INSTITUTE OF
INFORMATION AND COMMUNICATION
TECHNOLOGIES

INSTITUTE OF MATHEMATICS AND INFORMATICS

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MOLECULAR MECHANISM OF THE ANTI-INFLAMMATORY ACTION OF HEPARIN: A COMPUTATIONAL PERSPECTIVE OF THE COVID-19 CASE

In collaboration with

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BIOMATH Conference & School for Young Scientists
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BIOMATH'21

The COVID-19 Pandemic

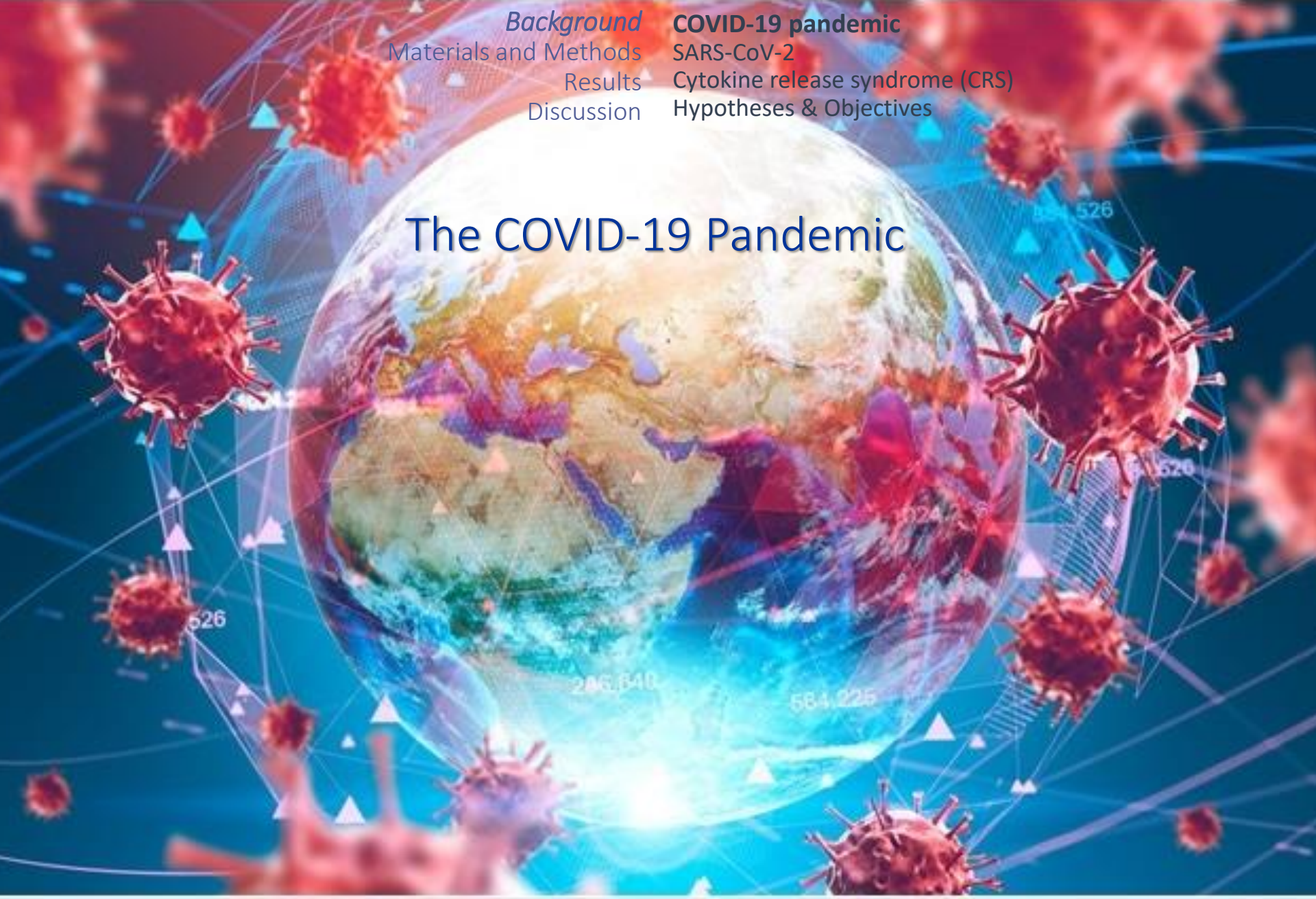
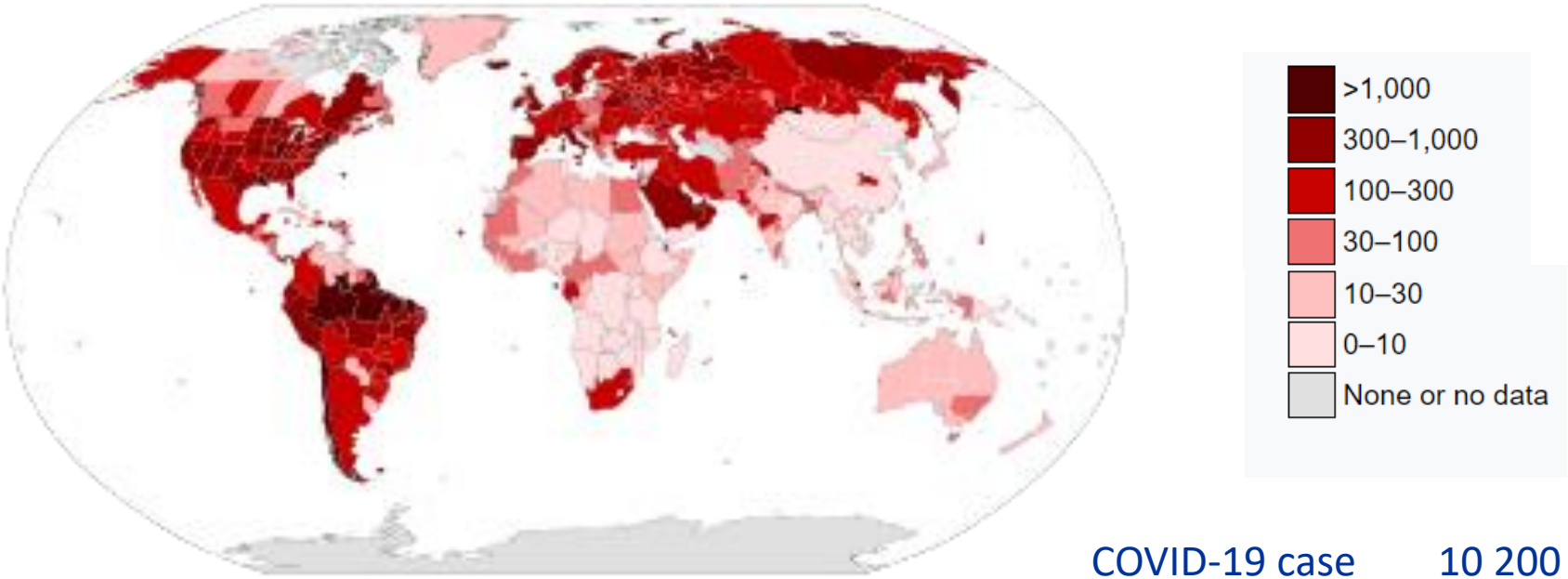


Image Credit: ImageFlow / Shutterstock

The COVID-19 Pandemic

Confirmed cases per 100 000 population as of 27 June 2020



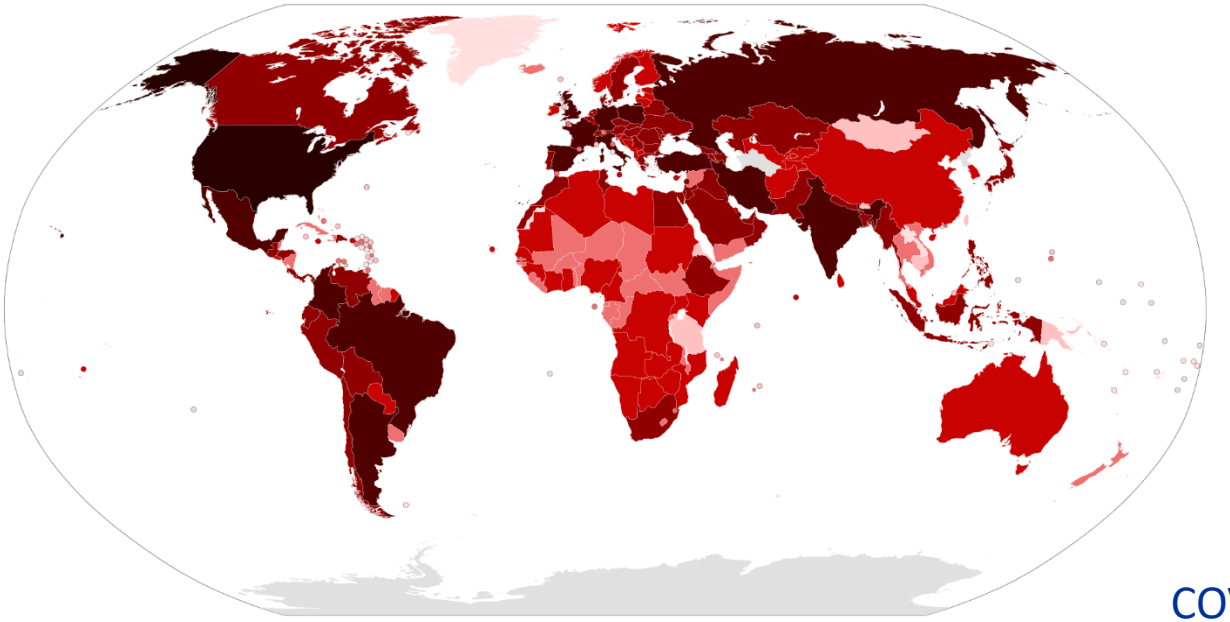
December 2019 Wuhan (reported)
30 January 2020 emergency
11 March 2020 pandemic

