Dynamics of a tethered polymer under shear flow

Rafael Delgado Buscalioni
Departamento de Ciencias y Técnicas Fisicoquimicas
Universidad de Educación a Distancia
Madrid

The film

Foreword and some applications

- The dynamics of tether chains under flow are relevant for many **technological applications**: stabilisation of colloidal suspensions, lubrication, chromatography, adhesion or drag reduction.

- Tethers are relevant in several **biological processes**, such as the ligand-receptor binding [Science **293**, 465, 2001] whereby the chains need to extend well beyond their equilibrium conformation to promote the adhesion of adjacent cells. Interestingly, the efficiency of this process depends more on the long time dynamics of the tethering chain via the occurrence of "rare" extended conformations, rather than on its equilibrium conformation. As shown in recent simulations, flow disturbances may also have a significant effect as the radius of gyration of the tether can dramatically increase if an external oscillatory force with a low enough frequency, is imposed in the normal-to-wall direction [J. Chem. Phys. **122**, 194912 (2005)].
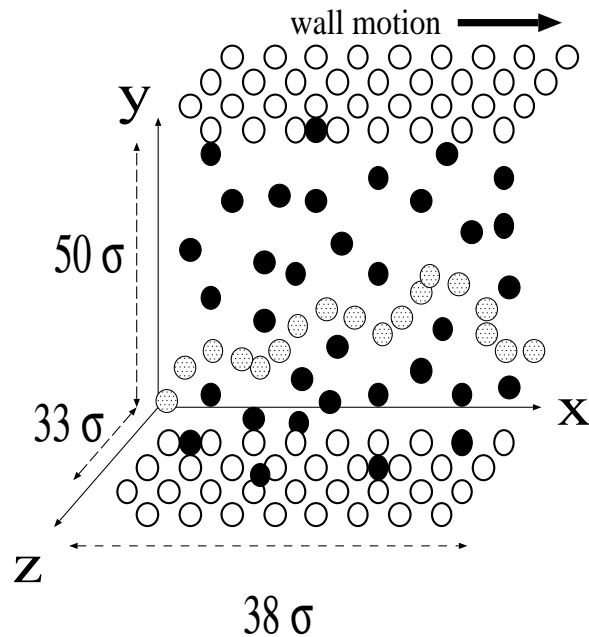
Foreword and some applications

- The ability to visualise individual polymers via fluorescent staining [Science **281**, 1335, 1998] has revealed that many non-trivial macroscopic properties of polymeric fluids can not be inferred from ensemble averages alone, but rather from the **individual chain dynamics** [Phys. Rev. Lett. **399** 564 (2000)]. Strikingly, the behaviour of individual chains in non-equilibrium can greatly differ from one to another, providing extremely rich dynamics when exposed to shear or [Nature **399** 564, 1999] elongational flow. One related fact is that the chain-flow interaction can be articulated over long characteristic times, much longer than the natural relaxation time of the chain.

- **Long characteristic times** are directly related to many properties of polymeric systems such as fluid memory or the strong resonance observed in polymer brushes [Nature **352**, 143, (1991)], single tethers [JCP, **243** 2001] or proteins [Science **309** 1096 (2005)] under periodic external perturbations.
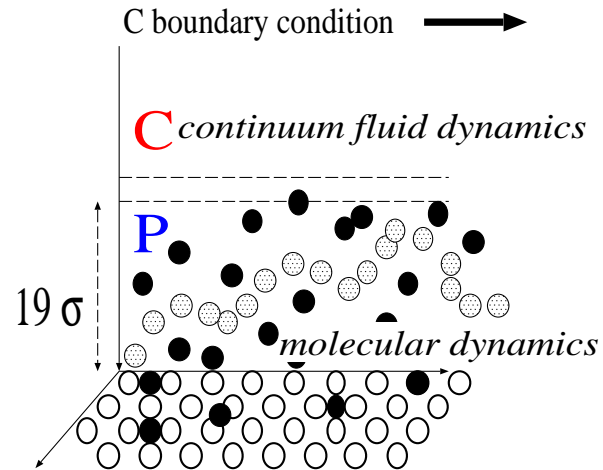
Outline of the talk

- Numerical modelling: methods.
- Non dimensional parameters.
- Structure of the polymer under shear
  - Probabily densities
  - Mean extensions in flow, gradient and vorticity direction
  - Variance of polymer extension
- Dynamics
  - Longest relaxation time
  - Short-time dynamics: Rouse modes analysis (brief)
  - Long-time dynamics: Memory and *cyclic dynamics*
- Conclusions and future work

Set-up and modelling

Molecular Dynamics

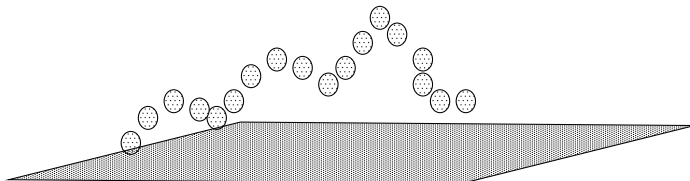wall motion →

y

50 σ

33 σ

z

38 σ

x

Hybrid model

C boundary condition →

C *continuum fluid dynamics*

P

19 σ

molecular dynamics

Flexible chain: LJ atoms with FENE potential, N=6
Explicit solvent (LJ). $\rho = 0.8\sigma^{-3}$, $T = 1.0\varepsilon/k_B$
Wall: two layers of LJ atoms, hexagonal lattice

Brownian Dynamics

Flexible chain: LJ atoms with FENE potential,
N = {20, 30, 60}
Implicit solvent, **free draining**
Wall: effective potential, $V\_wall \sim y^{-9}$

model I: **Molecular dynamics**

Equations of motion for N particles, Newtonian dynamics.

$$\dot{\boldsymbol{r}}_i = \mathbf{v}_i, \quad \dot{\mathbf{v}}_i = \mathbf{f}_i = -\nabla_{\boldsymbol{r}} V$$

Solved via standard molecular dynamics (MD) using the velocity-Verlet algorithm with a time step $\Delta t \simeq 10^{-3}\tau$, where $\tau = (m\sigma^2/\epsilon)^{1/2}$.

Solvent and monomers: Interparticle potential: $V_{LJ}(r) = \psi_{LJ}(r) - \psi_{LJ}(r_o)$
with $\psi_{LJ}(r) = 4\epsilon_f^{-1}\left[(\sigma/r)^{12} - (\sigma r)^6\right]$

Interaction with wall: $\epsilon_w = 1.7\epsilon_f$ (this guarantees no-slip).

Chain beads (bead-spring model of Kremer and Grest). A non-extensible chain formed by N monomers linked by the FENE potential $V_{FENE}(r_{ij}) = -\frac{1}{2}kR_0^2 \ln\left[1 - (r_{ij}/R_0)^2\right]$ (where r_{ij} is the distance between neighbouring beads, $R_0 = 1.5\sigma$, $k = 30\epsilon/\sigma^2$).

Standard LJ Units: The mass, length, energy and time: m, σ, ϵ and $\tau = \sigma\sqrt{m/\epsilon}$.

model II: The hybrid particle-continuum model
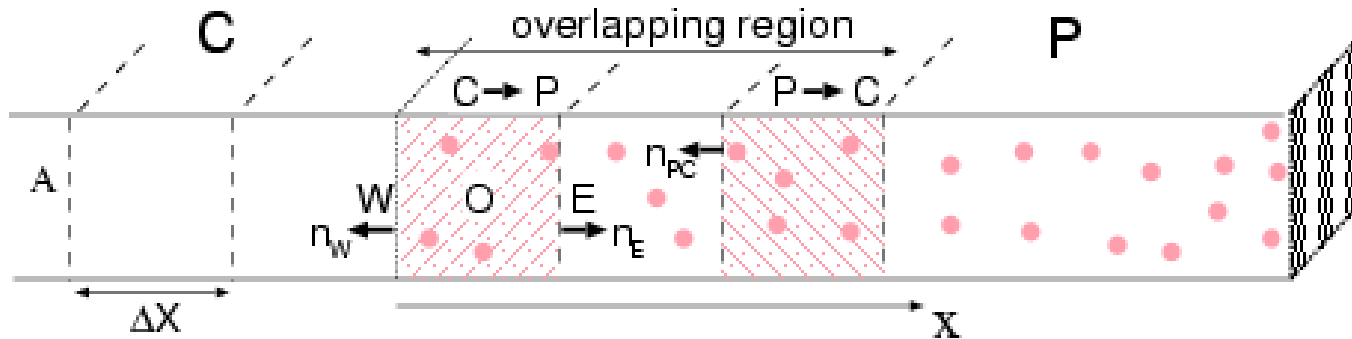
- The "P" region is solved via MD.
- The "C" region is solved via CFD (finite volume method).
- Both regions are dynamically coupled via the exchange fluxes of conserved quantities: mass, momentum, energy.

