INVENTING FOR LIFE

Enabling Technologies for Organic Chemistry (ETOC) Symposium

February 24-25, 2022

@AlexandraCSun

Vision-Guided, High-Throughput Liquid-Liquid Extraction Screening

presented by

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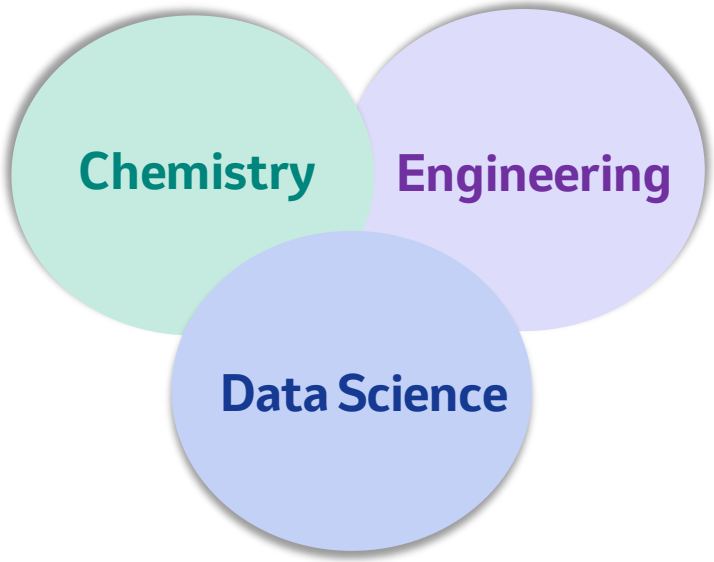

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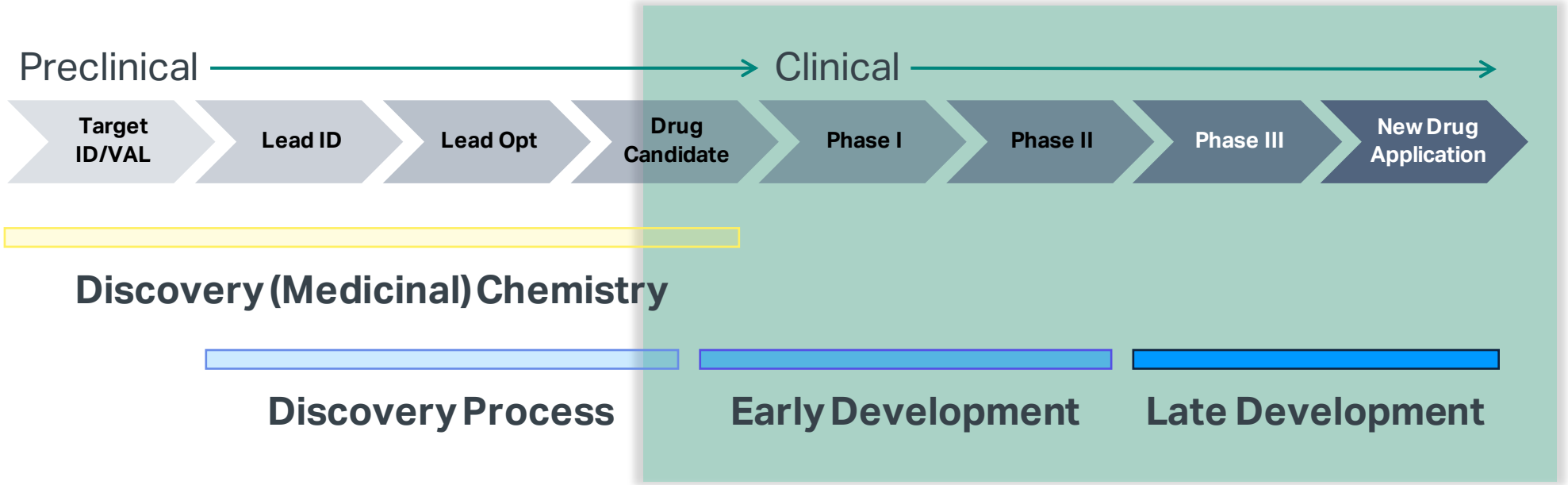
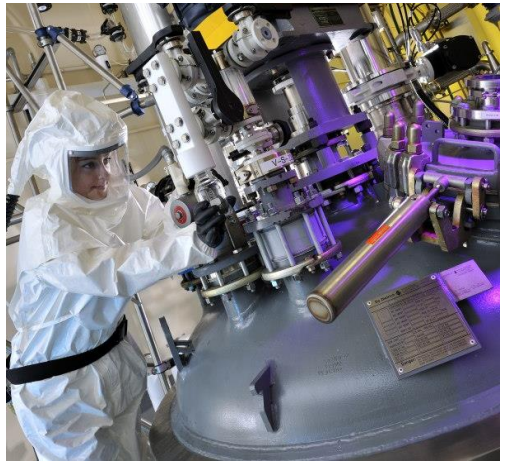
Leveraging DRE for small molecule process development



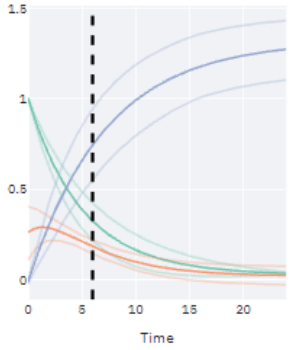


Leveraging DRE for small molecule process development

Reaction

High-Throughput Reaction Screening

Process Analytical Technologies

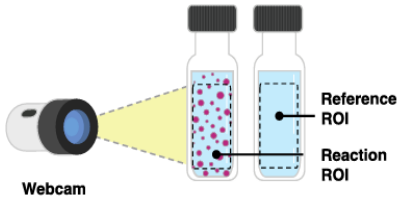


Predictive Process Understanding

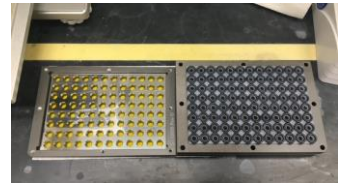
Workup

?

Crystallization



Automated Solubility Screening

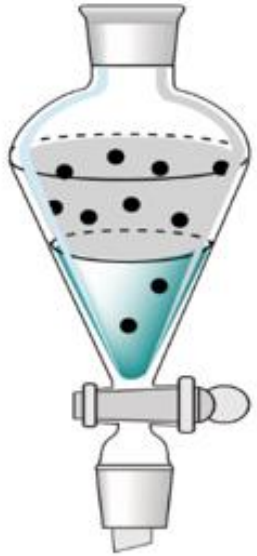


High-Throughput Powder X-ray Diffraction

How can we use DRE to develop more robust and sustainable workup processes?

iScience, **2021**, *24*, 3.

