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Kinetic analysis of IgG antibodies to beta-amyloid oligomers with surface plasmon resonance

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IgG binding kinetics with beta-amyloid oligomers

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ABSTRACT: Surface plasmon resonance was used to investigate the kinetics, affinity, and specificity of binding between anti-A β IgG antibodies and oligomeric A β . Two factors were needed to accurately characterize the IgG binding kinetics. First, a bivalent model was necessary to properly fit the kinetic association and dissociation sensograms. Second, a high concentration of IgG was necessary to overcome a significant mass transport limitation that existed regardless of oligomer density on the sensor surface. Using high IgG concentrations and bivalent fits, consistent kinetic parameters were found at varying sensor surface ligand densities. A comparison of binding specificity, affinity, and kinetic flux between monoclonal and natural human anti-A β IgG antibodies revealed the following findings: (1) Monoclonal antibodies 6E10 and 4G8 single-site binding affinity is similar between A β oligomers and monomers; (2) Natural human anti-A β IgG binding readily binds A β oligomers but does not bind monomers; (3) Natural human anti-A β IgG binds A β oligomers with a higher affinity and kinetic flux than 6E10 and 4G8. Both the present analytical methodology and antibody binding profiles are important for advances in antibody drug development and kinetic biomarker applications for Alzheimer's Disease.

Introduction

Recently, passive and active immunization therapies that target different forms of the beta-amyloid peptide ($A\beta$) have been investigated as treatments for Alzheimer's Disease (AD) [1-4]. While early clinical and preclinical studies of these treatments appeared promising, larger Phase II and III trials of these treatments did not significantly slow AD progression [2,3,5,6]. Despite the failure to meet study endpoints, two Phase III studies using passive antibody treatments showed a small reduction in cognitive decline in select subgroups [2,6]. The first of these antibody drugs is an engineered monoclonal IgG antibody Solanezumab [2]. The other antibody drug is IVIG, a natural polyclonal IgG antibody mixture purified from pooled human blood [6]. Both antibody products have been shown to bind soluble oligomeric forms of $A\beta$ ($OA\beta$) [7-10]. Thus, it may be possible to increase these clinical effects with an optimal modification of the binding thermodynamics and kinetics with $OA\beta$.

AD antibody drugs may yield meaningful clinical results if they can mimic natural anti- $A\beta$ antibody properties [8,10,11]. At present, this hypothesis is difficult to address because the affinity and kinetics of natural antibody binding to linear epitopes of monomeric $A\beta$ ($MA\beta$) and conformational neo-epitopes of soluble oligomers ($OA\beta$) or insoluble fibrils ($FA\beta$) are not well characterized. Until reliable methods are designed to measure these parameters, a rigorous comparison between natural and engineered antibodies is not possible. For reference throughout the present article, $A\beta$ will refer to $MA\beta$, $OA\beta$ and $FA\beta$ states collectively and $A\beta^+$ IgG designates an IgG antibody species that binds one or more of these states.

An initial question is whether the binding affinity and kinetics differ between natural and engineered $A\beta^+$ IgG [12]. Enzyme-linked immuosorbent assay (ELISA) methodologies are common approaches used to measure $A\beta^+$ IgG concentrations [8,9,11,13]. However, thermodynamic K_D measurements by natural $A\beta^+$ IgG by ELISA are challenging for a number of reasons. First, the standard ELISA is performed under conditions of high ligand immobilization where minimal IgG analyte remains equilibrated in solution. Second, natural human IgG preparations contain very high background signal due to polyvalent antibodies [8,14]. Third, MA β peptides have been shown to adopt OA β and FA β conformations when immobilized on ELISA plates and affinity column matrices [11]. Also, standard ELISA requires overnight procedures and are not well-suited for fast kinetic measurements. Thus, other approaches are necessary to obtain accurate equilibrium K_D and kinetic rate constants.

The relative affinities of different $A\beta^+$ IgG species has been inferred with high sensitivity ELISA, chaotropic salt titration, and kinetic off-rates by SPR [9,11,15]. These affinity studies have indicated that natural $A\beta^+$ IgG is of comparable affinity to commercial monoclonal antibodies, such as 6E10 and 4G8. However, a rigorous thermodynamic and kinetic analysis has not been achieved as of yet.

Surface plasmon resonance (SPR) has demonstrated the ability to accurately determine affinity and kinetic parameters of monoclonal antibodies binding to MA β . Based on this success, SPR may also prove to be a good method to perform more challenging binding kinetic measurements between natural $A\beta^+$ IgG and aggregated A β states [12,16]. In contrast to ELISA experiments, SPR measurements showed minimal binding from polyvalent IgG in IVIG [8,10,14].

Natural A β ⁺ IgG does not appear to bind MA β in SPR studies, in contrast with inconsistent findings of A β ⁺ IgG binding with MA β in ELISA studies [8-11,17,18]. These inconsistent findings may result from the high polyvalent background or possibly from unwanted conversion of MA β to FA β conformations on ELISA plates [8,9,11]. While SPR measurements have been used to measure either association or dissociation kinetics of natural A β ⁺ IgG binding, a global analysis of both simultaneous has not been achieved [9,10].

The challenges of A β ⁺ IgG kinetic analysis by SPR were highlighted in a recent study with monoclonal A β ⁺ antibodies (mAbs) [12]. Global kinetic fitting were successful when MA β was bound to immobilized mAbs but not when mAbs were bound to immobilized MA β and OA β /FA β . Also, local 1:1 fits of binding between mAbs and OA β /FA β exhibited high χ^2 values, suggesting a more complex binding model was needed. Nonetheless, this study provided an invaluable first step towards using SPR for kinetic analysis of natural A β ⁺ IgG binding to immobilized A β . In particular, it illustrates the need for a good model and/or experimental conditions that enable successful fitting of mAb binding to immobilized MA β and OA β . If this can be achieved for monoclonal antibodies, this model and/or conditions can be applied to SPR studies of natural polyclonal A β ⁺ IgG.

