Engineered novel cytokine receptors IFNγR1, IL10Rα and IFNαR1/2 cis-dimers significantly enhance the binding of the relevant cytokines IFNγ, IL-10 and IFNα

Shixia Wang, Christofer Walsh, Timothy Smith, Richard Parent, Alyssa Ball, Joshua Lubner, Jean Qiu
Conigen Bioscience, Inc., 17 Briden Street, Worcester, MA 01605

Abstract

Cytokines initiate signaling on target cells by engaging the ectodomains (ECDs) of cell surface receptors. Cytokine receptor dimeric forms are critical for cytokine ligand binding. To mimic the dimeric receptor conformation, we engineered novel cis-homodimers for human Type II interferon IFNγ receptor 1 (IFNγR1) and interleukin 10 receptor alpha (IL10Rα), and the cis-heterodimer for human Type I interferon IFNα receptor 1/2 (IFNαR1/2). To generate the IFNγR1 or IL10Rα homodimer, its ECD was fused with a homodimer motif at the C-terminus; to create IFNαR1/2 heterodimer, IFNαR1 and IFNαR2 ECDs were fused with a heterodimer motif at the C-terminus of each chain. These soluble dimeric receptor proteins were expressed/purified from HEK293T cells and could potently bind to the specific antibodies as measured by ELISA. We further investigated the dimeric or monomeric receptor protein binding to the cytokine ligands. Notably, the dimeric forms of IFNγR1, IL10Rα or IFNαR1/2 dramatically enhanced the binding to the relevant cytokine IFNγ, IL-10 or IFNα with >10-fold increases of binding potency based on determined EC50 values, compared to the monomeric receptor proteins. The findings imply that these engineered novel cis-homodimer and cis-heterodimer cytokine receptor proteins mimic the native conformation with better bioactivities. These innovative soluble cis-dimer cytokine receptor proteins can be very useful new molecules for basic immunology research and drug discovery.

Materials and Methods

Recombinant proteins: IFNγR1 homodimer His-tag (Conigen Bioscience, CSP-24015), IL10Rα homodimer His-tag (Conigen Bioscience, CSP-24018), IFNαR1/R2 heterodimer His-tag (Conigen Bioscience, CSP-24025-A1B1), IL10Rα momomer His-tag, IFNγR1 monomer His-tag, IFNαR1 monomer His-tag, IFNαR2, IFNγ, Type I IFN. IFNγR1 UniProt# P15260, IL10Rα UniProt #: Q13651, IFNαR1 UniProt# P17181, IFNαR2 UniProt# P48551.
SDS-PAGE: SDS-PAGE with reducing and non-reducing conditions were used to detect the dimer protein and its purity relative to monomer species.
Antibody binding ELISA: IFNγR1, IL10Rα and IFNαR proteins were coated on 96-well microtiter ELISA plates (2 μg/ml) and detected by each cytokine receptor-specific antibody at a serial dilution.
Receptor/Ligand binding ELISA: IFNγ was coated on 96-well microtiter plate (2 μg/ml) to bind to IFNγR1 at a serial dilution. IL10 was coated on 96-well microtiter plate (2 μg/ml) to bind to IL10Rα at a serial dilution. Universal Type I interferon was coated on 96-well microtiter plate (2 μg/ml) to bind to IFNαR1/R2 at a serial dilution. The bound IFNγR1 or IFNαR1/2 were further detected by a His-tag-specific antibody.
Label-free kinetics assay: The dissociation constant (Kd) was determined using a Nicoya Alto localized surface plasmon resonance (LSPR) instrument by directly immobilizing the IFNγR1, IFNαR1/R2 dimer or monomer receptor protein to a Carboxyl Cartridge and measuring binding of 5 serial dilutions of analyte IFNγ cytokine with regeneration of the ligand between each dilution (Multi-cycle) or Type I interferon 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.

