The University of Nottingham

A combined approach to size, heterogeneity, conformation & flexibility of bio-macromolecules

Steve Harding
National Centre for Macromolecular Hydrodynamics

NCMH

Size (mol wt.) & heterogeneity:

- SEC-MALLs – mol wt distributions
- AUC sedimentation equilibrium analysis – M* & distributions
- AUC sedimentation velocity analysis – g*(s) & distributions

- Size (mol wt.) and heterogeneity: **SEC-MALLs**

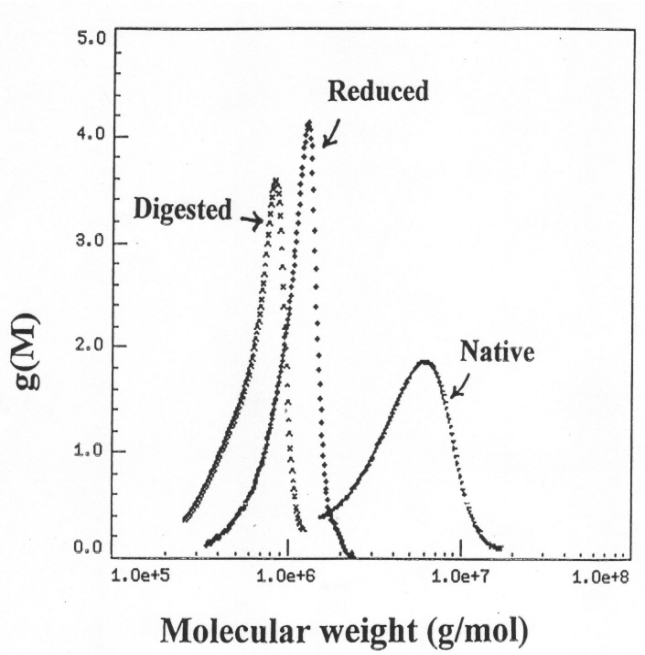
Biochemical Society Transactions 19 (1991) 510-511

Gel permeation chromatography–multi-angle laser light scattering characterization of the molecular mass distribution of 'Pronova' sodium alginate

J. C. Horton, S. E. Harding and J. R. Mitchell

University of Nottingham, Department of Applied Biochemistry and Food Science, School of Agriculture, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, U.K.

A relatively recent innovation in total intensity laser light scattering has been to replace the isolated fers of ionic strengths (I) of 0.1 M and 0.3 M with concentrations (c) in the range 0.5–5.0 mg/ml.



- Size (mol wt.) and heterogeneity: **SEC-MALLs**

Biochemical Society Transactions 19 (1991) 510-511

Gel permeation chromatography–multi-angle laser light scattering characterization of the molecular mass distribution of 'Pronova' sodium alginate

J. C. Horton, S. E. Harding and J. R. Mitchell
University of Nottingham, Department of Applied Biochemistry and Food Science, School of Agriculture, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, U.K.

A relatively recent innovation in total intensity laser light scattering has been to replace the isolated fers of ionic strengths (I) of 0.1 M and 0.3 M with concentrations (c) in the range 0.5–5.0 mg/ml.

ELSEVIER

International Journal of Biological Macromolecules
18 (1996) 133–139

INTERNATIONAL JOURNAL OF Biological Macromolecules STRUCTURE, FUNCTION AND INTERACTIONS

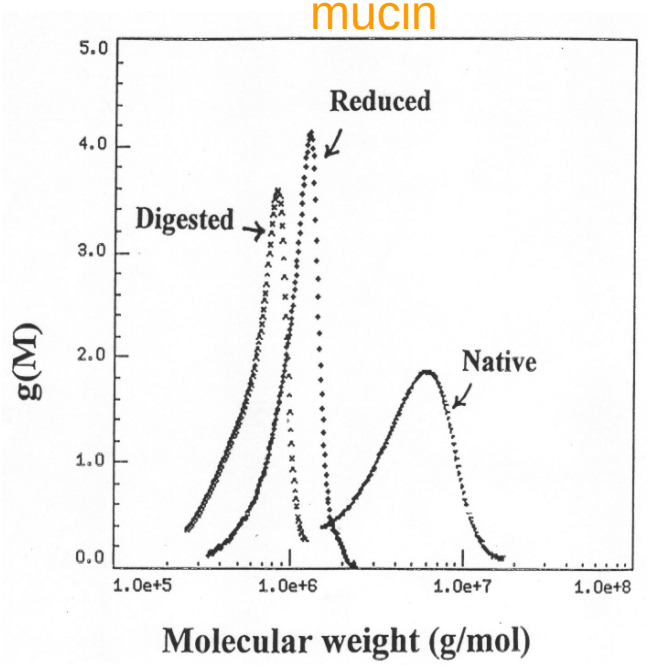
Rapid size distribution and purity analysis of gastric mucus glycoproteins by size exclusion chromatography/multi angle laser light scattering

Kornelia Jumel*[a], Immo Fiebrig[b], Stephen E. Harding[a]

[a]*University of Nottingham, Department of Applied Biochemistry and Food Science, Sutton Bonington, Loughborough, LE12 5RD, UK*
[b]*University of Nottingham, Department of Pharmaceutical Sciences, University Park, Nottingham, NG7 2RD, USA*

Received 10 July 1995; revision received 29 August 1995; accepted 5 September 1995

mucin



- Size (mol wt.) and heterogeneity: **SEC-MALLs**

Biochemical Society Transactions 19 (1991) 510-511

Gel permeation chromatography–multi-angle laser light scattering characterization of the molecular mass distribution of 'Pronova' sodium alginate

J. C. Horton, S. E. Harding and J. R. Mitchell
University of Nottingham, Department of Applied Biochemistry and Food Science, School of Agriculture, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, U.K.

A relatively recent innovation in total intensity laser light scattering has been to replace the isolated fers of ionic strengths (I) of 0.1 M and 0.3 M with concentrations (c) in the range 0.5–5.0 mg/ml.

ELSEVIER

International Journal of Biological Macromolecules
18 (1996) 133–139

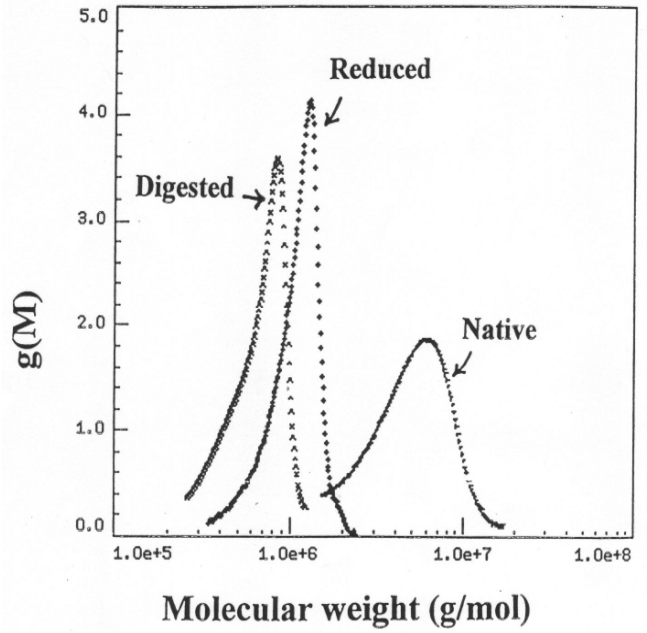
INTERNATIONAL JOURNAL OF
Biological Macromolecules
STRUCTURE, FUNCTION AND INTERACTIONS

Rapid size distribution and purity analysis of gastric mucus glycoproteins by size exclusion chromatography/multi angle laser light scattering

Kornelia Jumel*[a], Immo Fiebrig[b], Stephen E. Harding[a]

[a]*University of Nottingham, Department of Applied Biochemistry and Food Science, Sutton Bonington, Loughborough, LE12 5RD, UK*
[b]*University of Nottingham, Department of Pharmaceutical Sciences, University Park, Nottingham, NG7 2RD, USA*

Received 10 July 1995; revision received 29 August 1995; accepted 5 September 1995

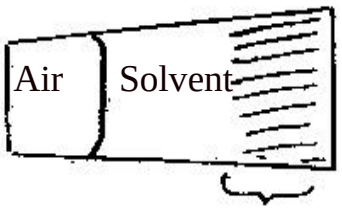


- Size (mol wt.) and heterogeneity: **Analytical ultracentrifuge**

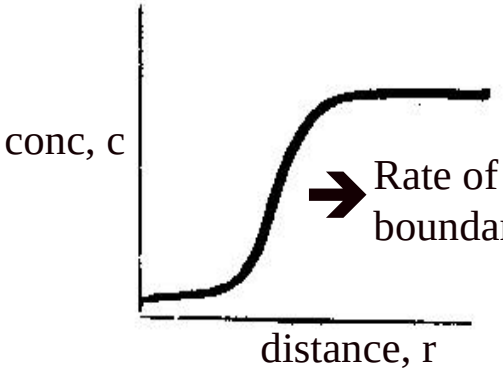
- Size (mol wt.) and heterogeneity: **Analytical ultracentrifuge**

Sedimentation Velocity

Centrifugal force →



Top view, sector of centrifuge cell

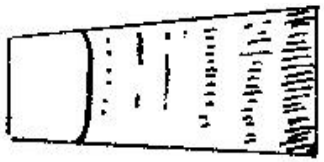


→ Rate of movement of boundary → sed. coeff

$s^{o}_{20,w}$

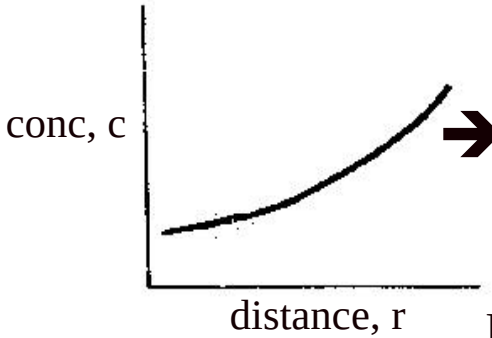
$1S=10^{-13}$sec

Sedimentation Equilibrium



Centrifugal force →

←Diffusion



→ STEADY STATE PATTERN

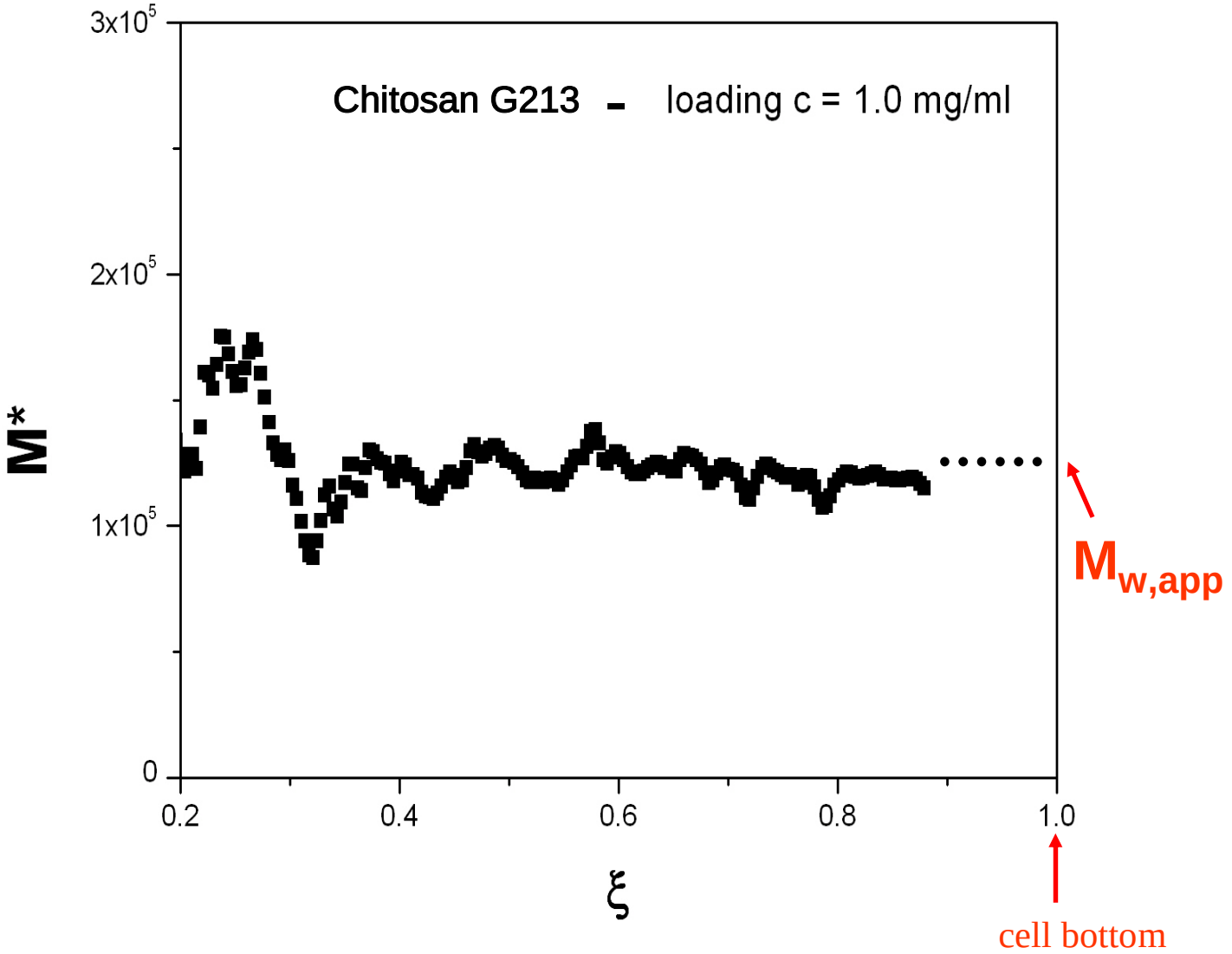
FUNCTION <u>ONLY</u> OF MOL. WEIGHT PARAMETERS

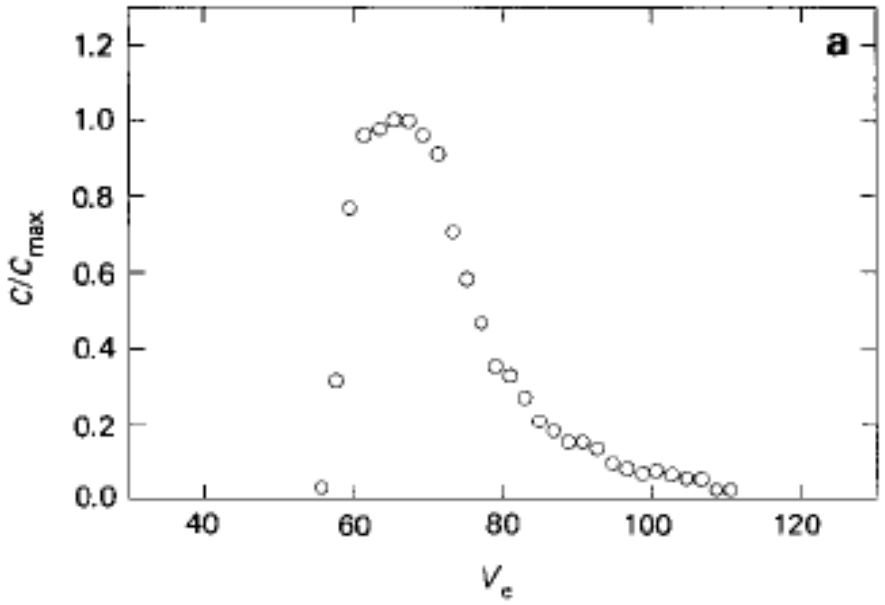
M analysis of sedimentation equilibrium*

