

# Chapter 19

## $\beta_2$ -Microglobulin Amyloidosis

Dorthe B. Corlin and Niels H. H. Heegaard

**Abstract** Dialysis-related amyloidosis (DRA) is a clinical syndrome of pain, loss of function and other symptoms due to the deposition of amyloid consisting of  $\beta_2$ -microglobulin ( $\beta_2m$ ) in the musculoskeletal system. The condition is seen in patients who suffer from chronic kidney disease and are treated with hemodialysis for a long time. Even though  $\beta_2m$  easily can be manipulated to form amyloid in laboratory experiments under non-physiological conditions the precise mechanisms involved in the formation of  $\beta_2m$ -amyloid in patients with DRA have been difficult to unravel. The current knowledge which is reviewed here indicates that conformational fluctuations centered around the D-strand, the DE-loop, and around the *cis*-configured Pro32 peptide bond are involved in  $\beta_2m$  amyloidosis. Also required are highly increased concentrations of circulating  $\beta_2m$  and possibly various post-translational modifications mediated by the pro-inflammatory environment in uremic blood, together with the influence of divalent metal ions (specifically  $Cu^{2+}$ ), uremic toxins, and dialysis-enhanced redox-processes. It seems plausible that domain-swapped  $\beta_2m$  dimers act as building blocks of  $\beta$ -spine cross- $\beta$ -sheet fibrils consisting of otherwise globular, roughly natively folded protein. An activated complement system and cellular activation perpetuate these reactions which due to the affinity of  $\beta_2m$ -amyloid for the collagen of synovial surfaces result in the DRA syndrome.

**Keywords** Dialysis-related amyloidosis ·  $\beta_2$ -microglobulin ·  $A\beta_2m$

### Abbreviations

AA	Amino Acid
$A\beta_2m$	$\beta_2$ -microglobulin amyloidosis
$\beta_2m$	$\beta_2$ -microglobulin
DRA	Dialysis-Related Amyloidosis
GAG	Glycosaminoglycan
GFR	Glomerular Filtration Rate

---

N. H. H. Heegaard (✉) · D. B. Corlin  
Department of Clinical Biochemistry and Immunology,  
Division of Microbiology and Diagnostics, Statens Serum Institut,  
Bldg. 85/240, Artillerivej 5, 2300 Copenhagen S, Denmark  
e-mail: NHE@ssi.dk

D. B. Corlin  
e-mail: dbcw@novonordisk.com

MHC Major Histocompatibility Complex

SAP Serum Amyloid P component

## 19.1 Introduction

Despite normally being a small soluble protein in the circulation or in complex with membrane proteins on cell surfaces,  $\beta_2$ -microglobulin ( $\beta_2$ m) is prone to form amyloid under specific conditions *in vitro* as well as *in vivo*. Thus,  $\beta_2$ m is the fibrillating culprit in  $A\beta_2$ m, a systemic type of amyloidosis that is first and foremost associated with chronic hemodialysis in a clinical syndrome named dialysis-related amyloidosis (DRA). Due to its small size ( $M_r = 11,729$ )  $\beta_2$ m is present in the glomerular filtrate of the normal kidney and  $\beta_2$ m was originally discovered as a component present in the urine of patients with tubular proteinuria, i.e. patients with defects in the reabsorption of  $\beta_2$ m in the proximal tubules of the kidneys (Berggård 1965; Berggard and Bearn 1968). While the DRA syndrome was defined earlier (Assenat et al. 1980; Charra et al. 1984; Kuntz et al. 1984; Warren and Otieno 1975) it was not until 1985 that it was discovered that  $\beta_2$ m is the major constituent of the amyloid deposits in osteoarticular tissues encountered in long-term hemodialysis patients with DRA symptoms (Gejyo et al. 1985; Gorevic et al. 1985, 1986).

In addition to its importance for patients with DRA  $\beta_2$ m is also an easily accessible model protein for *in vitro* studies of protein amyloidogenesis. Many of these aspects have been covered in several recent reviews (Drueke and Massy 2009; Eichner and Radford 2011; Heegaard 2009; Yamamoto et al. 2009). While Chap. 8 of this book focuses on the experimental aspects of  $\beta_2$ m misfolding and fibrillogenesis *in vitro* the present chapter is focused on the molecular pathology of  $\beta_2$ m under *in vivo* conditions where  $\beta_2$ m amyloidosis is encountered.

## 19.2 $\beta_2$ -Microglobulin in Physiology

$\beta_2$ m is present on the surface of all nucleated cells where it is the invariant chain in complex with and stabilizing membrane-anchored peptide- and lipid-presenting polypeptide chains (MHC class I and CD1 complexes) and Fc-receptors. It is also found as a conformationally less restricted (Hodkinson et al. 2009; Verdone et al. 2002) free monomer in blood and other biofluids, including synovial fluid (Gobezie et al. 2007), at low concentration (1.5–3  $\mu\text{g/ml}$  (128–256 nM) in serum) (Karlsson et al. 1980). The normal physiological function, if any, of the freely circulating  $\beta_2$ m is unknown.

### 19.2.1 $\beta_2$ -Microglobulin in the Healthy Organism

$\beta_2$ m associates non-covalently with MHC class I and CD1 complex heavy chains during synthesis to form MHC molecules that present peptides of 8–10 residues

processed from intracellular proteins for cytotoxic T-lymphocytes (Doherty and Zinkernagel 1975) or antigenic lipids for natural killer T-cells in the case of the CD1 complex (Gumperz 2006; Kang and Cresswell 2002).  $\beta_2$ m is required for efficient transport of nascent MHC class I heavy chains to the cell surface (Williams et al. 1989; Zijlstra et al. 1990).  $\beta_2$ m is also present in association with other types of MHC Class I-like cell receptors, such as the IgG Fc receptor (Praetor and Hunziker 2002) and the HFE hemochromatosis membrane protein (Feder et al. 1997).  $\beta_2$ m is critical for the stability and function of these receptors but is not directly involved in their binding of ligands (Otten et al. 1992).

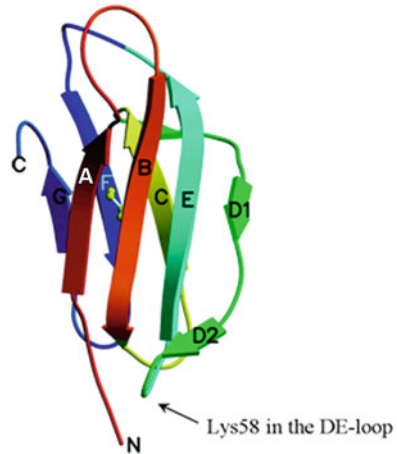
A variable number of MHC class I molecules are expressed on cell membranes from different cells. In the case of human lymphocytes it has been estimated that there are  $10^5$ – $10^6$   $\beta_2$ m-molecules/cell (Bjorck et al. 1979). When MHC is degraded, the MHC-associated  $\beta_2$ m is released to the circulation (Cresswell et al. 1974). This results in a quite constant production of free (monomeric)  $\beta_2$ m at a level of  $0.13 \text{ mg/h} \times \text{kg body weight}$  under normal conditions. More than 99.9 % of this circulating  $\beta_2$ m is filtered through the kidney glomeruli into the proximal tubules where it is reabsorbed by pinocytosis or by binding to the endocytic multiligand receptor megalin. Subsequently, it is degraded by the tubular cells (Karlsson et al. 1980; Saito et al. 1994). As a result, less than 0.1 % of the glomeruli-filtered  $\beta_2$ m is excreted in the urine under normal conditions. A half-time for free  $\beta_2$ m in the circulation of only about 60 min can be estimated on the basis of its serum concentration and production rate and the normal glomerular filtration rate and discounting exchange with cell-surface associated  $\beta_2$ m. These parameters fluctuate only minimally under normal physiological conditions (Karlsson et al. 1980). In renal disorders (e.g., cadmium poisoning) where proximal tubular function is destroyed and tubular proteinemia ensues, the GFR and  $\beta_2$ m serum concentrations are normal, but  $\beta_2$ m is lost in the urine. Urine from patients suffering from defects in the function of proximal tubules is therefore a convenient source of  $\beta_2$ m that can be purified to homogeneity by a combination of size-exclusion chromatography and chromatofocusing (Nissen et al. 1987, 1997).

## 19.2.2 $\beta_2$ -Microglobulin Structure

A detailed review of  $\beta_2$ m conformational structure and dynamics is provided by Chap. 7 by Esposito et al. Here, the features that are most relevant and important for the generation of  $\beta_2$ m amyloid *in vivo* are summarized.

$\beta_2$ m is a 99 residue protein and has a molecular weight of 11,729 Da. It folds natively into a seven-stranded antiparallel  $\beta$ -sandwich consisting of two  $\beta$ -sheets linked covalently by one intramolecular disulfide bond between Cys25 and Cys80. One sheet contains four strands (A, B, E and D) and the other sheet three (C, F and G) (Fig. 19.1). Native  $\beta_2$ m as well as amyloid  $\beta_2$ m are highly structured, tightly packed proteins. Strands B and F, connected by the disulfide bond, are the most protected part of the protein. Strands A, C, E, and G are moderately protected, leaving the

**Fig. 19.1** Schematic  $\beta_2m$  structure (PDB: 1DUZ) (Saper et al. 1991). The A-G  $\beta$ -strands are labelled and the DE-loop with the Lysine-58 cleavage point is marked by the arrow



D-strand connecting the two  $\beta$ -sheets of the  $\beta_2m$  sandwich as the most exposed part of the monomeric native protein (Villanueva et al. 2004). The D-strand interacts with the  $\alpha$ -heavy chain in the MHC class II cell membrane complex (Khan et al. 2000; Saper et al. 1991). The loop between the D and E strands is a strained but dynamic part of the molecule that may switch between several conformations (Okon et al. 1992; Verdone et al. 2002).

