

**8.13 P****Synthetic Peptides target ATP translocase of ‘*Candidatus Liberibacter asiaticus*’ to block ATP uptake**

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As an obligate intracellular pathogen, ‘*Candidatus Liberibacter asiaticus*’ (Las) may act as an “energy parasite” by importing ATP from its host’s cells. We previously demonstrated that the Las translocase NttA (gb|ACX71867.1) is functional in *Escherichia coli* and enables the direct import of ATP/ADP into the cell. Similar to other translocases, NttA was predicted to contain 12 transmembrane domains with 6 loops residing on the outer surface of the membrane. Using this structure, a 25-mer peptide was selected for synthesis based on the transmembrane (TM) hidden Markov model (HMM) and used as the target for the randomized 7-mer Ph.D.<sup>TM</sup> phage display library (New England Biolabs, USA) in an attempt to block the ability of NttA import ATP. Of the selected clones, 11 shared the HWGMWSY sequence, five shared the sequence SILPYPY, and four had unique 7-mer sequences. An ELISA was performed against the 25-mer using members of the two most highly represented sequences and all of the unique sequences. Of these phage sequences, six appeared to have binding capacities. Radiolabeled ATP uptake assays were performed on *E. coli* expressing NttA using 10 small synthetic peptides based on the phage sequences, and the results indicated that peptides from the HWGMWSY grouping showed the most potential for blocking ATP uptake. Two peptides, HS-6 covering the first six aa (+1 charge) and GY-5 (neutral) covering the last five aa, were selected. At a concentration of 0.23 mM, both HS-6 and Gy-5 peptides decreased the amount of [ $\alpha$ -<sup>32</sup>P] ATP in the cells by 29.9 and 27.9%, respectively, while at a peptide concentration of 2.3 mM the amount of intercellular [ $\alpha$ -<sup>32</sup>P] ATP declined by 57.6 and 70.6%, respectively. Since NttA shares limited amino acid identity with other known proteins, we expect to have Las-specific inhibition when these peptides are expressed in citrus plants.