Integrating ^{19}Focused Screening with Make-on-Demand Chemical Spaces for Enhanced Fragment Follow-Up

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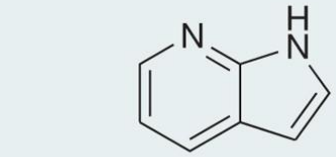
Fragment screening

Fragment-based drug design arose as an alternative approach to high-throughput screening.

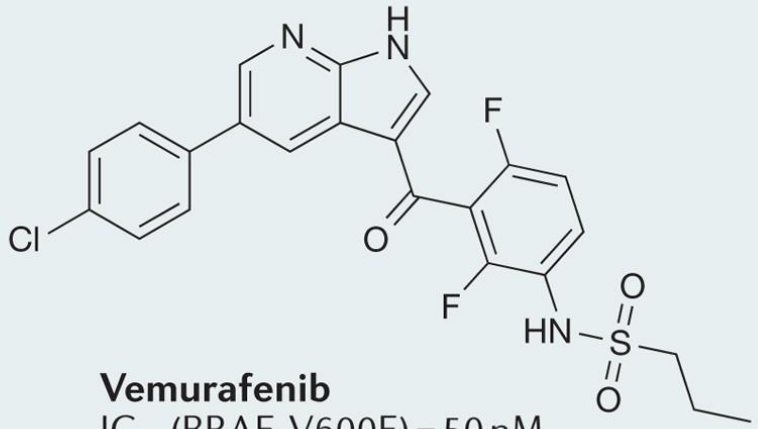
Fragment screening achieves:

- **Higher hit rates**
- **Lower MW hits**
- **Hits with higher solubility**

... but at lower affinity



Lead fragment
IC_{50} (PIM1) >200 µM

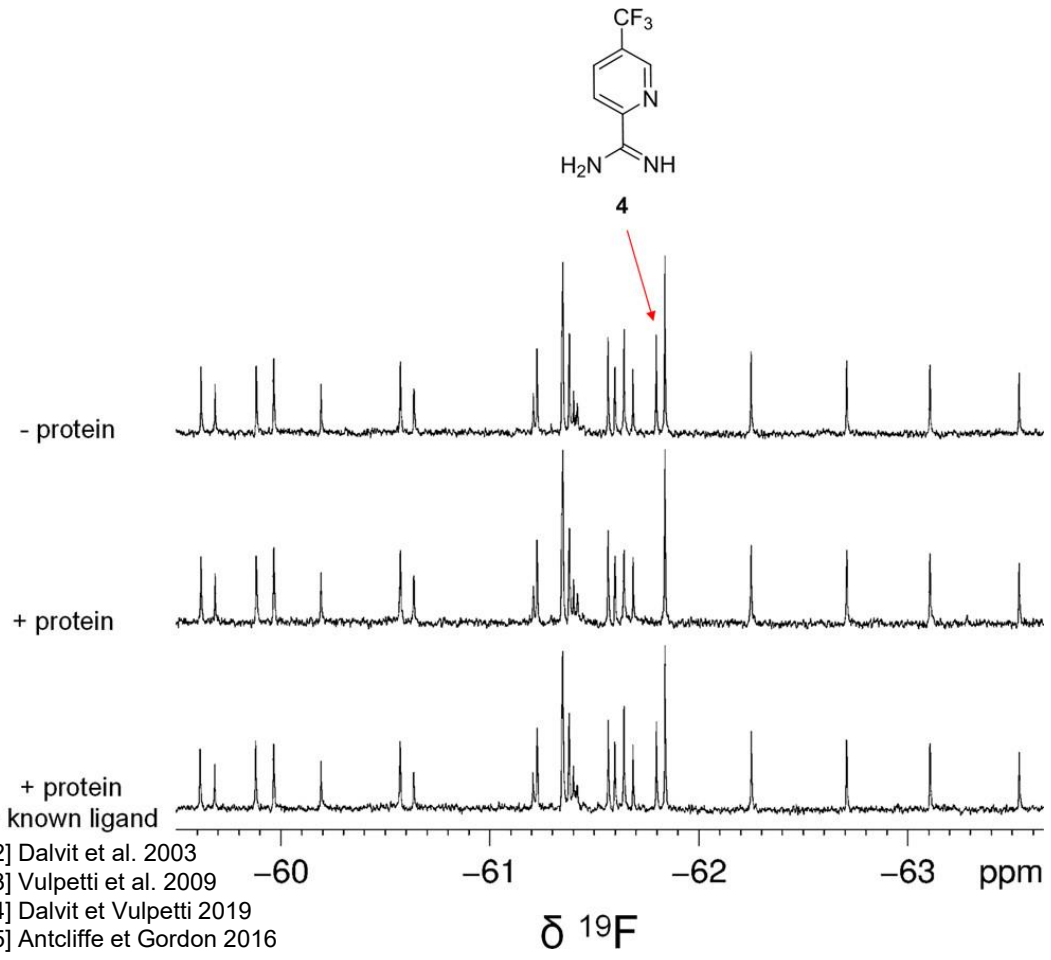


Vemurafenib
IC_{50} (BRAF-V600E) = 50 nM

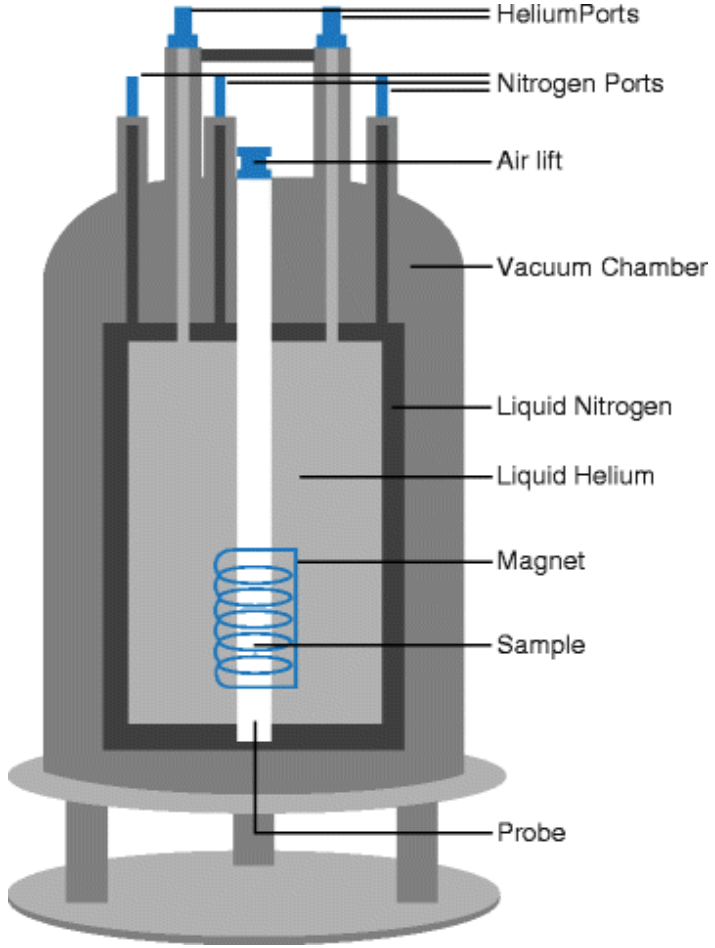
Nature Reviews | Drug Discovery

[1] Erlanson et al. 2016

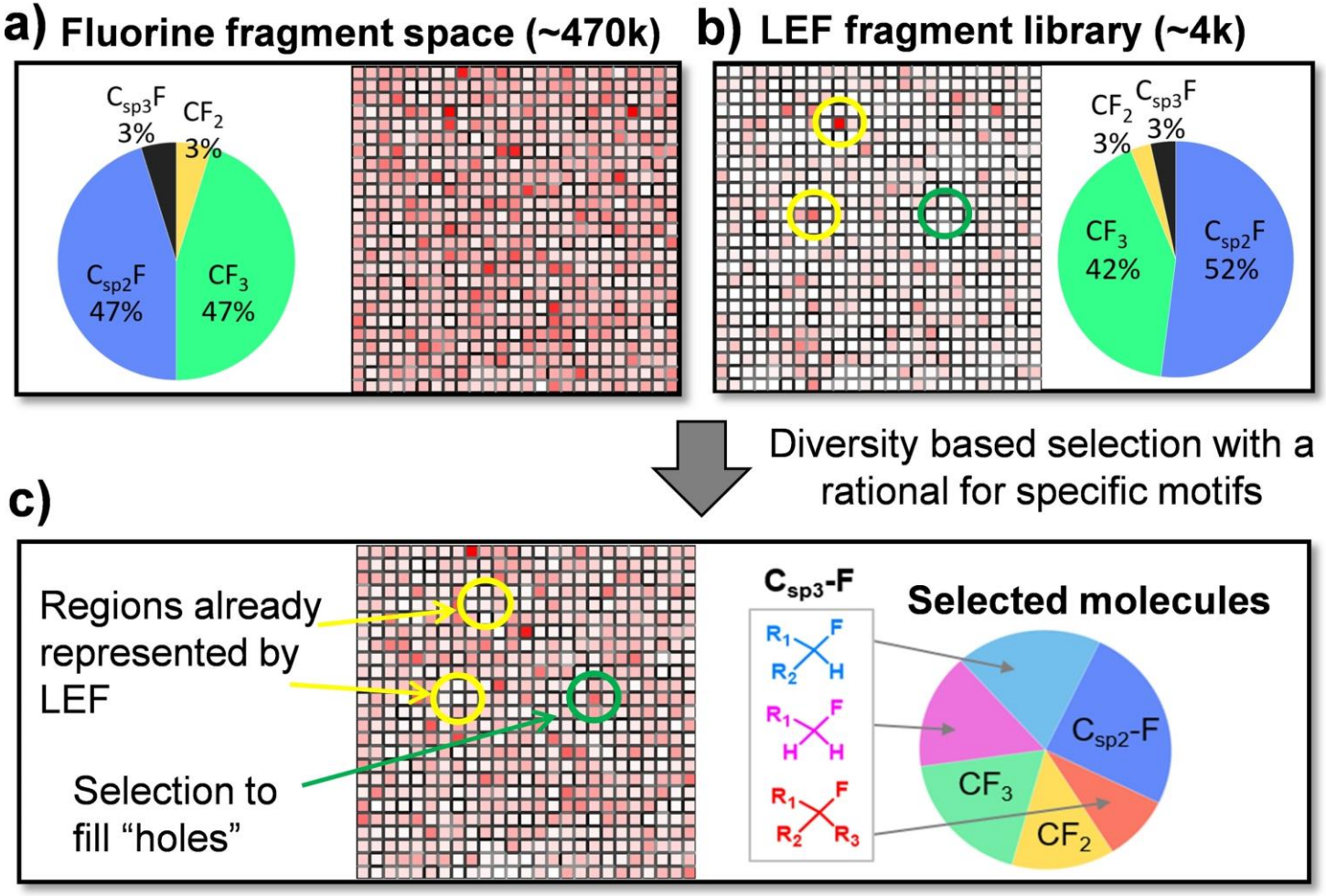
^{19}F NMR fragment screening (FAXS)



[2] Dalvit et al. 2003
[3] Vulpetti et al. 2009
[4] Dalvit et Vulpetti 2019
[5] Antcliffe et Gordon 2016

