Electronic Supplementary Material (ESI) for Nanoscale.

Diruthenium(II,III) metallodrugs of ibuprofen and naproxen encapsulated in intravenously injectable polymer-Lipid nanoparticles exhibit enhanced activity against breast and prostate cancer cells

Samara R. Alves Rico[a,b], Azhar Z. Abbasi[b,ǂ], Geise Ribeiro[a ǂǂ], Taksim Ahmed[b], Xiao Yu Wu[b*], Denise de Oliveira Silva[a*].

[a] Laboratory for Synthetic and Structural Inorganic Chemistry - Bioinorganic and Metallodrugs, Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, B2T, 05508-000, São Paulo, SP, Brazil.

[b] Advanced Pharmaceutics and Drug Delivery Laboratory, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, M5S 3M2, Canada.

[ǂ] Present address: 144 College Street, Toronto, ON, Canada M5S 3M2
[ǂǂ] Present address: Instituto de Física e Quimica, Universidade Federal de Itajubá, Av BPS 1303, 37500-903, Itajubá, MG, Brazil.

Authors email addresses:
samara.alves@usp.br, az.abbasi@utoronto.ca, geise_r@yahoo.com.br, taksim.ahmed@mail.utoronto.ca, sxy.wu@utoronto.ca, deosilva@iq.usp.br

*Corresponding authors

*Dr. Denise de Oliveira Silva, Laboratory for Synthetic and Structural Inorganic Chemistry - Bioinorganic and Metallodrugs of Institute of Chemistry, University of São Paulo. Av. Prof. Lineu Prestes, 748, B2T, 05508-000, São Paulo, SP, Brazil. e-mail: deosilva@iq.usp.br Tel: +55 (11) 3091-9124.

*Dr. Xiao Yu Wu, Advance Pharmaceutics & Drug Delivery Laboratory Leslie Dan Faculty of Pharmacy University of Toronto. 144 College street, Toronto, ON, M5S 3M2. e-mail: xywu@phm.utoronto.ca Tel: +1 (416) 978-5272 Fax: +1 (416) 978-8511

SI. 1. Temperature dependant cellular uptake

To study the cell uptake mechanism, cellular uptake was observed at two different temperatures. For this purpose, 3 mL of cell suspension (1 x 10^5 cells mL^{-1}) was added to two different Petri dishes, and maintained at 37 °C for 24 h (in 5 % CO_2, 95 % humidified air). After 24 h, one of the dishes was placed at 4°C for 1 h, while the other remained at 37 °C. After one hour, the medium from each dish was aspired and 2 mL of fresh growth medium containing fluorescent dye labeled SPLN (1 mg mL^{-1}) was added. The dishes were incubated again at 37 °C or 4°C for another 2 h. After the required time, the cells were washed twice with PBS, and the cell nuclei were stained by incubating cells with 2 mL growth medium containing Hoechst33342 (0.5 µg mL^{-1}) at 37 °C, for 10 min. Cells were then washed twice with growth medium, incubated again in fresh growth medium, and then, cell uptake was observed using the fluorescence microscope EVOS AMG.

