

**Folate Receptor-Targeted, ROS-Responsive Dextran-Block-Poly  
(propylene sulfide) Copolymer Nanoparticles: A  
Bioinformatics-Guided Approach for Prostate Cancer Therapy**

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## ABSTRACT

Utilizing big data to advance nanomedicine, especially via folic acid-receptor (FR) interactions for targeted cancer treatment, underscores a novel approach in oncology. Reactive oxygen species (ROS)-responsive nanocarriers, in particular, can rapidly release drugs in response to ROS stimulation, making them highly effective for tumor therapy. In this study, we fabricated a novel amphiphilic conjugate composed of folic acid (FA) decorated dextran-block-poly copolymer (FA-Dex-*b*-PPS) to serve as ROS-responsive nanocarriers for the treatment of prostate cancer. The chemical structure of FA-Dex-*b*-PPS was confirmed using Fourier transform infrared spectroscopy and proton nuclear magnetic resonance. The self-assembling behavior of FA-Dex-*b*-PPS into ROS-responsive nanoparticles and their degradation was characterized using fluorescence spectroscopy, dynamic light scattering, and transmission electron microscopy. Therapeutic nanocarriers encapsulating doxorubicin (Dox) were formed via dialysis. The FA-Dex-*b*-PPS nanoparticles demonstrated excellent oxidant-triggered Dox release ability *in vitro*. The outcomes from the cytotoxicity assays indicate that the FA-Dex-*b*-PPS nanocarriers exhibit high biocompatibility, with cell survival rates exceeding 85 % even at concentrations of 400 µg/mL. The utilization of confocal laser scanning microscopy for analysis demonstrated that the FA-Dex-*b*-PPS-Dox nanoparticles were efficiently internalized by PC3 cells through FR, in contrast to the non-FA coated drug-containing nanoparticles (Dex-*b*-PPS-Dox). Furthermore, both *in vitro* and *in vivo* xenograft mouse model analyses consistently demonstrated that FA-Dex-*b*-PPS-Dox nanoparticles exhibited the best anti-tumor effect toward PC3 cells compared to non-targeted Dex-*b*-PPS-Dox nanoparticles and free Dox at the same Dox dosage. These combined data suggest that nanocarriers with tumor-targeting capabilities and ROS response have greater potential for application in tumor therapy.

## 1. Introduction

Cancer ranks as a predominant factor contributing to mortality worldwide[1] . Prostate cancer (PCa) is frequently diagnosed among men across various cancer types,

characterized by high recurrence, metastatic rates, and mortalities. Effective therapies for PCa remain an unmet clinical need. Consequently, identifying targeted therapeutic approaches has become one of the pressing challenges in current clinical medical research. By deeply analyzing the pathogenesis and clinical characteristics of PCa, researchers are striving to explore novel therapeutic strategies and methods to enhance treatment efficacy and improve patient prognosis[2]. Concurrently, the advancement of science and technology has introduced emerging treatment modalities and techniques, offering new hope and possibilities for PCa therapy[3].

Conventional chemotherapy is one of the treatments for PCa; however, the clinical application of chemotherapeutic agents is constrained by their limited water solubility, absence of specificity, and elevated toxicity[4]. In order to surmount these challenges, scientists have engineered advanced drug delivery platforms including liposomes, hydrogels, and exosomes, with nanomedicine at the forefront of this research area[5]. Extensive research has been conducted on polymeric nanoparticles owing to their desirable attributes, which include biocompatibility, stability, and a substantial drug-loading capacity[6]. Unfortunately, most polymeric drug carriers lack the ability to actively target tumors and release drugs rapidly, which reduces the effectiveness of chemotherapy. Consequently, there exists an urgent requirement to engineer sophisticated polymeric nanoparticles that possess both tumor-targeting and rapid drug release functionalities for the treatment of PCa.

With the rapid development of big data analysis technologies, integrating these technologies to advance nanomedicine research has become a new trend in medical research[7]. Specifically, the specific interactions between folic acid (FA) and its receptors have recently become a focal point in academic research on targeted delivery of nanomedicines. FOLH1, also known as FA, is a gene that encodes a type II transmembrane glycoprotein of the M28 peptidase family. FA is a crucial molecule involved in several biological processes, such as RNA and DNA preparation, methylation, and modification. FA serves as the principal binding agent to the FR, a protein that is excessively expressed in the majority of cancerous cells[8, 9]. The

specific binding of FA and the folic acid receptor has been utilized as the basis for many targeted therapies to achieve precise drug delivery[10]. For example, FA-modified poly( $\epsilon$ -caprolactone) block poly(2-methacryloxyethyl phosphorylcholine) copolymers have shown higher cellular uptake compared to non-FA-modified counterparts in some cancer cells[11]. Moreover, studies have documented that the modification of FA-DABA-SMA copolymers is capable of decreasing the expression of HES1 and NOTCH1 proteins, thereby enhancing the levels of FR $\alpha$  expression in breast cancer cells[12]. In recent years, researchers have utilized FA conjugation to nanoparticles to achieve targeted cancer therapy. For instance, mixed miRNAs-loaded FR-conjugated PLGA nanoparticles have been designed to eliminate cancer cells from spermatogonia stem cells[13]. Similarly, Dox-conjugated glycol-block copolymer coated multiwall carbon nanotubes decorated with FR have been used to enrich Dox accumulation in tumor tissue and achieve targeted therapy of breast cancer[14]. In summary, the conjugation of FA has demonstrated potential in facilitating targeted drug delivery for the treatment of cancer. The exploitation of the interaction between FA and its receptor for targeted therapeutic approaches holds significant promise for the advancement of future strategies in cancer treatment. However, current research on the application of clinical data analysis in the bioinformatics study of FA in pan-cancer or PCa is still inadequate. Moreover, there is a notable lack of nanomedicine designed and constructed based on bioinformatics analysis results for the targeted treatment of PCa.

In recent years, the domain of pharmaceuticals and biomedicine has witnessed a growing trend in the development of smart drug delivery systems that respond to various stimuli[15]. Researchers have designed and synthesized a variety of stimuli-responsive polymeric nanoparticles to achieve targeted release of chemotherapy drugs and improve the antitumor effect, based on the characteristics of tumor tissue such as temperature, pH, ultrasound, reactive oxygen species (ROS), and enzyme stimulation[16, 17]. It is well known that ROS, including H<sub>2</sub>O<sub>2</sub>, superoxide, hydroxyl radical, and others, are highly expressed in cancer cells. ROS-responsive

polymeric nanoparticles, as drug delivery systems, have developed as an effective strategy for precise therapy, based on the higher ROS concentration in tumor tissues compared with normal tissues[18-20]. Drug delivery from ROS-responsive polymeric nanoparticles occurs mainly through two different pathways, namely, hydrophobic-hydrophilic transition and the degradation of polymeric chain segments[21, 22]. On one hand, ROS can oxidize hydrophobic polymers, which have chalcogen elements (such as S, Se, Te) in their backbone or side chain, into hydrophilic polymers. This hydrophobic-hydrophilic transition leads to dissociation of the polymer self-assembly and ultimately results in the rapid release of the drug. On the other hand, ROS-sensitive chemical structures, such as boronic acid esters, proline oligomers, thioketal, or diselenide groups, can be introduced into the polymer structure. The presence of ROS can then degrade the polymer, enabling rapid release of the drug. Therefore, ROS-responsive polymeric nanoparticles have great potential as drug delivery platforms in the future.

Polypropylene sulfide (PPS) is regarded as an ideal ROS-responsive polymer. The rationale for this lies in the facile synthesis of PPS through an anion-initiated ring-opening polymerization of propylene sulfide. Additionally, under the influence of ROS, the hydrophobic sulfur atoms within the PPS backbone can undergo oxidation, transitioning into hydrophilic sulfoxide or sulfone groups[23, 24]. This allows the polymer to undergo a hydrophobic-hydrophilic transition for rapid and controlled release of the drug[25]. Currently, polyethylene glycol-block-polypropylene sulfide (PEG-*b*-PPS) amphiphilic block copolymers are widely used as ROS-sensitive nanocarriers for the treatment of tumors, inflammation, and other diseases[26, 27]. This is mainly due to the fact that PEG is already approved by the FDA for improving the pharmacokinetics and bioavailability of drugs[28]. In addition, PEG-*b*-PPS can be easily synthesized by an anionic ring-opening polymerization reaction using PEG as a macromolecular initiator[29]. Nonetheless, it is important to acknowledge that polyethylene glycol (PEG) is a non-biodegradable biomaterial, and the existence of anti-PEG antibodies in patients treated with PEG-conjugated pharmaceuticals could

potentially diminish the therapeutic efficacy[30, 31]. Furthermore, the lack of functional groups on the PEG segment makes it difficult to introduce targeting ligands on PEG-*b*-PPS. Overall, PEG-*b*-PPS amphiphilic block copolymers remain a promising platform for drug delivery. Nevertheless, further research is required to address the limitations of PEG and optimize their performance for specific applications.

