CYCLOLAB

The Cyclodextrin Company

Cyclodextrins:

Applications in chemical analyses

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May 7th, 2015

CYCLONHIT Workshop

Research Center of Natural Sciences of Hungarian Academy of Sciences, Budapest

The ultimate purpose

Control volatility and sublimation

Guest specific interactions

Analytical purposes

Influence chemical stability

Increase bio-availability

Physical isolation of incompatible compounds

Catalysis

Reduce or eliminate side-effects and irritations

Aqueous solubility enhancement

Fields of CD enabled chemical analysis

Everything comes down to supramolecular complex formation

- Chiral separation
- Chiral columns
- Single molecule sensors
- Sample preparation
- Non-chiral separation
- Selective recognition of analytes
- Sensitivity improvement
- Determination of complex stability
- Complex stoichiometry
- Complex structure

Fields of CD enabled chemical analysis

Everything comes down to supramolecular complex formation

Determination of complex stability

Complex stoichiometry

Complex structure

Determination of complex stability
Capillary electrophoresis

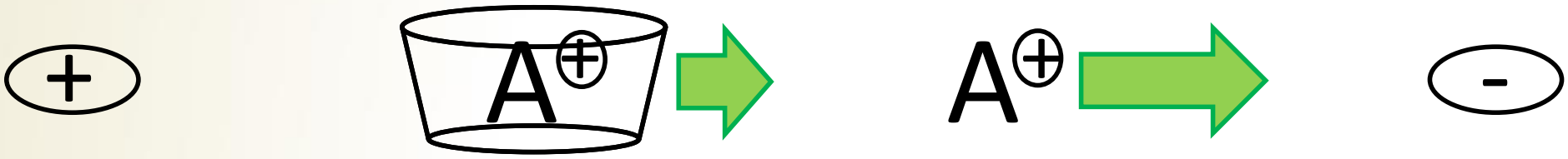
Short analysis, easy to optimize, minute material consumption - costs

Great performance, highly variable parameters, simple sample preparation

Automatization, aqueous/non-aqueous

UV active analytes are preferred, dependent on protonation state

Poor robustness of the methods

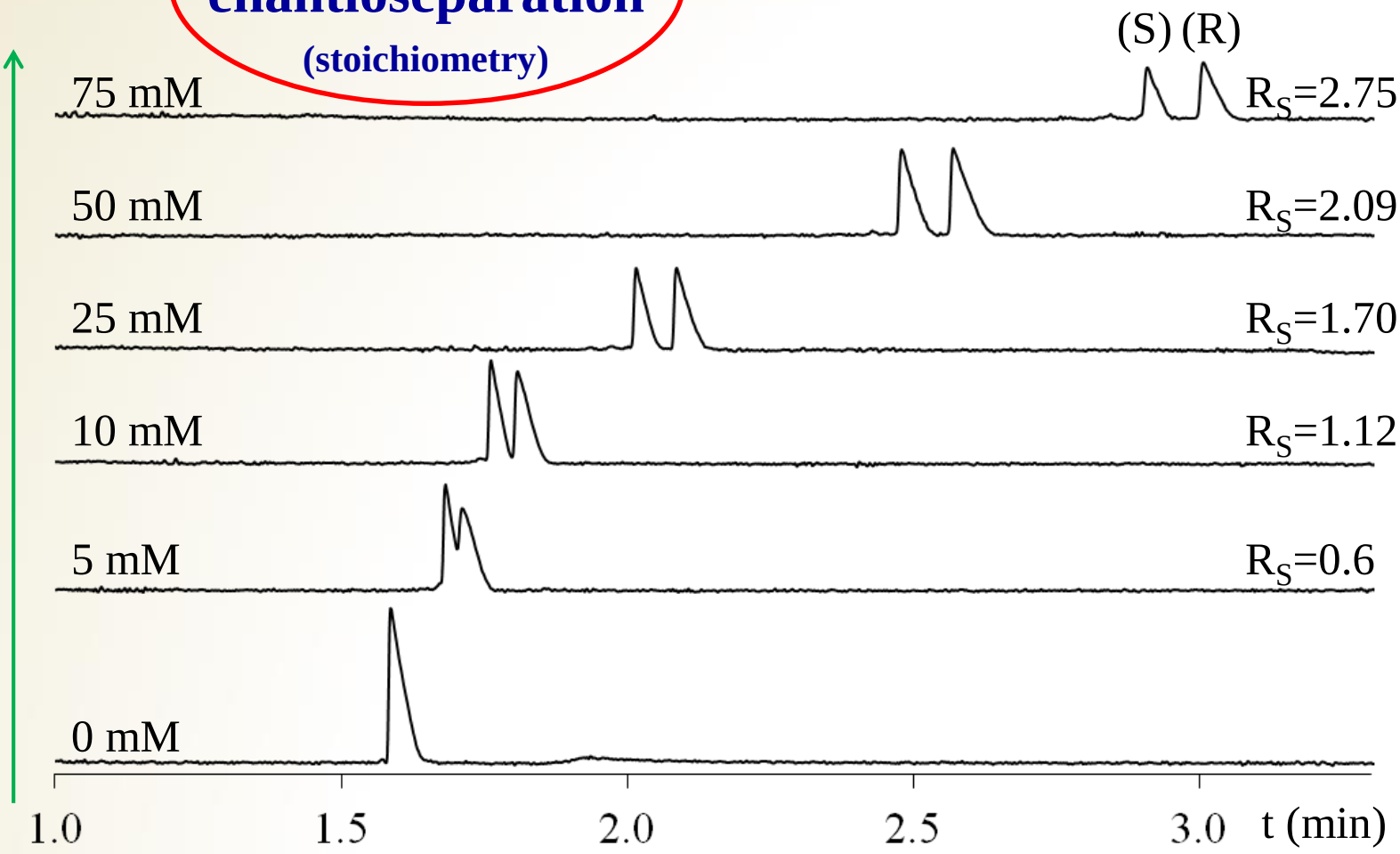


Determination of apparent stability constants

vincadifformine – RAMEB

binding and enantioseparation

(stoichiometry)



T. Sohajda et al, J Pharm Biomed Anal, 53, 1258-1266, 2010

Screening results
(Dapoxetine)

CD derivative	Binding constant (1/M), Resolution value
RAMEB-CD	K = 510 (1)
RAMEG-CD	K_S = 360 (2) K_R = 590 (8) R_S = 3,32
CM-β-CD DS~3	K < 5
CM-γ-CD	K_S = 54 (5) K_R = 61 (3) R_S = 1,38
CE-β-CD	K < 5
SP-α-CD DS~2	K < 5
SP-β-CD DS~4	K_S = 280 (6) K_R = 310 (8) R_S = 1,01
SP-γ-CD DS~2	K < 5
SHP-γ-CD DS~3	K < 5
SB-α-CD DS~4	K = 28 (1)
SB-β-CD DS~4	K_S = 610 (1) K_R = 690 (2) R_S = 0,62

