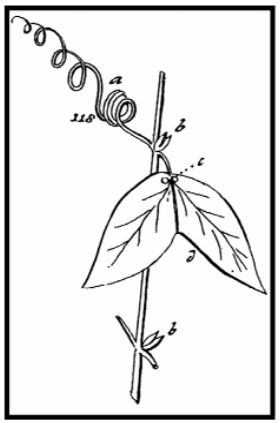
Extracting DNA twist rigidity from single molecule experiments

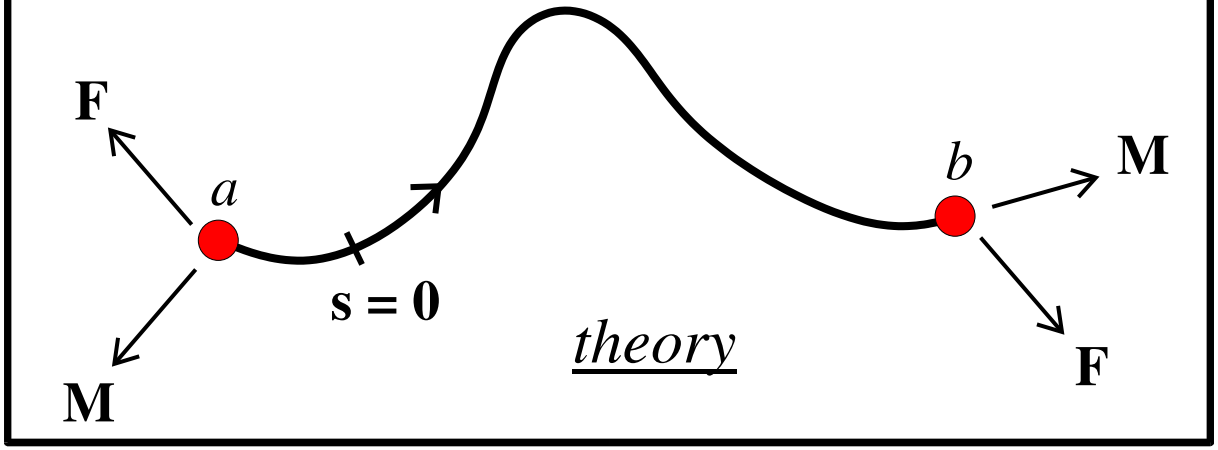
Sébastien Neukirch *CNRS & Université Paris 6 : Lab. de Modélisation en Mécanique*

Twisted Rods	Michael Thompson, Gert van der Heijden, Geoff Goss *Centre for Nonlinear Dynamics, Univ. College London, UK*
Continuation algorithm	Michael Henderson IBM, T.J. Watson Center, New York, USA
Mechanics of DNA	John H. Maddocks *Mathematics Institute, Ecole Polytechnique Fédérale, Lausanne*
Single molecule experiments	Vincent Croquette, David Bensimon, Gilles Charvin *Lab. Physique Statistique, Ecole Normale Supérieure, Paris*

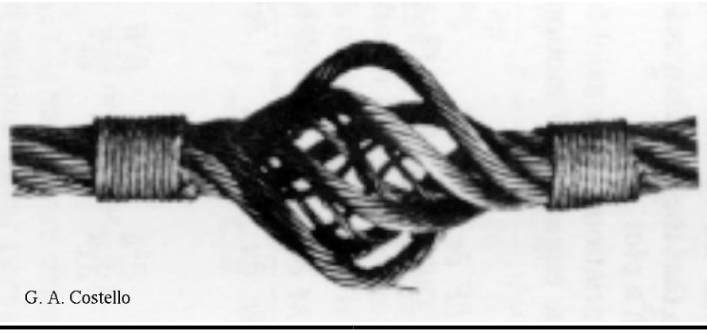
Elastic filaments

climbing plants

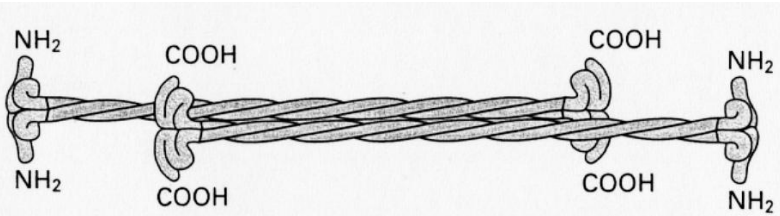




cables



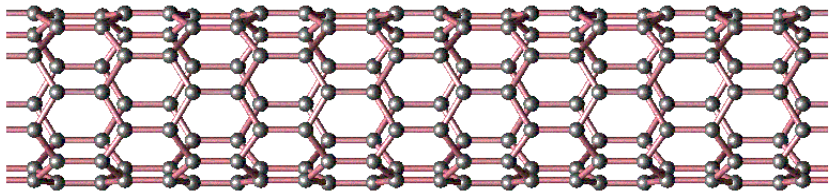
fibrous proteins



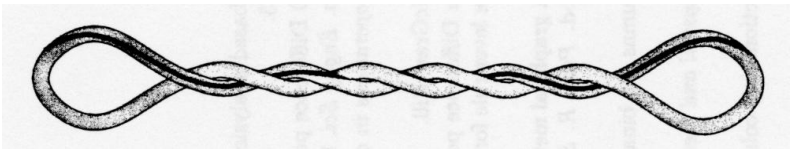
tétramère fait de deux dimères superenroulés étagés

applications

carbone nanotubes

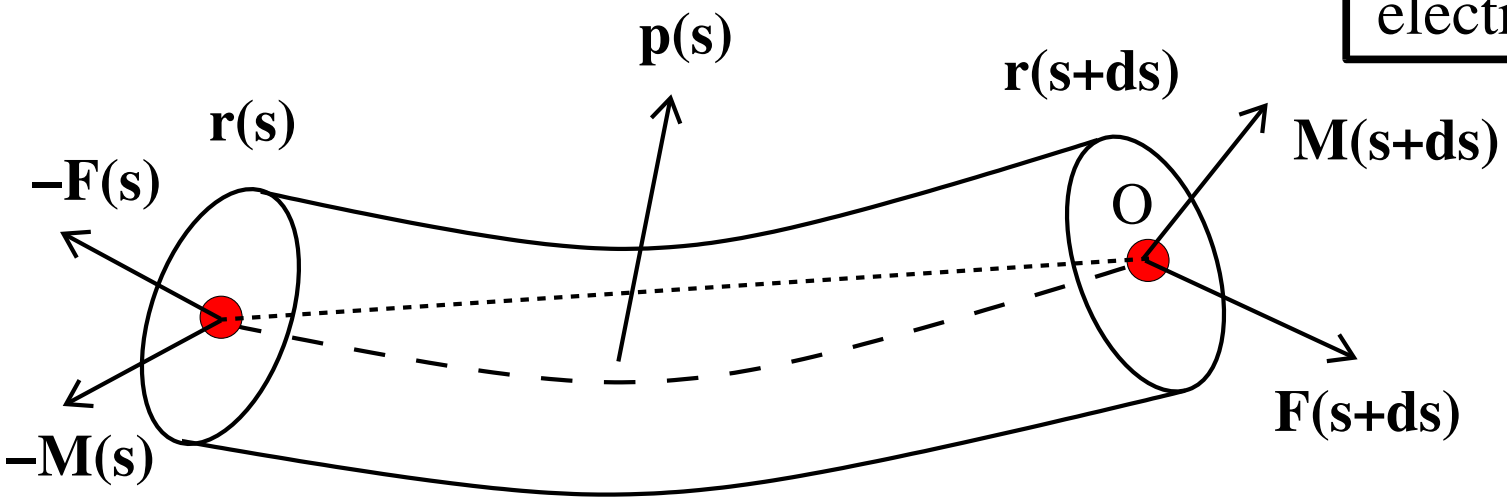


DNA super-coiling



Elastic rod in equilibrium

p(s) = external force
gravity, contact,
electrostatic, ...



force balance

$$p(s)\delta s + F(s+\delta s) - F(s) = 0$$

$$\boxed{p(s) + F'(s) = 0}$$

momentum balance

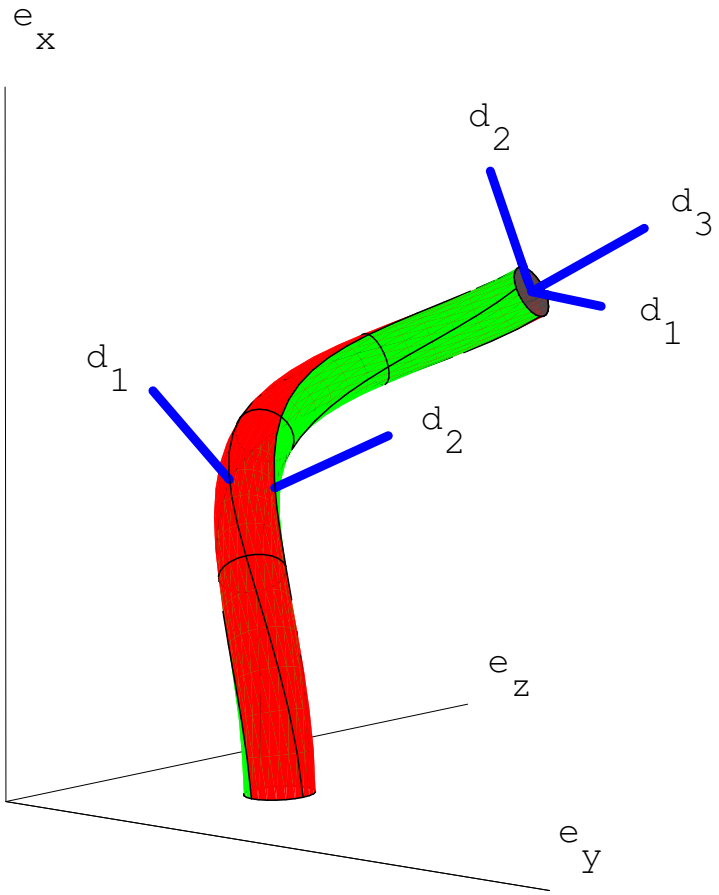
$$M(s+\delta s) - M(s) + \Pi_O[F(s+\delta s)] + \Pi_O[-F(s)] = 0$$

$$M(s+\delta s) - M(s) + 0 + (r(s) - r(s+\delta s)) \wedge (-F(s)) = 0$$

$$\boxed{M'(s) + r'(s) \wedge F(s) = 0}$$

Cosserat model for 1D elasticity

3 directors $\vec{d}_1, \vec{d}_2, \vec{d}_3$ on the top of $\vec{r}(s)$



no shear
no extension

$$\vec{r}\,'(s) = \vec{d}_3(s)$$

$$\begin{cases} \vec{d}_1\,'(s) = \vec{u}(s) \wedge \vec{d}_1 \\ \vec{d}_2\,'(s) = \vec{u}(s) \wedge \vec{d}_2 \\ \vec{d}_3\,'(s) = \vec{u}(s) \wedge \vec{d}_3 \end{cases}$$

evolution in SO(3)

$$\vec{u}(s) = \{u_1,\ u_2,\ u_3\}_{\vec{d}_1, \vec{d}_2, \vec{d}_3}$$

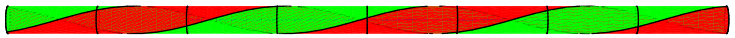
$$\vec{u}(s) = \{\kappa_1,\ \kappa_2,\ \tau\}_{\vec{d}_1, \vec{d}_2, \vec{d}_3}$$

u_1 , u_2 : curvature and u_3 : twist

Linear Constitutive Relations

Bending stiffness : K_0

$$\vec{M}\cdot\vec{d}_1 = K_0\, u_1$$
$$\vec{M}\cdot\vec{d}_2 = K_0\, u_2$$



Twist stiffness : K_3

$$\vec{M}\cdot\vec{d}_3 = K_3\, u_3$$

$K_0 = E\, I$

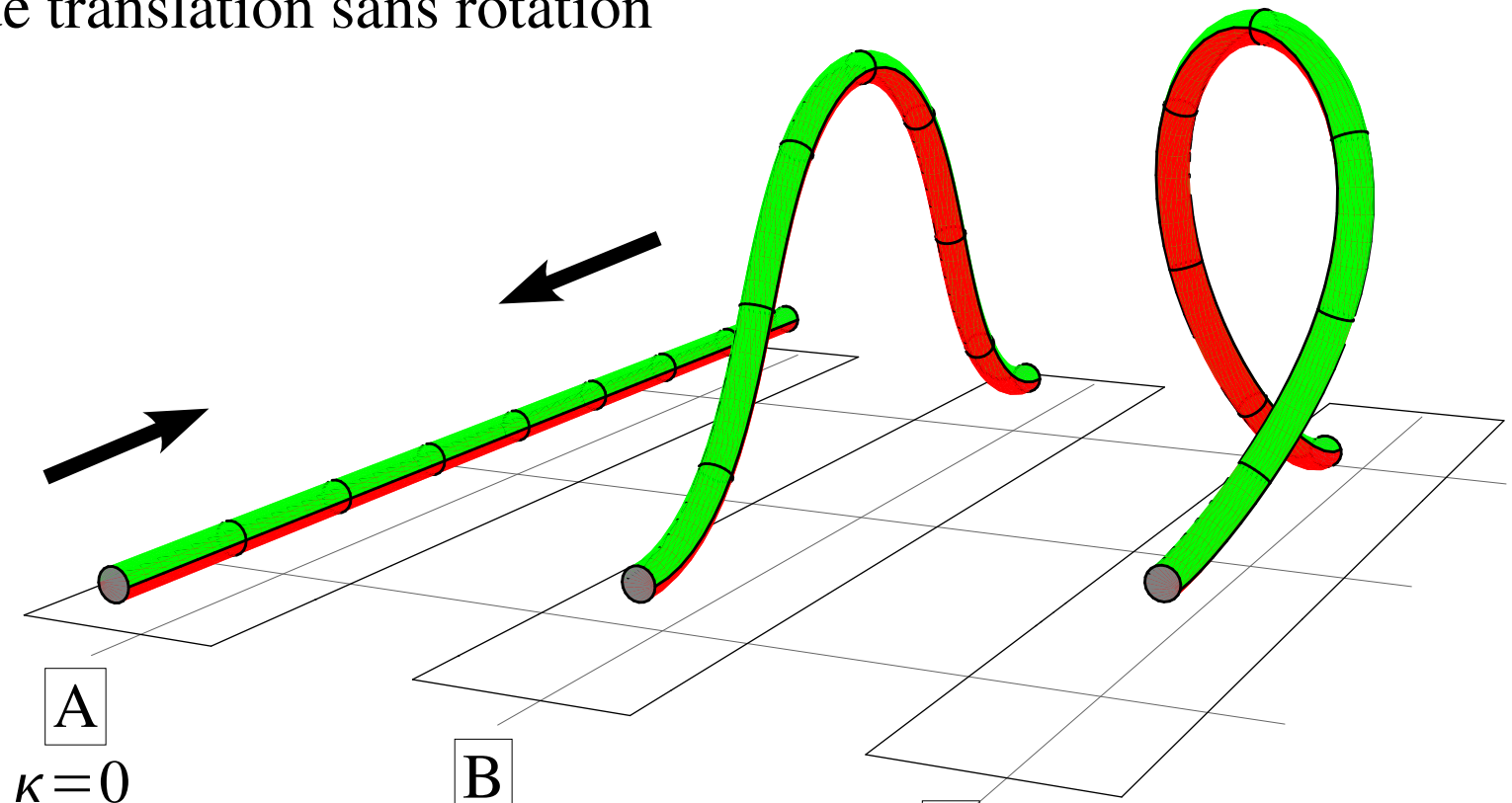
I : moment of inertia

E : Young's modulus

filament	E
Microtubule	1 GPa
DNA	1 GPa
Actine	2 GPa
Collagen	2 GPa
Rubber	2 GPa
Steel	200 GPa

Courbure et torsion sont liées

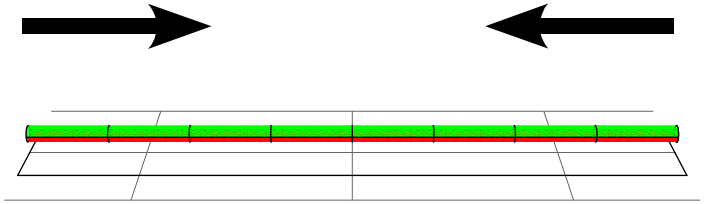
Expérience de translation sans rotation



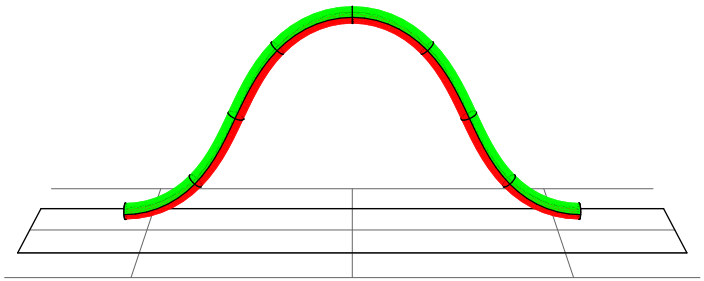
Courbure et torsion sont liées

Expérience de translation sans rotation

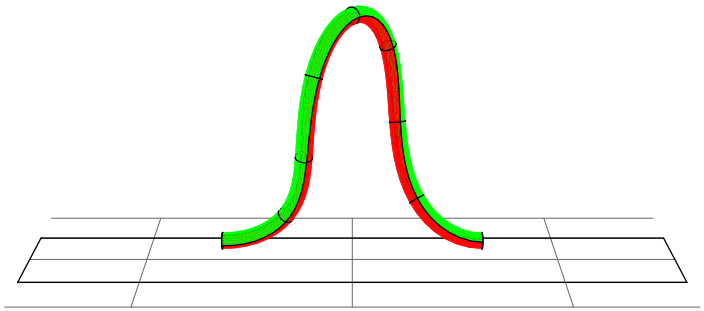
A $\kappa = 0$ $\tau = 0$



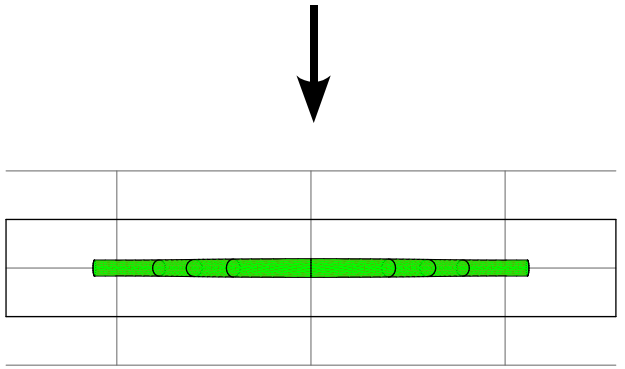
B $\kappa > 0$ $\tau = 0$ 2D

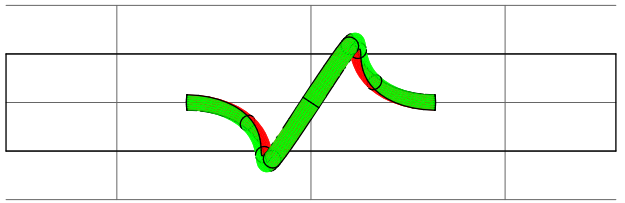


C $\kappa > 0$ $\tau > 0$ 3D



vues de dessus





Kirchhoff equations

21 ODEs with variable : s

ordinary differential equations

$$\frac{d}{ds}\vec{F}=\vec{p}$$

$$\frac{d}{ds}\vec{M}=\vec{F}\wedge\vec{d}_3$$

$$\frac{d}{ds}\vec{r}=\vec{d}_3$$

$$\frac{d}{ds}\vec{d}_i=\vec{u}\wedge\vec{d}_i$$

$$m_i=K_i u_i$$

linear elasticity

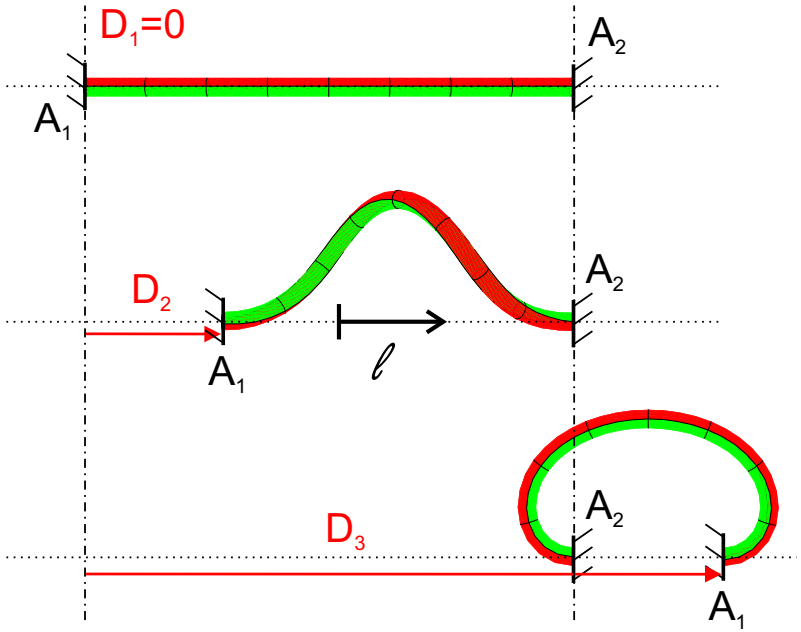
21 unknowns

$$\vec{F}(s)$$
$$\vec{M}(s)$$
$$\vec{r}(s)$$
$$\vec{d}_3(s)$$
$$\vec{d}_2(s)$$
$$\vec{d}_3(s)$$
$$\vec{u}(s)$$

