

Molecular aspects of drug development

Modeling of chimeric proteins

IFN α_{2b} -Fc

Sergeeva Daria

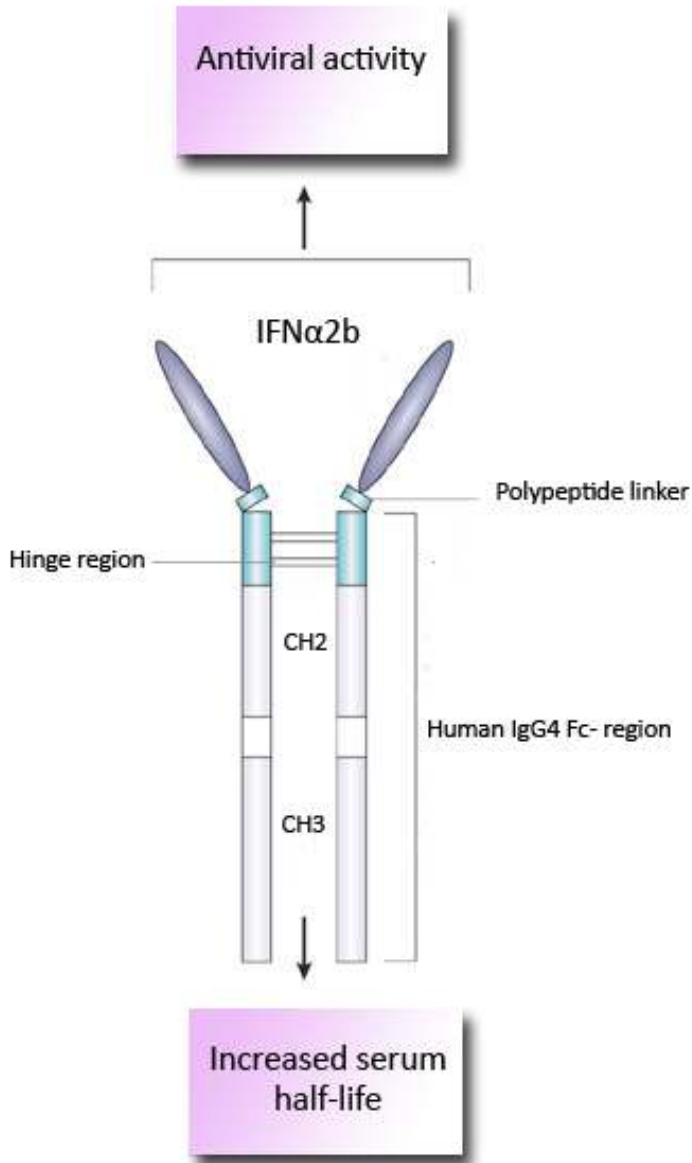
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Expression and study of biological activity of chimeric molecules IFN α 2b-Fc

IFN α 2b linked with Fc-fragment of IgG4

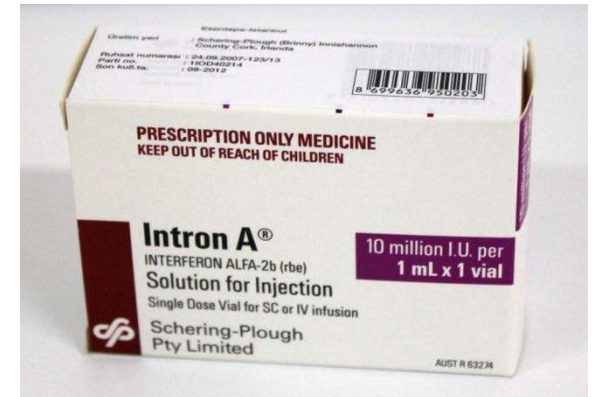


Other forms of IFN α 2b



PEG-IFN

IntronA



Problems of recombinant IFNs:

- short serum half-life
- frequent administration
- high dosage
- low efficacy
- side effects

