

Svedberg 125th anniversary

**Advances in conformational analysis
in heterogeneous systems**

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Conformation in heterogeneous systems

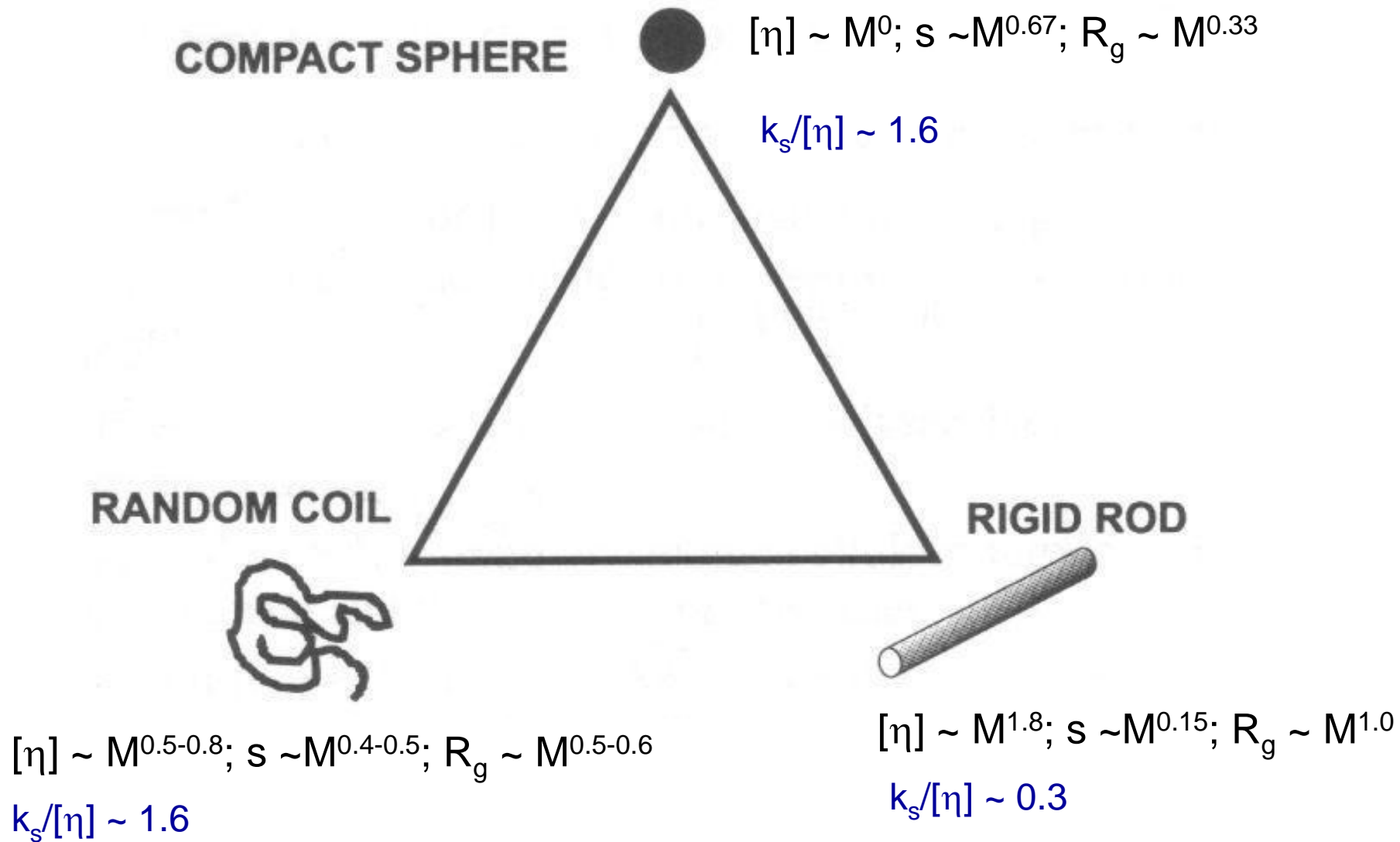
```
graph LR; A[Conformation in heterogeneous systems] --> B["Continuous:  
polysaccharides  
mucins"]; A --> C["Discrete:  
aggregated protein"]
```

