Supplementary Material (ESI) for *Soft Matter*
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Electronic Supplementary Material (ESI) for Soft Matter

Core-Shell Microgels as "Smart" Carriers for Enzymes

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Materials and Methods

Theoretical analysis of the ITC data

This section briefly explains the underlying model which was used to determine the binding constant K, the total number of adsorbed proteins N and the enthalpy change of the overall process ΔH_{itc} from the experimental raw ITC data. For a more detailed description see ref [1]:

ITC measures stepwise heat changes ΔQ_i during the course of the titration experiment where the protein solution is stepwise injected into the microgel dispersion. Before the heat data can be subjected to an equilibrium binding model the integrated heat change after each injection must be corrected by the integrated heat of dilution of the protein and microgel solution.

For the adsorption of lysozyme on oppositely charged microgels a one-site model was applied with N identical binding sites as the sigmoid shape of the ITC curve does no imply different kind of binding sites. Therefore the binding constant is given by the Langmuir-Isotherm:

$$K = \frac{\Theta}{(1-\Theta)[P]_u} = \frac{\Theta}{(1-\Theta)([P]_t - N\Theta[M]_t)} \qquad (1)$$

where Θ is the fraction of sites containing bound protein and $[P]_u$ is the concentration of unbound protein. The unknown value of $[P]_u$ can expressed by the total protein and microgel concentrations $[P]_t$ and $[M]_t$.

The amount of heat Q evolved on addition of protein depends on the amount of bound protein which is given by N, Θ, $[M]_t$ and the total volume V and the molar enthalpy change ΔH_{itc}:

$$Q = N\Theta[M]_t V \Delta H_{itc} \qquad (2)$$

Combining of equation (1) and (2) leads to an expression which relates Q to the binding

constant K and the total protein concentration $[P]_t$:

$$Q = \frac{N[M]_t V \Delta H_{itc}}{2}\left[1 + \frac{[P]_t}{N[M]_t} + \frac{1}{NK[M]_t} - \sqrt{\left(1 + \frac{[P]_t}{N[M]_t} + \frac{1}{NK[M]_t}\right)^2 - \frac{4[P]_t}{N[M]_t}}\right] \quad (3)$$

The differential heat ΔQ_i, i.e. the change of heat from injection i-1 to i is given by eq. (4):

$$\Delta Q_i = Q_i - Q_{i-1} + \frac{dV_i}{V}\left(\frac{Q_i + Q_{i-1}}{2}\right) \quad (4)$$

In this model the parameters N, K and ΔH_{itc} were determined by least square curve fitting using equation (3) and (4). On this way, the initial values of N, K and ΔH_{itc} are improved by standard Marquardt methods until no further improvement in the fit occurs with continued iteration.

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Figures and Tables

Table S1. Thermodynamic and binding parameters for lysozyme adsorption on negatively charged core-shell microgels in 10 mM MOPS pH 7.2 at different salt concentrations.

T [K]	Buffer (all contain 2 mM NaN_3)	N_b	K [M^{-1}]	ΔG_{bind} [kJ mol^{-1}]	ΔH_{itc} [kJ mol^{-1}]
288	10 mM MOPS	57300 ±210	$1.83x10^6$ $\pm1.1x10^5$	-34.5 ±0.1	49.4 ±0.2
293	10 mM MOPS	57400 ±190	$2.24x10^6$ $1.4x10^5$	-35.6 ±0.2	53.4 ±0.3
298	10 mM MOPS	60100 ±190	$2.62x10^6$ $\pm1.7x10^5$	-36.6 ±0.2	60.7 ±0.3
303	10 mM MOPS	60100 ±220	$2.84x10^6$ $\pm2.2x10^5$	-37.4 ±0.2	71.6 ±0.4
288	10 mM MOPS 10 mM NaCl	50200 ±390	$3.54x10^5$ $\pm2.0x10^4$	-30.6 ±0.1	58.4 ±0.6
293	10 mM MOPS 10 mM NaCl	50900 ±340	$4.81x10^5$ $\pm2.8x10^4$	-31.2 ±0.1	61.2 ±0.5
298	10 mM MOPS 10 mM NaCl	52800 ±260	$6.25x10^5$ $\pm3.0x10^4$	-33.1 ±0.1	66.1 ±0.4
303	10 mM MOPS 10 mM NaCl	54700 ±370	$7.55x10^5$ $\pm5.5x10^4$	-34.1 ±0.2	74.0 ±0.6
288	10 mM MOPS 25 mM NaCl	34300 ±200	$1.33x10^5$ $\pm2.7x10^3$	-28.3 ±0.1	69.7 ±0.6
293	10 mM MOPS 25 mM NaCl	39500 ±220	$1.71x10^5$ $\pm4.3x10^3$	-29.4 ±0.1	65.7 ±0.5
298	10 mM MOPS 25mM NaCl	37600 ±360	$1.85x10^5$ $\pm8.1x10^3$	-30.1 ±0.1	83.8 ±1.0
303	10 mM MOPS 25 mM NaCl	45900 ±220	$2.90x10^5$ $\pm8.8x10^3$	-31.7 ±0.1	73.3 ±0.5

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Table S2. Thermodynamic and binding parameters for lysozyme adsorption on negatively charged core-shell microgels in 5 mM PIPES pH 7.2 at different temperatures.

