Supplementary data for:

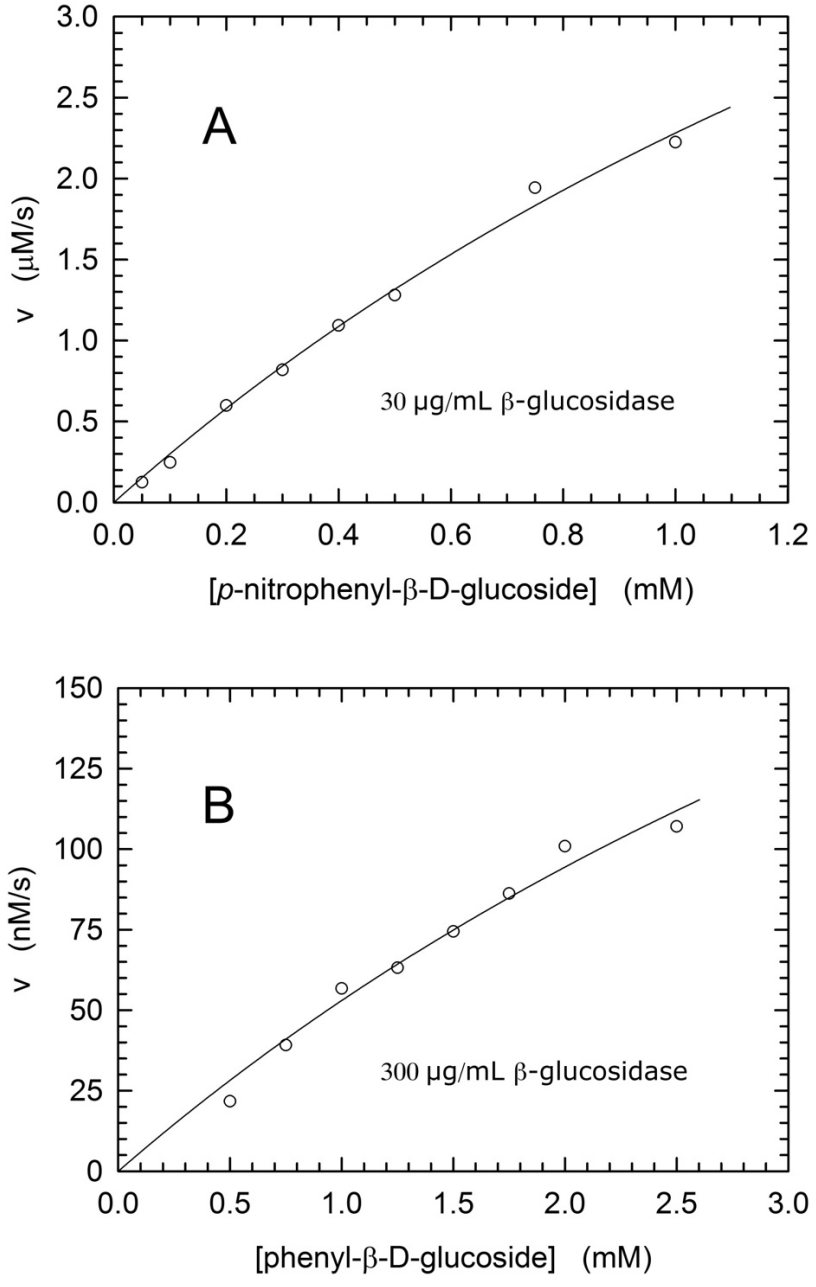
# The catalytic action of enzymes exposed to charged substrates outperforms the activity exerted on their neutral counterparts

