

## Boron Rich Nanotube Drug Carrier System Is Suited for Boron Neutron Capture Therapy – Supplemental Information

**\*Fabian Heide**<sup>a</sup> - Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

**Matthew McDougall**<sup>a</sup> - Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

**Candice Harder-Viddal** - Department of Chemistry and Physics, Canadian Mennonite University, Winnipeg, MB R3P 2N2, Canada

**Roy Roshko** - Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

**David Davidson** - Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

**Jiandong Wu** - Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

**Camila Aprosoff** - Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

**Aniel Moya-Torres** - Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

**Francis Lin** - Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

**\*Jörg Stetefeld** - Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

\*Corresponding Authors:

Jörg Stetefeld - +1(204)474-9731, Jorg.Stetefeld@umanitoba.ca

Fabian Heide - +1(204)332-0853, Heidef@myumanitoba.ca

<sup>a</sup> These authors have contributed equally and share first authorship.

## Crystallographic Data Table

Table S1. Crystallographic data table of RHCC-NT in complex with *o*-carborane (PDB ID 7R6H).

| <i>RHCC-NT o-carborane</i>            |                             |
|---------------------------------------|-----------------------------|
| <i>Wavelength</i>                     | 1.542                       |
| <i>Resolution range</i>               | 18.57 - 2.2 (2.279 - 2.2)   |
| <i>Space group</i>                    | P 21 21 21                  |
| <i>Unit cell</i>                      | 34.45 55.92 110.23 90 90 90 |
| <i>Total reflections</i>              | 22424 (2218)                |
| <i>Unique reflections</i>             | 11221 (1110)                |
| <i>Multiplicity</i>                   | 2.0 (2.0)                   |
| <i>Completeness (%)</i>               | 98.01 (98.66)               |
| <i>Mean I/sigma(I)</i>                | 14.97 (8.33)                |
| <i>Wilson B-factor</i>                | 21.6                        |
| <i>R-merge</i>                        | 0.02599 (0.06346)           |
| <i>R-meas</i>                         | 0.03676 (0.08975)           |
| <i>R-pim</i>                          | 0.02599 (0.06346)           |
| <i>CC1/2</i>                          | 0.999 (0.994)               |
| <i>CC*</i>                            | 1 (0.999)                   |
| <i>Reflections used in refinement</i> | 11174 (1105)                |
| <i>Reflections used for R-free</i>    | 826 (84)                    |
| <i>R-work</i>                         | 0.2217 (0.2502)             |
| <i>R-free</i>                         | 0.2666 (0.2782)             |
| <i>CC(work)</i>                       | 0.966 (0.922)               |
| <i>CC(free)</i>                       | 0.898 (0.836)               |
| <i>Number of non-hydrogen atoms</i>   | 1720                        |
| <i>macromolecules</i>                 | 1625                        |
| <i>ligands</i>                        | 72                          |
| <i>solvent</i>                        | 59                          |
| <i>Protein residues</i>               | 205                         |
| <i>RMS(bonds)</i>                     | 0.003                       |
| <i>RMS(angles)</i>                    | 0.42                        |
| <i>Ramachandran favored (%)</i>       | 100                         |
| <i>Ramachandran allowed (%)</i>       | 0                           |
| <i>Ramachandran outliers (%)</i>      | 0                           |
| <i>Rotamer outliers (%)</i>           | 0.54                        |
| <i>Clashscore</i>                     | 3.9                         |
| <i>Average B-factor</i>               | 35.23                       |
| <i>macromolecules</i>                 | 35.26                       |
| <i>ligands</i>                        | 30.11                       |
| <i>solvent</i>                        | 37.63                       |
| <i>Number of TLS groups</i>           | 4                           |

## Detailed Materials and Methods

### Molecular Dynamics Simulations

Molecular dynamics simulations were performed with the GROMACS molecular dynamics simulation package <sup>1</sup> using the AMBER force field parm94, and the TIP3P water model. All simulations were performed on a RHCC-NT-C<sub>2</sub>B<sub>10</sub>H<sub>12</sub> complex in which all four cavities were simultaneously occupied by a single carborane molecule. The complex model was prepared by inserting the ligand into each cavity of the measured structure of the RHCC-NT (PDB: 1FE6) <sup>2</sup>, crystallized at T = 298 K. Force field parameters (Amber 99) for o-carborane were obtained from Sarosi et al. <sup>3</sup> which were adapted and optimized for bond lengths, torsion parameters and bonded terms using GAFF values by Timofeeva et al. <sup>4</sup> and Gamba et al. <sup>5</sup>. The partial charges for the ligand were generated using Gaussian electrostatic potential fitting and simultaneously calculated at the HF/6-31+G\* level. Long-range electrostatics were employed through the Ewald particle mesh method with a non-bonded cut-off range of 1.0 nm.