Continuous:
polysaccharides
mucins

Discrete:
aggregated protein

Haug triangle and “scaling relations” or power law coeffs

k_s : Gralén coefficient

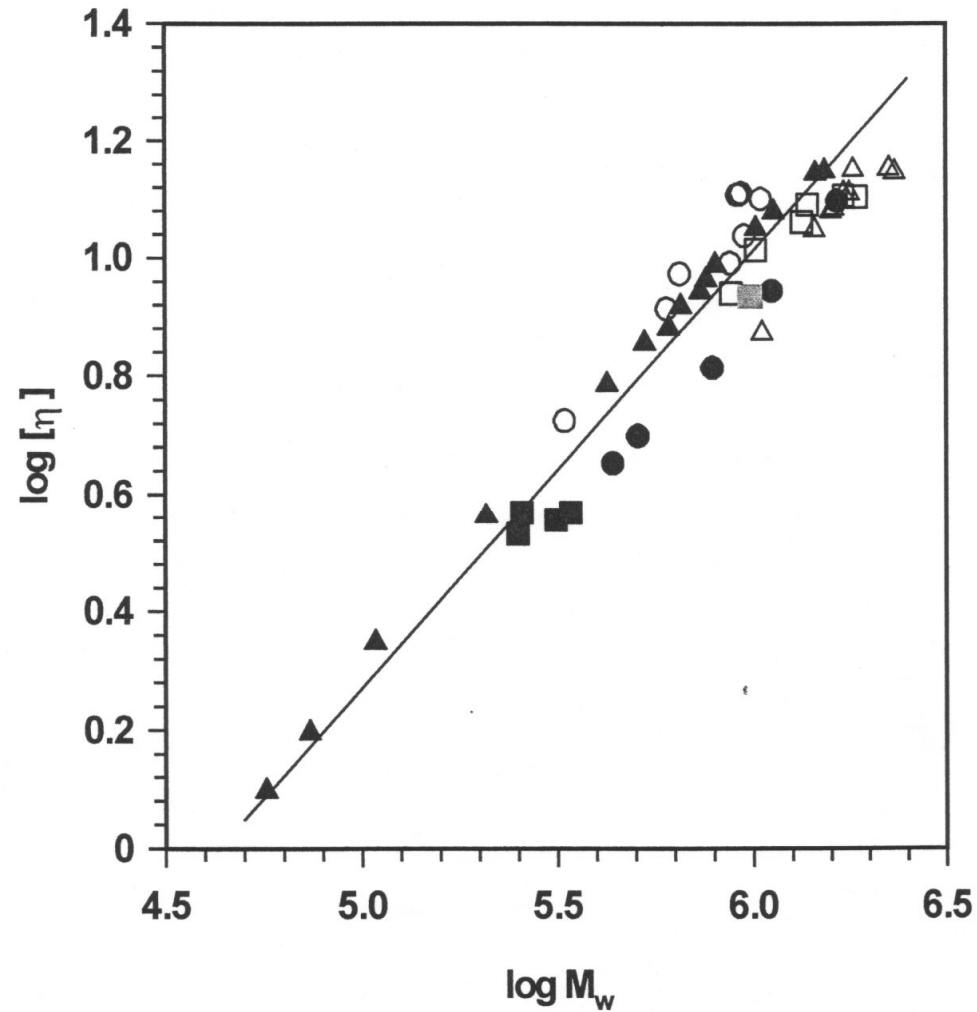


Power law plot - example

$$[\eta] \sim M^a$$

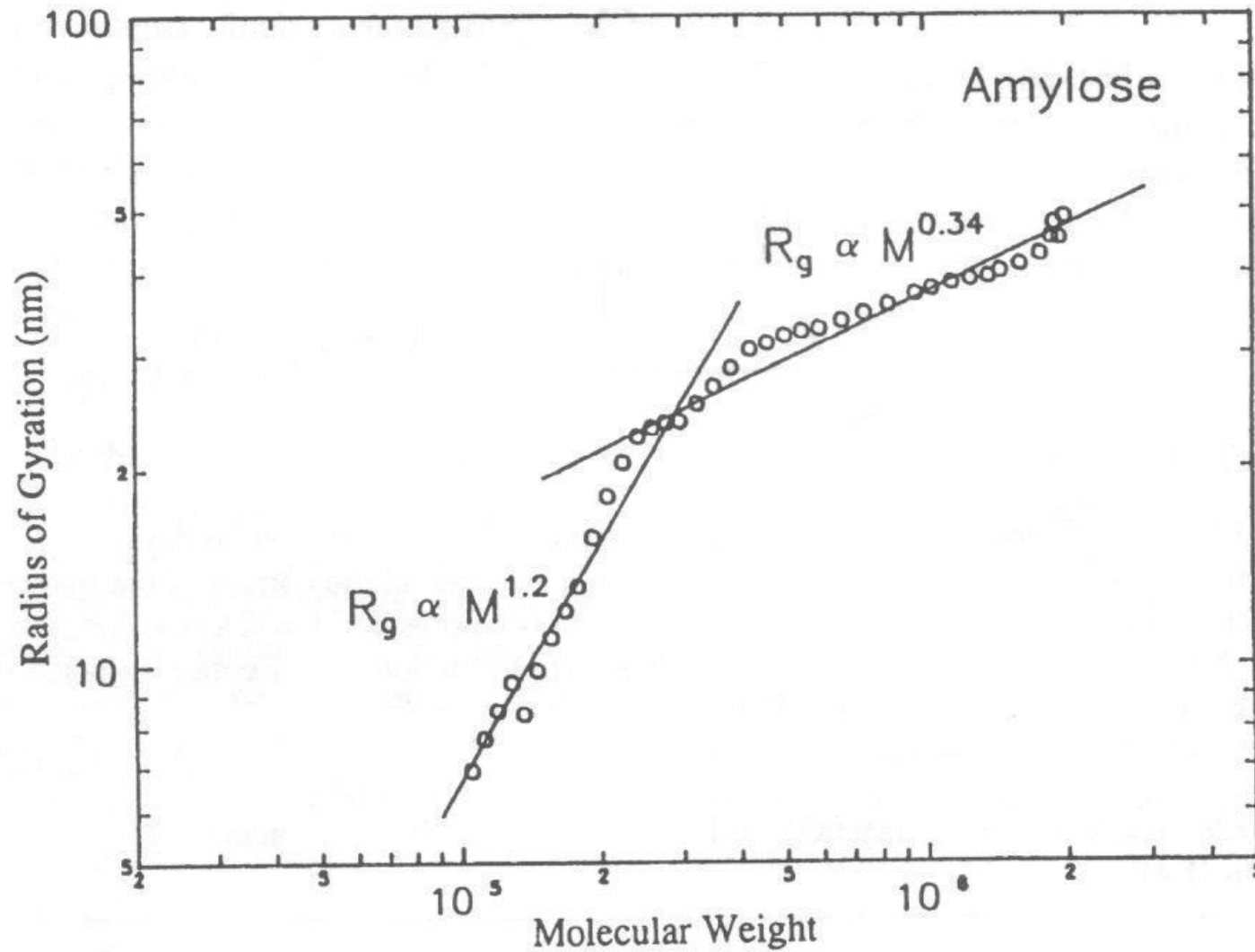
Galactomannans

$$a = 0.74 \pm 0.01$$



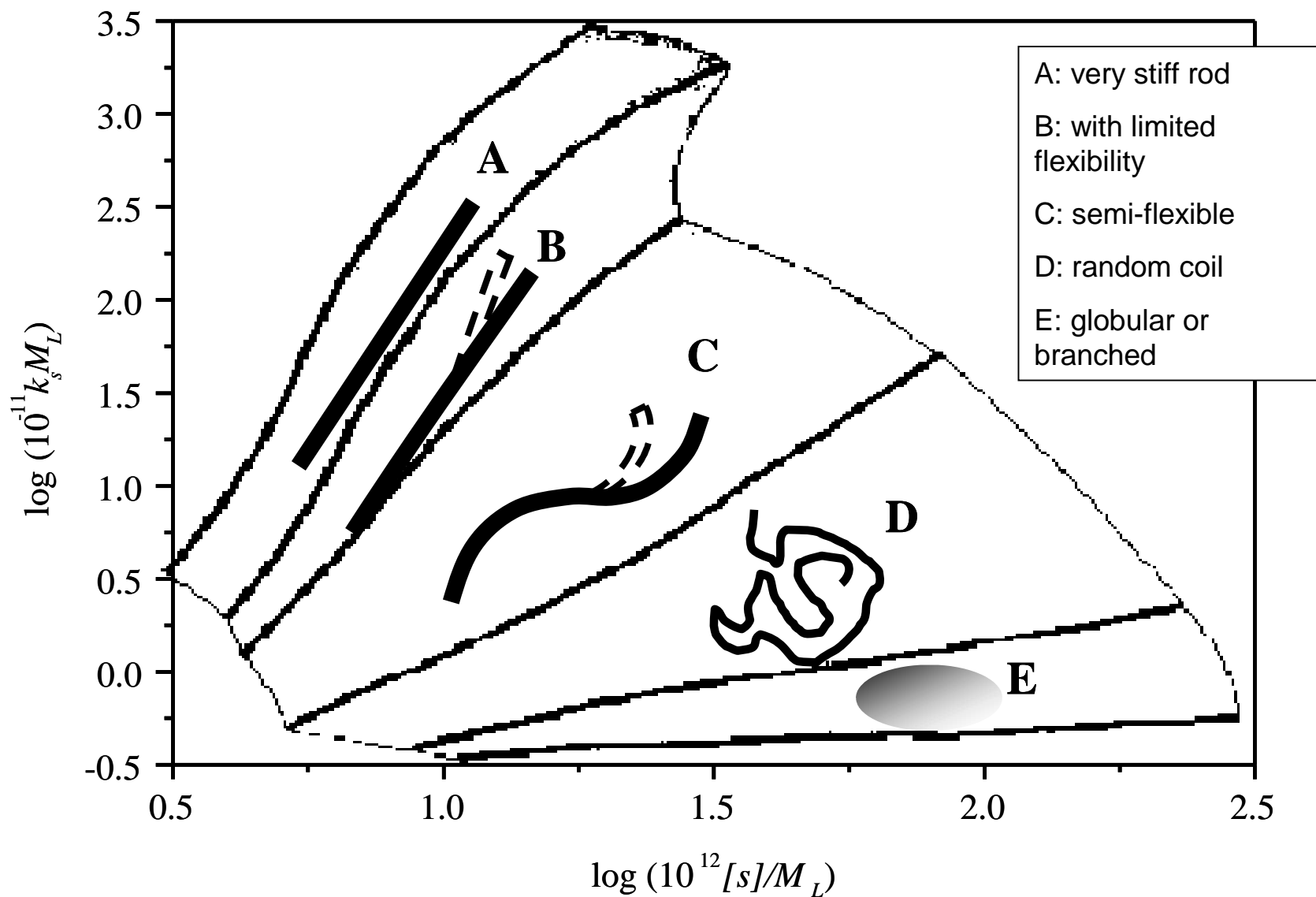
Picout, Ross-Murphy, Jumel & Harding (2002)
Biomacromolecules **3**, 761-767

Change in Conformation



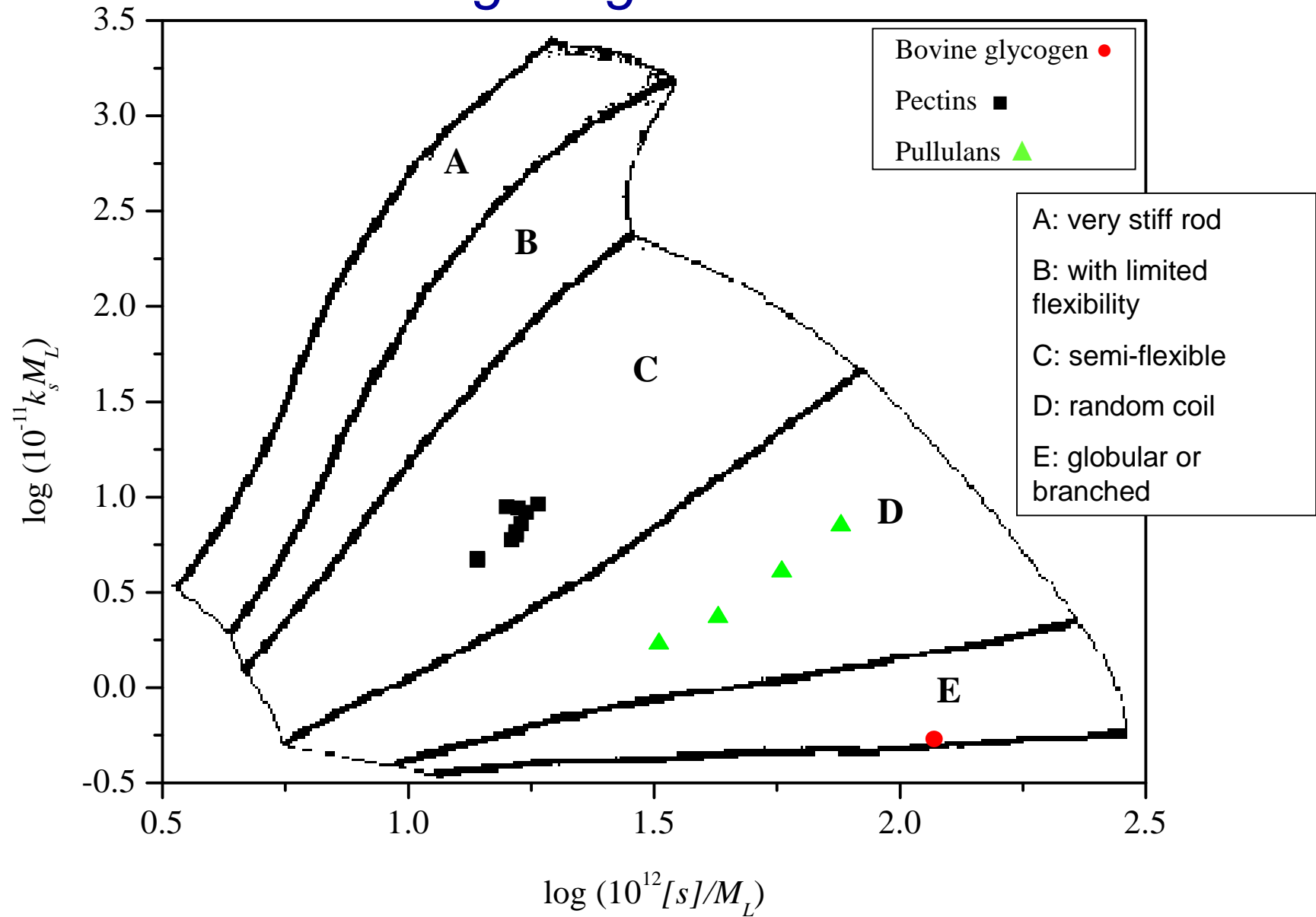
Rollings J (1992) in *Laser Light Scattering in Biochemistry* (Harding, Sattelle & Bloomfield eds)

