Congestive feedback uniformly partitions red blood cells in the zebrafish microvasculature

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Vascular networks are widely thought to be organized to traffic oxygen and dissolved chemicals to tissues as efficiently as possible. Yet the kinetics of oxygen disassociation require that red blood cells travel through each capillary at approximately the same rate, and it is not known how vascular networks realize uniform flow across fine vessels distributed at different distances from the heart. Here we study the trunk vessels of developing zebrafish embryos as a model for red blood cell partitioning in real vascular systems. Experimental measurements in a live zebrafish embryo show that fluxes are highly uniform between different vessels. Red blood cells partially clog the vessels that they flow through, so there is congestive feedback between the number of cells in a vessel and the flux into that vessel. Precisely controlling the congestive feedback creates uniform flow across distant segmental vessels. Although eliminating congestion is a key element in the design of efficient transport networks, this work suggests that microvasculature networks may target uniformity, rather than efficiency, and that they do so by creating and controlling congestion.
Plants and animals rely on vascular networks for transport of oxygen and nutrients \[^1,3^\]. In animals gas exchange within the network occurs primarily in peripheral vessels, including capillaries \[^1^\]. Measurements have shown remarkable consistency in flow rates between capillaries in different tissues and organisms \[^4,6^\]. Indeed if flow rate is too low, tissue surrounding a capillary will not receive enough oxygen, but if too high, then the total energy cost of transport will increase. Models of vascular networks as symmetric branching trees \[^7,9^\] place all capillaries the same distance from the heart. In a symmetric vascular network all vessels of the same radius receive identical fluxes, but in real asymmetric networks capillaries are placed at different distances from the heart; no existing theory explains then how flow is uniformly divided between capillaries. We studied the asymmetric vasculature of the embryonic zebrafish trunk as a model for the partitioning of red blood cells (RBCs) among micro-vessels since the architecture \[^10^\] and RBC fluxes \[^6^\] through the entire vascular network can be completely mapped.

**Results**

Oxygenated RBCs flow into the zebrafish trunk via the dorsal aorta (DA) and flow out via the posterior cardinal vein (PCV). The peripheral network consists of a series of intersegmental vessels (Se) spanning aorta and cardinal vein like the rungs of a ladder (Fig. 1A). Ses are broken into SeAs (arteries) and SeVs (veins) depending on whether they connect to the DA or PCV. Although embryonic tissues receive oxygen primarily by diffusion through the skin \[^11, 12^\], vascular transport becomes essential to embryo development after 2.5 weeks \[^13^\].

The first Se is much closer to the heart than the last Se so existing theory predicts that it will short-circuit the network. Since flow is laminar within the vessels \[^14, 15^\], we can model the trunk vessels as an hydraulic resistor network \[^2^\]. In this network the resistance, \( R \), of each vessel in the network is related to its length, \( \ell \) and radius \( r \) by the Hagen-Poiseuille law \[^16^\]:

\[
R = \frac{8\mu_{wb} \ell}{\pi r^4},
\]

where \( \mu_{wb} \) is the whole blood viscosity \( \mu_{wb} \approx 5 \text{ cP} \). \[^17^\] Solving for the flows in the network requires calculating the pressures at each point where a segmental artery branches off from the aorta. These pressures are obtained by applying Kirchoff’s First Law at each branching point. The full calculation is given in the Supplementary Information (SI) but we can derive an approximate analytic expression for the pressures and fluxes by assuming all SeAs have the same radius and length, as do all DA segments between Se. Then if the
respective conductances are \( \kappa_1 \) for DA segments and \( \kappa_2 \) for SeAs, flux conservation at the \( j_{th} \) branching point gives

\[
-k_1 p_{j-1} + (2k_1 + k_2)p_j - k_1 p_{j+1} = 0, \quad j = 2, ..., n
\]

(S1)

where \( p_j \) is the pressure where the \( j_{th} \) SeA branches off from the aorta. The solutions of this equation are of form

\[
p_j = C_+ \xi_j^+ + C_- \xi_j^- \quad \text{where} \quad C_\pm \quad \text{are constants and} \quad \xi_\pm \quad \text{are roots of the characteristic polynomial} \quad -1 + (1 + \frac{k_2}{k_1})\xi - \xi^2 = 0.
\]

Since \( \xi_+ \xi_- = 1 \), and \( \xi_+ + \xi_- > 2 \), we can order the roots so that \( 0 < \xi_- < 1 < \xi_+ \). \( \xi_+ \) gives exponentially increasing pressure, so the \( C_+ \xi_+^j \) term can only be non-negligible for a small number of distal Se. Thus the model predicts that fluxes decay exponentially with distance along the trunk (Fig. 1B). Since \( \kappa_2 \ll \kappa_1 \), the decay factor is approximately \( 1 - \sqrt{\frac{2\kappa_2}{2\kappa_1}} = 0.86 \) per Se. A model that incorporates the detailed geometry of the vascular network shows the same exponential decay, with a 21 fold difference between the first (rostral) and last (caudal) SeA (Fig. 1B). A model of tissue oxygenation[18] shows corresponding decrease in tissue partial oxygen pressures from rostral to caudal trunk (Fig. 1C and SI).

RBC fluxes are nearly uniform in real zebrafish intersegmental vessels. We measured the flow of DsRed-tagged RBCs in a 4 day post-fertilization transgenic Tg(gata1:dsRed; fli1a:EGFP) zebrafish that was anesthetized in 0.016% tricaine and embedded in 1% low gelling temperature agarose on a microscope slide. RBC movements were recorded under 10× magnification using a Zyla sCMOS camera on a Zeiss Axio Imager A2 fluorescent microscope. Although fluxes in individual vessels varied in time, mean fluxes varied little from vessel to vessel (Fig. 1D, slope 95% confidence interval is [-0.2590,0.0345]). The discrepancy between the hydraulic resistor network model and data from real zebrafish suggests that additional physical effects control red blood cell partitioning between Se.

We hypothesized that there is congestive feedback between the number of RBCs in a vessel and the flux of new RBCs entering the vessel. RBCs partially clog the Se vessels (vessel and cell diameters are both = 6 \( \mu \)m). To model this congestion we adopted a model previously used for the traffic of droplets in microfluidic channels[19, 20]. Specifically the resistance of the capillary with \( n \) RBCs is modeled as

\[
R = \bar{R} + n\frac{2\mu_{pl}\ell_{RBC}}{r^3\pi d}.
\]

(S2)

(See SI Appendix for a derivation of Eqn. (S2)) where \( \bar{R} \) is the original resistance given by Hagen-Poiseuille law, \( n \) is the number of RBCs, \( \mu_{pl} \approx 1 \) cP is the plasma viscosity [17], \( \ell_{RBC} \) is the length of a RBC, \( r \) is the radius of SeA, and \( d \) is the distance between the RBC and the endothelial wall. Eqn (S2) predicts negative feedback: if constant
pressure is maintained across the Se, then the probability of further cells entering the vessel decreases as \( n \) increases (Fig. 2A).

