Elucidating the Native Architecture of the YidC

Kedrov, Alexej; Sustarsic, Marko; de Keyzer, Jeanine; Caumanns, Joost; Wu, Zht Cheng; Driessen, Arnold; Nogales, E.

Published in:
Journal of Molecular Biology

DOI:
10.1016/j.jmb.2013.07.042

Publication date:
2013

Supplementary information

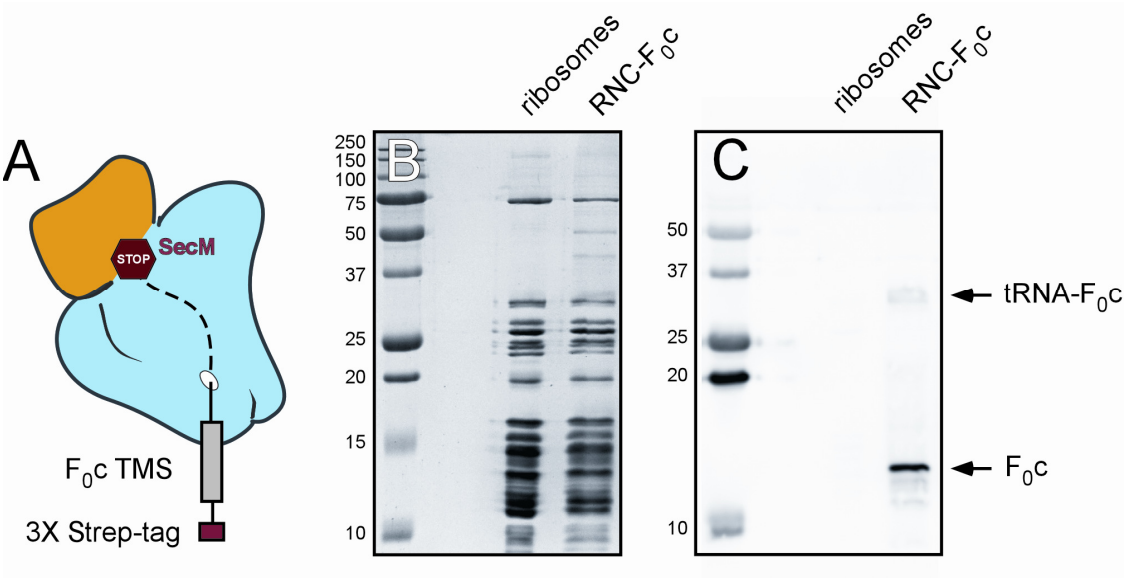

Figure S1. Purification of programmed and non-programmed ribosomes. (A) Organization of the programmed ribosome:nascent chain complex (RNC). Emerging polypeptide chain of the F_0c protein is fused to a regulatory SecM sequence at the C-terminal end, so the ribosome translation can be stalled in a defined state, with the first F_0c transmembrane segment (TMS) being fully exposed from the ribosome tunnel. The N-terminal end of the F_0c is conjugated to the triple Strep tag. (B) Isolated ribosomes and RNCs are visualized on SDS-PAGE stained with Coomassie. Multiple bands for non-translating ribosomes and RNC samples correspond to ribosomal proteins. Molecular masses (kDa) of protein markers are indicated on the left. (C) The presence of the stalled F_0c nascent chain in translating RNCs at 13 kDa is confirmed by Western blot using antibodies against the Strep-tag. The band at 35 kDa corresponds to the F_0c polypeptide complexed with tRNA. Molecular masses of biotinylated protein markers are indicated on the left.

