Structure and Formation of Antigen-Antibody Complexes

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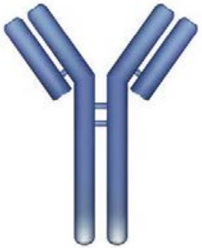
Immunoglobulin G

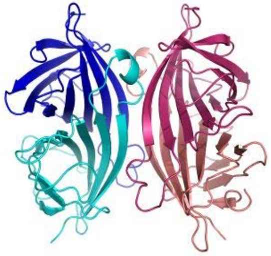
- Type of antibody found in human circulation
- Protects body from infection by targeting and binding to antigens
- Different subclasses
  - IgG1, IgG2, IgG3, IgG4
  - Differ in number of disulfide bonds



http://images.digopaul.com/wp-content/uploads/related_images/2015/09/10/igg_1.jpg

Streptavidin

- A protein produced by the bacterium Streptomyces avidinii
- Tetrameric
- Extremely high affinity for biotin



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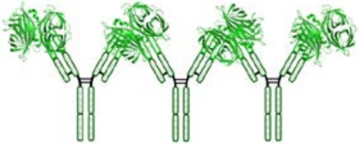
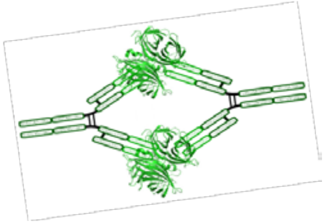
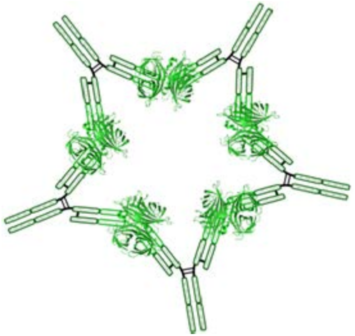
Motivation



- Structure of antigen-antibody complexes is important for understanding how they form and their effect in the immune system
    - Goal: to analyze how two subclasses of immunoglobulin G, IgG1 and IgG2, interact with the protein streptavidin and determine the structure of complexes
- A software framework developed at the NCNR lacks experimental data for its docking module
    - Goal: to provide substantial data & results to improve functionality

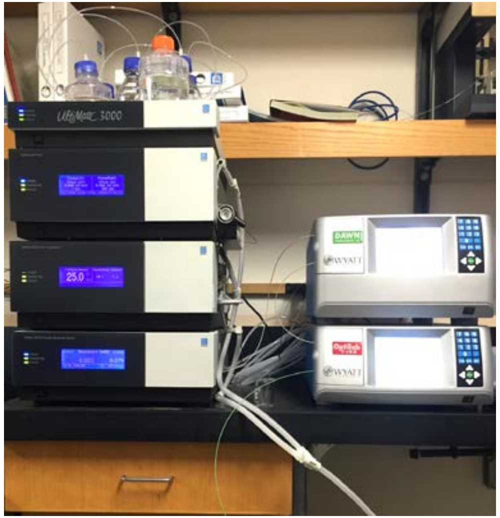


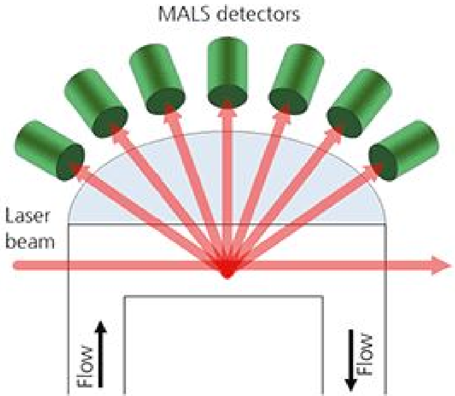


https://commons.wikivet.net/images/thumb/d/db/LH_IgG.png/250px-LH_IgG.png

Size-exclusion chromatography with multiangle light scattering (SEC-MALS)

- Varying concentration ratios, pH to evaluate best conditions for complex formation
- Series of buffer exchanges