model III: Brownian dynamics

$$0 = \boldsymbol{f}_i^{FENE} + \boldsymbol{f}_i^{drag} + \boldsymbol{f}_i^{wall}(Y_i) + \boldsymbol{f}_i^{rand}$$

$$\boldsymbol{f}_i^{drag} = \xi\left(\boldsymbol{v}_f(\boldsymbol{R}_i) - \frac{d\boldsymbol{R}_i}{dt}\right), \quad \text{with } \boldsymbol{v}_f(\boldsymbol{R}_i) = \dot{\gamma}Y_i\boldsymbol{e}_x$$

$$\xi\frac{d\boldsymbol{R}_i}{dt} = \boldsymbol{f}_i^{FENE} + \xi\dot{\gamma}Y_i + \boldsymbol{f}_i^{wall}(Y_i) + \boldsymbol{f}_i^{rand}$$

- Solvent velocity: $\boldsymbol{v}_f$; friction coefficient ξ; **bead diffusion constant**: $D = (k_BT/\xi) = 0.42$ (in LJ units).
- Position of the ith bead: $\mathbf{R}_i = X_i\boldsymbol{e}_x + Y_i\boldsymbol{e}_y + Z_i\boldsymbol{e}_z$
- Random force: $\langle \mathbf{f}_i^{rand}\rangle = 0$ and $\langle \boldsymbol{f}_i^{rand}(t_1)\cdot\boldsymbol{e}_\alpha \boldsymbol{f}_j^{rand}(t_2)\cdot\boldsymbol{e}_\beta\rangle = 2k_BT\xi\delta(t_1 - t_2)\delta_{ij}\delta_{\alpha\beta}$
- **Polymer:** Flexible chain, number of beads $N = \{20, 30, 60\}$ beads.
- **Solvent:** Free draining (no hydrodynamic interactions)
- **Walls:** Mean potential dependent on normal-to-wall particle coordinate $\boldsymbol{f}_i^{wall} \sim Y_i^{-9}\boldsymbol{e}_y$.

Langevin equation for the Rouse modes

The bead positions of the tethered polymer: $\boldsymbol{R}_n, \ n = \{0, N\}$ with
<div>fixed end $\boldsymbol{R}_0(0) = 0$</div>
<div>free end $\frac{\partial \boldsymbol{R}(N)}{\partial n} = 0$</div>

The normal coordinates (linear approximation) $\hat{\boldsymbol{R}}_p(t)$ are, [Macromol.,**28**, 985 (1995)]

$$\hat{\boldsymbol{R}}_p(t) = (1/N) \sum_{n=1}^{N} \boldsymbol{R}_n(t) \sin\left(\frac{(p+1/2)\pi n}{N}\right)$$

The Langevin equation for the normal coordinates:

$$\xi \frac{d\hat{\boldsymbol{R}}_p}{dt} = \alpha_p^2 \boldsymbol{K}\hat{\boldsymbol{R}}_p + \xi\dot{\gamma}\boldsymbol{M}\hat{\boldsymbol{R}}_p + \boldsymbol{f}_p$$

- **The eigenvalues**: $\alpha_p^2 \equiv (p+1/2)^2\pi^2/N^2$.
- **The flow matrix** $\mathbf{M}$ corresponds to a shear flow: $\mathbf{M} = \mathbf{e_x}\,\mathbf{e_y}$.
- **The brownian force**: $\langle \mathbf{f_p} \rangle = 0$ and $\langle f_p^\alpha(t_1) f_q^\beta(t_2) \rangle = 2k_B T \xi \delta(t_1 - t_2) \delta_{pq} \delta_{\alpha\beta}$
- **The friction coefficient**: $\xi = 3\pi\eta_s b$ (solvent viscosity $= \eta_s$)
- **The effective spring constant**: $K_{\alpha\beta} = k_\alpha \delta_{\alpha\beta}$.

For an strectched polymer [PRL,**82**, 3548 (1999)] $k_x = (\partial F(x)/\partial x)_{x=X}$ Along the flow direction
     $k_t = F(X)/X$ Along the transversal directions .

Longest relaxation time under no mean flow ($\dot{\gamma} = 0$).

Numerical results for $N = 60$

	MD and Hybrid	BD-EV	BD
$\tau_0(0)$	$(2000 \pm 500)\tau$	$(4000 \pm 200)\tau$	$(1200 \pm 200)\tau$

Theoretical estimation (free draining limit)

$$\tau_p(0) = \frac{\xi}{k}\alpha_p^{-2} = \tau_{blob}\frac{N^2}{\pi^2(p+1/2)^2}$$

with $\tau_{blob} = \xi/k$, also $\tau_{blob} = \eta_s b^3/(k_B T) \simeq 1.75$ (where $b = 0.961$ is the bead-to-bead distance).

(at $\dot{\gamma} = 0$ the spring is linear: $\partial F/\partial x = F/x$. So $k = k_x = k_t$.)

For the 0_{th} mode: $\tau_0(0) = \tau_{blob}\frac{4N^2}{\pi^2} = 2261$

Non-dimensional parameters

Dimensional variables

- Shear (Couette) Flow: $\boldsymbol{v} = v_x(y)\boldsymbol{e}_x$. Shear rate, $\dot{\gamma} = \frac{dv_x}{dy}$.

- Position of the ith bead: $\mathbf{R}_i = X_i\boldsymbol{e}_x + Y_i\boldsymbol{e}_y + Z_i\boldsymbol{e}_z$
- Maximum elongation $\boldsymbol{X}(t) = \max\{X_i\} - \min\{X_i\}$, etc...
- Contour length: $L = Nb$, where the bead-to-bead distance is $b = 0.961$ (for FENE potential)
- Longest relaxation time of the polymer $\tau_0(\dot{\gamma})$.

Non-dimensional variables and parameters

- Extensional parameter: $\epsilon \equiv 1 - X/L$
- Strain (shear rate $\times$ time): $\dot{\gamma}\,t$
- **Weissemberg number** : $\mathrm{Wi} = \dot{\gamma}\tau_0(0)$ (where $\tau_0(0)$ is the longest relaxation time at $\dot{\gamma} = 0$)

The governing parameter: Weissemberg number

- The dynamics of a single polymer under flow is determined by the Weissenberg number, $\mathrm{Wi} \equiv \dot{\gamma}\tau_0(0)$, which gives the ratio of the polymer relaxation time at equilibrium $\tau_0(0)$ and the flow characteristic time $1/\dot{\gamma}$.

- At $\mathrm{Wi} > 1$ the flow start to stretch the polymer average configuration because the fluid elements deform faster than the polymer can possibly relax.

- The **longest polymer relaxation time** was estimated from simulations by fitting the autocorrelation of the normal coordinate in flow direction $\hat{X}_0 \equiv \hat{\mathbf{R}}_0 \cdot \mathbf{i}$ to a single exponential function (similar relaxation times, within error bars, were obtained from the decorrelation time of the end-to-end vector.)

