Dynamics of IgG1 glycoforms and interaction with FcγR1

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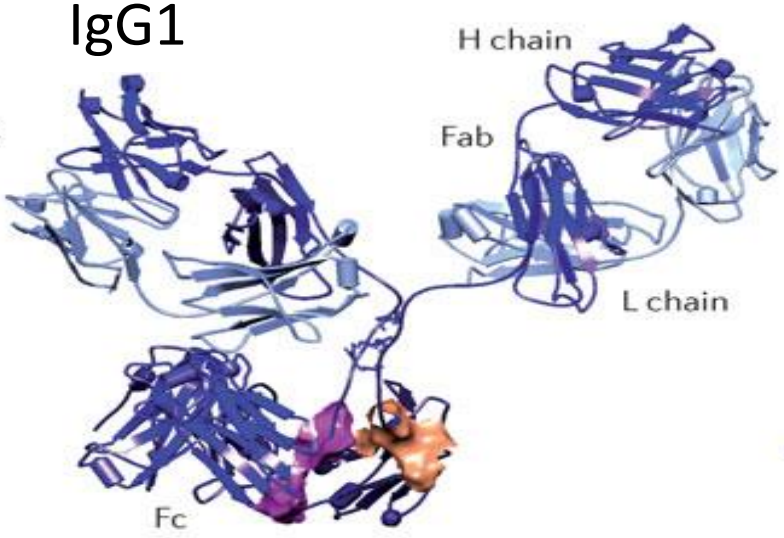
Glycan Effects on IgG1 Dynamics

- aIL8-hFc is a humanized IgG1 that targets chemokine Interleukin 8
- FcγR1a (CD64) is a high affinity receptor for Fc of IgG
- N-glycan structures of Fc can affect pharmacokinetics and immune effector functions → critical quality attribute

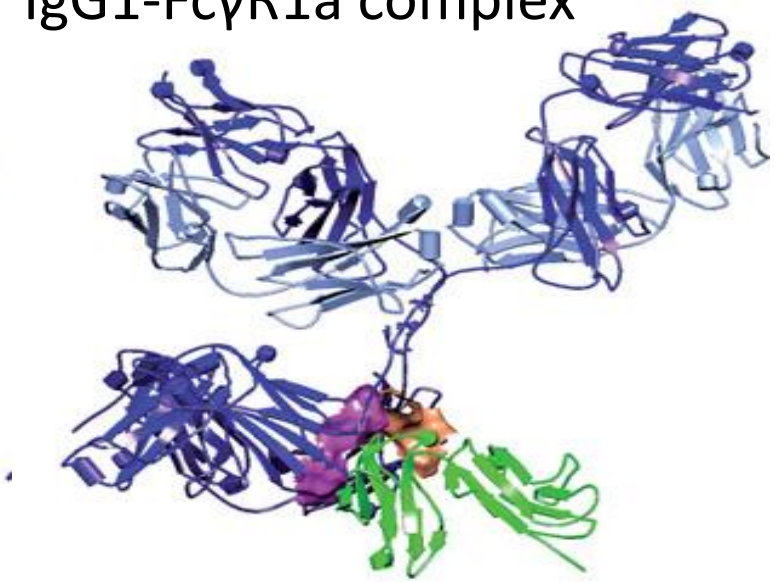
FcγR1a

Solubilized
(Sino Biological)

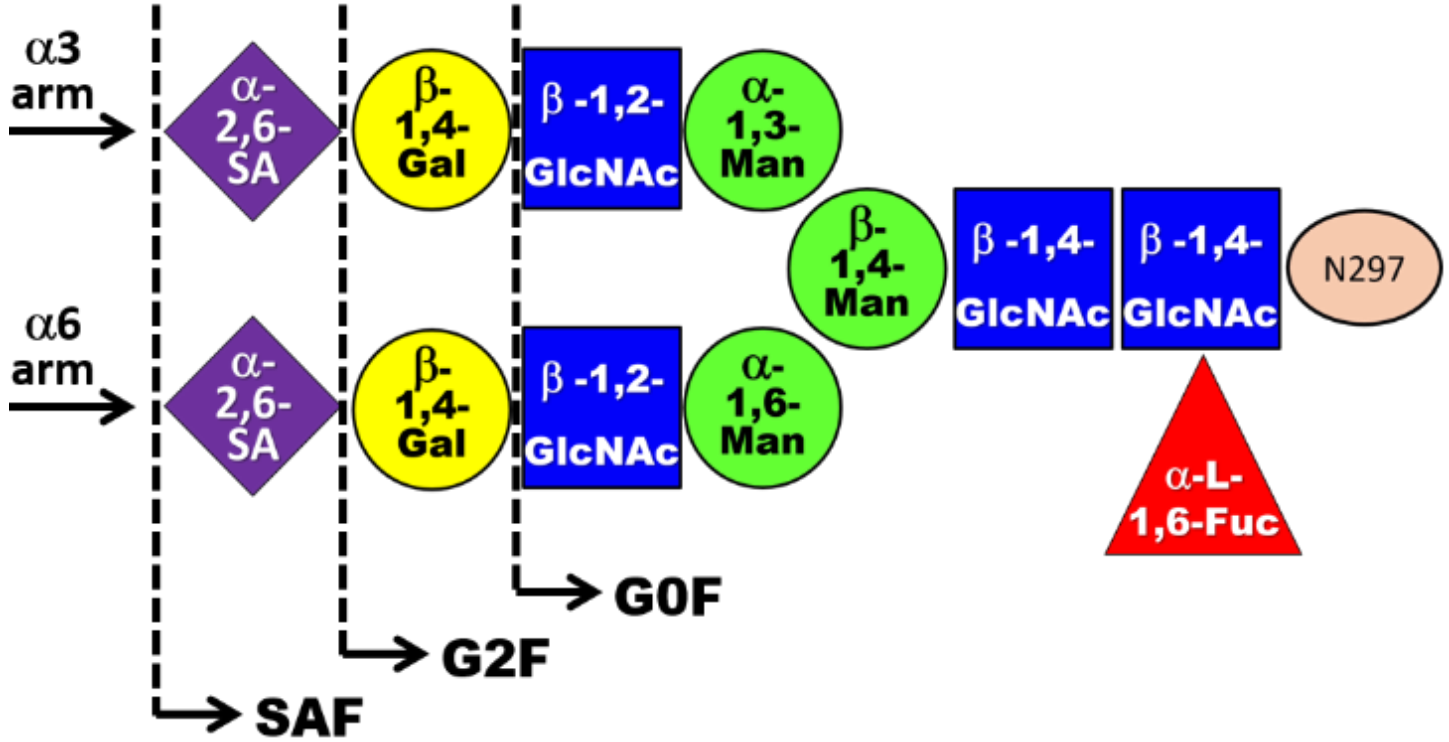
IgG1



IgG1-FcγR1a complex



Preparation of aIL8-hFc Glycoforms for Analysis



- CHO-DP12 cells used to produce aIL8-hFc
- aIL8-hFc bound to Protein A for solid-phase enzymatic remodeling

