Supplementary information

Encapsulation of FRET-based glucose and maltose biosensors to develop functionalized silica nanoparticles

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Experimental

Chemicals and proteins

Chemicals for the biochemical characterization of the sugar biosensors were purchased from Sigma Aldrich (Buchs, Switzerland). In particular, D-(+)-Maltose monohydrate BioUltra, ≥99.0% (HPLC) (product nr. 63418) and D-(+)-Glucose monohydrate for microbiology, ≥99.0% (Fluka) (product nr. 49159) were purchased from Sigma Aldrich (Buchs, CH) and used as analytes. All commercially available chemical reagents and solvents were used without further purification. Triton X-100 and tetraethyl orthosilicate (TEOS) were purchased from Sigma Aldrich (Buchs, Switzerland). Proteins were expressed and purified as described elsewhere[1] and stored in 20 mM MOPS, pH 7.4 at -20°C.

FRET-based sugar biosensors

The glucose biosensor pRSET FLII^{12}Pglu600µ [2, 3] and the maltose biosensor pRSET FLIPmal-25µ[4] were from Addgene (http://www.addgene.org). Their expression, purification, and sequences are described in[1].

Formation of silica-sensor nanoparticles

The encapsulation procedure of the protein biosensors was carried out similarly to the protocol described by Cao et al.[5] In brief, 2 ml of the respective sugar biosensor solution (1 mg/ml initial concentration) in 20 mM MOPS (pH 7.4) were used for the encapsulation process and 2.0 mg of $CaCl_2$ as well as 200 µL tetraethyl orthosilicate (TEOS) solution added. Separately, a mixture of 10 ml cyclohexane, 2 ml Triton X-100 and 2 ml *n*-hexanol was prepared and mixed mechanically (1400 rpm). Subsequently, the biosensor protein solution was added and the mixture was continuously stirred for 15 min before 100 µL of aqueous 25% ammonium hydroxide solution were injected to start the silica formation. After stirring over night at room temperature, 30 ml of acetone were added and the mixture centrifuged at 2000 rpm. The particles were redispersed in aqueous solution and again precipitated and centrifuged. The procedure was repeated 2 times and the precipitate finally redispersed in 2 ml of water containing 0.1wt.% of SDS.

Microscopy of the encapsulated biosensors

For light microscopy, the biosensors in the silica nanoparticles were diluted ten-folds in 20 mM MOPS (pH 7.4) and imaged with a Leica DM6000 microscope (Leica, United Kingdom) equipped with a digital camera (Leica Digital camera DFC350 FX, Germany) using a fluorescence microscope under bright light and with a GFP filter for fluorescence (λ_{ex} = 450-490nm, λ_{em} = 425-550 nm) or YFP (λ_{ex} = 500-520nm, λ_{em} = 530-535 nm).

Scanning transmission electron microscopy (STEM) was performed on a Hitachi S-4800 (Hitachi High technologies, Canada). For sample preparation, an aqueous dispersion of biosensor protein-encapsulated silica nanoparticles as obtained after synthesis was diluted with deionized water (1:50) and drop-casted on 300 mesh carbon coated cupper grids and sputtered with gold (Polaron Equipment, SEM coating Unit E5100, Kontron AG, Switzerland, 5 nm thick coating) before imaging.

Biochemical characterization of the encapsulated biosensors

Fluorescence measurements were performed with a Cary Eclipse Fluorescence Spectrophotometer equipped with a multiwall-plate reader (Varian) in black 96-well half-area plates at room temperature using a 100 µl sample. Typically, 10 µl of encapsulated biosensor or 5 µl of the free biosensor (1 mg/ml) were incubated in 20 mM MOPS (pH 7.4), in the presence of various concentrations of maltose or glucose, or urea for 2 hours. For temperature stability studies, the samples were incubated at 70°C without stirring. Fluorescence spectra were recorded with a λ_{ex} = 428 nm and λ_{em} = 450–600 nm. Changes in the biosensor were calculated as variations in the FRET ratio calculated as the ratio between the maximum fluorescence emitted at 528 nm (YFP, Citrine) and at 485 nm (ECFP, λ_{ex} = 428 nm).

