Electronic Supplementary Material (ESI) for RSC Advances.

Switched reaction specificity in polyesterases towards amide bond hydrolysis by enzyme engineering

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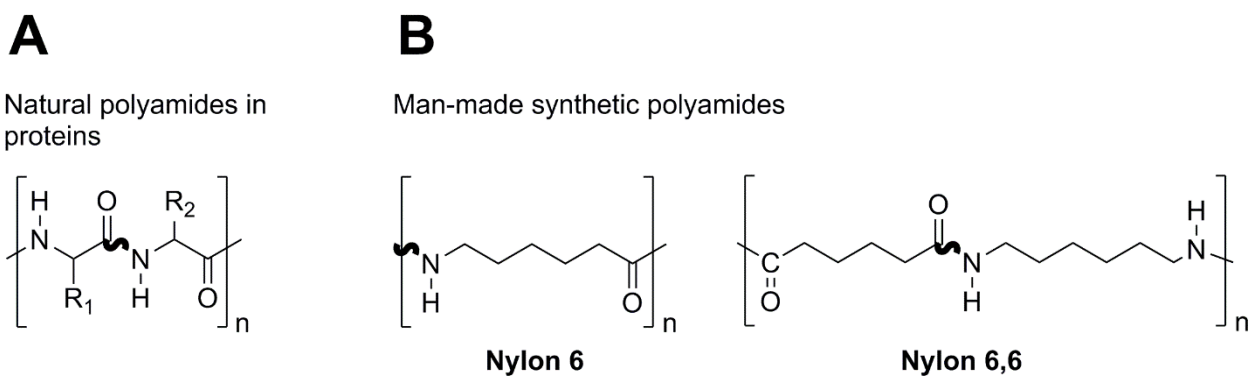


Fig. S1. Proteins (**A**) and synthetic polyamides represented by Nylon 6 (**B** *left*) and Nylon 6,6 (**B**, *right*) display significantly different backbone architectures. The reacting amide bond during hydrolysis is represented by the *Tilde*.

