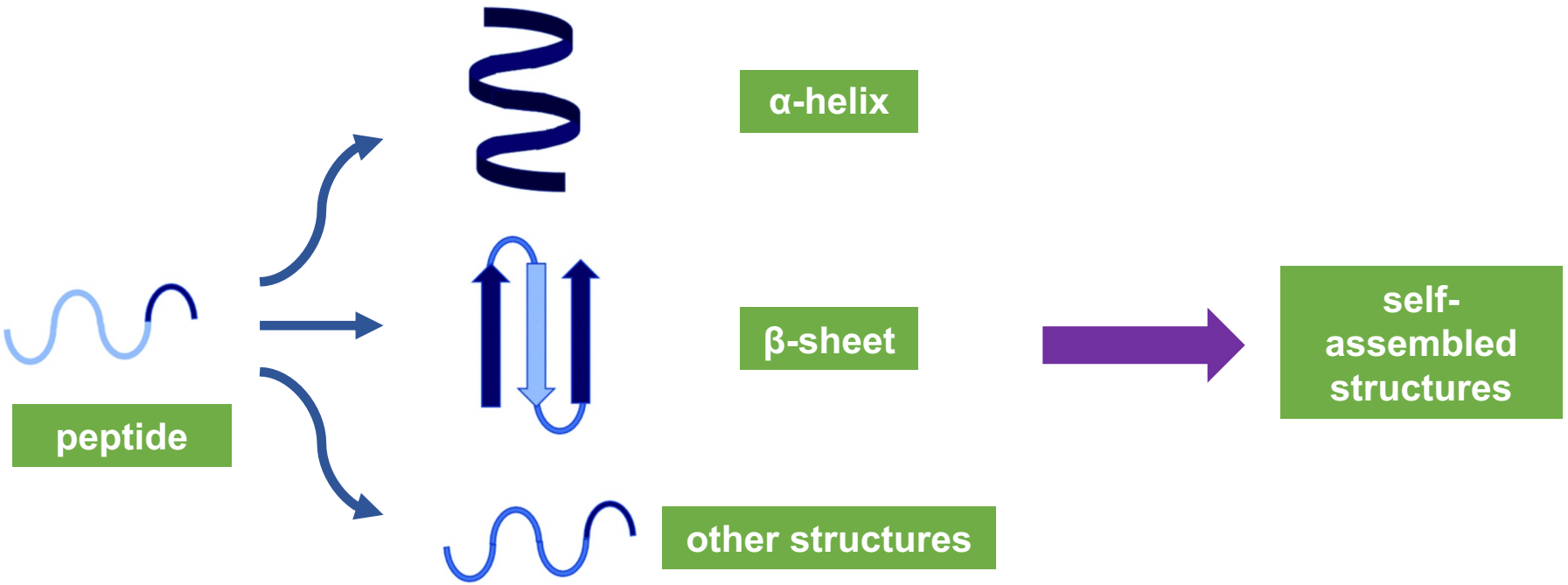
Targeting-Recruitment Self-Assembly (TRA) Strategy

Literature Seminar
2022/12/24
Lin Yuanqi

Contents

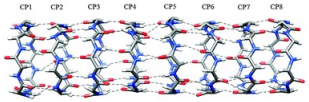
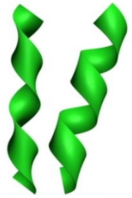
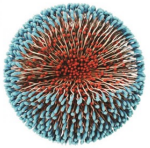
Self-Assembling Peptide

Peptide self-assembly is a process in which peptides spontaneously form ordered aggregates.



1) Lee, S.; Trinh, T. H.; Yoo, M.; Shin, J.; Lee, H.; Kim, J.; Ryou, C. *Int. J. Mol. Sci.* **2019**, *20*, 5850.

Self-Assembled Structures and Affected Factors

nanostrctures	
nanofibers	
nanotubes	
nanotapes	
nanoparticles	
hydrogels	

factors	
pH	hydrogen bonds, salt bridges, …
temperature	temperature-responsive
light	infrared (IR)-responsive

1) Lee, S.; Trinh, T. H.; Yoo, M.; Shin, J.; Lee, H.; Kim, J.; Ryou, C. *Int. J. Mol. Sci.* **2019**, *20*, 5850.

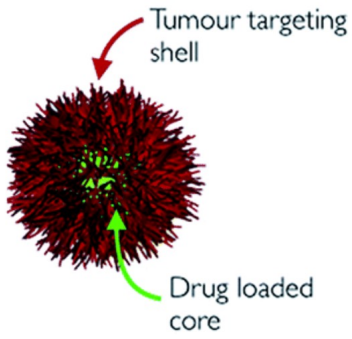
Application in Cancer Treatment

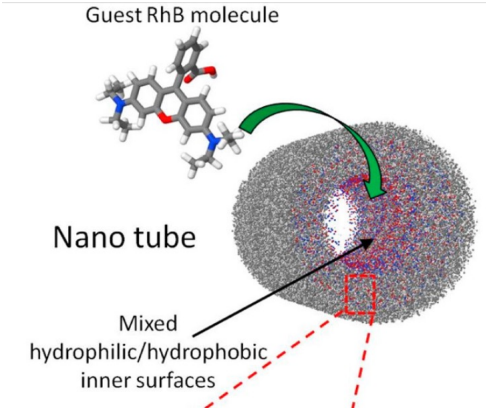
targeting

LyP-1 (CGNKRTRGC) recognizes lymphatic metastatic tumors and exerts cytotoxic activity.
LyP-1-conjugated PEG–PLGA nanoparticles (LyP-1-NPs) showed increased cellular uptake, thus showed good targeting efficiency to cancer cells.

drug delivery

Nanomaterials as drug delivery carriers have many advantages including high efficiency for drug loading, a low ratio for drug loss, and high stability to avoid body clearance.





1) Lee, S.; Trinh, T. H.; Yoo, M.; Shin, J.; Lee, H.; Kim, J.; Ryou, C. *Int. J. Mol. Sci.* **2019**, *20*, 5850.
2) Fan, T.; Yu, X.; Shen, B.; Sun, L. *J. Nanomater.* **2017**, *2017.*

Contents

Professor Hao Wang

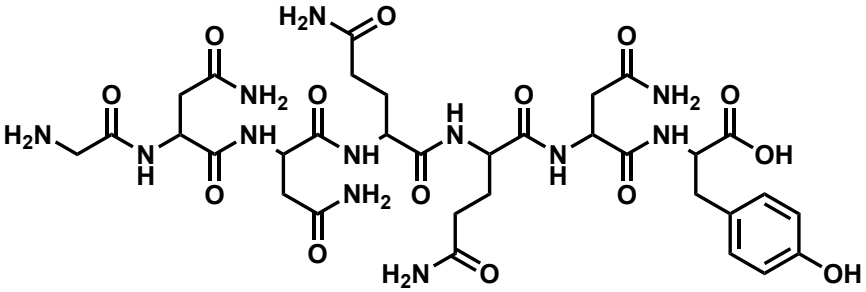
1996-2000	B.S. in Chemistry @Nankai University (Prof. Yu Liu)
2000-2005	Ph.D. in Chemistry @Nankai University (Prof. Yu Liu)
2006-2007	Alexander von Humboldt (AvH) Fellow @University of Würzburg (Prof. Frank Würthner)
2007-2010	Postdoctoral Fellow in Molecular and Medical Pharmacology @University of California (Prof. Hsian Rong Tseng)
2010-2011	Associate Professor in Molecular and Medical Pharmacology @University of California (Prof. Hsian Rong Tseng)
2011-	Professor @National Center for Nanoscience and Technology of China

nanomaterial

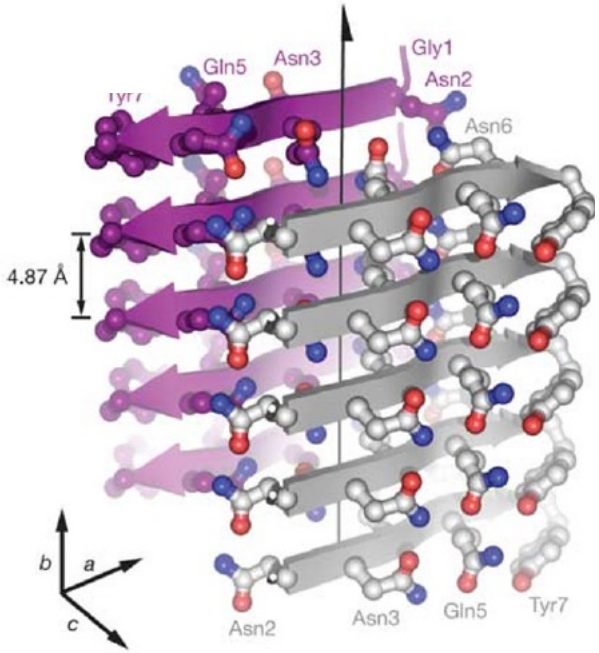
- development of “in vivo self-assembly” technology in physiological/pathological conditions
- study of bio-effect and further regulation of biological behavior
- exploration of self-assembled biomaterials for bioimaging, drug delivery, immunotherapy, etc

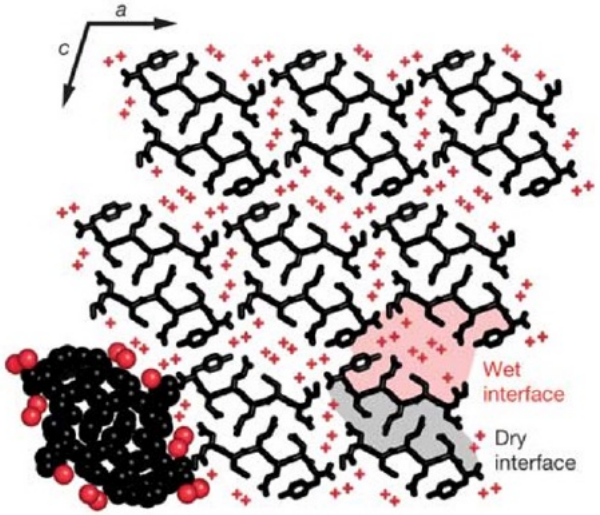
An Assembly Peptide: G7

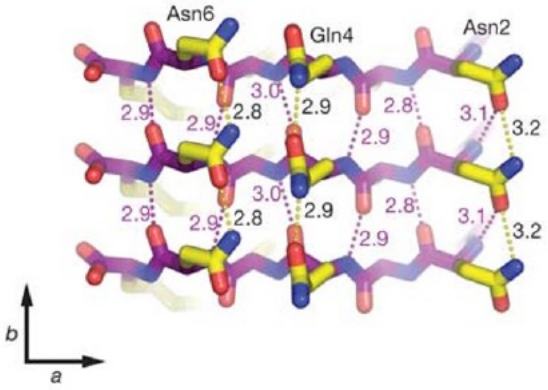
self-assembly motif **G7: GNNQQNY**



- derived from yeast protein Sup35
- reported to form aggregate
- self-assemble into β-sheet nanofibers at high concentrations



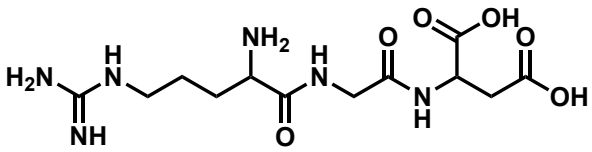




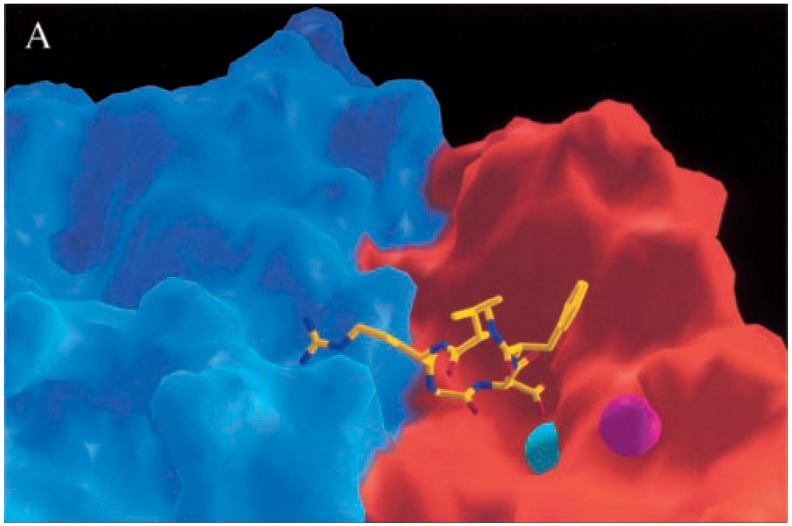
Nelson, R.; Sawaya, M. R.; Balbirnie, M.; Madsen, A. Ø.; Riekel, C.; Grothe, R.; Eisenberg, D. *Nature*. **2005**, *435*, 773.

