RTH
CENTER FOR NON-CODING RNA IN TECHNOLOGY AND HEALTH

ENSAFE

UNIVERSITY OF COPENHAGEN

Peptide pesticides

off-target binding prediction

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Center for non-coding RNA in Technology and Health (RTH)

Faculty for Health and Medical Sciences

University of Copenhagen

Why does off-target assessment of biopesticides matter?

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Increased global food demand

Effective crop protection

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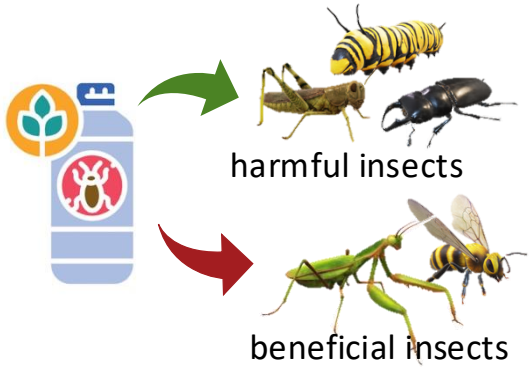
Effective crop protection



Novel biopesticides

Lack of EU regulations

Why does off-target assessment of biopesticides matter?

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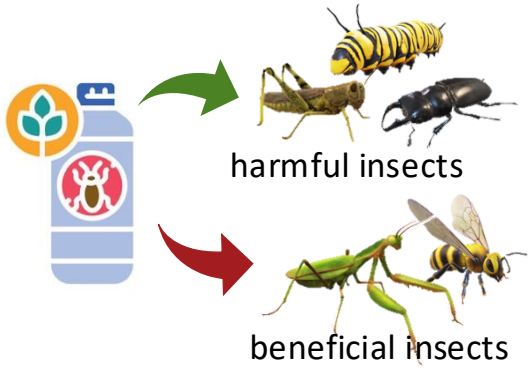


Novel biopesticides

Lack of EU regulations

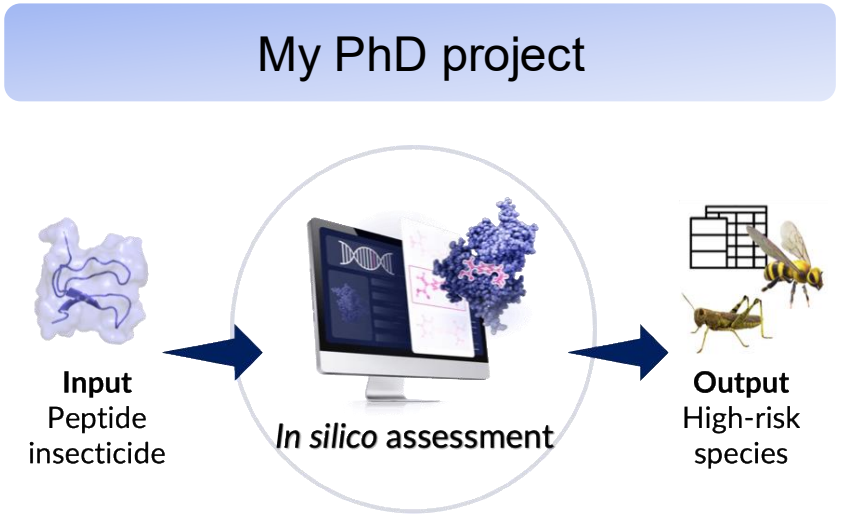
novo nordisk foundation
Novo Nordisk Foundation Challenge
Grant no. NNF24OC0087112

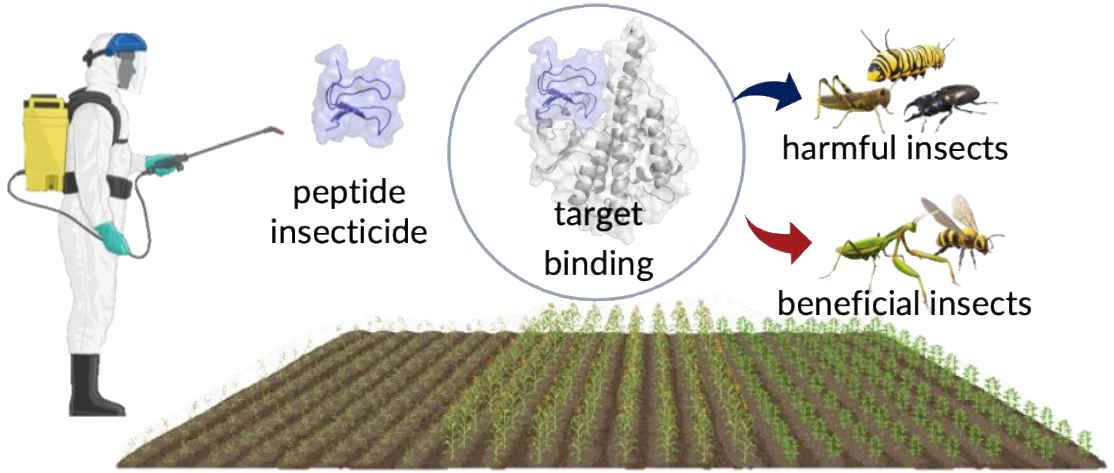
ENSAFE is an environmental risk assessment framework for siRNA- and peptide-based pesticides: integrating *in silico*, *in vitro*, and *in vivo* approaches.