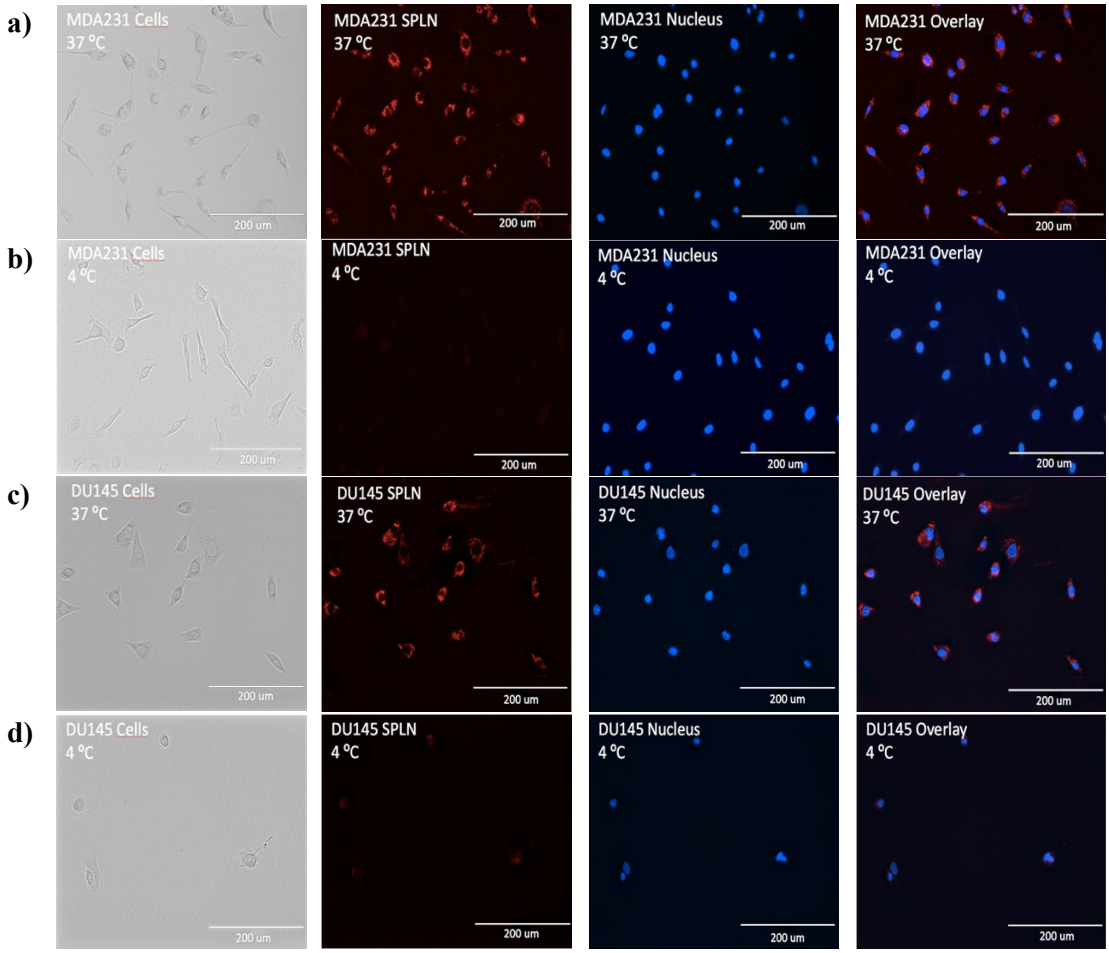

Figure 1: Fluorescence microscope images of MDA-MB-231 cells (a and b), DU145 cells (c and d). Images a), c) and e) displays uptake of SPLN after 2h of incubation at 37°C. Images b), d) and f) displays uptake of SPLN after 2h of incubation at 4 °C. Images from left to right, Transmission light channel, Red channel for Nile Red labeled SPLN, blue channel for stained nuclei and overlay of SPLN and cell nuclei.

SI. 2. Temperature dependant cellular uptake

Murine EMT6 breast cancer, human MDA-MB-231 breast cancer and DU145 prostate cancer cells were cultured in growth medium at a density of 1 x 10^5 cells mL^{-1} in small petri dishes made for confocal microscopy and maintained at 37 °C for 24 h in 5% CO_2. After 24 h, the medium from each dish was aspirated and replaced with 2 mL of growth medium containing fluorescent dye labeled SPLN (1 mg mL^{-1}), and cells were incubated at 37 °C for another 2 h. Cells were then washed twice with PBS buffer, and the cell nuclei were stained by incubation with 2 mL of growth medium

containing Hoechst33342 (0.5 μg mL^{-1}) at 37 °C for 10 min. Cells were then washed twice with growth medium and finally incubated in growth medium. Cellular internalization of SPLNs was confirmed using confocal laser scanning microscope (CLSM) (Zeiss LSM 510) by taking the Z-stack images up to 10 μm depth using a 40x objective lens.

