# Chimeric Polypeptide-Doxorubicin Conjugates Self-Assemble into Nanoparticles and Abolish Tumors after A Single Injection

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**Statement of Purpose:** Packaging clinically approved drugs into nanoscale delivery vehicles is of particular interest for cancer therapy. A practical drug carrier should: (1) be easy to synthesize; (2) self-assemble into monodisperse nanoparticles; (3) exhibit favorable pharmacokinetics and biodistribution; (5) release the drug with controlled kinetics; (6) lead to a therapeutic response; and (7) be biodegradable. However, most current carriers do not satisfy all these criteria. To address these requirements, we designed a new polymer delivery system called chimeric polypeptides (CPs) that consist of two segments: a hydrophilic, biodegradable elastin-like polypeptide (ELP) and a short segment for the attachment of chemotherapeutics such as Doxorubicin (Dox). We chose ELPs as one component of CPs because they meet some of criteria above; they are non-toxic, biodegradable, and display good pharmacokinetics [1-2]. Herein, we investigated whether the attachment of multiple copies of a hydrophobic drug, Dox, would impart sufficient amphiphilicity to the CP to drive its self-assembly into nanoparticles in the sub-100 nm size range that are desirable for cancer chemotherapy. This hypothesis is based on our previous observation that amphiphilic ELP block copolymers self-assemble into nanoparticles, driven by selective desolvation of one block [3]. We further investigated whether CP-Dox conjugates the improve biodistribution and in vivo efficacy of Dox.

**Methods:** Dox was conjugated to a CP by n-ß-maleimidopropionic acid hydrazide tri-fluoroacetic acid (BMPH). The CP-Dox conjugates were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Murine C26 and human HT-29 colon carcinoma lines were used for an *in vitro* cytotoxicity assay and in vivo as tumor xenografts in BALB/c mice and athymic mice, respectively. Cellular uptake and trafficking of CP-Dox conjugates in C26 cells were studied using LSM5 upright confocal microscope. Affymetrix mouse genome 430A 2.0 arrays were used to analyze gene expression of C26 tumor tissues responding to the treatments.

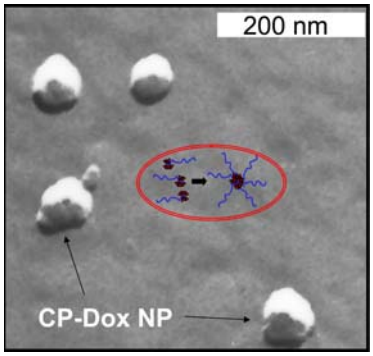


Figure 1. Freeze-fracture TEM of CP-Dox nanoparticles. Attachment of Dox triggers self-assembly of CP into ~40 nm diameter nanoparticles.

**Results:** Spherical particles of the CP-Dox conjugate were observed by freeze-fracture TEM upon conjugation of Dox to the CP (Fig 1). DLS showed these nanoparticles had a mean hydrodynamic radius of 21.1 ± 1.5 nm and a critical aggregation concentration (CAC) < 3 μM. This effect is not restricted to Dox, as a range of small hydrophobic molecules also exhibited this behavior when conjugated to the CP. CP-Dox nanoparticles improved the pharmacokinetics of Dox, as seen by the increase in the area-under-curve (AUC) by 150-fold over free drug. The nanoparticles also reduced Dox accumulation in the heart by 2.6-fold while increasing Dox accumulation in the tumor by 3.5-fold. Cellular uptake of CP-Dox, intracellular release of Dox from CP and trafficking of Dox to the nuclei were observed by confocal fluorescence microscopy. A single dose treatment eight days after tumor inoculation led to complete regression of C26 xenografts treated with CP-Dox. Dox in contrast, had a modest effect on tumor size compared to the PBS control (Fig. 2). Fifteen days after tumor inoculation, CP-Dox-treated mice had a mean tumor volume of 13 $mm^3$ versus 329 $mm^3$ for PBS-($p$=0.00002) and versus 166 $mm^3$ for free Dox- ($p$=0.03) treated control (Fig. 2). A single dose of CP-Dox also resulted in significant growth delay of HT-29 xenografts. In both cases, a substantial increase of animal survival was observed. Results of gene expression analysis suggested that CP carriers down regulate expression of genes responsible for DNA repair, which appears to be a new mode of tumor toxicity that is not observed with free drug.

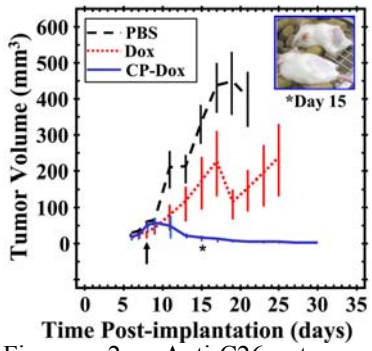


Figure 2. Anti-C26 tumor activity of CP-Dox.

**Conclusions:** This is the first example of genetically engineered CPs that self-assemble into near-monodisperse, sub-100 nm size nanoparticles upon drug attachment, and provide striking antitumor activity in multiple tumor models to a chemotherapeutic that is only modestly effective when delivered as free drug. The generality and simplicity of the CP formulation make it a uniquely attractive system to deliver cancer therapeutics.

**References:** [1] Urry DW. J of Bioactive and Compatible Polymers. 1991;6:263-282. [2] Liu W. J Control Release. 2006;116:170-178. [3] Dreher MR. J Am Chem Soc 2008;130:687-694