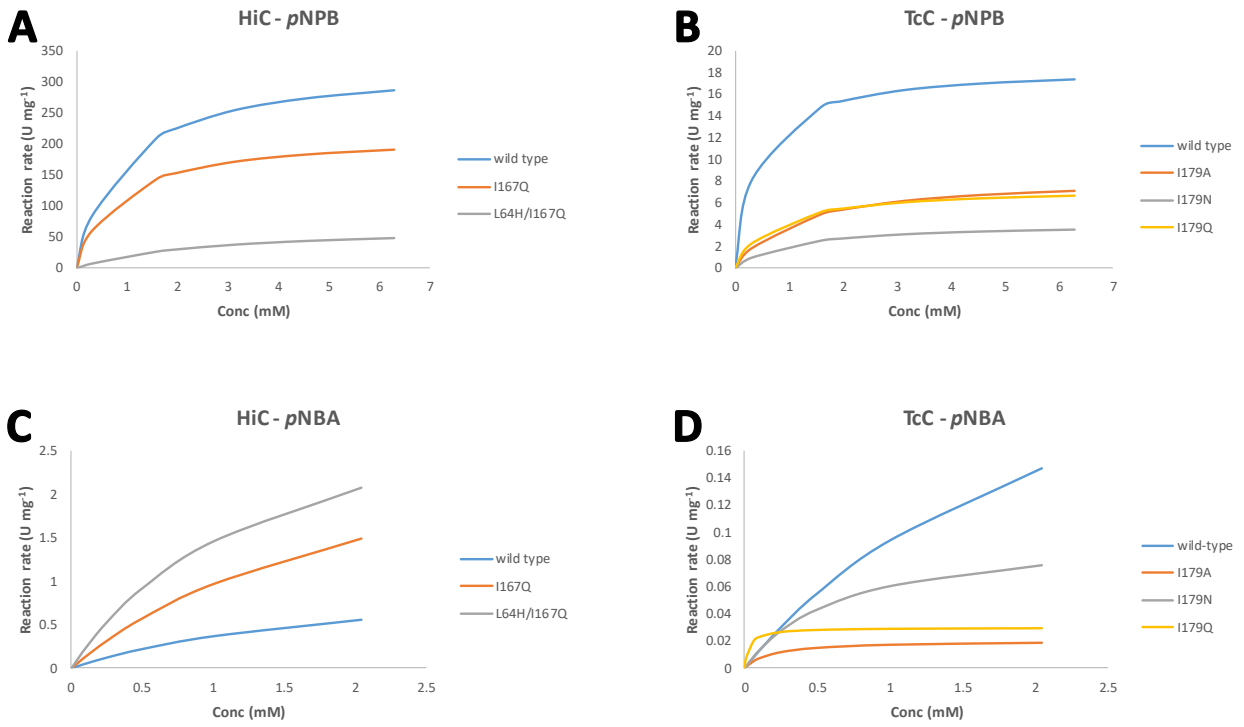


Fig. S2. Michaelis-Menten plot of HiC and TcC wild-type and variants. Analysis with the substrates *para*-nitrophenyl butyrate (*p*NPB) (**A** and **B**) and *para*-nitrobutyranilide (**C** and **D**), in a concentration range 0.3 - 6.3 mM and 0.01 - 2.04 mM, respectively. (**A** and **C**) HiC wild-type and variants: HiC wild type (*blue*), HiC I167Q (*orange*), and L64H/I167Q (*grey*). (**B** and **D**) TcC wild-type and variants: TcC wild type (*blue*), TcC I179A (*orange*), TcC I179N (*grey*), TcC I179Q (*yellow*). Standard deviation was lower than 5%.

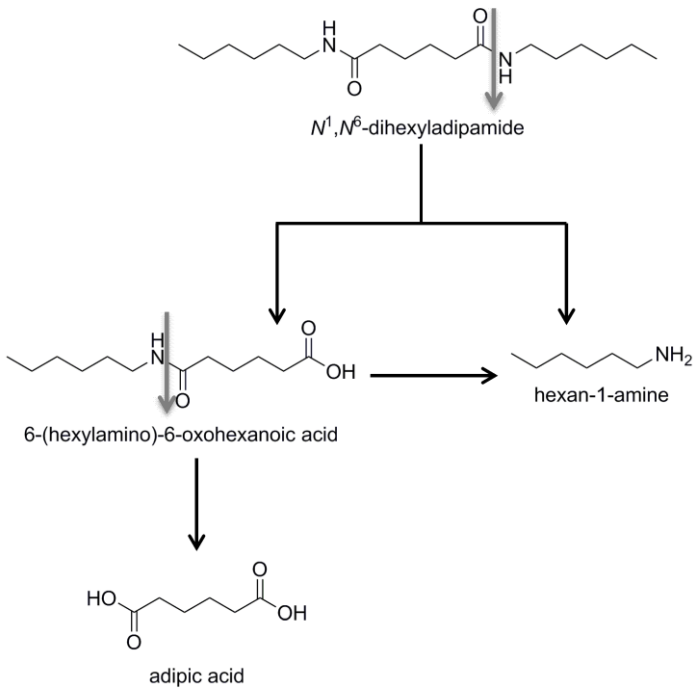


Fig. S3. Hydrolysis pathway of 3PA 6,6. The degradation of the substrate N^1-N^6-dihexyladipamide (3PA 6,6) was revealed by means of GC-FID.

