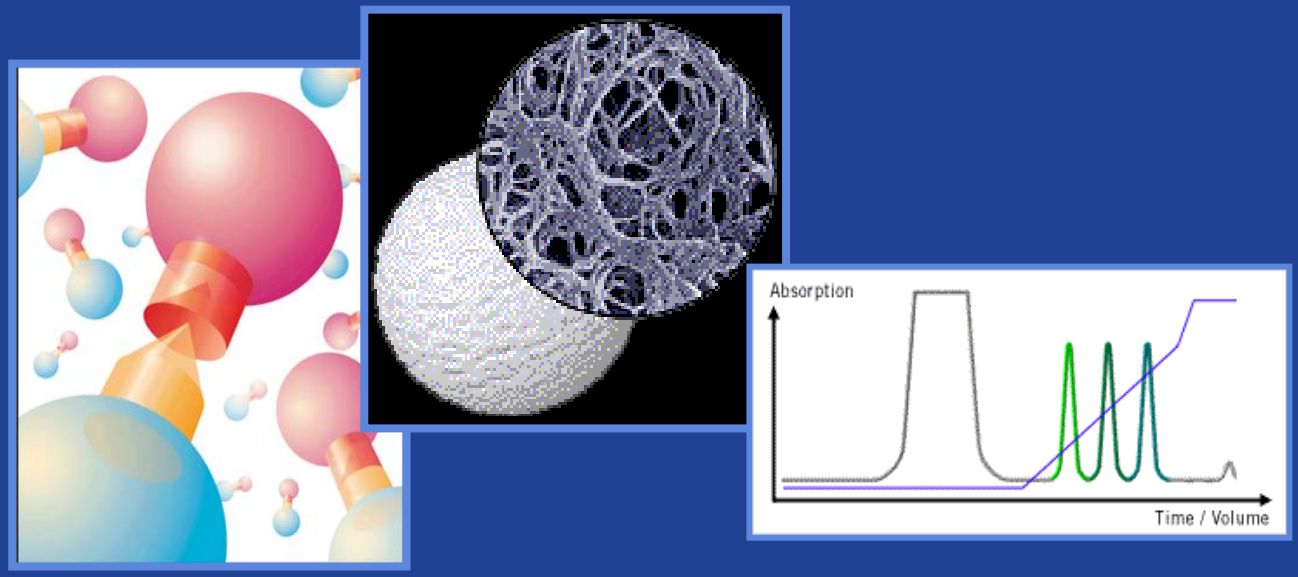
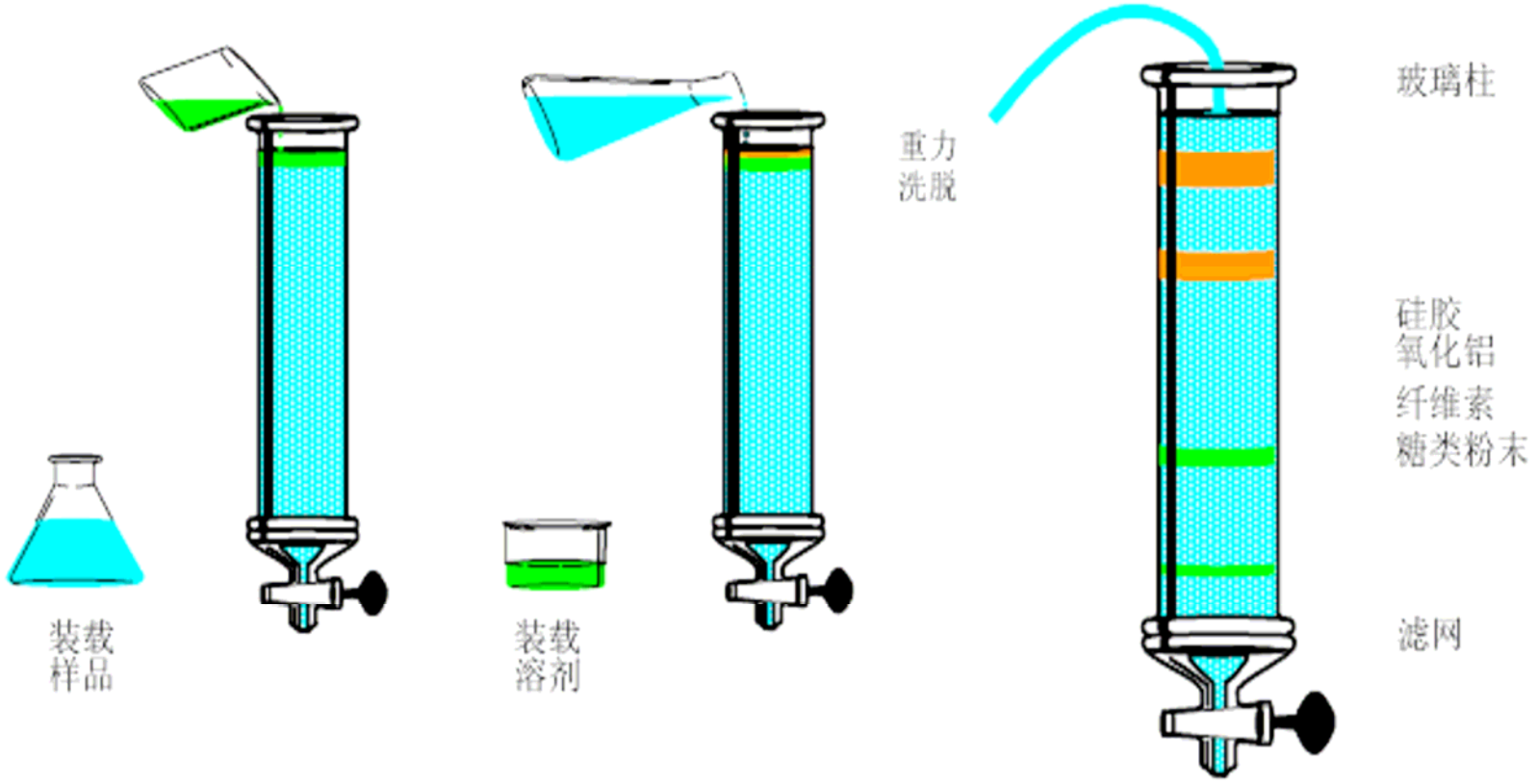
Chromatography terms
层析术语