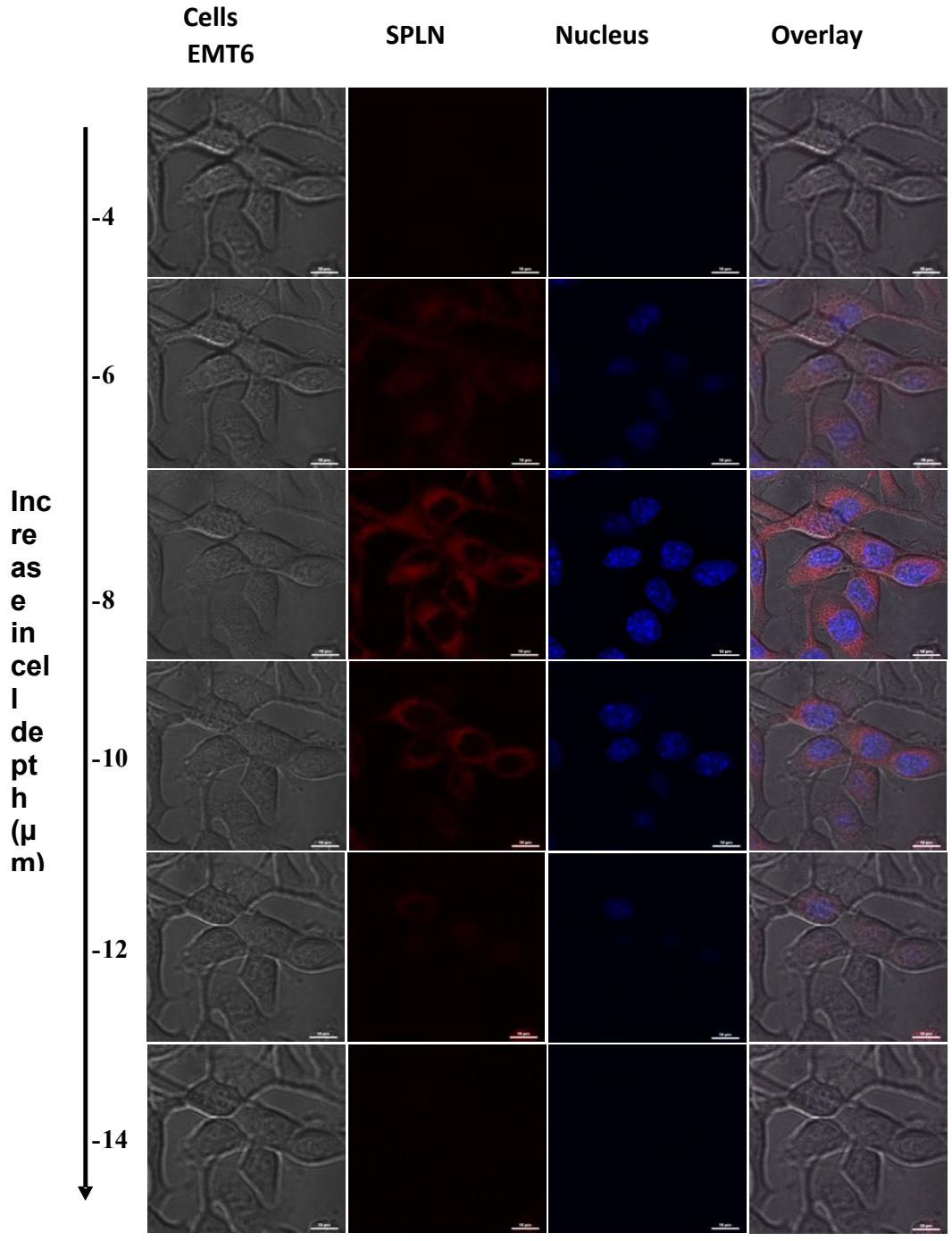

Figure 2: Confocal images of EMT6 showing the internalization of drug free SPLN after 2 h of incubation at 37 °C. The cellular uptake can be noticed by overlaying images. Images from left to right, bright field channel, red channel for SPLN, blue channel for stained nuclei and overlay of cells, SPLN and cell nuclei. The images were obtained in the z-Stack mode within a total depth of 14 μm, with 1 μm depth of each slice. Scales bar correspond to 10 μm.

