Supporting Information

A Versatile Carbonic Anhydrase IX Targeting Ligand Functionalized Porous Silicon Nanoplatform for Dual Hypoxia Cancer Therapy and Imaging

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Materials and methods

Fabrication of PSi: The formation of the porous structure was done using monocrystalline, boron doped p+ Si ⟨100⟩ wafers with a resistivity of 0.01–0.02 Ω·cm with a 1:1 (v/v) hydrofluoric acid (38%)–ethanol electrolyte, by electrochemical anodization. The application of pulsed low/high etching current repeated provided a multilayer structure on the Si wafers, with high porosity fracture planes for nanoparticle generation. By increasing the etching current abruptly, the multilayer film was lifted off from the substrate. The multilayer PSi films were stabilized by thermal hydrocarbonization (THCPSi), under exposure to a N_2/acetylene (1:1) flow at 500°C, and further functionalization of the THCPSi film was performed by a thermal treatment in undecylenic acid (UnTHCPSi) for 16 h at 120°C. The UnTHCPSi films were then wet milled in a 5 vol. % undecylenic acid–dodecane solution. The desired particle size was obtained by centrifugation. The surface carboxylic groups provided by the undecylenic acid were used as linkers for further modification.[1]

The selection of PEG linker: NH2-PEG-NH-Di-tert-butyl pyrocarbonate (Boc) linker was chosen because of its chemical groups (amine and Boc protected amine), which acted as linker to conjugate particles and VD11-4-2. Particles functionalized with carboxyl group were coupled with amine group of PEG via EDC/NHS reaction, and Boc protected the other end of PEG from crosslinking with another particle. After PEG conjugation, the Boc was removed from PSi-PEG and the amine group from the other side was exposed. Subsequently, VD11-4-2 was conjugated with PEG via amine group using CDI based chemical reaction. PEG with the molecular weight of 5000 was chosen because it had long enough chain in order to ensure VD11-4-2 will reach the active center, because of its stereometric properties.

*Quantitative Conjugating Efficacy***:** A series of solutions of VD11-4-2 with different concentrations were prepared by dissolving VD11-4-2 in EtOH, and their fluorescence intensity were measured at λ_{ex} = 360 nm and λ_{em} = 500 nm, and a standard curve between

concentration and fluorescence intensity was plotted. A certain amount of VD-PSi was mixed with NaOH solution (at pH value of 12) to dissolve the nanoparticles, then use ethyl acetate to extract the VD11-4-2 from the solution (3 × 4 mL), after evaporating the solution, the residue was re-dissolved in EtOH to measure its conjugation efficacy.

Fluorescent Thermal Shift Assay: Fluorescent Thermal Shift Assay (FTSA) is also called ThermoFluor or differential scanning fluorimetry.[2,3] Samples contained 8 μM carbonic anhydrase IX (CA IX); 0 to 40 μM of VD11-4-2 or 4 μM of CA IX and 0 to 0.3 mg ml^{-1} of VD-PSi, 100 μM sovatochromic dye 8-anilino-1-naphtalensulfonate, 10 % of ethanol and 50 mM of phosphate buffer with 50 mM of NaCl (pH 7.5). Samples were placed in a Corbett Rotor-Gene 6000 (Qiagen Rotor-Gene Q) instrument and heated from 25 °C to 99 °C applying heating rate of 1°/min. Fluorescence was observed using the blue channel (λ_{ex} = 365 ± 20 nm; λ_{em} = 460 ± 15 nm). Melting temperature of each sample was determined using sigmoidal model.[4]

Supplementary figures

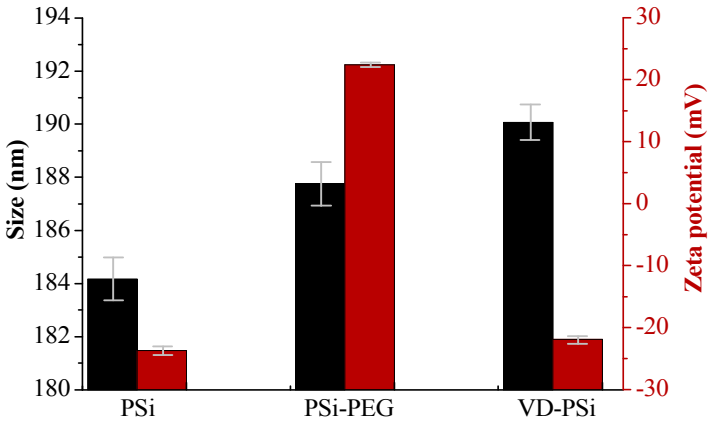

Figure S1. Particle size and zeta-potential changes during the synthesis.

