Transport properties of electrospun hydrogel nanofilaments: perspective use for drug delivery and tissue repair

Tomasz A. Kowalewski
Sylwia Pawłowska, Filippo Pierini
Department of Biosystems and Soft Matter
IPPT PAN, Warszawa

POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

The University of Illinois at Chicago September 5-12, 2019

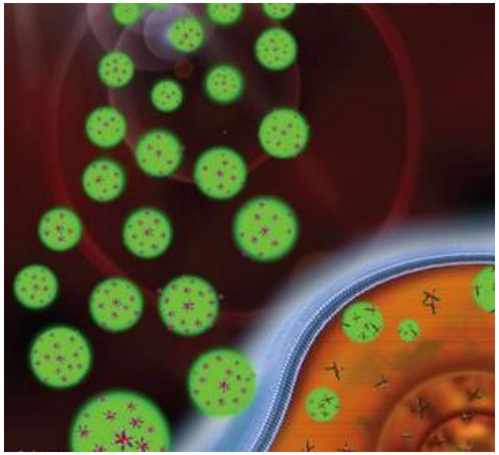
Drug Delivery System Challanges

Targeted drug delivery: systems allow selective targeting of the drug to a specific tissue, organ or specific cells inside the body to achieve a targeted drug action.

Controlled release drug delivery: systems capable to mantain the adequate end desired release of drug over an extended period of time.

Nanomedicine

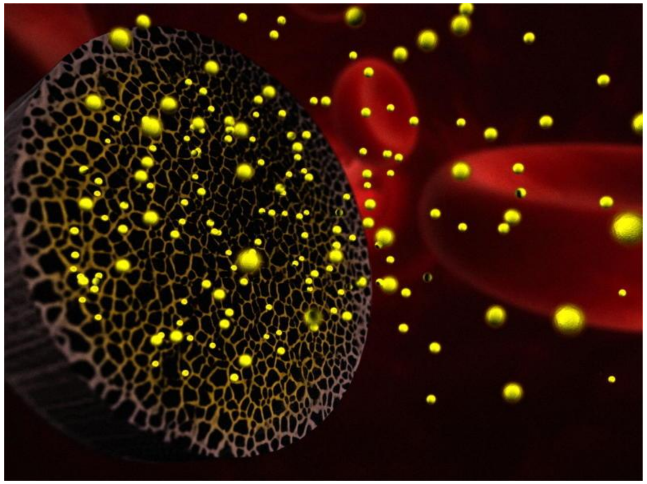
- Nanocarriers
- Drug
- Target
- Environment



INTRODUCTION

Nanomaterial-based Drug Delivery System

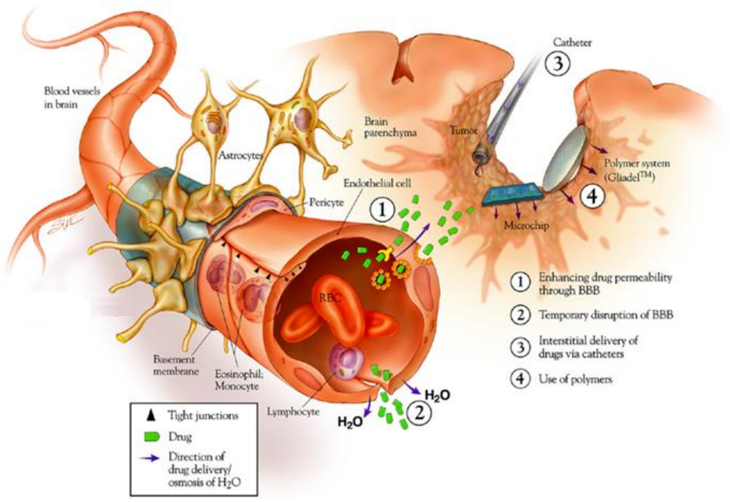
- Capable of achieving high drug loading
- Inert
- Biocompatible
- Mechanically strong
- Comfortable for the patient
- Readily processable
- Safe from accidental release
- Simple to administer
- Easy to fabricate and sterilize
- Free of leachable impurities



INTRODUCTION

Barriers to nanovehicle transport

- Hemo-Rheology
- Endothelial and Epithelial Barrier
- Reticuloendothelial System
- Blood–brain barrier
- Cell Membrane
- Nuclear membrane
- Ionic & Molecular Pumps



INTRODUCTION

Hydrogel characteristics

- three dimensional networks of hydrophilic polymers that are insoluble but can swell in water
- solid like and liquid like properties in one material
- biocompatibility
- controlled drug release

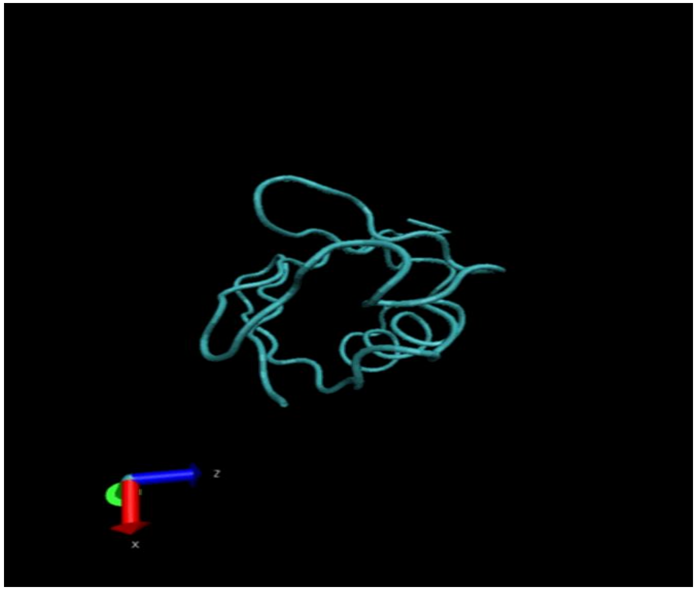
Mechanical properties are influenced by:

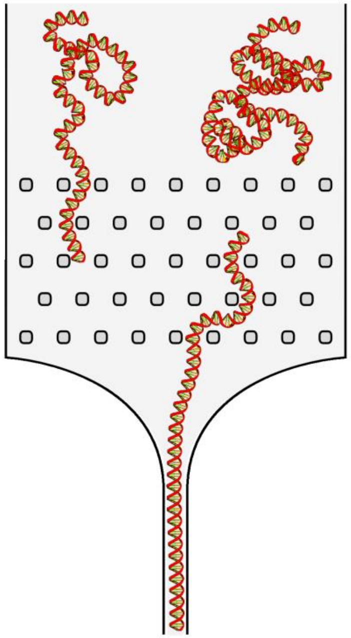
- type and composition of monomers
- cross-linking
- environmental factors (e.g. temperature, pH, ionic strength, light, electric and magnetic fields)

INTRODUCTION

Nanofilaments

- High deformability
- Coiling/uncoiling properties
- Capability to pass through crowded environments end pores





SCOPE

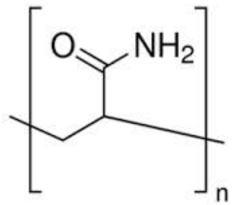
Production of flexible nanofilaments which can be used as drug carriers, with deformability and elasticity resembling those of long DNA chains

Mechanical characterization of highly deformable hydrogel nanofilaments by using AFM nanoindentation, Brownian motion and bending dynamic techniques.

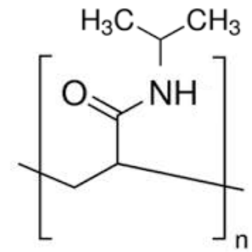
MATERIALS PRODUCTION

Hydrogels

Polyacrylamide



Poly(N-isopropylacrylamide)

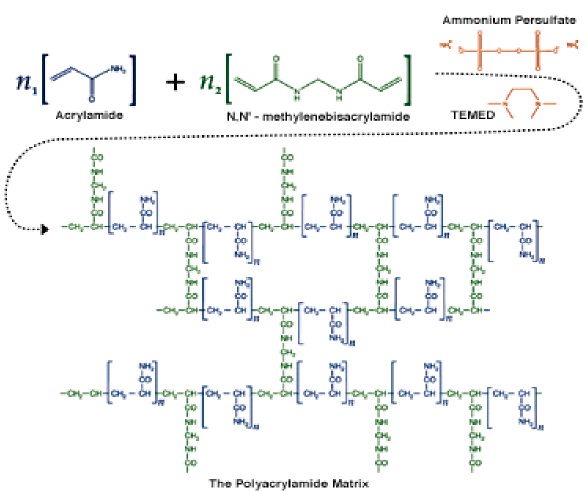


Matrerials:
Acrylamide (Aam)
N,N'-methylene bisacrylamide (BIS-Aam)
ammonium persulfate (APS)
N,N,N',N'-tetramethylethylenediamine (TEMED)
Bovine Serum Albumin with fluoresceine (BSA-FITC)

Samples:
- EA1 mass ratio of AAm/BIS-AAm (w/w): 37.5:1
- EA2 mass ratio of AAm/BIS-AAm (w/w): 20:1
- EA3 mass ratio of AAm/BIS-AAm (w/w): 4:1

Matrerials:
N,N-isopropylacrylamide (NIPAAm),
N,N'-methylene bisacrylamide (BIS-Aam)
ammonium persulfate (APS)
N,N,N',N'-tetramethylethylenediamine (TEMED)
Bovine Serum Albumin with fluoresceine (BSA-FITC)

Samples:
- EN1 mass ratio of NIPAAm/BIS-AAm (w/w): 37.5:1
- EN2 mass ratio of NIPAAm/BIS-AAm (w/w): 20:1
- EN3 mass ratio of NIPAAm/BIS-AAm (w/w): 4:1

