DNA coby polyelektrolyt: Manningova teorie kondenzace protiiontů

F5351 Základy molekulární biofyziky
Masarykova Univerzita
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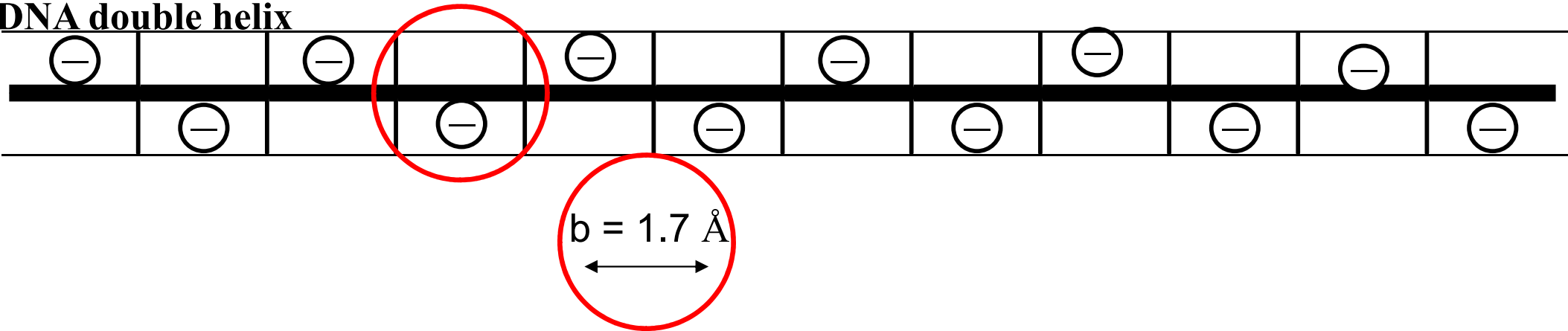24.11.2023

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Manning, G. S. (1978). "The molecular theory of polyelectrolyte solutions with aplications to the electrostatic properties of polynulceotides." *Quart. Rev. Biophys.* **11:** 179-246.

Double-stranded DNA can be considered as a linear long rod composed of segments, each carrying one unit charge. The segments repel each other. The repulsion is diminished if positively charged counter-ions associate with each segment on the DNA surface.

1 segment: charge -1e

DNA double helix

b = 1.7 Å

The distance b between segments harboring a unit charge (1 q_e=1.60x10^{-19} C) defines the <u>charge density</u> of the polymer.

For single-stranded DNA, b = 3.4 Å
For double-stranded DNA, b =1.7 Å

Before we continue, let us learn about two definitions:

Bjerrum* length l_B: the distance between two elementary charges (q_e) at which their repulsion energy is equal to the thermal energy $k_B T$

$$k_B T = \frac{k_e q_e^2}{\epsilon l_B} \implies l_B = \frac{k_e q_e^2}{\epsilon k_B T}$$
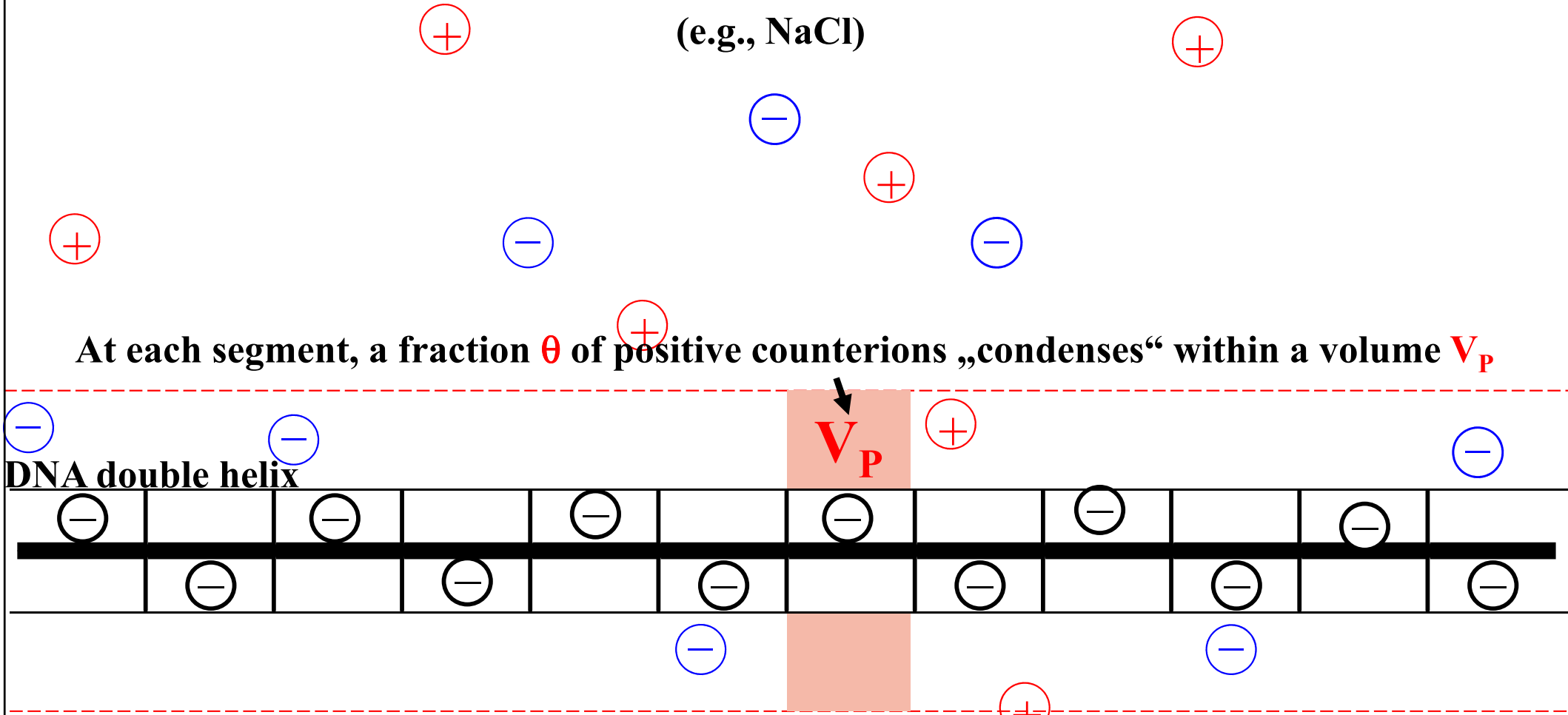
*Niels Jannicksen Bjerrum (1879-1958), dánský chemik

