



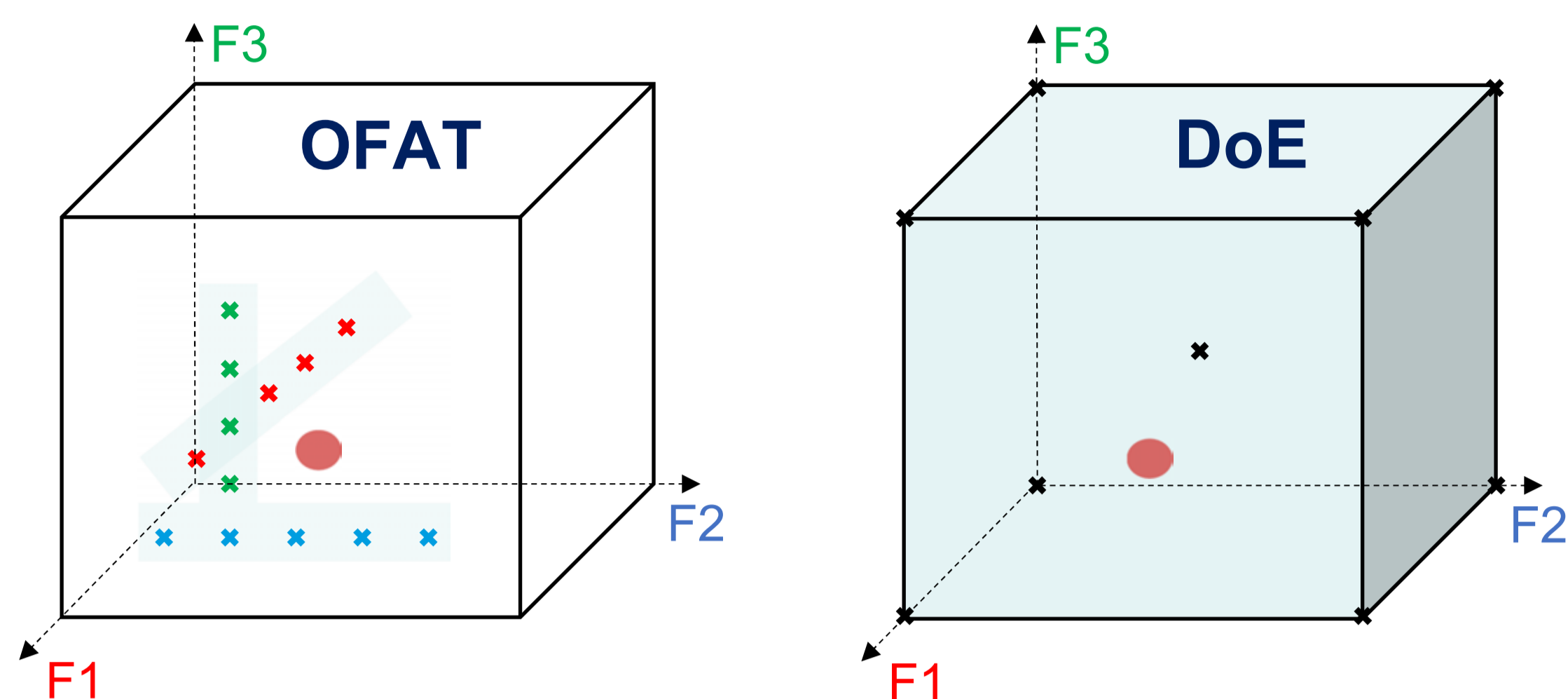
Design of Experiments as Powerful Tool for Peptide Cleavage Development



