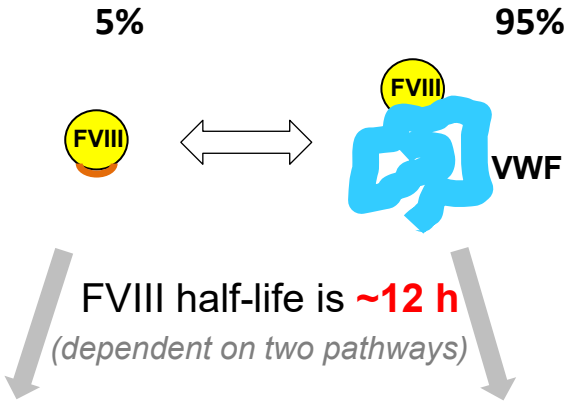
U.S. FOOD & DRUG ADMINISTRATION

Characterization of FVIII binding sites for LRP1 using hydrogen-deuterium exchange mass spectrometry and *in silico* docking

Haarin Chun, Ph.D.
Division of Hemostasis
Office of Plasma Protein Therapeutics
Center for Biologics Evaluation and Research

Introduction: Pathways of FVIII clearance

- Deficiency in blood coagulation factor VIII (FVIII) results in life-threating bleeding, Hemophilia A
- FVIII plasma half-life is ~12 h, which requires frequent FVIII infusions (2-3 times per week in prophylaxis)
- Both FVIII and VWF is catabolized by low density lipoprotein receptor-related protein 1 (LRP1).
- To improve disease treatment, understanding complicated FVIII-LRP1 interaction is important.



Fast clearance

FVIII is cleared in free form

FVIII half-life is **~2 h**

(in VWF-deficient humans)

Slow clearance

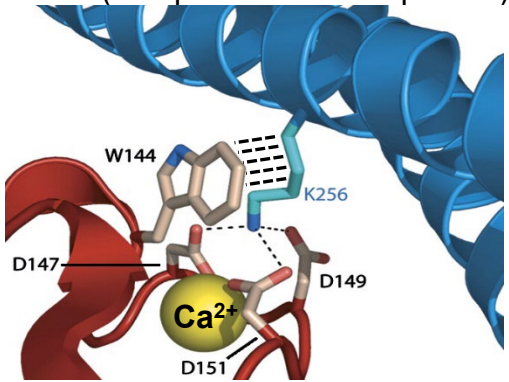
FVIII is cleared in complex with VWF

VWF half-life is **~20 h**

(biochemical barrier of FVIII half-life)

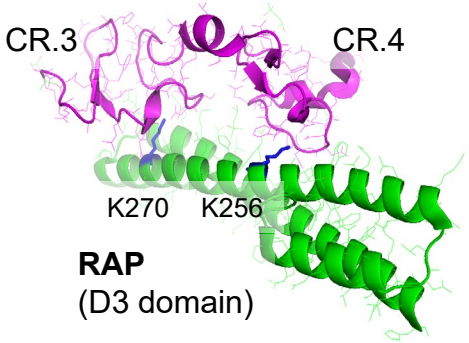
Introduction: General mode of ligands recognition by the LDLR family

RAP (receptor-associated protein)



CR.4 of LDLR

LDLR



Fisher et al, 2006, Molecular Cell 22, 277–83
PDB, 2FCW

RAP

- Two lysines are involved (termed “critical”).
- The interaction was termed bivalent.

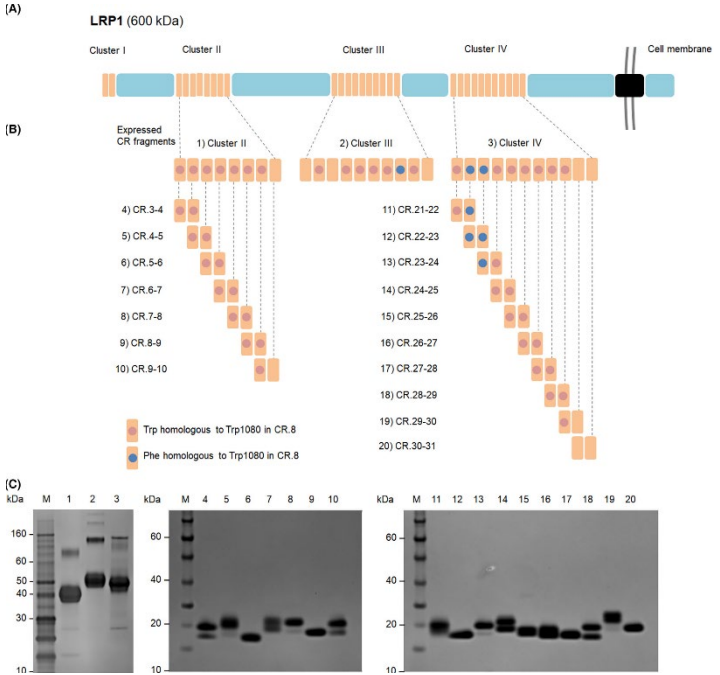
FVIII

- Two charged determinants are involved.
- The interaction was also termed bivalent.
- The canonical binding mode has not been demonstrated.

