INSTITUTE OF
INFORMATION AND COMMUNICATION
TECHNOLOGIES

INSTITUTE OF MATHEMATICS AND INFORMATICS

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IN SILICO STUDY OF THE MOLECULAR MECHANISM OF LMWH ANTI-INFLAMMATORY ACTION WITHIN THE COVID-19 CONTEXT

In collaboration with

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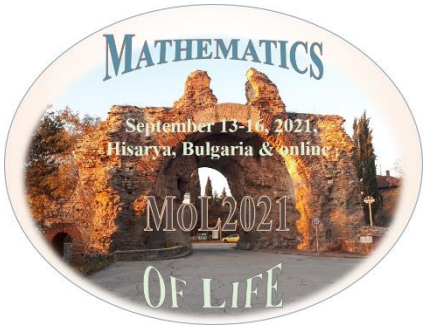
Bulgarian Academy of Sciences

IBER

N. Todorova



MATHEMATICS OF LIFE (MoL 2021)

September 13-16, 2021, Hisarya, Bulgaria & online

The COVID-19 Pandemic

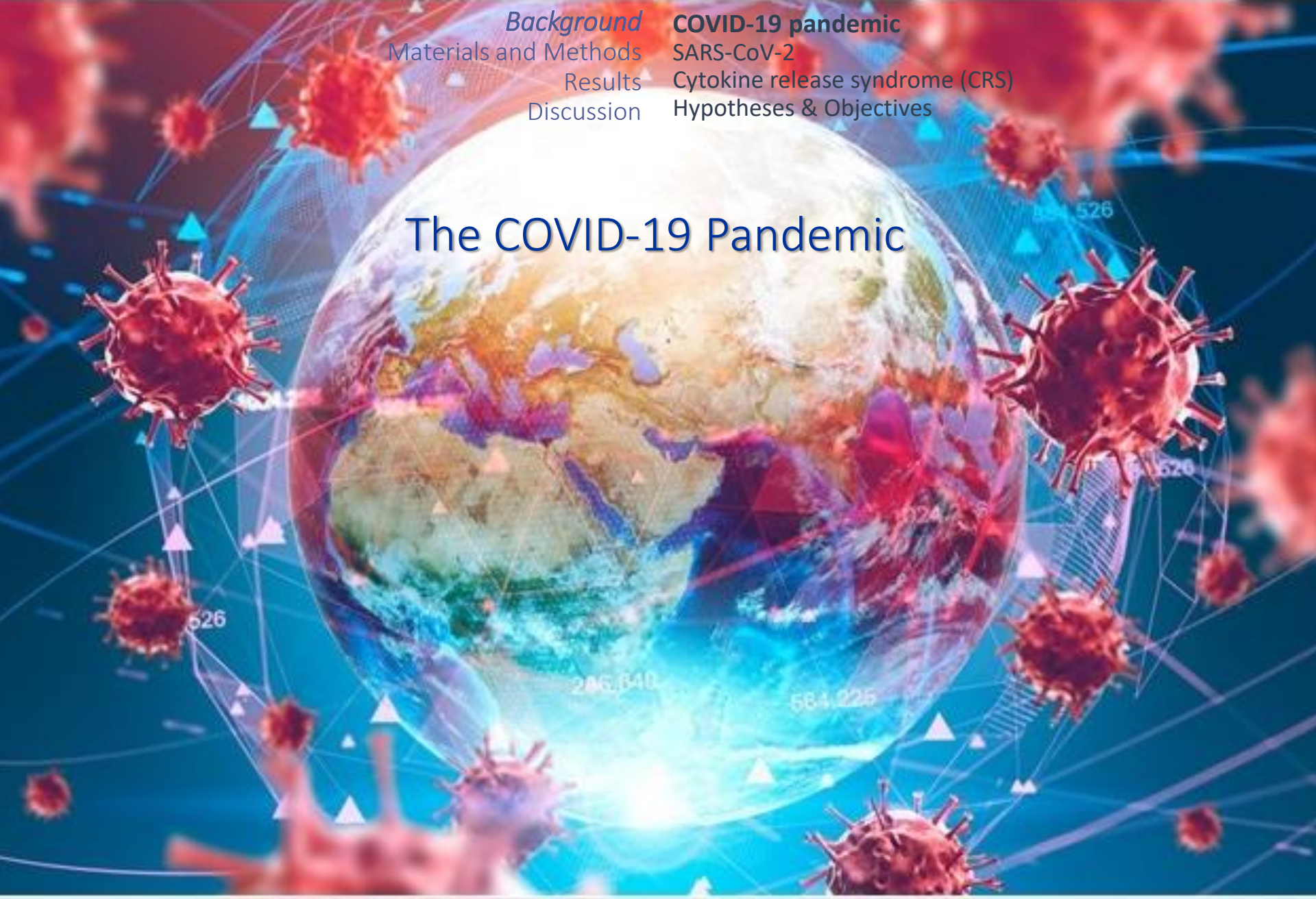
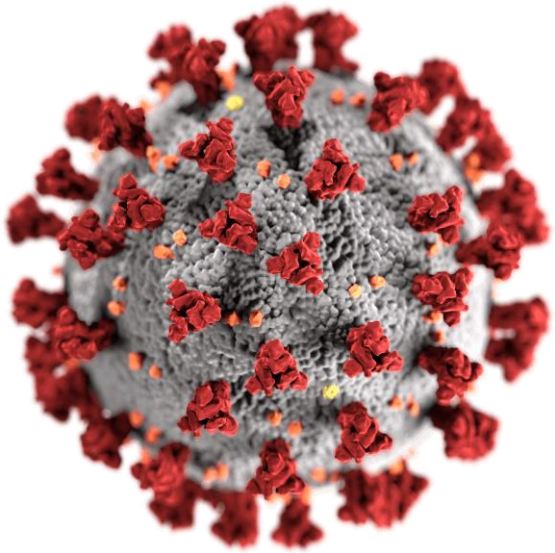


Image Credit: ImageFlow / Shutterstock

SARS-CoV-2

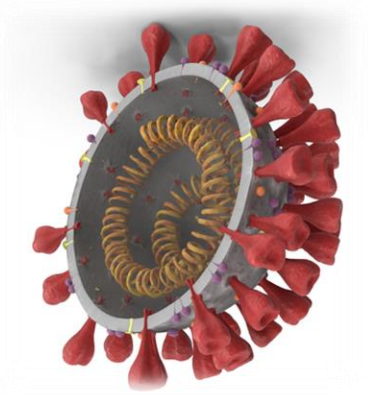


7th known "human" coronavirus
SARS-CoV [2002-2004; 11% CFR]
MERS-CoV [2012; ~34% CFR]
SARS-CoV-2 polybasic cleavage site

β-coronavirus
~ 30 000 bases
12 open reading frames (ORFs)
29 encoded proteins
29 viral proteins interact with 332 host-cell proteins

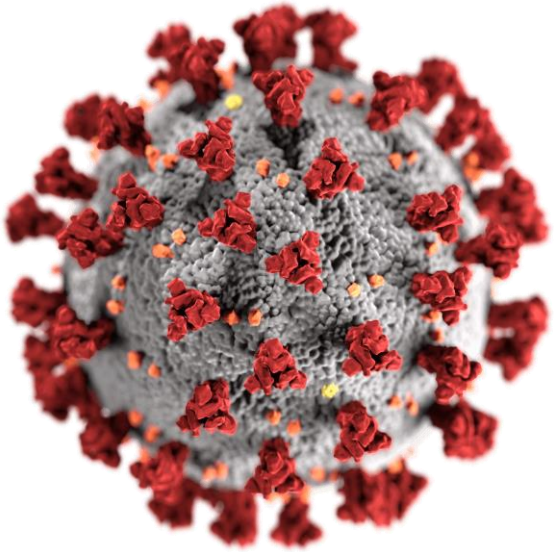
Virions measures
~ 100 nm in diameter
~ 10^3 Mda ≈ 1.6 fg

10 hours needed to burst ~ 1000 virions



https://phil.cdc.gov/Details.aspx?pid=23312
Alissa Eckert, MS & Dan Higgins, MAMS

SARS-CoV-2



A comprehensive understanding of how the virus **hijacks** the host and **inactivates its immune response** at the initial stage, how this relates to **the delayed (over)reaction of the immune system** and **how this overreaction can be tamed** is indispensable for

- devising **therapeutic strategies** to counteract SARS-CoV-2 infection
- developing **new drugs**, or
- **repurposing** existing ones

https://phil.cdc.gov/Details.aspx?pid=23312
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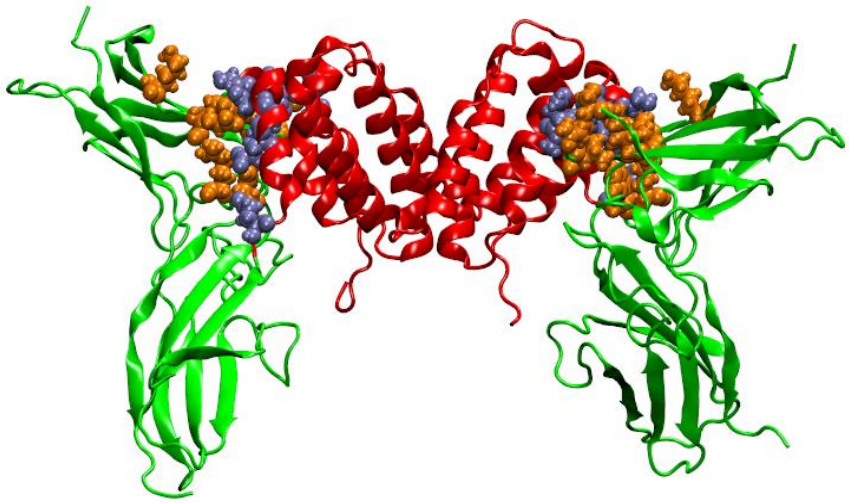
Cytokine release syndrome (CRS)

