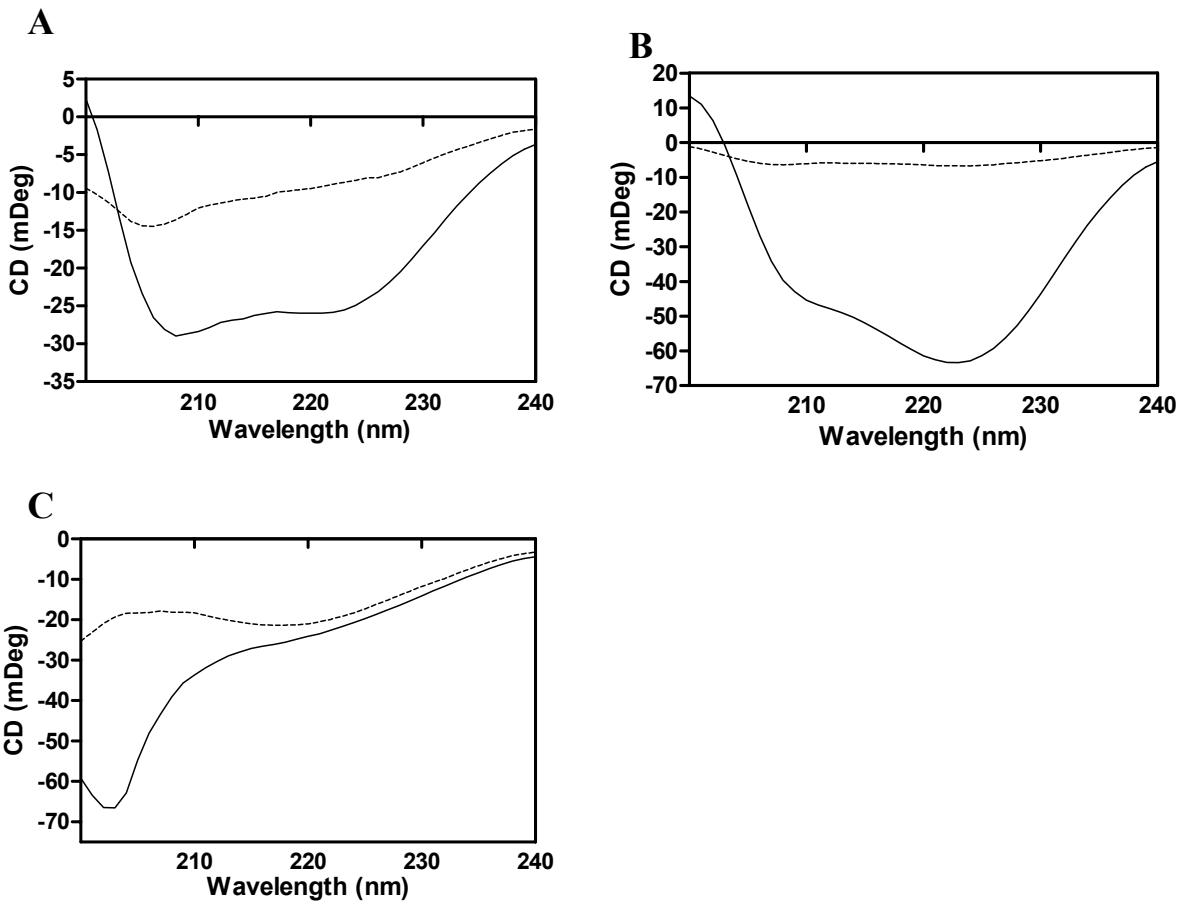
Supplemental Data

Supplemental Table 1

Fluorescence of Thioflavin T and Congo red by various proteins is indicative for protein misfolding.

