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Research towards automated continuous flow peptide synthesis

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Why peptides?

Peptides play a crucial role in human physiology, as hormones, neurotransmitters, growth factors and antibacterial agents

- Since 2000, at least 28 new, non-insulin peptide drugs
- As of 2016, more than 50 peptide drugs marketed worldwide, around 170 in clinical development and more than 200 in pre-clinical development
- In 2014, worldwide sales of peptide-based therapeutics exceeded $1 trillion for the first time

A. Henninot, J. C. Collins, J. M. Nuss, *J. Med. Chem*, **2017**, DOI: 10.1021/acs.jmedchem.7b00318

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Challenges in peptide synthesis

Solid phase peptide synthesis – not a solved problem

- Racemisation/condensation
- Other side product formation
- Low yield couplings
- Solubility issues
- Aggregation
- Difficult sequences
- Difficulty in monitoring

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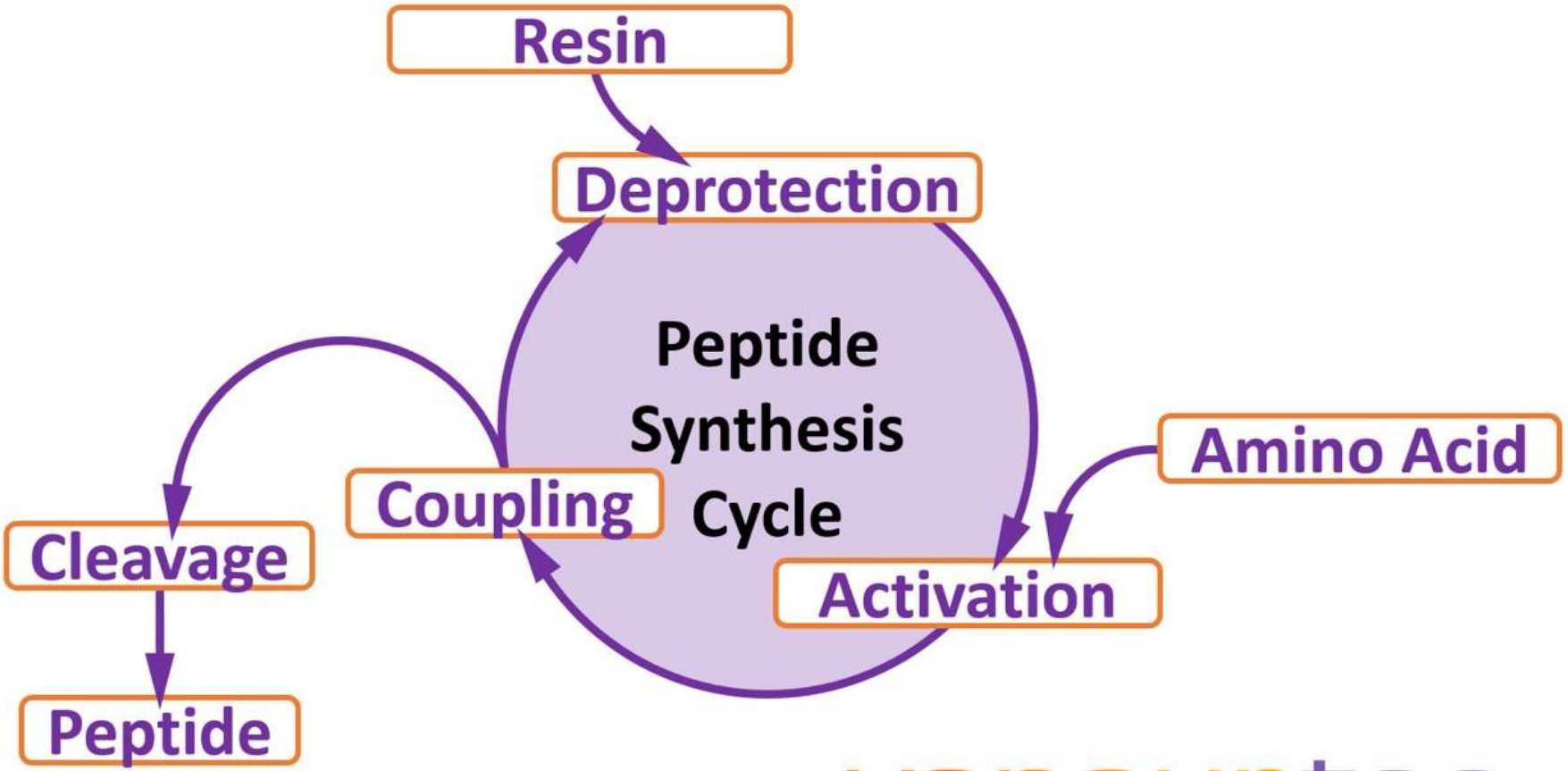
Peptide synthesis is usually a sequential deprotection/coupling process:



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Many of the problems occur during the activation step:

- Traditional methods for activating amide formation are not optimal for peptide synthesis.
- New activating agents reduce the reactivity of the activated species
- Can be low yielding/require longer reaction times

If the activation and coupling steps can be separated, and their environment controlled accurately, much more reactive species can be used without the risk of side reactions

