Structural Characterization and Comparability Between a Therapeutic Protein and its Novel Fc Fusion Counterpart

George Bou-Assaf

Biophysical Characterization – Analytical Development – Biogen

CASSS HOS meeting – Providence RI
10 APR 2018

Historical Overview

Syntonix
PHARMACEUTICALS

Biogen acquires Syntonix Pharmaceuticals in 2007

Biogen

Biogen spins off hemophilia business into independent company in 2017

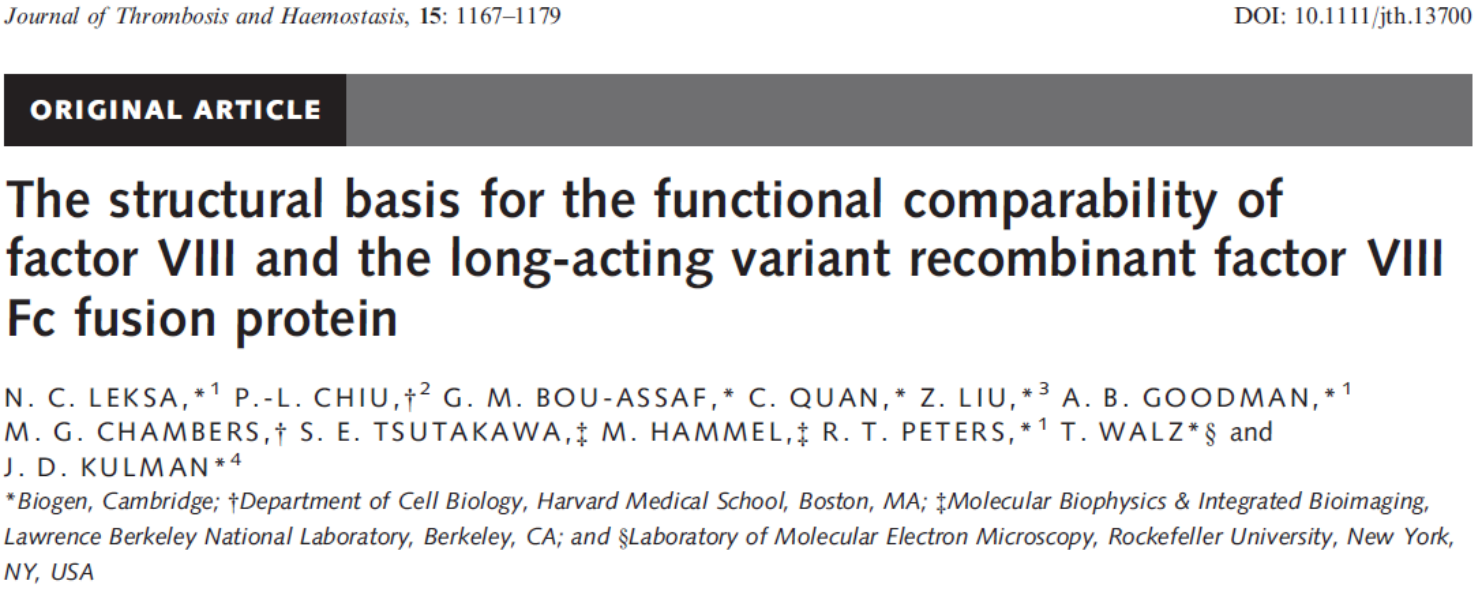
Bioverativ™

Sanofi acquires Bioverativ in 2018

SANOFI

Challenge: How do you get legal approval to present at a conference?

Publish!



Journal of Thrombosis and Haemostasis, **15**: 1167–1179 DOI: 10.1111/jth.13700

ORIGINAL ARTICLE

The structural basis for the functional comparability of factor VIII and the long-acting variant recombinant factor VIII Fc fusion protein

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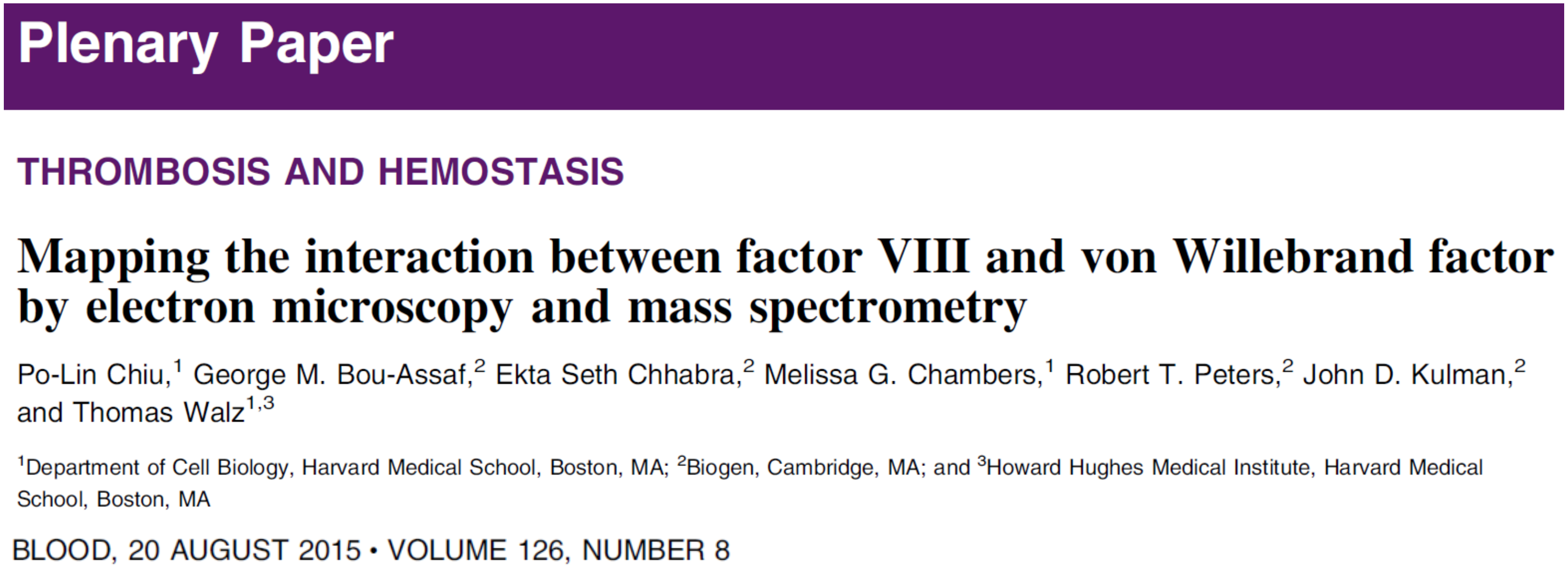

Plenary Paper

THROMBOSIS AND HEMOSTASIS

Mapping the interaction between factor VIII and von Willebrand factor by electron microscopy and mass spectrometry

Po-Lin Chiu,[1] George M. Bou-Assaf,[2] Ekta Seth Chhabra,[2] Melissa G. Chambers,[1] Robert T. Peters,[2] John D. Kulman,[2] and Thomas Walz[1,3]

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BLOOD, 20 AUGUST 2015 • VOLUME 126, NUMBER 8

Hemophilia A background

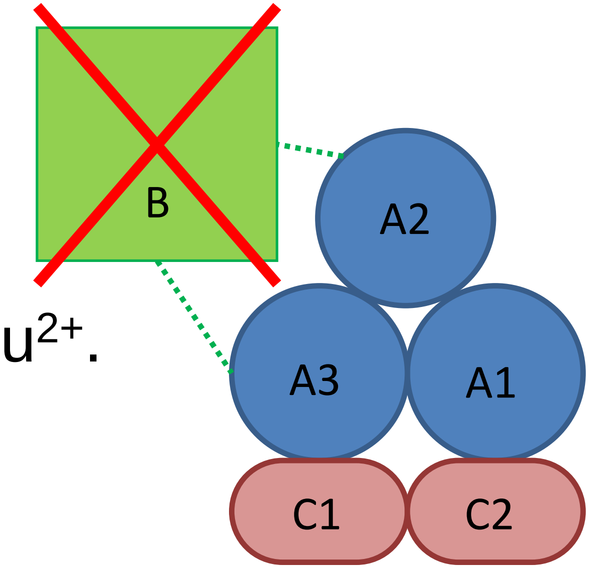
- Bleeding disorder caused by deficiency in clotting factor VIII (FVIII).
- In the absence of prophylaxis, patients suffer from spontaneous or traumatic bleeding.
- Current therapies employ intravenous injections 2-3 times per week to replace deficient FVIII.
- Several drugs on the market are either plasma-derived or recombinant FVIII products.
- Recombinant factor VIII Fc (rFVIIIFc) is a long-lasting therapy that has the potential to reduce treatment burden.

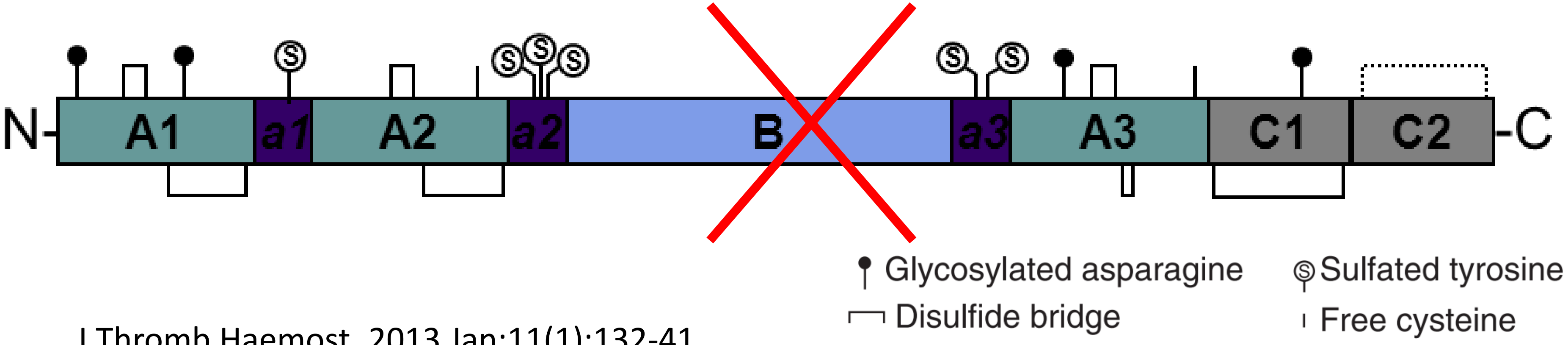
Blood. 2012 Mar 29;119(13):3024-30.

Blood. 2012 Mar 29;119(13):3031-7.

Recombinant FVIII background

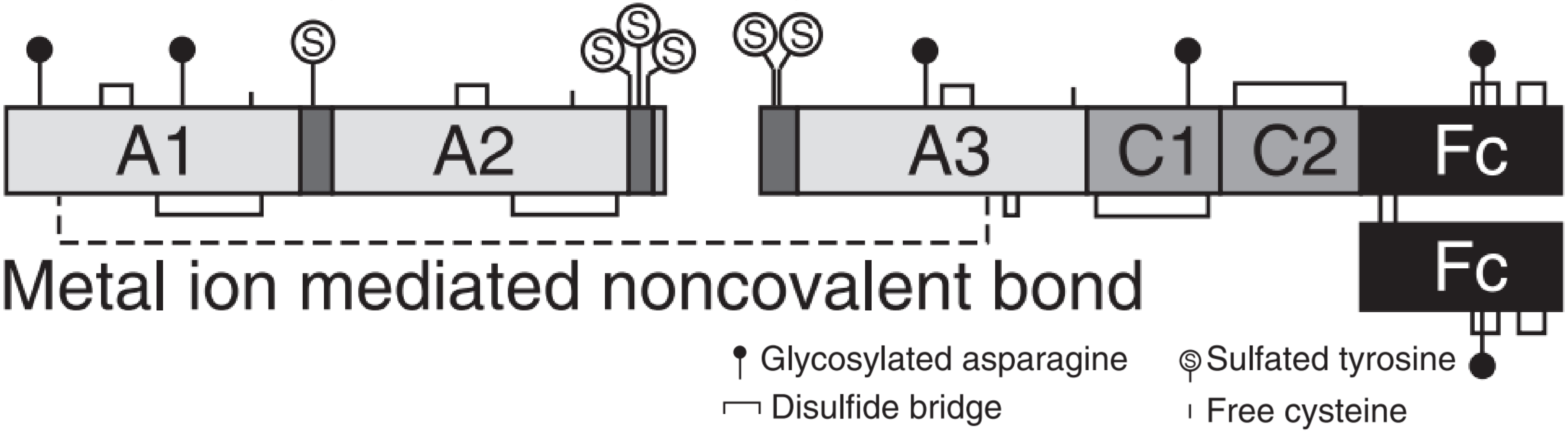
- FVIII is a ~300 kDa protein.
- FVIII is processed inside the cell to produce heavy and light chains held together non-covalently by Ca^{2+} and Cu^{2+}.
- B domain function is unknown and deletion does not affect function.
- Protein is rich in PTMs: N-glycosylation and Y sulfation.



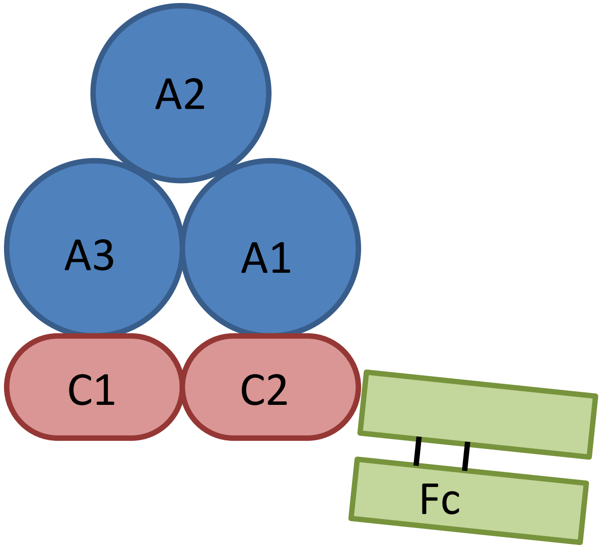


J Thromb Haemost. 2013 Jan;11(1):132-41.
Proc Natl Acad Sci U S A. 1986 Aug;83(16):5939-42.

Recombinant FVIIIFc background

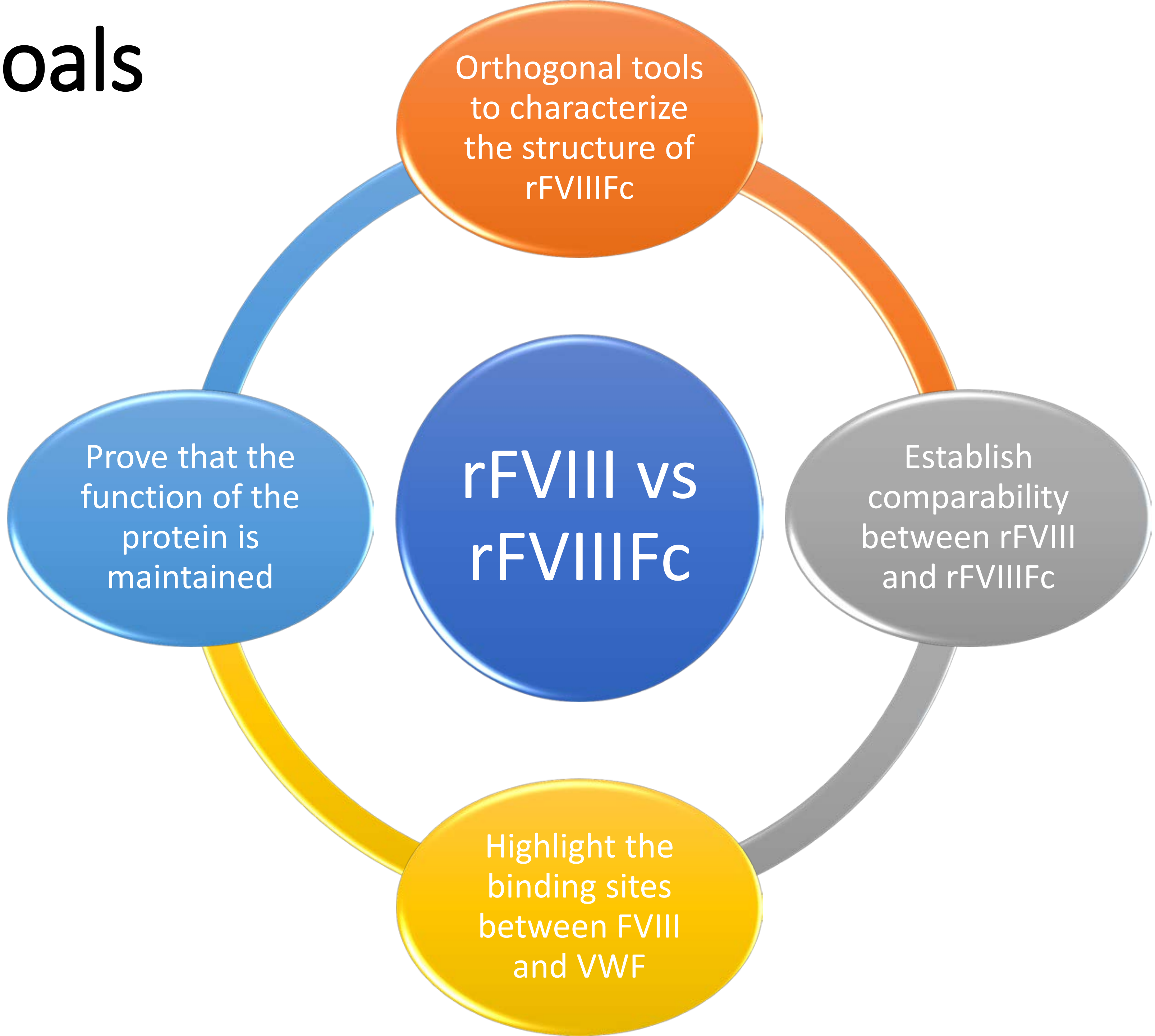


- rFVIIIFc is 220 kDa (195 kDa of unique sequence)[1]
- Fc allows binding to FcRn (similar to IgG), therefore protecting from degradation and extending half-life of FVIII
- 1.5- to 1.7-fold increase in half life.[2,3]



