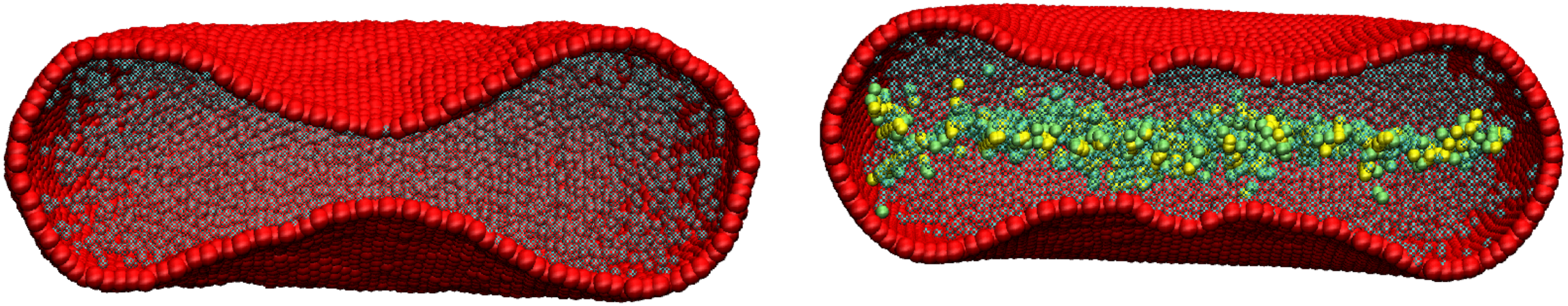
Multiscale Modeling of Sickle Cell Anemia



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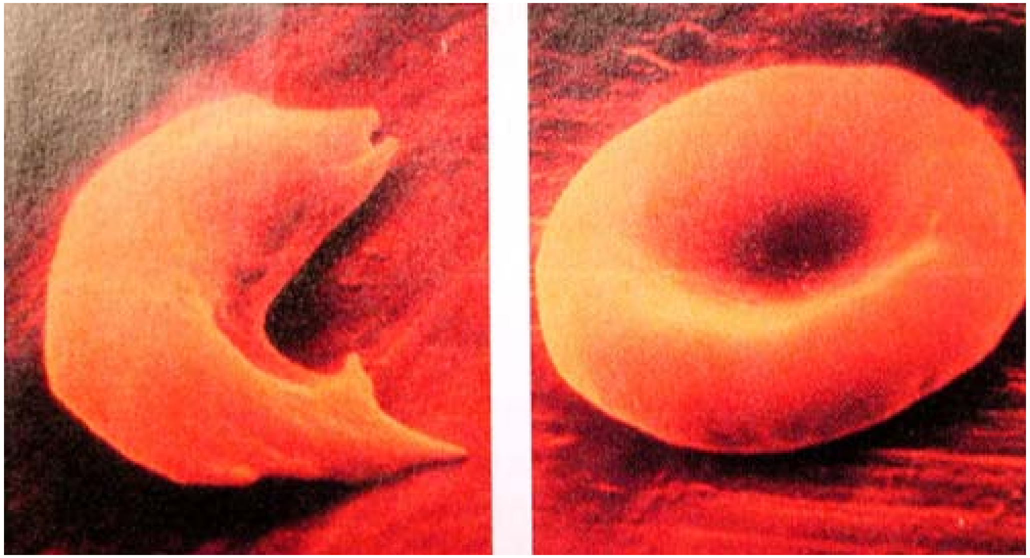
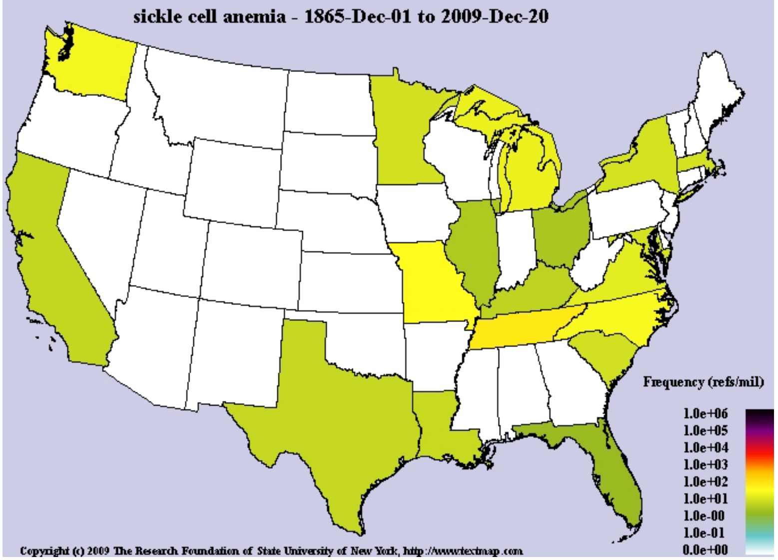
The CRUNCH group: www.cfm.brown.edu/crunch

Sickle cell anemia

- Sickle cell anemia is a genetic blood disorder affecting mainly Americans of Sub-Saharan African descent
- In the United States, about 1 out of 500 African-American children born will have sickle-cell anemia
- Life expectancy of the patients with sickle cell anemia is around 50 years





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[1] J. B. Herrick, *Arch. Intern. Med.*, 6:517–521, 1910

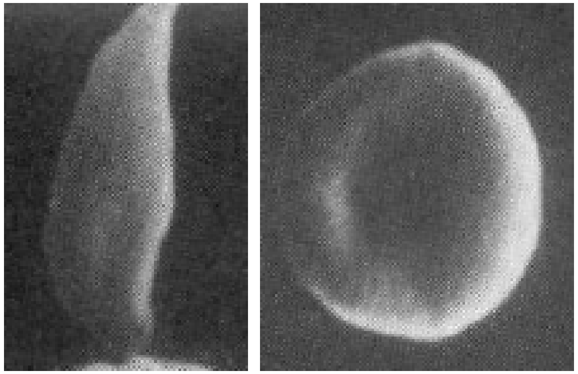
[2] L. Pauling, H. A. Itano, S. J. Singer, and I. C. Wells, *Science*, 110:543–548, 1949.

Sickle cell anemia

Physiological background

- Heterogeneous irregular cell shapes: *sickle, granular, maple, biconcave, etc.*

Oxygenated state

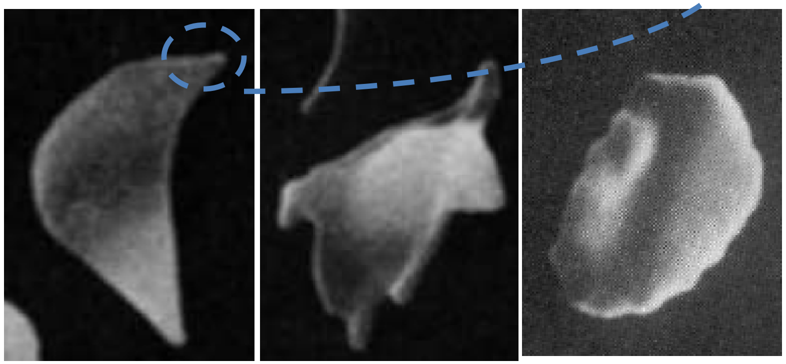


Irreversible sickle cell

biconcave

de-oxygenation →

Deoxygenated state



sickle (SS2) maple (SS3/SS4) granular(SS3/SS4)

- Heterogeneous cell membrane **rigidities**

Oxygenated state

SS4 > SS3 > SS2 ≈ Healthy

Deoxygenated state

Cell **rigidity** increases sharply (O(10^2) to O(10^4))

- Vaso-occlusion is the major cause of mortality in patients with sickle cell anemia.

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[1] D. K. Kaul and H. Xue, *Blood*, 77:1353–1361, 1991

[2] D. K. Kaul, M. E. Fabry, *etc., Journal of Clinical Investigation*, 72(1):22–31, 1983.

Sickle cell anemia - Outline

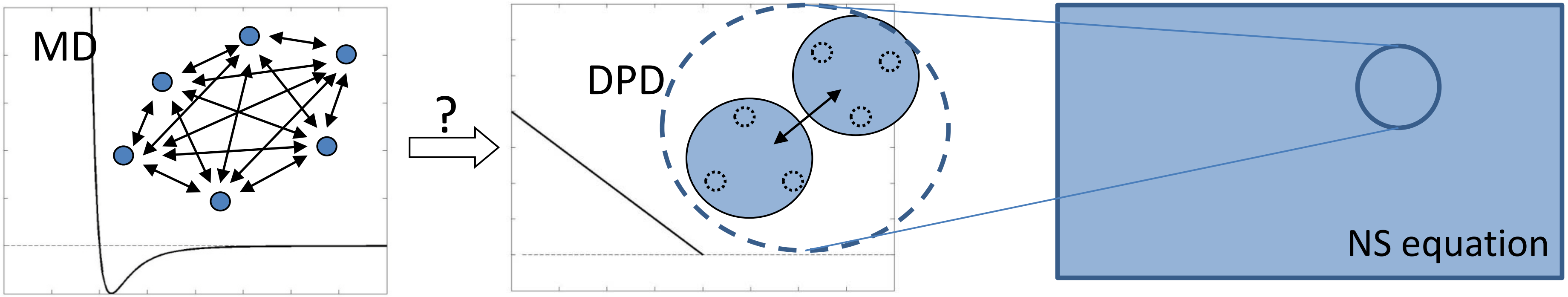
- Heterogeneous sickle cell morphology
  - Molecular interactions, coarse-graining, self-assembly
  - Effect of chirality and confinement
  - Kinetic model for the growth of the sickle hemoglobin (HbS) polymer

- Rheology of sickle cell suspensions
  - A multi-scale model of sickle red blood cell
  - Effect of cell rigidity and morphology

- Vaso-occlusion crisis induced by sickle cell anemia
  - Effect of cell-endothelium adhesive interaction
  - Interplay of multiple cell groups

Introduction

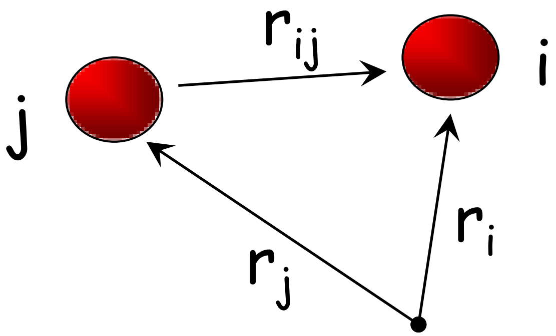
Dissipative Particle Dynamics



$$\vec{F}_{ij}^{C} = F_{ij}^{(c)}(r_{ij})\vec{e}_{ij}$$

$$\vec{F}_{ij}^{D} = -\gamma\omega^{D}(r_{ij})(\vec{v}_{ij}\cdot\vec{e}_{ij})\vec{e}_{ij}$$

$$\vec{F}_{ij}^{R} = \sigma\omega^{R}(r_{ij})\xi_{ij}\vec{e}_{ij}$$



- Does there exist a direct mapping between DPD and MD systems?
- How to impose proper boundary conditions to simulate meso-scale hydrodynamics?
- Applications to blood flow systems

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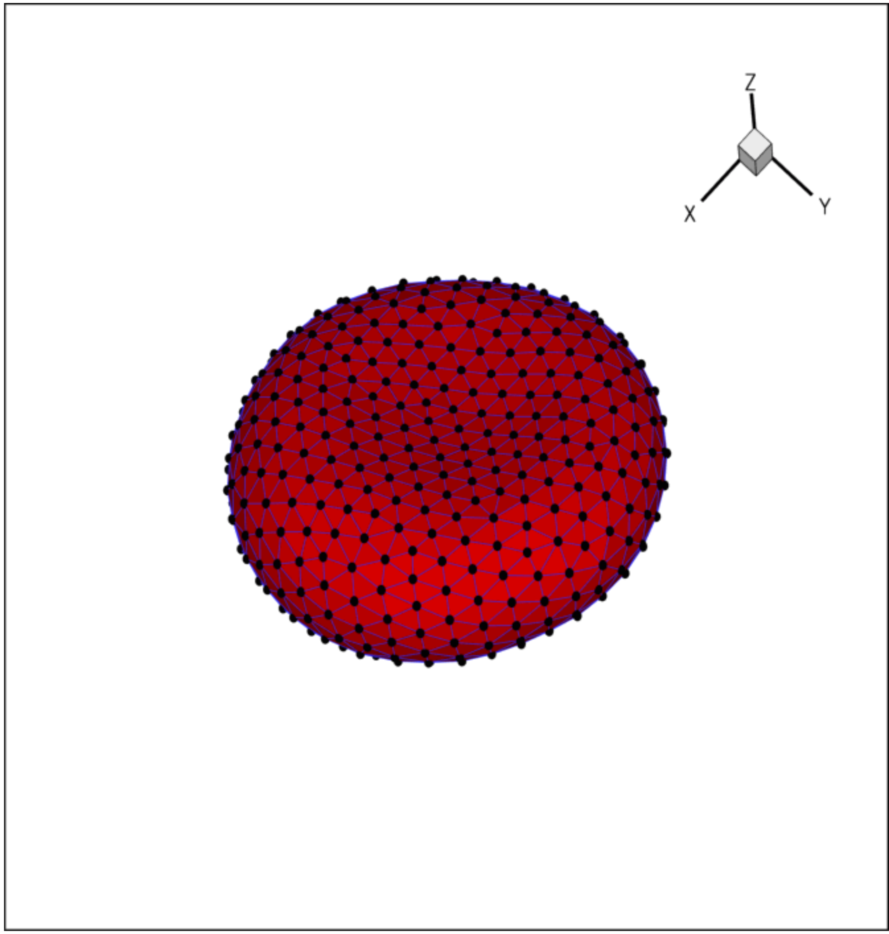
[1] Hoogerbrugge & Koelman, *Europhys Lett*, 19:155, 1992
[2] Espanol & Warren, *Europhys Lett*, 30:191, 1995

Multi-scale Red Blood Cell Model

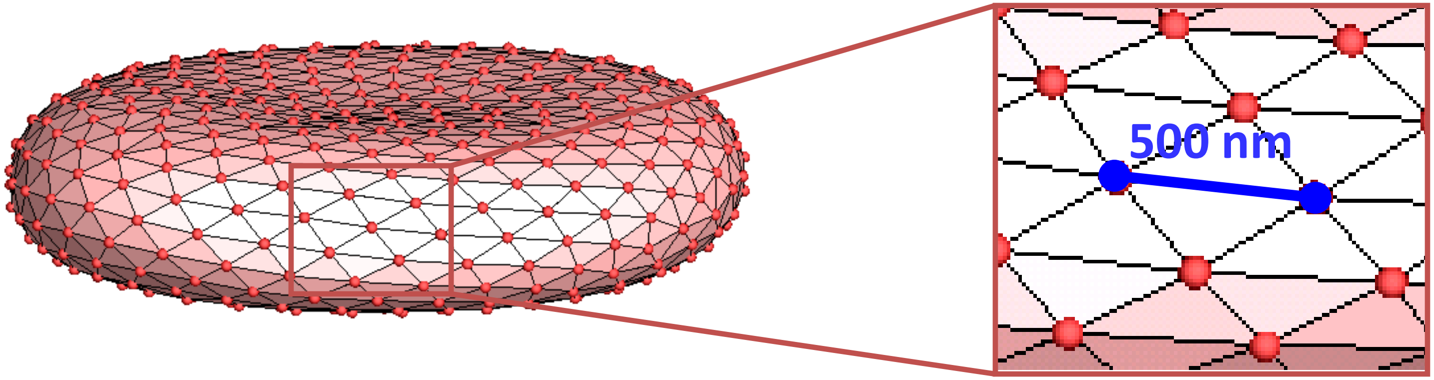
Main features:

Triangular mesh:
1) each vertex – a DPD particle
2) each edge – a viscoelastic spring
3) bending energy between faces
4) constant surface area (local or global)
5) constant volume