COVID-19 phases	Immune response
Prolonged incubation period (4-14 days): SARS-CoV-2 may have developed countermeasures against the immune system	
Non-severe stages	Specific innate immune response Adaptive immune response: Before the peak of the viral load
Acute phase: ARDS, CRS	IFN-stimulated genes with pro-inflammatory activity ***Proinflammatory cytokines***

Timely control of the cytokine storm in its early stage through immunomodulators and cytokine antagonists

Interferon-gamma (IFNγ)

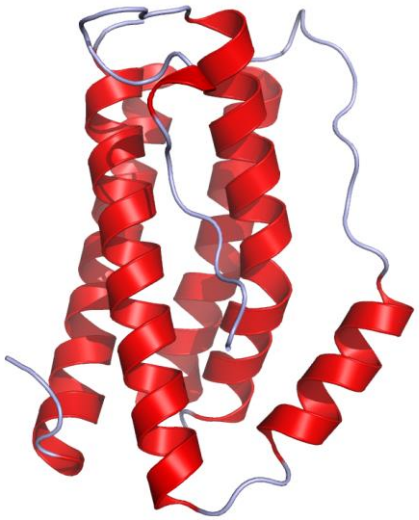


- Pleiotropic cytokine
- 2 x 143 aa; 25 kDa
- Key role in immune signaling and modulation of the innate and adaptive immune response
- Overexpression associated with certain autoimmune diseases

- **Inhibition of superfluous IFNγ / IFNγ signaling pathway:**
  - "blocking" the receptor via inactive mutated forms
  - blocking the cytokine binding sites

L. Litov et al. *A new approach to cope with autoimmune diseases: computer simulations and laboratory tests.* Radiotherapy and Oncology 102 (Suppl. 1) (2012) S134-S135

Interleukin 6 (IL-6)



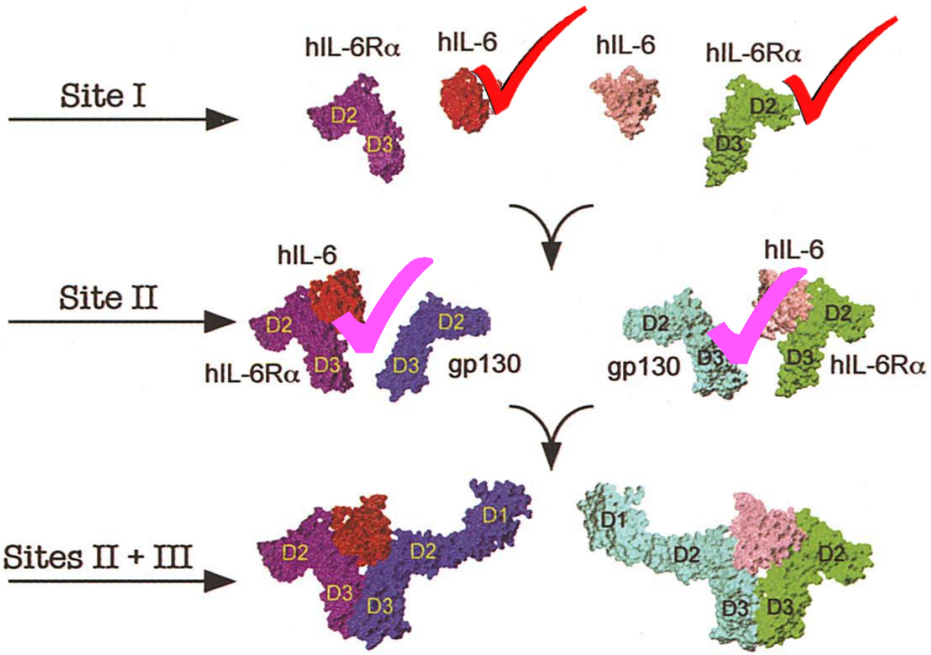
- Member of the IL-6 family of cytokines
- 212 aa; 23.7 kDa
- Secreted by many cell types during infection, inflammation or cancer
- Very low levels under normal conditions

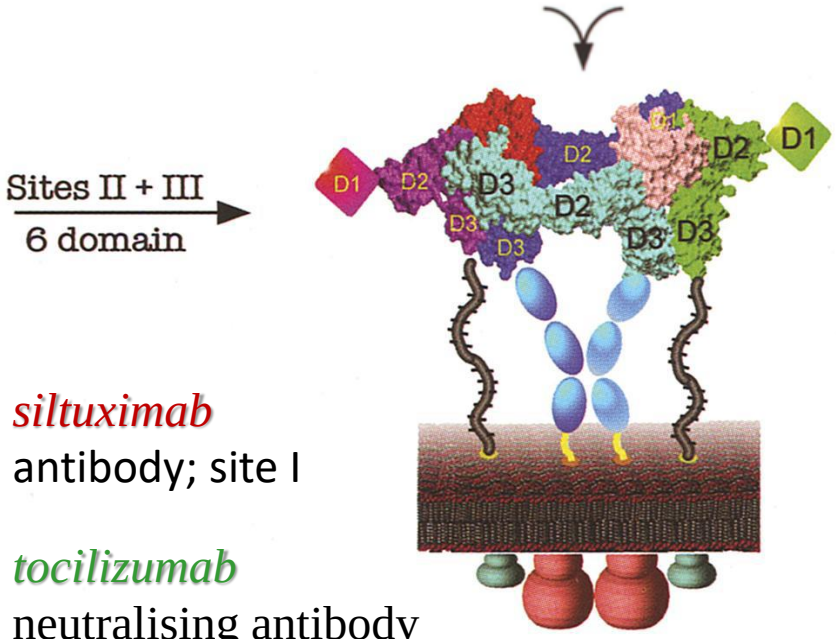
  -> biomarker
- Modulates the balance between humoral and cell-based immune responses
- Involved in B- and T-cells regulation
- Inhibition of IL-6 signaling:

  therapeutic potential in cancer, autoimmune diseases, infections and Covid-19

Interleukin 6 (IL-6) signaling

IL-6 → IL-6/IL-6R → IL-6/IL-6R/gp130





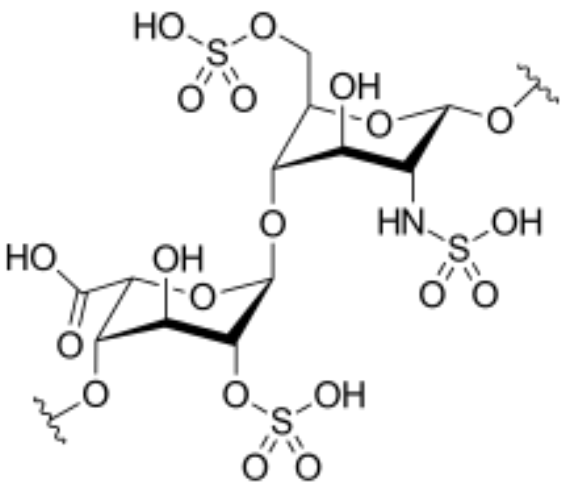
siltuximab
antibody; site I

tocilizumab
neutralising antibody

Side effects, increased risk of infections, lack of specificity

Heparin

Feasible alternative: GAGs, e.g. LMWH



Heparin (UFH)

- Polymer (polysaccharide), 3 to 30 kDa
- Variably sulfated repeating disaccharide unit
- High-density negative charge (x -2)
- High biological activity:
  binds to >700 proteins

Low molecular weight heparin (LMWH)

- >60% short chains < 8 kDa
- More predictable pharmacokinetics

Objective & Hypotheses

Understand the molecular mechanism of the antiinflammatory action of heparin

Heparin binding impairs the formation of the two biologically active complexes (IFNγ / IFNγRα and IL-6 / IL-6Rα / gp130) and by this – the activation of the respective signaling pathways

The *In Silico* Experiments

- Reconstruction of the full-length IFNγhomodimer
- Simulation of heparin/IFNγ complex
- Equilibration of the X-ray structure IL-6/IL-6Rα/gp130
- Identification of contact areas and binding sites
- Simulation of heparin/IL-6 binding
- Simulation of (IL-6/IL-6R)+heparin
- Complex-structure analysis
- Visualisation

Experimental evidence:
heparin binds to IFNγ, IL-6 and to the complex IL-6/IL-6Rα

Mummery, R.S. and Rider, C.C. The Journal of Immunology, 165/10 (2000)5671-5679

Molecular Dynamics

Simulation protocol:

- GROMACS 5.0.7
- explicit solvent
- time step 2 fs; Leap-frog algorithm
- rectangular box with –d 2.0 nm
- all cutoff radii 0.9 nm
- PME electrostatics: 1.2 nm
- T-control: Berendsen (in PR)

v-rescale, T = 310K and τ_t = 0.1ps

- P-control: Berendsen (in PR)

Parrinello-Rahman, τ_p = 0.5ps

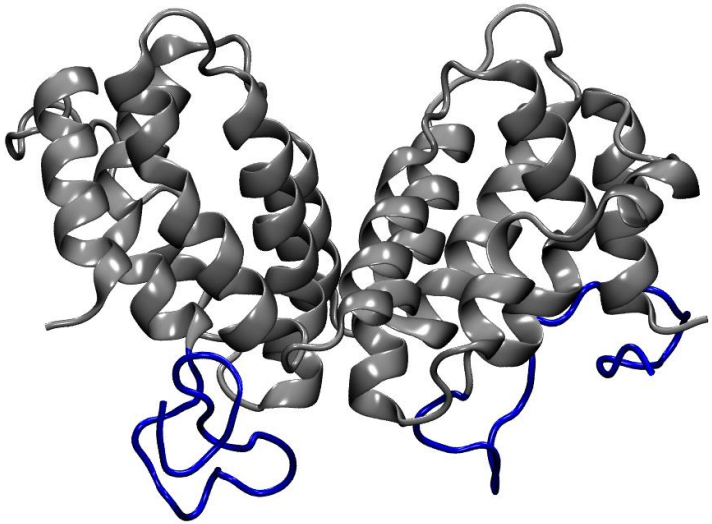
GROMACS
FAST. FLEXIBLE. FREE.

