Models Systems for Flavoenzyme Activity. Recognition and Redox Modulation of Flavin Mononucleotide in Water using Nanoparticles

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Supporting Information

Part I. Electrochemistry of FMN (flavin mononucleotide) in presence of salt.

Part II. Calculation of Binding Constant from Fluorescence titrations

Part III. Thermodynamic Cycle

Part IV. Experimental Section

PartI. Electrochemistry of FMN (flavin mononucleotide) in presence of salt.

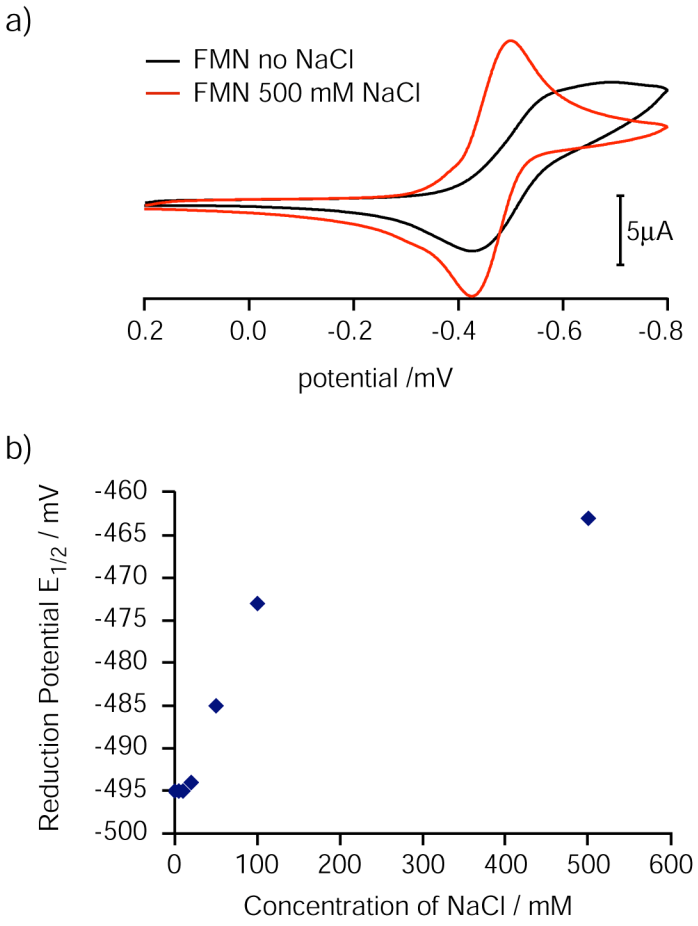

Fig. S1 a) Cyclic voltammetry of $5x10^{-4}$ M FMN and upon addition of 500 mM NaCl at *pH* = 8 water at 23°C; scan rate, 10mV/s vs. Ag/AgCl. b) Half-wave reduction potentials of FMN at different NaCl concentrations.

We have done cyclic voltammetry (CV) experiments of FMN in presence of differing concentrations of NaCl. FMN by itself has half-reduction potential of -492 mV, as the salt concentration is increased it shifts to less negative potentials. This shift in reduction potential could be due to interaction of free FMN with the salt ions. At the final step, when salt concentration was brought to 500 mM NaCl, half-wave reduction potential of FMN shifted to -465 mV.

Part II. Calculation of Binding Constant from Fluorescence titrations:

To simplify the calculations, we assumed that one nanoparticle has *n* identical and independent binding sites that are able to bind one FMN molecule each, the complexation of FMN with nanoparticles could be expressed by equation S1,

$$\text{NPSite} + \text{FMN} \underset{}{\overset{K_S}{\rightleftharpoons}} \text{NPSite:FMN} \qquad \text{(S1)}$$

where K_S denotes the microscopic binding constant. Because the fluorescence intensity decrease of FMN is attributed to the complexation with nanoparticles, the fluorescence intensity difference (_Z) is assumed to be proportional to the concentration of complexed FMN, i.e. _Z=__[NPSite:ChT]. The proportionality coefficient _ reflects the fluorescence intensity difference of unit FMN before and after complexation. Then K_S could be defined as:

$$K_s = \frac{[\text{ NPSite:FMN }]}{[\text{ NPSite }][\text{ FMN }]} = \frac{\Delta Z / \alpha}{([\text{NPSite}]_o - \Delta Z / \alpha)\,([\text{FMN}]_o - \Delta Z / \alpha)} \qquad \text{(S2)}$$

Where $[\text{NPSite}]_o$ and $[\text{FMN}]_o$ denote the initial concentrations of binding sites and FMN, respectively. The relationship between the concentrations of binding sites and nanoparticles is describable by $[\text{NPSite}]_o = n[\text{NP}]_o$. After a few manipulation, equation S2 is solved for _Z to give equation S3:

$$\Delta Z = \alpha/2 \left\{ ([\text{FMN}]_o + n[\text{NP}]_o + 1/K_s) - \{([\text{FMN}]_o + n[\text{NP}]_o + 1/K_s)^2 - 4n[\text{FMN}]_o[\text{NP}]_o\}^{1/2} \right\} \qquad \text{(S3)}$$

On the basis of equation S3, microscopic binding constants (K_S) and binding ratios (i.e. the number of nanoparticles' binding sites) could be readily determined by using the nonlinear least-squares curve-fitting analysis. The curve-fitting analysis was done on PC using Origin 7.0 program (OriginLab Co., Northampton, USA).