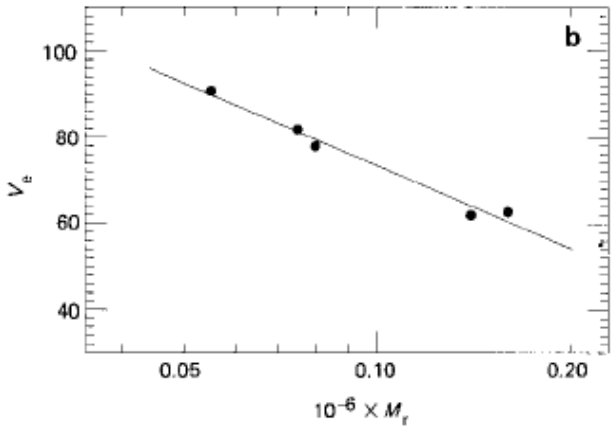
$$M^*(r) = j(r) / \left\{ kJ(a)(r^2 - a^2) + 2k \int_a^r r \cdot j(r)\, \mathrm{d}r \right\}$$

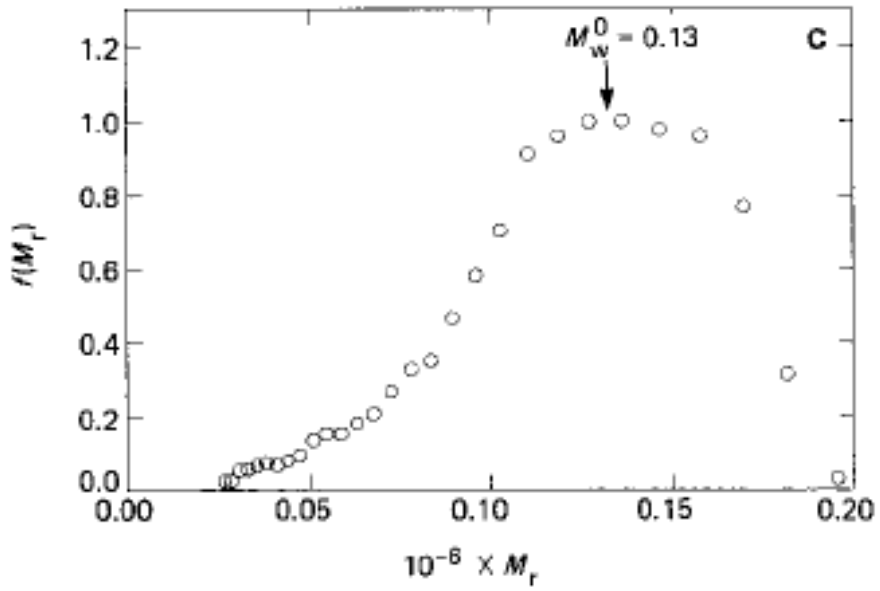
Creeth JM & Harding SE (1982) *J. Biochem. Biophys*. **7**, 25-34

Sedimentation equilibrium M plot*









SEC - sedimentation equilibrium mol. wt distribution: alginate

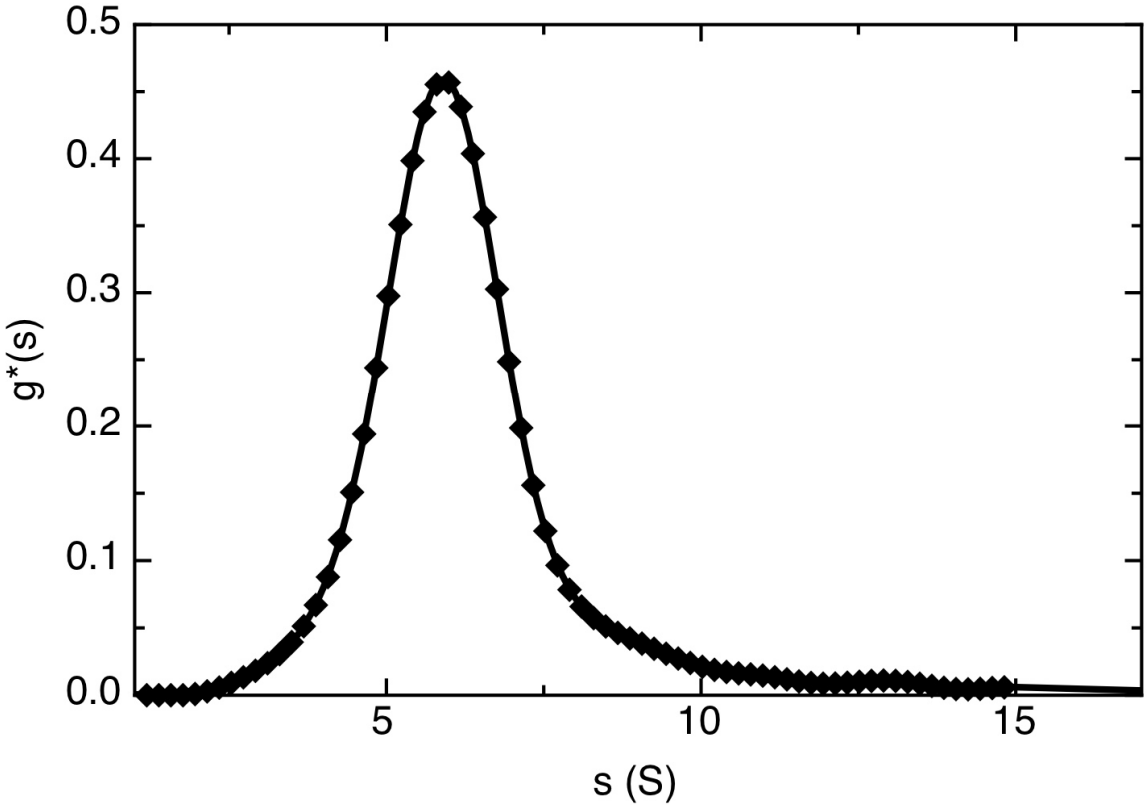
Ball, Harding & Mitchell (1988)

Sedimentation velocity g(s) plot*

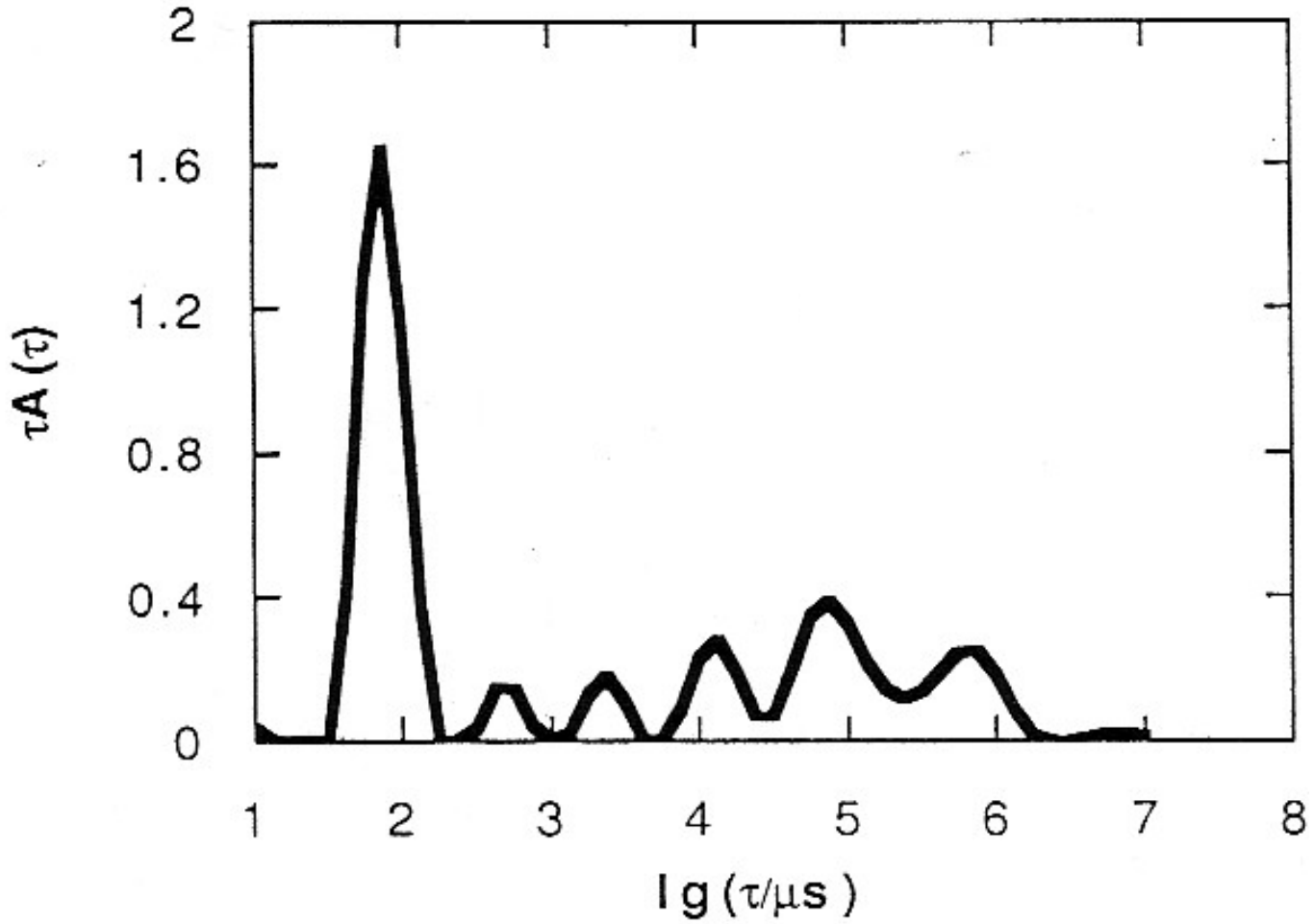
Lamm (1923) equation:

$$\frac{\partial \chi}{\partial t} = (1/r) \cdot \frac{\partial}{\partial r}\left[rD\frac{\partial \chi}{\partial r} - s\omega^2 r^2 \chi\right]$$

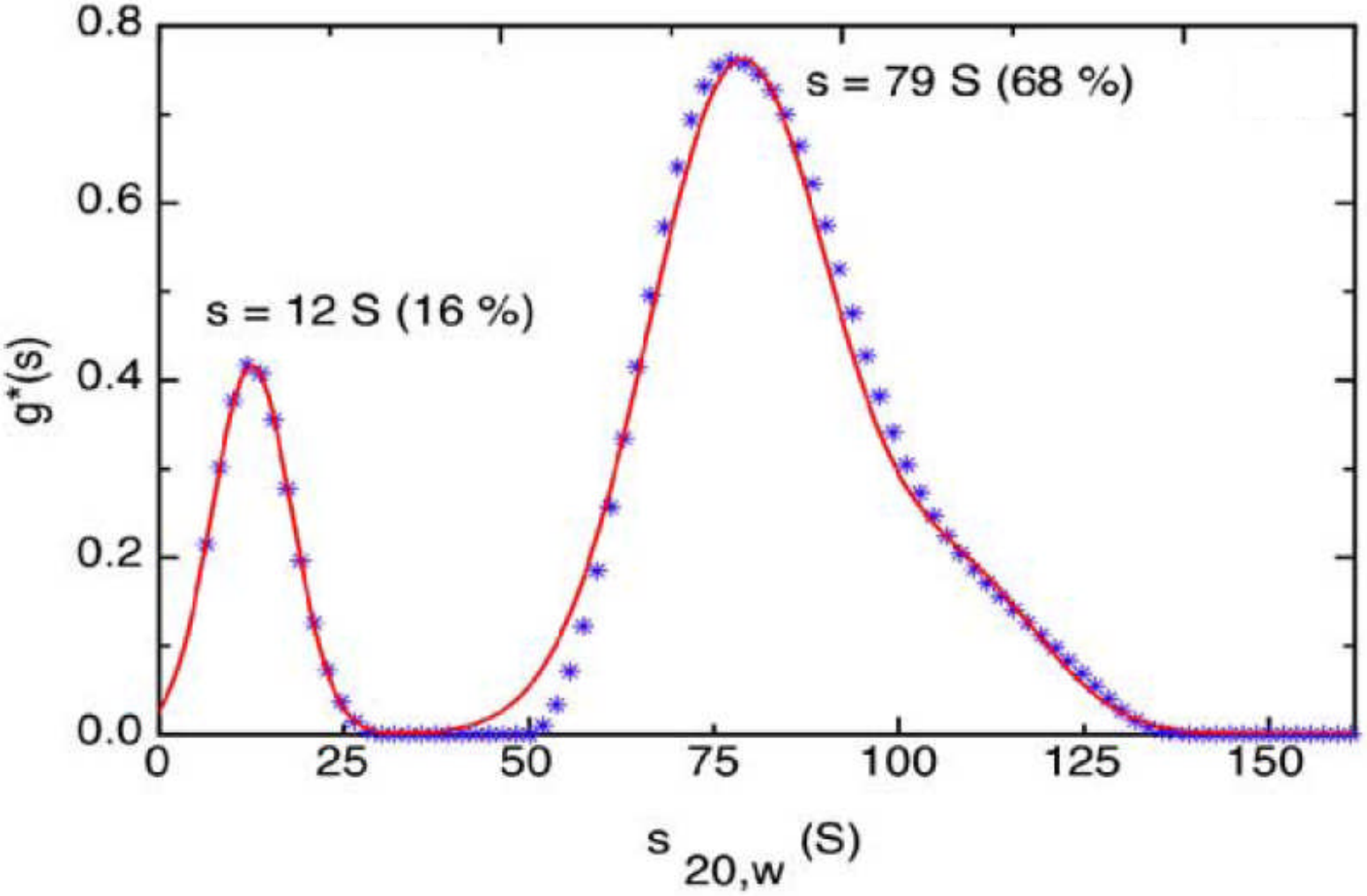
Numerical solutions to the Lamm equation by Claverie et al (1975) & implemented by Todd & Haschemayer (1981)



