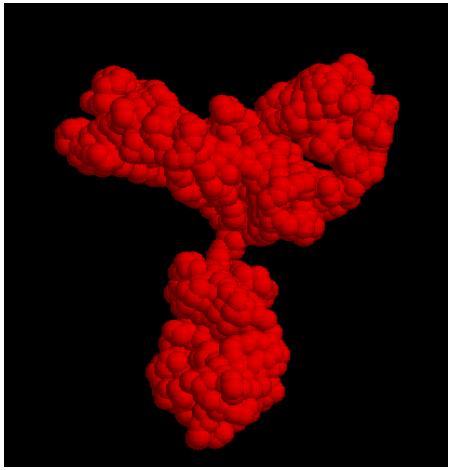
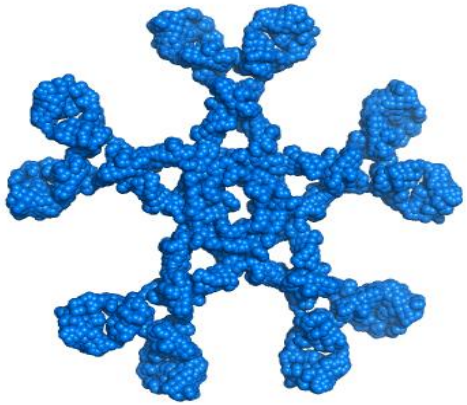
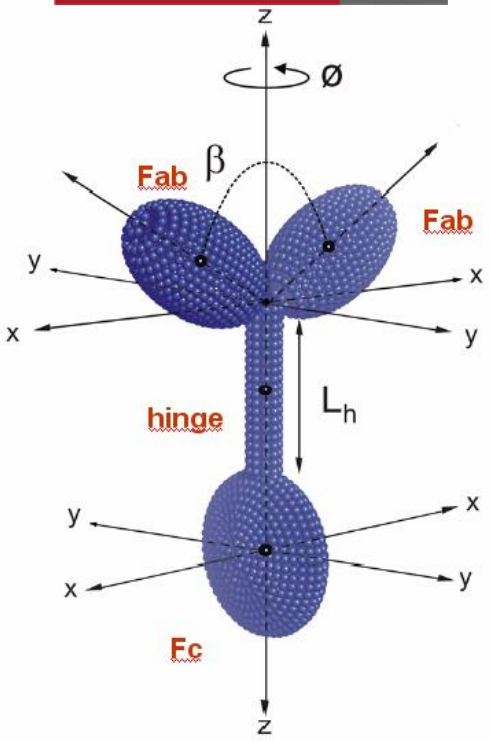
Dilute-solution properties of biomacromolecules as indicators of macromolecular structure and interactions

José García de la Torre,

Departament of Physical Chemistry
University of Murcia, Spain



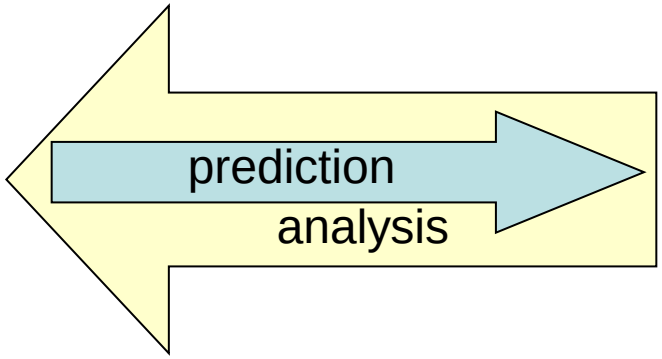




jgt@um.es
http://leonardo.inf.um.es/macromol

Our goals…

STRUCTURE:
** Size, shape
**Conformation, flexibility



Dilute-solution properties:
** Intrinsic viscosity $[\eta]$, other hydrodynamic properties: D, s, scattering Rg, $I(q)$, *etc*
** rotational/internal dynamics

Development of theoretical schemes, computational tools for:

- Prediction

- *RIGID* : (rigorous-HI rigid-body hydrodynamics) : HYDRO++, HYDROPRO, HYDROSUB, HYDRONMR, etc
- *FLEXIBE*: MONTEHYDRO (Monte Carlo), BROWFLEX (rigorous-HI Brownian dynamics simulation)

- Analysis: Single- , Multi-HYDFIT , HYDROFIT, …

(Also, experimental work: analytical ultracentrifugation, capillary viscometry, rheometry, multi-detection SEC, DLS+Z-potential., …)

Solution viscosity, η. Intrinsic viscosity, $[\eta]$

Concentration dependence of η ??? Series (polynomial) expansion :

$$\eta = A_0 + A_1 c + A_2 c^2 + A_3 c^3 + \cdots$$

Solvent viscosity: $A_0 = \eta_0$ $\qquad [\eta] = \dfrac{A_1}{\eta_0}$ $\qquad$ Huggins const.: $k_H = \dfrac{A_2}{\eta_0 [\eta]^2}$

