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Supporting Information

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hIAPP forms toxic oligomers in plasma

Diana C. Rodriguez Camargo,[a,b,c,d,*] Divita Garg,[c,d] Katalin Buday,[e] Andras Franko,[f,g] Andres Rodriguez Camargo,[h] Fabian Schmidt,[d] Sarah Cox,[b] Saba Suladze,[d] Martin Haslbeck,[d] Yonatan G. Mideksa,[a,c] Gerd Gemmecker,[d] Michaela Aichler,[i] Gabriele Mettenleiter,[i] Michael Schulz,[f,g] Axel Karl Walch,[i] Martin Hrabě de Angelis,[f,g] Matthias Feige,[a,c] Cesar A. Sierra,[h] Marcus Conrad,[e] Konstantinos Tripsianes,[j] Ayyalusamy Ramamoorthy,[a,b] Bernd Reif[a,c,d]

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a Institute for Advanced Study, Technische Universität München, 85748 Garching, Germany
b Program in Biophysics, Department of Chemistry, University of Michigan, Ann Arbor, MI, 48109-1055, USA
c Munich Center for Integrated Protein Science (CIPS-M) at Department of Chemistry, Technische Universität München, 85747 Garching, Germany
d Helmholtz Zentrum München, Institut of Structural Biology (STB), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
e Helmholtz Zentrum München, Institut of Developmental Genetics (IDG), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
f Helmholtz Zentrum München, Institute of Experimental Genetics (IEG), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
g German Center for Diabetes Research (DZD e.V.), 85764 Neuherberg, Germany
h Departament of Chemistry, Universidad Nacional de Colombia, Carrera 30 # 45-03 (Ciudad Universitaria), 111321 Bogota, Colombia
i Helmholtz Zentrum München, Research Unit Analytical Pathology (AAP), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
j Central European Institute of Technology (CEITEC), Masaryk University, Kamenice 5, 62500 Brno, Czech Republic

Materials and Methods

Recombinant hIAPP expression and purification

Human IAPP was expressed and purified as described previously [1]. The peptide is fully oxidized amidated at the C-terminus. For all experiments, peptide poweder was dissolved into the buffer or plasma samples. Molecular biology reagents were obtained from Roche, Sigma-Aldrich St. Louis, MO, USA and New England Biolabs. Isotopically labeled nutrients were acquired from Cambridge Isotope Laboratories (CIL).

Animal studies

To study the interaction between hIAPP and plasma, human islet amyloid polypeptide transgenic mice (TG/TG, +/TG) as well as and healthy controls (+/+) were employed [2]. EDTA plasma was collected as published previously [3, 4].

Cell toxicity assay

To quantify cellular toxicity, cells of the pancreatic beta-cell line b-tc3 (obtained from Thermo Fisher Scientific) were plated into 96-well culture plates (1.500 cells/well), using Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum. After one day, cells were treated with sterile filtered compounds (hIAPP, LDL, glucose, fructose) in increasing concentration. The final volume of each well amounted to 100 µL. As 0 % and 100 % viability controls, 3 “no cell” and 3 “no compound” wells were included. Cells were incubated for 24 h at 37 °C, using a CO_2 incubator. Cell viability was measured using AquaBluer (MultiTarget Pharmaceuticals, LLC). For these experiments, the media in each well was replaced with the 100x diluted AquaBluer medium. The plates were subsequently returned for 4 h to the CO_2 incubator. Thereafter, the fluorescence intensity was measured (excitation: 540nm, emission: 590 nm). The method is based on NADH/NADPH oxidase activity as an indicator of cell viability. The average fluorescence value (RFU) of the no-cell control (background) was subtracted from all other RFU values. The test RFU values were converted to % viability using the formula:

% viability = (RFUtest / RFU of the no-compound wells) x 100

Hemolytic activity

Human erythrocytes (donated by a healthy 31-year-old female) were used to explore the hemolytic activity of the peptide. The blood was centrifuged and washed four times with a 0.9 % NaCl solution to dispose the plasma. hIAPP was dissolved in PBS buffer (pH 7.4), and added to the erythrocytes. Suspensions were incubated for 3 h at 37 °C. Samples were centrifuged, and the optical density of the supernatant was measured at 451 nm to determine the extent of hemolysis. Hypotonically lyzed erythrocytes were used as a standard for 100 % hemolysis. The experiment was repeated three times including positive and negative controls.