DLS analysis: Contin plot

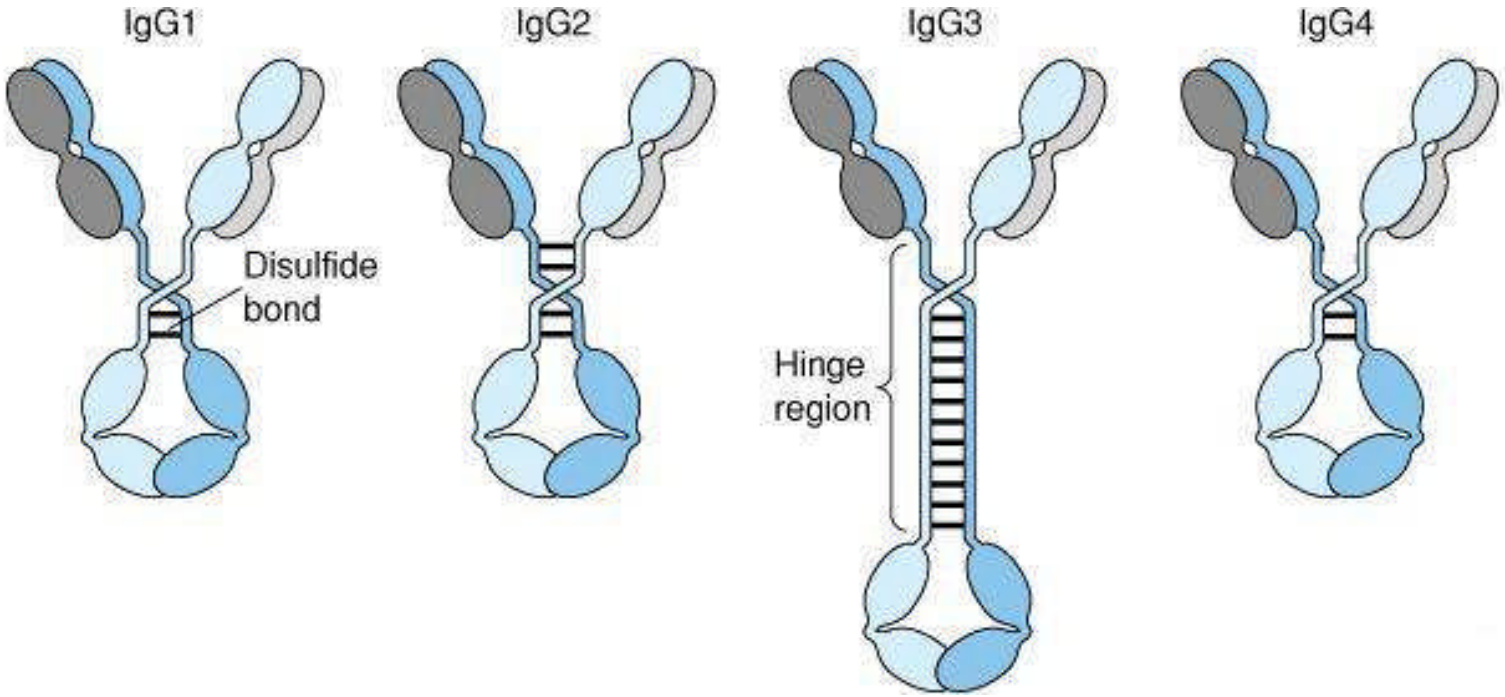


Sedimentation velocity g(s) plot: starch*



R. Tester, T Patel, S. Harding, Carbohydrate Research (2006)

……consider antibodies used in therapies, and their state of aggregation after bioprocessing

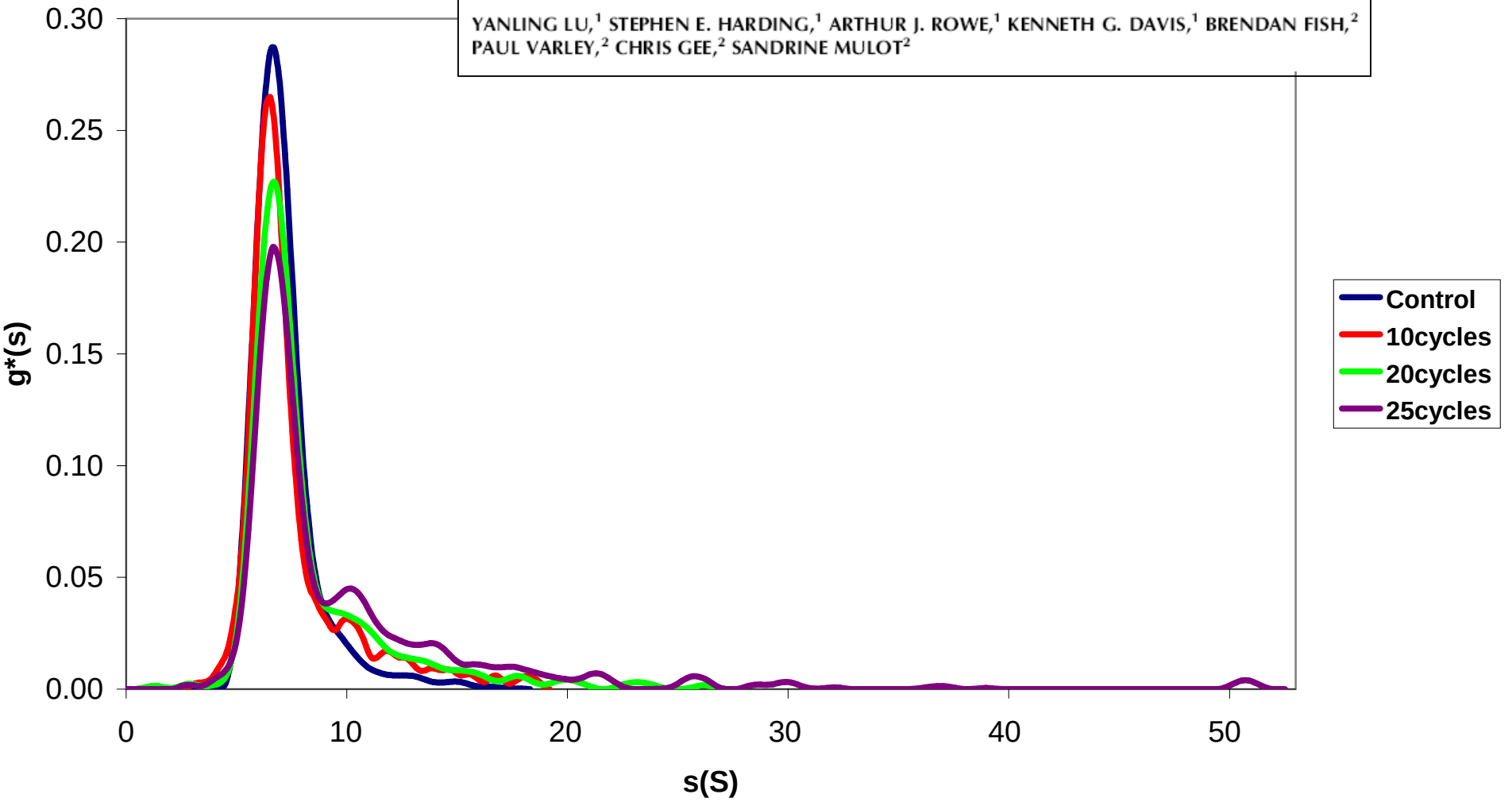


Size distribution by sedimentation velocity of a bioprocessed IgG4 antibody

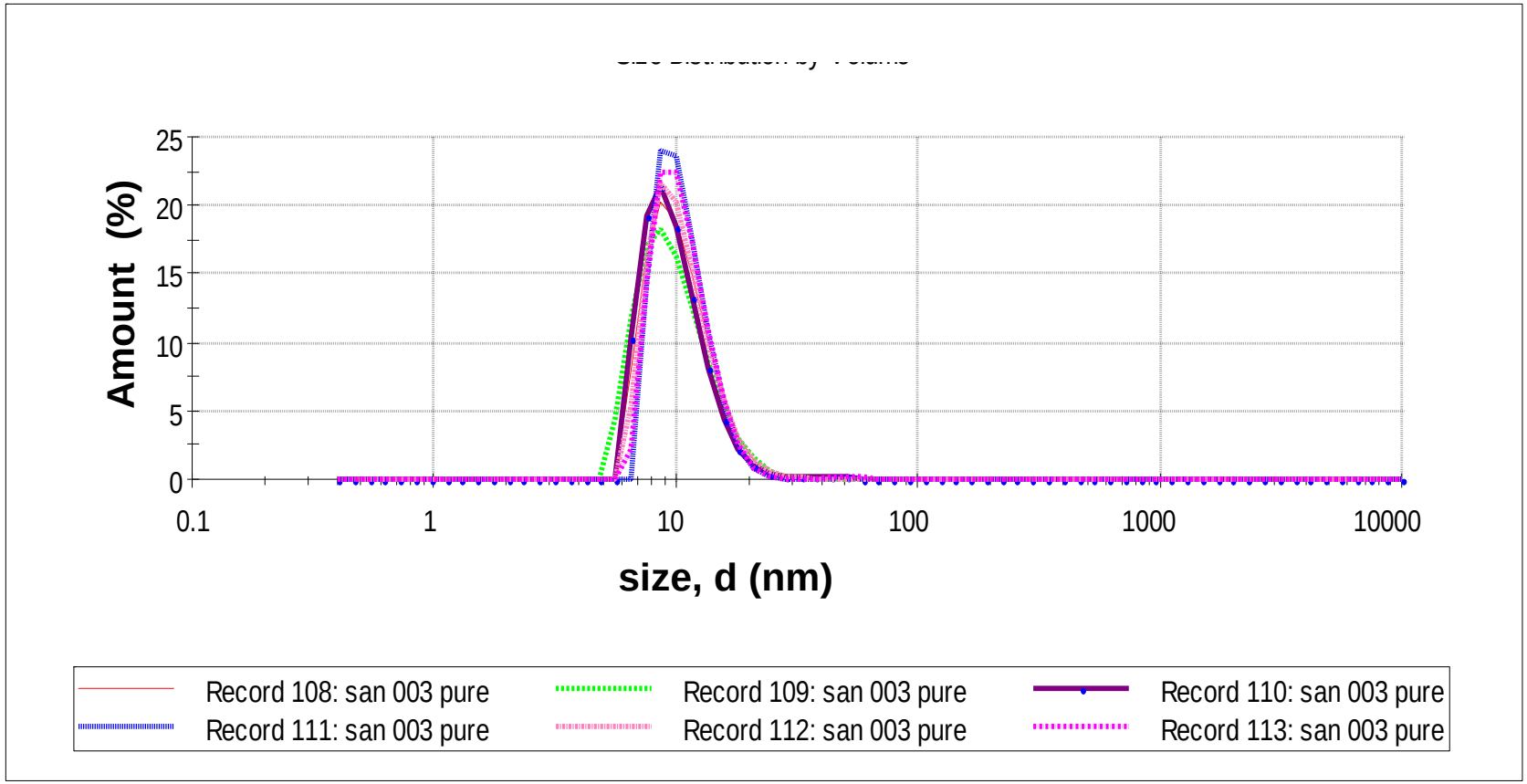
960 JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 97, NO. 2, FEBRUARY 2008

The Effect of a Point Mutation on the Stability of IgG4 as Monitored by Analytical Ultracentrifugation

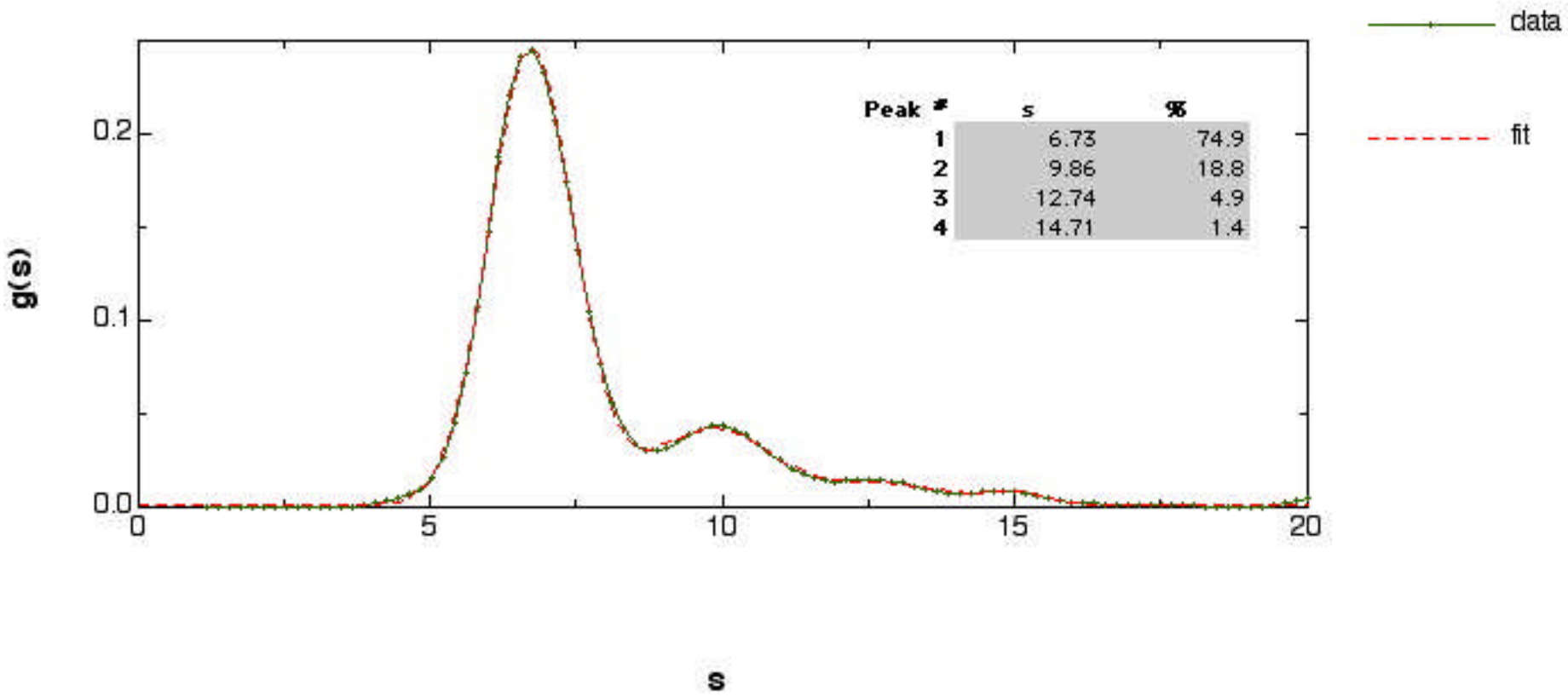
YANLING LU,[1] STEPHEN E. HARDING,[1] ARTHUR J. ROWE,[1] KENNETH G. DAVIS,[1] BRENDAN FISH,[2] PAUL VARLEY,[2] CHRIS GEE,[2] SANDRINE MULOT[2]



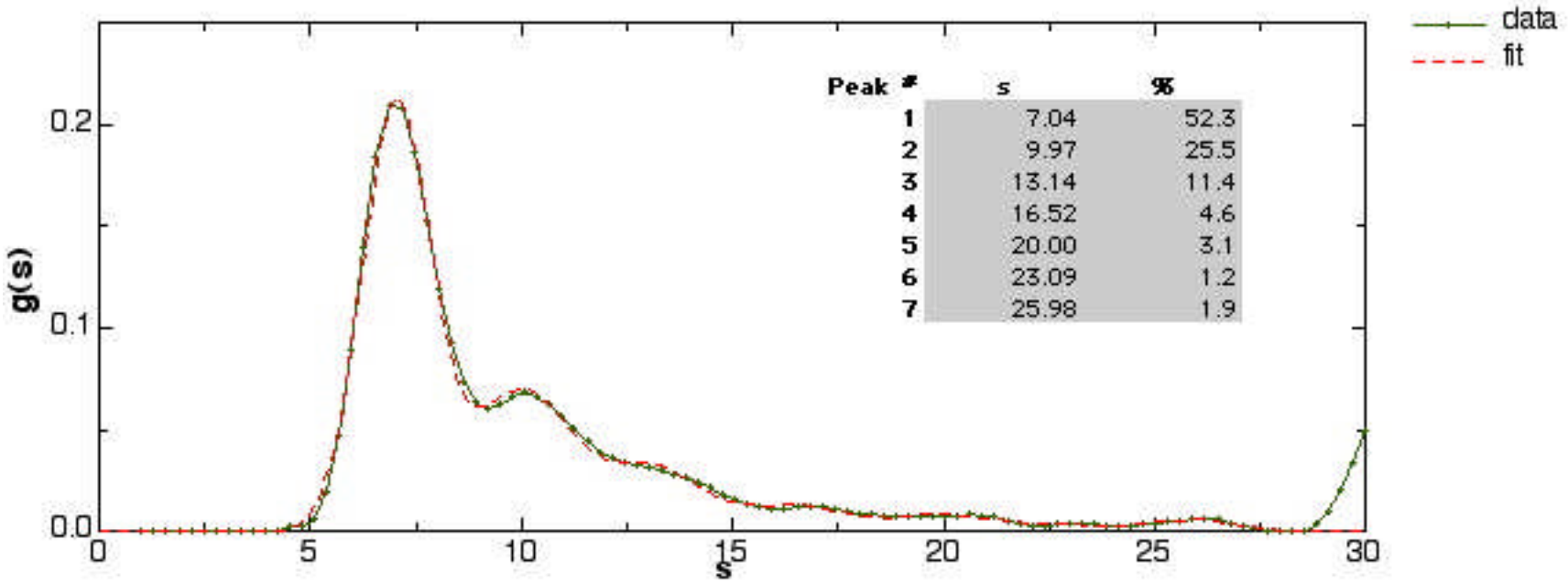
Size distribution by DLS of a bioprocessed IgG4 antibody