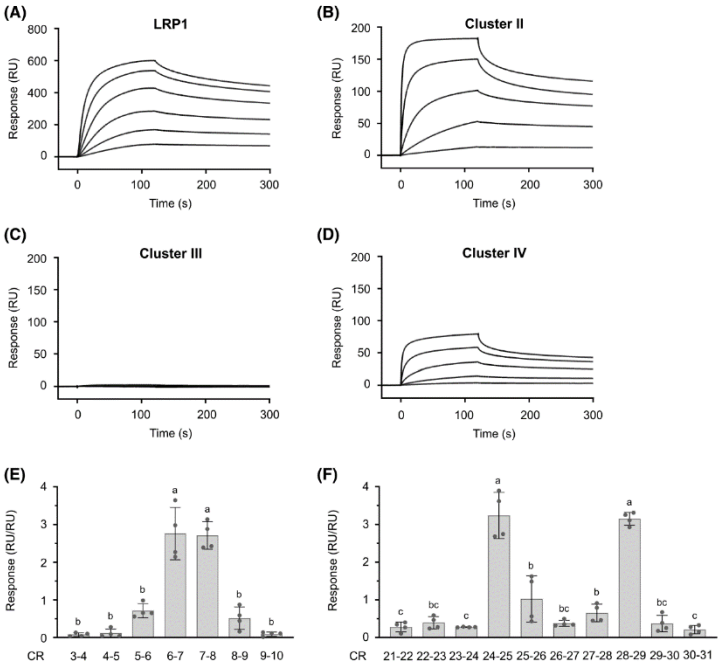
- RAP has two binding sites for LRP1 (with two pairs of “critical” lysine residues).
- FVIII has multiple lysine residues for LRP1 binding.
- LRP1 has multiple binding sites for RAP and FVIII.

Aims: Characterization of FVIII binding sites on LRP1 & Building a model for FVIII-LRP1 interaction

Results: Identification of FVIII binding sites on LRP1

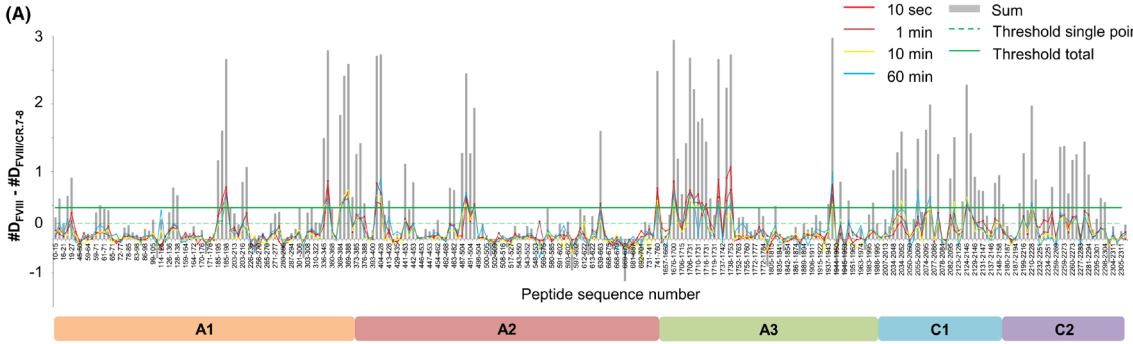


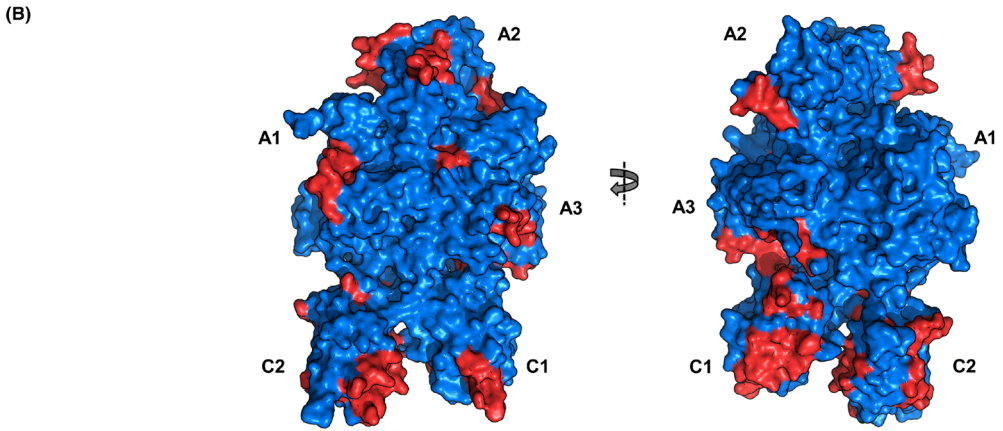
The clusters of complement-type repeat (CR) domains and adjacent two CR domains (CR doublets) of LRP1 were expressed in insect cells.



Expressed LRP1 fragments were tested for binding FVIII using surface plasmon resonance (SPR).

