# Computational Study of IL-6 Inhibition by Low-Molecular-Weight Heparin

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13th International Conference on "Large-Scale Scientific Computations"

June 10, 2021

# Overview

# Biological background

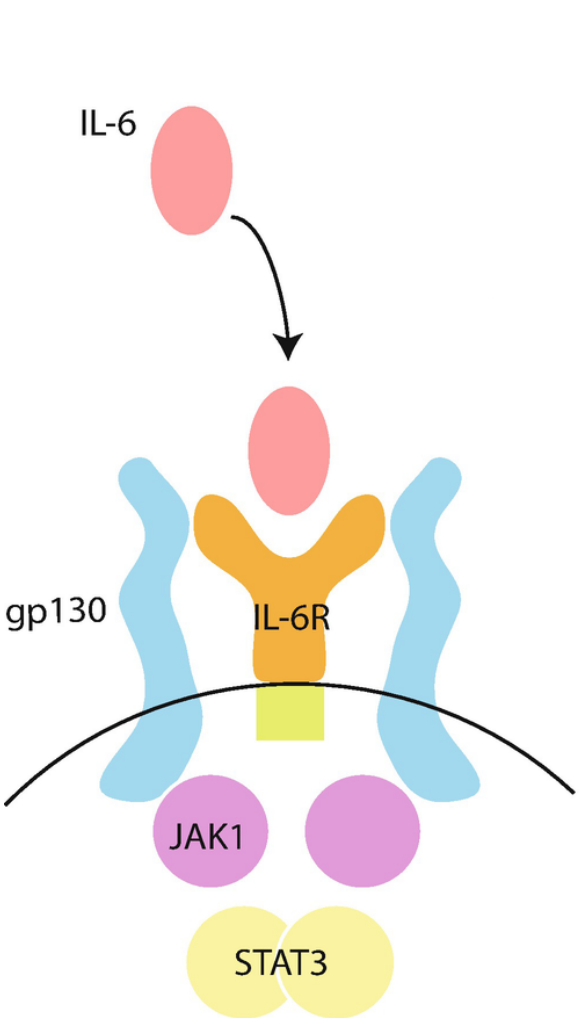
Moderate to severe cases of Covid-19 may suffer from Cytokine Release Syndrome (CRS):

- Large numbers of white blood cells are activated and release pro-inflammatory cytokines.
- This activates more white blood cells in a positive feedback loop of pathogenic inflammation.
- The dysregulated release of pro-inflammatory cytokines can be life-threatening and lead to systemic hyper-inflammation, hypotensive shock, and multi-organ failure.

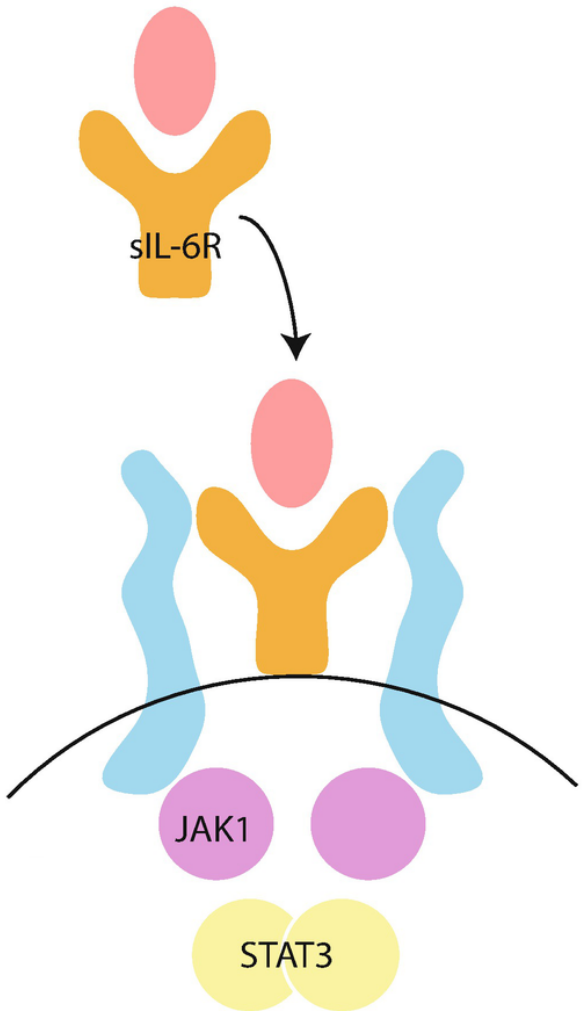
# Interleukine 6 (IL-6)

- Pleiotropic signalling molecule with both pro- and anti-inflammatory functions.
- Produced in response to tissue damage and infections.
- Involved in inflammation, including stimulation of acute phase protein synthesis, regulation of immune response and hematopoiesis.
- 212 amino acids, organized in an $\alpha$-helical bundle of 4 $\alpha$-helices and two long loops.
- Very low levels under normal conditions, but can raise many thousandfold during inflammation.
- Systemic overexpression of IL-6 playing pathological role in chronic inflammation, autoimmunity and cancer.