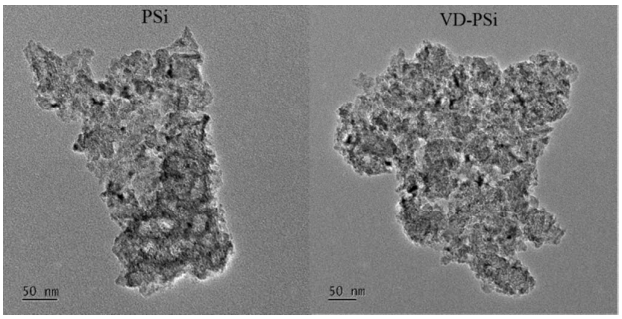

Figure S2. TEM images of PSi and VD-PSi.

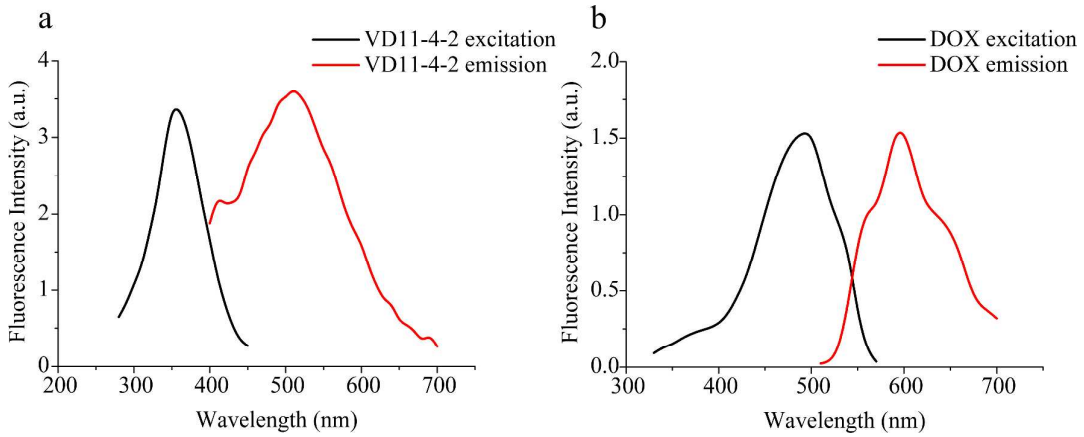

Figure S3. (a) VD11-4-2 fluorescence spectra. Excitation wavelength 360 nm and 500 nm is emission. (b) DOX fluorescence spectra. 490 nm and 600 nm are excitation and emission wavelengths, respectively.

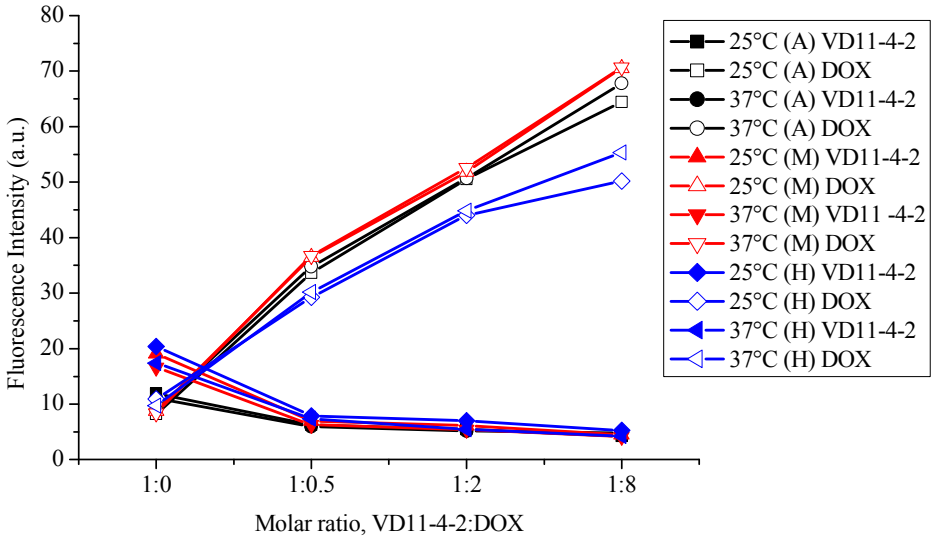

Figure S4. Fluorescence intensities of DOX (λ_{em} = 600 nm) and VD11-4-2 (λ_{ex} = 500 nm), when adding DOX into a fixed concentration of VD11-4-2 at a different pH and T (25 °C and 37 °C). A, M, H corresponds the type of buffer used, acetate (pH 4.97), MES (pH 5.31) and HEPES (pH 7.42), respectively.

