Supplementary Materials for

Structure-based Design of Peptides with High Affinity and Specificity to HER2 Positive Tumors

Lingling Geng, Zihua Wang, Xiaoliang Yang, Dan Li, Wenxi Lian, Zhichu Xiang, Weizhi Wang, Xiangli Bu, Wenjia Lai, Zhiyuan Hu*, Qiaojun Fang*

CAS Key Laboratory for Biomedical Effects of Nanomaterials & Nanosafety, National Center for Nanoscience and Technology, Beijing 100190, China

*Correspondence authors:

Qiaojun Fang, National Center for Nanoscience and Technology, Beijing 100190, China.

Phone: +86-10-82545562; Fax: +86-10-82545643; E-mail: fangqj@nanoctr.cn.

Zhiyuan Hu, National Center for Nanoscience and Technology, Beijing 100190, China.

Phone: +86-10-82545643; Fax: +86-10-82545643; E-mail: huzy@nanoctr.cn.

This file contains:

Supplementary Tables and Table legends

Table S1 Binding free energies and individual energy terms of complexes for HER2 and affibodies calculated by MM/GBSA (kcal/mol)

	ΔE_{vdw}	ΔE_{ele}	ΔG_{GB}	ΔG_{SA}	$-T\Delta S$	ΔG_{pred}	K_D[1-4]
HER2/Z_{wt}	-83.77±5.45	48.99±5.44	-30.05±4.93	-14.33±0.49	36.84±5.64	-42.33±5.45	>>50 nM[a]
HER2/ZHER2:4	-115.47±6.83	-115.32±9.12	128.74±8.80	-17.53±0.80	36.75±4.83	-82.84±5.89	50 nM
HER2/Z(HER2:342)	-126.77±6.63	-171.57±11.86	183.40±11.04	-19.16±0.64	38.47±3.55	-95.63±5.01	22 pM

ΔE_{vdw}, van der Waals contribution; ΔE_{ele}, electrostatic contribution; ΔG_{GB}, the polar contribution of desolvation; ΔG_{SA}, nonpolar contribution of desolvation; $-T\Delta S$, the conformational entropy at temperature T; ΔG_{pred}, the total binding free energy; K_D, dissociation equilibrium constant.

[a] So far, there is no reported K_D for HER2/Z_{wt} in the literatures because Z_{wt} does not bind to HER2 specifically. The affibody ZHER2:4 was screened and obtained based on Z_{wt} by phage display technology [1]. Therefore, wild type Z_{wt} should have a much higher K_D (>>50 nM) for its binding to HER2 protein.

Table S2 Binding free energies and individual energy terms of HER2 with pep27 and its mutations calculated by MM/GBSA (kcal/mol)

	ΔE_{vdw}	ΔE_{ele}	ΔG_{GB}	ΔG_{SA}	$-T\Delta S$	ΔG_{pred}
HER2/pep27	-100.49±6.63	-275.80±14.03	274.42±13.08	-15.08±0.75	40.93±5.01	-76.02±5.79
HER2/pep27-3E	-57.32±6.08	-253.47±17.46	248.92±16.83	-10.36±0.98	34.57±4.85	-37.66±5.49
HER2/pep27-10W	-69.64±5.97	-246.80±17.45	243.13±15.75	-10.74±0.82	36.23±3.92	-47.83±4.98
HER2/pep27-11Y	-83.86±5.98	-286.80±14.95	283.22±14.22	-13.52±0.80	38.19±5.11	-62.77±5.56
HER2/pep27-22M	-81.12±6.53	-262.00±7.80	258.84±7.03	-12.37±0.63	38.84±4.86	-57.81±5.54
HER2/pep27-24M	-115.95±8.93	-249.33±11.45	252.18±12.31	-17.25±1.29	39.25±4.90	-91.1±6.59
HER2/pep27-27M	-74.39±7.12	-288.77±10.99	284.50±10.26	-10.98±0.77	36.70±4.67	-52.94±5.88
HER2/pep27-27N	-97.94±7.06	-314.37±12.43	311.02±10.94	-15.22±0.71	37.95±5.13	-78.56±6.04
HER2/pep27-27R	-100.86±5.55	-365.66±13.22	361.93±12.31	-15.74±0.50	38.42±4.68	-81.9±5.18

ΔE_{vdw}, van der Waals contribution; ΔE_{ele}, electrostatic contribution; ΔG_{GB}, the polar contribution of desolvation; ΔG_{SA}, nonpolar contribution of desolvation; $-T\Delta S$, the conformational entropy at temperature T; ΔG_{pred}, the total binding free energy.