With Subra Suresh, MIT

General Spectrin-level and Multi-Scale RBC Models



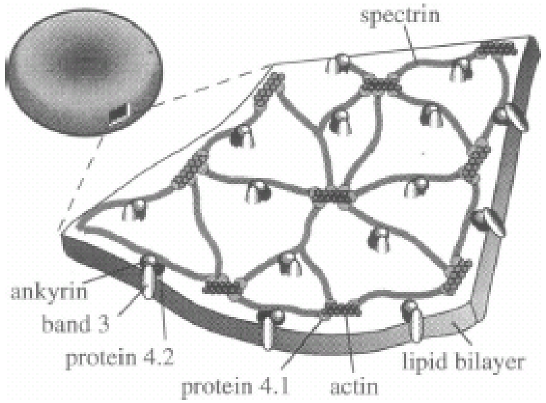
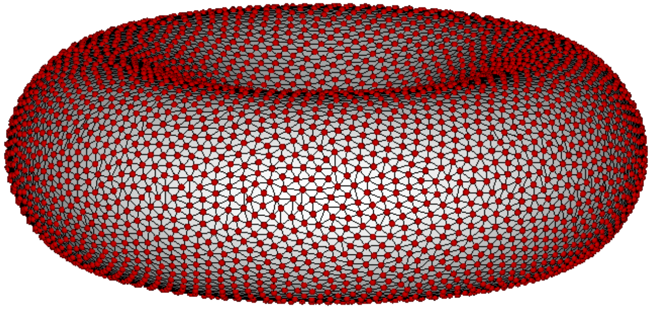
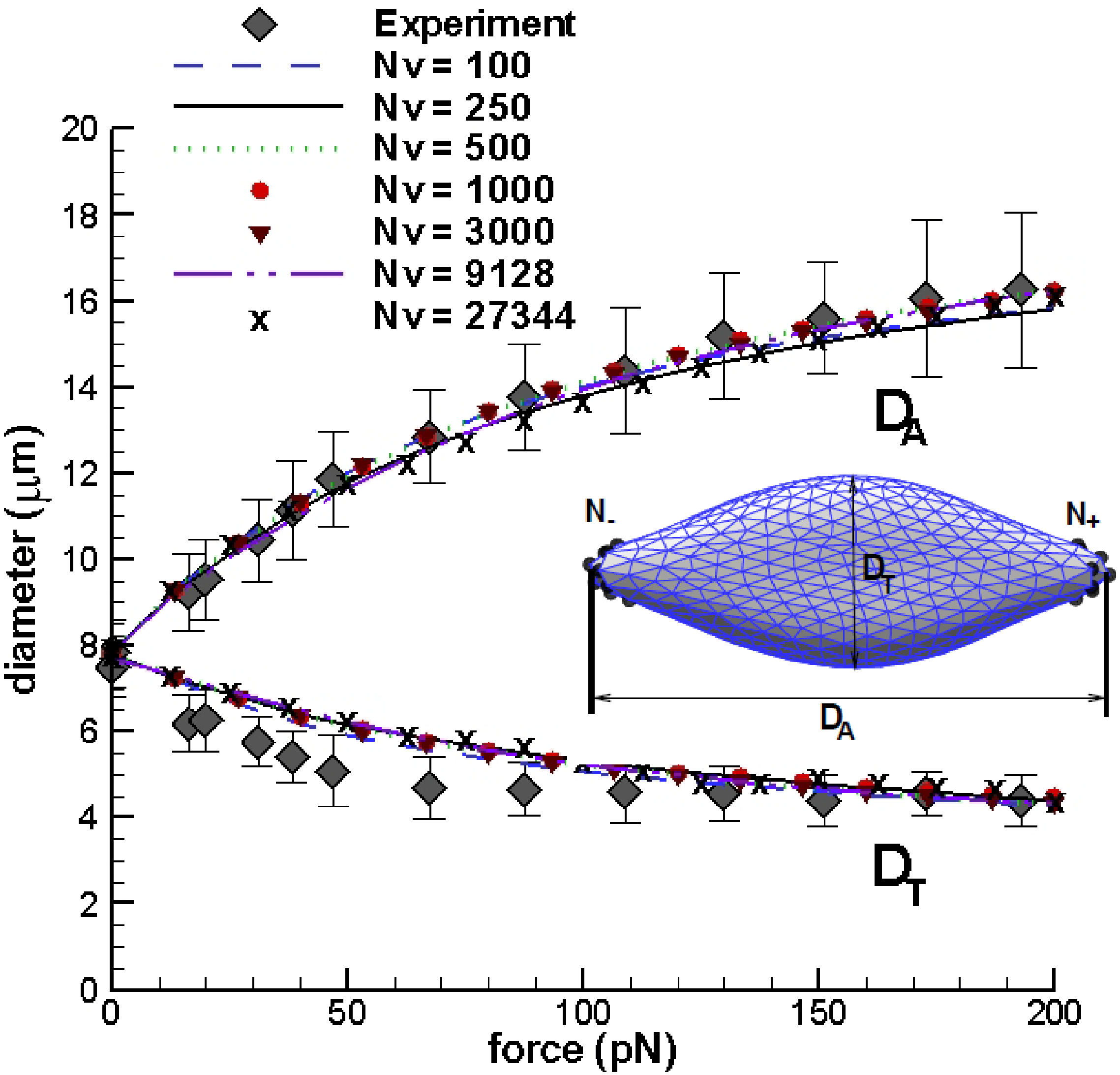


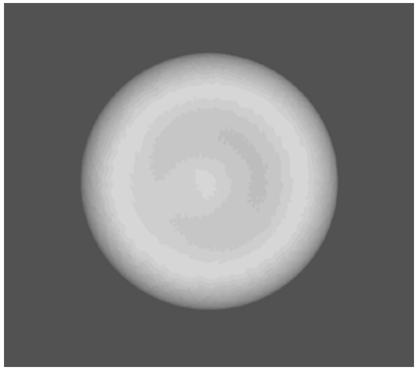
FIGURE 1 Arrangement of the major components of the RBC membrane skeleton.



1. **Pivkin & Karniadakis, PRL, 2008;**
2. **Fedosov, Caswell & Karniadakis, Biophys. J, 2010**

MS-RBC mechanics: healthy





$$\mu_0 = 6.3 \times 10^{-6} \frac{N}{m}$$

$$Y = 18.9 \times 10^{-6} \frac{N}{m}$$

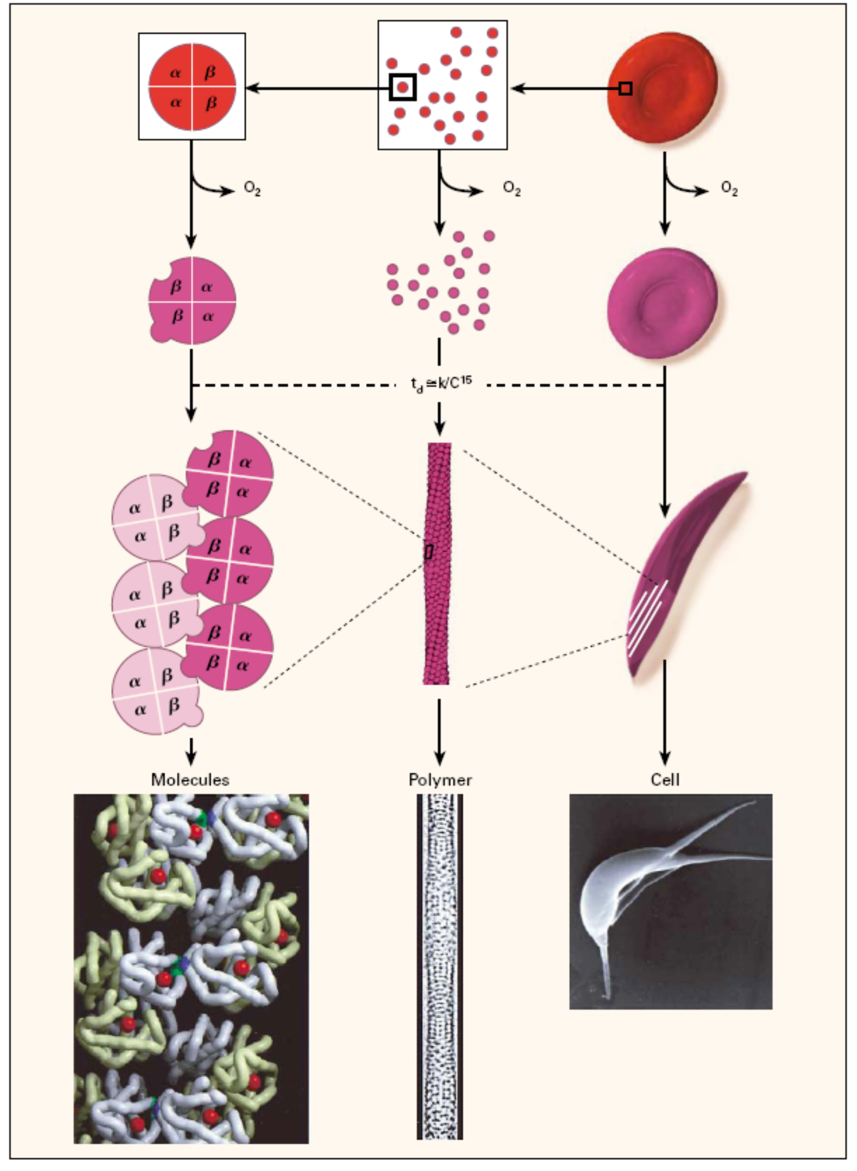
$$k_c = 2.4 \times 10^{-19} J$$

Experiment - Suresh et al., *Acta Biomaterialia*, 1:15-30, 2005

Key features of the RBC model

- RBC area and volume are constant
- Membrane elasticity
  - Based on the properties of the spectrin network
  - Coarse-graining procedure has no fitting parameters
  - No temperature dependence (Waugh, 1979; Mills, 2005)
- Membrane viscosity
  - Temperature dependent
- Cytosol viscosity (internal DPD fluid)
  - Temperature dependent
  - @ 25C = 9 times more viscous than surrounding fluid
  - @ 37C = 6 times more viscous than surrounding fluid
- Surrounding DPD fluid
  - Temperature dependent viscosity

Introduction: Molecular pathogenesis



Packaging of hemoglobin into RBCs requires that the protein be soluble.

Upon de-oxygenation:

Replacement of Glu at *β*6 with Val results in hydrophobic interaction (HI) with another hemoglobin molecule, causing aggregation into large polymers.

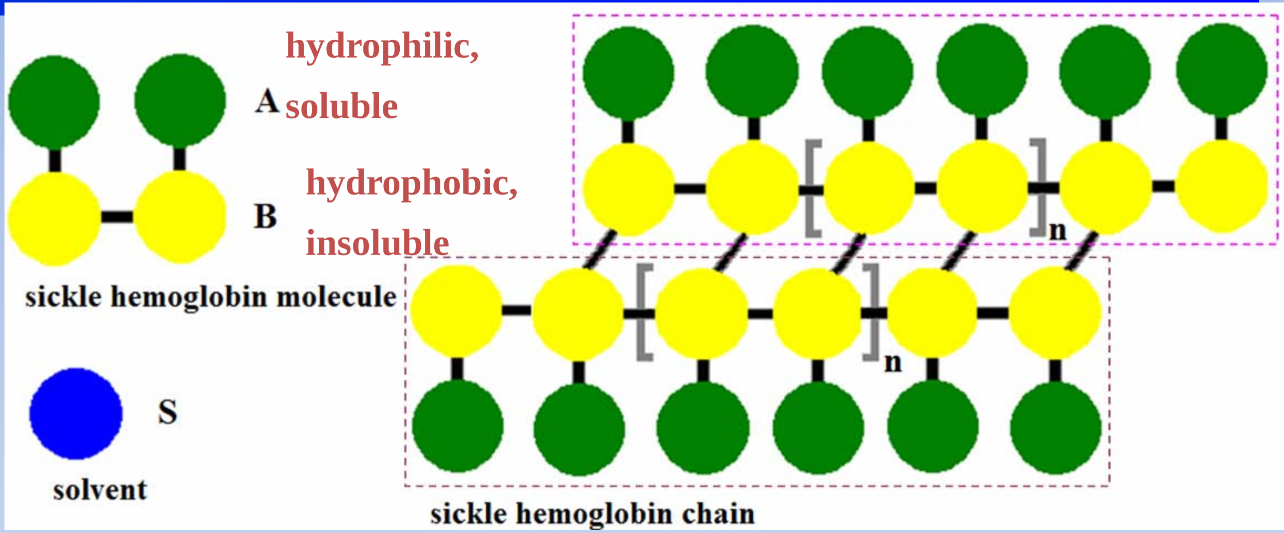
HI is necessary for the formation of polymers

Polymerization of deoxyhemoglobin and alignment of fibers result in a distortion of the shape of the RBCs and a marked decrease in its deformability.

H. F. Bunn, *N. Engl. J. Med*, 1997, *337*, 762

Coarse-grained HbS model

Normal RBC contains hemoglobin A that has 2 subunits denoted by α and 2 subunits denoted by β.



A schematic of coarse-grained model for sickle hemoglobin.

The packaging of a very high concentration of hemoglobin into RBCs requires that the protein be extraordinary **soluble**.

Change from charged to neutral hydrophobic amino acid causes aggregation upon de-oxygenation.

Dissipative Particle Dynamics (DPD) Method

DPD is a coarse-grained molecular dynamics method that models seamlessly soft matter and solvent.

Pairwise additive force：

$$\mathbf{F}_i = \sum_{i \neq j} \mathbf{F}_{ij}^{\mathrm{C}} + \mathbf{F}_{ij}^{\mathrm{D}} + \mathbf{F}_{ij}^{\mathrm{R}}$$

Conservative: fluid / system dependent

$$\mathbf{F}_{ij}^{\mathrm{C}} = a_{ij}\omega(r_{ij})\mathbf{n}_{ij}$$

Dissipative: frictional force, represents viscous resistance within the fluid

$$\mathbf{F}_{ij}^{\mathrm{D}} = -\gamma\omega^2(r_{ij})(\mathbf{n}_{ij} \cdot \mathbf{v}_{ij})\mathbf{n}_{ij}$$

Random: stochastic part, makes up for lost degrees of freedom eliminated after the coarse-graining

$$\mathbf{F}_{ij}^{\mathrm{R}} = \sigma\omega(r_{ij})\zeta_{ij}\Delta t^{-1/2}\mathbf{n}_{ij}$$

Dissipative and random forces form DPD thermostat

$$\sigma^2 = 2\gamma k_{\mathrm{B}} T$$

Dissipative Particle Dynamics Method

The force field is usually divided into two major parts: bonded and non-bonded potential terms:

$$V_{\text{tot}} = V_{\text{bonded}} + V_{\text{nonbonded}} = \left(V_{\text{str}} + V_{\text{bend}} + V_{\text{tors}}\right) + \left(V_{\text{vdw}} + V_{\text{es}} + \cdots\right)$$

Bonded interactions:

Hookean spring interaction (A-B and B-B):

$$V_{\text{str}} = k_{\text{str}}\left(r - r_0\right)^2$$

Bond-bending interaction (A-B-B and B-B-B in same chain):

$$V_{\text{bend}} = k_{\text{bend}}\left(\theta - \theta_0\right)^2$$

FENE interaction (A-B-B in different chains):

$$F_{\text{bend}} = k_{\text{bend}}\left(\frac{\theta - \theta_0}{1 - {}^{(\theta-\theta_0)}\!/_{\Delta\theta_{\max}}}\right)$$

Control the chain rigidity

Describe the chain chirality

Non-bonded interactions:

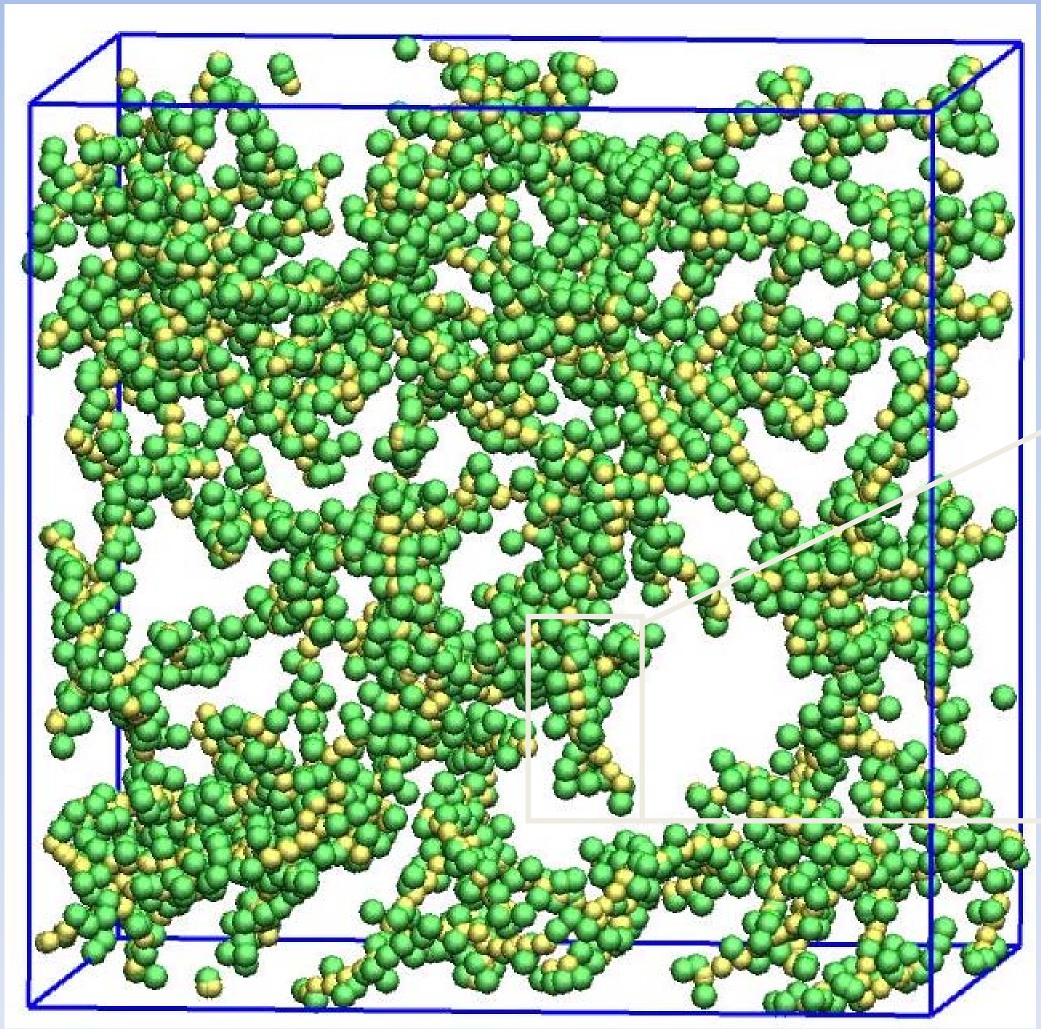
Pairwise conservative interaction:

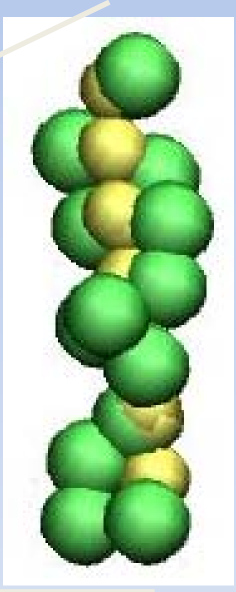
$$V_{\text{non-bonded}} = -\frac{a_{ij}}{2}\left(1 - r_{ij}/r_c\right)^2$$

