Plasminogen activation triggers transthyretin amyloidogenesis in vitro

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Systemic amyloidosis is a usually fatal disease caused by extracellular accumulation of abnormal protein fibrils, amyloid fibrils, derived by misfolding and aggregation of soluble globular plasma protein precursors. Both WT and genetic variants of the normal plasma protein transthyretin (TTR) form amyloid, but neither the misfolding leading to fibrillogenesis nor the anatomical localization of TTR amyloid deposition are understood. We have previously shown that, under physiological conditions, trypsin cleaves human TTR in a mechano-enzymatic mechanism that generates abundant amyloid fibrils in vitro. In sharp contrast, the widely used in vitro model of denaturation and aggregation of TTR by prolonged exposure to pH 4.0 yields almost no clearly defined amyloid fibrils. However, the exclusive duodenal location of trypsin means that this enzyme cannot contribute to systemic extracellular TTR amyloid deposition in vivo. Here, we therefore conducted a bioinformatics search for systemically active trypsin proteases with appropriate tissue distribution, which unexpectedly identified plasmin as the leading candidate. We confirmed that plasmin, just as trypsin, selectively cleaves human TTR between residues 48 and 49 under physiological conditions in vitro. Truncated and full-length protomers are then released from the native homotetramer and rapidly aggregate into abundant fibrils indistinguishable from ex vivo TTR amyloid. Our findings suggest that physiological fibrinolysis is likely to play a critical role in TTR amyloid formation in vivo. Identification of this surprising intersection between two hitherto unrelated pathways opens new avenues for elucidating the mechanisms of TTR amyloidosis, for seeking susceptibility risk factors, and for therapeutic innovation.

The in vivo processes responsible for misfolding of native precursors, for formation of amyloid fibrils, and for the anatomical localization of amyloid deposition are not known either for transthyretin (TTR) or for other types of systemic amyloidosis (1). The late onset of TTR amyloidosis, despite the abundance of circulating TTR from birth, is also mysterious.

In vitro studies suggest that TTR fibrillogenesis requires dissociation of the native tetramer, which is favored by the destabilizing mutations that are known to be amyloidogenic. Indeed the most aggressive, earlier onset forms of the disease are caused by highly destabilizing mutations whereas mutations that increase tetramer stability prevent amyloidosis (2). A single, selective, proteolytic cleavage in the loop interconnecting strands C and D dramatically destabilizes the native tetramer in the most unstable amyloidogenic S52P TTR (3) and the unusual Glu-51_Ser-52 duplicate variant (4) leading to abundant amyloid formation. In addition, mechanical forces, generated by a combination of physiological fluid flow and contact with hydrophobic surfaces, enhance susceptibility to this cleavage and thus uniquely promote formation of unequivocal amyloid

**The abbreviations used are:** TTR, transthyretin; w/w, weight for weight; ThT, thioflavin T; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; AFM, atomic force microscopy; buffer A, 20 mM Tris-HCl, pH 7.5 containing 150 mM NaCl and 5 mM CaCl2.
fibrils, both by other amyloidogenic variants that are more stable than S52P and by WT TTR (5).

Trypsin, which we have previously used to trigger TTR amyloid fibril formation in vitro, is synthesized only by the exocrine pancreas and secreted exclusively into the small bowel lumen. It is therefore unlikely to be involved in pathogenesis of systemic TTR amyloidosis. However, we show here that plasmin, identified in our comprehensive bioinformatics search for pathophysiologically plausible candidate proteases, effectively replicates the role of trypsin in in vitro TTR amyloidogenesis. Furthermore, the normal, ubiquitous, continuous, physiological activation of plasminogen is fully consistent with a key role of plasmin in TTR amyloidogenesis.

Results

Search for candidate trypsic proteases in the MEROPS database

There were 344 peptidases in the MEROPS database (6) able to cleave substrates with trypsic specificity, that is C-terminal to lysine (position P1), and with relevantly wider tissue distribution than trypsin itself. Seventy-five of them were both human and extracellular according to the curated UniProt protein database, the majority being either serine chymotrypsin–like or metallopeptidase types (Table 1). Among the four enzymes with specificity higher than 30% for lysine at P1 (Table 2), trypsin was excluded because of its exocrine location. Tryptase alpha did not trigger TTR amyloid formation in our fibrillogenesis assay (3) and kallikrein–related peptidase 12 had very modest activity (Fig. S1). In contrast, plasmin not only fulfilled our search criteria but its active site is also strikingly similar to that of trypsin (Fig. 1).

