

Dynamic Modulation of Binding Affinity as a Mechanism to Regulate Interferon Signaling

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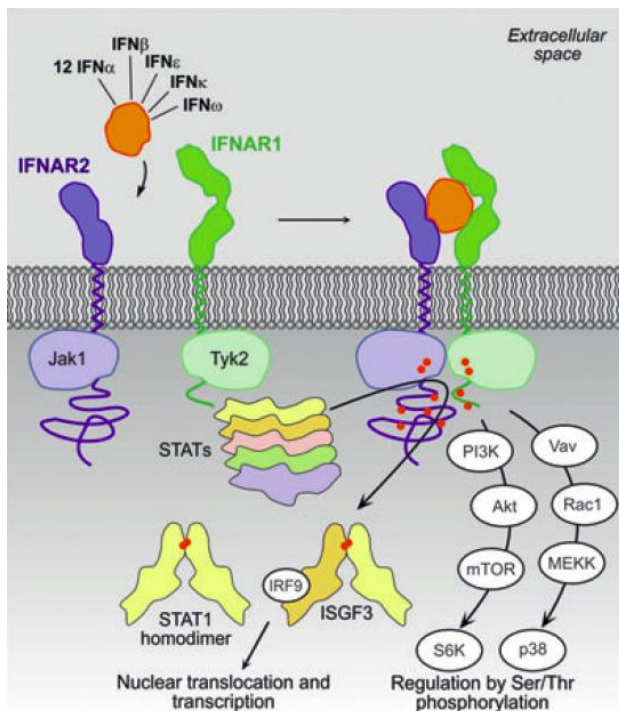
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Type I interferons (IFNs) and receptors (IFNAR1 and IFNAR2) and their signaling network

IFNs are members of the cytokine family mediating diverse biological responses: resistance to viral infections, modulation of cell survival, promotion of antitumor activities, regulation of immune response.



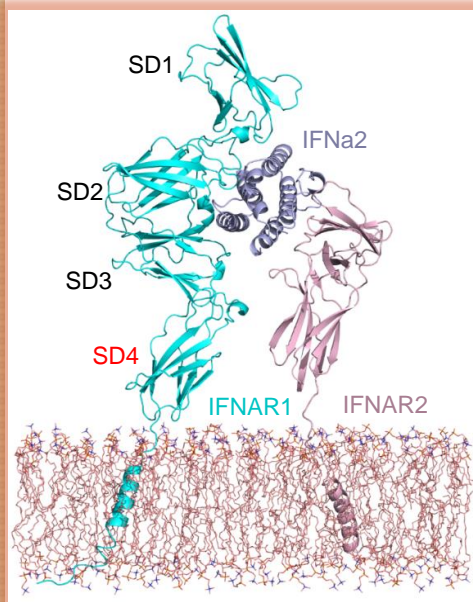
Weak, **transient binding** to a few receptors is sufficient to induce **robust activities** (such as antiviral).

Tight and **prolonged binding** to a large number of IFN receptors is required to induce the **tunable activities** (e.g., antiproliferative) .

The tyrosine kinase 2 (Tyk2) and Janus family kinases (Jak1) associated with IFNAR1 and IFNAR2, respectively, transphosphorylate each other, and phosphorylate-specific tyrosine residues of IFNAR1 and IFNAR2 (red dots).

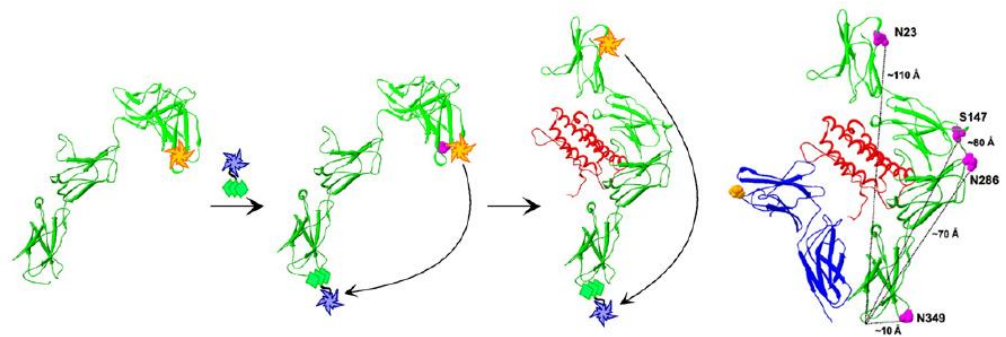
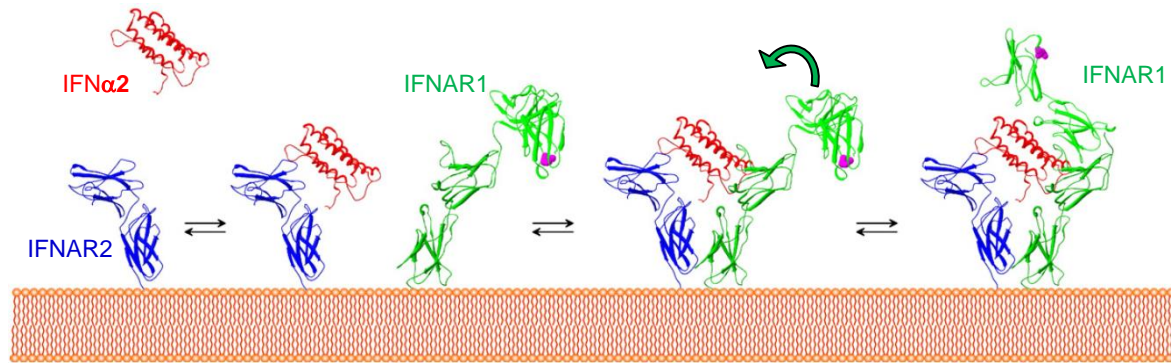
Upon phosphorylation, STAT1 and STAT2 form homo- and heterodimers, which translocate into the nucleus to activate transcription.