Manning** structural parameter ξ: the ratio between l_B and b, that is, the product of the Bjerrum length (a constant [Å]) and the linear charge density ([Å⁻¹]). **It is thus a dimensionless parameter proportional to the charge density**.

$$\xi = \frac{l_B}{b} = \frac{k_e q_e^2}{\epsilon k_B T b}$$

** Gerald S. Manning, professor emeritus, Rutgers University, New Jersey, USA

**Univalent salt in water
(e.g., NaCl)**

At each segment, a fraction θ of positive counterions „condenses" within a volume V_P

V_P

DNA double helix

The charge of 1 segment is reduced to $(-1+\theta)e$

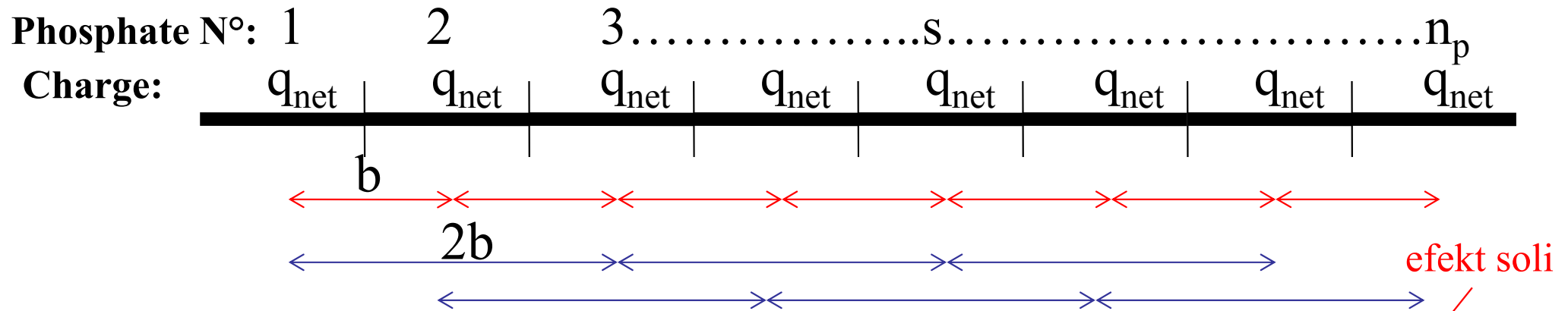**Gain in enthalpy ΔH, since repulsion between segments is diminished**

Loss in entropy ΔS, since order increases

Nature will „choose" such a θ fraction, that $\Delta G = \Delta H - T\Delta S$ is minimal

$\Downarrow$

$$\delta\Delta G(\theta, V_P)/\delta\theta = 0$$

**1 equation
2 unknowns**

Calculation of the electrostatic repulsion term ΔG_{el}

Phosphate N°: 1   2   3……………….s……………………………….n_p

Charge: q_{net} | q_{net} | q_{net} | q_{net} | q_{net} | q_{net} | q_{net} | q_{net}

b

2b

$q_{net} = q_e(1-\theta)$

efekt soli

Each pair contributes: $\Delta G_{ij} = \dfrac{k_e q_e^2 (1-\theta)^2}{\varepsilon |i-j| b} e^{-\frac{|i-j|b}{r_D}}$

efekt vody

Coulomb

Debye-Hückel screening factor

r_D = „Debye screening length" proportional to $c^{-1/2}$
c: concentration of univalent salt

if $n_p \gg s$

$|i-j| = 1$: number of pairs $= n_p-1 \approx n_p$

$|i-j| = 2$: number of pairs $= n_p-2 \approx n_p$

All pairs contribute:

.

.

$|i-j| = s$: number of pairs $= n_p-s \approx n_p$

$|i-j| > s$: interaction negligible $\Delta G_{ij} \approx 0$

Simplifying the sum over pairwise electrostatic repulsion terms

$$\Delta G_{el} = \frac{n_p L_{AV} k_e q_e^2 (1-\theta)^2}{\varepsilon b} \sum_{|i-j|=1}^{s} \frac{1}{|i-j|} e^{-\frac{|i-j|b}{r_D}}$$

$$\sum_{|i-j|=1}^{s} \frac{1}{|i-j|} e^{-\frac{|i-j|b}{r_D}} = e^{-\frac{b}{r_D}} + \frac{1}{2} e^{-\frac{2b}{r_D}} + \frac{1}{3} e^{-\frac{3b}{r_D}} + \dots$$

Looks like a Taylor series….