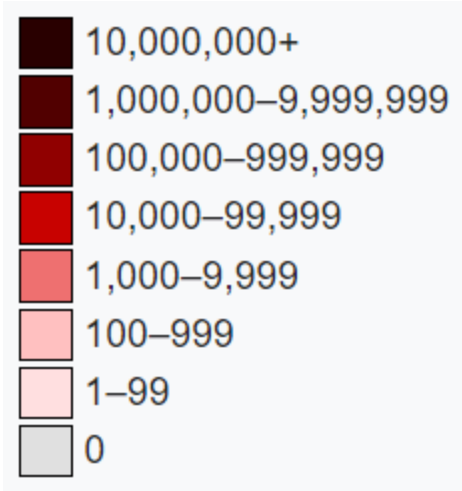
COVID-19 case	10 200 000
Deaths	503 000
Recovered	5 510 000
> 188 countries and territories	

https://en.wikipedia.org/wiki/COVID-19_pandemic

The COVID-19 Pandemic

Total number of confirmed cases as of 11 December 2020





December 2019 — Wuhan (reported)
30 January 2020 — emergency
11 March 2020 — pandemic

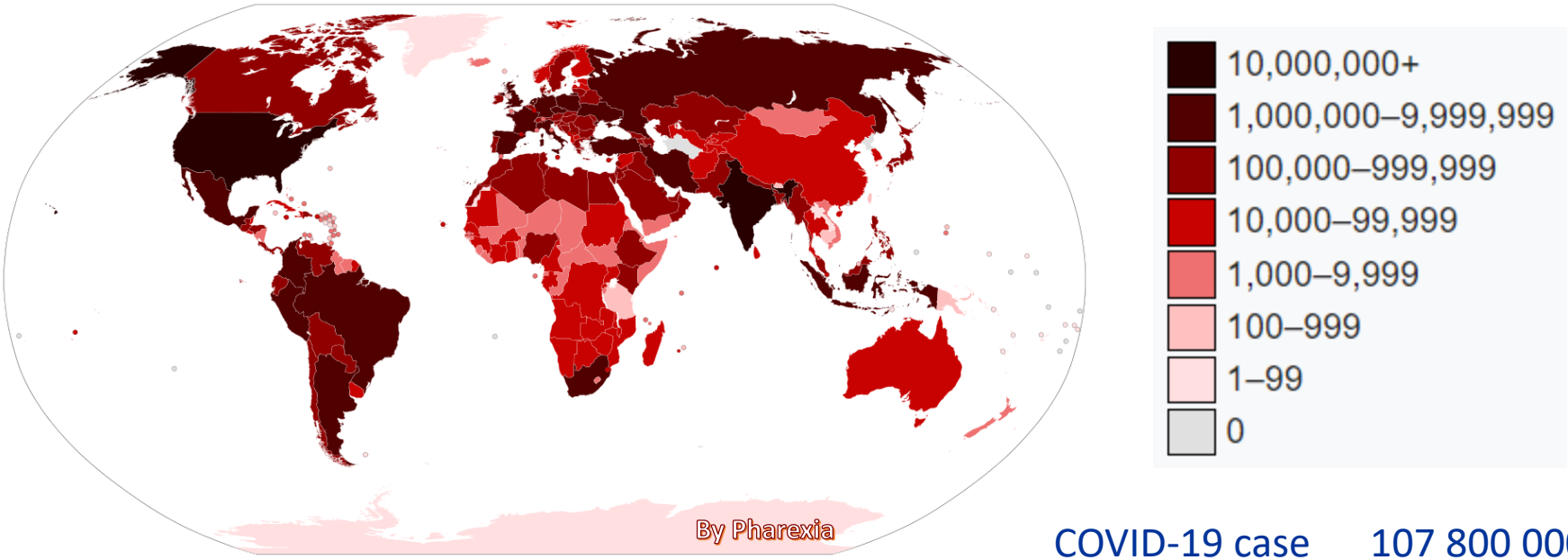
COVID-19 case	72 400 000
Deaths	1 616 000
Recovered	50 720 000

218 countries and territories

https://en.wikipedia.org/wiki/COVID-19_pandemic

The COVID-19 Pandemic

Total number of confirmed cases as of 10 February 2021

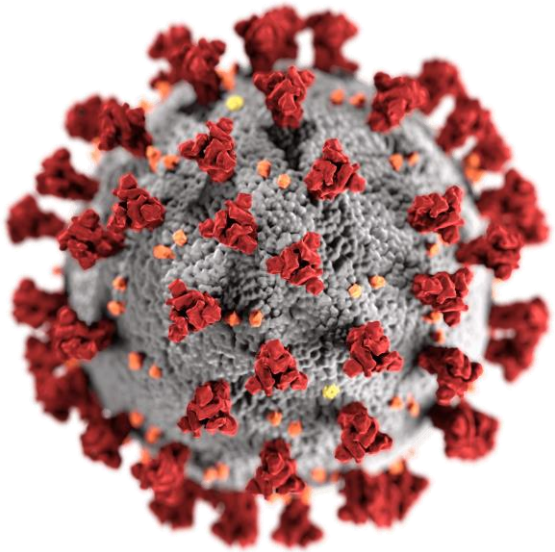


December 2019 — Wuhan (reported)
30 January 2020 — emergency
11 March 2020 — pandemic

COVID-19 case	107 800 000
Deaths	2 360 000
Recovered	79 650 000
219 countries and territories	

https://en.wikipedia.org/wiki/COVID-19_pandemic

SARS-CoV-2

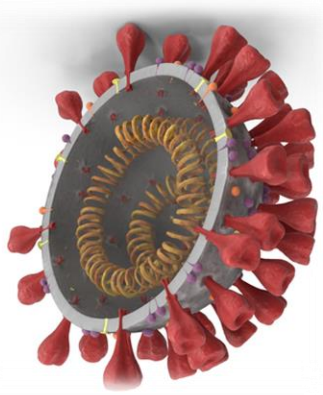


7th known "human" coronavirus
SARS-CoV [2002-2004; 11% CFR]
MERS-CoV [2012; ~34% CFR]
SARS-CoV-2 polybasic cleavage site

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

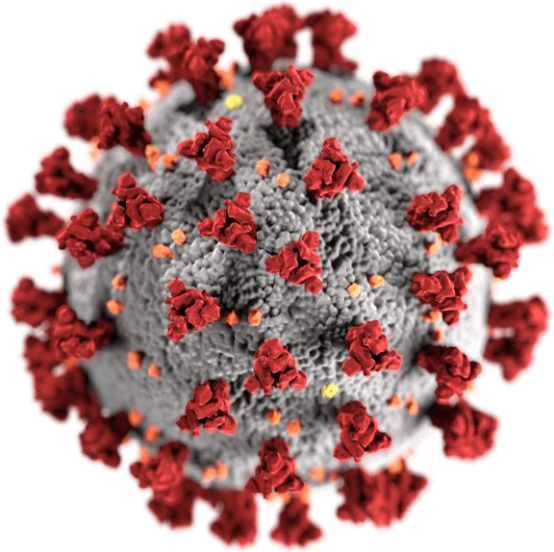
Virions measures
~ 100 nm in diameter
~ 10^3 Mda ≈ 1.6 fg

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

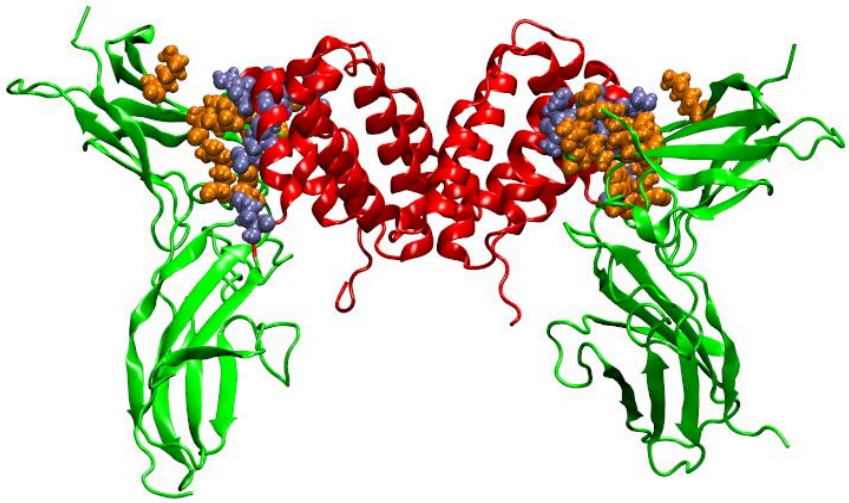
https://phil.cdc.gov/Details.aspx?pid=23312
Alissa Eckert, MS & Dan Higgins, MAMS

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: **IFNγ** and **IL-6**

Interferon-gamma (IFNγ)

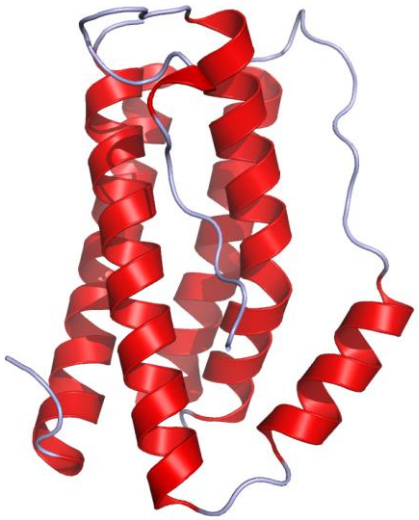


- Pleiotropic cytokine
- 2 x 143 aa; 25 kDa
- Key role in immune signaling and modulation of the innate and adaptive immune response
- Overexpression associated with certain autoimmune diseases

- **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)



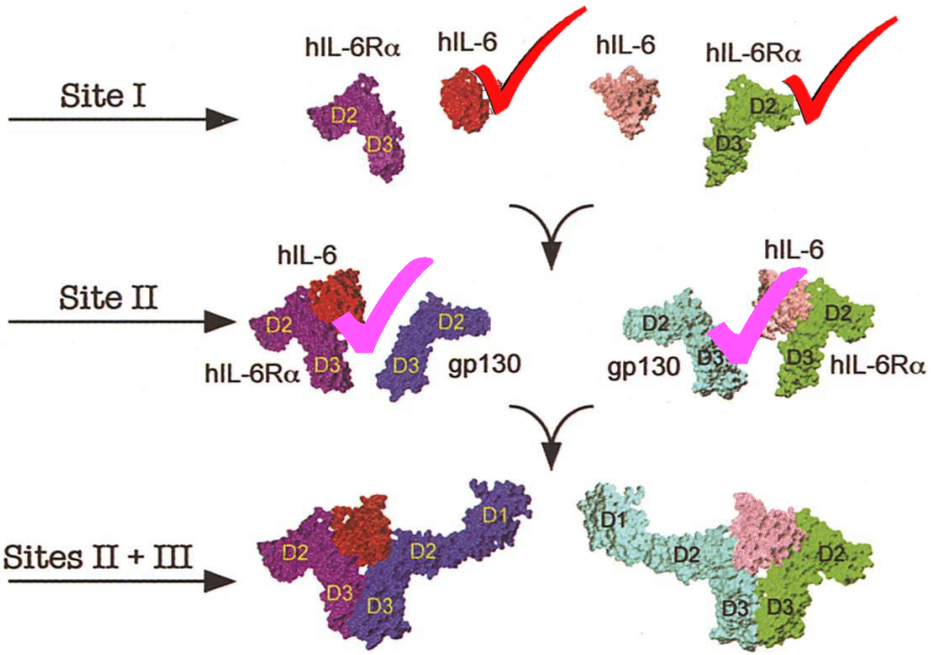
- Member of the IL-6 family of cytokines
- 212 aa; 23.7 kDa
- Secreted by many cell types during infection, inflammation or cancer
- Very low levels under normal conditions

