纳升液相色谱柱技术研究

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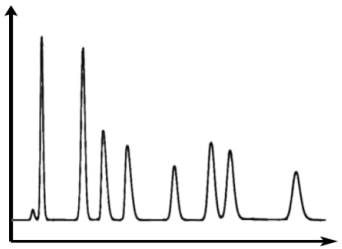
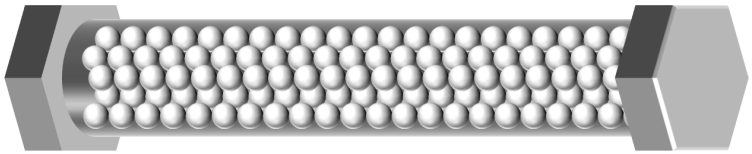
高质量色谱柱是实现高效分离的核心



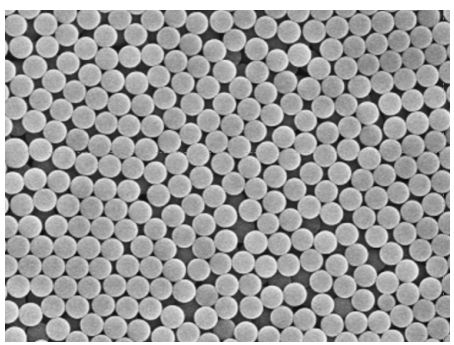
高质量分离



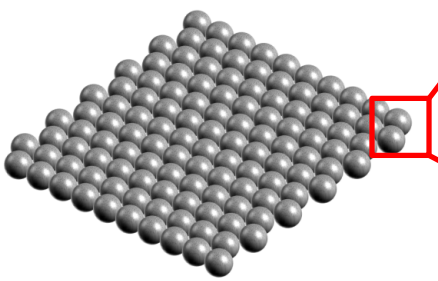
高质量色谱柱

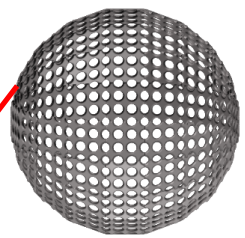
构筑有序结构的色谱填料是学界长期关注的重点

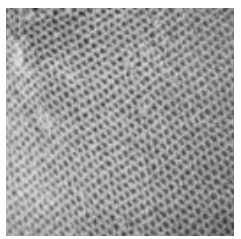
- 纳微公司
- 张丽华等, *Anal. Chem.*, **2022**, 94, 9525