Amyloidogenic cleavage of TTR by plasmin

Consistent with its known structure and proteolytic specificity, plasmin did indeed trigger TTR amyloid formation in vitro, although it was slightly less active than trypsin (Fig. 2). With S52P TTR in solution, stirred at physiological pH and ionic strength, and the same enzyme:TTR w/w ratio, the thioflavin T (ThT) signal increased more rapidly in the presence of trypsin than plasmin and reached a higher final value (Fig. 2A). Nevertheless, both samples contained abundant amyloid fibrils with the pathognomonic amyloid red-green birefringence after Congo Red staining when viewed in strong cross-polarized light, and showing typical fibrillar morphology in negative staining EM (Fig. 2, B and C). The crucial residue 49–127 fragment produced by the specific amyloidogenic cleavage was present after fibrillogenesis induced by plasmin but was slightly less abundant than with trypsin (Fig. 2, D and E), consistent with the longer lag phase and lower yield of fibrils (Fig. 2A). However, as in our previous studies with trypsin, TTR amyloid fibrillogenesis mediated by plasmin in the mechano-enzymatic process was accelerated by seeding with preformed TTR amyloid fibrils, which eliminated the lag phase and produced a

Table 1
Bioinformatics search for trypsin like protease(s)

Summary of the human extracellular proteases identified in the MEROPS database with lysine in position P1 of the substrate.

<table>
<thead>
<tr>
<th>Clan</th>
<th>Family</th>
<th>Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A01</td>
<td>Asp_pepsin_like</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>C01</td>
<td>Cys_papain_like</td>
<td>1</td>
</tr>
<tr>
<td>MA</td>
<td>M01</td>
<td>Aminopeptidase_like</td>
<td>2</td>
</tr>
<tr>
<td>MA</td>
<td>M10</td>
<td>Metallopeptidase</td>
<td>14</td>
</tr>
<tr>
<td>MA</td>
<td>M12</td>
<td>Astacin_like</td>
<td>7</td>
</tr>
<tr>
<td>MA–MC</td>
<td>M13–M43</td>
<td>Neprilysin_like; carboxypeptidase</td>
<td>7</td>
</tr>
<tr>
<td>PA</td>
<td>S01</td>
<td>Ser_chymotrypsin_like</td>
<td>38</td>
</tr>
<tr>
<td>SB</td>
<td>S08</td>
<td>Ser_subtilisin_like</td>
<td>2</td>
</tr>
<tr>
<td>SR</td>
<td>S60</td>
<td>Ser_lactoferrin</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>75</td>
</tr>
</tbody>
</table>

Table 2

Secreted peptidases with specificity for lysine in position P1 higher than 30%

Plasmin and tryptase have structural similarities with trypsin; the structure of kallikrein–related peptidase 12 is not known.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Specificity for Lys at P1</th>
<th>Primary localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>S01.151: trypsin 1</td>
<td>60</td>
<td>Intestinal tract</td>
</tr>
<tr>
<td>S01.143: tryptase alpha</td>
<td>56</td>
<td>Lung, stomach, spleen, heart, and skin</td>
</tr>
<tr>
<td>S01.020: kallikrein–related peptidase 12</td>
<td>55</td>
<td>Salivary glands, stomach, uterus, trachea, prostate, thymus, lung, colon, brain, breast, and thyroid</td>
</tr>
<tr>
<td>S01.233: plasmin</td>
<td>45</td>
<td>Plasma and many other extracellular fluids</td>
</tr>
</tbody>
</table>

Figure 1. Structural and functional similarities between trypsin and plasmin in complex with the peptide P3-P3' corresponding to sequence 46–51 of TTR. The backbones of trypsin (magenta; PDB ID: 3D65) and plasmin (green; PDB ID: 3UIR) are overlaid; the catalytic triad, in ball and stick, with the Asp residue, in sticks, that lead to the correct orientation of the Lys-substrate (Lys-48 in TTR) are specifically highlighted. The numbering refers to trypsin residues. The P3-P3' peptide backbone of textilinin-1 in the complex with plasmin is shown in cyan. The side chain of Lys in position P1 is also represented in sticks with the distances from Asp-189. For clarity the corresponding peptide complexed to trypsin is not shown.
higher final yield (Fig. S2). Plasmin-induced fibrillogenesis was inhibited by α2-antiplasmin, the natural inhibitor of the enzyme (Fig. 2F).

