In Silico Modeling of Shear-Stress-Induced Nitric Oxide Production in Endothelial Cells through Systems Biology

Andrew Koo,[†||] David Nordsletten,[¶] Renato Umeton,[‡] Beracah Yankama,[§] Shiva Ayyadurai,[†] Guillermo García-Cardeña,[||] and C. Forbes Dewey, Jr.[†‡]*

[†]Department of Biological Engineering, [‡]Department of Mechanical Engineering, and [§]Laboratory for Information and Decision Systems, Massachusetts Institute of Technology, Cambridge, Massachusetts; [¶]Department of Biomedical Engineering, King's College London, London, United Kingdom; and [||]Laboratory for Systems Biology, Center for Excellence in Vascular Biology, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

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Modeling Shear-Induced NO Production

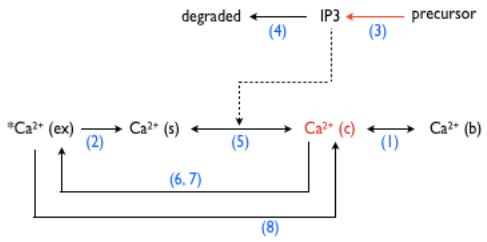
*Correspondence: cfdewey@mit.edu

SUPPORTING MATERIAL

Model Reactions and Parameters

Model 1: Shear-stress-induced calcium influx

Model Diagram (Figure legends at the end of appendix):



List of species:

Species	Name	Initial Amount	Ref.
Ca^{2+} (b)	Calcium complexed to intracellular binding proteins	3870 nM	(1)
Ca^{2+} (ex)	Extracellular calcium	$1.5 * 10^6$ nM	(1)
Ca^{2+} (s)	Calcium in intracellular storage	$2.83 * 10^6$ nM	(1)
Ca^{2+} (c)	Cytosolic calcium	117.2 nM	Steady state value for the integrated model under "no flow" condition
IP3	Inositol 1,4,5-triphosphate	0 nM	(1)

List of reactions:

#	Description	Rate equation	Ref.
1	$[Ca^{2+}$ (b)$] \leftrightarrow [Ca^{2+}$ (c)$]$	$k_7[Ca^{2+}(b)] - k_6[Ca^{2+}(c)] \cdot (B_T - [Ca^{2+}(b)])$	(1)
2	$[Ca^{2+}$ (ex)$] \rightarrow [Ca^{2+}$ (s)$]$	$k_{CCE} \cdot (\frac{fracK \cdot Ca_0^{2+}}{K3 + Ca_0^{2+}} - [Ca^{2+}(s)]) \cdot ([Ca^{2+}(ex)] - [Ca^{2+}(s)])$	(1)
3	$\phi \rightarrow$ [IP3]	$k_1 \cdot (R_T - \frac{R_T}{2} \cdot (e^{-t/\tau_I} + e^{-t/\tau_{II}}) + \left(\frac{\tau_I + \tau_{II}}{\tau_I - \tau_{II}}\right) \cdot (e^{-t/\tau_I} - e^{-t/\tau_{II}})) \cdot \left(\frac{[Ca^{2+}(c)]}{K_1 + [Ca^{2+}(c)]}\right)$	(1)
4	[IP3] $\rightarrow \phi$	$k_2[IP3]$	(1)
5	$[Ca^{2+}$ (s)$] \leftrightarrow [Ca^{2+}$ (c)$]$	$k_3 \frac{k_{CICR}[Ca^{2+}(c)]}{K_{CICR} + [Ca^{2+}(c)]} \cdot \left(\frac{[IP3]}{K_2 + [IP3]}\right)^3 \cdot [Ca^{2+}(s)] - k_4\left(\frac{[Ca^{2+}(c)]}{K_3 + [Ca^{2+}(c)]}\right)^2 + k_5\left([Ca^{2+}(s)]\right)^2$	(1)
6	$[Ca^{2+}$ (c)$] \rightarrow [Ca^{2+}$ (ex)$]$	$\dot{V}_{ex} \frac{[Ca^{2+}(c)]}{K_5 + [Ca^{2+}(c)]}$	(1)
7	$[Ca^{2+}$ (c)$] \rightarrow [Ca^{2+}$ (ex)$]$	$\dot{V}_p \frac{\left([Ca^{2+}(c)]\right)^2}{K_4^2 + \left([Ca^{2+}(c)]\right)^2} + \dot{V}_{hi} \frac{\left([Ca^{2+}(c)]\right)^4}{K_{hi}^4 + \left([Ca^{2+}(c)]\right)^4}$	(1)
8	$[Ca^{2+}$ (ex)$] \rightarrow [Ca^{2+}$ (c)$]$	$\dot{Q}_{shear}$	(2)

List of parameters:

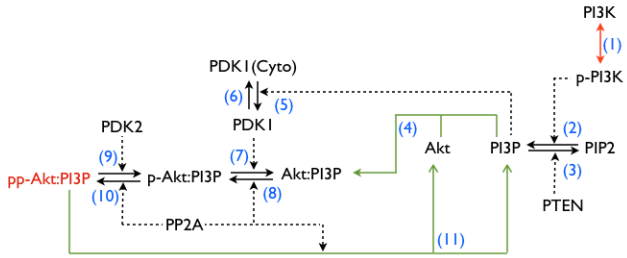
	Units	Ref.	Ref. value	Model value
R_T	# / cell	(1)	$4.4 * 10^4$	$4.4 * 10^4$
k_1	nM • s^{-1}	(1)	$1.2 * 10^{-3}$	$6.0 * 10^{-4}$
k_2	s^{-1}	(1)	2	1
k_3	s^{-1}	(1)	6.64	3.32
k_4	nM • s^{-1}	(1)	5000	2500
k_5	nM^{-1} • s^{-1}	(1)	$1.0 * 10^{-10}$	$5.0 * 10^{-11}$
k_6	nM^{-1} • s^{-1}	(1)	0.1	0.05
k_7	s^{-1}	(1)	300	150
K_1	nM	(1)	0	0
K_2	nM	(1)	200	200
K_3	nM	(1)	150	150
K_4	nM	(1)	80	80
K_5	nM	(1)	321	321
K_{hi}	nM	(1)	380	380
k_{CICR}	dimensionless	(1)	1	1
K_{CICR}	nM	(1)	0	0
k_{CCE}	nM^{-1} • s^{-1}	(1)	0	0
B_T	nM	(1)	$1.2 * 10^5$	$1.2 * 10^5$
Ca_0^{2+}	nM	(1)	100	100
$\dot{Q}_{shear}$	nM^{-1} • s^{-1}	(2), based on 10 dynes/cm^2	6000	3000
$\dot{V}_p$	nM^{-1} • s^{-1}	(1)	1630	815
$\dot{V}_{ex}$	nM^{-1} • s^{-1}	(1)	18330	9165
$\dot{V}_{hi}$	nM^{-1} • s^{-1}	(1)	4760	2380

