The Effects of Oxidation on Striated Muscle

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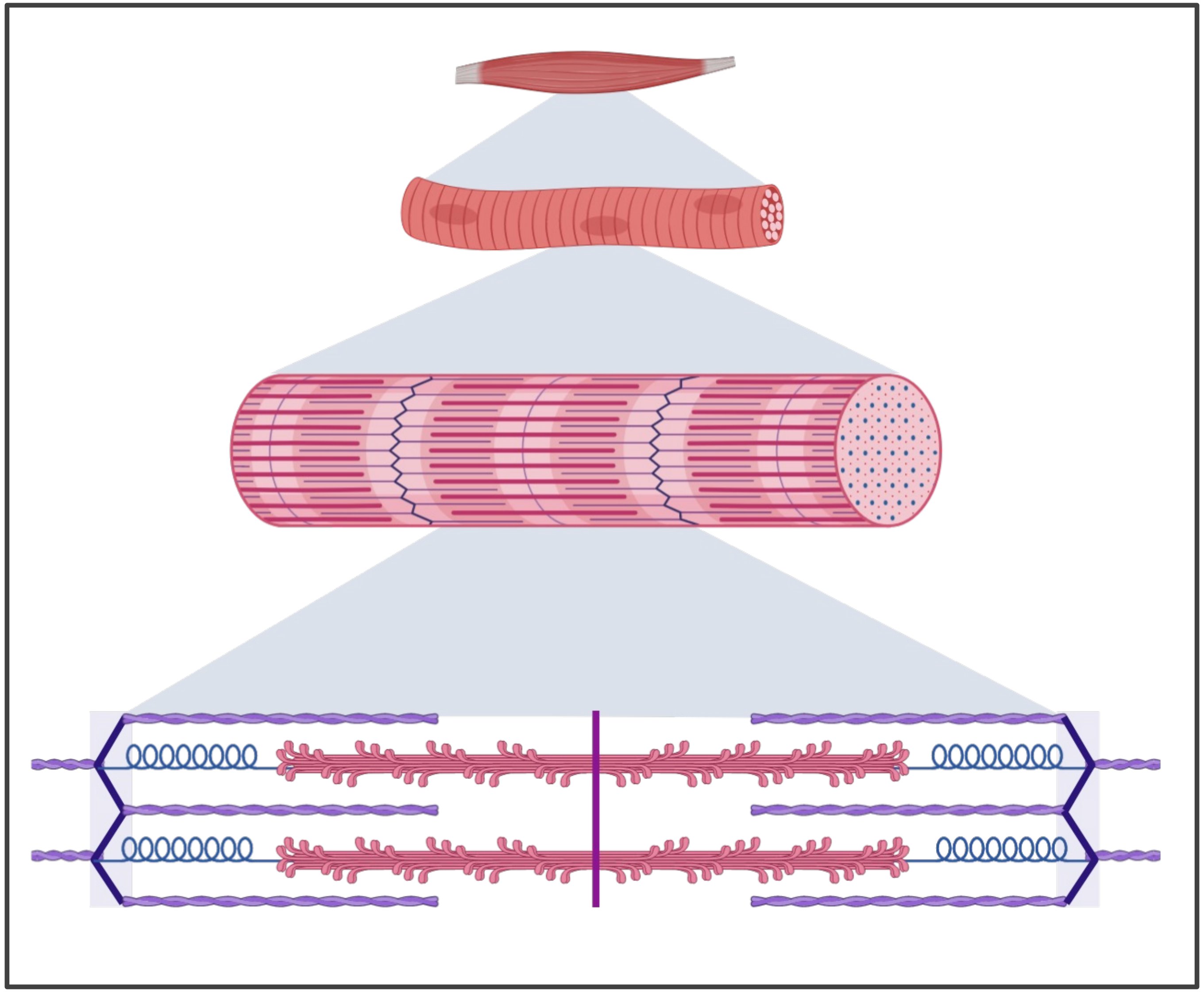
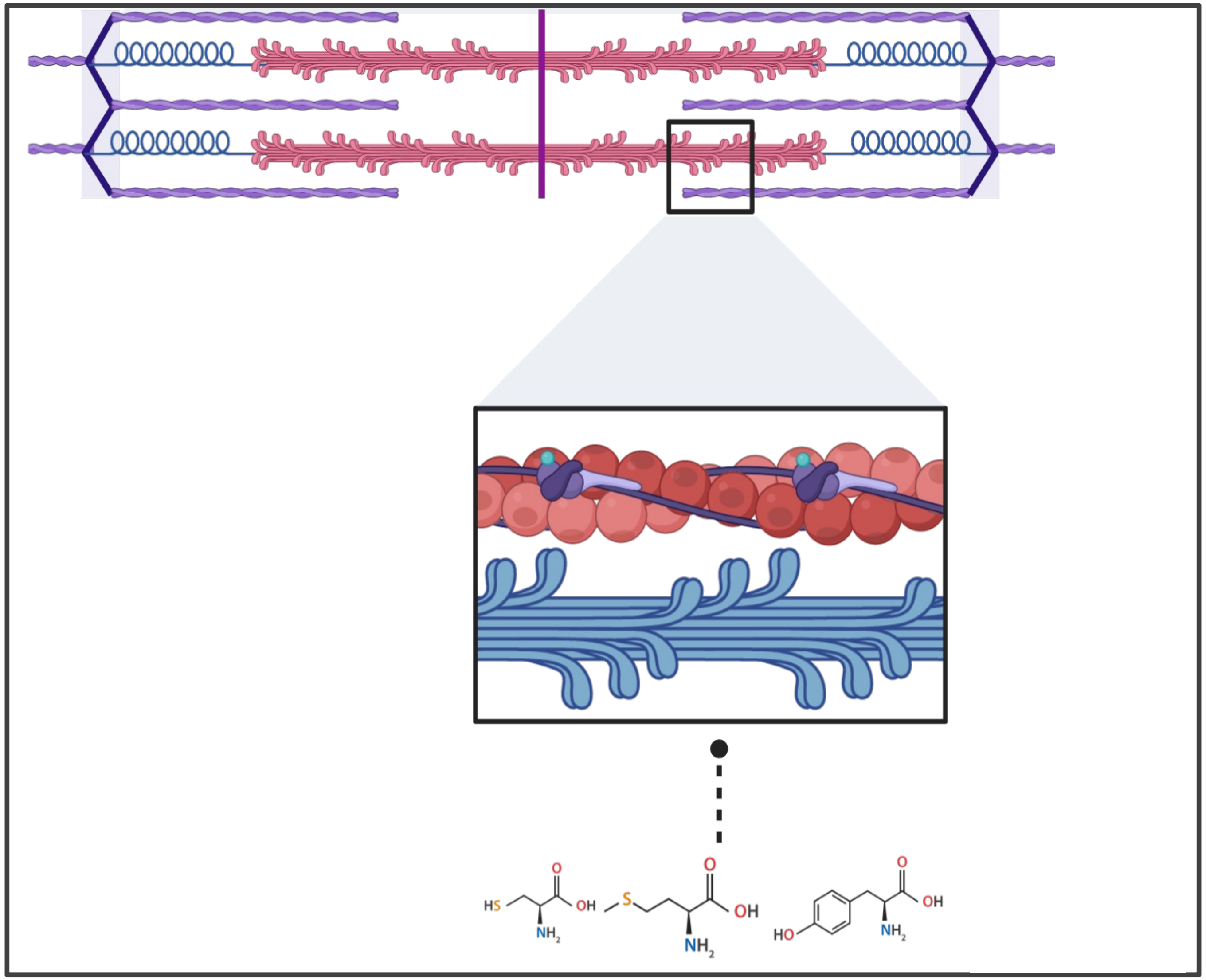
contractile Apparatus

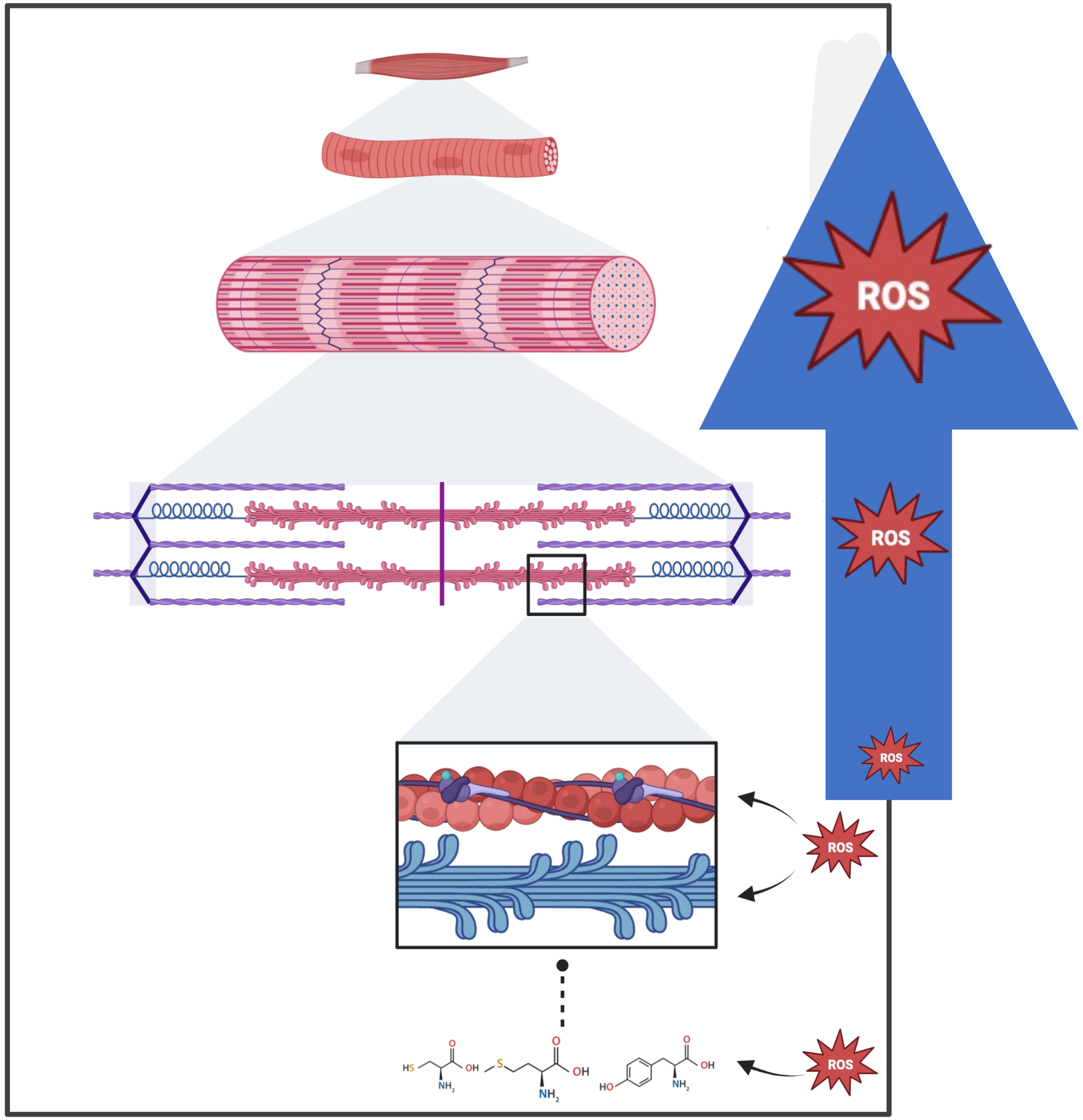


OXIDATION OCCURS AT THE AMINO-ACID LEVEL

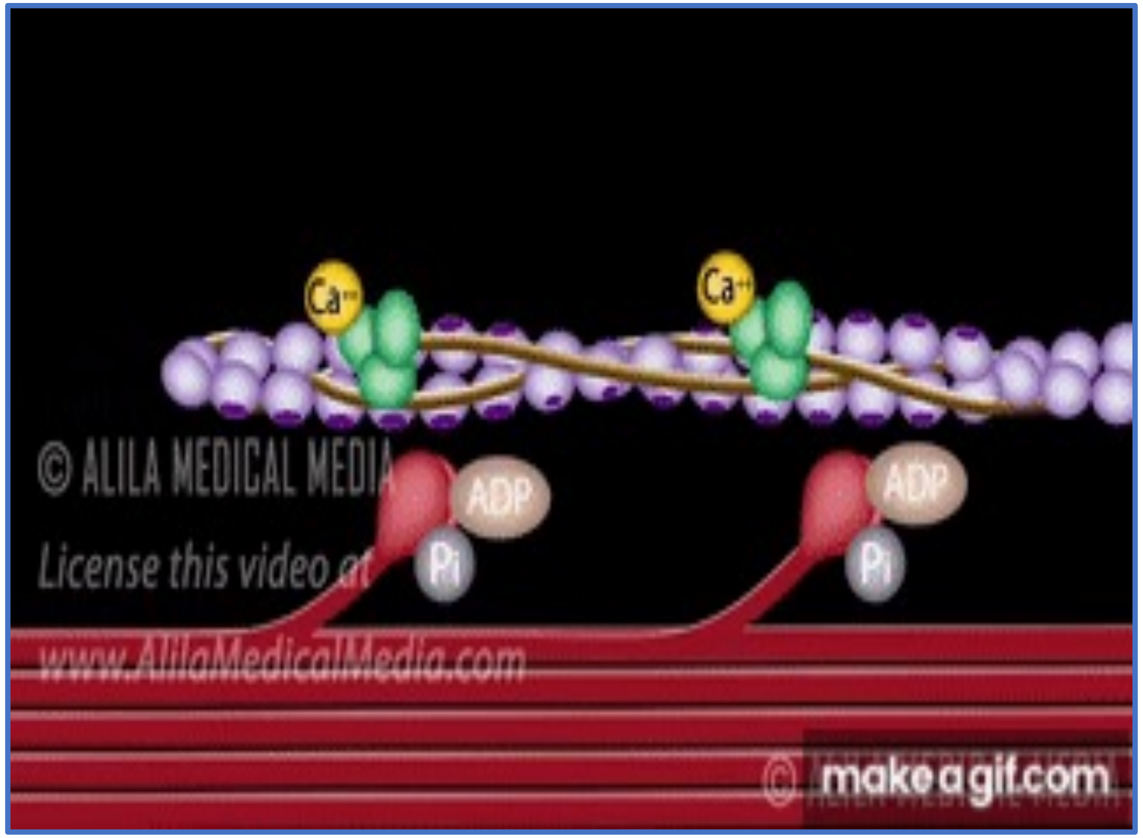


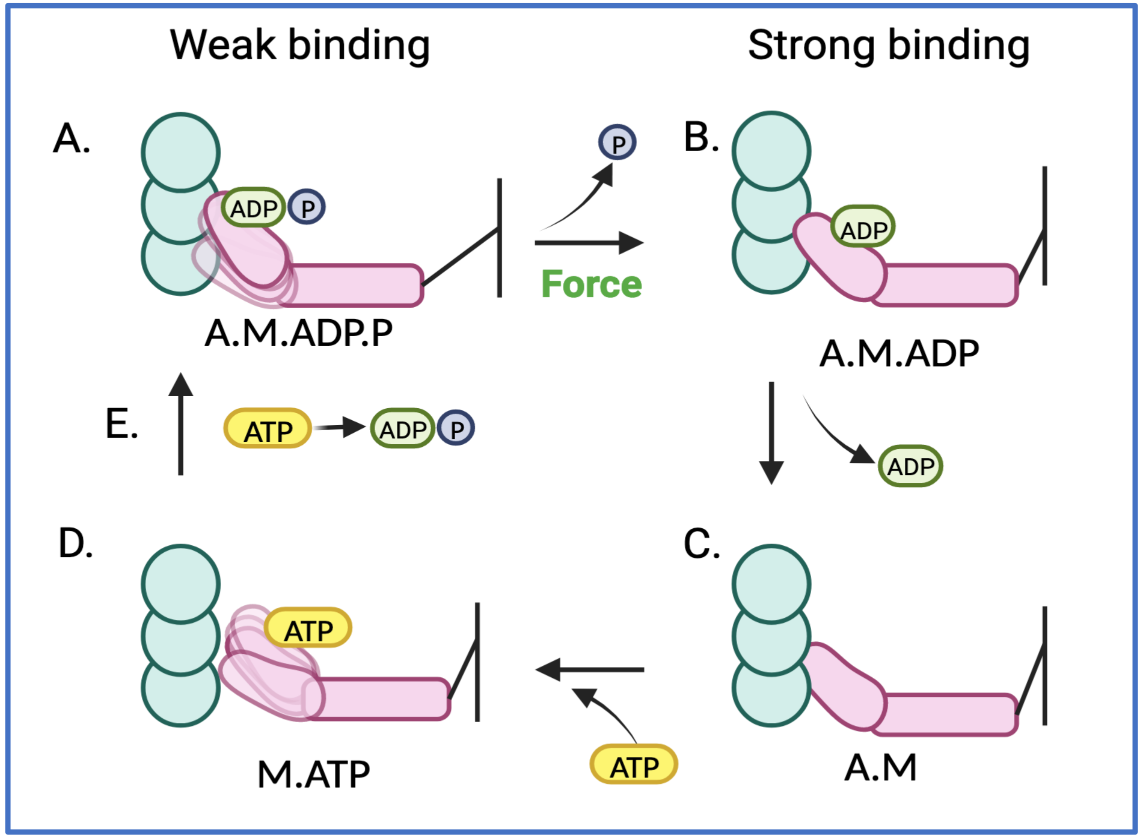
OXIDATION OCCURS AT THE AMINO-ACID LEVEL

- What is the extent of oxidation?
- What are effects of oxidation at different scales ?
- Are these effects consistent across these scales?