Western blot analysis

Purified hIAPP was dissolved into 30 µL of a 30 mM acetic acid solution (pH 5.3) and vortexed. Subsequently, 470 µL of plasma and 10 µL of SDS sample buffer were added. Glucose, fructose and LDL were prepared as concentrated stock solution and added directly to the peptide solution to yield the desired final concentrations. Samples were boiled for 5 min at 90 °C and separated by 12 % protein gels. The resolving gel was composed of: 3.3 mL dH2O, 4 mL 30% acrylamide mix (Roth Cat.Nr.: 3029.1), 2.5 mL 1.5 M Tris (pH : 8.8), 100 µL 10 % SDS, 100 µL 10 % ammonuim persulphate, 4 µL TEMED (Roth Cat.Nr.: 2367.3); The staking gel consisted of: 1.4 mL dH_2O, 330 µL 30 % acrylamide mix (Roth Cat.Nr.: 3029.1), 250 µL 1M Tris (pH 6.8), 20 µL 10% SDS, 20 µL 10% ammonium persulphate, 2 µL TEMED. The final running gel was transferred onto a Tnas-Blot-TurboTM Mini PVDF membrane with a Bio-Rad Trans-Blot TurboTM transfer system. The membrane was blocked in nonfat dry milk (Roth) with Tween-Tris-buffered saline with 5 % (w/v) for 1 hour and then incubated with the primary antibody A133 [5] in Tween-Tris-buffered saline with

a ratio 1:1000 overnight at 4 °C. Immunoreactivity was analyzed with an antirabbit peroxidase-conjugated secondary antibody (Serva) and chemiluminescence detection (Bio-Rad).

Protein-lipid overlay assay

Membrane Lipid Strips™ from Echelon (Cat.Nr.: P-6002) have been spotted with 100 pmol of fifteen different biologically important lipids. Membrane lipid strips were initially blocked with 5 mL of blocking buffer (PBS-T, 0.1 % v/v Tween-20; 3 % BSA free fat) and gently agitated for one hour at room temperature (RT). The blocking buffer was discarded. 2.5 nM of protein was dissolved in 5 mL PBS-T 3%. The membrane was incubated for 1 hr at RT with gentle agitation. Strips were washed three times with 5 mL PBS-T under gentle agitation for 5-10 min. Next, the primary antibody A133 [5] was added to 3 mL of a PBS-T 3% BSA blocking solution at a ratio 1:1000. The membrane was incubated for 1 h at RT with gentle agitation. The washing protocol was repeated and the membrane was finally incubated with the antirabbit peroxidase-conjugated secondary antibody (Serva) at a ratio of 1:5000 into PBS-T 3% BSA blocking solution followed by incubation at RT for 1 h. The membrane was finally washed again and blotted as in the WB experiments.

NMR experiments

All NMR experiments were performed using Bruker Avance 500, 600, 850 MHz spectrometers, equipped with cryo-probes. The ^{15}N and ^{13}C shifts were referenced indirectly. The proton chemical shift was referenced relative to the water resonance frequency. Backbone assignments were obtained via triple resonance experiments [6] employing a perdeuterated sample. The backbone chemical shifts (Cα, N, NH) were used to calculate the Random Coil Index (RCI), using webserver from the Wishart lab [7]. For the NMR data were processed using the software TopSpin (Bruker). Spectra were analyzed using ccpNMR analysis [8].

Transmission electron microscopy (TEM)

To prepare samples for TEM, 10 µL of each sample was placed on the EM grid for 1 min, followed by a drying procedure with filter paper. The grid was subsequently washed three times by adding a drop of water for 3 s, and drying it each time with filter paper. For staining, 10 µL of a 1% uranyl acetate solution was added for 30 s. The excess of the solution was dried with filter paper. Grids were purchased from Electron Microscopy Sciences (Hatfield, PA 19440, USA; Formvar/Carbon 300 mesh copper coated). Samples were measured immediately employing an EM 10 CR (Zeiss, Germany).