The study here presents the results of A β ⁺ mAb 6E10 and 4G8 binding with immobilized MA β and OA β by SPR. The key findings are as follows. First, the bivalent analyte model is optimal for kinetic fits. Second, a mass transfer limitation is shown to be present in IgG binding kinetics with OA β regardless of ligand concentration on the sensor surface [19]. Third, a high IgG analyte concentration is able to overcome the mass transfer limitation and enable consistent

kinetic parameter determination. Lastly, the first rigorous kinetic comparison between IVIG A β ⁺ IgG and mAbs 6E10 and 4G8 is shown. The present work establishes an SPR approach that enables accurate kinetic binding characterization for both natural and engineered A β ⁺ IgG.

Materials and Methods

Surface Plasmon Resonance Sensor Chip Preparation

Surface Plasmon Resonance sensor chip preparation and experiments were conducted on a Biacore T100 (GE Life Sciences) for monoclonal antibody studies and a Biacore X (GE Life Sciences) for IVIG studies and oligomer stability measurements. MA β or OA β was prepared as described in Ref. [20]. All MA β or OA β immobilizations on the sensor chips were performed within two hours after exhaustive dialysis (MW 3000 cut-off) into 10 mM sodium acetate buffer pH 4 immobilization buffer. For low MA β immobilization (< 100 RU), the stock solution was diluted 1:10 prior to dialysis. For immobilization levels of OA β higher than 100 RU, the SEC purified OA β solution was concentrated 20-fold prior to dialysis (Amicon, 10 kD filter).

CM5 sensor activation was performed using a mixture of 25 mM N-hydroxysuccinimide (NHS) and 0.1 M N-ethyl-N-dimethylaminopropylcarbodiimide (EDC) for 7 min at 5 μ L/min. MA β immobilization was performed for 7 min with a 5 μ L/min flow using either MA β preparation. OA β immobilization at low (< 100 RU) and moderate (500-1000 RU)

immobilization levels were achieved after 7 min of contact time at 5 $\mu\text{L}/\text{min}$ flow using the unconcentrated or 20-fold concentrated OA β preparation respectively. OA β immobilization at high (2000-4000 RU) and very high (6000-8000 RU) immobilization levels were achieved after 21 and 42 min of contact time respectively at 5 $\mu\text{L}/\text{min}$ flow using the 20-fold concentrated OA β preparation. Residual activated sites after each immobilization were capped using 1.0 M ethanolamine (pH 8.5) for 7 min at 5 $\mu\text{L}/\text{min}$. For each immobilization, the reference flow cell was also activated and capped. Prior to experiments, HBS-EP (0.01 M HEPES, 0.15 M NaCl, 0.05 mM EDTA, 0.05% surfactant P20, pH 7.4) running buffer was run at 30 ml/min for no less than 30 minutes to establish a stable signal.

The initial immobilization of OA β consisted of native OA β (nOA β). Regeneration of the sensor after A β^+ IgG binding required 0.5% SDS. Thus, for each sensor chip prepared, only one binding measurement of IgG to the initial nOA β could be performed before it was exposed to 0.5% SDS regeneration. An exhaustive regeneration procedure involving ten 30 second and six 60 second 0.5% SDS treatments yielded 40-60% of the nOA β peptide that was stable to further SDS exposure. The resulting SDS-stable OA β (sOA β) product on the sensor chip could then be used in repeated binding studies of different IgG analytes at varying concentrations. In the present study, IgG binding kinetics with both sOA β and nOA β are reported. Having defined sOA β and nOA β states, the term OA β refers to both states inclusively.

Surface Plasmon Resonance Experiments

SPR experiments were performed in HBS-EP buffer at a flow rate of 30 $\mu\text{L}/\text{min}$ at 25 $^{\circ}\text{C}$ temperature. Both mAb antibodies 4G8 and 6E10 were dialyzed into HBS-EP and prepared at concentrations ranging from 20-400 nM. Gamunex Immune Globulin Intravenous (Human), 10% (Talecris Biotherapeutics Inc, Research Triangle Park NC) was used undiluted after dialysis in HBS-EP buffer (approximately 500 μM total IgG).

SPR studies involved an initial measurement of IgG binding to immobilized “native” nOA β . The necessary regeneration condition of 0.5% SDS also removed some SDS-labile oligomer components (denoted as xOA β). Ultimately 40-60% of nOA β remained as SDS-stable oligomers (sOA β). A complete conversion of nOA β into sOA β on the sensor surface was achieved with ten 30 second pulses and six 60 second pulses of 0.5% SDS. Thus, for a given sensor chip preparation, the initial measurement of IgG binding kinetics was performed against nOA β whereupon all subsequent measurements after the initial regeneration were conducted on the remaining sOA β .

Monoclonal antibody 6E10 and 4G8 binding to MA β and OA β included a 60 s association followed by 1800 s of dissociation before regeneration. IVIG binding to MA β and OA β included a 60 s association followed by a minimum of 400 s dissociation before regeneration. Regeneration of the MA β and OA β surfaces after A β^+ IgG binding required an extensive regeneration protocol consisting of ten 30 s and six 60 s pulses of 0.5% SDS. While longer than typical regeneration procedures, this regeneration protocol enabled both removal of the IgG analyte and full retention of immobilized sOA β ligand after each binding measurement.

Other regeneration protocols that were tested either failed to remove IgG from the surface or degraded sOA β on the sensor surface.

SPR Analysis

Three binding models were used to fit full SPR sensograms of IgG analyte binding to immobilized A β ligands using either Biacore T100 evaluation software or BIAevaluation Version 4.1 for Biacore X data. Both global and local fits were performed. Based on published ELISA results and SPR Calibration Free Concentration Analysis (Kwon et. al., PLOS ONE, in press), the natural A β ⁺ IgG concentration in IVIG used for fitting was 60 nM [8,21].

The simplest model was 1:1 Langmuir binding between free analyte A and site x on immobilized ligand L shown in Eq. (1) [22].

A more complex model used was the bivalent analyte fit where A can bind two of the same epitope that are found in close proximity on the sensor surface, shown in the two-step reaction shown in Eq. (2) [23].

