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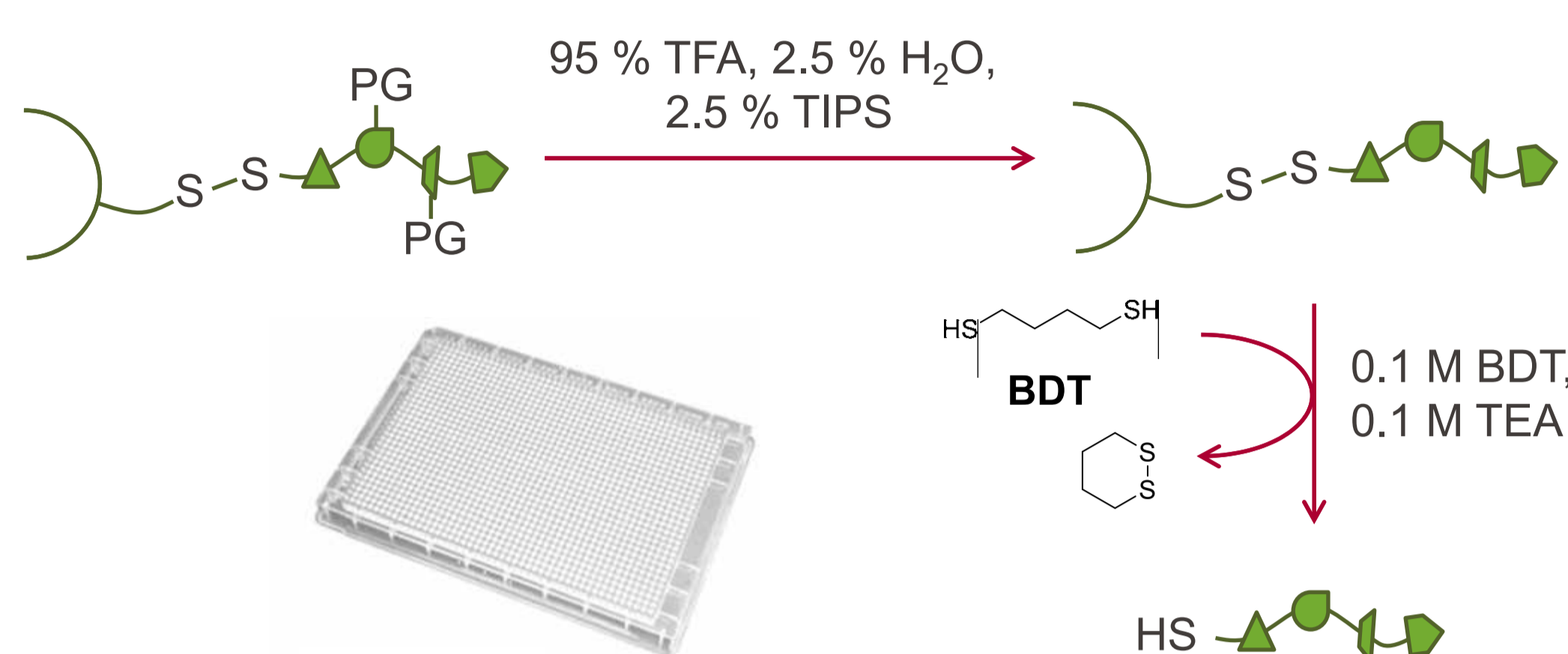
<https://doi.org/10.17952/37EPS.2024.P2124>



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## Established method to synthesize thiol peptides

Special resin for solid phase peptide synthesis that yields C-terminal thiol [1]