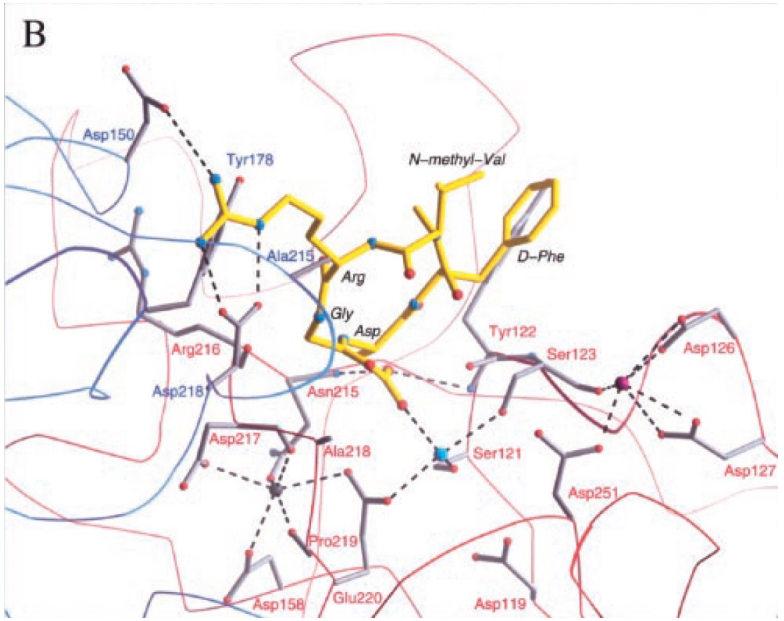
RGD Motif

RGD (receptor: integrin $\alpha_V\beta_3$)



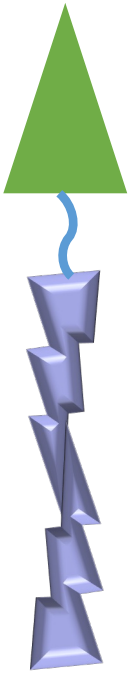
Integrin $\alpha_V\beta_3$ -targeting peptides with an exposed arginine-glycine-aspartate (RGD) sequence play a crucial role in targeted anticancer drug delivery.



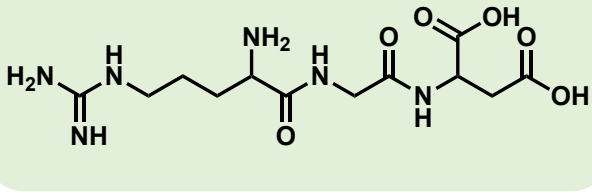


Xiong, J. P.; Stehle, T.; Zhang, R.; Joachimiak, A.; Frech, M.; Goodman, S. L.; Arnaout, M. A. *Science* **2002**, *296*, 151.

Assembly Sequence: G7-RGD

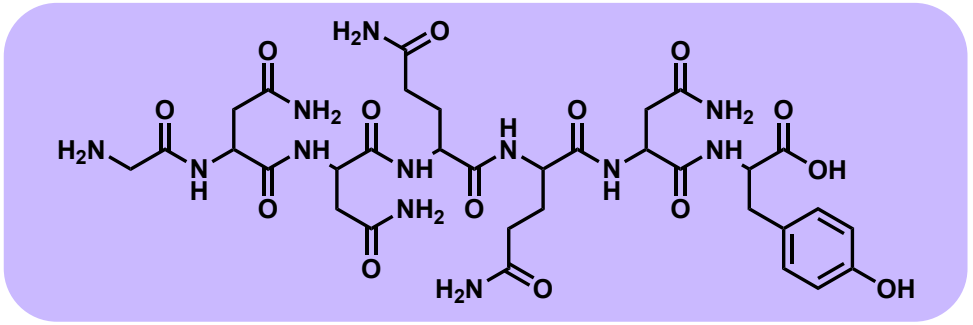


targeting motif



RGD (receptor: integrin $\alpha_V\beta_3$)

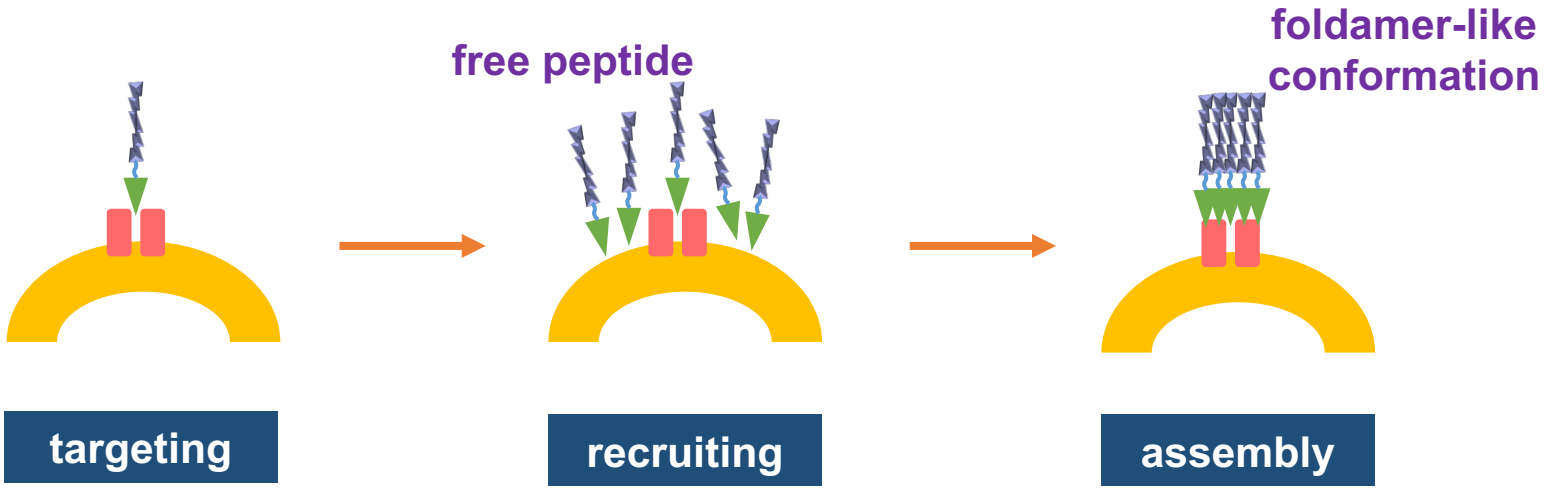
self-assembly motif



G7: GNNQQNY

G7-RGD (GNNQQNYRGD)
bind the tumor-overexpressed protein receptor integrin $\alpha_V\beta_3$ on the cell membrane

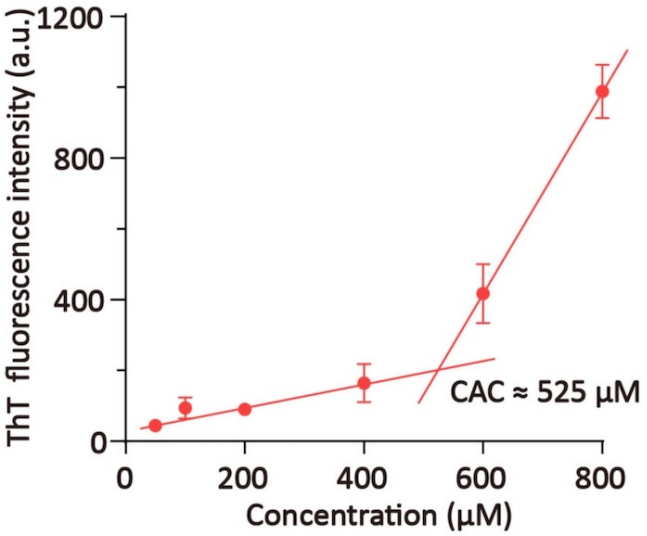
Targeting-Recruitment Self-Assembly (TRA) Strategy



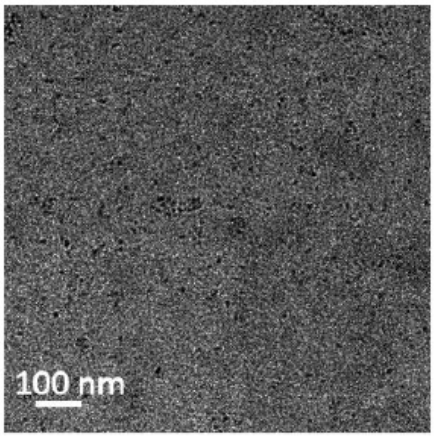
The TRA strategy anchors self-assembling peptides to proteins, stabilizing peptide structures and triggering reduction in the activation energy of assembly. The immobilized peptides recruit surrounding monomers to drive the self-assembly process, which further mediates receptor oligomerization.

