Supporting Material

Aggregation modulators interfere with membrane interactions of β_2-microglobulin fibrils

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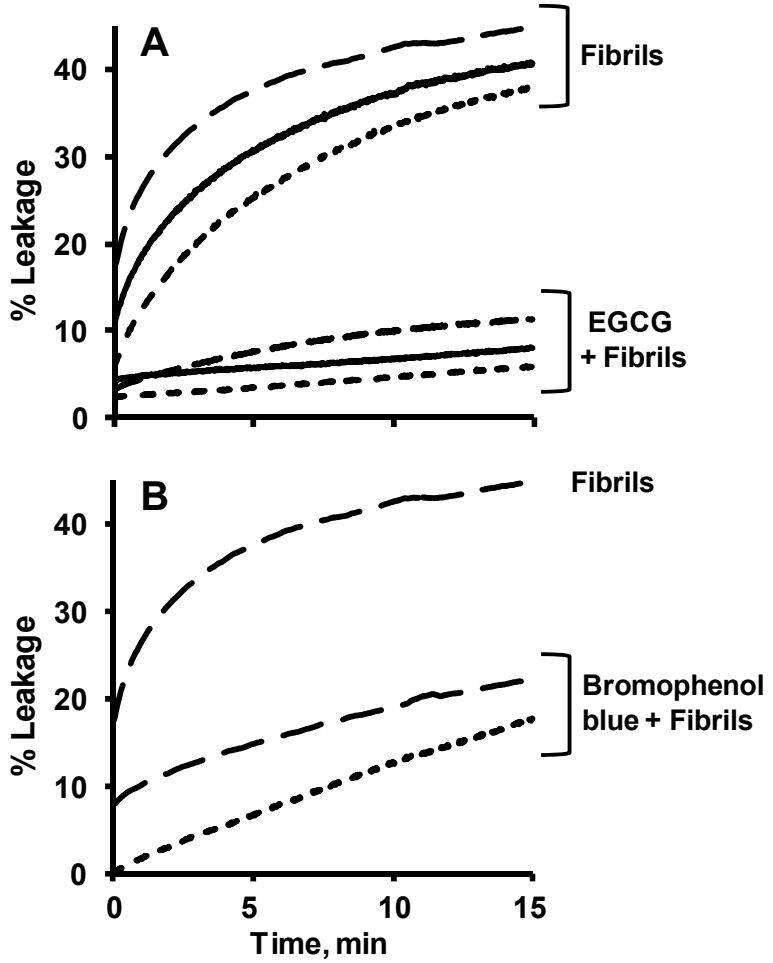
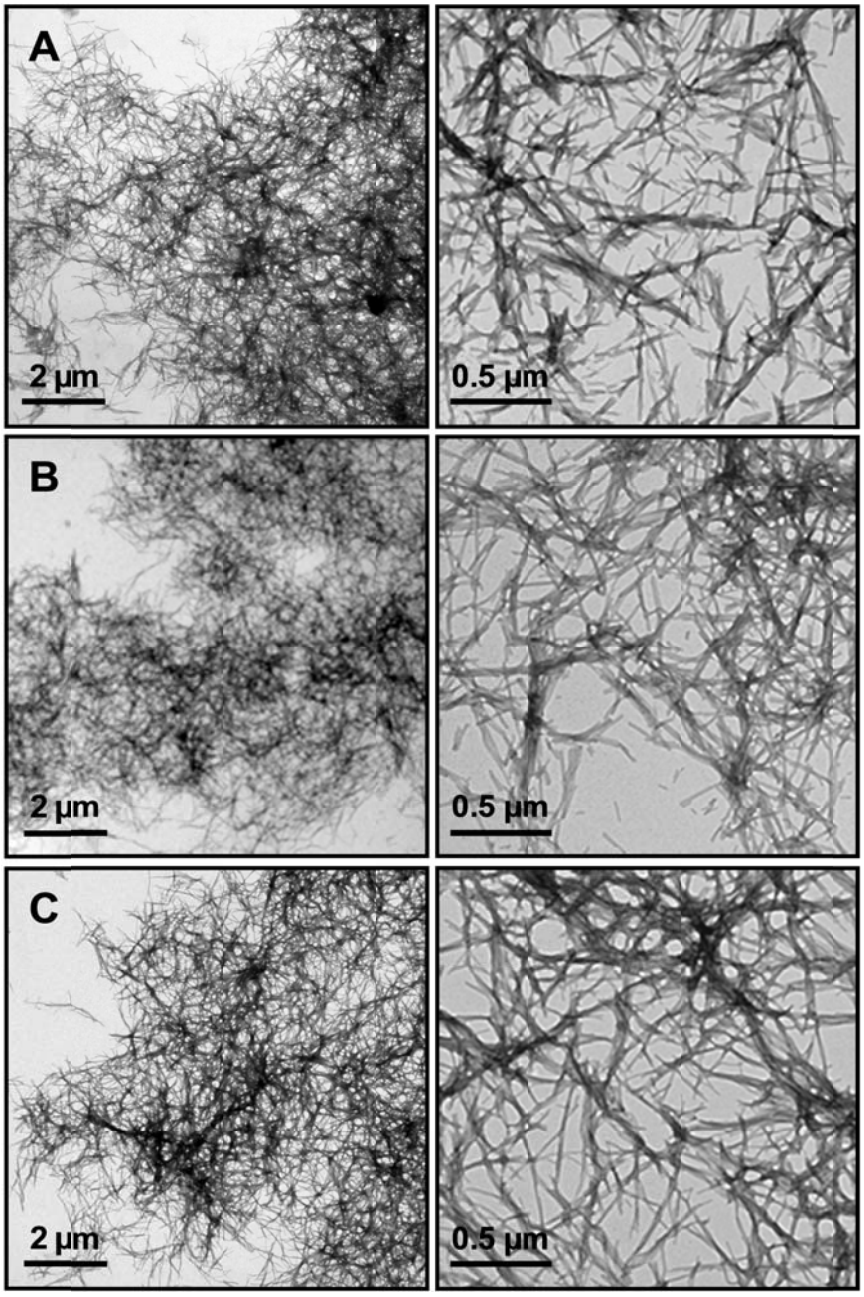


