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Self-assembly of glutamic acid linked paclitaxel dimers into nanoparticles for chemotherapy

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ABSTRACT

In this work, a glutamic acid linked paclitaxel (PTX) dimer (**Glu-PTX$_2$**) with high PTX content of 88.9 wt% was designed and synthesized. **Glu-PTX$_2$** could self-assemble into nanoparticles (**Glu-PTX$_2$** NPs) in aqueous solution to increase the water solubility of PTX. **Glu-PTX$_2$** NPs were characterized by electron microscopy and dynamic light scattering, exhibiting spherical morphology and favorable structural stability in aqueous media. **Glu-PTX$_2$** NPs could be internalized by cancer cells as revealed by confocal laser scanning microscopy and exert potent cytotoxicity. It is envisaged that **Glu-PTX$_2$** NPs would be an alternative formulation for PTX, and such amino acid linked drug dimers could also be applied to other therapeutic agents.

Cancer remains a leading cause of death,[1,2] and chemotherapy is indispensable for most cancer cases among various treatments, although it always induces high incidence of side effects.[3–6] This promotes the development of innovative and more efficient formulations for such drug molecules. Paclitaxel (PTX) is a potent chemotherapeutic agent, but the poor water solubility poses a challenge for clinical application of it.[7–12] Taxol, in which PTX is administered in a vehicle containing ethanol and Cremophor EL®, has increased the solubility, while it is considered to cause some hypersensitivity reactions.[9,13] Even if some nanoscale formulations of PTX, such as Abraxane and Genoxol-PM can improve the therapeutic index of PTX by decreasing systemic toxicity and improving antitumor efficacy,[14–19] the PTX loading contents are usually low (less than 20 wt%).[20–24] Therefore, novel drug formulations that combine high water solubility with high drug loading are desired.

Instead of utilizing drug carriers, nanomedicines prepared directly from small organic molecules via supramolecular self-assembly could realize high drug loadings.[25–29] It is well-known that amphiphilic organic molecules could self-assemble into nanoparticles. For example, Yan et al. reported some nanodrugs assembling from amphiphilic drug-drug or targeting ligand-drug conjugates.[3,30–33] Cui and coworkers synthesized drug amphiphiles by coupling short peptides with hydrophobic anticancer drugs to form nanomedicines.[34–38] Some hydrophobic molecules could also self-assemble into nanoparticles. For instance, Wang and He et al. demonstrated that disulfide bond bridge could turn hydrophobic anticancer prodrugs into self-assembled nanomedicines.[39] Recently, our group has also reported the self-assembly of several organic dimers for cellular imaging or cancer therapy.[40–43]

In this work, a PTX dimer (**Glu-PTX$_2$**) was synthesized by choosing Boc-l-glutamic acid (Boc-l-Glu) as the linker, in place of alkane chains used in our previous works.[42,43] The reason for the choice is that glutamic acid is one of the essential amino acids of the human body and Boc-l-Glu shows great biocompatibility. **Glu-PTX$_2$** could self-assemble into nanoparticles (**Glu-PTX$_2$** NPs) in water. The self-assembly behavior and stability of the nanoparticles were investigated in detail. **Glu-PTX$_2$** NPs could be internalized by cancer cells as evidenced by confocal laser scanning microscopy (CLSM) and showed high cellular proliferation inhibition against human cervical carcinoma (HeLa) and human breast cancer (MCF-7) cells.

Glu-PTX$_2$ was synthesized through one-pot condensation reaction of PTX and Boc-l-Glu in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (DMAP) (Fig. 1). The structure of

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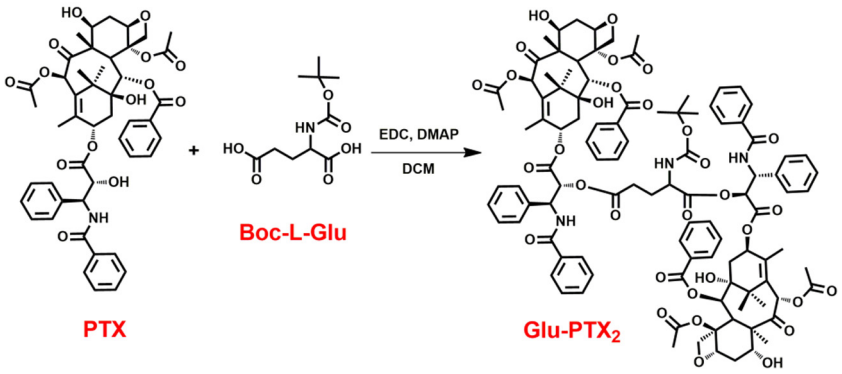

Fig. 1. The synthetic route of **Glu-PTX$_2$**.