IgG1 samples prepared by V. Tayi & M. Butler, Dept. Microbiology, U. Manitoba

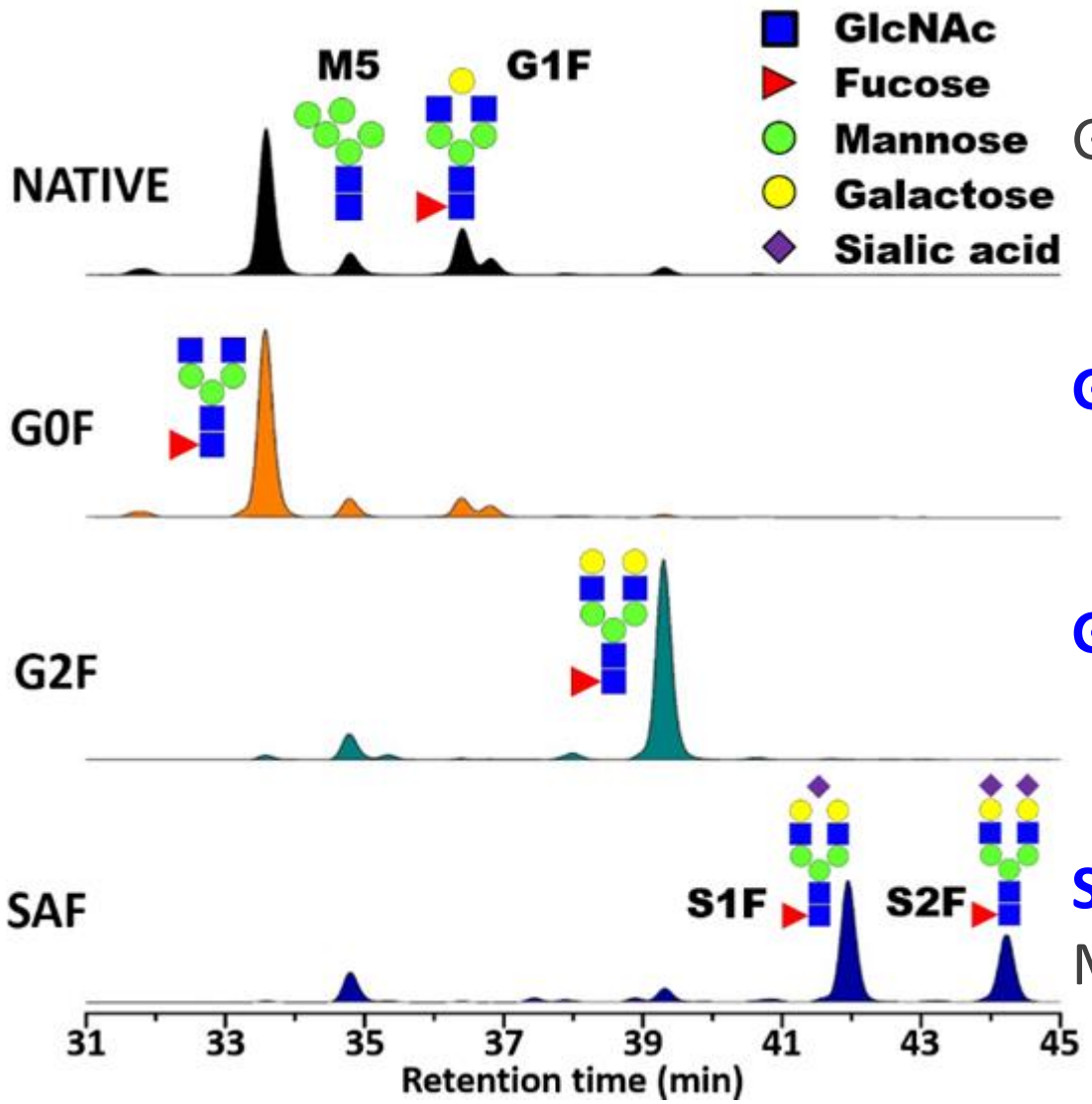
Glycosylation Profiles of Four aIL8-hFc Samples

