BIOCHEMISTRY
including biophysical chemistry & molecular biology
Addition and Correction

Domain Interactions and Close Vicinity of *trans* Reentrant Loops in the Na^+-Citrate Transporter CitS of *Klebsiella pneumoniae* [(2010) *Biochemistry 49,* 4509. DOI: 10.1021/bi100336s]. Adam Dobrowolski, Fabrizia Fusetti, and Juke S. Lolkema*

Page 4509. F.F. is at the Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, Netherlands Proteomics Centre and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands.

Page 4511. *Mass Spectrometry Analysis*. Partially purified split CitS variants treated with sodium tetrathionate (NaTT) were separated by SDS−PAGE, using a 12% gel, and stained with Coomassie Brilliant Blue. Selected bands were cut from the gel. The pieces of gel were fragmented and destained in 50 mM ammonium bicarbonate with 50% acetonitrile, Reduction and alkylation of cysteine residues were achieved via incubation with 100 mM dithiothreitol, followed by iodoacetamide treatment. Acetonitrile-dehydrated pieces of the gel were reswollen via addition of 10 μL of a 10 ng/μL trypsin solution and incubated overnight at 37 °C. Tryptic peptides were extracted twice with 30 μL of 60% acetonitrile in 1% trifluoroacetic acid (TFA) in water and vacuum-dried.

Dried peptides were resuspended in 0.1% TFA and separated on a C18 capillary column (C18 PepMap 300, 75 μm × 150 mm, 3 μm particle size, LC-Packing, Amsterdam, The Netherlands) mounted on an UltiMate 3000 nanoflow liquid chromatography system (LC-Packing). Aqueous solutions of 0.05% TFA (A) and 80% acetonitrile with 0.05% TFA (B) were used for elution. A gradient from 4 to 40% B over 50 min was used at a flow rate of 300 nL/min. Column effluent was mixed in a 1:4 (v/v) ratio with a solution of 2.3 mg/mL α-cyano-4-hydroxycinnamic acid (LaserBio Laboratories, Sophia-Antipolis, France) in a 60% ACN/0.07% TFA mixture. Fractions of 12 s were spotted on a blank MALDI target with a Probot MALDI spotter system (Dionex). Mass spectrometric analysis was conducted with a MALDI-TOF/TOF 4800 Proteomics Analyzer (Applied Biosystems) in the range of m/z 600−4000, in positive ion mode. Peptides with signal-to-noise levels of > 50 were selected for MS/MS fragmentation. Matching of the MSMS spectra to the CitS sequences was performed with Mascot, version 2.1 (Matrix Science, London, U.K.).

Page 4515. **Acknowledgment.** We thank Wim Huibers for help with preparing the samples for mass spectrometry.

DOI: 10.1021/bi101746j
Published on Web 11/08/2010