The self-assembled microstructures

Bond-bending and torsional interactions among the hydrophilic and hydrophobic particles excluded

$k_{\text{bend}} = 0.0 \quad (\text{A} - \text{B} - \text{B})$





The self-assembled small aggregates

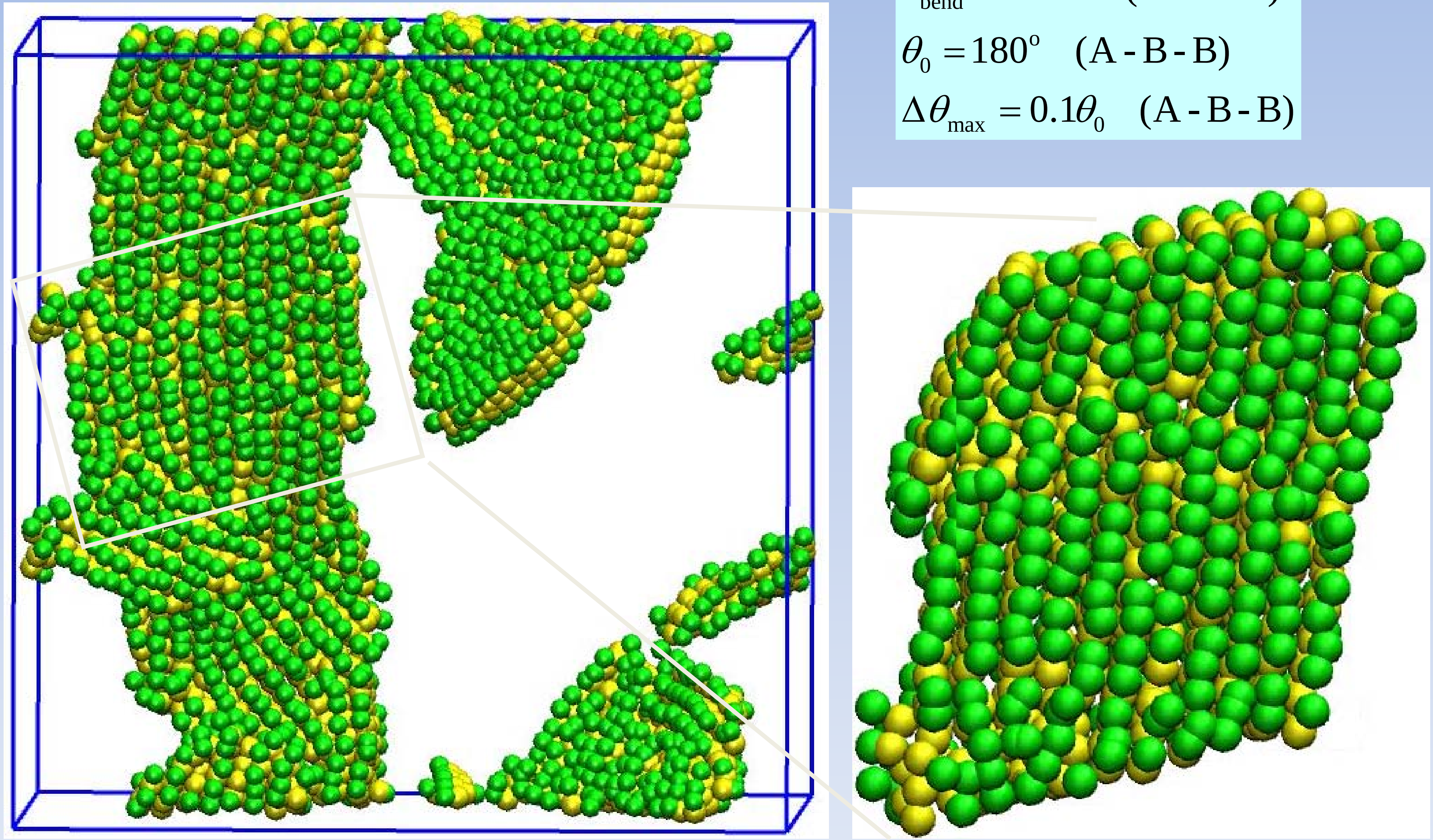
The self-assembled microstructures

Bond-bending and torsional interactions among the hydrophilic and hydrophobic particles included

$k_{\mathrm{bend}} = 200.0 \quad (\mathrm{A\text{-}B\text{-}B})$

$\theta_0 = 180^{\circ} \quad (\mathrm{A\text{-}B\text{-}B})$

$\Delta\theta_{\max} = 0.1\theta_0 \quad (\mathrm{A\text{-}B\text{-}B})$



The self-assembled elongated sheet-like microstructures

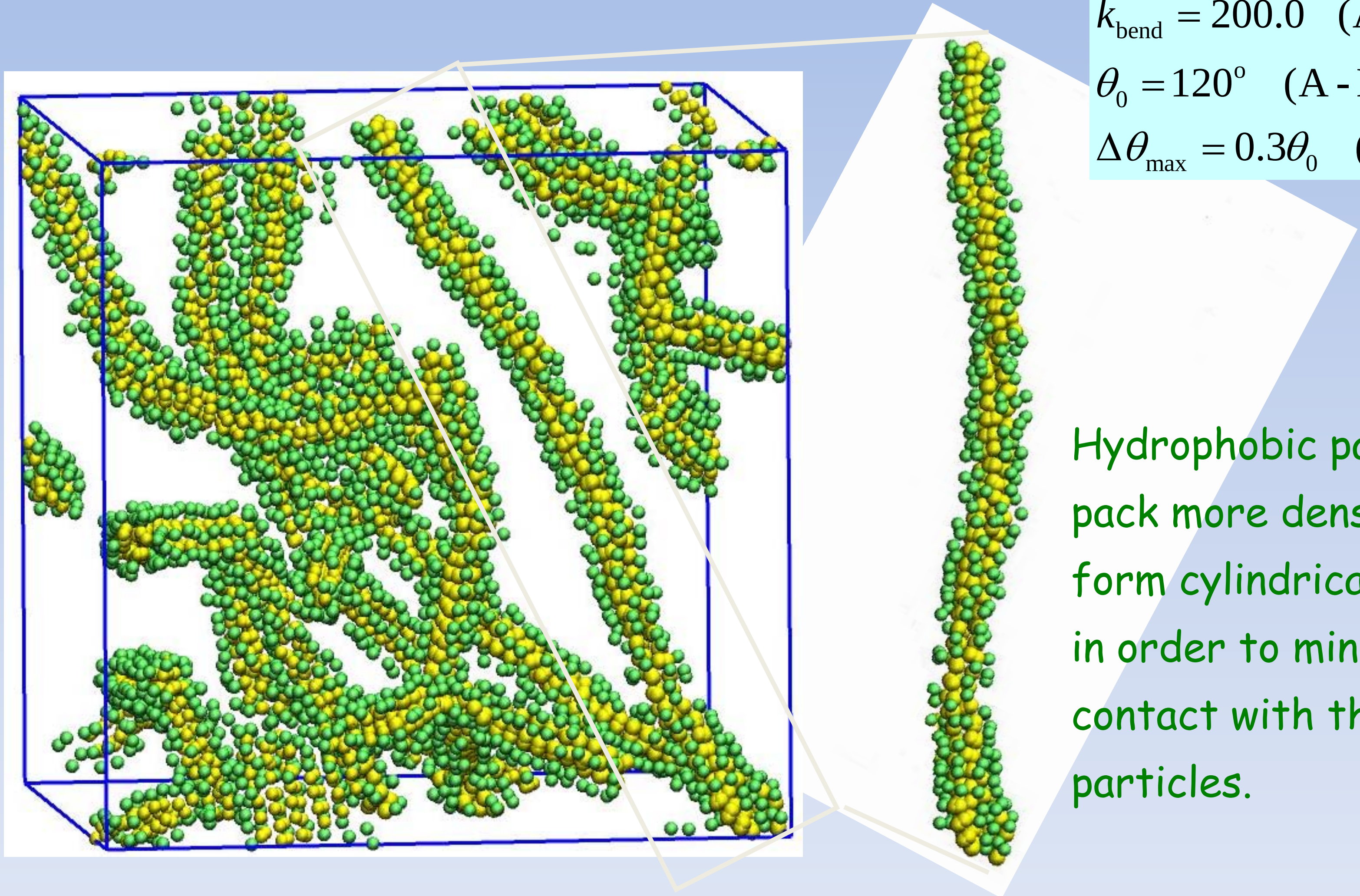
The self-assembled microstructures

Bond-bending and torsional interactions among the hydrophilic and hydrophobic particles included

$$k_{\text{bend}} = 200.0 \quad (\text{A-B-B})$$

$$\theta_0 = 120^{\circ} \quad (\text{A-B-B})$$

$$\Delta\theta_{\max} = 0.3\theta_0 \quad (\text{A-B-B})$$



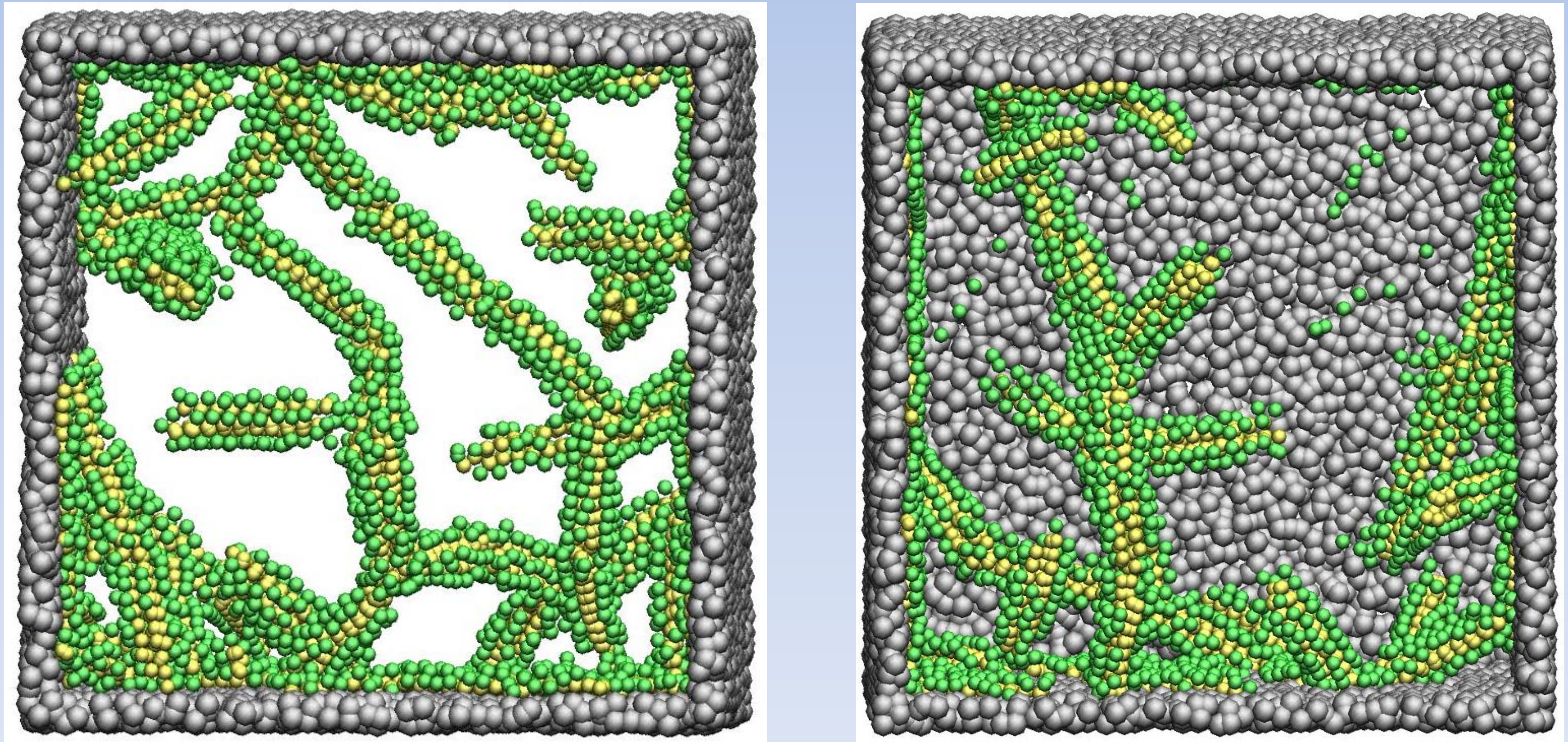
Hydrophobic particles pack more densely and form cylindrical micelles in order to minimize contact with the solvent particles.

The self-assembled elongated step-like bundle microstructures

HbS self-assembly in hard confinement

Confinement has an influence on the self-assembled morphologies of biopolymer or soft matter;

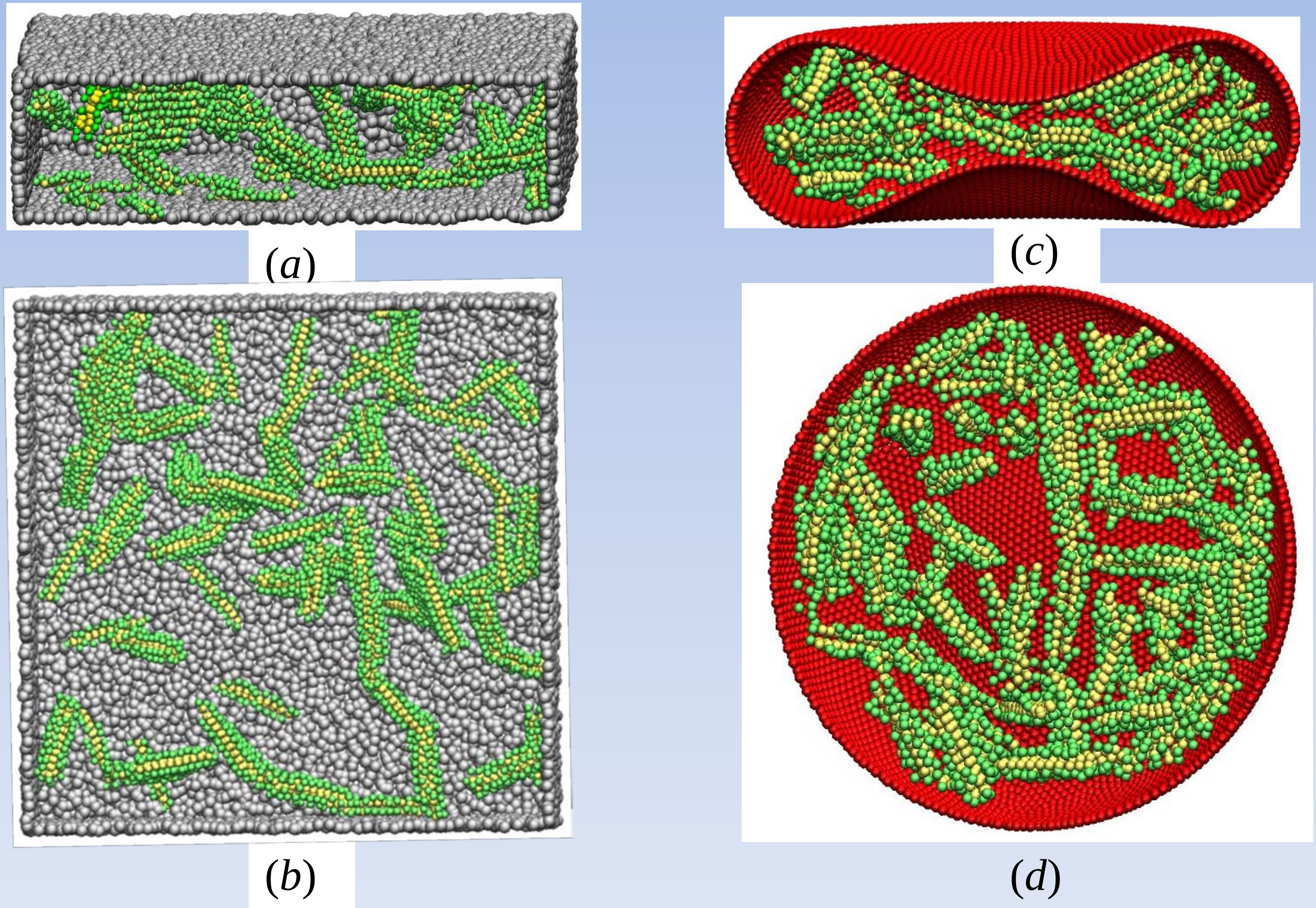
To illustrate the effect of the confinement on microstructure formation, we simulate the self-assembly of HbS in hard confinement;



Self-assembled microstructures of HbS molecules in cube box with 2D (*left*) and 3D (*right*) confinement

HbS self-assembly in hard confinement

Effect of the aspect ratio of the geometry


(*a*) (*b*) (*c*) (*d*)

Self-assembled microstructures of HbS molecules in a cubiod box (left) and inside a rigid RBC (right).