1. J Thromb Haemost. 2013 Jan;11(1):132-41.
2. Blood. 2012 Mar 29;119(13):3024-30.
3. Blood. 2012 Mar 29;119(13):3031-7.

Goals



rFVIII vs rFVIIIFc

Orthogonal tools to characterize the structure of rFVIIIFc

Establish comparability between rFVIII and rFVIIIFc

Highlight the binding sites between FVIII and VWF

Prove that the function of the protein is maintained

Structural toolbox

HDX-MS

X-ray crystallography

SPR

Biogen

- Nina Leska
- Chao Quan
- Ekta Seth Chhabra
- Zhan Liu
- Abby Goodman
- Rob Peters
- John Kulman
- George Bou-Assaf

EM

Harvard Medical School

- Po-Lin Chiu
- Melissa Chambers
- Thomas Walz

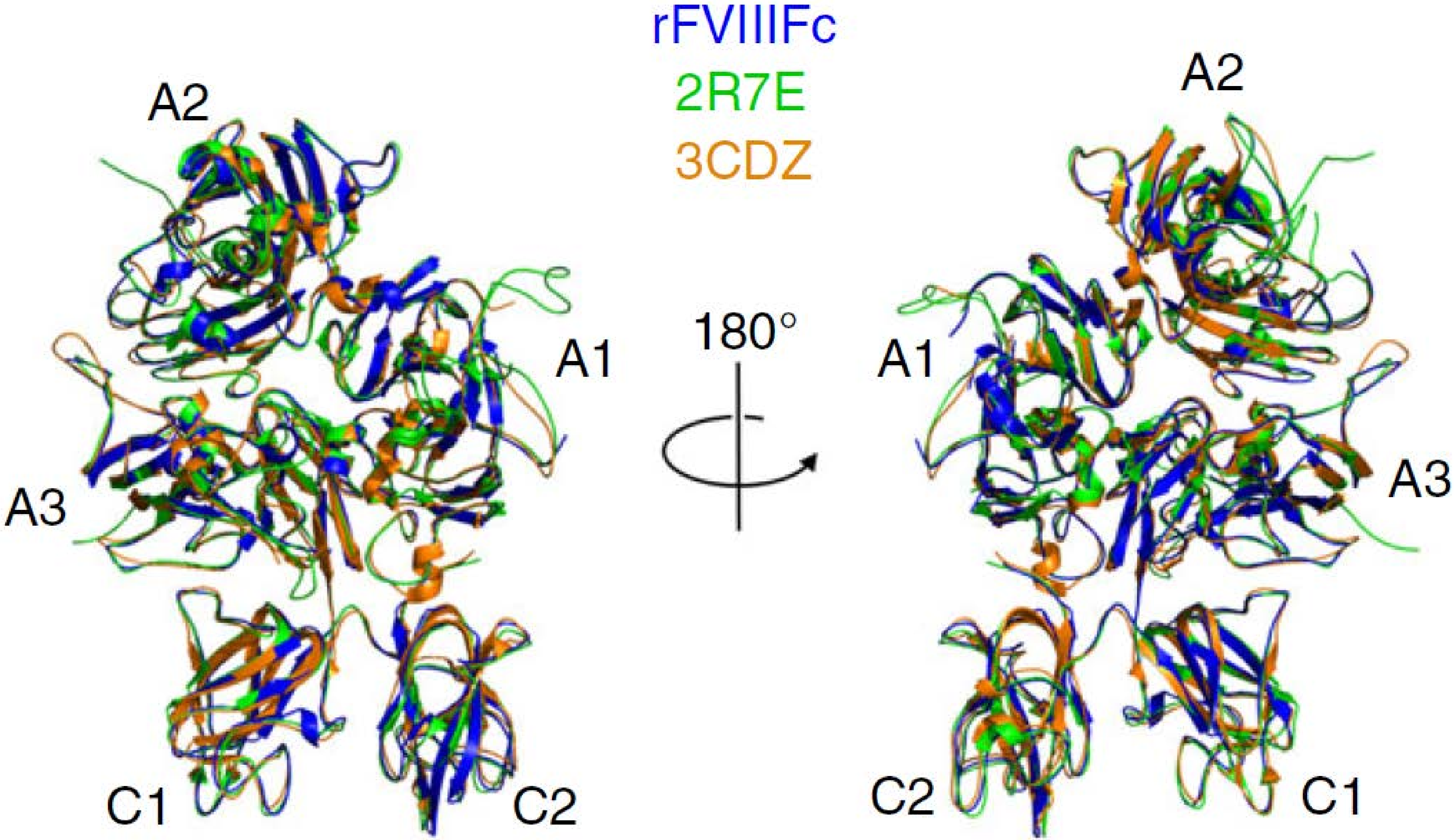
SAXS

Lawrence Berkeley National Lab

- Susan Tsutakawa
- Michal Hammel

Goal 1: Structural Characterization of rFVIIIFc and comparison to rFVIII

X-ray crystallography



- FVIII part of rFVIIIFc overlays well with prior structures of rFVIII
- Fc electron density map was not resolved which indicates high flexibility

X-ray crystallography

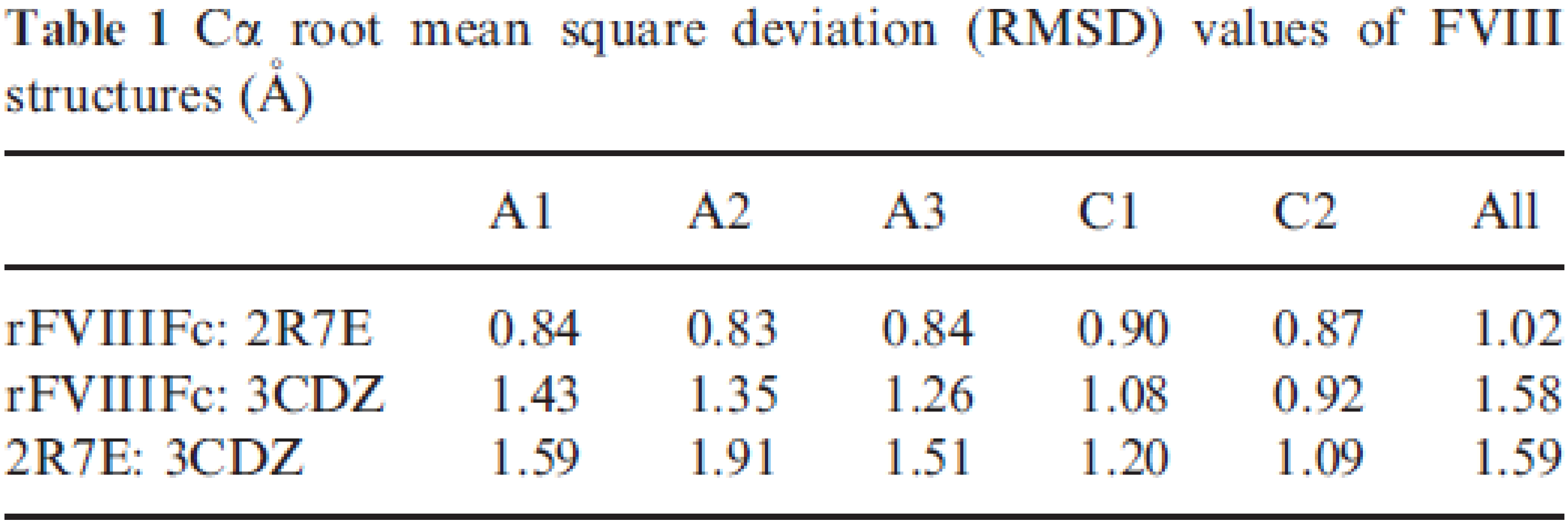
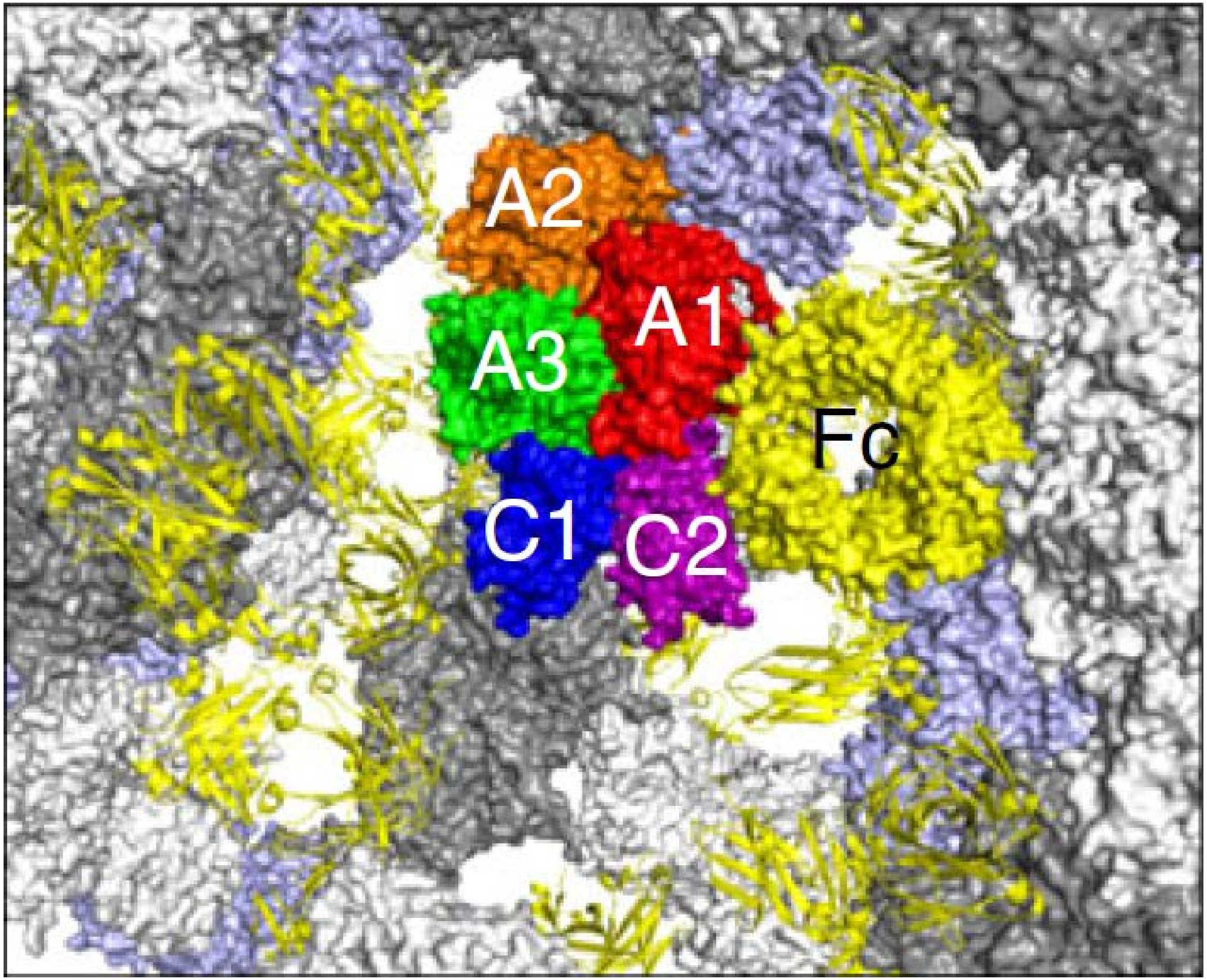


Table 1 Cα root mean square deviation (RMSD) values of FVIII structures (Å)

	A1	A2	A3	C1	C2	All
rFVIIIFc: 2R7E	0.84	0.83	0.84	0.90	0.87	1.02
rFVIIIFc: 3CDZ	1.43	1.35	1.26	1.08	0.92	1.58
2R7E: 3CDZ	1.59	1.91	1.51	1.20	1.09	1.59

- Low RMSD values indicate high level of structural comparability between the FVIII component of rFVIIIFc and existing rFVIII structures.

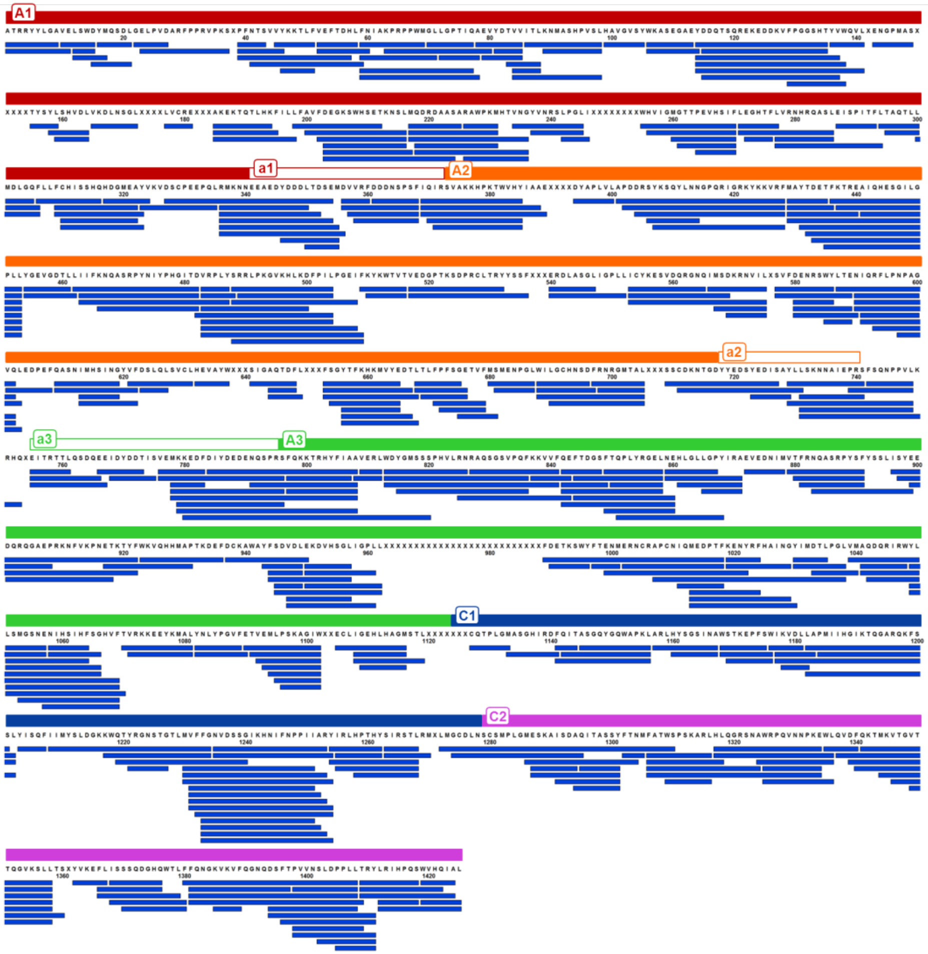
X-ray crystallography



- Large solvent channel points to putative location of a highly flexible Fc domain in rFVIIIFc
- Modeled IgG1 Fc domain (1HZH) fits nicely in the solvent channel