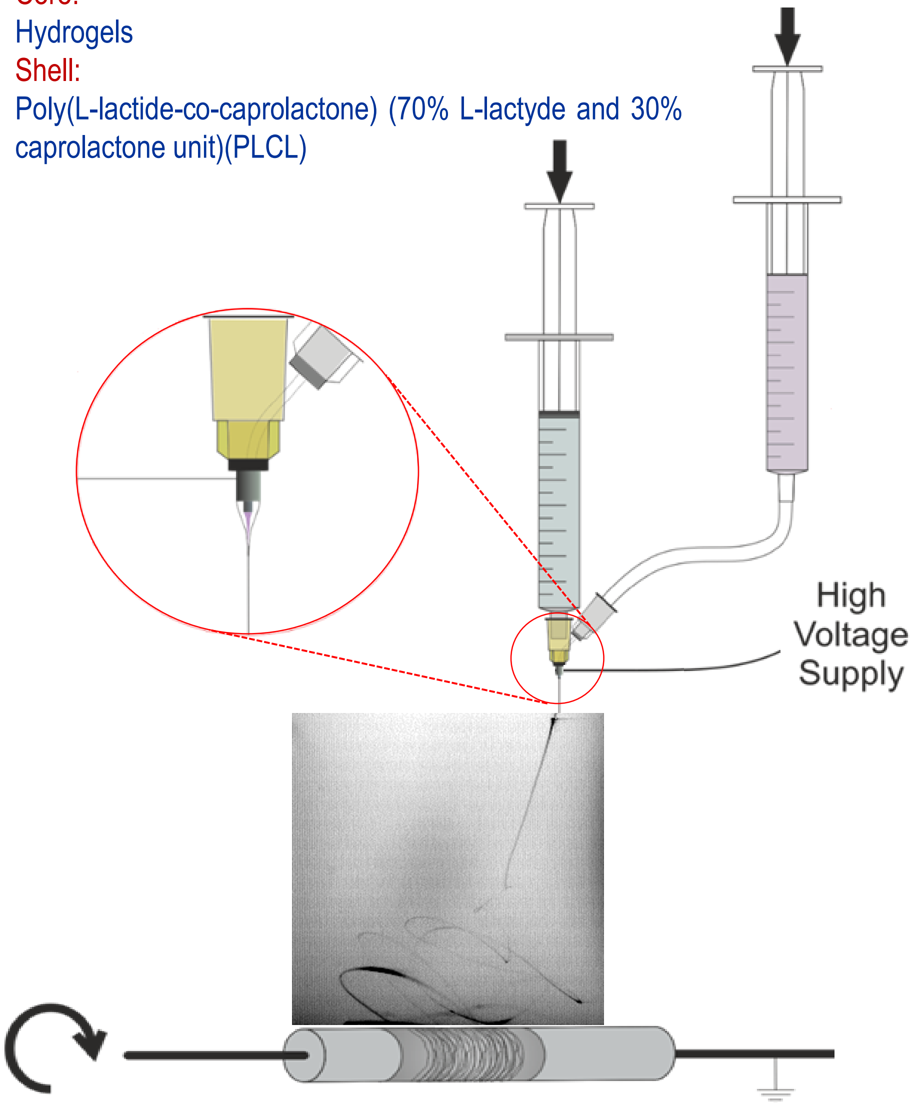


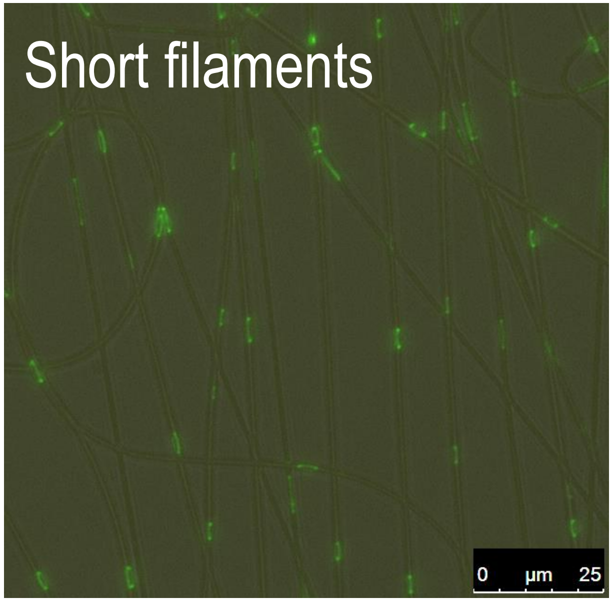
Polymerization

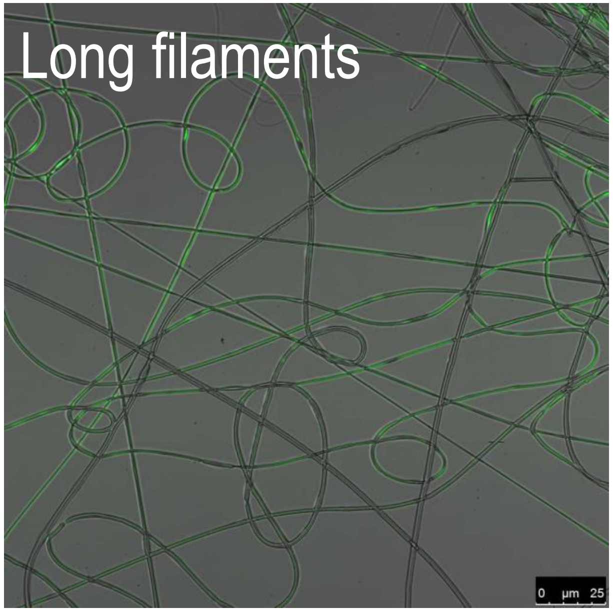
MATERIALS PRODUCTION

Core-Shell Electrospun Fibers

Core:
Hydrogels
Shell:
Poly(L-lactide-co-caprolactone) (70% L-lactyde and 30% caprolactone unit)(PLCL)

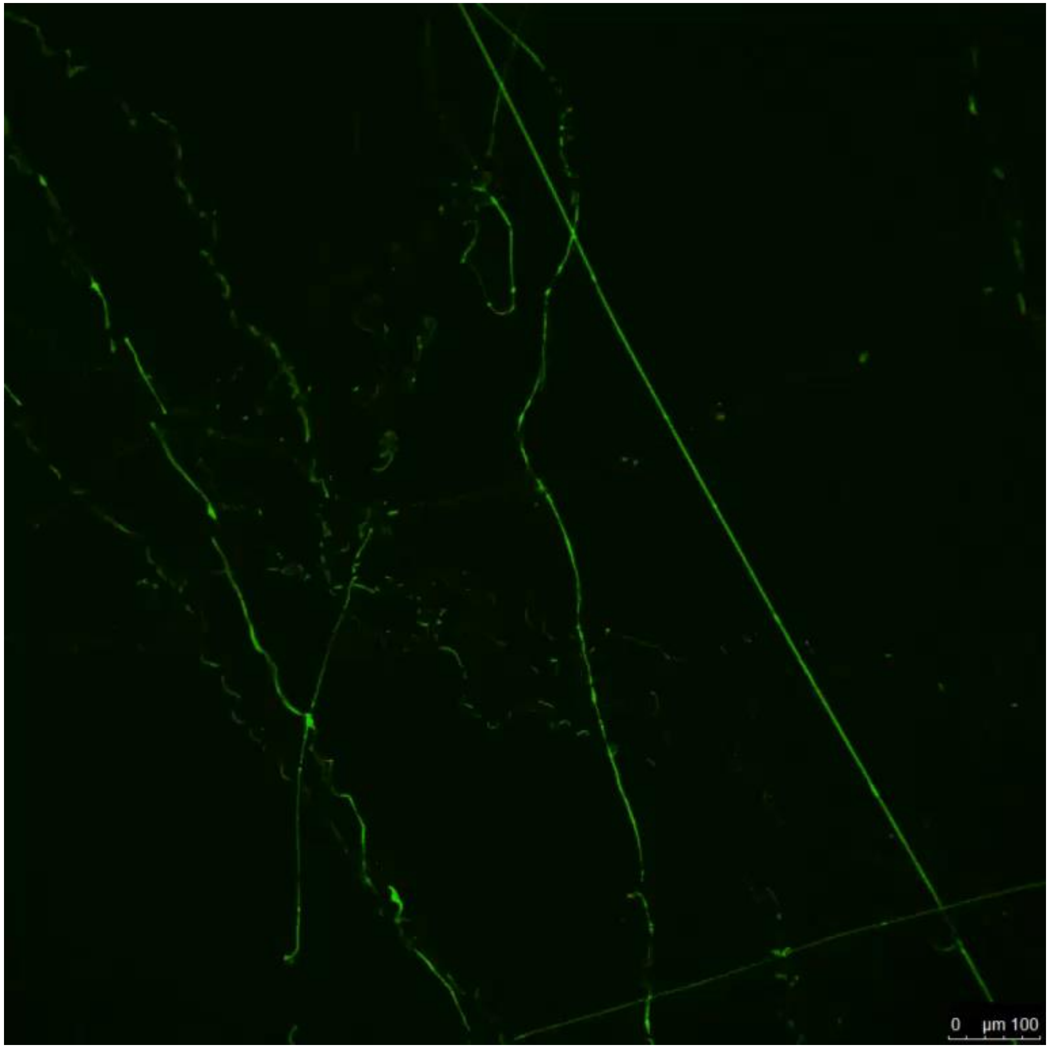






MATERIALS PRODUCTION

Shell Dissolution



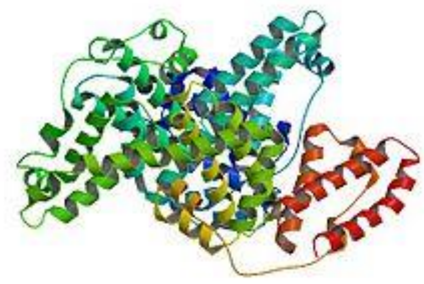
Sheel dissolution and filaments extraction in
N,N-dimethylformamide (DMF)

CHARACTERIZATION

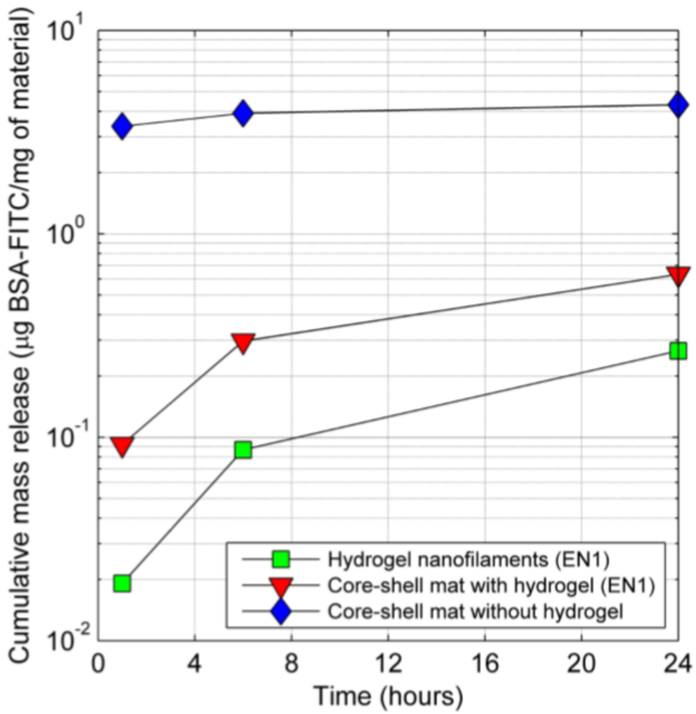
Protein Release

Matrerials:
N,N-isopropylacrylamide (NIPAAm),
N,N'-methylene bisacrylamide (BIS-Aam)
ammonium persulfate (APS)
N,N,N',N'-tetramethylethylenediamine (TEMED)
Bovine Serum Albumin with fluoresceine (BSA-FITC)

Samples:
- EN1 mass ratio of NIPAAm/BIS-AAm (w/w): 37.5:1
  0.7% $_{w/w}$ of BSA-FITC in hydrogel