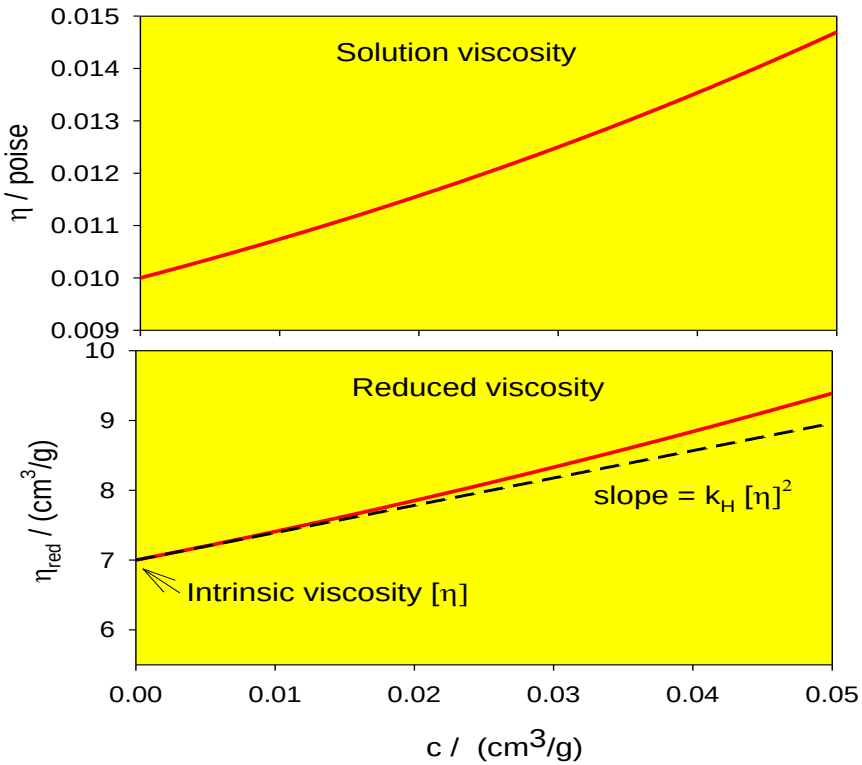
$$\eta = \eta_0 + \eta_0 [\eta] c + \eta_0 k_H [\eta]^2 c^2 + \ldots$$

Reduced viscosity $\eta_{red} = \dfrac{\eta - \eta_0}{\eta_0 c}$

$$\eta_{red} = [\eta] + k_H [\eta]^2 c + \ldots$$

Intrinsic viscosity $[\eta] = \lim_{c \to 0} \eta_{red}$

Some IgG1 antibody: $[\eta]$ = 7.0 cm^3/g ; $k_H \cong 0.8$



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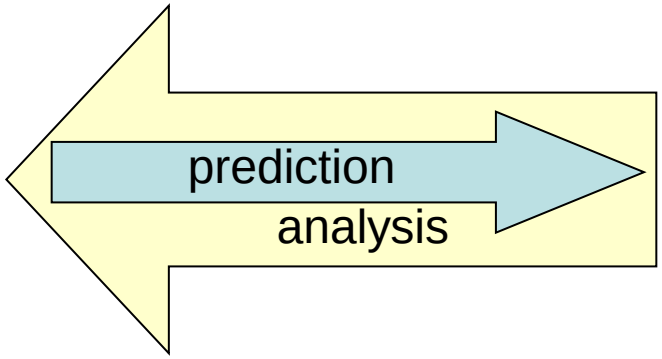

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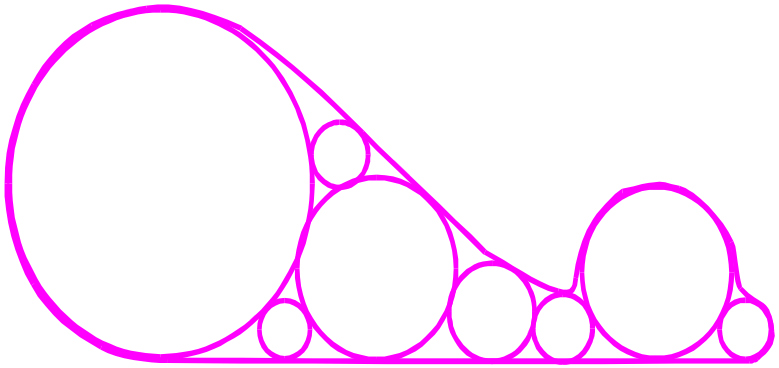
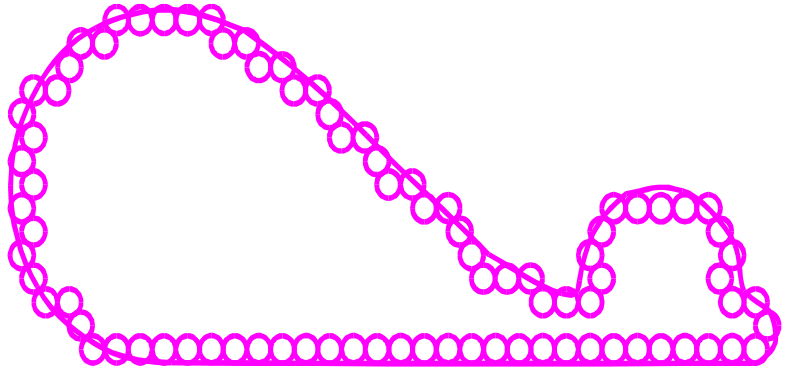
Rigid-body hydrodynamics: Bead and shell models

Particle modeled as an array of N spherical elements (beads). Size and shape of the particle is reproduced by the model.



