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Plate-based Chemistry/Direct-to-Biology for Accelerated Drug Discovery

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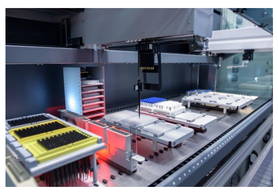
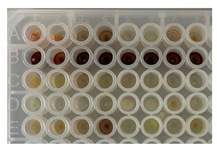
Background & Introduction

The optimization of molecules in drug discovery is commonly a tedious and expensive process. Implementing automation and miniaturization coupled with direct application in biological assays is an attractive solution to accelerate synthesis, safe expensive reagents, and bypass the need of extensive single compound purification and handling. This innovative approach is frequently known as plate-based chemistry or direct-to-biology.[1,2] Besides classical small molecules, this principle has also been applied to new modalities, such as PROTACs.[3]

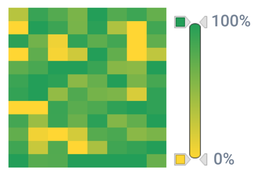
Here, we present a proof-of-principle case study that combines automated reaction miniaturization of amide couplings followed by direct biological readouts of unpurified reaction mixtures. We demonstrate the compatibility of crude reaction mixtures with a fluorescence resonance energy transfer assay (FRET) and surface plasmon resonance spectroscopy (SPR). In addition, we show the automation and miniaturization of SNAr reactions for the hit expansion of fragment screening hits of a membrane protein in combination with ASMS.



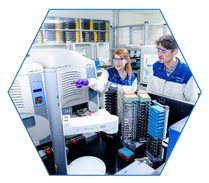
Automated synthesis



Reactions in microtiter plates
<1 mg material per well



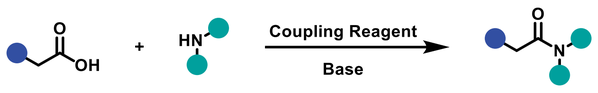
Heat map after LCMS analysis
→ cherry picking for assays