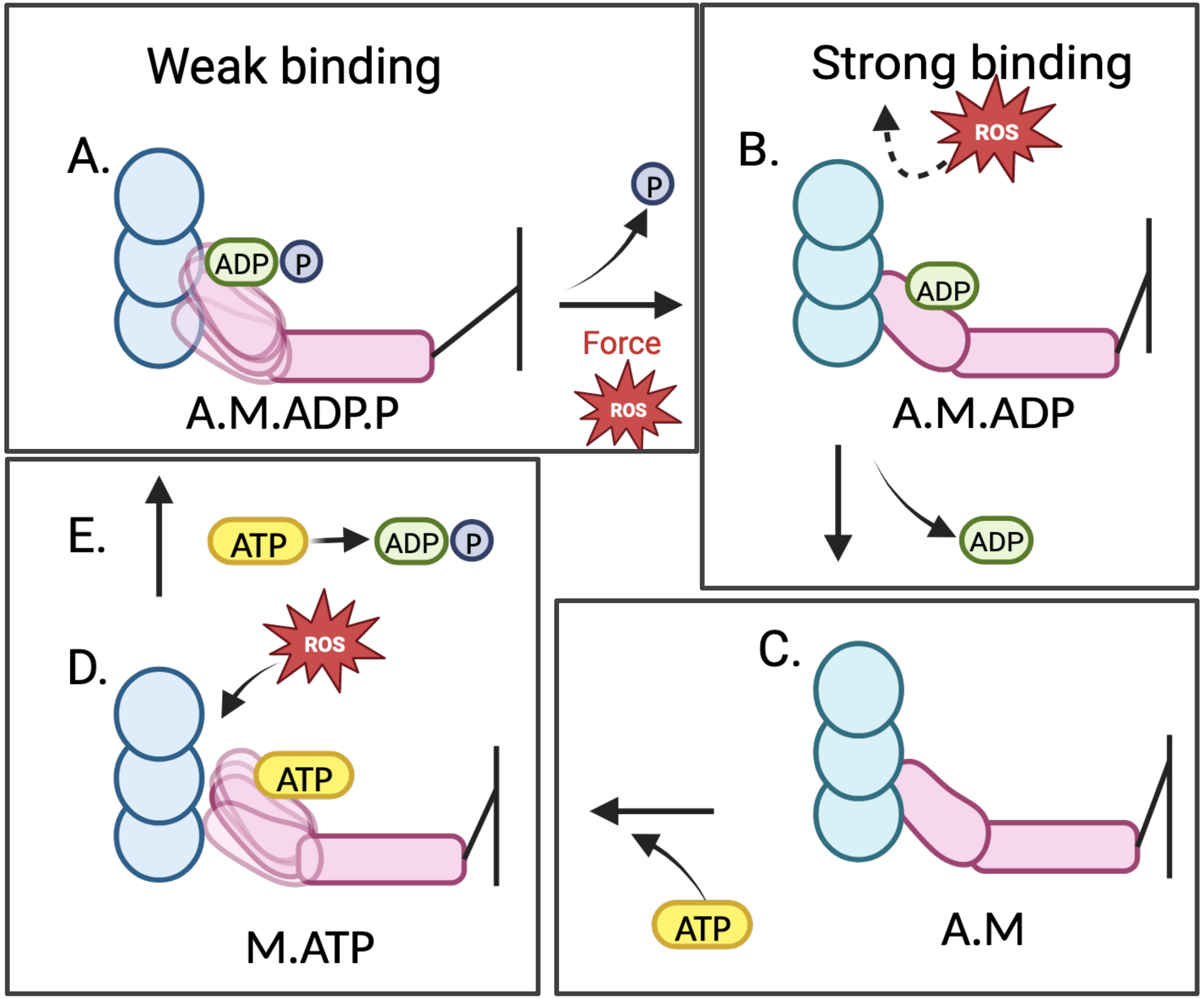
ROS = Reactive oxygen species





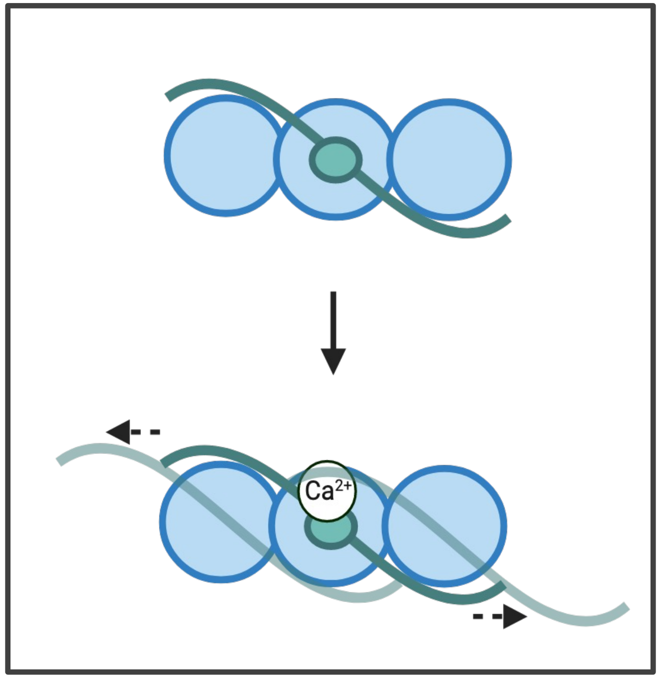
Contraction cycle

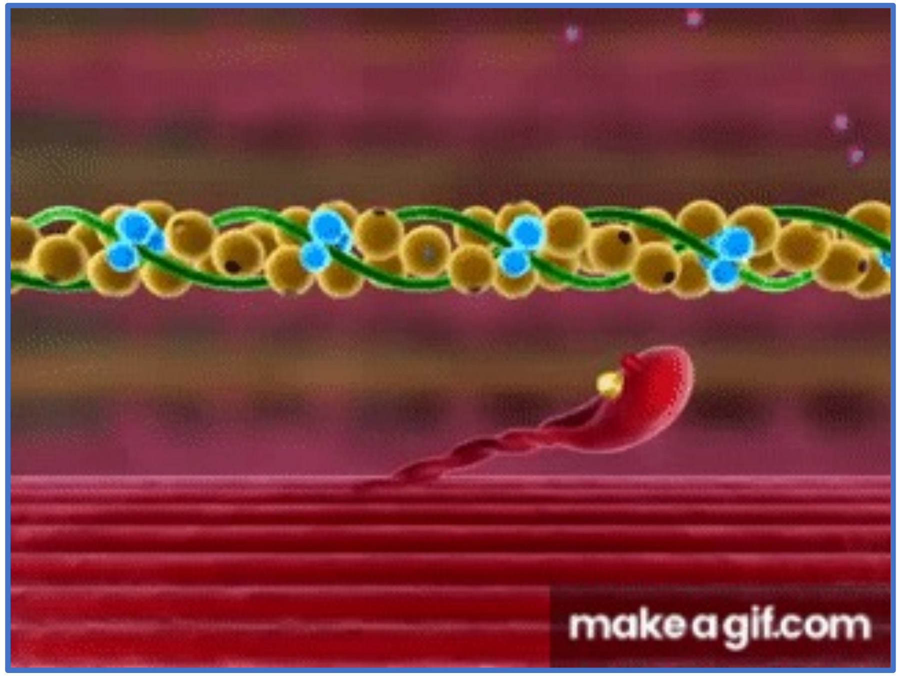
oxidation affects many steps in contraction cycle

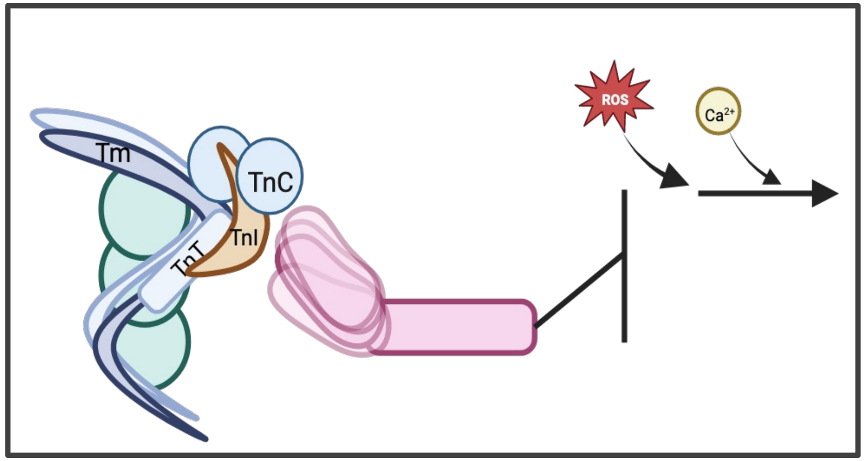


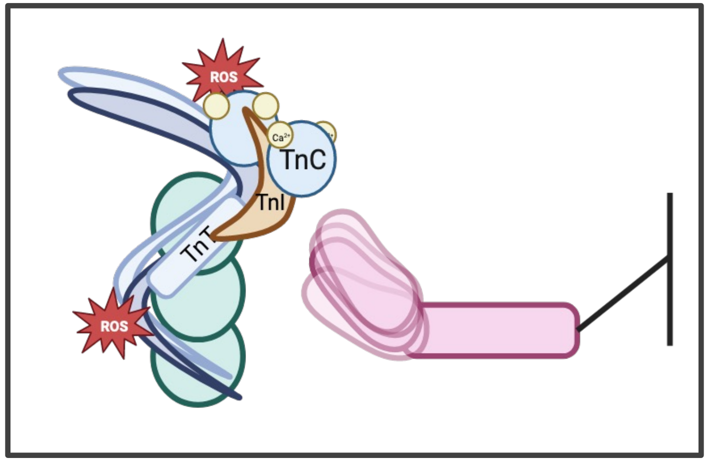
How does oxidation affect the A.M interaction?

Oxidation also affects regulated A.M interactions

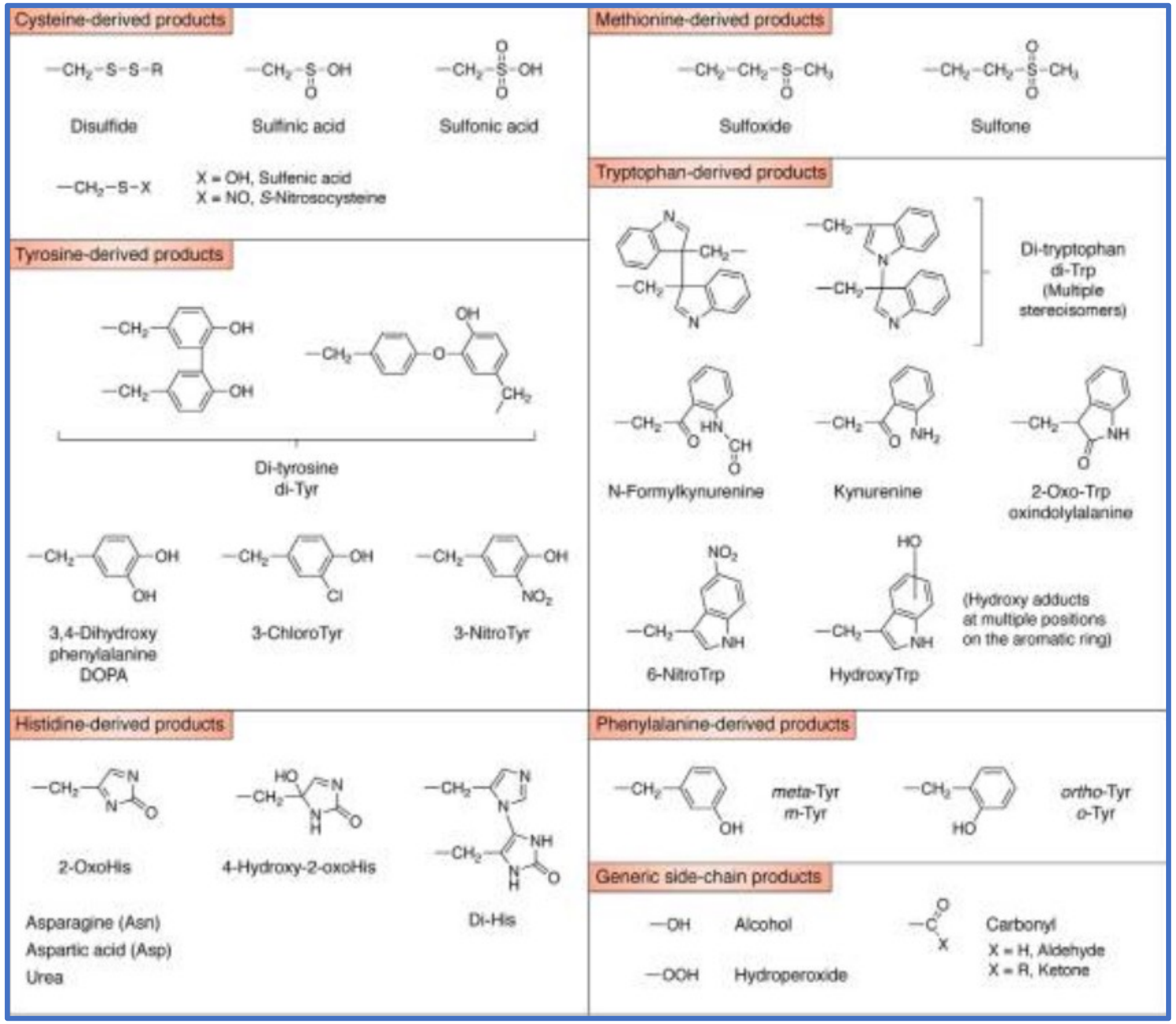






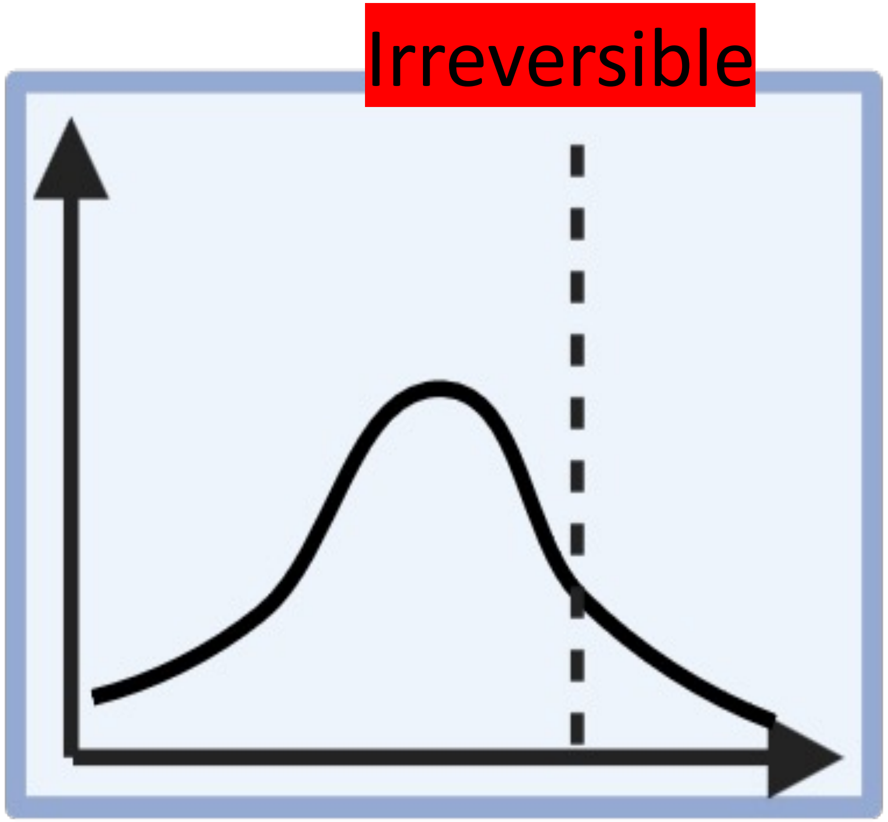


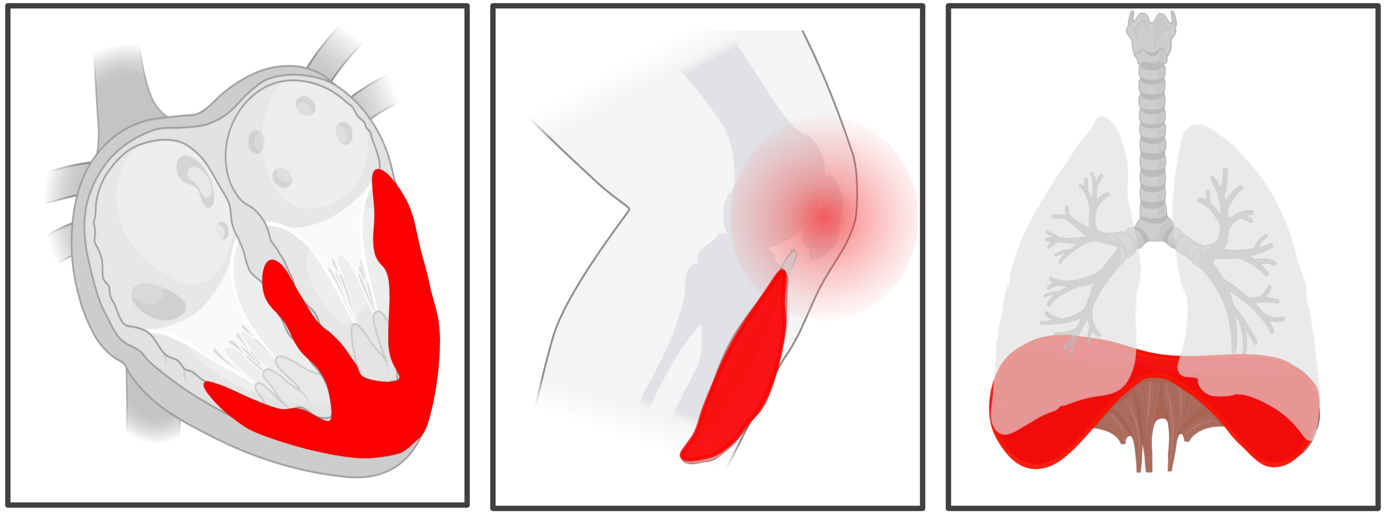
Oxidation is complicated by variable dynamics