Ligand-binding induces conformational change in IFNAR1, which propagate to its membrane-proximal domain



Ternary complex of IFN α 2, IFNAR1 and IFNAR2 in the presence of the POPC lipid bilayer.

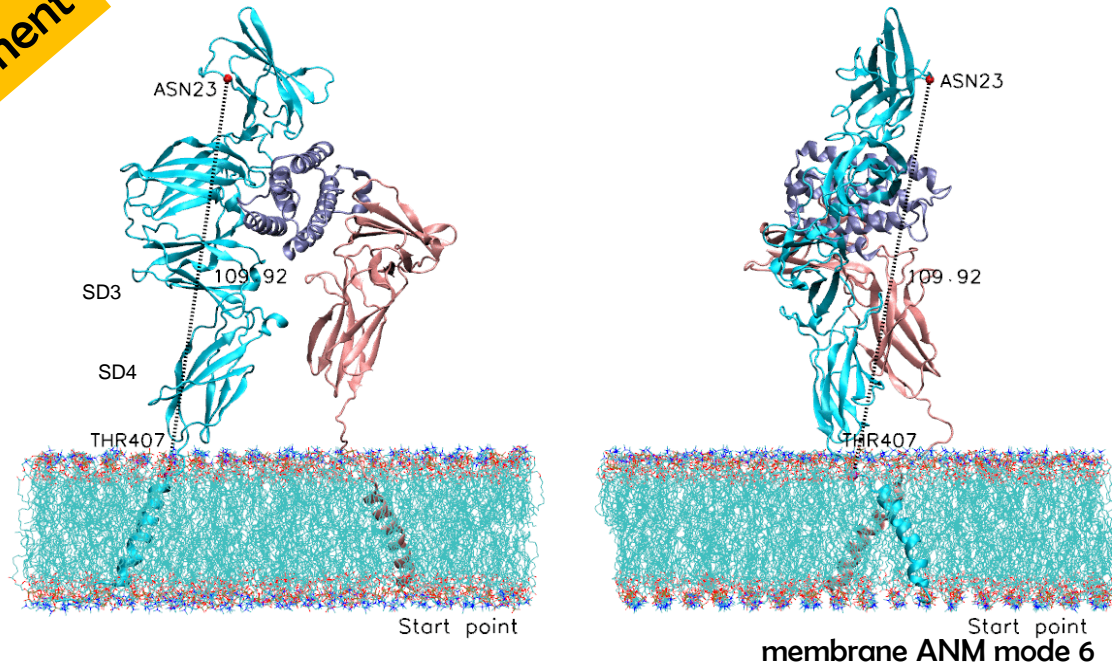
PDB 3SE3 + modelled IFNAR1-SD4 domain and two transmembrane (TM) helices on membrane.



Conformational change in IFNAR1 was observed by FRET, which showed an increase of ~ 12 Å in the distance between the C and N-terminal domains upon ligand binding.

Intrinsic ability of bound form IFNAR1 on membranes to adapt its conformation to functional interactions (observed in experiments)