T [K]	Buffer (all contain 2 mM NaN_3)	N_b	K [M^{-1}]	ΔG_{bind} [kJ mol^{-1}]	ΔH_{itc} [kJ mol^{-1}]
288	5 mM PIPES	58100 ±310	$1.04x10^6$ $\pm 7.4x10^4$	-33.2 ±0.2	46.3 ±0.3
293	5 mM PIPES	60500 ±440	$1.26x10^6$ $1.4x10^5$	-34.2 ±0.3	49.1 ±0.4
298	5 mM PIPES	61400 ±450	$1.38x10^6$ $\pm 1.6x10^5$	-35.0 ±0.3	56.2 ±0.5
303	5 mM PIPES	63100 ±450	$1.82x10^6$ $\pm 2.3x10^5$	-36.3 ±0.3	60.7 ±0.6

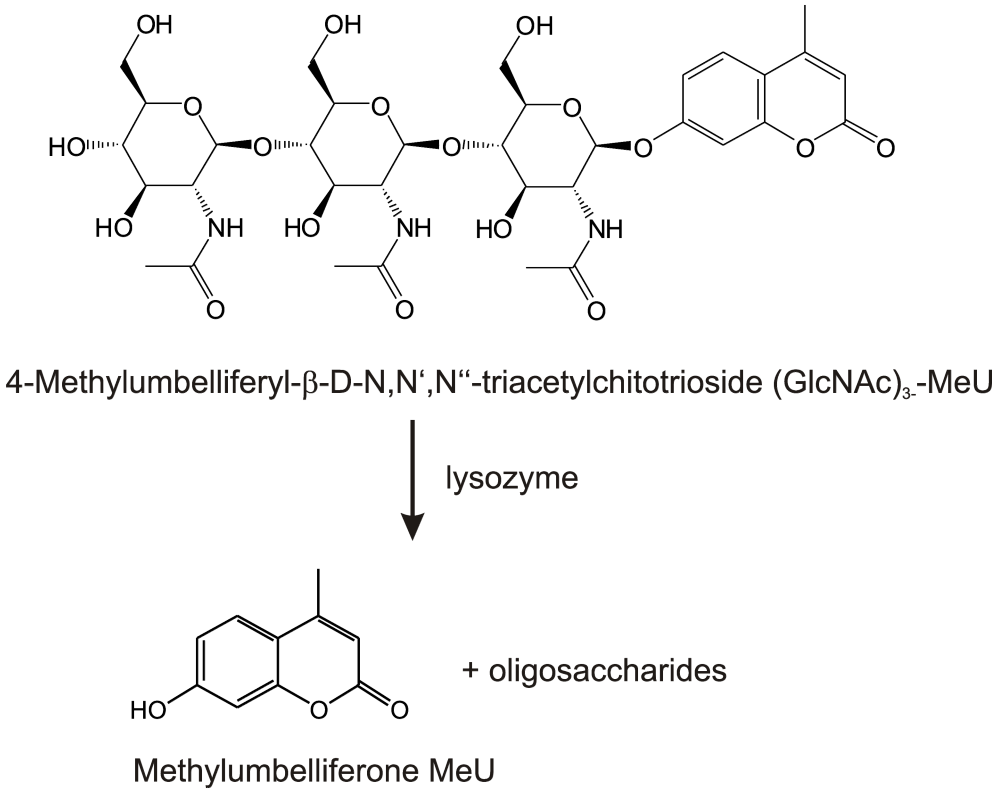

Figure S1. Reaction equation for the hydrolysis of $(GlcNAc)_3$-MeU catalyzed by lysozyme.

