

Mathematical Modelling and Analysis of Membrane-Enhanced Liquid-Phase Oligonucleotide Synthesis on Homostars

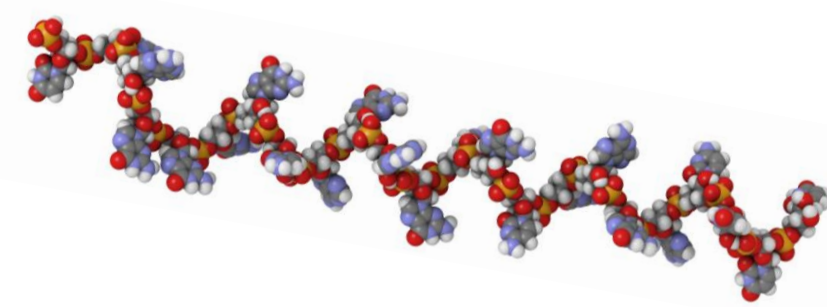
Alberto Saccardo,
Benoît Chachuat*

The Sargent Centre for Process Systems Engineering,
Department of Chemical Engineering, Imperial College London,
UK

* Corresponding Author: b.chachuat@imperial.ac.uk

OLIGONUCLEOTIDES THERAPEUTICS

Oligonucleotides and peptides (TIDES):
high specificity and selectivity against
hard-to-treat diseases



Rise in chronic diseases 2020-2050:
2x for U.S. 50+ [1]

TIDES market: **USD 42B in 2022** → **USD 69B by 2028**



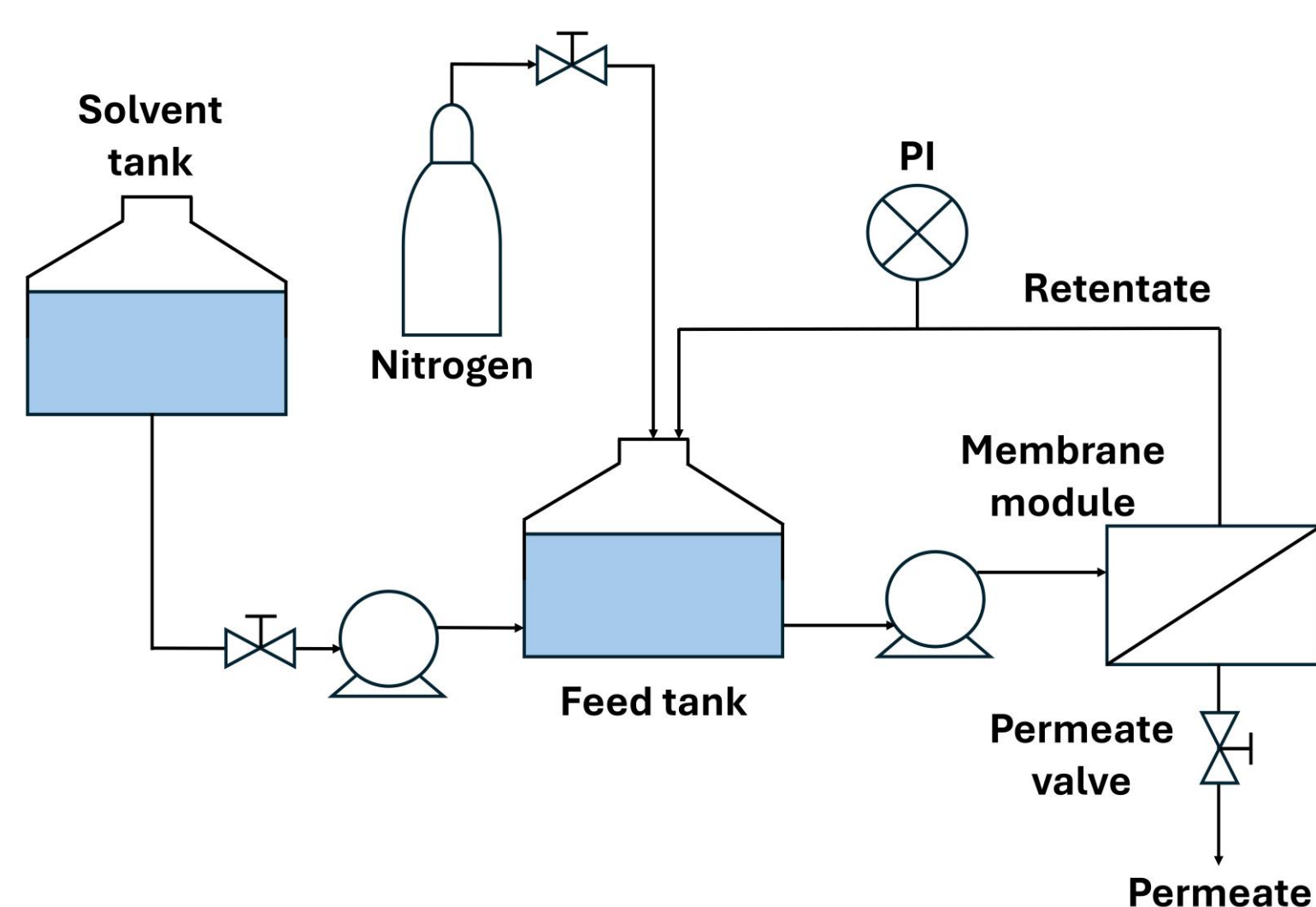
Unsustainable industrial production
(Solid Phase Synthesis)
15000 kg of waste per kg of product [2]