# IL-6 signaling




Cis-signaling




Trans-signaling

# Unfractionated heparin

- Naturally occurring glycosaminoglycan;
- Used as an anticoagulant to prevent the formation of clots and extension of existing clots within the blood;
- Native heparin molecular weight ranges from 3 to 30 kDa, but pharmacological unfractionated heparin weights 12–15 kDa;
- Heparin is a polymer of repeating disaccharide units of $1 \rightarrow 4$ linked uronic acid ($\beta$-D-glucuronic or $\alpha$-L-iduronic acid) and D-glucosamine (N-acetylated or N-sulfated);
- Each saccharide monomer can be further 2-, 3- or 6-O-sulfated;
- This makes heparin one of the biological macromolecules with the highest charge density in the body.

# Low Molecular Weight derivatives of Heparin (LMWH)

- Low molecular weight heparins are produced by chemical or enzymatic depolymerization of unfractionated heparin;
- Average chain molecular weight ranges from 3 to 8 kDa;
- Different manufacturing processes produce structural variations at the reducing and non-reducing ends of the carbohydrate chains;
- In addition to their anticoagulant properties, in recent years LMWH attract research interest for their anti-inflammatory effects.

# Molecular dynamics

Integrate Newton's equations of motion:

$$m_i \frac{d^2 \mathbf{r}_i}{dt^2} = \mathbf{F}_i \tag{1}$$

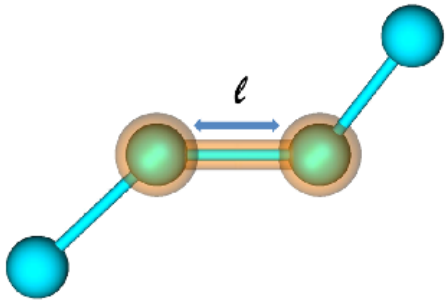
$$\mathbf{F}_i = -\nabla_i U(\{\mathbf{r}_i\}) \tag{2}$$

The potential energy function is called force field:

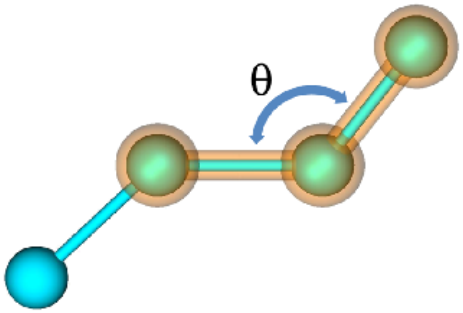
$$\begin{aligned} U(\{\mathbf{r}_i\}) = & \sum_{bond} K_l (l - l_0)^2 + \sum_{angle} K_\theta (\theta - \theta_0)^2 + \\ & + \sum_{torsion} K_\phi (1 + cos(n\phi - \delta)) \\ & + \sum_{i=1}^{N} \sum_{j=i+1}^{N} \left( 4\varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \right) \end{aligned} \tag{3}$$

