Chirality of coiled-coils : elasticity matters

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joint work with

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Globular proteins / Fibrous proteins

D. A. Parry & J. M. Squire, J. Struct. Biol. (1998)

1991 : crystal structure of coiled-coil protein (GCN4)

Site-directed mutagenesis :
replace specific residues => effect on the structure
                     (two-stranded coiled-coil -> three-stranded coiled-coil)

Design

« This was a veritable revelation.
The simpler fibrous proteins had thus become a *superb vehicle*
by which the precise factors specifying protein structure could be
recognized. »

=> Similar approach for globular proteins ? (not yet)

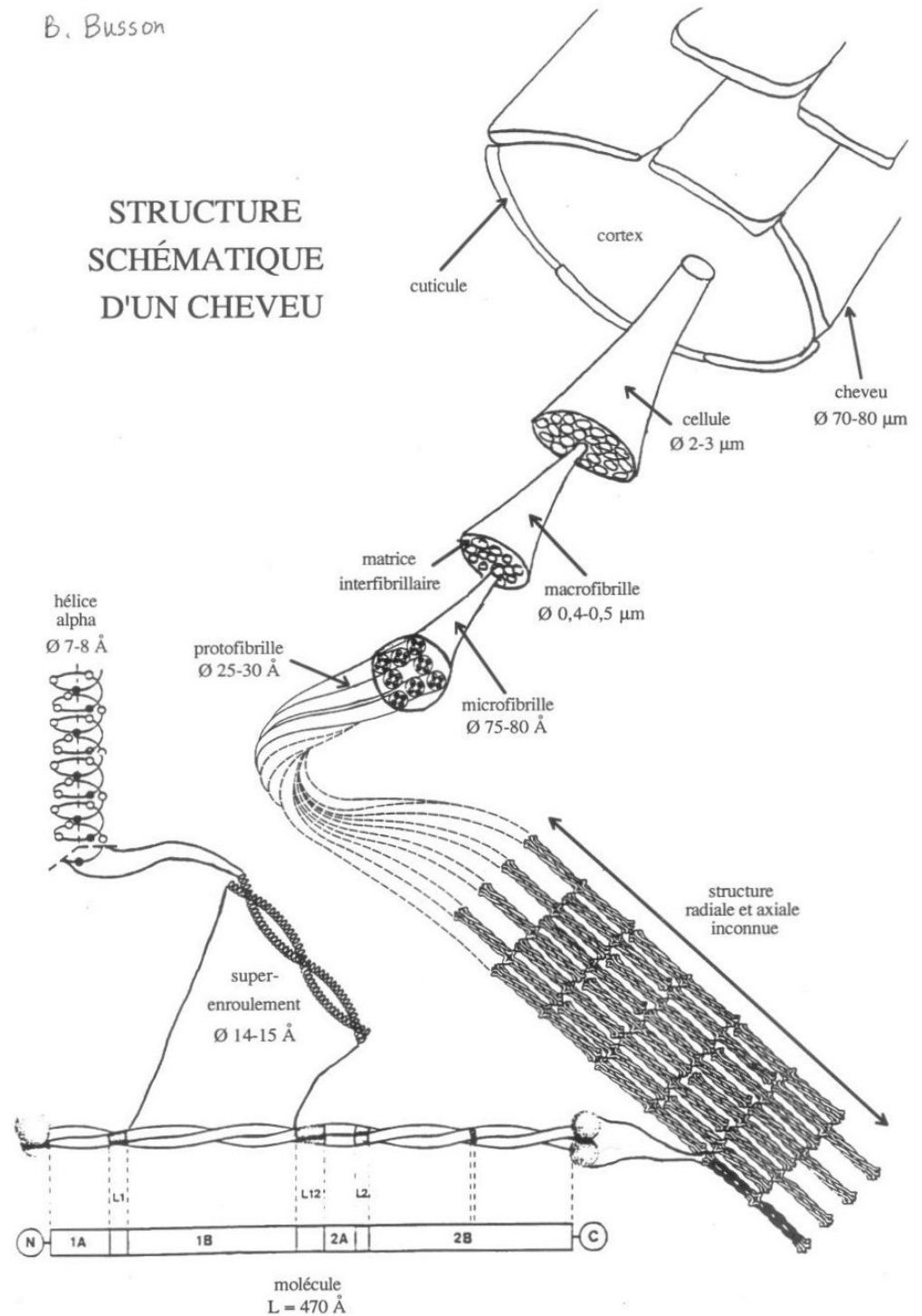Examples of fibrous proteins :
keratins (nails, hair, skin, ...)
collagens (bones, tendons, ...)
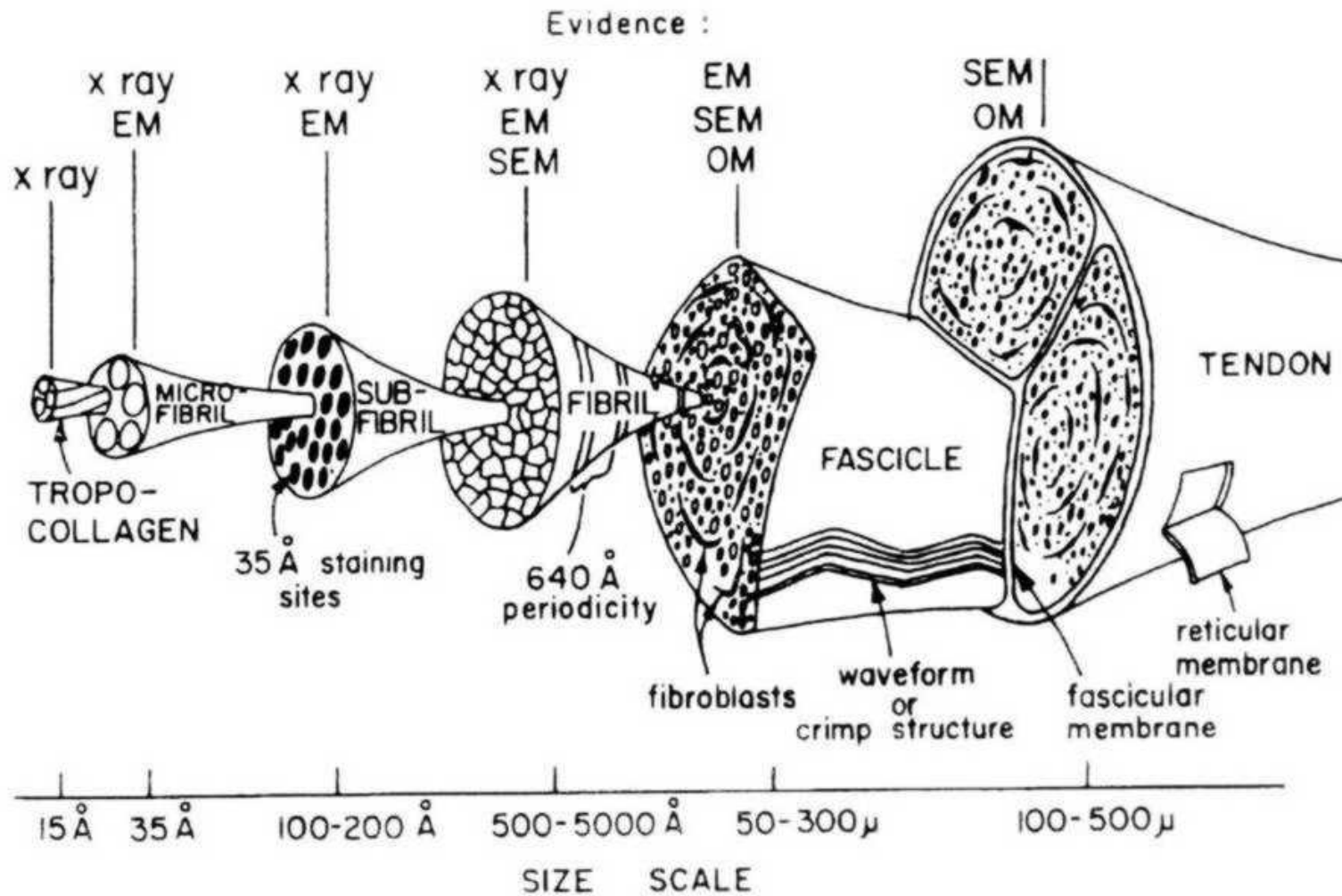
Keratin

hierarchical structure

X-Ray Diffraction:
Jean Doucet
Fatma Briki
Bertrand Busson
(LPS - Orsay)

B. Busson

STRUCTURE
SCHÉMATIQUE
D'UN CHEVEU

cuticule

cortex

cheveu
Ø 70-80 µm

cellule
Ø 2-3 µm

matrice
interfibrillaire

macrofibrille
Ø 0,4-0,5 µm

hélice
alpha
Ø 7-8 Å

protofibrille
Ø 25-30 Å

microfibrille
Ø 75-80 Å

structure
radiale et axiale
inconnue

super-
enroulement
Ø 14-15 Å

N 1A 1B 2A 2B C

L1 L12 L2

molécule
L = 470 Å

Collagen

high tensile strength : 4000 kg to rupture the Achille's tendon of a horse (Yamada 1970)