Conformation Zoning Diagram



Pavlov, Rowe & Harding (1997). *Trends in Analytical Chemistry*, **16**, 401-405.

Conformation Zoning Diagram



HYDFIT plot – flexibility determination, L_p

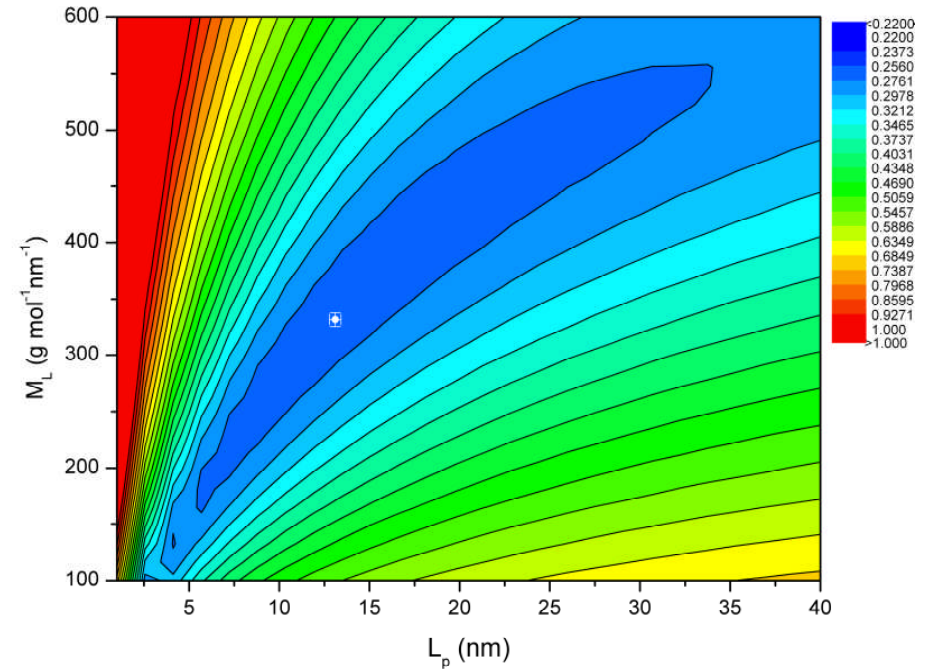
Garcia de la Torre & Ortega (2007), *Biomacromolecules* 8, 2462-2475

“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

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



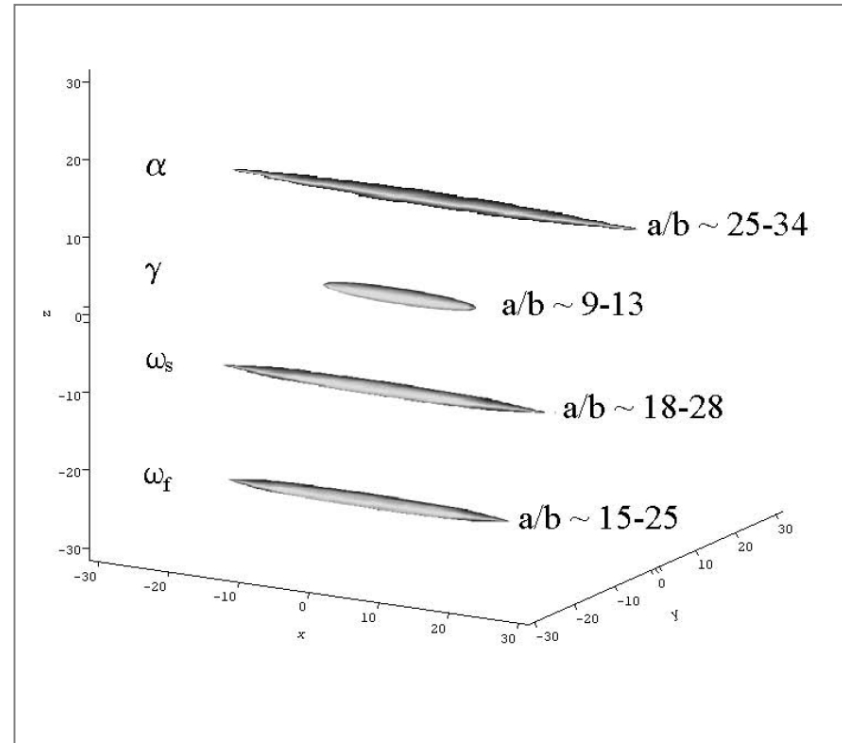
Konjac glucomannan, $L_p \sim 13\text{nm}$
(Kok et al, 2009)

