Engineered Novel CD155 and Nectin-4 Homodimer Proteins Dramatically Enhance Binding Affinities to Their Receptor TIGIT Compared to Respective Monomers

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Abstract

Understanding immune checkpoint receptor and ligand interactions is crucial for immuno-oncology. Nectin-4 and CD155 are part of the Nectin/Nectin-like family ligands of TIGIT (an inhibitory receptor on lymphocytes), can form homodimers on the cell membrane, and are often over-expressed on tumor cells. Nectin-4 and CD155 are type 1 integral membrane proteins. The ectodomain (ECD) containing 3 Ig-like domains (1 Ig-V followed by 2 Ig-C) is responsible for TIGIT interaction. To better mimic the native dimer conformation and quaternary structure, we designed novel soluble Nectin-4 and CD155 cis-homodimers with the ECD 3 Ig-like domains (ECD-3D) fused to a dimer motif at the C-terminus. These dimeric proteins expressed/purified from HEK293T cells demonstrated potent binding to the specific antibodies. Very interestingly, these Nectin-4 and CD155 dimeric proteins dramatically enhanced the binding to their receptor TIGIT by >50 fold compared to the respective monomer proteins, as measured by ELISA. The Nectin-4 dimer DNA immunization scheme elicited potent antibody responses in mice. AlphaFold 2 predicted that the Nectin-4 ECD-3D dimeric protein structure was well-superimposed with the known ECD-2D crystal structure from the RCSB Protein Data Bank. The findings support that the engineered novel Nectin-4 and CD155 cis-dimer proteins mimic native conformations and can be potentially used as advanced bioactive antigens for immuno-oncology research and anti-tumor drug discovery.

Materials and Methods

Recombinant Proteins: TIGIT-Fc dimer (Conigen Bioscience, CSP-24028) Nectin-4 dimer His-tag (Conigen Bioscience, CSP-24016), CD155 dimer His-tag (Conigen Bioscience, CSP-24029), Nectin-4 monomer His-tag (AcroBiosystems, Cat# NE4-H52H3), CD155 monomer His-tag (AcroBiosystems, Cat# CD5-H5223). TIGIT UniProt# Q495A1, CD155 UniProt# P15151, Nectin-4 UniProt# Q96NY8.
Antibody binding assays: CD155 or Nectin-4 specific antibody binding was measured by ELISA. CD155 and Nectin-4 dimer or monomer proteins were coated on 96-well microtiter plates at 2 μg/ml and detected by CD155 or Nectin-4 specific antibodies at a series of concentrations.
Ligand/Receptor binding assays: ELISA and Label-free kinetics assays were performed to evaluate the CD155 and Nectin-4 dimer or monomer binding potencies to the TIGIT receptor. **ELISA:** CD155 or Nectin-4 dimer or monomer proteins were coated on 96-well microtiter plates at 2 μg/ml and detected by TIGIT-Fc dimer at a series of concentrations.
Label-free kinetics assay: The dissociation constant (Kd) was determined using a Nicoya Alto localized surface plasmon resonance (LSPR) instrument. TIGIT-Fc dimer was directly immobilized to a carboxyl cartridge and interacted with 5 serial dilutions of analyte (CD155 dimer, CD155 monomer, Nectin-4 dimer, or Nectin-4 monomer) in a titration (Single-cycle) binding kinetics assay.
Computational modeling: Structures were predicted using the ColabFold implementation of AlphaFold2. All predictions were run using PDB100 template mode, MMseqs2 (Uniref + environmental), AlphaFold2 multimer v3 model, and 12 recycles. Crystal structure studies (Xtal) were retrieved from the RCSB-PDB, and all alignments and visualizations were created with PyMOL3.