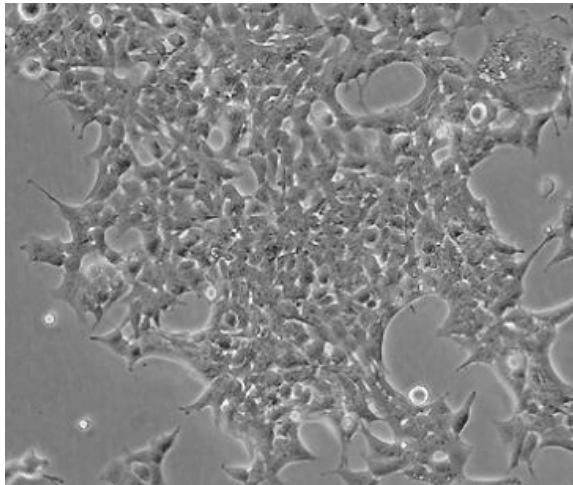
4 variants of chimeric molecules - different activity?

I: IFN - hinge - Fc

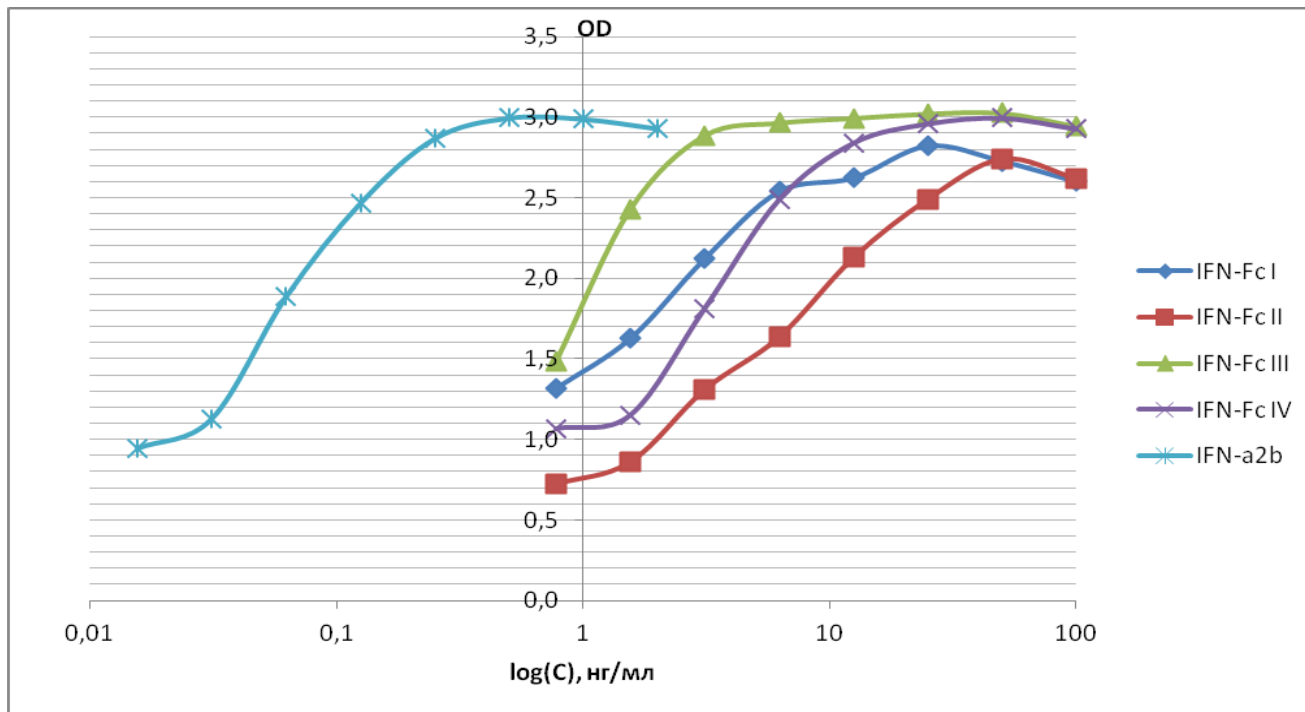
II: IFN - **GGGS** - hinge - Fc

III: IFN - **GGSGGGGS** - hinge - Fc

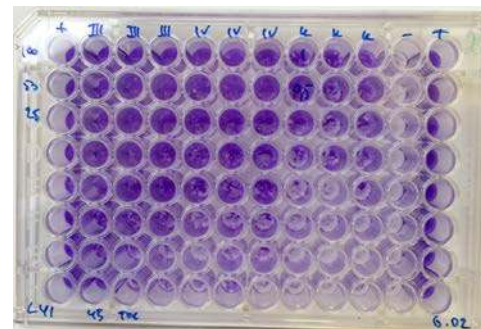
IV: IFN - **GGSGGSGGGGSGGGGS** - hinge - Fc



Antiviral activity



$\text{IFN}\alpha_{2b}\text{-Fc-I} - 14,0 \pm 2,7\%$
 $\text{IFN}\alpha_{2b}\text{-Fc-II} - 3,4 \pm 0,5\%$
 $\text{IFN}\alpha_{2b}\text{-Fc-III} - 20,9 \pm 3,5\%$
 $\text{IFN}\alpha_{2b}\text{-Fc-IV} - 12,1 \pm 3,7\%$



How linker length affects molecule flexibility and biological activity?

Tasks:

- create models of fusion proteins
- find different conformations of linker regions and study their flexibility
- compare modeling results and *in vitro* experimental data

