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Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy, October 2012

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ABSTRACT

Dipeptide Binding Protein A (DppA) is a member of a family of ABC proteins and is involved in the transportation of potentially beneficial dipeptides as nutrient source through the periplasmic space and into cell. DppA was successfully cloned into expression vectors and over expressed in *Escherichia coli*, extracted, purified, and characterized. DppA was subjected to biophysical characterization usingmass spectrometry. Mass spectrometry (MS) analysis and Analytical ultra centrifugation was used to evaluate the recombinant DppA's molecular weight. Optimized Isothermal Titration Calorimetry (ITC) and MS analysis were carried out to assess the biophysical properties of DppA-dipeptide interaction. DppA have shown they bind their ligands with different degrees of specificity and affinity. This study has demonstrated that DppA binds with a stoichiometry of dipeptide per protein molecule and has a preference for small and polar dipeptides. Protein mutation studies have shown that specific amino acid residues located in the binding site are vitally important for both the stability of DppA as well as its ability to bind its ligands. The removal of the conserved residues in DppA has a major impact on the binding specificity demonstrating the significance of the residues in controlling ligand selection and uptake. ITC analysis for both the wild type and mutant-proteins have been obtained, which have enabled structural changes associated with ligand binding to be monitored in detail. This study also revealed thatsmall residues did not bind very well to the protein which appears to be cause by the shape, size and charge properties of the binding site that act as selectors allowing the interaction of dipeptides and binding site of DppA. The findings presented within this thesis highlight the physiological importance of

the DppA-dipeptide interaction, and reflect the differences in affinity and specificity of the binding due to types of amino acid residues present in the dipeptides.