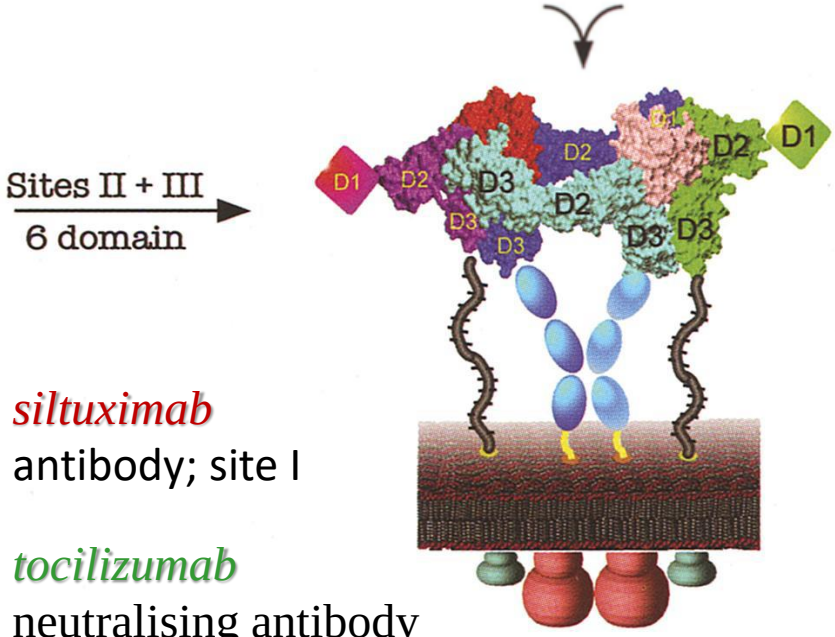
  -> biomarker
- Modulates the balance between humoral and cell-based immune responses
- Involved in B- and T-cells regulation
- Inhibition of IL-6 signaling:

  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





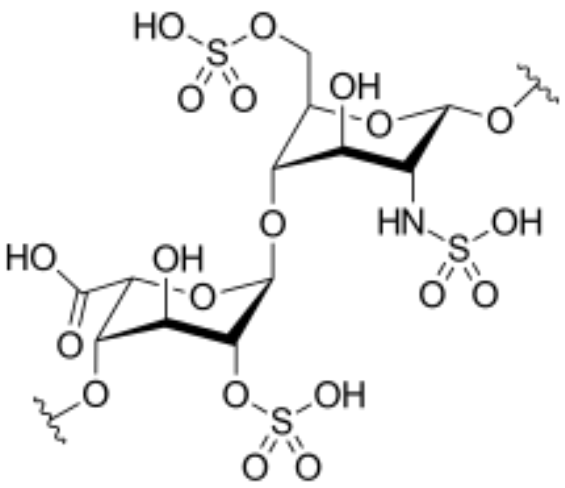
siltuximab
antibody; site I

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

Hypotheses & Objectives

Inhibition of IFNγ and IL-6 activity by GAGs like heparin can significantly suppress or even reverse the development of the cytokine storm

Finding means to prevent the development of the cytokine storm in the acute phase of the disease through the inhibition of IL-6 and IFNγ activity

M ∪ B

Molecular Dynamics

- Classical description of the systems;
- Empirical parameterisation of the interaction potential between atoms and molecules – molecular force field;
- The force field is conservative, depending on atoms positions only, pair-additive (*NB: cut-offs, boundary conditions*)

$$V = \sum_i V_s + \sum_i V_a + \sum_i V_t + \sum_i V_v + \sum_i V_e + \ldots$$

Bond strength | *Bond angle* | *Torsion* | *VdW interactions* | *Coulomb interaction*

- Numerical solution of classical equations of motion with a finite step Δt

$$m_i \frac{d^2 \mathbf{r}_i}{dt^2} = \mathbf{F}_i$$

$$\mathbf{F}_i = -\nabla_i U(\{\mathbf{r}_i\})$$

Molecular Dynamics

- Deterministic (contrary to MC-based methods) (time- and momentum-dependent phenomena and properties; conformation-space sampling)
- Average over microcanonical ensemble *(NVE)*

$$(\bar{A})_{MD} \simeq \frac{1}{n}\sum_{k=1}^{n} A\left[p^N(t_k), r^N(t_k)\right]$$

$$(\bar{A})_{MD} \simeq \langle A \rangle_{microcan}$$

- Experimental verification → observable macroscopic characteristics of the system (energy, temperature, pressure, etc.)

Molecular dynamics (MD) provides often experimentally inaccessible information for the equilibrium and transport properties of the investigated systems → ***in silico* experiments**

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

The *In Silico* Experiments

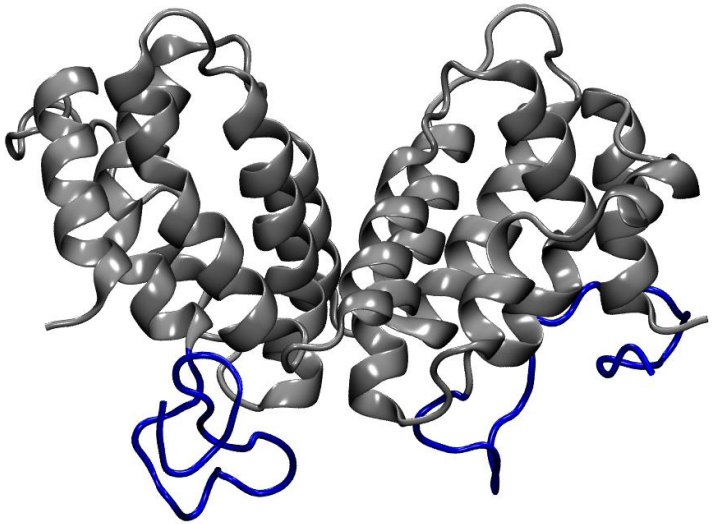
- Reconstruction of the full-length IFNγhomodimer
- Simulation of heparin/IFNγ complex
- Equilibration of the X-ray structure IL-6/IL-6Rα/gp130
- Identification of contact areas and binding sites
- Simulation of heparin/IL-6 binding
- Simulation of (IL-6/IL-6R)+heparin
- Complex-structure analysis
-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

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

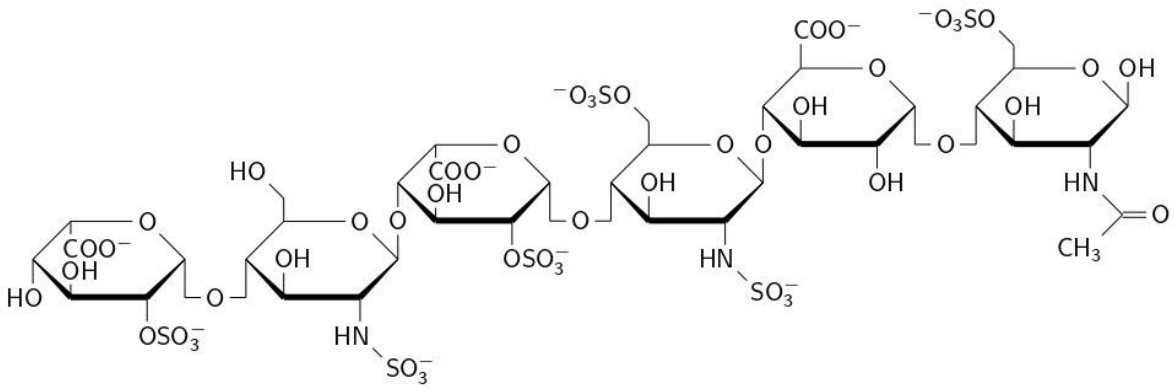
- Protein Data Bank: **PDB ID 1P9M**
- Structure parameterized with the CHARMM36m force field
- Solvation, neutralization, equilibration
- 10 ns production MD to get the initial structure

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

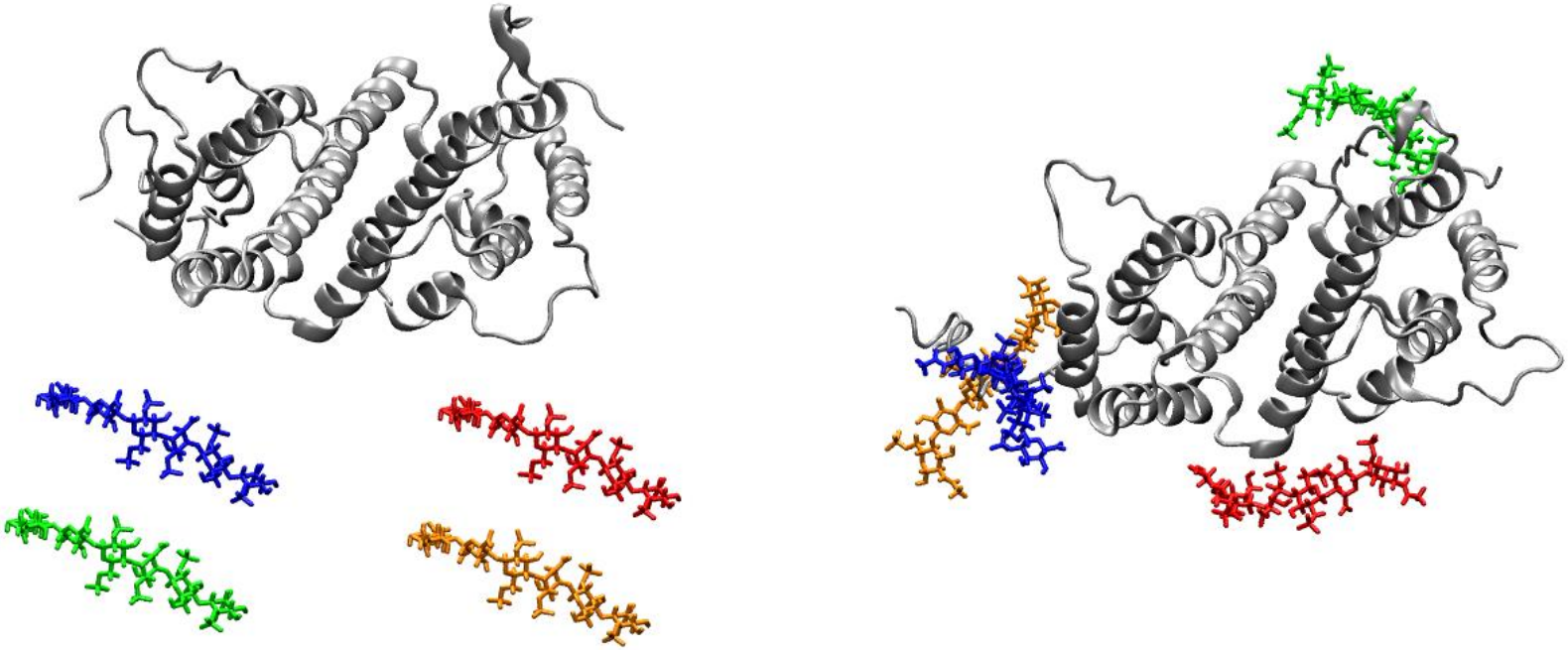
α-L-IdoA(2S) (1→4) β-D-GlcNS (1→4) α-L-IdoA(2S) (1→4) β-D-GlcNS(6S) (1→4) β-D-GlcA(1→4) β-D-GlcNAc(6s).