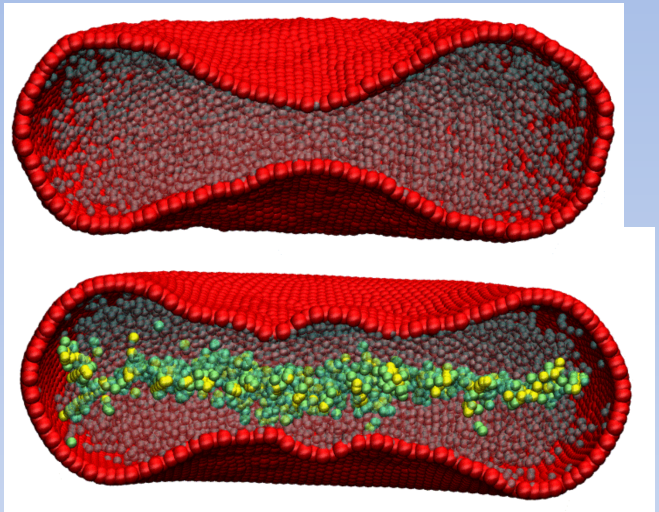
Shape deformation of RBC induced by HbS fibers

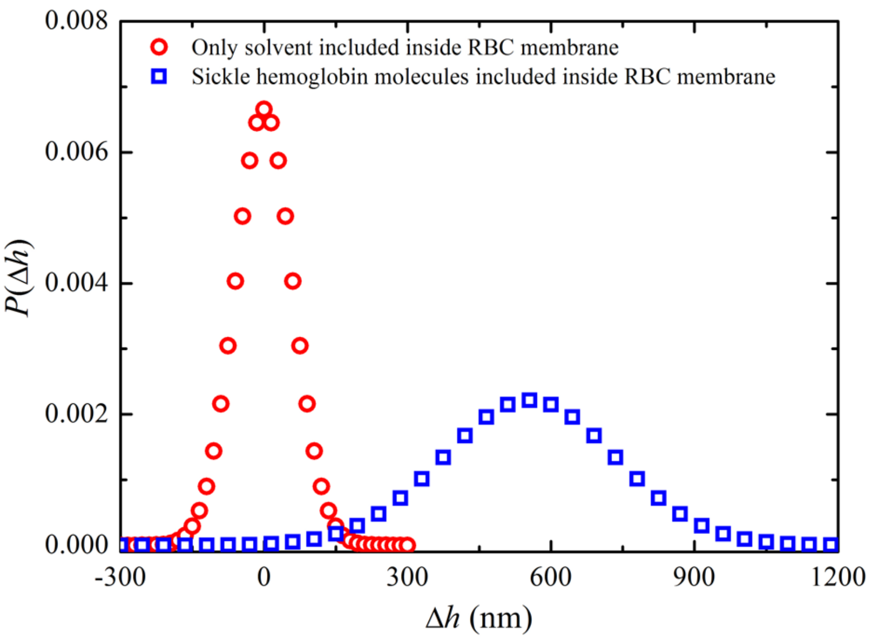
RBC responses for DPD particles inside the membrane

RBC can keep its shape when only the solvent particles are included inside the RBC membrane.

Strong RBC membrane fluctuations take place when we include the HbS molecules into the RBC.

The HbS molecules cannot self-assemble into elongated HbS fiber inside the RBC.



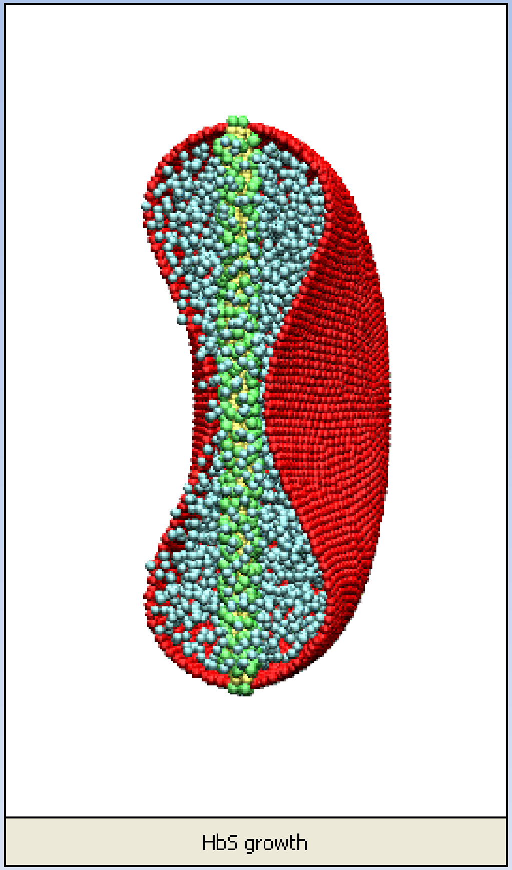


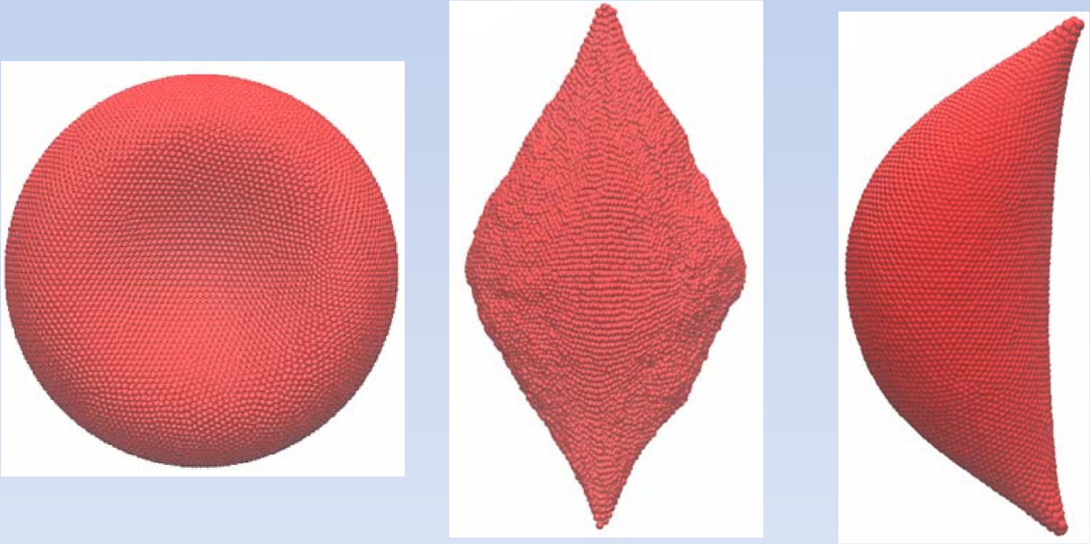
Shape deformation of RBC induced by HbS fibers

To simulate the growth of a HbS fiber, we use a linear spring model described by,

$$F_{\text{str}} = k_{\text{str}} \left(l_{\text{ref}} - l \right)$$

$$l_{\text{ref}} = l_0 \left\{ 1 - \left[\left(\frac{l_{\text{target}}}{l_0} \right)_{\max} - 1 \right] \left[\frac{p_{O_2\_\text{target}} - p_{O_2}}{p_{O_2\_\text{target}}} \right] \right\}$$





Biconcave shape   Holly leaf shape   Sickle shape

Shape deformation of RBC induced by the growth of HbS fiber in DPD simulation

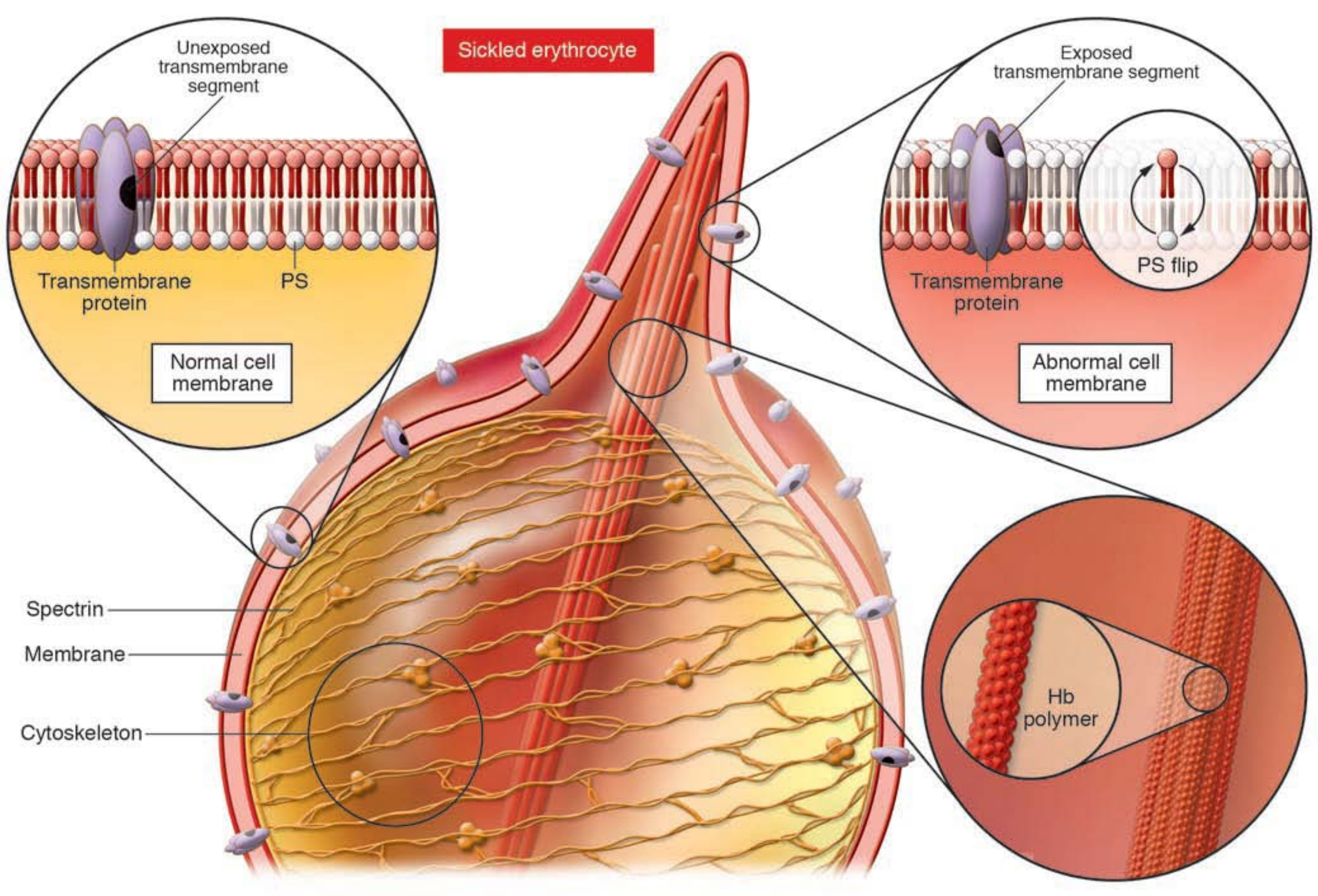
Summary

Self-assembled complex microstructures are obtained from HbS in 3D DPD simulations;

Hydrophobic interactions are demonstrated to be necessary with chirality being the main driver for the formation of HbS fibers;

Linear elongation and bond-bending interactions of HbS fibers lead to sickle-shaped cells.

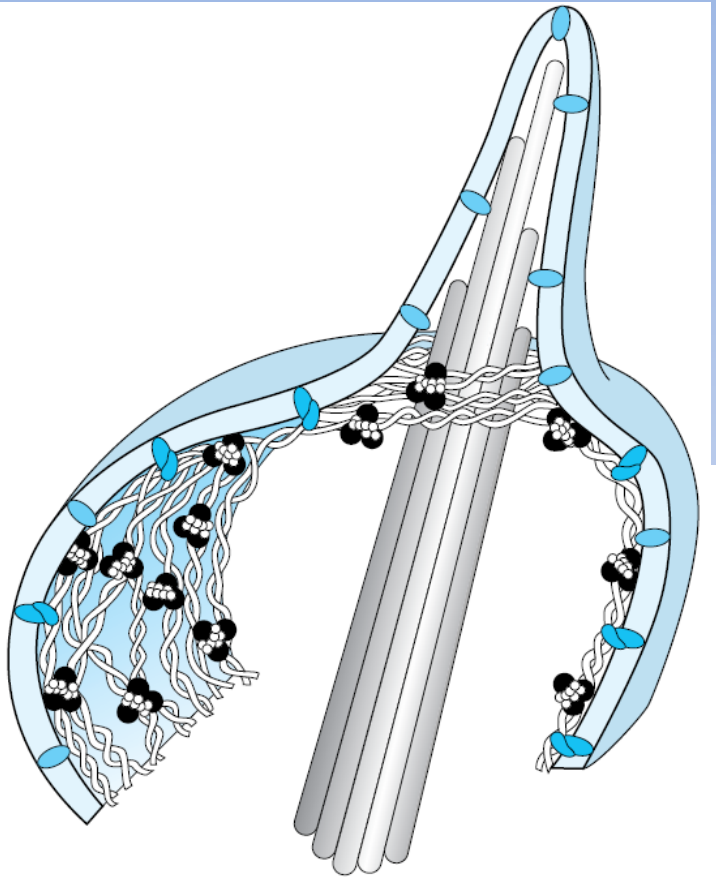
Penetration and destruction of the RBC membrane



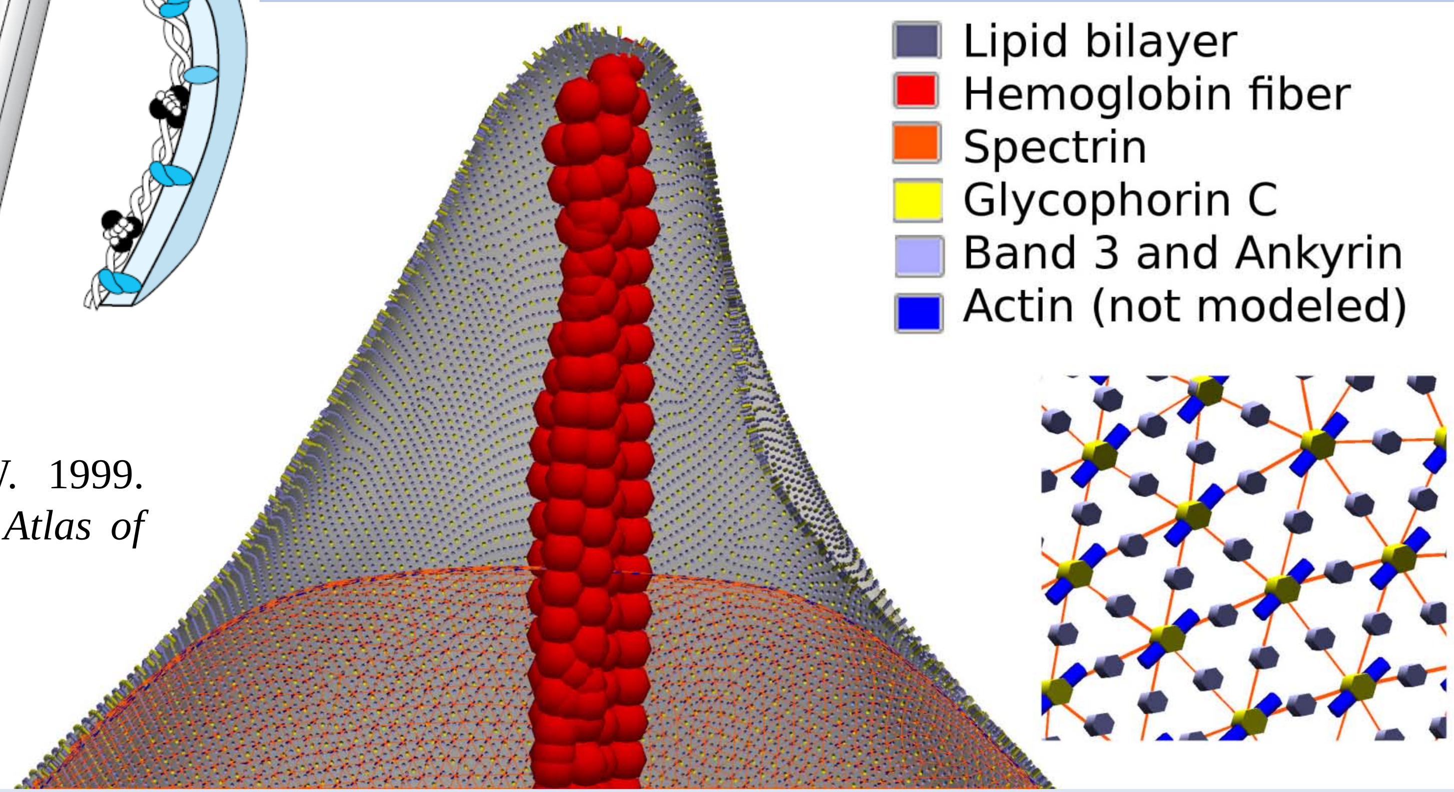
Alteration of the RBC membrane by polymerization of sickle hemoglobin. The membrane is penetrated and destroyed by the intracellular formation of sickle hemoglobin polymers, resulting in spicule formation.