HDX-MS sequence coverage

FVIII



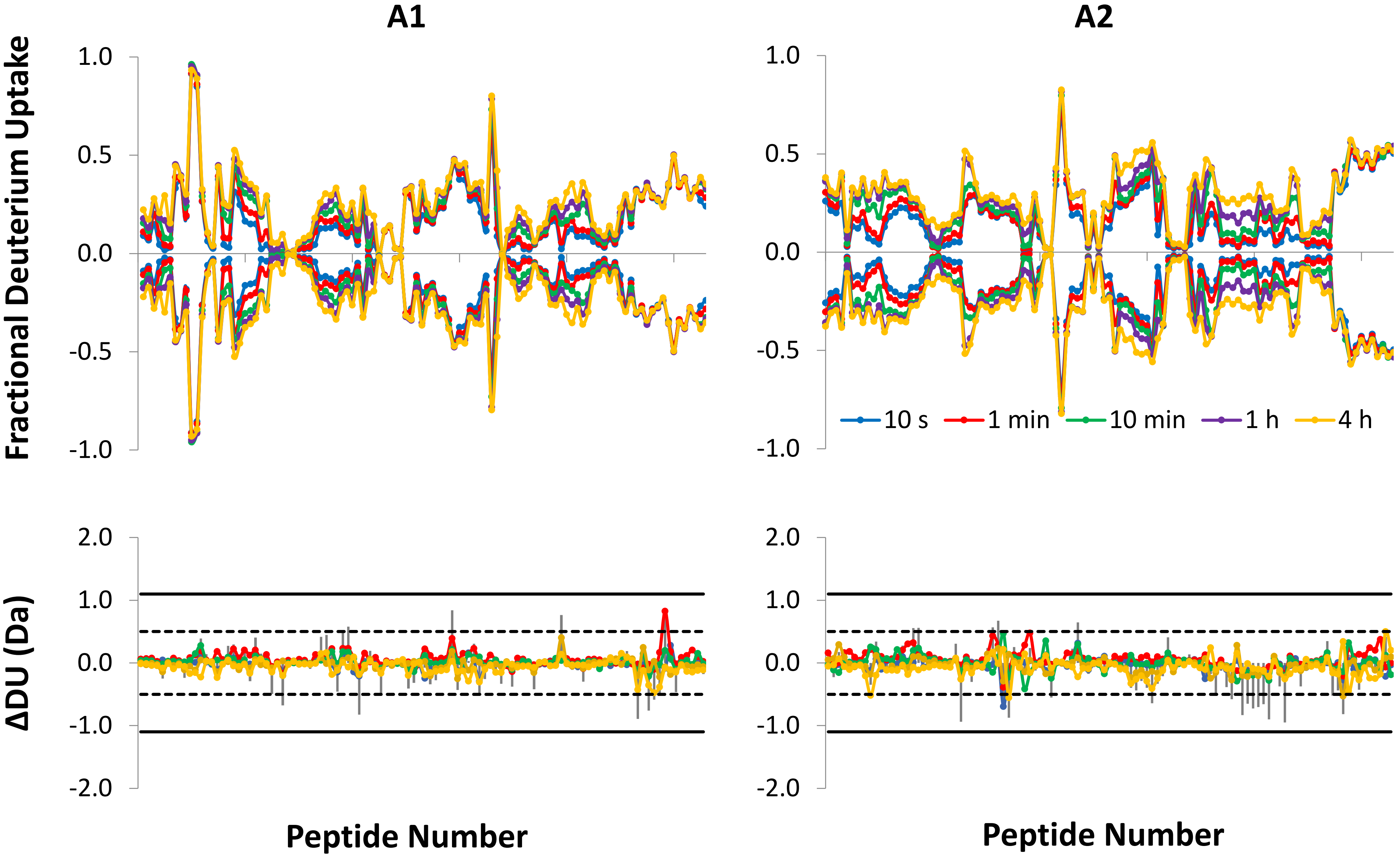
Fc

	Number of peptides	Sequence coverage
FVIII	416	94%
Fc	50	84%

- Adjusted gradient (longer than usual) to identify more peptides
- Areas of poor coverage correspond to heavy PTMs