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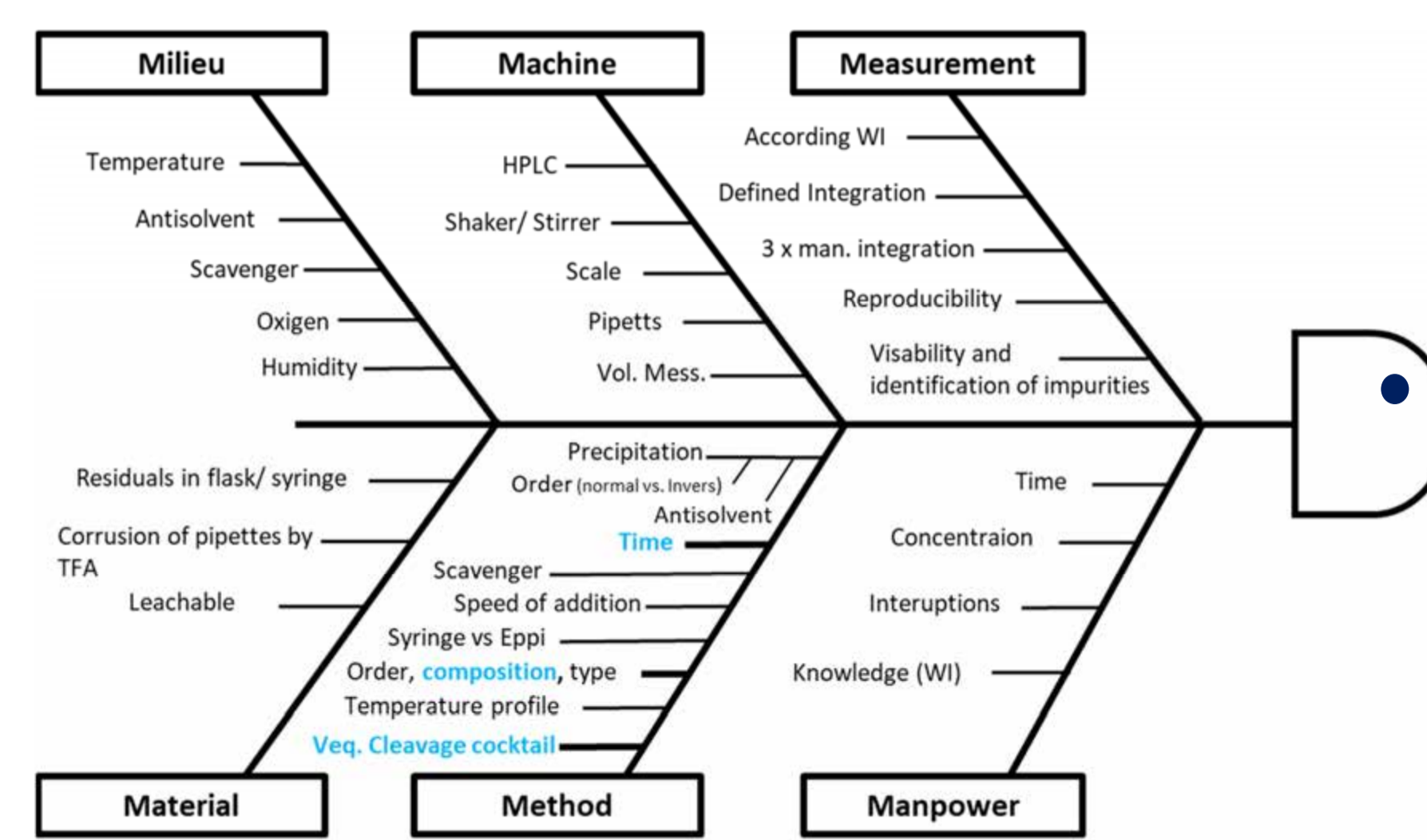
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OFAT vs. DoE



Which factors to screen?

- Ishikawa or brainstorming
- Identify most critical factors
- Focus on quantitative factors