# Molecular mechanics force field

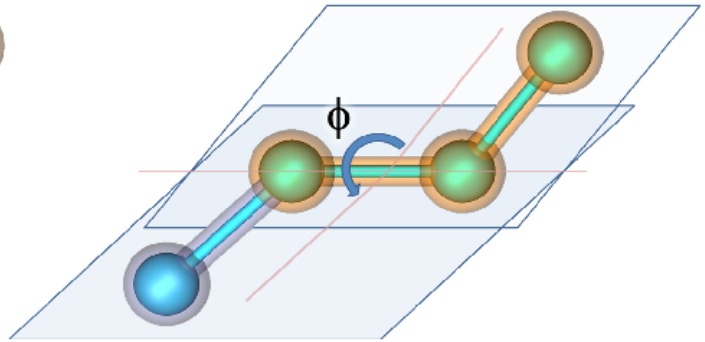
$$V_b = k_b (l - l_0)^2$$

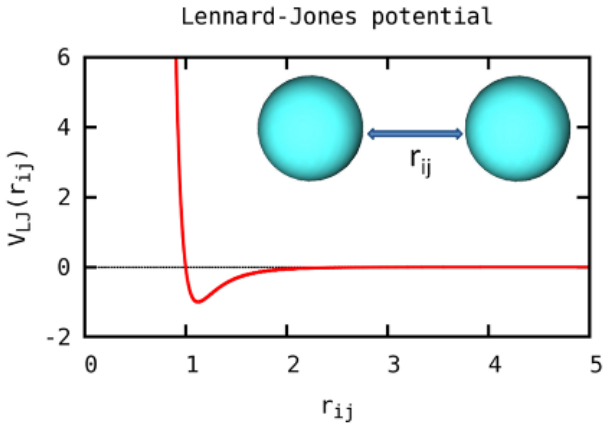


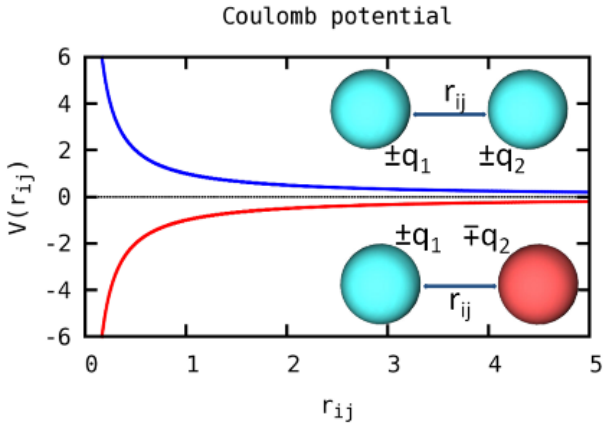

$$V_a = k_a (\theta - \theta_0)^2$$




$$V_t = 1/2 V_n [1 + cos(n\phi - \phi_0)]$$



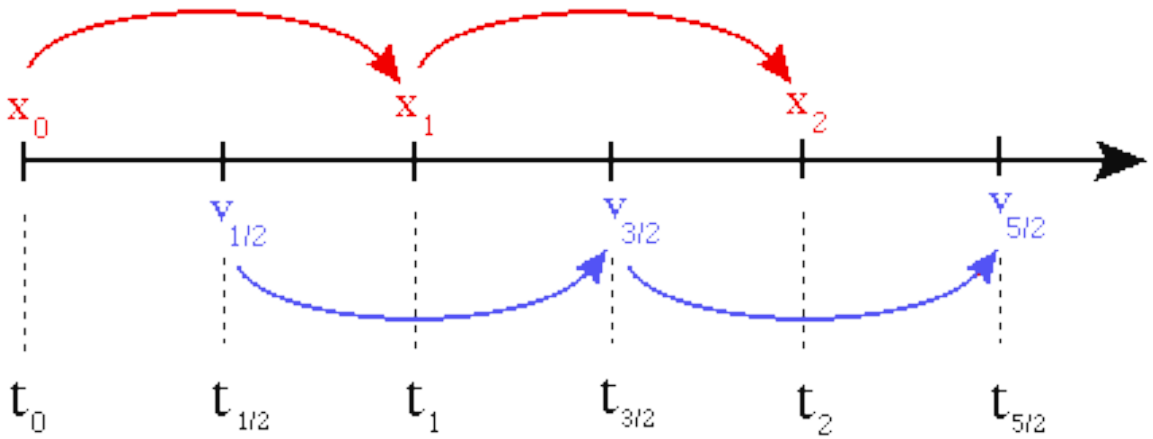




$$V_{vdw} = 4\varepsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^{6} \right]$$

$$V_{coul} = \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}$$

# Leap frog integrator

$$\mathbf{v}_i^{n+1/2} = \mathbf{v}_i^{n-1/2} + \frac{\mathbf{F}_i^n}{m_i}\Delta t + O\left(\Delta t^3\right)$$
$$\mathbf{r}_i^{n+1} = \mathbf{r}_i^n + \mathbf{v}_i^{n+1/2}\Delta t + O\left(\Delta t^3\right) \quad (4)$$



# Protein structures

IL-6:

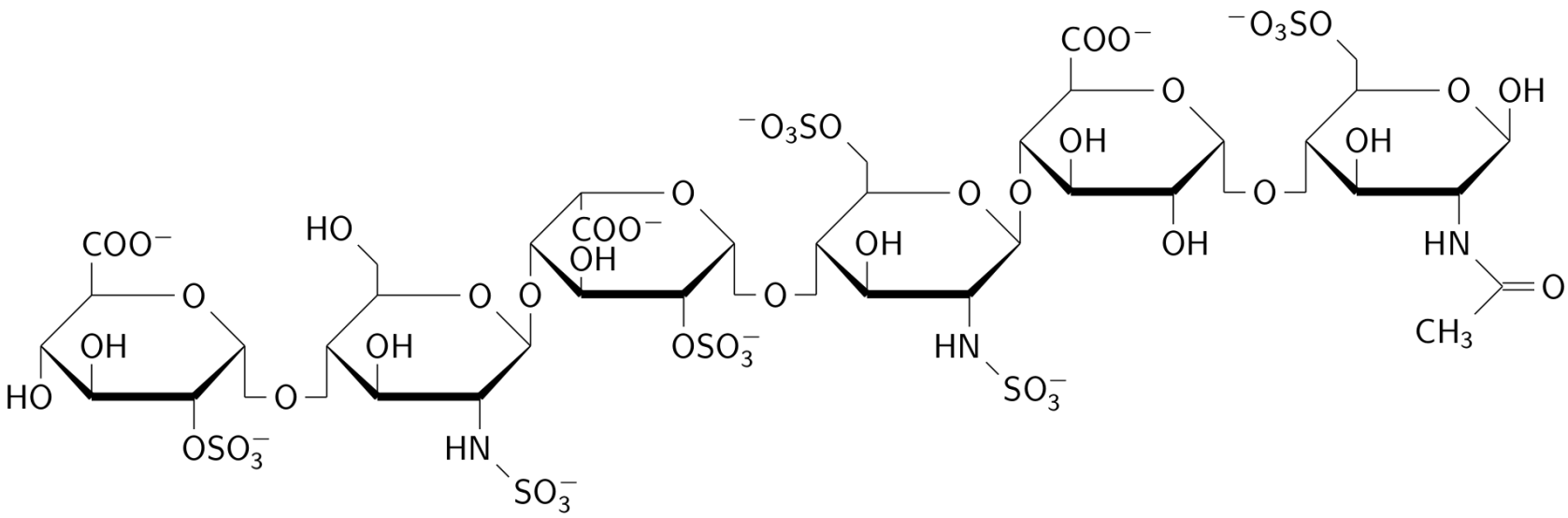
- PDB ID 1ALU;
- The missing residues, $^{52}$SerSerLysGluAlaLeuAlaGluAsn$^{60}$, added using the loop-modeling interface to MODELLER of UCSF Chimera;
- 10 ns equilibration.