Table S3. Comparison of molecular weights of pep27, pep27-24M and ZHER2:4 as well as their kinetic parameters of binding to HER2.

Peptide	**M_W (kDa)**	**K_D[a] (nM)**	**k_a[b] ($M^{-1}s^{-1}$)**	**k_d[c] (s^{-1})**
ZHER2:4 [4]	8.7	～50	～1.8×10^5	～9.9×10^{-3}
pep27	3	～346	～3.1×10^4	～10.7×10^{-3}
pep27-24M	3	～293	～4.11×10^4	～12.1×10^{-3}

[a]Dissociation equilibrium constant. [b]Association rate constant. [c]Dissociation rate constant. M_W, molecular weight.

Supplementary Figures and Figure legends

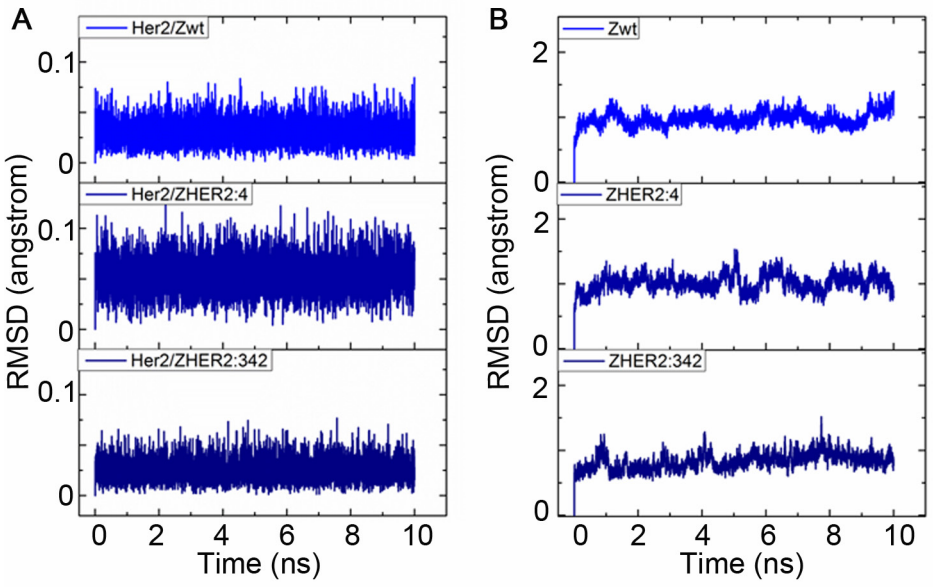

Figure S1. Root-mean-square deviation (rmsd) of backbone atoms of structures in MD trajectories from the starting structures for the three complexes (A) and their ligands (B).

pep27	N K F N K G M R G Y W G A L G G G N G K R G I R G Y D
pep27-3E	N K E N K G M R G Y W G A L G G G N G K R G I R G Y D
pep27-10W	N K F N K G M R G W W G A L G G G N G K R G I R G Y D
pep27-11Y	N K F N K G M R G Y Y G A L G G G N G K R G I R G Y D
pep27-22M	N K F N K G M R G Y W G A L G G G N G K R M I R G Y D
pep27-24M	N K F N K G M R G Y W G A L G G G N G K R G I M G Y D
pep27-27M	N K F N K G M R G Y W G A L G G G N G K R G I R G Y M
pep27-27N	N K F N K G M R G Y W G A L G G G N G K R G I R G Y N
pep27-27R	N K F N K G M R G Y W G A L G G G N G K R G I R G Y R

Figure S2. Alignment of the sequence of pep27 and the single mutated sequences.

