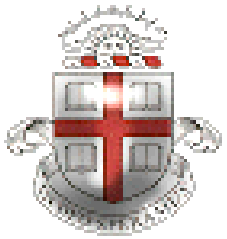
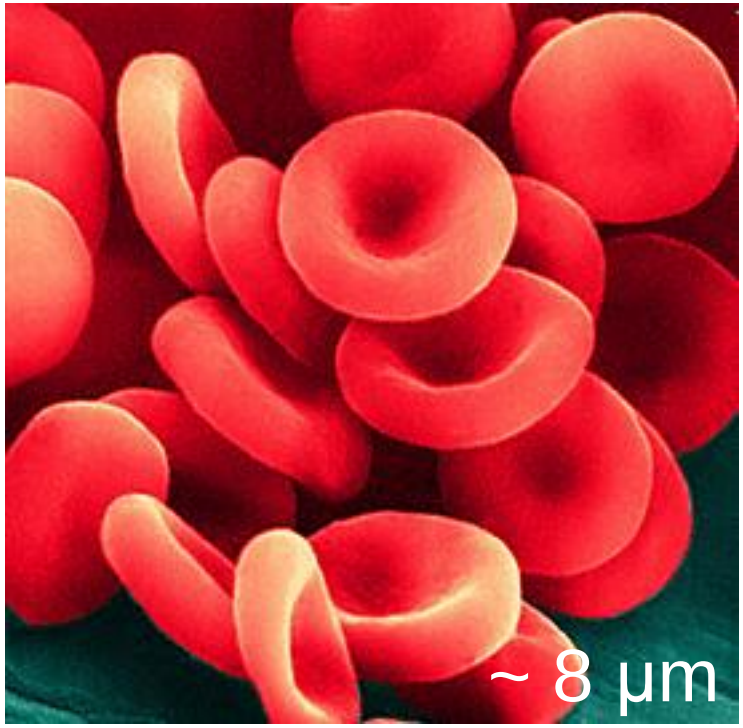
Mechanics of traversal of immature and diseased red blood cells in human spleen and consequences for hereditary blood disorders

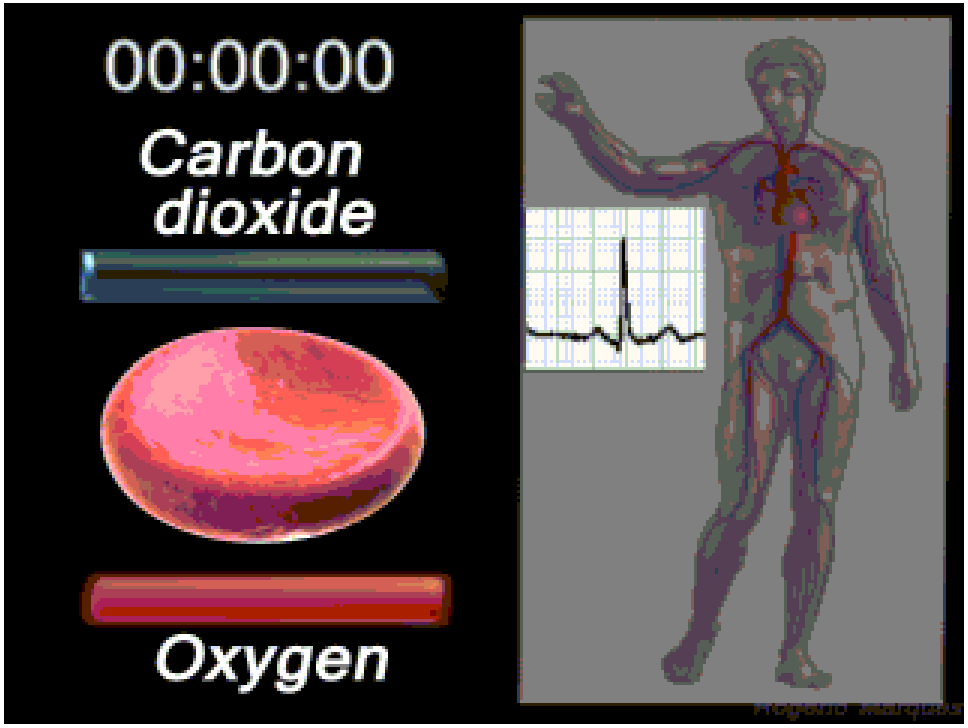
He Li
Division of Applied Mathematics
Brown University



Mature Human Erythrocytes



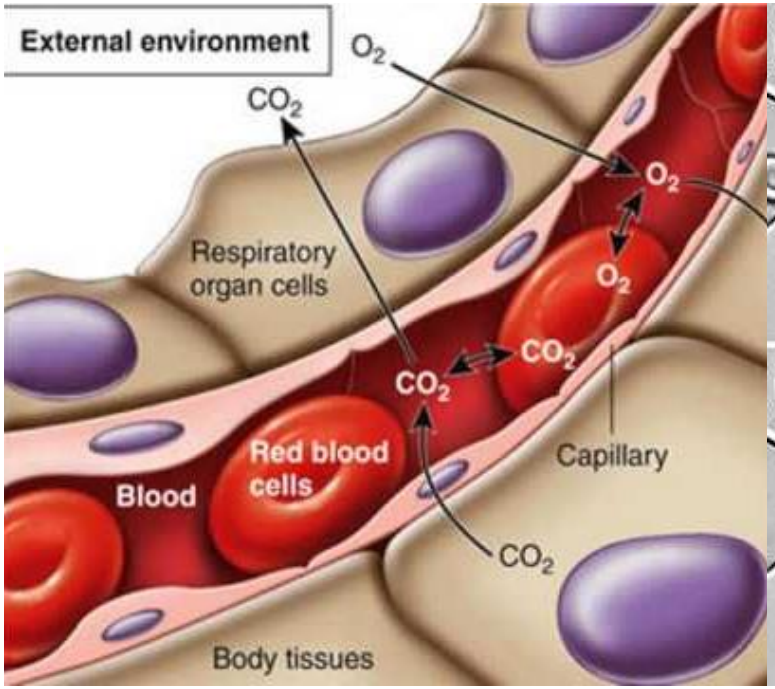
Erythrocytes or Red blood cells (RBCs) have 7 to 8-micron diameter discoid shape. They make ~500,000 passages through the circulation during its lifespan of ~120 days. Travel ~300 miles. 20s per circulation in Human body.



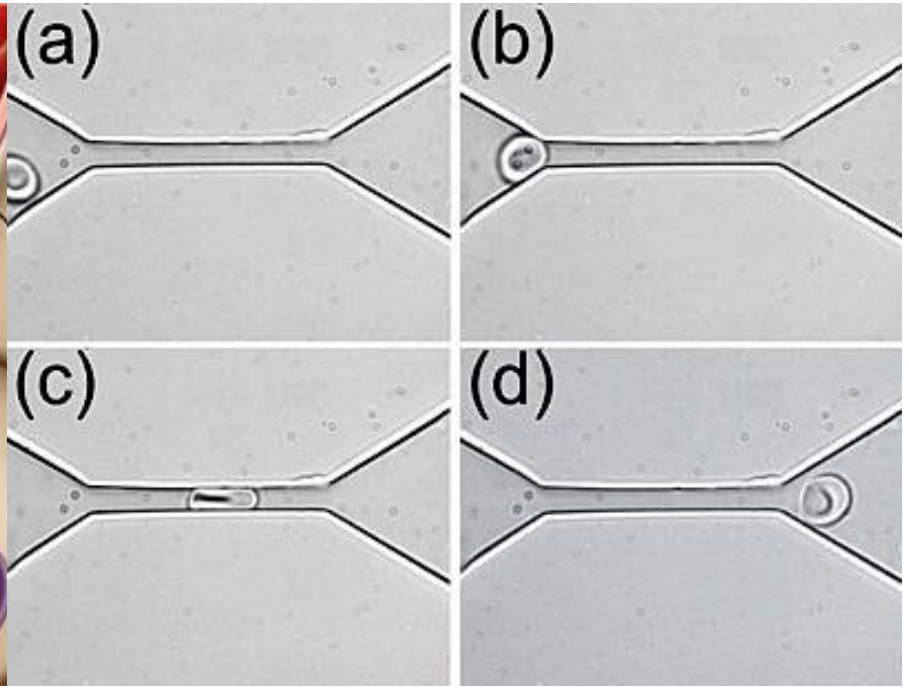
By Rogeriopfm - Own work, partly based on File: Grafik blutkreislauf.jpg by Sansculotte., CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=8626685

