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Computational Dissection of the Cooperative Effects of Fc Glycosylation and Light Chain Isotype on IgG1::FcγRIIIa Recognition

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Immunoglobulins are glycoproteins composed of:

- Two identical **heavy chains** (HCs)
- Two identical **light chains** (LCs)

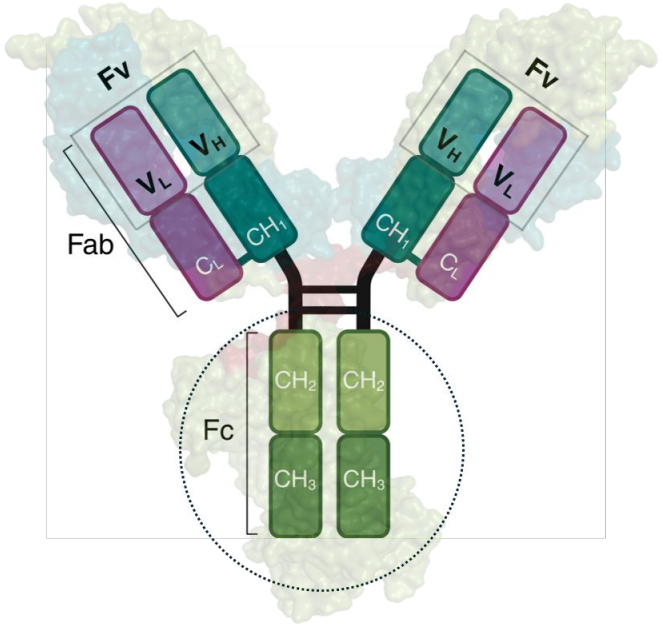
linked through **disulphide bonds** between conserved cysteines

Functional components:

- two identical fragment antigen-binding (Fab) domains
- the fragment crystallizable (Fc) region

The ability of IgGs to activate immune system by interaction with FcγRs is regulated by **N-glycosylation** occurring in the Fc.

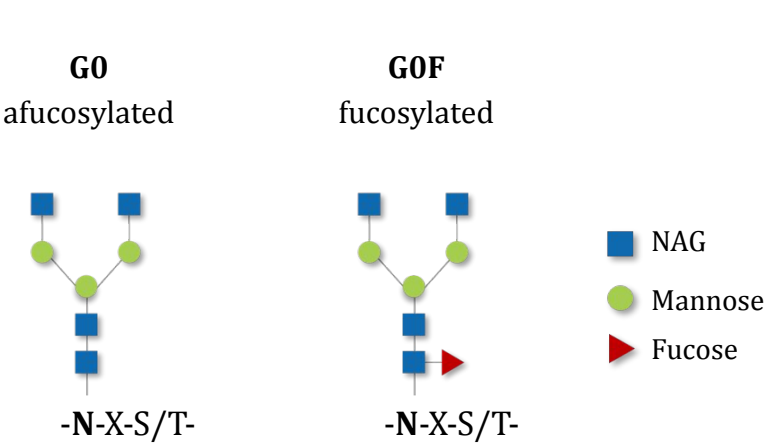
Target recognition

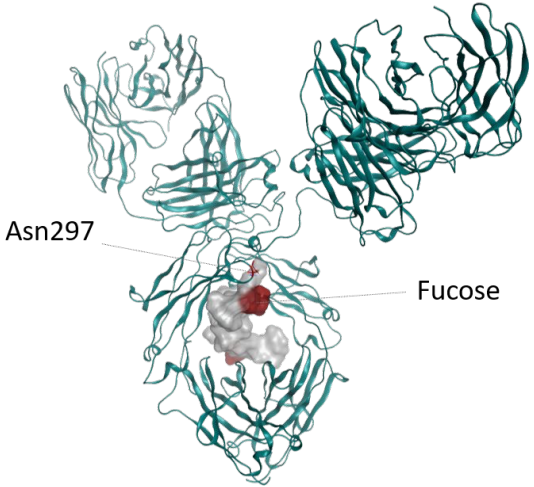


Interaction with FcγRs

Introduction: The Fc region and N-glycosilation

- **Asn297** is a universally conserved N-linked glycosylation site since it is located in a specific N-X-S/T motif.
- The composition of Asn297 glycans influence the quaternary structure of the Fc.
- **Core fucosylation decreases the binding affinity of IgG1s to FcγRIIIa**.