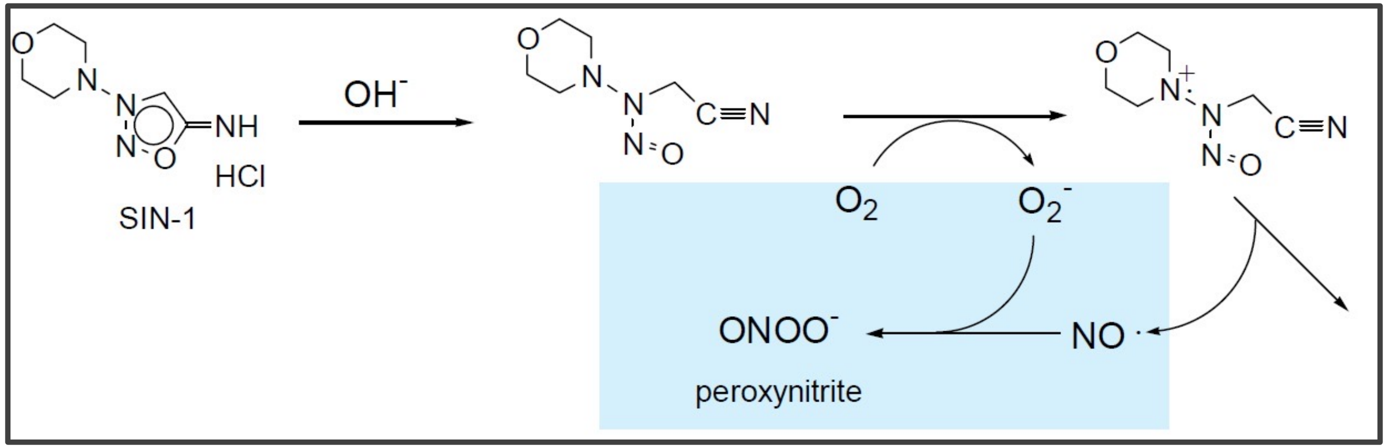


Hawkins & Davies, 2019

Redox balance & Disease







Central question: How does oxidation impact muscle protein interactions and functionality across different biophysical scales, and what is the consistency of these oxidative effects from the molecular to the macroscopic level?

Study questions

Study I:

- How does oxidation influence actin-myosin interactions?
- What are the implications for muscle contraction?

Study II:

- How does oxidation alter regulated actin-myosin interactions?
- How can we detect oxidation on contractile proteins?

Study III:

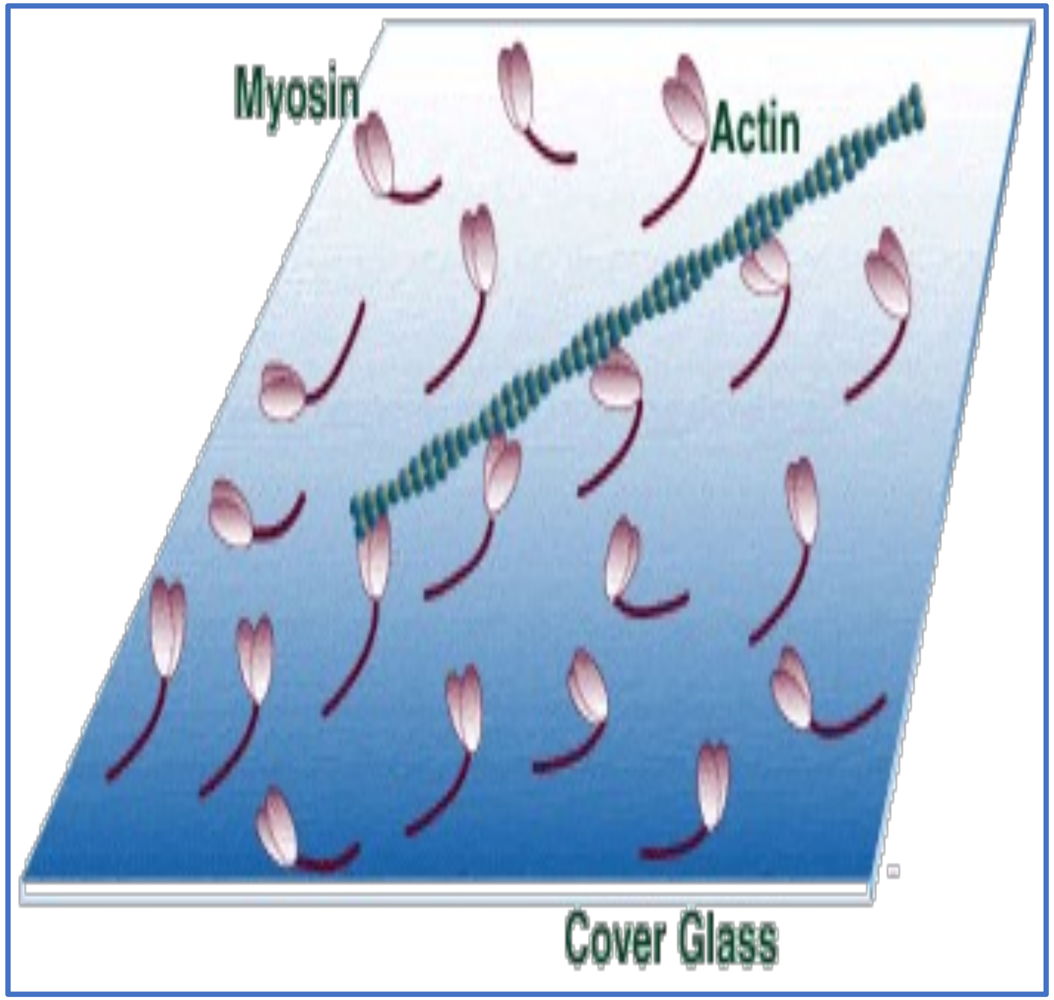
- Does oxidation alter the A.M crossbridge directly?
- How do the observed effects compare to computational predictions?

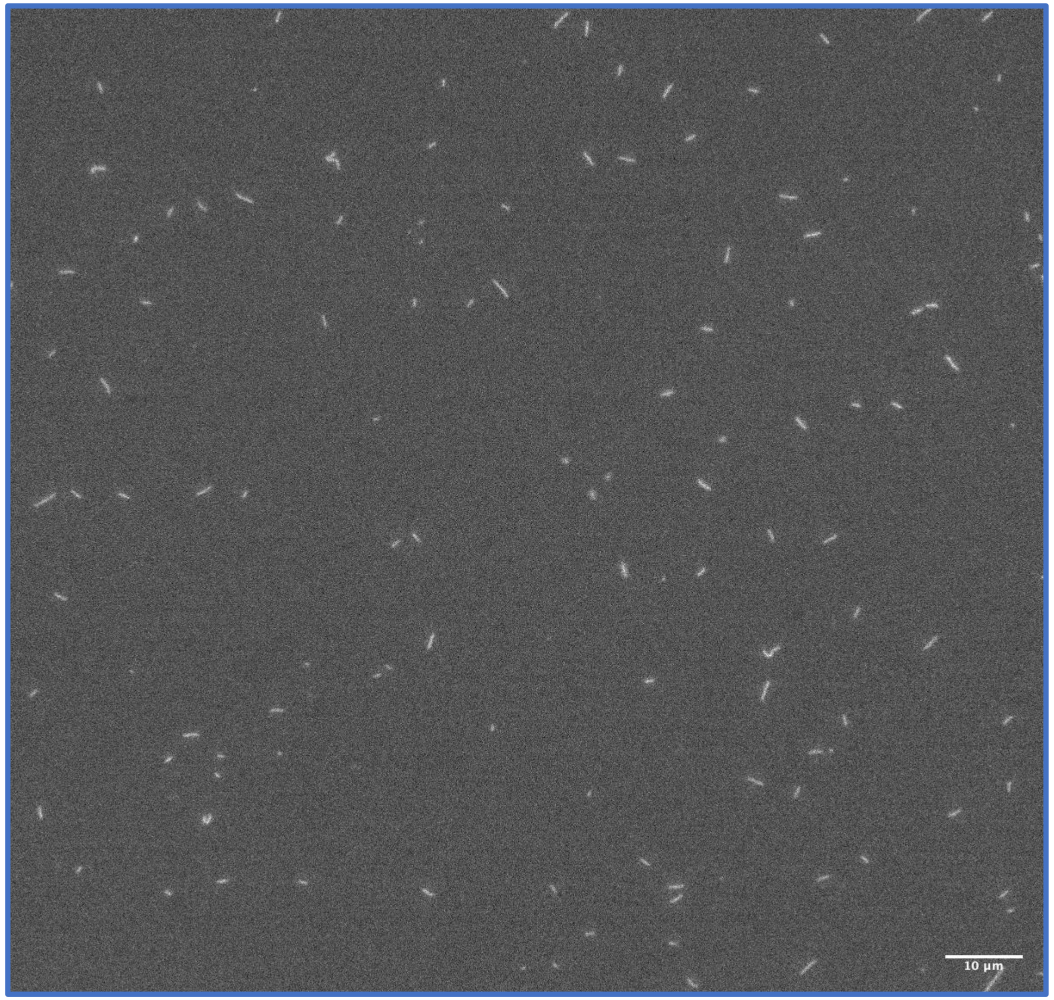
Study I: Oxidation alters actin-myosin interaction

Study I:
- How does oxidation influence actin-myosin interactions?
- What are the implications for muscle contraction?

Hypothesis: If A.M are oxidized, then the A.M force and velocity will be diminished.

In vitro motility assay (IVMA)



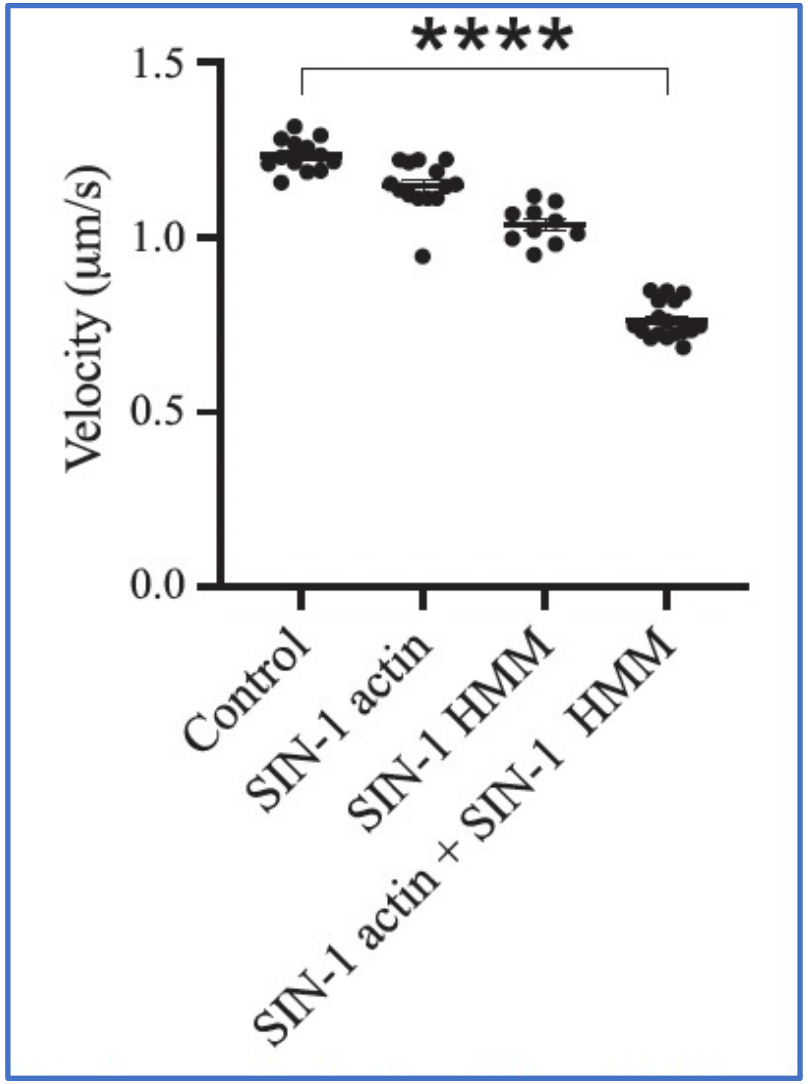


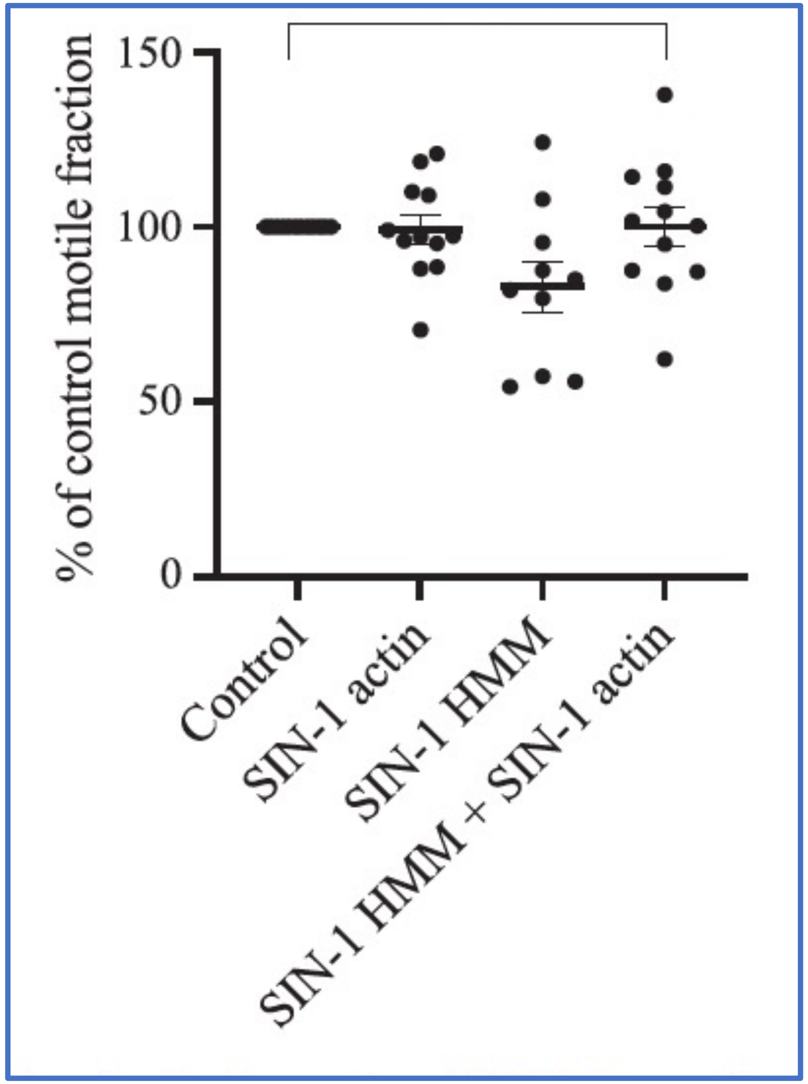
Oxidation reduces actin sliding velocity

