1. Detailed description of human erythrocyte band 3 clustering model.

The main body of our band 3 clustering model is based on a previously published kinetic model of human erythrocyte (RBC) metabolism [1]. The minimal reactions associated with the antioxidant pathway shown below, were extracted from the original whole RBC metabolism model.

![Schematic representation of metabolic reactions included in our model (minimal model)](image)

1-1. Initial and steady-state concentrations of all substrates used in the model and details of the enzymatic reactions

Abbreviations of all reactions and reactants expressed in the developed minimal model are shown in Table 1.
Table 1: Steady-state concentrations of metabolic intermediates in the RBC model CYTOPLASM

| Variable                          | Abbreviation | Concentration [mM] |
|-----------------------------------|--------------|--------------------|
| Glucose                           | GLC          | 5.00E-03           |
| Glucose 6-phosphate               | G6P          | 6.00E-02           |
| Gluconolactone 6-phosphate        | GL6P         | 5.30E-06           |
| Carbon dioxide                    | CO2          | 1.20E+00           |
| Glutathione (reduced)             | GSH          | 3.30E+00           |
| Glutathione (oxidized)            | GSSG         | 4.70E-03           |
| Nicotinamide adenine phosphate    | NADP         | 6.50E-05           |
| Nicotinamide adenine phosphate    | NADPH        | 6.50E-02           |
| Free 2,3-Diphosphoglycerate       | f23DPG       | 2.30E+00           |
| Free adenosine diphosphate        | MgADP        | 1.10E+01           |
| Free adenosine triphosphate       | MgATP        | 1.30E+01           |
| Free guanosine diphosphate        | GDP          | 9.60E-02           |
| Free orthophosphate               | Pi           | 1.00E+00           |

Below (Table 2) is a list of processes that structure the model.

Table 2: Enzymatic reactions included in the models

| Enzyme / Process | Abbreviation | Substrates               | Products               |
|------------------|--------------|--------------------------|------------------------|
| Hexokinase       | HK           | GLC + MgATP              | → G6P + MgADP          |
| Glucose 6-phosphate dehydrogenase | G6PDH | G6P + NADP              | → GL6P + NAPDH         |
| Glutathione reductase | GSSGR | GSSG + NADPH            | → GSH + MgADP + Pi     |
| Glutathione turnover | OX         | 2GSH                   | → GSSG                 |

1-2. Kinetic equations and parameters of metabolic reactions used in the model.

Below we present a detailed description of the kinetic equations and parameters used in the model. The abbreviations of enzymes and metabolites correspond to those shown in Table 1.
HK

\[
V = \frac{e_f \left( \frac{\text{the} K_{\text{catf}} \ AB}{K_{i, B} K_{m, A}} - \frac{\text{the} K_{\text{catr}} \ PQ}{K_{i, Q} K_{m, P}} \right)}{1 + \frac{A}{K_{i, A}} + \frac{B}{K_{i, B}} + \frac{AB}{K_{i, B} K_{m, A}} + \frac{P}{K_{i, P}} + \frac{Q}{K_{i, Q}} + \frac{PQ}{K_{i, Q} K_{m, Q}} + \sum_{j=1}^{4} \frac{J_{ij}}{K_{j,i} K_{i, B}}} 
\]

(S1)

\[
\text{the} K_{\text{catf}} = \frac{1.662 \times 10^{9.55} \times \text{pH}^{-7.02}}{1 + 10^{-7.02} + 10^{-9.55}} 
\]

(S2)

\[
\text{the} K_{\text{catr}} = \frac{1.662 \times 10^{9.55} \times \text{pH}^{-7.02}}{1 + 10^{-7.02} + 10^{-9.55}} 
\]

(S3)

Symbols: A, MgATP; B, GLC; P, G6P; Q, MgADP; I, Pi, 2,3-BPG and GDP

| Parameter                  | Value     |
|----------------------------|-----------|
| \(e_f\) (M)               | 2.50E−08  |
| \(K_{m,\text{MgADP}}, K_{i,\text{MgADP}}\) (M) | 1.00E−03  |
| \(K_{m,\text{MgATP}}, K_{i,\text{MgATP}}\) (M) | 1.00E−03  |
| \(K'_{i,2\text{BPG}}\) (M) | 2.70E−03  |
| \(K'_{i,\text{GSH}}\) (M) | 3.00E−03  |
| \(K'_{i,\text{GDP}}\) (M) | 1.00E−05  |
| \(K'_{i,\text{GP}}\) (M) | 1.00E−05  |
| \(K_{\text{GGLC}}\) (M) | 4.70E−05  |
| \(K_{m,\text{GP}}, K_{i,\text{GP}}\) (M) | 4.70E−05  |
| \(k_{\text{catf}}\) (s\(^{-1}\)) | 180       |
| \(k_{\text{catr}}\) (s\(^{-1}\)) | 1.16      |

Parameter values were taken from [2].

