Comparison of polysaccharide stationary phases in supercritical fluid chromatography

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Why supercritical fluid chromatography ?

Packed column SFC uses a **carbon dioxide** – **solvent** mobile phase

Viscosity is lessened : higher flow rates are possible and column efficiency is improved (peaks are thiner)

Solvent consumption is usually reduced as compared to HPLC

Collected fractions are more concentrated in prep

Overall productivity is usually improved

Current state of chiral SFC

Similarly to HPLC:

Limited understanding of the factors favouring successful separation

Screening strategies are the only ones used everywhere

- time-optimized strategies: SFC can be very fast with short columns, gradient elution and parallel screening

- usually optimized in terms of complementarity of the systems screened

Is it good enough?

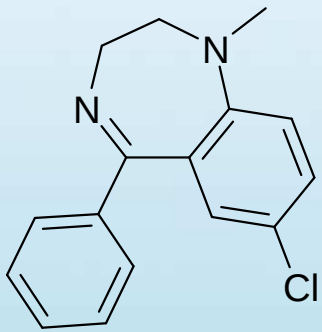
Can we do anything better?

Objectives of our study

To propose a classification of chiral stationary phases for SFC use

The knowledge acquired in HPLC is not sufficient

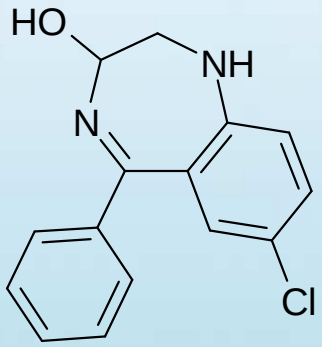
HPLC-SFC transfer is not always immediate



diazepam

α (LC) = 1

α (SFC) = 8.09



oxazepam

α (LC) = 1.39

α (SFC) = 1

Lux Cellulose-1, CO_2-iPrOH or HPT-iPrOH 90:10 (v/v), 25°C, SFC : 150 bar 3 mL/min, HPLC : 1 mL/min

Objectives of our study

To propose a classification of chiral stationary phases for SFC use

To propose compound-tailored screening strategies rather than generic strategies

To possibly predict the outcome of a separation

To improve our understanding of enantioseparation mechanisms

How to proceed?

Need for consistent data to compare chromatographic systems

Litterature is a mess !!!

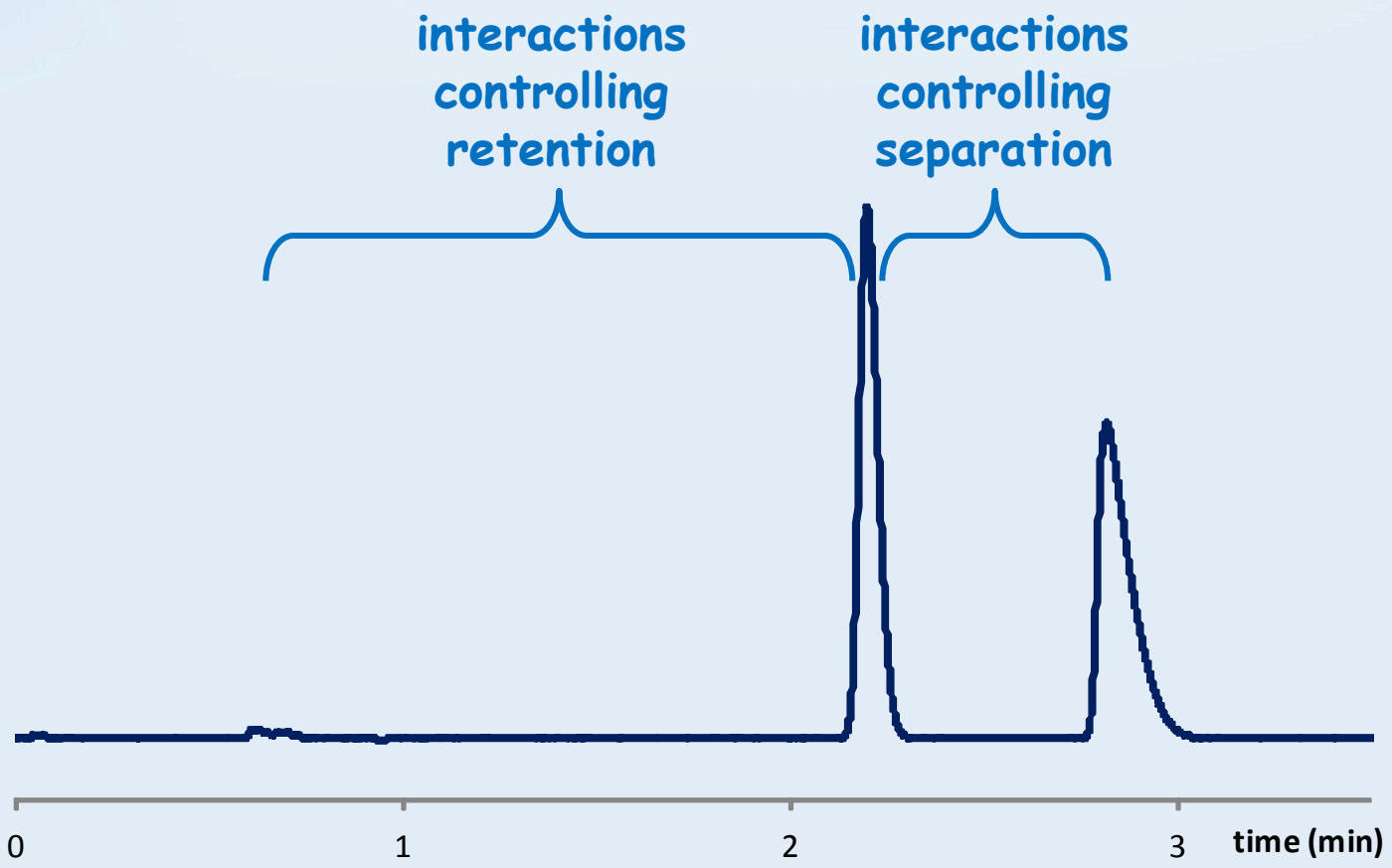
Large set of compounds with structural diversity

Identical operating conditions for all columns

CO_2-MeOH 90:10 (v/v), 25°C, 150 bar, 3 mL/min

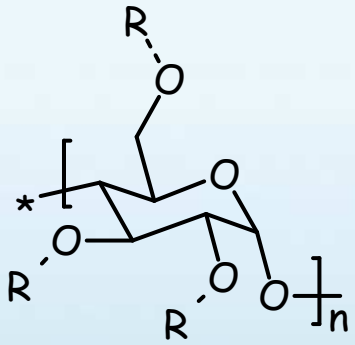
How to proceed?

Need to distinguish between

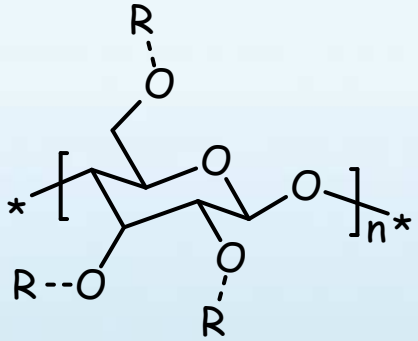


Modified polysaccharide chiral stationary phases

Amylose

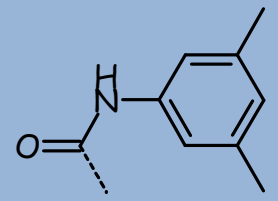


Cellulose

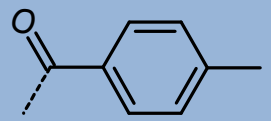


Phenomenex chiral stationary phases (CSP)

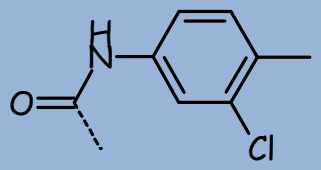
4 CSPs based on Cellulose



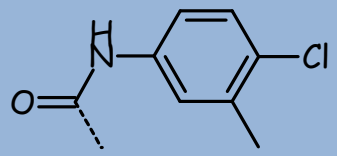
Lux Cellulose-1 (LC1)



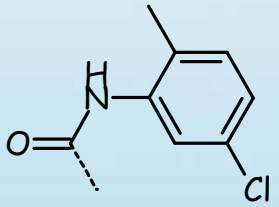
Lux Cellulose-3 (LC3)



Lux Cellulose-2 (LC2)

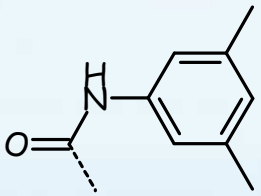


Lux Cellulose-4 (LC4)

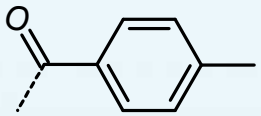


Lux Amylose-2 (LA2)

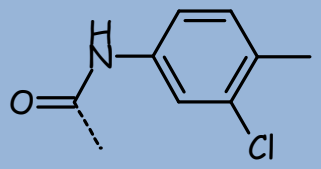
Phenomenex chiral stationary phases (CSP)



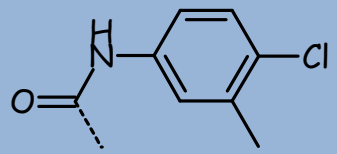
Lux Cellulose-1 (LC1)



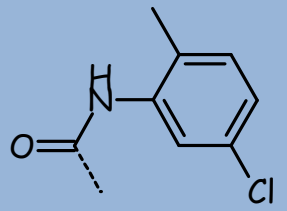
Lux Cellulose-3 (LC3)



Lux Cellulose-2 (LC2)



Lux Cellulose-4 (LC4)

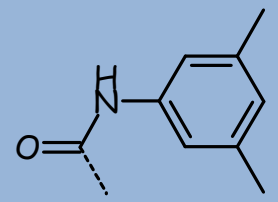


Lux Amylose-2 (LA2)

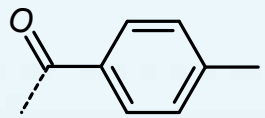
3 chlorinated CSPs

Phenomenex chiral stationary phases (CSP)

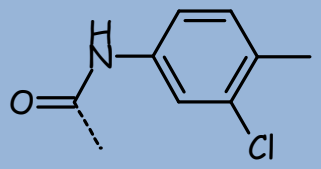
1 ester ligand



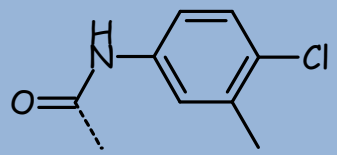
Lux Cellulose-1 (LC1)



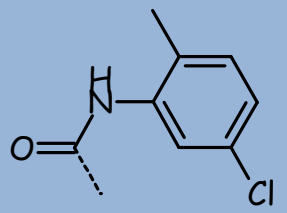
Lux Cellulose-3 (LC3)



Lux Cellulose-2 (LC2)



Lux Cellulose-4 (LC4)



Lux Amylose-2 (LA2)