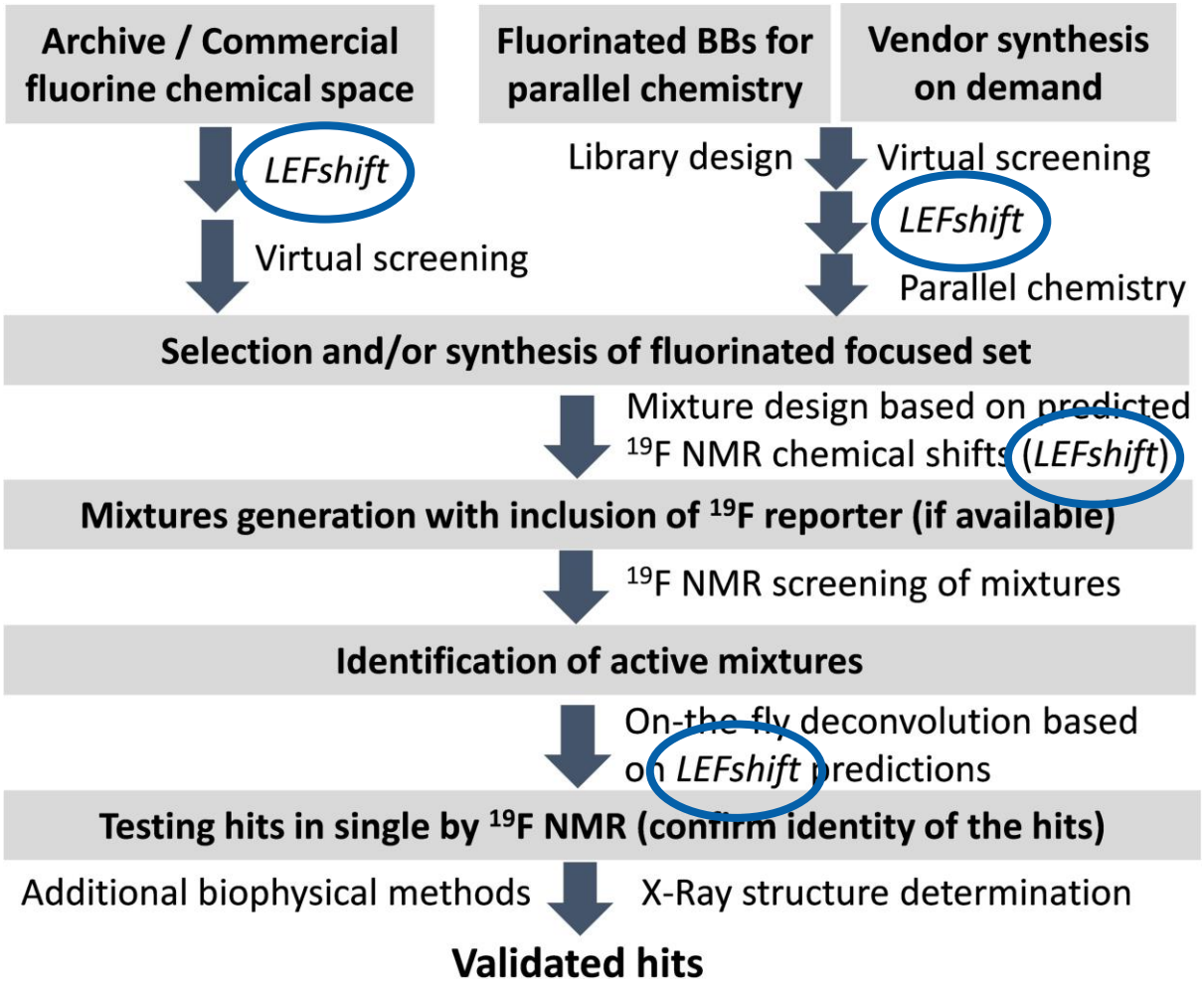


Local environment of fluorine library (LEF5500)