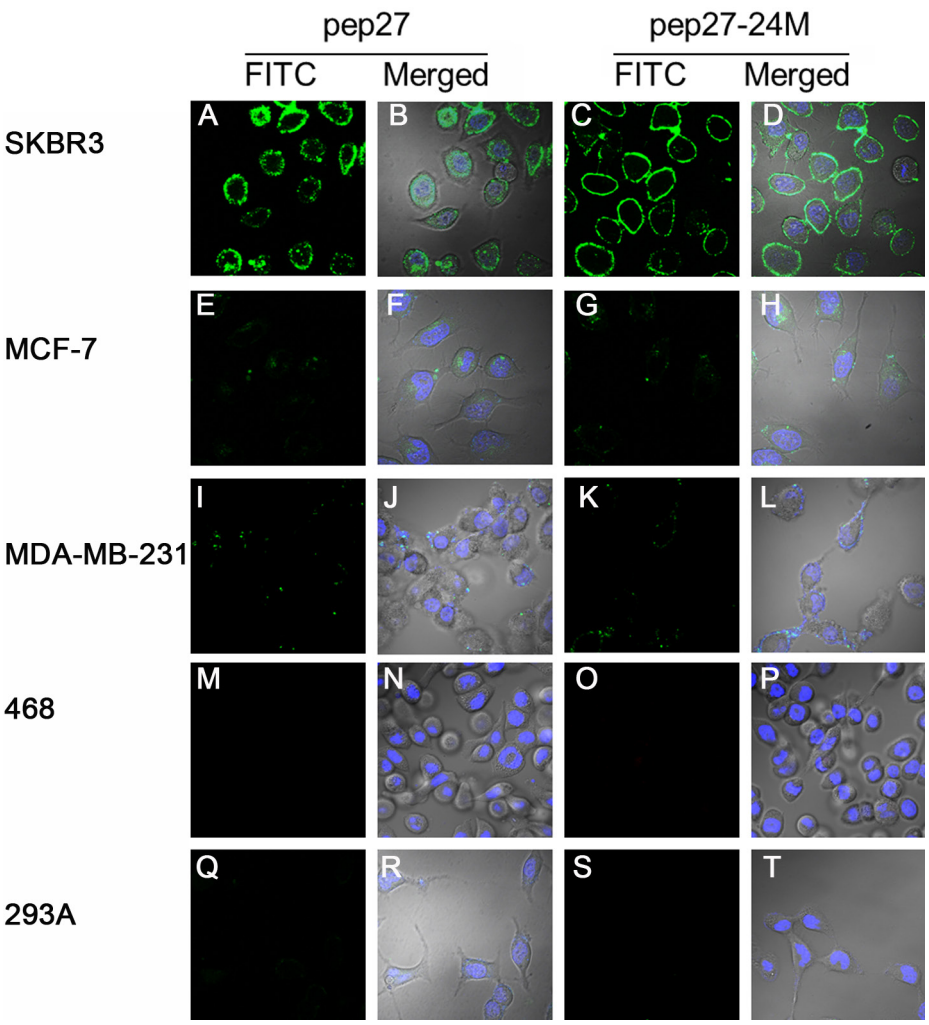

Figure S3. Immunocytochemistry analysis of FITC labeled peptides binding to HER2 in cell lines with HER2 high (SKBR3), medium (MCF-7 and MDA-MB-231), and low (468 and 293A) expression. Both peptides showed significant fluorescence signals in SKBR3 cell membrane (A-D), weak signals in MCF-7 and MDA-MB-231 cells (E-L) but no signals in 468 and 293A cells (M-T). The Hoechst (blue), FITC (green) and Bright field are merged in panels.

References

1. Wikman M, Steffen AC, Gunneriusson E, Tolmachev V, Adams GP, Carlsson J, et al. Selection and characterization of HER2/neu-binding affibody ligands. Protein Eng Des Sel. 2004; 17: 455-62.

2. Steffen AC, Orlova A, Wikman M, Nilsson FY, Stahl S, Adams GP, et al. Affibody-mediated tumour targeting of HER-2 expressing xenografts in mice. Eur J Nucl Med Mol Imaging. 2006; 33: 631-8.

3. Orlova A, Magnusson M, Eriksson TL, Nilsson M, Larsson B, Hoiden-Guthenberg I, et al. Tumor imaging using a picomolar affinity HER2 binding affibody molecule. Cancer Res. 2006; 66: 4339-48.

4. Steffen AC, Wikman M, Tolmachev V, Adams GP, Nilsson FY, Stahl S, et al. In vitro characterization of a bivalent anti-HER-2 affibody with potential for radionuclide-based diagnostics. Cancer Biother Radiopharm. 2005; 20: 239-48.