Bead model
(in strict sense)



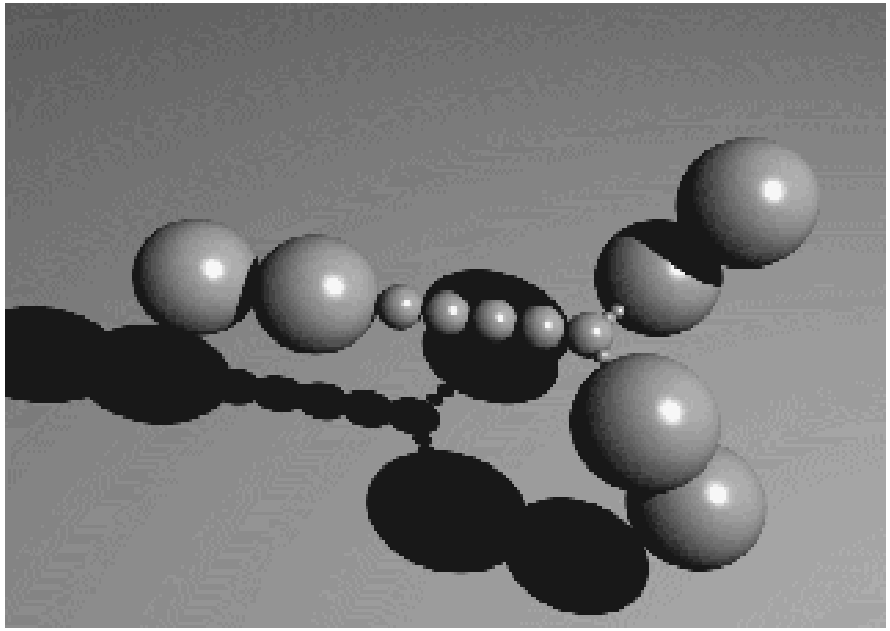
Shell model
bead-shell model

Program HYDRO++

TRV, c.a. 1980; Biophys. J, 1994; J. Phys. Chem, 2007.

Example: Human antibody molecule IgG3:

Davies…Burton et at, *Molec. Immunology* (1984)



```
Antibody Igg3 !Title
hydroigg3     !output files
hydroigg3.dat !Structural filename
293.          !Temperature, Kelvin
0.010         !Solvent viscosity
158000.       !Molecular weigth
0.760         !Specific volume
1.0           !Solution density
... (distances, scattering, covolume-A2,…)
*             !End of file
```

hydroigg3.dat →

```
1.E-07,     !Unit of length (10 A)
15,         !Number of beads
-12.9000   0.0000   0.0000   2.05
 -8.8      0.       0.       2.05
 -6.0      0.       0.       0.75
 -4.5      0.       0.       0.75
 -3.0      0.       0.       0.75
 -1.5      0.       0.       0.75
  0.       0.       0.       0.75
  0.5      0.       0.8660   0.25
  0.75     0.       1.2990   0.25
  0.5      0.      -0.8660   0.25
  0.75     0.      -1.2990   0.25
  1.9      0.      -3.2909   2.05
  3.95    -3.5507  -3.2909   2.05
  1.9      0.       3.2909   2.05
  3.95     3.5507   3.2909   2.05
```

Output of HYDROxxx programs: HYDRO++ for IgG3

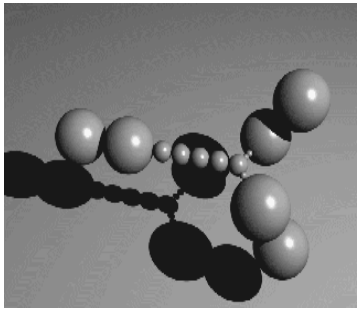
hydroigg3-res.txt

```
Translational diffusion coefficient:  3.757E-07 cm2/s
      Stokes (translational) radius:  5.711E-07 cm
                  Radius of gyration:  7.492E-07 cm
                              Volume:  2.256E-19 cm3
Rotational diffusion coefficient:  5.946E+05 s-1
 Harm. mean relax.(correl.) time:  2.803E-07 s
                Relaxation time (1):  4.395E-07 s
                Relaxation time (2):  3.503E-07 s
                Relaxation time (3):  3.343E-07 s
                Relaxation time (4):  2.058E-07 s
                Relaxation time (5):  2.057E-07 s
                Intrinsic viscosity:  9.823E+00 cm3/g
     Sedimentation coefficient:  5.850E+00 svedberg

                      ......
  Calculation of scattering form factor, P(q) vs h
                    . . . . . .
        Distribution of distances p(r)
                    . . . . . .
           Longest distance :  2.163E-06 cm

                        Covolume:  5.787E-18 cm3
```

Also:
* Scattering function
* Distances distribution
* SAXS, SANS
* covolume, 2nd virial



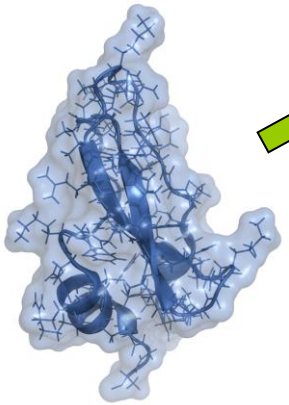
HYDROPRO

(Biophys. J. 2000; Biophys. J. 2012)

