

Congratulations to New ICFO PhD Graduate

Dr Sarah Keary graduated with a thesis entitled i¿½Spatiotempor l organisation of protein nanoclusters in adhesion complexesi¿½

February 28, 2022

We congratulate Dr Sarah Keary who defended her thesis today in ICFO's auditorium with online participations.
Dr Keary obtained her MSc degree in Optics and Photonics from the Karlsruhe Institute of Technology, Germany. She joined the Single Molecule Biophotonics research group led by ICREA Prof Dr Maria Garcia-Parajo to carry out her PhD studies. Dr Keary's thesis entitled ?Spatiotemporal organisation of protein nanoclusters in adhesion complexes' was supervised by ICREA Prof Dr Maria Garcia-Parajo and Dr Felix Campelo.

ABSTRACT:

The main goal of this thesis was to contribute to the understanding of the nanoscale lateral organisation of key proteins in adhesion complexes. For this, we exploited single molecule

localisation-based super-resolution microscopy STORM to visualise the lateral organisation of five key proteins of the adhesion complex: the integrins, ?5?1 and ?v?3, and three of their adaptor proteins: paxillin, talin, and vinculin.
We first established that these proteins form nanoclusters of around 50nm size that are preserved across all five proteins. Interestingly, these nanoclusters have similar size and number of localisations regardless of their localisation on the membrane, i.e., in the different adhesion structures studied, namely, FA and fAs as well as outside, and were maintained for different cell seeding times, from 90 min to 24 h. These results suggest that nanoclustering constitutes a general mechanism of adhesion protein organisation, creating nanohubs of functional activity. When studying how protein organisation in nanoclusters changes as a function of adhesion time, we revealed a two- and a four-fold increase in the density of ?5?1 and ?v?3 clusters, respectively, for cells that spread for 24 h as compared to those that spread for 90 min. Further analysis suggests that the increase in density of integrin nanoclusters is due to selective targeting of new integrin nanoclusters to the basal membrane.
Following on from this, we then focus on mapping the distribution of these nanoclusters, first by measuring the nearest neighbour distance; (NND) between clusters of the same protein, and second by considering the shortest distance between clusters of different proteins. We found a clear physical segregation of nanoclusters of the same protein around ~55 nm, which is established at early time points after cell seeding for ?5?1 and the adaptors and maintained after 24 h. Interestingly, ?v?3 nanoclusters exhibited a more random distribution at earlier seeding times and progressively reached similar lateral segregation at 24 h. Concomitant with this lateral segregation, we observed an enriched of all proteins at distances between 100-200 nm. Our observations are in line with the existence of a critical distance spacing between integrins needed for support adhesion and stabilisation of focal adhesions.
Furthermore, we found that the relative distribution of nanoclusters of different proteins is predominantly random, with the exception of ?5?1 and paxillin, which organise with a separation of 50 nm. Such an unexpected random distribution between integrins and their adaptors might reflect the dynamic and short-live active state of integrins.
Finally, we evaluated and described the mesoscale organisation of nanoclusters inside adhesions. Specifically, we computed the shortest distance between a nanocluster and the edge of the adhesion and studied how the distance to the edge depends on the NND between clusters of different proteins. Remarkably, we found a preference for ?5?1 nanoclusters to be at the edge of the adhesions and in close proximity to its adaptors in a peripheral belt region of the adhesions.
Altogether, the results of this thesis demonstrate a clear lateral and hierarchical organisation of integrins and their adaptors inside focal adhesions. Based on our results (together with extensive literature in the field), we propose that one population of ?5?1 nanoclusters and their adaptors preferentially localise close to the edge of adhesion complexes regulating the

process of adhesion. A second population of ?5?1 and most of the ?v?3 nanoclusters organise more randomly at the centre of the adhesions, with dynamic and brief engagement to their adaptors, likely playing a role in mechanotransduction. As a whole, we postulate that the lateral nano- and meso-scale organisation of adhesion proteins is strictly related to and important for the functions of adhesion, mechanosensing and mechanotransduction.

Thesis Committee

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