

## Folic acid-tagged protein nanoemulsions with controllable size for cancer therapy

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Cancer is one of the most devastating diseases and the conventional chemotherapeutics agents distribute nonspecifically in the body, inducing a number of drawbacks [1, 2]. Protein-based nanoparticles have gained considerable interest as drug delivery devices due to their exceptional characteristics [3, 4]. Additionally, protein-based nanoparticles can also be easily amenable for surface modification and covalent attachment of drugs and targeting ligands [3, 5].

The aim of this work was the development of albumin nanoemulsions as drug delivery systems for cancer therapy. The production of albumin nanoemulsions was achieved by high pressure homogenization of an aqueous solution with an organic solvent (vegetable oil), subjecting the mixture to varying number of homogenization cycles at high pressure. In order to determine the best formulation for therapeutic applications, physicochemical and biological (*in vitro* and *in vivo*) characterizations were performed.

Albumin nanoemulsions were produced by high pressure homogenization with and without a tri-block copolymer (Poloxamer 407), which presents a central hydrophobic chain of polyoxypropylene (PPO) and two identical lateral hydrophilic chains of polyethylene glycol (PEG). We observed a linear correlation between tri-block copolymer concentration and size – the use of 5 mg/mL of Poloxamer 407 yields nanoemulsions smaller than 100 nm. Molecular dynamics and fluorescent tagging of the tri-block copolymer highlight their mechanistic role on the size of emulsions. Folic acid (FA)-tagged protein nanoemulsions were shown to promote specific folate receptor (FR)-mediated targeting in FR positive cells. Carbon monoxide releasing molecule-2 (CORM-2) was incorporated in the oil phase of the initial formulation. FA-tagged nanoemulsions loaded with CORM-2 exhibited a considerable antitumor effect and an increased survival of BALB/c mice bearing subcutaneous A20 lymphoma tumors (Figure 1). The developed nanoemulsions also demonstrated to be well tolerated by these immunocompetent mice.

The novel strategy presented here enables the construction of highly stable, size controlled, functionalized protein-based nanoemulsions with excellent characteristics for active targeting in cancer therapy.

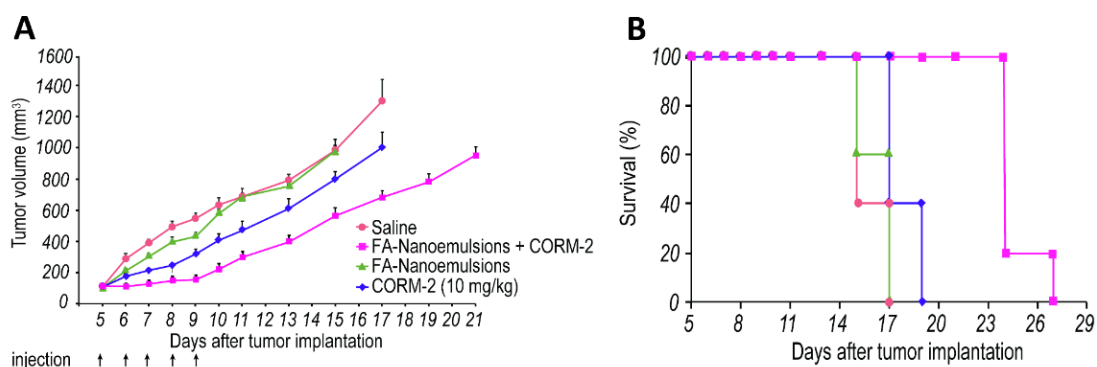


Figure 1. (A) Tumor growth curves of immunocompetent BALB/c mice bearing subcutaneous A20 lymphoma tumors treated intravenously with FA-tagged nanoemulsions loaded CORM-2, empty FA-tagged nanoemulsions, CORM-2 or saline. Data represent mean tumor volumes ( $\pm$ SE). (B) Survival curves of mice treated with FA-tagged nanoemulsions loaded CORM-2 and control groups.

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