Molecular modeling of IFNa2b-Fc

Homology modeling

Phyre2, HHpred, RatporX, Swiss-Model, I-Tasser



Quality estimation

ModFOLD



Homodimeric structure modeling

ClusPro2, PyMOL

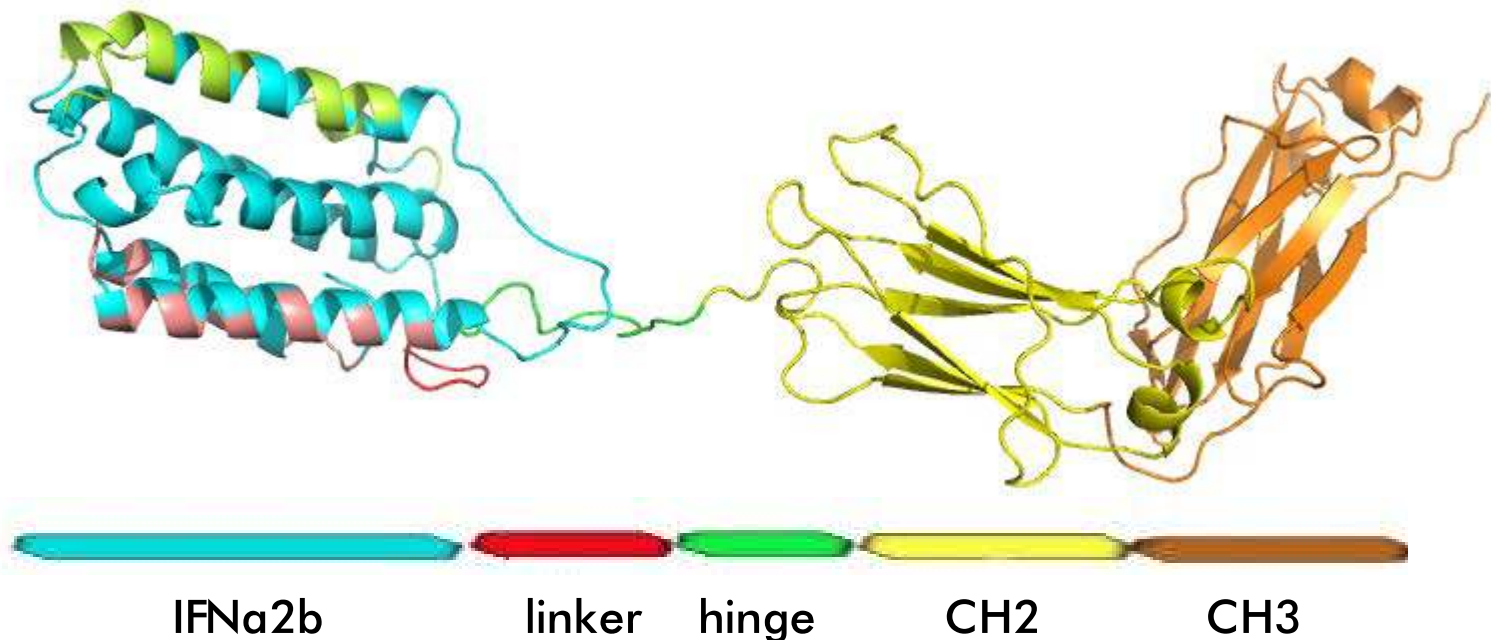


Molecular dynamics

CABS-flex

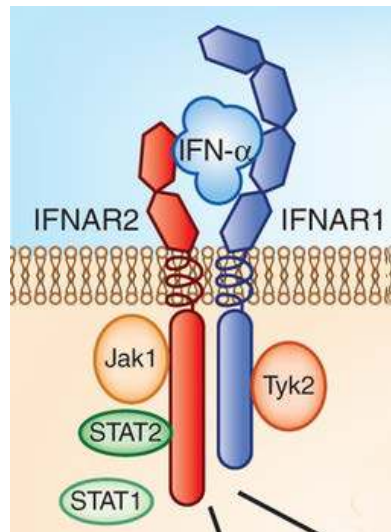
Structure modeling

- Monomer models for 4 variants of IFNa2b-Fc were built by homology modeling
- Best quality score was demonstrated by HHpred tool
- Linker regions were constructed by *ab initio* modeling