Legend

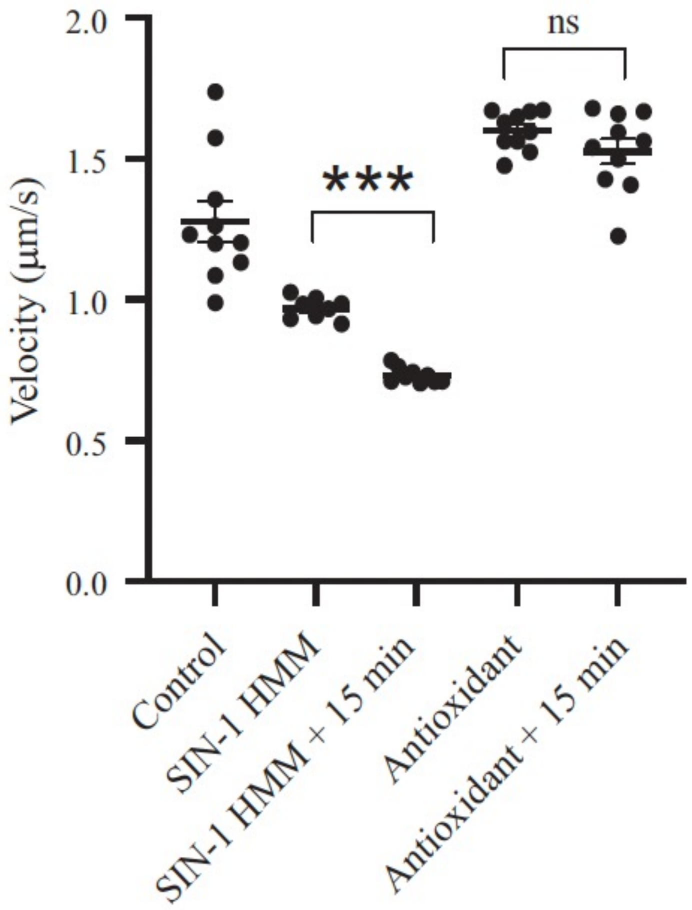
HMM: functional region of myosin

SIN-1: oxidized condition

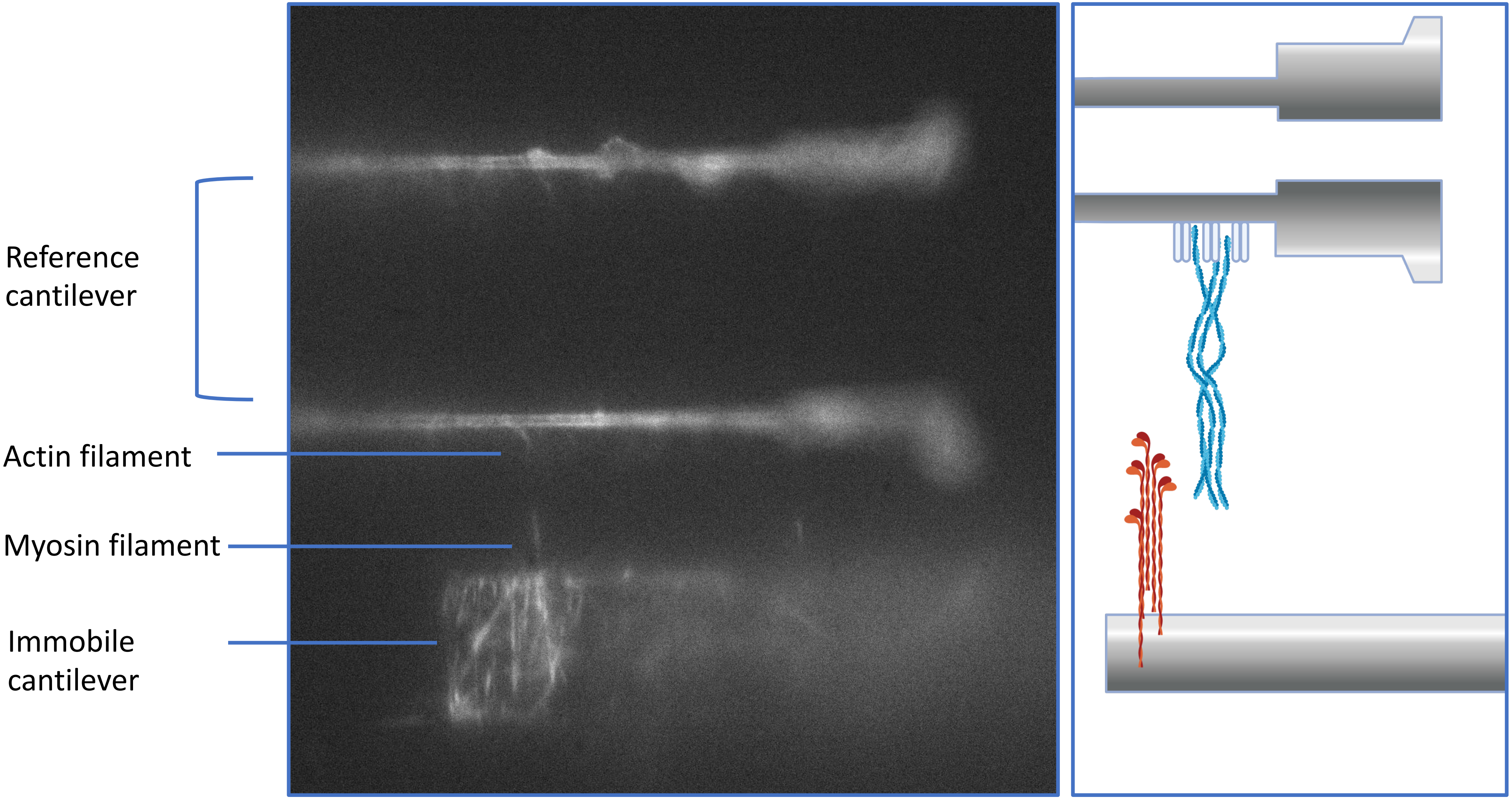




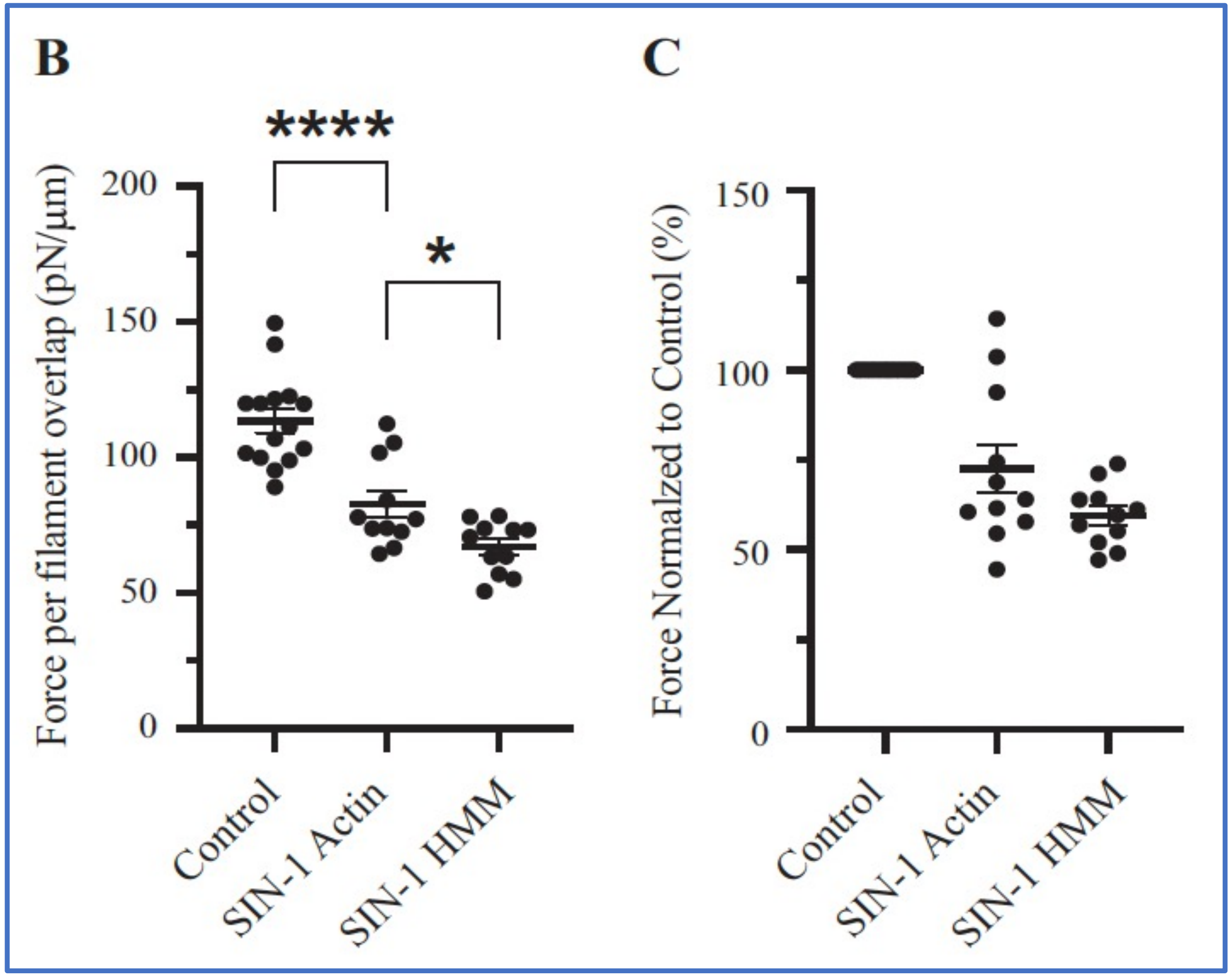
Antioxidants prevent velocity decrease



Filament Force Measurement System (FFMS)



Force is decreased when actin or myosin are oxidized



Study I summary

Experiment	Metric	Result	Interpretation
IVMA	Velocity	Decrease	Crossbridge turnover, attachment time
FFMS	Force	Decrease	Weak to strong transition, conformational change

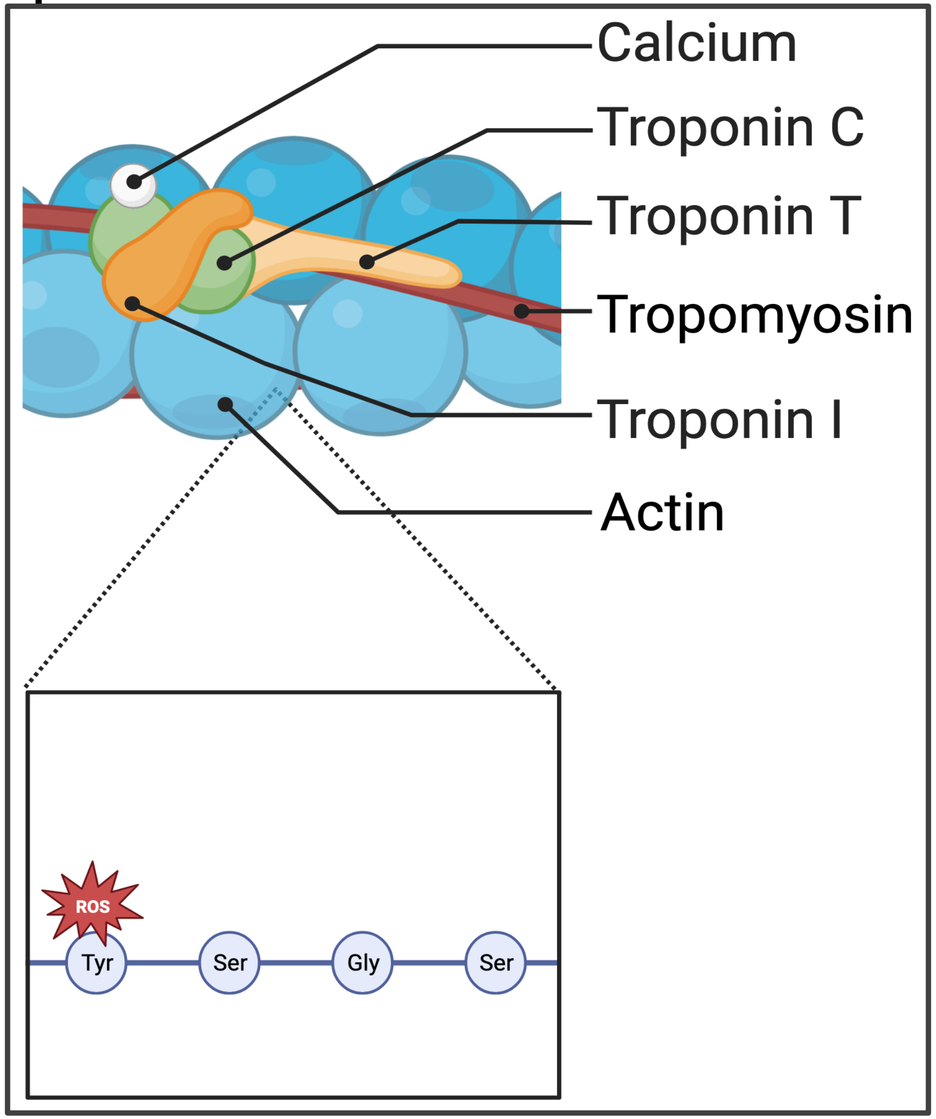
Study II. Oxidation of regulated actin filaments

Study II:

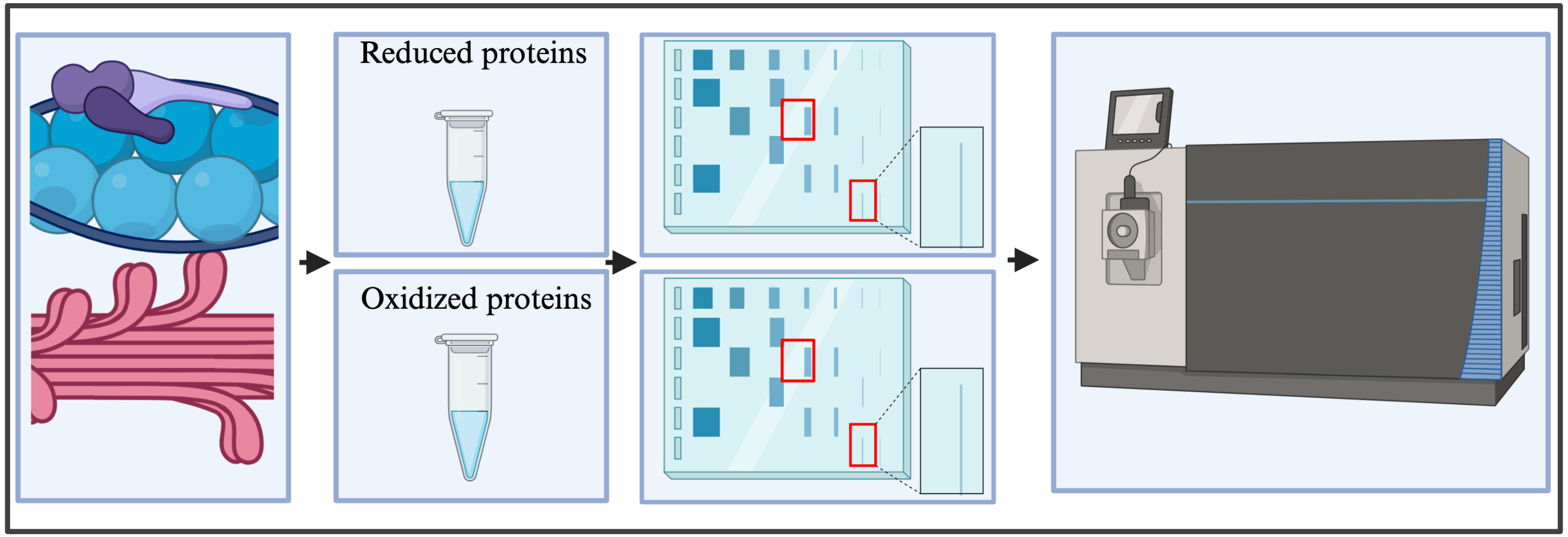
- How does oxidation alter regulated actin-myosin interactions?
- How can we detect oxidation on contractile proteins?

Hypothesis: I. If regulated actin thin filaments are oxidized, then there will be a measurable decrease in their sliding velocity and regulation by the regulatory complex. **II.** If regulated actin thin filaments are oxidized, then mass spectrometry will detect specific oxidative modifications on these proteins.

Did SIN-1 treatment oxidize regulatory proteins?