Figure S1: The effect of longer pre-incubation of polyphenols with β_2m fibrils on the fibril-induced vesicle leakage. β_2m fibrils were incubated in pH 7.4 buffer **(A-B)** alone; with **(A)** EGCG or **(B)** bromophenol blue for the following time periods: ***Long dash:*** 3min; ***solid:*** 15 min; ***short dash***: 30 min.

Prolonged incubation of β_2m fibrils in pH 7.4 buffer with no additives reduced the extent of vesicle leakage by ~ 7% relative to the 3 min incubation, presumably due to formation of larger fibrillar aggregates at this pH [52]. Fibrils treated with either EGCG or bromophenol blue for 30 min gave rise to comparable reduction of the extent of dye leakage. Thus, the longer incubation time does not improve the inhibitory properties of these polyphenols.


A
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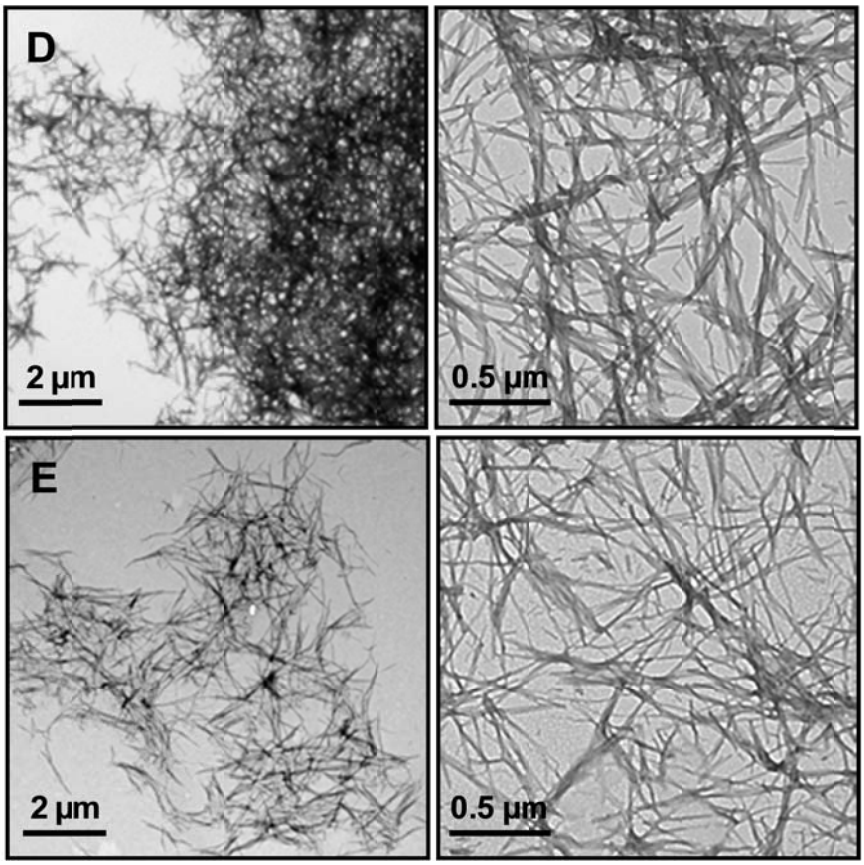

Figure S2: TEM images of β_2m fibrils incubated with polyphenols and GAGs in the absence of lipid vesicles. (A) β_2m fibrils in pH 7.4 buffer (control); β_2m fibrils incubated for 5 min with (**B**) EGCG; (**C**) bromophenol blue; (**D**) resveratrol or (**E**) full-length heparin. Each image is shown on two different scales.