Table S2. Molecular Dynamics simulation parameters.

| Parameter                                  | Value                     |
|--|---------------------------|
| Solvation Box                              | 5.2 nm x 5.2 nm x 10.4 nm |
| SPC water molecules                        | 8560                      |
| Ions                                       | 16 Na+                    |
| E <sub>min, initial</sub> <sup>1</sup>     | 1000 kJ/mol               |
| E <sub>min, convergence</sub> <sup>1</sup> | 40-50 kJ/mol              |
| Pressure                                   | 1 atm                     |
| Temperature <sup>2</sup>                   | 300 K                     |
| Time <sup>2</sup>                          | 2 ns                      |

<sup>1</sup> Energy minimization was achieved through the method of steepest descent.

<sup>2</sup> Parameters during production run using a Berendsen barostat and a velocity-rescaling thermostat.

## Results

Table S3. Standard free energies<sup>1</sup> in kJ/mol to transfer  $C_2B_{10}H_{12}$  from solvent to the four cavities of RHCC-NT.

| Cavity | $\Delta G_{cav \rightarrow gas}^2$ | $\Delta G_r^0$ | $\Delta G_{sol \rightarrow gas}^2$ | $\Delta G_{trans} = \Delta G_{sol \rightarrow gas} - \Delta G_{cav \rightarrow gas} - \Delta G_r^0$ |
|--------|------------------------------------|----------------|------------------------------------|---|
| 1      | $+16.9 \pm 2.7$                    | -1.5           | $+8.7 \pm 0.8$                     | $-6.7 \pm 3.5$  |
| 2      | $+33.2 \pm 1.1$                    | -1.5           | $+8.7 \pm 0.8$                     | $-23.0 \pm 1.9$   |
| 3      | $+22.5 \pm 0.7$                    | -1.5           | $+8.7 \pm 0.8$                     | $-12.3 \pm 1.5$   |
| 4      | $+19.1 \pm 1.3$                    | -1.5           | $+8.7 \pm 0.8$                     | $-8.9 \pm 2.1$  |

<sup>1</sup> Free energies were calculated using the method of double-decoupling with a flat bottom harmonic well (FBHW) restraint potential<sup>6,7</sup>.

<sup>2</sup> Values were computed using the multi-configurational thermodynamic integration (MCTI) method<sup>6,7</sup>.