Solutions?

LIQUID PHASE OLIGONUCLEOTIDES SYNTHESIS (LPOS)

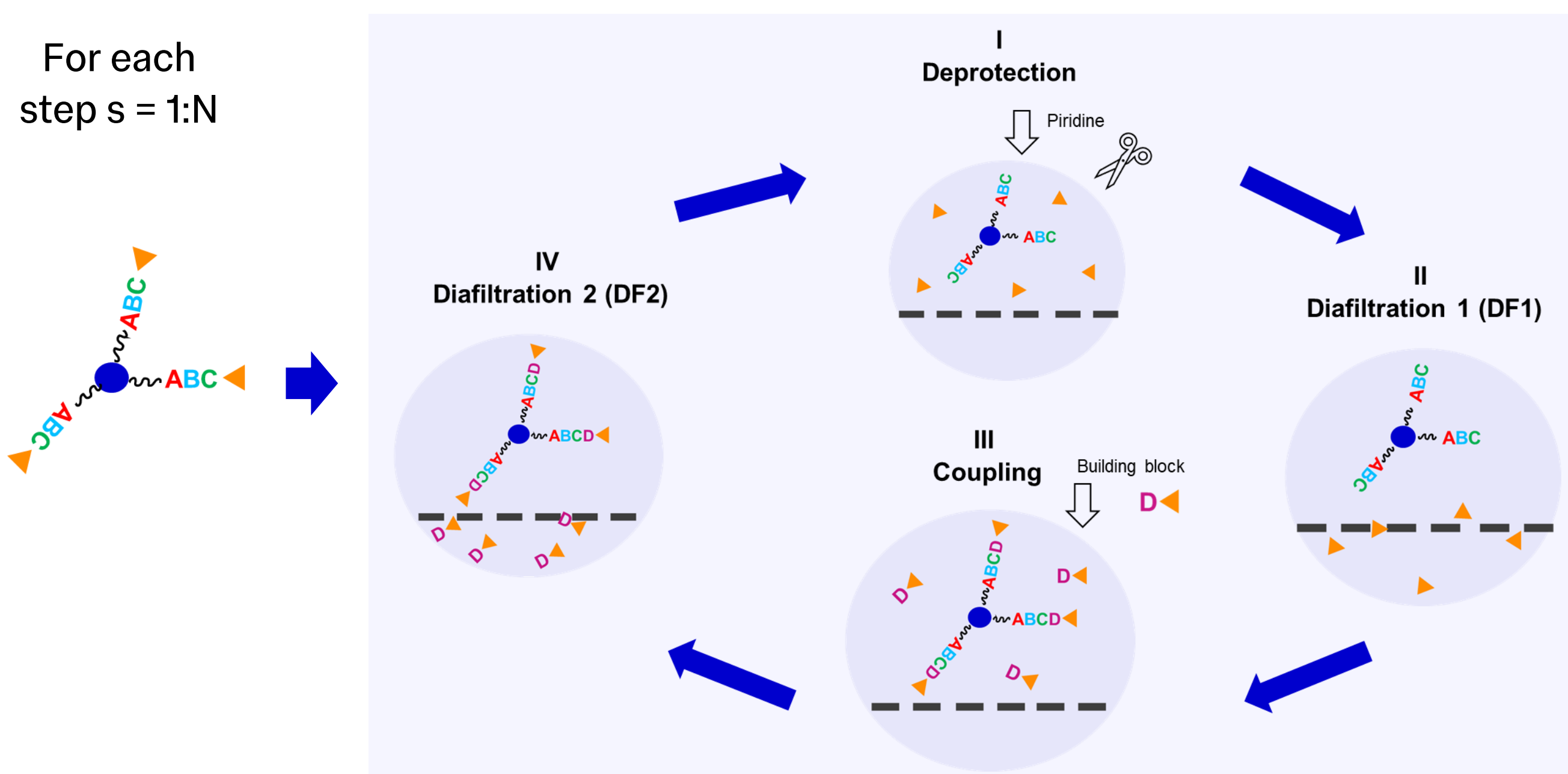
Membrane-Enhanced Liquid Phase Oligonucleotides Synthesis (LPOS)

