George R. Bousfield

Dr. L.M. Jones Distinquished Professor,
Department of Biological Sciences

August 10, 2015

# Influence of Glycans on Biological Activities of Pituitary Gonadotropins: Follicle-Stimulating and Luteinizing Hormone

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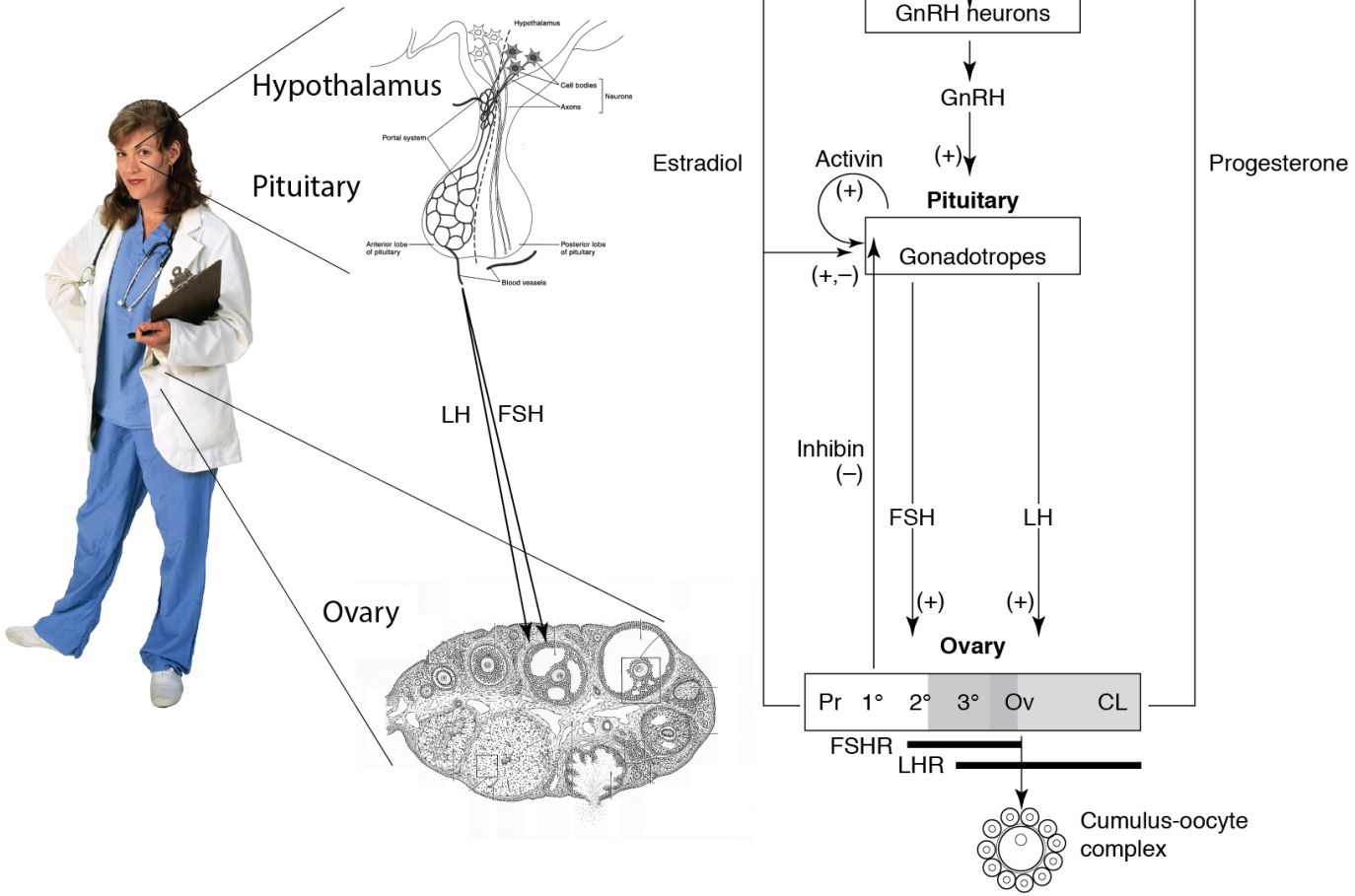
# Hypothalamo-Pituitary-Gonadal Axis



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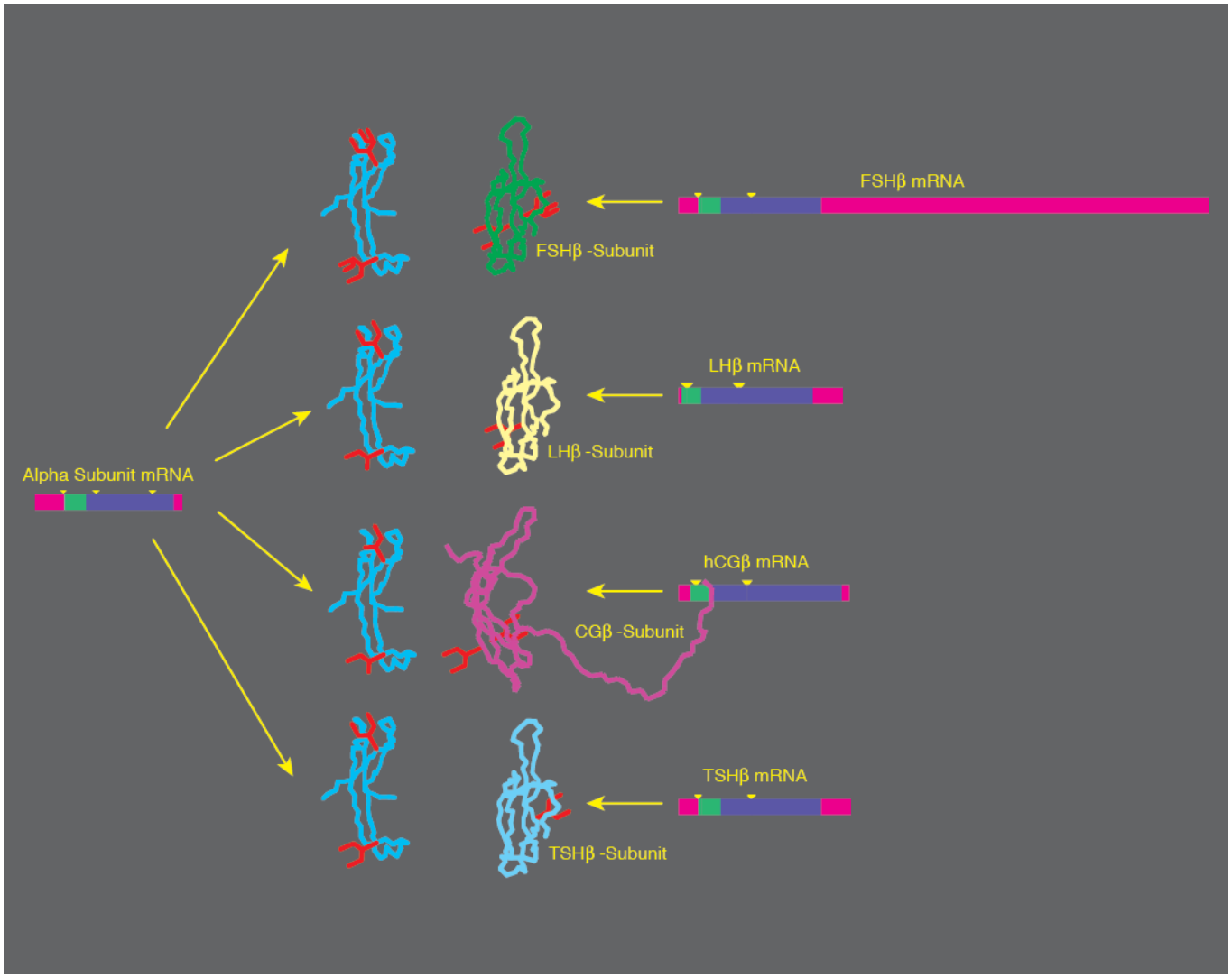
# LH, FSH & Glycoprotein Hormone Family



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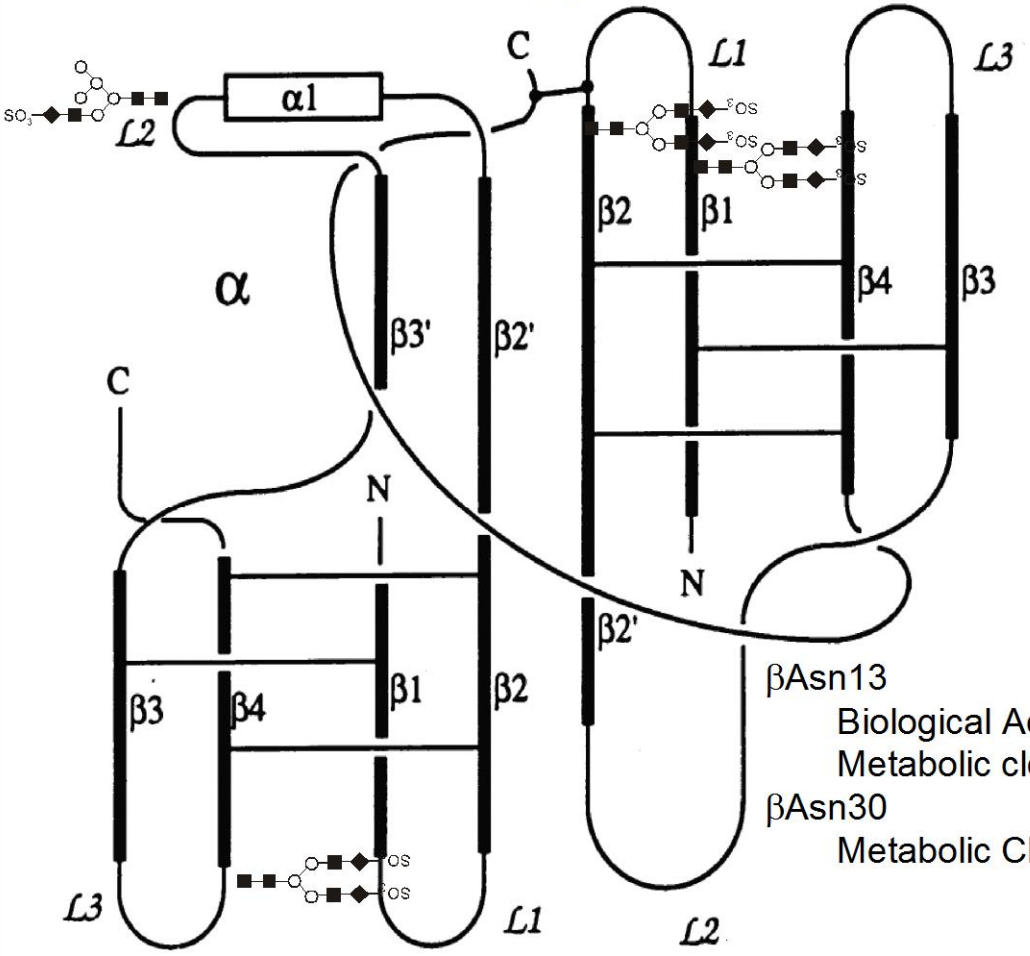
# α-Subunit Embraced by β Seatbelt Loop

Stability of some, but not all hormones
Inhibits LH receptor-binding by steric hindrance






A.J. Lapthorn, et al., Nature, 369 (1994) 455-461. H. Wu, et al., Structure, 2 (1994) 545-558.

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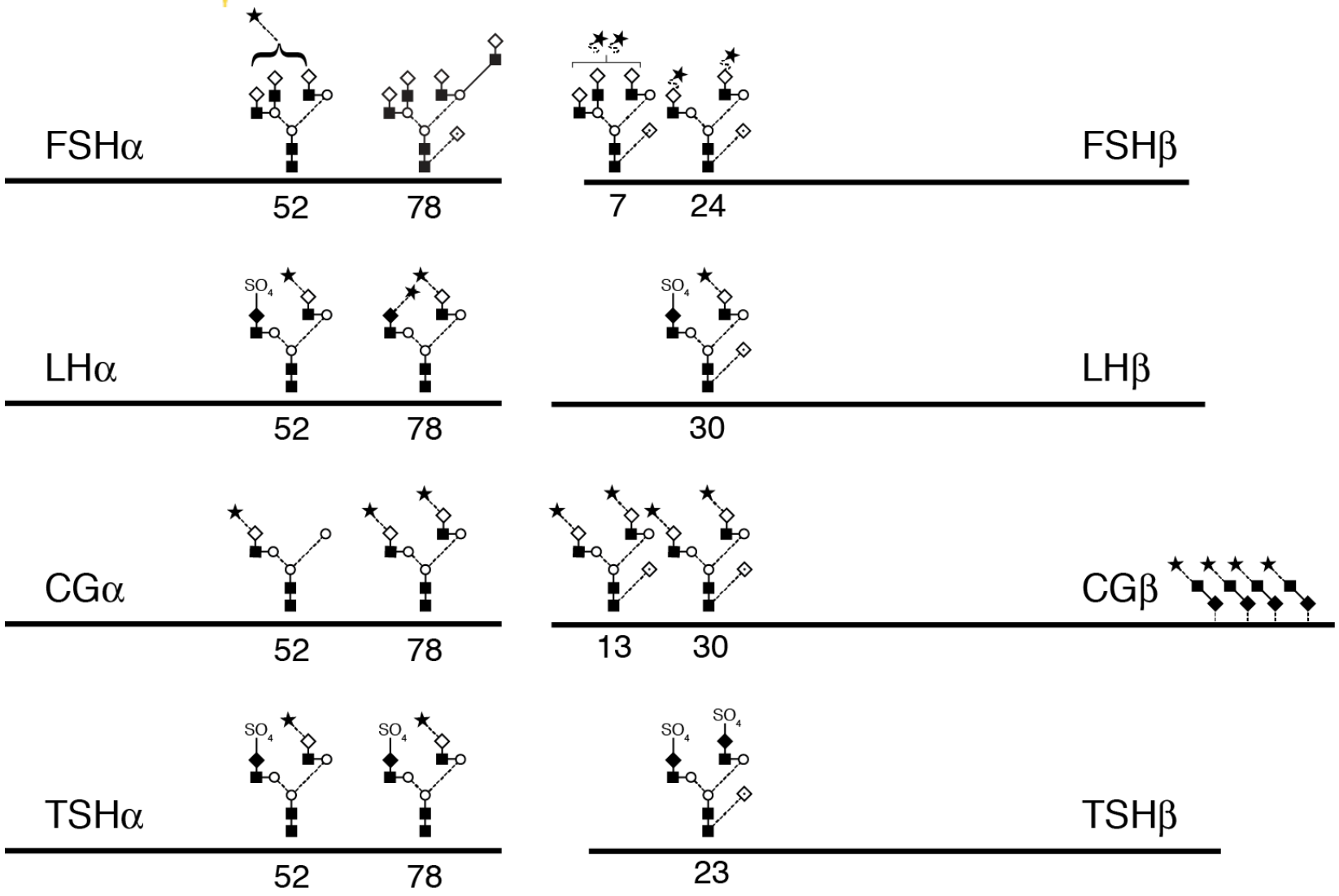
# Hormone-specific glycosylation

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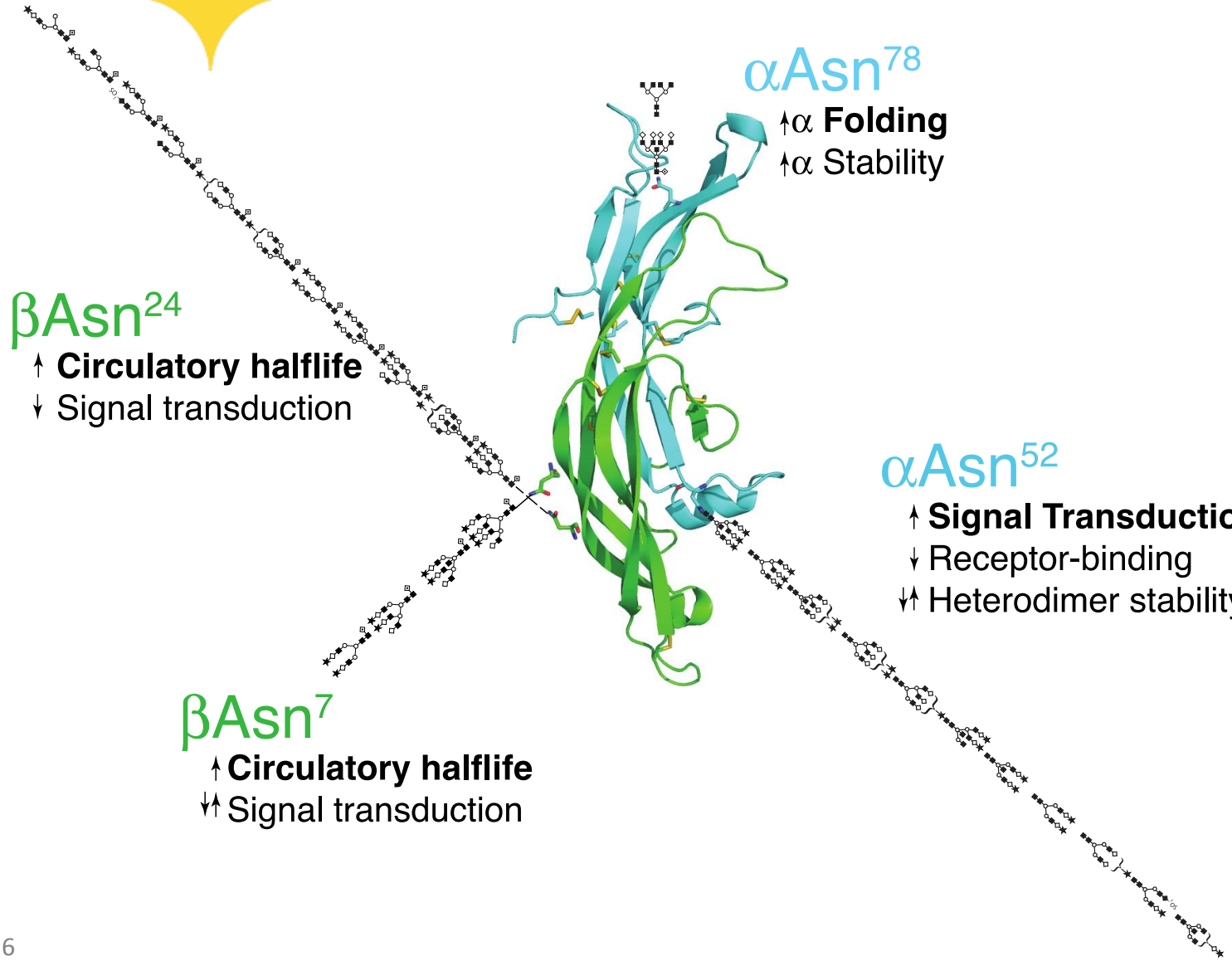
# LH/FSH Glycan Functions



αAsn$^{78}$
- ↑α **Folding**
- ↑α Stability

βAsn$^{24}$
- ↑ **Circulatory halflife**
- ↓ Signal transduction

αAsn$^{52}$
- ↑ **Signal Transduction**
- ↓ Receptor-binding
- ↓↑ Heterodimer stability