IL-6/IL-6R$\alpha$ complex:

- PDB ID 1P9M;
- 10 ns equilibration.

# LMW heparin model

- Based on a literature review, the following hexasaccharide sequence was chosen as a model molecule for general LMWH: GlcNAc(6S) (1→4) GlcA (1→4) GlcNS(6S) (1→4) IdoA(2S) (1→4) GlcNS (1→4) GlcA(2S).
- Each oligosaccharide chain has a net charge of -9*e*.



# LMW heparin model

- The Glycan Reader & Modeler module of the CHARMM-GUI server was used for generation of a three-dimensional structure, corresponding to the chosen carbohydrate sequence, as well as topology using the latest version of the CHARMM36 carbohydrate force field.
- The topology was converted to a GROMACS-compatible topology using the parmed module of Ambertools 16.

# LMWH-protein starting structures

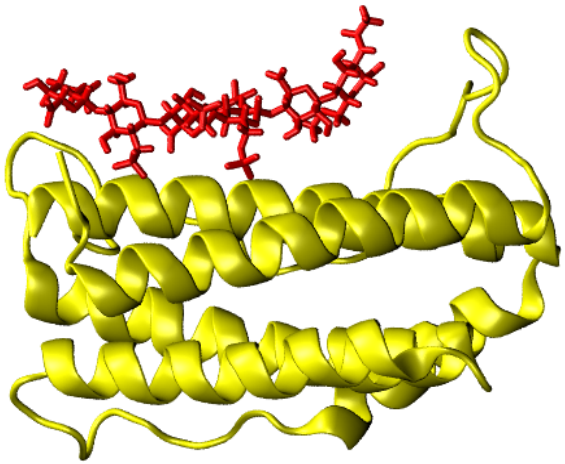
- The MOE software package used for preparation of the initial structures of the complexes of IL-6 and LMWH, and IL-6/IL6R$\alpha$ and LMWH.
- The oligosaccharide was docked in IL-6 and IL-6/IL6R$\alpha$ complex in proximity to IL-6 binding sites I and II.

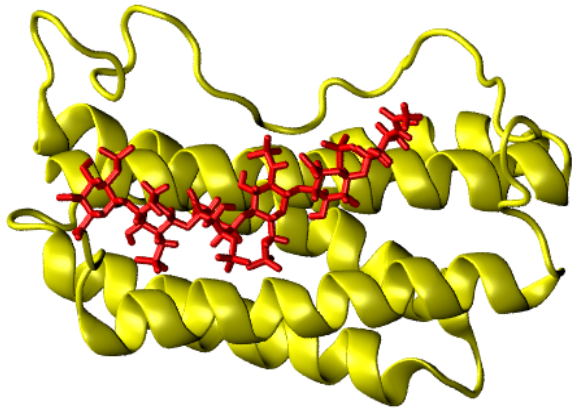
# Production run protocol

- GROMACS MD simulation package, version 2016.3;
- CHARMM36 force filed + modified TIP3P water model;
- Rectangular simulation boxes with 2 nm to the edges;
- Periodic boundary conditions;
- Constraints on all bonds;
- Leapfrog integrator with 2 fs timestep;
- NPT ensemble at 310K (v-rescale, $\tau_T = 0.25 ps^{-1}$) and 1 atm (Parrinello-Rahman, $\tau_P = 1.0 ps^{-1}$);
- PME electrostatics ($r_{coloumb}$=1.2nm) + shifted VdW ($r_{switch}$=0.1 nm, $r_{vdw}$=1.2 nm);

# IL-6–LMWH Complex

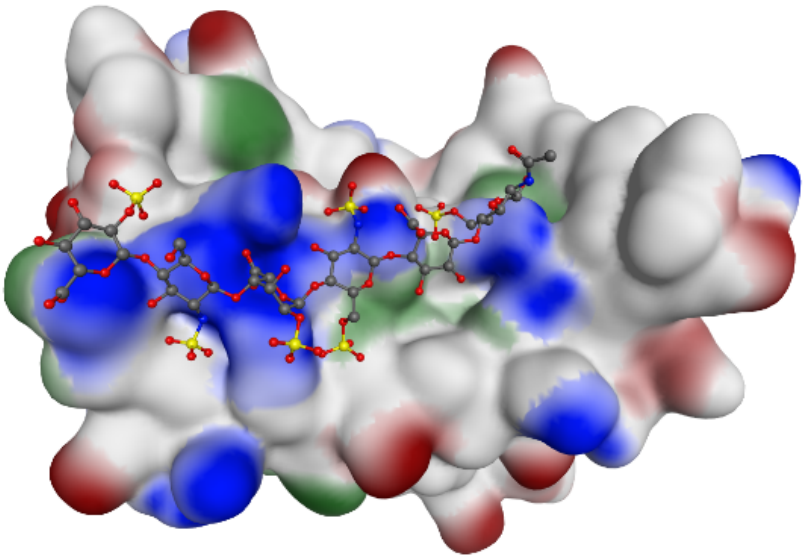
- A stable complex between the cytokine and the oligosaccharide throughout the 250 ns MD run.
- The carbohydrate binds to IL-6 at $Arg^{24}$, $Lys^{27}$, $Arg^{30}$, $Leu^{33}$, $Ser^{37}$, $Arg^{40}$, $Cys^{50}$, and $Glu^{51}$ from helix A and $Lys^{171}$, $Gln^{175}$, $Arg^{179}$ and $Arg^{182}$ from helix D.