Structural bioinformatics approaches and off-target binding prediction

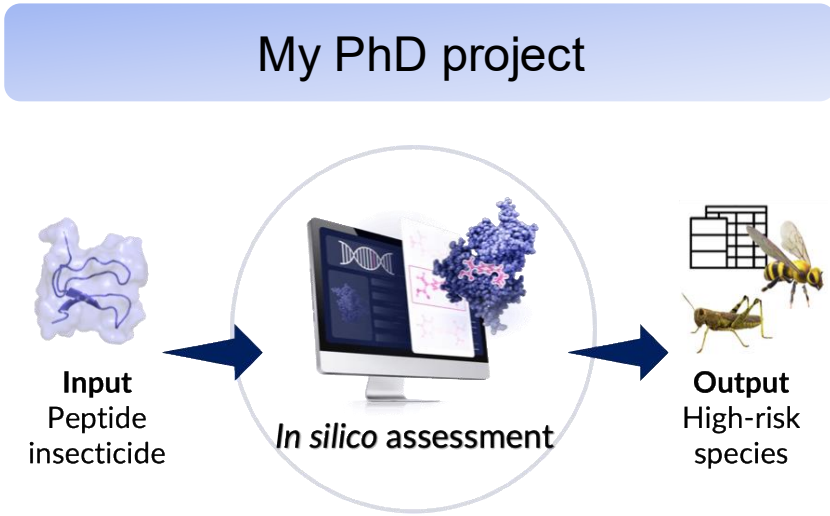
My PhD project

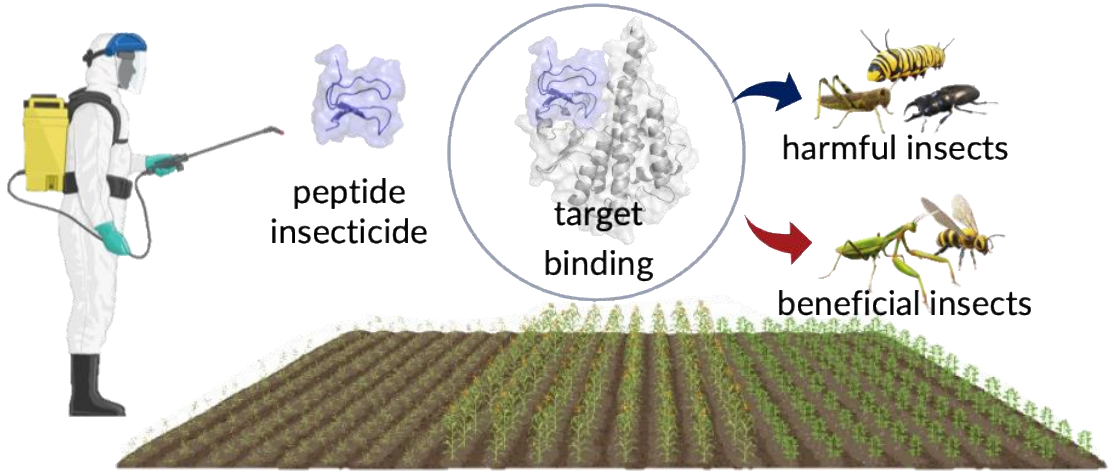
Structural bioinformatics approaches and off-target binding prediction





Structural bioinformatics approaches and off-target binding prediction





Hypothesis

- Off-target effects are through promiscuous target binding, which can be predicted from on-target
  - Conserved binding motifs.
  - Off-target structural plausibility.

Off-target binding prediction through conserved binding motifs and structural plausibility

- Prediction workflow

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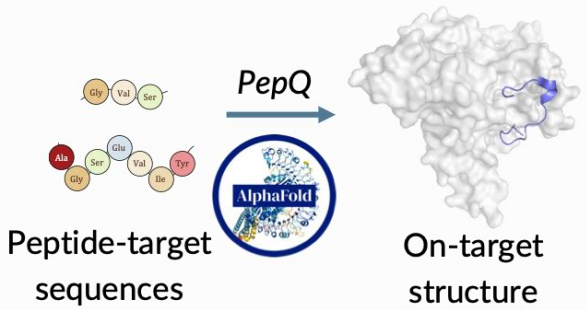
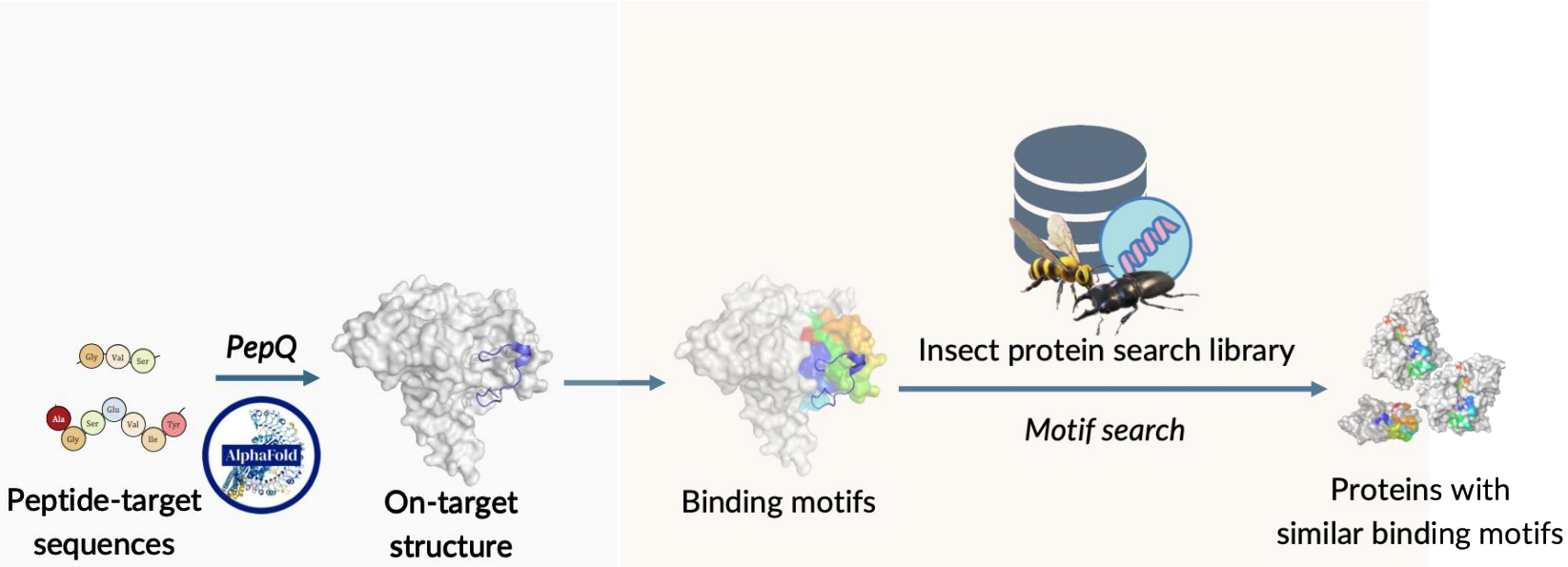