βAsn$^{7}$
- ↑ **Circulatory halflife**
- ↓↑ Signal transduction



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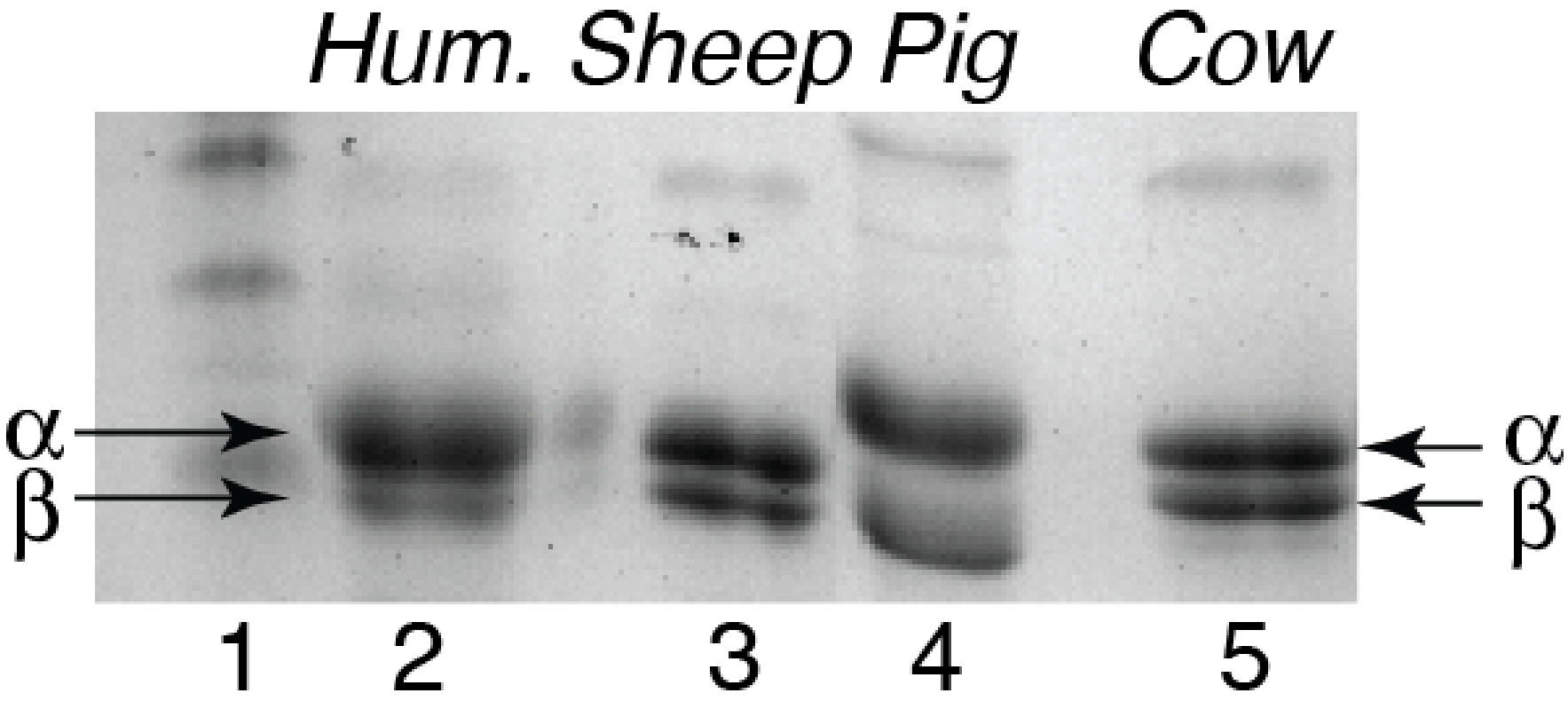
# LH Preparations



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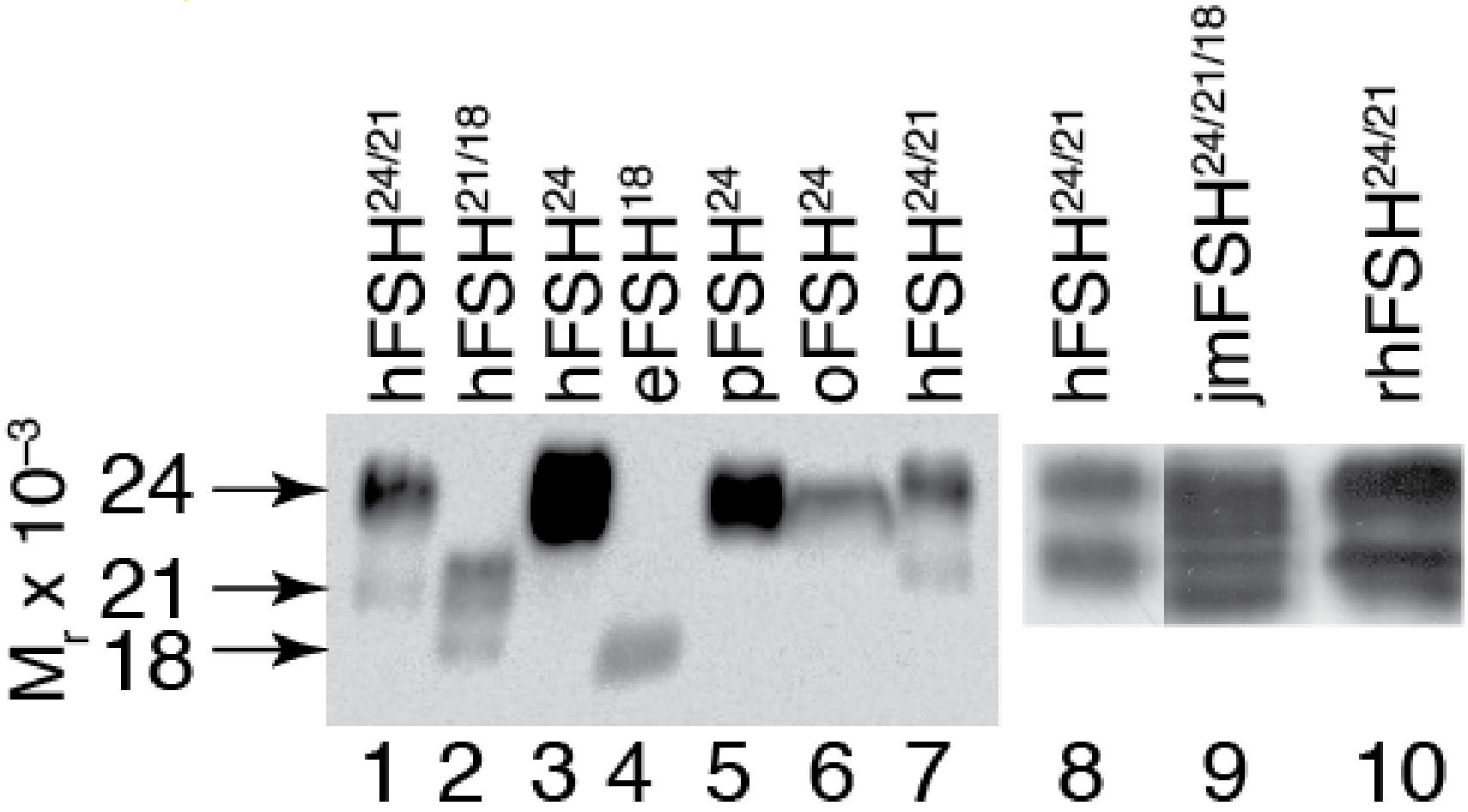
# Mammalian FSHβ Western Blots



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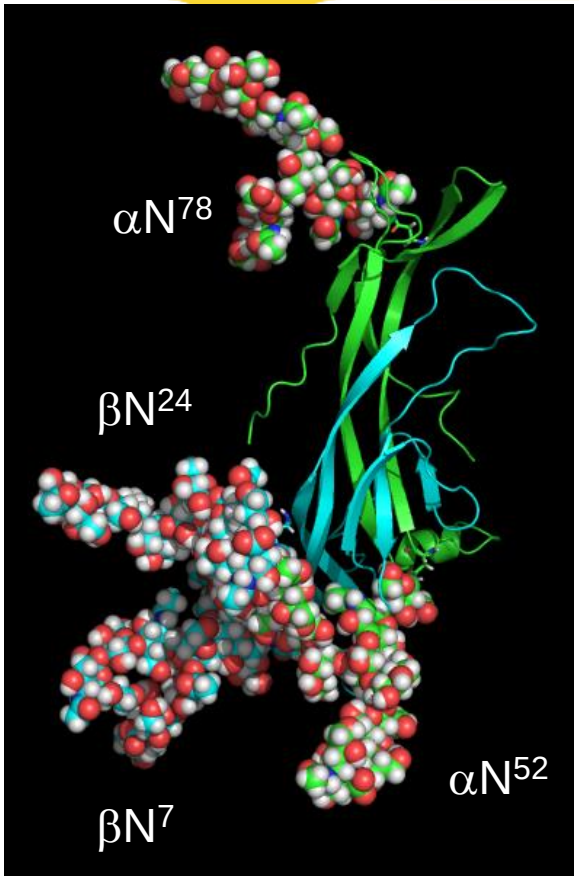
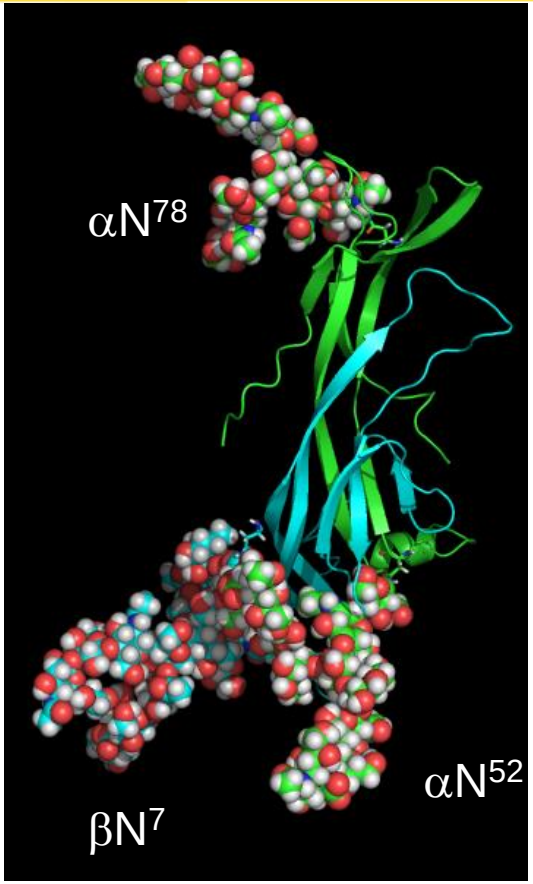
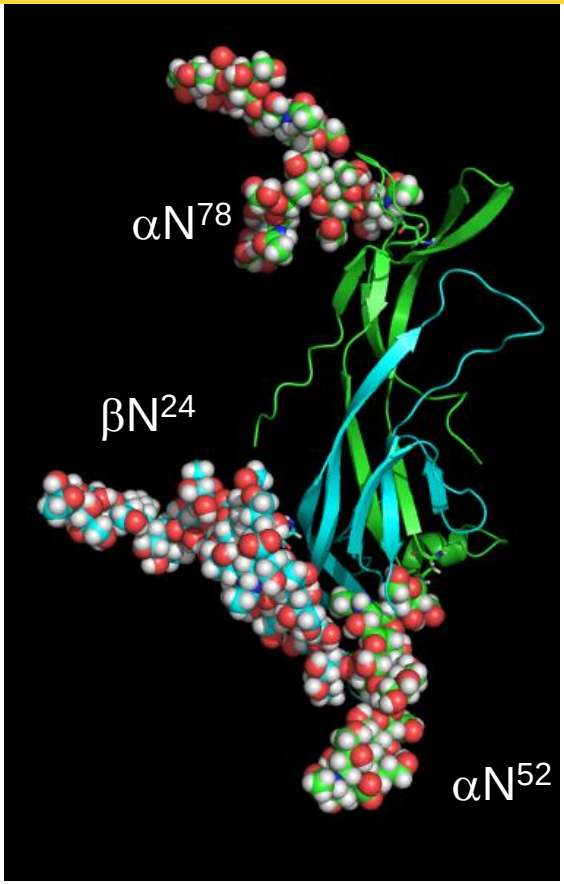
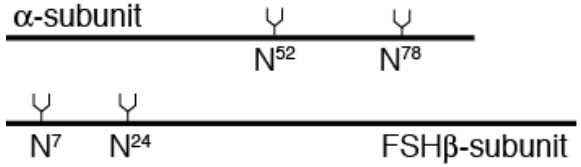
# Human FSH Glycoforms

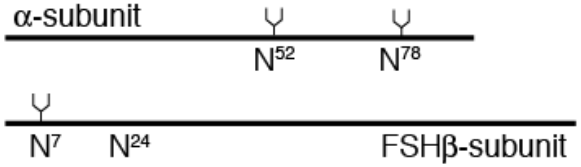


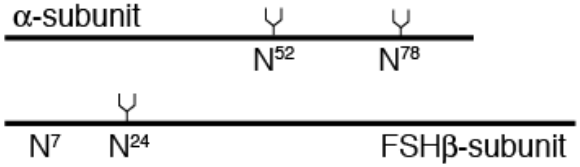




FSH$^{24}$




FSH$^{21}$




FSH$^{18}$



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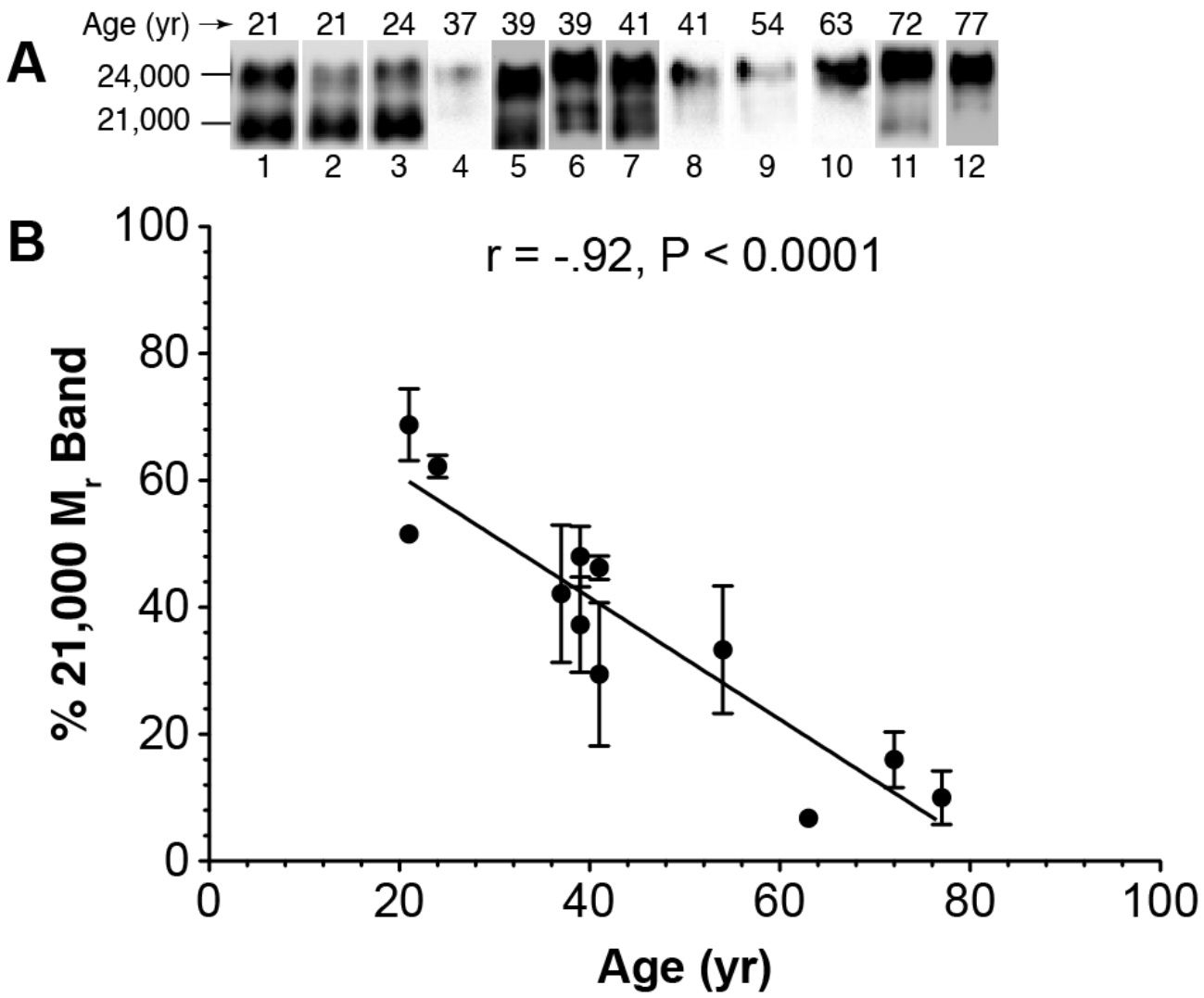
# Age-related decline in pituitary hFSH[21]



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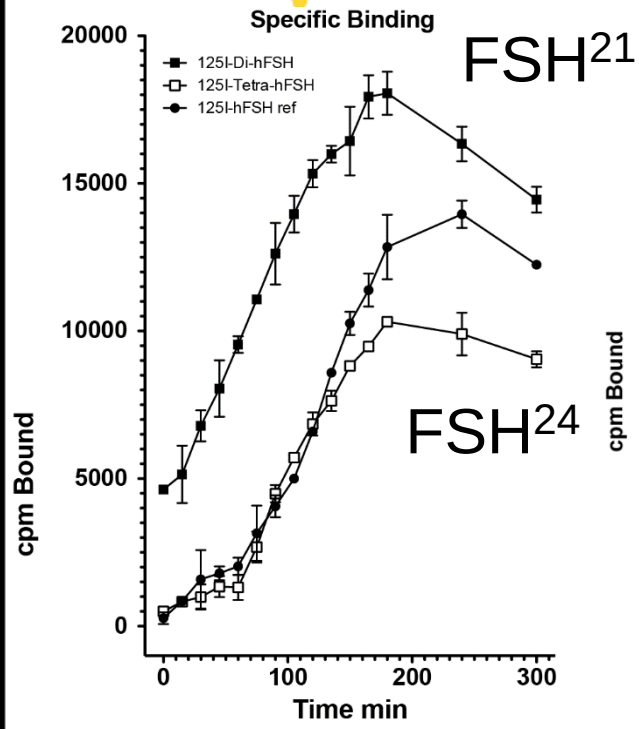
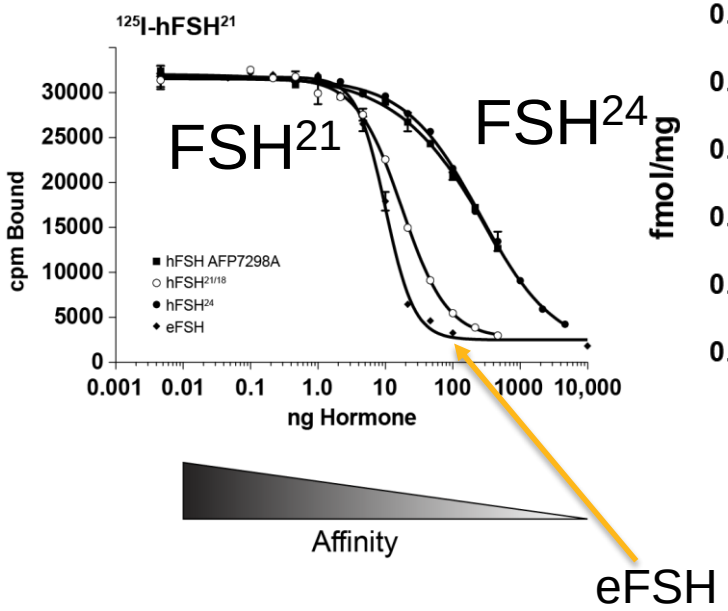
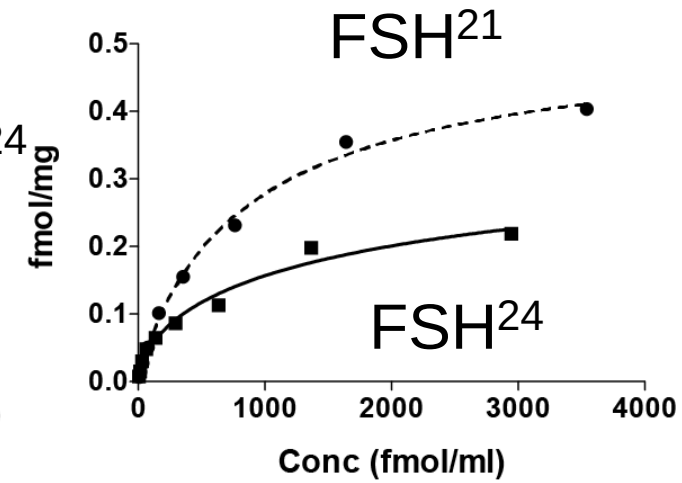
# FSH$^{21}$ More Active Than FSH$^{24}$