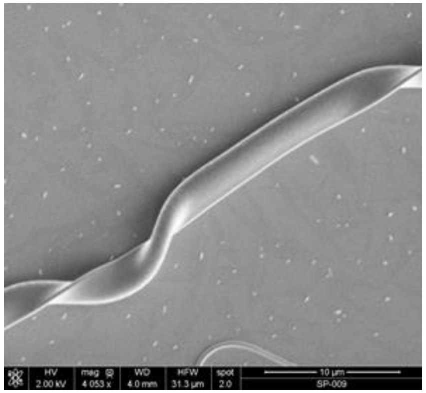
Bovine Serum Albumin



CHARACTERIZATION

Morphology

SEM

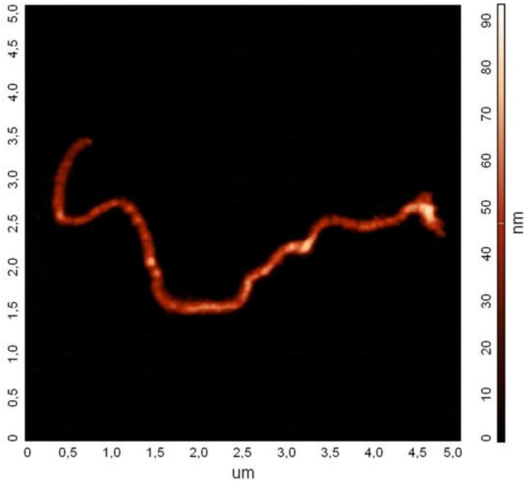


Ribbon-like core-shell
PLCL/PAAm nanofibre (EA3)

TIRF

Fluorescent nanofilaments
extracted as core from nanofibre

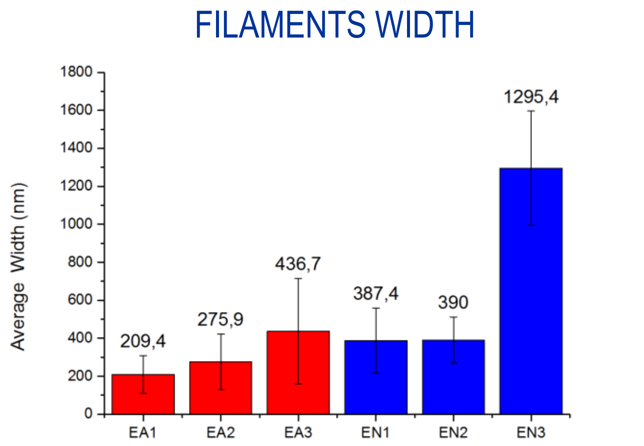
AFM

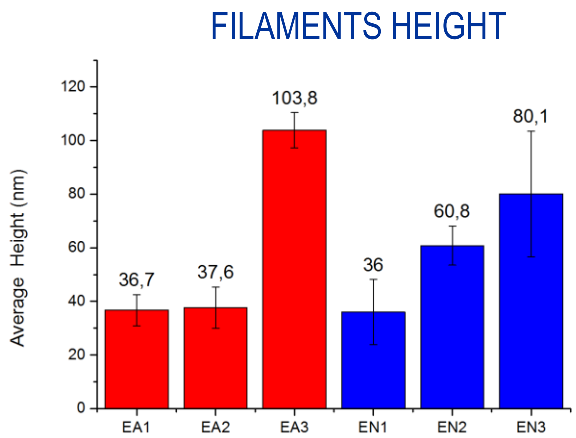


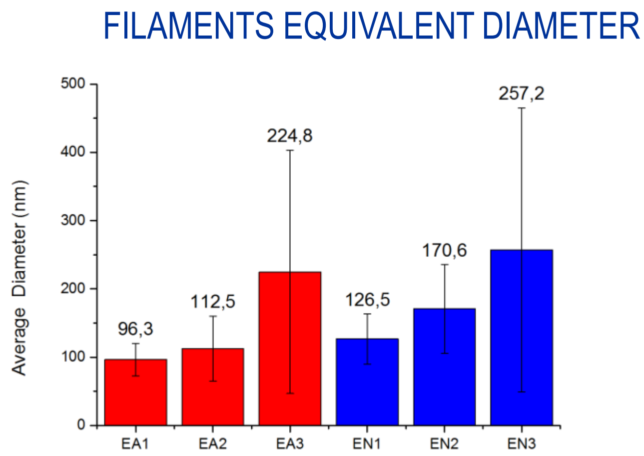
Ribbon-like nanofilament (EA1): contour length 7 µm, width 128 nm, height 39 nm

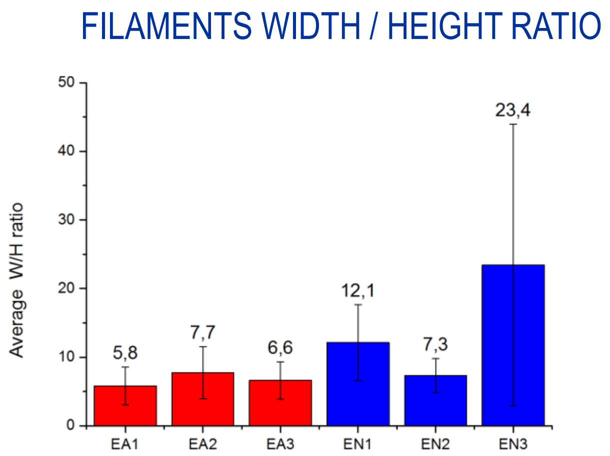
CHARACTERIZATION

Morphology



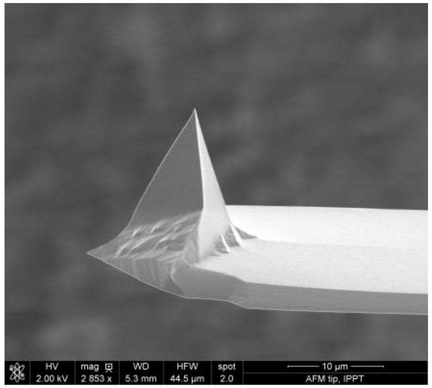


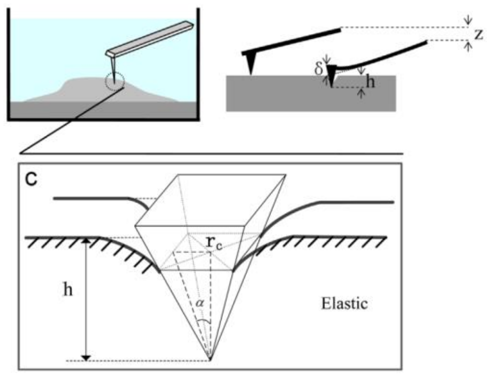


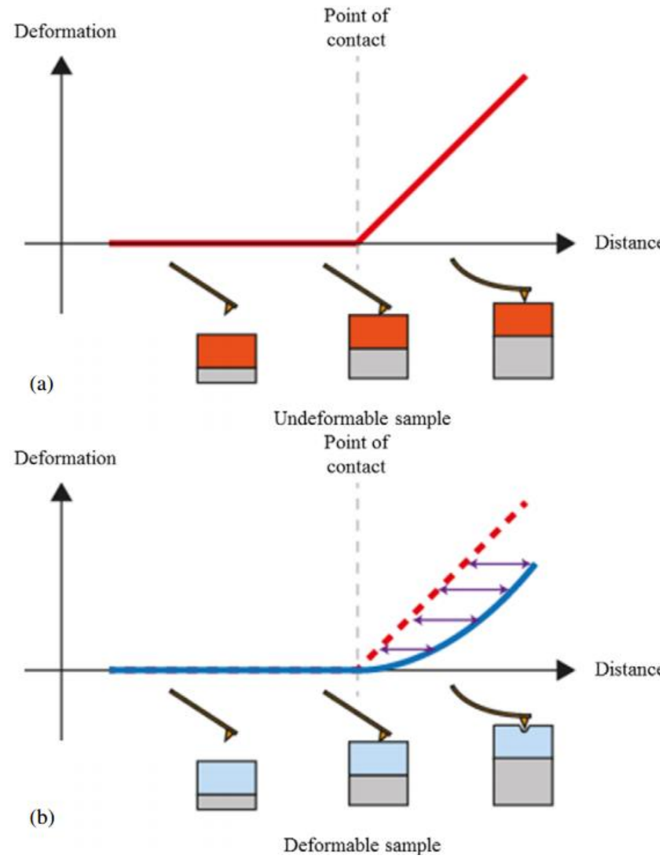


CHARACTERIZATION

AFM Nanoindentation







Hertz Model

$$E = F \cdot \frac{3}{4} \cdot \frac{1 - \nu^2}{\sqrt{r} \cdot \delta^{\frac{3}{2}}}$$

where F is the applied force, E is elastic modulus of the sample, v is the Poisson`s ratio of the sample, δ is the indentation depth and r is the equivalent radius for a spherical indenter

CHARACTERIZATION

Thermal Fluctuations

Diffusion coefficients:

$$D_a = \frac{k_B T[ln(L/R) - 0.5)]}{2\pi\eta_s L}$$

$$D_b = \frac{k_B T[ln(L/R) + 0.5)]}{4\pi\eta_s L}$$

$$D_\vartheta = \frac{3k_B T[ln(L/R) - 0.5)]}{\pi\eta_s L^3}$$

k_B is Boltzmann's constant
T is the absolute temperature
η_s is the viscosity of the water
θ is the rotation angle
L, R are the length and radius of the filament

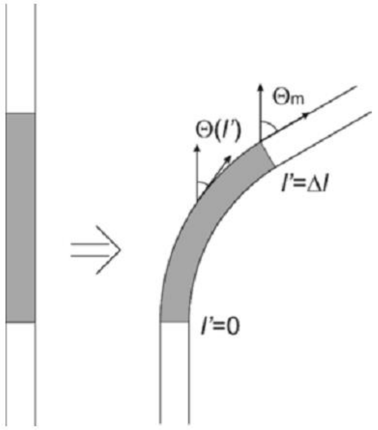
Cosine correlaction method

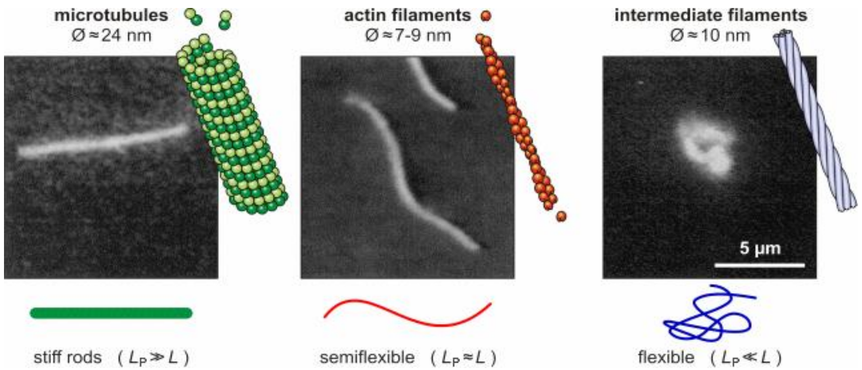
$$\langle cos\theta(s)\rangle = e^{-L/2L_p}$$