Given constant pressure difference across the Se, Eqn \[S2\] predicts that if \( u_c \) is the velocity of RBCs within the SeA, \( 1/u_c \) should increase linearly with \( n \). In real vessels the heart beat and complex vascular geometry lead to variations in the pressure difference, creating large fluctuations in the cell velocities. However, observed time-averaged flows in each of the 12 intersegmental arteries (SeA) well support the linear fit (Fig. 2B). The increase in \( 1/u_c \) per cell gives a measure of the resistance per cell, which we denote henceforth as feedback strength \( \alpha_c \equiv \frac{2\eta \ell_{AN} C}{\pi d} \). A priori estimation of \( \alpha_c \) is difficult because it depends sensitively on \( d \). \( d \) represents the thickness of the lubricating layer of plasma between RBC and vessel wall. Although lubricating layers feature in many models of RBC movement through microchannels[21, 24], their thickness can not be measured directly. Instead we use the regression lines for each SeA to measure \( \alpha_c \) and thus \( d \). We found that \( \alpha_c \) decreases from the rostral to caudal SeA (Fig. 2C), and \( d \) increases from 12.3 nm to 1.15 \( \mu \)m (Fig. 2C, inset), consistent with measured range of thicknesses of the glycocalyx coating[25, 26].

To probe how variation in the amount of congestion between Se vessels effects RBC partitioning we studied a reduced model of the vascular network. We built a mean field model for the flows in a model network including only the first and last Se (Fig. 3A). Suppose in each capillary the RBCs are well mixed and they divide in proportion to flow rates at branching points. Then the red blood cell concentration (\#/volume) will be the same in all vessels. Let \( R_i \) be the modified resistance of \( i_{th} \) vessel according to Eqn \[S2\]. Then conserving fluxes at each node of the network gives:

\[
F = \frac{p_1 - p_2}{R_1} + \frac{p_1}{R_2} \frac{p_1 - p_2}{R_1} = \frac{p_1}{R_1} \frac{p_2}{R_3} + \frac{p_2}{R_4},
\]

where \( F \) is the influx into the first node. We can solve Eqn \[S3\] by Crammer’s rule. Defining a matrix determinant

\[
\Delta = - \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \left( \frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4} \right) + \frac{1}{R_1^2},
\]

the fluxes in each vessel are given by

\[
Q_1 = \frac{-F}{\Delta R_1} \left( \frac{1}{R_3} + \frac{1}{R_4} \right),
Q_2 = \frac{-F}{\Delta R_2} \left( \frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4} \right),
Q_3 = \frac{-F}{\Delta R_3 R_1},
Q_4 = \frac{-F}{\Delta R_4 R_1},
\]
Here $R_2, R_4$ are modified resistances of vessels 2, 4, $R_1, R_3$ are resistances of vessels 1, 3. Of particular interest is the ratio of the flux between the last and first Se:

$$
\frac{Q_4}{Q_2} = \frac{\bar{R}_2 + V_2 \rho \bar{\alpha}_2}{\bar{R}_4 + V_4 \rho \bar{\alpha}_4} \left(1 + \frac{R_1}{R_3} + \frac{R_1}{R_4 + V_4 \rho \alpha_4}\right)^{-1}
$$

(S6)

where $\alpha_2, \alpha_4$ are respectively the values of $\alpha_c$ in the first and last Se, $\bar{R}_2, \bar{R}_4$ are the uncongested resistance calculated by the Hagen-Poiseuille law in vessels 2, 4, and $V_i$ is the volume of vessel $i$. We use $Q_4/Q_2$ as a measure flux uniformity.

Many of the parameters in Eq. (S6) are tightly constrained: the dimensions of the two Se are similar (i.e. $\bar{R}_2 \approx 2\bar{R}_4$ and $V_2 \approx 2V_4$), moreover, since the vessel network extends during the the growth of the embryo and supplies the tail fin in adult zebrafish[27, 28], the caudal DA has to maintain the same radius as the rostral DA, leading to $R_1 \approx 11R_3$. Thus the second factor $(1 + \frac{R_1}{R_3} + \frac{R_1}{R_4 + V_4 \rho \alpha_4})^{-1}$ has an upper bound $\frac{1}{12}$ independent of $\alpha_4$. Therefore the only parameters that can be used to increase $Q_4/Q_2$ and eliminate the short-circuit are the relative sizes of $\alpha_2$ and $\alpha_4$. In particular $Q_4/Q_2$ is largest if $\alpha_2 \gg \alpha_4$. To meet this condition the lubrication layer must be thinner in the first Se, preventing this Se from short-circuiting the network. Thus uniformization of flow requires smaller lubrication layer thicknesses in vessels close to the heart. However there is then a trade-off between uniformity and the transport efficiency, measured by the dissipation:

$$
D_{\text{network}} = D_{\text{aorta}} + D_{\text{SeA}} + D_{\text{RBC}}
$$

(S7)

(See SI Appendix for derivation) Here $l_i$ is the length of the vessel $i$, $r_a$ is the radius of DA, and $r_c$ is the radius of SeA. To compare equivalent networks as we vary $\alpha_2$ we also vary $F$ to keep the total flux through the SeAs ($Q_2 + Q_4$) constant. Since total flux is held constant $D_{\text{RBC}}$, the dissipation within the lubricating layers surrounding each cell increases with $\alpha_2$, driving up the network dissipation. Thus increasing uniformity by increasing $\alpha_2$ decreases the transport efficiency of the network (Fig. 3B).

**Discussion**

Incorporating congestive feedback that varies in strength from first to last Se leads to optimally uniform distribution of fluxes, matches real zebrafish data (Fig. 1D), and leads to uniform oxygen perfusion in the trunk (Fig. 4A). To
test whether variation in congestive feedback is adaptive, we modeled different variations in feedback (that is varying the total change in $\alpha_c$ between first and last Se) and computed for each network the coefficient of variation of the RBC fluxes. The simulation is based on a discrete droplet model\[19]. Initially all RBCs are placed equi-distantly in the aorta. At each step we calculate the resistance for each capillary by Eqn (S2), and solve for each of the flow rates $Q_j, j = 1, \ldots, 2n$ for each vessel. Then we let all RBCs travel according to the predicted plasma speed. If a RBC arrives at a node it stops and decides which capillary to enter by a Bernoulli process with probability determined by the flow rate ratio of the capillaries. Flows are then recomputed. If a RBC arrives at the end of the tail or any SeA it returns to the first node. We found that near uniform flux can be achieved only over a narrow range of feedback variations. Too little variation, and the first Se short-circuits the network. Too much, and the caudal vessels receive more flow than rostral vessels. Optimal variation depends on hematocrit (concentration of RBCs) and defines a curve in $(\alpha_c, \rho)$ space. We found that the empirical feedback strength is close to the optimal value for the real zebrafish hematocrit\[29] (Fig. 4B).