- High scalability
- High yield and purity
- Easy reaction monitoring
- Long sequences



LPOS process for a product composed by N building blocks

For each step $s = 1:N$



MATHEMATICAL MODELLING

We developed a novel mathematical model for LPOS[3]

- **DIAFILTRATION** $\frac{dX}{dt} = -\frac{Q}{V} \cdot (1 - r_X) \cdot X$
- **DEPROTECTION** $\frac{dOD_k}{dt} = -\frac{dOP_k}{dt} = k_D \cdot OP_k \cdot MSA$
- **COUPLING** $\frac{dOP_{k+1}}{dt} = -\frac{dOD_k}{dt} = k_C \cdot BB_n \cdot OD_k \cdot DCI \cdot CSO$

Correct coupling reaction at step s $ABC + D \rightarrow ABCD$

Sequence errors

Truncation errors

ABC

Repeated sequences

ABCC

Neglected: C is quenched and washed out in two diafiltration steps

Combinatorial error propagation! In our model

Desired product: ABCDEF
• Longest sequence
• Exact composition known

VS

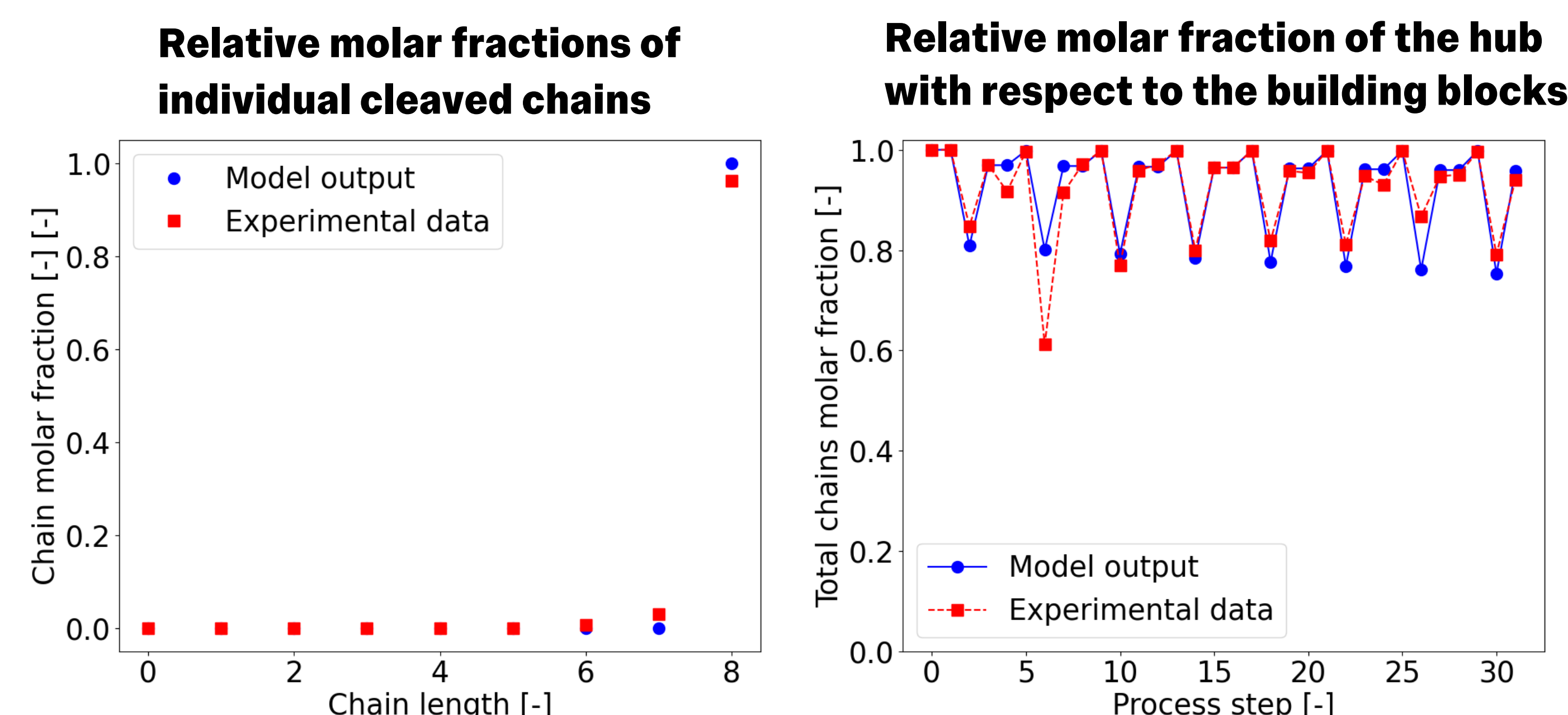
Impurities:
• Overall concentration known



