Using large cohorts and CRISPR/dCas9 epitoolbox to identify & validate genes regulating IgG glycome

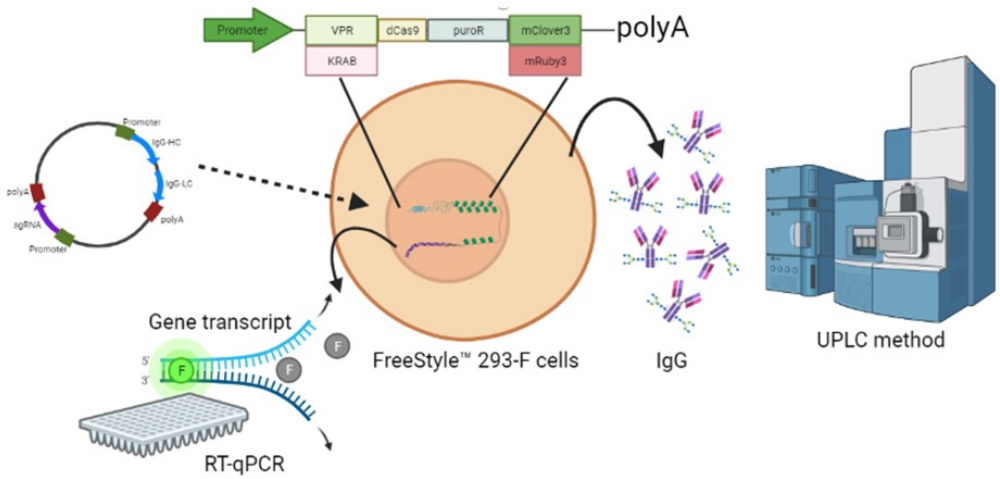
Gordan LAUC [1], Vlatka ZOLDOS [2]

[1] University of Zagreb Faculty of Pharmacy and Biochemistr; Zagreb, Croatia; [2] University of Zagreb Faculty of Science, Zagreb, Croatia

glauc@pharma.hr
vzoldos@biol.pmf.hr

Glycosylation of IgG is an essential regulator of the immune system, but our understanding of this process is still limited. Significant efforts have been invested in studying the role of glycosyltransferases in IgG glycosylation, but today it is clear that enzyme expression is not the key regulatory mechanism. By performing large genome-wide association studies (GWAS) we mapped a network of over 40 genes that associate with IgG glycome composition and are in pleiotropy with different inflammatory diseases.

These includes many transcriptional factors, immune specific signalling proteins, chromatin regulators, solute carriers, cytoskeleton and Golgi and ER associated proteins. However, GWAS is just a hypothesis generating tool, thus GWAS hits need to be functionally validated. To approach the functional role of GWAS hits in IgG glycosylation we developed a transient expression system HEK293FreeStyle with stably integrated CRISPS/dCas9 system in the cell genome. The *in vitro*system enables manipulation of a gene of interest and subsequent analysis of the effect of gene activation/silencing on IgG glycan phenotype in a single experiment. We have also developed a new method to evaluate glycosylation of both Fab and Fc regions of recombinant IgG at the same time. Proof of principle for this approach was provided by analysing effects of several GWAS hits, including genes downstream of the oestrogen receptor, on glycosylation of IgG *in vitro*.



Expression system for production of IgG with stably integrated dCas9-VPR and dCas9-KRAB cassettes for up- and down-regulation of glyco-genes

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