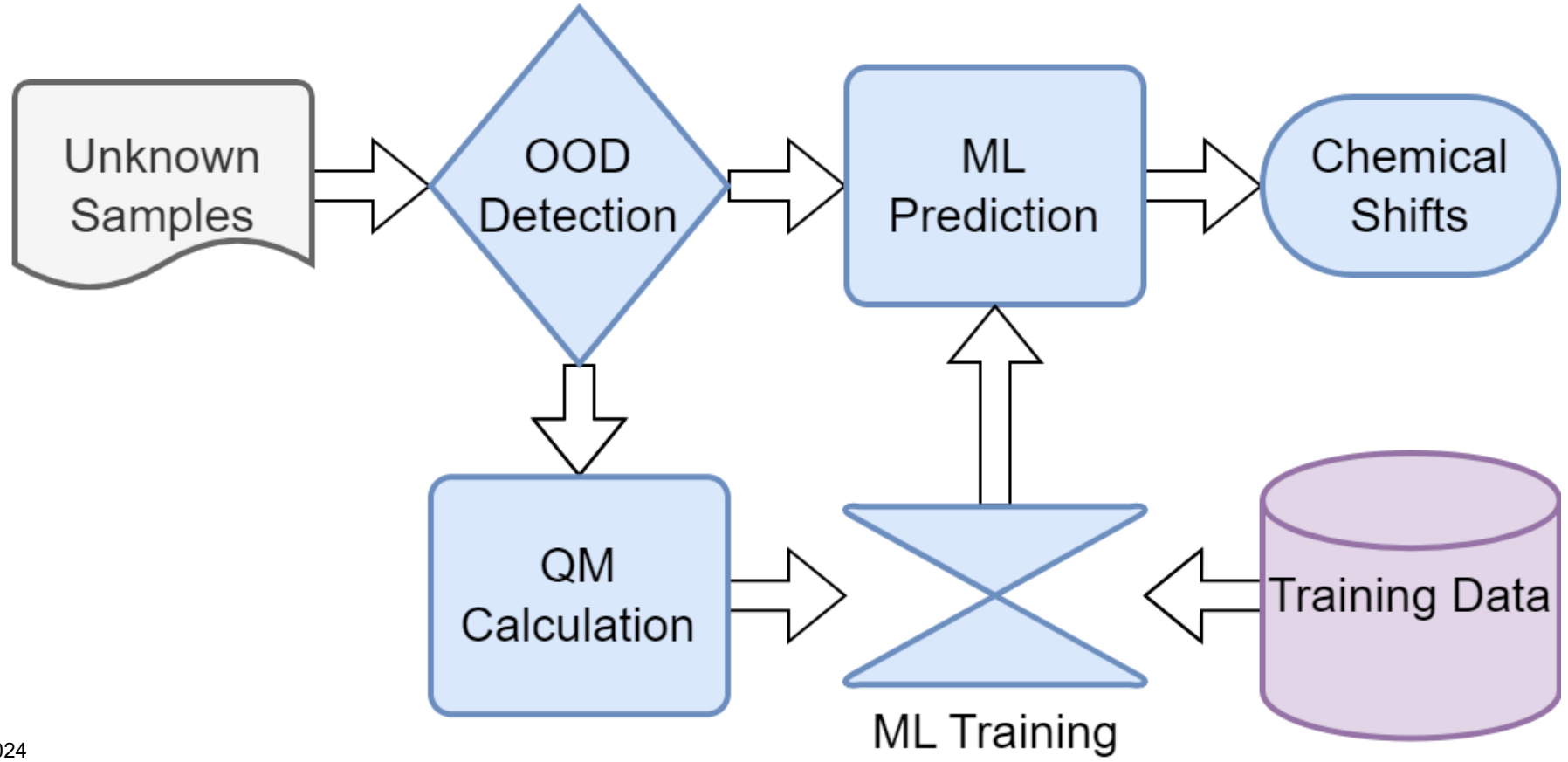
[6] Vulpetti et al. 2022

^{19}Focused screening



[6] Vulpetti et al. 2022
[7] Vulpetti et al. 2024

QM assisted ML workflow



[8] Penner et Vulpetti 2024

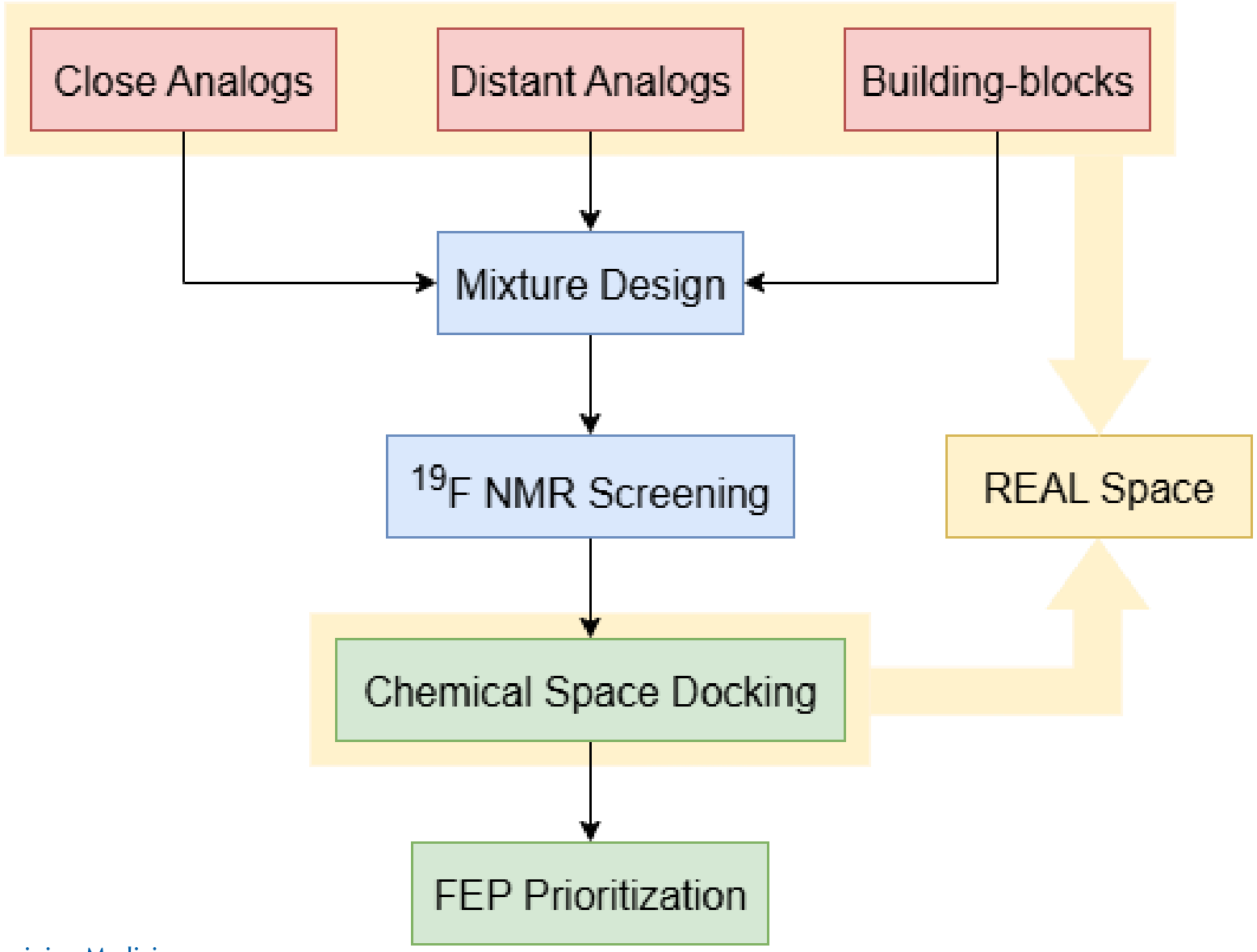
Enamine REAL Space

“... comprises 76.9B make-on-demand molecules and is currently the largest offer of commercially available compounds.”