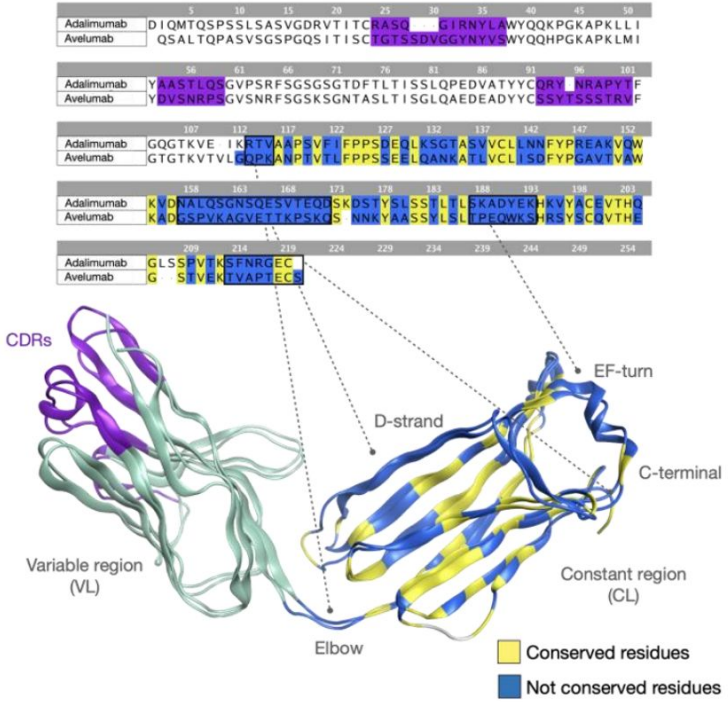


Introduction: The light chain (LC) isotype

Two LC isotypes have been identified: **κ** and **λ**.

- κ and λ LCs share **similar 3D structures, but they exhibit several differences**: e.g., different physical-chemical properties within the CDRs.

- Previous computational works showed indeed that the LC isotype can modulate the Fab rotation and Fc opening.



Aim and workflow of the study

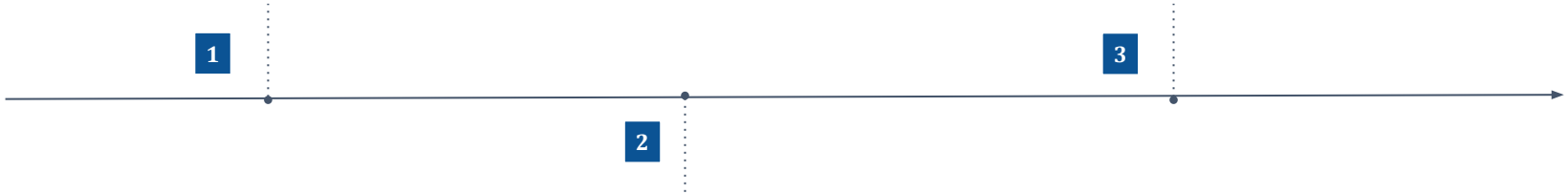
The aim of this work is to study, through *in silico* approaches, the impact of core fucosylation and LC isotypes on the interaction between IgG1s and FcγRIIIa.

Case study:

Adalimumab (directed against TNF-α) and **avelumab** (directed against PD-L1) in both **G0** and **G0F** states.

Isolation and selection of antibody structures suitable for FcγRIIIa binding

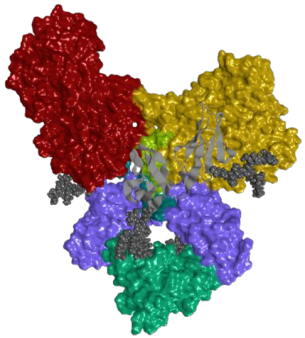
Classical MD simulations & analysis of the trajectories



