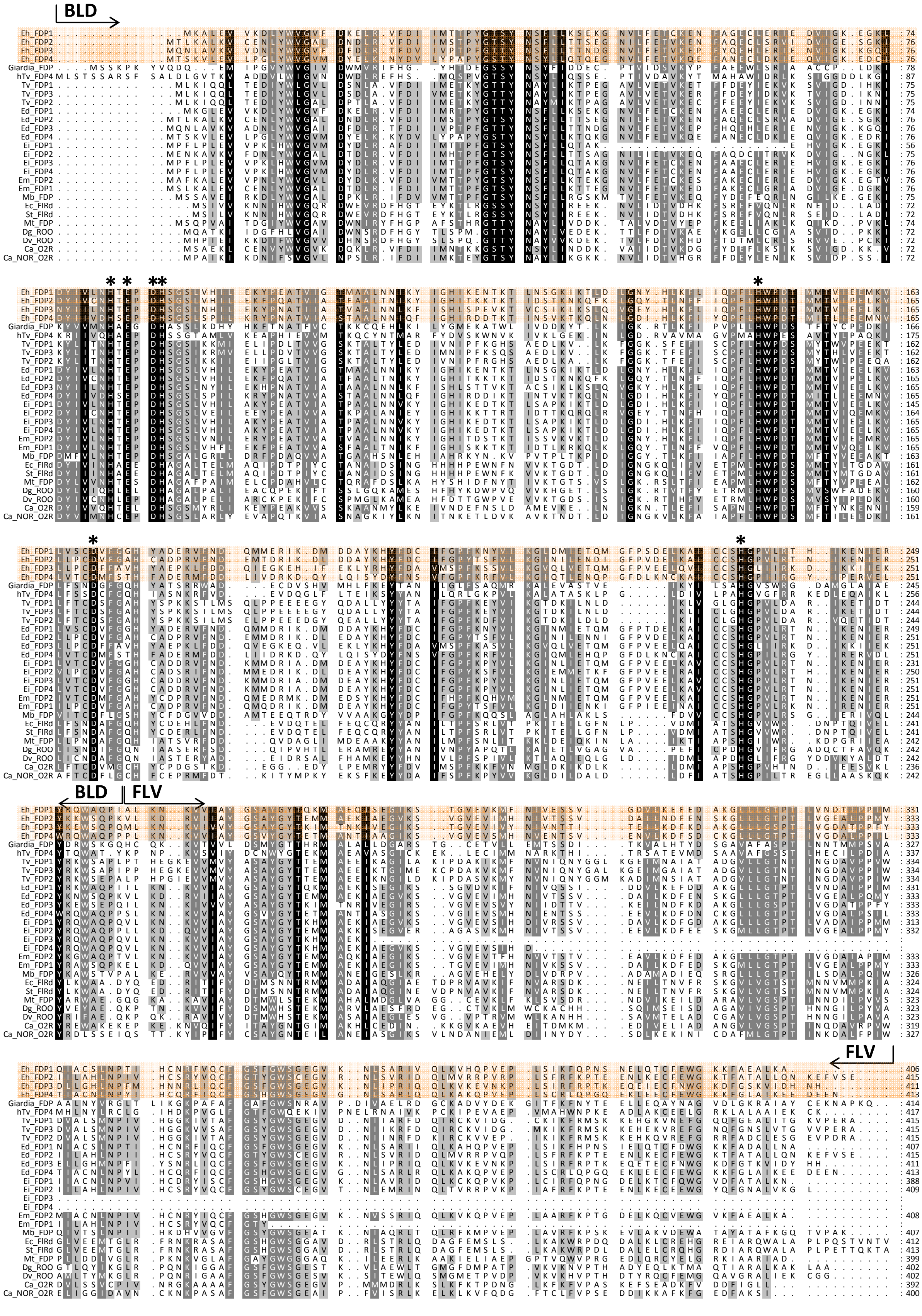

Supplem. Fig. 1

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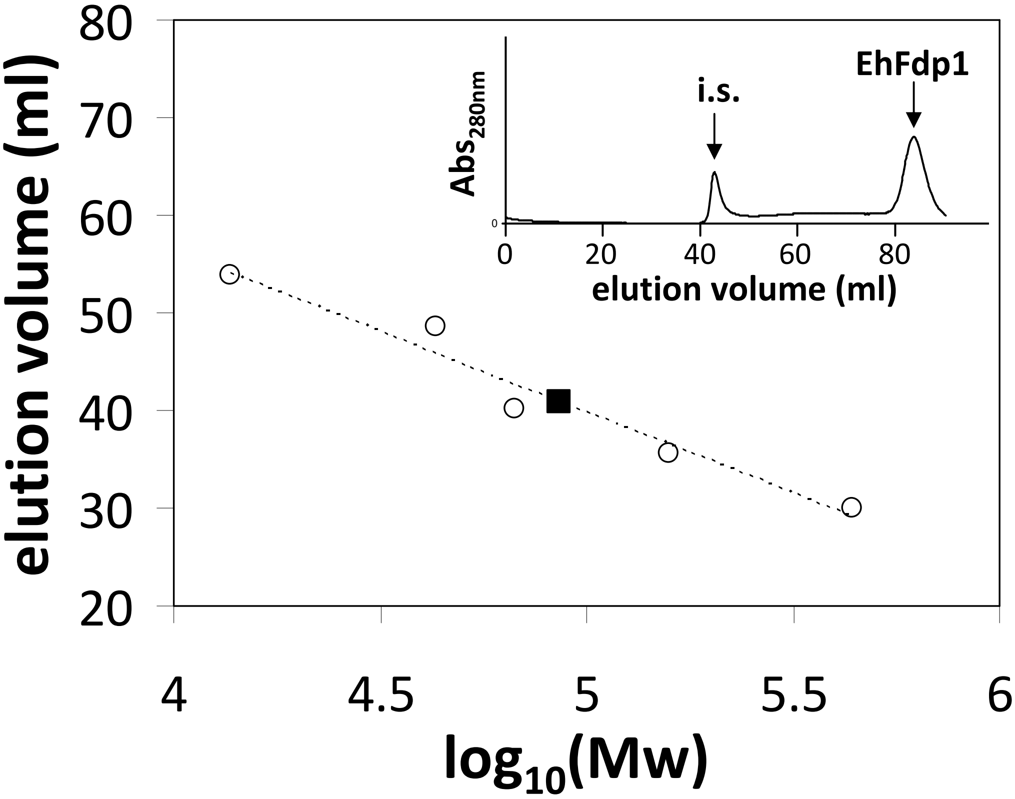
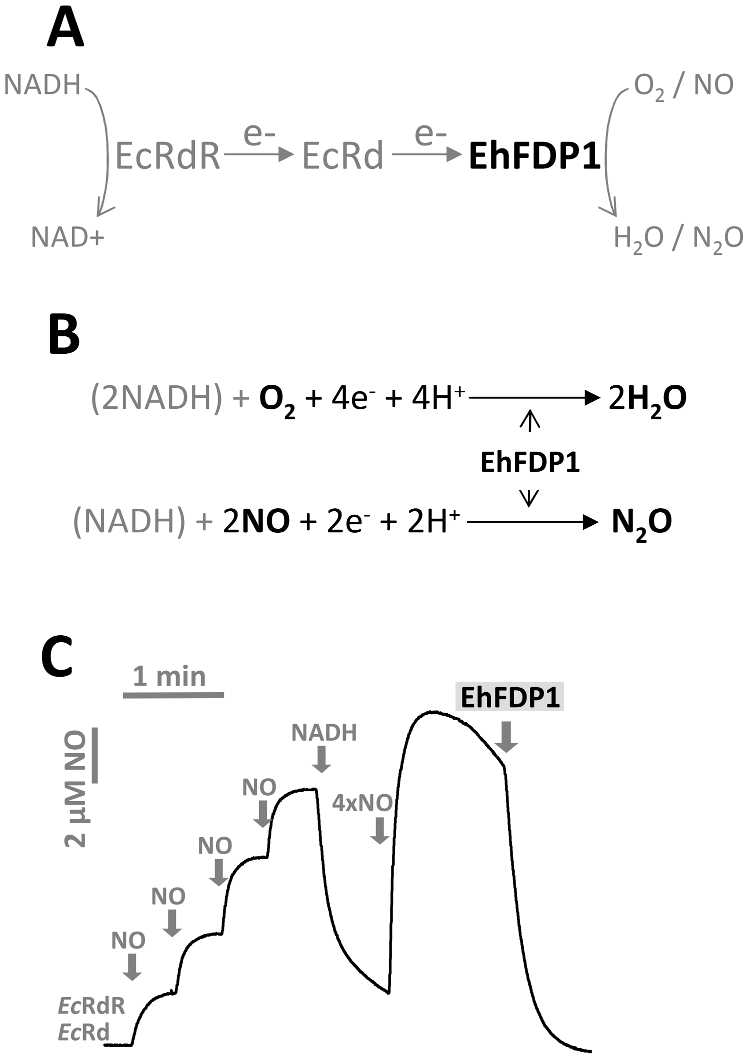
Supplementary Figure 1: Aminoacid sequence alignment between *Entamoeba histolytica* flavodiiron proteins and other protozoan FDPs, as well as prokaryotic models. Sequence alignment obtained with Clustal X. Eh_FDP1, EhFDP2, Eh_FDP3 and EhFDP4, the four *Entamoeba histolytica* flavodiiron proteins (XP_656946, XP_654023, XP_651815, XP_651627); Giardia_FDP – *Giardia intestinalis* flavodiiron protein (XP_001707670); hTv_FDP4, *Trichomonas vaginalis* hydrogenosomal flavodiiron protein (XP_001583562); Tv_FDP1, Tv_FDP2 and Tv_FDP3, *Trichomonas vaginalis* flavodiiron proteins (XP_001315422, XP_001317833, XP_001322980); Ed_FDP1, Ed_FDP2, Ed_FDP3 and Ed_FDP4, *Entamoeba dispar* flavodiiron proteins (XP_001736248, XP_001740812, XP_001738262, XP_001735719); Ei_FDP1, Ei_FDP2, Ei_FDP3 and