Module 1. On-target structure generation

Jumper, J *et al.* (2021). Highly accurate protein structure prediction with AlphaFold. Nature, 596(7873), 583-589.
Evans, R., *et al.* (2022). Protein complex prediction with AlphaFold-multimer. bioRxiv [Preprint](2022).

Off-target binding prediction through conserved binding motifs and structural plausibility

- Prediction workflow



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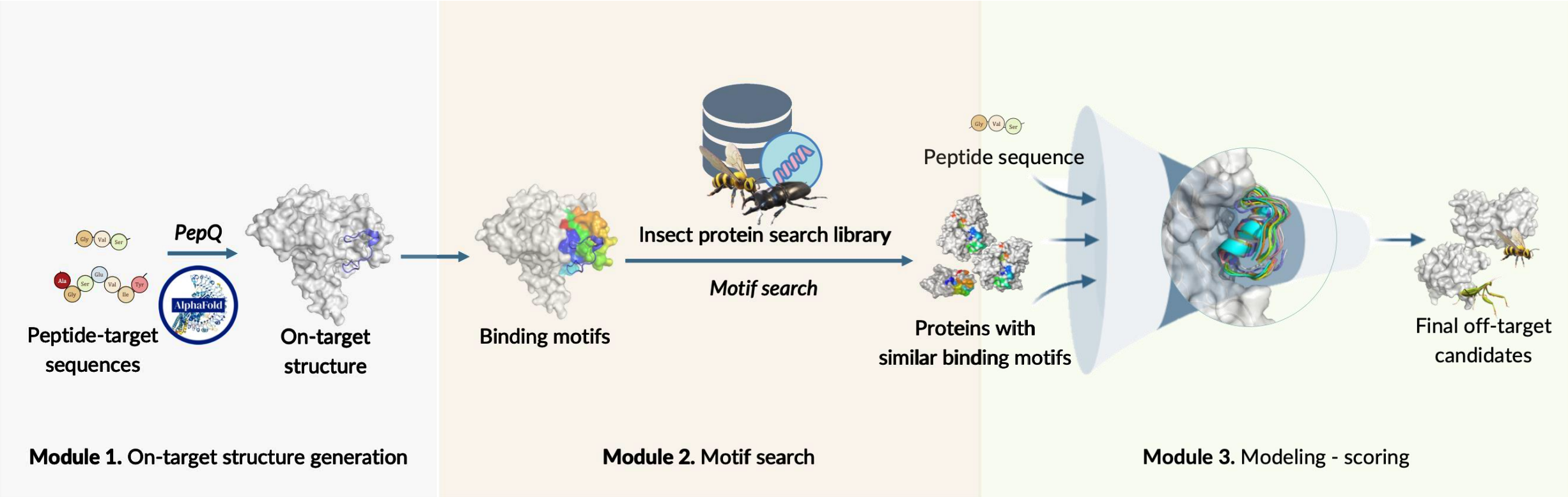

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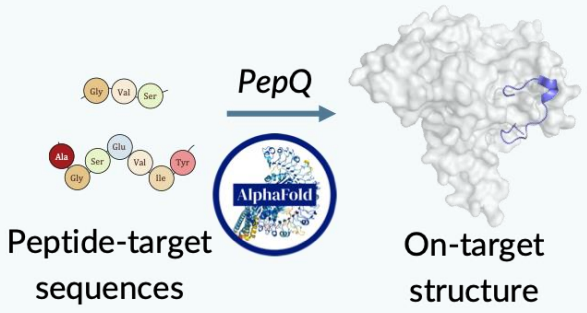
PepQ: peptide-protein model quality assessment



Module 1. On-target structure generation

- Generating the on-target structure generation using ColabFold
  - 5 models per run.
  - Confidence scores.

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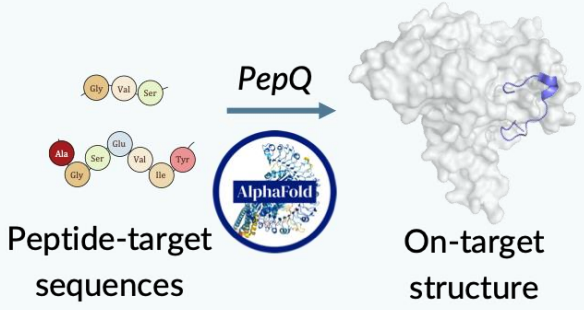

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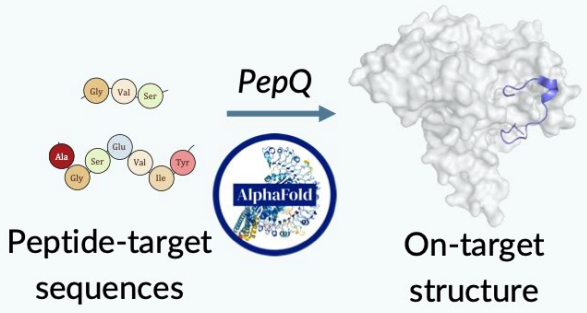
How to assess and select complexes with high quality?