Fluorescence experiments

The fluorescence experiments were performed using a spectrofluorimeter PTI QuantaMaster™ 40, using Quartz cells with a path length of 5 nm. Samples were freshly prepared and transferred directly into the cell while stirring at RT. Experiments were carried out using a constant excitation wave length of 305 nm. The emission was scanned in the range between 200 to 600 nm. Solutions were prepared by adding NMR buffer (30 mM d-acetic acid, pH 5.3) to the dried peptide immediately prior to the measurements, keeping the solution on ice at all the times. The final hIAPP concentration amounted to 40 µM. The data were analyzed using the software Origin (OriginLab Corporation, Northampton, MA 01060, USA).

Light microscopy

DIC and fluorescence images were acquired using a Leica DMi8 CS Bino widefield fluorescence microscope using GFP and DAPI filter sets or DIC optics and recorded with a cooled charge-coupled device camera. A Leica oil immersion 100x objective was used with an ND of 1.49.

In the fluorescence images, the green and blue fluorescence was detected using a GFP filter (excitation at 450 nm - 490 nm; emission at 500 nm - 550 nm) and a DAPI filter (excitation at 325 nm - 375 nm; emission at 435 nm - 485 nm), respectively.

Thioflavin-T assay

To monitor the aggregation kinetics of hIAPP in presence and absence of LDL, fructose and glucose, we performed Thioflavin-T (ThT) assays. The peptide was dissolved in NMR buffer (30 mM d-acetic acid, pH 5.3) at a maximum concentration of 150 μM and maintained on ice at all times. For the experiments, this stock solution was diluted into the appropriate buffers (10 μM ThT; 20 mM PO_4, pH 7.4, or 30 mM acetate, pH 5.3) to a final peptide concentration of 10 μM. To test the influence of cosolvents that are enriched in the plasma of diabetic patients, buffers contained 2 μM LDL, 35 mM glucose or fructose. Samples were subsequently plated in triplicate on uncoated Fisherbrand 96-well polystyrene plates. Readings were taken on a Biotek Synergy two microplate reader. Samples were incubated at 25 °C for 24 h in the instrument with continuous, slow orbital shaking. Wells were read from the bottom with an excitation wavelength of 440 nm (30 nm bandwidth) and an emission wavelength of 485 nm (20 nm bandwidth) at 3-min intervals. Following data acquisition, the raw fluorescence traces were background corrected and normalized. Normalized curves were subsequently plotted using the software Origin (OriginLab Corporation, Northampton, MA 01060, USA).

Competing financial interests.

The authors declare no competing financial interests.

Author Contributions

D.C.R.C., B.R., D.G., and A.R.C. designed the experiments. D.C.R.C. expressed, purified and characterized hIAPP. D.C.R.C., K.T., R.S., F.S., and G.G. performed NMR experiments and analyzed the data. A.F, M.S., and M.H.d.A. extracted and processed the mouse plasma samples. M.H., S.C., A.R.C. and C.A.S. performed the fluorescence and ThT experiments and analysis. S.S. performed the DLS experiments. D.C.R.C., Y.M. and M.F. contributed the DIC microscopy experiments and analysis. D.C.R.C., K.B. and M.C. performed the western blot analysis, lipid blot assay and cell toxicity. D.C.R.C. performed the hemolytic activity assay. D.C.R.C., M.A., G.M. and A.K.W. carried out EM experiments and analysis. D.C.R.C., D.G, F.S., A.F., A.R. and B.R. wrote the manuscript with contributions from all authors.

Conflicts of interest

The authors declare no conflicts of interest.

