# Investigating the influence of O-linked glycosylation on the proteolytic stability and dimerization propensity of insulin using molecular dynamics

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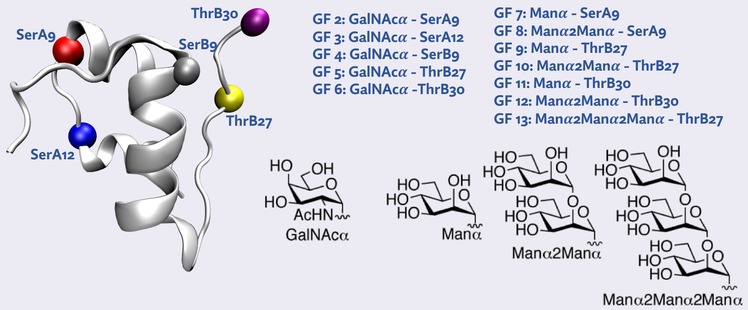
## Oral insulin drugs are highly desirable, but their development is challenging

- People disfavor insulin delivered via subcutaneous injections due to inconvenience and side effects.
- Major challenges of oral insulin:
  - Poor gastrointestinal stability
  - Poor absorption in the small intestine

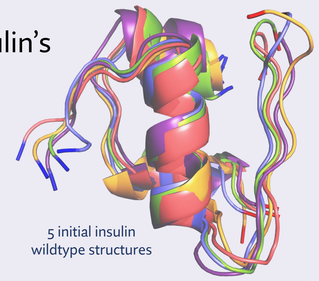
## Certain O-glycosylation patterns were shown to improve insulin's properties

- Better insulin properties for oral delivery:
  - High proteolytic stability
  - Low dimerization propensity
- 12 insulin glycoforms (GF) were studied systematically.
  - Proteolytic stability: GF 13 > GF 10 > GF 9 ≈ Wild type
  - Dimeric propensity: GF 9 > GF 13 > GF 10 > Wild type




## Aim: Develop metrics for screening insulin GFs with improved properties for oral delivery

- The mechanism of how O-glycosylation influences insulin's properties remains elusive.
- A computational screening method is desirable.
  - Inputs: 12 GFs, 10 $\mu$s MD pooled from 5 wild types

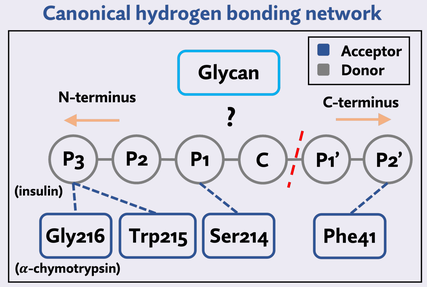



## Solvent exposure is partially predictive of proteolytic stability

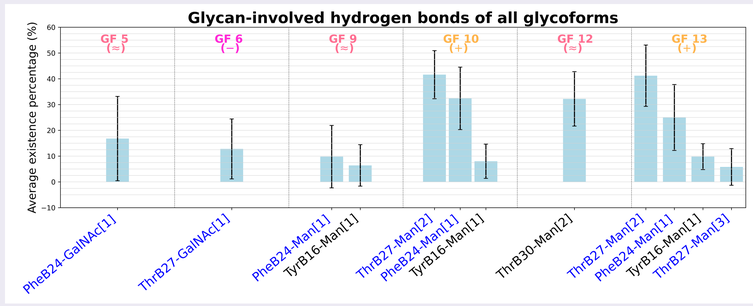
- **Metric 1: SASA of the scissile bonds**
  - Previous finding: PheB25 and TyrB26 are the most susceptible cleavage sites by $\alpha$-chymotrypsin.
  - Hypothesis: Lower solvent exposure of B25 and B26 leads to higher proteolytic stability.
- **Metric 2: SASA of the P1 sites**
  - Previous finding: The P1 sites need to be solvent exposed to bind with $\alpha$-chymotrypsin.
  - Hypothesis: Lower solvent exposure of the P1 sites (B24 and B25) leads to higher proteolytic stability.
- **Results**: Kendall's tau coef. between -0.35 and -0.55.

## Stable glycan-involved H-bonds are indicative of high proteolytic stability

- Hypothesis: The glycan competes with $\alpha$-chymotrypsin as the acceptors and disrupts the H-bond network, hence increasing the proteolytic stability.

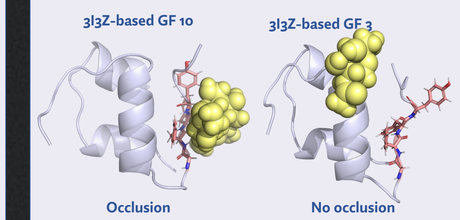


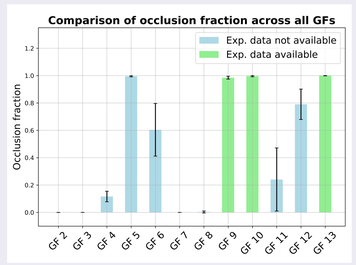

- **Results**:
  - Proteolytically unstable GFs (GFs 2, 3, 4, 7, 8, 11) do not have any glycan-involved H-bond.
  - Proteolytically stable GFs all have stable H-bonds between the glycan and PheB24 or ThrB27.




## Frequent glycan-dimer occlusion is indicative of low dimerization propensity

- Previous finding: The dimer interface includes GlyB23-ThrB26 of the two interacting insulin monomers.
- Hypothesis: Frequent glycan-dimer occlusion disfavors the dimerization process.
- **Results**: Glycan-dimer occlusion is predictive of dimerization propensity.




## Some metrics were rejected as not predictive

- Conformational flexibility and $\beta$-sheet propensity of the binding sites are not predictive of proteolytic stability.
- The secondary structures of the tail region do not correlate with dimerization propensity.

## Our work eases the screening of insulin GFs with improved properties for oral delivery

- Useful predictors:
  - Glycan-involved H-bond for proteolytic stability
  - Glycan-dimer occlusion for dimerization propensity
- Future work:
  - Apply the framework in a blind challenge
  - Apply deep-learning methods (DiffNets) to identify more subtle structural signatures.

Scan the QR codes for more information about our paper and authors! ☺

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