- IFNγ/LMWH 500 ns +1.5 μs
- IL-6 150 ns
- IL-6/LMWH 250 ns
- IL-6/IL-6Rα/LMWH 250 ns
- IL-6/IL-6Rα/LMWH + Mg^{2+} 500 ns

M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess, E. Lindahl, GROMACS: *High performance molecular simulations through multi-level parallelism from laptops to supercomputers,* SoftwareX 1 (2015) pp. 19-25

Structure modelling

Interferon-gamma (IFNγ)



- Protein Data Bank: PDB ID 1FG9
- Missing C-termini reconstructed (the centroid of the largest cluster after ρ-fitting of three independent 500 ns folding trajectories from the largest-cluster centroid of an initial 200 ns folding trajectory)

D.J. Thiel DJ, et al. Structure **8** (2000) 927-936
P. Petkov, E. Lilkova, N. Ilieva, ….. In: Lecture Notes in Computer Science, Vol. **10655** (2018) 544-551
E. Lilkova, P. Petkov, N. Ilieva, ….. Journal of Molecular Modeling **25** (2019) 127

Structure modelling

Interleukin 6 (IL-6)

- Protein Data Bank: **PDB ID 1ALU**
- Missing residues reconstructed with loop-modelling interface of CHIMERA
- Structure parameterized with the CHARMM36m force field
- Solvation, neutralization, equilibration
- 10 ns production MD to get the initial structure

IL6 / IL6-Rα / gp130 complex

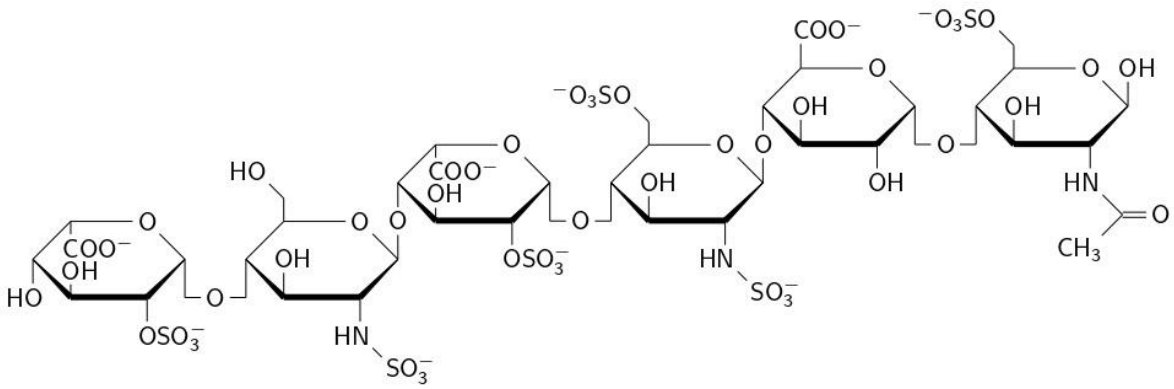
- Protein Data Bank: **PDB ID 1P9M**
- Structure parameterized with the CHARMM36m force field
- Solvation, neutralization, equilibration
- 10 ns production MD to get the initial structure

H.M. Berman et al. The Protein Data Bank. Nucleic Acids Research, **28** (2000) 235
E.F. Pettersen et al. UCSF Chimera (…) J. Comput. Chem. **25**/13 (2004) 1605
Z. Yang et al. UCSF Chimera: MODELLER, and IMP (…) J. Struct. Biol. **179** (2012) 269

Structure modelling

GAG structure: hexasaccharide as a general LMWH

- Literature-based hexasaccharide sequence; net charge -9e
- 3D structure generation with Glycan Reader & Modeler module of the CHARMM-GUI server
- Structure parameterized with CHARMM36m carbohydrate force field
- GROMACS-compatible topology with the parmed module of Ambertools 16



Structure modelling

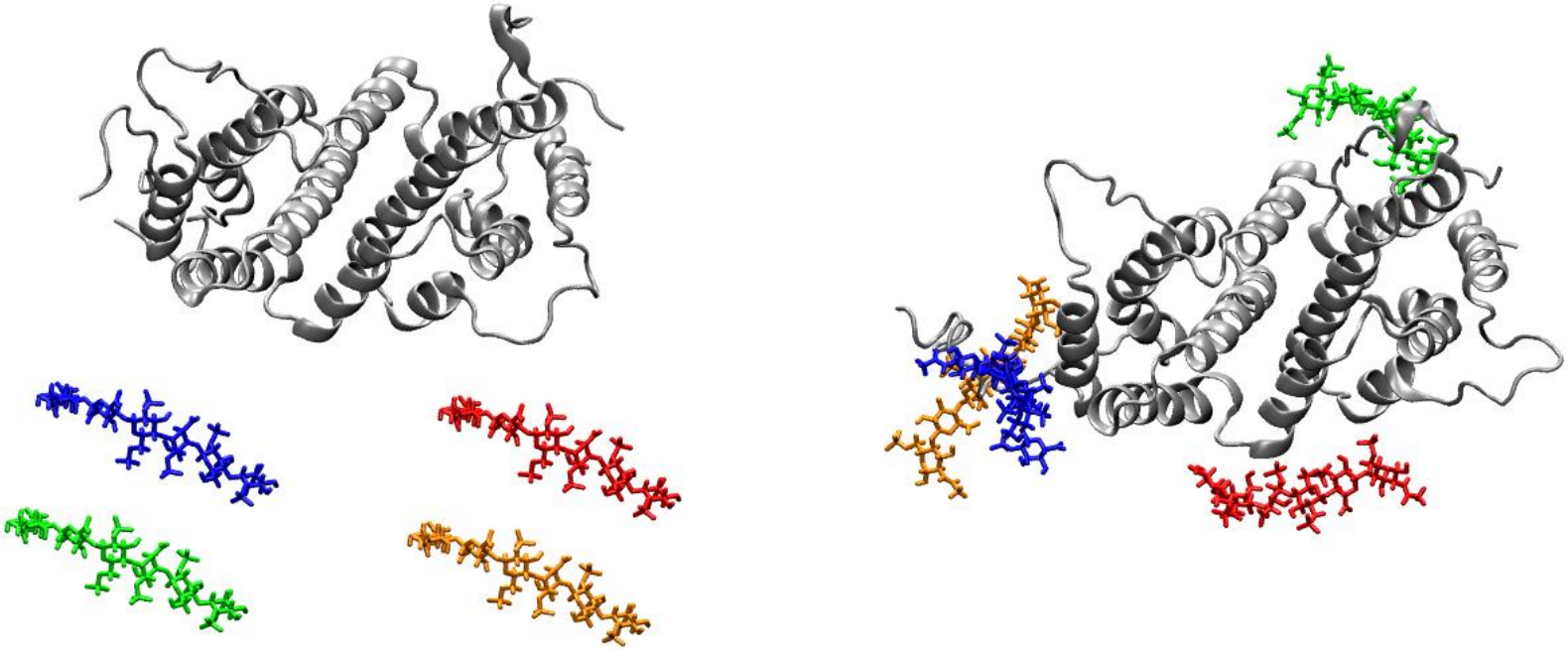
α-L-IdoA(2S) (1→4) β-D-GlcNS (1→4) α-L-IdoA(2S) (1→4) β-D-GlcNS(6S) (1→4) β-D-GlcA(1→4) β-D-GlcNAc(6s).

GAG/protein complex

- Molecular Operating Environment (MOE): GAG molecules docked in IL6 and IL6-IL6Rα complex based on the SAS structure

K. Mazák et al. Carbohydrate Research **384** (2014) 13–19
S-J. Park et al. CHARMM-GUI Glycan Modeler (…). Glycobiology, **29** (2019) 320–331
S. Jo et al. CHARMM-GUI (…). J. Comput. Chem. 29 (2008) 1859-1865
(MOE) https://www.chemcomp.com/Products.htm