Liquid-liquid extraction (LLE) optimization



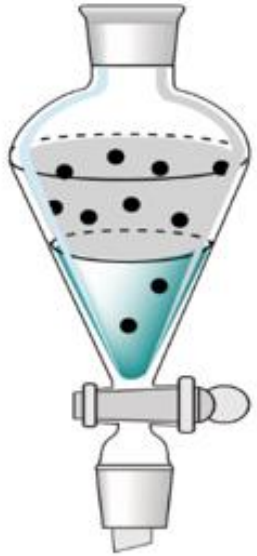
Benefits of Liquid-Liquid Extractions:

1. Isolation of API from hydrophilic impurities
2. Robust to changes to reaction conditions
3. Scale-up is thermodynamically controlled and not equipment or scale-dependent

Liquid-liquid extraction (LLE) optimization

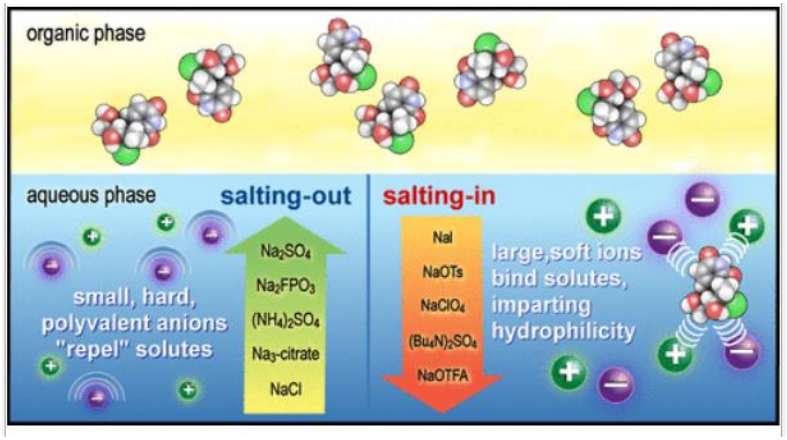
Screening Parameters (Input)

- ✓ Organic Solvents
- ✓ Organic/Aqueous Phase Ratios
- ✓ Temperature, pH
- ✓ Salts and Additives



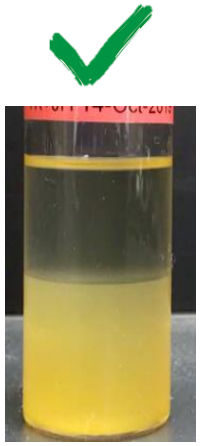
Analysis Parameters (Output)

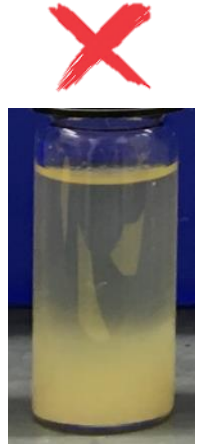
- ✓ Distribution coefficient
- ✓ **Interface quality**
- ✓ **Phase ratio**



Salting-out strategies informed by the Hofmeister series

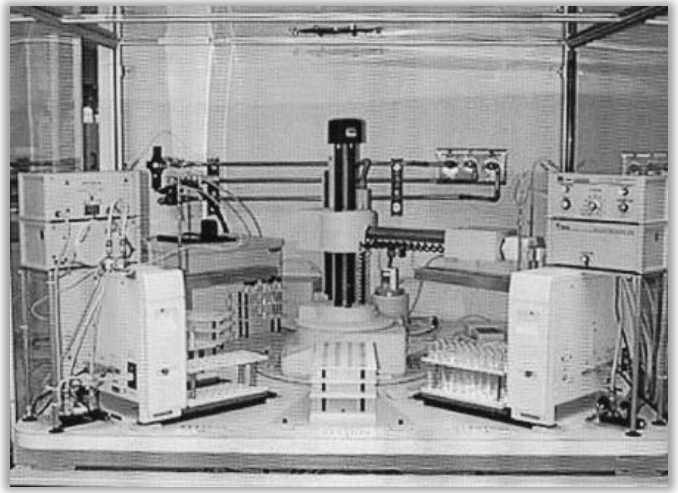
Org. Process Res. Dev. **2017**, *21*, 1355-1370





HTE platforms for LLE

LLE Robot (Abbot, 2000)



Interface detection using refractometer flow cell

80 samples per screen (15 mL)

High-Throughput LLE (BMS, 2016)

Visual analysis performed manually using visualization plate

24 samples per screen (2-4 mL)

Automated LLE Screening (GSK, 2021)

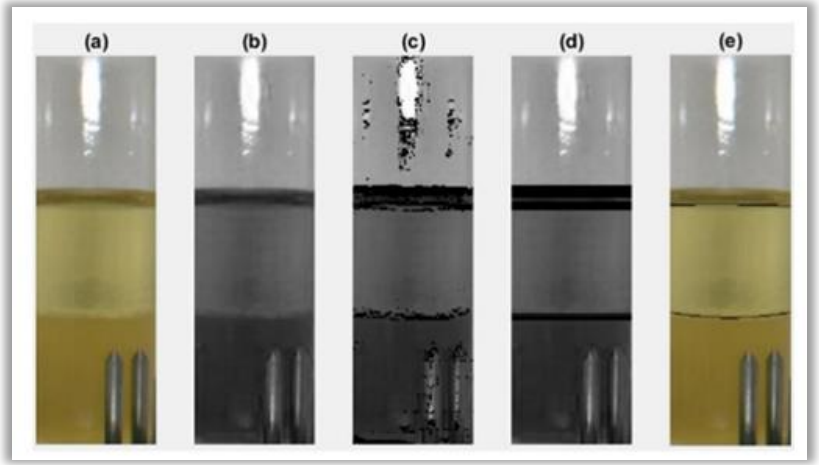


Image analysis algorithm enables automated visual analysis

24 samples per screen (2-4 mL)

JAMMC, **2000**, *22*, 187-194.; *Org. Process. Res. Dev.* **2016**, *20*, 1728-1737.; *Org. Process. Res. Dev.* **2021**, *25*, 2738-2746.

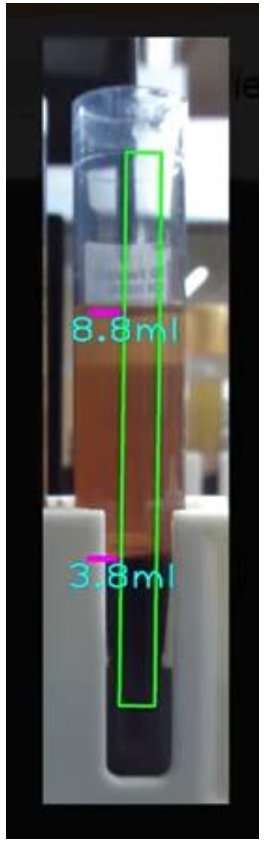
How can we increase screening throughput?