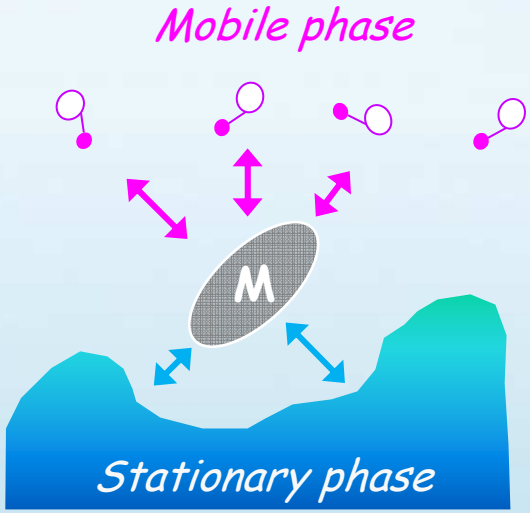
4 carbamate ligands

A method to compare stationary phases

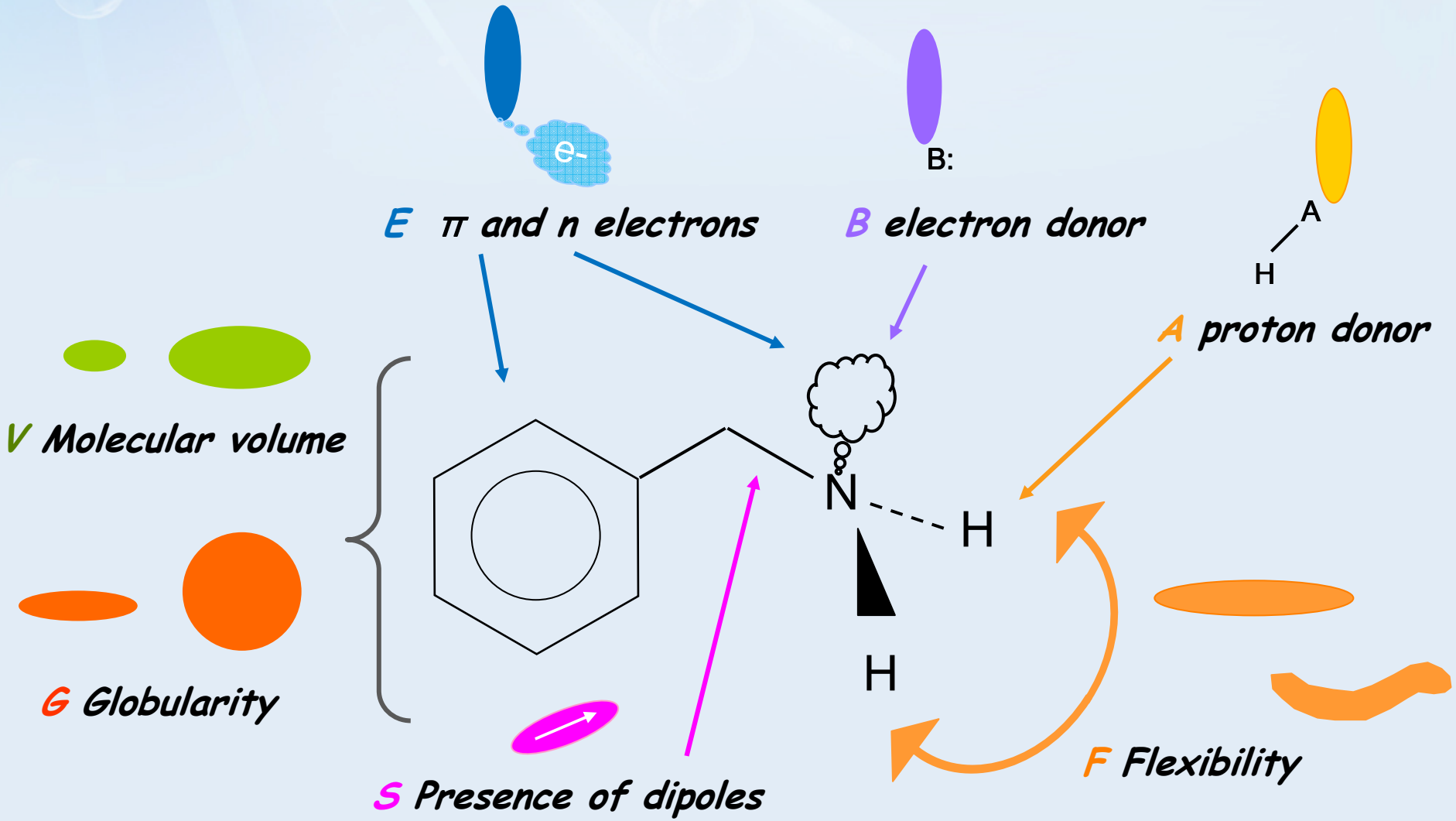
The analyte interacts with the stationary phase and the mobile phase

depending on molecular characteristics inducing different capabilities for interaction

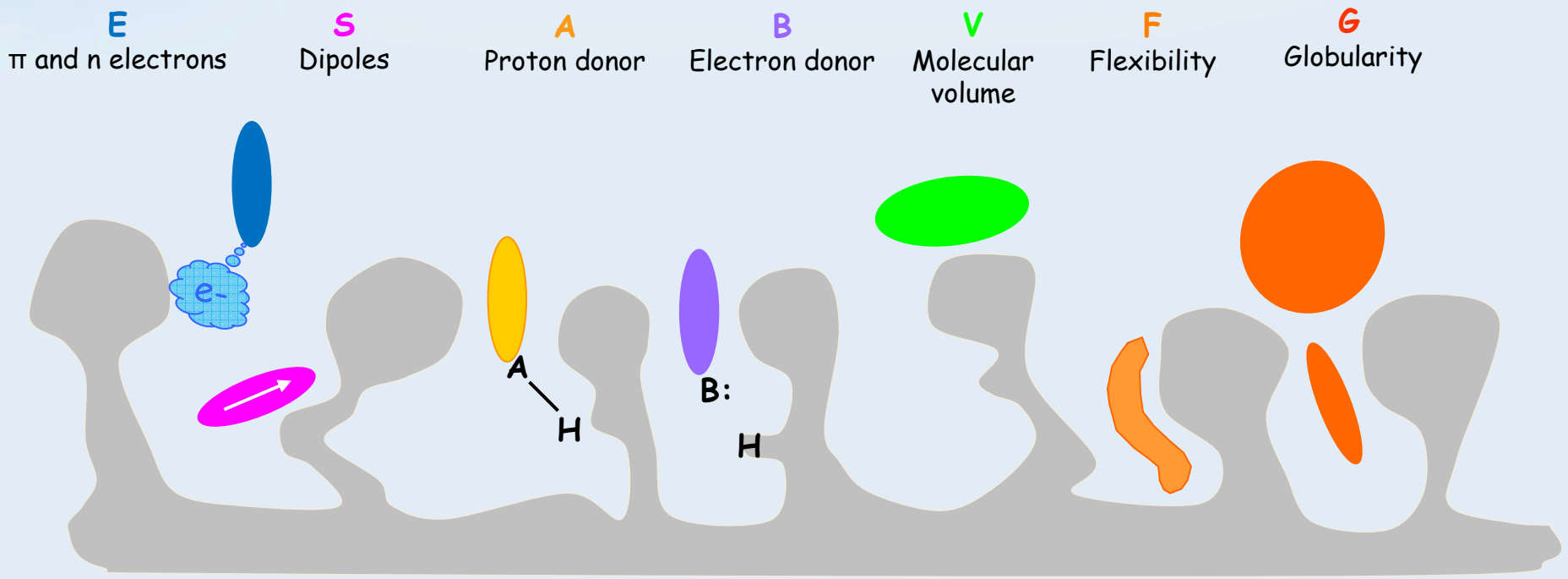
Example: an acidic compound can interact via hydrogen-bonding



A method to compare stationary phases
Analyte capabilities for interaction



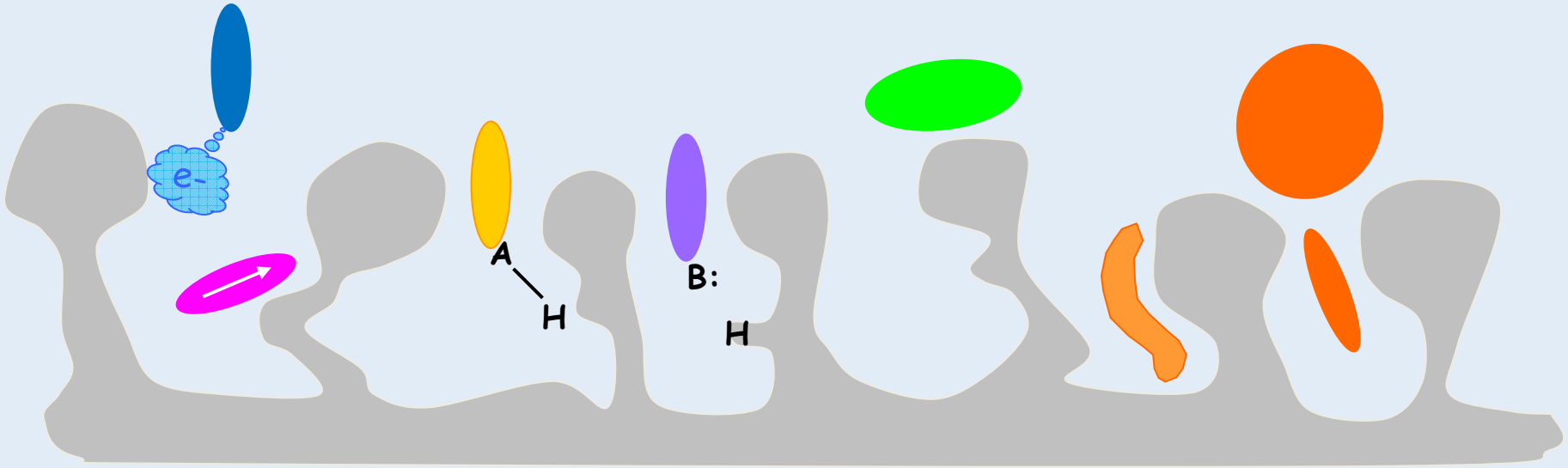
A method to compare retention properties
Interactions in the chromatographic system



A method to compare retention properties
Modified solvation parameter model

$$\log k = c + eE + sS + aA + bB + vV + fF + gG$$

E	S	A	B	V	F	G
π and n electrons	Dipoles	Proton donor	Electron donor	Molecular volume	Flexibility	Globularity



e	s	a	b	v	f	g
π-π interactions	dipole-dipole interactions	H-bonding	H-bonding	dispersive interactions	flexible adaptation	steric resistance to insertion

A method to compare retention properties
Modified solvation parameter model

$$\log k = c + eE + sS + aA + bB + vV + fF + gG$$

Determine the properties of a group of probe analytes (>200): *E*, *S*, *A*, *B*, *V*, *F* and *G* with a dedicated software (or litterature data: 4000 compounds)

Measure retention *k* for each of them in the chromatographic system you wish to characterize

Do a multiple linear regression to determine the system coefficients: *e*, *s*, *a*, *b*, *v*, *f* and *g*.

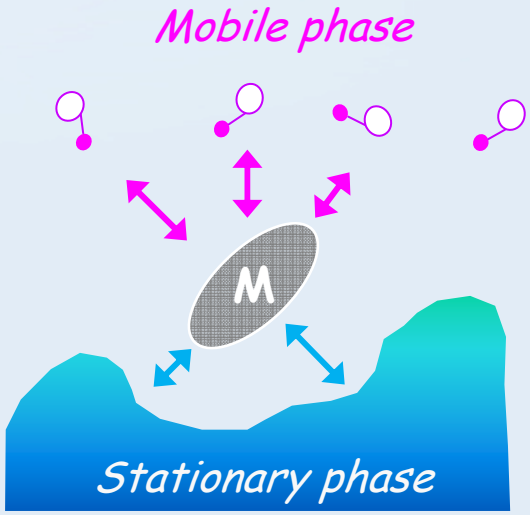
(*e*, *s*, *a*, *b*, *v*, *f*, *g*)
represent retention in the chromatographic system

A method to compare retention properties
Modified solvation parameter model

$$\log k = c + eE + sS + aA + bB + vV + fF + gG$$

For each coefficient x:

$$x = x_{stationary} - x_{mobile}$$

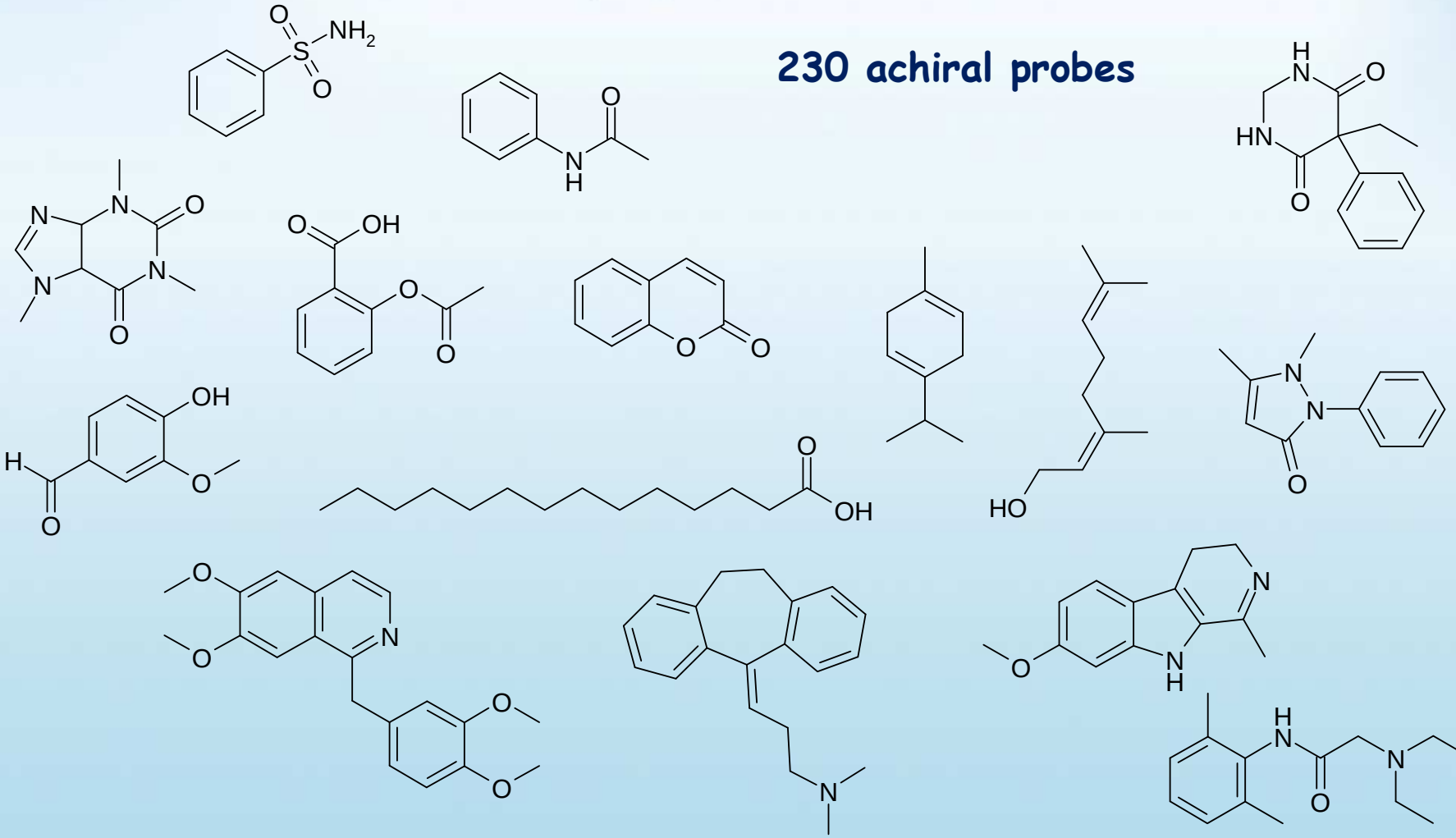


If $x_{stationary}$ is constant, ***mobile phases can be compared***

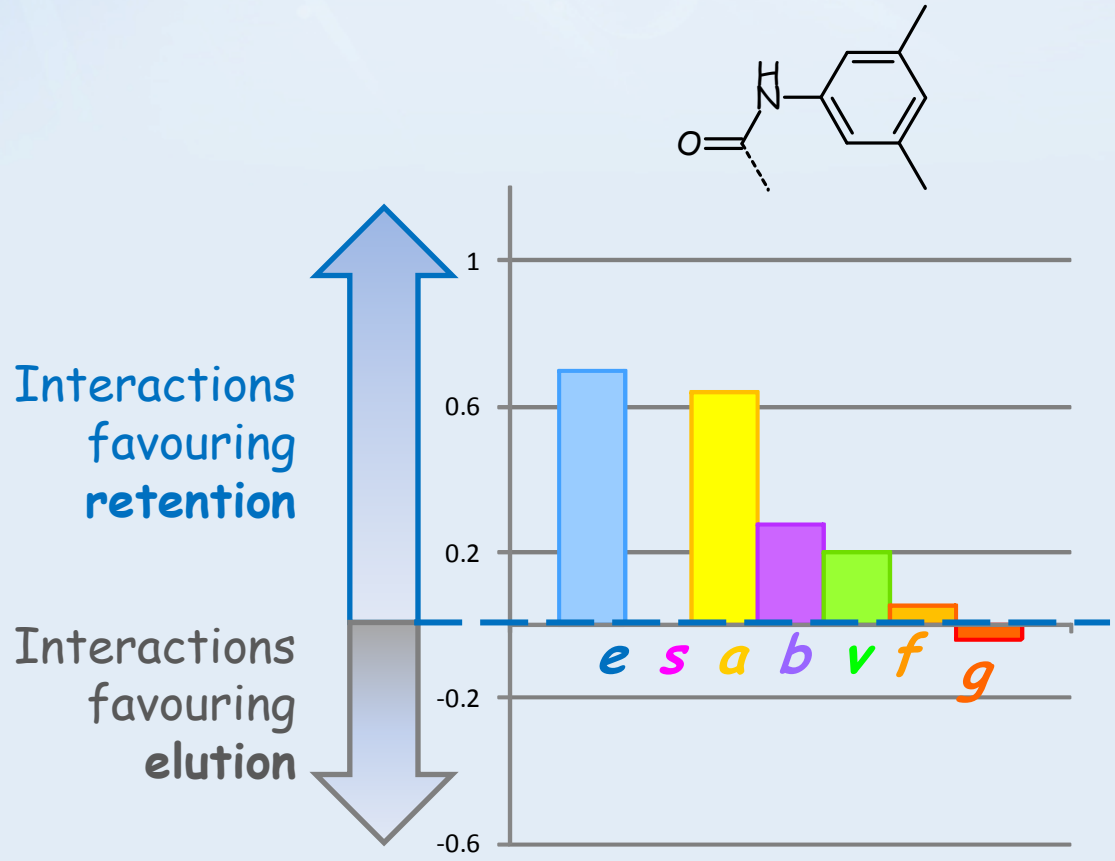
If x_{mobile} is constant, ***stationary phases can be compared***

A selection of achiral probe analytes

230 achiral probes



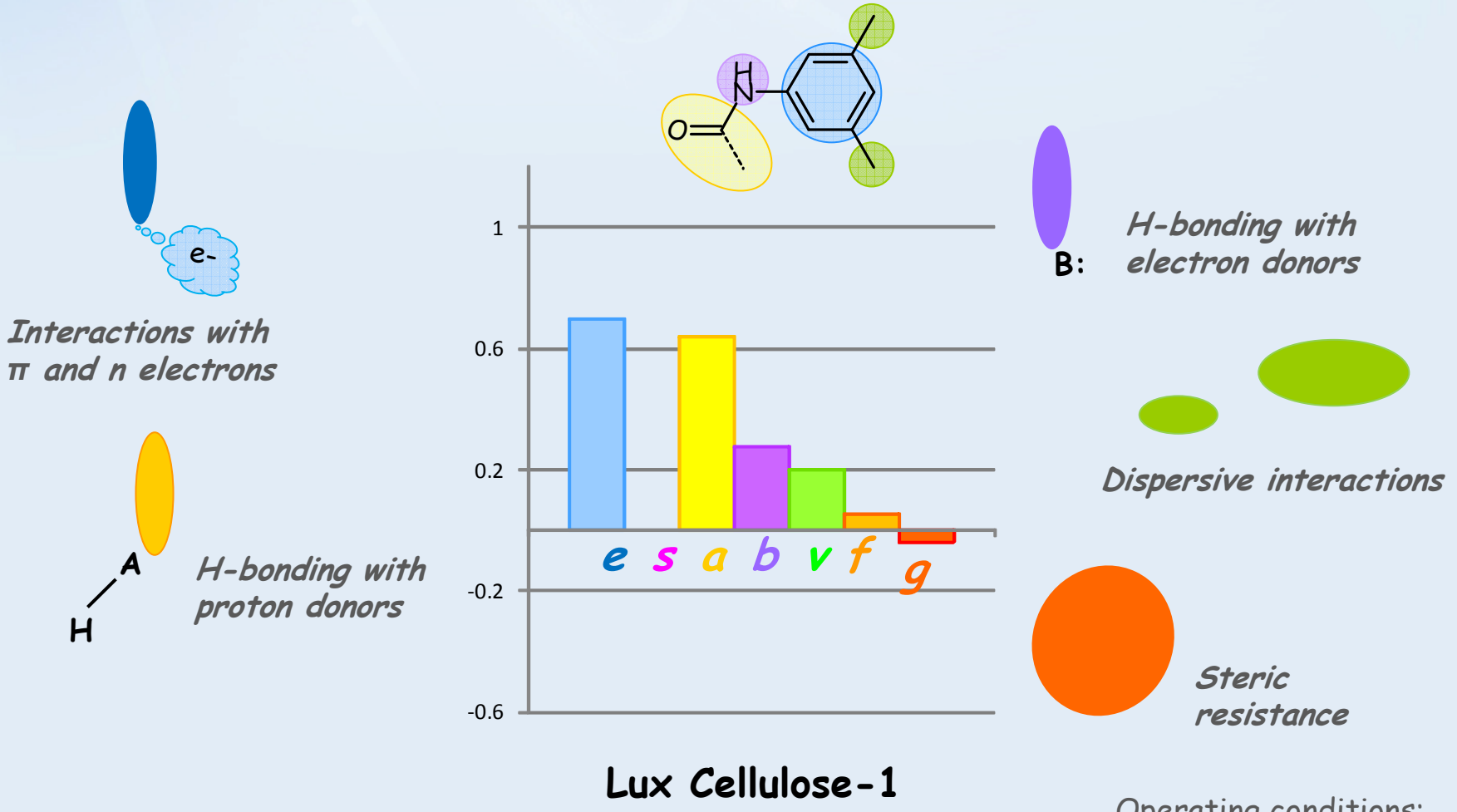
A method to compare stationary phases
The solvation parameter model



Lux Cellulose-1

Operating conditions:
CO_2-MeOH 90:10 (v/v)
25°C, 150 bar 3 mL/min

A method to compare stationary phases
The solvation parameter model



Operating conditions:
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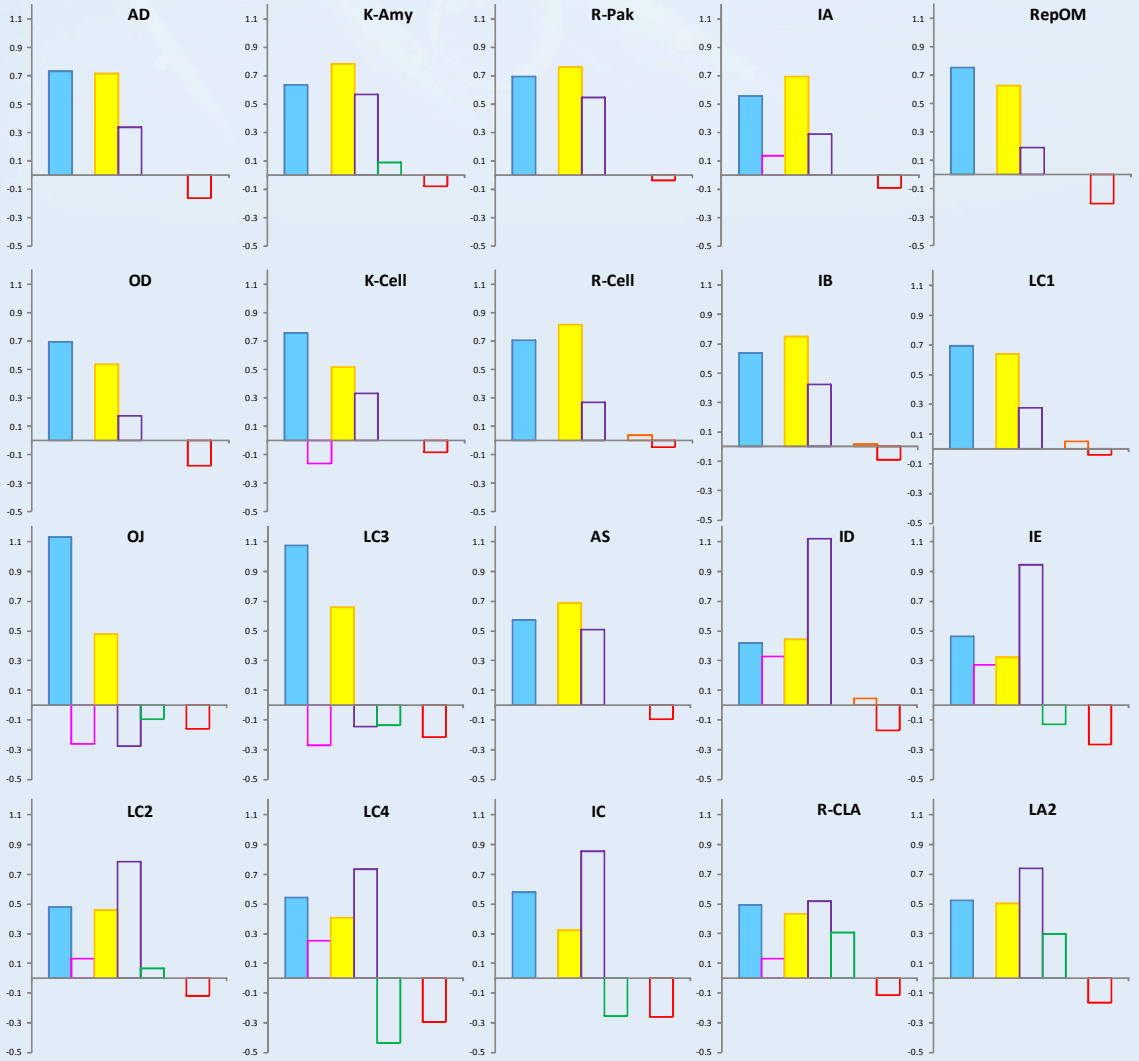
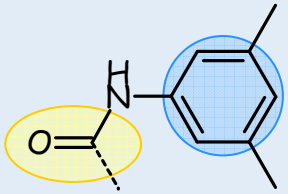
A method to compare retention properties
Modified solvation parameter model