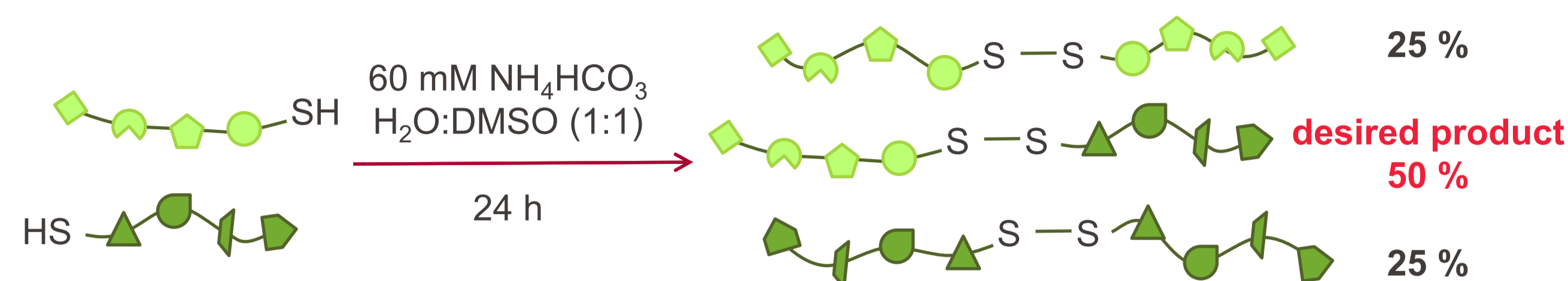


Peptide synthesizer with upgrade for parallel synthesis of 1536 peptides[2]

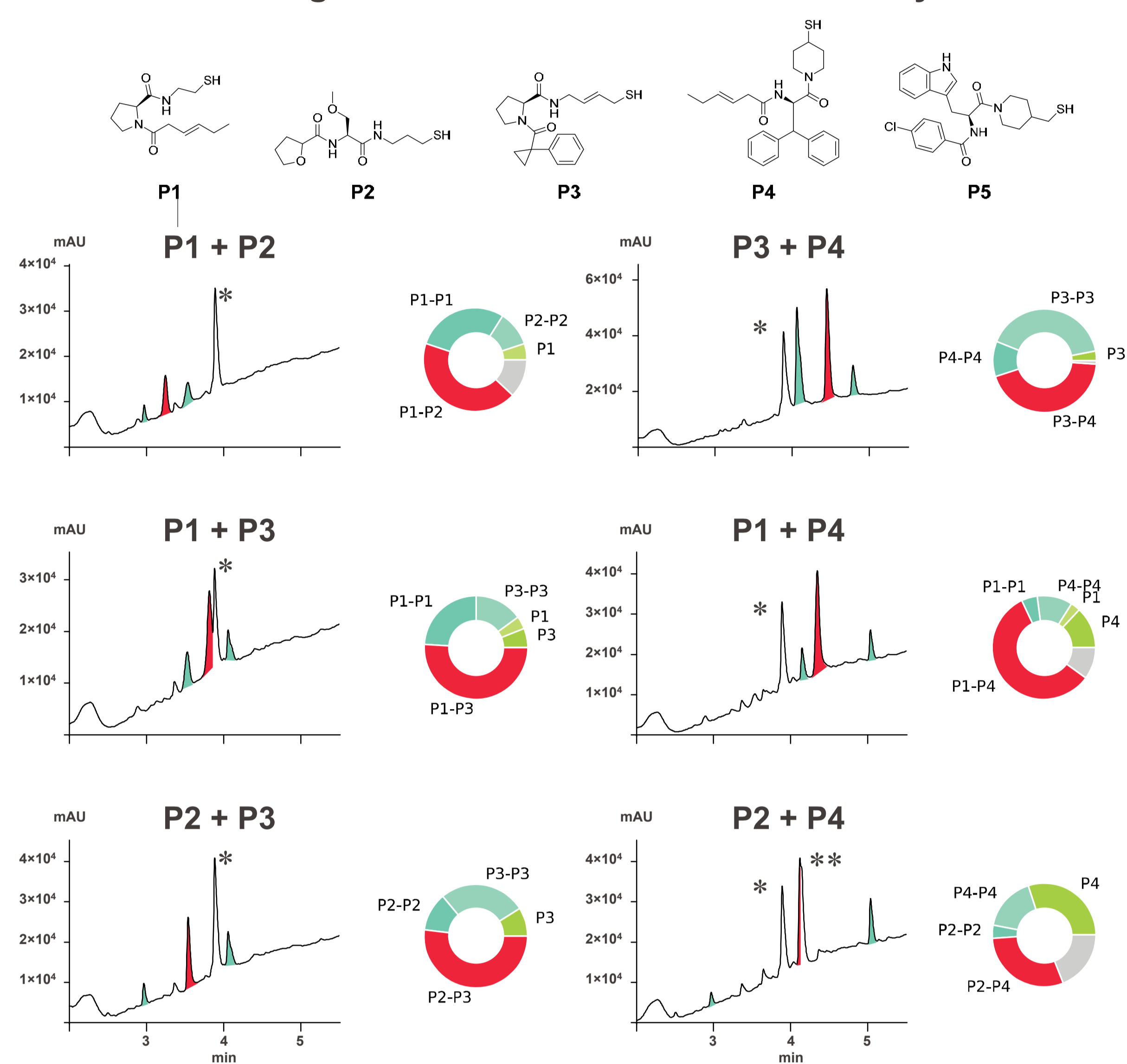
Reduced thiol peptides are stored in 1536 microwell plate

