Synthesis of Cobalt Crosslinked Albumin Nanoparticles and *In Vitro* Evaluation of Macropinocytic Uptake in Gastric Carcinoma Cells

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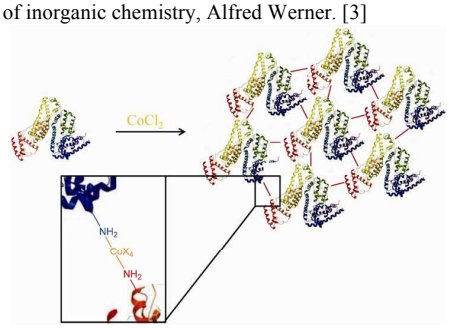
Purpose:
Recent research in the development of bioconjugation has allowed for widespread use in the field of medicinal chemistry research. By conjugating chemical functionality with a bioactive species, acceleration to the drug development process is evident allowing for an increase in the capability to aid in both the treatment and diagnosis of diseases. [1] In this study, albumin nanoparticles (NPs) utilizing a cobalt crosslink to lysine residues on adjacent proteins were synthesized. By incorporating a fluorescently labeled marker to our cobalt crosslinked albumin NP (Co-Alb-FITC), we are able to report on *in vitro* characterization as well as preliminary cytotoxicity data.

Materials & Methods:
Recently, we reported a novel strategy that can be used to crosslink protein to form nanoparticles ranging from 10 to 500 nm in size. [2] The method utilizes a labile Co^{2+} complex to crosslink lysine residues on adjacent proteins that can then be "locked" into conformation by oxidation to an exchange inert Co^{3+} complex. The coordination chemistry itself has ties dating back to the father of inorganic chemistry, Alfred Werner. [3]



Bovine serum albumin and cobalt (II) chloride hexahydrate were combined to make an aqueous solution. Through a series of steps involving sonication, centrifugation, and addition of hydrogen peroxide, Co(III)-crosslinked albumin (Co-Alb) NPs were synthesized. Dynamic light scattering (DLS) measurements determined our NPs to be 10-500 nm in diameter.
NP stability was tested by dispersing NPs in 10 mM PBS or RPMI-1640 (ATCC) containing 10 % fetal bovine serum (FBS). Cytotoxicity studies were conducted on SNU-5 cells and Jurkat T lymphocyte cells. NP uptake was measured by image-based flow cytometry.

Results:
As mentioned previously, DLS measurements showed NPs utilized in these studies had an average diameter of ~500 nm. (Fig. 1)

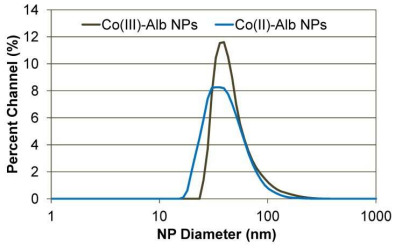


Figure 1. Nanoparticle size of Co-Alb NPs

Rapid particle uptake was observed with virtually all cells exhibiting an increase in FITC emission after just 30 min of exposure to Co-Alb-FITC NPs (Fig. 2A). Representative images of events from the dot plots (Fig. 2B) visually confirmed results, while uptake was observed to increase steadily during the first ~9 h at which point it appeared to reach saturation and no further uptake was observed upon prolonging exposure to particles (Fig. 2C). 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA), a known inhibitor of micropinocytosis, was used to pre-treat cells for 5 min prior to Co-Alb-FITC NPs dosing and displayed significant reduced uptake (Fig. 3A-B). The relative proportion of cells exhibiting high uptake was reduced from 73% in the absence of EIPA to 16% in the presence of 75 μM EIPA. EIPA reduced uptake in a dose-dependent manner with cells incubated in the absence of inhibitor exhibiting >8 times more macropinocytic uptake relative to cells pre-incubated with 100 μM EIPA (Fig. 3C).

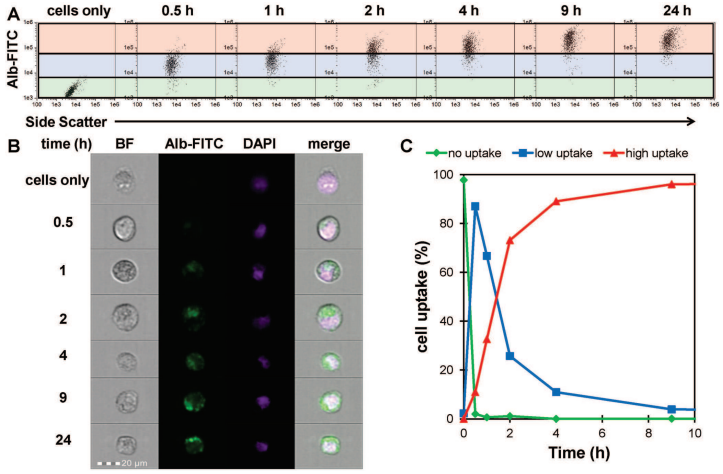


Figure 2. Co-Alb-FITC NPs kinetic uptake and FlowSight images.

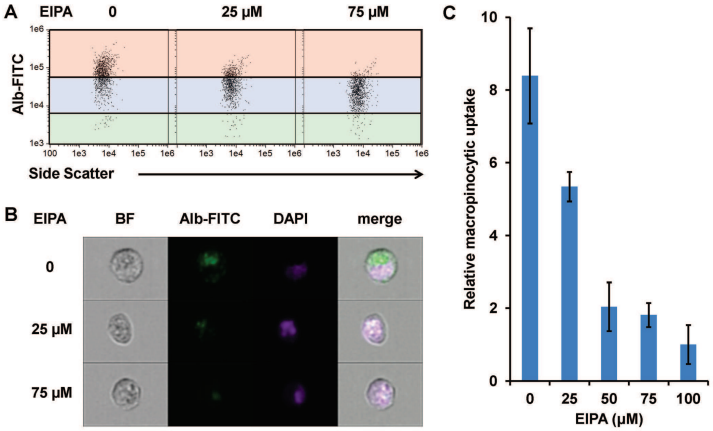


Figure 3. Co-Alb-FITC NPs uptake study w/ introduction of EIPA

Conclusion:
Accordingly, this study demonstrated *three* key outcomes: first, cancer cells efficiently internalize Co-Alb NPs while displaying high levels of macropinocytic uptake; second, Co-Alb NPs show biocompatibility and potential as a drug delivery vector through demonstrating no toxcity; third, because SNU-5 cells are not known to harbor Ras mutations, this study shows that some non-Ras mutated tumor types also rely on macropinocytosis as a mechanism of cell survival.

References:
[1] Fox, M. E., Szoka, F. C.; Fréchet, J. M. J. Soluble Polymer Carriers for the Treatment of Cancer: The Importance of Molecular Architecture. *Accounts Chem Res* **2009,** 42, (8), 1141-1151.
[2] Nguyen, D., Cavazos, R.J., Harris, A., Petros, R.A. Utilizing Cobalt Coordination Chemistry for "Traceless" Stimuli-Responsive Bioconjugation Involving Therapeutic Nanoparticles, Protein PEGylation, and Drug-(Bio)Polymer Conjugates. *Comment Inor Chem* **2014,** in press, 10.1080/02603594.2014.940103.
[3] Werner, A., A contribution to the constitution of inorganic compounds. *Z Anorg Allg Chem* **1893,** 3, 267.

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