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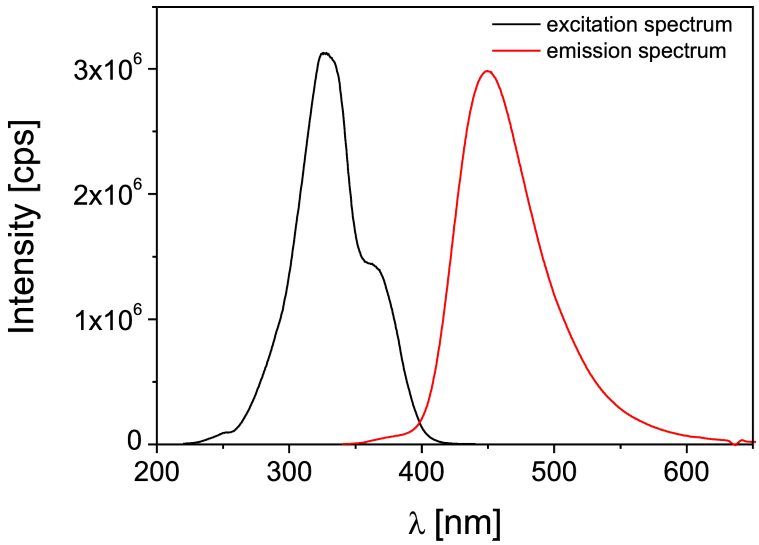

Figure S2. Excitation and emission spectra of MeU (1.24×10^{-6} M) in 10 mM MOPS pH 7.2 at 293 K. The excitation wavelength of the emission spectrum was 360 nm.

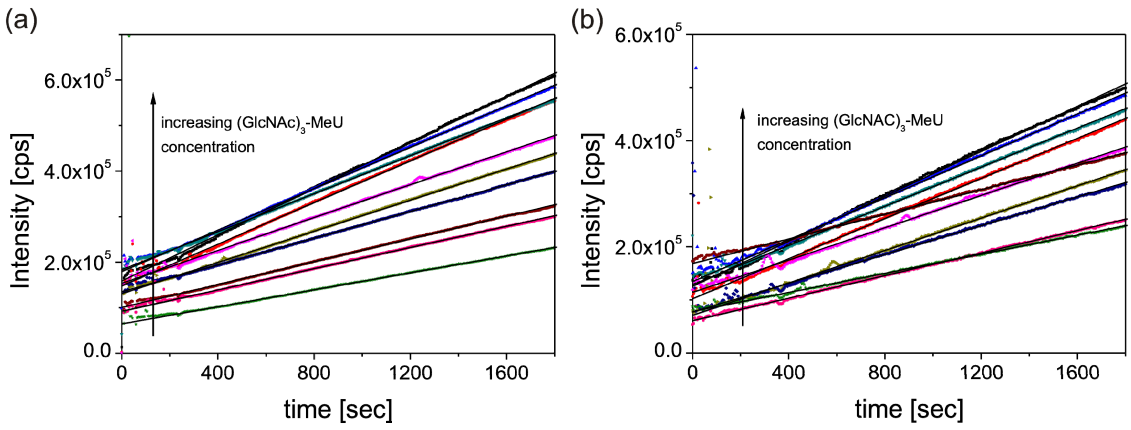

Figure S3. Fluorescence intensity as function of time for the conversion of $(GlcNAc)_3$-MeU for concentrations ranging from 1.6×10^{-5} to 1.3×10^{-4} M at 315 K in 10 mM MOPS pH 7.2. The excitation wavelength λ_{ex} was 360 nm and the emission wavelength λ_{em} was 450 nm. The reaction was catalyzed by a) 0.05 g/L free lysozyme and b) 0.02 g/L adsorbed lysozyme.

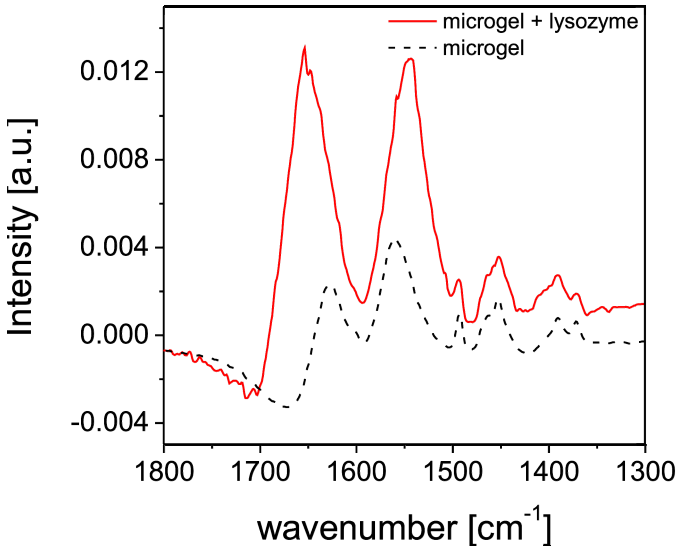

Figure S4. FT-IR spectra of the charged core-shell microgel before and after adsorption of lysozyme in 10 mM MOPS pH 7.2 at 298 K. The spectra were normalized to a microgel concentration of 1 wt-%. Microgel particles with immobilized lysozyme carry 660 mg protein per gram microgel.

1 L. Indyk and H. F. Fisher, *Method Enzymol*, 1998, 295, 350-364.