1536 highly diverse thiol peptides Ready to screen, no purification needed!

## Testing dimerization reaction

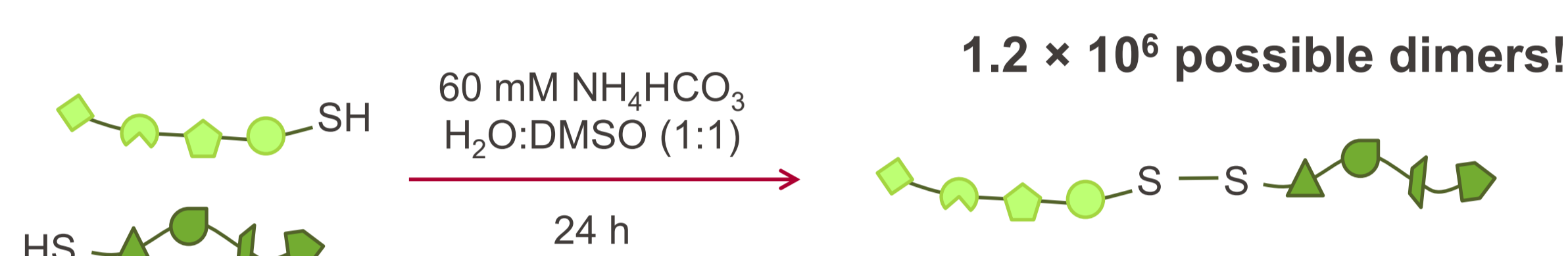


LCMS chromatograms of test dimerizations and analysis of results



Transfer of 2 nmol per peptide, final concentration of peptides 100 μM  
\* Impurity on the column, \*\* overlapping product peak