**Faster Association**

**Higher Affinity**

**Greater Occupancy**



Bousfield, G. R., et al., Mol. Cell. Endocrinol. **382**: 989-997, 2014

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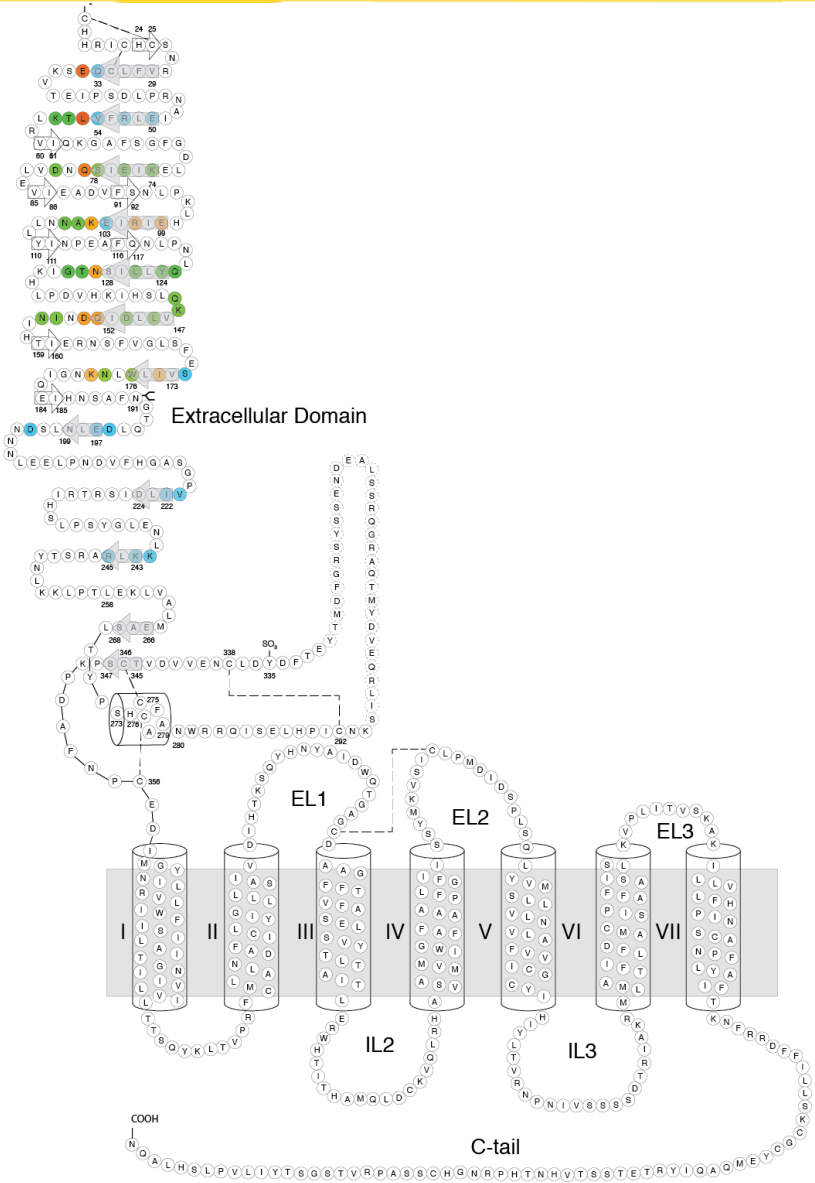
# FSH receptor



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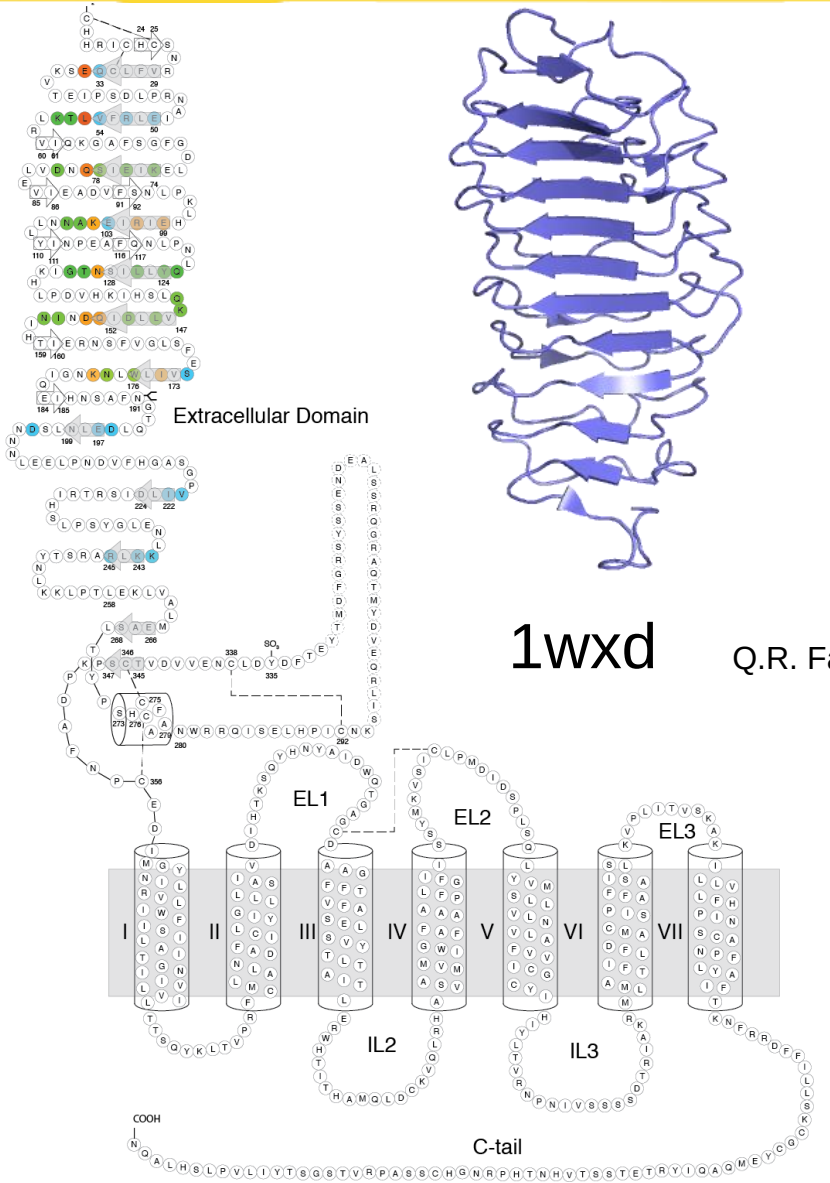
# FSH receptor hormone-binding domain






1wxd

Q.R. Fan, et al., Nature, 433 (2005) 269-277.



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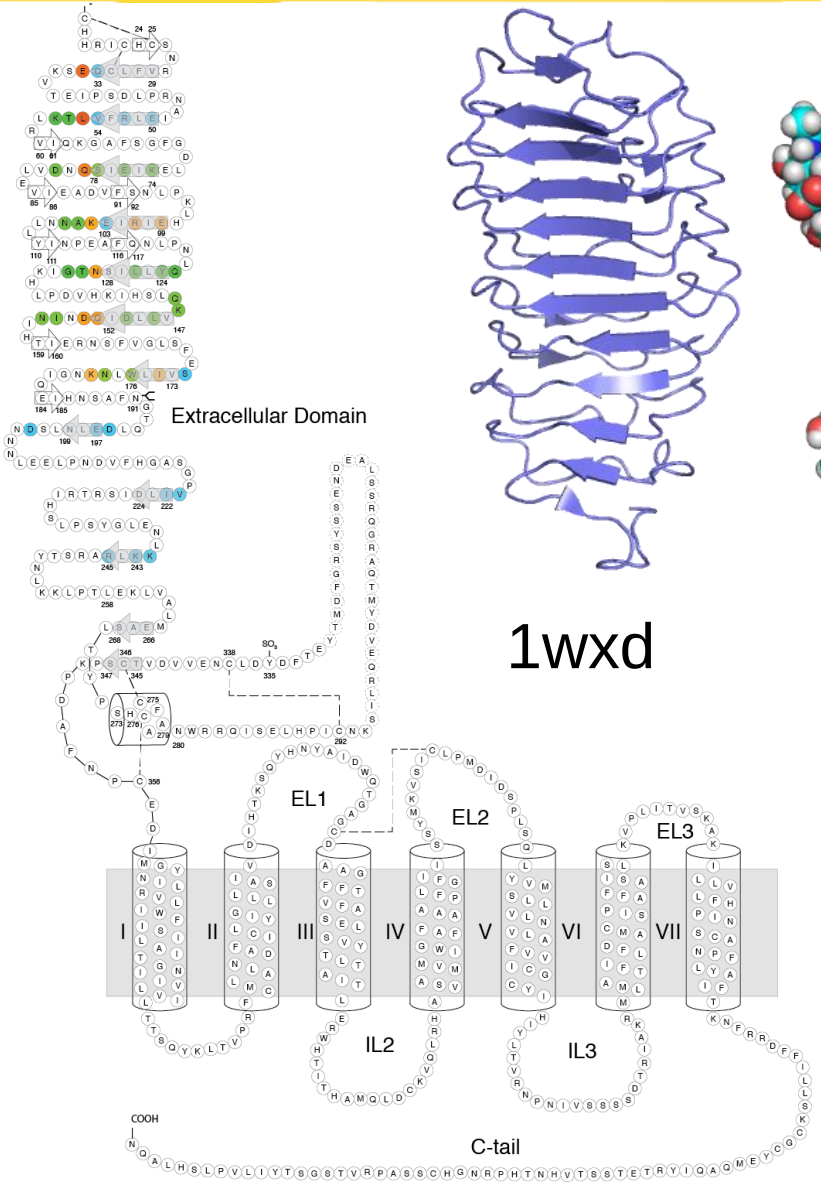
# FSHR–FSH$^{24}$ Complex



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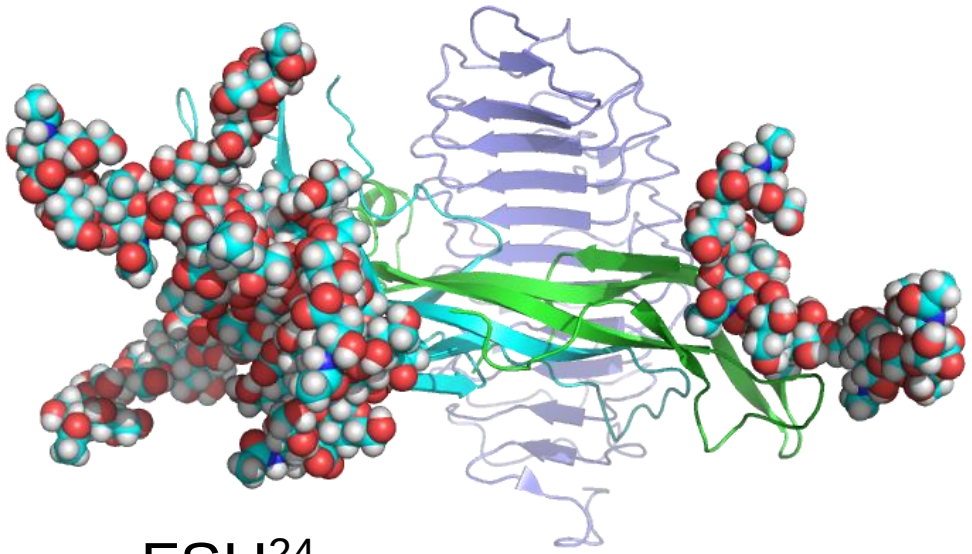

FSH$^{24}$

1wxd

Glycam model of FSH$^{24}$



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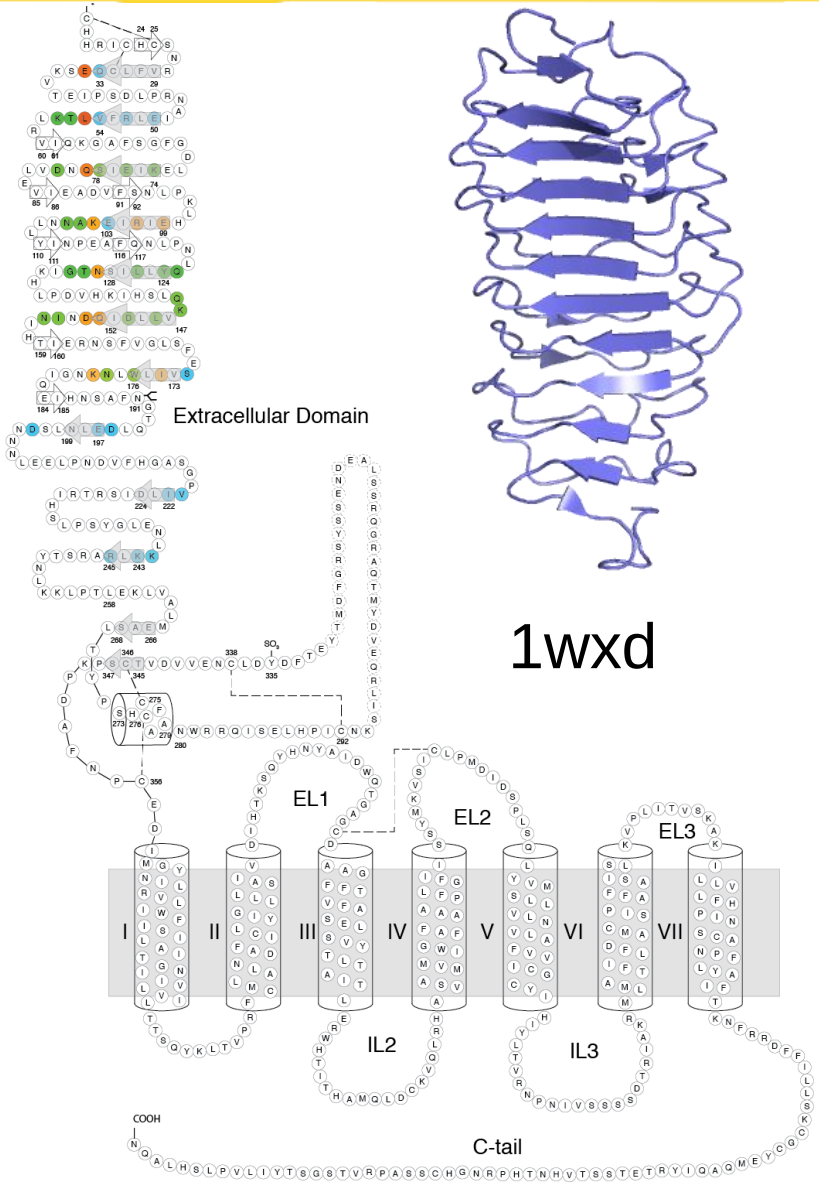
# FSH receptor hormone-binding domain



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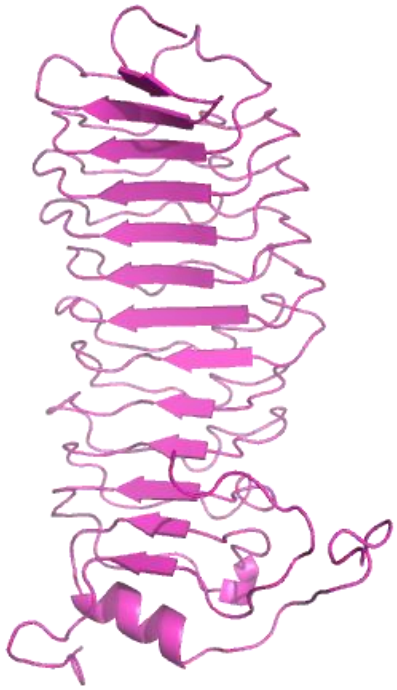

4ay9

X. Jiang, et al., Proc. Natl. Acad. Sci. USA, 109 (2012) 12491-12496.



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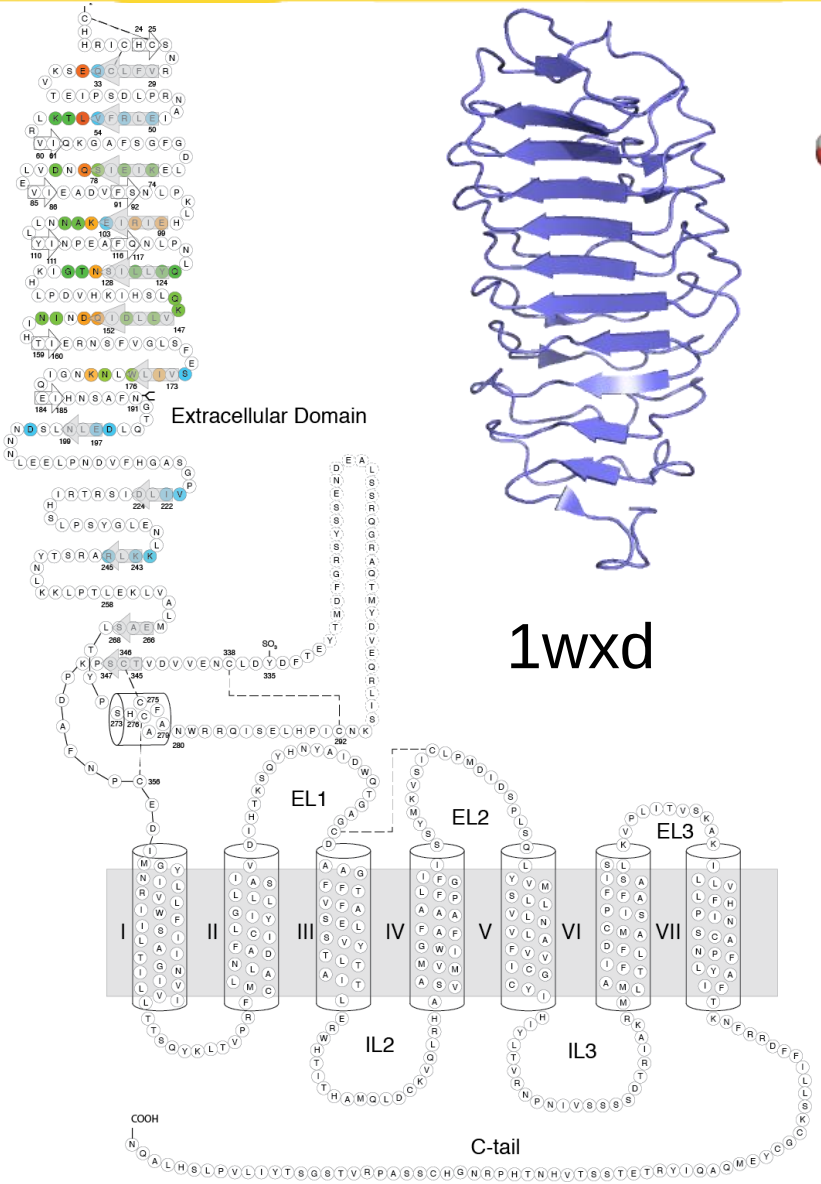
# FSH receptor hormone-binding domain