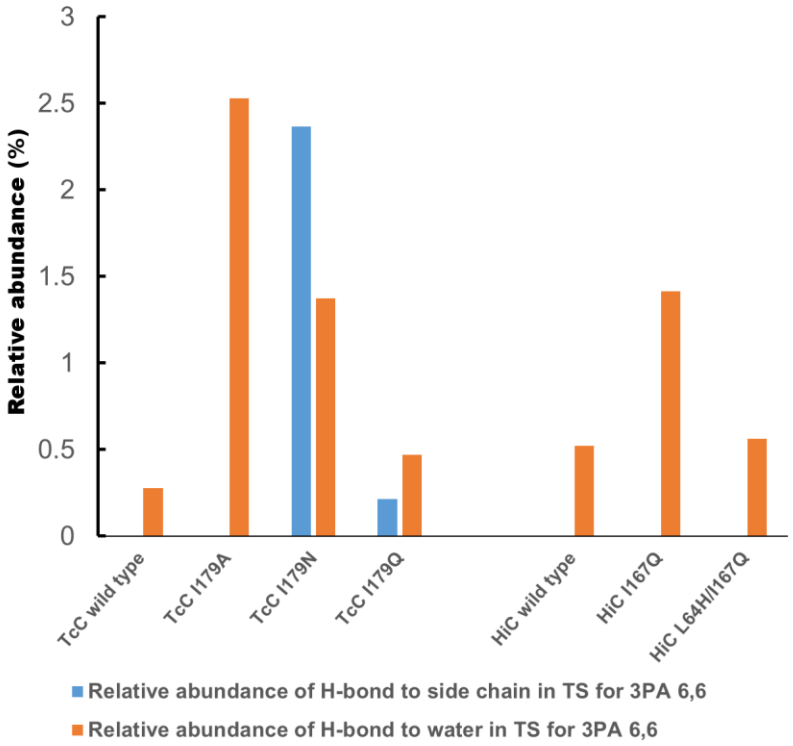


Fig. S4. The preferred hydrogen bond acceptor in TS is a water molecule. The data presented corresponds to weak hydrogen bonds and is based on analysis of 100 ns MD simulations that were run in duplicate. For each trajectory, 38000 and 40000 snapshots were analyzed for TcC and HiC respectively.

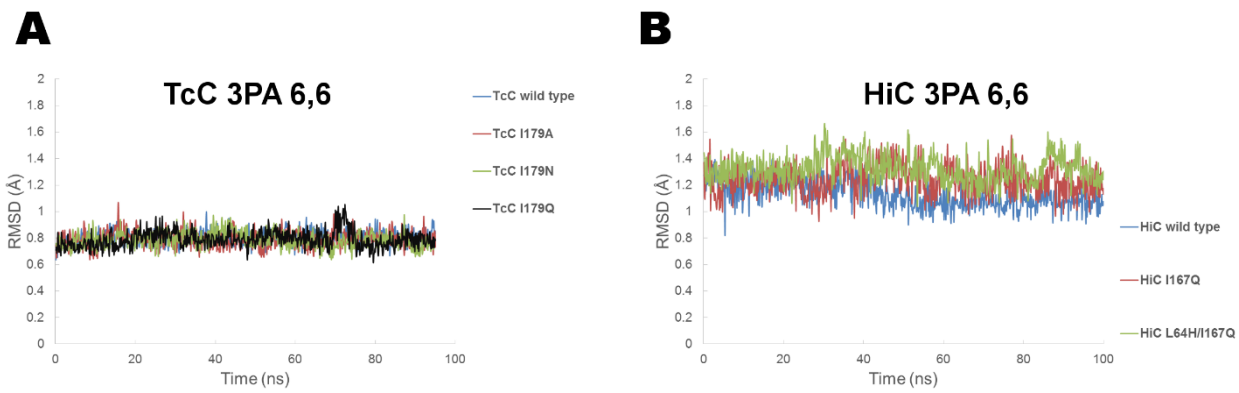


Fig. S5. Production phase used for analysis of MD simulations corresponded to 95 ns for TcC wild type and variants (**A**), and 100 ns for HiC (**B**).

