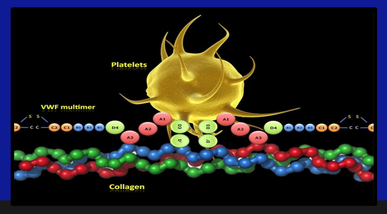
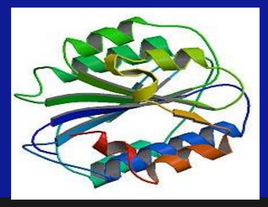


Unfolding the Mysteries of vWF Year 2:

Molecular dynamics simulation of vWF A2 domain and comparison of computational and experimental results

Prathysha Kothare: Parkland High School

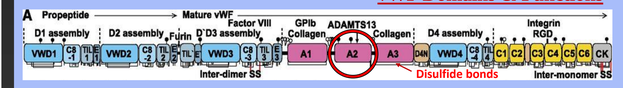


BACKGROUND INFORMATION

vWF Multimeric Protein

vWF is a polypeptide plasma glycoprotein whose adhesive quality, multi-functional domains, and domain mechanosensitivity enable its regulation of hemostasis.

vWF Domains & Functions

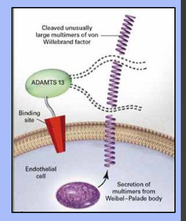


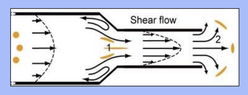
A1 Domain: Binds to GPIb-receptor on platelets to initiate coagulation
A3 Domain: Binds to exposed collagen to secure vWF multimers to rupture site
A2 Domain: In the vWF monomer structure, most domains are stabilized by structural disulfide bonds. However, A2 is not and therefore can unfold under shear flow forces.

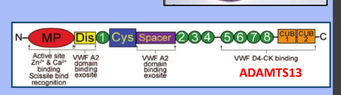
ADAMTS13 A2 Cleavage

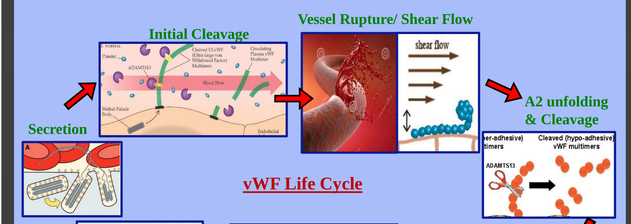
ADAMTS13: Proteolytic enzyme responsible cleaving and regulating vWF multimer length.

Ultra-long vWF multimers remain in the blood following endothelial secretion. vWF elongates and A2 domains unfold under high shear forces induced by vessel rupture or blockage. ADAMTS13 recognizes Tyr1605-Met1606 proteolytic site exposed through A2 unfolding and cleaves vWF to render fragmented proteins, thus normalizing hemostatic responses.





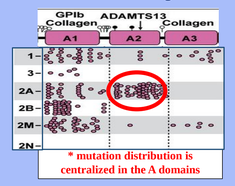




vWD: General Mutations & Q1541R

Genetic mutations in vWF induce abnormal hemostatic patterns including thrombosis or hemophilia. vWF anomalies are categorized as von Willebrand's Disease (vWD).

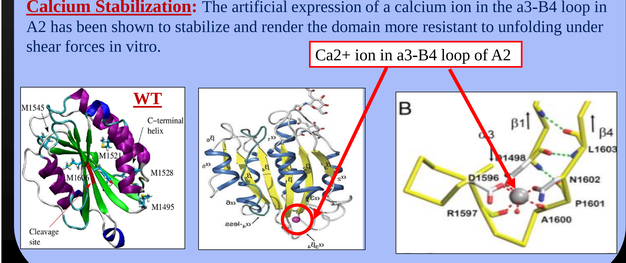
Q1541R Type 2A: Type 2A vWD is categorized by fragmented vWF multimers that cannot properly facilitate clotting and induce hemophilia. These disintegrated vWF multimers form because a mutation in A2 causes them to unfold prematurely, exposing Tyr1605-Met1606 and activating excessive ADAMTS13 cleavage.