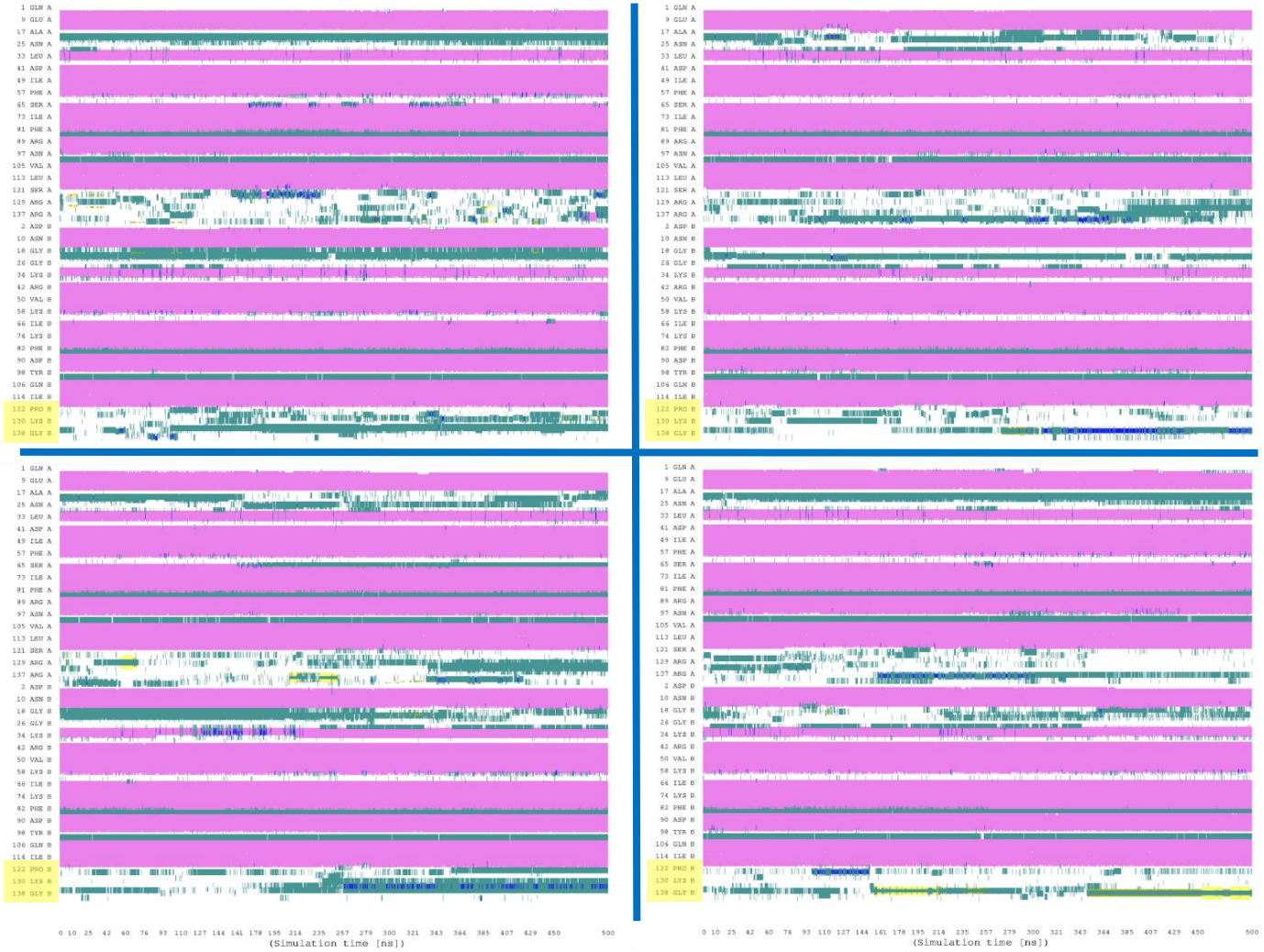
IFNγ/heparin complex



Initial and final conformations: IFNγ & four hexasaccharides (a representative LMWH structure) after 500 ns MD simulation

E. Lilkova, N. Ilieva, P. Petkov, M. Rangelov, and L. Litov, AIP Conference Proceedings **2302** (2020) 020003

IFNγ/heparin complex

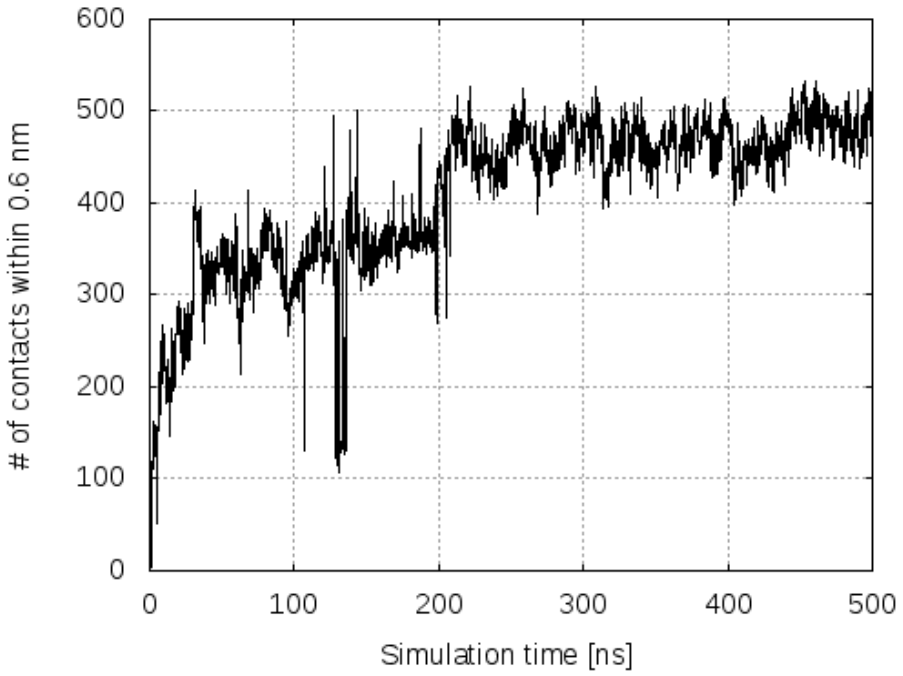


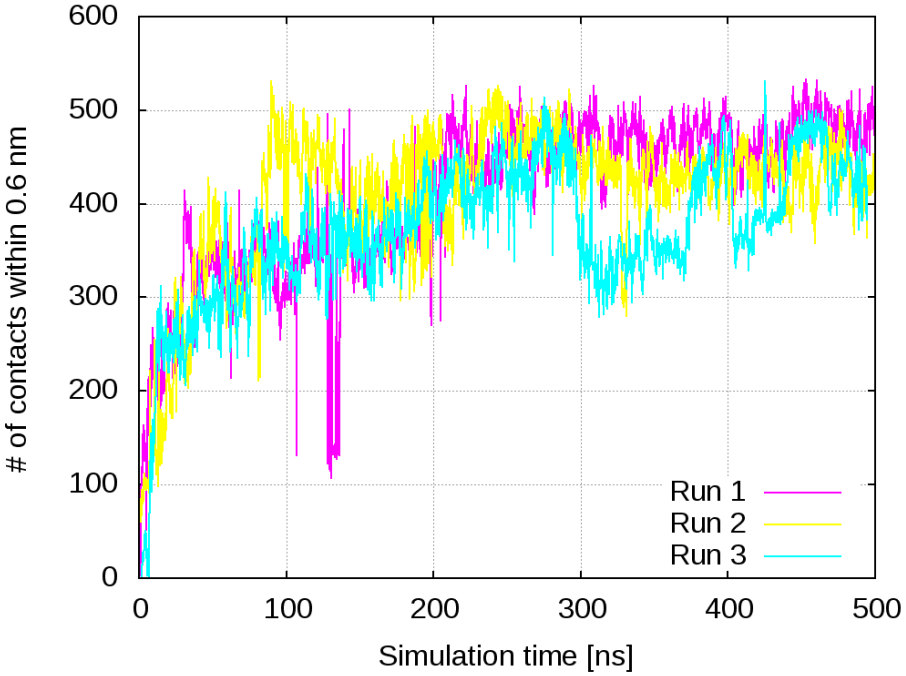
LMWH binding:
No influence on the cytokine's globule

Secondary-structure plot of IFNγ: *apo form (top left) & three independent binding simulations: globule (amino acids 1-121) & C-termini (amino acids 122-143)*

α-helix | extended-β | 3-10 helix
coil | turn

IFNγ/heparin complex

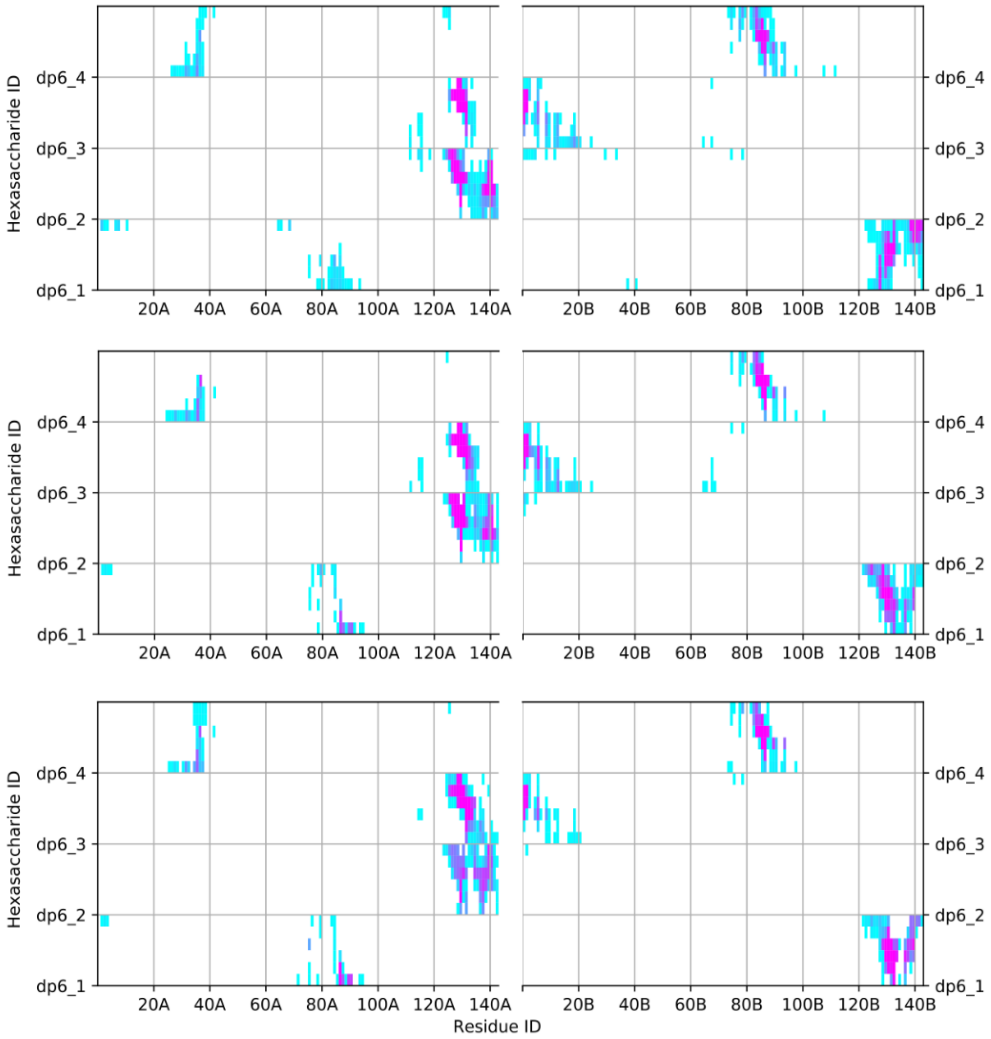




Pair contacts between IFNγ and the hexasaccharides. *Number of contacts (averaged over the three independent simulations, and for the three independent runs) as a function of the simulation time between any pair of atoms of IFNγ and any of the four hexasaccharides within 0.6 nm*

* With three hexasaccharides attached, the complex already has a **negative net charge**.