Structure

Probability densities: monomers spatial distribution

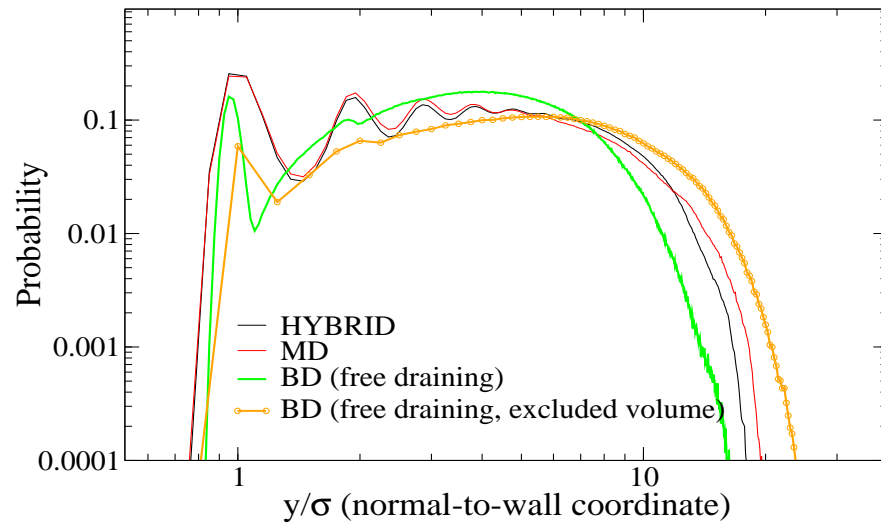| Flow direction	Gradient direction	Vorticity direction
x	y	z
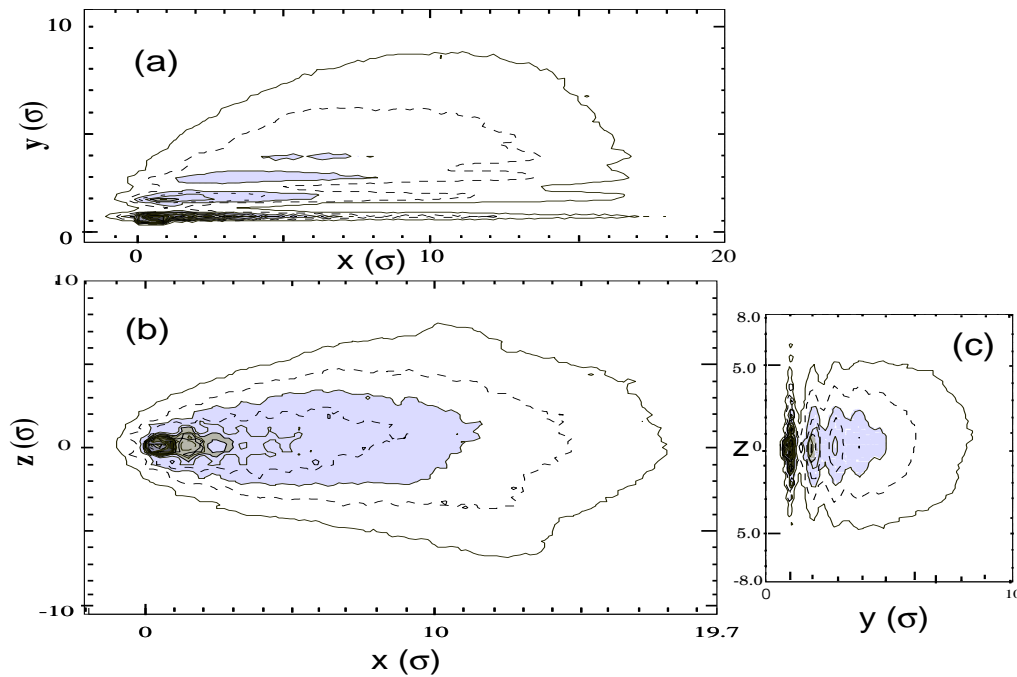
Marginal probabilities of finding any monomer with x,y and z coordinates

Results for $\dot{\gamma} = 0.001$ (Wi=2.0).
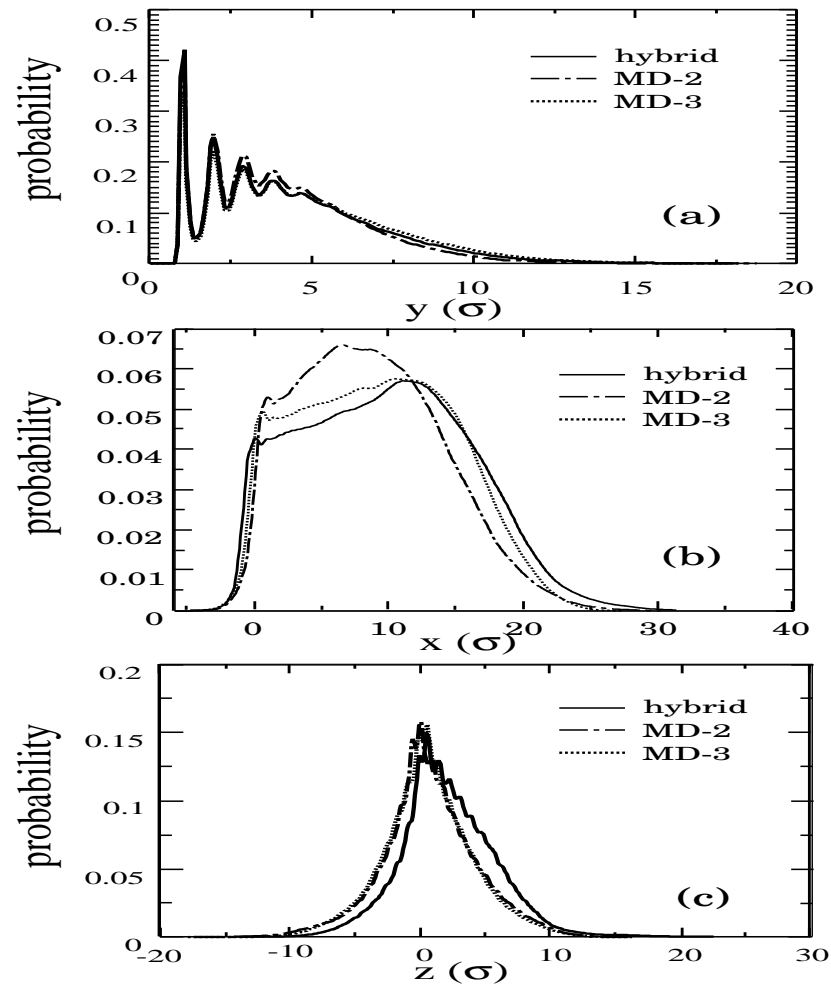
Theoretical analysis: Mean and variance
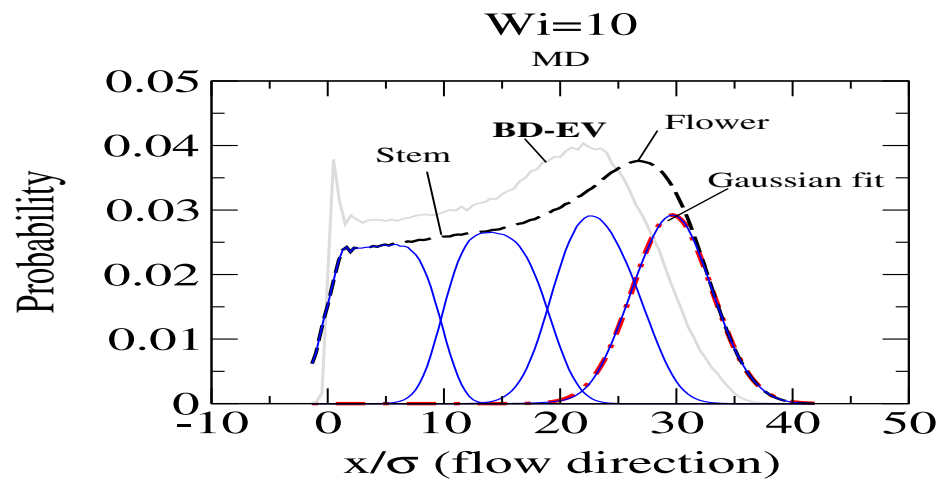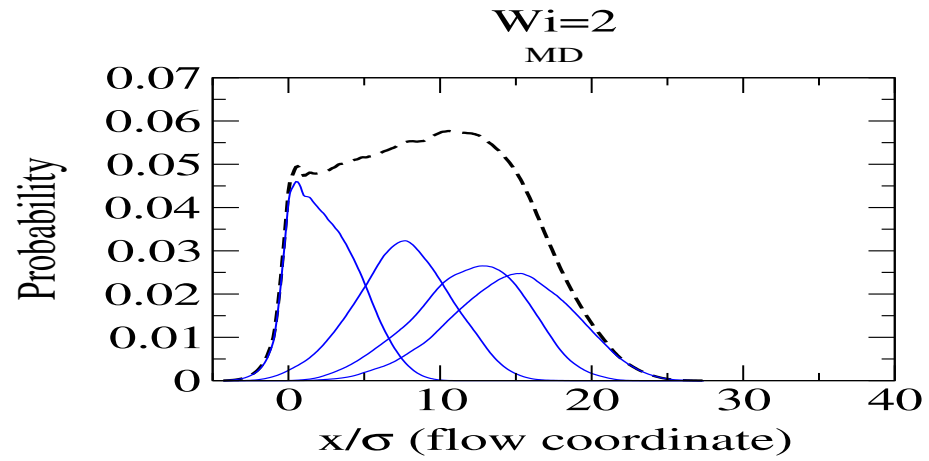
Struture at equilibrium (no flow)

Struture under shear flow

Wi=2
MD

Wi=10
MD

Theoretical scalings: Force *versus* extension $F(X)$.