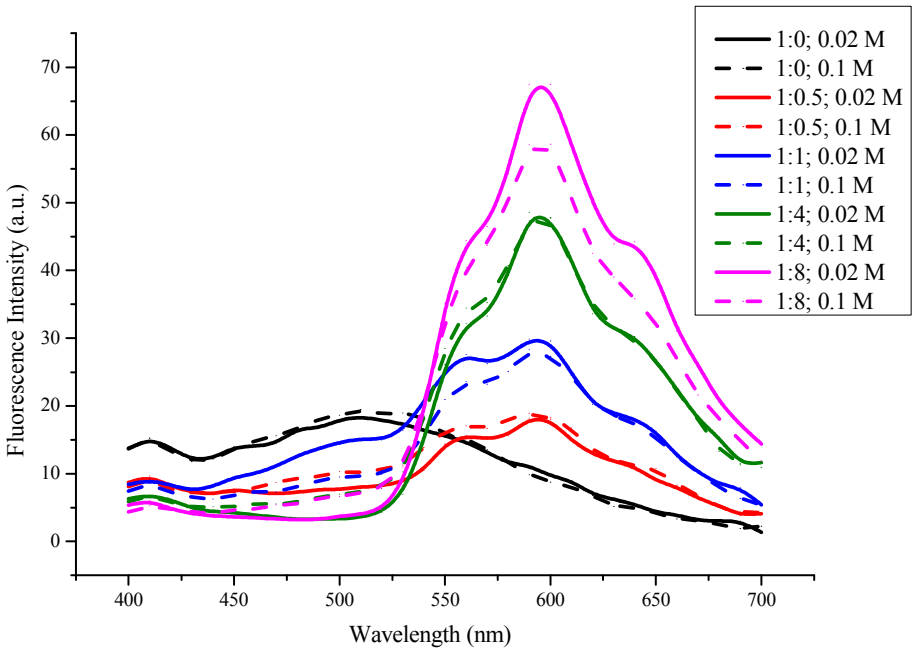

Figure S5. FRET effect and ionic strength (0.02 M and 0.1 M of NaCl). DOX was added to a fixed concentration of VD11-4-2 (λ_{ex} = 360 nm).

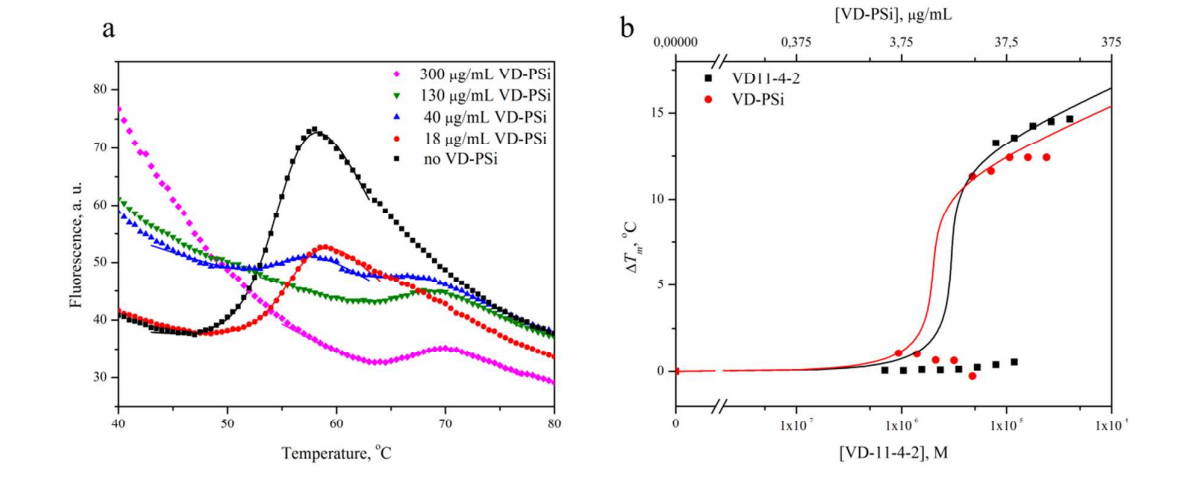

Figure S6. Binding of CA IX protein was determined by the fluorescent thermal shift assay (FTSA). (a) Protein unfolding curves with different amounts of VD-PSi added. Data points are observed fluorescence intensities during gradient temperature increase, and curves correspond to sigmoid model used for melting temperature determination.[4] (b) FTSA ligand dosing curves. Data points show the ΔTm of CA IX and CA IX with different concentrations of VD11-4-2 and VD-PSi, curves represent Kd determination model.[4]

