PolyLC INC.
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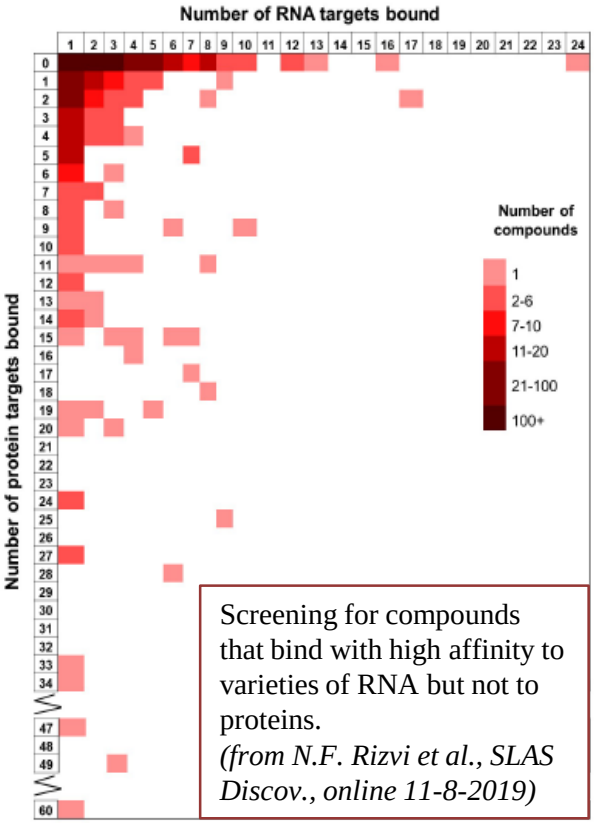
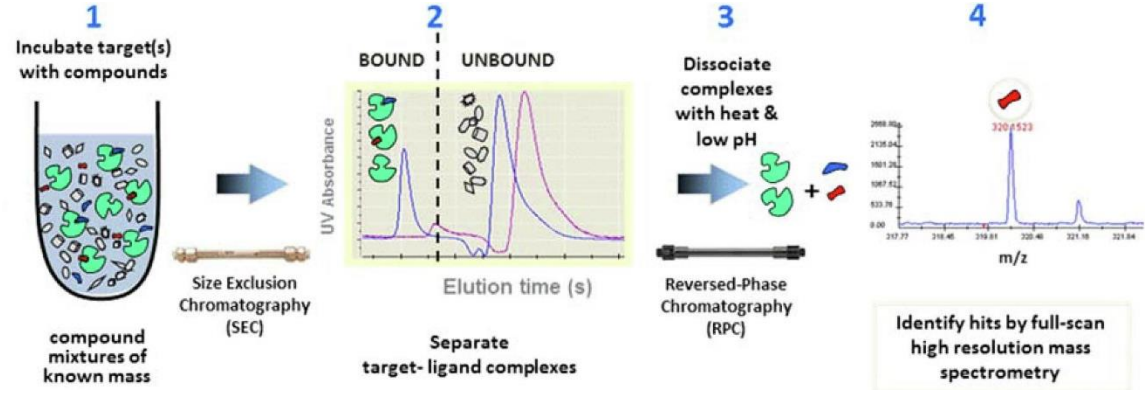
High-Throughput Screening (HTS)

For the past 19 years, pharmaceutical companies have successfully used our SEC columns to screen combinatorial libraries of up to 2,000 components per run to identify small molecules that bind with high affinity to a large target entity. No derivatization or special equipment is needed. The target can be a protein, a whole organelle such as a ribosome, a riboswitch or the folded DNA of a promoter region. Strongly bound molecules migrate through the SEC column with the target and elute in the Vo peak instead of in the Vt peak with the rest of the small molecules. The small molecules in the Vo peak are then identified and a new library is synthesized with features in common with the high-affinity subset. Several such iterations may produce a drug candidate with very high affinity (Kd < 100 nM) and selectivity.

The SEC must be completed in less than one minute or even high-affinity molecules will start to diffuse off the target. PolyLC's SEC columns can separate the Vo and Vt peaks to baseline under these conditions, which is essential for preventing false positives.



