

Appendix

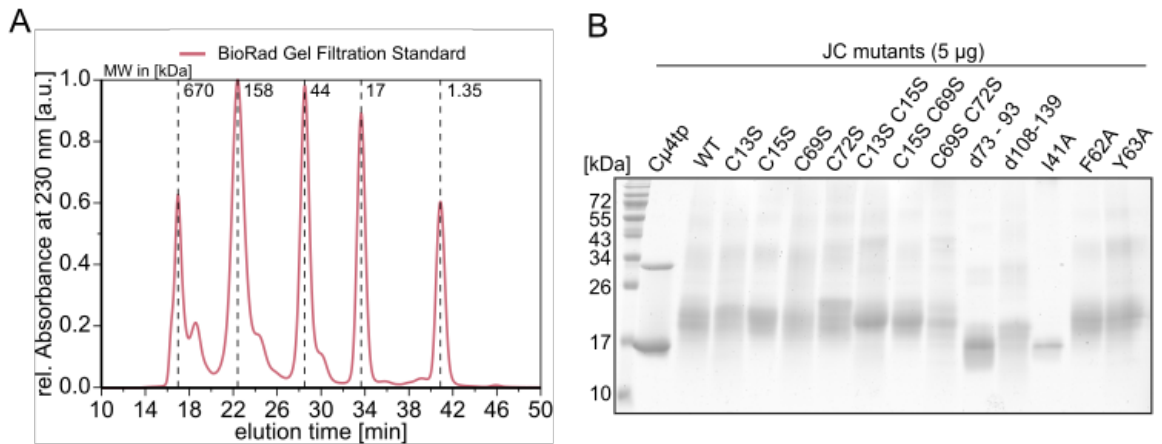
How J-chain ensures the assembly of immunoglobulin IgM pentamers

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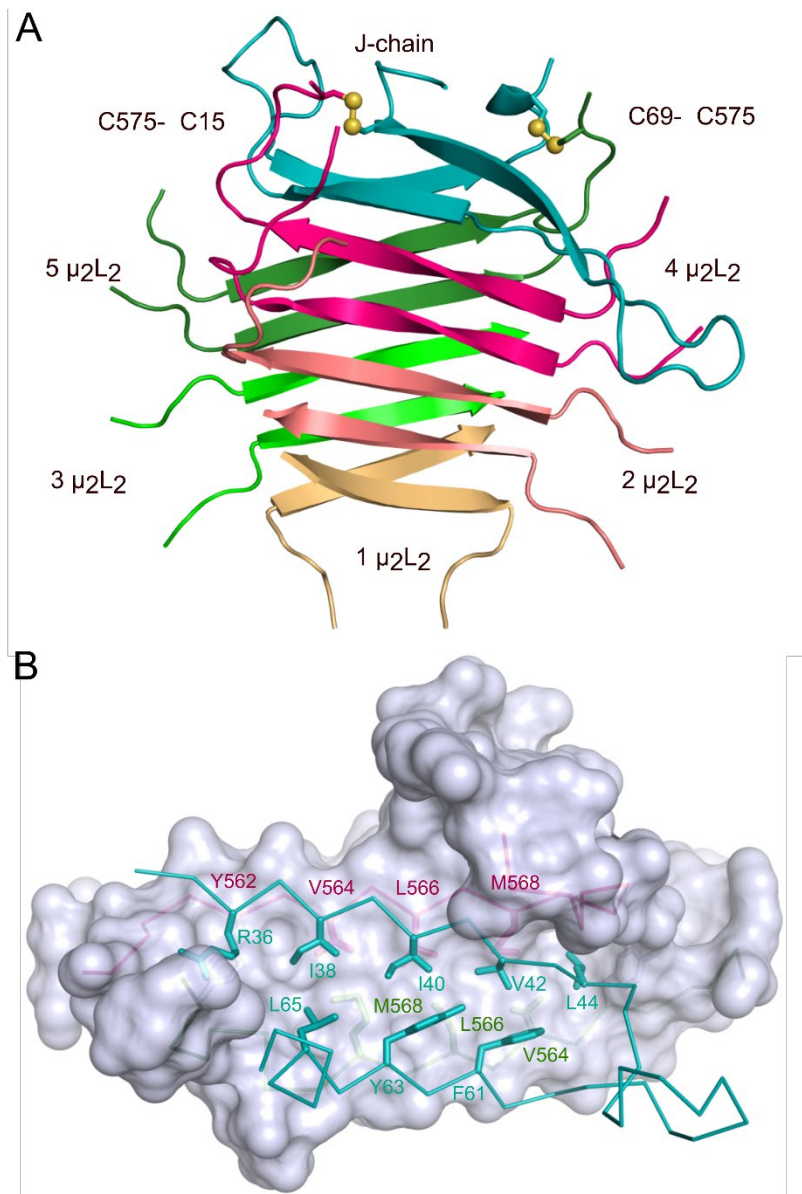


Appendix Figure S2 - Quality control of recombinant proteins

Panel **A**: Chromatogram of molecular weight standards: Thyroglobulin (bovine): 670 kDa, γ -globulin (bovine): 158 kDa, ovalbumin (chicken): 44 kDa, myoglobin (horse): 17 kDa, vitamin B12: 1.350 kDa.

Panel **B**: JC is predominantly monomeric on non-reducing SDS-PAGE.

Most recombinant JC forms monomers after refolding and oxidation, which are visible as smear with an apparent mass of > 17 kDa on non-reducing SDS-PAGE. Covalently bridged species form small amounts of oligomers visible above the monomer bands. Cp4tp is present as monomers (< 17 kDa), which can be oxidized to dimers (< 34 kDa) in the absence of reducing agents like DTT.



Appendix Figure S3 - Organization of the μ tps in μ_2L_2 subunits of pentameric IgM.

Panel **A** shows a front view of the core of pentameric IgM, with the two disulfide bonds between JC and C575 highlighted as yellow spheres). Note the opposite μ tp arrangements in subunit 1 (in orange, bottom part of the figure) compared to the other four. Note also how the two **beta-strands from the J chain are mimicking the μ tps arrangements in the pentamer core, extending the β -sandwich structure.**

Panel **B** highlights the different arrangements of the μ tps that interface with JC.

The protein surface formed by subunits 4 and 5 upon interaction with a JC is portrayed in grey. The images were generated using PyMOL (The PyMOL Molecular Graphics System) are extrapolated from the Cryo-EM structure of human IgM-Fc in complex with the J chain and the ectodomain of pIgR.