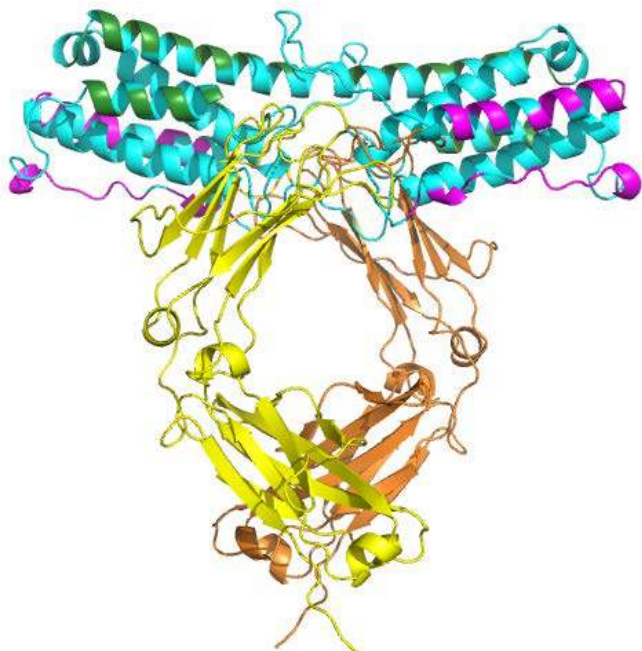


Homodimerization of IFNa2b-Fc

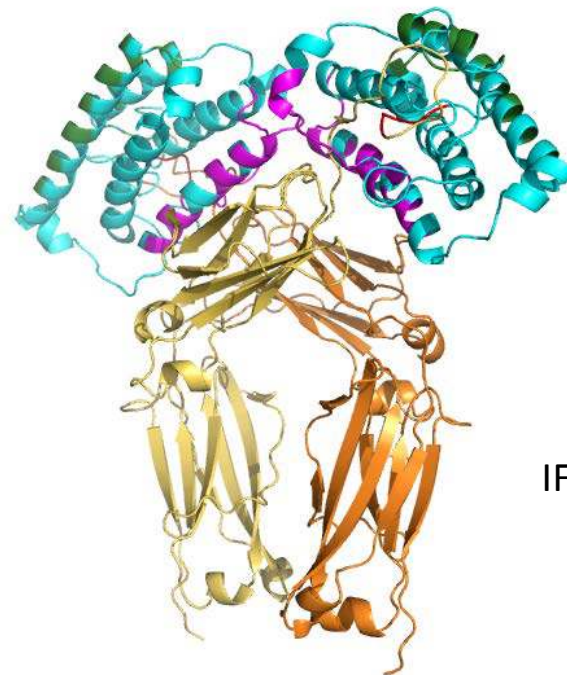
- Possible dimer structures were predicted by protein-protein docking with ClusPro2
- Molecules were aligned against dimeric Fc-region of IgG by PyMOL
- Binding sites for IFNAR1 and IFNAR2 receptors were defined