Renewable and biodegradable natural polysaccharides, including starch, dextran, hyaluronic acid, and cellulose, have attracted substantial interest due to their potential utility in the biomedical field[32]. Dextran stands out as a polysaccharide of choice for in-depth exploration, largely due to its desirable characteristics including aqueous solubility, compatibility with biological systems, and a reduced potential to elicit an immune response[33]. While dextran-based block polymers have not been extensively studied, recent literature reports have focused on the preparation of dextran-containing amphiphilic block polymers using click reactions[34, 35]. This method offers meticulous regulation of the material's architecture and attributes, enabling the integration of dextran's benefits with those of other substances. Moreover, the hydroxyl groups on the dextran allow for further modifications by coupling various targeting ligands, fluorescent molecules, and drugs[36-38]. Despite the promising applications of dextran-based block polymers, there is currently no research on dextran-block-polypropylene sulfide copolymers. This is an exciting area of potential research, as polypropylene sulfide is another biodegradable material that exhibits favorable properties for biomedical applications.

In this study, we initially extracted the expression data of FA across 33 pan-cancer types from the publicly available repository, The Cancer Genome Atlas (TCGA). Thereafter, we analyzed the role of FA in tumor cell stemness. Progressing from this, our study explored the relationship between FA and PCa in terms of TNM staging, prognostic survival, and tumor infiltrating immune cells. Building on the critical theoretical foundation of FA's high expression in PCa, we report the synthesis of FA decorated dextran-block-polypropylene sulfide polymer using click chemistry and

EDCI/HOBT-catalyzed esterification reactions. The obtained amphiphilic polymer's chemical structure was characterized using Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). Furthermore, the self-assembly behavior of the amphiphilic block polymer was demonstrated using dynamic light scattering (DLS), transmission electron microscopy (TEM), and fluorescence spectroscopy. Additionally, Dox-loaded FA-Dex-*b*-PPS nanoparticles were found to exhibit ROS-responsive drug release behavior under high ROS environments (Scheme 1). *In vitro* and *in vivo* experiments were conducted to compare the anti-tumor effect of FA-Dex-*b*-PPS-Dox nanoparticles, non-targeted Dex-*b*-PPS-Dox nanoparticles, and Free Dox, all at the same Dox dosage. The results demonstrated that FA-Dex-*b*-PPS-Dox nanoparticles exhibited the best anti-tumor growth effect towards PC3 cells. Drawing from both *in vitro* and *in vivo* experimental outcomes, we observed that employing FA as a tumor-targeting ligand can augment the intracellular drug concentration and potentiate the antitumor efficacy of Dox-loaded nanoparticles.

## 2. RESULTS AND DISCUSSION

### 2.1 Pan-Cancer Analysis of FOLH1 Transcriptomic Expression and Its Correlation with Tumor Stemness and Prostate Cancer Pathology

To thoroughly investigate the transcriptomic expression pattern of FOLH1 across various cancers, we utilized pan-cancer data encompassing 34 common cancer types from the TCGA database. As depicted in Figure 1A, FOLH1 expression was found to be significantly altered in multiple cancer types, such as UCEC, LUAD, ESCA, STES, KIPAN, COAD, COADREAD, PCPG, PRAD, KIRC, LUSC, THCA, OV, PAAD, UCS, LAML, GBM, GBMLGG, LGG, BRCA, KIRP, STAD, LIHC, WT, SKCM, KICH, and CHOL. These findings underscore the pivotal role of FOLH1 in cancer pathogenesis (all P values <0.05). Then, to investigate the role of the FOLH1 gene in tumor stemness, we calculated the Spearman correlation between FOLH1 gene expression and tumor stemness scores within each cancer type. As depicted in Figure 1B, significant correlations were observed in 18 cancer types, with 4 of these demonstrating a significant positive correlation. These included

BRCA(N=1080)(R=0.148942256393485,P=8.80956467409179e-7),PRAD(N=491)(R=0.258044667531986,P=6.55838181611533e-9),LUSC(N=483)(R=0.206506468183196,P=0.0000047371602794732),ACC(N=76)(R=0.289905397385117,P=0.0110771226466697). In contrast, a significant negative correlation between FOLH1 gene expression and tumor stemness scores was found in 14 other cancer types, such as LGG(N=507)(R=-0.123664750206764, P=0.00529765414468569), CESC(N=301)(R=-0.19327883282091, P=0.000748542829133704), COAD(N=281)(R=-0.314768065937981, P=7.02241656987141e-8), COADREAD(N=369)(R=-0.280195437770192, P=4.3960481873053e-8), SARC(N=253)(R=-0.153905971298841, P=0.014460193903918), KIPAN(N=860)(R=-0.589272769954414, P=1.55736718515943e-81), UCEC(N=177)(R=-0.265404997445562, P=0.000356694282028656), KIRC(N=512)(R=-0.407254021627045, P=7.08589079849258e-22), THYM(N=119)(R=-0.264175478120932, P=0.00369422930699814). These findings provide crucial insights for further exploration of the mechanism by which the FOLH1 gene influences tumor stemness. It is evident that FOLH1 is highly expressed in prostate cancer, rendering it a promising target for future investigative research.

In light of the above bioinformatics analysis of FOLH1 across pan-cancer datasets and drawing upon the foundation of our previous research efforts, we have chosen PCa as the tumor type for our subsequent studies. The relationship between FOLH1 and pathologic T stage, pathologic N stage, clinical M stage from TCGA-PRAD dataset were further analyzed. As illustrated in Figures 1C and 1D, PCa patients with high levels of FOLH1 expression are correlated with more severe pathological T stages (T3 and T4) and elevated pathological N stages (N1). However, there was no significant association between FOLH1 expression status in clinical M stage (Figure 1E). The area under the receiver operating characteristic (ROC) curve (AUC) for FOLH1 reached 0.828 (Figure 1F), signifying that FOLH1 expression possesses considerable diagnostic utility in the context of PCa.

The collective bioinformatics findings indicate that the strategic design of



nanomedicines targeting FOLH1 harbors significant potential for therapeutic advancement. FOLH1, also known as the folate receptor, has been the subject of numerous studies wherein folate-conjugated nanotherapeutics have been employed to target tumor cells expressing the folate receptor, thereby facilitating precision-targeted therapy.

## 2.2 Preparation and Characterization of FA-Dex-*b*-PPS

The number of polysaccharide-based block polymers is significantly lower when compared to polyethylene glycol-based block polymers. This is mainly due to the large number of reactive groups on the polysaccharides need to protect, the lower activity of the terminal reducing groups and difficult to find good solvent for both blocks that limit the preparation of polysaccharide-based block polymers[39]. Currently, chemical conjugation is widely used to synthesis polysaccharide-based block polymers. However, the reaction efficiency of coupling reactions between two large molecular blocks is usually low. Therefore, coupling reactions with high efficiency and selectivity are the basic requirements for the preparation of polysaccharide-based block polymers. Cu(I)-catalyzed "alkyl/azide" click reactions occurring under mild reaction conditions are highly selective, efficient, and reliable and play a crucial role in the preparation of polysaccharide block polymers[35]. In this study, dextran/poly(propylene sulphide) block polymer was prepared by using the Cu(I)-catalyzed "alkyl/azide" click reactions. To achieve this,  $\alpha$ -azide-dextran was synthesized using the reductive amine reaction and  $\alpha$ -alkyl-PPS was synthesized through anionic ring-opening polymerization. The dextran/poly(propylene sulphide) amphiphilic block polymer was then prepared by click reaction. Finally, folic acid molecule was grafted onto the dextran chain segment by esterification to enable the block polymer the ability to target the folic acid receptor, as shown in Scheme 2.

The chemical structure of the polymers was characterized by infrared spectroscopy, as illustrated in Figure 2A. For the  $\alpha$ -azide Dex, the stretching vibration absorption peak of the hydroxyl group on the polysaccharide was located at 3500  $\text{cm}^{-1}$ , and the characteristic peak of the azide group appeared at 2106  $\text{cm}^{-1}$ . This indicated

that the azide group was successfully introduced to the reducing-end of the dextran via the reductive amine reaction. The C-H stretching vibration of PPS was observed in the region of 2850-3000  $\text{cm}^{-1}$ . The spectrum for FA-Dex-*b*-PPS clearly showed that the azide group absorption peak at 2106  $\text{cm}^{-1}$  had completely disappeared, indicating that the product was successfully prepared through the click reaction. Furthermore, the hydroxyl absorption peak on the dextran chain segment appeared at 3500  $\text{cm}^{-1}$  and the absorption peak located at 1430  $\text{cm}^{-1}$  was attributed to PPS block.

The molecular structure of  $\alpha$ -alkyne-PPS was elucidated using NMR spectroscopy, with the resulting data depicted in Figure 2B. The chemical shift of the H-atom of the methyl group on the initiator n-butyl mercaptan was located at 0.90 ppm. The peak attributed to the methyl groups of the propylene sulphide repeated units appeared at 1.35 ppm, and the H-atom attributed to the methylene group was located at 2.52-2.65 ppm. The peak located at 2.85-3.00 ppm belonged to the hypomethyl group. By comparing the integrated area of the methyl group on the repeat unit of PPS with that of the initiator methyl group, we were able to calculate the polymerization degree of PPS to be approximately 78, and the molecular weight to be approximately 5911 g/mol, which was calculated based on the following equation:

$$M_{\text{n-butane thiol}} + M_{\text{propylene sulfide}} \times 78 + M_{\text{Propargyl bromide}} - M_{\text{HBr}} = 90.19 \text{ g/mol} + 74.14 \text{ g/mol} \times 78 + 118.96 \text{ g/mol} - 80.91 \text{ g/mol} = 5911 \text{ g/mol} \quad (3),$$

where  $M$  represents the molar mass.

