Supporting Information

Derivation and application of equations (1; here equal to 4) and (2; here 7)

In order to extract an approximate time constant from the quenching curves given in Figure 1, we applied the Stern-Volmer equation:

$$\frac{F}{F_0} = \frac{1}{1 + K_q \times c_q} \quad (1)$$

where F/F_0 is the relative fluorescence as a function of the quencher concentration c_q and a Stern-Volmer constant K_q. c_q is the time-dependent concentration of the quencher at the nanowell's bottom in a parallel position to the bottom porphyrin. The differential equation, which would describe the restricted movement to reach this position is Fick's second law using an average diffusion constant for a one-dimensional diffusion into a membrane. Its solution is deduced from the equation for a one-dimensional diffusion of a solute out of a membrane[9] and is represented by a damped Fourier series:

$$c(x,t) = C_0 - \sum_n \frac{4c_0}{n\pi} \times \sin(\frac{n\pi}{l}x) \times e^{-\frac{n^2\pi^2}{l^2}Dt} \quad \text{(n=1,3,5...)} \qquad (2)$$

c_o corresponds to the concentration of the solute in the bulk solution and l is the height of the nanowell. The time-dependent concentration c_q of equation 1 is the concentration c(l/2,t) at x = l/2:

$$c(\tfrac{l}{2},t) = c_0 - \frac{4c_0}{\pi}\left[e^{-\frac{\pi^2}{l^2}Dt} - \frac{1}{3}e^{-\frac{9\pi^2}{l^2}Dt} + \frac{1}{5}e^{-\frac{25\pi^2}{l^2}Dt} ... \right] \quad (3)$$

There is a conceptional difficulty with this formula in our case. According to Fick's law, diffusion follows a concentration gradient, but in our experiments the porphyrin concentration in bulk solution is 10^{-6} M, whereas one porphyrin in one nanowell means 0.2 M. In order to keep the "diffusion" going we assume irreversible trapping at the bottom and an average concentration of the not-yet trapped porphyrins over all the wells. Trapped porphyrins are not counted. In this manner, the concentration within the nanowells never exceeds the bulk concentration and combination of the Fourier series with

the Stern-Volmer equation leads to equations (4) for the time-dependent fluorescence decay in nanowells with the height l.

$$\frac{F(t)}{F_0} = \frac{1}{1 + K_q\left[c_0 - \sum_n f_n \times e^{-\frac{n^2\pi^2}{l^2}Dt}\right]} \quad (4)$$

or more generally: $$F(t) = \frac{F_0}{y_0 - \sum_n A_n \times e^{-t/t_n}} \quad (5)$$

Many of the experimental quenching curves in Figure 1 can indeed be described by this formula (see Fig. 2a,b). A_n are the amplitudes of the exponential functions with the time constants t_n, which are correlated by n^2(6). Thus one decay function is characterized by one time constant t_1 only (7):

$$t_n = \frac{l^2}{n^2 \times \pi^2 \times D} \quad (6) \qquad t_1 = \frac{l^2}{\pi^2 D} \quad (7)$$

Equation (7) corresponds to the law of Einstein-Smoluchowsky and can be used to calculate the diffusion constant D. Applying equation (5) we have taken into account three terms of the Fourier series for all the curves of Figure 1. We have also tried an additional exponential term with $t_m = t_1/2$, which corresponds to the term c_q^2, appropriate for irreversible trapping. Fitting of the curves did, however, not improve. We therefore skipped this complication and applied the simpler function as given by (5). This equation with one independent time constant t_1 only gave excellent fits for the water-soluble porphyrins **3a** and **3b**; especially the sigmoid decay in case of the 15 Å-deep wells was reproduced perfectly. The curve of Figure 2a for porphyrin **3a** was calculated with a time constant of 21000 s and a diffusion constant of $D = 10^{-23}$ m^2s^{-1} was determined. In the cases where the fitting with one time constant only was less perfect, the calculated curves were always reasonably close and we got time constants between 1300 and 37000 (Figure 2a). It also turned out that the Einstein-Smoluchowsky law for unrestricted diffusion (eq. 6) is not strictly valid. We nevertheless used (6) to

calculate diffusion constants D between $6x10^{-24}$ m^2s^{-1} and $3x10^{-23}$ m^2s^{-1}. D thus varied only by a factor of 5 using different quencher molecules, solvents and depths of nanowells. The order of magnitude is clearly 10^{-24} – 10^{-23} m^2s^{-1}. It was found that relative values of D clearly responded to minor changes of the diameter of the quencher molecules. D for the *meta*-methylpyridinium isomer **3b**, for example, was larger by a factor of two as compared to the larger *para*-isomer **3a**. The viscosity of the solvent also played a role[6] ($D_{CHCl3} > D_{EtOH} > D_{H2O}$; compare Figure 1), but different sizes and solubilities of the applied porphyrins in different solvents did not allow for meaningful comparisons. A hint comes from the temperature dependence of the time constant (Fig. 2c). A higher temperature accelerates the quenching process and decreases the amplitude of the slow phase. Since the latter effect is not included in formula (6), we determined only the half time of the fast phase. Its temperature dependence followed an Arrhenius function with an activation energy of 2.7 kcal/Mol.