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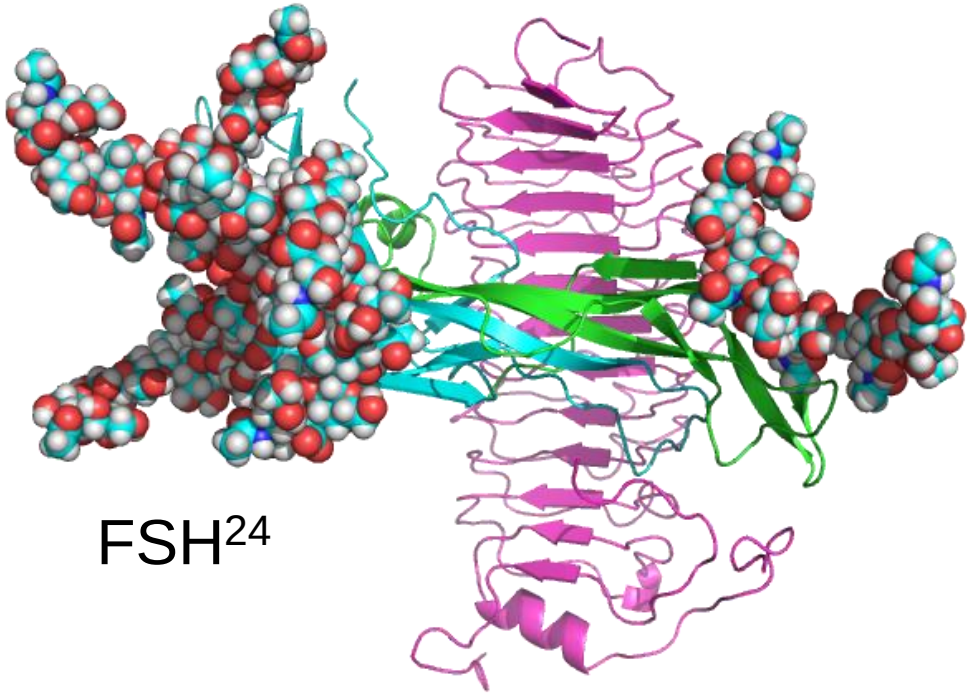

FSH$^{24}$

4ay9

Glycam model of FSH$^{24}$



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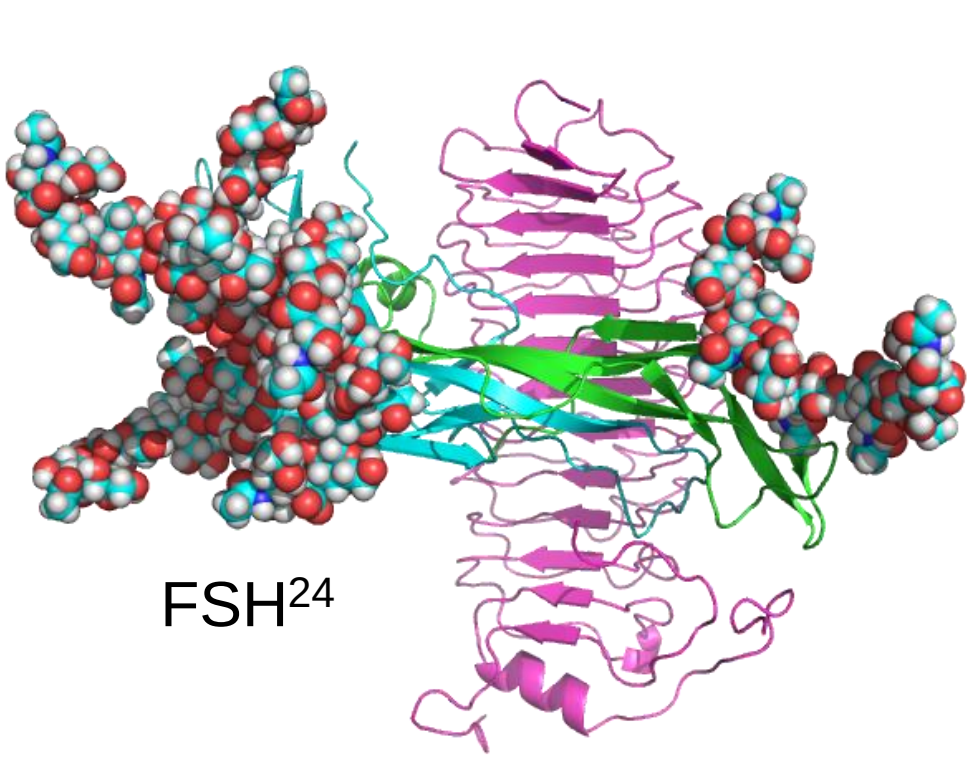
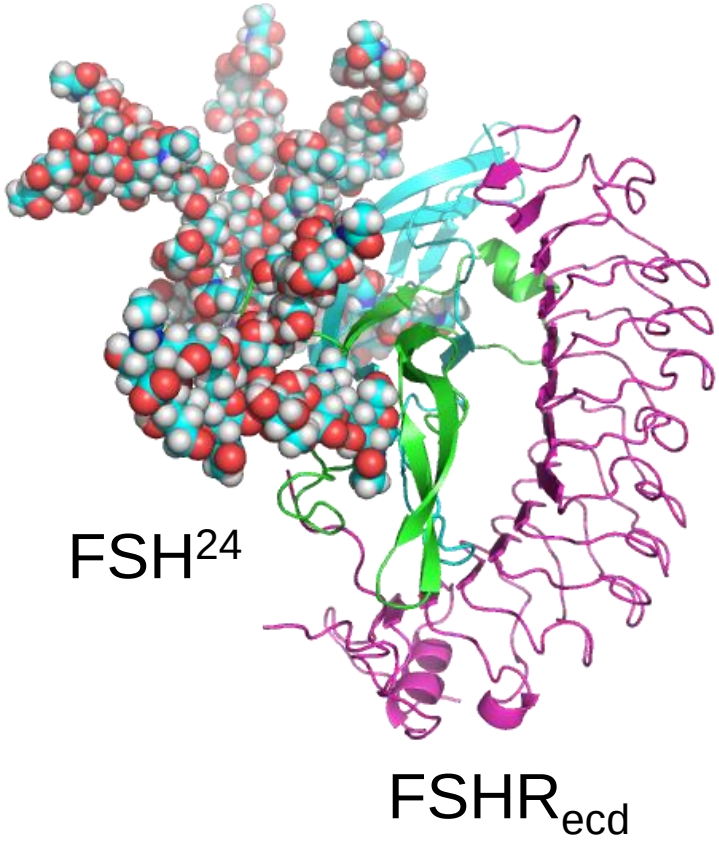
# FSH glycans and $FSHR_{ecd}$




Glycam models of $FSH^{24}$



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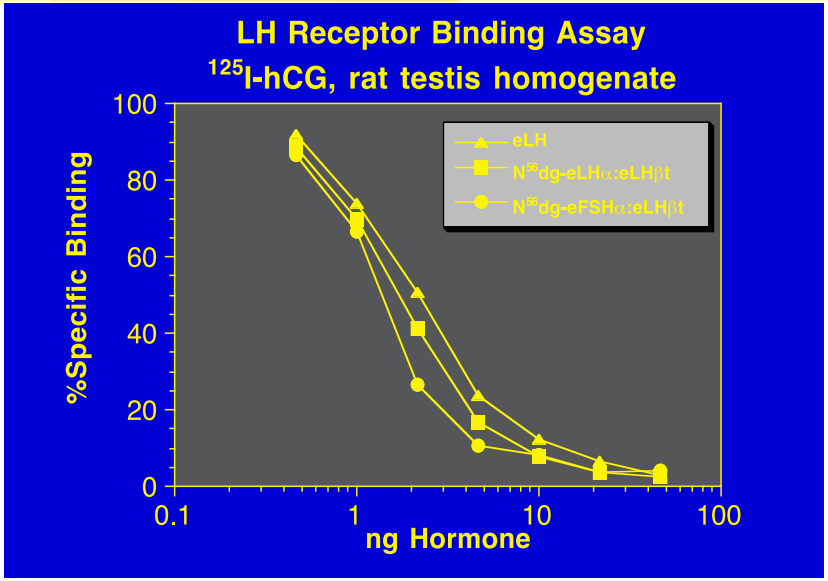
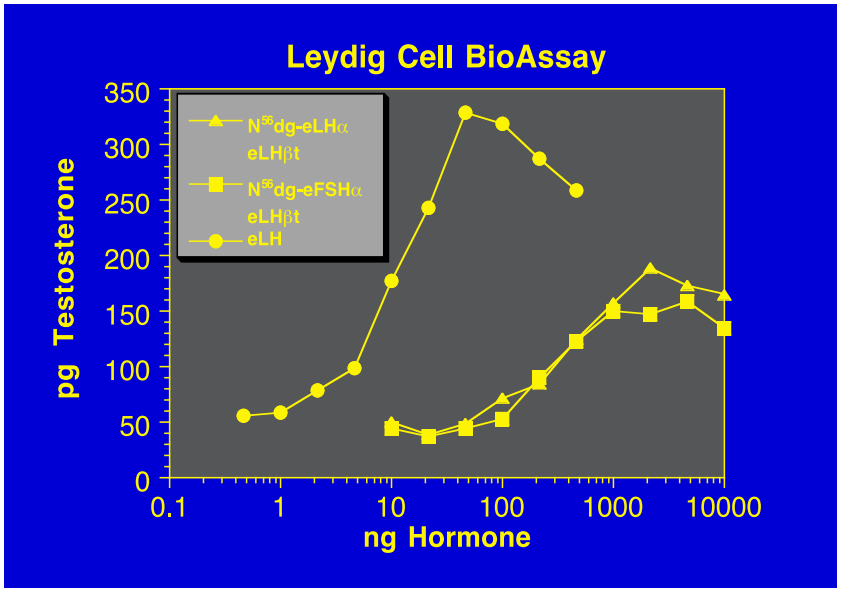
# αAsn$^{52}$ Glycan Necessary for Biological Activity



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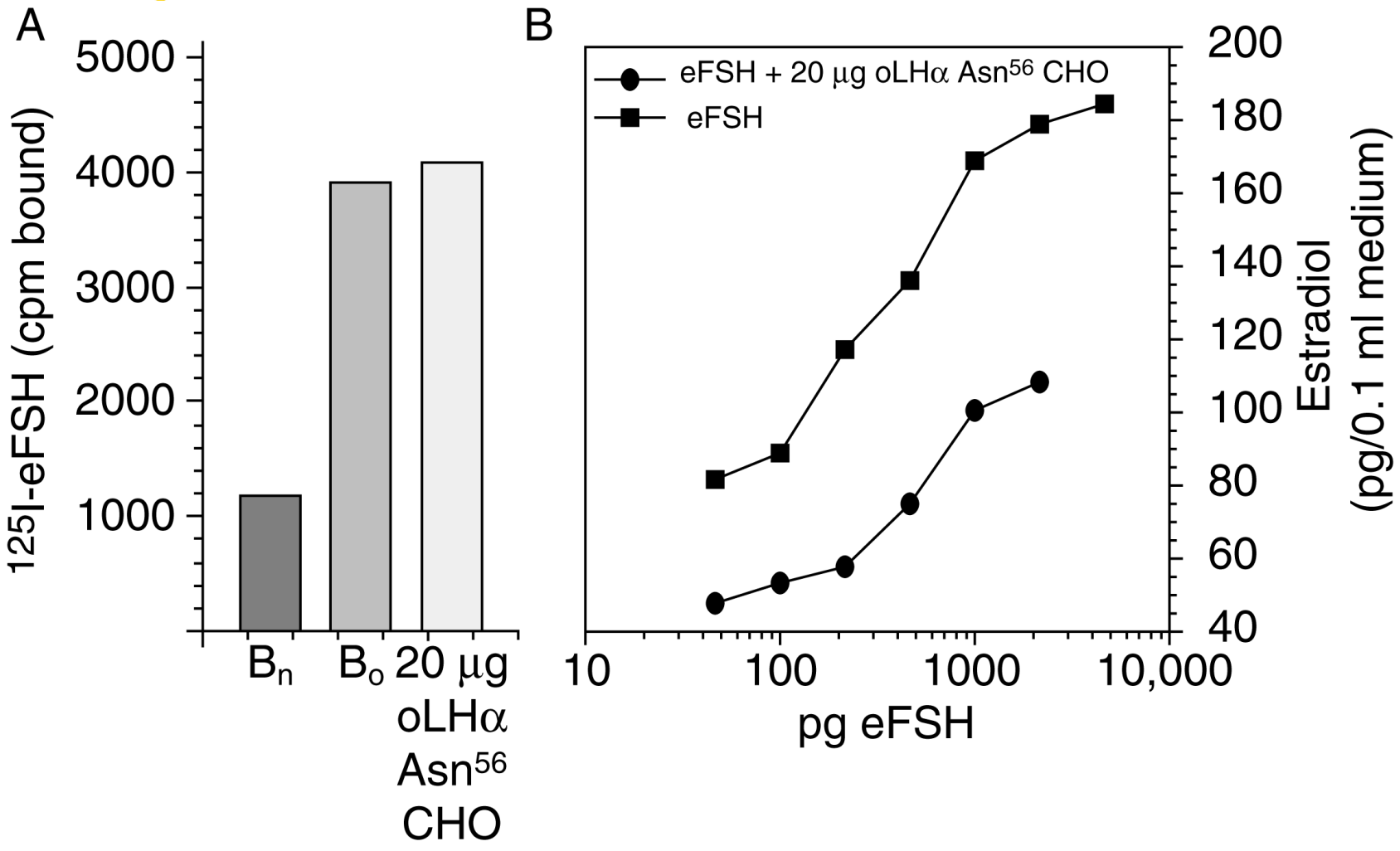
# Oligosaccharide Inhibition of FSH-stimulated steroidogenesis



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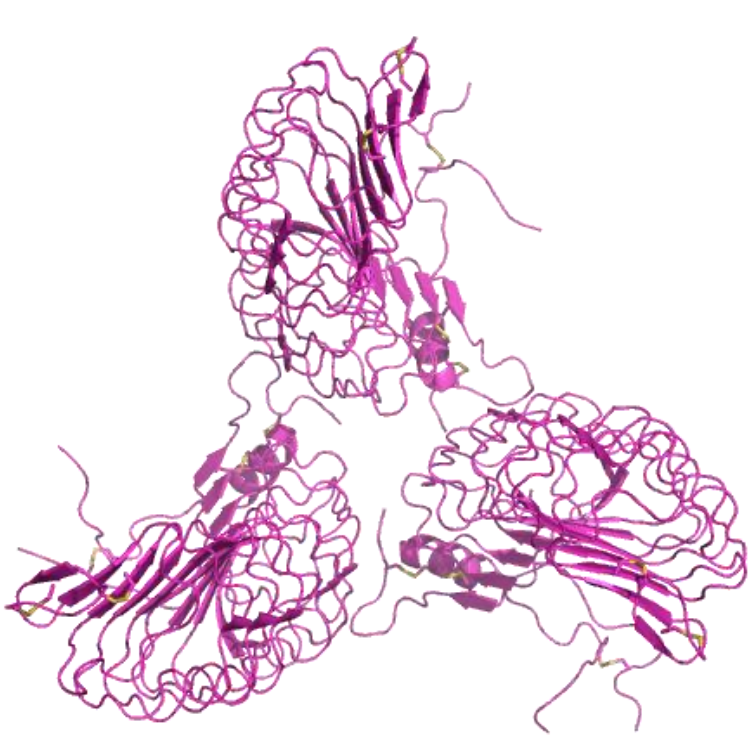
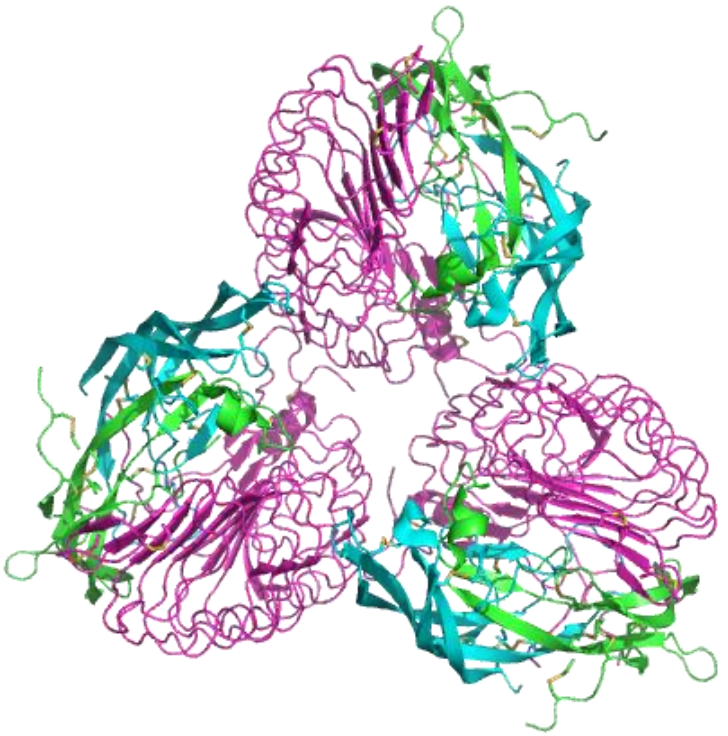
# FSH Receptors as Oligomers







Jiang, X., et al., (2012) Proc. Natl. Acad. Sci. USA **109**(31): 12491-12496.

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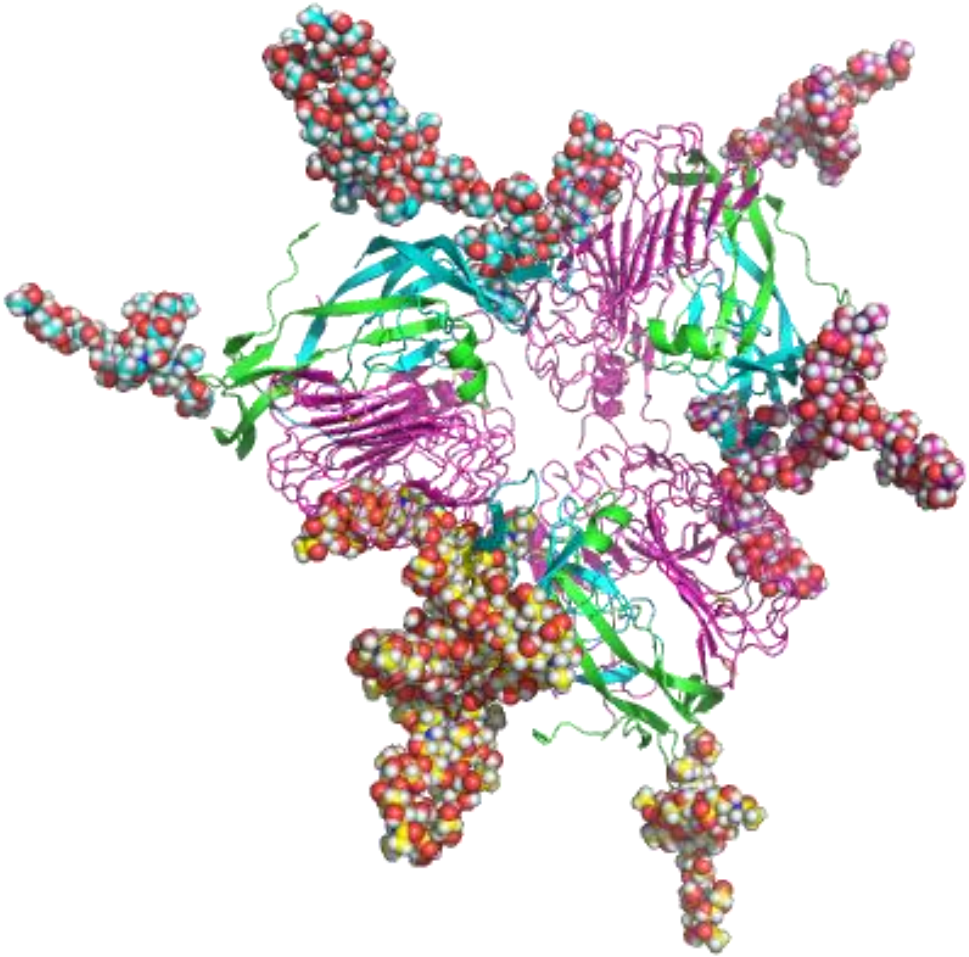
# FSH Receptors as Oligomers



Glycam models of FSH



Jiang, X., et al., (2012) Proc. Natl. Acad. Sci. USA **109**(31): 12491-12496.