L is the contour filament length
L_p is the persistence lenght
θ is the angle between two unit tangent vectors

Persistence length is the length over which correlations in the direction of the tangent are lost. Lp is correlated to the Young`s Modulus (E):

$$L_p = \frac{E\,I}{k_B T}$$





Thermal Fluctuations

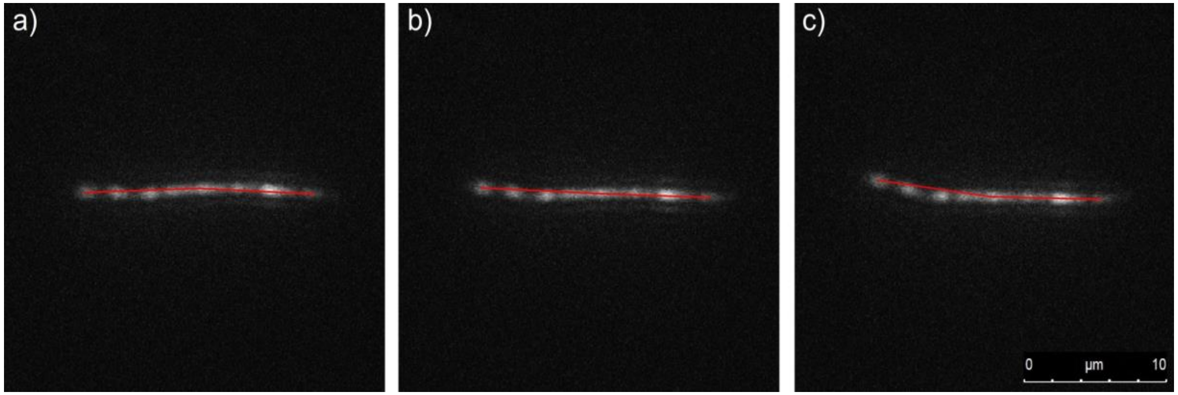
Contour length: 15.6 μm
Persistance length: 598 μm

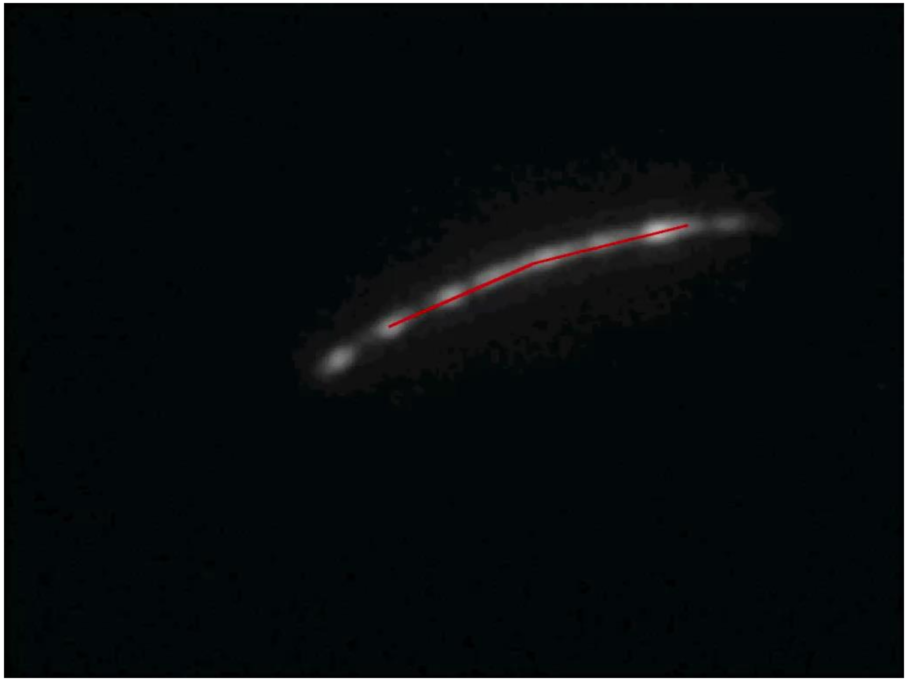
Experimental diffusion coefficients:
$D_a = 0.266\ \mu m^2$
$D_b = 0.070\ \mu m^2$
$D_{rot} = 0.0023\ rad^2$

Theoretical diffusion coefficients:
$D_a = 0.182\ \mu m^2$
$D_b = 0.116\ \mu m^2$
$D_{rot} = 0.0044\ rad^2$

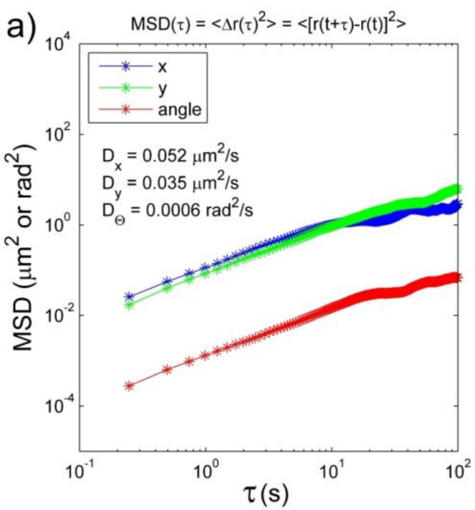
Thermal Fluctuations

Bending dynamics of a nanofilament of contour length 21.5 μm. Red lines indicate arms of the fibre starting from the centre of the fibre mass. The angle between the red lines was measured to assess flexibility. The time interval between frames is t = 0.25 s.

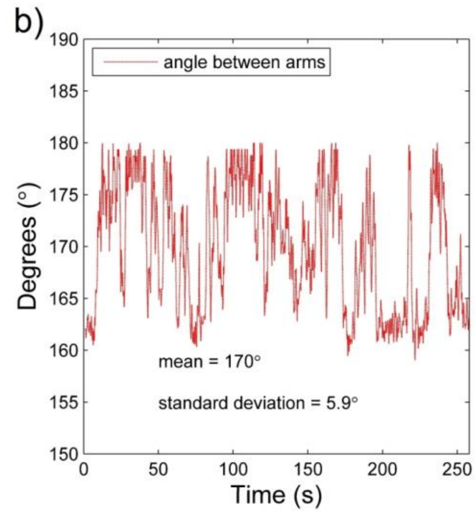




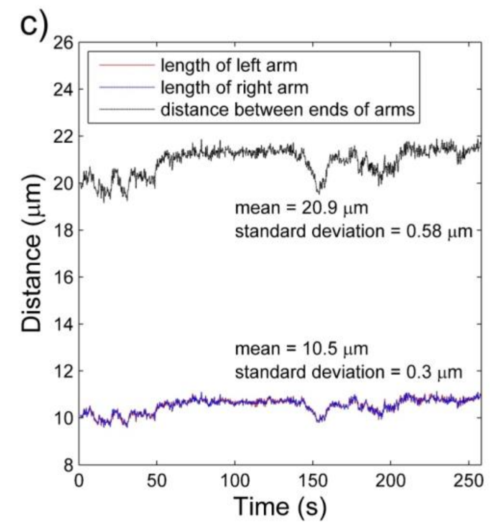
Thermal Fluctuations



Mean square displacement of a filament of contour length 21.5 μm as a function of lag time



Angle between arms of the bending filament as a function of time



Length of left and right arm of the bending filament, and distance between both ends of the arms.

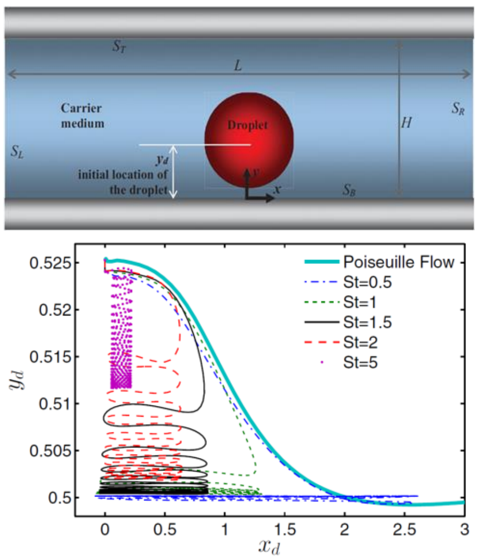
Mechanical Properties

	Young modulus E (kPa)		
Monomer mass ratio	AFM indentation	Cosine correlation	Flow
NIPAAm:BIS-AAm			
37.5:1	–	4.5±0.4	2.3
20:1	8.50±1.19	3.1±1.7	–
4:1	18.11±4.85	3.8±1.1	–
AAm:BIS-AAm			
37.5:1	4.06±1.18	6.1±2.6	–
20:1	15.80±2.77	5.0±1.1	–
4:1	55.82±5.64	5.8±0.8	–