PepQ: peptide-protein model quality assessment



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Reference structure is present

DockQ

Similarities models vs. reference

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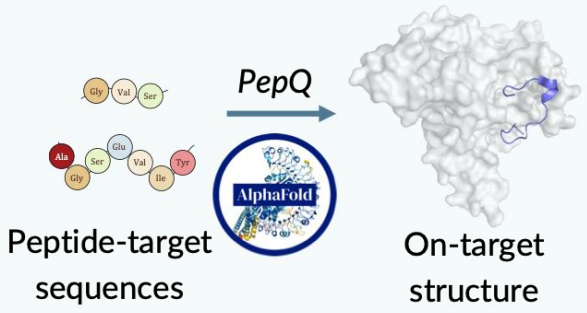

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Reference structure is present	Reference structure is absent
DockQ Similarities models vs. reference	**pdockq, pdockq2** Predicted DockQ for protein-protein models

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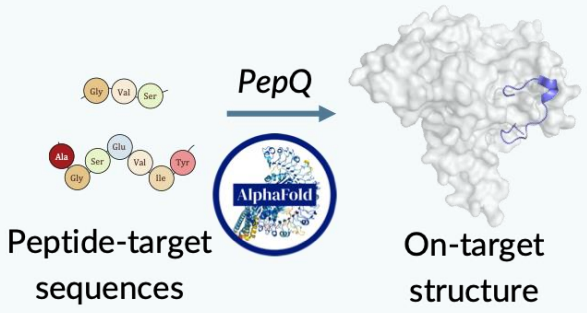

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No available quality assessment for peptide-protein models

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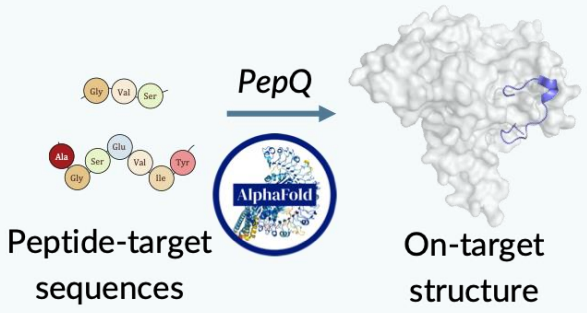

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PepQ

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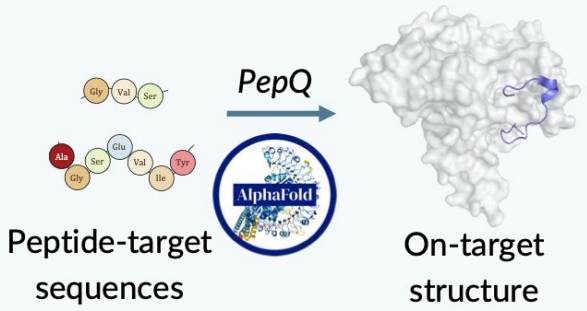

Module 1. On-target structure generation

Data

Query peptide-protein complexes from Protein Data Bank with the criteria regarding resolution, canonical amino acids, lengths, and redundancy filter.

- Training set: complexes released before the release date of AlphaFold-Multimer.
- Test set: complexes released after the release date of AlphaFold-Multimer.
- Similarity filtering: any complexes in the test set that are similar to those in the training set were removed

Bryant, P. *et al.* (2022). Improved prediction of protein-protein interactions using AlphaFold2. Nature communications, 13(1), 1265.
Zhu, W. *et al.* (2023). Evaluation of AlphaFold-Multimer prediction on multi-chain protein complexes. *Bioinformatics*, *39*(7), btad424.

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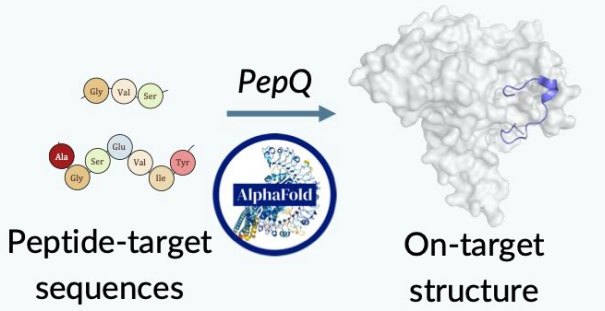

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Competing scores

- pdockq: predicted protein-protein complex quality score (Bryant, 2022)
- pdockq2: predicted protein-protein complex quality score (Zhu, 2023)
- AlphaFold-Multimer internal ranking scores.

Model architecture

- Multi-head attention: regression head (Huber), ranking head (ranking loss)

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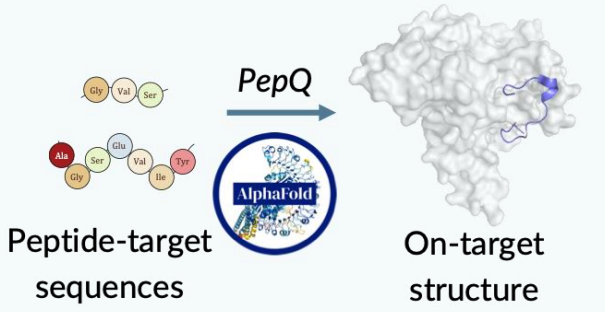
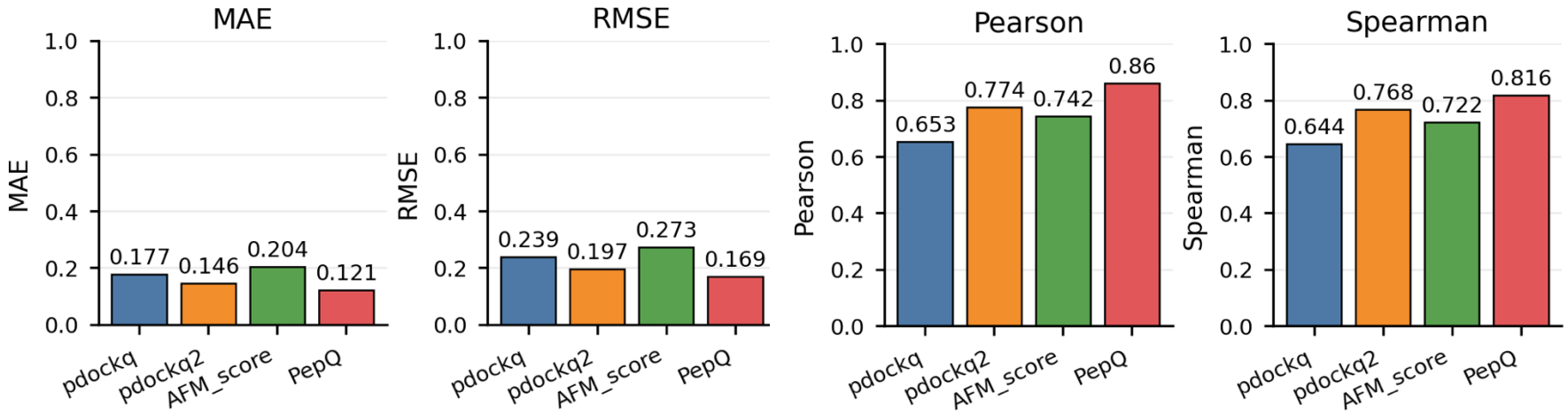


