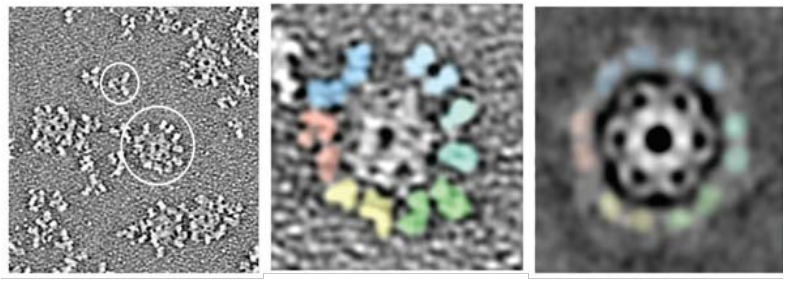


Diebolder CA, et al. Complement is activated by IgG hexamers assembled at the cell surface. Science 2014; 343:1260–3.

IgG Cooperativity

Learning new tricks from old dogs

CASSS 2018

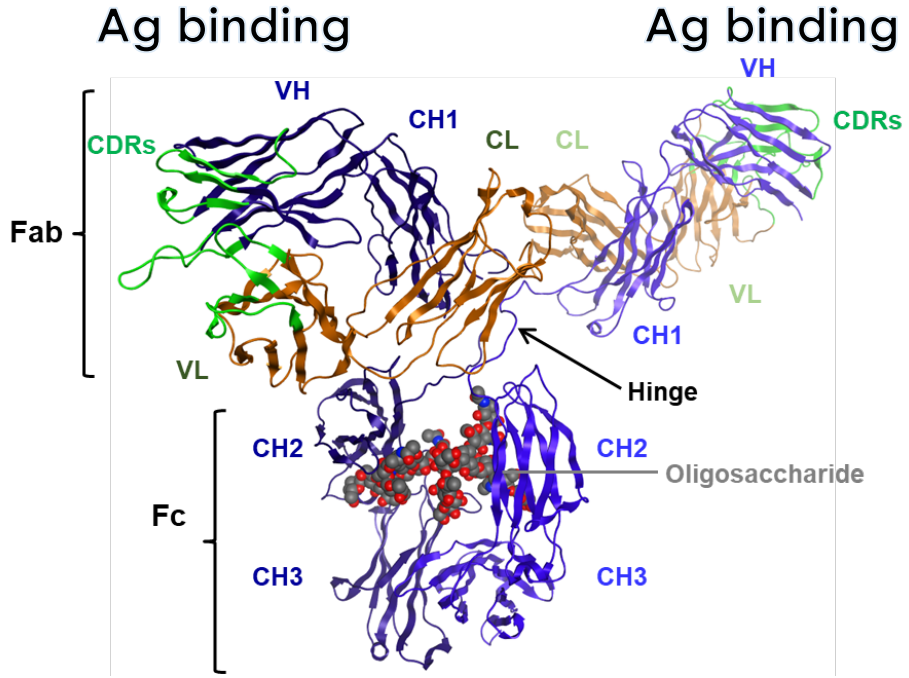
Danlin Yang

Yang, et al. (2017) "IgG cooperativity - Is there allostery? Implications for antibody functions and therapeutic antibody development." MAbs 9:1231-1252.

Yang, et al. (in press) "Weak IgG self-and hetero-association characterized by fluorescence analytical ultracentrifugation" Protein Sci.

Outline

- Cooperativity- some rules
  - Mechanisms of cooperativity
- IgG cooperativity
  - Experimental system
  - Self-association and cooperativity
- Functional implications

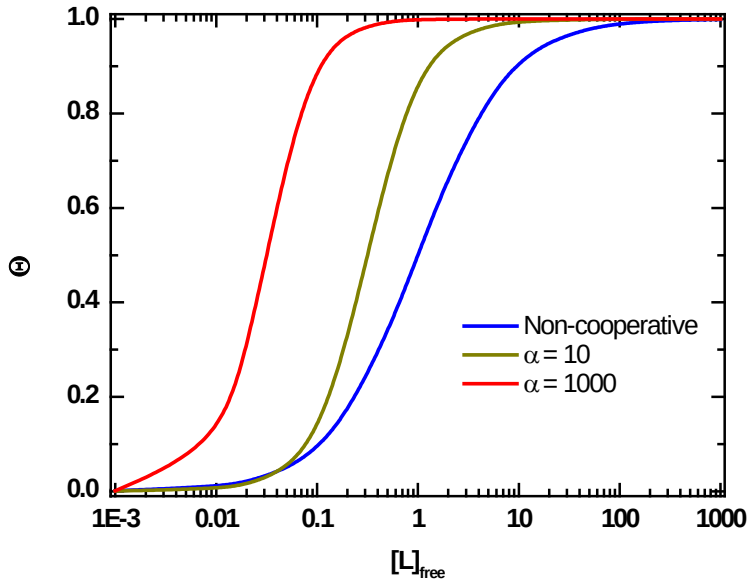


Effector functions
FcR binding
C1q binding

Cooperativity- some rules

Cooperative free energy: α

$$P + L \overset{K_1}{\leftrightarrow} PL + L \overset{\alpha K_1}{\longleftrightarrow} PL_2$$