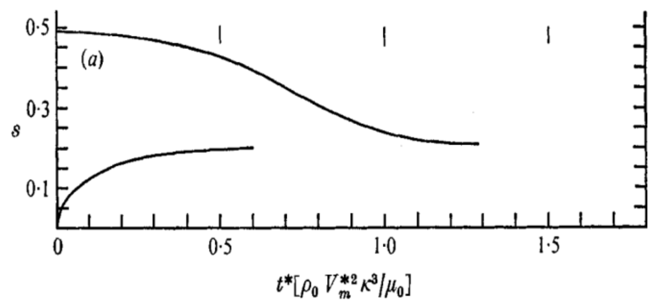
Understanding transport of nano-objects

Lateral migration of particles in a tube flow

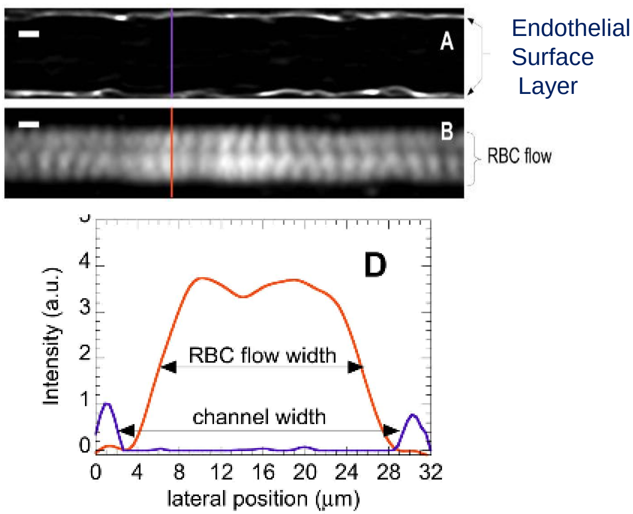
Numerical, droplet



Theoretical, particle



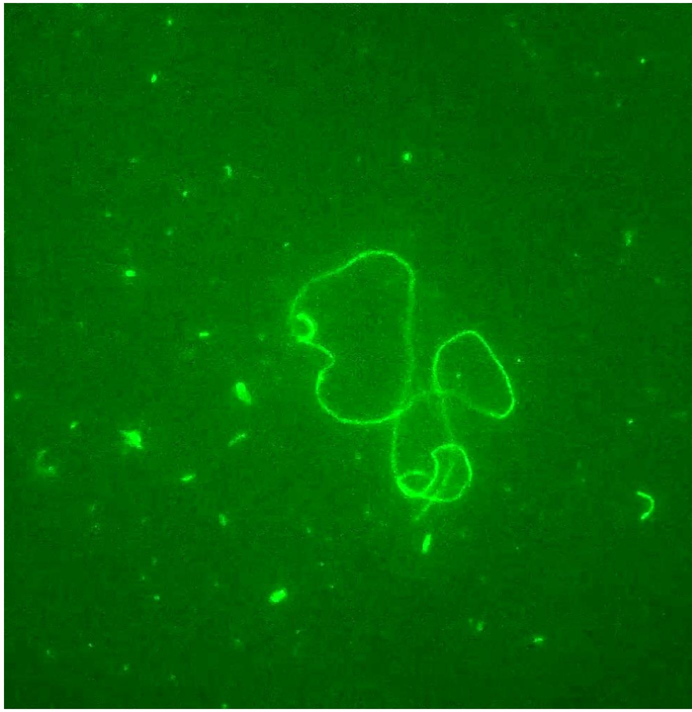
Experiment, RBC



Tsvirkun D., Grichine A., Duperray A., Misbah C., Bureau L.:Microvasculature on a chip: study of the Endothelial Surface Layer and the flow structure of Red Blood Cells. Nature Scientific Reports (2017), 7:45036.
Ho B. P., Leal L. G.: Inertial migration of rigid spheres in two-dimensional unidirectional flows. J. Fluid Mech. (1974), 65(2), pp. 365-400.
Chaudhury K., Mandal S., Chakraborty S.: Droplet migration characteristics in confined oscillatory microflows. Physical Review E (2016), 93, 023106.

Proposed experimental model of long polymeric chains

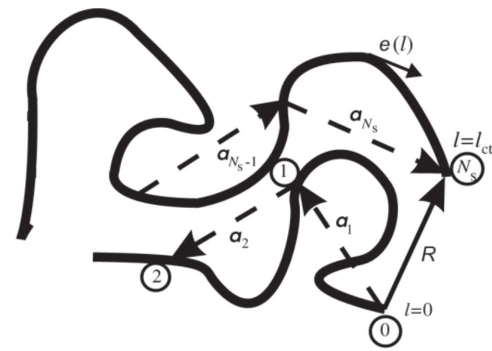
Highly deformable polymer nano-object undergoing a strong deformation under the influence of mechanical and physical phenomena.



- ➢Verification of the existing theoretical models
- ➢Checking their potential application

Mechanical parameters of the filament
Persistence Length

Thermal fluctuations

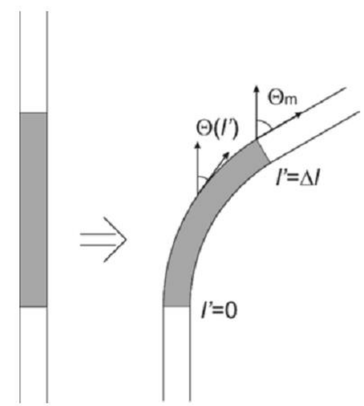


Persistence length

$$L_p = \frac{E\,I}{k_B T}$$

$I = \pi R^4/4$ – moment of inertia

E – Young modulus

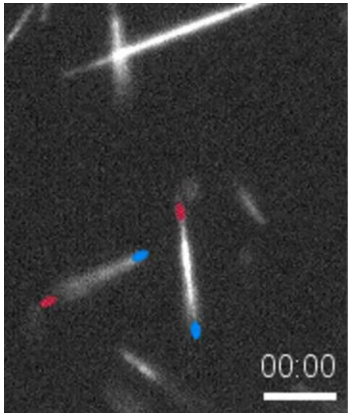
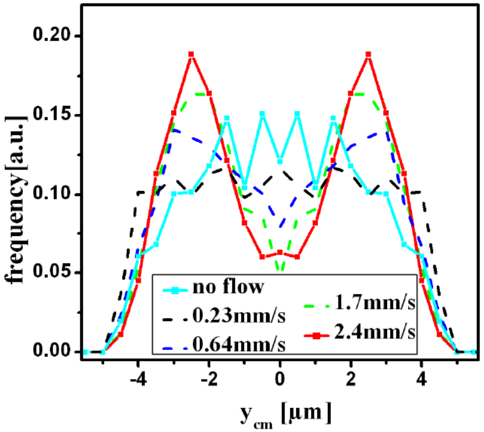
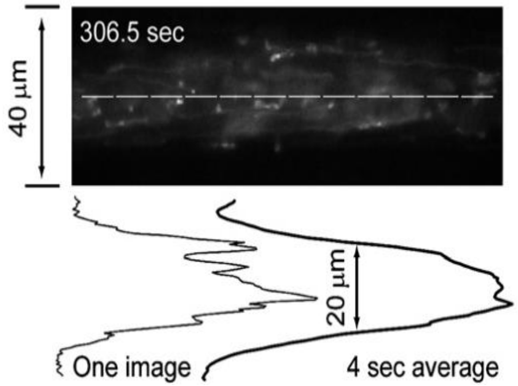


Cosine correlation method

$$\langle cos\theta(s)\rangle = e^{-L/2L_p}$$

Warm Like Chain – Kratky & Porod 1949

Cross-flow migration of long polymer objects

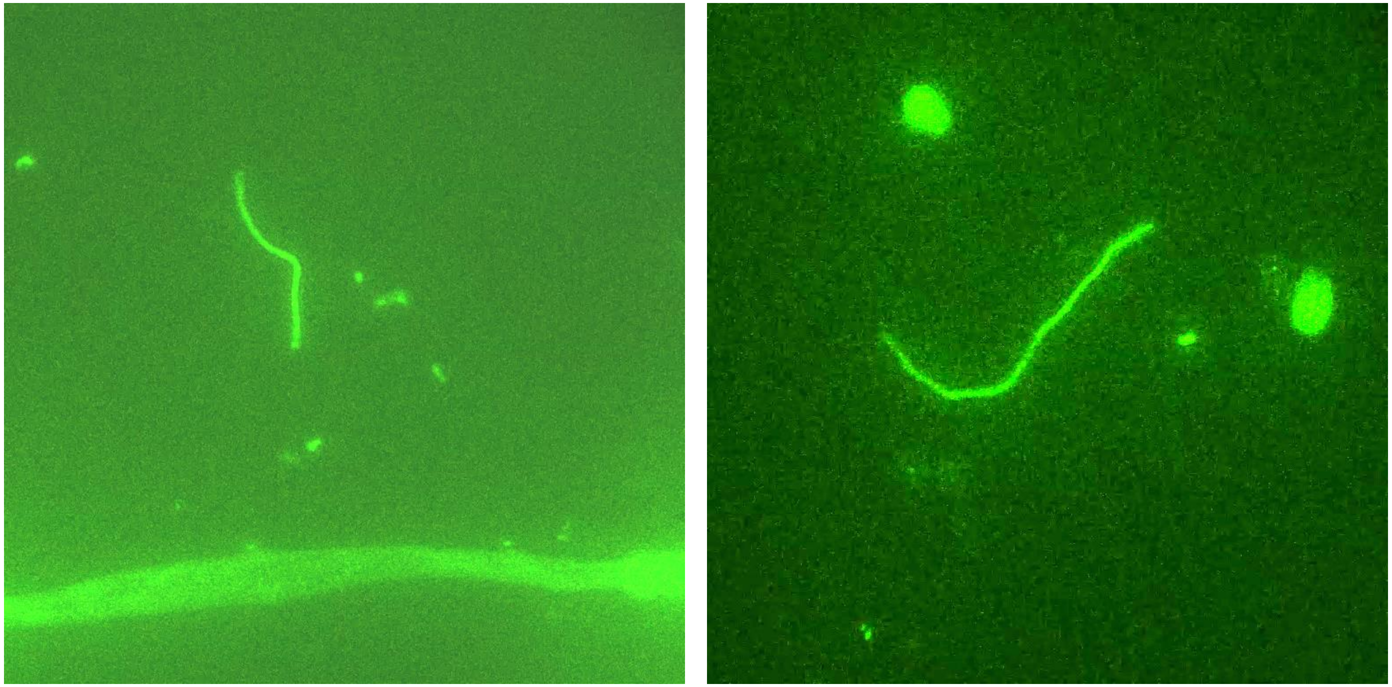
Stiff filaments kinesin microtubules *Lp/L* > 1	Semiflexible filaments actin filaments *Lp/L* ~ 1	Flexible filaments DNA *Lp/L* < 1
		
to channel wall	between wall and center of the channel	channel center

Sanches T. et al., Spontaneous motion in hierarchically assembled active matter, Nature 491 (2012)
Saintillan D., et al. Effect of flexibility on the shear-induced migration of short-chain polymers in parabolic channel flow, Journal of Fluid Mechanics (2006), 557, 297-306
Steinhauser D.R.: Actin filaments and bundles in flow, PhD thesis, Gottingen (2008)
Jo K., Chen. Y.L., De Pablo J.J., Schwartz D.C.: Elongation and migration of single DNA molecules in microchannels using oscillatory shear flows, Lab on a Chip (2009), 9, 2348-2355.

Bending dynamics of nanofilaments, radius 70nm thermal fluctuations => evaluating flexibility *A*

Contour length: 42.3 µm
Persistence length: 31 µm

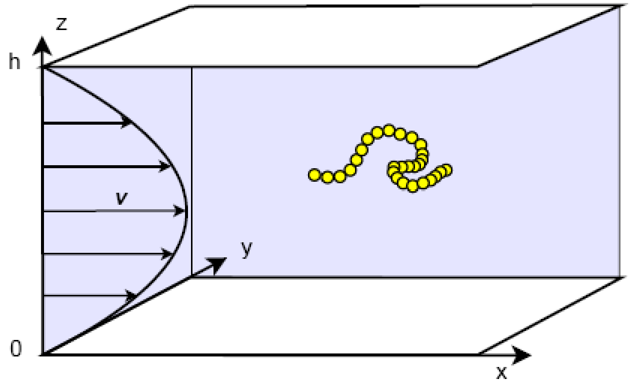
Contour length: 126.1 µm
Persistence length: 56 µm

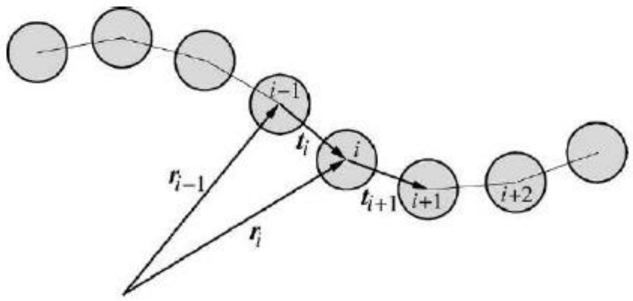


Simplified Hydrodynamics

$$\begin{aligned} \eta\nabla^2\mathbf{v} - \nabla p &= \mathbf{0}, \\ \nabla \cdot \mathbf{v} &= 0, \end{aligned}$$