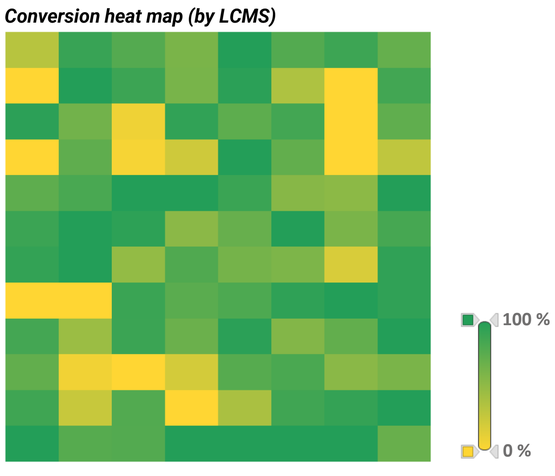
Assays for readout:
- ASMS
- SPR
- FRET

Case Study 1: Proof-of-Principle Study using Amide Coupling as Model Reaction

1. Chemistry

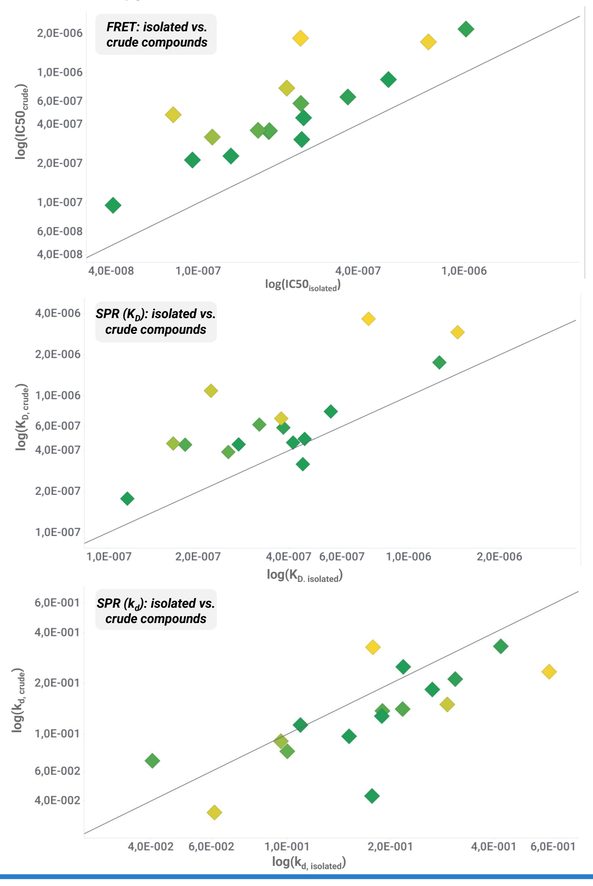


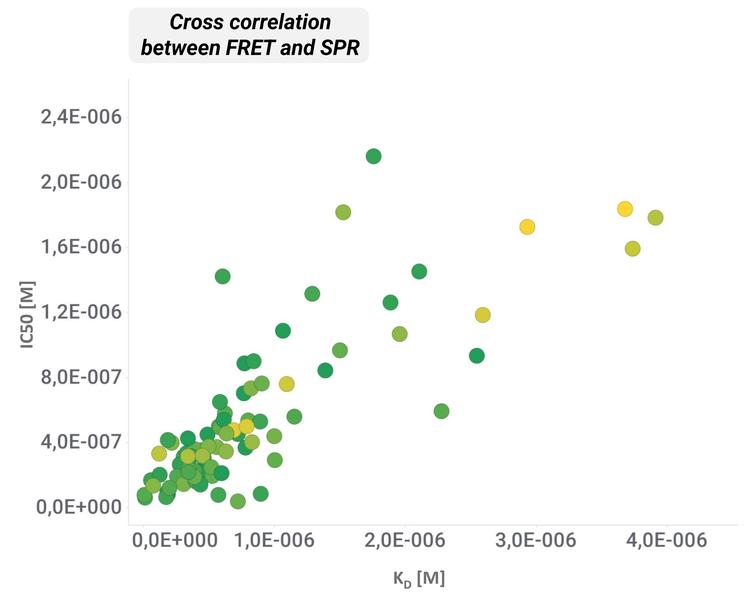
Conversion heat map (by LCMS)



- Amide coupling as model reaction: acid coupled with 96 diverse amines. Building blocks were selected by enumeration of the properties of products.
- Reactions were set-up by a Tecan liquid handling robot <1 mg per reaction. Conversion was determined by LCMS analysis.
- 91% of reactions show conversion to product and were further analyzed in FRET as well as SPR assays.

2. Biology

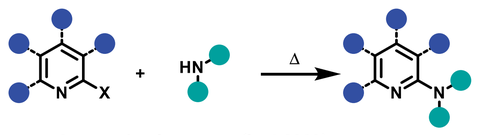




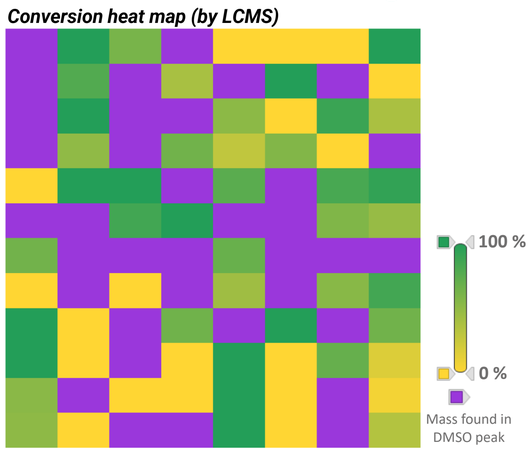
- 16 representative products were isolated as references based on potency and yield of automated synthesis. Assay data for isolated and crude products were collected.
  → Correlation for IC_{50}, K_D and k_d
- Cross correlation between FRET and SPR data for all crude mixtures
  → Biochemical and biophysical assay tolerate crude mixtures for accelerated first SAR generation