G6PDH

\[
V = \frac{V_m \frac{AB}{K_{m, A} K_{m, B}}}{1 + \frac{B}{K_{m, B}} \left( 1 + \frac{A}{K_{m, A}} \right) + \frac{P}{K_{m, P}} + \frac{\text{ATP}}{K_{\text{ATP}}} + \frac{2,3\text{-BPG}}{K_{2,3\text{-BPG}}} + \sum_{j=1}^{4} \frac{J_{ij}}{K_{j,i} K_{m, B}}} 
\]

(S4)

Symbols: A, G6P; B, NADP; P, NAPDH
Parameter values were taken from [3].

**GSSGR**

\[
v = \frac{e_v (N_1 AB - N_2 P^2 Q)}{GSSGR_{rd}} \tag{S5}
\]

\[
\text{GSSGR}_{rd} = D_1 + D_2 A + D_3 B + D_4 P + D_5 Q + D_6 AB + D_7 AP + D_8 BQ + D_9 P^2 + (D_{10} + D_{11}) PQ + (D_{12} + D_{13}) ABP + D_{14} AP^2 + D_{15} BPQ + D_{16} P^2 Q + D_{17} ABP^2 + D_{18} BP^2 Q
\]  

- \[N_1 = k_1 k_4 k_9 k_{10} k_{11}\]
- \[N_2 = k_1 k_4 k_9 k_{10} k_{12}\]
- \[D_1 = k_2 k_4 k_{11} (k_5 k_6 + k_4 k_7 + k_4 k_8)\]
- \[D_2 = k_4 k_9 k_{11} (k_5 k_6 + k_4 k_7 + k_4 k_8)\]
- \[D_3 = k_4 k_9 k_{11} \]
- \[D_4 = k_4 k_9 k_{11} \]
- \[D_5 = k_4 k_9 k_{11} \]
- \[D_6 = k_4 k_9 k_{11} \]
- \[D_7 = k_4 k_9 k_{11} \]
- \[D_8 = k_4 k_9 k_{11} \]
- \[D_9 = k_4 k_9 k_{11} \]
- \[D_{10} = k_4 k_9 k_{11} \]
- \[D_{11} = k_4 k_9 k_{11} \]
- \[D_{12} = k_4 k_9 k_{11} \]
- \[D_{13} = k_4 k_9 k_{11} \]
- \[D_{14} = k_4 k_9 k_{11} \]
- \[D_{15} = k_4 k_9 k_{11} \]
- \[D_{16} = k_4 k_9 k_{11} \]
- \[D_{17} = k_4 k_9 k_{11} \]
- \[D_{18} = k_4 k_9 k_{11} \]

Symbols: A, NADPH; B, GSSG; P, GSH; Q, NADP
Parameter values were taken from [4].

**OX**

\[ \nu = k S \]  
(S5)

S: substrate of the reaction

| Parameter     | Value      |
|---------------|------------|
| \( k (s^{-1}) \) | 2.38E−05   |

The parameter value was adjusted to achieve the appropriate steady-state concentration of metabolites.

1-3. **Comparison of minimal model and model with whole RBC metabolism**

The minimal model with the above reactions and the whole RBC model both showed similar steady state values for metabolites.

Table 2. Comparison of steady state concentration of metabolites of original whole RBC model [1] and minimal model (t=10000 sec).
| Name     | Whole RBC model [1] (mM) | Simplified partial model (mM) |
|----------|--------------------------|-------------------------------|
| GSH      | 3.25                     | 3.53                          |
| GSSG     | $0.46 \times 10^{-2}$    | $0.44 \times 10^{-2}$         |
| GLC1     | 5.00 (fixed)             | 5.00 (fixed)                  |
| MgATP    | 1.49                     | 0.98                          |
| MgADP    | 1.19                     | 0.8                           |
| f23DPG   | 2.13                     | 2.48                          |
| G6P      | 0.73                     | 0.74                          |
| GDP      | 0.1                      | 0.1                           |
| GL6P     | $5.24 \times 10^{-6}$    | $5.70 \times 10^{-6}$         |

1-4. Descriptions of diamide-mediated reactions

The following reactions represent the reactions that occur when the RBC is treated with diamide, thiol group oxidant. Simulation settings were set to 30% hematocrit, similar to the experimental study ([5]), by constructing a RBC compartment (where internal diamide is present) nested within a larger environment compartment (where external diamide, or ext_diamide is present). A was set to 0 at steady state, and was set to 1 to model the incubation of cells with diamide.

\[
v = k \times [\text{ext \_ diamide}] \times \frac{3}{7} \times A \quad (S7)
\]

\[
k = 1.27 \times 10^{-3} \quad ([6])
\]

Once inside the cell, diamide rapidly reactions with GSH to form GSSG. (main text, Table 1 equation 2; [7]).

![Reaction Diagram](S8)

When first simulating the model with the given reactions, we found that GSH was only slightly decreased, and it quickly returned to its initial levels (Figure 2, grey). Since the rate of direct diamide reduction with GSH was high, we assumed that there
was an alternative diamide-mediate pathway that created a long-lasting effect on GSH metabolism.