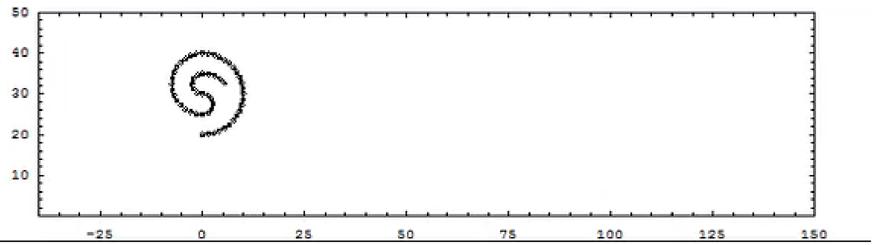
- Chain of spheres in Stokes approximation (*Re=0*)
- Neglecting Brownian fluctuations
- Time is parameter (step by step solution)

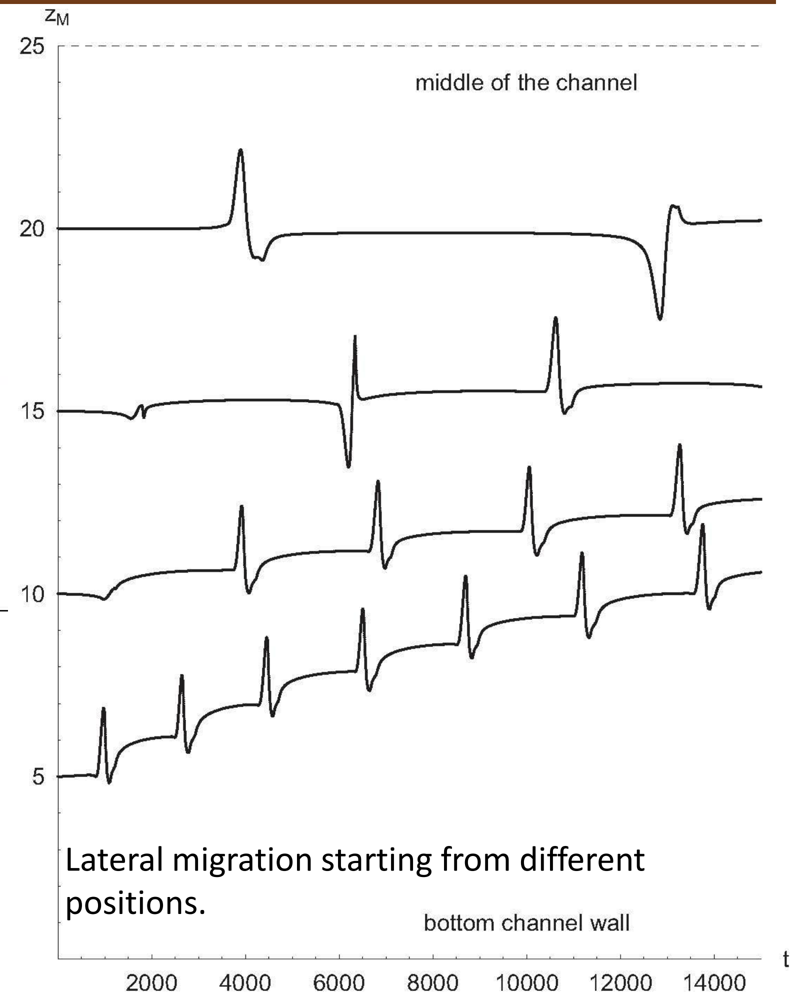




Bead-Spring hydrodynamic model

Flipping and tumbling of fiber conveyed by channel flow.
Number of beads *N=20*;
Relative Flexibility ***A* =0.5**;
Relative Extensibility ***k* =10**.





Lateral migration starting from different positions.

K. Sadlej, E. Wajnryb, M.L. Ekiel-Jezewska, D. Lamparska, and T.A.. Kowalewski, Int. J. Heat Fluid Flow 31, 996–1004 (2010).

Bead-Spring Hydrodynamic Model

Flipping and tumbling => Is it related to long molecules hydrodynamics ?

Beads model:	DNA 100kbp experiment
$N = 5 – 20$ (**d/L > 0.05**)	=> **$d/L < 10^{-4}$**
Relative flexibility ***$\underline{A}$ = 0.5 – 20***	=> **$\underline{A}$ = 2.5 10^4**
Relative extensibility ***$\underline{k}$ =10***	=> ***$\underline{k}$ = 52-100***