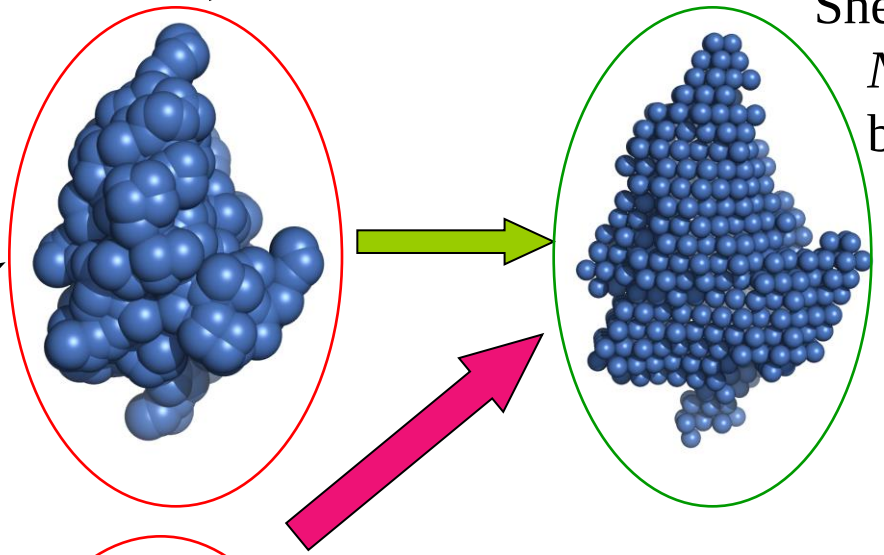
Atomic-level primary bead model,
$N = N_a = 533$ atoms

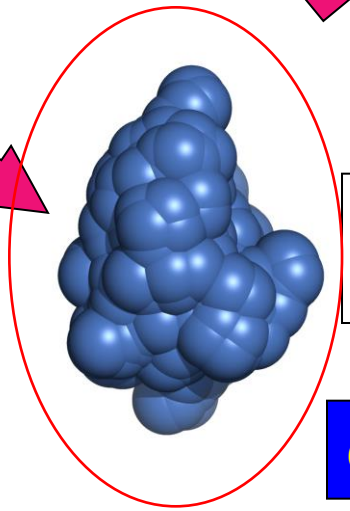
Shell model,
$N \cong 2000$
beads

HYDROPRO v7, 2005



BPTI (PDB: 1pti)





HYDROPRO v10, 2011

Residue-level primary bead model,
$N = N_r = 58$ residues

Computing time proportional to N^3

HYDROPRO:

** Tipical accuracy: usually better than:

❖ 4% for Rg, and translational properties (diffusion, D; sedimentation, s ; Stokes-hydrodynamic radius, a_T)

❖ 10% for intrinsic viscosity $[\eta]$ (4% for Einstenian-hydrodynamic radius, a_I)

** Computing time: a few seconds, in a simple PC

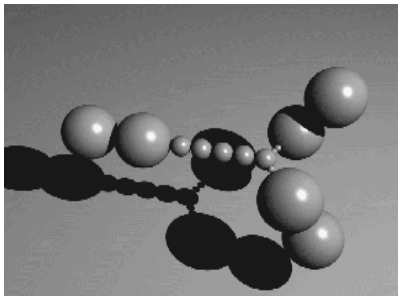
** Applicability:

❖ > 500 literature references to *Biophys J* 2000 (first version)
❖ > 40 literature references to *Biophys J* 2012, published Nov. 2012 (last version)

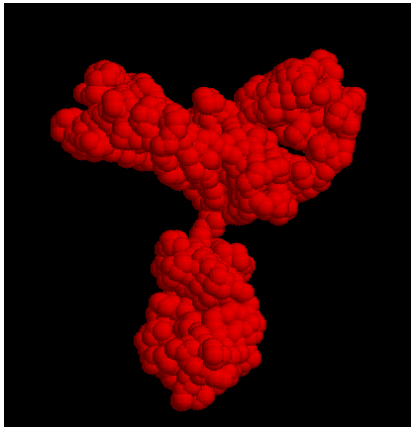
“Crystallohydrodynamics” approach: HYDROSUB

Harding, Garcia de la Torre et al, Biophys.Chem (2001)

A. Ortega A., J. García de la Torre, “ Crystallohydrodynamics of IgG“. In Roberts G. (Ed.) *Encyclopedia of Biophysics* (2013)



Too simple…

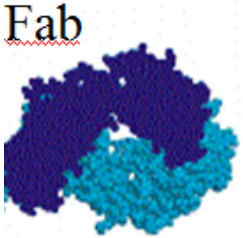


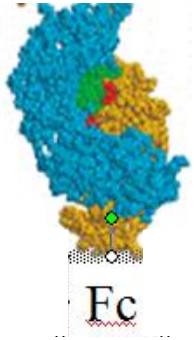
Too much detail…
… or not available.

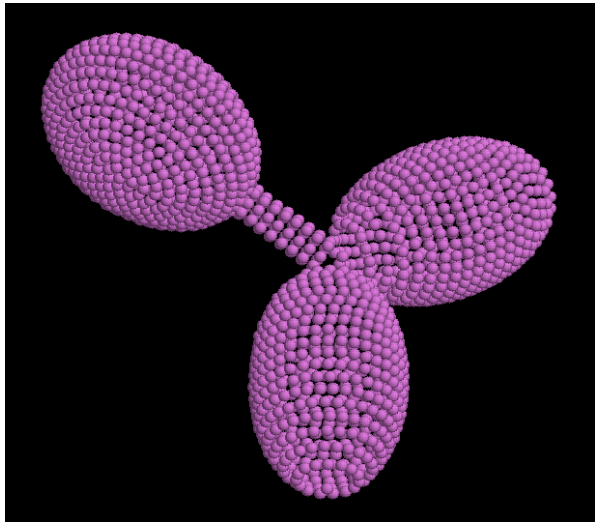
Subunits: represented as ellipsoids or cylinders

- Dimensions $(2a,2b)$ or (L, d) from solution properties: D, s, Rg, $[\eta]$… and simple theoretical expressions.