A method to compare retention properties
Modified solvation parameter model

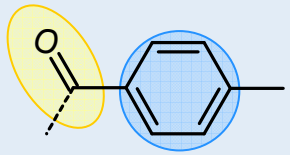


A method to compare retention properties
Modified solvation parameter model

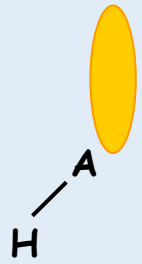
Interactions with π and n electrons



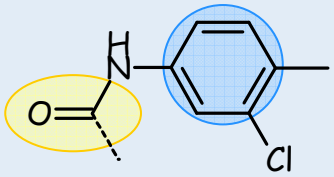
Lux Cellulose-1 (LC1)



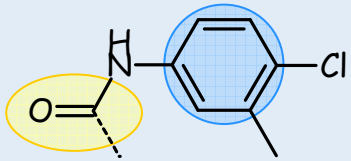
Lux Cellulose-3 (LC3)



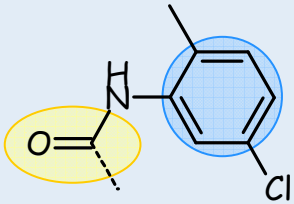
H-bonding with proton donors



Lux Cellulose-2 (LC2)

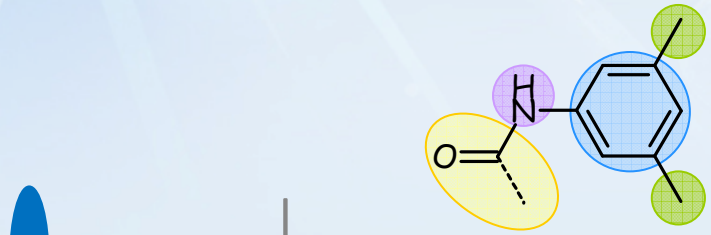


Lux Cellulose-4 (LC4)



Lux Amylose-2 (LA2)

A method to compare stationary phases
The solvation parameter model

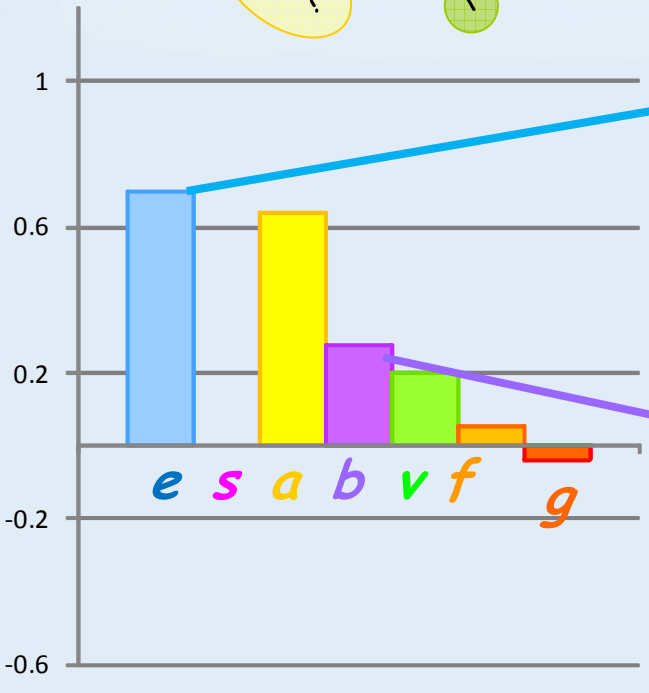


e-

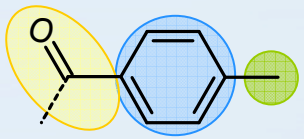
Interactions with π and n electrons

B:

H-bonding with electron donors



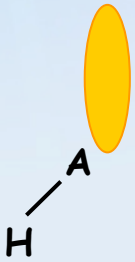
Lux Cellulose-1



Lux Cellulose-3

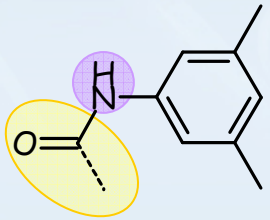
Operating conditions:
CO_2-MeOH 90:10 (v/v)
25°C, 150 bar 3 mL/min

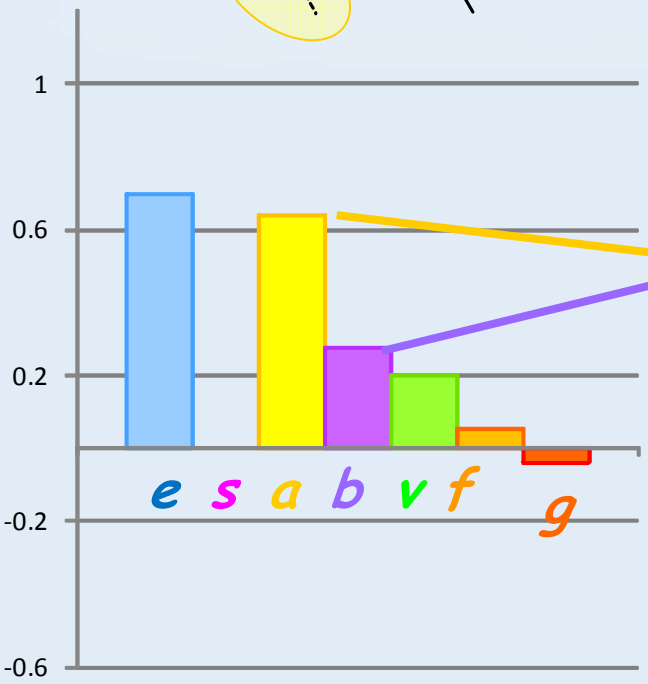
A method to compare stationary phases
The solvation parameter model



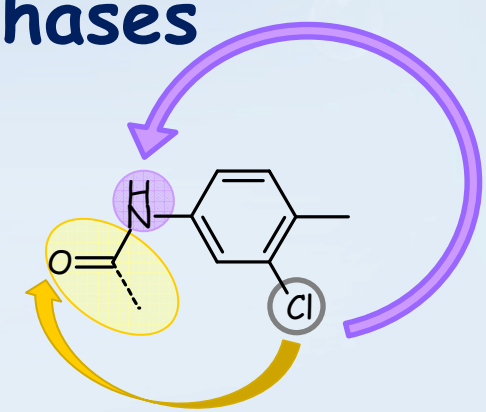
H-bonding with proton donors

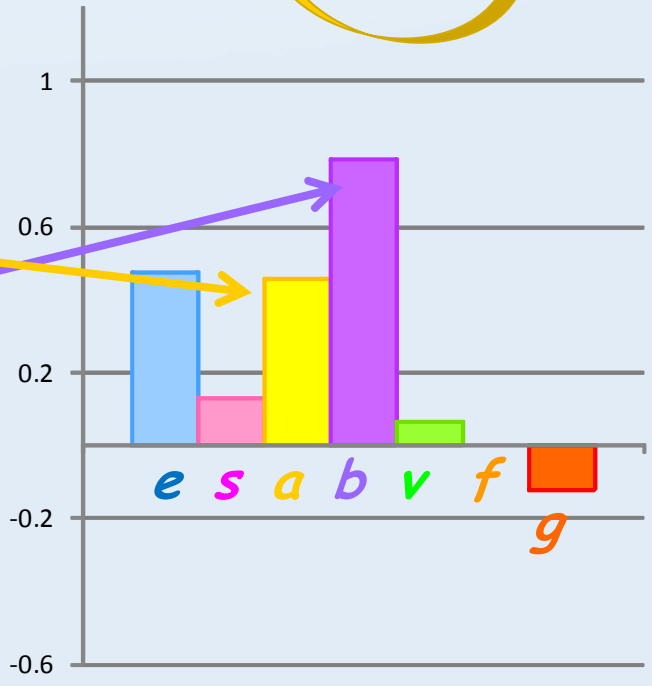
H-bonding with electron donors





Lux Cellulose-1



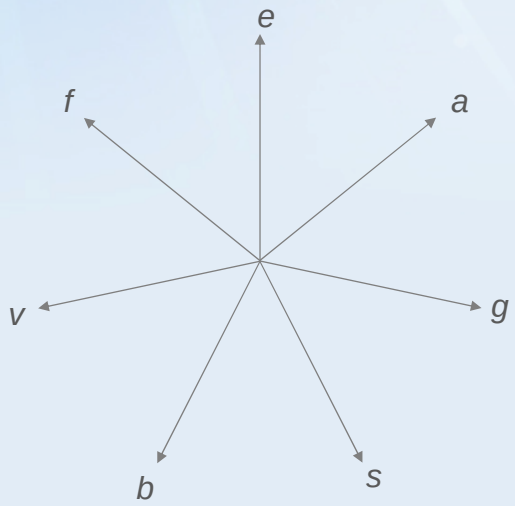


Lux Cellulose-2

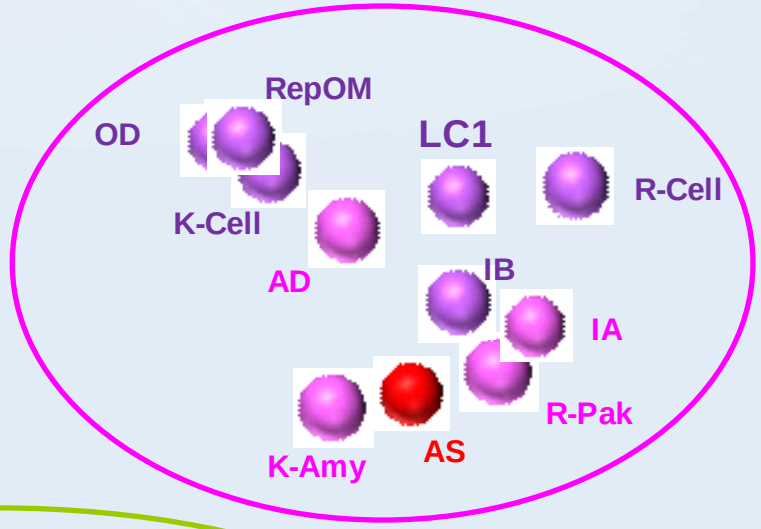
Operating conditions:
CO_2-MeOH 90:10 (v/v)
25°C, 150 bar 3 mL/min

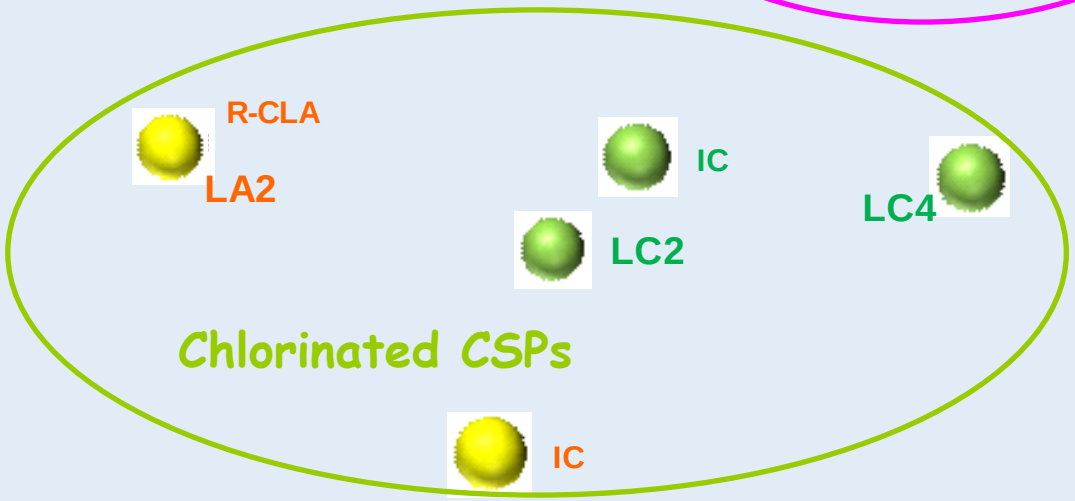
A method to compare retention properties
Modified solvation parameter model