More uniform partitioning of RBCs between SeAs is possible but altering physiological parameters creates trade-offs in the transport efficiency. For example decreasing $\rho$ increases RBC uniformity, but at the cost of increasing the dissipation. On the other hand, the zebrafish vascular network is far from minimizing transport costs: in particular either increasing $\rho$ or decreasing the feedback variation would reduce dissipation three-fold or more (Fig. 4C). Thus transport efficiency alone does not appear to be the main objective function for the zebrafish trunk network.

**Conclusion**

Our direct measurements showed that there is a 93 fold variation in the thickness of the plasma-layer surrounding RBCs between first and last Se, allowing wide control of the amount of congestion created by RBCs in different vessels. This variation is consistent with previous measurements of the thickness of glycocalyx\[26] - the protein coat that is thought to lubricate RBC flow in fine vessels, suggesting that glycocalyx thickness tuning may provide a general mechanism for uniformizing RBC fluxes\[26].

Additionally our mechanistic analysis shows the sensitivity of RBC partitioning to different forms of perturbation, which in real micro vascular networks may include morphological mutations\[13], infarcts\[30], and micro-aneurysms\[31]. We studied RBC partitioning under variation in the spacing of Se\[10] and the introduction of a DA-PCV shunt\[32]. We found that under a wide range of Se spacing variability, RBC fluxes remained uniform (SI). However, when a
shunt is introduced between DA and PCV close to the heart the absence of congestive feedback in the shunt causes it to short-circuit the vascular network (SI). Shunt formation is lethal in embryos, and our model shows that it creates conditions under which congestive re-distribution of RBCs is impossible.

In conclusion, the observed uniform flow in the zebrafish trunk within Se vessels results from the tuned congestive feedback of RBCs. Vascular networks have been extensively studied as models of optimal networks that minimize transport costs. Congestion within transport networks hinders efficient transport, and extensive work has targeted congestion-elimination in human-built networks for transporting data or vehicle traffic. Here we have shown that tuning, rather than eliminating, congestion is a key feature of how uniform flow is maintained at the level of the finest vessels; Moreover by exhibiting direct trade-offs that exist between achieving uniformity and minimizing the cost of transport, we show that efficient transport does not, though uniformity of flux may, provide a central organizing principle at the finest scales of the vascular network.

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Author contributions

S-S.C., S.T., V.S., S-P.L.H. and M.R. designed research and wrote the paper. S-S.C., S.T., Y-H.L., S-P.L.H. and M.R. performed the research.

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Fig. 1

(A)

(B)

(C)

(D)
Microvessels in the zebrafish trunk network exhibit uniform flow in violation of existing hydraulic models. (A) 3 day post-fertilization zebrafish embryo trunk network and wiring diagram. (B) Uniform perfusion of the trunk requires that each vessel should receive the same RBC flux, but a hydrodynamic model for the flow of whole blood through this network predicts that fluxes through Se decrease exponentially with distance from the heart. Shown: numerical solution using real zebrafish geometric parameters (black curve), and from a reduced analytical model (gray curve). (C) Decreasing fluxes between Se leads to non-uniform oxygen supply across the embryo from the Se vessels according to a PDE model for oxygen diffusion. Bands of high $ppO_2$ correspond to Se locations. Zebrafish CT image reproduced from\[33\] (D) Measured RBC fluxes in the zebrafish embryo are almost uniform across all Se (gray line shows regression). A model incorporating feedback variation agrees well with the data (black curve). The simulation is performed with 990 cells circulating for 1000 seconds, and with feedback that decreases linearly from rostral to caudal Se, matching the rostral and caudal $\alpha_c$ measurements in Figure 2. Shown: mean flux over 1s intervals from 38.2 s interval per Se (black circle). Black bars denote 95% confidence intervals on fluxes.
Fig. 2

(A) RBC number in Se

(B) RBC number

(C) Se No.
Fig. 2. RBCs clog vessels creating congestive feedback on flow. (A) As the number of RBCs in a single Se increases so does the resistance (gray curve), which lowers the probability (black curve) of additional RBCs entering the Se. (B) Lubrication theory predicts a linear relation (gray line) between reciprocal of RBC velocity and the number of RBCs in each Se. The $y$-intercept is determined from the theoretical plasma velocity in a network with no RBCs. Shown: data from the 11th SeA (black dots). (C) In real zebrafish the resistance per RBC ($\alpha_c$) decreases from rostral to caudal Se. Corresponding gap thicknesses, $d$, range between 12.3 nm and 1.15 $\mu$m (inset panel). Gray line: linear regression of $\alpha_c$ against vessel number. Shown: data with 95% confidence intervals calculated by bootstrapping with 1000 samples.
Fig. 3

(A) Diagram showing a network of paths with labels $2l_2$, $2l_4$, $Q_1$, $Q_2$, $Q_3$, and $Q_4$.

(B) Graph plotting normalized dissipation against resistance per cell ($10^{-5}$ g/μm$^4$ s) with two lines indicating $Q_4/Q_2$ and normalized dissipation.
Fig. 3. A reduced vascular network model exposes trade-offs between uniformity and transport efficiency. (A) Diagram of the reduced model of the network showing vessel lengths $l_i$, fluxes $Q_i$, and radii $r_i$. (B) Increasing the feedback strength $\alpha_2$ increases flux uniformity, measured by the ratio of fluxes in the last and the first Se (black curve), but also increases dissipation (gray curve), if the total flux through all SeA is maintained.
Fig. 4. Congestive feedback is tuned to uniformize flow across different Se. (A) A model of oxygen diffusion predicts uniform oxygen concentrations when feedback is incorporated. Bands of high $ppO_2$ correspond to Se locations. (B) Dependence of flux uniformity upon controllable parameters is explored by allowing hematocrit, $\rho$, and feedback variation between first and last Se, $\Delta$feedback, to vary independently and computing the coefficient of variation (CV) for flow in all Se. Flux uniformity is achieved only within a narrow manifold of values of hematocrit and feedback variation. The empirical values (red dot) lie close to this optimal manifold. (C) Higher uniformity can be achieved if $\rho$ is decreased (white arrow) but doing so leads to rapid increase of the normalized dissipation, decreasing transport efficiency. In this calculation we altered $\rho$ and feedback variation, and varied the total flux into the trunk to keep the total RBC flow through the fine vessels constant. The dissipation is normalized by its value for the real zebrafish parameters.
Supplementary Information for: “Congestive feedback uniformly partitions red blood cells in the zebrafish microvasculature”