### 19.3 $\beta_2$ -Microglobulin in Pathology

Levels of plasma  $\beta_2m$  increase in lymphoproliferative disorders, in chronic renal failure (with decreased filtration), in inflammation and infection and in other conditions with high cell turnover (Wu 2006). A relationship between tumor burden in certain lymphoproliferative disorders, particularly multiple myeloma, and serum levels of  $\beta_2m$  thus has been noted.  $\beta_2m$  is lost in the urine in conditions where proximal tubular function is impaired.

While elevated plasma  $\beta_2m$  levels are absolutely necessary for the development of DRA it is not sufficient since increased circulating levels of  $\beta_2m$  where the kidney function is normal e.g. in chronic immunoinflammatory conditions, in infections and hematopoietic malignancies (Bartl et al. 1989; Bhalla et al. 1985; Bourantas et al. 1999) do not lead to  $\beta_2m$  amyloidosis. In agreement with this there are no signs of  $\beta_2m$  amyloidosis in engineered mice models with highly increased human  $\beta_2m$  in the circulation (Zhang et al. 2010). A sustained increase in  $\beta_2m$  concentration in the circulation combined with specific abnormalities (e.g. inflammation and oxidative stress (Annuk et al. 2005; Terawaki et al. 2004)) encountered in renal insufficiency and/or the process of hemodialysis thus are necessary for the development of DRA.

## 19.4 $\beta_2$ -Microglobulin in Dialysis-Related Amyloidosis

The incidence of DRA is seemingly declining with the increased use of high-flux dialysis, with changes in dialysate composition, and as the components of dialysis machines that come into contact with patient blood (dialysis membranes and tubings) have become more biocompatible (Fujimori 2011; Schwalbe et al. 1997; Winchester et al. 2003).

### 19.4.1 *The Clinical Syndrome*

DRA results from the deposition and accumulation of  $\beta_2$ m amyloid (Floege and Ehlerding 1996; Gejyo et al. 1985; Gorevic et al. 1985). Deposition primarily takes place in osteoarticular tissue. The clinical manifestations of DRA include erosive and destructive osteoarthropathy, destructive spondyloarthropathy, cystic bone lesions, carpal tunnel syndrome, and other neuropathies (Kiss et al. 2005) (Fig. 19.2) leading to joint pain, decreased function, and fractures.

In addition,  $\beta_2$ m amyloid deposits may be found in extra-osteoarticular tissues, including the heart, gastrointestinal tract, ovaries, liver, ureter and subcutaneous tissue (Buchholz et al. 1995; Kawano et al. 1998; Mogyorosi and Schubert 1999; Mount et al. 2002; Shimizu et al. 2003). Amyloid deposits outside the musculoskeletal system are often asymptomatic but may cause serious complications such as heart failure or gastrointestinal bleeding. Extensive deposition of  $\beta_2$ m amyloid in the small blood vessels in the myocardium may cause calcification of the heart and cardiac dysfunction, thereby contributing to the occurrence of heart disease in long-term dialysis patients (Takayama et al. 2001).

The prevalence of DRA may also be influenced by patient age and duration of dialysis treatment (Jadoul 1998; Jadoul et al. 1997). Recent studies, however, fail to demonstrate a clear correlation between clinical manifestations (carpal tunnel syndrome) and duration of end-stage kidney disease and hemodialysis (Kwon et al. 2011). Furthermore, the type of hemodialysis, e.g. high-flux/low-flux dialyzers, membrane type, dialysate composition and purity, and biocompatibility of the material in contact with the biofluids are all factors that may influence the development of amyloid deposits (Baz et al. 1991; Campistol et al. 1999; Locatelli et al. 1999; van Ypersele et al. 1991) even though there are no clear effects on circulating oxidative stress parameters (Schneider et al. 2011). There is no direct correlation between the efficiency of  $\beta_2$ m removal and the rate of progress of DRA (Danesh and Ho 2001; Kwon et al. 2011).

### 19.4.2 *$\beta_2$ -Microglobulin Aggregation, Fibrillation, and Amyloidogenesis*

A great number of *in vitro* conditions have been shown to induce amyloid fibrillation of  $\beta_2$ m (cf. Chap. 8). These conditions include extremes of pH, amino acid



**Fig. 19.2** Magnetic resonance imaging of humeral erosions in a 63-year-old man on hemodialysis for 31 years for chronic glomerulonephritis with biopsy-proven dialysis-related amyloidosis. Coronal T1-weighted MR image (470/12) shows osteolysis in superior-posterior humeral head, which communicates with joint (*arrow*). Low-signal-intensity tissue representing amyloid appears within lesion. Amyloid deposits are also visible within subdeltoid bursa between deltoid muscle and humerus (*arrowheads*). (Reproduced with permission from Fig. 4 in Kiss et al. 2005)

substitutions, anionic detergent, addition of trifluoroethanol, ultrasonication, agitation, heat treatment, incubation with  $\text{Cu}^{2+}$  (Morgan et al. 2001; Ohhashi et al. 2005; Sasahara et al. 2007; Yamamoto et al. 2004a) and other factors (Heegaard 2009). Most of these conditions are unlikely to be encountered *in vivo* except for slightly decreased pH at inflammatory sites (Treuhaft and McCarty 1971), increased divalent cations (especially  $\text{Cu}^{2+}$ ), Proline-32 *cis*-to-*trans* isomerisation, and Lysine-58 cleaved forms of  $\beta_2\text{m}$  (cf. Table 19.1) (Eichner and Radford 2011; Heegaard 2009).

Some of the fibrillation pathways do not necessarily involve major conformational changes (Calabrese et al. 2008; Eakin et al. 2006; Liu et al. 2011). This is in accord with the lack of correlation between thermodynamic stability and tendency to amyloid formation of  $\beta_2\text{m}$  variants (Smith et al. 2003). Thus, highly conformationally unstable  $\beta_2\text{m}$  variants may readily unfold and aggregate but do not in every case form amyloid. Amyloid fibrillation appears to be linked to the conformational dynamics of retained globular folds e.g. leading to domain swapped dimers as the building blocks in fibril formation (Hafner-Bratkovic et al. 2011; Liu et al. 2011; Sambashivan et al. 2005; Wahlbom et al. 2007; Domanska et al. 2011; Mendoza et al. 2011). Such mechanisms are compatible with limited structural rearrangements e.g. *cis*-to-*trans* isomerisation of the Pro-32 bond or rearrangement or perturbations of the DE-loop in the globular fold (Barbet-Massin et al. 2010; Colombo et al. 2011;

**Table 19.1** Modifications reported on A $\beta_2$ m *in vivo* (based on Table I in Heegaard 2009)

Modification	Circulating $\beta_2$ m	Amyloid $\beta_2$ m	Comments	References
Advanced glycation end products (AGE)	Yes	Yes	By mass spectrometry, and by immunochemical methods. Primary amino groups may be AGE modified. AGE (such as 3-deoxyglucosone) may then mediate crosslinks e.g. between lysine and arginine side chains. AGE-adducted $\beta_2$ m inhibits fibril extension <i>in vivo</i> (Hashimoto et al. 1999)	Brancaccio et al. 2000; Miyata et al. 1993, 1996; Niwa et al. 1996, 1997; Argiles et al. 1995; Niwa and Tsukushi 2001; Tang et al. 1996
Dimerization, oligomerization	Yes	Yes	Size-exclusion chromatography and SDS-PAGE, 2D-PAGE	Garcia-Garcia et al. 1997; Gorevic et al. 1986; Niwa et al. 1996 Liu et al. 2011
Disulfide exchanged and domain swapped higher oligomers with intermolecular disulfides	No	Yes	$\beta_2$ m fibrillation <i>via</i> 3D domain swapping with intermolecular disulfide linkages requires breaking of intramolecular Cys(25)-Cys(80) bond. Disulfide rearrangement has been observed in other amyloidogenic proteins (Domanska et al. 2011; Knaus et al. 2001; Lee and Eisenberg 2003; Nilsson et al. 2004; Wahlboom et al. 2007)	
N-terminal truncations ( $\Delta$ N3, $\Delta$ N6, $\Delta$ N19)	No	Yes	Also partial deamidation of Asn17 and Asn42 and oxidation of Met99 in fibrillar $\beta_2$ m. $\Delta$ N6 constitutes up to 26% of <i>ex vivo</i> $\beta_2$ m amyloid	Linke et al. 1989; Stoppini et al. 2000, 2005
$\Delta$ K58	Yes	No	Examined in fibril extracts from two different patients. Found circulating in sera from some dialysis patients, not in controls. The variant fibrillates spontaneously at near-physiological pH (Corfin et al. 2009)	Corlin et al. 2005; Giorgetti et al. 2007
Solubility	No	Yes	Amyloid fibril heterogeneity results in different aqueous extractability	Bellotti et al. 1998; Stoppini et al. 2005
Acidic isoforms (pI 5.7, 5.3, and 4.5–5.0)	Yes (serum and urine)	Yes	pI 5.3 isoform found as both monomer and dimer in sera from patients	Argiles et al. 1992, 1995; Miyata et al. 1993; Ogawa et al. 1988;
Deamidation (Asn17 $\Rightarrow$ Asp17)	(Yes)—in ultrafiltrate	No	May be <i>in vitro</i> artifact (Vincent et al. 1994)	Vincent et al. 1994 Gorevic et al. 1985; Odani et al. 1990
Acidic isoforms and deamidation $\Rightarrow$ Asp42	No	Yes		Gorevic et al. 1985
Complexation with $\alpha_2$ -macroglobulin	Yes	Yes		Argiles et al. 1989; Motomiya et al. 2003; Gouin-Charnet et al. 2000

Eichner and Radford 2009; Eichner and Radford 2011; Mimmi et al. 2006; White et al. 2009) and has recently also been shown to be a plausible mechanism involved in  $\Delta N6\beta_2m$  amyloid formation (Domanska et al. 2011). While increasingly supported by experimental data these mechanisms have not yet been conclusively tested with *in vivo*-generated material.