Rules

More than one binding site (cooperative unit)

ΔG_{coop} = -RT ln(α), value is small

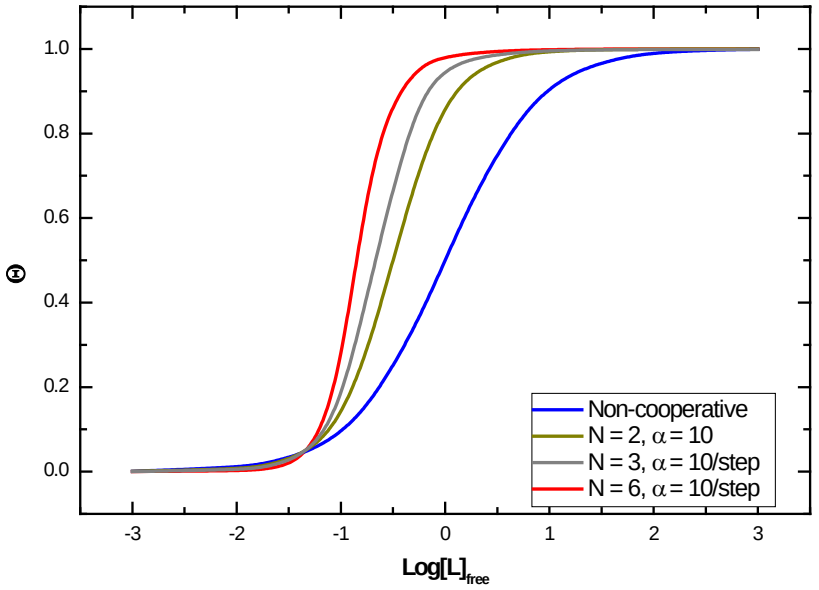
$\alpha < 1$ Negative cooperativity $R_{90:10} > 81$

$\alpha > 1$ Positive cooperativity $R_{90:10} = 18$

Shape unchanged, just onset

Number of cooperative units: N

$$P + L \overset{K_1}{\leftrightarrow} PL + L \overset{\alpha K_1}{\longleftrightarrow} PL_2 + L \overset{\alpha^2 K_1^2}{\longleftrightarrow} PL_3 + L \ldots n$$



Rules

Increasing N sharpens curve

N = 1 $R_{90:10}$ = 81

N = 2 $R_{90:10}$ = 18

N = 3 $R_{90:10}$ = 10

N = 6 $R_{90:10}$ = 8

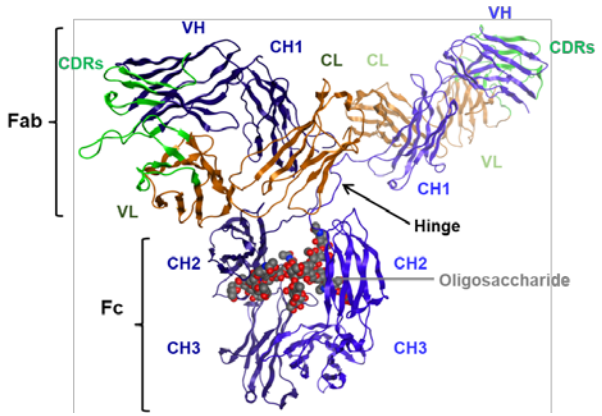
Mechanisms of cooperativity

Mechanisms are non-exclusive

- Intramolecular: allostery
  - Conformational
    - 2º or 3º structural changes
  - Configurational- covalent change
    - Kinase/phosphatase PO_4^{2-}
    - Class/Subclass swapping
      - E.g. $IgG_1 \rightarrow IgG_4$

Conformational

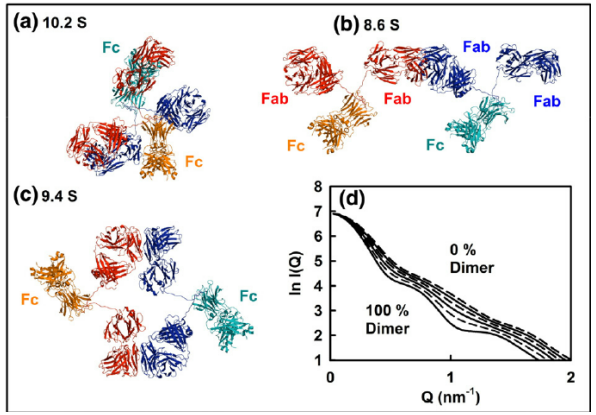
Fab:Fab or Fab:Fc



Configurational

Subclass switching

- Intermolecular: associative
  - Mechanisms
    - Weak self-association
    - Allostery on binding
  - IgG::C1q assembly
  - Fc:FcR binding