Mass Spectrometry



Many residues in multiple proteins

	Name	Reduced	Oxidized
Protein	ACTA_BOVIN	5.976E+11	1.395E+12
Peptide	AGFAGDDAPR	7.682E+07	1.346E+11
Peptide	AGFAGDDAPR[15.995]	4.993E+05	5.148E+07
Peptide	AVFPSIVGRPR	1.885E+10	2.894E+10
Peptide	HQGVM[Oxid]VGM[Oxid]GQK	2.397E+09	6.935E+07
Peptide	HQGVMVGMGQK	2.019E+09	2.010E+09
Peptide	HQGVM[Oxid]VGMGQK	4.080E+08	9.438E+08
Peptide	HQGVMVGM[Oxid]GQK	4.080E+08	9.438E+08
Peptide	HQGVM[Oxid]VGM[Oxid]GQKDSYVGDEAQSK	8.005E+06	3.278E+07
Peptide	HQGVM[Oxid]VGMGQK[Hydroxyl]	1.024E+09	5.319E+09
Peptide	HQGVMVGM[Oxid]GQK[Hydroxyl]	1.372E+09	6.999E+09
Peptide	DSYVGDEAQSK	4.713E+10	8.991E+10
Peptide	DSYVGDEAQSKR	1.558E+09	2.637E+09
Peptide	DSYVGDEAQSK[Hydroxyl]R	0.000E+00	1.337E+06
Peptide	RGILTLK	1.135E+08	4.987E+07
Peptide	C[Carboxymethyl]DIDIRK	3.309E+08	2.495E+08
Peptide	C[Carboxymethyl]PETLFQPSFIGM[Oxid]ESAGIHETTYNSIM[Oxid]K	3.169E+08	1.253E+09
Peptide	C[Carboxymethyl]PETLFQPSFIGM[Oxid]ESAGIHETTYNSIMK	3.956E+08	6.868E+08
Peptide	C[Carboxymethyl]PETLFQPSFIGM[Oxid]ESAGIHETTYNSIMK[Hydroxyl]	3.169E+08	1.253E+09
Peptide	C[Carboxymethyl]PETLFQPSFIGMESAGIHETTYNSIM[Oxid]K	3.956E+08	6.868E+08
Peptide	C[Carboxymethyl]PETLFQPSFIGMESAGIHETTYNSIMK	1.555E+08	1.524E+08
Peptide	DLY[28.990]ANNVLSGGTTM[Oxid]YPGIADR	1.591E+08	4.195E+08
Peptide	DLYANNVLSGGTTM[Oxid]Y[28.990]PGIADR	1.591E+08	3.561E+08
Peptide	DLYANNVLSGGTTM[Oxid]YPGIADR	1.050E+10	3.181E+10
Peptide	DLYANNVLSGGTTM[Oxid]YPGIADR[15.995]	6.364E+07	9.068E+08
Peptide	DLYANNVLSGGTTMYPGIADR	7.469E+09	1.029E+10
Peptide	DLTDYLM[Oxid]K	8.716E+09	3.940E+10
Peptide	DLTDYLMK	7.107E+09	1.047E+10
Peptide	DLTDYLM[Oxid]KILTER	3.537E+06	2.058E+07
Peptide	DLTDYLM[Oxid]K[Hydroxyl]	9.534E+07	5.753E+08
Peptide	DLTDYLMK[Hydroxyl]	8.716E+09	3.940E+10
Peptide	EK[Hydroxyl]LC[Carboxymethyl]YVALDFENEM[Oxid]ATAASSSSLEK	1.206E+07	1.191E+07
Peptide	EK[Hydroxyl]LC[DoubleOxid]YVALDFENEM[Oxid]ATAASSSSLEK	0.000E+00	1.219E+08
Peptide	EK[Hydroxyl]LC[DoubleOxid]YVALDFENEMATAASSSSLEK	2.472E+06	1.234E+07
Peptide	EKLC[Carboxymethyl]YVALDFENEM[Oxid]ATAASSSSLEK	1.184E+09	2.682E+09
Peptide	EKLC[Carboxymethyl]YVALDFENEMATAASSSSLEK	3.362E+08	2.631E+08
Peptide	FRC[Carboxymethyl]PETLFQPSFIGM[Oxid]ESAGIHETTYNSIM[Oxid]K	1.755E+08	9.440E+08
Peptide	FRC[Carboxymethyl]PETLFQPSFIGM[Oxid]ESAGIHETTYNSIMK[Hydroxyl]	1.755E+08	9.440E+08
Peptide	FRC[Carboxymethyl]PETLFQPSFIGMESAGIHETTYNSIM[Oxid]K	7.460E+07	3.070E+08
Peptide	SYELPDGQVITIGNER	1.161E+11	2.312E+11
Peptide	SY[28.990]ELPDGQVITIGNER	2.237E+09	1.954E+09
Peptide	SYELPDGQVITIGNER[15.995]	1.180E+06	3.223E+07
Peptide	GYSFVTTAER	2.134E+08	2.630E+08
Peptide	M[Oxid]QKEITALAPSTM[Oxid]K	4.769E+05	1.382E+07
Peptide	MQK[Hydroxyl]EITALAPSTM[Oxid]K	4.769E+05	1.382E+07
Peptide	EITALAPSTMK	1.199E+10	1.520E+10
Peptide	EITALAPSTM[Oxid]K	2.323E+08	2.052E+08
Peptide	EITALAPSTMK[Hydroxyl]	1.681E+10	6.340E+10
Peptide	EITALAPSTMKIK	2.587E+06	5.264E+06
Peptide	EITALAPSTM[Oxid]K[Hydroxyl]	2.175E+08	2.197E+09
Peptide	ILTERGYSFVTTAER	1.181E+07	1.498E+07
Peptide	IIAPPER	2.744E+10	4.484E+10
Peptide	IIAPPERK	1.739E+08	4.784E+08
Peptide	IWHHSFYNELR	5.589E+07	1.191E+08
Peptide	K[Hydroxyl]DLYANNVLSGGTTM[Oxid]YPGIADR	4.664E+07	3.156E+08
Peptide	K[Hydroxyl]DLYANNVLSGGTTMYPGIADR	4.869E+09	1.810E+10
Peptide	KDLY[28.990]ANNVLSGGTTM[Oxid]YPGIADR	9.221E+07	3.697E+08
Peptide	KDLYANNVLSGGTTM[Oxid]Y[28.990]PGIADR	8.912E+07	4.876E+07
Peptide	KDLYANNVLSGGTTM[Oxid]YPGIADR	1.858E+08	1.921E+10
Peptide	KDLYANNVLSGGTTM[Oxid]YPGIADR[15.995]	4.711E+07	3.156E+08
Peptide	KDLYANNVLSGGTTMYPGIADR	4.533E+09	5.905E+09
Peptide	LC[Carboxymethyl]YVALDFENEM[Oxid]ATAASSSSLEK	1.610E+09	1.514E+08
Peptide	LC[Carboxymethyl]YVALDFENEMATAASSSSLEK	3.353E+08	3.318E+08
Peptide	QEYDEAGPSIVHR	4.059E+10	9.298E+10
Peptide	QEYDEAGPSIVHRK	2.193E+06	0.000E+00
Peptide	TTGIVLDSGDGVTHNVPIYEGY[28.990]ALPHAIM[Oxid]R	8.182E+08	1.986E+09
Peptide	TTGIVLDSGDGVTHNVPIYEGYALPHAIM[Oxid]R	1.382E+10	6.933E+10
Peptide	TTGIVLDSGDGVTHNVPIYEGYALPHAIM[Oxid]R[15.995]	9.503E+07	9.317E+08
Peptide	TTGIVLDSGDGVTHNVPIYEGYALPHAIMR	1.502E+10	2.497E+10
Peptide	VAPEEHPTLLTEAPLNPK	2.176E+11	3.838E+11
Peptide	Y[28.990]PIEHGIITNWDDM[Oxid]EK	1.593E+08	3.920E+08
Peptide	YPIEHGIITNWDDM[Oxid]EK	1.153E+07	8.667E+07
Peptide	YPIEHGIITNWDDMEK	1.485E+07	1.301E+08
Peptide	YPIEHGIITNWDDMEK[Hydroxyl]	9.672E+06	8.198E+07

	Name	Reduced	Oxidized
peptide	TPM1_BOVIN	3.446E+11	9.487E+10
peptide	AADESER	1.488E+08	6.988E+05
peptide	AELSEGK[Hydroxyl]C[DoubleOxid]AELEEELK	1.673E+06	2.966E+07
peptide	AELSEGKC[Carboxymethyl]AELEEELK	0.000E+00	4.048E+06
peptide	AEQAEADK	7.415E+06	6.648E+04
peptide	AEQAEADKK	7.459E+07	2.178E+04
peptide	AEQAEADKKAAEDR	9.815E+04	1.345E+06
peptide	AISEELDHALNDM[Oxid]TSI	6.955E+07	1.532E+09
peptide	AISEELDHALNDMTSI	1.600E+08	1.402E+07
peptide	AQK[Hydroxyl]DEEKMEIQEIQLKEAK	0.000E+00	7.190E+02
peptide	AQKDEEK	7.179E+06	0.000E+00
peptide	AQKDEEK[Hydroxyl]M[Oxid]EIQEIQLK	1.211E+08	5.535E+07
peptide	AQKDEEKM[Oxid]EIQEIQLK	1.284E+10	5.368E+09
peptide	AQKDEEKM[Oxid]EIQEIQLKEAK	2.353E+07	0.000E+00
peptide	AQKDEEKMEIQEIQLK	8.678E+08	7.412E+07
peptide	ATDAEADVASLNR	2.322E+10	6.795E+09
peptide	ATDAEADVASLNRR	1.432E+09	5.677E+08
peptide	ATEDELDK	6.636E+08	4.709E+08
peptide	ATEDELDKYSEALK	4.860E+07	3.185E+07
peptide	ATEDELDKYSEALKDAQEK	2.448E+10	4.683E+09
peptide	C[Carboxymethyl]AELEEELK	9.031E+09	2.249E+09
peptide	CAELEEELK	3.631E+06	1.303E+06
peptide	DAQEKLELAEK	3.294E+05	3.066E+08
peptide	DEEK[Hydroxyl]M[Oxid]EIQEIQLK	2.677E+07	1.375E+07
peptide	DEEK[Hydroxyl]MEIQEIQLK	2.637E+09	1.376E+09
peptide	DEEKM[Oxid]EIQEIQLK	3.522E+09	1.841E+09
peptide	DEEKMEIQEIQLK	5.605E+08	4.552E+07
peptide	EDKYEEEIK	7.326E+09	3.132E+09
peptide	GM[Oxid]KVIESR	0.000E+00	0.000E+00
peptide	HIAEDADR	1.527E+10	3.423E+07
peptide	HIAEDADRK	0.000E+00	1.528E+07
peptide	HIAEDADRKYEEVAR	5.691E+05	1.966E+07
peptide	IQLVEEELDR	4.990E+10	1.264E+10
peptide	IQLVEEELDRAQER	1.924E+08	7.982E+07
peptide	K[Hydroxyl]ATDAEADVASLNR	0.000E+00	3.554E+06
peptide	K[Hydroxyl]LVIIESDLER	0.000E+00	1.714E+04
peptide	KATDAEADVASLNR	2.144E+10	5.560E+09
peptide	KATDAEADVASLNRR	5.877E+08	2.407E+08
peptide	KLVIIESDLER	1.198E+07	7.234E+07
peptide	KLVIIESDLERAEER	3.801E+06	0.000E+00
peptide	KYEEVAR	1.705E+10	5.485E+09
peptide	LATALQKLEEAEK	2.885E+07	4.462E+07
peptide	LATALQKLEEAEKAADESER	1.228E+07	7.213E+06
peptide	LEEAEKAADESER	0.000E+00	1.690E+07
peptide	LELAEKK	3.403E+05	1.835E+07
peptide	LKATEDELDK	0.000E+00	7.532E+07
peptide	LKATEDELDKYSEALK	1.328E+09	5.394E+08
peptide	LKATEDELDKYSEALKDAQEK	3.749E+06	7.131E+08
peptide	LKEAETR	2.913E+04	0.000E+00
peptide	LVIIESDLER	2.214E+10	5.761E+09
peptide	LVIIESDLERAEER	0.000E+00	9.376E+06
peptide	M[Oxid]EIQEIQLK	1.222E+09	1.326E+09
peptide	MEIQEIQLK	4.631E+09	6.320E+08
peptide	MEIQEIQLKEAK	0.000E+00	0.000E+00
peptide	QLEDELVSLQK	3.756E+10	8.338E+09
peptide	QLEDELVSLQKK	0.000E+00	9.848E+06
peptide	R[15.995]IQLVEEELDR	6.707E+05	0.000E+00
peptide	RIQLVEEELDR	1.725E+07	2.079E+07
peptide	SIDDLEDELYAQK	3.942E+10	8.830E+09
peptide	SIDDLEDELYAQK[Hydroxyl]	1.788E+08	2.376E+05
peptide	SIDDLEDELYAQKLK	2.857E+07	0.000E+00
peptide	SIDDLEDELYAQKLKYK	0.000E+00	0.000E+00
peptide	SKQLEDELVSLQK	5.114E+09	1.584E+09
peptide	SKQLEDELVSLQKK	0.000E+00	1.321E+07
peptide	SLEAQAEK	1.729E+10	6.429E+09
peptide	SVTKLEK	6.001E+05	0.000E+00
peptide	TVTNNLK	6.227E+08	2.474E+07
peptide	TVTNNLKSLEAQAEK	2.192E+07	0.000E+00
peptide	VLSDKLK	0.000E+00	6.009E+06
peptide	Y[28.990]SQKEDKYEEEIK	7.393E+07	2.791E+07
peptide	YSEALKDAQEK	0.000E+00	2.825E+08
peptide	YSEALKDAQEKLELAEK	1.159E+08	4.004E+07
peptide	YSQKEDK	6.456E+06	0.000E+00
peptide	YSQKEDKY[28.990]EEEIK	8.450E+06	0.000E+00
peptide	YSQKEDKYEEEIK	2.308E+10	7.370E+09

	Name	Reduced	Oxidized
Protein	TNNI3_BOVIN	3.499E+10	5.630E+10
Peptide	AKETLDLR	8.682E+08	1.301E+09
Peptide	AYATEPHAK	1.224E+09	1.370E+09
Peptide	EDTEKENR	3.196E+06	4.209E+06
Peptide	EVGDWRK	5.163E+05	3.461E+08
Peptide	ISADAM[Oxid]M[Oxid]QALLGAR	7.694E+06	7.750E+07
Peptide	ISADAM[Oxid]M[Oxid]QALLGAR[15.995]	5.020E+08	1.567E+09
Peptide	ISADAM[Oxid]MQALLGAR	1.934E+08	1.993E+07
Peptide	ISADAMM[Oxid]QALLGAR	1.934E+08	1.350E+08
Peptide	ISADAMMQALLGAR	7.194E+06	1.117E+04
Peptide	KEDTEKENR	4.343E+07	4.355E+07
Peptide	KNIDALSGM[Oxid]EGR	3.033E+04	2.869E+08
Peptide	KNIDALSGM[Oxid]EGRK	0.000E+00	1.205E+06
Peptide	KNIDALSGMEGR	9.731E+06	5.036E+06
Peptide	NIDALSGM[Oxid]EGR	7.482E+09	1.084E+10
Peptide	NIDALSGM[Oxid]EGR[15.995]	1.306E+08	1.544E+08
Peptide	NIDALSGM[Oxid]EGRK	0.000E+00	2.271E+07
Peptide	NIDALSGMEGR	4.801E+08	1.798E+08
Peptide	NIDALSGMEGR[15.995]	1.225E+08	1.084E+10
Peptide	NIDALSGMEGR[15.995]K[Hydroxyl]	0.000E+00	8.343E+05
Peptide	NITEIADLNQK	1.893E+10	2.013E+10
Peptide	RSSANYR	8.109E+06	3.974E+07
Peptide	SGGSTAGDTVPAPPPVR	8.894E+06	3.481E+06
Peptide	SGGSTAGDTVPAPPPVRR	7.738E+03	5.924E+08
Peptide	TLM[Oxid]LQIAK	3.723E+09	6.813E+09
Peptide	TLM[Oxid]LQIAKQELER	0.000E+00	3.526E+06
Peptide	TLMLQIAK	1.055E+09	3.786E+08
Peptide	VDEERYDVEAK	8.563E+03	2.150E+08
Peptide	VDKVDEER	0.000E+00	3.803E+08
Peptide	VDKVDEERYDVEAK	0.000E+00	5.334E+08
Peptide	VRISADAM[Oxid]M[Oxid]QALLGAR	0.000E+00	4.411E+06
Peptide	VTKNITEIADLNQK	2.986E+06	1.894E+07

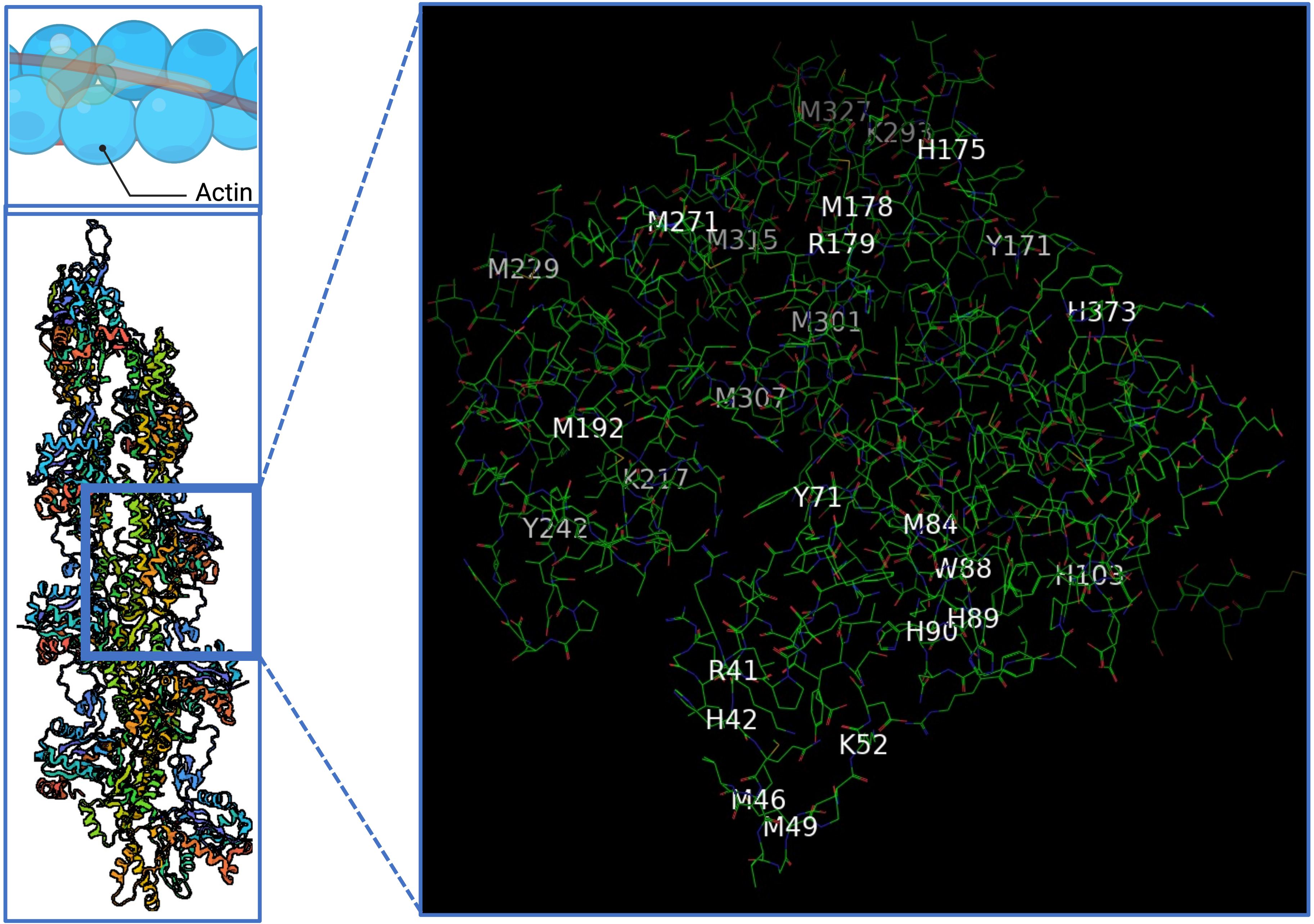
Mass spec

	Name	Reduced	Oxidized
Protein	TNNC1_BOVIN	1.120E+10	1.475E+10
Peptide	AAVEQLTEEQK	1.707E+09	2.664E+09
Peptide	AAVEQLTEEQK[Hydroxyl]NEFK	4.319E+05	5.598E+06
Peptide	AAVEQLTEEQKNEFK	7.013E+09	1.093E+10
Peptide	GKSEEELSDLFR	5.750E+08	2.489E+08

