Supplementary Table 1. Effect of M^+ binding on the charge of the M^+ ligating atoms. The model systems were based on the Na^+ replete β-galactosidase structure 1DP0 and the K^+ replete structure 4TTG. The systems included the side chains of Tyr 100, Asp 201, and Asn 604. To obtain a covalently closed system, the Cα of each of these residues was converted to methyl groups. The system also included the Phe 601 – Cys 602 peptide and the Phe 601 side chain. The N of Phe 601 and the Cα of Cys 602 were converted to methyl groups. Finally, the system contained the M^+ coordinating waters, and, optionally, the M^+. Partial atomic charges were calculated based on Mulliken and Natural Bond Orbital (NBO) analysis for systems with or without the M^+. Calculations were performed with Gaussian09 at the B3LYP/QZVP level of theory.

Mulliken Charges

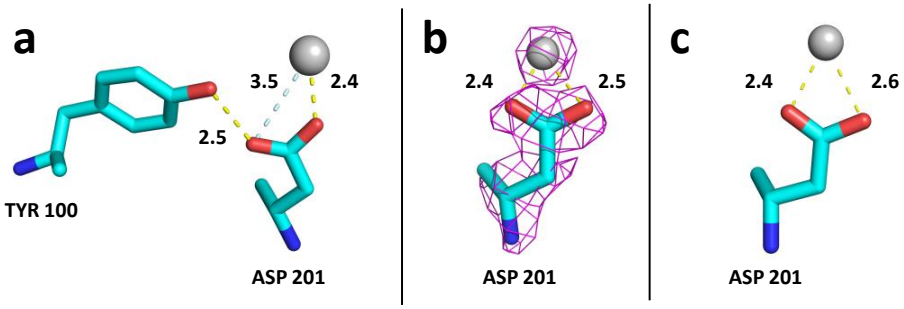
	- Na	+ Na	Δ	%
Na	-	0.931	-	
Asp 201 OD2	-0.399	-0.534	-0.135	33.7%
Asn 604 OD1	-0.242	-0.527	-0.285	117.7%
Phe 601 O	-0.310	-0.429	-0.119	38.4%
H2O 1 O	-0.719	-0.882	-0.163	22.7%
H2O 2 O	-0.663	-0.748	-0.084	12.7%

	- K	+ K	Δ	%
K	-	0.627	-	
Asp 201 OD2	-0.546	-0.599	-0.053	9.7%
Asn 604 OD1	-0.402	-0.488	-0.086	21.4%
Phe 601 O	-0.444	-0.592	-0.148	33.3%
H2O 1 O	-0.306	-0.37	-0.064	20.9%
H2O 2 O	-0.308	-0.359	-0.051	16.6%
H2O 2 O	-0.326	-0.363	-0.037	11.3%

NBO Charges

	- Na	+ Na	Δ	%
Na	-	0.717	-	
Asp 201 OD2	-0.706	-0.761	-0.055	7.8%
Asn 604 OD1	-0.536	-0.615	-0.079	14.7%
Phe 601 O	-0.589	-0.675	-0.086	14.5%
H2O 1 O	-0.843	-0.896	-0.053	6.2%
H2O 2 O	-0.884	-0.894	-0.010	1.1%

	- K	+ K	Δ	%
K	-	0.599	-	
Asp 201 OD2	-0.736	-0.767	-0.031	4.2%
Asn 604 OD1	-0.576	-0.631	-0.055	9.5%
Phe 601 O	-0.626	-0.685	-0.059	9.4%
H2O 1 O	-0.878	-0.899	-0.021	2.4%
H2O 2 O	-0.865	-0.878	-0.013	1.5%
H2O 2 O	-0.872	-0.893	-0.021	2.4%



Supplementary Figure 1. Computational studies of Y100A substituted β-galactosidase showing the role Tyr 100 has orienting the Asp 201–M^+ interaction. Coordination distances are in Å.

A. The native protein coordination structure. Coordinates are from the Na^+ replete crystal structure 1DP0. Coordination distances are averaged over all 4 monomers of the tetramer.

B. Y100A substituted β-galactosidase modeled by molecular dynamics. A hydrated system based on the Na^+ replete β-galactosidase structure 1DP0 was constructed with Ala substituted for Tyr 100. The system consisted of a protein monomer, contained 94014 atoms and was approximately 84 × 84 × 129 Å in size. The simulations were performed with additive force-fields (CHARMM-27) and with parameters indicated in the main paper. The system was equilibrated for 0.25 ns and the production run was 0.5 ns. Asp 201 to Na^+ coordination distances are averaged over the production run. The mesh outlines the average positions of the Na^+ and Asp 201 side chain in the production run, contoured at 1 standard deviation.

C. Y100A substituted β-galactosidase modeled by QM/MM. A system based on the Na^+ replete β-galactosidase structure 1DP0 was constructed with Ala substituted for Tyr 100. For QM/MM minimizations, SCCDFT-TB methods were employed. Atom represented with this method were the M^+, the side chains of Tyr 100, Asp 201, Asn 604, and Try 568, the main chain atoms (side chains excluded) from Gln 600 C to Cys 602 C, and the waters within 4 Å of the M^+. Molecular mechanics atoms were represented with CHARMM-27 force fields. Protein atoms further than 10 Å from the QM region were fixed in place and water molecules further than 4 Å from the M^+ were deleted from the system. Further details were as described for the QM-MM models in the main article. Similar calculations with the wild-type β-galactosidase maintained the experimental structure.