Mature Human Erythrocytes

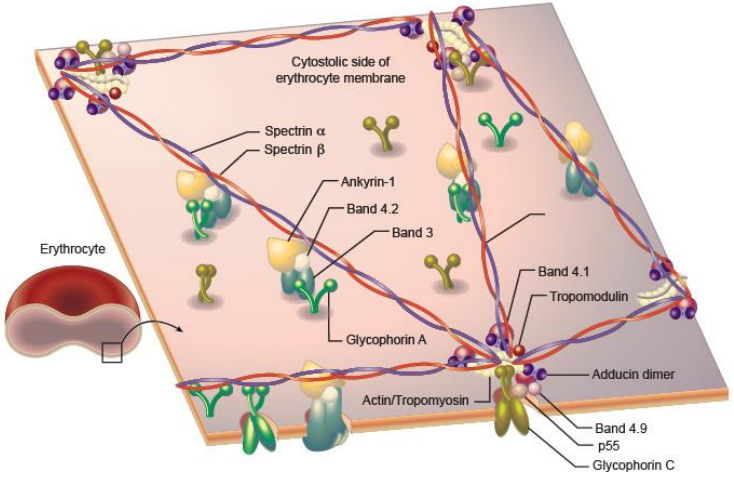
Transiting in capillaries



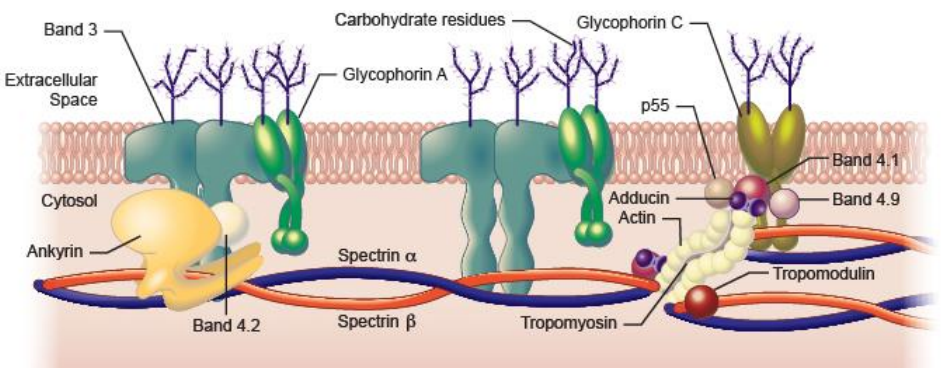
Passage through microfluidic channel



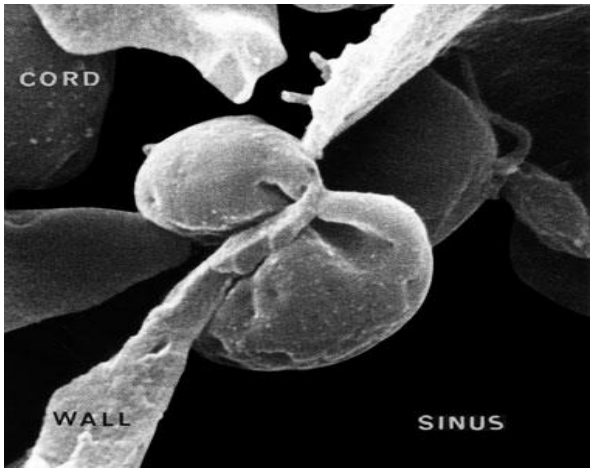
Li et al. (2008)



Membrane Structure

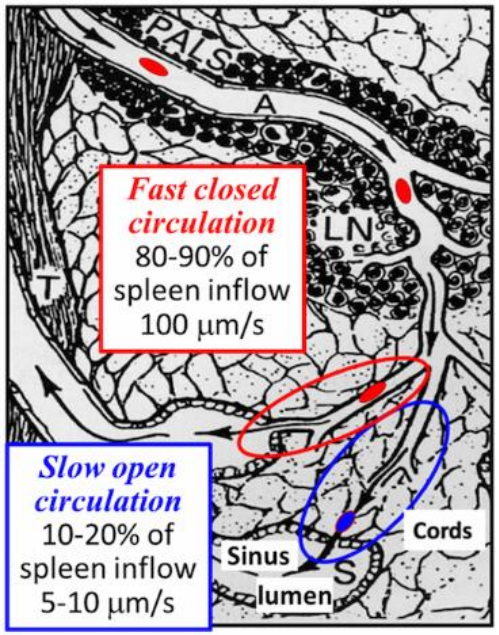


Human Spleen

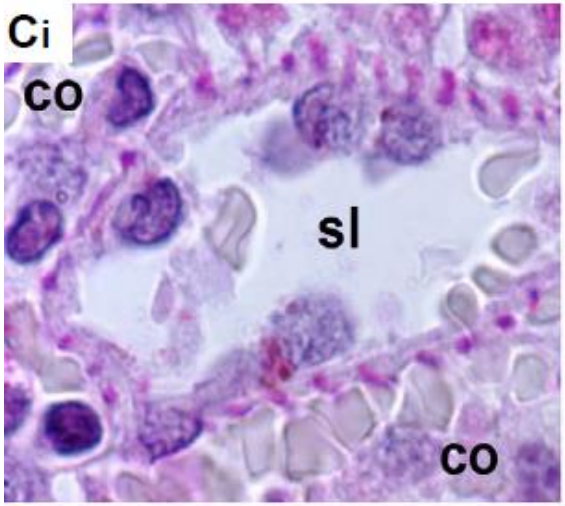


An and Mohandas (2008)

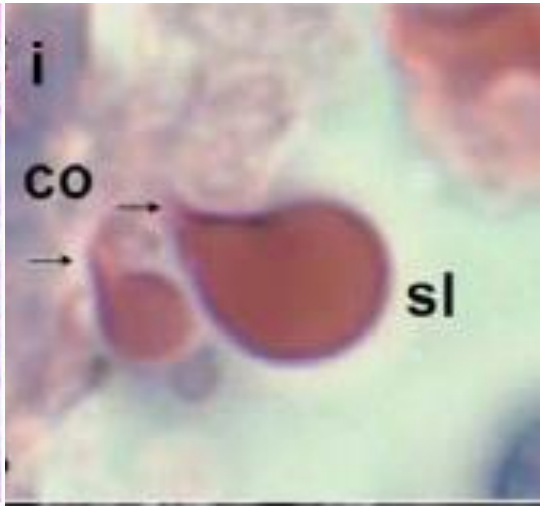
The spleen is the largest organ in the lymphatic system and it consists of two compartments, the white pulp and the red pulp. The white pulp tissue consists of immune cells (T cells and B cells) to fight infection. The red pulp tissue filters the blood and removes old or damaged RBCs. Five Liters of blood circulate through heart every minutes, with 5% going to the spleen.



Groom (1987)



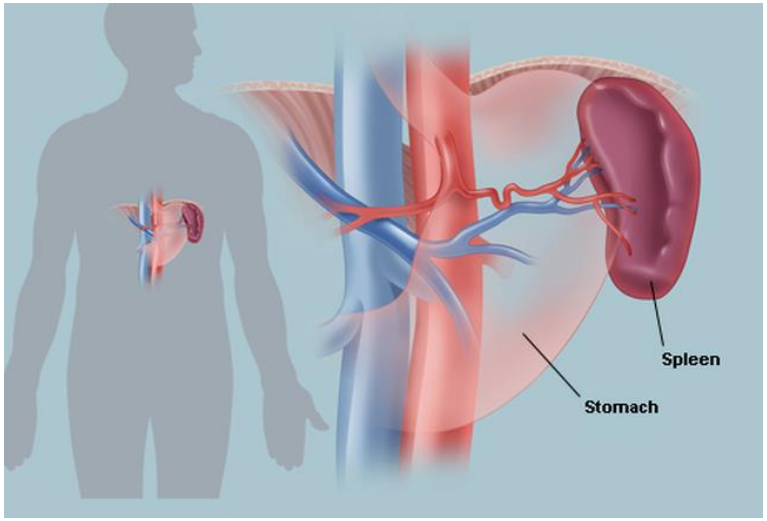
Buffet et al. (2011)



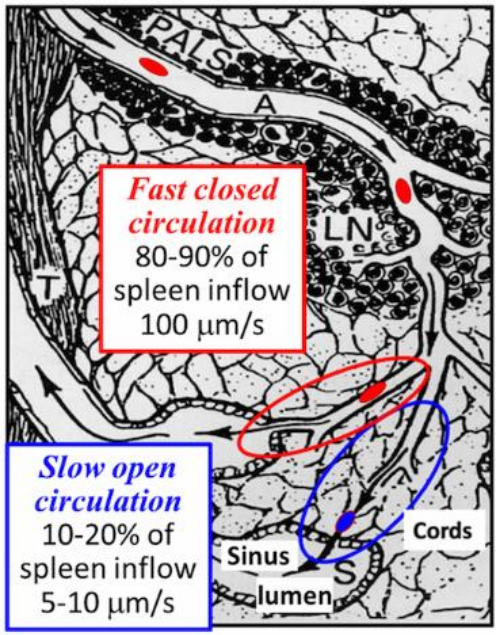
Deplaine et.al (2005)

RBCs have to squeeze though the interendothelial slit (~1 µm) to return to circulation.

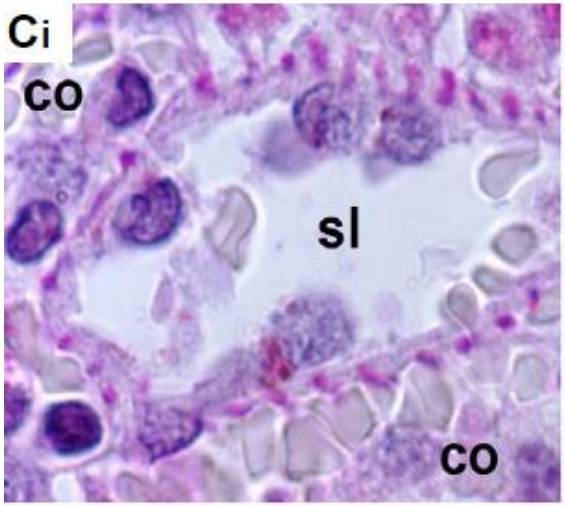
Human Spleen



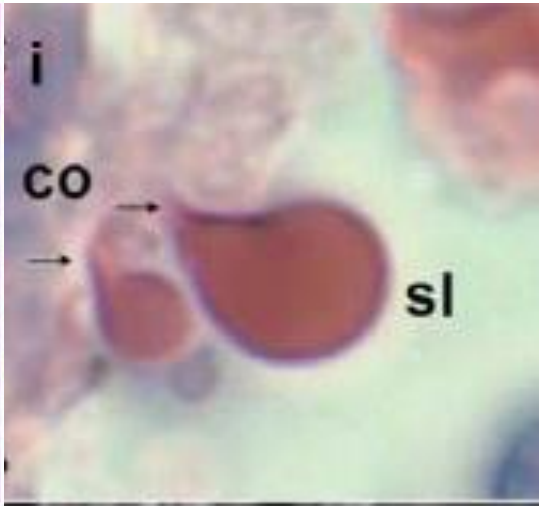
The spleen is the largest organ in the lymphatic system and it consists of two compartments, the white pulp and the red pulp. The white pulp tissue consists of immune cells (T cells and B cells) to fight infection. The red pulp tissue filters the blood and removes old or damaged RBCs. Five Liters of blood circulate through heart every minutes, with 5% going to the spleen.