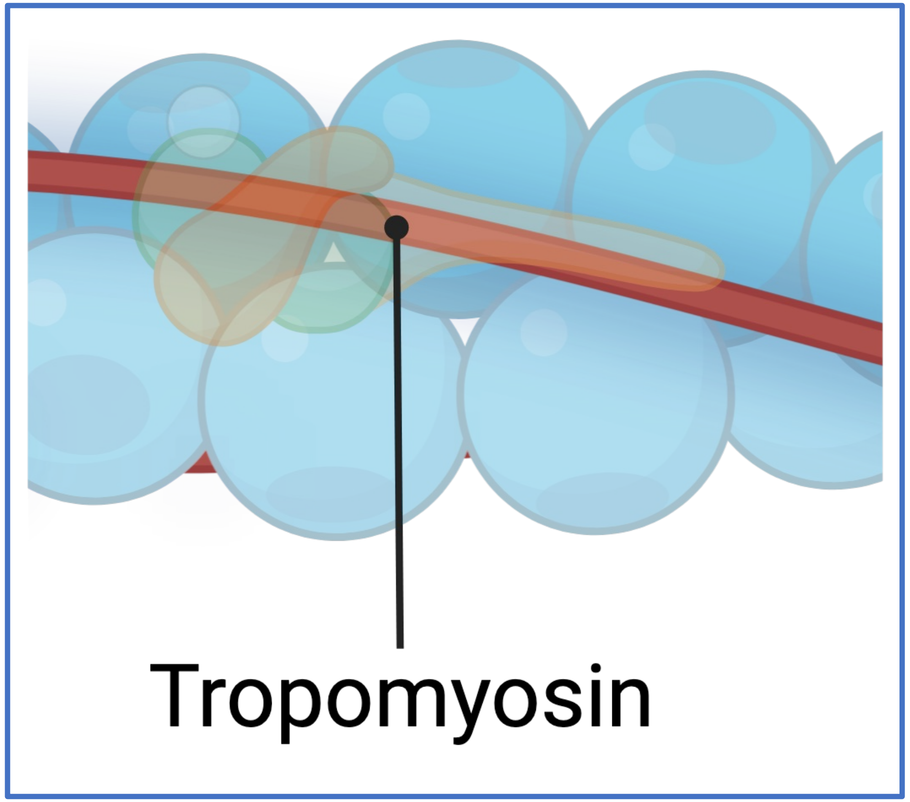
Peptide sequence

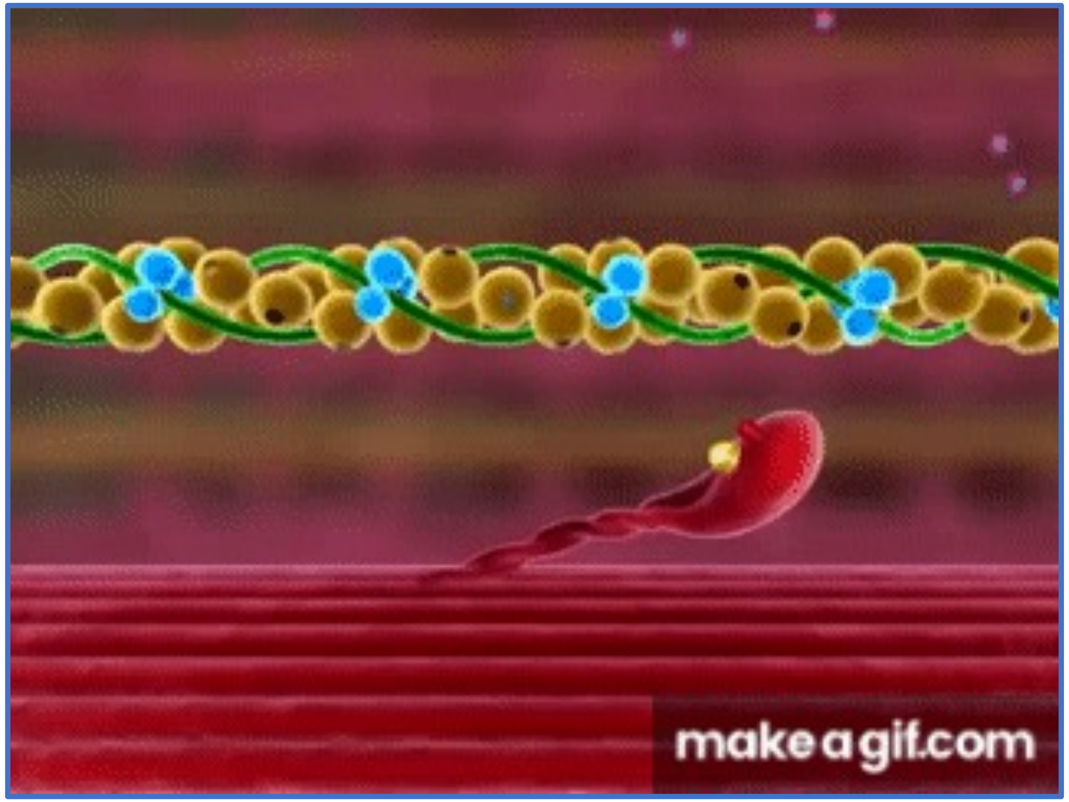
Spectral count in reduced or oxidized samples

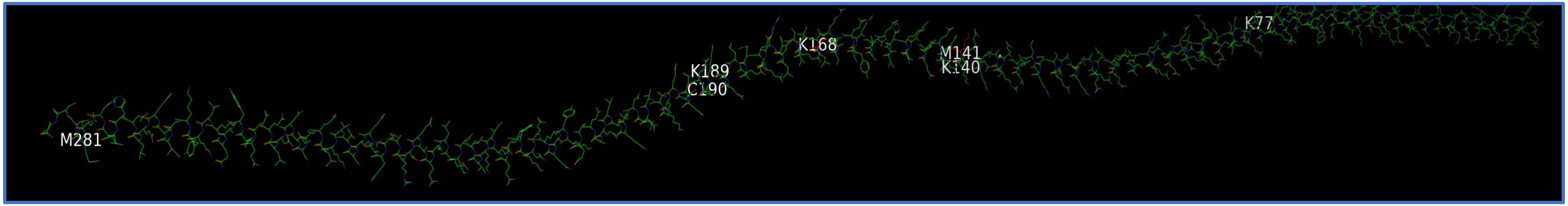
Globular Actin (g-actin)



Tropomyosin (TPM1)



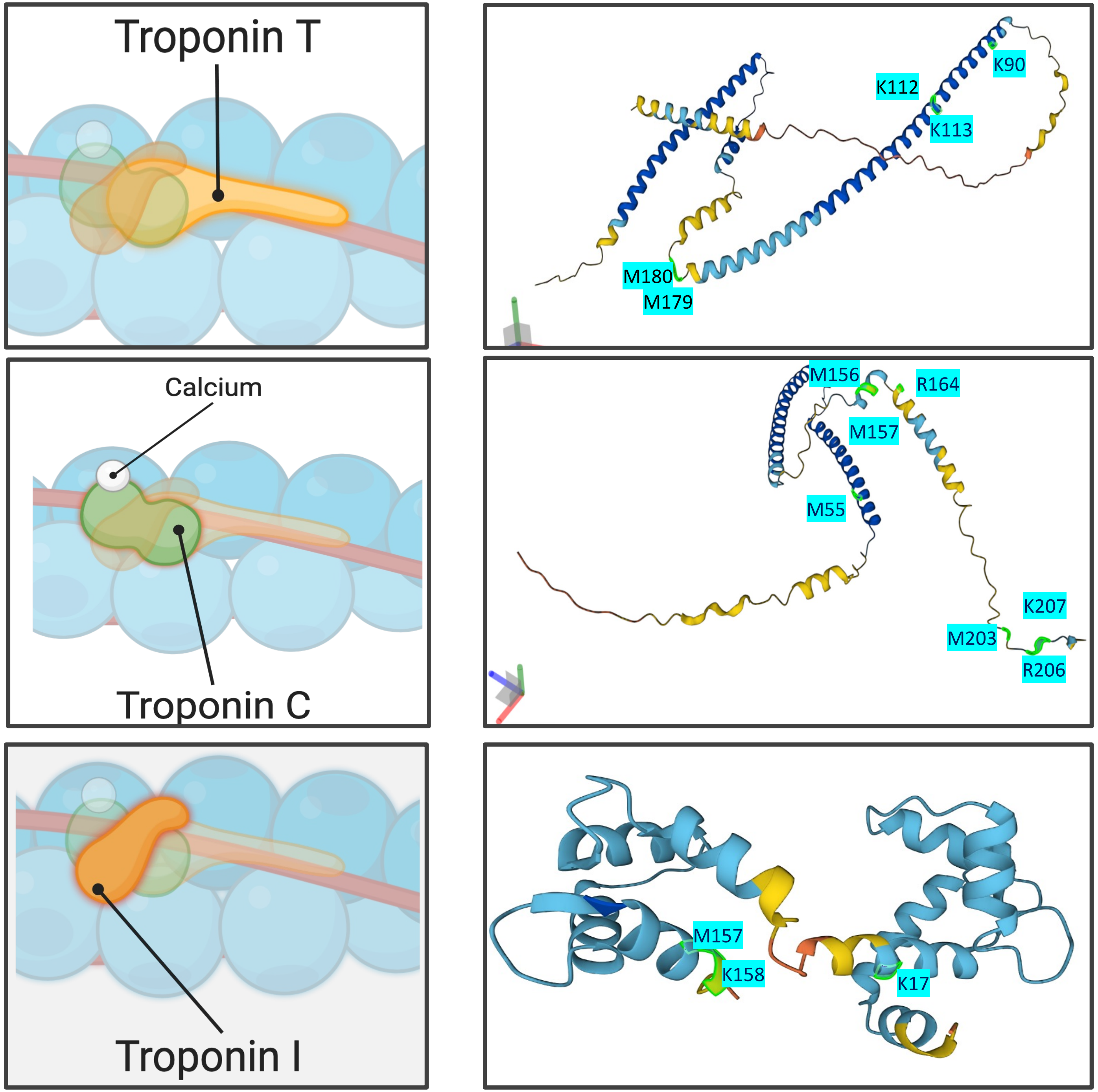




TnT

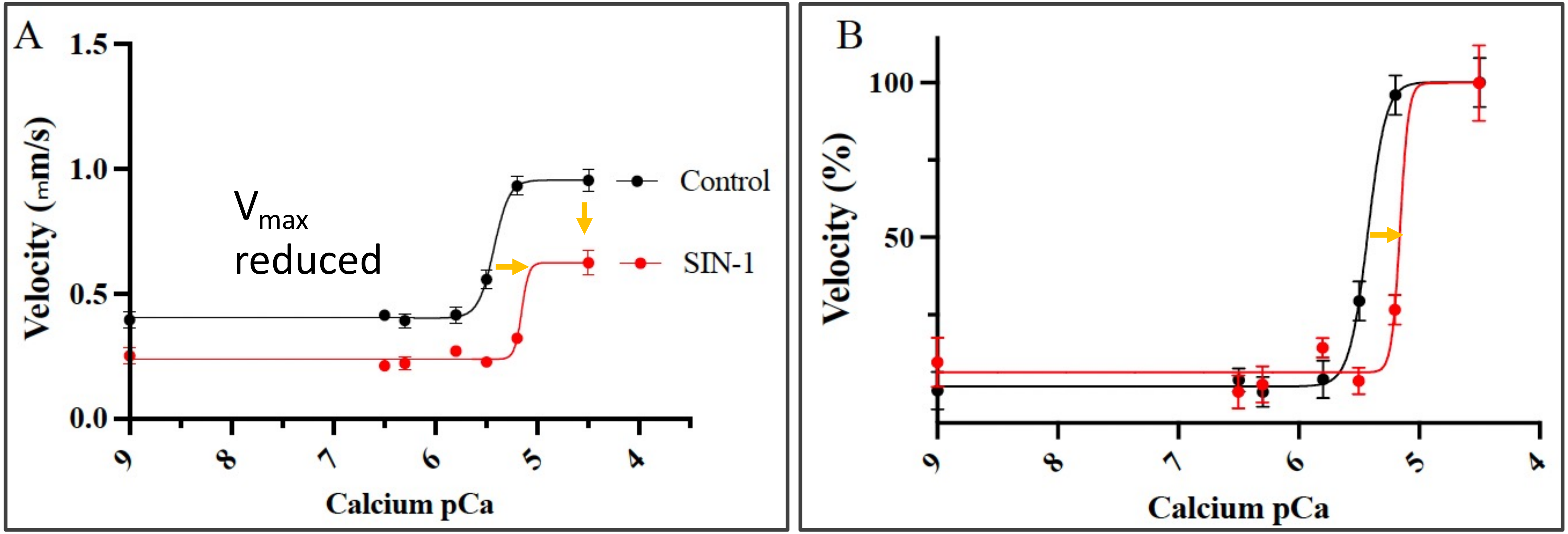
tnc

tnI



Troponin T
K90
K112
K113
M180
M179
Calcium
Troponin C
M156
R164
M157
M55
K207
M203
R206
Troponin I
M157
K158
K17

Decreased velocity, ca sensitivity



Ca^{2+} sensitivity reduced (pCa where V_{max} is half)
Indicates that more calcium is required to fully activate the filament

Study II summary

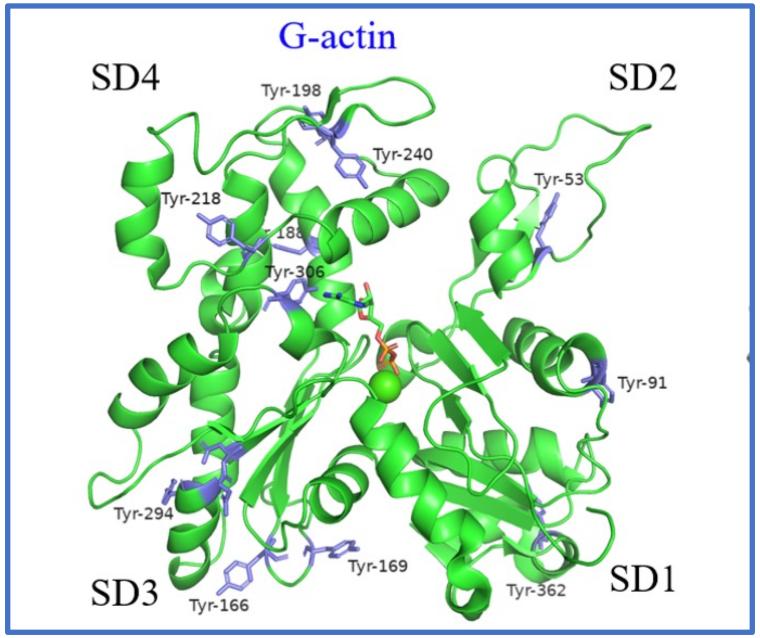
Experiment	Metric	Result	Interpretation
IVMA	Ca^{2+} sensitivity	Decrease	Sensitivity to calcium for filament activation, calcium sensing, other factors?
MS	Oxidation location	Increase	Multiple locations susceptible to oxidation, non-specific. High background oxidation, other residues may be somewhat resistant to oxidation?

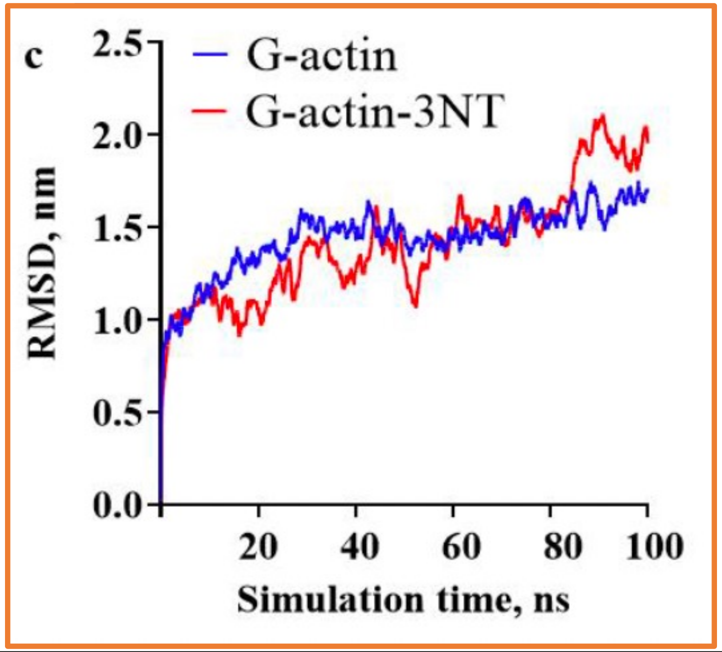
Study III. Oxidation-induced structural changes in actin and myosin evaluated by computational simulation and high-speed AFM

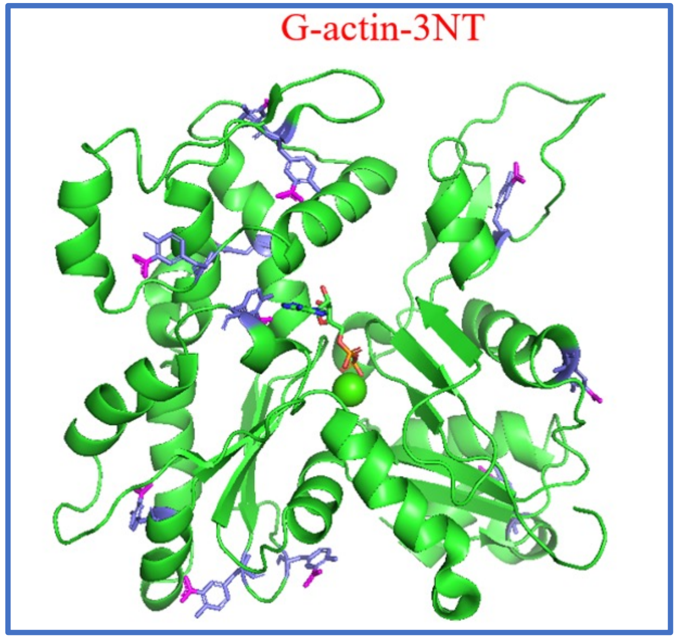
Study III:
- Does oxidation alter the A.M crossbridge directly?
- How do the observed effects compare to computational predictions?