$i=1,2,3$

boundary conditions

- how the rod is held
- few solutions are admissibles



$$\vec{d}_3(A_1)=\vec{d}_3(A_2)$$

$$\vec{r}(A_2)-\vec{r}(A_1)=k\,\vec{d}_3(A_2)$$

$$(D=L-k)$$

Find admissible equilibrium solutions : shooting method

initial conditions

$r(0)=(0,0,0)$

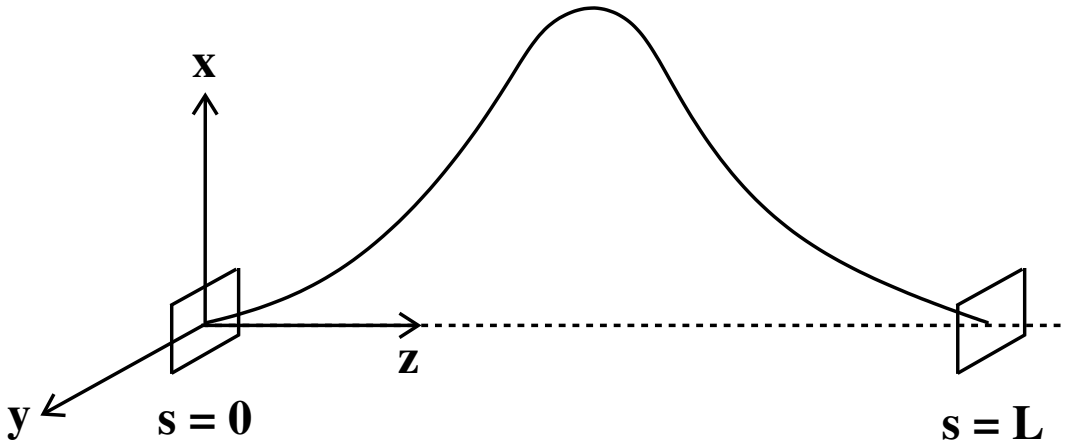
$d_1(0)=(1,0,0)$

$d_2(0)=(0,1,0)$

$d_3(0)=(0,0,1)$

parameters

$\vec{F}(0), \vec{M}(0)$



end conditions

$$\left.\begin{array}{l} x(L)=0 \\ y(L)=0 \\ d_3 x(L)=0 \\ d_3 y(L)=0 \end{array}\right\} \phi$$

solution of ODEs

$$\phi(\overbrace{\vec{F}(0), \vec{M}(0)}^{u})=0$$

$$\Leftrightarrow \phi(u)=0$$

$\phi \in \mathbb{R}^L$

$u \in \mathbb{R}^P$

this defines a *P-L* solution manifold

1D solution manifold : path following predictor-corrector scheme

1D solution manifold

$$\begin{cases}\phi_1(u_1,u_2,u_3)=0\\ \phi_2(u_1,u_2,u_3)=0\end{cases}$$

At each point :

1-(predictor)

we take a guess : Z_i

2-(corrector)

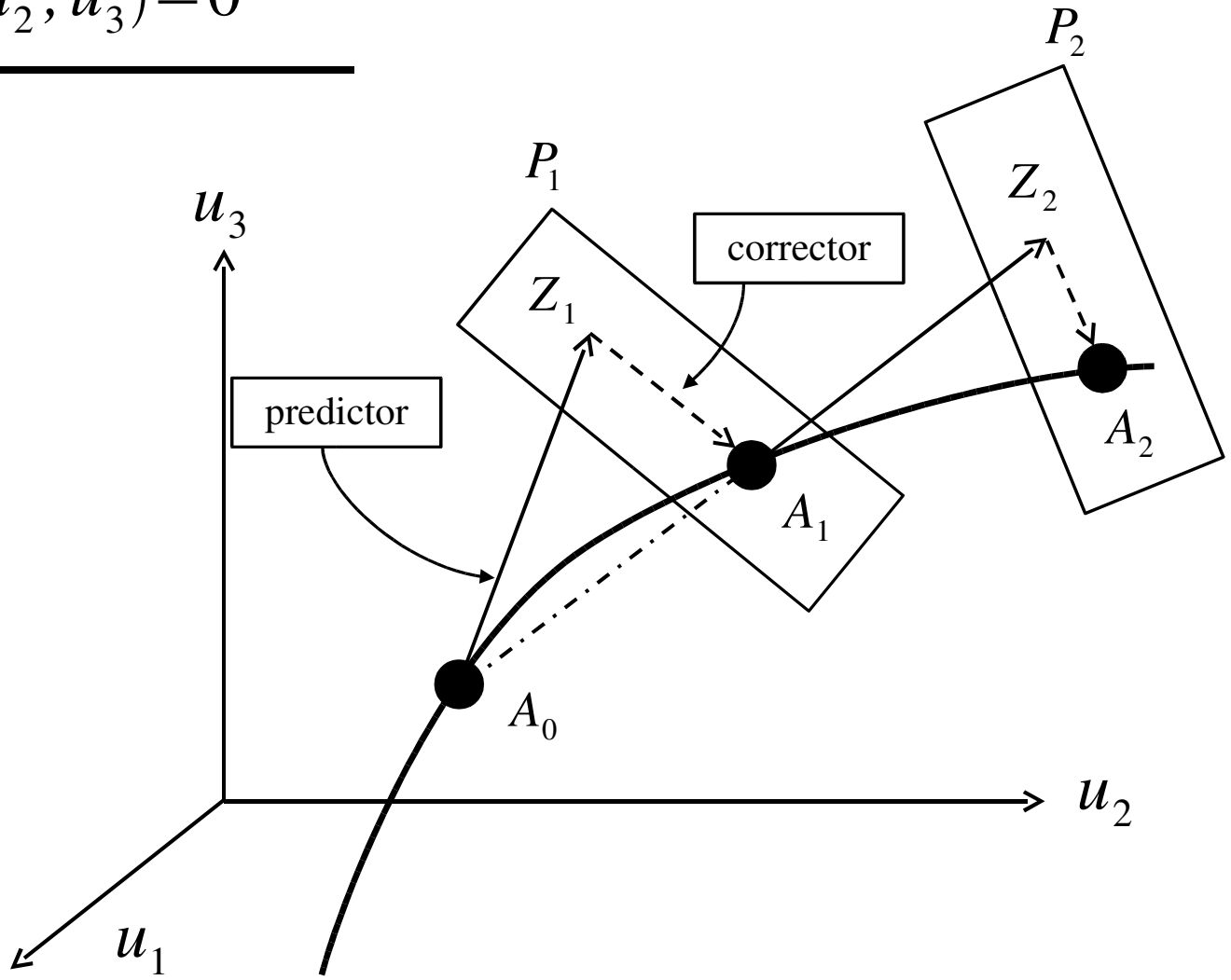
we define a projection :

$$P_i(u_1,u_2,u_3)=0$$

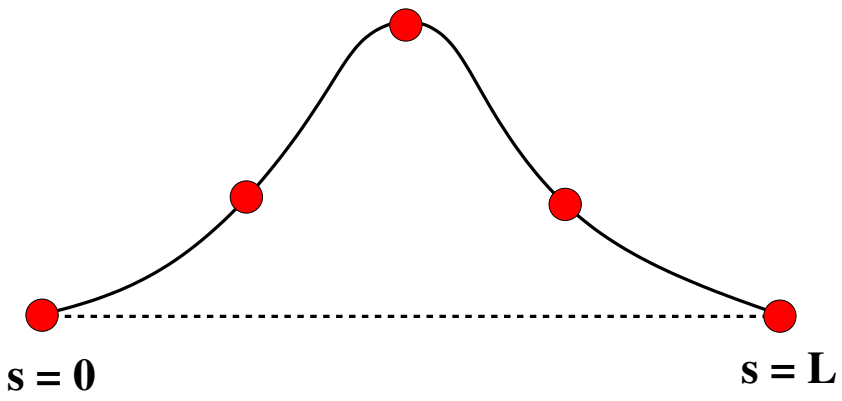
and we solve :

$$\begin{cases}\phi_1(u_1,u_2,u_3)=0\\ \phi_2(u_1,u_2,u_3)=0\\ P_i(u_1,u_2,u_3)=0\end{cases}$$

to obtain A_i



Find admissible equilibrium solutions : discretization methods

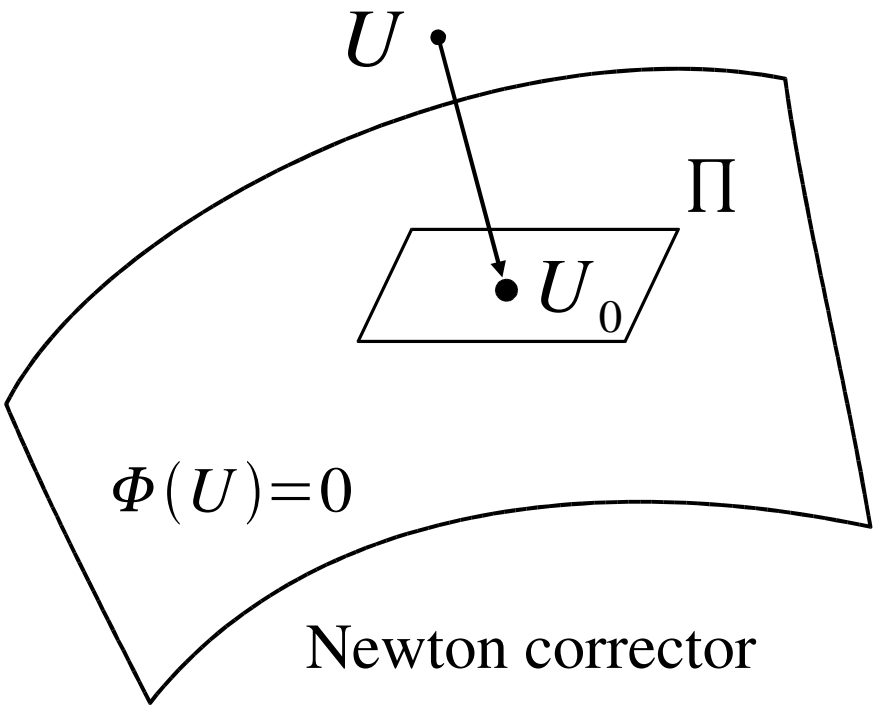


discretization over N intervals

$$\Phi(U)=0$$

- boundary conditions
- matching conditions

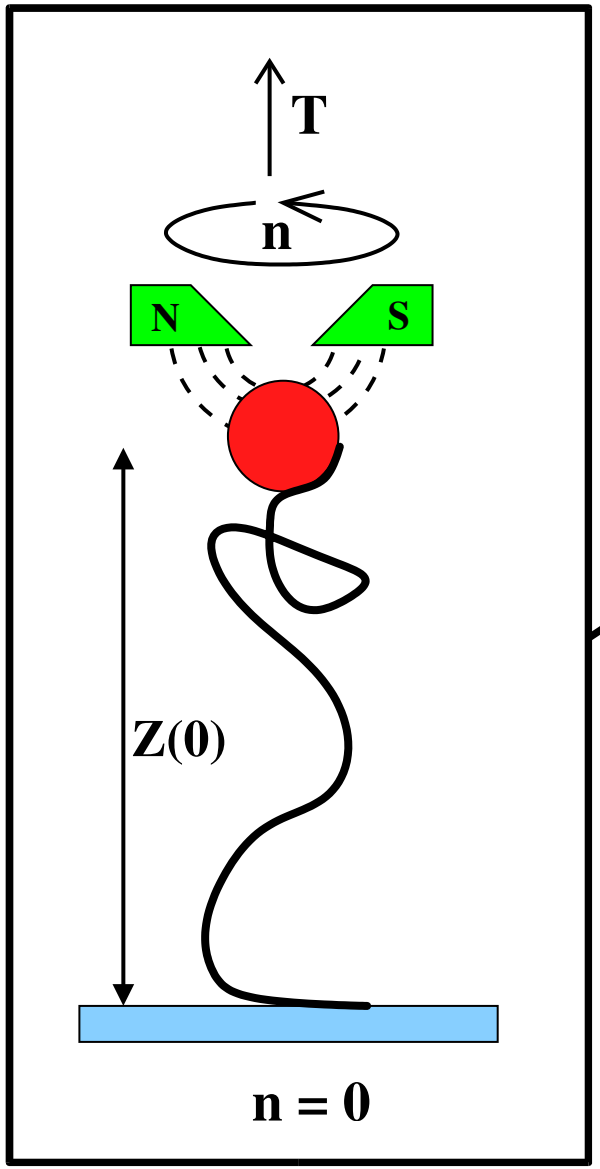
system of nonlinear algebraic equations

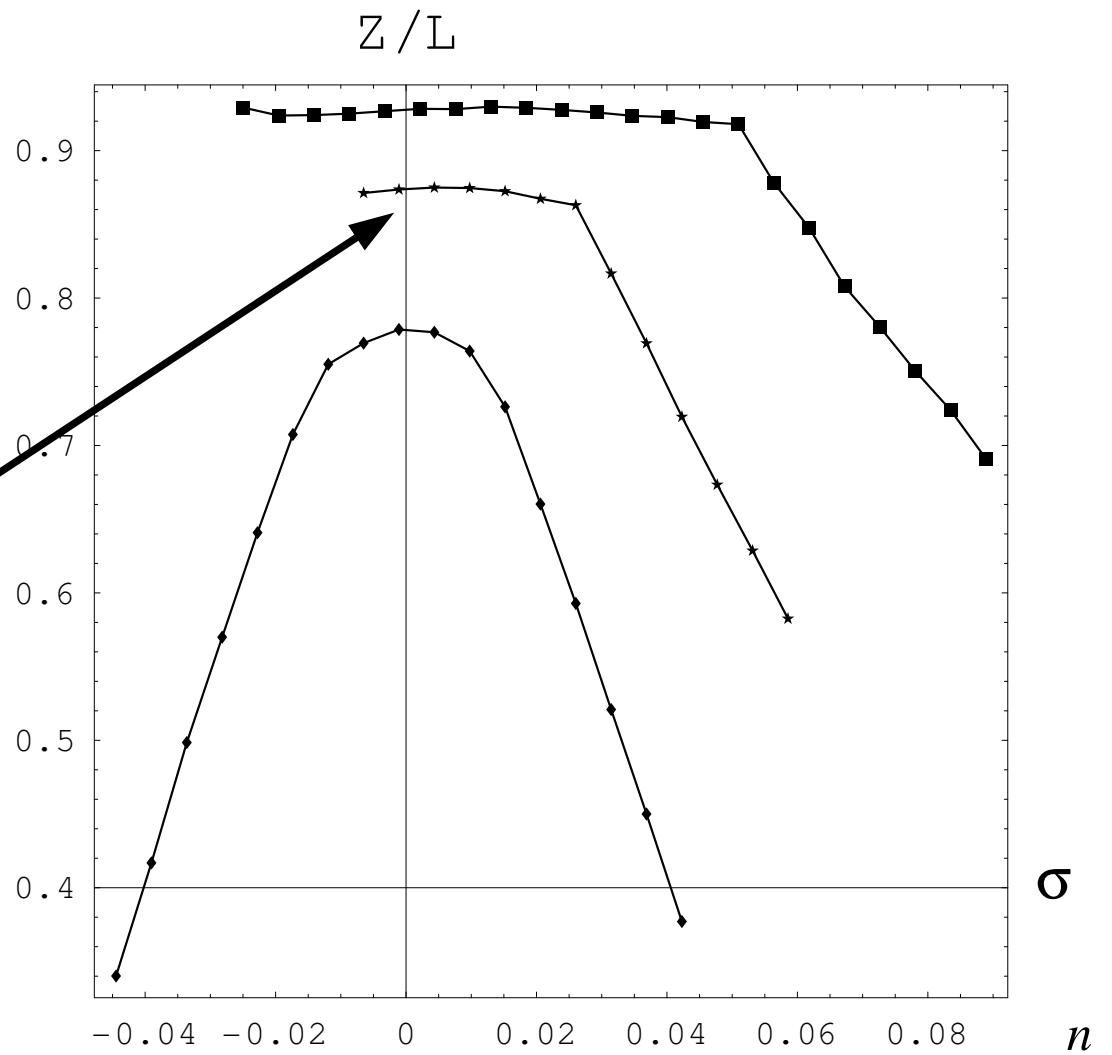


$$\underbrace{\Phi(U_0)}_{=0}=\Phi(U)+\frac{D\Phi}{DU}(U_0-U)+\ldots$$

1-we take a point U
2-compute Jacobian
3-kernel is tangent plane Π
4-we project orthogonaly : $U \rightarrow U_0$