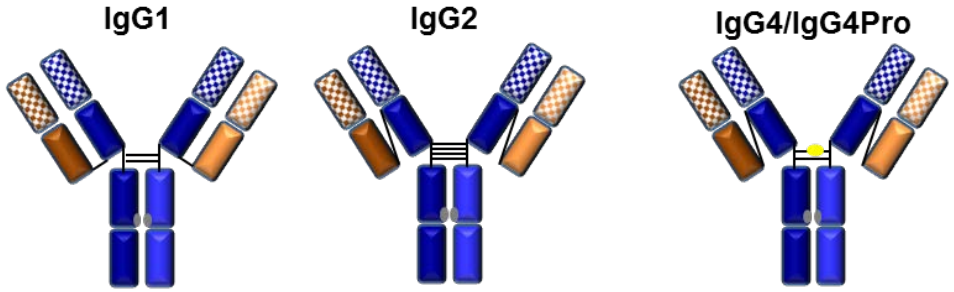


Rayner, et al. (2012) "The Solution Structure of Rabbit IgG Accounts for Its Interactions with the Fc Receptor and Complement C1q and Its Conformational Stability" JMB 425

Experimental system

12 anti-IL13 mAbs + Protein A purified Human IgG

V-region 3 Paratopes	**C-region** 4 subclasses
mAb 1	IgG1
	IgG2
	IgG4
	IgG4Pro



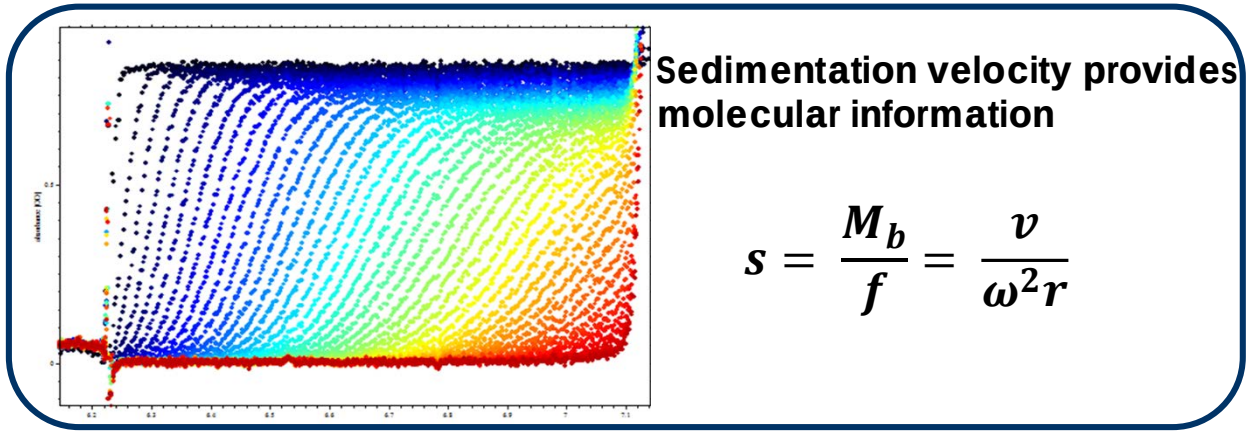
No evidence for V-V or C-V conformational or configurational cooperativity

mAb 3	IgG1
	IgG2
	IgG4
	IgG4Pro
Hu p-IgG	IgG1,2,4

Purified F(ab')^2 and HL-Fc for each mAb prepared

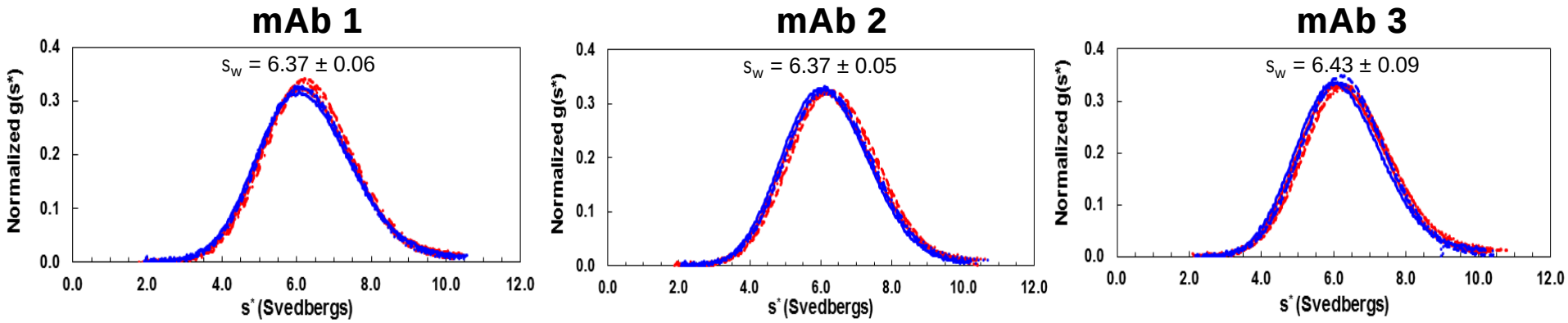
Stable cell lines for mAbs are available