* mutation distribution is centralized in the A domains

- missense substitution: Glutamine to Arginine

Calcium Stabilization: The artificial expression of a calcium ion in the a3-B4 loop in A2 has been shown to stabilize and render the domain more resistant to unfolding under shear forces in vitro.



RESEARCH QUESTION AND HYPOTHESIS

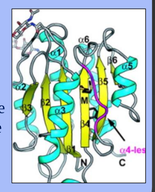
IN VITRO EXPERIMENTATION: When using Optical Tweezers (OT) to characterize vWF's mechanosensitive A2 domain, how does the 2A 1541 mutation influence the monomer unfolding force? How do these forces and the work performed compare to those needed for the A2 wildtype (WT)?

MD SIMULATIONS: When CHARMM and NAMD software are programmed to model vWF A2 domain in shear flow, how does the simulated unfolding behavior compare to that in OT?

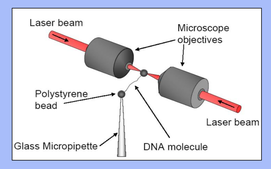
- How do **Q1541R** and **Calcium ions** influence A2 unfolding force and work, linearization rate, and beta-sheet unfolding sequence?

HYPOTHESIS:

- If the 2A 1541 mutation is present, then in vitro OT data will validate last year's results in that the mutant has smaller unfolding extension.
- If a 2A 1541 mutation is expressed in A2 MD simulation, then the extension force required will be less than that for the WT, and the domain's B4 sheet will be exposed under lower forces and work.
- If a Ca+2 is expressed in the A2 a3-B4 fold in MD simulation, then the required unfolding force and work performed will be significantly greater than that for the WT.

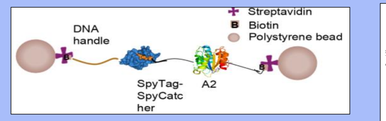


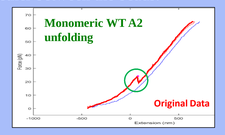
OPTICAL TWEEZERS EXPERIMENTATION



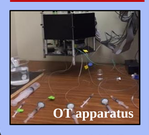
Optical tweezers use laser traps to manipulate nanoscale objects. While one micro-bead is trapped by a laser, the other is secured by a micropipette. The force at which A2 unfolds is quantified by tethering a domain between two beads and pulling them apart to quantify the monomer unfolding force and extension.

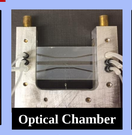
Polystyrene beads coated with streptavidin and anti-Dig antibodies allow DNA handles coupled to the monomer to form disulfide bonds and tether between the beads.

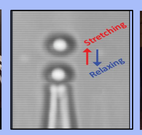




OT MACHINERY

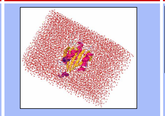
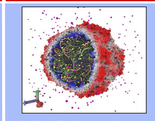




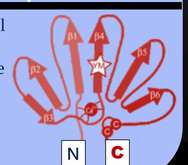


*** See packet for monomer construction and OT procedure

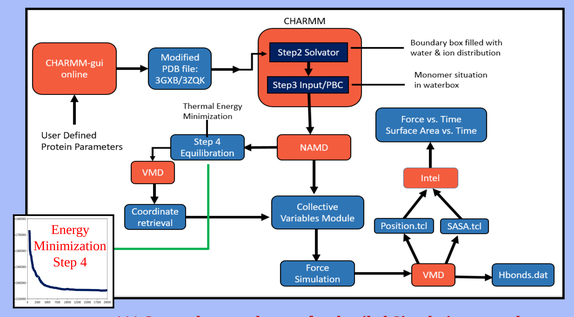
MOLECULAR DYNAMICS SIMULATION (MDS)

Protein Construction and Simulation Setup	Force Simulation Software	Control & Programming	Analysis, Display, Graphing Software
			
