Supplementary Information

Super-resolution imaging reveals eightfold symmetry of gp210 proteins around the nuclear pore complex and resolves the central channel with nanometer resolution

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Figure S1. Alexa647 labeled gp210 protein in the nuclear envelope of *Xenopus laevis* oocytes. (a) Conventional widefield fluorescence image, (b) dSTORM image. Scale bar 5 µm.
**Figure S2.** Nuclear envelope resolved by two different microscopy methods. (a) Electron micrograph of a negatively stained nuclear envelope of a *Xenopus laevis* oocyte. (b) dSTORM image of gp210 proteins immunostained with X222 antibodies and Alexa647 F(ab)$_2$ fragments. (c) WGA-Alexa647 labeled N-acetylglucosamine modified nucleoporins. Scale bars 500 nm.
Figure S3. dSTORM image of the nuclear envelope of a *Xenopus laevis* oocyte with a density of 9 pores µm$^{-2}$. (a, b) WGA-Alexa647 was used to label N-acetylglucosamine modified nucleoporins, e.g. Nup62, in the central channel of the NPCs. (b, c) Higher magnifications to highlight that the inner central channel can be efficiently resolved by dSTORM. Scale bars 1 µm (a), 250 nm (b), 50 nm (c).
Figure S4. dSTORM image of dual-stained NPCs. (a) Alexa647 immunostained nucleoporin and gp210 in the nuclear envelope of a Xenopus laevis oocyte and (b, c) high magnification views. Scale bars 1 µm (a), 200 nm (b), 100 nm (c).
**Figure S5.** Part of a dSTORM image of immunolabeled gp210 proteins. For the generation of an average super-resolution image potential pores were automatically found (green rectangles). First a template matching is performed to identify suitable pores. Pores in too dense areas are automatically discarded. Scale bar 200 nm.
Figure S6. Combined localizations from dSTORM images of immunolabeled gp210 proteins. 426 individual pores were aligned to a model of an eightfold ring. From the combined ~160,000 localizations the radius of the ring is estimated to 164 ± 7 nm and indicated in the figure. The crosses show an eightfold symmetry and are spaced by 45 degrees exactly. One pixel corresponds to 5.2 nm.
Figure S7. Angular average of all combined localizations from Fig. S5 of immunolabeled gp210 proteins. The fit to the distribution matches very well. The FWHM of the distribution is 35.8 nm. The peak position is to be corrected by \(-0.5s^2/R^2\) due to the angular average of normal distributed data off center. Here \(s\) is the standard deviation of the distribution and \(R\) the peak position. The average radius is 82 nm.
Figure S8. Two-color dSTORM images of NPCs using WGA-ATTO520 and Alexa647 labeled F(ab)_2 fragments binding to X222 antibodies directed against gp210. (a, b) Conventional widefield fluorescence images and (c, d) corresponding dSTORM images. (e) The vector field used for correction of chromatic aberrations by transforming c. (f) Aligned two-color dSTORM image. Scale bar 5 µm.
Figure S9. Image smoothing for display. (a) dSTORM image of immunolabeled gp210 proteins, (c) dSTORM image of WGA labeled N-acetylglucosamine modified nucleoporins. (b, d) Convolution of (a) and (c) with a Gaussian function with a standard deviation of 1 pixel which corresponds to 7 nm (gp210; a, b) and 5 nm (WGA; c, d). Scale bars 50 nm.