Assembly Concentration (CAC) in Solution

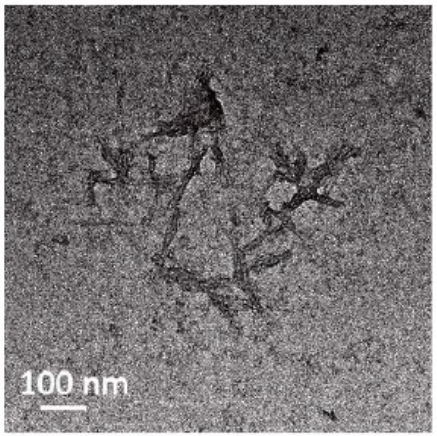
G7-RGD only



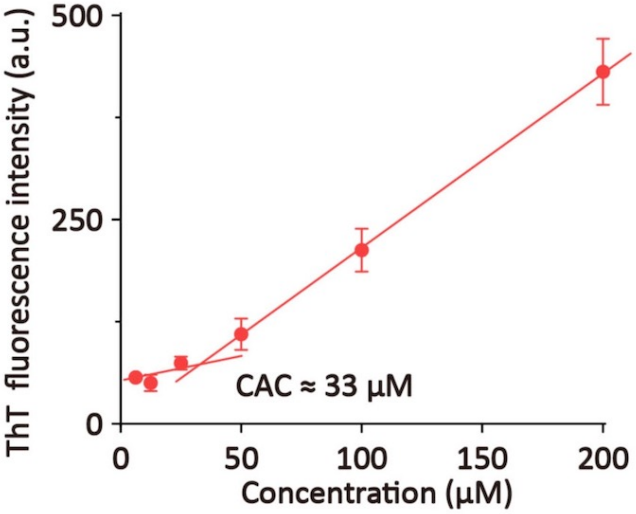
400 μM G7-RGD in saline solution



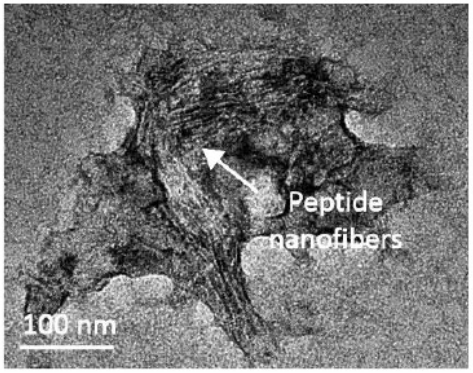
800 μM G7-RGD in saline solution



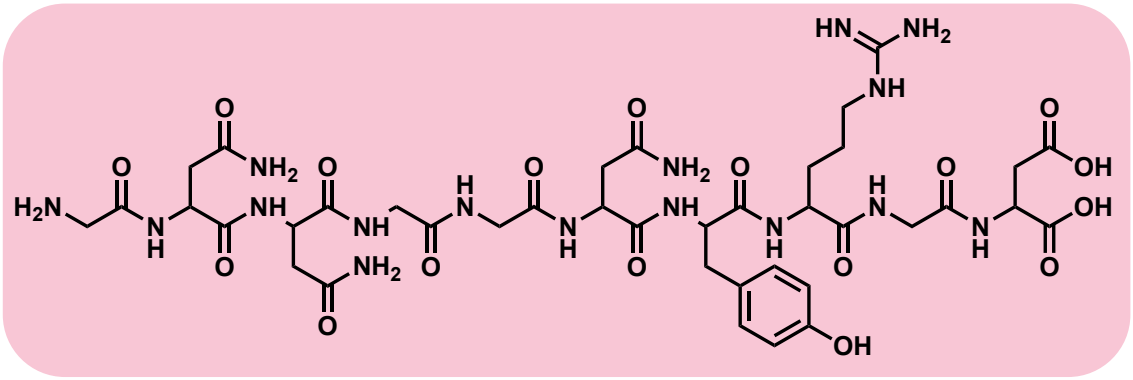
G7-RGD + integrin $\alpha_V\beta_3$



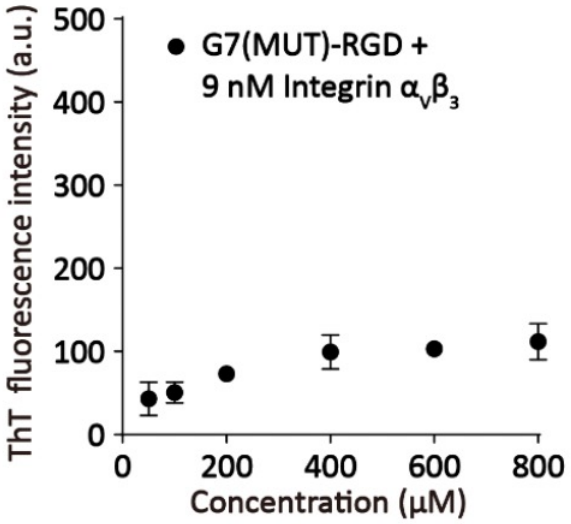
100 μM G7-RGD + 9 nM integrin $\alpha_v\beta_3$ in saline solution



Model Experiment In Solution



G7(MUT)-RGD:GNNGGNY-RGD

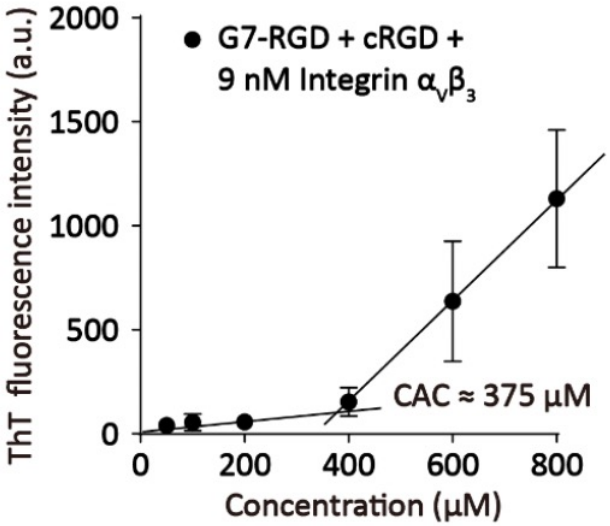


not trigger self-assembly

add cRGD (10 nM)

as an integrin $\alpha_V\beta_3$ inhibitor

add G7-RGD



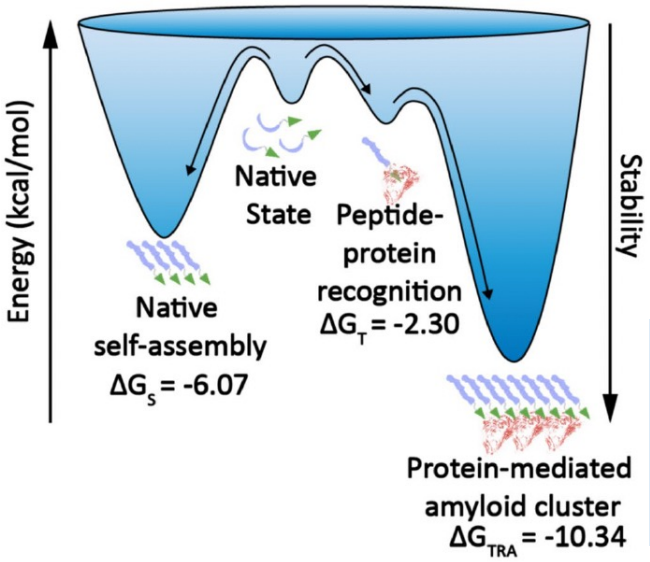
CAC of G7-RGD did not decrease prominently

Thermodynamics Investigation Using Surface Plasmon Resonance Imaging (SPRi)

K_D: dissociation constant ΔG:Gibbs free binding energy

lower K_D → higher affinity higher ΔG → higher higher stability

	K_D (mol/L)	ΔG (kcal/mol)
recognition between G7-RGD and integrin	2.05×10^{-2}	$\Delta G_T = -2.30$
self-assembly of G7-RGD without integrin	3.54×10^{-5}	$\Delta G_S = -6.07$
self-assembly of G7-RGD with integrin	1.26×10^{-6}	$\Delta G_{TRA} = -10.34$



ΔG_T: the binding energy G7-RGD targeting integrin $\alpha_V\beta_3$

ΔG_S: the energy of G7-RGD self-assembly

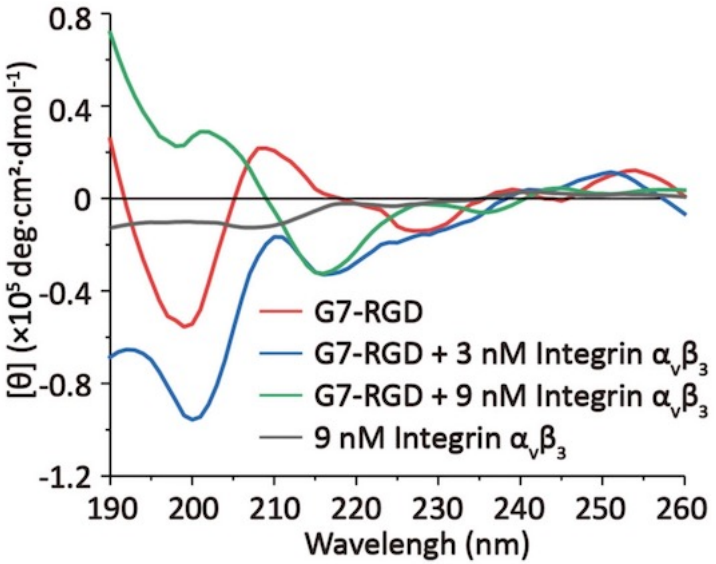
ΔG_{TRA} : the binding energy of targeted recruitment self-assembly

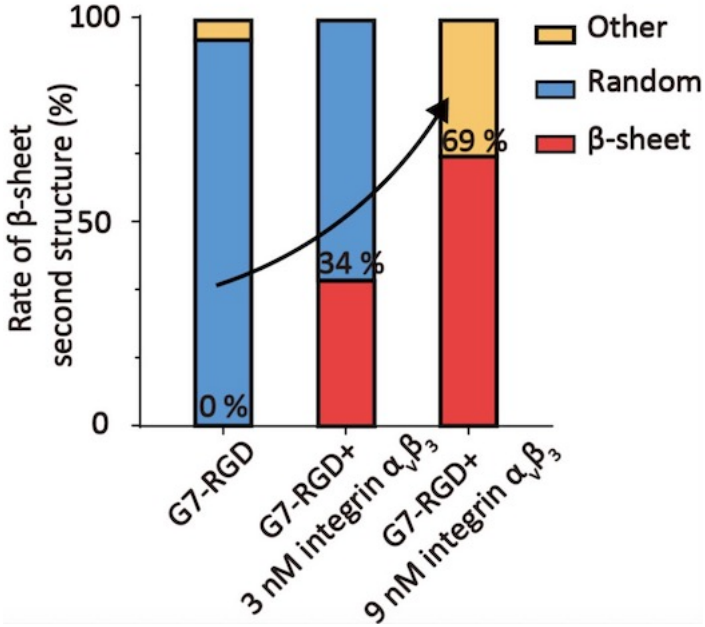
$\Delta G_{TRA} = \Delta G_T + \Delta G_{RA}$

SPR readouts manifested that the targeting motif RGD upraised binding affinity and further induced self-assembly of TRA peptide to form aggregates with higher stability.