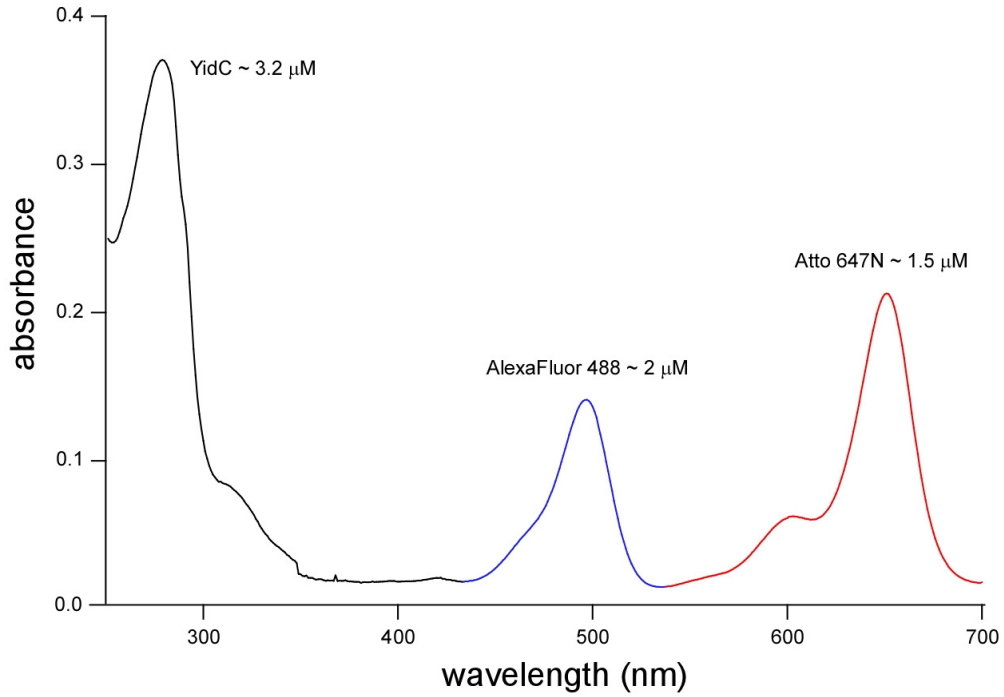

Figure S2. Stochastic labeling of YidC with spectrally different fluorophores. Detergent-solubilized YidC bearing a single cysteine was incubated in presence of 200 µM AlexaFluor 488-C_5-maleimide and Atto 647N-maleimide to achieve equal labeling of YidC protomers. Labeled YidC was purified as described and manifested overall labeling of 110-115 %, including limited unspecific labeling.

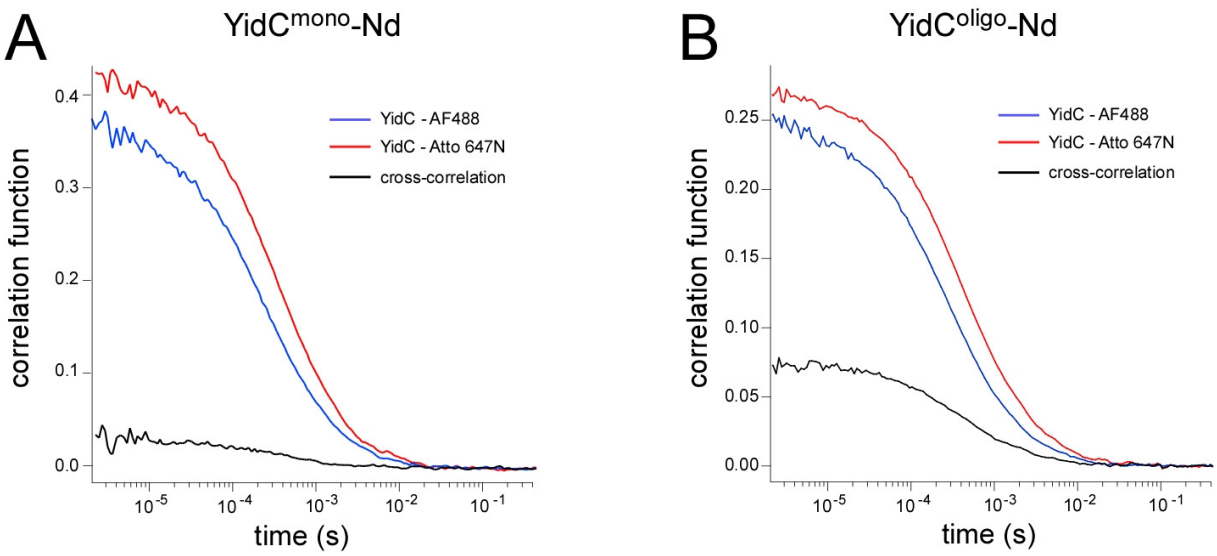

Figure S3. FCCS analysis of the number of YidC molecule incorporated into nanodiscs. Differently labeled YidC molecules were mixed at AlexaFluor 488 to Atto 647N ratio of 1:1 and reconstituted into nanodiscs at low (A) and high (B) densities to achieve single and multiple copies of YidC within a single disc, respectively. The fraction of nanodiscs containing multiple YidC molecules was determined from the ratio of the cross-correlation signal to the amplitude of the auto-correlation curves.