P. S. Frenette and G. F. Atweh. *J. Clin. Invest.* **117**:850–858 (2007)

Penetration and destruction of the RBC membrane

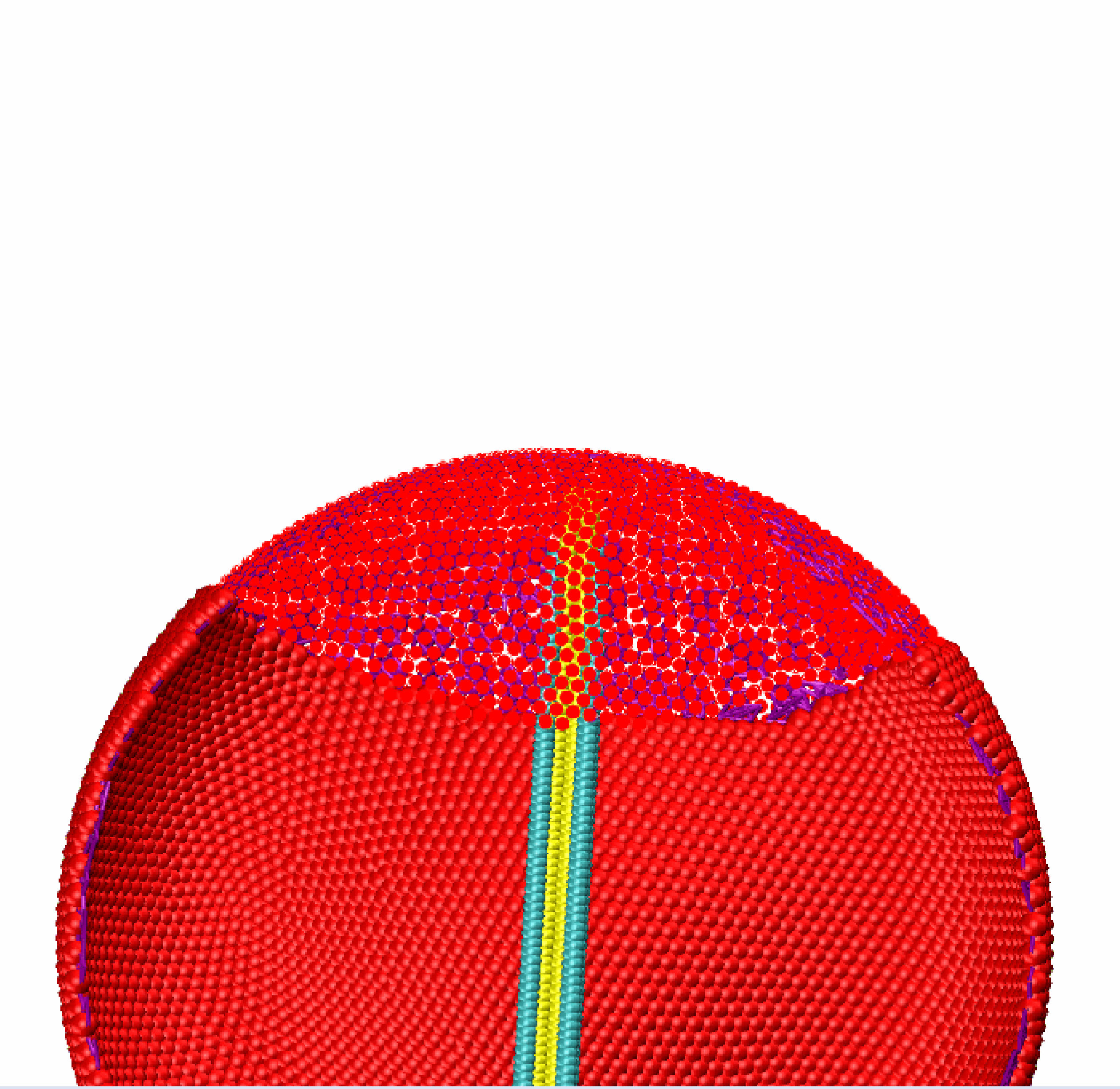


Statius van Eps, L.W. 1999. Sickle cell disease. In *Atlas of diseases of the kidney*.



DPD simulation

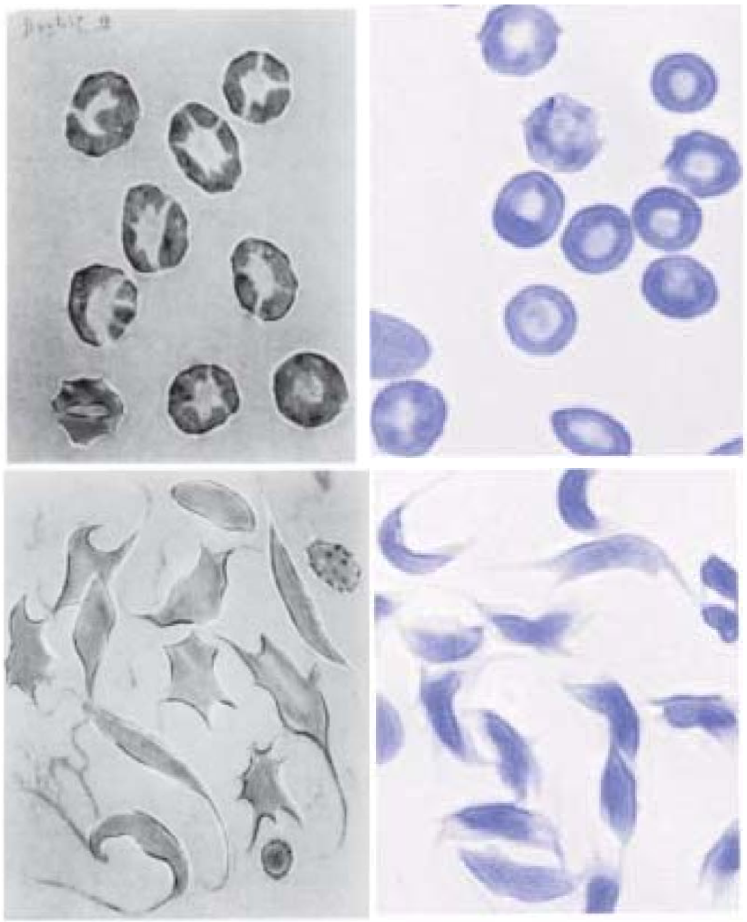
Penetration and destruction of the RBC membrane

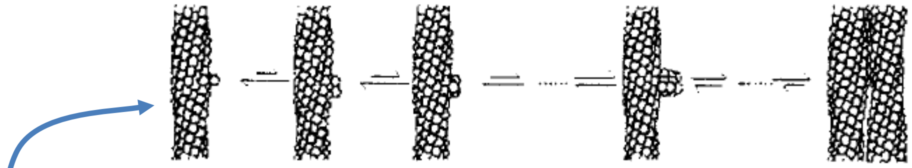


DPD simulation

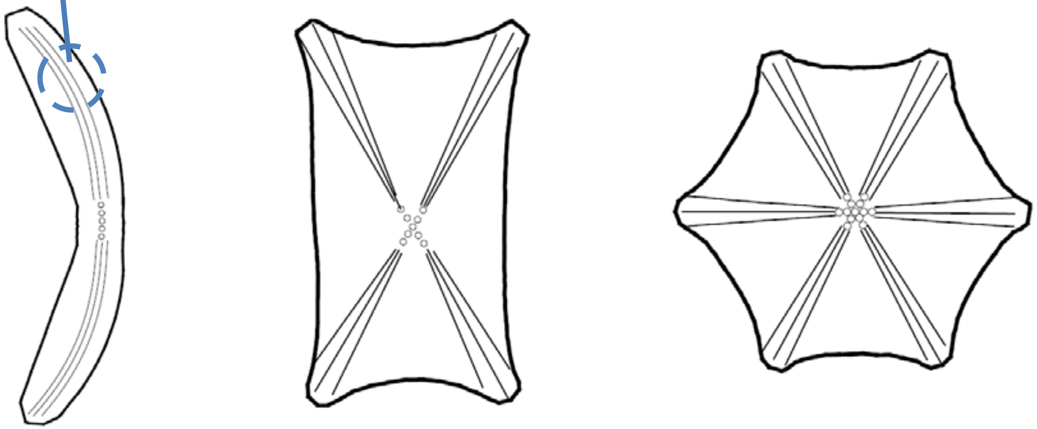
Sickle cell morphology

Intracellular HbS polymer domain





Sketches of the typical intracellular HbS polymer configuration



- With moderate mean corpuscular hemoglobin concentration (MCHC) , the HbS polymer tends to grow along one direction.
- High MCHC environment favors isotropic growth directions with spherulite polymer configuration

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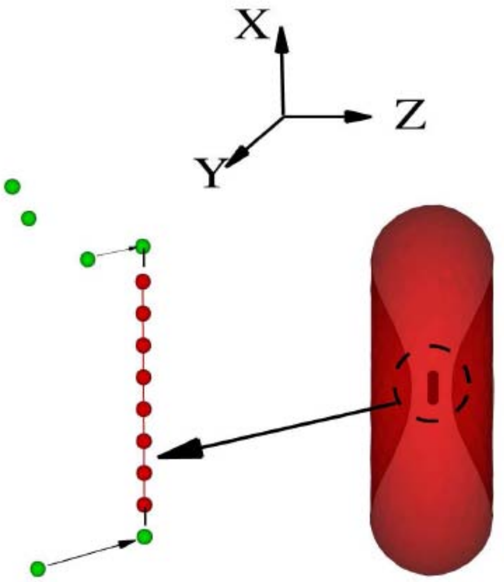
[1] F. A. Ferrone, J. Hofrichter, and W. A. Eaton, *Journal of Molecular Biology*, 183, 611, 1985.
[2] G. W. Christoph, J. Hofrichter, and W. A. Eaton, *Biophysical Journal,* 88, 1371, 2005.
[3] H. Lei and G. E. Karniadakis, *Soft Matter,* vol. 8, p. 4507, 2012.

Sickle cell morphology

Intracellular aligned HbS polymer



- Model of the aligned HbS polymer

$$V_{bond} = \frac{k_b(3x_{ij}^2 - 2x_{ij}^3)}{(1 - x_{ij})} + \frac{k_p}{l_{ij}}$$

$$V_{angle} = k_a(\theta - \theta_0)^2$$

- One polymer chain represents N_f HbS fibers

$$\kappa = N_f^2 \kappa_0 \qquad \kappa_0 = 1.0 \times 10^{-24} Nm^2$$

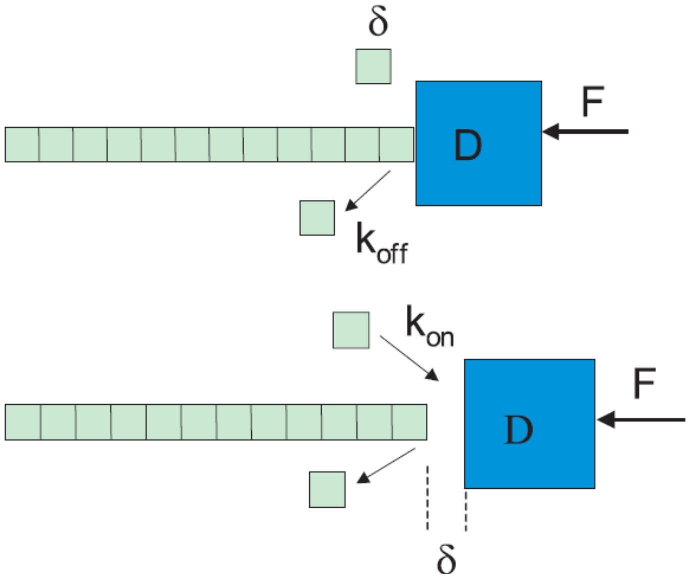
$$Y = Y_0 \qquad Y_0 = 0.1 GPa$$

- Growth rate of the HbS polymer domain

$$P_t = 1 - e^{-k_t \Delta t}$$

$$k_t = k_{on} e^{-(\mathbf{f_s} \cdot \hat{\mathbf{e}})\delta / k_B T} - k_{off}$$

$$k_{on} = \frac{N_f k_+ \gamma_c c \delta}{l_0}; \qquad k_{off} = \frac{N_f k_- \delta}{l_0}$$



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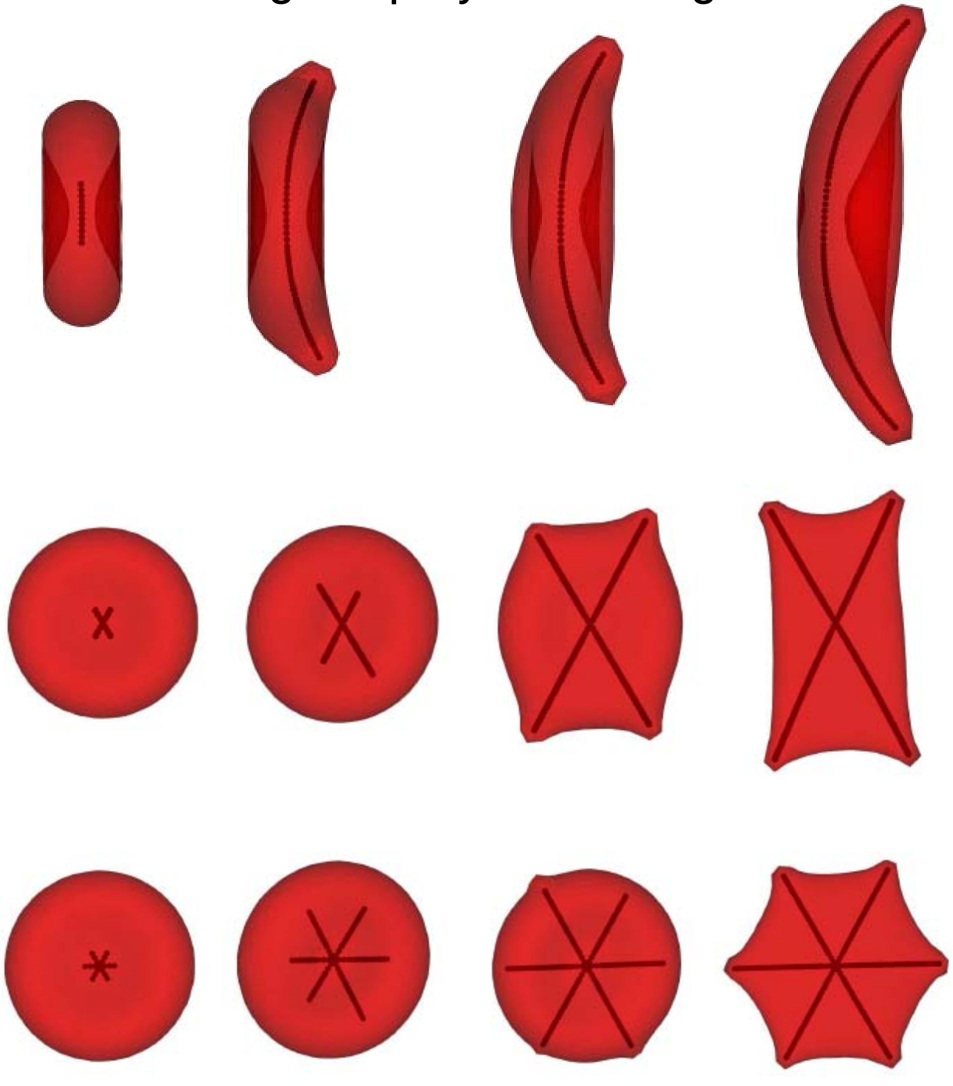
[1] C. S. Peskin, G. M. Odell, and G. F. Oster, *Biophysical Journal*, 65:316–324, 1993.

Sickle cell morphology

Effect of the aligned polymer configuration



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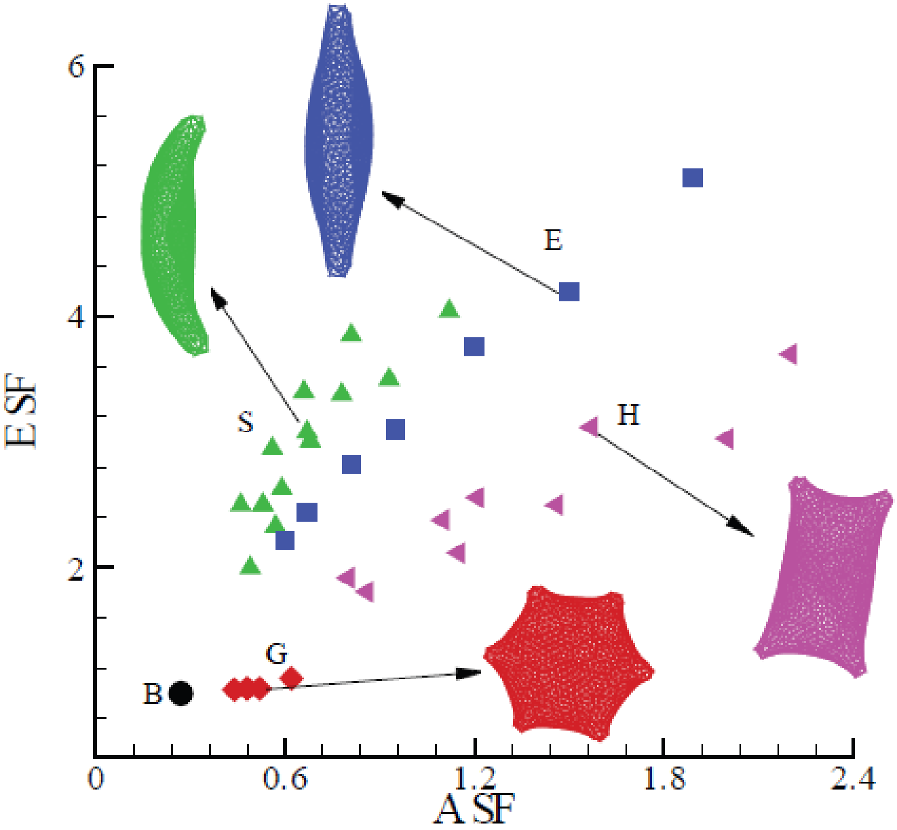
[1] J. D. Corbett, W. E. Mickols, and M. F. Maestre. *J. Biol. Chem.,* 270:2708–2715, 1995.