- Worm-like chain (WLC); semi-flexible (DNA) [Macromol. **28**, 3548 (1995)]:

$$F(X) = \frac{k_B T}{2b} \left\{ \left(\frac{L}{2X} \right)^2 - \frac{1}{4} + X/L \right\}$$

- Freely jointed chain (FJC); flexible:

$$F(X) = -3k_B T b \left\{ \frac{(X/L)}{1 - \left(\frac{X}{L} \right)^2} \right\}$$

.

For an streched polymer, $X/L \sim 1$ and $\epsilon \equiv 1 - X/L \sim 0$.

$$\text{WLC} \quad F_{WLC} \sim \epsilon^{-2}$$

$$\text{FJC} \quad F_{FJC} \sim \epsilon^{-1}$$
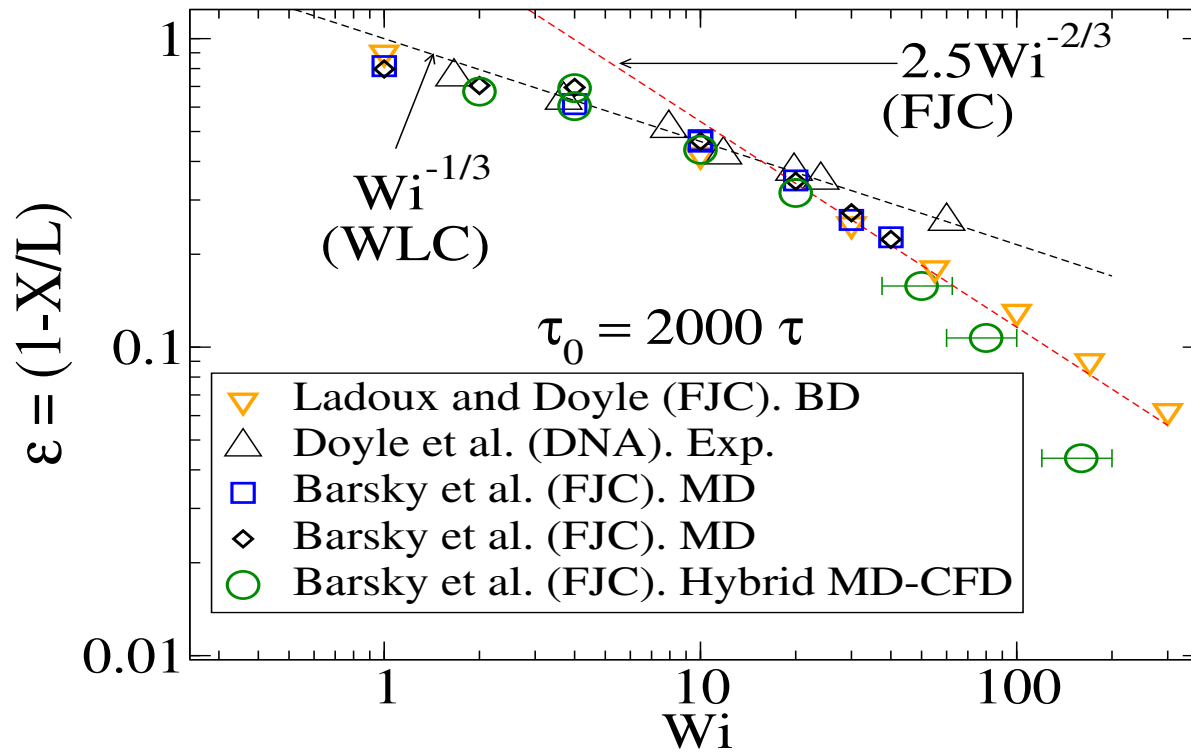
Theoretical scalings: Polymer elongation.

Transversal fluctuations of the chain	Spring energy $\sim$ Thermal energy
	$\frac{1}{2}k_t Y^2 \quad \sim \quad k_B T$

Extension in the flow direction	Spring force $\sim$ Drag force
	$F \quad \sim \quad \xi \dot{\gamma} Y$

Effective spring constant	$k_t = F/X$

Theoretical scalings

Worm-like-chain -WLC- (semiflexible)	$(F \sim \epsilon^{-2})$	$Y \sim \epsilon^{-1}$	$\epsilon \sim \mathrm{Wi}^{-1/3}$
Freely jointed chain -FJC- (flexible)	$(F \sim \epsilon^{-1})$	$Y \sim \epsilon^{-1/2}$	$\epsilon \sim \mathrm{Wi}^{-2/3}$

Polymer extension in flow direction: numerical and experimental results

$$\epsilon = \begin{cases} \mathrm{Wi}^{-1/3} & \text{for Wi} < 10 \\ 2.2\mathrm{Wi}^{-2/3} & \text{for Wi} > 10 \end{cases}$$

Theoretical scalings: Polymer elongation in transversal directions (II).

Step random walk argument: Hatfield and Quake, PRL, 82, 3548 (1999)

Total number of steps	N
Steps in the longitudinal (flow) direction	$N\,X/L$
Steps in the transversal direction	$N\,(1 - X/L) = N\epsilon$
Hence, the radius of giration in the transversal direction is	$R_g^{(t)} = N^{1/2}\epsilon^{1/2}$.

This simple argument works well in the **vorticity direction**
(neutral direction) but not so well in gradient direction.

Theoretical scalings: Polymer elongation in gradient direction (III).

Scalings based on the analogy with the Graetz-Leveque analysis for thermal boundary layers

- Diffusion-advection balance:

$$D_{eff}\frac{\partial^2 Y}{\partial y^2} \simeq v_x \frac{\partial Y}{\partial x}$$

- Order of magnitude analysis: $\frac{\partial^2 Y}{\partial y^2} \sim \frac{\langle Y \rangle}{\langle Y^2 \rangle} \sim \langle Y \rangle^{-1}$ and $v_x \frac{\partial Y}{\partial x} \sim \dot{\gamma} \langle Y \rangle^2 / L$

- Thus $\langle Y \rangle \sim (D_{eff}/\dot{\gamma})^{1/3}$

Different scalings for the *effective* bead diffusion D_{eff} are proposed, leading to small differences in $\langle Y \rangle$.