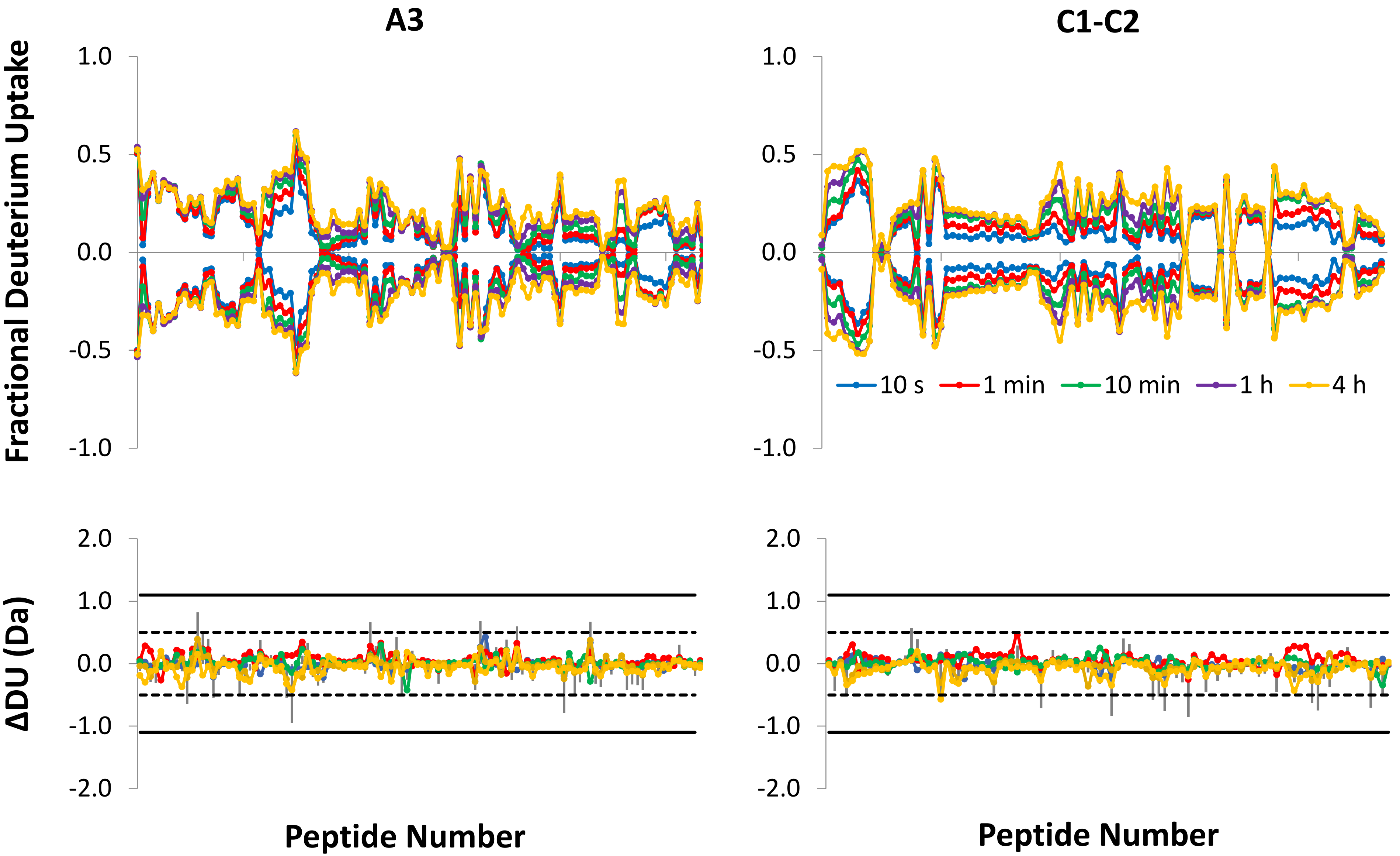
HDX-MS results

Heavy chain peptides in A1 and A2 domains



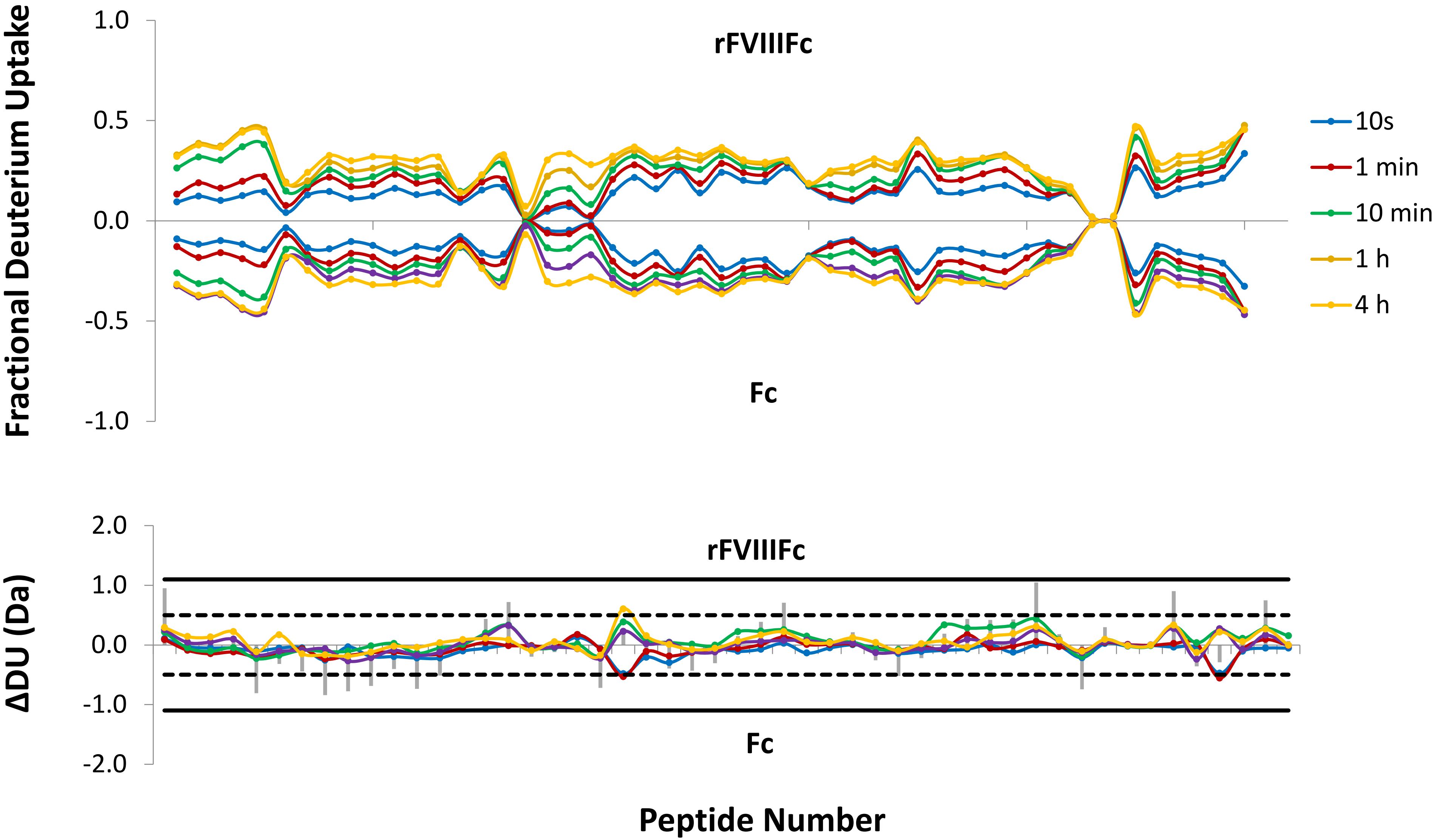
HDX-MS results

Light chain peptides in A3, C1 and C2 domains



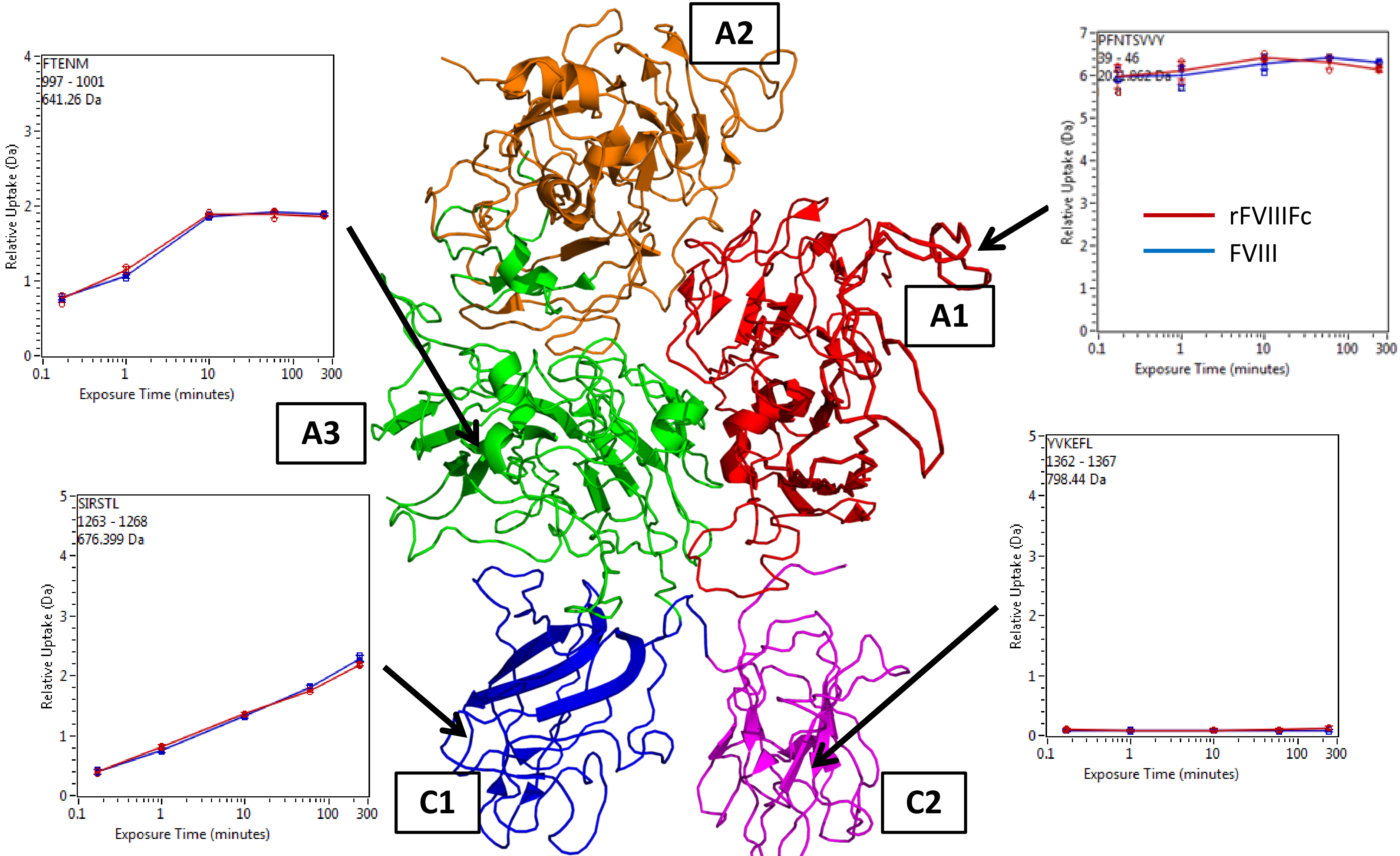
HDX-MS results

Free Fc vs Fc in rFVIIIFc



HDX-MS results

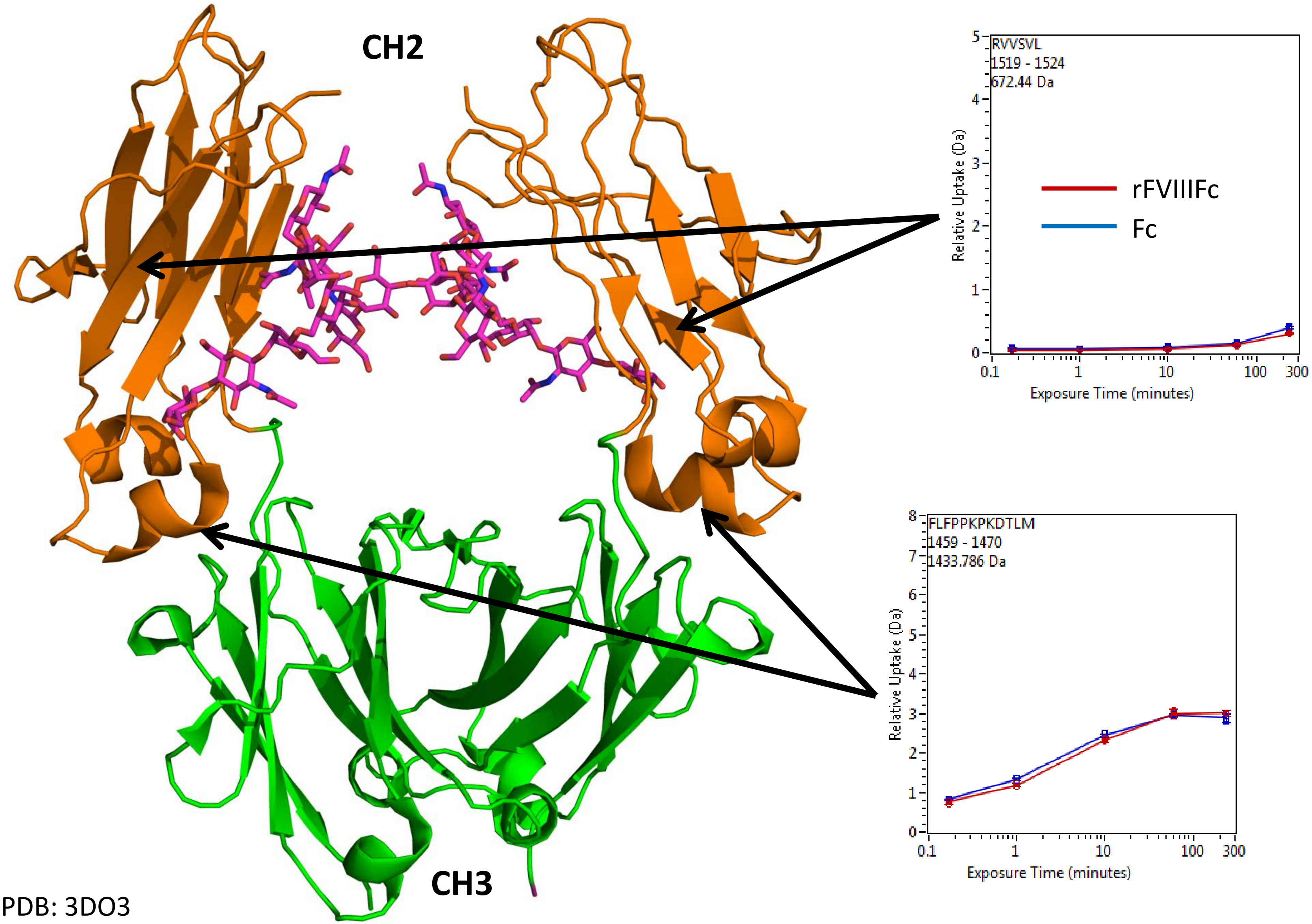
Correlation with crystal structure



PDB: 2R7E

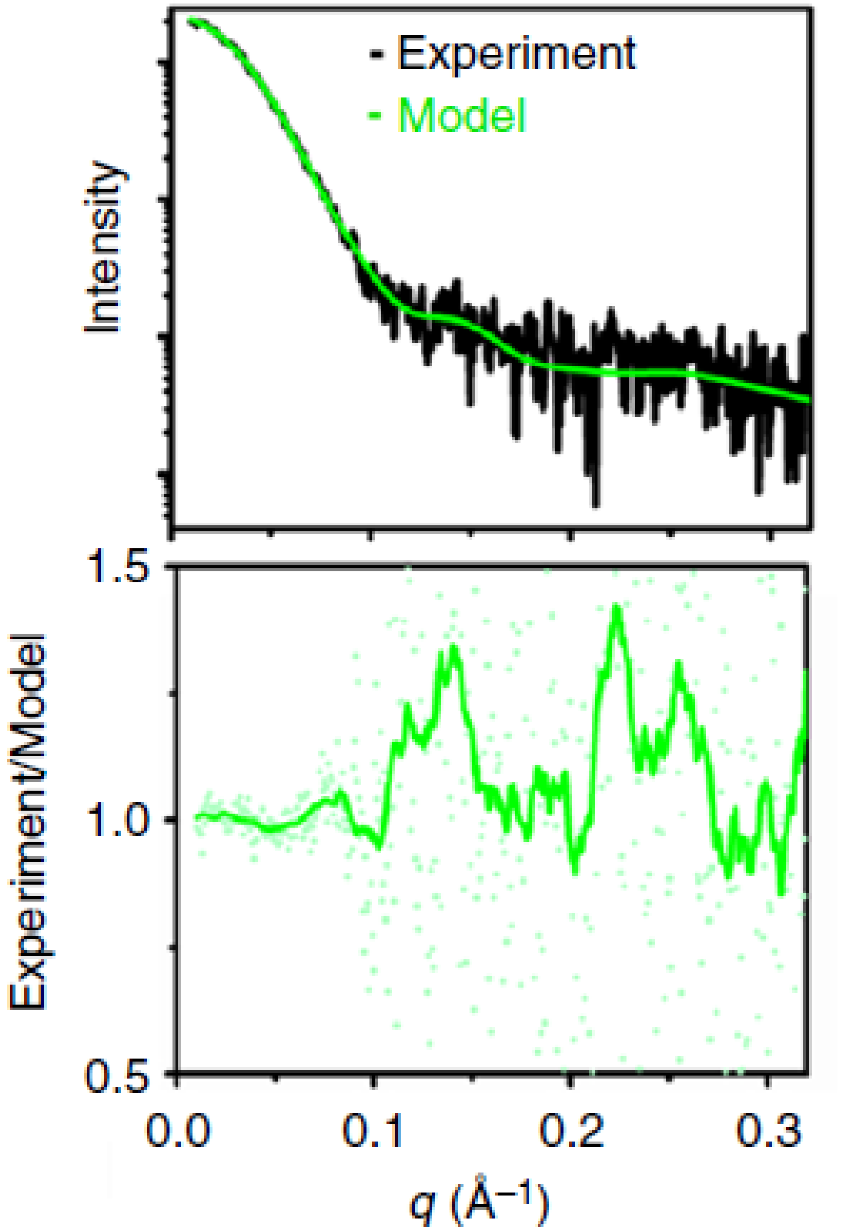
HDX-MS results

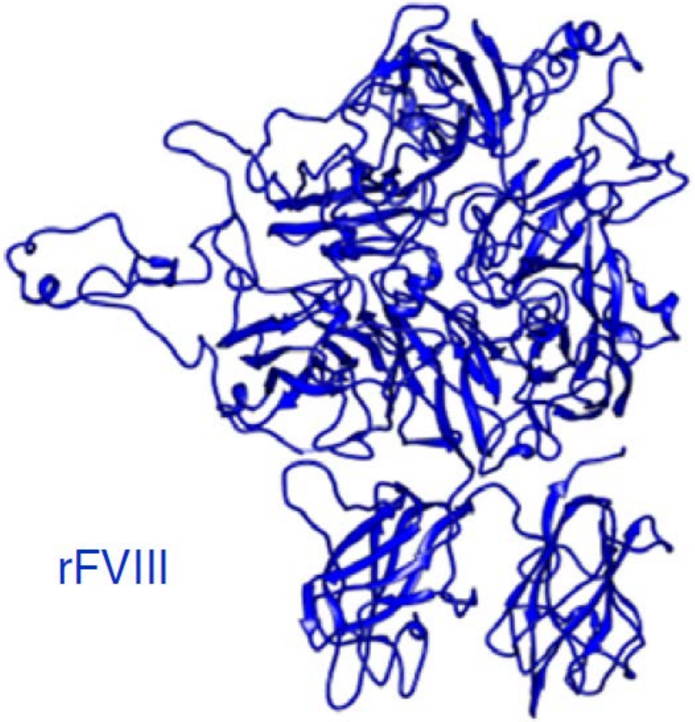
Correlation with crystal structure



PDB: 3DO3

SAXS of rFVIII

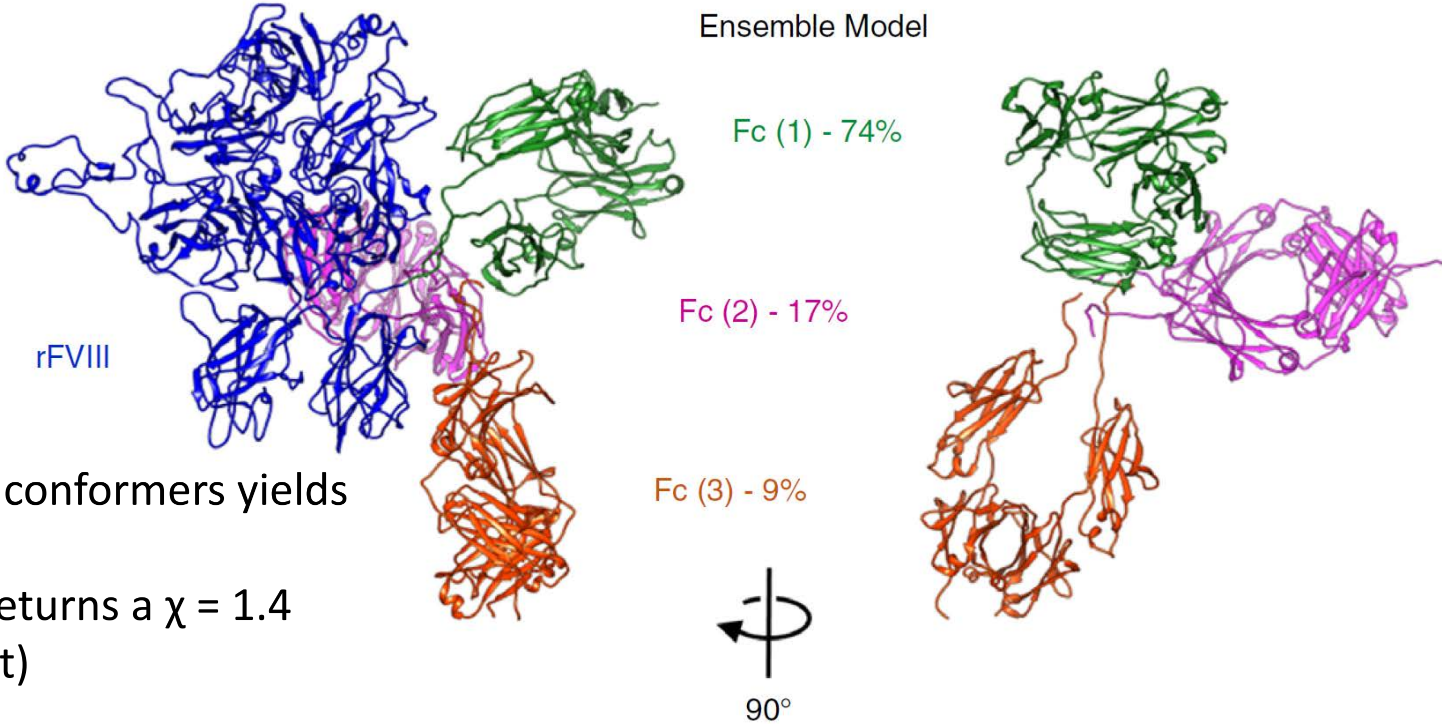




- Model intensity plot (green, based on crystal structure) fits nicely with the experimental scattering data (black)
- $\chi = 1.1$

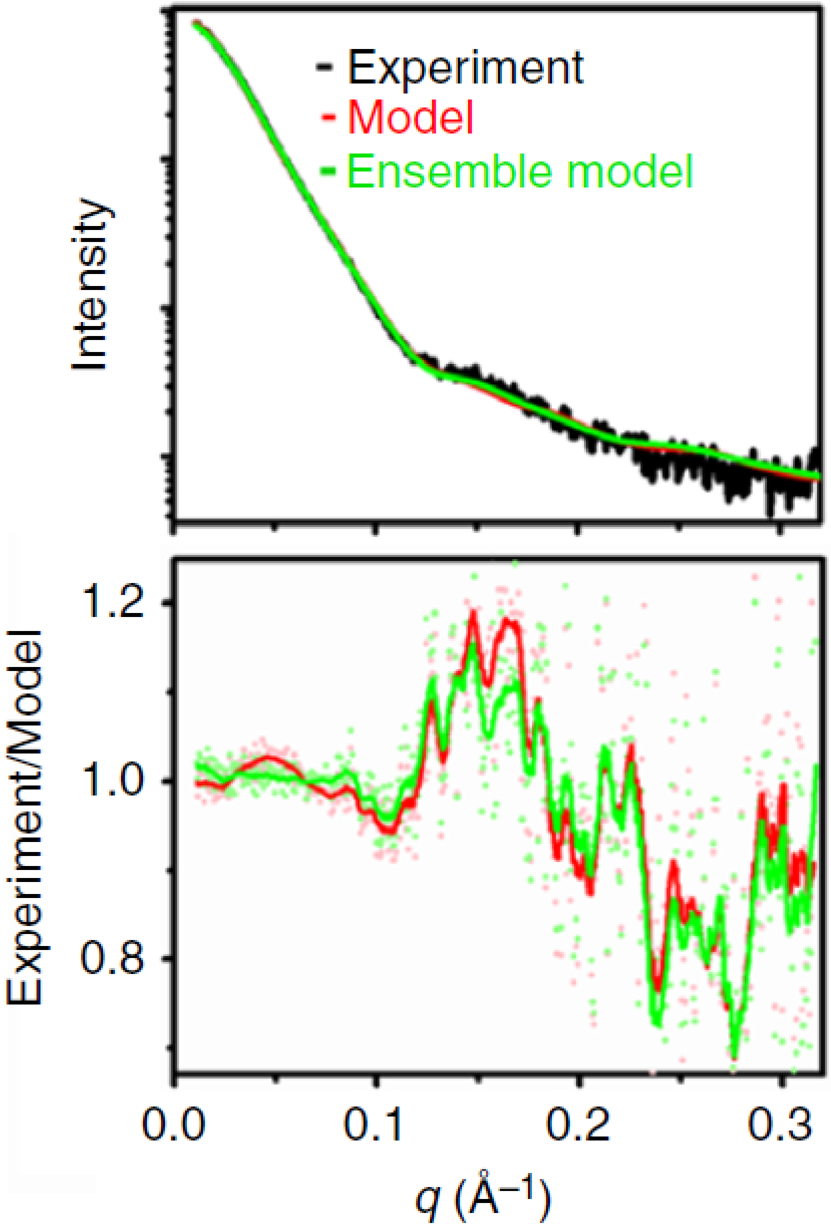
SAXS of rFVIIIFc

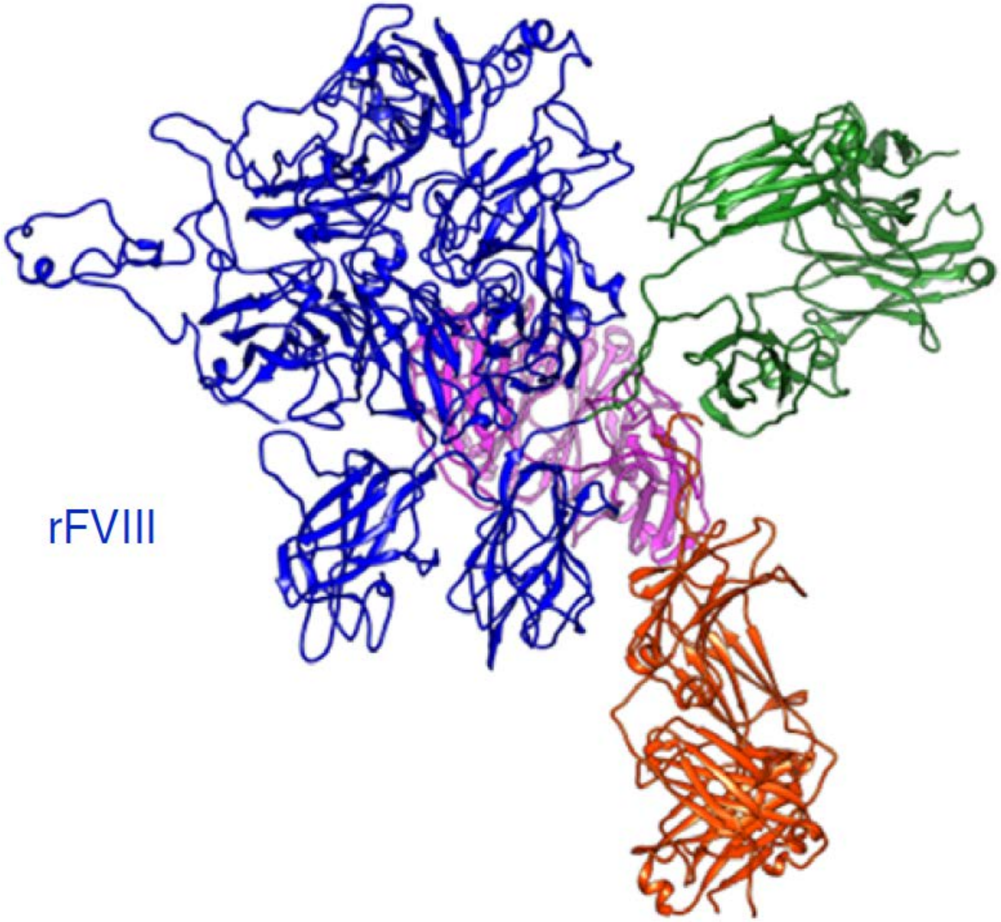
- Crystal structure of rFVIIIFc did not resolve the Fc domain
- Model the structure by tethering the known crystal structure of Fc to that of rFVIII and allowing them to move as rigid bodies relative to each other.
- Conformers were averaged and initial model returns a $\chi = 1.6$



- Cluster analysis of conformers yields 3 populations
- Ensemble model returns a $\chi = 1.4$ (13% improvement)