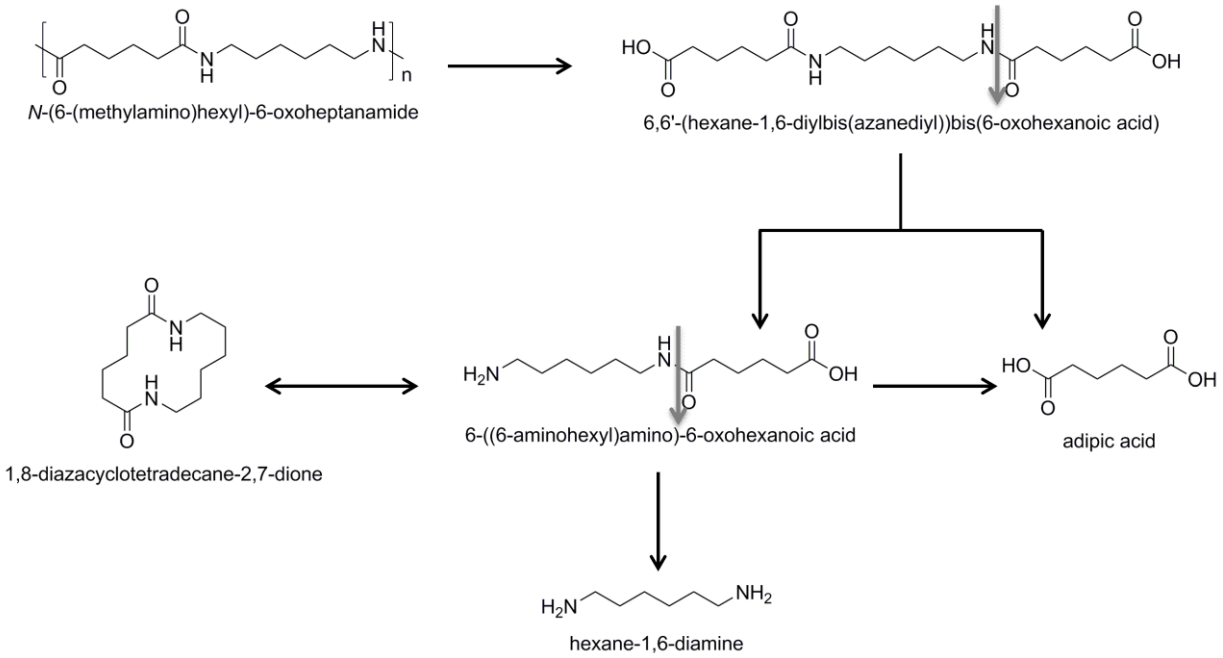
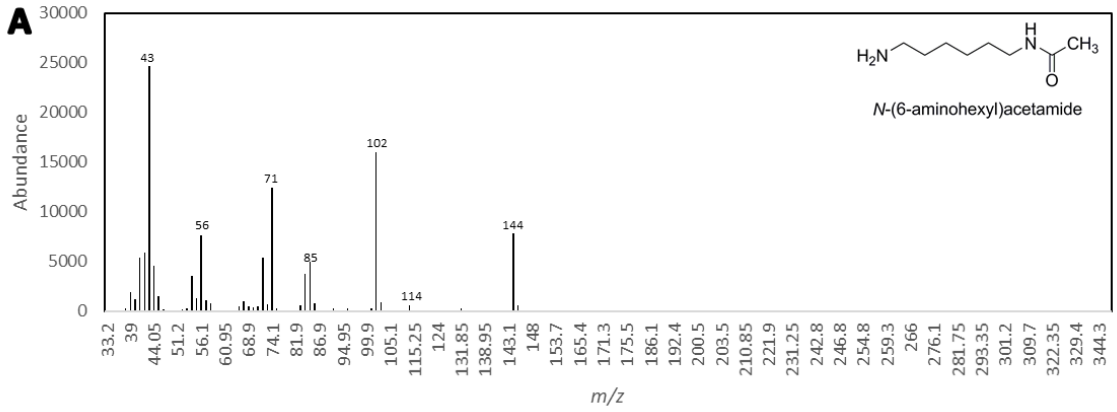
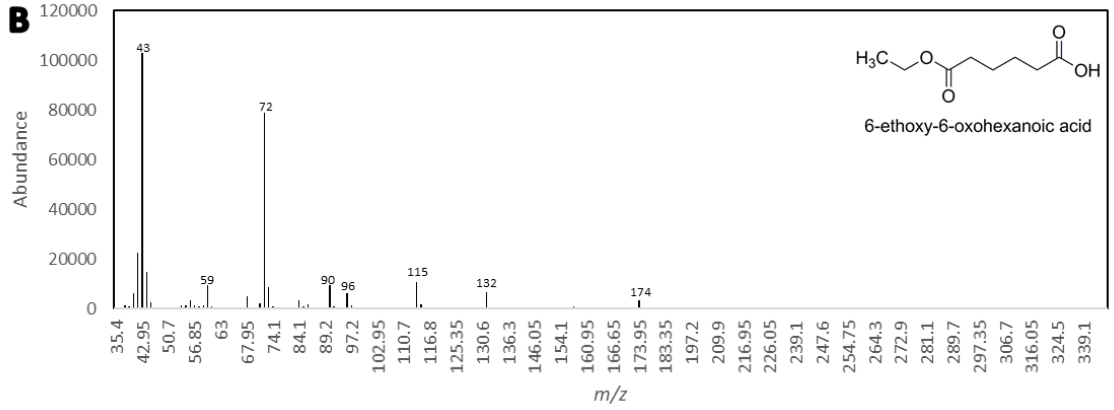
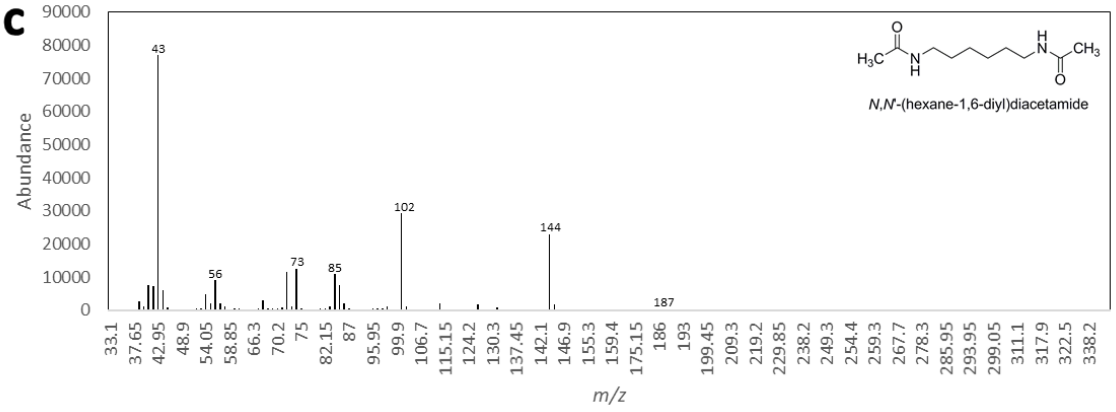