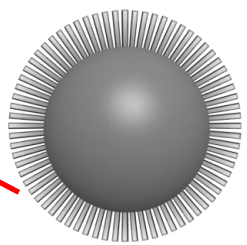
单分散微球

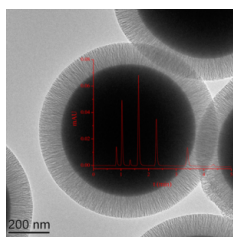






有序介孔

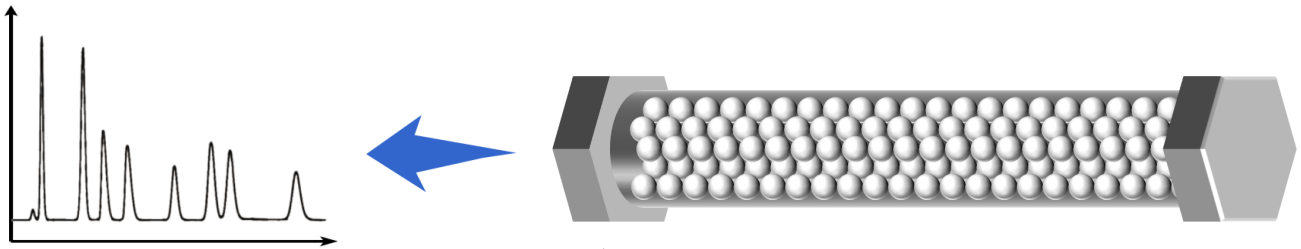




壳核径向孔

- 瞿其曙等, *Anal. Chem.*, **2015**, 87, 9631
- 安捷伦公司

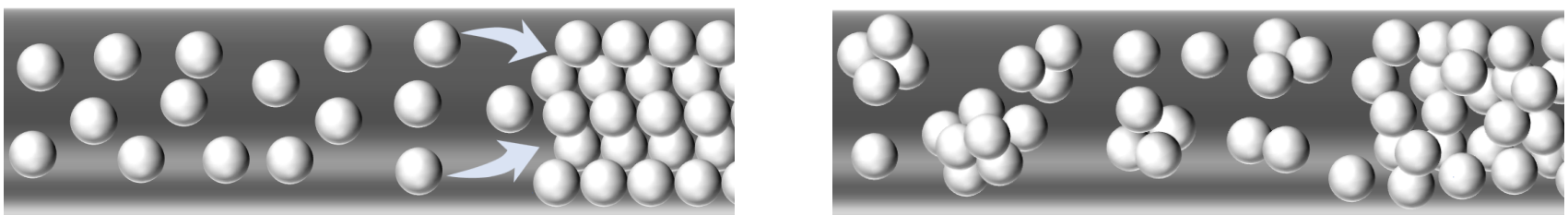
高质量色谱柱是实现高效分离的核心



高质量分离

高质量色谱柱

有序的色谱柱床同样显著影响色谱的分离质量！

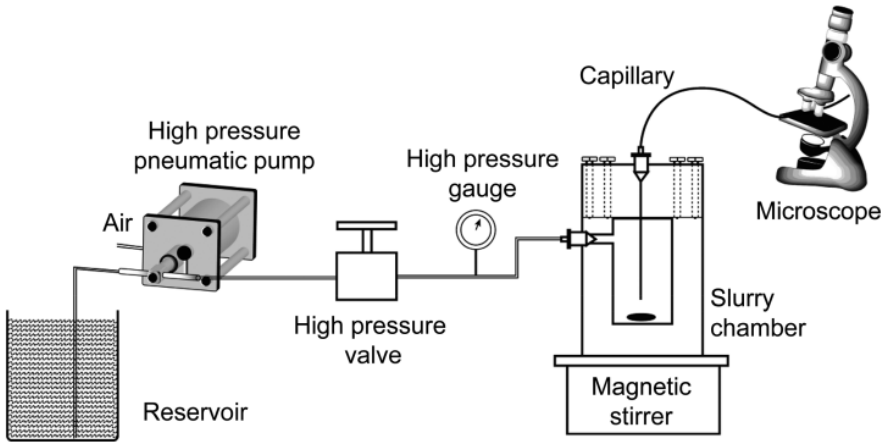


理想情况：有序组装

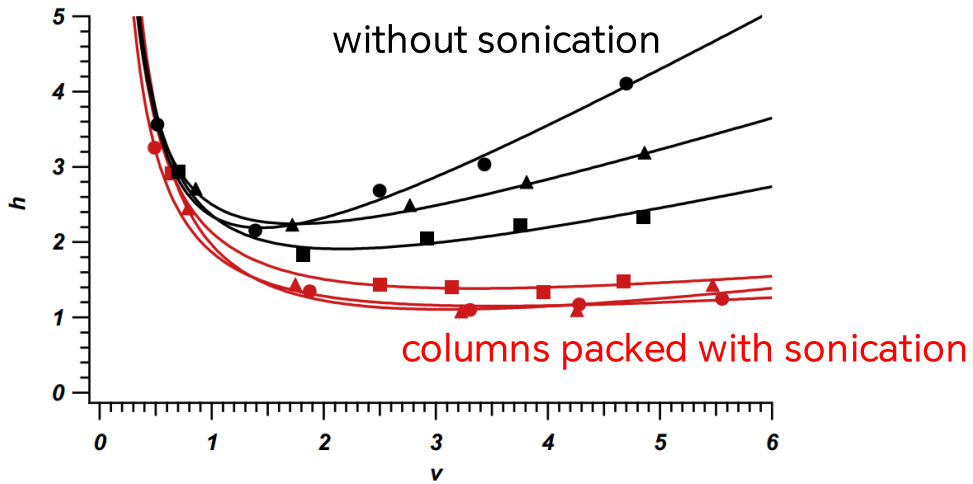
实际情况：随机填充

挑战：将颗粒填料的有序性转化为填充柱床的有序性

经典的高压匀浆填充技术



利用**磁力搅拌**来分散匀浆



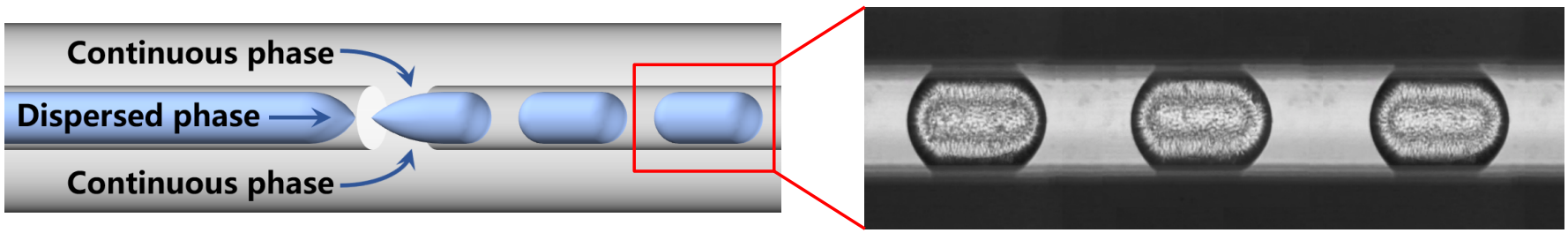
借助**超声处理**获得更好的分散性

维持填料分散难　　**柱间重现性差**　　**难以实现有序性的转化**

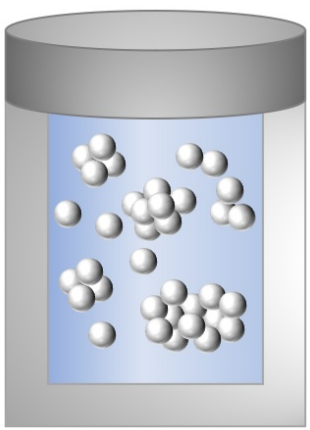
Armstrong et al., *Anal. Chem.*, **2017**, 89, 8177
Jorgenson et al., *J. Chromatogr. A*, **2016**, 1462, 165