imagination at work

History of Chromatography
层析技术的历史

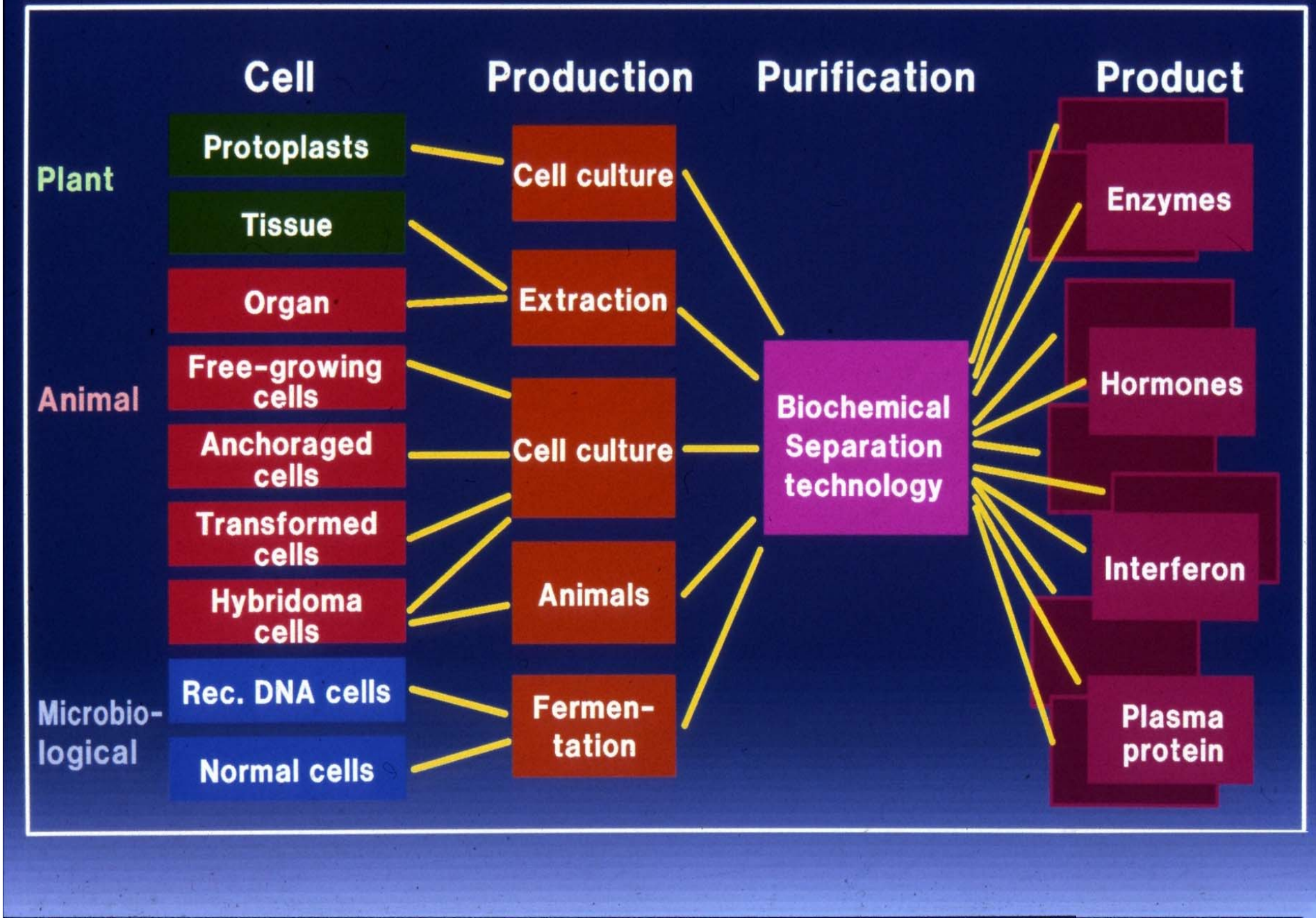


1906 - Mikhail Semenovich Tswett (1872-1919)

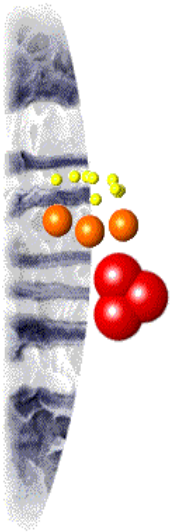
植物色素分离：碳酸钙吸附剂/石油醚

imagination at work

Purification - a key technology

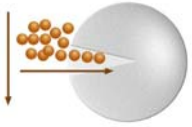


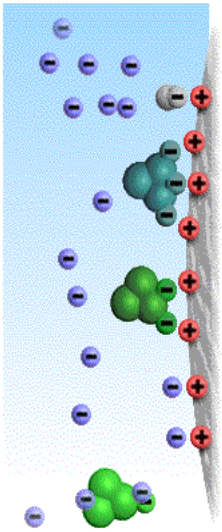
Principles of operation for chromatography techniques 层析原理