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

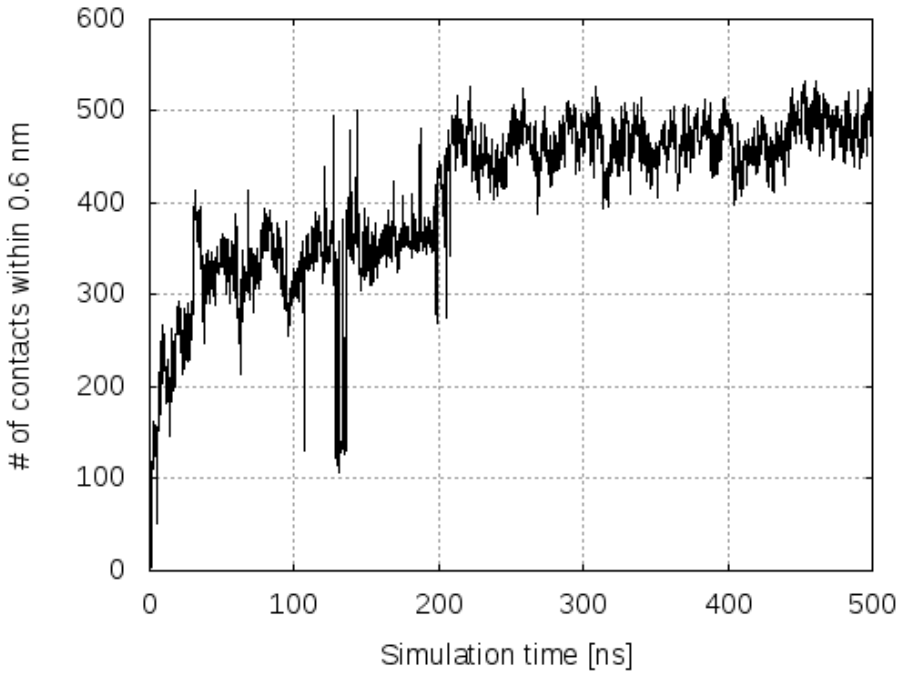
IFNγ/heparin complex

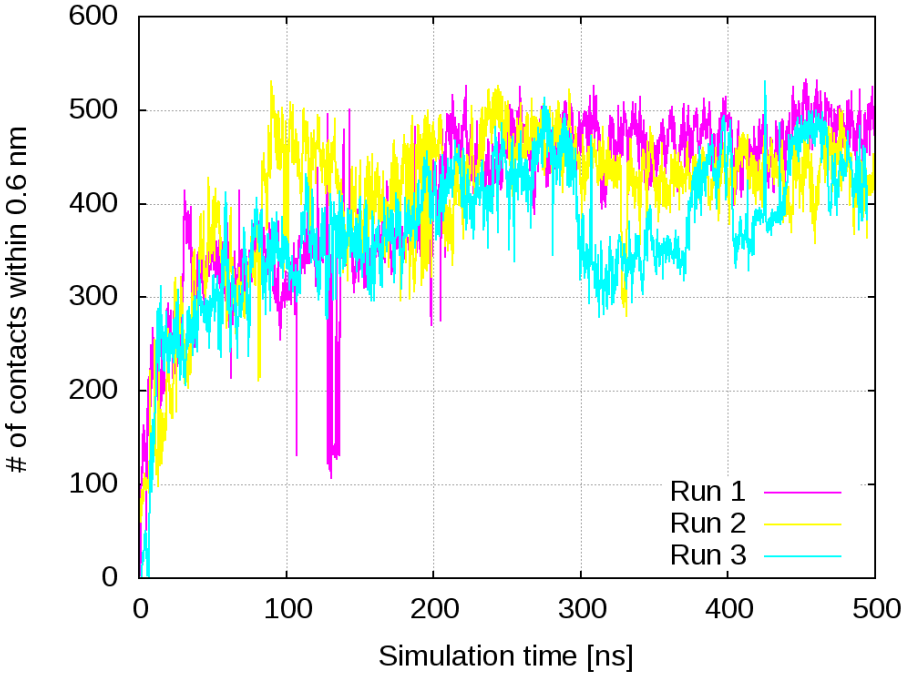


Initial and final conformations: IFNγ & four hexasaccharides (a representative LMWH structure) after 500 ns MD simulation

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

IFNγ/heparin complex

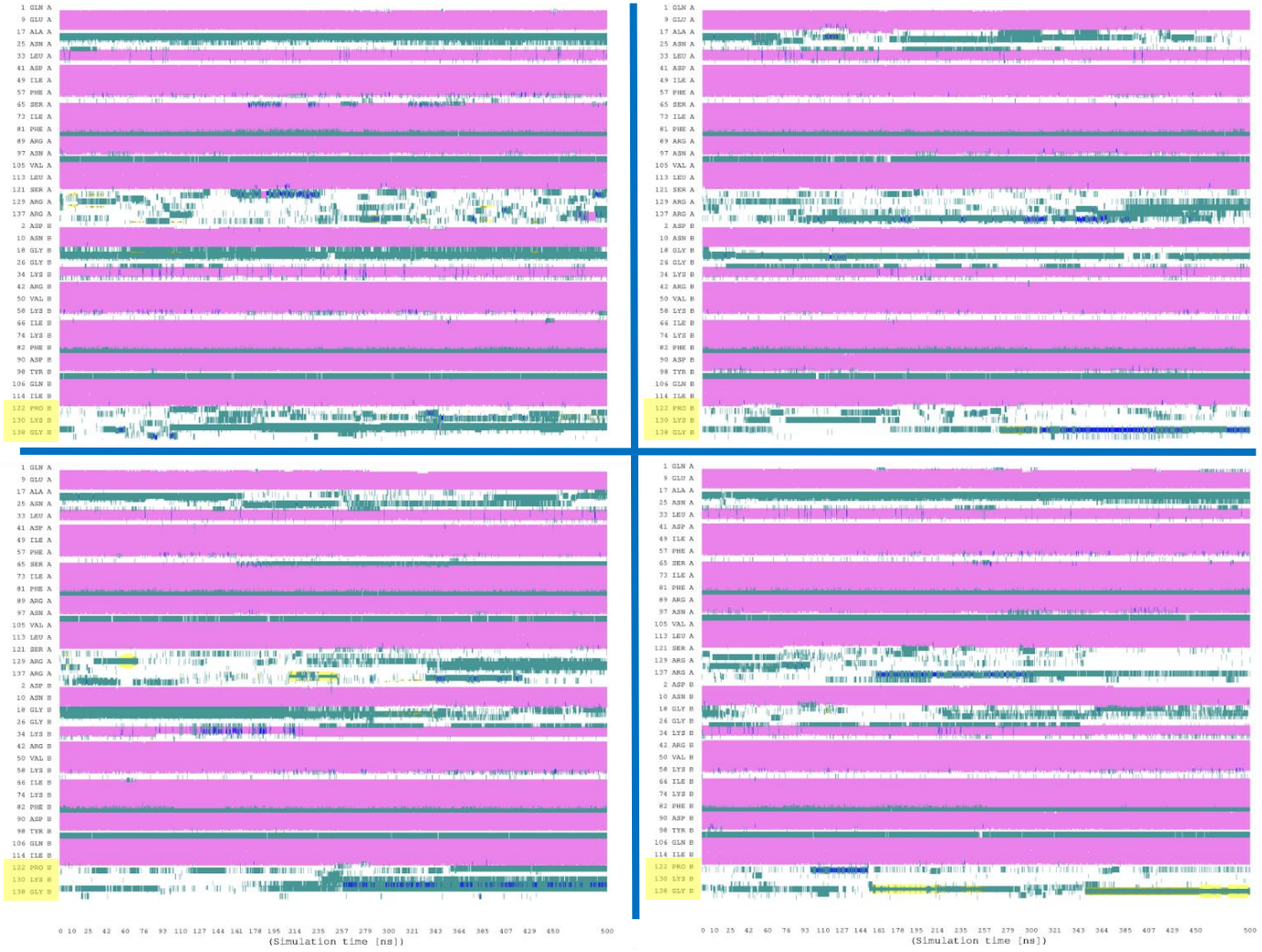




Pair contacts between IFNγ and the hexasaccharides. *Number of contacts (averaged over the three independent simulations, and for the three independent runs) as a function of the simulation time between any pair of atoms of IFNγ and any of the four hexasaccharides within 0.6 nm*

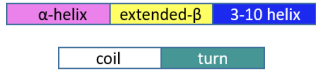
* With three hexasaccharides attached, the complex already has a **negative net charge**.