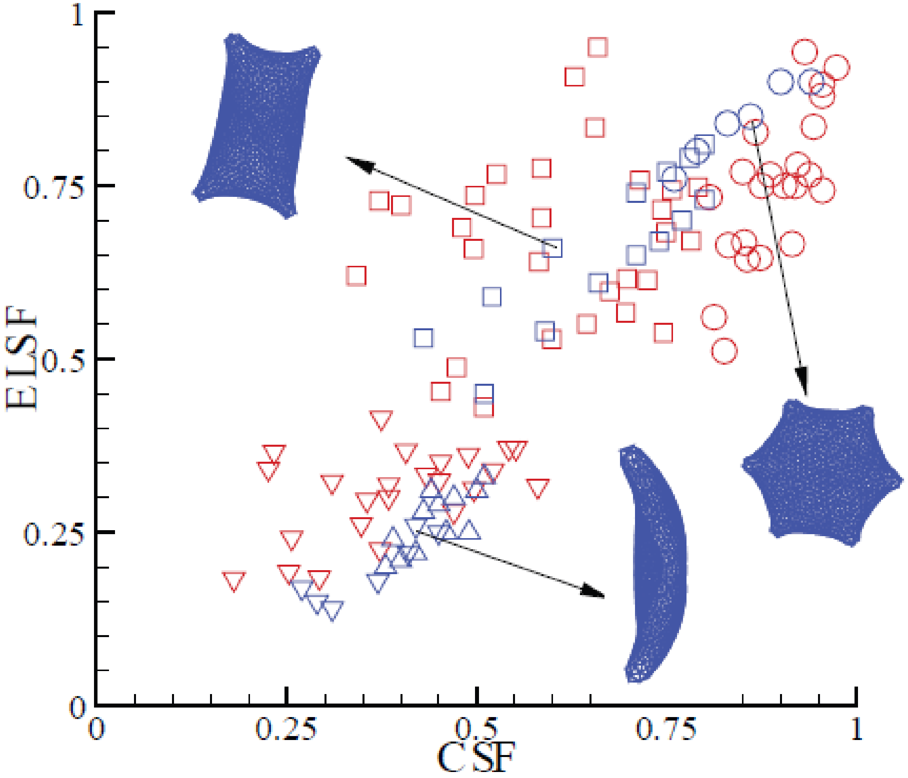
Sickle cell morphology

3D and 2D structural factors

$$\mathrm{CSF} = 4\pi\ area/(perimeter)^2$$

$$\mathrm{ELSF} = D_b/D_a,$$





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[1] K. Horiuchi, J. Ohatak, Y. Hirano, and T. Asakura, *J. Lab. Clin. Med.*, 115:613, 1990.

Sickle cell morphology

Remarks

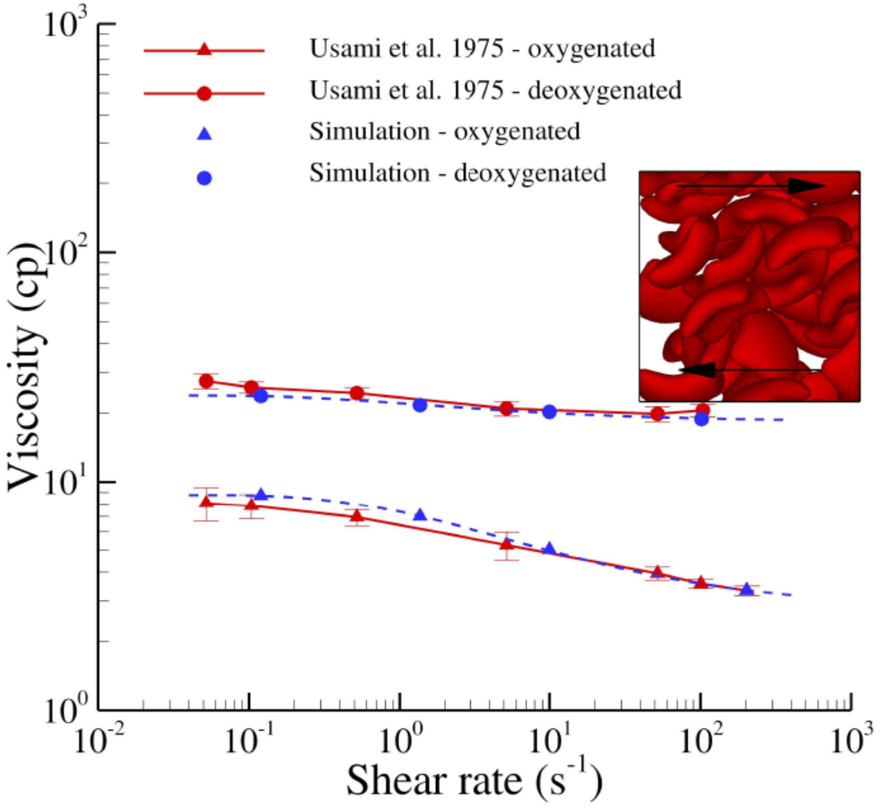
- Heterogeneous sickle cell morphologies observed in experiments are successfully predicted by a coarse-grained model of aligned sickle hemoglobin polymers.

- SS-RBCs are *primarily* determined by the angular width of the aligned hemoglobin polymer domain, but it also depends, *to a lesser degree*, on the polymer growth rate and the cell rigidity.

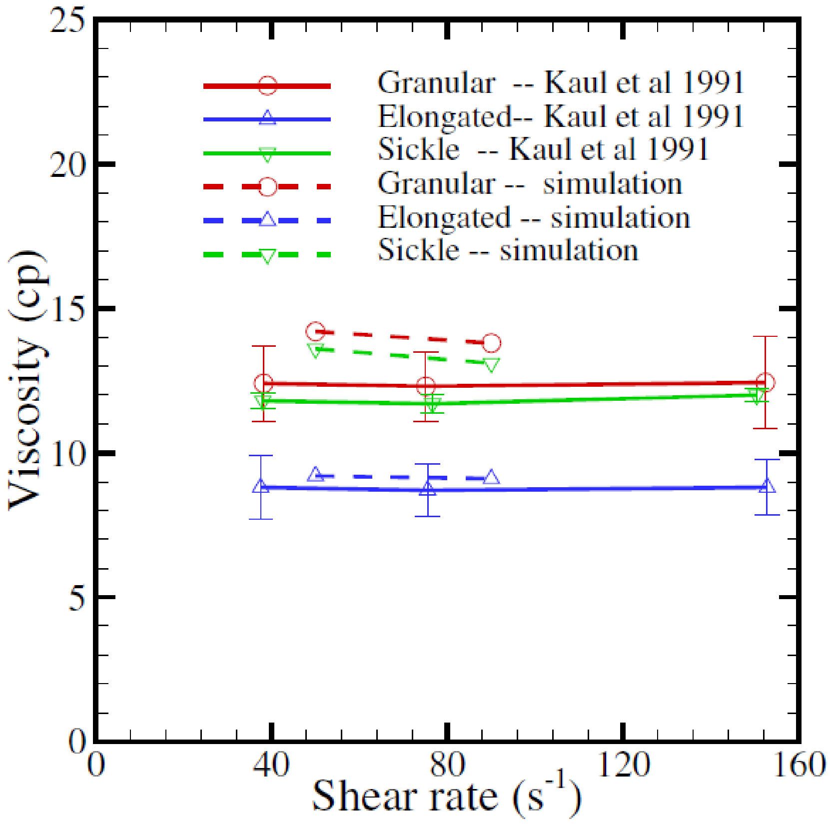
- In *in vivo* microcirculation, the cell morphology is a dynamic process, which is further influenced by the hypoxic conditions in blood vessel, adhesive interaction with endothelium cell, and is often accompanied with vaso-occlusion crisis.

Rheology of sickle cell suspension

Shear viscosity



Ht = 45%



Ht = 40%

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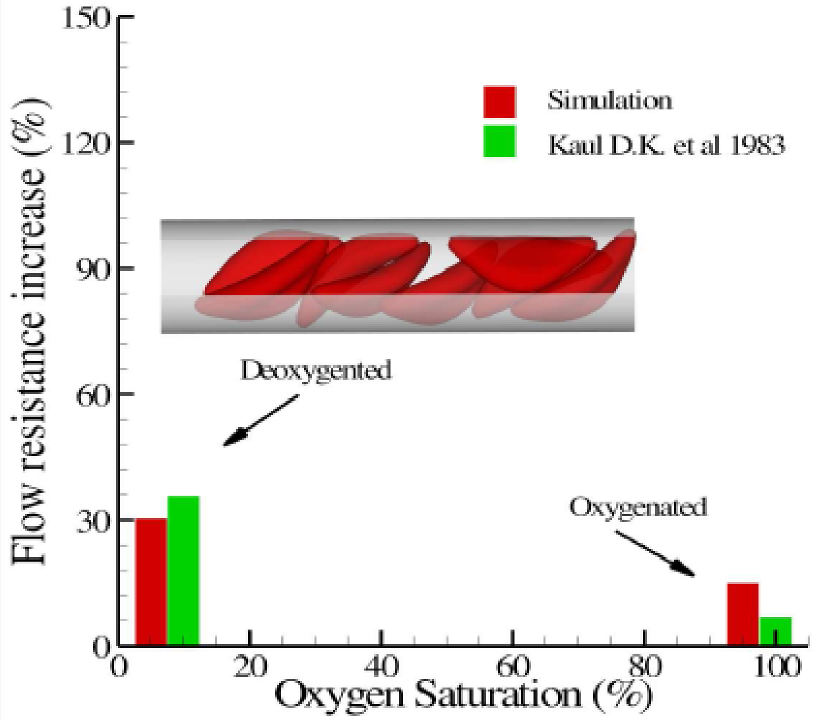
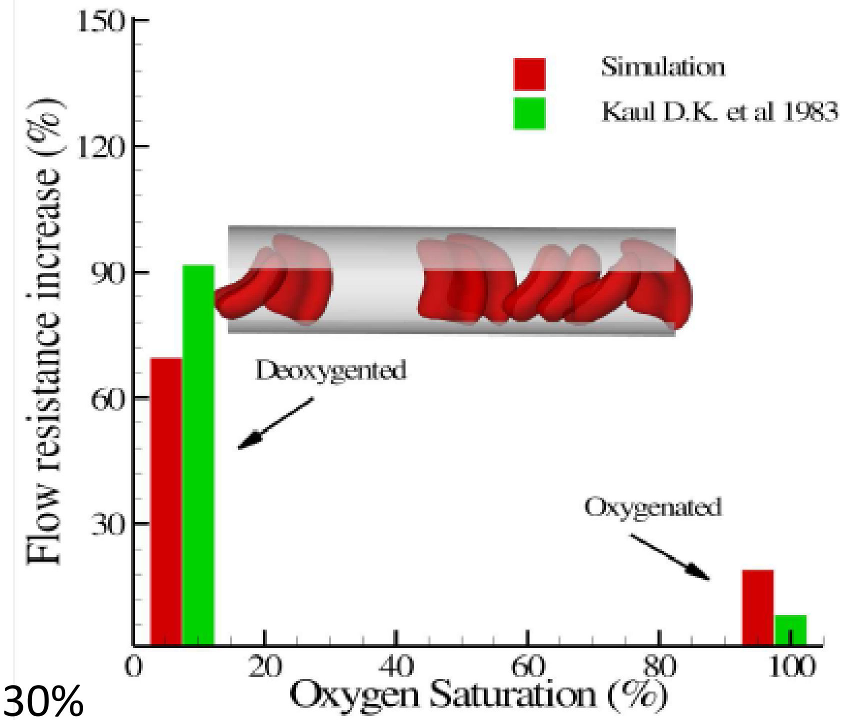
[1] Usami, S., S. Chien, P. M. Scholtz, and J. F. Bertles, *Microvascular Research* 9:324, 1975
[2] DK. Kaul and H Xue, *Blood*, 77, 1353-1361, 1991

Rheology of sickle cell suspension

Hemodynamics in pipe flow



Ht = 30%



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[1] DK Kaul, ME Fabry, P Windisch, S Baez and RL Nagel, J. Clin. Invest, 1983, 22-31

Rheology of sickle cell suspension

Remarks

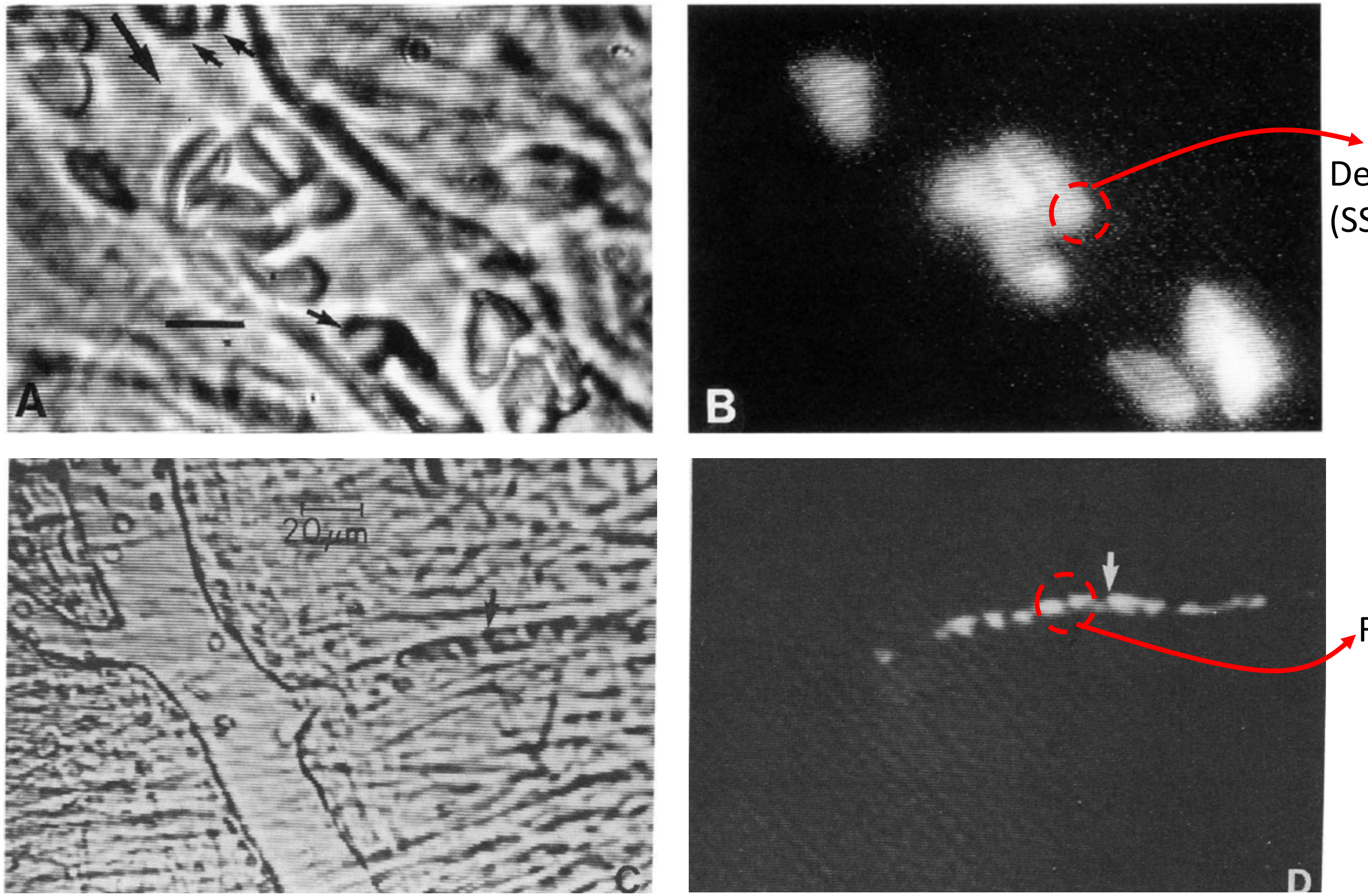
- The present model captures the major rheological properties of sickle cell suspension.

- Cell morphology affects the shear viscosity and flow resistance.