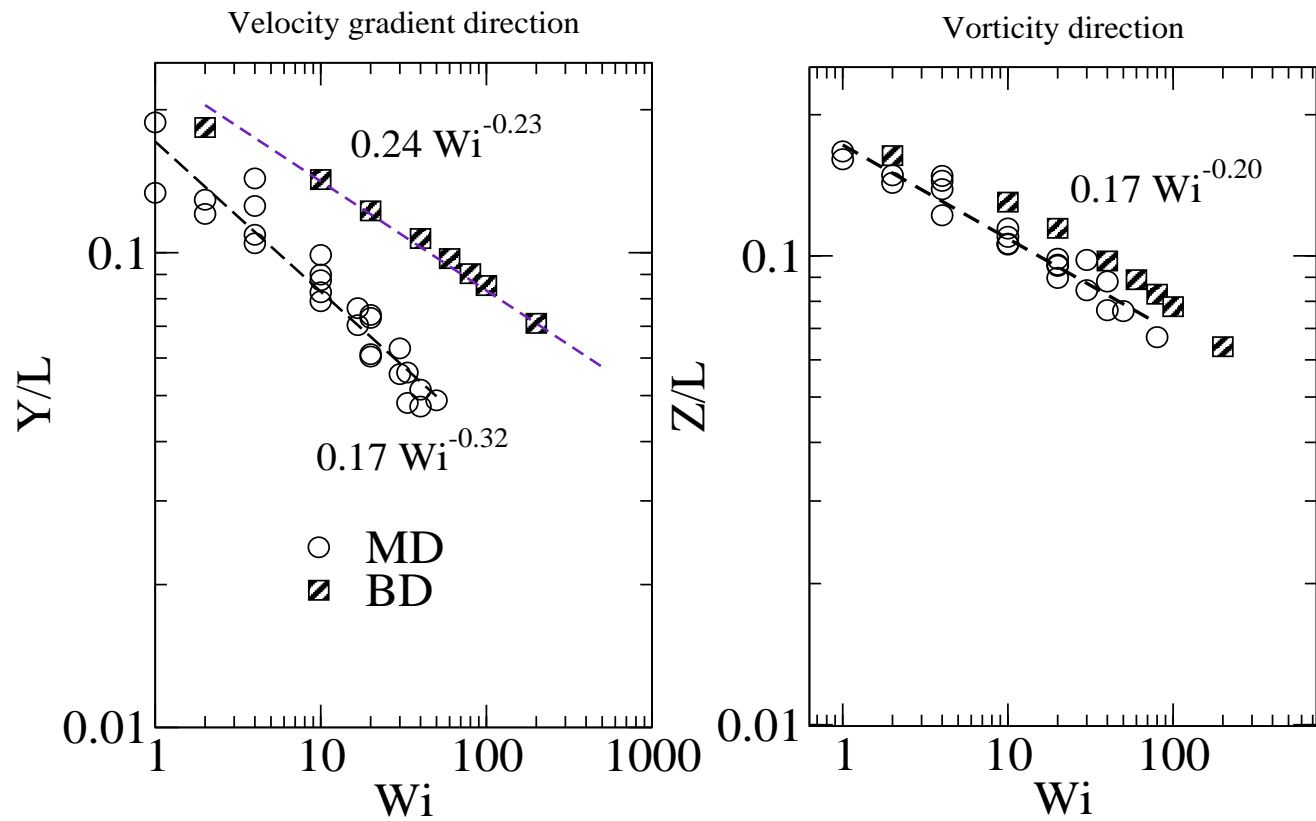|		
$D_{eff} \sim D$	$Y \sim \dot{\gamma}^{-1/3}$	The bead diffusion near the wall is the same as in bulk. [J.Chem.Phys. **106**, 7752, 2002]
$D_{eff} \sim Y^{-2/3}$	$Y \sim \dot{\gamma}^{-3/11}$	The effective diffusion depends on the gradient coordinate, and was calculated from BD simulations [J.Fluid.Mech. **334**, 251, 1997]
$D_{eff} \sim D/Y$	$Y \sim \dot{\gamma}^{-1/4}$	The column of beads above one monomer needs to be displaced before one monomer can possibly move this direction [J.Rheol. **44**, 713, 2000]

The above scalings depends on hydrodynamic interactions, on the chain flexibility, on the excluded volume? Not yet clear.

However differences are small and the Graetz-Leveque scaling provides good results.

Velocity gradient direction

$0.24 \, \mathrm{Wi}^{-0.23}$

$0.17 \, \mathrm{Wi}^{-0.32}$

○ MD
◪ BD

Y/L

Wi

Vorticity direction

$0.17 \, \mathrm{Wi}^{-0.20}$

Z/L

Wi

Experiments with DNA strands, free flexible chains in shear flow:

$\langle Y \rangle \sim \mathrm{Wi}^{-0.26}$ [Texeira *et al.* Macromolecules **38** (2005)].

$\langle Y \rangle \sim \mathrm{Wi}^{-0.30}$ [Schroeder *et al.* Macromolecules **38** (2005)] ($\mathrm{Wi} > 100$)

$\langle Z \rangle \sim \mathrm{Wi}^{-0.17}$ [Schroeder *et al.* Macromolecules **38** (2005)] (random growth theory: $\mathrm{Wi}^{-0.166}$)

Variance of the chain exension.

Experimental facts: shear driven enhanced fluctuation [Doyle *et al.* PRL (2000)].
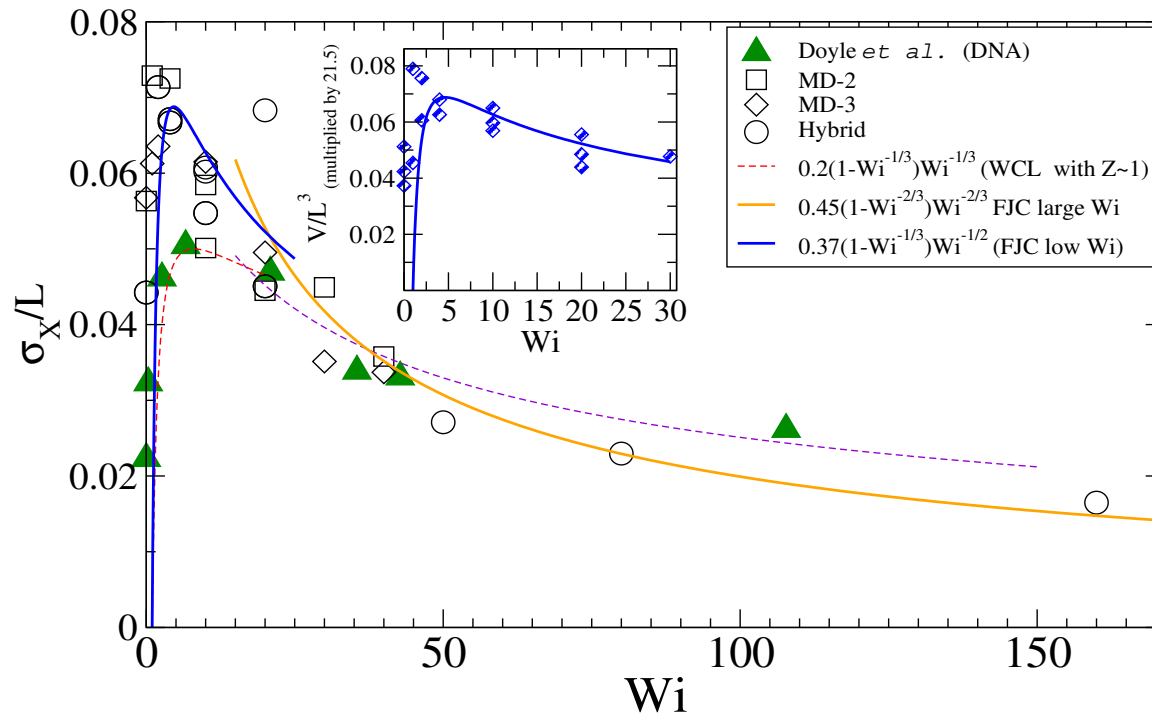
Theoretical scalings: variance of the chain exension

Hypothesis: Fluctuation in polymer extension scale like the polymer accesible volume [R.D-B & P Coveney, Physica A (2005)]