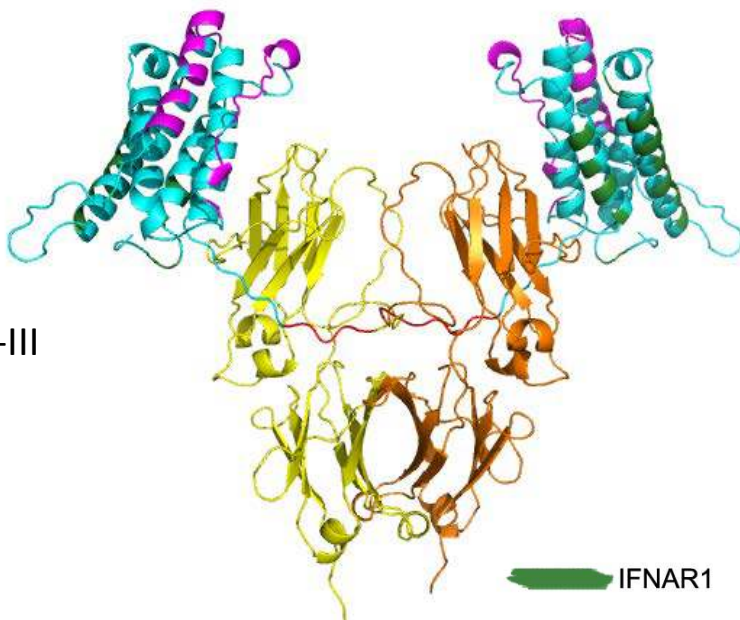
IFNa_{2b}-Fc-I



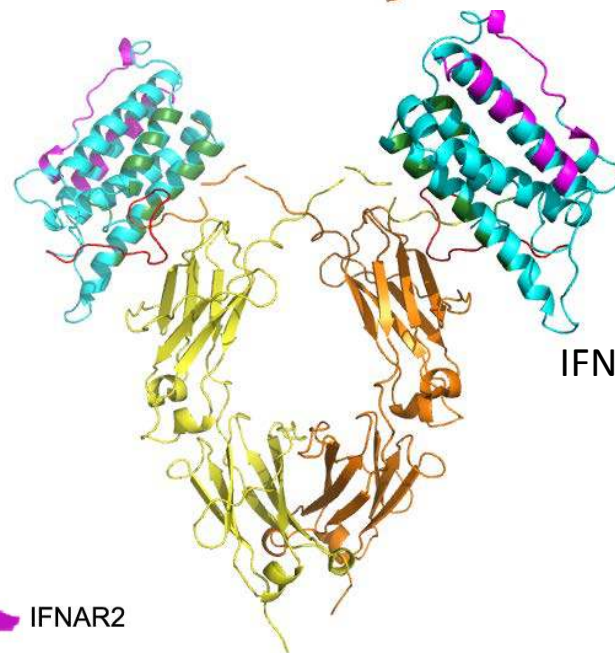
IFNa_{2b}-Fc-II