It should be noted that k_{a2} , in Eq. (2) is a bimolecular rate but with units of $\text{RU}^{-1} \text{s}^{-1}$ to reflect the effective concentration of Lx on the two-dimensional sensor surface. To compare the second association step between flow cells with different R_{max} values, the value of τ_{a2} , shown in Eq. (3), is used.

$$\tau_{a2} = \frac{1}{k_{a2} * R_{\text{max}}} \quad (\text{units of seconds}) \quad (3)$$

The most parameterized model tested was the 1:2 heterogenous ligand model, in which Eq. (1) is modeled simultaneously with a second independent binding site y on ligand L that competes for A (Eq. (4)).

In all three models, the mass transfer coefficient t_c was also fitted in these models to correct for any mass transfer limitation (MTL) effects on k_{a1} and k_{a2} in Eq. (1-4) [19,24].

The fitted value of t_c in Eq. (5) is only appropriate to correct for a small MTL effect and is not appropriate to identify or compensate for large MTL effects on kinetics [19]. An accurate measurement of MTL in an SPR binding reaction was accomplished by measuring the slope (m)

of the best linear fit to analyte association with the sensor surface under a low (m_l) and high (m_h) running buffer flow using Eq. (6) [19,21].

$$MTL = \frac{\frac{m_h}{m_l} - 1}{\left(\frac{f_h}{f_l}\right)^{1/3} - 1} \quad (6)$$

In Eq. (6), f_h and f_l are the respective values of the high and low flow (in $\mu\text{L}/\text{min}$) selected for the experiment.

The most appropriate model and fitting conditions were determined using the reduced chi-squared (χ^2) statistic, randomness in the fit residuals, and the consistency of the fitted parameters across different IgG analyte concentrations and A β ligand surface densities [25]. Unless a more complex model could be shown to significantly (i.e. > 2-fold) improve the fit quality and consistency, the simpler model was used.

Results

The stability of nOA β to SDS was measured using SPR in Fig. 1. SDS-PAGE analysis shows nOA β to be partially stable to SDS treatment (Fig. 3b in Ref. [20]). However, some fragments of nOAb produced from SDS-PAGE conditions migrate near the monomer (Fig. 3b in

Ref. [20]). It is therefore unclear if prolonged exposure to SDS would eventually denature nOA β completely back to MA β . The nOA β stability was assessed by immobilizing SEC-purified nOA β on the SPR sensor and treating this ligand with a series of 30 second 0.5% SDS pulses (Fig. 1a). Fig. 1a shows that five 0.5% SDS treatments reduce the initial nOA β to 50% of its initial value, with diminishing reduction after each subsequent pulse. For the sensor in Fig. 1a, five additional 30 second pulses plus six 60 second pulses of 0.5% SDS produced 45% of the initial ligand remaining as sOA β that resists further reduction by SDS (not shown). This final sOA β percentage varied from 40-60% for different flow cell preparations.

The reduction in nOA β after each SDS pulse occurs because most A β peptides in immobilized nOA β are not covalently bound to the surface. When SDS disrupts their hydrophobic interactions with nOA β , they detach from nOA β and flow out of the cell. However, approximately 45% of nOA β peptides have nonbonded interactions that resist SDS denaturation. The fraction of nOA β peptides that adopt SDS-resistant states are denoted as sOA β . One model of nOA β includes a stable “core” of sOA β conformations and an SDS-labile “outer shell” of A β peptide conformations (denoted as xOA β). Fig. 1b shows that the normalized rate of xOA β loss after pulses 1, 3, and 5 follows an exponential decay process with similar time constants. Thus, the kinetic stability of the each xOA β “shell” is similar and suggests a uniform structure throughout the xOA β layers.

*A β * IgG binding to OA β involves bivalent interactions and is an intrinsically diffusion-limited process*

Association and dissociation kinetics of the N-terminal specific A β ⁺ mAb 6E10 were measured with low levels of immobilized MA β (8 RU) and sOA β (15 RU) on the sensor surface (Fig. 2). 6E10 concentration was varied from 33-166 nM and the binding fit globally using the 1:1 model. This global 1:1 fit of 6E10 binding to MA β was successful (Fig. 2a), indicating that 6E10 binds MA β exclusively with either a single site or entirely with two sites. Consistent with previous work, the global 1:1 fit of 6E10 binding to immobilized sOA β was of poor quality (Fig. 2b) [12]. The parameters from the global 1:1 fits are shown in Table 1.

Given the two Fab domains of IgG, the bivalent analyte model was the most logical model to investigate to improve the fit quality of 6E10 binding to sOA β [23]. Because the 1:1 fit of 6E10:MA β binding was already quite good (Fig. 2a), only 4% improvement in χ^2 was observed with the bivalent model (Fig. 2c). For sOA β , the bivalent analyte model was shown to significantly reduce the χ^2 of the global fit by 33% (Fig. 2d). While clearly an improvement over the 1:1 model, the bivalent fit continued to exhibit a poor fit quality by visual inspection and a relatively high χ^2 value. The 1:2 heterogeneous ligand model performed slightly worse than the bivalent analyte model. Table 2 shows parameters from the bivalent analyte fit and comparison ratios of χ^2 with the 1:1 and 1:2 models.

Of note in Table 2 is the a particularly low value of t_c for sOA β binding (2.9×10^5) consistent with a mass transfer limitation. A mass transport limitation (MTL), or diffusion-limitation, is best described as a specific case where $k_a[L] > t_c$ in Eq. (5). In the extreme case where $k_a[L] \gg t_c$, the association is a first order reaction that is determined entirely by the

diffusion rate. The presence of an MTL would account for the poor global fit for 6E10 binding to sOA β . However, an MTL is unexpected given the low surface density of L (15 RU of sOA β) used in the SPR experiments.

Local bivalent analyte fits were then analyzed to confirm the presence of an MTL (Fig. 3). Fig. 3a and 3b show a comparison of local 1:1 and bivalent analyte fits to a representative sensograms of 6E10:MA β and 6E10:sOA β binding respectively. Fig. 3c and 3d show the respective residuals of 6E10:MA β and 6E10:sOA β fits. As with the global fits, both the 1:1 and bivalent model provided good quality fits of 6E10:MA β binding reaction in Fig. 3a and 3c. A significant reduction in χ^2 and residuals was observed when the bivalent fit was used for 6E10:sOA β binding in Fig. 3b and 3d.