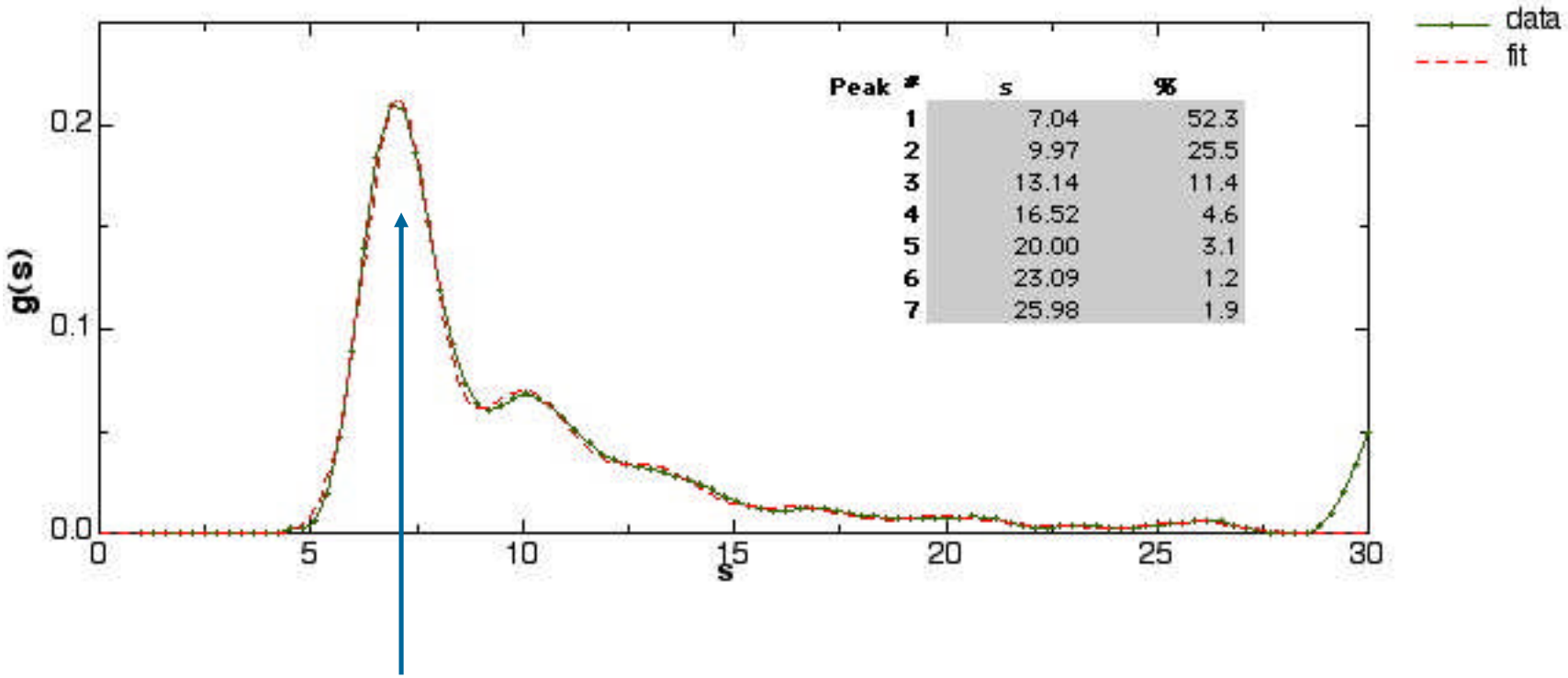


…provides an excellent assessment of the quality/ the extent of heterogeneity



…this preparation is even worse!





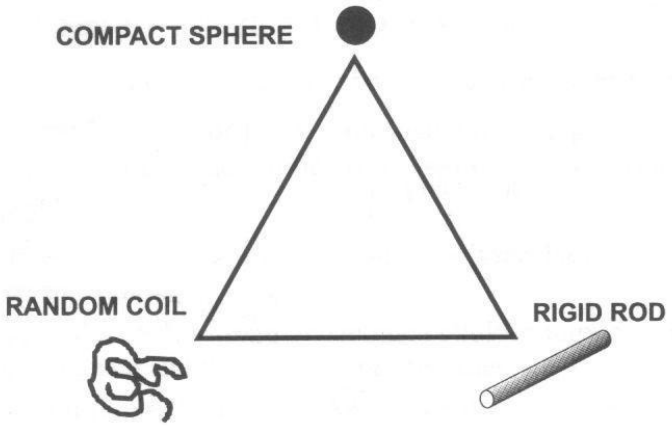
Also, changes in sedimentation coefficient of monomer may reflect change in conformation

Molecular conformation & flexibility:

- general conformation – conformation zoning
- flexibility – persistence length using global methods
- whole body or ellipsoid representations
- bead model representations


AMVn
Anton Paar
Automated Micro Viscometer
Menu
Eval
F1
F2
F3
F4
F5

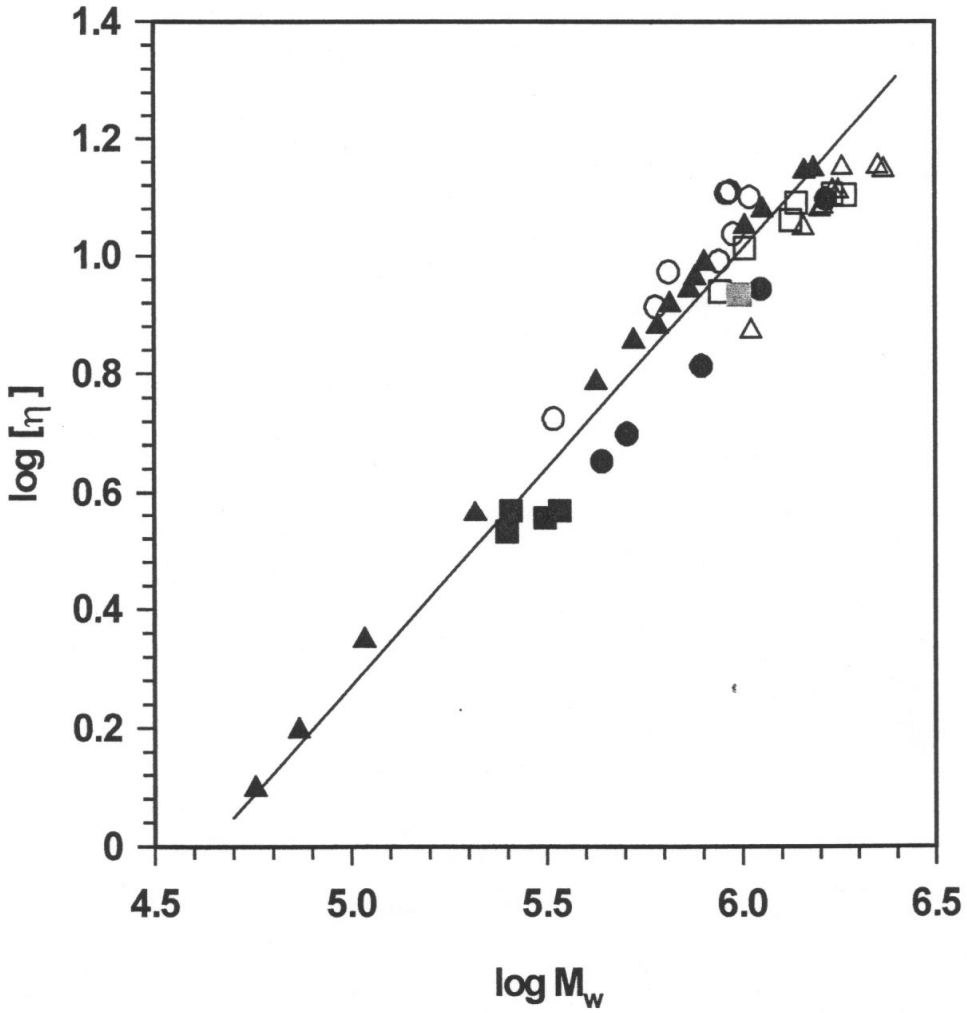
• General molecular conformation: Haug triangle and power law coeffs



Sphere	*Rod*	*Coil*
$[\eta] \sim M^{0}$	$[\eta] \sim M^{1.8}$	$[\eta] \sim M^{0.5\text{-}0.8}$
$s^{o}_{20,w} \sim M^{0.67}$	$s^{o}_{20,w} \sim M^{0.15}$	$s^{o}_{20,w} \sim M^{0.4\text{-}0.5}$
$R_g \sim M^{0.33}$	$R_g \sim M^{1.0}$	$R_g \sim M^{0.5\text{-}0.6}$

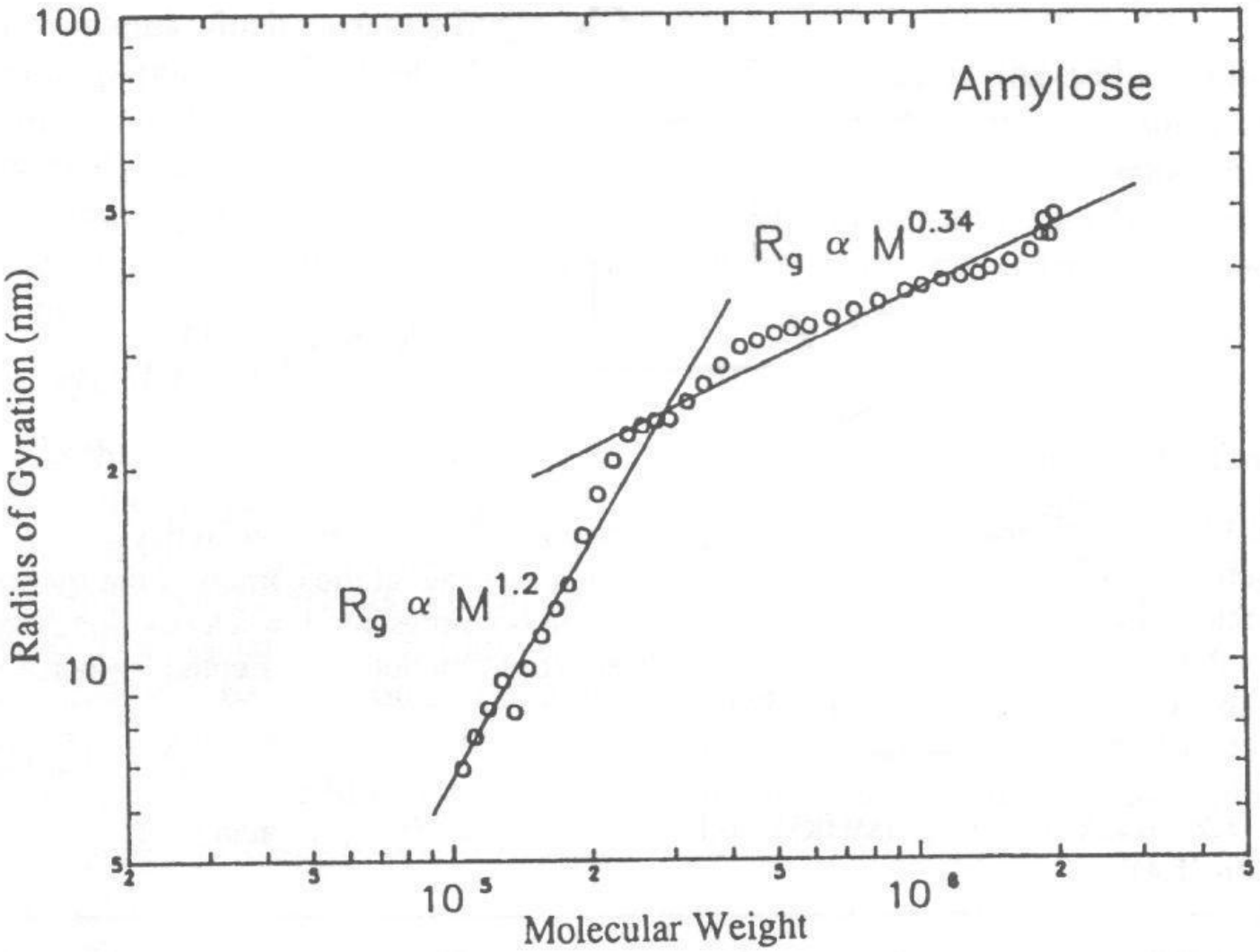
Mark-Houwink-Kuhn-Sakurada Power law plot