基于微液滴的填柱技术

我们的工作：微液滴技术精准制造色谱柱床

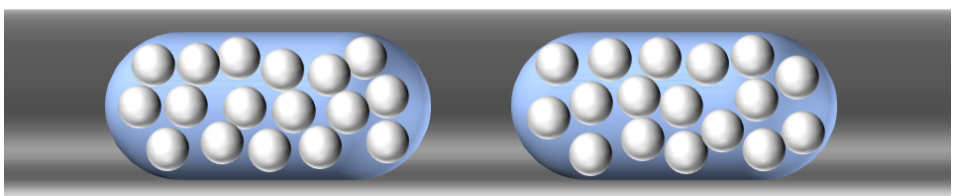


微液滴作为微纳尺度的匀浆罐



传统匀浆填充

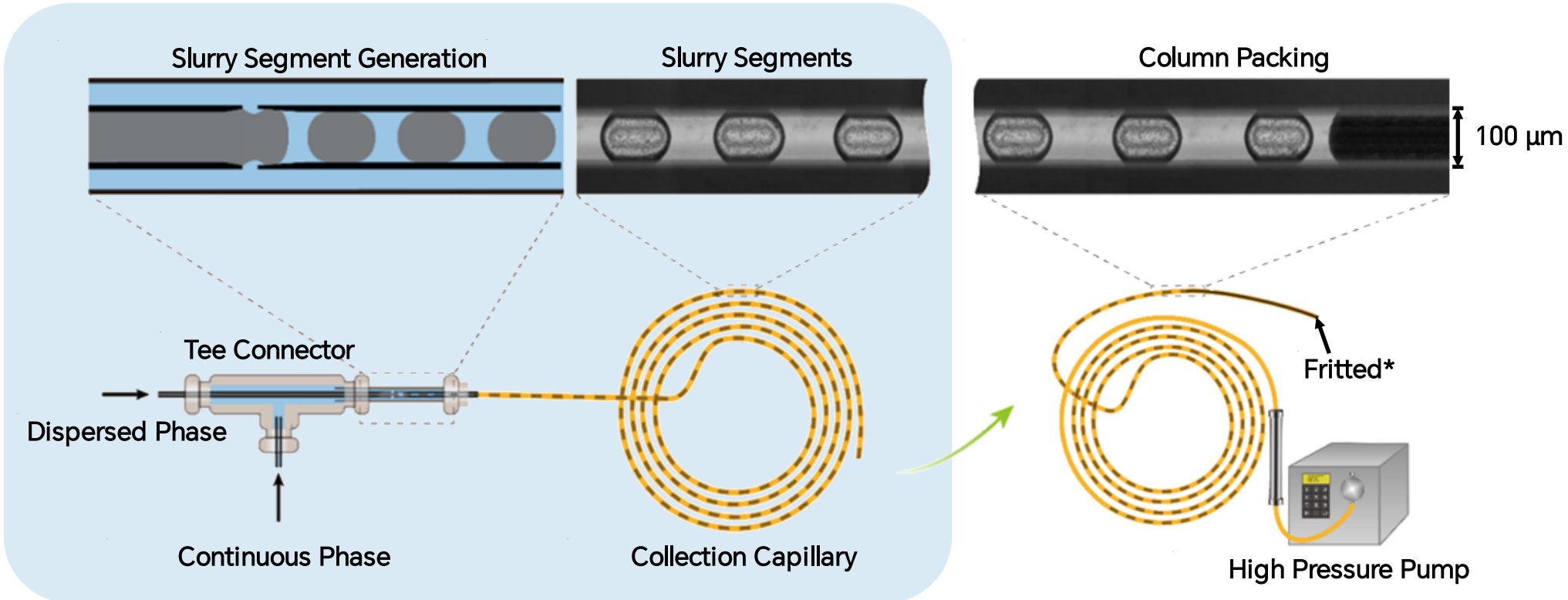
- 填料沉降和聚集问题



微液滴填充

- nL-pL级的匀浆罐
- 液滴内部对流可以维持匀浆分散
- 避免填料的聚集

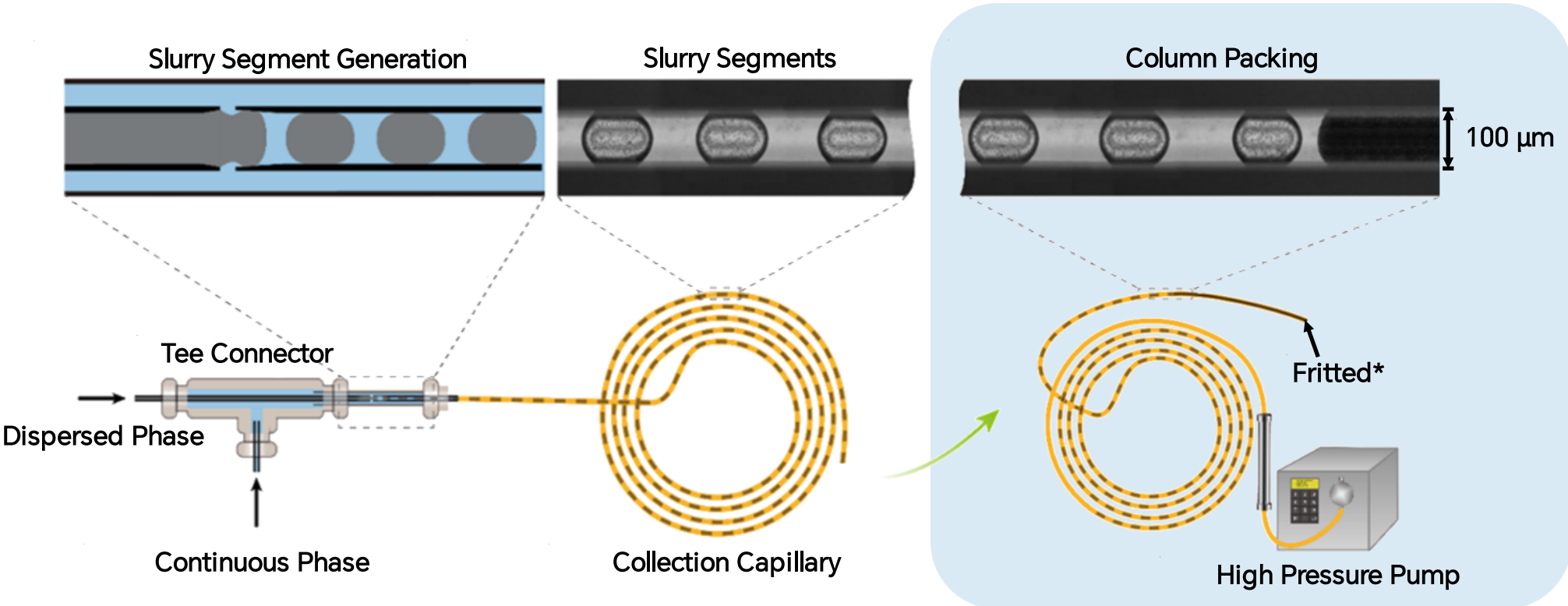
基于微液滴的填柱技术



Step 1：微液滴的生成与收集

Anal. Chem., **2021**, 93, 8450

基于微液滴的填柱技术



Step 2：色谱柱装填（*单颗粒塞）

* *J. Chromatogr. A*, **2021**, 1648, 462218
Anal. Chim. Acta, **2019**, 1062, 147
Anal. Chim. Acta, **2018**, 1033, 205