The kinetics of *in vitro* generation of amyloid-like fibrils displays characteristic traits in which a lag phase precedes a period of exponential growth. During this lag phase the early aggregates or nuclei are generated, and the subsequent rapid fibril elongation corresponds to the exponential growth period. The majority of *ex vivo*  $\beta_2m$  amyloid (i.e. material isolated from clinical specimens and studied in the laboratory) appears to consist of aggregated full-length, wild-type  $\beta_2m$  (Gorevic et al. 1986). However, natively folded  $\beta_2m$  is not prone to self-aggregate at neutral pH even at vastly supraphysiological concentrations (Eakin and Miranker 2005; Kad et al. 2001; Myers et al. 2006) and, as mentioned above, *in vivo* fibrillogenesis will not be initiated simply by the presence of high  $\beta_2m$  plasma concentrations. It is likely that the assembly of  $\beta_2m$  monomers into *bona fide* amyloid fibril requires encounters between partially unfolded molecules. Under native conditions this is extremely rare, and possibly explains why the generation of amyloid *in vivo* is a process that occurs over prolonged periods of time, usually several years.

#### 19.4.2.1 $\beta_2m$ Post-Translational Modifications

The conformational dynamics of  $\beta_2m$  has been studied using H/D-exchange experiments monitored by e.g. mass spectrometry (Heegaard et al. 2005; Hodkinson et al. 2009; Jørgensen et al. 2007). These experiments show the capability of  $\beta_2m$  to undergo transient regional unfolding-refolding under physiological conditions and also document the increased rate of unfolding—even though the molecule has the same overall conformation as the wild-type (Mimmi et al. 2006)—of a cleaved variant of  $\beta_2m$  termed  $\Delta K58\text{-}\beta_2m$  in which Lys58 has been proteolytically removed (Heegaard et al. 2005). This post-translationally modified  $\beta_2m$  has been found in the circulation of many (20–40 %) hemodialysis patients (Corlin et al. 2005), and serum levels are markedly higher in patients dialyzed with the less biocompatible, copper containing low-flux membranes (Cuprothane), than in those treated with synthetic high-flux membranes.  $\Delta K58\text{-}\beta_2m$  is thought to be generated *in vivo* as a consequence of the activation of the complement system by cleavage of  $\beta_2m$  by the serine protease C1s between Lys58 and Asp59 in the DE-loop followed by the rapid removal of Lys58 by carboxypeptidase B activities (Nissen et al. 1990). The removal of Lys58 changes the protein from a single chain molecule with an intrachain disulphide bond to two chains held together by an interchain disulphide bond. The two chains consist of residues 1–57 and 59–99 of native  $\beta_2m$ . The removal of Lys58 only results in minor global conformational changes (Mimmi et al. 2006) but a positively charged residue that otherwise could contribute to electrostatic repulsion between  $\beta_2m$  molecules is lost. This makes the AA59–70 region of the molecule more hydrophobic and aggregation-prone. Also, the molecule is considerably more conformationally unstable as shown



by capillary electrophoresis experiments (Heegaard et al. 2002). Thus the cleavage of  $\beta_2$ m induces significantly accelerated (about 10 times) rates of cooperative unfolding at physiological temperature, increasing molecular aggregation and the ability to generate fibrils with amyloid features (Heegaard et al. 2005).

Other posttranslationally modified species e.g.  $\beta_2$ m with deletions of N-terminal residues (Esposito et al. 2000) (Table 19.1) are also of interest for the *in vivo* situation because catalytic amounts (1 %) are sufficient to induce conversion of native  $\beta_2$ m into  $A\beta_2$ m *in vitro* (Eichner and Radford 2011). A catalytic effect was also shown for fibrils of Lys-58 cleaved  $\beta_2$ m formed in the presence of heparin sulfate (Corlin et al. 2010). N-terminally truncated  $\beta_2$ m has been found to be a significant constituent of  $A\beta_2$ m fibrils *ex vivo* (Stoppini et al. 2000, 2005) (cf. below). The  $\Delta$ N6 truncation of  $\beta_2$ m (Bellotti et al. 1998; Linke et al. 1987, 1989; Stoppini et al. 2000, 2005) results in structural rearrangements of the protein rendering the N-terminal region more disordered at the same time as the  $\beta$ -bulge of strand D, the successive loop, and strand E all adopt a less flexible conformation. The  $\Delta$ N6 $\beta_2$ m-variant has been shown to have a reduced free energy of stabilization as compared to native  $\beta_2$ m and to possess an enhanced tendency to precipitate and self-aggregate (Esposito et al. 2000). At pH lower than 7,  $\Delta$ N6 $\beta_2$ m generates amorphous aggregates and short fibrillar structures, and at neutral pH this variant possesses the ability to further extend *ex vivo*  $\beta_2$ m fibrils. Moreover, examination of a putative role of collagen on  $\beta_2$ m fibril formation showed that the presence of  $\Delta$ N6 $\beta_2$ m together with collagen induced the generation of  $\beta_2$ m amyloid-like fibrils (Canale et al. 2011; Relini et al. 2006).

Posttranslational modifications in the form of truncations and cleavages thus significantly decrease  $\beta_2$ m solubility at physiological conditions and are likely to occur *in vivo*. Measurements suggest that up to 30 % of the  $\beta_2$ m in amyloid fibers extracted from *ex vivo* deposits are present as N-terminally modified or truncated forms while Lys-58 cleaved  $\beta_2$ m could not be detected in the same extracts (Bellotti et al. 1998; Floege and Ehlerding 1996; Giorgetti et al. 2007; Linke et al. 1986). Conversely, Lys-58 cleaved  $\beta_2$ m has been demonstrated in the circulation of many dialysis patients while N-terminally truncated  $\beta_2$ m variants have only been demonstrated in amyloid deposits.

#### 19.4.2.2 $\beta_2$ m Pro32-Isomers and Conformers

In native, wild-type  $\beta_2$ m the peptide bond preceding Pro32 attains the less common, thermodynamically unfavorable *cis* configuration (Jahn et al. 2006). Engineered mutations changing Pro32 to glycine or alanine, causing the peptide bond to stay in the *trans* configuration, result in markedly increased rates of amyloid fiber growth and in a repacking of the hydrophobic core of the protein wherein the backbone changes in the BC loop affect the conformation of strand D and the DE loop (Jahn et al. 2006; Eakin et al. 2006). Thus, a  $\beta_2$ m folding intermediate in which Pro32 is kept in *trans* configuration does not bind the MHC heavy chain well (Esposito et al. 2008) and is much more prone to aggregation than the wild-type  $\beta_2$ m molecule (Jahn et al. 2006). Since the isomerization of this bond is the slowest step in the folding

of native  $\beta_2m$  (Eakin et al. 2006; Eichner and Radford 2009; Kameda et al. 2005), a population of protein intermediates in the non-native *trans* configuration may exist and be of significance in fibril formation. Interestingly, chelation of  $Cu^{2+}$  by His31 promotes Pro32 isomerization (Eakin et al. 2002).

A  $\beta$ -bulge in this D strand is straightened out in infrequently encountered  $\beta_2m$  structures and this may favour oligomerization by edge strand docking (Richardson and Richardson 2002) possibly facilitating amyloid formation (Trinh et al. 2002). However, recent data do not support a role for the  $\beta$ -bulge in protecting against amyloid formation (Azinas et al. 2011).

#### 19.4.2.3 $\beta_2m$ - $Cu^{2+}$ Interactions

Many proteins are known to have specific binding sites for metal ions, and binding of ions can significantly alter the properties of the protein.  $\beta_2m$  binds metal cations, in particular  $Cu^{2+}$ , and this binding will, under the proper experimental conditions, result in the generation of fibrous  $\beta_2m$  aggregates (Eakin et al. 2002). The binding of  $Cu^{2+}$  to  $\beta_2m$  is specific, while other divalent cations such as  $Ca^{2+}$  and  $Zn^{2+}$  bind nonspecifically (Morgan et al. 2001). Studies have indicated that binding of  $Cu^{2+}$  to  $\beta_2m$  lower the energy barrier of transition (Deng et al. 2006) to a partially unfolded state. The binding of  $Cu^{2+}$  (chiefly by His31) promoting these local molecular rearrangements has also been proposed to enhance the *cis-trans* isomerization at Pro32 leading to a partial unfolding of the hydrophobic core (Eakin et al. 2004) and thus increasing conformational instability (De Lorenzi et al. 2008). From a pathophysiological point of view, the relevance of  $Cu^{2+}$  to  $\beta_2m$  amyloidogenesis in DRA is evident, not only because of the former use of the Cuprophane membranes containing copper, but also since a dialysis patient is exposed to more than 100 L of dialysate during each dialysis session (Cheung et al. 2006). The dialysate contains copper to a maximum level of 1.6  $\mu M$  (Vorbeck-Meister et al. 1999), being within a factor of two of the measured affinity of  $Cu^{2+}$  and  $\beta_2m$  (Eakin et al. 2002).