Gel Filtration

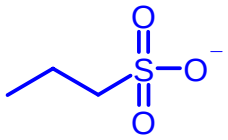
分子筛

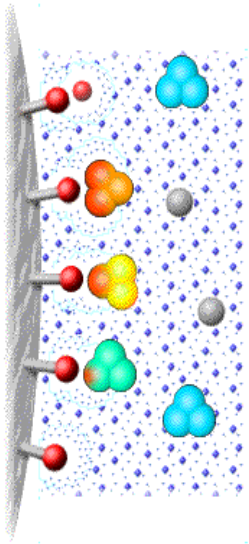




Ion Exchange

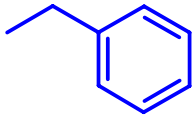
离子交换

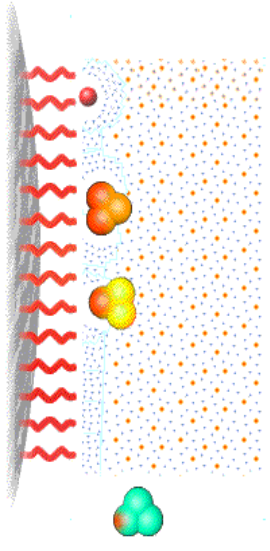




Hydrophobic interaction

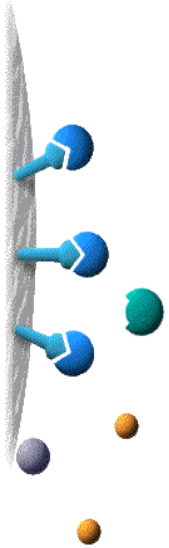
疏水层析





Reversed phase

反相



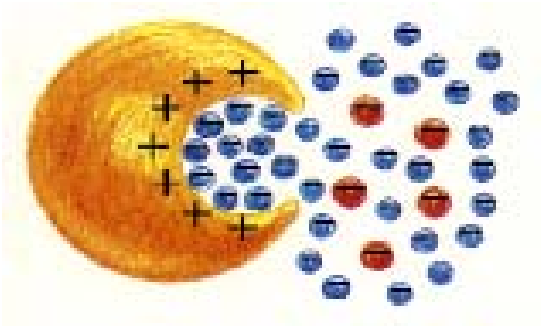
Affinity

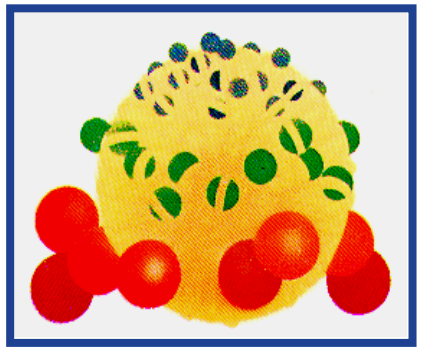
亲和

imagination at work

Important terms & parameters
重要的术语和参数

- Separation volumes 分离体积
- Resolution 分辨率
- Selectivity 选择性
- Efficiency 柱效
- Symmetry 对称性
- Linear flow rate 线性流速
- Capacity 载量





Separation volumes 分离体积

V_o = void volume 外水体积

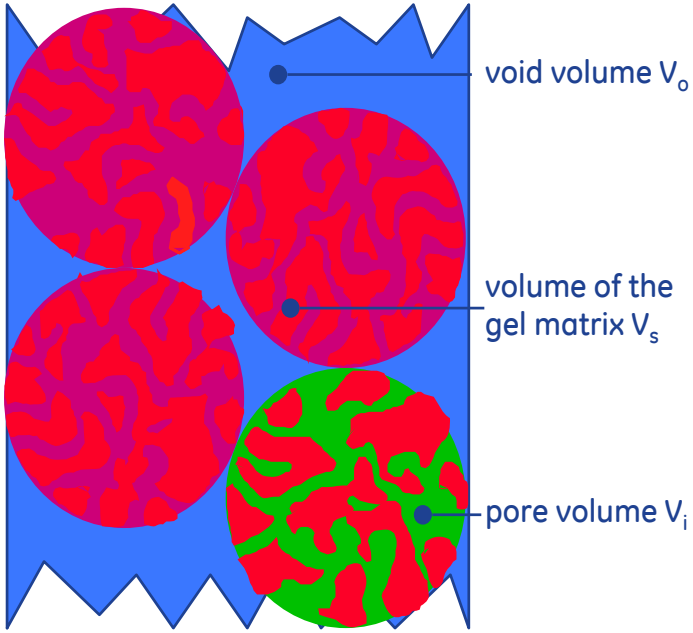
V_R = retention volume or elution volume 保留体积/洗脱体积

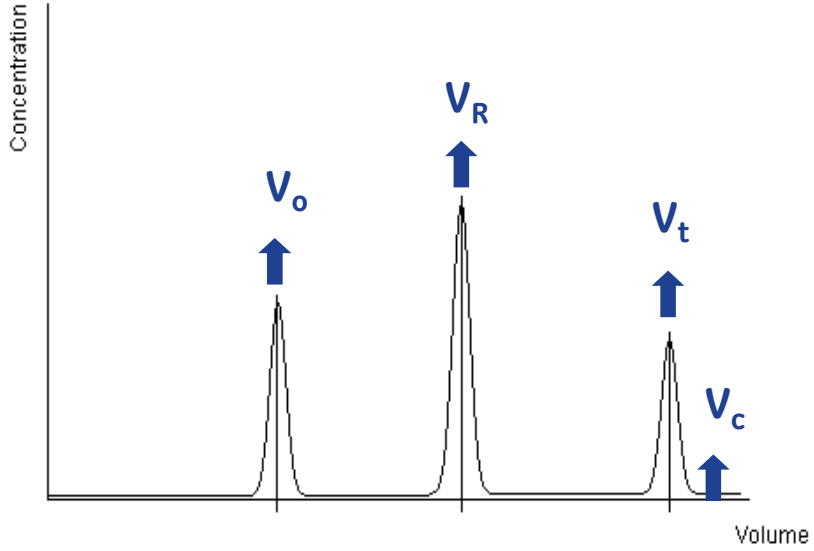
V_t = total liquid volume of the bed 总液体体积