Fig. S6. Possible Nylon 6,6 hydrolysis pathway. The release products present at the end of each path were used as standard to identify released products by means of GC-MS (Fig. S9). Hexane-1,6-diamine shown in the spectra A and C, adipic acid shown in the spectra B and 1,8-diazacyclotetradecane-2,7-dione shown in the spectra D (fig. S7). The grey arrow shows the possible target of the enzyme.


A
Abundance
m/z
N-(6-aminohexyl)acetamide


B
Abundance
m/z
6-ethoxy-6-oxohexanoic acid


C
Abundance
m/z
N,N'-(hexane-1,6-diyl)diacetamide

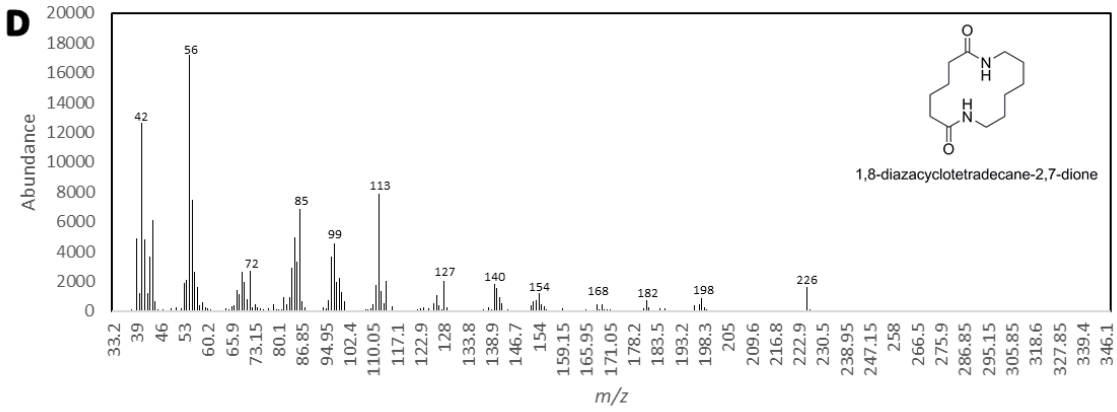

D
Abundance
m/z
1,8-diazacyclotetradecane-2,7-dione

Fig S7. Compounds identified by comparison of the fragment spectrum against those reported in the library NIST. (**A**) *N*-(6aminohexyl)acetamide (MW: 158 g mol^{-1}) with a retention time of 13.15 min; (**B**) 6-ethoxy-6-oxohexanoic acid (MW: 174 g mol^{-1}) with a retention time of 15.5 min; (**C**) *N,N'*-(hexane-1,5-diyl)diacetamide (MW: 200 g mol^{-1}) with a retention time of 15.9 min; (**D**) 1,8-diazacyclotetradecane-2,7-dione (MW: 226 g mol^{-1}) with a retention time of 28.5 min.