#### 19.4.2.4 $\beta_2m$ -amyloid Seeding

Addition of nucleating seeds (preformed  $\beta_2m$  fibrils) to a solution of native  $\beta_2m$  will result in extensive fibrillation by elongation of the existing fibrils. In a study by Jones et al. (2003), a range of peptides of  $\beta_2m$  were examined for their ability to assemble into amyloid-like fibrils *in vitro* and for their ability to act as seeds for full-length  $\beta_2m$ . The study showed that only peptides having a sequence corresponding to strand E in wild-type  $\beta_2m$  (E peptides) were capable of fibrillation, even when studied over a wide pH range. These fibrils also had the ability to seed the formation of fibrils in a solution of full-length  $\beta_2m$ . The E peptides contain a very large number of aromatic amino acids, i.e., the aromatic residues Phe56, Trp60, Phe62 and Tyr63 which are all important for hydrophobic interactions of  $\beta_2m$  with the heavy chain in the MHC class I complex (Saper et al. 1991). The importance of the DE-loop region is further underscored by a substantial number of experiments showing the

effect of mutations and posttranslational modifications in the D-strand and DE-loop region (AA50–63) (Azinas et al. 2011; Santambrogio et al. 2010) including other nucleation/elongation experiments strongly implicating this aromatic-rich region in amyloidogenic interactions (Platt et al. 2008). The region was shown directly by 2D NMR measurements to be the longest contiguous region involved in intermolecular contacts in low pH-generated fibrils (Debelouchina et al. 2010) and is also central in the putative amyloidogenic structure involved in domain-swapped propagating dimers (see below).

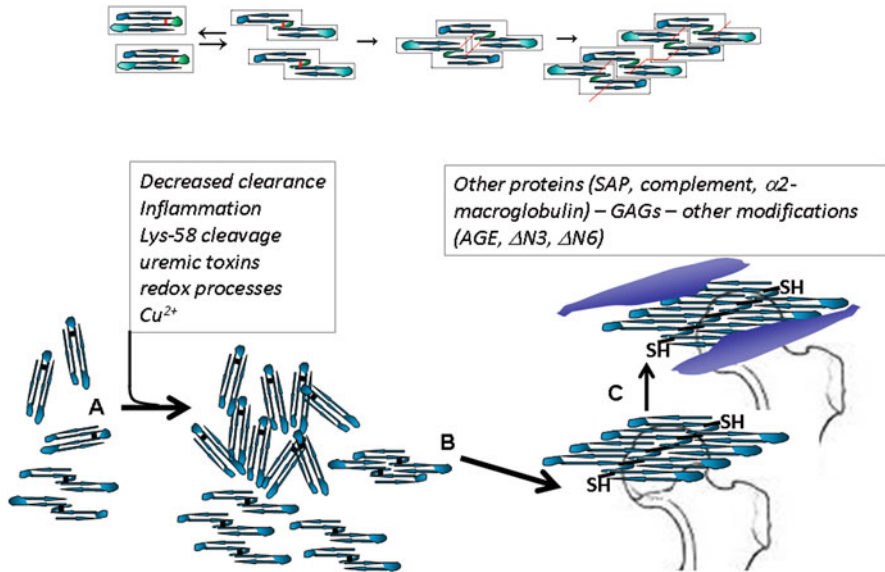
#### 19.4.2.5 Structure of $\beta_2$ m Amyloid Fibrils

All amyloid fibrils, despite having rather different morphologies, share a common cross- $\beta$  spine structure (Jahn et al. 2010). Albeit not fully understood, the propensity of a protein or peptide to form amyloid fibrils is dependent on an interplay between secondary structure, charge, sequence and hydrophobicity.

Experiments using low pH and high salt have shown that in the early aggregation processes  $\beta_2$ m monomers first assemble into dimers and tetramers, then as  $\beta_2$ m aggregates over time, changes in secondary structure can be observed indicative of the emergence of antiparallel intermolecular  $\beta$ -sheet structures (Fabian et al. 2008). In a study using H/D exchange of amide protons combined with NMR analysis the core of the  $\beta_2$ m amyloid fibril was mapped. The result was that in addition to the regions protected from exchange in the native monomeric  $\beta_2$ m, the residues in the native loops also become highly protected in the fibrillar state indicating an increase in the hydrogen bond network in the fibrils, leaving only the N- and C-terminal ends unprotected from exchange. Also, the CD spectrum of  $\beta_2$ m amyloid fibrils showed increased  $\beta$ -sheet content, supporting the suggestion that both the native loops and the native  $\beta$ -strands are transformed into  $\beta$ -sheets in the  $\beta_2$ m amyloid fibrils (Hoshino et al. 2002).

The structure of  $\beta_2$ m amyloid fibrils has been suggested to involve the assembly of six protofilaments, arranged in pairs of three protofilaments, wherein each protofilament is built from globular subunits in a dimer-of-dimers packing (Mendoza et al. 2011; White et al. 2009). Domain-swapped dimers are also at the heart of a model for the  $\beta_2$ m fibril core formation stabilized by disulfide exchange leading to intermolecular disulfide bridges. This leads to a “steric zipper” arrangement with the AA54–59 segment most likely involved and thereby forming the  $\beta$ -spine with retention of the globular features of the other parts of the protein that participates in the amyloid fibril structure (Liu et al. 2011). In another model tetramers are formed by dimer-dimer interactions involving D- and G-strands from the two different dimer-units (Mendoza et al. 2011) while D-D interactions were found unlikely to form dimer interfaces (Mendoza et al. 2010).

Three-dimensional domain swapping is emerging as a common mechanism for amyloid fibril formation (Ecroyd et al. 2010; Hafner-Bratkovic et al. 2011) and entails disulfide exchange to get covalently linked dimer  $\beta_2$ m building blocks forming the steric-zipper spine of amyloid fibrils (Fig. 19.3). This is consistent with the accelerating effect of reducing agents on A $\beta_2$ m formation *in vitro* at physiological



**Fig. 19.3** Schematic model of  $\beta_2m$  amyloidogenesis *in vivo* in chronic hemodialysis patients. Upper panel (based on Liu et al. 2011) shows the disulfide exchange involved in domain-swapped  $\beta_2m$  dimer formation and the proposed structure of a runaway domain-swapped oligomer with inter-dimer disulfide bonds indicated. Lower panel shows a schematic of amyloid formation by  $\beta_2m$ . Three stages are envisioned: (A), normal conditions, monomeric  $\beta_2m$  displays conformational fluctuations (depicted as closed and open domain forms) but is present at normal concentrations; (B) Increased concentrations in renal disease lead to formation of  $\beta_2m$  dimers possibly stabilized by intermolecular disulfide bonding between swapped domains (cf. upper panel); (C) Association, organization and consolidation on collagen of modified and/or dimerized  $\beta_2m$  and then further oligomerization with runaway dimerization as proposed by Eisenberg et al. (Liu et al. 2011) on the seeding surface of collagen-attached  $\beta_2m$

pH in some studies (Liu et al. 2011) and with the fiber polymorphism characteristic of various amyloids. Disulfide rearrangement is well known to occur *in vivo* e.g. in immunoglobulin arm rearrangements (Liu et al. 2010) and in some amyloidogenic proteins (Knaus et al. 2001; Nilsson et al. 2004). The precise mechanisms associated with this model *in vivo* and its relevance for DRA remain to be settled. Domain swapping and stabilization by disulfide exchange have not been shown *ex vivo*  $A\beta_2m$ , and some studies actually show that thiol compounds (reductants) inhibit  $\beta_2m$  amyloid fibril formation at neutral pH (Yamamoto et al. 2008). Also, while there is no doubt that chronic kidney disease and hemodialysis lead to a proinflammatory and oxidatively stressed environment in the circulation (possibly further enhanced by oxidizing  $Cu^{2+}$ -ions) (Lee et al. 2011) the factors associated with chronic kidney disease and hemodialysis that would be especially conducive to breaking intramolecular disulfide bonds in the core of a tightly packed globular protein remain to be characterized.

### 19.4.3 Accessory Molecules

In the attempt to elucidate the early fibrillating events in DRA a number of co-factors have been studied for their ability to elicit and facilitate  $\beta_2$ m fibrillogenesis (Table 19.2). Glycosaminoglycans (GAGs) are long unbranched polysaccharide chains consisting of repeating disaccharide units. Heparan sulfate is a glycosaminoglycan which is expressed on cell surfaces and binds non-covalently to a variety of proteins (Jackson et al. 1991). In many of the amyloidoses heparan sulfate has been shown to be a universal component of amyloid (Jackson et al. 1991; Magnus et al. 1991; Snow et al. 1987, 1988, 1991; Snow and Kisilevsky 1985; Young et al. 1989, 1992) and thus this GAG has been proposed to play an active role in amyloid generation by promoting fibrillogenesis (Castillo et al. 1998; Cohlberg et al. 2002; Goedert et al. 1996) rather than being passively accumulated. Heparan sulfate and heparin (which is commonly administered as an anticoagulant during hemodialysis treatment) have been shown to promote  $\beta_2$ m amyloid formation *in vitro* (Borysik et al. 2007) and to exert a stabilizing effect on such  $\beta_2$ m fibrils (Myers et al. 2006; Yamaguchi et al. 2003; Yamamoto et al. 2004b). Both uremic serum and synovial fluid also have amyloid-enhancing effects in seeding experiments (Myers et al. 2006). Interestingly, a mouse human  $\beta_2$ m model with vastly supraphysiological circulating  $\beta_2$ m levels did not show  $\beta_2$ m-amyloid formation even in the cases where preformed amyloid fibril seeds were injected (Zhang et al. 2010). This again shows that the specific uremic environment of kidney failure patients is an indispensable prerequisite for the triggering of A $\beta_2$ m.

Collagen fibers, which are found in the joint environment, have also been shown to promote  $\beta_2$ m fibrillogenesis (Relini et al. 2006) and could be relevant for DRA pathology. The positively charged collagen molecules are proposed to act as an immobilized surface on which the  $\beta_2$ m molecules bind and become oriented in a fashion facilitating fibril formation. Also apolipoprotein E (ApoE) which is a cholesterol transport protein has been suggested to be of relevance for amyloidogenesis, since it has been found to be ubiquitously co-localized with amyloid deposits in both systemic and localized amyloidoses. However, reports are contradictory showing both promoting and inhibitory effects of ApoE on fibril formation (Naiki et al. 1997; Wisniewski et al. 1994).

