Supporting Information

Nontoxic tumor-targeting optical agent for intraoperative breast tumor imaging

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Supplemental data

Figure S1

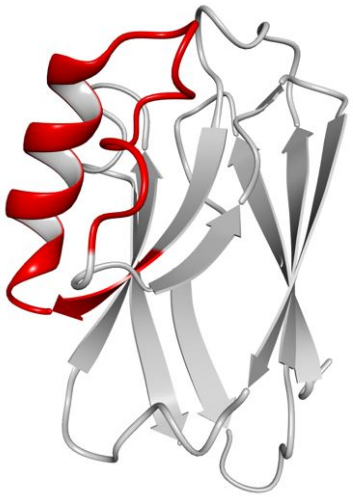


Figure S1. Full-length azurin. The α-helical domain incorporated into p28 (aa 50-77) is highlighted in **red**. The β-barrel structure is oriented vertically.

Figure S2

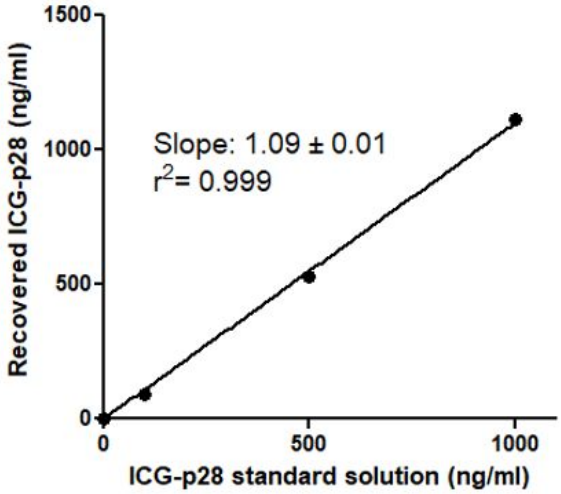
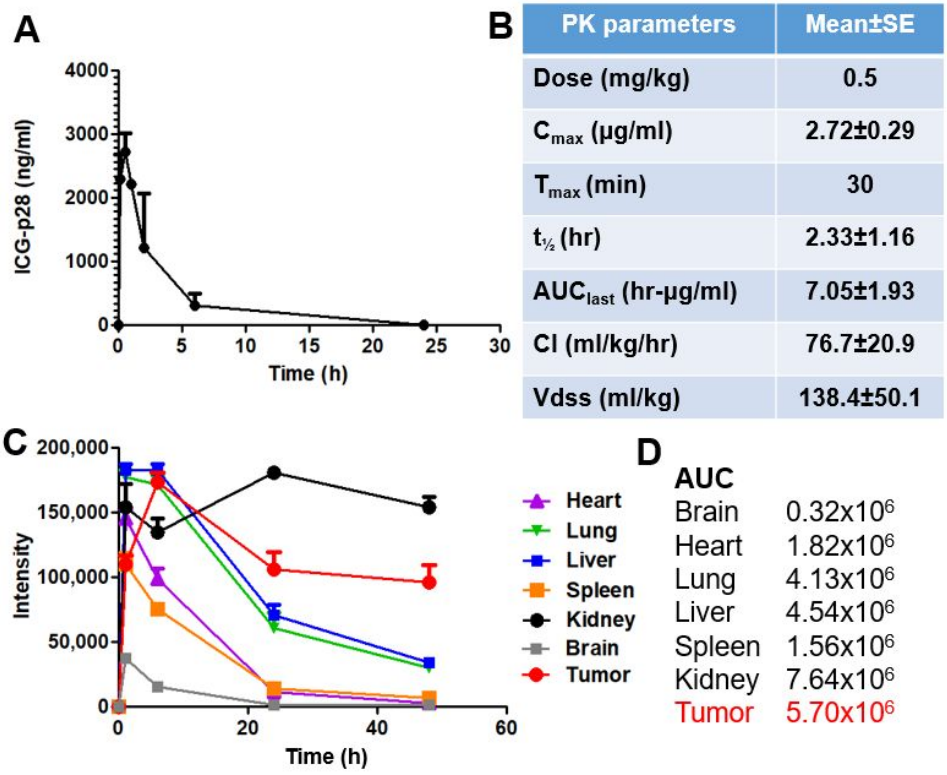


Figure S2. The representative calibration standard of ICG-p28 recovered from mouse serum. Each sample was analyzed by LC/MS/MS. r^2=0.999.