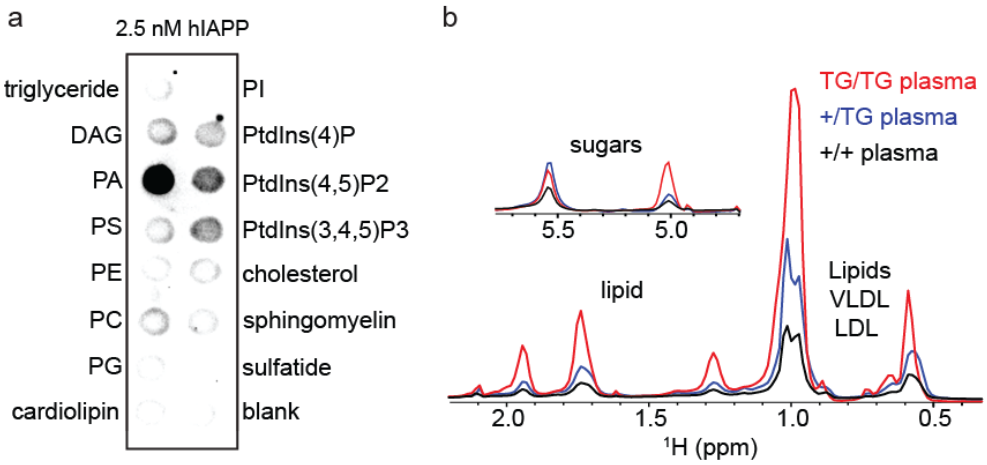

Figure S1. *a) Lipid strip blot assay performed with a 2.5 nM hIAPP sample. hIAPP was identified using the hIAPP specific antibody A133* [5]. *We find that hIAPP interacts weakly with triglycerides, cholesterol, phospholipids, while a stronger interaction is observed for phosphatidylglycerides and in particular with phosphatidic acid. b) Comparison of the* ^{1}*H- NMR spectra of hIAPP in the plasma of TG/TG (red), +/TG (blue) and +/+ mice (black) with assignments of the major plasma components. In agreement with previous resuls, we find that the amount of lipids (VLDL and LDL) as well as lactate, valine, and sugars is strongly increased in the TG/TG plasma* [9].