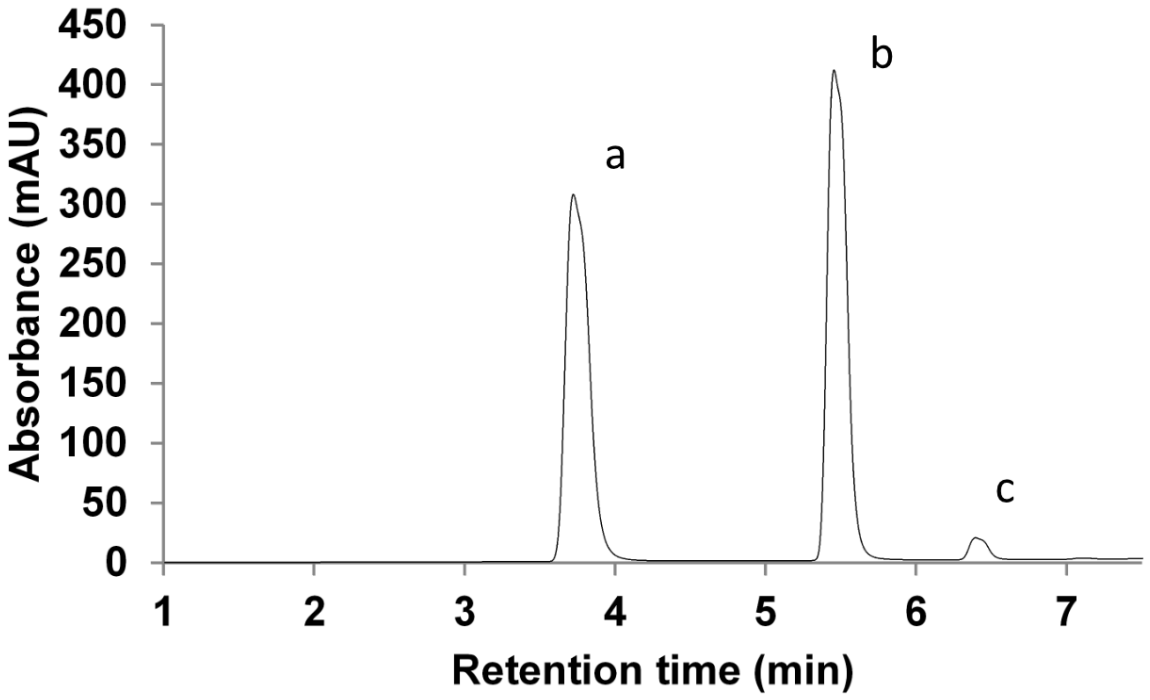


Fig. S8. HPLC chromatogram of release products from PET hydrolysis. The chromatogram was acquired at 241 nm. (**a**) Terephthalic acid (Ta) at 3.75 min; (**b**) mono-(2-hydroxyethyl) terephthalate (MHET) at 5.45 min; (**c**) bis(2-hydroxyethyl) terephthalate (BHET) at 6.4 min.

Table S1. Water-restructuring mutations. Primers used for the two-stage site-directed mutagenesis are shown with mismatched nucleotides underlined.

Primer name	**Nucleotide sequences**
TcC Ile179Asn_Fwd	5'-CGC TGA TCA TCG GGG CCG ACC TCG ACA CAA A<u>A</u>CG CGC CGG TCG CCA CGC ACG CGA AAC CG-3'
TcC Ile179Asn_Rev	5'-CGG TTT CGC GTG CGT GGC GAC CGG CGC G<u>T</u>T TGT GTC GAG GTC GGC CCC GAT GAT CAG CG-3'
TcC Ile179Ala_Fwd	5'-CGC TGA TCA TCG GGG CCG ACC TCG ACA CA<u>G C</u>CG CGC CGG TCG CCA CGC ACG CGA AAC CG-3'
TcC Ile179Ala_Rev	5'-CGG TTT CGC GTG CGT GGC GAC CGG CGC G<u>GC</u> TGT GTC GAG GTC GGC CCC GAT GAT CAG CG-3'
TcC Ile179Gln_Fwd	5'-CGC TGA TCA TCG GGG CCG ACC TCG ACA CA<u>C AG</u>G CGC CGG TCG CCA CGC ACG CGA AAC CG-3'
TcC Ile179Gln_Fwd	5'-CGG TTT CGC GTG CGT GGC GAC CGG CGC <u>CTG</u> TGT GTC GAG GTC GGC CCC GAT GAT CAG CG-3'

Table S2. Average distance and angle between the reacting NH-group of the 3PA 6,6 substrate and the engineered side chain.