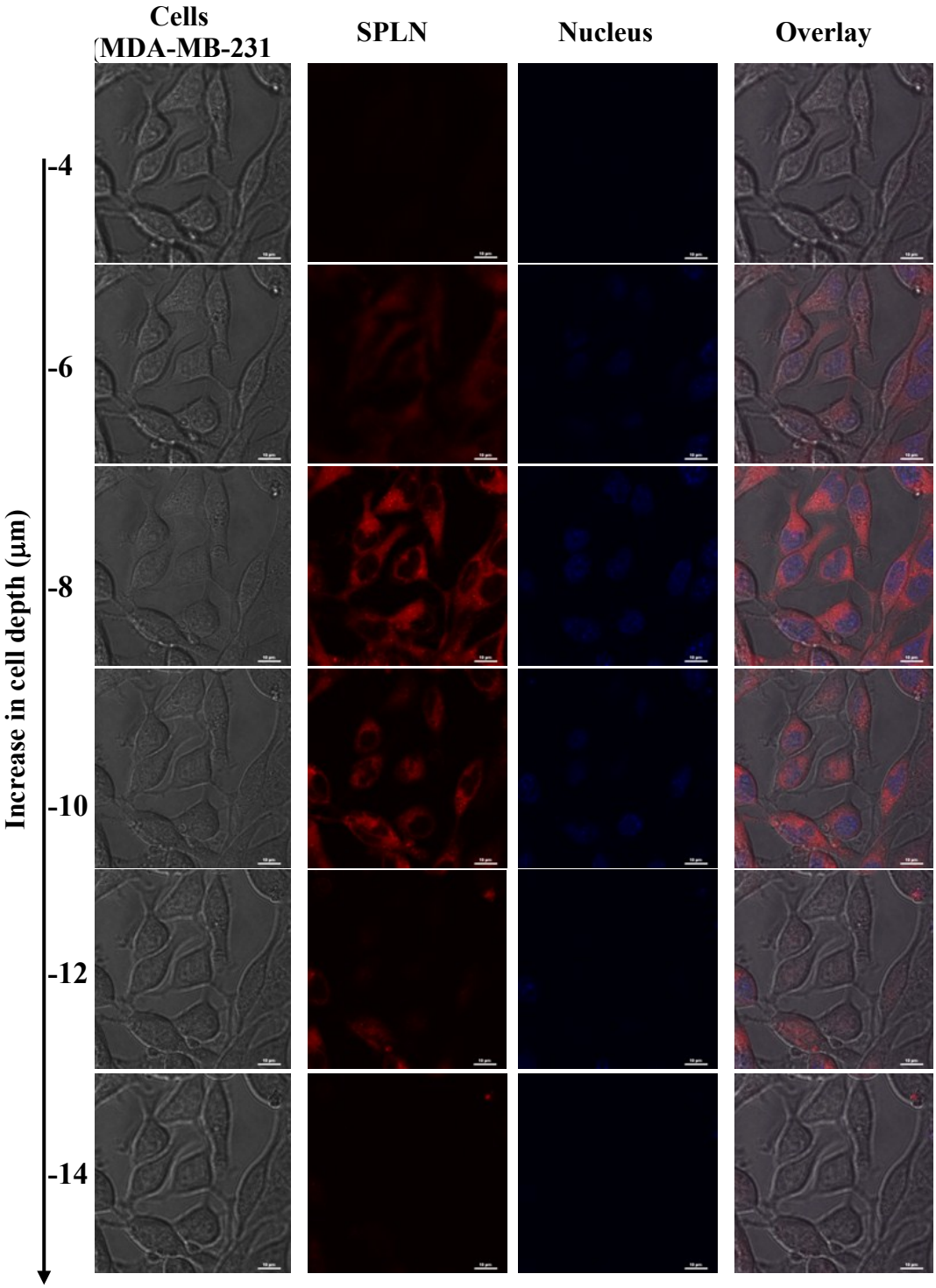

Figure 3: Confocal images of MDA-MB-231 showing the internalization of drug free SPLN after 2 h of incubation at 37 °C. The cellular uptake can be noticed by overlaying images. Images from left to right, bright field channel, red channel for SPLN, blue channel for stained nuclei and overlay of cells, SPLN and cell nuclei. The images were obtained in the z-Stack mode within a total depth of 14 μm, with 1 μm depth of each slice. Scales bar correspond to 10 μm.