Apparent average binding constants

Preferred cavity size/DS/susbtituent for complexation

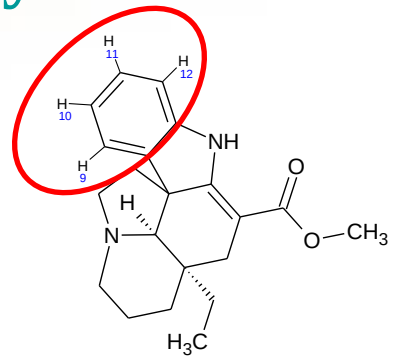
Enantioselectivity

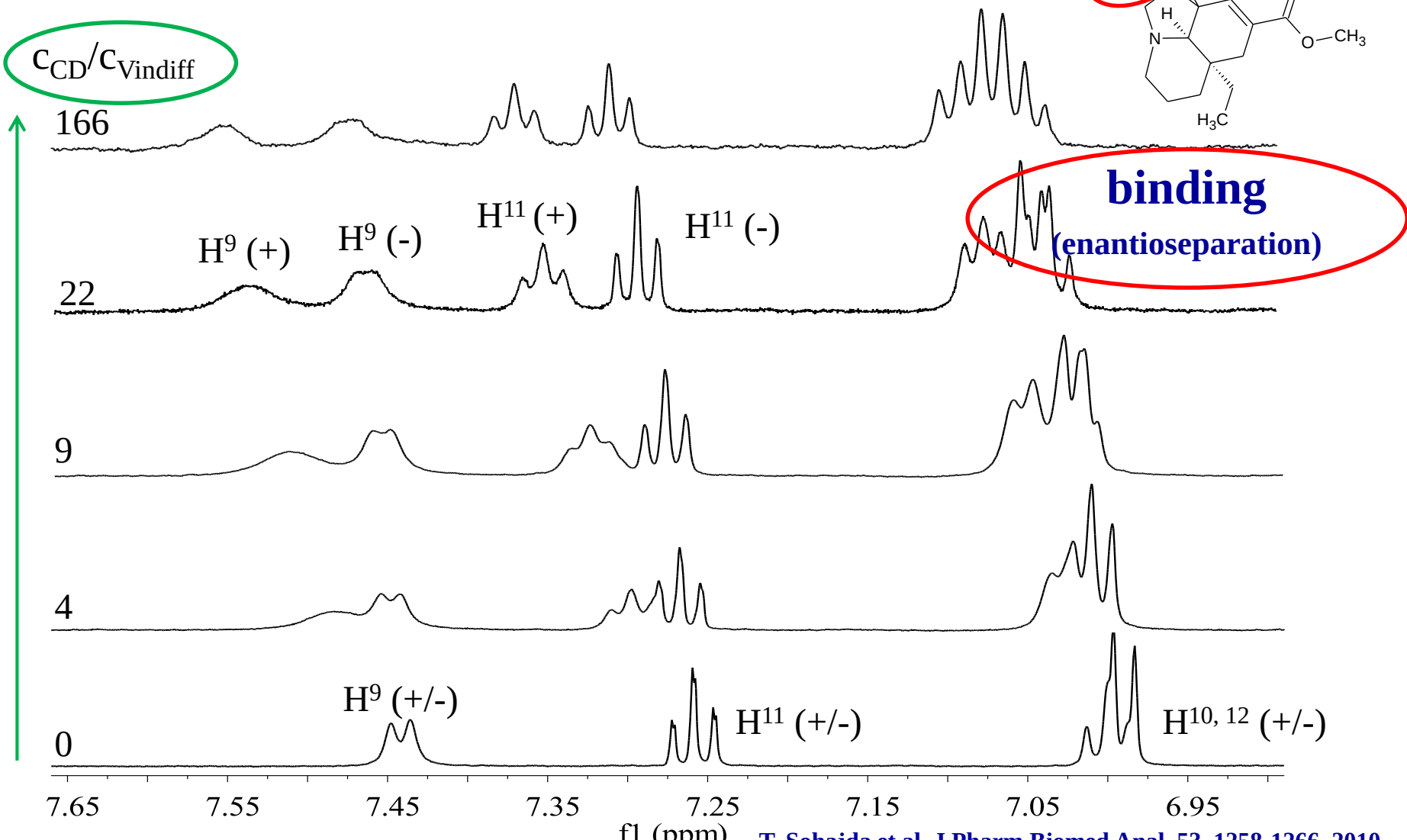
Enantiomer Migration Order

G. Neumajer et al, J Pharm Biomed Anal, 62, 42-47, 2012

Determination of apparent stability constants

vincadifformine – HPGCD

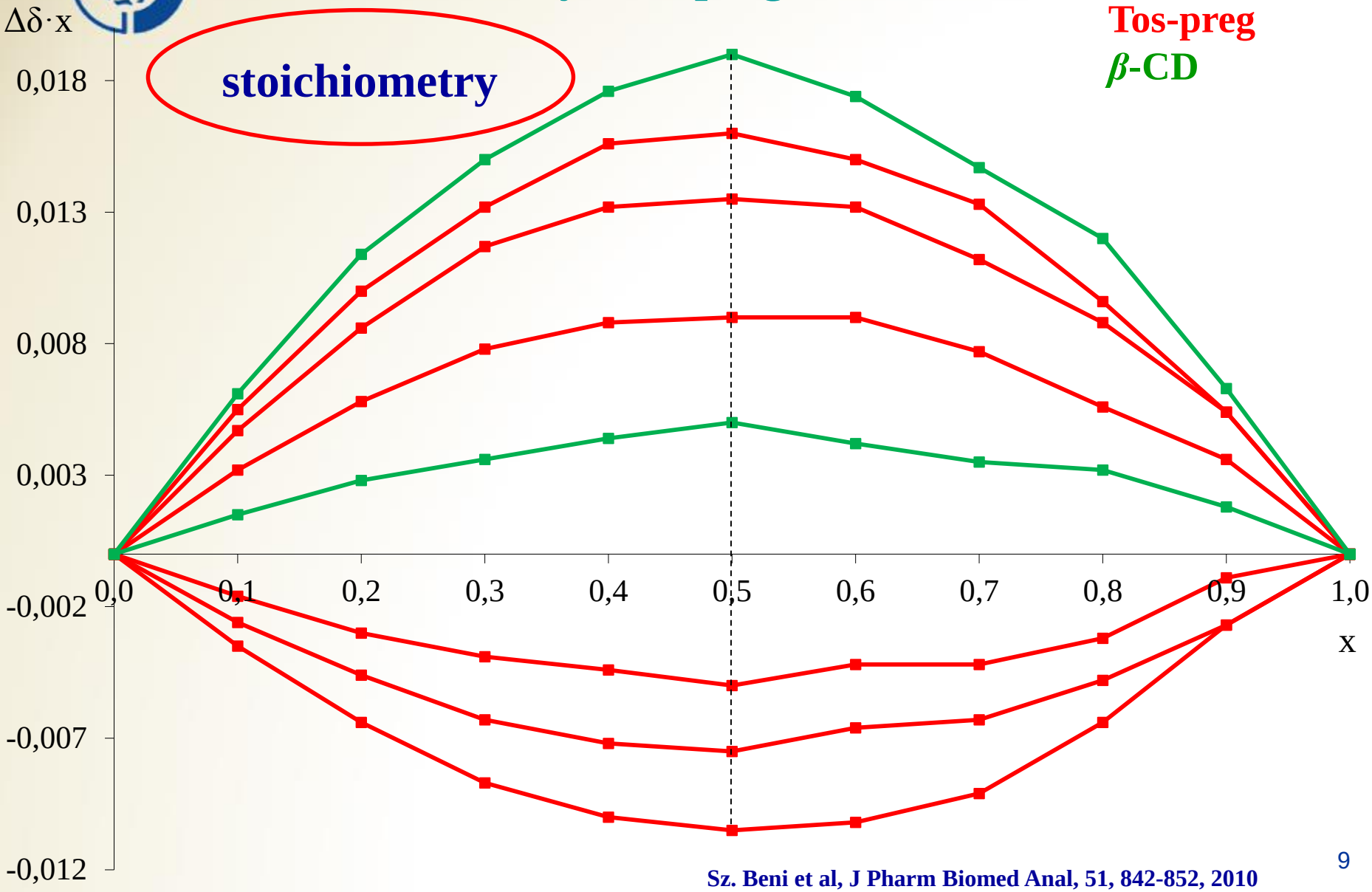




T. Sohajda et al, J Pharm Biomed Anal, 53, 1258-1266, 2010

Complex stoichiometry Job's plot 1H NMR

Tosylated pregabalin - BCD

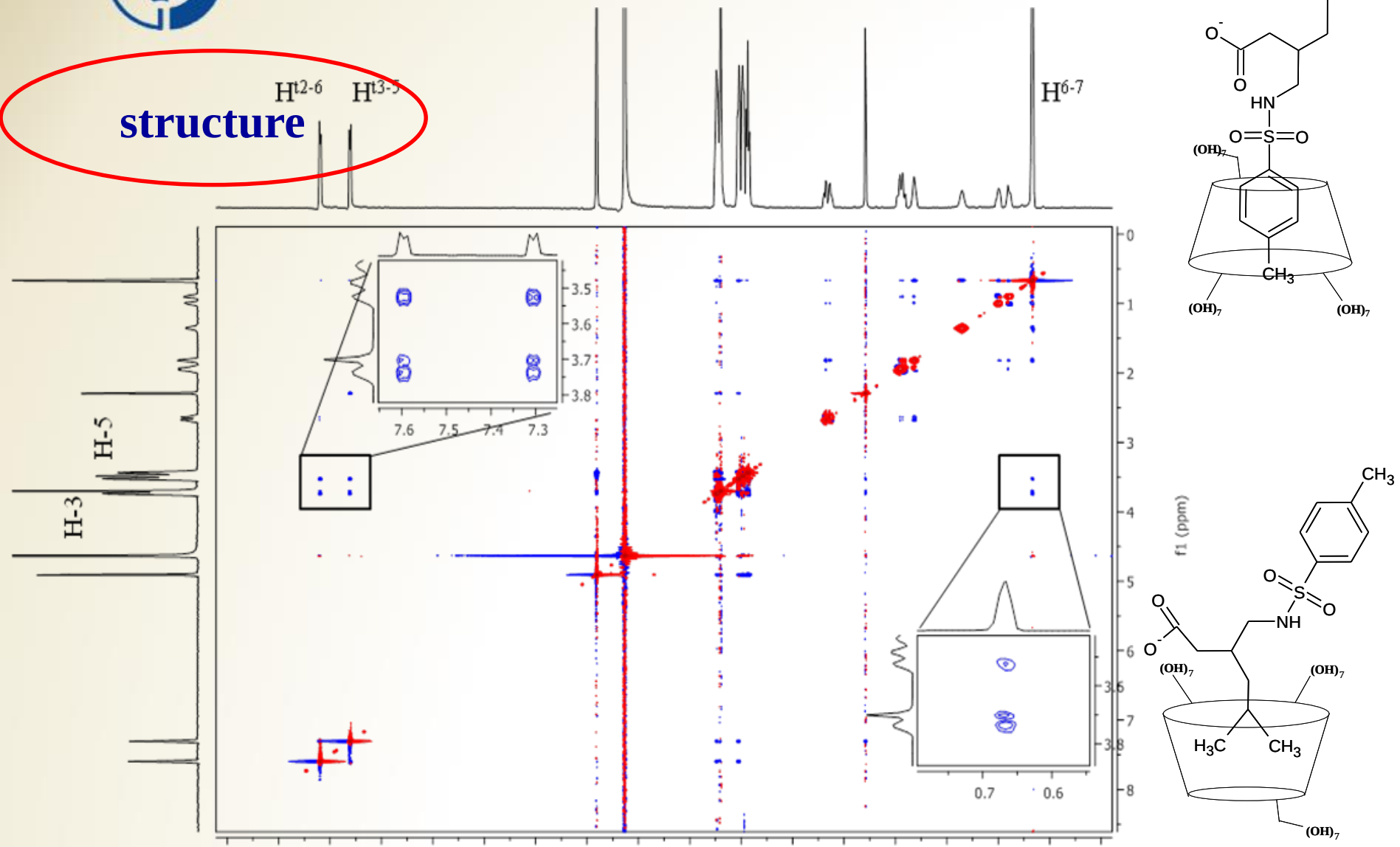


Sz. Beni et al, J Pharm Biomed Anal, 51, 842-852, 2010

Complex structure – 2D ROESY

Tosylated pregabalin - BCD

structure



Sz. Beni et al, J Pharm Biomed Anal, 51, 842-852, 2010

Comparison

Method	Costs	Time	Material need	Analytes	Information quality
Capillary electrophoresis					
NMR spectroscopy					
Phase solubility					