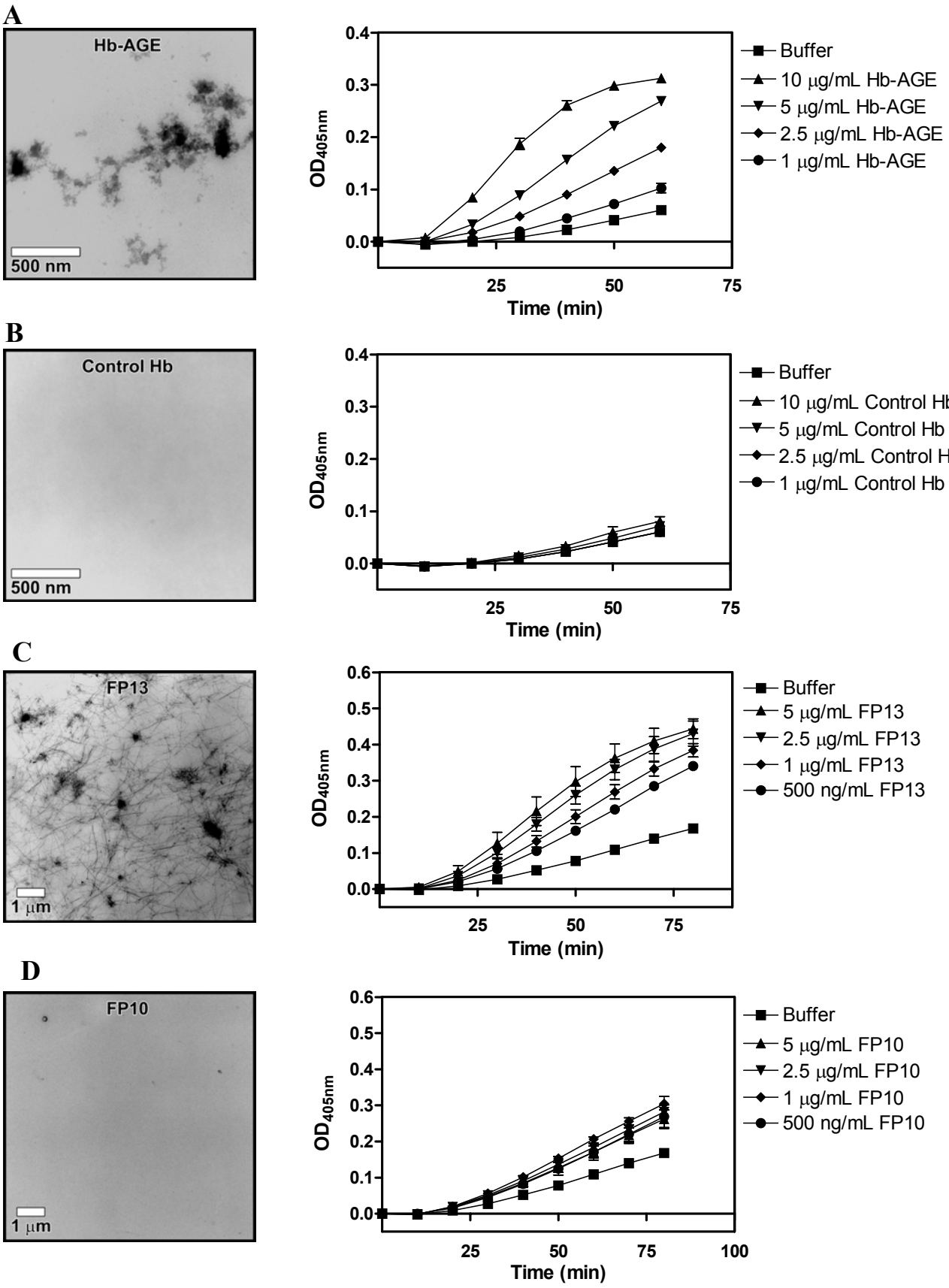
Protein /peptide	Thioflavin T	Congo Red
Aβ (1-42) Fresh	1.13 +/- 0.09	0.18 +/- 0.01
Aβ (1-42) Fibrils	4.16 +/- 0.14	0.94 +/- 0.00
TTR11 Fibrils	0.71 +/- 0.00	0.03 +/- 0.02
TTR-wt pH=2.0	1.61 +/- 0.03	ND
TTR-wt	0.20 +/- 0.11	ND
Bence-Jones protein	0.70 +/- 0.03	ND
dOVA	2.11 +/- 0.04	1.38 +/- 0.01
nOVA	0.24 +/- 0.01	0.02 +/- 0.03
Endostatin Urea	0.38 +/- 0.00	1.22 +/- 0.02
Endostatin Native	0.11 +/- 0.00	0.43 +/- 0.01
BSA-AGE	ND	ND
BSA Fresh	0.02+/-0.01	0.00+/-0.02
FP13	0.15+/-0.06	0.42+/-0.01
FP10	0.03+/-0.01	0.01+/-0.01
Hb-AGE	ND	ND
Hb Fresh	ND	ND
IAPP (1-37) Fibrils	2.10 +/- 0.12	0.64 +/- 0.12

Fluorescence of Thioflavin T and Congo red was assayed in the presence of 100 µg/mL of protein. ND = not determined. Fibril formation of Aβ (1-42) led to a large increase in both assays, indicative of amyloid formation. Similar increases in ThT and CR fluorescence with lower absolute values were seen after treatment of native ovalbumin (nOVA), native Endostatin (nEndo) and transthyretin, that caused them to form aggregates. No ThT and CR fluorescence could be determined with BSA-AGE and Hb-AGE since it displayed autofluorescence at the wavelengths of both assays. Differences in protein structure were therefore determined by far UV circular dichroism, as is displayed in Supplemental Figure 1.