Fig. 3e and 3f show all local bivalent analyte fit parameters at individual 6E10 concentrations used in binding MA β and sOA β respectively. If an MTL is present in either binding, the fitted association constant k_{a1} would be expected to correlate inversely with 6E10 concentration [19,21]. For MA β , k_{a1} did not show a significant trend across the different 6E10 concentrations (Fig. 3e). In contrast, the k_{a1} with sOA β drops 10-fold as 6E10 concentration was increased 6-fold, consistent with a MTL (Fig. 3f). In addition, the lowest 6E10 concentrations in Fig. 3f (33 nM) yields a $k_{a1} \sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$ near the IgG diffusion limit reported in previous experimental and computational studies [26,27]. In summary, the results in Fig. 3f indicate that 6E10:sOA β binding is partially mass transport limited at low ligand surface densities of sOA β .

If the binding of 6E10 to 15 RU of immobilized sOA β is partially diffusion-limited, a change in fitted kinetic parameters is expected if the reaction becomes completely diffusion limited

via an increased sOA β ligand density [19,26]. Specifically for IgG, an upward shift of k_{a1} towards $10^6 \text{ M}^{-1} \text{ s}^{-1}$ is expected when the sOA β ligand density is increased [19,26]. To test this possibility, sOA β flow cells with ligand densities of 406 RU and 1870 RU were used to increase the MTL of 6E10 binding kinetics (Fig. 4a,c,e) [28]. In addition, the mid-terminal A β^+ antibody 4G8 was also tested to confirm if the observed outcomes were epitope-dependent (Fig. 4b,d,f). Fig 7a and 7b show local 1:1 and bivalent fits to representative 6E10 and 4G8 binding measurements with sOA β . Fig 7c and 7d show the respective residuals for these 6E10 and 4G8 fits. The improvement in fit quality of the bivalent model over the 1:1 model is apparent for both 6E10 and 4G8 in Fig. 4a-d. Thus, the bivalent fit was used for further analysis of 6E10 and 4G8 binding to sOA β .

Fig. 4e and Fig. 4f show the bivalent fit parameters for 20-400 nM 6E10 and 4G8 respectively at 406 and 1870 RU of immobilized sOA β . Comparison of Fig. 4e and 4f shows that the overall kinetic parameter trends are remarkably similar between the two IgG analytes and also between the two sOA β densities. Similar to Fig. 3f, the value of k_{a1} in Fig. 4e/4f drops as the concentration is increased. The parameters τ_{a2} , k_{d1} , and k_{d2} also change as the concentration is increased. In general, all kinetic parameters ultimately reach stable concentration-independent values at 400 nM of IgG analyte. Exponential extrapolation of each parameter trajectory indicates that the parameter values at 400 nM are all within 20% of their value at an “infinite” IgG analyte concentration. In all cases, the relationship between k_{a1} and IgG concentration is consistent with a partial MTL that is reduced at higher analyte concentrations [26]. In this scenario, the fitted kinetic parameters more accurately reflect their true values at higher IgG concentrations.

A surprising finding in Fig. 4e and 4f is that the surface density of sOA β had little or no effect on any of the fitted values of k_{a1} , regardless of concentration. The expected increase of k_{a1} towards $10^6 \text{ M}^{-1} \text{ s}^{-1}$ at 1870 RU of immobilized sOA β was not observed at either 30 nM or 400 nM IgG [28]. By contrast, a small decrease in $6 \times 10^5 k_{a1}$ was noted for 1870 RU sOA β in Fig. 4e. This minor shift is opposite that expected under partial MTL and is more likely due to slightly different IgG preparations in the two experiments.

A hypothesis that reconciles this result is that IgG binding to immobilized sOA β is inherently mass transport limited due to a high local concentration of binding sites on the oligomer. A depletion layer exists around each oligomer where the binding flux is very high if an IgG antibody is near. Since the MTL is similar between low and high values of immobilized sOA β , the local MTL effect must outweigh the MTL contributions resulting from increased sOA β immobilization on the sensor surface between 0-1870 RU. This is possible if sOA β is large enough where the IgG epitopes do not uniformly distribute on the sensor surface and are instead found in sporadic but highly dense “islands”.

To test this hypothesis, the mass transport limitation was directly measured by varying the flow of analyte in the flow cell and measuring of the association slopes (Fig. 5) [21]. Eq. (6) was then used to quantitatively determine the MTL [21,28]. Fig. 5a and 5b show the clear deviation in association slopes at 10 and 60 $\mu\text{L}/\text{min}$ at low IgG analyte concentration (20 nM). The MTL is the same for low (15 RU in Fig. 5a) and high (406 RU in Fig. 5b) sOA β surface densities. It is also of sufficient magnitude (~ 0.22) to produce a fitting error for the true kinetic parameters [29]. At IgG concentrations of 400 nM, the MTL was eliminated in both the low surface density flow

cell (Fig. 5c) and the high density flow cell (Fig. 5d). These results provide strong support for the hypothesis that $A\beta^+$ IgG binding to sOA β is inherently mass transport limited.

Varying analyte concentration (Fig. 3-4), ligand density (Fig. 4-5), and flow (Fig. 5) provides strong evidence of the inherent MTL of $A\beta^+$ IgG binding to immobilized sOA β . For immobilized nOA β , this rigorous array of measurements was cost-prohibitive because only one measurement was possible per flow cell because SDS regeneration reduced the nOA β to sOA β . That said, a 400 nM concentration of 6E10 was selected for use in binding measurements in three independent flow cells with 33 RU, 804 RU, and 3320 RU nOA β . Assuming similar 6E10 binding kinetics between sOA β and nOA β , there would be minimal MTL in 400 nM 6E10 binding to nOA β based on Figs. 3-5. With accurate kinetic parameters for 400 nM 6E10 binding to both sOA β and nOA β , a comparison of the two oligomer states was possible.