Exemplary Standard Approaches

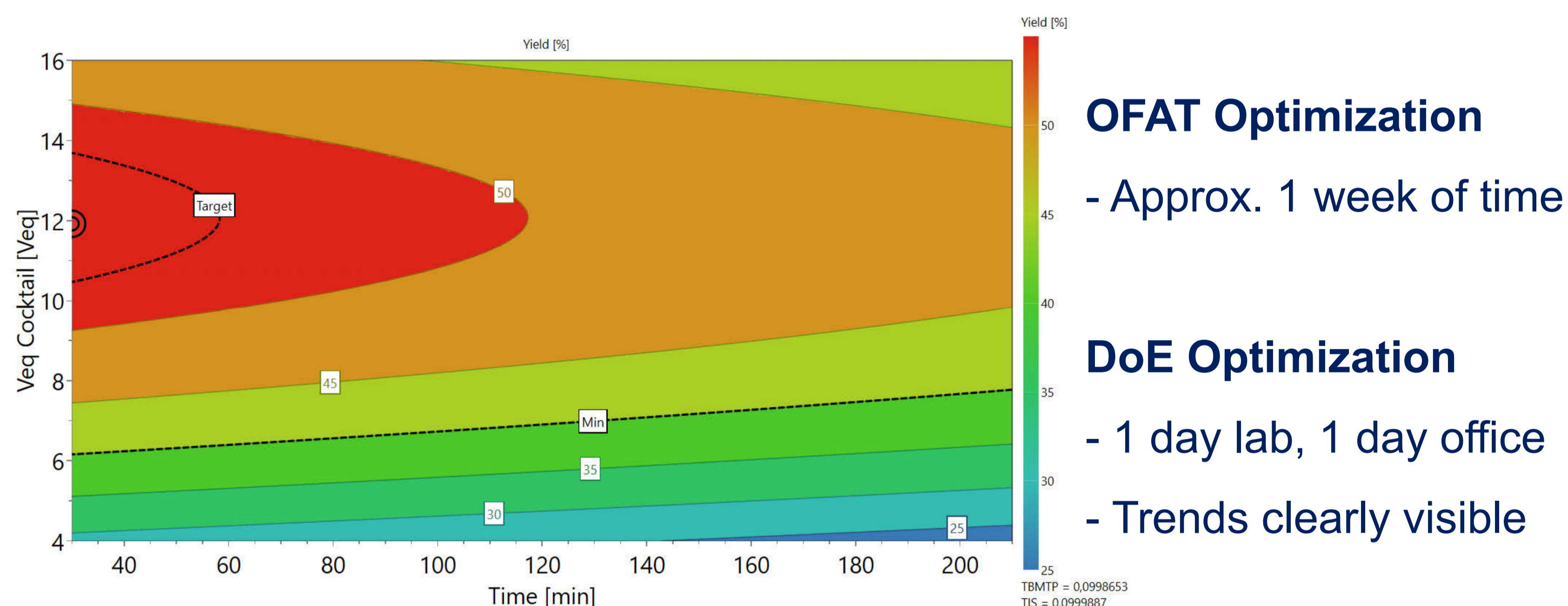
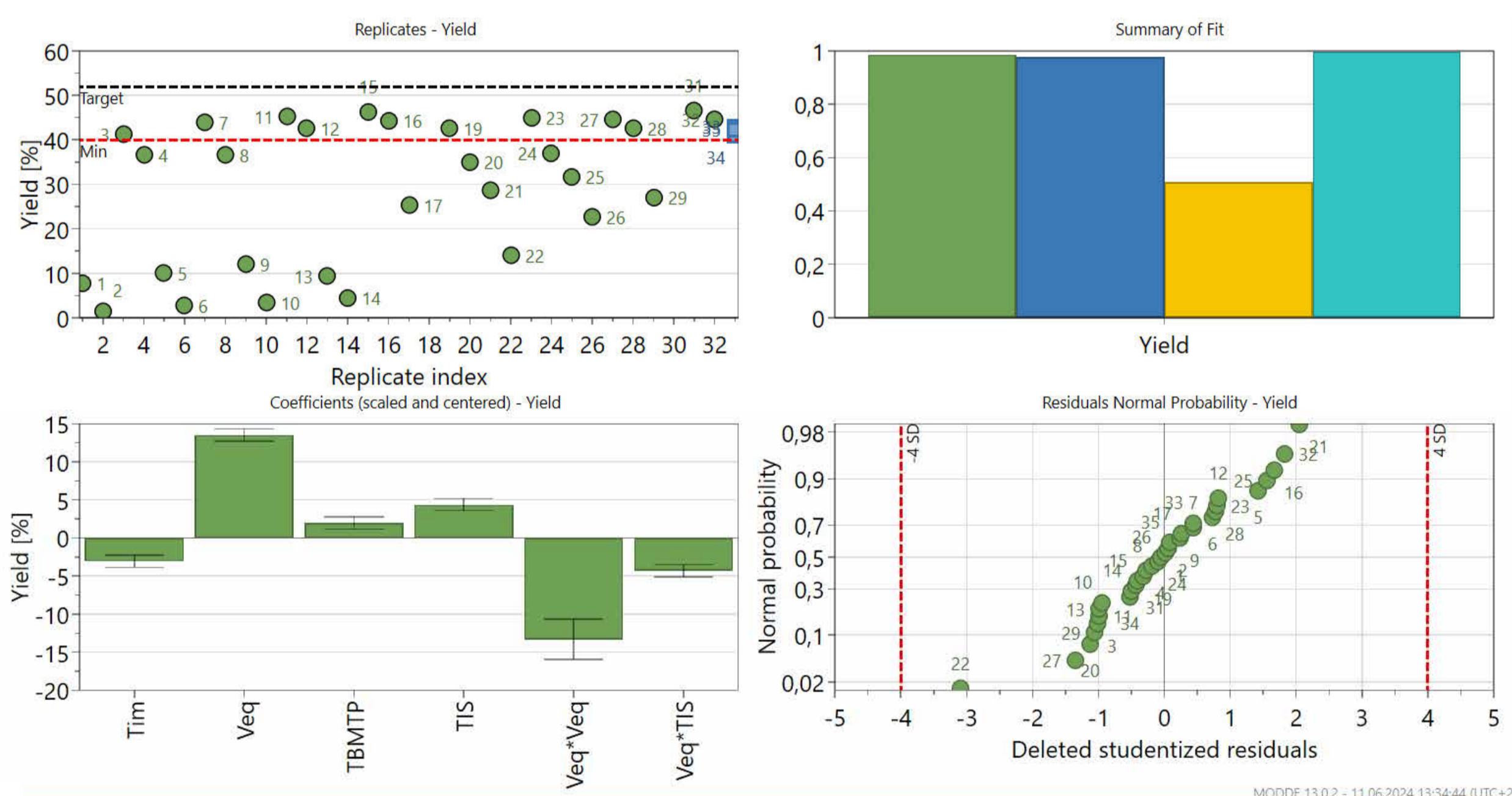
OFAT: 3 factors, 5 levels
3 × 5 settings - 2 replicates = 13 experiments