funnel-shaped potential energy landscape

Secondary Structure Study By CD Analysis

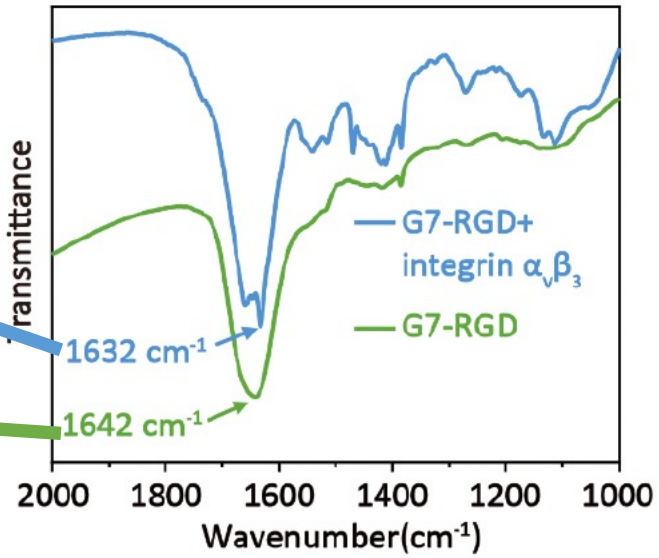




FTIR spectra

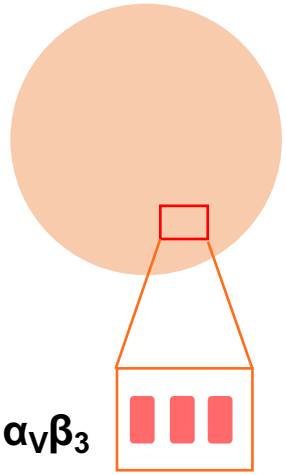
indication of the parallel β-sheet structure

a characteristic peak of the monomer state



TRA Design in Biological System

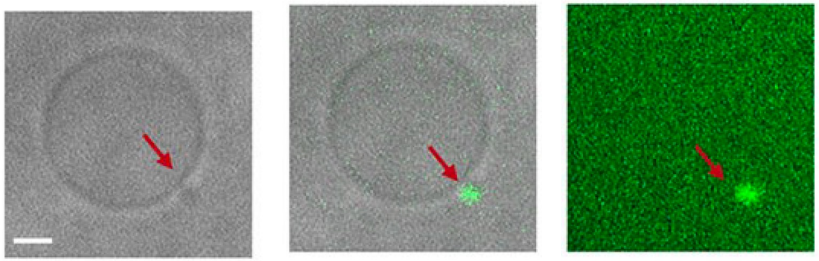
giant plasma membrane vesicles (GPMVs)



integrin $\alpha_V\beta_3$

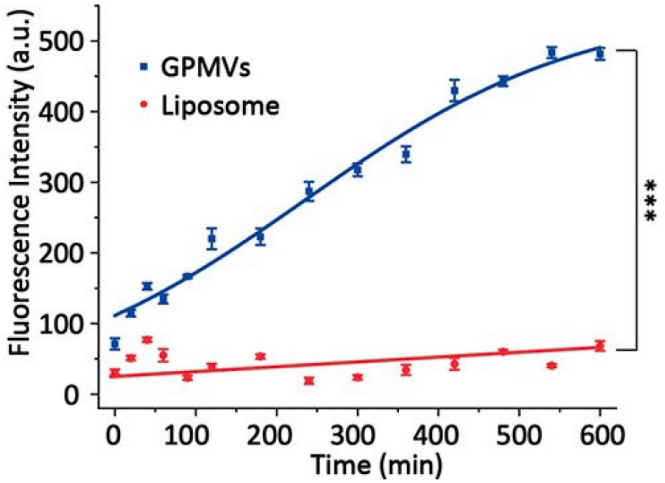
\+

fluorophore-labled G7-RGD

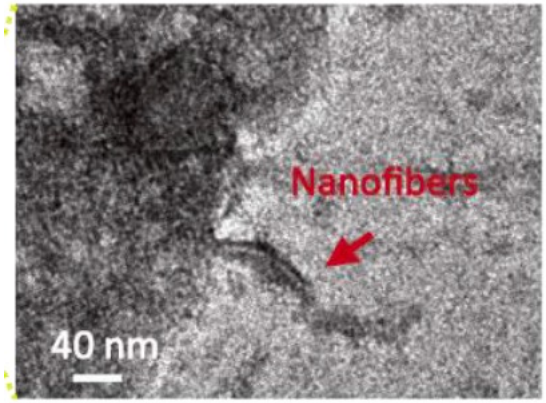


G7-RGD self-assembled on the GPMV membrane

kinetic fluorescence curve

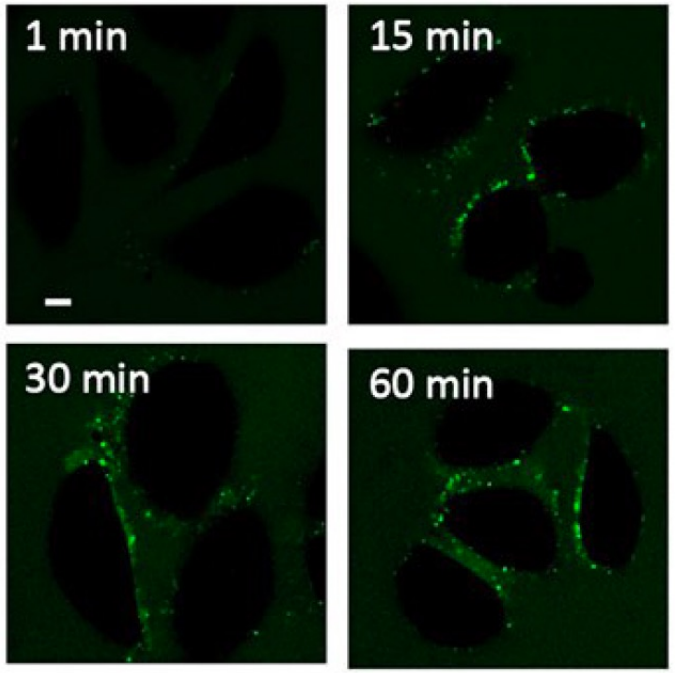


TEM images

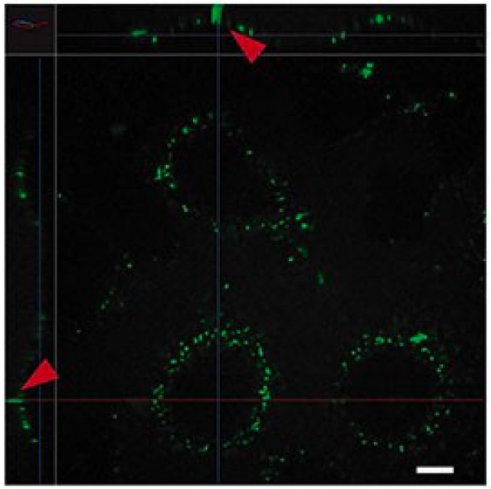


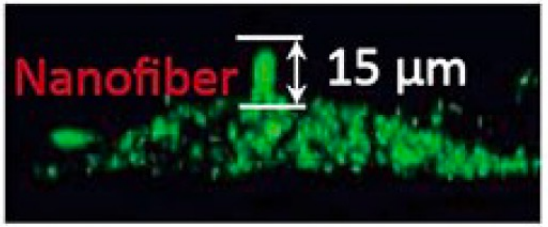
Self-Assembly on MCF-7 Membranes

confocal images

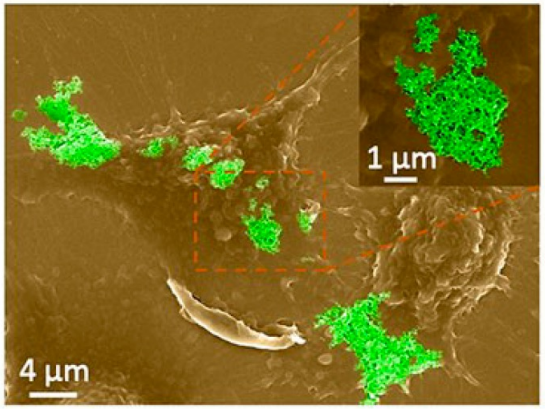


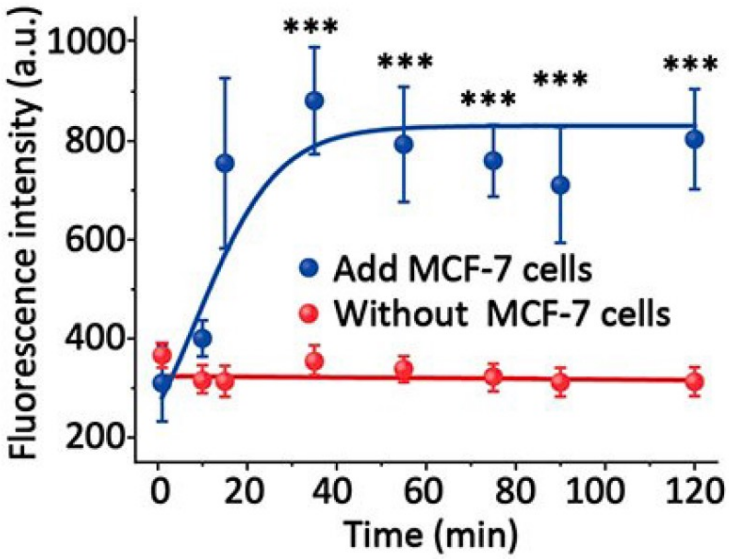
3D images





SEM images





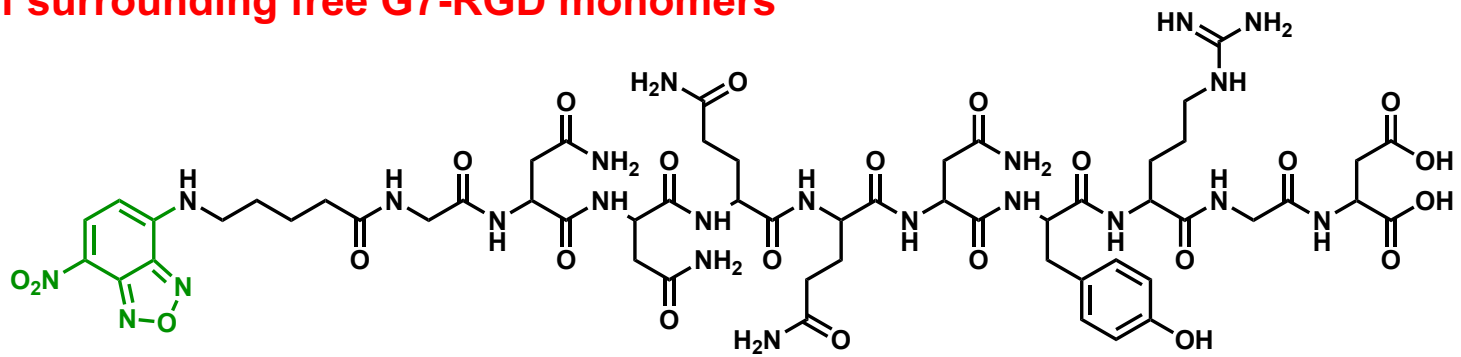
confirmation of self-assembly of G7-RGD self-assembly on the MCF-7 membrane

Probe Design

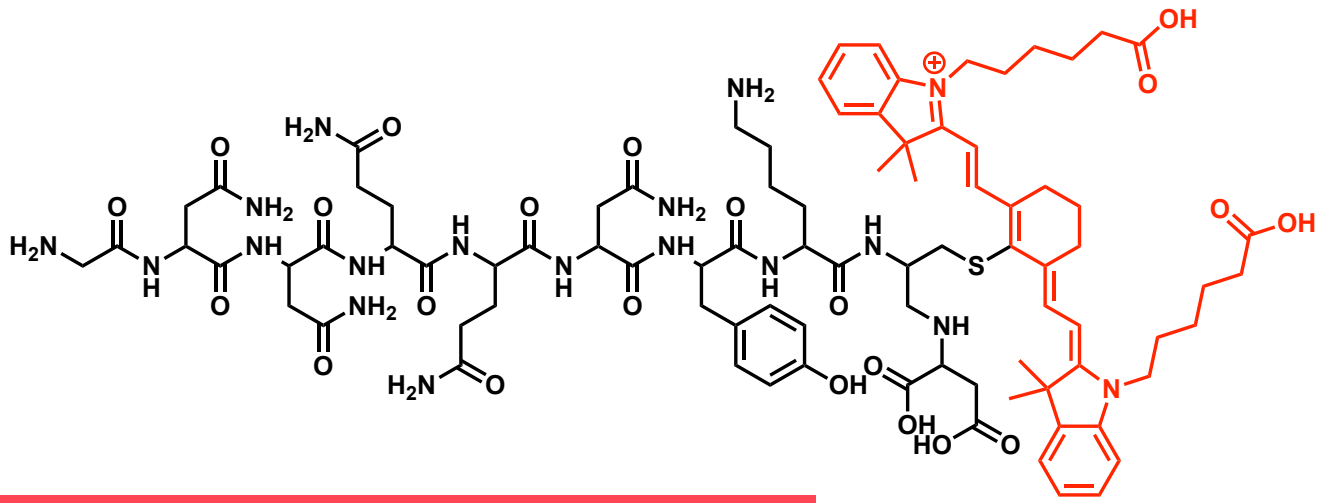
G7-RGD rapidly self-assembled binding to integrin $\alpha_V\beta_3$