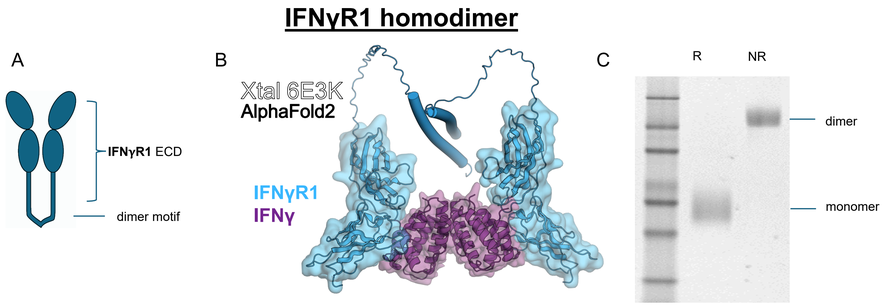

Fig. 1. IFNγR1 homodimer protein design and expression. (A) The ectodomain of human IFNγR1 was genetically fused to a proprietary linker and dimerization motif to promote soluble dimer formation. The recombinant protein was expressed in HEK293T cells and purified. (B) Predicted structure of the recombinant IFNγR1 homodimer bound to IFNγ agrees with Xtal. (C) SDS-PAGE analysis of the purified IFNγR1 homodimer under non-reducing (NR) and reducing (R) conditions.

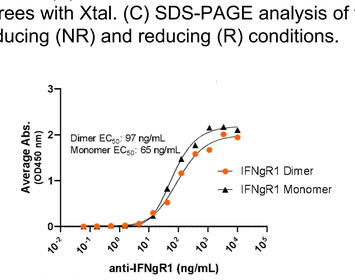

Fig. 2. IFNγR1 dimer and monomer protein binding to IFNγR1-specific monoclonal antibody, as detected by ELISA.

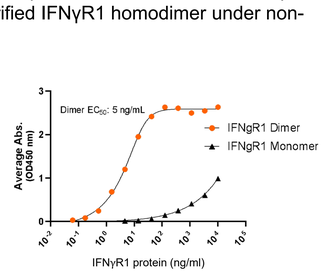

Fig. 3. IFNγR1 dimer or monomer protein binding to its ligand IFNγ cytokine, as measured by ELISA.

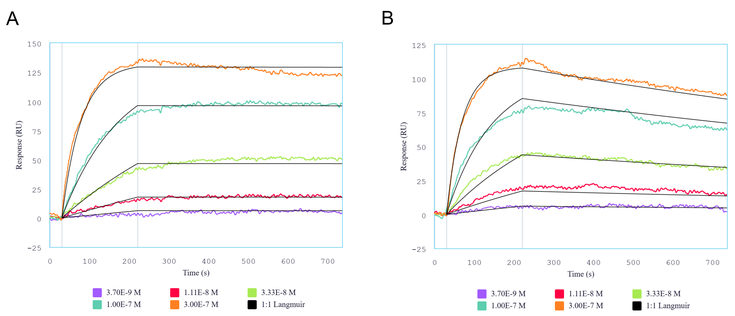

Fig. 4. IFNγR1 receptor dimer or monomer protein binding to its ligand IFNγ cytokine as measured by localized surface plasmon resonance (LSPR). (A) IFNγR1 dimer/ IFNγ binding Kd: **0.049 nM**; (B) IFNγR1 monomer/ IFNγ binding Kd: **5.54 nM**. The IFNγR1 dimer binding affinity to IFNγR1 was **113-fold** higher than IFNγR1 monomer.

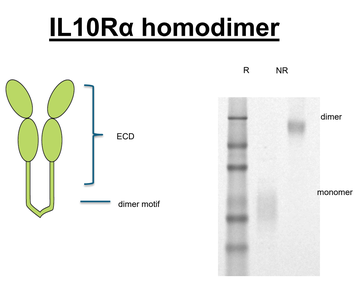

Fig. 5. IL10Rα homodimer protein design and expression. (A) The ectodomain of human IL10Rα was genetically fused to a proprietary linker and dimerization motif to promote soluble dimer formation. (B) SDS-PAGE analysis of the purified IL10Rα homodimer under non-reducing (NR) and reducing (R) conditions.