DoE: 3 factors, 2 levels
2³ + 3 replicates = 11 experiments

OFAT <u>O</u> ne <u>F</u> actor at <u>a</u> <u>T</u> ime	- Small designed space - Iterative experiments - No information about factor interactions
DoE <u>D</u> esign of <u>E</u> xperiments	- Large designed space covered - Parallelization of experiments - Information about factor impact and interactions

Case study of peptide with ~20 AA, linear, Cysteine contained

Factor	Generic	OFAT	OFAT ^{TBMTP}	Exp. SP ^{TBMTP}	Exp. SP ^{TBMTP}	Rob. SP
Cocktail Veq.	20.0 Veq.	15.0 Veq.	15.0 Veq.	13.0 Veq.	13.0 Veq.	12.8 Veq.
Time	120 min	90 min	90 min	90 min	90 min	90 min
Water	2.5%	2.0%	2.0%	5.5%	5.5%	5.6%
TIPS	2.5%	8.0%	8.0%	7.0%	7.0%	7.0%
Thioscavenger	2.5%	6.0% (DTT)	6.0% (TBMTP)	7.5% (TBMTP)	7.5% (TBMTP)	7.5% (TBMTP)
TFA	92.5%	84.0%	84.0%	80.0%	80.0%	79.9%
Yield	30.4 mAU·min	53.7 mAU·min	51.3 mAU·min	51.8 mAU·min	51.8 mAU·min	47.6 mAU·min
Purity	64.0%AN	71.2%AN	69.8%AN	71.5%AN	71.5%AN	75.9%AN
Theo. Yield			45.3 mAU·min			47.6 mAU·min
Theo. Purity			73.0%AN			75.9%AN