A method to compare stationary phases
A retention map



Ester CSPs

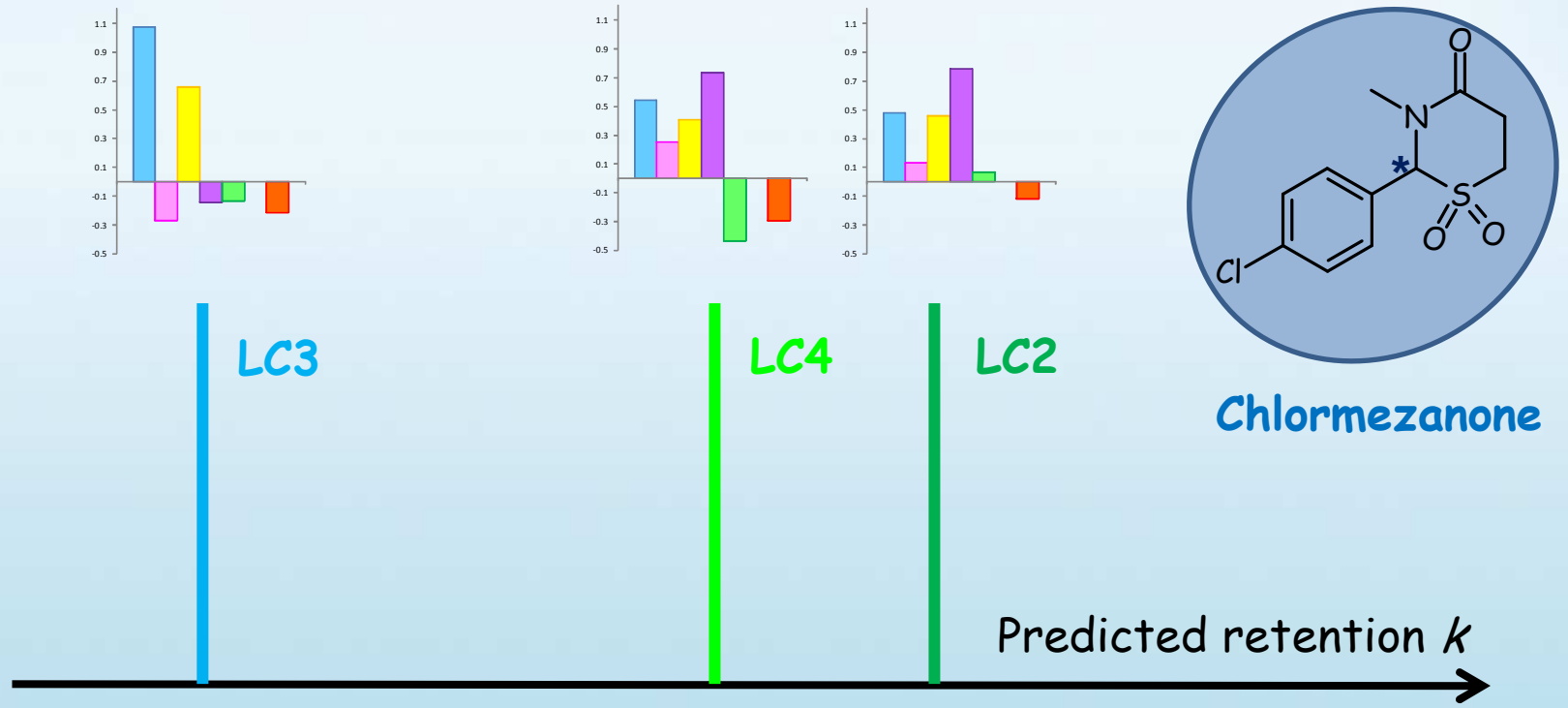




The solvation parameter model
As a tool to predict retention

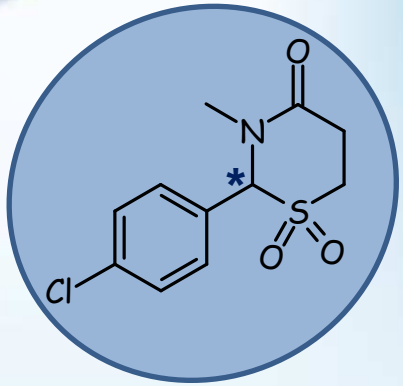
Retention models + molecular descriptors

E, S, A, B, V, F, G



Chlormezanone

The solvation parameter model
As a tool to predict retention



Chlormezanone

Operating conditions:
CO_2-MeOH 90:10 (v/v)
25°C, 150 bar 3 mL/min

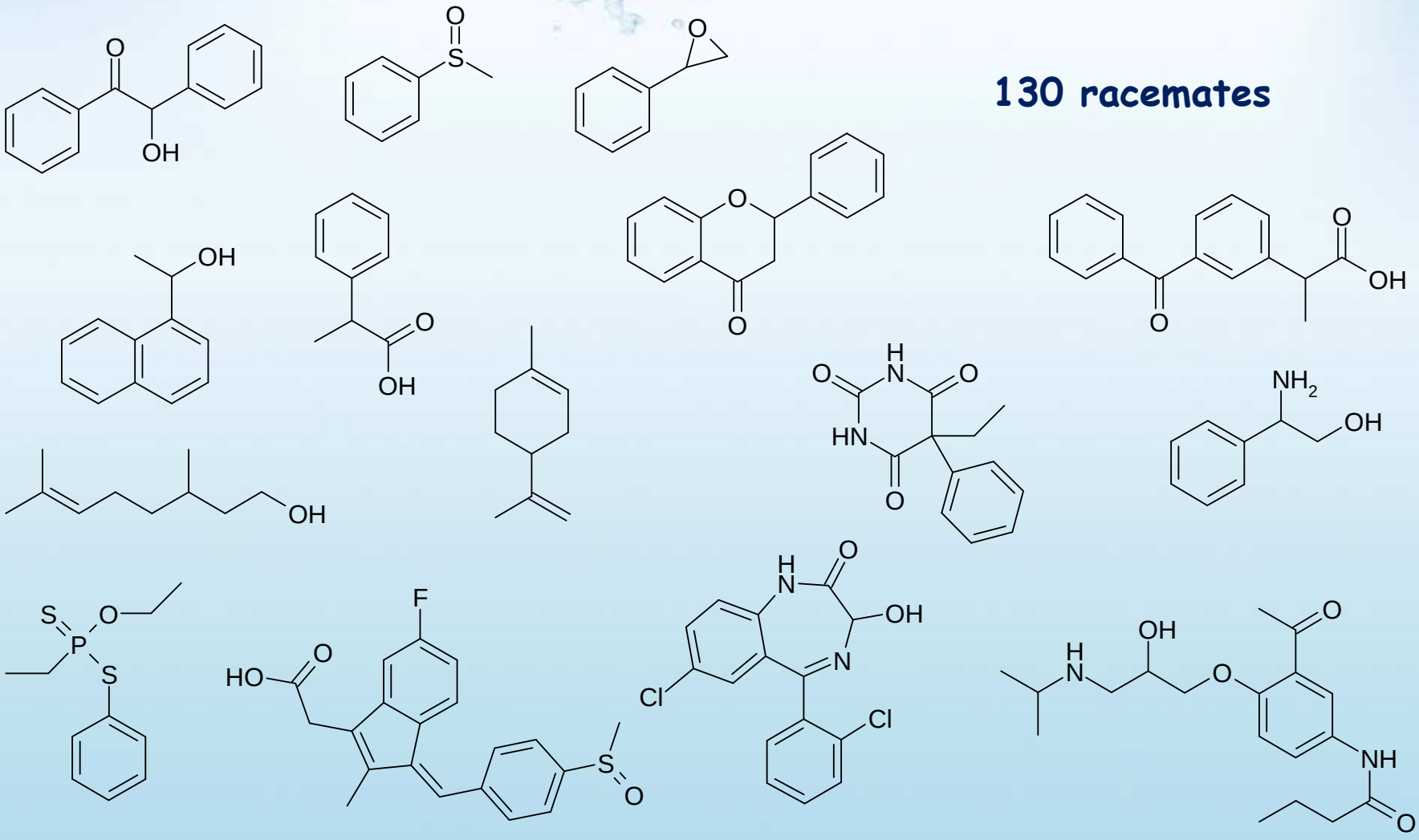
Now what about separation ?

How can we compare the separation capabilities of different CSPs ?

Most frequent method encountered in the literature :
analyze a large group of chiral probe compounds

Compare success rate

A selection of chiral probe analytes



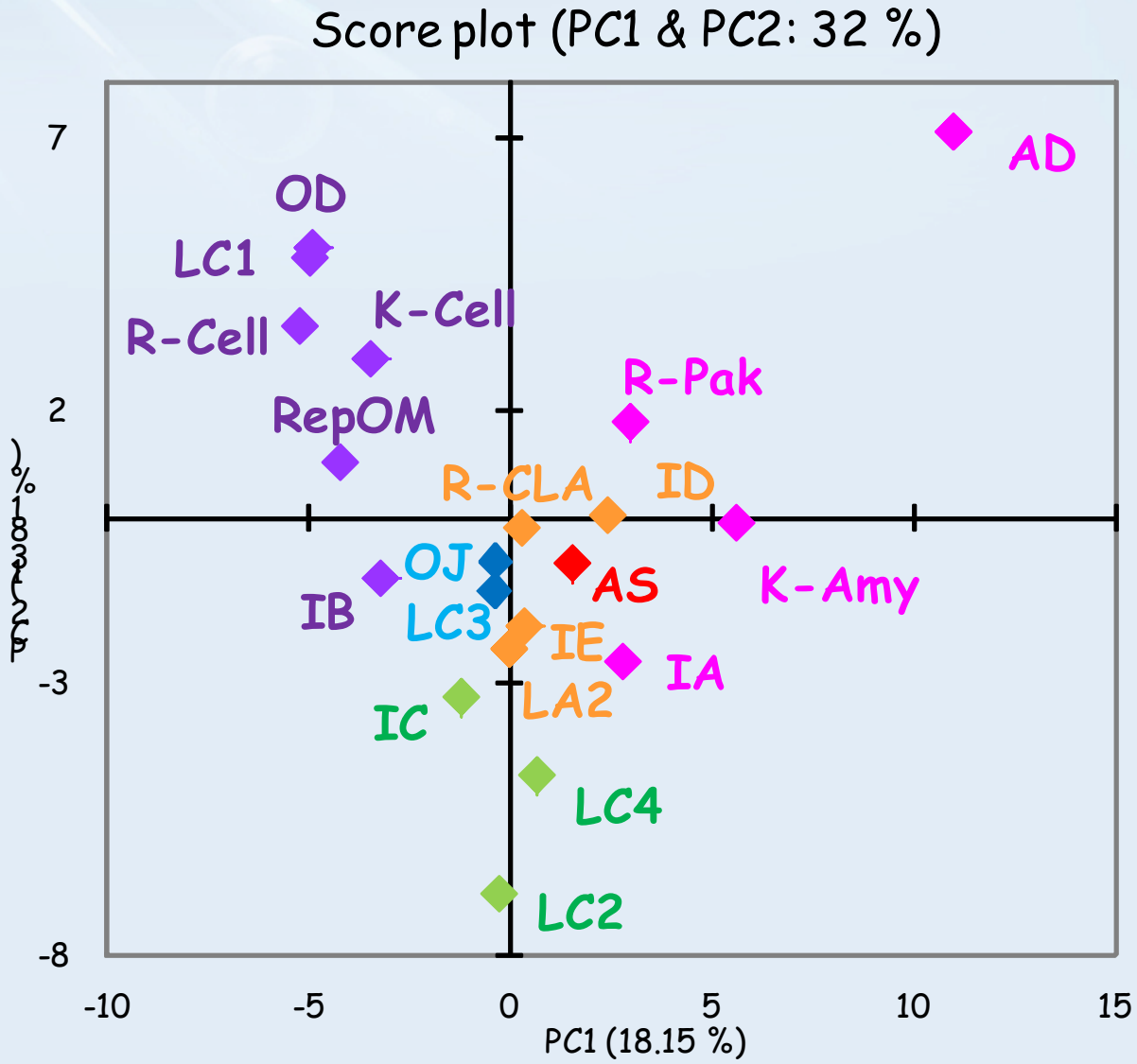
Comparison based on sucess rate

So what ??

