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Supporting Information

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Periodic Iron Nanomineralisation in Human Serum Transferrin Fibrils

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Figures S1 – S13

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Supporting Information

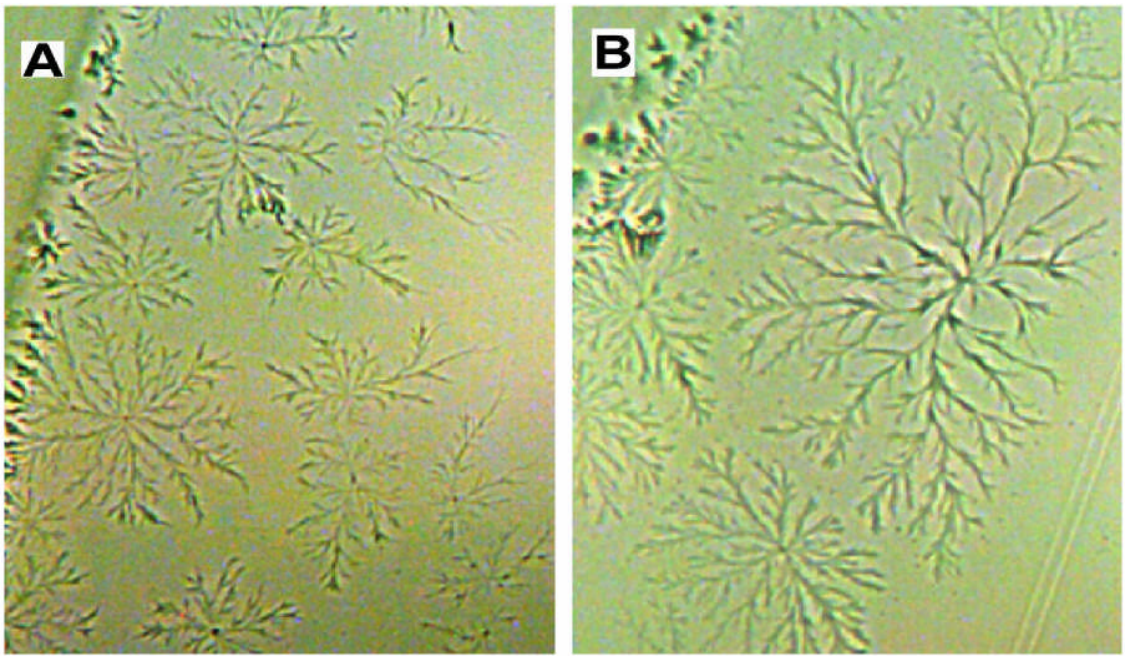

Fig. S1. Optical microscopic image of holo-hTf on a glass surface after (A) 1 day and (B) 2 days incubation at 37 $^{\circ}$C.