Protein A

PNGase F

2-AB labeling

HILIC HPLC



G0F 77%, G1F 16%, M5 7%,

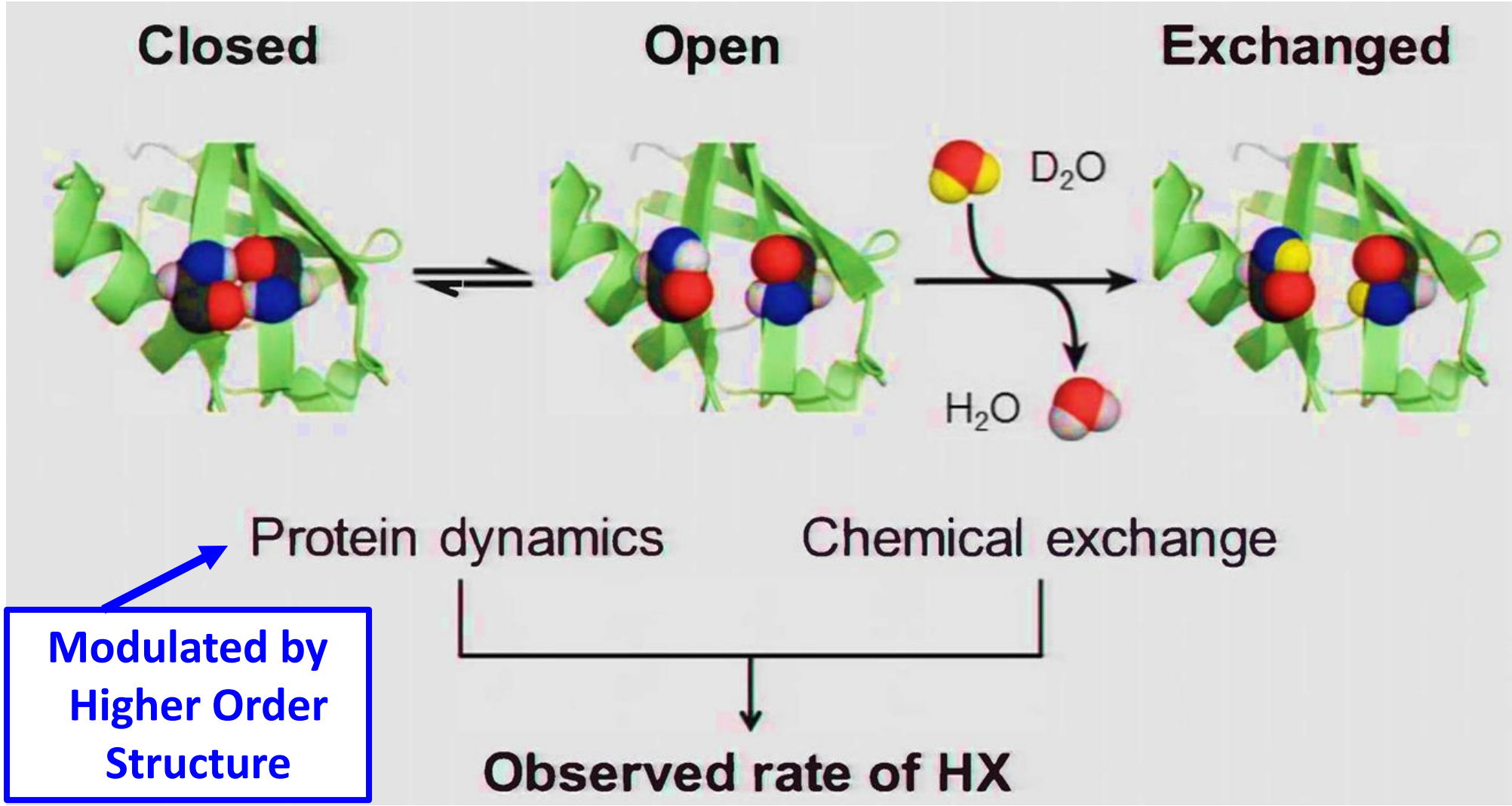
G0F 82%, G1F 11%, M5 7%

G2F 93%, M5 7%

S1F 54%, S2F 31%,
M5 11%, G2F 4%

IgG1 samples prepared by V. Tayi & M. Butler, Dept. Microbiology, U. Manitoba

H/D Exchange Theory: Linderstrøm-Lang Mechanism



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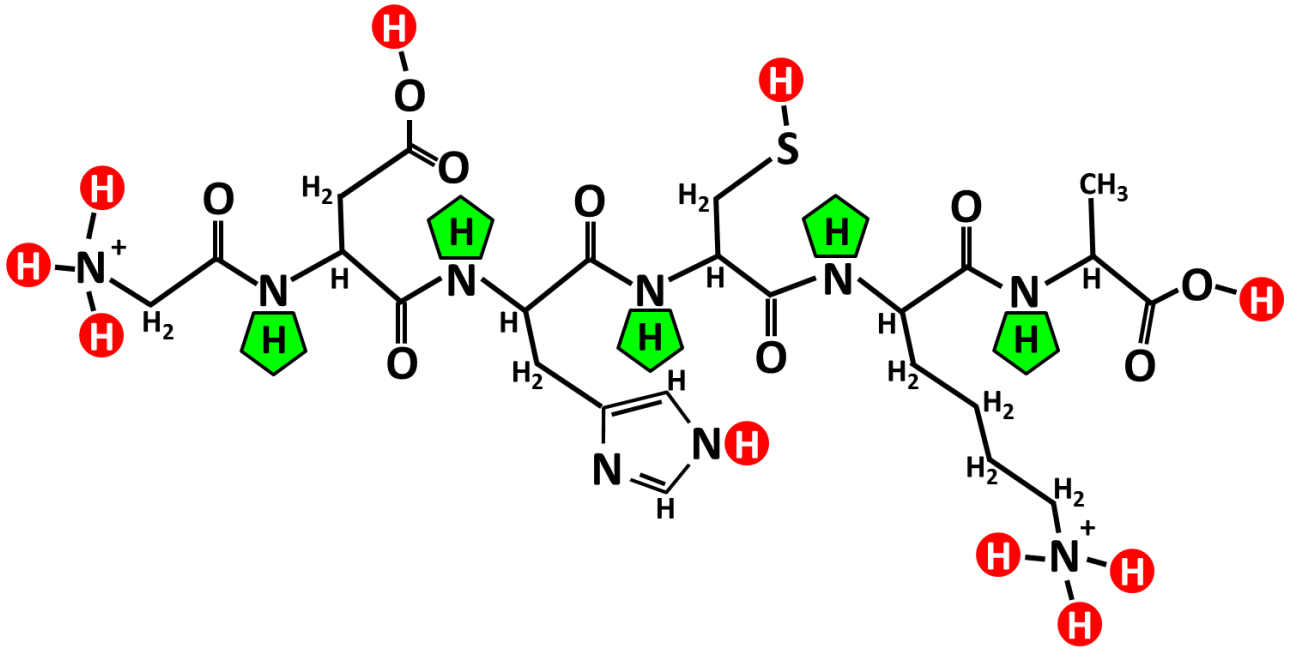
Hydrogen-Deuterium Exchange Rate