Comparison based on similar separations (α)

Pincipal component analysis furnishes a view of columns based on separation factors

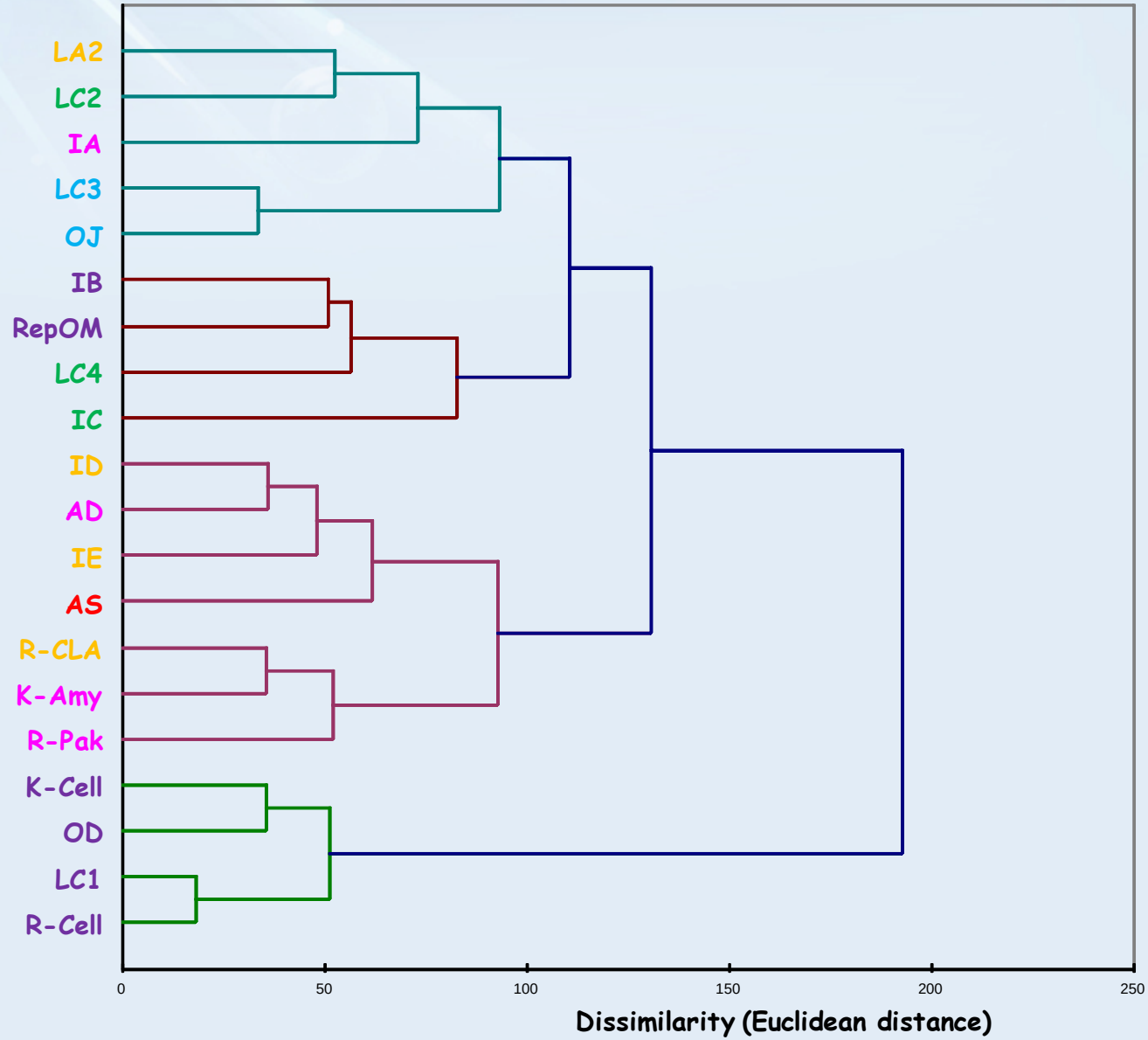
So what ??



Comparison based on similar separations (α)

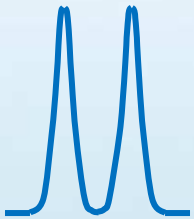
Cluster analysis furnishes a classification of columns based on separation factors

So what ??



Looking for another (more informative) method

For each chromatographic system, divide the racemates into two classes

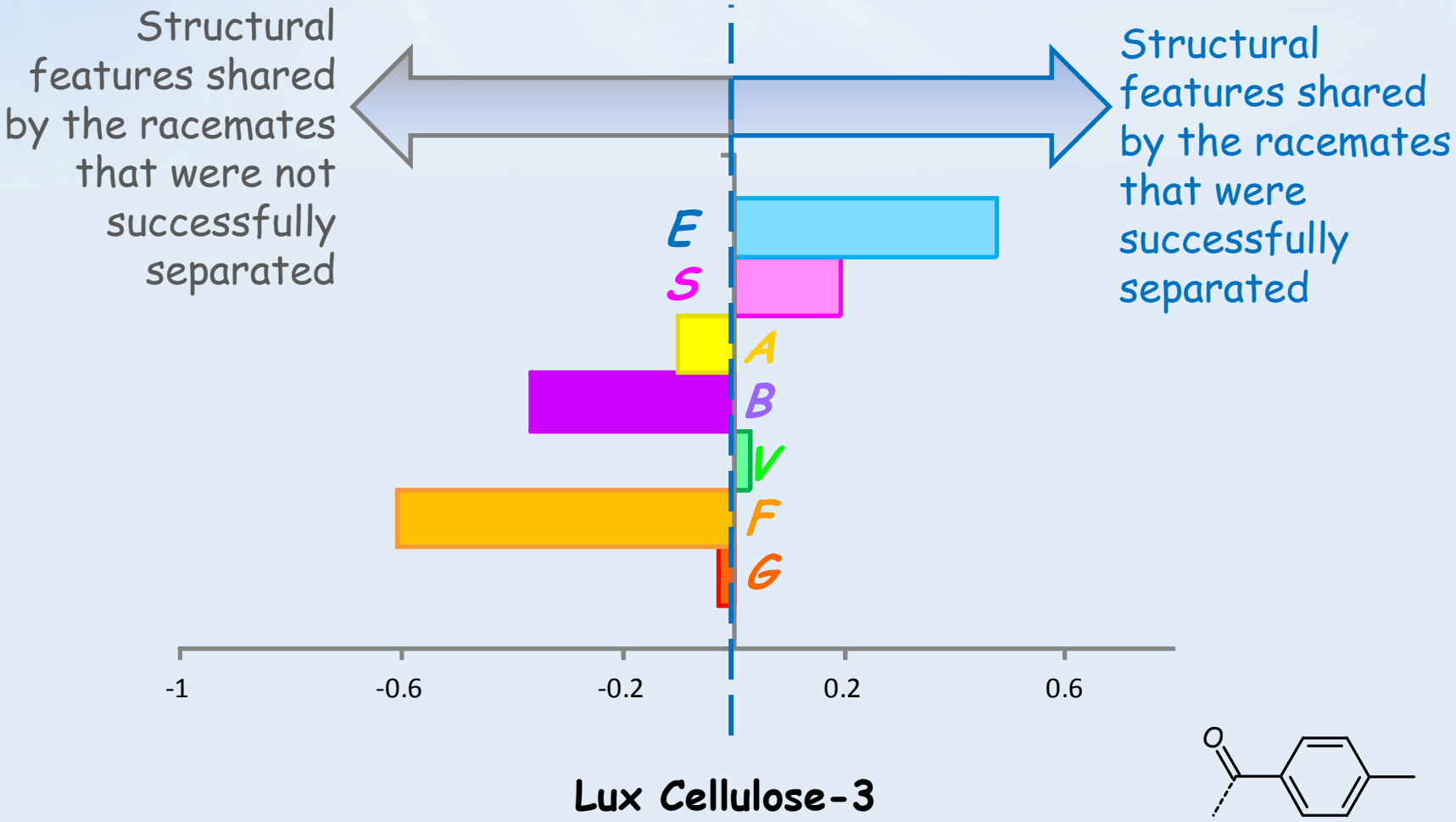


Separated

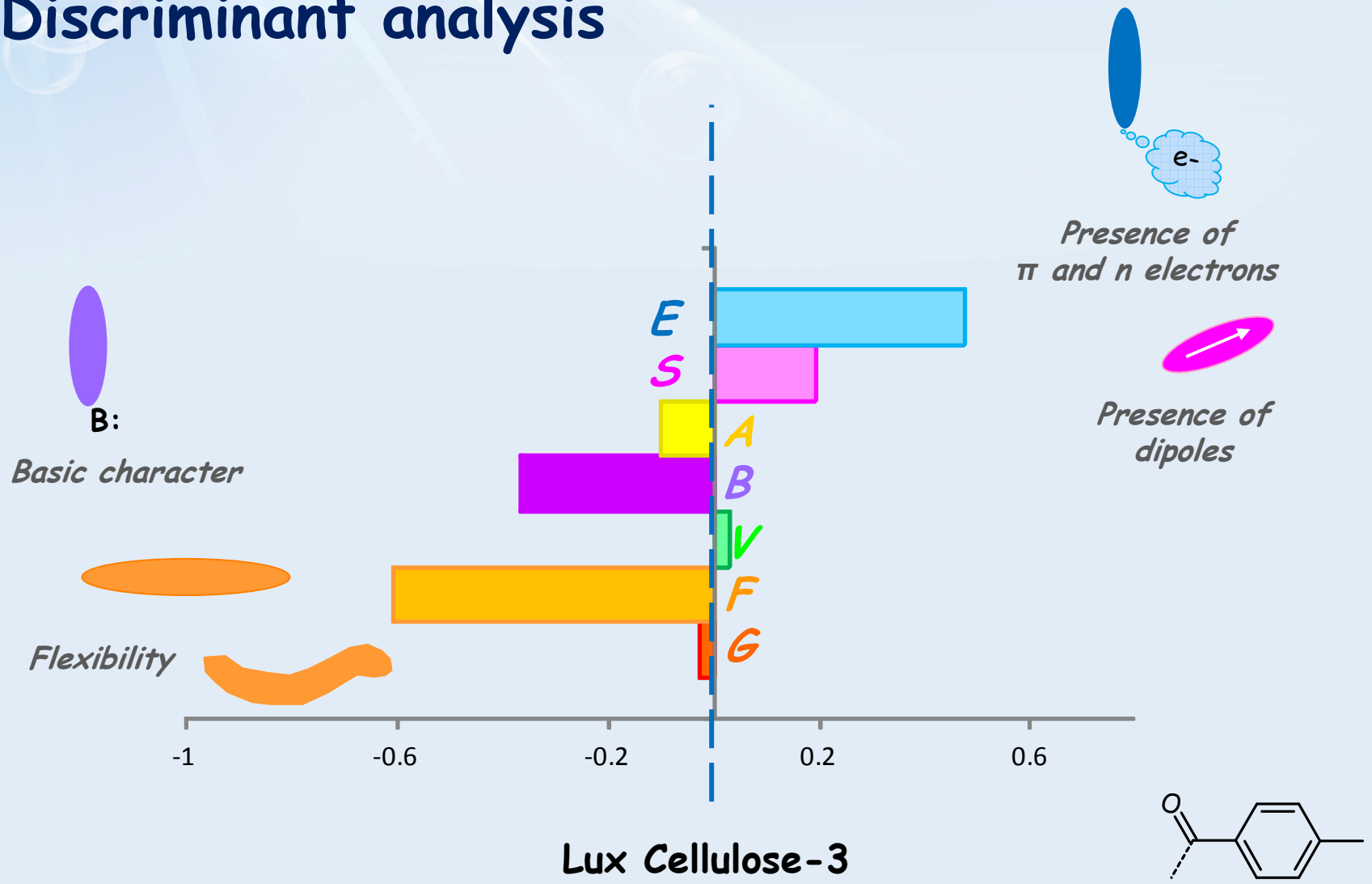
Not separated

Calculate factorial discriminant analysis to determine the features that are common to the racemates in each class