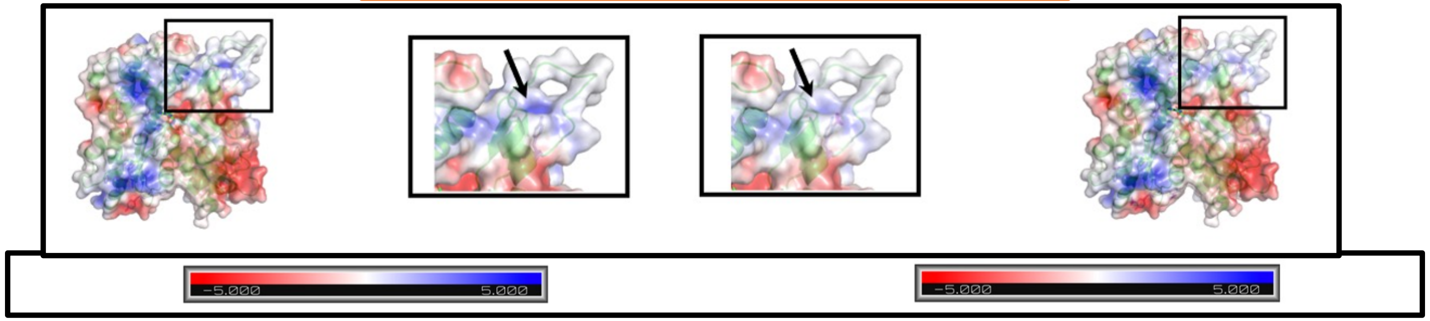
Hypothesis: If actin and myosin are oxidized, then computational simulations and HS-AFM will reveal structural alterations, such as changes in protein conformation and decreased stability, compared to those in non-oxidized conditions.