V_i = inner pore volume = $V_c - V_s - V_o$ 内孔体积/内水体积

V_c = total geometric volume of the column 柱体积

V_s = volume of gel matrix 固相凝胶的体积

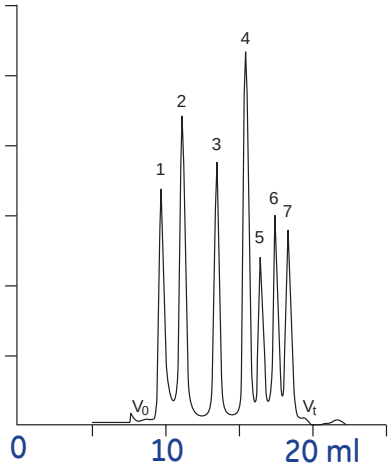




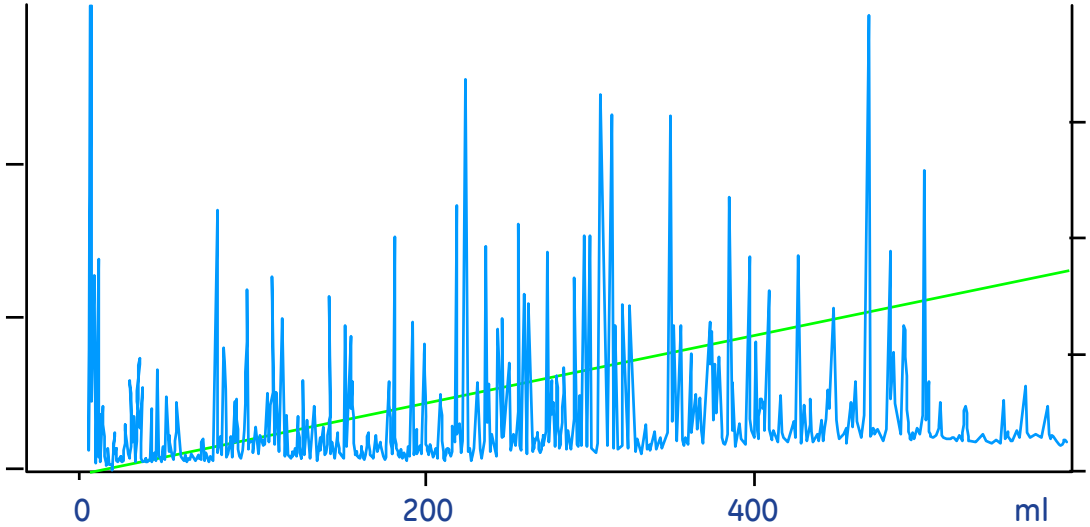
imagination at work

Resolution 分辨率（分离峰的能力）
the power to separate peaks

Maximum number of peaks in gel filtration ca 15



Maximum number of peaks in RPC > 150

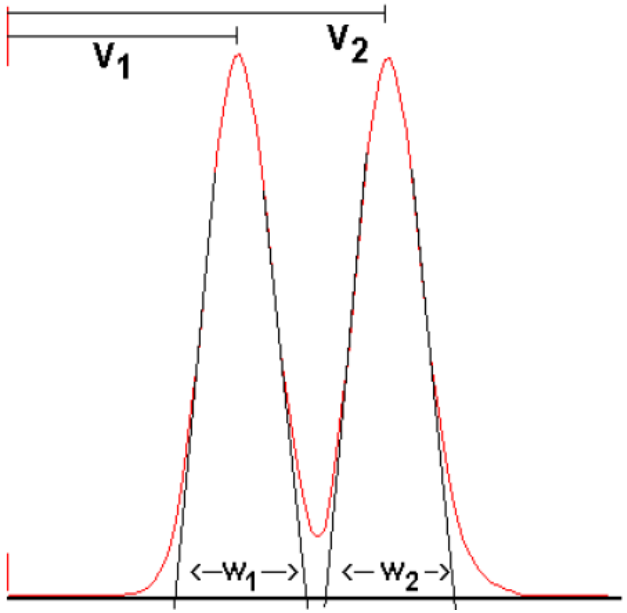


Remember: we are not purifying peaks, but proteins & peptides!

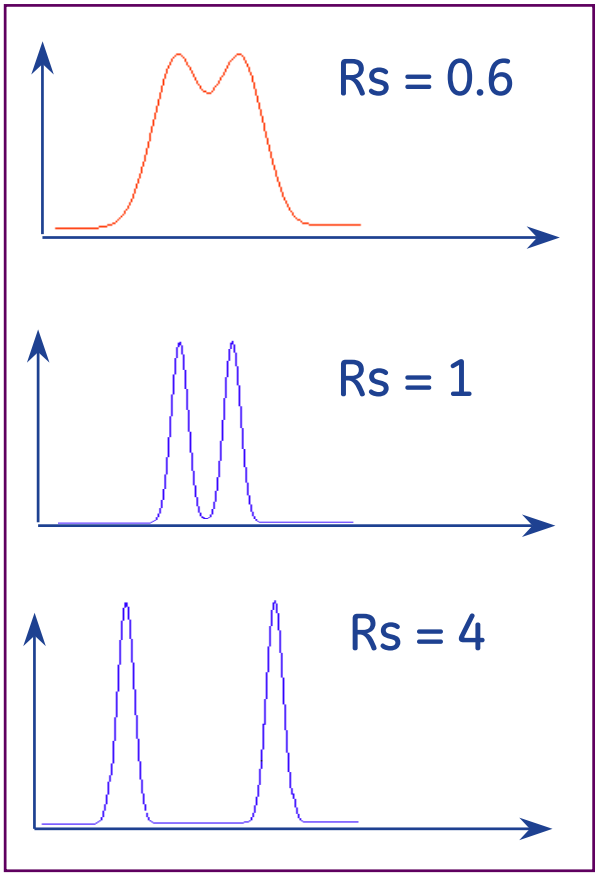
注意：我们不是在纯化峰，而是纯化蛋白质和多肽！

imagination at work

Resolution factor, R_s 分辨率

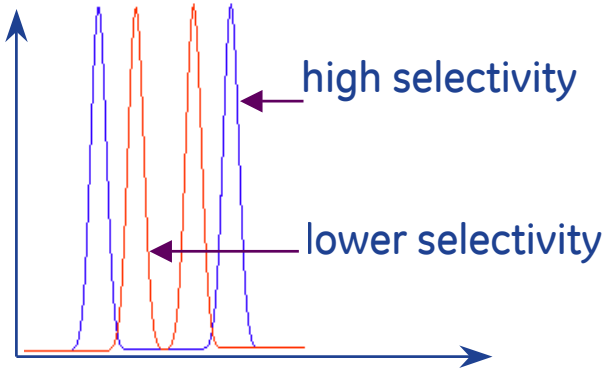


$$R_s = \frac{V_2 - V_1}{(W_1 + W_2) / 2}$$



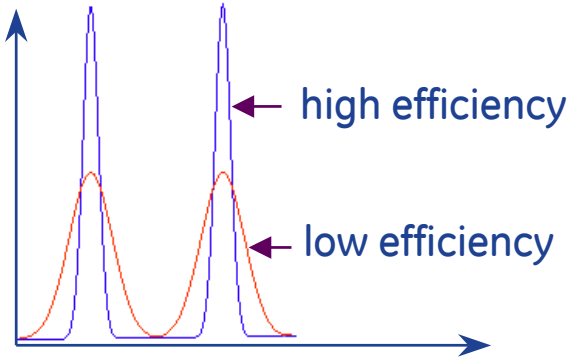
两峰相对于其峰宽而言, 分开的有多远

Resolution: depends on 分辨率取决于 selectivity 选择性 and efficiency 柱效



Selectivity 选择性:

- 峰分离的量度
- 高选择性可以达到基线分离
- 高选择性可以弥补低的柱效
  - 峰体积有可能增加



Efficiency 柱效:

- 峰宽的量度
- 柱效越高峰越窄
- 高柱效需要良好的柱填装技术
- 高柱效有时可弥补选择性不足

imagination at work

Selectivity 选择性

To maximize the differences between proteins !
使得不同蛋白之间的差异最大化!