Supporting Information

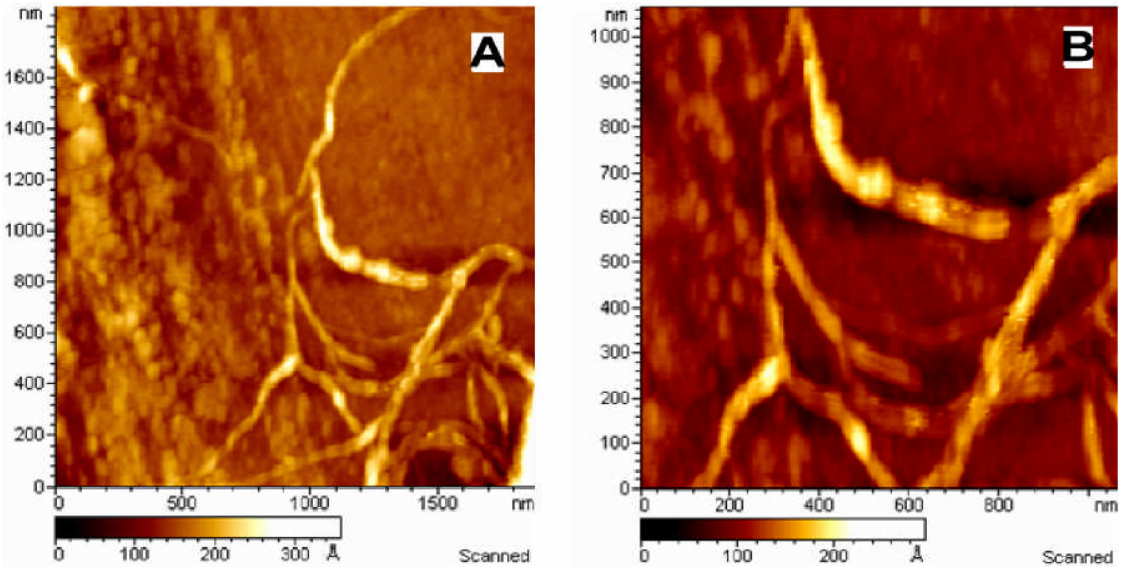

Fig.S2. AFM images of a fresh solution of holo hTf (1 µM) in 25 mM $NaHCO_3$ (pH= 8.21) on a freshly cleaved mica surface; (A) Fibrous network partially masked by $NaHCO_3$ salt clusters; (B) magnified view of the fiber network.