Conformation in heterogeneous systems

Continuous:
polysaccharides
mucins

Discrete:
aggregated protein

Conformation analysis in a polydisperse protein system – gliadin

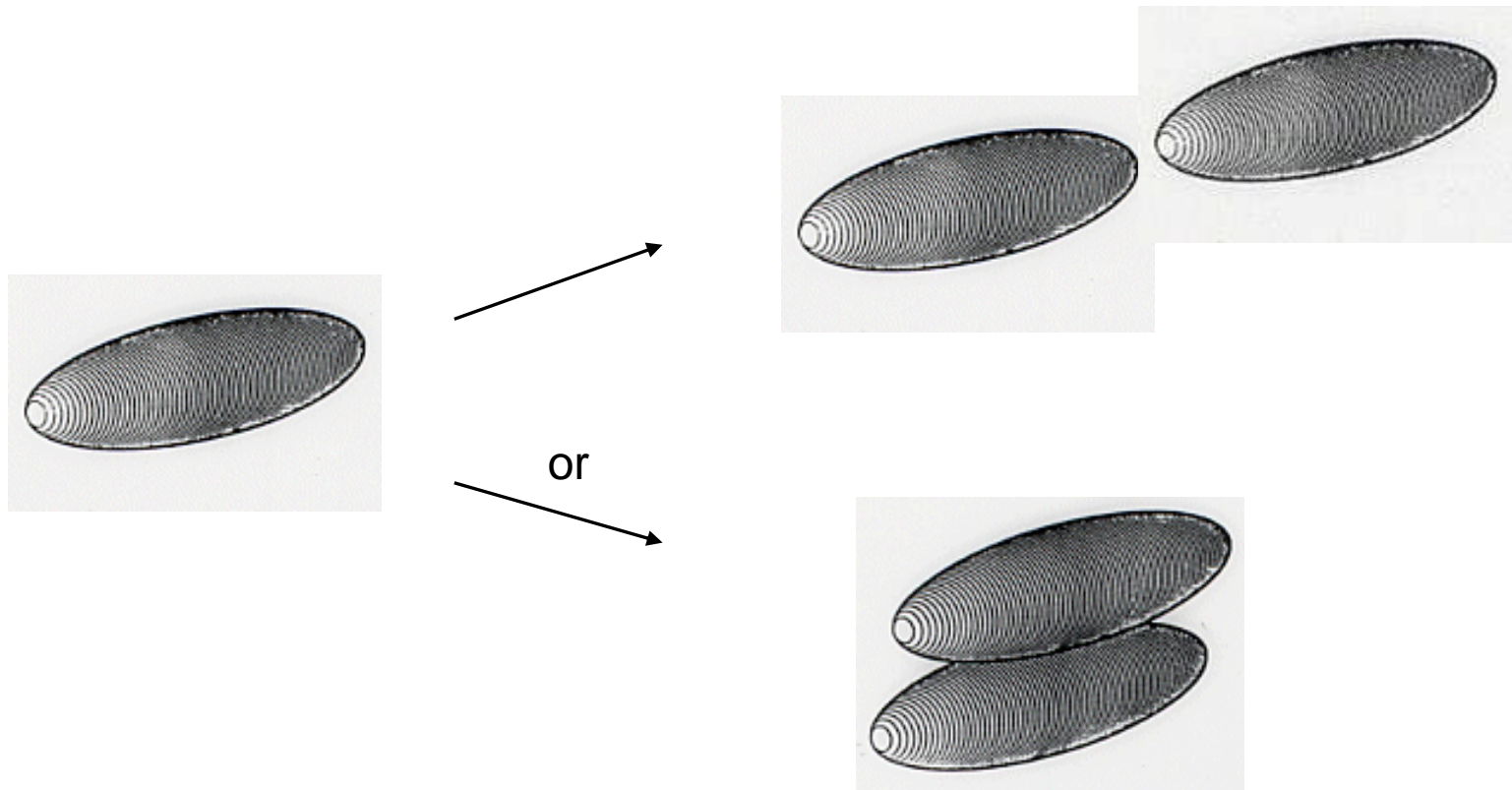


**Structure and heterogeneity of
gliadin: a hydrodynamic evaluation**
S. Ang et al, *Eur. Biophys. J.* (2009)

ELLIPS1

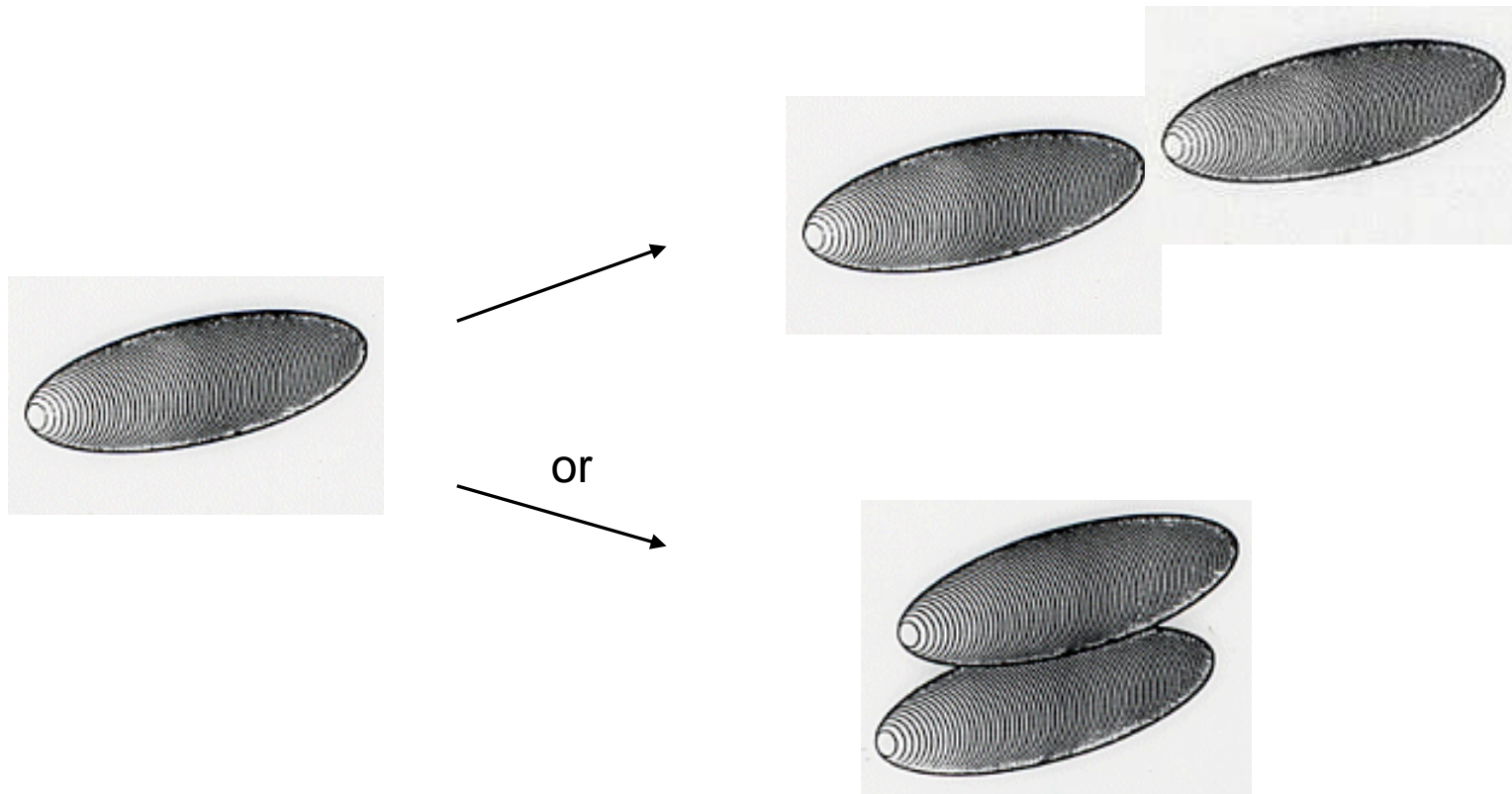
www.nottingham.ac.uk/ncmh

Conformation analysis in a dimerising protein system – neurophysin



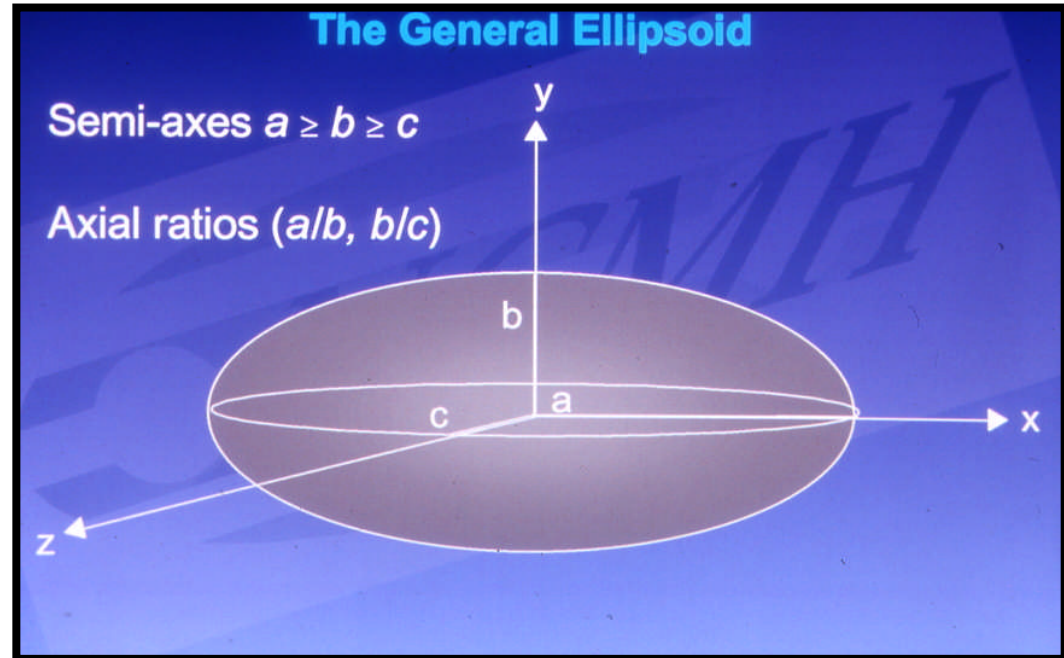
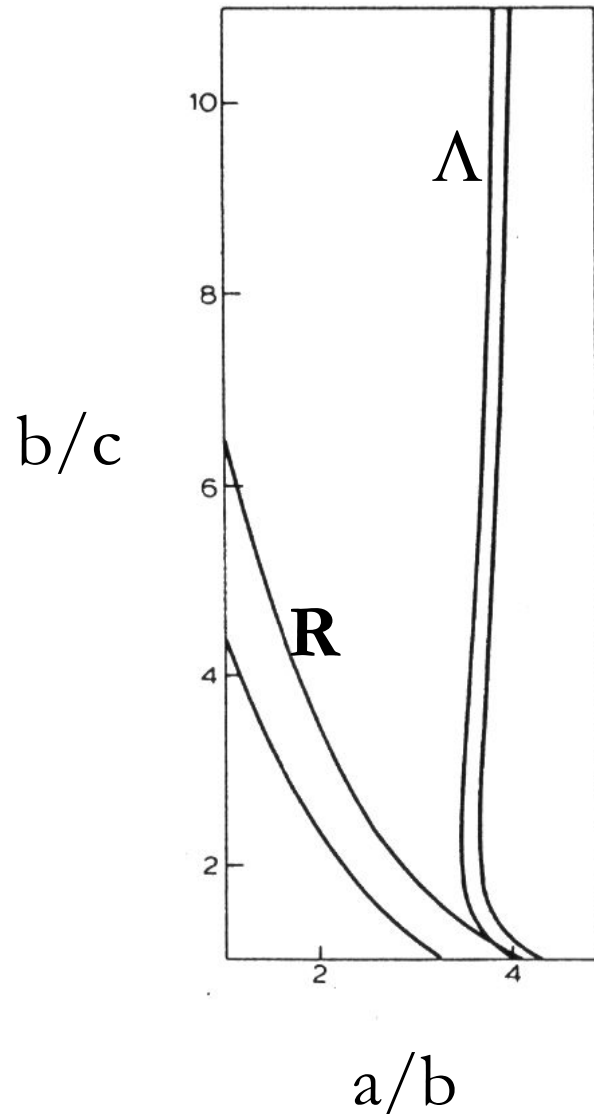
?

