**Figure 4:** **Connectivity analysis of assembloids.** **(a)** formed by a Midbrain Organoid (MO) and a Striatum Organoid (StrO), over a MEA chip with 64 electrodes. A red square inset shows the area represented in a scheme at the right. Rows of electrodes are exposed to neurite growth in microtunnels of 10 µm width. (**b)** Rasterplot of each single spike detected per electrode, five periods of synchronized activity can be seen in a total duration of 3 mins. (**c)** Spike Time Tiling Coefficient (STTC) is calculated by the probabibility of spiking in electrode 2 (e2, red spikes) within a temporal window (Δt=10ms, blue bars) around spikes in electrode 1 (e1). (**d)** Crosscorrelation of the time offset between spikes for electrode pairs in the upper left representation of the grid. Arrows in the histogram point to the significant peak of probability (Prob.; mean+5std) used to assess the direction and the resultant speed. (**e)** Signal propagation across the area recorded. Black arrows are the vectorial sum of all the connections found by STTC>0.8 with a given direction as in C). (**f)** From 12 assembloids, 11 (92%) formed a stable connection from MO to StrO (see Methods) within 40 days in vitro as shown by the cumulative probability of connection. (**g)** The average vertical directionality (y, directed in e) for the white reference coordinates) was calculated for each assembloid; early (DIV<40), intermediate (DIV40-80) and late (DIV>80) stages of maturation were used for statistics (\*\*=p<0.001, t-Student; post hoc Kruskal-Wallis all groups p=5.4\*10-6). (**h)** The absolute value of averaged lateral directionality (|x|, again as refered in e) ) also yielded significance between early stage compared to intermediate and late stages (\*\*\*=p<0.00001, t-Student; post hoc Kruskal-Wallis all groups p=2.3\*10-6).