?

self-assembly of G7-RGD on the membrane was a consequence of recruitment of surrounding free G7-RGD monomers

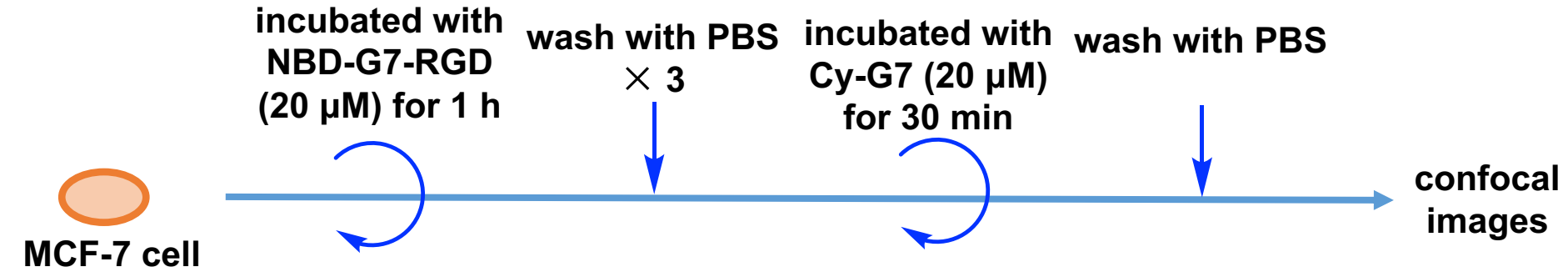


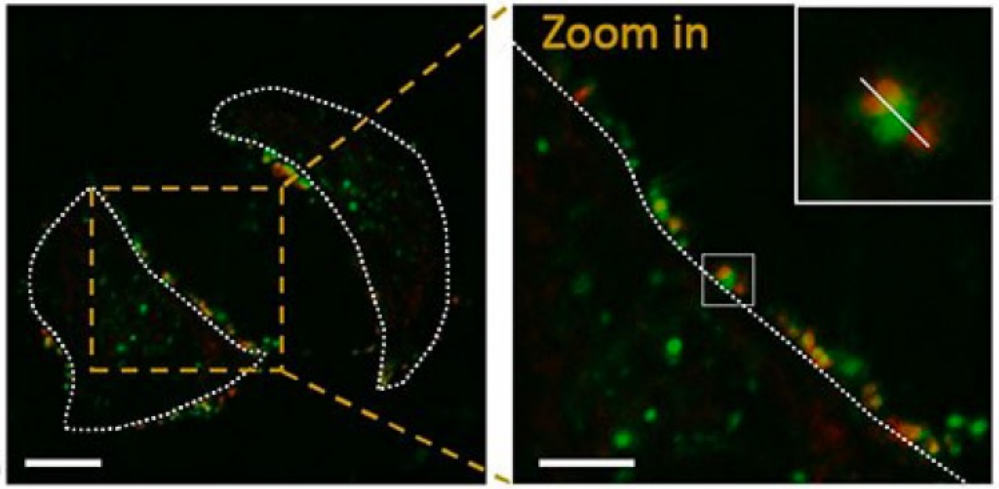
NBD-G7-RGD: NBD-X-GNNQQNY-RGD emitting green fluorescence



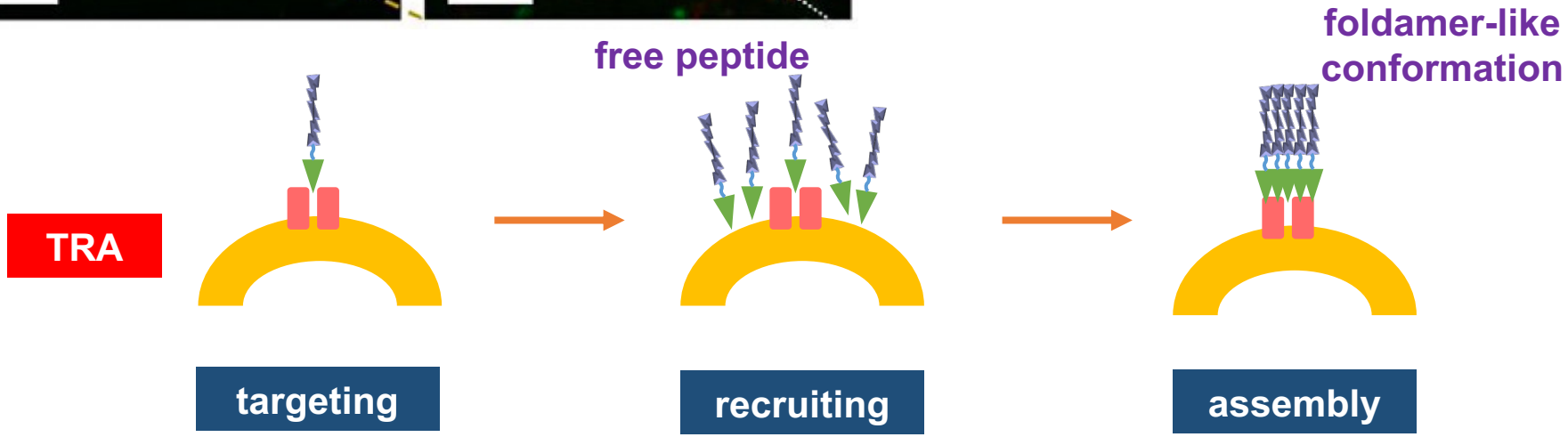
Cy-G7: GNNQQNY-KC(Cy)D emitting red fluorescence

Self-Assembled NBD-G7-RGD (1)



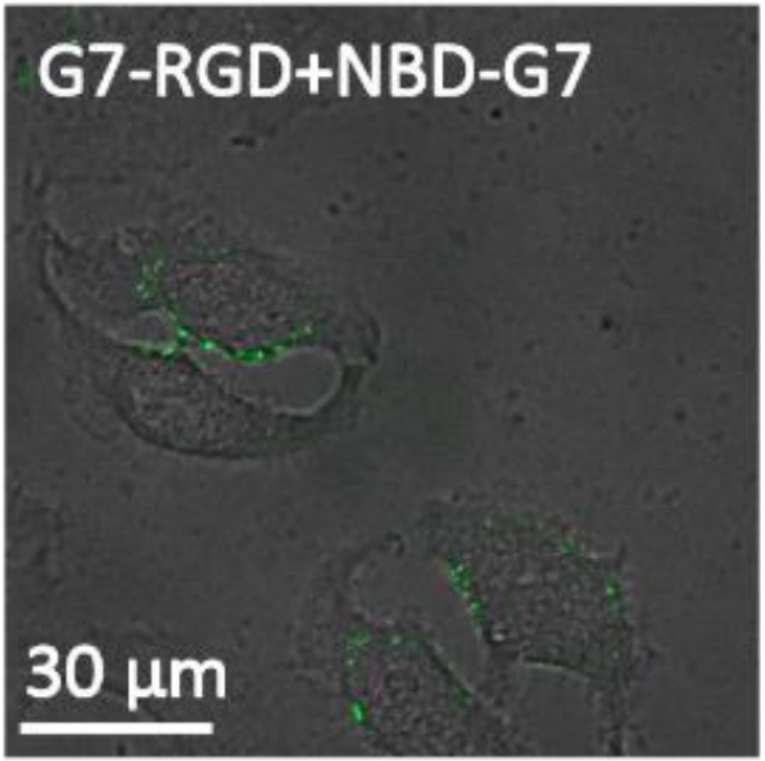


NBD-G7-RGD initially self-assembled on the cell membrane to form aggregates, and then Cy-G7 monomers were recruited as building blocks.

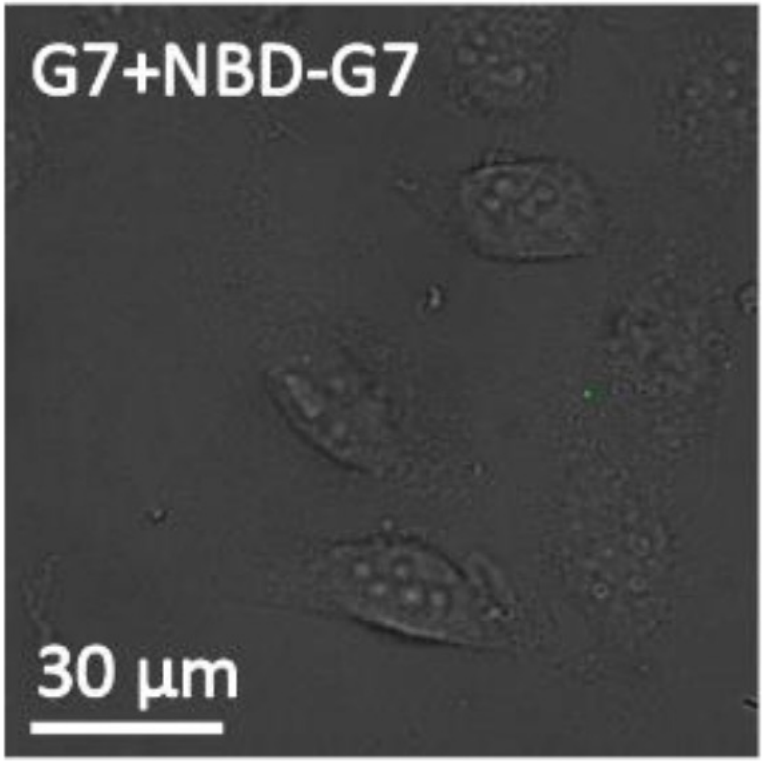


Self-Assembled NBD-G7-RGD (2)

adding G7-RGD prior to NBD-G7



adding G7 prior to NBD-G7

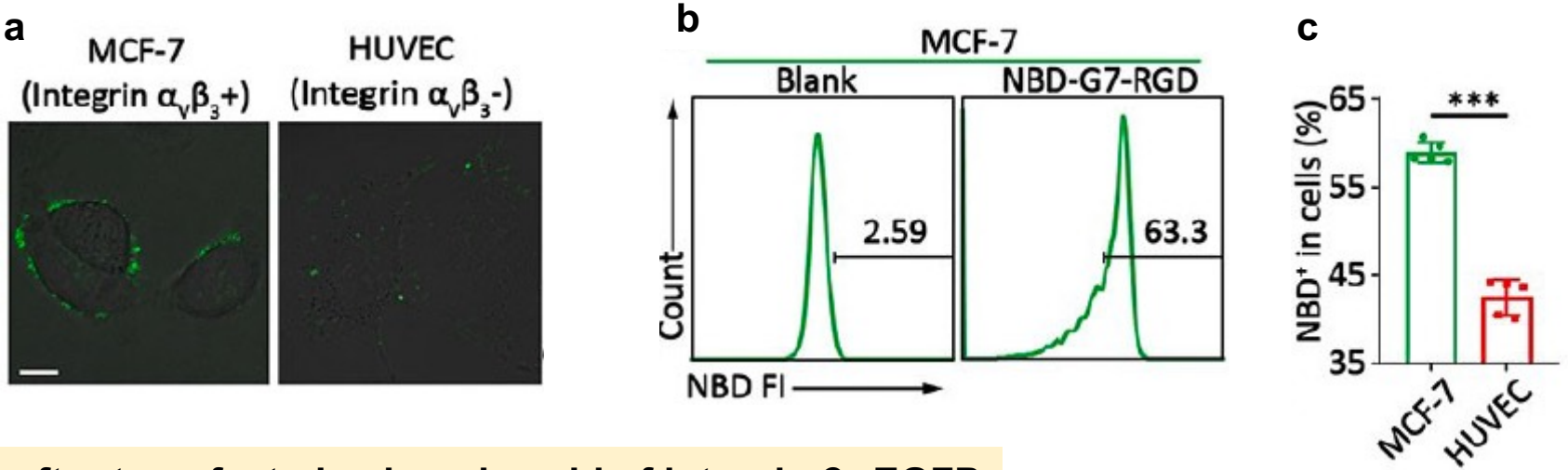


G7-RGD acted as the pre-assembly unit to target MCF-7 cells, followed by self-assembly and recruitment of the fluorescent building block NBD-G7.