Cvičení 1: odvoďte Taylorův rozvoj pro funkci f(x)=ln(1+x)
(vyžadováno je řešení, nikoliv jen výsledek)

Taylorův rozvoj :

$$f(x) = f(a) + \frac{f'(a)}{1!}(x-a) + \frac{f''(a)}{2!}(x-a)^2 + \frac{f^{(3)}(a)}{3!}(x-a)^3 + \cdots.$$

$$f(x) = f(a) + \frac{f'(a)}{1!}(x-a) + \frac{f''(a)}{2!}(x-a)^2 + \frac{f^{(3)}(a)}{3!}(x-a)^3 + \cdots.$$

List of Taylor series of some common functions

$$e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} \qquad \text{for all } x$$

$$\ln(1+x) = \sum_{n=0}^{\infty} \frac{(-1)^n}{n+1} x^{n+1} \qquad \text{for } |x| < 1$$

$$\sqrt{x+1} = \sum_{n=0}^{\infty} \frac{(-1)^n (2n)!}{(1-2n)n!^2 4^n} x^n \qquad \text{for } |x| < 1$$

$$\frac{x^m}{1-x} = \sum_{n=m}^{\infty} x^n \qquad \text{for } |x| < 1$$

http://en.wikipedia.org/wiki/Taylor_series

Simplifying the sum over pairwise electrostatic repulsion terms

$$\Delta G_{el} = \frac{n_p L_{AV} k_e q_e^2 (1-\theta)^2}{\varepsilon b} \sum_{|i-j|=1}^{s} \frac{1}{|i-j|} e^{-\frac{|i-j|b}{r_D}}$$

Cvičení 2: vypočtěte sumu
↓

$$\sum_{|i-j|=1}^{s} \frac{1}{|i-j|} e^{\frac{-|i-j|b}{r_D}} = e^{\frac{-b}{r_D}} + \frac{1}{2} e^{\frac{-2b}{r_D}} + \frac{1}{3} e^{\frac{-3b}{r_D}} + ... =$$

$$\ln(1+x) = \sum_{n=0}^{\infty} \frac{(-1)^n}{n+1} x^{n+1} \quad \text{for } |x| < 1$$

$$e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} \quad \text{for all } x \qquad e^x = 1 + x + \frac{x^2}{2} + \frac{x^3}{6} + \frac{x^4}{24} + ...$$

$$e^x = 1 + x \quad \text{for } x<<1 \qquad -\ln(1 - e^{\frac{-b}{r_D}}) =$$

Cvičení 3: zjednodušte (r_D>>b)

Simplifying the sum over pairwise electrostatic repulsion terms

$$\Delta G_{el} = \frac{n_p L_{AV} k_e q_e^2 (1-\theta)^2}{\varepsilon b} \sum_{|i-j|=1}^{s} \frac{1}{|i-j|} e^{-\frac{|i-j|b}{r_D}}$$

Sum has become a simple logarithmic term!

$$\sum_{|i-j|=1}^{s} \frac{1}{|i-j|} e^{-\frac{|i-j|b}{r_D}} = e^{-\frac{b}{r_D}} + \frac{1}{2} e^{-\frac{2b}{r_D}} + \frac{1}{3} e^{-\frac{3b}{r_D}} + ... = -\ln(\frac{b}{r_D})$$

$$\Delta G_{el} = -\frac{n_p L_{AV} \boxed{k_e q_e^2} (1-\theta)^2}{\boxed{\varepsilon b}} \ln(\frac{b}{r_D})$$

with $\xi = \dfrac{k_e q_e^2}{\varepsilon b} \dfrac{1}{kT}$

ξ is the dimensionless «Manning structural factor» proportional to charge density

$$\Delta G_{el} = -\xi R T n_p (1-\theta)^2 \ln(\frac{b}{r_D}) \qquad \ln(\frac{b}{r_D}) < 0; \quad \Delta G_{el} > 0$$

