Kinetic Characterization of the C1q-Antibody-Antigen Complex by Surface Plasmon Resonance (SPR)

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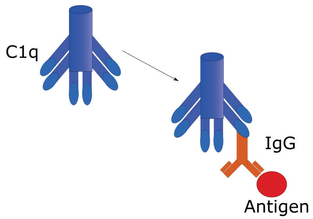
1 Abstract

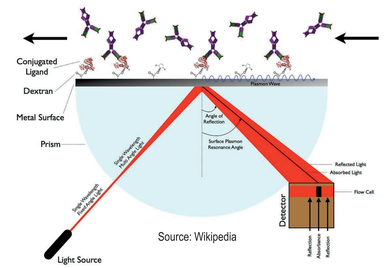
In the growing landscape of immunological therapeutics (such as IgGs raised against a pharmacologically relevant target) it is important to develop effective assays to determine their biological impact. While *in vitro* assays lack biological context, they provide refined, reproducible methods that generate highly informative and quantitative data. One technique that provides such data is Surface Plasmon Resonance (SPR), which utilizes plasmon effects in thin gold layers to track protein binding in real time. With the use of SPR we have developed and optimized a methodology that allows the detection of the human protein C1q complex binding IgG antibodies in the presence and absence of their antigen. Notably, C1q is a difficult protein to work with, displaying poor immobilization behaviour and nonspecific binding (NSB). However, through immobilization of the antibody of interest and use of a lightly methylated surface we have optimized an assay that allows for kinetic and thermodynamic assessment of C1q binding an antigen free or bound antibody. We note 0.1-1 nM binding in the case of this antibody, however we do not observe differences due to antigen be present. This advancement in methodology has overcome some of the traditional hurdles in applying C1q to SPR experiments, and can provide kinetic-rich data in complementation to existing cell-based assays.

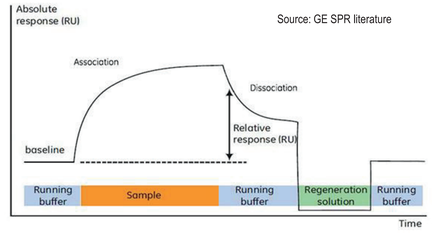
2 Introduction

The classic complementation pathway is a vital part in the cytotoxic clearance functions of the immune system. It allows for recognition of pathogens to induce cell death, which is vital for immune recognition. In the initial stage of this recognition, binding of an IgG raised against an antigen displayed by the pathogen is recognized by protein complex C1q. Thus, understanding the nature of this interaction is vital in the growing world of immunological therapeutics. However, protein C1q, likely in part due to its size, can be difficult to test directly, in an isolated format, outside of the in-vivo or cellular environment. In this work the Biophysical Characterization group at Charles River Laboratories has developed a *in vitro* methodology using Surface Plasmon Resonance (SPR) to gain refined kinetic and thermodynamic insight and parameters.

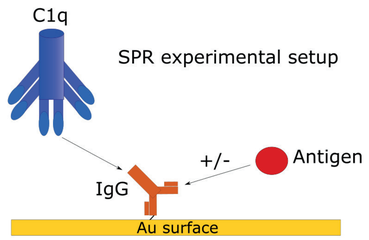


3 Surface Plasmon Resonance (SPR)



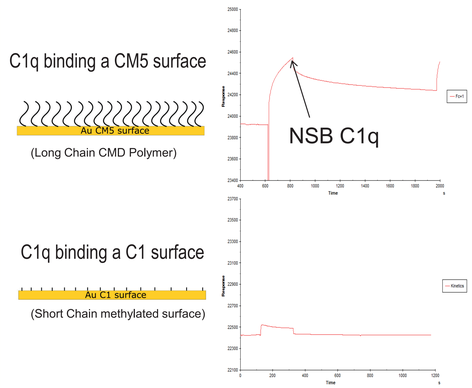


4 Methods

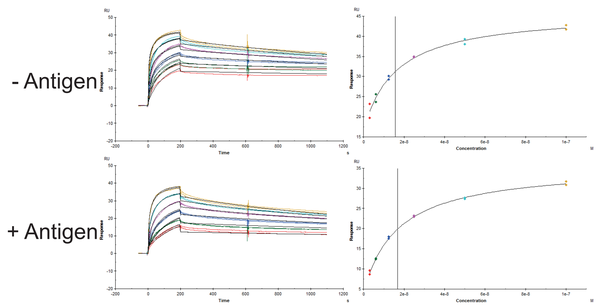


The optimal SPR assay shown above is as follows: The IgG of interest is immobilized to the sensor surface prior via EDC/NHS crosslinking to 60 RU. Either C1q or C1q + antigen were injected for 200 s at 100 µl/min, then allowed to dissociate for an additional 900 s. Blank antigen injection was used to subtract antigen binding signal. Immobilized C1q is antibody-binding incompetent (No binding observed, not shown). and the antigen is unable to be immobilized to the SPR surface directly.

5 Eliminating C1q NSB



6 C1q binding assay



Antigen	k_a (1/Ms)	k_d (1/s)	K_D (kinetic, M)	K_D (thermo, M) ± SE
-	1.0146E6 ± 1.73E3	3.264E-4 ± 8.4E-7	3.213E-10	1.566E-8 ± 5.9E-9
+	4.533E5 ± 6.1E2	5.463E-4 ± 1.2E-6	1.205E-9	1.68E-8 ± 1.3E-9

It should be noted a number of optimizations make this assay possible. This binding orientation was decided on after trying all three combinations (immobilization of antigen, C1q, and antibody). Antigen immobilization worked poorly and yielded no binding of the IgG. Also as a more broadly applicable assay to various IgG-antigen interactions the antigen will vary greatly. C1q can be readily immobilized but cannot bind antibody when crosslinked to the sensor. Additionally, C1q binds to CM5 SPR dextran sensor surfaces with high NSB. To overcome this we used the lightly methylated C1 SPR surface. We find this eliminates C1q binding without introducing notable NSB (a problem with this surface otherwise). We also do not observe a difference in C1q-antibody binding due to saturation of the IgG with antigen (IgG-antigen binding data not shown). Also kinetic fitting indicates bivalent binding is occurring, likely why K_D agreement from kinetic and thermodynamic derivations is 10x different. However this can be easily overcome with sparser immobilization of the IgG in future experiments.