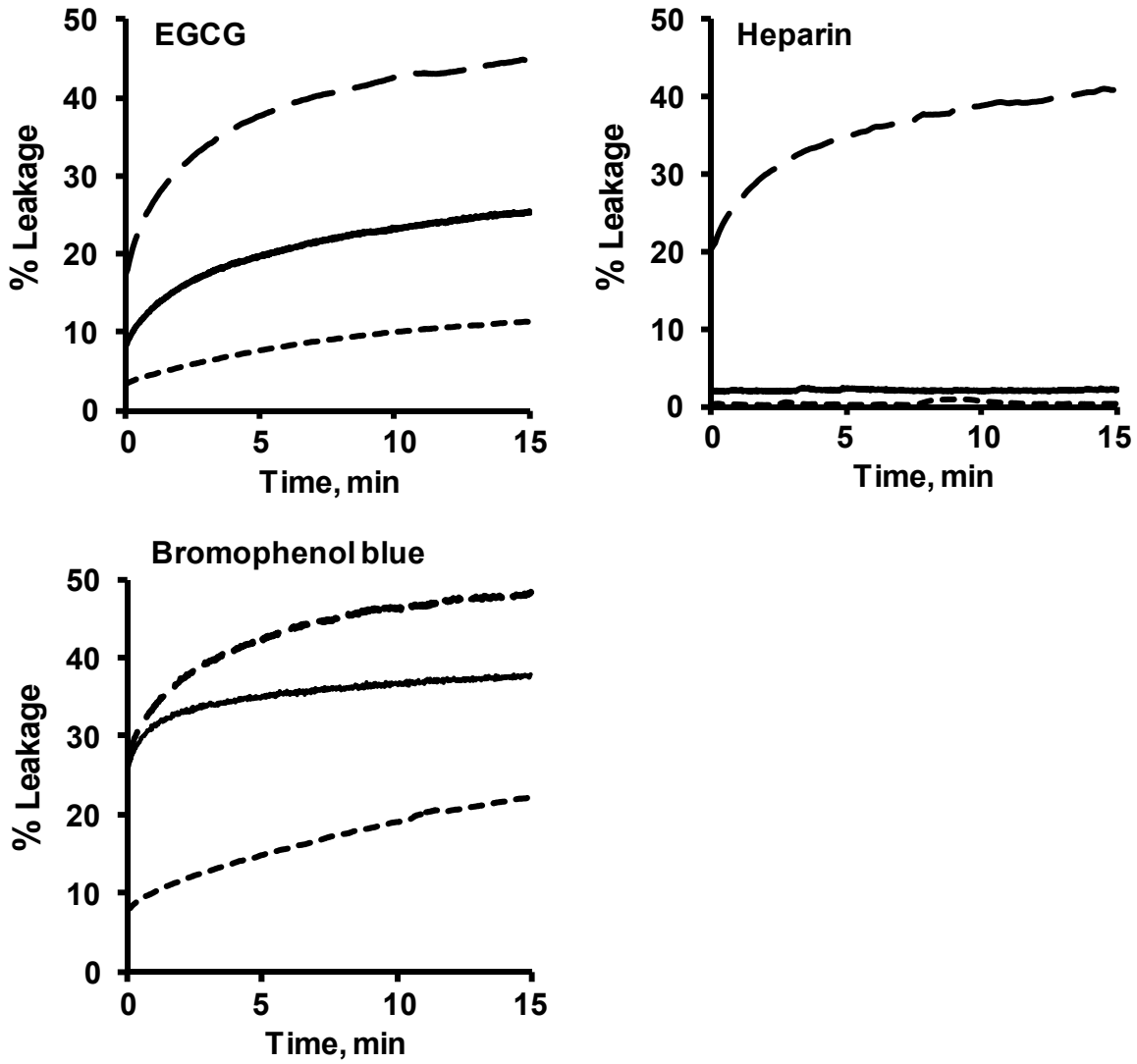

Figure S3: Kinetic curves showing the effect of vesicle pre-incubation with different additives on β_2m-fibril induced membrane leakage. ***Long dashed:*** β_2m fibrils alone (no fibrillation modulators added). ***Solid line:*** fibrillation modulators incubated with vesicles for 30 min before addition of fibrils. ***Short dashed:*** fibrillation modulators incubated with β_2m fibrils for 3 min prior to addition to the vesicles.