To test the performance at different pH conditions the sugar biosensors either in free form or entrapped in nanoparticles (10 µl) were incubated for 1 hr at 250 rpm at room temperature with 20 mM MOPS solution (90 µl) at pH values between 4 and 8. Data analysis was performed with SigmaPlot (Systat Software Inc.).

Zeta-potential measurement of the encapsulated biosensors

For zeta potential measurements an aqueous dispersion of the silica nanoparticles with encapsulated sugar biosensors was diluted (1:100) with a 1 mM KCl solution. The resulting dispersion was measured on a Zetasizer Nano ZS90 (Malvern Instruments).

Figures

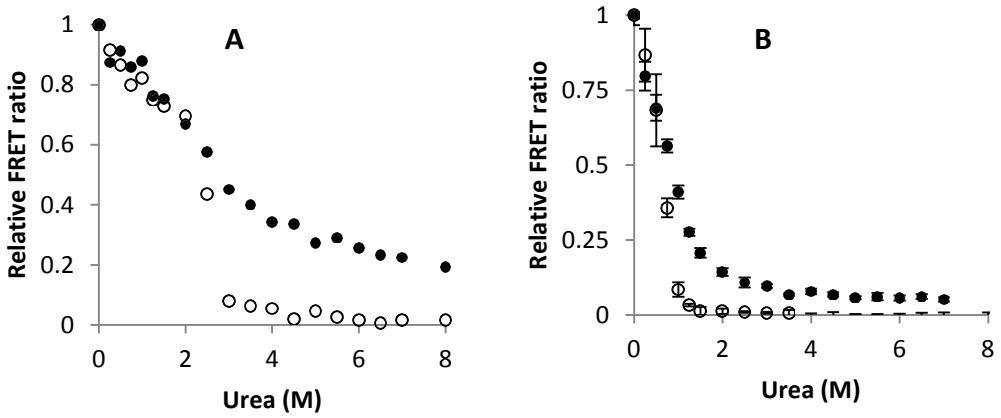

Fig. S1 Relative changes in FRET ratio of the maltose biosensor (A) and glucose (B) biosensor at increasing urea concentrations. Values are reported as average±standard deviation.