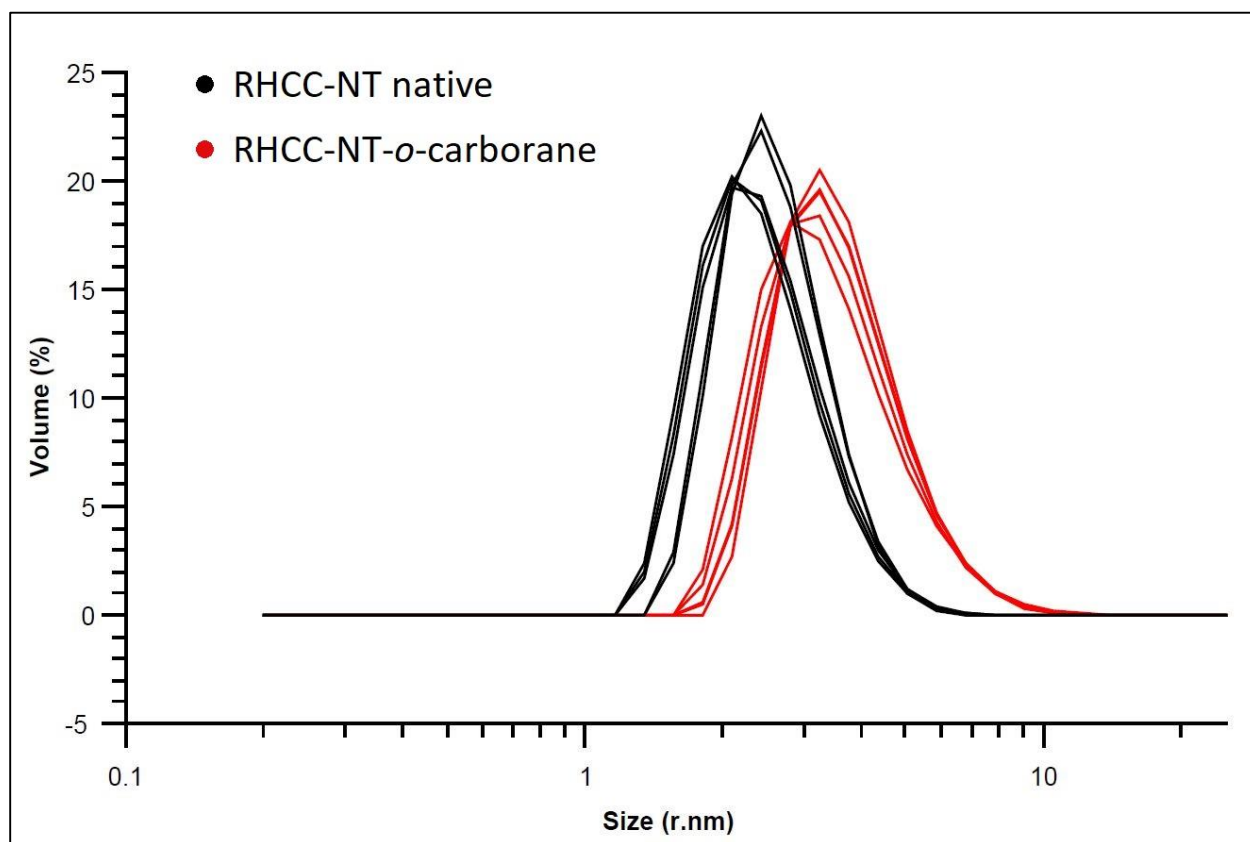


Figure S1. Size distribution profile of native RHCC-NT (black) and *o*-carborane incubated RHCC-NT (red). The hydrodynamic radius is shifted slightly indicating ligand uptake and possible coiled coil conformational changes.