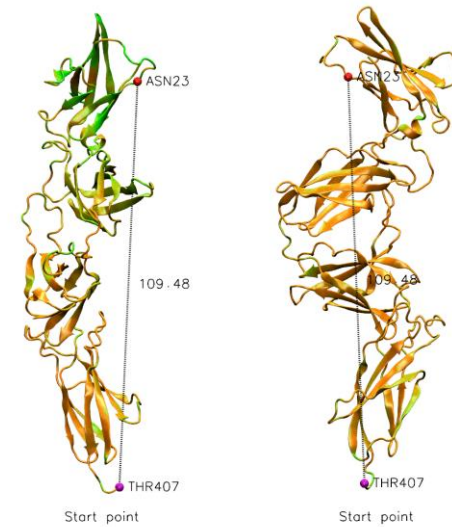
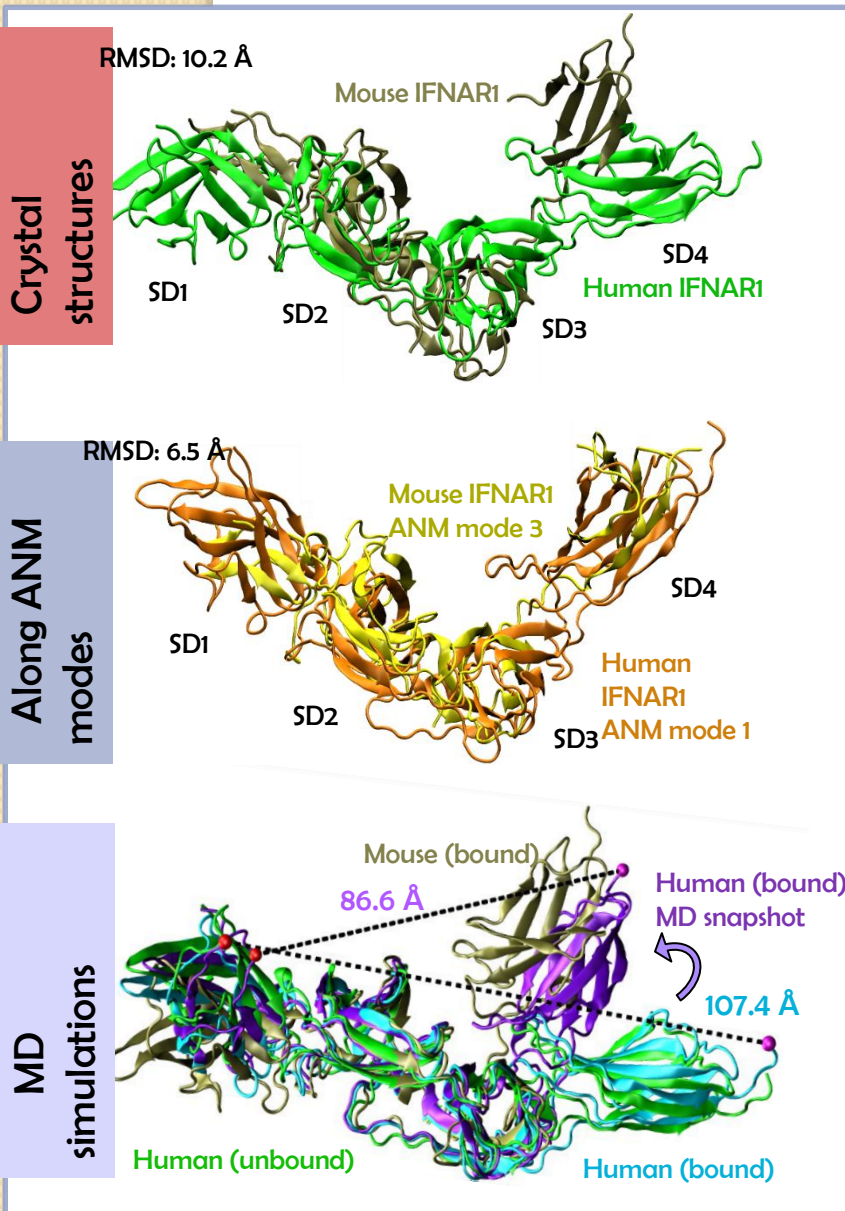
Methodological development



Coupled movements of the ternary complex and membrane

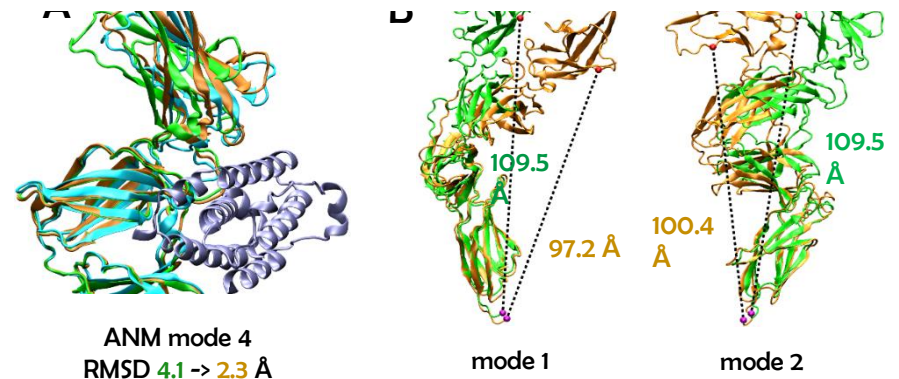
The SD3 and SD4 domains moved close to each other. The distance between residues N23 (*red sphere*) and T407 (*magenta sphere*) decreased by ~ 10 Å. IFNAR1, IFN α 2 and IFNAR2 in the ternary complex are colored *cyan*, *light blue* and *pink*, respectively.