Fields of CD enabled chemical analysis

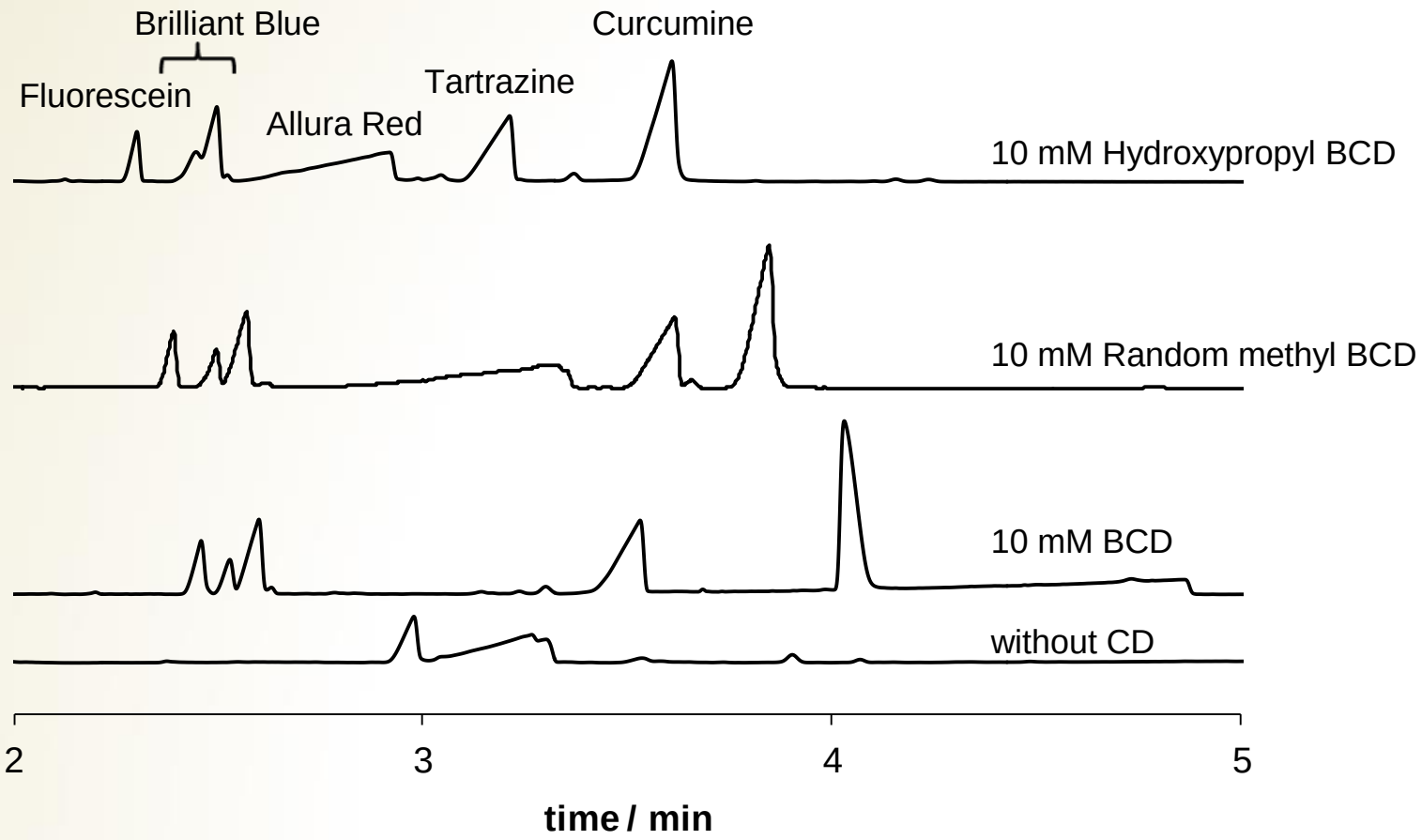
Everything comes down to supramolecular complex formation

- Chiral separation
- Non-chiral separation
- Sensitivity improvement
- Selective recognition of analytes
- Single molecule sensors
- Chiral columns
- Sample preparation

Non-chiral separations

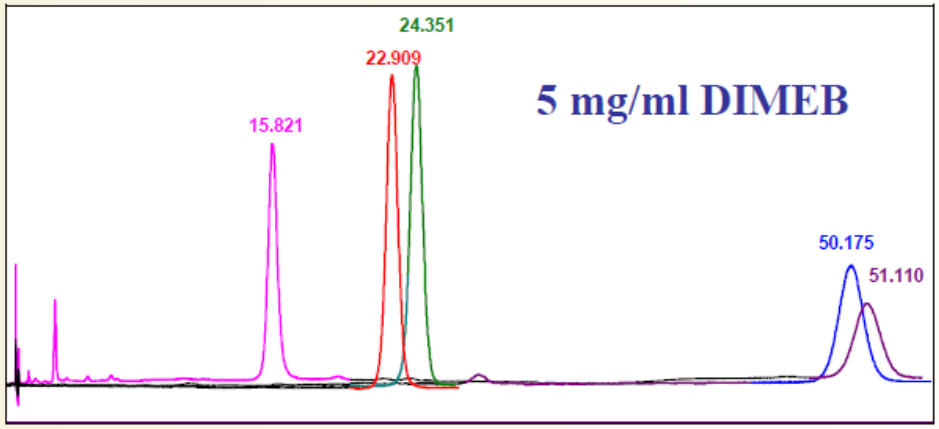
Dye trace analysis – textile industry

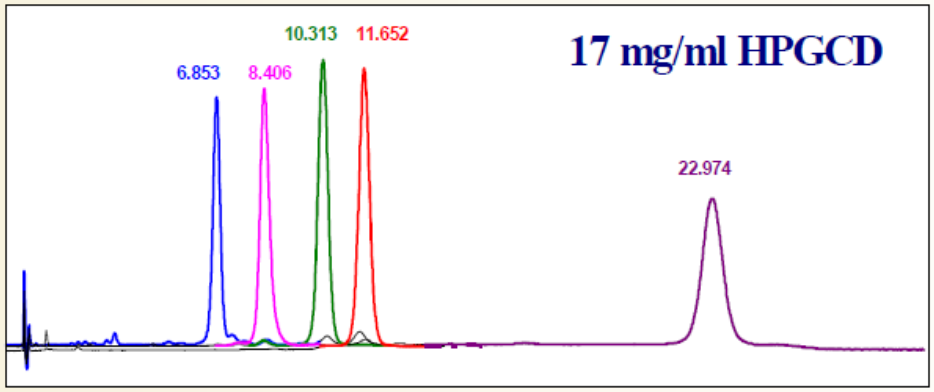
Using the right CD at the right concentration the selectivity can be enhanced!



Non-chiral separations

Using the right CD at the right concentration the selectivity can be enhanced!