- Can use more reactive activation
- Increase yields of each coupling
- Accelerate reaction time

Technology to solve a chemical problem

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Continuous flow SPPS

- Activation and coupling occur separately
- Activated amino acid passed over deprotected end of peptide
- Temperature of activation independent of coupling
- Can optimise activation for each amino acid
- Significant opportunities for real-time monitoring

Fixed-bed for SPPS

No channelling
- Accurate control over residence time distribution
- A challenge – resin swelling due to peptide growth

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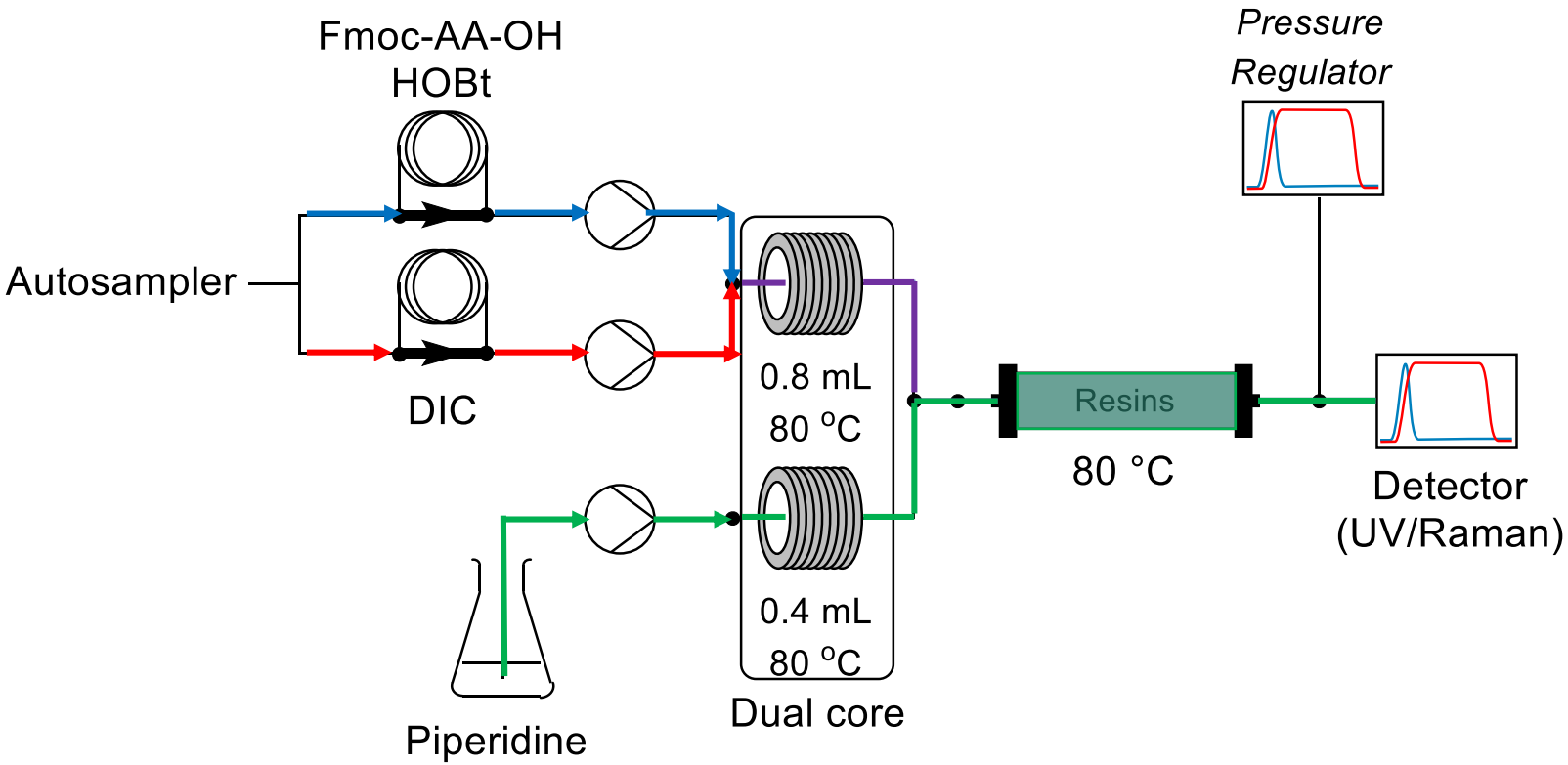
CF-SPPS