Peter Fratzl (Universität Wien, Österreich)
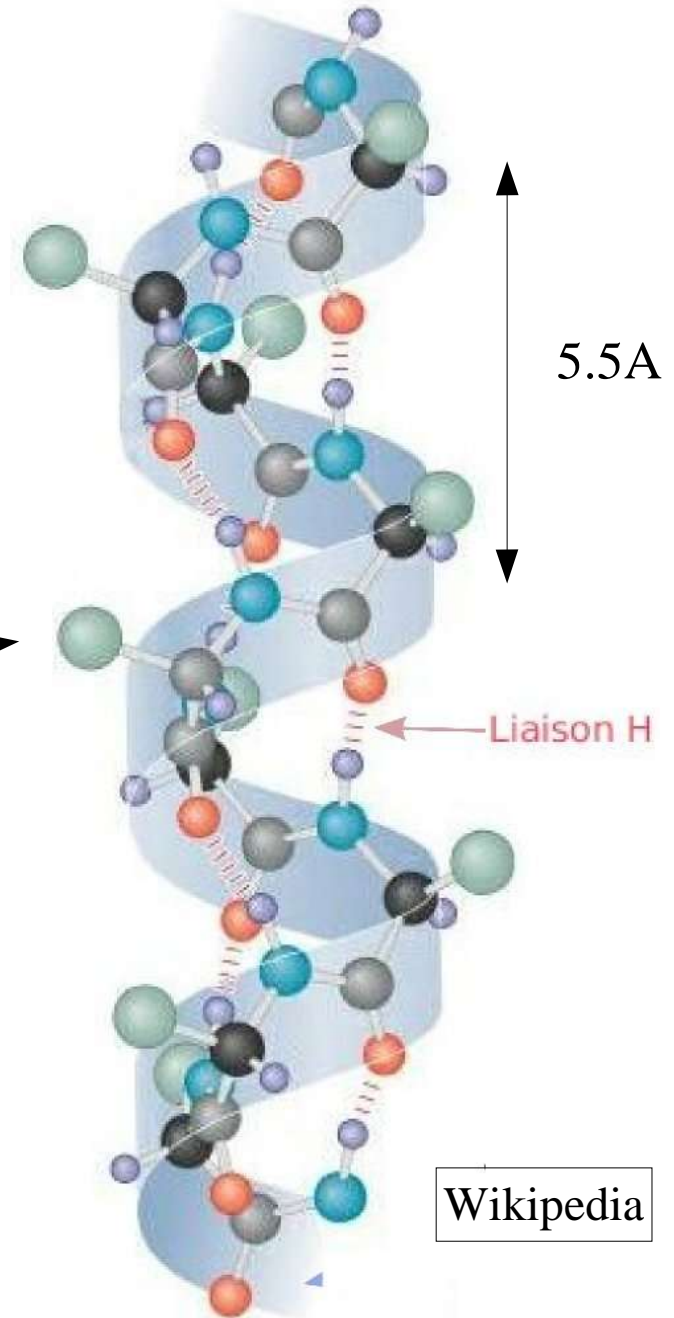
The Alpha Helix

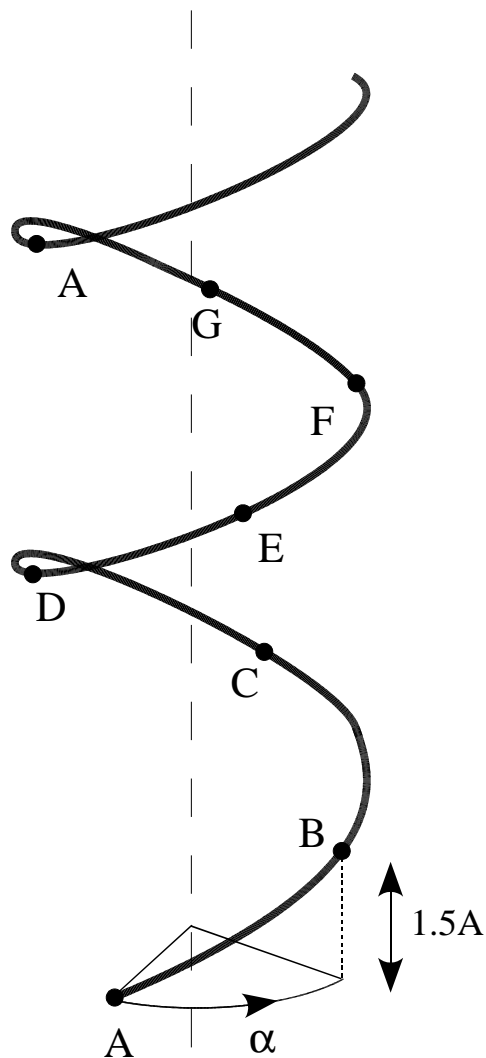**primary structure**

secondary structure

one amino acid

NH

HCR₁

OC

NH

HCR₂

OC

NH

peptide plane

residue

Pauling, Corey & Branson (1951)

- peptide bond is planar
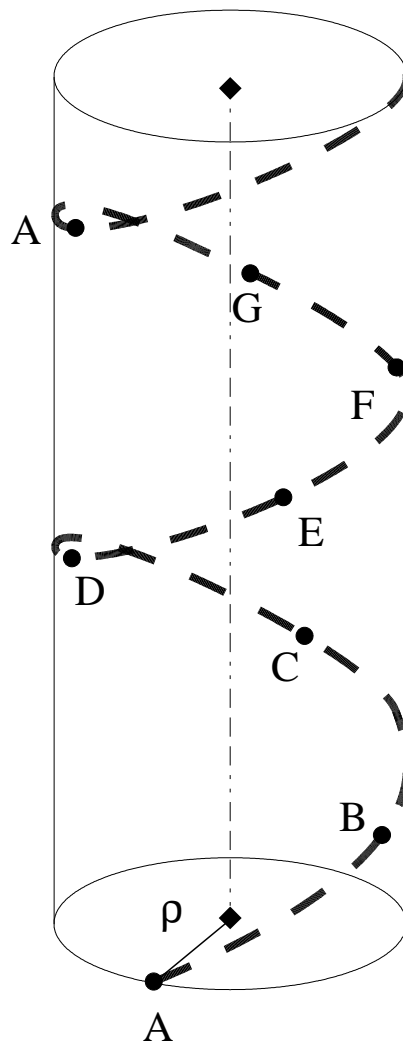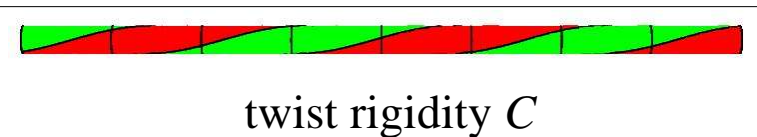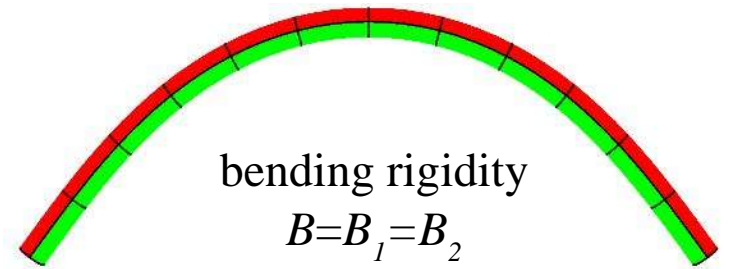- hydrogen bonds
- non-integer 3.6 residues / turn

5.5A

Liaison H

● N

● ● C

● O

● H

Wikipedia

alpha helix => elastic rod

bending rigidity
$B=B_1=B_2$

twist rigidity C
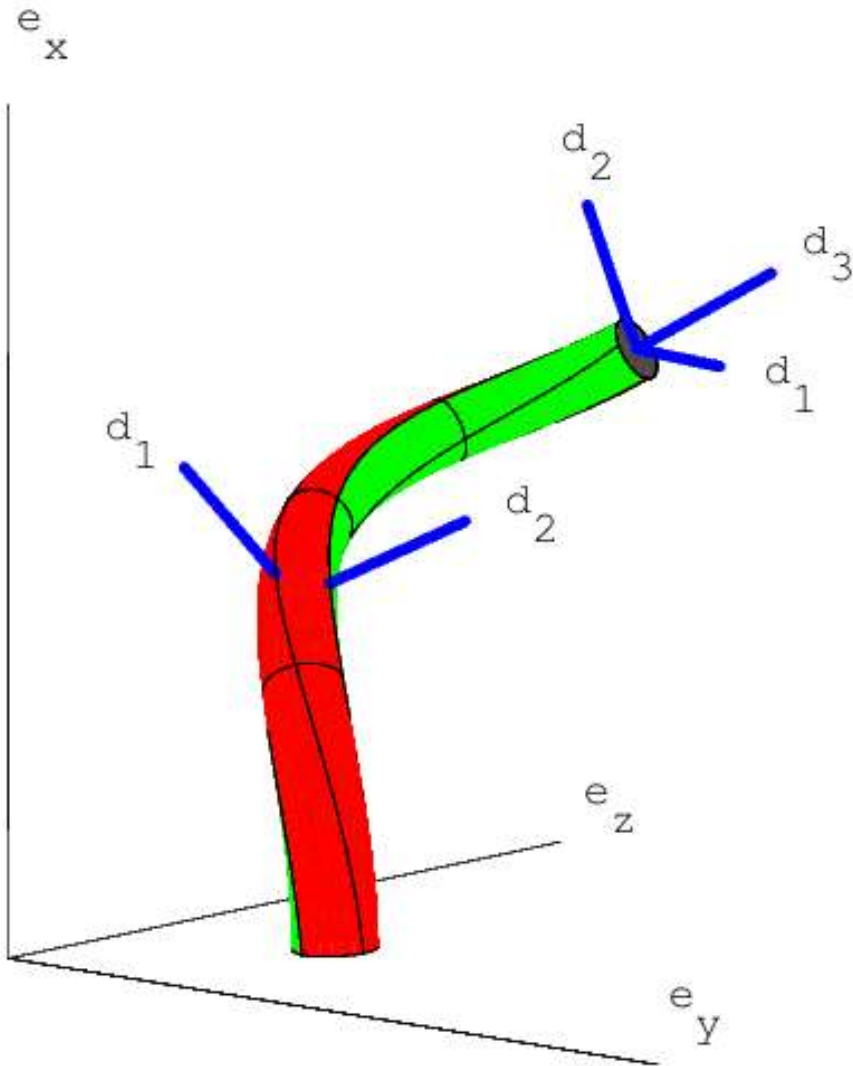
hydrogen bonds
+ planar peptide bonds
= inextensible

molecular dynamics:

$B \simeq 100 \ nm\,kT$ $\qquad C \simeq ?$

S. Choe & S. Sun, J. Chem. Phys. 2005

A
G
F
E
D
C
B
1.5A
A
α

heptad motif

α = 100°

A
G
F
E
D
C
B
ρ

A

ρ = 2.2A

(Cosserat) material frame

$\{\vec{e}_x, \vec{e}_y, \vec{e}_z\}$ fixed frame

$\vec{r}(s)$ rod central line

$\vec{t}(s)$ tangent

$\{\vec{d}_1(s), \vec{d}_2(s), \vec{d}_3(s)\}$
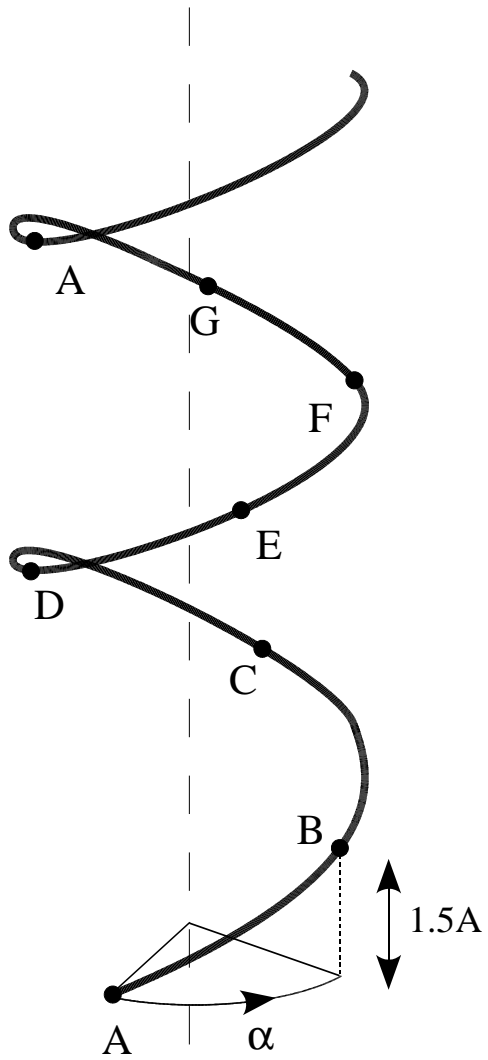
moving frame (Cosserat directors)
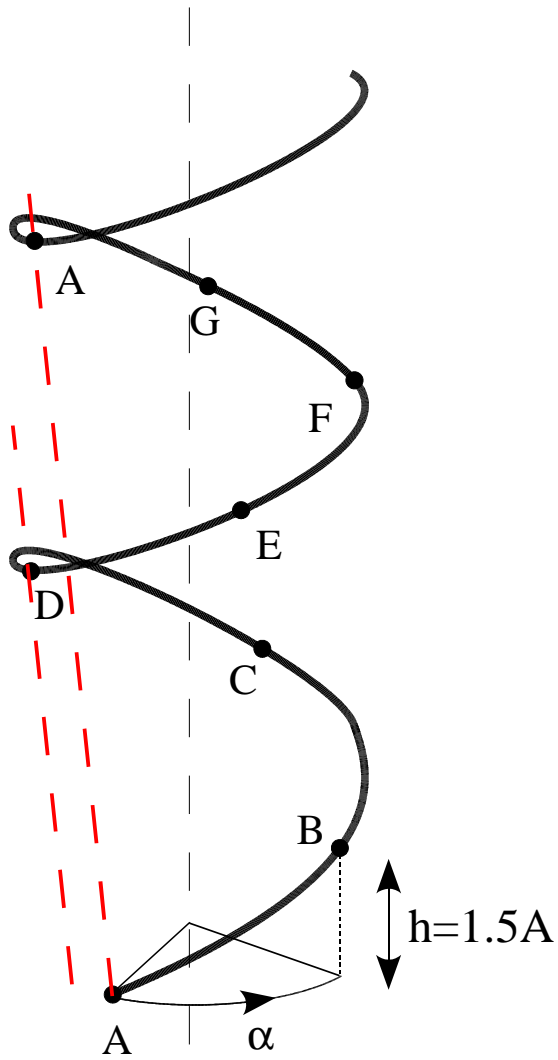
$$\vec{d}_3(s) \equiv \vec{t}(s)$$

But $\vec{d}_1(s) \neq \vec{n}(s)$
$\vec{d}_2(s) \neq \vec{b}(s)$
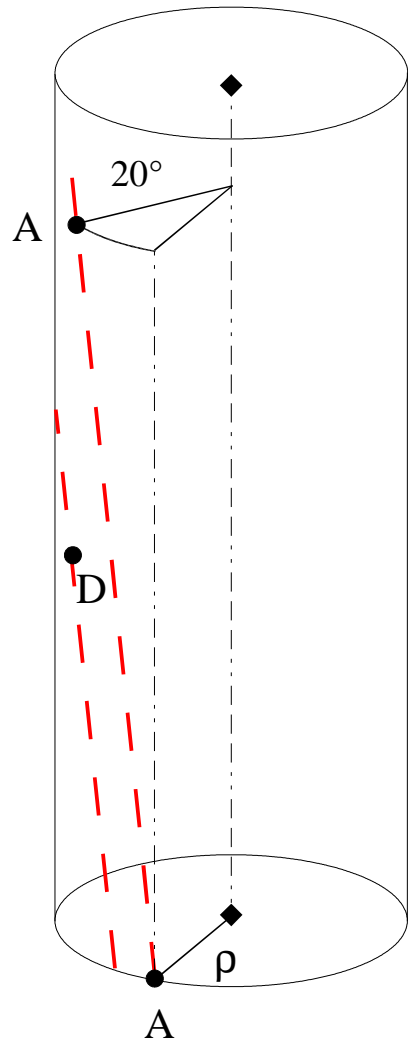
Primitive helix & hydrophobic strip
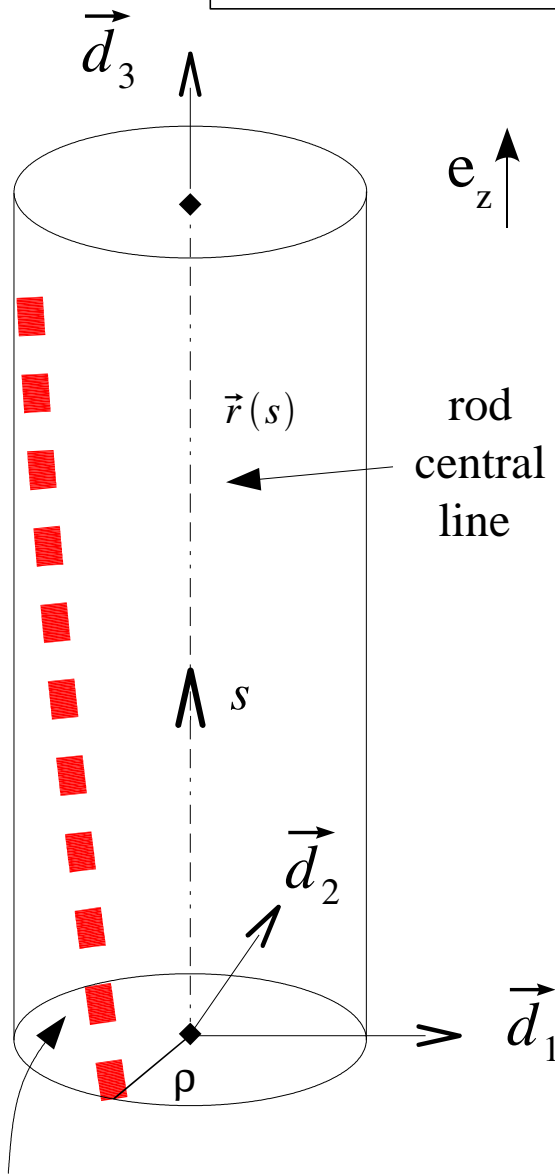
Primitive helix & hydrophobic strip

h=1.5A

α

A and D are hydrophobic residues

Primitive helix & hydrophobic strip

e_z

$20°$

A

D

A

ρ

20° shift for a height of 7 $h = 10.5$ A

Primitive helix & hydrophobic strip

Hydrophobic strip :

$$\overrightarrow{d_{HP}} = \cos(\hat{\tau} s)\overrightarrow{d_1}(s) + \sin(\hat{\tau} s)\overrightarrow{d_2}(s)$$

20° shift for a height of $7\,h = 10.5$ A

$$\hat{\tau} = -0.033\,rad/A$$

Important :

d_{HP} is fixed w. r. t. d_1 and d_2

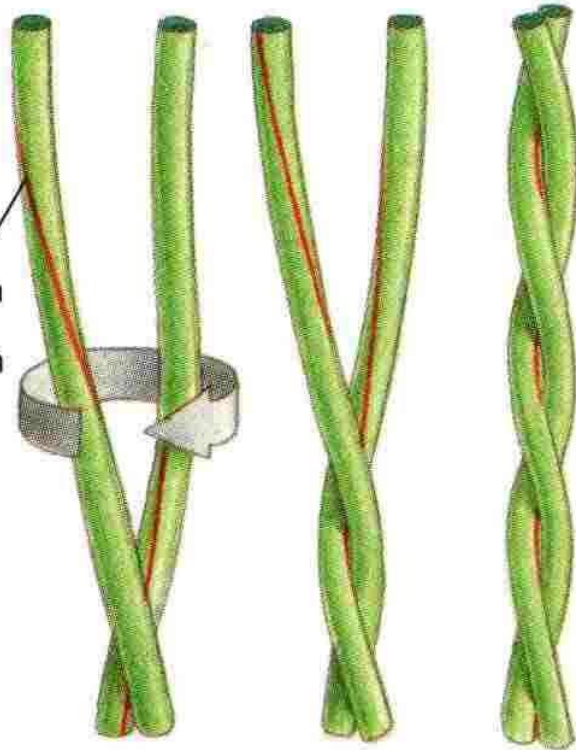i.e. $\hat{\tau}$ is fixed (same for straight or bent config.)

