Determination of interaction parameters for reversibly self-associating antibodies: A comparative analysis

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Skaggs School of Pharmacy and Pharmaceutical Sciences

Discovery. Development. Delivery.

INTRODUCTION

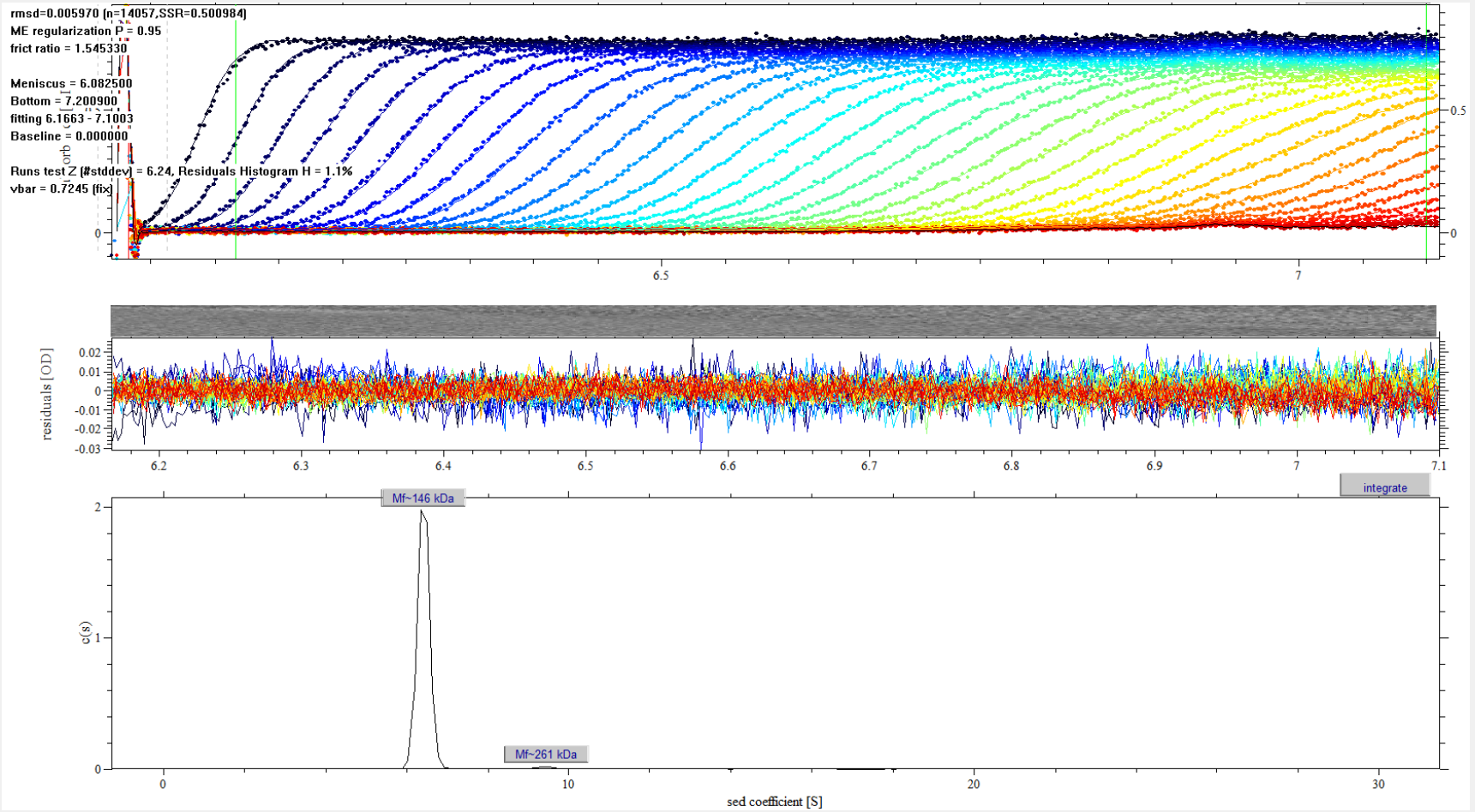
Purpose

- Long-term: Gain a more predictive and mechanistic understanding of reversible self-association (RSA) in therapeutic proteins
- Immediate: Define stoichiometric assembly pathways, interaction affinities, and nonideality terms
- Key points:
  - RSA and nonideality mask one another at sufficiently high concentrations
  - Globally fit concentration-dependent data sets with direct boundary fitting
  - This approach allows for determination of molecular parameters

Hopkins MM, et al (2018) *J Pharm Sci*, In Press.

INTRODUCTION

Sedimentation Velocity (SV) AUC



mAb A one concentration (1 mg/mL) *c*(*s*) analysis

Schuck P (2000) *Biophys J* 78: 1606-1619

INTRODUCTION

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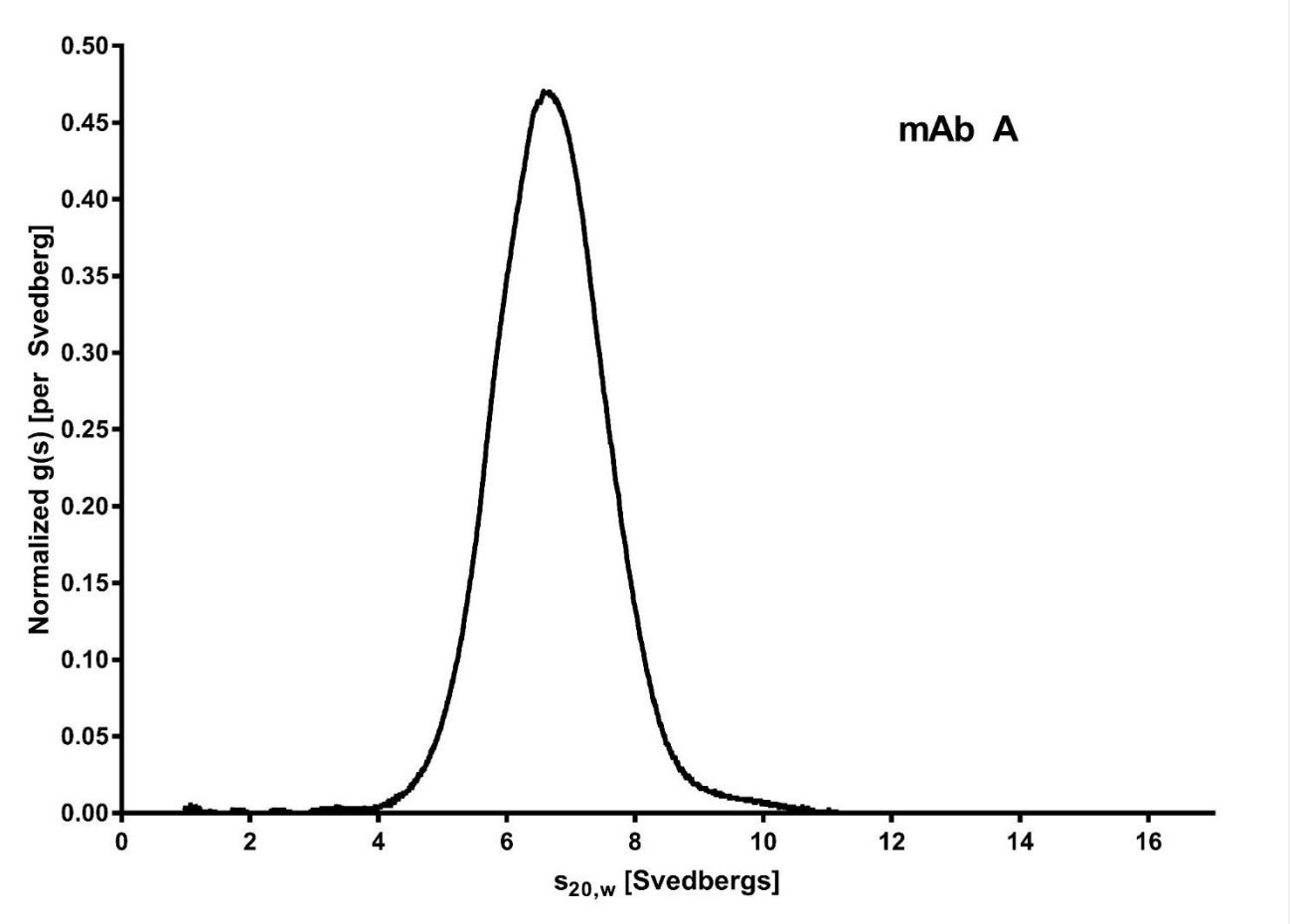