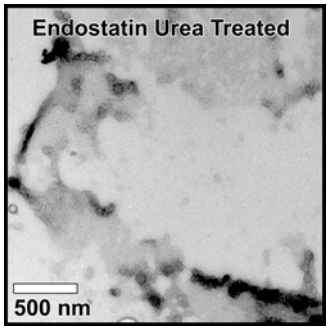


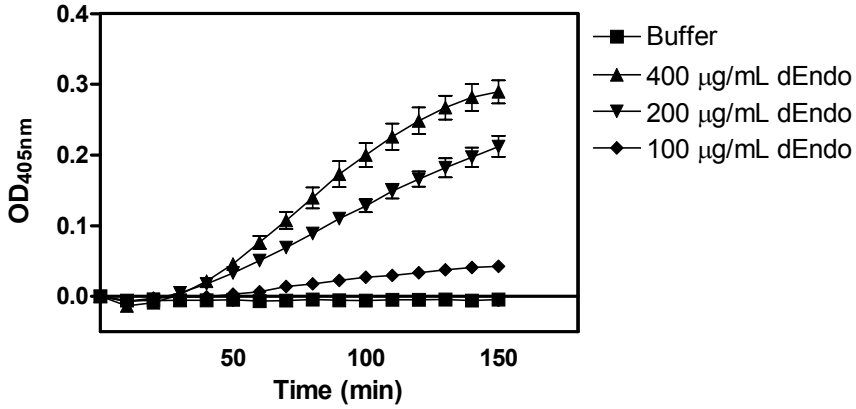
Supplemental Figure 1

Far UV Circular Dichroism detects the ellipticity of peptide bonds in a protein, which gives insight into secondary structure. Far UV spectra of BSA-AGE (**A**), Hb-AGE (**B**) and FP13 (**C**) indicate structural differences compared to control preparations. The secondary structures of BSA-AGE (**A**; dotted line), Hb-AGE (**B**; dotted line) and fibrin peptide FP13 (**C**; dotted line) were compared to those of control BSA (**A**; solid line) control Hb (**B**; solid line) and the control peptide FP10 (**C**; solid line). In all cases, significant conformational differences were seen that indicate loss of secondary structure.

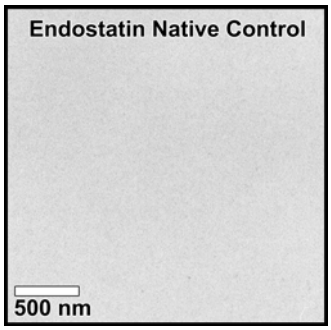

A
Hb-AGE
500 nm
OD405nm
Time (min)
Buffer
10 μg/mL Hb-AGE
5 μg/mL Hb-AGE
2.5 μg/mL Hb-AGE
1 μg/mL Hb-AGE
B
Control Hb
500 nm
OD405nm
Time (min)
Buffer
10 μg/mL Control Hb
5 μg/mL Control Hb
2.5 μg/mL Control Hb
1 μg/mL Control Hb
C
FP13
1 μm
OD405nm
Time (min)
Buffer
5 μg/mL FP13
2.5 μg/mL FP13
1 μg/mL FP13
500 ng/mL FP13
D
FP10
1 μm
OD405nm
Time (min)
Buffer
5 μg/mL FP10
2.5 μg/mL FP10
1 μg/mL FP10
500 ng/mL FP10

E


Endostatin Urea Treated
500 nm


0.4
0.3
0.2
0.1
0.0
OD405nm
50
100
150
Time (min)
Buffer
400 μg/mL dEndo
200 μg/mL dEndo
100 μg/mL dEndo

F


Endostatin Native Control
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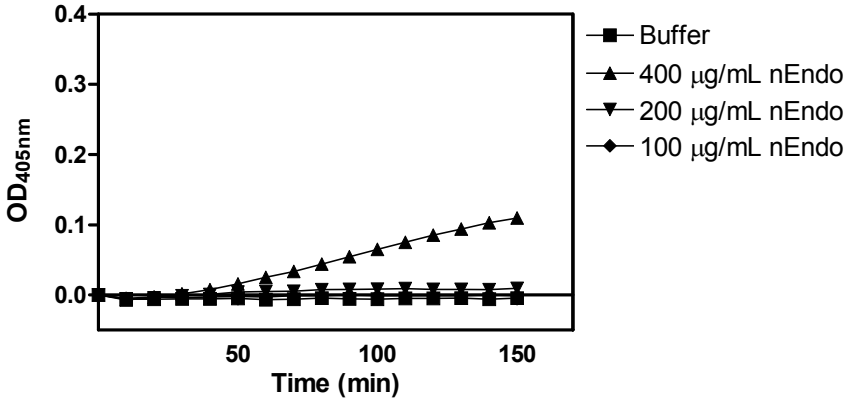
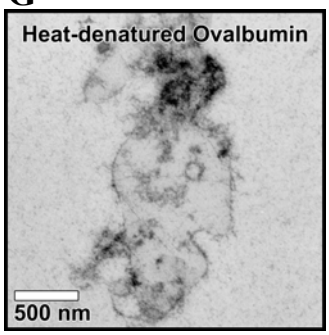

0.4
0.3
0.2
0.1
0.0
OD405nm
50
100
150
Time (min)
Buffer
400 μg/mL nEndo
200 μg/mL nEndo
100 μg/mL nEndo