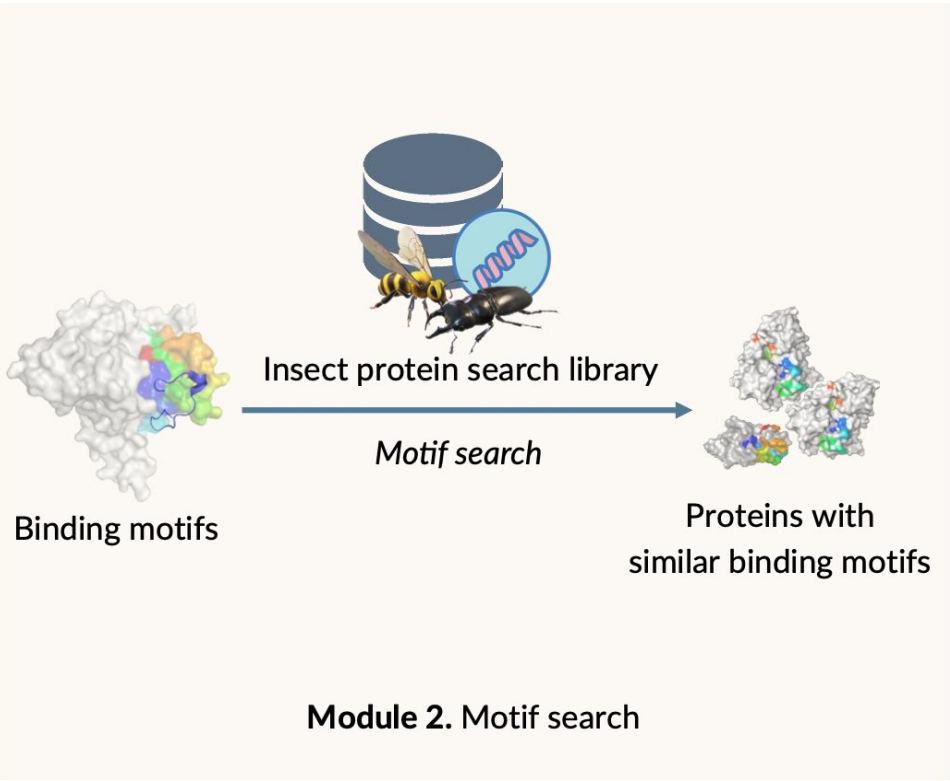
Module 1. On-target structure generation

Performance comparison on test set



- pdockQ and pdockQ2 are logarithmic regression models
  - pdockQ relies on inter-chain contact counts and interface pLDDT.
  - pdockQ2 enhances this framework by incorporating PAE.
- PepQ further integrates peptide-specific confidence features, allowing more accurate assessment of peptide-protein interfaces.

Structure-based motif search



Module 2. Motif search

Search tool

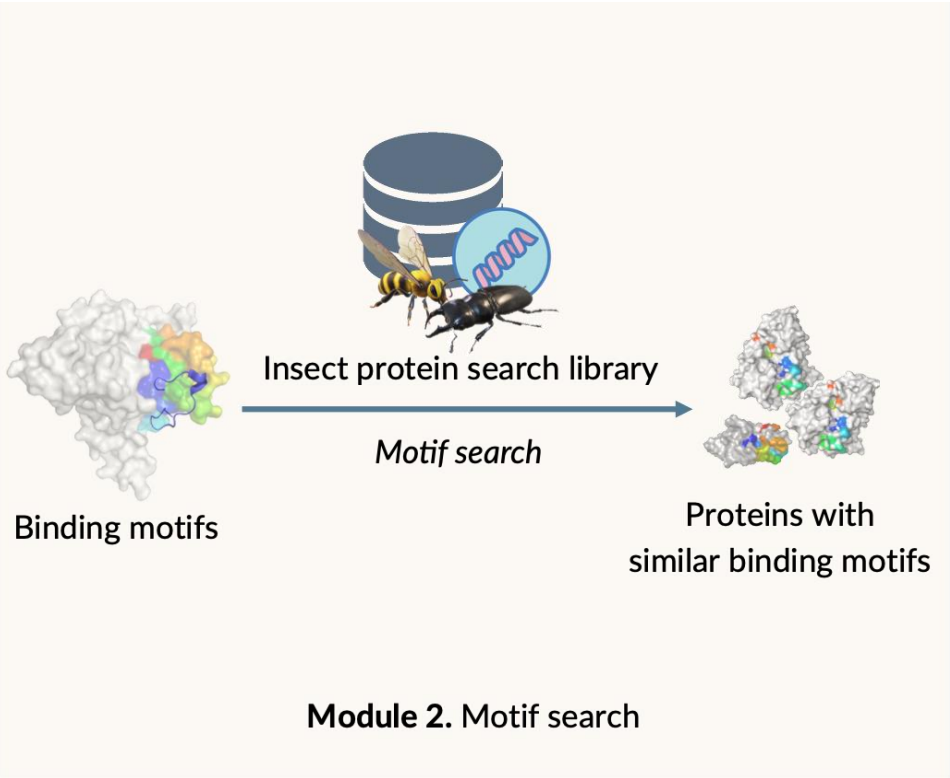
- Folddisco
  - Amino-acid identity, interatomic distances, and backbone/side-chain angular features.
  - Fast lookups against large structural databases

Protein library

- AlphaFold Protein Structure Database
- Indexed

Kim, H., *et al.* (2025). Structural motif search across the protein-universe with Folddisco. *bioRxiv*, 2025-07.

