Bispecific DNA-Peptide Conjugates for Targeting Cancer Cells

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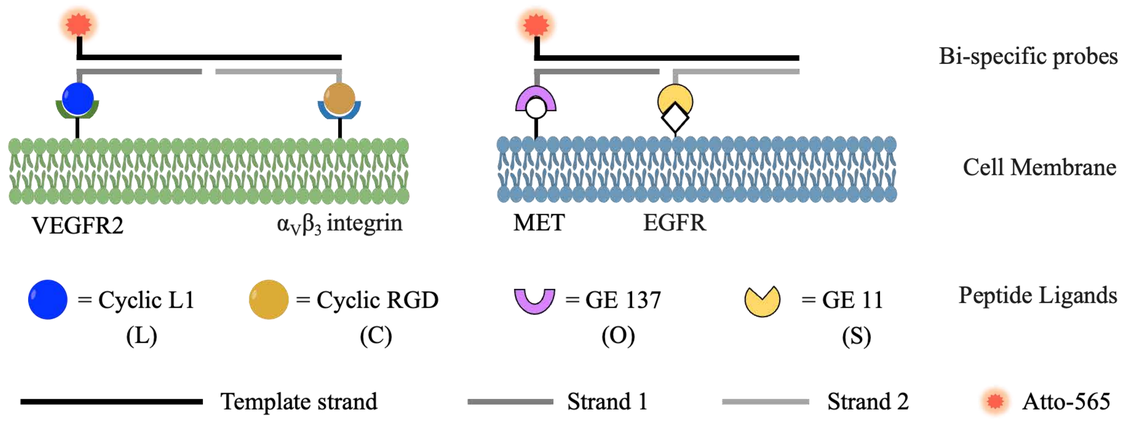
Introduction

Cells of a given tissue express a unique "fingerprint" of surface receptors. By targeting two oncogenic receptors that are uniquely overexpressed in a cancer cell of interest and displaying their peptide ligands in the proper arrangement, it is possible to achieve high-avidity, highly selective binding to cancerous cells over surrounding healthy cells. Herein, we demonstrate that the programmable base-pairing interactions make DNA an ideal scaffold[1-3] to direct the presentation of peptide ligands binding pairs of receptors, such as the VEGFR/$\alpha_v\beta_3$ integrin and the EGFR/MET pairs.

Aim



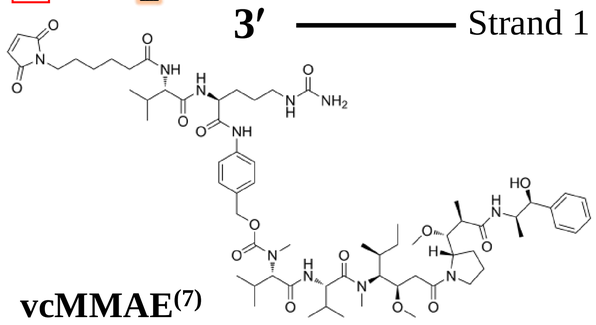
Designing the probes

5′ —— Template Strand —— 3′

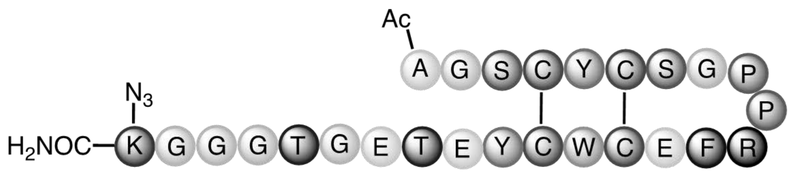
Dye/MMAE-GCGTAAGGAGATCTGGAATGA-GTCGAGCTCCATAATAAGCG

CGCATTCCTCTAGACCTTACT CAGCTCGAGGTATTATTCGC

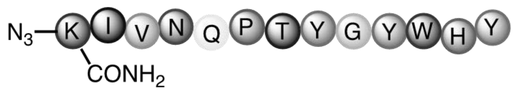
3′ —— Strand 1 —— 5′ 3′ —— Strand 2 —— 5′



vcMMAE[7]

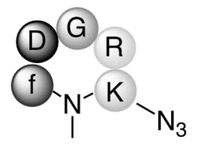


GE137[6]

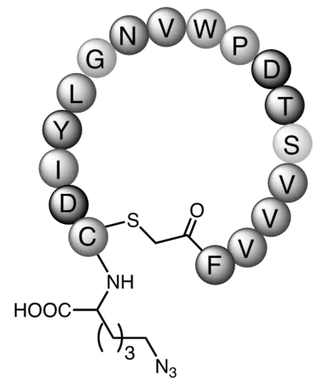


GE11[6]