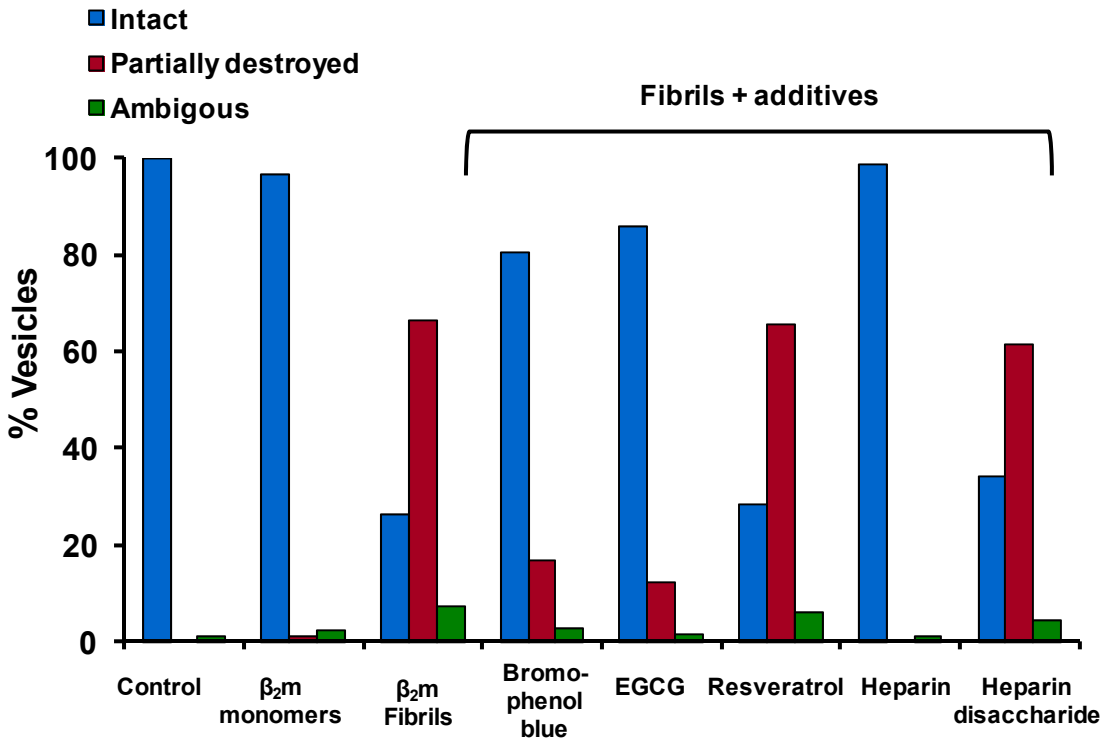

Figure S4: Counting statistics of GV distortion by β_2m fibrils and the effect of different additives. GV counting was acquired from confocal images (representative data are shown in Fig. 3) for the following samples: GVs in buffer (control), GVs incubated with: β_2m monomers; β_2m fibrils or fibril/additives mixtures. Total number of vesicles counted in each category is indicated in Table S1. Vesicles were classified as intact; partially destroyed (exhibiting remaining spherical structure as in Fig. 3D(i)) or ambiguous (GVs that appeared in close proximity to fibrillar aggregates and thus could not be reliably ascribed to either intact or partially destroyed). Note that completely disintegrated vesicles were not included in these counts since it is difficult to estimate their quantity.

Compound	Total vesicle counts	N of fibrillar aggregates coated by lipids
Control (vesicles only)	109	N/A
β_2m monomers	90	N/A
β_2m fibrils	95	39
Bromophenol blue + fibrils	108	15
EGCG + fibrils	122	7
Resveratrol + fibrils	81	29
Heparin + fibrils	90	0
Heparin disaccharide + fibrils	88	36

Table S1: Counting statistics of GV disintegration by β_2m fibrils. GV and fibrillar aggregates counts were acquired from confocal images (representative data are shown in Fig. 3). Only fibrillar aggregates coated by lipids but not bound to vesicles were taken into account (examples are shown in Fig. 3D(ii) by arrows). Note that lipids extracted by a fibrillar aggregate could be derived from a single or multiple vesicles. Note that completely destroyed GVs were not included in total vesicle counts.