Case Study 2: Hit Expansion after Fragment Screening by ASMS

1. Chemistry

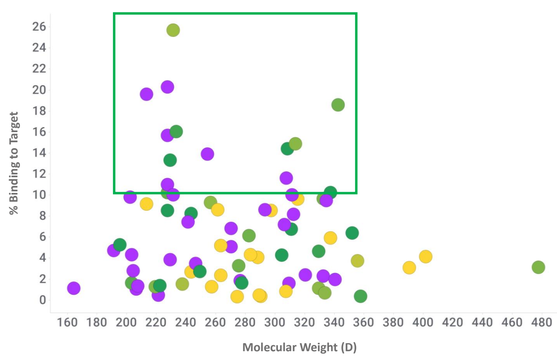


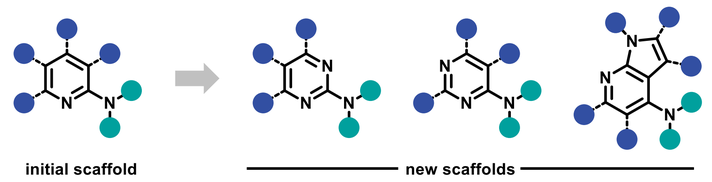
Conversion heat map (by LCMS)



- Aminopyridine cluster was found by ASMS fragment screening
  → Easy access by S_NAr chemistry
- 96 heterocyclic building blocks suitable for functionalization by S_NAr selected by enumeration of the properties of products
- 80% of reactions show conversion to product. Conversion was determined by LCMS analysis.
- Compounds were pooled and further analyzed by ASMS

2. Biology

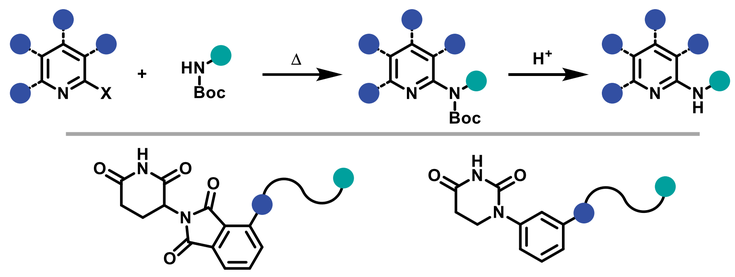




- ASMS gives several improved hits - i.e. high % binding to target
- Diverse building block selection allows fast growing and early scaffold hops to accelerate fragment optimization
- Second optimization round for variation of amines is currently in progress

Conclusions and Outlook

- We have shown a proof-of-principle study and show tolerance of crude mixtures with a FRET assay, SPR and ASMS
- FRET and SPR data of representative isolated compounds and crude mixtures correlate
- Two step procedures (i.e. reaction + Boc deprotection) are under investigation[4]
- A PROTAC library with E3 ligases connected to different linkers is in preparation[3]



References

[1] N. J. Gesmundo, B. Sauvagnat, P. J. Curran, M. P. Richards, C. L. Andrews, P. J. Dandliker, T. Cernak, *Nature* **2018**, *557*, 228.
[2] S. Kitamura, Q. Zheng, J. L. Woehl, A. Solania, E. Chen, N. Dillon, M. V. Hull, M. Kotaniguchi, J. R. Cappiello, S. Kitamura, V. Nizet, K. B. Sharpless, D. W. Wolan, *J. Am. Chem. Soc.* **2020**, *142*, 10899.
[3] C. E. Hendrick, J. R. Jorgensen, C. Chaudhry, I. I. Strambeanu, J.-F. Brazeau, J. Schiffer, Z. Shi, J. D. Venable, S. E. Wolkenberg, *ACS Med. Chem. Lett.* **2022**, *13*, 1182.
[4] N. Gesmundo, K. Dykstra, J. L. Douthwaite, Y.-T. Kao, R. Zhao, B. Mahjour, R. Ferguson, S. Dreher, B. Sauvagnat, J. Saurí, T. Cernak, *Nat. Synth.* **2023**, *2*, 1082.

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