IFNAR1 – Extreme Flexibility/Adaptability due to modularity

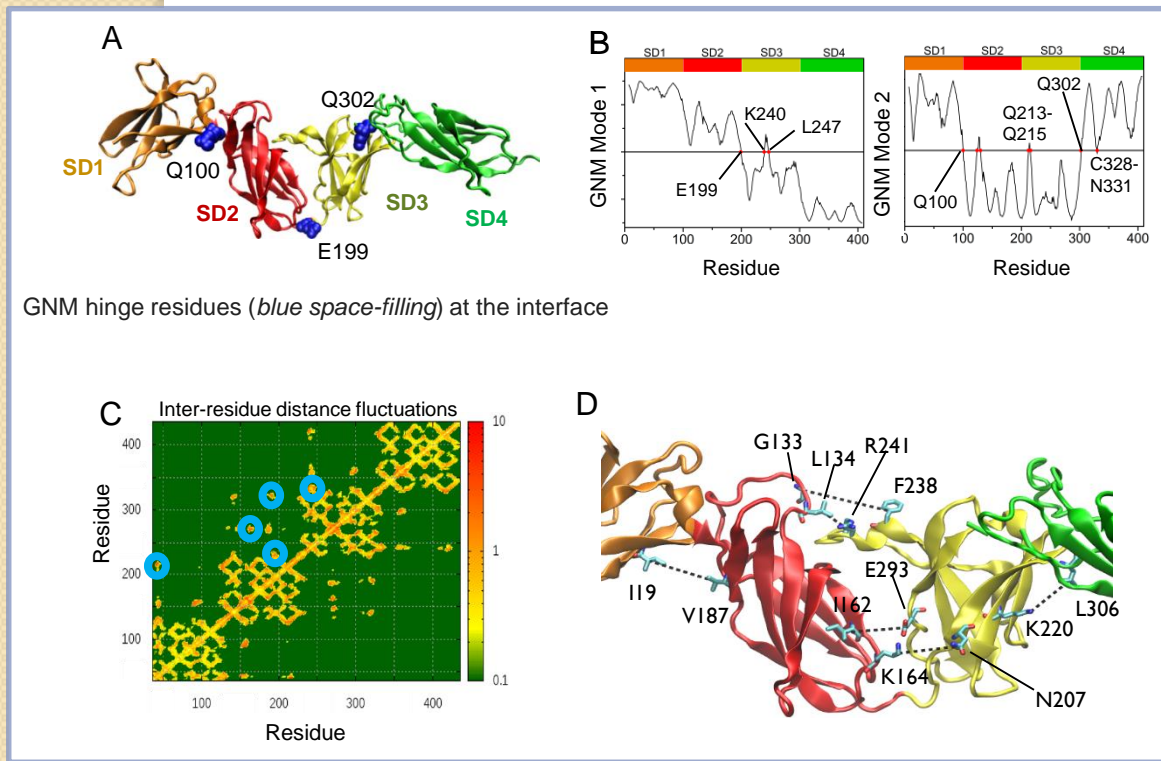


Human IFNAR1, ANM mode 1

Bound crystal structure / Unbound crystal structure / ANM-prediction, starting from unbound

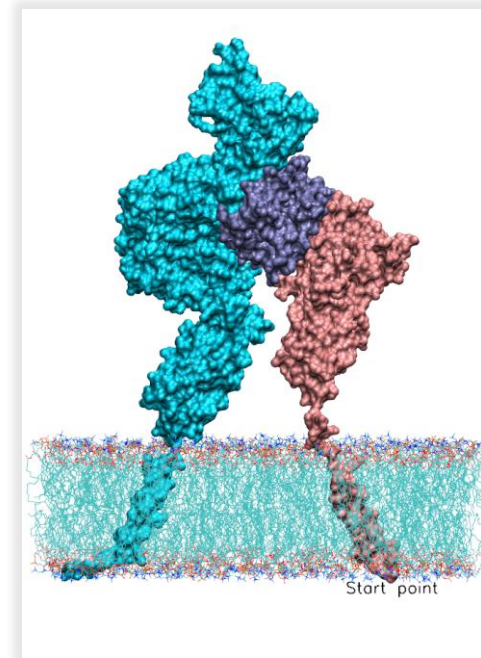


GNM-based identification of IFNAR1 hinge sites



GNM hinge residues (*blue space-filling*) at the interface

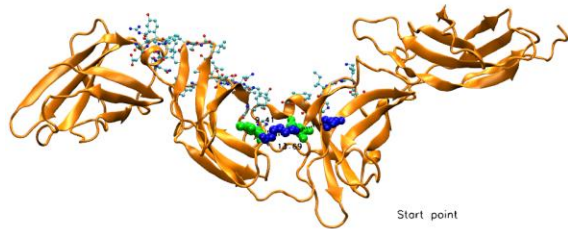
Blue circles highlight sequentially distant inter-subdomain interactions. They are subject to large ANM distance fluctuations that permit them to come into close proximity to form disulfide bridges even if in the equilibrium state they are not so close to each other, and they are located on opposite sides of a GNM hinge.