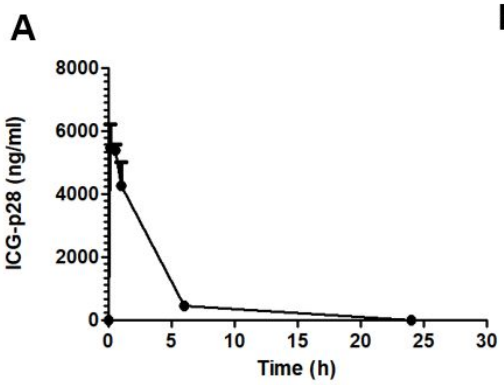
Figure S3



PK parameters	Mean±SE
Dose (mg/kg)	0.5
C_{max} (μg/ml)	2.72±0.29
T_{max} (min)	30
$t_{½}$ (hr)	2.33±1.16
AUC_{last} (hr-μg/ml)	7.05±1.93
Cl (ml/kg/hr)	76.7±20.9
Vdss (ml/kg)	138.4±50.1

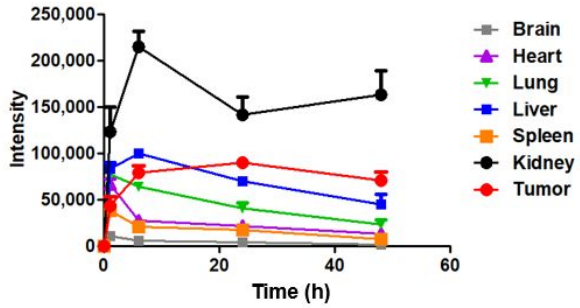
Figure S3. PK parameters and biodistribution of ICG-p28 in MDA-MB-231 xenografted mice. **A.** Levels of ICG-p28 in MDA-MB-231 cells were determined by LC/MS. **B.** PK parameters were calculated from the levels of p28 (**A**). **C.** The fluorescence intensities of each organ from animals bearing MDA-MB-231 xenografts were determined at each exposure time, and the AUC was analyzed based on the intensities (**D**). Mean+SD.

Figure S4



B

PK parameters	Mean±SE
Dose (mg/kg)	0.5
C_{max} (μg/ml)	5.70±0.51
T_{max} (min)	18.6
$t_{½}$ (hr)	1.23±0.67
AUC_{last} (hr-μg/ml)	11.7±2.45
Cl (ml/kg/hr)	44.9±9.44
Vdss (ml/kg)	82.6±10.9



D

AUC	
Brain	$0.20x10^6$
Heart	$1.12x10^6$
Lung	$2.09x10^6$
Liver	$3.39x10^6$
Spleen	$0.81x10^6$
Kidney	$7.76x10^6$
Tumor	$3.76x10^6$

Figure S4. PK parameters and biodistribution of ICG-p28 in IOWA-1T xenografted mice. A. Levels of ICG-p28 in IOWA-1T cells were determined by LC/MS. **B.** PK parameters were calculated from the levels of p28 (**A**). **C.** The fluorescence intensities of each organ from animals bearing IOWA-1T xenografts were determined at each exposure time, and the AUC was analyzed based on the intensities (**D**). Mean+SD.