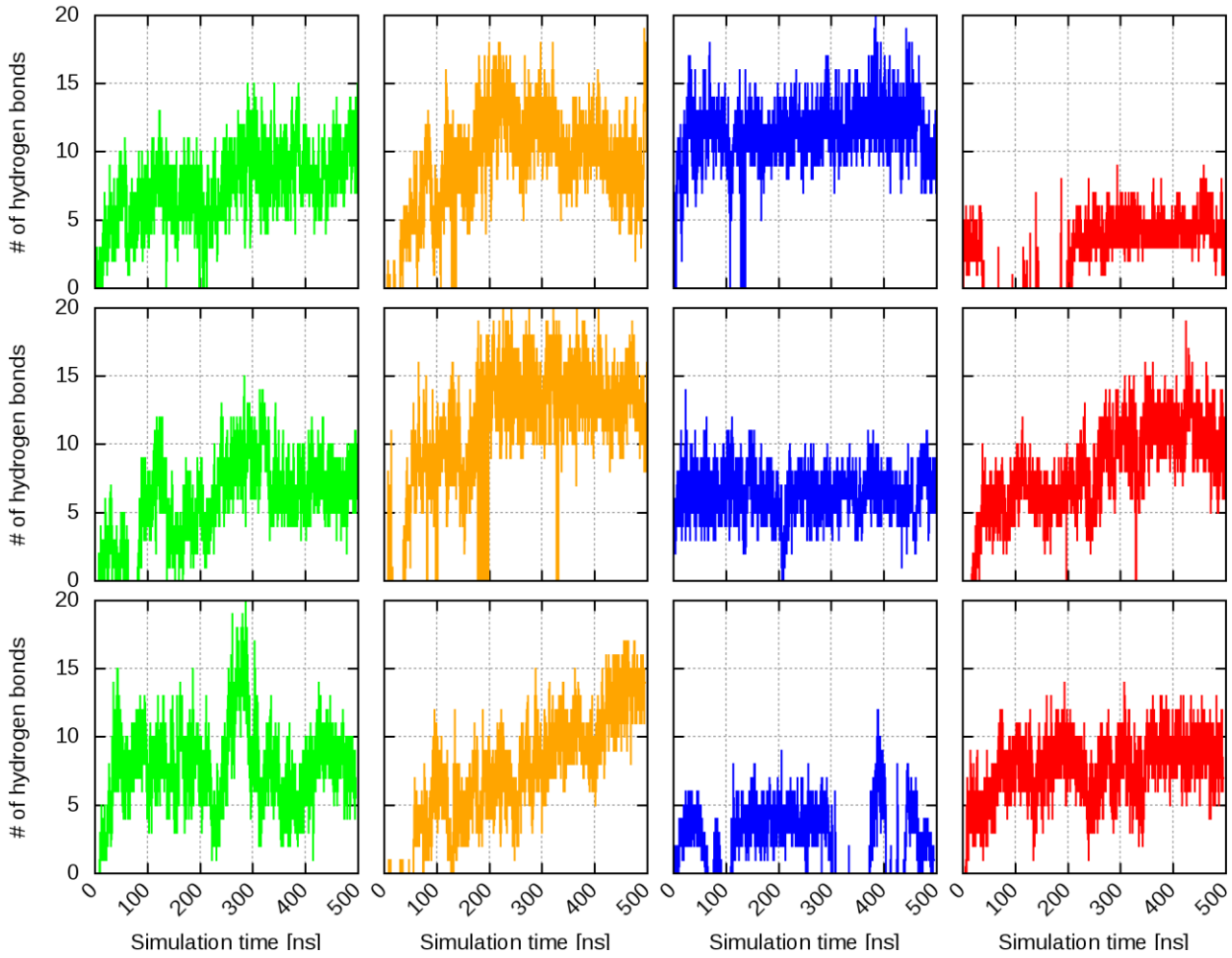
IFNγ/heparin complex



LMWH binding:
Interaction expected with the
positively charged parts of IFNγ
- Leu^{120} -Gln^{143}
- $^{86}LysLysLysArg^{89}$

Contact map of the IFNγ/LMWH complex: *Contacts within 0.6 nm between each of the four hexasaccharides and the two monomers of IFNγ; contact occupancy within the last 250 ns of the three simulations, ranges from 0 to 1*

IFNγ/heparin complex

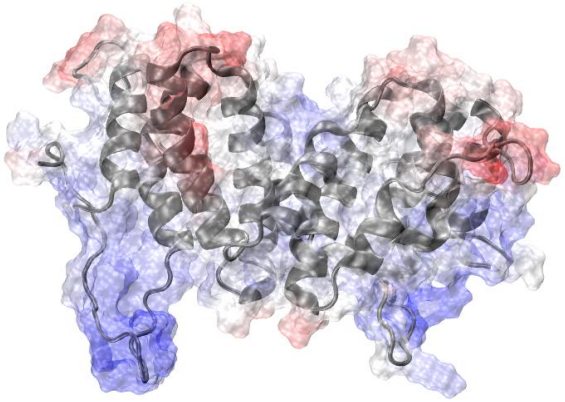


LMWH binding:
Very stable complexes
- Leu^{120} -Gln^{143}
- $^{86}LysLysLysArg^{89}$

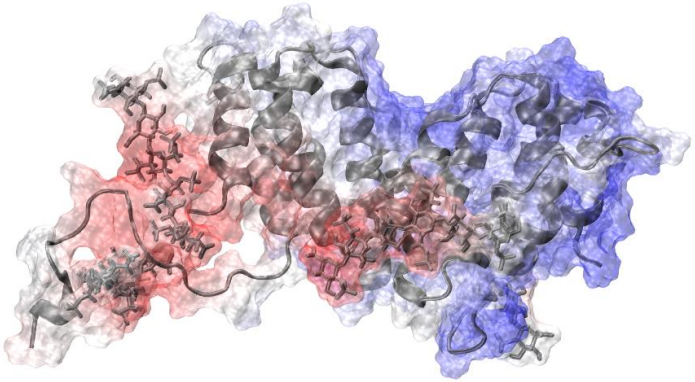
7-14 H-bonds

Hydrogen bonds *between IFNγ and each of the four LMWH in the three runs*

IFNγ/heparin complex

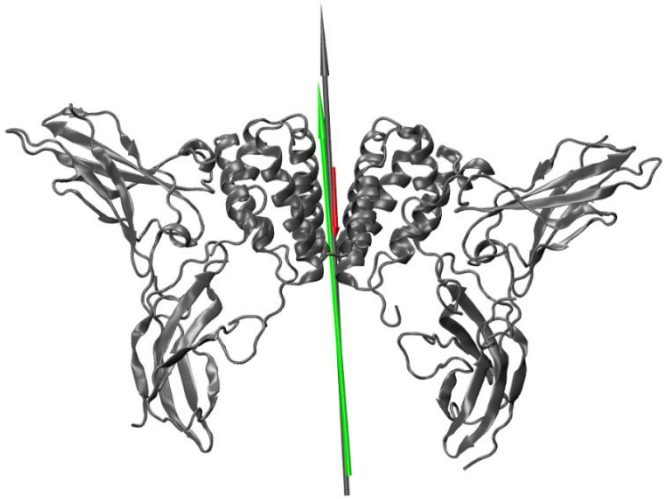


Electrostatic potential surface of hIFNγ alone and in complex with LMWH molecules



