COMMUNICATION

Supporting information:

Biotin interaction with human erythrocytes: Contact on membrane surface and formation of self-assembled fibrous structures

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Supporting Figures:



- ¹⁰ Figure S1. Contact mode atomic force micrograph of biotin solutions incubated with erythrocytes in fresh and aged conditions. (a, b) 3 days aged 2D and 3D micrograph of biotin bound erythrocyte clearly showing the dimension of biotin bounded erythrocytes and biotin fibers, (c, d) AFM deflection mode liquid cell imaging of biotin bound 3 days aged erythrocyte
- 15 and corresponding 3D micrograph (in PBS buffer) (with absolute scale bar supporting the main text figure 4).



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Figure S2: Contact mode AFM micrograph of fresh and 3 days aged RBC fixed on the glass surface: (a) micrograph of fresh RBC's, (b) magnified micrograph of fresh RBC (c) micrograph of 3 days aged RBC's and (d) ²⁰ magnified micrograph of 3 days RBC (*without color contour, supporting the main text figure 1*).



Figure S3: For control; Contact mode AFM micrograph of (a) PBS buffer, (b) 3 days aged biotin solution of biotin in PBS buffer, (c) 2 days 2D micrograph of erythrocytes in PBS solution and corresponding (d) 3D micrograph of erythrocytes.



Figure S4: Cross section of healthy RBC's in contact mode(*with absolute ss scale bar supporting the main text figure la*).







Figure S6: Non-contact (a,b) (ACAFM) and contact (c-f) mode 2D and corresponding 3D AFM micrograph of fresh erythrocytes. (a) 2D and (b)
so corresponding 3D ACAFM mode micrograph of fresh erythrocytes, (c) 2D and (d) corresponding 3D contact mode friction micrograph of erythrocytes showed the stability of erythrocytes with resonant frequency [f] of 85.9 kHz and (e) 2D and (f) corresponding 3D topograph of erythrocytes.

HABA- avidin assay: A 10⁻⁵ M solution of HABA in phosphate buffered saline was prepared and saturated with avidin solution. The UV visible spectrum was recorded before and after saturation. 10 μL biotin solution in PBS (1mM) was added to the HABA saturated avidin solution and the ⁹⁵ UV visible spectrum was recorded. The absorbance due to HABA-avidin complex decreased (Figure S7) as expected due to the release of HABA molecules from their binding sites in avidin by biotin binding[Ref. 1-3]. The 1mM solution of biotin in PBS was separately incubated with erythrocytes in two separate microcentrifuge tubes and the freshly
incubated biotin solution with RBC's was centrifuged to remove cells by pelleting. The supernatant containing unbound biotin was filtered through a 0.22µm millipore filter and 10 µL of the supernatant was added to the



¹⁴⁵ **Figure S7.** UV-Vis titration of avidin-HABA complex with biotinerythrocyte incubated solution, under fresh and aged conditions. Biotin alone was used as a control.



Figure S8. AFM images of RBC (a,b) fresh and (c,d) aged with actual height(Z) scale along with X and Y scale. The digits are not visualized ¹⁶⁵ clearly therefore we inserted the digits manually into the images.



Figure S9: Diameter (in μ m) distribution chart of four different (fresh and aged) RBC's.

Additional References:

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HABA-avidin solution.

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