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Abstract

This Appendix contains the following Supplementary Information:

I Hydraulic network model of red blood cell fluxes in the absence of hydrodynamic feedbacks

II Model for oxygen perfusion

III Model for hydrodynamic feedback due to passage of a red blood cell through a Intersegmental vessel

IV Mean field model for a two-vessel network

V Effect of network perturbation upon red blood cell partitioning

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1 HYDRAULIC NETWORK MODEL OF RED BLOOD CELL FLUXES IN THE ABSENCE OF HYDRODYNAMIC FEEDBACKS

I. HYDRAULIC NETWORK MODEL OF RED BLOOD CELL FLUXES IN THE ABSENCE OF HYDRODYNAMIC FEEDBACKS

FIG. S1: Topology of the zebrafish trunk vascular network.

To solve for flow rates in a system without red blood cells (RBC), we model the trunk vasculature as having a ladder-topology with the two rails of the ladder representing the dorsal aorta (DA) and posterior cardinal vein (PCV) of the trunk, spanned by rungs representing the intersegmental vessels. Another vessel, the dorsal longitudinal anastomotic vessel (DLAV) bridges between intersegmental vessels, but in our segments few RBCs were observed travelling through the DLAV so we neglect it in our model. Since the network is symmetric across the midplane of the rungs, we can assume that the fluid pressure is the same at the mid-point of each rung. We can therefore build a model that represents just one half of the network, comprising the DA and the segmental arteries only. Our model contains $2n$ blood vessels, indexed $i = 1, 2, 3 \ldots 2n$, with odd numbers indexing successive segments of the DA, and the even numbers representing intersegmental arteries (SeA). For a 4 day post fertilization (4 dpf) zebrafish embryo $n = 12$. Denote by $r_i$, $l_i$, and $R_i$ be the radius, length, and the resistance of the $i$-th vessel. Then since the flow in the network is laminar, the flux through each vessel is given by the Hagen-Poiseuille law\cite{1}, namely the total flux of blood through a vessel is proportional to the pressure difference between its two ends, $\Delta p_i$: $Q_i = \Delta p_i / R_i$. The constant of proportionality, $R_i$, is called the hydraulic resistance of the vessel and is related to its dimensions and the blood viscosity, $\mu$, by:

$$R_i = \frac{8 \mu l_i}{\pi r_i^4} \quad (S1)$$

Here we use the whole blood viscosity $\mu \approx 5 \text{ cP} \quad [2]$. Let $p_j$, $j = 1, 2, \ldots n$ be the pressures at the $j$th junction of the network (i.e. where vessel $2j - 1$ and $2j$ branch). Assume that the total flux of blood into the trunk is $F$. All of the intersegmental arteries and the terminal
segment of the DA have the same pressure where they meet the symmetry axes, without loss of generality we can set this common pressure to be 0.

According to Hagen-Poiseuille law the whole blood flux, \( Q_i \), within the \( i \)th vessel is given by formulas:

\[
Q_{2i-1} = \frac{p_i - p_{i+1}}{R_{2i-1}}, \quad i = 1, 2, \ldots n
\]
\[
Q_{2i} = \frac{p_i}{R_{2i}}, \quad i = 1, 2, \ldots n.
\]

(S2) with different formulas for the segments of the DA and for intersegmental vessels. To compute the unknown nodal pressures we apply Kirchoff’s first law (conservation of mass) at each branch point:

At the 1st node conservation of mass gives:

\[
F = Q_1 + Q_2
\]

(S3) while at the \( j \)th node, \( 2 \leq j \leq n \) conservation of mass gives:

\[
Q_{2j-3} = Q_{2j-1} + Q_{2j}.
\]

(S4) If we substitute Eq.(S1,S2) into Eq.(S3,S4) we will get \( n \) equations for the \( n \) unknown nodal pressures: \( p_1, \ldots, p_n \). Then we can solve for \( p_j, \ j = 1, \ldots, n \) and obtain the flow rates \( Q_i \) by Eq.(S1,S2). Vessel lengths were measured from a 4 day post-fertilization transgenic \( Tg(gata1:dsRed; fli1a:EGFP) \) zebrafish that was anesthetized in 0.016% tricaine and embedded in 1% low gelling temperature agarose on a microscope slide and analyzed under 10× magnification using a Zyla sCMOS camera on a Zeiss Axio Imager A2 fluorescent microscope:
### I HYDRAULIC NETWORK MODEL OF RED BLOOD CELL FLUXES IN THE ABSENCE OF HYDRODYNAMIC FEEDBACKS

| \(i\) | \(l_i (\mu \text{m})\) | \(i\) | \(l_i (\mu \text{m})\) |
|---|---|---|---|
| 1 | 150 | 2 | 151 |
| 3 | 183 | 4 | 141 |
| 5 | 178 | 6 | 156 |
| 7 | 174 | 8 | 160 |
| 9 | 155 | 10 | 172 |
| 11 | 175 | 12 | 166 |
| 13 | 169 | 14 | 163 |
| 15 | 166 | 16 | 156 |
| 17 | 174 | 18 | 146 |
| 19 | 168 | 20 | 138 |
| 21 | 168 | 22 | 123 |
| 23 | 169 | 24 | 113 |

We found little variation in radius between different intersegmental vessels and between different segments of the DA. Accordingly, we set \(r_{2i-1} = 7 \mu \text{m}\) and \(r_{2i} = 3 \mu \text{m}\). Solving the system of linear equations numerically gives the result shown in Fig. 1B.

