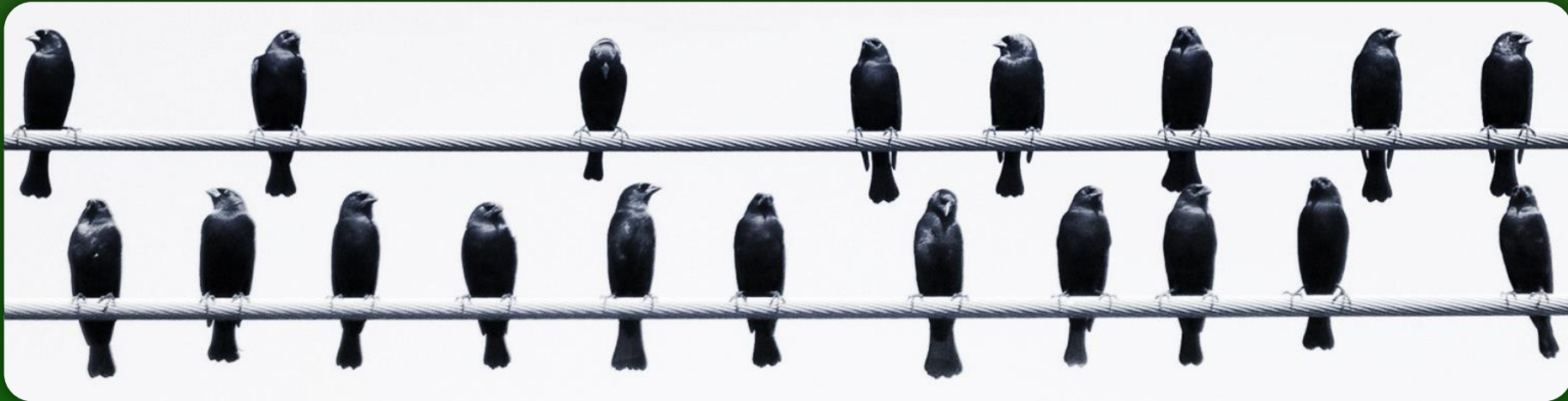# Znáš svého souseda?

aneb

studium retenčního mechanismu

sekvemerů ultrakrátkých oligonukleotidů

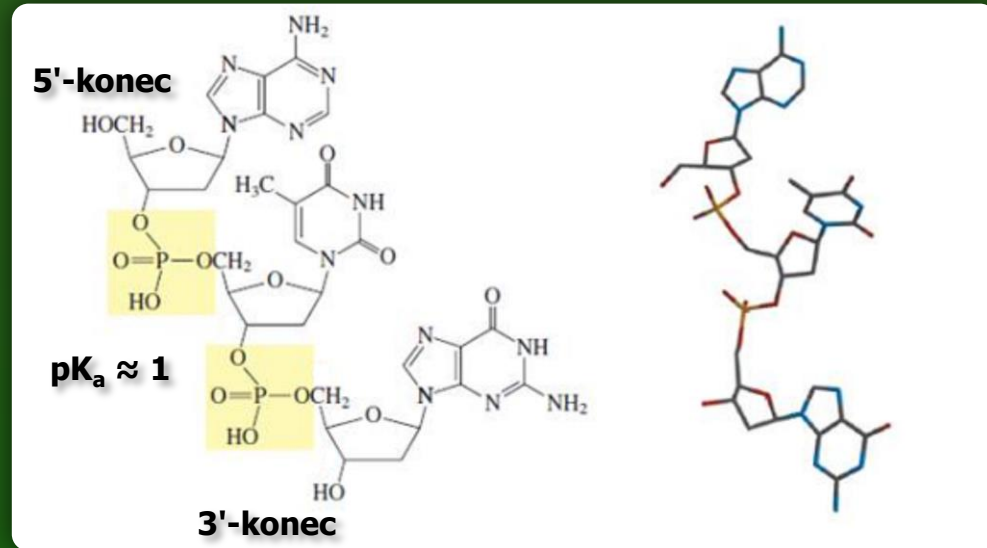**Miroslava Bittová**

Sekvemery

obecná struktura trinukleotidu

: 5′-XXX-3′
  : A, G, T, C, U
  : neobsahuje 5′-koncový fosfát

5'-konec

$pK_a \approx 1$

3'-konec

sekvemer (sekvenční izomery)