取决于

- Separation technologies 分离技术
- Chromatography media screen 填料筛选
- Separation conditions screen 参数优化

Efficiency 柱效

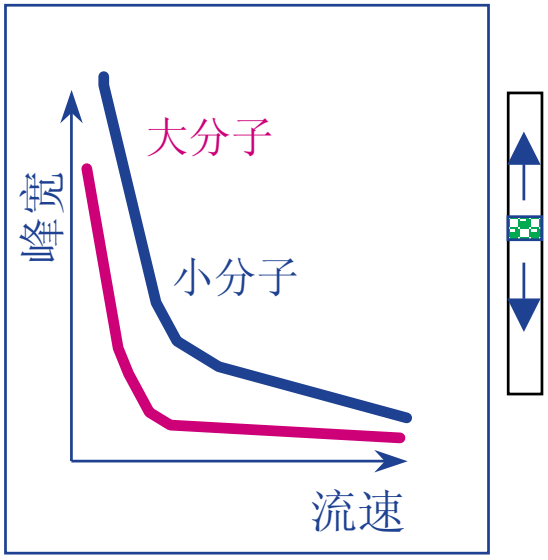
A powerful tool to improve Resolution !
提高分辨率的有力工具 !

取决于

- **flow rate** 流速
- particle size of matrix 粒径大小
- particle size distribution of matrix 粒径分布
- packing quality of the column 层析柱的装填
- sample volume and viscosity 样品体积和黏度

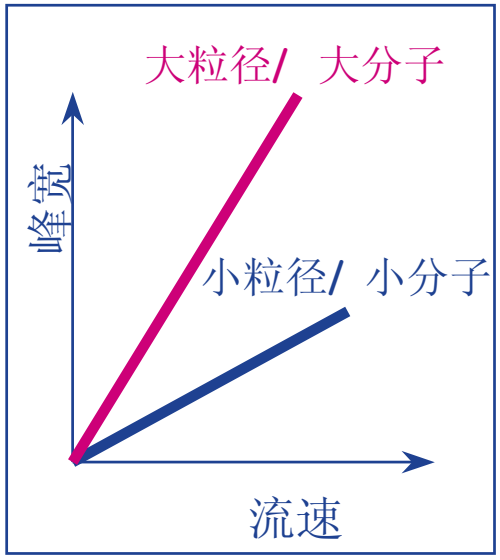
Band broadening effects
峰展宽的影响因素

Diffusion along column (轴向扩散)



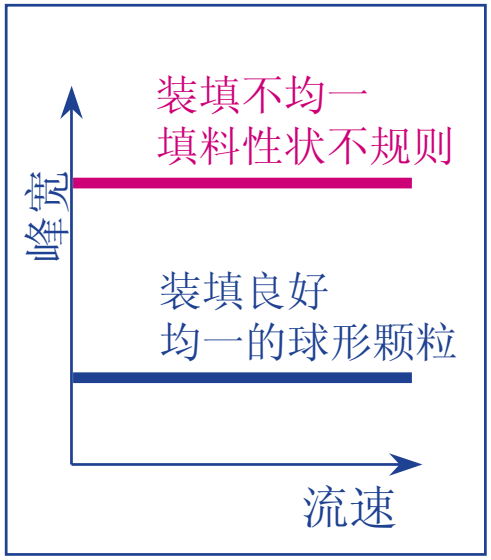
增加流速

Mass transfer (传质阻力)



降低流速

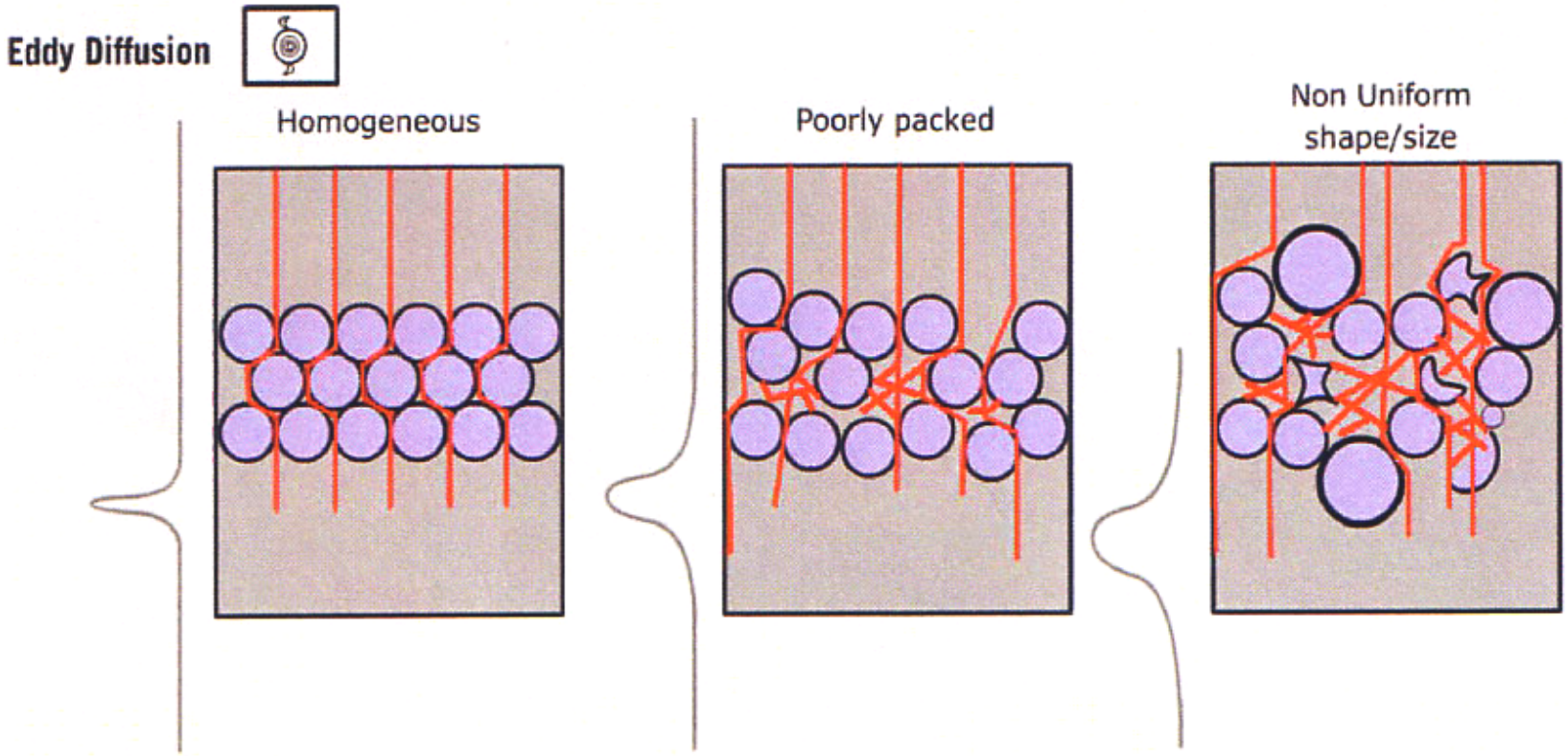
Eddy diffusion (涡流扩散)