FLN: Flunisolide

BRFLN: Bromoflunisolide

FLNAL: Flunisolide alcohol

DS: Desonide

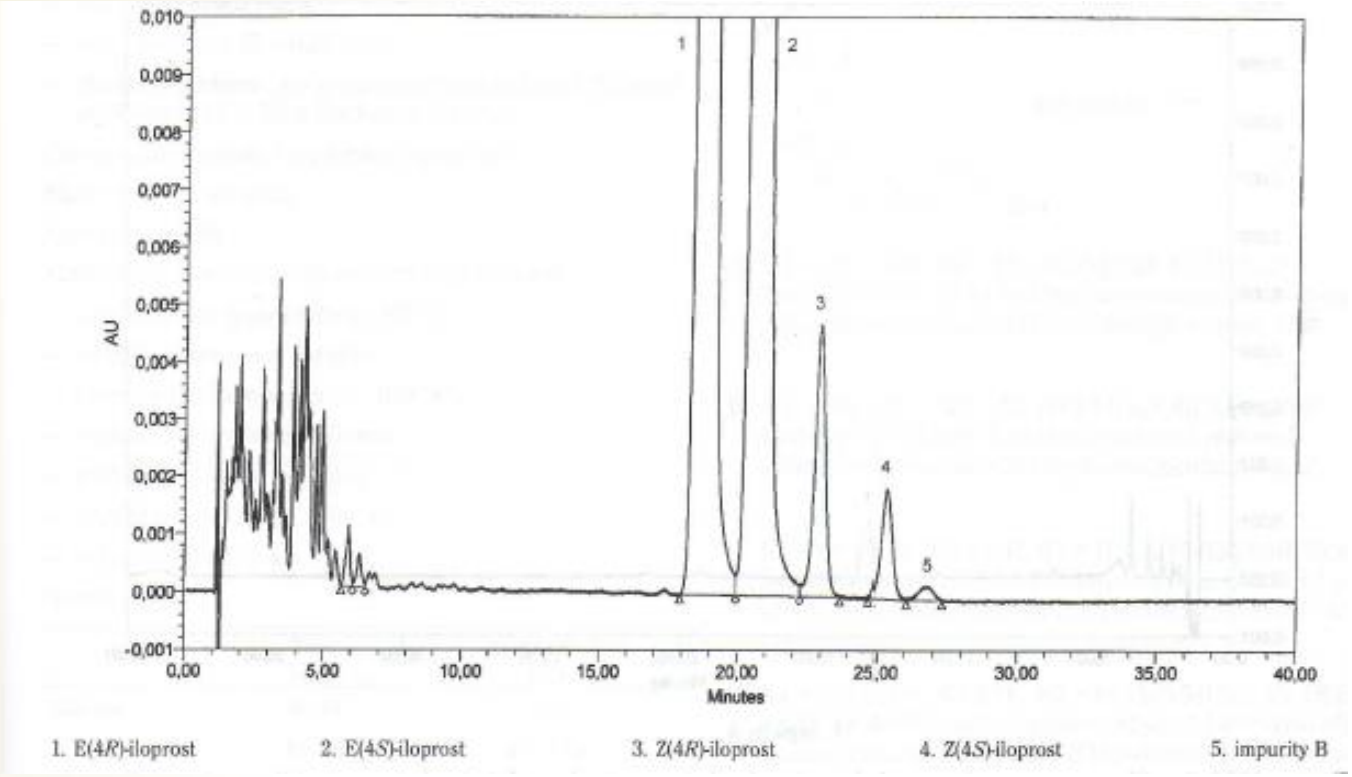
CLDS: Chlorodesonide

Non-chiral separations

Pharmacopeial example

Iloprost monograph, polar related substances:

Mobile phase: mix 330 m of acetonitrile R1 and 670 ml of **12 g/l BCD** adjusted to pH 2.0 with phosphroic acid R1.



Non-chiral separations

Pros:

Selector type, quality and concentration is flexible

Cheap columns and additives

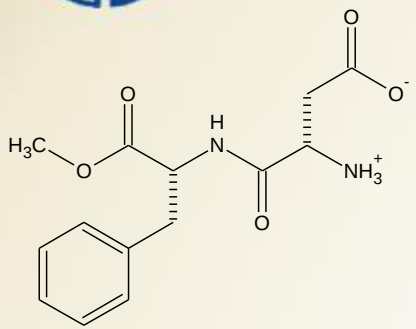
Cons:

The selector may affect detection (UV, MS)

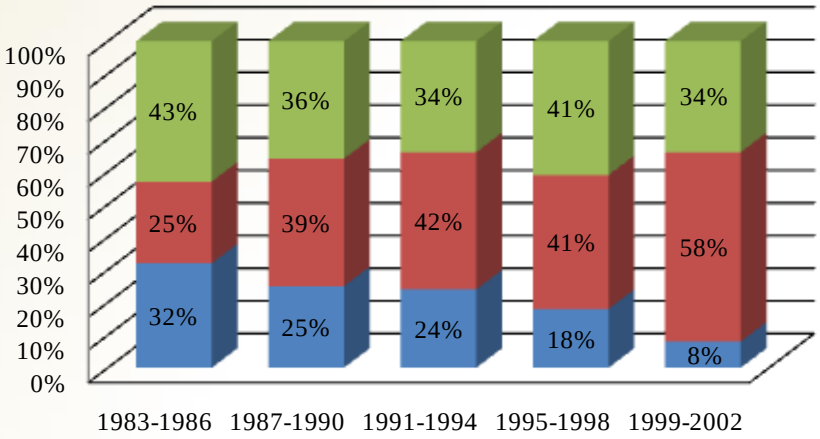
High material need

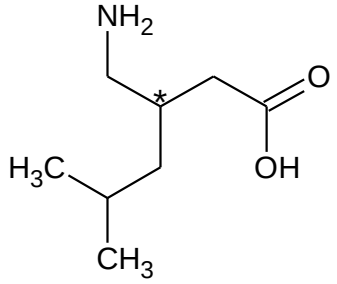
Reliable selector quality?

Chiral separations

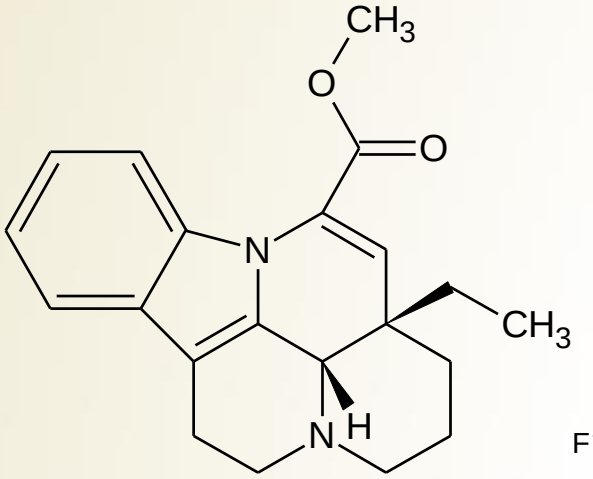


aspartame (Asm)
(Nutrasweet®)

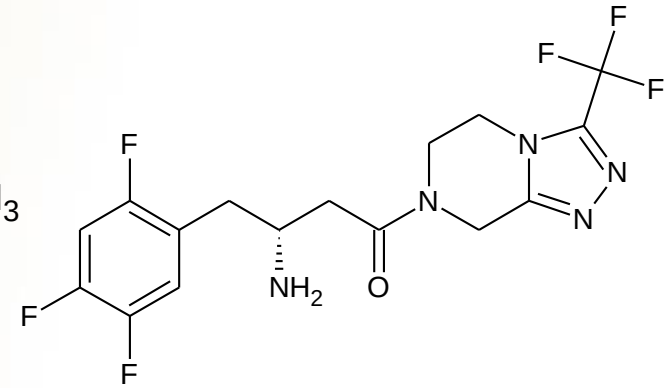




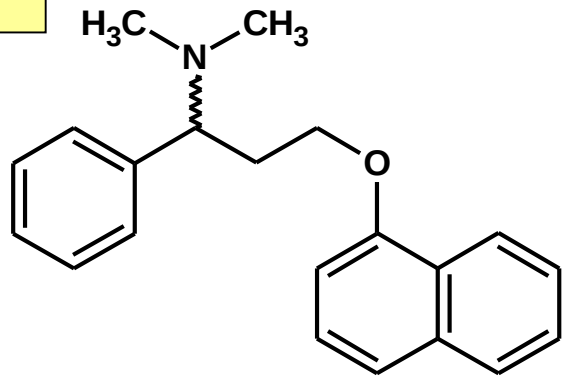
pregabalin (Preg)
(Lyrica®)



vinpocetin



sitagliptin (Sgli)
(Januvia®)



dapoxetine (Dpx)
(Priligy®)