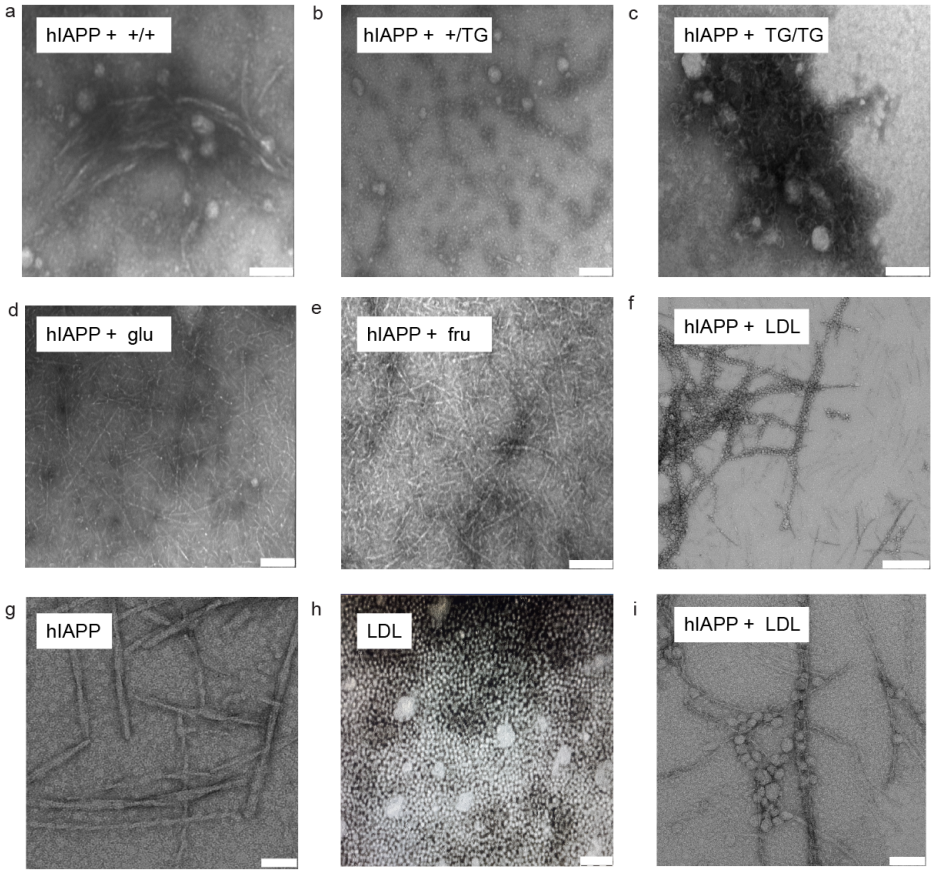

Figure S2. ***Transmission electron microscopy (EM) shows that the hIAPP aggregation state is affected by plasma, LDL, glucose and fructose.*** *hIAPP in the presence of* ***a*** *+/+ blood plasma,* ***b*** *+/TG plasma,* ***c*** *TG/TG plasma,* ***d*** *35 mM glucose,* ***e*** *35 mM fructose,* ***f*** *2 μM LDL.* ***g*** *hIAPP fibrils control (100 μM, pH 7.5).* ***h*** *2 μM LDL control.* ***i*** *hIAPP in presence of 2 μM LDL (different region of the grid). EM images have been recorded after completion of the NMR experiments shown in Figure 1. Scale bars represent a length of 200 nm.*