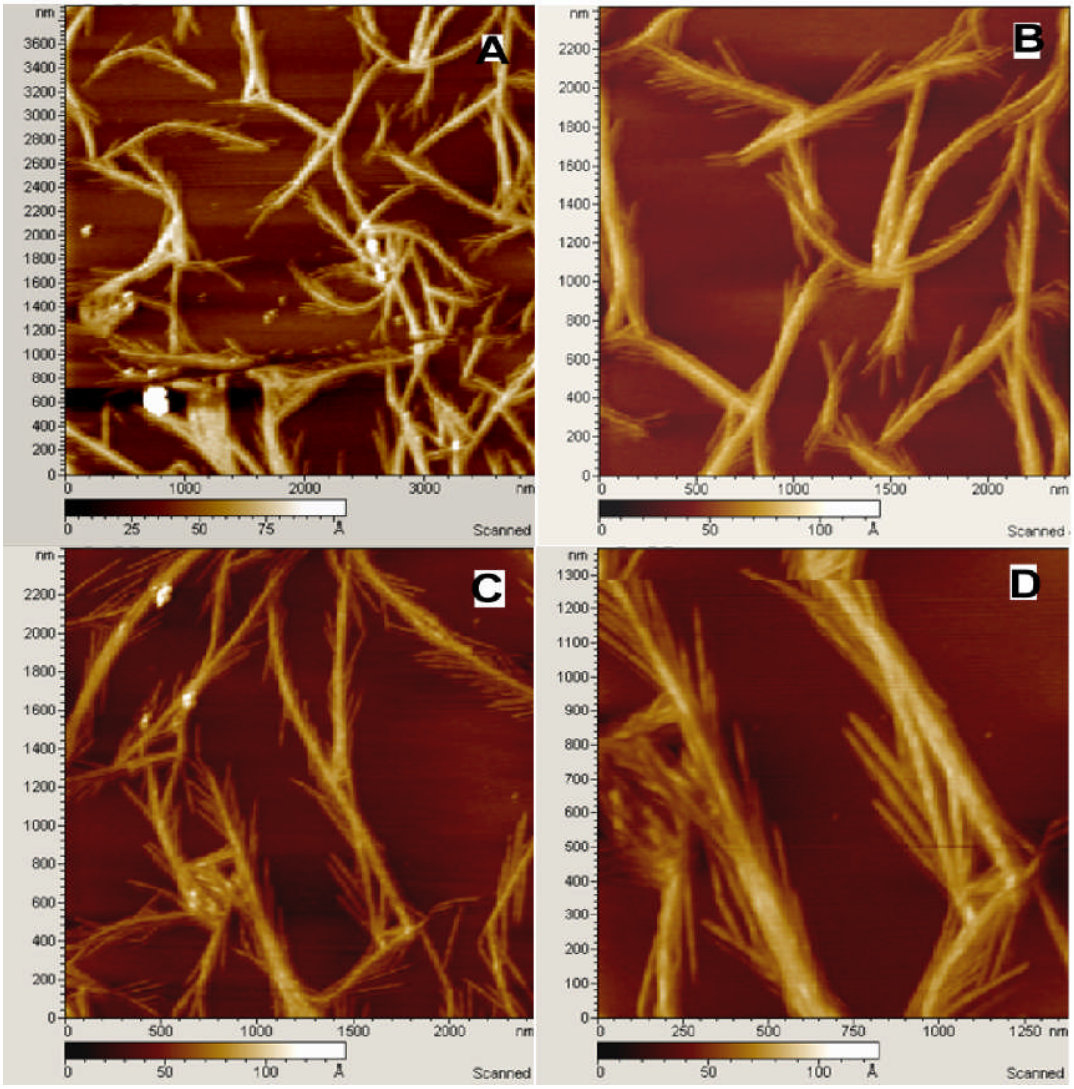

Fig. S3. Typical AFM images of aqueous holo-hTf (1 µM) on a freshly-cleaved mica surface.

Supporting Information

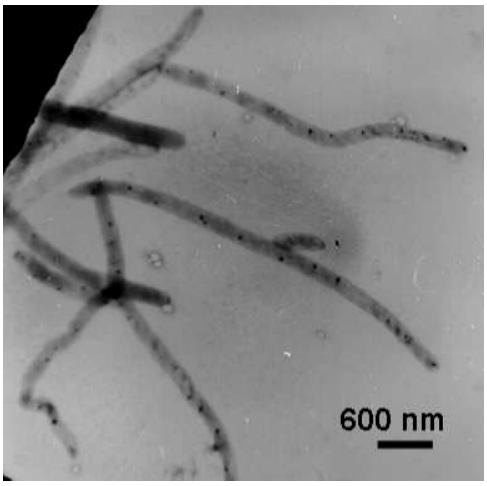

Fig. S4. TEM image from aqueous holo-hTf (1 µM) showing formation of multiple fibers.

