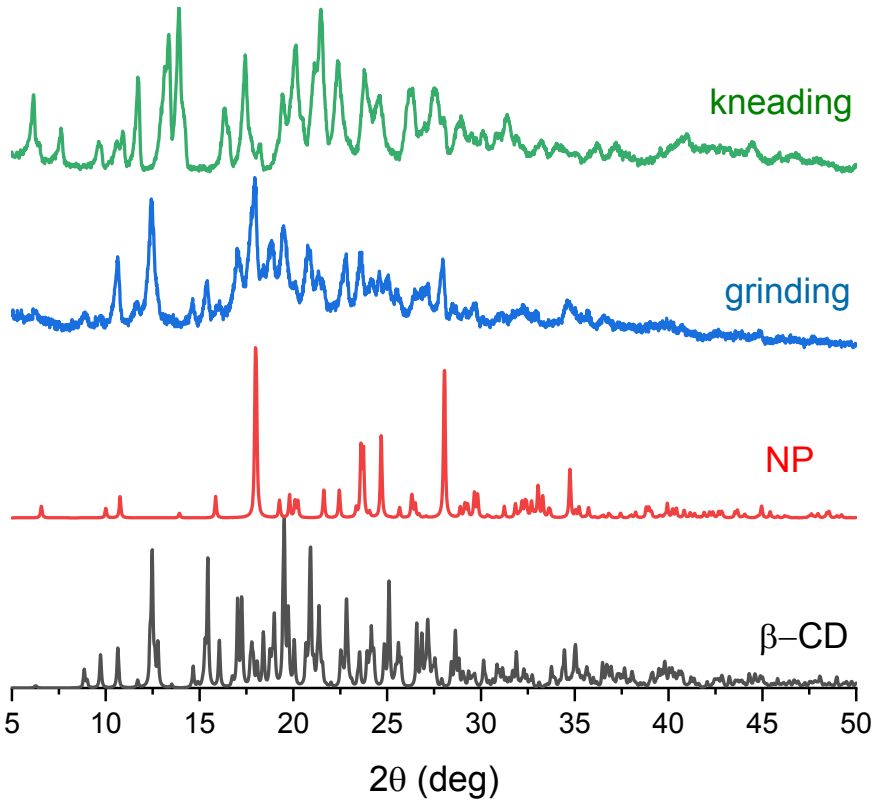
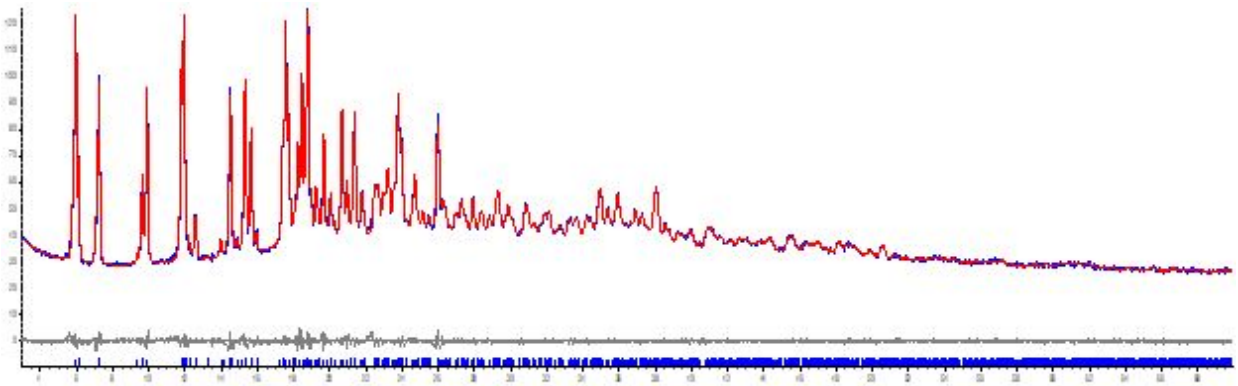



**Fig. S1.** Comparison of the XRPD patterns of β-CD·NP synthesized by kneading (green line) with the starting materials β-CD (black line) and NP (red line): the powder pattern of the complex is clearly distinct from the diffractograms of the reagents and from the one of the grinding mixture (blue line).



| S.G. | a (Å) | b (Å) | c (Å) | β (°) | V (Å)$^3$ | **Rwp** |
|---|---|---|---|---|---|---|
| **C2** | 19.0117 | 24.4573 | 15.7788 | 110.2143 | 6884.93 | **3.387** |

**Fig. S2**. Pawley refinement plot with the corresponding unit cell parameters and FOM for the inclusion complex β-CD·NP synthesized via mechanochemistry.