G


Heat-denatured Ovalbumin
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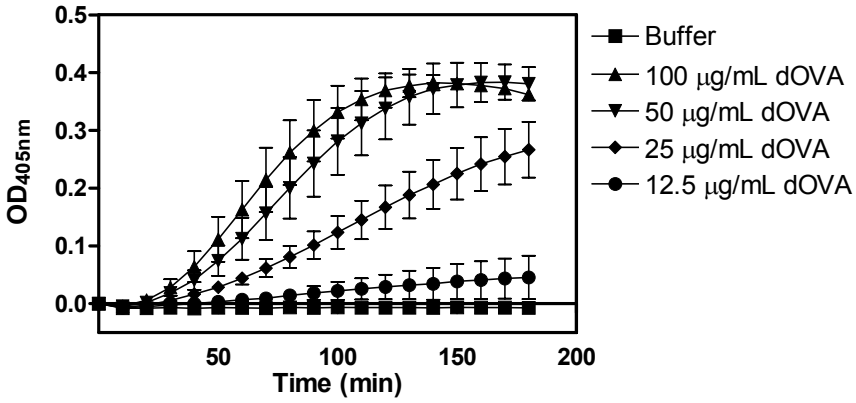
0.5
0.4
0.3
0.2
0.1
0.0
OD405nm
50
100
150
200
Time (min)
Buffer
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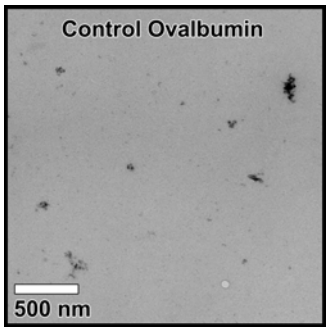
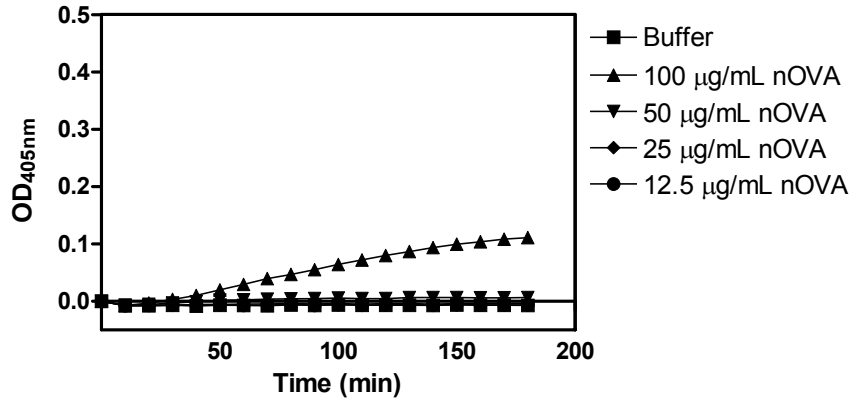
25 μg/mL dOVA
12.5 μg/mL dOVA