Solution hydrodynamics by AUC



Sedimentation velocity provides molecular information

$$s = \frac{M_b}{f} = \frac{v}{\omega^2 r}$$



Red: pH 5 acetate Blue: pH 7.4 PBS

- **mAb hydrodynamics are comparable**
  - **Slight differences between subclasses expected/observed**
- **Labeled IgG indistinguishable from unlabeled**

Solution charge (Z_{DHH}) by MCE

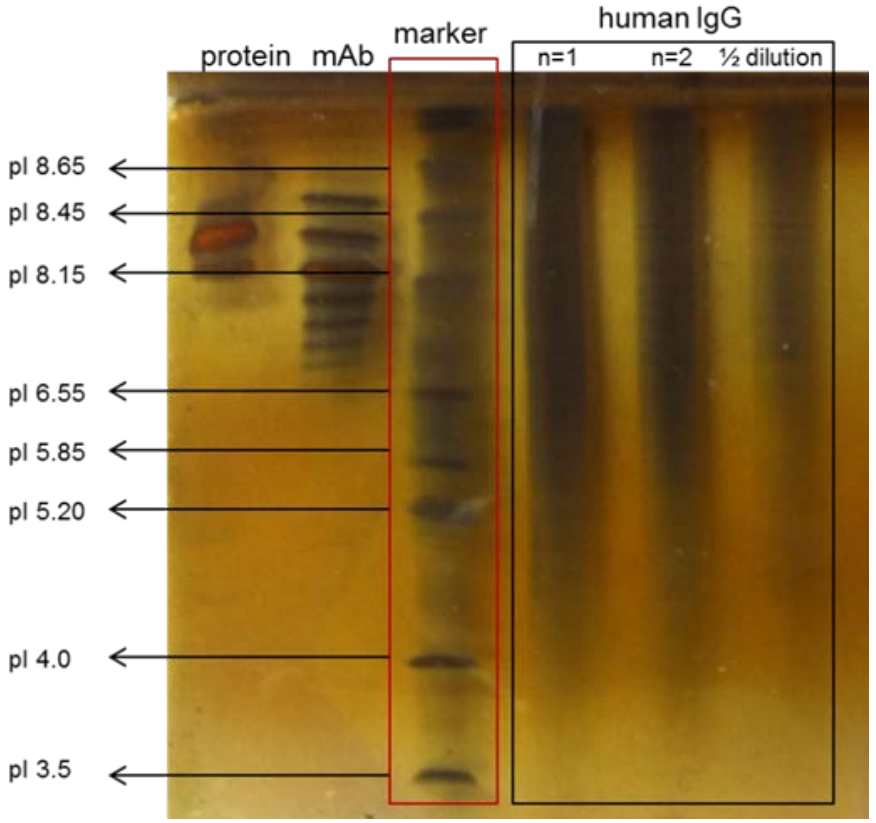
- pH 5 Z_{calc} off by ~50
  - Z Fc off by ~30
  - Z depends on mAb
  - Z depends on subclass
  - Z consistent within subclass
    - i.e. IgG4 & 4Pro
- pH 7.4 Z_{DHH} 0 to -11
  - Varies by mAb
  - Varies by subclass
  - Varies by $F(ab')^2$
  - Not predicable from Z_{calc}
  - Not sum of $F(ab')^2$ + Fc

Yang D, et al. (in press) "Weak IgG self- and hetero-association characterized by fluorescence analytical ultracentrifugation" Protein Sci.