Conformation analysis in a dimerising protein system – neurophysin



ELLIPS3

Conformation analysis in a dimerising protein system – neurophysin

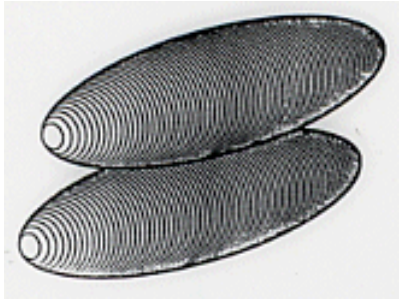
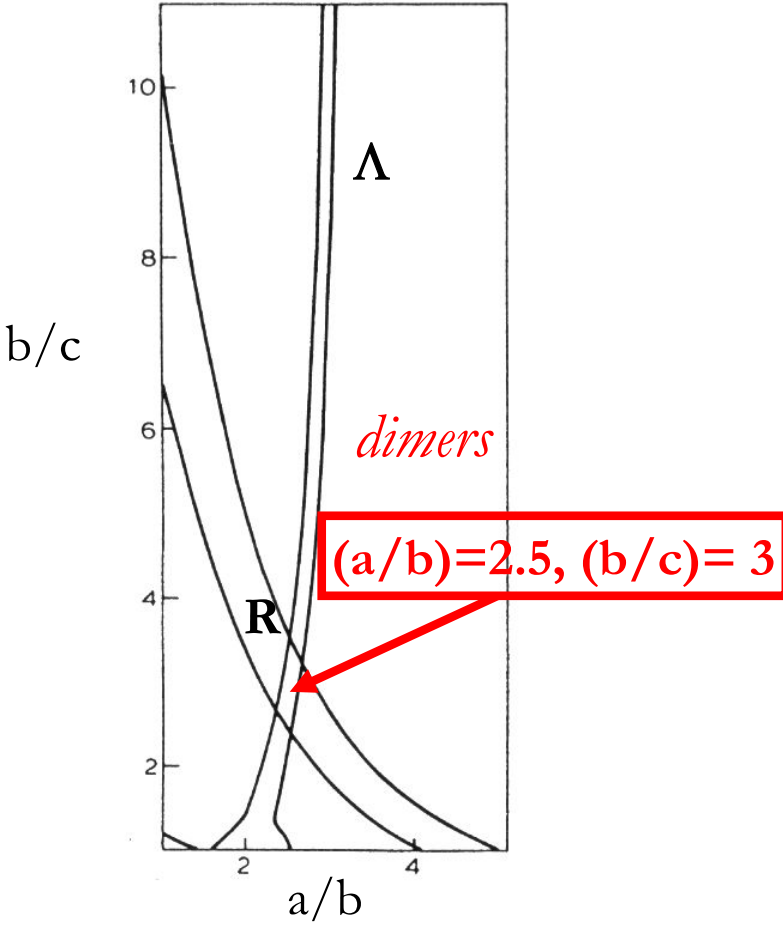
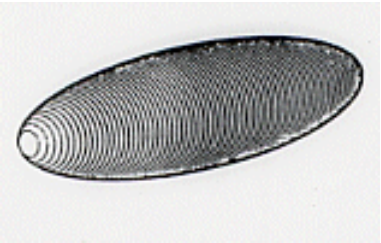
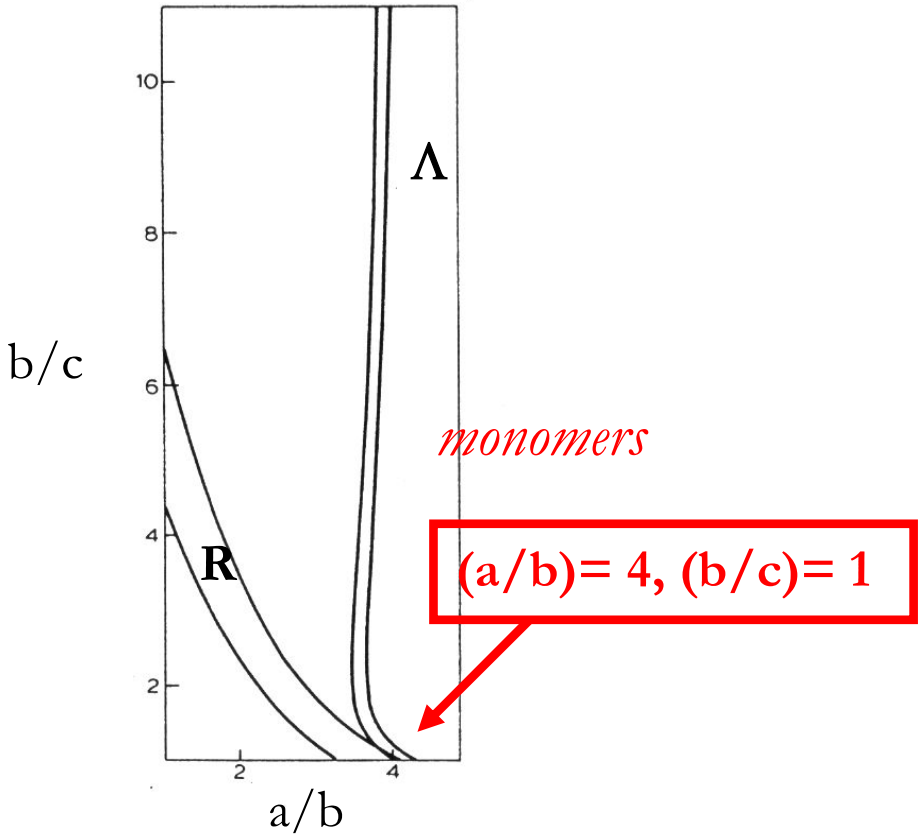


Shape parameters R and Λ are from sedimentation, viscosity and fluorescence measurements

$$\Lambda = \{3\eta_o[\eta]M\}/\{N_A kTt_h\}$$

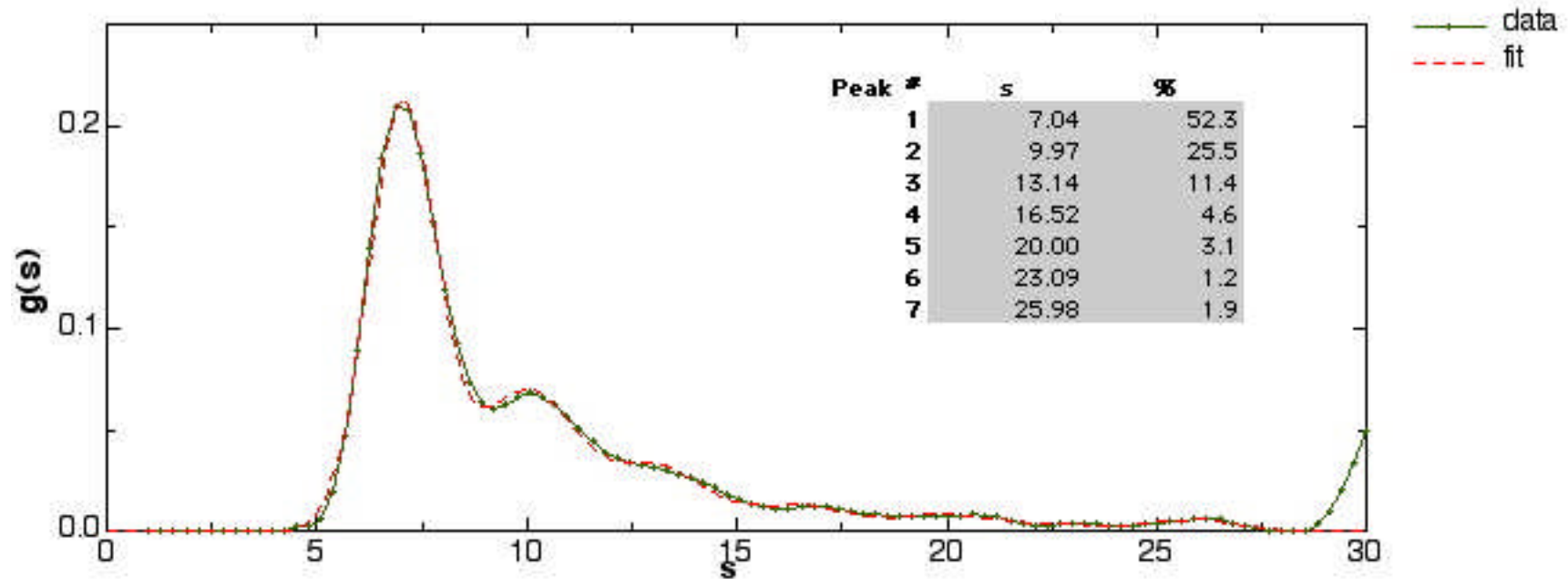
$$R = k_s/[\eta]$$

Neurophysin dimerises – here's what happens



Conformation analysis in an aggregated monoclonal antibody system – effect of bioprocessing