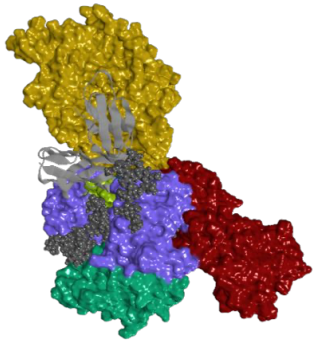
Generation of mAb::FcγRIIIa complexes
starting from aMD-derived simulations

Generation of mAb::FcγRIIIa complexes

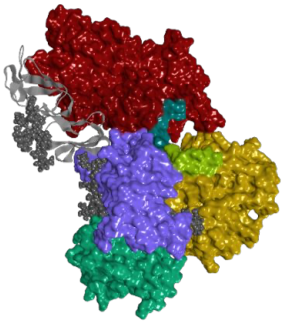
The mAb::FcγRIIIa complexes have been generated by structural superposition of the Fc region to the Fc-FcγRIIIa structure previously identified by Ferrara *et al.* in 2011.



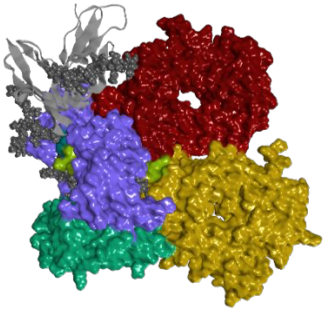
G0 adalimumab::FcγRIIIa



G0F adalimumab::FcγRIIIa



G0 avelumab::FcγRIIIa



G0F avelumab::FcγRIIIa

Fab1 Fab2 Hinge1 Hinge2 CH2 CH3

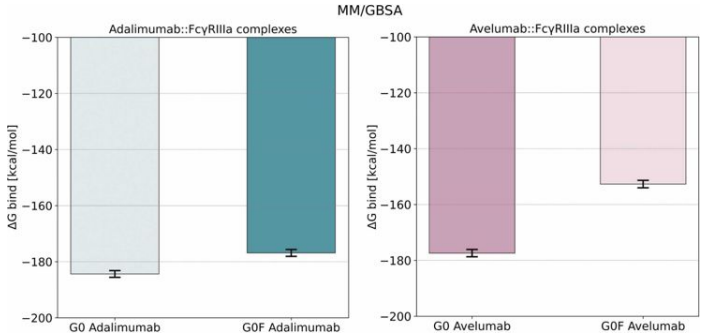
Glycans

Ferrara C, et al. Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcγRIII and antibodies lacking core fucose. Proc Natl Acad Sci U S A. 2011;108(31):12669-12674. doi:10.1073/pnas.1108455108

Complexes characterization

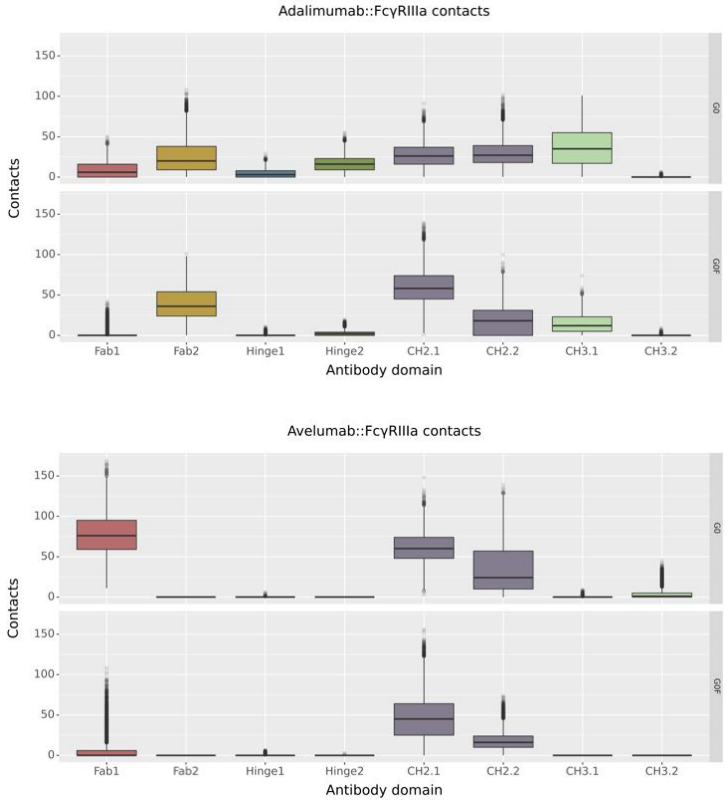
LC impact

- FcγRIIIa establishes a **broad set of contacts with adalimumab**,
- Avelumab: FcγRIIIa interacts only with one Fab and the CH2 domain, indicating reduced interface diversity and potentially lower complex stability