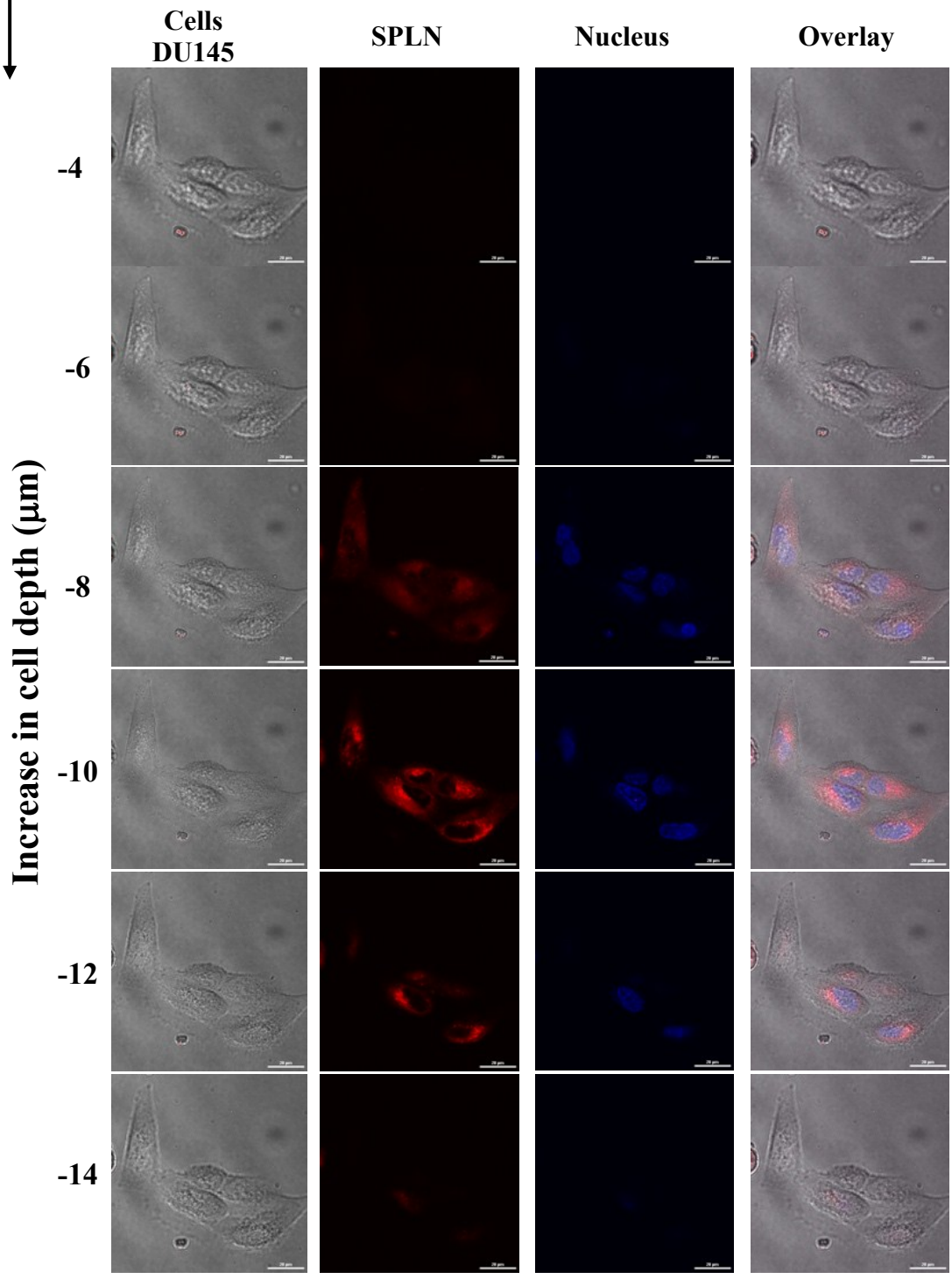

Figure 4: Confocal images of DU145 showing the internalization of drug free SPLN after 2 h of incubation at 37 °C. The cellular uptake can be noticed by overlaying images. Images from left to right, bright field channel, red channel for SPLN, blue channel for stained nuclei and overlay of cells, SPLN and cell nuclei. The images were obtained in the z-Stack mode within a total depth of 14 µm, with 1 µm depth of each slice. Scales bar correspond to 20 µm.