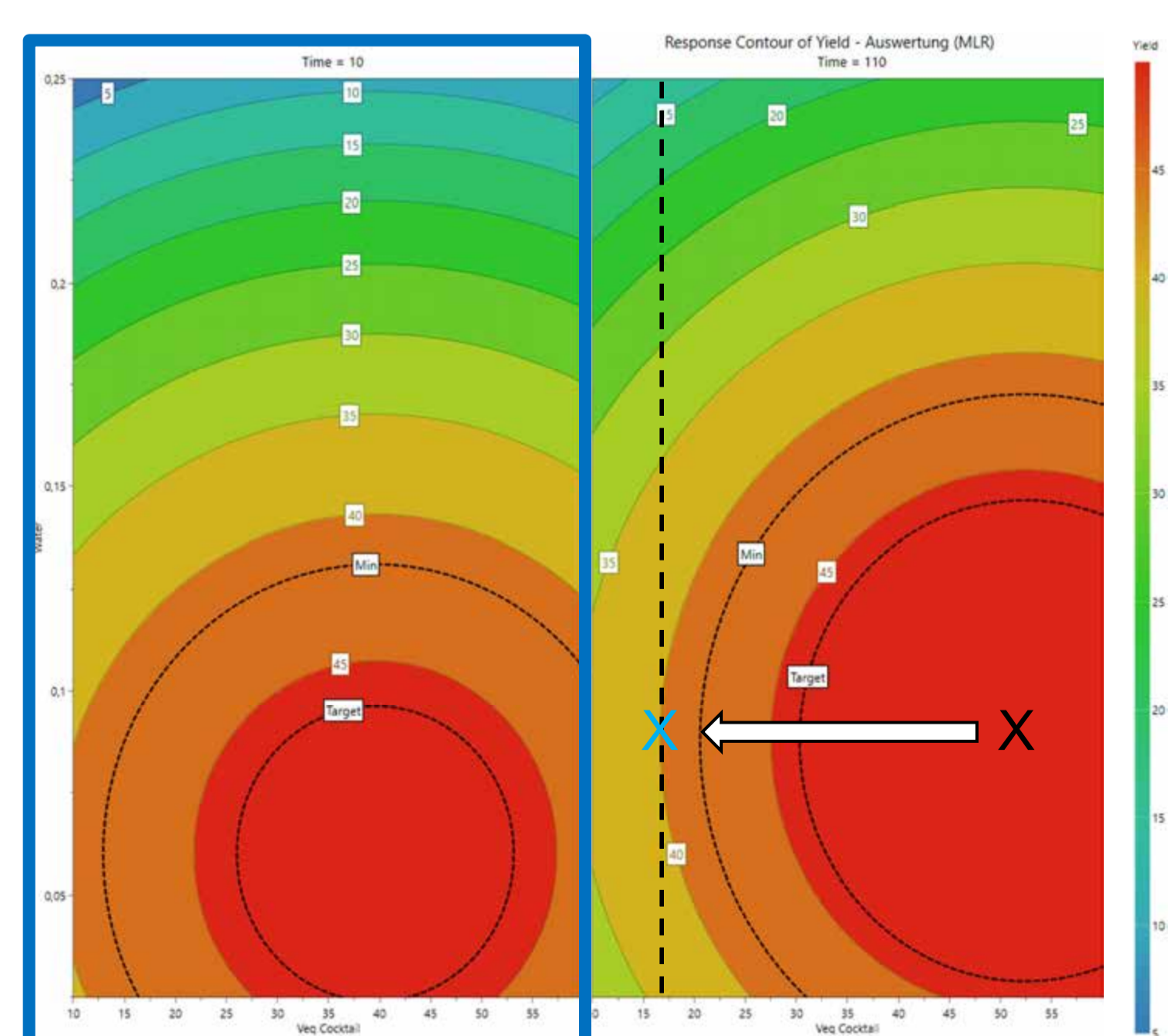


PMI* / Cost

- Reaction time of 10 min is too short for upscale
- $\frac{1}{3}$ PMI for $\frac{4}{5}$ yield compared to optimum

Red: High yield
Orange / yellow: Good to moderate yield
Green to blue: Low yield

*PMI: Process Mass Intensity



Conclusion

- DoE for in-depth evaluation results
- Qualitative factors preferable with OFAT in addition to DoE
- Similar number to less experiments than with OFAT
- Not as intuitive as OFAT
- Less time consuming
- Very positive customer feedback

- Main DoE expertise in peptidyl resin cleavage in **early-stage development** (50 mg scale)
- **Upscale successfully performed** with adaptations according to 'RGT' rule
- Other **process** steps in peptide synthesis were also **successfully optimized**
E.g. (on-resin) cyclization, deprotections, reduction

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