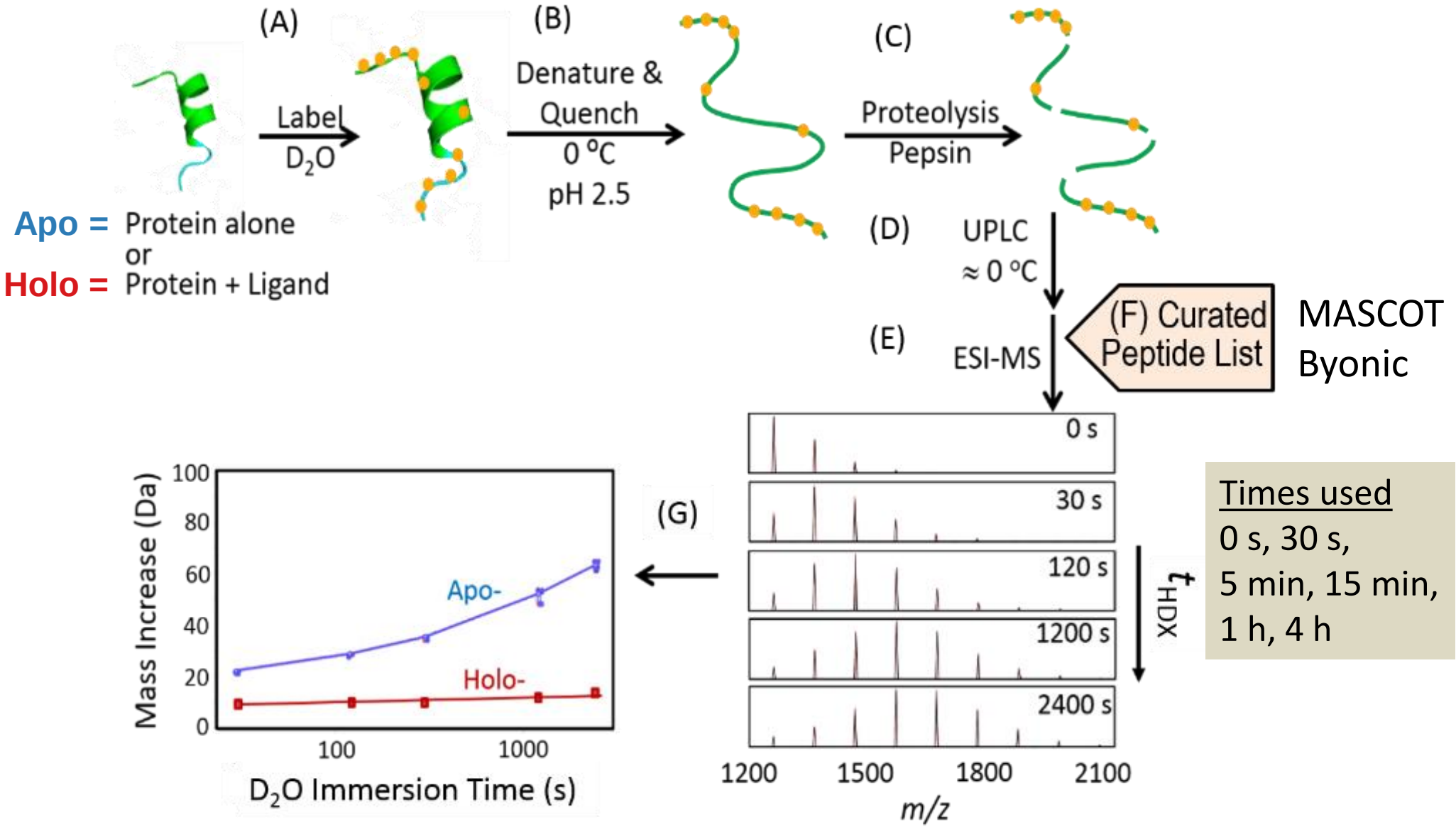


Gly – Asp – His – Cys – Lys – Ala

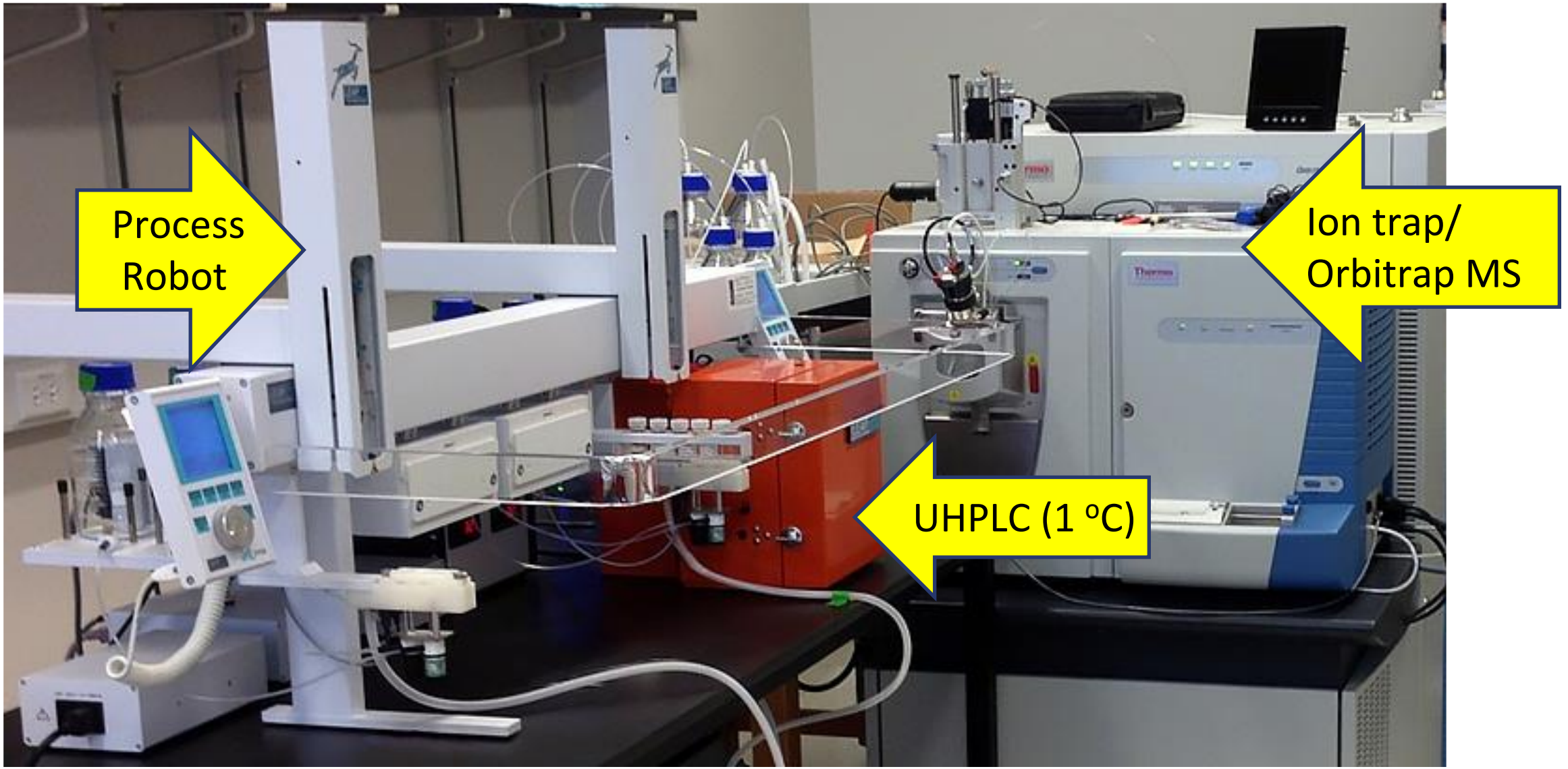
H/D exchange rate of side chains is too fast (t_{ex} < 1 ms)

H/D exchange rate of amide backbone is suitable (t_{ex} = 10 s to days)