Coiled-coils (of alpha helices)

NH$_2$

Δt

Streifen aus hydrophoben „a" und „d" Aminosäuren

$3.6\, h = 5.5\, A$

0,5 nm

$= 2\, \rho$

Alberts et al. (1998)

- X-Ray : 5.15A reflexion (and not 5.5A)
- Hydrophobic residues are hidden from solvent

=> Crick (1953) : super-helical structure

Fraser & McRae (1973) : super-helical pitch as a function of the twist of the hydrophobic strip:

11 nm $= P$

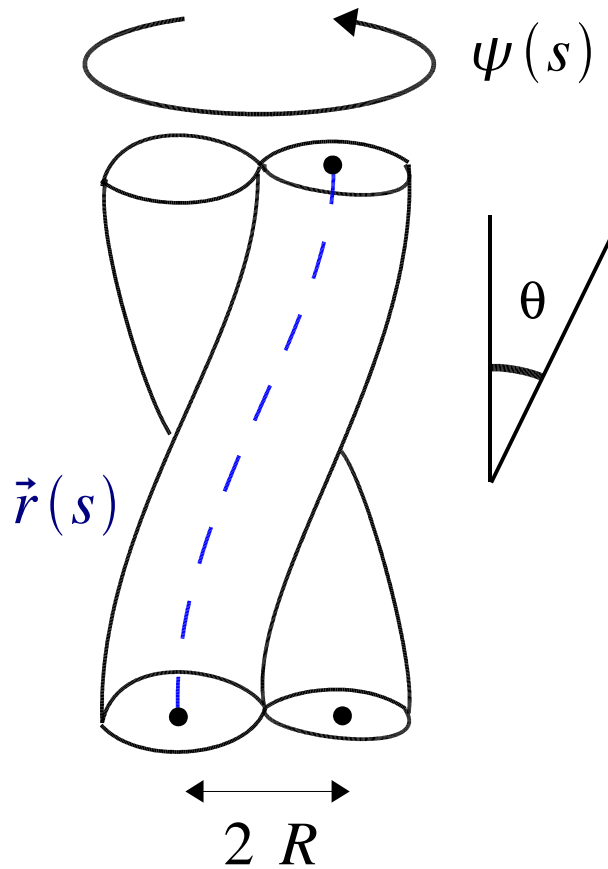$$P = \frac{2\,\pi}{\Delta t} \sqrt{H^2 - R^2\, (\Delta t)^2}$$

$$\boxed{H = h \cos \theta}$$

$2R$

(verified) prediction :
11 residues motif => right-handed coiled-coil

A. Lupas (1996)

Coiled-coil configurations : central line

$\vec{r}(s)$ spans an helix of pitch $P = \dfrac{2\pi R}{\tan\theta}$

$$\vec{r}(s) = \begin{vmatrix} +R\ \sin\psi(s) \\ -R\ \cos\psi(s) \\ s\ \cos\theta \end{vmatrix} \quad \text{with} \quad \psi(s) = s\ \frac{\sin\theta}{R}$$

R super-helical radius $(R \neq \rho)$

θ helical angle ($\theta < 0$: left-handed coiled-coil)
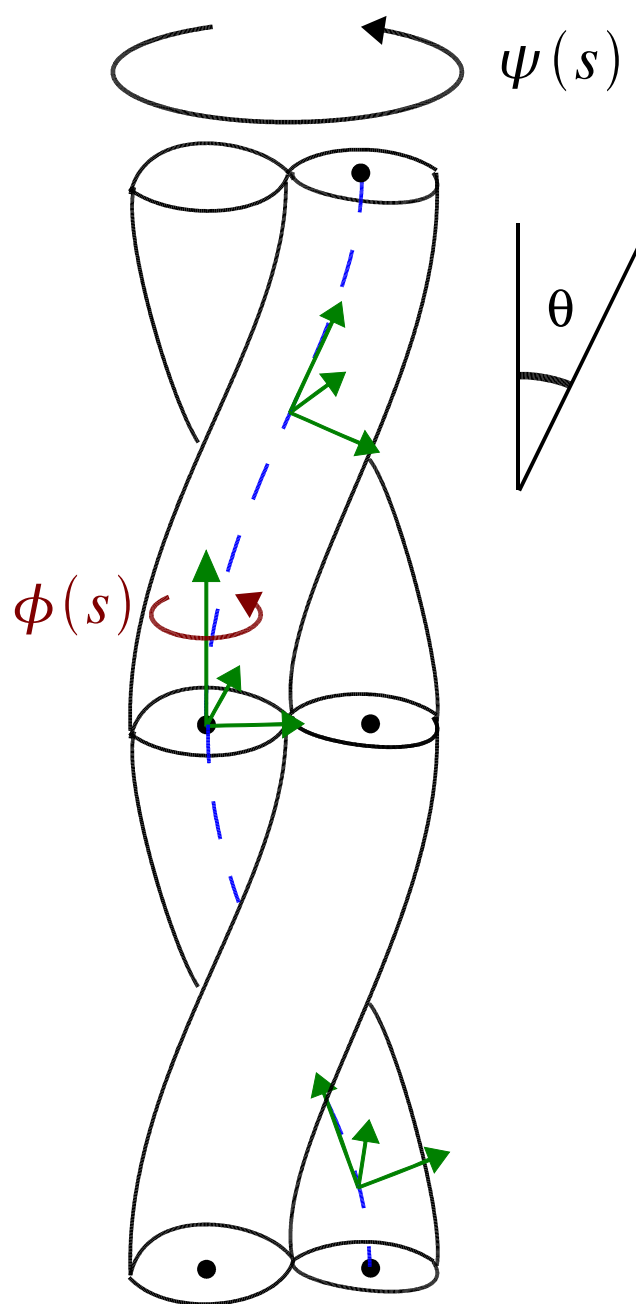
$\psi(s)$ equatorial angle

Frenet frame

$$\vec{t}(s) = \begin{vmatrix} \sin\theta\ \cos\psi(s) \\ \sin\theta\ \sin\psi(s) \\ \cos\theta \end{vmatrix} \qquad \vec{n}(s) = \begin{vmatrix} -\sin\psi(s) \\ +\cos\psi(s) \\ 0 \end{vmatrix} \qquad \vec{b}(s) = \begin{vmatrix} -\cos\theta\ \cos\psi(s) \\ -\cos\theta\ \sin\psi(s) \\ \sin\theta \end{vmatrix}$$

Coiled-coil configurations : directors

$\psi(s)$

θ

$\phi(s)$

rotation of an angle φ(s) about the tangent t(s)

$$\vec{d}_1(s) = \sin\phi \ \vec{n}(s) - \cos\phi \ \vec{b}(s)$$
$$\vec{d}_2(s) = \cos\phi \ \vec{n}(s) + \sin\phi \ \vec{b}(s)$$
$$\left(\vec{d}_3(s) = \vec{t}(s)\right)$$
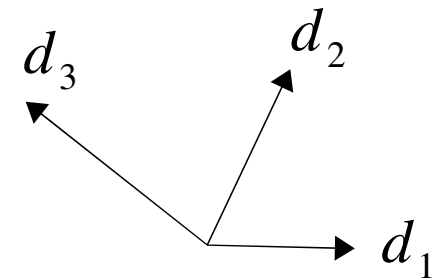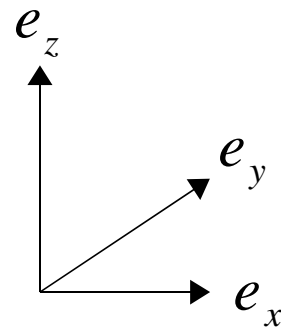
$\phi = \phi(s)$ internal twist

3D central line => Frenet torsion
and material twists around central line => internal twist

3 *Euler angles* $\{\theta, \psi, \phi\}$

$\{\vec{e}_x, \vec{e}_y, \vec{e}_z\}$ => rotation => $\{\vec{d}_1, \vec{d}_2, \vec{d}_3\}$
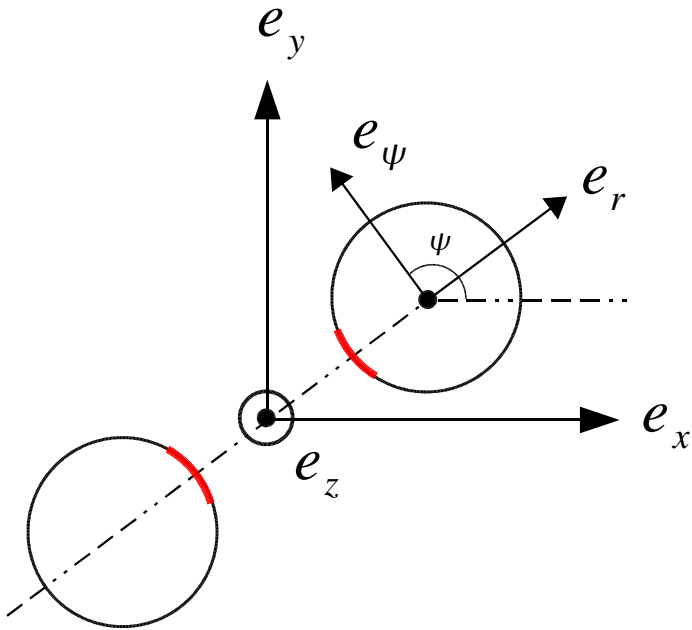
e_z

e_y

e_x

d_3

d_2

d_1

Hydrophobic constraint

Cost to bury 1 hydrophobic residue ~ 5 kT   (Tanford 1962)
Cost to bend helix along 7/2 residues ~ 0.16 kT

(R=5A , $\theta = 0.2$ rad)

Hypothesis : geometrical constraint: $d_{HP} = - e_r$

$$\overrightarrow{d_{HP}}=\cos(\hat{\tau}s)\overrightarrow{d}_1(s)+\sin(\hat{\tau}s)\overrightarrow{d}_2(s)=-\vec{e}_r$$

$$\left|\begin{array}{l}\overrightarrow{d}_1(s)=\sin\phi\ \vec{n}(s)-\cos\phi\ \vec{b}(s)\\ \overrightarrow{d}_2(s)=\cos\phi\ \vec{n}(s)+\sin\phi\ \vec{b}(s)\end{array}\right|$$

$$\cos(\hat{\tau}s+\phi)=0\ \Rightarrow\ \phi(s)=\frac{\pi}{2}-\hat{\tau}s$$

Remark : **this geometrical constraint tells nothing about θ (i.e. chirality)**

A continuous familly of possible coiled-coils

M

0

$2\,M$

$> 2\,M$

'possible'
means
hydrophobic strip
buried

Mechanics of straight n-plies

who	# strands	θ (or pitch)	external forces
Stump & Fraser (1998)	2	const.	no
Coleman & Swigon (2000)	2	var.	no
Gaspar & Nemeth (2001)	2	var.	no
Thompson, Heijden & Neukirch (2002)	2	var.	yes
Neukirch & Heijden (2002)	3	const.	yes