Alessandra Stefan*°, Alejandro Hochkoeppler*°$

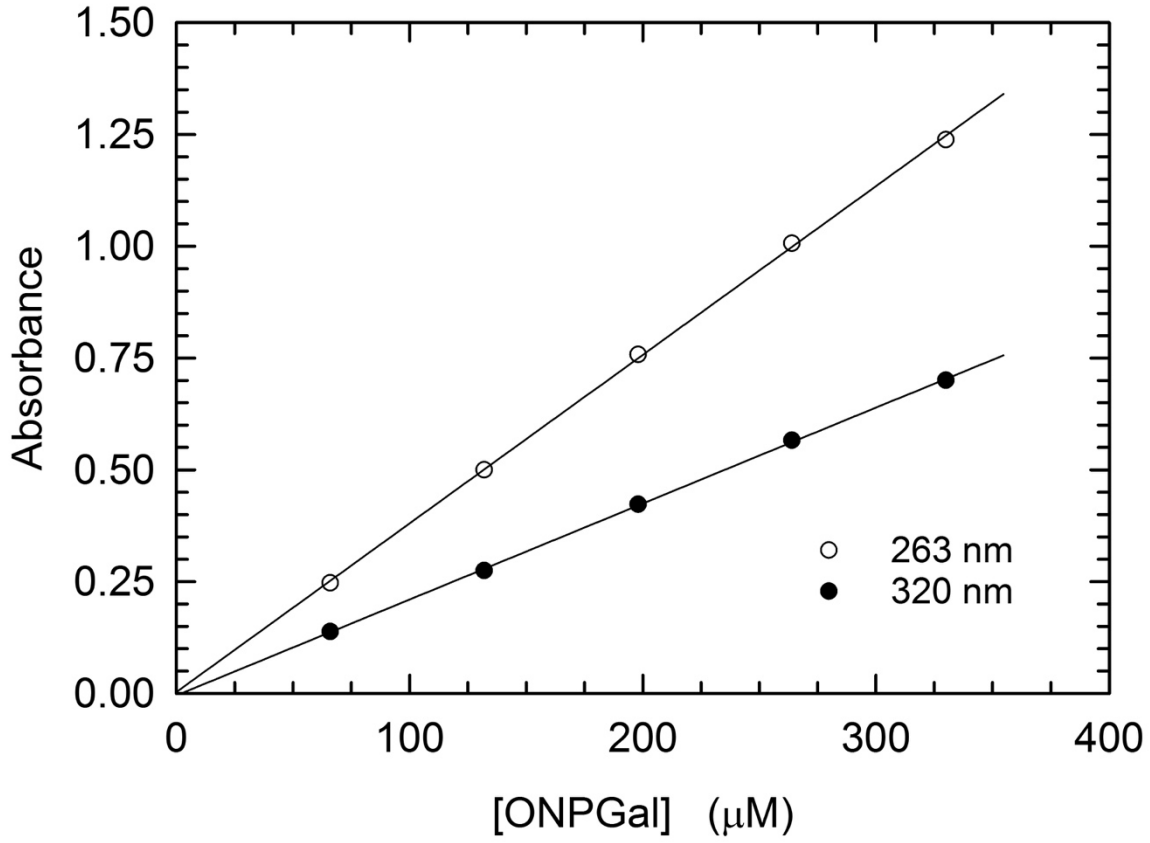
*Department of Pharmacy and Biotechnology, University of Bologna, Viale Risorgimento 4, 40136 Bologna, Italy