Screening for compounds that bind with high affinity to varieties of RNA but not to proteins.
(from N.F. Rizvi et al., SLAS Discov., online 11-8-2019)



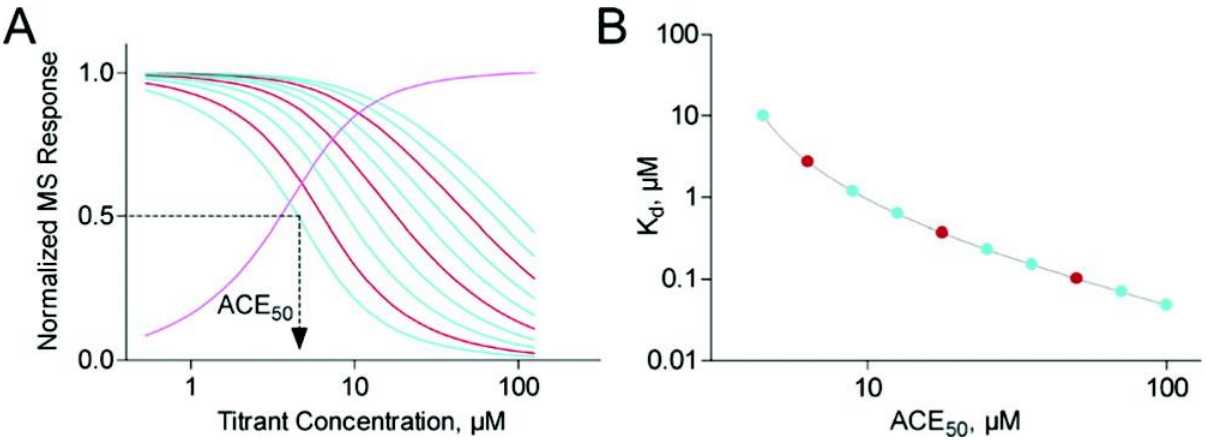
(from N.F. Rizvi and E.B. Nickbarg, Methods 167 (2019) 28)

This method is usually implemented with the following PolyHYDROXYETHYL A™ columns (all 5-µm, 60-Å):

ITEM#	SIZE	PRICE (US$)
051HY05006	50x1.0-mm	475.00
3.54HY05006	35x4.6 mm	400.00
052HY05006	50x2.1 mm	415.00 [***most popular***]

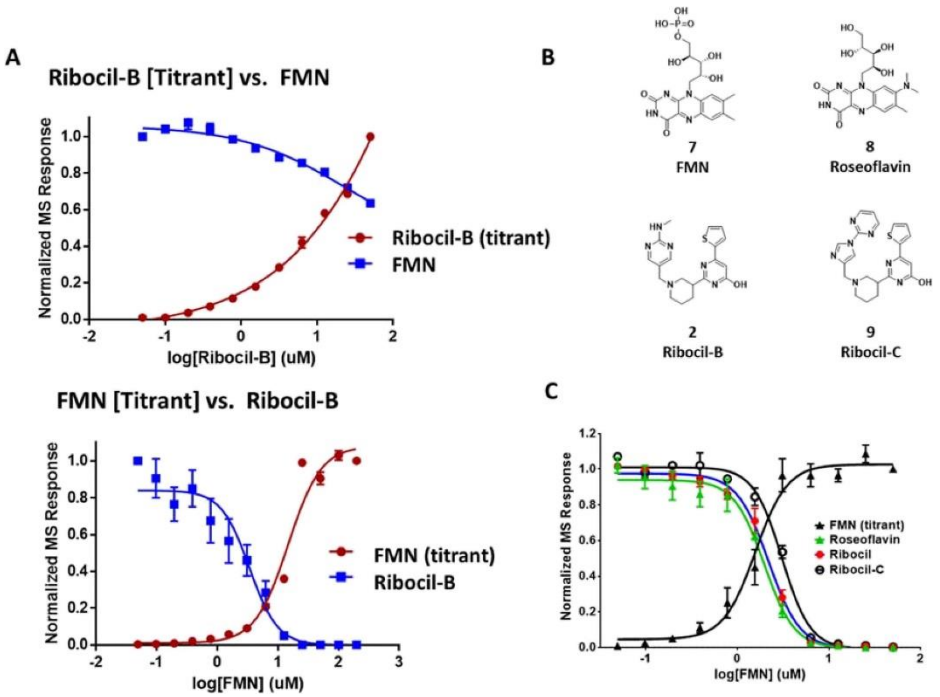
Also available: Other column dimensions, guard cartridges, and 3-µm material.
Contact us at: info@polylc.com

The SEC method can also be used to measure binding constants, effects of cofactors on binding, competitive and noncompetitive binding, and other ligand interactions with target proteins:



Affinity **C**ompetition **E**xperiments enable protein−ligand binding affinity measurements in compound mixtures. As simulated in (A), a library of compounds of varying affinity (blue) is embedded with calibrant ligands of known *K*d (red) and titrated with a compound of known *K*d (purple) to yield MS-measured ACE_{50} curves. (B) A calibration curve generated from the calibrants' ACE_{50} and *K*d values yields the other mixture components' *K*ds.

from: D.A. Annis et al., Anal. Chem. ***2007,*** *79, 4538-4542.*



Here, the affinity of FMN for the FMN riboswitch was compared to that of various compounds discovered in an ALIS (HTS) screen.

(from N.F. Rizvi and E.B. Nickbarg, Methods 167 (2019) 28)