Glu-PTX$_2$ was validated by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Fig. S1, Supporting Information). All the protons of the molecule could be clearly resolved in the ^{1}H NMR spectrum. The signal at m/z 1940.7 (Fig. S1, Supporting Information) is ascribed to [**Glu-PTX$_2$** + Na]$^+$, which further confirmed the successful synthesis of **Glu-PTX$_2$** with high PTX content of 88.9 wt%.

Glu-PTX$_2$ NPs could be obtained from the self-assembly of **Glu-PTX$_2$** in aqueous solution, which increased the water solubility of PTX. For the preparation of **Glu-PTX$_2$** NPs, distilled water was added dropwise to **Glu-PTX$_2$** solution in methanol under stirring. After evaporation of methanol and dialysis, **Glu-PTX$_2$** NPs were obtained. The size distribution and morphology of the nanoparticles were characterized by transmission electron microscopy (TEM) (Fig. 2a), scanning electron microscopy (SEM) (Fig. 2b) and dynamic light scattering (DLS) (Fig. 2c). The TEM (Fig. 2a) and SEM (Fig. 2b) images indicate the formation of spherical nanoparticles. The average hydrodynamic diameter of **Glu-PTX$_2$** NPs determined by DLS is about 376.6 nm with a polydispersity index (PDI) of 0.117. The particle size measured by TEM or SEM is smaller than that determined by DLS, probably due to the volume shrinkage during sample drying.[44] The zeta potential of **Glu-PTX$_2$** NPs in aqueous solution is negative (−30.3 mV), which is beneficial for their stability due to the electrostatic repulsion. The diameter and PDI measured by DLS showed little changes within 12 days (Fig. S2, Supporting Information), demonstrating that **Glu-PTX$_2$** NPs were stable in water. Furthermore, the negligible changes of diameter and PDI after storage in phosphate-buffered saline (PBS, pH 7.4) containing 10% fetal bovine serum (FBS) for 24 h (Fig. 2d) validated that the nanoparticles also possessed good stability in physiological environment.

For exploring the mechanism of self-assembly, the X-ray diffraction spectra of PTX, **Glu-PTX$_2$** and **Glu-PTX$_2$** NPs were investigated. As shown in Fig. S3, sharp and intense peaks of PTX are presented, suggesting that it is in crystalline form. In contrast, **Glu-PTX$_2$** molecule and freeze-dried powder of **Glu-PTX$_2$** NPs lack of obvious sharp characteristic peaks, indicating that **Glu-PTX$_2$** was in an amorphous state. Therefore, the lower crystallinity of **Glu-PTX$_2$** compared with that of PTX might be the possible reason for

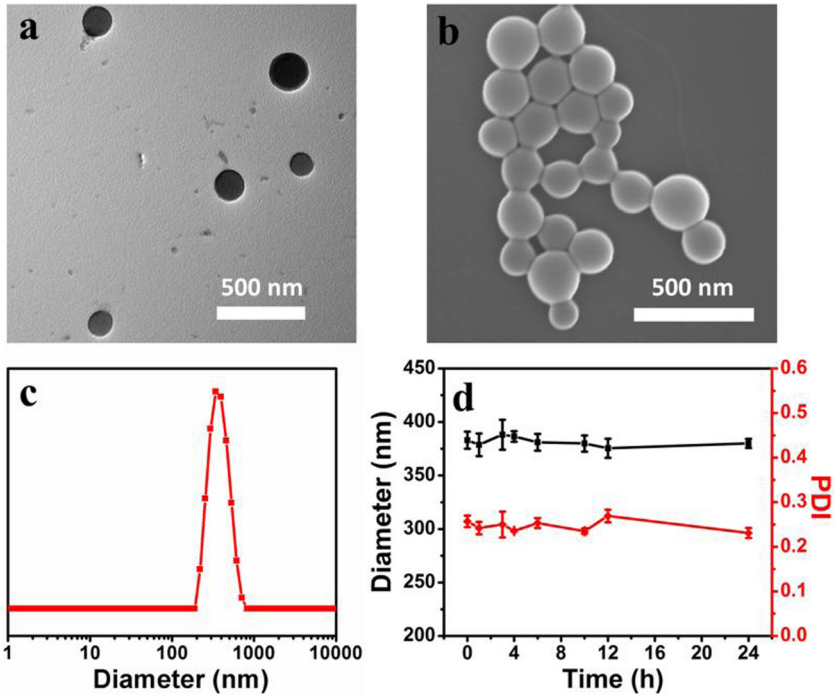

Fig. 2. (a) TEM and (b) SEM images of **Glu-PTX$_2$** NPs. (c) Size and size distribution of **Glu-PTX$_2$** NPs. (d) Changes of the diameter and PDI of **Glu-PTX$_2$** NPs incubated in PBS (pH 7.4) containing 10% FBS as a function of time measured by DLS.