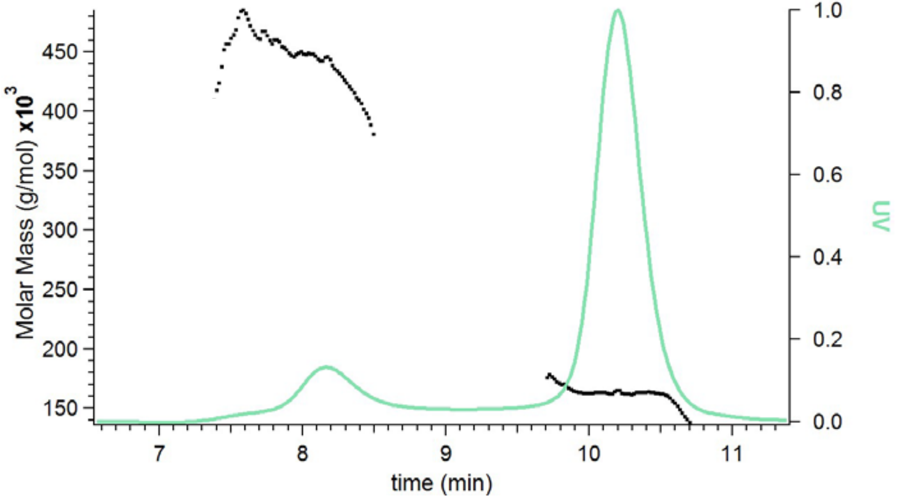




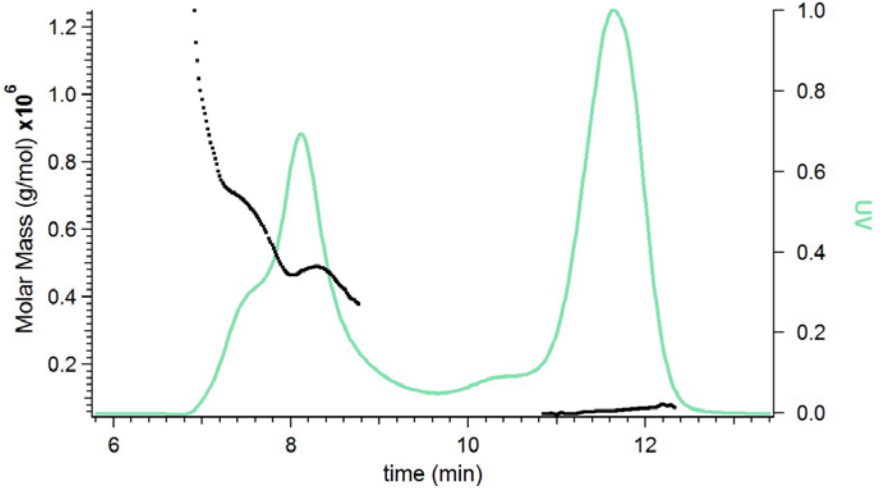
http://www.materials-talks.com/wp-content/uploads/2014/08/MALS-mutli-angle-light-scattering-in-flow.jpg