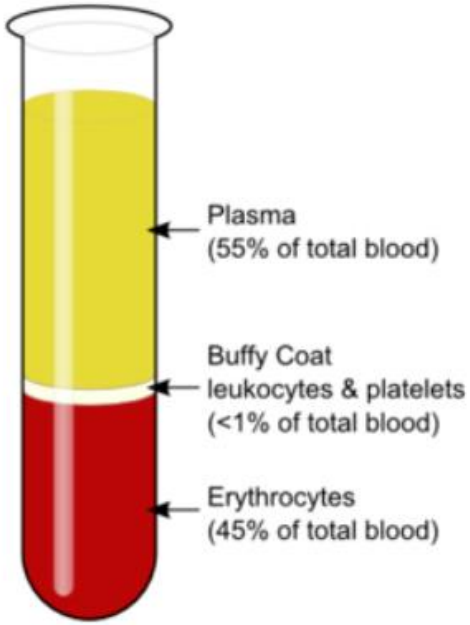
<u>This work:</u>
Automated LLE Screening using the Tecan Platform

Automated image analysis using TubeEyeX camera

96 samples per screen (0.5 – 1 mL)

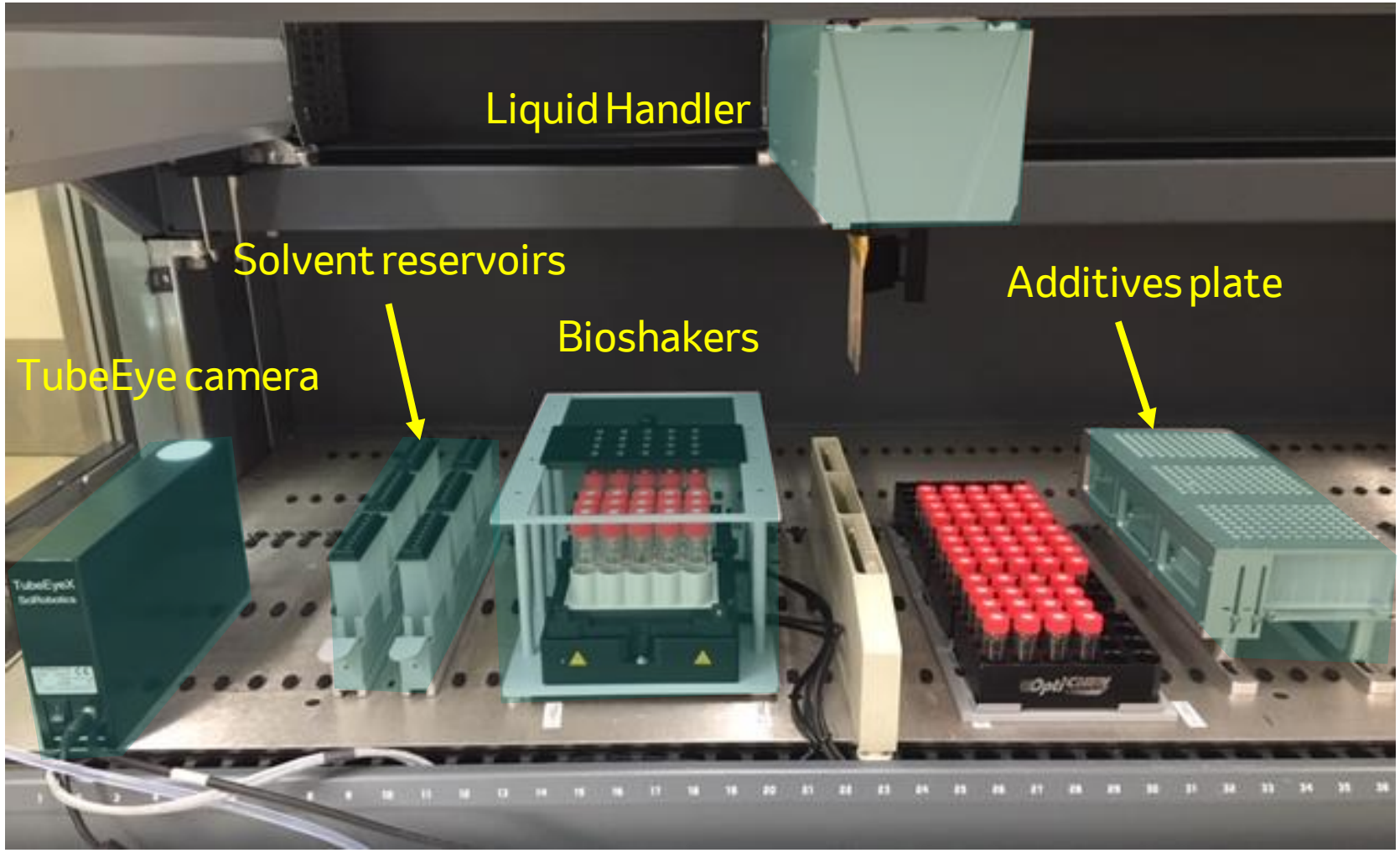
TubeEyeX camera:
Automated buffy coat extractions