The critical importance of protease specificity for TTR amyloid formation was exemplified by the failure of three different, potent, proteolytic enzymes, thrombin, chymotrypsin, and proteinase K, to trigger any amyloidogenesis (Fig. 2A). On the other hand, all the amyloidogenic TTR variants tested so far, as well as WT TTR, were cleaved by plasmin in our in vitro mechanoenzymatic system. They all formed unequivocal amyloid fibrils, although the yields were lower with V30M, L55P, and V122I TTR than with S52P and were lowest with WT TTR (Fig. 3). Crucially, the known, superstable, T119M TTR variant was not cleaved at all (Fig. 3). These observations are fully consistent with the usually earlier onset and more aggressive phenotypes in carriers of amyloidogenic TTR mutations, compared with the late onset of WT TTR amyloidosis, and with the protection against TTR amyloidosis in carriers of amyloidogenic TTR gene mutations afforded by co-inheritance of the gene for the T119M variant.

In contrast to the susceptibility of native TTR to cleavage by plasmin, which was greatly enhanced by mechanical forces, preformed TTR amyloid fibrils were completely resistant to degradation by plasmin (Fig. S3). This differs from Aβ-amyloid fibrils that are digested by plasmin, which has been suggested to be a putative protective mechanism against amyloid formation in Alzheimer’s disease (7).

From fibrin to fibril formation

To study the amyloidogenicity of plasmin in a more physiological environment, we created a model fibrin clot on which fibrinolysis was initiated in the presence of either the highly amyloidogenic unstable S52P TTR or the superstable nonamyloidogenic T119M variant. Polymerization and depolymerization were monitored by nonspecific light scattering at 350 nm (Fig. 4A) and by the specific spectrofluorimetric signal of ThT binding to amyloid fibrils (Fig. 4B).

In phase I, fibrinogen was converted into fibrin by addition of thrombin, monitored by the rapid increase in turbidity. Once the clot was formed, tissue plasminogen activator (tPA), plas-
minogen, and TTR were gently layered on the clot surface, at
the time point shown (arrow) in Fig. 4, A and B, producing
physiological fibrinolysis of the clot as indicated by the rapid
decline in turbidity in phase II. When S52P TTR was present,
the initial fall in light scattering was swiftly followed by a sharp
rise that correlated with the appearance and increase in the
ThT amyloid fibril signal (Fig. 4B). In the presence of T119M
TTR, which is not susceptible to cleavage by plasmin and does
not form amyloid fibrils (5) (Fig. 3), there was no secondary rise
in turbidity and no ThT signal (Fig. 4, A–C).

Atomic force microscopy analysis of the reactants at the end
of the experiment with the stable T119M or the pathogenic
S52P variant TTR (that is 4 and 5 in Fig. 4, A–C) showed
remarkably different structures, consistent with the spectrom-
etry results. S52P TTR produced morphologically typical
mature amyloid fibrils, 4–7 nm in height (Fig. 4D) emerging
from a thick layer of short fibrils. No fibrillar material was seen
either with T119M TTR (Fig. 4E) or in the absence of any added
TTR (Fig. 4F). Only single globular structures and short beaded
chains were observed.

Discussion

The spectrum of systemic TTR amyloidosis comprises the
many very rare hereditary forms caused by different mutations
(8), the cardiac amyloidosis caused by the V122I variant in in-
dividuals of African origin (9) and cardiac amyloidosis, mostly in
elderly men, caused by WT TTR (10). Recent advances in imaging
have shown that the latter is substantially more prevalent than previously recognized (11). There are no licensed treat-
ments that arrest disease progression and TTR amyloidosis is thus an important unmet medical need. Current trials of TTR
gene expression knockdown by experimental siRNA (12) and
antisense oligonucleotide (ASO) drugs (13) have shown prom-
ising results. However, elucidation of the mechanism underly-
ing the in vivo transition of native, soluble, globular, tetrameric
TTR into insoluble, polymeric, amyloid fibrils is crucial for
understanding the natural history of the disease and for design
of other effective therapies.