Chiral separations

Requirements

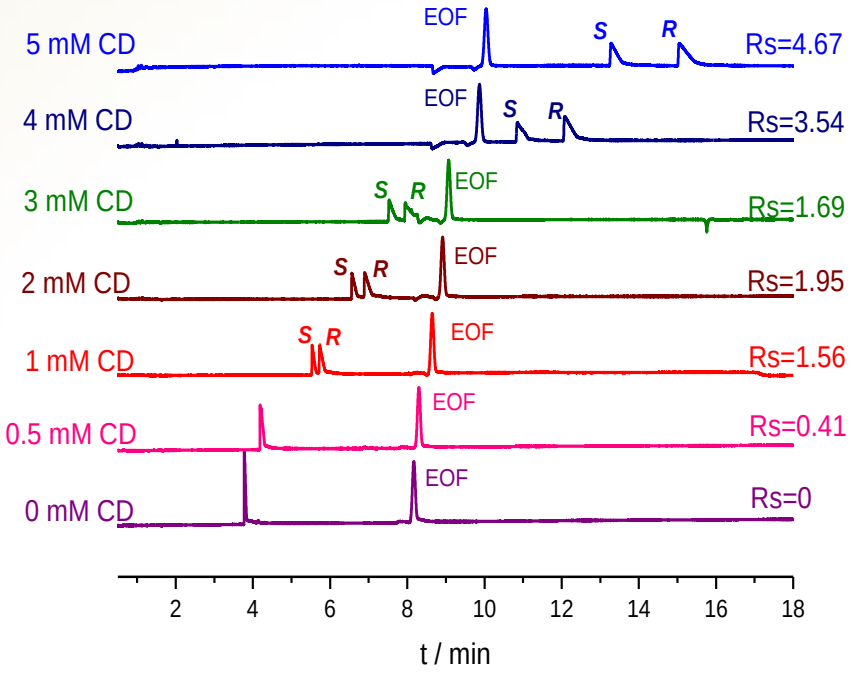
- **At least for one enantiomer:**

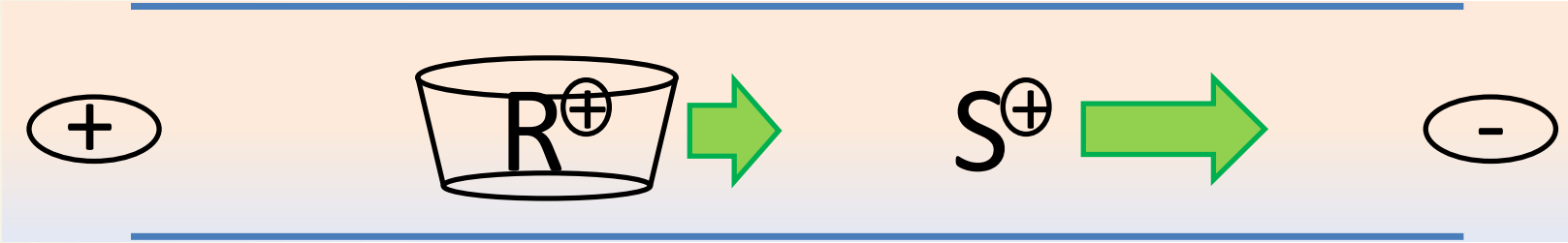
$$\mu_{free} \neq \mu_{complex}$$

- **For the complexes formed:**

$$\mu_{S,cplx} \neq \mu_{R,cplx} \; (K_S \neq K_R)$$

Alogliptin + SBECD

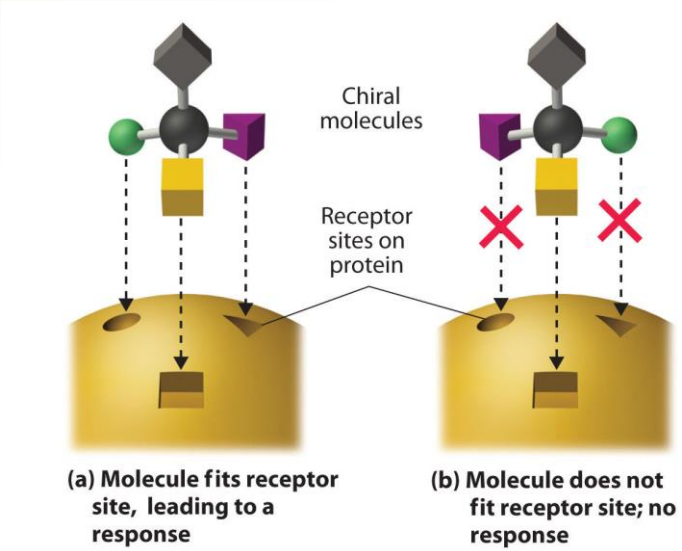




I. Fejos et al, Electrophoresis, 35, 2885-2891, 2014

Application fields for chiral separations

Different biological activity



Chiral analysis of natural compounds

Follow-up analytics of enantiosele s

Control of Active Pharmaceutical

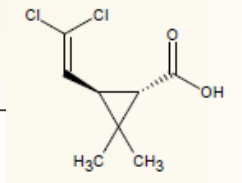
Agrochemistry (herbicides)

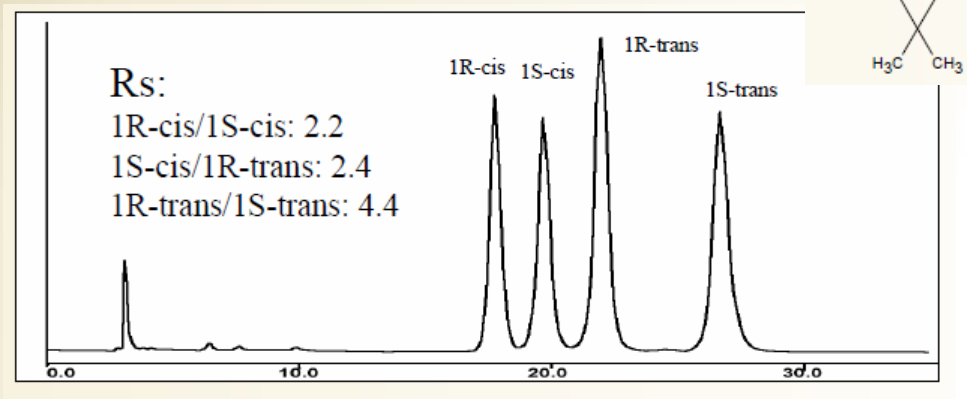
Application fields for chiral separations

Cosmetics (fingerprint)

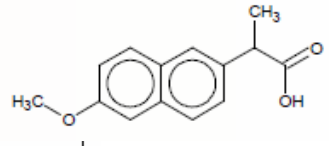
CD-bound chiral columns

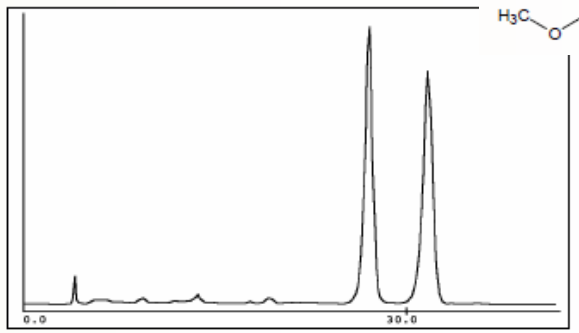
Permethrinic acid



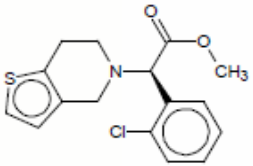


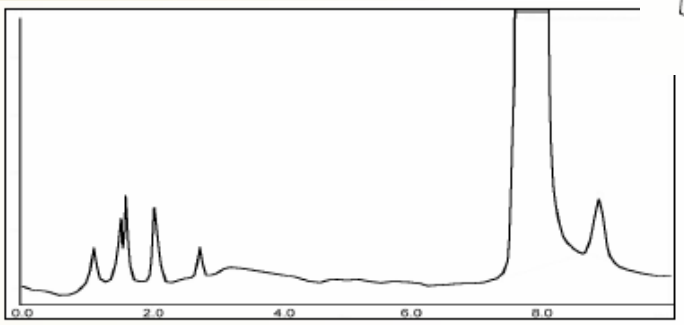
Naproxen





Clopidogrel – 0.5% (R) impurity





CD-bound chiral columns

Pros:

The selector does not affect detection

Possibility for semi-preparative separations

Cons:

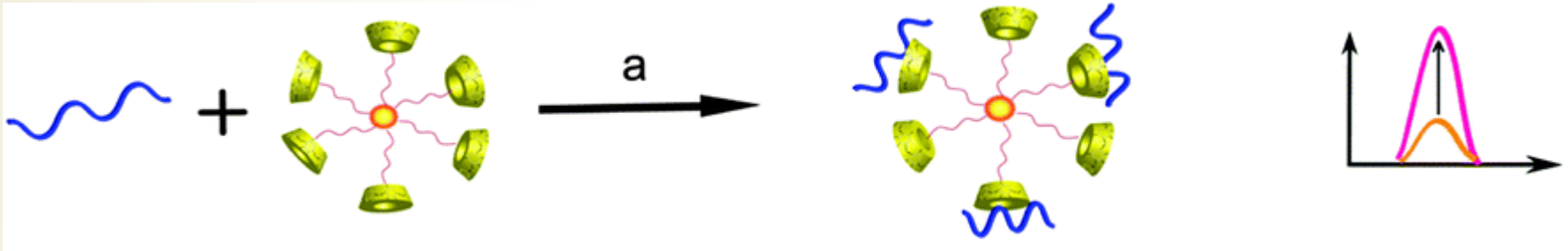
Expensive and very specific columns

Sensitivity improvement

In the CD cavity the analyte microenvironment suffers a significant and temporary change.

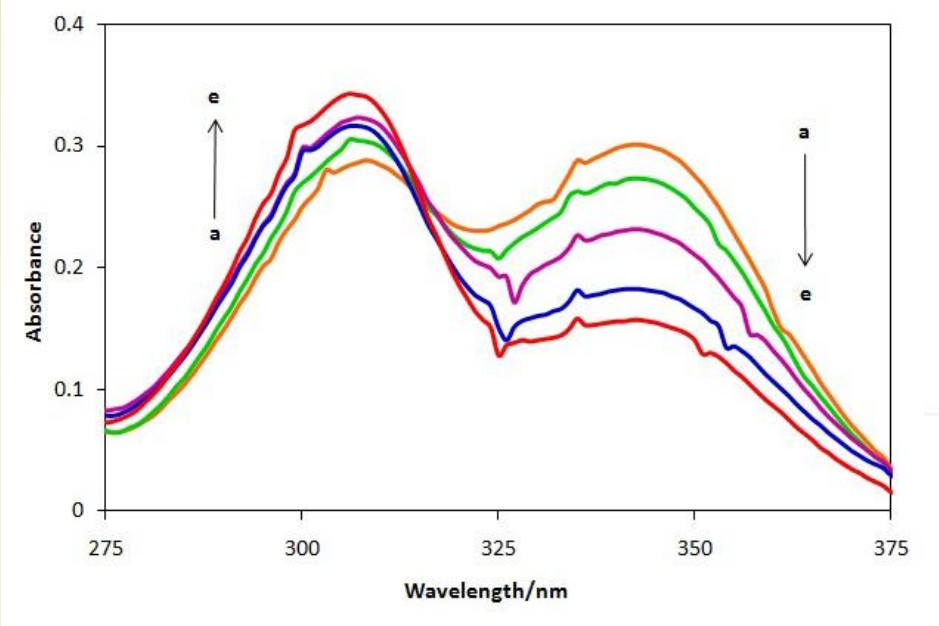
Enhanced spectral response, fluorescence or phosphorescence by lipophilic environment

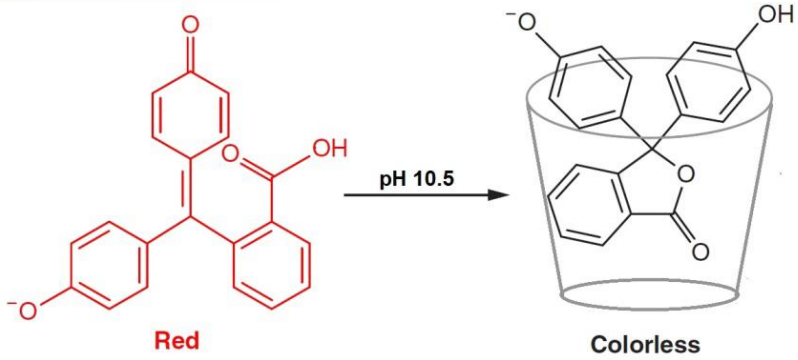
Protection of the fluorescing/phosphorescing state by the CD cavity from quenchers