Proposed hydrogel nanofilaments as experimental model

d = 120-150 nm; L = 5 μm – 100 μm; E = 2 kPa ; L_p = 5 μm – 10 μm

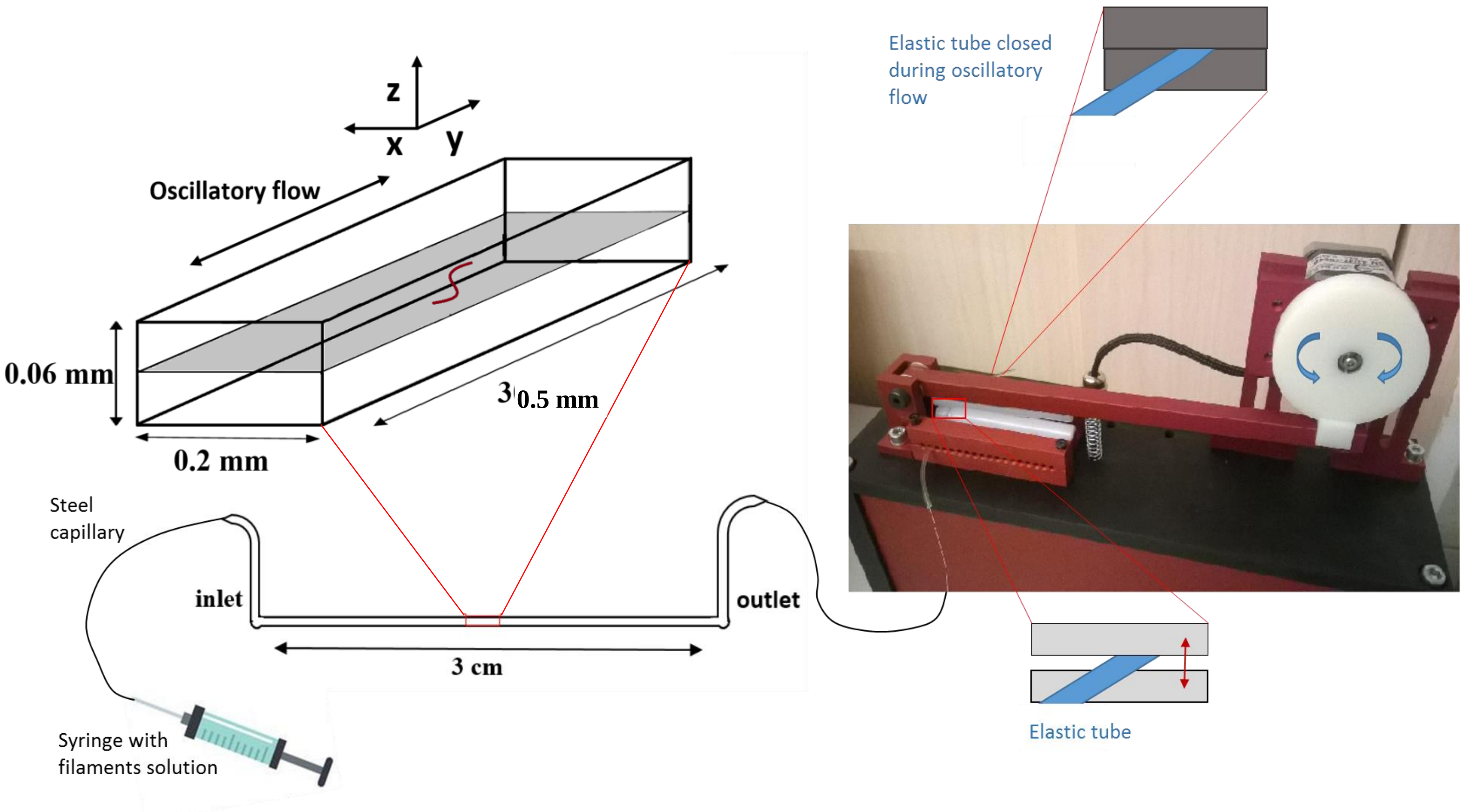
d/L = 0.03 – 0.005

$\underline{A}$ = 10 – 160

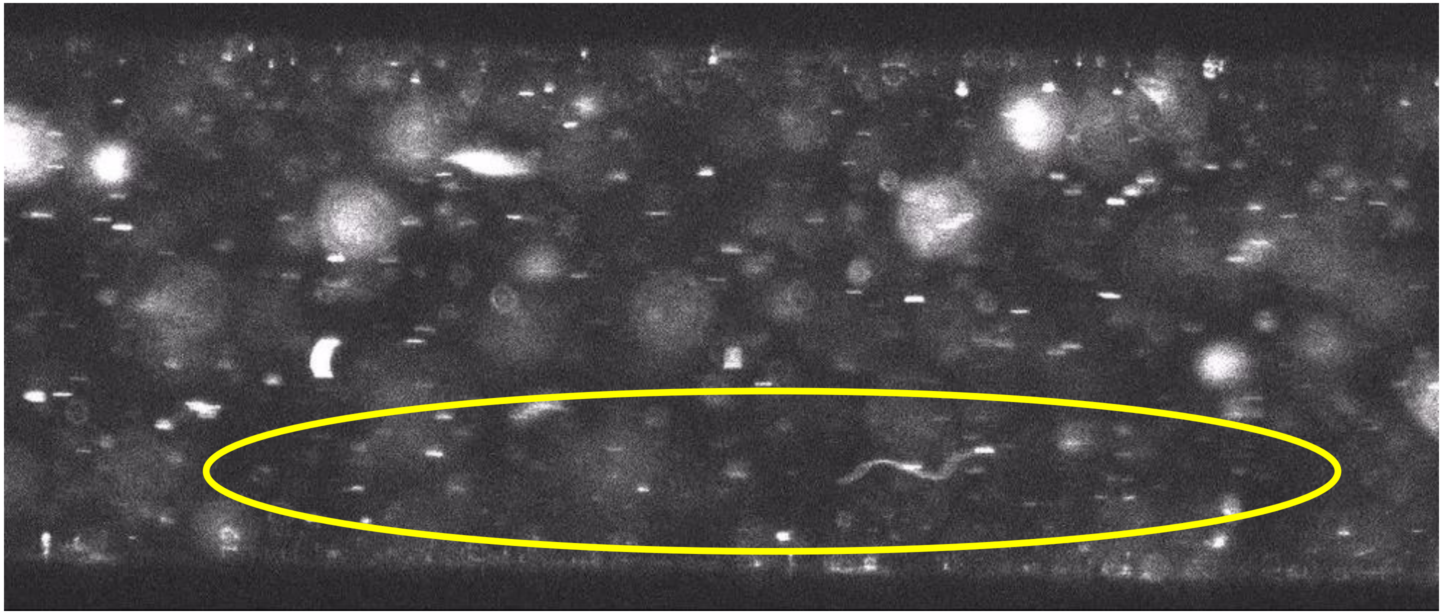
$\underline{k}$ = 16 – 350

Includes Brownian fluctuations

Oscillating flow system

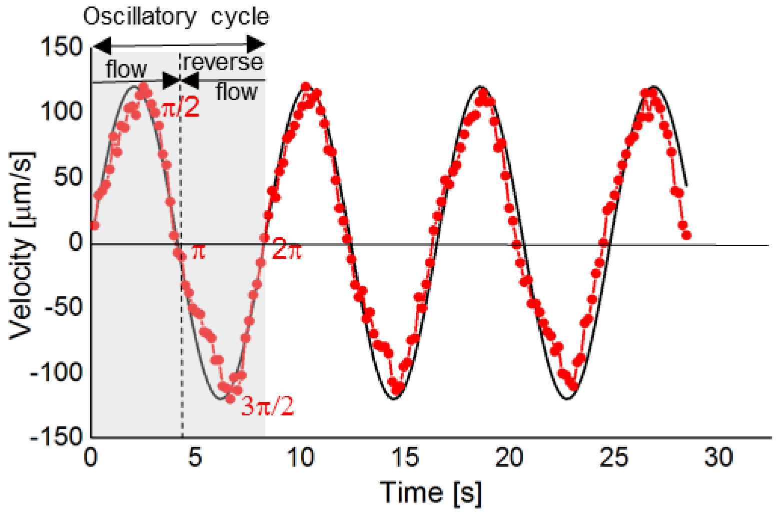


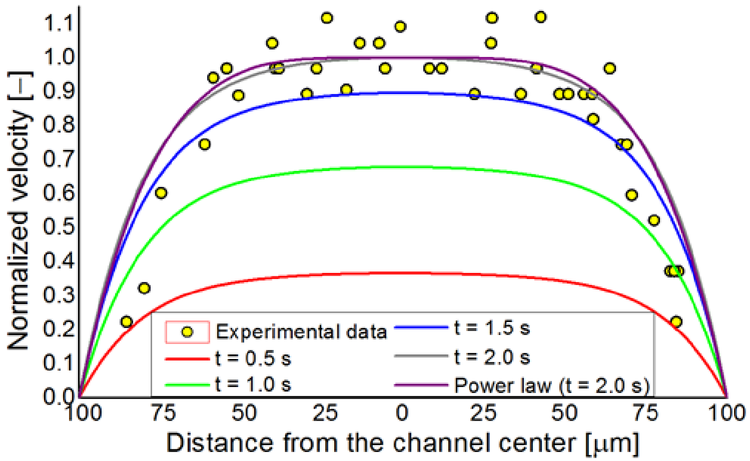
Hydrogel nanofilaments in oscillating flow



Vav [um/s]	L [um]	R [um]	Re (Dchannel)	Lp [um]	E [kPa]	Sp
68.87	61	0.067	3.11.10-2	7.51	2	47

Flow profile during oscillating flow

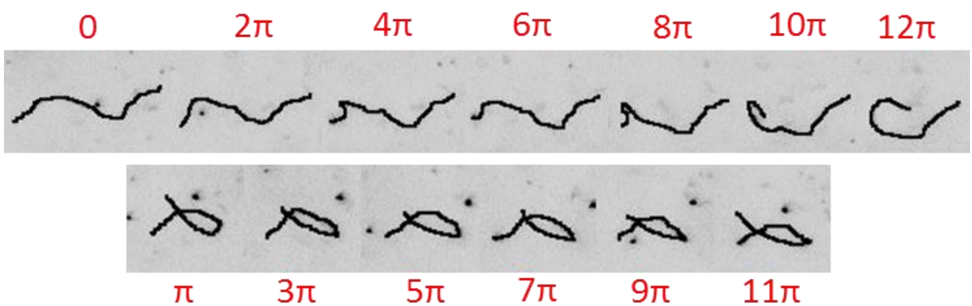




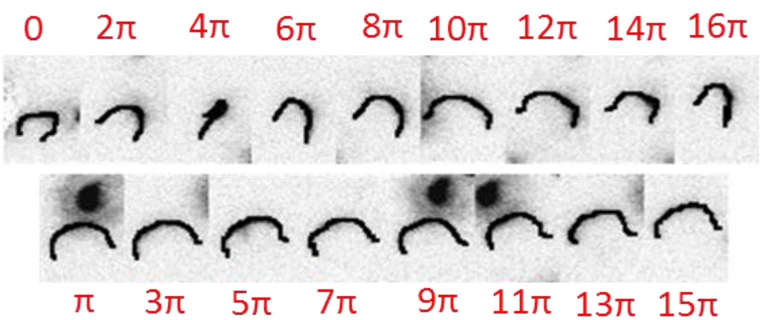
$$V(x,t) = V_{max}\left[1 - \left|\left(\frac{x}{w_h}\right)^{4.5}\right|\right] \cdot \sin(2\pi f t)$$

Bahaviour of nanofilament under oscillating flow

Bent-like filaments

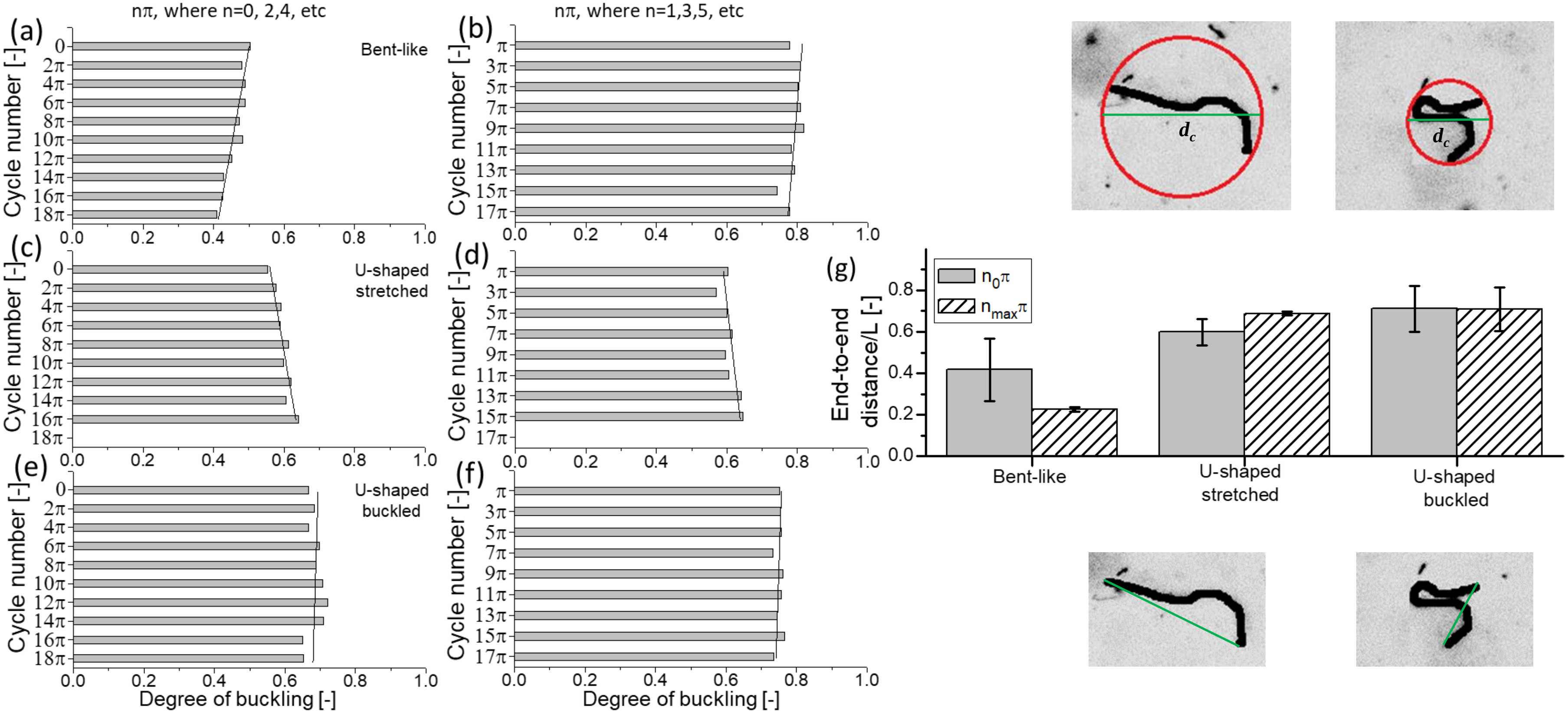


U-shaped filaments

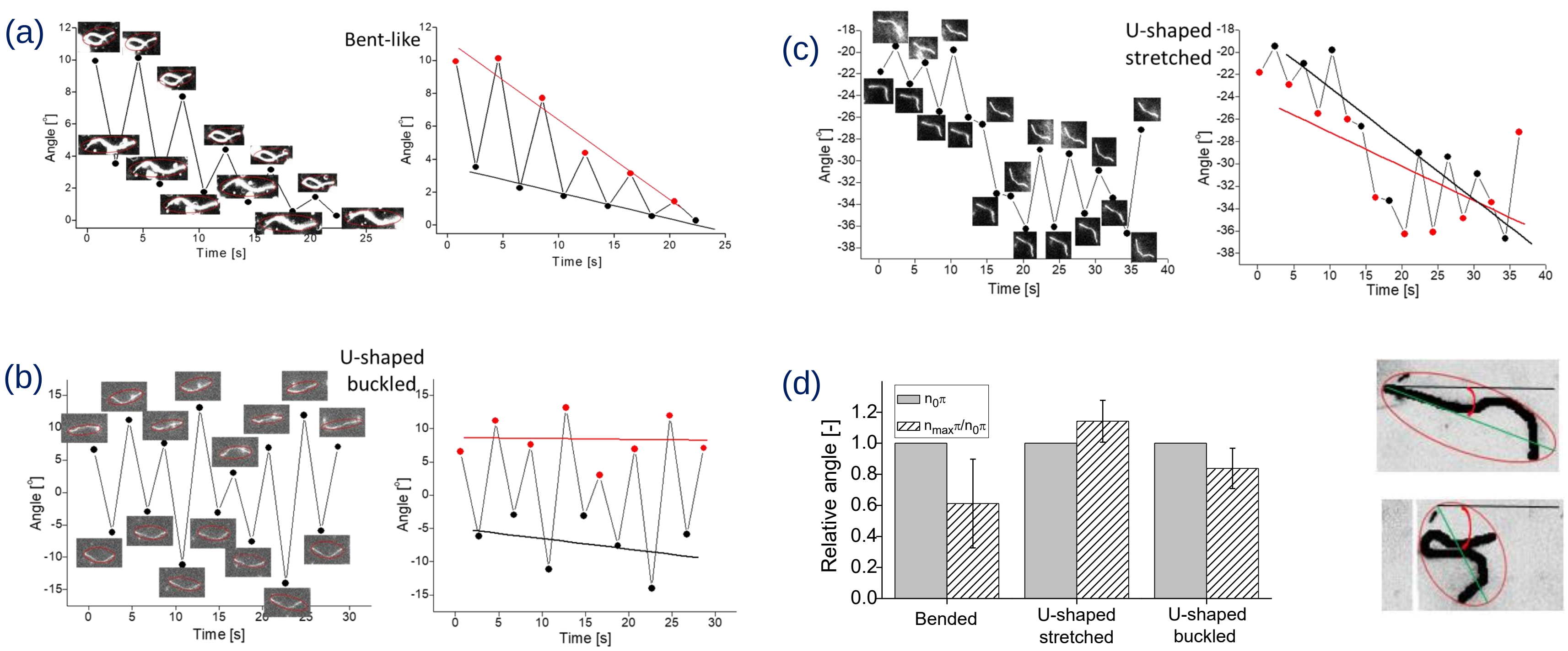


d	140±19 nm	128±27 nm
L	65±17 μm	31±5 μm
L_p	10±4.3 μm	7.5±7 μm
L_p/L	0.15±0.04	0.25±0.2
V_r	0.001±0.0007	0.001±0.0008