Galactomannans
a=0.74±0.01

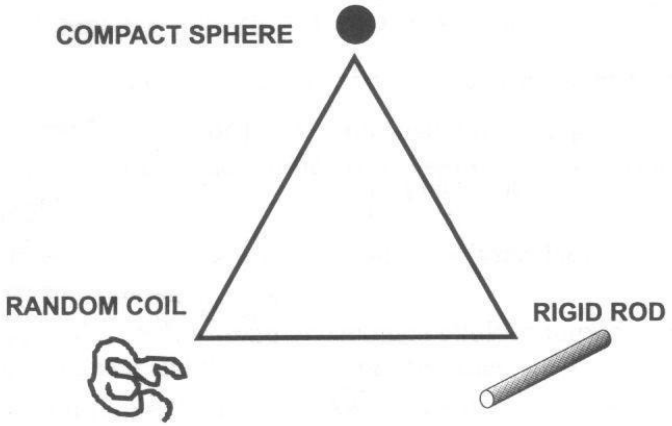


Picout et al (2003) *Biomacromolecules* **2**, 1301-1309

Change in Conformation

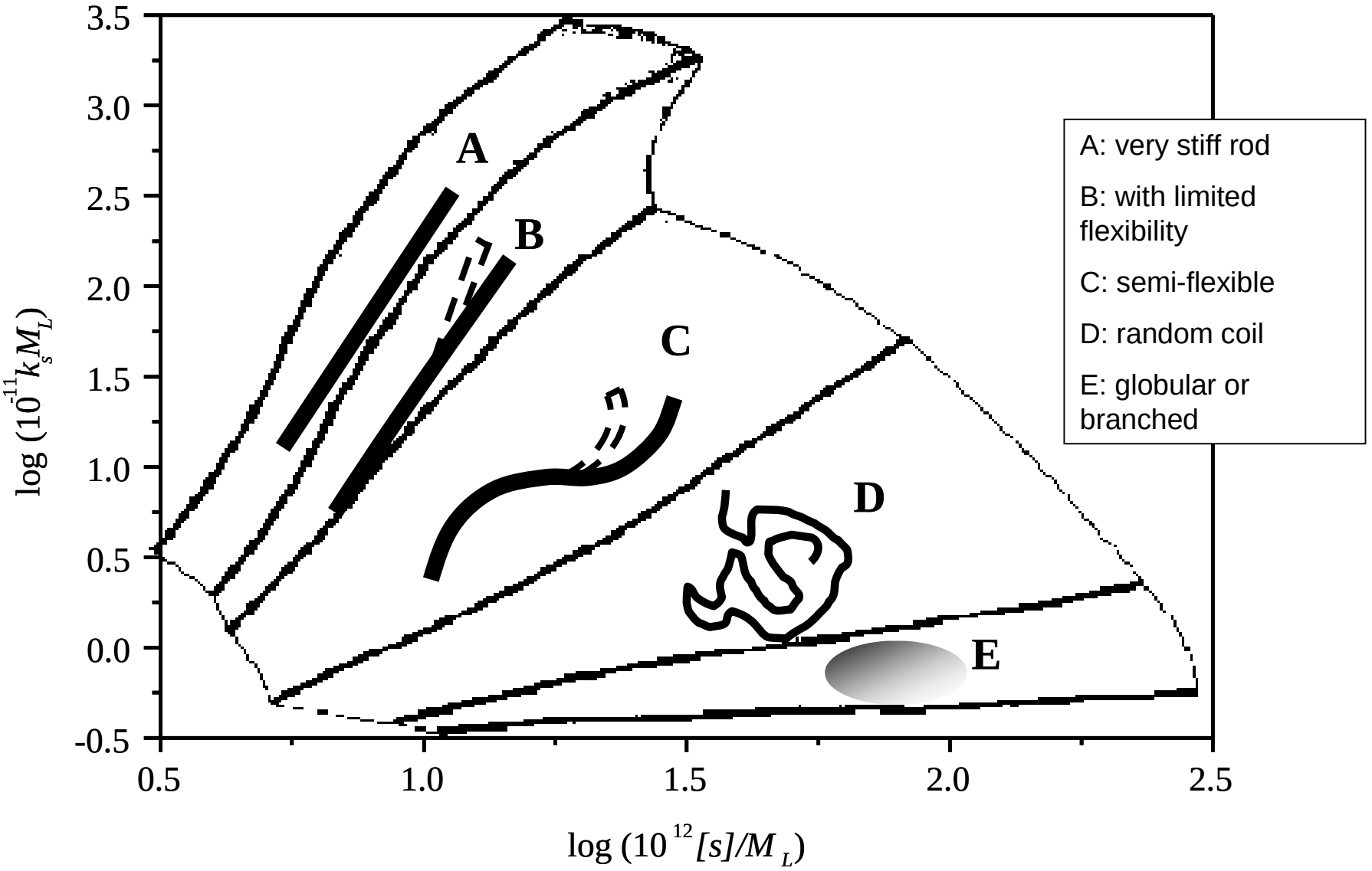


Rollings J (1992) in *Laser Light Scattering in Biochemistry* (Harding, Sattelle & Bloomfield eds)

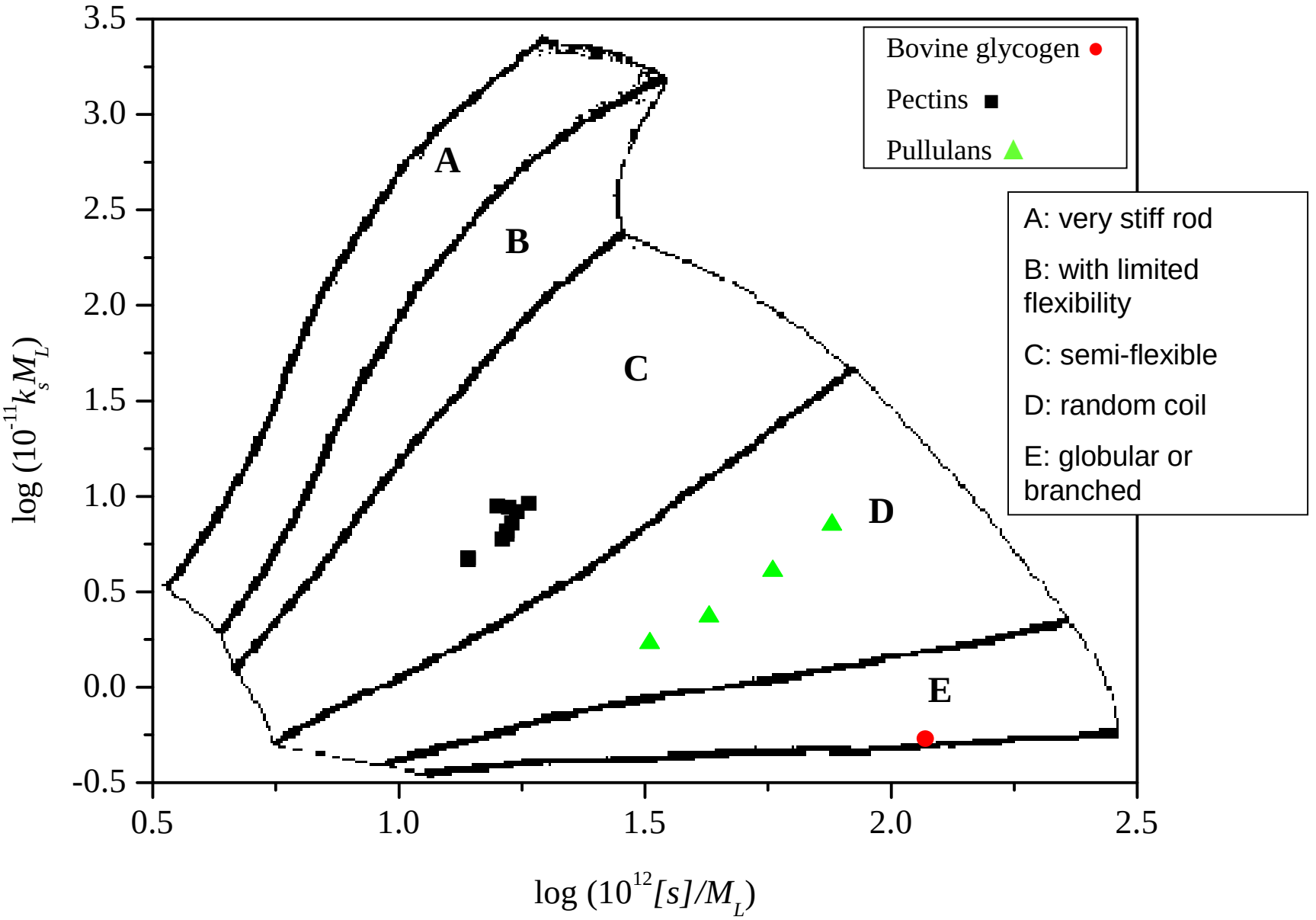


Sphere	Rod	Coil
$[\eta] \sim M^{0}$	$[\eta] \sim M^{1.8}$	$[\eta] \sim M^{0.5\text{-}0.8}$
$s^{o}_{20,w} \sim M^{0.67}$	$s^{o}_{20,w} \sim M^{0.15}$	$s^{o}_{20,w} \sim M^{0.4\text{-}0.5}$
$R_g \sim M^{0.33}$	$R_g \sim M^{1.0}$	$R_g \sim M^{0.5\text{-}0.6}$
$k_s/[\eta] \sim 1.6$	$k_s/[\eta] < 1$	$k_s/[\eta] \sim 1.6$

Conformation Zoning:



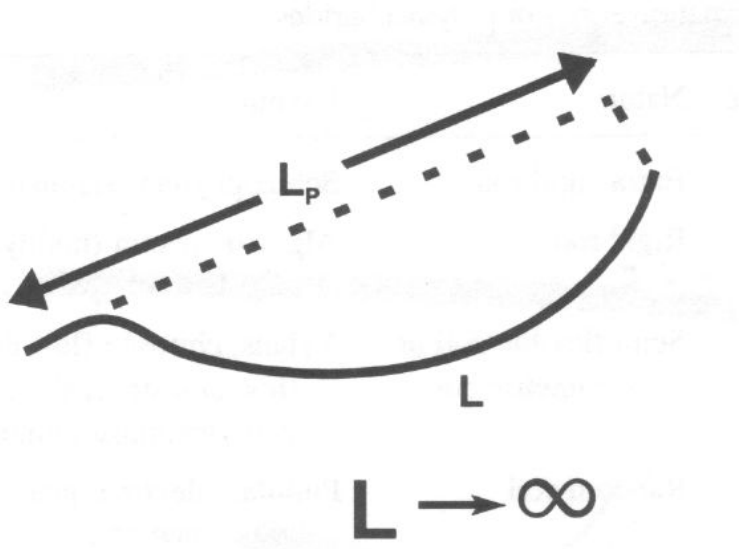
Pavlov et al. (1997). *Trends in Analytical Chemistry*, **16**, 401-405.


Bovine glycogen
Pectins
Pullulans
A: very stiff rod
B: with limited flexibility
C: semi-flexible
D: random coil
E: globular or branched
A
B
C
D
E
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.5
1.0
1.5
2.0
2.5
log (10^-11 k_s M_L)
log (10^12 [s]/M_L)

Measure of flexibility: persistence length L_p

Theoretical limits: 0 (random coil) → ∞ (perfect rod)

Practical limits ~ 2nm → 200nm



“Bushin-Bohdanecky” relation

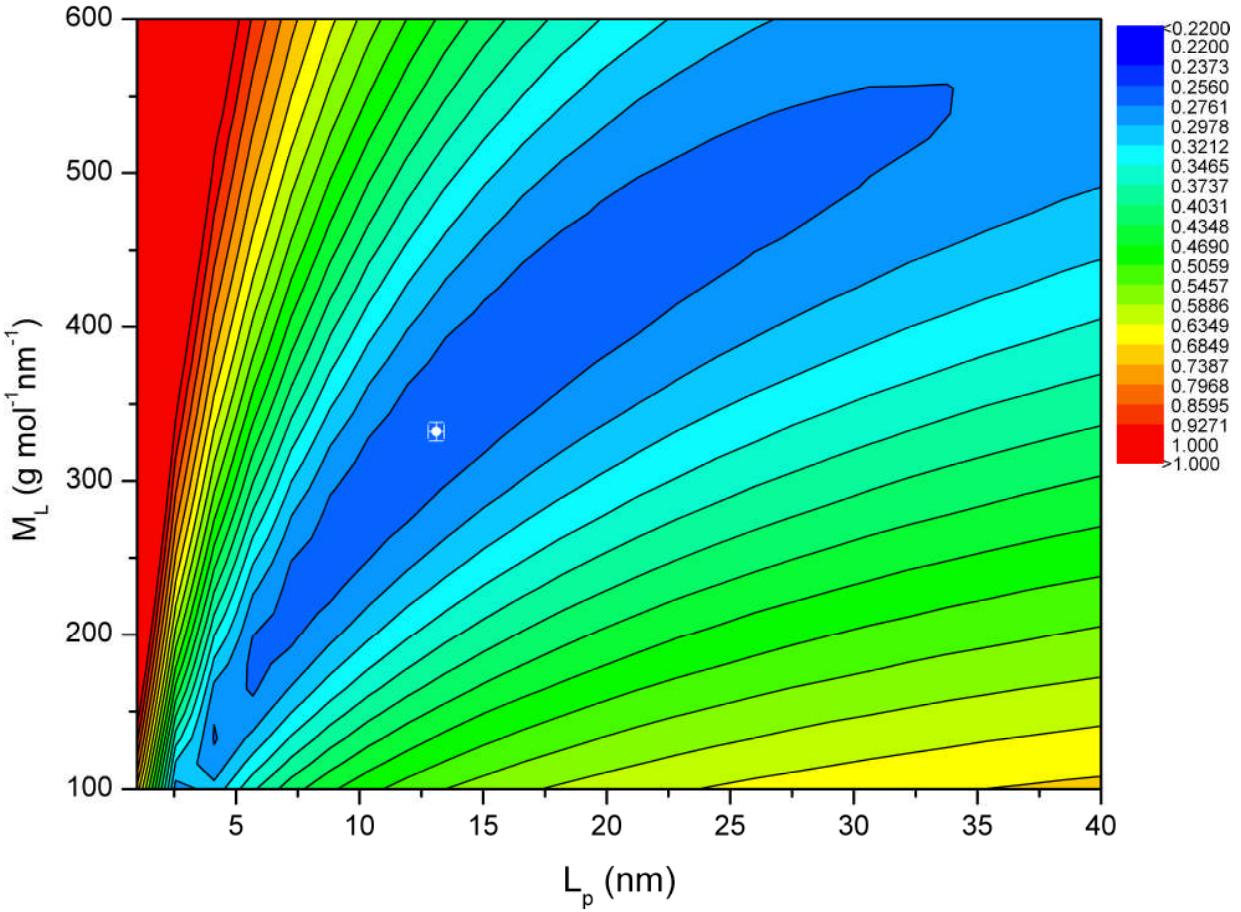
$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3} \left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2}$$

“Yamakawa-Fujii” relation

$$s^0 = \frac{M_L\left(1-\bar{v}\rho_0\right)}{3\pi\eta_0 N_A} \times \left[1.843\left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3\left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + \ldots.\right]$$

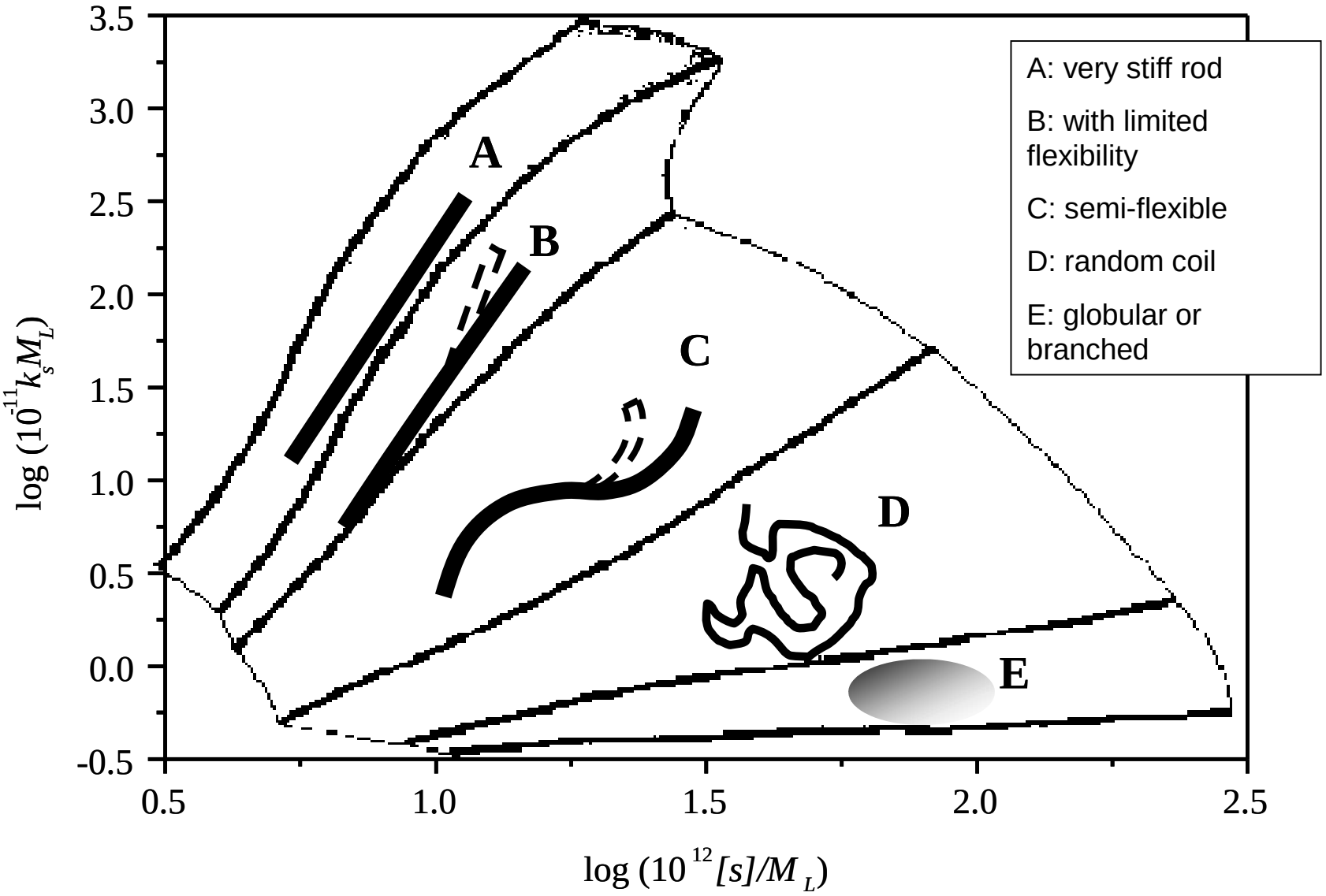
• Molecular conformation: flexibility analysis