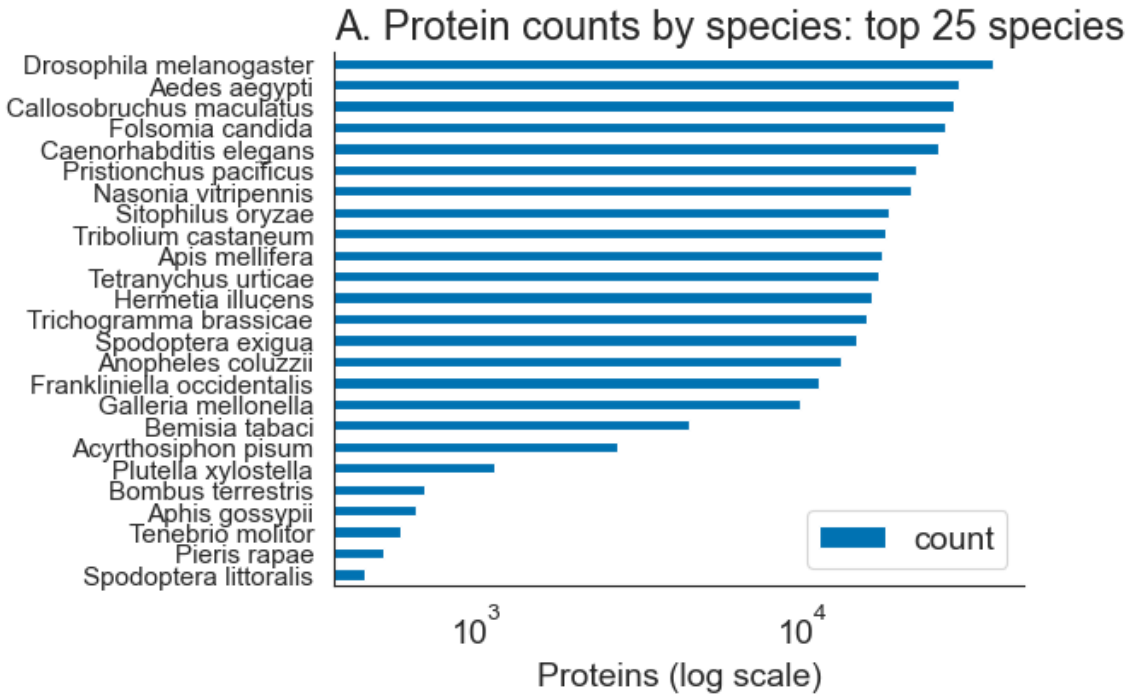
Protein library



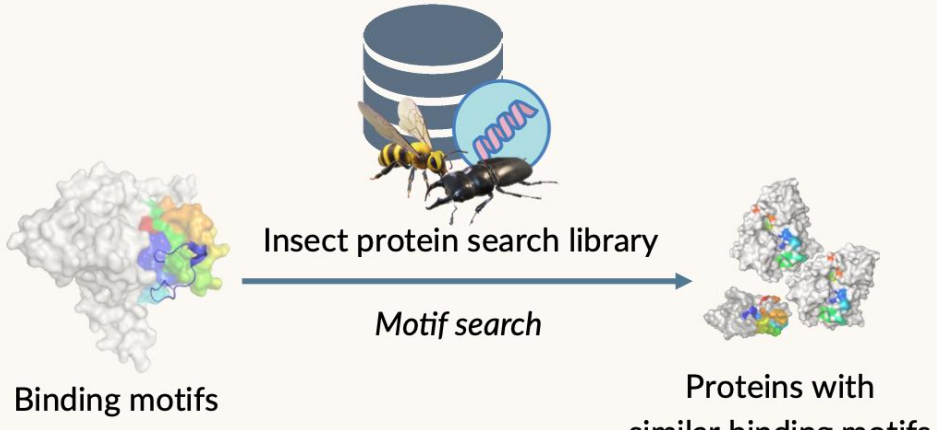
Module 2. Motif search

Pre-indexed, ready-to-use library

- 367 142 proteins
- 99 species of interest



Future steps



Module 2. Motif search

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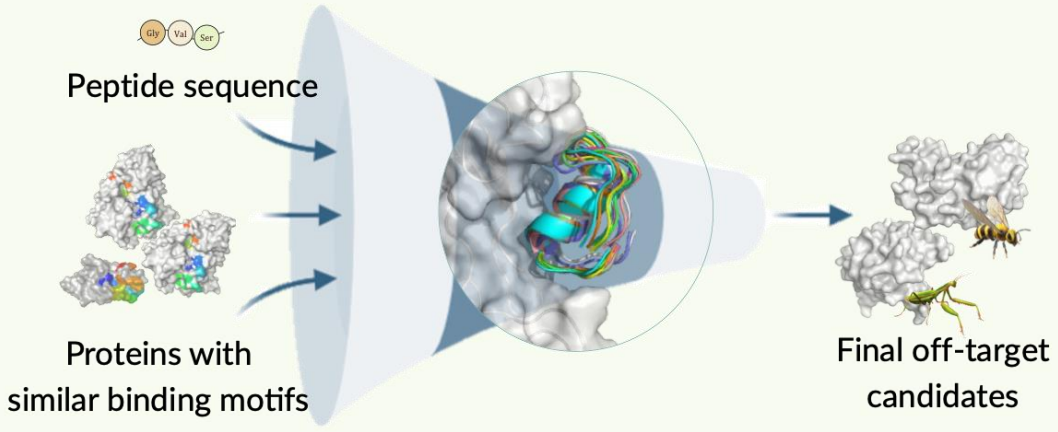
- Improve quantity and quality of protein library.