Charmm-gui: web-based interface used to create MDS systems and input files for force simulations **CHARMM:** MDS program consisting of physical chemistry force fields and data analysis capabilities	**NAMD:** MDS parallel programming model for interactive force simulations **SOL:** Parallel computing platform with remote login capability via MobaXTerm; Super-computer to conduct protein force simulations	**MobaXTerm:** (UNIX)Windows application for remote computing • **SSH:** Encrypted remote login session for secure client-server connection • **SFTP:** Network protocol for file transfer and modification	**VMD:** Software for display, animation, and analysis of biomolecular systems **TecPlot & Excel:** Graphing & Data Analysis software **VideoMach:** Dynamic visualization software for protein morphology

Macro-Molecular Dynamic Simulation enables theoretical characterization of A2 atomic behavior. Specifically, CHARMM and NAMD were used to build and induce the domain to shear flow forces. MDS subjected both monomer C-N termini to 50 ang/ns pulling speeds with a 5 pN/ang spring constant. All parameters were entered in Python and run through UNIX in MobaXTerm.



MD SIMULATION SCHEMATICS



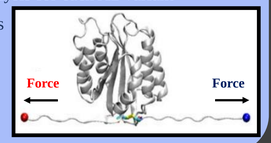
*** See packet supplement for detailed Simulation procedure

ANALYTICAL GOALS & VARIABLES

INDEPENDENT VARIABLE: A2 variants = Ca2+ ion and 1541 mutant
DEPENDENT VARIABLES: Unfolding force, Extension rate, Beta-sheet unfolding
CONTROL GROUP: WT A2 domain in OT and MD Simulation

OBJECTIVES:

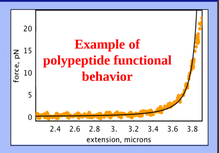
- Comparison of A2 WT to 1541 unfolding in OT using Worm-Like Chain elasticity model (validate & expand last year's results)
- Analysis of MD Force-Time data of each terminus in Ca2+, 1541, and WT
- Hydrogen bond & beta-sheet extension analysis for each A2 variant
- Characterizing morphological discrepancies between variants
- Drawing conclusions between MD and OT results



OT ANALYSIS: WT AND Q1541R

vWF POLYPEPTIDE ELASTICITY

Many biological polypeptides demonstrate elastic qualities through which they exhibit rigid behavior under low forces but significant extension under high tension. A2 possesses such elasticity and therefore exhibits a similar force-extension relationship.



$$\frac{F(x)\cdot L_p}{k_B T} = \frac{1}{4}\left(1-\frac{x}{L_c}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c}$$

WLC MODEL

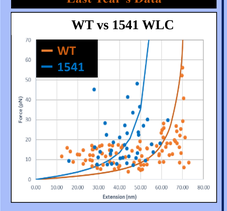
The Worm-Like-Chain (WLC) is a first-principles model used to characterize semi-flexible polypeptide elasticity.

OriginPro and Excel non-linear solvers were used to fit the WLC to OT data. Key parameters include:

- L_c : Maximum unfolding A2 extension (A2 WT= ~70 nm)
- L_p : Measure of protein rigidity (A2 WT= ~0.5-1 nm)

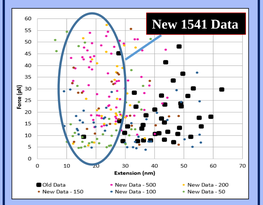
Variable	Definition	Units	Classification
F(x)	Pulling force	pN	Independent
x	Extension	nm	Dependent
T	Absolute temperature	K	Constant
k_B	Boltzmann's Constant	pN.nm/K	Constant
L_c	Contour Length	nm	Parameter
L_p	Persistence Length	nm	Parameter

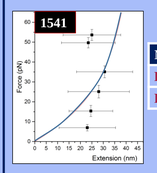
Last Year's Data



WT (nm)	1541 (nm)
L_c = ~75.4-82.4	L_c = ~64.4
L_p = ~0.82-1.05	L_p = ~0.638

*** See packet for data analysis procedure



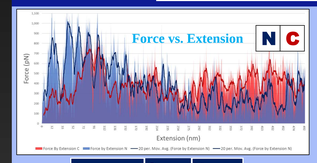


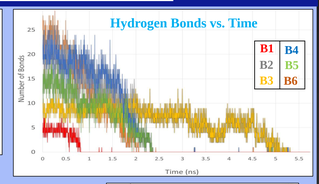
New 1541 WLC
L_c = ~ 58.91 nm
L_p = ~0.275 nm