- In pipe flow, the flow resistance measured by simulation results is lower than the experimental measurements.
  - ➢ *effect of the cell adhesion?*

Vaso-occlusion induced by sickle cell anemia

Physiological Background



Deformable discocytes (SS2 cells)

Rigid ISCs (SS4 cells)

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[1] D. K. Kaul, M. E. Fabry, R. L. Nagel, *PNAS*, 86, pp3356, 1989

[2] D. K. Kaul, D. Chen, J. Zhan, *Blood*, 83, pp3006, 1994

Vaso-occlusion crisis

Physiological Background

Deformable SS2 cells adhere to endothelium cells in post cappillary / inflammation activated leukocytes adhere to the venule

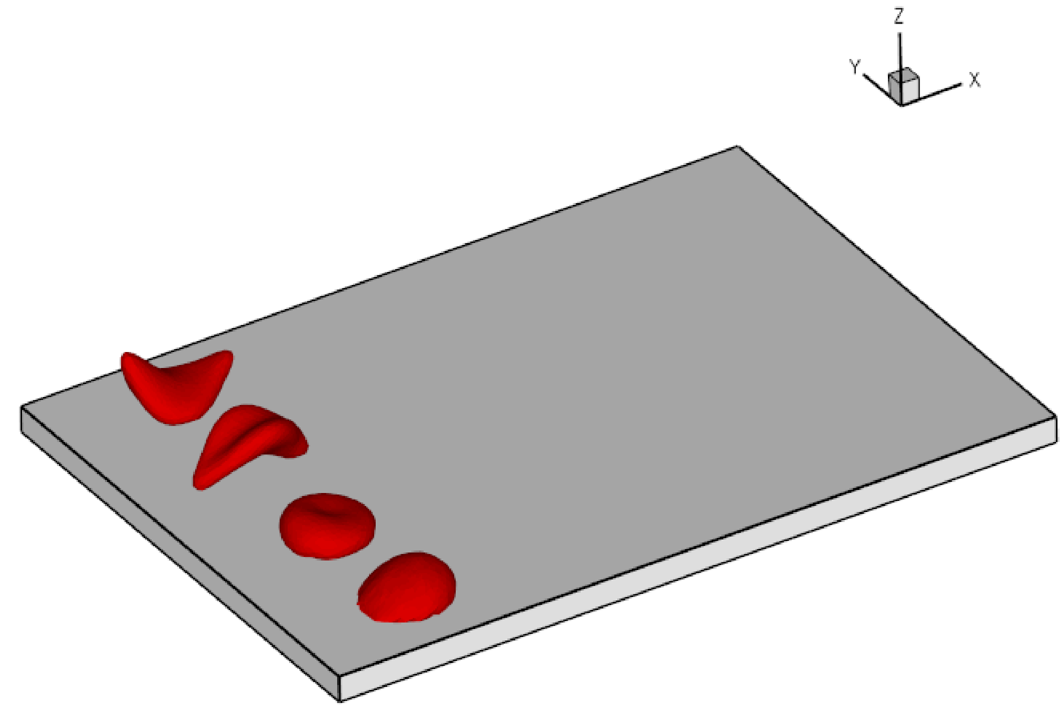
Trap rigid SS4 cells (mostly Irrersible sickle cells)

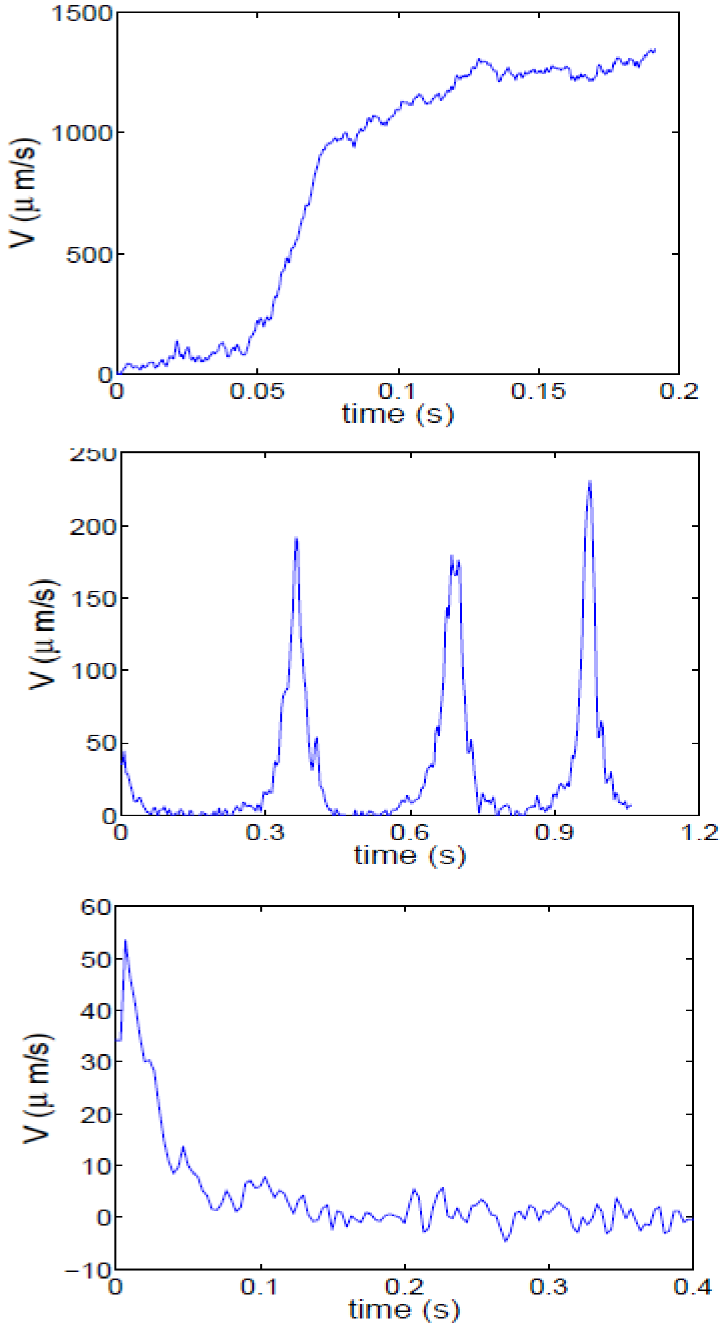
Blood occlusion in post cappillary

- In post capillaries, a specific pattern (adherent SS2+ trapped SS4) appears in occluded sites.
- Why the SS2 groups are more adherent than ISC (SS4) groups?
- Most occlusion sites occur in post-capillary with diameter less than 10 micrometers.
- Adherent Leukocytes also contribute to the vaso-occlusion in venular flow.

Vaso-occlusion

Shear flow response





$$k_{on} = k_{on}^{0} \exp\left(-\frac{\sigma_{on}(l-l_0)^2}{2k_BT}\right),$$

$$k_{off} = k_{off}^{0} \exp\left(\frac{\sigma_{off}(l-l_0)^2}{2k_BT}\right),$$

Same adhesive parameters applied to the sickle cells

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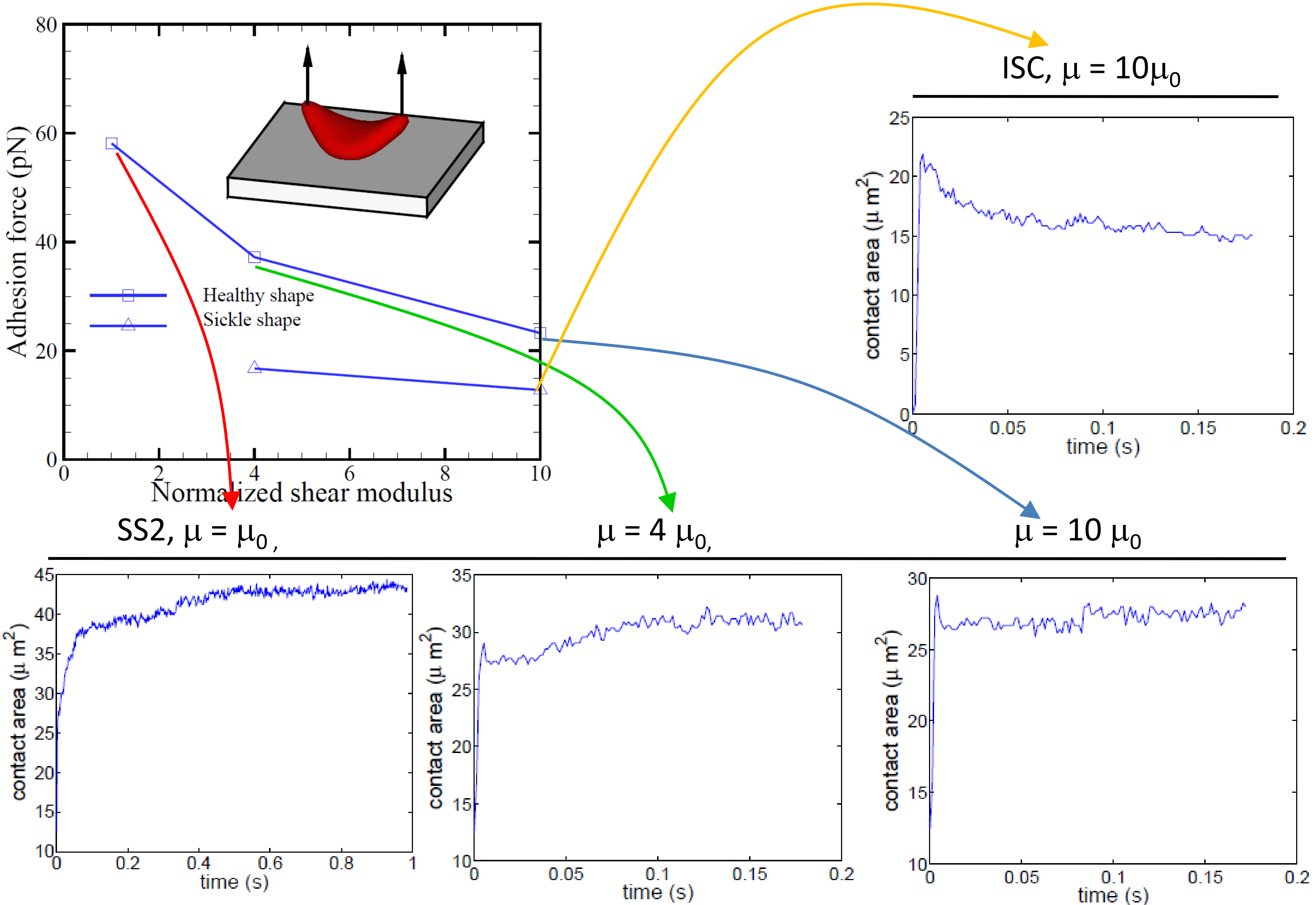
[1] D. A. Fedosov, B. Caswell, and G. E. Karniadakis, *Biophysical Journal*, 100, 2084, 2011.

Vaso-occlusion



ISC, $\mu = 10\mu_0$

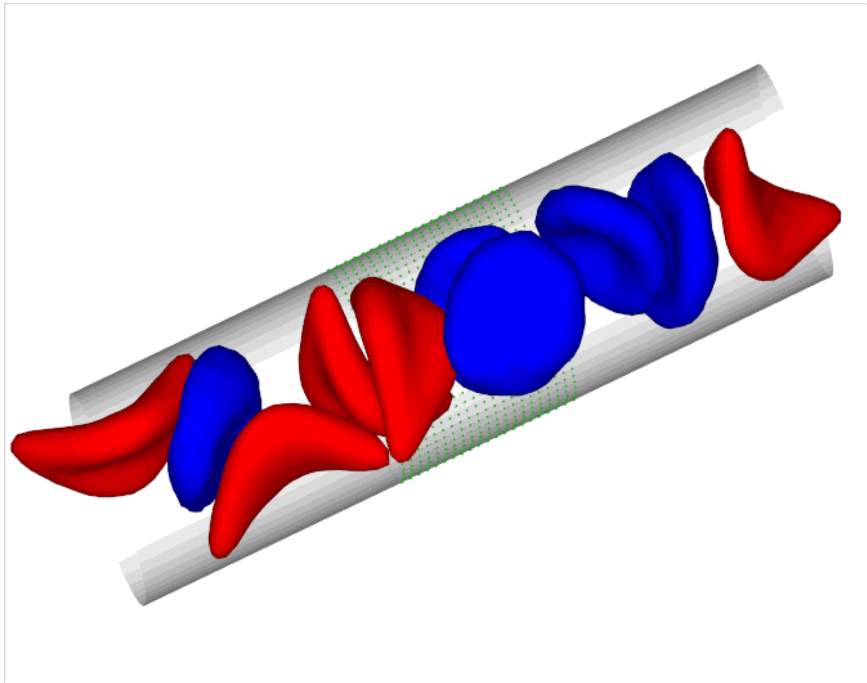
SS2, $\mu = \mu_0$,

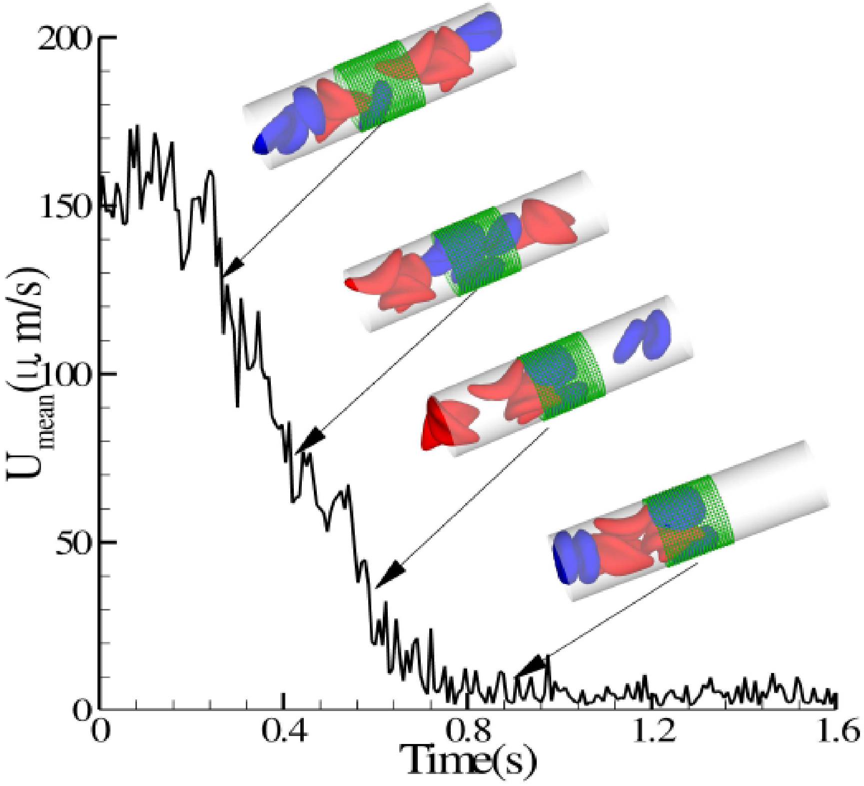
$\mu = 4\ \mu_0$,

$\mu = 10\ \mu_0$

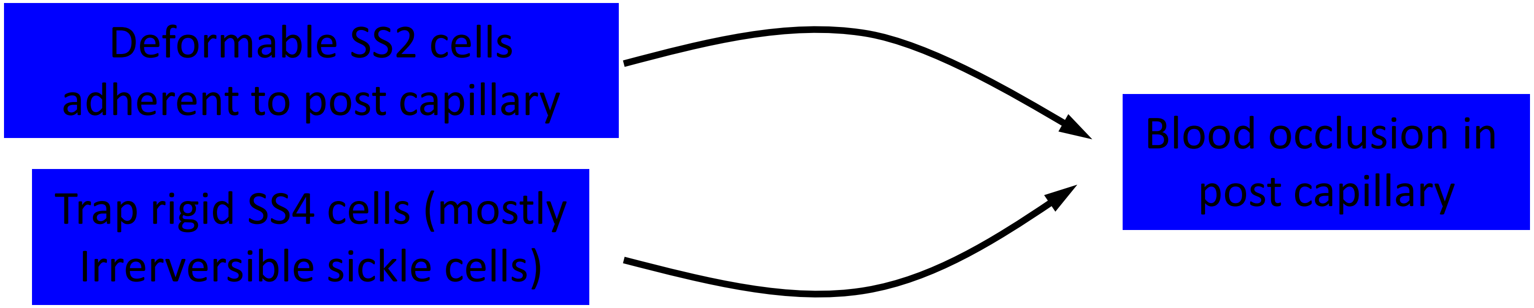
Vaso-occlusion

Pipe flow (SS2 + SS4)





Same adhesive parameters applied



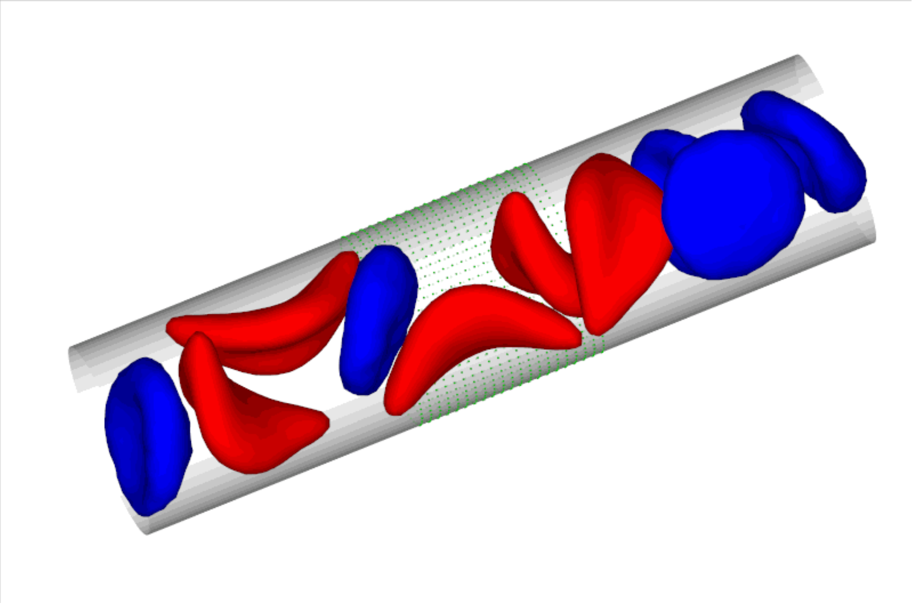
Deformable SS2 cells adherent to post capillary

Trap rigid SS4 cells (mostly Irrerversible sickle cells)