IFNγ/heparin complex

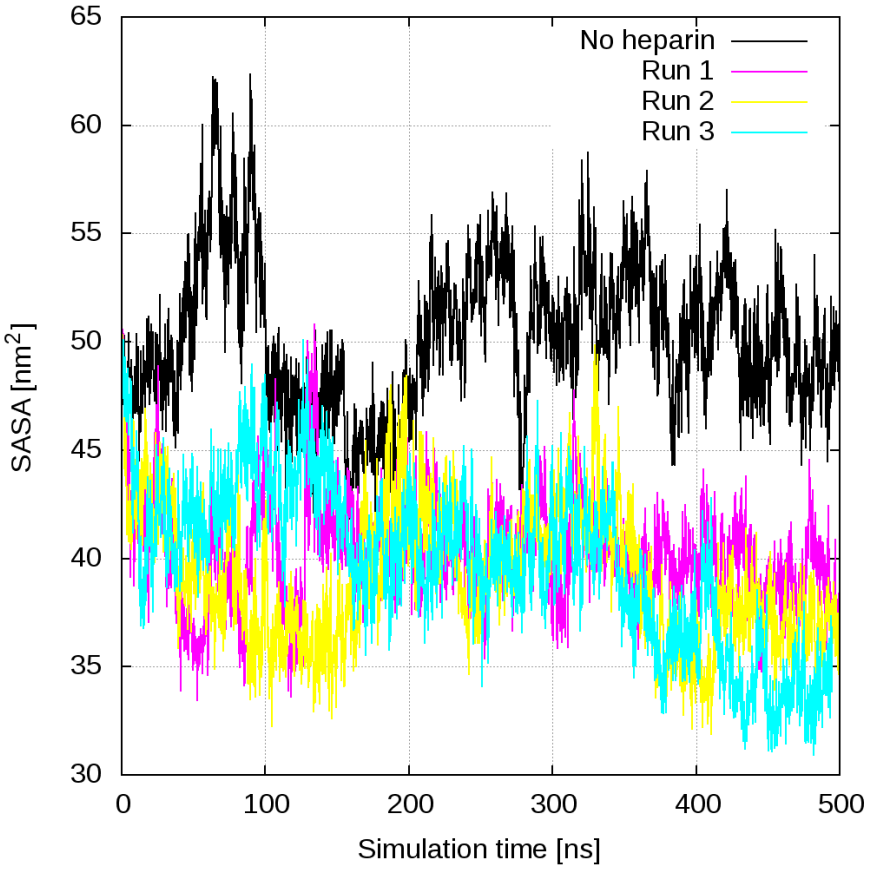


LMWH binding:
No influence on the cytokine's globule

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



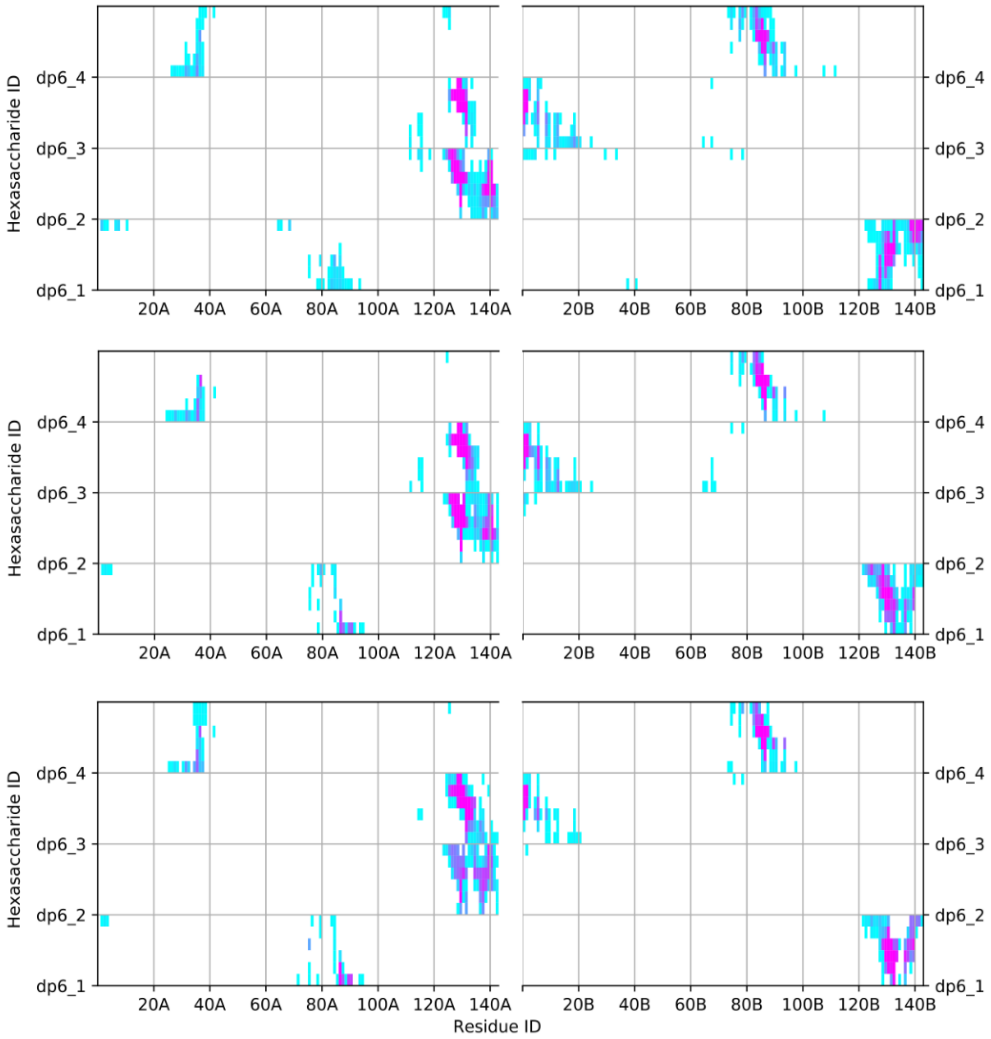
IFNγ/heparin complex



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*

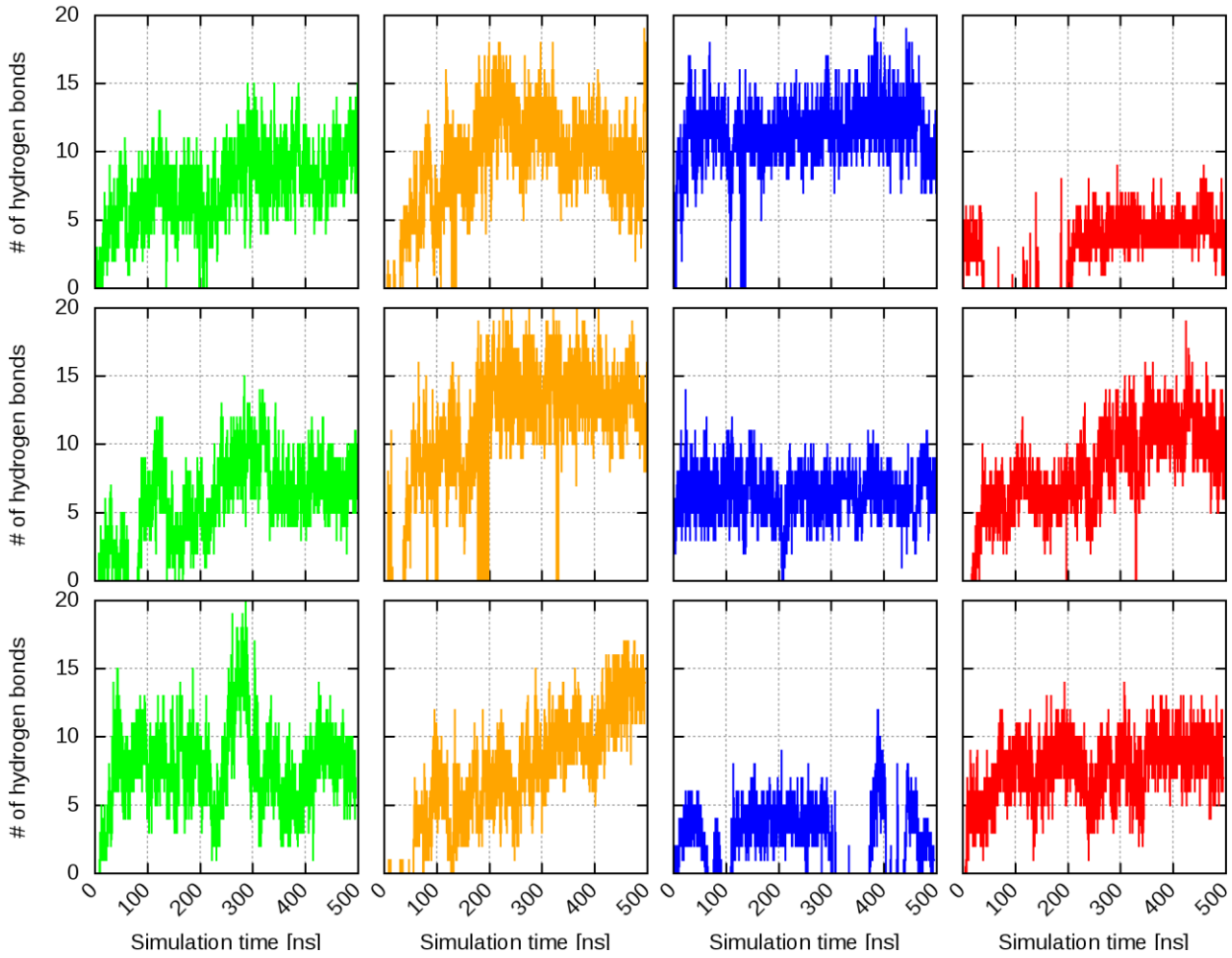
IFNγ/heparin complex



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

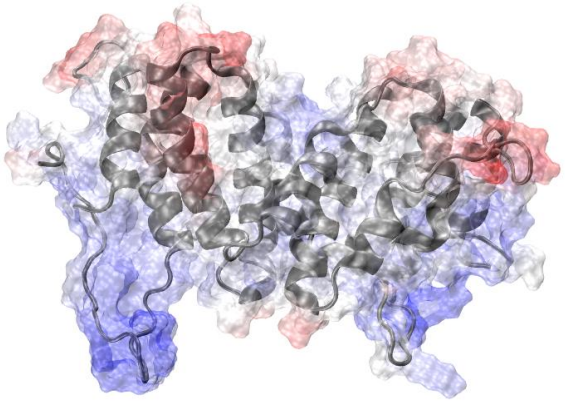


LMWH binding:
Very stable complexes
- Leu^{120} -Gln^{143}
- $^{86}LysLysLysArg^{89}$