Groom (1987)



Buffet et al. (2011)

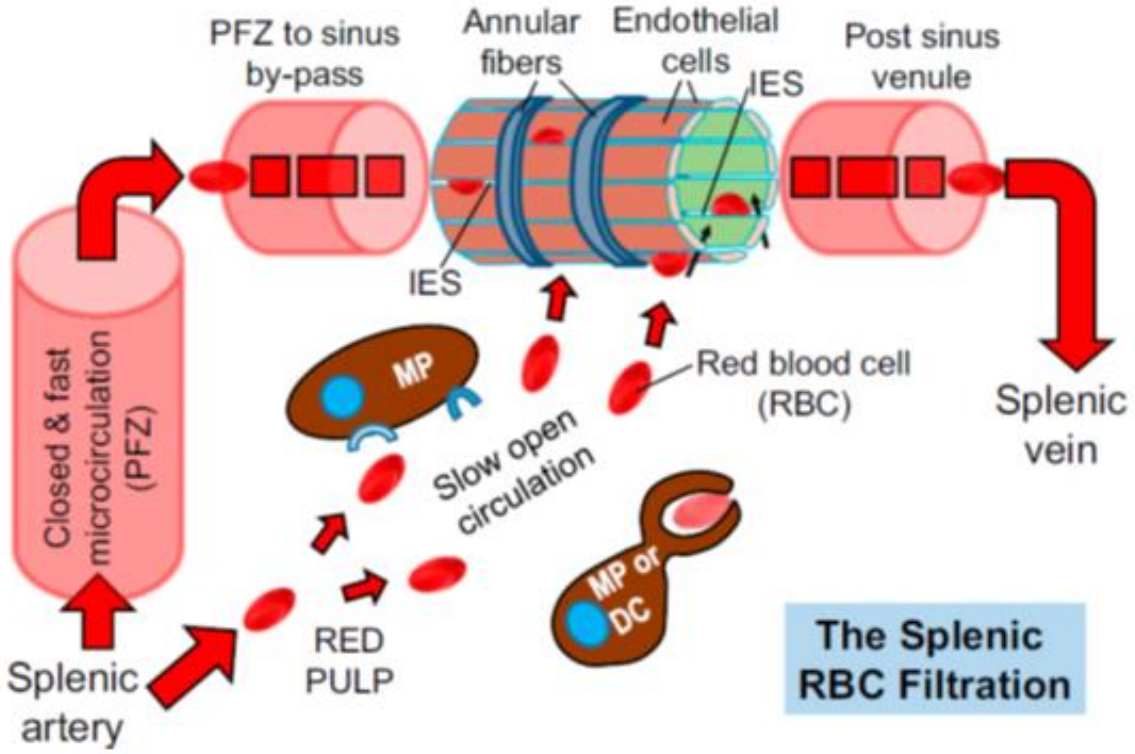


Deplaine et.al (2005)

RBCs have to squeeze though the interendothelial slit (~1 µm) to return to circulation.

Spleen and Interendothelial Slit (IES)

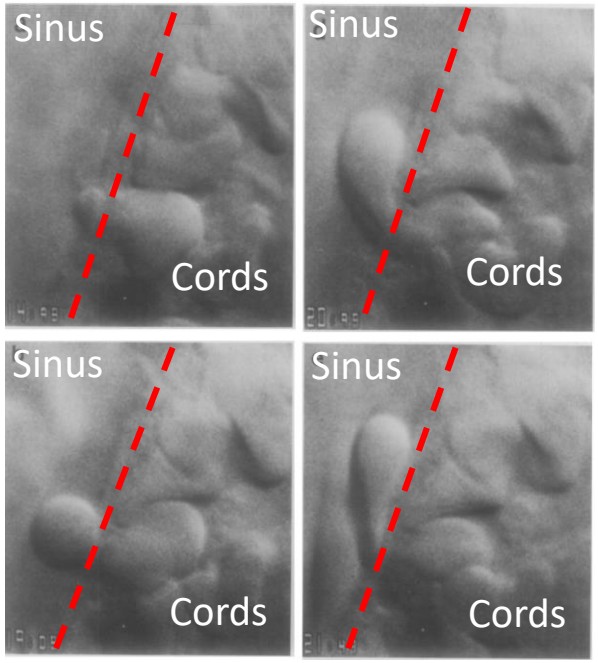
The interendothelial slit (IES) is the narrowest circulatory pathway in the human spleen with a function of filtering the aged and diseased red blood cells (RBCs).



Extensive work has been done in elucidating the function of spleen in sensing and clearing RBCs with alternations in their size, shape and deformability, through *in vivo* (Macdonald et al. (1987), Groom et al. (1991)), *ex vivo* (Buffet et al. (2006), Safeukui et al. (2012)), *in vitro* (Buffet et al. (2011), Gambhire et al.(2017)) experiments, and numerical modeling (Pivkin et al. (2016), Salehyar et al. (2016)).

Passage of RBCs through IES

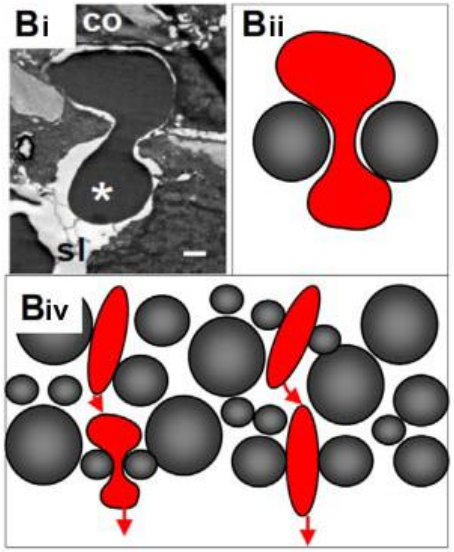
In vivo



MacDonald et al. (1987)

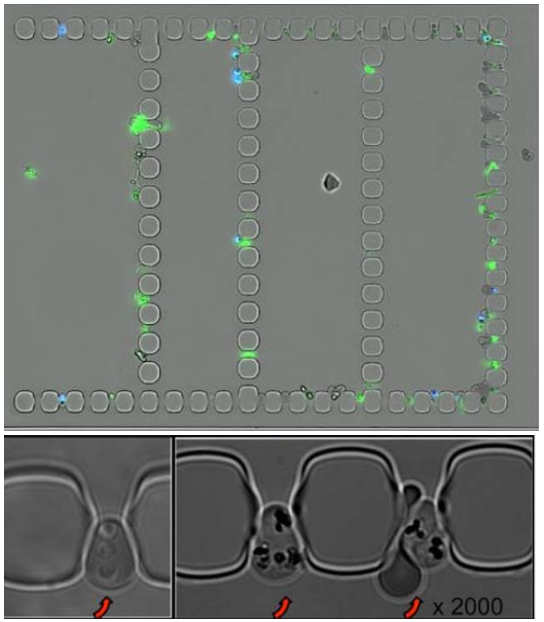
In vitro

Microbeads filtration



Deplaine et al. (2005)

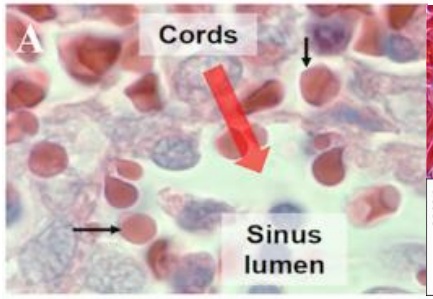
Microfludics



Picot et al. (2015)

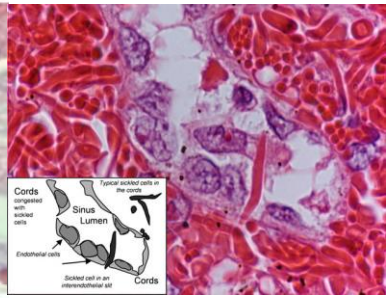
Ex vivo

Malaria-infected RBCs

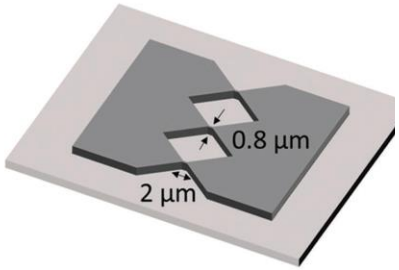


Buffet et al. (2005)

Sickle cells

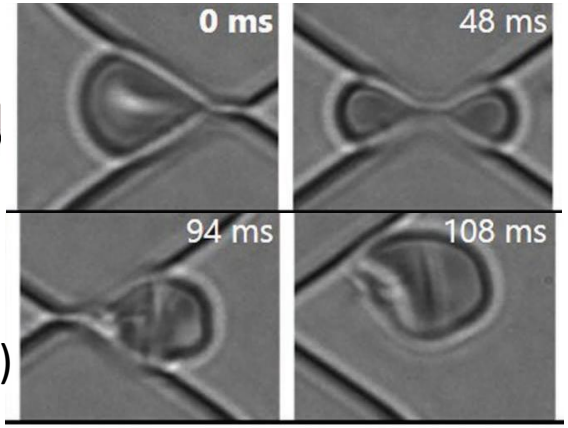


Brousse et al. (2014)