Automated LLE screening workflow

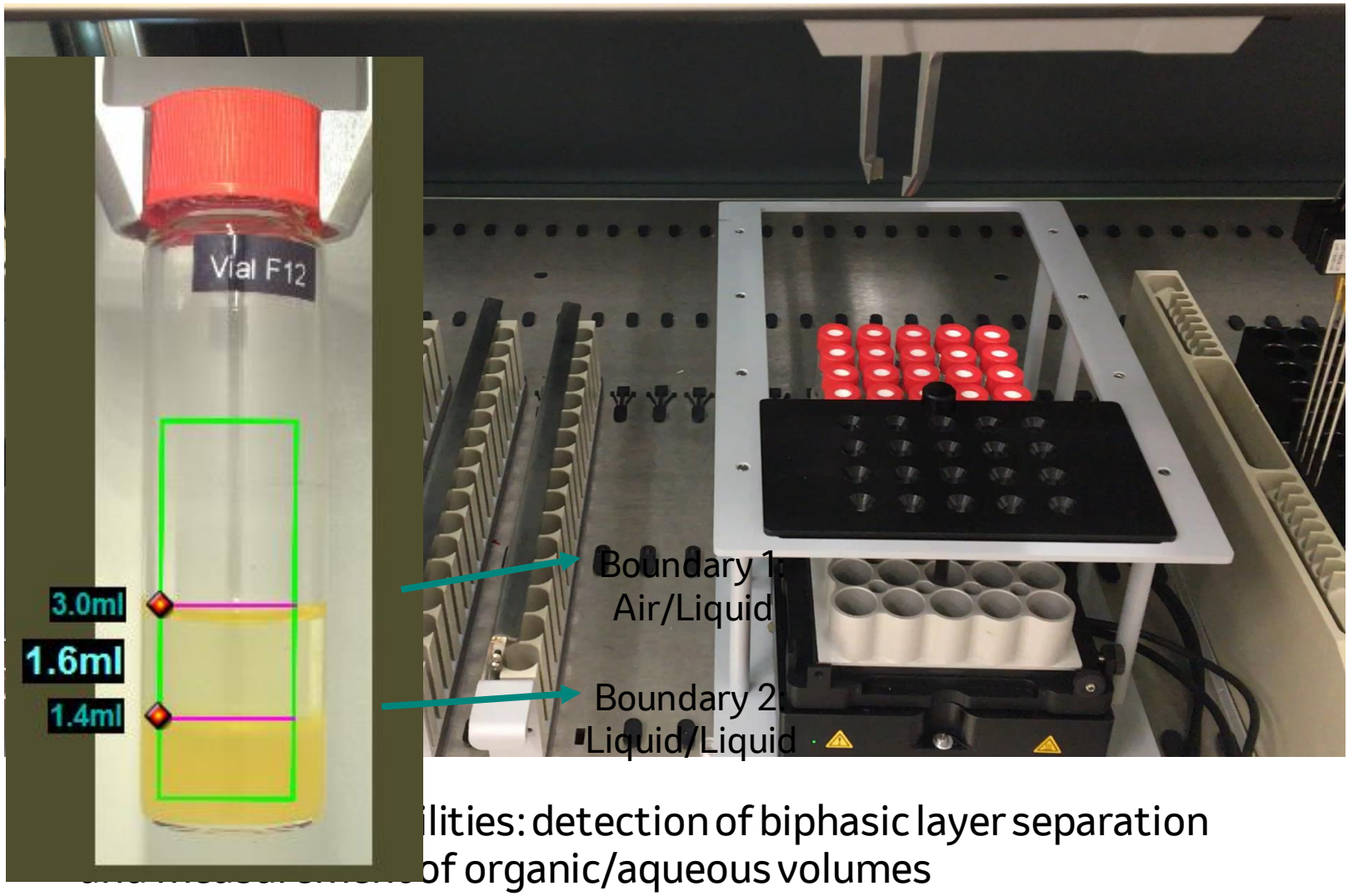
- Sample preparation
- Agitation and settling
- Image capturing and hit identification
- Organic and aqueous layer sampling
- Data processing and visualization



Throughput: 96 samples per run, 4-5 h run time

LLE screening automation workflow

- Sample preparation
- Agitation and settling
- Image capturing and hit identification
- Organic and aqueous layer sampling
- Data processing and visualization