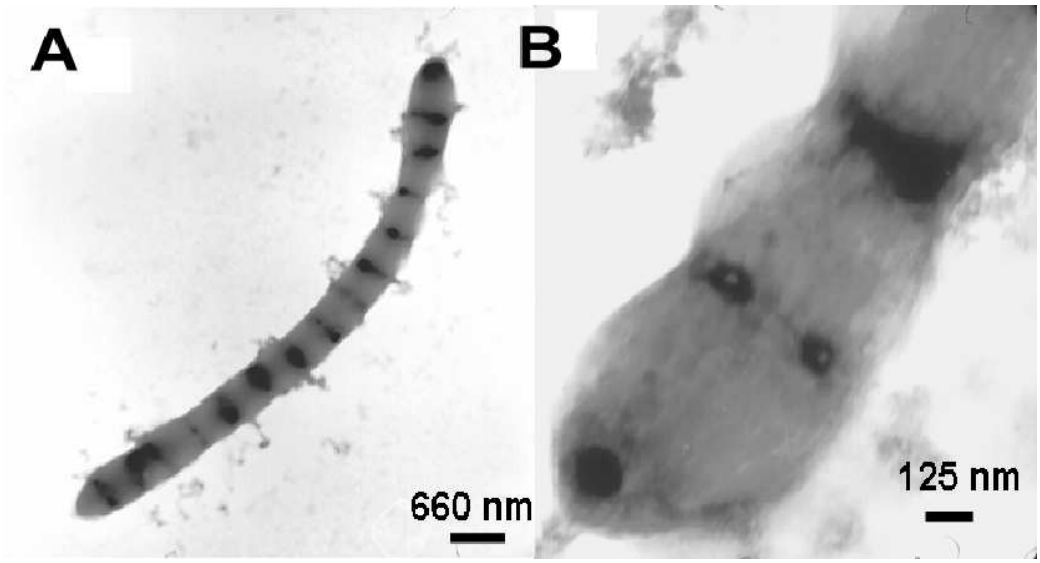

Fig. S5. TEM images from holo-hTf (1 µM) in 1 mM sodium bicarbonate solution (pH 7.22); (A) showing the periodic deposits along the length of the fiber; (B) magnified view of a fiber showing that deposits can occur as banding or can be more localized (spacings of ca. 400 nm).

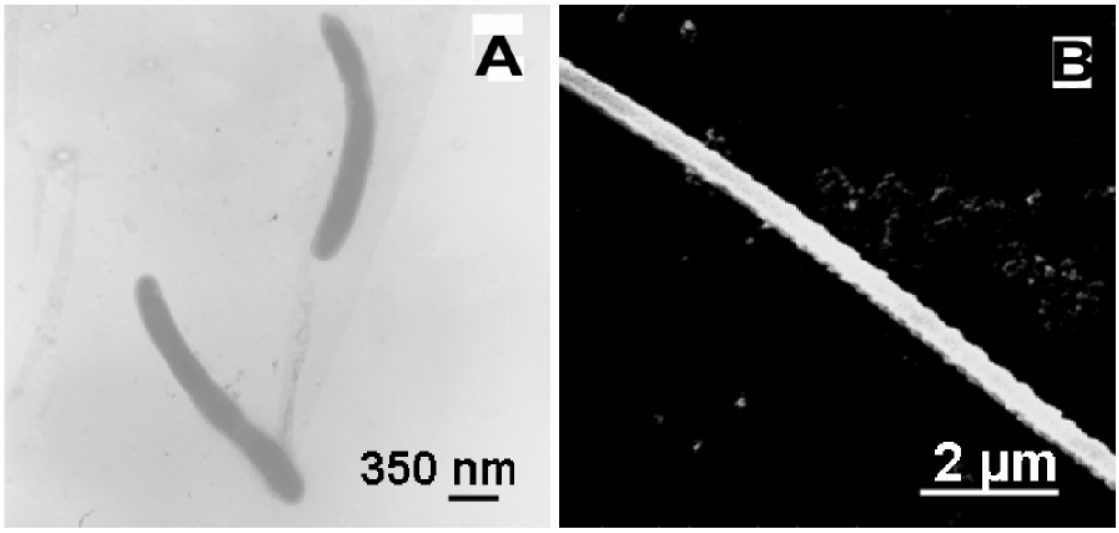

Fig. S6. (A) A TEM image and (B) SEM image from aqueous apo-hTf. No black deposits are seen in the TEM image.