Results

CD155 and TIGIT

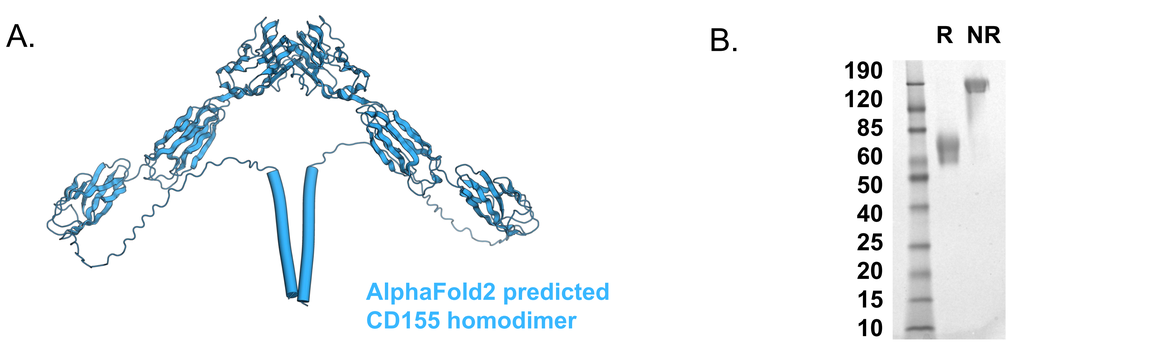


Fig. 1. CD155 homodimer protein design and expression. The ectodomain of human CD155 was genetically fused to a proprietary linker and dimerization motif to promote soluble dimer formation. The recombinant protein was expressed in HEK293T cells and purified. (A) Predicted structure of the recombinant CD155 homodimer. (B) SDS-PAGE analysis of the purified CD155 homodimer under non-reducing (NR) and reducing (R) conditions.

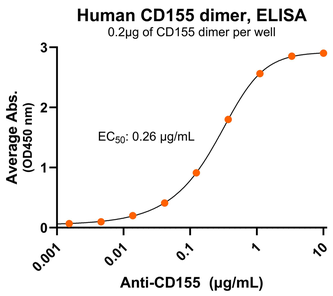


Fig. 2 CD155 protein dimer binding to CD155 specific monoclonal antibody, as detected by ELISA.

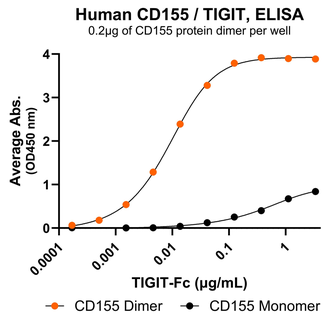
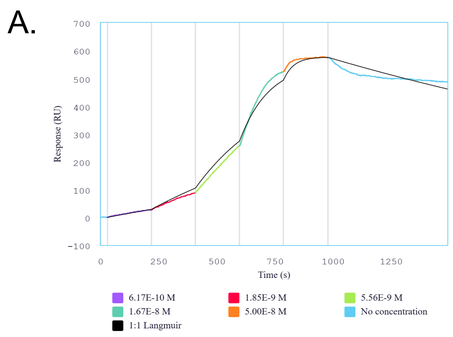


Fig. 3 CD155 dimer or monomer protein binding to its receptor TIGIT-Fc dimer, as measured by ELISA.



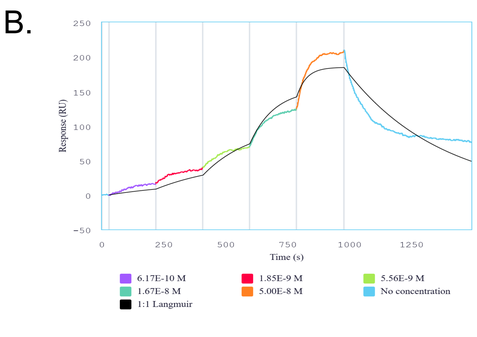


Fig. 4. CD155 dimer or monomer protein binding to its receptor TIGIT-Fc dimer, as measured by localized surface plasmon resonance (LSPR). (A) CD155 dimer/TIGIT binding Kd: **0.1 nM**; (B) CD155 monomer/TIGIT binding Kd: **5.7 nM**. The CD155 dimer binding affinity to TIGIT was **57-fold** higher than the CD155 monomer.