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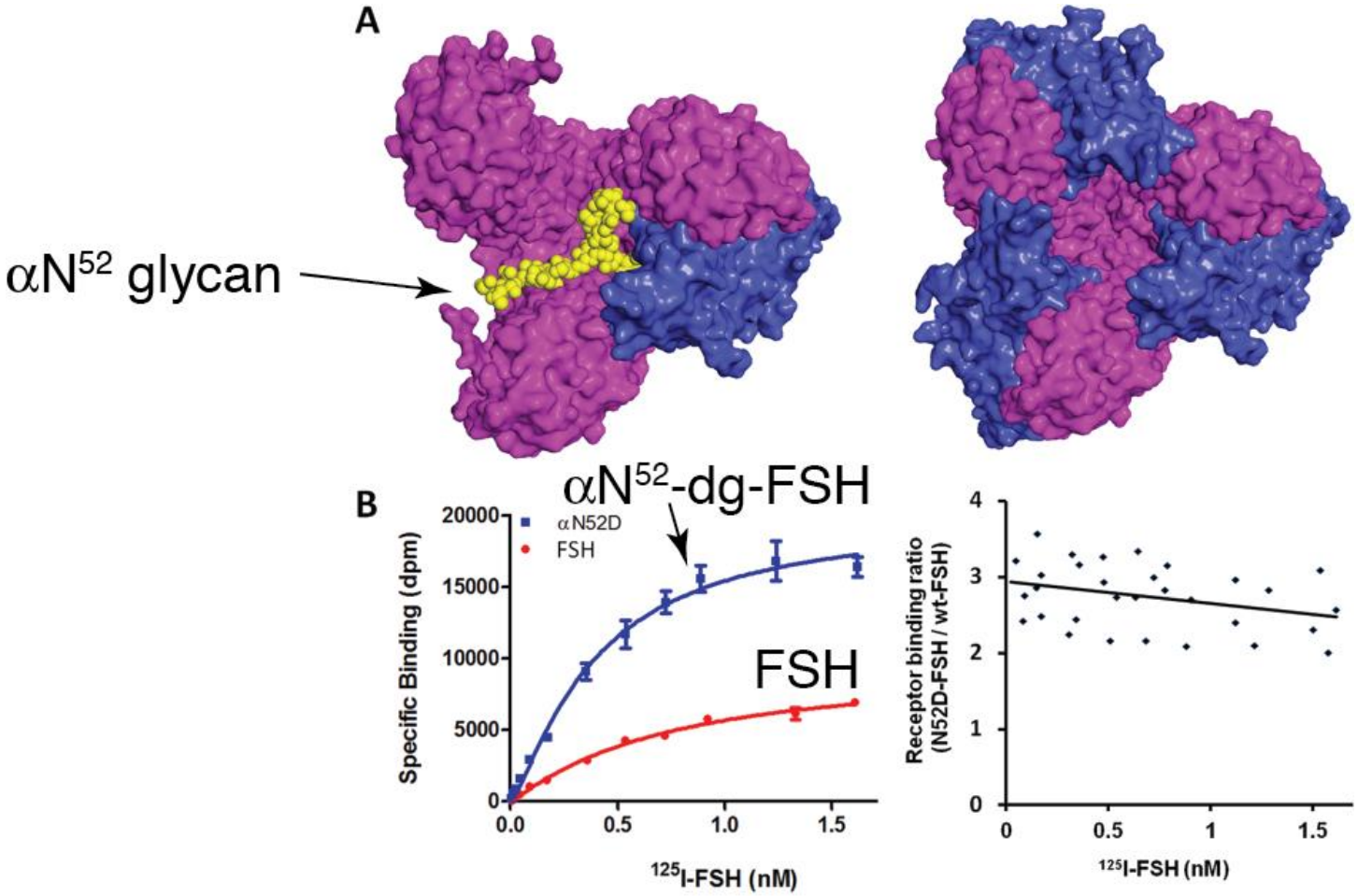
# αN52 glycan potentially reduces binding




Jiang, X., et al., (2014) J. Biol. Chem. 289(20): 14273-14282.

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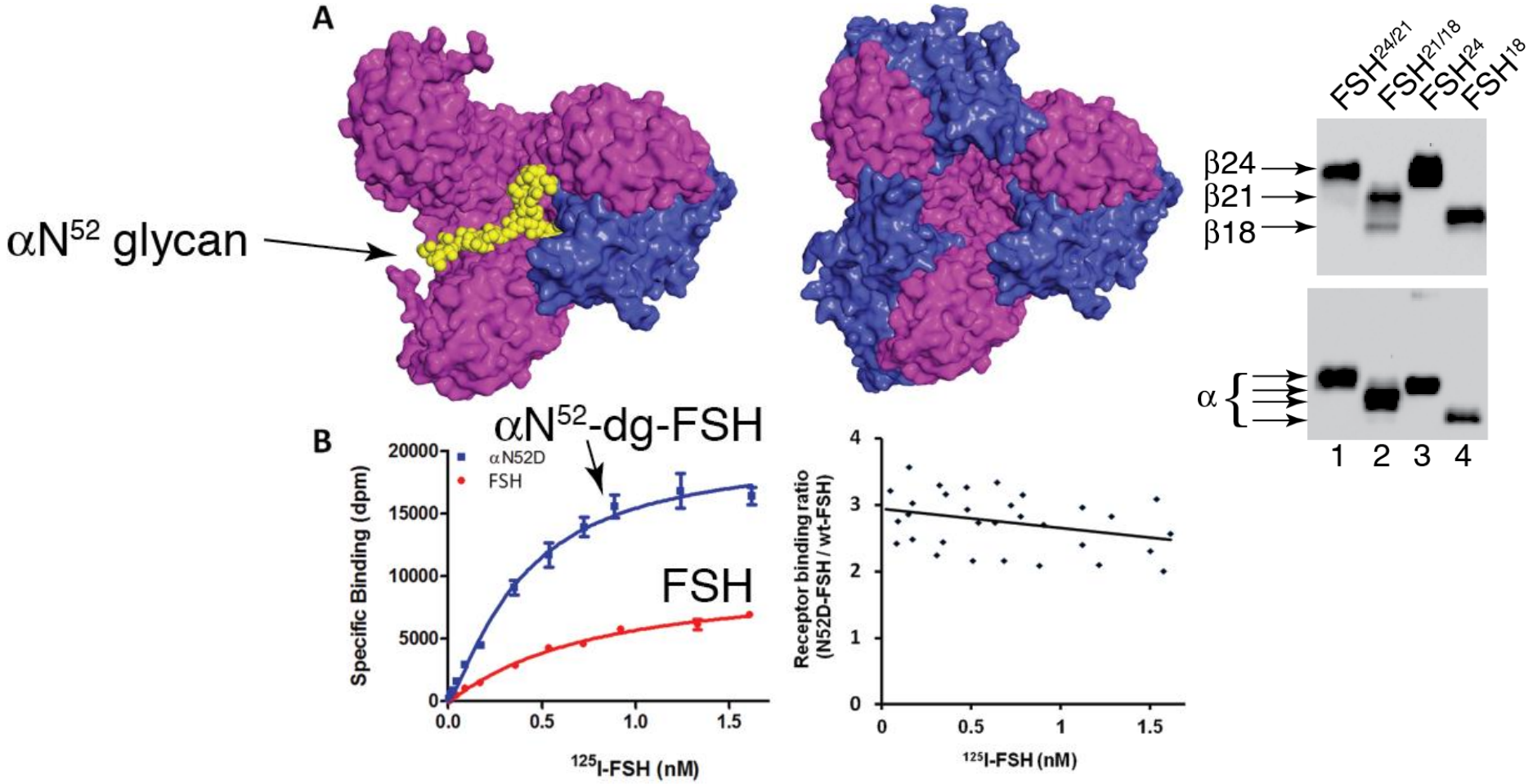
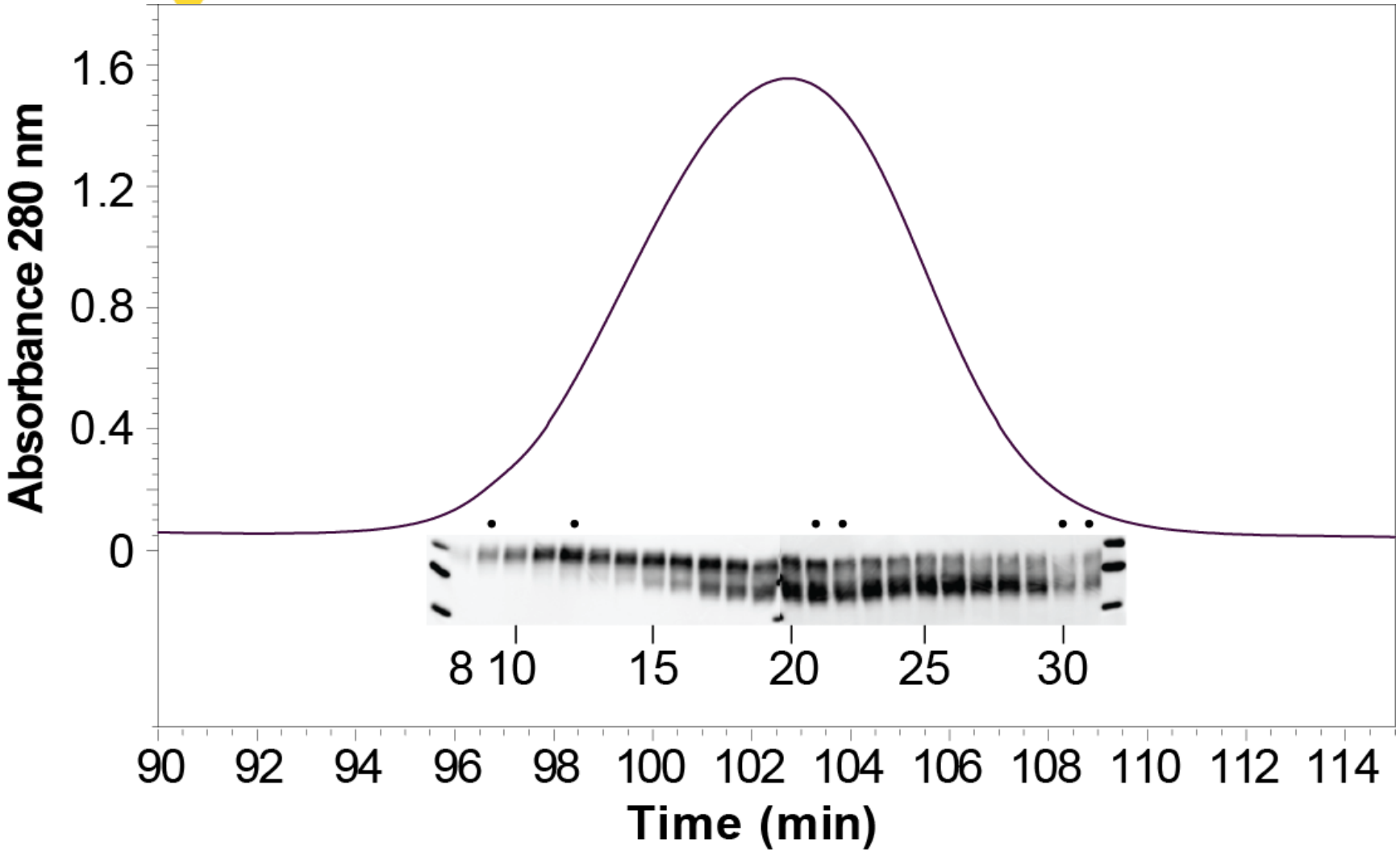
# αN52 glycan potentially reduces binding




Jiang, X., et al., (2014) J. Biol. Chem. 289(20): 14273-14282.

# FSH Glycoform Isolation



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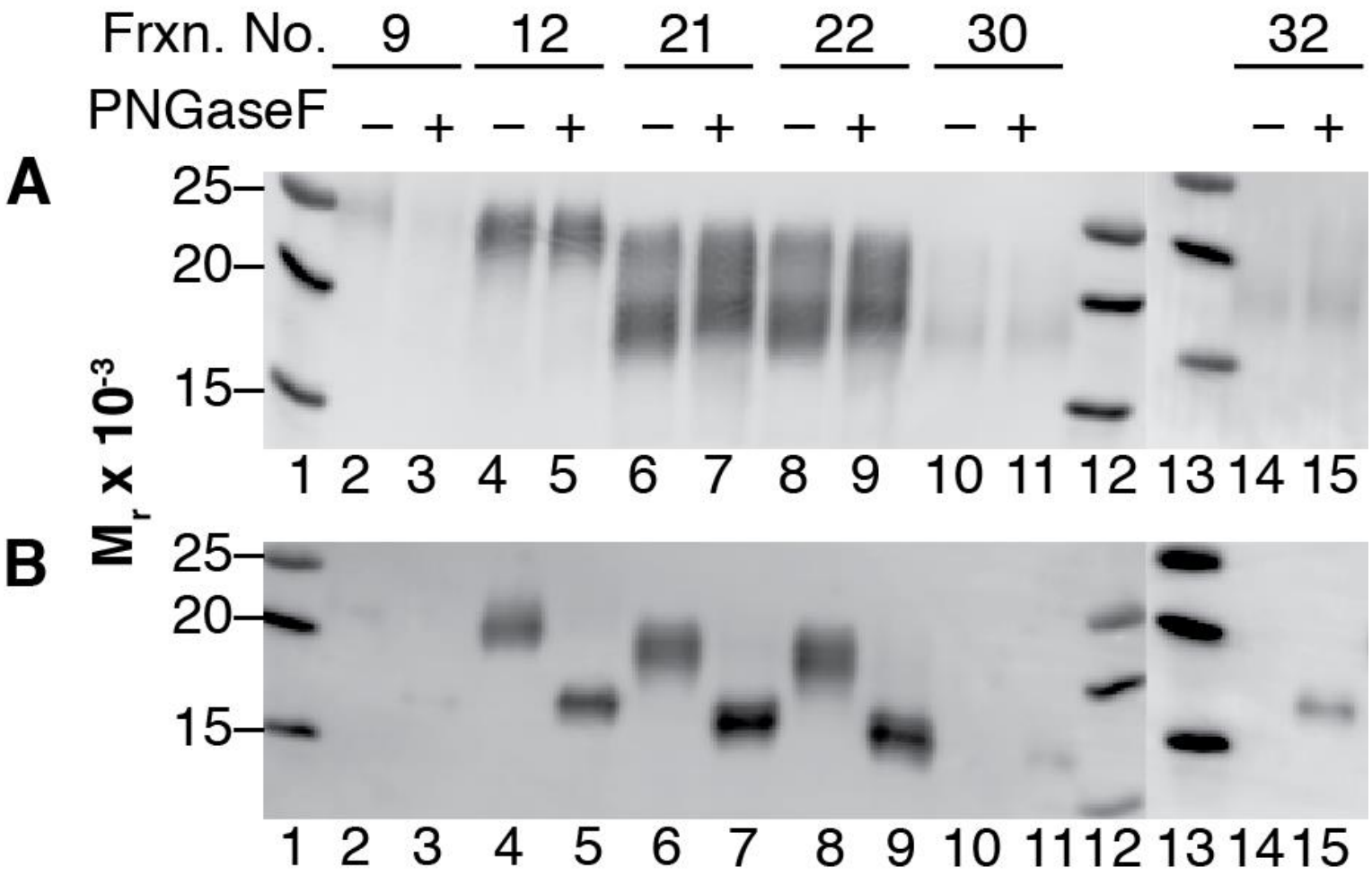
# Selective αAsn$^{52}$ Glycan Removal



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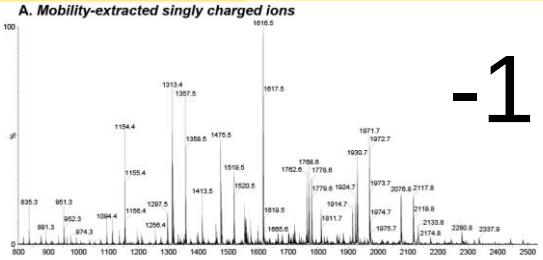
# ESI-MS vs Ion Mobility + MS

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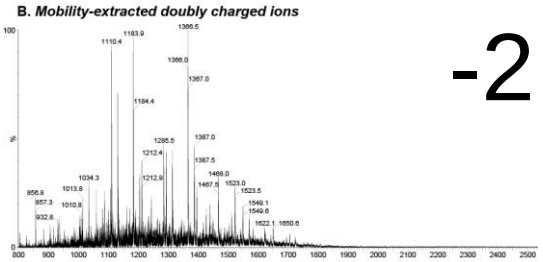


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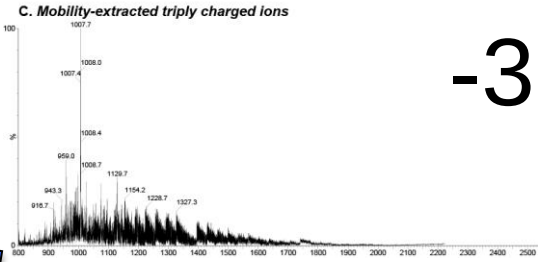


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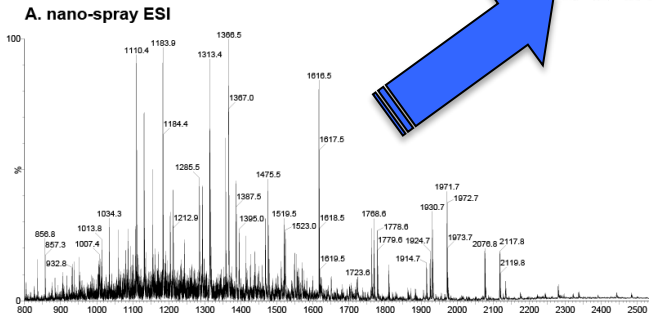