Serum amyloid P component (SAP) is a common component of extracellular matrix in the microfibrillar mantle of elastic fibers and in the glomerular basement membrane. SAP binds to amyloid in a calcium-dependent manner and is a universal constituent of all amyloid deposits, comprising up to  $\sim 15\%$  of the amyloid tissue mass (Skinner et al. 1980). SAP itself is highly resistant to proteolysis (Kinoshita et al. 1992) and possesses the ability to also prevent proteolysis of the amyloid fibrils to which it binds (Tennent et al. 1995), thereby possibly contributing to their persistence *in vivo*. Due to its specificity and affinity for amyloid deposits SAP may be used for radioimaging *in vivo* of some systemic amyloidoses (Pepys 2006).

**Table 19.2** Factors with  $\beta_2$ m amyloid-enhancing or inhibiting effects that may be encountered or exploited *in vivo*

Enhancement	Inhibition
Cu(II) (Eakin et al. 2002, 2004)	Small sulfonated drugs/dyes (Regazzoni et al. 2011)
Glycosaminoglycans (seed stabilization) (Myers et al. 2006)	Chaperones, e.g. $\alpha_2$ -macroglobulin (Ozawa et al. 2011a)
Low pH (McParland et al. 2000)	AGE-adducts <i>in vitro</i> (Hashimoto et al. 1999)
Lysophosphatidic acid, non-esterified fatty acids, SDS (Hasegawa et al. 2008; Kardos et al. 2011)	Tetracyclin antibiotics (Giorgetti et al. 2011)
Reductants-oxidation, disulfide exchange (Liu et al. 2011)	Reductants (dithiothreitol, cysteine, tris-(2-carboxyethyl) phosphine) (Ahn et al. 2010; Yamamoto et al. 2008)
Folding intermediates with non-native cis-Pro32-isomers (Eakin et al. 2006)	
Collagen (Myers et al. 2006; Relini et al. 2006)	
$\Delta$ N6- $\beta_2$ m binds more strongly to collagen (Giorgetti et al. 2005)	
$\beta_2$ m cleaved after Lysine-58 (Heegaard et al. 2002)	

#### 19.4.4 Treatment of $\beta_2$ -microglobulin Amyloidosis

Treatment of amyloidosis is difficult. The general strategy is to treat symptoms secondary to organ damage and to reduce the production of amyloid by restricting the production of precursor molecules (Pepys 2006). In the case of  $A\beta_2$ m there is no curative treatment except for reconstitution of renal function by renal transplantation (Pepys 2006). This will halt DRA progression but does not revert established  $\beta_2$ m amyloid deposits (Labriola et al. 2007; Mourad and Argiles 1996) that must be treated by surgery. It is worth noting that amyloid deposits are not static entities but rather go from a more reversible state when newly formed to much more stable irreversibly consolidated fibril formations over time (Kardos et al. 2011). Dye molecules and GAG analogues have been used experimentally based on the notion of inhibiting interactions with accessory molecules. Screening of sulfonated small molecule, tetracycline analogies, and other antibiotics-based potential fibrillogenesis inhibitors (Giorgetti et al. 2011; Regazzoni et al. 2011) has identified promising compounds that may divert aggregation into non-amyloidogenic pathways (Woods et al. 2011). Also tetracycline (Giorgetti et al. 2011) and proteins such as  $\alpha_2$ -macroglobulin (Ozawa et al. 2011a) and compounds stabilizing native protein structures have been launched, especially for treating the transthyretin amyloidoses (Adamski-Werner et al. 2004; Sacchetti and Kelly 2002) but have not yet been explored in DRA. In DRA most work is directed at reducing disease progression by depleting the precursors of  $\beta_2$ m amyloid by optimized dialysis including  $Cu^{2+}$ -free dialysis membranes (Miura et al. 1992; van Ypersele et al. 1991).

Experimental therapies may be derived from preliminary reports of amyloid fibril destruction by laser irradiation of thioflavin T-fibril complexes which has been shown *in vitro* both for keratoepithelin and  $\beta_2$ m-derived peptide fibrils (Ozawa et al. 2011b; Ozawa et al. 2009). The clinical implementation of fibril destruction by amyloid dye-excitation, however, is far from straightforward.

## 19.5 Conclusions and Future Directions

The list of factors that have been shown to influence the conformation of intact  $\beta_2$ m is very long. In DRA-patients, the increased serum concentration is important, but there is no simple correlation between DRA-severity and circulating  $\beta_2$ m concentration (Gejyo et al. 1986), and  $\beta_2$ m-amyloid has not been observed in other diseases where there are sustained, elevated  $\beta_2$ m concentrations. Furthermore, many of the *in vitro* conditions that are highly favorable for amyloid formation from normal  $\beta_2$ m such as very low pH and very high ionic strength are not encountered *in vivo*. Some of the factors that may be relevant enhancers and inhibitors of  $\beta_2$ m amyloidogenicity *in vivo* are listed in Table 19.2. In this regard the catalysis of amyloidogenic conformations by divalent cations, especially  $\text{Cu}^{2+}$  and its relationship to Pro-32 *trans-to-cis* conversion, at neutral pH (Eakin et al. 2002, 2004, 2006) is very interesting. Most, if not all, amyloid proteins have affinities for divalent cations, and metal ions have been proposed as a triggering factor in Alzheimer's disease (Atwood et al. 2000) even though the evidence linking  $\text{Cu}^{2+}$  to enhancement or inhibition of amyloid formation by amyloid  $\beta$ -peptides is ambiguous (Pedersen et al. 2011). Metals are also involved in Parkinson's disease (Uversky et al. 2001), immunoglobulin light chain (Davis et al. 2001), and prion protein amyloidogenesis (Jackson et al. 2001). Furthermore, the presence of a subfraction of cleaved  $\beta_2$ m-species in the amyloid deposits (Linke et al. 1986, 1987, 1989; Stoppini et al. 2000) and of another cleaved variant in the circulation of hemodialysis patients—especially those treated with  $\text{Cu}^{2+}$ -containing, complement-activating dialysis membranes and the demonstration of the decreased conformational stability *in vitro* of such truncated variants (Corazza et al. 2004; Esposito et al. 2000; Heegaard et al. 2005) together with their catalytic effects on amyloidogenesis (Eichner and Radford 2011) provide strong indications of the pathogenetic importance of post-translationally modified  $\beta_2$ m for the development of  $\beta_2$ m amyloidosis in dialysis patients.

The possibility that several factors may interplay in different ways *in vivo* and yet lead to the  $\beta_2$ m amyloidosis syndrome DRA is unlikely since the syndrome is limited to such a well-defined group of patients. In that regard it is an interesting model disease for all types of amyloid.

With a normal half-life in solution of only one hour it is likely that monomeric  $\beta_2$ m may be regarded as a conformationally unstable waste product with unfavorable exposure of hydrophobic side chains in the solvent exposed DE-loop which is otherwise designed to be stabilized by fitting into the MHC class I receptor complex in which  $\beta_2$ m fulfils its proper physiological function.

## References

- Adamski-Werner SL, Palaninathan SK, Sacchetti JC, Kelly JW (2004) Diflunisal analogues stabilize the native state of transthyretin. Potent inhibition of amyloidogenesis. *J Med Chem* 47:355–374
- Ahn M, Kang S, Koo HJ, Lee JH, Lee YS, Paik SR (2010) Nanoporous protein matrix made of amyloid fibrils of beta2-microglobulin. *Biotechnol Prog* 26:1759–1764
- Annuk M, Soveri I, Zilmer M, Lind L, Hulthe J, Fellstrom B (2005) Endothelial function, CRP and oxidative stress in chronic kidney disease. *J Nephrol* 18:721–726.
- Argiles A, Mourad G, Axelrud-Cavadore C, Watrin A, Mion C, Cavadore JC (1989) High-molecular-mass proteins in haemodialysis-associated amyloidosis. *Clin Sci (Lond)* 76:547–552
- Argiles A, Derancourt J, Jauregui-Adell J, Mion C, Demaille JG (1992) Biochemical characterization of serum and urinary beta 2 microglobulin in end-stage renal disease patients. *Nephrol Dial Transplant* 7:1106–1110
- Argiles A, Garcia-Garcia M, Derancourt J, Mourad G, Demaille JG (1995) Beta 2 microglobulin isoforms in healthy individuals and in amyloid deposits. *Kidney Int* 48:1397–1405
- Assenat H, Calemard E, Charra B, Laurent G, Terrat JC, Vanel T (1980) Hémodialyse, syndrome du canal carpien et substance amyloïde. *Nouv Presse Med* 24:1715
- Atwood CS, Scarpa RC, Huang X, Moir RD, Jones WD, Fairlie DP, Tanzi RE, Bush AI (2000) Characterization of copper interactions with alzheimer amyloid beta peptides: identification of an attomolar-affinity copper binding site on amyloid beta1–42. *J Neurochem* 75:1219–1233
- Azinas S, Colombo M, Barbiroli A et al (2011) D-strand perturbation and amyloid propensity in beta-2 microglobulin. *FEBS J* 278:2349–2358
- Barbet-Massin E, Ricagno S, Lewandowski JR, Giorgetti S, Bellotti V, Bolognesi M, Emsley L, Pintacuda G (2010) Fibrillar vs crystalline full-length beta-2-microglobulin studied by high-resolution solid-state NMR spectroscopy. *J Am Chem Soc* 132:5556–5557
- Bartl R, Frisch B, Diem H, Mundel M, Fateh-Moghadam A (1989) Bone marrow histology and serum beta 2 microglobulin in multiple myeloma—a new prognostic strategy. *Eur J Haematol Suppl* 51:88–98
- Baz M, Durand C, Ragon A, Jaber K, Andrieu D, Merzouk T, Purgus R, Olmer M, Reynier JP, Berland Y (1991) Using ultrapure water in hemodialysis delays carpal tunnel syndrome. *Int J Artif Organs* 14:681–685
- Bellotti V, Stoppini M, Mangione P, Sunde M, Robinson C, Asti L, Brancaccio D, Ferri G (1998) Beta2-microglobulin can be refolded into a native state from ex vivo amyloid fibrils. *Eur J Biochem* 258:61–67
- Berggård I (1965) Identification and isolation of urinary proteins. In: Peeters H (ed) *Protides of the biological fluids (Proceedings of the 12th Colloquium, Bruges, 1964)*, Elsevier, Amsterdam, pp 285–291
- Berggard I, Bearn AG (1968) Isolation and properties of a low molecular weight beta-2-globulin occurring in human biological fluids. *J Biol Chem* 243:4095–4103
- Bhalla RB, Safai B, Pahwa S, Schwartz MK (1985) Beta 2-microglobulin as a prognostic marker for development of AIDS. *Clin Chem* 31:1411–1412
- Bjorck L, Akerstrom B, Berggard I (1979) Occurrence of beta 2-microglobulin in mammalian lymphocytes and erythrocytes. *Eur J Immunol* 9:486–490
- Borysik AJ, Morten IJ, Radford SE, Hewitt EW (2007) Specific glycosaminoglycans promote unseeded amyloid formation from beta2-microglobulin under physiological conditions. *Kidney Int* 72:174–181
- Bourantas KL, Hatzimichael EC, Makis AC, Chaidos A, Kapsali ED, Tsiara S, Mavridis A (1999) Serum beta-2-microglobulin, TNF-alpha and interleukins in myeloproliferative disorders. *Eur J Haematol* 63:19–25
- Brancaccio D, Gallieni M, Niwa T, Braidotti P, Coggi G (2000) Ultrastructural localization of advanced glycation end products and beta2-microglobulin in dialysis amyloidosis. *J Nephrol* 13:129–136