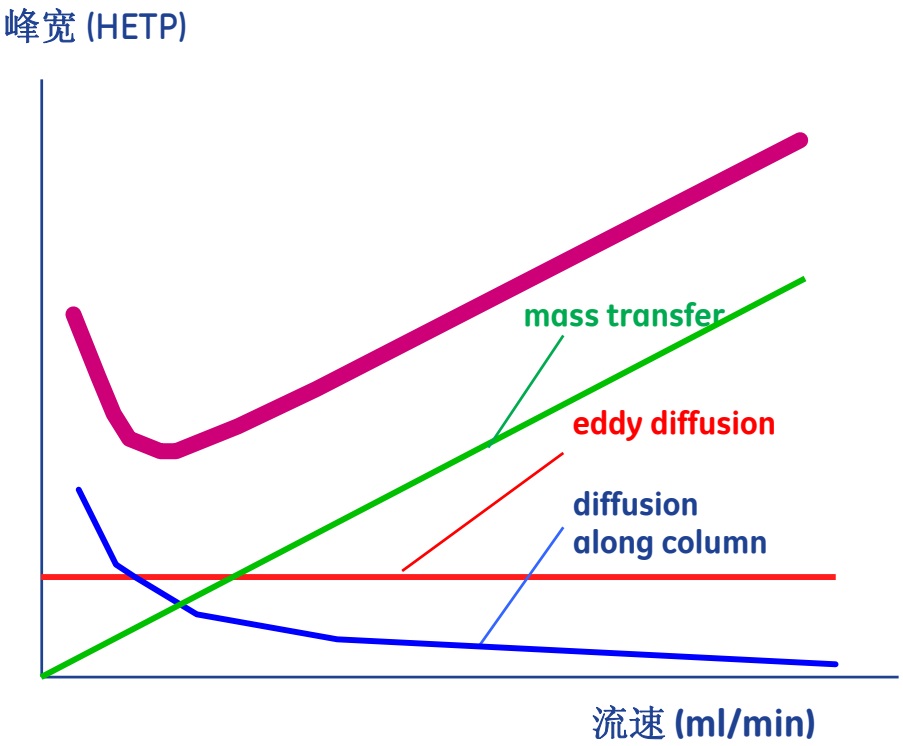
均一的填料
良好填装

Eddy diffusion (涡流扩散)

Particles distribution & packing
填料粒径分布 & 柱装填质量



流速(Flow)对峰宽(Peak width)的影响



- Diffusion along column (轴向扩散):
  小分子扩散速度快，高流速下峰较窄
- Mass transfer (传质阻力):
  小分子传质速度快，低流速下峰较窄
- Eddy diffusion (涡流扩散):
  装填良好，均一的球形颗粒使峰较窄

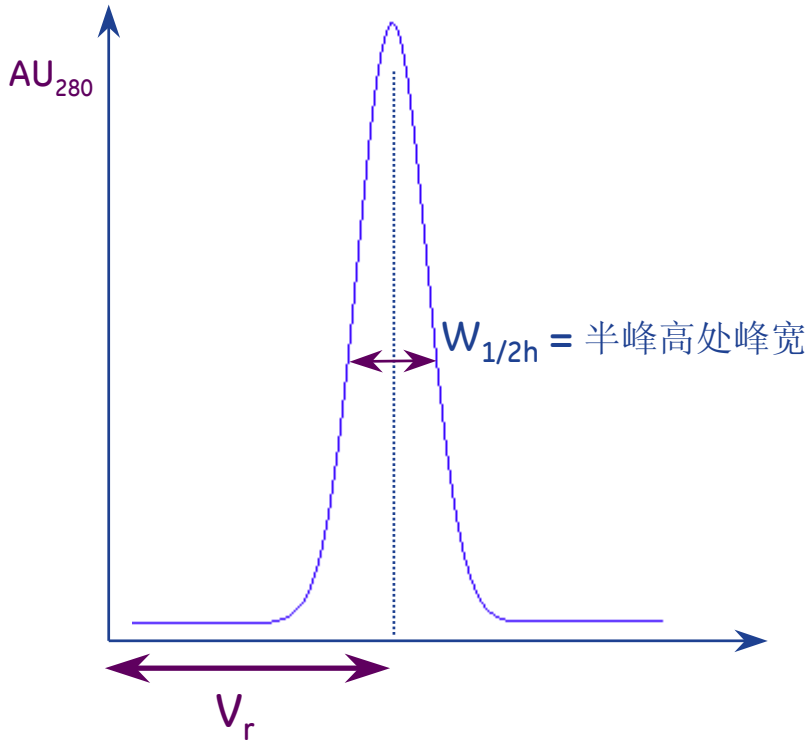
$$H = Ad_p + B/u + Cd_p^2u \qquad \text{(层析速率方程)}$$