Reaction between C and D neglected: MW of BB << than the one of the hubs

EXPERIMENTAL VALIDATION

Good agreement between simulations and experimental data for an 8-mer



FEASIBLE OPERATING SPACE ANALYSIS

Process performance variables

- Yield** $Y = \frac{OP_N(t_{F2,N}^-)}{HS \cdot NB}$ → Desired sequence at the end of the process
- Purity** $P = \frac{OP_N(t_{F2,N}^-)}{OT(t_{F2,N}^-)}$ → Residual building blocks before coupling
- Residue** $R = \max_{n=1 \dots N} \frac{\sum_{k=1}^n BB_k(t_{C,n}^-)}{OT(t_{C,n}^-)}$ → Total oligonucleotides before coupling

What input combinations lead to good process performance?

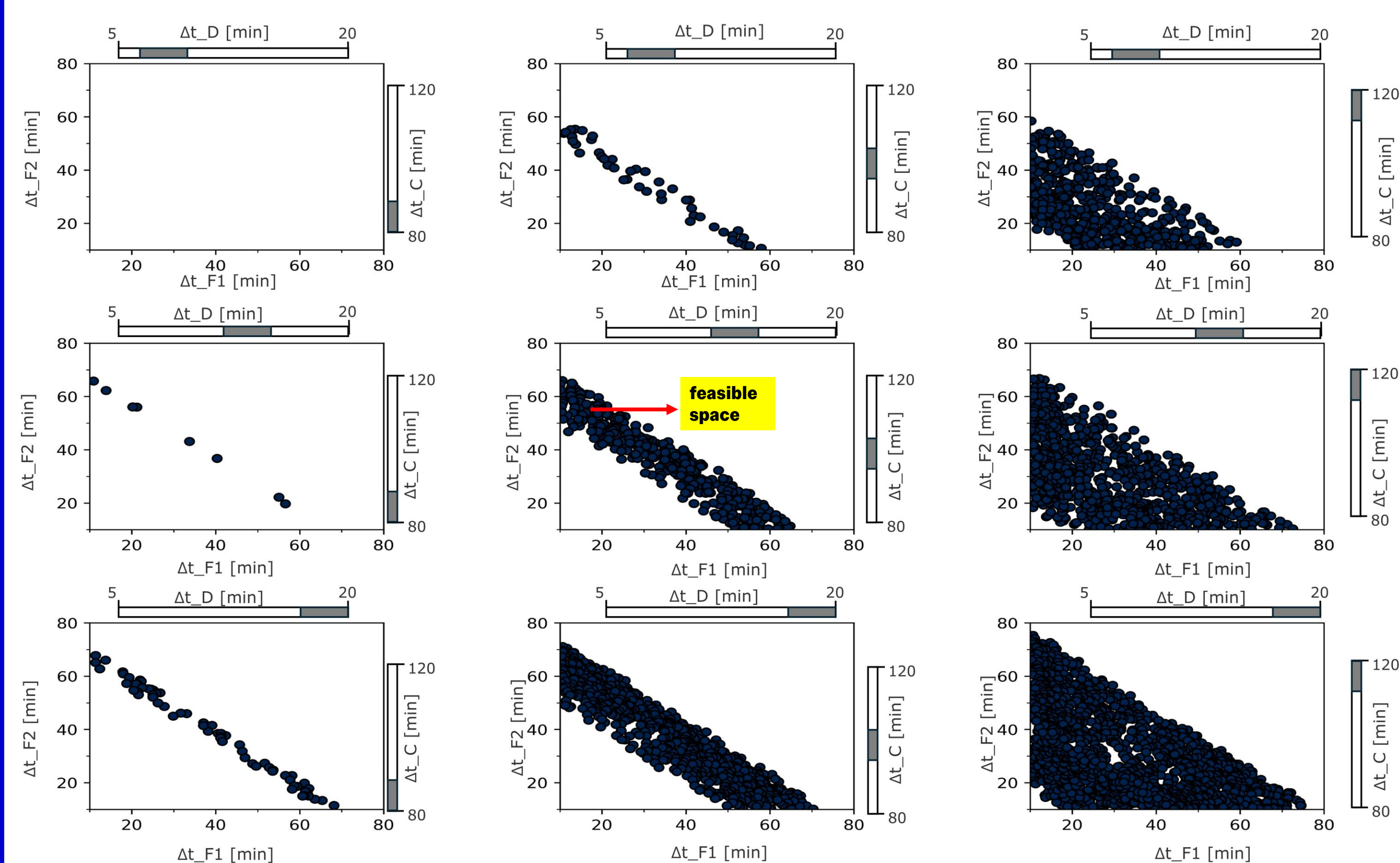
→ Nested Sampling Algorithm to explore **feasible operational regions**

Inputs

- Diafiltration times $\Delta t_{F1}, \Delta t_{F2}$
- Coupling and deprotection times $\Delta t_C, \Delta t_D$

“Good scenario”

- **Yield (Y):** $\geq 90\%$
- **Purity (P):** $\geq 90\%$
- **Residual building blocks (R):** $\leq 1\%$