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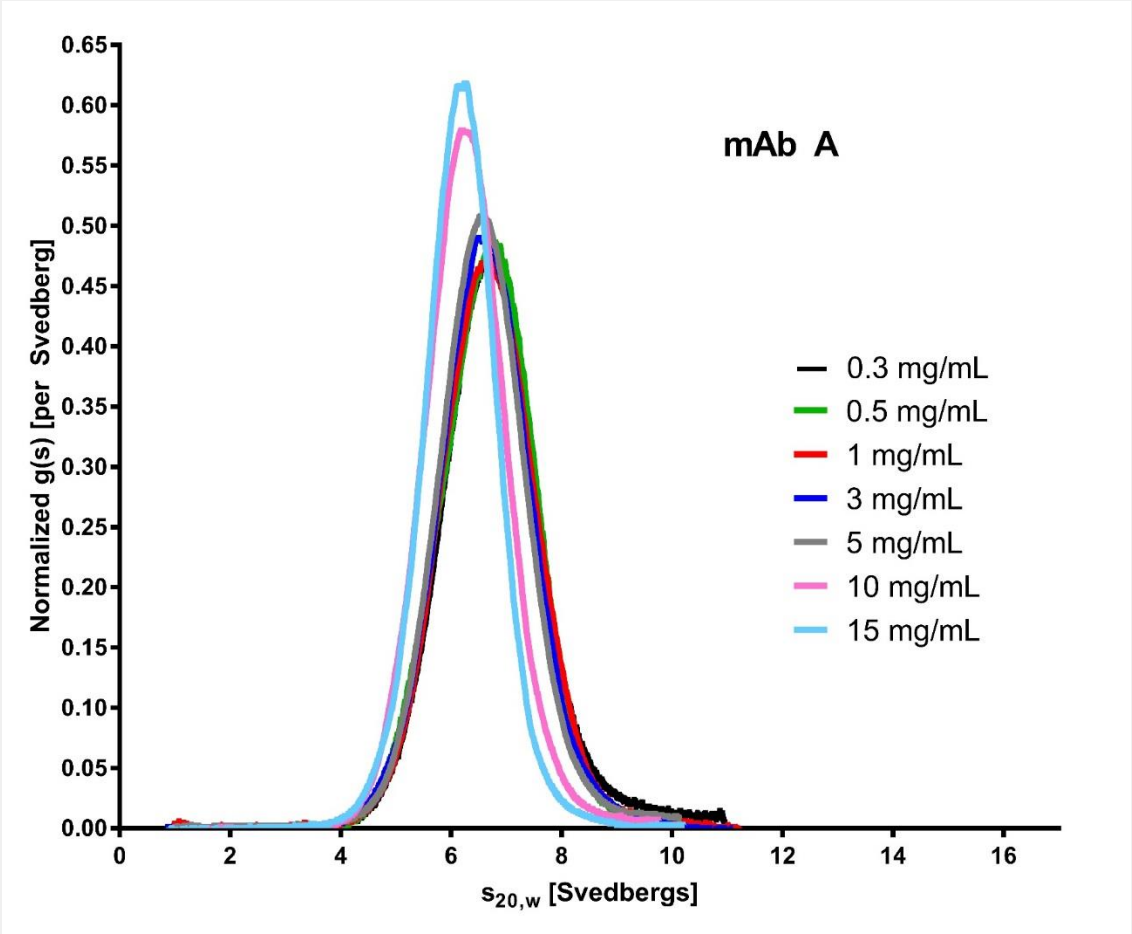
Philo JS (2006) *Anal Biochem* 354: 238-246