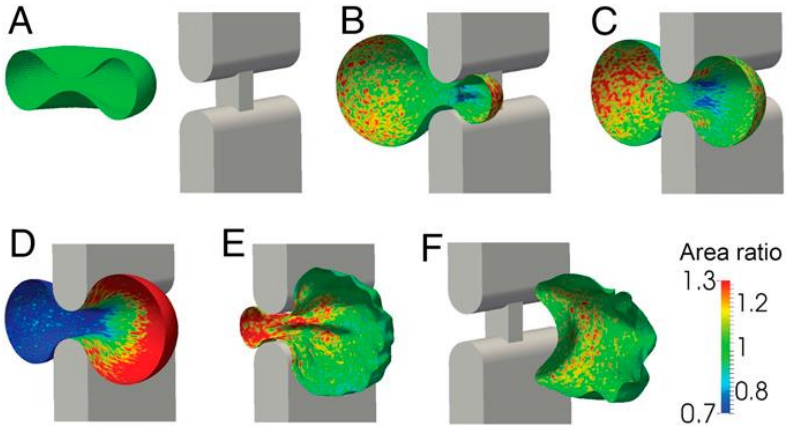
Pattern obtained on silicon

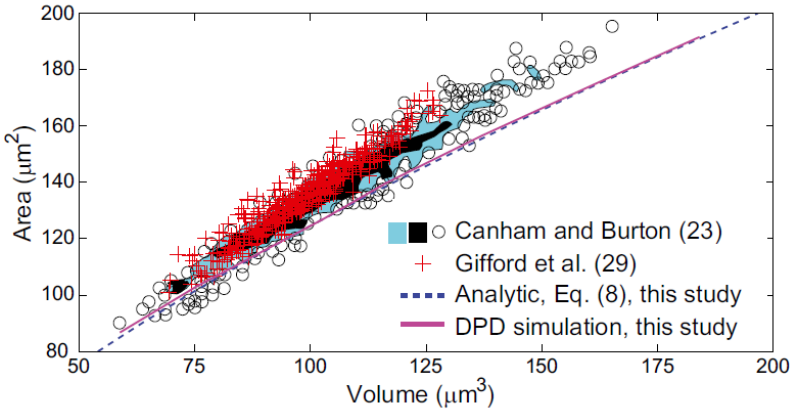
Gambhire et al. (2017)



Passage of RBCs through IES

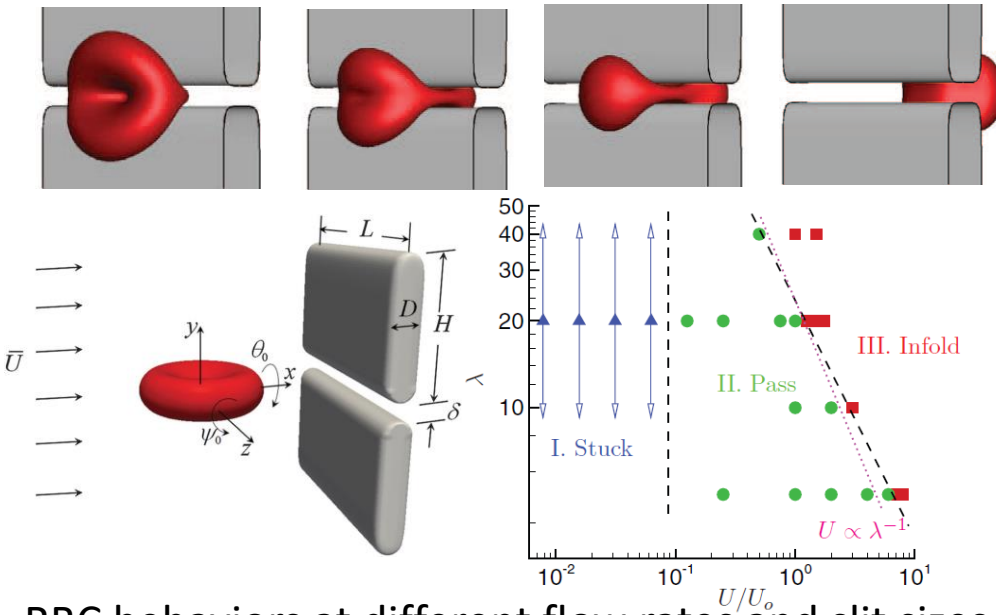
DPD RBC model



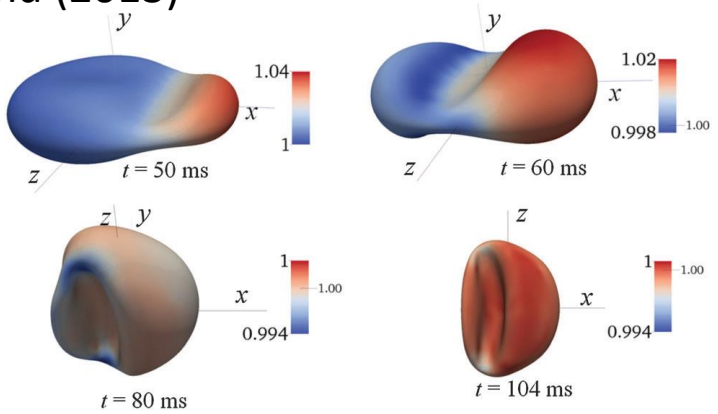


Quantifying biophysical limits for RBCs to pass through the IES. Pivkin et al. (2016)

Boundary integral method



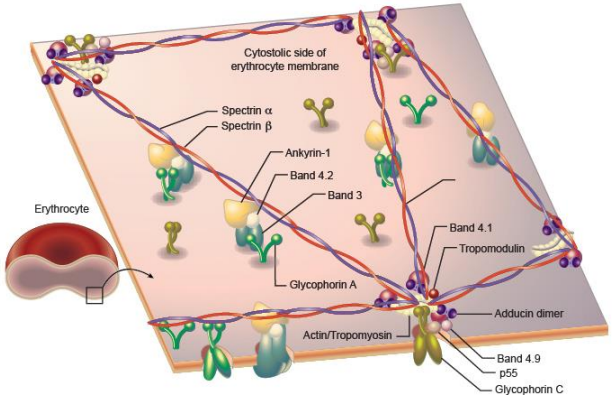
RBC behaviors at different flow rates and slit sizes. Freund (2013)

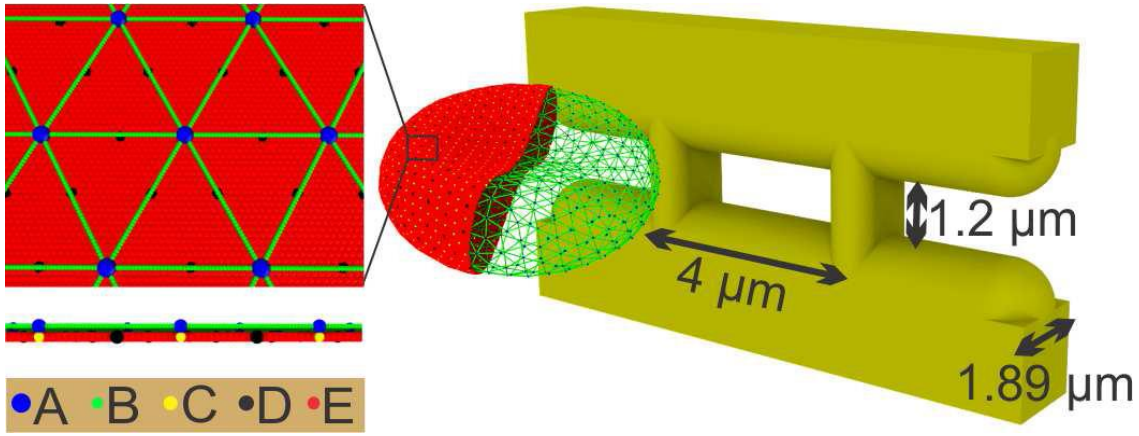


RBC deformation during the passage. Salehyar et al. (2016)

RBC and IES Model

1. The role of IES in filtering RBCs with significant membrane protein defects, such as seen in hereditary spherocytosis (HS) and elliptocytosis (HE), has not been addressed.
2. How the spleen facilitates the maturation of reticulocytes has not been investigated in sufficient detail. For example, whether the spleen plays a role in defining and determining the size and shape of maturing reticulocytes is not clear.