- 172 well-validated parallel synthesis protocols
- 181 288 building blocks
- Success rate of over 80%

[9] REAL Space – Enamine

Workflow overview



Three conceptual approaches to assemble the ^{19}Focused set

Close analogs

- In the Enamine REAL Space product space
- Substructure searching methodology (**SpaceMACS**)

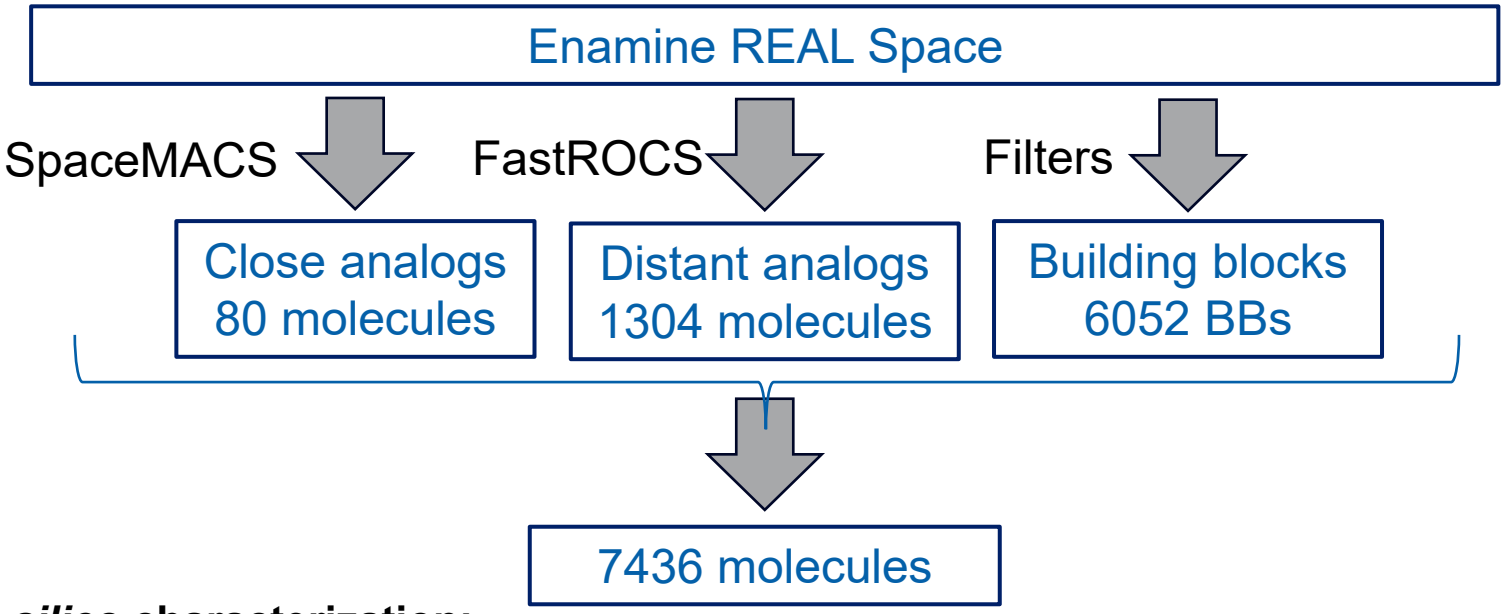
Distant analogs

- In the Enamine REAL Space product space
- Methods with scaffold hopping potential (**FastROCS**)
- Structurally more different from known chemical matter
- Focus on novelty

Building-blocks

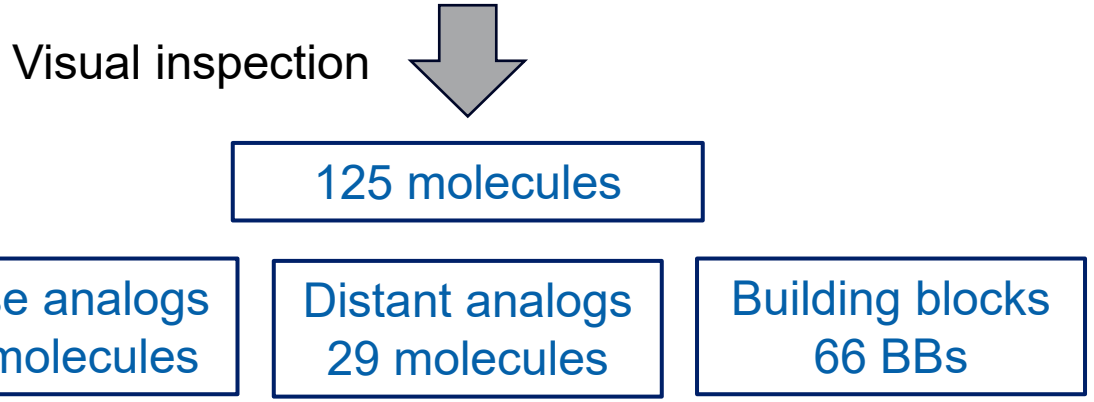
- In the Enamine REAL building-blocks
- Fragment-like, diverse motifs
- Focus on follow-up potential (**reaction handles**) and novelty

In silico workflow



***In silico* characterization:**

- Descriptor calculation by RDKit (MW, HBA, HBD, tPSA, RotB)
- logP/logD, pKa, Solubility (global Novartis models)
- Docking



Pre-screening

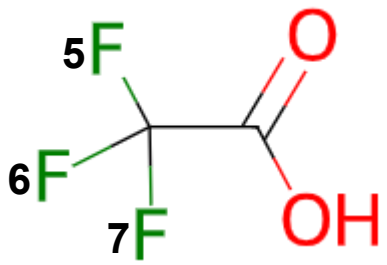
- Screened all compounds by SPR and DSF
- 4 out of around 30 compounds in the close and distant analog sets were active
- 1 compound from the building-blocks set was active