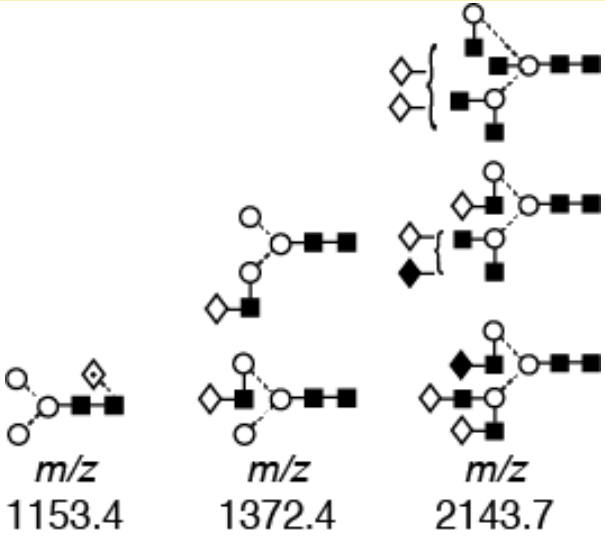

*m/z* 1153.4

*m/z* 1372.4

*m/z* 2143.7



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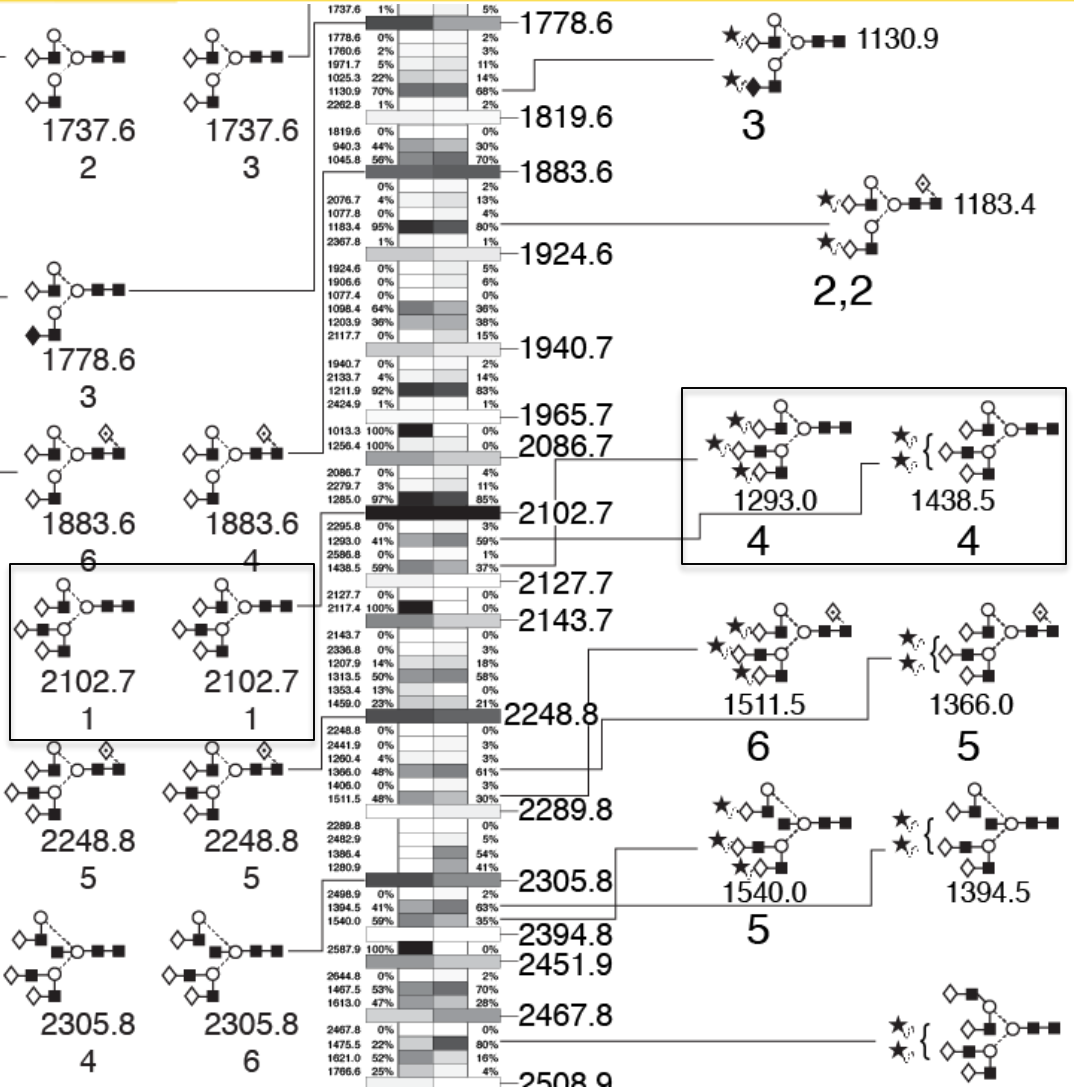
# Compare FSH preparation glycans

| | | |
|---|---|---|
| 1372.5 | 0.76 | 0.23 |
| 1413.5 | 0.21 | 0.00 |
| 1518.5 | 0.62 | 1.27 |
| 1575.5 | 0.22 | 0.11 |
| 1721.6 | 0.26 | 0.09 |
| 1737.6 | 9.97 | 11.56 |
| 1778.6 | 9.33 | 5.83 |
| 1819.6 | 0.52 | 0.20 |
| 1883.6 | 8.05 | 10.71 |
| 1924.6 | 1.70 | 1.24 |
| 1940.7 | 4.83 | 4.88 |
| 1965.7 | 0.16 | 0.00 |
| 2086.7 | 4.88 | 3.24 |
| 2102.7 | 10.95 | 13.71 |
| 2127.7 | 0.50 | 0.00 |
| 2143.7 | 6.25 | 2.89 |
| 2248.8 | 9.20 | 10.23 |
| 2289.8 | 0.00 | 0.87 |
| 2305.8 | 9.26 | 7.54 |
| 2394.8 | 0.01 | 0.00 |
| 2451.9 | 5.33 | 3.52 |
| 2467.8 | 2.25 | 6.38 |
| 2508.9 | 0.49 | 0.00 |



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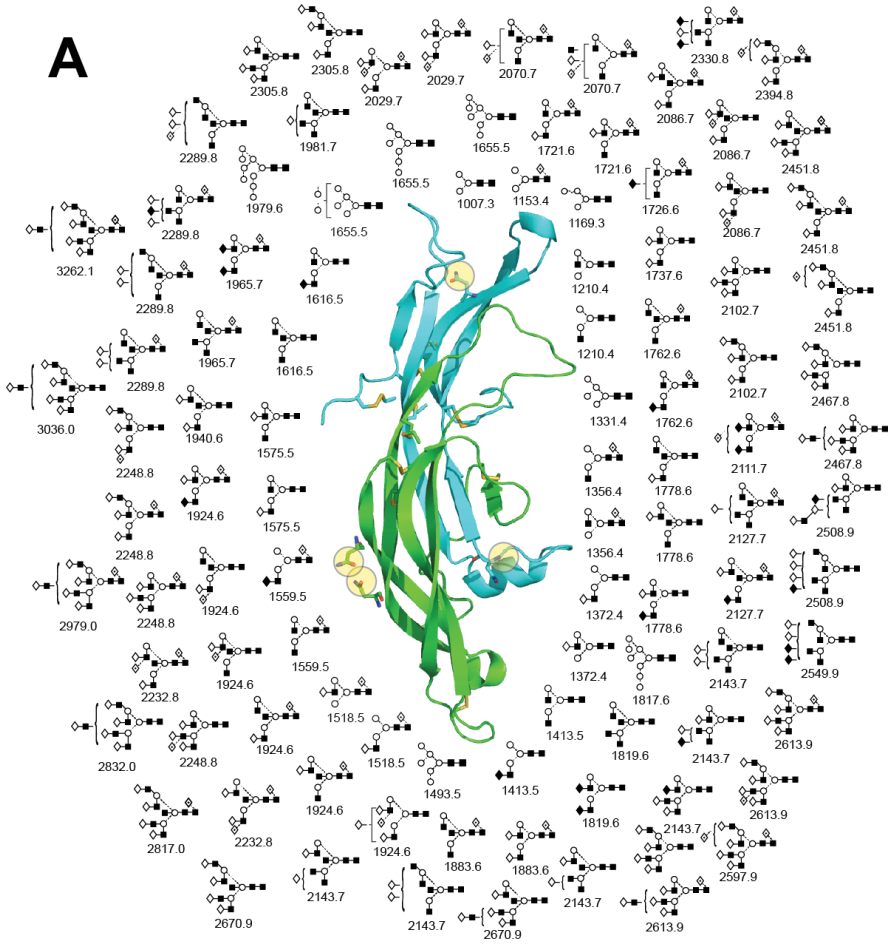
# FSH Total Glycan Microheterogenity



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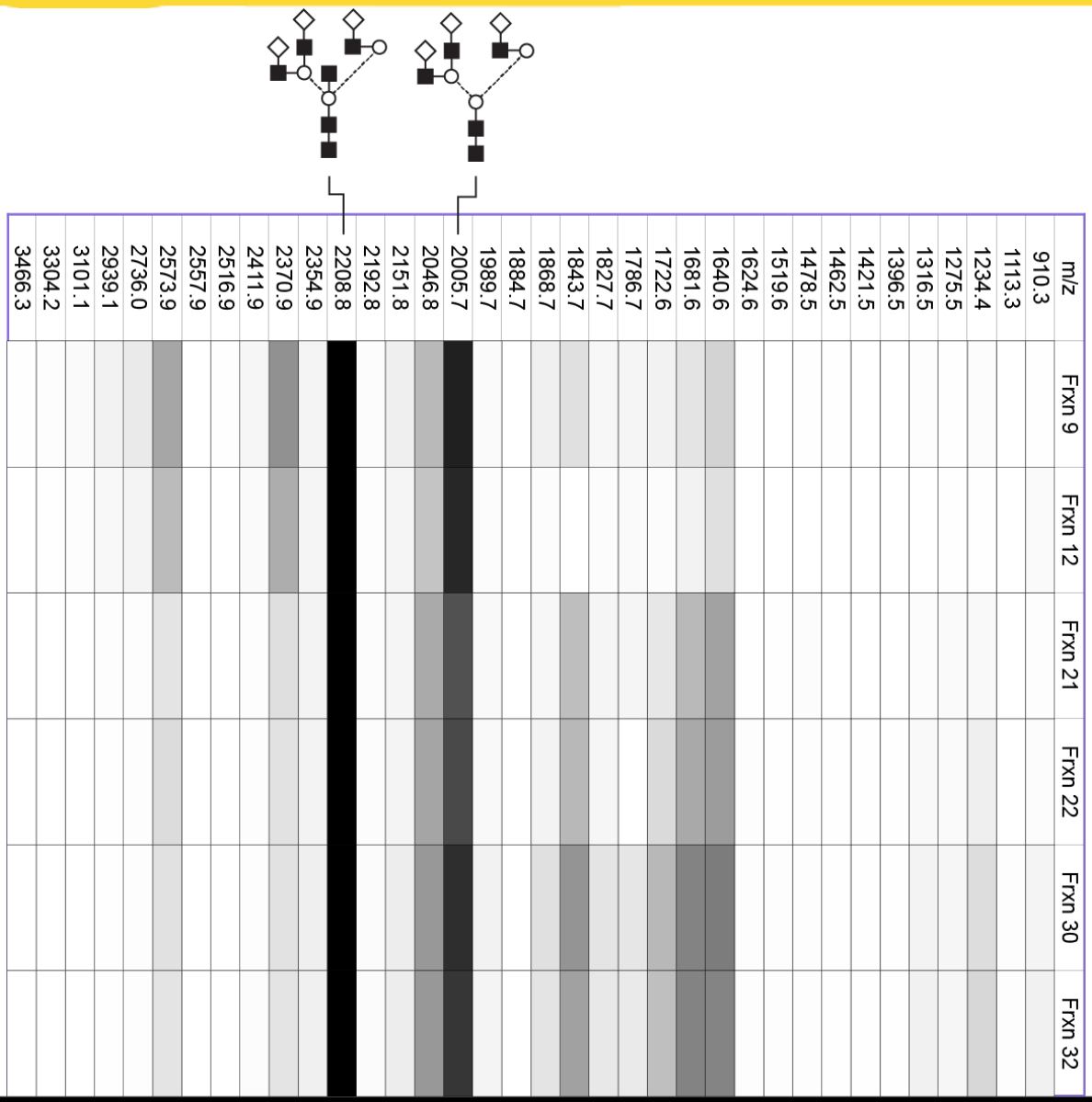
# FSHα N52 Glycans



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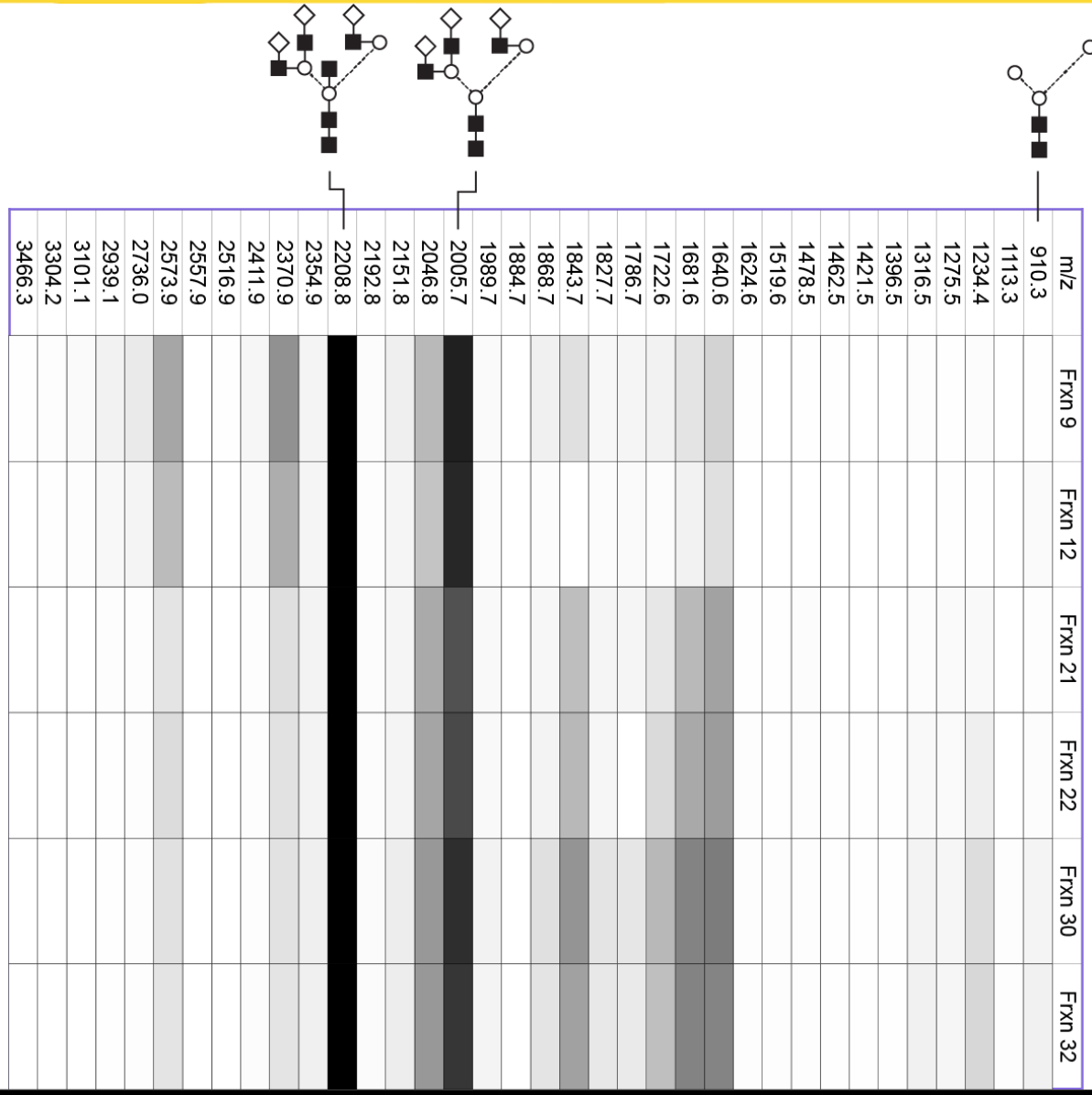
# FSHα N52 Glycans



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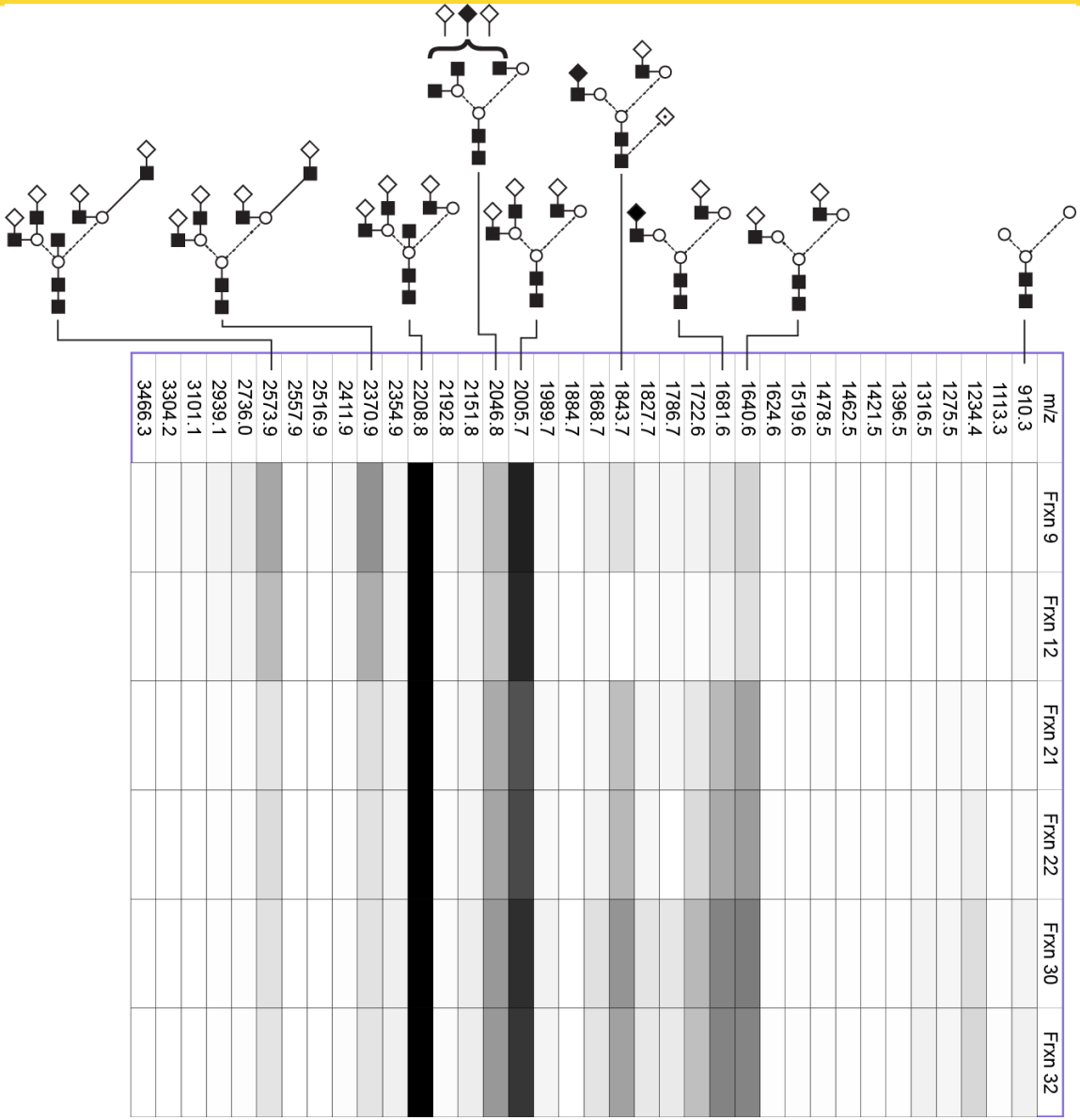
# FSHα N$^{52}$ Glycans



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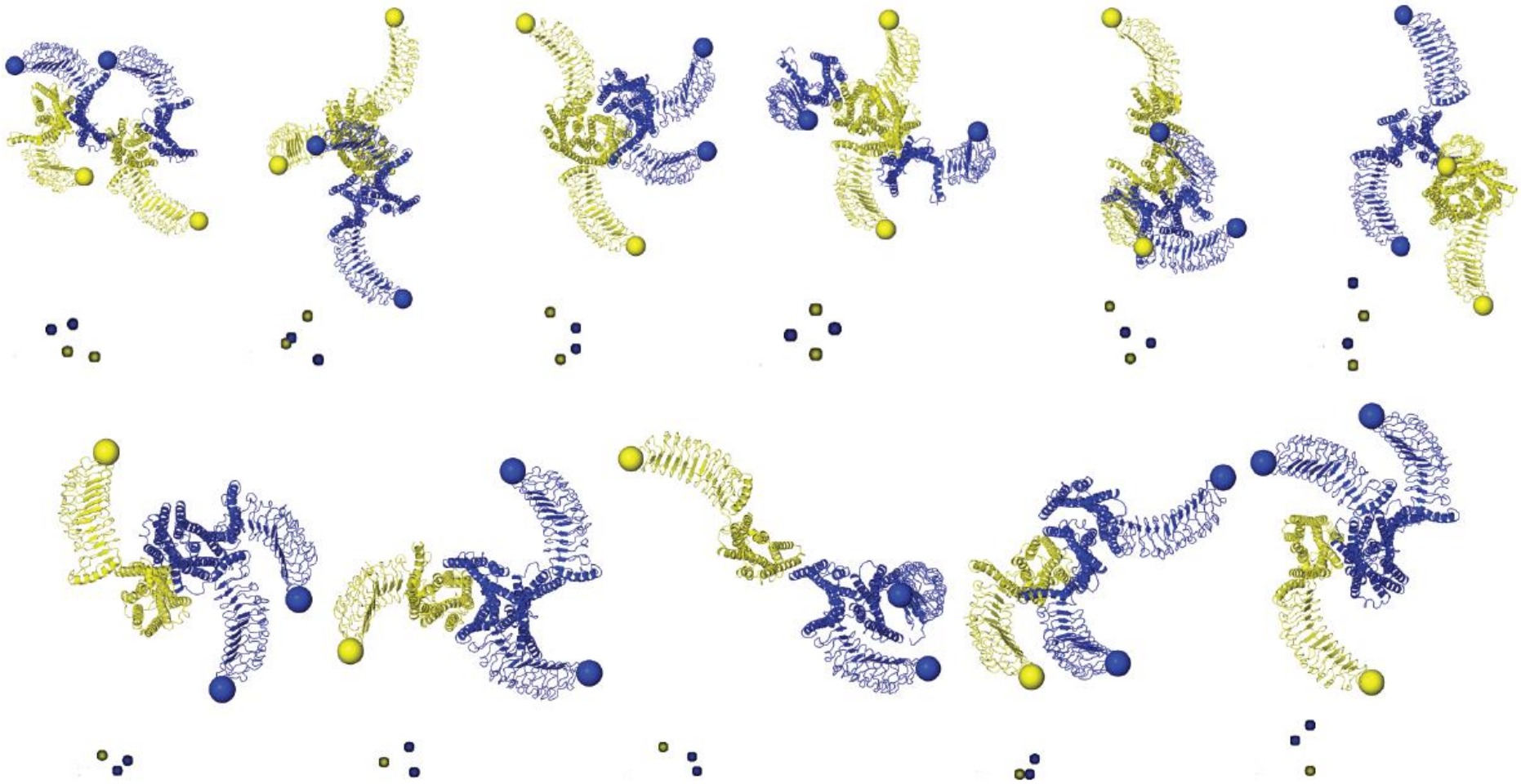
# LH receptors as variety of oligomers





Jonas, K. C., et al. (2015). J. Biol. Chem. **290**: 3875-3892.