Elastic energy

$$V = 2 \left[\frac{1}{2} \int_0^\sigma B\kappa^2 + C\tau^2 \, ds \right] - FL - M\alpha$$

$$L = \sigma \cos\theta$$

$$\alpha = \frac{\sigma}{R}\sin\theta$$

σ : total contour-length of one strand

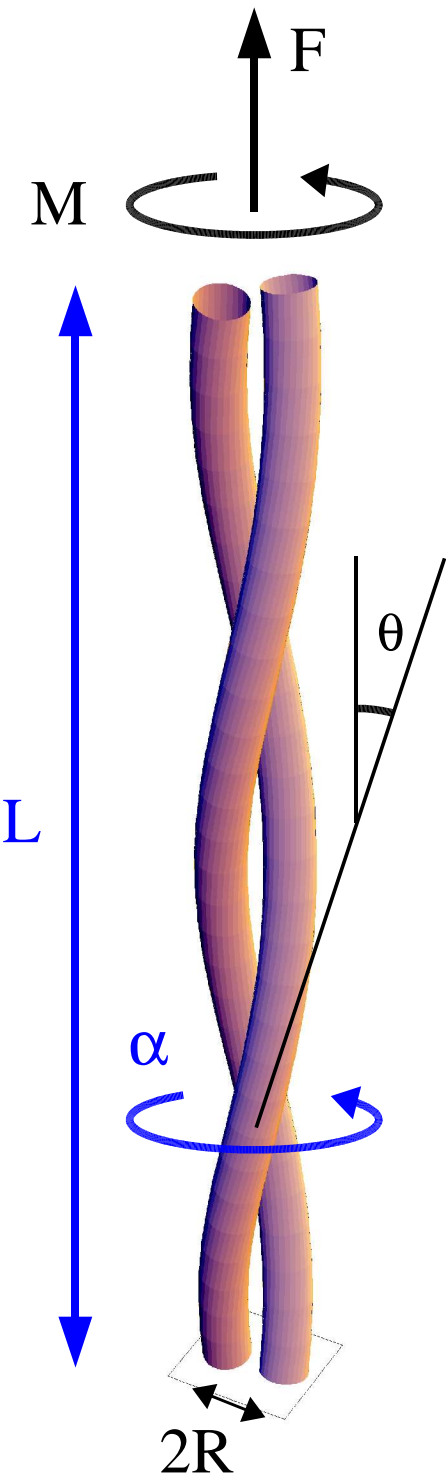Hypotheses : α-helix conserved under constraint

θ constant of s

extensibility not included

curvature $\qquad \kappa = \dfrac{\sin\theta \sin\theta}{R}$

total twist $\qquad \tau = \dot{\phi} + \tau_F$

with $\begin{cases} \dot{\phi} = -\hat{\tau} & \text{(hydrophobic constraint)} \\[2ex] \tau_F = \dfrac{\sin\theta \cos\theta}{R} & \text{(Frenet torsion of central line)} \end{cases}$

Equilibrium of the coiled-coil structure

$$V = V[\theta] \implies \frac{dV}{d\theta} = 0$$

$$4\,B\sin^3\theta\cos\theta + 2\,R\,C\cos(2\theta)\left(-\hat{\tau} + \frac{\sin\theta\cos\theta}{R}\right) + R^2\,F\sin\theta - R\,M\cos\theta \;=\; 0$$

$$\text{si } F = 0 = M: \quad -\frac{2\,B\sin^3\theta\cos\theta}{R\,C\cos(2\theta)} = \underbrace{-\hat{\tau} + \frac{\sin\theta\cos\theta}{R}}_{} \quad (=\tau:\ \text{total twist})$$

$$\text{for } \theta \ll 1: \quad \theta \simeq \hat{\tau}\,R \qquad (\text{ Fraser \& McRae (1973) })$$

mechanical equilibrium

coiled-coil handedness $=$ hydrophobic strip handedness

Comparaison with structural data

X-ray data from Harbury et al, *Nature* (1994)
GCN4 leucine-zipper

	X-ray data			model
GNC4	res/turn	rise/res		$\hat{\tau}$ (rad/A)
dimer	3.62	1.51 A		-0.039
trimer	3.60	1.53 A		-0.033
tetramer	3.59	1.52 A		-0.030

hydrophobic strip twist $\hat{\tau}$

Comparaison with structural data

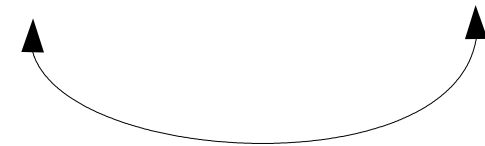X-ray data from Harbury et al, *Nature* (1994)
GCN4 leucine-zipper

GNC4	X-ray data			model	
	res/turn	rise/res	super-helix R	$\hat{\tau}$ (rad/A)	2θ
dimer	3.62	1.51 A	4.9 A	-0.039	-22°
trimer	3.60	1.53 A	6.7 A	-0.033	-26°
tetramer	3.59	1.52 A	7.6 A	-0.030	-27°

$$\theta \simeq \frac{1}{2} \arcsin\left(2 \ \hat{\tau} R\right)$$

Comparaison with structural data

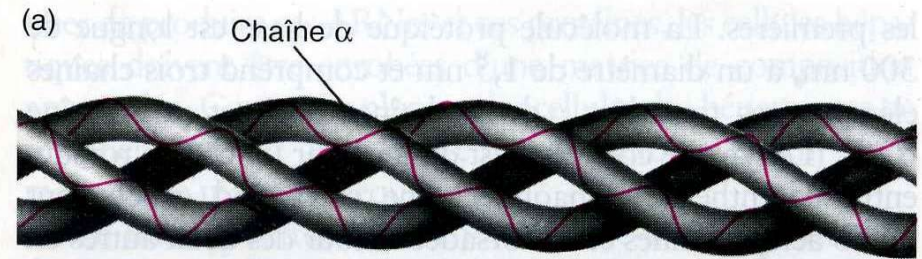X-ray data from Harbury et al, *Nature* (1994)
GCN4 leucine-zipper

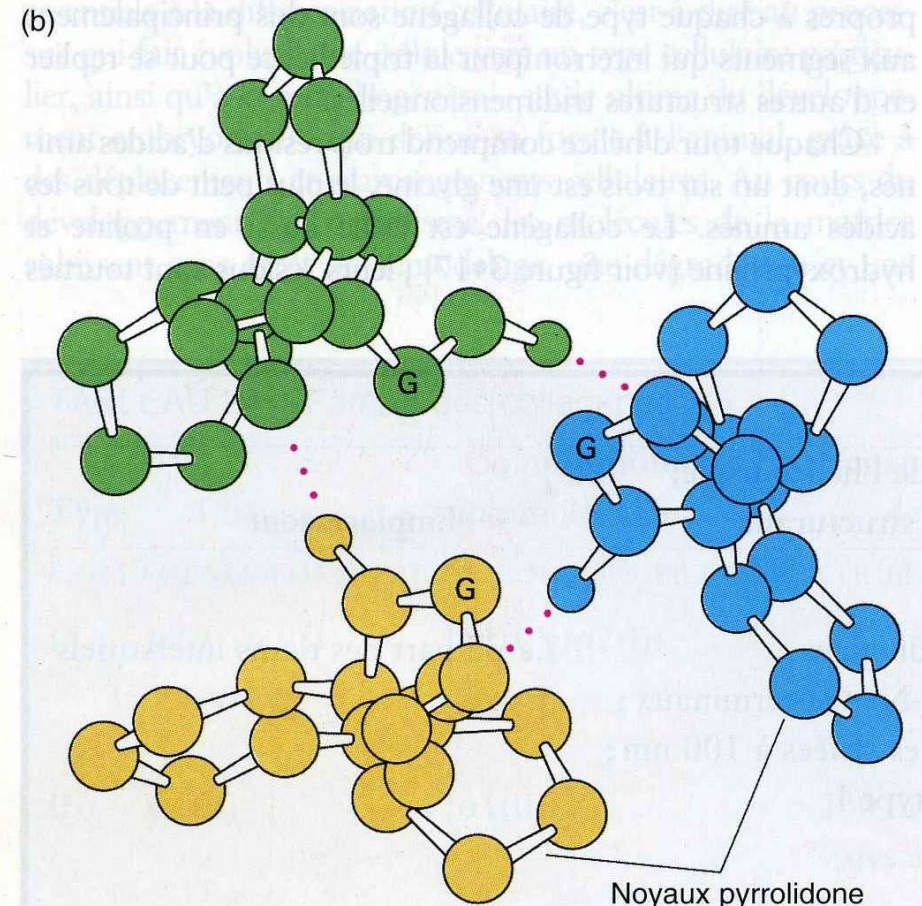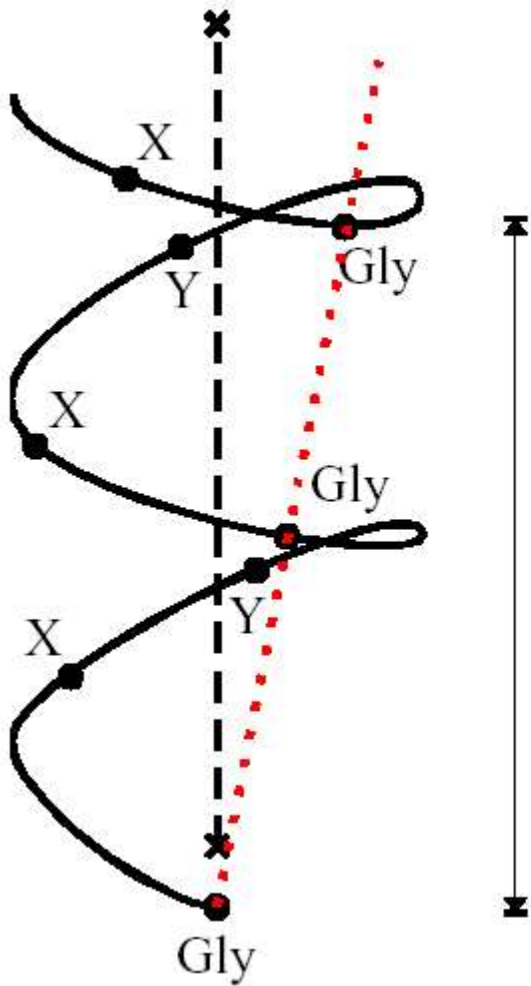| GNC4	X-ray data				model	
	res/turn	rise/res	super-helix R	super-helix 2θ	$\hat{\tau}$ (rad/A)	2θ
dimer	3.62	1.51 A	4.9 A	-23.4°	-0.039	-22°
trimer	3.60	1.53 A	6.7 A	-26.8°	-0.033	-25°
tetramer	3.59	1.52 A	7.6 A	-26.0°	-0.030	-26°

good agreement

Collagen triple helix

➜Fibrous protein (bone, tendon).

➜25% of total proteinic mass
  of human body.