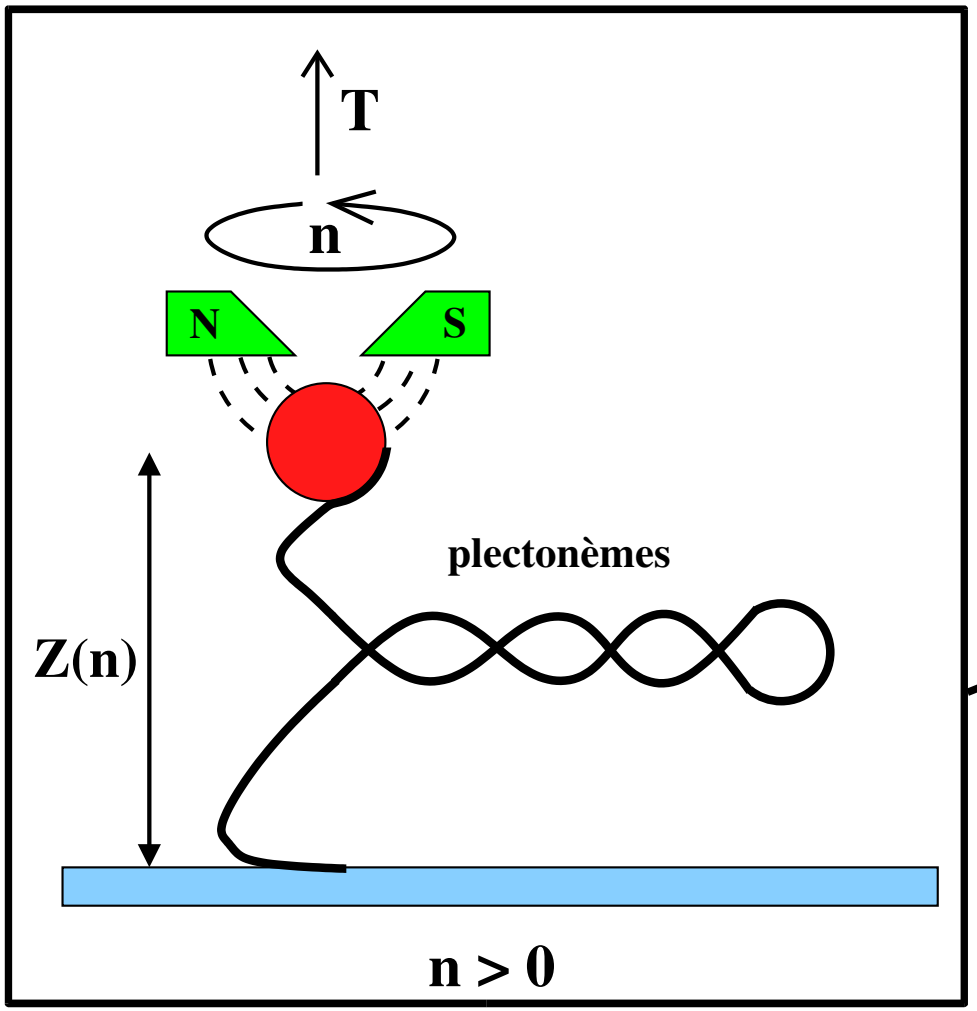
Pulling and twisting DNA

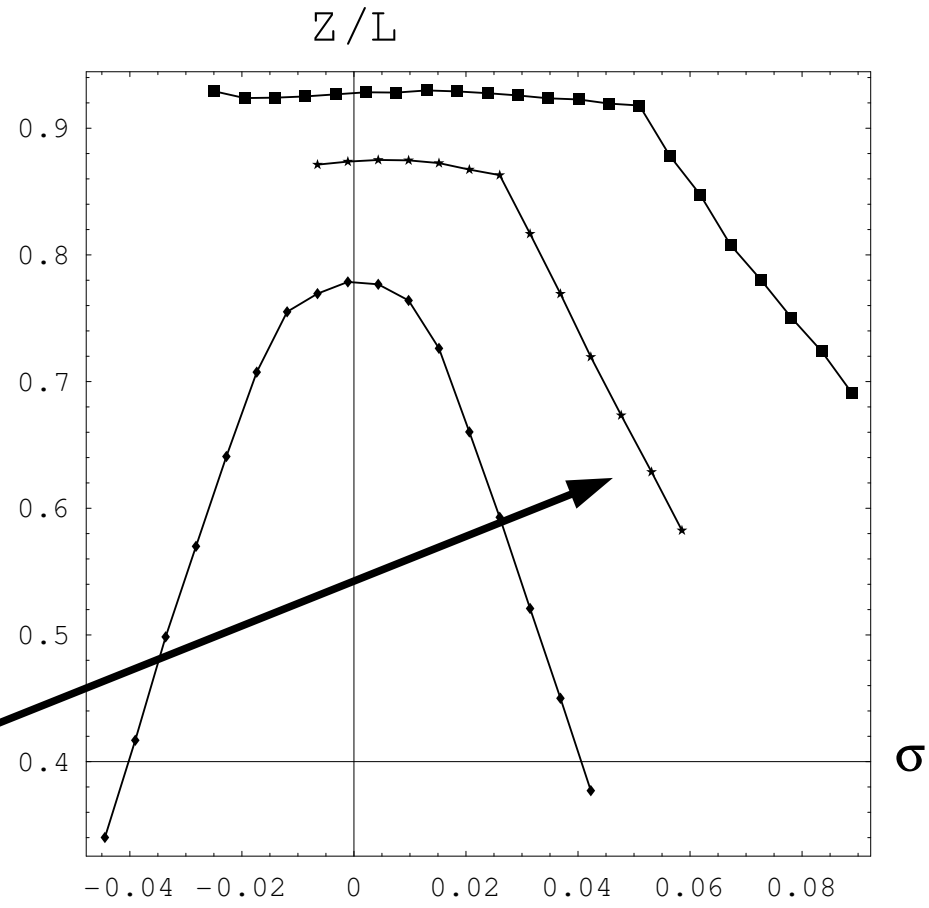




data from Gilles Charvin (LPS-ENS)

Pulling and twisting DNA

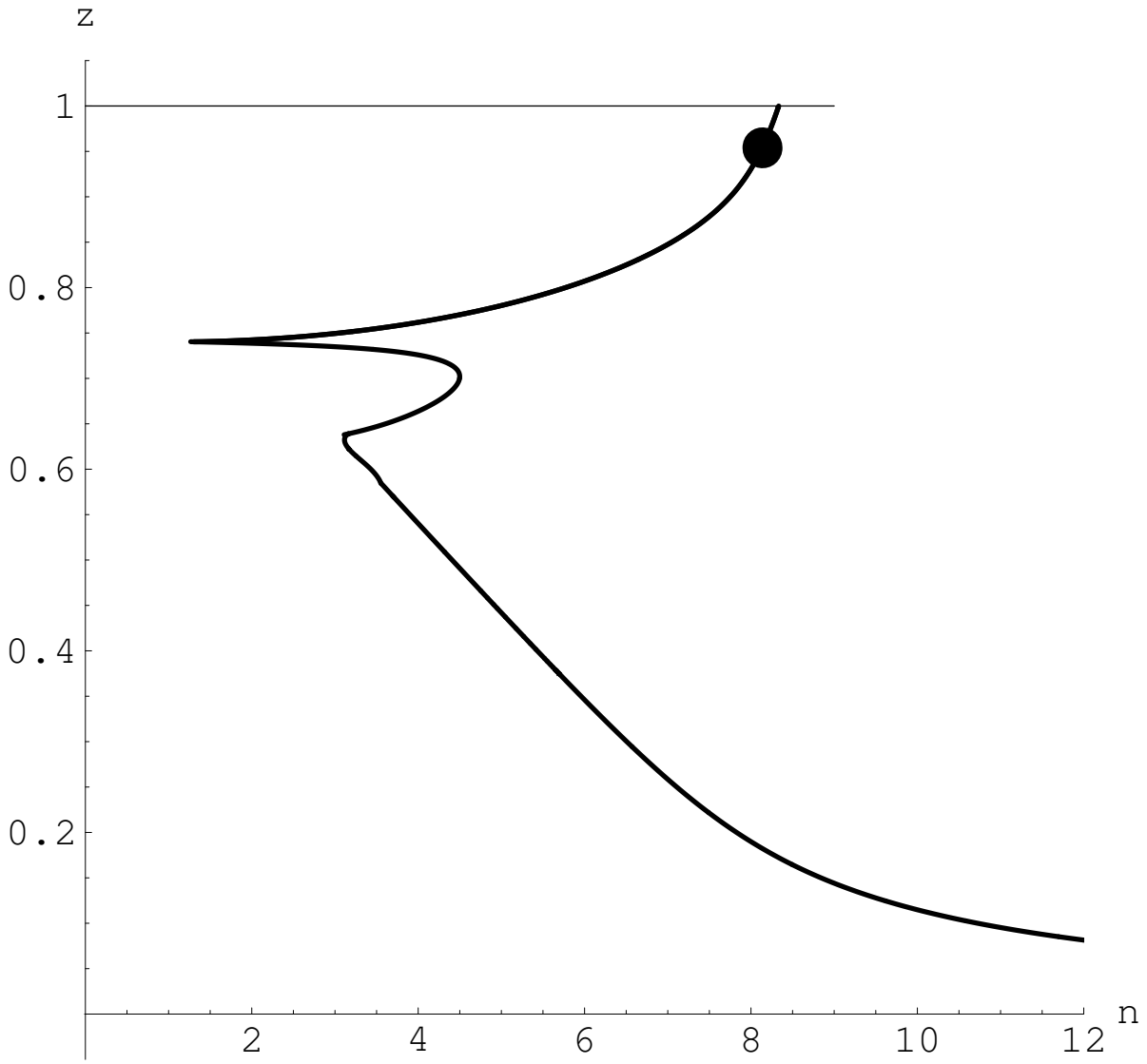




data from Gilles Charvin (LPS-ENS)

$$\sigma = \frac{n}{n_0}$$

Results : how a twisted rod coils



$$\frac{L}{2R} = 170$$

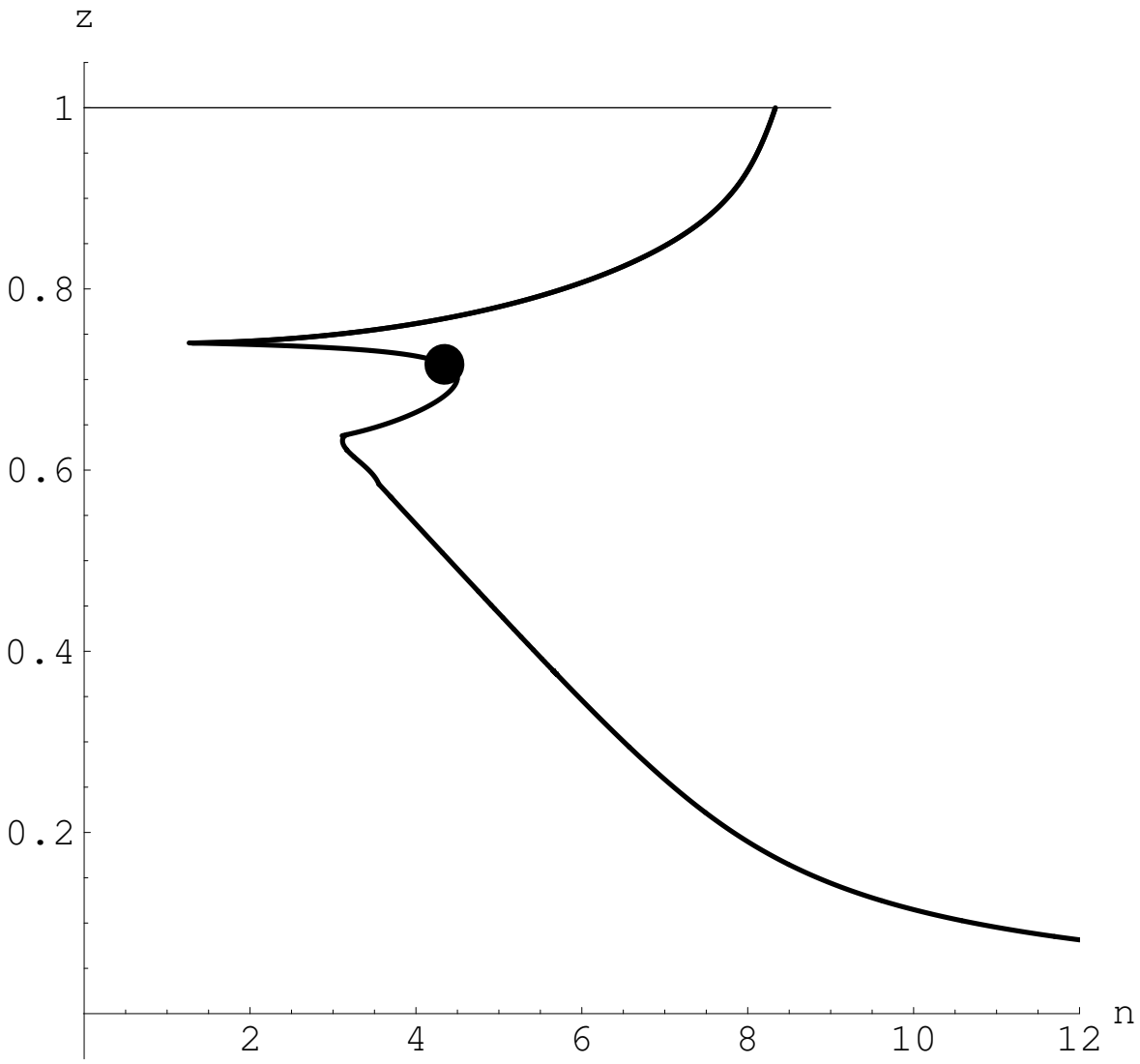
$$t = \frac{TL^2}{4\pi^2 K_0} = 6$$

0 contact(s)

$$\frac{Z}{L} = 0.95$$

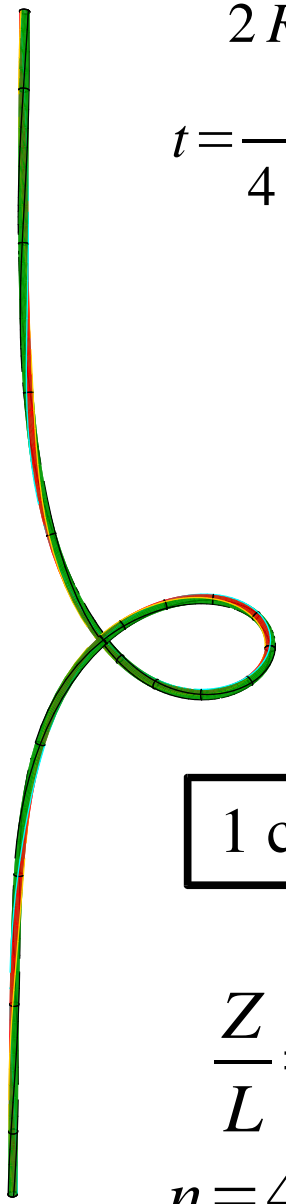
$$n = 8.1 \; turns$$

Results : how a twisted rod coils



$$\frac{L}{2R} = 170$$

$$t = \frac{TL^2}{4\pi^2 K_0} = 6$$

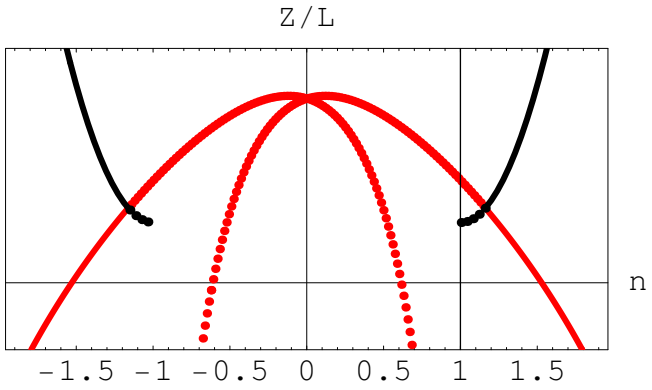


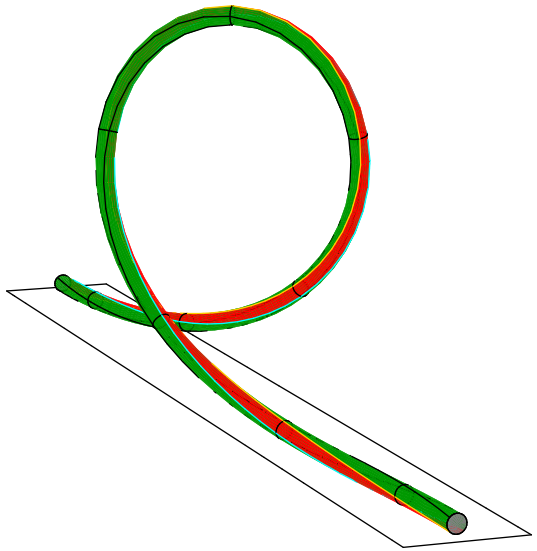
1 contact(s)