INTRODUCTION

Sedimentation Velocity (SV) AUC

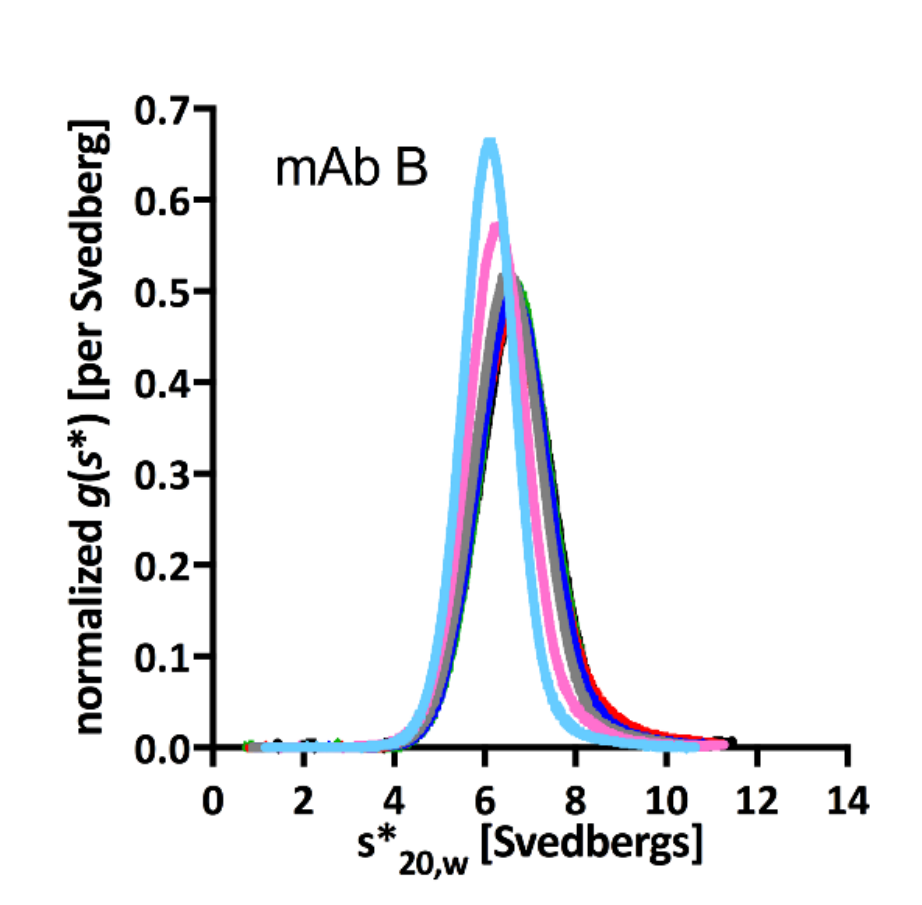


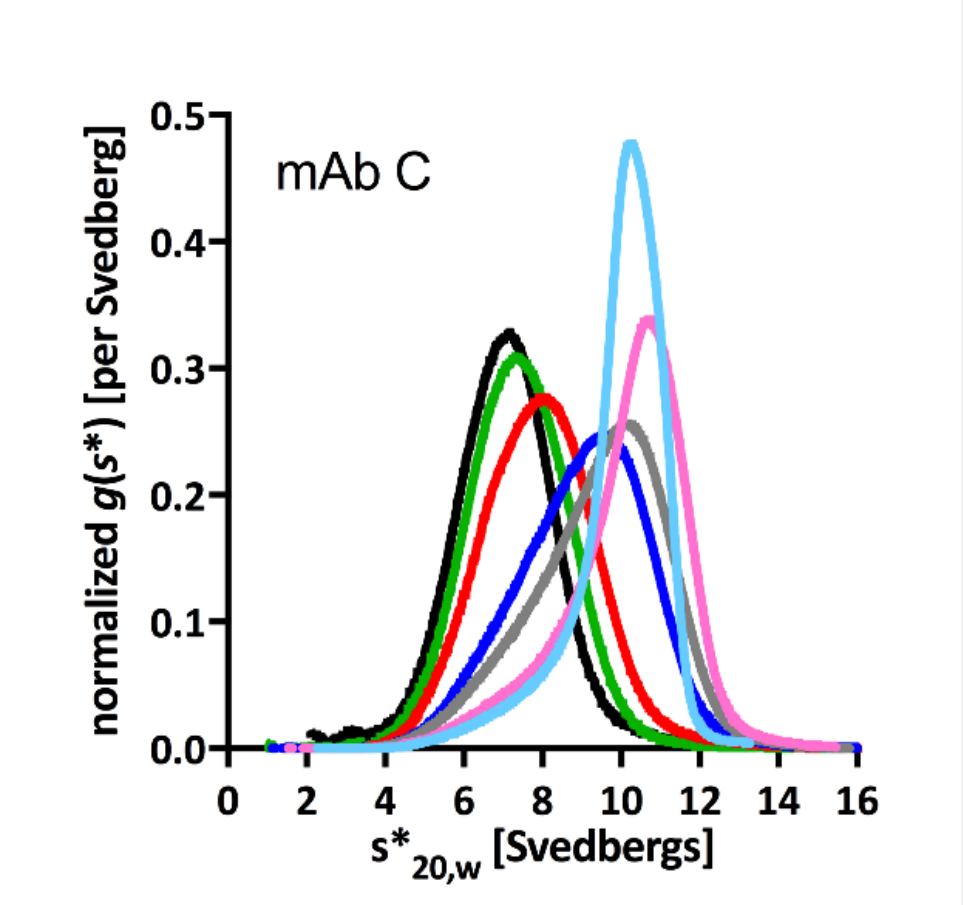
mAb A concentration-dependent g(s) analysis

Hopkins MM, et al (2018) J Pharm Sci In Press

RESULTS

g(*s**) plots reveal nonideality and RSA





Hopkins MM, et al (2018) *J Pharm Sci* In Press

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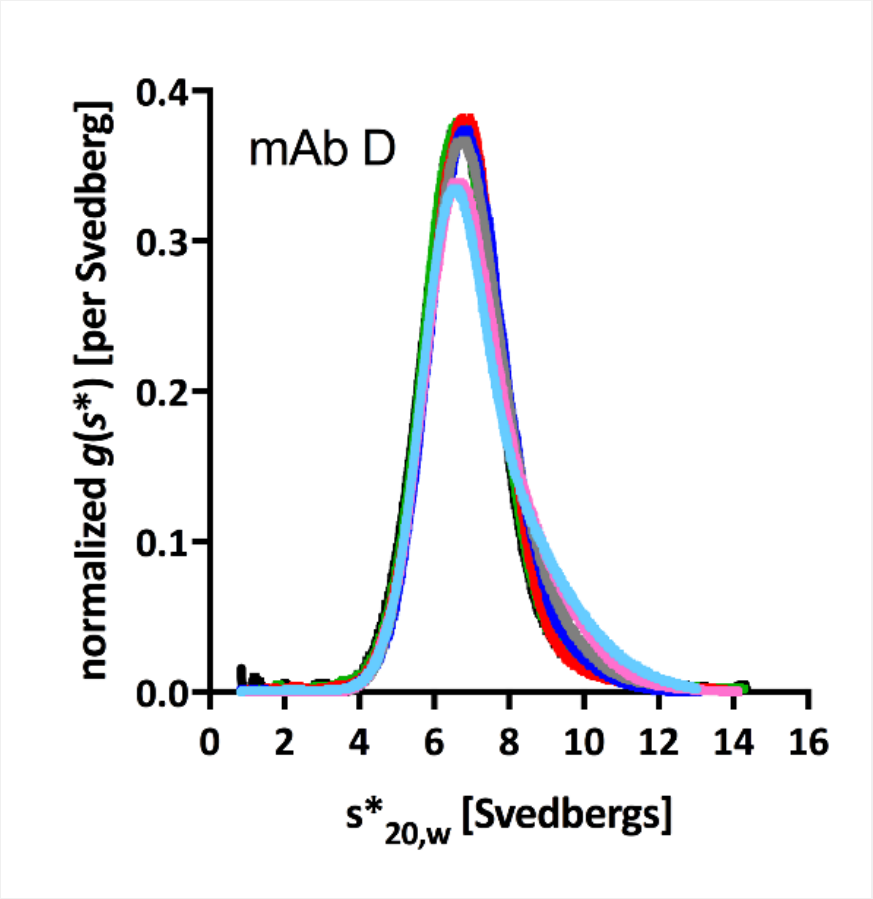