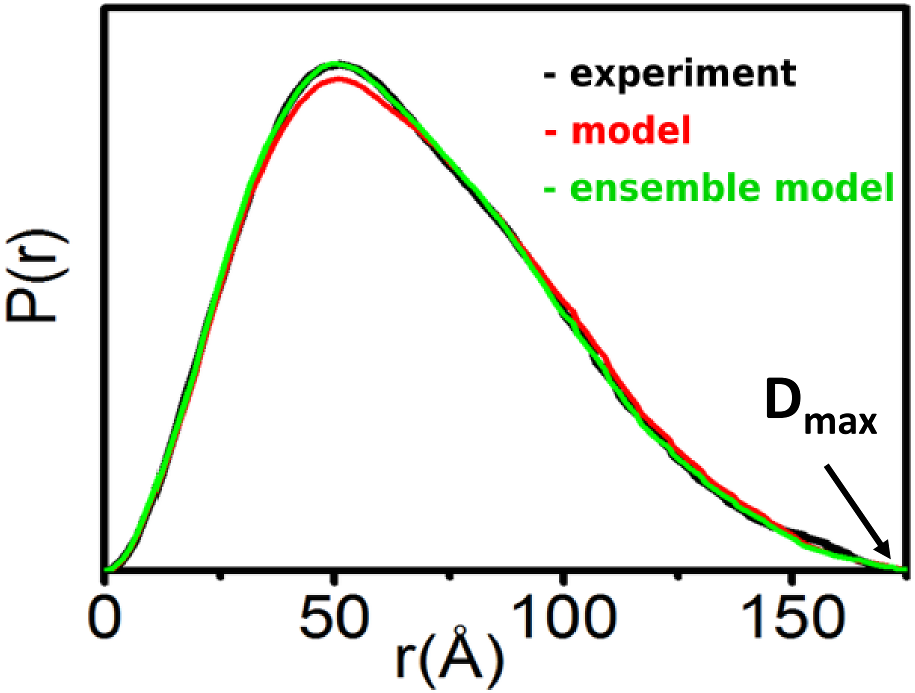
SAXS of rFVIIIFc



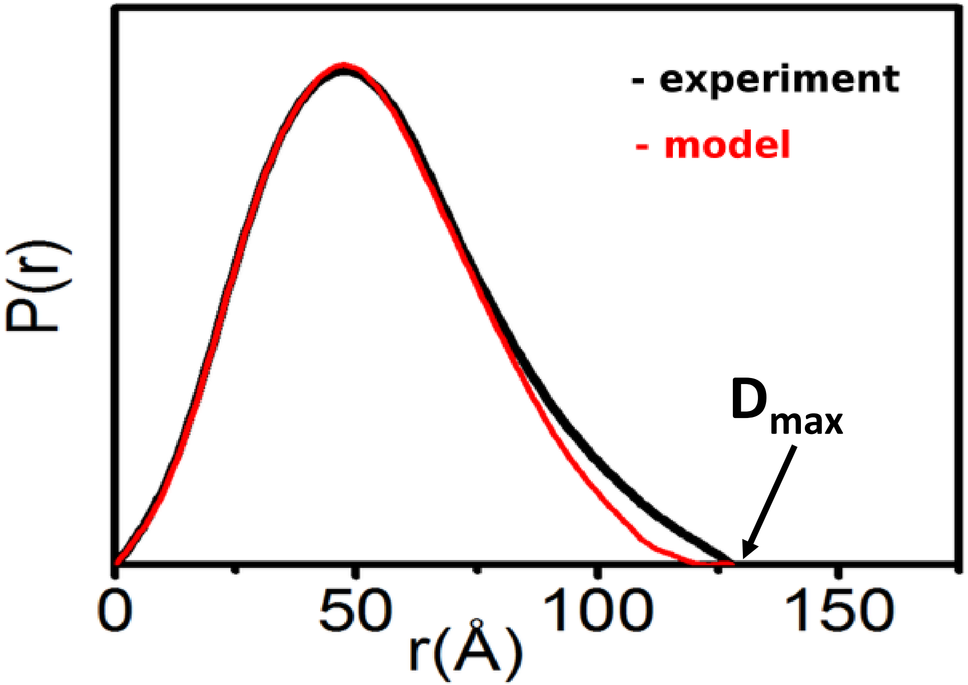


- Ensemble model fits experimental data better than average model

SAXS of rFVIIIFc compared to rFVIII



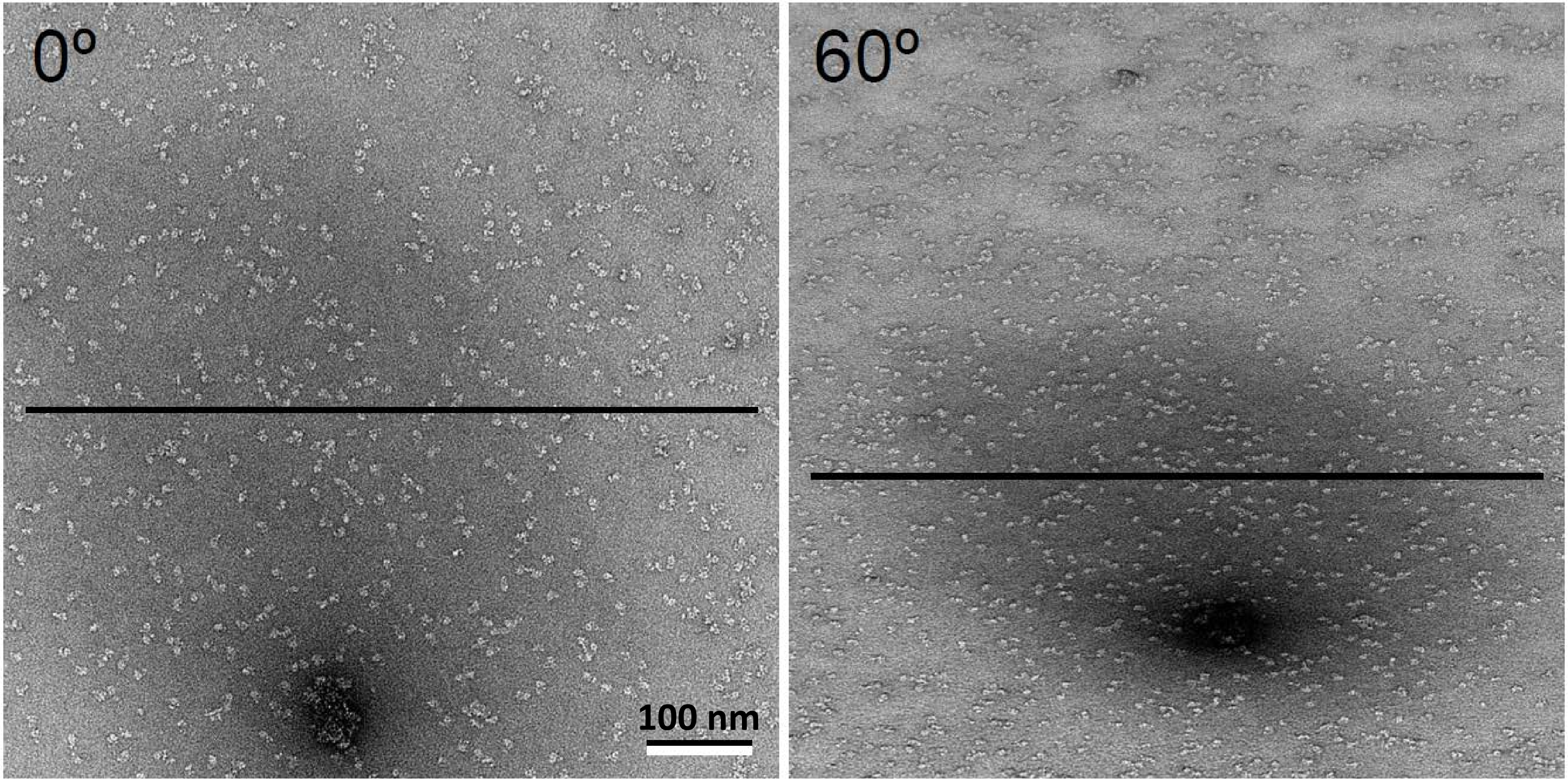
rFVIIIFc



rFVIII

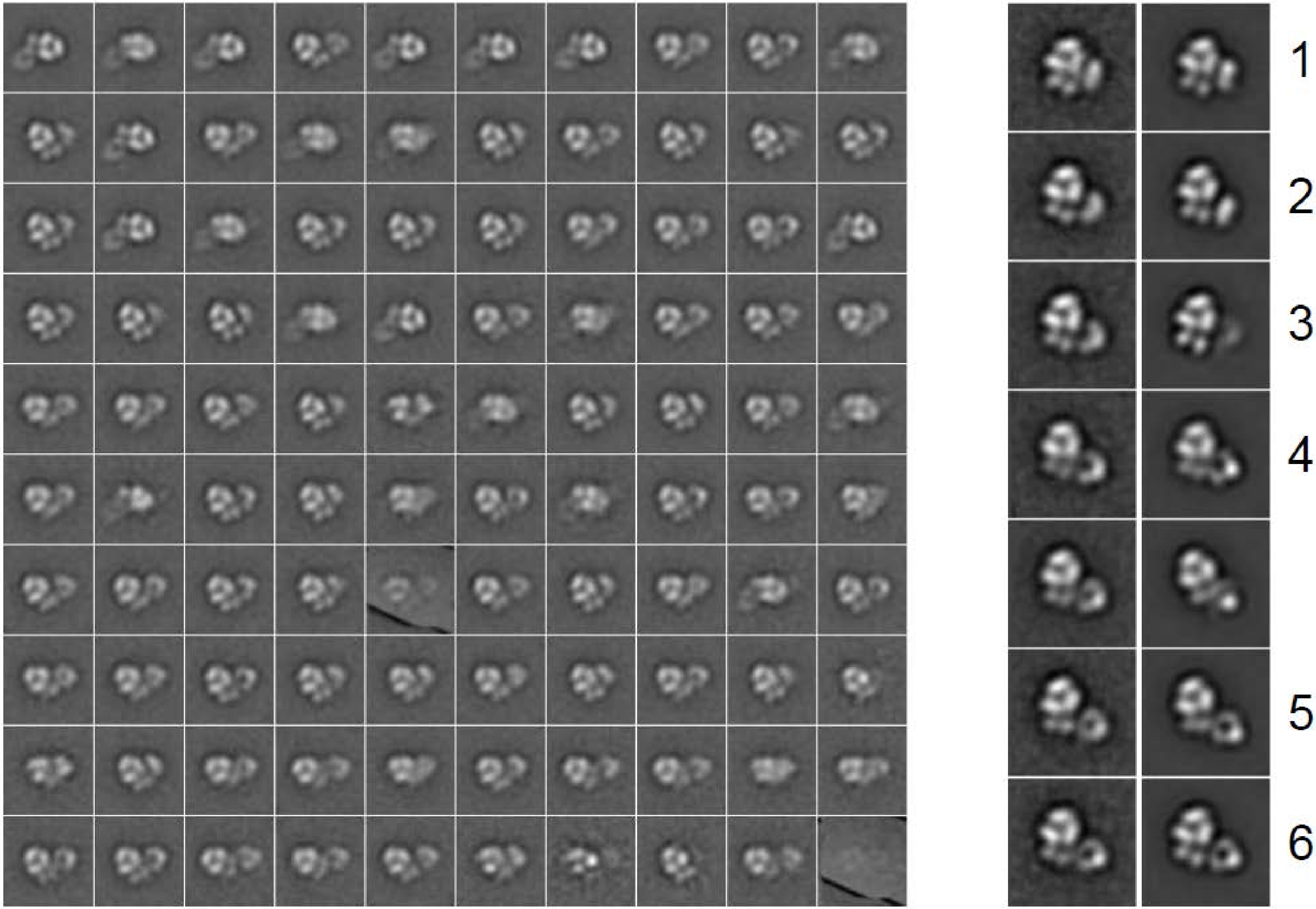
- Pair distance distribution function is wider for rFVIIIFc compared to rFVII and the maximum protein dimension is larger because of the appended Fc moiety
- Ensemble model fits experimental data better than average model

SP cryo-EM of rFVIIIFc



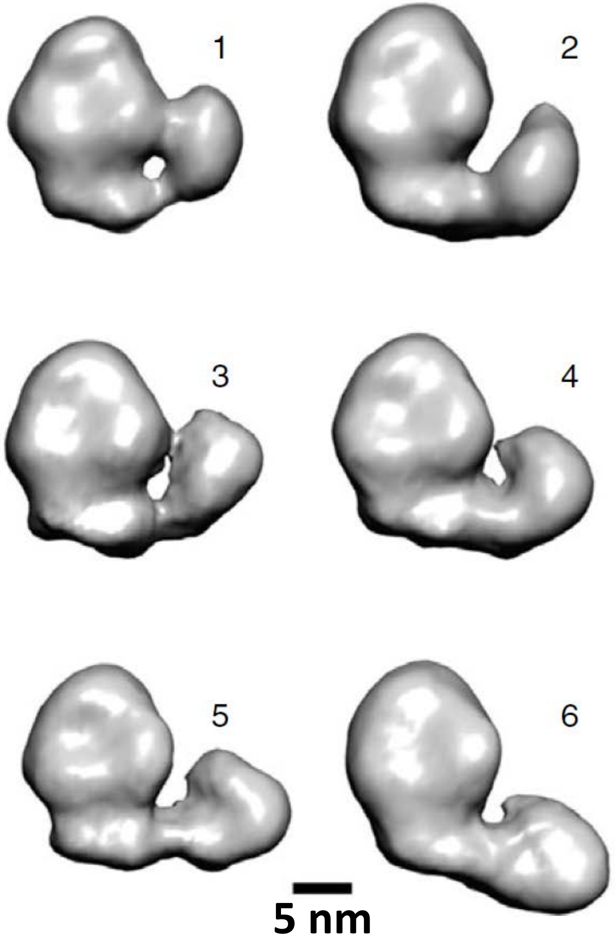
- SP-cryo EM data was collected at 0 and 60° tilt angle to enable 3D reconstruction.

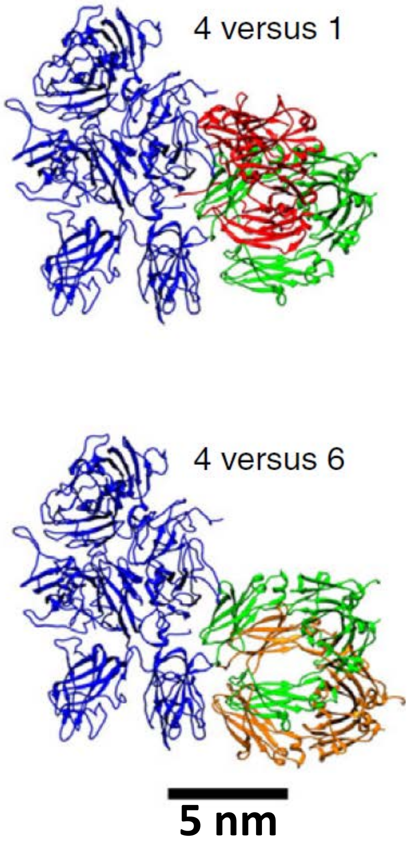
SP cryo-EM of rFVIIIFc

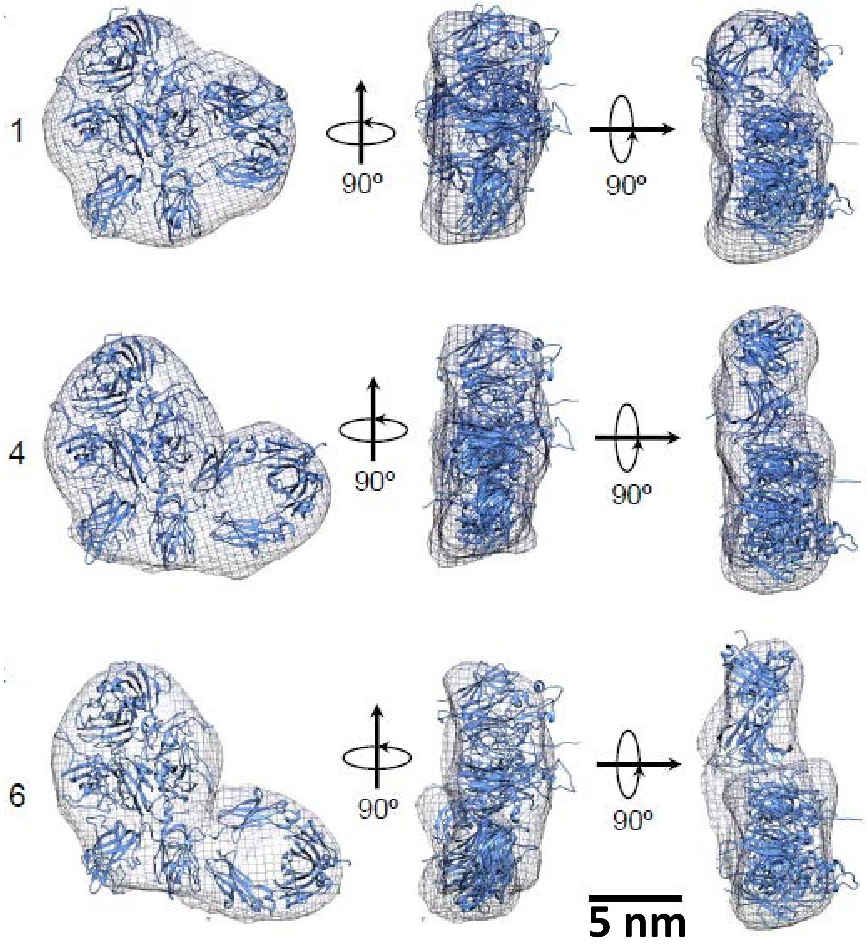


- More than 16,000 images analyzed, oriented, classified, and averaged

SP cryo-EM of rFVIIIFc



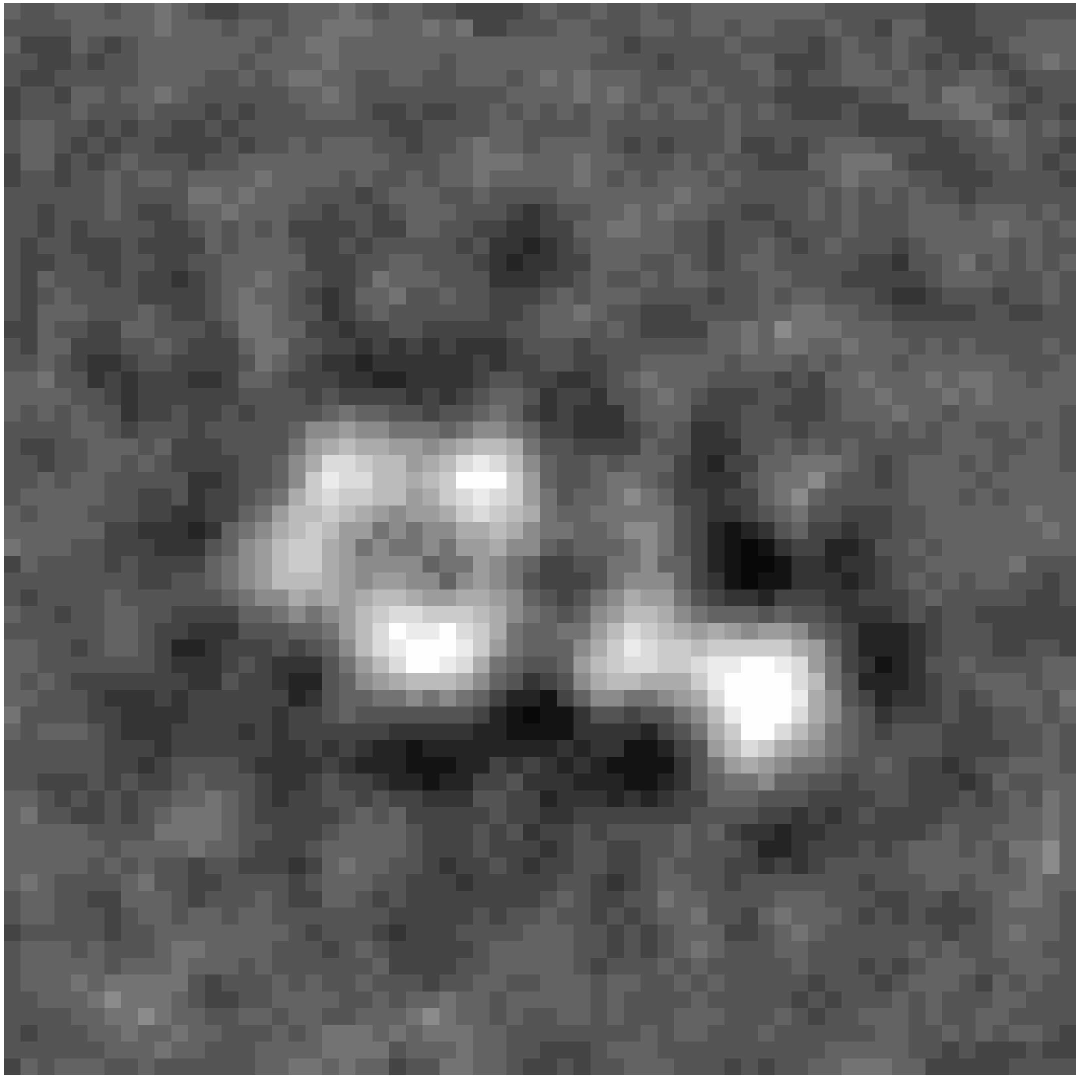




- Crystal structure models fit nicely inside the electron density maps
- The Fc domain adopts multiple orientations relative to the FVIII part of rFVIIIFc indicating not only flexibility of the linker but also free rotation around the linker