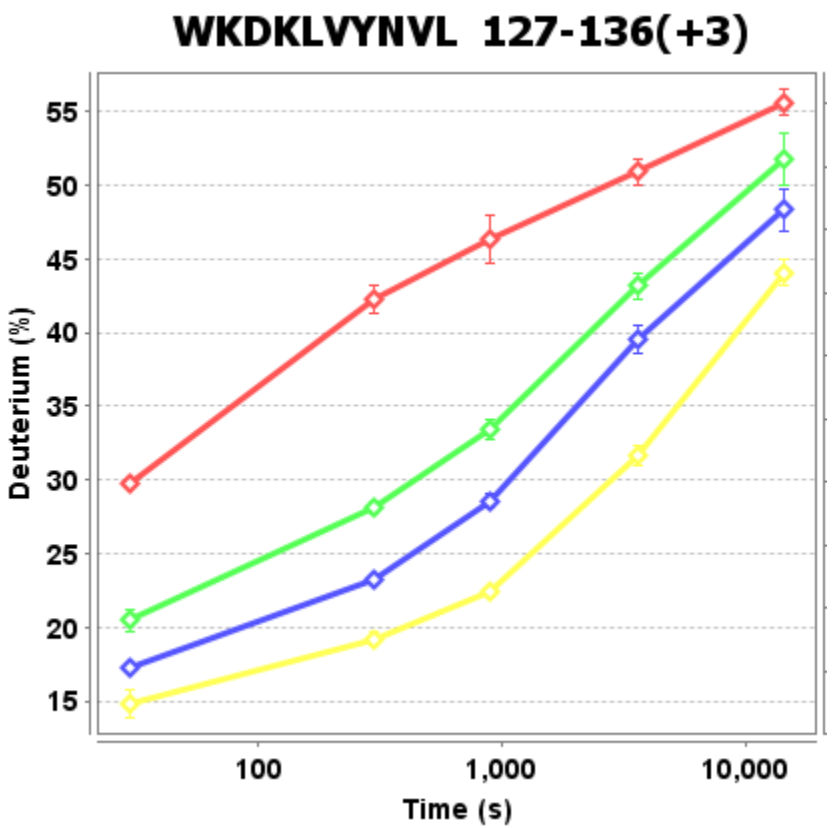
HDX-MS Overview

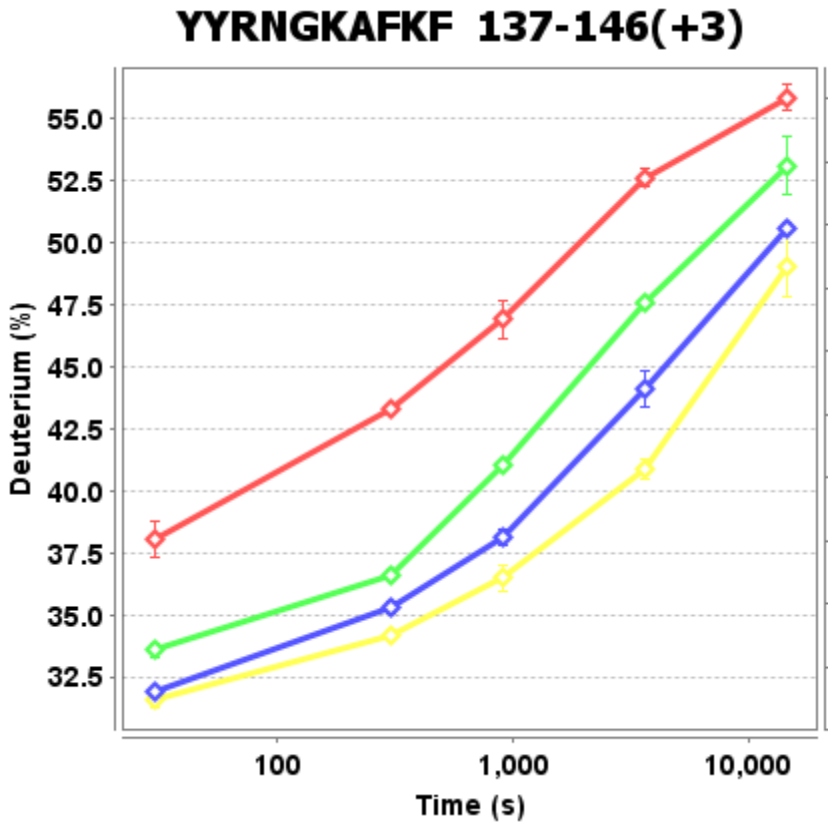


HDX-MS Instrument



Deuterium Uptake Plots





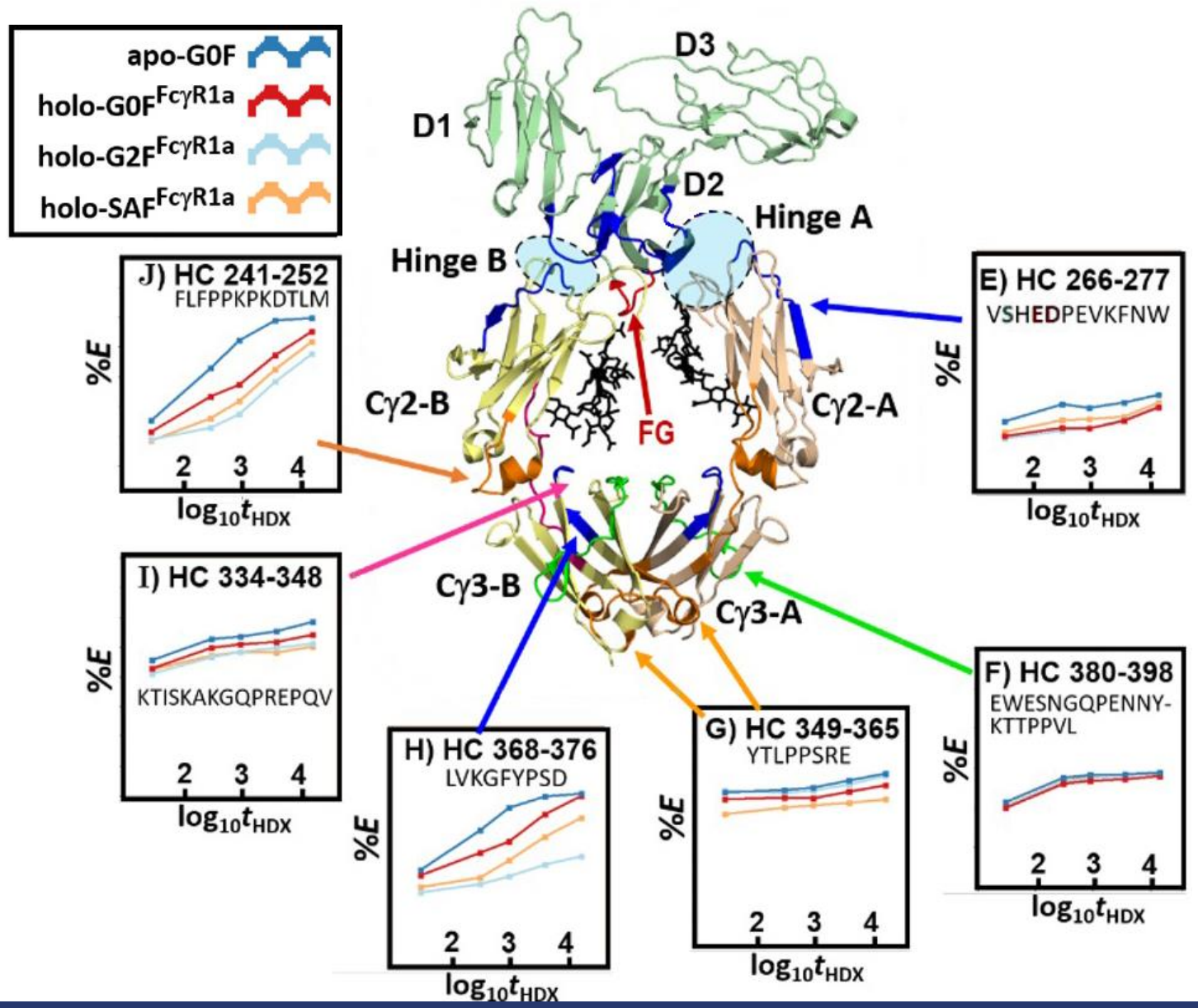
apo-FcγR1a

holo-FcγR1a^{G0F}

holo-FcγR1a^{G2F}

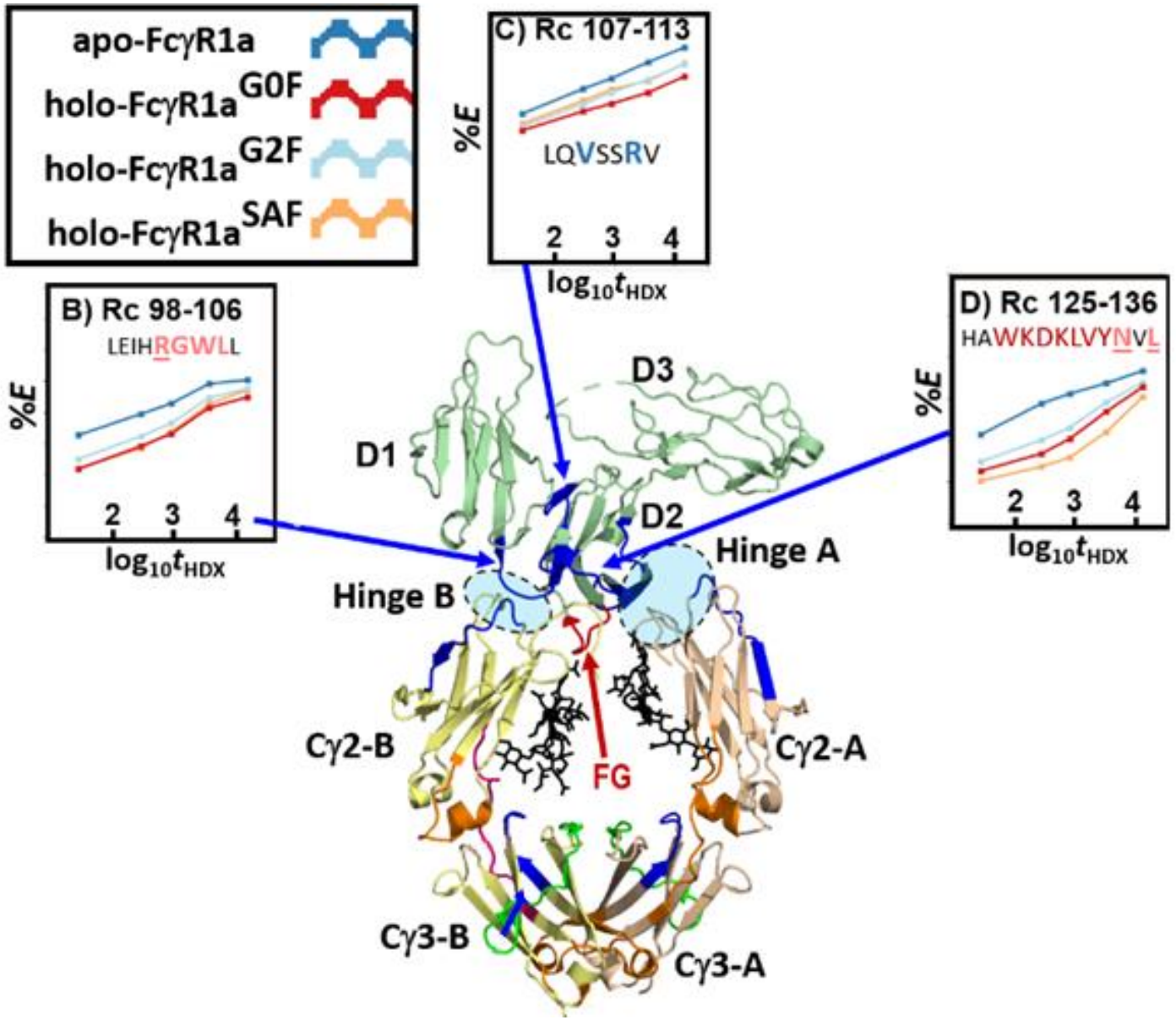
holo-FcγR1a^{SAF}

Deuterium Uptake in aIL8-hFc



- apo aIL8-hFc is typically more dynamic than holo forms
- Dynamics for holo proteins are distinct for G0F, G2F, and SAF binding interactions
- Glycan volume does not fully account for changes in dynamics