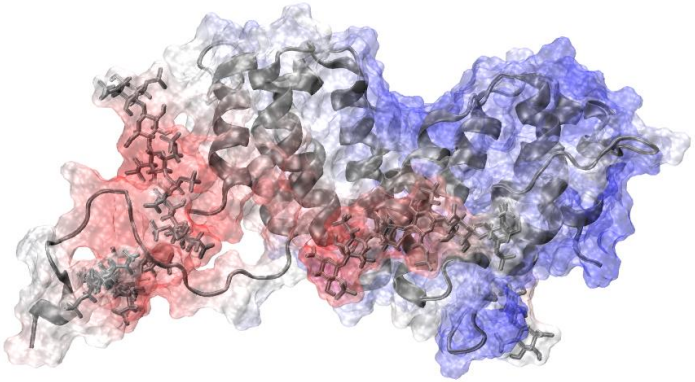
7-14 H-bonds

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

IFNγ/heparin complex

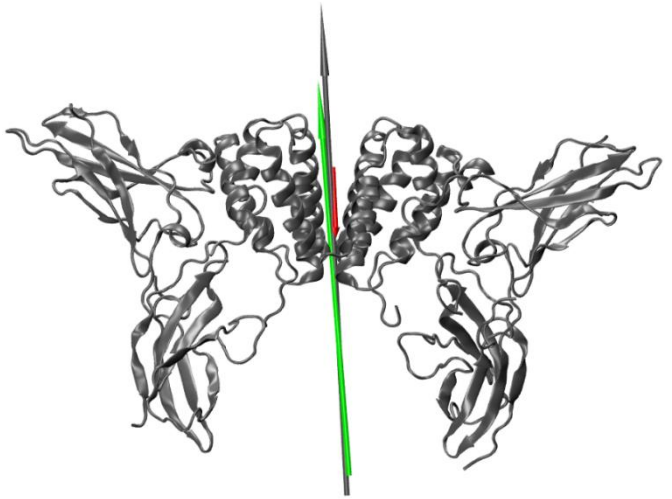


Electrostatic potential surface of hIFNγ alone and in complex with LMWH molecules



LMWH binding:

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



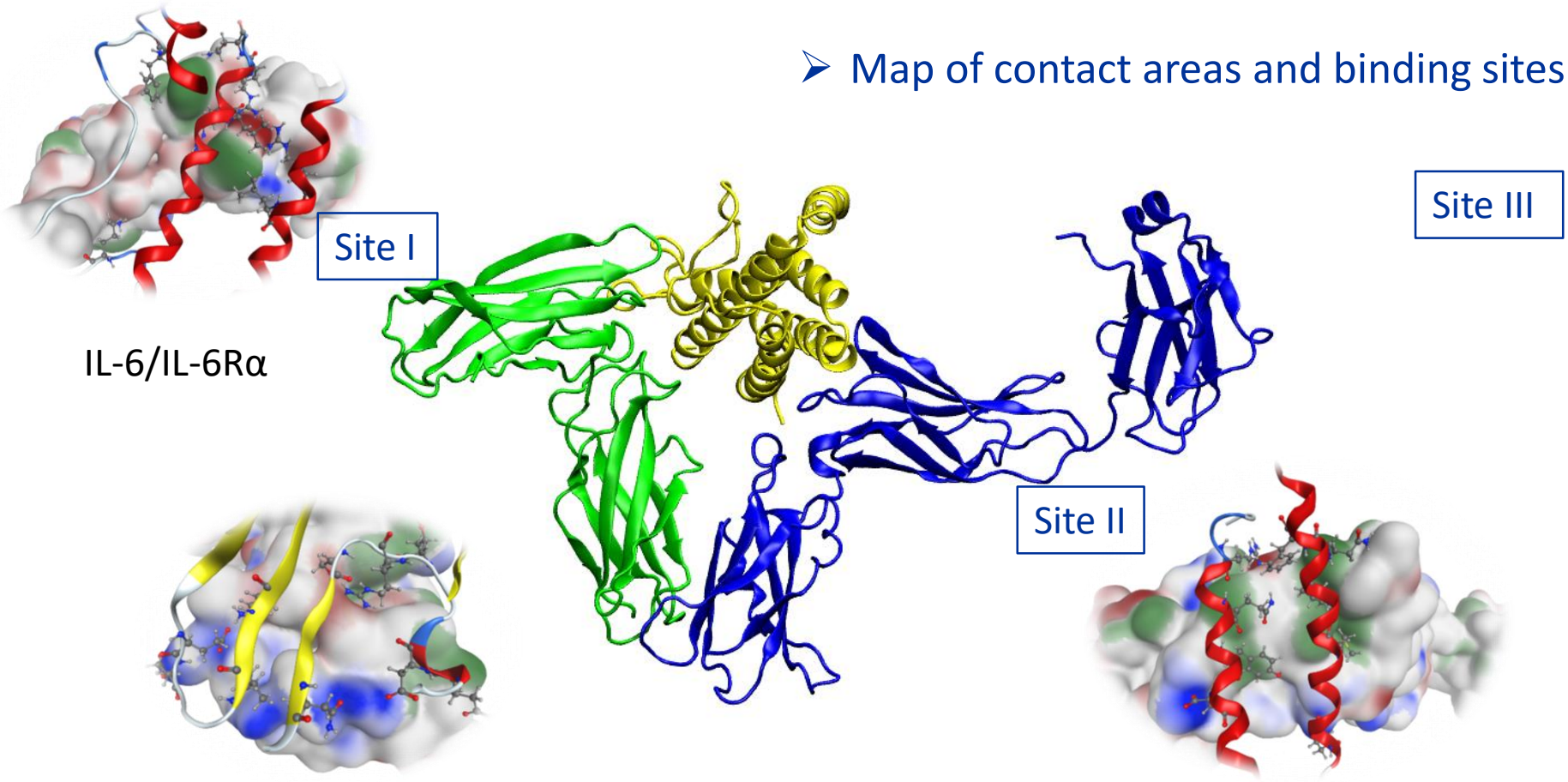
Dipole moments of the hIFNγ–hIFNγR1 complex

IFNγ/heparin complex

LMWH binds to the C-termini of IFNγ with high affinity, forming very stable complexes due to the strong electrostatic attraction. The resulting complex gradually changes its net charge from positive to negative. This impedes further interaction of the cytokine with the extracellular part of the IFNγR1 (also negatively charged) which is the first necessary step in the IFNγ transduction pathway.

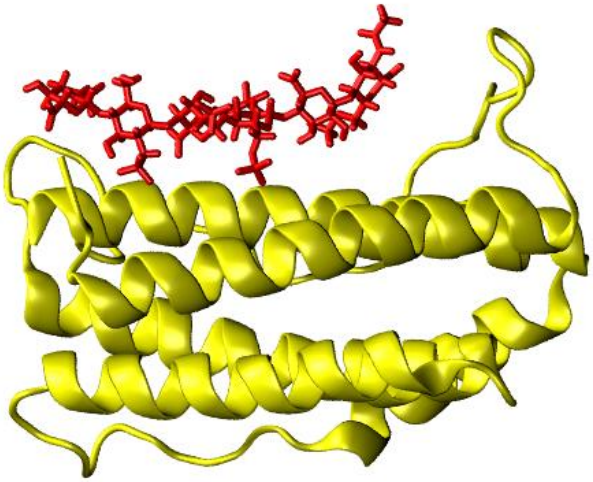
E. Lilkova, N. Ilieva, P. Petkov, M. Rangelov, and L. Litov, AIP Conference Proceedings **2302** (2020) 020003
L. Litov et al., *Heparin as an Anti-Inflammatory Agent.* bioRxiv-223859 (2020) 20 pp.

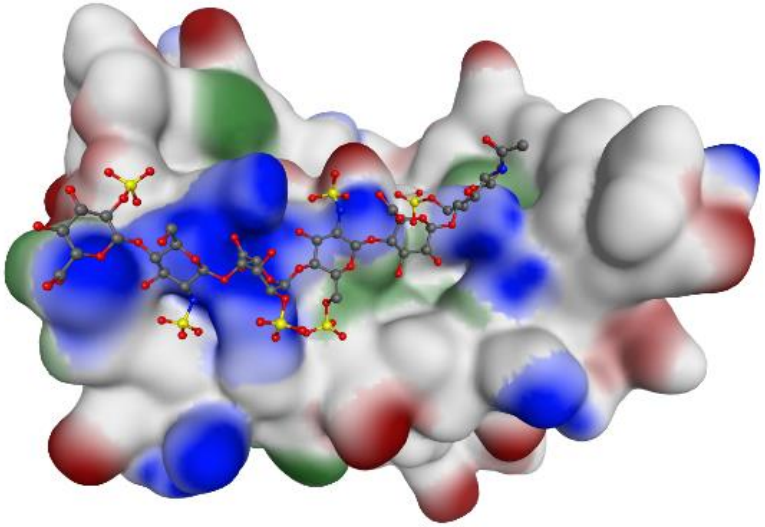
IL-6/IL-6Rα/gp130 complex

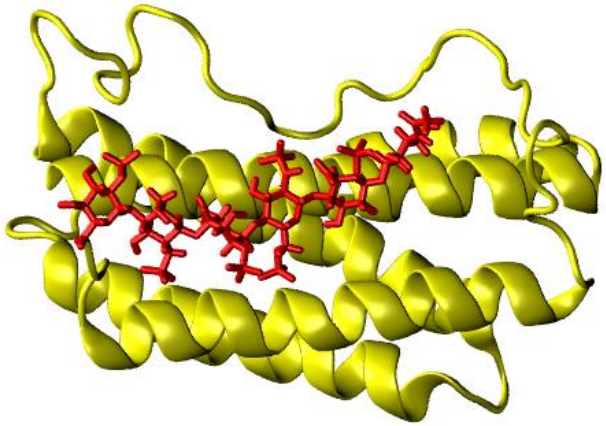


➢ Map of contact areas and binding sites

IL-6/heparin complex



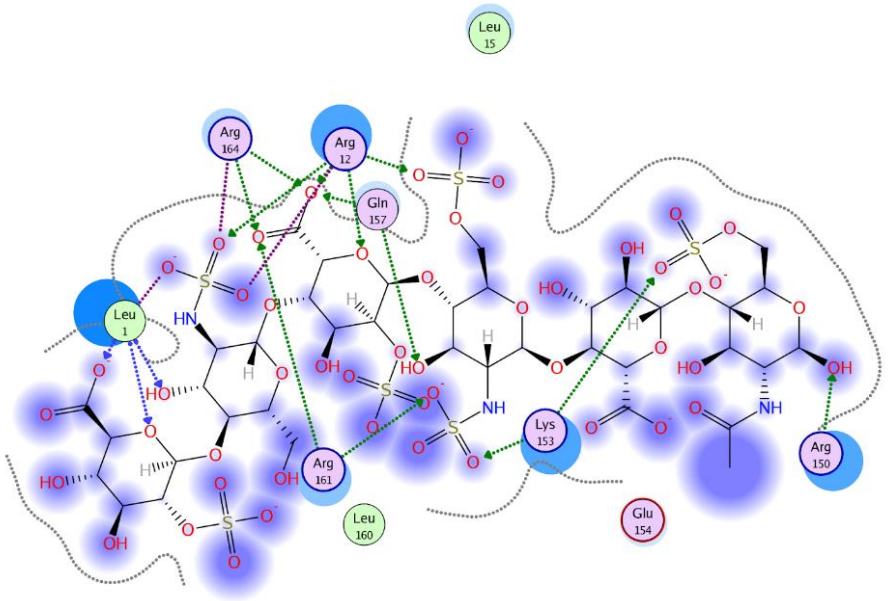




- LMWH binds to IL-6
- No substantial structural changes in IL-6
- Polar interaction, determined by the charge distribution
- Position with high chances for inhibiting the triple complex building