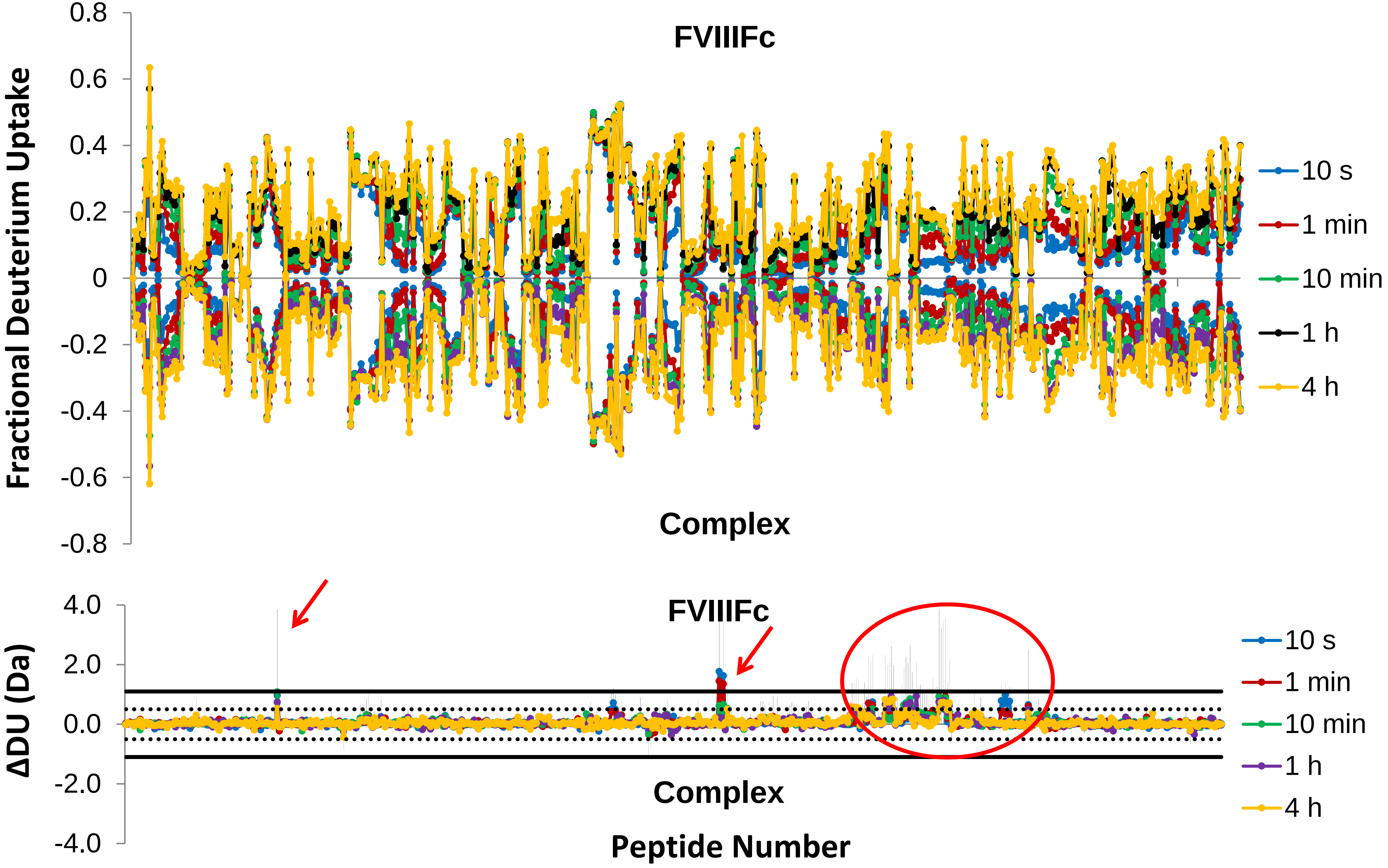
SP cryo-EM of rFVIIIFc

A picture is worth 1000 words, but a movie is priceless…



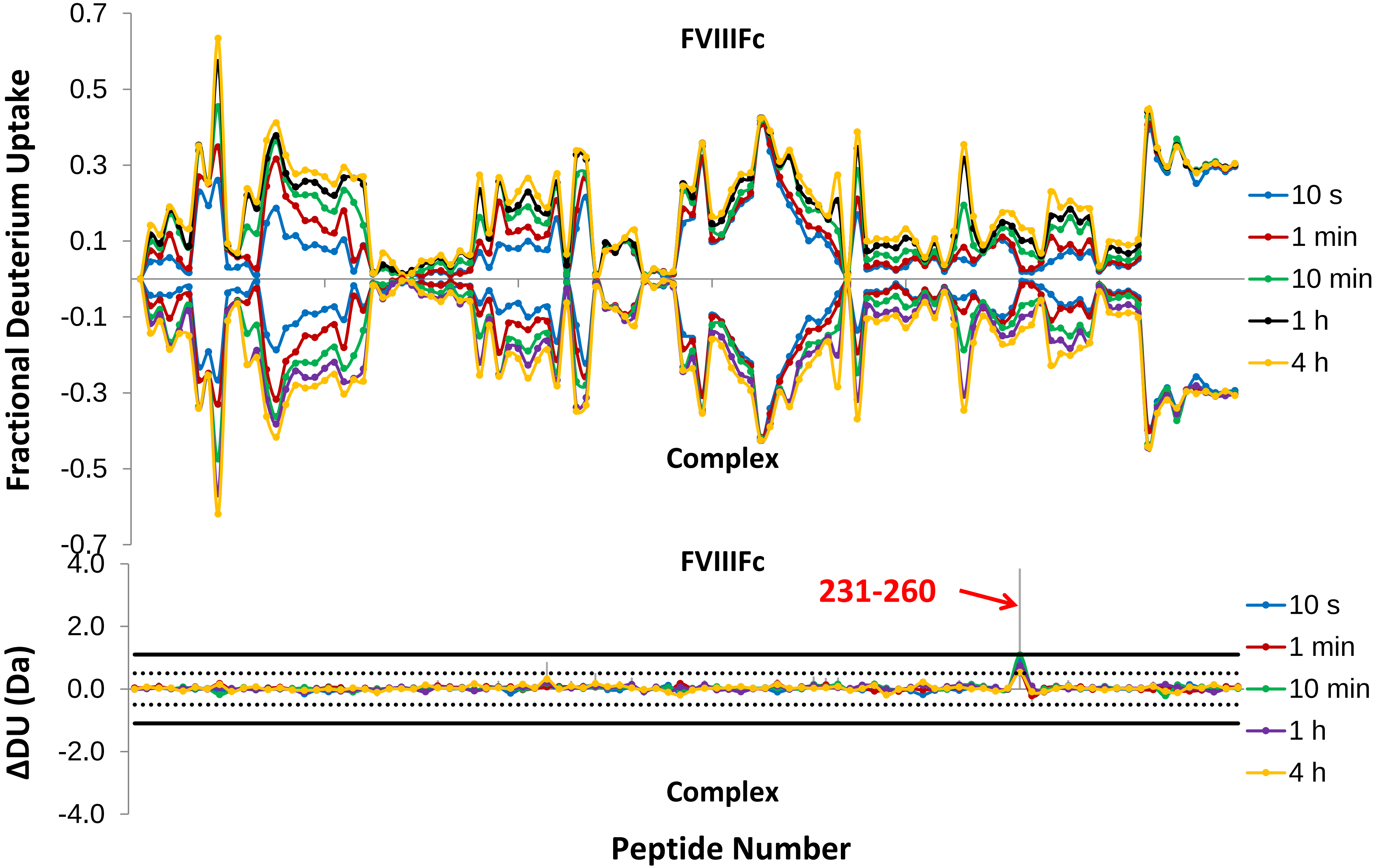
Goal 2: Revealing the interaction sites between rFVIII(Fc) and VWF

HDX of rFVIIIFc ± D'D3 (VWF)



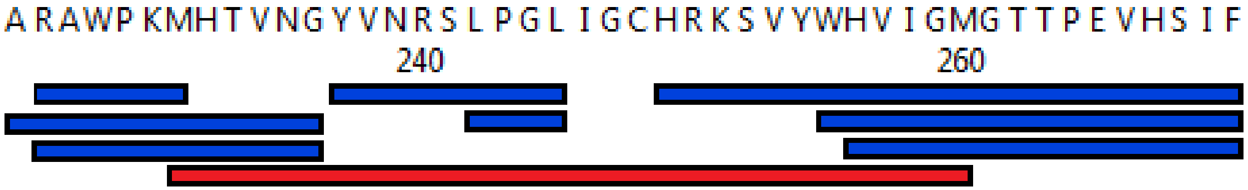
HDX of rFVIIIFc ± D'D3 (VWF)

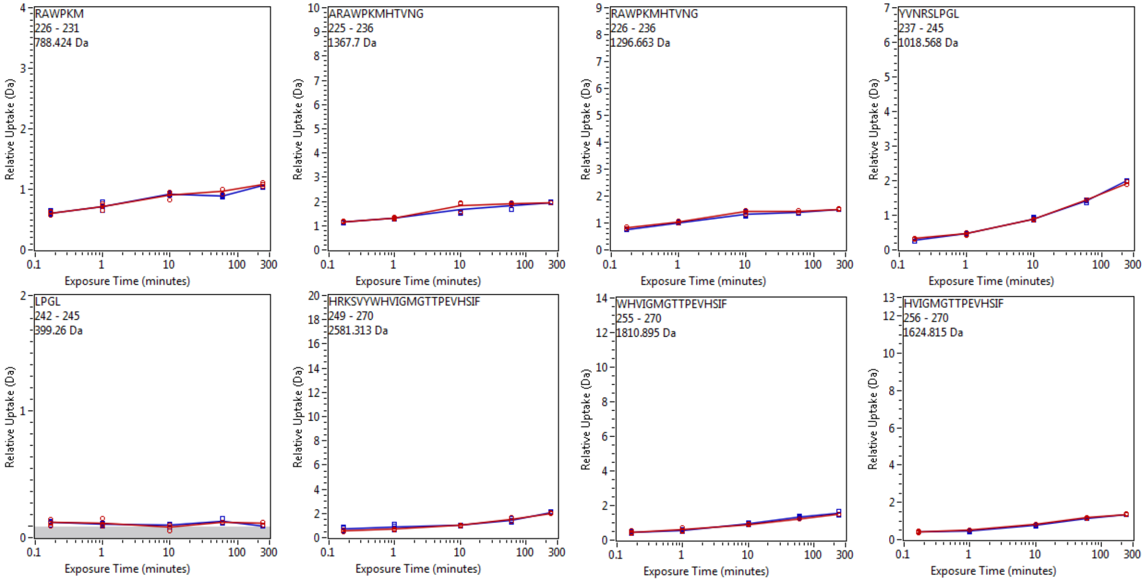
A1 domain

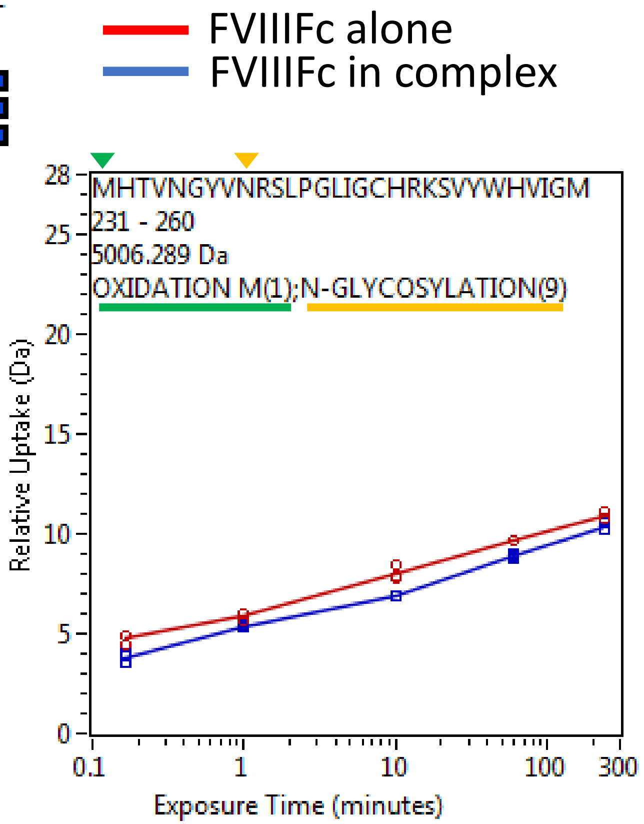


HDX changes in peptide 231-260

A1 domain



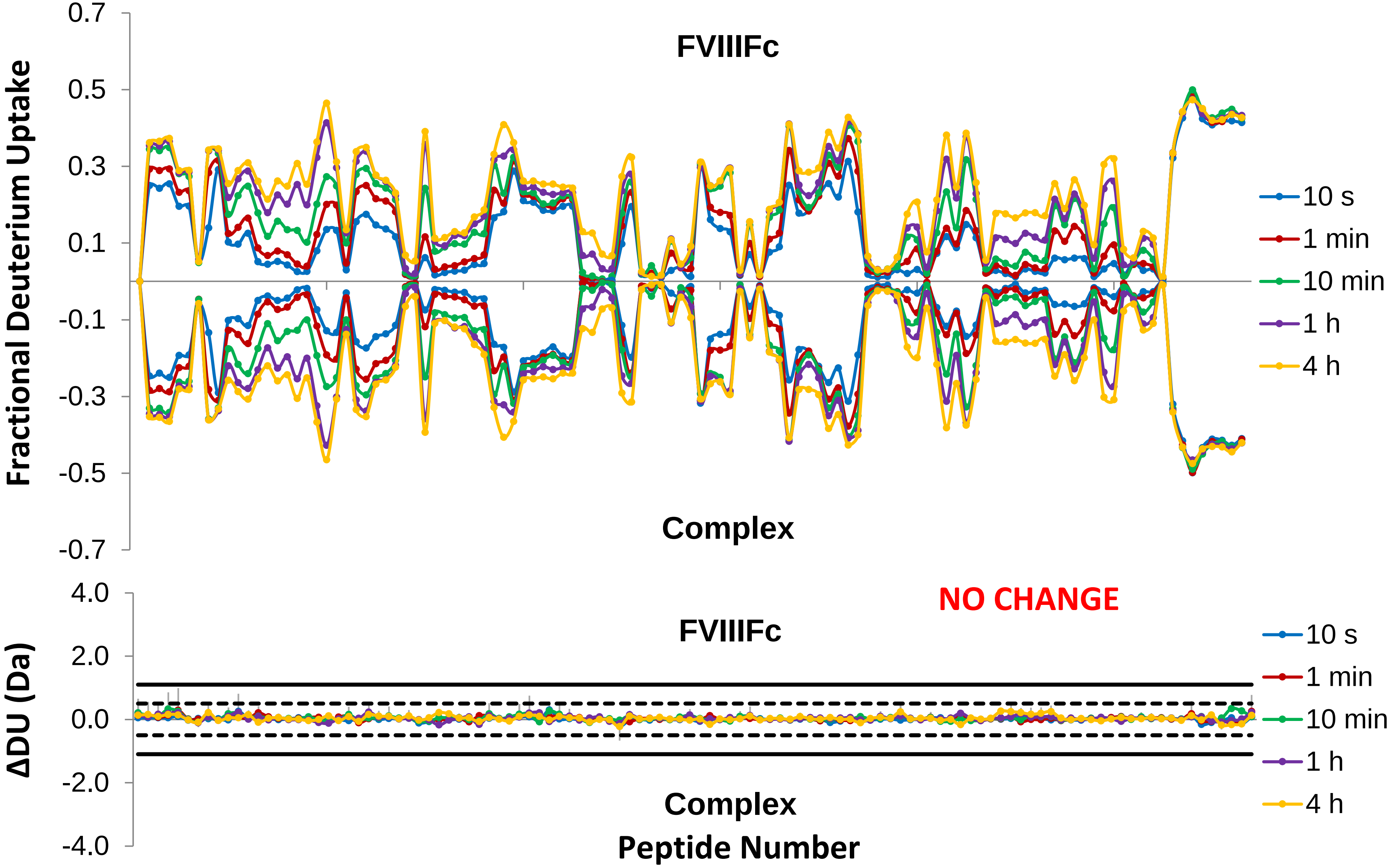




- None of the partially or fully overlapping peptides exhibit any changes in deuterium uptake uptake upon binding of D’D3
- Changes observed in peptide 231-260 could therefore be attributed to changes in the amide hydrogens of the carbohydrate and not to those of the protein backbone