- Sets biased by available chemistry were more successful
- It was encouraging to see a building-block hit < 100 µM
- We wanted to follow up on challenging hits

Set	Compounds active in SPR (<100 µM)
Close Analogs	4
Distant Analogs	4
Building-blocks	1

QM Predictions

- **QM predictions were added to LEFShift to handle compounds far outside of the training data**
- **Validation showed that neither an ML-based method or a QM-based method performed strictly better**
- **QM predictions are more stable for distant compounds**
- **Handling molecules for QM is a bit different than for other methods**
  - V2000 Mols truncate coordinates to 4 decimal places, which affects results



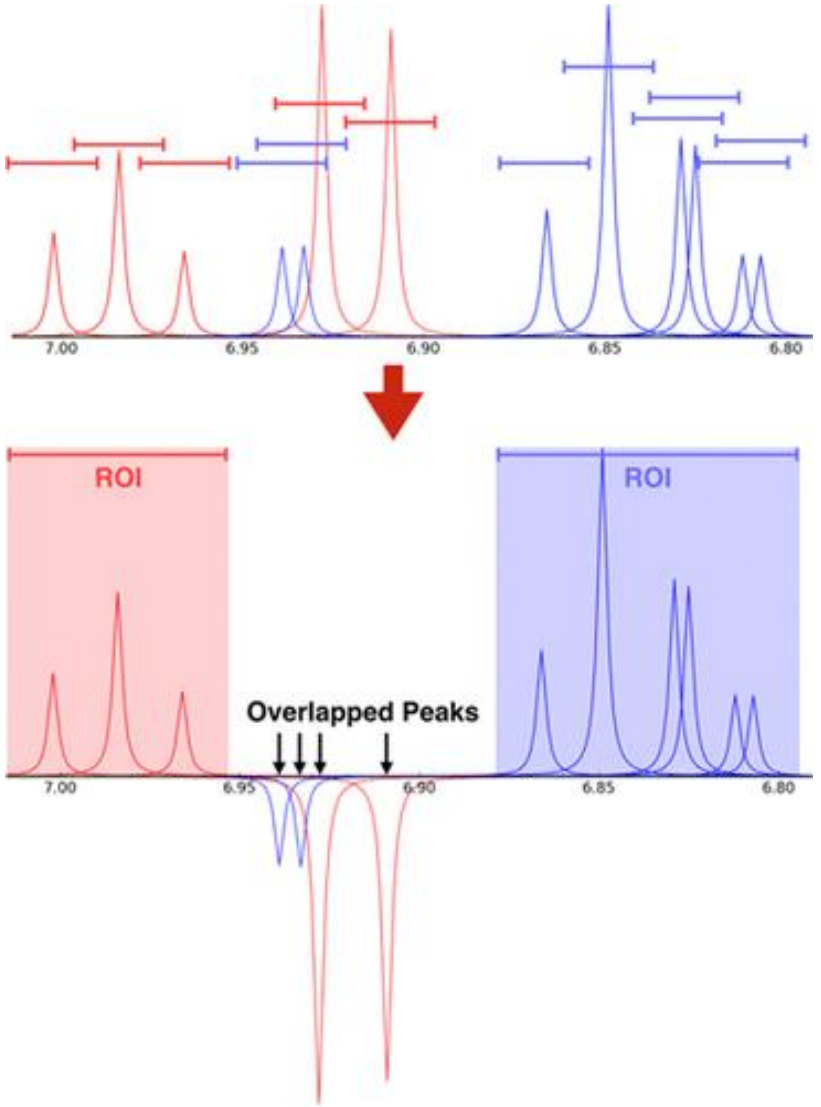
Fluorine	Shieldings from Truncated Coordinates	Shieldings from Full Coordinates
Fluorine 5	254.4190	254.4423
Fluorine 6	252.8276	252.8554
Fluorine 7	252.8237	252.8515
Trifluoromethyl Average	253.3568	253.3831

The experimental precision for ^{19}F chemical shift is ~ 0.003 ppm[11]

[8] Penner et Vulpetti 2024
[11] Rosenau et al. 2018

Mixture design

- **Optimizing mixture design can be a challenging algorithm**
  - This is a lot easier in ^{19}F NMR
- **A simple approach proved pragmatic**
  - Sorting by predicted chemical shift and periodically assigning to mixtures
  - The simplicity facilitates human intervention
- **We tried to separate problematic compounds**
  - Uncertain predictions
  - Solubility risks
  - etc.