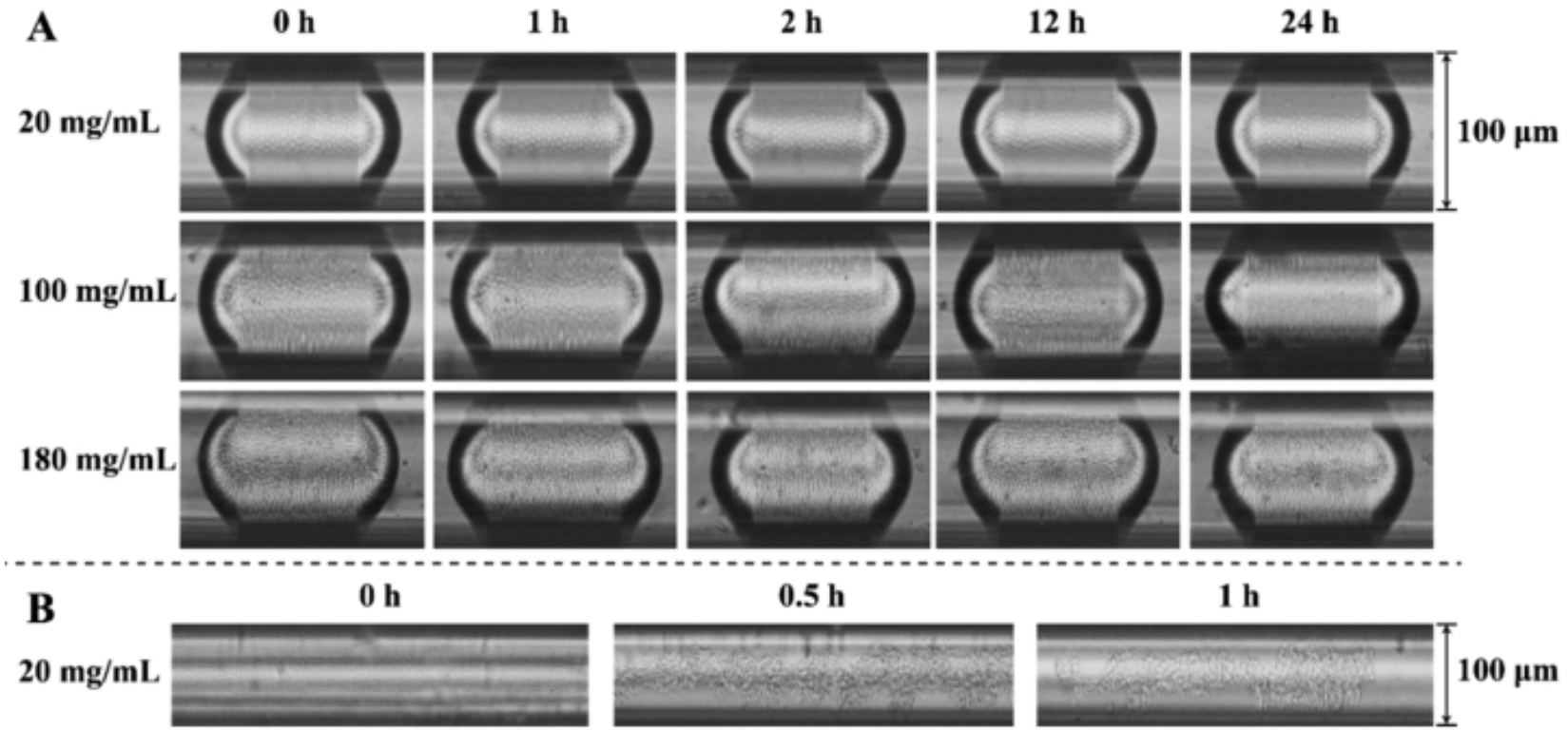
Anal. Chem., **2021**, 93, 8450

基于微液滴的填柱技术

匀浆稳定性表征

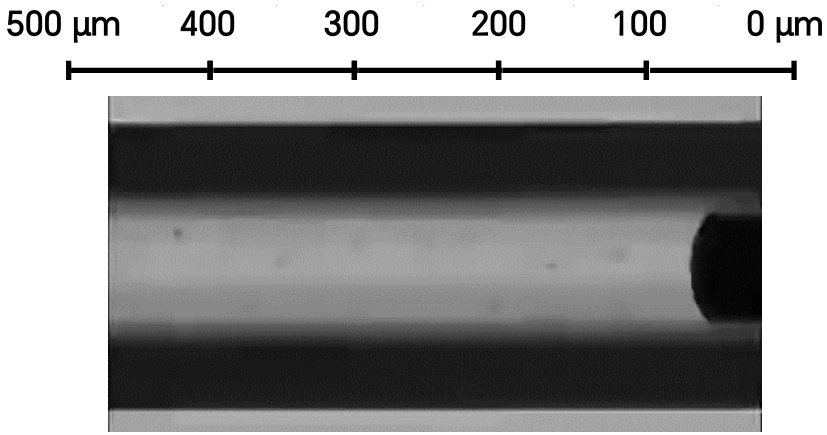
微液滴内的填料分散

传统匀浆法的填料分散

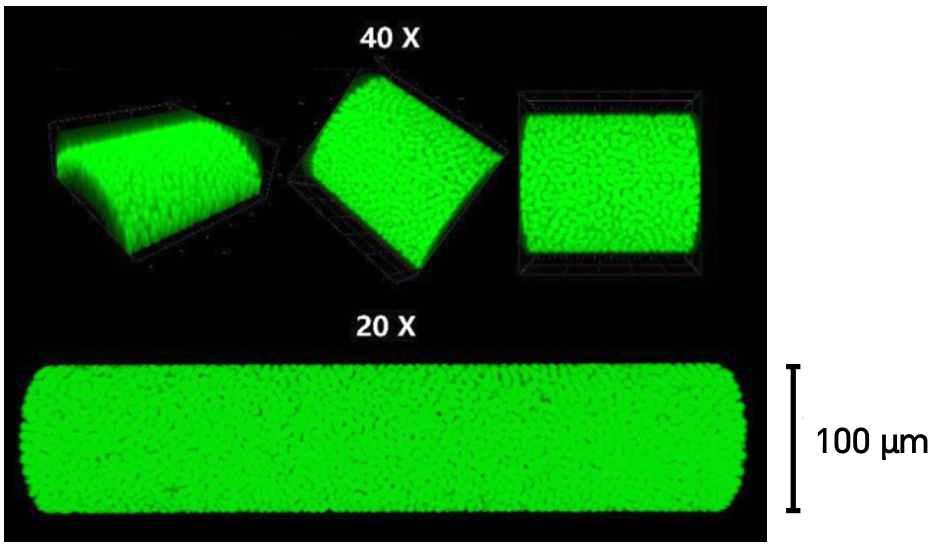


即使在180 mg/mL的高匀浆浓度下，微液滴内部的填料也能**长时间稳定分散**

柱床的填充和表征



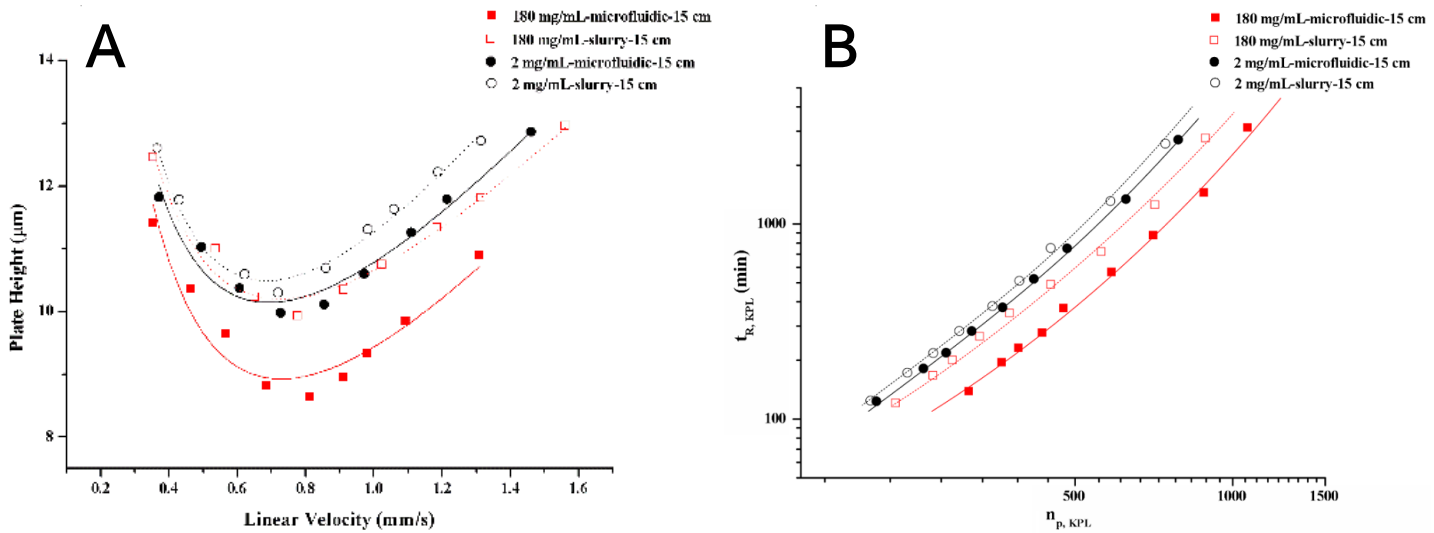
柱床形成（4倍延迟）



荧光重构柱床的三维形貌

- 以50 μm的分辨率逐层**精准组装**色谱柱床
- 在180 mg/mL的高匀浆浓度下未发现明显的空腔

色谱动力学评价

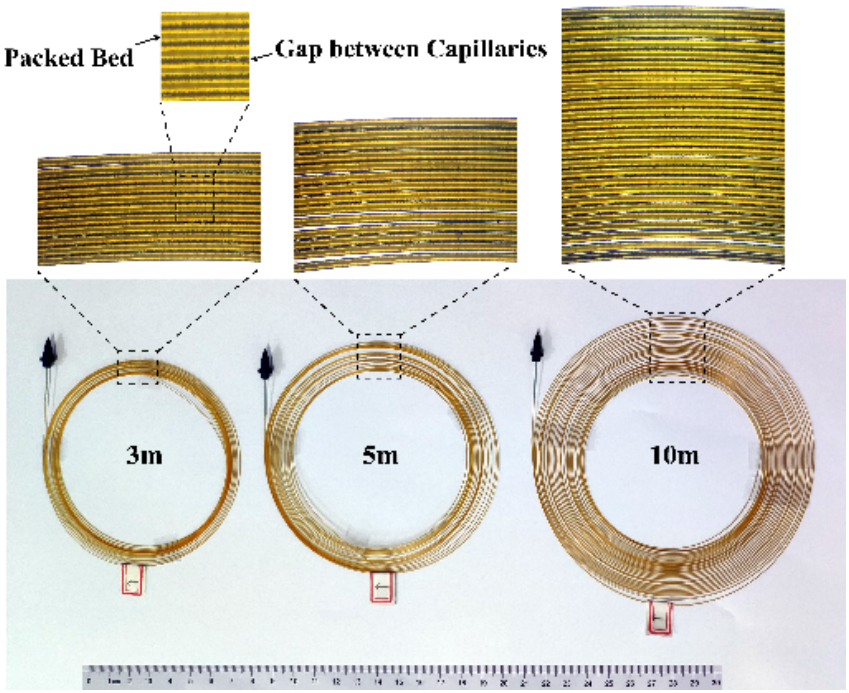


基于微液滴和传统匀浆填充毛细管柱的Van Deemter曲线（A）和Desmet曲线（B）

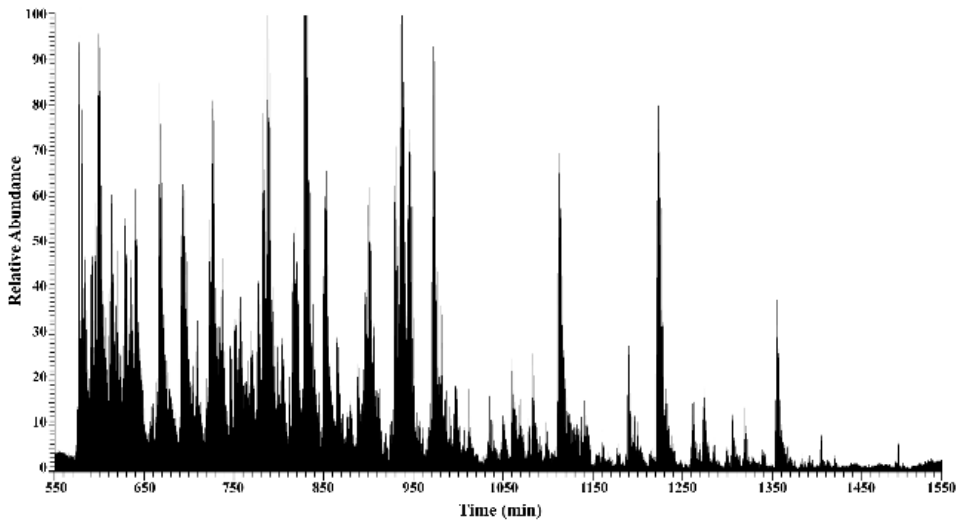
- 柱效高达116,000 plates/m（提升13%）
- 分离阻抗减小2800（改善40%）

基于微液滴的填柱技术

超长色谱柱制备



微液滴技术填充制备超长毛细管柱

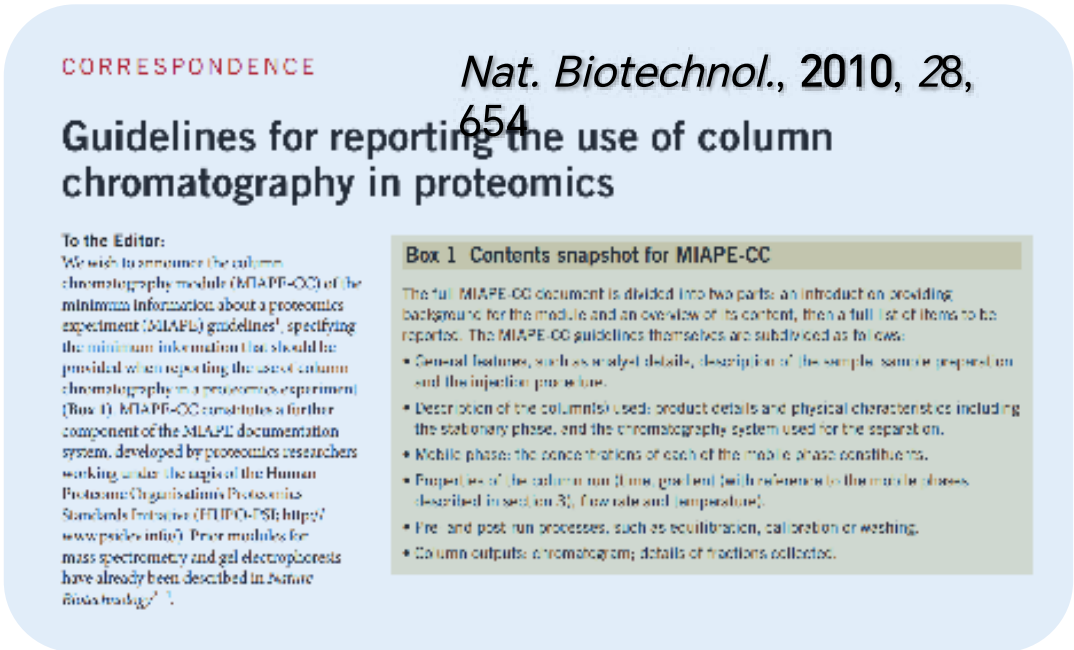


5 m超长色谱柱分离HeLa蛋白酶解物的色谱图

	蛋白鉴定数	多肽鉴定数
5 m超长色谱柱	3922	53752
10 cm色谱柱	2152	14892

工业蛋白质组学需要高通量的制柱技术

- In 2010, Nature Biotechnology published guidelines for column chromatography by Human Proteome Organization's Proteomics Standards Initiative, which highlighted the demand for high quality microcolumns towards the standardization of proteomic analysis.



CORRESPONDENCE

Nat. Biotechnol., **2010**, *28*, 654

Guidelines for reporting the use of column chromatography in proteomics

To the Editor:

We wish to announce the column chromatography module (MIAPE-CC) of the minimum information about a proteomics experiment (MIAPE) guidelines[1], specifying the minimum information that should be provided when reporting the use of column chromatography