Triggered Release of Pharmacophores from [Ni(HAsO$_3$)]-loaded Polymer-Caged Nanobin Enhances Pro-apoptotic Activity: A Combined Experimental and Theoretical Study

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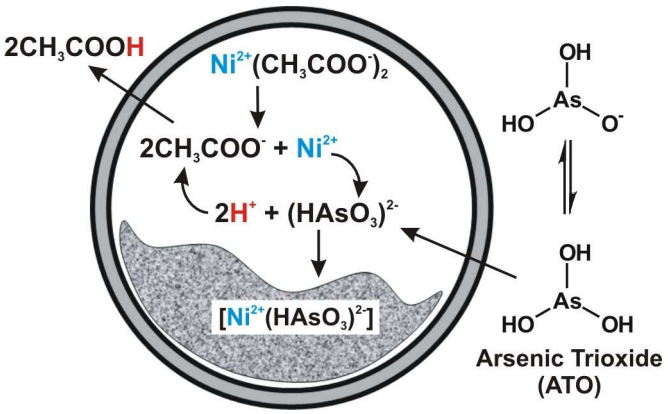

Scheme S1. Schematic drawing of the IGM loading of As^{III} using acetate ion gradient.[S1]

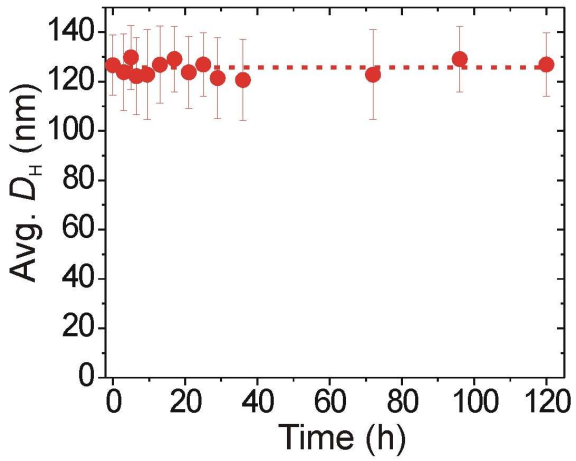

Figure S1. Time-dependent average hydrodynamic diameter (D_H) changes of polymer-caged nanobins (PCNs) that were incubated in 10 mM HEPES buffer (pH = 7.4, 150 mM NaCl) at 37 ^{o}C. D_H was measured by dynamic light scattering (DLS) at pH 7.4 and 25 ^{o}C.

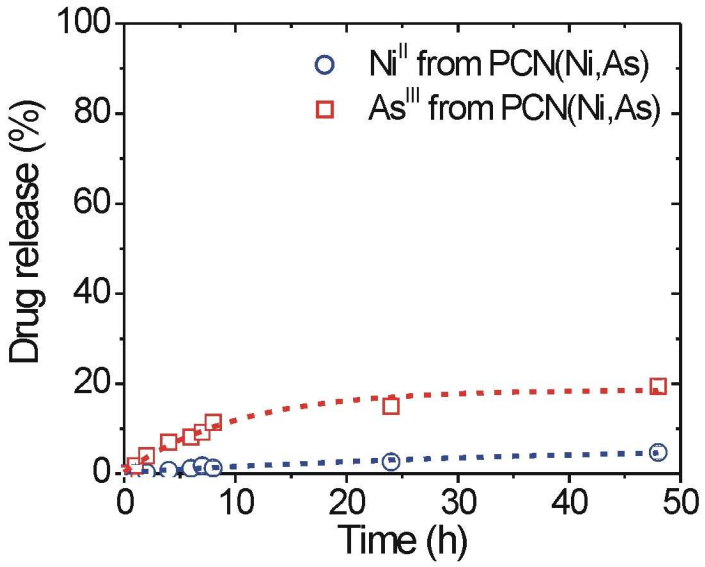

Figure S2. Cumulative-release profiles of Ni^{II} and As^{III} from PCN(Ni,As) when incubated in 10 mM HEPES buffer (pH = 7.4, 150 mM NaCl) at 37 ^{o}C.

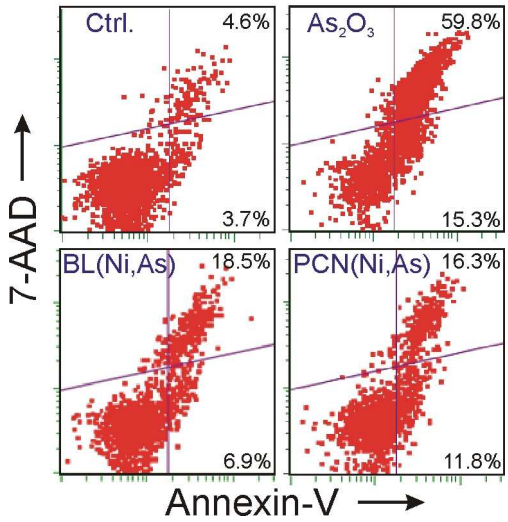

Figure S3. Fluorescence-activated cell sorter (FACS) plots of the responses to ATO-drug formulations using Annexin-V/7-AAD and *in vitro* cytotoxicity profiles. Cells were incubated with drug-free media (Ctrl.), 10 μM solution of free ATO, 10 μM [As^{III}] solution of BL(Ni,As), and 10 μM [As^{III}] solution of PCN(Ni,As). Incubation was carried out for 48 h before the cells were analyzed with Guava Nexin™ assay. In each FACS plot, the lower-left (Annexin-V^-, 7-AAD^-), lower-right (Annexin-V^+, 7-AAD^-), and upper-right (Annexin-V^+, 7-AAD^+) quadrants represent the populations of live cells, apoptotic cells, and necrotic/dead cells, respectively. The numerical percentage values for the latter two populations are indicated in the appropriate quadrants.