Cvičení 4: Vypočtěte ξ pro dvojšroubovicovou DNA (b = 1.7 Å) při 25 °C

$$\xi = \frac{k_e q_e^2}{\varepsilon b}\frac{1}{kT}$$

$k = 1.38\text{x}10^{-23}\ \text{J.K}^{-1}$　　　$T = 298\ \text{K}$
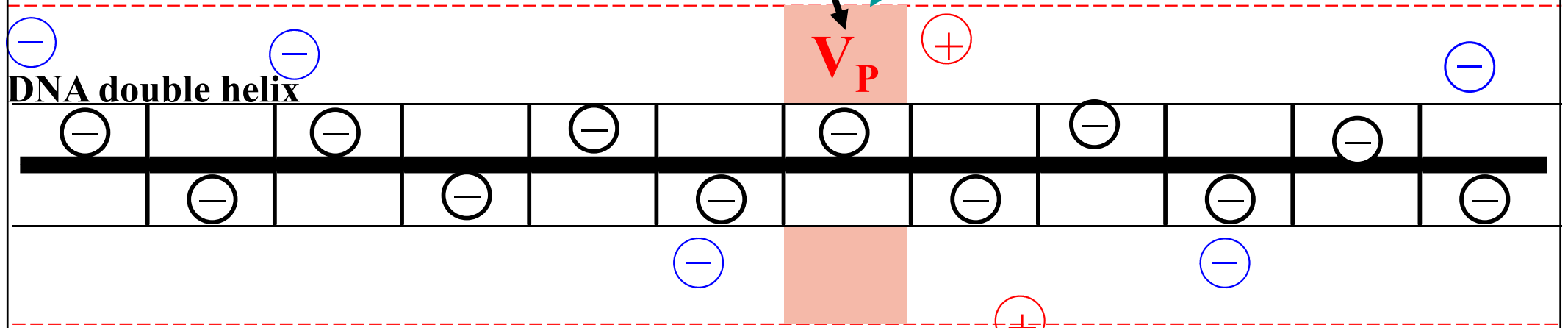
$k_e = 9.0\text{x}10^9\ \text{Nm}^2\text{C}^{-2}$　　　$\varepsilon = 78$　　　　$q_e = 1.60\text{x}10^{-19}\ \text{C}$

c_{bulk}

$c_{local} = n_P \theta (n_P V_P)^{-1} = \theta V_P^{-1}$

A fraction θ of positive counterions „condenses" within a volume V_P

V_P

DNA double helix

Loss in entropy ΔS, since order increases

Mixing entropy term dependent on θ*

$$\Delta G_{mix} = \theta n_P RT \ln\left(\frac{c_{local}}{c_{bulk}}\right) = \theta n_P RT \ln\left(\frac{\theta V_P^{-1}}{c}\right)$$

number of moles of couterions that is confined from c_{bulk} to c_{local}

$c_{bulk} = c$ = concentration [mol/L] of univalent salt KNOWN

V_P [L/mol] = volume to which the θ counterions/phosphate are confined

V_p UNKNOWN → HOPELESS CALCULATION???

Let's minimize the total free energy with respect to θ

$$\partial(\Delta G)/\partial\theta = 0$$

$$\Delta G = \Delta G_{el} + \Delta G_{mix} = RT n_P \left[-\xi(1-\theta)^2 \ln\left(\frac{b}{r_D}\right) + \theta \cdot \ln\left(\frac{\theta V_P^{-1}}{c}\right)\right]$$

* i.e., loss in entropy when the fraction θ of counterions associates with each segment

$$f(x) = \ln\left(\frac{1}{1+2x}\right)$$

Minimizing total free energy

$$\Delta G = \Delta G_{el} + \Delta G_{mix} = RTn_P[-\xi(1-\theta)^2\ln(\frac{b}{r_D}) + \theta \cdot \ln(\frac{\theta V_P^{-1}}{c})]$$

$$\frac{\delta(\Delta G)}{\delta\theta} =$$

Cvičení 6: vypočtěte derivaci. Naznačte postup výpočtu.

Dependence of ΔG_{mix} on c

Dependence of ΔG_{el} on c

From Debye-Hückel Theory of electrolytes

$$\frac{\delta(\Delta G)}{\delta\theta} = RTn_P[2\xi(1-\theta)\cdot\ln(\frac{b}{r_D}) + 1 + \ln(\frac{\theta V_P^{-1}}{c})] = 0$$

$$r_D\;[m] = \sqrt{\frac{\varepsilon_0\varepsilon RT}{2(L_{AV}q_e)^2}\frac{10^{-3}}{c}} \qquad\qquad b = \frac{k_e q_e^2}{\varepsilon\xi}\frac{1}{kT}$$

$$\frac{b}{r_D} = \frac{k_e q_e^2}{\varepsilon\xi}\frac{1}{kT}\left(\frac{2L_{AV}^2 q_e^2 c}{\varepsilon_0\varepsilon L_{AV}kT\cdot 10^{-3}}\right)^{\frac{1}{2}} = \left(\frac{2k_e^2 q_e^6 L_{AV}c}{\xi^2\varepsilon_0(\varepsilon kT)^3\cdot 10^{-3}}\right)^{\frac{1}{2}}$$

$$2\xi(1-\theta)\cdot\frac{1}{2}\ln\left[\frac{2k_e^2 q_e^6 L_{AV}c}{\xi^2\varepsilon_0(\varepsilon kT)^3\cdot 10^{-3}}\right] + 1 - \ln(\frac{c}{\theta V_P^{-1}}) = 0$$

$$\xi(1-\theta)\cdot\ln\left[\frac{2k_e^2 q_e^6 L_{AV}c}{\xi^2\varepsilon_0(\varepsilon kT)^3\cdot 10^{-3}}\right] + 1 - \ln(\frac{c}{\theta V_P^{-1}}) = 0$$

$$\xi(1-\theta) \cdot \ln\left[\frac{2k_e^2 q_e^6 L_{AV} c}{\xi^2 \varepsilon_0 (\varepsilon kT)^3 \cdot 10^{-3}}\right] + 1 - \ln\left(\frac{c}{\theta V_P^{-1}}\right) = 0$$

We want to know θ at the low concentration limit, $c \rightarrow 0$

$\xi(1-\theta) \ln c$

$\downarrow$

$-\infty$

$\ln c$

$\downarrow$

$-\infty$

$\rightarrow$Singular terms containing ln c must vanish!

$$\xi(1-\theta) \qquad\qquad - 1 = 0$$

Now we have got a second equation!