We employed NMR spectroscopy to determine the detailed chemical structure of the dextran/poly(propylene sulphide) block polymer synthesized via the click reaction in DMSO. As exhibited in Figure 2C, all the H-atom peaks attributed to dextran and PPS blocks were identified. Moreover, the H-atom on the triazole ring which was produced after the click reaction was located at 8.10 ppm. The presence of the triazole ring provided compelling evidence for the successful preparation of dextran/poly(propylene sulphide). Using the ratio between the integrated area of the methyl H-atom signal of PPS and the methyl H-atom of dextran, we calculate that the molar ratio of the hydrophobic and hydrophilic repeating units in the polymer is about

2.22:1, which corresponded to a mass fraction of hydrophobic chain segments of approximately 50 %. We cannot provide GPC data for dextran/poly(propylene sulphide) mainly due to the block copolymer can only be dissolved in DMSO, however, we do not have a GPC with DMSO as the mobile phase.

The folic acid molecule was grafted onto the dextran block via the EDCI/HOBT-catalyzed esterification. Since folic acid contains unsaturated structures such as benzene rings, the H on this type of unsaturated structure appears at high chemical shifts. As shown in Figure 2D, the characteristic peaks of unsaturated cyclic structures on folic acid were appeared at 6.50-8.50 ppm, confirming the successful synthesis of target product. The degree of substitution of folic acid was calculated from the NMR spectra as approximately 1.7 folic acid molecules per dextran block by the ratio of the integrated area of the H-atom signal of the benzene ring on the folic acid to the integrated area of the methyl H-atom signal on the n-butyl mercaptan.

### 2.3 Preparation and characterization of ROS sensitive-nanoparticles

The FA-Dex-*b*-PPS block polymer composed of hydrophilic dextran block and hydrophobic PPS block, allowing it to self-assembly into nanoparticles in selective solvents. In this study, FA-Dex-*b*-PPS based nanoparticles were obtained through a nanoprecipitation process. It's well known that the size of nanoparticles plays an important role in drug delivery, as it determines whether the drug can reach the tumor site by passthrough the biological barriers and escaping clearance by the immune system during *in vivo* circulation[40]. Figure 3A shows the DLS result of the blank nanoparticles in aqueous solution. The size of the nanoparticles was approximately 101 nm when the nanoparticles were dispersed at a pH=7.4 aqueous solution mimicked the human physiological environment and in the absence of H<sub>2</sub>O<sub>2</sub>. The morphology of the nanoparticles was observed by transmission electron microscopy (TEM). As shown in Figure 3C, the nanoparticles were spherical in structure. Subsequent, we investigated the particle size and morphology of nanoparticles after encapsulated with the hydrophobic antitumor drug Dox. From Figure 3B, we could find that after loading with hydrophobic drug, the particle size of the nanoparticles

was increased from 101 nm to 130 nm. This is mainly due to the encapsulation of hydrophobic drug in the hydrophobic core of the nanoparticles through hydrophobic interaction[41]. As Figure 3D shows that the drug-loaded nanoparticles maintained their spherical like morphology, and exhibited a larger size than the blank nanoparticles.

The stability of nanoparticles is crucial in *in vivo* drug delivery since it prevents drug premature release, extends circulation time of drug in the blood vessel, and enhances drug accumulation at the tumor site[42]. Critical micelle concentration is a widely used parameter to characterized the stability of nanoparticles. Pyrene can act as fluorescent probe to measure the cmc value. Figure 4C shows the fluorescence spectroscopy of pyrene at different polymer concentrations at pH 7.4 and 0  $\mu\text{M}$   $\text{H}_2\text{O}_2$ . As the polymer concentration increases, a noticeable red-shift in the fluorescence spectroscopy of pyrene was occurs. This phenomenon is due to the gradual formation of nanoparticles with hydrophobic cores as the polymer concentration increases. The hydrophobic pyrene is encapsulated within the hydrophobic cores of the nanoparticles through physical interactions, causing a change in their chemical environment and resulting in a red-shift in their fluorescence spectra[43]. The cmc value of FA-Dex-*b*-PPS was approximately 0.0093 mg/mL, calculated from  $I_{337}/I_{335}$  versus the logarithm of the concentration (Figure 4C). However, when the polymeric nanoparticle in a solution with pH 6.5 and 100  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , the amphiphilic polymer was oxidized to a fully hydrophilic polymer. Consequently, even though the polymer concentration increase, it is impossible to form nanoparticles. The fluorescence spectra of pyrene at different polymer concentrations under these conditions (Figure 4B) indicate that the fluorescence intensity of pyrene is weak, and there is no red-shift in the spectra. As a result, the cmc value cannot be obtained.

#### **2.4 Drug loading and ROS triggered *in vitro* release**

Doxorubicin is a broad-spectrum chemotherapeutic agent; however, its therapeutic utility is compromised by its poor aqueous solubility and the occurrence of toxicological effects on non-targeted organs. Amphiphilic block polymers are widely

used as drug nanocarriers to enhance the water solubility of antitumor drugs and minimize their side effects. In this study, Dox was encapsulated within the hydrophobic core of FA-Dex-*b*-PSS nanoparticles using a nano-precipitation method. The drug loading content was approximately 6.3 %, indicating the potential of FA-Dex-*b*-PSS as a promising nano-drug delivery system for tumor therapy.

Further, the release of the encapsulated drug from the drug-laden nanoparticles was investigated *in vitro*. As shown in Figure 4D, under normal physiological conditions (pH=7.4, 0.0  $\mu$ M H<sub>2</sub>O<sub>2</sub>), Dox was slowly released from the nanoparticles, and the cumulative release was no more than 20 % over 72 h. At a weak acid environment (pH=6.5, without H<sub>2</sub>O<sub>2</sub>), the release rate of Dox increased slightly, and the cumulative release of approximately 30 % at the same period. A faster drug release rate was observed under a pH=4.5 acidic conditions (without H<sub>2</sub>O<sub>2</sub>), and almost 70 % of the Dox was released over 72 h. This phenomenon could be attributed to the improved solubility of Dox under acidic conditions[44]. Under simulate human physiological conditions (pH 7.4 contain with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>), the cumulative 72 h release of Dox was less than 20 %. Maintaining the same pH of 7.4 and increase the H<sub>2</sub>O<sub>2</sub> concentration to 500  $\mu$ M did not significantly increase the release of Dox, with the cumulative drug release only approaching 28 %. This indicated that PPS could not be oxidized only in the presence of H<sub>2</sub>O<sub>2</sub>. However, when the pH value was lowered to 6.5 and H<sub>2</sub>O<sub>2</sub> concentration was set at 100  $\mu$ M, approximately 60 % of the Dox was released from the nanoparticles. As mentioned above, the PPS block could be oxidized under weak acidic conditions, containing 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>, resulting in a hydrophobic to hydrophilic transition. Hence, under this condition, a faster release of Dox phenomenon could be observed.

## **2.5 The antitumor effects of FA-Dex-*b*-PPS-Dox and cellular uptake on prostate cancer cells in vitro**

It has also been reported that targeted therapy of prostate cancer can be achieved by targeting PC3 cells with folic acid decorated nanoparticles[45, 46]. These results suggest that we can achieve prostate cancer precise therapy by targeting folate

receptor of PC3 cells. Hence, PC3 cells were selected as our research cells in this study based on the above basis. Firstly, the biocompatibility of the synthesized FA-Dex-*b*-PPS was evaluated by CCK-8 assay in vitro. When the concentration of FA-Dex-*b*-PPS reached 400 µg/mL, the PC3 cells survival rate was still more than 80 %, indicating there was good biocompatibility of FA-Dex-*b*-PPS as nanocarriers (Figure 5A). Then, after FA-Dex-*b*-PPS encapsulating with Dox, we quantified it with Dox as the gradient concentration to evaluate the inhibition of cell proliferation by CCK-8 assay. After 24 h incubation, the cell viability of FA-Dex-*b*-PPS-Dox group was  $41.46 \pm 0.31$  % compared with the control group, while cell viability dropped to only  $17.61 \pm 0.47$  % after 48 h incubation. FA-Dex-*b*-PPS-Dox inhibited the growth of PC3 cells more obviously than Free Dox and Dex-*b*-PPS-Dox (Fig. 5B). Furthermore, the invasion and migration with different treatment on PC3 cells were also evaluated by transwell and wound healing assays. As shown in Figure 5C, 5D, 5E, and 5F, different treatments could inhibit the invasion and migration on PC3 cells. FA-Dex-*b*-PPS-Dox exhibited great stronger inhibition both invasion and migration among them.