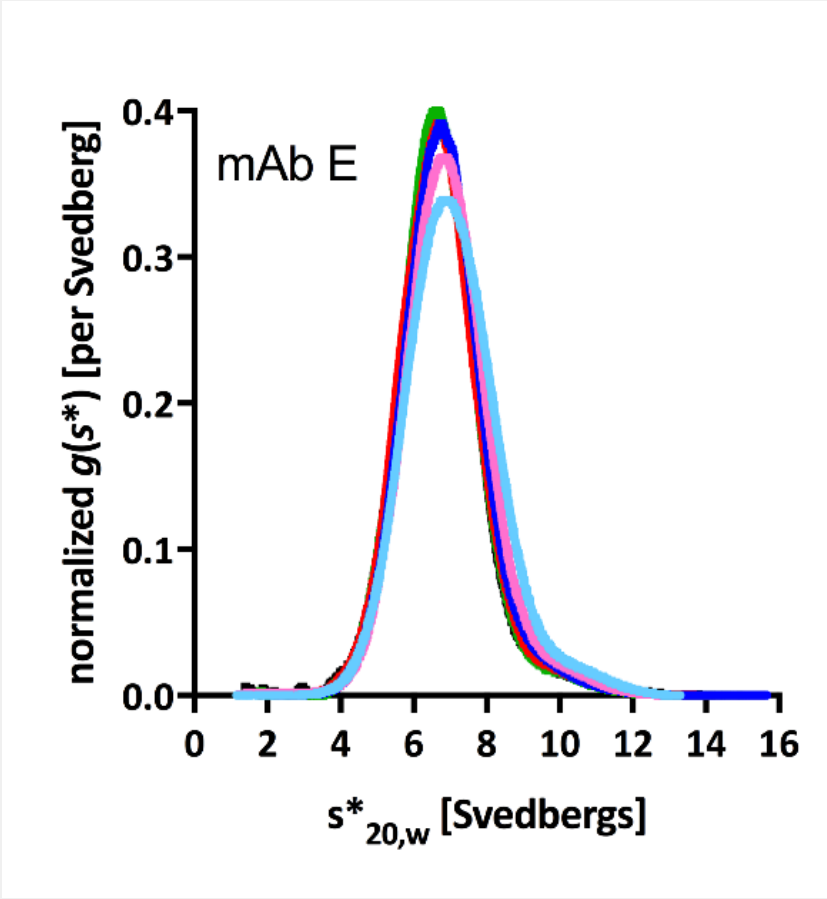

Hopkins MM, et al (2018) *J Pharm Sci* In Press

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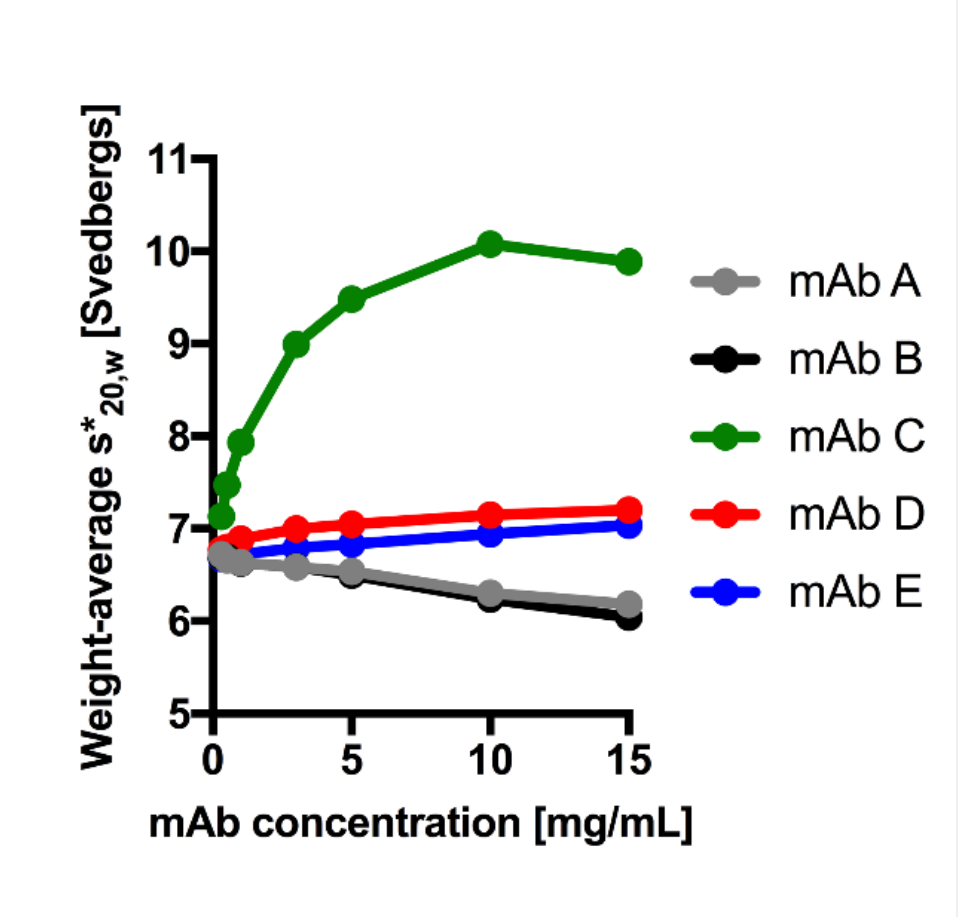

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RESULTS

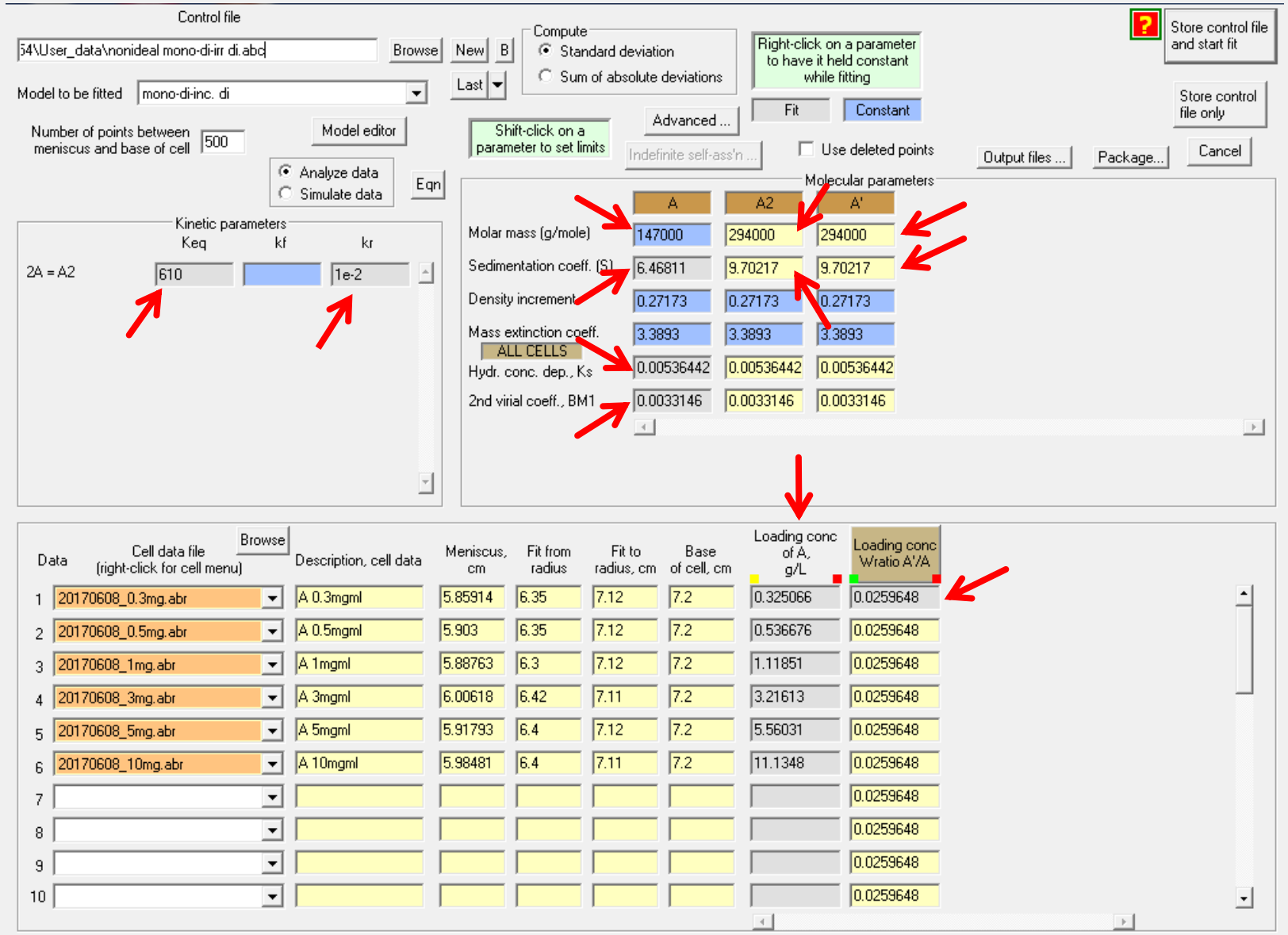
Direct fitting with SEDANAL

- Allows global fitting of multispecies and multicomponent systems
- Curve-fitting algorithm using nonlinear least-squares (NLLS) analysis
- Resolution of interaction rate constants (k_{off}, k_{on}), equilibrium constants (K_{eq}, K_d), hydrodynamic (k_s) and thermodynamic (BM_1) nonideality by direct fitting of data
  - » $s_{20,w}$, MW, % irreversible species, initial loading concentrations, fringe offset