$$\theta = 1 - \xi^{-1}$$

k   Boltzmann's constant

k_e electrostatic constant

q_e electron charge

ε_0 vacuum permittivity

L_{AV} Avogadro's number

ξ dimensionless charge density of polymer

c concentration of univalent electrolyte

ε dielectric coefficient of electrolyte

T absolute temperature

} knowns

θ fraction of counterions condensed

V_P volume to which θ counerions condense

} unknowns

$$\xi(1-\theta) \cdot \ln[\frac{2k_e^2 q_e^6 L_{AV} c}{\xi^2 \varepsilon_0 (\mathcal{E}kT)^3 \cdot 10^{-3}}] + 1 - \ln(\frac{c}{\theta V_P^{-1}}) = 0$$

We want to know θ at the low concentration limit, c→0

$\xi(1-\theta) \ln c$

↓

-∞

ln c

↓

-∞

→Singular terms containing ln c must vanish!

$$\xi(1\text{-}\theta) \qquad \qquad - 1 = 0$$

Now we have got a second equation!

$$\theta = 1 - \frac{1}{\xi}$$

DNA in water:

$\xi = 4.2 \quad \theta = 0.76$

$\xi < 1 \rightarrow \theta < 0$ no association of counterions

$\xi > 1 \rightarrow \theta > 0$ association of counterions

76% of the negative charge of the phosphates is neutralized by condensed counterions!

Now we can determine V_P, the volume to which the θ counterions/nucleotide condense in double-stranded DNA

$$\xi(1-\theta)\cdot\ln\left[\frac{2k_e^2 q_e^6 L_{AV} c}{\xi^2 \varepsilon_0 (\mathcal{E}kT)^3 \cdot 10^{-3}}\right] + 1 - \ln\left(\frac{c}{\theta V_P^{-1}}\right) = 0$$

base of natural logarithms

$$\xi(1-\theta)\cdot\ln(c) + \xi(1-\theta)\cdot\ln\left[\frac{2k_e^2 q_e^6 L_{AV} e}{\xi^2 \varepsilon_0 (\mathcal{E}kT)^3 \cdot 10^{-3}}\right] - \ln(c) + \ln\left(\frac{\theta}{V_P}\right) = 0$$

$$\underbrace{\xi(1-\theta)}_{=1}\cdot\underbrace{\ln\left[\frac{2k_e^2 q_e^6 L_{AV} e\cdot\theta}{\xi^2 \varepsilon_0 (\mathcal{E}kT)^3 \cdot 10^{-3} V_P}\right]}_{=0} = 0$$

$$\frac{2k_e^2 q_e^6 L_{AV} e\cdot\theta}{\xi^2 \varepsilon_0 (\mathcal{E}kT)^3 \cdot 10^{-3} V_P} = 1$$

$$V_P = \frac{2k_e^2 q_e^6 L_{AV} e\cdot\theta}{\xi^2 \varepsilon_0 (\mathcal{E}kT)^3 \cdot 10^{-3}}$$

$$V_P = \frac{2k_e^2 q_e^6 L_{AV} e \cdot \theta}{\xi^2 \varepsilon_0 (\varepsilon kT)^3 \cdot 10^{-3}}$$