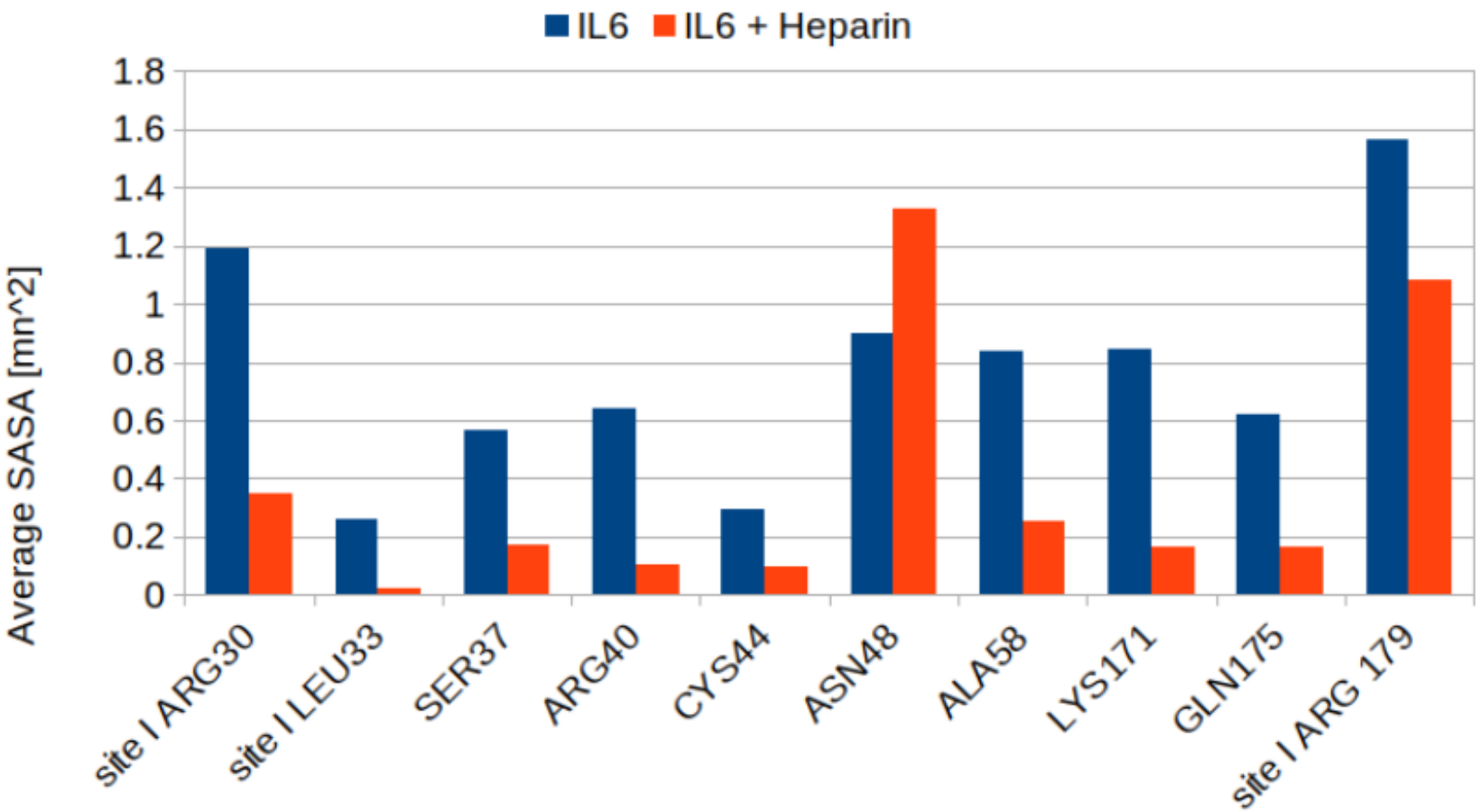
# IL-6 Electrostatic Potential upon LMWH binding

The complex is stabilised through a large number of polar interactions between the positively charged amino acids in the cytokine and the negatively charged sulphates in the LMWH chain.



