

**Table S1.** List of initial concentration of pool values taken in simulation of comprehensive network of eNOS activation pathway.

S.NO	Molecular Species	Concentration ( $\mu\text{M}$ )
1	G protein	1 <sup>[1]</sup>
2	Adenylyl Cyclase	0.015 <sup>[1]</sup>
3	PKA	0.5 <sup>[1]</sup>
4	ATP	5000 <sup>[1]</sup>
5	eNOS	1 <sup>[2]</sup>
6	PI3-kinase	0.1 <sup>[3]</sup>
7	PIP2	7 <sup>[3]</sup>
8	PTEN	0.27 <sup>[3]</sup>
9	Akt	0.2 <sup>[4]</sup>
10	PDK	1 <sup>[4]</sup>
11	PIP3-PDK2	0.003 <sup>[4]</sup>
12	PP2A	0.15 <sup>[4]</sup>
13	VEGF	2e-06 <sup>[5]</sup>
14	VEGFR2	0.0024 <sup>[6]</sup>
15	PLC $\gamma$	0.82 <sup>[7]</sup>
16	PLC $\beta$	0.8 <sup>[7]</sup>
17	PKC	1 <sup>[7]</sup>
18	Calcium	0.08 <sup>[8]</sup>
19	Ca_Sequester	6.3 <sup>[1]</sup>
20	IP3R	0.0166 <sup>[1]</sup>
21	Calmodulin	20 <sup>[7]</sup>
22	L-Arginine	100 <sup>[9]</sup>

**Table S2. List of kinetic parameters and biochemical reactions considered in shear and VEGF induced eNOS activation.**

S.NO	Specification	Binding Reaction	$k_f$ ( $\mu\text{M}^{-1} \text{s}^{-1}$ )	$k_b$ ( $\text{s}^{-1}$ )
1	Activate_Gs	$\text{GDP-}\alpha\beta\gamma \rightarrow \text{GTP.Gs-}\alpha + \text{Gs-}\beta\gamma$	$0.004^*$	0
2	Trimerise_Gs	$\text{GTP.Gs-}\alpha \rightarrow \text{GDP.Gs-}\alpha$	$1.25\text{e-}06^{[1]}$	$0^{[1]}$
3	GTPase	$\text{GDP.Gs-}\alpha \rightarrow \text{GDP-}\alpha\beta\gamma$	$0.25^{[1]}$	$0^{[1]}$
4	GTP.Gs-bind-AC	$\text{GTP.Gs-}\alpha + \text{AC} \leftrightarrow \text{GTP.Gs-}\alpha - \text{AC}$	$4.8\text{e-}07^*$	$1^*$
5	PKA-activation	$\text{cAMP} + \text{Inactive-PKA} \leftrightarrow \text{PKA-Active}$	$7.5\text{e-}08^*$	$10^*$
6	eNOS1-phosphorylation	$\text{PKA-Active} \leftrightarrow \text{eNOS-Po4}$	$4.24\text{e-}07^*$	$8^*$
7	PI3K-bind-Gs-by	$\text{Gs-}\beta\gamma + \text{PI3K} \leftrightarrow \text{Gs-}\beta\gamma\text{-PI3K}$	$9.9\text{e-}08^*$	$2.5^*$
8	Activated-PI3K	$\text{Gs-}\beta\gamma\text{-PI3K} \leftrightarrow \text{PI3K-Active}$	$0.039^*$	$0.01^*$
9	PDK1Translocation	$\text{PIP3} + \text{PDK1} \leftrightarrow \text{PIP3-PDK1}$	$7.8\text{e-}08^{[4]}$	$1^{[4]}$
10	PIP3bindAKT	$\text{PIP3} + \text{Akt} \leftrightarrow \text{PIP3-Akt}$	$7.8\text{e-}08^{[4]}$	$0.19^{[4]}$
11	VEGF binding VEGFR2	$\text{VEGF} + \text{VEGFR-2} \leftrightarrow \text{VEGF-VEGFR2}$	$4.98\text{e-}05^{[10,11]}$	$0.51^{[10,11]}$
12	Dimerization	$\text{VEGF-VEGFR2} \leftrightarrow \text{VEGFR2-dimer}$	$0.166^{[12,13]}$	$0.036^{[12,13]}$