τ_I	s	(1)	33	66
τ_{II}	s	(1)	0.005	0.01
fracK	dimensionless	(1)	$7.1 * 10^6$	$7.1 * 10^6$

References for model 1:
(1, 2)

Model 2: Shear-stress-induced AKT phosphorylation

Model Diagram:



List of species:

Species	Name	*Initial Amount	Ref.
PI3K	PI 3-kinases	99.97 nM	Total PI3K concentration 100 nM (3)
p-PI3K	Phosphorylated PI 3-kinases	0.03 nM	---
PIP2	Phosphatidylinositol-4,5-biphosphate	6967.27 nM	Total PIP2 concentration 7000 nM (3)
PI3P	Phosphatidylinositol-3,4,5-triphosphate	0.35 nM	---
PTEN	Phosphatase and tensin homolog	0.1 nM	Constant (3)
Akt	Akt, or Protein Kinase B	167.62 nM	Total Akt concentration 200 nM (3)
Akt: PI3P	Membrane bound Akt	29.2 nM	---
p-Akt:PI3P	Monophosphorylated Akt	1.46 nM	---
pp-Akt:PI3P	Biphosphorylated Akt	1.72 nM	---
PDK1 (cyto)	Cytosolic phosphoinositide-dependent kinase-1	999.75 nM	Total PDK1 concentration 1000 nM (3)
PDK1	Phosphoinositide-dependent kinase-1	0.25 nM	---
PDK2	Phosphoinositide-dependent kinase-2	3 nM	Constant (3)
PP2A	Protein phosphatase 2	150 nM	Constant (3)

*** Initial amounts were obtained by simulating the model under "no flow" condition, with the reference value as initial concentrations, for a sufficient amount of time to reach steady state.**

List of reactions:

#	Description	Rate equation	Ref.
*1	[PI3K] ↔ [p-PI3K]	$\exp(1-\left(\frac{t}{15}\right)^{1.8})\cdot 0.907\cdot t^{0.8}\cdot(1-\left(\frac{t}{15}\right)^{1.8})$	(4)
2	[PIP2] → [PI3P]	$k_2\frac{[p-PI3K][PIP2]}{K_{m2}+[PIP2]}$	(3)
3	[PI3P] → [PIP2]	$k_3\frac{[PTEN][PI3P]}{K_{m3}+[PI3P]}$	(3)
4	[Akt] + [PI3P] ↔ [Akt:PI3P]	$k_4[PI3P][Akt]-k_{r4}[Akt:PI3P]$	(3)
5	[PDK1 (cyto)] → [PDK1]	$k_5[PI3P][PDK1]$	(3)
6	[PDK1] → [PDK1 (cyto)]	$k_6[PDK1]$	(3)
7	[p-Akt:PI3P] → [Akt:PI3P]	$k_7\frac{[PP2A][p-Akt:PI3P]}{K_{m7}+[p-Akt:PI3P]}$	(3)

8	[Akt:PI3P] → [p-Akt:PI3P]	$k_8 \frac{[PDK1][Akt:PI3P]}{K_{m8}+[Akt:PI3P]}$	(3)
9	[pp-Akt:PI3P] → [p-Akt:PI3P]	$k_9 \frac{[PP2A][pp-Akt:PI3P]}{K_{m9}+[pp-Akt:PI3P]}$	(3)
10	[p-Akt:PI3P] → [pp-Akt:PI3P]	$k_{10} \frac{[PDK2][Akt:PI3P]}{K_{m10}+[Akt:PI3P]}$	(3)
11	[pp-Akt:PI3P] → [Akt] + [PI3P]	$k_{11} \frac{[PP2A][pp-Akt:PI3P]}{K_{m11}+[pp-Akt:PI3P]}$	(3)

*** Time-dependent function describing PI3K activation was generated from the experimental data in [4]. The shear stress waveform used in this paper is a laminar flow of 5 dynes/cm^2.**

List of parameters:

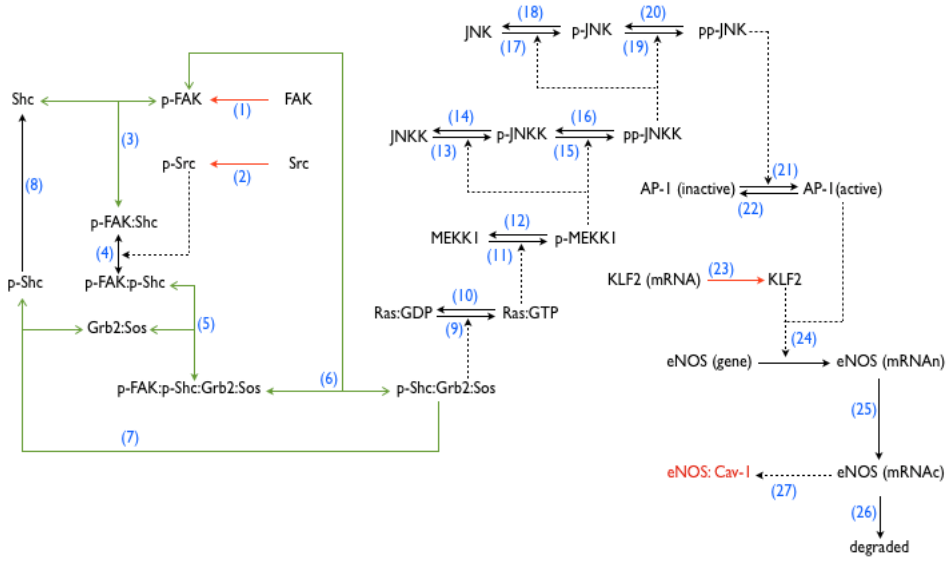
	Units	**Ref.**	**Ref. value**	**Model value**
k_2	s^{-1}	(3)	0.05	0.2
K_{m2}	nM	(3)	6170	6170
k_3	s^{-1}	(3)	5.5	7.5
K_{m3}	nM	(3)	80.9	80.9
k_4	$nM^{-1} \bullet s^{-1}$	(3)	0.045	0.045
k_{r4}	s^{-1}	(3)	0.089	0.089
k_5	$nM^{-1} \bullet s^{-1}$	(3)	0.0007	0.0007
k_6	s^{-1}	(3)	0.98	0.98
k_7	s^{-1}	(3)	0.037	0.037
K_{m7}	nM	(3)	8800	8800
k_8	s^{-1}	(3)	20	20
K_{m8}	nM	(3)	80000	80000
k_9	s^{-1}	(3)	0.04	0.04
K_{m9}	nM	(3)	48000	48000
k_{10}	s^{-1}	(3)	20	20
K_{m10}	nM	(3)	80000	80000
k_{11}	s^{-1}	(3)	0.163	0.163
K_{m11}	nM	(3)	48000	48000

References for model 2:

(3, 4)

Model 3: Shear-stress-induced eNOS expression

Model Diagram:



List of species:

Species	**Name**	***Initial Amount**	**Ref.**
FAK	Focal adhesion kinase	57 nM	Total FAK concentration 80 nM (7)
p-FAK	Phosphorylated FAK	0.605 nM	---
Src	Src kinase	72 nM	Total Src concentration 90 nM (7)
p-Src	Phosphorylated Src	18 nM	---
Shc	Shc adaptor protein	819.25 nM	Total Shc concentration 1000 nM (8)
p-FAK:Shc	Protein complex	0.857 nM	---
p-FAK:p-Shc	Protein complex	15.962 nM	---
Grb2:Sos	Grb2:Sos adaptor protein	3.23 nM	Total Grb2:Sos concentration 10 nM (8)
p-FAK:p-Shc:Grb2:Sos	Protein complex	5.577 nM	---
p-Shc:Grb2:Sos	Protein complex	1.193 nM	---
p-Shc	Phosphorylated Shc	157.162 nM	---
Ras:GDP	Ras protein (GDP state)	119.384 nM	Total Ras concentration 120 nM (8)
Ras:GTP	Ras protein (GTP state)	0.616 nM	---
MEKK1	MEKK1 kinase	98.514 nM	Total MEKK1 concentration 100 nM (10)
p-MEKK1	Phosphorylated MEKK1	1.486 nM	---
JNKK	JNK-activated kinase	299.706 nM	Total JNKK concentration 300 nM (10)
p-JNKK	Monophosphorylated JNKK	0.288 nM	---
pp-JNKK	Biphosphorylated JNKK	0.006 nM	---
JNK	c-Jun N-terminal kinases	299.997 nM	Total JNK concentration 300 nM (10)
p-JNK	Monophosphorylated JNK	0.003 nM	---
pp-JNK	Biphosphorylated JNK	0 nM	---
eNOS (mRNAn)	Nuclear eNOS mRNA	0.09 nM	Estimate
eNOS (mRNAc)	Cytosolic eNOS mRNA	3.214 nM	Estimate
eNOS: Cav-1	eNOS (inactive due to Cav-1 binding)	34.98 nM	See model 4

AP-1 (inactive)	Activator Protein-1 (inactive)	50 nM	Total AP-1 concentration 50 nM (estimate)
AP-1 (active)	Activator Protein-1 (active)	0 nM	---
KLF2 (protein)	Krueppel-like factor 2	10 nM	Estimate

*** Initial amounts were obtained by simulating the model under "no flow" condition, with the reference value as initial concentrations, for a sufficient amount of time to reach steady state.**

List of reactions:

#	Description	Rate equation	Ref.
*1	[FAK] ↔ [p-FAK]	$\exp(1-\left(\frac{t}{60}\right)^{0.35})\cdot 4\cdot t^{-0.65}\cdot(1-\left(\frac{t}{60}\right)^{0.35})$	(5)
*2	[Src] ↔ [p-Src]	$\exp(1-\left(\frac{t}{540}\right)^{1.3})\cdot 0.026\cdot t^{0.3}\cdot(1-\left(\frac{t}{540}\right)^{1.3})$	(6)
3	[p-FAK] + [Shc] ↔ [p-FAK:Shc]	$k_3[p-FAK][Shc]-k_{r3}[p-FAK:Shc]$	(8)
4	[p-FAK:Shc] ↔ [p-FAK:p-Shc]	$k_4[p-Src][p-FAK:Shc]-k_{r4}[p-FAK:p-Shc]$	(7, 8)
5	[p-FAK:Shc] + [Grb2:Sos] ↔ [p-FAK:Shc:Grb2:Sos]	$k_5[p-FAK:Shc][Grb2:Sos]-$ $k_{r5}[p-FAK:p-Shc:Grb2:Sos]$	(8)
6	[p-FAK:p-Shc:Grb2:Sos] ↔ [p-FAK] + [p-Shc:Grb2:Sos]	$k_6[p-FAK:p-Shc:Grb2:Sos]-$ $k_{r6}[p-FAK][p-Shc:Grb2:Sos]$	(8)
7	[p-Shc:Grb2:Sos] → [p-Shc] + [Grb2:Sos]	$k_7[p-Shc:Grb2:Sos]$	(8)
8	[p-Shc] → [Shc]	$\frac{V_8[p-Shc]}{K_{m8}+[p-Shc]}$	(8)
9	[Ras:GDP] → [Ras:GTP]	$k_9\frac{[p-Shc:Grb2:Sos][Ras:GTP]}{K_{m9}+[Ras:GTP]}$	(8)
10	[Ras:GTP] → [Ras:GDP]	$\frac{V_{10}[Ras:GTP]}{K_{m10}+[Ras:GTP]}$	(8)
11	[MEKK1] → [p-MEKK1]	$k_{11}\frac{[Ras:GTP][MEKK1]}{K_{m11}+[MEKK1]}$	(10)
12	[p-MEKK1] → [MEKK1]	$\frac{V_{12}[p-MEKK1]}{K_{m12}+[p-MEKK1]}$	(10)
13	[JNKK] → [p-JNKK]	$k_{13}\frac{[p-MEKK1][JNKK]}{K_{m13}+[JNKK]}$	(10)
14	[p-JNKK] → [JNKK]	$\frac{V_{14}[p-JNKK]}{K_{m14}+[p-JNKK]}$	(10)
15	[p-JNKK] → [pp-JNKK]	$k_{15}\frac{[p-MEKK1][p-JNKK]}{K_{m15}+[p-JNKK]}$	(10)
16	[pp-JNKK] → [p-JNKK]	$\frac{V_{16}[pp-JNKK]}{K_{m16}+[pp-JNKK]}$	(10)
17	[JNK] → [p-JNK]	$k_{17}\frac{[pp-JNKK][JNK]}{K_{m17}+[JNK]}$	(10)
18	[p-JNK] → [JNK]	$\frac{V_{18}[p-JNK]}{K_{m18}+[p-JNK]}$	(10)
19	[p-JNK] → [pp-JNK]	$k_{19}\frac{[pp-JNKK][p-JNK]}{K_{m19}+[p-JNK]}$	(10)

20	[pp-JNK] → [p-JNK]	$\frac{V_{20}[pp-JNK]}{K_{m20}+[pp-JNK]}$	(10)
21	[AP-1 (inactive)] → [AP-1 (active)]	$k_{21}\frac{[pp-JNK][AP-1_{(inactive)}]}{K_{m21}+[AP-1_{(inactive)}]}$	Assum.
22	[AP-1 (active)] → [AP-1 (inactive)]	$\frac{V_{22}[AP-1_{(active)}]}{K_{m22}+[AP-1_{(active)}]}$	Assum.
*23	ϕ → [KLF2]	$\frac{\exp(0.55(5-t/3600))/(3600\cdot 29.256)}{(1+2\cdot\exp(0.55(5-t/3600))+\exp(1.1(5-t/3600))}$	(12)
24	ϕ → [eNOS (mRNAn)]	$k_{24t1}[AP-1_{(active)}]+k_{24t2}[KLF2]$	Assum.
25	[eNOS (mRNAn)] → [eNOS (mRNAc)]	$k_{25}[eNOS_{mRNAn}]$	(9)
26	[eNOS (mRNAc)] → ϕ	$k_{26}[eNOS_{mRNAc}]$	(11)
27	ϕ → [eNOS:Cav-1]	$\frac{V_{27}[eNOS_{mRNAc}]}{K_{m27}+[eNOS_{mRNAc}]}$	Assum.

*** Time-dependent functions describing FAK, Src activation, and KLF2 expression were generated from the experimental data in (5, 6, 12). The shear stress waveform used in (5, 6) is a laminar flow of 12 dynes/cm^2, the shear stress waveform used in (12) is an oscillatory (1 Hz) flow of 12±4 dynes/cm^2.**

List of parameters:

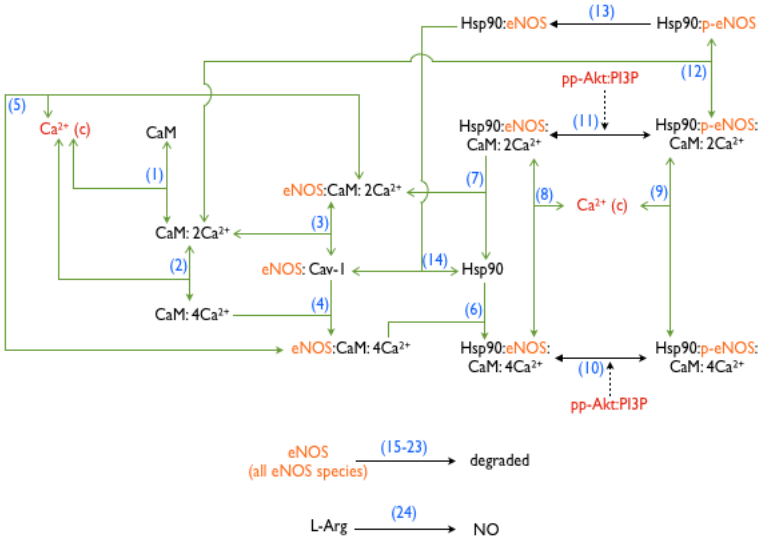
	Units	**Ref.**	**Ref. value**	**Model value**
k_3	$nM^{-1} \bullet s^{-1}$	(8)	0.1	0.1
k_{r3}	s^{-1}	(8)	1.0	1.0
k_4	$nM^{-1} \bullet s^{-1}$	(7)	8.33	8.33
k_{r4}	s^{-1}	(8)	5.0	5.0
k_5	$nM^{-1} \bullet s^{-1}$	(8)	60	60
k_{r5}	s^{-1}	(8)	546	546
k_6	s^{-1}	(8)	2040	2040
k_{r6}	$nM^{-1} \bullet s^{-1}$	(8)	15700	15700
k_7	s^{-1}	(8)	40.8	40.8
V_8	$nM \bullet s^{-1}$	(8)	0.0154	154
K_{m8}	nM	(8)	340	340
k_9	s^{-1}	(8)	0.222	0.222
K_{m9}	nM	(8)	0.181	0.181
V_{10}	$nM \bullet s^{-1}$	(8)	0.289	0.289
K_{m10}	nM	(8)	0.0571	0.0571
k_{11}	s^{-1}	Estimated from (10)	---	0.035
K_{m11}	nM	(10)	10	10
V_{12}	$nM \bullet s^{-1}$	(10)	0.25	0.125
K_{m12}	nM	(10)	8.0	8.0
k_{13}	s^{-1}	(10)	0.025	0.005
K_{m13}	nM	(10)	15.0	15.0
V_{14}	$nM \bullet s^{-1}$	(10)	0.75	0.375
K_{m14}	nM	(10)	15.0	15.0
k_{15}	s^{-1}	(10)	0.025	0.005
K_{m15}	nM	(10)	15.0	15.0
V_{16}	$nM \bullet s^{-1}$	(10)	0.75	0.375
K_{m16}	nM	(10)	15.0	15.0
k_{17}	s^{-1}	(10)	0.025	0.002
K_{m17}	nM	(10)	15.0	30.0

V_{18}	nM• s^{-1}	(10)	0.5	0.05
K_{m18}	nM	(10)	15.0	15.0
k_{19}	s^{-1}	(10)	0.025	0.002
K_{m19}	nM	(10)	15.0	30.0
V_{20}	nM• s^{-1}	(10)	0.5	0.05
K_{m20}	nM	(10)	15.0	15.0
k_{21}	s^{-1}	Estimated from fitting exp. data	---	$4.0 * 10^{-5}$
K_{m21}	nM	Estimated from fitting exp. data	---	25.0
V_{22}	nM• s^{-1}	Estimated from fitting exp. data	---	0.002
K_{m22}	nM	Estimated from fitting exp. data	---	5
k_{24t1}	s^{-1}	Estimated from fitting exp. data	---	$1.2 * 10^{-4}$
k_{24t2}	s^{-1}	Estimated from fitting exp. data	---	$9.0 * 10^{-6}$
k_{25}	s^{-1}	(9)	0.001	0.001
k_{26}	s^{-1}	Estimated from (11)	$1.1 * 10^{-5}$	$2.8 * 10^{-5}$
V_{27}	nM• s^{-1}	Estimated from fitting exp. data	---	0.02824
K_{m27}	nM	Estimated from fitting exp. data	---	16

References for model 3:
(5-12)

Model 4: Shear-stress-induced NO production

Model Diagram:



List of species:

Species	**Name**	***Initial Amount**	**Ref.**
Ca^{2+} (c)	Cytosolic calcium	117.2 nM	See Model 1
pp-Akt:PI3P	Biphosphorylated Akt	1.72 nM	See Model 2
CaM	Calmodulin	7635.36 nM	Total CaM concentration 8000 nM (estimate)
CaM:2Ca^{2+}	2 calcium bound calmodulin	347.52 nM	---
CaM:4Ca^{2+}	4 calcium bound calmodulin	2.83 nM	---
eNOS: Cav-1	eNOS (inactive due to Cav-1 binding)	34.98 nM	Total eNOS concentration 50 nM (estimate)
eNOS:CaM:2Ca^{2+}	eNOS protein complex	2.12 nM	---
eNOS:CaM:4Ca^{2+}	eNOS protein complex with calcium/calmodulin-induced activation	0.04 nM	---
Hsp90	Heat shock protein 90	199987 nM	Total Hsp90 concentration 200000 nM (estimate)
Hsp90:eNOS: CaM:2Ca^{2+}	eNOS protein complex	10.98 nM	---
Hsp90:p-eNOS: CaM:2Ca^{2+}	Phosphorylated eNOS protein complex	0.11 nM	---
Hsp90:eNOS: CaM:4Ca^{2+}	eNOS protein complex with calcium/calmodulin-induced activation	1.04 nM	---
Hsp90:p-eNOS: CaM:4Ca^{2+}	Phosphorylated eNOS protein complex with calcium/calmodulin-induced activation	0.01 nM	---
Hsp90:eNOS	eNOS protein complex	0.08 nM	---
Hsp90:p-eNOS	Phosphorylated eNOS protein complex	0.64 nM	---
NO	Nitric Oxide	0 (relative	---

		scale)	

*** Initial amounts were obtained by simulating the model under "no flow" condition, with the reference value as initial concentrations, for a sufficient amount of time to reach steady state.**

List of reactions:

#	Description	Rate equation	*Ref.
1	[CaM] ↔ [CaM:2Ca^{2+}]	$k_1 \cdot [CaM][Ca^{2+}] - k_{1r} \cdot [CaM:2Ca^{2+}]$	Assum.
2	[CaM:2Ca^{2+}] ↔ [CaM:4Ca^{2+}]	$k_2 \cdot [CaM:2Ca^{2+}][Ca^{2+}] - k_{2r} \cdot [CaM:4Ca^{2+}]$	Assum.
3	[CaM:2Ca^{2+}] + [eNOS:Cav-1] ↔ [eNOS:CaM:2Ca^{2+}]	$k_3 \cdot [CaM:2Ca^{2+}][eNOS:Cav-1] - k_{3r} \cdot [eNOS:CaM:2Ca^{2+}]$	Assum.
4	[CaM:4Ca^{2+}] + [eNOS:Cav-1] → [eNOS:CaM:4Ca^{2+}]	$k_4 \cdot [CaM:4Ca^{2+}][eNOS:Cav-1]$	Assum.
5	[eNOS:CaM:4Ca^{2+}] ↔ [eNOS:CaM:2Ca^{2+}]	$k_5 \cdot [eNOS:CaM:4Ca^{2+}] - k_{5r} \cdot [eNOS:CaM:2Ca^{2+}][Ca^{2+}]$	Assum.
6	[eNOS:CaM:4Ca^{2+}] + [Hsp90] → [Hsp90:eNOS:CaM:4Ca^{2+}]	$k_6 \cdot [eNOS:CaM:4Ca^{2+}][Hsp90]$	Assum.
7	[Hsp90:eNOS:CaM:2Ca^{2+}] → [eNOS:CaM:2Ca^{2+}] + [Hsp90]	$k_7 \cdot [Hsp90:eNOS:CaM:2Ca^{2+}]$	Assum.
8	[Hsp90:eNOS:CaM:4Ca^{2+}] ↔ [Hsp90:eNOS:CaM:2Ca^{2+}]	$k_8 \cdot [Hsp90:eNOS:CaM:4Ca^{2+}] - k_{8r} \cdot [Hsp90:eNOS:CaM:2Ca^{2+}][Ca^{2+}]$	Assum.
9	[Hsp90:p-eNOS:CaM:4Ca^{2+}] ↔ [Hsp90:p-eNOS:CaM:2Ca^{2+}]	$k_9 \cdot [Hsp90:p-eNOS:CaM:4Ca^{2+}] - k_{9r} \cdot [Hsp90:p-eNOS:CaM:2Ca^{2+}][Ca^{2+}]$	Assum.
10	[Hsp90:eNOS:CaM:4Ca^{2+}] ↔ [Hsp90:p-eNOS:CaM:4Ca^{2+}]	$\frac{k_{10}[pp-AKT:PI3P][Hsp90:eNOS:CaM:4Ca^{2+}]}{K_{m10}+[Hsp90:eNOS:CaM:4Ca^{2+}]} - \frac{V_{10r}[Hsp90:p-eNOS:CaM:4Ca^{2+}]}{K_{m10r}+[Hsp90:p-eNOS:CaM:4Ca^{2+}]}$	Assum.
11	[Hsp90:eNOS:CaM:2Ca^{2+}] ↔ [Hsp90:p-eNOS:CaM:2Ca^{2+}]	$\frac{k_{11}[pp-AKT:PI3P][Hsp90:eNOS:CaM:2Ca^{2+}]}{K_{m11}+[Hsp90:eNOS:CaM:2Ca^{2+}]} - \frac{V_{11r}[Hsp90:p-eNOS:CaM:2Ca^{2+}]}{K_{m11r}+[Hsp90:p-eNOS:CaM:2Ca^{2+}]}$	Assum.
12	[Hsp90:p-eNOS:CaM:2Ca^{2+}] ↔ [Hsp90:p-eNOS] + [CaM:2Ca^{2+}]	$k_{12} \cdot [Hsp90:p-eNOS:CaM:2Ca^{2+}] - k_{12r} \cdot [Hsp90:p-eNOS][CaM:2Ca^{2+}]$	Assum.
13	[Hsp90:p-eNOS] → [Hsp90: eNOS]	$\frac{V_{13}[Hsp90:p-eNOS]}{K_{m13}+[Hsp90:p-eNOS]}$	Assum.
14	[Hsp90:eNOS] → [Hsp90] + [eNOS:Cav-1]	$k_{14} \cdot [Hsp90:eNOS]$	Assum.
15	[eNOS:Cav-1] → ϕ	$k_D \cdot [eNOS:Cav-1]$	Assum.
16	[eNOS:CaM:2Ca^{2+}] → ϕ + [CaM:2Ca^{2+}]	$k_D \cdot [eNOS:CaM:2Ca^{2+}]$	Assum.
17	[eNOS:CaM:4Ca^{2+}] → ϕ + [CaM:4Ca^{2+}]	$k_D \cdot [eNOS:CaM:4Ca^{2+}]$	Assum.
18	[Hsp90:eNOS:CaM:2Ca^{2+}] → ϕ + [CaM:2Ca^{2+}] + [Hsp90]	$k_D \cdot [Hsp90:eNOS:CaM:2Ca^{2+}]$	Assum.
19	[Hsp90:eNOS:CaM:4Ca^{2+}] → ϕ + [CaM:4Ca^{2+}] + [Hsp90]	$k_D \cdot [Hsp90:eNOS:CaM:4Ca^{2+}]$	Assum.
20	[Hsp90:p-eNOS:CaM:2Ca^{2+}] → ϕ + [CaM:2Ca^{2+}] + [Hsp90]	$k_D \cdot [Hsp90:p-eNOS:CaM:2Ca^{2+}]$	Assum.
21	[Hsp90:p-eNOS:CaM:4Ca^{2+}] → ϕ + [CaM:4Ca^{2+}] + [Hsp90]	$k_D \cdot [Hsp90:p-eNOS:CaM:4Ca^{2+}]$	Assum.
22	[Hsp90:p-eNOS] → ϕ + [Hsp90]	$k_D \cdot [Hsp90:p-eNOS]$	Assum.

23	[Hsp90:eNOS] $\rightarrow \phi$ + [Hsp90]	$k_D \cdot [Hsp90:eNOS]$	Assum.
24	$\phi \rightarrow$ [NO]	$k_{CaM} \cdot [Hsp90:eNOS:CaM:4Ca^{2+}] +$ $k_{CaM} \cdot [Hsp90:p-eNOS:CaM:4Ca^{2+}] +$ $k_{CaM} \cdot [eNOS:CaM:4Ca^{2+}] +$ $k_p \cdot [Hsp90:p-eNOS:CaM:2Ca^{2+}] +$ $k_p \cdot [Hsp90:p-eNOS]$	Assum.