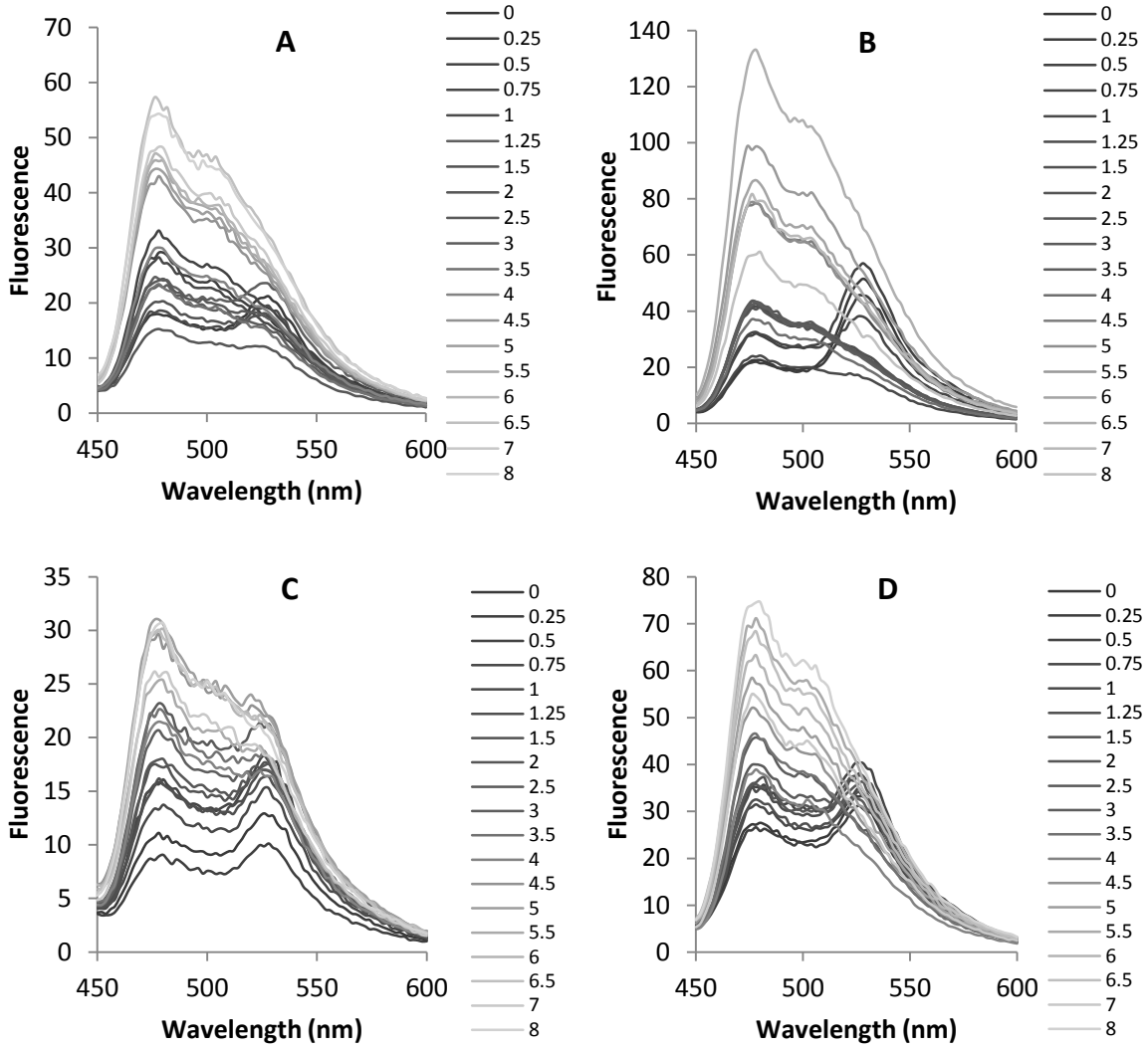

Fig. S2 Fluorescence emission spectra of the glucose (A,B) and maltose (C,D) biosensors in encapsulated (A,C) and free form (B,D) in the presence of urea (reported as molar concentration in the legends). A) Functionalized silica particles containing the glucose biosensor, B) glucose biosensor in free form, C) functionalized silica particles containing the maltose biosensor, D) maltose biosensor in free form.

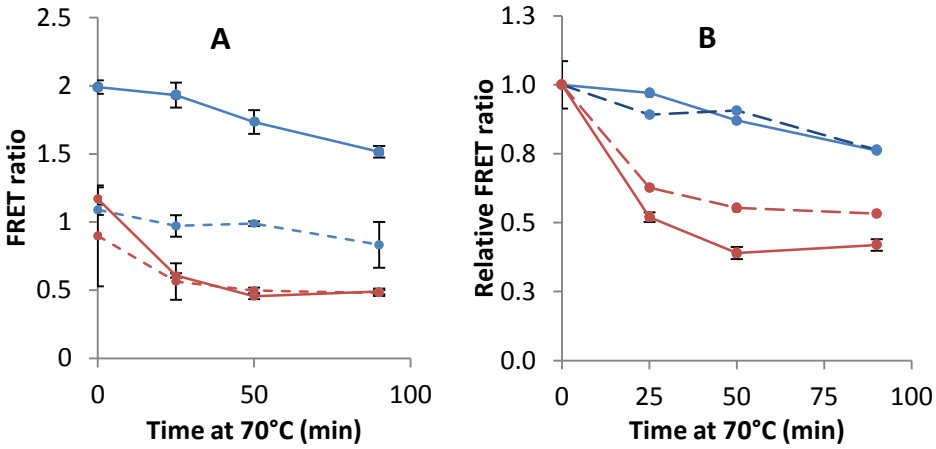

Fig. S3 Absolute (A) and relative (B) FRET ratio changes upon incubation at 70°C for the glucose (blue lines) or maltose (red lines) biosensor as free form (continuous line) or functionalized nanoparticle (dashed line). Values are reported as average±standard deviation.