H: 等板高度, d_p: 粒径, **u** : linear flow 线速度

Ad_p: eddy , **B/u**: diffusion along column, Cd_p^2u : mass transfer

imagination at work

Efficiency 柱效测定方法



$$N = 5.54 \left(\frac{V_r}{W_{1/2h}} \right)^2$$

$$HETP = L/N$$

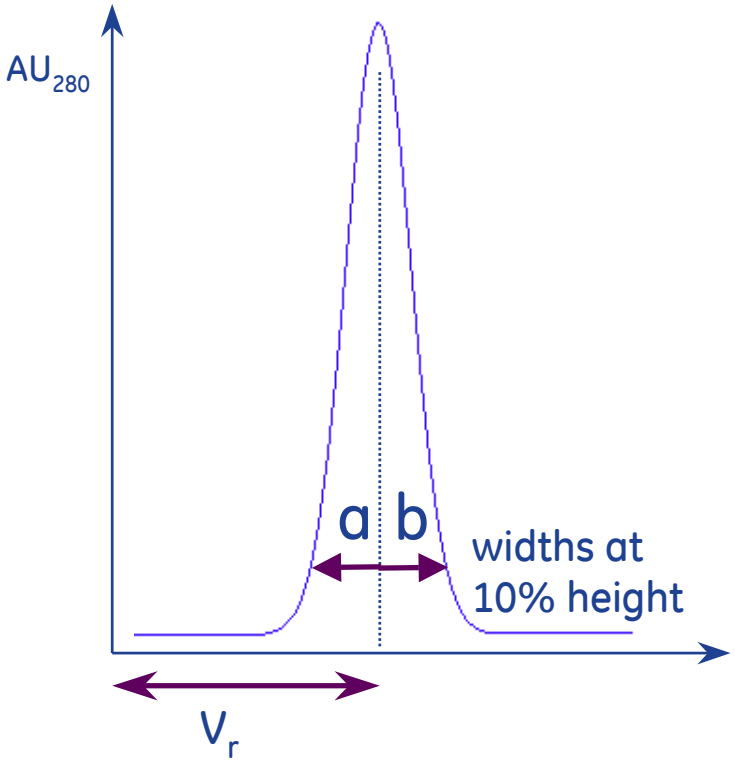
N = 理论塔板数；　　L = 柱高

Test: 1% 丙酮溶液

- 大约 0.5% 的柱体积， 0.2 AUFS 在 280 nm
- 或者用 2 M NaCl 用电导检测

建议在检查柱效和寿命时检测

Symmetry: 对称性
the shape of your peak 峰的形状



$$A_s = \frac{b}{a}$$

A_s =1 is perfect shaped Gauss curve

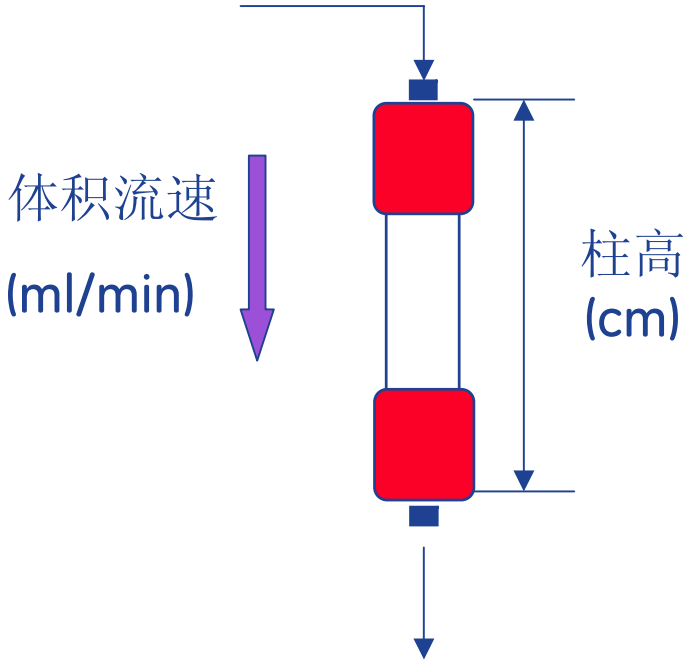
A_s >1 peak is tailing 拖尾

A_s <1 peak is leading 前倾

A_S 0.8~1.5 一般可以接受！

imagination at work

线性流速 (linear flow rate)



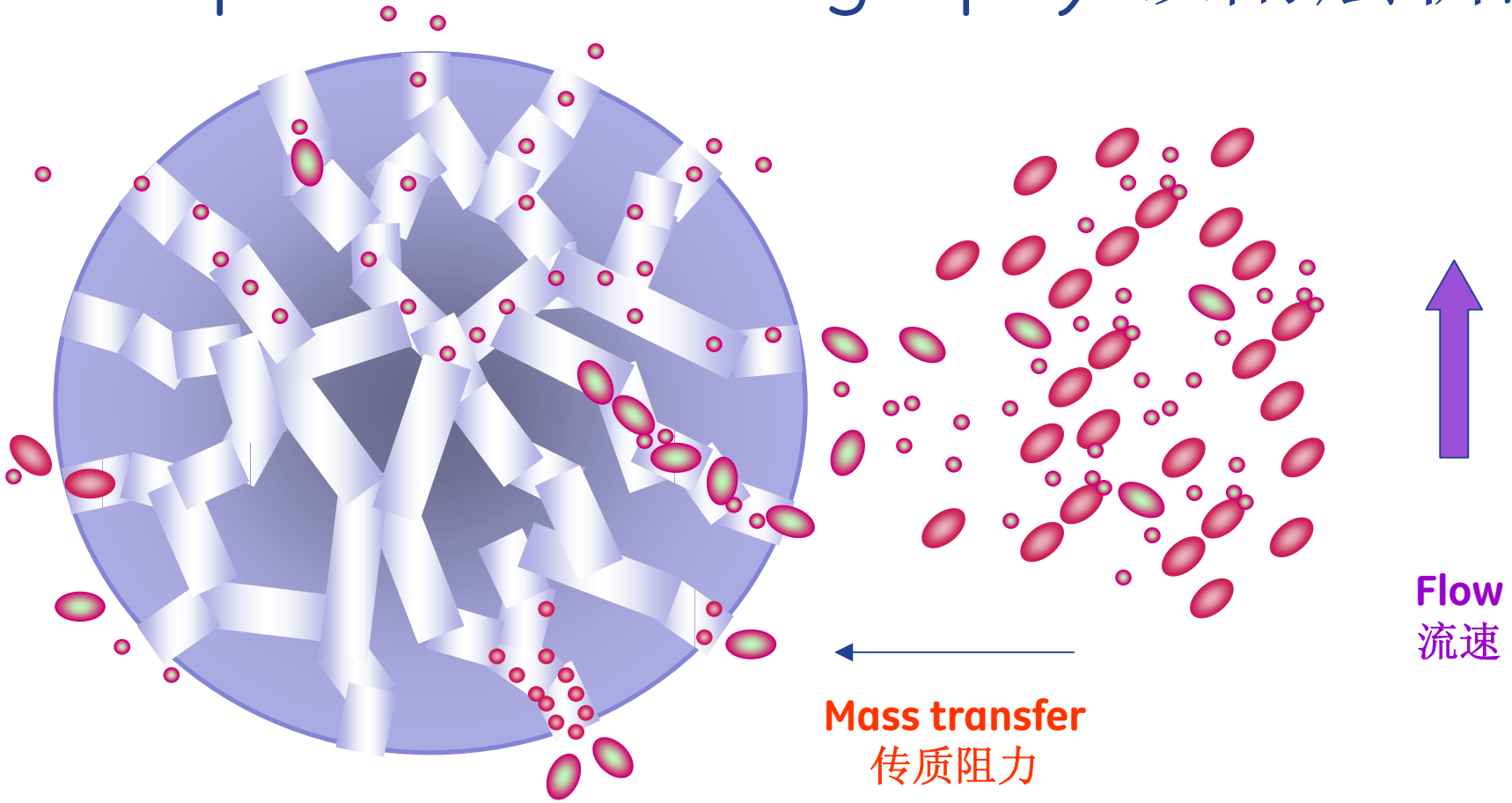
线性速度：

流体前沿沿柱管向下的迁移速度 **(cm/hr)**

$$\text{线性流速 (cm/hr)} = \frac{\text{体积流速 (ml/min)} \times 60}{\text{截面积 (cm)}^2}$$

imagination at work

Capacity 载量
(Adsorption chromatography 吸附层析)



imagination at work

Capacity depends on 载量取决于:

- Protein properties 蛋白性质

  -MW分子量, shape形状, charge电荷, hydrophobicity疏水性, etc

- Media properties 填料性质

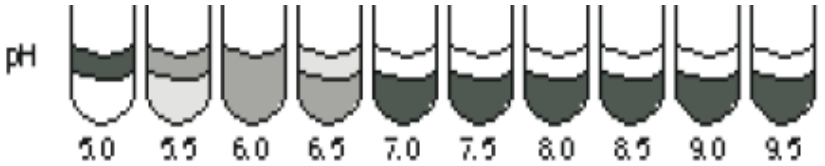
  -开孔率,粒径, 配基密度,etc

- Operation parameters 操作条件

  -pH, salt conc.盐浓度, additives添加剂, protein conc.蛋白浓度, temp温度, flow流速

Capacity 载量(mg protein/ml beads)

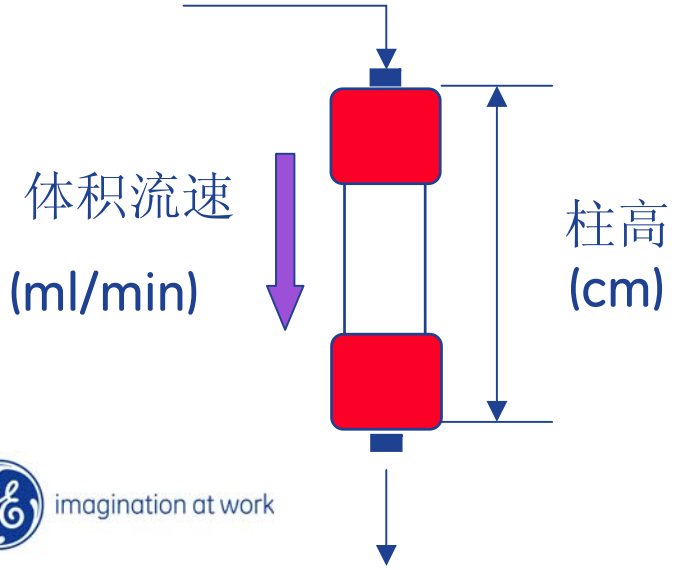
-Static capacity 静态载量



-Shake & Incubate 混合 & 孵育

-**Dynamic capacity** 动态载量(DBC): 质量传递

-Flow,residence time (停留时间) calculation

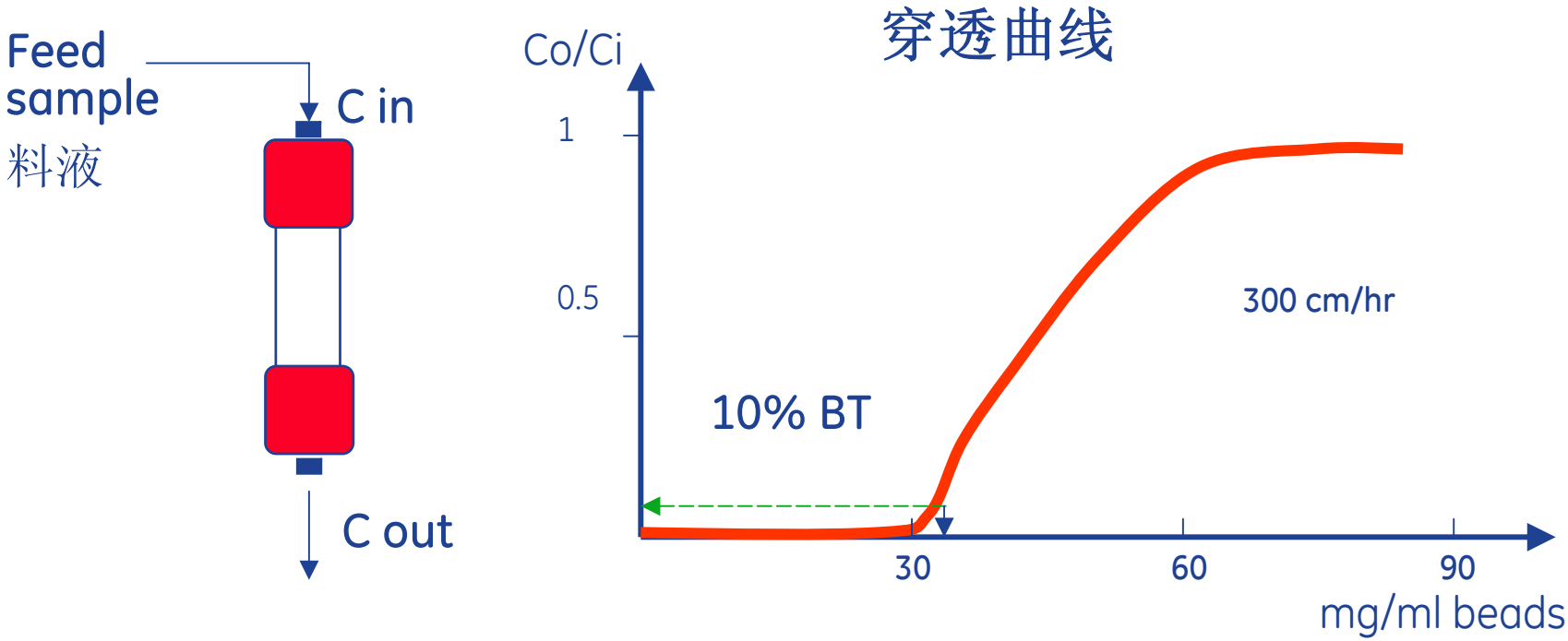


$$\text{线性流速 (cm/h)} = \frac{\text{体积流速 (ml/min)} \times 60}{\text{截面积 (cm}^2\text{)}}$$

$$\textbf{停留时间 Tr (min)} = \frac{\text{柱高 (cm)} \times 60}{\text{线性流速 (cm/hr)}}$$

imagination at work

Dynamic Capacity (mg protein/ml beads)
动态载量



- Residence time停留时间 (bed height柱高, linear flow线性流速)
-真实样品 (protein conc.蛋白浓度, buffer缓冲液, impurities杂质成分, temp温度)
- Good column efficiency 好的柱效

imagination at work

Q & A ?

Please visit:

www.gehealthcare.com/protein-purification

imagination at work