Next, to visualize distribution of the nanoparticles, PC3 cells were incubated with 5 µg/mL Dox of nanoparticles for predetermined time durations. Then specificity and cellular accumulation of Free Dox or nanoparticles by PC3 cell lines were confirmed by confocal laser scanning microscopy (CLSM) through intracellular Dox accumulations (red-fluorescence) (Figure 5G). The cell nuclei were labeled with Hoechst dye, exhibiting a blue fluorescence. Notably, following incubation, the free form of Dox preferentially accumulated within the cell nuclei, having been transported into the cells via osmosis. This is consistent with previous literature reports that the uptake of Dox was mainly dependent on a diffusion mechanism[47]. Compared with Free Dox, it is of more accumulation of Dex-*b*-PPS-Dox nanoparticles in cytoplasm. While FA-Dex-*b*-PPS-Dox distributed diffusely both cell nuclei and cytoplasm of cells, which was speculated to the uptake of Dox through the folic acid receptor-mediated endocytosis process, leading to its uniform distribution in

the whole cell[48]. All the above results demonstrates that FA-Dex-*b*-PPS-Dox has a good antitumor effect *in vitro* and is a kind of nanoparticles with great potential value.

## **2.6 FA-Dex-*b*-PPS-Dox induced apoptosis of PC3 cells through ROS production**

As research in nanotechnology for cancer treatment intensifies and its applications broaden, the design of ROS-responsive nanodrug delivery system has become an important platform for cancer diagnosis and therapy[49]. NADPH oxidase 4 (NOX4) is a member of the NADPH oxidase, which can catalyze the reduction of molecular oxygen to various ROS[50]. The ROS produced by NOX4 are involved in various biological functions such as cell apoptosis, differentiation and tumor growth[51]. Dox, a kind of the most common cancer chemotherapeutic drugs, is widely used in multiple clinical cancer therapy. It was reported that Dox can induce the increase  $H_2O_2$  levels, breaking the balance of oxidative stress in cells by regulating the NOX4 expression levels[52]. These indicated that Dox could be used as a potential drug to enhance  $H_2O_2$  levels of cells. In this study, FA-Dex-*b*-PPS nanocarriers were used to load traditional chemotherapy drug Dox to form a novel nanomedicine (FA-Dex-*b*-PPS-Dox). On the one hand, Dox exerted traditional chemotherapy effects. On the other hand, Dox increased the production of intracellular  $H_2O_2$  through NOX4 pathway, which further oxidized the PPS block, thereby inducing a hydrophobic-hydrophilic transition, leading to the dissociation of the polymer self-assembly and ultimately results in the rapid release of the drug. As shown in Figure 6A and 6B, both Dex-*b*-PPS-Dox and FA-Dex-*b*-PPS-Dox could increase the expression levels of NOX4. Further, flow cytometry (Figure 6C) and CLSM (Figure 6D) were used to evaluate qualitatively and quantitatively the production of intracellular ROS, respectively. FA-Dex-*b*-PPS-Dox significantly increases intracellular ROS levels, demonstrating the targeted enhancement of Dex-*b*-PPS-Dox with FA modification. In addition, glutathione (GSH) and superoxide dismutase (SOD) levels reflect the antioxidant abilities of intracellular cells. As shown in Figure 6E and 6F, compared with control group, Free Dox could increase both intracellular GSH and SOD levels. FA-Dex-*b*-PPS-Dox significantly reduced GSH

and SOD levels, while there were no significant SOD levels differences after Dex-*b*-PPS-Dox treatment. These results illustrated cell antioxidant status of different treatments. Due to a series of intracellular redox chemical reactions after FA-Dex-*b*-PPS-Dox treatment, all those reduced the antioxidant capacity of cancer cells, resulting the reduction of GSH and SOD levels. In general, FA-Dex-*b*-PPS-Dox elevated intracellular ROS levels, while depleted GSH and SOD levels.

Next, we further evaluated cell apoptosis using flow cytometry and western blot assays, including the expression levels of Cleaved Caspase-3, a key factor of apoptosis. The results, as shown in Figure 6G, 6H, and 6I, revealed that FA-Dex-*b*-PPS-Dox treatment significantly induced apoptosis of PC3 cells when compared with the control group. Additionally, the expression levels of Cleaved Caspase-3 were significantly increased after Free Dox and FA-Dex-*b*-PPS-Dox treatment, while Dex-*b*-PPS-Dox showed no significant difference. These findings demonstrate that FA-Dex-*b*-PPS-Dox induces apoptosis of PC3 cells through ROS production. Significantly, the findings of the present investigation imply that FA-Dex-*b*-PPS-Dox may represent a potential therapeutic strategy for targeting PC3 tumor cells.

## 2.7 The antitumor efficacy of FA-Dex-*b*-PPS-Dox *in vivo*

To assess the efficacy of various treatments *in vivo*, male nude mice harboring PC3 tumors were employed as the experimental animal model. The animal tumor models were established by subcutaneously injecting  $5 \times 10^6$ /mL PC3 cells. When the tumor volume reached approximately 50-100 mm<sup>3</sup>, all mice were randomly divided into 4 groups, namely Control, Free Dox, Dex-*b*-PPS-Dox, and FA-Dex-*b*-PPS-Dox, and were injected through the tail vein with a dosage of 5 mg/kg Dox. Figure 7A and 7B display the tumor volume and weight after different treatments. The tumor volume in mice treated with FA-Dex-*b*-PPS-Dox decreased notably compared to the control groups, but there was no difference observed in the tumor volume of FA-Dex-*b*-PPS-Dox compared with Dex-*b*-PPS-Dox. However, there was a statistically significant difference between the tumor weight of FA-Dex-*b*-PPS-Dox



and Dex-*b*-PPS-Dox, indicating that FA-Dex-*b*-PPS-Dox effectively inhibited tumor growth. This phenomenon could be explained by the specific absorption of FA-Dex-*b*-PPS-Dox by the folic acid receptor on tumor cells.

The antitumor efficacy of FA-Dex-*b*-PPS-Dox was further validated through hematoxylin-eosin and immunohistochemical staining of the tumor tissues, as demonstrated in Figure 7C. Representative images showed a greater number of obviously necrotic regions in tumor tissue after FA-Dex-*b*-PPS-Dox treatment using hematoxylin-eosin staining. In addition, compared to the other groups, the ratio of positive cells of Cleaved Caspase-3 was elevated, and the ratio of negative cells of proliferating cell nuclear antigen (PCNA) was reduced after FA-Dex-*b*-PPS-Dox treatment using immunohistochemistry staining. These findings indicate that FA-Dex-*b*-PPS-Dox is capable of triggering apoptosis and inhibiting the proliferation of cancer cells. Moreover, there were no significant changes in the body weight of the four groups, as demonstrated in Figure 8A. Notably, the survival quality of mice after Free Dox treatment was very poor, and the mortality rate of conventional chemotherapy drugs Free Dox treatment was also high, with obvious hepatorenal toxicity, as shown in Figure 8B and 8C.

To investigate the related toxicity of these nanomedicines *in vivo*, the major metabolic organs, including the heart, liver, spleen, lung, and kidney, were evaluated using hematoxylin-eosin staining. There was significant inflammatory cell infiltration in the liver and kidney after Free Dox or Dex-*b*-PPS-Dox treatments, while FA-Dex-*b*-PPS-Dox showed no obvious abnormalities in these major metabolic organs. This indicates that the modification of FA could enhance the tumor targeting of drugs and reduce accumulation in metabolic organs through the folic acid receptor-mediated endocytosis process.

### 3. Conclusion

We report on the development of ROS-responsive Dex-*b*-PPS copolymers for targeted oncotherapy. The Dex-*b*-PPS copolymers exhibit strong ROS responsiveness and high loading capacity for Dox, and after modification with FA, they are capable

of targeted oncotherapy. Our studies have demonstrated that FA-Dex-*b*-PPS micelles release Dox faster in response to 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>. *In vitro* assays such as the CCK-8 assay, transwell assay, and wound healing assay, along with *in vivo* experiments on xenograft mouse models, demonstrate that FA-Dex-*b*-PPS-Dox exhibits greater antitumor effects compared to non-targeted Dex-*b*-PPS-Dox. These results suggest that folic acid decorated Dex-*b*-PPS copolymers are a promising class of therapeutic block copolymer nanoparticles that are ROS-responsive and have great potential in the field of oncotherapy.

#### 4. EXPERIMENTAL SECTION

*Materials:* 1-buthane thiol, 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) and propylene sulfide (PS) (>96 %) were purchased from Tokyo Chemical Industry (TCI, Japan). Propargyl bromide (99 %) was supplied by Alfa Aesar and used as received. Dextran (Mn = 6600 g/mol) and Sodium azide (NaN<sub>3</sub>, 99 %) were obtained from Sigma-Aldrich. Sodium cyanoborohydride (NaBH<sub>3</sub>CN, 98 %) and 3-chloropropylamine hydrochloride (98 %) were acquired from Aladdin Chemical Company (Shanghai, China). Copper sulfate pentahydrate (CuSO<sub>4</sub>•5H<sub>2</sub>O, 99 %) and sodium ascorbate (NaAsc, 99 %) were acquired from Macklin Chemical Company (Shanghai, China). Doxorubicin hydrochloride (Dox•HCl) was purchased from Meilunbio (Dalian, China). Tetrahydrofuran (THF, chromatographic grade, >99.9 %) and dichloromethane (DCM, Chromatographic grade, >99.9 %) were obtained from J&K Chemical Reagent Inc (Beijing, China) and used as received without further purification. All additional chemicals were utilized in their as-received state without undergoing any additional purification steps.