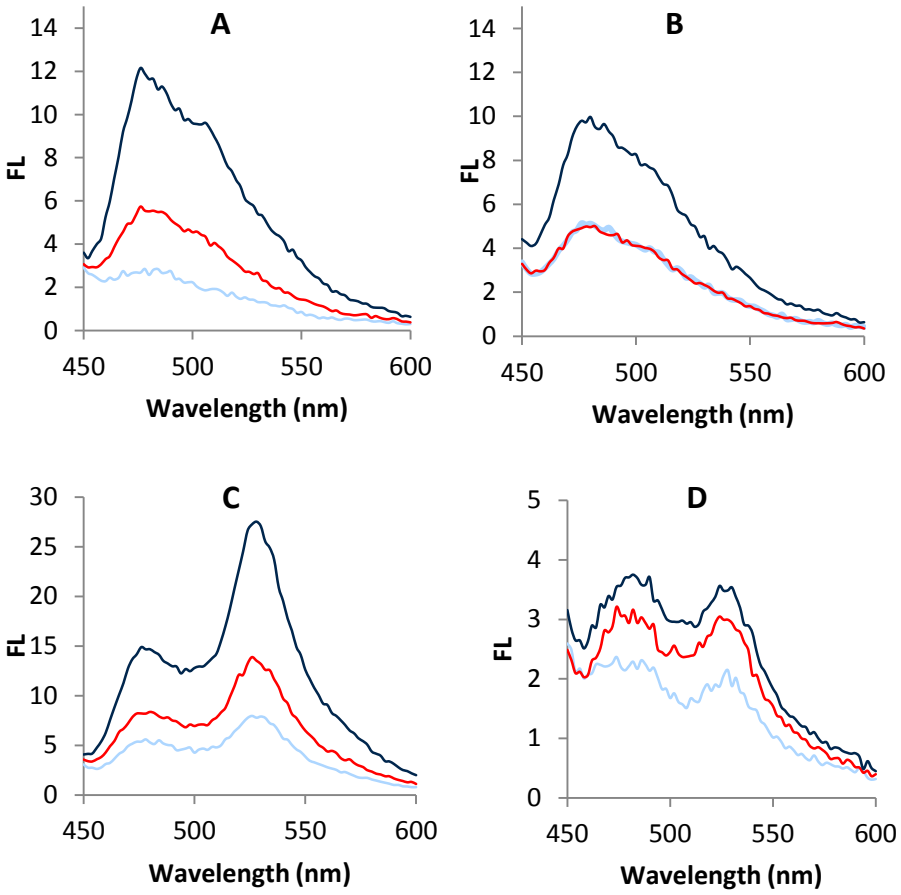

Fig. S4 Fluorescence emission spectra of the glucose and maltose biosensors as functionalized silica nanoparticles or in free form after incubation at 70°C for 25 (dark blue line), 50 (red line), or 90 (light blue line) min. A) Functionalized silica particles containing the glucose biosensor, B) glucose biosensor in free form, C) functionalized silica particles containing the maltose biosensor, D) maltose biosensor in free form.

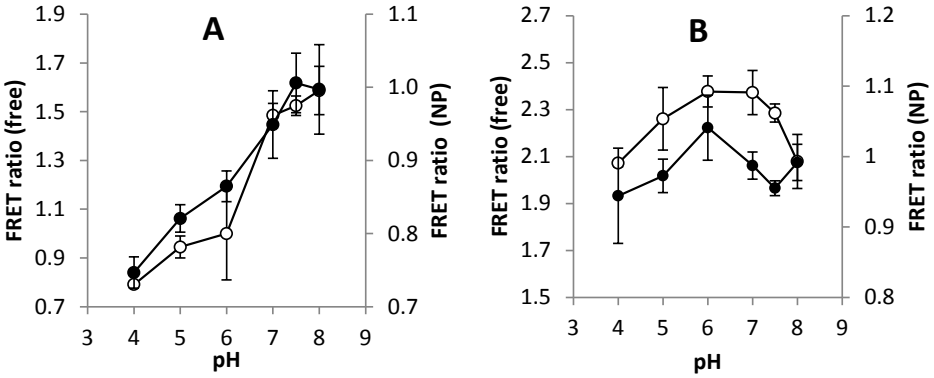

Fig. S5 FRET ratio dependency on the pH of the biosensor environment of the maltose (A) and glucose (B) biosensors in the free (empty dots) and the nanoparticles (filled dots) form. Values are reported as average ± standard deviation.