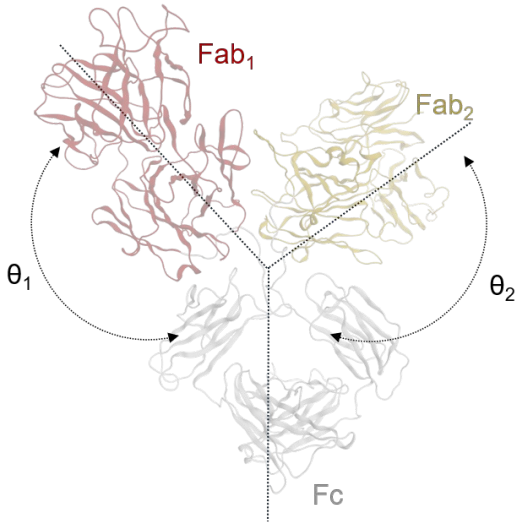


Glycosylation impact

- Based on MM/GBSA, fucosylated antibodies appear to be less stable than the afucosylated ones

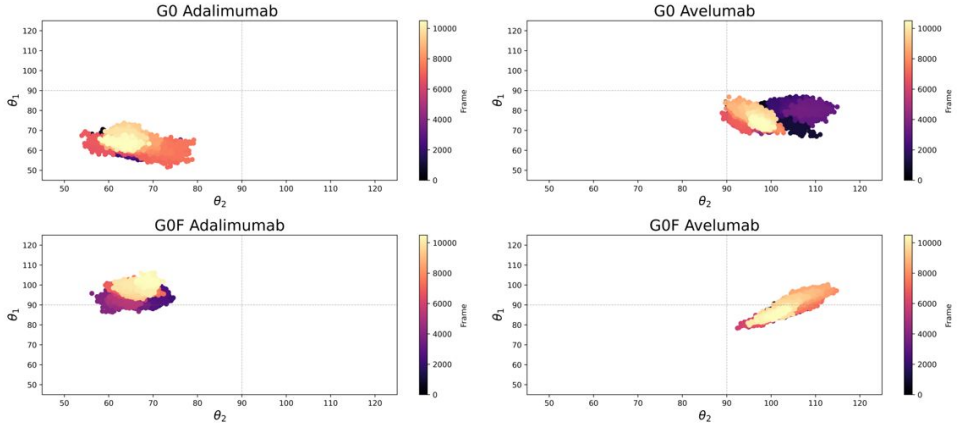


Not only Fc but also **Fab domains positively contribute to the stability of the interaction with FcγRIIIa**.



θ < 90° Y conformation

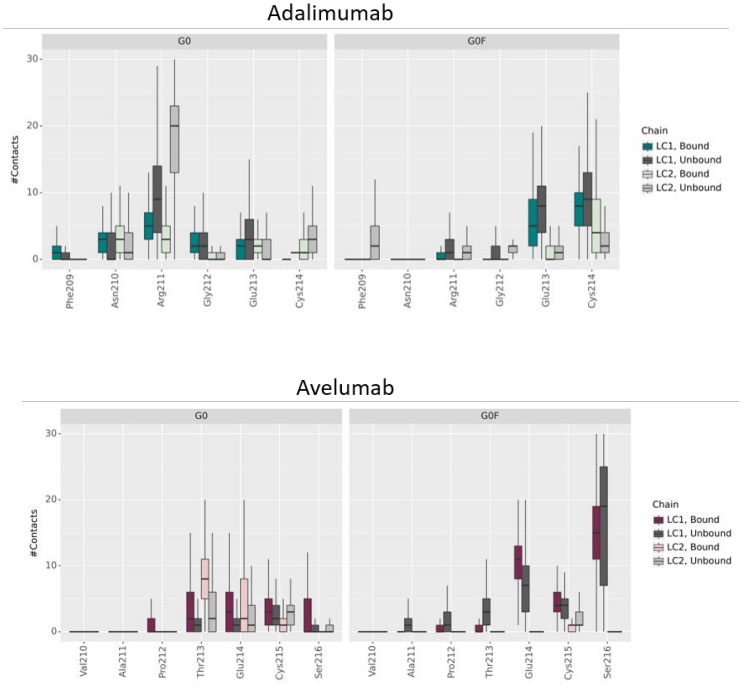
θ > 90° T conformation



θ_1 and θ_2 remained stable for all mAbs:

