Realgymnasium Rämibühl

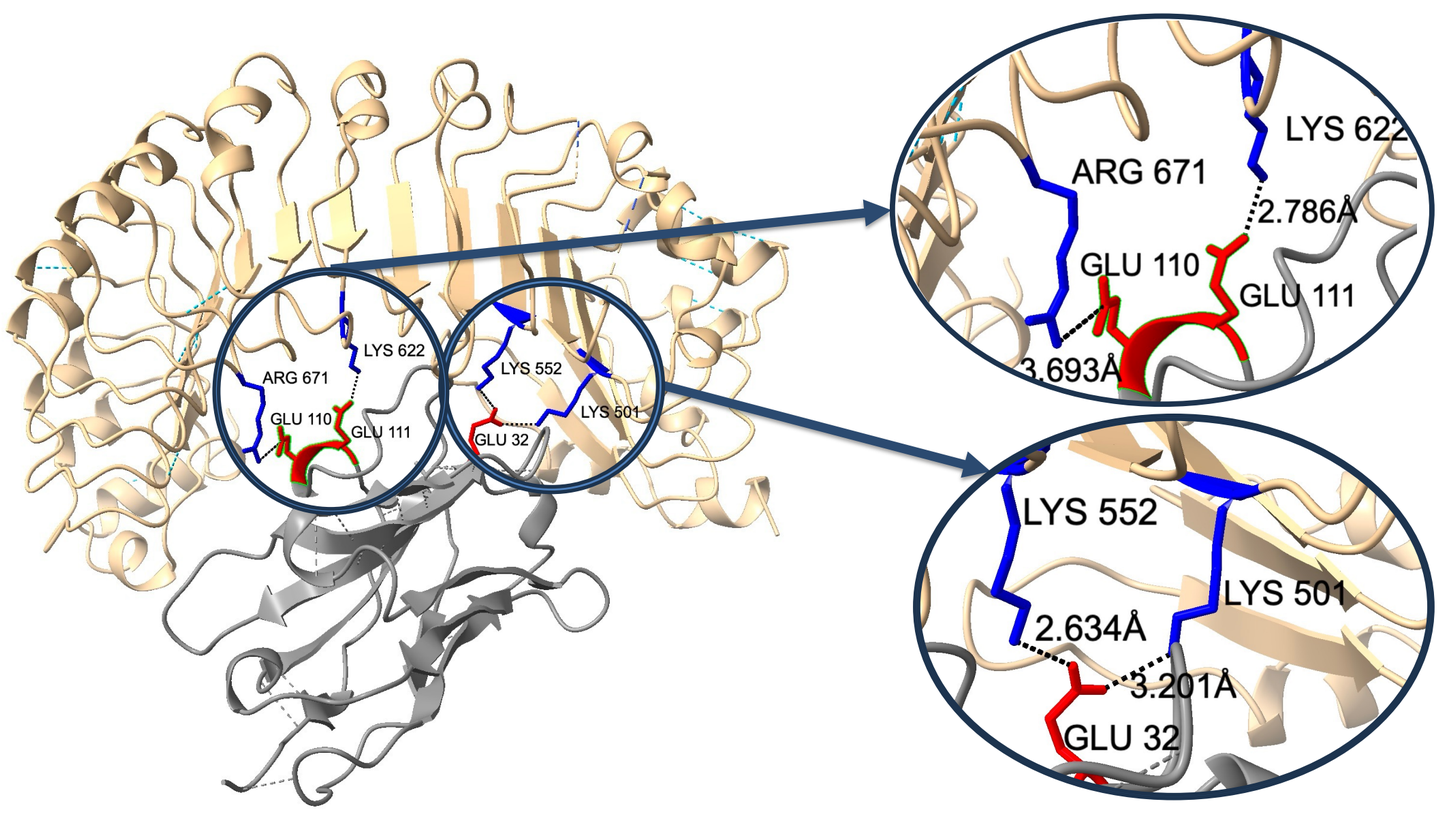
# How to open the channel?

## Finding modulation sites of the Volume Regulating Anion Channel through a synthetic ligand, a potential supplementary drug for the chemotherapeutics cisplatin and carboplatin by Arthur Petrov