HDX of rFVIIIFc ± D’D3 (VWF)

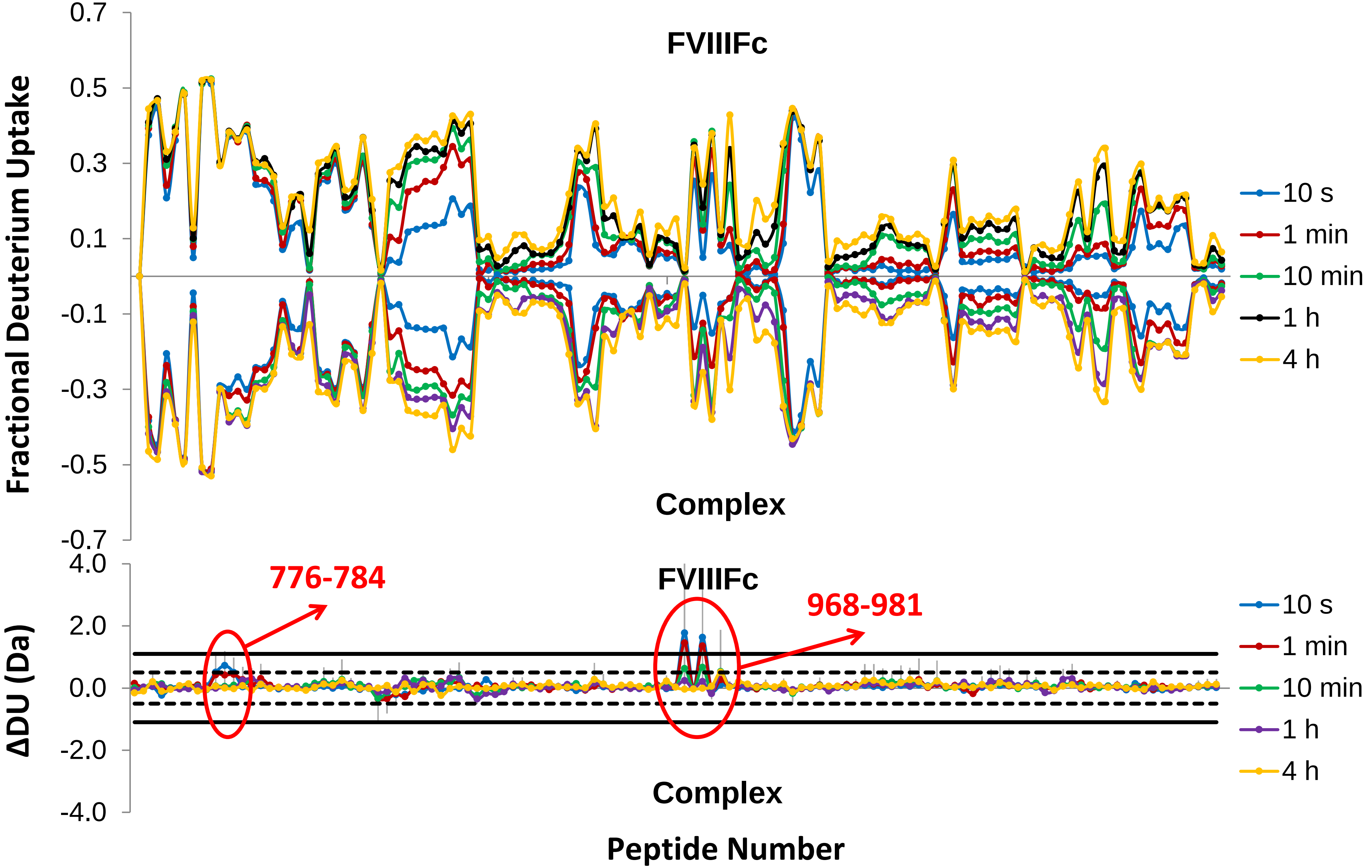
A2 domain



NO CHANGE

HDX of rFVIIIFc ± D’D3 (VWF)

A3 domain

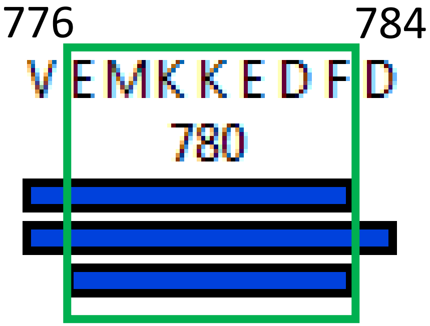


776-784

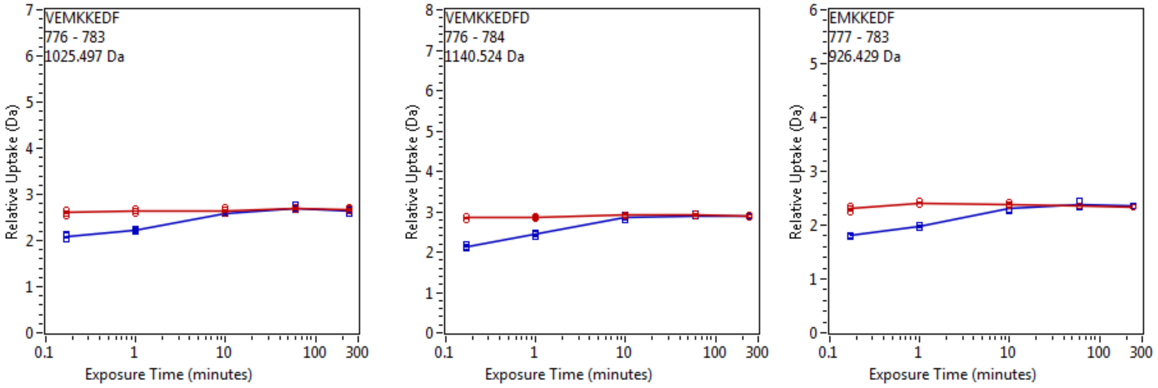
968-981

HDX changes in A3 domain

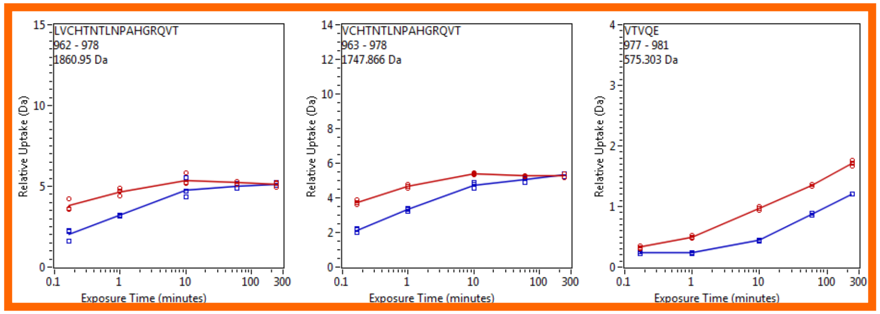
Peptides 776-784 and 968-981

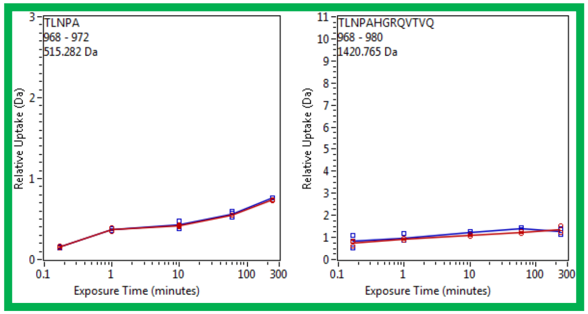


🟥 FVIIIFc alone
🟦 FVIIIFc in complex



- The same extent of changes in DU are observed in all three overlapping peptides. Therefore V776 and D784 are not involved in the interaction

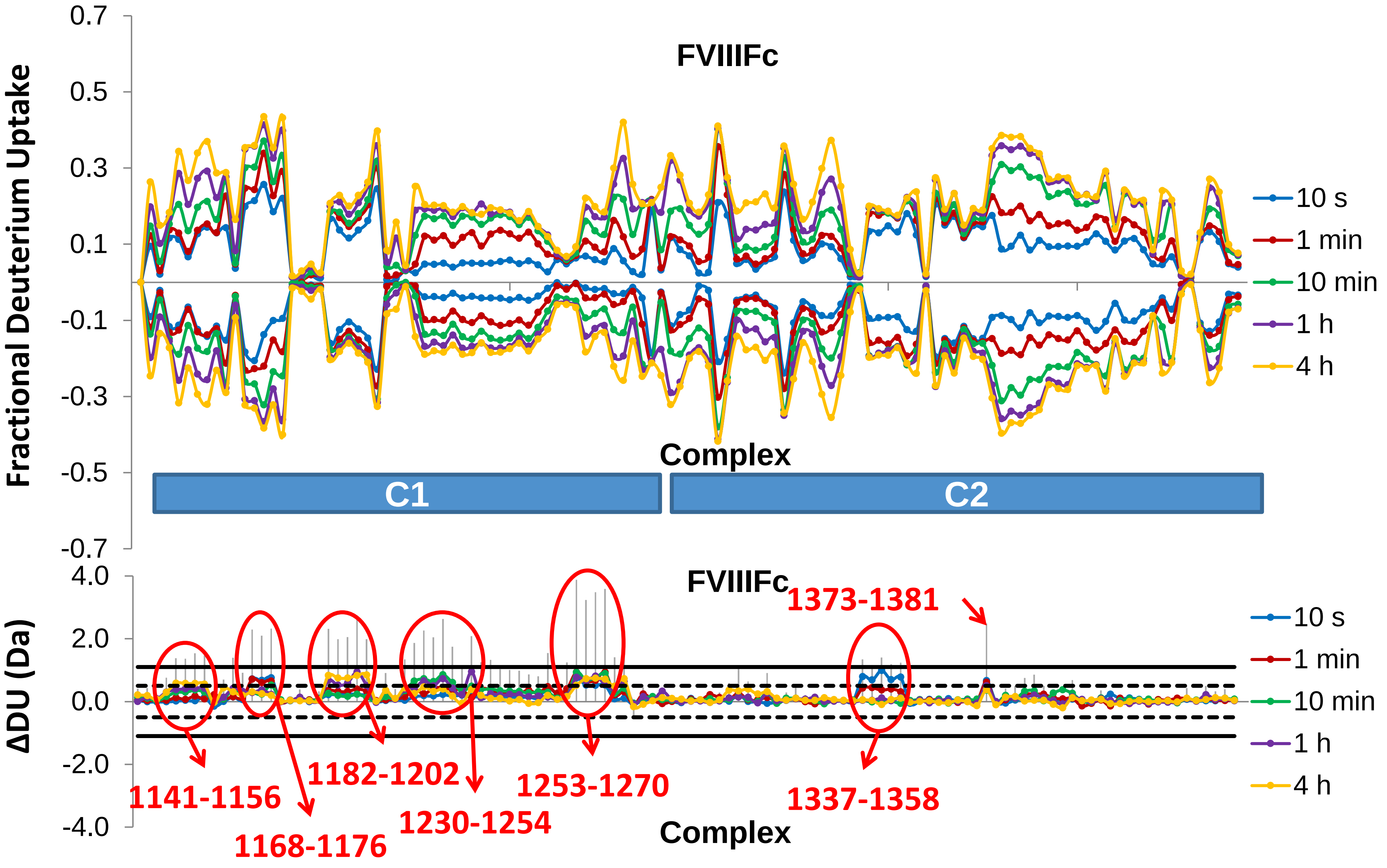




- Because peptides in green do not exhibit any change in DU, the changes observed in peptides in orange are localized to the amino acids highlighted in red.

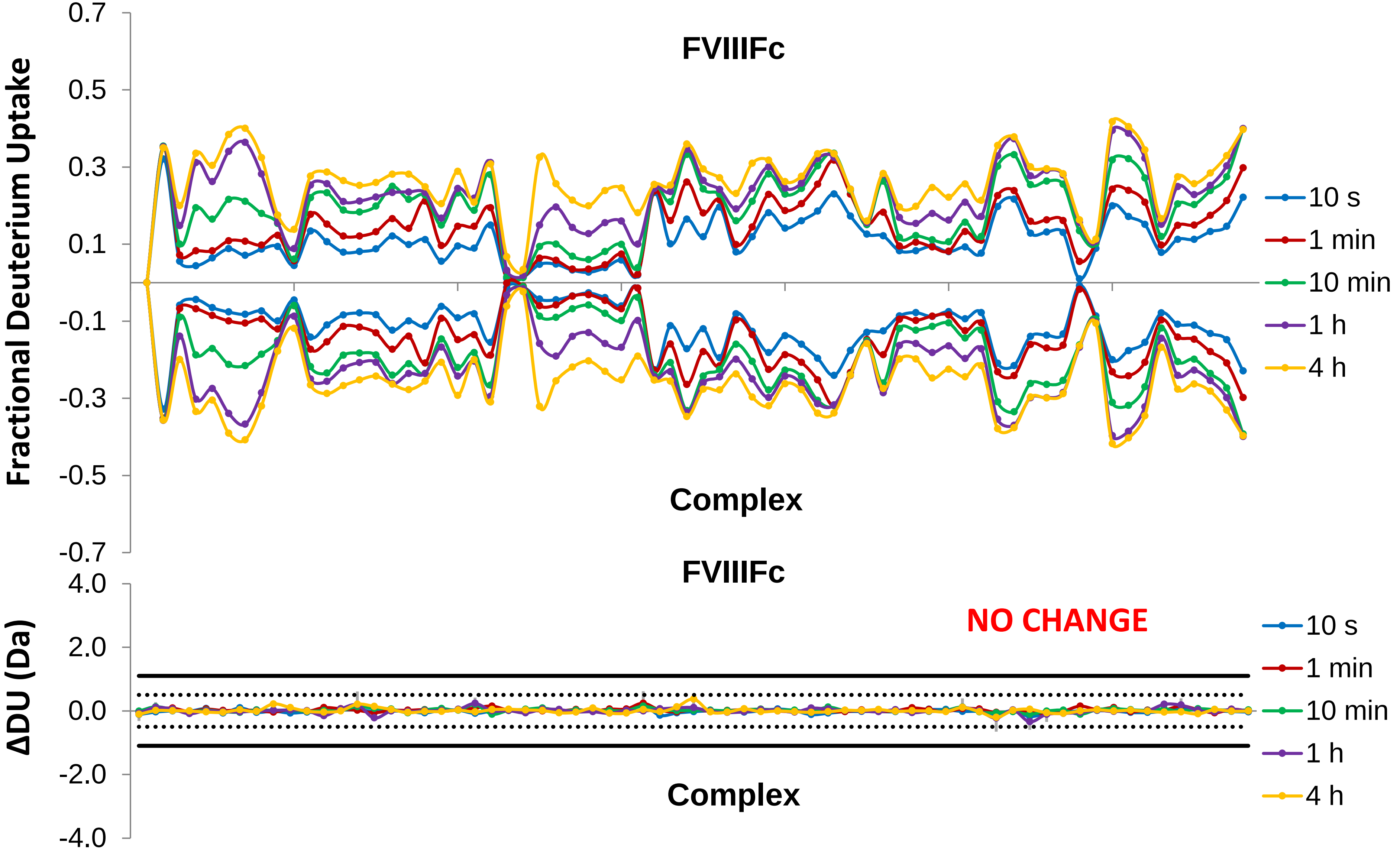
HDX of rFVIIIFc ± D’D3 (VWF)

C1-C2 domains



HDX of rFVIIIFc ± D’D3 (VWF)