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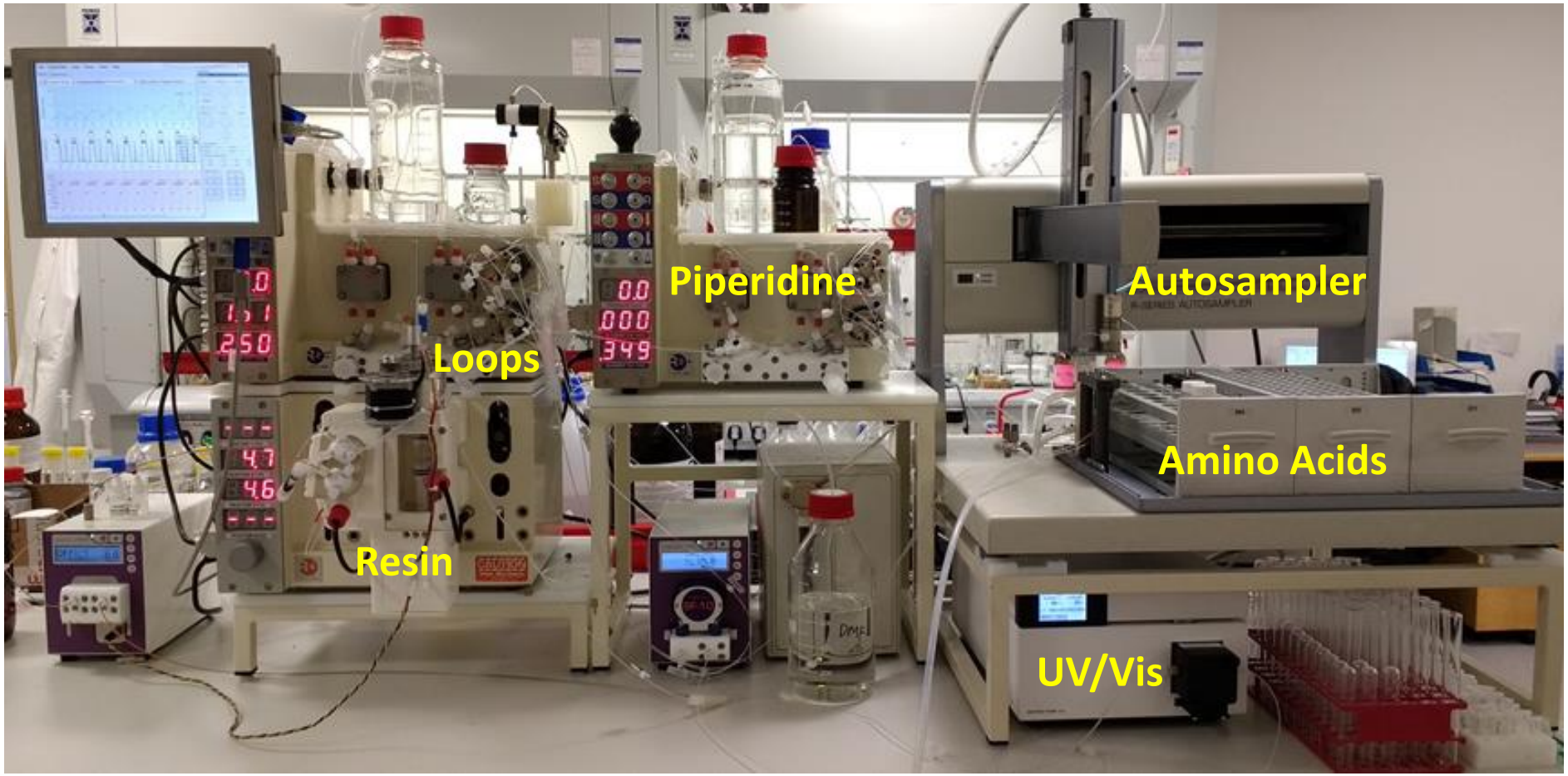