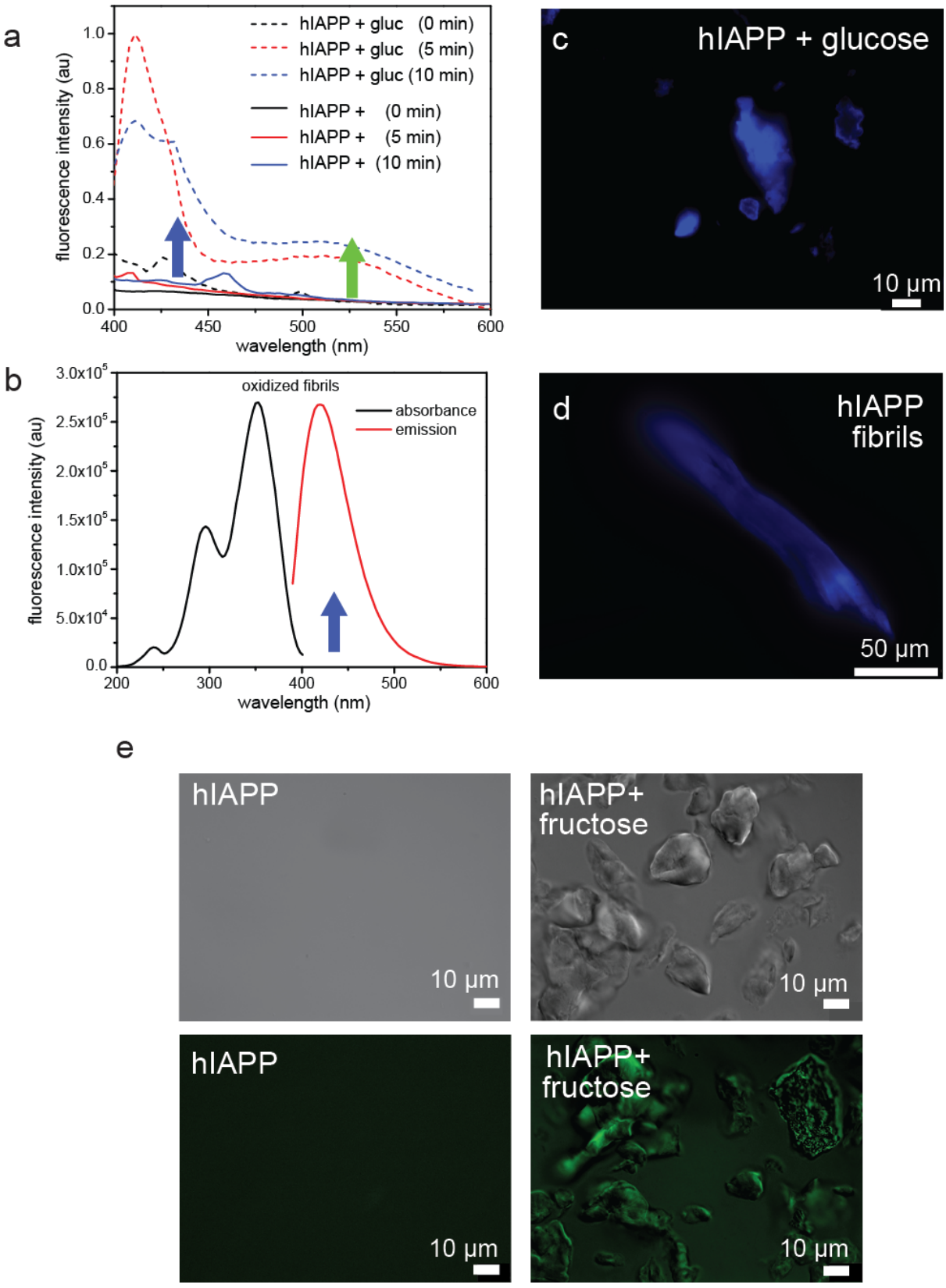

***Figure S3**. **Intrinsic fluorescence of glucose and LDL induced hIAPP oligomeric assemblies. a** Fluorescence emission spectra of a 40 µM hIAPP solution in the presence and absence of 35 mM glucose monitored over the time (excitation at 305 nm, emission at 400-600 nm). The emission signal is increasing in the presence of glucose as a function of time, suggesting that the interaction between hIAPP and sugars is very dynamic. The solutions were prepared using NMR buffer (30 mM d-acetic*

acid, pH 5.3). The blue and green arrow indicate emission signal passing through the GFP and DAPI filter, respectively. ***b*** *Absorbance (black) and emission (red, with excitation at 305 nm) spectra of preformed oxidized hIAPP fibrils (in 30 mM d-acetic acid, pH 5.3).* ***c*** *Fluorescence image of a freshly dissolved solution containing 40 µM hIAPP and 35 mM glucose, using a DAPI filter.* ***d*** *Fluorescence image of preformed oxidized hIAPP fibrils using a DAPI filter (excitation at 325 nm - 375 nm; emission at 435 nm - 485 nm). We propose that sugars capture the peptide in a colloidal like state, in which the hIAPP retains a high degree of flexibility, but is structured enough to yield intrinsic fluorescence which is presumably due to the an extension of the conjugation of the peptide bond.* ***e*** *DIC and GFP-filtered fluorescence microscopy images of hIAPP in the absence and presence of fructose.*

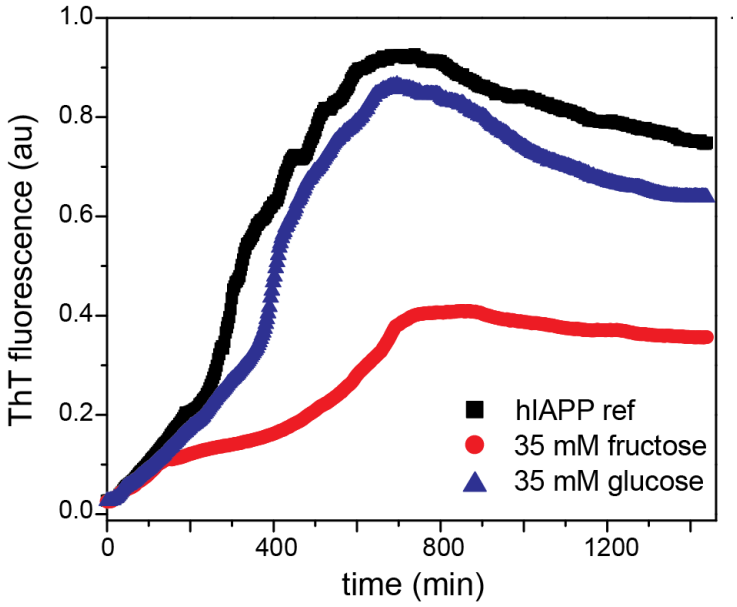

Figure S4*. Thioflavin T (ThT) aggregation kinetics after incubation of hIAPP with 35 mM glucose (blue) and 35 mM fructose (red). The strongest effect on inhibiton of aggregation is seen in the presence of fructose.*

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