## Workflow for synthesizing dimer libraries



Direct screening in dimerization plate

protein of interest



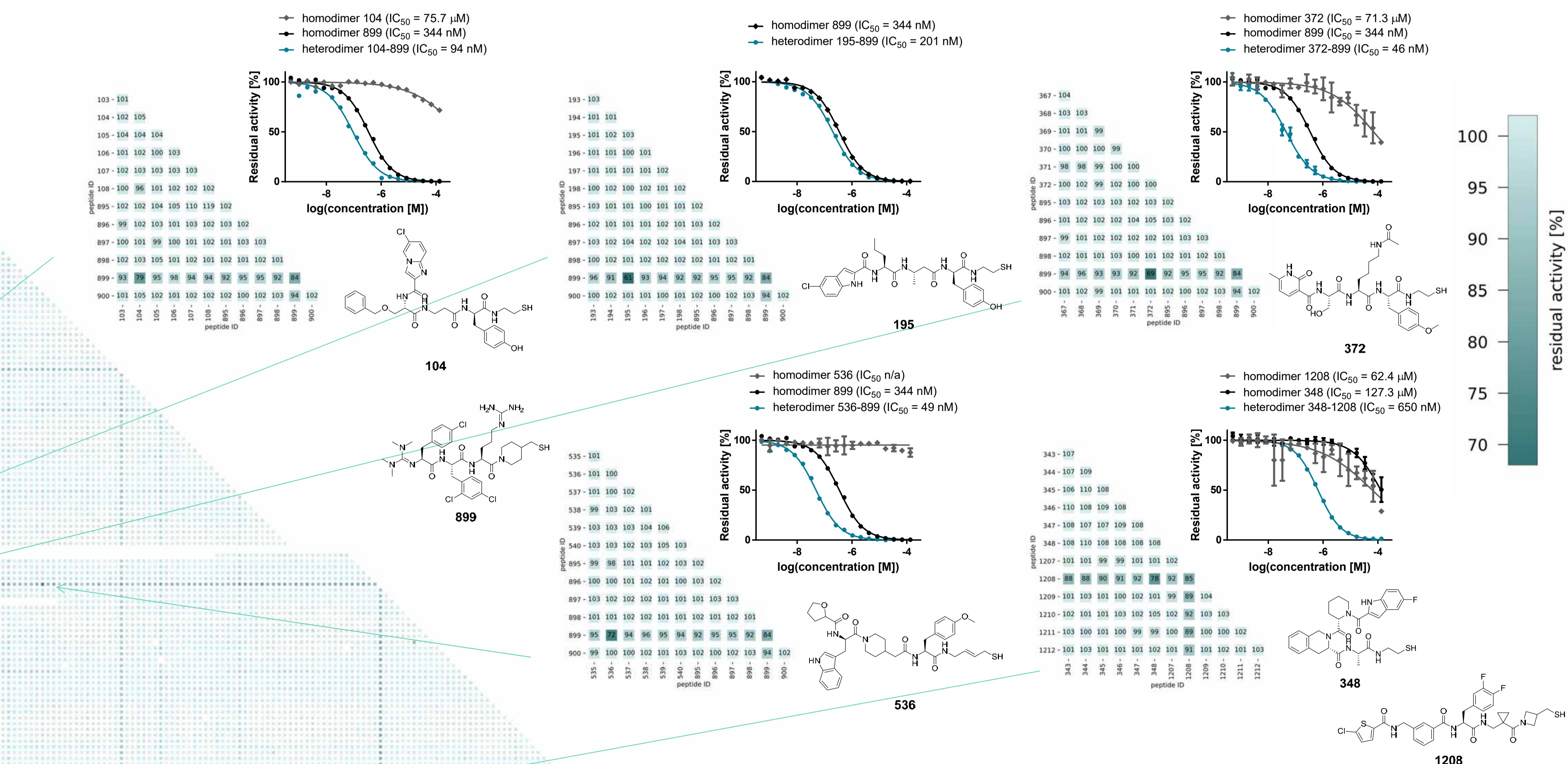
Dimerization and screening plate

Acoustic droplet ejection to transfer nanoliter volumes of peptides to dimerization plate

## Synthesis of dimer library and screening against thrombin

- To facilitate screening of a large library, dimers are screened in mixtures. Peptides are organized in groups of 6. In each screening well, two groups are pooled resulting in a mixture of 12 peptides forming 72 dimers. In total a library of 853 471 dimers was screened.
- Mixtures that decreased the proteolytic activity of thrombin in the first step are deconvoluted by resynthesizing and screening all 72 dimers of the mixture in parallel.
- After identification of the active dimer in the mixture, the compound is resynthesized and purified for characterization and IC<sub>50</sub> determination.

218 pools of 6 peptides per pool



218 pools of 6 peptides per pool

Large screen: Transfer of 10 pmol per peptide → screening concentration for each individual dimer ~ 100 nM  
Deconvolution: Transfer of 2.5 pmol per peptide → screening concentration for each individual dimer ~ 170 nM

### References

- [1] Schüttel, M., et al. "Solid-phase peptide synthesis in 384-well plates." *Journal of Peptide Science* 30.4 (2024): e3555.  
[2] Bogner, Z., et al. "Solid-phase peptide synthesis on disulfide-linker resin followed by reductive release affords pure thiol-functionalized peptides." *Organic & Biomolecular Chemistry* 20.29 (2022): 5699-5703.

## Conclusion

- New method to easily and purification-free synthesize peptide libraries.
- A library of 853 471 peptides was screened against the model target thrombin.
- Nanomolar thrombin inhibitors were identified.