*Methods—Bioinformatics analysis:* The Cancer Genome Atlas (TCGA) pan-cancer dataset, which encompasses RNA-sequencing data and curated clinical phenotypes for 34 prevalent cancer types, was obtained from the Pan-Cancer Atlas Hub within the University of California Santa Cruz (UCSC) Xena dataset (<https://xenabrowser.net/>). Specifically, transcriptome data for the FOLH1 gene were extracted from 34 tumor types, including glioblastoma (GBM, t=153, n=1157),

glioblastoma multiforme (GBMLGG, t=662, n=1157), lower grade glioma (LGG, t=509, n=1157), uterine corpus endometrioid carcinoma (UCEC, t=180, n=23), breast invasive carcinoma (BRCA, t=1092, n=292), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC, t=304, n=13), lung adenocarcinoma (LUAD, t=513, n=397), esophageal squamous cell carcinoma (ESCA, t=181, n=668), stomach and intestinal stromal tumor (STES, t=595, n=879), kidney renal papillary cell carcinoma (KIRP, t=288, n=168), kidney and pancreas cancer (KIPAN, t=884, n=168), colorectal adenocarcinoma (COAD, t=288, n=349), colorectal and rectal adenocarcinoma (COADREAD, t=380, n=159), prostate adenocarcinoma (PRAD, t=495, n=152), stomach adenocarcinoma (STAD, t=414, n=211), head and neck squamous cell carcinoma (HNSC, t=518, n=44), kidney renal clear cell carcinoma (KIRC, t=530, n=168), lung squamous cell carcinoma (LUSC, t=498, n=397), liver hepatocellular carcinoma (LIHC, t=369, n=160), Wilms tumor (WT, t=120, n=168), skin cutaneous melanoma (SKCM, t=102, n=558), bladder urothelial carcinoma (BLCA, t=407, n=28), thyroid carcinoma (THCA, t=504, n=338), rectal adenocarcinoma (READ, t=92, n=10), ovarian serous cystadenocarcinoma (OV, t=419, n=88), pancreatic adenocarcinoma (PAAD, t=178, n=171), testicular germ cell tumor (TGCT, t=148, n=165), uterine corpus sarcoma (UCS, t=57, n=78), acute lymphoblastic leukemia (ALL, t=132, n=337), acute myeloid leukemia (LAML, t=173, n=337), pheochromocytoma and paraganglioma (PCPG, t=177, n=3), adenocarcinoma of the breast (ACC, t=77, n=128), kidney chromophobe (KICH, t=66, n=168), cholangiocarcinoma (CHOL, t=36, n=9), as well as relevant paracancerous tissues.

The dataset for this study was sourced from the Uniform Standardized Pan-Cancer dataset provided by the University of California, Santa Cruz (UCSC) database (<https://xenabrowser.net/>) – the TCGA Pan-Cancer (PANCAN), which encompasses 10,535 samples and 60,499 genes. Building upon this foundation, we extracted the expression data for the ENSG00000086205 (FOLH1) gene across various samples. Subsequently, we further refined our selection to include samples derived from

primary blood-derived cancers (Peripheral Blood) and primary tumors. Following the methodology outlined in the previous study (Malta, T. M. et al, 2018), we computed the RNAss tumor stemness scores corresponding to the mRNA characteristics of each tumor. Thereafter, we integrated the stemness indices with gene expression data and applied a  $\log_2(x+1)$  transformation to each expression value. After excluding cancer types with fewer than three samples, we ultimately obtained expression data for 37 cancer types.

*Methods—Methods and Instrumentation:* The infrared absorption spectra of the samples were measured using a Perkin-Elmer Paragon 1000 infrared spectrometer, USA. The chemical structure of the polymers was analyzed using a Bruker NMR instrument (Bruker AV-500). All deuterated solvents were purchased from Sigma and tetramethylsilane (TMS) was used as an internal standard. Particle size and particle distribution index (PDI) were determined by using a Malvern particle sizer (Malvern Nano-ZS/ZEN-3600 Zetasizer). The dry-state morphology of the nanoparticles was examined utilizing a transmission electron microscope (TEM, JEM-2100F, JEOL, Tokyo, Japan). A minute quantity of the nanoparticle suspension was deposited onto a copper grid, and following a one-minute interval, the excess solution was meticulously blotted away using filter paper. Subsequently, the specimens were subjected to negative staining with a 1 wt% phosphotungstic acid solution. A small phosphotungstic acid solution droplet was cover the sample, after 20 seconds the excess phosphotungstic acid solution was carefully blotted off with filter paper. Prior to TEM observation, samples are stored in a desiccator to remove excess moisture. The critical micelle concentration (cmc) for the block copolymer was ascertained through the application of the widely employed pyrene fluorescence probe technique. Briefly, 50  $\mu\text{L}$  of acetone solution containing pyrene  $6 \times 10^{-5}$  mol/L was added to a 10 mL EP tube, and the acetone was removed via evaporation. Various concentrations of the block polymer's aqueous solutions were introduced into EP tubes, which were subsequently agitated in the absence of light for 24 h at 37 °C on a temperature-controlled shaker before conducting the tests. The tests were carried out

using a Shimadzu RF-5301PC fluorescence spectrophotometer. The excitation spectrum was scanned in the wavelength range of 300-350 nm, with an emission wavelength of 373 nm and a slit width of 5 nm. The fluorescence spectra of pyrene in the nanoparticle solution were recorded separately under two different conditions, that is, PBS (50 mM, pH 7.4) without any H<sub>2</sub>O<sub>2</sub> or PBS (50 mM, pH 6.5) with 100 μM H<sub>2</sub>O<sub>2</sub>. The intensity ratio of fluorescence intensity at 337 nm and 335 nm was used as the vertical coordinate and the logarithm of micelle concentration was used as the horizontal coordinate. The concentration value corresponding to the intersection of the data points is the measured cmc. The concentration of Dox in the aqueous solution was measured using a Shimadzu UV-3150 UV-Vis spectrometer and the absorbance intensity at 480 nm was recorded. A working curve was established by measuring the UV absorption at 480 nm for a series of known concentrations of aqueous Dox solutions.

*Methods—Preparation of α-alkyne Poly(Propylene sulfide) (alkyne-PPS):* α-alkyne poly(propylene sulfide) can be synthesized via a combination of anionic polymerization of propylene sulfide and termination with propargyl bromide. Prior to anionic polymerization, the monomer propylene sulfide is first purified by distillation. The anionic polymerization process of propylene sulfide is based on the method previously reported in the literature[23]. A 100 mL flame-dried round-bottom flask was filled with DBU (4 mmol, 0.60 mL) and 20 mL of ultra-dry THF, and then degassed with argon gas for 30 minutes. The flask was placed in a cold trap to keep the solution temperature below 0 °C. The pre-degassed THF solution containing 1-buthane thiol (1.0 mmol, 0.07 mL) was then slowly dripped into the flask via a dropping funnel, and the reaction was continued for 30 minutes to activate the initiator. The degassed propylene sulfide (80 mmol, 6.24 mL) monomer was then added to the round-bottom flask in one go using a syringe, and the reaction was stirred for 2 h at a temperature of no more than 0 °C. Propargyl bromide (2 mmol, 0.24 g) was added to the reaction system to terminate the anionic polymerization reaction and to introduce an alkyne group. After 12 h of reaction, the resulting salt was removed by filtration,

and most of the THF was distilled out under reduced pressure. The mixture was then dropped into cold anhydrous ether to give a white precipitate, which was collected, washed three times with ether, and dried under vacuum to yield a white viscous polymer. <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>, δ): 0.9-0.95 (t, CH<sub>3</sub>CH<sub>2</sub>), 1.35-1.41 (s, CH<sub>3</sub>CH), 1.45-1.52 (s, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.53-1.60 (m, CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.50-2.56 (t, CH≡C-), 2.56-2.70 (s, CHCH<sub>2</sub>S), 2.83-2.98 (t, SCHCH<sub>2</sub>).

*Methods—Preparation of α-azide Dextran (azide-Dex):* The azide functionality was incorporated into the reducing end of dextran by employing a previously documented procedure, albeit with slight modifications [53]. Initially, a mixture consisting of 2 g of dextran and 40 mL of deionized water was prepared in a 100 mL round-bottomed flask. After complete dissolution of the dextran, 15 g of 3-chloropropylamine hydrochloride and 2 g of NaBH<sub>3</sub>CN were added to the flask. The reaction vessel was secured with a rubber stopper throughout the process. The reaction solution was agitated in an oil bath maintained at 50 °C for a duration of 7 days, during which time 3 g of NaBH<sub>3</sub>CN was added daily. Upon completion of the reaction, the product was decanted into a dialysis membrane (1000 Da) and subjected to dialysis against deionized water for a period of three days to remove residual inorganic contaminants. It should be highlighted that throughout the dialysis procedure, the volume of the mixed solution in the dialysis tube increases significantly, and the collected dialysate must be concentrated before the next reaction. The aqueous fraction of the mixture was largely evaporated through vacuum distillation, resulting in a volume of approximately 30 mL of solution. Subsequently, 2 g of sodium azide were introduced into the solution, followed by stirring in an oil bath heated to 80 °C for a duration of 24 h. The product was then subjected to dialysis against deionized water over a three-day period to eliminate surplus inorganic salts. The process was concluded with the acquisition of a white powder, achieved by lyophilizing the dialysate.