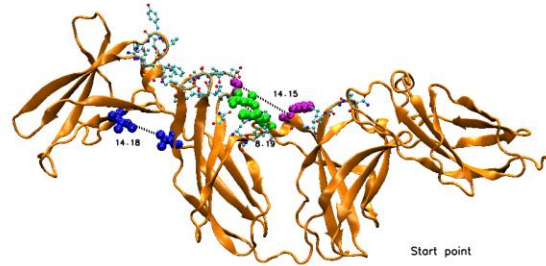
Membrane ANM mode 6

The structural mobility of IFNAR1 allows selected residues pairs to come into proximity and form disulfide bridges.

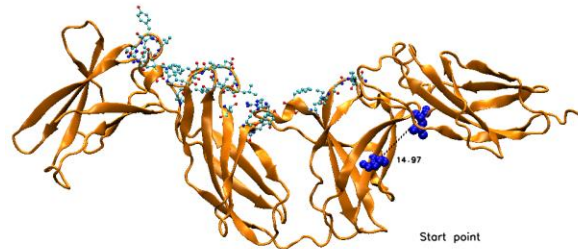
Mode 1 I162E293 (green) and K164N207 (blue)



Mode 4 G133F238 (purple), L134R241 (green) and I19V187 (blue)

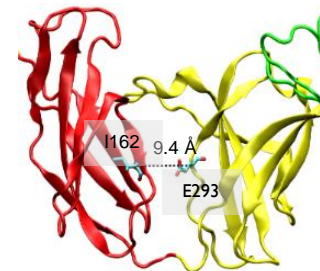
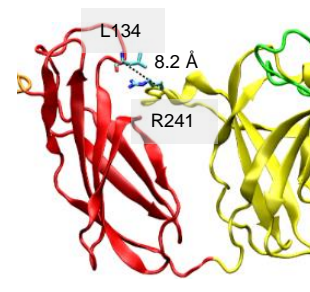
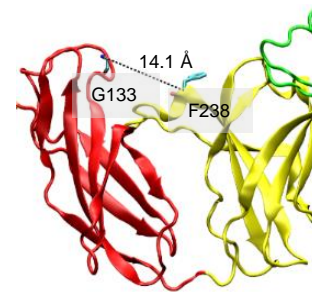


Mode 2 K220L306 (blue)

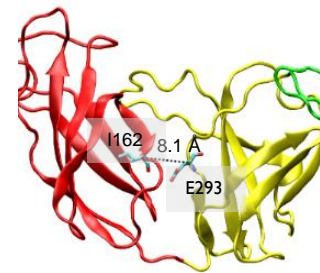
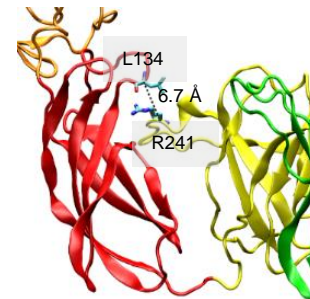
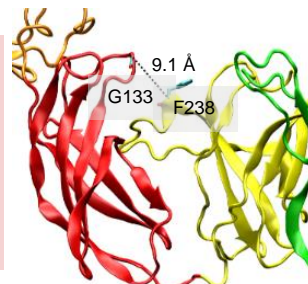


SD1 --> SD4

Crystal structures



Along ANM modes



Mode 4 G133-F238

Mode 4 L134-R241

Mode 1 I162-E293

Residue pairs located on opposite sides of GNM hinges selected for cross-linking experiments and functional assays

Set I – residue pairs predicted by ANM soft modes to alter global dynamics if cross-linked									
Subdo- mains	Res 1	Res 2	Dist ^a (Å)	ANM results					
				Mode	MaxVar (Å) ^b	Max d (Å)	Min d (Å)	E-Min d ^c (Å)	E2-Min d ^d (Å)
SD2-SD3	G133	F238	14.1	4	5.1	17.0	11.9	11.6	9.1
	L134	R241	8.2	4	2.1	9.4	7.3	7.4	6.7
	I162	E293	9.4	1	2.7	10.8	8.1	8.3	8.1
	K164	N207	13.7	1	4.5	16.1	11.6	8.9	8.4
SD3-SD4	K220	L306	14.9	2	4.6	17.4	12.9	14.5	12.6
SD1-SD2	I19	V187	14.2	4	5.2	17.1	11.9	10.7	9.7
Set II – pairs located in the vicinity of hinge centers									
SD2-SD3	Y163	E293	7.6	2	0.7	8.2	7.5	6.0	6.2
	E111	F290	7.5	1	1.3	8.2	6.9	5.8	5.6
SD1-SD2	D15	T123	6.5	2	0.3	6.7	6.4	5.9	6.0
	D16	V127	6.4	4	0.2	6.6	6.4	6.5	6.6
SD3-SD4	C268	C328	6.8	4	0.4	7.1	6.7	8.2	8.3