- Accesible volume $= \langle X \rangle \langle Y \rangle \langle Z \rangle$
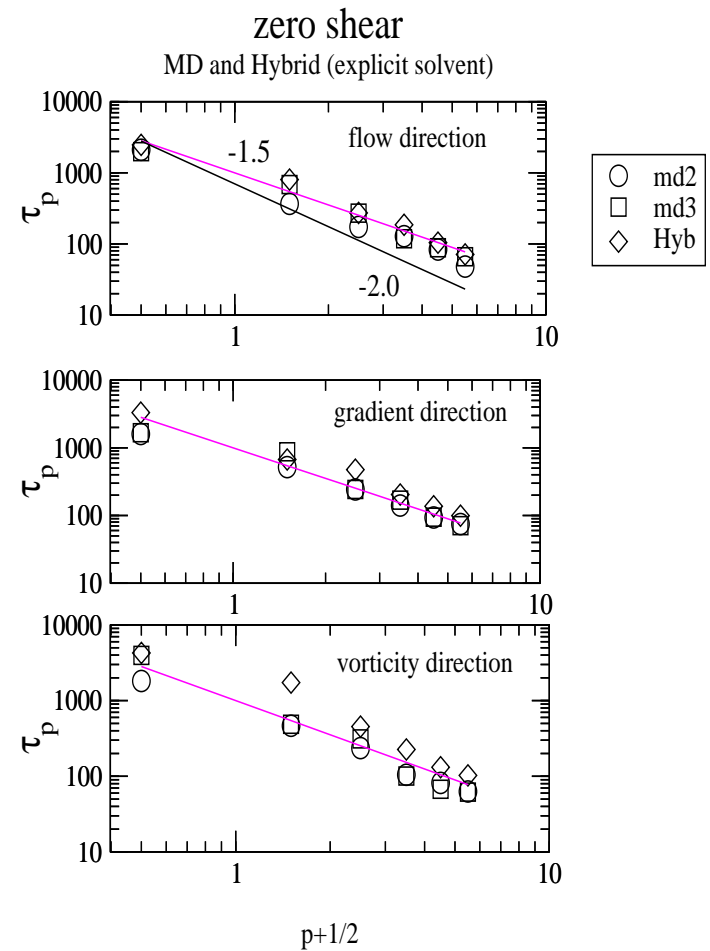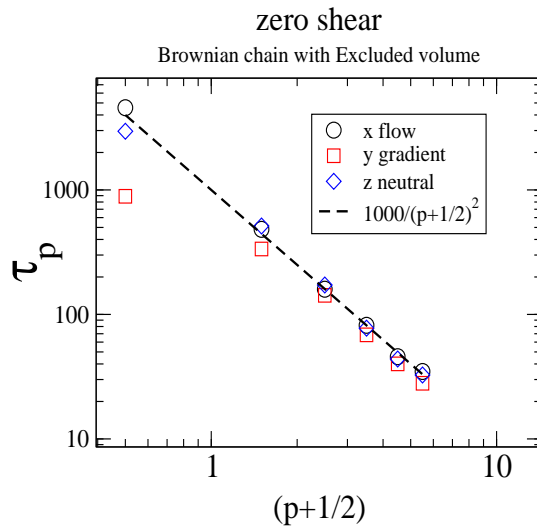- Standard deviation of chain extension: $\sigma_X = \sqrt{\langle X^2 \rangle - \langle X \rangle^2}$
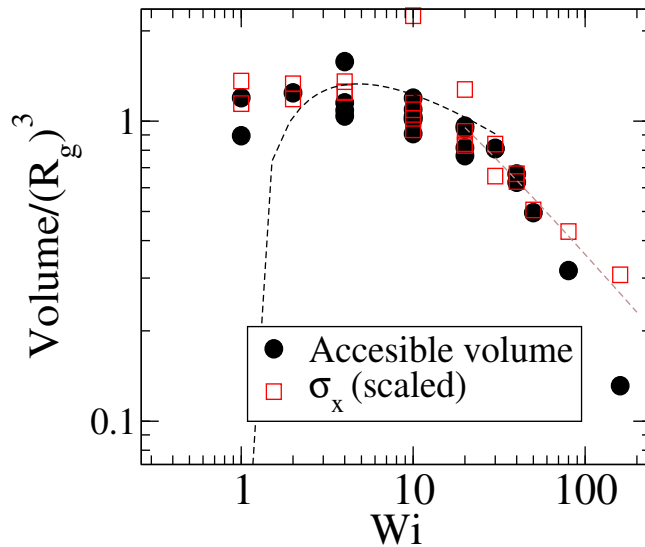
Dynamics

Polymer dynamics at short-time scales: relaxation of Rouse modes

BD-EV (FD): $\tau_p \sim (p+1/2)^{-2}$ MD: $\tau_p \sim (p+1/2)^{-1.6}$

Dynamics: order of magnitude (OM) analysis

Objetive: The scaling for the longest relaxation time under flow $\tau_0 \sim \epsilon^{\alpha_\tau}$.

Method: The OM analysis is based on force balance. Forces are estimated from the Langevin equation. All relevant variables are expressed in terms of the (small) elongation parameter ϵ, assuming a power-law form, e.g. $X \sim \epsilon^{\alpha_X}$.

Viscous force	$\xi \hat{R}_0 / \tau_{0,R} \sim \quad \epsilon^{\alpha_R + \alpha_\xi - \alpha_\tau}$
Brownian force	$(k_B T k_r)^{1/2} \sim \quad \epsilon^{\alpha_k/2}$
Spring force	$k_r \hat{R}_0 \sim \quad \epsilon^{\alpha_R + \alpha_k}$
Flow drag (along x direction)	$\xi \dot{\gamma} \hat{Y}_0 \sim \quad \epsilon^{\alpha_\xi + \alpha_{\dot{\gamma}} + \alpha_Y}$

Exponents assumed for a flexible chain at large Wi ($\epsilon << 1$)

$\alpha_{\dot{\gamma}} = -3/2$	$\alpha_\xi = 0$ (assumption)
$\alpha_k = -2$ for flow direction	$\alpha_k = -1$ for transversal directions
$\alpha_X = 0$	$\alpha_Z = 1/2, \; \alpha_Y = 1/2 \;\boxed{?}$

Relaxation time at zero shear rate, $\dot{\gamma} = 0$

Spring force balances Brownian force.

$$\tau_p(0) = \frac{\xi}{k}\alpha_p^{-2} = \tau_{blob}\frac{N^2}{\pi^2(p+1/2)^2}$$

where $\tau_{blob} = \xi/k$, also $\tau_{blob} = \eta_s b^3/(k_B T) \simeq 1.75$.

Note: at $\dot{\gamma} = 0$ the spring is linear: $\partial F/\partial x = F/x$. So $k = k_x = k_t$.

Longest relaxation time: for the 0_{th} mode ($p = 0$).

$$\tau_0(0) = \tau_{blob}\frac{4N^2}{\pi^2} = 2261$$

Result from simulations, $\tau_0(0) = 2000 \pm 500$.

Relaxation time under shear flow: the flow direction

Balance of viscous and drag force: time scale for convection-induced chain stretching.

$$\xi \tau_0^{-1}(\dot{\gamma}) \langle \hat{X}_0 \rangle \simeq \xi \dot{\gamma} \langle \hat{Y}_0 \rangle$$

This leads to,

$$\frac{\tau_0(0)}{\tau_0(\mathrm{Wi})} \sim \mathrm{Wi} \frac{\langle \hat{Y}_0 \rangle}{\langle \hat{X}_0 \rangle}$$

At large Wi,

$$\tau_0(\mathrm{Wi}) \sim (\mathrm{Wi} \langle Y \rangle)^{-1}$$

.

WLC	$Y \sim \epsilon$	$\epsilon \sim \mathrm{Wi}^{-1/3}$	$\tau_{0,x} \sim \mathrm{Wi}^{-2/3}$
FJC	$Y \sim \epsilon^{1/2}$	$\epsilon \sim \mathrm{Wi}^{-2/3}$	$\tau_{0,x} \sim \mathrm{Wi}^{-2/3}$

Relaxation time in the transversal directions, Y and Z

Balance of viscous and Brownian forces

$$\frac{\xi R_0}{\tau_0} \sim (k_B T k_t)^{1/2}$$

With $R_0 = \{Y_0, Z_0\}$.