Future steps



Module 3. Modeling - scoring

Module 2. Motif search

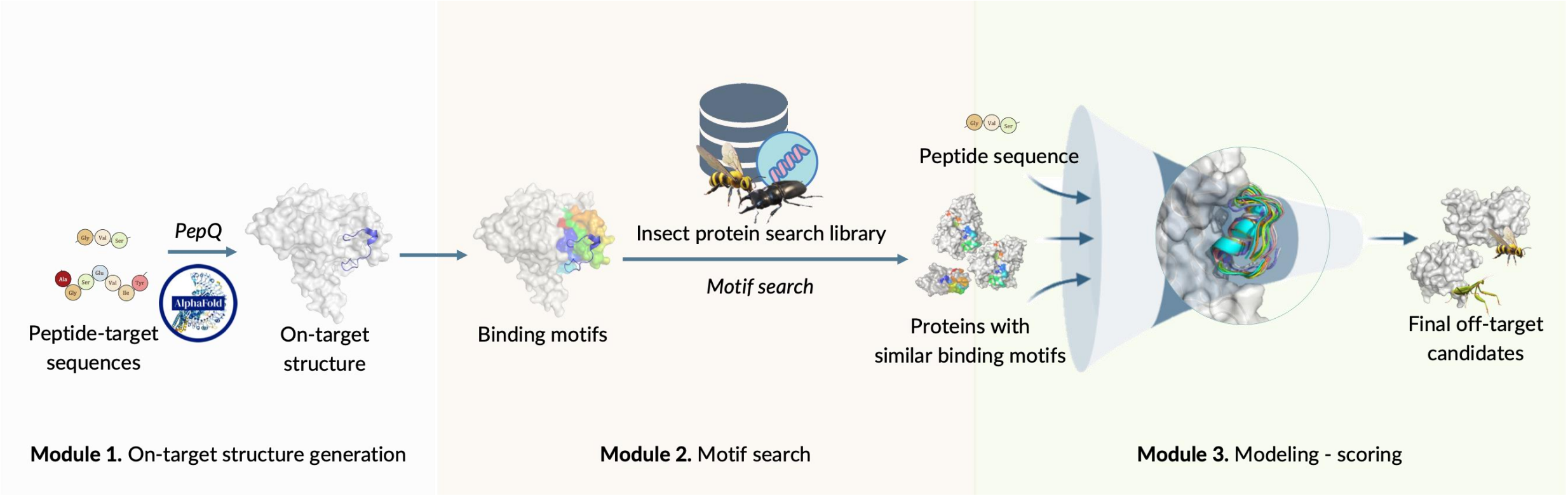
- Improve quantity and quality of protein library.

Module 3. Modelling - scoring

- Curate and generate training datasets reflecting off-target binding.
- Develop models to predict off-target binding probability.

Off-target binding prediction through conserved binding motifs and structural plausibility

- Prediction workflow



Acknowledgements

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  - Mikkel Frier Wolfhagen
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  - Stefan E. Seemann
  - Stefano Roncelli
  - Gül S. Demircan
  - **Jan Gorodkin**
- **Collaborators**
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  - ENSAFE consortium
- **Funding**
  - Novo Nordisk Foundation

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Novo Nordisk Foundation Challenge

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Thank you for your attention!

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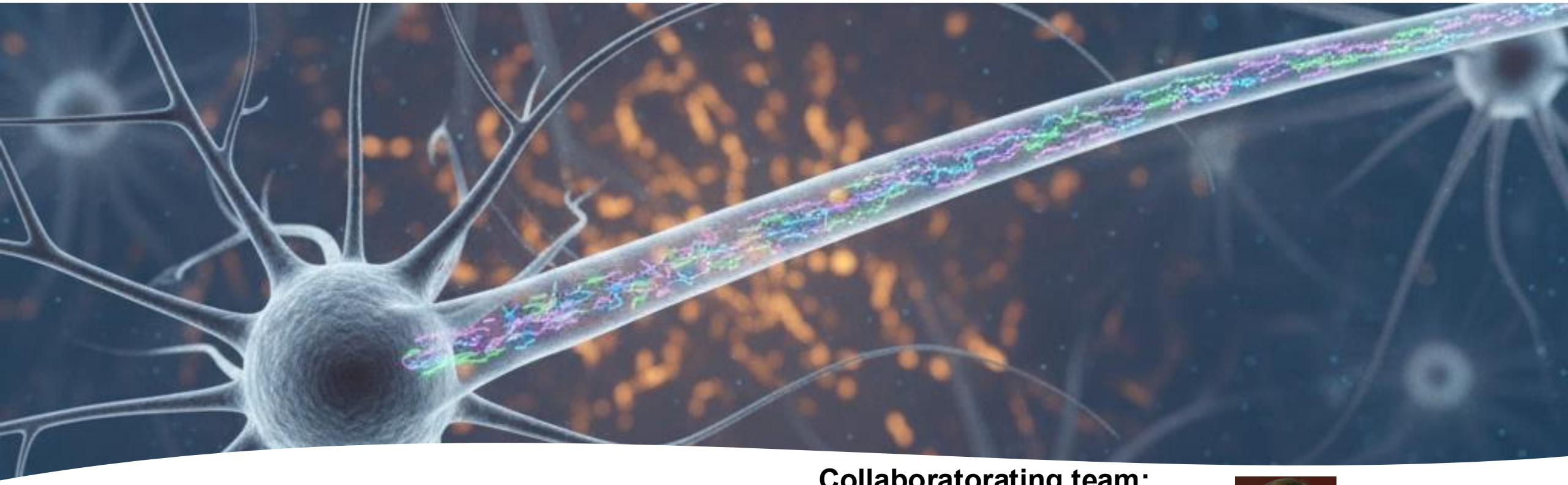
Upcoming PhD or postdoc position at RTH
Talk to Jan