- Buchholz NP, Moch H, Gasser TC, Linke RP, Thiel GT, Mihatsch MJ (1995) Ureteral amyloid deposits of beta 2-microglobulin origin in both kidney recipients of 1 donor. *J Urol* 153:399–401
- Calabrese MF, Eakin CM, Wang JM, Miranker AD (2008) A regulatable switch mediates self-association in an immunoglobulin fold. *Nat Struct Mol Biol* 15:965–971
- Campistol JM, Torregrosa JV, Ponz E, Fenollosa B (1999) Beta-2-microglobulin removal by hemodialysis with polymethylmethacrylate membranes. *Contrib Nephrol* 125:76–85
- Canale C, Relini A, Gliozzi A (2011) Atomic force microscopy of ex vivo amyloid fibrils. *Methods Mol Biol* 736:81–95
- Castillo GM, Cummings JA, Yang W, Judge ME, Sheardown MJ, Rimvall K, Hansen JB, Snow AD (1998) Sulfate content and specific glycosaminoglycan backbone of perlecan are critical for perlecan's enhancement of islet amyloid polypeptide (amylin) fibril formation. *Diabetes* 47:612–620
- Charra B, Caemard F, Uzan M, Terrat JC, Vanel T, Laurent G (1984) Carpal tunnel syndrome, shoulder pain and amyloid deposits in long-term hemodialysis patients. *Proc Eur Dial Transplant Assoc* 21:291–295
- Cheung AK, Rocco MV, Yan G et al (2006) Serum beta-2 microglobulin levels predict mortality in dialysis patients: results of the HEMO study. *J Am Soc Nephrol* 17:546–555
- Cohlberg JA, Li J, Uversky VN, Fink AL (2002) Heparin and other glycosaminoglycans stimulate the formation of amyloid fibrils from alpha-synuclein in vitro. *Biochemistry* 41:1502–1511
- Colombo M, Ricagno S, Barbiroli A, Santambrogio C, Giorgetti S, Raimondi S, Bonomi F, Grandori R, Bellotti V, Bolognesi M (2011) The effects of an ideal beta-turn on beta-2 microglobulin fold stability. *J Biochem* 150:39–47
- Corazza A, Pettirossi F, Viglino P et al (2004) Properties of some variants of human beta2-microglobulin and amyloidogenesis. *J Biol Chem* 279:9176–9189
- Corlin DB, Sen JW, Ladefoged S, Lund GB, Nissen MH, Heegaard NH (2005) Quantification of cleaved b2-microglobulin in serum from patients undergoing chronic hemodialysis. *Clin Chem* 51:1177–1184
- Corlin DB, Johnsen CK, Nissen MH, Heegaard NH (2009) A beta2-microglobulin cleavage variant fibrillates at near-physiological pH. *Biochem Biophys Res Commun* 381:187–191
- Corlin DB, Johnsen CK, Nissen MH, Heegaard NH (2010) Glycosaminoglycans enhance the fibrillation propensity of the beta2-microglobulin cleavage variant—DeltaK58-beta2m. *Biochem Biophys Res Commun* 402:247–251
- Cresswell P, Springer T, Strominger JL, Turner MJ, Grey HM, Kubo RT (1974) Immunological identity of the small subunit of HL-A antigens and beta2-microglobulin and its turnover on the cell membrane. *Proc Natl Acad Sci U S A* 71:2123–2127
- Danesh F, Ho LT (2001) Dialysis-related amyloidosis: history and clinical manifestations. *Semin Dial* 14:80–85
- Davis DP, Gallo G, Vogen SM, Dul JL, Sciarretta KL, Kumar A, Raffin R, Stevens FJ, Argon Y (2001) Both the environment and somatic mutations govern the aggregation pathway of pathogenic immunoglobulin light chain. *J Mol Biol* 313:1021–1034
- De Lorenzi E, Colombo R, Sabella S, Corlin DB, Heegaard NH (2008) The influence of Cu(2+) on the unfolding and refolding of intact and proteolytically processed beta(2)-microglobulin. *Electrophoresis* 29:1734–1740
- Debelouchina GT, Platt GW, Bayro MJ, Radford SE, Griffin RG (2010) Intermolecular alignment in beta(2)-microglobulin amyloid fibrils. *J Am Chem Soc*. [Epub ahead of print]
- Deng NJ, Yan L, Singh D, Cieplak P (2006) Molecular basis for the Cu<sup>2+</sup> binding-induced destabilization of beta2-microglobulin revealed by molecular dynamics simulation. *Biophys J* 90:3865–3879
- Doherty PC, Zinkernagel RM (1975) H-2 compatibility is required for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. *J Exp Med* 141:502–507
- Domanska K, Vanderhaegen S, Srinivasan V et al (2011) Atomic structure of a nanobody-trapped domain-swapped dimer of an amyloidogenic beta2-microglobulin variant. *Proc Natl Acad Sci U S A* 108:1314–1319

- Drueke TB, Massy ZA (2009) Beta2-microglobulin. *Semin Dial* 22:378–380
- Eakin CM, Miranker AD (2005) From chance to frequent encounters: origins of beta2-microglobulin fibrillogenesis. *Biochim Biophys Acta* 1753:92–99
- Eakin CM, Knight JD, Morgan CJ, Gelfand MA, Miranker AD (2002) Formation of a copper specific binding site in non-native states of beta-2-microglobulin. *Biochemistry* 41:10646–10656
- Eakin CM, Attenello FJ, Morgan CJ, Miranker AD (2004) Oligomeric assembly of native-like precursors precedes amyloid formation by beta-2 microglobulin. *Biochemistry* 43:7808–7815
- Eakin CM, Berman AJ, Miranker AD (2006) A native to amyloidogenic transition regulated by a backbone trigger. *Nat Struct Mol Biol* 13:202–208
- Ecroyd H, Thorn DC, Liu Y, Carver JA (2010) The dissociated form of kappa-casein is the precursor to its amyloid fibril formation. *Biochem J* 429:251–260
- Eichner T, Radford SE (2009) A generic mechanism of beta2-microglobulin amyloid assembly at neutral pH involving a specific proline switch. *J Mol Biol* 386:1312–1326
- Eichner T, Radford SE (2011) Understanding the complex mechanisms of beta(2) -microglobulin amyloid assembly. *FEBS J* 278:3868–3883
- Esposito G, Michelutti R, Verdone G et al (2000) Removal of the N-terminal hexapeptide from human beta2-microglobulin facilitates protein aggregation and fibril formation. *Protein Sci* 9:831–845
- Esposito G, Ricagno S, Corazza A et al (2008) The controlling roles of Trp60 and Trp95 in beta2-microglobulin function, folding and amyloid aggregation properties. *J Mol Biol* 378:887–897
- Fabian H, Gast K, Laue M, Misselwitz R, Uchanska-Ziegler B, Ziegler A, Naumann D (2008) Early stages of misfolding and association of beta2-microglobulin: insights from infrared spectroscopy and dynamic light scattering. *Biochemistry* 47:6895–6906
- Feder JN, Tsuchihashi Z, Irrinki A et al (1997) The hemochromatosis founder mutation in HLA-H disrupts beta2-microglobulin interaction and cell surface expression. *J Biol Chem* 272:14025–14028
- Floege J, Ehlerding G (1996) Beta-2-microglobulin-associated amyloidosis. *Nephron* 72:9–26
- Fujimori A (2011) Beta-2-microglobulin as a uremic toxin: the Japanese experience. *Contrib Nephrol* 168:129–33. [Epub 2010, Oct 7]
- Garcia-Garcia M, Gouin-Charnet A, Mourad G, Argilés A (1997) Monomeric and dimeric b2-microglobulin may be extracted from amyloid deposits *in vitro*. *Nephrol Dial Transplant* 12:1192–1198
- Gejyo F, Yamada T, Odani S et al (1985) A new form of amyloid protein associated with chronic hemodialysis was identified as beta 2-microglobulin. *Biochem Biophys Res Commun* 129:701–706
- Gejyo F, Homma N, Suzuki Y, Arakawa M (1986) Serum levels of beta 2-microglobulin as a new form of amyloid protein in patients undergoing long-term hemodialysis. *N Engl J Med* 314:585–586
- Giorgetti S, Rossi A, Mangione P et al (2005) Beta2-microglobulin isoforms display a heterogeneous affinity for type I collagen. *Protein Sci* 14:696–702
- Giorgetti S, Stoppini M, Tennent GA et al (2007) Lysine 58-cleaved beta2-microglobulin is not detectable by 2D electrophoresis in ex vivo amyloid fibrils of two patients affected by dialysis-related amyloidosis. *Protein Sci* 16:343–349
- Giorgetti S, Raimondi S, Pagano K et al (2011) Effect of tetracyclines on the dynamics of formation and destructure of beta2-microglobulin amyloid fibrils. *J Biol Chem* 286:2121–2131
- Gobezie R, Kho A, Krastins B, Sarracino DA, Thornhill TS, Chase M, Millett PJ, Lee DM (2007) High abundance synovial fluid proteome: distinct profiles in health and osteoarthritis. *Arthritis Res Ther* 9:R36
- Goedert M, Jakes R, Spillantini MG, Hasegawa M, Smith MJ, Crowther RA (1996) Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. *Nature* 383:550–553
- Gorevic PD, Casey TT, Stone WJ, DiRaimondo CR, Prelli FC, Frangione B (1985) Beta-2 microglobulin is an amyloidogenic protein in man. *J Clin Invest* 76:2425–2429