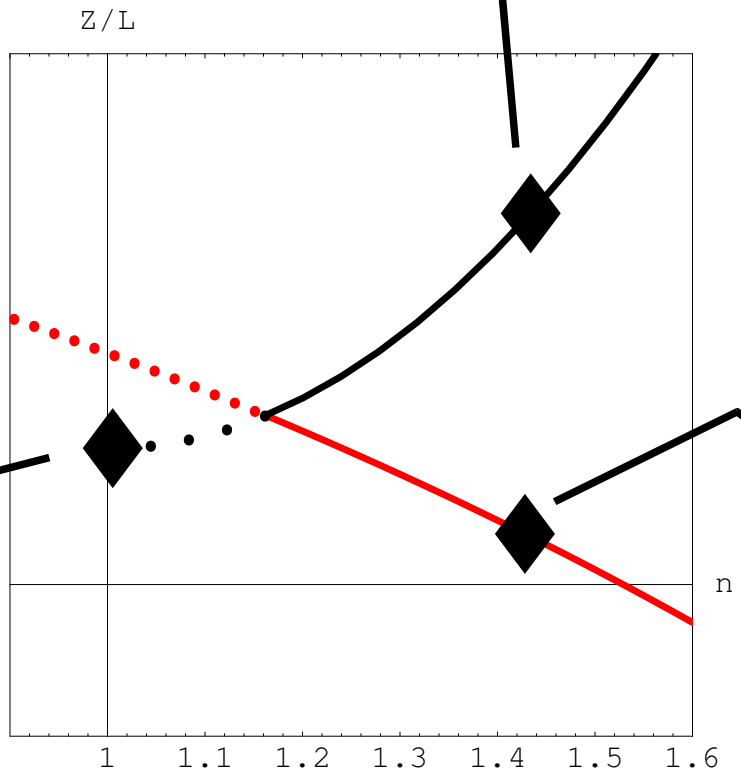
$$\frac{Z}{L} = 0.72$$

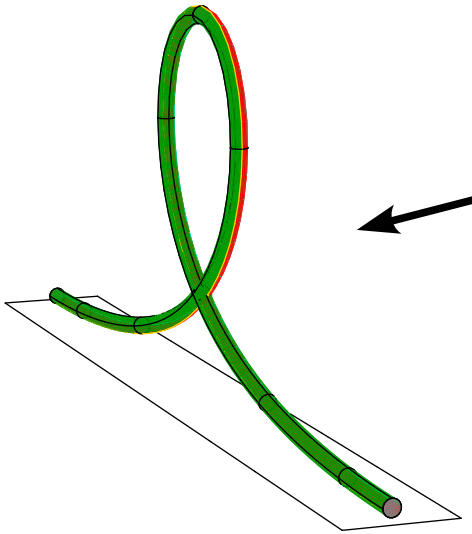
$$n = 4.3 \text{ turns}$$

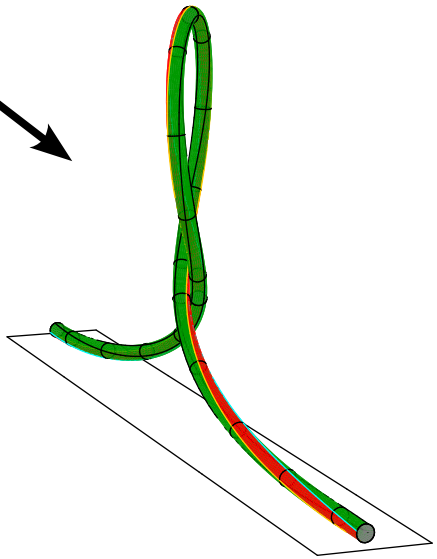
Bifurcation : 0 contact -> 1 contact



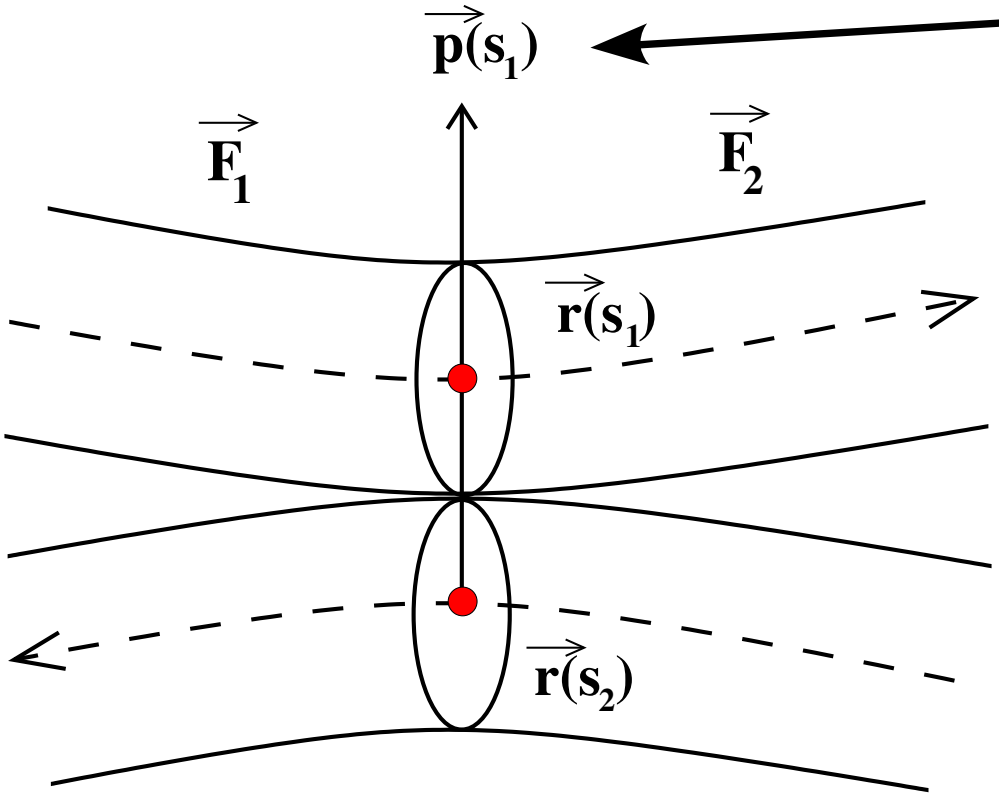








Hard-wall contact, no friction



force from strand at s_2
acting on strand at s_1

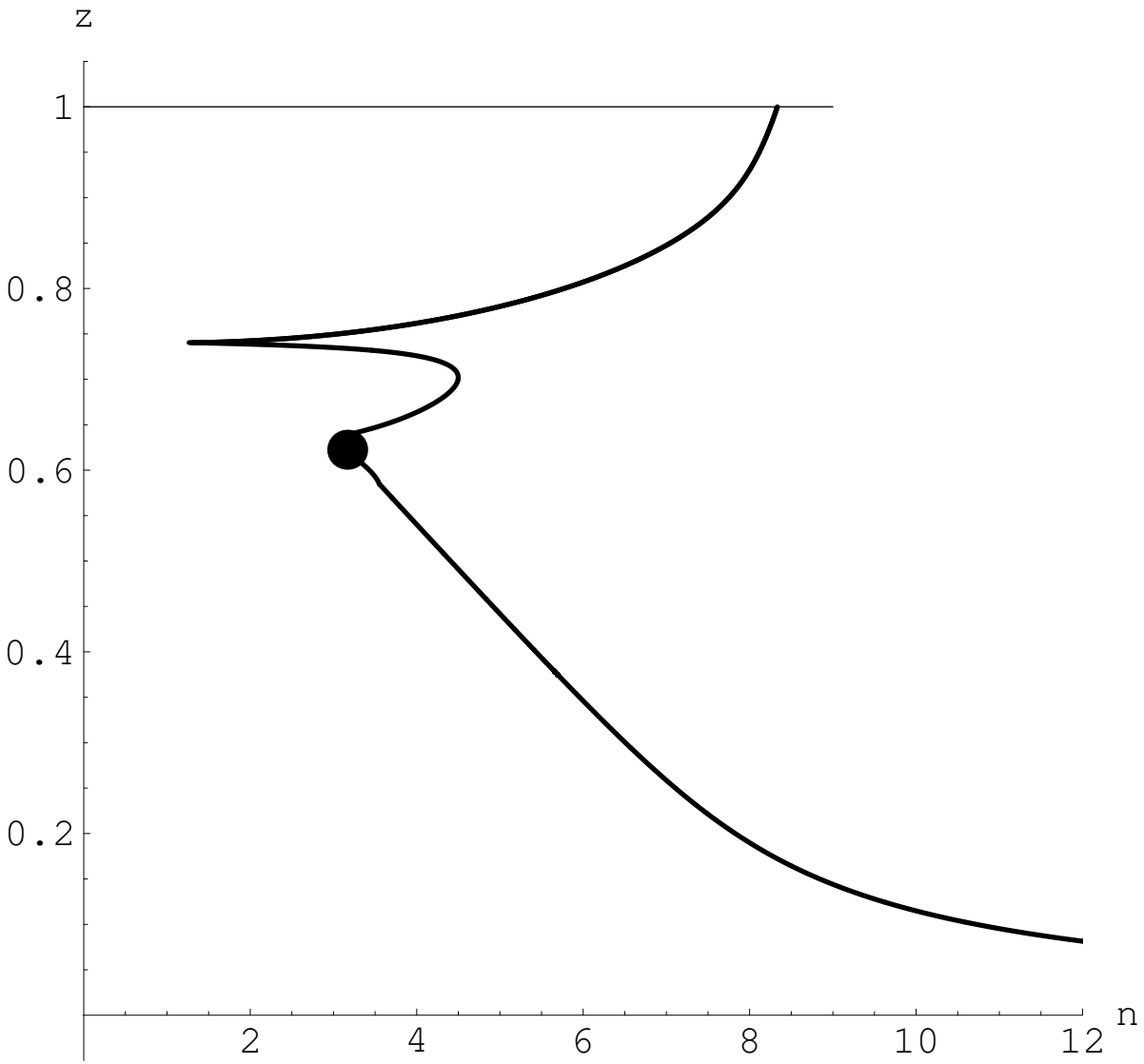
$$\vec{F}_1 = \vec{p} + \vec{F}_2$$

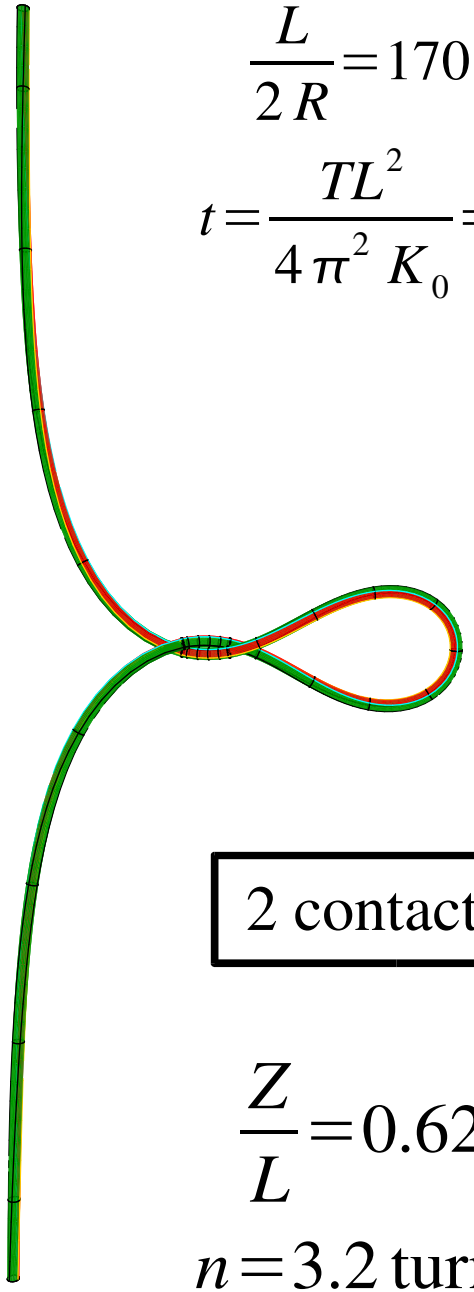
$$\vec{p} = p\,\frac{\vec{r}(s_1) - \vec{r}(s_2)}{\left|\vec{r}(s_1) - \vec{r}(s_2)\right|}$$

touching conditions :

$$\begin{cases} \left|\vec{r}(s_1) - \vec{r}(s_2)\right| = \text{thickness} \\ \left(\vec{r}(s_1) - \vec{r}(s_2)\right) \perp \vec{d}_3(s_1) \\ \left(\vec{r}(s_1) - \vec{r}(s_2)\right) \perp \vec{d}_3(s_2) \end{cases}$$

Results : how a twisted rod coils





$$\frac{L}{2R} = 170$$

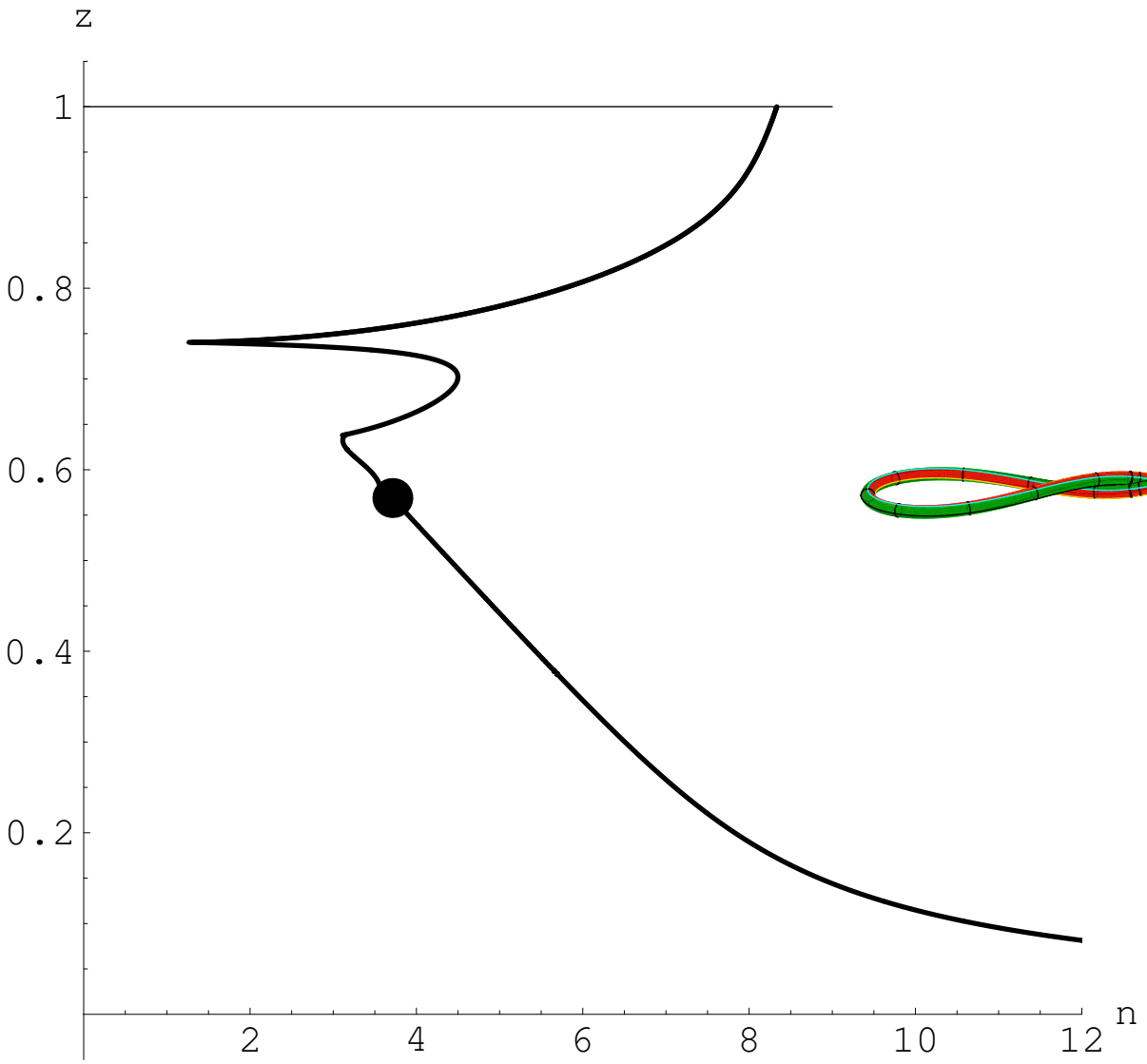
$$t = \frac{TL^2}{4\pi^2 K_0} = 6$$

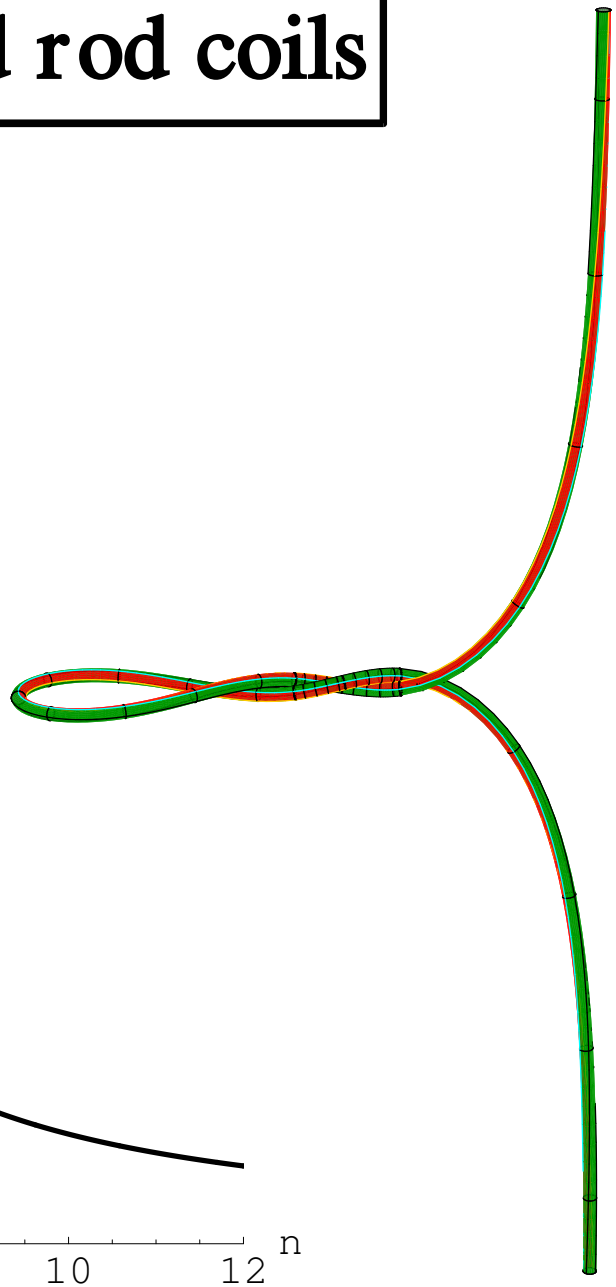
2 contact(s)

$$\frac{Z}{L} = 0.62$$

$$n = 3.2 \text{ turns}$$

Results : how a twisted rod coils





$$\frac{L}{2R}=170$$

$$t=\frac{TL^2}{4\pi^2 K_0}=6$$

3 contact(s)