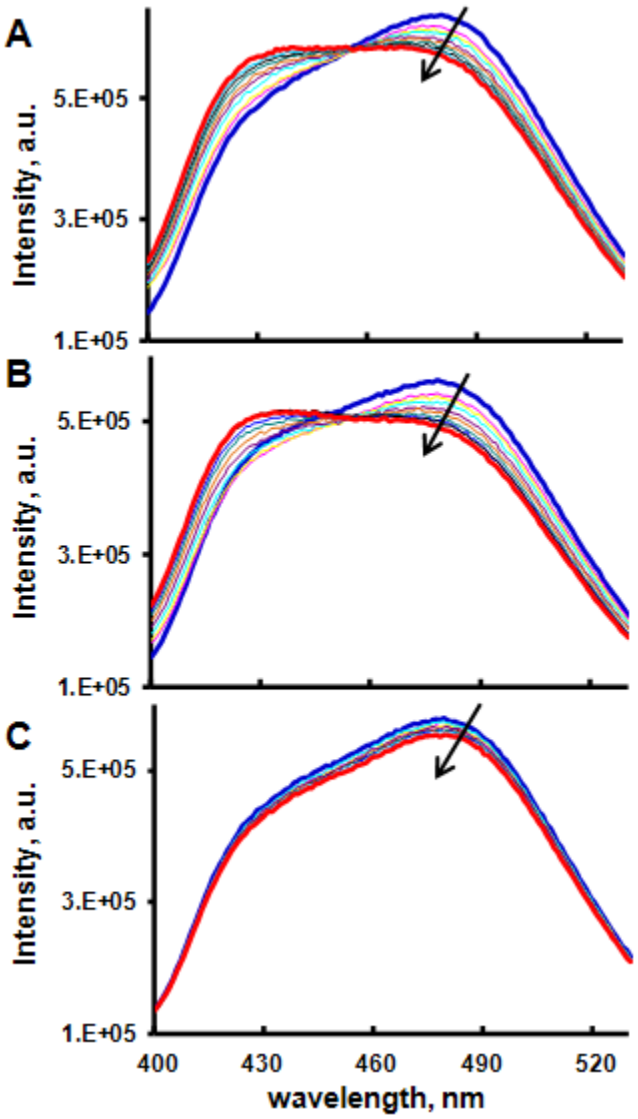

Figure S5: Time course of Laurdan fluorescence shift. Emission spectra of Laurdan within PC/PG (1/1) LUVs were recorded over 20 min after addition of the following compounds to the vesicles: **(A)** β_2m fibrils; (**B**) β_2m fibrils pre-incubated for 3 min with full length heparin; (**C**) β_2m monomers. Arrows indicate time progression at the following intervals: 0, 0.5, 1, 2, 4, 7, 10, 13, 16 and 20 min. The first and the last time points are highlighted in blue and red, respectively.

Supporting Methods

Transmission Electron Microscopy (TEM).

β_2m fibrils were incubated with polyphenols and GAGs in the absence of vesicles as follows. Aliquots from β_2m fibril stock solution (120 μM monomer equivalent concentration) were incubated for 3 min with the required amount of the tested compound to obtain the following β_2m-to-inhibitor ratios: β_2m/heparin - 1:0.4 (*w/w*); β_2m/polyphenols (EGCG, bromophenol blue, resveratrol) – 1:1 (*w/w*). The samples were then diluted 10-fold by the liposome buffer (pH 7.4) and further incubated for 5 min prior to deposition on 300 mesh copper grids covered by a carbon-stabilized Formvar film. Following 30 sec incubation, excess solutions were removed and the grids were negatively stained for 20 sec with a 1 % (*w/v)* uranyl acetate solution. For the control sample, the liposome buffer was used instead of inhibitors. Samples were viewed with a FEI Tecnai G2 F20 field emission gun (FEG) electron microscope, and the images were recorded using Gatan Ultrascan 4000 CCD camera at 120 kV.

Preparation of rhodamine-labeled β_2m monomer:

The fluorescent dye 5-(and-6)- carboxytetramethylrhodamine-SE (TMR) was dissolved in DMSO at 1 mg/ml and added dropwise to a stirred β_2m solution (10 mg/ml of protein in 10 mM sodium bicarbonate buffer, pH 9.4) in the dark for 1 hour. A ten-fold molar excess of the dye over β_2m was used. A PD10 desalting column (GE Healthcare, Little Chalfont, UK) was equilibrated using 25 ml of 100 mM Tris-HCl pH 8.0. Subsequently, 2.5 ml of the TMR-β_2m conjugate was loaded onto the column and eluted using 3.5 ml of 100 mM Tris-HCl, pH 8.0. The TMR-labelled β_2m was dialyzed against double-distilled water, lyophilized and stored at -20 °C. The protein was labelled with 2, 3, 4 or 5 TMR molecules per monomer with a ratio of ~1:2:2:1 as determined by ESI-MS. TMR-labelled fibrils were prepared by mixing unlabeled and labeled monomers such that the final preparation contained 10% of TMR-bound monomer.

Preparation of Large Unilamellar Vesicles (LUVs):

The egg PC/PG 1/1 (molar ratio) lipid mixture was supplemented with 0.24 % (molar ratio) of rhodamine-PE for determination of lipid concentration. All lipid components were dissolved in chloroform/ethanol (1:1, v/v) and dried together *in vacuo* for 4 hours. The resulting lipid films were hydrated in the liposome buffer (50 mM Hepes, 110 mM NaCl, 1 mM EDTA, 0.02 % (*w/v*) NaN_3, pH 7.4) by vortexing lipid samples for 2 hours. LUVs were prepared by extruding the lipid suspensions through a polycarbonate filter with 400 nm pore size and annealing for 30 min at room temperature. The vesicles were stored at +4 °C and used within 1 day.