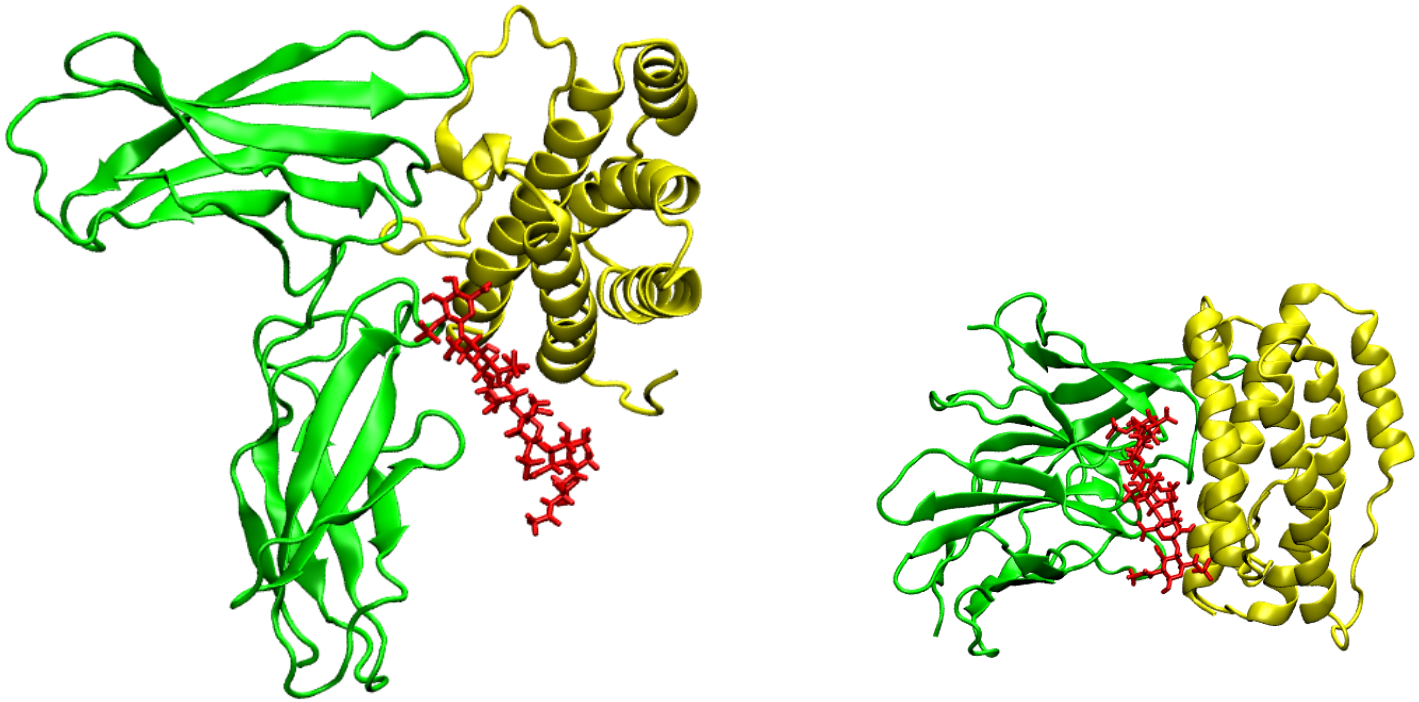
| aa | d [Å] | E [kcal/mol] |
|---|---|---|
| $Leu^{19}$ | 2.29 | -11.4 |
| $Arg^{182}$ | 2.31 | -11.2 |
| $Arg^{30}$ | 2.35 | -10.6 |
| $Lys^{171}$ | 2.36 | -10.5 |
| $Arg^{30}$ | 2.39 | -10.1 |
| $Lys^{171}$ | 2.44 | -9.5 |
| $Arg^{30}$ | 2.46 | -9.3 |
| $Arg^{179}$ | 2.48 | -9.0 |
| $Arg^{182}$ |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

# IL-6 SASA upon LMWH binding



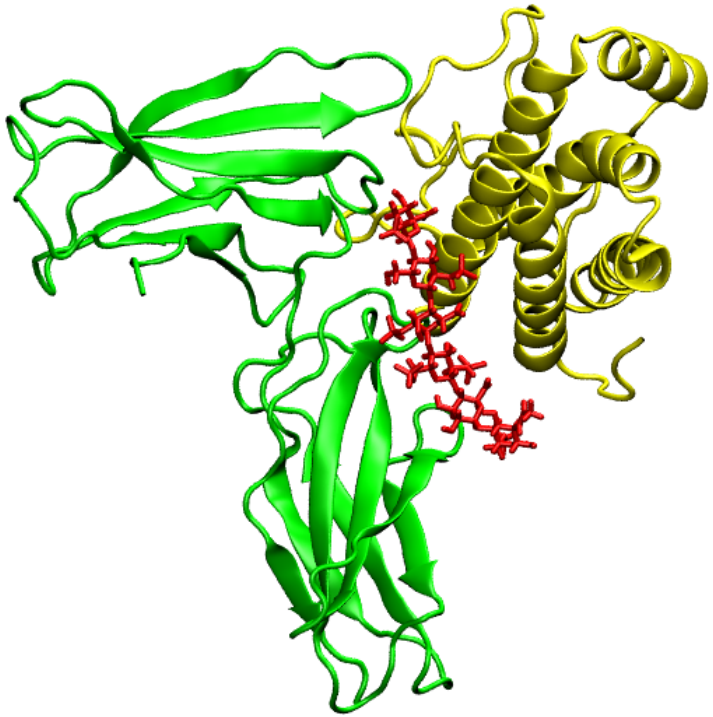
# IL-6/IL-6R$\alpha$–LMWH complex position 1

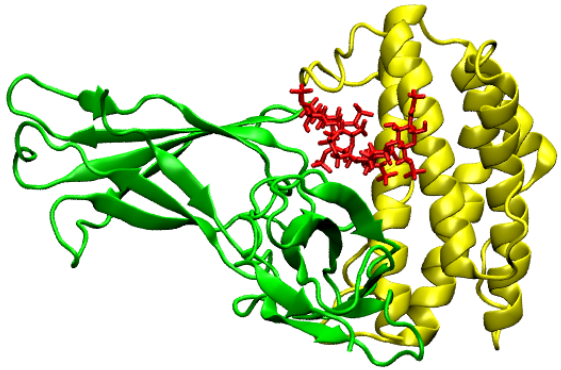
The oligosaccharide is strongly bound to residues $Arg^{40}$, $Lys^{41}$, and $Arg^{168}$ of IL-6, which are not among the direct participants in the complex formation.