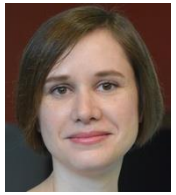


Decoding RNA journey:

Predictive models for the subcellular RNA fate in neurons

Collaboratorating team:

Chekulaeva (UCPH / Berlin)



Ulitsky (Weizmann Institute)

Gorodkin (UCPH)

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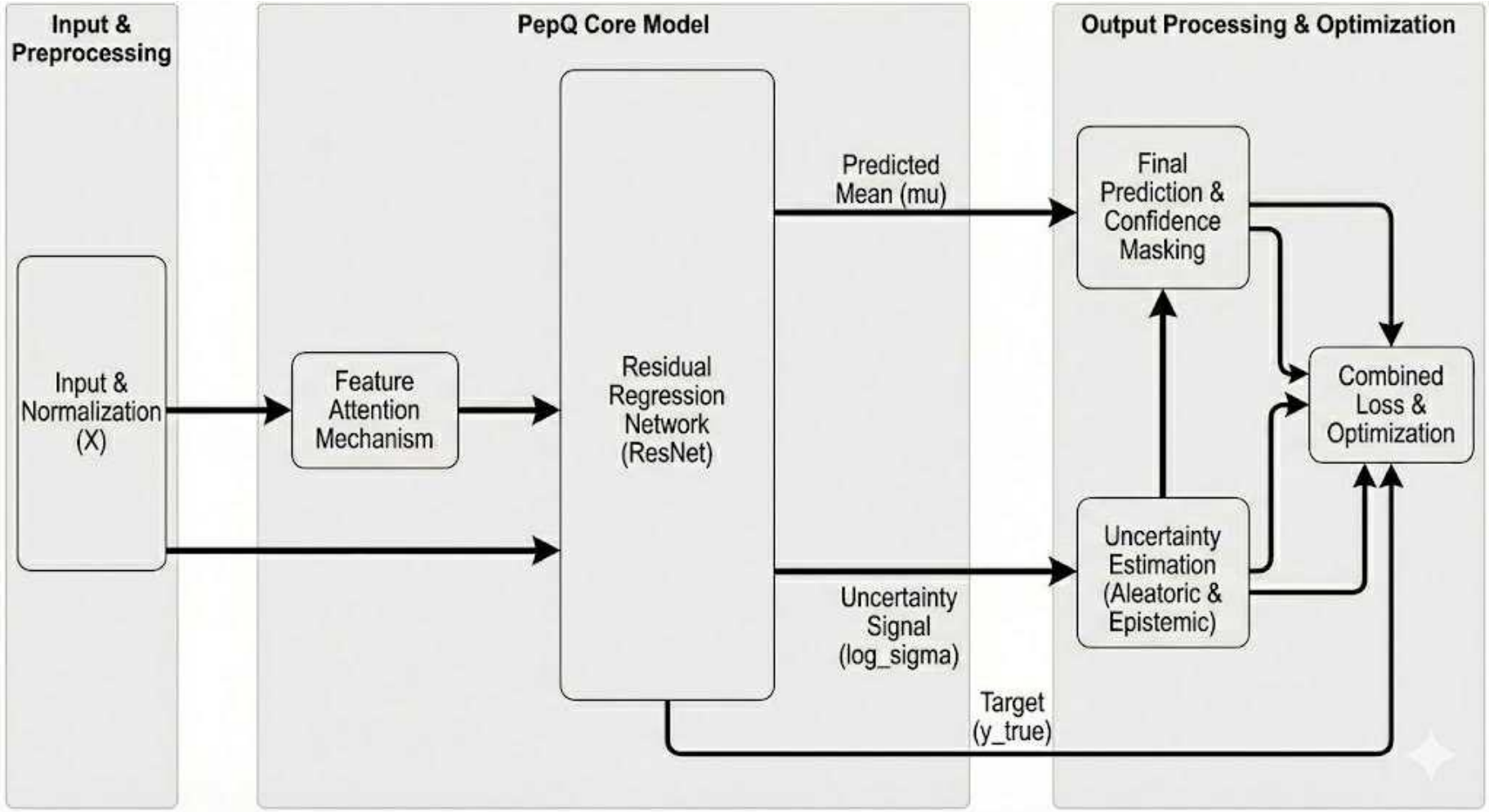

PepQ: peptide-protein model quality assessment

Full Objective

$$L(\theta; e) = w_{reg}\ \mathcal{L}_{reg} + w_{rank}(e)\,\mathcal{L}_{rank} + w_{nll}\ \mathcal{L}_{nll} + w_{attn\_entropy}\,\mathcal{L}_{ent} + w_{gate\_l2}\,\mathcal{L}_{gate}$$

Regression Loss: $\mathcal{L}_{reg} = \sum_{i=1}^{B} (y_i - \mu_i)^2$ *or* Huber Loss

Ranking Loss: $\mathcal{L}_{rank} = w_{rank}(e) \sum_{i,j \in P}^{P} w_{ij} \log\left(1 + \exp\left(-s_{ij}(\Delta\mu_{ij} - m)\right)\right).$

Gaussian NLL Loss: $\mathcal{L}_{nll} = \sum_{i=1}^{B} \left(\log \sigma_i + \frac{1}{2}\left(y_i - \mu_i\right)^2\right),$

Attention Entropy Loss: $\mathcal{L}_{ent} = w_{attn\_entropy}\, H = w_{attn\_entropy} \frac{H}{1}{BH} \sum_{i=1}^{B} \sum_{H=1} \sum_{f=1}^{H} - a_{i,h,f} \log a_{i,h,f}$

Gate L2 Loss: $\mathcal{L}_{gate} = w_{gate\_l2} \sum_{f=D}^{B} \left(g_{i,f} - 1\right))^2.$