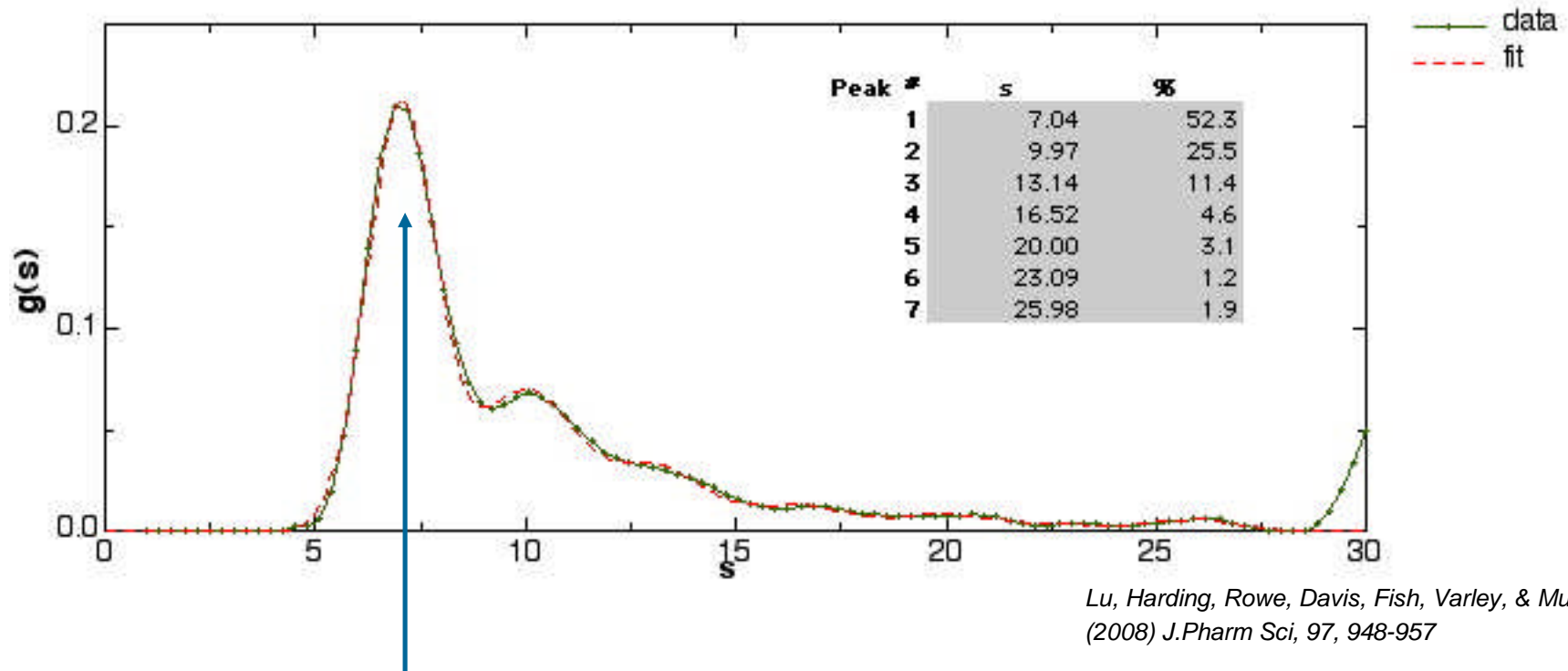
Freeze-thaw bioprocessed IgG₄



Lu, Harding, Rowe, Davis, Fish, Varley, & Mulot,
(2008) *J.Pharm Sci*, 97, 948-957

Conformation analysis in an aggregated monoclonal antibody system – effect of bioprocessing

Freeze-thaw bioprocessed IgG₄

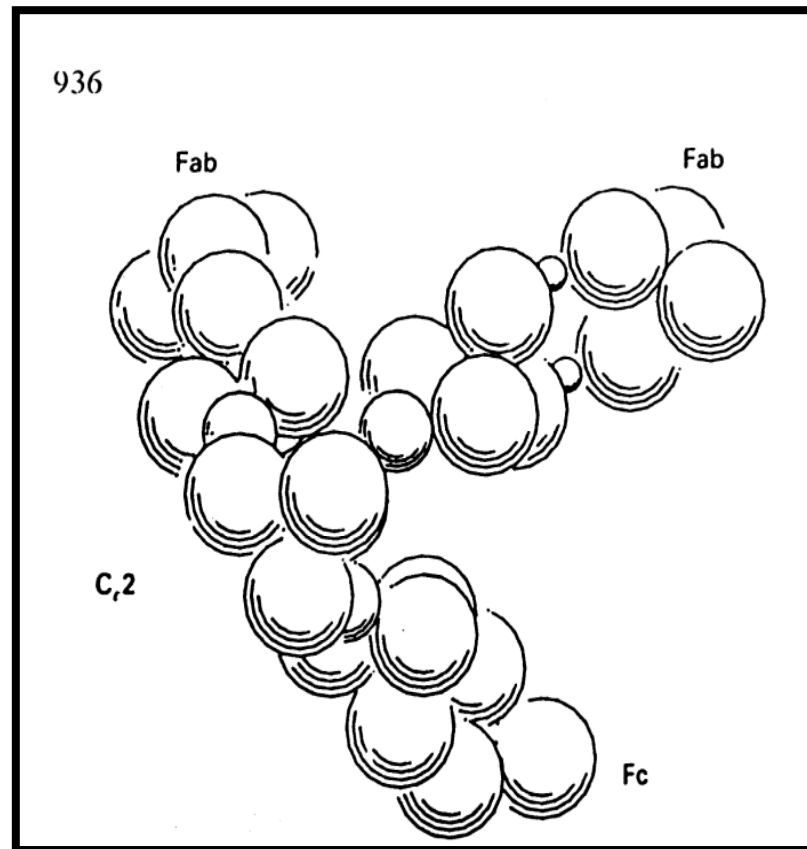


Monomer – is there a link between conformation change and aggregation? – need s and other data to answer this

Bead model – “cusp” shape for IgE, 1990

*Modelled on $s=7.26S$,
 $R_g= 6.8nm$*

*.. and iterated from crystal
structure of a hinge deleted
IgG mutant*



936

BIOCHEMICAL SOCIETY TRANSACTIONS

1990

A model for the solution conformation of rat IgE

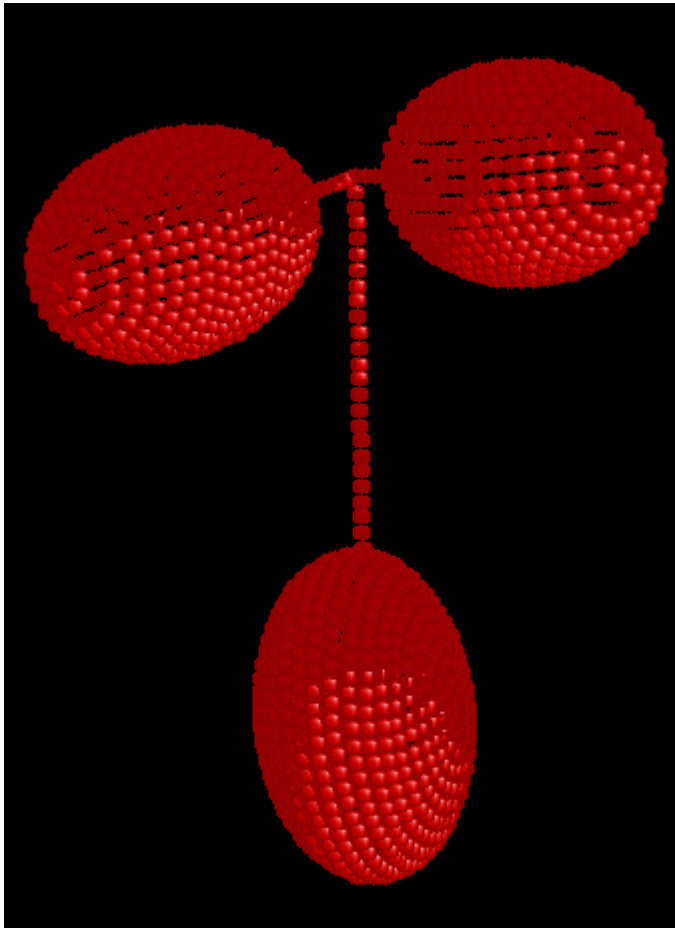
KENNETH G. DAVIS,* MARTIN GLENNIE,†
STEPHEN E. HARDING* and DENNIS R. BURTON‡

**Department of Applied Biochemistry and Food Science,
Nottingham University Agricultural School, Sutton
Bonington, Loughborough LE12 5RD, U.K., †Tenovis
Research Laboratory, Southampton General Hospital,*

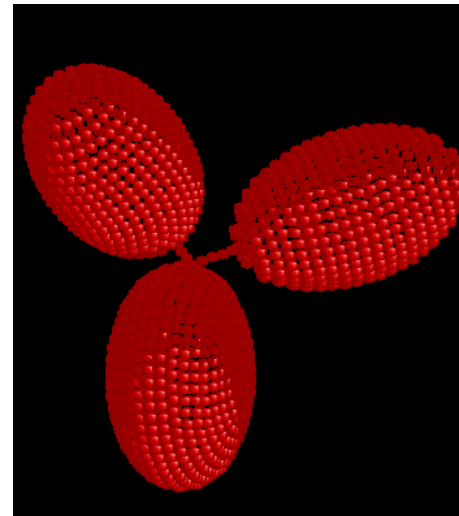
then tried to reproduce the experimental parameters from models which incorporate as many of the known structural and immunological properties of antibodies as possible.