*Methods—Synthesis of dextran-block-poly(propylene sulfide) copolymer by Husigen Cycloaddition:* Copper(I)-catalyzed azide/alkyne Husigen cycloaddition has

been widely utilized to prepare block polymers that incorporate polysaccharide chain segments[35]. In this work, a polysaccharide-based diblock copolymer was synthesized via the Huisgen cycloaddition between alkyne-PPS and azide-Dex. In a representative procedure, 0.16 g of azide-modified dextran was transferred into a 50 mL round-bottomed flask, which already held 25 mL of DMSO. Viscous PPS is hard to dissolve directly in DMSO, so 0.1 g of alkyne-PPS was dissolved in 4 mL of THF and then slowly added dropwise to the DMSO solution. To prevent the oxidation of cuprous ions by oxygen dissolved in the DMSO, a high-purity argon gas was continuously passed through the DMSO to remove oxygen.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and NaAsc were subsequently added, and the flask was further maintained under an argon atmosphere for 10 minutes. The flask was sealed with a rubber stopper, and the mixture was stirred in an oil bath at 50 °C for 3 days. After the reaction, the mixture was transferred to dialysis tubes (50 kDa) and dialyzed with deionized water containing 0.01 % EDTA-Na for 2 days, followed by dialysis with deionized water for another 2 days to remove excess inorganic salts and azide-Dex. The dialysate was lyophilized to obtain white powder.

*Methods—Preparation of folic acid decorated dextran-block-poly(propylene sulfide) (FA-Dex-b-PSS) copolymer via esterification reaction:* The hydroxyl groups present on dextran provides an opportunity for esterification with the carboxyl group of folic acid. In this study, folic acid decorated Dex-b-PSS was synthesized following a previously reported procedure[54]. Briefly, 0.02 g of FA was dissolved in 15 mL of anhydrous DMSO. After the folic acid had fully dissolved, to the flask, 0.1 g of Dex-b-PSS was introduced, after which EDCI (0.017 g, 0.09 mmol) and HOBT (0.012 g, 0.09 mmol) were sequentially added. Subsequently, the flask was sealed, wrapped in aluminum foil, and agitated at ambient temperature for a period of two days. After the reaction was complete, the reaction mixture was packed into dialysis bags (6 kDa) and dialyzed for three days to remove excess folic acid and DMSO. The dialysate was lyophilized, yielding a yellow powder. The degree of substitution of folic acid can be calculated using methods previously described in literature[54].

*Methods— ROS sensitive-nanoparticles preparation and characterization:* The FA-Dex-*b*-PSS amphiphilic copolymer is capable of undergoing self-assembly to produce nanoparticles in chosen solvents. Then, 10 mg of FA-Dex-*b*-PSS was dissolved in 1.5 mL of warm DMSO under continuous stirring. The combined solution was incrementally introduced into 5 mL of deionized water under continuous stirring for 1 h. Subsequently, the mixture was transferred to a dialysis sack with a 6 kDa molecular weight cutoff and subjected to dialysis against deionized water for a duration of 2 days to eliminate the organic solvents. Following this, the dialysate was filtered through a 0.22 µm pore-size syringe filter. The dimensions and dispersal of the synthesized nanoparticles were assessed utilizing a Malvern particle size analyzer. The dried state morphology of the nanoparticles was examined with a JEOL JEM-2100F transmission electron microscope. The critical micelle concentration of the block polymer was ascertained using the established pyrene fluorescence probe technique, employing a Shimadzu RF-5301PC fluorescence spectrophotometer.

*Methods— Dox loaded and in vitro release:* To incorporate Dox within the internal compartment of the nanoparticles, a solution containing 10 mg of FA-Dex-*b*-PSS and 1 mg of Dox•HCl was prepared by dissolving them in warm DMSO. Triethylamine was added in an equivalent amount to neutralize the hydrochloride, and the resulting mixture was dropped into deionized water with continuous stirring. Following this, the solution was decanted into a dialysis tube and subjected to dialysis against deionized water for a period of 24 h. The resulting dialysate was then passed through a needle filter. The concentration of Dox was determined using an established standard curve, and the drug loading content (DLC) was calculated using the following formula:

$$\text{DLC(\%)} = w_1/w_2 \times 100 \quad (1)$$

where  $w_1$  is the weight of Dox loaded in the nanoparticles and  $w_2$  represents the weight of FA-Dex-*b*-PSS.

*In vitro* drug release was conducted under various conditions, including pH 7.4 without H<sub>2</sub>O<sub>2</sub>, pH 6.5 without H<sub>2</sub>O<sub>2</sub>, pH 4.5 without H<sub>2</sub>O<sub>2</sub>, pH 7.4 with 500 µM H<sub>2</sub>O<sub>2</sub>,



pH 7.4 with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>, and pH 6.5 with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>. To begin, 3 mL of drug-loaded nanoparticle solution was placed into a dialysis bag and transferred to a centrifuge tube containing 27 mL of PBS at different pH and H<sub>2</sub>O<sub>2</sub> concentrations. The tubes were then sealed and placed into a 37 °C water bath, where they were agitated at 100 rpm. At predetermined time intervals, 3 mL of solution outside the dialysis bag was withdrawn and replaced with 3 mL of fresh buffer solution. The drug release experiment was repeated three times, and the Dox concentration was measured at 480 nm using a UV-Vis spectrometer (UV-3150, Shimadzu, Japan). The accumulated percentage of released Dox was calculated using the following equation:

$$E_r(\%) = \frac{V_e \sum_{i=1}^{n-1} C_i + V_0 C_n}{m_{\text{DOX}}} \times 100\% \quad (2)$$

In which  $m_{\text{DOX}}$  denotes the mass of doxorubicin within the micelles,  $V_0$  signifies the total volume of the release medium ( $V_0$  is 30 mL),  $V_e$  is the volume of the medium exchanged ( $V_e = 3$  mL), and  $C_i$  is the concentration of DOX in the  $i$ th aliquot.

*Methods—CCK-8 assay:* Human prostate cancer cell lines PC3 was maintained in RPMI-1640 medium (Hyclone, United States) that contained 10 % fetal bovine serum (FBS) and 1 % penicillin-streptomycin, within a 37°C incubator under a 5% CO<sub>2</sub> environment. CCK-8 assay was conducted to evaluate the in vitro biocompatibility of FA-Dex-*b*-PPS and the inhibition of cell proliferation of Free Dox, Dex-*b*-PPS-Dox, and FA-Dex-*b*-PPS-Dox to PC3 cells. Briefly, cells were plated into 96-well plates at a density of approximately  $1 \times 10^4$  cells/well. After 24 h or 48 h incubation with different treatments, 10  $\mu$ L solution of different treatments concentrations were added to the appropriate wells for appropriate time in a 5 % CO<sub>2</sub> incubator at 37 °C. Subsequently, 10  $\mu$ L of the CCK-8 reagent were added to each well, and the plate was further incubated for a period of 2 to 4 h. The optical density of each well was then quantified at a wavelength of 450 nm using a multimode plate reader.

*Methods—Transwell assay:* The upper surface of the lower membrane in the transwell chamber was coated with a solution of Matrigel at a concentration of 50 mg/L, diluted 1:8. The cells were incubated in serum-free RPMI 1640 medium for a duration of 12 h, and the  $5 \times 10^5$  PC3 cells density of cell suspension was adjusted. A

200  $\mu$ L volume of cell suspension was introduced into the upper compartment of the transwell insert, while 600  $\mu$ L of medium supplemented with 10% FBS was placed in the lower chamber of the 24-well culture plate. The 24 well culture plates were placed in 5 % CO<sub>2</sub> incubator at 37 °C for 24 h. Afterward, the cells in the upper layer of the cell membrane were wiped with a cotton swab, and the rest of cells were fixed with 4 % paraformaldehyde for 20 minutes and dyed with crystal violet solution for 15 minutes. After the chambers were dried, pictures were taken under the microscope and conducted comparative analysis. Each sample is randomly counted for 10 visual fields, and the average value is taken.

*Methods—Wound healing assay:* PC3 cells were plated and grown to 90% confluence in a six-well plate overnight. Following this, wounds were created using a 200- $\mu$ L sterile pipette tip, and any cellular debris was subsequently cleared away. The cells were incubated in the medium solution of different treatments: Control (PBS), Free Dox, Dex-*b*-PPS-Dox, and FA-Dex-*b*-PPS-Dox. The images of distance migrated by the cells were taken at 0 and 24 h after wound scratching through a microscope (Leica, Germany) in the same position of the plate. The experiments were conducted in triplicate independently.

*Methods—Cellular uptake:* PC3 cells were treated with PBS, Free Dox, Dex-*b*-PPS-Dox, and FA-Dex-*b*-PPS-Dox maintaining Dox content 5  $\mu$ g/mL for 8 h with Hoechst for 10 min after washing with PBS. Then cellular uptake of different group by confocal laser scanning microscope.