Ei_FDP4, *Entamoeba invadens* flavodiiron proteins (EIN_289520+, EIN_295740+, EIN_311980+, EIN_312270+); Em_FDP1 and Eh_FDP2, *Entamoeba moshkovskii* flavodiiron proteins (CAI11386, CAI11385); Mb_FDP, *Mastigamoeba balamuthi* flavodiiron protein (Q2PCA4); Ec_FlRd, *Escherichia coli* flavorubredoxin (NP_417190); St_FlRd, *Salmonella typhimurium* flavorubredoxin (NP_461761); Mt_FDP, *Moorella thermoacetica* flavodiiron protein (Q9FDN7); Dg_ROO, *Desulfovibrio gigas* rubredoxin:oxygen oxidoreductase (Q9F0J6); Dv_ROO, *Desulfovibrio vulgaris* rubredoxin:oxygen oxidoreductase (YP_012395); Ca_O2R, *Clostridium acetobutylicum* O2-reducing flavodiiron protein (AAK79003); Ca_NOR_O2R, *Clostridium acetobutylicum* NO- and O2-reducing flavodiiron protein (NP_349063). +, gene accession number from amoebaDB website (http://amoebadb.org/amoeba/). *, ligands for the diiron active site; BLD, β-lactamase-like domain; FLV, flavodoxin-like domain. The *E. histolytica* FDP sequences are highlighted.