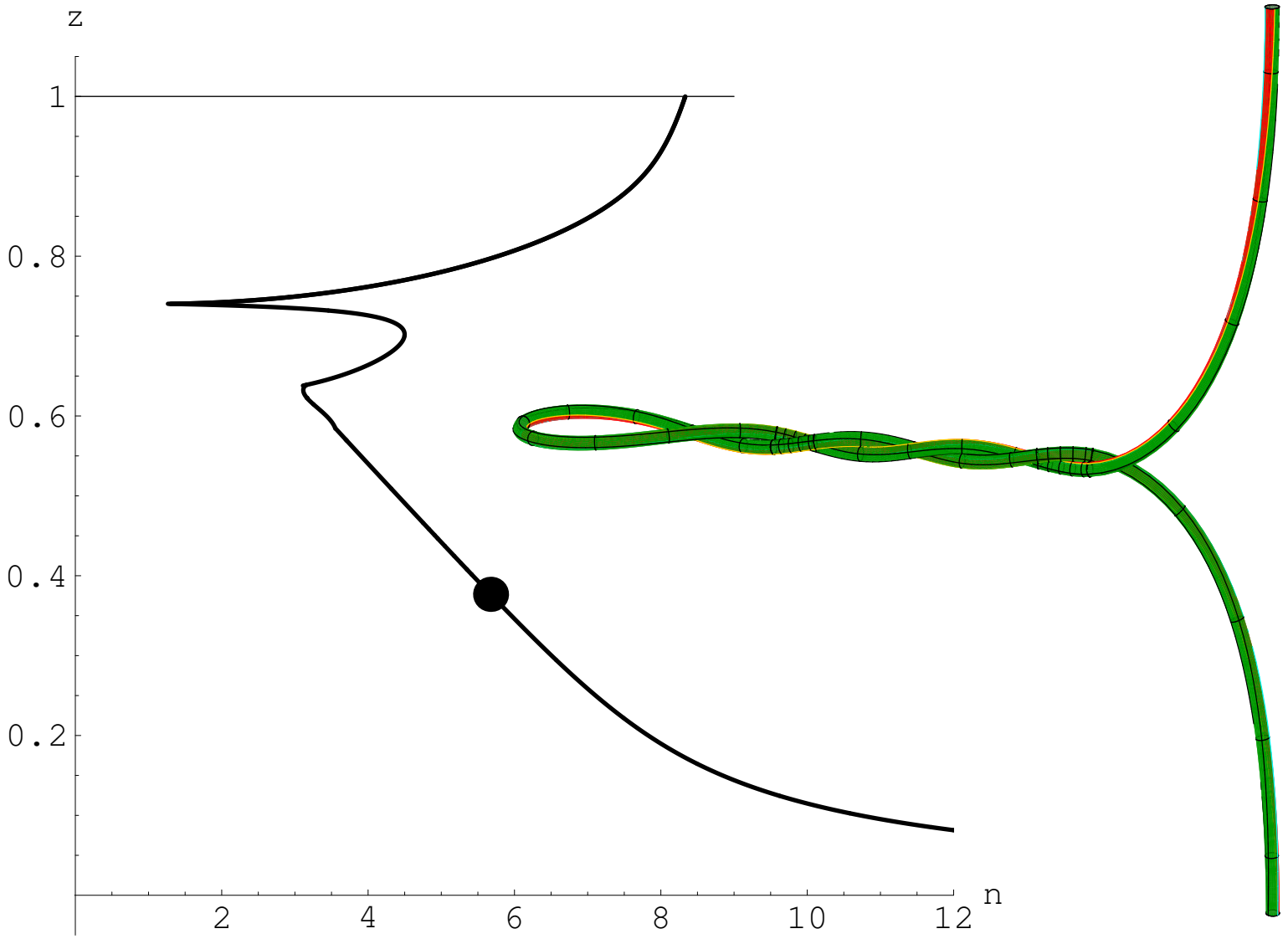
$$\frac{Z}{L}=0.57$$

$$n=3.7 \text{ turns}$$

Results : how a twisted rod coils

$$\frac{L}{2R} = 170$$

$$t = \frac{TL^2}{4\pi^2 K_0} = 6$$



1L1 contact(s)

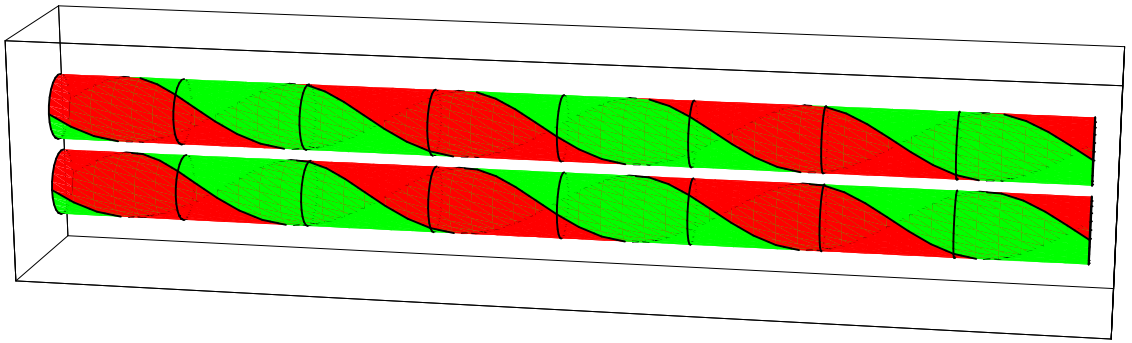
$$\frac{Z}{L} = 0.38$$

$$n = 5.7 \text{ turns}$$

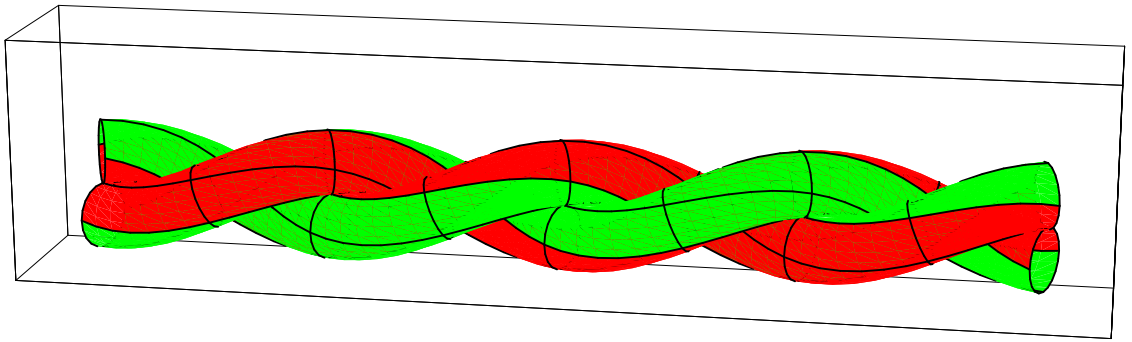
Filaments coiled in helical structures

Previous work : Fraser & Stump (1998) , Coleman & Swigon (2000)

Lk = 6
Tw = 6
Wr = 0



Lk = 6
Tw = 2.35
Wr = 3.65

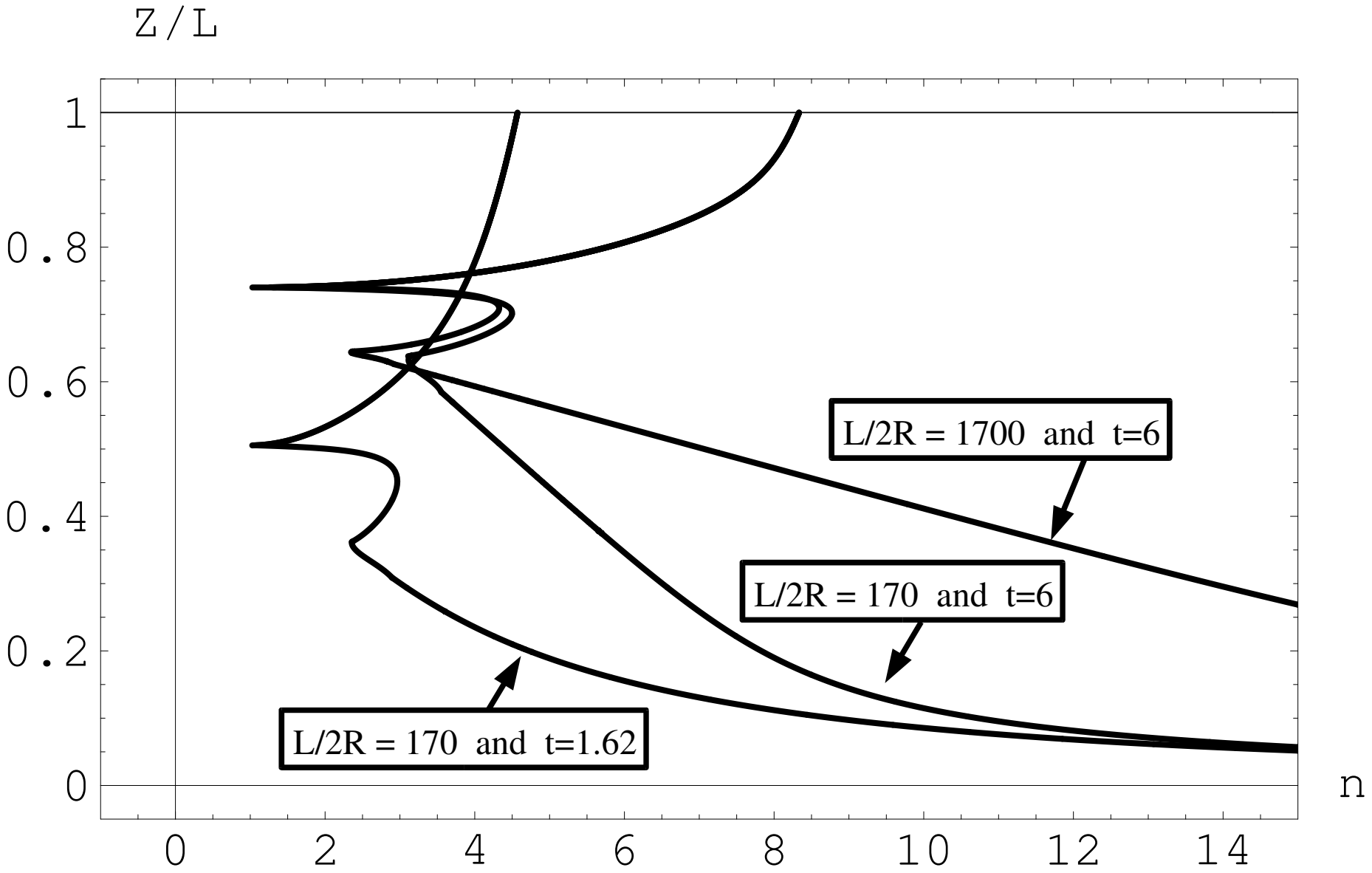


$$2\ K_0\, n \sin^3\theta \cos\theta + \epsilon\, n\, K_3\, R\tau \cos 2\theta + R^2\, F \sin\theta - \epsilon\, R\, M \cos\theta = 0$$

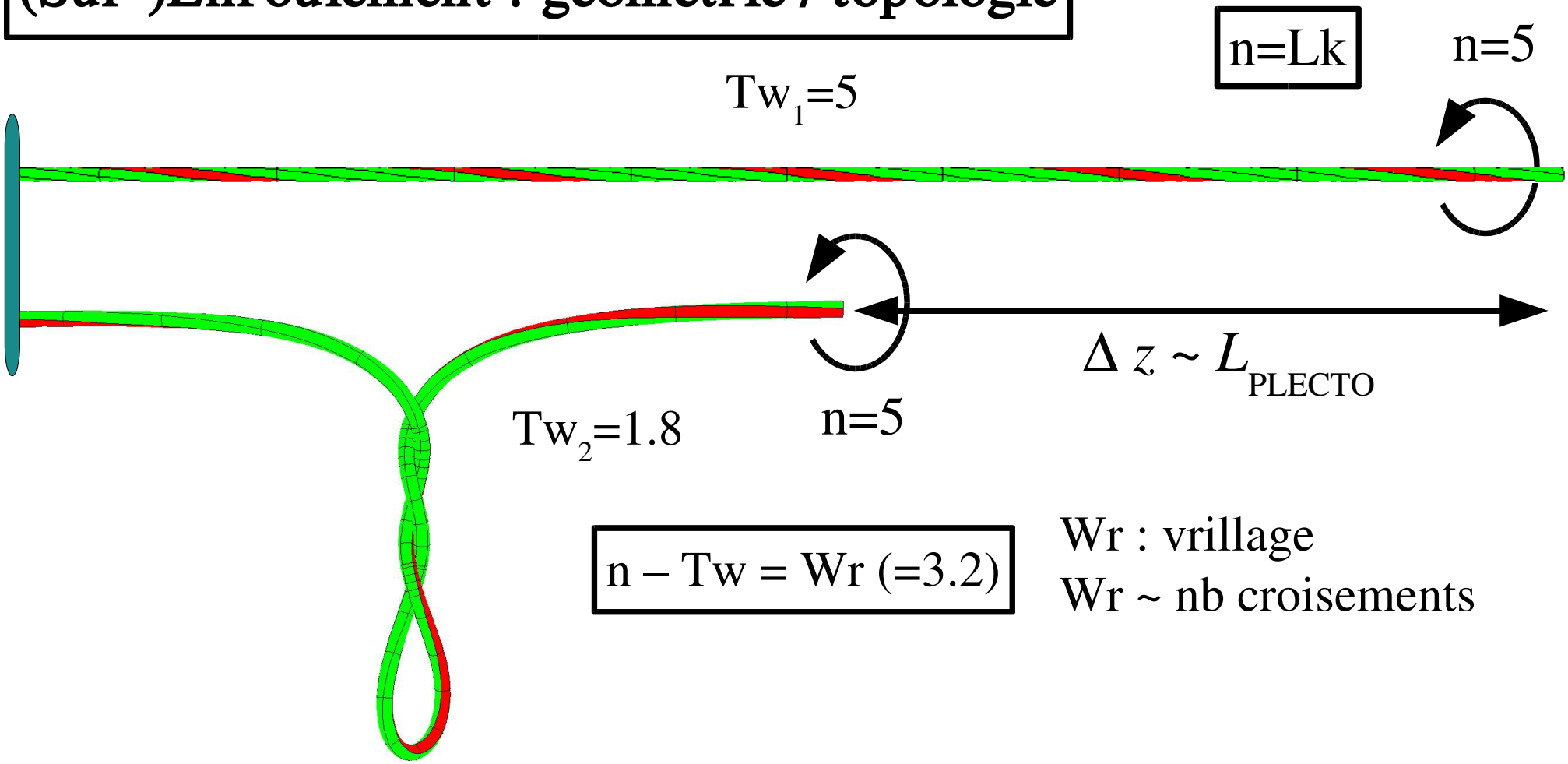
$$p R^3 = \frac{\sin^2\theta}{\cos 2\theta}\left(K_0 \sin^2\theta + \frac{R^2 F}{n}\cos\theta - \epsilon \frac{R M}{n}\sin\theta \right)$$

ε=+-1 : handedness
n : nb of strands
F, M : external stress

Slope of linear part : fonction of *t* and *L/R*



(Sur-)Enroulement : géométrie / topologie



n – Tw = Wr (=3.2)

Wr : vrillage
Wr ~ nb croisements

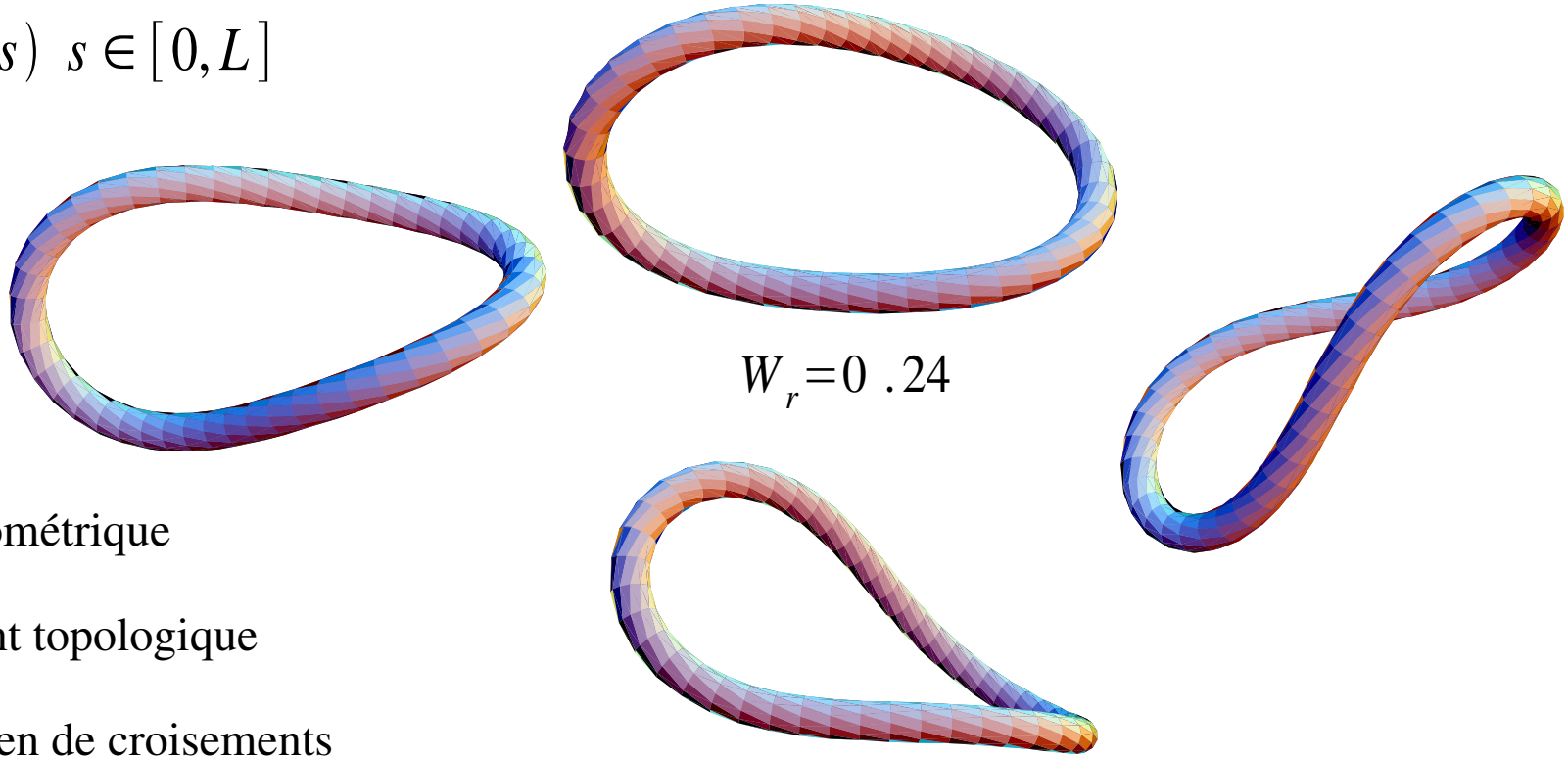
$$\frac{1}{2\pi}\int_0^L \dot{\psi}(1-\cos\theta)\,ds = 1.2 = Wr \ \underline{mod}\ 1$$

$$\frac{1}{2\pi}\int_0^L (\dot{\psi}+\dot{\phi})\,ds = 3 = Lk \ \underline{mod}\ 1$$

Vrillage d'une courbe : writhe

$$W_r(C) = \frac{1}{4\pi} \oiint \frac{\left(r(s_1) - r(s_2)\right) \cdot \left(t(s_1) \times t(s_2)\right)}{|r(s_1) - r(s_2)|^3} \, ds_1 \, ds_2$$

$$C : s \to r(s) \quad s \in [0, L]$$



$W_r = 0.24$

- $W_r \in R$
- invariant géométrique
- PAS invariant topologique
- nombre moyen de croisements vus sous tous les angles

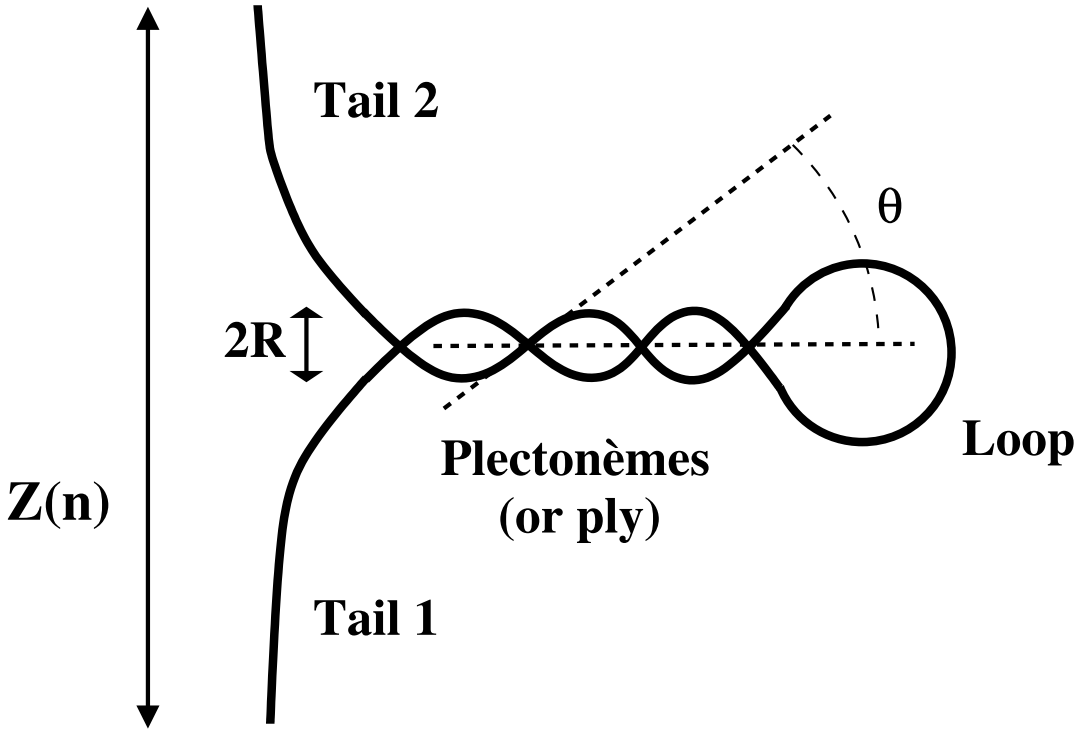
Topology : Writhe

Wr is given by a double integral, hence not additive (a priori).