- **Y-shaped conformation** for G0 adalimumab
- **T-shaped conformation** for the G0F state, as well as in avelumab regardless of glycosylation state.

The hinge behavior is modulated by the LC isotype



Hinge-LC interaction network

- κ-LC: hinge-LC contacts decrease → hinge rearrangement facilitates FcγRIIIa engagement.
- λ-LC: mAbs maintain or increase these contacts → rigid architecture that limits hinge mobility.

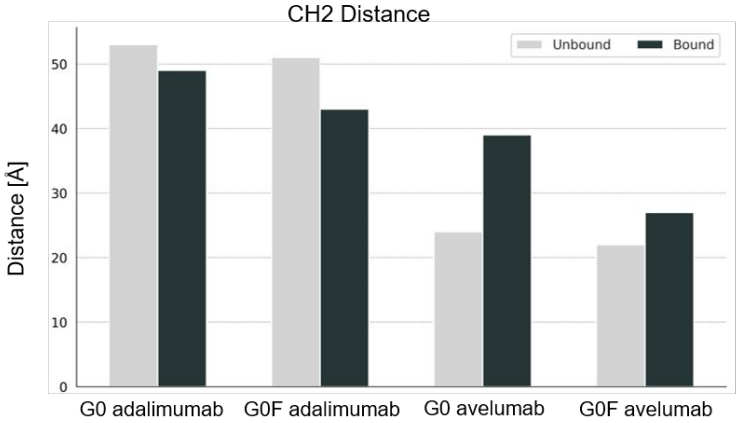
Changes in solvent exposure

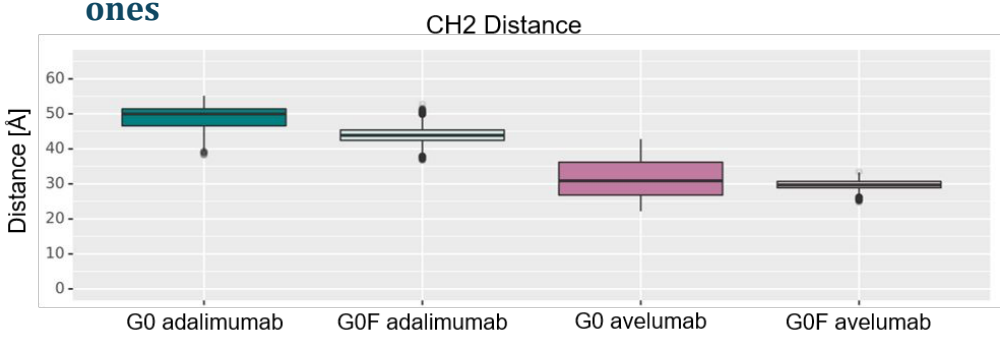
- Adalimumab: the lower exposure is due to the interaction with FcγRIIIa,
- Avelumab: SASA values are related to the antibody conformation, that hinders the interaction with FcγRIIIa.

Conformational analysis

Despite the change in the conformation upon binding, **the Fc of the G0F antibodies is more closed compared to G0 ones**

- The distance between the CH2 domains is higher in adalimumab compared to avelumab, with differences among the glycosylation states in adalimumab.