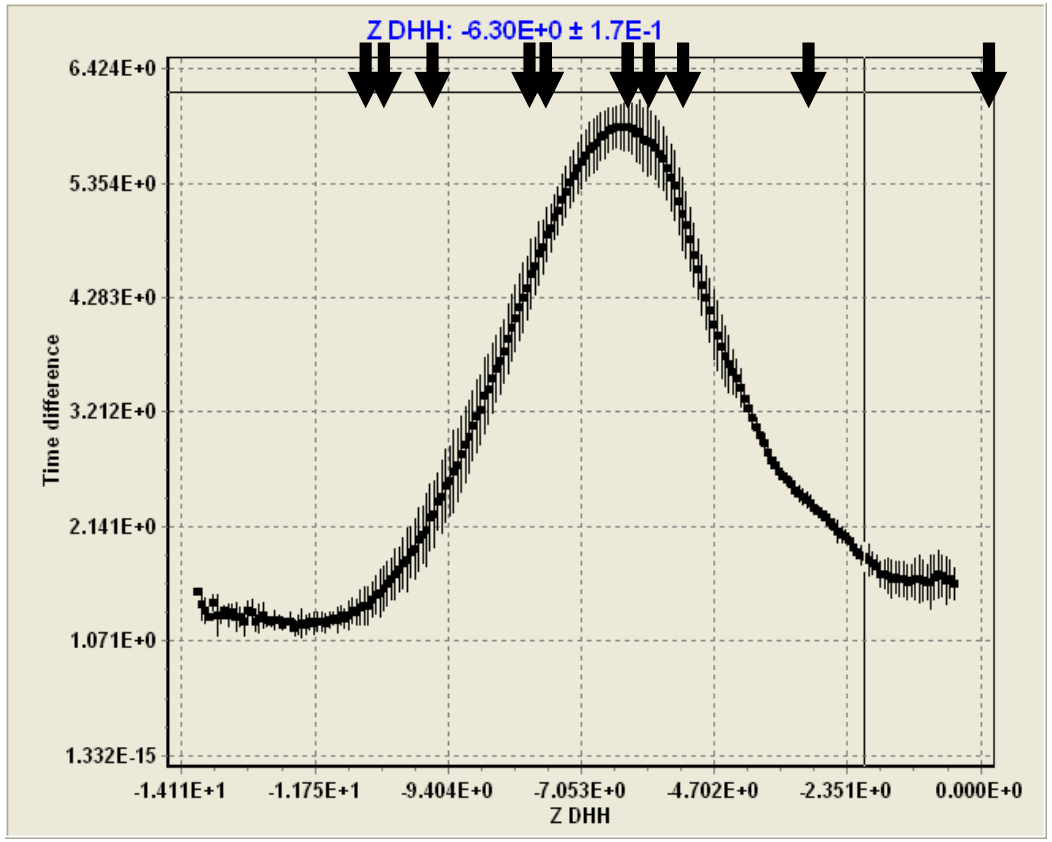
Human poly-IgG

- Physiological charge distribution narrow
- Some mAbs outside "normal" range
- IEF tells you nothing about charge

IEF



Z_{DHH} distribution from MCE PBS

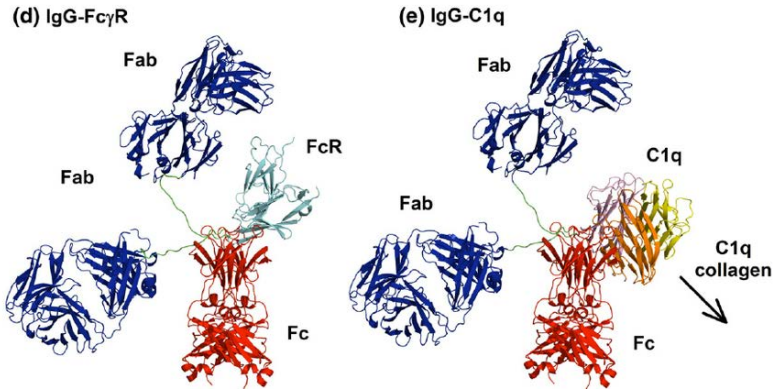


Yang D, et al. (in press) "Weak IgG self- and hetero-association characterized by fluorescence analytical ultracentrifugation" Protein Sci.

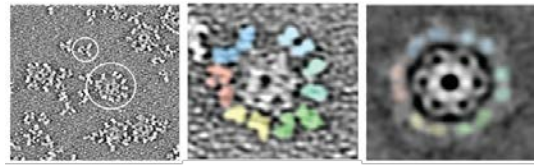
Intermolecular cooperativity

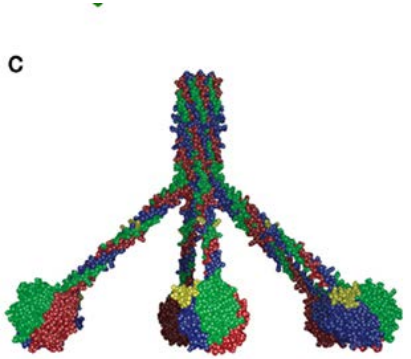
IgG effector functions

- Fc binding
  - FcγR
  - Cellular binding & transport
  - RI, RIIA,RIIB,RIIIA,RIIIB, Rn



- C1q binding sites
  - Adaptive & innate immune coupling
  - "Self" versus "foreign"
    - Tolerance versus immunity





