

## **MOLECULAR ELASTICITY OF SINGLE SPECTRIN REPEATS STUDIED BY FORCED UNFOLDING USING AFM**

**Stephan M. Altmann**<sup>1</sup>, Gruenberg Raik<sup>2</sup>, Pierre-Francois Lenne<sup>3</sup>, Jari Ylaenne<sup>4</sup>, Michael Nilges<sup>2</sup>, Heinrich J.K. Hoerber<sup>1</sup>, Arnt Rae<sup>5</sup>; <sup>1</sup>EMBL, Meyerhofstr.1, Heidelberg, 69117 Germany, <sup>2</sup>Institute Pasteur, France, <sup>3</sup>Domaine Universitaire Saint Jerome, France, <sup>4</sup>University of Oulu, Finland, <sup>5</sup>University of Bergen, Norway

Forced unfolding experiments of single modular proteins have been a big step forward to understand how proteins consisting of repeated domains behave under mechanical stress. It has become widely accepted that single domains of such multi-domain proteins will unfold in an all-or-none fashion when stretched e.g. between a surface and an AFM tip. We recently reported forced unfolding experiments of spectrin repeats with results different from all previously performed forced unfolding studies, containing two distinct populations of peak-to-peak distances. To examine these findings further we have now engineered two mutants of this protein. In one mutant we replaced two amino acids to stabilize repeats against external force. In the other we crosslinked the first and the second helix via a sulfur-bridge by introducing two properly positioned cysteine residues. In addition to experimental forced unfolding measurements with a special type of AFM we also studied the forced unfolding of the wild type and the mutant repeats using molecular dynamics simulation methods. The results of the mutation experiments and the computer simulations support that the wild type spectrin repeat unfolds in a manner that is decidedly different from Titin Ig like domains.