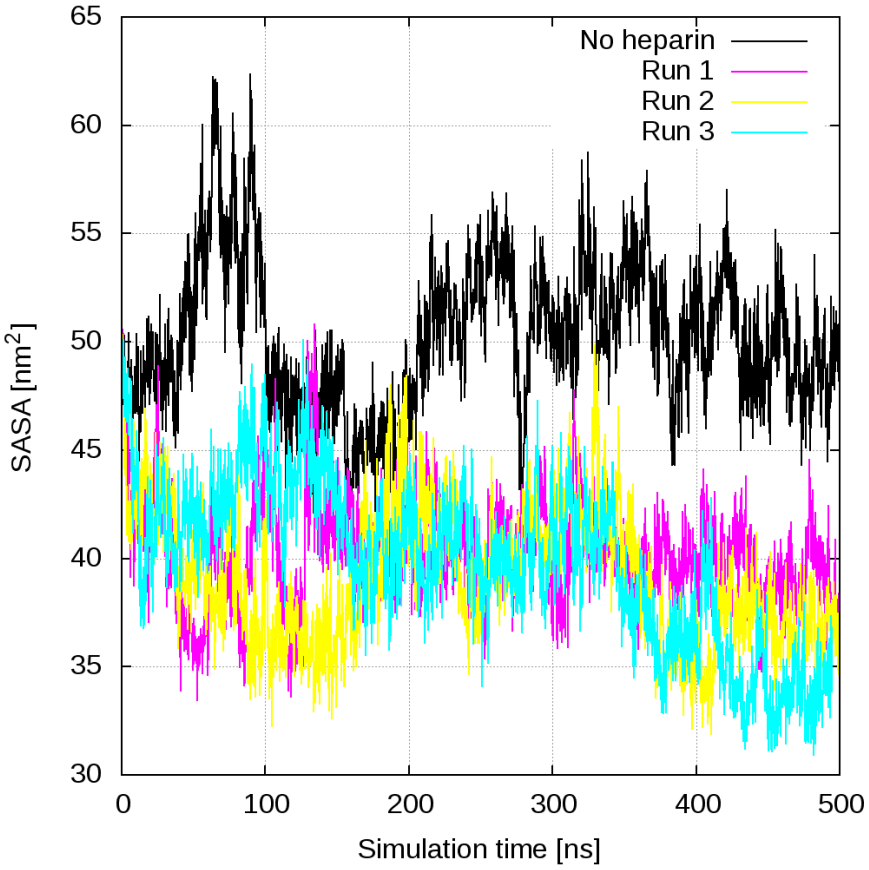
LMWH binding:

- ***Electrostatic attraction*** between IFNγ and its receptor
- ***Not possible with a net negative charge***



Dipole moments of the hIFNγ–hIFNγR1 complex

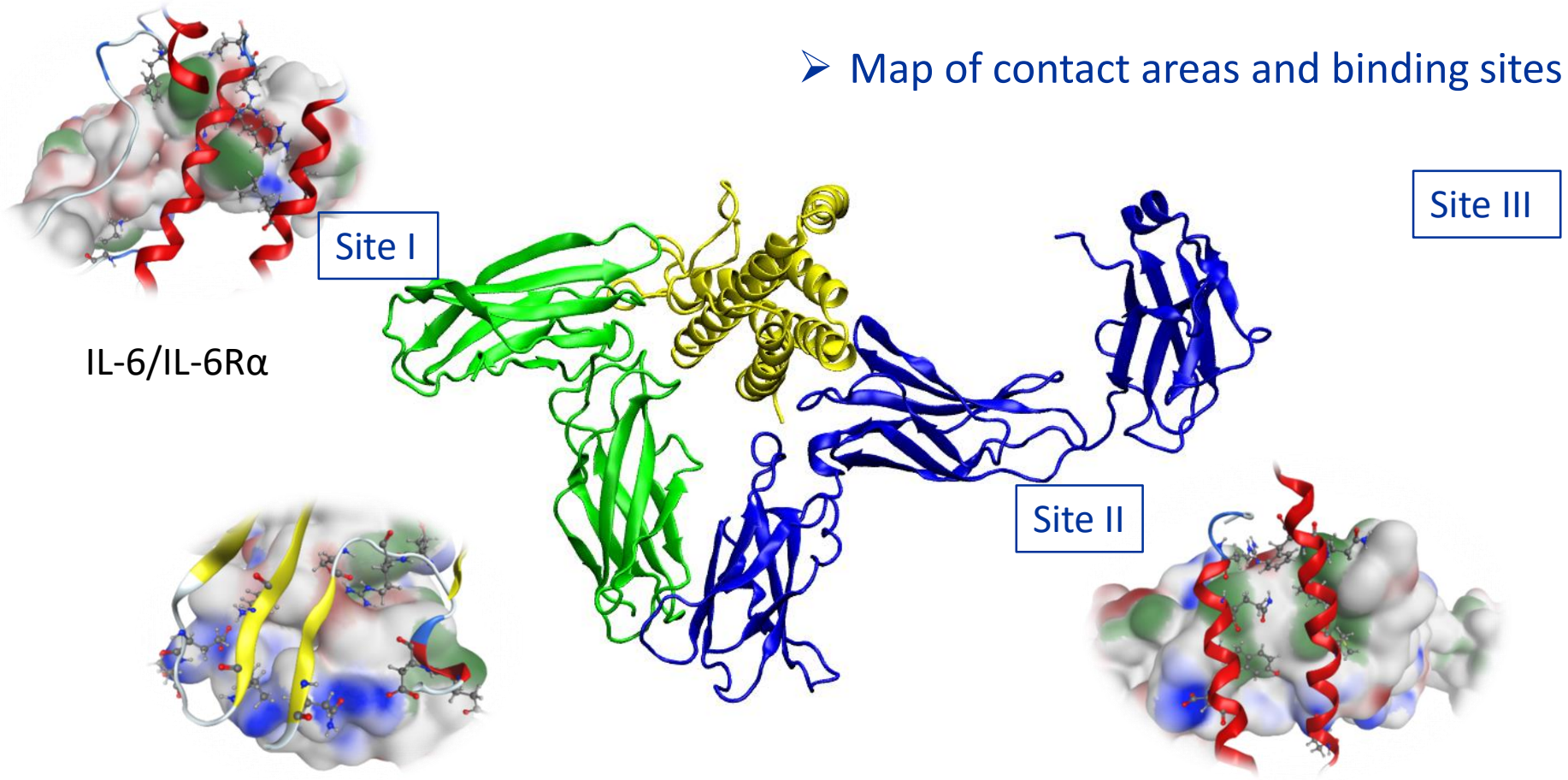
IFNγ/heparin complex



LMWH binding:
Impairment of the binding affinity of IFNγ to its receptor to be expected

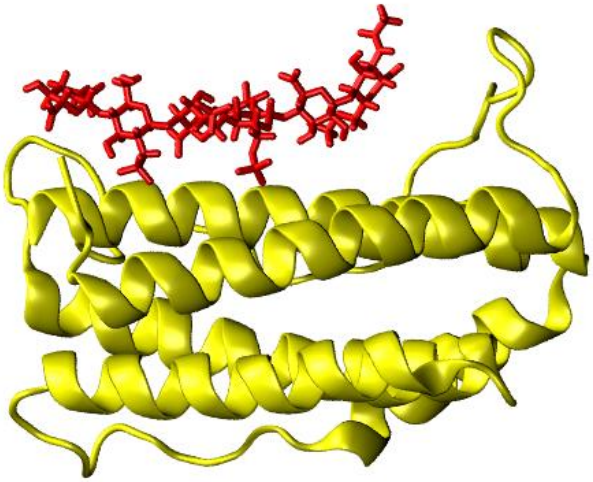
C-termini solvent-accessible surface area (SASA)*: IFNγ reference simulation (in black) and the three independent binding simulations*

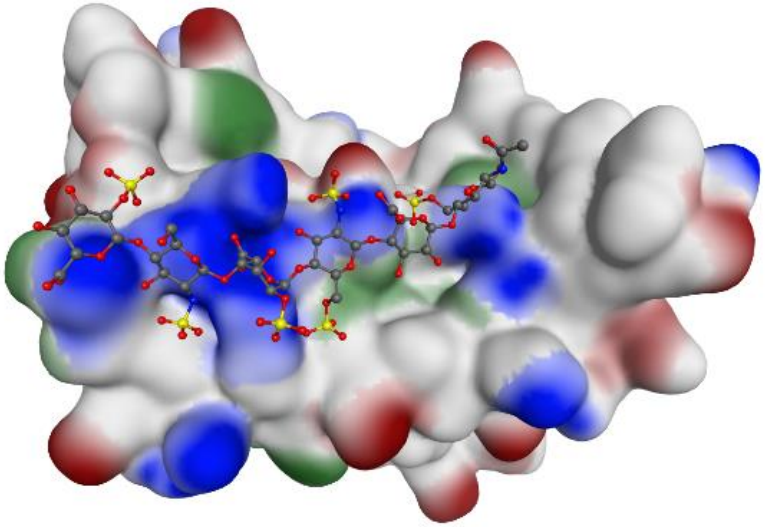
IL-6/IL-6Rα/gp130 complex

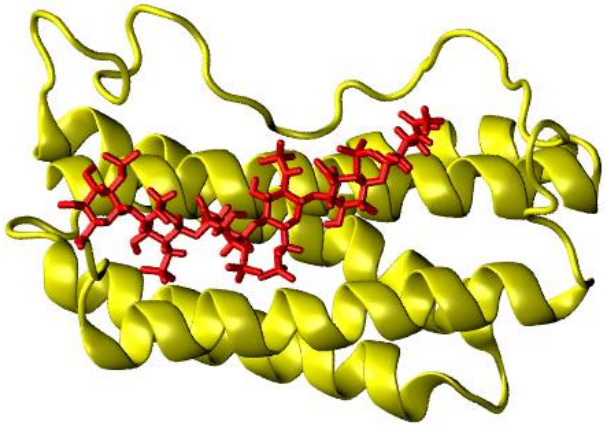


➢ Map of contact areas and binding sites

IL-6/heparin complex



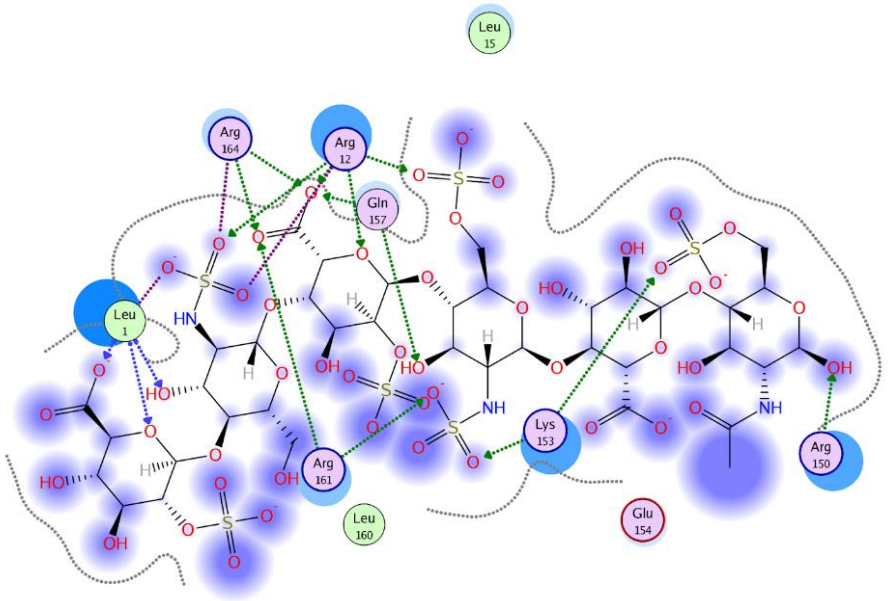




- LMWH binds to IL-6
- No substantial structural changes in IL-6
- Polar interaction, determined by the charge distribution
- Position with high chances for inhibiting the triple complex building

