SUPPORTING INFORMATION

Efficient Nitrogen-13 Radiochemistry catalyzed by a Highly Stable Immobilized Biocatalyst

Eunice S. da Silva [a], Vanessa Gómez-Vallejo[b], Jordi Llop [a]* and Fernando López-Gallego[c,d,*]

[a] *Radiochemistry and Nuclear Imaging. CIC biomaGUNE, Paseo Miramon 182, 20009, San Sebastian (Spain)*

[b] *Radiochemistry Platform, Molecular Imaging Unit. CIC biomaGUNE, Paseo Miramon 182, 20009, San Sebastian (Spain)*

[c] *Biofunctional nanomaterials unit. CIC biomaGUNE, Paseo Miramon 182, 20009, San Sebastian (Spain)*

[d] *IKERBASQUE, Basque Foundation for Science, Bilbao (Spain)*

Supplementary information

Fluorescence studies. We analyzed the fluorescence spectrum at 25 ºC of soluble eNR and immobilized eNR on Ag-DEAE without incubation at 40ºC, and soluble and immobilized eNR on Ag-DEAE incubated at 40ºC for 15 minutes (Figure S3). The fluorescence spectrum of the soluble enzyme remained unaltered after the thermal incubation in spite of the activity decay induced by the thermal treatment. However, the fluorescence spectrum of the thermally incubated eNR immobilized on Ag-DEAE dramatically changed. The incubated enzyme showed higher intensity for the tryptophan fluorescence peak (314 nm) and lower intensity for the flavin fluorescence peak (532 nm) than the non-incubated immobilized enzyme. Moreover, the enzyme immobilized on Ag-DEAE presented a 10 nm-shift of the Trp-fluorescence peak (324 nm) regarding to the soluble enzyme (314 nm), but when the immobilized enzyme was incubated at 40º C, such fluorescence peak shifted back 10 nm to the UV, presenting the same value than the soluble enzyme. These results point out that tryptophan residues are more accessible to the media in the thermally incubated immobilized enzyme. Moreover, the thermal incubation seems to trigger the shielding of the FAD cofactor because its fluorescence was quenched after thermal incubation. Therefore, the shift of Trp-fluorescence peak and the differences in both Trp and FAD^+ fluorescence intensities reveal that the immobilization itself causes some structural rearrangement; interestingly, the temperature also induces further structural reorganization in the immobilized enzyme that can explain the enhancement in both activity and stability of the immobilized eNR incubated at 40ºC.

Supplementary figures

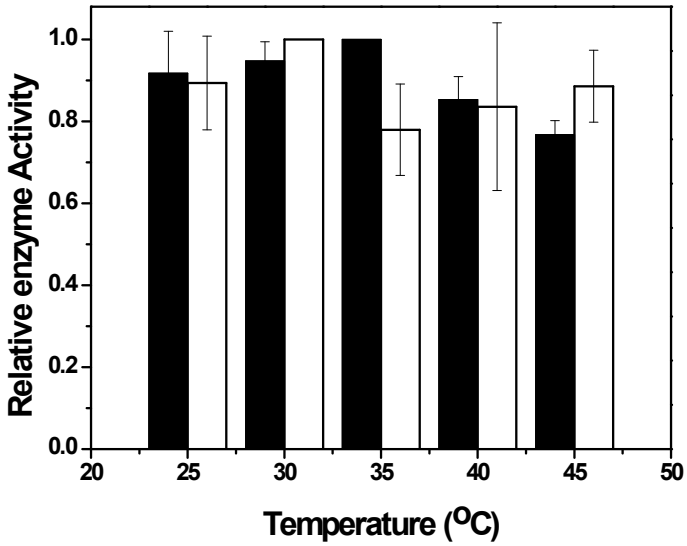

Figure S1- Activity/temperature profile of both soluble and immobilized eNR. Reductase activity of eNR immobilized on Ag-DEAE (white bars) and soluble eNR (black bars) was assayed at different temperatures at pH 7.5.