<b>13</b>	Autophosphorylation	VEGFR2-dimer $\leftrightarrow$ VEGFR-PO4	0.38 <sup>[14]</sup>	0.23 <sup>[14]</sup>
<b>14</b>	Internalization	VEGFR-PO4 $\rightarrow$ Internalized	2.8e-15 <sup>[15]</sup>	0
<b>15</b>	PI3K-Binding-VEGFR-PO4	VEGFR-PO4+PI3K_inactive $\leftrightarrow$ PI3K- VEGFR-PO4	9.6e-08 <sup>*</sup>	0.089 <sup>*</sup>
<b>16</b>	Activates_PI3K	PI3K- VEGFR-PO4 $\leftrightarrow$ PI3K-Active	0.014 <sup>*</sup>	0.004 <sup>*</sup>
<b>17</b>	PLC $\gamma$ _Activation	Inactive_ PLC $\gamma$ + VEGFR-PO4 $\leftrightarrow$ PLC $\gamma$ _Active	9.79e-08 <sup>*</sup>	1.55 <sup>*</sup>
<b>18</b>	IP3-binding-IP3R	IP3+IP3+IP3+IP3R $\leftrightarrow$ IP3-IP3R_chan	3.08e-23 <sup>[1]</sup>	0.87 <sup>[1]</sup>
<b>19</b>	PKC-act-Ca	Ca_cytosol+PKC $\leftrightarrow$ PKC_Ca	6.039e-10 <sup>[1]</sup>	5 <sup>[1]</sup>
<b>20</b>	PKC-act-DAG	PKC_Ca+DAG $\leftrightarrow$ PKC_Ca_DAG	3.57e-07 <sup>[1]</sup>	7.67 <sup>[1]</sup>
<b>21</b>	PKC_Active	PKC_Ca_DAG $\leftrightarrow$ PKC_Active	2.4 <sup>[1]</sup>	0.0034 <sup>[1]</sup>
<b>22</b>	PKC-bind-eNOS	PKC_Active +eNOS-Thr497 $\leftrightarrow$ eNOS-Thr497-Po4	6.82e-08 <sup>*</sup>	0.49 <sup>*</sup>
<b>23</b>	DAG-Degradation	DAG $\rightarrow$ dag_degraded	0.015 <sup>[7*]</sup>	0
<b>24</b>	IP3-degradation	IP3 $\rightarrow$ IP3_degraded	0.0318 <sup>[7*]</sup>	0
<b>25</b>	Ca-bind-CaM	Ca_cytosol+CaM $\leftrightarrow$ Ca-CaM	6.43e-10 <sup>[1*]</sup>	8.2 <sup>[1*]</sup>
<b>26</b>	Ca-CaM-bind-eNOS	Ca-CaM+eNOS-ca-CaM $\leftrightarrow$ eNOS Ca-Cam Active	4.58e-07 <sup>[16]</sup>	0.18 <sup>[16]</sup>
<b>27</b>	Ca-bind- Inactive_ PLC $\gamma$	Ca_cytosol+ Inactive_ PLC $\gamma$ $\leftrightarrow$ PLC $\gamma$ _Ca	7.24e-08 <sup>[7]</sup>	15 <sup>[7]</sup>
<b>28</b>	Ca-bind- Active_	Ca_cytosol+ Active_	1.08e-08 <sup>[7]</sup>	15 <sup>[7]</sup>

