

SUPPORTING INFORMATION

Table S1. Wavelengths used to monitor the release of methylumbelliferone at various pH values.

| pH | Wavelength (nm) | $\Delta\epsilon$ ($\text{cm}^{-1} \text{M}^{-1}$) |
|------|-----------------|---|
| 3.83 | 347 | 1936 |
| 4.25 | 347 | 1921 |
| 4.78 | 347 | 1834 |
| 5.25 | 347 | 1830 |
| 5.61 | 347 | 1753 |
| 5.70 | 348 | 1809 |
| 6.14 | 348 | 1977 |
| 6.59 | 349 | 2140 |
| 7.15 | 354 | 2695 |
| 7.60 | 370 | 2966 |
| 8.03 | 380 | 2992 |
| 8.62 | 388 | 2999 |
| 8.92 | 390 | 2999 |

Table S2. Wavelengths used to monitor hydrolysis of aryl sialosides at 37 °C and pH 5.25.

| Leaving Group | Wavelength (nm) | $\Delta\epsilon$ ($\text{cm}^{-1} \text{M}^{-1}$) |
|-----------------------|-----------------|---|
| 4-nitrophenol | 340 | 1840 |
| 4-methylumbelliferone | 347 | 1830 |
| 4-cyanophenol | 262 | 663 |
| 3-nitrophenol | 348 | 750 |
| 3-chlorophenol | 280 | 646 |
| 3-methoxyphenol | 281 | 574 |
| phenol | 270 | 1013 |

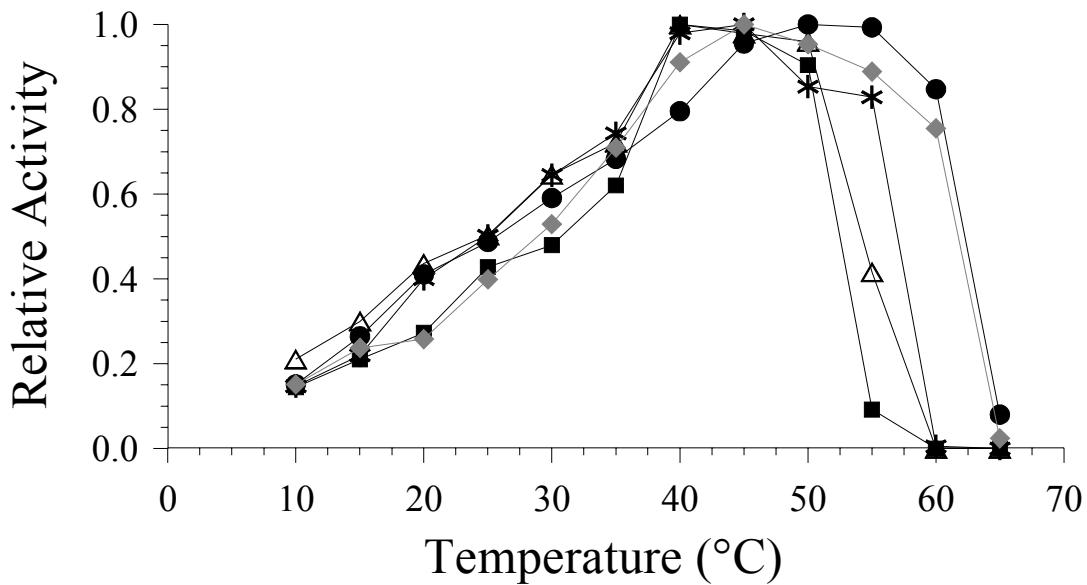


Figure S1. Effect of temperature on the relative rates of sialidase-catalyzed hydrolysis of MU- α Neu5Ac at pH 5.25 for the wild-type enzyme (●) and mutants Y370A (*), Y370D (Δ) and Y370G (■).