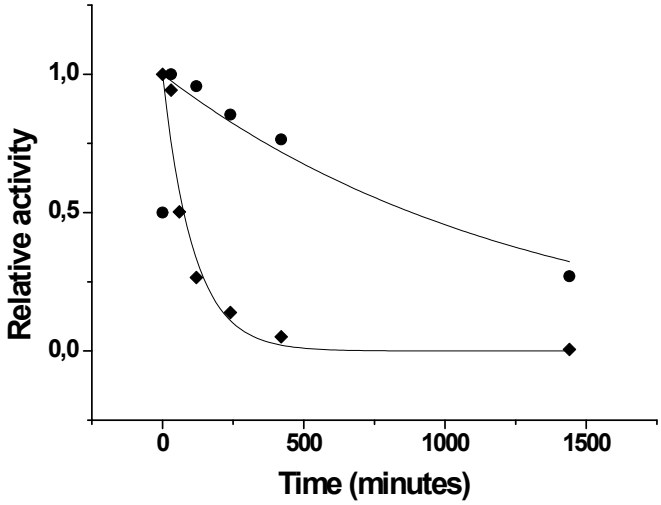

Figure S2- Thermal inactivation courses of *Aspergillus niger* nitrate reductase immobilized onto Ag-DEAE at 40°C. The preparation of *A. niger* eNR insoluble derivative was carried out as described in Section *Material and Methods*. Each insoluble and soluble preparations were incubated in 10mM sodium phosphate at pH 7.5 and 40ºC. The *A. niger* eNR preparations used to perform these studies were: immobilized on Ag-DEAE (●) and soluble (♦).

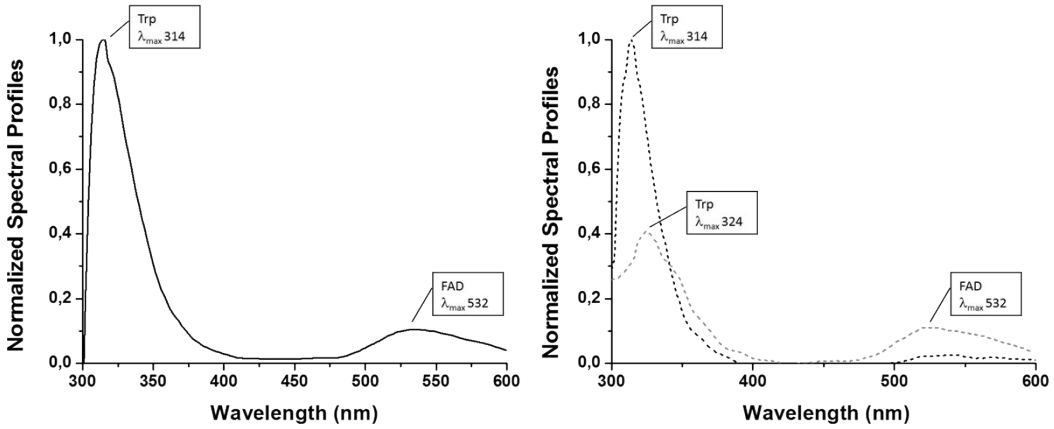

Figure S3 – Emission fluorescence spectra of *A. niger* eNR (soluble and insoluble). (A) Fluorescence spectra of *A. niger* eNR soluble. The fluorescence spectrum of soluble enzyme incubated 15 minutes at 40º C perfectly overlapped to the non incubated enzyme. (B) The fluorescence spectra of *A. niger* eNR immobilized on Ag-DEAE activated (dash black) and no-activated (dash grey). The preparation of *A. niger* eNR insoluble derivative was carried out as described in Section *Material and Methods*. Both insoluble and soluble preparations were incubated in 10mM sodium phosphate at pH 7.5 and 25ºC and 40ºC.