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



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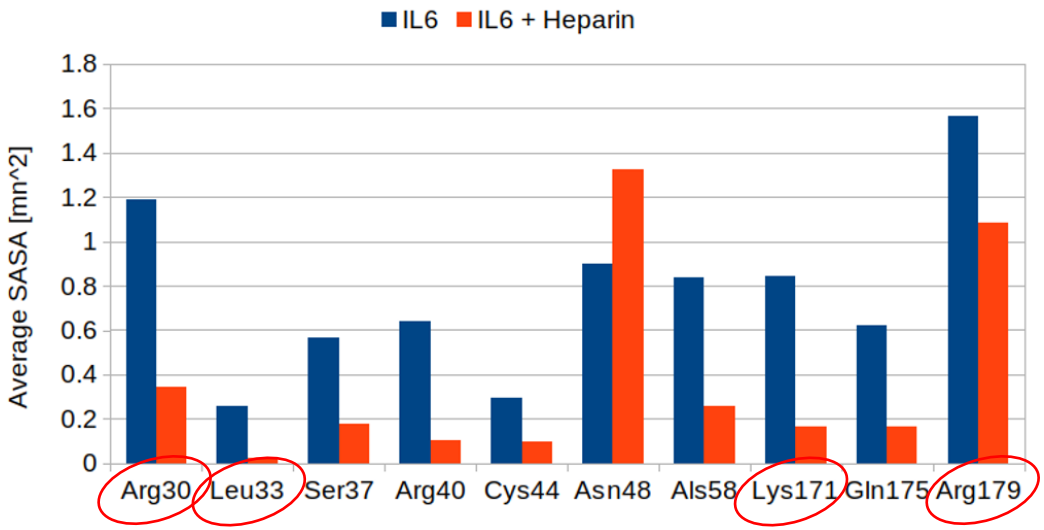
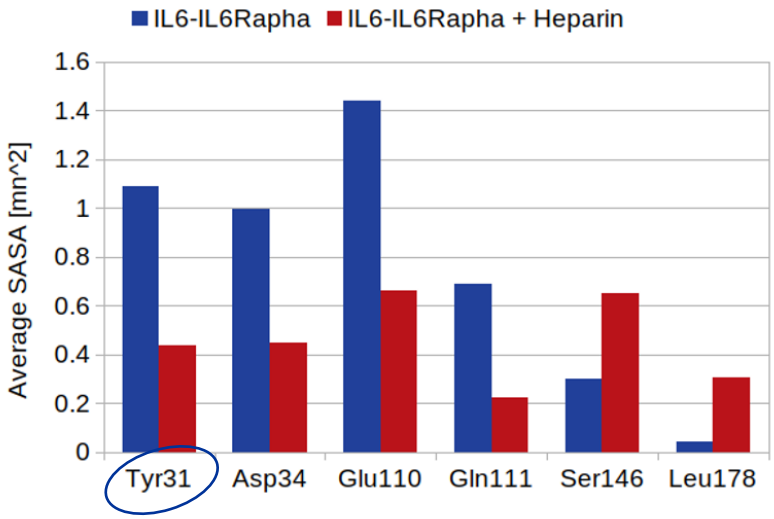
- Position with high chances for inhibiting the triple complex building

IL-6/heparin complex



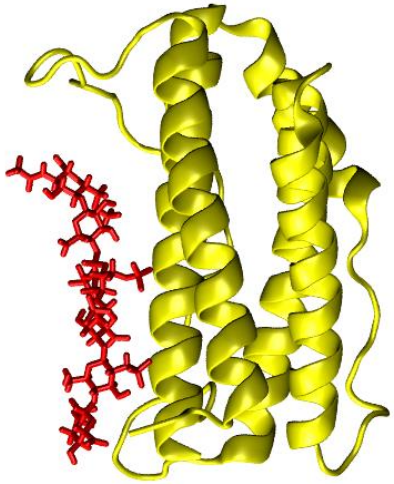


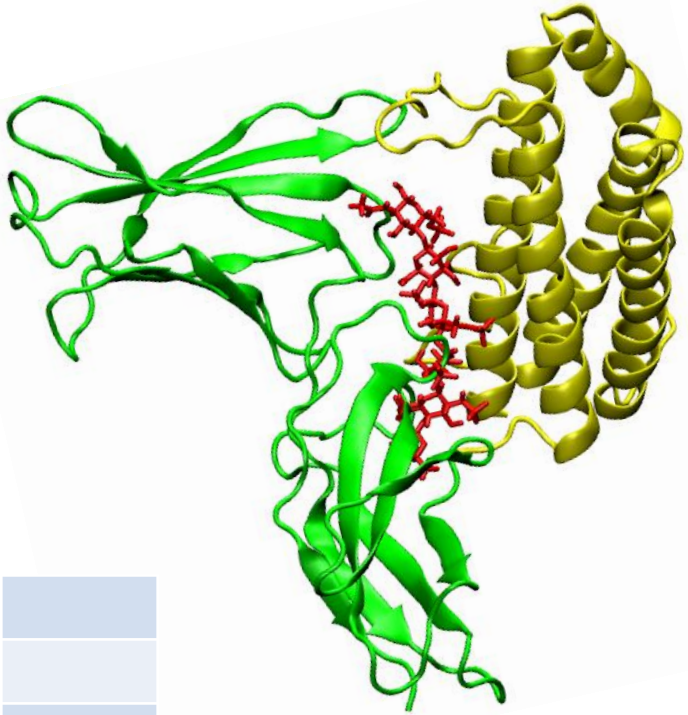
Average SASA values for the most affected through LMWH binding residues *(SASA change exceeds the standard deviation)*

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/heparin/IL-6Rα complex

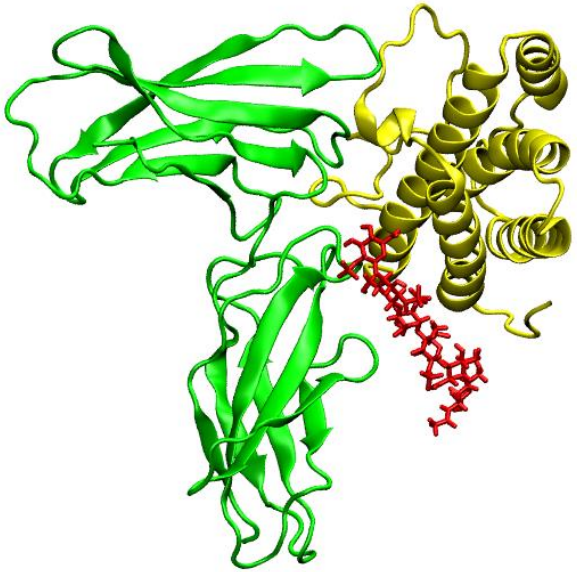


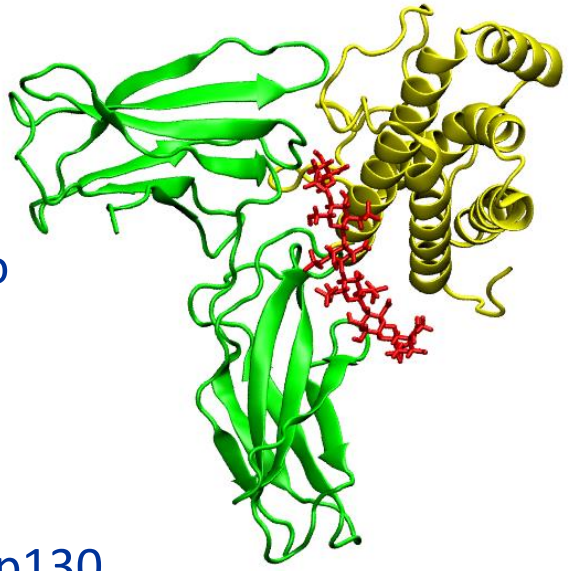


I	IL-6/IL-6R	30Arg	54Lys	33Leu		
	IL-6/IL-6R	178Leu	179Arg	182Arg	183Gln	
II	IL-6/gp130	24Arg	28Gln	31Tyr	34Asp	
	IL-6/gp130	110Glu	117Met	121Val	124Gln	125Phe
	HP/IL-6	19Leu	40Arg			
	HP/IL-6	171Lys	179Arg	182Arg		