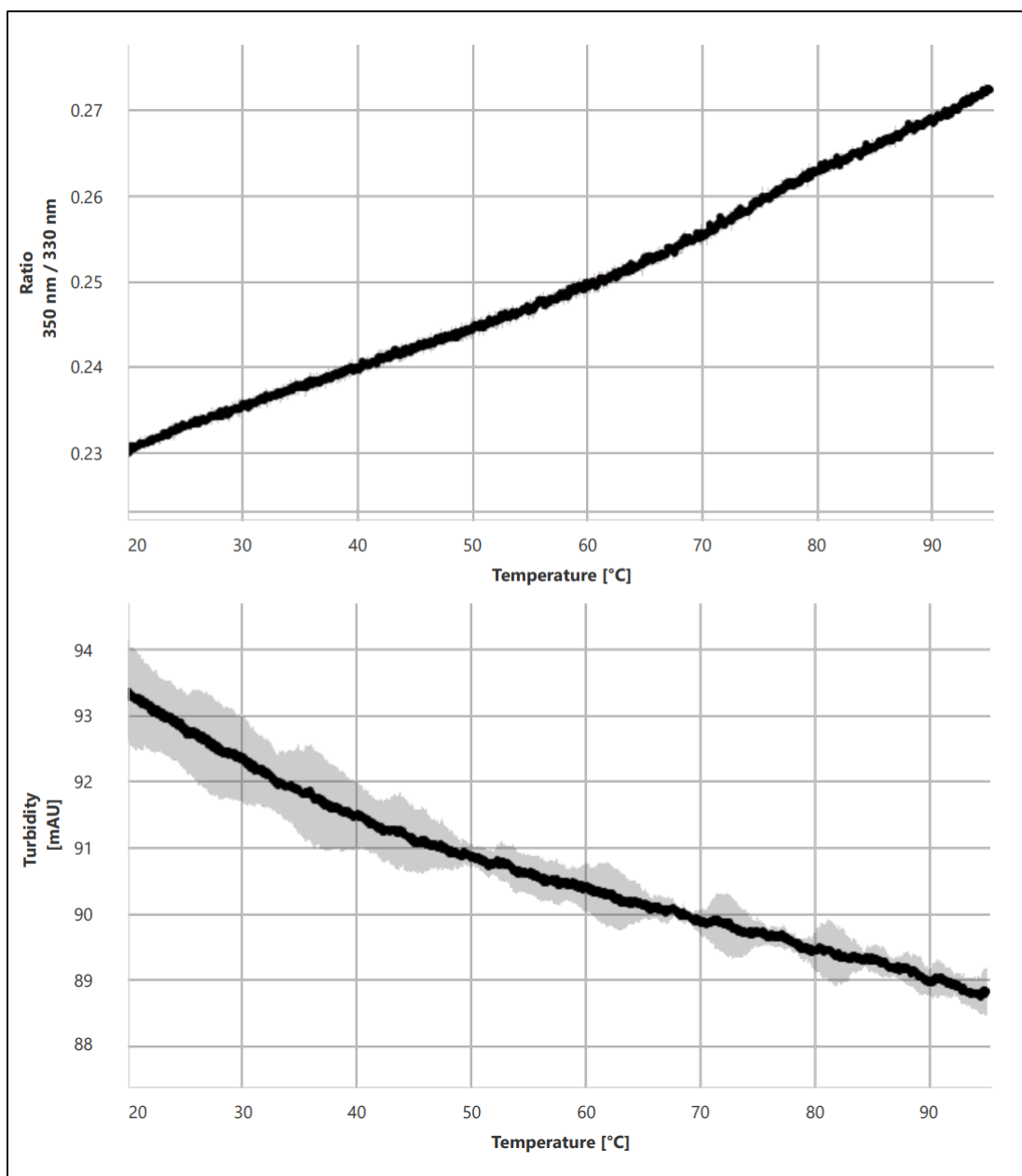


Figure S2. Thermal unfolding analysis of RHCC-NT containing *o*-carborane. The 350 nm/330 nm ratio, which measures the internal Trp/Tyr fluorescence, does not show a shift from a folded to an unfolded state. In addition, turbidity measurements do not show any large changes in aggregation states of the sample, indicating a folded state of the RHCC-NT across the temperature gradient. Protein samples were measured in triplicates.