- Gorevic PD, Munoz PC, Casey TT, DiRaimondo CR, Stone WJ, Prelli FC, Rodrigues MM, Poulik MD, Frangione B (1986) Polymerization of intact  $\beta_2$ -microglobulin in tissue causes amyloidosis in patients on chronic hemodialysis. *Proc Natl Acad Sci U S A* 83:7908–7912
- Gouin-Charnet A, Laune D, Granier C, Mani JC, Pau B, Mourad G, Argiles A (2000) Alpha2-macroglobulin, the main serum antiprotease, binds beta2-microglobulin, the light chain of the class I major histocompatibility complex, which is involved in human disease. *Clin Sci (Lond)* 98:427–433
- Gumperz JE (2006) The ins and outs of CD1 molecules: bringing lipids under immunological surveillance. *Traffic* 7:2–13
- Hafner-Bratkovic I, Bester R, Pristovsek P et al (2011) Globular domain of the prion protein needs to be unlocked by domain swapping to support prion protein conversion. *J Biol Chem* 286:12149–12156
- Hasegawa K, Tsutsumi-Yasuhara S, Ookoshi T, Ohhashi Y, Kimura H, Takahashi N, Yoshida H, Miyazaki R, Goto Y, Naiki H (2008) Growth of beta(2)-microglobulin-related amyloid fibrils by non-esterified fatty acids at a neutral pH. *Biochem J* 416:307–315
- Hashimoto N, Naiki H, Gejyo F (1999) Modification of beta 2-microglobulin with D-glucose or 3-deoxyglucosone inhibits A beta 2M amyloid fibril extension in vitro. *Amyloid* 6:256–264
- Heegaard NH (2009) Beta(2)-microglobulin: from physiology to amyloidosis. *Amyloid* 16:151–173
- Heegaard NH, Roepstorff P, Melberg SG, Nissen MH (2002) Cleaved beta 2-microglobulin partially attains a conformation that has amyloidogenic features. *J Biol Chem* 277:11184–11189
- Heegaard NHH, Jørgensen TJD, Rozlosnik N, Corlin DB, Pedersen JS, Tempesta AG, Roepstorff P, Bauer R, Nissen MH (2005) Unfolding, aggregation, and seeded amyloid formation of Lysine-58-cleaved  $\beta_2$ -microglobulin. *Biochemistry* 44:4397–4407
- Hodkinson JP, Jahn TR, Radford SE, Ashcroft AE (2009) HDX-ESI-MS reveals enhanced conformational dynamics of the amyloidogenic protein beta(2)-microglobulin upon release from the MHC-1. *J Am Soc Mass Spectrom* 20:278–286
- Hoshino M, Katou H, Hagihara Y, Hasegawa K, Naiki H, Goto Y (2002) Mapping the core of the beta(2)-microglobulin amyloid fibril by H/D exchange. *Nat Struct Biol* 9:332–336
- Jackson RL, Busch SJ, Cardin AD (1991) Glycosaminoglycans: molecular properties, protein interactions, and role in physiological processes. *Physiol Rev* 71:481–539
- Jackson GS, Murray I, Hosszu LL, Gibbs N, Waltho JP, Clarke AR, Collinge J (2001) Location and properties of metal-binding sites on the human prion protein. *Proc Natl Acad Sci U S A* 98:8531–8535
- Jadoul M (1998) Dialysis-related amyloidosis: importance of biocompatibility and age. *Nephrol Dial Transplant* 13(Suppl 7):61–64
- Jadoul M, Garbar C, Noel H, Sennesael J, Vanholder R, Bernaert P, Rorive G, Hanique G, van Ypersele de SC (1997) Histological prevalence of beta 2-microglobulin amyloidosis in hemodialysis: a prospective post-mortem study. *Kidney Int* 51:1928–1932
- Jahn TR, Parker MJ, Homans SW, Radford SE (2006) Amyloid formation under physiological conditions proceeds via a native-like folding intermediate. *Nat Struct Mol Biol* 13:195–201
- Jahn TR, Makin OS, Morris KL, Marshall KE, Tian P, Sikorski P, Serpell LC (2010) The common architecture of cross-beta amyloid. *J Mol Biol* 395:717–727
- Jones S, Manning J, Kad NM, Radford SE (2003) Amyloid-forming peptides from beta2-microglobulin—Insights into the mechanism of fibril formation in vitro. *J Mol Biol* 325:249–257
- Jørgensen TJD, Cheng L, Heegaard NHH (2007) Mass spectrometric characterization of conformational preludes to  $\beta_2$ -microglobulin aggregation. *268:207–216*
- Kad NM, Thomson NH, Smith DP, Smith DA, Radford SE (2001) Beta(2)-microglobulin and its deamidated variant, N17D form amyloid fibrils with a range of morphologies in vitro. *J Mol Biol* 313:559–571
- Kameda A, Hoshino M, Higurashi T, Takahashi S, Naiki H, Goto Y (2005) Nuclear magnetic resonance characterization of the refolding intermediate of beta2-microglobulin trapped by non-native prolyl peptide bond. *J Mol Biol* 348:383–397
- Kang SJ, Cresswell P (2002) Calnexin, calreticulin, and ERp57 cooperate in disulfide bond formation in human CD1d heavy chain. *J Biol Chem* 277:44838–44844