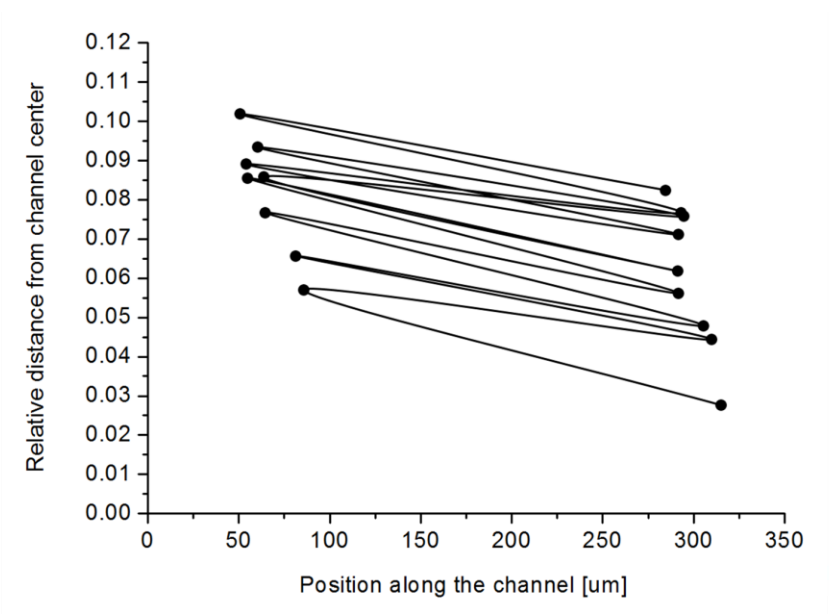
Elongation parameter

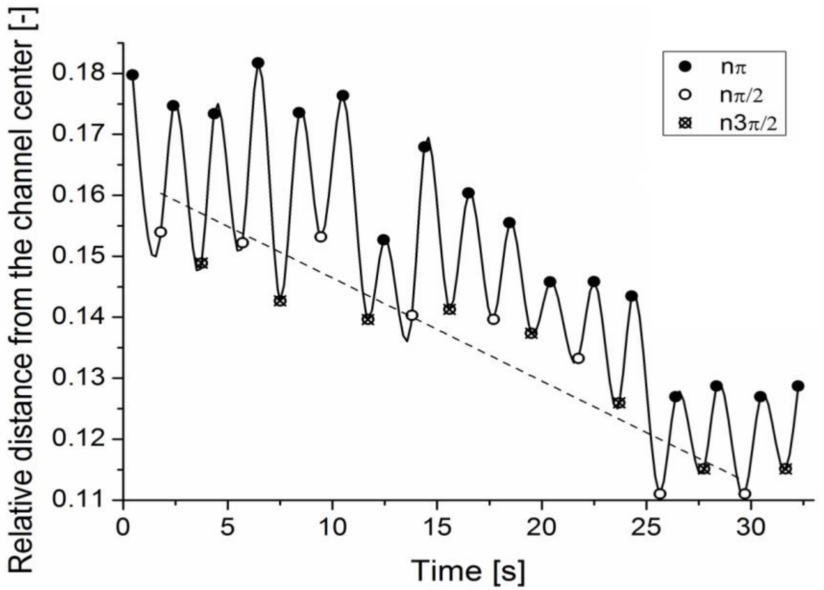


Orientation – inclination angle



Lateral migration

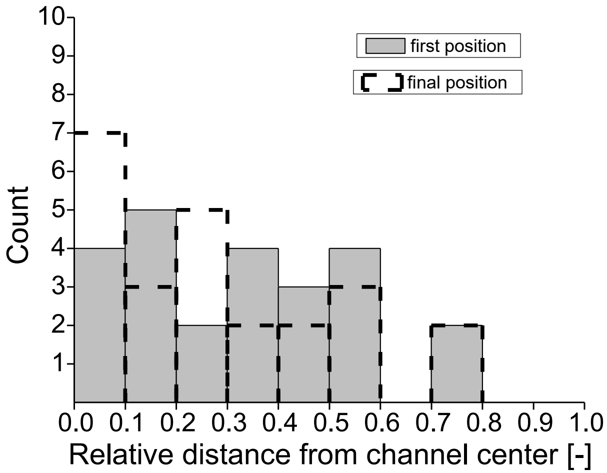




R = 90 nm; L = 32 μm; L_p = 25 μm; Re = 0.07; Sp =3; V_r = 0.0016

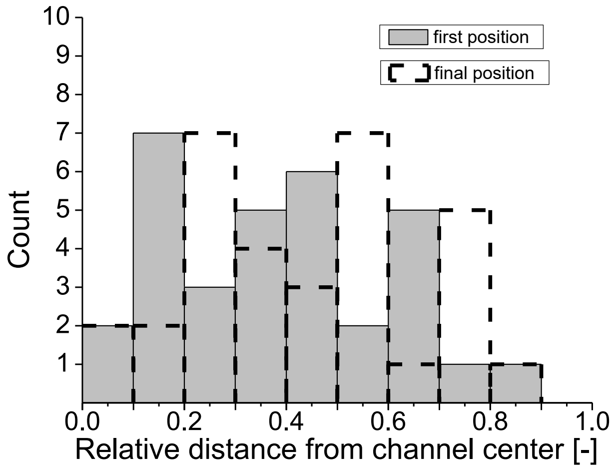
R = 53 nm; L = 41 μm; L_p = 2.89 μm; Re = 0.06; Sp =10; V_r = 0.0021

Lateral migration of hydrogel nanofilaments



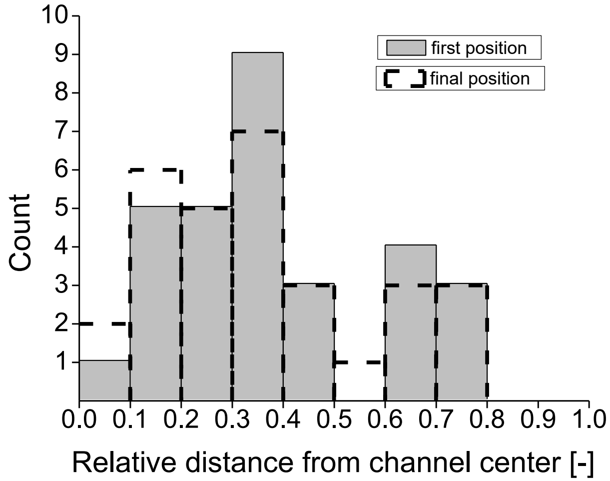
Bent-like filaments

V_{max} = 145 μm/s



U-shaped stretched filaments

V_{max} = 125 μm/s



U-shaped buckled filaments

V_{max} = 250 μm/s

Conclusions

Electrospinning technique is very good method for closing hydrogel material in the form of filament.

Mobility of nanofilaments depends on several factors like flow velocity, persistence length, contour length, local shear stress, buckling-bending properties.

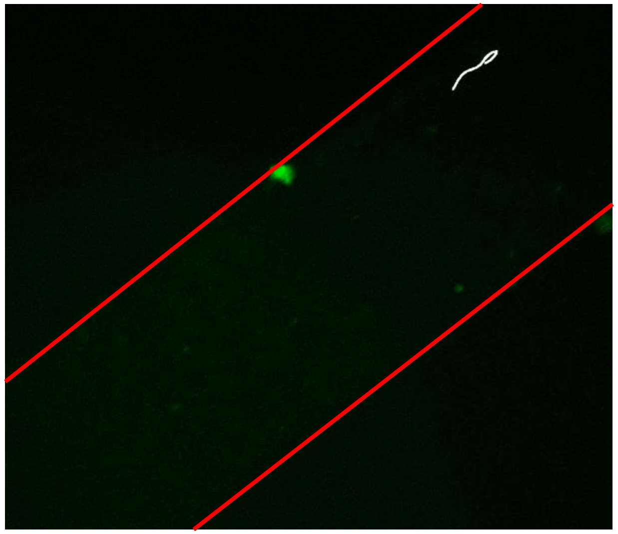
Confirmed lateral migration of deformable filaments

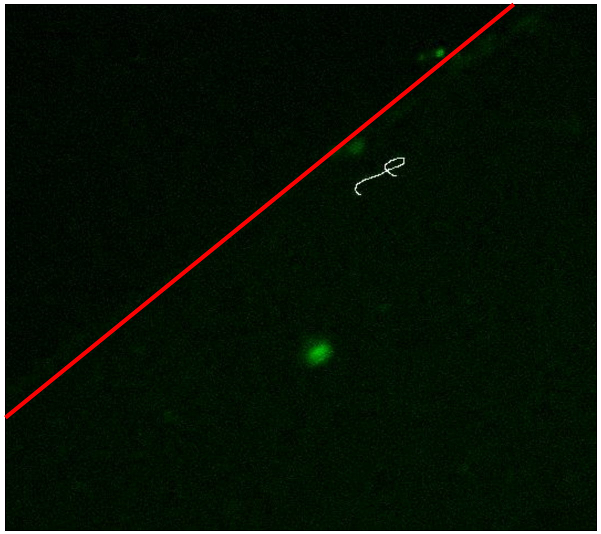
There is no evidence of predicted by the Stokesian model fiber flipping, i.e. sudden changes of orientation

Brownian fluctuations have rather secondary effects, stochasticity observed only at stop-flow time steps

Proposed experimental model may help to elucidate role of hydrodynamic interactions in describing physical phenomena responsible for the processes of folding and bending of long molecular chains (e.g. proteins, DNA).

Thank you for your attention!





U= 45µm/s, L =25µm, R = 50nm, D = 100µm, $Re_D=4 \cdot 10^{-3}$