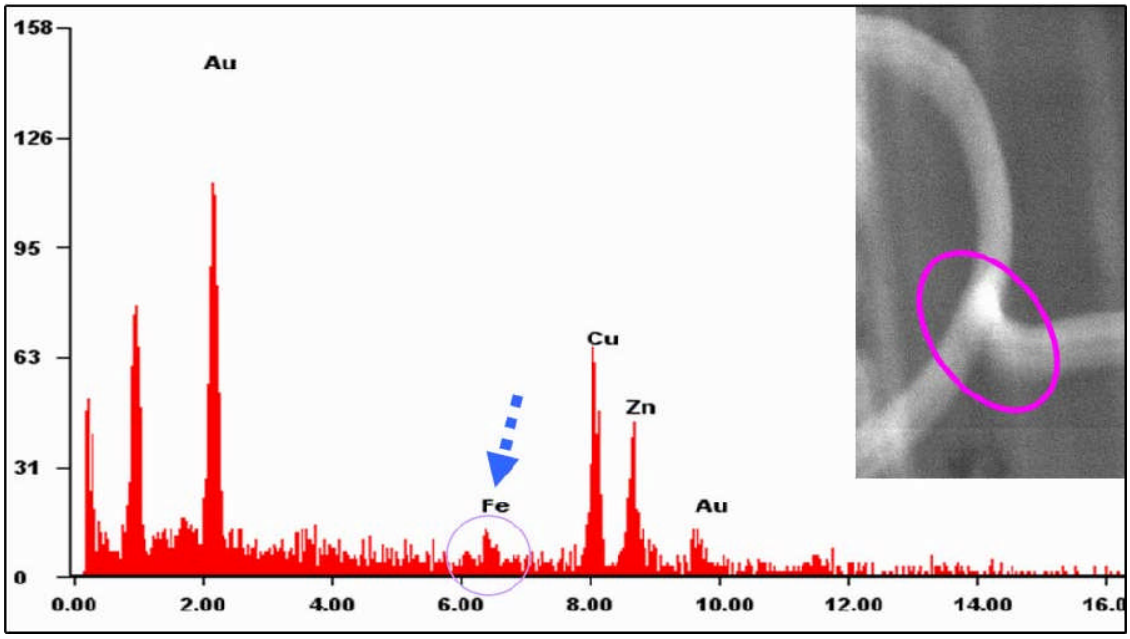

Fig. S7. EDAX spectrum from the indicated area of a junction of a branched fiber of holo-hTf showing a weak peak for Fe. The remaining peaks arise from the grid and its coating.

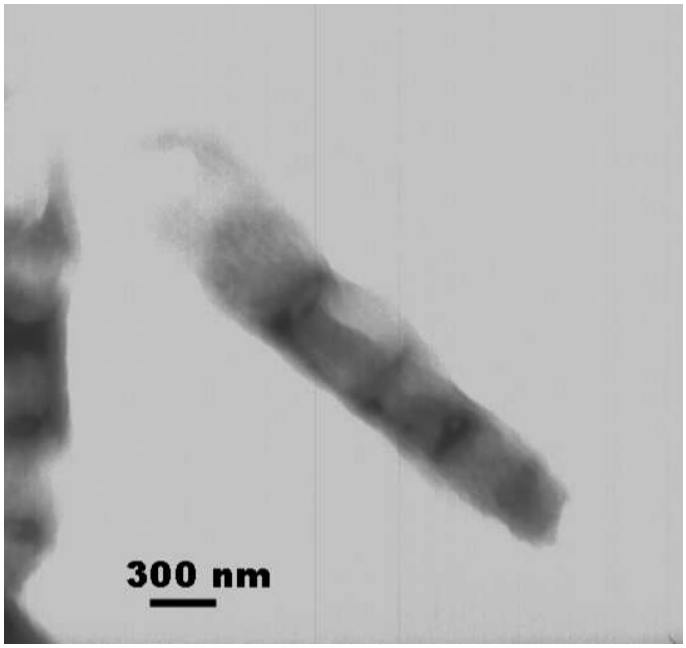

Fig. S8. TEM images of Mn-hTf fibers from a 5 mM sodium bicarbonate solution showing periodic black banding.