**Asymptotic formula for the flux in intersegmental vessels**

We noticed that in our numerical solution of the linear system in the previous section the largest segmental vessel flux was in the first intersegmental vessel, with successive intersegmental vessels receiving a smaller and smaller fraction of the flow. Correspondingly we developed an asymptotic formula for the flux in each vessel, in which the analytical dependence of flow upon parameters like length and radius would be clear, by assuming that each segment of the DA has the same hydraulic resistance, and that each Se has the same resistance. Specifically, we assign to each segment of the DA a resistance calculated by the average spacing of SeAs, 169 \(\mu \text{m}\), and assigned to each SeA the average SeA length 149 \(\mu \text{m}\). It will prove useful to work with the vessel conductances, which are simply the reciprocal of their resistance. In our asymptotic model all segments of the DA have the same conductance \(\kappa_1 = 9.4 \times 10^5 \mu \text{m}^4 \text{s/g}\), and all Se arteries have the same conductance \(\kappa_2 = 3.9 \times 10^4 \mu \text{m}^4 \text{s/g}\).
Then Eq.(S4) reads:

$$-\kappa_1 p_{i-1} + (2\kappa_1 + \kappa_2) p_i - \kappa_1 p_{i+1} = 0, \quad i = 2, \ldots, n$$  \hspace{1cm} (S5)

This is a second order recursion relation with constant coefficients. Its general solution is:

$$p_i = C_+ \xi_+^i + C_- \xi_-^i,$$  \hspace{1cm} (S6)

where $\xi_\pm$ are the roots of the auxiliary polynomial $\xi^2 - (2 + \lambda)\xi + 1 = 0$ in which there is a single dimensionless parameter, representing the relative conductivity of DA segments and SeAs

$$\lambda = \frac{\kappa_2}{\kappa_1} = 0.04.$$  \hspace{1cm} (S7)

This equation has two roots, with $\xi_+ > 1$ and $\xi_- < 1$. In general $C_+$ and $C_-$ must both be non-zero to satisfy our boundary conditions (namely $p_{n+1} = 0$ and $F = \kappa_2 p_1 + \kappa_1 (p_1 - p_2)$). However the two components give rise to exponentially growing and decaying pressures respectively, and since exponentially increasing pressure is unphysical, we may assume that the first term is negligible, except potentially in a small boundary layer region consisting of the very last nodes in the network. Therefore over most of the nodes we may assume that:

$$p_i = C_- \xi_-^i,$$  \hspace{1cm} (S8)

where (from our auxiliary equation) $\xi_- = 0.81$. Then by the Hagen-Poiseuille law, the whole blood fluxes in the intersegmental vessels are given by the formula:

$$Q_{2i} = \kappa_2 p_i \equiv C \xi_-^i$$  \hspace{1cm} (S9)

for some constant $C \equiv C_-$, denoting exponential decreasing fluxes between successive Se. $C$ can be computed from Eqn.(S3). Despite the simplification in geometry, the analytic formula agrees very well with the solution to the full system of linear equations (Fig. 1B in the main text). Additionally, since for any $\lambda > 0$, it is impossible to organize an auxiliary polynomial without one having one root $\xi_- < 1$, we see that exponential decay in fluxes is an inescapable feature of the ladder-like geometry of the trunk vasculature.

II. MODEL FOR OXYGEN PERFUSION

It is thought that diffusion of oxygen through the skin is generally sufficient to supply zebrafish tissues with oxygen[3]. However, circulatory system looping defects are typically
lethal by 17.5 d.p.f.[4], suggesting oxygen transport by the circulatory system contributes to oxygen supply relatively early in embryonic development. To determine whether oxygen diffusion through tissues might compensate for unequal partitioning of RBCs between microvessels, we directly model the diffusive transport of oxygen through the zebrafish torso using an oxygen reaction-diffusion model[5]. Within the zebrafish trunk the oxygen partial pressure, $P$, obeys a reaction diffusion equation:

$$D \alpha \nabla^2 P = -C + S$$  \hspace{1cm} (S10)

where $S$ represents the distribution of oxygen sources, and $C$ the rate of oxygen consumption per volume of tissue, $\alpha$ is the solubility of oxygen and $D$ is the diffusivity of dissolved oxygen. We solved this Partial Differential Equation by creating a Finite Element Model with first order tetrahedral elements implemented in Comsol Multiphysics (Comsol, Los Angeles). We extracted the geometry of the trunk muscles from the Zebrafish Anatomy Portal [6], and the distribution of intersegmental vessels within the trunk from the Zebrafish Vascular Atlas [7]. The parameter $D\alpha$, sometimes called the oxygen permeability, was measured by [5] to be: $D\alpha = 8 \times 10^{-14}$ m$^2$/s (mmHg). We modified the oxygen consumption rate found by [5] to: $C = 5.1 \times 10^{-4} P / 40$ ml oxygen/ml tissue mmHg). This formula agrees with the rate measured by [5] when $P = 40$ mmHg, but is smaller at lower oxygen partial pressures, representing the regulation of tissue oxygen consumption with oxygen availability. The source term represents the rate of oxygen release from blood, and is compactly supported on the intersegmental vessels. We used the following conversion factors: assuming that a RBC enters a vessel with all of its hemoglobin molecules bound to oxygen, and that all of this oxygen is released, the total oxygen release from each intersegmental vessel can be computed from:

$$\text{rate of oxygen release (ml/s)} = 1.39 \times 10^{-10} \times \text{RBC flux (in cells/s)}$$  \hspace{1cm} (S11)

we distribute this flux uniformly across the length of each segmental artery. We apply no-flux boundary conditions on the boundaries of the trunk. By applying this boundary condition, our model represents only the contribution of oxygen transport in the circulatory system to tissue oxygen levels. Oxygen diffusing through the skin of the fish will increase the oxygen partial pressure everywhere by a constant amount, but does not affect the absolute differences in partial pressure that our model is designed to measure.
III. MODEL FOR HYDRODYNAMIC FEEDBACK DUE TO PASSAGE OF A RED BLOOD CELL THROUGH A INTERSEGMENTAL VESSEL

![Diagram of hydrodynamic feedback model](image)

**FIG. S2:** Diagram for the hydrodynamic feedback model.

Red blood cells (RBCs) are large enough to almost plug the intersegmental vessels that they pass through. This leads to a large increase in the hydraulic resistance of a Se that contains RBCs and corresponding decrease in fluxes. To model the decrease in flux, we adapted a model created by [8] to describe the effect of droplet traffic upon the resistance of a microfluidic channel. More complex models for movement of red blood cells in capillary have been devised [8-11], but similarly predict hydrodynamic feedback that increases with the number of RBCs in the capillary.