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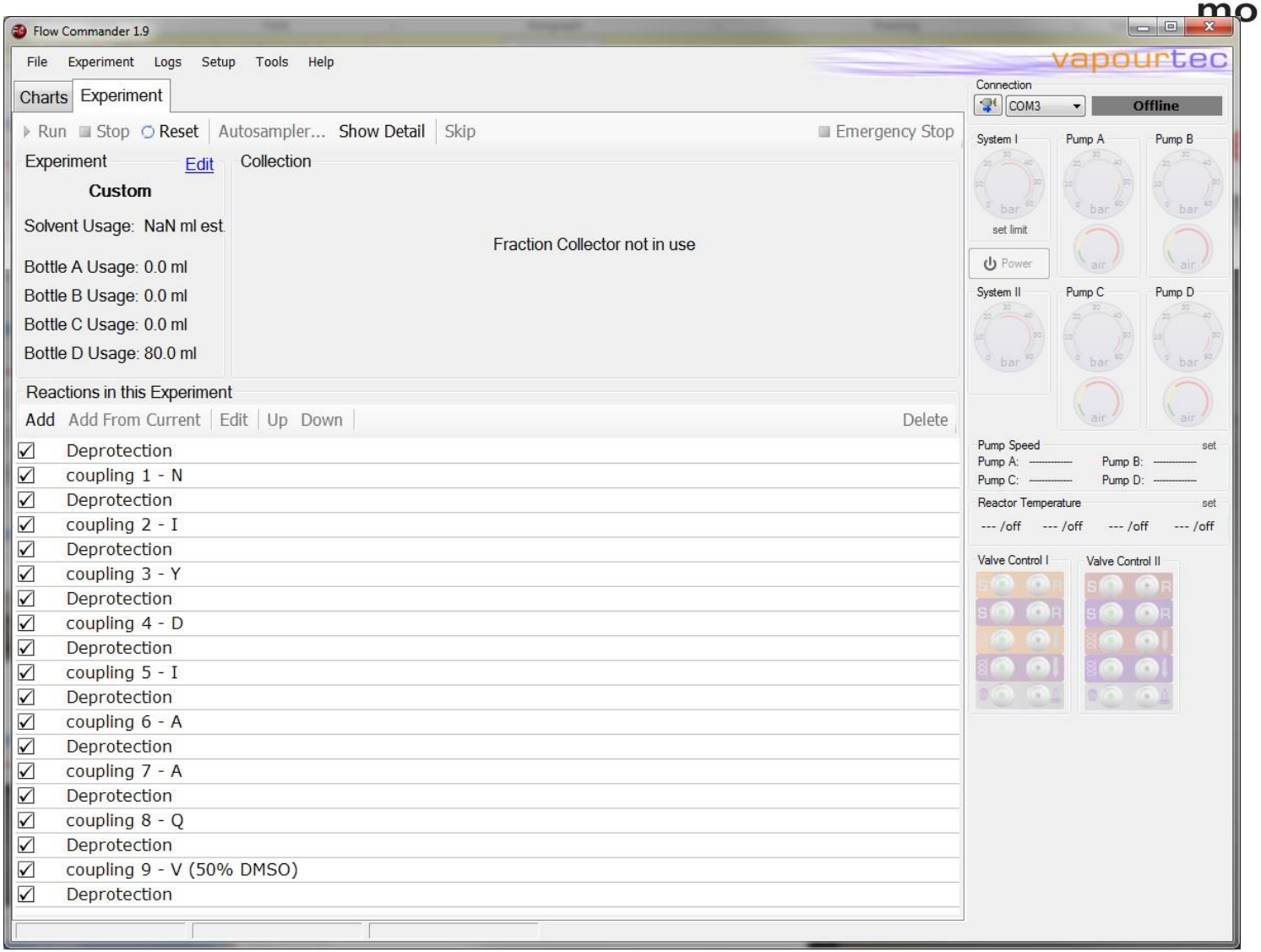

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Advantages:

1. Use fewer equivalents of amino acid (2-4 eq.)

2. Many activating agents can be used (DIC/HOBt)

3. Difficult sequences can be optimised quickly by detecting the problematic steps

4. Sensitive monomers (e.g. N-linked glycans) can be handled more efficiently

5. The peptide can be scaled up linearly

6. Current deprotection/coupling cycle 10-15 min

7. Reduced volume of solvent required for washing

8. Real-time UV-monitoring of Fmoc cleavage: monitoring conversion

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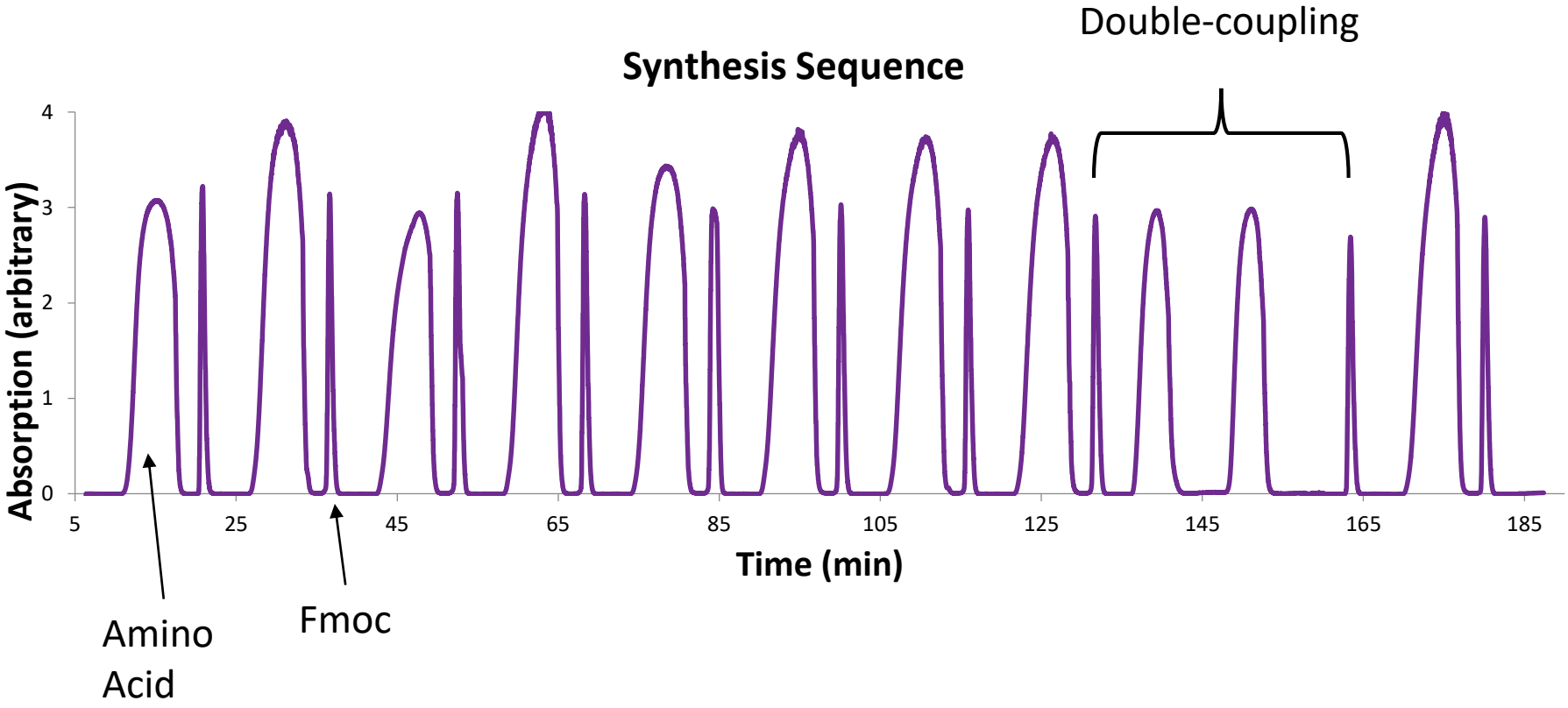