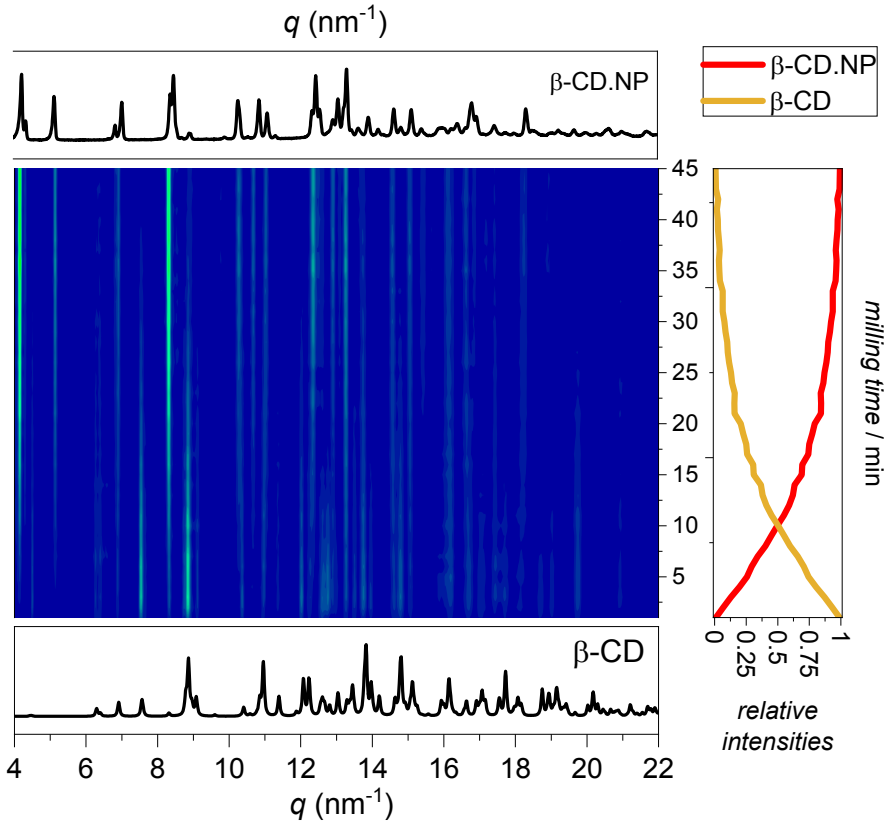



**Fig. S3.** 2D plot as in Figure 2 of the main text, but without the red and orange boxes.

**Raman spectroscopy**

**Table S2**. Raman wavenumbers ($cm^{-1}$) and assignments for the main bands of β-CD,[1] NP[2–4] and β-CD·NP [For sake of clarity, NP and β-CD are indicated here as N and B, respectively].

| β-CD | NP | β-CD/NP | Assignments |
|---|---|---|---|
| | 3133 | 3138 | CH stretching (N) |
| | 3073 | 3083 | CH stretching (N) |
| 2943 | | 2945 | CH stretching (B) |
| 2908 | | 2905 | CH stretching (B) |
| | 1564 | 1568 | ring stretching + CH in plane bending (N) |
| 1466 | | 1462 | CH bending (B) |
| 1453 | | 1456 | CH bending (B) |
| | 1434 | 1434 | ring stretching + CH in plane bending (N) |
| | 1401 | 1401 | ring stretching + CH in plane bending (N) |
| 1340 | | 1340 | OH in plane bending (B) |

