

Supporting Information for

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4 **Influence of nitrogen limitation on the bioaccumulation kinetics**
5 **of hematite nanoparticles in the freshwater alga *Euglena***
6 ***intermedia***

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8 pages, including 3 Tables and 4 Figures

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23 **Table S1** Chemical components and their concentrations in HUT.

Components	Final concentration (mg L ⁻¹)	Components	Final concentration (mg L ⁻¹)
proteose peptone	600	CH ₃ COONa	400
yeast extract	400	potassium citrate	40
KH ₂ PO ₄	20	Vitamin B ₁	0.0004
MgSO ₄ • 7H ₂ O	25	Vitamin B ₁₂	0.0005

25 **Table S2** Chemical components and their concentrations in AF-6.

Components	Final concentration (mg L ⁻¹)	Components	Final concentration (mg L ⁻¹)
NH ₄ NO ₃	198.1	Na ₂ EDTA	0.75
CaCl ₂ ·2H ₂ O	147	FeCl ₃ ·6H ₂ O	0.097
MgSO ₄ ·7H ₂ O	246.7	MnCl ₂ ·4H ₂ O	0.041
KH ₂ PO ₄	34	ZnCl ₂ ·7H ₂ O	0.005
CaCO ₃	10	Na ₂ MoO ₄ ·2H ₂ O	0.004
Citric acid	2	CoCl ₂ ·6H ₂ O	0.002
Fe-citrate	2		

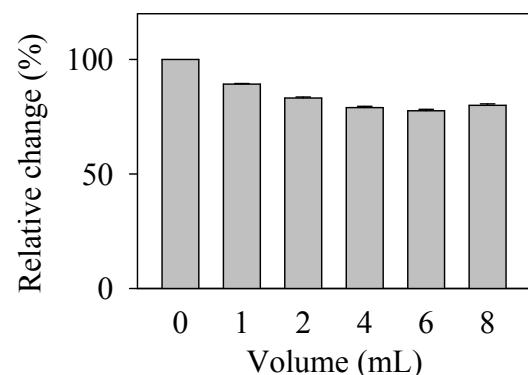
27 **Table S3** Full names of the abbreviations used throughout the text.

Abbreviation	Full name
HemNPs	polyacrylate-coated hematite (α -Fe ₂ O ₃) nanoparticles
QDs	quantum dots
-N	nitrogen-depleted
NE	nutrient-enriched
TEM	transmission electron microscopy
DLS	dynamic light scattering particle sizer
RCF	relative centrifugal force
[HemNPs] _{cell}	HemNP concentration in the cells (pg-Fe μm^{-3})
[HemNPs] _{med}	HemNP concentration in the experimental medium (mg-Fe L ⁻¹)
[HemNPs] _{ads}	cell-surface-adsorbed concentration of HemNPs (pg-Fe μm^{-3})
[HemNPs] _{intra}	intracellular concentration of HemNPs (pg-Fe μm^{-3})
k_u	uptake rate constant of HemNPs (L μm^{-3} h ⁻¹)
k_e	efflux rate constant of HemNPs (h ⁻¹)
μ	cell-specific growth rate (h ⁻¹)
F _v /F _m	maximum photosynthetic system II quantum yield
Water-PAM	Water Pulse Amplitude Modulated Fluorometer
GF/F membrane	glass fiber/fine membrane
SSC	side scatter
[PS] _{surf}	amount of polysaccharide attached on the cell surface (fg-Xanthan _{eq} μm^{-3})
[CHO] _{cell}	cellular carbohydrate concentration (μmol μm^{-3})
[PS] _{dis}	amount of polysaccharides released into the culture medium (μM)
ANOVA	analysis of variance
EDX	energy dispersive X-ray spectrometry