in a proteomics experiment (Box 1). MIAPE-CC constitutes a further component of the MIAPE documentation system, developed by proteomics researchers working under the aegis of the Human Proteome Organisation's Proteomics Standards Initiative (HUPO-PSI; http://www.psidev.info). Prior modules for mass spectrometry and gel electrophoresis have already been described in *Nature Biotechnology*[2,3].

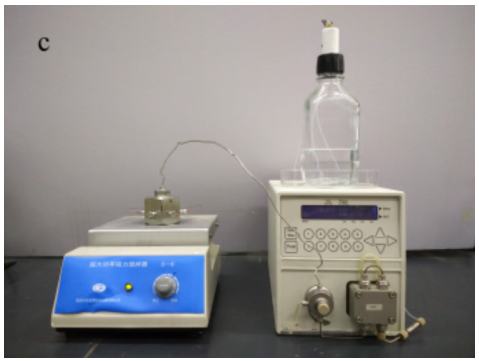
Box 1 Contents snapshot for MIAPE-CC

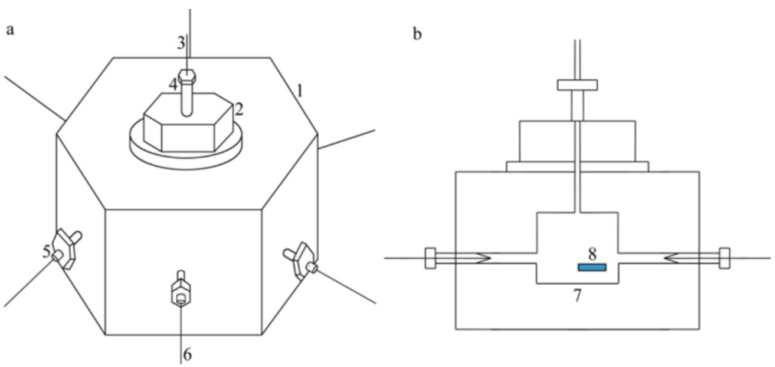
The full MIAPE-CC document is divided into two parts: an introduction providing background for the module and an overview of its content, then a full list of items to be reported. The MIAPE-CC guidelines themselves are subdivided as follows:

- General features, such as analyst details, description of the sample source preparation and the injection procedure.
- Description of the column(s) used: product details and physical characteristics including the stationary phase, and the chromatography system used for the separation.
- Mobile phase: the concentrations of each of the mobile phase constituents.
- Properties of the column run (time gradient [with reference to the mobile phases described in section 3], flow rate and temperature).
- Pre- and post-run processes, such as equilibration, calibration or washing.
- Column outputs: a chromatogram; details of fractions collected.