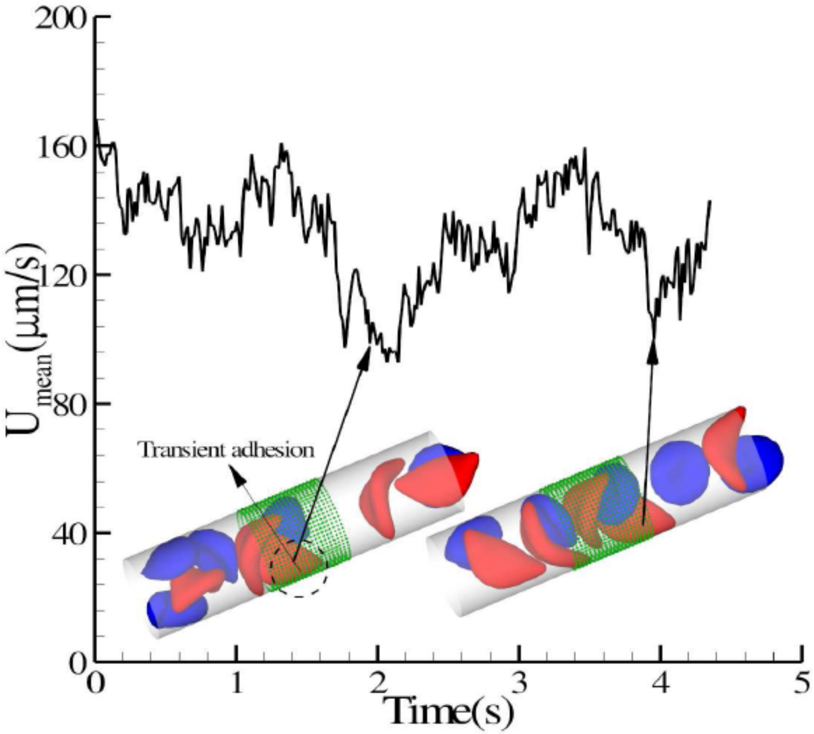
Blood occlusion in post capillary

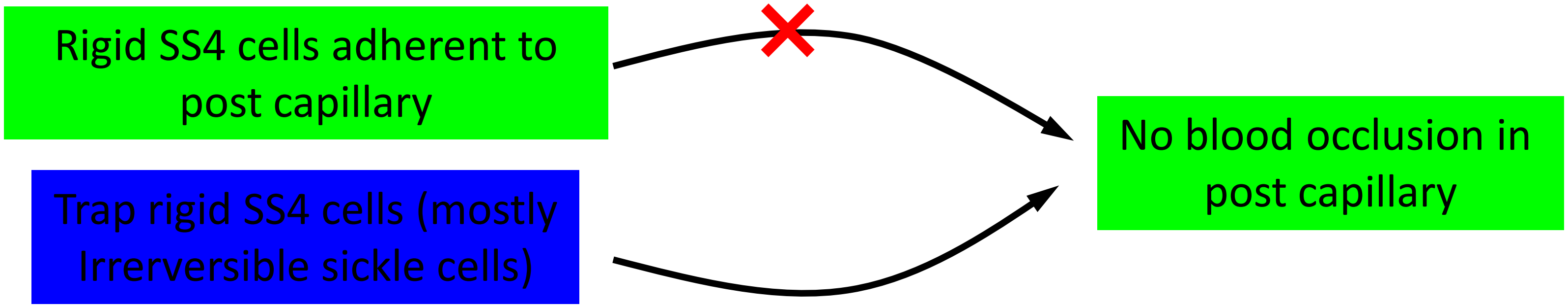
Vaso-occlusion

Pipe flow (SS2 + SS4)



Adhesive interaction only applied to the irreversible sickle cells (red color)





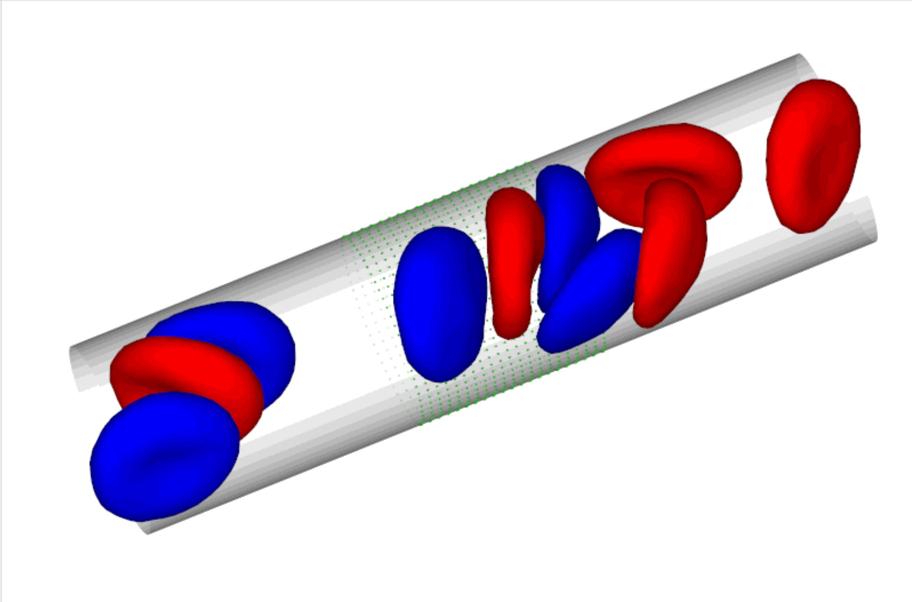
Rigid SS4 cells adherent to post capillary

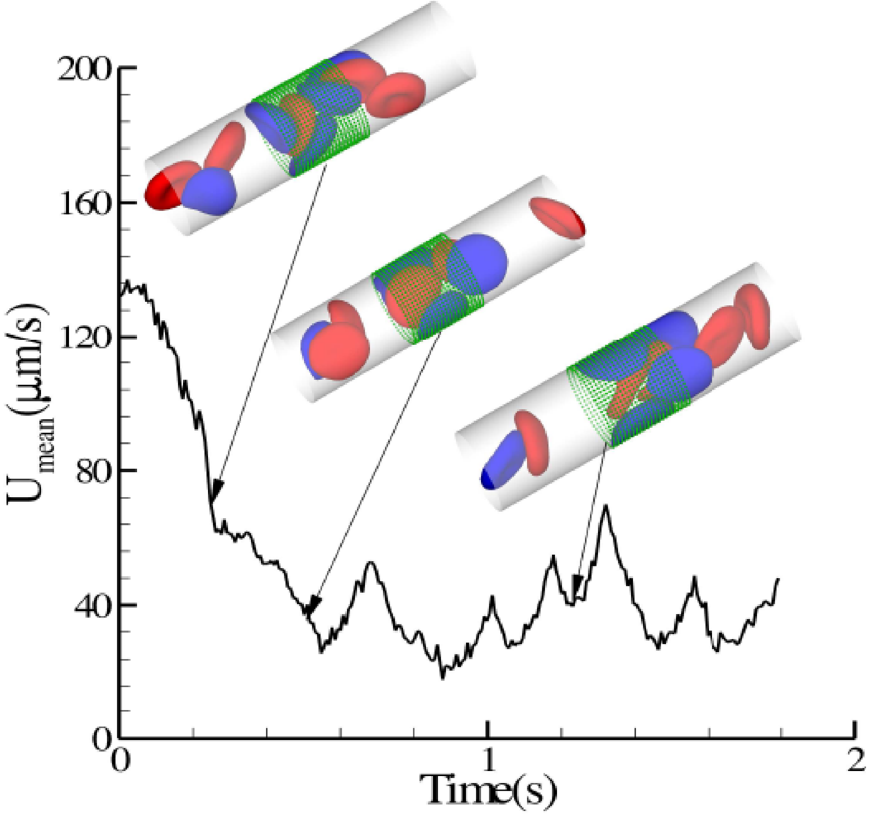
Trap rigid SS4 cells (mostly Irrerversible sickle cells)

No blood occlusion in post capillary

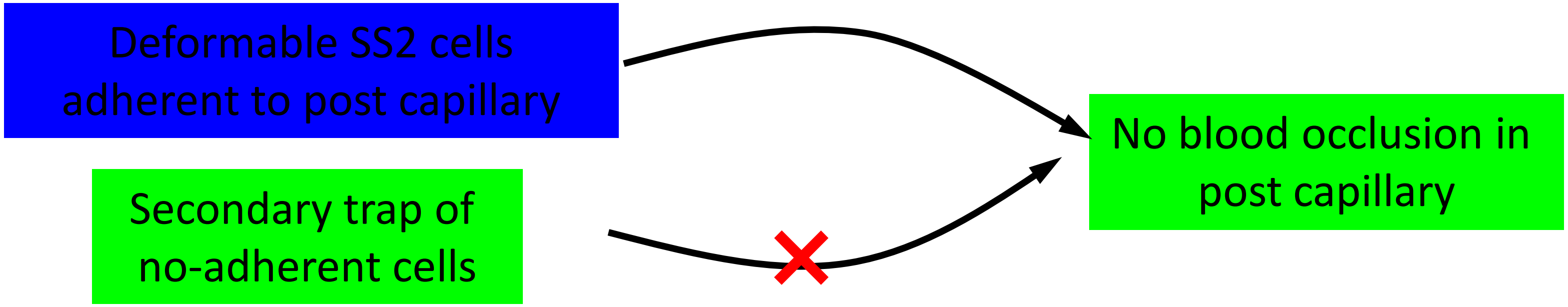
Vaso-occlusion

Pipe flow (SS2 + healthy)





Adhesive interaction only applied to the SS2 sickle cells (blue)



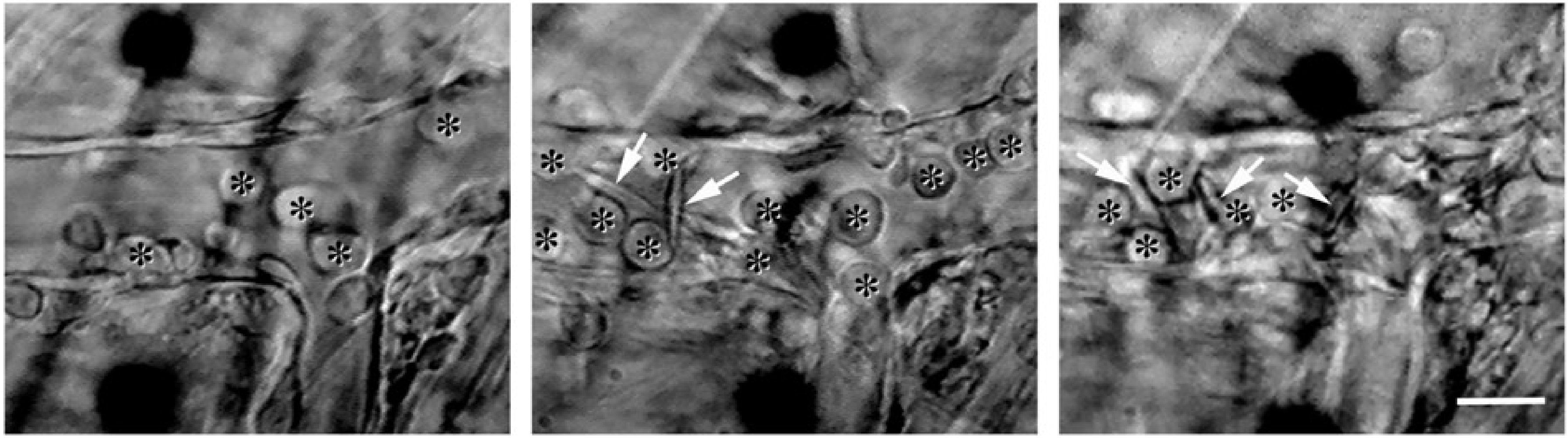
Deformable SS2 cells adherent to post capillary

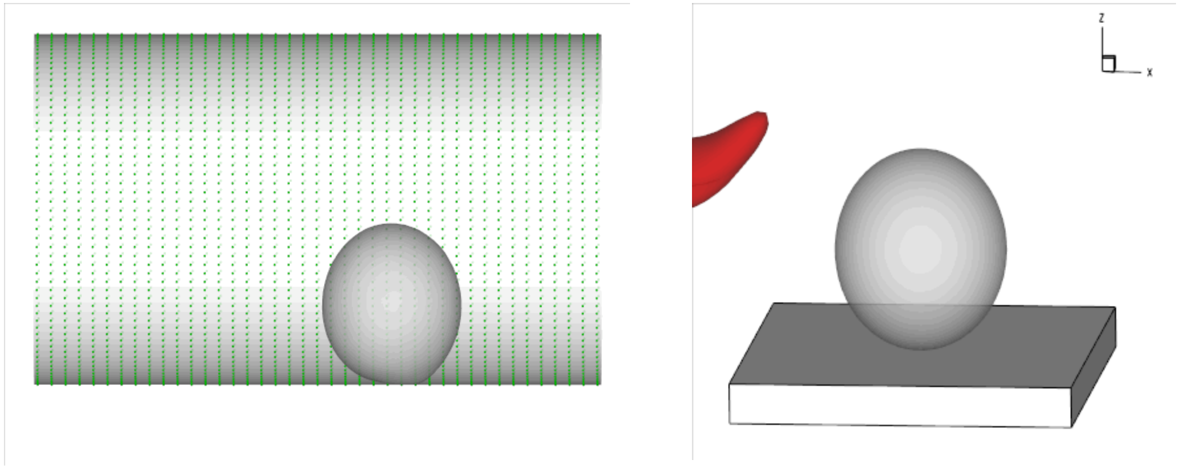
Secondary trap of no-adherent cells

No blood occlusion in post capillary

Vaso-occlusion

Second paradigm : inflammation activated Leukocytes





WBC and Sickle cell interaction:

$$U_M(r) = D_e[e^{2\beta(r_0-r)} - 2e^{\beta(r_0-r)}]$$

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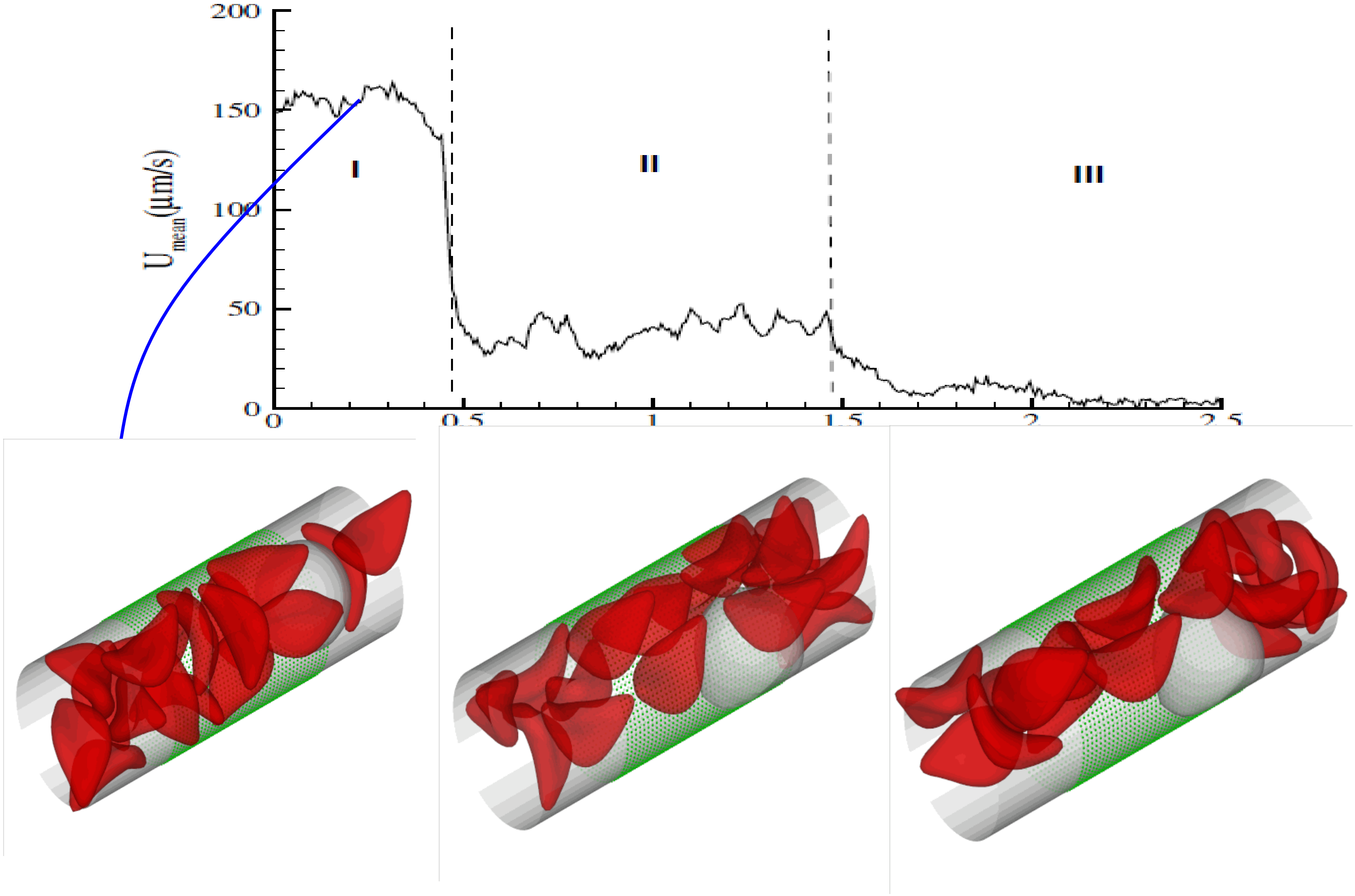
[1] A. Turhan, L. A. Weiss, N. Mohandas, B. S. Coller and P. S.Frenette, *PNAS*, 99, 3047 , 2002

Vaso-occlusion

Multiple-step process



Vaso-occlusion

Remarks

- Sickle cells groups exhibit heterogeneous adhesive characteristics in post-capillaries due to the heterogeneous membrane rigidity and cell morphology.

- Deformable SS2 cell group exhibits larger adhesive interaction in post-capillary, which may trap the ISC group with larger stiffness and more irregular shape, resulting in the *specific* cell pattern in occluded sites.

- For post-capillaries of diameter larger than 10um, the combination of SS2 and ISC cells results in sluggish flow while full occlusion is not observed. However, in venular flow of larger diameter, the inflammation activated leukocytes may result in vaso-occlusion states.

References

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- H. Lei, G. Karniadakis, “Quantifying the rheological and hemodynamic characteristics of sickle cell anemia”, *Biophysical Journal*, vol. 102, p. 185, 2012
- H. Lei and G. E. Karniadakis, “Predicting the morphology of sickle red blood cells using coarse-grained models of intracellular aligned hemoglobin polymers” *Soft Matter*, vol. 8, p. 4507, 2012.
- X. Li, B. Caswell, and G. E. Karniadakis, “Effect of chain chirality on the self-assembly of sickle hemoglobin”, *Biophysical Journal*, vol. 103, p. 1, 2012.