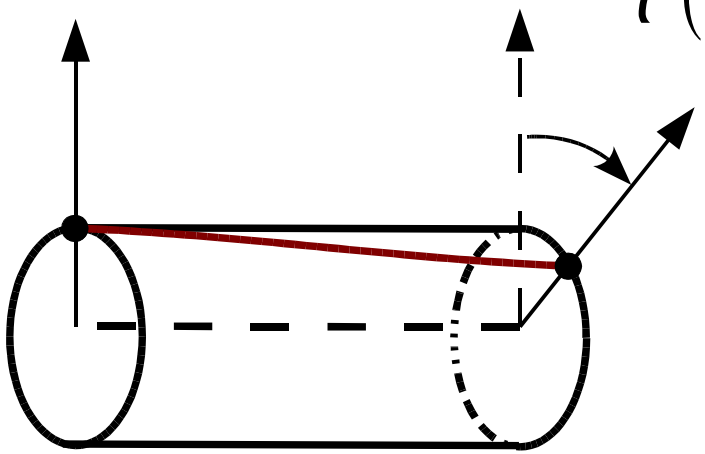
But there is Fuller theorem (1978) : $\int_0^L \int_0^L \cdots ds\, ds' \to \int_0^L \cdots ds$

$$Wr = Wr_{Plecto} + Wr_{Loop} + Wr_{Tails}$$ (we neglect Wr_{Loop} et Wr_{Tails})

$$Wr \approx Wr_{Plecto} = -\epsilon \frac{L_{Plecto}}{4\,\pi R} \sin(2\theta)$$



Twist of a curve



$\tau(s)$ rotation of the section arount the tangent

$$Tw = \frac{1}{2\pi}\int_0^L \tau\, ds = \frac{1}{2\pi}\tau\, L$$

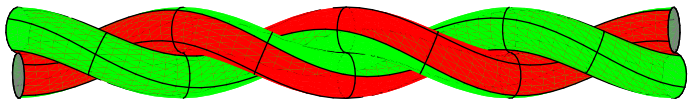
$Tw \in \mathbb{R}$

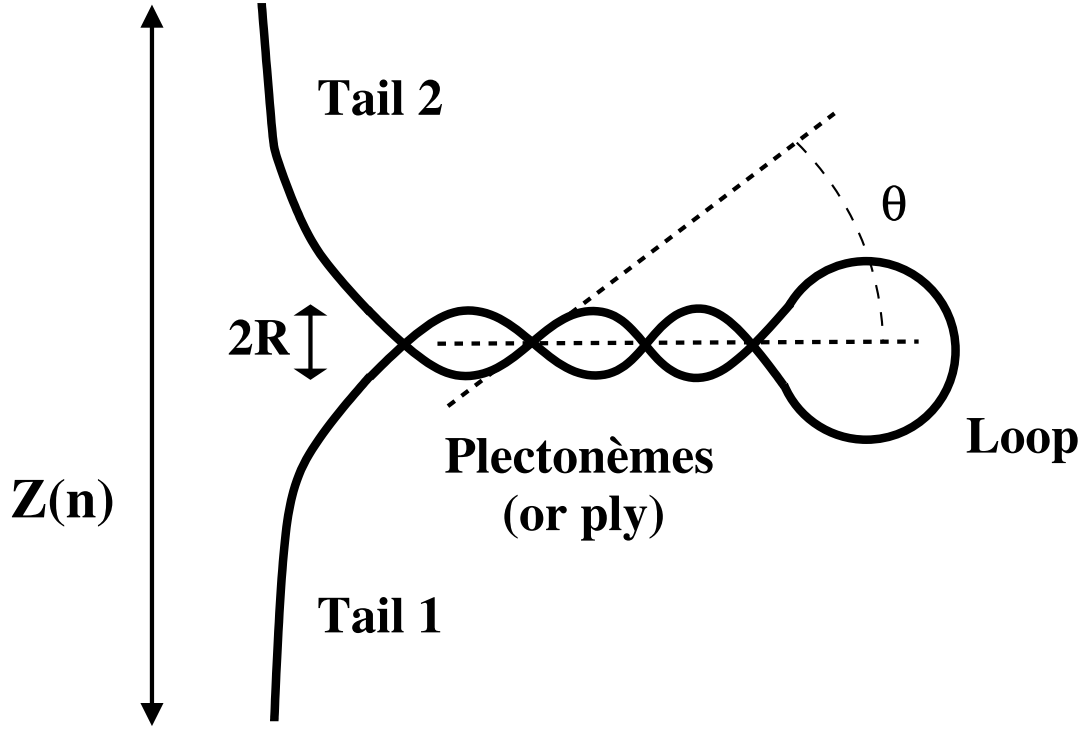
Tw not topologically invariant
Tw is additive (single integrale)

Topology : Twist

$$Tw = \frac{1}{2\pi}\int_0^L \tau\, ds = \frac{1}{2\pi}\tau L \qquad (\tau \text{ constant } \%\ s)$$

Equilibrium of plectonemic part yields : $\tau = -\epsilon \frac{1}{2R}\frac{K_0}{K_3}\left(\tan 2\theta - \sin 2\theta\right)$





Nombre d'enlacements d'un ruban : Link

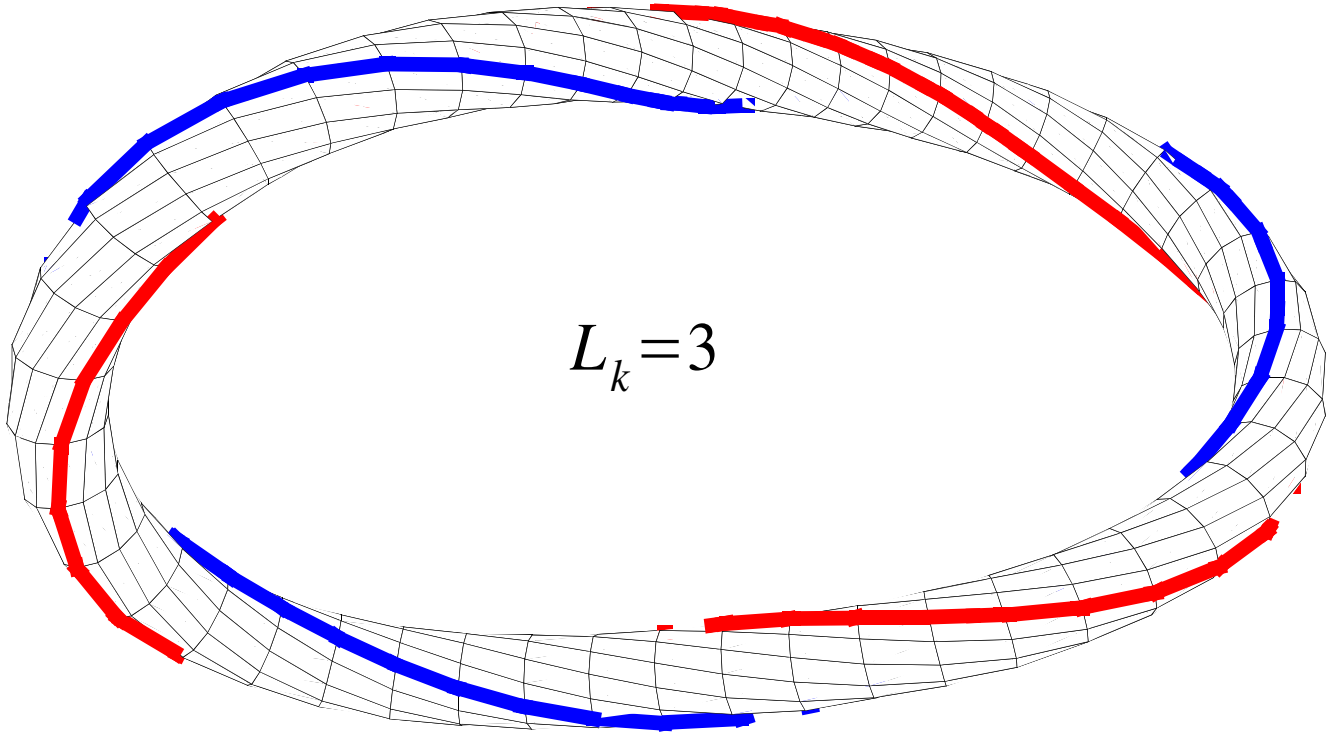
$$L_k(C_1, C_2) = \frac{1}{4\pi} \oiint \frac{(r_1(s_1) - r_2(s_2)) \cdot (t_1(s_1) \times t_2(s_2))}{|r_1(s_1) - r_2(s_2)|^3} \, ds_1 \, ds_2$$

C_1 et C_2 : bords du ruban

$C_1 : s \to r(s) + \epsilon \, d_1(s)$

$C_2 : s \to r(s) - \epsilon \, d_1(s)$

$s \in [0, L]$



- normalement $L_k \in Z$
- invariant géométrique
- invariant topologique
- nombre de rotations d'une boucle autour de l'autre

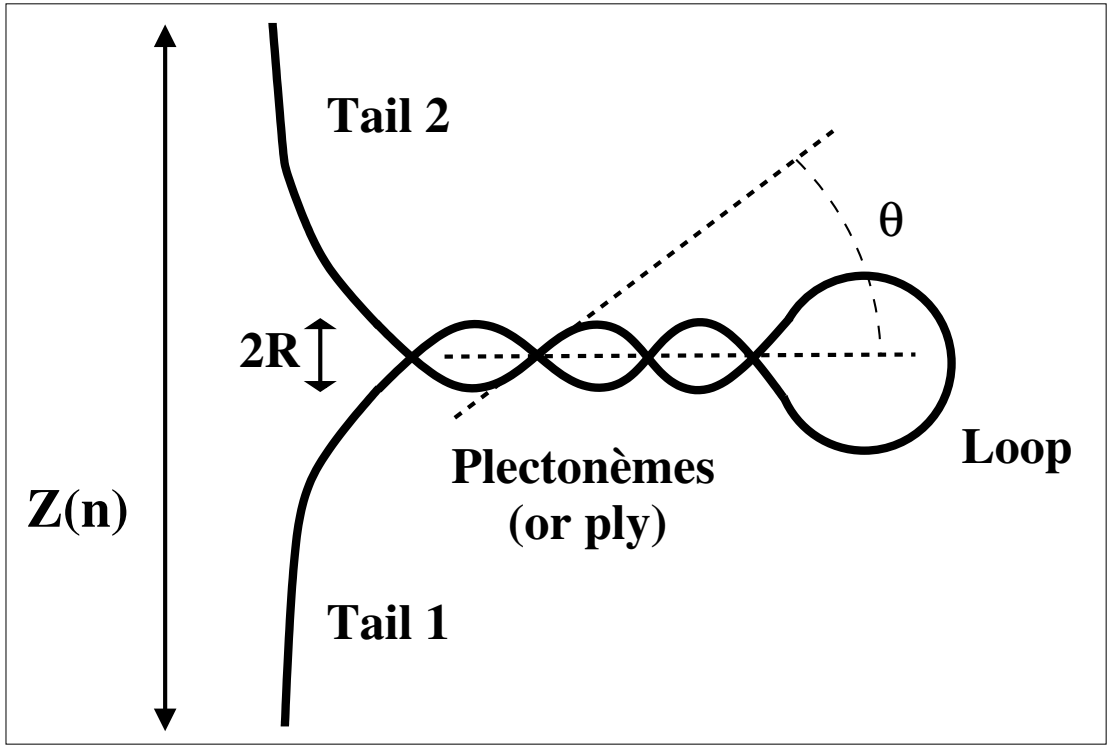
Topology : Twist, Writhe and Link

Calugareano, White, Fuller

$n = Lk = Tw + Wr$

$$Tw = -\epsilon \frac{L}{4\pi R} \frac{K_0}{K_3} \left(\tan 2\theta - \sin 2\theta\right)$$

$$Wr \approx -\epsilon \frac{L_{Plecto}}{4\pi R} \sin(2\theta)$$



$$L = L_{Plecto} + \cancel{L_{Loop}} + L_{Tails}$$

$$L_{Tails} = Z \cdot \frac{L}{Z(0)}$$

$$n = \frac{L}{4\pi R}\left\{\frac{K_0}{K_3}\left(\tan 2\theta - \sin 2\theta\right) + \sin 2\theta\left(1 - \frac{Z}{Z(0)}\right)\right\}$$

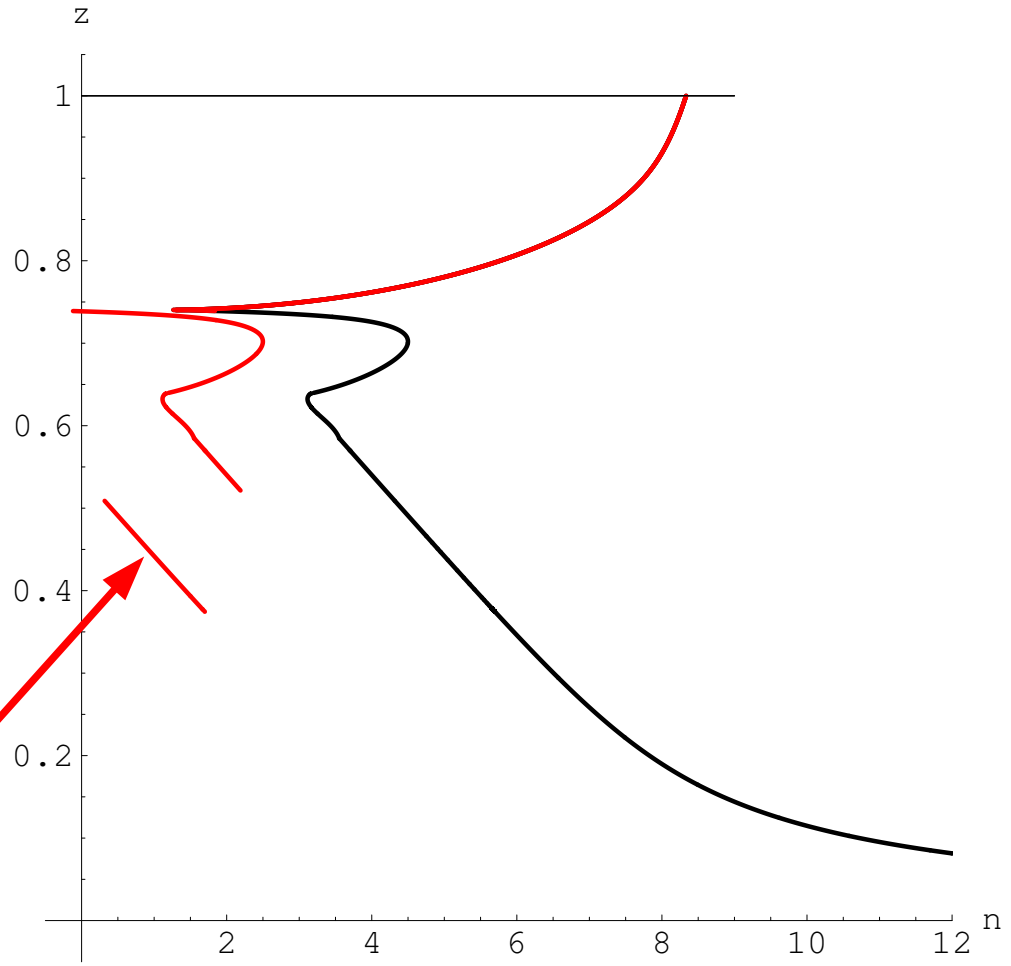
$(\epsilon = -1)$

Writhe (and link): wrong formula

slenderness ratio: $\frac{L}{2R} = 170$

constant tension: $t = \frac{TL^2}{4\pi^2 K_0} = 6$

$$n = Lk = \frac{1}{2\pi}\int_0^L (\dot{\psi} + \dot{\phi})\, ds \ \underline{mod}\ 1$$

