Abstract

Integrin mediated cell-matrix adhesion plays an important role in cellular attachment and migration, signal transduction and control of the cytoskeleton and is therefore of major interest for the treatment of cancer metastasis and developmental disorders. More than 100 proteins, collectively called the integrin adhesome, are localized in adhesion sites and have a cytosolic fraction. In order to understand how adhesion sites are assembled and maintained, it is essential to study the state of their components in the cytosolic pool.

Using FCCS, the pairwise associations of 13 key proteins were quantified, thereby revealing a high extent of interconnections between them in the cytosol. FRAP measurements show a rapid exchange of material between focal adhesions and the cytosol. By combining these methods, the cytosolic pool was characterized to consist of diverse building blocks that are confined in size.

In steady state focal adhesion exchange material symmetrically with the cytosol, releasing proteins in the same interaction and phophorylation state as they entered. This ensures a standardized cytosolic pool of building blocks and prevents a gradient of altered components around adhesion sites that could have lead to communication between focal adhesions. In contrast, rapidly disassembling focal adhesions release their material asymmetrically in large complexes, as was determined by perturbating the actomyosin contractility of cells.

To enable the detection of high order complexes, a novel correlation spectroscopy approach was developed. By separating fluorophores by both their fluorescence lifetime and spectrum, associations between three components per complex were resolved simultaneously.