The bivalent fit parameters for 400 nM 6E10 binding to sOA β and nOA β are shown in Fig. 6a. The bivalent fit parameters for 400 nM 4G8 binding to sOA β is shown in Fig. 6b. Fig. 6a and 6b show that the binding parameters k_{a1} and k_{d1} are largely insensitive to both ligand surface density or whether the binding pair was 6E10:sOA β , 6E10:nOA β or 4G8:sOA β . Binding parameters τ_{a2} and k_{d2} for 6E10 do not show clear trends but are highly variable, so a rigorous comparison of τ_{a2} and k_{d2} was not performed.

Fig. 6c and 6d show the χ^2 ratio of the bivalent fit as compared to 1:1 and 1:2 fitting models. In this comparison, lower χ^2 ratios below 1 reflect an increased benefit of the bivalent fit over the 1:1 or 1:2 model. The conclusions from Fig. 6c and 6d are the same for 6E10:sOA β , 6E10:nOA β and 4G8:sOA β binding. First, the bivalent fit improves over the 1:1 fit and this

improved fit quality is more pronounced at higher OA β surface densities. This is likely due to increased signal resolution. Second, the χ^2 ratio of the bivalent and 1:2 model is close to one. Therefore, the more complex 1:2 fitting model is not appropriate here and the simpler bivalent fit is the preferred model [25].

Anti-A β IgG in IVIG is oligomer-specific with greater affinity and faster binding kinetics than commercial monoclonal antibodies

Fig. 7a shows representative sensograms of IVIG binding in the reference flow cell and in a flow cell with 1020 RU of immobilized sOA β . Despite dialysis into HBS-EP running buffer, a high refractive index shift is evident in the reference flow cell. However, very little nonspecific binding from IVIG is present when the association step is complete after 60 sec and the refractive index returns to baseline. At 70 seconds, the reference cell signal is approximately 3% of that found in the sOA β flow cell. A β^+ IgG binding after reference subtraction, along with 1:1 and bivalent analyte fits, is shown in Fig. 7b with fit residuals in Fig. 7c. Fits in Fig. 7b and 7c clearly show an improved fit when the bivalent analyte model is used.

Fig. 8 shows the selectivity of A β^+ IgG in IVIG for MA β , sOA β , and nOA β . Fig 8a shows significant binding of natural human A β^+ IgG to both sOA β and nOA β but no measurable binding to MA β . Fig 8b shows a comparison of A β^+ IgG binding to MA β , sOA β , and nOA β after normalization with the surface ligand density. In Fig. 8b, the relative A β^+ IgG binding response to sOA β and nOA β is shown to be similar while no binding to MA β was found after normalization.

Because no binding to MA β was found, A β^+ IgG in IVIG could be more precisely defined as OA β^+ /FA β^+ IgG.

Fig. 9 shows a comparison of fitted bivalent analyte parameters k_{a1} , k_{d1} , and K_{D1} between 6E10, 4G8, and natural human OA β^+ /FA β^+ IgG. The high variability in 6E10 and IVIG parameters τ_{a2} and k_{d2} , shown by errors in Table 3, prevented a rigorous comparison of these variables. Comparison of k_{a1} in Fig. 9a and k_{d1} in Fig. 9b indicates that natural human OA β^+ /FA β^+ IgG associates and dissociates from OA β faster than 6E10 and 4G8 in the initial binding step. Fig. 9c shows that the K_{D1} of natural A β^+ IgG is 2-fold lower than that of 6E10 and 3-fold lower than that of 4G8. Despite a similar K_{D1} , natural A β^+ IgG binding parameters k_{a1} , and k_{d1} are slightly higher (1.3-fold) with nOA β than with sOA β (Table 3). Fig. 9d shows that the χ^2 of the bivalent fit of natural human OA β^+ /FA β^+ IgG binding is significantly reduced versus the 1:1 fit and is similar to the 1:2 fit. As with 6E10 and 4G8, the bivalent analyte model was the simplest and most logical model to fit IVIG antibody binding to OA β . Table 3 summarizes the fitted bivalent model parameters, with errors, for binding between the IgG analytes and A β ligands measured under experimental conditions where MTL is minimized at high IgG analyte concentrations.

Discussion

From a clinical standpoint, it is not known if antibodies that bind OA β are capable of preventing or treating AD [30]. To address this question from a biophysical perspective, it is

important to determine if natural and engineered A β ⁺ antibodies differ in their binding kinetics to A β . The present study has provided a method capable of achieving this needed analysis.

Earlier studies have demonstrated that A β ⁺ IgG binding to immobilized OA β /FA β was poorly defined by 1:1 mechanism [12]. Close inspection of the fits from this study show that biphasic dissociation was present, which the 1:1 model could not account for. With sufficient signal resolution at higher IgG analyte and OA β ligand concentrations, the bivalent analyte model used here consistently shows improvement over the 1:1 model for OA β (Fig. 2-4, 6, 7, 9).

The most reliable parameters of the bivalent analyte fit are k_{a1} and k_{d1} , which characterize the monovalent IgG binding with a single epitope. The high precision of k_{a1} and k_{d1} in Fig. 9 and in Table 3 owes to the fact that the association and early dissociation are well-captured in the timeframe of SPR measurements. By contrast, fitted values of k_{a2} and k_{d2} are much less precise as they would require many days of measurement to resolve the second dissociation phase adequately. While the values of k_{a2} and k_{d2} may be uncertain, including these parameters in the bivalent fit enables a more reliable and accurate determination of k_{a1} and k_{d1} .

In addition to improved fits with OA β , the use of the bivalent model also helps address the problematic binding of IgG to immobilized MA β . 6E10 binding to immobilized MA β did show a reasonable 1:1 global fits in the present study (Fig. 2a). However, bivalent binding of 6E10 to immobilized MA β has been demonstrated previously because the k_d of 6E10:MA β binding was much lower with immobilized MA β ($< 10^{-4} \text{ s}^{-1}$) than when 6E10 was immobilized ($\sim 10^{-3} \text{ s}^{-1}$) [12]. Consistent with this previous study, the k_d of the 1:1 fit for 6E10 with immobilized MA β in Table 1 was also quite slow ($1.8 \times 10^{-4} \text{ s}^{-1}$) in the present study. In both studies, the binding of 6E10 to

immobilized MA β likely includes an influence from bivalent dissociation such that the fitted k_d is lower than the true k_d .