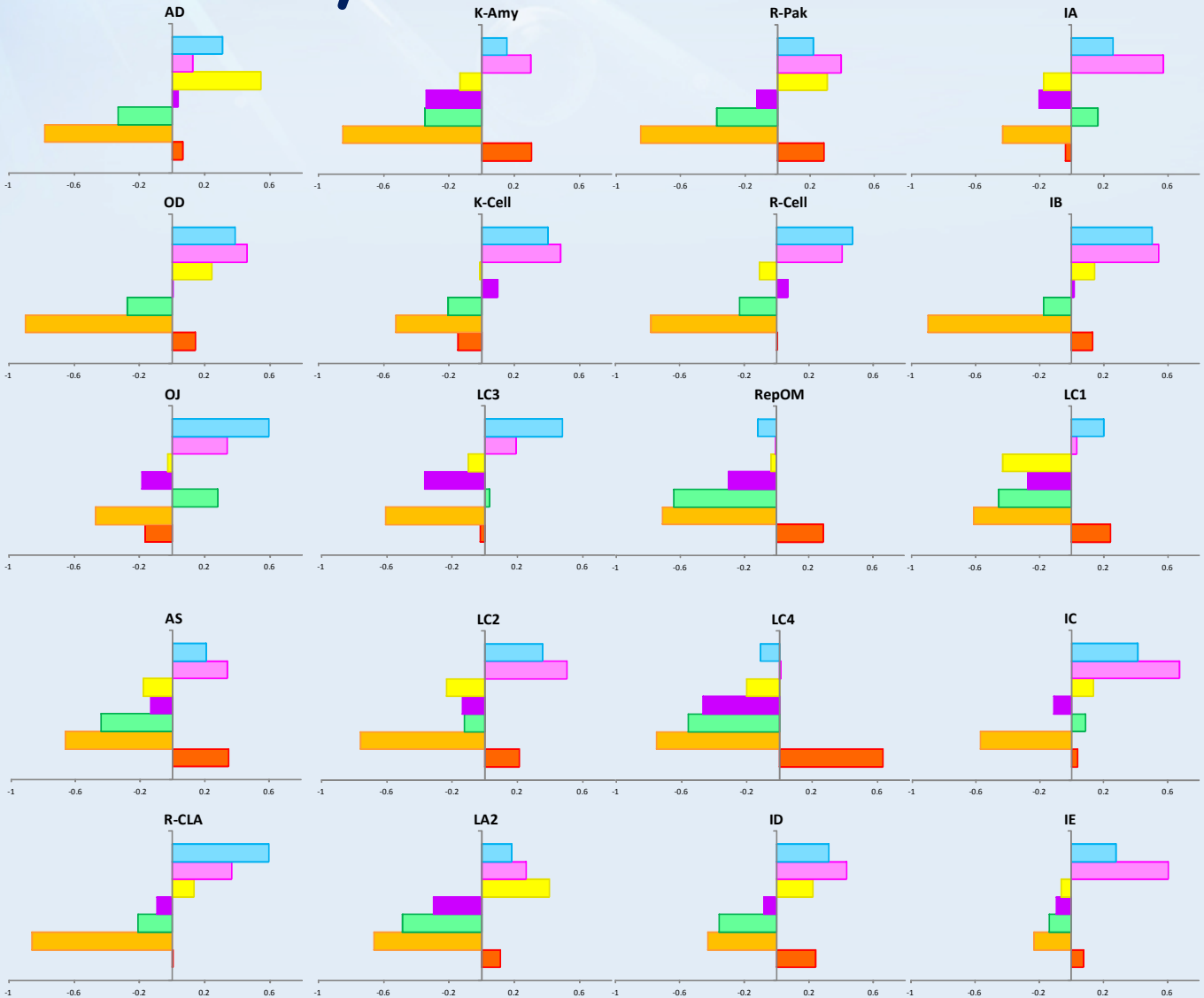
A method to compare separation capabilites
Discriminant analysis



A method to compare separation capabilities
Discriminant analysis



A method to compare separation capabilities Discriminant analysis

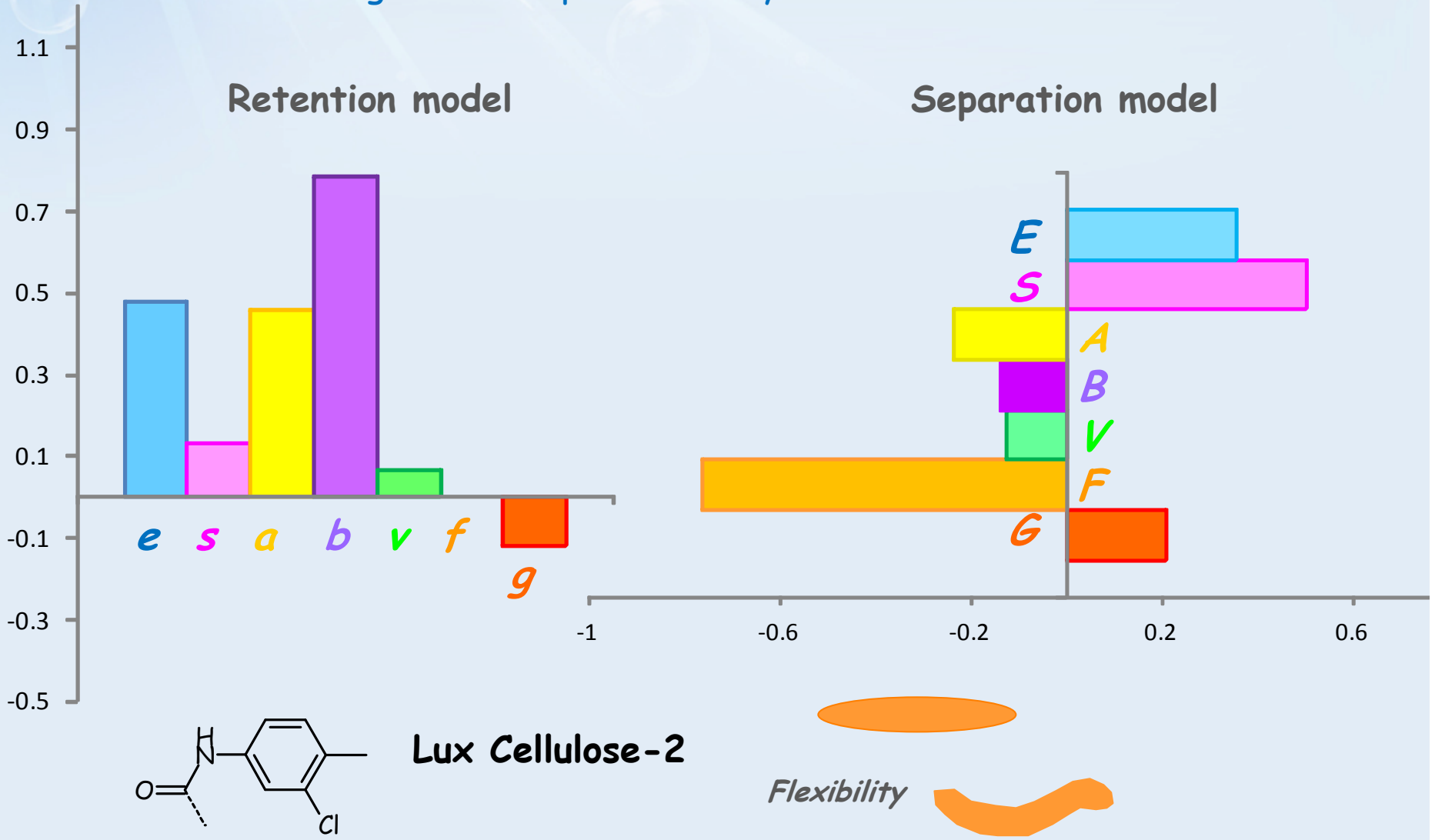


A method to compare separation capabilities Discriminant analysis

Flexibility

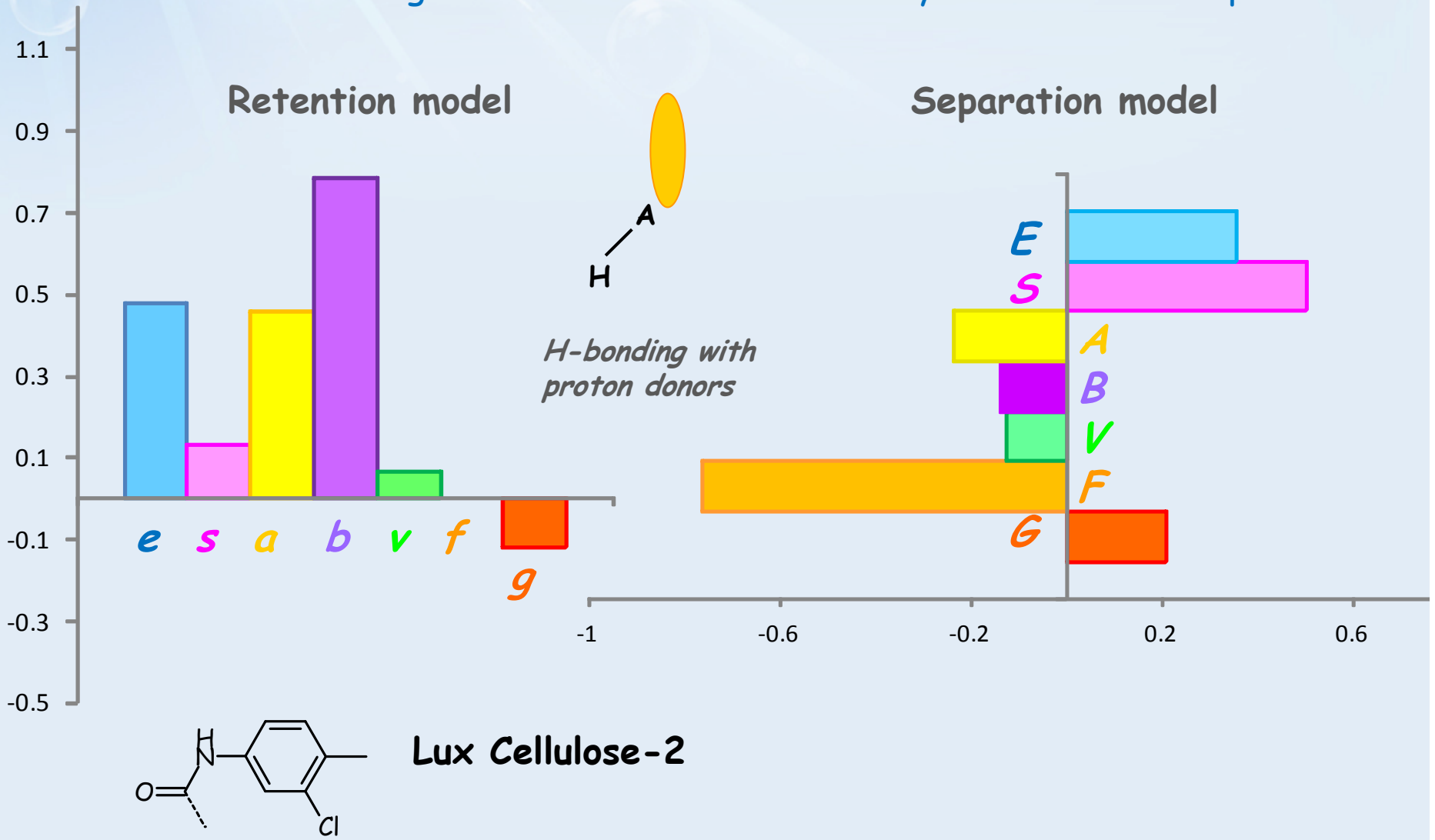
A method to compare separation capabilities

The factors affecting enantioseparation may have little influence on retention



A method to compare stationary phases

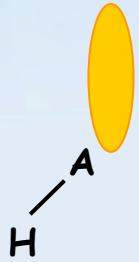
The interactions causing retention are not necessarily favourable to separation



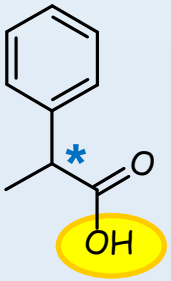
Lux Cellulose-2

A method to compare stationary phases

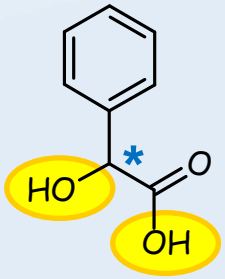
The interactions causing retention are not necessarily favourable to separation