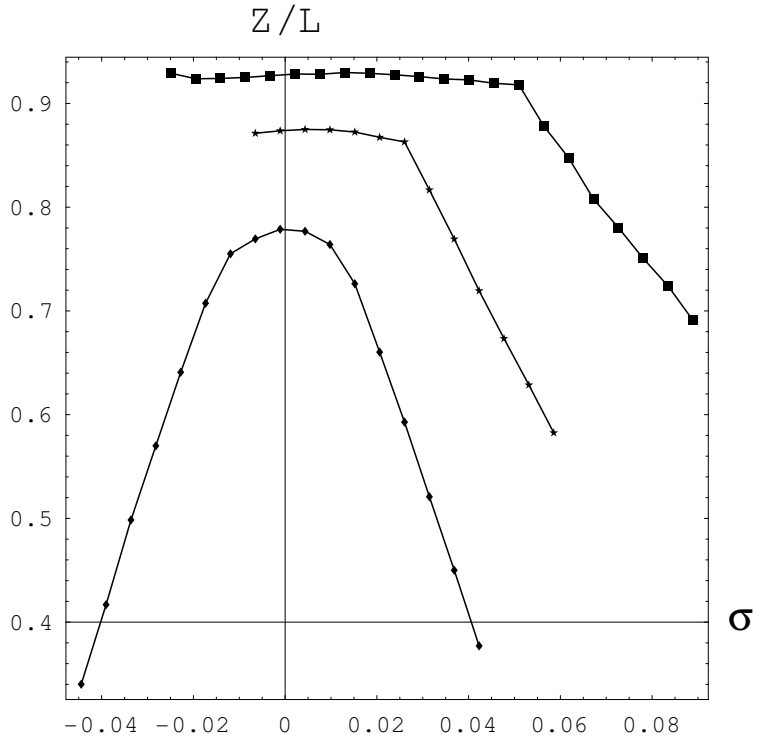


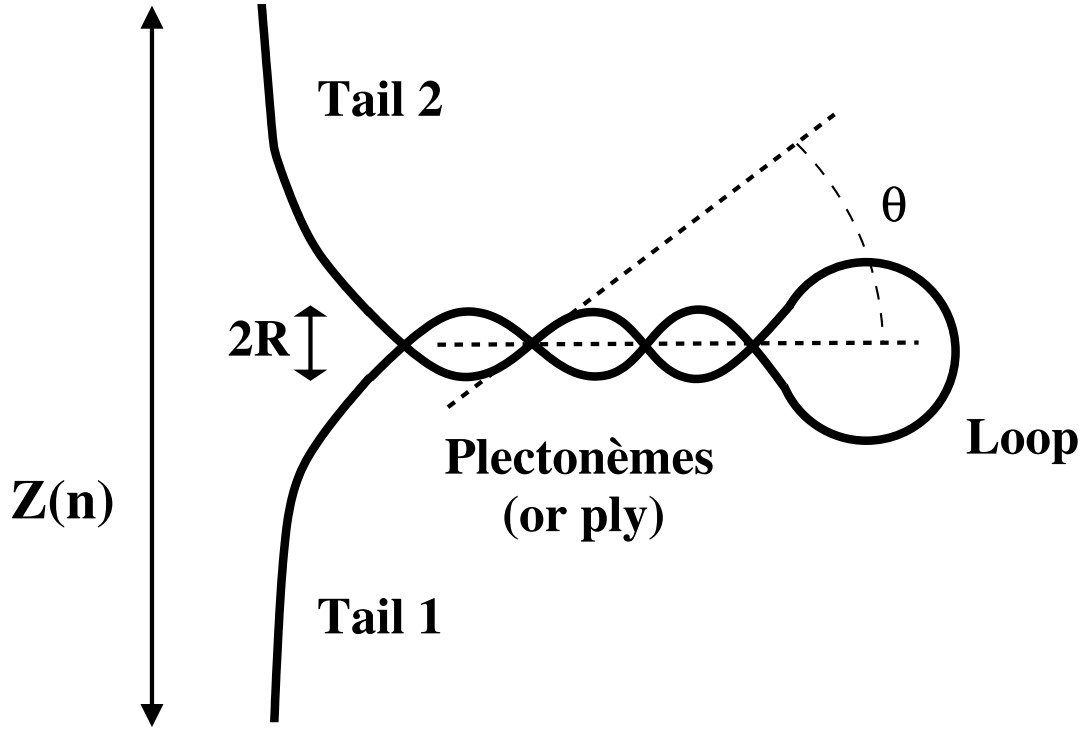
Helical angle θ : fonction of *t* and *L/R*

Helix angle θ is almost constant for all configurations in the linear part of the response curve (same for Twist)

θ does not depend on K_3 (centre line **r**(s) does not depend on K_3)

$\theta = \theta(t\ ,\ L/R)$

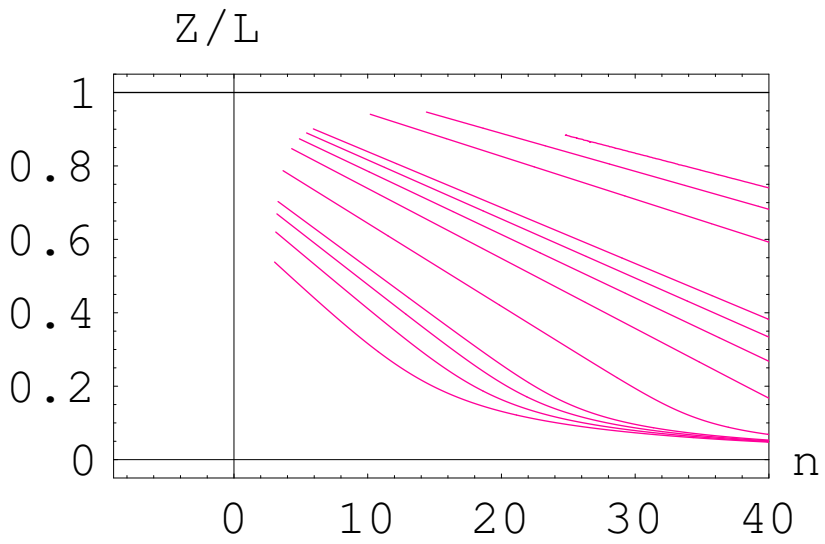


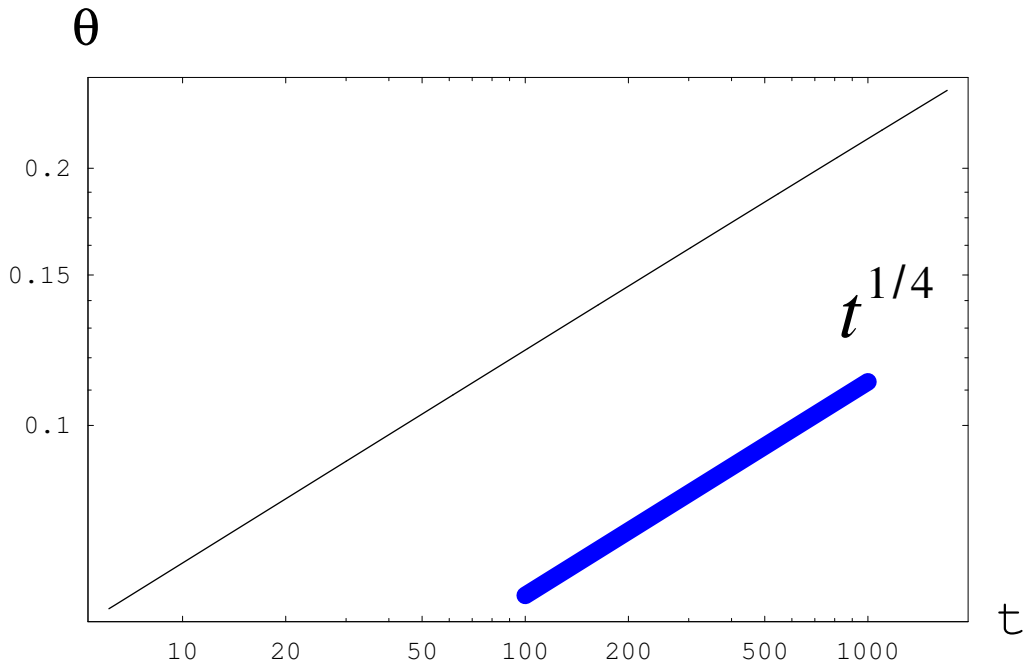


Helical angle θ : fonction of *t* and *L/R*

we vary *t* while keeping L / R fixed

$$\frac{L}{2R} = 1700$$



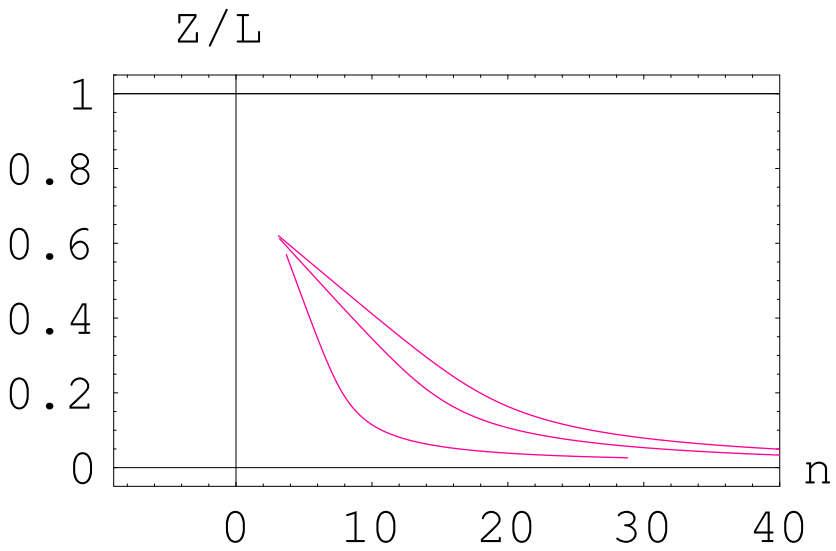


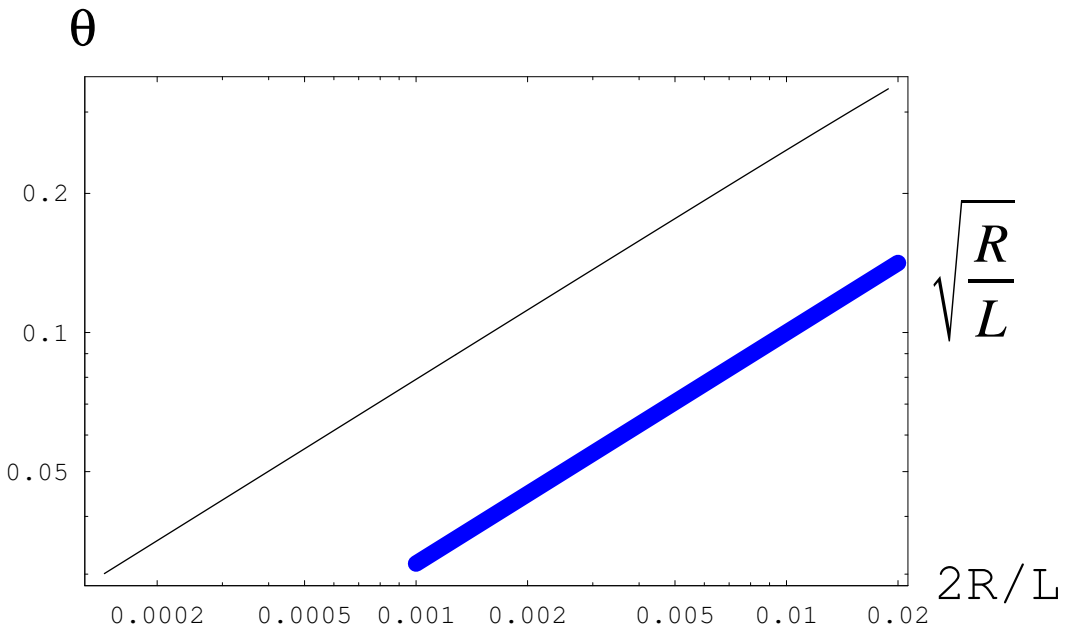
$$t = \frac{T L^2}{(2\pi)^2 K_0}$$

Helical angle θ : fonction of *t* and *L/R*

we vary L / R while keeping *t* fixed

$$t = \frac{T\,L^2}{(2\,\pi)^2\,K_0} = 6$$





polynomial interpolation : $\dfrac{R^2 T}{K_0} = \varphi(\theta) = 1.66\ \theta^4$

Approximate equation for the linear part

$$z = \underbrace{z(0)\left\{1+\frac{K_0}{K_3}\left(\frac{1}{\cos 2\theta}-1\right)\right\}}_{z_\beta} - \underbrace{\frac{4\,\pi R}{\sin 2\theta}\,\frac{z(0)}{L}}_{\alpha}\, n$$

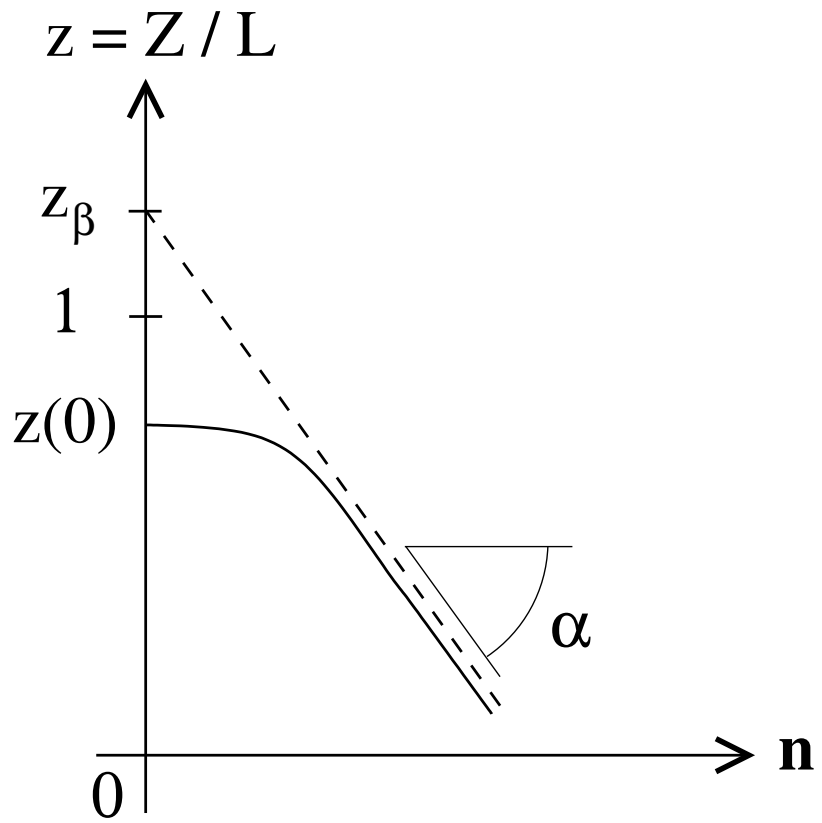
$$\frac{\sigma}{H}=\frac{n}{L}$$

H = 3.57 nm

this is not a fit !

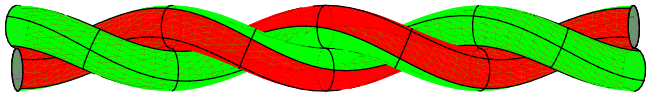
$$\begin{cases} \dfrac{R^2 T}{K_0} = \varphi(\theta) = 1.66\,\theta^4 \\ \alpha = \dfrac{4\,\pi R}{\sin 2\theta}\,\dfrac{z(0)}{L} \\ z_\beta = z(0)\left\{1+\dfrac{K_0}{K_3}\left(\dfrac{1}{\cos 2\theta}-1\right)\right\} \end{cases}$$

3 unknowns : (R , θ , K_0 / K_3)



Repulsive force in the plectonemic part

Mechanical equilibrium implies $\frac{p R^3}{K_0} = \frac{\sin^4 \theta}{\cos 2\theta}$



with $\frac{R^2 T}{K_0} = 1.66\,\theta^4$ this yields $p \simeq \frac{T}{1.66\,R}$

careful : T and R are related

How does this compare with Debye Huckel electrostatic repulsion of two charged (straight) helices ?

$$p \simeq 4\ k_B T \frac{L_B}{\lambda_D} \nu^2\ \frac{e^{(-2\ R/\lambda_D)}}{\sqrt{2\,R/\lambda_D}}$$

Ubbink + Odijk [1999] also in Marko+Siggia [1995]

L_B : *Bjerrum length* $(0.7\,nm)$

λ_D : *Debye length* $(0.8\,nm)$

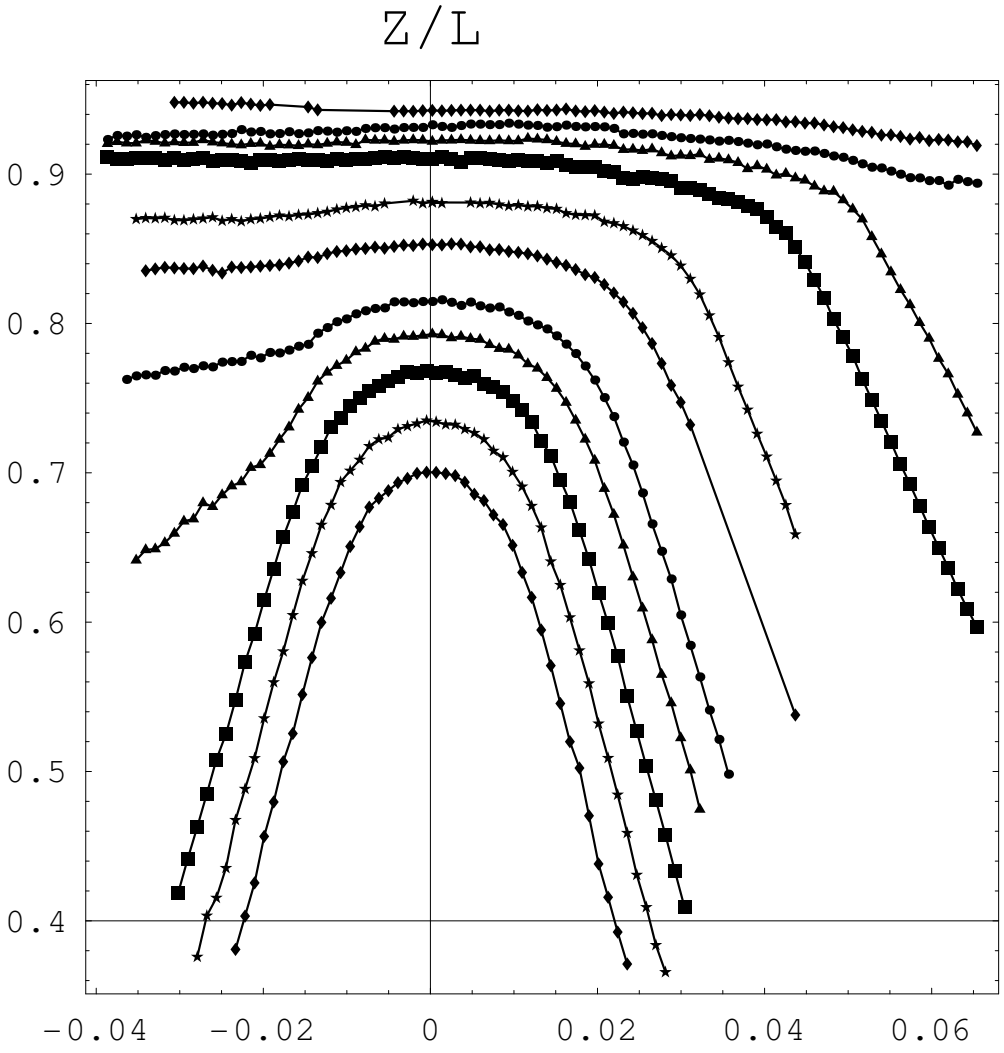
ν : *Poisson − Boltzmann effective charge per unit length* $10-40\ nm^{-1}$

Results

buffer solution = monovalent salt (phosphate) 10mM

molecule ~ 48kb

$$B = \frac{K_0}{k_B T} = 51\ nm$$ (worm-like chain)



data from Vincent Croquette (LPS-ENS)

T (pn)	θ (rad)	R (nm)	C/B	P pN/µm
0,25	0,42	6,73	1,75	28
0,33	0,44	6,46	1,71	39
0,44	0,45	6,02	1,72	56
0,57	0,45	5,33	1,64	82
0,74	0,48	5,37	1,71	108
1,10	0,47	4,10	1,62	208
1,31	0,45	3,54	1,41	284
2,20	0,48	3,11	1,40	556