ilities: detection of biphasic layer separation
of organic/aqueous volumes

LLE screening automation workflow

Sample preparation

↓

Agitation and settling

↓

Image capturing and hit identification

↓

Organic and aqueous layer sampling

↓

Data processing and visualization

LLE screening automation workflow

Sample preparation

↓

Agitation and settling

↓

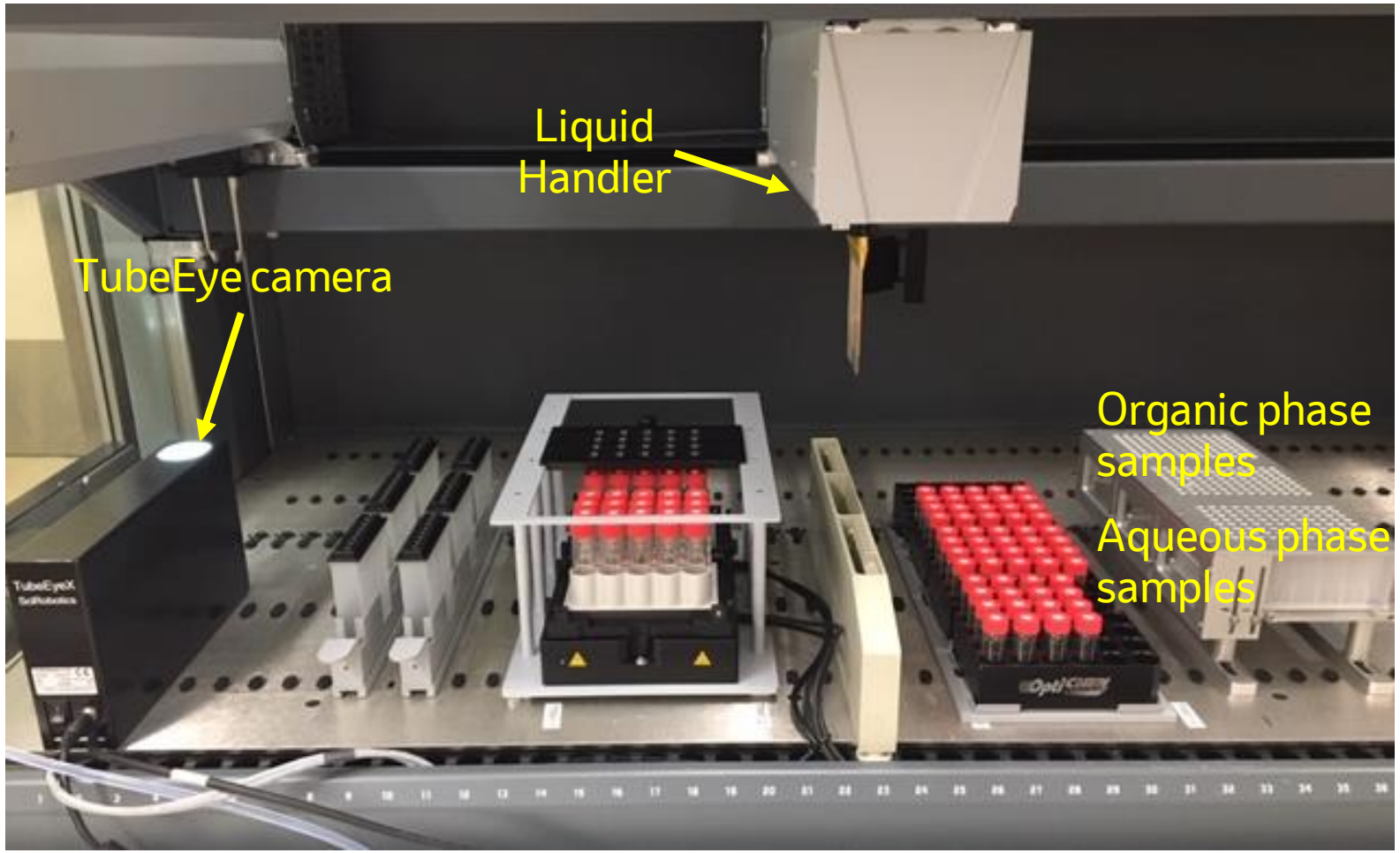
Image capturing and hit identification

↓

Organic and aqueous layer sampling

↓

Data processing and visualization



- Automated preparation of organic and aqueous phase LC samples

LLE screening automation workflow – automated data analysis

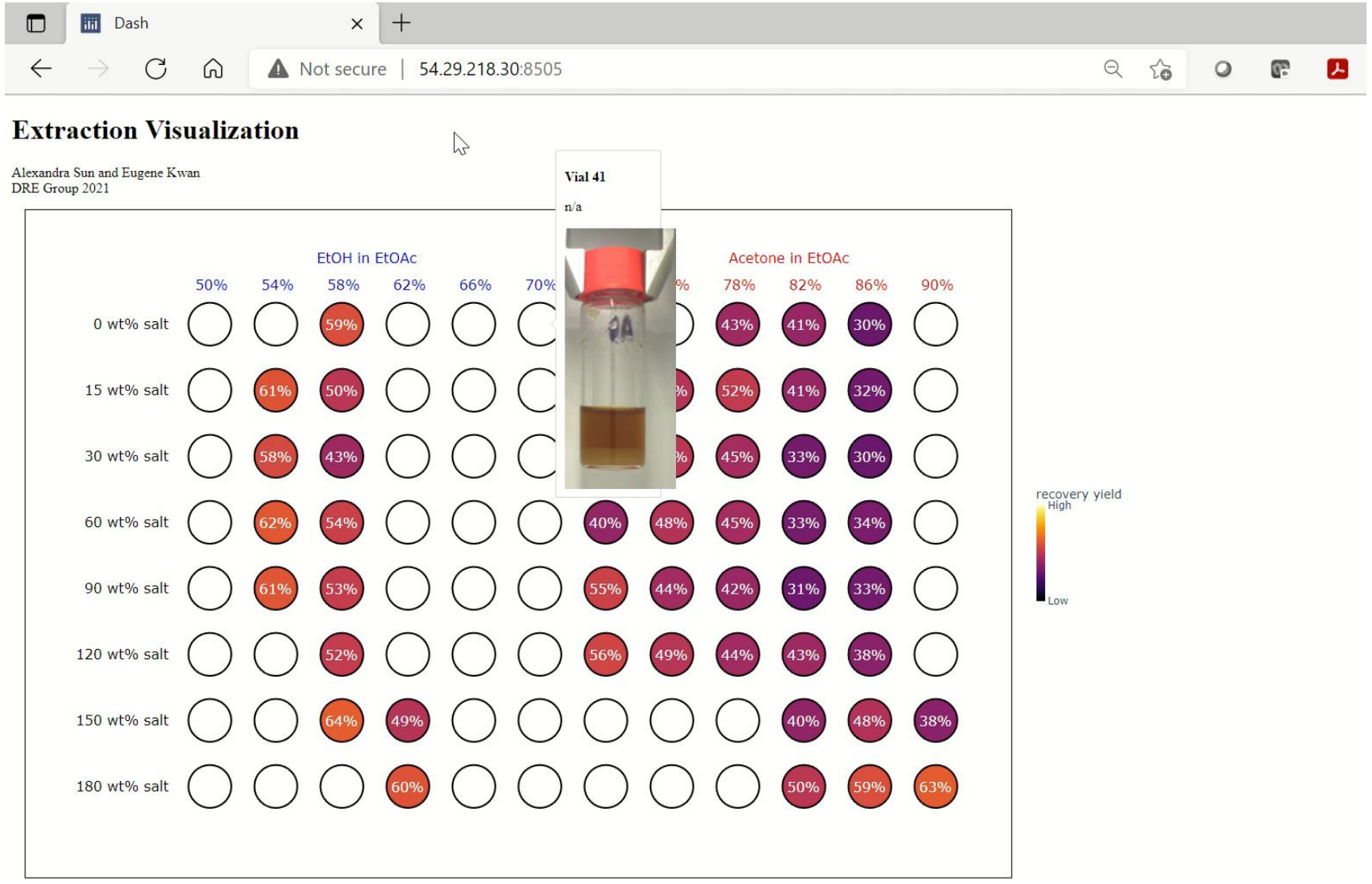