Stafford and Sherwood (2004) *Biophys Chem* 108:231-243

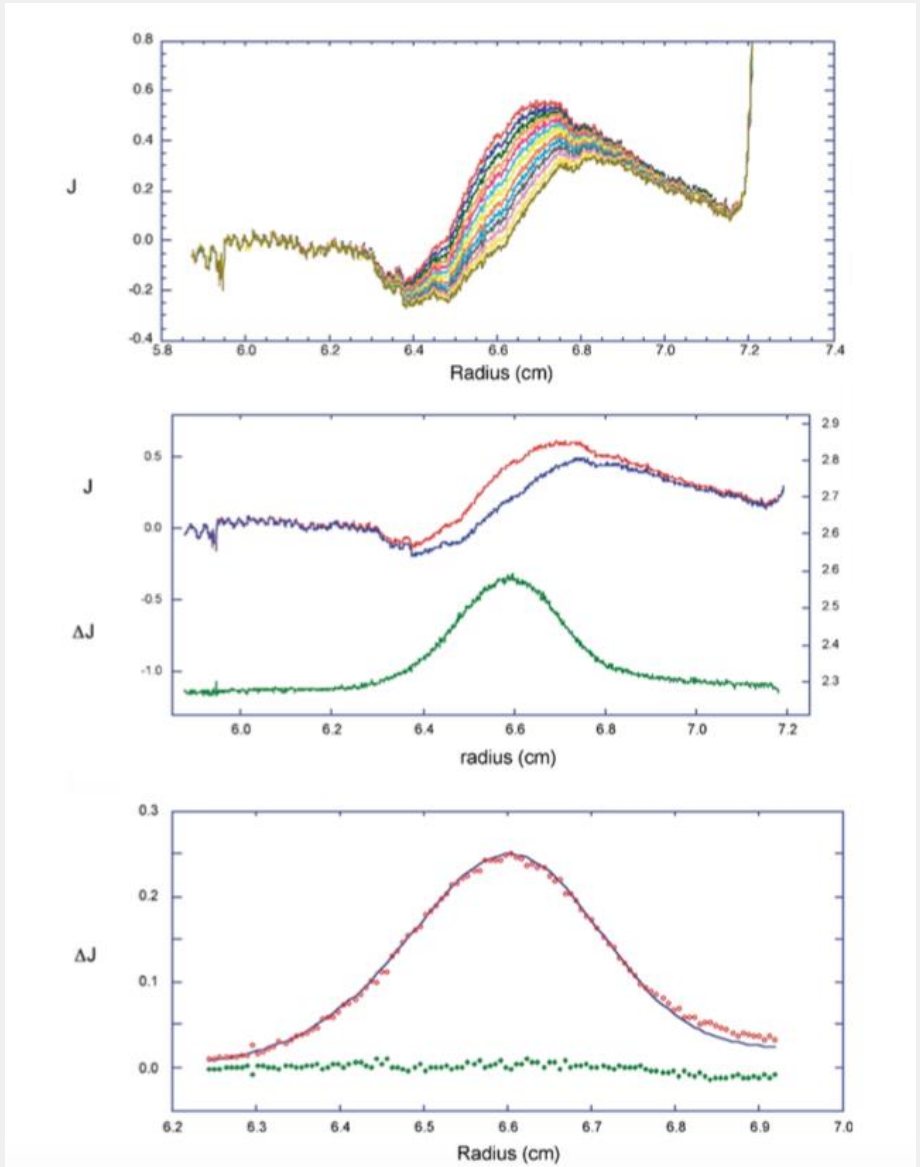
RESULTS

Direct fitting with SEDANAL



RESULTS

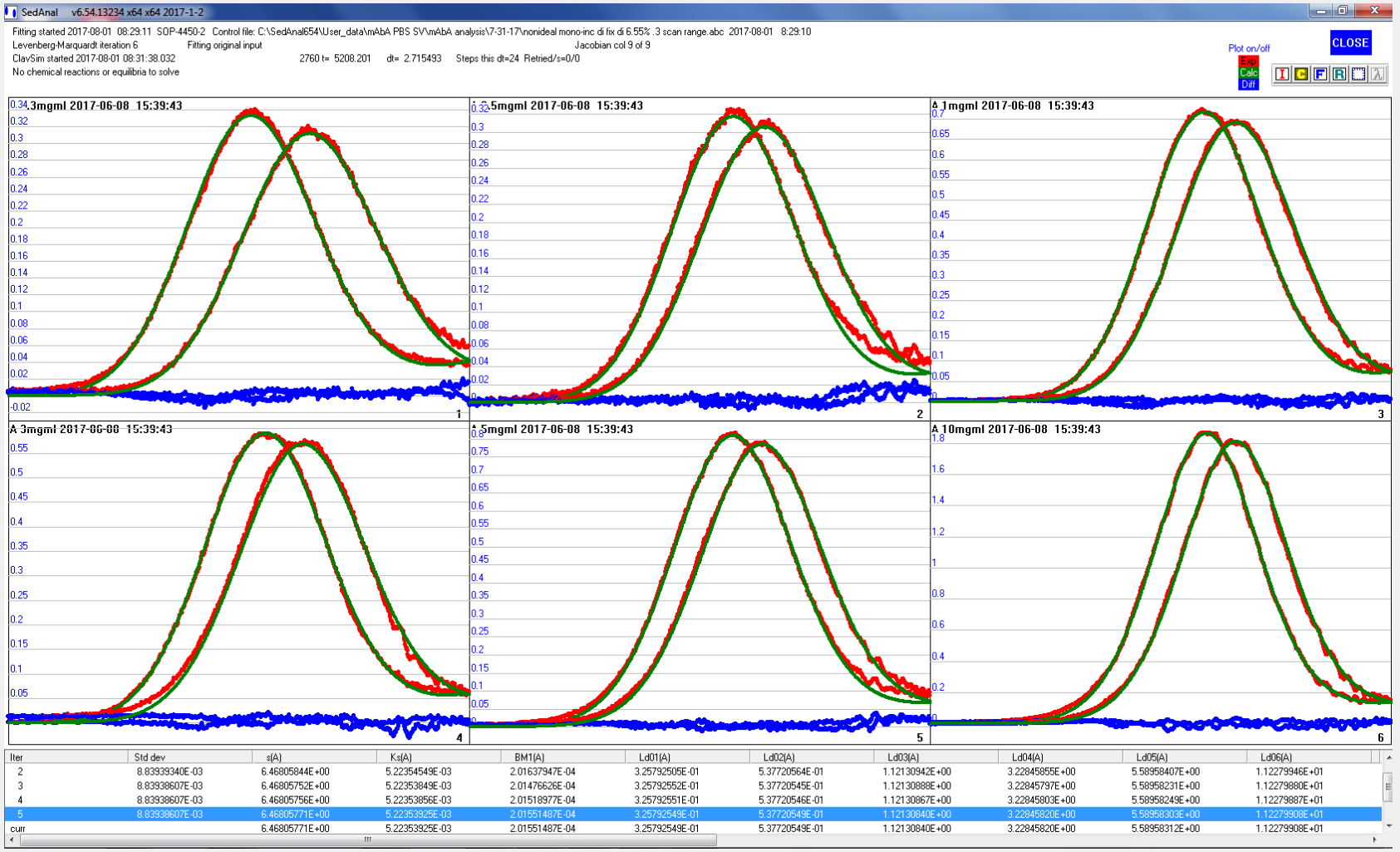
SEDANAL fits time-difference curves to remove systematic error



Stafford and Sherwood (2004) *Biophys Chem* 108:231-243

RESULTS

SEDANAL global fitting of concentration-dependent time difference curves



Iter	Std dev	s(A)	Ks(A)	BM1(A)	Ld01(A)	Ld02(A)	Ld03(A)	Ld04(A)	Ld05(A)	Ld06(A)
2	8.83933340E-03	6.46805844E+00	5.22354549E-03	2.01637947E-04	3.25792505E-01	5.37720564E-01	1.12130942E+00	3.22845855E+00	5.58958407E+00	1.12279946E+01
3	8.83938607E-03	6.46805752E+00	5.22353849E-03	2.01476626E-04	3.25792552E-01	5.37720545E-01	1.12130888E+00	3.22845797E+00	5.58958231E+00	1.12279880E+01
4	8.83938607E-03	6.46805756E+00	5.22353856E-03	2.01518977E-04	3.25792551E-01	5.37720546E-01	1.12130867E+00	3.22845803E+00	5.58958249E+00	1.12279887E+01
5	8.83938607E-03	6.46805771E+00	5.22353925E-03	2.01551487E-04	3.25792549E-01	5.37720549E-01	1.12130840E+00	3.22845820E+00	5.58958303E+00	1.12279908E+01
curr		6.46805771E+00	5.22353925E-03	2.01551487E-04	3.25792549E-01	5.37720549E-01	1.12130840E+00	3.22845820E+00	5.58958312E+00	1.12279908E+01