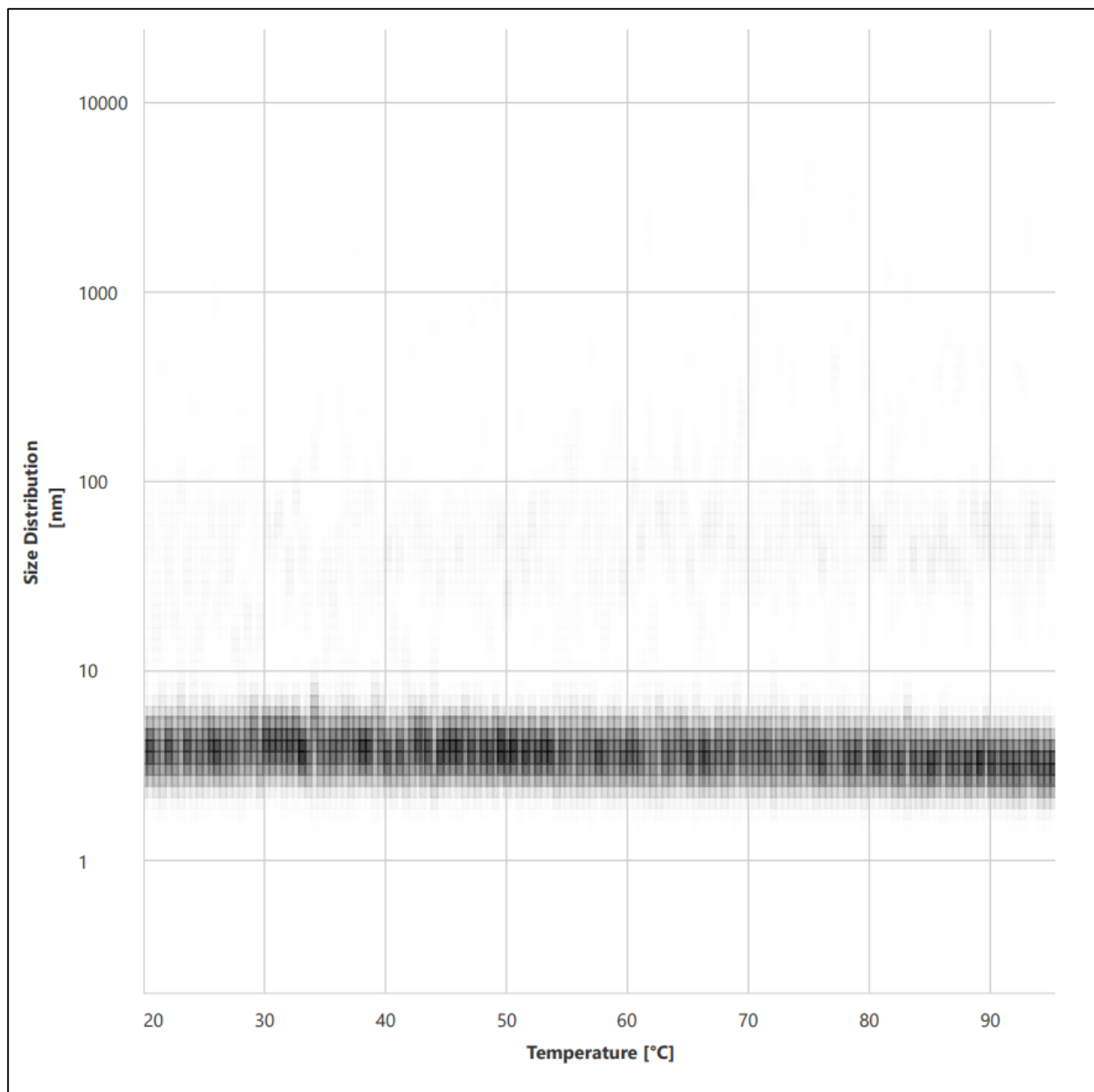


Figure S3. Size distribution analysis of RHCC-NT containing *o*-carborane over a temperature gradient. The size distribution of RHCC-NT does not change with increasing temperature, indicating a stable and intact nanotube structure. Samples were measured in triplicates.

## References

- (1) Van Der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. GROMACS: Fast, Flexible, and Free. *J. Comput. Chem.* **2005**, *26* (16), 1701–1718. <https://doi.org/10.1002/jcc.20291>.
- (2) Stetefeld, J.; Jenny, M.; Schulthess, T.; Landwehr, R.; Engel, J.; Kammerer, R. A. Crystal Structure of a Naturally Occurring Parallel Right-Handed Coiled Coil Tetramer. *Nat. Struct. Biol.* **2000**, *7* (9), 772–776. <https://doi.org/https://doi.org/10.1038/79006>.
- (3) Sárosi, M.-B.; Lybrand, T. P. Molecular Dynamics Simulation of Cyclooxygenase-2 Complexes with Indomethacin Closo -Carborane Analogs. *J. Chem. Inf. Model.* **2018**, *58* (9), 1990–1999. <https://doi.org/10.1021/acs.jcim.8b00275>.
- (4) Timofeeva, T. V.; Suponitsky, K. Y.; Yanovsky, A. I.; Allinger, N. L. The MM3 Force Field for 12-Vertex Boranes and Carboranes. *J. Organomet. Chem.* **1997**, *536–537*, 481–488. [https://doi.org/10.1016/S0022-328X\(96\)06819-2](https://doi.org/10.1016/S0022-328X(96)06819-2).
- (5) Gamba, Z.; Powell, B. M. The Condensed Phases of Carboranes. *J. Chem. Phys.* **1996**, *105* (6), 2436–2440. <https://doi.org/10.1063/1.472111>.
- (6) Gilson, M. K.; Given, J. A.; Bush, B. L.; McCammon, J. A. The Statistical-Thermodynamic Basis for Computation of Binding Affinities: A Critical Review. *Biophys. J.* **1997**, *72* (3), 1047–1069. [https://doi.org/10.1016/S0006-3495\(97\)78756-3](https://doi.org/10.1016/S0006-3495(97)78756-3).
- (7) Hamelberg, D.; McCammon, J. A. Standard Free Energy of Releasing a Localized Water Molecule from the Binding Pockets of Proteins: Double-Decoupling Method. *J. Am. Chem. Soc.* **2004**, *126* (24), 7683–7689. <https://doi.org/10.1021/ja0377908>.