Sample preparation

Agitation and settling

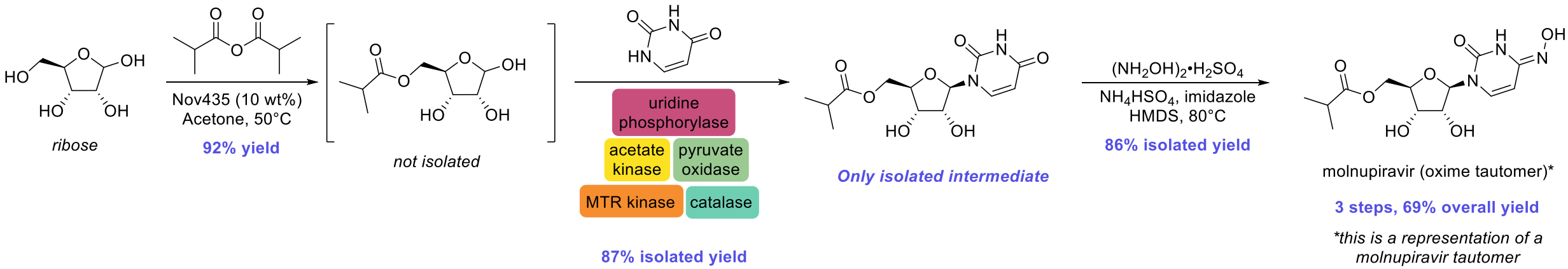
Image capturing and hit identification

Organic and aqueous layer sampling

Data processing and visualization



Case Study: Biocatalytic synthesis of Molnupiravir

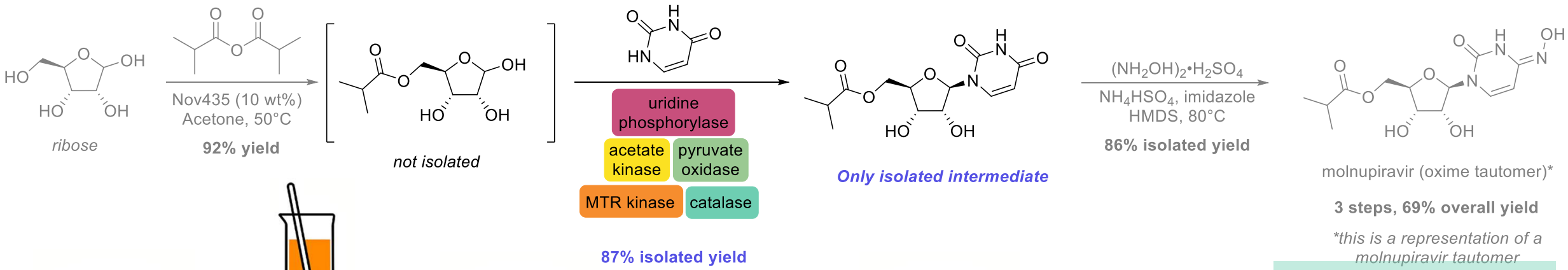


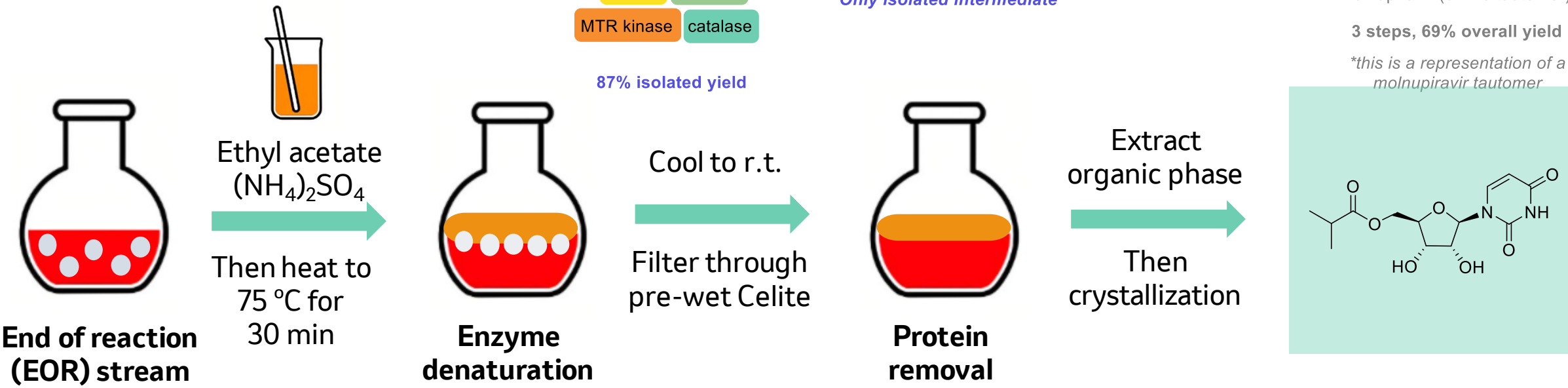
Innovative chemistry enables a 3-step route to molnupiravir

Developed and piloted on 100 kg scale in 6 months

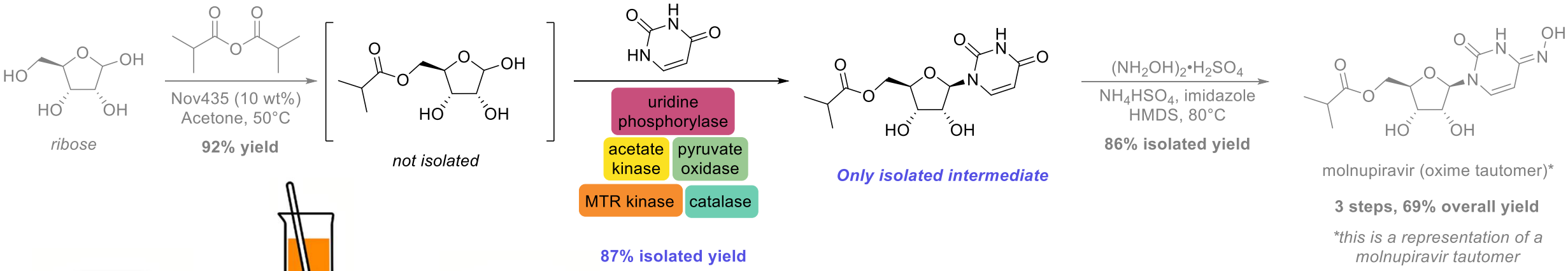
ACS Cent. Sci. **2021**, *7*, 1980-1985.

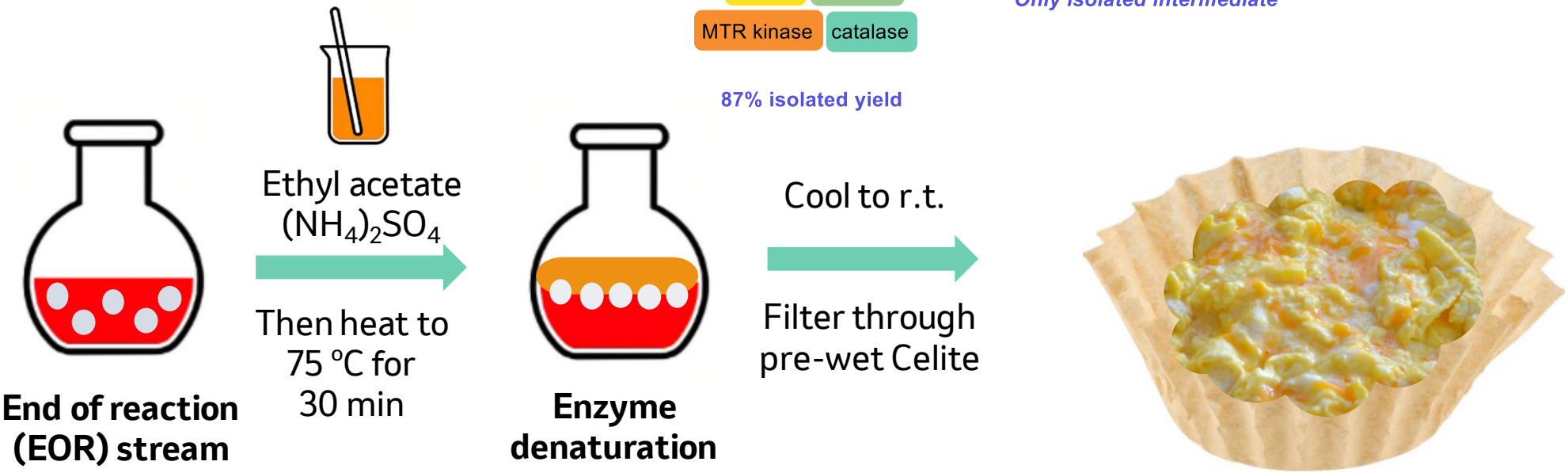
How do we remove enzyme at the end of a biocatalytic reaction?





How do we remove enzyme at the end of a biocatalytic reaction?