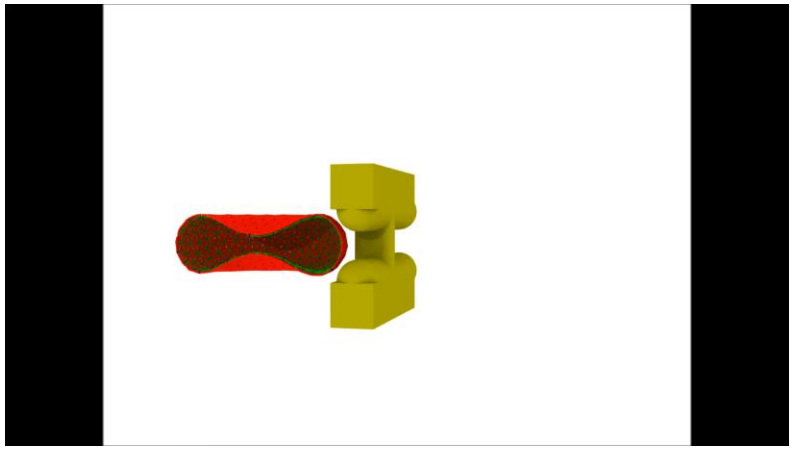


A: actin junctions D: band 3 particles
B: spectrin particles C: glycophorin particles E: lipid particles

Pivkin et al. (2016)

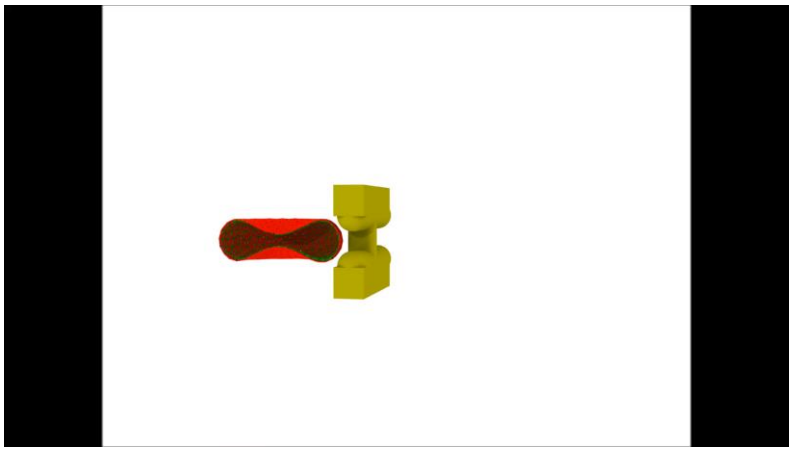
Healthy and Aged RBCs

Retention



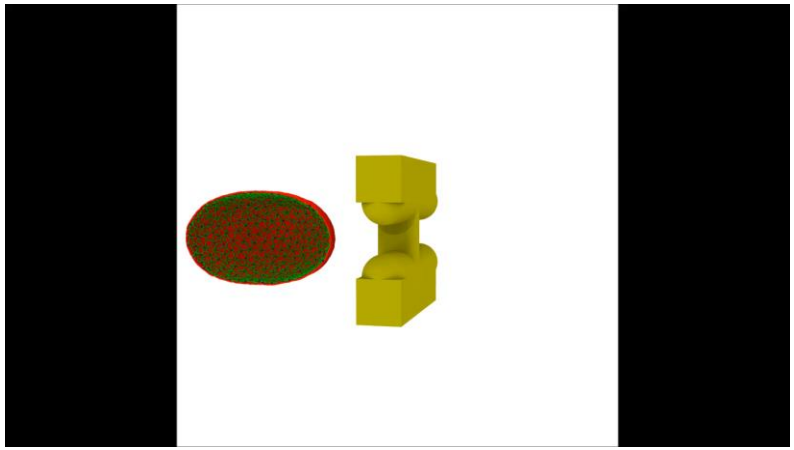
Area 140 μm^2, volume 90 μm^3, 3 Pa/μm

Pass through



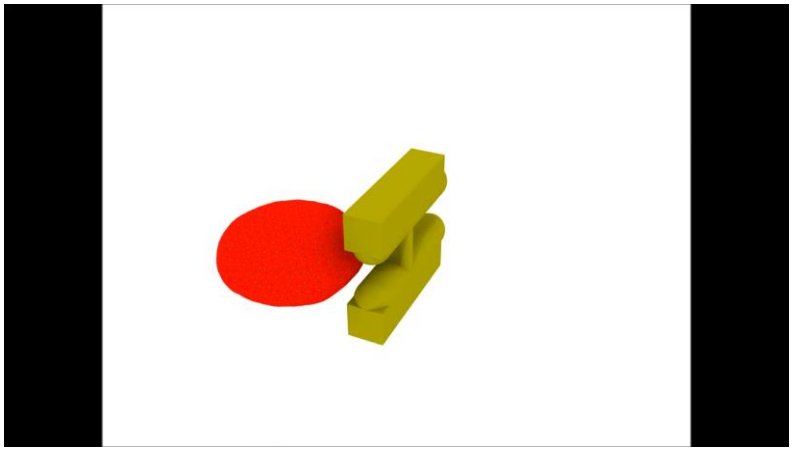
Area 140 μm^2, volume 90 μm^3, 5 Pa/μm

Retention



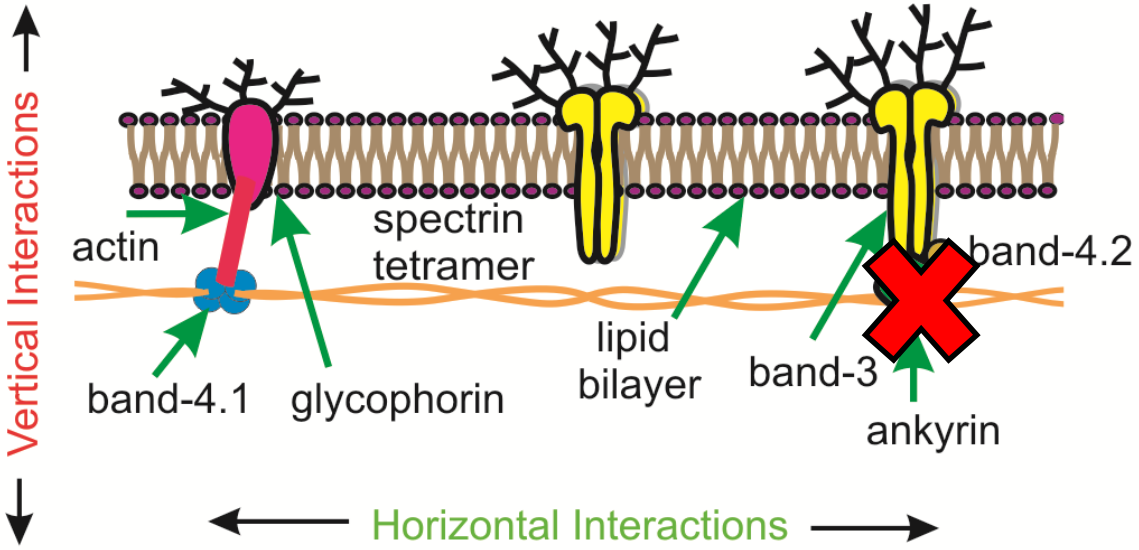
Area 110 μm^2, volume 90 μm^3, 8 Pa/μm

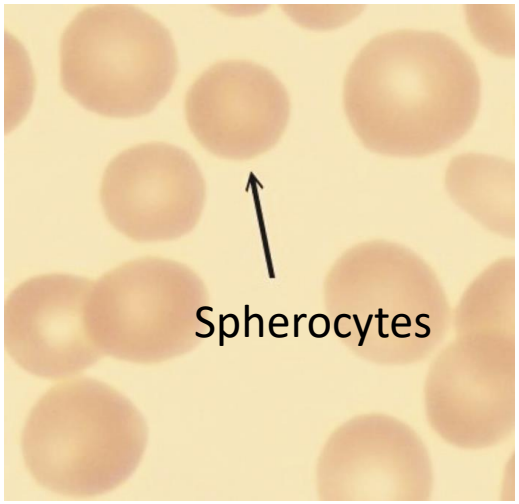
Lysis

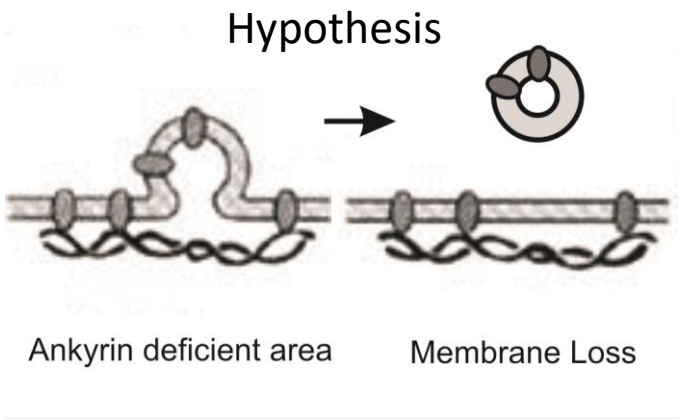


Area 110 μm^2, volume 90 μm^3, 10 Pa/μm

Hereditary Spherocytosis (HS)







Estimated frequencies of mutations in membrane proteins

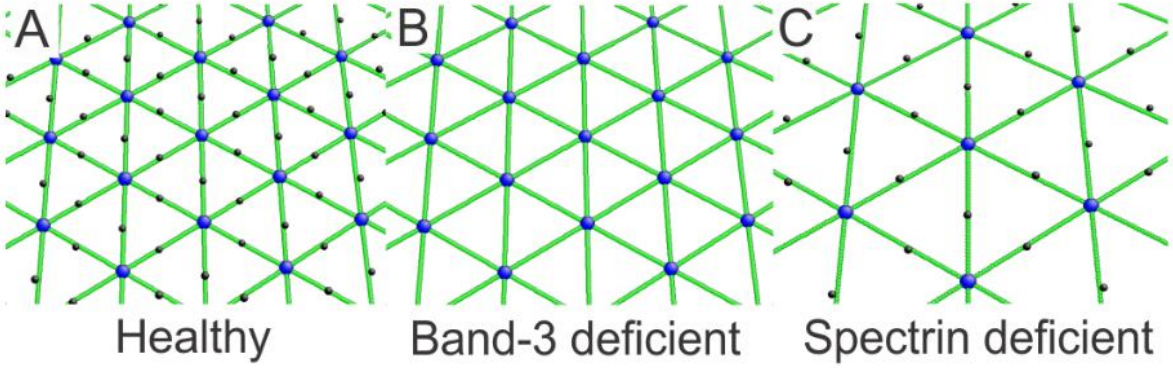
Hereditary Spherocytosis:

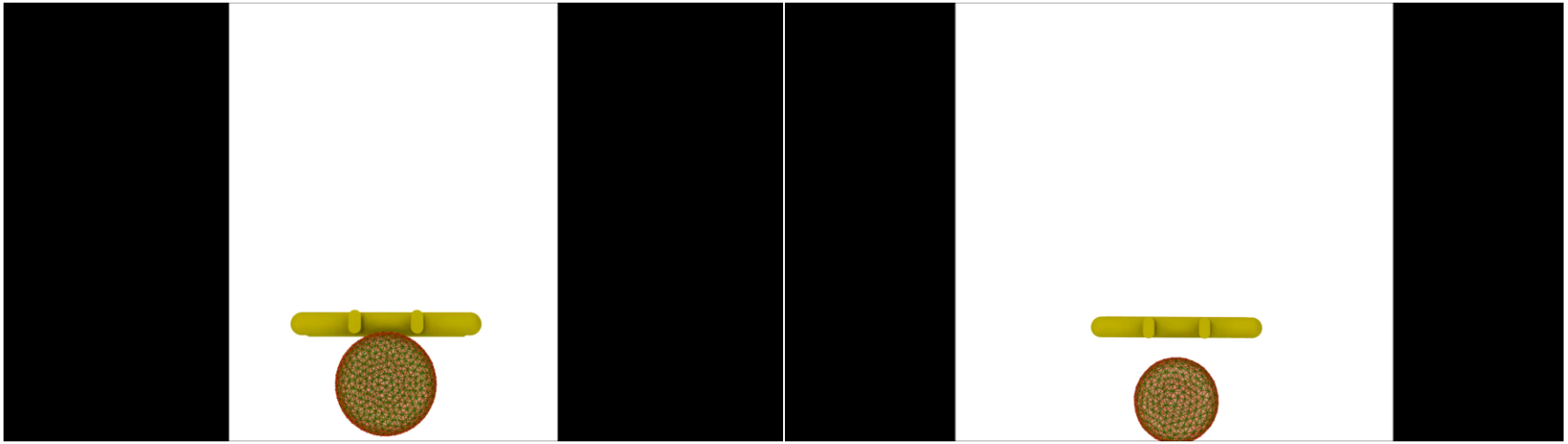
Ankyrin	40-65%
β-spectrin	20-35%
Band 3	15-30%
Protein 4.2	<5%

Perrotta et al. (2008)

RBCs in Hereditary Spherocytosis

The band-3 or protein 4.2 deficiency leads to reduced vertical connectivity of RBC membrane, whereas the spectrin or ankyrin-deficiency results in reduced spectrin network density.





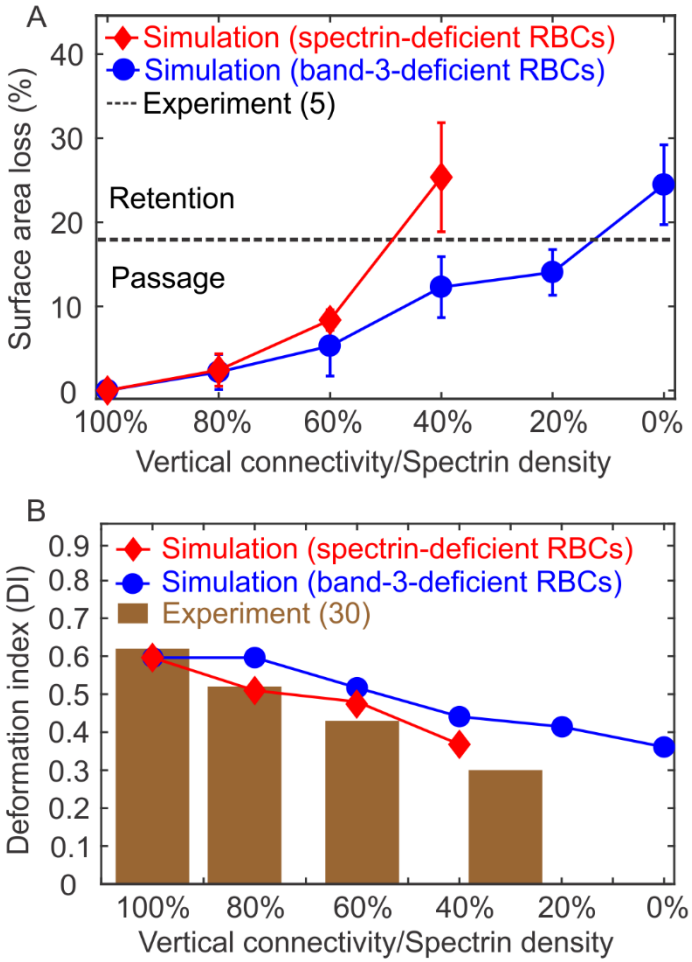
60% vertical connectivity (top view)

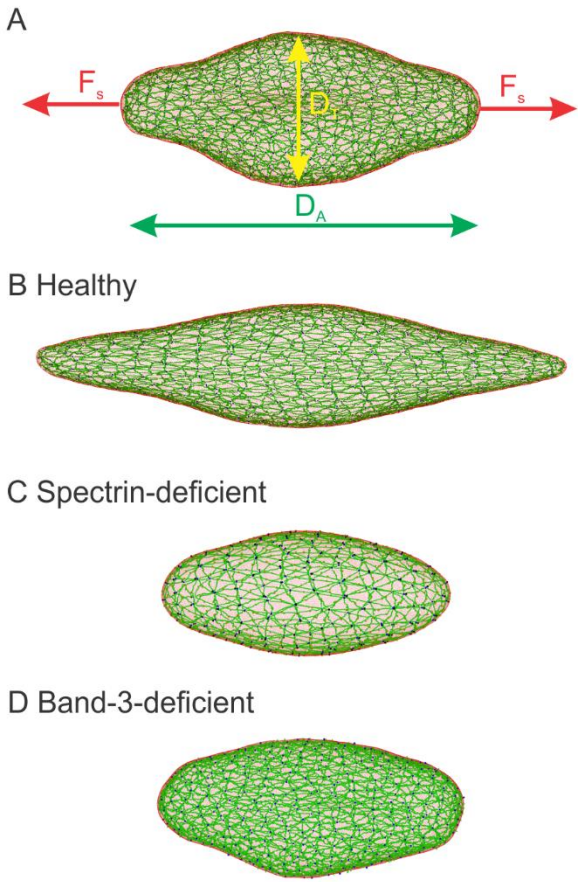
60% spectrin density (top view)

Traversing IES causes intrasplenic vesiculation of RBCs in HS.

RBCs in Hereditary Spherocytosis

RBCs shed more surface area as protein deficiency increases, spectrin-deficient RBCs lose more surface area than band-3-deficient RBCs. The corresponding DI of RBCs illustrates a progressive decrease as the degree of protein deficiency increases.

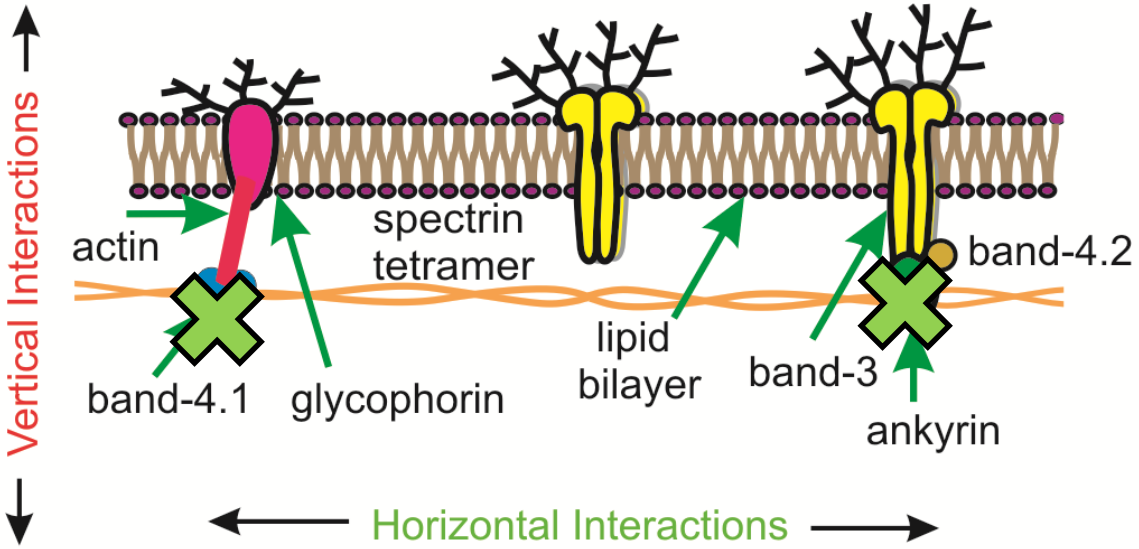


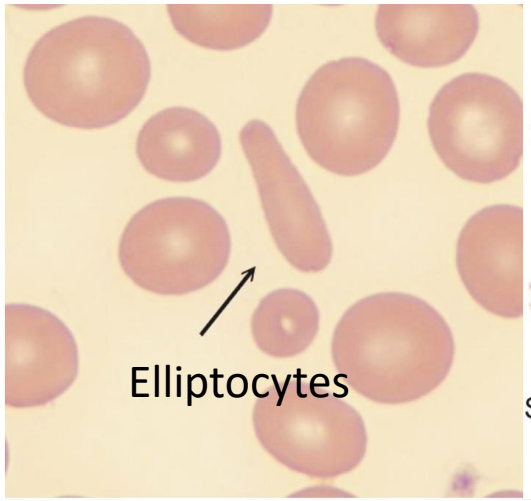


Safeukui, et al. (2012), Walensky et al. (2003)