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

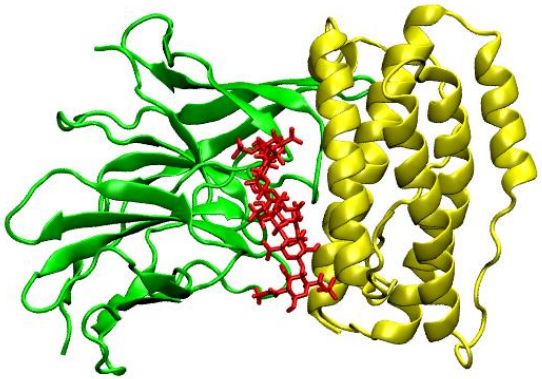
IL-6/IL-6Rα + heparin complex

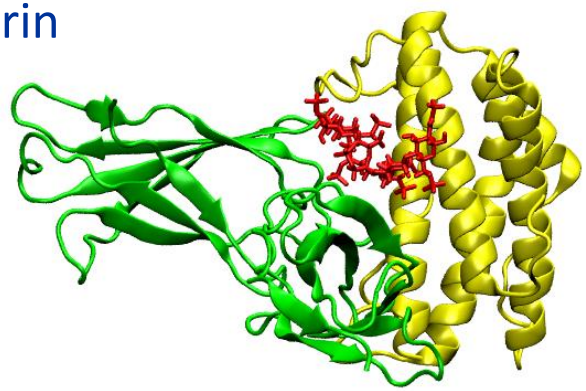




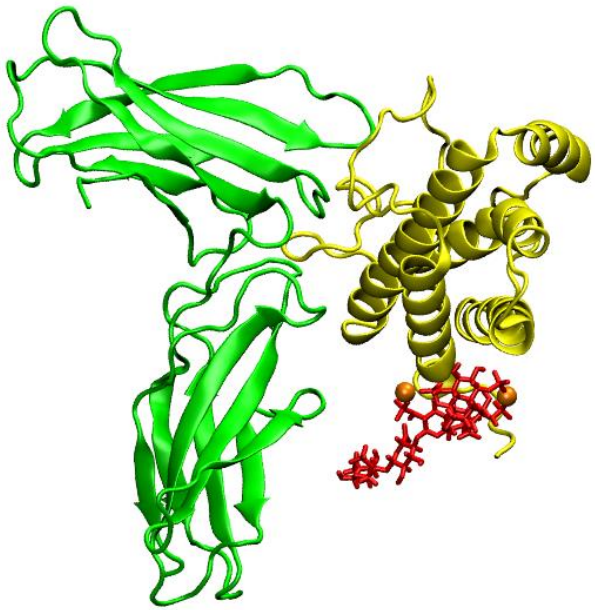
- Molecular docking to place the heparin
- MD simulation
- Structure analysis

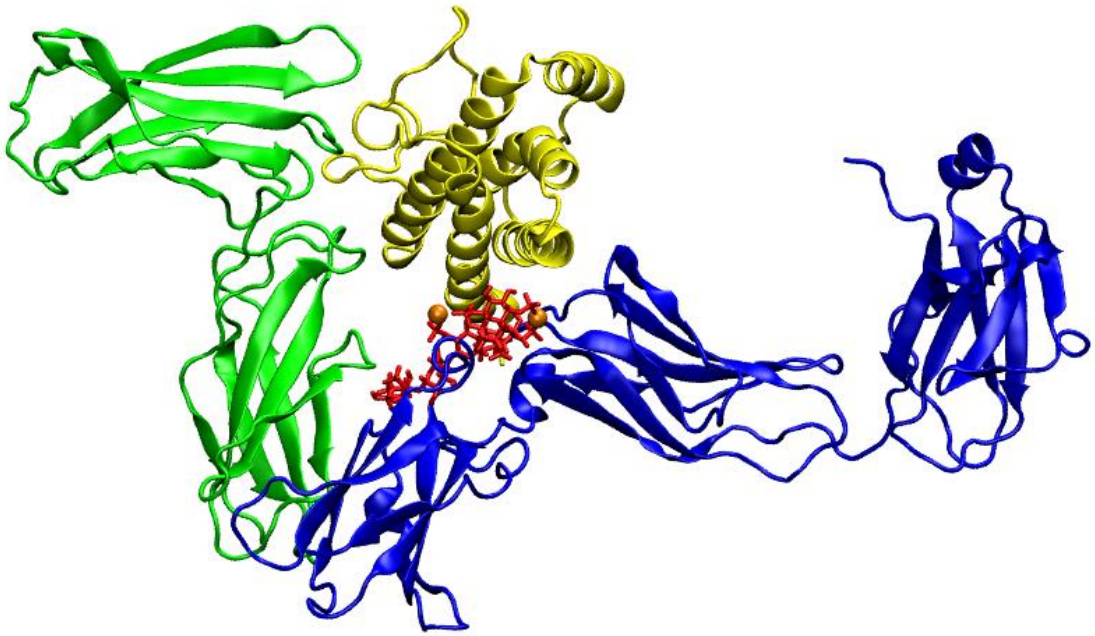
Option I for IL-6/IL-6R/gp130 in the presence of heparin





IL-6/IL-6Rα + heparin + Mg (+ gp130)



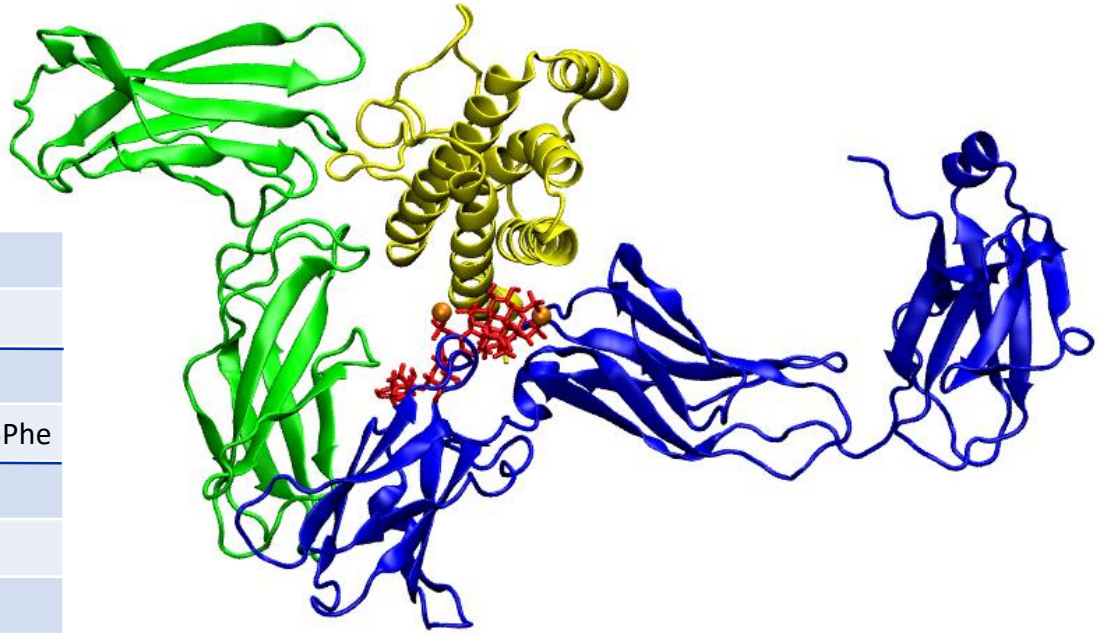


Heparin, in the presence of Mg ions, blocks binding site II (IL-6/gp130) and being positioned in front of helix A, effectively prevents the formation of the biologically active triple complex with gp130

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	



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

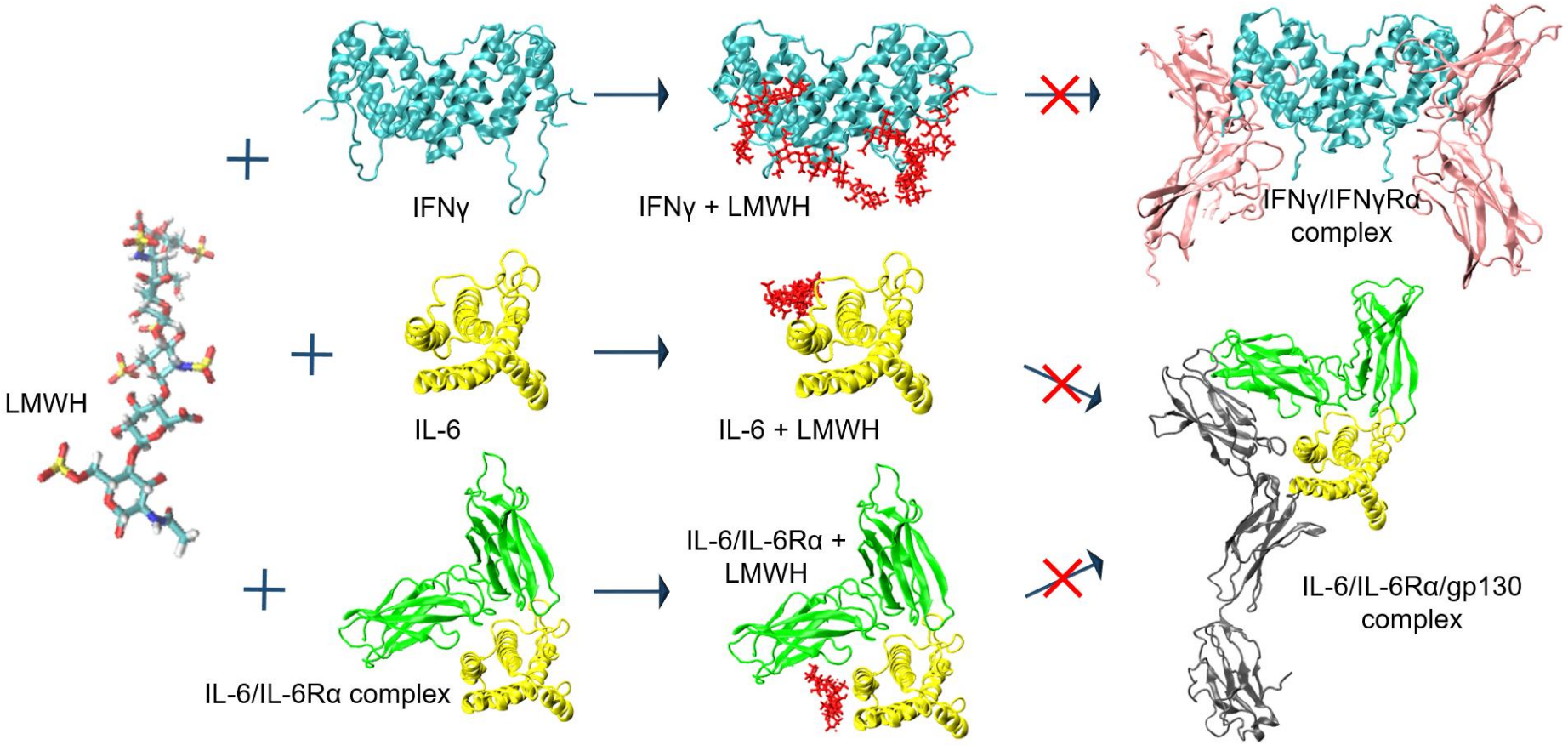
Conclusions

- 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



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

Acknowledgements

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Thank you
for your attention!