^a Distances between the two residues (based on C α atoms) in the human IFNAR1 crystal structure, unbound form (PDB id: 3s98) with modeled SD4

^b MaxVar is the difference between the maximal and minimal distances (Max d and Min d) between the two residues in the examined mode (based on C α atoms) using the scaling parameter $\alpha = 27$;

^c E-Min d is the value for Min d after energy minimization;

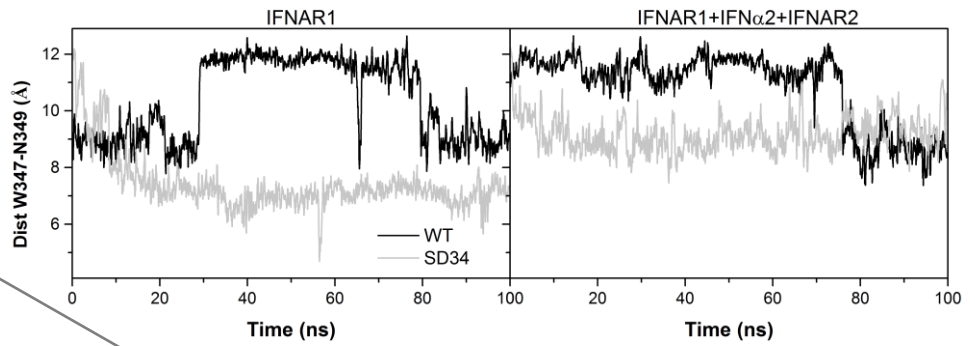
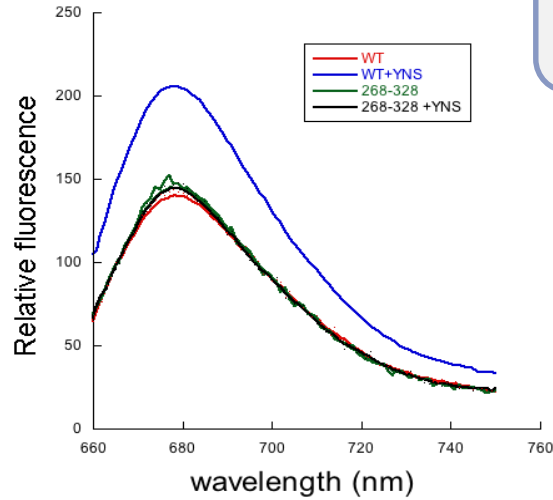
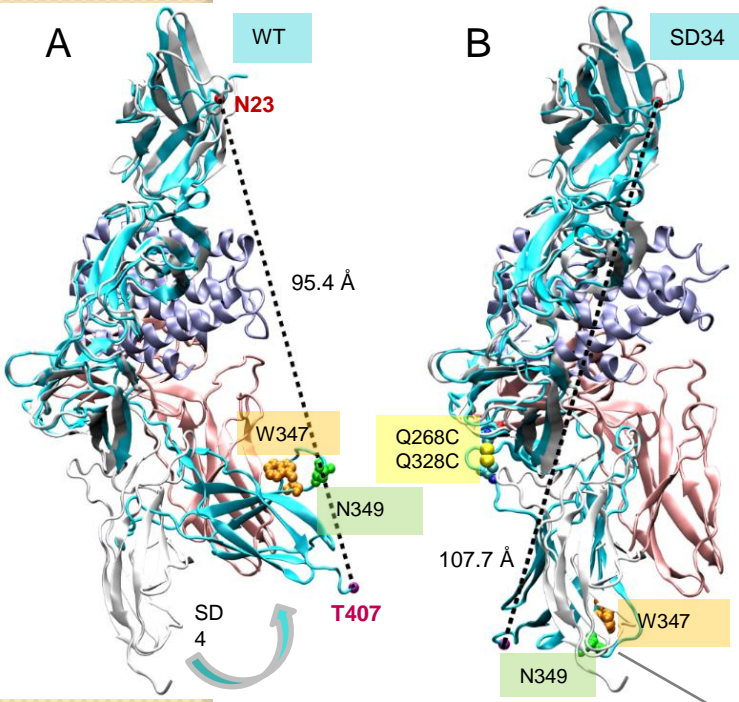
^d E2-Min d is the closest separation attained after a 2nd round of displacement along the same modes and energy minimization.

Disulfide bridge formation between 268-328 (SD34) and its restrictive effect on the dynamics of IFNAR1 SD4.

The 268-328 disulfide bond hinders de-quenching of the fluorescence of Trp 347 upon IFN binding, suggesting inhibition of SD4 movement upon IFN binding.