The bivalent model reconciles these disparate findings with immobilized and free MA β binding to 6E10 (Table 3). Specifically, the bivalent fit parameters for the first association step for 6E10 binding to immobilized MA β ($k_{a1} = 5.8 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, $k_{d1} = 11 \times 10^{-5} \text{ s}^{-1}$, and $K_{D1} = 19 \text{ nM}$) are consistent with the 1:1 parameters reported for MA β binding to immobilized 6E10 ($k_{a1} = 3.8 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, $k_{d1} = 8.5 \times 10^{-5} \text{ s}^{-1}$, and $K_{D1} = 22 \text{ nM}$) [12]. This agreement demonstrates that it is possible for SPR to provide consistent kinetic parameters regardless of whether IgG or MA β is immobilized. However, the bivalent model must be used if MA β is immobilized.

Having established the bivalent model as ideal for IgG binding to immobilized A β , the thermodynamic affinity of 6E10 for OA β was compared to MA β . In both previous and the present studies, 1:1 fits of IgG binding with OA β report a much lower k_{d1} and K_{D1} than with MA β [12]. Net antibody dissociation from OA β is indeed quite slow in the present study and in other SPR studies [12,31]. However, this slow dissociation likely results from more prevalent bivalent binding rather than increased affinity for the epitope. The results of the present study help address this question.

Table 3 shows that 6E10 monovalent epitope affinity with OA β ($K_{D1} \sim 45\text{-}47 \text{ nM}$) is actually 2-fold lower than for MA β due to an increased k_{d1} . Compared to published values of 4G8 binding to MA β ($k_{a1} = 2.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$; $k_{d1} = 8.1 \times 10^{-3} \text{ s}^{-1}$; $K_D = 30 \text{ nM}$), 4G8 affinity for sOA β ($K_{D1} = 69 \text{ nM}$) is also 2-fold lower [12]. However, the 4G8 affinity difference results from a 6-fold decrease in k_{a1} ($3.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$) offset by a 3-fold decrease in k_{d1} ($2.6 \times 10^{-3} \text{ s}^{-1}$). Taken

together, these results show that overall affinity of A β ⁺ mAbs for their linear epitopes weakens slightly in binding interactions with oligomeric A β . Thus, the slow dissociation of 6E10 and 4G8 from immobilized OA β results from bivalent interactions and not from higher affinity binding to the epitope.

The present study also revealed that the binding of analytes with OA β is an intrinsically diffusion-limited process. Unlike most ligands, this mass transport limitation cannot be overcome by reducing the OA β immobilization on the sensor chip. A search of the literature did not reveal other ligands that exhibited this behavior in SPR studies. Thus, an intrinsic MTL could be a novel property of large oligomeric assemblies of amyloid proteins.

Taken together, these results help establish accurate experimental conditions for future SPR kinetic studies of A β ⁺ IgG. High concentrations of IgG analytes (≥ 400 nM) are necessary to reduce the MTL and obtain successful kinetic fits of OA β binding [32]. Although not used here, increasing the analyte flow from 30 to 100 μ l/min will also reduce the MTL by approximately 30% [21].

Having established SPR experimental conditions and an appropriate fitting model for 4G8 and 6E10, a comparison with natural A β ⁺ IgG was possible. Natural A β ⁺ IgG from IVIG show both higher affinity and faster kinetic interactions to OA β relative to engineered antibodies (Fig. 9 and Table 3). This result reconciles conflicting reports in the literature that show natural A β ⁺ IgG to be both higher in affinity but lower in kinetic stability than 6E10 [9,15]. It is not yet clear if higher affinity and kinetic flux are clinically relevant to AD. But such binding properties do appear different between natural and engineered A β ⁺ IgG.

Another notable result is the complete lack of binding between natural A β ⁺ IgG in IVIG and MA β (Fig. 8). This finding confirms a previous SPR experiment but contrasts somewhat with ELISA studies that indicate low levels of anti-MA β IgG binding are present [8-11,17,18]. This result does support the hypothesis that A β adopts a FA β conformation when immobilized on ELISA plates and affinity columns [11]. Regardless of whether this hypothesis is true, IVIG does not appear to bind MA β in SPR studies [10]. To clarify this antigen preference, we further specified natural A β ⁺ IgG in IVIG as OA β ⁺/FA β ⁺ IgG.

Previous ELISA studies measured IVIG binding to different A β fragments and have identified the C-terminus of A β as the preferred epitope of natural A β ⁺ IgG [10,13]. The lack of binding affinity for MA β by IVIG in the present study indicates that natural IgG does not bind linear epitopes of A β . To reconcile these two findings, it is proposed that natural OA β ⁺/FA β ⁺ IgG only bound the C-terminal A β fragments with a high propensity to oligomerize and adopt the high-affinity neo-epitope in the ELISA experiment.

Although binding kinetics were slightly faster with nOA β , OA β ⁺/FA β ⁺ IVIG affinity was similar between nOA β and sOA β (Fig. 8b). SDS treatment did not significantly alter the antigenic neo-epitope formed when nOA β assembled from monomeric precursors. It has been proposed that the increased binding propensity of natural IgG to OA β versus MA β is mediated through β -sheet pairing between OA β and Fc domains [33]. While this is an intriguing possibility, this model would need to account for the biphasic dissociation found in both mAb (Fig. 3) and IVIG (Fig. 7) binding to OA β . To facilitate future investigation into this and other hypotheses, the present

study has established optimal SPR experimental and fitting procedures for A β ⁺ IgG binding to immobilized OA β .

In summary, this work provides an improved kinetic characterization of the binding between A β oligomers and IgG antibodies. This method can be used to assess different A β ⁺ IgG kinetic binding fluxes of antibody drugs used to treat AD. In addition, this knowledge may help design methods of that quantify engineered and natural A β ⁺ IgG in biological fluids by their kinetic profiles.