- Solution properties:
  - Experimentally determined
  - Predicted from available crystal structures (HYDROPRO)







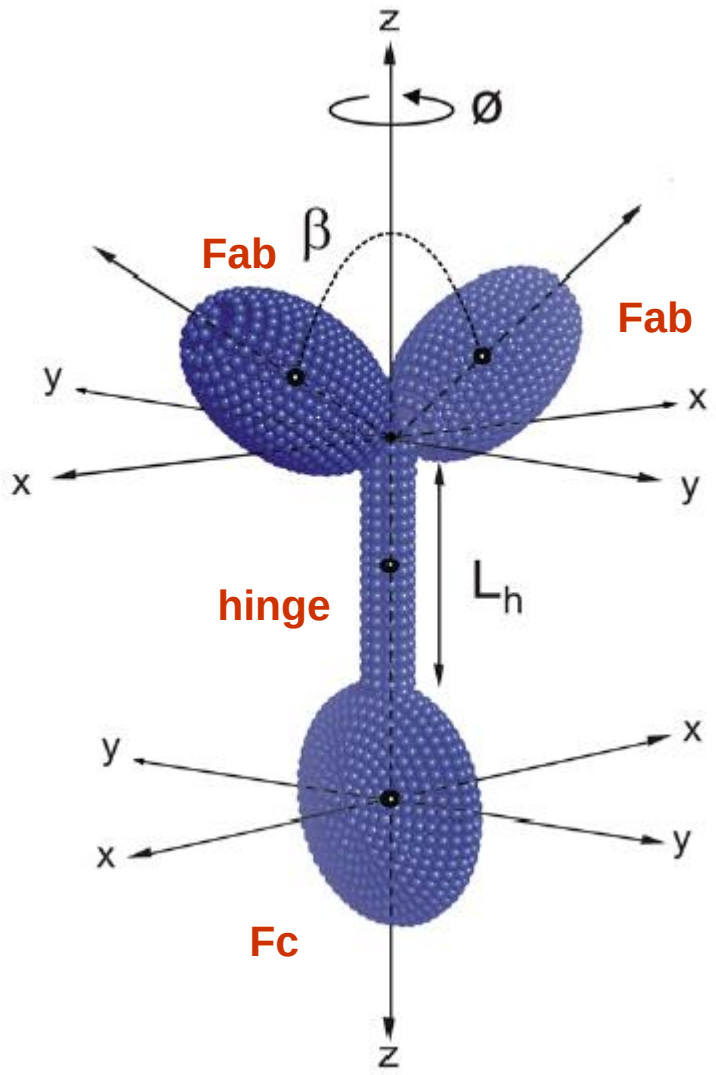
HYDROSUB – MultiSUB - HYDROFIT/ Antibodies (1)

a) Suite of HYDROxxx programs: Rigid particles of arbitary shape, bead/shell models. User-constructed model -- HYDROSUB

b) MULTIxxx : Programs for multiple model construction (changing structural parameters), produce HYDROxxx input files -- MULTISUB

c) HYDROFIT : Fit experimental data / Optimize structural parameters using calc./exptal.-deviation, target funcion Δ({params})

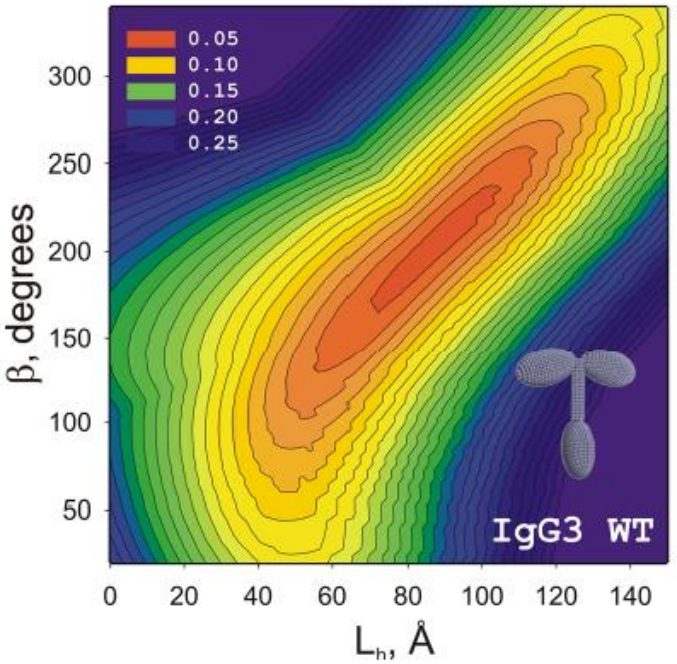
$$\Delta(L_h, \beta, \phi)$$



HYDROSUB / HYDROFIT Antibodies (2)

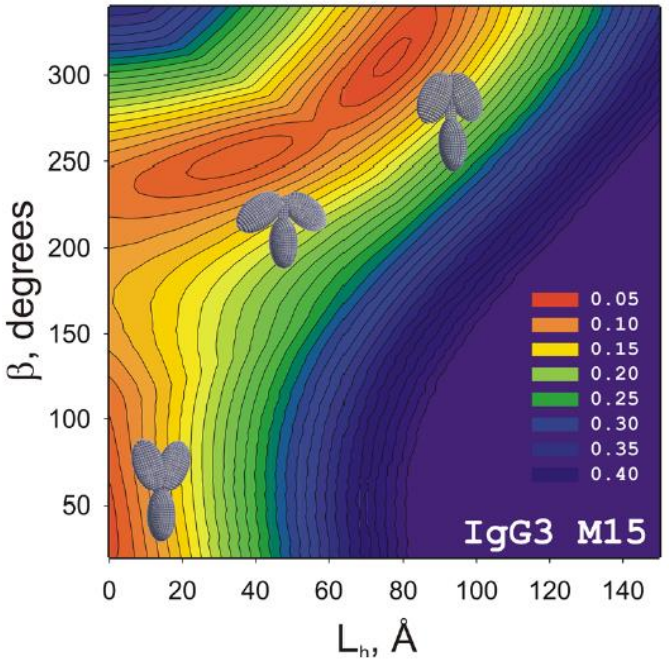
IgG3 - Exptal. Data: ***M, s, D, Rg, [η]*** Target: $\Delta(L_h, \beta)$