The influential original model of TTR denaturation and
aggregation at low pH (14) demonstrated that tetramer disas-
sembly is crucial, and that analogues of thyroxine, the natural
ligand of TTR, can inhibit this process (15). The observations
led to design, development, and clinical testing of tafamidis (16)
and diflunisal (17), compounds that stabilize TTR against acid
denaturation, for use as inhibitors of TTR amyloidogenesis,
mimicking the trans-suppressive effect of the TTR-stabilizing
T119M variant (2). Despite the capacity of tafamidis to increase
the stability of TTR in plasma through the occupancy of just
one of the two binding sites (18), its clinical use does not halt
disease progression in a substantial proportion of patients (19).
The limited clinical efficacy probably reflects the fact that the
low pH model does not represent the actual pathophysiological
mechanism of TTR amyloid fibrillogenesis. Indeed there is no
relevant in vivo location in which TTR could be exposed to the
acid conditions used in vitro.

We have recently demonstrated that specific proteolytic
cleavage of the residue 48–49 bond in the flexible loop con-
necting strands C and D, in just a single TTR protomer within
the native tetrameric TTR assembly, causes rapid dissociation
into cleaved and uncleaved protomers. Under physiological
conditions in vivo, these then swiftly form abundant TTR
fibrils, which are indistinguishable from ex vivo TTR amyloid
fibrils (3, 5). The whole process occurs in the presence of phys-
iological scale mechanical forces provided by stirring and by
exposure to hydrophobic surfaces. Discovery of the critical role
of proteolysis explains the almost universal presence of the
TTR residue 49–127 fragment in ex vivo TTR amyloid fibrils
(20). Other features consistent with the mechano-enzymatic
mechanism operating in vivo include the presence of a lag-
phase preceding fibrillogenesis, and acceleration of fibril for-
mation when preformed fibril seeds are present. We have also
shown that binding of small ligands by the intact TTR tetramer
significantly reduces its susceptibility to cleavage and aggrega-
tion. However, maximum inhibition is only achieved by ligands
that simultaneously occupy both the two binding sites and the
central channel between them in the core of the TTR molecule
(21).

A crucial question about the mechano-enzymatic mecha-
nism has hitherto been the identity of the tryptic protease
responsible for TTR amyloidosis in vivo. The present demon-
stration of the efficacy of plasmin in vitro highlights it as
an extremely plausible candidate. Other potent proteolytic
enzymes were completely inactive in triggering TTR amyloid
formation in vitro. Kallikrein-related peptidase 12, which has
only very transient activity in vivo, did produce a small ThT
signal of amyloid formation with S52P TTR, the most unstable
and amyloidogenic TTR variant, but there was a long lag
phase and very modest yield. Plasmin mediates the essential
specific cleavage in TTR much more potently and, with clas-
sical kinetic phases of nucleation and elongation, it generates
abundant fibrils that are identical to ex vivo TTR amyloid
fibrils. The relative lower activity of plasmin compared with
trypsin cannot be easily explained. The remarkable self-di-
gestion of plasmin, once activated, may reduce its activity
and therefore delay the formation of the first fibrillar nuclei
thus contributing to a reduced yield of fibrils. A complete

Figure 3. Plasmin-mediated fibrillogenesis. Relative ThT emission fluores-
cence intensities of TTR samples at 1 mg/ml after 25 h incubation with shak-
ing in the presence of plasmin at an enzymesubstrate ratio of 1:50. Mean ±
S.D. of three replicates is shown.
characterization of the kinetics of all processes together with the determination of the TTR–plasmin structure should clarify the differences that we have observed. The several amyloidogenic TTR variants tested so far and the WT protein are all cleaved by plasmin, with varying efficiency replicating the findings with trypsin, whereas the stable, nonpathogenic, protective T119M variant is resistant. Furthermore, plasmin is ubiquitously and continuously activated in vivo to provide for essential fibrinolysis on the vascular wall and also in the extracellular matrix, precisely where TTR amyloid is deposited.