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Figure Legends

Fig. 1. *A portion of nOA β is stable to SDS denaturation.* (a) SPR sensogram of immobilized nOA β exposed to five 30 second pulses of 0.5% SDS resulting in 50% OA β remaining. The final resting value of SDS-stable oligomers (sOA β) is produced with ten 30 s pulses and six 60 s pulses and is 45% (1020 RU) of the initial value (2264 RU). (b) SPR measurements of the nOA β decay following pulse 1 (●), 3 (○), and 5 (●). Also shown are single exponential fits of decays after pulse 1 (—), 3 (-----), and 5 (—). Fitted decay time constants are indicated in the Figure legend.

Fig. 2. *At low A β immobilization levels, global fits of 6E10 binding succeed for monomeric A β but fail with oligomeric sOA β .* SPR sensograms (grey data points) and global fits (—) are shown for 1:1 fits of monomeric A β (a), 1:1 fits of oligomeric sOA β (b), bivalent fits of monomeric A β (c) and bivalent fits of oligomeric sOA β (d). 6E10 concentrations shown are 33, 66, 100, and 166 nM.

Fig. 3. *Mass transport limitations are observed in sOA β kinetics but not with MA β .* Local bivalent fits and parameters are shown for 6E10 binding to MA β and sOA β . Sensogram raw data (black data points), 1:1 local fit (—) and bivalent analyte local fit (—) of 200 nM 6E10 binding 8 RU of MA β ligand (a) and 15 RU of sOA β ligand (b). Fit residuals for the local 1:1 fit

(grey data points), and the bivalent analyte local fit (black data points) of 200 nM 6E10 binding 8 RU of MA β ligand (c) and 15 RU of sOA β ligand (d). Fitted parameters k_{a1} , k_{d1} , τ_{a2} , k_{d2} from local bivalent fits of 6E10 binding to MA β (e) and sOA β (f). In Panels e and f, black squares represent bivalent parameters k_{a1} (upper ■) and k_{d1} (lower ■). Open circles represent τ_{a2} (upper ○) and k_{d2} (lower ○).

Fig. 4. *Kinetics of mAb binding are independent of sOA β surface density. Bivalent fits and parameters are shown for mAb binding to sOA β . Sensogram raw data (black data points), 1:1 local fit (—) and bivalent analyte local fit (—) of 400 nM 6E10 binding 406 RU of sOA β ligand (a) and 400 nM 4G8 binding 1870 RU of sOA β ligand (b). Fit residuals for the local 1:1 fit (grey data points), and the bivalent analyte fit (black data points) of 400 nM 6E10 binding 406 RU of sOA β ligand (c) and 400 nM 4G8 binding 1870 RU of sOA β ligand (d). Fitted parameters k_{a1} , k_{d1} , τ_{a2} , k_{d2} from bivalent fits of 6E10 (e) and 4G8 (f). In Panels e and f, black symbols (●, ○, ■) indicate data from 406 RU sOA β and grey symbols (●, ○, ■) indicate data from 1870 RU sOA β . Squares represent bivalent parameters k_{a1} (upper ■, ■) and k_{d1} (lower ■, ■). Open circles represent τ_{a2} (○, ○). Closed circles represent k_{d2} (●, ●).*

Fig. 5. *The kinetics of anti-A β IgG binding to OA β are inherently mass-transport limited and independent of immobilized OA β . Association sensograms are shown for 6E10 (black data) and 4G8 (grey data) analytes at 20 nM with 15 RU of immobilized OA β (a), at 20 nM with 406 RU of immobilized OA β (b), at 400 nM with 15 RU of immobilized OA β (c), and at 400 nM with*

406 RU of immobilized OA β (**d**). For each pair of association sensograms, the upper line was collected at 60 μ l/min and the lower line was collected at 10 μ l/min. The mass transport limitation calculated from each pair of association sensograms is shown next to the data.

Fig. 6. *Mass-transport limitations are absent at high anti-A β IgG concentrations.* Local bivalent fit parameters are shown at varying OA β immobilization levels for binding of 400 nM analytes 6E10 (**a**) and 4G8 (**b**). In Panels **a** and **b**, squares represent bivalent parameters k_{a1} (upper $\blacksquare$) and k_{d1} (lower $\blacksquare$). Open circles represent τ_{a2} (upper $\circ$) and k_{d2} (lower $\circ$). The ratio of χ^2 reduction between the bivalent analyte fit and either the 1:1 fit ($\blacktriangle$) or the 1:2 heterogeneous ligand fit (Δ) are shown for 6E10 (**c**) and 4G8 (**d**). The χ^2 reduction values below 1 indicate the degree which the bivalent model χ^2 is better while χ^2 reduction values above 1 indicate the degree which the bivalent model χ^2 is worse. In panels **a,b,c,d**, black symbols ($\blacksquare$, $\circ$, $\blacktriangle$, Δ) indicate data from mAb binding to sOA β and grey symbols ($\blacksquare$, $\circ$, $\blacktriangle$, Δ) indicate data from 6E10 binding to “native” nOA β .

Fig. 7. *Binding kinetics between natural A β^+ IgG and sOA β obeys a bivalent analyte model.* (**a**) SPR raw sensograms of 10% Gammunex IVIG binding to a flow cell with 1020 RU of immobilized sOA β (—) and a reference flow cell (—). (**B**) Reference cell corrected SPR sensogram (**b**) and fit residuals (**c**) of natural A β^+ IgG binding to sOA β (black data points) with a 1:1 fit (—) and bivalent analyte fit (—).

Fig. 8. *IVIG exhibits high binding to native nOA β and SDS-treated sOA β with no binding to monomeric MA β .* (a) Raw and (b) surface-normalized adjusted SPR sensograms of 10% IVIG binding to 7526 RU of nOA β , (grey data points/line) 3203 RU of sOA β (black data points/line), and 474 RU of MA β (dashed line). Also shown are the bivalent analyte fits and residuals (**b** inset) for sOA β and nOA β .