: heteropolymery se shodnými podjednotkami, ale jejich jiným pořadím

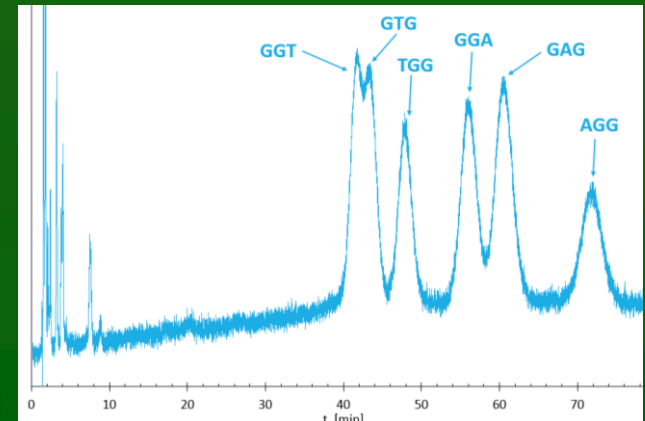|	
:: GAG, GGA, AGG	(A,2G)
TAT, TTA, ATT	(A,2T)
GTG, GGT, TGG	(2G,T)
TGT, TTG, GTT	(G,2T)
AGA, AAG, GAA	(2A,G)
ATA, AAT, TAA	(2A,T)
:: TAG, TGA, GAT, AGT GTA, ATG	(A,T,G)

2

Iontová interakční kapalinová chromatografie na obrácené fázi

(II-RPLC, *ion-interaction reversed-phase liquid chromatography*)

stínění náboje analytu a **expozice polárních částí** interakci se SF

: u II-RPLC se SF interaguje výhradně polární část analytu

: u IP-RPLC i nepolární část surfaktantu
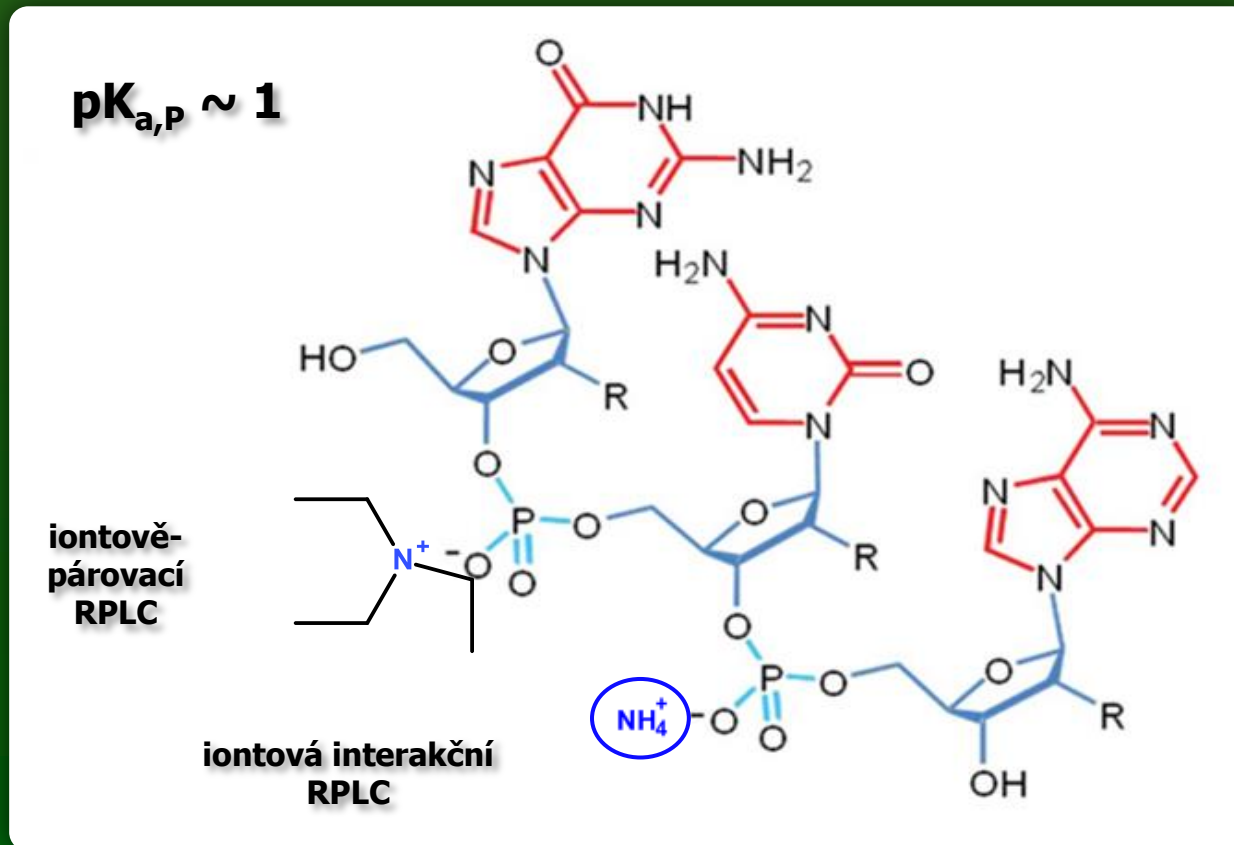
Co může ovlivnit retenci sekvemerů v II-RPLC a tím něco říct o retenčním mechanismu?

