|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***C1* (%)** | ***C2* (%)** | ***k1off* (s-1)** | ***k2off*(s-1)** | ***k1on*(s-1)** | ***k2on* (s-1)** | ***k1* on/off** | ***k2* on/off** | ***T1off* (s)** | ***T2off* (s)** | ***T1on* (s)** | ***T2on* (s)** |
| **AUX1-YFP** | 7.0 | 92.7 | 0.033 | 0.0002 | 0.86 | 0.08 | 26 | 337 | 30 | 4421 | 1.2 | 13.1 |
| **AUX1-YFP LatB** | 7.2 | 92.6 | 0.028 | 0.0002 | 1.10 | 0.13 | 40 | 512 | 36 | 4047 | 0.9 | 7.9 |
| **AUX1-YFP Ory** | 8.3 | 91.5 | 0.026 | 0.0002 | 0.92 | 0.08 | 35 | 391 | 38 | 4739 | 1.1 | 12.1 |
| **PIN1-GFP** | 14.9 | 84.9 | 0.071 | 0.0010 | 5.17 | 0.44 | 73 | 416 | 14 | 953 | 0.2 | 2.3 |
| **PIN1-GFP LatB** | 15.2 | 84.1 | 0.047 | 0.0006 | 0.97 | 0.07 | 21 | 115 | 21 | 1717 | 1.0 | 14.9 |
| **PIN1-GFP Ory** | 17.0 | 82.5 | 0.056 | 0.0010 | 2.20 | 0.19 | 39 | 190 | 18 | 1009 | 0.5 | 5.3 |

**Supplementary Figure 1**: List of parameters calculated from the application of two phase exponential fit to normalized FRAP data. *C1* and *C2* are representations of two states of protein aggregations (%), *k1* and *k2* are rates of binding (*on*) and unbinding (*off*) and T1 and T2 are mean times of binding (*on*) unbinding (*off*).



**Supplementary Figure 2**: FRAP in two-day-old tobacco BY-2 cells expressing PIN1-GFP or AUX1-YFP in ROIs applied on the whole cells, comparison with FRAP performed in ROIs applied to the transversal PM. **a:** Fluorescence of cells before bleach. **b:** Kinetics of FRAP for PIN1-GFP (black circles) and AUX1-YFP (red triangles) in ROIs applied to whole cells (full symbols). For comparison FRAP in rectangular ROIs applied to the transversal PM (empty symbols) is shown. Error bars = SEM (n=10).

a



b

|  |  |  |
| --- | --- | --- |
|  | **V-to-H** | ***tau* (ms)** |
| **AUX1-YFP ± SD** | 0.6±0.04 | 10.31±1.37 |
| **AUX1-YFP LatB ± SD** | 0.57±0.03 | 7.85±1.08 |
| **AUX1-YFP Ory ± SD** | 0.55±0.03 | 7.81±1.69 |
| **AUX1-YFP Fil ± SD** | 0.51±0.04 | 6.65±0.89 |
| **PIN1-GFP ± SD** | 0.39±0.02 | 5.36±0.66 |
| **PIN1-GFP LatB ± SD** | 0.59±0.08 | 10.99±3.93 |
| **PIN1-GFP Ory ± SD** | 0.52±0.08 | 8.12±0.52 |
| **PIN1-GFP Fil ± SD** | 0.43±0.07 | 6.81±2.16 |

**Supplementary Figure 3:** **a:** Vertical (blue) and horizontal (red) cross-sections of the RICS correlation curves fitted with either free diffusion model or model considering binding. The quality of fit for free diffusion model was very low, therefore fitting for binding-governed motion was used. **b**: Values of parameter tau (τ) for AUX1-YFP and PIN-GFP calculated from the application of binding-governed motion model. Note that PIN1-GFP spends shorter time in trapped state then AUX1-YFP and that interference with actin (LatB) and microtubular (Ory) cytoskeleton increases this rather "bound" pool of PIN1-GFP, while AUX1-YFP seems to be rather unaffected or even slightly faster. In contrast, plasma membrane sterol disruption with filipin decreases the time of binding for AUX1-YFP, but not for PIN1-GFP.