Assaying Small Molecule Partitioning into Biomolecular Condensates

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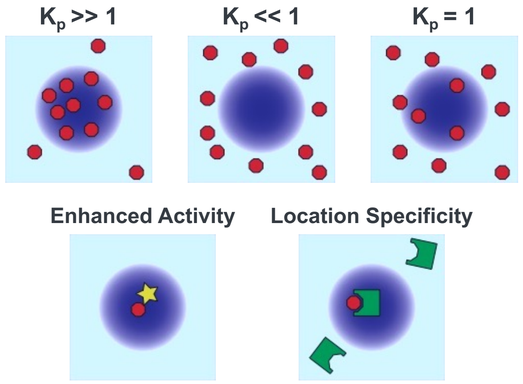
Introduction

The cellular milieu contains a dense, diverse complement of proteins, nucleic acids, and small molecules. Biomolecular condensates are structures that form by the phase separation of a subset of these molecules from the surrounding cellular solution. Condensates can form and dissolve dynamically and play essential roles in many cellular processes. Misregulation of condensates can drive multiple diseases. Therefore, finding drugs that can restore condensate homeostasis represents a new treatment frontier. We call these drugs condensate modifying drugs (c-mods).

We introduce (1) a method to measure drug partitioning into *in vitro* reconstituted condensates, and (2) a high-throughput screen (HTS) pipeline to identify small molecules that modulate FUS-containing stress granules in cultured cells. Together, these two approaches can be used to identify and optimize c-mod activity within the target condensate.

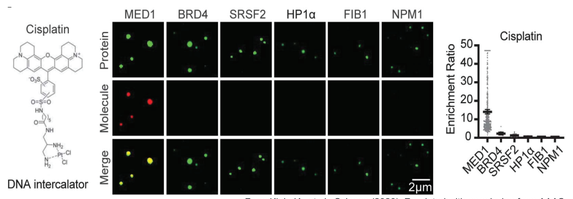
Background

- Biomolecular condensates can concentrate, exclude, or have no impact on small molecules. Each scenario has important implications for drug design.
- Condensate environment may affect activity
- Small molecule partitioning (Kp) can localize the effect to a condensate.



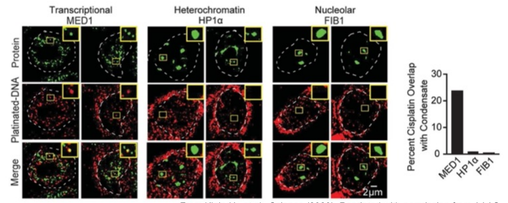
Small Molecule Partitioning

- Antineoplastic drugs can partition into transcriptional condensates (Klein IA, et al., *Science*, 2020).
- Cisplatin partitions only with Med1 condensates.



From Klein IA, et al., Science (2020). Reprinted with permission from AAAS.

- Cisplatin partitioning results in DNA associated with Med1 transcriptional condensates to be preferentially platinated.



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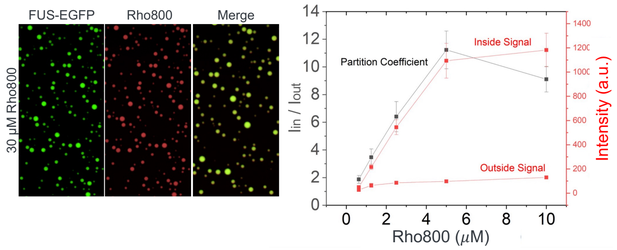
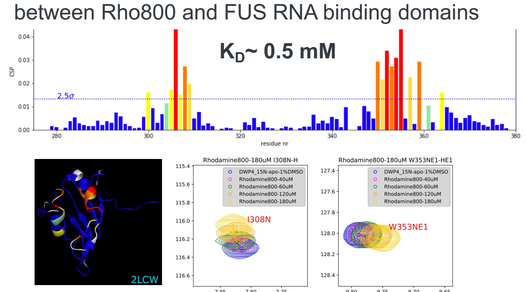
Weak Interactions are Sufficient to Drive Small Molecule Partitioning

- Rhodamine dyes partition into FUS condensates in vitro.

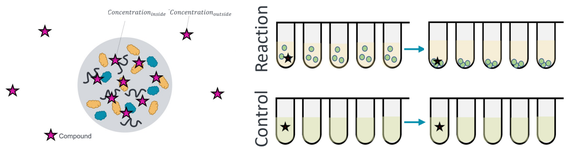


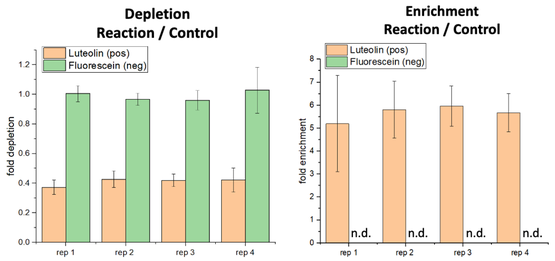
- NMR measurements reveal low affinity interactions between Rho800 and FUS RNA binding domains



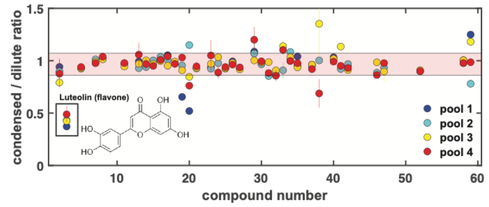
Label-free Quantification of Small Molecule Partitioning

- Mass spectrometry can quantify depletion or enrichment of small molecules in the dense phase





- Mass spectrometry depletion can be scaled for HTS

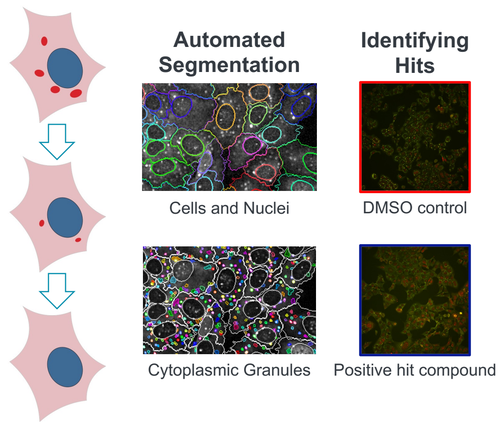


Acknowledgements

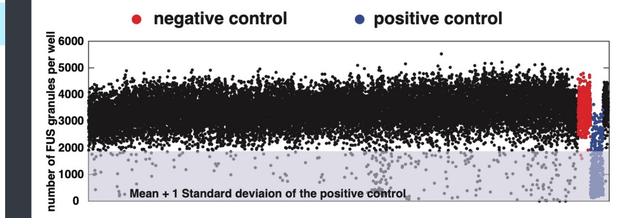
ZoBio (https://zobio.com) - NMR experiments
Nuvisan (https://www.nuvisan.com) - Mass spectrometry

High-Throughput Screening

- U2OS cells expressing fluorescently-labeled FUS were treated with small molecules (20 k library) and stressed with arsenite to induce stress granule formation
- High content-imaging was used to identify changes in stress granule morphology.
- 1.5% of compounds had stress granules reduced to levels commensurate with the positive control.



- Quantification of compound-treated wells reveals many compounds that reduce the number of FUS-containing stress granules



Conclusions

- Phenotypic HTS in cultured cells is an efficient approach to identify c-mods
- Partitioning can impact activity of drug molecules
- Incorporating partitioning optimization can improve efficacy by delivering the c-mod to the target condensate.