Oxidation alters electrostatics in specific locations of G-actin

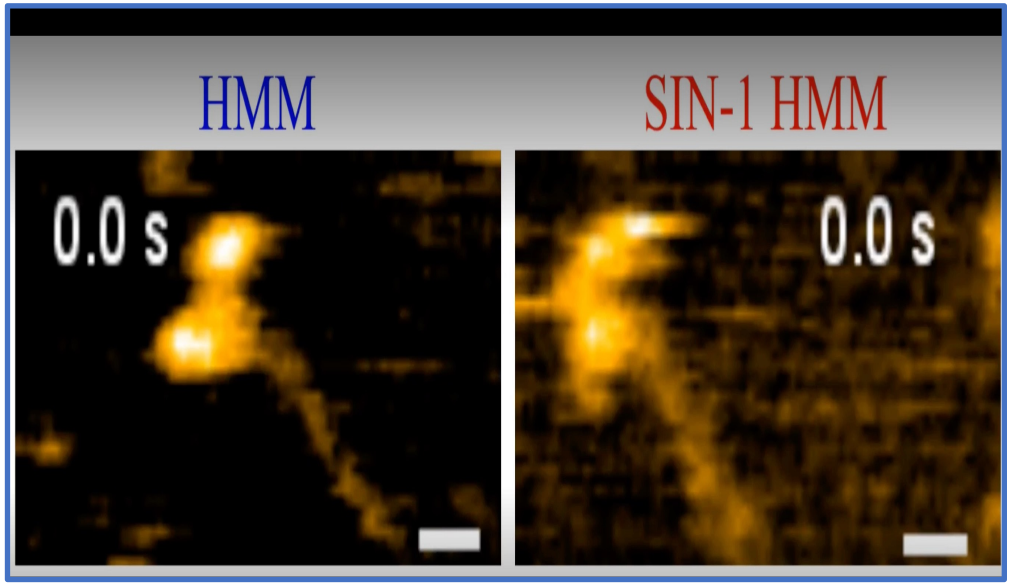


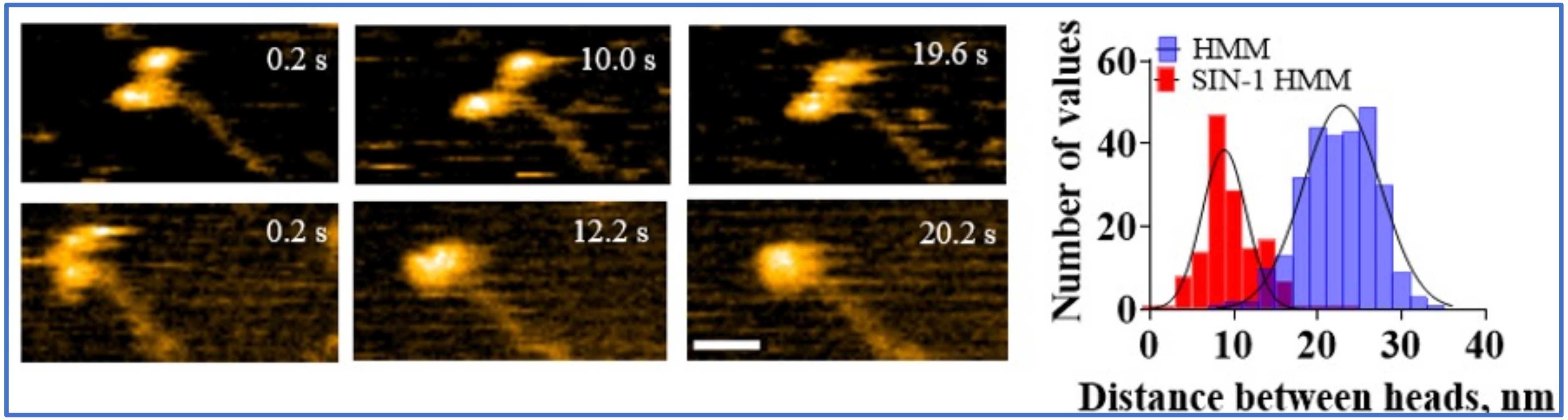




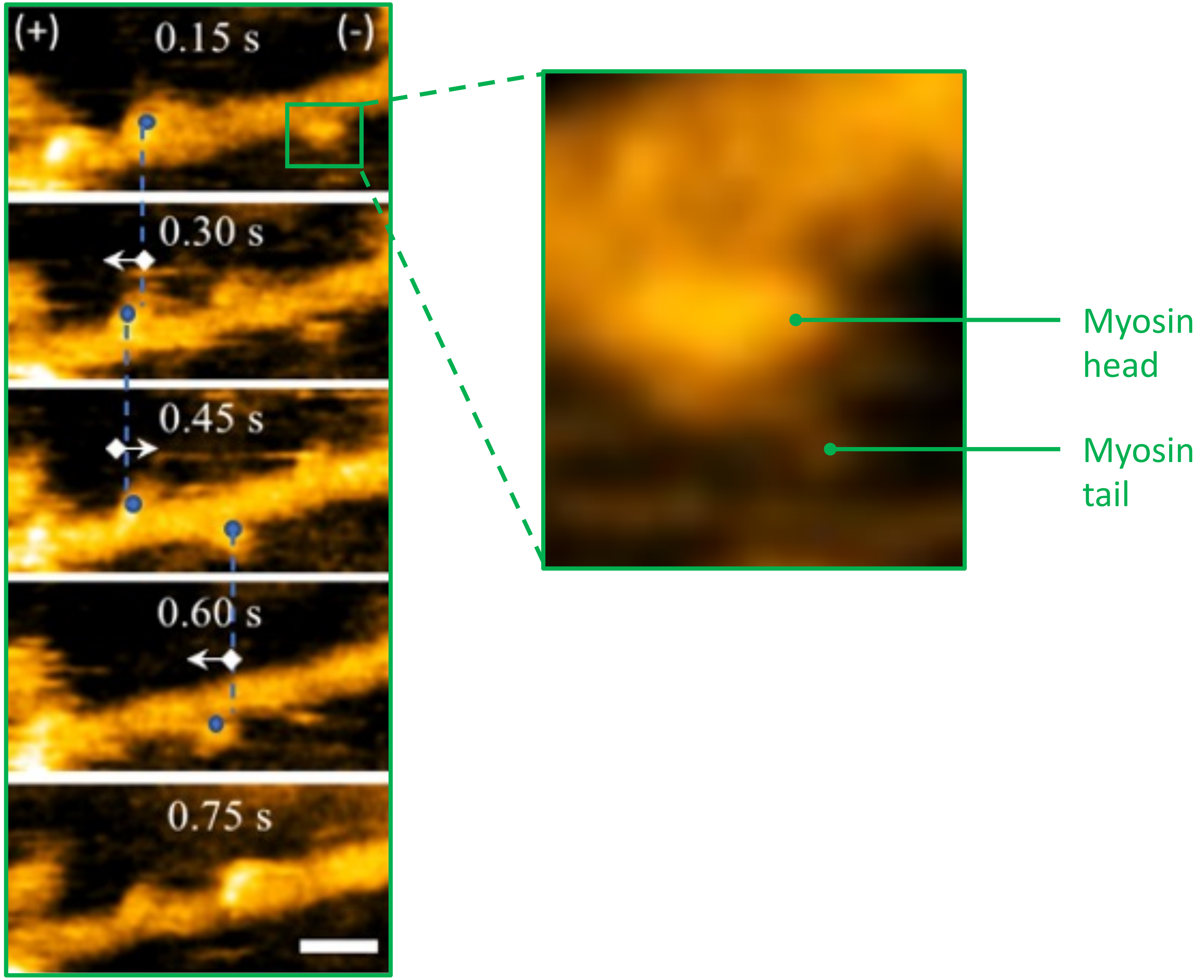


Oxidation of HMM increases hmm self-affinity

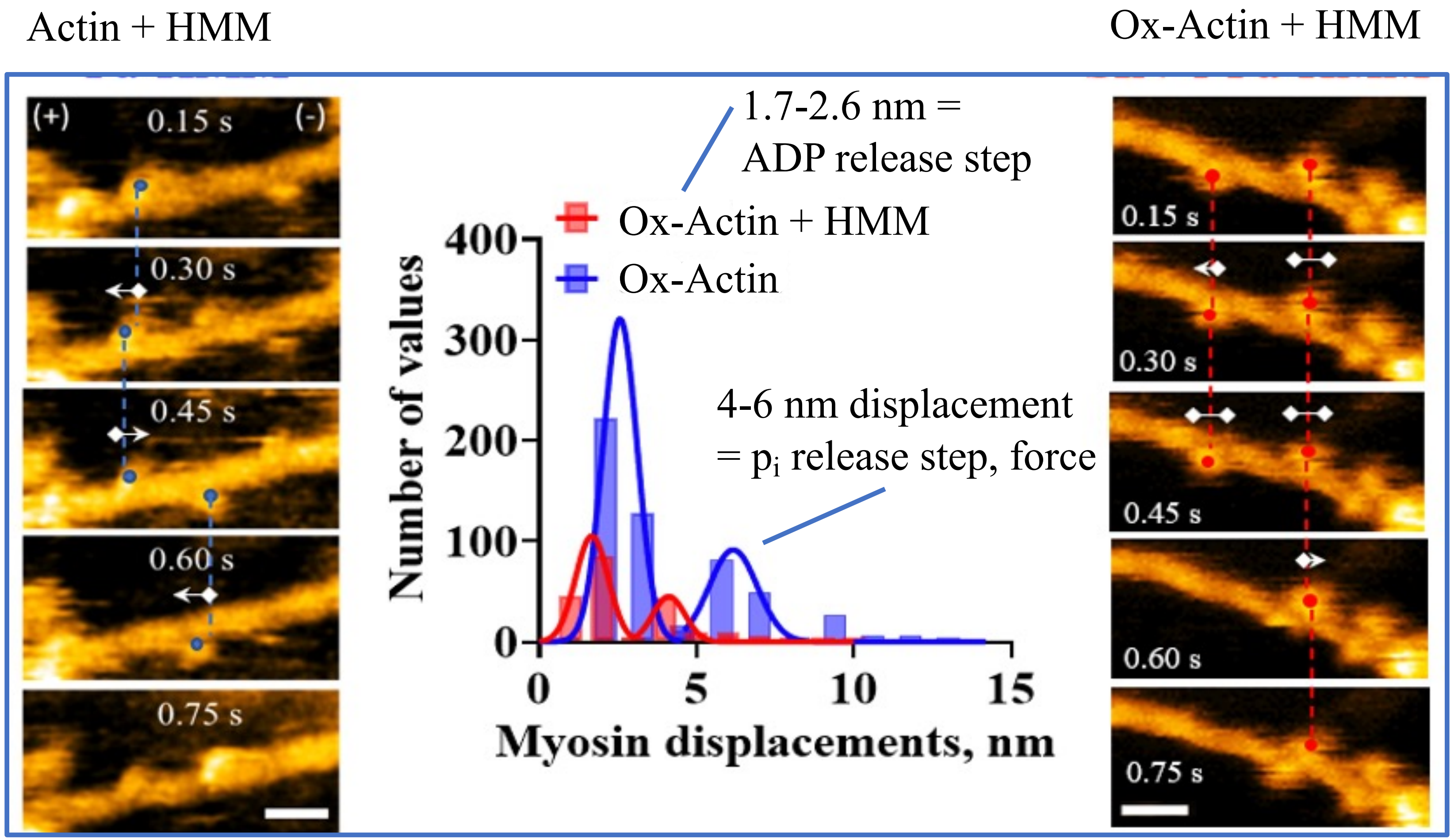




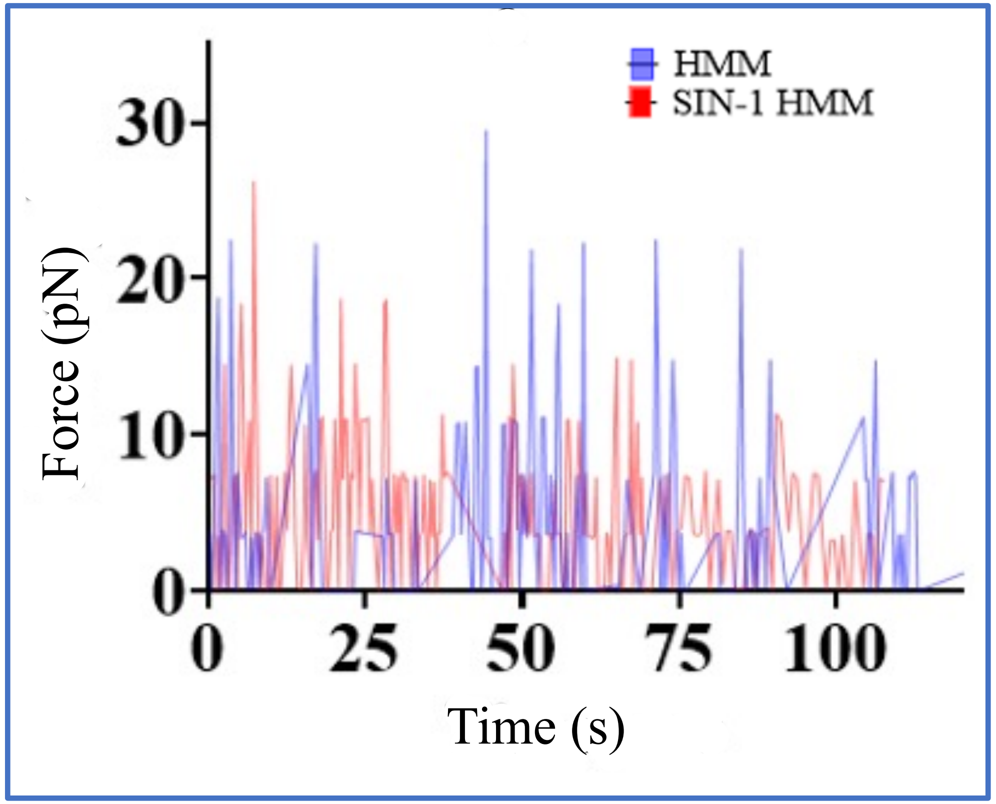
Oxidized actin-myosin interactions

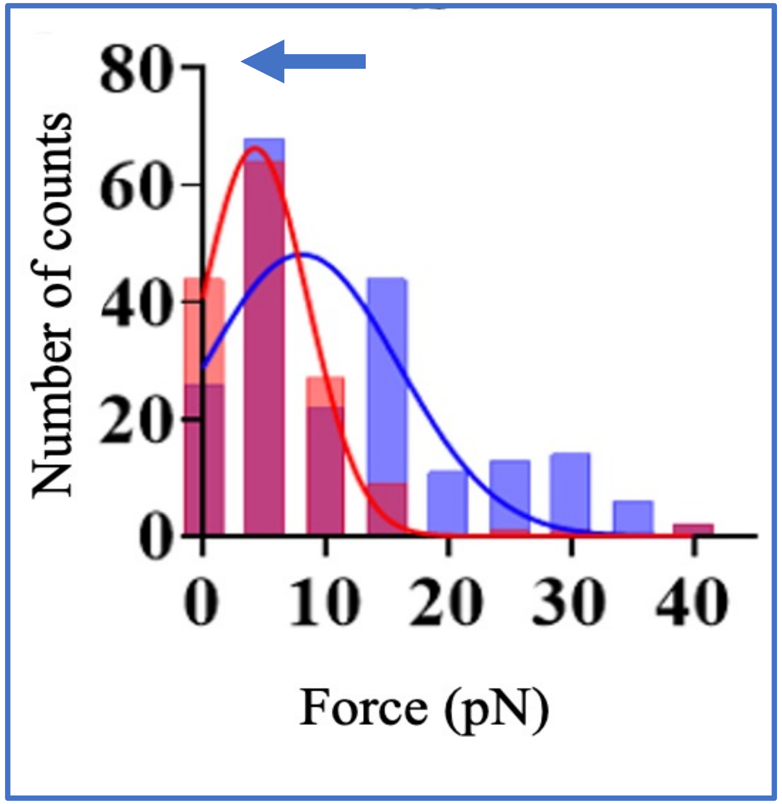


Reduced myosin displacement = AM binding, attachment /detachment

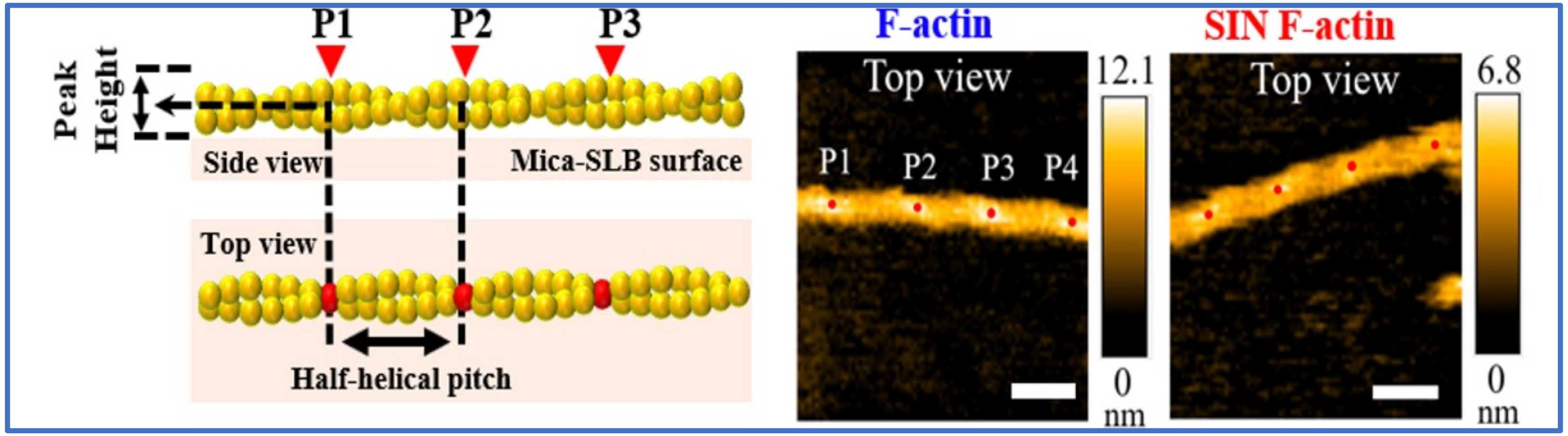


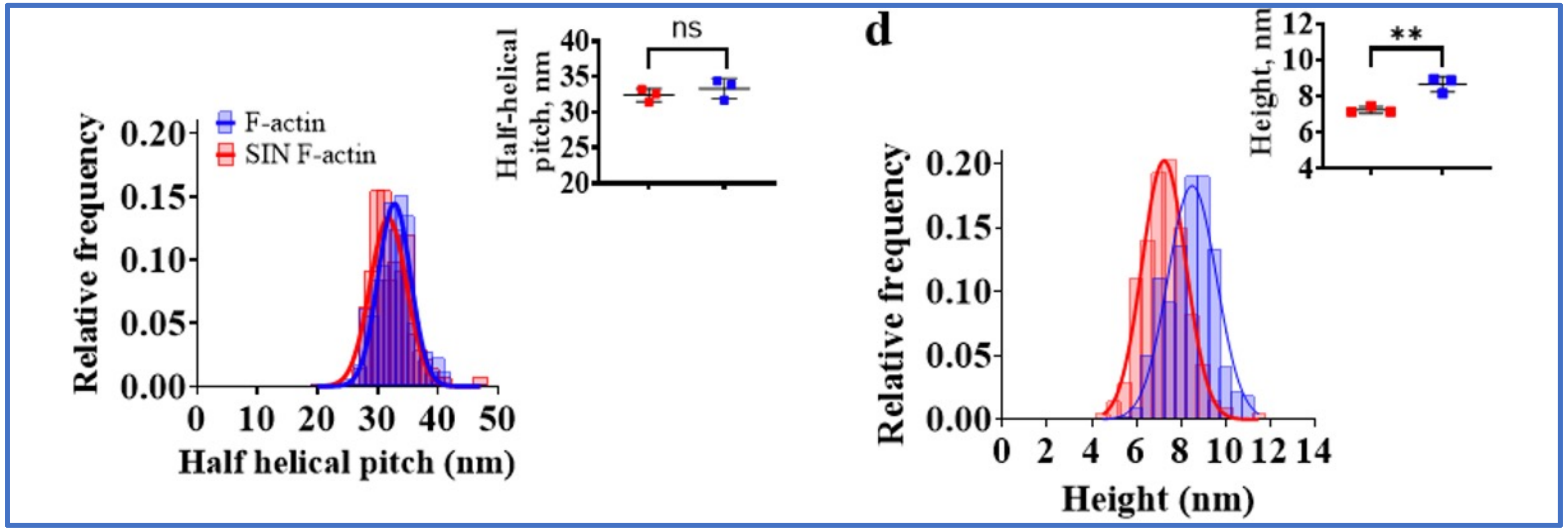
Oxidized actin implies Reduced AM force





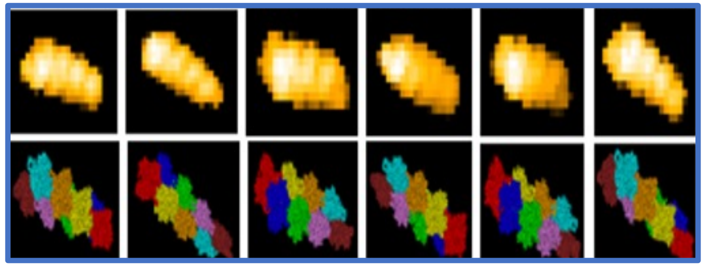
Actin morphology is altered



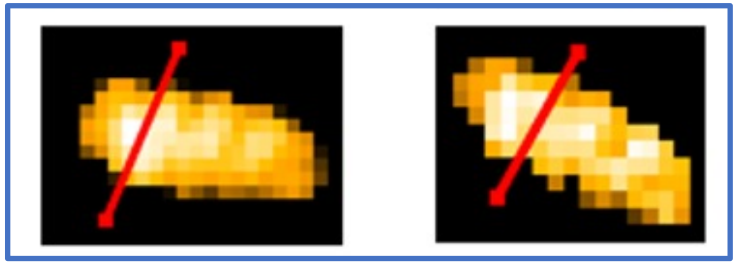


Peak height is reduced in experimental and SIM data

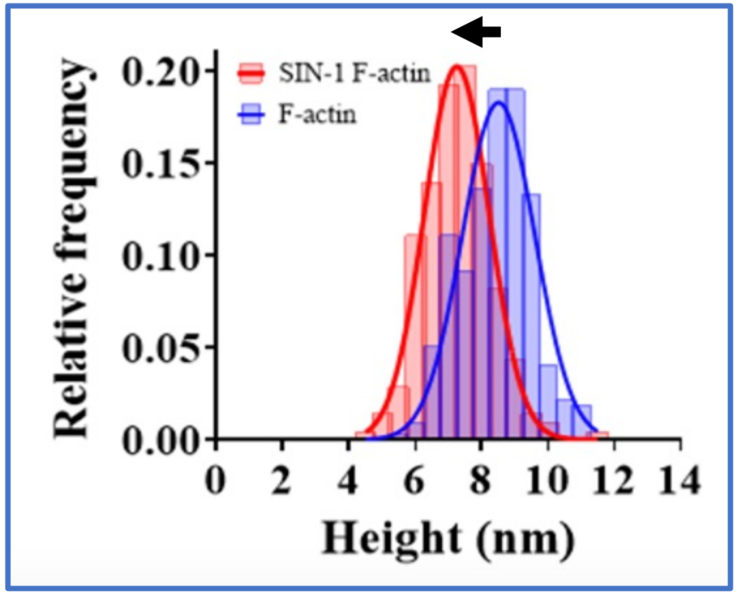
Simulated multiple orientations



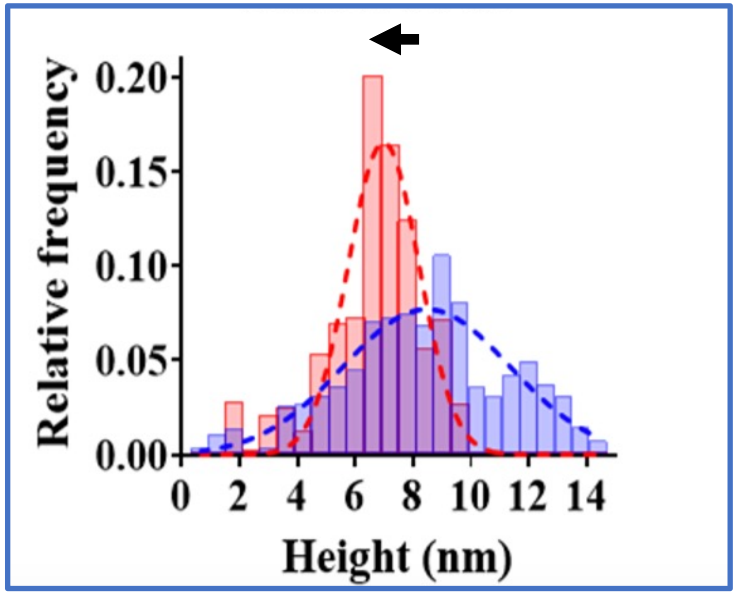
Measured multiple angles



Experimental

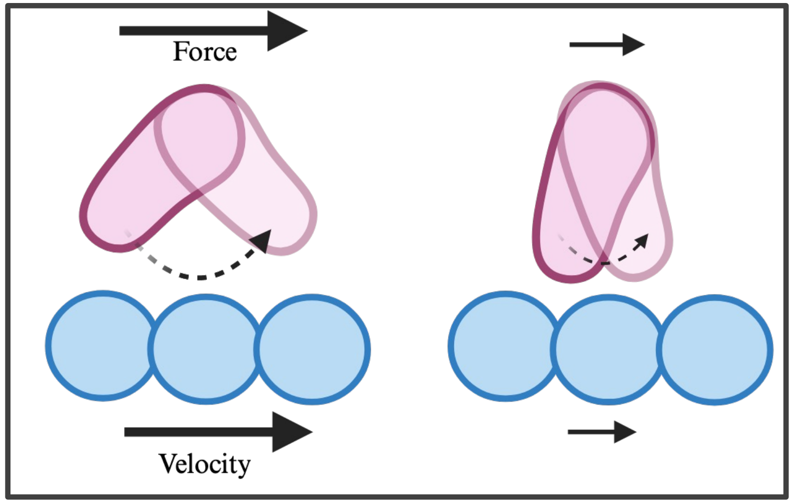


Simulated

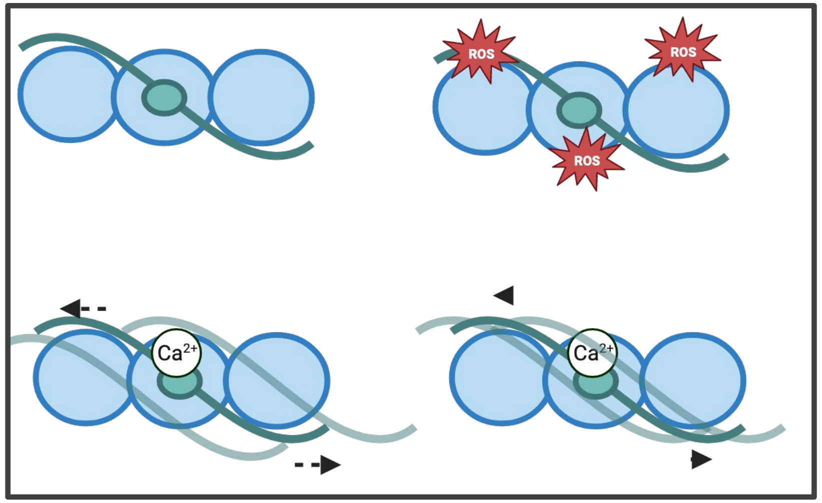


summary

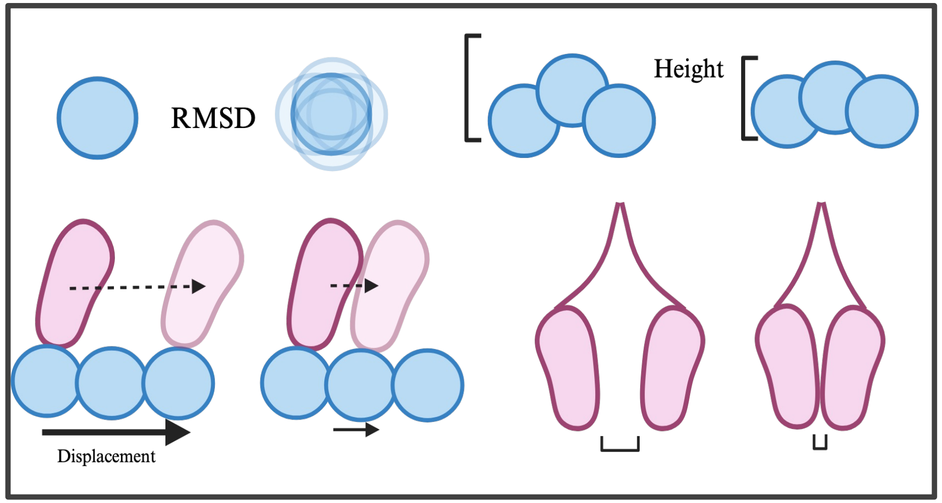
Study I



Study II



Study III



- Crossbridge function is altered
- Reduced filament force
- Reduced filament velocity

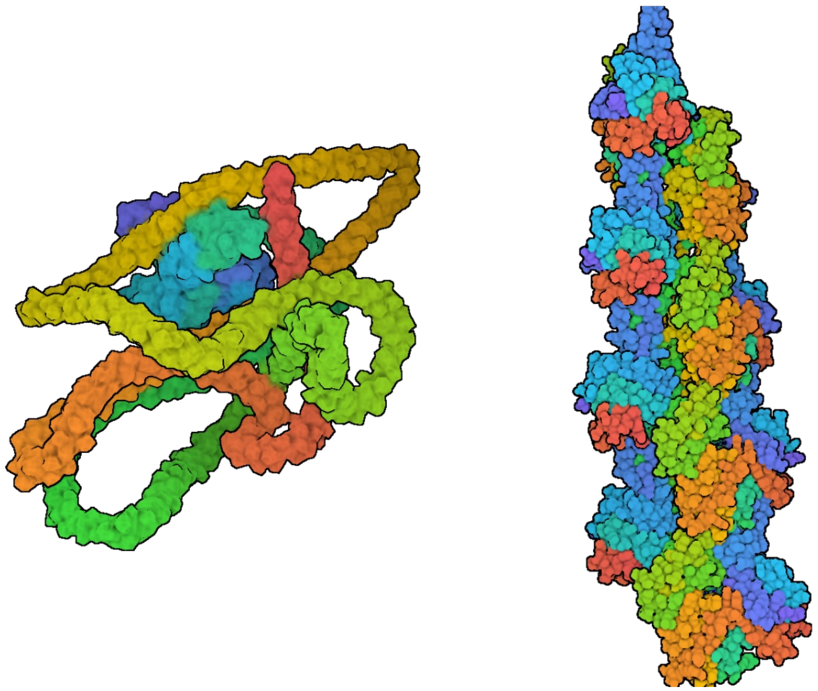
- A.M regulation is altered
- Reduced calcium sensitivity
- Oxidation located on all TF proteins

- Structure and function of actin & myosin are altered individually and in combination
- Increased RMSD, reduced displacement
- Myosin binding

Findings

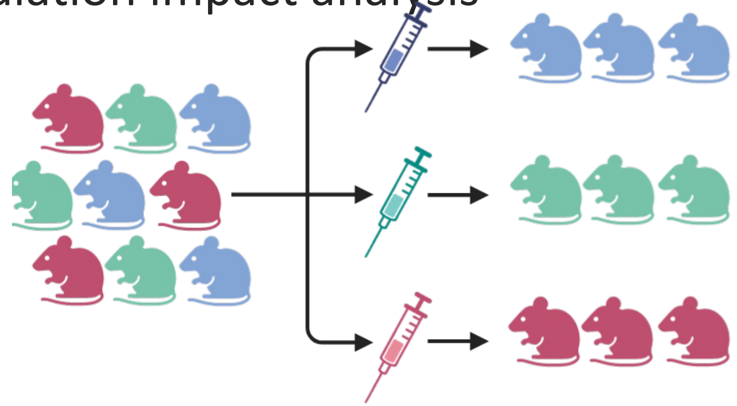
This study:

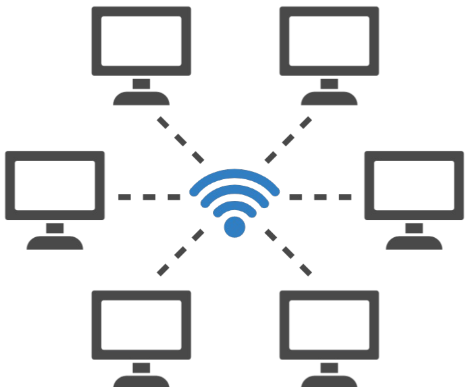
- Mechanistic understanding of contraction
- May inform therapeutic development



Prospective studies:

- Computational models X experimental studies
- Single molecule studies, value prop or population impact analysis





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FIN