	PLC $\gamma$	PLC $\gamma \leftrightarrow$ PLC $\gamma$ _Ca_Active		
<b>29</b>	PLC $\gamma$ _Ca_Active-dephosphorylation	PLC $\gamma$ _Ca_Active $\rightarrow$ PLC $\gamma$ _Ca	5e-06 <sup>[7]</sup>	0
<b>30</b>	Activate_Gq	GDP- $\alpha\beta\gamma \rightarrow$ Gq- $\alpha$ + Gq- $\beta\gamma$	0.0005 <sup>*</sup>	0
<b>31</b>	Trimerise_Gq	Gq- $\alpha \rightarrow$ GDP.Gq- $\alpha$	3.62e-07 <sup>*</sup>	0
<b>32</b>	GTPase_q	GDP.Gq- $\alpha \rightarrow$ GDP- $\alpha\beta\gamma$	0.24 <sup>*</sup>	0
<b>33</b>	Gq- $\alpha$ binds PLC_ $\beta$	Gq- $\alpha$ + PLC_ $\beta$ _Inactive $\leftrightarrow$ PLC_ $\beta$ -Active	9.79e-08 <sup>[17*]</sup>	1.55 <sup>[17*]</sup>
<b>34</b>	eNOS binds L-Arg	eNOS <sup>conserved</sup> + L-arg $\leftrightarrow$ Nitric Oxide + L-citrulline	3.84e-07 <sup>[18]</sup>	9.8e-06 <sup>[18]</sup>
<b>35</b>	Ca_release	IP3IP3R_chan+Ca_sequester $\leftrightarrow$ Ca_cytosol	gmax 0.1 <sup>[1]</sup>	Perm 19 <sup>[1]</sup>

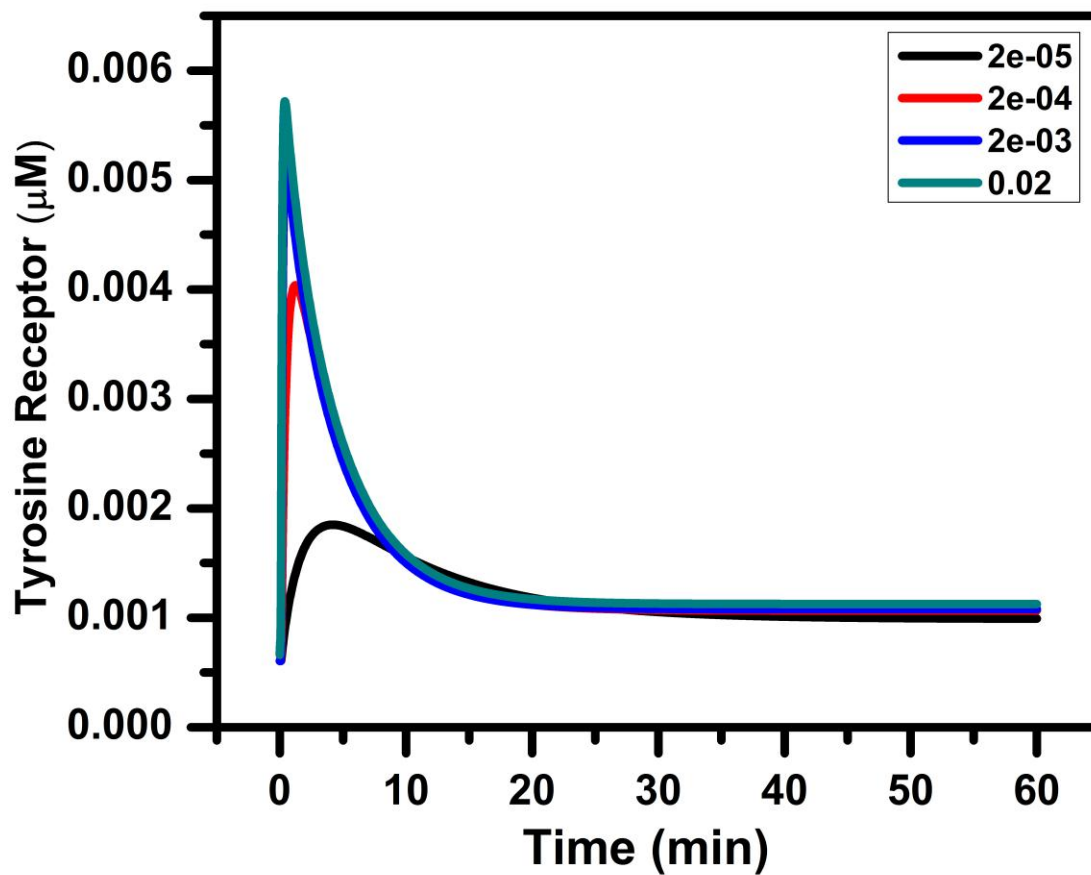
**Table S3. List of enzyme kinetic parameters involved in the eNOS signalling cascade**