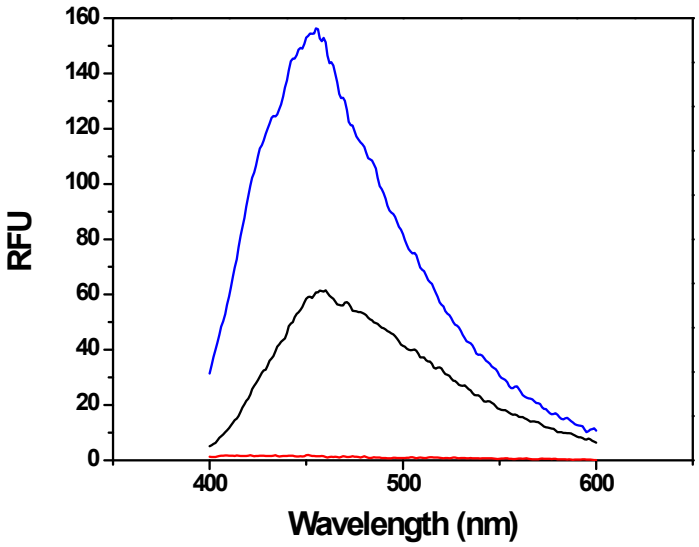

Figure S4 – Emission fluorescence spectrum of NADPH soluble and trapped into Ag-DEAE. The samples were excited at 360 nm and fluorescence emission was scanned from 400-600 nm. Soluble NADPH (black line). eNR immobilized on DEAE (red line) and eNR immobilized on DEAE and equilibrated with NADPH 1 mM (blue line).

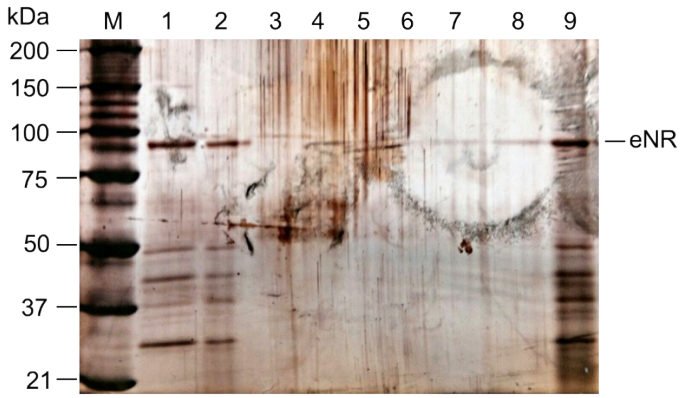

Figure S5- SDS-PAGE / silver staining of *Aspergillus niger* nitrate reductase. Lane M: molecular weight marker; Lane 1: eNR soluble; lane 2: eNR immobilized before any reaction; lane 3: non bound protein after immobilization; lane 4: cycle 1; lane 5: cycle 2; lane 6: cycle 3; lane 7: cycle 4; lane 8: cycle 5; and lane 9: eNR immobilized after 5 reaction cycles.

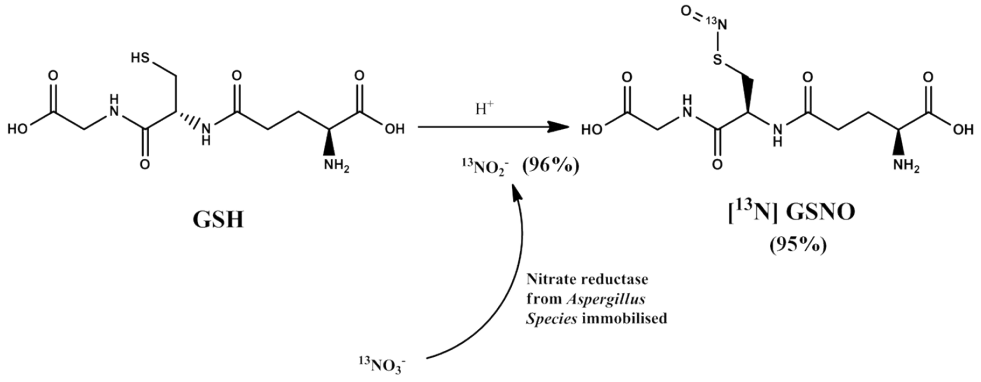

Figure S6 - [^{13}N]GSNO synthesis using $^{13}NO_2^-$ produced by enzymatic catalysis.