大规模制柱挑战：高速、高重现

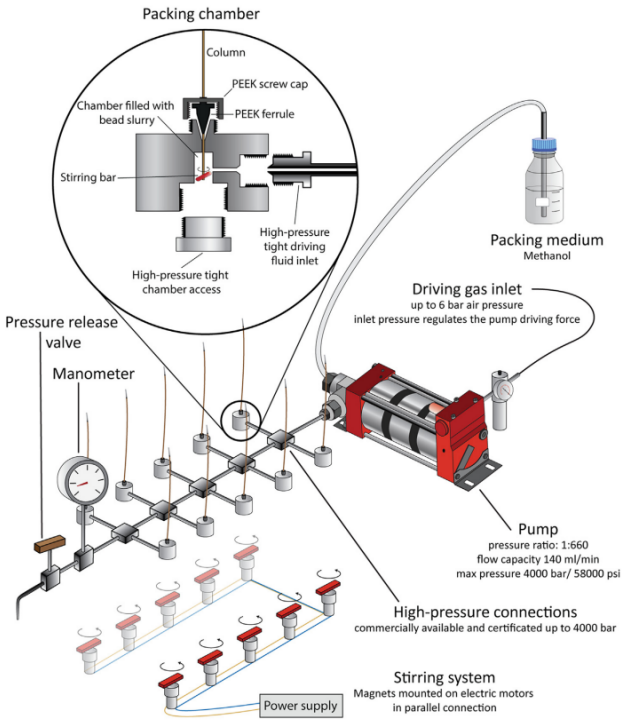
高通量制柱技术的发展





多通道填柱装置同时装填6根色谱柱

张养军等, *色谱*, **2015**, 33, 1155

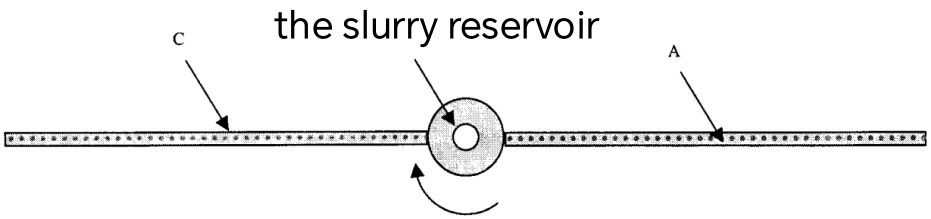


并联高压填柱装置同时装填10根色谱柱

Mann et al., *Mol. Cell. Proteomics*, **2021**, 20, 100082

基于离心填柱的高通量制造

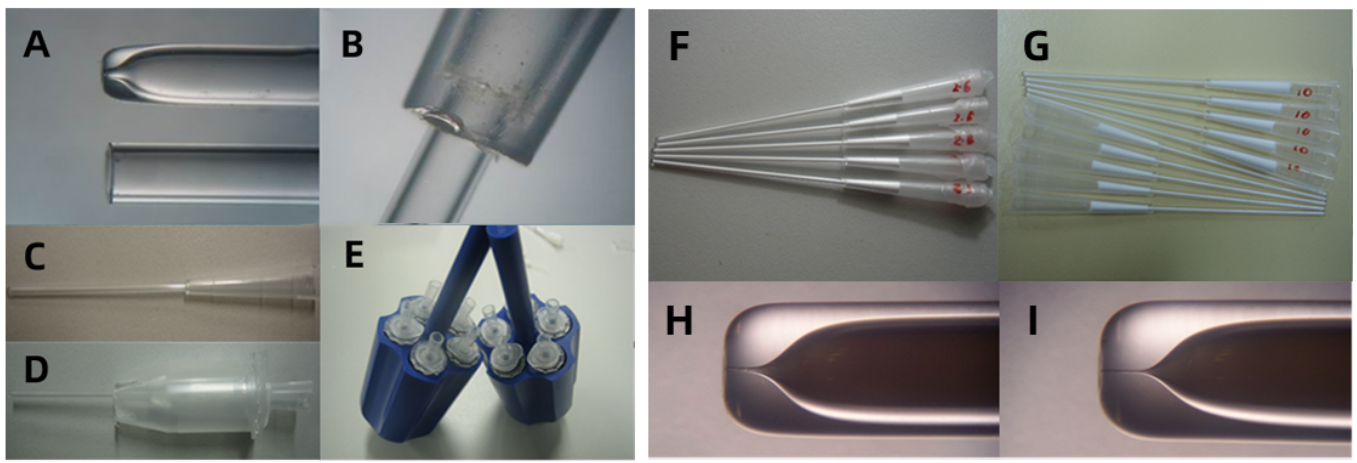
离心填柱的早期探索



用于毛细管电色谱的离心填充色谱柱

Colon et al., *J. Microcolumn Sep.*, **1998**, 10, 439
Electrophoresis, **1999**, 20, 2360
J. Chromatogr. A, **2000**, 887, 43