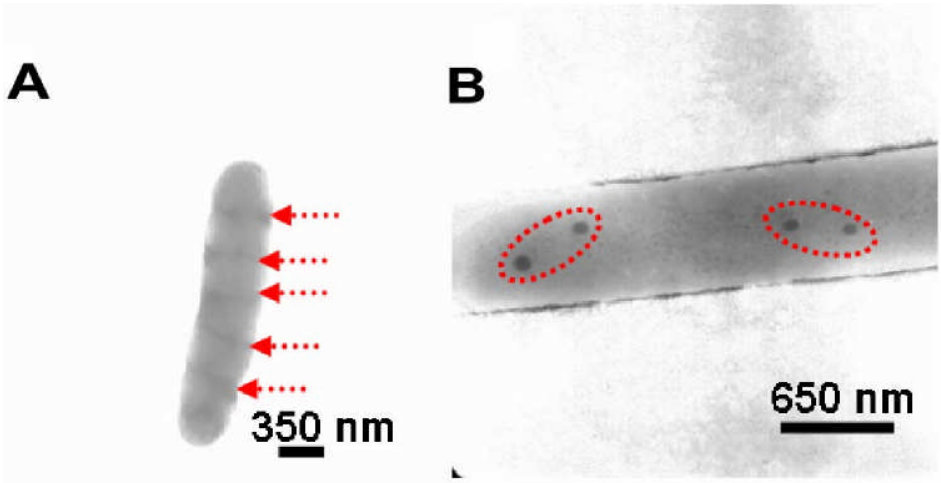

Fig. S9. TEM images of Bi-hTf fibers from a 5 mM sodium bicarbonate solution showing localized black deposits.

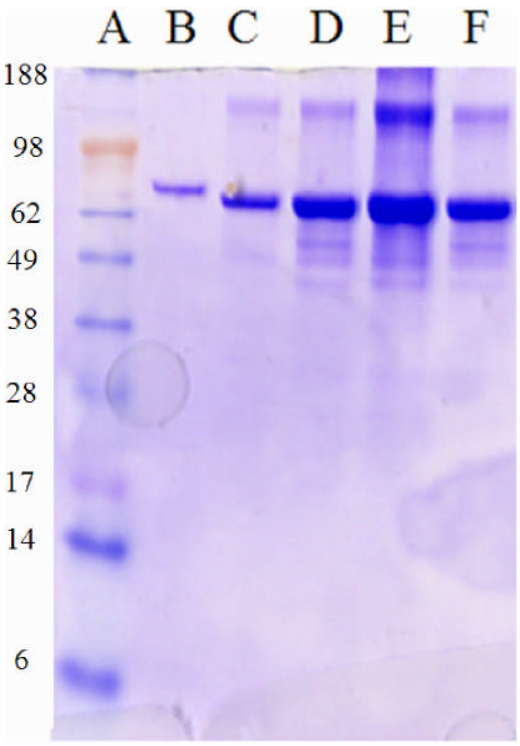

Fig. S10. SDS-PAGE showing deglycosylation of human transferrin using PNGase F (12% bis-Tris precast SDS gel run in MES buffer stained with Coomassie blue). Lane A: molecular weights were estimated using the Seebluplus-2 standard molecular weight marker. Lane B: pure holohTf. Lane C: Non-glycosylated recombinant N413D/N611D holo-hTf (kind gift from Dr Anne Mason, University of Vermont). Lane D: holo hTf + PNGase F in 50 mM phosphate pH 8.5, 5 µM DTT, 4 days. Lane E: holo hTf + PNGase F in 50 mM phosphate pH 8.5, 2 µM DTT, 6 days. Lane F: holo hTf + PNGase F in 50 mM phosphate pH 8.5, 5 µM DTT, 8 days.

