**Supplementary Material**

**High-Speed Atomic Force Microscopy Reveals Structural Dynamics of -Synuclein Monomers and Dimers**

Yuliang Zhang1,2, Mohtadin Hashemi1, Zhengjian Lv1,3, Benfeard Williams4, Konstantin Popov5, Nikolay V. Dokholyan5 and Yuri L. Lyubchenko1,\*.

1*Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE, 69198, USA.*

2*Biology and Biotechnology Division, Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA.*

3*Bruker Nano Surfaces Division, 112 Robin Hill Road, Goleta, Santa Barbara, CA 93117, USA.*

4*Curriculum in Bioinformatics and Computational Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.*

5*Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599;*

\**E-mail: ylyubchenko@unmc.edu*



**Figure S1.** Analysis of tail contour of -syn particles. A trace was recorded along the tail profile and fitted with parametric splines. Blue open circles indicate the original trace along the tail. Red solid line is the fitting curve, which is used for the further analysis.



**Figure S2.** Analysis of volume for -syn particles. The raw AFM images, saved in ASCII format, was imported into the Femtoscan software ([www.femtoscanonline.com](http://www.femtoscanonline.com)), the compact spherical features were contoured using the isoline function, and the volume of the molecule was calculated using the volume measurement feature of the software.



**Figure S3.** HS-AFM images of wild-type -syn monomers. Representative images showing the three types of compact monomers identified during experiments: globular (A), one-tailed (B), and two-tailed (C). The scale bar is 5 nm. (D) The distribution of length of tails obtained from both one- and two-tailed structures.

****

**Figure S4.** Aggregation propensity profile of -syn based on Zagg calculation at pH 7. The three different regions of a-syn are indicated by: Green, N-terminal; Blue, NAC region; Red, C-terminal region. The dashed line at is used to determine the aggregation-prone regions.