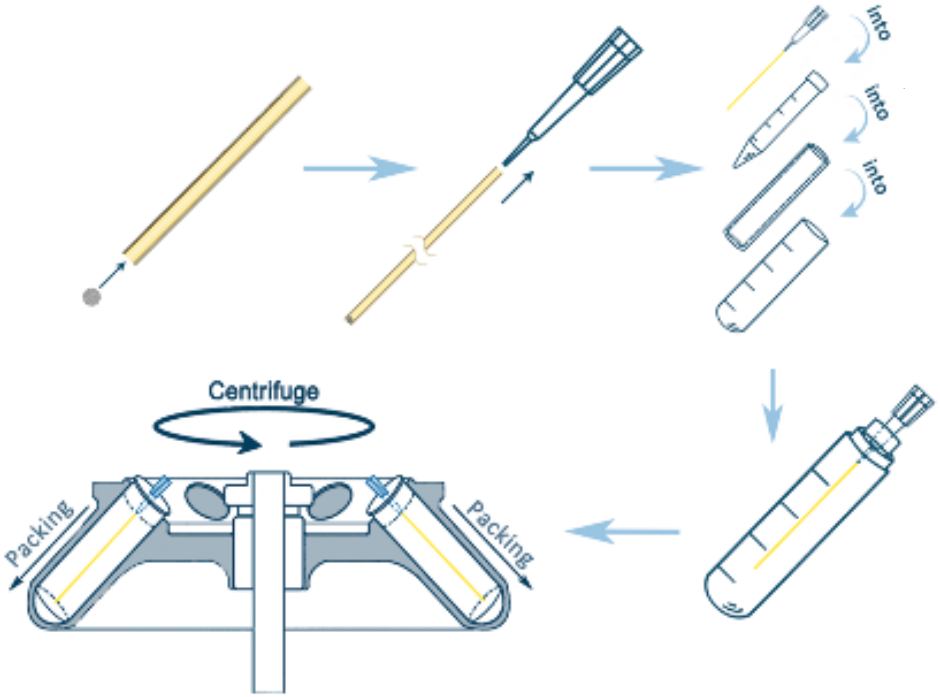
我们早期的工作：尝试利用离心技术高通量制备玻璃小柱



- **每3分钟装填10根色谱柱**

J. Sep. Sci., **2009**, 32, 1831

基于离心填柱的高通量制造



Step 1：微柱填充装置组装

微柱填充装置由实验室常见的配件组装而成

Step 2：色谱柱离心填充

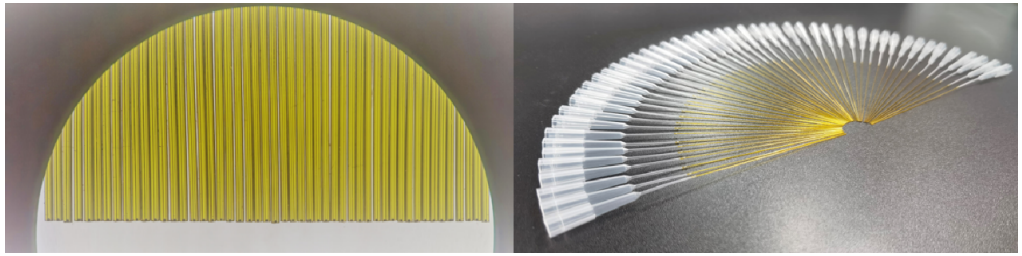
毛细管色谱柱可以在几分钟内填充完毕

- **离心填柱的技术指标**
  - ✔ 毛细管内径：150 μm, 100 μm, 75 μm, 50 μm, etc.
  - ✔ 颗粒尺寸：5 μm, 3 μm, sub-2 μm, etc.
  - ✔ 填料质地：SiO_2, TiO_2, ZrO_2, polymer, etc.