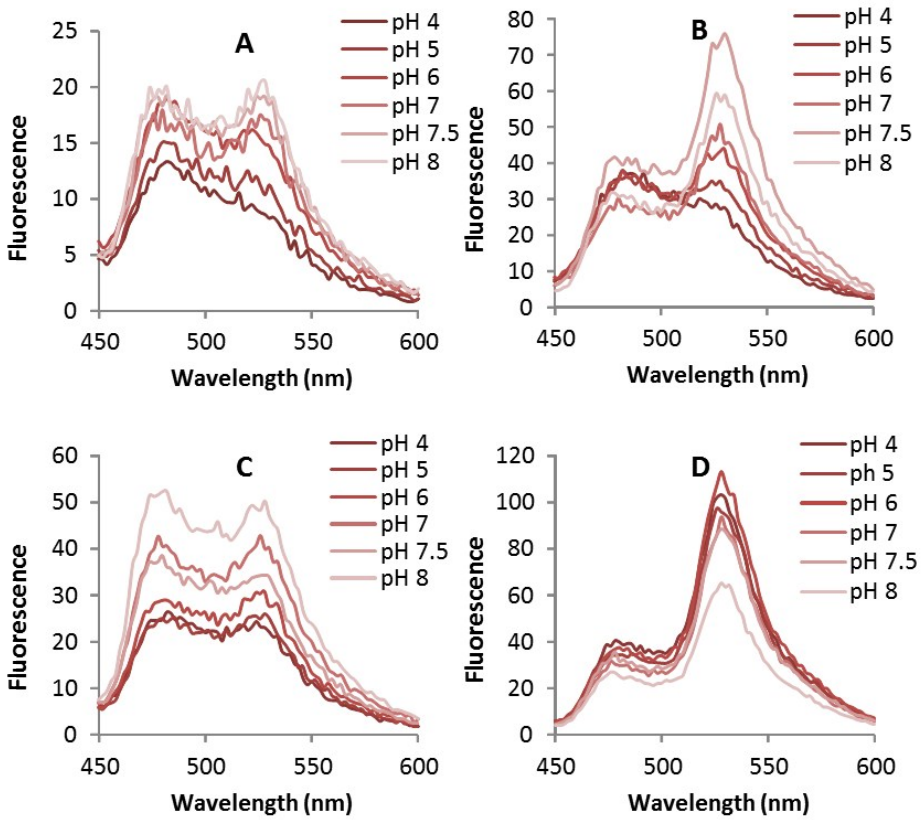

Fig. S6 Fluorescence emission spectra of glucose and maltose biosensors in free or as protein-silica nanoparticles under different pH conditions. A) Functionalized silica particles containing the maltose biosensor, B) maltose biosensor in free form, C) functionalized silica particles containing the glucose biosensor, D) glucose biosensor in free form.

References

1 R. Moussa, A. Baierl, V. Steffen, T. Kubitzki, W. Wiechert and M. Pohl, *J. Biotechnol.,* 2014, **191**, 250-259.

2 K. Deuschle, S. Okumoto, M. Fehr, L. L. Looger, L. Kozhukh and W. B. Frommer, *Protein Science,* 2005, **14**, 2304-2314.

3 M. Fehr, S. Okumoto, K. Deuschle, I. Lager, L. L. Looger, J. Persson, L. Kozhukh, S. Lalonde and W. B. Frommer, *Biochem. Soc. Trans.,* 2005, **33**, 287-290.

4 M. Fehr, W. B. Frommer and S. Lalonde, *Proceedings of the National Academy of Sciences,* 2002, **99**, 9846-9851.

5 A. Cao, Z. Ye, Z. Cai, E. Dong, X. Yang, G. Liu, X. Deng, Y. Wang, S. Yang, H. Wang, M. Wu and Y. Liu, *Angewandte Chemie International Edition,* 2010, **49**, 3022-3025.