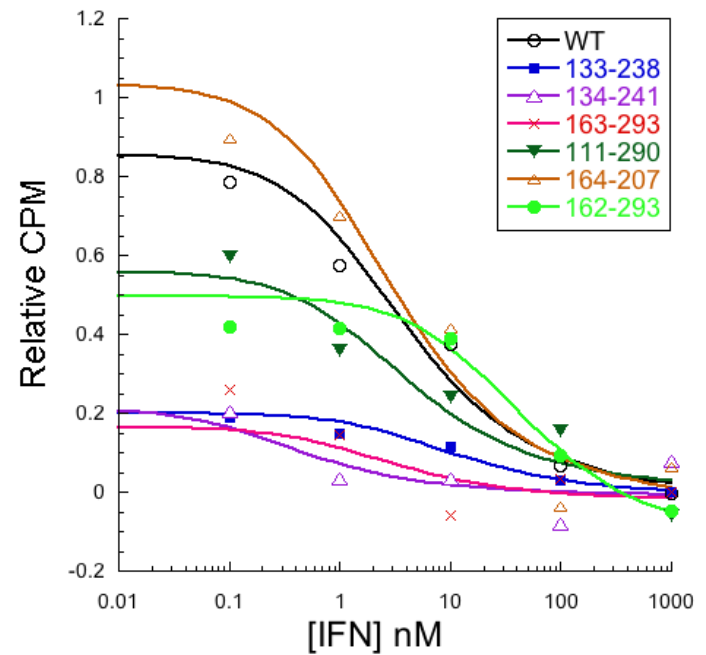
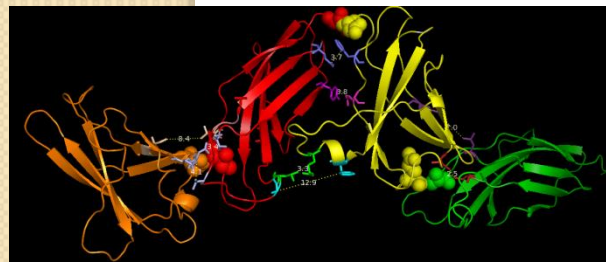
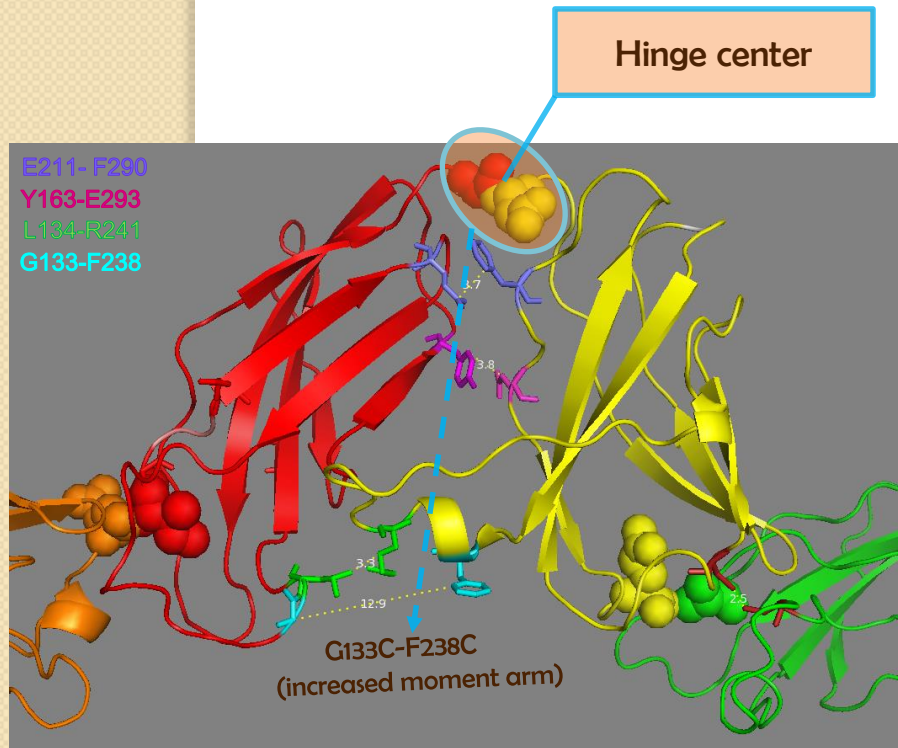
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The distances (W347-N349) in SD34 (gray) are in low level (strong quenching effects), compared to the WT (black).

SD4 movement is severely restricted in the double mutant SD34 (B), compared to the WT (A).

White : initial IFNAR1 conformation
Cyan : MD sampled IFNAR1 conformation



The cross-links (G133-F238 and L134-R241) with increasing distance from the global hinge center have stronger effects to the binding, due to **increased moment arm**.

Conclusion:

Dynamics modulates binding affinity, which in turn, modulates biological activity.