1. WT, wild type



L_h, ≈ 100 Å , β ≈ 180º
Long hinge

2. M15, mutant

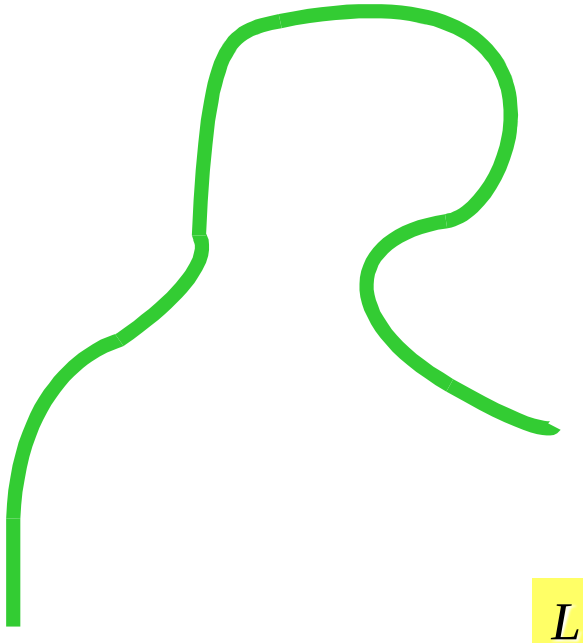


L_h, ≈ 0 , β ≈ 60º
hingeless

but not (physically) unique !!

Amorós, Hardíng and García de la Torre, *Eur. Biophys. J* (2010)

The wormlike chain



- Contour length, L
- Persistence length, P
- Hydrodynamic diameter, d

P and d are characteristic of the macromolecule; e.g. $P \cong 50$ nm, $d \cong 2$ nm for double-helical B-DNA

L is related to molecular weight: $L = M / M_L$, M_L =1950 Da/nm for DNA

Aspect / Conformation; Limits:

** $L>>P$, x = $L/P >>1$, very large: fully flexible coil

** $L<<P$, x = $L/P <<1$, very small: nearly rigid rod

Wormlike chains : Good fit for 147 data points !!!
DNA from 8 to 200 000 base pairs !!!

Amorós, Ortega and García de la Torre, *Macromolecules* (2012)

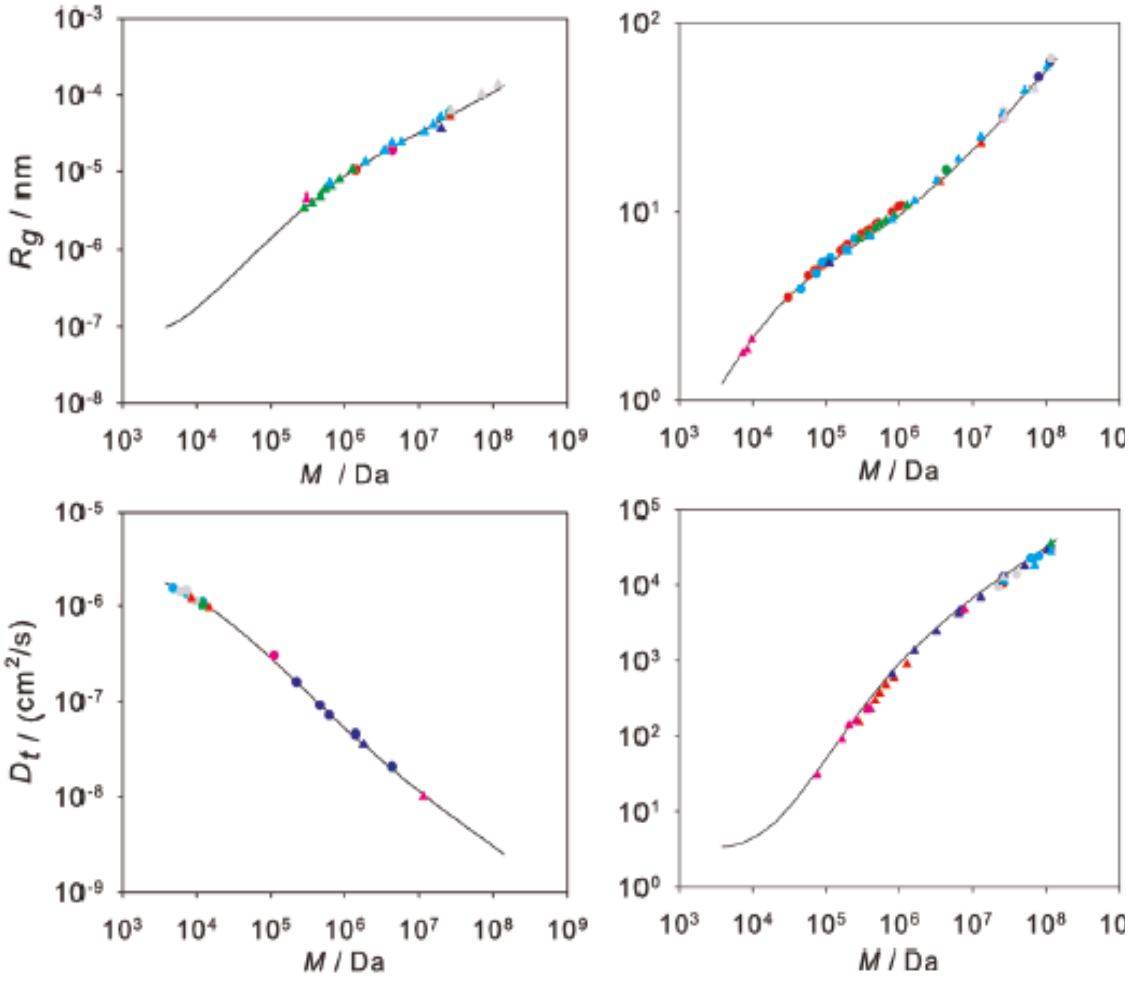
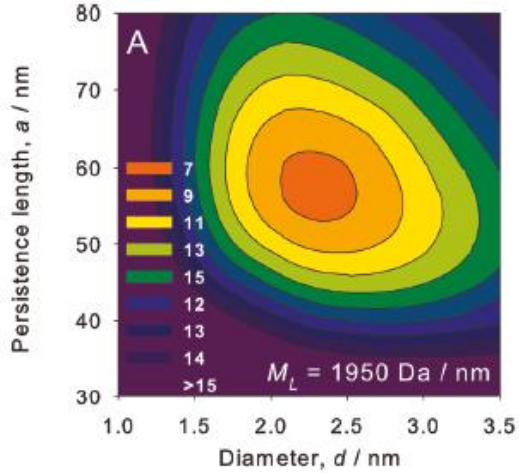
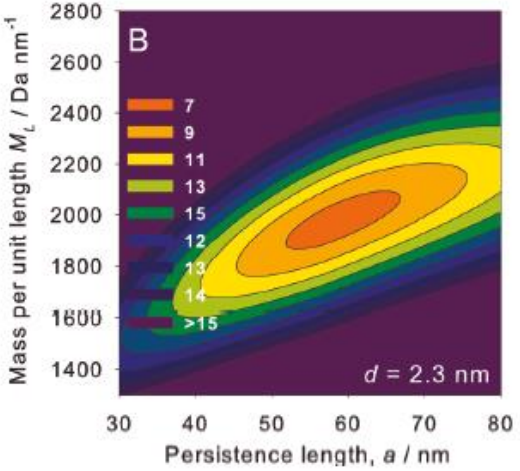