ACP carrier fragment (65-74)

H_2N-VQAAIDYING-$CONH_2$

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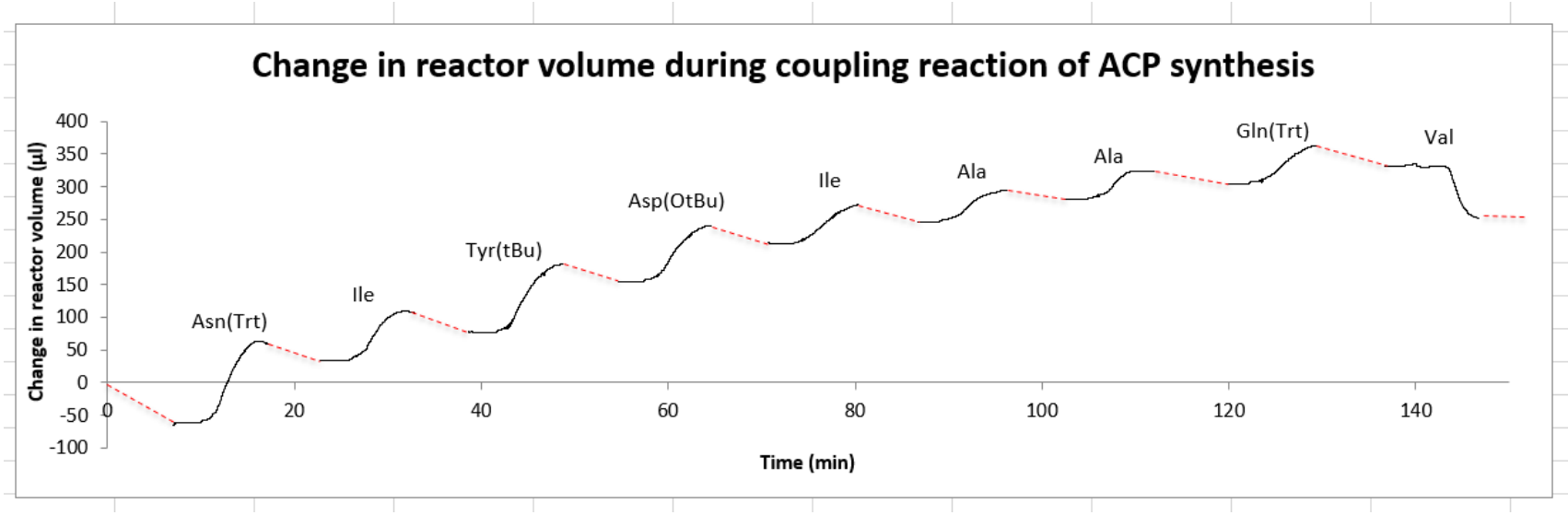
Resin swelling and peptide growth

As the peptide chain grows in length, the volume it occupies increases. As a result, the fixed bed “grows”

We have developed a reactor that detects and measures changes to the volume occupied by the peptide chain

Each amino acid has a typical volume change associated with coupling, and this is repeatable