H


Control Ovalbumin
500 nm


0.5
0.4
0.3
0.2
0.1
0.0
OD405nm
50
100
150
200
Time (min)
Buffer
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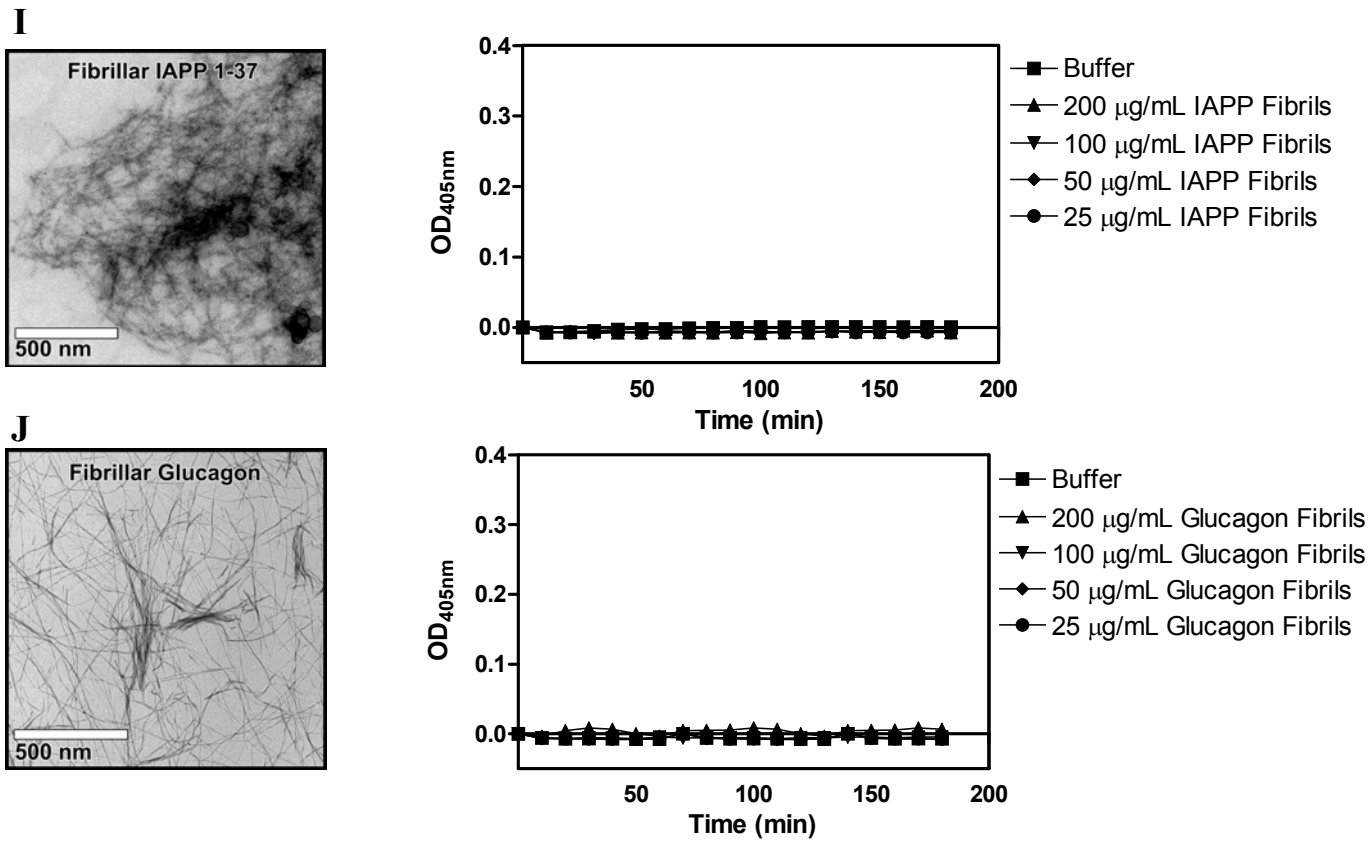
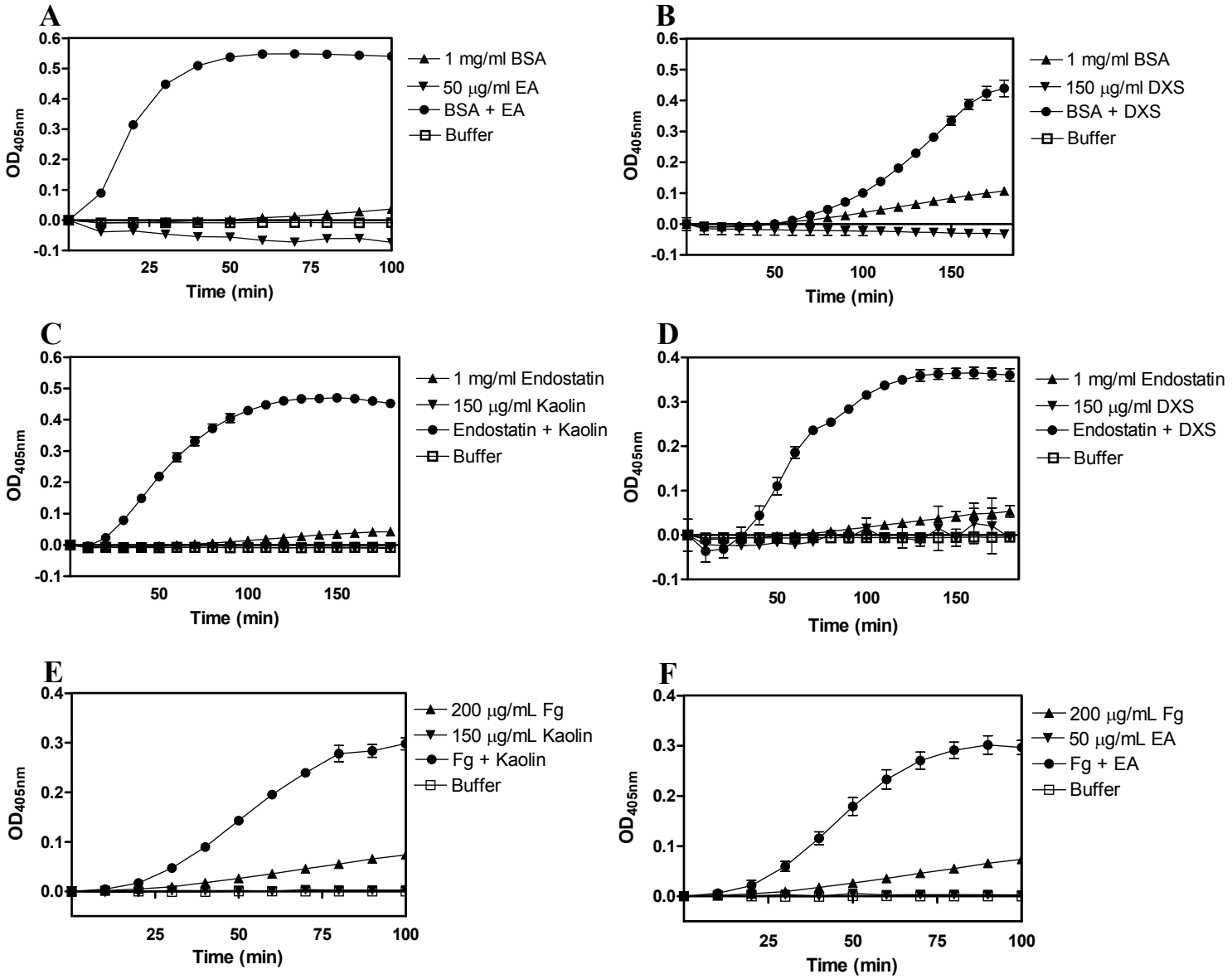
50 μg/mL nOVA
25 μg/mL nOVA
12.5 μg/mL nOVA