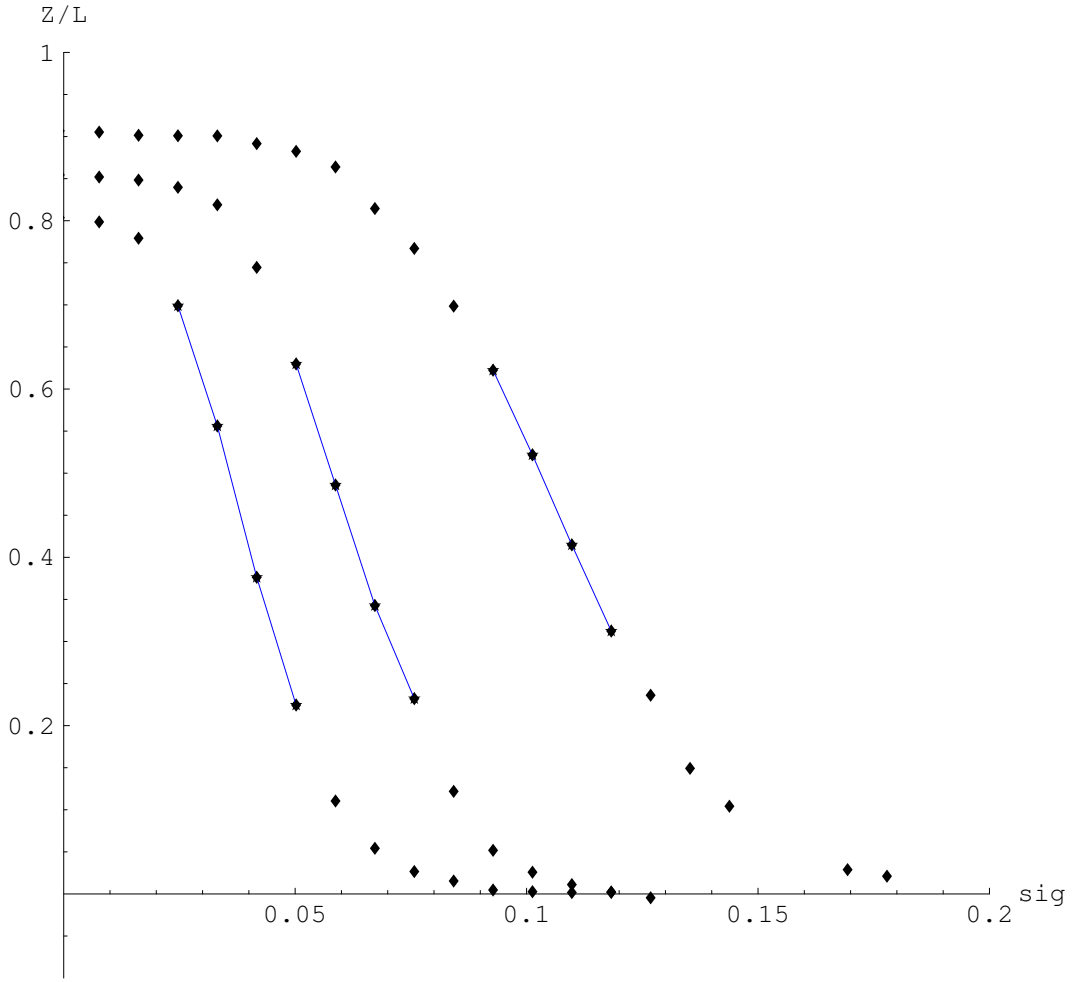
$$\sigma = \frac{n}{n_0}$$

$$C = \frac{K_3}{k_B T} = 80 \pm 10\ nm$$

Results

buffer solution = monovalent salt (PB) 10mM

molecule ~ 6kb



$$B = \frac{K_0}{k_B T} = 46\ nm$$ (worm-like chain)

T (pn)	θ (rad)	R (nm)	C/B	P pN/µm
0,45	0,43	5,03	1,13	64
0,90	0,46	4,14	0,98	145
3,00	0,54	3,37	1,21	646

$$C = \frac{K_3}{k_B T} = 50 \pm 5\ nm$$

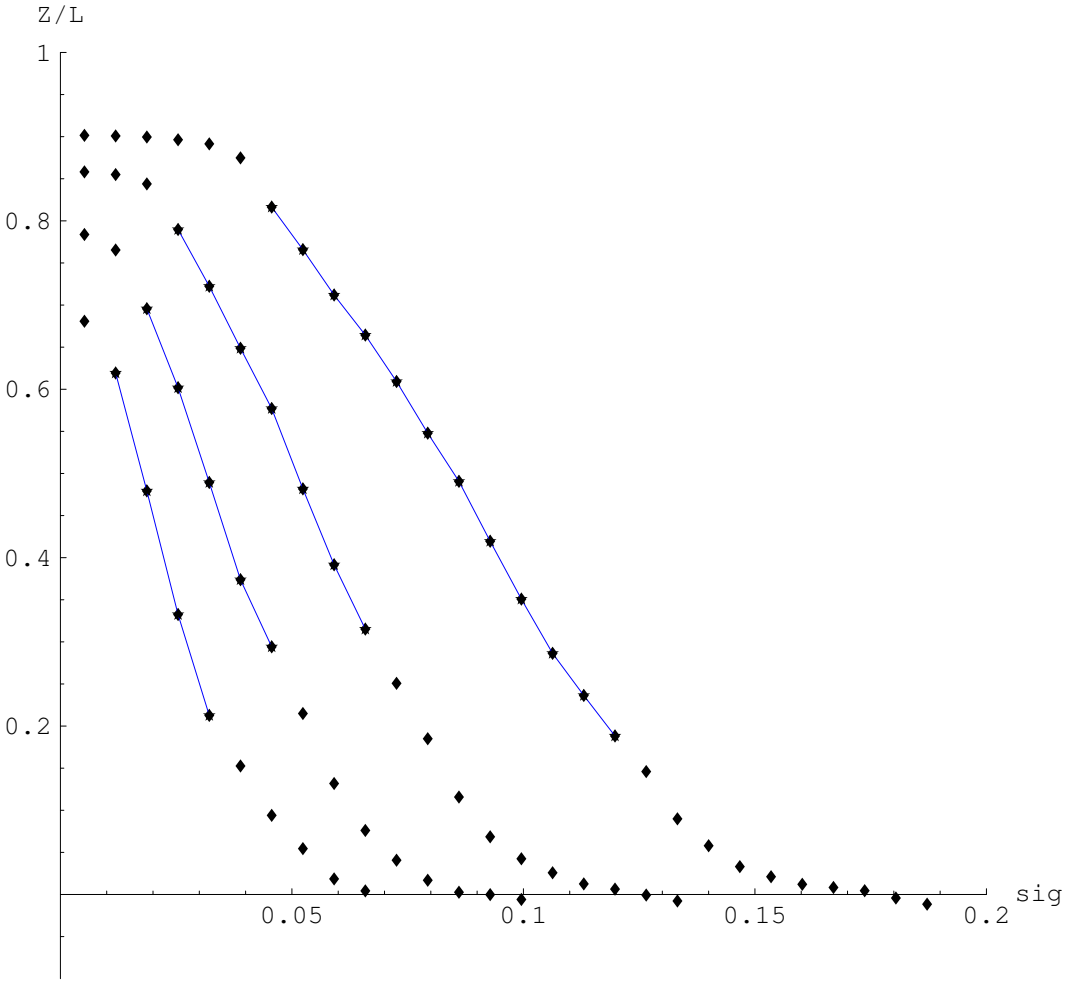
data from Gilles Charvin (LPS-ENS)

$$\sigma = \frac{n}{n_0}$$

Results

buffer solution = monovalent salt (PB) 100mM

molecule ~ 6kb



$$B = \frac{K_0}{k_B T} = 49\ nm$$ (worm-like chain)

T (pn)	θ (rad)	R (nm)	C/B	P pN/µm
0,20	0,37	5,56	1,35	26
0,45	0,37	3,72	1,31	87
0,90	0,37	2,67	1,19	242
3,00	0,44	2,12	1,54	1 007

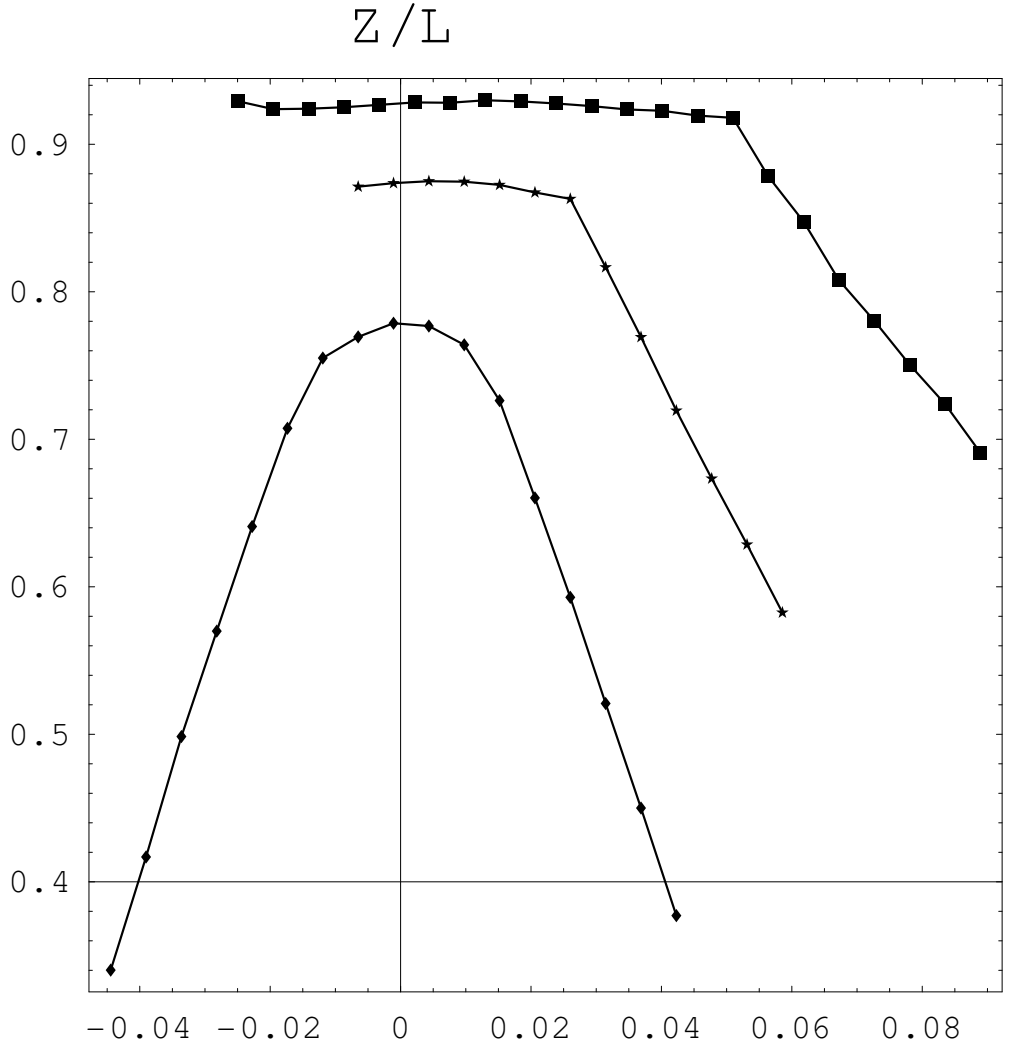
$$C = \frac{K_3}{k_B T} = 66 \pm 8\ nm$$

$$\sigma = \frac{n}{n_0}$$

data from Gilles Charvin (LPS-ENS)

Results

buffer solution = monovalent salt (phosphate) 150mM
+ divalent salt (magnésium) 5mM



data from Gilles Charvin (LPS-ENS)

molecule ~ 11kb

$$B = \frac{K_0}{k_B T} = 57\ nm$$ (worm-like chain)

T (pn)	θ (rad)	R (nm)	C/B	P pN/µm
0,45	0,31	2,81	1,08	114
1,45	0,32	1,68	1,01	615
4,30	0,35	1,16	0,99	2 690

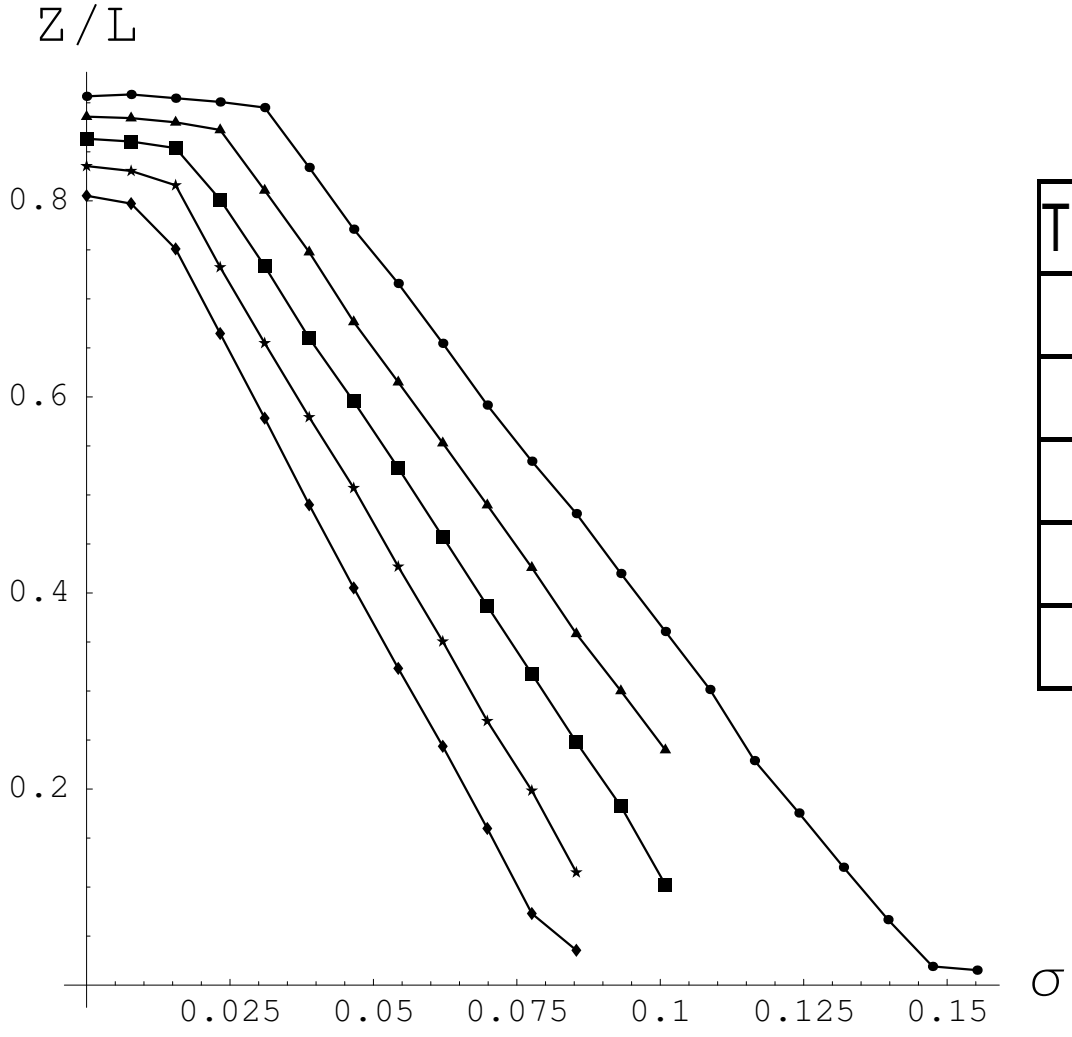
$$C = \frac{K_3}{k_B T} = 59\ \pm 2.5\ nm$$

σ

$$\sigma = \frac{n}{n_0}$$

Results

buffer solution = monovalent salt (NaCl) 500mM



data from par R. Fulconis (Institut Curie)

molecule ~ 9kb

$$B = \frac{K_0}{k_B T} = 40\ nm$$

(worm-like chain)

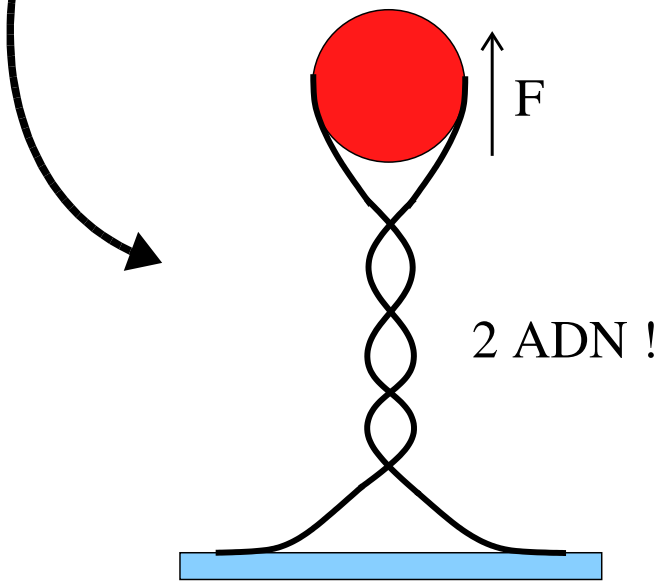
T (pn)	θ (rad)	R (nm)	C/B	P pN/µm
0,62	0,34	2,38	2,02	187
0,95	0,36	2,23	2,12	310
1,30	0,37	1,95	2,02	485
1,80	0,38	1,81	1,90	729
2,70	0,41	1,76	1,92	1 150

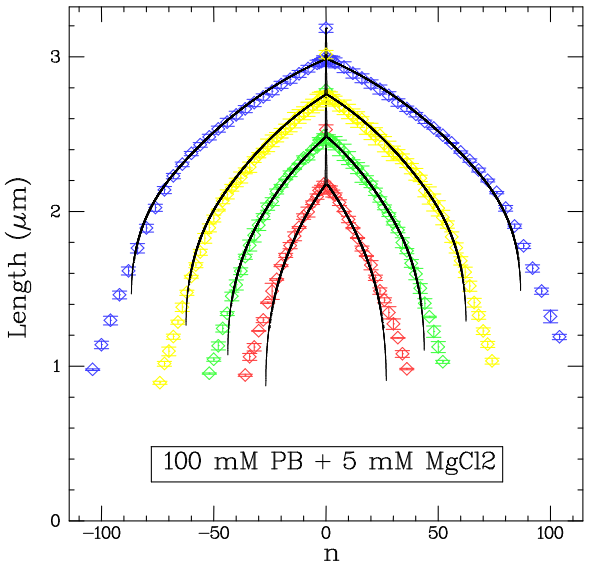
$$C = \frac{K_3}{k_B T} = 80 \pm 4\ nm$$

$$\sigma = \frac{n}{n_0}$$

Futur work

- Include :
  - chirality and twist-extension coupling
  - electrostatic repulsion
- Model other experiments :
  - DNA braids (Gilles Charvin)
  - Chromatin (10 nm) fiber (A. Bancaud & J.-L. Viovy)
  - DNA+RecA complex (R. Fulconis & J.-L. Viovy)





Relation between n and σ

$$\sigma = \frac{n}{n_0} = n\frac{H}{L}$$

$$L = 0.34\ nbp\ nm$$

σ : super-coiling ratio

n : number of turns applied on the magnetic bead

$n_0 = L / H$: intrinsic twist of the DNA double helix

H = 3.57 nm : pitch of the DNA double helix

L : total contour length of the DNA molecule