H-bonding with proton donors



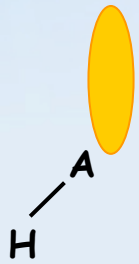
Phenylpropionic acid



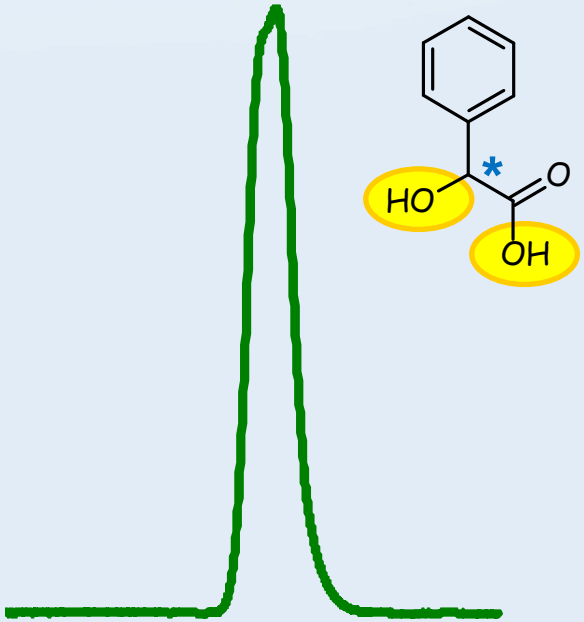
Mandelic acid

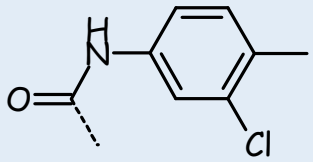
A method to compare stationary phases

The interactions causing retention are not necessarily favourable to separation



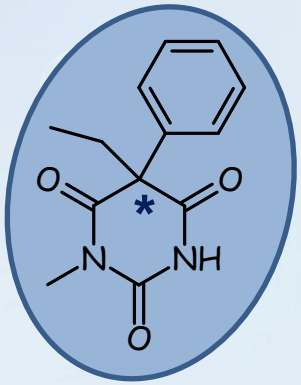
H-bonding with proton donors





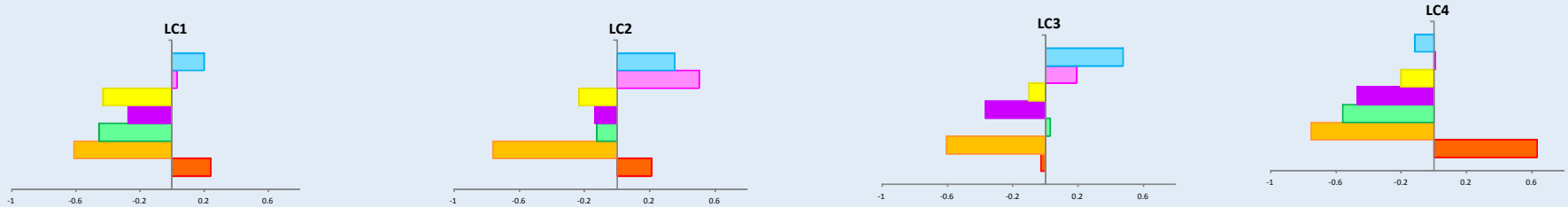
Lux Cellulose-2

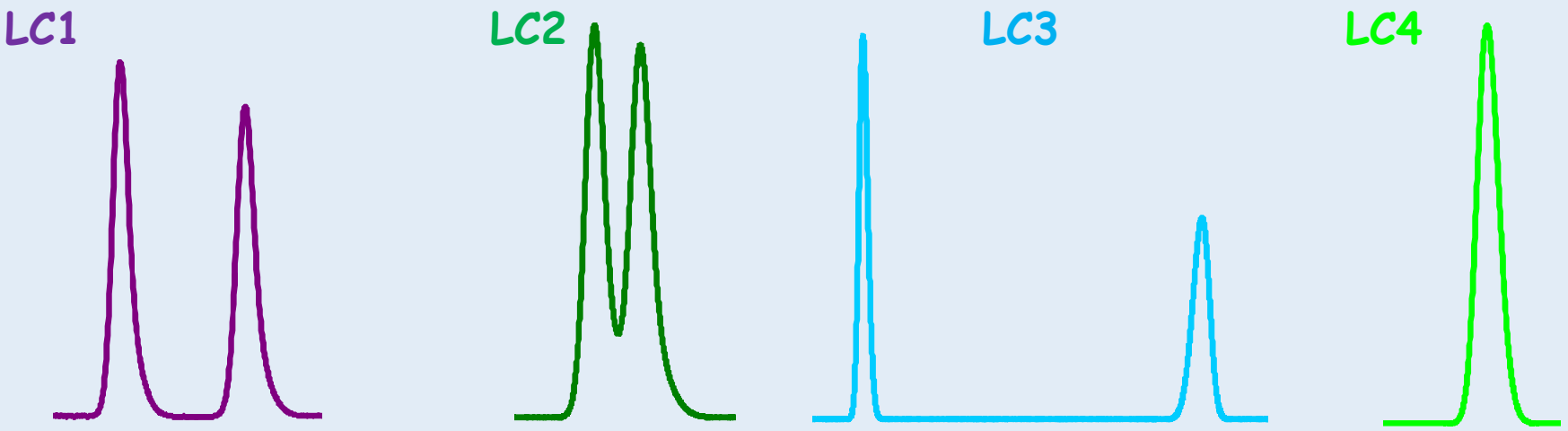
A method to compare stationary phases



Mephobarbital has little **flexibility** :
rigidity is favourable to separation on all columns

But it is **globular** (not a planar molecule) : unfavourable on LC4

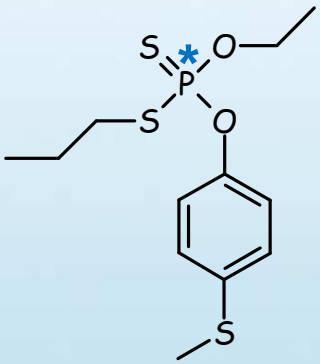




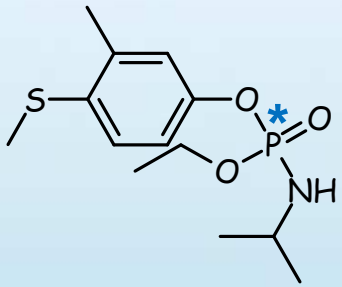
What else can we do ?

Is it possible to use the separation models

to predict the outcome of a separation ?



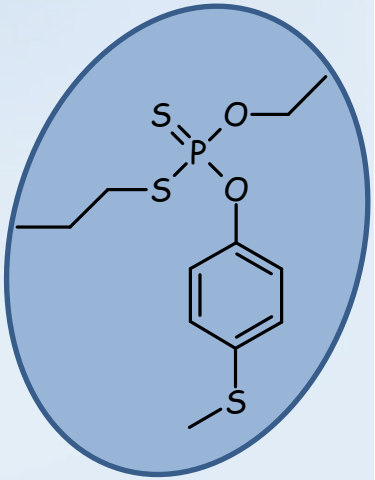
Sulprofos

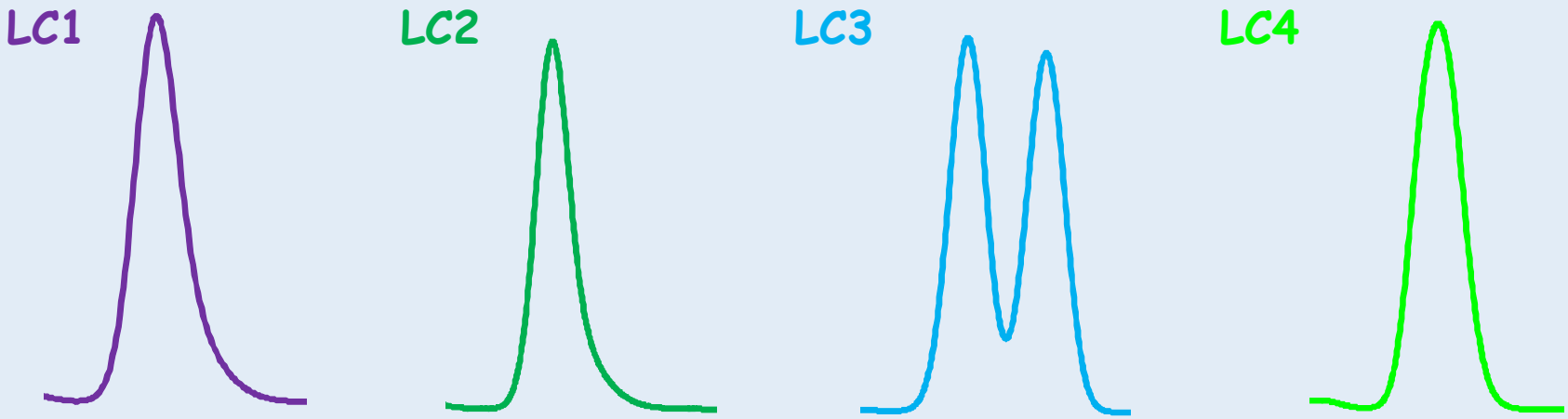


Fenamiphos

Can we predict the outcome of a separation ?

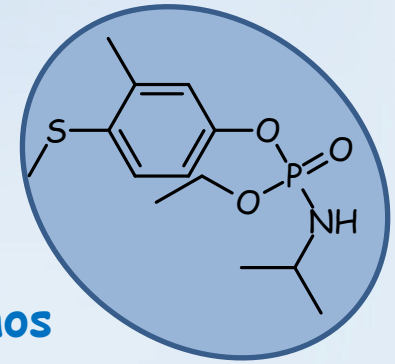
Sulprofos



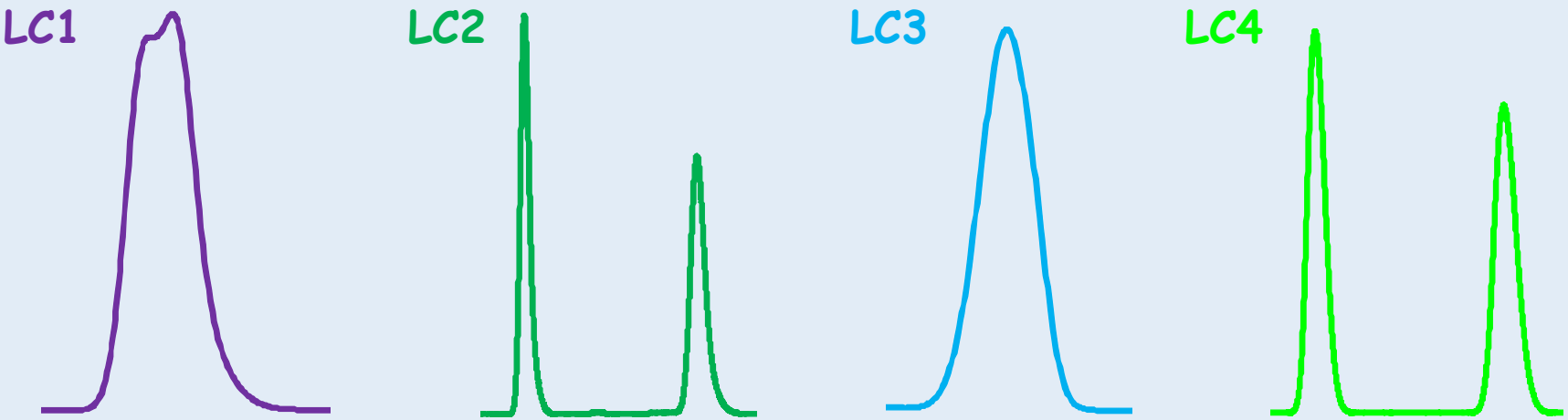


Predicted probability of success on each stationary phase

Can we predict the outcome of a separation ?



Fenamiphos



Predicted probability of success on each stationary phase

What else can we do ?

Is it possible to use the separation models

to predict the outcome of a separation ?

The method is not perfect (≈ 70% success)
needs some refinement

Molecular descriptors are the weakest link

Conclusions

Multivariate data analyses provide some insight into

- Retention mechanisms
- Separation mechanisms

Prediction is

- Quite good for retention
- Possible but not always successful for separation

A bit of reading…

C. West, A. Bouet, I. Gillaizeau, G. Coudert, M. Lafosse, E. Lesellier, Chirality, 22 (2010) 242

C. West, Y. Zhang, L. Morin-Allory, J. Chromatogr. A, 1218 (2011) 2019

C. West, G. Guenegou, Y. Zhang, L. Morin-Allory, J. Chromatogr. A, 1218 (2011) 2033

C. West, A. Bouet, S. Routier, E. Lesellier, J. Chromatogr. A, 1269 (2012) 325

C. West, M. Cieslikiewicz-Bouet, K. Lewinski, I. Gillaizeau, Chirality, 25 (2013) 211

S. Khater, Y. Zhang, C. West J. Chromatogr. A, 1303 (2013) 83

C. West, Current Anal. Chem., 10,1 (2014) 99 (review)

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