Fig. 9. *Natural A β^+ IgG binds OA β with higher affinity and faster kinetics than 6E10 or 4G8.* Comparison of bivalent analyte parameters k_{a1} (**a**), k_{d1} (**b**), and K_{D1} (**c**) between natural A β^+ IgG binding to sOA β ($\bullet$), natural A β^+ IgG binding to nOA β ($\circ$), 6E10 binding to sOA β (—), and 4G8 binding to sOA β (—). Natural A β^+ IgG parameters, indicated by “IVIG”, are plotted as a function of the OA β immobilization level. In panels **a,b,c**, the value of each line shown for 6E10 and 4G8 is the averaged k_{a1} , k_{d1} , or K_{D1} value across all sOA β immobilization levels in Fig. 11. Panel (**d**) shows the ratio of χ^2 reduction between the bivalent analyte fit and either the 1:1 fit ($\blacktriangle, \Delta$) or the 1:2 heterogeneous ligand fit ($\blacktriangle, \Delta$) for natural A β^+ IgG binding to sOA β (closed triangles $\blacktriangle, \blacktriangle$) or to nOAb (open triangles Δ, Δ). The χ^2 reduction values below 1 indicate the degree which the bivalent model χ^2 is better while χ^2 reduction values above 1 indicate the degree which the bivalent model χ^2 is worse. Error bars show the standard deviation (n=3) of natural A β^+ IgG binding to sOA β (closed symbols). No statistics were determined for nOA β as only a single measurement could be performed (n=1).

a **b**

a **b** **c** **d**

a **b** **c** **d**

a **b**

Table 1. Global fit parameters for 1:1 Langmuir binding with mAb 6E10.

<u>Ligand</u>	<u>Immobilized</u>	<u>k_a (M⁻¹ s⁻¹)</u>	<u>k_d (s⁻¹)</u>	<u>K_D (M)</u>	<u>R_{max} (RU)</u>	<u>t_c (s⁻¹)</u>	<u>χ^2 (RU²)</u>
MA β	8 RU	1.8 x 10 ⁵	1.8 x 10 ⁻⁴	1.2 x 10 ⁻⁹	5.2	3.0 x 10 ⁻¹⁸	0.0462
OA β	15 RU	4.2 x 10 ⁵	4.2 x 10 ⁻⁴	1.4 x 10 ⁻¹⁰	8.3	1.7 x 10 ⁻¹⁴	0.241

Table 2. Global fit parameters for 1:1 bivalent analyte fit of mAb 6E10 binding.

<u>Ligand</u>	<u>Immobilized</u>	<u>$k_{a1}(\text{M}^{-1} \text{s}^{-1})$</u>	<u>$k_{d1}(\text{s}^{-1})$</u>	<u>$K_{D1}(\text{M})$</u>	<u>$k_{a2}(\text{RU}^{-1} \text{s}^{-1})$</u>	<u>$k_{d2}(\text{s}^{-1})$</u>	<u>$R_{\text{max}}(\text{RU})$</u>	<u>$\tau_{a2}(\text{s})$</u>	<u>$t_c(\text{s}^{-1})$</u>	<u>$\chi^2_{\text{bv}}(\text{RU}^2)$</u>	<u>$\chi^2_{\text{bv}}/\chi^2_{1:1}$</u>	<u>$\chi^2_{\text{bv}}/\chi^2_{1:2}$</u>
MA β	8 RU	6.0×10^4	3.4×10^{-4}	5.6×10^{-9}	7.7×10^{-4}	6.1×10^{-4}	5.2	250	6.3×10^7	0.0445	0.96	1.0
OA β	15 RU	2.3×10^5	1.1×10^{-4}	4.8×10^{-10}	3.2×10^{-3}	1.1×10^{-3}	8.3	38	2.9×10^5	0.160	0.66	0.72

Table 3. Local bivalent analyte fit parameters for IgG binding to immobilized A β ligands under minimal MTL^a.

<u>Ligand</u>	<u>Analyte</u>	<u>k_{a1}</u> (10 ⁴ M ⁻¹ s ⁻¹)	<u>k_{d1}</u> (10 ⁻³ s ⁻¹)	<u>K_{D1}</u> (10 ⁻⁹ M)	<u>τ_{a2}</u> (s)	<u>k_{d2}</u> (10 ⁻⁴ s ⁻¹)	<u>K_{D2}</u> (10 ⁻⁴)	<u>t_c</u> (RU M ⁻¹ s ⁻¹)
MA β ^b	6E10 ^h	5.8	1.1	19	14	3.3	47	1.0 x 10 ⁸
sOA β ^c	6E10 ⁱ	5.3 (3.0)	2.0 (0.3)	47 (23)	6.5 (7.4)	1.1 (1.1)	5.8 (6.6)	2.3 (0.9) x 10 ⁸
nOA β ^d	6E10 ⁱ	6.0 (3.2)	2.3 (0.7)	45 (17)	9.3 (13.3)	4.1 (6.4)	6.9 (8.6)	1.8 (1.3) x 10 ⁸
sOA β ^e	4G8 ⁱ	3.8 (0.5)	2.6 (0.3)	69 (1)	7.3 (2.1)	1.9 (0.6)	15 (9)	1.3 (0.7) x 10 ¹⁴
sOA β ^f	IVIG ^j	21 (3)	4.4 (0.5)	21 (2)	24 (9)	0.4 (1.0)	8.8 (19.6)	7.4 (5.2) x 10 ⁸
nOA β ^g	IVIG ^j	35	5.9	17	0.46	180	84	3.4 x 10 ¹²

^a Standard deviations are indicated in parenthesis. No standard deviation is shown if n=1.

^b One measurement at 8 RU of immobilized MA β (n=1).

^c Three measurements at 15, 406, and 1870 RU of immobilized sOA β (n=3).

^d Three measurements at 33, 804, and 3320 RU of immobilized nOA β (n=3).

^e Two measurements at 406 and 1870 RU of immobilized sOA β (n=2).

^f Two measurements at 1020 and 3203 RU of immobilized sOA β (n=2).

^g One measurement at 7526 RU of immobilized nA β (n=1).

^h 200 nM

ⁱ 400 nM

^j 10% Gammunex dialyzed into HBS-EP running buffer.