Peptide Ligands



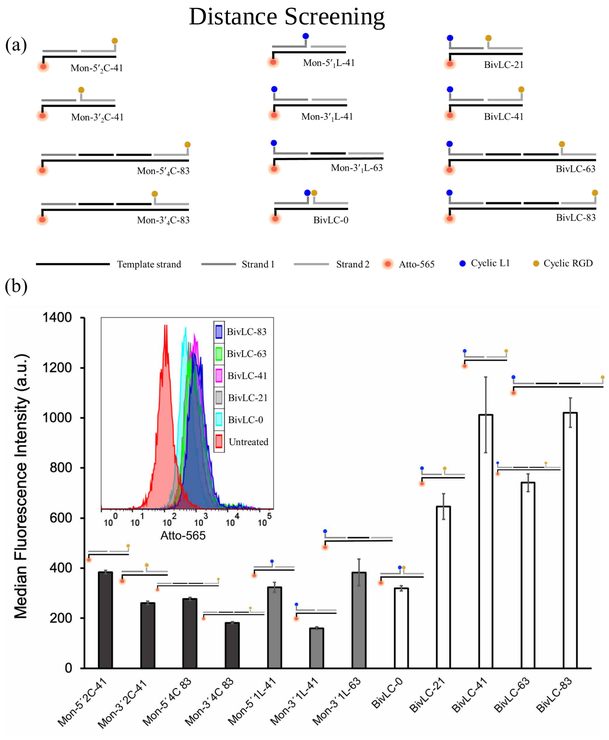
Cyclic RGD[4]



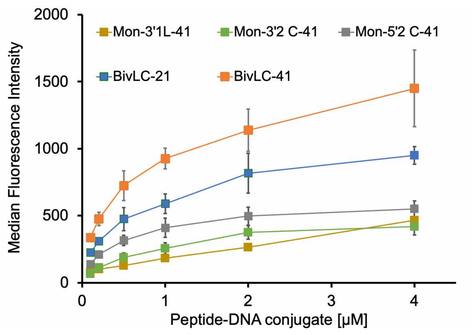
Cyclic L1[5]

Targeting VEGFR2-$\alpha_v\beta_3$

Distance Screening



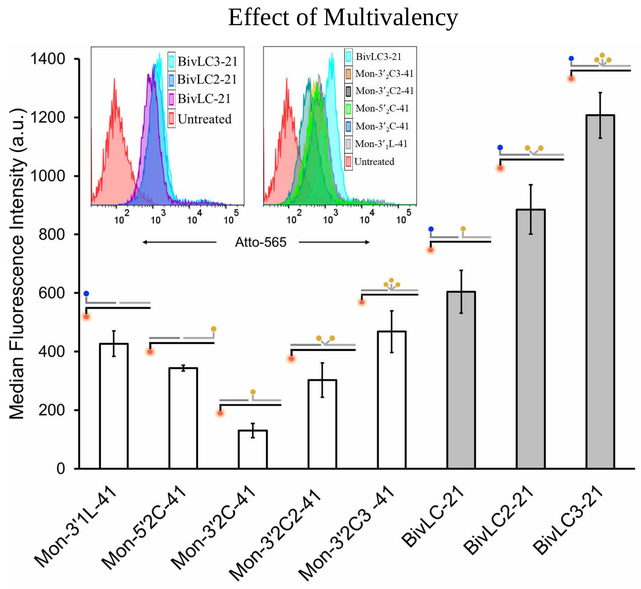
Concentration-dependent Flow Cytometry



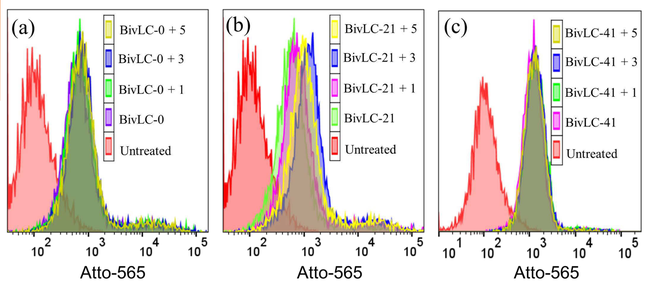
- **Mon-3'$_1$L-41:** 0.01916 x 10^{-6} M#
- **Mon-3'$_2$C-41:** 0.7105 x 10^{-6} M
- **Mon-5'$_2$C-41:** 0.3042 x 10^{-6} M
- **BivLC-21:** 0.2560 x 10^{-6} M
- **BivLC-41:** 0.2098 x 10^{-6} M