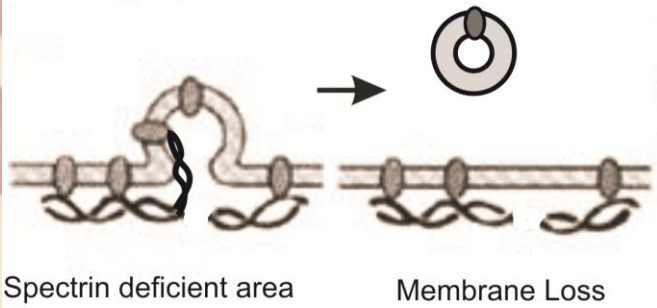
$DI = (D_A - D_T)/(D_A + D_T)$

Hereditary Elliptocytosis (HE)





Hypothesis



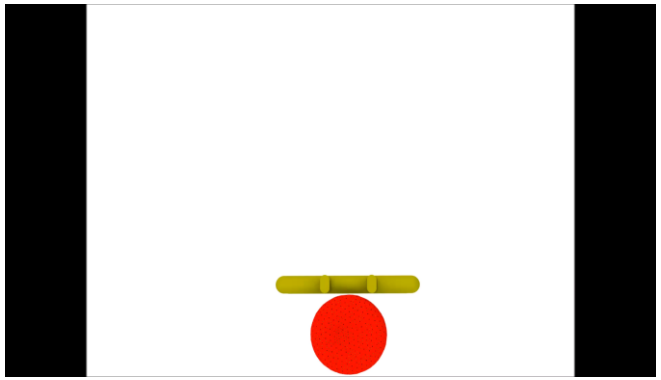
Estimated frequencies of mutations in membrane proteins

Hereditary Elliptocytosis:

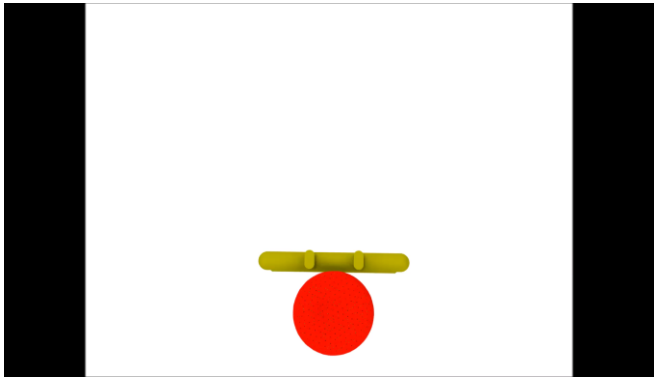
α-spectrin	~65%
β-spectrin	~30%
Protein 4.1	~5%

An et al. (2008)

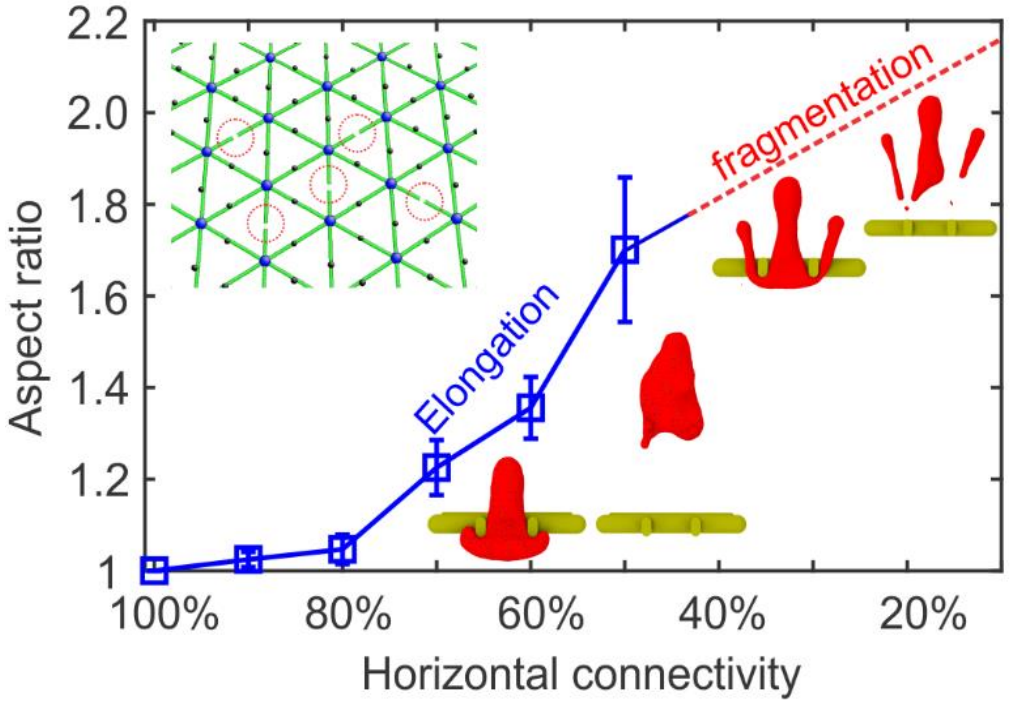
RBCs in Hereditary Elliptocytosis



Horizontal connectivity of 50% (top view)



Horizontal connectivity of 20% (top view)

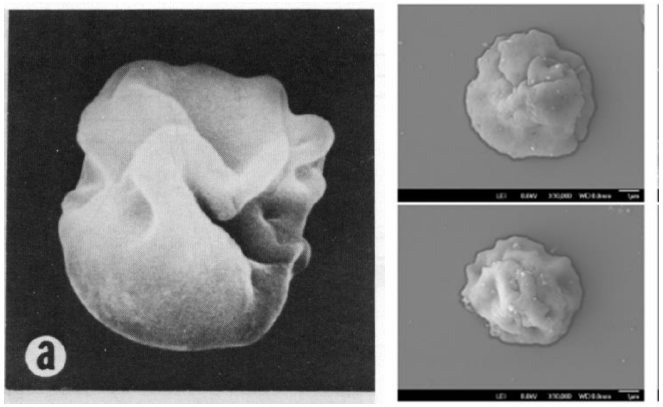


Aspect ratio = D_A/D_T

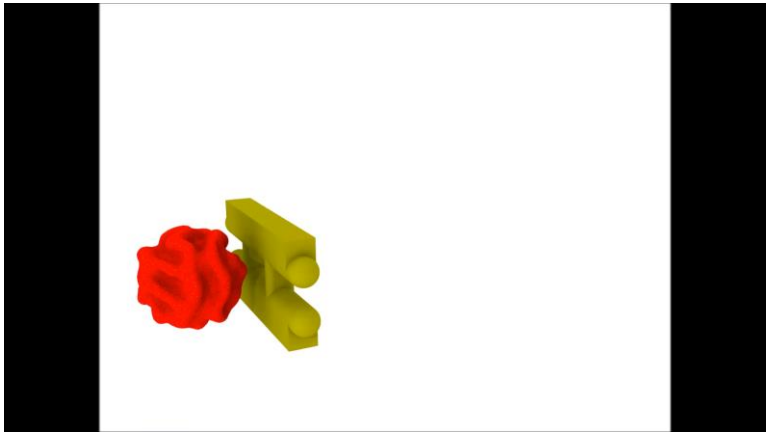
Traversing IES causes shape transition and fragmentation of RBCs in hereditary elliptocytosis.

Traversal of Reticulocytes

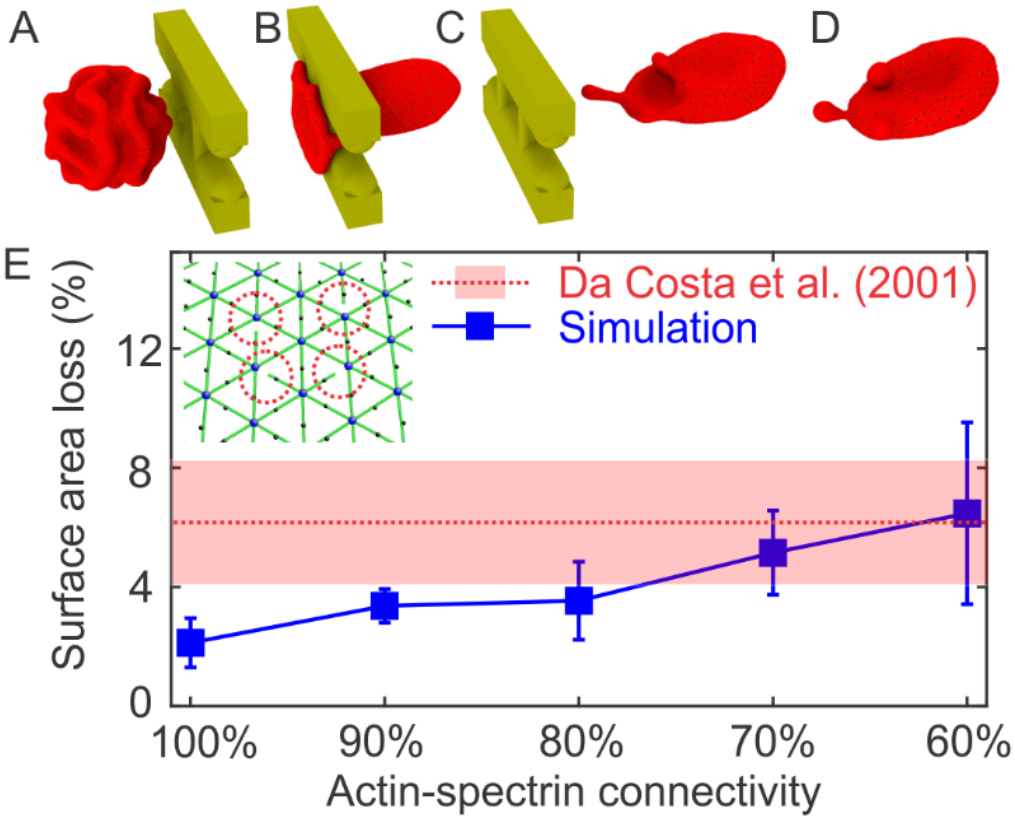
We examine how IES in spleen facilitates the maturation of young RBCs (reticulocytes).