To model the change of hydrodynamic resistance in a vessel due to a RBC we assume that the RBC within the SeA deforms into the shape of a cylinder with length \( l \) and radius \( a - d \) where \( a \) is the inner radius of the capillary and \( d \) is the thickness of the lubricating layer of fluid between the RBC and vessel wall (Fig. S2). We assume that \( d \ll a \), i.e. that the RBC almost fills the vessel. Suppose the RBC is moving with speed \( u_c \) and the mean velocity of plasma in front of and behind the RBC is \( u_p \). Then the flow rate in the segmental vessel is: \( Q = \pi a^2 u_p \). We consider a frame moving with the RBC: if, in this frame, the average speed of the blood plasma contained in the gap between RBC and vessel wall is \( u_g \), then
IV MEAN FIELD MODEL FOR A TWO-VESEL NETWORK

conservation of mass requires that:

\[(u_p - u_c)\pi a^2 = (\pi a^2 - \pi(a - d)^2)u_g \approx 2\pi ad u_g\]  \hspace{1cm} (S12)

If \(d \ll a\), then this formula can be satisfied only if:

\[u_p \approx u_c\]  \hspace{1cm} (S13)

i.e. the speed of the cell matches the mean velocity of plasma within the vessel.

Now let \(\Delta p\) be the pressure across the entire vessel and \(\Delta p_c\) the difference in pressure between the rear and front of the RBC. We can think of \(\Delta p_c\) as the pressure that is utilized in moving the RBC through the tightly fitting vessel. Balancing the total pressure force on the RBC against the viscous drag on the side walls of the RBC, we determine that:

\[\Delta p_c \pi a^2 \approx \frac{2\pi a \ell_{RBC} \mu u_c}{d} \Rightarrow \Delta p_c = \frac{2\mu \ell_{RBC} u_c}{ad} = \frac{2\mu \ell_{RBC} u_p}{ad}.\]  \hspace{1cm} (S14)

Since we modeling cell movement and plasma flow separately, the viscosity in this formula should be the viscosity of plasma: \(\mu \approx 1\) cP [2]. Now suppose we have \(n\) cells in the vessel. Then the pressure drop available to drive fluid through the vessel is \(\Delta p - n\Delta p_c\) (note that since \(\Delta p_c\) depends on the speed of the cells, it will also vary with \(n\)). Since the resistance of the vessel is given by Eqn (S1) the flux may be written as:

\[Q = \pi a^2 u_p = \frac{\Delta p - n\Delta p_c}{\bar{R}}\]  \hspace{1cm} (S15)

where \(\bar{R}\) denotes the resistance with no RBC inside the vessel, and \(R\) denotes the resistance adjusted by the effect of RBCs. In deriving this formula we use the fact that \(n\ell_{RBC}\), the total length of the vessel occupied by RBCs is much less than the vessel length \(\ell\), so the resistance of the parts of vessel not occupied by RBCs is essentially identical to the resistance of a vessel that contains no RBCs.

Finally we substitute for \(\Delta p_c\) from Eqn (S14) to obtain:

\[Q = \pi a^2 u_p = \frac{\Delta p}{\bar{R} + n\frac{2\mu \ell_{RBC}}{a^2 \pi d}}.\]  \hspace{1cm} (S16)

IV. MEAN FIELD MODEL FOR A TWO-VESEL NETWORK

To understand the role of variations in congestive feedback between SeAs, we develop a mean field model in which only two of the intersegmental vessels are modeled. Specifically
we model the first and last SeA. The reduced network consists of 4 blood vessels indexed 1 (the segment of DA between the two SeAs), 3 (the segment of DA after the last Se, which connects directly to the PCV) and 2, 4 being the two SeAs (Fig. 3A in main text). We define variables \( n_i, l_i, S_i, V_i, Q_i, \bar{R}_i, R_i \ i = 1, 2, 3, 4 \) to be the number of cells contained in vessel \( i \), its length, cross-section area, volume, total flow rate, resistance in the absence of RBCs, and resistance modified by the presence of cells. Finally we define \( p_j, j = 1, 2 \) to be the unknown pressures at the two branching points within the network (just as in the model without feedbacks, the symmetry of the network allows us to assign the same pressure value, \( p = 0 \) to the end of the SeA and the end of the DA). We make a continuum or mean field approximation for the effect of the RBCs contained in each vessel. Specifically, we assume that RBCs in each vessel are uniformly dispersed through that vessel. Then, in steady state we can balance the flux of RBCs into each vessel with the flux of RBCs out of each vessel. For example, for vessel 1, the flux of RBCs (number/time) out of the vessel is equal to the volume of blood leaving the vessel in unit time \( Q_1 \) multiplied by the density (number/volume) of RBCs: \( n_1/V_1 \). Similarly the flux of RBCs into the vessel is given by the total flux of RBCs entering the network through node 1, in unit time (since the total number of RBCs in the network is constant, RBCs leaving the network in unit time is equal to the flux of \( n_2 Q_2/V_2 + n_3 Q_3/V_3 + n_4 Q_4/V_4 \)) multiplied by the fraction of flow passing through node 1 that goes into the first vessel (which, provided cells are partitioned in the same fraction as flows will be: \( \frac{Q_1}{Q_1 + Q_2} \)). Thus in steady state:

\[
\left( \frac{n_2 Q_2}{V_2} + \frac{n_3 Q_3}{V_3} + \frac{n_4 Q_4}{V_4} \right) \frac{Q_1}{Q_1 + Q_2} = \frac{n_1 Q_1}{V_1} \tag{S17}
\]

Similar conservation statements for each of the other 3 vessels in the network give:

\[
\left( \frac{n_2 Q_2}{V_2} + \frac{n_3 Q_3}{V_3} + \frac{n_4 Q_4}{V_4} \right) \frac{Q_2}{Q_1 + Q_2} = \frac{n_2 Q_2}{V_2} \tag{S18}
\]

\[
\frac{n_1 Q_1}{V_1} \frac{Q_3}{Q_3 + Q_4} = \frac{n_3 Q_3}{V_3} \tag{S19}
\]

\[
\frac{n_1 Q_1}{V_1} \frac{Q_4}{Q_3 + Q_4} = \frac{n_4 Q_4}{V_4} \tag{S20}
\]

Note that these equations require that:

\[
\frac{n_1}{V_1} = \frac{n_2}{V_2} = \frac{n_3}{V_3} = \frac{n_4}{V_4} \equiv \rho \tag{S21}
\]

i.e. the concentration (number/volume) of RBCs is the same in every edge. The concentration \( \rho \) is related to the hematocrit \( H \) by \( H = \rho \times V_{\text{cell}} \) where \( V_{\text{cell}} \) is the volume of a RBC.
mean field model for a two-vessel network

fact constant concentration is a corollary of the assumption that the partitioning of RBCs at a junction is identical to the partitioning of blood flow rates at that junction.