"Hydfit" or Global analysis: Garcia de la Torre & Ortega, Biomacromolecules (2007)



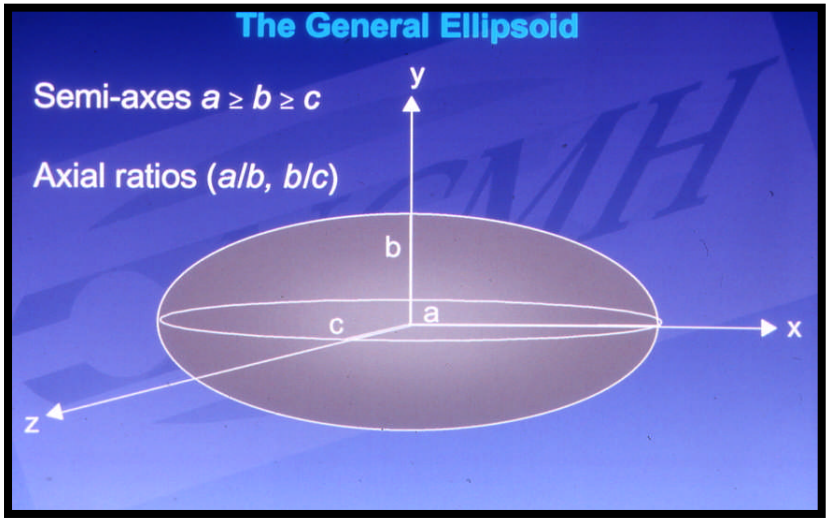
Konjac glucomannan, L_p ~ 13nm (Kok et al, 2009)

Conformation Zoning:



Pavlov et al. (1997). *Trends in Analytical Chemistry*, **16**, 401-405.

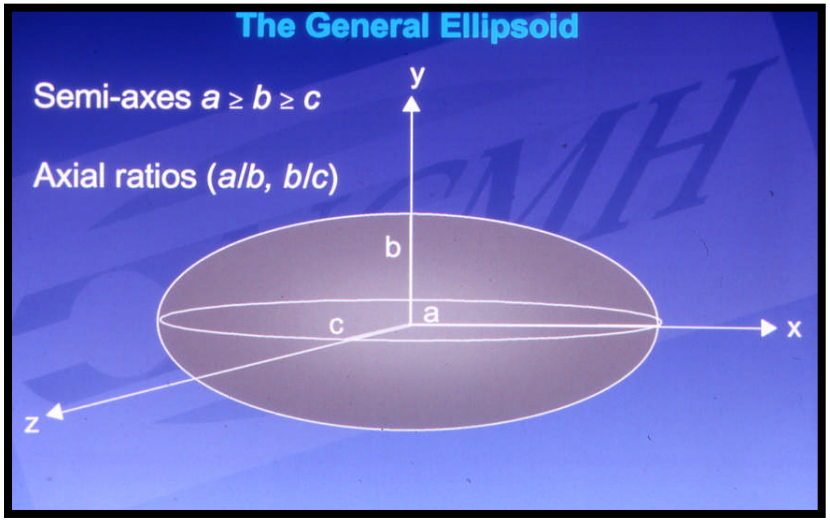
• Molecular conformation: ellipsoid representation



ELLIPS algorithms www.nottingham.ac.uk/ncmh

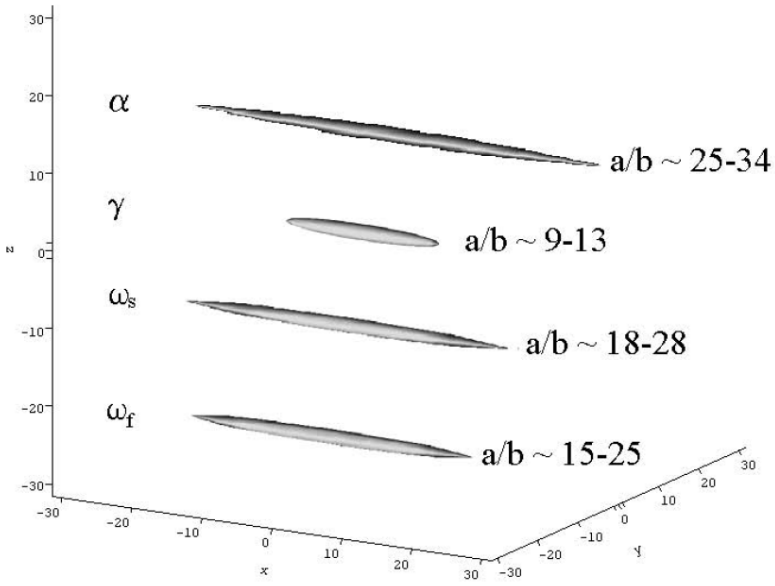
Universal_Param: Calculates shape parameter from s, D, [η], B
ELLIPS1 Evaluates a/b for prolate or oblate ellipsoid from shape parameter
ELLIPS2 Evaluates shape parameter from (*a*,*b*,*c*) or (*a*/*b*, *b*/*c*)
ELLIPS3 Evaluates (*a*/*b*, *b*/*c*) from combinations of hydration independent shape functions.
ELLIPS4 Evaluates (*a*/*b*, *b*/*c*) from electro-optic decay combined with other hydrodynamic data.
ELLIPSDRAW 3D plot of ellipsoid from (a/b, b/c)
COVOL Evaluates B from (a/b, b/c)

• Molecular conformation: ellipsoid representation



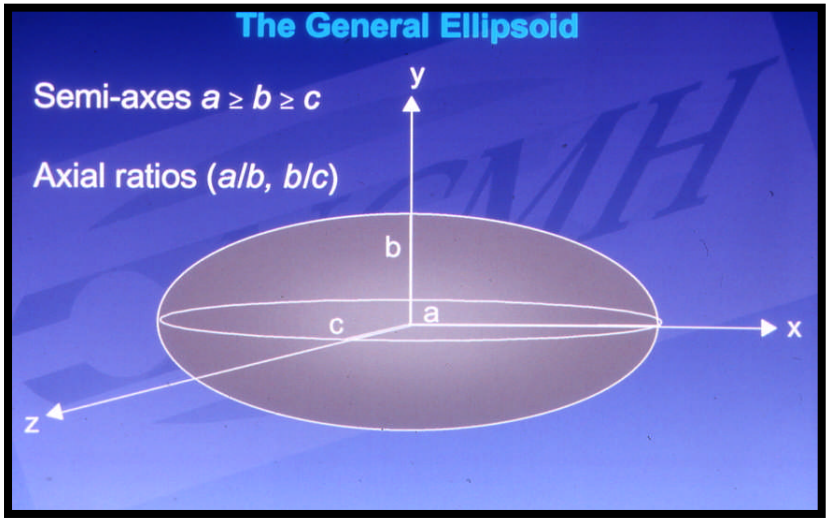
ELLIPS algorithms www.nottingham.ac.uk/ncmh

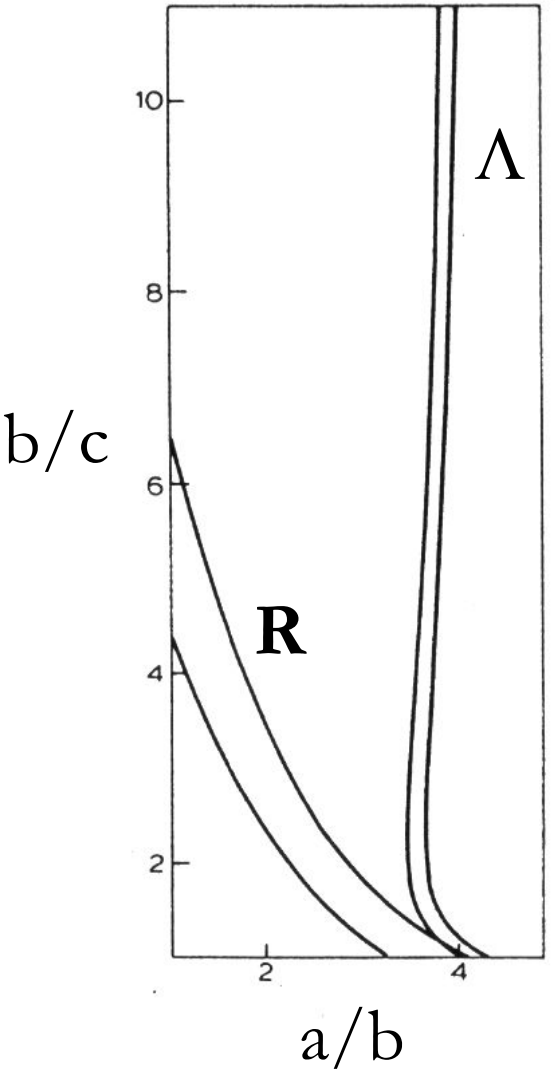
Universal_Param: Calculates shape parameter from s, D, [η], B
ELLIPS1 **Evaluates a/b for prolate or oblate ellipsoid from shape parameter**
ELLIPS2 Evaluates shape parameter from (*a*,*b*,*c*) or (*a*/*b*, *b*/*c*)
ELLIPS3 Evaluates (*a*/*b*, *b*/*c*) from combinations of hydration independent shape functions.
ELLIPS4 Evaluates (*a*/*b*, *b*/*c*) from electro-optic decay combined with other hydrodynamic data.
ELLIPSDRAW 3D plot of ellipsoid from (a/b, b/c)
COVOL Evaluates B from (a/b, b/c)



Structure and heterogeneity of gliadin: a hydrodynamic evaluation
S. Ang et al, Eur. Biophys. J. (2009)

• Molecular conformation: ellipsoid representation

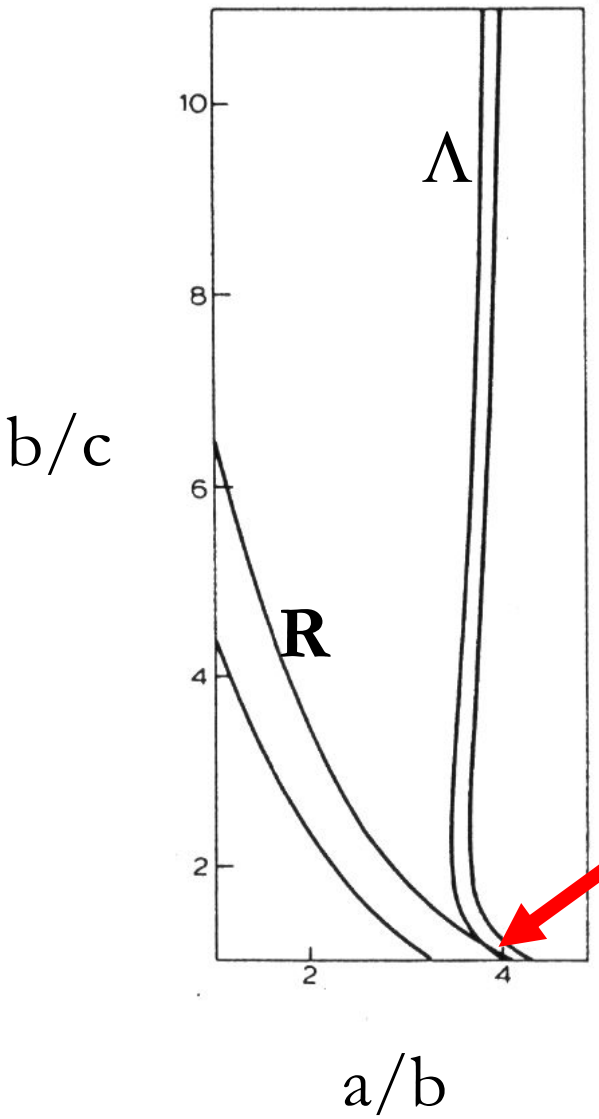


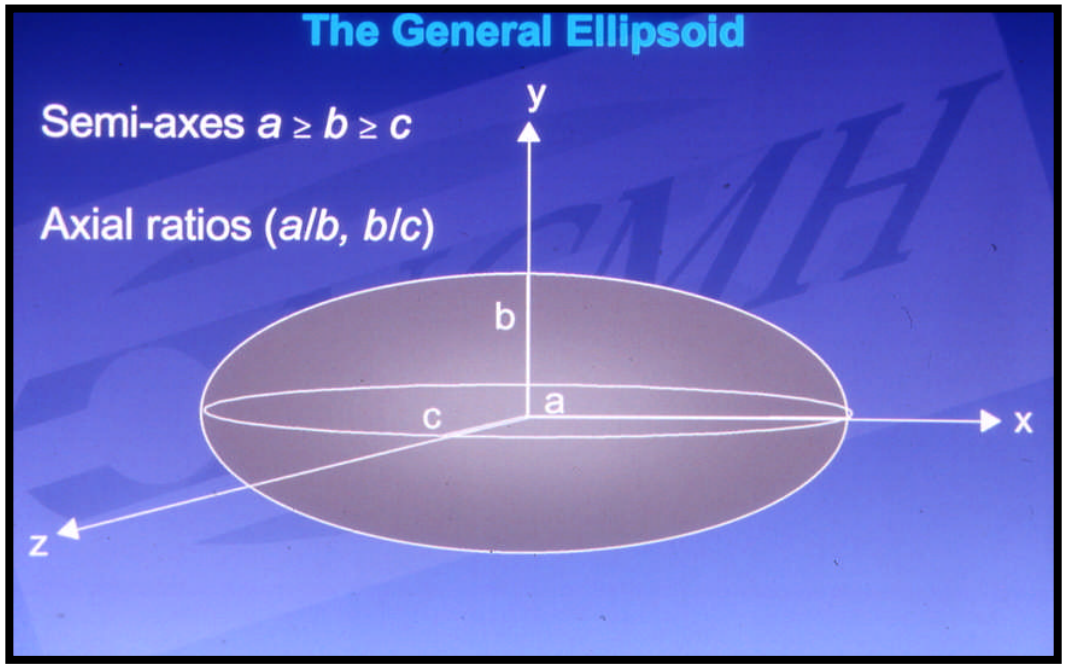


ELLIPS algorithms www.nottingham.ac.uk/ncmh

Universal_Param: Calculates shape parameter from s, D, [η], B
ELLIPS1 Evaluates a/b for prolate or oblate ellipsoid from shape parameter
ELLIPS2 Evaluates shape parameter from (*a*,*b*,*c*) or (*a/b*, *b/c*)
ELLIPS3 **Evaluates (*a/b*, *b/c*) from combinations of hydration independent shape functions.**
ELLIPS4 Evaluates (*a/b*, *b/c*) from electro-optic decay combined with other hydrodynamic data.
ELLIPSDRAW 3D plot of ellipsoid from (a/b, b/c)
COVOL Evaluates B from (a/b, b/c)