°CSGI, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, FI Italy

$Corresponding Author. e-mail: a.hochkoeppler@unibo.it

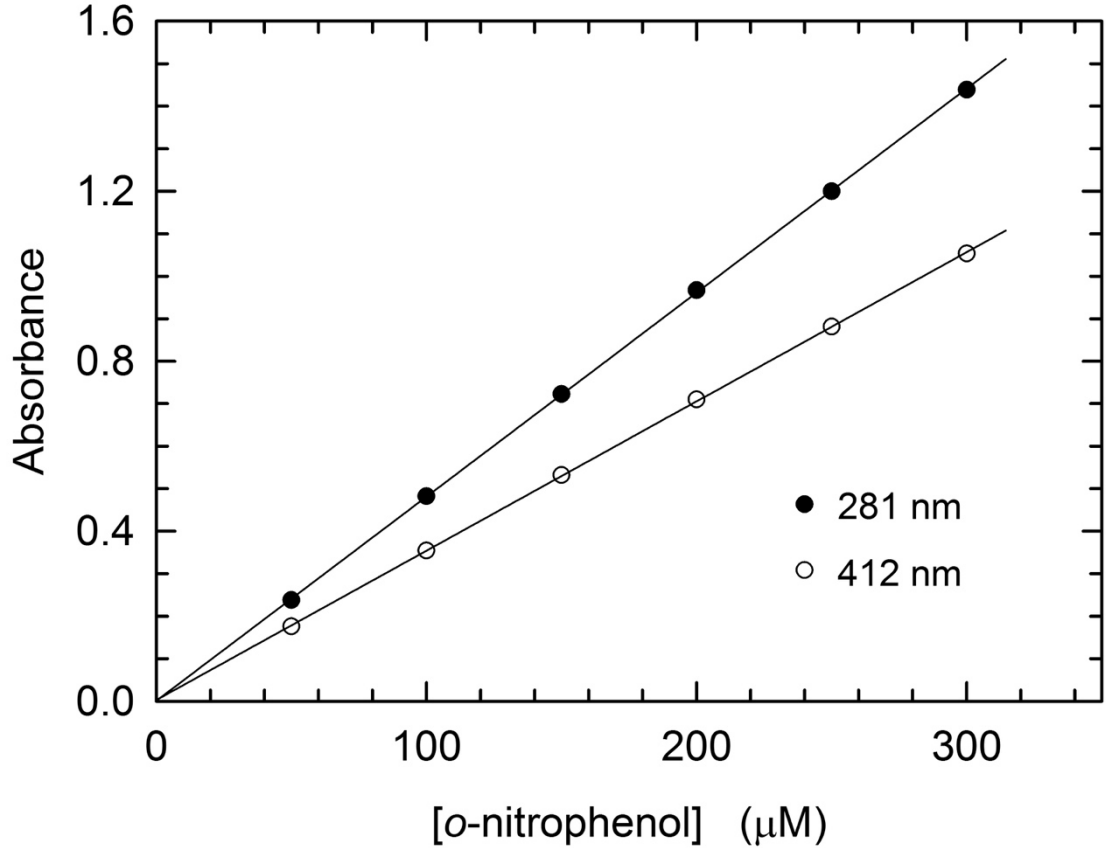



**Supplementary Figure S1. Catalytic action of *Prunus dulcis* β-glucosidase at the expense of *p*-nitrophenyl-β-D-glucoside and phenyl-β-D-glucoside.**
Initial reaction velocities of the hydrolysis of *p*-nitrophenyl-β-D-glucoside (A) and phenyl-β-D-glucoside (B) catalyzed by almond β-glucosidase at 30 (A) and 300 μg/mL (B). Both reactions were assayed at pH 5.5 (50 mM MES buffer).



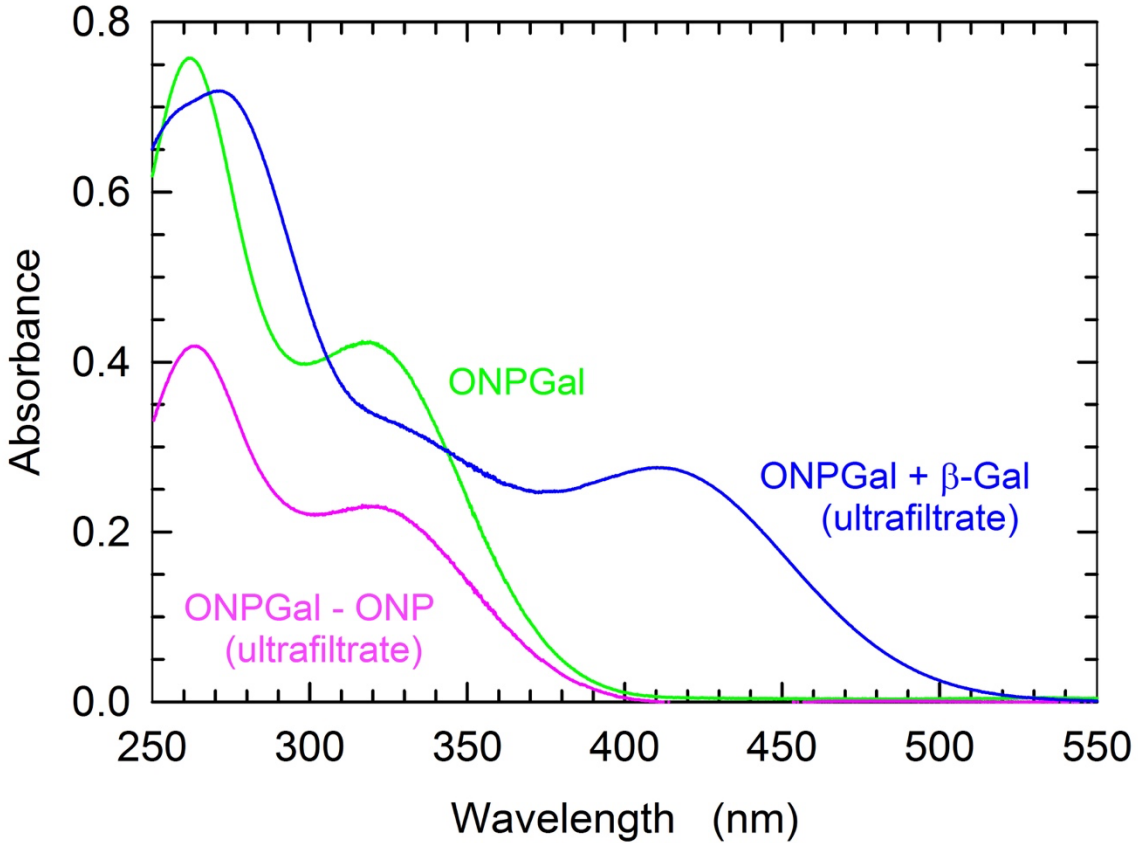

**Supplementary Figure S2. Determination of the molar absorption coefficient of *o*-nitrophenyl-β-D-galactopyranoside at 263 and 320 nm.**