| | | | |
|---|---|---|---|
| | 1281 | 1271 | CH in plane bending (N) |
| 1258 | | 1261 | OH in plane bending (B) |
| 1248 | | 1245 | OH in plane bending (B) |
| | 1209 | 1215 | ring stretching (N) |
| | 1159 | 1143 | ring stretching (N) |
| | 1093 | 1113 | CH in plane bending + $CCl_3$ asymmetric stretching (N) |
| 1083 | | 1089 | coupled CC and CO stretching (B) |
| 1042 | | 1046 | coupled CC and CO stretching (B) |
| | 988 | 988 | ring breathing (N) |
| 948 | | 948 | ring vibration (B) |
| | 931 | 927 | CH out of plane bending (N) |
| | 810 | 817 | CH out of plane bending (N) |
| | 776 | 762 | $CCl_3$ symmetrical deformation mode (N) |
| | 633 | 633 | $CCl_3$ asymmetrical deformation mode (N) |
| 587 | | 594 | ring vibration (B) |
| 576 | | 576 | ring vibration (B) |
| 480 | | 480 | ring vibration (B) |
| 440 | | 440 | ring vibration (B) |
| | 411 | 411 | $C_{ring}$-Cl stretching (N) |

With regard to β-CD, the main variations are attributable to CH stretching (change in the band profile at about 3000-2900 $cm^{-1}$), CH bending (at about 1400-1380 $cm^{-1}$), OH in plane bending (at about 1330 $cm^{-1}$), coupled CC and CO stretching (in the 1050−1030 $cm^{-1}$ range), and ring and skeletal vibrations (600−400 $cm^{-1}$). It is interesting to note that the bands assignable to β-CD appear strengthened.

The main bands of NP that undergo changes are C-H stretching (at about 3100 $cm^{-1}$), CH bending (around 1400 $cm^{-1}$), ring stretching (at about 1200-1100 $cm^{-1}$) and C-Cl stretching (411 $cm^{-1}$). It can be observed that some NP bands noticeably vary in intensity because of the interaction with β-CD. These results prove the occurrence of interactions between NP and β-CD because they indicate a change in the chemical environment experienced by both components upon formation of the inclusion complex.

**Inhibition tests**

**Table S3.** Full data related to Figure 6 in the main text.

$O_2$ consumption by *N. europaea* in the absence of inhibitors, in the presence of 100 µM NP or in the presence of 100 µM β-CD·NP complex, at t = 0 h

| | Mean (%) | SD (%) |
|---|---|---|
| *Nitrosomonas europaea* | 100 | 0.3 |
| +NP 100 µM | 52.4 | 0.3 |
| + β-CD·NP 100 µM | 57.7 | 0.6 |

$O_2$ consumption after 2 h incubation of *N. europaea* in the absence of inhibitors, in the presence of 100 µM NP or in the presence of 100 µM β-CD·NP complex, at t = 2 h

| | Mean (%) | SD (%) |
|---|---|---|
| *Nitrosomonas europaea* | 100 | 0.2 |
| +NP 100 µM | 15.4 | 0.5 |
| + β-CD·NP 100 µM | 15.7 | 0.2 |

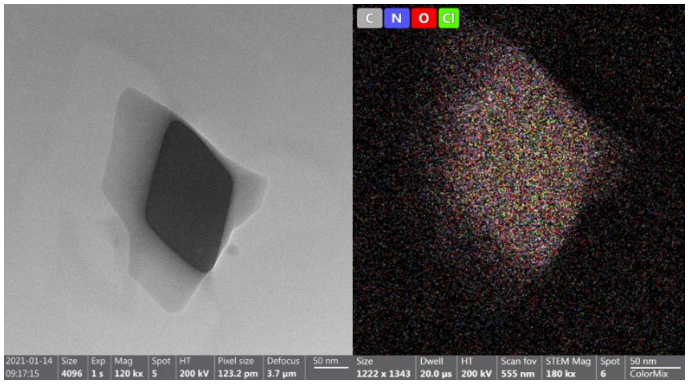
Nitrite production by *N. europaea* in the absence of inhibitors, in the presence of 100 µM NP or in the presence of 100 µM β-CD·NP complex, at t = 0 h

| | Mean (%) | SD (%) |
|---|---|---|
| *Nitrosomonas europaea* | 100 | 0.03 |
| +NP 100 µM | 113.2 | 0.03 |
| + β-CD·NP 100 µM | 118.9 | 0.03 |