$k = 1.38 \times 10^{-23}$ J.K^{-1}

$k_e = 9.0 \times 10^9$ Nm^{2}C^{-2}

$q_e = 1.60 \times 10^{-19}$ C

$\varepsilon_0 = 8.85 \times 10^{-12}$ C^{2}N^{-1}m^{-2}

$L_{AV} = 6.02 \times 10^{23}$ mole^{-1}

$e = 2.718$

DNA in water at 25°C
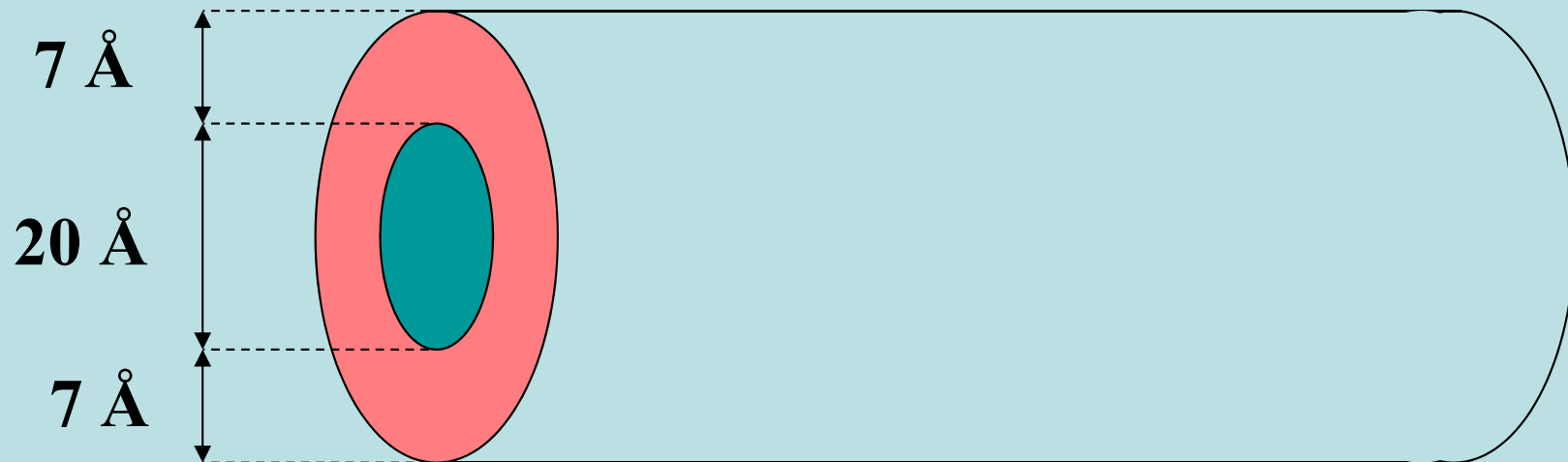
$\xi = 4.2$

$\varepsilon = 78$

$T = 298$ K

$\theta = 0.76$

The volume to which the condensed univalent counter-cations are confined, V_P
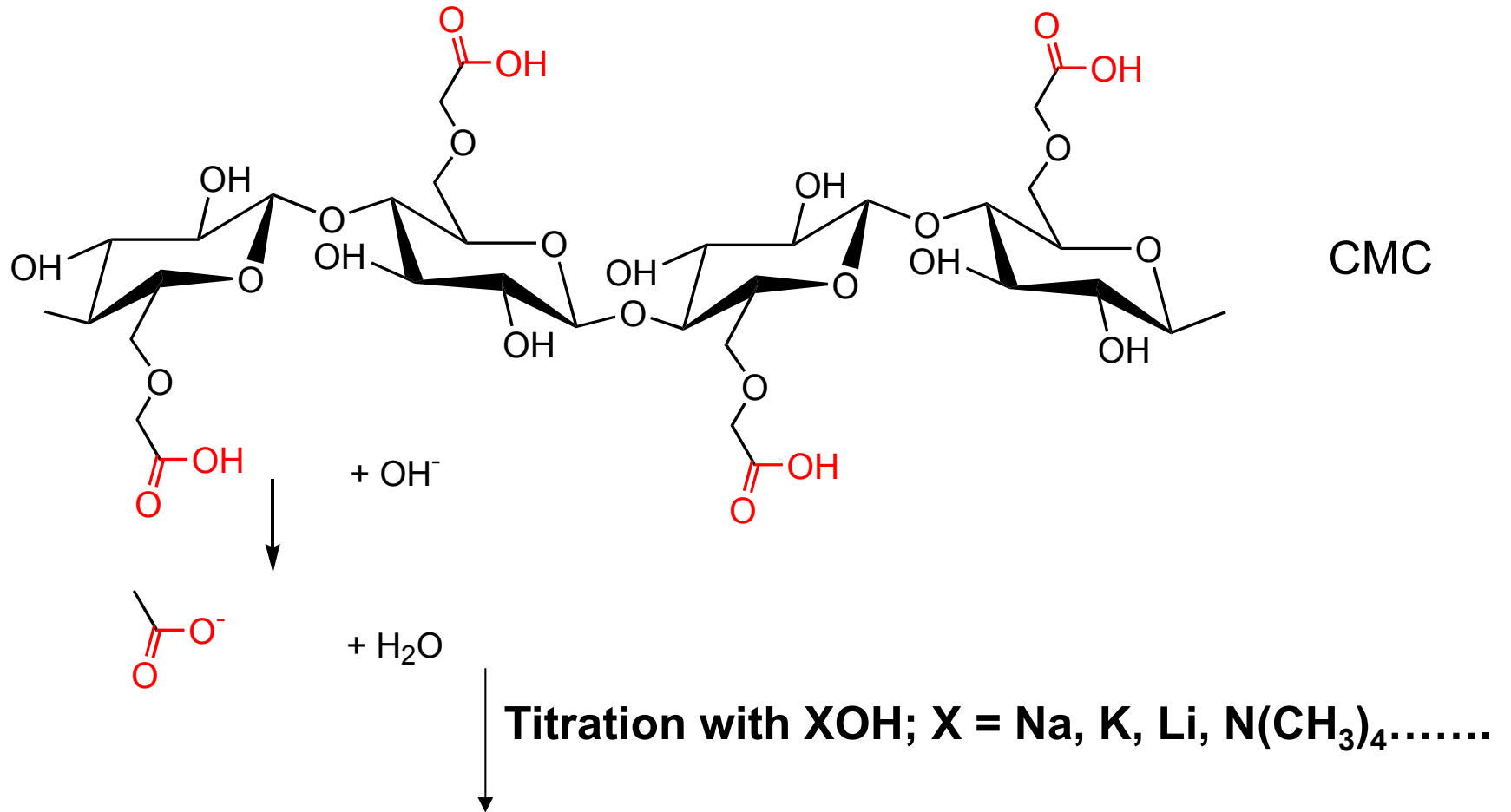
Per mole: 646 cm^3

Per nucleotide: 1073 Å^3

This corresponds to a layer of ~7 Å around the DNA double-helix (which corresponds ~to a cylinder with a diameter of 20 Å)