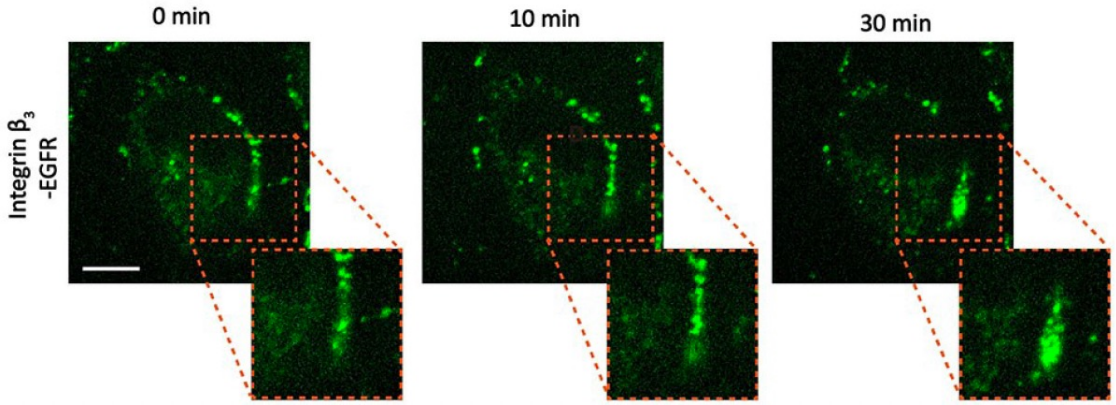
Receptor Oligomerization

? G7-based self-assembly → physical drag force → receptor oligomerization

confocal microscopy + flow cytometry



after transfected using plasmid of integrin β_3-EGFP

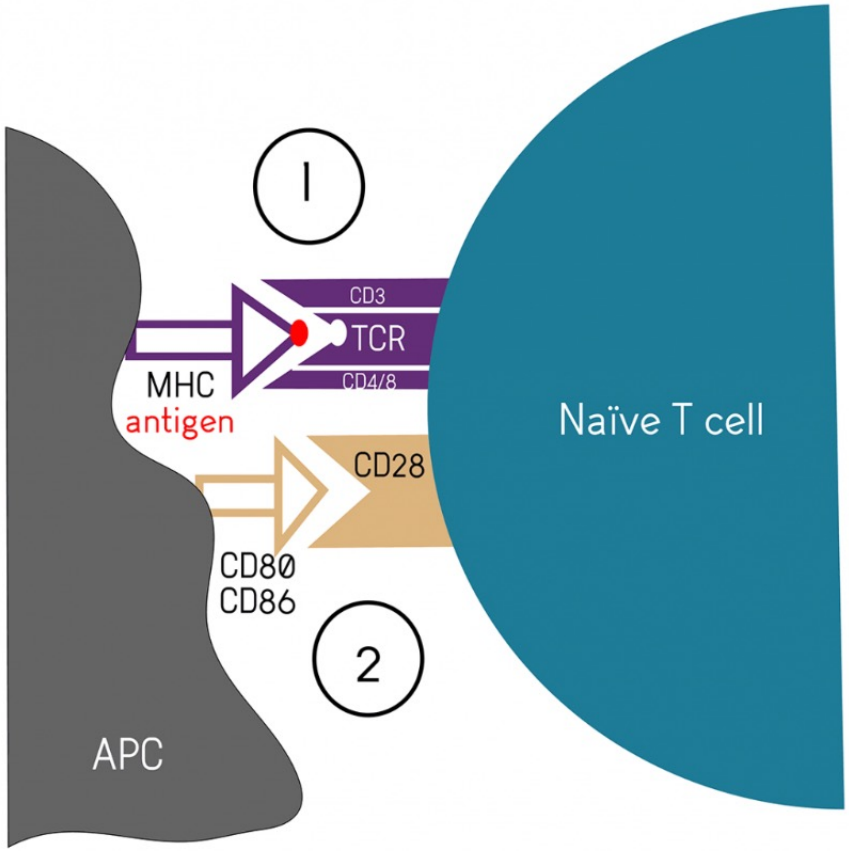


G7-RGD assembly caused oligomerization propensity of integrin $\alpha_V\beta_3$.

T-cell Activation

CD3: a highly effective T cell membrane marker

TCR-CD3 complex recognizes the antigen that binds to the major histocompatibility complex (pMHC) to initiate antigen-specific T cell activation.



The T cell activation needs two signals:

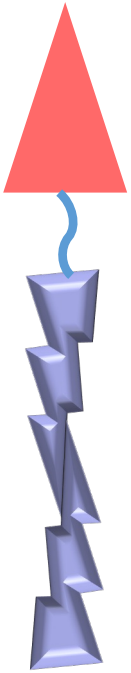
The first one is the engagement of the antigen specific T Cell Receptor (TCR) with the antigen.

The second one is needed to activate helper CD4+ and cytotoxic CD8+ T cells.

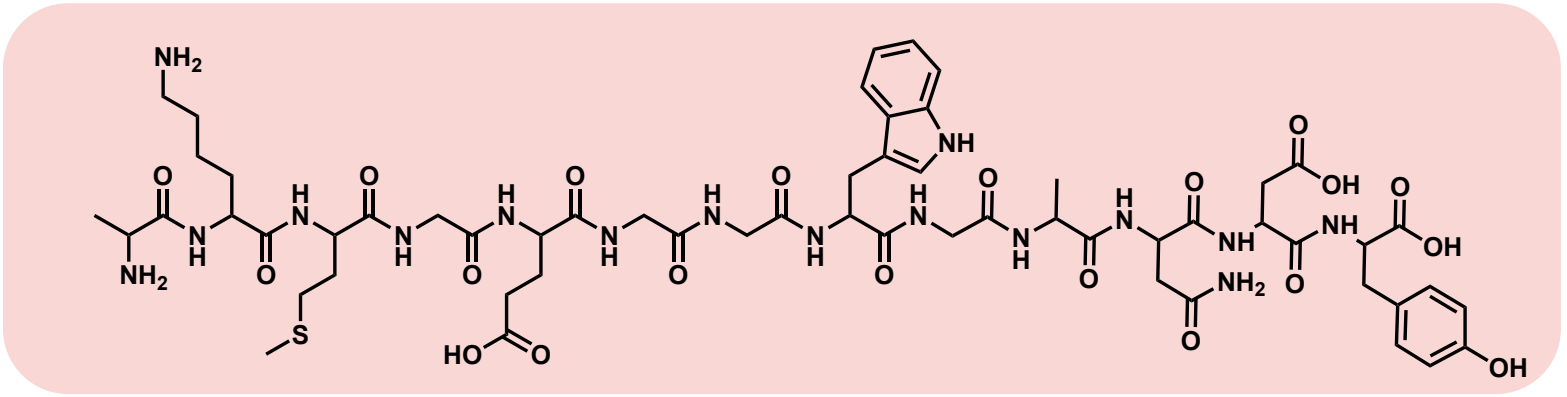
During the process, CD28 which provides co-stimulatory signals and secreted interleukin-2 (IL-2) are necessary.

Smith-Garvin, J. E.; Koretzky, G. A.; Jordan, M. S. *Annu. Rev. Immunol.* **2009**, *27*, 591.

Assembly Sequences: G7-antiCD3

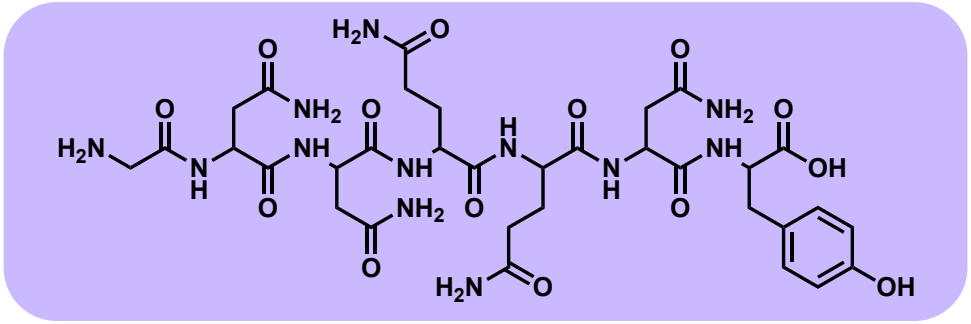


targeting motif



AKMGEGGWGANDY (receptor: CD3)

self-assembly motif



G7: GNNQQNY

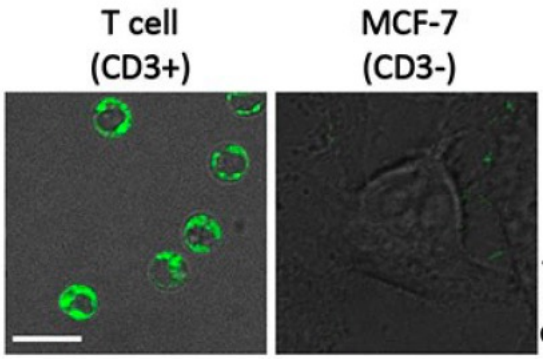
Oligomerization of CD3

targeting motif to CD3

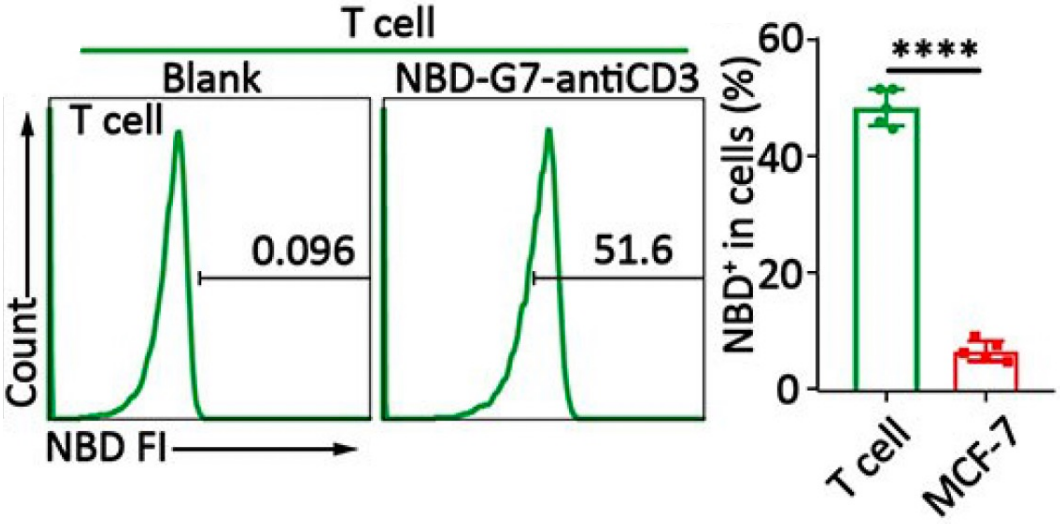
G7-antiCD3: AKMGEGGWGANDY-GNNQQNY

(G7-antiCD3 containing 2 % NBD-G7-antiCD3)