Figure 3. Plots of the experimental and calculated values, with
a = 56 nm, d = 2.3 nm, and M_L = 1950 Da nm^{-1} for the four prope
of DNAs.

Contour plots for
typical percent deviation



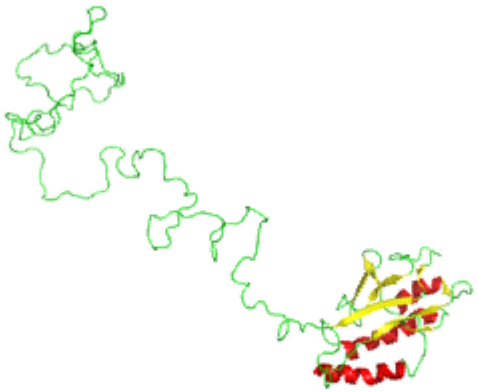


Intrinsically disordered proteins: ***ZipA***

D. Amorós, A.Ortega, J. Garcia de la Torre "Prediction of hydrodynamic and other solution properties of partially disordered proteins with a simple, coarse-grained model" *Journal of Chemical Theory and Computation*, 9, 1678-1685 (2013).

Structure

- Globular domain:
  140 aa's
- Flexible tail:
  183 aa's



Simulation vs. experimental results

Accurate exptal. sedimentation coeff. from SV :

***s* = 2.2 S**

G. Rivas, personal communicaton;

Oshashi *et al.* J. Bacteriology. 184 (2002)

Calculated:

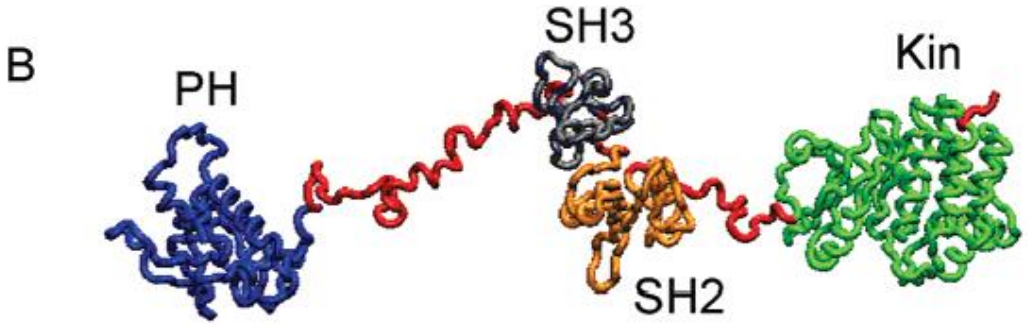
**** BROWFLEX Brownian dynamics**

**** MONTEHYDRO Monte Carlo**

***s* = 1.9 – 2.1 S**

ZipA.avi

Intrinsically disordered proteins Burton tyrosine kinase (BTK)



659 residues: 4 globular domains, 3 linkers
PH/169 – linker/50 – SH3/55 – linker/7 – SH2 – linker/21 – Kin/258