For the FENE potential (FJC): $k_t = F/X \sim \epsilon^{-1}$.

$$\left| \text{ Vorticity and gradient direction (z) } \right| Z, Y \sim \epsilon^{1/2} \left| \tau_{0,Z} \sim \epsilon \sim \text{Wi}^{-2/3} \right|$$

# Longest relaxation times in different directions: *"relaxation anisotropy"*
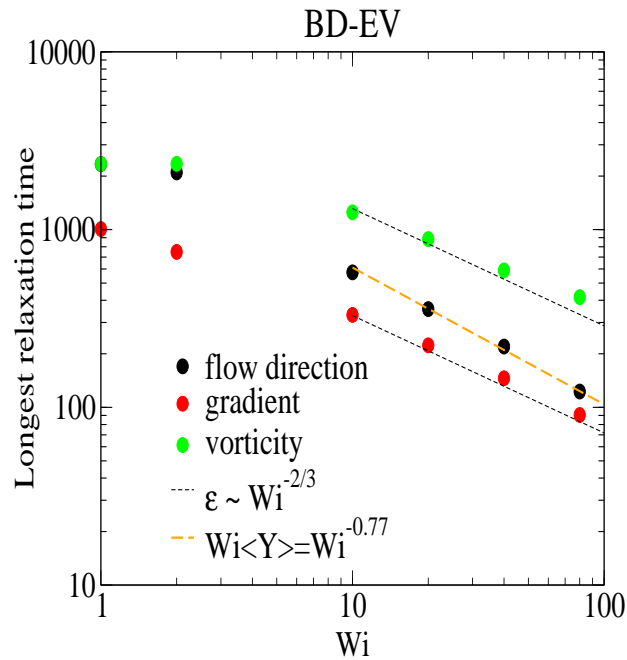
	Flow	Gradient	Vorticity direction
MD	$3600\mathrm{Wi}^{-0.7(1)}$	$2400\mathrm{Wi}^{-0.5(5)}$	$4600\mathrm{Wi}^{-0.5(0)}$
Theory (OM)	$\mathrm{Wi}\langle Y\rangle \sim \mathrm{Wi}^{-0.68}$	$\epsilon \sim \mathrm{Wi}^{-0.66}$	$\epsilon \sim \mathrm{Wi}^{-0.66}$

Long-time dynamics: the cyclic motion A recent experimental study on the dynamics of individual DNA chains under steady shear, by Doyle *et al.* [Phys. Rev. Lett. **399** 564 (2000)], demonstrated that the tethered chain in shear flow performs cyclic dynamics arising from a complex chain-flow interaction. These authors suggested that the cyclic dynamics are aperiodic, as the power spectrum of the chain extension along flow direction does not exhibit distinct peaks.
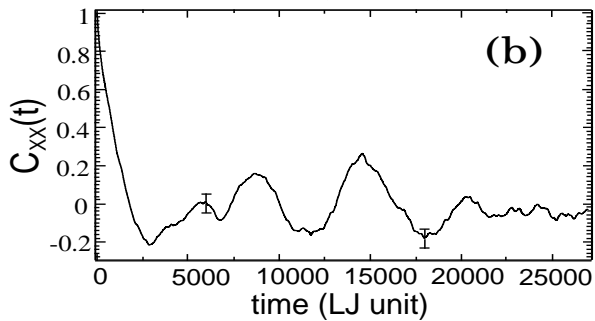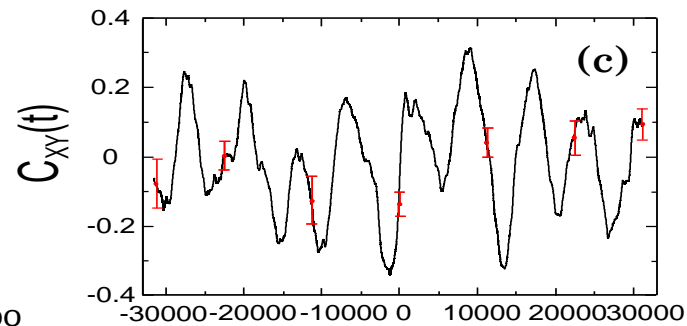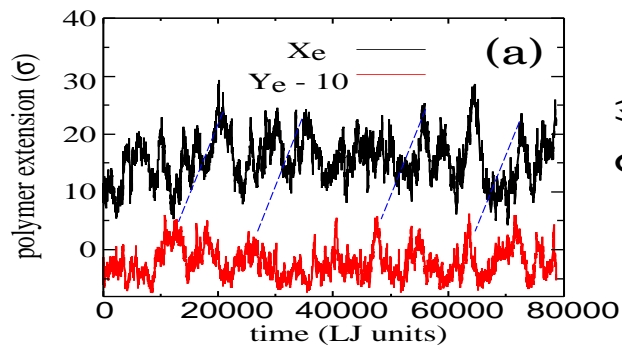
Cyclic dynamics

$\mathrm{Wi} = 2$ time in arbitrary units
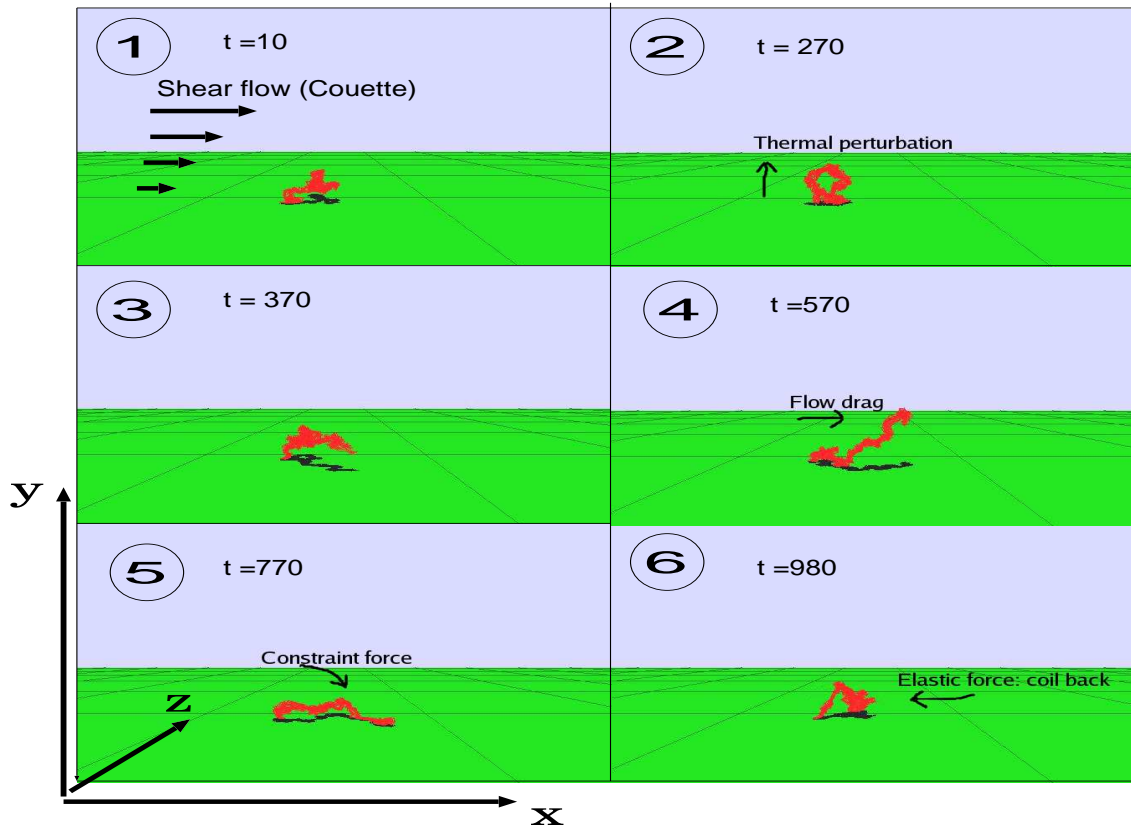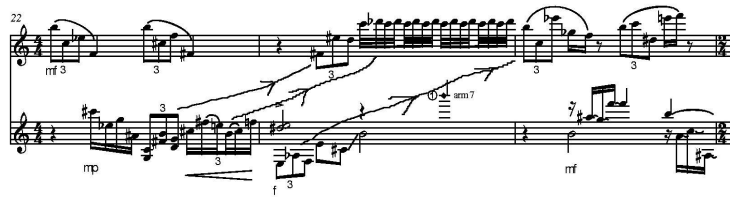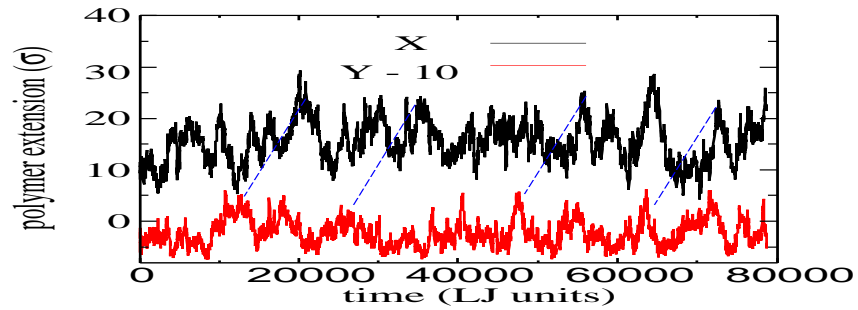
Cyclic dynamics