intermolekulární interakce solut – solvent – stacionární fáze

: solvent – organická složka MF

:: různé typy interakcí – dipól-dipól, proton-donorní či proton-akceptorní

: vytipování a využití několika různých rozpouštědel a jejich obsahu v MF

: analýzu získaných dat pomocí rovnice Snydera-Soczewińského

:: závislost retenčního faktoru na polaritě MF- upřesnění vlivu rozpouštědla

$$\ln k = \ln k_0 - a \cdot \varphi$$

Snyderova-Soczewińského rovnice

a – parametry každého analytu v systému
k$_0$ – extrapolovaná hodnota *k* pro vodu jako MF
φ – objemový zlomek organické fáze

výsledkem snažení by mělo být dovození **povahy interakcí** při separaci a tím i **retenčního mechanismu**

ABSTRACT

We studied sequence-dependent retention properties of synthetic 5′-terminal phosphate absent trinucleotides containing adenine, guanine and thymine through reversed-phase liquid chromatography (RPLC) and QSRR modelling. We investigated the influence of separation conditions, namely mobile phase composition (ion interaction agent content, pH and organic constituent content), on sequence-dependent separation by means of ion-interaction RPLC (II-RPLC) using two types of models: experimental design–artificial neural networks (ED-ANN), and linear regression based on molecular dynamics data. The aim was to determine those properties of the above-mentioned analytes responsible for the retention dependence of the sequence.

Our results show that there is a deterministic relation between sequence and II-RPLC retention properties of the studied trinucleotides. Further, we ... agent in the mobile phase, the more promine... the polar component of solvation energy in Q... vacuum to water, and the non-polar compo... best describe the retention properties of trin... sequences 5′-NAN-3′, 5′-ANN-3′, 5′-TGN-3′... Their role is still unknown, but since linear ... higher observable variance coverage than th... solvent-analyte interactions are responsible ... cleotides and some intramolecular interactio... 5′-NGA-3′ trinucleotides.

Research Article

Toward reading the sequence of short oligonucleotides from their retention factors obtained by means of hydrophilic interaction chromatography and ion-interaction reversed-phase liquid chromatography

Retention characteristics of selected synthetic 5′-terminal phosphate absent penta-nucleotides containing adenine, guanine, and thymine were studied in relation to their sequence by hydrophilic interaction chromatography and ion-interaction reversed-phase liquid chromatography. The organic solvent content, pH, and buffer concentration in mobile phases were evaluated as influential separation conditions. Data demonstrate that both compared chromatographic modes can be used to separate synthetic penta-nucleotides according to their nucleotide composition. Moreover, reversed-phase liquid chromatography allows separation according to their sequence. We have found a simple linear additive model to describe the retention order in both separation modes in regard to their sequence. In hydrophilic interaction chromatography, the retention behavior is controlled primarily by the hydrophilicity of involved nucleotides and minimally by their sequence position. For reversed-phase liquid chromatography, the nucleotide hydrophobicity plays an important role in their retention properties and the influence of their location in sequence on the retention increases toward the center and decreases toward the termini. Our results show that the penta-nucleotide sequence, and thus its spatial arrangement induced by the surrounding environment, is highly related to the retention properties, so it may be hypothetically used to read the sequence from the retention properties acquired under particular separation conditions.

Kontakt:

Miroslava Bittová

bittova@sci.muni.cz

Spolupráce:

Jan Havliš

NCBR

5

to boldly go where
no man has gone before !

Děkuji za pozornost