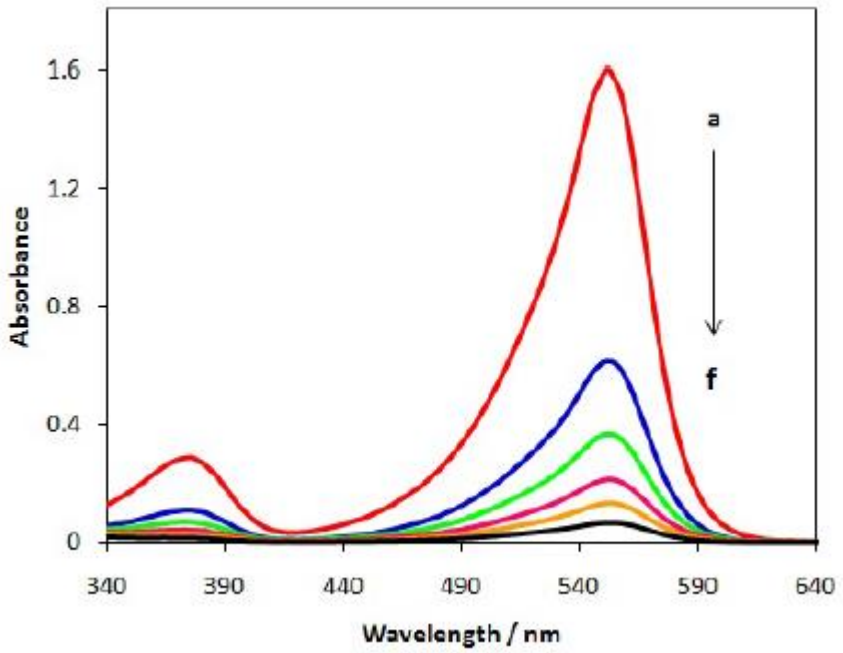


L Szente and J Szeman, Anal Chem, 85, 8024-8030, 2013

Spectroscopic methods





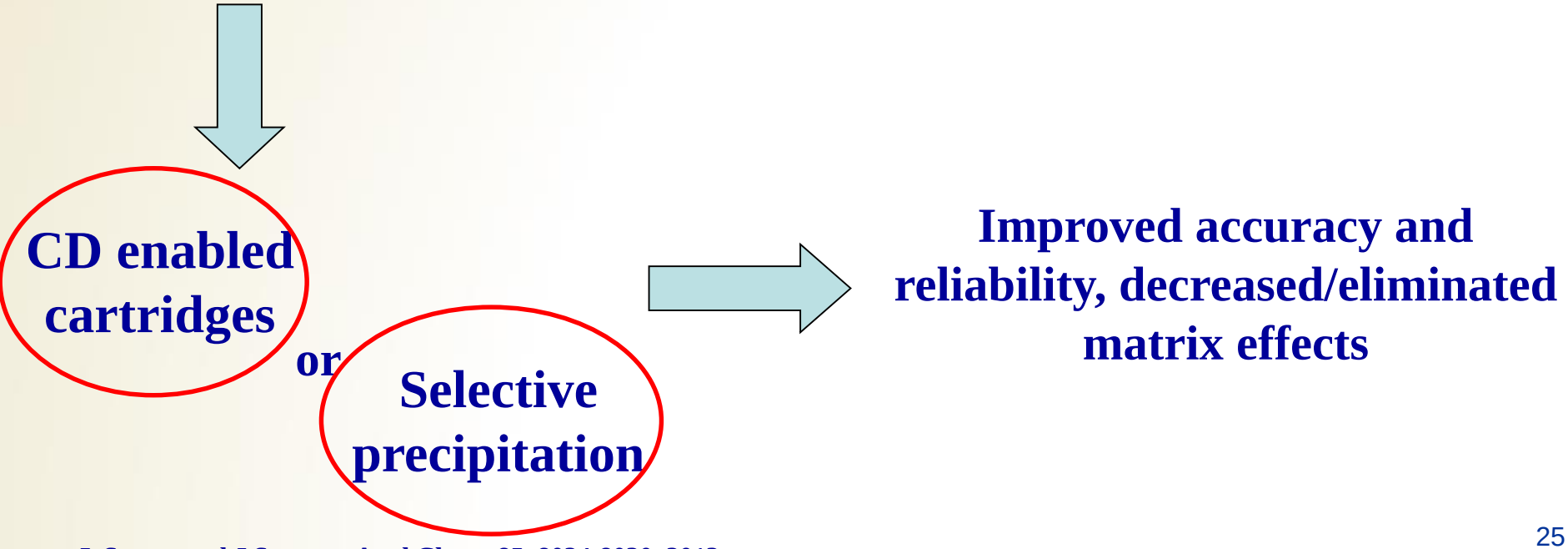


Taguchi K, J Am Chem Soc, 108, 2705-2709, 1986

Sample preparation

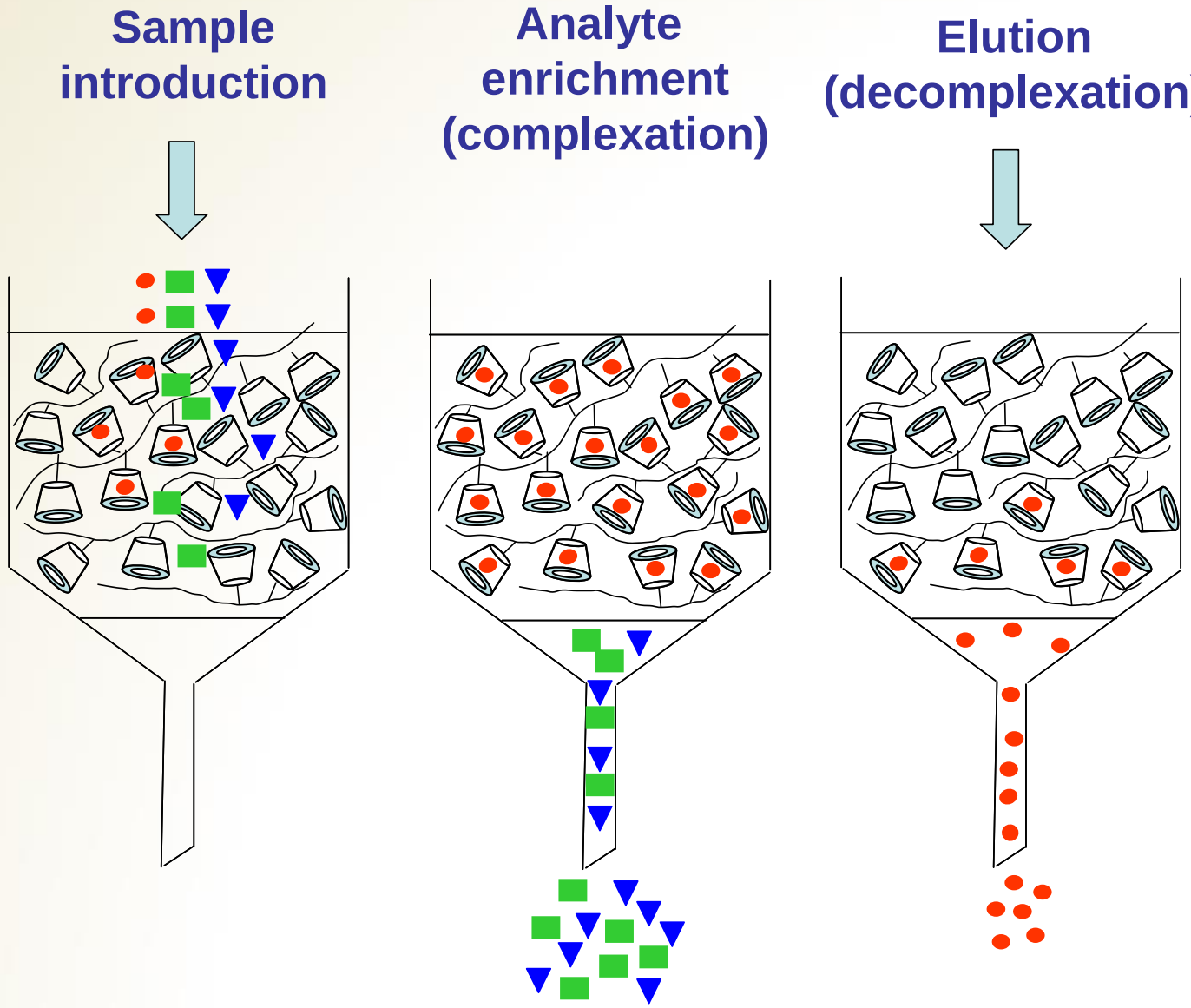
Aim: to concentrate the compontent of interest to an adequate level

How: Selective trapping and removal of the analyte or the interfering matrix using CDs