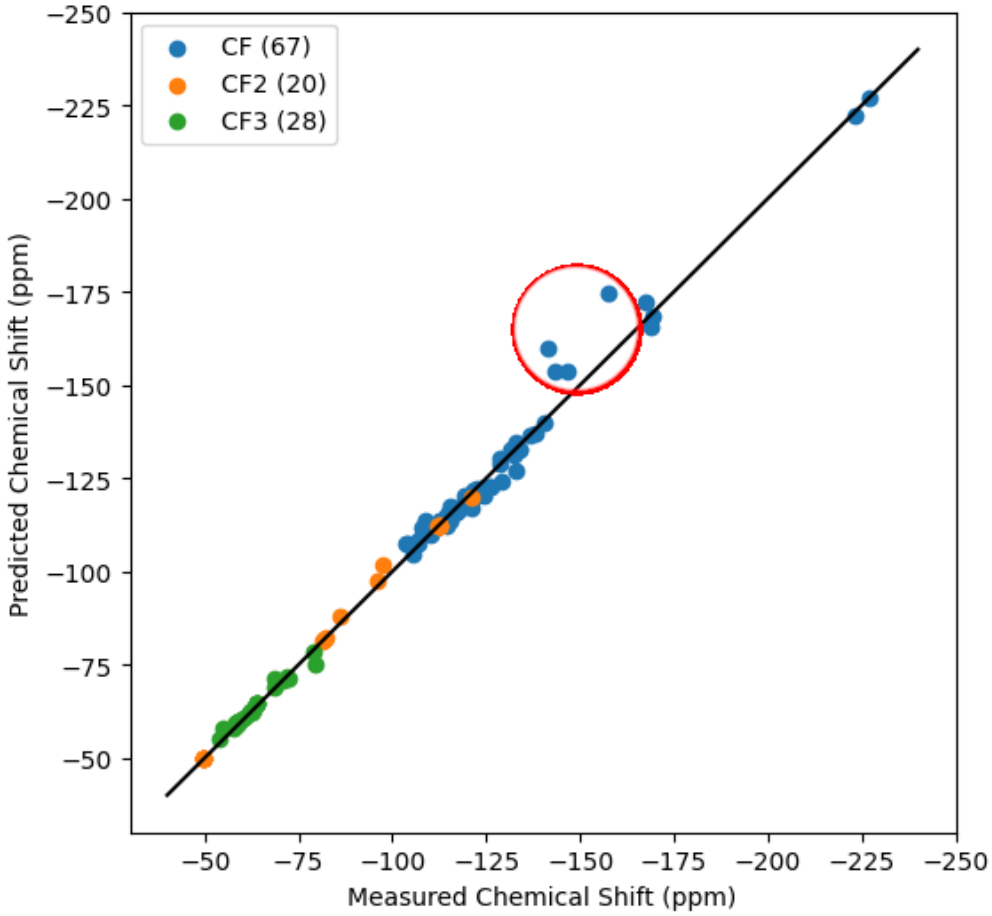
[10] Stark et al. 2016

^{19}F chemical shift prediction overview

- Errors are largely in the expected range
- The four predictions with the largest error were all declared as uncertain
- Unassigned stereocenters made QM difficult
- Because the prediction had marked them as uncertain, we could separate them into different mixtures
- They were assignable by exclusion

Fluorine Motif	MAE[a] (ppm)	RMSE[b] (ppm)
CF (n = 67)	2.174	3.977
CF_2 (n = 20)	0.583	1.133
CF_3 (n = 28)	0.875	1.339

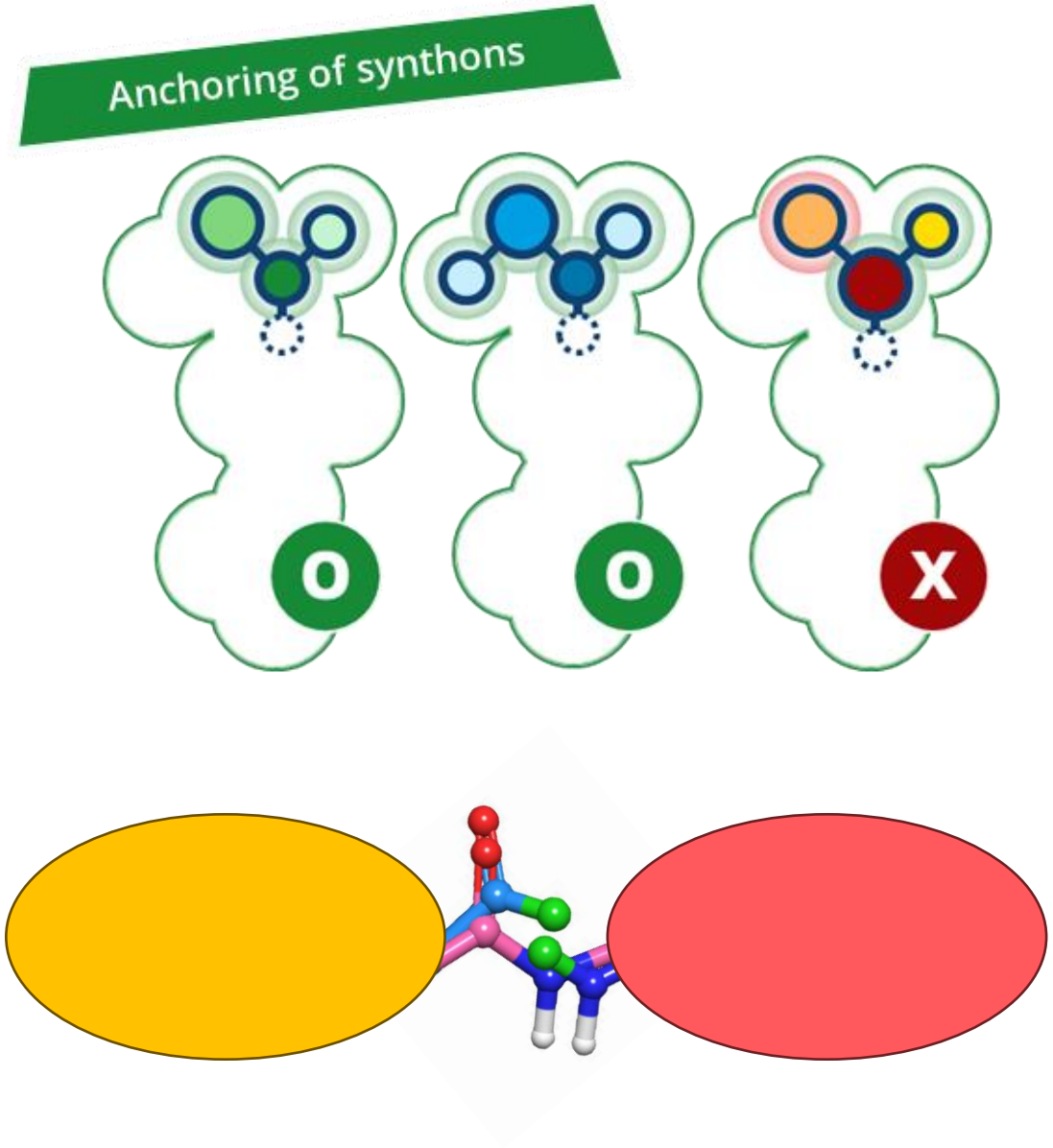
[a] mean absolute error [b] root-mean-square error