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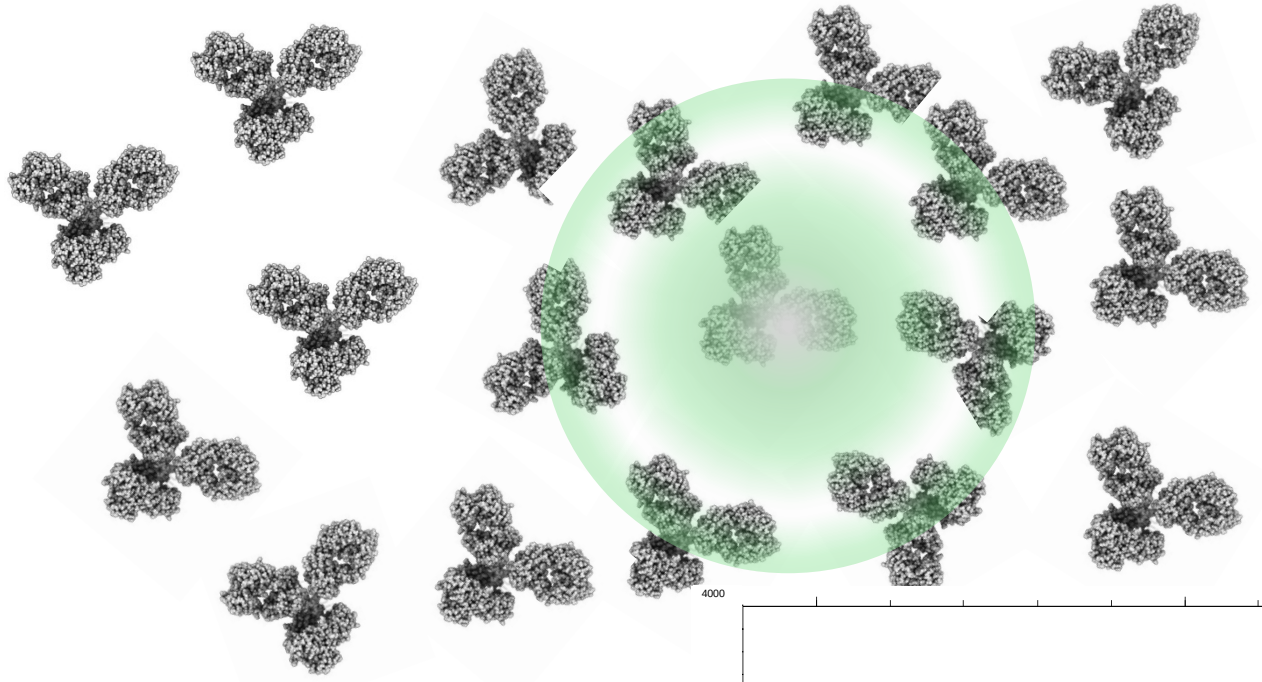
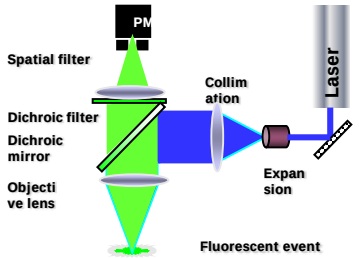
Rayner, et al. (2012) "The Solution Structure of Rabbit IgG Accounts for Its Interactions with the Fc Receptor and Complement C1q and Its Conformational Stability" JMB 425

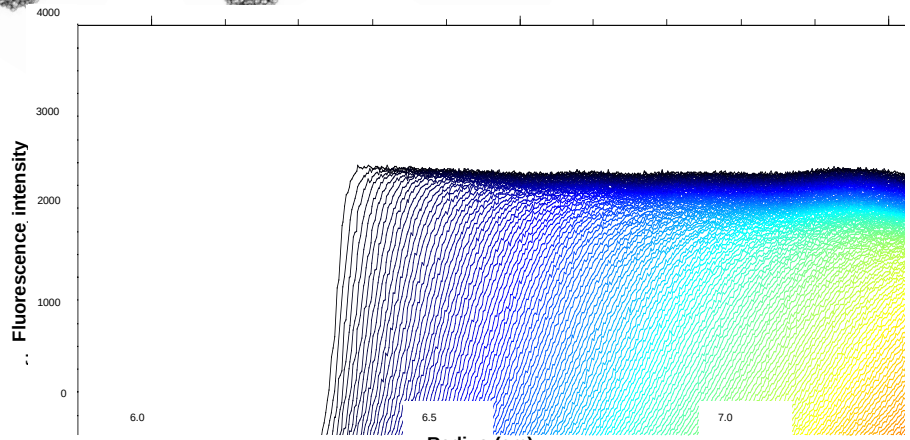
Gaboriaud, et al. (2012) "The Human C1q Globular Domain: Structure and Recognition of Non-Immune Self Ligands" Front. Immuno. 2:92

Weak Ab:Ab association?