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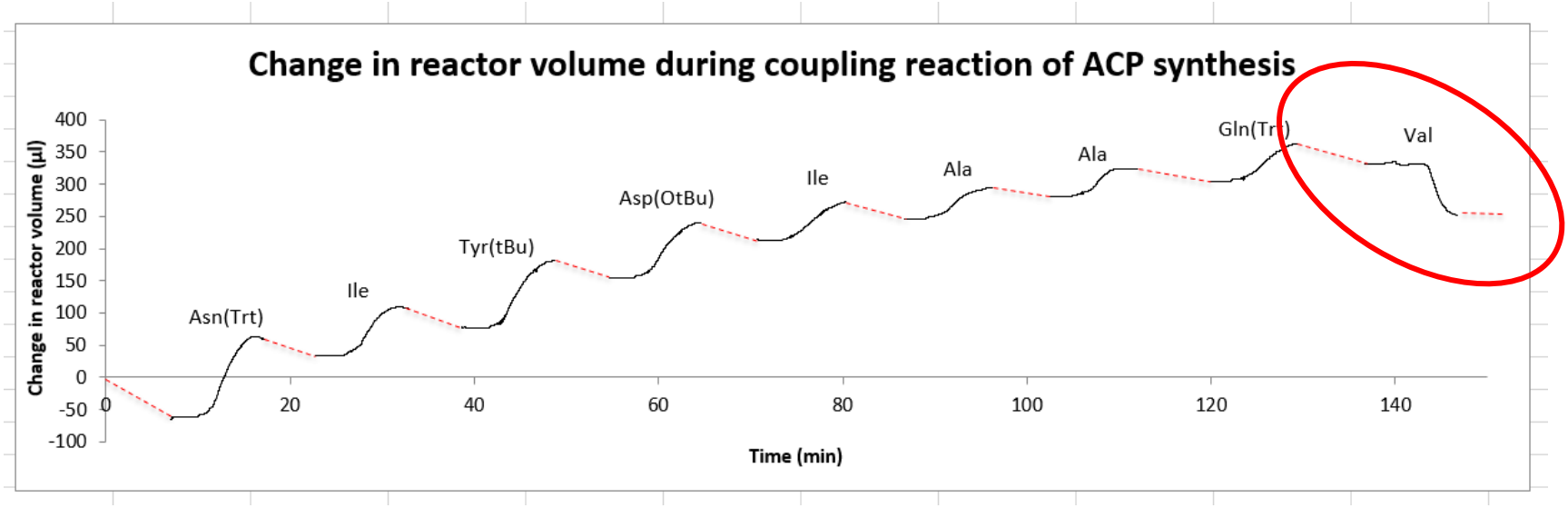
Resin swelling and peptide growth

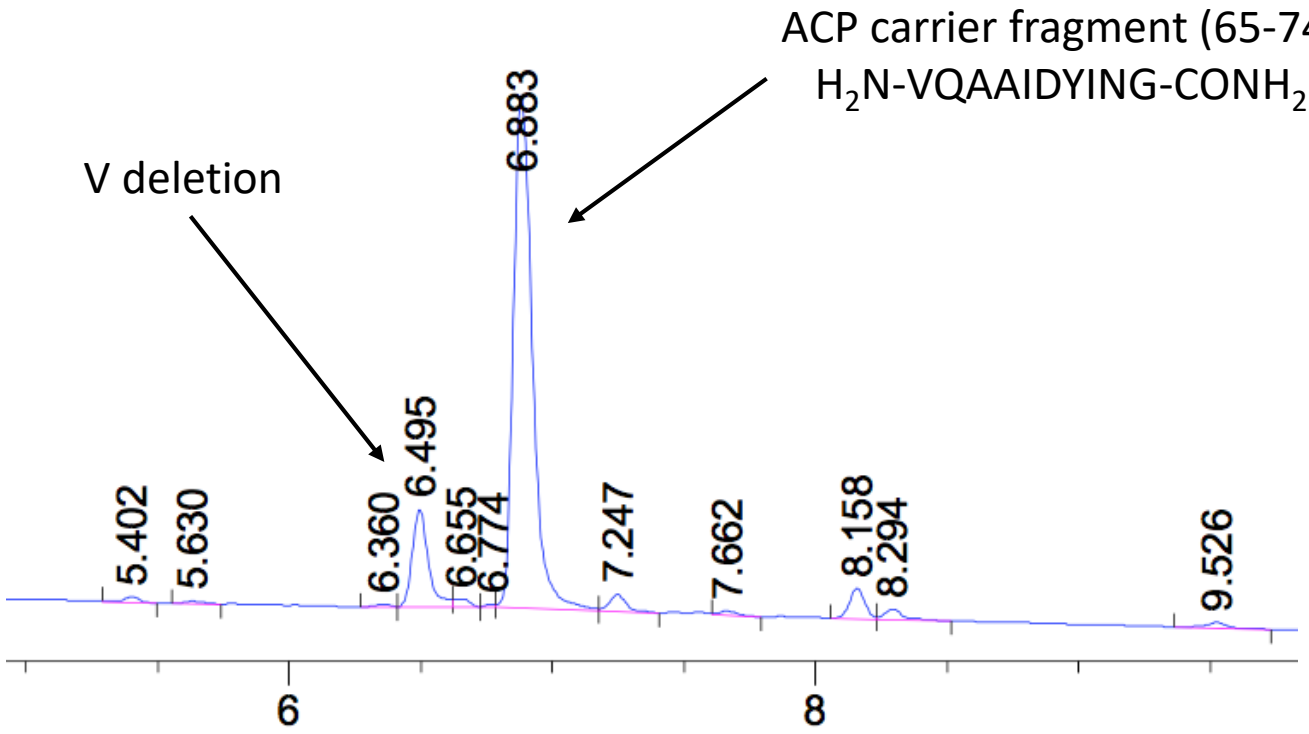
Using these data, each coupling step can be optimised

During synthesis, the coupling efficiency of each step is monitored in real-time

A failed coupling, or coupling that is inefficient can be detected

Collapse of the resin in an aggregation event has resulted in a poor coupling of valine