IFNa_{2b}-Fc-III

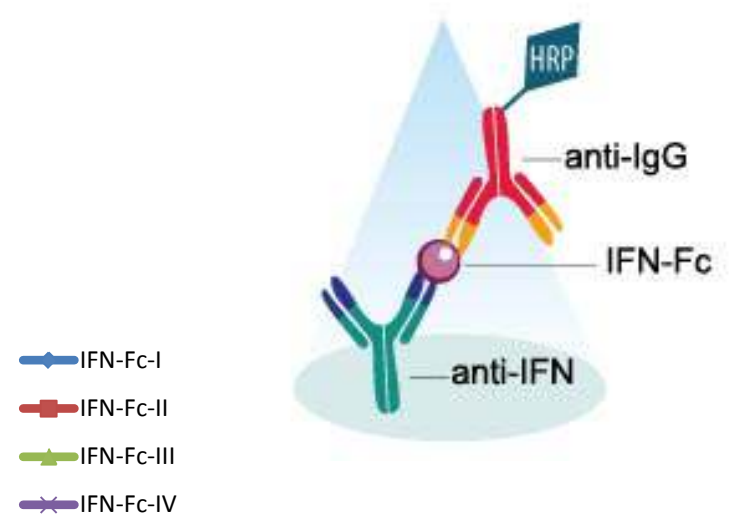
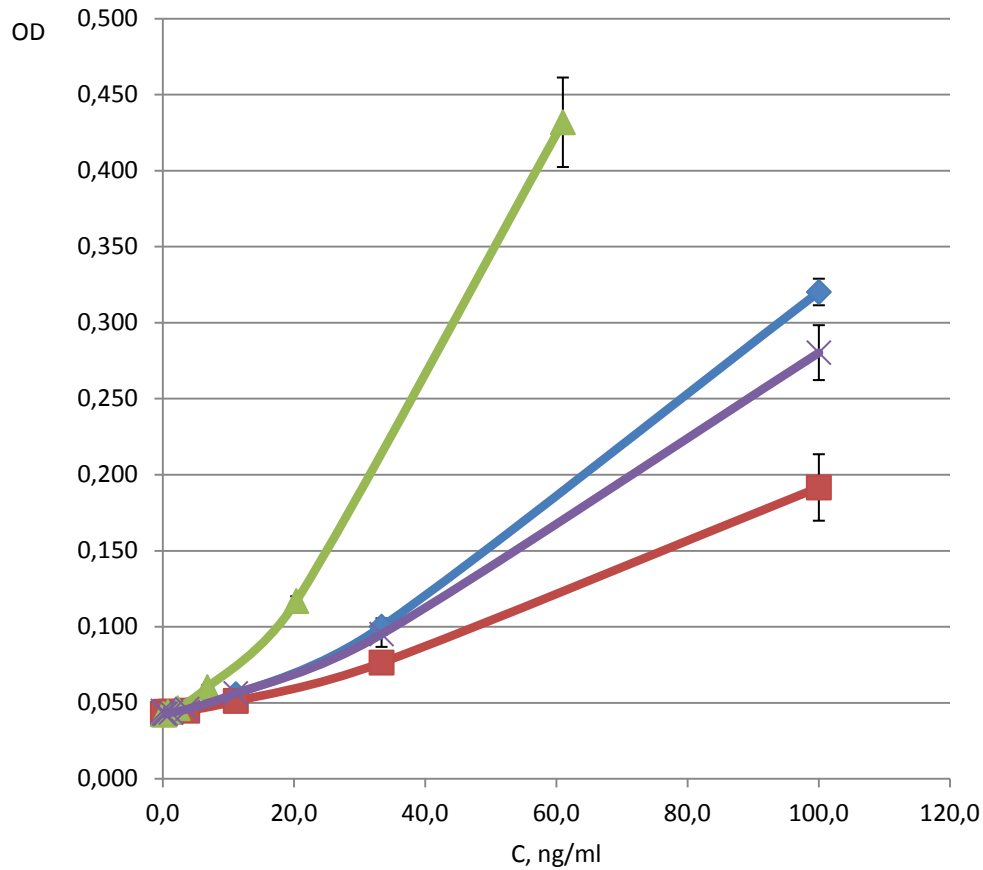


