Supporting information

Concentration dependence of TRX peaks S and H

Peak H. We measured peak H1 of TRX in 50 mM McIlvain buffer, pH 7.5. 50 nM TRX yielded well-developed peak H at accumulation time (t_A) 120 s and stripping current (I_{str}) -20 μA. Between 50 and 400 nM TRX, area of this peak (A_p) increased linearly with TRX concentration (Fig. S-1).

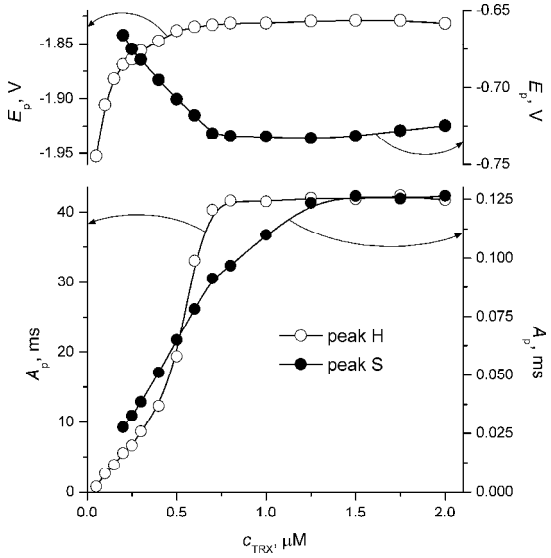

Figure S-1. Dependence of H and S peaks potentials, E_p and areas, A_p on TRX concentration. 50 mM McIlvain buffer, pH 7.5; initial potential +0.1V; I_{str} -20 μA; 120 s accumulation at open current circuit.

In the concentration range from 400 to 800 nM steeper increase of A_p was observed. Peak potential (E_p) shifted to less negative values up to about 600 nM TRX. Further increase of TRX concentration, from 0.8 to 2.0 μM, had no significant influence on peak H area and its potential.

Peak S. Under the given conditions well resolved peak S appeared on the TRX chronopotentiograms starting from about 200 nM TRX. Peak S yielded two ranges of its A_p linearly dependent on TRX concentration (Fig. S-1). From 200 to 700 nM TRX A_p increased with TRX concentration and E_p was shifted to more negative potentials. Between 0.7 and 1.25 μM TRX A_p increased less steeply but E_p did

not change. At 1.25 μM TRX limiting values of peak S area were attained suggesting full electrode coverage; from 1.25 to 2.0 μM concentration its E_p almost did not change (Fig. S-1). More details will be published elsewhere.

Advantages of CPS in studies of electrocatalytic signals of proteins

At present fast voltammetric methods are available affording comparable or even faster potential changes than in CPS (when working with the intensities of polarizing currents used in this paper). To our knowledge such voltammetric methods are not usual in studies of biomacromolecules, they suffer from some limitations (e.g. regarding the size of the working electrode) and have not been used in the analysis of non-conjugated proteins. Particularly, these methods can hardly be applied in the analysis of compounds yielding electrocatalytic signals. It has been shown that electrocatalytic voltammetric signals, decrease with increasing scan rate. Thus at very high scan rates the advantage of high electron yield in electrocatalytic processes can be lost; at high scan rates electrocatalytic voltammetric peaks may become smaller than those due to few electron redox processes. Such changes were observed[1,2] even at 1 V/s scan rates (good for redox processes but inconvenient for electrocatalysis) as compared to 100 mV/s (convenient for electrocatalysis). In CPS the changes of potential are delayed during the electrode process what makes it possible to obtain well-developed electrocatalytic signals even at relatively high intensities of the polarizing currents.

Fluorescence emission spectra of reduced and oxidized TRX

100 μM TRX was oxidized by 2 mM azodicarboxylic acid bis(dimethylamide) for 1 h at laboratory temperature. Reduced form of TRX was prepared by treatment with 2 mM tris(2-carboxyethyl)phosphine under the same conditions. The reduction/oxidation of TRX was performed to achieve fully reduced/oxidized state of TRX. Fig. S-2 shows a large increase in fluorescence quantum

yield accompanying reduction of TRX indicating a significant change in the environment of one of the two tryptophan residues.

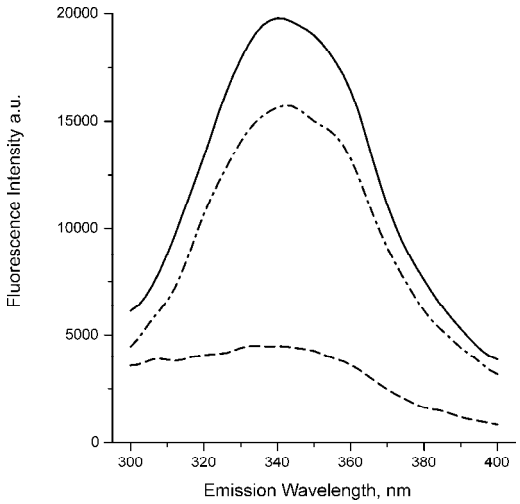

Figure S-2. Fluorescence emission spectra of 5 μM reduced (solid line), untreated (dash-dotted line) and oxidized (dashed line) forms of TRX. Fluorescence measurements were performed in 0.2 M McIlvain buffer, pH 7 with the excitation wavelength 280 nm using PC1™ Photon Counting Spectrofluorimeter (ISS, Champaign, Illinois, USA).

Similar results were obtained earlier by Stryer et al.[3]. These authors concluded that there was a localized conformational change on reduction of TRX. Earlier we reduced oxidized decapeptide SS38[4] in the same way as TRX and we also observed a significant difference in the fluorescence spectra of its reduced and oxidized form.

REFERENCES

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(4) Dorcak, V.; Palecek, E. *Electroanalysis* **2007**, *19*, 2405-2412.