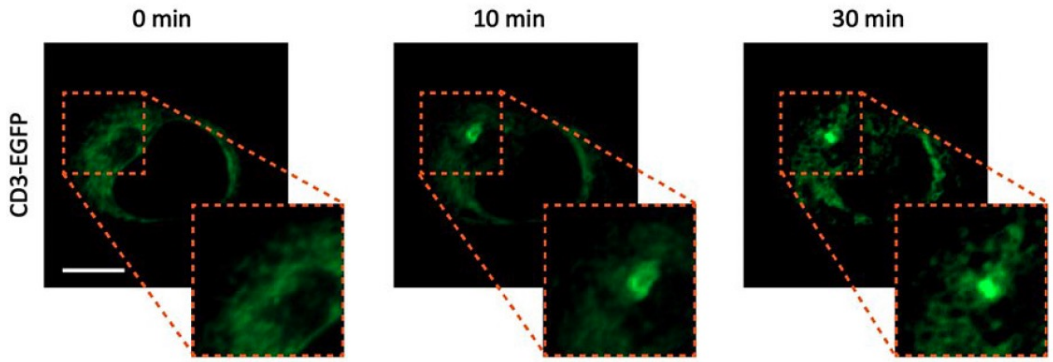
confocal images



flow cytometry histogram



confocal images

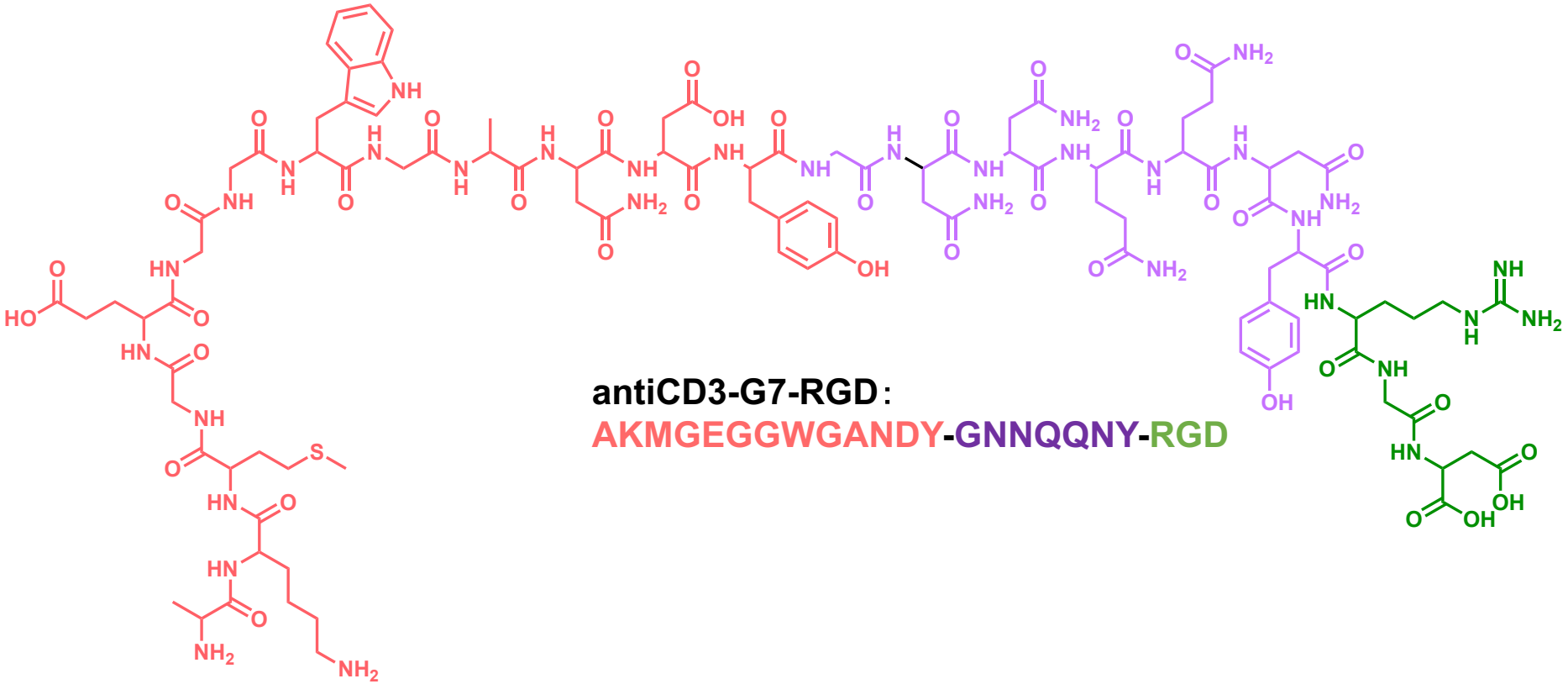


The CD3-EGFP receptor was subjected to aggregation mediated by G7-antiCD3.

TRA Design

TRA strategy

a promising approach for potential biomedical applications

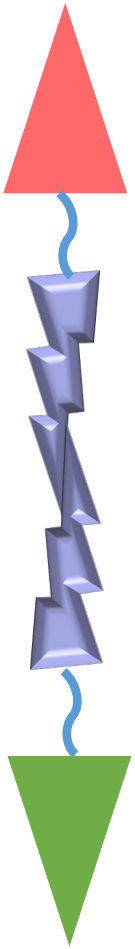


antiCD3-G7-RGD:
AKMGEGGWGANDY-GNNQQNY-RGD

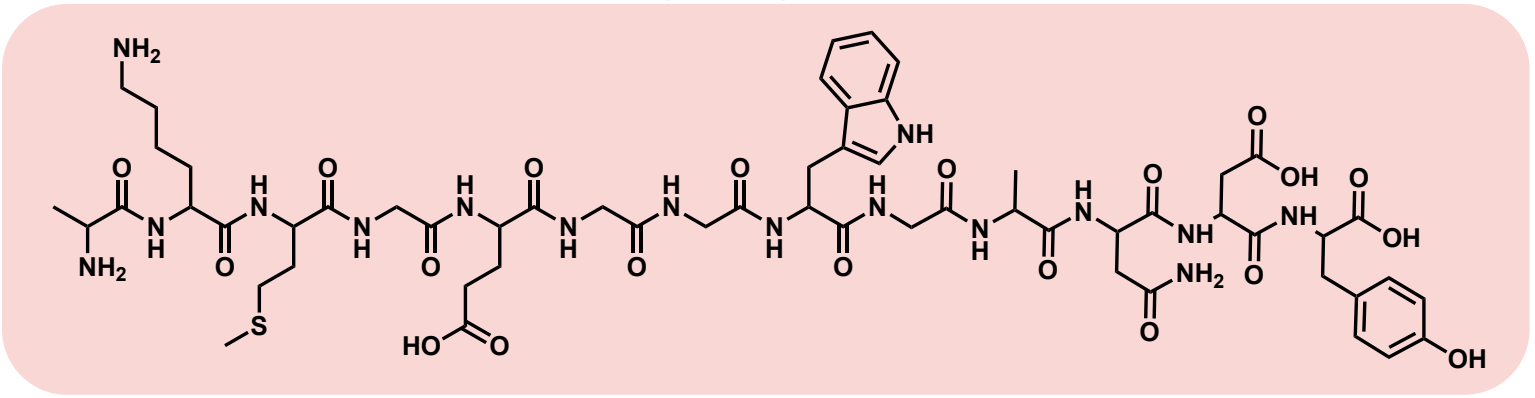
bispecific conjugate → **activate T cells**

bispecific conjugate → **mediate tumor cytolysis**

Assembly Sequence: AntiCD3-G7-RGD

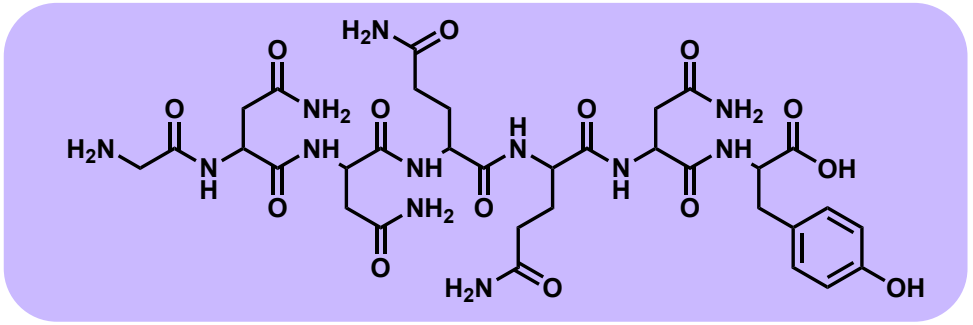


targeting motif



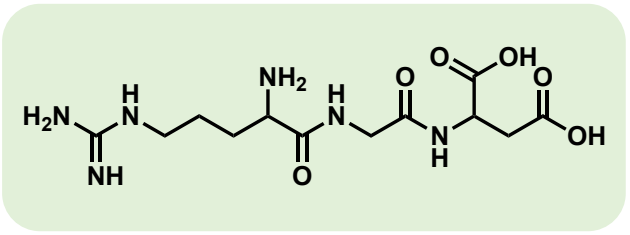
AKMGEGGWGANDY (receptor: CD3)