# IL-6/IL-6R$\alpha$–LMWH complex position 2

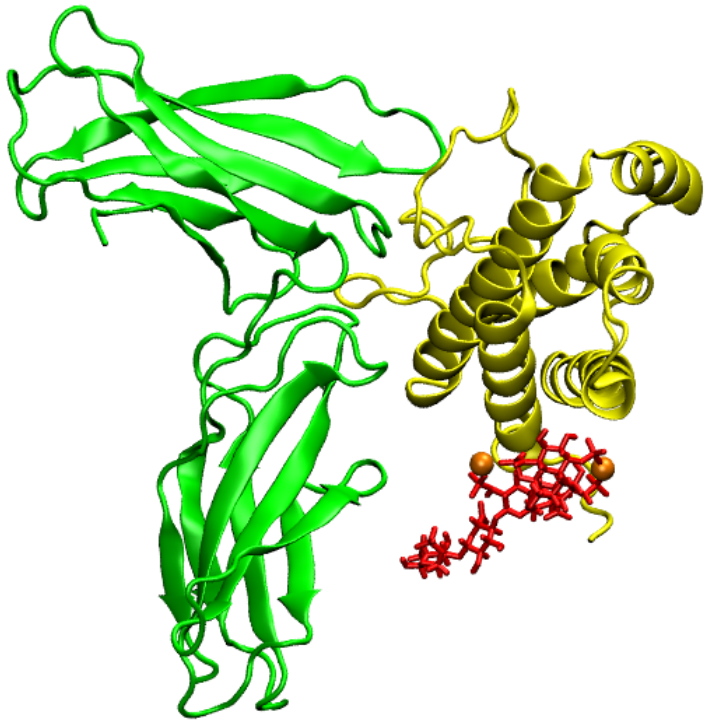
The oligosaccharide binds to residues Arg$^{30}$ of IL-6 helix A, Lys$^{252}$ of IL-6R$\alpha$ and comes in close proximity to Tyr$^{31}$ of IL-6 helix A. This position is unstable during a 250 ns MD run.