The possible in vivo scenario of plasmin-mediated TTR fibrillogenesis is summarized in Fig. 5. Plasminogen can be activated by tPA within the clot and also by urokinase plasminogen activator (uPA) in the extracellular matrix. Sufficient proteolysis of the TTR tetramer by plasmin may then provide the critical concentration of both truncated and full-length TTR protomers required for nucleation of fibrils. Once nucleation has occurred, the elongation of fibrils can progress at lower concentrations of monomers provided by either of the plasminogen activating pathways. Plasmin activity is finely regulated by activators, including tPA and uPA, and inhibitors, including α2-plasmin inhibitor and plasminogen activator inhibitor. The conditions for critical TTR cleavage, sufficient to initiate amyloidogenesis, may thus only arise rarely but there is certainly scope for variation in this complex system. For example, physiological fibrinolysis is notably affected by the intensity of normal physical activity (22). All these features of plasmin, combined with the importance of mechanical forces, are consistent with the prevalence of TTR amyloid deposition in the heart and carpal tunnel, both of which are notable sites of continuous repetitive vigorous movement. The pathogenetic significance of plasmin also opens a broad and completely novel perspective for investigation of factors that may determine individual susceptibility and the natural history of the familial and acquired forms of TTR amyloidosis, including the initiation, progression, and tissue distribution of amyloid deposition. In addition, the wholly unexpected and surprising confluence of the fibrinolysis pathway, the physiological remodeling of the extracellular matrix regulated by urokinase (23), and the pathogenesis of TTR amyloidosis are of considerable fundamental interest.

Figure 4. From fibrin to fibrils. A and B, spectrophotometric absorbance/light scattering at 350 nm (A) and amyloid-specific ThT emission fluorescence (B) during clotting of fibrinogen to fibrin (phase I) followed by fibrinolysis in the presence of S52P TTR (red) or of the highly stable T119M TTR variant (blue) (phase II). Following fibrinolysis, increase in turbidity and ThT were observed in the presence of S52P TTR whereas neither of these signals increased when T119M TTR was present instead. Arrows indicate addition of tPA, plasminogen, and TTR. The results shown are the mean ± S.D. of three independent experiments. C, wells containing a solution of fibrinogen in the presence of 1) thrombin and 2) fibrin clot; 3) a solution of tPA, plasminogen, and TTR layered over the clot surface; 4) fibrinolysis with no further aggregation in the presence of T119M TTR; 5) fibrinolysis in the presence of S52P TTR showing the turbidity of amyloid fibril formation. D–F, surface plots of topographic tapping mode AFM images showing (D) the presence of fibrillar structures in the sample containing clot, tPA, plasminogen, and S52P TTR; (E) and (F) the presence of globular structures in samples containing clot, tPA, and plasminogen (E) in the presence of T119M TTR or (F) in the absence of any TTR isoform.
Experimental procedures

Reagents

Recombinant TTR variants were expressed and purified as described previously (21). Human fibrinogen was isolated from citrate-heparin–treated human plasma by affinity chromatography on recombinant clamping factor 221–559 fragment (24) and was absorbed with lysine-Sepharose 4B and gelatin-Sepharose 4B to remove traces of plasminogen and fibronectin, respectively. Enzymes purchased from Sigma-Aldrich were plasmin (P1867), proteinase K (P2308), chymotrypsin (C2160000), tPA (T0831), plasminogen (SRP6518), thrombin (T7572), and tryptase (T7063). Tryptase was purchased from Promega (V5280) and recombinant human kallikrein 12 from Biotechne (3095-S.E.). All the enzymes used were able to cleave the C-terminal end of Lys in the D-Val-Leu-Lys 4-nitroanilide dihydrochloride peptide (Sigma-Aldrich, V0882) following the manufacturer’s instructions. All other reagents including α2-antiplasmin (SRP6313) were purchased from Sigma-Aldrich unless otherwise stated.

MEROPS database search

MEROPS (https://www.ebi.ac.uk/merops/) is a manually annotated database with information on more than 4000 peptidases classified according to families and clans. The residues of the proteolytic substrate are designated Pn—P4—P3—P2—P1—Pm, with Pm indicating the scissile bond. Substrate specificity was based on the frequency of Lys at position P1.