Tracer IgG in different IgG backgrounds

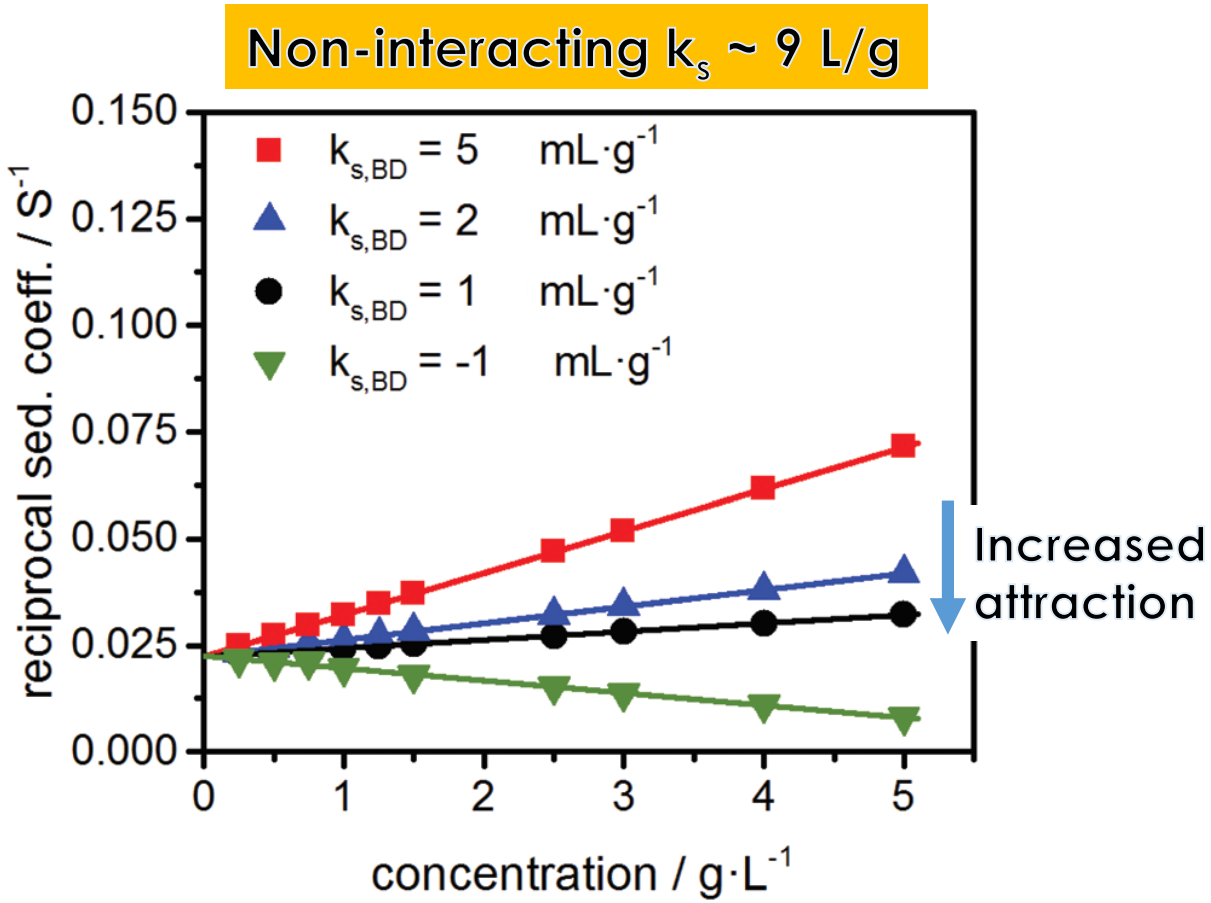






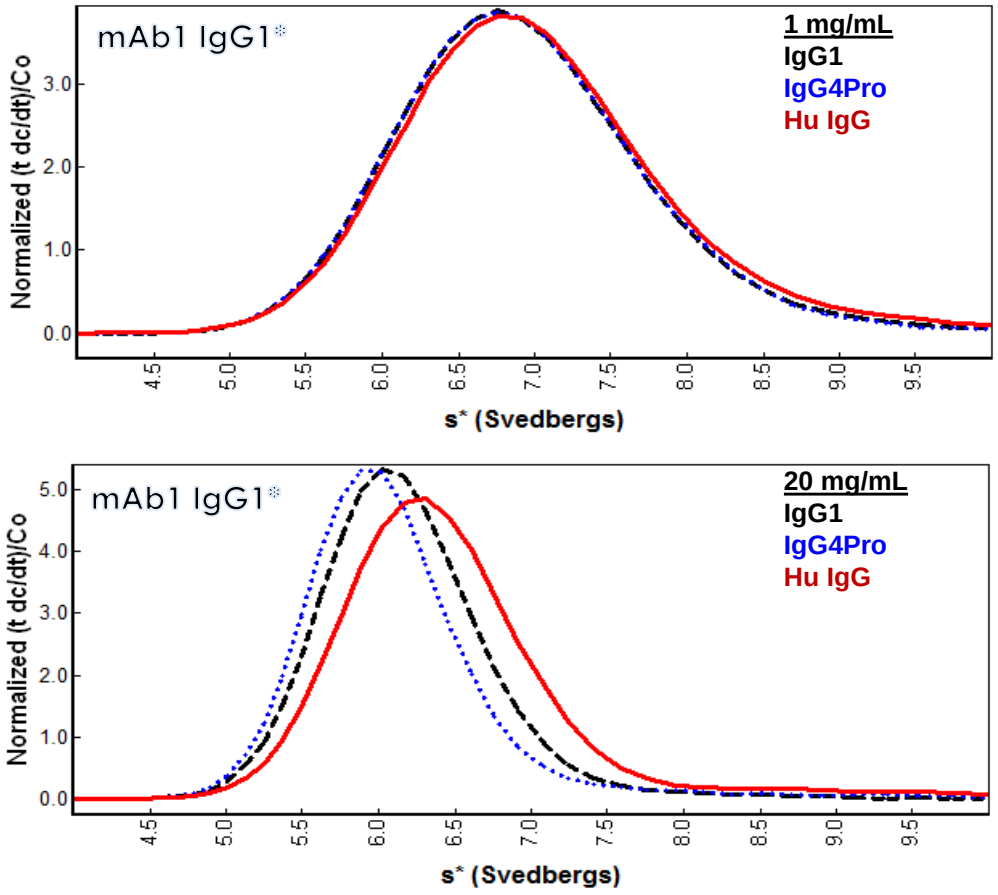
Hydrodynamic nonideality: k_s

- **Concentration-dependent nonideality**
  - **Sedanal: fit for k_S, B, K_a**
- **k_s reflects interactions between adjacent molecules**
  - **k_s = 9 → non-interacting spheres**
  - **k_s > 9 → repulsion or asymmetry**
  - **k_s < 9 → attractive interactions**



Uttinger, et. al (2017) Nanoscale, 9:17770 - 17780

Results- all show weak attraction



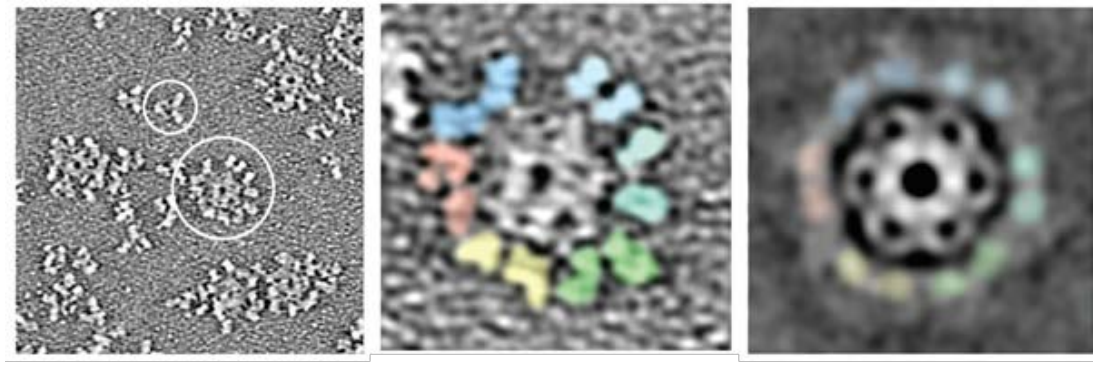
mAb ID	Tracer	Background		
		IgG1	IgG4Pro	Human IgG
mAb 1	IgG1	7.0 ± 0.4	8.0 ± 0.4	4.3 ± 0.2
	IgG4Pro	8.4 ± 0.4	9.1 ± 0.5	5.4 ± 0.3
human IgG		7.3 ± 0.4	8.0 ± 0.4	5.1 ± 0.3
mAb 2	IgG1	5.2 ± 0.3	5.3 ± 0.3	3.2 ± 0.2
	IgG4Pro	5.5 ± 0.3	5.6 ± 0.3	4.4 ± 0.2
human IgG		6.2 ± 0.3	6.5 ± 0.3	5.1 ± 0.3
mAb 3	IgG1	6.4 ± 0.3	7.5 ± 0.4	4.5 ± 0.2
	IgG4Pro	6.0 ± 0.3	8.3 ± 0.4	5.1 ± 0.3
human IgG		7.2 ± 0.4	8.6 ± 0.4	5.1 ± 0.3

- Smaller k_S → strongest attraction
- Depends on mAb
  - IgG4:IgG4 weaker attraction
  - Human poly IgG stronger

Implications for antibody functions

- **IgG:IgG interactions source of cooperative free energy for effector functions**

- **Generality of cooperative interactions allows wider array of epitope spacing to initiate complement activation**



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Summary

- 12 mAbs produced and characterized
  - Stable cell lines available
- No evidence for intramolecular cooperativity
  - No allostery
  - Sub-class swap had no effect on Ag binding
- Charge must be measured
  - pI of Hu poly-IgG ranges from < 4 to > 10
  - Z_{DHH} Hu poly-IgG is -6±3
- All IgGs exhibit weak self-association
  - K_d ~100 – 2000
  - IgG_4 weakest

Thanks to

- Danlin Yang
- Sue Chase
- Walt Stafford
- Jack Correia
- Chris Roberts
- David Hayes
- Boehringer-Ingelheim
- BITC