Supplem. Fig. 1 **Vicente 2012**



Supplementary Figure 2: *Entamoeba histolytica* flavodiiron protein EhFdp1 is homodimeric. Oligomeric profile of EhFdp1 determined by size exclusion chromatography. Standards and sample loaded (separately) onto a 120-ml Superdex S-200 (GE Healthcare) connected to an Åkta Prime FPLC System, previously equilibrated and ran at 0.5 ml/min with 20 mM Tris-HCl buffer, 18% glycerol, 150 mM NaCl, pH 7.5. Internal standard (i.s.): dextran blue (Mw 1000 kDa); molecular mass standards: ribonuclease (13.7 kDa), ovalbumin (44 kDa), albumin (67 kDa), aldolase (158 kDa) and ferritin (440 kDa). Calibration curve obtained by linear regression of elution volume vs $\log_{10}$(standard molecular mass). Hollow circles, molecular mass standards; black square, EhFdp1. Inset, chromatogram obtained for EhFdp1.



Supplementary Figure 3: *Entamoeba histolytica* flavodiiron protein EhFdp1 has low nitric oxide reductase activity. Amperometric measurement using a Clark-type nitric oxide electrode. **(A)** Electron delivery to EhFDP1 achieved by assembling a hybrid electron transfer chain composed by NADH, *Escherichia coli* NADH:(flavo)rubredoxin oxidoreductase (EcRdR) and *Escherichia coli* flavorubredoxin rubredoxin domain (EcRd). **(B)** Reaction stoichiometries for oxygen reduction to water or nitric oxide reduction to nitrous oxide, at the expense of NADH oxidation. **(C)** Amperometric assay for nitric oxide reduction by EhFdp1 in anaerobiosis. Reaction mixture volume 1 ml; measurements done at room temperature, in 50 mM Tris-HCl, 18% glycerol, pH 7.5. Anaerobic conditions achieved by thorough degassing of reaction buffer with argon, and by adding glucose oxidase (4 U/ml), glucose (2 mM) and catalase (130 U/ml) to the assay. Sequential addition of 2 µM EcRdR, 4 µM EcRd, 4x 2 µM NO, 1 mM NADH, 8 µM NO, and 320 nM EhFdp1. Nitric oxide depletion upon addition of sub-stoichiometric EhFdp1 proceeds through non-linear kinetics.

Supplementary Table 1 – Annotation of flavodiiron protein-encoding genes in *Entamoeba histolytica* genome.

Flavodiiron protein abbreviation	*.m annotation*	*EHI annotation*
EhFdp1	6.m00467[a]	EHI_096710 EHI_152650
EhFdp2	155.m00084	EHI_159860
EhFdp3	146.m00121	EHI_129890
EhFdp4	65.m00171	EHI_064530

a - the gene identified as 6.m00467 has two identical equivalents in the most recent annotation, which are both herein represented by EhFdp1.

Supplementary Table 2 – Redox mediators used to obtain the binuclear diiron site in a mixed-valence state, monitored by electron paramagnetic resonance (EPR) spectroscopy.

Redox mediator[a]	*Eº (mV, vs. SHE)*
1,2-napthoquinone-4-sulphonic acid	+215
1,2-napthoquinone	+180
trimethylhydroquinone	+115
1,4-napthoquinone	+60
menadione	0
plumbagin	-40
indigo trisulphonate	-70
indigo disulphonate	-110
2-hydroxy-1,4-napthoquinone	-152
anthraquinone-2-suhphonate	-225

a - redox mediators (40 µM each) were incubated for 30 minutes with 190 µM EhFdp1 under anaerobic conditions prior to addition of sodium dithionite.