*Methods—Reactive oxygen species generation:* The intracellular levels of ROS were quantified using a dedicated ROS assay kit (beyotime, China). The attached PC3 cells were treated with different treatments (Control, Free Dox, Dex-*b*-PPS-Dox, and FA-Dex-*b*-PPS-Dox) for 8 h. After the incubation, harvested cells were treated with 1  $\mu$ L DHE (S0033, Beyotime Biotechnology, Shanghai, China) at 37 °C with CO<sub>2</sub> in the dark for 30 minutes. Subsequently, the cells underwent two PBS washes and were then disaggregated into a uniform single-cell suspension. The fluorescent intensity was analyzed by flow cytometry (BD Bioscience).

*Methods— Apoptosis assay:* The Annexin-V apoptosis detection kit was utilized to assess the apoptotic status of PC3 cells that had been subjected to Control (PBS), Free Dox, Dex-*b*-PPS-Dox, and FA-Dex-*b*-PPS-Dox. PC3 cells were seeded and incubated for 24 h to reach 80 % confluency in a six-well plate. Then, the cells were treated with different treatments for 24 h at 37 °C in a CO<sub>2</sub> incubator. Subsequently, the cells were harvested and rinsed with chilled PBS, and then redispersed in 1× binding buffer at a concentration of 1×10<sup>6</sup> cells per milliliter. The cell suspension was then incubated with Annexin-V at ambient temperature in a light-shielded setting. Whereafter, before being analyzed by flow cytometry, the cells were washed, re-suspended, and PI was added. Using the FACS Calibur flow cytometer, the fluorescence intensity was analyzed and repeated three times. All analysis was carried out using FlowJo software.

*Methods— Western blot analysis:* PC3 cells subjected to various treatments underwent two PBS washes and were subsequently lysed using an ice-cold Radio-Immunoprecipitation Assay (RIPA) buffer containing 1mM PMSF, from which total protein extracts were obtained. The concentration of total protein was quantified using the BCA protein assay kit. Separation of proteins was achieved through electrophoretic techniques utilizing 10% SDS-polyacrylamide gel matrices, followed by the complete transfer of the desired protein fractions onto polyvinylidene difluoride membranes. Subsequent to this, the membranes were subjected to a blocking step using 5% non-fat dry milk in TBST buffer at ambient temperature for a duration of 1 h. This was followed by an overnight incubation at 4°C with specific primary antibodies against NOX4, Cleaved Caspase-3, and β-actin. After thorough washing with TBST buffer on three separate occasions, the membranes were then probed with horseradish peroxidase-conjugated secondary antibodies for an hour at 37°C. β-actin served as the internal reference for normalization purposes. All experiments were carried out with three replicates. Blots were determined using chemiluminescence detection system membranes. Image J software (version 1.8.0, NIH, USA) was used to quantify the intensity of the immunoreactive bands.

*Methods— In vivo antitumor study:* Animal studies were conducted with BALB/c mice aged between 4 to 6 weeks. All experiments were handled based on the relevant principles of the Guide for the Care and Use of Laboratory Animals and approved by the ethics committee of the South China University of Technology (Approval no. S-2023-078-01). Experimental animals were allocated to various groups in a random fashion. For tumor induction, approximately  $5 \times 10^6$  PC3 cells were harvested and administered subcutaneously to the right flanks of nude mice, and tumor growth was monitored until the volume reached 50-100 mm<sup>3</sup>. The test animals received a single intravenous dose of the therapeutic agent at 5 mg/kg body weight, administered every other day for a total of five injections. In contrast, the control group received an equivalent volume of PBS via the same route. Tumor dimensions and the body weight of the mice were recorded throughout the study. Following the completion of the treatment regimen, mice were euthanized via cervical dislocation. Subsequent to euthanasia, hematoxylin and eosin (H&E) staining was utilized to examine the histological features of tumor tissues as well as the primary organs, including the heart, liver, spleen, lungs, and kidneys.

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## Conflict of Interest

The authors report no conflict of interest in the present study.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability Statement

All the data associated with this study are presented in the paper or in the Supporting Information.

## Keywords

Folic acid, Copolymer nanoparticles, Prostate cancer.

## References

1. Siegel, R. L.; Miller, K. D.; Fuchs, H. E.; Jemal, A., *CA: a cancer journal for clinicians* **2022**, *72* (1), 7-33. DOI 10.3322/caac.21708.
2. Yu, L.; Sui, B.; Fan, W.; Lei, L.; Zhou, L.; Yang, L.; Diao, Y.; Zhang, Y.; Li, Z.; Liu, J.; Hao, X., *Journal of extracellular vesicles* **2021**, *10* (3), e12056. DOI 10.1002/jev2.12056.
3. Sridaran, D.; Bradshaw, E.; DeSelm, C.; Pachynski, R.; Mahajan, K.; Mahajan, N. P., *Cell reports. Medicine* **2023**, *4* (10), 101199. DOI 10.1016/j.xcrm.2023.101199.
4. Allen, T. M.; Cullis, P. R., *Science (New York, N.Y.)* **2004**, *303* (5665), 1818-22. DOI 10.1126/science.1095833.
5. Sharifi-Azad, M.; Fathi, M.; Cho, W. C.; Barzegari, A.; Dadashi, H.; Dadashpour, M.; Jahanban-Esfahlan, R., *Cancer cell international* **2022**, *22* (1), 196. DOI 10.1186/s12935-022-02605-y.
6. Lee, J.; Kim, K.; Kwon, I. C.; Lee, K. Y., *Advanced materials (Deerfield Beach, Fla.)* **2023**, *35* (10), e2207342. DOI 10.1002/adma.202207342.
7. Liu, P.; Wu, Y.; Xu, X.; Fan, X.; Sun, C.; Chen, X.; Xia, J.; Bai, S.; Qu, L.; Lu, H.; Wu, J.; Chen, J.; Piao, J.-G.; Wu, Z., *Nano Research* **2023**, *16* (7), 9688-9700. DOI 10.1007/s12274-023-5541-1.
8. Donadoni, E.; Siani, P.; Frigerio, G.; Di Valentin, C., *Nanoscale* **2022**, *14* (33), 12099-12116.

- DOI 10.1039/d2nr02603a.
9. Dominguez-Martinez, I.; Joaquin-Ovalle, F.; Ferrer-Acosta, Y.; Griebenow, K. H., *Pharmaceutics* **2022**, *14* (3). DOI 10.3390/pharmaceutics14030490.
  10. Zhu, J.; Li, H.; Xiong, Z.; Shen, M.; Conti, P. S.; Shi, X.; Chen, K., *ACS applied materials & interfaces* **2018**, *10* (41), 34954-34964. DOI 10.1021/acsami.8b12355.
  11. Du, W.; Lu, Q.; Zhang, M.; Cao, H.; Zhang, S., *ACS applied bio materials* **2021**, *4* (4), 3246-3255. DOI 10.1021/acsabm.0c01612.
  12. DeCarlo, A.; Malardier-Jugroot, C.; Szewczuk, M. R., *Bioconjugate chemistry* **2021**, *32* (3), 512-522. DOI 10.1021/acs.bioconjchem.0c00625.
  13. Shams, A.; Shabani, R.; Asgari, H.; Karimi, M.; Najafi, M.; Asghari-Jafarabadi, M.; Razavi, S. M.; Miri, S. R.; Abbasi, M.; Mohammadi, A.; Koruji, M., *Nanomedicine (London, England)* **2022**, *17* (8), 531-545. DOI 10.2217/nnm-2021-0210.
  14. Omurtag Ozgen, P. S.; Atasoy, S.; Zengin Kurt, B.; Durmus, Z.; Yigit, G.; Dag, A., *Journal of materials chemistry. B* **2020**, *8* (15), 3123-3137. DOI 10.1039/c9tb02711d.
  15. Wang, X.; Li, C.; Wang, Y.; Chen, H.; Zhang, X.; Luo, C.; Zhou, W.; Li, L.; Teng, L.; Yu, H.; Wang, J., *Acta pharmaceutica Sinica. B* **2022**, *12* (11), 4098-4121. DOI 10.1016/j.apsb.2022.08.013.
  16. Lin, J. T.; Du, J. K.; Yang, Y. Q.; Li, L.; Zhang, D. W.; Liang, C. L.; Wang, J.; Mei, J.; Wang, G. H., *Materials science & engineering. C, Materials for biological applications* **2017**, *81*, 478-484. DOI 10.1016/j.msec.2017.08.036.
  17. Ding, H.; Tan, P.; Fu, S.; Tian, X.; Zhang, H.; Ma, X.; Gu, Z.; Luo, K., *Journal of controlled release : official journal of the Controlled Release Society* **2022**, *348*, 206-238. DOI 10.1016/j.jconrel.2022.05.056.
  18. Ge, C.; Zhu, J.; Wu, G.; Ye, H.; Lu, H.; Yin, L., *Biomacromolecules* **2022**, *23* (6), 2647-2654. DOI 10.1021/acs.biomac.2c00399.
  19. Zhang, Y.; Li, Y.; Huang, S.; Zhang, H.; Lin, Q.; Gong, T.; Sun, X.; Zhang, Z.; Zhang, L., *Nanoscale* **2021**, *13* (36), 15267-15277. DOI 10.1039/d1nr02964a.
  20. Su, M.; Xiao, S.; Shu, M.; Lu, Y.; Zeng, Q.; Xie, J.; Jiang, Z.; Liu, J., *Colloids and surfaces. B, Biointerfaces* **2020**, *193*, 111067. DOI 10.1016/j.colsurfb.2020.111067.
  21. Lee, S. H.; Gupta, M. K.; Bang, J. B.; Bae, H.; Sung, H. J., *Adv Healthc Mater* **2013**, *2* (6), 908-15. DOI 10.1002/adhm.201200423.
  22. Ye, H.; Zhou, Y.; Liu, X.; Chen, Y.; Duan, S.; Zhu, R.; Liu, Y.; Yin, L., *Biomacromolecules* **2019**, *20* (7), 2441-2463. DOI 10.1021/acs.biomac.9b00628.
  23. Gupta, M. K.; Martin, J. R.; Werfel, T. A.; Shen, T.; Page, J. M.; Duvall, C. L., *Journal of the American Chemical Society* **2014**, *136* (42), 14896-902. DOI 10.1021/ja507626y.
  24. Cerritelli, S.; O'Neil, C. P.; Velluto, D.; Fontana, A.; Adrian, M.; Dubochet, J.; Hubbell, J. A., *Langmuir : the ACS journal of surfaces and colloids* **2009**, *25* (19), 11328-35. DOI 10.1021/la900649m.
  25. Allen, S. D.; Liu, Y. G.; Kim, T.; Bobbala, S.; Yi, S.; Zhang, X.; Choi, J.; Scott, E. A., *Biomaterials science* **2019**, *7* (2), 657-668. DOI 10.1039/c8bm01224e.
  26. Velluto, D.; Demurtas, D.; Hubbell, J. A., *Molecular pharmaceutics* **2008**, *5* (4), 632-42. DOI 10.1021/mp7001297.
  27. Wu, W.; Chen, M.; Luo, T.; Fan, Y.; Zhang, J.; Zhang, Y.; Zhang, Q.; Sapin-Minet, A.; Gaucher, C.; Xia, X., *Acta biomaterialia* **2020**, *103*, 259-271. DOI 10.1016/j.actbio.2019.12.016.
  28. Zeng, Z.; Chen, S.; Chen, Y., *ChemMedChem* **2023**, *18* (20), e202300245. DOI