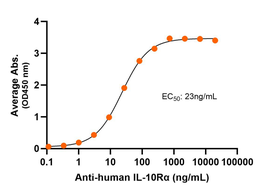

Fig. 6. IL10Rα dimer protein binding to IL10Rα-specific monoclonal antibody, as detected by ELISA.

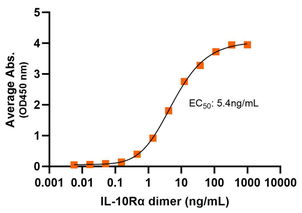

Fig. 7. IL10Rα dimer protein binding to its ligand IL10 cytokine, as detected by ELISA. IL10 cytokine binding was not detected by IL10Rα monomer.

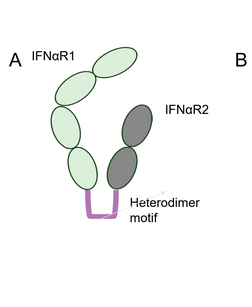

Fig. 5. IFNαR1/R2 heterodimer protein design and expression. (A) The ectodomains of human IFNαR1/R2 were genetically fused to a proprietary linker and dimerization motif to promote soluble heterodimer formation. The recombinant protein was expressed in HEK293T cells and purified. (B) Predicted structure of the recombinant IFNαR1/R2 heterodimer bound to IFN2α agrees with Xtal. (C) SDS-PAGE analysis of the purified IFNαR1/R2 heterodimer under non-reducing (NR) and reducing (R) conditions.

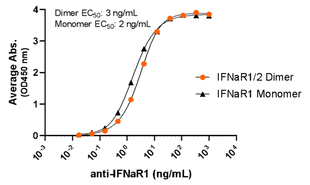

Fig. 6. IFNαR1/R2 dimer and IFNαR1 monomer protein binding to IFNαR1-specific monoclonal antibody, as detected by ELISA.

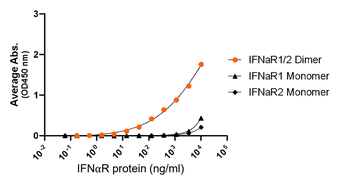

Fig. 7. IFNαR1/R2 dimer, IFNαR1 or IFNαR2 monomer protein binding to its ligand Type I interferon cytokine, as measured by ELISA.

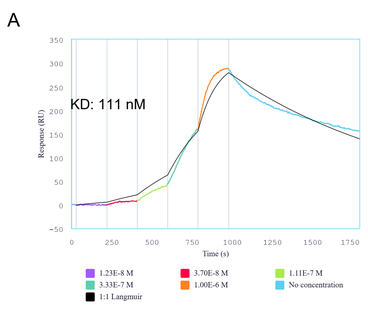

Fig. 8. IFNαR1/R2 receptor heterodimer or IFNαR1 monomer protein binding to its ligand universal Type I interferon cytokine as measured by localized surface plasmon resonance (LSPR). (A) IFNαR1/R2 dimer/Type I interferon binding Kd: **111 nM**; (B) IFNαR1 monomer/Type I interferon **no binding** detected.

Conclusions

- The soluble dimeric cytokine receptors IFNγR1 homodimer, IL10Rα homodimer and IFNαR1/R2 heterodimer proteins were designed to mimic the native dimer structures as predicted.
- The recombinant IFNγR1 homodimer, IL10Rα homodimer and IFNαR1/R2 heterodimer proteins expressed from HEK293T cells were purified with high purity and demonstrated specific potent binding to the respective antibodies.
- The IFNγR1 homodimer, IL10Rα homodimer and IFNαR1/R2 heterodimer proteins significantly increased the binding potencies to their respective cytokine ligands Type II interferon, IL10 and Type I interferon cytokines compared to the respective monomeric receptor proteins.
- These novel IFNγR1 homodimer, IL10Rα homodimer and IFNαR1/R2 heterodimer proteins can be potentially very useful for immunology research and drug discovery.

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