Proteolysis and fibrillogenesis of S52P TTR

Recombinant S52P TTR, 100 μl volumes at 0.5 mg/ml in 20 mM Tris–HCl containing 150 mM NaCl, 5 mM CaCl2, pH 7.4, containing 10 μM ThT (25) was incubated at 37 °C in Costar 96-well black plates in the presence of a protease at an enzyme:substrate ratio of 1:50. Plasmin, trypsin, thrombin, proteinase K, chymotrypsin, trypase alpha, and kallikrein 12 were tested. The plate was sealed with clear sealing film and subjected to 900 rpm double-orbital shaking. Bottom fluorescence was recorded at 500-s intervals (BMG LABTECH, FLUOstar Omega). Homogenous 15% SDS-PAGE (GE Healthcare) under reducing conditions was performed in a 15% polyacrylamide gel.
conditions was used to analyze protein composition before and after fibril formation. After electrophoretic separation, samples treated and untreated with trypsin or plasmin were blotted onto an activated PVDF membrane. Western blotting was developed with polyclonal sheep anti-human TTR (6 ng/μl, The Binding Site, United Kingdom/code AU066X) and polyclonal rabbit anti-sheet peroxidase conjugate (1.3 μg/ml, Dako, Denmark/code P0163) as primary and secondary antibodies, respectively. Peroxidase activity was visualized using a precipitating substrate containing 3,3′-diaminobenzidine and urea hydrogen peroxide (SigmaFAST DAB tablets, Sigma-Aldrich).

**Effect of α2-antiplasmin on TTR fibril formation**

Recombinant S52P TTR in 200 μl volumes at 1 mg/ml in 20 mM Tris-HCl at pH 7.5, containing 150 mM NaCl, 5 mM CaCl2, 10 μM ThT, was incubated at 37 °C in sealed Costar 24-well black-wall plates, together with 20 ng/μl of plasmin while subjected to 900 rpm double-orbital shaking in the presence of 0.09, 0.18, 0.36, and 0.72 μM α2-antiplasmin and in its absence. Based on an average molecular mass of 55 kDa for plasmin, the selected inhibitor concentrations corresponded to molar ratios to plasmin of 0.25:1, 0.5:1, 1:1, and 2:1, respectively. ThT fluorescence emission was monitored using a BMG LABTECH FLUOstar Omega plate reader. Data were normalized to the ThT signal plateau reached in the samples without the plasmin inhibitor. All experiments were conducted in triplicate.

**Fibrillogenesis of TTR variants and WT TTR**

Recombinant S52P, V30M, L55P, V122I, WT and T119M TTR in 500 μl volumes at 1 mg/ml in 20 mM Tris-HCl containing 150 mM NaCl, 5 mM CaCl2, 10 μM ThT, pH 7.5 were incubated at 37 °C in sealed Costar 24-well black-wall plates, together with 20 ng/μl of plasmin while subjected to 900 rpm double-orbital shaking. ThT fluorescence emission was monitored until it reached a plateau. All experiments were conducted in triplicate.

**Preparation of amyloid seeds from S52P TTR with plasmin**

S52P TTR at 1 mg/ml in 20 mM Tris-HCl, 150 mM NaCl, 5 mM CaCl2, 10 μM ThT, pH 7.5 was incubated at 37 °C with plasmin at an enzyme:substrate ratio of 1:50 w/w in volume of 200 μl in a 96-well black-wall plate. The plate was sealed with clear sealing film, subjected to 900 rpm double-orbital shaking and bottom fluorescence was recorded (BMG LABTECH FLUOstar Omega). Aliquots of the final ThT positive material were deposited on freshly cleaved mica and dried under mild vacuum. Samples in which no pellet was present were diluted and deposited as described above, but after drying they were rinsed with water to remove excess salts. Tapping mode AFM images were acquired in air using a multimode scanning probe microscope equipped with an “E” scanning head (maximum
scan size, 10 μm) and driven by a NanoScope V controller (Digital Instruments, Bruker). Single-beam uncoated silicon cantilevers (type OMCL-AC160TS, Olympus and TESPA-Digital Instruments, Bruker). Single-beam uncoated silicon

References


Plasmin primes TTR amyloidogenesis

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Plasminogen activation triggers transthyretin amyloidogenesis in vitro

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