Deuterium Uptake in FcγR1a



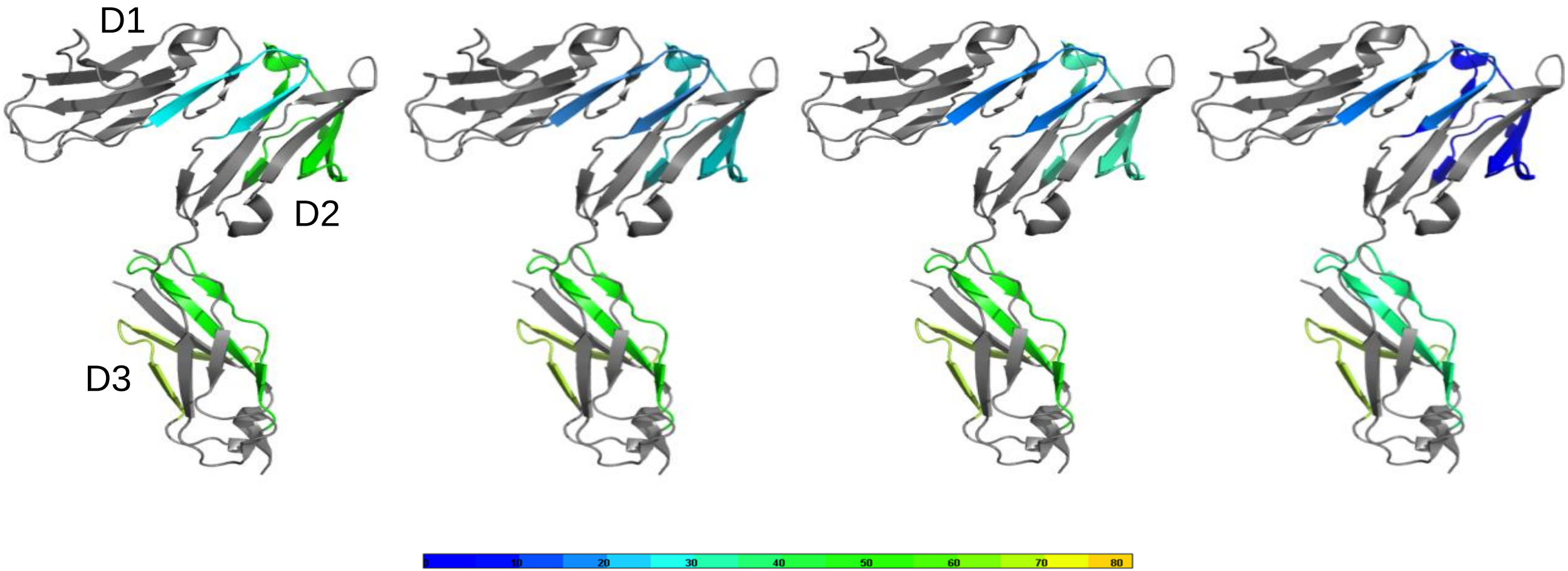
D-uptake of FcγR1a after 30 seconds

FcγR1a

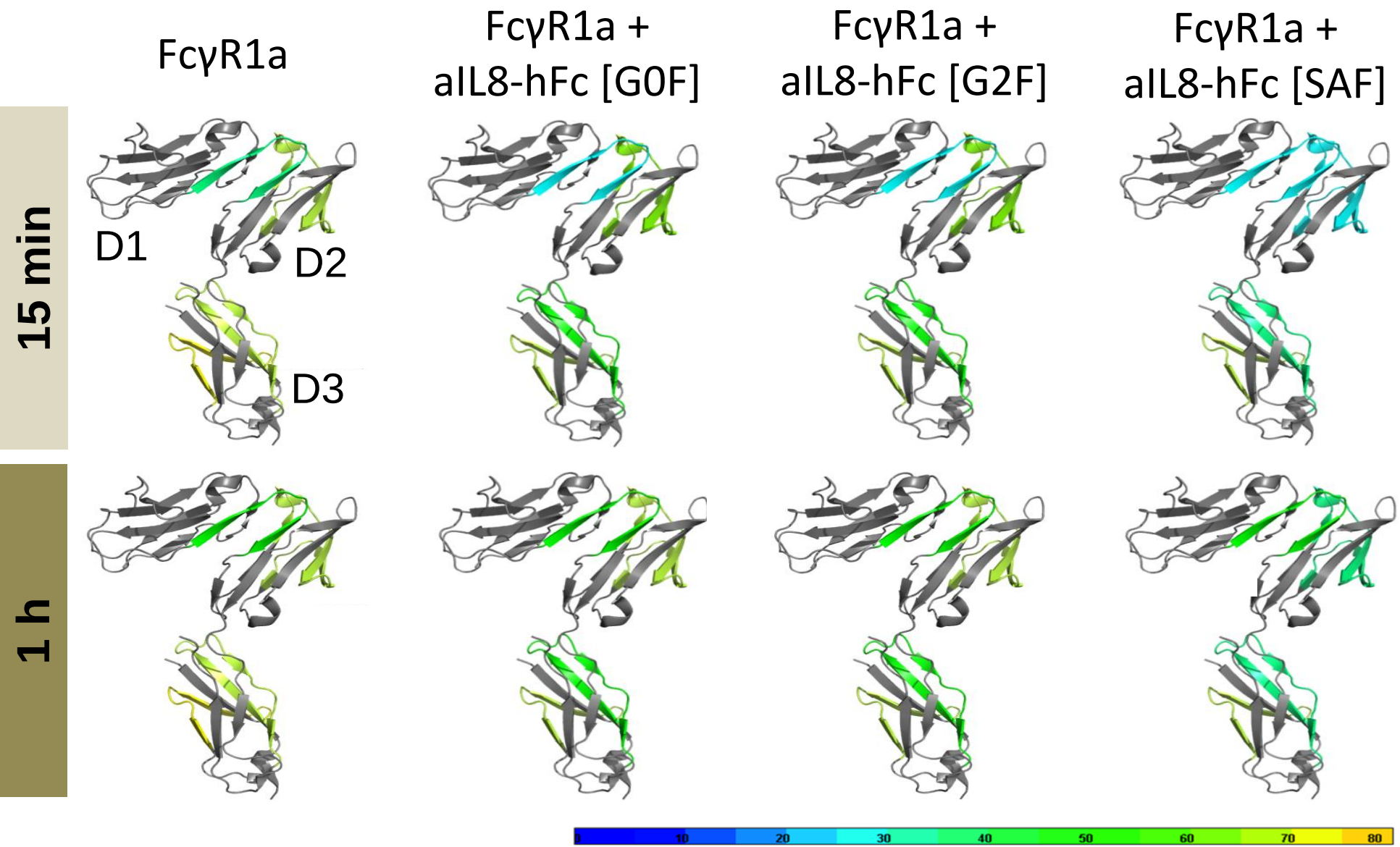
FcγR1a + aIL8-hFc [G0F]

FcγR1a + aIL8-hFc [G2F]