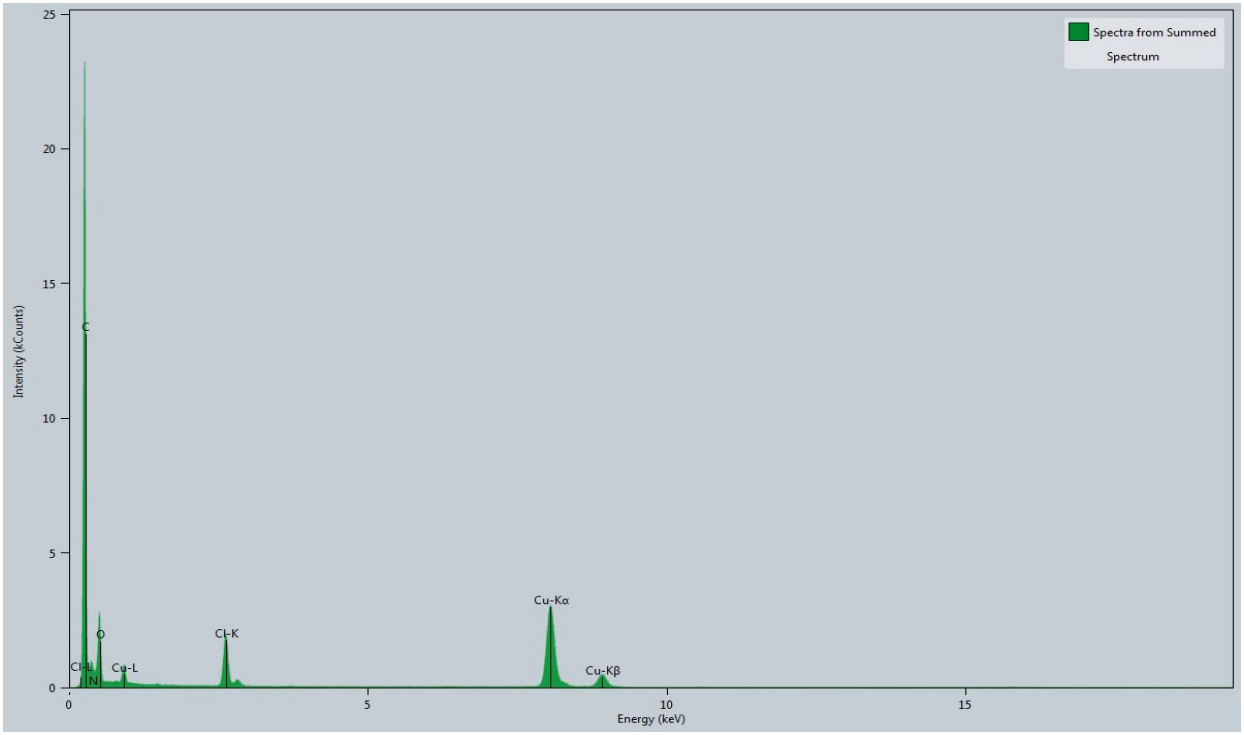
Nitrite production after 2 h incubation of *N. europaea* with increasing amount of NP or β-CD·NP complex

| | Mean (%) | SD (%) |
|---|---|---|
| *Nitrosomonas europaea* | 100 | 0.012 |
| +NP 10 µM | 79.6 | 0.004 |
| +NP 25 µM | 24.0 | 0.006 |
| +NP 50 µM | 0.05 | 0.014 |
| +NP 100 µM | 0.01 | 0.022 |
| *Nitrosomonas europaea* | 100 | 0.012 |
| + β-CD·NP 10 µM | 79.6 | 0.012 |
| + β-CD·NP 25 µM | 26.6 | 0.006 |
| + β-CD·NP 50 µM | 0.05 | 0.020 |
| + β-CD·NP 100 µM | 0.01 | 0.006 |

**TEM and EDX**




(a)




(b)

**Fig. S4.** (a) Elemental mapping on a single crystal of the inclusion complex β-CD·NP, and (b) the corresponding spectrum resulting from the contribution of each element in the sample: C, N, Cl and O atoms are all detectable.

**4. $^1$H-NMR spectroscopy**[5]

β-CD·NP powder was dissolved in $D_2O$ and an $^1$H-NMR spectrum was recorded on a Varian INOVA 400 (400 MHz) spectrometer. Chemical shifts are reported in ppm, with tetramethylsilane as internal reference standard.