ACP carrier fragment (65-74)
H2N-VQAAIDYING-CONH2
V deletion
6.883
6.495
5.402
5.630
6.360
6.655
6.774
7.247
7.662
8.158
8.294
9.526
6
8

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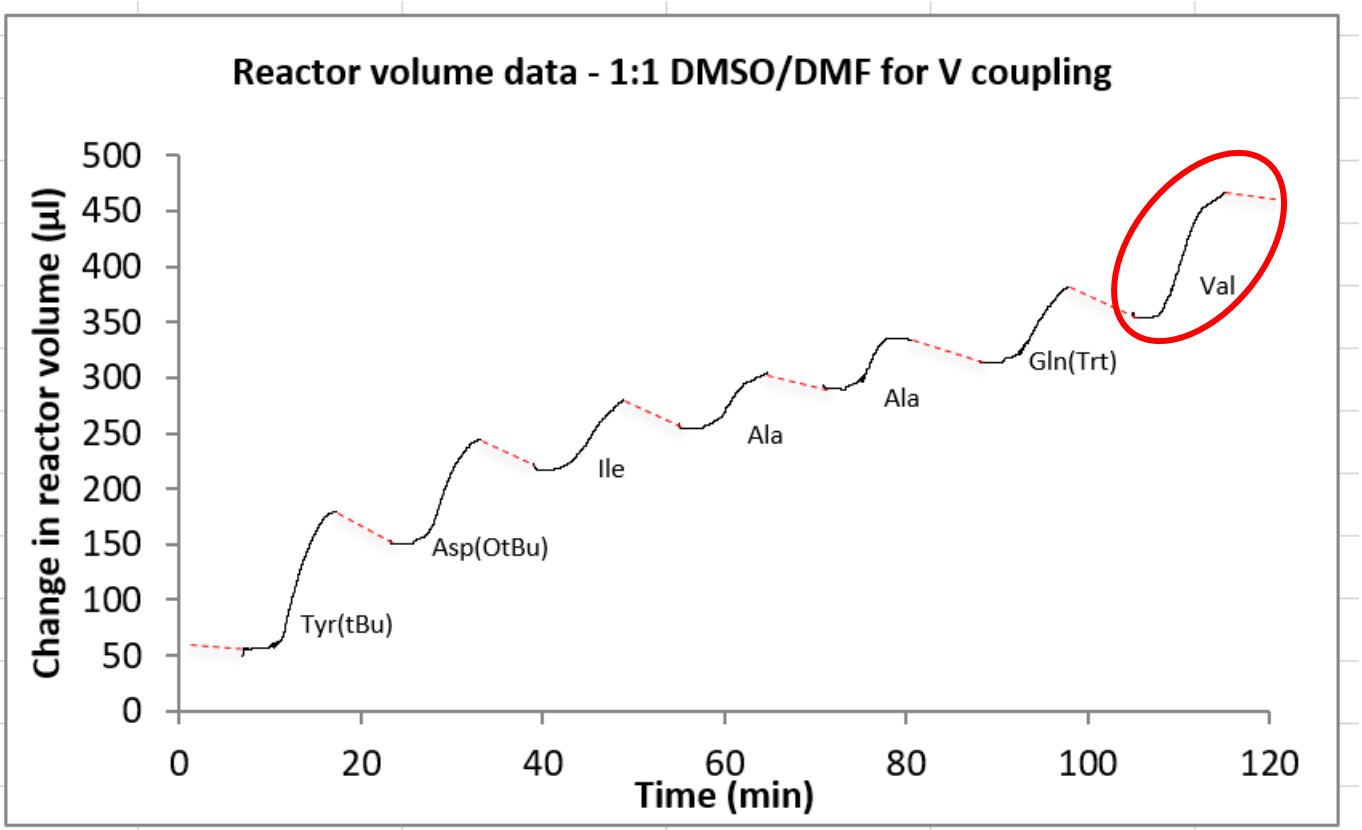
Literature suggested that aggregation events can be disrupted by DMSO addition of DMSO prevented aggregation, and the coupling was successful