Obtain a “pure and monodisperse” complex

Results Fitting



Results Fitting



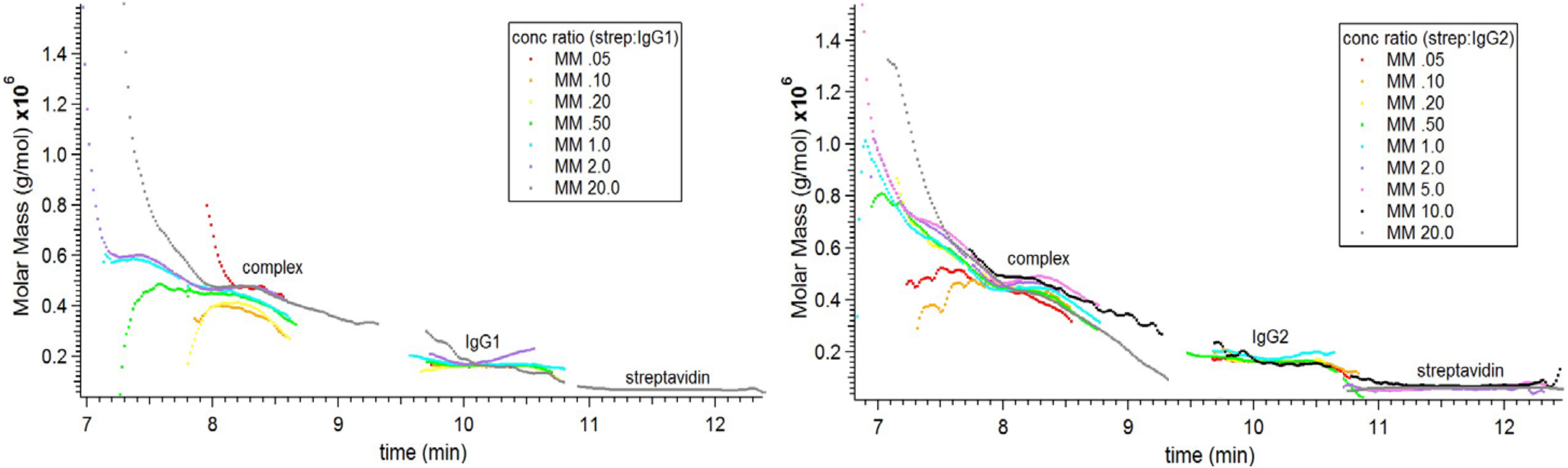
pH 6.5 buffer

Concentration Ratio .50 (strep:IgG1)

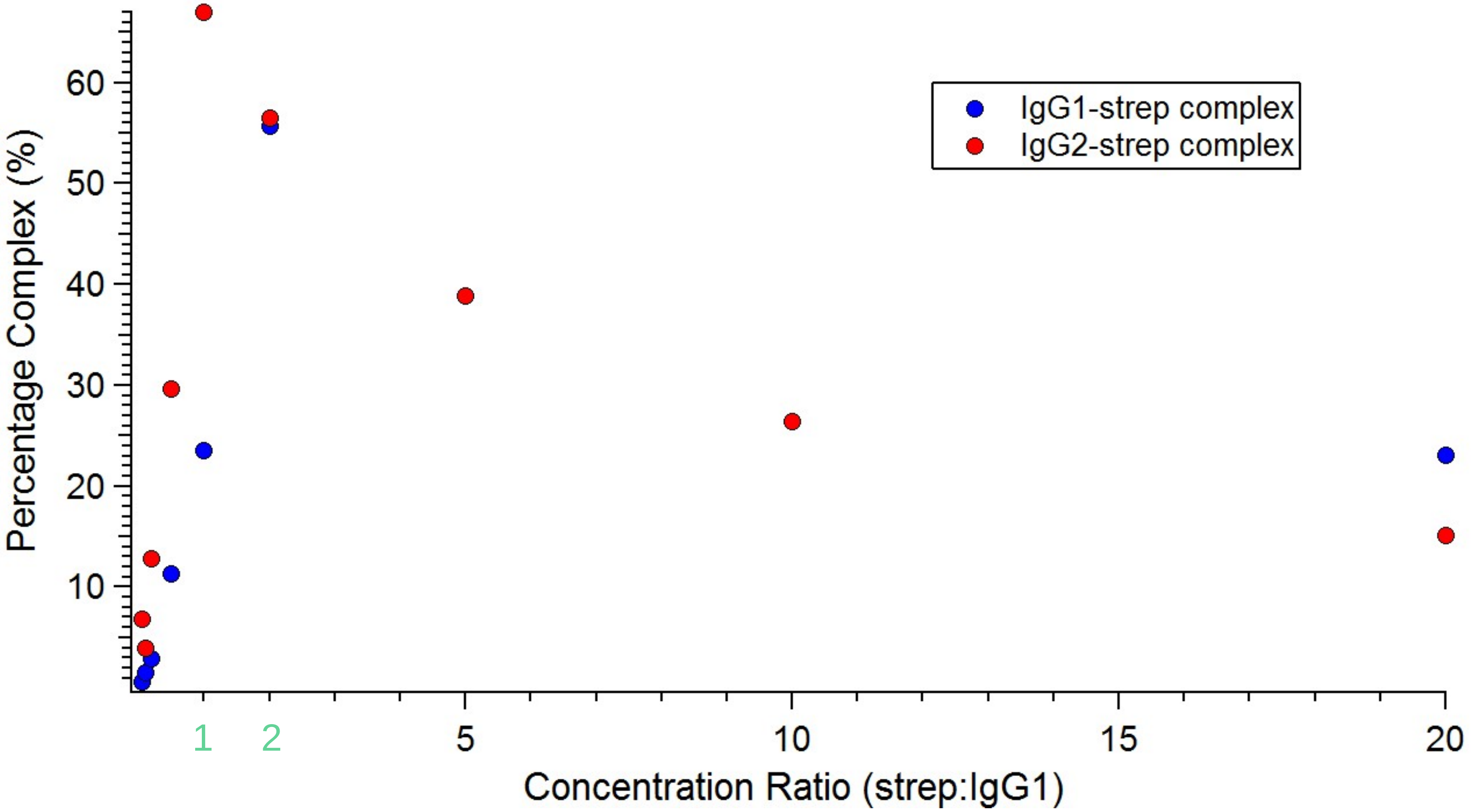
Concentration Ratio 5.0 (strep:IgG2)