Variant	NH---O=$C_{acceptor}$ distance (Å)[a]	NH---O=$C_{acceptor}$ angle (°)[a]
TcC wild type[b]	-	-
TcC I179A[b]	-	-
TcC I179N[c]	4.53	94.1
TcC I179Q[d]	5.15	107.2
HiC wild type[b]	-	-
HiC I167Q[e]	5.75	79.8
HiC L64H/I167Q[f]	7.42	63.3

[a] Based on 100 ns MD-simulations that were run in duplicate. For each trajectory of wild type and variants, 38000 snapshots were analyzed for TcC and 40000 snapshots for HiC. The acceptor refers to the introduced side chain in each case.

[b] No H-bond possible.

[c] Relative abundance of weak hydrogen bond 2.37%. Relative abundance of strong hydrogen bond 1.13%.

[d] Relative abundance of weak hydrogen bond 0.21%. Relative abundance of strong hydrogen bond 0.028%.

[e] Relative abundance of weak hydrogen bond 0%. Relative abundance of strong hydrogen bond 0%.

[f] Relative abundance of weak hydrogen bond 0%. Relative abundance of strong hydrogen bond 0%.

Table S3. Average distance and angle between the reacting NH-group of the 3PA 6,6 substrate and water networks.

Variant	NH---O_{wat} distance (Å)[a]	NH---O_{wat} angle (°)[a]
TcC wild type[b],[c]	5.06	67.9
TcC I179A[d],[e]	3.96	90.2
TcC I179N[f],[g]	3.88	90.9
TcC I179Q[h],[i]	4.62	84.8
HiC wild type[j],[k]	4.01	118.4
HiC I167Q[l],[m]	3.27	108.3
HiC L64H/I167Q[n],[o]	3.96	120.8

[a] Based on 100 ns MD-simulations that were run in duplicate. For each trajectory of wild type and variants, 38000 snapshots were analyzed for TcC and 40000 snapshots for HiC

[b] 24.8% relative probability to find at least one water molecule within 6 Å of the reacting NH-group of the substrate. [c] Relative abundance of weak hydrogen bond 0.28%. Relative abundance of strong hydrogen bond 0.0026%.

[d] 49.1% relative probability to find at least one water molecule within 6 Å of the reacting NH-group of the substrate. [e] Relative abundance of weak hydrogen bond 2.53%. Relative abundance of strong hydrogen bond 0.060%.

[f] 36.1% relative probability to find at least one water molecule within 6 Å of the reacting NH-group of the substrate. [g] Relative abundance of weak hydrogen bond 1.37%. Relative abundance of strong hydrogen bond 0.023%.

[h] 17.6% relative probability to find at least one water molecule within 6 Å of the reacting NH-group of the substrate. [i] Relative abundance of weak hydrogen bond 0.47%. Relative abundance of strong hydrogen bond 0.0066%.

[j 8.3% relative probability to find at least one water molecule within 6 Å of the reacting NH-group of the substrate. [k] Relative abundance of weak hydrogen bond 0.52%. Relative abundance of strong hydrogen bond 0.0023%.

[l] 25.1% relative probability to find at least one water molecule within 6 Å of the reacting NH-group of the substrate. [m] Relative abundance of weak hydrogen bond 1.42%. Relative abundance of strong hydrogen bond 0.0087%.

[n] 4.5% relative probability to find at least one water molecule within 6 Å of the reacting NH-group of the substrate. [o] Relative abundance of weak hydrogen bond 0.5637%. Relative abundance of strong hydrogen bond 0.010%.

Table S4. Average number of water molecules in the active site and average number of hydrogen bonds per water molecule. The given values are the averages from two independently run 100 ns MD-simulations using 3PA 6,6. For each trajectory of wild type and variants, 38000 snapshots were analyzed for TcC and 40000 snapshots for HiC.

Variant	Average number of waters in active site[a]	Average number of hydrogen bonds per water
TcC wild type	6.1	2.68
TcC I179A	10.1	2.77
TcC I179N	7.8	2.86
TcC I179Q	4.6	2.59
HiC wild type	4.6	2.81
HiC I167Q	8.0	2.77
HiC L64H/I167Q	3.5	2.38

[a] The number of waters within 8 Å of the scissile NH-group of the substrate.