Flux conservation at each node, plus the resistance-to-cell number relationship in Eqn. (S16) then allows us to compute the fluxes $Q_i$. Specifically, suppose the fluid inflow is $F$ into the first node. Then the Hagen-Poiseuille’s law:

$$Q_1 = \frac{p_1 - p_2}{R_1}, \quad Q_2 = \frac{p_1}{R_2}, \quad Q_3 = \frac{p_2}{R_3}, \quad Q_4 = \frac{p_2}{R_4}.$$  \hspace{1cm} (S22)

while conserving fluxes at the two nodes gives:

$$F = \frac{p_1 - p_2}{R_1} + \frac{p_1}{R_2}$$  \hspace{1cm} (S23)

$$\frac{p_1 - p_2}{R_1} = \frac{p_2}{R_3} + \frac{p_2}{R_4}$$  \hspace{1cm} (S24)

We can solve for the nodal pressures $p_1, p_2$ by linear algebra. Define a matrix determinant:

$$\Delta = \begin{vmatrix} \frac{1}{R_1} + \frac{1}{R_2} & -\frac{1}{R_1} \\ \frac{1}{R_1} & -\left(\frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4}\right) \end{vmatrix} = -\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \left(\frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4}\right) + \frac{1}{R_1^2}.$$  \hspace{1cm} (S25)

Then Cramer’s rule gives:

$$p_1 = \frac{1}{\Delta} \begin{vmatrix} F & -\frac{1}{R_1} \\ 0 & -\left(\frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4}\right) \end{vmatrix} = \frac{-F\left(\frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4}\right)}{\Delta}$$  \hspace{1cm} (S26)

$$p_2 = \frac{1}{\Delta} \begin{vmatrix} \frac{1}{R_1} + \frac{1}{R_2} & F \\ \frac{1}{R_1} & 0 \end{vmatrix} = \frac{-F}{\Delta}.$$  \hspace{1cm} (S27)

From the formulas for the nodal pressures $p_i$ we can use Eqn. (S22) to calculate the fluxes in each vessel, $Q_i$:

$$Q_1 = \frac{-F}{\Delta R_1} \left(\frac{1}{R_3} + \frac{1}{R_4}\right), \quad Q_2 = \frac{-F}{\Delta R_2} \left(\frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4}\right), \quad Q_3 = \frac{-F}{\Delta R_3 R_1}, \quad Q_4 = \frac{-F}{\Delta R_4 R_1}.$$  \hspace{1cm} (S28)

Although these represent volume fluxes (volume/time), the RBC flux in each vessel can then be computed by multiplying by the cell concentration, $\rho$.

To analyze flows within the network we focused on two measures: (i) ratio of RBC fluxes in the two intersegmental vessels:

$$\text{flux ratio} = \frac{\rho Q_2}{\rho Q_4} = \frac{Q_2}{Q_4} = \frac{R_1 R_4}{R_2} \left(\frac{1}{R_1} + \frac{1}{R_3} + \frac{1}{R_4}\right)$$

$$= \frac{R_4 + V_4 \rho \alpha_4}{R_2 + V_2 \rho \alpha_2} \left(1 + \frac{R_1}{R_3} + \frac{R_1}{R_4 + V_4 \rho \alpha_4}\right).$$  \hspace{1cm} (S29)
(ii) We also compute the viscous dissipation within the network when the useful RBC flux is fixed. First we calculate the dissipation for the Poiseuille’s flow. The flow profile is[1]

$$u_z(r) = \frac{2Q}{\pi a^4} (a^2 - r^2).$$  \hspace{1cm} (S30)

where $a$ is the radius of the pipe and $Q$ is the flow rate. We can calculate the strain tensor $E$, and the only non-zero element is $[1]$:

$$e_{zr} = -\frac{2Qr}{\pi a^4}.$$  \hspace{1cm} (S31)

Thus the dissipation is

$$D = 2\mu \int \Omega E : E$$

$$= 2\mu L \cdot 2\pi \int_{0}^{a} \frac{8Q^2 r^2}{\pi^2 a^8} r dr$$

$$= 2\mu L \cdot 2\pi \cdot \frac{8Q^2}{\pi^2 a^8} \cdot \frac{a^4}{4} = \frac{8\mu LQ^2}{\pi a^4}$$  \hspace{1cm} (S32)

where $L$ is the length of the pipe, and $\Omega$ is the control volume inside the pipe, and $\mu$ is the viscosity. Now for the zebrafish trunk network we split the dissipation into three parts $D_{\text{aorta}}, D_{\text{Se}}, D_{\text{RBC}}$ corresponding to dissipation resulting from the aorta segments, the SeA, and the fluid in the lubricating layer surrounding RBCs. By Eqn. (S32) we have

$$D_{\text{aorta}} = \frac{8\mu_{wb}}{\pi r_a^4} \sum_{i=1}^{2} l_{2i-1} Q_{2i-1}^2$$  \hspace{1cm} (S33)

$$D_{\text{Se}} = \frac{8\mu_{pl}}{\pi r_c^4} \sum_{i=1}^{2} l_{2i} Q_{2i}^2$$  \hspace{1cm} (S34)

where $\mu_{wb} \approx 5$ cP is the whole blood viscosity[2], $\mu_{pl} \approx 1$ cP is the plasma viscosity[2], $r_a$ is the radius of aorta, and $r_c$ is the radius of SeA. To evaluate $D_{\text{RBC}}$ consider the gap $\Omega_g$ between the RBC and the endothelial wall. The velocity $u$ here has two components $u^{\text{Poiseuille}}, u^{\text{Couette}}$ resulting from the Poiseuille and Couette flow. By the Stokes equation[1] and Eqn. (S14) as $d \rightarrow 0$

$$\frac{\mu u^{\text{Poiseuille}}}{d^2} \sim \frac{\Delta p_c}{\ell_{RBC}} \sim \frac{\mu u_c}{\ell_{RBC} d} \Rightarrow u^{\text{Poiseuille}} \sim \frac{du_c}{\ell_{RBC}}.$$  \hspace{1cm} (S35)
On the other hand

$$u_{\text{Couette}} \sim u_c \quad (S36)$$

as $d \to 0$. Thus the dissipation from $u_{\text{Poiseuille}}$ is negligible compared with the dissipation from $u_{\text{Couette}}$ when the gap is narrow. The velocity profile for the Couette flow within the gap is

$$u_{\text{Couette}}(r) = (-r + r_c) \frac{u_c}{d}. \quad (S37)$$

The only non-zero component of the strain tensor $E$ is

$$e_{rr} = -\frac{u_c}{2d}. \quad (S38)$$

Calculating the dissipation as before and substituting for $u_c$ by Eqn. (S13,S16) we get for a single SeA with $n$ RBCs:

$$D = \frac{n \mu_p \ell_{\text{RBC}} u_c^2}{d} (2r_c - d) \approx \frac{2nr_c \mu_p \ell_{\text{RBC}} Q^2}{\pi^2 r_c^4 d} = \frac{2nr_c \mu_p \ell_{\text{RBC}} Q^2}{\pi r_c^3 d} = n\alpha_c Q^2 \quad (S39)$$

where $\alpha_c$ is the feedback strength. Thus the dissipation resulting from RBCs is

$$D_{\text{RBC}} = \rho \sum_{i=1}^{2} V_i \alpha_2 Q_{2i}^2 \quad (S40)$$

where $V_i$ is the volume of the vessel $i$.