➜Extra-cellular filaments
  (unlike actin, keratin).

➜Hierarchical structure

➜Tropo-collagen : triple-helix.

➜Each strand has 1050 residues.
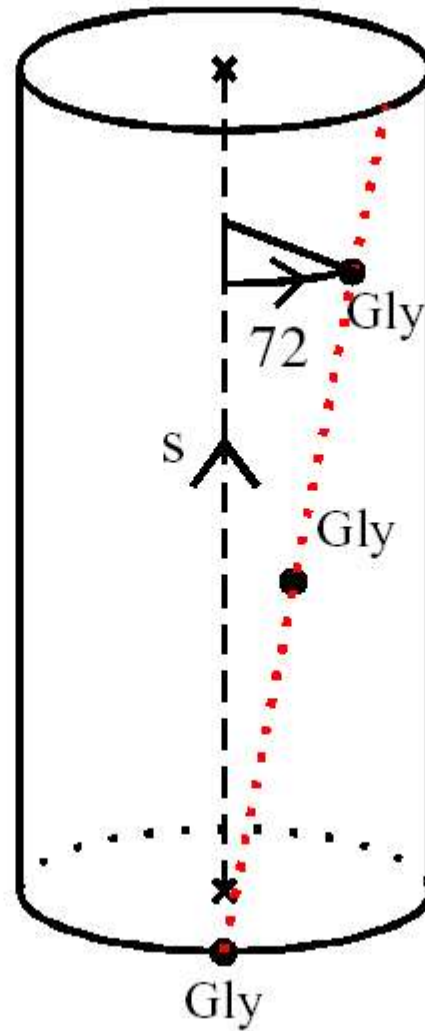
➜Each strand is itself an helix
  polymer (Gly-X-Y)$_n$

(a) Chaîne α

Fragment d'une molécule de collagène, une hélice triple de pas droit

(b)

G

G

G
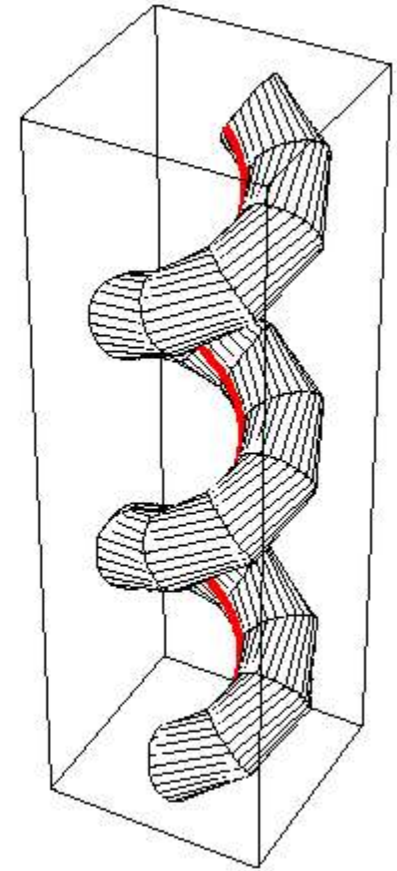
Noyaux pyrrolidone

Lodish et al. (1997)

Collagen: the glycine residues

3.3 residues / turn

$\hat{\tau} = +0.0732 \;\; rad/A$

$\phi' = -\hat{\tau}$

Comparaison with structural data

X-ray data from Bella et al (1994)

collagen trimer	X-ray data			model	
	res/turn	rise/res	super-helix R	$\hat{\tau}$ (rad/A)	2θ
	3.3	2.86 A	2.8 A	+0.073	+24°

$$\theta \simeq \frac{1}{2} \arcsin\left(2\,\hat{\tau}\,R\right)$$

Comparaison with structural data

X-ray data from Bella et al (1994)

collagen trimer	X-ray data				model	
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Comparaison with structural data

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	3.3	2.86 A	2.8 A	+23°	+0.073	+24°

$$\theta \simeq \frac{1}{2}\arcsin(2\,\hat{\tau}\,R)$$

$$(\sin(2\theta) = 2\,\hat{\tau}\,R)$$

Equilibrium equation: $\quad -\dfrac{2\,B\sin^3\theta\cos\theta}{R\,C\cos(2\theta)} = -\hat{\tau} + \dfrac{\sin\theta\cos\theta}{R}$

$$\frac{C}{B} = 2.3$$

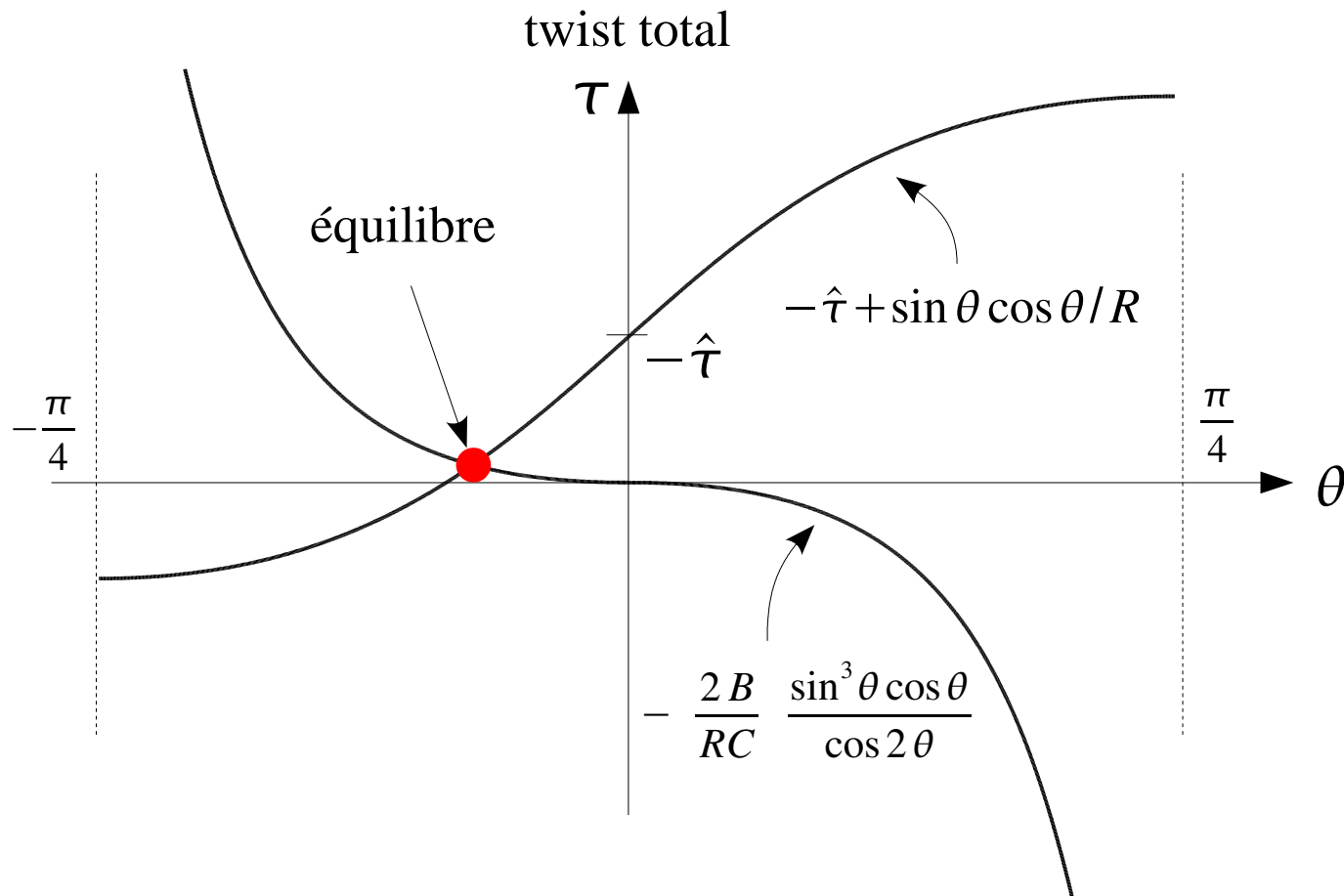elasticity : $\quad C\,/\,B < 1$

(GXY)$_n$ helix is easier to bend than to twist

ADN : $\quad 1 < C/B < 1.5$

Résolution graphique de l'équation d'équilibre

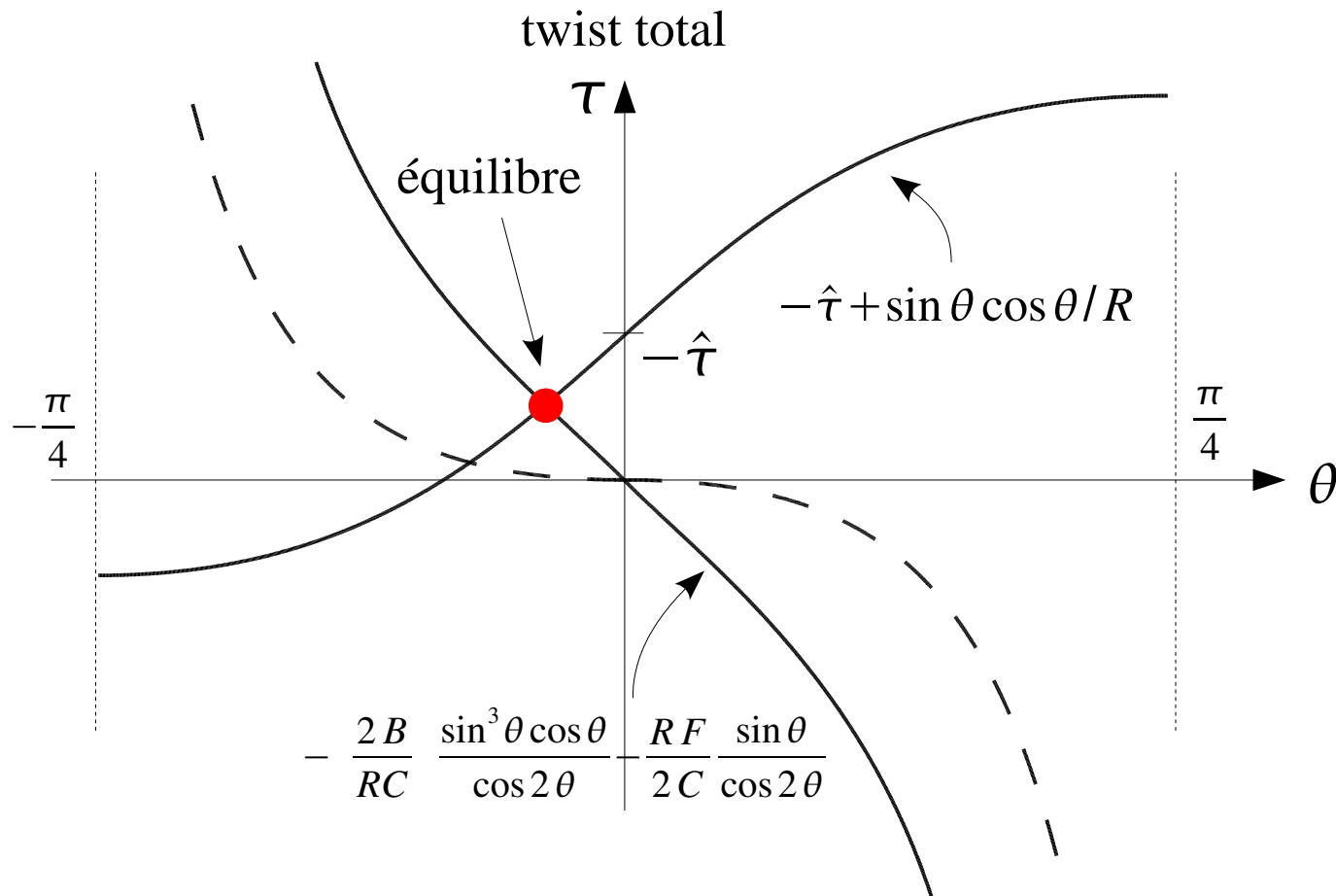super-enroulement isolé : $F = 0$ & $M = 0$