7 Å

20 Å

7 Å

Counterion Condensation Theory: Manning, G. S. (1978) *Quart. Rev. Biophys.* **11:** 179-246
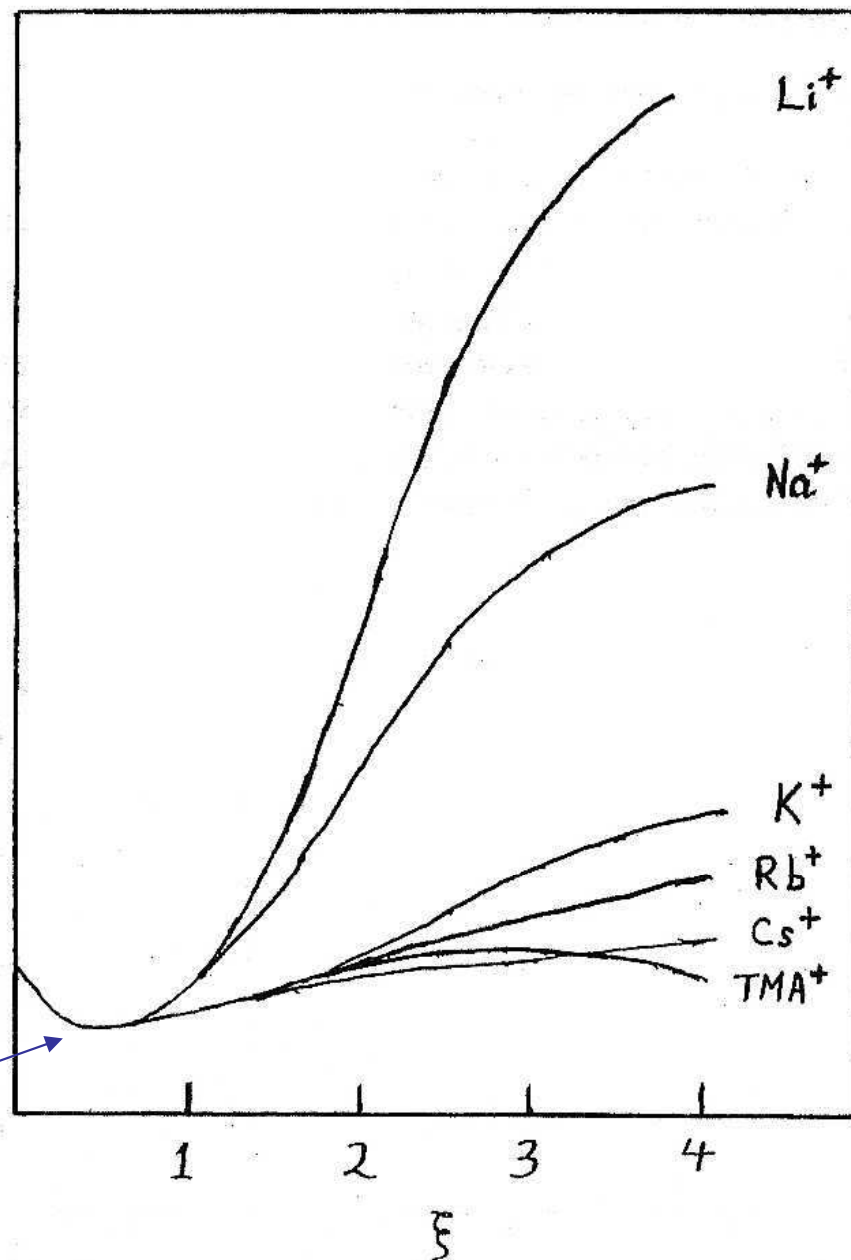
Experimental and theoretical results supporting the Counterion Condensation Theory

1. Titration of Carboxymethylcellulose (CMC) with different hydroxides monitored by ultrasonic absorption

CMC

+ OH⁻

+ H₂O

Titration with XOH; X = Na, K, Li, N(CH₃)₄.......