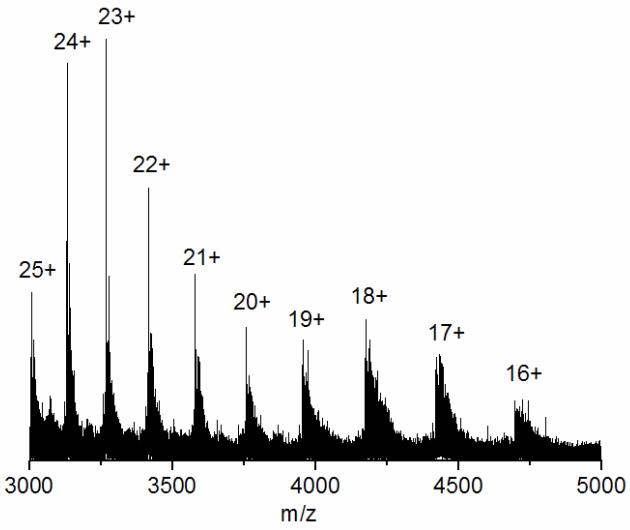

Fig. S11. ESI mass spectrum of 10 µM deglycosylated human holo transferrin purified on immobilized concanavalin-A and exchanged in 10 mM ammonium acetate and 0.01% HCOOH + 10% isopropanol. Under the acidic conditions used for MS, only the apo-protein is detected (Calc. for apo-deglycosylated-hTf, 75141 Da; Obs. 75144 Da).

