Affinity selection discovery of *N*-methylated cyclic peptide inhibitors of prokaryotic glycolytic mutases

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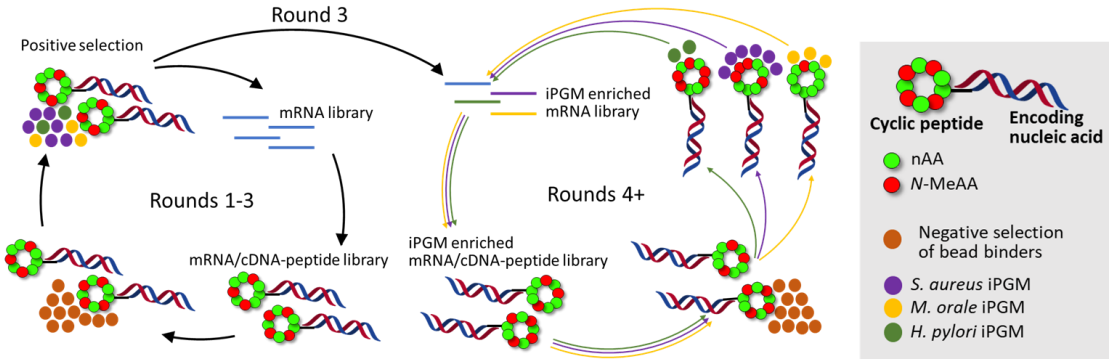
N-methylated amino acids (*N*-MeAA) are privileged residues in natural bioactive peptides critical to bioactivity and metabolic stability[1]. However, *de novo* discovery of these peptides through utilizing affinity selection methodologies is limited by low EF-Tu affinity of the *N*-methyl-aminoacyl-tRNA, causing poor ribosomal incorporation of *N*-methylated amino acids into the nascent peptide chain[2]. By modifying the tRNA's T-stem region to compensate and tune the EF-Tu affinity[3], we conducted a mRNA display-based screen using a macrocyclic peptide (MCP) library that contains six different *N*-MeAAs. Utilizing a "pool-and-split" enrichment strategy (see figure) we identified *N*-methylated MCPs against three orthologues of prokaryotic, metal ion-dependent phosphoglycerate mutases[4]. The identified MCPs reached upwards to 57% *N*-methylation in the random region with up to three consecutively incorporated *N*-MeAAs, rivalling natural products. Potent nanomolar inhibitors strongly mediated by *N*-methylation and ranging in ortholog-selectivity were identified. Co-crystal structures reveal both an active site metal ion-coordinating cysteine lariat-shaped MCP, architecturally similar to ipglycermide Ce-2[5-6], however, functionally dependent on two trans *N*-Me backbone amides, as well as a metal ion-independent inhibitor chemotype that acts as a 3-phosphoglycerate mimetic.



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