RESULTS

Non-interacting example: mAb A

Monomer (A) with irreversible dimer (A') and nonideality

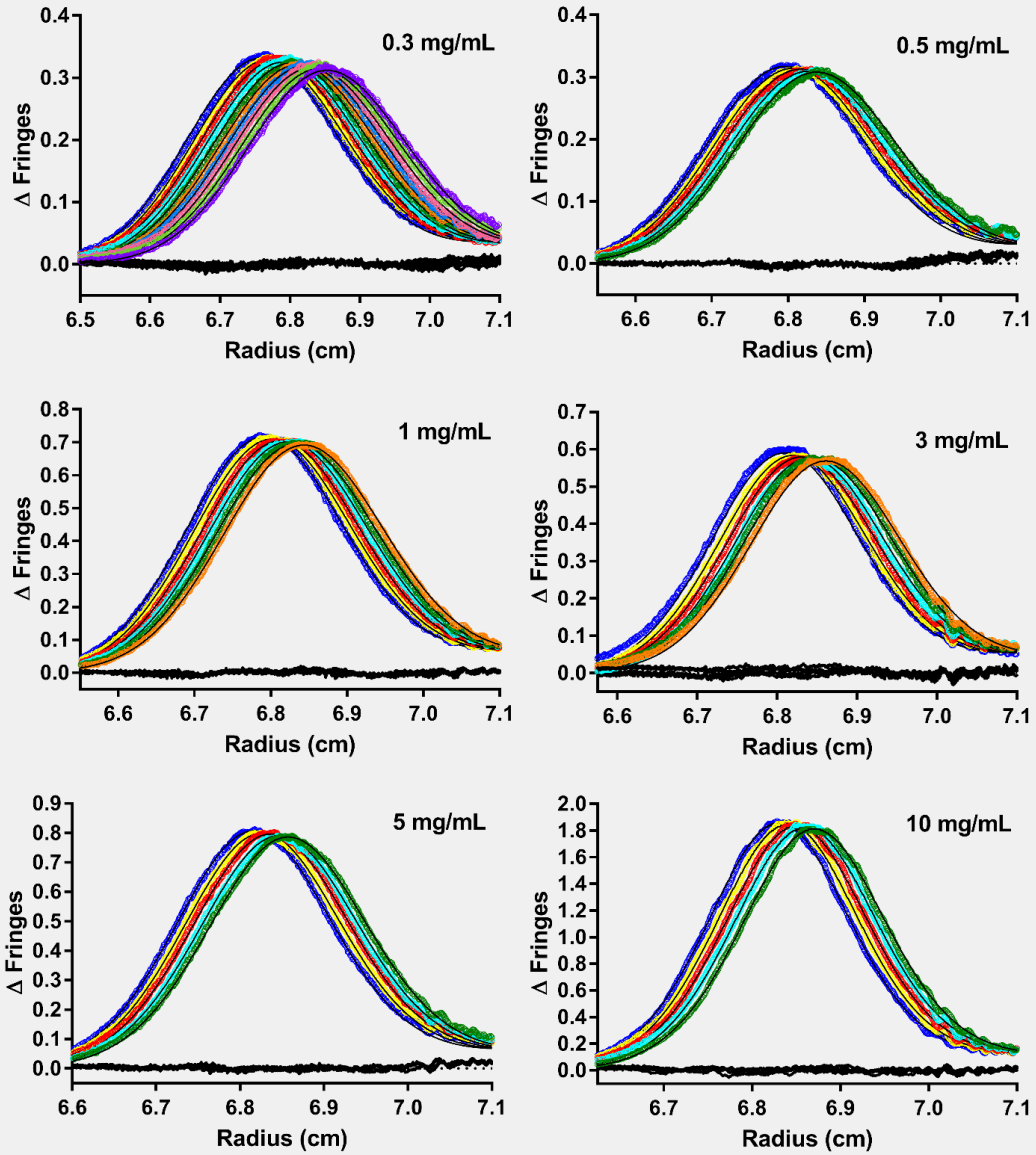
RMSD = 8.84E-03 fringes

Resolved parameters:

$s_{20,w}$: 6.47
k_s: 5.22 mL/g
BM_1: 0.20 mL/g
A': 2 %

Fixed or constrained:

MW: A & A'
$s_{20,w}$: A'



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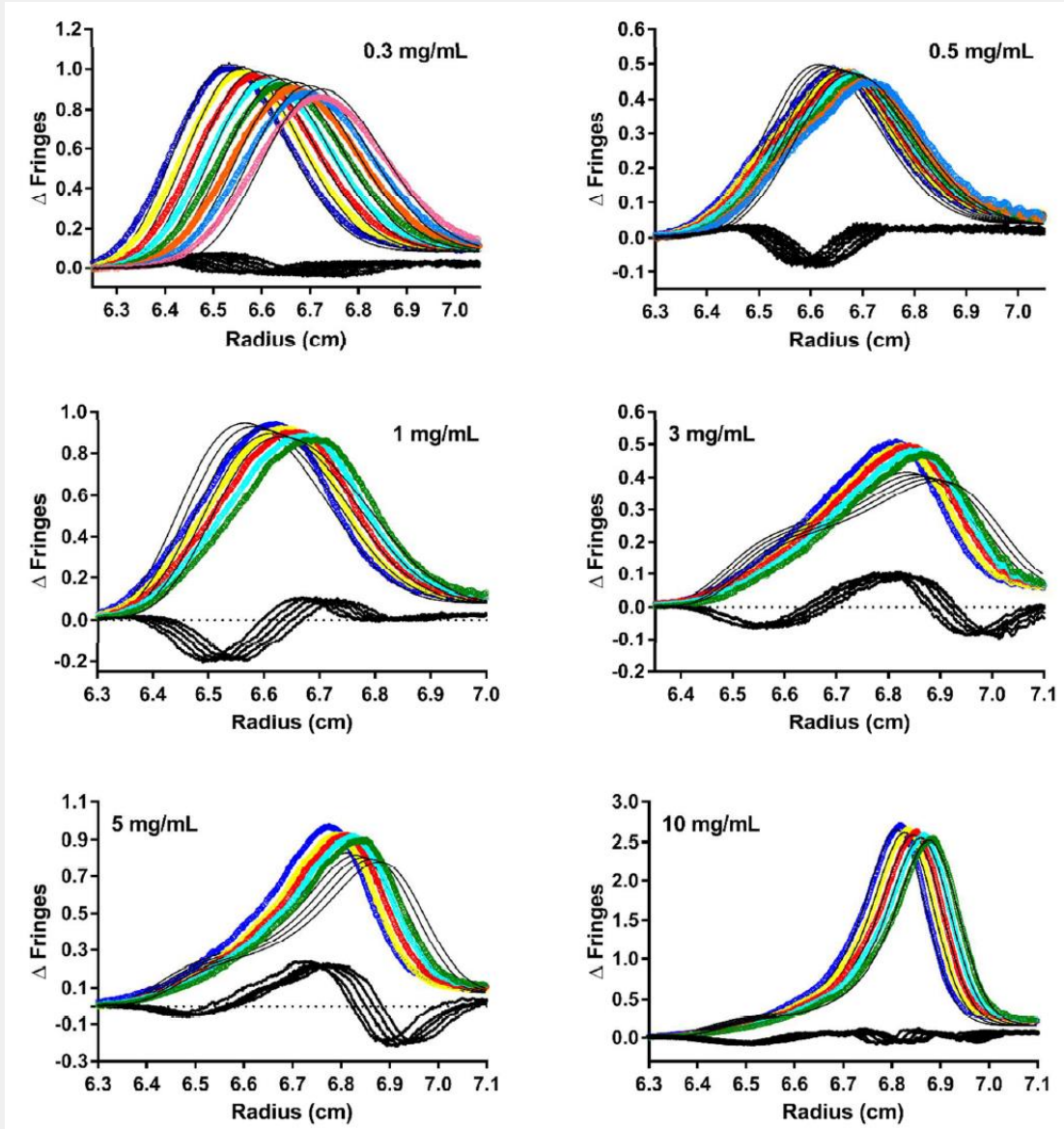
Interacting example: mAb C