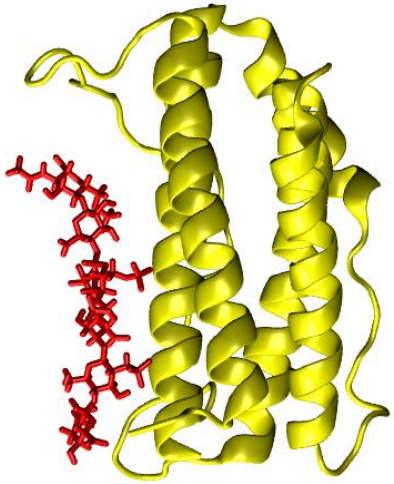
IL-6/heparin complex

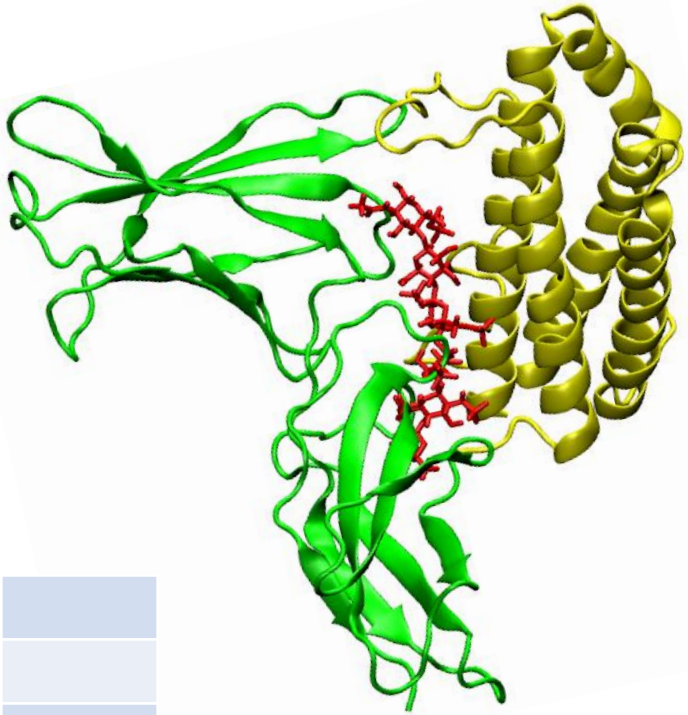
aa	d [Å]	E [kcal/mol]
Leu 19	2.29	-11.4
Arg 182	2.31	-11.2
Arg 30	2.35	-10.6
Lys 171	2.36	-10.5
Arg 30	2.39	-10.1
Lys 171	2.44	-9.5
Arg 30	2.46	-9.3
Arg 179	2.48	-9.0
Arg 182	2.49	-9.0



- LMWH binds to IL-6
- No substantial structural changes in IL-6
- Polar interaction, determined by the charge distribution
- Position with high chances for inhibiting the triple complex building

IL-6/heparin/IL-6Rα complex

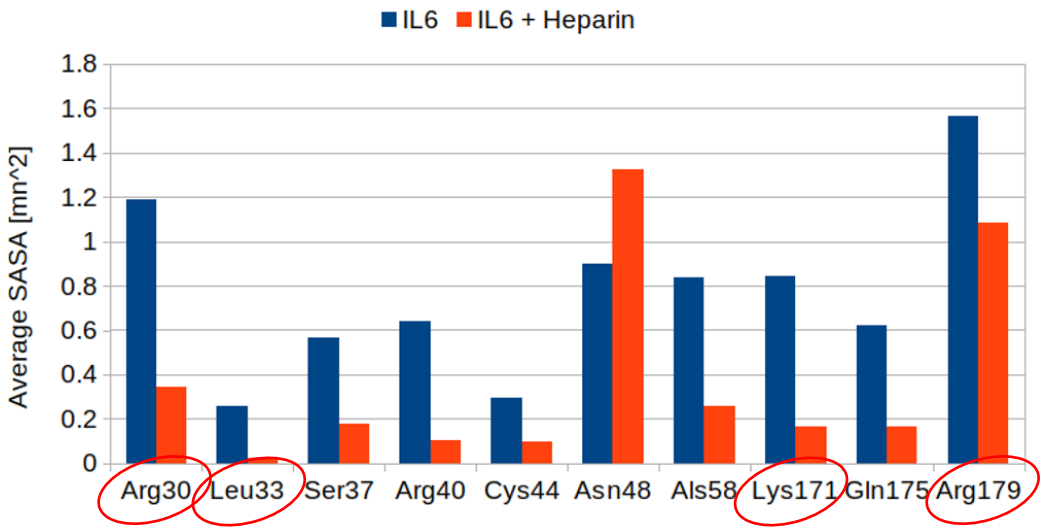


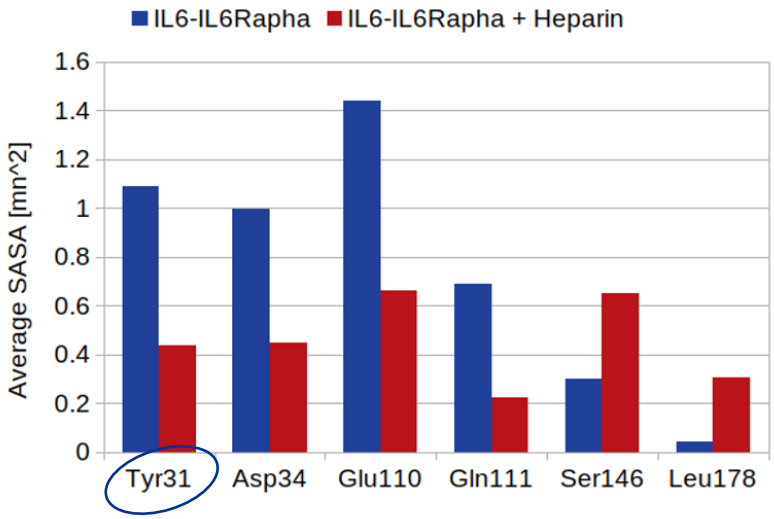


IL-6/IL-6R	30Arg	54Lys	33Leu		
IL-6/IL-6R	178Leu	179Arg	182Arg	183Gln	
IL-6/gp130	24Arg	28Gln	31Tyr	34Asp	
IL-6/gp130	110Glu	117Met	121Val	124Gln	125Phe
HP/IL-6	19Leu	40Arg			
HP/IL-6	171Lys	179Arg	182Arg		

Heparin blocks binding site 1 (IL-6/IL-6R), thus disabling the binding of IL-6 to its receptor