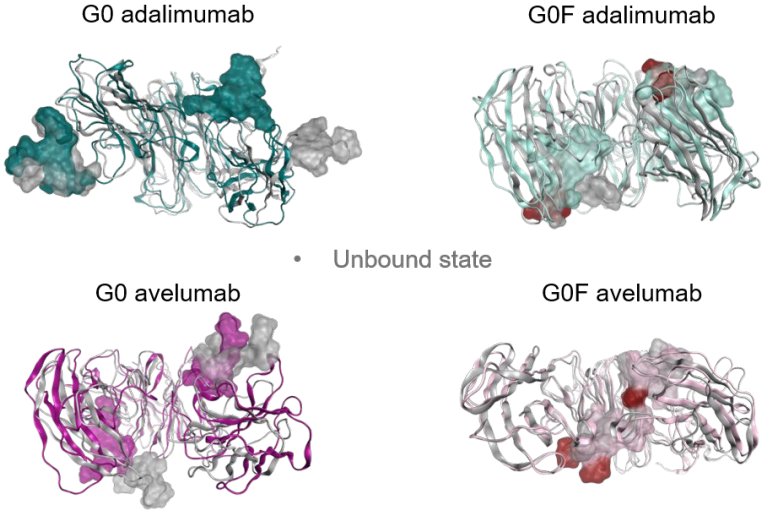
Upon FcγRIIIa binding,

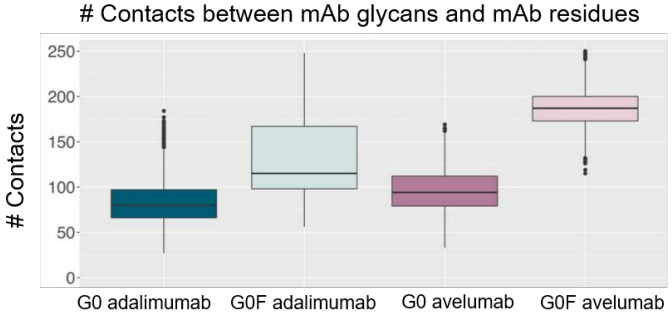
- CH2 distance decreases in adalimumab, whereas it increases in avelumab, meaning that **to stabilize the complex, the conformational rearrangement is needed in the Fc** as coordinated movement of CH2 and CH3 domains.

Conformational analysis

Structural rearrangements in the CH2 domains are accompanied by **rearrangements in the glycan chains**

- Upon receptor binding, in G0 mAbs one of the two glycan chains shifts inside of the cavity. No change was observed in the G0F species.





- G0 mAbs show the lowest number of contacts between the sugars and the antibody residues, allowing for a higher mobility of the glycans

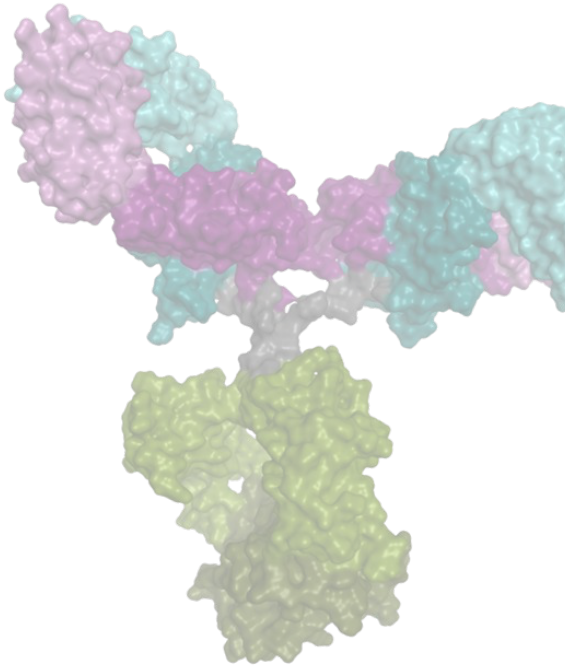
Conclusions

Interaction patterns

- Adalimumab: FcγRIIIa binding region is extended to the portion surrounding the hinge and CH2 domains as well as to the Fabs
- Avelumab: the interaction is more restricted, involving Fab residues only in G0 form.

Fc motions

- Adalimumab: Fc closes, leading to a more stable receptor interaction.
- Avelumab: Fc opens, especially in G0F, resulting in the least stable complex.



Fucosylation and λ-LC combination

G0 forms are consistently more stable than G0F. The strongest destabilization upon fucosylation occurs in avelumab, showing that λ-LC and fucose synergistically limit FcγRIIIa binding.

Acknowledgements

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Thank you for your attention!

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