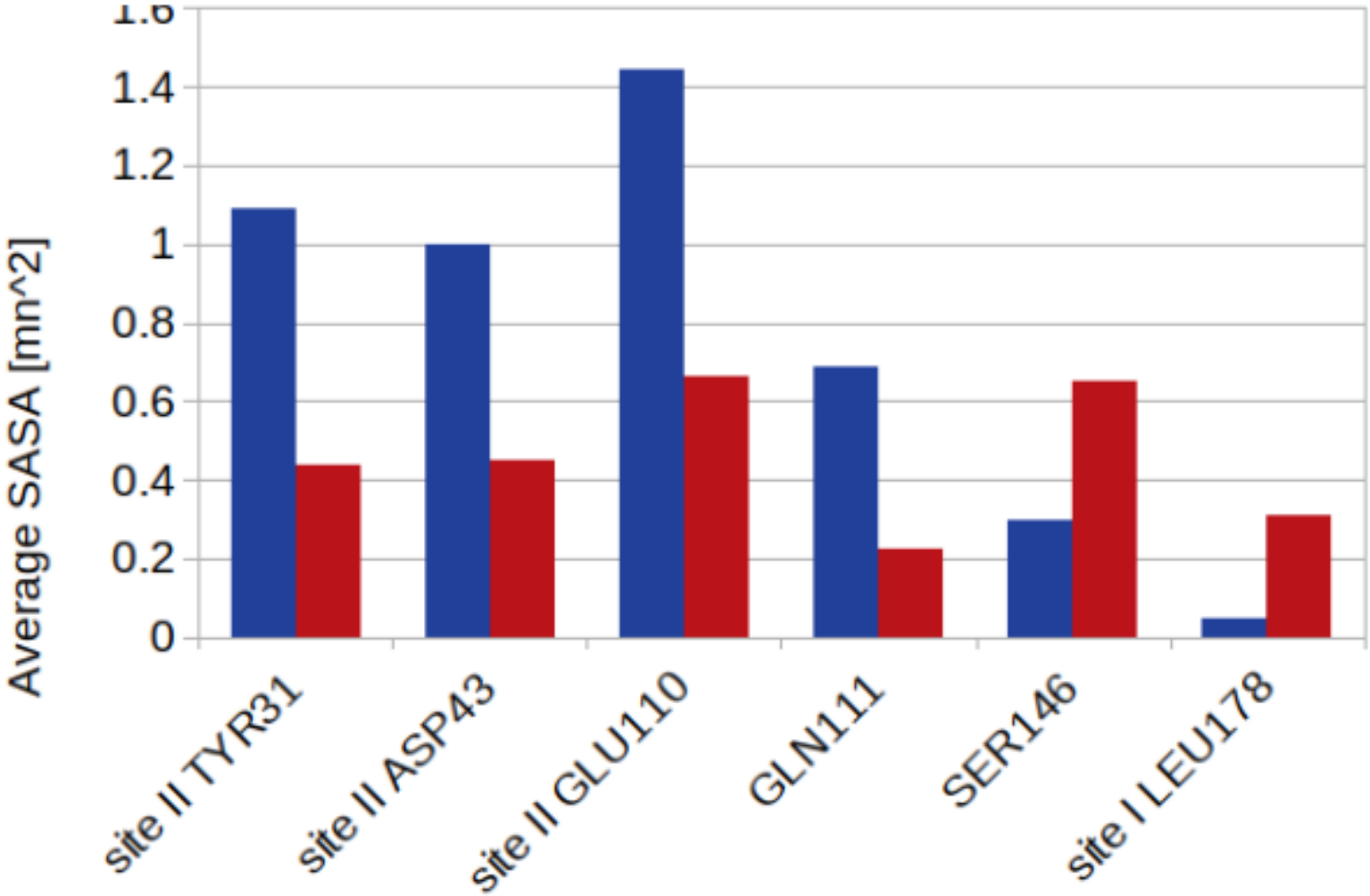


# IL-6/IL-6R$\alpha$–$Mg^{2+}$–LMWH complex

The complex is stabilised when a divalent ion is added to the system, in this case a $Mg^{2+}$.

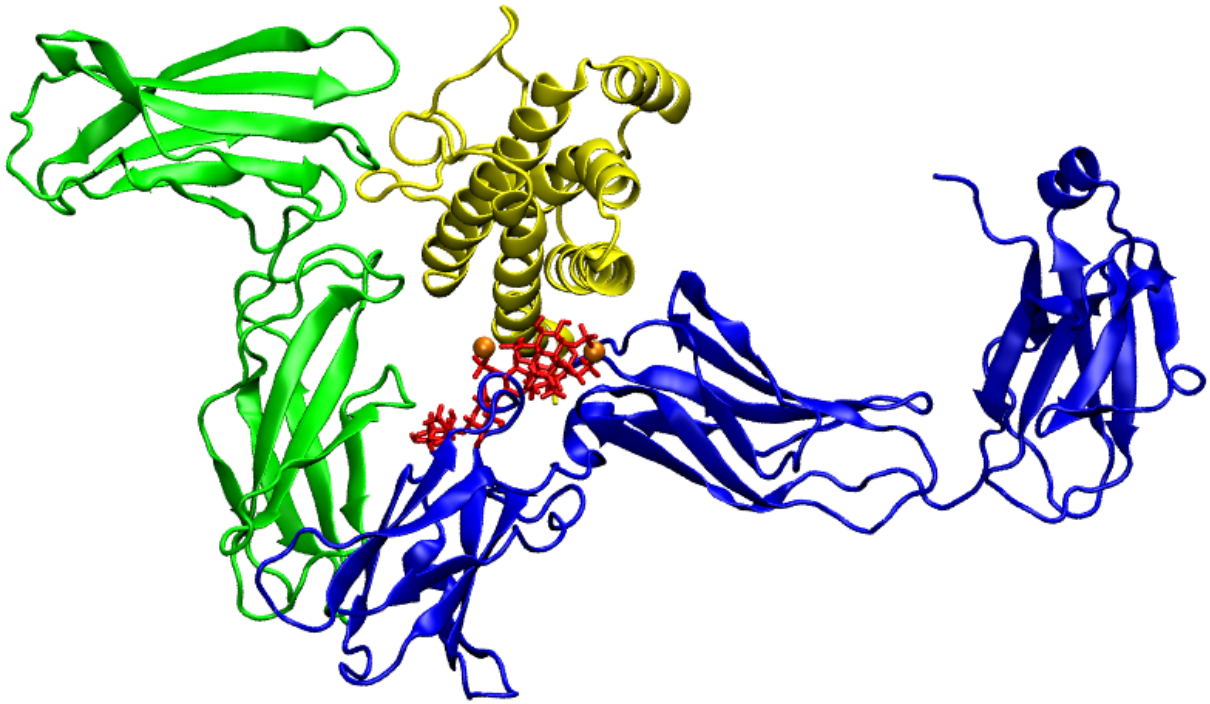


# IL-6 SASA upon LMWH binding to the IL-6/IL-6R$\alpha$/$Mg^{2+}$ complex



# Inhibition of recruitment of gp130 to the IL-6/IL-6R$\alpha$–$Mg^{2+}$–LMWH complex

LMWH, 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 complex with gp130.



# Conclusions

- LMWH oligosaccharides interact with IL-6 by binding to 4 of the 7 aa residues in binding site I, blocking that way its binding to the receptor IL-6R$\alpha$.
- LMWH oligosaccharides interact with the complex IL-6/IL-6R$\alpha$, which prevents further binding of this complex to gp130.
- Hence, the computational modelling results indicate that LMWH could be useful in the treatment of a cytokine storm by inhibiting IL-6 activity, especially in the context of the trans-signalling mechanism.

# Acknowledgments

This work was supported in part by the Bulgarian Science Fund (Grant KP-06-DK1/5/2021) and by the Bulgarian Ministry of Education and Science (contract D01–205/23.11.2018) under the National Scientific Program "Information and Communication Technologies for a Single Digital Market in Science, Education and Security (ICTinSES)", approved by DCM # 577/17.08.2018.

Computational resources were provided by the BioSim HPC Cluster at the Faculty of Physics at Sofia University "St. Kl. Ohridski".


# Thank You!