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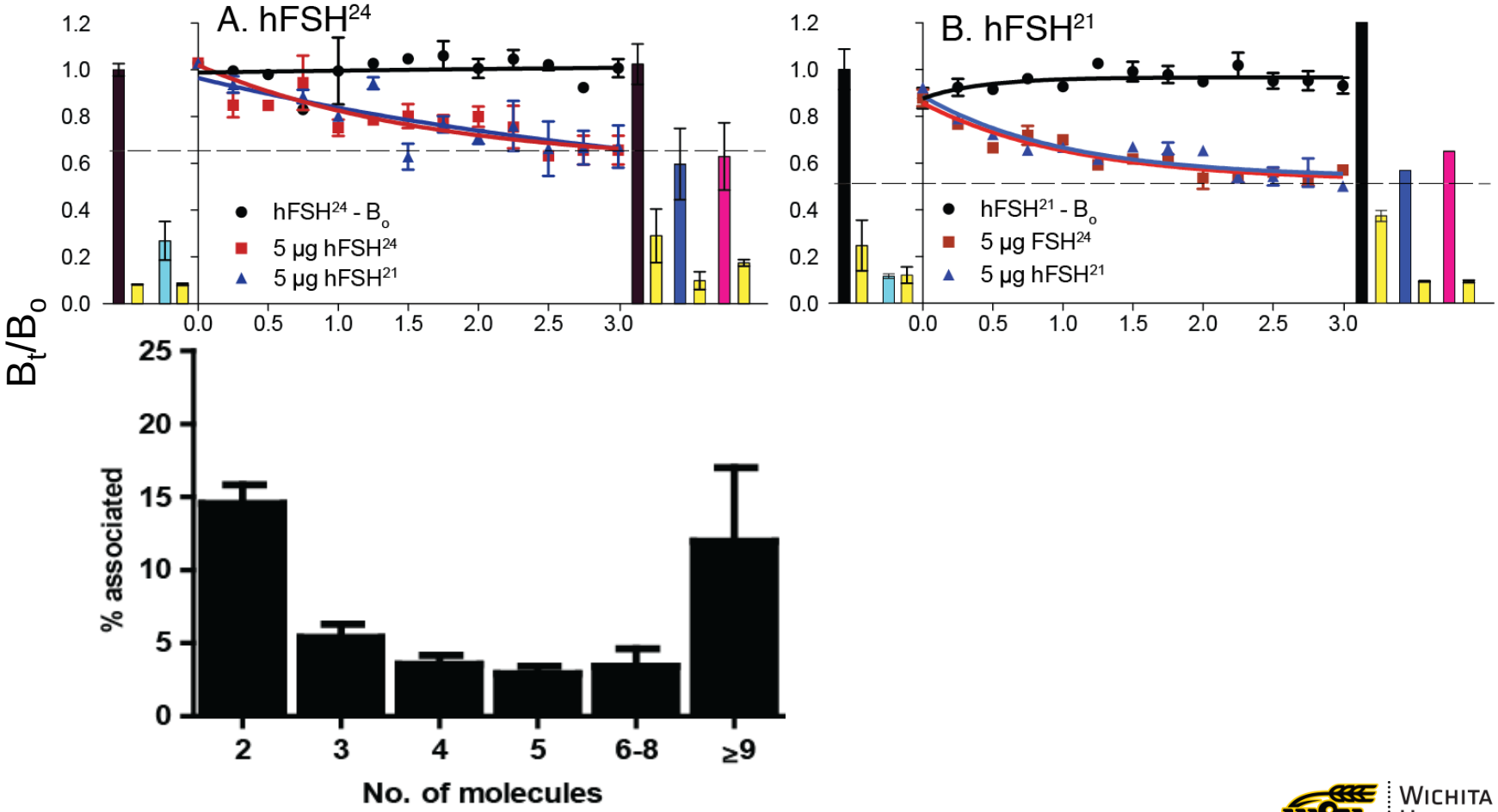
# FSHR Dissociation Reveals Negative Cooperativity for Both FSH$^{21}$ and FSH$^{24}$

Calf testis membranes



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# Acknowledgements


## WSU
Wendy Walton
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Hy-Vong Ha
Katie Norton
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Vladimir Butnev (WSU)
Viktor Butnev (WSU)
Bin Shuai (WSU)

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P01 AG029531
P20 RR016475
P20 RR-17708




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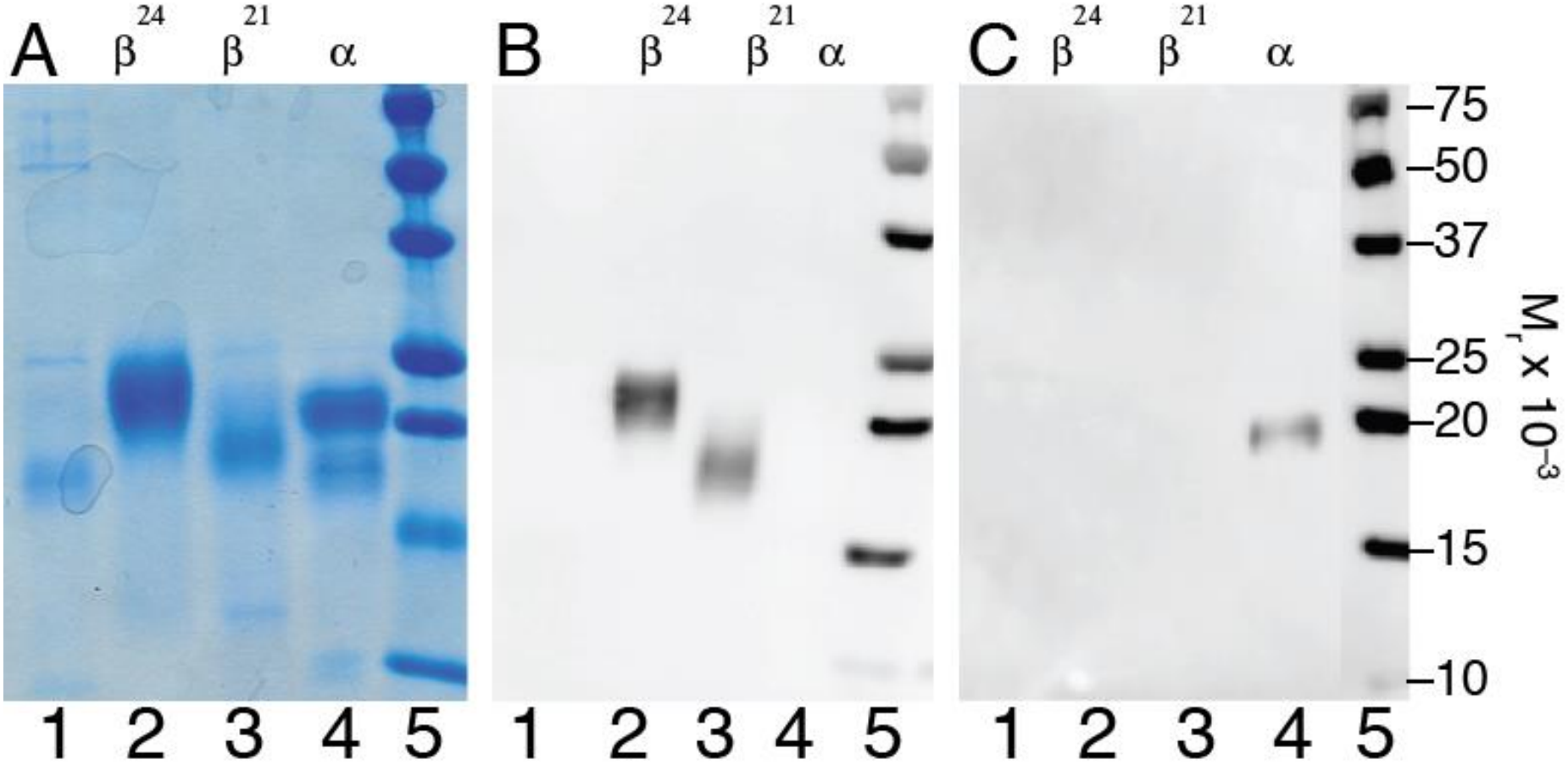
# Isolation of FSHβ subunit glycoforms




Coomassie Blue anti-FSHβ anti-FSHα



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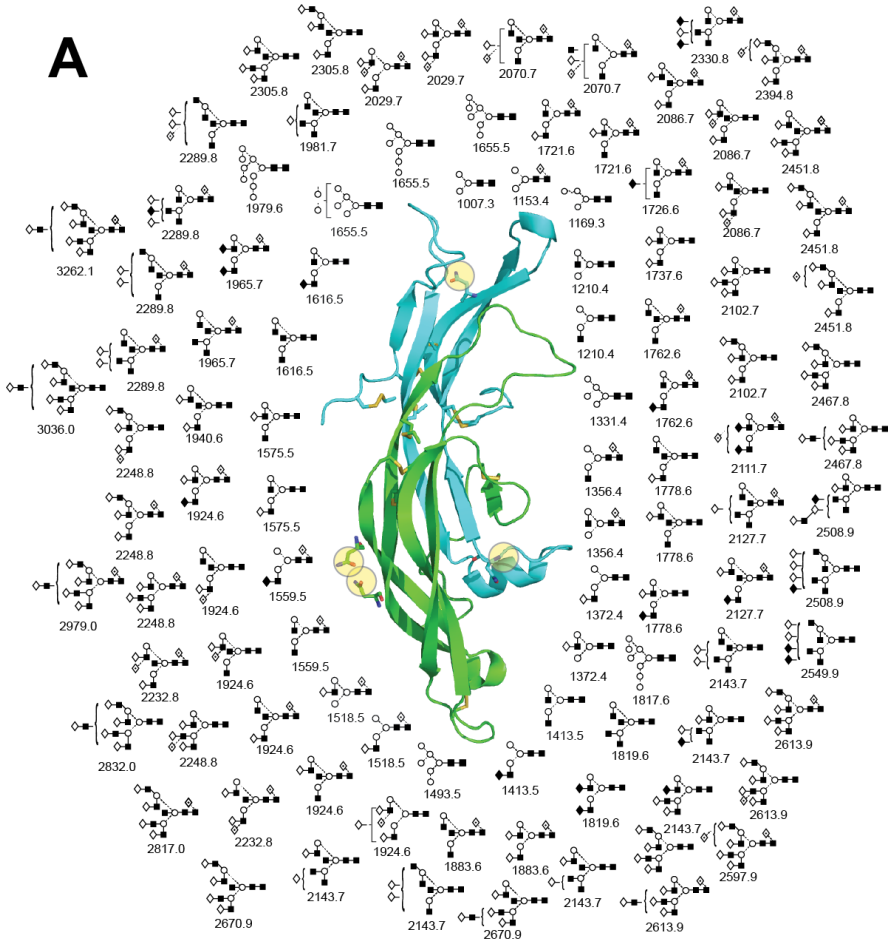
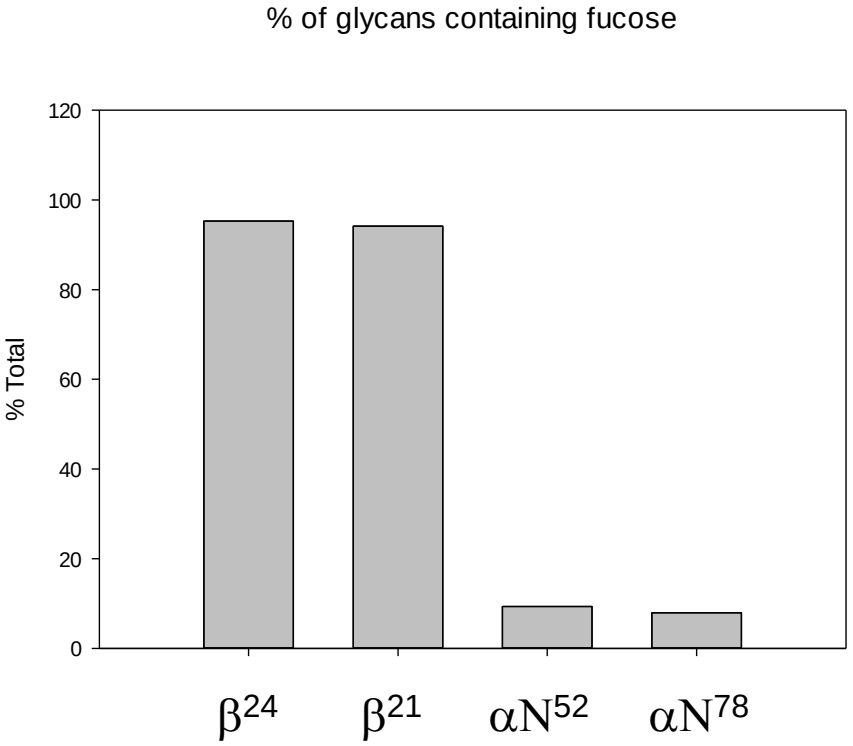
# FSH Total Glycan Microheterogenity



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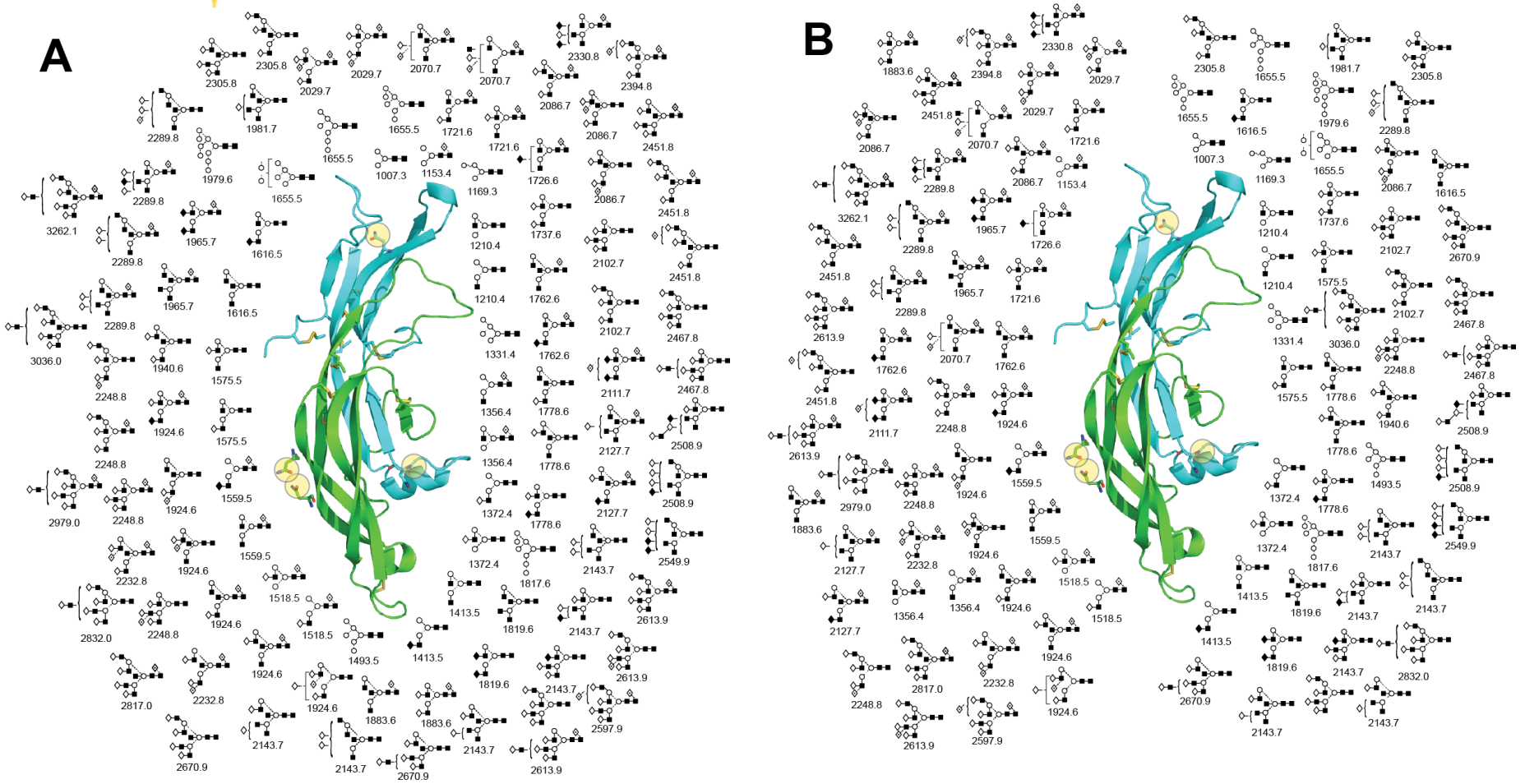
# FSH Total Glycan Microheterogenity



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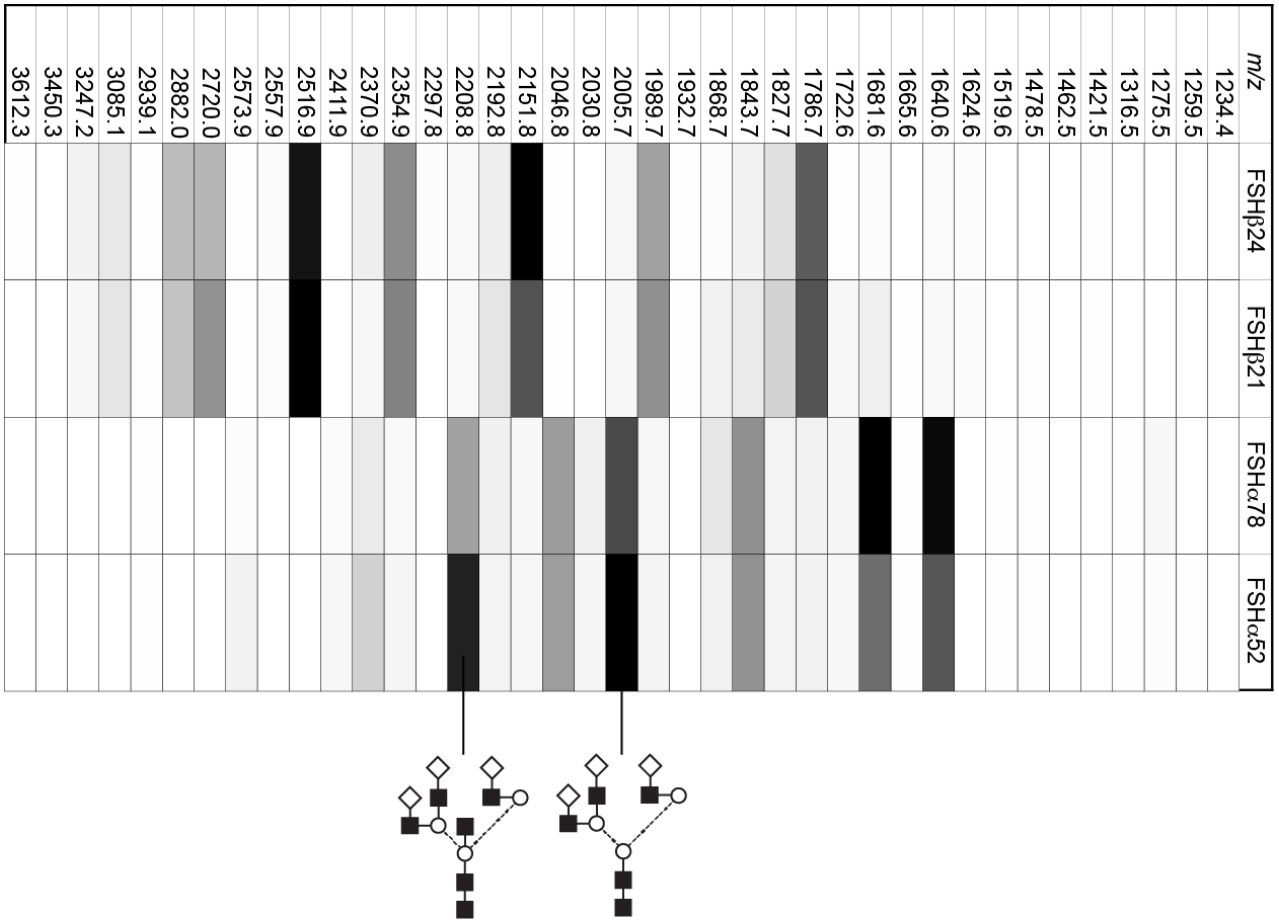
# Site-Specific FSH Glycosylation αAsn$^{52}$