10.1002/cmdc.202300245.

29. Napoli, A.; Tirelli, N.; Kilcher, G.; Hubbell, J. A., *Macromolecules* **2001**, *34*, 8913-8917.

30. McSweeney, M. D.; Wessler, T.; Price, L. S. L.; Ciociola, E. C.; Herity, L. B.; Piscitelli, J. A.; Zamboni, W. C.; Forest, M. G.; Cao, Y.; Lai, S. K., *Journal of controlled release : official journal of the Controlled Release Society* **2018**, *284*, 171-178. DOI 10.1016/j.jconrel.2018.06.002.

31. Hong, L.; Wang, Z.; Wei, X.; Shi, J.; Li, C., *Journal of pharmacological and toxicological methods* **2020**, *102*, 106678. DOI 10.1016/j.vascn.2020.106678.

32. Yazdi, M. K.; Sajadi, S. M.; Seidi, F.; Rabiee, N.; Fatahi, Y.; Rabiee, M.; Dominic, C. D. M.; Zarrintaj, P.; Formela, K.; Saeb, M. R.; Bencherif, S. A., *Progress in polymer science* **2022**, *133*. DOI 10.1016/j.progpolymsci.2022.101590.

33. Song, S. G.; Oh, C.; Yoo, S.; Cho, J. Y.; Kim, K. S.; Song, C.; Premkumar, T., *International journal of biological macromolecules* **2023**, *253* (Pt 5), 127069. DOI 10.1016/j.ijbiomac.2023.127069.

34. Yang, H.; Zhang, L. M., *International journal of biological macromolecules* **2021**, *181*, 1243-1253. DOI 10.1016/j.ijbiomac.2021.05.101.

35. Schatz, C.; Louguet, S.; Le Meins, J. F.; Lecommandoux, S., *Angewandte Chemie (International ed. in English)* **2009**, *48* (14), 2572-5. DOI 10.1002/anie.200805895.

36. Soleymani, M.; Poorkhani, A.; Khalighfard, S.; Velashjerdi, M.; Khori, V.; Khodayari, S.; Khodayari, H.; Dehghan, M.; Alborzi, N.; Agah, S.; Alizadeh, A. M., *Scientific reports* **2023**, *13* (1), 13560. DOI 10.1038/s41598-023-40627-2.

37. Ngo, L.; Knothe Tate, M. L., *Scientific reports* **2023**, *13* (1), 9119. DOI 10.1038/s41598-023-30322-7.

38. Tian, H.; Yu, L.; Zhang, M.; He, J.; Sun, X.; Ni, P., *Colloids and surfaces. B, Biointerfaces* **2023**, *228*, 113400. DOI 10.1016/j.colsurfb.2023.113400.

39. Schatz, C.; Lecommandoux, S., *Macromolecular rapid communications* **2010**, *31* (19), 1664-84. DOI 10.1002/marc.201000267.

40. Yue, Y.; Li, H.; Wang, X.; Zhang, B.; Li, Y.; Liu, Y.; Ma, X.; Liu, G.; Zhao, X.; Shi, J., *ACS applied materials & interfaces* **2023**, *15* (37), 44175-44185. DOI 10.1021/acsami.3c06674.

41. Yang, X.; Chen, Y.; Yuan, R.; Chen, G.; Blanco, E.; Gao, J.; Shuai, X., *Polymer* **2008**, *49* (16), 3477-3485. DOI <https://doi.org/10.1016/j.polymer.2008.06.005>.

42. Reed, G. A., *Current protocols in pharmacology* **2016**, *75*, 7.6.1-7.6.12. DOI 10.1002/cpph.16.

43. Wilhelm, M.; Zhao, C. L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J. L.; Riess, G.; Croucher, M. D., *Macromolecules* **1991**, *24* (5), 1033-1040. DOI 10.1021/ma00005a010.

44. Chang, L.; Deng, L.; Wang, W.; Lv, Z.; Hu, F.; Dong, A.; Zhang, J., *Biomacromolecules* **2012**, *13* (10), 3301-10. DOI 10.1021/bm301086c.

45. Choi, K. H.; Nam, K. C.; Cho, G.; Jung, J. S.; Park, B. J., *Nanomaterials (Basel, Switzerland)* **2018**, *8* (9). DOI 10.3390/nano8090722.

46. Au, K. M.; Satterlee, A.; Min, Y.; Tian, X.; Kim, Y. S.; Caster, J. M.; Zhang, L.; Zhang, T.; Huang, L.; Wang, A. Z., *Biomaterials* **2016**, *82*, 178-93. DOI 10.1016/j.biomaterials.2015.12.018.

47. Lee, Y.; Park, S. Y.; Mok, H.; Park, T. G., *Bioconjugate chemistry* **2008**, *19* (2), 525-31. DOI 10.1021/bc700382z.

48. Yang, X.; Grailer, J. J.; Rowland, I. J.; Javadi, A.; Hurley, S. A.; Matson, V. Z.; Steeber, D. A.; Gong, S., *ACS nano* **2010**, *4* (11), 6805-17. DOI 10.1021/nn101670k.

49. Xu, X.; Saw, P. E.; Tao, W.; Li, Y.; Ji, X.; Bhasin, S.; Liu, Y.; Ayyash, D.; Rasmussen, J.; Huo, M.; Shi,

931 J.; Farokhzad, O. C., *Advanced materials (Deerfield Beach, Fla.)* **2017**, *29* (33). DOI  
932 10.1002/adma.201700141.

933 50. Muzza, M.; Pogliaghi, G.; Colombo, C.; Carbone, E.; Cirello, V.; Palazzo, S.; Frattini, F.; Gentilini,  
934 D.; Gazzano, G.; Persani, L.; Fugazzola, L., *Cancers* **2022**, *14* (23). DOI 10.3390/cancers14235857.

935 51. Yang, X.; Yu, Y.; Wang, Z.; Wu, P.; Su, X.; Wu, Z.; Gan, J.; Zhang, D., *Frontiers in oncology*  
936 **2022**, *12*, 968043. DOI 10.3389/fonc.2022.968043.

937 52. Zheng, H.; Xu, N.; Zhang, Z.; Wang, F.; Xiao, J.; Ji, X., *Frontiers in pharmacology* **2022**, *13*,  
938 823975. DOI 10.3389/fphar.2022.823975.

939 53. Rosselgong, J.; Chemin, M.; Almada, C. C.; Hemery, G.; Guigner, J. M.; Chollet, G.; Labat, G.;  
940 Da Silva Perez, D.; Ham-Pichavant, F.; Grau, E.; Grelier, S.; Lecommandoux, S.; Cramail, H.,  
941 *Biomacromolecules* **2019**, *20* (1), 118-129. DOI 10.1021/acs.biomac.8b01210.

942 54. Tang, Y.; Li, Y.; Xu, R.; Li, S.; Hu, H.; Xiao, C.; Wu, H.; Zhu, L.; Ming, J.; Chu, Z.; Xu, H.; Yang, X.;  
943 Li, Z., *Nanoscale* **2018**, *10* (36), 17265-17274. DOI 10.1039/c8nr04657c.

944