Time signal of the polymer extensions, autocorrelation and cross-correlations

$$C_{AB}(t) = \frac{\langle A(t)B(0)\rangle}{\left(\langle A(0)^2\rangle\langle B(0)^2\rangle\right)^{1/2}}$$
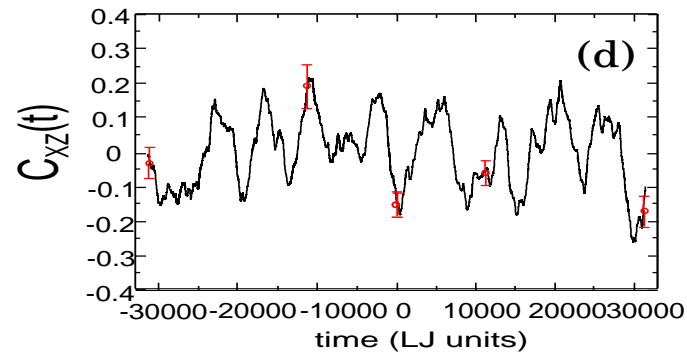
Canon for a tethered chain.
Be flat.

(musical joke)

Cyclic dynamics: Cross-power spectral density (CPSD)

$$P_{XY}(\omega) = \int_{-\infty}^{\infty} \langle X(t)Y(0) \rangle \exp(-I\omega t)dt \tag{1}$$

$$P_{XY}(\omega) = P_X(\omega)P_Y^*(\omega) \ \ \text{Parseval theorem} \tag{2}$$
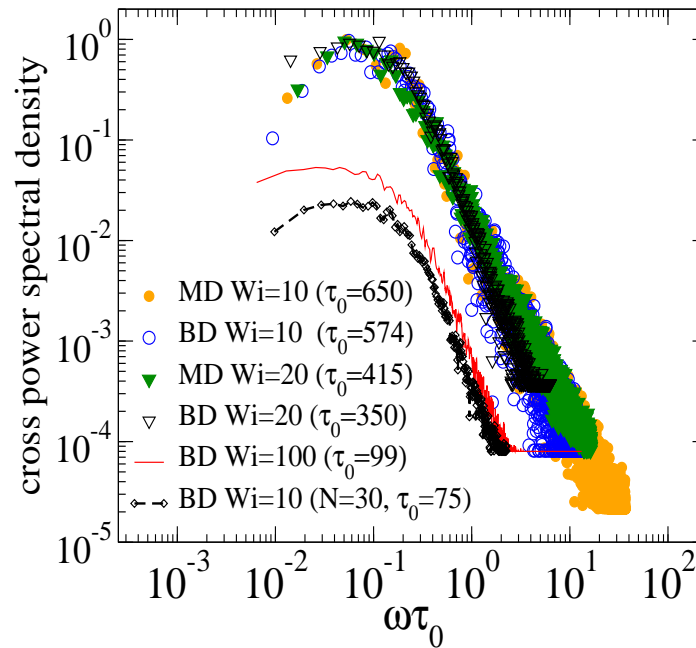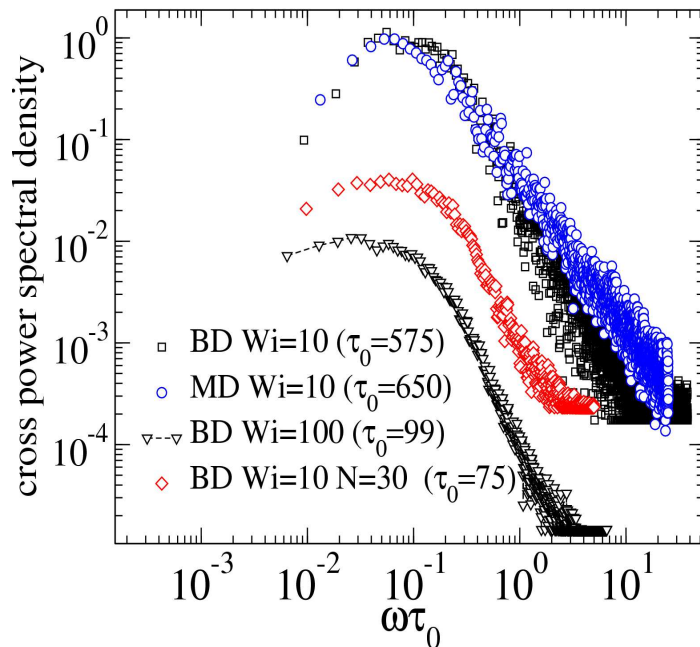
- The study of low-frequency chain dynamics $\omega\tau_0 << 1$ requires `looong` simulations
- Present results were obtained from runs over a grand-total of $\sim 1000\tau_0(0)$ for the BD chains (month) and $\sim 500\tau_0(0)$ in the MD case (several months).

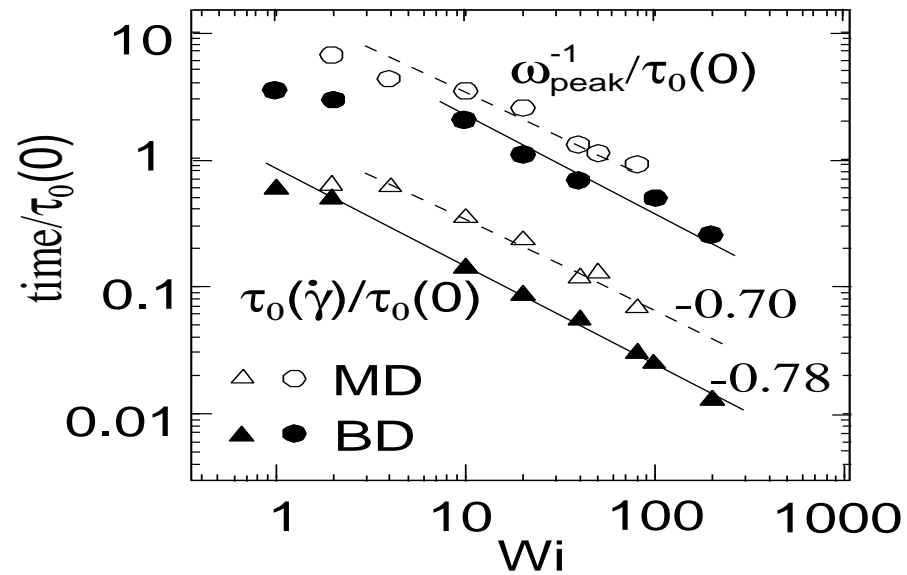# Cyclic dynamics: Cross-power spectral density (CPSD)

$$\omega_{peak}\tau_0(\text{Wi}) = 0.08 \pm 0.02$$

CPSD from **polymer extensions**   From 0th **normal coordinate**

Cyclic dynamics: peak frequency and longest relaxation time under flow

Conclusions

- We found that the cyclic motion of grated polymers under shear flow presents a characteristic time, clearly revealed as a frequency peak (ω_{peak}) in the cross-spectral density associated to the polymer extension in flow and gradient directions.

- The characterisctic time, $T_{cycle} = \omega_{peak}^{-1}$ is more than 10 times larger than the longest relaxation time of the chain under flow $\tau_0(\mathrm{Wi})$.

- It scales like the time needed to stretch the polymer by convection.

- As an indication of the generality of this phenomenon, the ratio between the cycling and relaxation time (about 14) appears to be independent on the presence of hydrodynamic interactions and on the chain lenght.

- This coherent recursive motion introduces long memory in the fluid and suggests resonance effects under periodic external forcing.

References of the present research

- R. Delgado-Buscalioni, Cyclic motion of a grafted polymer under shear flow, *Phys. Rev. Lett.*, to appear (March 2006)
- R. Delgado-Buscalioni and P. V. Coveney, Structure of a tethered polymer under flow using molecular dynamics and hybrid molecular-continuum simulations, *Physica A*, in press (2005).
- S. Barsky, R. Delgado-Buscalioni and P.V. Coveney, Comparison of molecular dynamics to hybrid molecular dynamics-computational fluid dynamics for a single tethered polymer in solution, *J. Chem. Phys.* **121** 2403 (2004)