Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary information

Quantitative epitope analysis reveals drastic 63% reduced immuno-affinity and 60% enhanced transmissibility for SARS-CoV-2 variants

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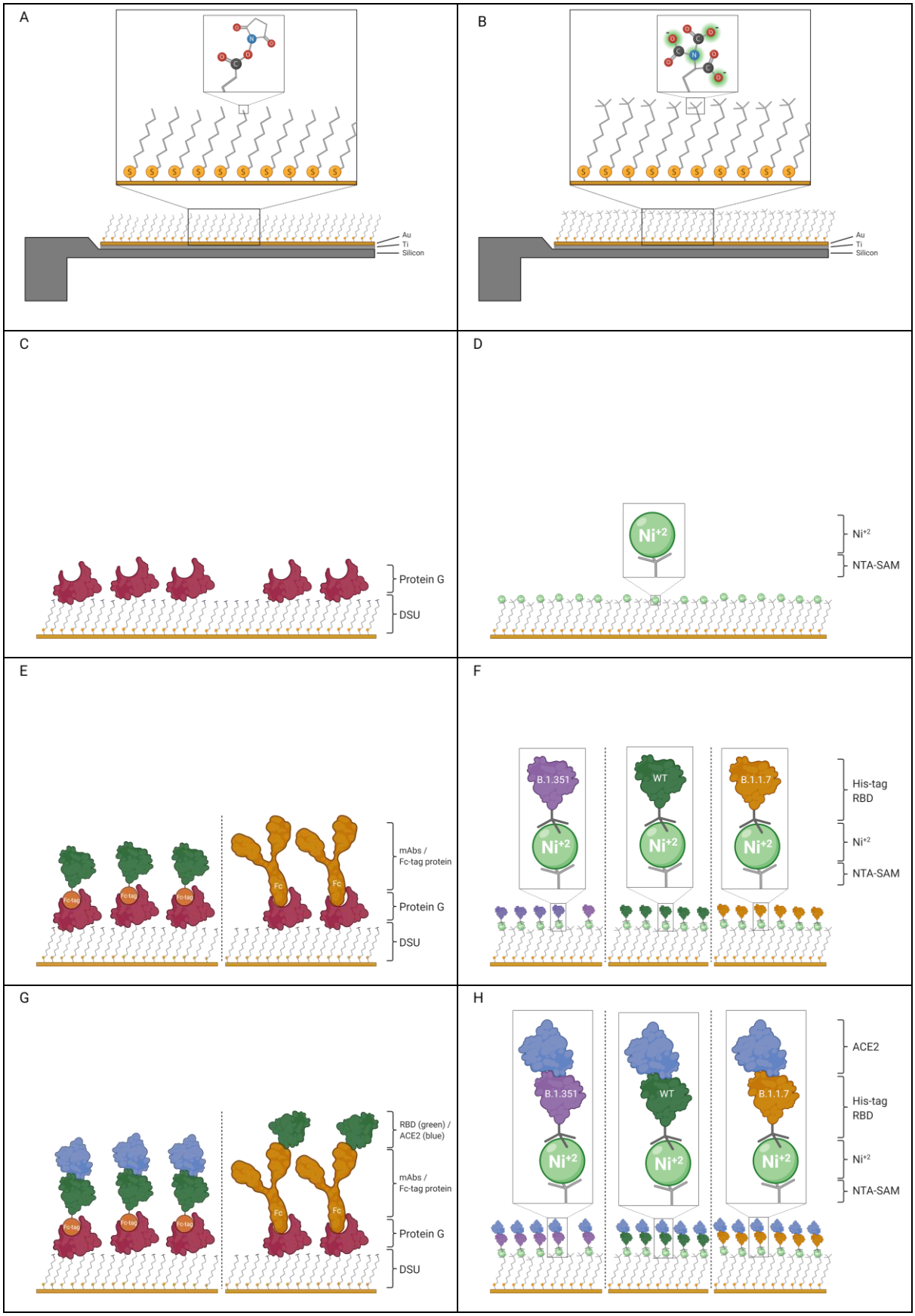

Fig. S1 Protein G/Fc-tag and NTA/His-tag functionalization process. Each cantilever used in the present study is coated with 3 nm of Titanium and 23/33 nm of Gold (top and bottom side, respectively) via metal evaporation (in **A**, **B** only the top side coating is shown in order to facilitate a simplified graphical representation). **A** Incubation of the sensitive surface in