Absorbance values determined at 263 and 320 nm as a function of *o*-nitrophenyl-β-D-galactopyranoside concentration. These values were obtained from the spectra (reported in Figure 4A of the main text) of solutions buffered at pH 7.5 using 10 mM Tris-HCl. The continuous lines represent the best fit of a linear equation to the experimental observations. By means of these fits, the extinction coefficients at 263 and 320 nm were determined as equal to 3800±43 and 2100±20 $M^{-1}cm^{-1}$, respectively.



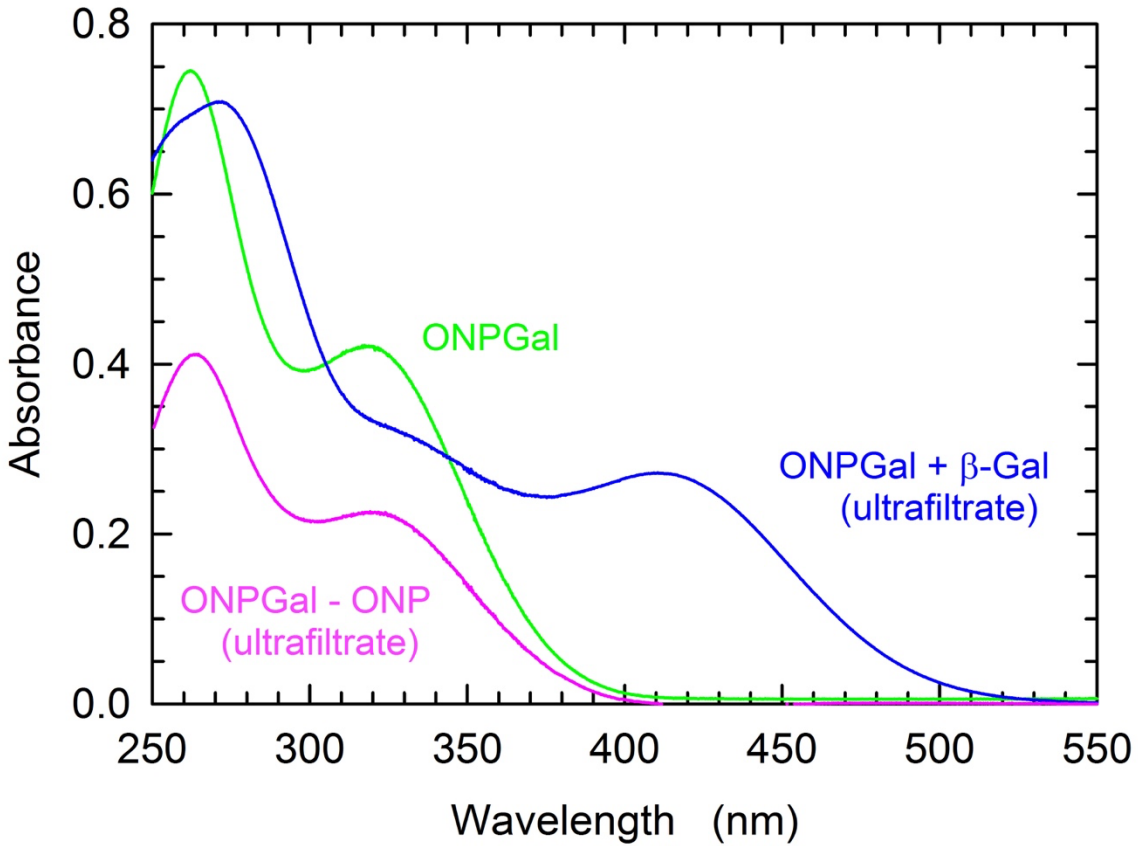

**Supplementary Figure S3. Determination of the molar absorption coefficient of *o*-nitrophenol at 281 and 412 nm.**

Absorbance values determined at 281 and 412 nm as a function of *o*-nitrophenol concentration. These values were obtained from the spectra (reported in Figure 4B of the main text) of solutions buffered at pH 7.5 using 10 mM Tris-HCl. The continuous lines represent the best fit of a linear equation to the experimental observations. By means of these fits, the extinction coefficients at 281 and 412 nm were determined as equal to 4800±18 and 3500±15 $M^{-1}cm^{-1}$, respectively.