Part III. Thermodynamic Cycle

The interdependence of redox processes and recognition events between **TMA-NP** and FMN coenzyme molecules demonstrated graphically with the thermodynamic cycle can also be expressed mathematically:

$$K_a(\text{red}) = K_a(\text{ox}).\exp\{[E_{1/2}(\text{b}) - E_{1/2}(\text{u})].nF/RT\}$$

where, K_a(red) and K_a(ox) are the association constants in the oxidized and reduced form, and $E_{1/2}$(u) and $E_{1/2}$(b) are standard reduction potentials in the unbound and bound-gold nanoparticles form.

Part IV. Experimental Section

Materials and General Methods

11-bromoundecane, trimethylamine 40% (w/w) in water, thiolacetic acid, 2,2'-azobis-isobutyronitrile (AIBN), octanethiol, mercaptoundecanoic acid, sodium borohydride, tetraoctylammonium bromide and Flavin mononucleotide (riboflavin 5'-phosphate sodium salt) were purchased from Sigma-Aldrich. Hydrogen tetrachloroaurate (III) hydrate was obtained from Strem Chemicals, Inc. and used without further purification. Hydrochloric acid concentrated was purchased from Fisher. All the solvents were purchased from Pharmco and were used as received unless specified otherwise.

Synthesis

*N,N,N*Trimethyl (11-mercaptoundecyl) ammoniumchloride, trimethylammonium thiolate functionalized gold nanoparticles (**TMA-NP**),[1] and the carboxylate functionalized nanoparticles (**MUA-NP**),[2] were synthesized using previously published procedures. For the synthesis of MMPCs, Murray's place exchange reaction is used. We have place exchanged octane thiolate capped gold nanoparticles with *N,N,N*Trimethyl (11-mercaptoundecyl) ammoniumchloride in 1:1 feeding ratio to obtain **TMA-NP**s. ^{1}H-NMR end group analysis revealed that 60% of monolayer was composed of trimethylammonium thiolate. To synthesize **MUA-NP**s, octanethiolate capped gold nanoparticles were place exchanged with mercapto-undecanoic acid ligands with 1:3 feeding ratio to increase the solubility of resulting MMPCs. Analysis of **MUA-NP**s showed that 85% of monolayer is made up of carboxylate thiolate groups and remaining 15% is octane thiolate ligands.

Fluorescence

Experiments were performed on a Shimadzu RF-5301 PC spectrofluorophotometer using a 10 mm cuvette. The samples were excited at 445 nm, and the emission spectra were recorded from 465 to 650 nm with λ_{max} = 522 nm. FMN (0.5 μM) was titrated with **TMA-NP** (1 μM) and the FMN concentration was kept constant during the titrations at *pH* = 8 water. The *pH* of solution was kept constant during titrations. To determine the degree of signal intensity loss due to absorption by nanoparticles, the fluorescence intensity signal was normalized with control measurements using **TMA-NP** (1 μM) particles in presence of 100mM NaCl salt.

Cyclic Voltammetry (CV)

All electrochemical experiments were carried out on a BASC3 potentiostat equipped with BAS Epsilon-EC- Ver1.40.67NT. In the CV studies, a 1 mm carbon disk and platinum wire electrode were utilized as working and auxiliary electrodes, respectively and Ag/AgCl electrode was used as reference electrode in 10 ml single compartment electrochemical cell. The cell resistance was compensated by the BAS Epsilon software. In all experiments the sweep rate was 10 mV/s unless otherwise stated. The *pH* of solutions were set to 8 and they were degassed by bubbling argon through them for 5 min before each measurement and argon atmosphere was maintained over the solution throughout the experiments. **TMA-NP** ($1x10^{-4}$ M) was added into flavin mononucleotide sodium salt ($5 x10^{-4}$ M) solution and then 0.5 M NaCl was added into this mixture at *pH* = 8 water at 23°C. As a control noninteracting **MUA-NP**s ($1x10^{-4}$ M) were added into FMN ($5 x10^{-4}$ M) solution at the same conditions. Cyclic voltammograms were observed after each addition.

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[1] C. M. McIntosh, E. A. Esposito, A. K. Boal, J. M. Simard, C. T. Martin, V. M. Rotello, *J. Am. Chem. Soc.,* 2001, **123**, 7626

[2] J. Simard, C. Briggs, A. K. Boal, V. M. Rotello, *Chem. Commun.,* 2000, 1943.