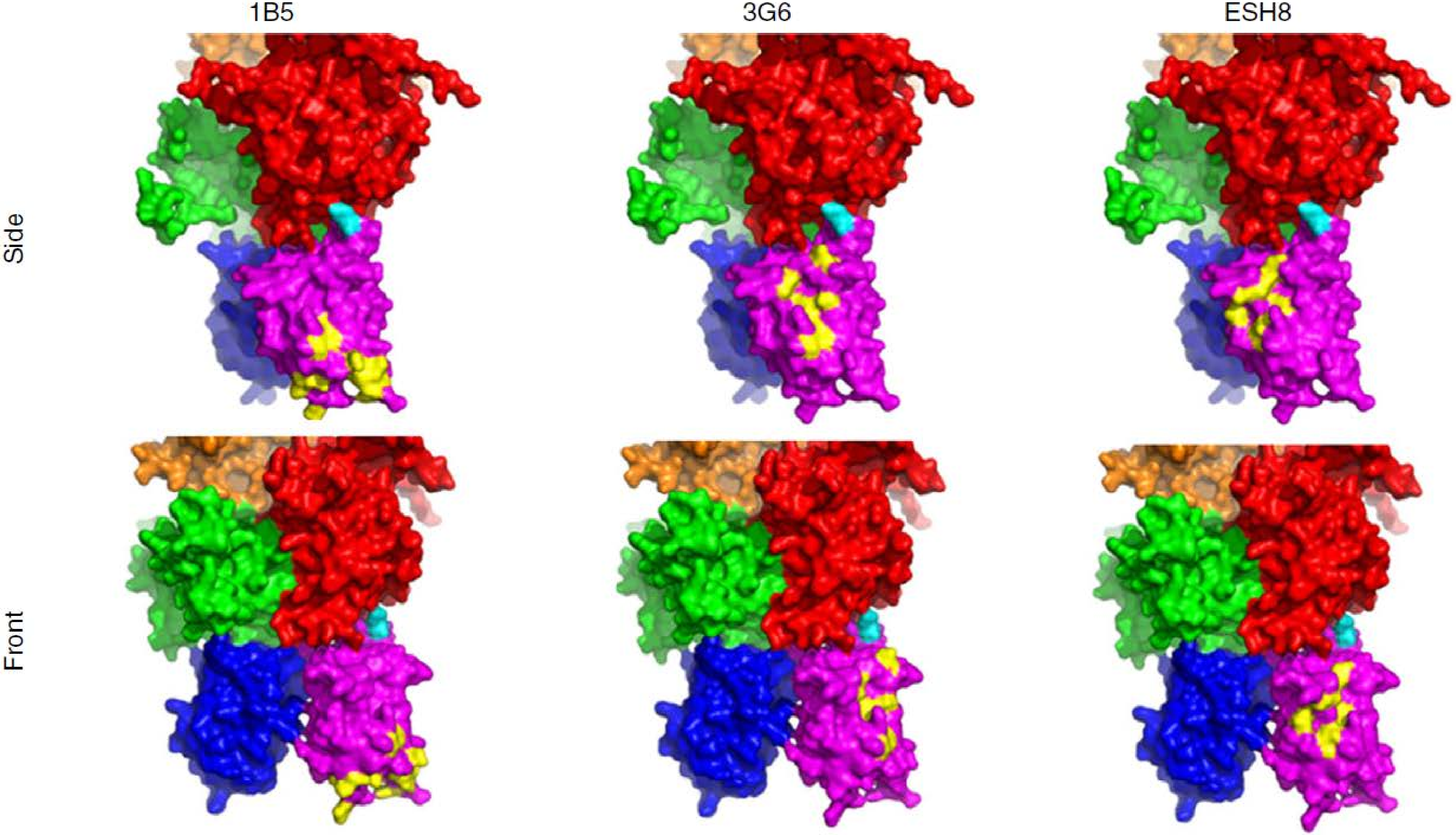
Fc



Goal 3: Ensuring that the function of FVIII is not altered by the Fc fusion

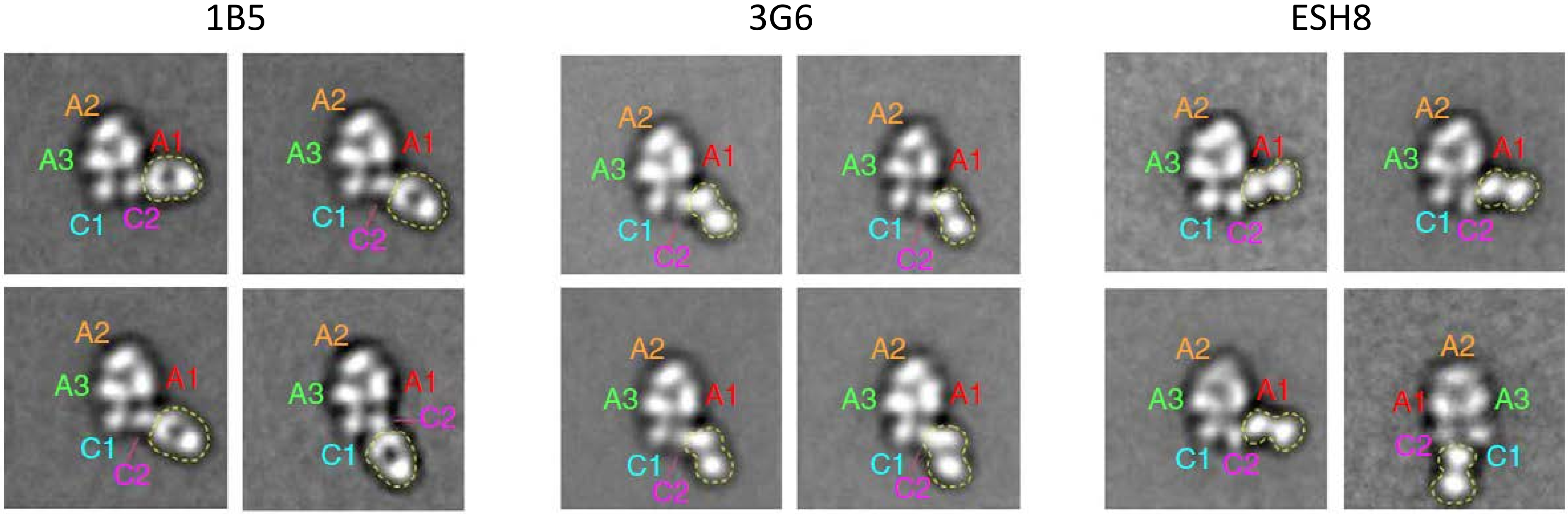
Ab binding to C2 domain

FVIII epitopes



Ab binding to C2 domain

EM images of FVIII-Fab complexes

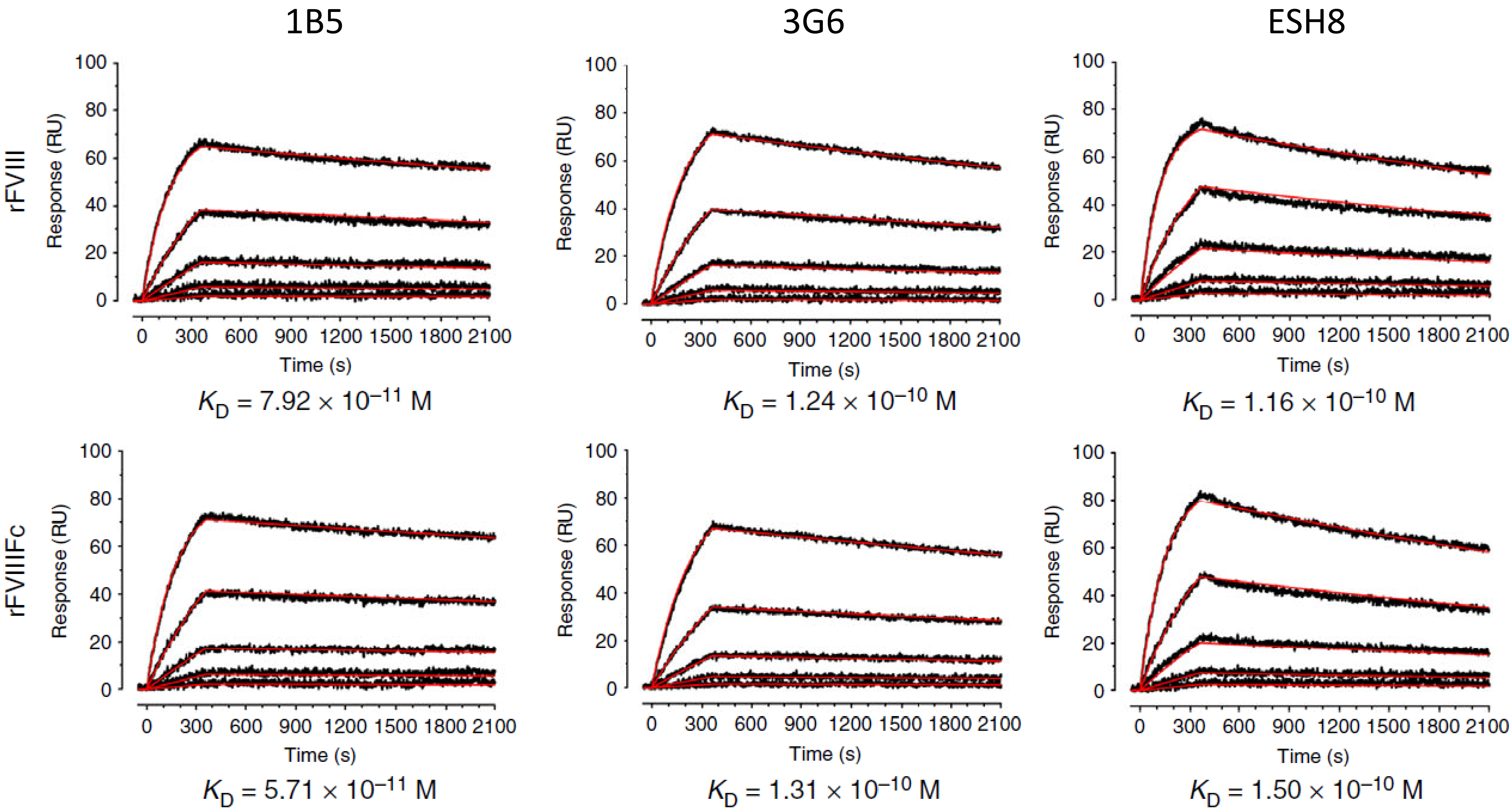


1B5 3G6 ESH8

- Prior EM data shows that Fabs bind C2 domain of rFVIII in areas that could be occupied by the Fc
- To confirm that Fc does not get in the way, SPR was performed on these 3 antibodies with rFVIII and rFVIIIFc

Ab binding to C2 domain

SPR



$K_D = 7.92 \times 10^{-11}$ M

$K_D = 1.24 \times 10^{-10}$ M

$K_D = 1.16 \times 10^{-10}$ M

$K_D = 5.71 \times 10^{-11}$ M

$K_D = 1.31 \times 10^{-10}$ M

$K_D = 1.50 \times 10^{-10}$ M

Ab binding to C2 domain

SPR

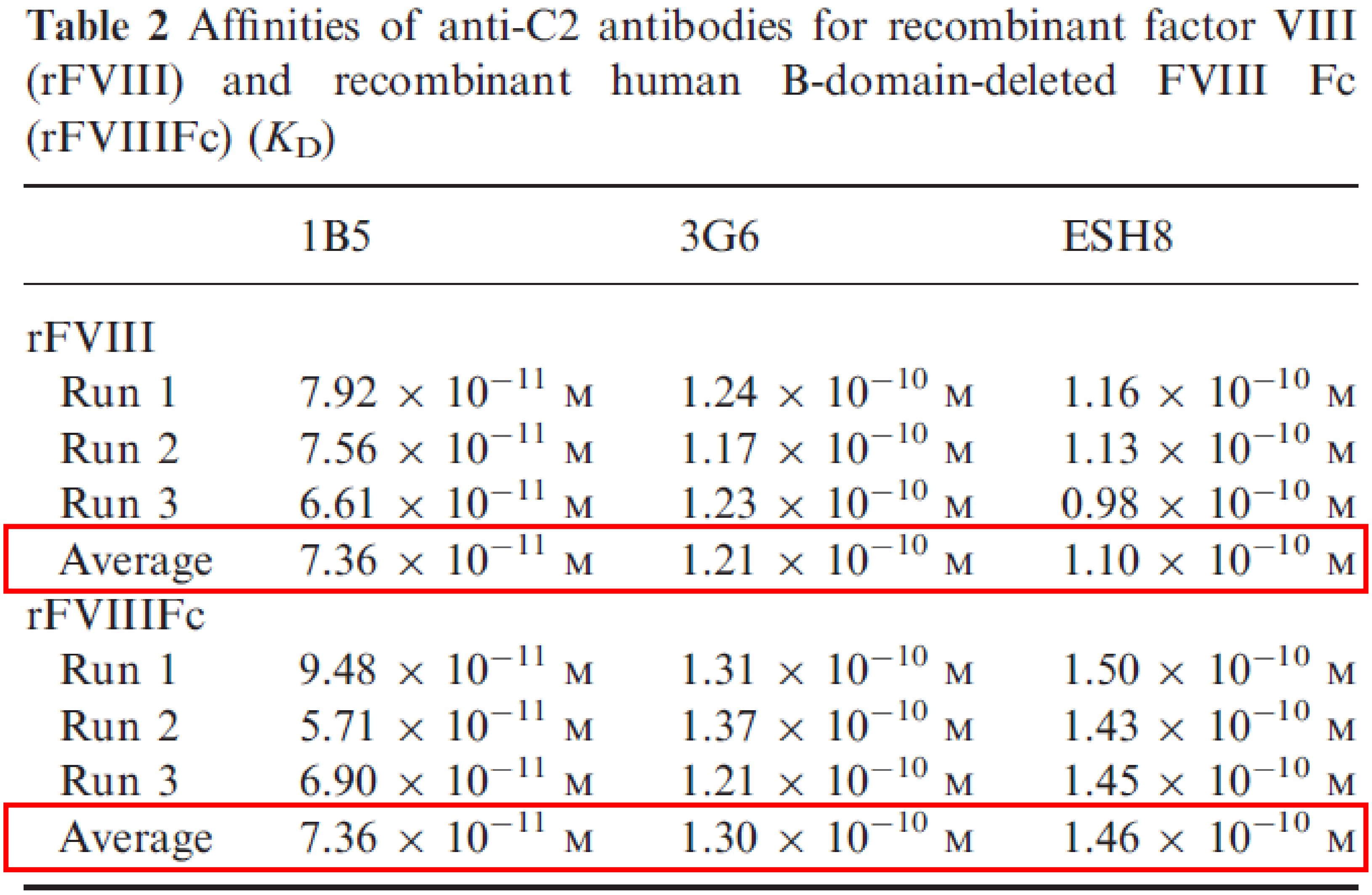


Table 2 Affinities of anti-C2 antibodies for recombinant factor VIII (rFVIII) and recombinant human B-domain-deleted FVIII Fc (rFVIIIFc) (K_D)

	1B5	3G6	ESH8
rFVIII			
Run 1	7.92×10^{-11} M	1.24×10^{-10} M	1.16×10^{-10} M
Run 2	7.56×10^{-11} M	1.17×10^{-10} M	1.13×10^{-10} M
Run 3	6.61×10^{-11} M	1.23×10^{-10} M	0.98×10^{-10} M
Average	7.36×10^{-11} M	1.21×10^{-10} M	1.10×10^{-10} M
rFVIIIFc			
Run 1	9.48×10^{-11} M	1.31×10^{-10} M	1.50×10^{-10} M
Run 2	5.71×10^{-11} M	1.37×10^{-10} M	1.43×10^{-10} M
Run 3	6.90×10^{-11} M	1.21×10^{-10} M	1.45×10^{-10} M
Average	7.36×10^{-11} M	1.30×10^{-10} M	1.46×10^{-10} M

Resolution

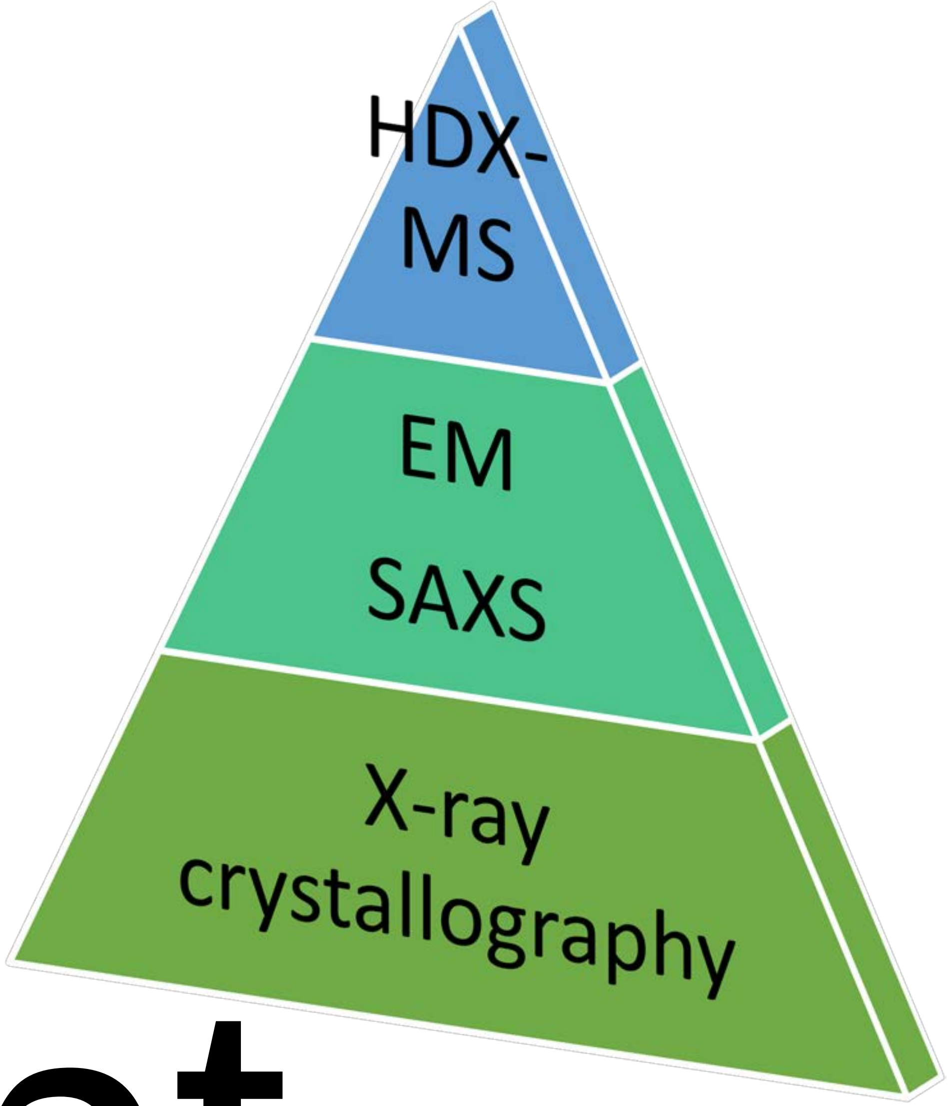
10 pt

20 pt

40 pt

80 pt

160 pt



Thank you!