Figure S5

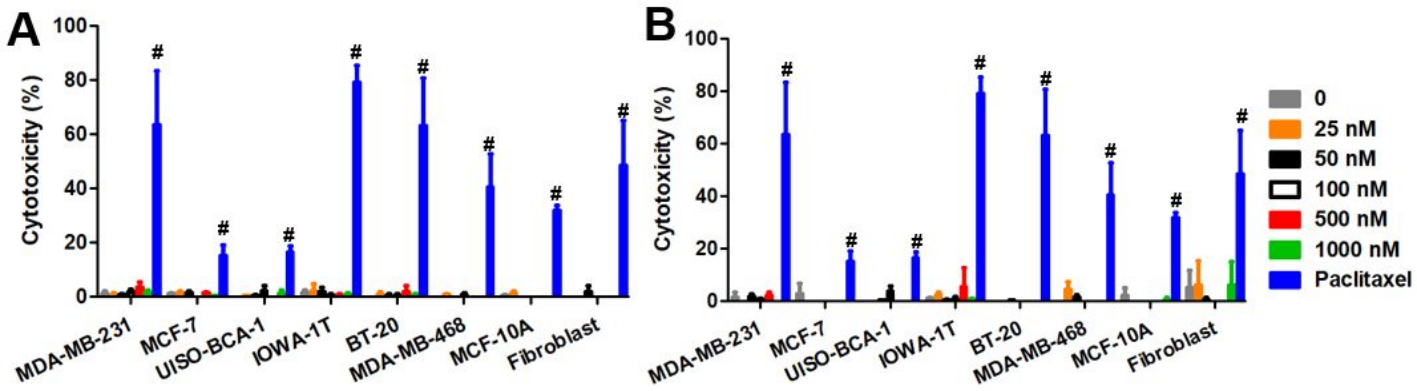


Figure S5. Membrane toxicity. Either ICG alone (**A**) or p28 alone (**B**) was applied to human breast cancer cell lines and normal cell lines to determine membrane toxicity via LDH leakage assays. Paclitaxel was used as a positive control compound. Mean+SD. #: P<0.001 (ANOVA).

Figure S6

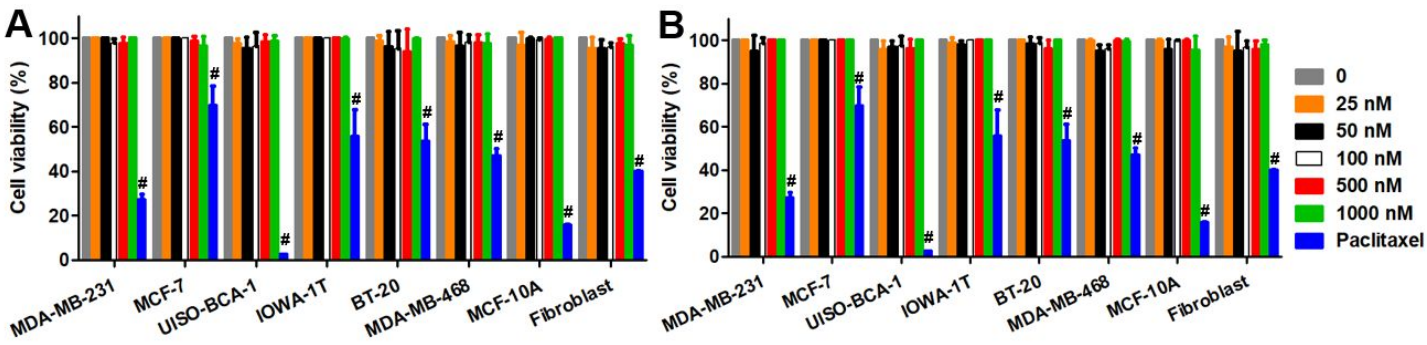


Figure S6. Cellular toxicity. Either ICG alone (**A**) or p28 alone (**B**) was applied to human breast cancer cell lines and normal cell lines to determine cellular toxicity via MTT assays. Paclitaxel was used as a positive control compound. Mean+SD. #: P<0.001 (ANOVA).

Figure S7

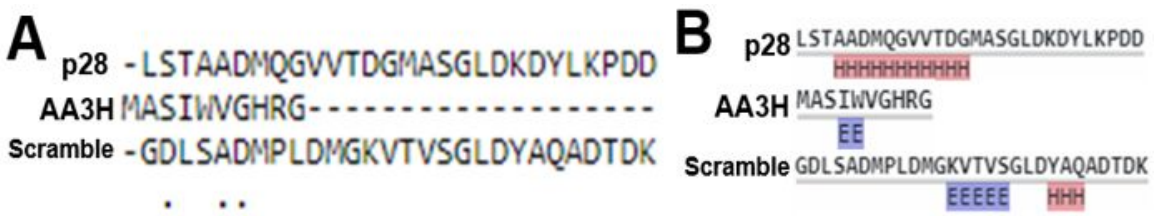


Figure S7. Primary and secondary sequence comparisons among p28, AA3H and scramble p28 peptides. Multiple sequence alignment via Clustal W 2.1 (**A**) and secondary structural analysis via Quick2D (H: α-helix, E: β-sheet) (**B**).