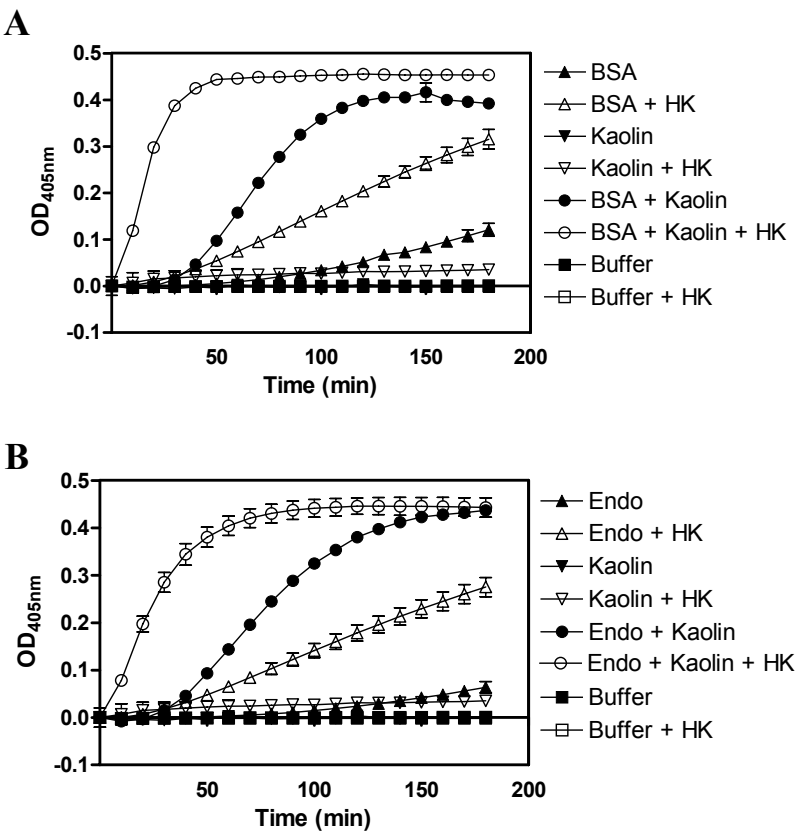
Supplemental Figure 2

FXII is activated by misfolded protein aggregates, but not by amyloid fibrils, in vitro. Activation of FXII was measured in vitro by a chromogenic assay for FXII dependent kallikrein generation. The conversion of 0.3 mM Chromozym PK in the presence of 7.7 nM PK was determined in presence and absence of 0.97 nM FXII. None of the protein preparations tested was capable of converting Chromozym PK in the presence of PK without FXII present (not shown). In agreement with the data of Figure 2, amorphous aggregates of glycated hemoglobin (Hb-AGE), but not native hemoglobin, activated FXII-dependent kallikrein generation (A, B; experiment performed in blocked wells). The fibrin peptide FP13, but not FP10, had formed a mixture of both small fibrils and dense amorphous aggregates and was also found to activate FXII-dependent kallikrein generation (C, D; experiment performed in blocked wells). Indeed, both aggregates of Endostatin and ovalbumin that had formed during artificial denaturation (E, G, respectively) induced kallikrein generation, whereas their native counterparts could not (F, H). In addition to findings with fibrillar Aβ and TTR11 peptides, amyloid fibrils of IAPP and glucagon were incapable of inducing kallikrein generation (I, J).



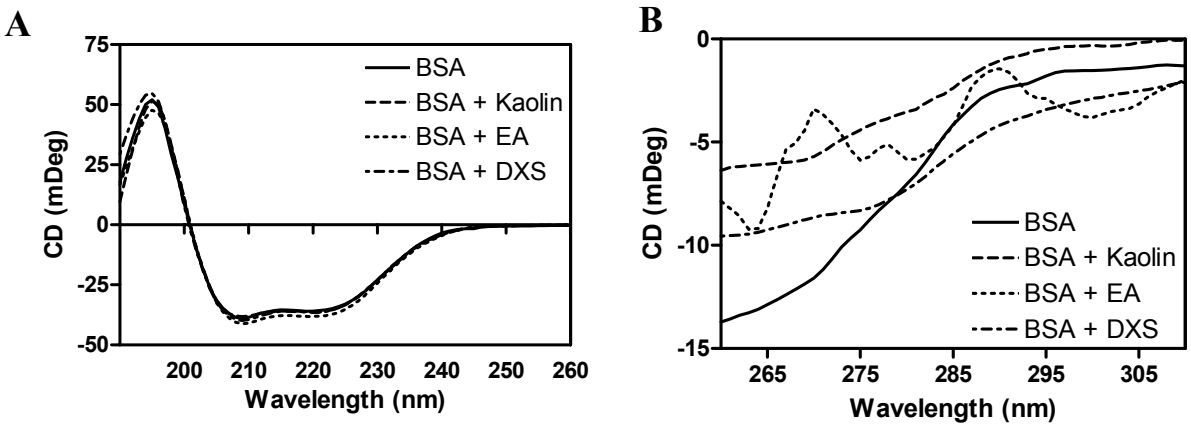
Supplemental Figure 3

FXII-dependent kallikrein generation by surfaces is modulated by cofactor proteins. Surfaces were incubated for 5 minutes at 37 ºC with three different proteins, BSA, Endostatin and Fibrinogen (Fg) and analyzed for their ability to induce FXII-dependent kallikrein generation. It was found that addition of either of these proteins was required for FXII activation by kaolin, ellagic acid (EA) and dextran sulfate (500 kDa; DXS).