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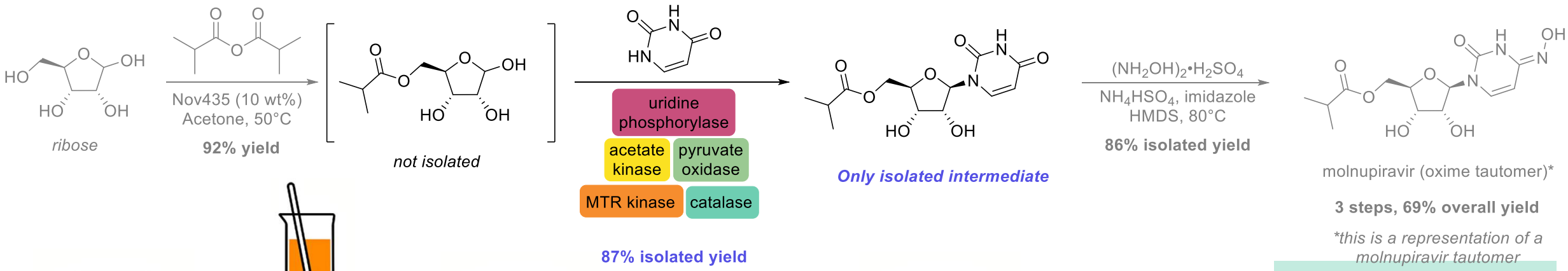
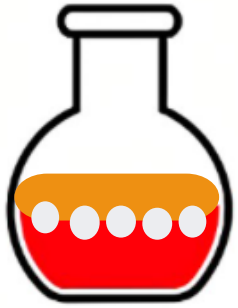


End of reaction (EOR) stream

Ethyl acetate
$(NH_4)_2SO_4$

Then heat to 75 °C



Enzyme denaturation

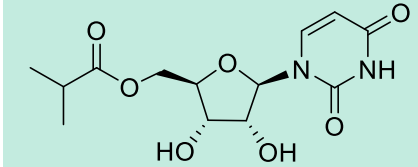
Cool to r.t.

Filter through pre-wet Celite

Protein removal

Extract organic phase

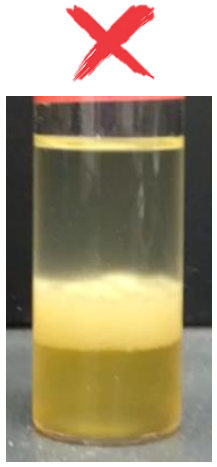
Then crystallization



105 mg/mL water solubility

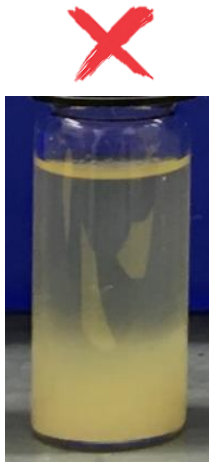
Challenge: High water solubility of 5'-isobutyryl uridine ***and*** enzyme rag layer formation prevents development of a direct extraction strategy

Developing an LLE strategy for protein removal

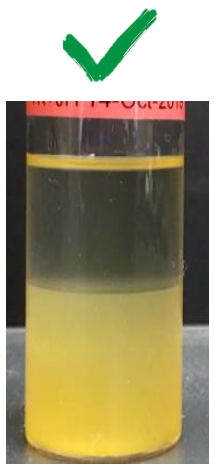


Protein rag

Cloudy organic



Miscible



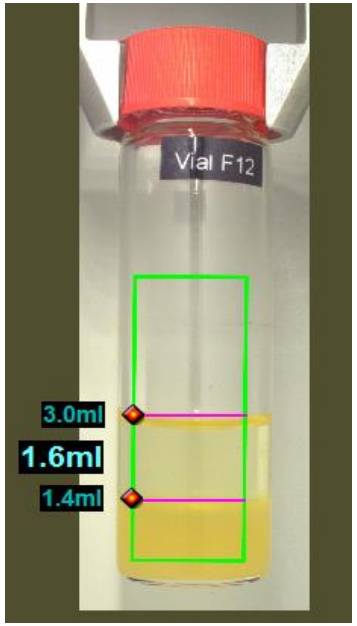
Clean Interface

Objective: Identify direct extraction conditions for **enzyme removal** and **>80% recovery** of 5'-isobutyryl uridine **after a single extraction**

LLE Screening strategy: Additive libraries

Hampton Research Additive Plates

Additives classes screened:
- Inorganic salts
- Amino acids
- Dissociating agents
- Linkers
- Polymers
- Polyamines/chelating agents
- Carbohydrates
- Detergents
- Organic solvents
- And many more….



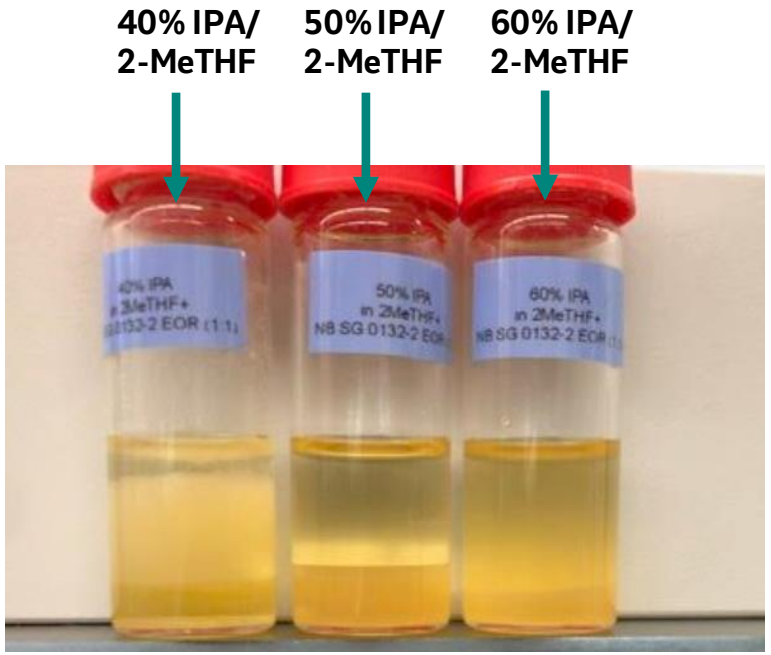
- Only **<u>one</u> PPG400 additive** yielded layer separation with 50% EtOAc/EtOH
- Extraction efficiency of ~60% required further optimization

LLE Screening strategy: Organic solvents

2-MeTHF/Alcohol co-solvent

0% alcohol → 100% alcohol

t-amylOH										
iprOH						71%				
iBuOH										
nBuOH										



- Most co-solvents provided inseparable layers
- **Only 50% IPA/2-MeTHF** yielded a phase split with 71% recovery

LLE Screening strategy: Inorganic salts

Sodium Sulfate

Ammonium Sulfate

40% 2-MeTHF/IPA

60% 2-MeTHF/IPA

wt% salt relative to substrate

0

300

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

*Acyl Uridine extraction yield expressed as % in Organic Layer.