S.No	Specification	Enzymatic Reaction	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{s}^{-1}$ )
1	GTP.Gs-a-bind-AC-Cyclase	$\text{GTP.Gs-}\alpha - \text{AC} + \text{ATP} \leftrightarrow$ $\text{GTP.Gs-}\alpha - \text{AC} - \text{ATP} \rightarrow \text{cAMP}$	20*	18*
2	Activated PI3k kinases	$\text{Active-PI3Kinase} + \text{PIP2} \leftrightarrow \text{PI3K-}$ $\text{PIP2} \rightarrow \text{PIP3}$	38 <sup>[1]</sup>	10 <sup>[1]</sup>
3	PTEN Phosphatase	$\text{PIP3} + \text{PTEN} \leftrightarrow \text{PIP3-PTEN} \rightarrow \text{PIP2}$	0.8 <sup>[1]</sup>	6.2 <sup>[1]</sup>
4	PIP3-PDK1 phospho-T308	$\text{PIP3-AKT} + \text{PIP3-}$ $\text{PDK1} \leftrightarrow \text{PIP3AKT-}$ $\text{PIP3PDK1} \rightarrow \text{PIP3-Akt-T308}$	0.023 <sup>[4]</sup>	5.8 <sup>[4]</sup>
5	PIP3-PDK2 phospho-S473	$\text{PIP3-Akt-T308} + \text{PIP3-}$ $\text{PDK2} \leftrightarrow \text{PIP3AktT308-}$ $\text{PIP3PDK2} \rightarrow \text{PIP3-Akt-S473}$	0.99 <sup>[4]</sup>	9.7 <sup>[4]</sup>
6	PP2A Dephospho-T308	$\text{PIP3-Akt-}$ $\text{S473} + \text{PP2A} \leftrightarrow \text{PIP3AktS473-}$ $\text{PP2A} \rightarrow \text{PIP3-Akt-T308}$	0.26 <sup>[4]</sup>	1.2 <sup>[4]</sup>
7	PP2A Dephospho-S473	$\text{PIP3-Akt-T308} + \text{PP2A} \leftrightarrow$ $\text{PIP3AktT308-PP2A} \rightarrow \text{PIP3-AKT}$	220 <sup>[4]</sup>	19.2 <sup>[4]</sup>
8	PIP3-AKT-T308-S473-eNOS-phospho	$\text{PIP3-Akt-S473} + \text{eNOS1} \leftrightarrow \text{PIP3-}$ $\text{Akt-S473-eNOS1} \rightarrow \text{eNOS2-po4}$	0.1*	0.1*
9	PLC $\gamma$ _hydrolysis	$\text{PLC}\gamma_{\text{Active}} + \text{PIP2} \leftrightarrow \text{PLC}\gamma_{\text{-}}$ $\text{PIP2} \rightarrow \text{DAG} + \text{IP3}$	9.9 <sup>[7*]</sup>	10 <sup>[7*]</sup>
10	Inactive_PLC $\gamma$ _Ca_hydrolysis	$\text{PLC}\gamma_{\text{Ca}} + \text{PIP2} \leftrightarrow \text{PLC}\gamma_{\text{-Ca-}}$ $\text{PIP2} \rightarrow \text{DAG} + \text{IP3}$	97 <sup>[7*]</sup>	14 <sup>[7*]</sup>
11	Active_PLC $\gamma$ _Ca_hydrolysis	$\text{PLC}\gamma_{\text{Ca Active}} + \text{PIP2} \leftrightarrow \text{PLC}\gamma_{\text{-}}$ $\text{Ca-Active_PIP2} \rightarrow \text{DAG} + \text{IP3}$	19 <sup>[7*]</sup>	57 <sup>[7*]</sup>

12	PLC_β-hydrolysis	PLC_β-Active+PIP2 ↔ PLC_β - PIP2 → DAG+IP3	98 <sup>[16*]</sup>	5 <sup>[16*]</sup>
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Kinetic parameters taken from indicated references are optimized and adapted to the present simulation conditions, hence may slightly vary from the literature value. Parameters indicated in [\*] are results of fitting under the model as described in Materials and Methods.

**Figure. S1.** Dose dependent effect of VEGF on tyrosine receptor phosphorylation



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