Streptavidin-IgG1 Complex Samples

Streptavidin-IgG2 Complex Samples

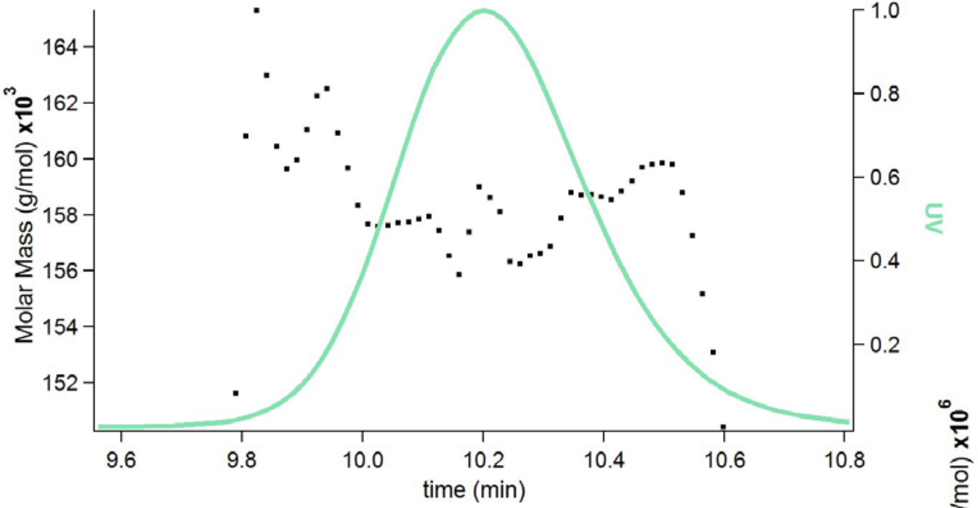


pH 6.5 buffer


IgG1-strep complex
IgG2-strep complex
Percentage Complex (%)
60
50
40
30
20
10
1
2
5
10
15
20
Concentration Ratio (strep:IgG1)