ELLIPS3 *applied to neurophysin monomers*

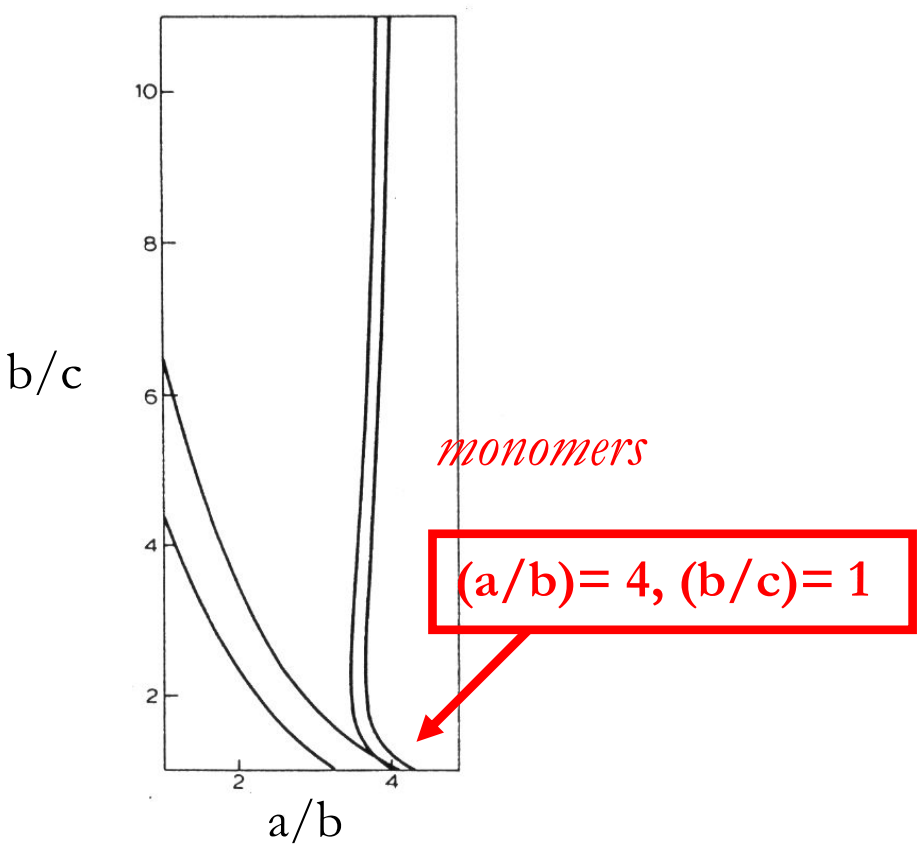


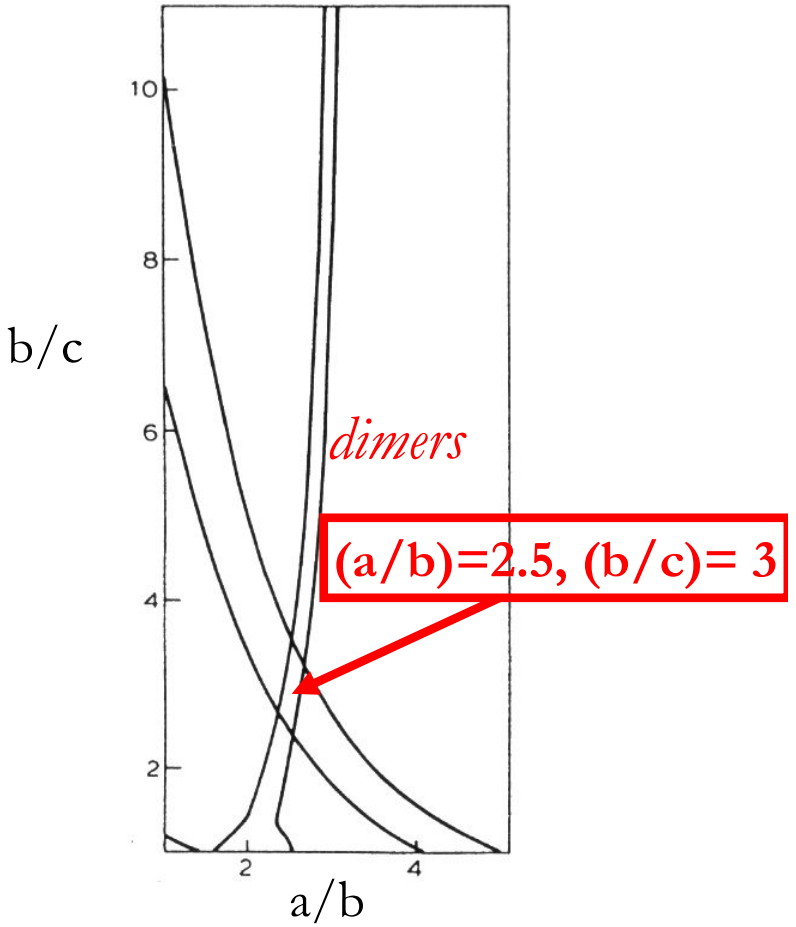


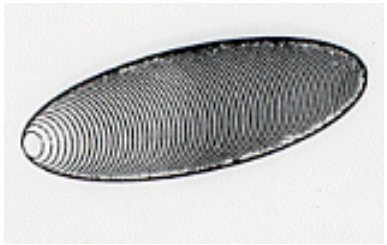
(a/b) = 4, (b/c) = 1

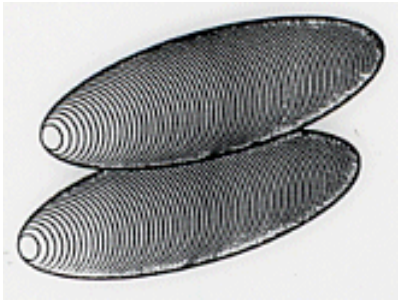
Shape parameters R and Λ are from sedimentation, viscosity and fluorescence measurements

Neurophysin dimerises – here's what happens

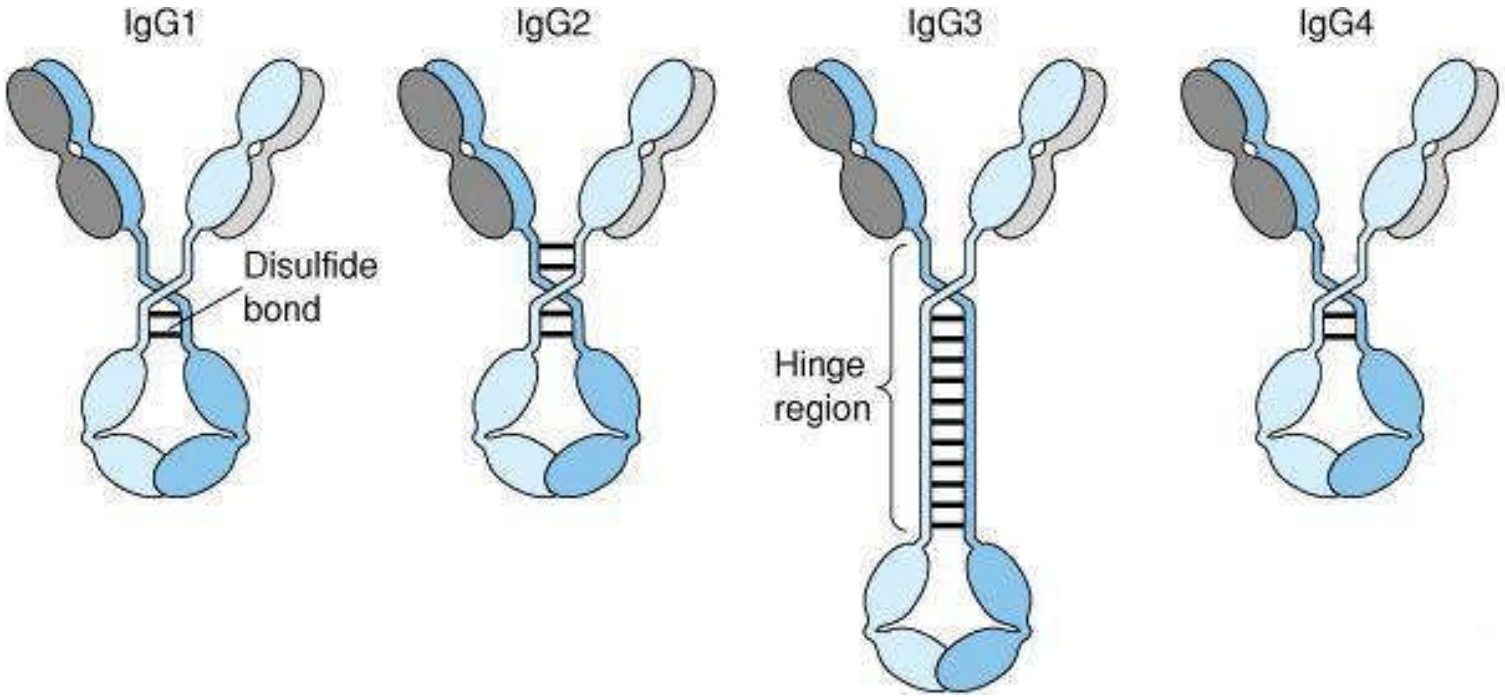




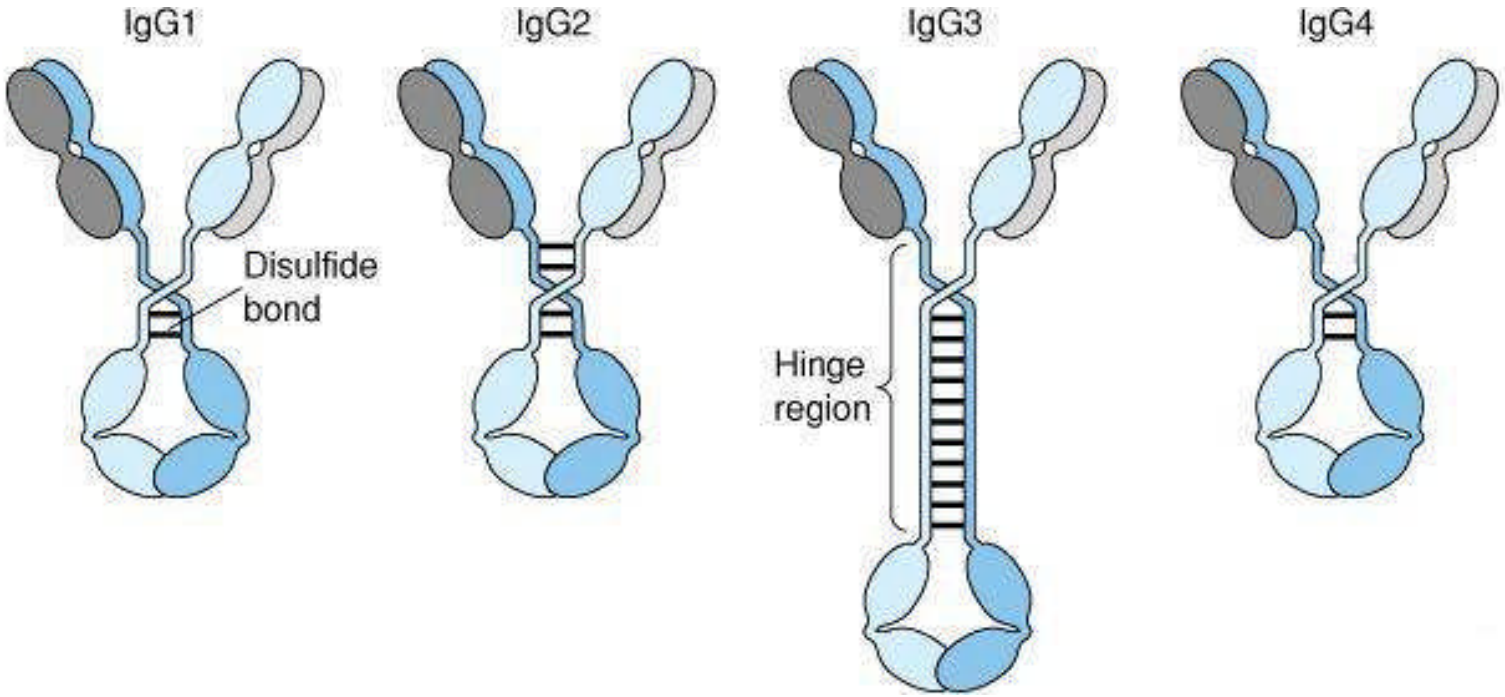




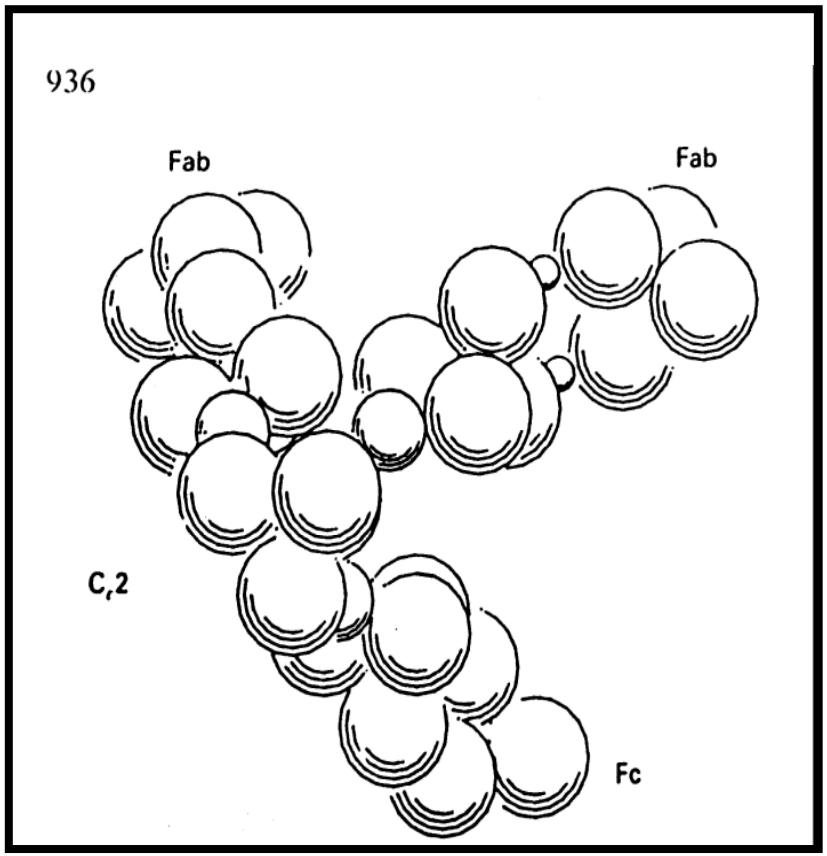
……whole-body ellipsoids won't do for complicated shapes like antibodies……