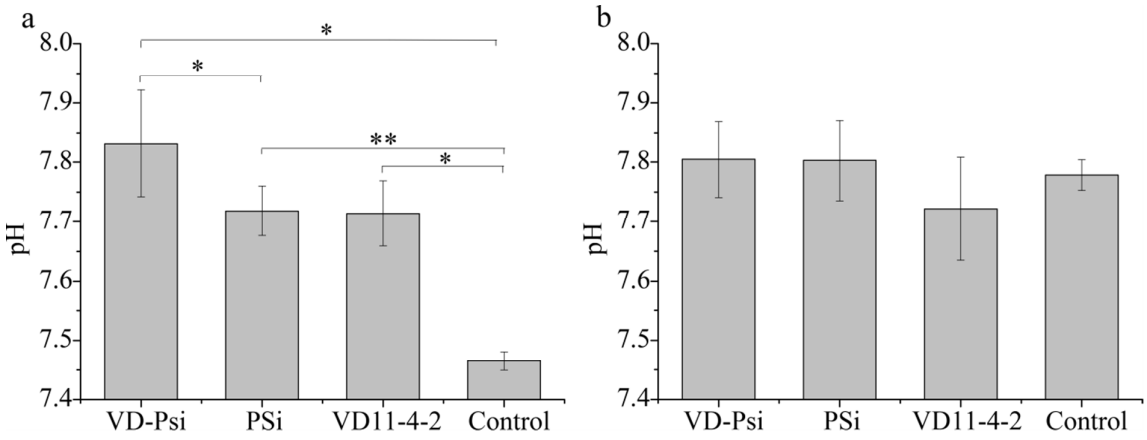

Figure S7. Results of extracellular pH of MCF-7 breast cancer cells measured by pH-meter in (a) hypoxia and (b) normoxia. Cells were treated with VD-PSi and PSi at the concentration of 100 μg ml^{-1} and 6.4 μg ml^{-1} (IC_{50}) VD11-4-2. All the data sets are compared with the control (MCF-7 cells cultured in growth medium without particles or compound). Data are shown as mean ± s.d. ($n \geq 3$).

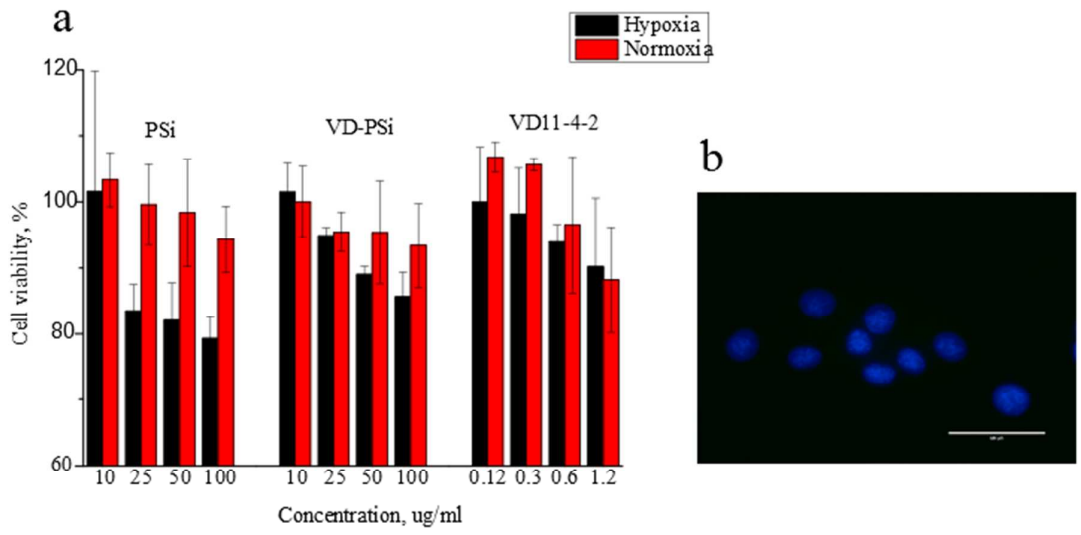

Figure S8. (a) Normoxia (red) and hypoxia (black) cell viability results of MCF-7 breast cancer cells treated with different concentrations of nanocarriers (PSi and VD-PSi) and CA IX inhibitor VD11-4-2 for 48 h evaluated by an ATP-based luminescent cell viability assay. All the data sets are compared with the controls. The concentration indicates the amount of PSi particles tested; other compound concentrations are equivalent to the corresponding concentration within VD-PSi. (b) Negative control of immunofluorescence analysis. Scale bar is 50 μm.

References

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