OT CONCLUSIONS:

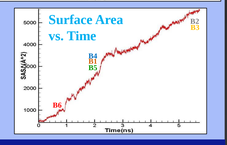
- New 1541 results suggest unfolding phenomena in low extension ranges
- L_c for 1541 is 78% less than that of WT
- Results suggest premature or double unfolding of 1541
- This would allow early proteolytic site exposure of the mutated domain, thereby rendering shorter hemophilic fragments

MDS: WILDTYPE ANALYSIS



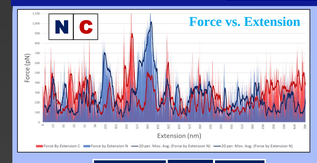


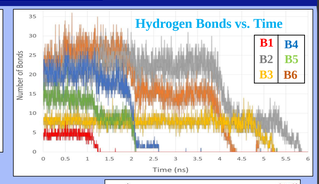
Average	C	N
Force (pN)	394.2	391.2
Work (aJ)	19.4	19.3



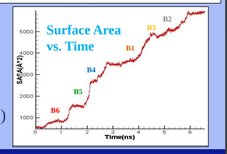
- Fully linearized at ~ 5.3 ns
- ~ Same average force for both termini
- 28% more average work to extend C terminus
- B1,B5,B4 lost all Hbonds at same time (~2.2 ns)
- WT showed linear SA trend (smooth unfolding)

1541 ANALYSIS



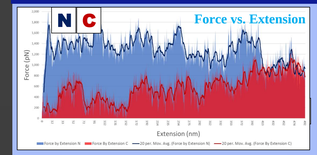


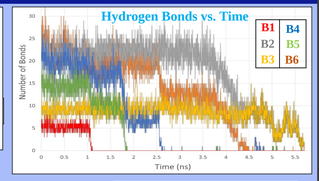
Average	C	N
Force (pN)	265.6	261.6
Work (aJ)	13.1	12.9



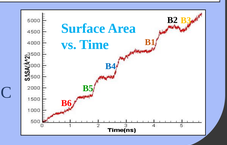
- Fully linearized at ~ 5.9 ns
- ~ Same average force and work for both termini
- B1, B2, B4, B5 showed double unfolding
- B2 sheet was last to lose all Hbonds (B2 has mutation)
- SA showed distinct step change increases

CALCIUM ANALYSIS



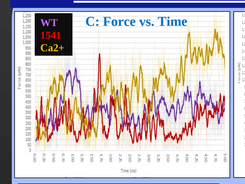


Average	C	N
Force (pN)	576.6	1284.8
Work (aJ)	29.1	64.7



- Fully linearized at ~ 5.7 ns
- N terminus required > 2x unfolding force/work than C
- B1, B2, B4, B5 showed double unfolding
- SA showed distinct step change increases

MDS COMPARISON AND CONCLUSIONS



Compared to WT
Avg Force/Work
Ca2+ = 240%
1541 = 67%

OBSERVATIONS:

- Order of full-extension: WT, Ca2+, 1541
- WT = smooth trend; 1541 & Ca2+ = step change extension
- B1, B4, B5 beta-sheet cascade observed in WT, while Ca2+ & 1541 followed staggered sheet unfolding sequence
- Peak force for 1541occurred later than that of WT
- Mutation in B2 caused it to unfold last in 1541
- B4 for Ca2+ unfolded later than in WT and 1541

PROJECT CONCLUSIONS:

MD Simulations
- ✓ Calcium ion stabilized A2
- ✓ 1541 required lower average force and work to unfold
- ✓ Variants affected protein morphology during extension

OT Experiments
- ✓ OT suggests that 1541 unfolds prematurely or twice
- ✓ The L_c for the mutant was less than that of the WT

THUS … While OT and MDS are not directly comparable, both studies converged in proving that the ease of unfolding 1541 is greater than that of the WT, which would explain excessive cleavage and hemophilia. Simulations also proved Calcium's role in stabilizing A2.