……whole-body ellipsoids won't do for complicated shapes like antibodies…… so use ***bead modelling***



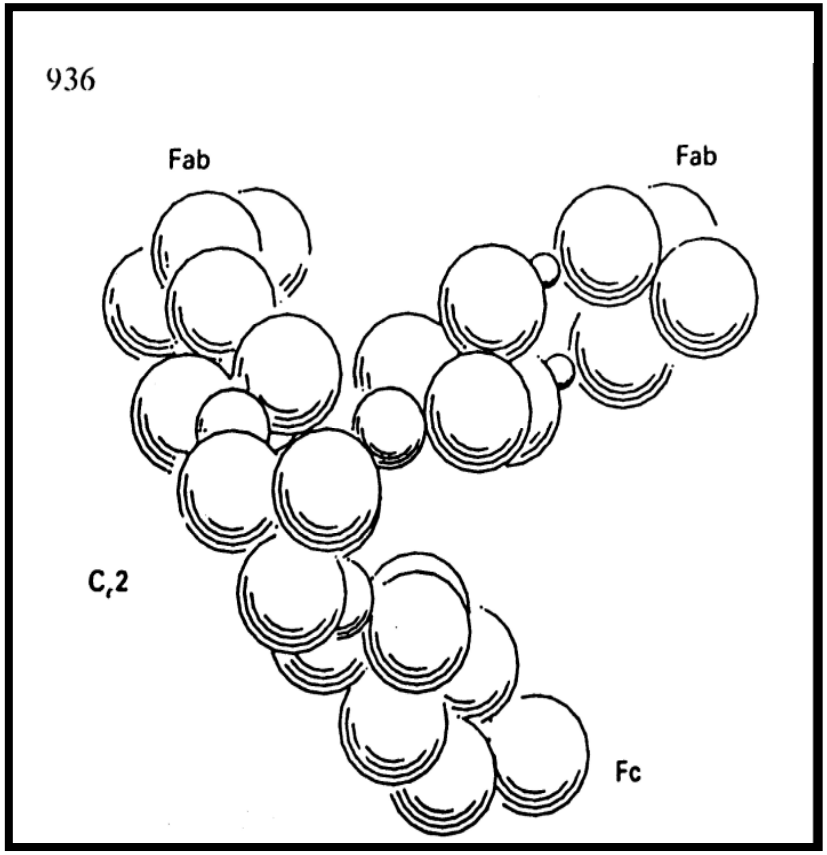
• Molecular conformation: bead modelling



• Molecular conformation: bead modelling

1st demonstration that IgE is cusp shaped, 1990

Bead model: s=7.26S,
R_g= 6.8nm



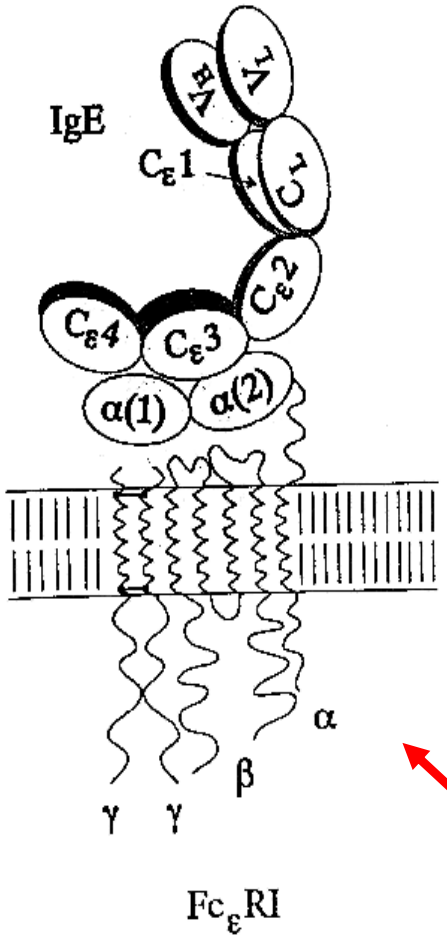
A model for the solution conformation of rat IgE

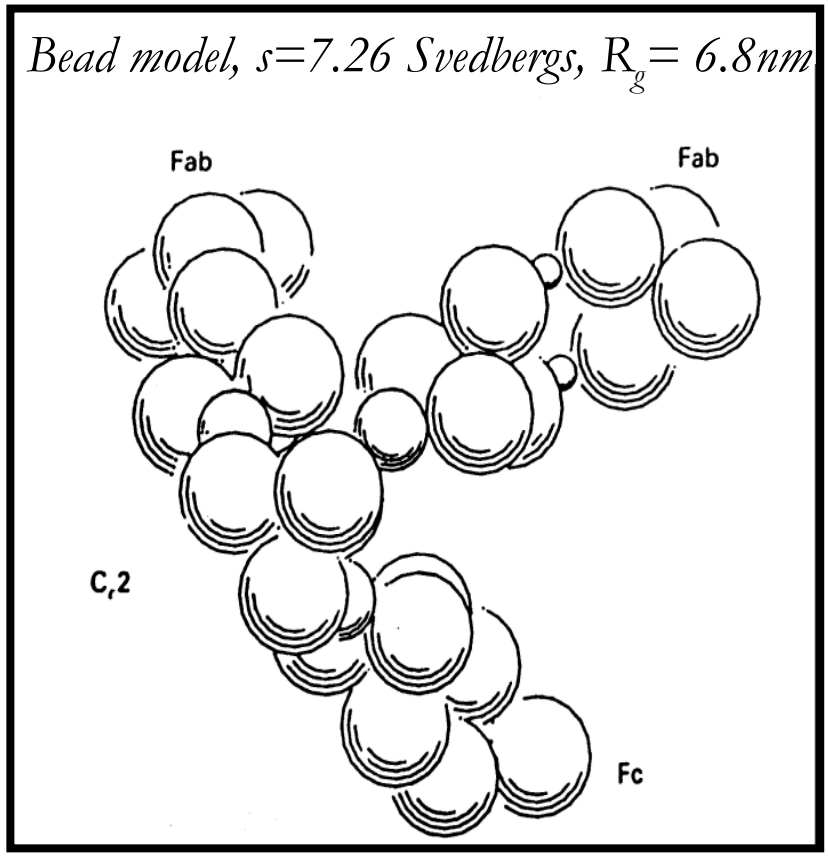
KENNETH G. DAVIS,* MARTIN GLENNIE,† STEPHEN E. HARDING* and DENNIS R. BURTON‡
**Department of Applied Biochemistry and Food Science, Nottingham University Agricultural School, Sutton Bonington, Loughborough LE12 5RD, U.K., †Tenovus Research Laboratory, Southampton General Hospital,*

then tried to reproduce the experimental parameters from models which incorporate as many of the known structural and immunological properties of antibodies as possible.

Immunoglobulin E (IgE) is found in monomeric form in serum and possesses five immunoglobulin domains in its heavy chain as well as being glycosylated at various sites. Two

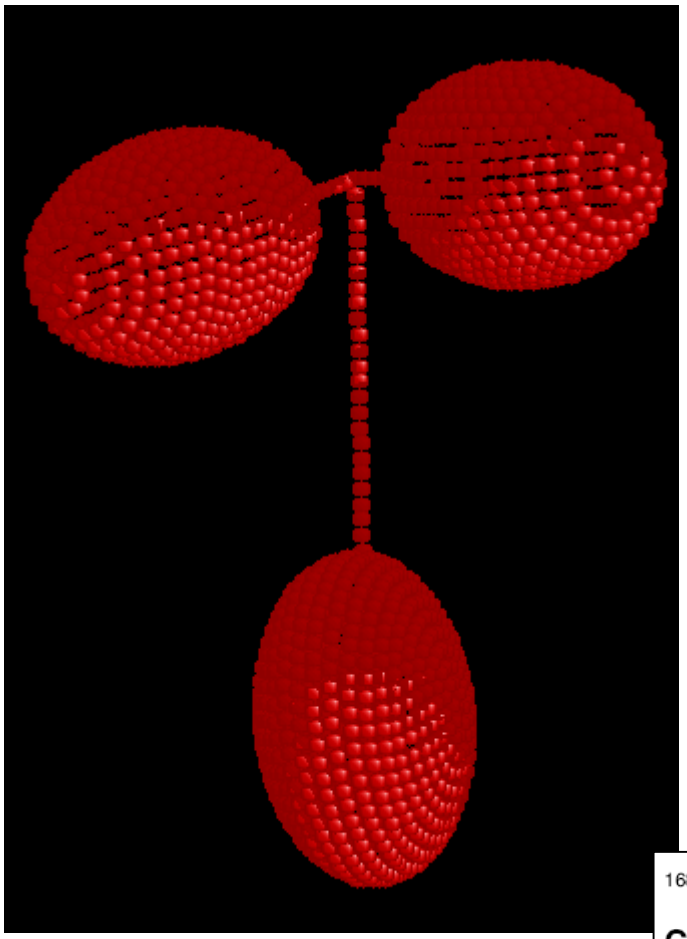
Consistent with function….



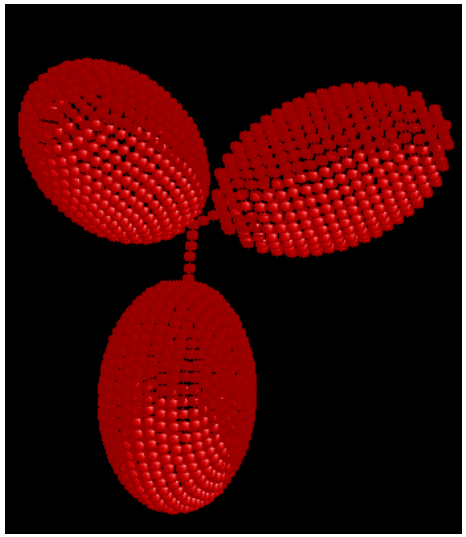


High Affinity Receptor

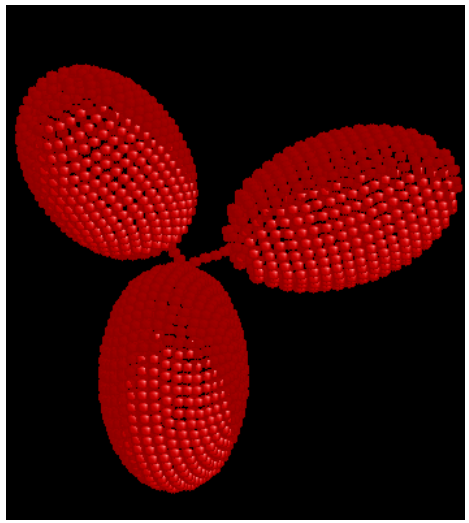
Conformation of engineered antibodies from s, R_g, D_{max}, [η] and crystal structure of the domains



A model of chimeric IgG3 wild type



A model of chimeric IgG3 m15 with 15aa in hinge.



A model of chimeric hinge deleted IgG3 HM5.

1688 Biophysical Journal Volume 91 September 2006 1688–1697

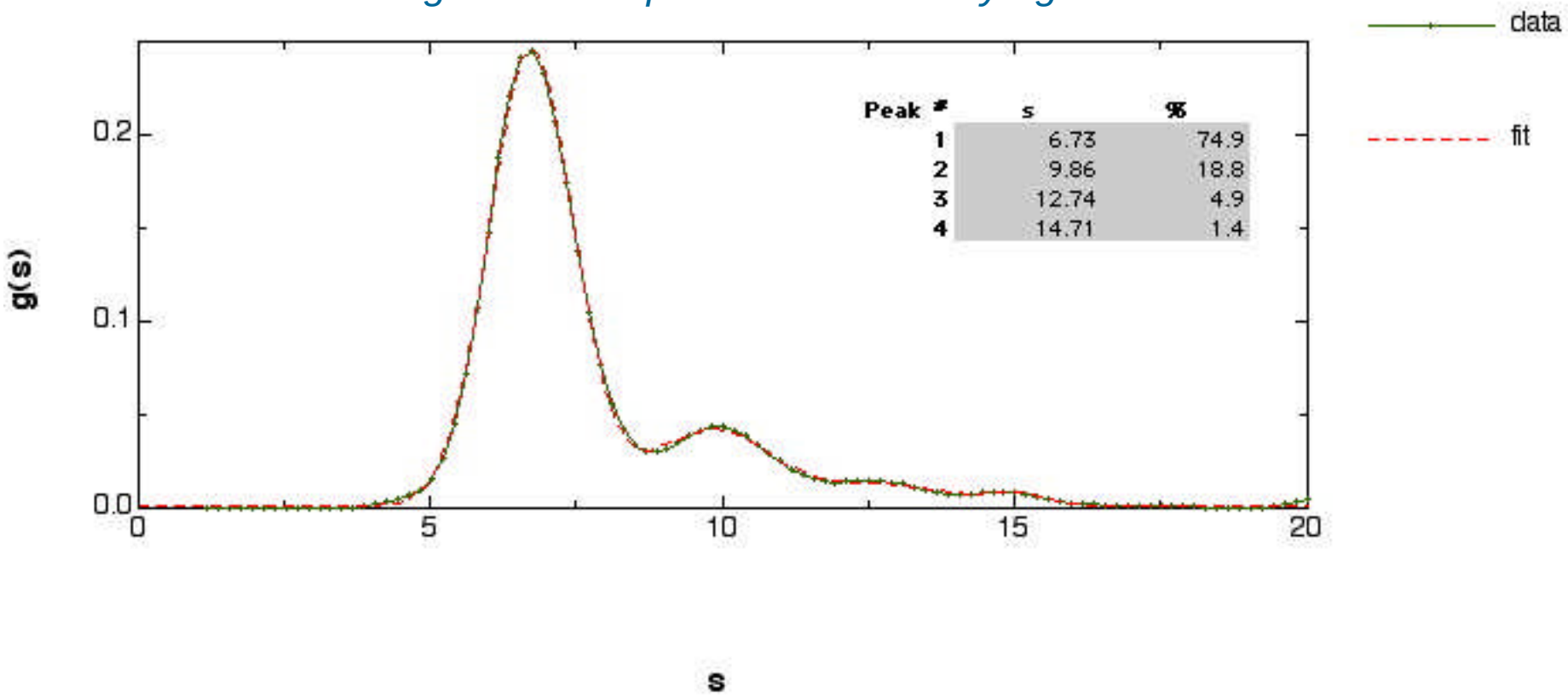
Crystallohydrodynamics of Protein Assemblies: Combining Sedimentation, Viscometry, and X-Ray Scattering

Yanling Lu,* Emma Longman,* Kenneth G. Davis,* Álvaro Ortega,† J. Günter Grossmann,‡ Terje E. Michaelsen,§ José García de la Torre,† and Stephen E. Harding*

*National Centre for Macromolecular Hydrodynamics, University of Nottingham, School of Biosciences, Sutton Bonington, England; †Departamento de Quimica Fisica, Universidad de Murcia, Murcia, Spain; ‡CCLRC Daresbury Laboratory, Synchrotron Radiation

Challenge: conformation determination in mixed systems

… here's our heterogeneous bioprocessed antibody again



Thanks to:

Professors Arthur Rowe, Jose Garcia de la Torre, Simon Ross-Murphy, Georges Pavlov & Drs. Dave Scott & Gordon Morris