The plateau was not reached within the concentration range

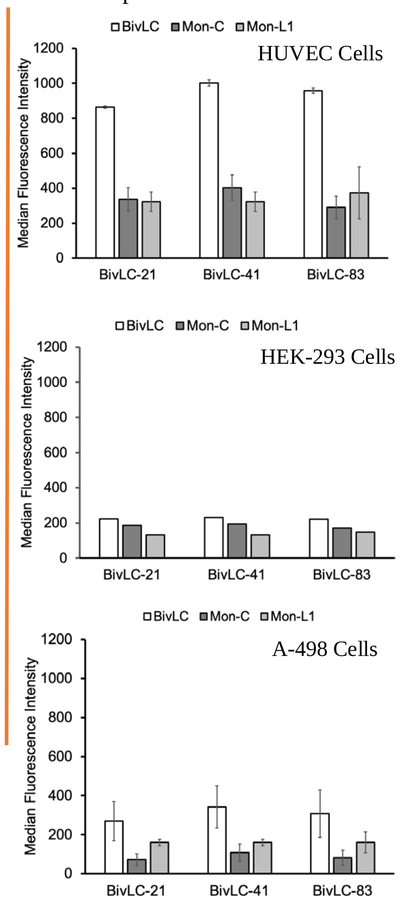
Effect of Multivalency



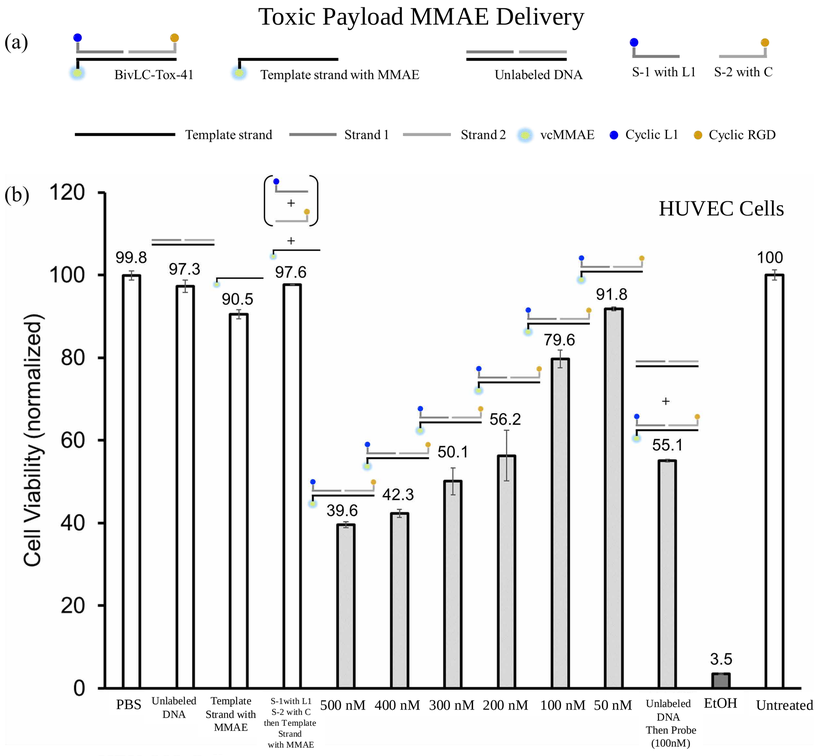
Spacer Nucleotide Effect Analysis



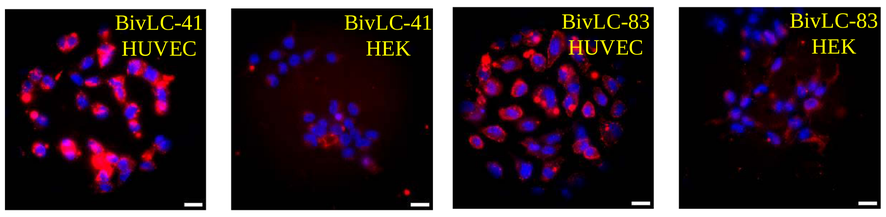
Bi-Specific Probes with Cells



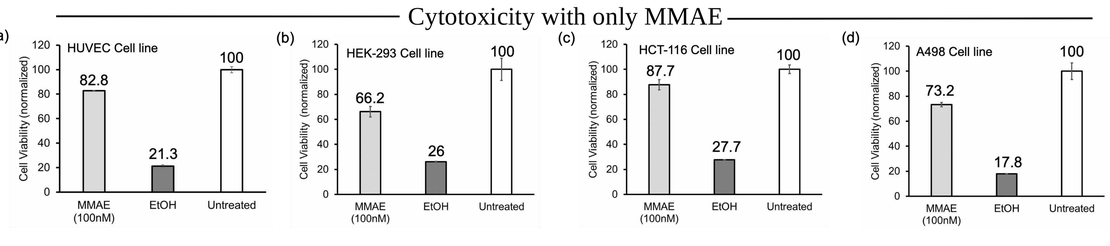
Toxic Payload MMAE Delivery



Fluorescence Microscopy Data

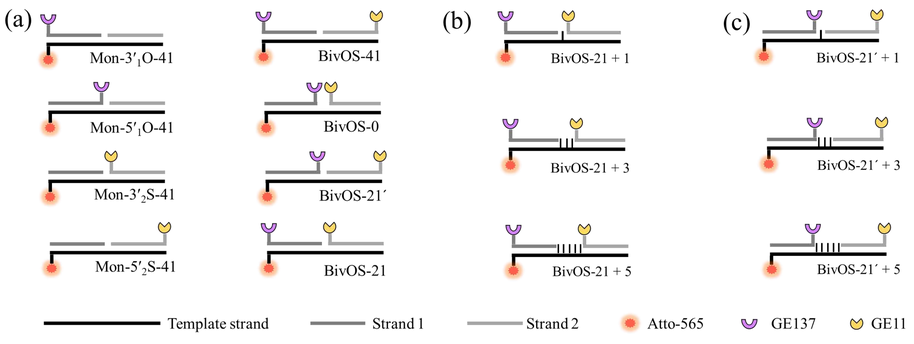


Cytotoxicity with only MMAE

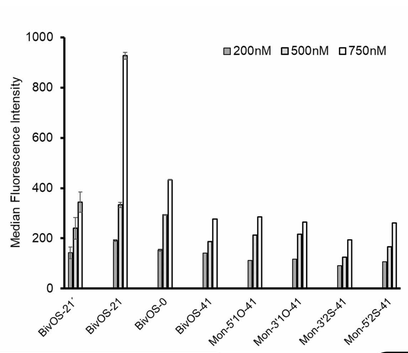


Targeting EGFR-MET

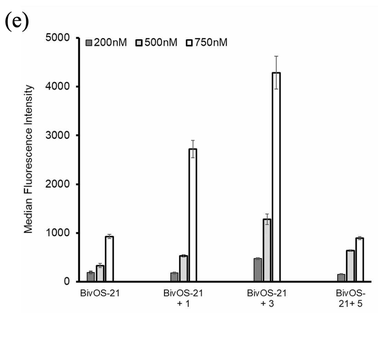
Monovalent and Heterobivalent G137 (O) / GE11 (S) conjugates



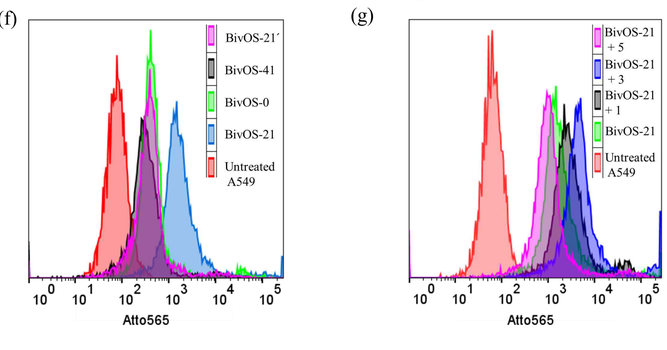
Distance Screening



Flexibility Screening



Flow Cytometry Histograms



Conclusions

- Distance-controlled arrangement of peptide-DNA conjugates enhances the specificity of cancer cell recognition.
  - VEGFR2-$\alpha_v\beta_3$ (HUVEC): high affinity for distances > 135 Å
  - EGFR-MET (A549): high affinity for distance ≈ 70 Å
- Bispecific DNA-peptide probes can deliver cytotoxic agent (MMAE) selectively to cells expressing oncogenic receptor pairs

References and Acknowledgement

(1) Seitz et al. Angew. Chem. Int. Ed. 2011, 50, 4146 –4150; **(2)** Seitz et al. Angew. Chem. Int.Ed. 2019, 58,907 –911; **(3)** Seitz et al. Angew. Chem. 2020, 132,21202 –21208; **(4)** Appella et al, Nat Commun. 2012, 3, 614; **(5)** Murakami et al, ACS Chem. Biol. 2013, 8, 1205-1214; **(6)** Hardwick et al, Nat. Med. 2015, 21, 955-961; **(7)** Parren et al, Nat. Med. 2018, 24, 203-212.

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