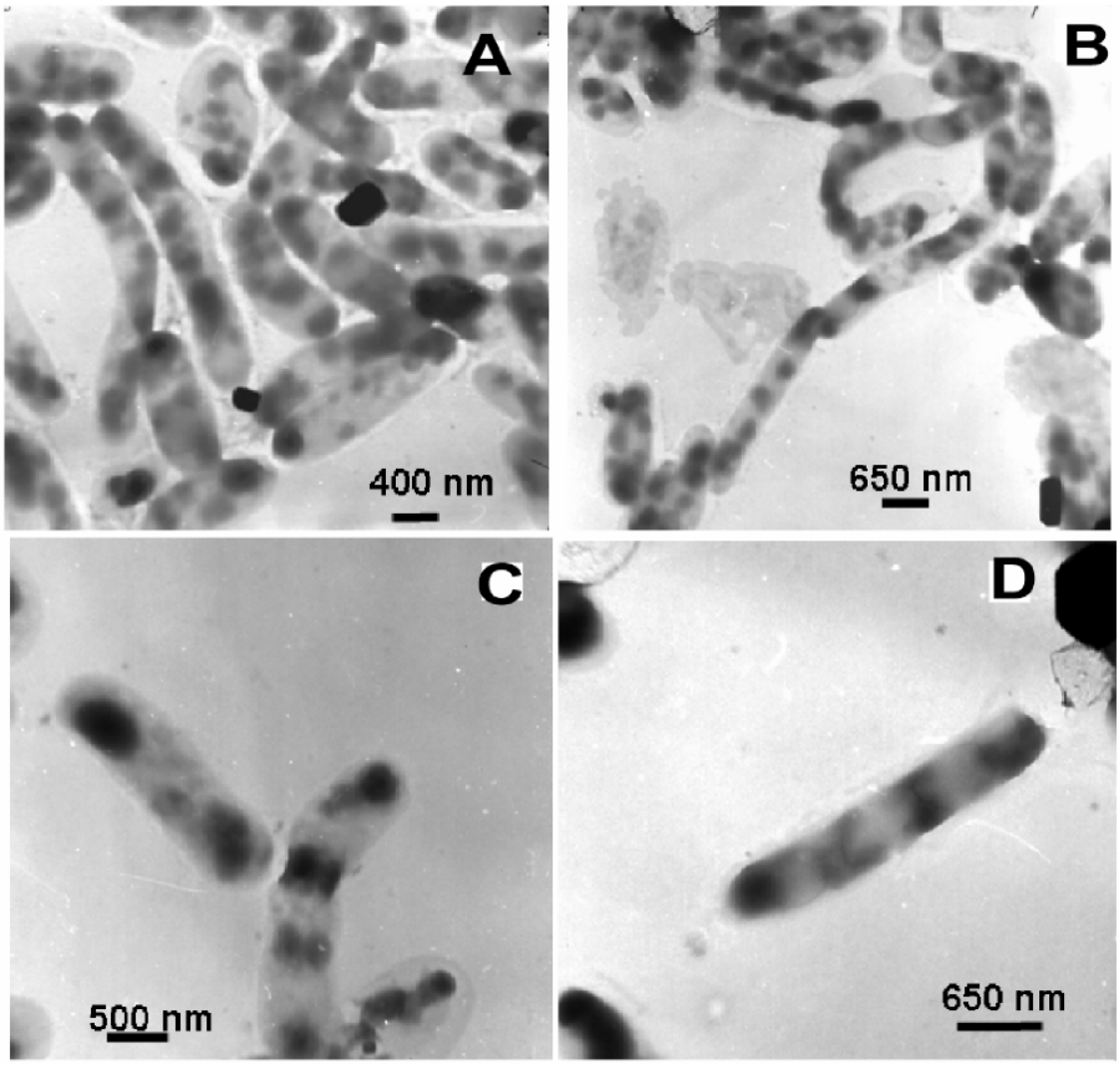

Fig. S12. TEM images from deglycosylated holo-hTf (1 µM in 5 mM NH_4HCO_3, pH 7.4).

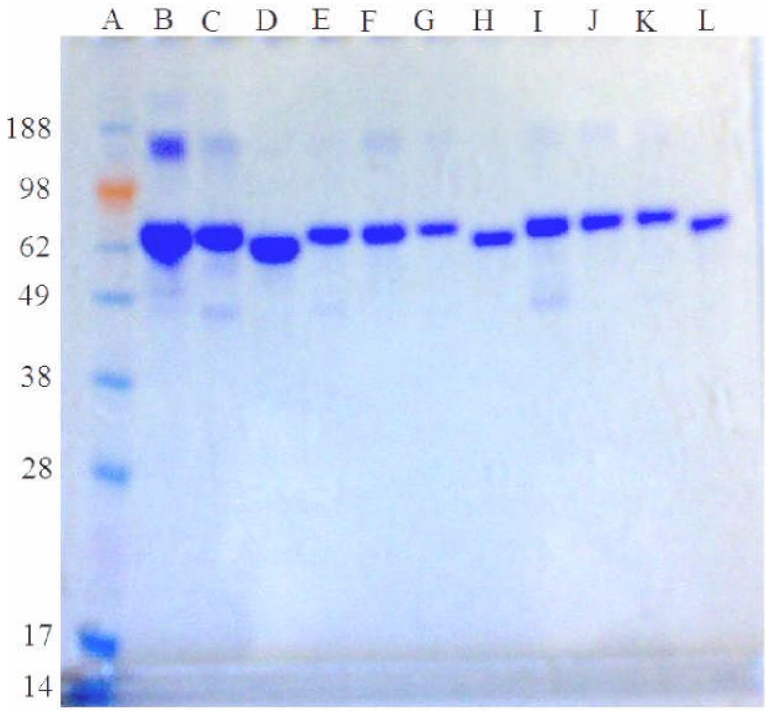

Fig. S13. SDS-PAGE of samples of 5 µM human transferrin in 5 mM NH_4HCO_3, shows that drying on a glass surface does not significantly affect the integrity of the protein. The electrophoresis was performed on a 12% bis-tris precast SDS gel in MES buffer stained with Coomassie blue. Lane A: Molecular weights were estimated using the Seebluplus-2 standard molecular weight markers. Lane B: holo-hTf incubated at 37 °C for 48 h. Lane C: Mn-loaded-hTf incubated at 37 °C for 48 h. Lane D: deglycosylated holo-hTF incubated at 37 °C for 48 h. Lane E: apo-hTF dried on a glass plate at 20 °C. Lane F: holo-hTf dried on a glass slide at 20 °C. Lane G: Mn-loaded-hTf dried on a glass plate at 20 °C. Lane H: deglycosylated holo-hTf dried on a glass plate at 20 °C. Lane I: apo-hTf dried on a glass plate at 37 °C. Lane J: holo-hTf dried on a glass slide at 37 °C; Lane K: Mn-loaded-hTf dried on a glass plate at 37 °C; Lane L: deglycosylated holo-hTf dried on a glass plate at 37 °C. The molecular weight of the deglycosylated protein is ca. 4 kDa lower than that of the native protein.