V. EFFECT OF NETWORK PERTURBATION UPON RED BLOOD CELL PARTITIONING

Real zebrafish vascular networks, and microvascular networks generally vary from individual to individual[7, 12]. Some forms of anatomical variation lead to embryo death, while others do not affect embryo viability at all. We used the model of hydrodynamic feedback to determine whether uniform RBC fluxes could be achieved in two previously studied forms of vascular network variability.
A. Variation in spacing between intersegmental vessels

In real vascular networks the intersegmental arteries are not evenly spaced. In our model (and supported by visualization of the dsRed-tagged RBC movements), we assume that the Se vessels alternate artery-vein-artery-... Real trunk vasculature does not always follow this pattern of strict alternation; in fact arteries and veins can be ordered in many different ways, and the particular ordering of vessels seems to have little impact on embryo growth[7], we therefore infer that it does not affect oxygen perfusion through the trunk. To investigate whether the feedback mechanism robustly uniformizes RBC fluxes, independently of the ordering of arteries and veins, we simulated RBC partitioning between intersegmental vessels in zebrafish with large variations in SeA spacing. Specifically, we define a vector \{Pa(i) : i = 1, ..., 11\} of normalized intersegmental distances. The entries of Pa is normalized such that \[ \sum_{i=1}^{11} Pa(i) = 11.\] The lengths \[l_{2i-1}, i = 1, ..., 11\] of the DA segments are then given by

\[l_{2i-1} = l_{\text{aorta}}Pa(i),\]

where \[l_{\text{aorta}} = 169 \mu\text{m}\] is the mean intersegmental vessel spacing in a 4 dpf zebrafish. Fix the length of the last DA segment (between the final Se and the direct connection to the PCV) to be \[l_{23} = 169 \mu\text{m}\]. We create a network with high variation in the intersegmental vessel spacing by setting \[Pa(2i - 1) = 1.692, i = 1, ..., 6\] and \[Pa(2i) = 0.1692, i = 1, ..., 5,\] so that successive spacings differ by a factor of 10. Just as in Fig. 4, we assume a linearly decreasing feedback strength (i.e. a linear form for the resistance per cell \(\alpha_i\)), in which the resistance per cell in the \(i\)-th SeA is given by a formula:

\[\alpha_i = \frac{(\alpha_{\text{min}} - \alpha_{\text{max}})}{n - 1} + \alpha_{\text{max}} - \frac{\alpha_{\text{min}} - \alpha_{\text{max}}}{n - 1}, \quad i = 1, ..., n\]

where \[\alpha_{\text{max}} = 2.334 \times 10^{-5} \text{ g/\mu m}^4\text{ s}\] is the feedback strength within the first SeA from the data and \[\alpha_{\text{min}} = 1.01 \times 10^{-6} \text{ g/\mu m}^4\text{ s}\] is that of the last Se. \(\alpha_{\text{max}}\) and \(\alpha_{\text{min}}\) are obtained from linear regression on the measured feedback strengths (see main-text, Fig. 2C). We then used a direct numerical simulation of the RBC dynamics in this network (see Fig. 4 in the main text) to calculate the partitioning of RBCs in the modified network. We estimate the uniformity of flows for each set of network parameters by computing the Coefficient of Variation (CV) of the RBC flux. The CV is 0.2538 in the uneven spacing case, which indicates a lower uniformity compared to a network with the empirically determined Se
spacings (with CV value 0.1815, see Fig. S3). However, if the feedback strength varies with distance along the DA, (i.e. with vessel distance from the heart, rather than simply being a function of vessel index), namely:

$$\alpha_i = (\alpha_{\text{max}} - \alpha_{\text{min}}) \frac{\sum_{j=1}^{n-1} Pa(j)}{\sum_{j=1}^{n-1} Pa(j)} + \alpha_{\text{min}}$$  \hspace{1cm} (S43)

then our computed RBC partitioning CV, under the same simulation condition as in Fig. 1D, is 0.1827, which is almost identical to the unperturbed network.

![Graph](image)

**FIG. S3:** Predicted RBC fluxes in wildtype zebrafish due to variability in SeA spacing variant. The wildtype RBC flux (star) becomes oscillatory under variant spacing (circle), but shows similar overall uniformity. If the feedback variation is adjusted then uniform partitioning of RBCs is restored (cross).

**B. DA-PCV shunt**

*mibta52h* mutant zebrafish have altered differentiation of vessels into arteries or veins. In particular the mutant trunk vasculature includes a circulatory loop (shunt) between aorta and posterior cardinal vein in the middle of the trunk [13]. The mutation is lethal by two weeks post fertilization [13]. To simulate the effect of *mibta52h* upon the partitioning of RBCs through the zebrafish trunk vasculature we created a model of the network, by starting with the same wild type network geometry as in Fig. S1 but with the 6th SeA being assigned a radius 7 \( \mu \text{m} \) (identical to the aorta) and a length 17 \( \mu \text{m} \), based on vessel measurements from [13]. The length of the DA vessel segments on either side of the shunt connection were
set to be one half of the mean aorta vessel length, mimicking the DA malformation seen around the shunt in real zebrafish [13]. The RBC flux in the shunt, 39 s\(^{-1}\), is much greater than the mean RBC flux in all the SeA, 0.3 s\(^{-1}\). Because RBCs have smaller radii than the shunt connection, no hydrodynamic feedback occurs within this vessel, and the feedback mechanism is unable to redistribute RBCs to other SeA (Fig. S4). We expect the low RBC fluxes in the other vessels to be associated with low oxygen transport to the rest of the trunk, which may contribute to the lethality of the mit^{ta52b} mutation.

![Graph of RBC flux vs. Se No.]

FIG. S4: Predicted RBC fluxes in mit^{ta52b} mutant zebrafish due to the appearance of a DA-PCV shunt. The shunt creates a short-circuit in the network, which due to the large radius of the shunt is non-recoverable by hydrodynamic feedback. A shunt introduced at the location of the 6th SeA leads to lower and less uniform fluxes (circle) compared to wild type embryos (star), and there is almost no RBC flux posterior to the shunt location.

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