Cytoadherence of erythrocytes invaded by *Plasmodium falciparum*: Quantitative contact–probing of human malaria receptors

Supplementary Materials

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1. Force spectroscopy troubleshooting

Critical steps during the experimental measurements:

- Precise attachment of a RBC or an iRBC to the cantilever requires prior immobilization of the cell on the glass slide through PDL mediation (Step B in Figure 1). Otherwise the RBC and iRBC tend to roll under the tipless cantilever during the attachment engagement. To promote a sufficiently strong adhesion of the RBC and iRBC on the glass slide, the PDL coating should be dried before pouring the cell suspension.
- The RBC and iRBC do not adhere to the glass slide in regions where PDL has been exposed to the CHO cells culture buffer. Culturing of the CHO cells must hence be confined to a small central region of the slide (100–150 μl drop) leaving a significant fraction of the PDL coated glass slide unexposed to the buffer necessary for the operation of iRBC or RBC attachment to the cantilever. Furthermore, the CHO cells culture buffer must be gently washed (3x with PBS) to confine its influence prior to RBC and iRBC pouring.
- After a hold time of typically 10 min, the RBC and iRBC not attached to the glass slide must be gently washed (3x with PBS/BSA) to avoid perturbation of the force-displacement curves during acquisition.
- The concentration of BSA in the washing and testing buffer must be carefully regulated since without this protein the RBC and iRBC tend to transform into echinocytes within 10-20 min. On the other hand, it was experimentally verified that concentrations as low 10 g/ml tended to promote rolling of RBC and iRBC during engagement impairing the attachment of the probe to the tipless cantilever.

- The concentration of ConA must be carefully regulated so that is can strongly mediate the probe attachment to the tipless cantilever without inducing visible deformation of the cell.
- The engagement operation must result in a precise attachment of the RBC or iRBC to the tipless cantilever. Due to its reduced dimensions, the cell probe must be positioned at the cantilever edge to prevent the ConA-incubated cantilever lower surface from contacting with the CHO cells.
- To minimize the mediators dissolution or binding of soluble molecules to the ConA incubated cantilever, that would subsequently impede probe attachment, thermalization of the system should be partly carried out after Step B (Figure 1).
- Thermalization should be kept to a minimum (10–20 min) to maximize cell/parasite viability during each measuring campaign. This limitation and the relatively fast motion of the cantilever/probe during the successive searches for adequate CHO cells induce local temperature variations that are translated into differential hydrodynamic effects during the force-displacement cycles. These effects prevent the extension/retraction curves from coinciding, and may also induce slight curvatures on the baselines resulting from thermal expansion mismatch in the two-layered (Au/SiN) cantilever. These effects can be corrected with polynomial functions up to 3rd degree. Nevertheless, since the trigger force is imposed as a deflection difference relative to the initial value, the hydrodynamic effects induce scattering on the effective trigger force, which must be treated statistically.
- The RBC membrane vibration and the rotating hemazoin in the parasitophorous vacuole may affect the cantilever deflection. The curves must be discarded whenever the effects are too intense to allow a correct measurement of the parameters related to the adhesion events.

2. Videos acquired during force spectroscopy experiments

- **Video A** - Force versus displacement cycle performed against the glass side (viewed from below). The rotating hemazoin crystals inside the parasitophorous vacuole are clearly visible.
- **Video B** - Force versus displacement cycle performed against a CHO cell (viewed from below).

3. Immunofluorescence following force spectroscopy

Immunofluorescence investigations carried out on slides used for force spectroscopy experiments attested to the presence of CSA on the membranes of the CHO cells (Figure S1).

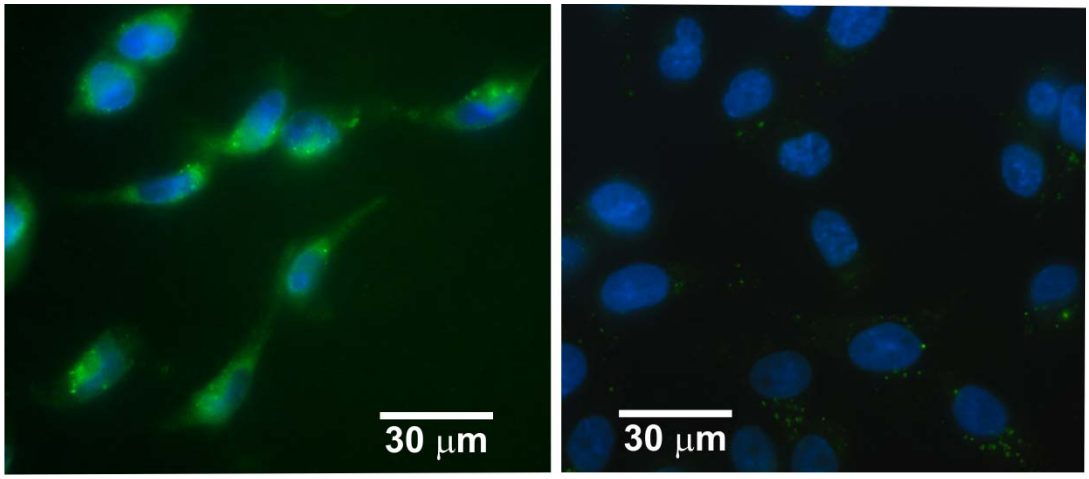

Figure S1 – (a) CSA expression (green label), (b) negative control showing CHO cells nuclei.

4. Adherence assays

The CSA–binding specificity of the FCR3–CSA parasite strain was confirmed through adherence assays that demonstrated the blocking effect of CSA in solution on the adhesion of iRBCs to CHO cells (Figure S2).

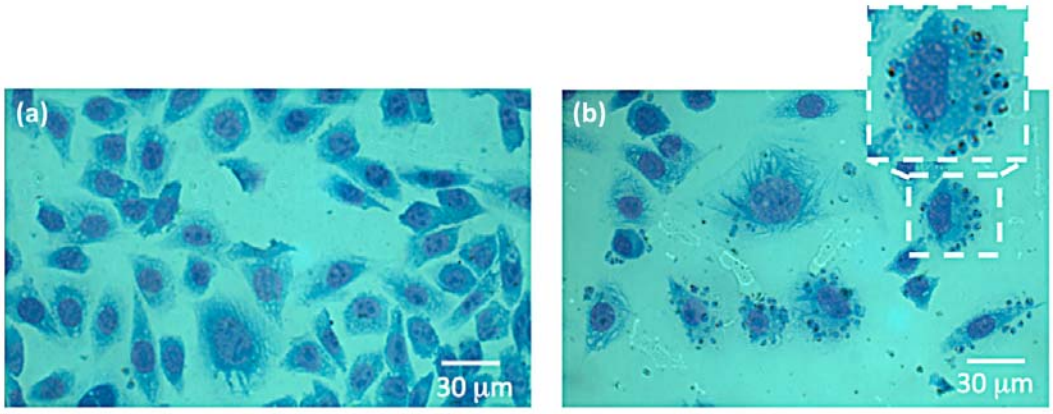

Figure S2 – Blocking effect of CSA in solution on the adhesion of iRBC to CHO cells: (a) 100 μg/ml CSA in solution hindered the adhesion of iRBC to CHO cells, (b) in the absence of CSA in solution iRBC tended to adhere to CHO cells.