Nectin-4 and TIGIT

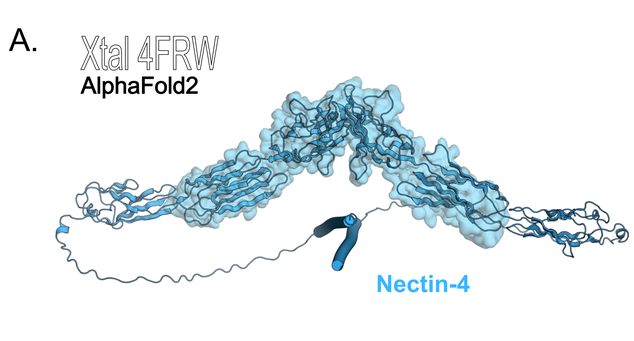


Fig. 5. Nectin-4 homodimer protein design and expression. The ectodomain of human Nectin-4 was genetically fused to a proprietary linker and dimerization motif to promote soluble dimer formation. The recombinant protein was expressed in HEK293T cells and purified. (A) Predicted structure of the recombinant Nectin-4 homodimer agrees with Xtal. (B) SDS-PAGE analysis of the purified Nectin-4 dimer under non-reducing (NR) and reducing (R) conditions.

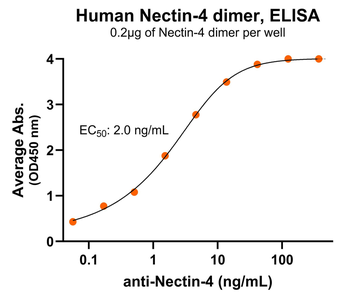


Fig. 6. Nectin-4 protein dimer binding to Nectin-4 specific monoclonal antibody, as detected by ELISA.

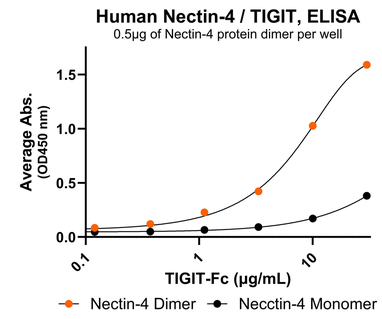
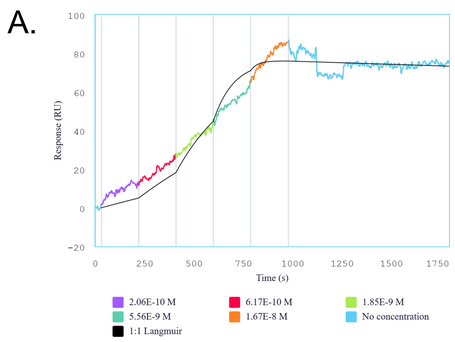


Fig. 7. Nectin-4 dimer or monomer protein binding to its receptor TIGIT-Fc dimer, as measured by ELISA.



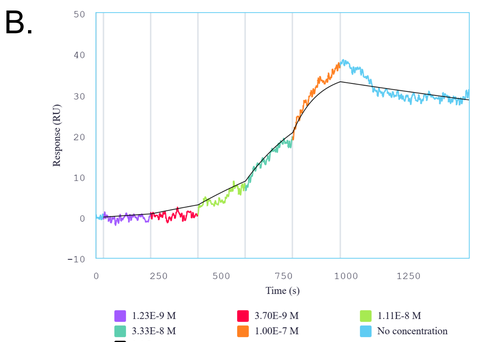


Fig. 8 . Nectin-4 dimer or monomer protein binding to its receptor TIGIT-Fc dimer, as measured by localized surface plasmon resonance (LSPR). (A) Nectin-4 dimer/TIGIT binding Kd : **0.02 nM**; (B) Nectin-4 monomer/TIGIT binding KD: **2.9 nM**. The Nectin-4 dimer binding affinity to TIGIT was **145-fold** higher than the Nectin-4 monomer.

Conclusions

- The novel designs of CD155 and Nectin-4 ectodomain homodimer proteins to mimic the native dimer structures are well-superimposed with the known CD155 and Nectin-4 crystal structures.
- The recombinant CD155 homodimer and Nectin-4 homodimer proteins expressed from HEK293T cells were purified with high purity and demonstrated specific potent binding to the respective antibodies.
- The CD155 homodimer and Nectin-4 homodimer significantly increased the binding potencies to their receptor TIGIT-Fc dimer with **>50 and >100 fold** compared to CD155 monomer and Nectin-4 monomer, respectively.
- These novel CD155, Nectin-4 and TIGIT-Fc homodimer proteins can be used as antigens and immunogens for research and drug discovery.
- The high affinity and wider receptor/ligand binding windows can serve as more sensitive tools for therapeutic molecule evaluations.

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