

“IgG’s: contending with aggregating circumstances”

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Opinion

IgG antibodies are in increasing demand for use as therapeutic humanized monoclonal antibodies (mAb’s),^{1,2} mass- tagged antibodies for spatial imaging of sectioned tissue biopsies^{3,4} and quantitative assays of disease markers and pathogens. During the production of such antibodies (typically as mAb’s), compromising aggregation events can be encountered during producer cell culture, antibody purification, storage and/or covalent labeling.^{5–8} Native structures and conformations of IgG’s achieve a minimal free-energy state dictated by composite electrostatic interactions, hydrogen bonds, Van der Waals effects, side-chain flexibility, and hydrophobic effects.⁸ As IgG monomer concentrations increase, inter-molecular interactions can occur to the extent of forming aggregated monomers. Here, unfolded states can expose hydrophobic cores as prime drivers of folding events.⁸ Unfolded states can also be triggered (and propagated) by solvents, and by the covalent labeling of antibodies. Needs therefore exist for not only approaches that limit IgG aggregation, but also for the informative assay of IgG aggregates.

The continued need to address IgG aggregation is underscored first by recent examples of approaches aimed at limiting aggregate formation. These include new surfactants for producer cell cultures such as Peptronic (a potential substitute for Pluronics);⁹ Protein A resins that (during the acidified elution of isolated antibodies) release isolated IgG’s at higher pH (e.g. pH 4.5);¹⁰ and advances in predicting and defining antibody domains as IgG motifs that initiate and propagate compromising seed conformations, leading to aggregation.^{11,12} This extends further to the development of protective cleavable antibody fusion proteins (e.g., Anticalin-IgG fusions),¹³ as well as the site-specific re-engineering of therapeutic antibodies to counter aggregation (e.g., CC49, an anti- AG-72 mAb).¹⁴ Parallel needs exist for tractable and informative approaches for assaying IgG aggregation, together with approaches for preparing aggregated IgG’s as comparative controls. For the latter (and as an IgG aggregation model), transient exposure to low pH (as relevant to producer cell culture and IgG purification) is commonly and effectively employed to induce IgG aggregation^{15–18} (with heat, agitation, shear force, and high pressure as additional options). Assays of aggregated IgG’s include first those which can be essentially 2-step, and report on relative frequencies of aggregates. These include fluorescent spin dye detection, dynamic light scattering, and differential scanning calorimetry. Additional useful approaches have been reviewed with excellent detail.¹⁹ In addition, MALDI TOF MS that employs a high MW dynode detector is also effective for directly and quantitatively assaying antibody aggregates, including mega-Dalton IgM species.²⁰

As described above, protein aggregation (including IgG’s) frequently involves conformational shifts that expose hydrophobic domains.^{5,8,19} This alters protease accessibility, and has led to the development of limited proteolysis as an aggregate assay that can additionally inform on shifts in protease accessibility within specific domains.^{5,21–23} This is to the extent that limited proteolysis (“LiP”) has been applied, together with LC-MS, to define aggregated protein profiles not only for target protein structural changes in neurodegenerative disease,^{22,23} but also for intact cell populations.²⁴

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For this Opinion report, one component goal is to draw attention to the suggested advantages of implementing a medium- throughput workflow for the assay of IgG aggregates that combines LiP with MALDI TOF MS.

LiP first is employed to generate peptides as hydrolyzed from aggregate- containing IgG samples, and from non-aggregated monomer IgG controls. For control samples, it’s noted that hydrophobic interaction chromatography (HIC) resins have been optimized that can effectively remove possible IgG aggregates (and yield monomeric populations)^{25,26} For the native proteolysis of IgG samples, IdeS together with Arg-C or trypsin (single- tube processing at a selected time-point and temperature) generate peptides suitable for MALDI analyses. Simultaneous (or prior) deglycosylation of IgG samples is an added compatible option (e.g., using Endo-S2). For highly aggregated IgG’s that may yield high MW peptides, samples can be processed (if necessary) via C4 resin tips (to remove possible large protein fragments prior to MALDI MS) with an option to reduce disulfide bonds prior to MALDI MS (or by the use of a reducing MALDI matrix). MALDI array multiplexing (e.g., 48-well plate) can readily be employed, and peptide m/z signatures for control IgG vs samples with suspected aggregates can be analyzed (vs positive control pH- aggregated standards) via straightforward open software such as Mass-Up [bio.tools/Mass-Up] (including principle component analyses). For antibodies with known sequences, added insight can be gained into IgG domains within aggregated IgG that may act as seed sites. Beyond rapidly providing specific quantitative peptide signatures for aggregated vs monomeric IgG antibodies, and especially for mAb’s with known sequences, this combined LiP plus MALDI MS approach promises to provide insight into antibody domains which become mis-folded to the extent of conformationally masking or revealing proteolytic sites.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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