*** All reactions from this model were generated based on our assumptions.**

List of parameters:

	Units	**Ref.**	**Ref. value**	**Model value**
k_1	$nM^{-1} \bullet s^{-1}$	Estimated from (13)	---	0.004
k_{1r}	s^{-1}	Estimated from (13)	---	10.3
k_2	$nM^{-1} \bullet s^{-1}$	Estimated from (13)	---	0.08
k_{2r}	s^{-1}	Estimated from (13)	---	1152
k_3	$nM^{-1} \bullet s^{-1}$	Initial estimate	---	$1.5 * 10^{-4}$
k_{3r}	s^{-1}	Initial estimate	---	1.5
k_4	$nM^{-1} \bullet s^{-1}$	Initial estimate	---	0.015
k_5	s^{-1}	Estimated from (13)	---	115.2
k_{5r}	$nM^{-1} \bullet s^{-1}$	Estimated from (13)	---	0.08
k_6	$nM^{-1} \bullet s^{-1}$	Estimated from (15)	---	0.002
k_7	s^{-1}	Estimated from (15)	---	1.5
k_8	s^{-1}	Estimated from (13)	---	115.2
k_{8r}	$nM^{-1} \bullet s^{-1}$	Estimated from (13)	---	0.08
k_9	s^{-1}	Estimated from (13)	---	115.2
k_{9r}	$nM^{-1} \bullet s^{-1}$	Estimated from (13)	---	0.08
k_{10}	s^{-1}	Estimated from fitting exp. data	---	0.1
K_{m10}	nM	Estimated from fitting exp. data	---	5
V_{10r}	$nM \bullet s^{-1}$	Estimated from fitting exp. data	---	4
K_{m10r}	nM	Estimated from fitting exp. data	---	20
k_{11}	s^{-1}	Estimated from fitting exp. data	---	0.1
K_{m11}	nM	Estimated from fitting exp. data	---	5
V_{11r}	$nM \bullet s^{-1}$	Estimated from fitting exp. data	---	4
K_{m11r}	nM	Estimated from fitting exp. data	---	20
k_{12}	s^{-1}	Initial estimate	---	1.5
k_{12r}	$nM^{-1} \bullet s^{-1}$	Initial estimate	---	$1.5 * 10^{-4}$
V_{13}	$nM \bullet s^{-1}$	Estimated from fitting exp. data	---	4
K_{m13}	nM	Estimated from fitting exp. data	---	20
k_{14}	s^{-1}	Estimated from (15)	---	1.5
k_D	s^{-1}	Estimated from half life (16)	$1.13 * 10^{-5}$	$9.45 * 10^{-5}$
k_{CaM}	s^{-1}	Estimated from (14)	---	17
k_p	s^{-1}	Estimated from (14)	---	5

References for model 4:
(13-16)

Time-dependent functions as model inputs

As described in the manuscript, time-dependent functions were used as model inputs throughout the NO systems as proxies for the mechanotransduction process. These time-dependent functions were either taken directly from existing models or fit from experimental data. The details of the five time-dependent functions are described below:

1) Production of IP3 (Model 1: Reaction 3)

$$\frac{d[IP3]}{dt} = k_1 \cdot (R_T - \frac{R_T}{2} \cdot (e^{-t/\tau_I} + e^{-t/\tau_{II}}) + \left(\frac{\tau_I + \tau_{II}}{\tau_I - \tau_{II}}\right) \cdot (e^{-t/\tau_I} - e^{-t/\tau_{II}})) \cdot \left(\frac{[Ca^{2+}(c)]}{K_1 + [Ca^{2+}(c)]}\right)$$

This equation was taken directly from the calcium dynamics model created by Wiesner et al. (1) through combining *Eq. 11* and *Eq. 16* of the original paper. This theoretical model was based on experimental measurement of human umbilical vein endothelial cells (HUVECs) assuming a laminar shear stress of 10 dynes/cm^2.

2) PI3K Activation (Model 2: Reaction 1)

The equation describing PI3K activation was generated by fitting the experimental data of Go et al. (4) (Figure 2B of the paper) to the following equation:

$$[p-PI3K]_t = 1 + a \cdot \left(\frac{t}{b}\right)^c \cdot \exp(1 - \left(\frac{t}{b}\right)^c)$$

Parameter values of a, b, c were optimized by curve-fitting using MATLAB. We then calculated the time-derivative of the above equation to obtain the time-dependent differential equation of PI3K activation (as shown in Model 2: Reaction 1):

$$\frac{d[p-PI3K]}{dt} = \exp(1 - \left(\frac{t}{15}\right)^{1.8}) \cdot 0.907 \cdot t^{0.8} \cdot (1 - \left(\frac{t}{15}\right)^{1.8})$$

In the Go et al. study, bovine aortic endothelial cells (BAECs) were exposed to a laminar shear stress of 5 dynes/cm^2. PI3K in the time-course experiments was isolated through immunoprecipitation and the PI3K activity was measured by a radioactive assay. Total PI3K concentration was assumed to be 100 nM based on the model of Koh et al. (3).

3) FAK Activation (Model 3: Reaction 1)

Similarly, the equation describing FAK activation was generated by fitting the experimental data of Li et al. (5) (Figure 2A of the paper) to the following equation:

$$[p-FAK]_t = 1 + a \cdot \left(\frac{t}{b}\right)^c \cdot \exp(1 - \left(\frac{t}{b}\right)^c)$$

Parameter values of a, b, c were optimized by curve-fitting using MATLAB. We then calculated the time-derivative of the above equation to obtain the time-dependent differential equation of FAK activation (as shown in Model 3: Reaction 1):

$$\frac{d[p-FAK]}{dt} = \exp(1 - \left(\frac{t}{60}\right)^{0.35}) \cdot 4 \cdot t^{-0.65} \cdot (1 - \left(\frac{t}{60}\right)^{0.35})$$

In the Li et al. study, BAECs were exposed to a laminar shear stress of 12 dynes/cm^2. FAK in the time-course experiments was isolated through immunoprecipitation and FAK activation was measured using a phophotyrosine-specific antibody. Total FAK concentration was assumed to be 80 nM based on the model of Yee et al. (7).

