Supplementary data for the article:

Menghiu, G.; Ostafe, V.; Prodanović, R.; Fischer, R.; Ostafe, R. A High-Throughput Screening System Based on Fluorescence-Activated Cell Sorting for the Directed Evolution of Chitinase A. *International Journal of Molecular Sciences* **2021**, *22* (6), 3041. https://doi.org/10.3390/ijms22063041.

Figure S1. Chemical structures of chitin and chitosan. (**a)** Chitin fragment comprising six *N*-acetyl-D-glucosamine residues. (**b)** Partially-deacetylated chitin (chitosan), here presented as six alternating *N*-acetyl-D-glucosamine and D-glucosamine residues. (**c)** Fully-deacetylated chitosan (poly-D-glucosamine) comprising six D-glucosamine residues. The arrows show the positions cleaved by ChiA, an endochitinase that cleaves the glycosidic bond randomly at internal sites in a chitin or chitosan polymer, downstream of an *N*-acetyl-D-glucosamine unit. The requirement for an *N*-acetyl-D-glucosamine unit means that ChiA cannot hydrolyze fully-deacetylated chitosan.

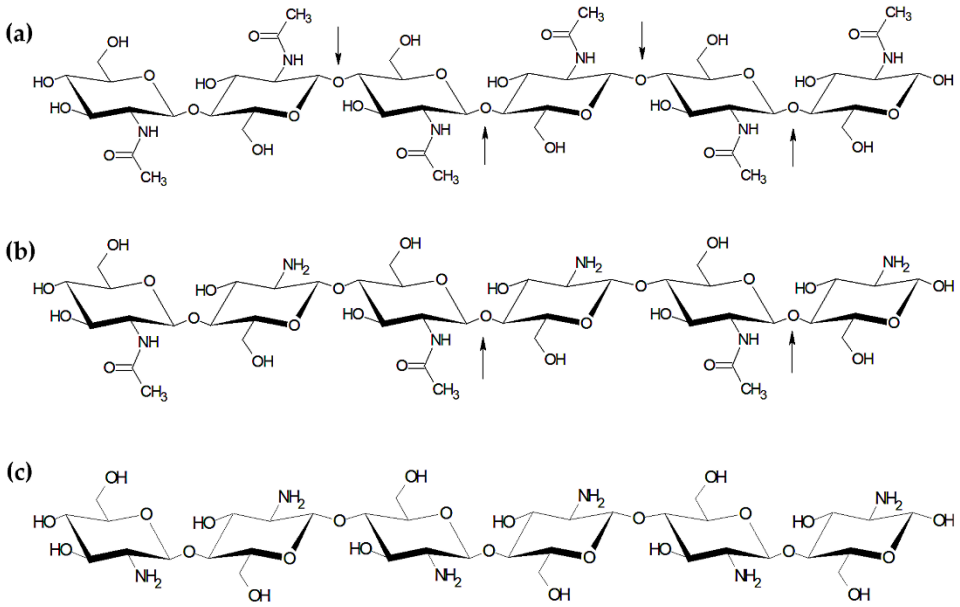

Figure S2. Chemical structures of the cyclodextrins used to prevent leakage of the fluorescent product 4MU. **(a)** Methyl-β-cyclodextrin (MCD). **(b)** 2-Hydroxypropyl-β-cyclodextrin (HCD). **(c)** Possible structure of methyl-β-cyclodextrin. **(d)** Possible structure of 2-hydroxypropyl-β-cyclodextrin.

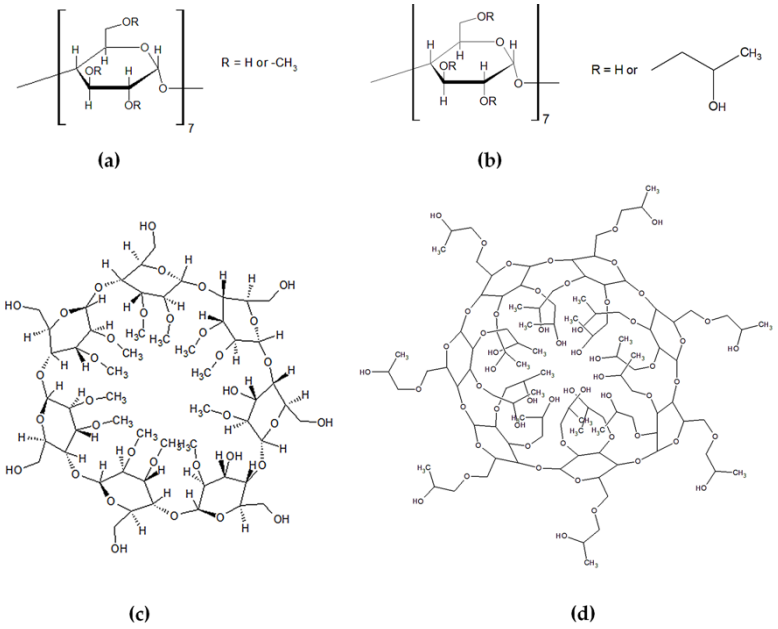

Figure S3. FACS response of 4MU, in the presence and absence of MCD/HCD, using a perfluorinated oil (PicoSurf) and SDS detergent, in double water-oil-water emulsions.