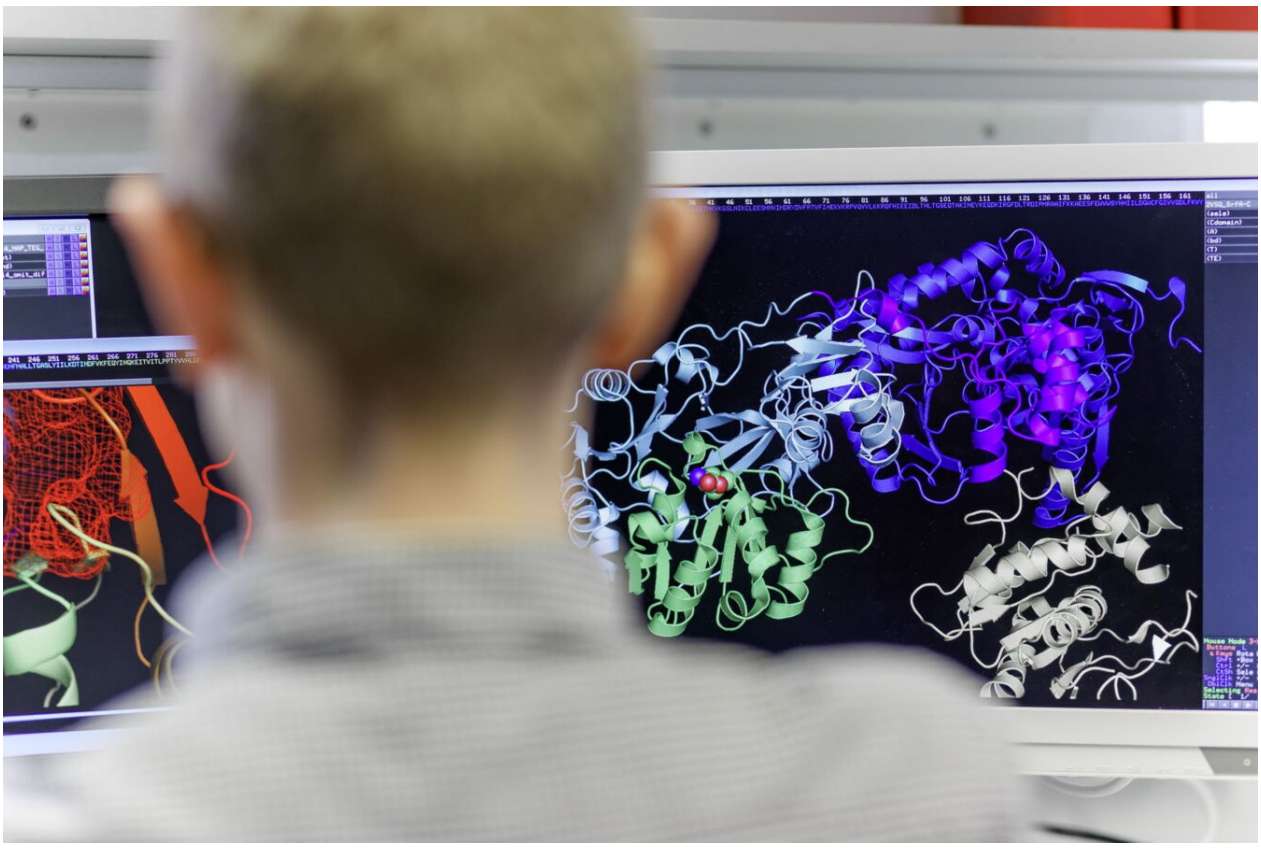
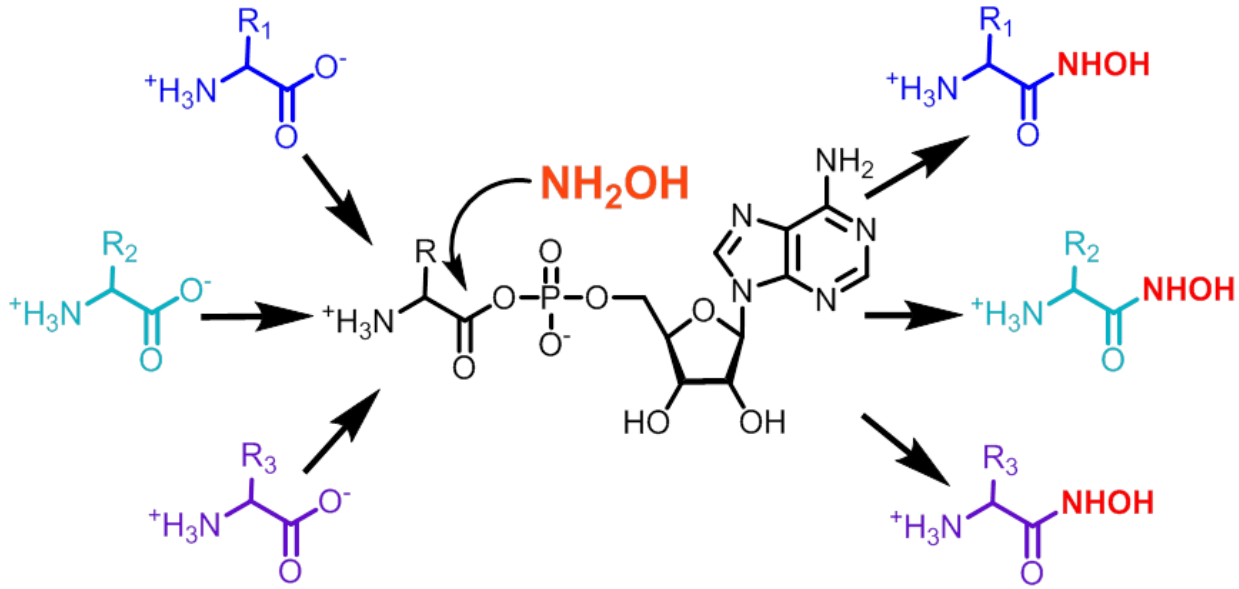
Enzymology

On the molecular level, nonribosomal peptide synthetases (NRPSs) are chains of enzymatic domains acting concertedly to assemble a peptide from amino acid building blocks. When enzyme engineers swap domains or modify preferences for building blocks, protein structures may be disrupted or individual steps slow down and peptide formation stalls. We analyse engineered NRPSs in order to identify kinetic bottlenecks and learn from failure. For this purpose, we employ spectrophotometric assays and UPLC-MS to identify reaction products, side products and intermediates bound to the enzyme. We have developed the HAMA assay to record specificity profiles of NRPS for dozens of substrates in parallel. Kinetic profiles are analysed in light of structural models. In the next step, kinetic bottlenecks can perhaps be removed by site-directed mutagenesis or directed evolution to generate more efficient designer NRPSs.

- 


R1
+H3N
O-
O
R2
+H3N
O-
O
R3
+H3N
O-
O
NH2OH
R
+H3N
O
O
P
O
O-
O
NH2
N
N
N
N
O
HO
OH
R1
+H3N
NHOH
O
R2
+H3N
NHOH
O
R3
+H3N
NHOH
O