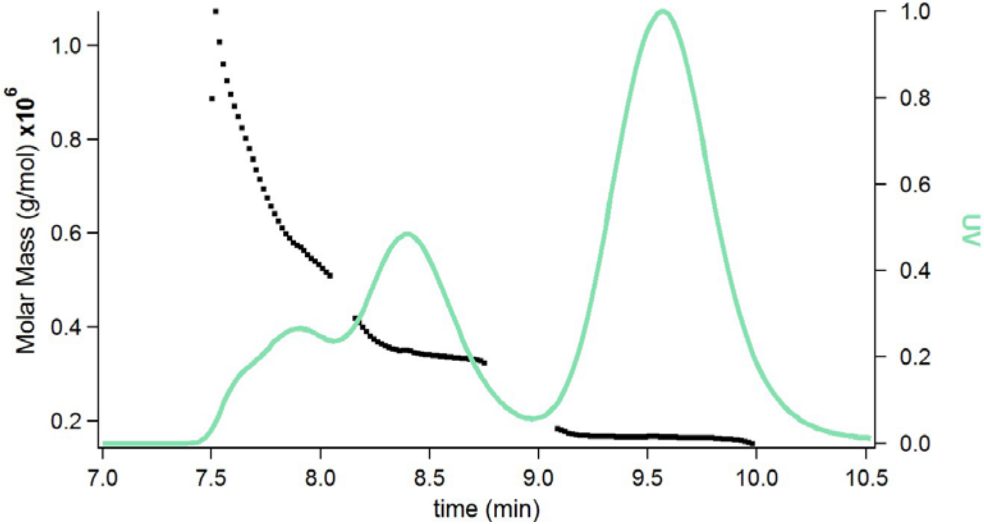
Effects of Different pH

pH 6.5 buffer



IgG2 Individual Sample

pH 3.0 buffer

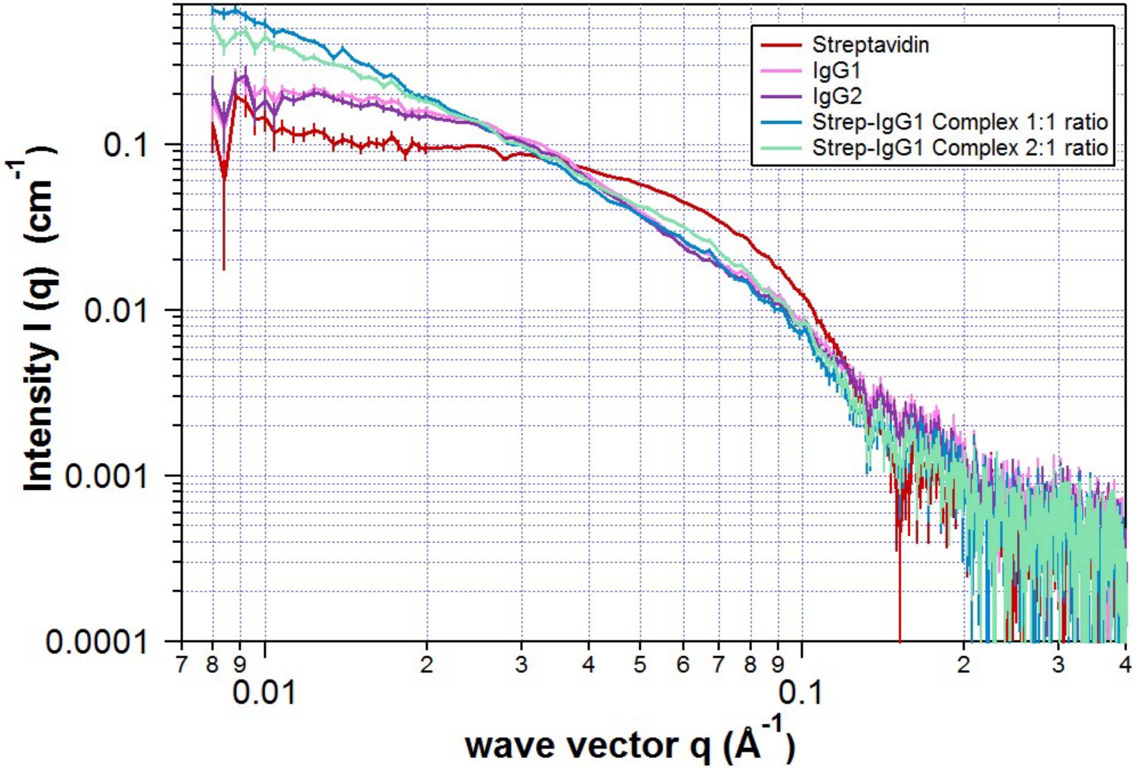


Aggregation occurs in pH 3.0 buffer

Conclusions from SEC-MALS

- Higher percentage of complex forms in a buffer with a more neutral pH
- 1.0 and 2.0 concentration ratios (strep:IgG) yield most complex
- Aggregation of IgG2 occurs in a more acidic environment

SAXS Profile



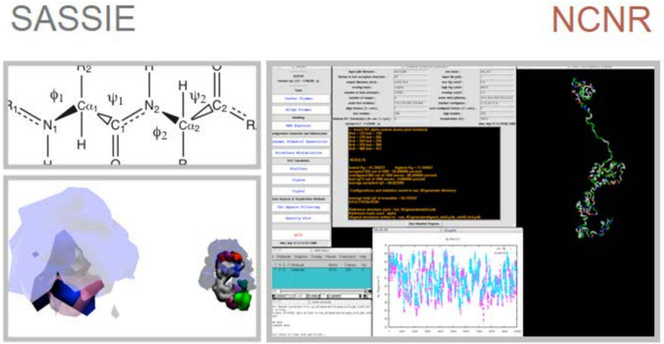
- Larger molecules scatter more at low intensity (q)
- Complex with 1:1 concentration ratio scatters more than 2:1 complex

Future Work

Performing more small-angle scattering

Testing SASSIE Docking Module

- Software framework designed to create atomistic models of molecular systems and to compare small-angle scattering data from these models directly to experimental data



Acknowledgements

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