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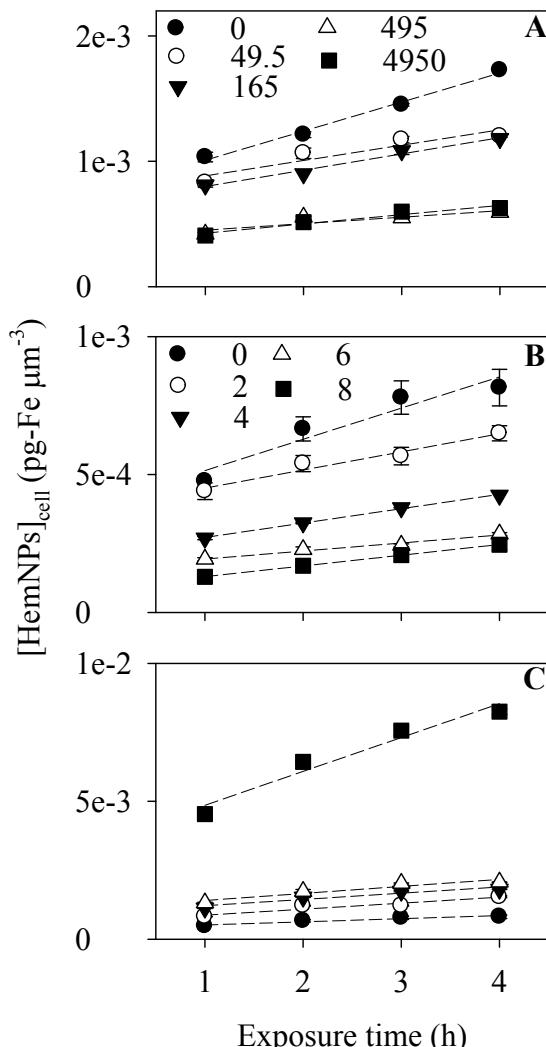
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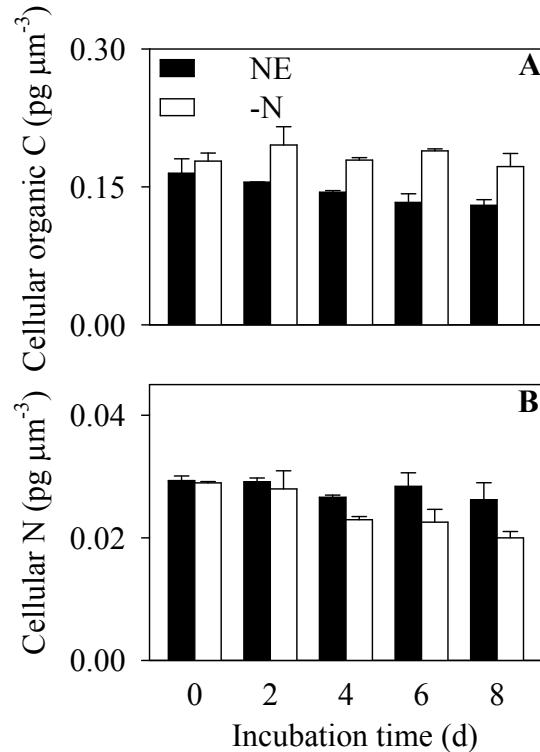


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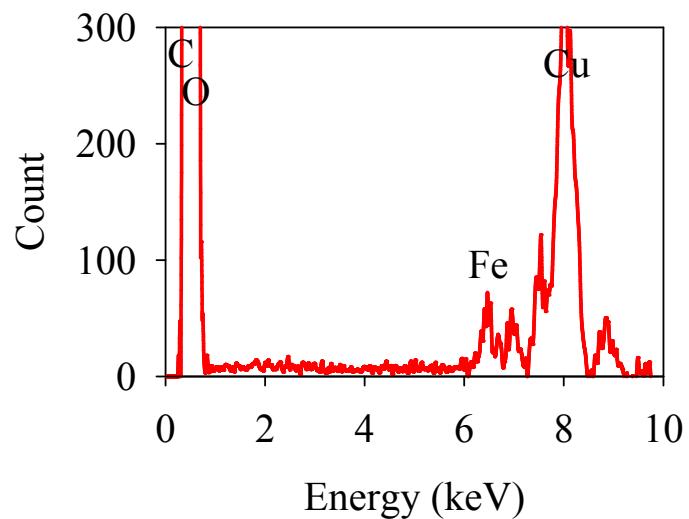
32 **Fig. S1** Relative change of the cellular HemNP concentration ($[\text{HemNPs}]_{\text{cell}}$) with increasing
33 volume of rinsing solution (i.e., AF-6).



36 **Fig. S2** The increase in the cellular HemNP concentration ($[HemNPs]_{cell}$) with increasing
 37 exposure time in *Euglena intermedia* pre-cultured (A) in the experimental medium with
 38 different concentrations of nitrogen (0, 49.5, 165, 495, and 4950 μM) for 2 days as well as in
 39 (B) the nutrient-enriched and (C) nitrogen-depleted media for 0, 2, 4, 6, and 8 days before the
 40 4-h uptake experiment. Dashed lines are the linear regression between $[HemNPs]_{cell}$ and
 41 exposure time. The data are reported as the mean \pm standard deviation ($n = 3$).



44 **Fig. S3** The cellular (A) organic carbon and (B) nitrogen concentrations in *Euglena intermedia*
 45 pre-cultured in the nutrient-enriched (NE) and nitrogen-depleted (-N) media for 0, 2, 4, 6, and
 46 8 days. The data are reported as the mean \pm standard deviation (n = 3).



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49 **Fig. S4** Representative elemental composition of the arrowed spots in the TEM images of Fig.

50 2B and C, as investigated by energy dispersive X-ray (EDX) spectrometry.