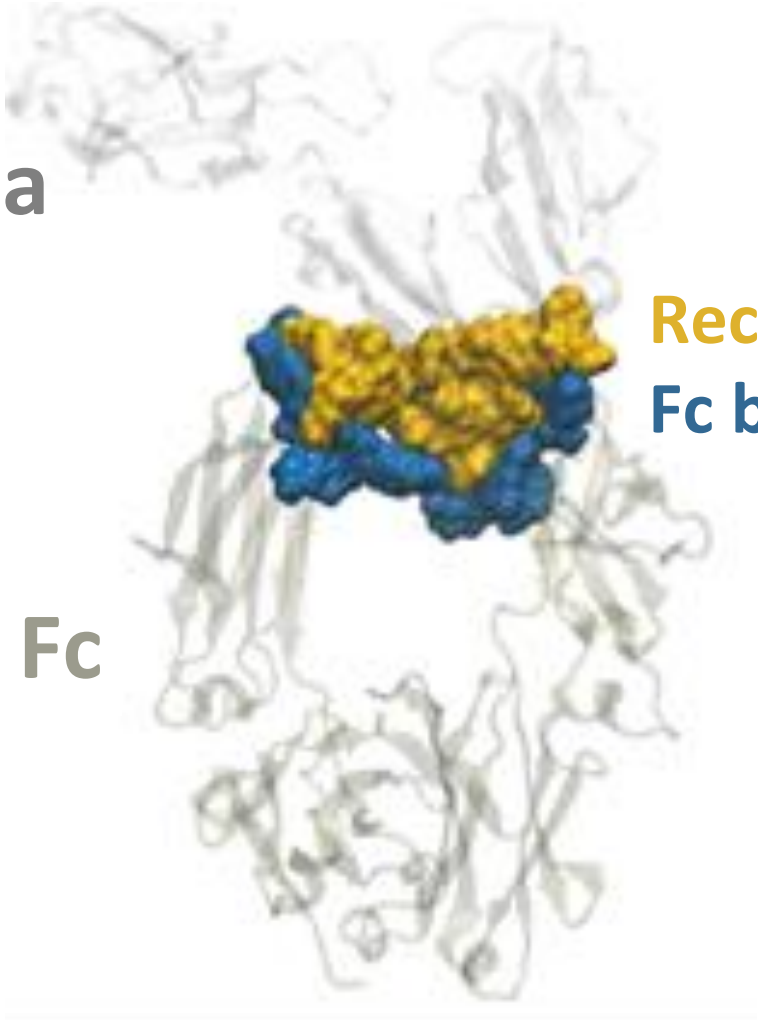
FcγR1a + aIL8-hFc [SAF]



D-uptake of FcγR1a



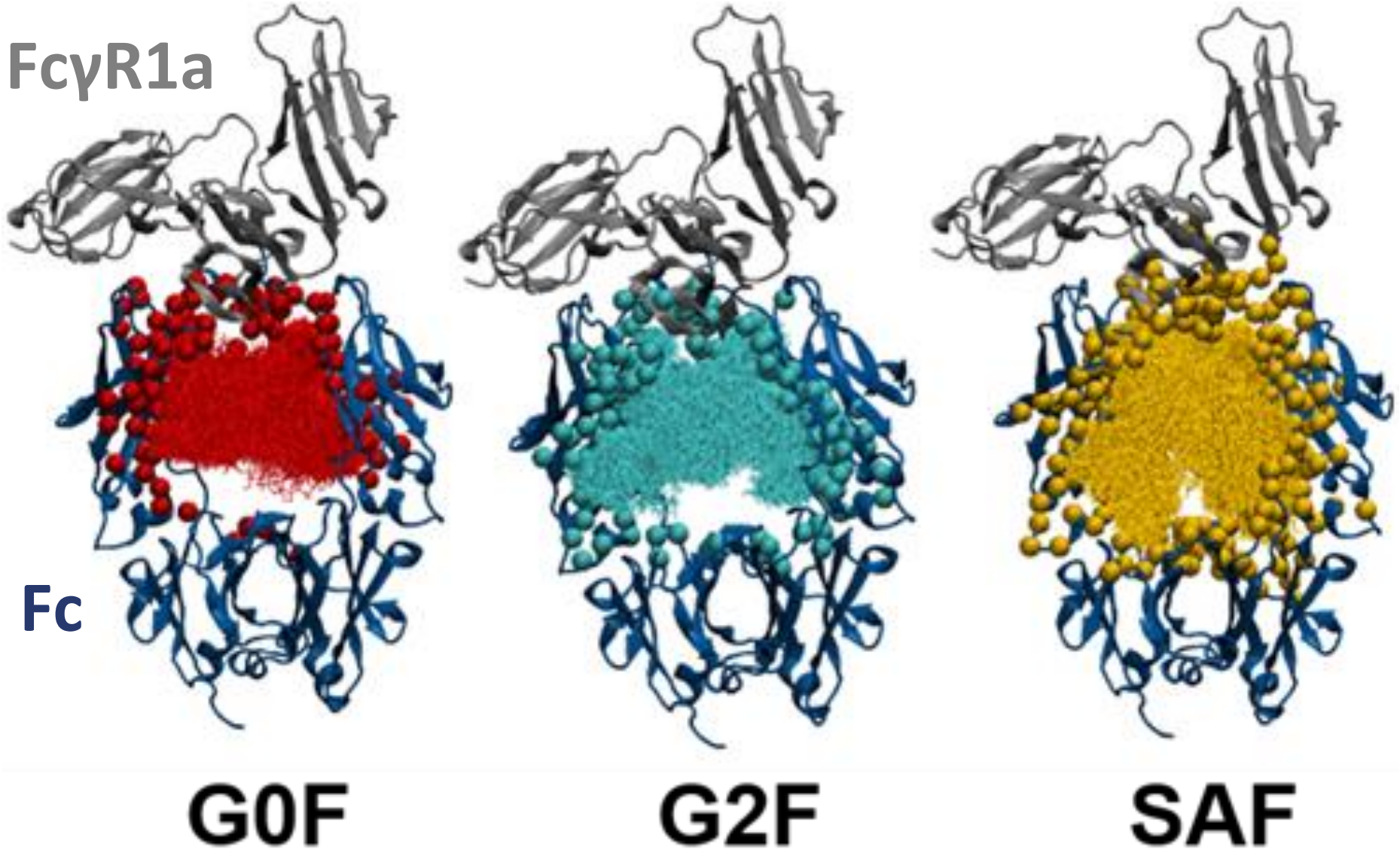
Surfaces of Binding



Receptor binding surface

Fc binding surface

Glycan Spread and Interactions



Conclusions

- Dynamics for holo proteins are distinct for G0F, G2F, and SAF
- Terminal glycan affects structural dynamics
- Glycan volume does not fully account for changes in dynamics
- Changes in dynamics of D3 of FcγR1a show transmission of signal toward ITAM

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