Coulombel, et al. (1979)



Malleret, et al. (2013)



Reticulocytes with a surface area of 161 μm^2, volume 103.5 μm^3 (Gifford, et al. (2006)), driven by a pressure gradient of 10 Pa/μm.

Summary

1. HS RBCs may lose surface area through shedding vesicles during their passage through IES due to the weakened cohesion between the cytoskeleton and the lipid bilayer. Loss of surface area from HS RBCs becomes more pronounced as degree of protein deficiency is elevated.

2. In HE, RBCs are elongated after traversing IES, contributing to the shape transition to elliptical shapes. In severe forms of HE, RBCs break into fragments during their passage of IES.

3. The above findings enlighten a new “incendiary firefighter” paradigm for the role of spleen in blood disorders. The spleen not only senses and clears RBCs with abnormal shapes and deformability, but also contributes to pathological alternations of RBCs in blood disorders

4. Reticulocytes releases redundant surface area during their passage of IES and spleen plays an important role in defining and determining the shape of RBCs.

Thank you for your time and attention.

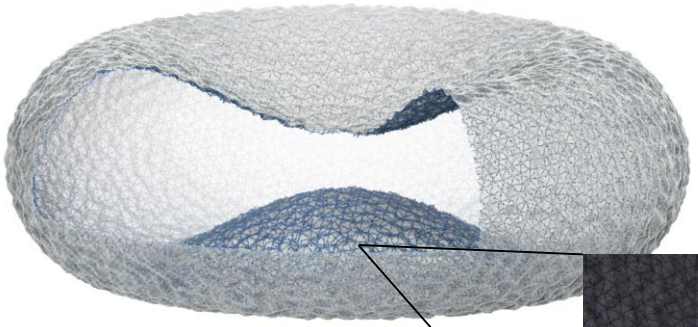
Acknowledgements:

Collaborators: Lu Lu, Xuejin Li, Pierre Buffet, Ming Dao, George Em Karniadakis, and Subra Suresh

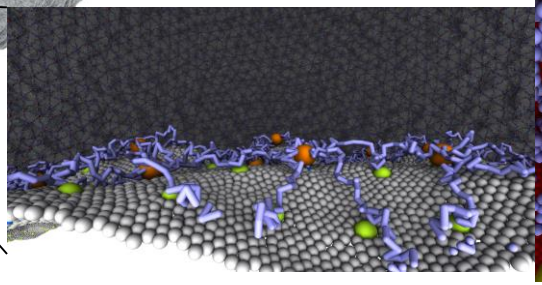
The work is supported by the NIH Grant U01HL114476

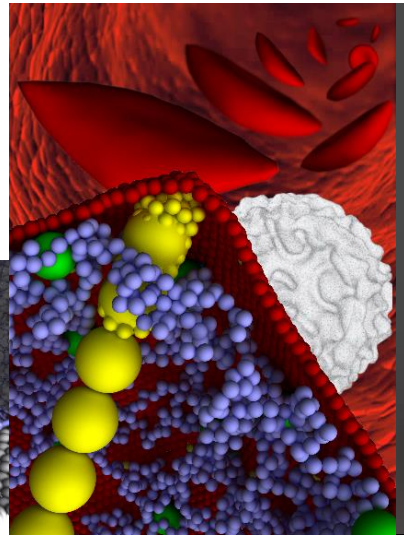
Multiscale Modeling of RBCs in Blood Diseases

CGMD RBC~4000000 particles

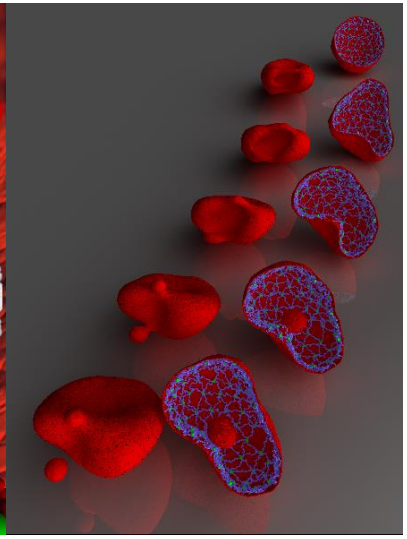


Tang et al. (2017)

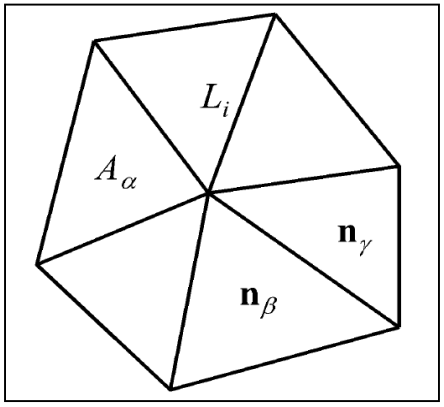


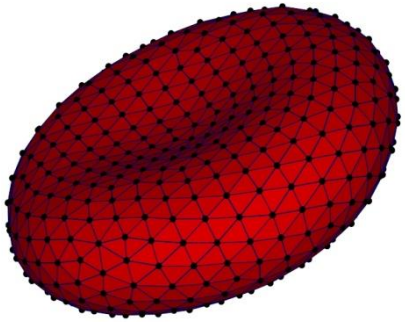


Lu et al. (2017)

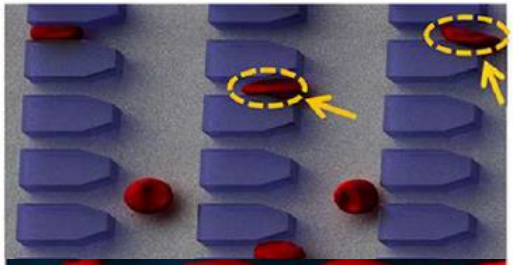


Li et al. (2018)





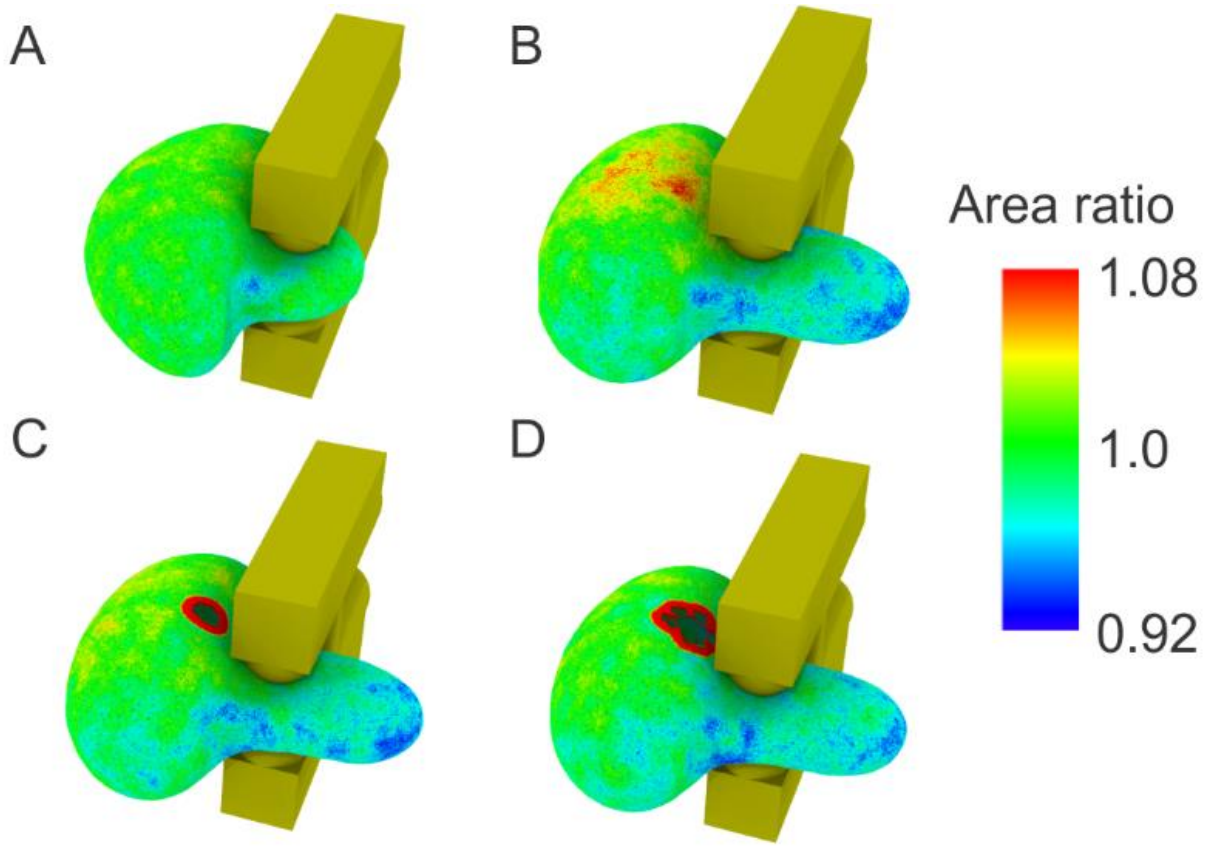
DPD RBC model consists of 23867 DPD particles.
Fedosov et al. (2010)



Li et al. (2017)

Lei et al. (2013)

Healthy and Aged RBCs



Area 110 μm^2, volume 90 μm^3, 10 Pa/µm

Lysis is initiated from the membrane area where the area expansion is maximized.