A survey on the effects of diamide revealed the following:

- A significant amount of GSH may be reversibly bound to protein, resulting in formation of mixed disulfides between GSH and protein sulfhydryl groups to form S-glutathionylated proteins (PSSG), under oxidative stress ([8])
- The formation of bonds is reversible after removal of oxidative stress ([9])
- In RBCs, diamide has been shown to induce mixed disulfide bonds between GSH and Hb, and also membrane skeletal proteins ([10], [11])

Given the above, we decided to incorporate these effects of diamide into the model so that there would be two pathways of GSH consumption. The reaction scheme is as shown below.

\[
\text{GSH} + \text{PSH} \xrightleftharpoons{k_3} \text{PSSG} \quad (S9)
\]
\[
\text{PSSG} + \text{GSH} \xrightarrow{k_4} \text{PSH} + \text{GSSG} \quad (S10)
\]

As the parameters were not available in literature, we estimated these parameters by conducting a parameter analysis and comparing it with the time course observed from the previous experiment for the control RBC treated with diamide ([5]).
Figure 2. Comparison of GSH behavior in diamide-treated RBCs. The y-axis represents the GSH concentration relative to the initial concentration. Grey solid lines represent simulation results of the model without reactions S9 and S10. Black solid lines represent the results of the model that have incorporated these reactions, and the black dotted lines represents the experimental results from [5].

2. Detailed description of the band 3 clustering model incorporating spectrin interactions

The prototype model for band 3 clustering was extended to include the cytoskeletal components that characterize the RBC membrane.

2-1. Descriptions of modeling the cytoskeletal spectrin network compartment

In the model incorporating the spectrin cytoskeletal network, in addition to regular vacant voxel species, we assigned certain vacant voxel species to be specific to holding spectrin/bound band 3 species (main text Figure S2). Therefore, for each band 3 state (e.g. band 3, oxidized band 3, phosphorylated band 3, clustered band 3) there exists different species. For example for band 3 of natural state, band 3 occupying a vacant voxel (freeBand3), band 3 occupying a vertex voxel (BoundBand3), and band 3
occupying an edge voxel (Spectrin_band 3) due to its infrequent hopping between adjacent compartments (hop diffusion) over spectrin, can exist.

Normally the diffusion of diffusing freeBand3 is confined within the spectrin domains, however occasionally such band 3 are known to hop into other compartments (Figure 3, top right). We have represented the hop-diffusion scheme with a two-step reaction involving formation of a spectrin associate band 3 species.

**Figure 3. Schematic representation of how molecules behave in spectrin model.** In the spectrin model, the initial spectrin meshwork mostly inhibits the free diffusion of spectrin non-bound band 3 (left).

However, at a low probability, band 3 diffusing in confined regions enters a space between the cytoskeleton (here represented as the creation of a species, Spectrin_band3), and passes through the spectrin fence. When oxidation occurs, since sequential phosphorylation promotes the dissociation of BoundBand3 from spectrin, and thus results in a break in the structure, there is an increase in the number of band 3 molecules that can freely diffuse over domains (bottom).
The reaction scheme, equations, and parameters for the hop-diffusion reaction are given below.

\[
\text{freeBand3} + \text{spectrin} \xrightarrow{k} \text{Spectrin\_Band3} \quad (S11)
\]
\[
\text{Spectrin\_Band3} \xrightarrow{k} \text{Spectrin} + \text{Band3} \quad (S12)
\]

| Parameter | Value   | Reference   |
|-----------|---------|-------------|
| \( p \)   | 0.008   | fitted to [12] |
| \( k \)   | 100 M s\(^{-1}\) | fitted to [12] |

Once the RBC is treated with diamide, band 3 species (included BoundBand3) are oxidized, phosphorylated, and BoundBand3 become freely diffusing Band3phos molecules which diffuse away from their original positions. Since the tethering BoundBand3 is absent and replaced by a vacant compartment (vertex), the neighboring spectrin is also removed from its original position and replaced by a vacant compartment (edge). Thus oxidation induces a zipper like removal of the spectrin network. The reaction scheme, equations, and parameters for these reactions after oxidation are given below.

\[
\text{spectrin} + \text{vertex} \xrightarrow{k_1} \text{edge} \quad (S13)
\]
\[
\text{edge} + \text{spectrin} \xrightarrow{k_2} \text{edge} \quad (S14)
\]

| Parameter | Value   | Reference   |
|-----------|---------|-------------|
| \( k_1 \) | 0.01    | approximated |
| \( k_2 \) | 0.0001  | approximated |

By incorporating these reactions representing the properties of the membrane at steady state, and the dissociation of the spectrin cytoskeleton upon oxidative treatment, we were able to more closely reproduce the effects of oxidation \textit{in silico}, and assess the
how band 3 clustering may be regulated by the spatial organization of the RBC membrane.

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