$$-\ \frac{2\ B\sin^3\theta\cos\theta}{R\,C\cos 2\,\theta}\ =\ -\hat{\tau}+\frac{\sin\theta\cos\theta}{R}\qquad(\ =\tau:\text{twist total}\ )$$

Résolution graphique de l'équation d'équilibre

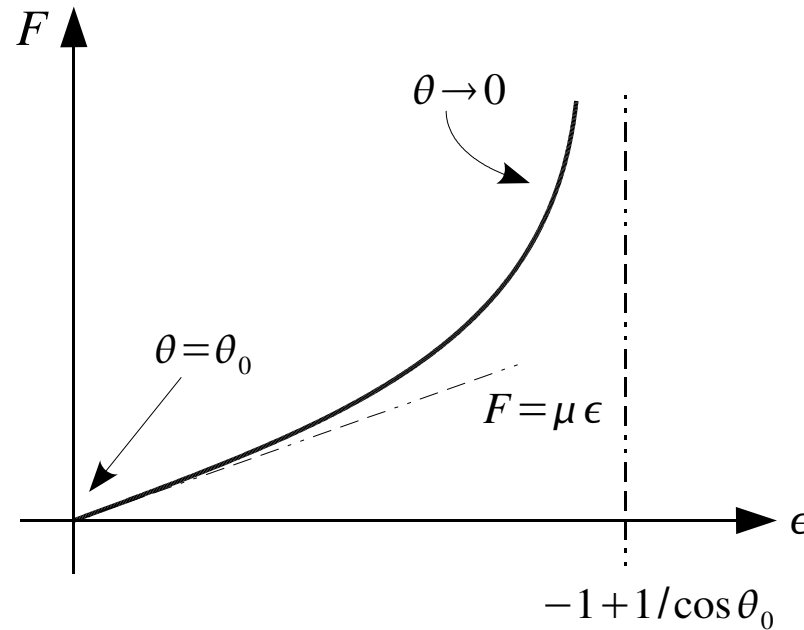super-enroulement sous tension : *F > 0 & M = 0*

$$-\frac{2\,B\sin^3\theta\cos\theta}{R\,C\cos 2\theta}-\frac{RF}{2\,C}\frac{\sin\theta}{\cos 2\theta}=-\hat{\tau}+\frac{\sin\theta\cos\theta}{R}\ (=\tau:\text{twist total})$$

Réponse en extension

$$F = \frac{2}{R^2} \frac{\cos 2\theta}{\sin\theta} \left[\frac{C}{2}\left(\sin 2\theta_0 - \sin 2\theta\right) + B\left(\tan 2\theta_0 \, \sin^2\theta_0 - \tan 2\theta \, \sin^2\theta\right)\right]$$

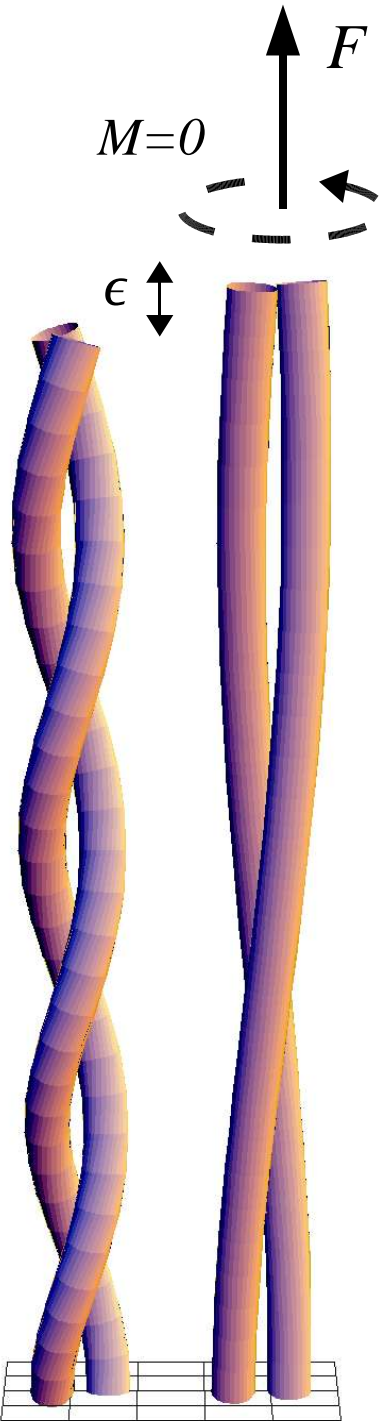$$\mu = \left.\frac{\partial F}{\partial \epsilon}\right|_{\theta_0} = \frac{2C}{R^2\,\theta_0^2}$$

$$\epsilon = \frac{\cos\theta}{\cos\theta_0} - 1$$

$$\left(\theta_0 = 10° \,\&\, R = 5\,A\right) \;\rightarrow\; \mu = 80\;nN\;\;(!) \qquad \epsilon = 1\% \;\rightarrow\; F = 800\;pN\;\;(!)$$

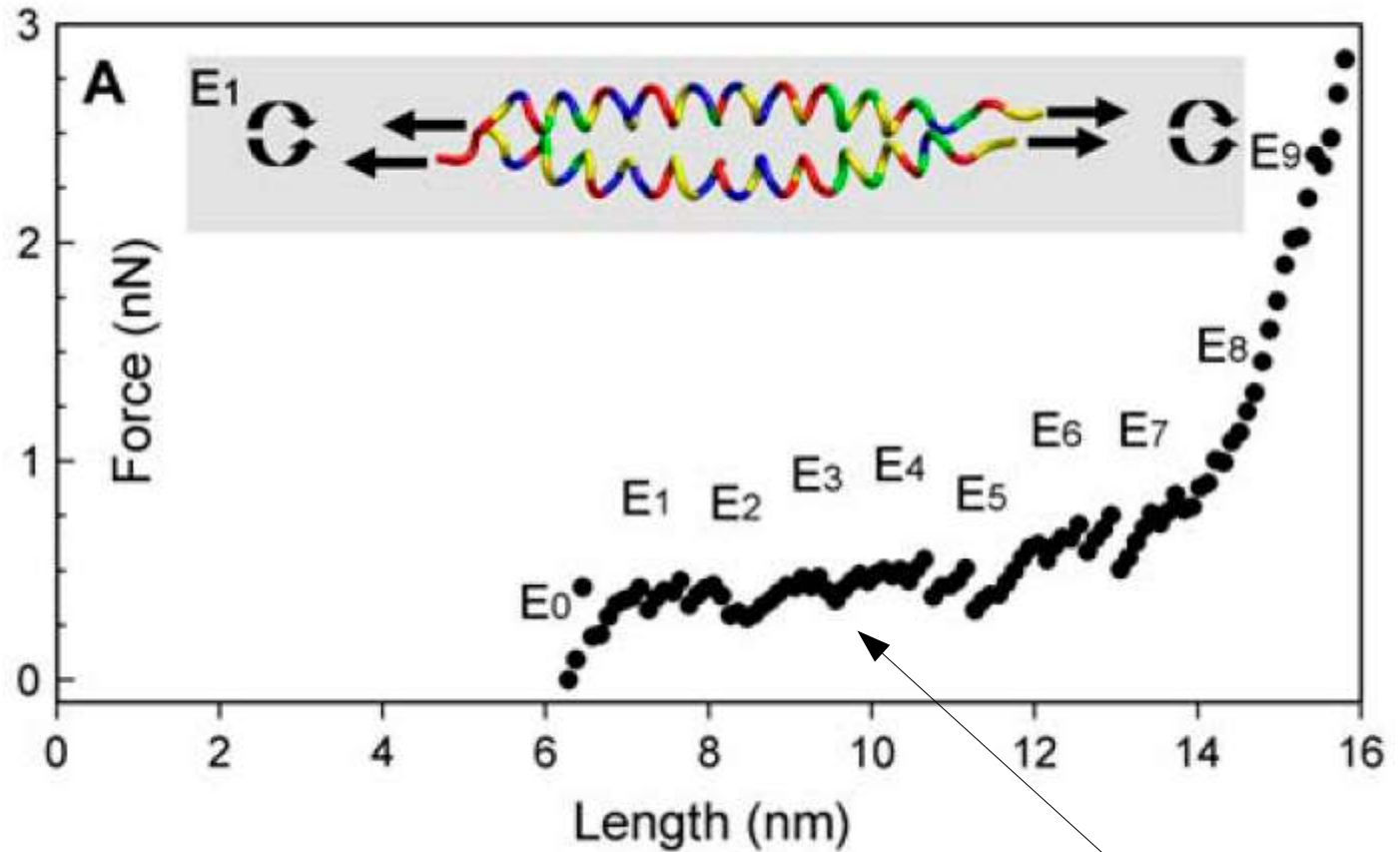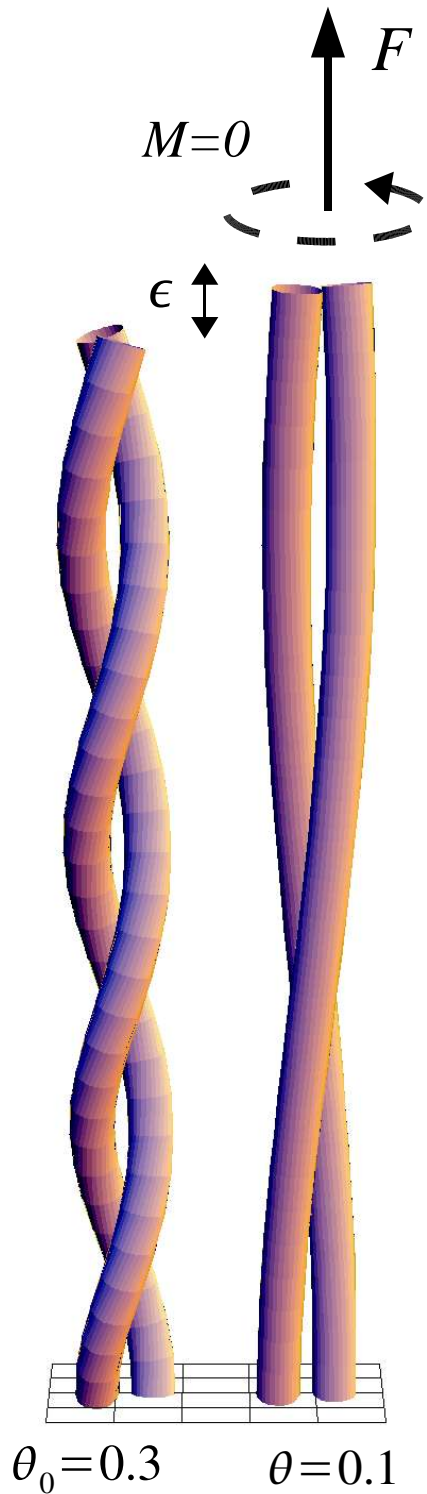liaisons hydrogène cassent bien avant