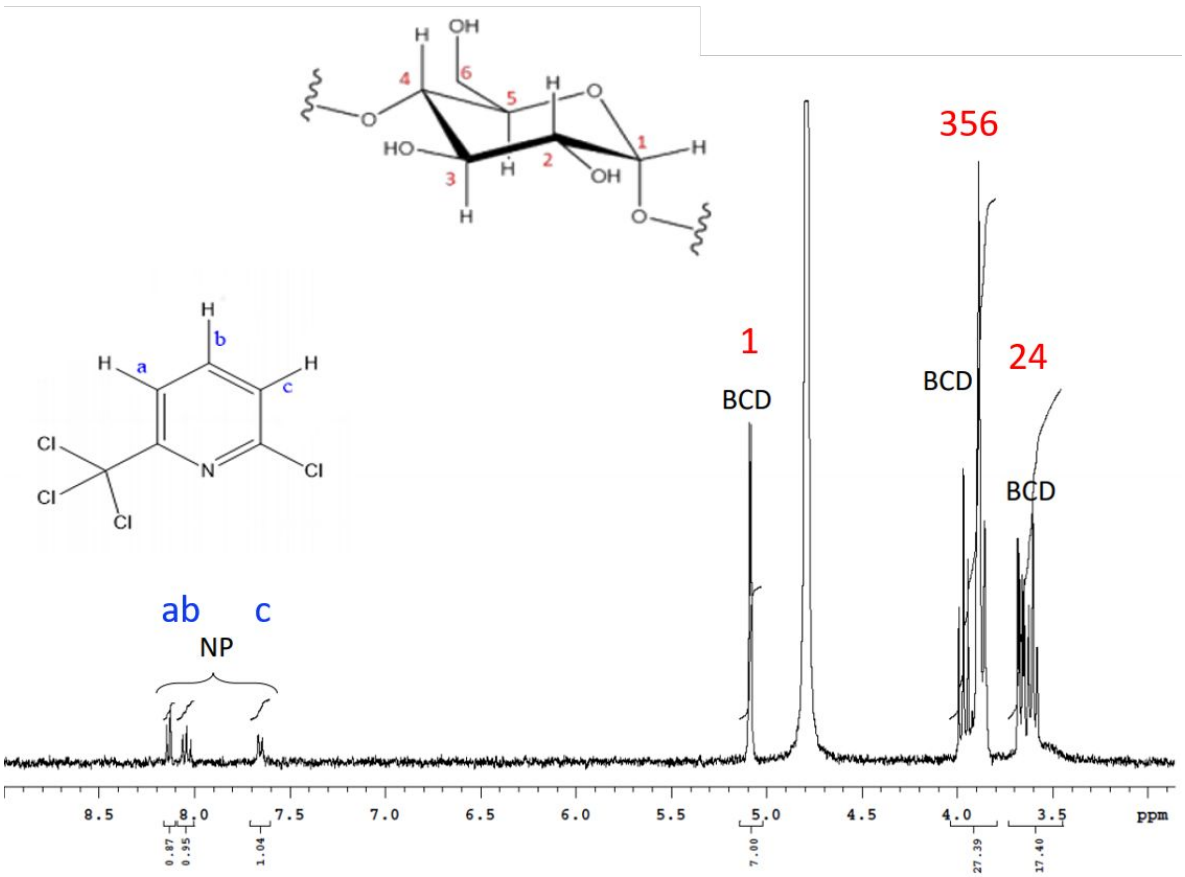



**Fig. S5**. $^{1}$H-NMR spectrum in $D_2O$ of the inclusion complex β-CD·NP mechanochemically synthesized (the purity of the sample was previously verified by PXRD analysis). Evidence of the inclusion complex formation is found in the signals of NP, which would otherwise be undetectable given its insolubility in water. By comparing the integration of the peaks corresponding to the two components, it was possible to calculate the host/guest stoichiometric ratio of the complex; integration of diagnostic peaks for β-CD (H1, 7.00 H, δ≈5.1 ppm) and nitrapyrin (Hc, 1.04 H, δ≈7.7 ppm) confirms the 1:1 stoichiometry.