[8] Penner et Vulpetti 2024

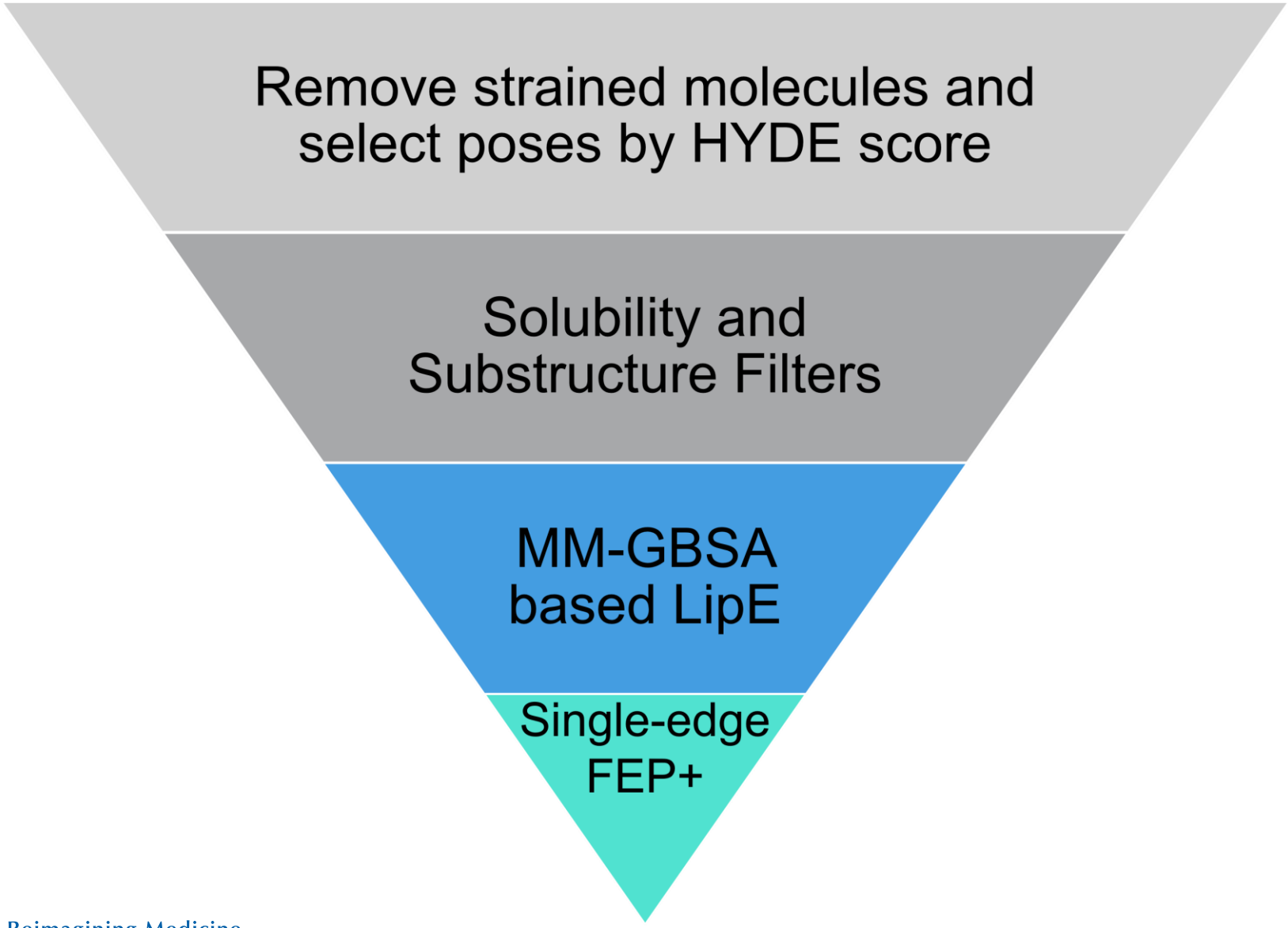
Chemical space docking

- **Synthon-based combinatorial docking approach**
- **The initial step involves determining an anchoring synthon**
- **We guarantee follow-up by starting in the Enamine chemical space**
- **Is that follow-up useful to SAR?**
  - Distant analogs can be split into their synthons
  - Building-blocks need to have reactive handles pointing in the right direction



[12] Beroza et al. 2022
[13] Mueller et al. 2022

Filtering cascade



Single-edge vs. Cycle Closure FEP+

- Compound 1 is the original ^{19}Focused screening hit
- Compounds 2-6 are hits from the follow-up (2-3 from SPR, 4-6 from DSF)
- The stronger hits were better predicted in single-edge FEP+
- Cycle closure FEP+ improves predictions for some compounds
- Many molecules were overpredicted in single-edge FEP+

Compound	SPR K_D (μM)	^{19}F NMR K_I (μM)	Single-edge FEP+ (μM)	Cycle Closure FEP+ (μM)
Compound 1	>100	870	n/a	1361
Compound 2	108	330	124	319
Compound 3	53	66.6	66.4	91.4
Compound 4	>100	748	347	705
Compound 5	>100	1170	202	195
Compound 6	>100	1538	378	1057

Summary

- **We tried different approaches to make-on-demand spaces**
  - Close analogs stay close to known chemistry and find quick hits
  - Distant analogs are also influenced by known chemistry but manage to explore into different chemotypes
  - Building-blocks were the riskiest approach but fundamentally address make-on-demand spaces
- **QM can be a helpful addition to property prediction**
  - Was better at handling novel chemistry
  - Is a high precision method and needs to be handled that way
- **Positioning the screening in a make-on-demand space facilitated follow-up**
  - Following up on the distant analog hits was the most successful
  - Full molecules may avoid issues with extension directions

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