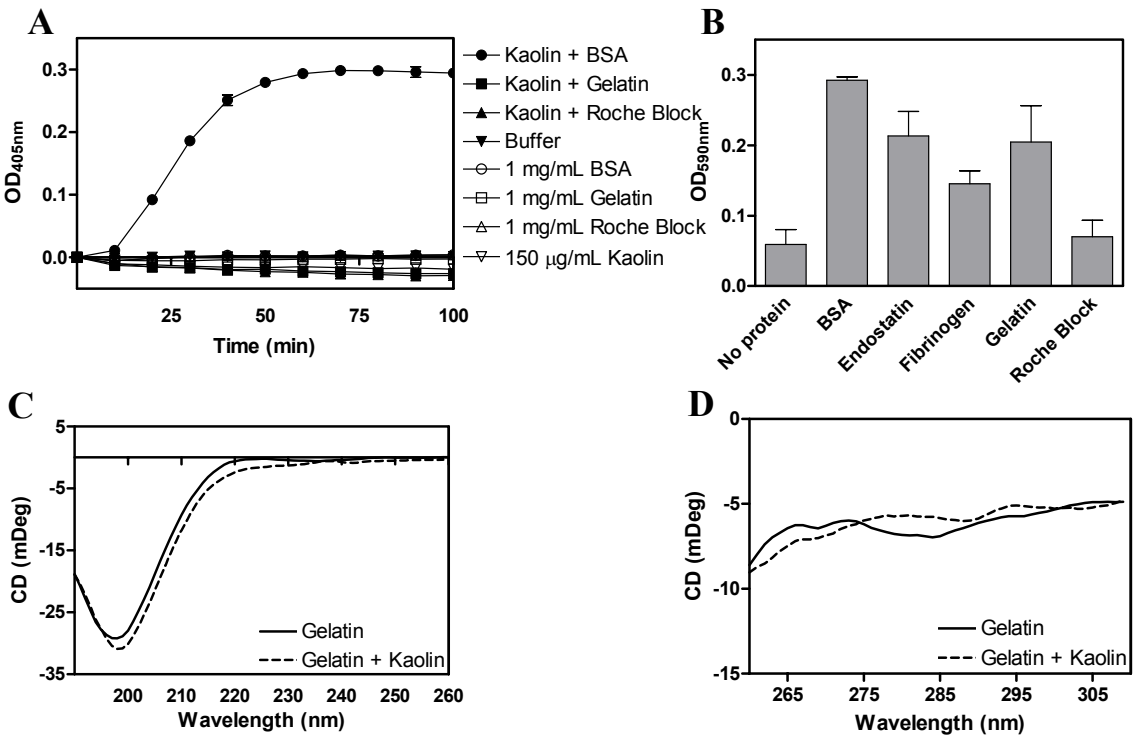
Supplemental Figure 4

HK accelerates FXII-dependent kallikrein generation by a protein cofactor. As described earlier, kallikrein generation was investigated by monitoring the conversion of 0.3 mM Chromozym PK in the presence of 7.7 nM PK and 0.97 nM FXII, with the presence and absence of 5.9 nM HK (Calbiochem). Absence of FXII resulted in no activation under all conditions tested. As described earlier, activation of FXII by 150 µg/mL of kaolin was dependent on the presence of 1 mg/mL of BSA (**A**) or Endostatin (**B**). HK enhanced FXII-dependent kallikrein generation by BSA and by BSA mixed with kaolin without enhancing the effect of kaolin alone.