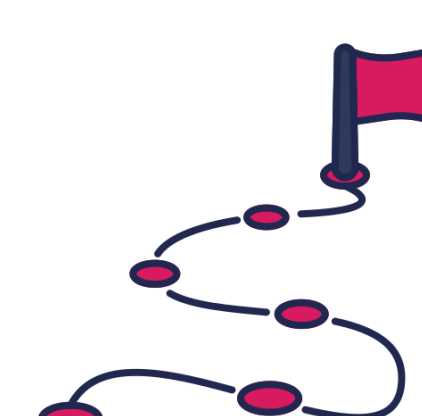
- Feasible operating space **bounded by** Δt_{F1} and Δt_{F2}
- **Longer** $\Delta t_C, \Delta t_D$: **greater flexibility**
- **Longer diafiltration durations:** > product losses
- **Shorter durations:** > building block residues

CONCLUSIONS

We developed the **first mathematical model** for Liquid Phase Oligonucleotides Synthesis, validated it with experimental data, and used it to analyse feasible process operations

Next steps

- Account for additional side reactions
- Account for parameter uncertainty in design space characterisation
- Model-Based Design of Experiments to improve prediction confidence



ACKNOWLEDGMENTS

This project received funding from the FASST (Flexible, Agile, Scalable and Sustainable Medicines Manufacturing) grant under the Innovate UK Transforming Medicines Manufacturing program. Saccardo and Chachuat gratefully acknowledge experimental support from Exactmer Ltd.