Monomer (A) – trimer (A3) – hexamer (A6)
1 – 3 – 6

RMSD = 5.19E-02

Reaction 1: 3A = A3
Reaction 2: 2A3 = A6



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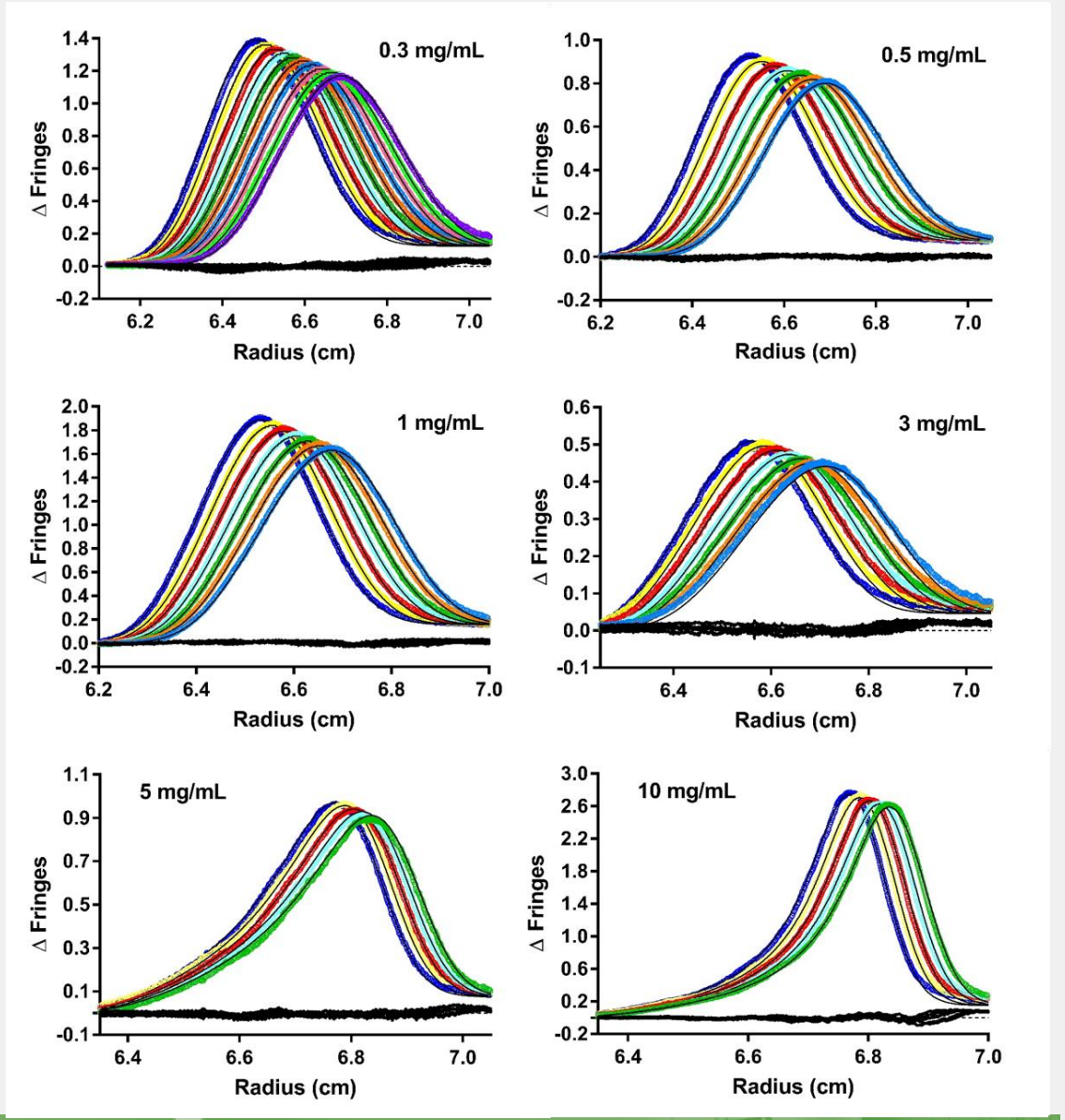
Interacting example: mAb C

Model	RMSD	$F_{calculated}$
1-3	6.51E-02	16.350
1-2-3	3.42E-02	4.512
1-2-4	2.83E-02	3.090
1-2-4-6	2.74E-02	2.896
1-3-6	5.19E-02	10.392
isodesmic	1.61E-02	1.000

$$F_{calculated} = \frac{RMSD_1{}^2}{RMSD_2{}^2}$$

$F_{critical}$ = 1.002



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RESULTS

Interacting example: mAb C

Isodesmic with nonideality

RMSD = 1.61E-02 fringes

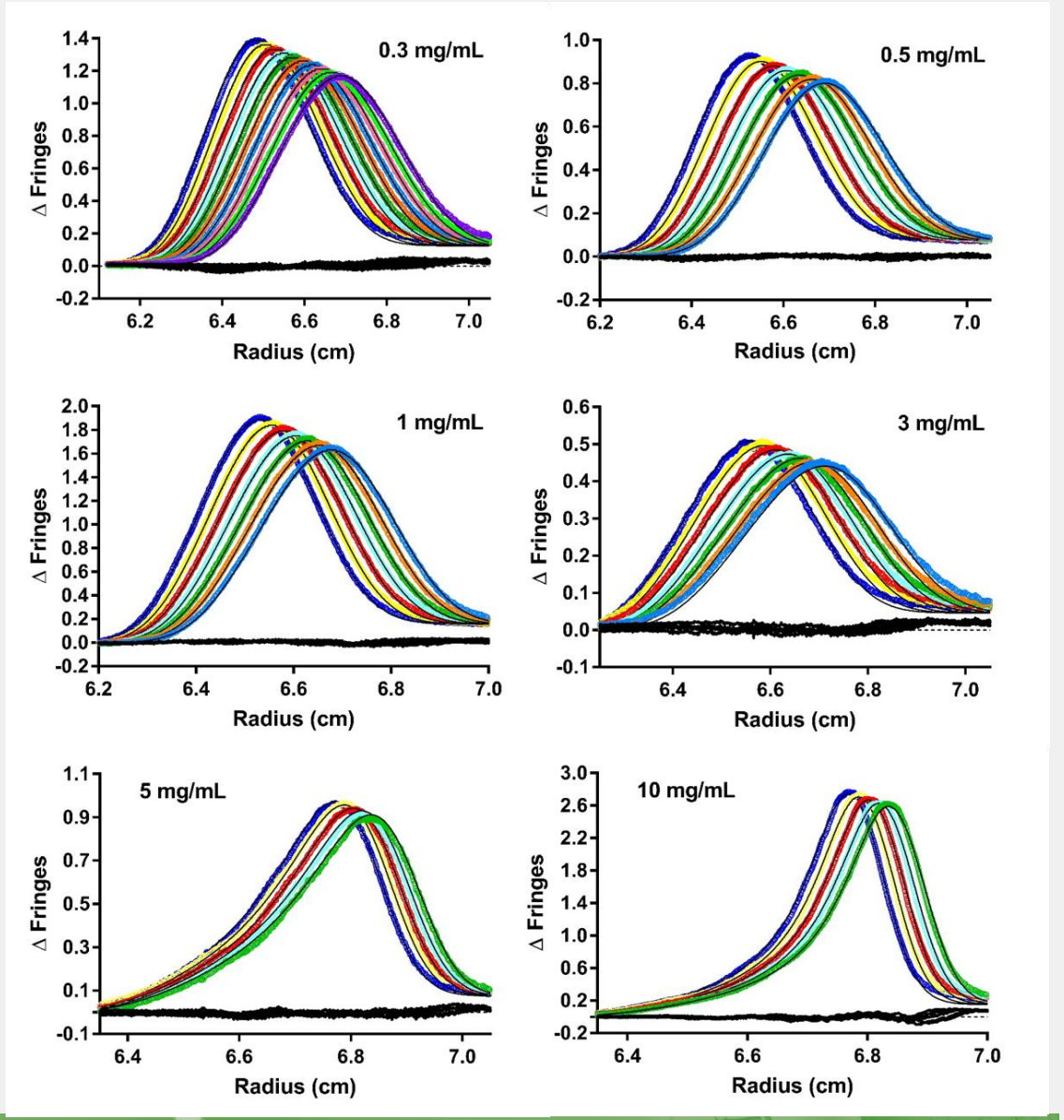
Resolved parameters:

K_d: 32.8 μM

$s_{20,w}$: 6.51

k_s: 35.4 mL/g

BM_1: 8.10 mL/g



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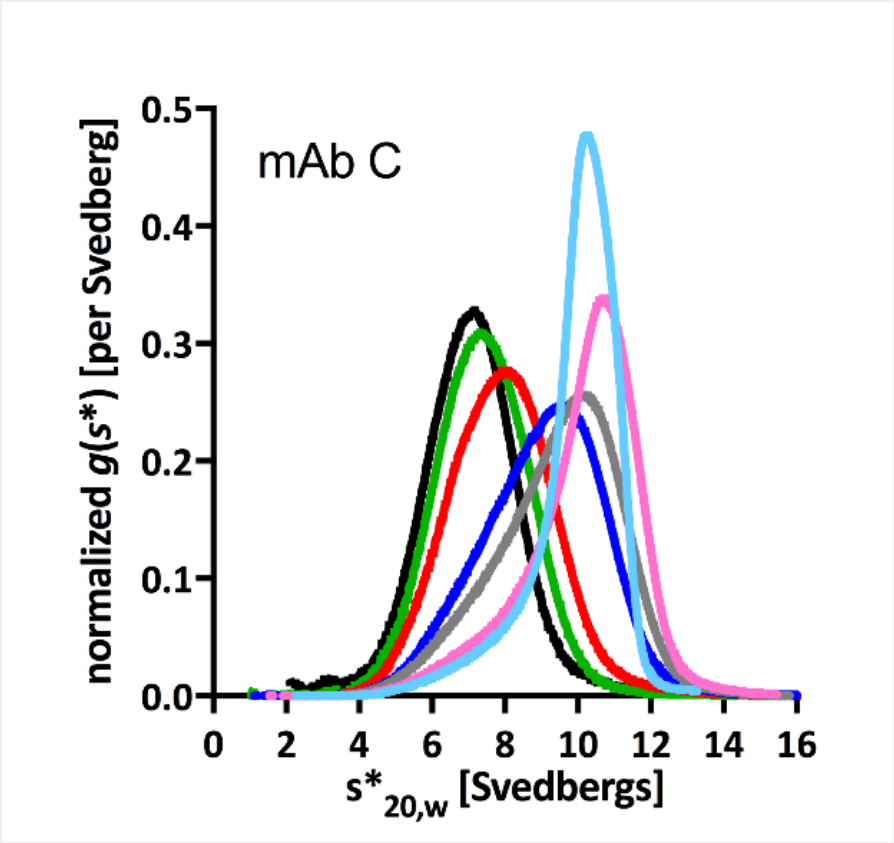
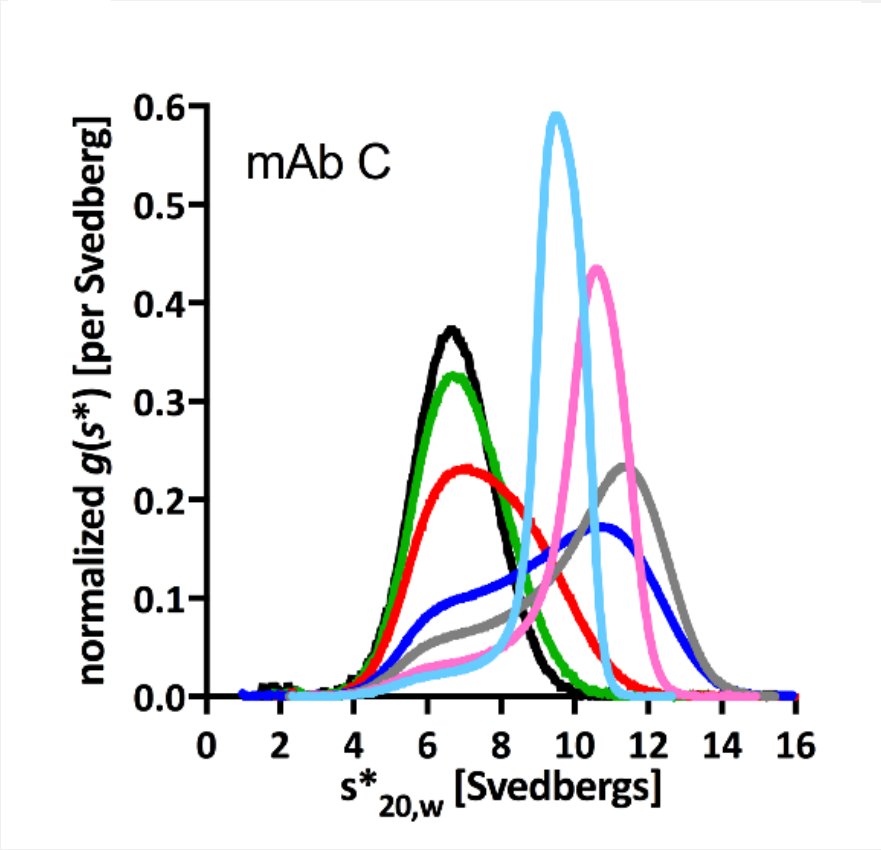
RESULTS

Comparison of simulated and experimental *g*(*s**) plots

Experimental results





Simulated results: isotropic

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DISCUSSION

Summary & Conclusions

- Direct boundary fitting of SV data allows for determination of stoichiometric binding models, interaction affinities, and nonideality terms, allowing for the deconvolution of RSA from nonideality
- Observed distinct kinetics, equilibrium constants ranging from micro- to millimolar, and stoichiometric models from monomer-dimer to isodesmic
- Investigating the accuracy and precision of nonideality terms, possibly due in part to cross-correlation

DISCUSSION

Future Directions

- Qualitative preliminary studies reveal strong temperature dependence, as well as sensitivity to pH and salt concentration
- Future studies will quantitatively address these findings in more detail using thermodynamic linkage analysis

Acknowledgements & Support

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MedImmune

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