IFNa_{2b}-Fc-IV

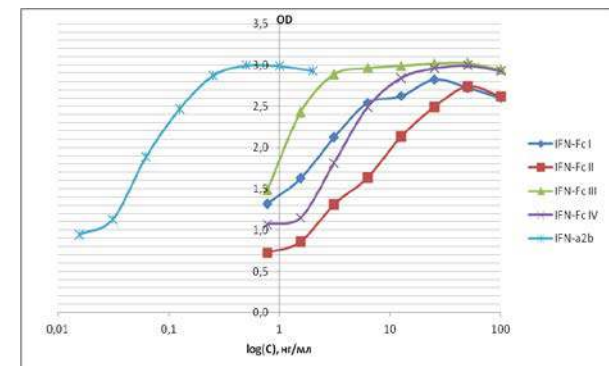


IFNAR1 IFNAR2

ELISA with neutralizing antibodies

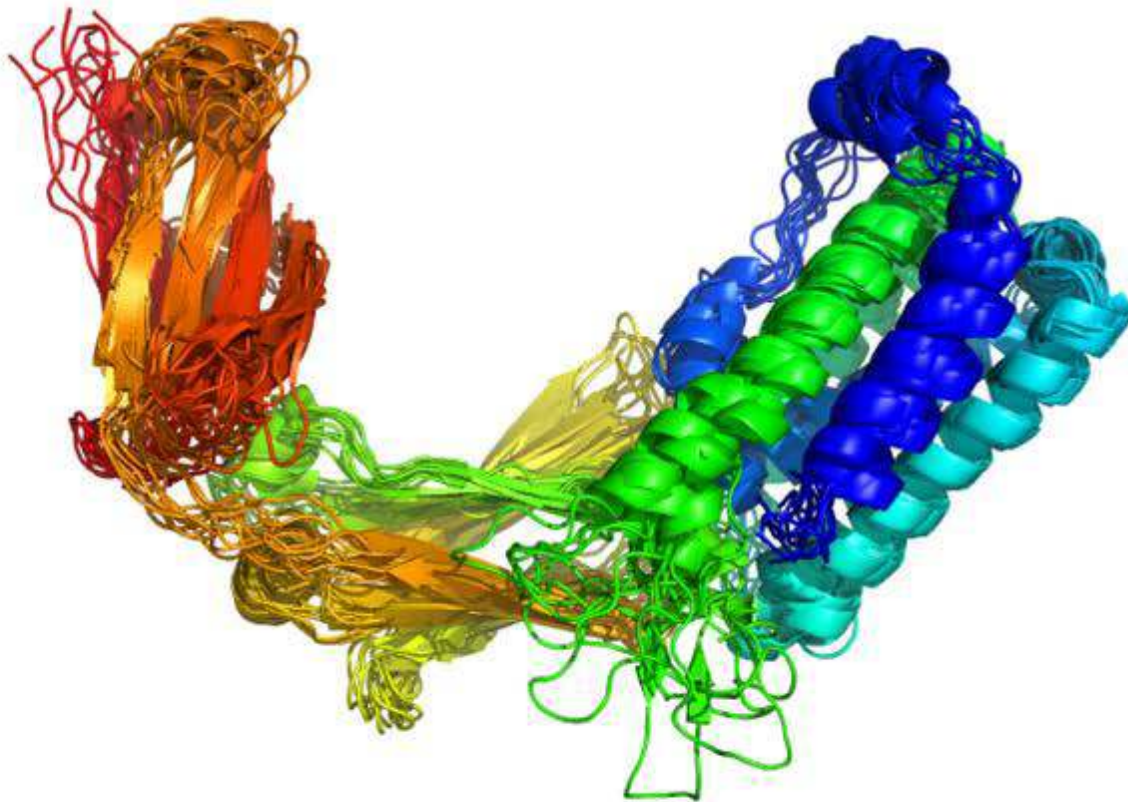


Antiviral activity

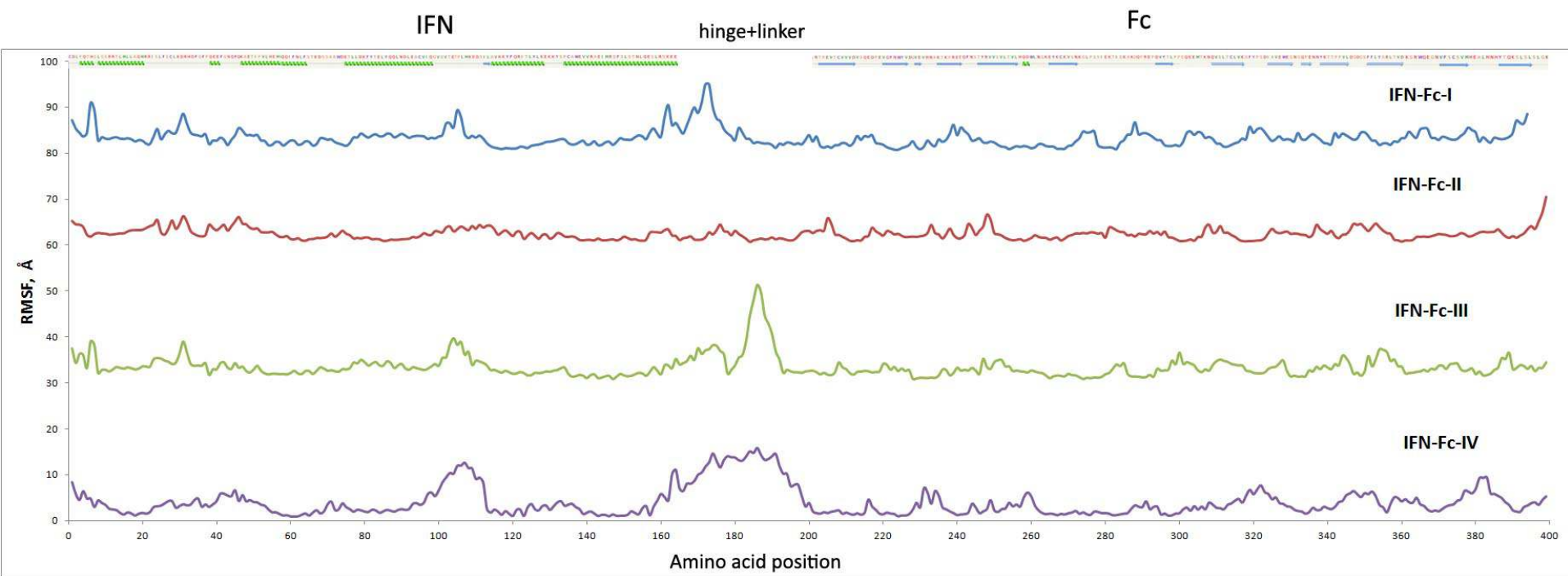


CABS-flex

Fast simulation of protein structure fluctuations



Amino acid fluctuation profile



Results

- Three-dimensional structure prediction can explain different activity of chimeric molecules IFN α 2b-Fc
- Different flexibility of linker region can affect biological activity

Molecular structure modeling -
useful tool for drug development!