self-assembly motif



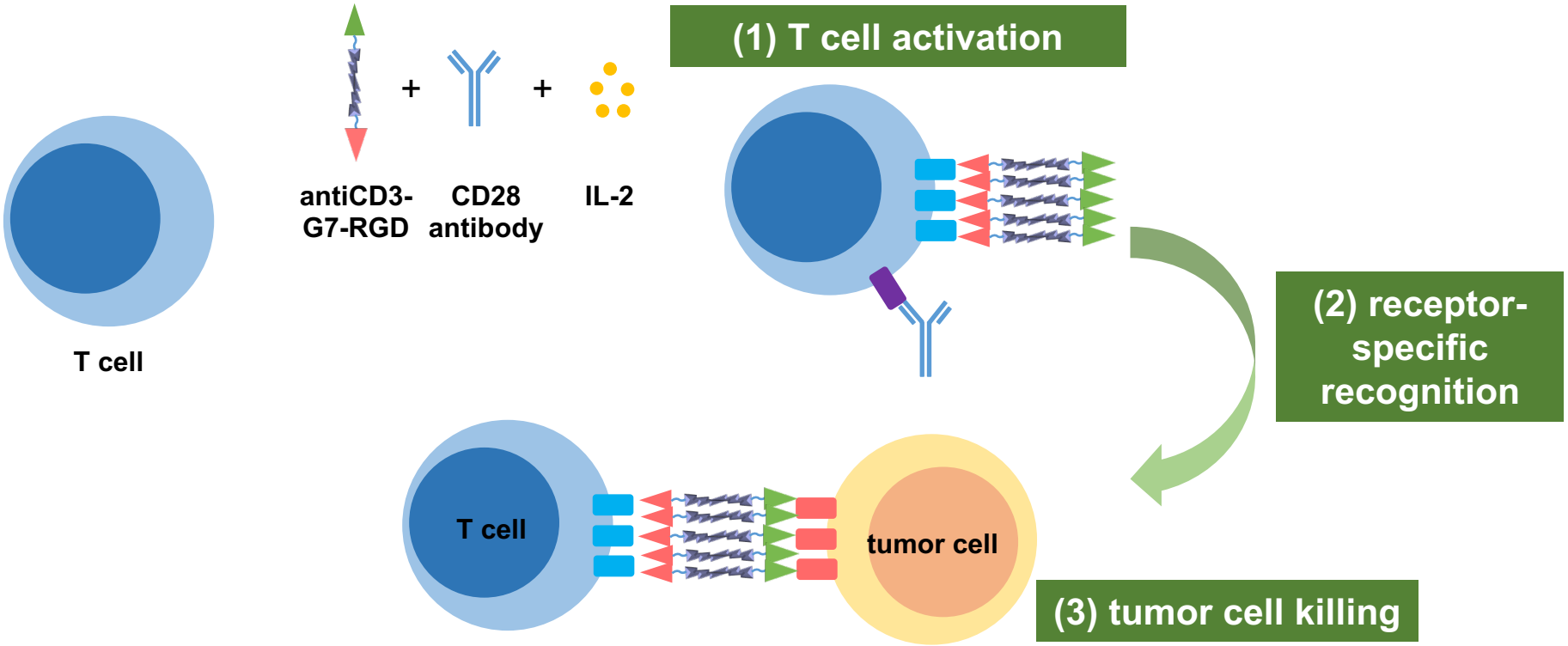
G7: GNNQQNY

targeting motif

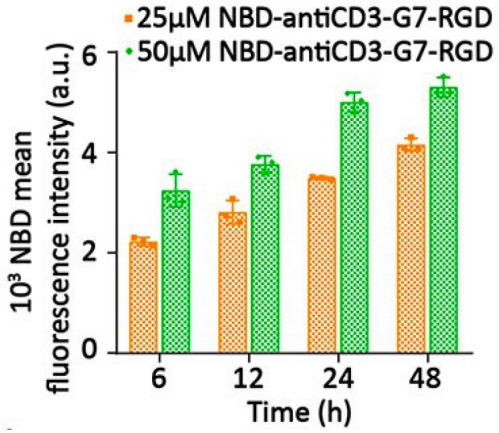


RGD (receptor: integrin $\alpha_V\beta_3$)

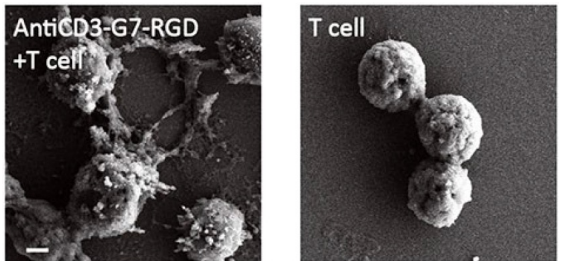
Aggregation of Self-Assembling Peptide on T Cells



flow cytometry quantification

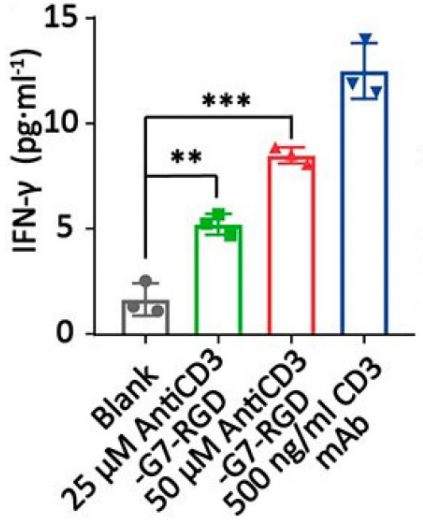


SEM images

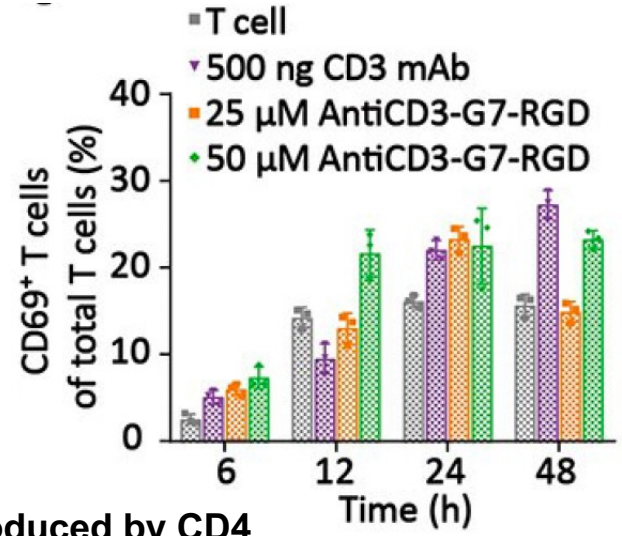


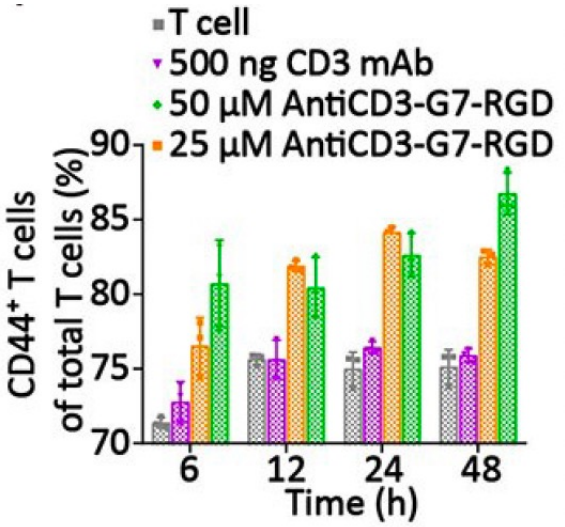
Tumor Cytolysis Mediated by TRA

ELISA measurement



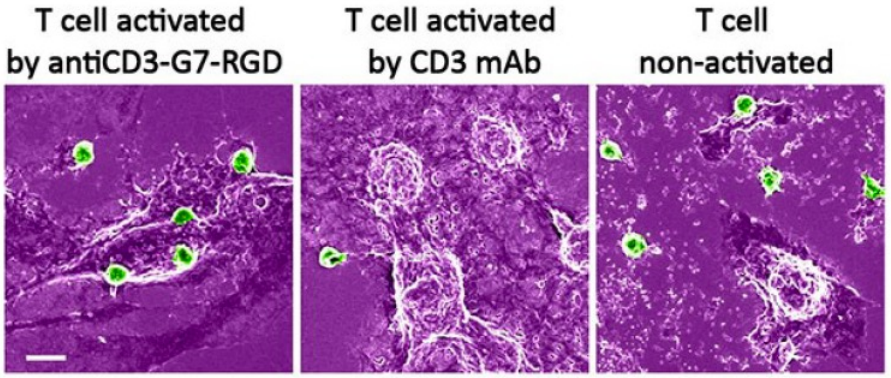
flow cytometry analysis

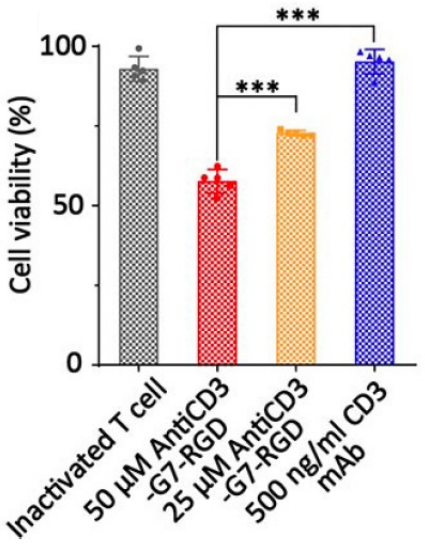




The IFN-γ protein is interferon produced by CD4 and CD8 T lymphocytes or activated NK cells.

SEM images

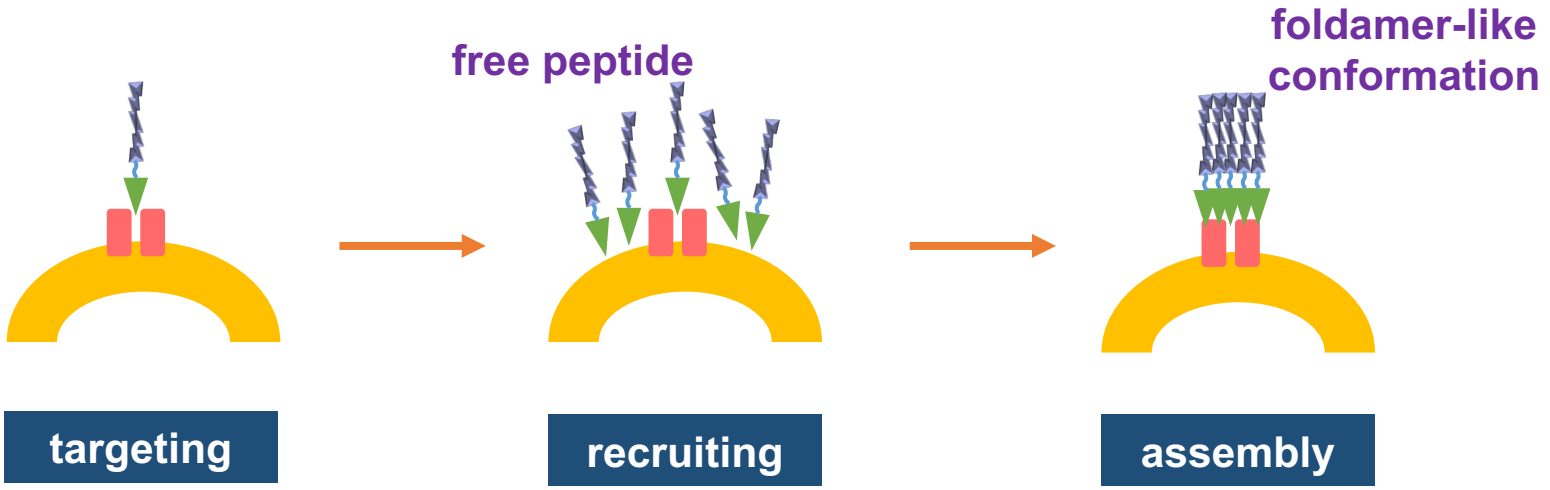




T cells were activated by the antiCD3-G7-RGD.

Conclusion and Perspectives

A specific and highly efficient in situ self-assembly strategy, TRA was developed.



A bispecific conjugate of antiCD3-G7-RGD was designed to not only promote oligomerization of CD3 receptors and activate T cells in vitro, but improve T cell-mediated tumor cytolysis.