IL-6/heparin complex



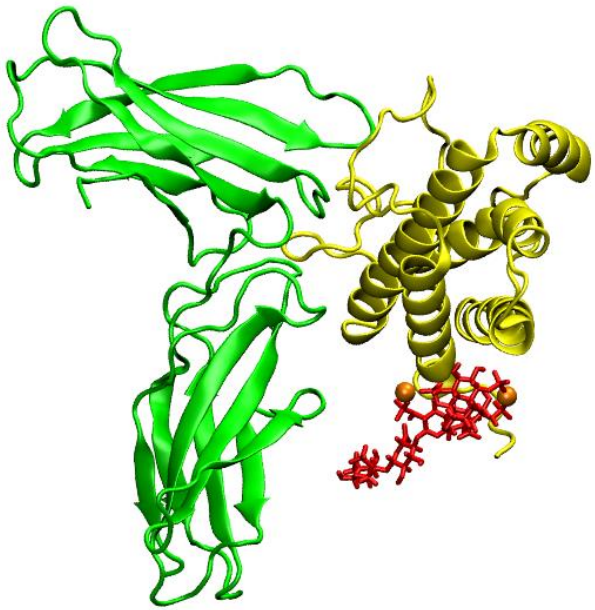


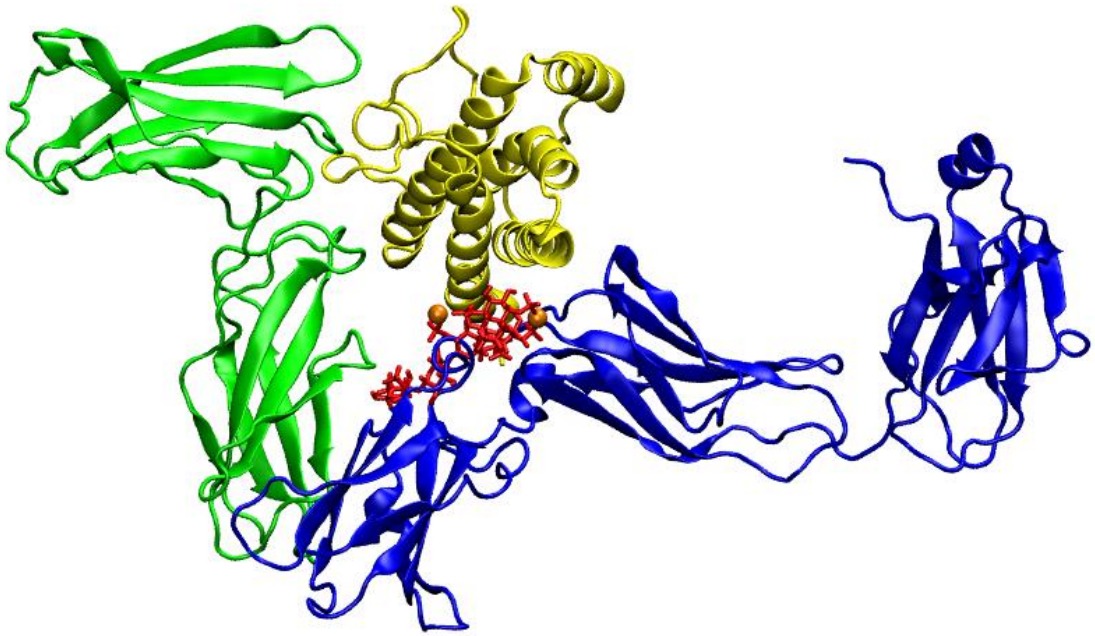
Average SASA values for the most affected through LMWH binding residues *(SASA change exceeds the standard deviation)*

LMWH binding:

- *affected all key residues from Site I* → *IL-6/IL-6Rα*
- *affected the only charged residue from Site II* → *IL-6/gp130*

IL-6/IL-6Rα + heparin + Mg (+ gp130)



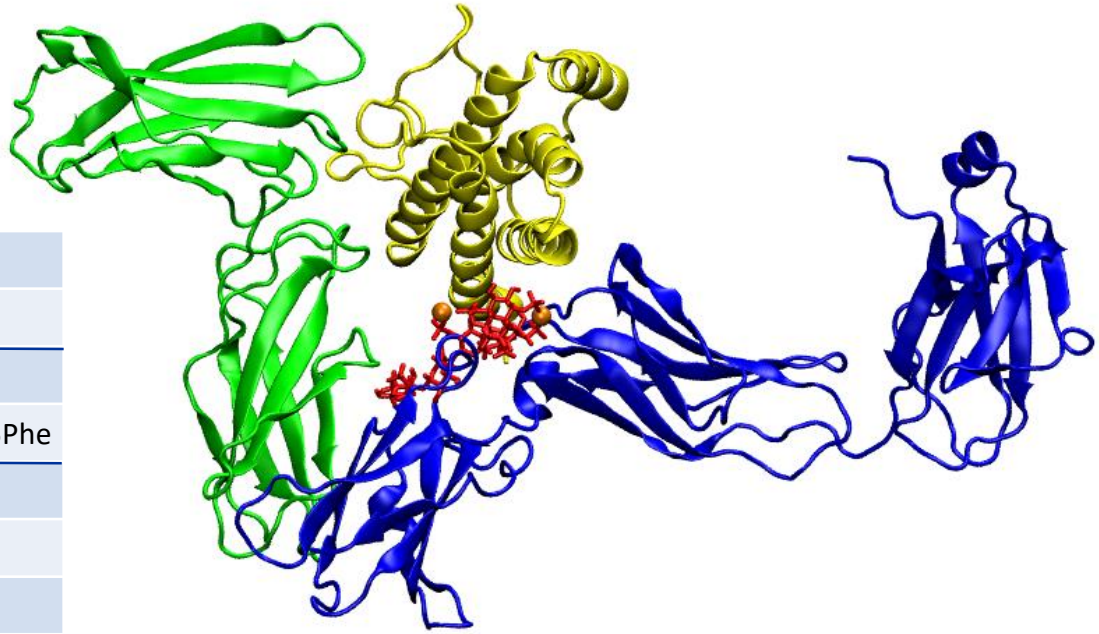


Heparin, in the presence of Mg ions, blocks binding site II (IL-6/gp130) and being positioned in front of helix A, effectively prevents the formation of the biologically active triple complex with gp130

IL-6/heparin/IL-6Rα complex

Contact residues and heparin binding

IL-6/IL-6R	30Arg	54Lys	33Leu		
IL-6/IL-6R	178Leu	179Arg	182Arg	183Gln	
IL-6/gp130	24Arg	28Gln	31Tyr	34Asp	
IL-6/gp130	110Glu	117Met	121Val	124Gln	125Phe
HP/**IL-6**/IL-6R	40Lys	41Lys	168Arg		
Mg	30Arg	31Tyr			
HP/IL-6/**IL-6R**	233Arg	281Gln	284Trp	276Gln	



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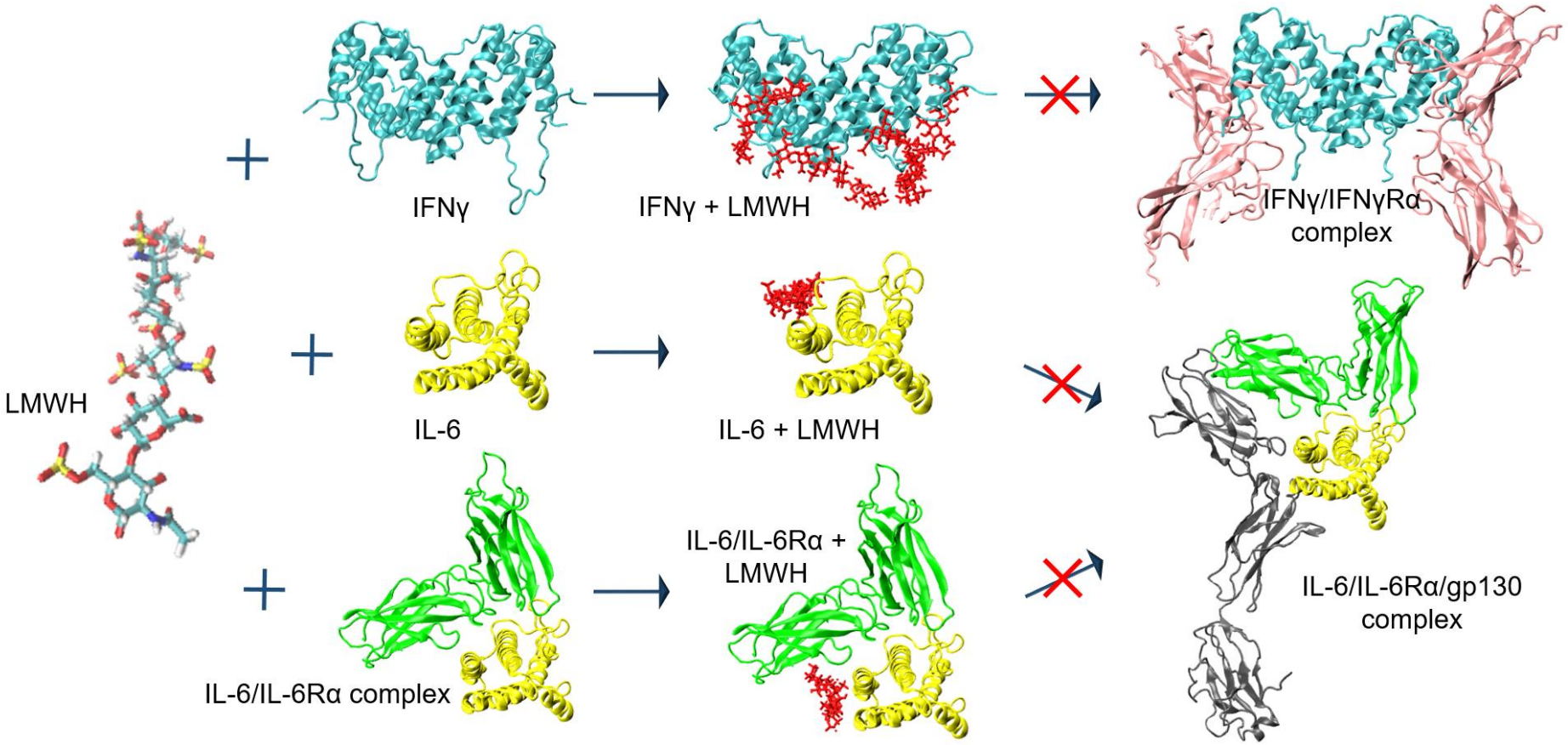
Conclusions



Conclusions

- LMWH binds with high affinity to IFNγ, fully inhibiting the interaction with its receptor
- LMWH interacts with IL-6, blocking that way its binding to the receptor IL-6Rα
- LMWH interacts with the complex IL-6/IL-6Rα and prevents further binding of this complex to gp130

Heparin inhibits two of the key players in the CRS (the cytokine storm) – IL-6 and IFNγ, which opens the possibility to stop and even to reverse its development.

L. Litov et al., *Heparin as an Anti-Inflammatory Agent.* bioRxiv-223859 (2020) 20 pp.

Conclusions

- Heparin is a potent anti-inflammatory agent, due to its ability to engage with two of the key cytokines in the development of the cytokine storm – IFNγ and IL-6.
- Heparin can influence favourably conditions characterised by an overexpression of certain cytokines (associated with autoimmune diseases, but also with uncontrolled inflammatory processes, in particular with COVID-19)
- Heparin's anti-inflammatory action does not depend on the virus type and, in general, the cause of the acute inflammatory process
- Threefold activity of heparin: anticoagulant, anti-inflammatory and antiviral
- An added benefit: heparin is a well-known and widely used medication

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Thank you
for your attention!