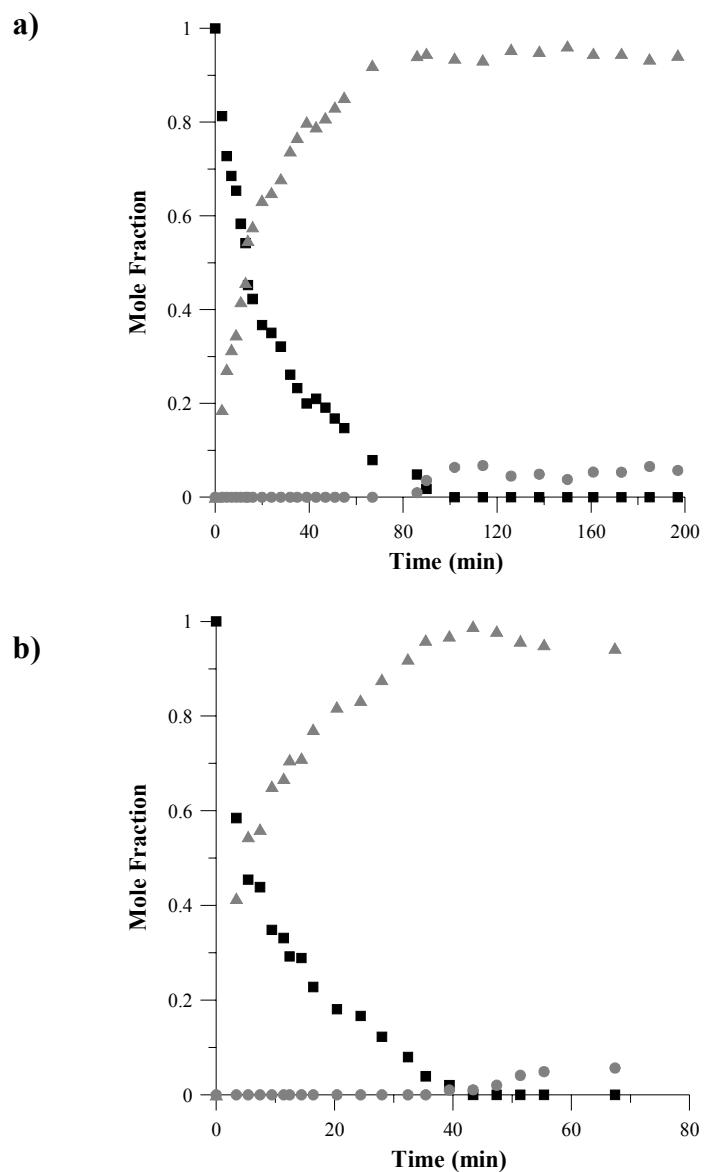


Figure S2. Mole fractions of substrate and products monitored over time. **a)** Y370A-catalyzed hydrolysis and **b)** Y370G-catalyzed hydrolysis. Values were calculated using the relative integrals of the H_{3eq} signals of MU- α Neu5Ac (■), α Neu5Ac (○) and β Neu5Ac (▲).

Table S3. Michaelis-Menten Parameters for Hydrolysis of α -D-Sialosides by the Wild-type Recombinant *M. viridifaciens* Sialidase, at pH 5.25 and 37 °C.

| Leaving Group | pK _a | k _{cat} (s ⁻¹) | k _{cat} /K _m ($\times 10^6$ M ⁻¹ s ⁻¹) |
|------------------------|-----------------|-------------------------------------|--|
| 4-nitrophenol | 7.18 | 145 ± 7 | 8.5 ± 1.7 |
| 4-methylumbellif erone | 7.80 | 52 ± 3 | 7.2 ± 1.7 |
| 4-cyanophenol | 7.96 | 236 ± 14 | 8.1 ± 1.7 |
| 3-nitrophenol | 8.27 | 203 ± 6 | 5.7 ± 0.7 |
| 3-chlorophenol | 9.09 | 235 ± 7 | 7.9 ± 1.0 |
| 3-methoxyphenol | 9.60 | 191 ± 6 | 2.6 ± 0.3 |
| Phenol | 9.92 | 230 ± 12 | 5.2 ± 1.0 |
| lactose (3-OH) | 13.6 | 138 ± 5 | 0.36 ± 0.06 |
| lactose (6-OH) | 13.8 | 207 ± 7 | 0.050 ± 0.009 |

Table S4. Michaelis-Menten Parameters for Hydrolysis of α -D-Sialosides by the Y370D Mutant *M. viridifaciens* Sialidase, at pH 5.25 and 37 °C.

| Leaving Group | pK _a | k _{cat} (s ⁻¹) | k _{cat} /K _m ($\times 10^3$ M ⁻¹ s ⁻¹) |
|------------------------|-----------------|-------------------------------------|--|
| 4-nitrophenol | 7.18 | 8.0 ± 0.3 | 240 ± 40 |
| 4-methylumbellif erone | 7.80 | 14.6 ± 0.6 | 101 ± 14 |
| 4-cyanophenol | 7.96 | 16.3 ± 1.4 | 220 ± 70 |
| 3-nitrophenol | 8.27 | 9.9 ± 0.2 | 91 ± 6 |
| 3-chlorophenol | 9.09 | 7.2 ± 0.2 | 35 ± 3 |
| 3-methoxyphenol | 9.60 | 2.1 ± 0.2 | 3.1 ± 0.8 |
| phenol | 9.92 | 1.34 ± 0.06 | 6.1 ± 1.2 |
| lactose (3-OH) | 13.6 | 0.017* | 0.0085 |
| lactose (6-OH) | 13.8 | 0.013* | 0.0043 |

* No error is associated with these values because they are estimates based on a single rate measurement at high concentration of substrate and enzyme.