Results: Identification of CR.7–8 binding sites on FVIII by HDX-MS

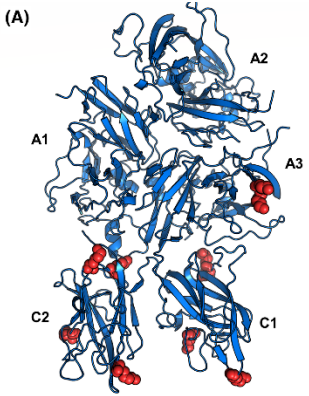




Hydrogen deuterium exchange-mass spectrometry (HDX-MS) of FVIII / CR.7-8 complex:

- 264 peptides covering 78% of the primary sequence of FVIII were detected.
- Peptides overlapping 20 distinct surface areas of FVIII showed decreased deuterium uptake in the presence of CR.7-8, indicating their protection by the CR doublet.
- The major areas include surface-exposed lysine residues and were mainly located on FVIII C1 domain and adjacent domains, consistent with previously observed in FVIII complex with cluster II by HDX-MS.

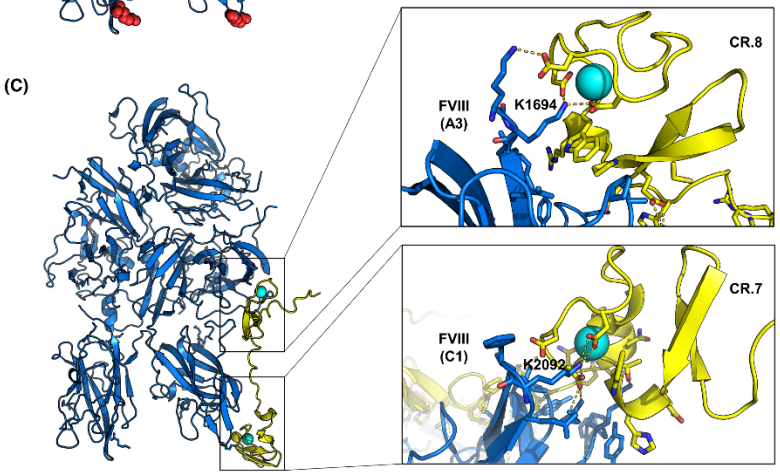
Results: Modeling interactions between FVIII and CR.7–8 *in silico*

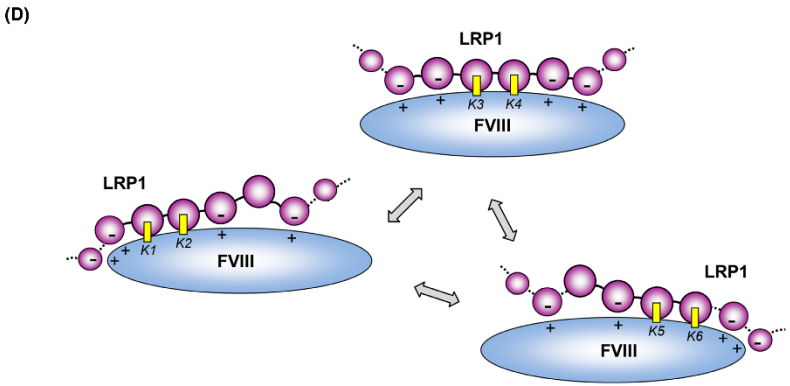


(B)

CR.8

CR.7 Lysine	1693	1694	2049	2092	2110	2111	2227	2236	2239	2249
1693		-11.0			-22.8	-19.0		-18.1		
1694	-20.9			-3.9	-24.9	-17.8		-7.4		
2049				-11.6	-18.4	-8.6	-12.9			-14.2
2092	-14.6	-25.0	-9.3		-13.5	-18.9				-16.2
2110	-16.6	-15.6	-16.6	-11.8		-21.6	-18.4			-21.3
2111	-17.2	-19.5	-10.7	-13.6	-17.1					
2227			-12.2	-4.7	-14.7			-13.0		-13.5
2236	-15.5	-12.5					-13.3			-16.1
2239										
2249	-14.7	-25.4	-13.6	-15.7	-15.5			-26.2		





Simulations of CR.7-8 docking to ten lysine residues on the FVIII (light chain) identified by HDX-MS:

- 90 docking pairs interacting in the canonical mode
- A representative model is shown for the docking of FVIII K1694 (A3 domain) and K2092 (C1 domain).
- The results support (i) multiplicity of FVIII binding sites for CR.7-8 and (ii) canonical mode of interaction.

Conclusion

LRP1 has multiple binding sites for FVIII

- Clusters II and IV form independent sites of LRP1 for binding FVIII.
- Within each cluster, multiple CR doublets bind FVIII in the canonical binding mode.
- Overlapping CR doublets of LRP1 may have distant binding sites on FVIII and bind FVIII alternatively.

FVIII has multiple sites for binding LRP1

The real-time binding unit of LRP1 is composed of more than two CR domains.

U.S. FOOD & DRUG ADMINISTRATION

Thank you!

Disclaimer: *The information in this presentation represents the opinions of the speaker and does not necessarily represent FDA's position or policy.*


FDA
U.S. FOOD & DRUG
ADMINISTRATION