LLE Screening strategy: Inorganic salts

Sodium Sulfate **Ammonium Sulfate**

40% 2-MeTHF/IPA **60% 2-MeTHF/IPA**

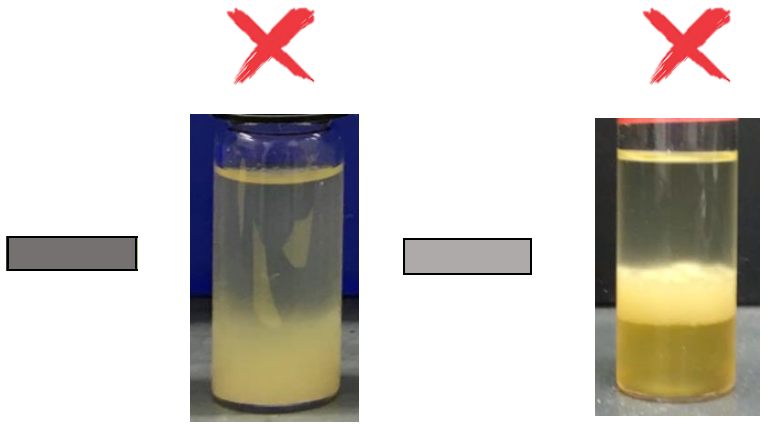
wt% salt relative to substrate

0

300

	1	2	3	4	5	6	7	8	9	10	11	12
A			73%						69%			
B												
C							60%	45%		45%		
D	60%						70%	79%				
E												
F												
G												
H												

*Acyl Uridine extraction yield expressed as % in Organic Layer.



Miscible Protein rag

LLE Screening strategy: Inorganic salts

Sodium Sulfate **Ammonium Sulfate**

40% 2-MeTHF/IPA **60% 2-MeTHF/IPA**

wt% salt relative to substrate

0 **300**

	1	2	3	4	5	6	7	8	9	10	11	12
A			73%						69%			
B												
C							60%	45%		45%		
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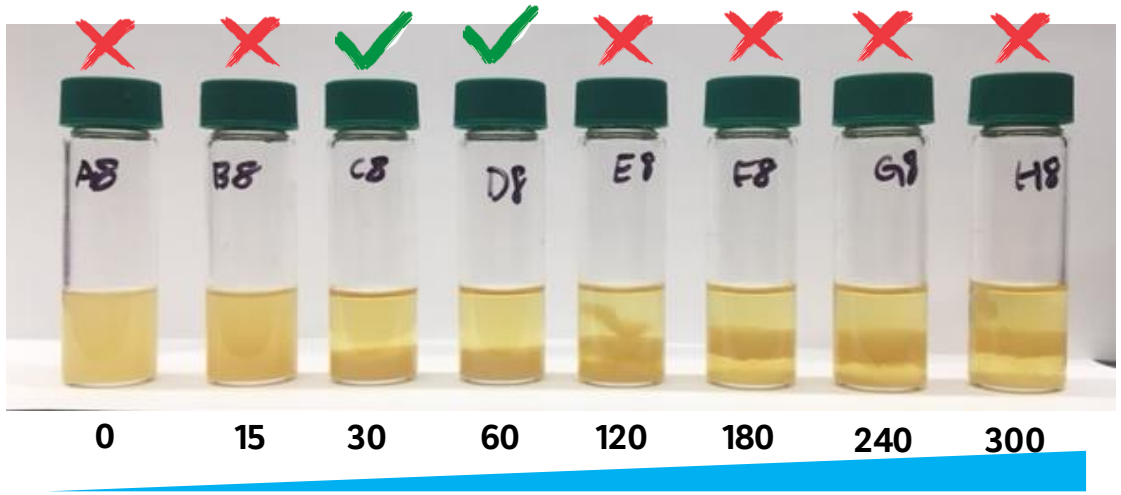


HTE-Enabled LLE optimization

Additives → 60% recovery → Solvents → 70% recovery → Salts → 80% recovery

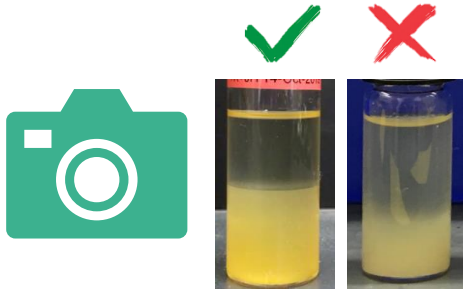
Product recovery: 90% after single extraction on 50 mL scale

Out of >700 conditions evaluated….
100 samples gave a phase split
6 samples gave a recovery yield >70%

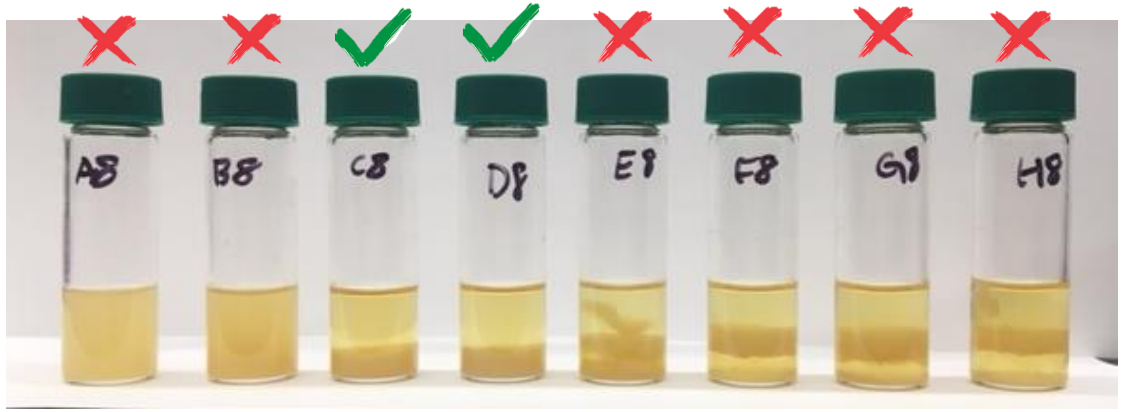
Final Extraction Conditions:
44% 2-MeTHF/IPA, 60 wt% ammonium sulfate

Summary

- Development of a vision-guided HTE LLE platform



- Successful pipeline application enables enzyme removal



- New technology can be implemented with sufficient pre-investment

- Cross-disciplinary collaboration enables innovation

Acknowledgements

Tecan Development

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Jacob Forstater

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Patrick Fier

Tetsuji Itoh

Umme Ayesa