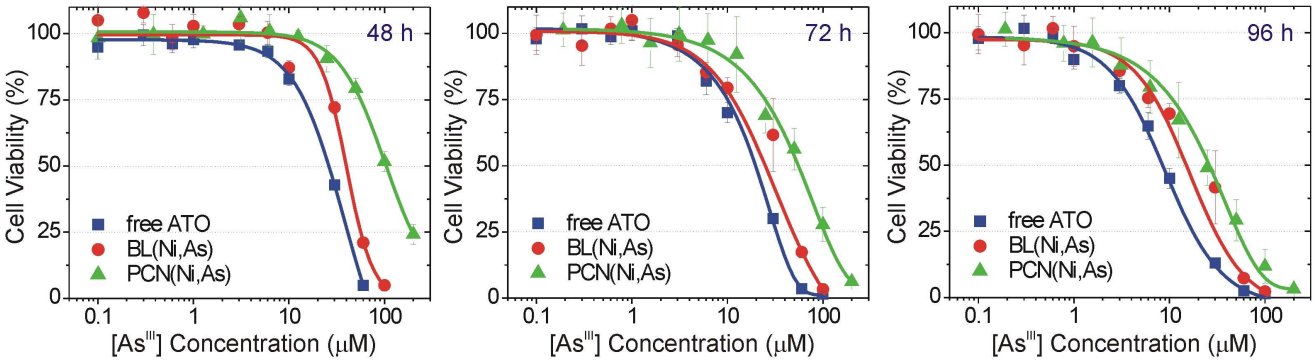

Figure S4. Time-dependent dose-response curves of HeLa human cervical cancer cells that were exposed to As^{III}-drug formulations. Cells were incubated with free ATO, BL(Ni,As),and PCN(Ni,As) at 37 °C. Under our exposure conditions, both PCN(Ni,As) and BL(Ni,As) are stable during the exposure periods, past 96 h. PCN has been shown to be highly stable when being incubated in HEPES buffer for as long as 5 days (Figure S1) and As^{III} leakage from BL(Ni,As) after 96 h was found to be less than 25%.[S1]

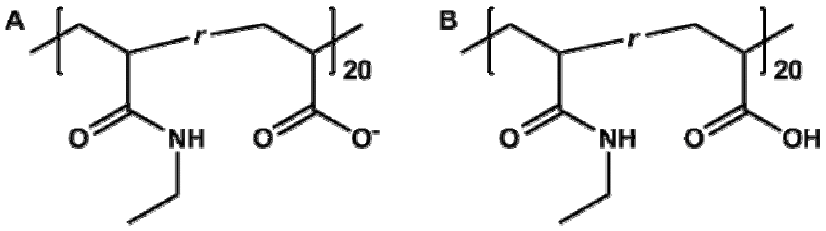

Figure S5. Chemical structures of the model poly(AAc-*r*-AAm) polymers that were used in the molecular simulation studies (both MC and MD) at virtual pH 7.4 (A) and 5.0 (B).

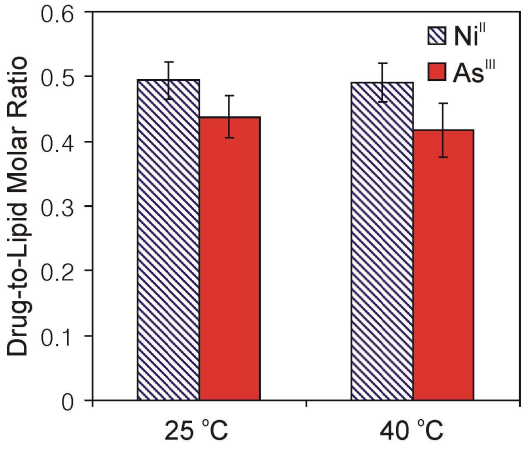

Figure S6. The Ni^{II}-to-lipid and As^{III}-to-lipid ratios in PCN(Ni,As) that was incubated at 40 °C for 2 h are the same as those for PCN(Ni,As) that was left at 25 °C, further confirming that there was minimum drug leakage from PCN(Ni,As) when its size becomes smaller at the higher temperature.

Author Contributions Audit. S.-M. L. and S. T. N. conceived the project. S.-M. L. prepared and characterized the materials, validated them *in vitro*, and wrote the initial drafts of this manuscript. O.-S. L. performed molecular modeling and wrote the initial modeling section in this manuscript. S.-M. L. and S. T. N. finalized the manuscript. S. T. N., G. C. S., and T. V. O. supervised the project and edited the final version of this manuscript.

References

S1. Chen, H.; MacDonald, R. C.; Li, S.; Krett, N. L.; Rosen, S. T.; O'Halloran, T. V., Lipid Encapsulation of Arsenic Trioxide Attenuates Cytotoxicity and Allows for Controlled Anticancer Drug Release. *J. Am. Chem. Soc.* **2006,** *128*, 13348-13349.