dithiobis(succinimidyl-undecanoate) (DSU) is providing a long-chain N-hydroxysuccinimide-ester functionalized self-assembled monolayer (SAM) (the zoom is highlighting the reactive group at the end of each chain), which is accessible for nucleophilic attack (e.g., amide bond formation with molecules containing amino-groups). **B, D** A nickel-chelated nitrilotriacetate (NTA) SAM is obtain using a metal chelate compound such as nitrilotriacetate linked to an aliphatic thiol in combination with Ni^{2+}, in order to create a reactive monolayer for incoming his-tag proteins (**A**, **B** The zoom-in highlights the self-assembly to a tightly packed monolayer arrangement). **C** Protein G incubation is linking the protein with an amide bond to the DSU, open functional groups are quenched with ethanolamine (T). **E**, **F** The incubation of the functionalized array in Fc-tag (**E**) or His-tag (**F**) protein solutions is producing a binding-oriented monolayer (**F** describes a functionalization pattern as designed for experiment in Fig. 5 main text). Highlighted in **E**, due to the presence of protein G binding sites, a monolayer of specific antibodies can be obtained. Panel **E** and **F** show the final functionalized probes' sensitive surface. **G** and **H** represent an example of protein-protein recognition as explored in this manuscript, specifically between RBD-ACE2 (left portion of **G** and **H**) and mAbs-RBD (right portion of **G**).

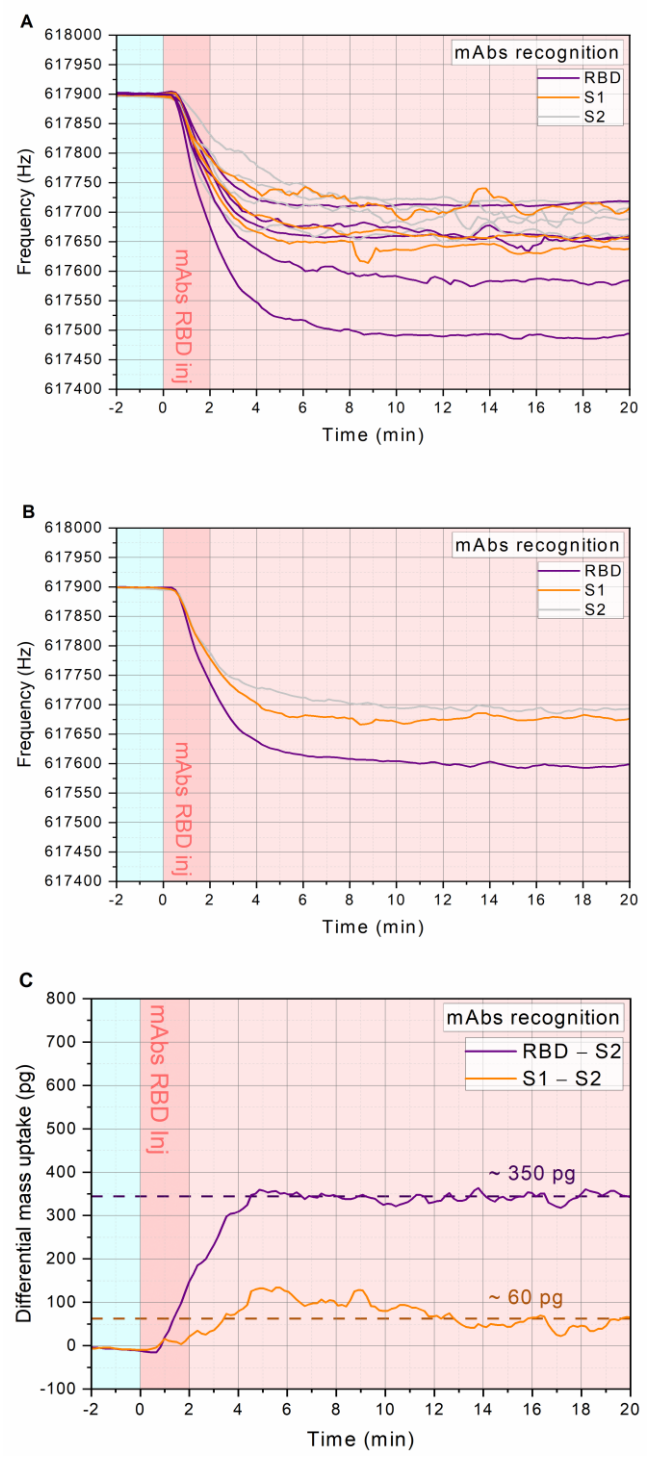

Fig. S2 From sample injection to mass differential analysis: data process flow.
A An automated fluidic system is injecting the sample of interest into the microfluidic chamber where the sensor functionalized surfaces are exposed. Light blue background indicates stabilization in buffer (PBS), the dark pink represents sample injection, and the light pink denotes signal stabilization in serum (no flow). The frequency shifts correlated with specific biological phenomena of interest (e. g. protein-protein interaction) are acquired and recorded. For each single resonator, the resonance frequencies are calibrated to a specific common value by taking the hydrodynamics and the sensor dimensions into account.
B The calibrated frequency shifts are averaged between resonators that share the same functionalization. This step is crucial to obtain a more robust outcome and reduce false positive or negative results.
C The frequency shifts of the averaged signals are converted in mass uptakes and the resulting differential analysis is shown. Each signal reported in c is representing the specific binding to the epitope involved. The differential analysis eliminates any unspecific binding contribution and enviromental convolution.