Upon titration, the COOH groups are successively deprotonated, become COO⁻, and the negative charge density increases. This modifies the structure of the water layer at the surface of the polymer, which can be monitored by measuring the absorption of ultrasound. If there is no association of cations with the polyanion, the structure of the water layer should be independent of the cation.
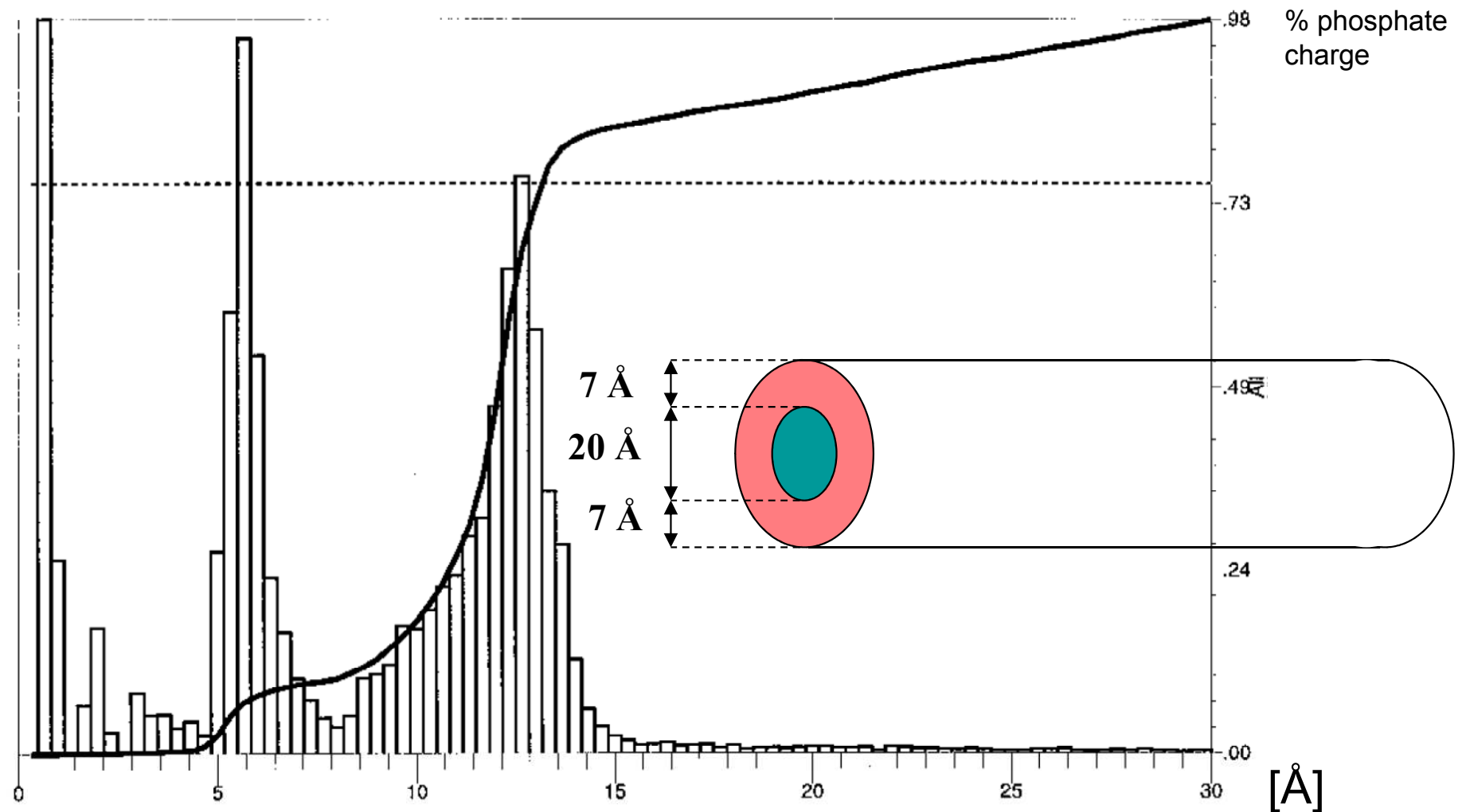
$\xi > 1$: great differences between the various hydroxides $\Rightarrow$ association between the counterions and the polymer, as predicted by the Manning theory

$\xi < 1$: no difference between the various hydroxides $\Rightarrow$ no association between the counterions and the polymer

Fig. 3
Ultrasonic absorption measurements on carboxymethylcellulose as a function of polymer charge density ξ for various counterions (TMA$^+$ = tetramethylammonium ion) [5]. The ordinate is a measure of the difference between the absorption of the solution and that of the solvent; for the ordinate scale and further detail, see the original reference [5]. The solid curves for each counterion were interpolated by the authors of [5] through their data points

R. Zana et al., J. Chim. Phys, 68, 1258-1266 (1971)

2. Molecular dynamics simulations of a solvated DNA duplex

Radial distribution of Na$^+$ counterions around a DNA duplex 12 base-pairs long, from a molecular dynamics simulation (Yang et al., <u>J. Am. Chem. Soc.</u> 1997, **119**, 59-69). The calculation predicts 82% of the phosphate charge to be neutralized with cations moving within a radius of 15 Å from the helix axis. Manning theory predicts 76% of cations within 17 Å from the helix axis.

Remember:

$$\theta = 1 - \xi^{-1}$$

In a diluted solution of counterions, θ is concentration-independent!

θ = fraction of monovalent cations predicted by Counterion Condensation Theory to condense on each unit charge of the poly-anion

If $\xi > 1$ then a fraction of $1-\xi^{-1}$ counterions condense at each unit charge

If $\xi < 1$, there is no counterion condensation.

duplex DNA in H_2O at 298 K: $\xi = 4.2$ single-stranded DNA: $\xi = 2.1$

Problems: Nucleotides and Nucleic Acids

1) Match the type of bond with the role below:

<u>Bond_type</u>
(a) phosphodiester
(b) *N*-glycosidic
(c) phosphate ester

(d) hydrogen bonds

<u>Role</u>
____ links base to pentose in nucleotide
____ joins adjacent nucleotides in one strand
____ join complementary nucleotides in two
strands
____ difference between a nucleoside and a
nucleotide

2) Describe briefly what is meant by saying that two DNA strands are complementary.

3) Describe briefly how noncovalent interactions contribute to the three-dimensional shapes of RNA molecules.

4) Describe qualitatively how the t_m for a double-stranded DNA depends upon its nucleotide composition.

Derivační vzorce a pravidla

Vzorce používající se pro výpočet derivací:

$(c)' = 0$

$(x^n)' = n*x^{n-1}$

$(c*u)' = c * (u)'$

$(u+v)' = (u)' + (v)'$

$(u*v)' = (u)'*v + u*(v)'$

$(u/v)' = ((u)'*v - u*(v)') / v^2$

$(ln(x))' = 1/x$

$(log(x))' = 1/ (x*ln10)$

$(1/v)' = -v' / v^2$

$(sin(x))' = cos (x)$

$(cos(x))' = -sin(x)$

$(tg(x))' = 1/ cos^2(x)$

$(cotg(x))' = -1/ sin^2(x)$

$(e^x)' = e^x$

$(a^x)' = $ **$(a^x) * lna$**

Derivační metody

Složené derivace- derivace složené funkce je rovna součinu derivací na než lze složená funkce rozložit.