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

More recent strategies use even more data: \underline{s} , \underline{R}_g ,
 \underline{D}_{max} , $[\eta]$ and crystal structure of the domains



A model of chimeric IgG3 wild type



*A model of chimeric
hinge deleted IgG3
HM5.*

Modelling
algorithm:
SOLPRO

1688

Biophysical Journal Volume 91 September 2006 1688–1697

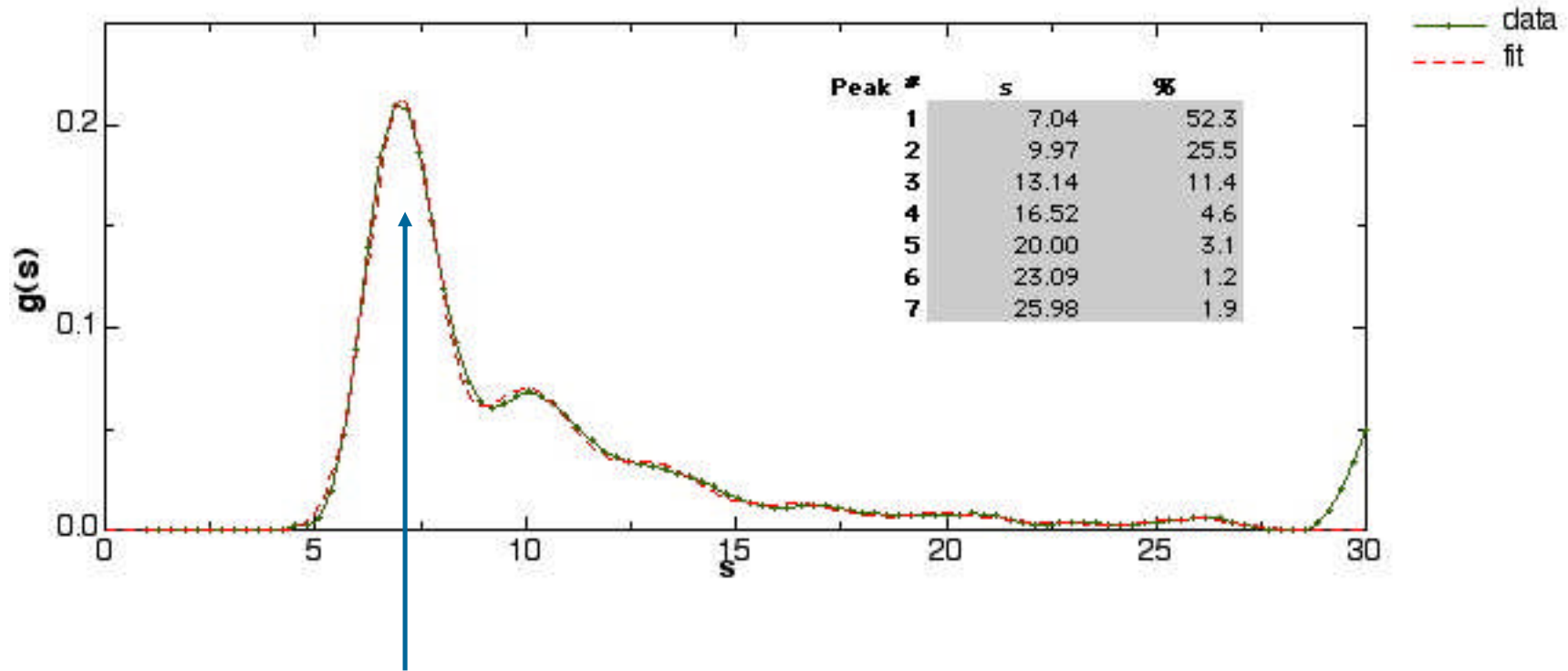
Crystallohydrodynamics of Protein Assemblies: Combining Sedimentation, Viscometry, and X-Ray Scattering

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 Química Física, Universidad de Murcia, Murcia, Spain; ‡CCLRC Daresbury Laboratory, Synchrotron Radiation

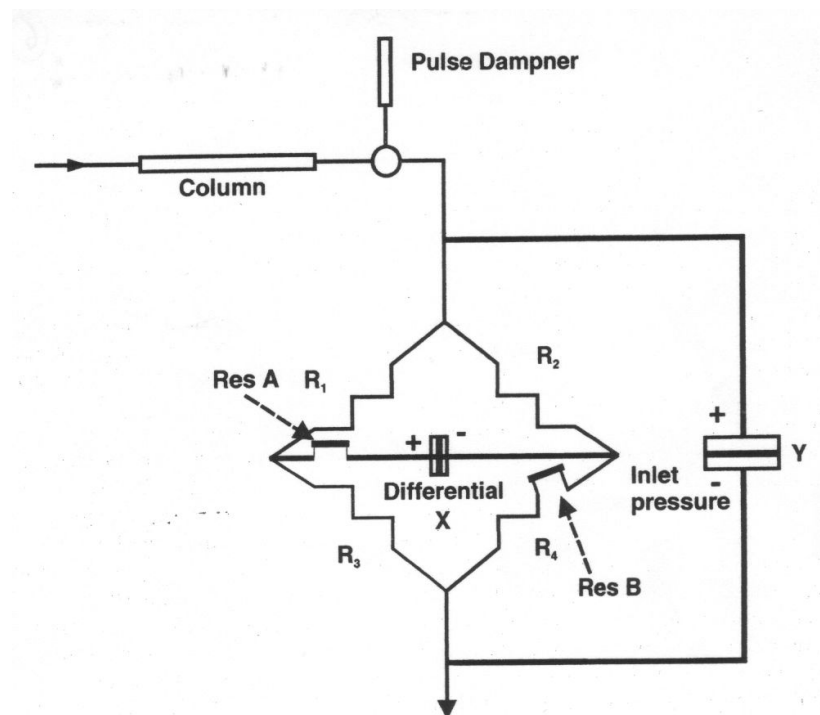
Conformation analysis in an aggregated monoclonal antibody system – effect of bioprocessing

Freeze-thaw bioprocessed IgG₄



Monomer – is there a link between conformation change and aggregation? – need s and other data to answer this

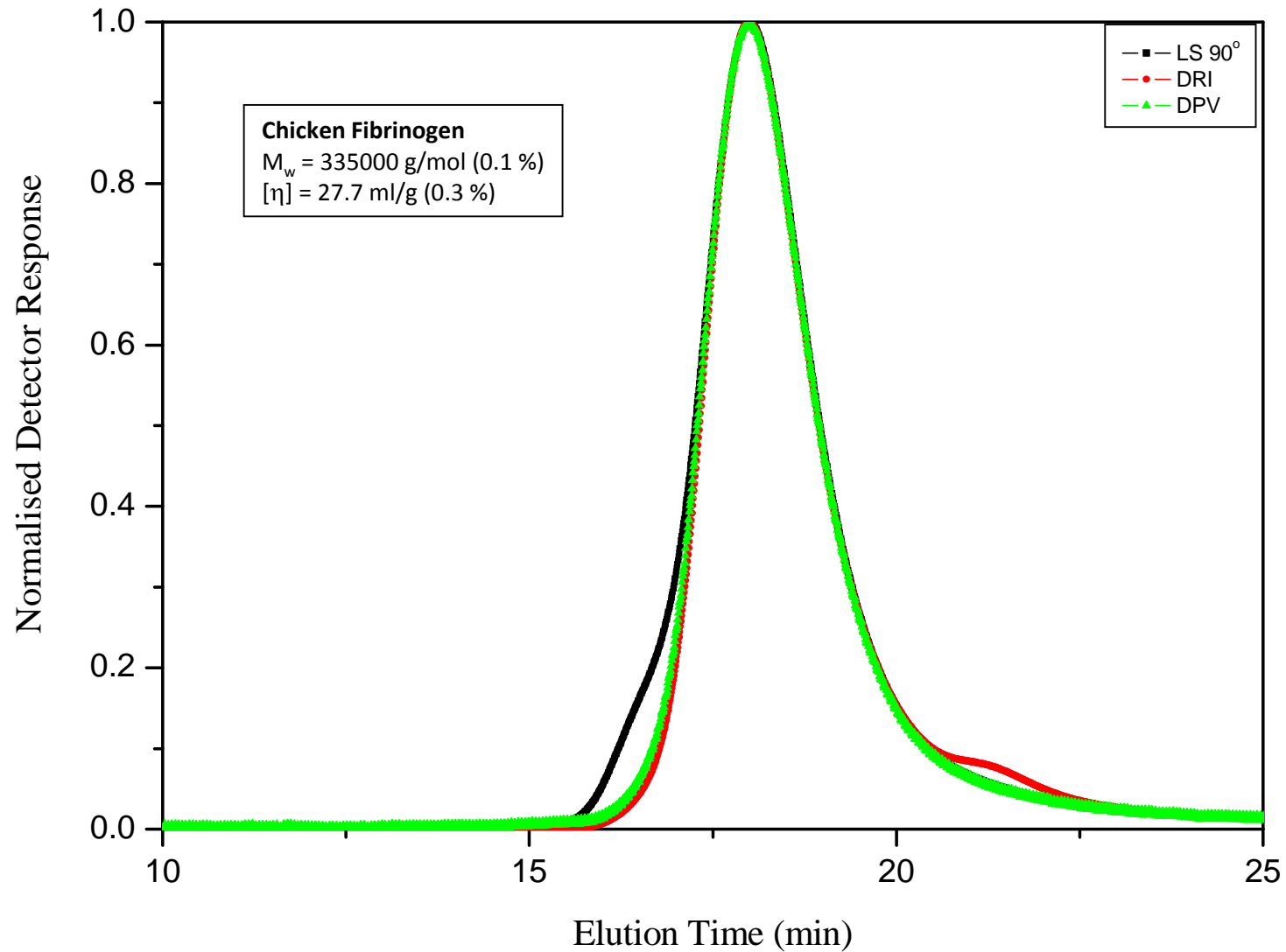
Differential pressure Viscometer:

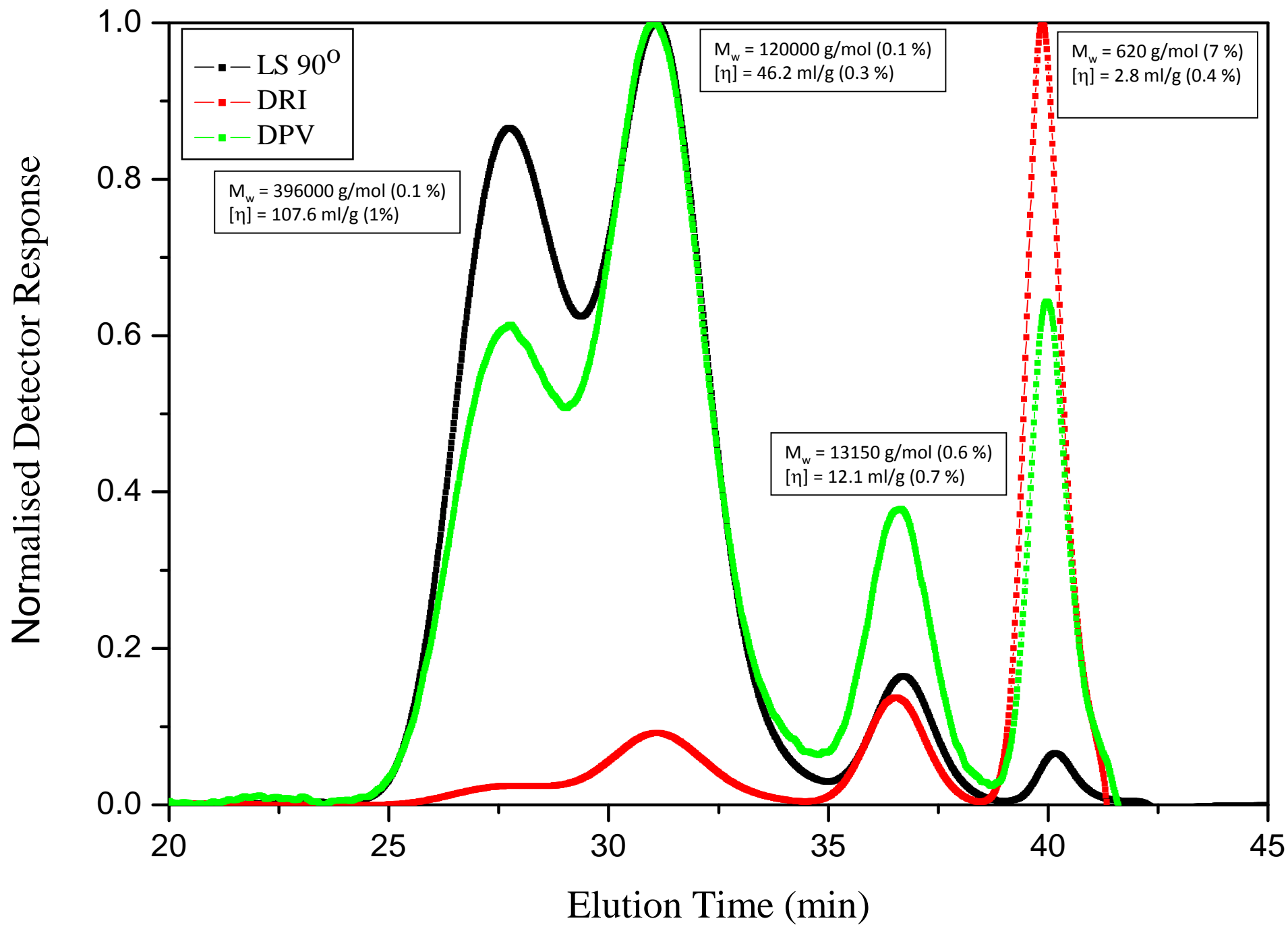


Viscotek (Malvern) or Viscostar (Wyatt)



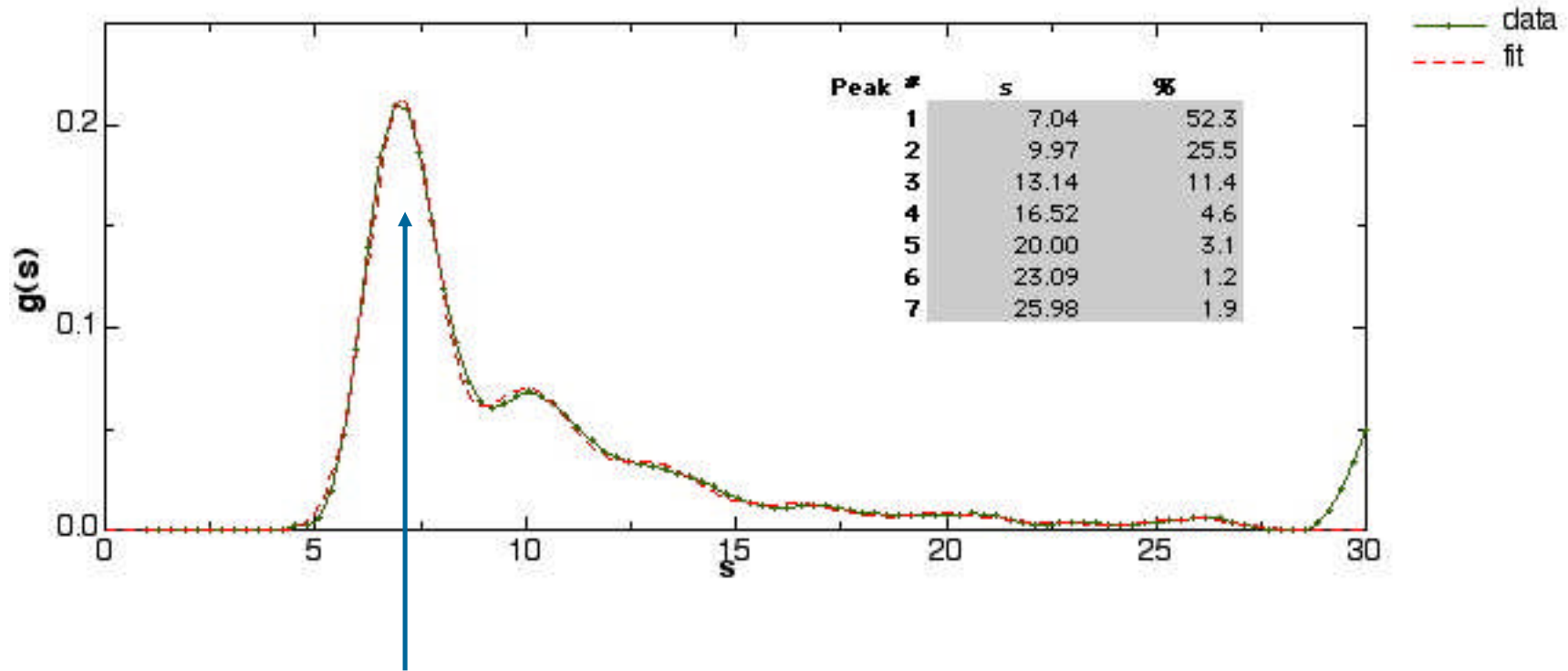
....on-line intrinsic viscosity measurement






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National Centre for
Macromolecular
Hydrodynamics



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