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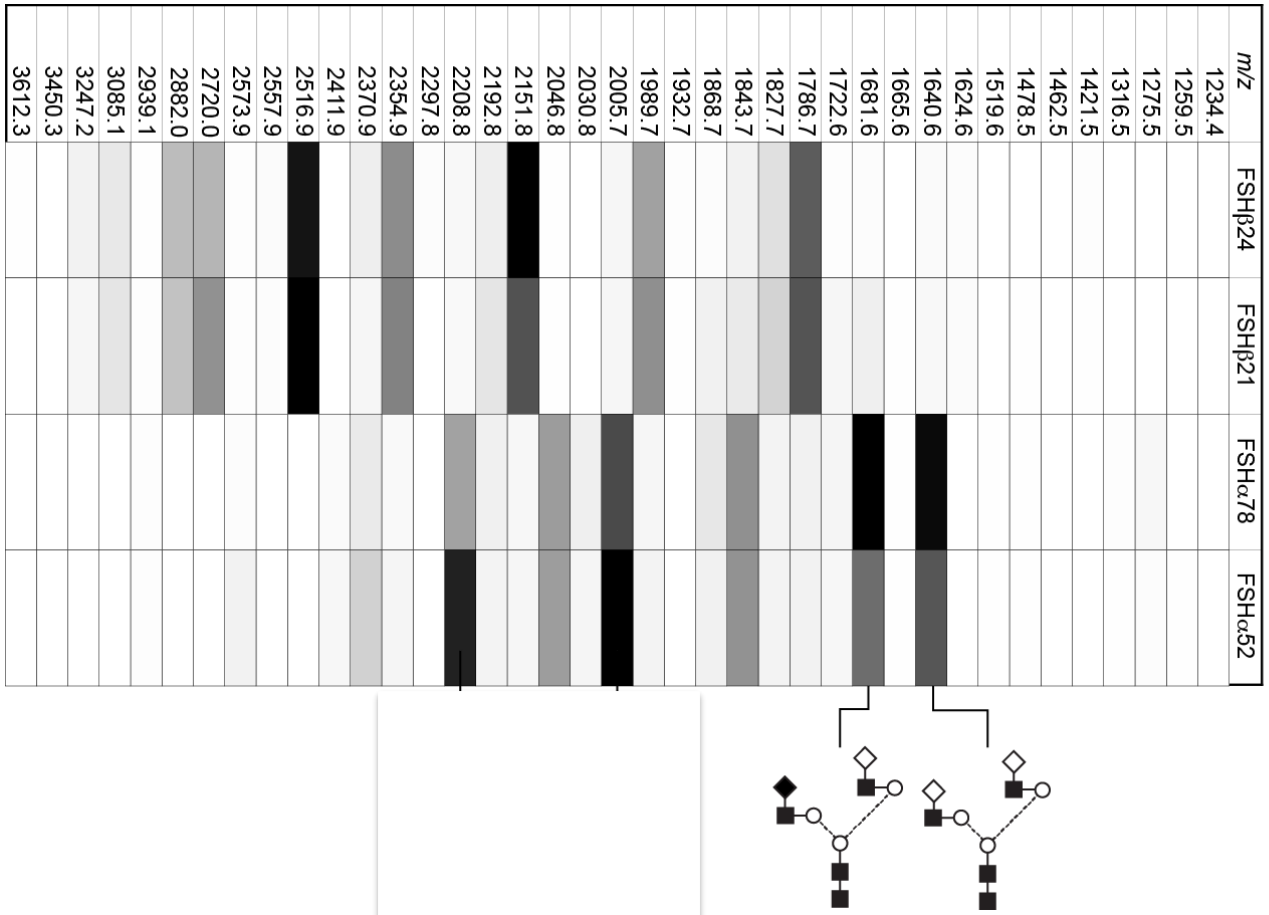
# Site-Specific FSH Glycosylation αAsn$^{78}$



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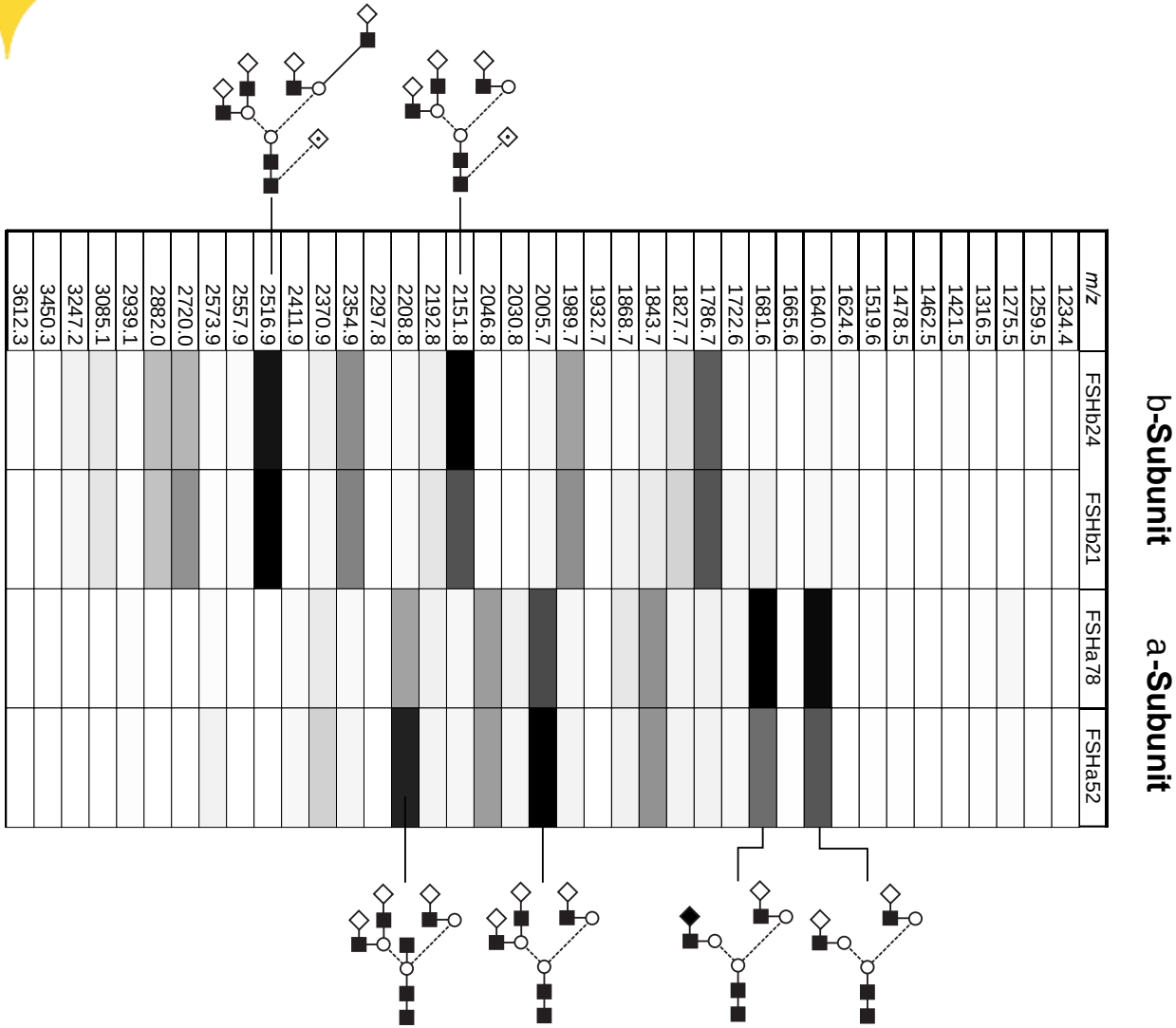
# Site-Specific FSH$^{24}$ Glycosylation βAsn$^{7/24}$



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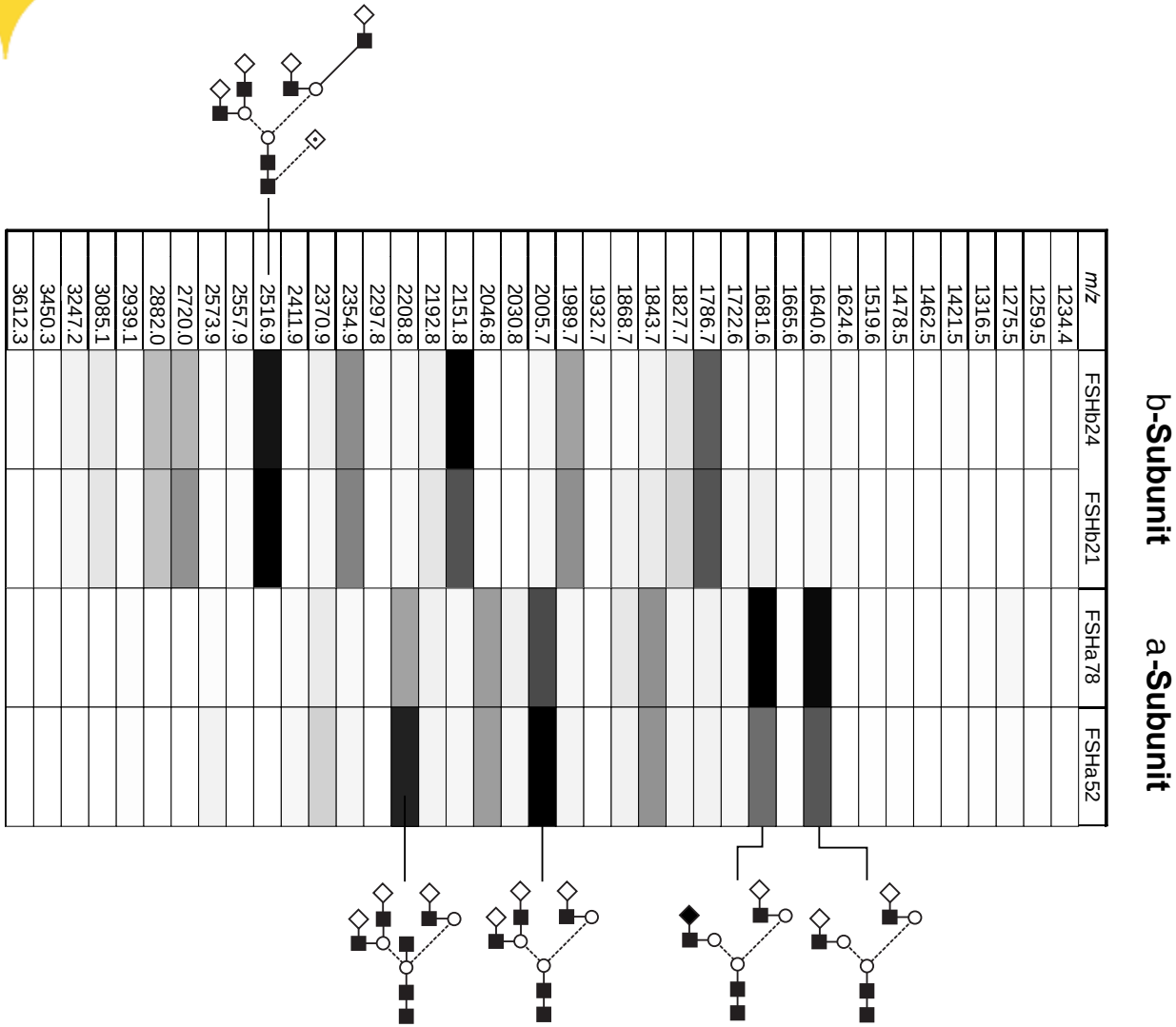
# Site-Specific FSH[21] Glycosylation βAsn[7]



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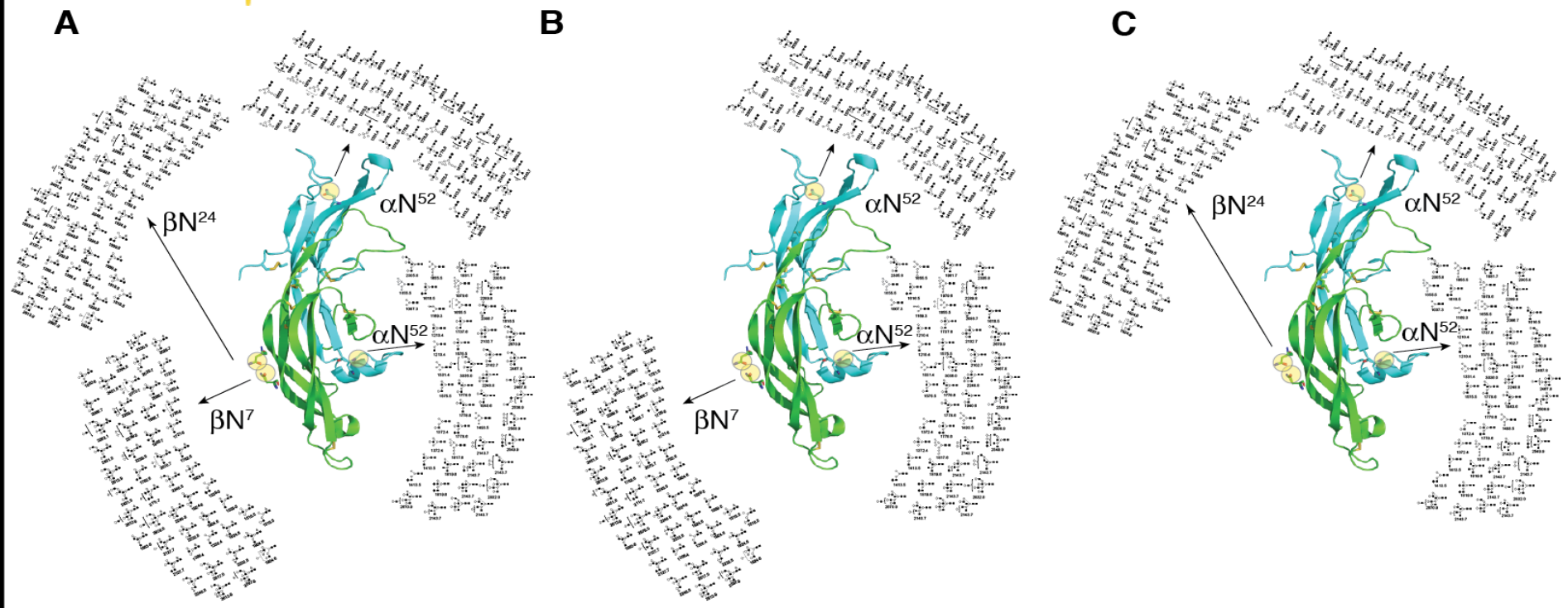
# Macro + Micro Heterogeneity in FSH




**FSH$^{24}$** **FSH$^{21}$** **FSH$^{18}$**



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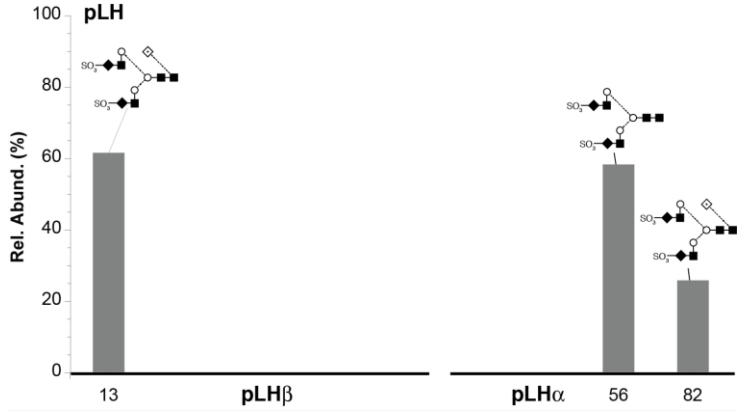
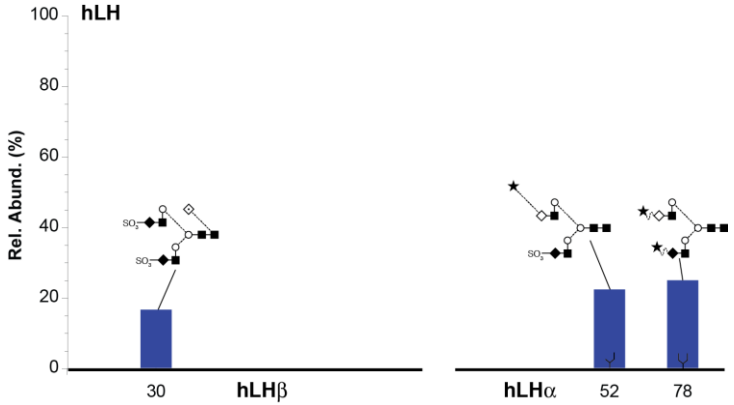
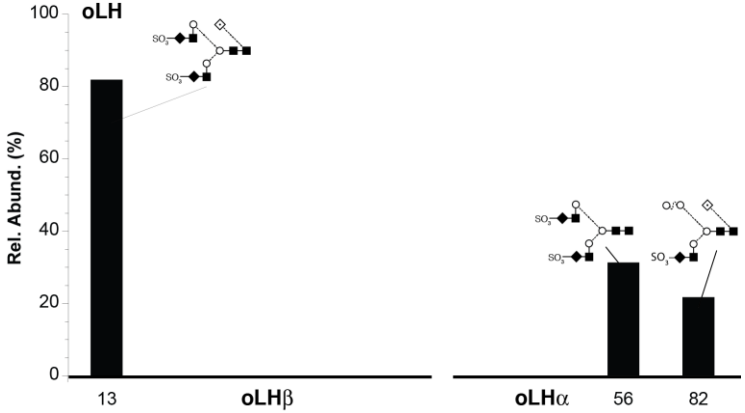
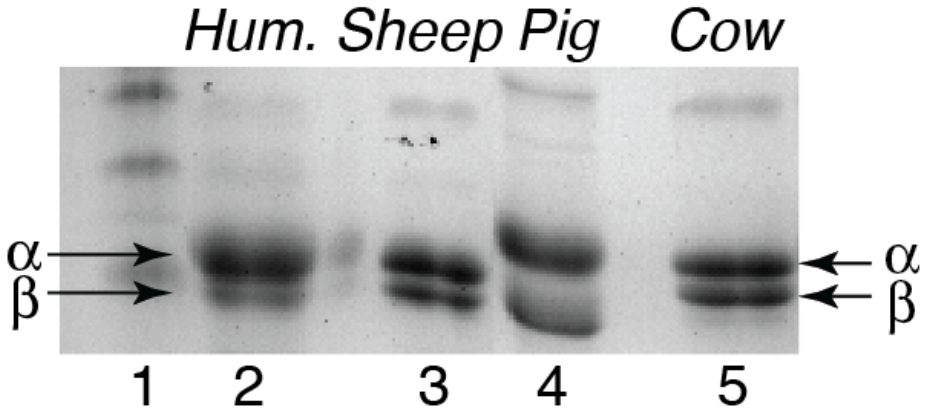
# LH Major N-glycans



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