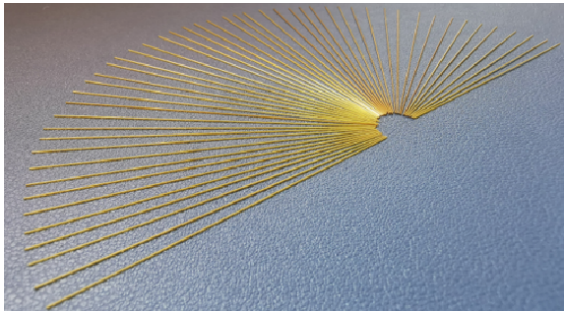
Anal. Chem., **2022**, 94,

基于离心填柱的高通量制造

微柱填充装置



填充完成的毛细管柱

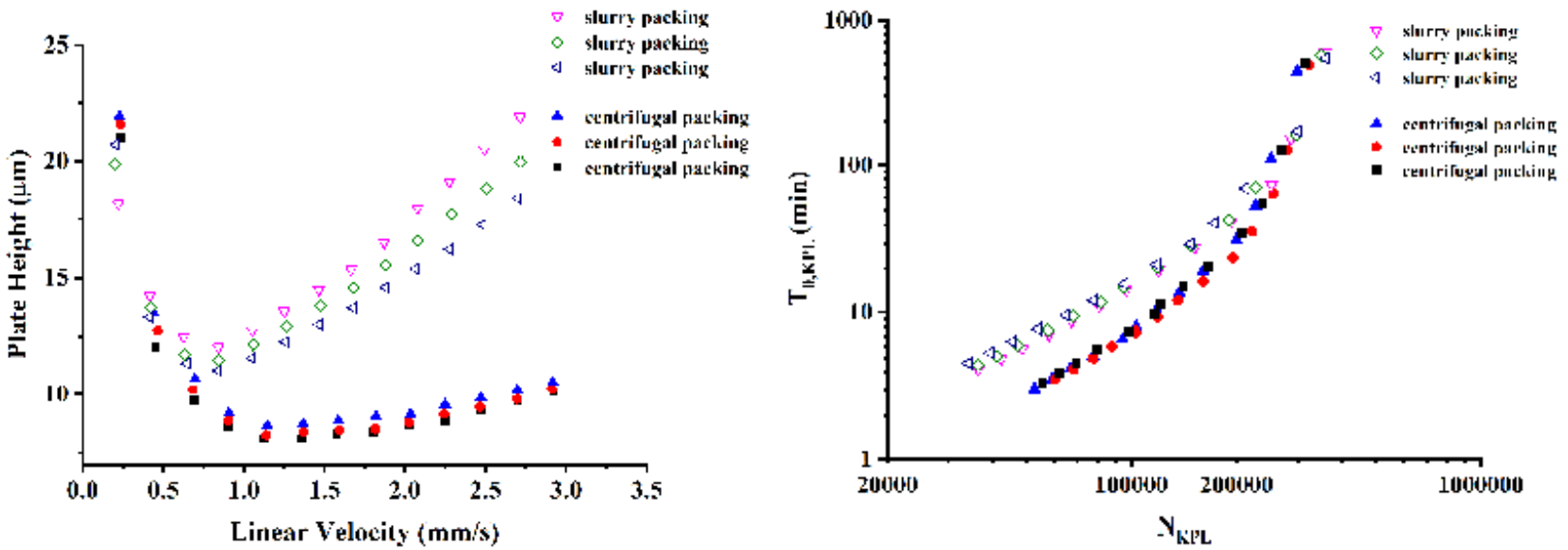


>200 columns/h

- 6×50 mL的小型台式离心机，可以轻松实现每天制备2800+根的毛细管柱
- 更大的离心机可以进一步提高制造通量

Anal. Chem., 2022, 94,

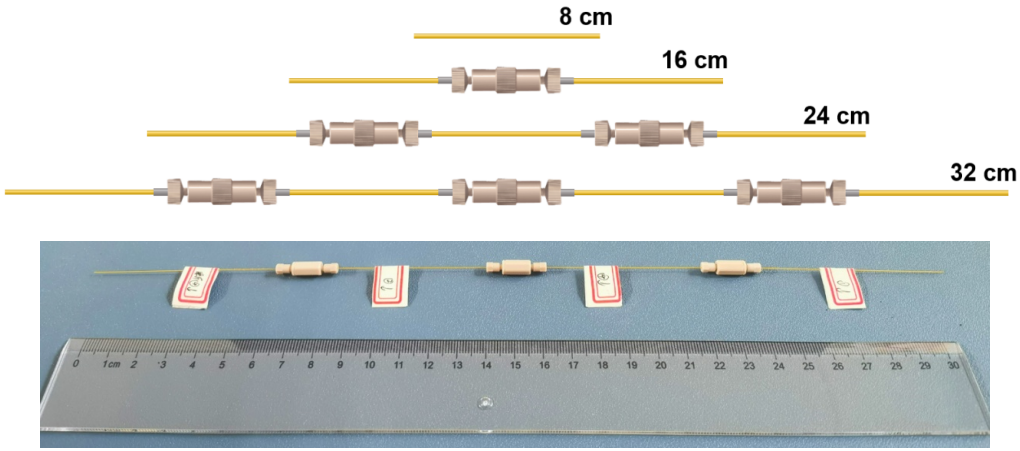
基于离心填柱的高通量制造



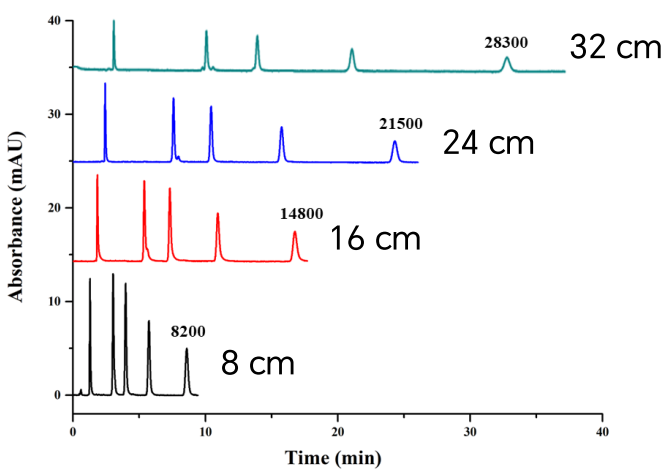
离心和传统匀浆填充毛细管柱的Van Deemter曲线和Desmet曲线

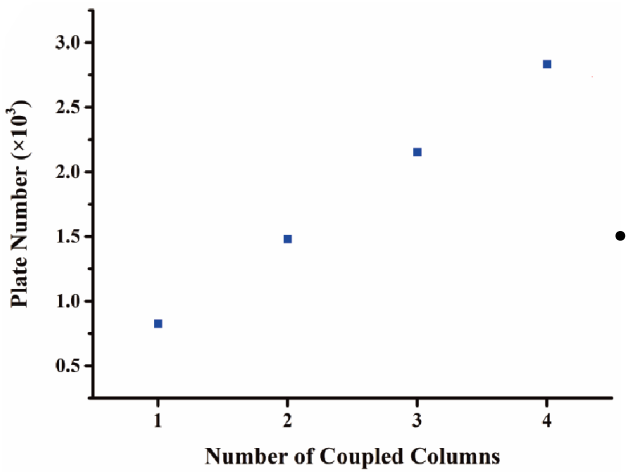
- 最低折合塔高度板 h_{min} = 1.6-1.7
- 动力学性能优于匀浆填充柱

基于离心填柱的高通量制造



- 离心机深度决定柱长度
- 零死体积接头串联多根毛细管柱组装出长柱

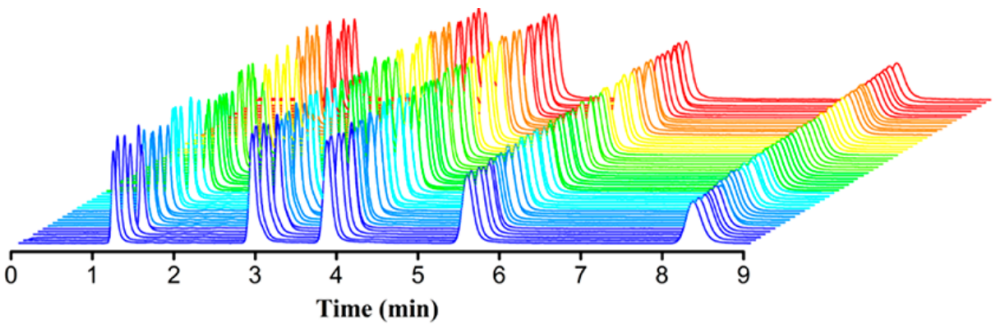




串联结构不会损耗柱性能

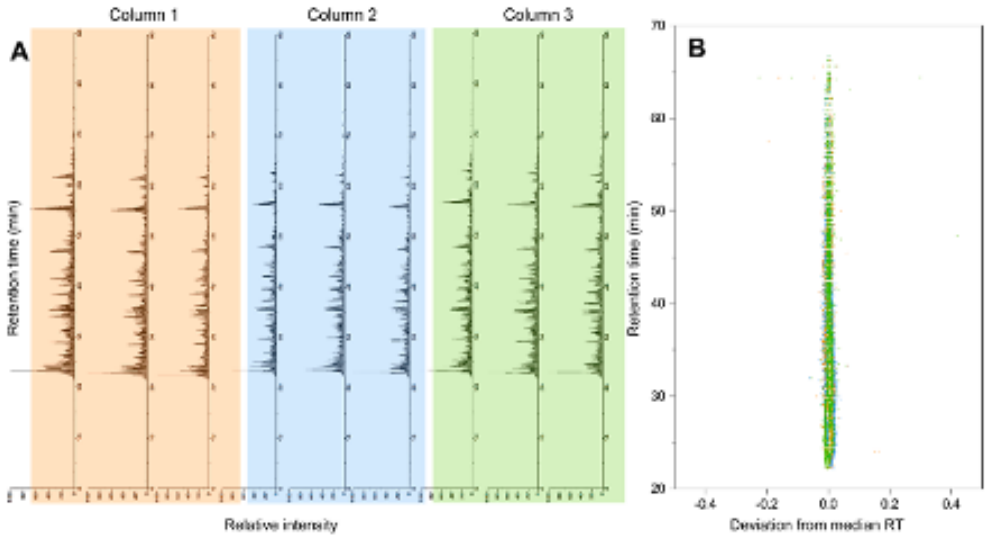
- 柱效与串联柱柱长具有良好的线性关系

离心填充柱具有出色的柱间重现性



50 根离心填充柱的苯系物分析谱图

	RSD (n=50)
保留时间	1.4-2.0%
峰面积	6.9-3.1%
柱效	5.1-3.1%



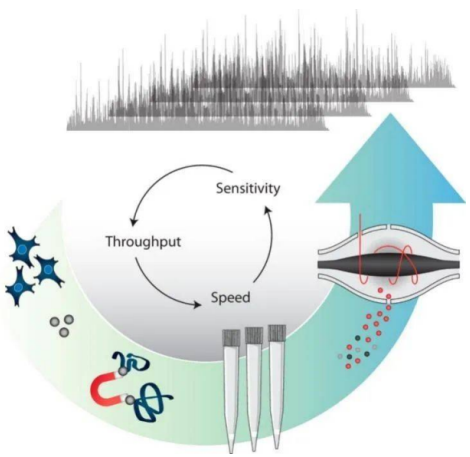
HeLa蛋白酶解物的次间/柱间重现性分析
(95.9%肽段的保留值偏差小于2%)

高重现性蛋白质组学分析

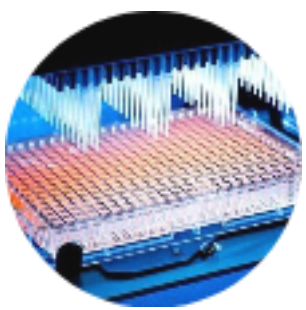
总结与展望

✔ 精准组装柱床　✔ 大规模制柱　✔ 高重现制柱

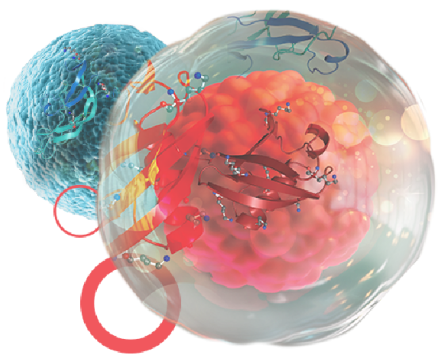
Supporting



工业蛋白质组学



大规模筛选分析



单细胞蛋白质组学

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