4) Src Activation (Model 3: Reaction 2)

Again, the equation describing Src activation was generated by fitting the experimental data of Jalali et al. (6) (Figure 1 of the paper) to the following equation:

$$[p-Src]_t = 1 + a \cdot \left(\frac{t}{b}\right)^c \cdot \exp\left(1 - \left(\frac{t}{b}\right)^c\right)$$

Parameter values of a, b, c were optimized by curve-fitting using MATLAB. We then calculated the time-derivative of the above equation to obtain the time-dependent differential equation of Src activation (as shown in Model 3: Reaction 2):

$$\frac{d[p-Src]}{dt} = \exp\left(1 - \left(\frac{t}{540}\right)^{1.3}\right) \cdot 0.026 \cdot t^{0.3} \cdot \left(1 - \left(\frac{t}{540}\right)^{1.3}\right)$$

In the Jalali et al. study, BAECs were exposed to a laminar shear stress of 12 dynes/cm^2. Src in the time-course experiment was isolated through immunoprecipitation and the Src activity was measured by a radioactive assay. Total Src concentration was assumed to be 90 nM based on the model of Yee et al. (7).

5) KLF2 Activation (Model 3: Reaction 23)

The equation describing KLF2 activation was generated by fitting the experimental data of Young et al. (12) (Figure 1B of the paper, assuming the translational process is fast and KLF2 protein expression corresponds well with mRNA expression) to the following equation (t/3600 to adjust the time from hours to seconds):

$$[KLF2]_t = 1 + \frac{a}{1 + \exp\left(b \cdot \left(c - \frac{t}{3600}\right)\right)}$$

Parameter values of a, b, c were optimized by curve-fitting using MATLAB. We then calculated the time-derivative of the above equation to obtain the time-dependent differential equation of KLF2 activation (as shown in Model 3: Reaction 23):

$$\frac{d[KLF2]}{dt} = \frac{\exp\left(0.55\left(5 - \left(\frac{t}{3600}\right)\right)\right)/(3600 * 29.256)}{\left(1 + 2 \cdot \exp\left(0.55\left(5 - \left(\frac{t}{3600}\right)\right)\right) + \exp\left(1.1\left(5 - \left(\frac{t}{3600}\right)\right)\right)\right)}$$

In the Young et al. study, HUVECs were exposed to an oscillatory (1 Hz) shear stress of 12±4 dynes/cm^2. mRNA from endothelial cells was isolated in the time-course experiments and relative mRNA expression was measured through qPCR. Initial KLF2 concentration was assumed to be 10 nM based on our experimental observation of low quantity presence in endothelial cells.

Parameter Optimization

The parameters of individual models were optimized in CellDesigner based on the following general iterative steps:

1. Conduct parameter sensitivity analysis to search for the high sensitive parameters (Simulation -> Control Panel -> Interactive Simulation -> Parameter Value).
2. Optimize the set of most sensitive parameters to fit the experimental observed values.

Model 1: Shear stress-induced calcium influx

It is observed through parameter scan that most kinetic parameters that govern reaction rates in this model (k_1, k_2, k_3, k_4, k_5, k_6, k_7, Q_{shear}, V_P, V_{ex}, V_{hi}, τ_I, τ_{II}) need to be adjusted simultaneously in order to achieve sensible simulation outcome. The experimental data used to optimize the parameters is from the study of Schwarz *et al.* (17).

Model 2: Shear stress-induced AKT phosphorylation

The parameters k_2 and k_3 were found to be the most sensitive parameters. These parameters were moderately optimized within the same order of magnitude to better fit the experimental data of Boo *et al.* (18).

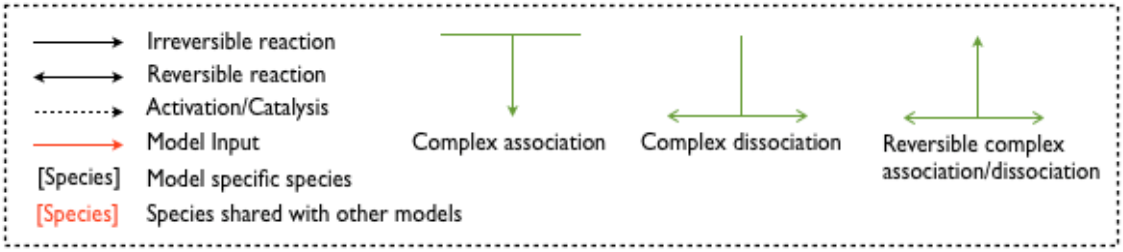
Model 3: Shear stress-induced eNOS expression

There was a big shift in V_8 due to modification of model structure compared to the reference model. Other parameters (including the eNOS degradation parameter k_D listed in the 4th model) were estimated or moderately optimized within the same order of magnitude to better fit the experimental data of our lab (eNOS mRNA time course) and the study of Li *et al.* (19) (eNOS protein time course).

Model 4: Shear stress-induced NO production

Most of the parameters in this model are direct estimation from experimental data without further optimization. The parameters governing eNOS phosphorylation (reaction 10 and 11) and dephosphorylation (reaction 13) were optimized according to eNOS phosphorylation time course data from Boo *et al.* (18). The two NO production parameters, k_{CaM} and k_p, were optimized to better fit the experimental observation of Florian *et al.* (20) under both static and shear stress conditions.

Model diagram legends:



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