Supplementary information

Exploring the use of leucine zippers for the generation of a new class of inclusion bodies for pharma and biotechnological applications

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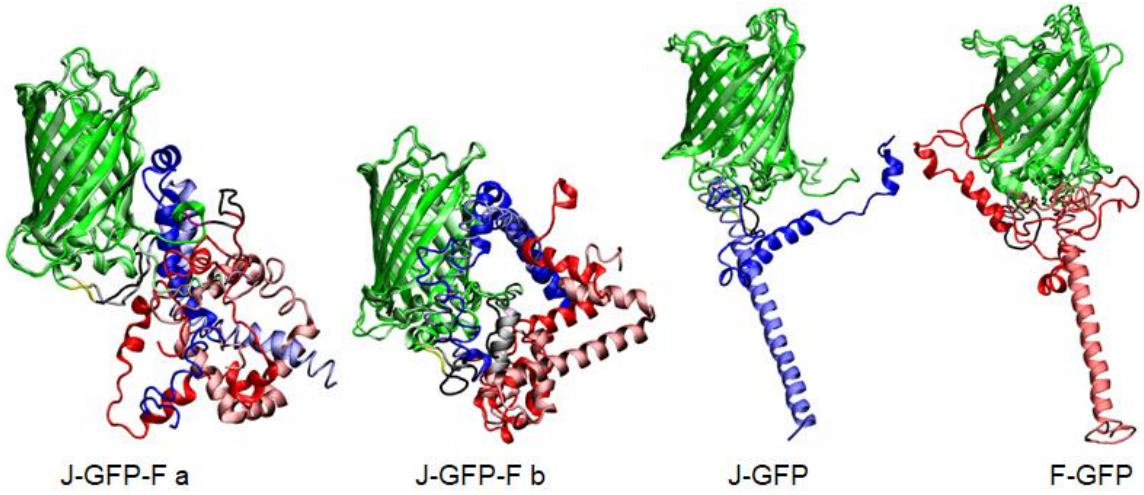
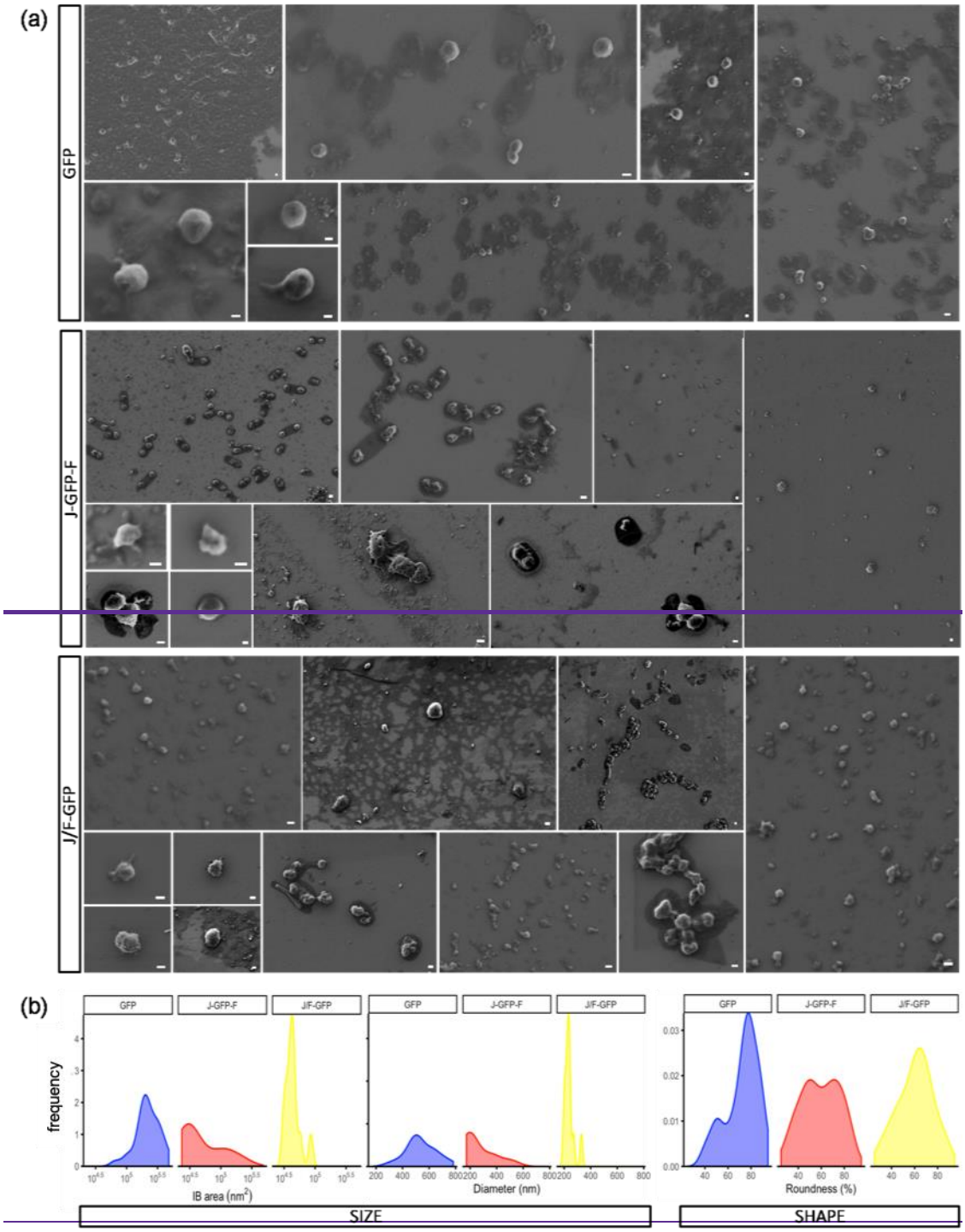
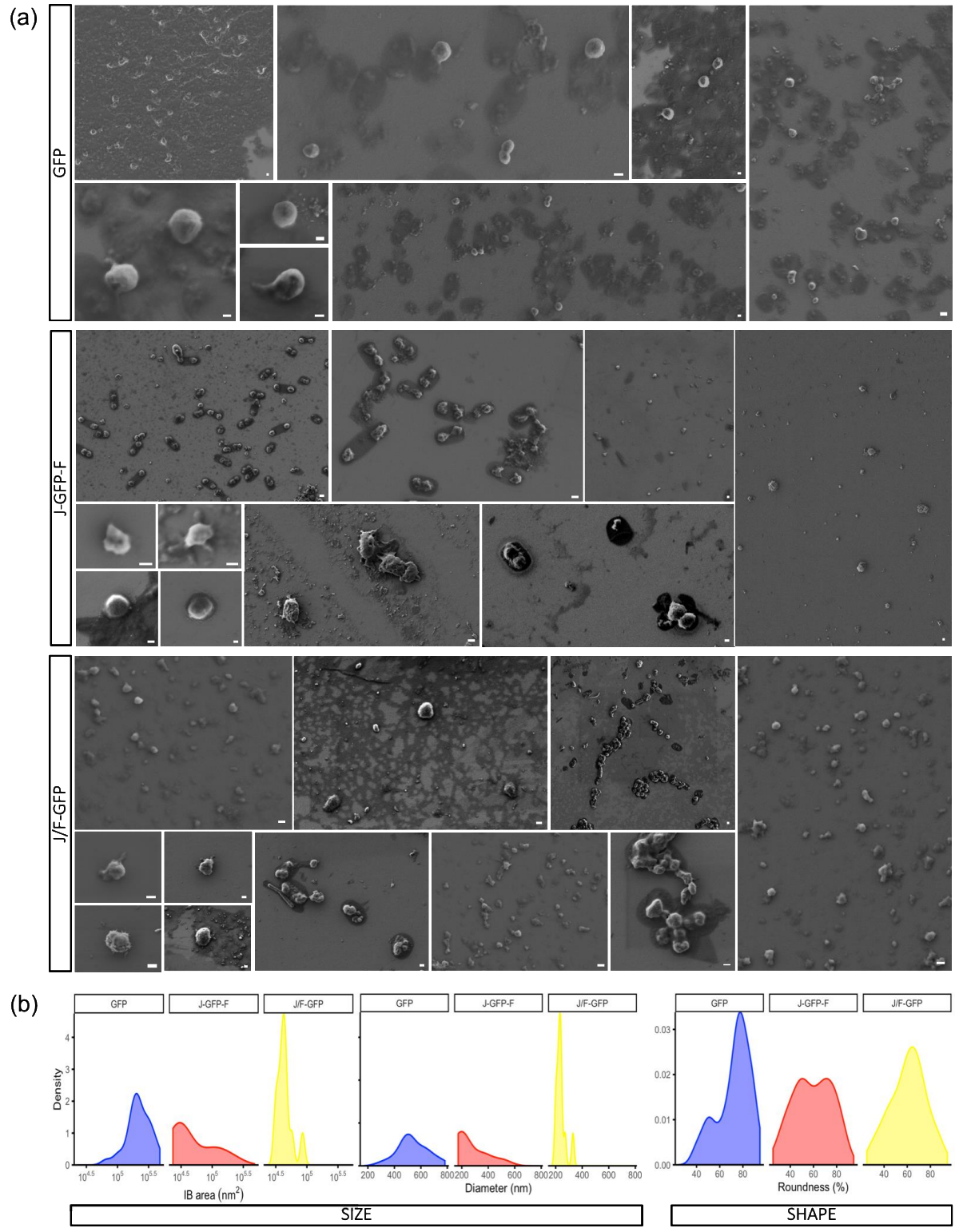
Figures