<u>Remarque</u> : structure extensible même si éléments inextensibles
couplage extension - rotation

$\theta_0 = 0.3 \qquad \theta = 0.1$

Réponse en extension

F

$M=0$

ϵ

A

E1 ⟳ ⟶ ⟶ ⟳ E9

E0

E1 E2 E3 E4 E5 E6 E7 E8 E9

Force (nN)

Length (nm)
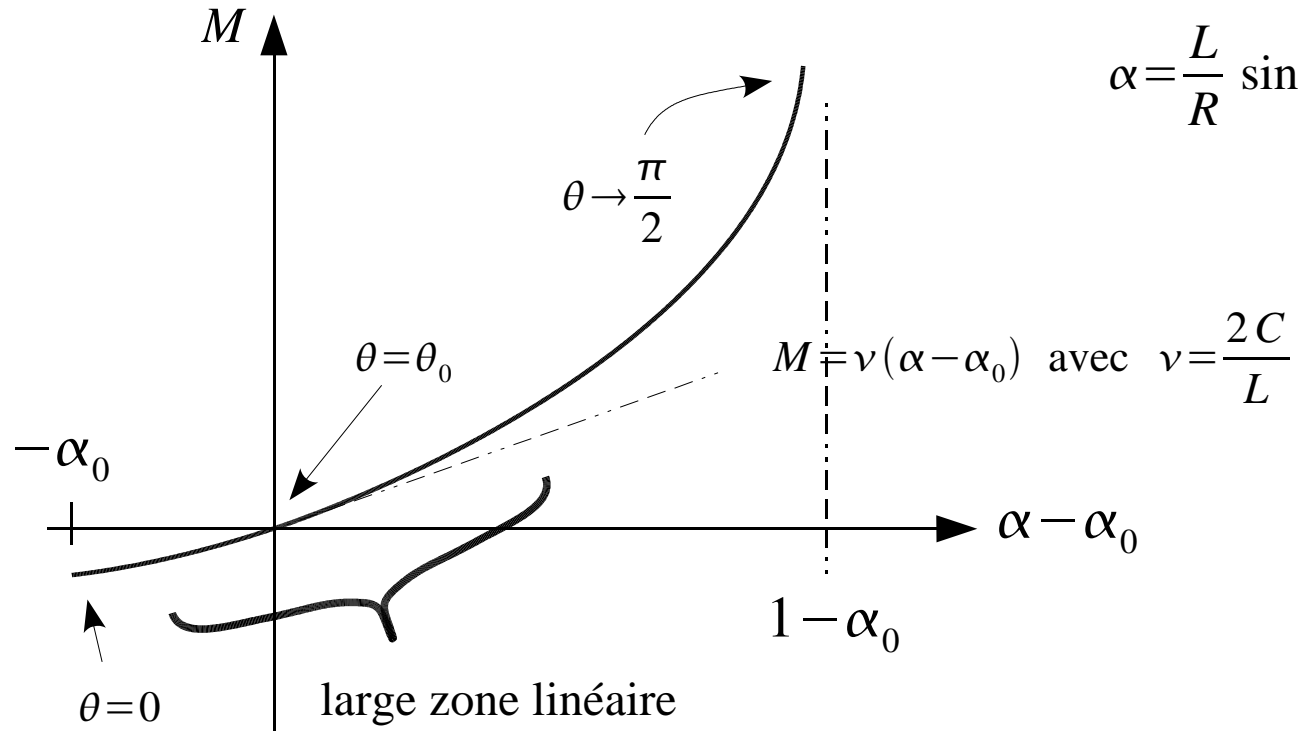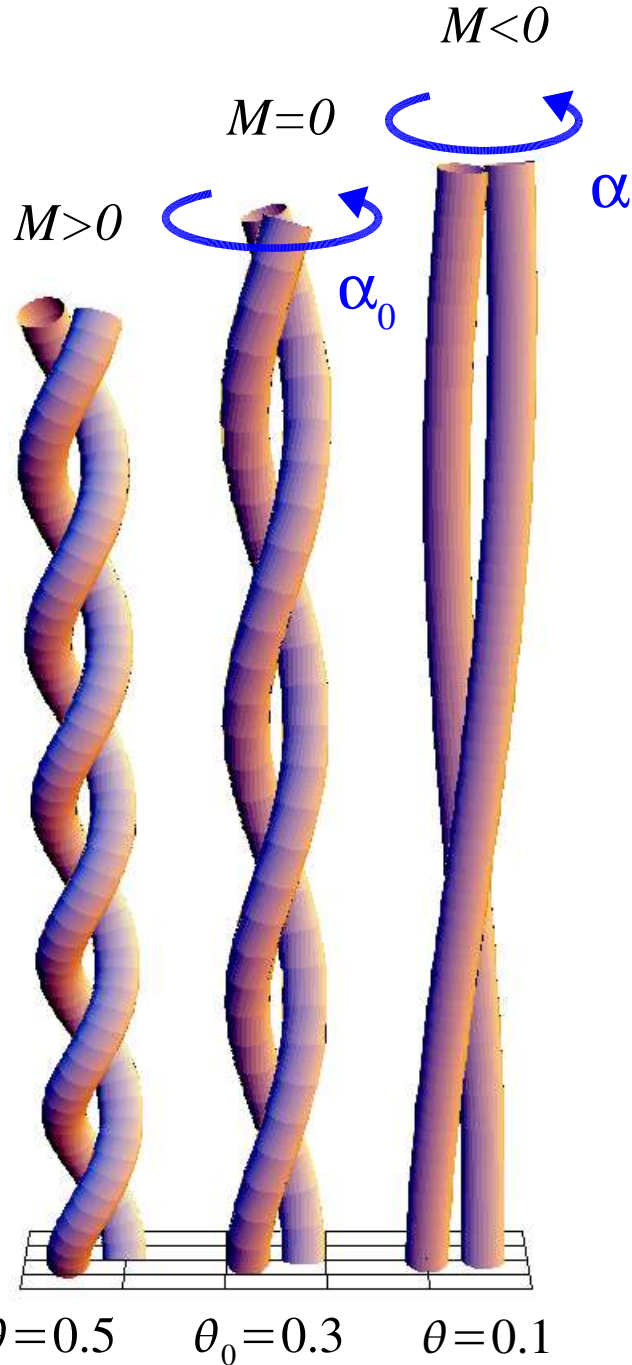
$\theta_0=0.3$ $\theta=0.1$

Simulations mécanique moléculaire sur la partie S2 de Beta-cardiac myosin (MYH7) Root et al, *Biophysical Journal* (2006)

dénaturation des hélices alpha

Réponse en rotation

$F=0$

$M>0$

$M=0$

$M<0$

α

α_0

$$M=-\ \frac{2}{R}\ \frac{\cos 2\theta}{\cos\theta}\ \left[\frac{C}{2}\left(\sin 2\ \theta_0 -\sin 2\ \theta\right)+B\left(\tan 2\ \theta_0\ \sin^2\theta_0 -\tan 2\ \theta\ \sin^2\theta\right)\right]$$

$$\alpha=\frac{L}{R}\ \sin\theta$$

M

$\theta\to\dfrac{\pi}{2}$

$\theta=\theta_0$

$M=\nu(\alpha-\alpha_0)$ avec $\nu=\dfrac{2C}{L}$

$-\alpha_0$

$\alpha-\alpha_0$

$1-\alpha_0$

$\theta=0$

large zone linéaire

$\theta=0.5$ $\theta_0=0.3$ $\theta=0.1$

Partie S2 de Beta-cardiac myosin (MYH7) :
$L = 6$ nm donc $\nu=140$ pN nm

=> $M = 3$ pN nm pour 1% de rotation

Diversité des protéines fibreuses

α-kératine

complexe actine-myosine

complexe de troponine
actine
tropomyosine
I C T

10 nm

(A) région α-hélicoïdale

(B) dimère superenroulé
48 nm

tétramère fait de deux dimères superenroulés étagés

force

extension (%)

μ-tubule

Dimère
tubuline β
tubuline α
GDP
GTP

8 nm

24 nm

Janmey et al., J. Cell Biol. (1991)

résistance rupture
rigide en extensibilité