**Supplementary Figure S4. Binding of *o*-nitrophenyl-β-D-galactopyranoside to multiple sites of β-galactosidase.**

Absorption spectra observed with: i) the initial concentration of *o*-nitrophenyl-β-D-galactopyranoside (200 µM) to be exposed to 1 µM β-galactosidase (green line); ii) the sample obtained by collecting the ultrafiltrate ($M_r$ cutoff 100 kDa) of a solution containing 1 µM enzyme and 200 µM substrate (blue line). The magenta line represents the difference absorption spectrum obtained by subtracting to the absorption of the ultrafiltrated sample (blue line) the contribution of the *o*-nitrophenol present (the concentration of which was determined with the band centered at 410 nm). All spectra were recorded at pH 7.5 (10 mM Tris-HCl).




**Supplementary Figure S5. Binding of *o*-nitrophenyl-β-D-galactopyranoside to multiple sites of β-galactosidase.**

Absorption spectra observed with: i) the initial concentration of *o*-nitrophenyl-β-D-galactopyranoside (200 μM) to be exposed to 1 μM β-galactosidase (green line); ii) the sample obtained by collecting the ultrafiltrate ($M_r$ cutoff 100 kDa) of a solution containing 1 μM enzyme and 200 μM substrate (blue line). The magenta line represents the difference absorption spectrum obtained by subtracting to the absorption of the ultrafiltrated sample (blue line) the contribution of the *o*-nitrophenol present (the concentration of which was determined with the band centered at 410 nm). All spectra were recorded at pH 7.5 (10 mM Tris-HCl).

| | Absorbance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 263 (nm) | 320 (nm) | 412 (nm) | [ONP]* (μM) | [ONPGal]** (μM) | [ONPGal]*** (μM) | Δ[ONPGal] (μM) | ONPGal-ONP (μM) |
| Sample 1 (Figure 4C) | | | | | | | | |
| ONPGal | 0.7698 | 0.4315 | - | | 203 | 205 | -90 | -9 |
| Sample | 0.7209 | 0.3459 | 0.2824 | 81 | | | | |
| Sample - ONP | 0.4244 | 0.2334 | - | | 117 | 111 | | |
| Sample 2 (Supplementary Figure S4) | | | | | | | | |
| ONPGal | 0.7563 | 0.4217 | - | | 199 | 201 | -90 | -11 |
| Sample | 0.7069 | 0.3389 | 0.2754 | 79 | | | | |
| Sample - ONP | 0.4187 | 0.2296 | - | | 110 | 109 | | |
| Sample 3 (Supplementary Figure S5) | | | | | | | | |
| ONPGal | 0.7440 | 0.4199 | | | 196 | 200 | -90 | -12 |
| Sample | 0.6954 | 0.3321 | 0.2713 | 78 | | | | |
| Sample - ONP | 0.4114 | 0.2244 | | | 108 | 107 | | |

**Supplementary Table ST1. Absorbance values at the indicated wavelengths of ultrafiltrated solutions and determination of their *o*-nitrophenyl-β-D-galactopyranoside concentration.**
The rows denoted as ONPGal, Sample, and Sample – ONP do respectively report: i) the Absorbance values and the concentration of ONPGal detected in ultrafiltrated solutions not exposed to β-galactosidase before ultrafiltration (ONPGal); ii) Absorbance values and the concentration of ONP detected in samples obtained by collecting the ultrafiltrate ($M_r$ cutoff 100 kDa) of a solution containing 1 μM enzyme and 200 μM substrate (Sample); iii) Absorbance values (to which the spectral contribution of *o*-nitrophenol was subtracted) and the concentration of ONPGal detected in samples obtained by collecting the ultrafiltrate ($M_r$ cutoff 100 kDa) of a solution containing 1 μM enzyme and 200 μM substrate (Sample – ONP).
*Determined with extinction coefficient for *o*-nitrophenol at 412 nm equal to 3500 $M^{-1}cm^{-1}$.
** Determined using an extinction coefficient for *o*-nitrophenyl-β-D-galactopyranoside at 263 nm equal to 3800 $M^{-1}cm^{-1}$.
*** Determined using an extinction coefficient for *o*-nitrophenyl-β-D-galactopyranoside at 320 nm equal to 2100 $M^{-1}cm^{-1}$.