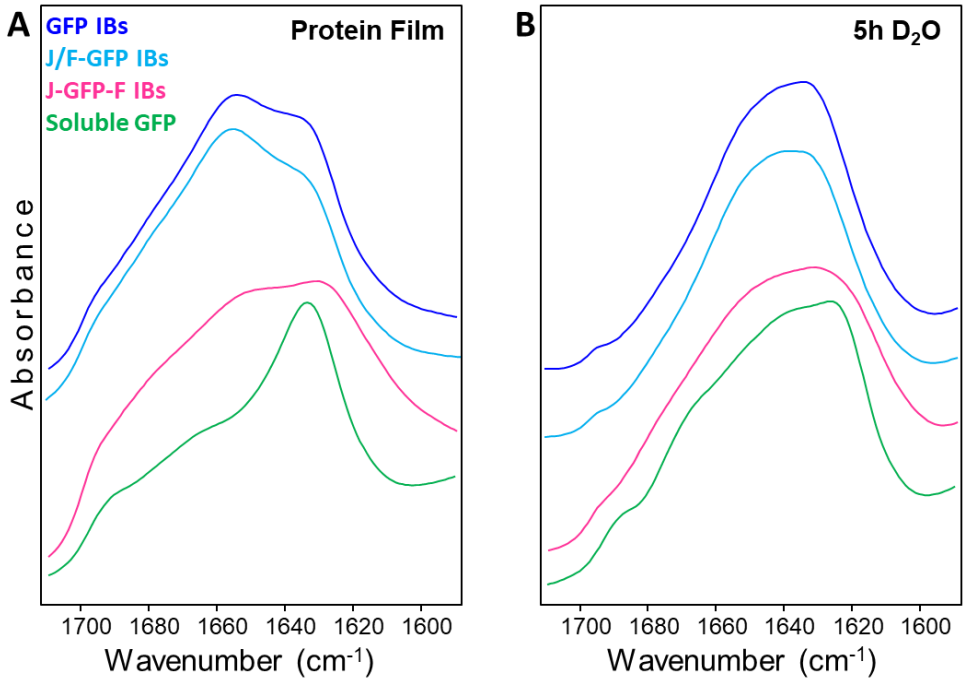


Supplementary Figure S1. Overlap between starting model (lighter shades) and final configuration (darker shades) of the J-GFP-F (two models: a and b) and J/F-GFP constructs (one model each), after 250 ns of molecular dynamics simulation. The generated models were minimized, placed in a cubic water box, minimized again, equilibrated and, for each construct, 250ns of molecular dynamics simulation were performed. Large rearragementsof the Jun/Fos domains were observed. Construct domains are color coded as follow: GFP (green), Jun (blue), Fos (red).


(a)
GFP
J-GFP-F
J/F-GFP
(b)
GFP
J-GFP-F
J/F-GFP
frequency
IB area (nm²)
Diameter (nm)
Roundness (%)
SIZE
SHAPE



Supplementary Figure S2. (a) Representative FESEM images of the isolated IBs for each construct: GFP IBs, J-GFP-F IBs and J/F-GFP IBs. Bars size represent 200 nm. (b) Frequency distribution of IBs ultrastructural morphometry quantification for each construct: size (area (nm^2) and diameter (nm)) and shape (roundness (%)).



Supplementary Figure S3. A) FTIR absorption spectra of the protein films. B) FTIR absorption spectra collected after re-hydration of the protein films with D_2O for 5 h. GFP and J/F-GFP IBs displayed similar absorption spectra both as film and after re-hydration, while J-GFP-F IBs showed distinct spectral features. As a control, the absorption spectra of the soluble GFP are also shown.

Tables

Supplementary Table 1. Statistics for the protein aggregation ratio (%) for each construct over time. (a) Aggregation ratio (%) differences between the three constructs and (b) aggregation ratio (%) differences for each construct over time. Different letters mean statistically significant difference (Post-hoc Tukey HSD (THSD) comparisons).

(a)

Protein	Aggregation ratio (%)	p-value
GFP	44.57 ± 7.71 [a]	0.0189
J-GFP-F	52.56 ± 7.36 [a, b]	
J/F-GFP	73.55 ± 3.59 [b]	

(b)

Protein	**GFP**			**J-GFP-F**			**J/F-GFP**			p-value
Time (h)	**1**	**3**	**5**	**1**	**3**	**5**	**1**	**3**	**5**	Time
Aggregation ratio (%)	29.18± 17.15 [a]	53.12± 18.85 [a]	51.40± 3.80 [a]	49.78± 26.98 [a]	41.43± 30.11 [a]	66.46± 2.73 [a]	69.71± 15.15 [a]	70.23± 10.89 [a]	70.23± 9.14 [a]	0.057