L Szente and J Szeman, Anal Chem, 85, 8024-8030, 2013

Sample preparation



L Szente and J Szeman, Anal Chem, 85, 8024-8030, 2013

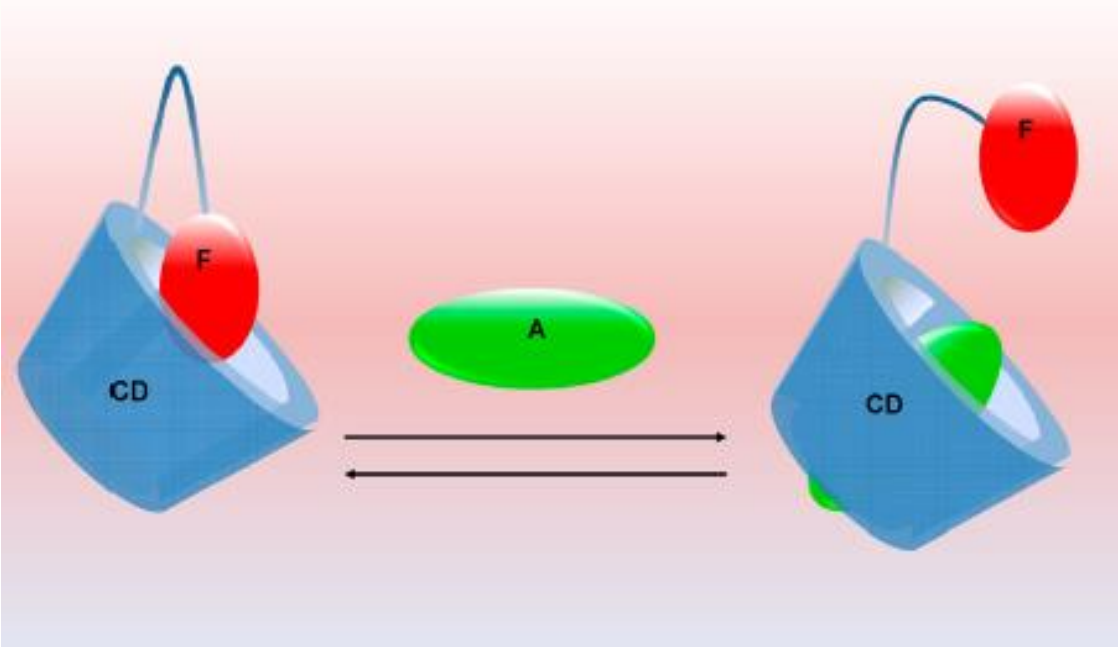
Fluorescence detection

CD enabled sensors

Chromophore or fluorophore tagged CDs

The presence of a competitive guest changes the fluorescence spectra

Selective to compounds with higher affinity towards the CD cavity than the fluorophore



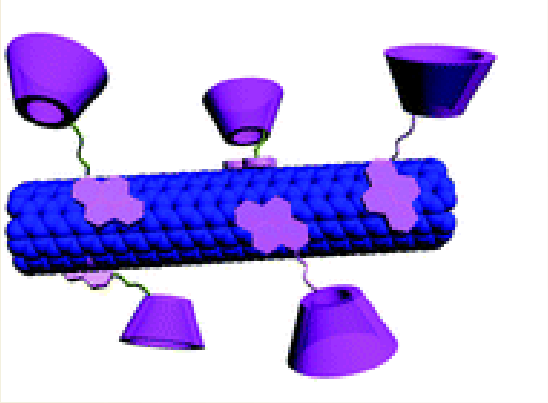
L Szente and J Szeman, Anal Chem, 85, 8024-8030, 2013

Future? applications
Diagnostic applications

Early diagnosis of Alzheimer disease, even prognosis

Determination of the amount of ethane and butane in the expired breath

„breath biomarkers”



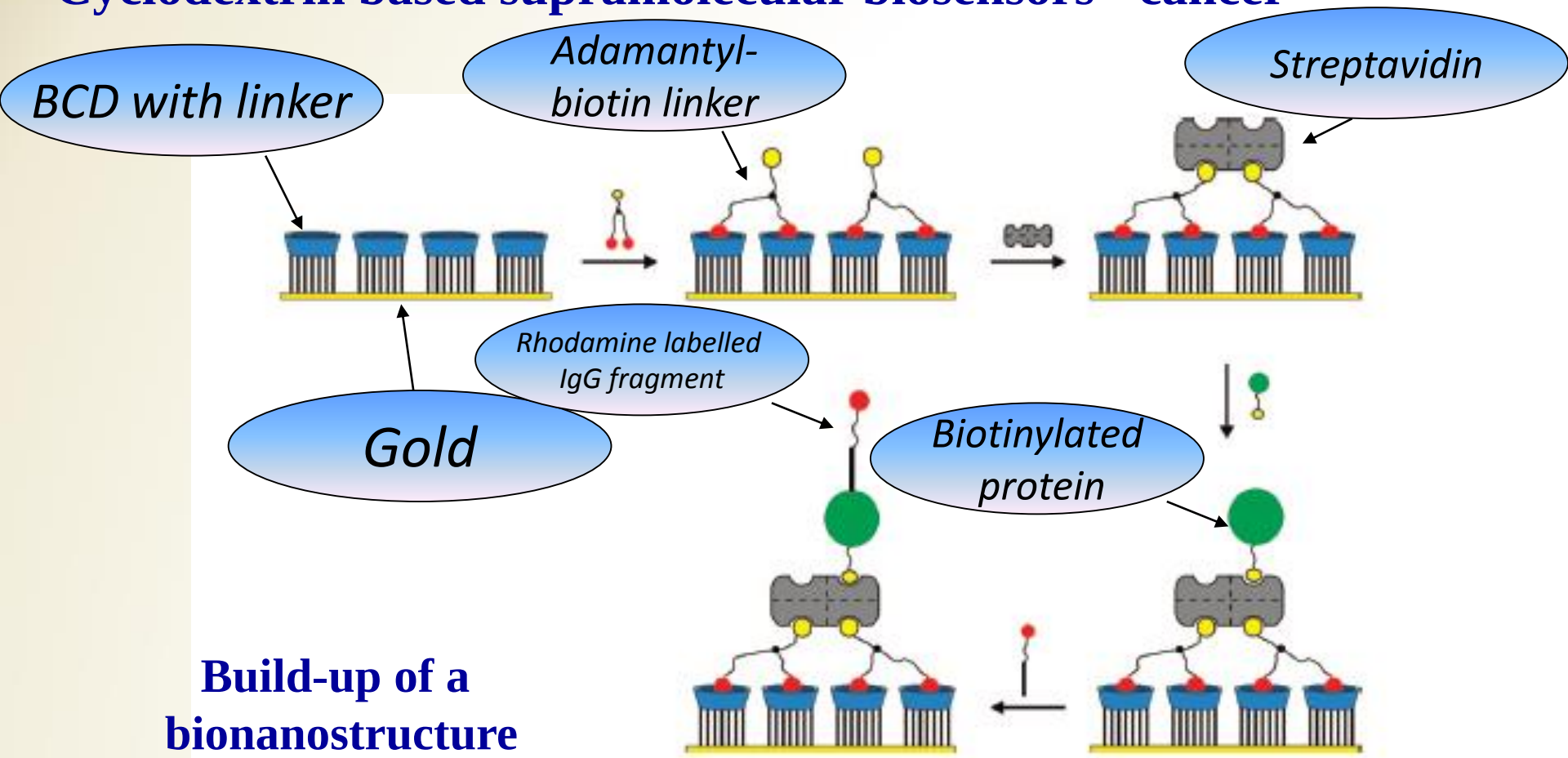
A network of carbon nanotubes coated with CDs and CD derivatives

Enhanced sensitivity for volatile biomarkers in breath

Q Ashton Acton, Parkinson's Disease: New Insights for the Healthcare Professionals, 2013