Experimental:
Márquez,…Svergun *EMBO J* (2003)

Results

Rg : exptal / calc -- 50 / 47 Å
s : exptal / calc -- 3.9 / 3.3 S

BTK.avi

Not-so… / semi… / dilute solutons

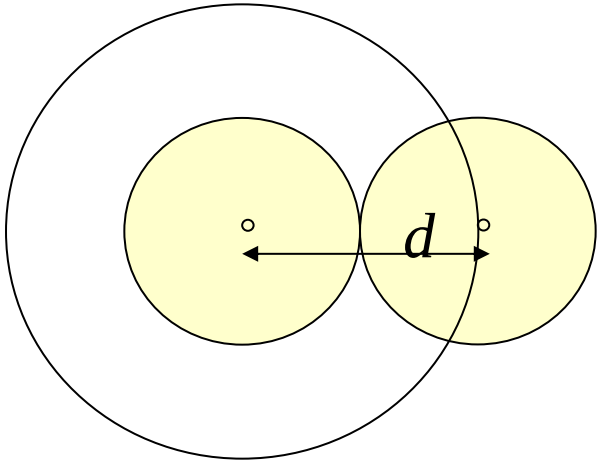
Molecular weight from non-very-dilute solutions

$$\frac{1}{M_{app}} = \frac{1}{M} + \xi B c + \cdots$$ 2nd virial coeff.: $B = B_{exc} + B_{elect}$

ξ: =1, Osmom. ; =2 LS, SE Abs. or Rayleigh; =4 SE Schlieren,...

No charge or high salt: $B \approx B_{exc} = \frac{u N_A}{2M^2}$ u : molecular covolume

Spherical particles: $u = \frac{4}{3}\pi d^3$



u, B_{exc} for rigid particles of arbitrary shape:
Calculated in all HYDROxxx programs.

Garcia de la Torre, Harding and Carrasco *Eur Biophys J* 1999.

Related to conc.-dependence of other properties

$$D_{app} = D(1+k_d c) \qquad \frac{1}{s_{app}} = \frac{1}{s}(1+k_s c) \qquad \frac{\eta_{sp}}{c} = [\eta](1+k_H c)$$

Harding and Johnson, *Biochem J* 1985 : $k_d = 2BM - \bar{v} - k_s$. What about k_H ???

Table 1 Values of k_H for several systems

System	k_H
Flexible chains	
In good solvents	0.2–0.4
In good solvents, theory	0.76
In theta solvents	0.4–0.7
In theta solvents, theory	0.6
Denaturated proteins	0.3–0.5
Oligomeric propylene glycol $M = 130$–940 g/mol	0.9–0.5
Poly(isobutylene) $M = 5{,}000$ g/mol	1.0
Globular particles	
Spheres (uncharged) theory	0.69–0.80
Silica spheres	1.0
Haemoglobin	0.81–0.93
Bovine serum albumin	0.89–1.42
Wormlike particles (from rod to coil)	
Xanthan polysaccharide	0.40–0.50
Schizophyllum commune polysaccharide	0.39–048
Poly(hexyl isocyanate)	0.39–0.59
Non-globular (multidomain) particles	
IgG antibody	0.74–0.84
Phycocianin (hexameric)	0.3
Myosin A	0.31
Fibrinogen	1.2
Aggregating, interacting macromolecules	
Folch-Pi protein	1.4, 8.4,...
IgG heteropolymer	0.6
Schizophyllan native/renaturated	0.2–0.6/1.6
Carboxymethylcellulose in acetonitrile-water-salts	2–25
Urease (polymerising)	40
Neurophysin	3
Silica rods	3.5–4.2
Chondroitin sulfates (possibly aggregating) $I = 0.15$ M	0.004–0.24
Rodlike particles	
Theoretical	0.75
Theoretical	0.4
Light meromyosin	0.64
Fd virus	1.54
Polyamide oligomers	1.0–1.7
Polyelectrolytes/Polysaccharides	
Alginate in varying ionic strength $I = 0.2$–0.005 M NaCl	0.35–0.55
Guar gum in monovalent ions/urea	0.5–0.6/0.9–1.3
Succinoglican 25/75 °C	0.34/0.44
Poly(styrene sulfonate) $M = 4.1 \times 10^5$ g/mol $I = 10^{-1} - 10^{-3}$ M	0.8–2.0
Poly(styrene sulfonate) $M = 1.1 \times 10^6$ g/mol $I = 10^{-1} - 3 \times 10^{-5}$ M	0.4–7.9
Chondroitin sulfates $I = 0.15$ M	0.004–0.24

Huggins constant, k_H

An extensive compilation of literature data:
Pamies,…and Garcia de la Torre, *Colloid Polym. Sci.* 2008

In the absence of
(a) Aggregation
(b) Strong electrostatic interaction,

$k_H \cong 0.3 - 1.2$ (spheres, rods, random coils,…)

Antibodies, $k_H \cong 0.8$

Conclusions:

** Dilute-solution props., particularly $[\eta]$, can be reliably calculated and/or analysed with our computational tools

** *c* - dependence of η of dilute solutions can predict aggregation behavior and abnormally high η at higher *c*

** The pharmacological relevance of the abnormally high η of biologics in moderately concentrated solutions should be a stimulus for new theoretical / computational developments

Thank you!

MIBio 2013

UNIVERSIDAD DE MURCIA

Group of Physical Chemistry of Macromolecules

Co-authors:

A. Ortega , D. Amoros, J.G.Hernández-Cifre, R. Pamies

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Germán Rivas (CIB, Madrid)

GOBIERNO DE ESPAÑA

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