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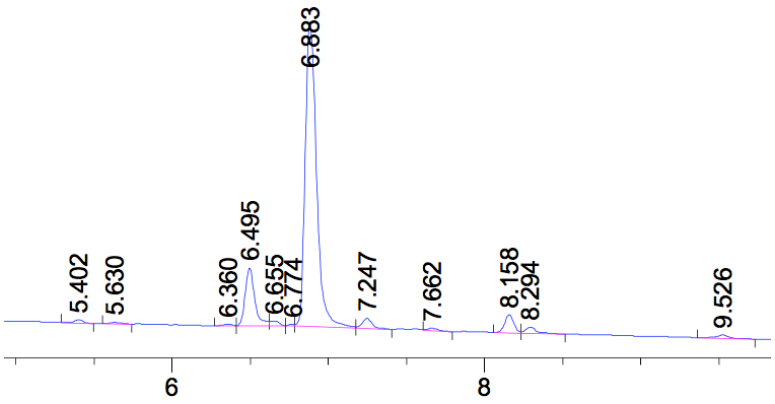
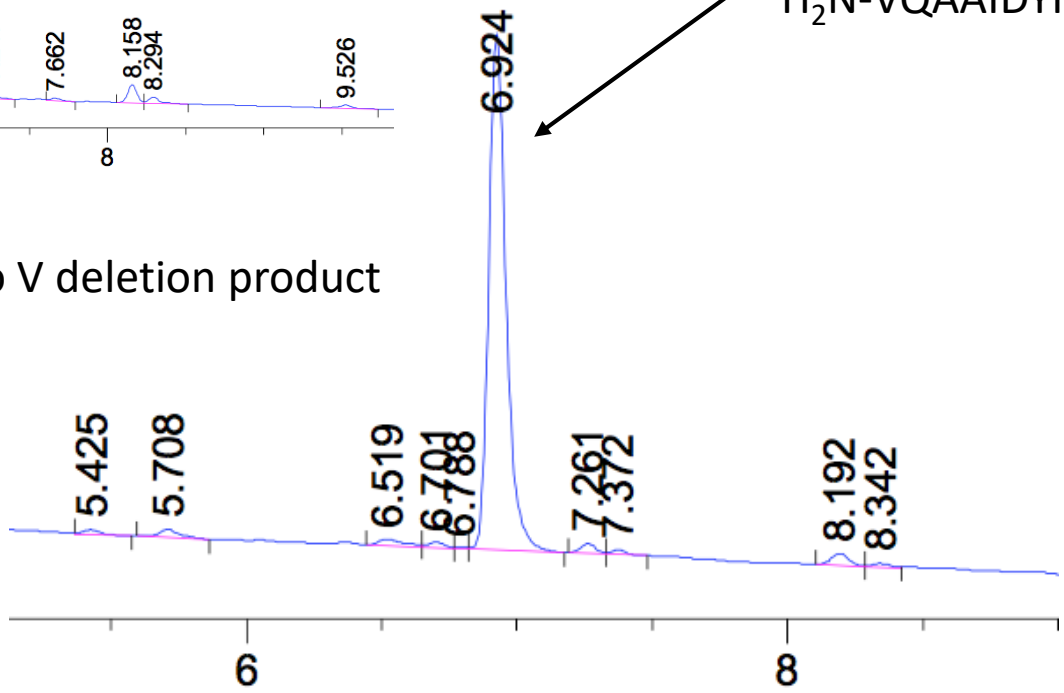


ACP carrier fragment (65-74)
H_2N-VQAAIDYING-$CONH_2$

No V deletion product



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Scaling up

Optimisation experiments carried out using 0.2 g Rink amide resin, 0.16 mmol of peptide

To increase throughput, the optimised conditions were applied to a reactor filled with 0.4 and 1.0 g of resin respectively (0.32 and 0.8 mmol of peptide)

The peptide was obtained crude, with a purity of **94%** in little over 2 hours

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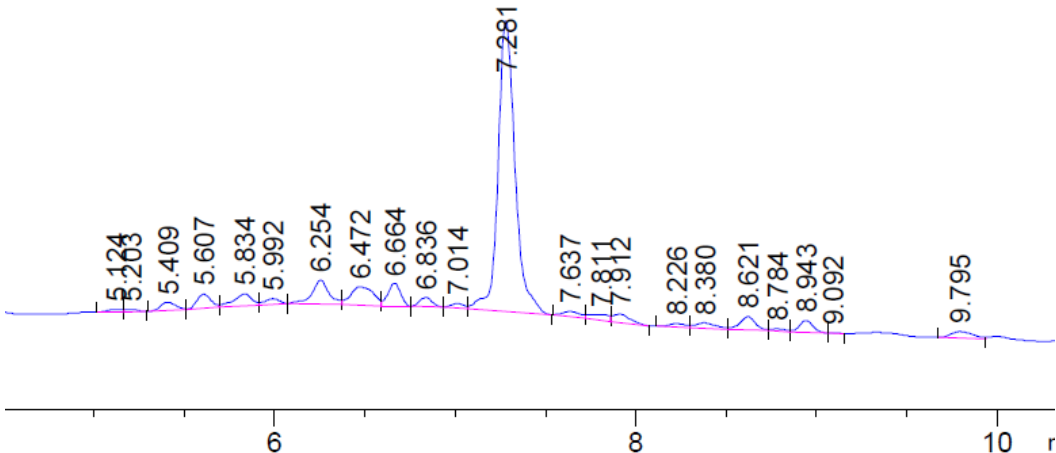
Longer peptides

- Synthesis of a peptide with the length around limits of linear SPPS (~40 aa)
- The project aims for large scale production of this peptide
- Crude from un-optimised gram scale synthesis (HPLC)
- Purity around 60%- observed impurities mostly due to post-synthesis Met oxidation
- Isolated (crude) yield >90%
- Cost of reagents and resin for 1g of crude: ~£50 (based on catalogue prices)
- Synthesis time around 11 hours



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Conclusions

- Modular platform built around the R-Series
- Development of the variable bed flow reactor allows detailed monitoring of coupling efficiency
- Effective optimisation on the small scale
- Direct translation of optimal conditions to increased scale
- Wide range of activating agents
- Can use different solvents/reagents for specific couplings

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