Future? applications
Diagnostic applications

Cyclodextrin based supramolecular biosensors - cancer

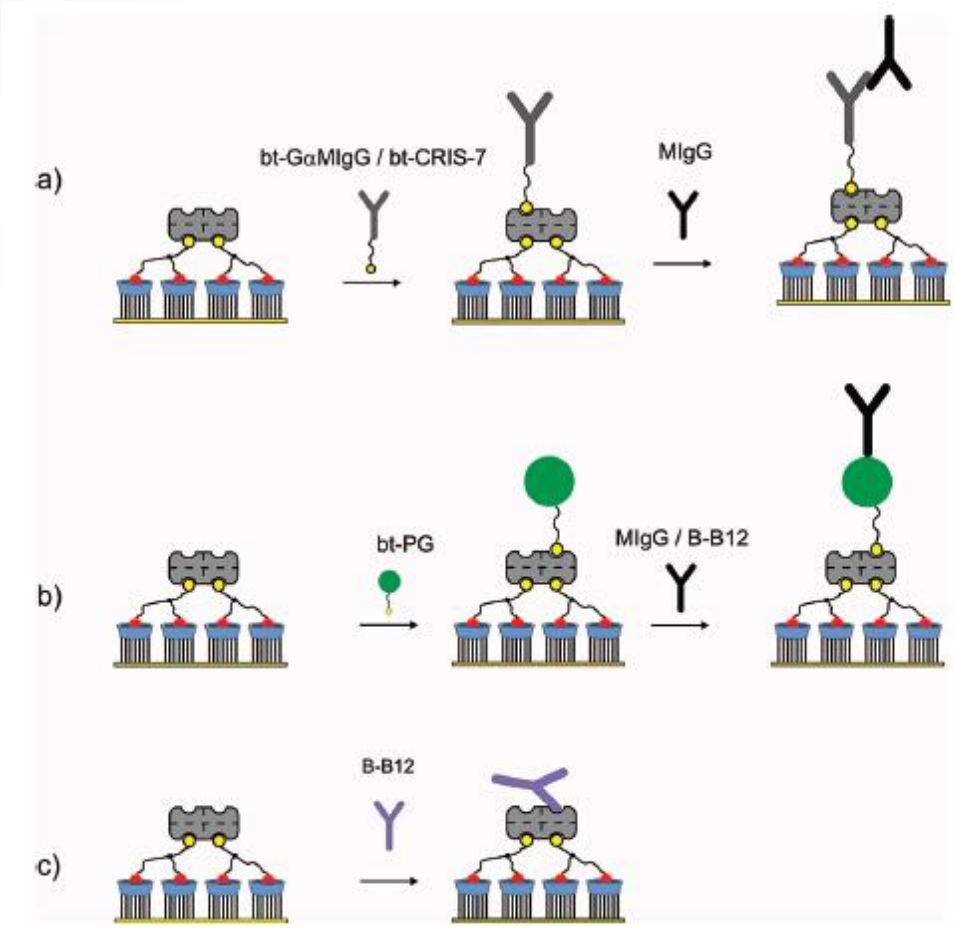


Build-up of a bionanostructure

Ludden et al, J Am Chem Soc, 130, 6964-6973, 2008

Future? applications
Diagnostic applications

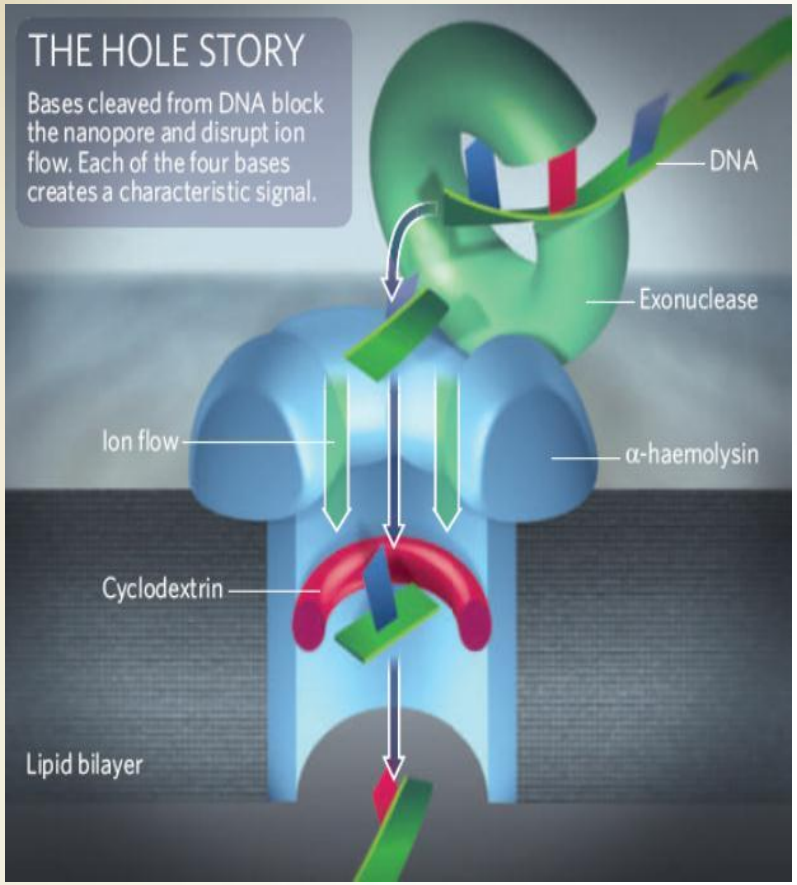
Cyclodextrin based supramolecular biosensors - cancer



Ludden et al, J Am Chem Soc, 130, 6964-6973, 2008

Future? applications

DNA sequencing – *Pore in the Hole*



Identifying each DNA base by changes in the ion current flowing across the pore

Individual human genome analysis

↑

Continuous DNA sequencer/reader

↑

Analyte recognizing sensor

↑

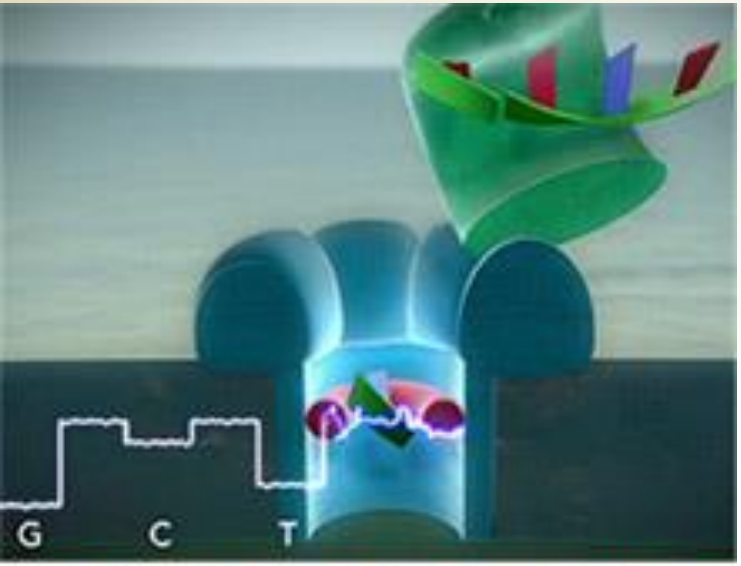
Protein nanopore with covalently tagged cyclodextrin adaptor

nature nanotechnology ARTICLES
PUBLISHED ONLINE: 22 FEBRUARY 2009 | DOI: 10.1038/NNANO.2009.12

Continuous base identification for single-molecule nanopore DNA sequencing

James Clarke[1], Hai-Chen Wu[2], Lakmal Jayasinghe[1,2], Alpesh Patel[1], Stuart Reid[1] and Hagan Bayley[2]*

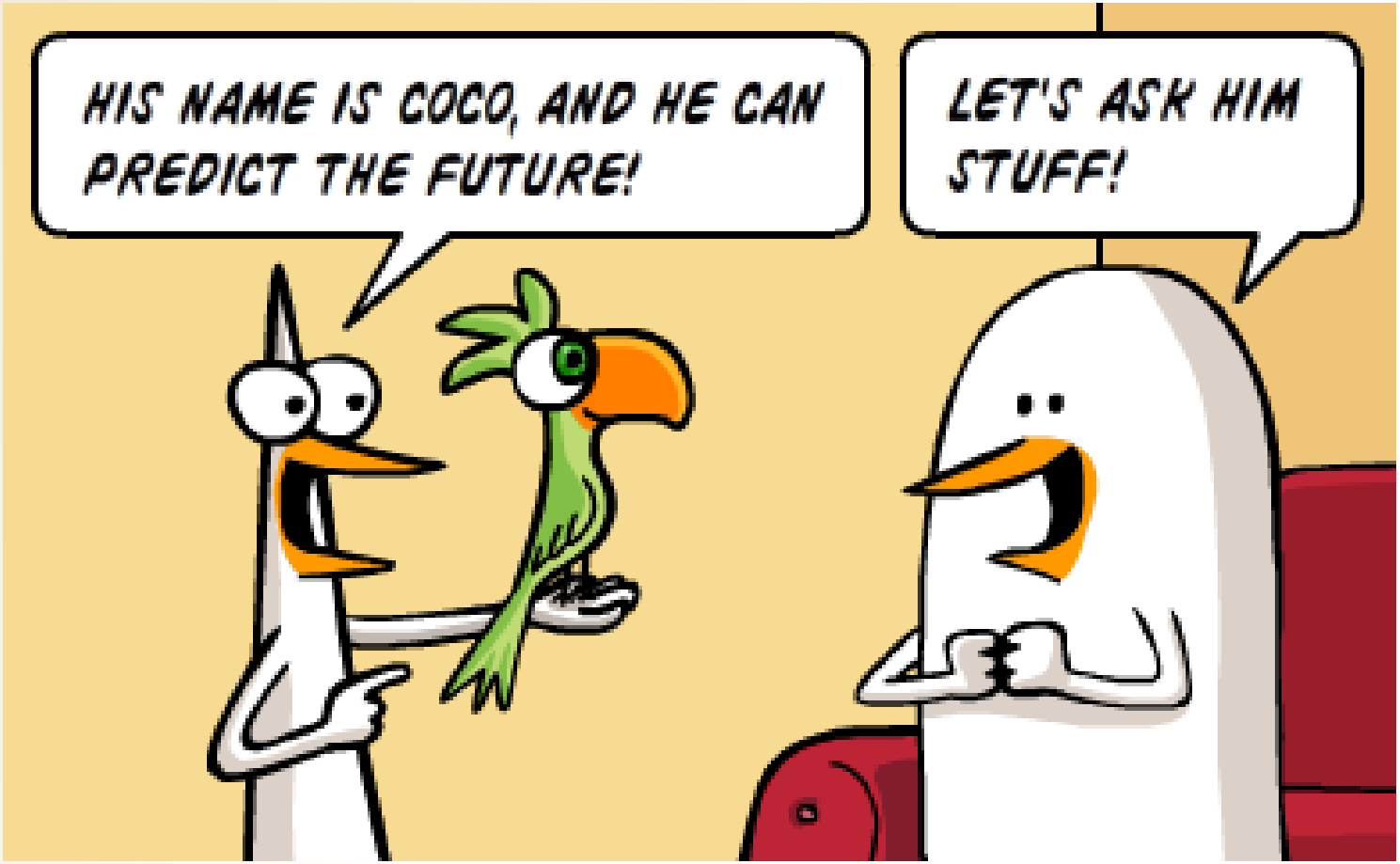
Future? applications
DNA sequencing



DNA array chip prototype with amino-β-cyclodextrin

550 nucleotide analyzed in 1 minute!

Future applications



Thanks for your attention