**5. Thermogravimetric Analysis (TGA)**

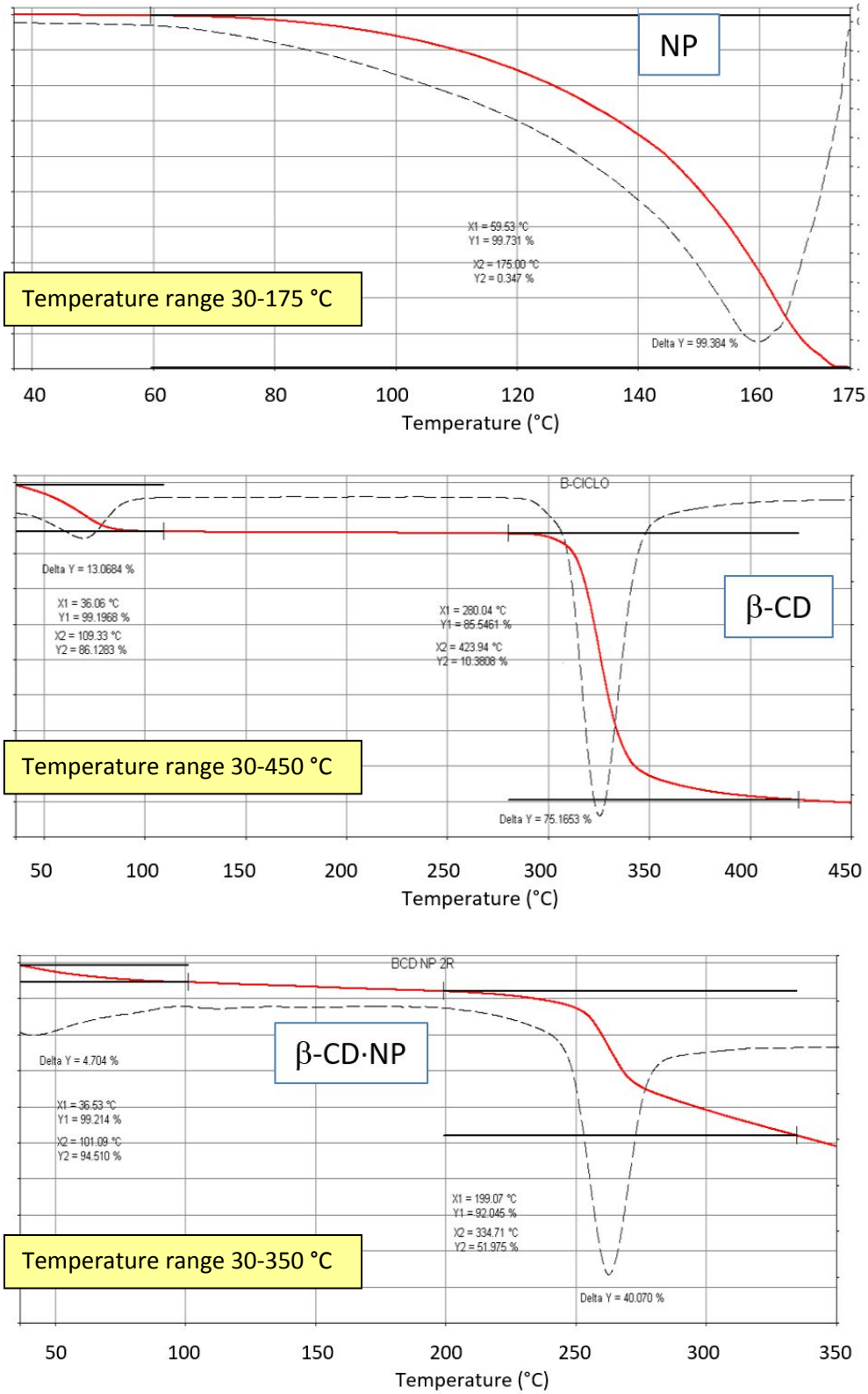



**Fig. S6.** TGA traces for NP (top), β-CD (middle) and β-CD·NP (bottom). The weight loss at T < 100 °C for the complex corresponds to ca. 3 water molecules per formula unit.

**References**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