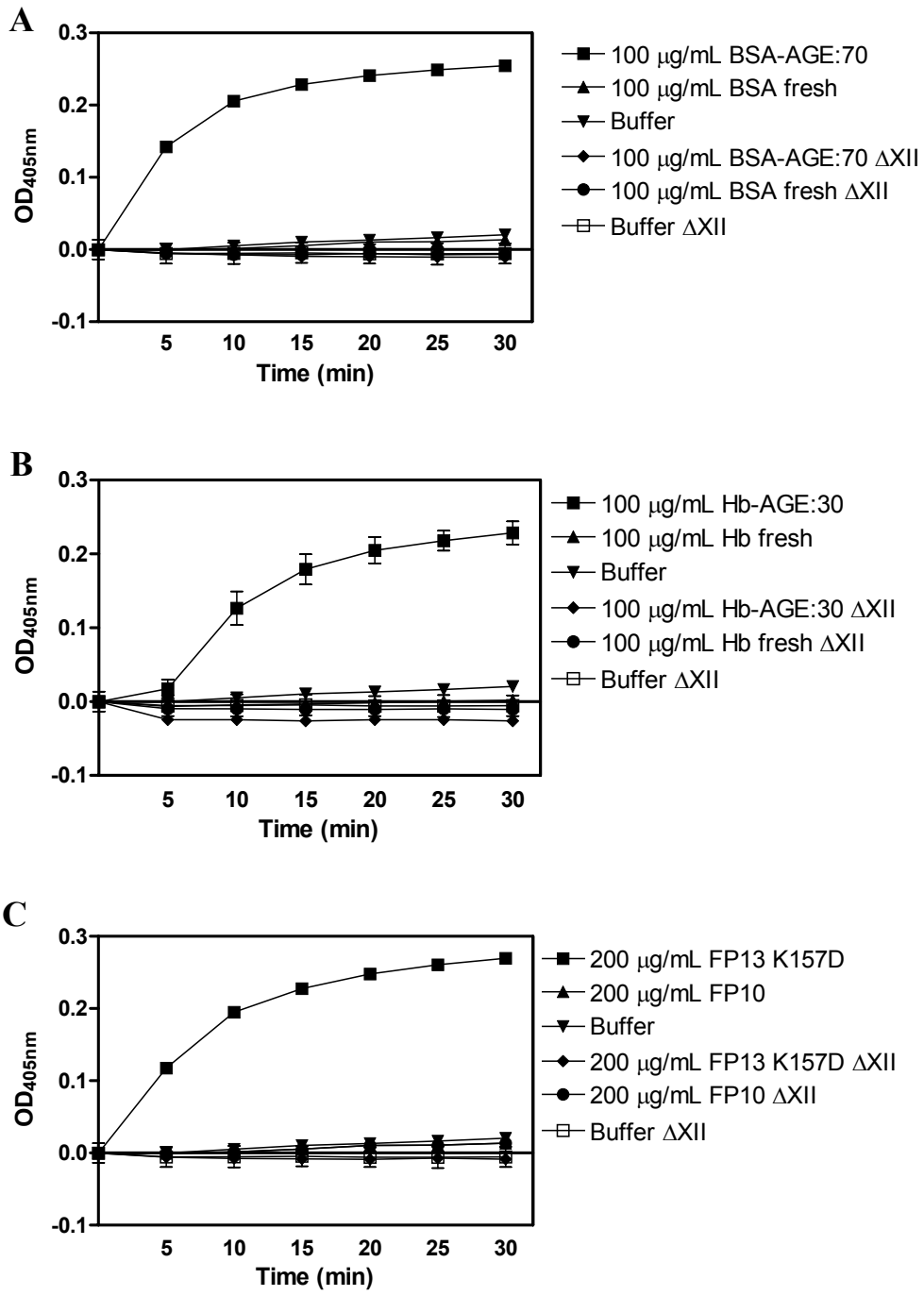
Supplemental Figure 5

Structure determinations by circular dichroism indicate changes in tertiary, but not in secondary structure of BSA. Far UV Circular Dichroism detects the ellipticity of peptide bonds in a protein, which gives insight into secondary structure. However, near UV spectra are used to give insight into changes in tertiary structure by monitoring ellipticity of aromatic amino acids in a protein. Far and near UV spectra were recorded of BSA in the presence and absence of kaolin, DXS or EA, but no significant changes were observed in secondary structure (**A**). Near UV spectra of BSA were significantly changed after addition of kaolin, DXS or EA, indicating structural perturbations leading to loss of tertiary structure (**B**). These findings are in good agreement with those found by others who studied structural changes that occur during protein adsorption (14).



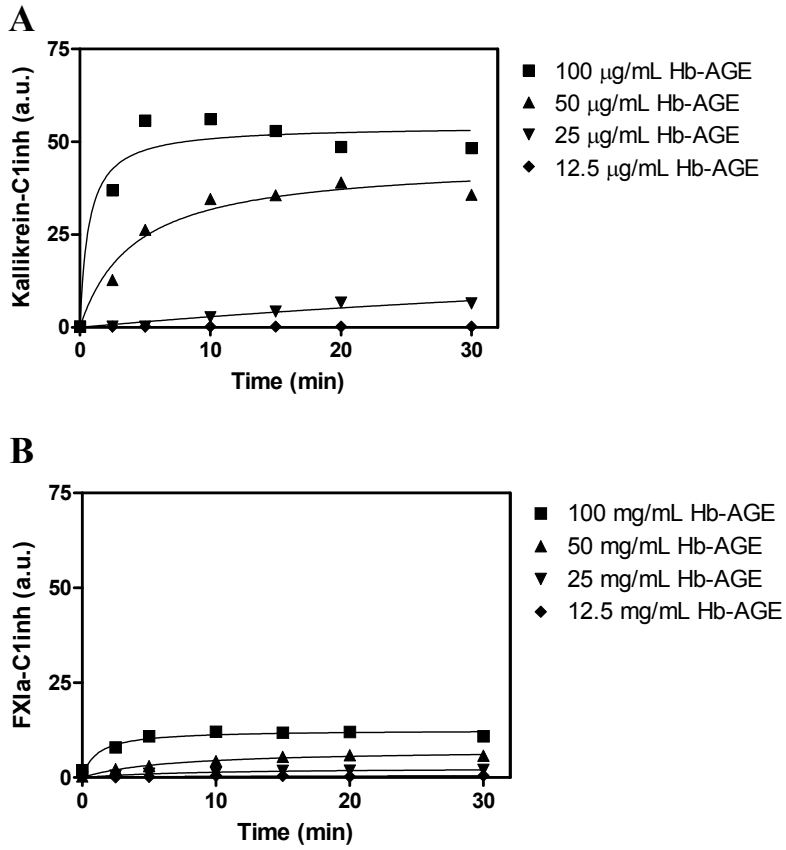
Supplemental Figure 6

Gelatin and Roche Blocking Reagent do not support FXII-dependent kallikrein generation in the presence of kaolin. In a chromogenic FXII-dependent kallikrein generation assay, BSA, but not gelatin or Roche Blocking Reagent could function as a cofactor for kallikrein generation in the presence of kaolin (**A**). In a pull-down experiment, gelatin, but not Roche Blocking Reagent bound to kaolin, explaining the obvious lack cofactor function of this peptide mixture (**B**). Gelatin was analyzed for its structural properties by circular dichroism spectroscopy when bound to kaolin and was found not to undergo an appreciable conformational change in the presence of this surface; its secondary structure had a large amount of random coil, which did not alter significantly when kaolin-bound (**C**). Additionally, the near UV CD spectrum (and thus the tertiary structure) of gelatin was not significantly altered in the presence of kaolin (**D**), which is most likely required for induction of FXII-dependent kallikrein generation.



Supplemental Figure 7

Misfolded protein aggregates stimulate kallikrein generation in plasma, in a FXII-dependent manner. Addition of misfolded protein aggregates of BSA-AGE (**A**), Hb-AGE (**B**) or FP13 (**C**) to 1:10 diluted plasma resulted in conversion of Chromozym PK, whereas native control preparations did not. This process was found to be completely dependent on the presence of FXII in plasma, since plasma deficient in FXII (ΔXII) did not respond to the presence of the aggregates.



Supplemental Figure 8

Dose-dependency of kallikrein-C1inh complex formation (**A**) and FXIa-C1inh complex formation (**B**), in plasma by Hb-AGE.