Nanodiscs (ND) are stable membrane bilayer nanoparticles of around 10-20 nm in diameter, stabilized by two molecules of amphiphilic artificial scalloped proteins (MSP) and homogenously distributed in solution. ND have bilayer characteristics comparable to usual liposomes and well known as a lipid membrane platform for structural and functional studies of transmembrane proteins but they can be also helpful for structural studies of membrane associated proteins.

Factor VIII (FVIII) is a multimeric blood plasma glycoprotein, which when activated FVIIIa (FVIII) acts as a co-factor to the Factor IXa (IXa). Activation of the Tenase complex (FVIIIa-Ag) onto a negatively charged activated plasma membrane amplifies thrombin generation more than 10^4 times, which is critical for normal blood coagulation. Detect or deficiency of FVIII causes Hemophilia A, a severe hereditary bleeding disorder, and intravenous administration of either human or porcine FVIII restores normal plasma function.

Despite the critical role of FVIII for coagulation process, the knowledge of its membrane-bound organization alone or within the Tenase complex is incomplete which hampers drug discovery for effective regulation of the complex activity and thus design of new pro- and anti-coagulant drugs. In this study we present our work on ND designed for structural studies of membrane-bound FVIII by transmission electron microscopy (TEM) and single-particle analysis (SPA).

Nanodiscs (ND) were prepared in saline HEPES buffer from Galactosyl Ceramides (GC) and Phospholipid/Choline (PS) with two forms of scaffolding protein – MSP1D1 (for discs ~9.5 nm diameter) and extended version MSP1E3D1 (for discs ~12.1 nm diameter) following the procedure described in Riche et al., 2003.

**Electron microscopy (EM)**

The samples were blotted down to 0.05 mg/ml MSP protein concentration and were deposited onto freshly carbon-coated and plasma treated hexagonal grid (300 mesh) and stained by 1% Uranyl acetate. Digital electron micrographs were recorded with a 4096 x 4096 pixel Ultrascan CCD camera at low electron dose conditions (20 electrons/Å2), a final magnification of 56,400 x but detached from grid with 180-200 Å lift-off using ultrasonic electron microscope operated at 200 kV.

The size of the ND was measured with the Digital Micrograph software (Gatan Inc.). 2D analysis and single particle reconstruction of the ND and FVIII-ND particles was carried out with the EMAN2 (Tan et al., 2007).

**FVIII + ND complexes preparation**

The FVIII-ND samples were prepared by mixing equal volumes of FVIII and ND solutions to obtain 1.1 molecular FVIII to ND ratio incubated 5 min at room temperature (21°C).

**Characterization of assembled ND by EM**

The size of the ND was measured with the Digital Micrograph software (Gatan Inc.). 2D analysis and single particle reconstruction of the ND and FVIII-ND particles was carried out with the EMAN2 (Tan et al., 2007).

**Electron microscopy of negatively stabilized FVIII bound to ND with different PS concentration.** The different organization of FVIII bound to the ND is marked as follows: star – FVIII bound to one side of the disc, triangle – FVIII bound to opposite sides of the ND, cross – multiple attachments of FVIII to the ND and double ND. Surface area class averages calculated from ~ 5,000 FVIII-ND particles data sets at different concentration of FVIII are shown illustrating the ND membrane organization. As the most homogenous membrane-bound organization was observed with the ND at 80 % PS we collected more EM micrographs at these conditions and boxed 13,022 FVIII-ND particles at 180 x 180 pixels (2.9 Å/pix) for further image processing.

**3D reconstruction**

3D reconstruction of FVIII bound to ND was calculated with the EMAN2 software. The authors acknowledged the Cryo-EM computing and research support facilities at the Sealy Center for Structural Biology (SCSB) at UTMB, the director of the SCSB, Dr. Montgomery Pethica for continuous support and constructive criticism and encouragement; Dr. Pasi Laitinen from Emory Hospital (Atlanta, Georgia) for support with the porcine FVIII characterization; Ms. Emily D. Elson senior student from Bald High Preparatory Academy (Galveston, TX) for help with the ND-PS particles extraction and Dr. Alyxey I. Krylov for helpful discussions regarding the SPA.

Authors disclosure information

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