### INTRODUCTION

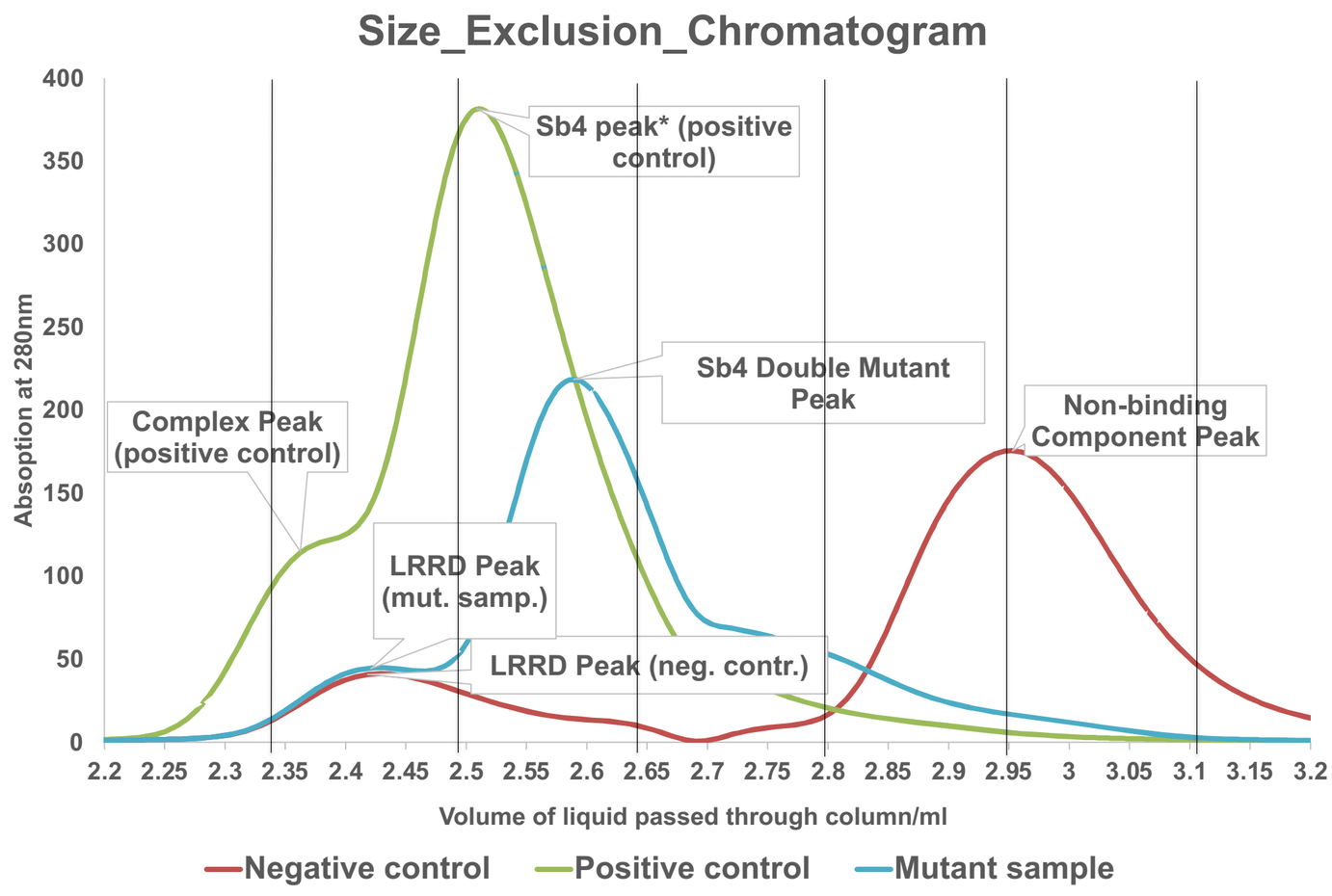
The Volume Regulating Anion Channel (VRAC) is a ubiquitous transmembrane channel involved in regulatory volume decrease to counter osmotic swelling. It is regulated by a protein binding the intracellular Leucine-Rich Repeat Domain (LRRD). The VRAC has also been involved in the uptake of anticancer chemotherapeutics cisplatin and carboplatin. An activating synthetic ligand (Sb4) binding the regulating LRRD was developed in 2021. This work set out to find the amino acids on Sb4 building the strongest bond to the LRRD. The strongest non-covalent bond is a salt bridge, a mix of an ionic and hydrogen bond. By changing these amino acids on the Sb4, its binding strength to the LRRD could be regulated. Introducing a Sb4 with adapted binding strength into cancer cells could increase the specificity of chemotherapy. It would optimize the drug uptake by cancer cells only.

### RESULTS



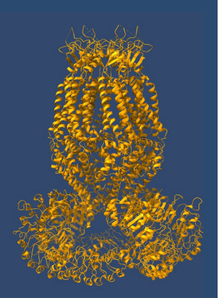

To the left, the crystal structure of the Sb4 (grey) binding the LRRD (yellow) is shown. Based on a salt bridge being at maximum 4Å long and having a certain angle, the following possibilities were identified: GLU110 forms one with ARG671, GLU111 with LYS662, and GLU32 with either LYS552 or LYS501. The sidechains of these amino acids are highlighted in blue (LRRD) and red (Sb4). Through point mutations of Sb4, replacing the interacting amino acids with alanine, these salt bridges were abolished, because alanine has a non-polar sidechain. These point mutations were done in two sets (double mutants), GLU32 + GLU110 and GLU110 + GLU111. Abolishing two such strong bonds should disrupt binding of the LRRD and Sb4.

The Size-Exclusion-Chromatography (SEC) tested this hypothesis. It separated molecules by size, where bigger molecules washed out first. If the LRRD and the Sb4 bound, they formed a big complex (complex peak) and washed out first, as shown by the positive control (green). If the components didn't bind, they had separate peaks, as shown by the negative control (red). The blue line shows two separate peaks, an LRRD peak and an Sb4 mutant peak. Thus, the SECs indicate that binding was disrupted by the double mutants. The Figure to the right shows the result for the GLU32 + GLU110 double mutant. The graphs of both double mutants coincide proving that binding was abolished.
*The Sb4 peak in the positive control is a result of overloading the sample with Sb4 to ensure it bound all available LRRDs.




### Conclusion

The results of the SECs and an SDS-PAGE (not shown) confirm that GLU32, GLU110 and GLU111 are amino acids involved in salt bridges critical for binding. Hence, they are implicated in opening the channel. These amino acids are likely modulation sites influencing the binding strength of the Sb4 and LRRD. The optimal binding strength is to be determined by further research.
Furthermore, the three confirmed salt bridges also create a profile for how the natural, currently unknown LRRD ligand could look, as it has to also build these salt bridges.
The introduction of the Sb4 mutants into cancer cells could occur through CRISPR systems or Antibody-Sb4-conjugates, where the Antibody is primed to the EGFR, a receptor strongly overexpressed in cancer cells, and the Sb4 is fused to the FC-Region of the Antibody.



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