Acknowledgments

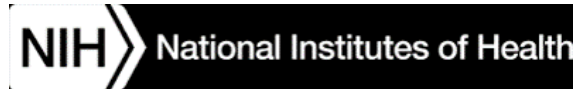


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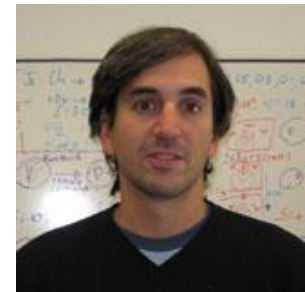
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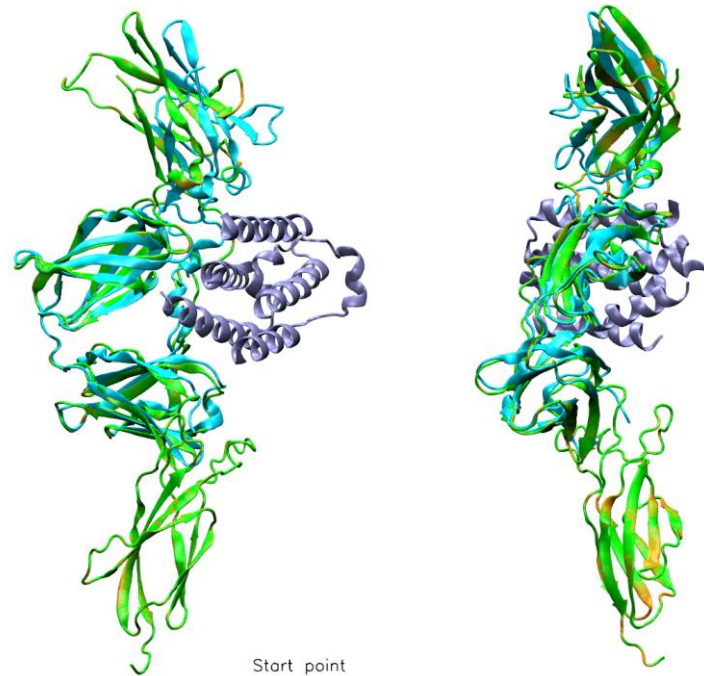
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Intrinsic ability of IFNAR1 to adapt its conformation to functional interactions (observed in experiments) upon movements along its structure-encoded global modes.



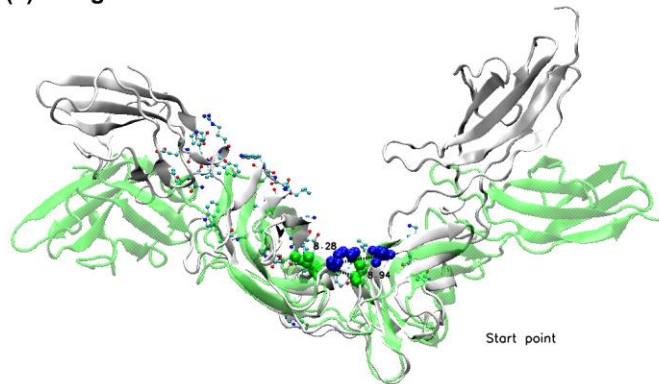
ANM Mode 4

The change observed experimentally between the unbound (*green*) and bound (*cyan*) structures of IFNAR1 agrees with the changes intrinsically favored by ANM.

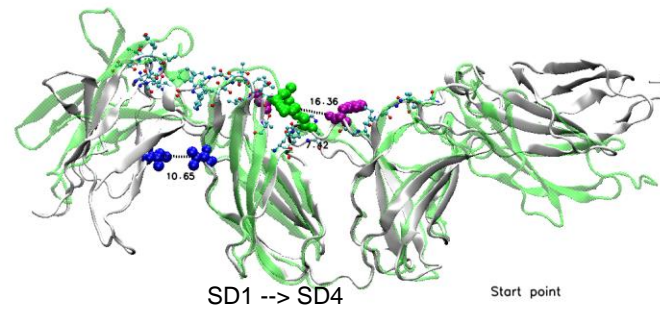
The diagram colored *orange* is obtained by starting from the unbound form (*green*) and deforming it along its intrinsically accessible ANM mode 4.

Enhanced Movements along the global ANM modes of IFNAR1 structures from human.

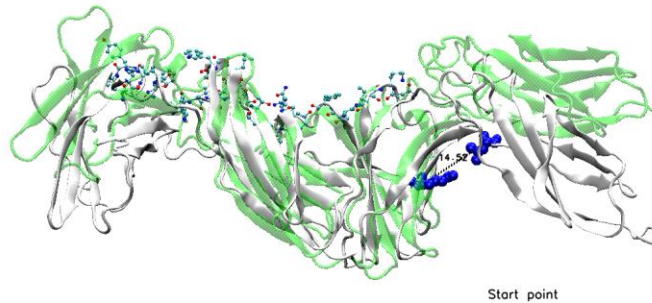
(a) 2nd generation Mode 1



(c) 2nd generation Mode 4



(b) 2nd generation Mode 2



2nd generation ANM:

green: crystal structure of human IFNAR1

yellow: 1st generation

gray: 2nd generation ANM