- Kardos J, Micsonai A, Pal-Gabor H, Petrik E, Graf L, Kovacs J, Lee YH, Naiki H, Goto Y (2011) Reversible heat-induced dissociation of beta2-microglobulin amyloid fibrils. *Biochemistry* 19(50):3211–3220
- Karlsson FA, Wibell L, Evrin PE (1980) b2-microglobulin in clinical medicine. *Scand J Clin Lab Invest* 40(Suppl 154):27–37
- Kawano M, Muramoto H, Yamada M, Minamoto M, Araki H, Koni I, Mabuchi H, Nonomura A (1998) Fatal cardiac beta2-microglobulin amyloidosis in patients on long-term hemodialysis. *Am J Kidney Dis* 31:E4
- Khan AR, Baker BM, Ghosh P, Biddison WE, Wiley DC (2000) The structure and stability of an HLA-A\*0201/octameric tax peptide complex with an empty conserved peptide-N-terminal binding site. *J Immunol* 164:6398–6405
- Kinoshita CM, Gewurz AT, Siegel JN, Ying SC, Hugli TE, Coe JE, Gupta RK, Huckman R, Gewurz H (1992) A protease-sensitive site in the proposed Ca(2+)-binding region of human serum amyloid P component and other pentraxins. *Protein Sci* 1:700–709
- Kiss E, Keusch G, Zanetti M, Jung T, Schwarz A, Schocke M, Jaszchke W, Czernak BV (2005) Dialysis-related amyloidosis revisited. *AJR Am J Roentgenol* 185:1460–1467
- Knaus KJ, Morillas M, Swietnicki W, Malone M, Surewicz WK, Yee VC (2001) Crystal structure of the human prion protein reveals a mechanism for oligomerization. *Nat Struct Biol* 8:770–774
- Kuntz D, Naveau B, Bardin T, Druke T, Treves R, Dryll A (1984) Destructive spondylarthropathy in hemodialyzed patients. A new syndrome. *Arthritis Rheum* 27:369–375
- Kwon HK, Pyun SB, Cho WY, Boo CS (2011) Carpal tunnel syndrome and peripheral polyneuropathy in patients with end stage kidney disease. *J Korean Med Sci* 26:1227–1230
- Labriola L, Garbar C, Jadoul M (2007) Persistence of beta2-microglobulin amyloidosis 20 years after successful kidney transplantation. *Am J Kidney Dis* 50:167–168
- Lee S, Eisenberg D (2003) Seeded conversion of recombinant prion protein to a disulfide-bonded oligomer by a reduction-oxidation process. *Nat Struct Biol* 10:725–730
- Lee DM, Jackson KW, Knowlton N, Wages J, Alaupovic P, Samuelsson O, Saeed A, Centola M, Attman PO (2011) Oxidative stress and inflammation in renal patients and healthy subjects. *PLoS ONE* 6:e22360.
- Linke RP, Bommer J, Ritz E, Waldherr R, Eulitz M (1986) Amyloid kidney stones of uremic patients consist of beta 2-microglobulin fragments. *Biochem Biophys Res Commun* 136:665–671
- Linke RP, Hampf H, Bartel-Schwarze S, Eulitz M (1987) Beta 2-microglobulin, different fragments and polymers thereof in synovial amyloid in long-term hemodialysis. *Biol Chem Hoppe Seyler* 368:137–144
- Linke RP, Hampf H, Lobeck H, Ritz E, Bommer J, Waldherr R, Eulitz M (1989) Lysine-specific cleavage of beta 2-microglobulin in amyloid deposits associated with hemodialysis. *Kidney Int* 36:675–681
- Liu C, Sawaya MR, Eisenberg D (2011) beta-Microglobulin forms three-dimensional domain-swapped amyloid fibrils with disulfide linkages. *Nat Struct Mol Biol* 18:49–55
- Liu H, Chumsae C, Gaza-Bulseco G, Hurkmans K, Radziejewski CH (2010) Ranking the susceptibility of disulfide bonds in human IgG1 antibodies by reduction, differential alkylation, and LC-MS analysis. *Anal Chem* 82:5219–5226
- Locatelli F, Marcelli D, Conte F, Limido A, Malberti F, Spotti D (1999) Comparison of mortality in ESRD patients on convective and diffusive extracorporeal treatments. The Registro Lombardo Dialisi E Trapianto. *Kidney Int* 55:286–293
- Magnus JH, Stenstad T, Kolset SO, Husby G (1991) Glycosaminoglycans in extracts of cardiac amyloid fibrils from familial amyloid cardiomyopathy of Danish origin related to variant transthyretin Met 111. *Scand J Immunol* 34:63–69
- McParland VJ, Kad NM, Kalverda AP, Brown A, Kirwin-Jones P, Hunter MG, Sunde M, Radford SE (2000) Partially unfolded states of beta(2)-microglobulin and amyloid formation in vitro. *Biochemistry* 39:8735–8746
- Mendoza VL, Antwi K, Baron-Rodriguez MA, Blanco C, Vachet RW (2010) Structure of the preamyloid dimer of beta-2-microglobulin from covalent labeling and mass spectrometry. *Biochemistry* 49:1522–1532

- Mendoza VL, Baron-Rodriguez MA, Blanco C, Vachet RW (2011) Structural insights into the pre-amyloid tetramer of beta-2-microglobulin from covalent labeling and mass spectrometry. *Biochemistry* 50:6711–6722
- Mimmi MC, Jorgensen TJ, Pettrossi F et al (2006) Variants of beta-microglobulin cleaved at lysine-58 retain the main conformational features of the native protein but are more conformationally heterogeneous and unstable at physiological temperature. *FEBS J* 273:2461–2474
- Miura Y, Ishiyama T, Inomata A, Takeda T, Senma S, Okuyama K, Suzuki Y (1992) Radiolucent bone cysts and the type of dialysis membrane used in patients undergoing long-term hemodialysis. *Nephron* 60:268–273
- Miyata T, Oda O, Inagi R, Iida Y, Araki N, Yamada N, Horiuchi S, Taniguchi N, Maeda K, Kinoshita T (1993) beta 2-Microglobulin modified with advanced glycation end products is a major component of hemodialysis-associated amyloidosis. *J Clin Invest* 92:1243–1252
- Miyata T, Taneda S, Kawai R, Ueda Y, Horiuchi S, Hara M, Maeda K, Monnier VM (1996) Identification of pentosidine as a native structure for advanced glycation end products in b2-microglobulin-containing amyloid fibrils in patients with dialysis-related amyloidosis. *Proc Natl Acad Sci U S A* 93:2353–2358
- Mogyorosi A, Schubert ML (1999) Dialysis-related amyloidosis: an important cause of gastrointestinal symptoms in patients with end-stage renal disease. *Gastroenterology* 116:217–220
- Morgan CJ, Gelfand M, Atreya C, Miranker AD (2001) Kidney dialysis-associated amyloidosis: a molecular role for copper in fiber formation. *J Mol Biol* 309:339–345
- Motomiya Y, Ando Y, Haraoka K, Sun X, Iwamoto H, Uchimura T, Maruyama I (2003) Circulating level of alpha2-macroglobulin-beta2-microglobulin complex in hemodialysis patients. *Kidney Int* 64:2244–2252
- Mount SL, Eltabbakh GH, Hardin NJ (2002) Beta-2 microglobulin amyloidosis presenting as bilateral ovarian masses: a case report and review of the literature. *Am J Surg Pathol* 26:130–133
- Mourad G, Argiles A (1996) Renal transplantation relieves the symptoms but does not reverse beta 2-microglobulin amyloidosis. *J Am Soc Nephrol* 7:798–804
- Myers SL, Jones S, Jahn TR, Morten IJ, Tennent GA, Hewitt EW, Radford SE (2006) A systematic study of the effect of physiological factors on b2-microglobulin amyloid formation at neutral pH. *Biochemistry* 45:2311–2321
- Naiki H, Gejyo F, Nakakuki K (1997) Concentration-dependent inhibitory effects of apolipoprotein E on Alzheimer's beta-amyloid fibril formation in vitro. *Biochemistry* 36:6243–6250
- Nilsson M, Wang X, Rodziewicz-Motowidlo S, Janowski R, Lindstrom V, Onnerfjord P, Westermarck G, Grzonka Z, Jaskolski M, Grubb A (2004) Prevention of domain swapping inhibits dimerization and amyloid fibril formation of cystatin C: use of engineered disulfide bridges, antibodies, and carboxymethylpapain to stabilize the monomeric form of cystatin C. *J Biol Chem* 279:24236–24245
- Nissen MH, Thim L, Christensen M (1987) Purification and biochemical characterization of the complete structure of a proteolytically modified beta-2-microglobulin with biological activity. *Eur J Biochem* 163:21–28
- Nissen MH, Roepstorff P, Thim L, Dunbar B, Fothergill JE (1990) Limited proteolysis of beta 2-microglobulin at Lys-58 by complement component C1s. *Eur J Biochem* 189:423–429
- Nissen MH, Johansen B, Bjerrum OJ (1997) A simple method for the preparation and purification of C1 complement cleaved beta 2-microglobulin from human serum. *J Immunol Methods* 205:29–33
- Niwa T, Katsuzaki T, Momoi T, Miyazaki T, Ogawa H, Saito A, Miyazaki S, Maeda K, Tatemichi N, Takei Y (1996) Modification of beta 2m with advanced glycation end products as observed in dialysis-related amyloidosis by 3-DG accumulating in uremic serum. *Kidney Int* 49:861–867
- Niwa T, Katsuzaki T, Miyazaki S, Momoi T, Akiba T, Miyazaki T, Nokura K, Hayase F, Tatemichi N, Takei Y (1997) Amyloid b2-microglobulin is modified with imidazolone, a novel advanced glycation end product, in dialysis-related amyloidosis. *Kidney Int* 51:187–194
- Niwa T, Tsukushi S (2001) 3-Deoxyglucosone and AGEs in uremic complications: inactivation of glutathione peroxidase by 3-deoxyglucosone. *Kidney Int Suppl* 78:S37–S41

- Odani H, Oyama R, Titani K, Ogawa H, Saito A (1990) Purification and complete amino acid sequence of novel b2-microglobulin. *Biochem Biophys Res Commun* 168:1223–1229
- Ogawa H, Saito A, Oda O, Nakajima M, Chung TG (1988) Detection of novel beta 2-microglobulin in the serum of hemodialysis patients and its amyloidogenic predisposition. *Clin Nephrol* 30:158–163
- Ohhashi Y, Kihara M, Naiki H, Goto Y (2005) Ultrasonication-induced amyloid fibril formation of beta2-microglobulin. *J Biol Chem* 280:32843–32848
- Okon M, Bray P, Vucelic D (1992) <sup>1</sup>H NMR assignments and secondary structure of human beta 2-microglobulin in solution. *Biochemistry* 31:8906–8915
- Otten GR, Bikoff E, Ribaldo RK, Kozlowski S, Margulies DH, Germain RN (1992) Peptide and beta 2-microglobulin regulation of cell surface MHC class I conformation and expression. *J Immunol* 148:3723–3732
- Ozawa D, Yagi H, Ban T, Kameda A, Kawakami T, Naiki H, Goto Y (2009) Destruction of amyloid fibrils of a beta2-microglobulin fragment by laser beam irradiation. *J Biol Chem* 284:1009–1017
- Ozawa D, Hasegawa K, Lee YH, Sakurai K, Yanagi K, Ookoshi T, Goto Y, Naiki H (2011a) Inhibition of beta2-microglobulin amyloid fibril formation by alpha2-macroglobulin. *J Biol Chem* 286:9668–9676
- Ozawa D, Kaji Y, Yagi H, Sakurai K, Kawakami T, Naiki H, Goto Y (2011b) Destruction of amyloid fibrils of keratoepithelin peptides by laser irradiation coupled with amyloid-specific thioflavin T. *J Biol Chem* 286:10856–10863
- Pedersen JT, Østergaard J, Rozlosnik N, Gammelgaard B, Heegaard NH (2011) Cu(II) mediates kinetically distinct, non-amyloidogenic aggregation of amyloid-beta peptides. *J Biol Chem* 286:26952–26963
- Pepys MB (2006) Amyloidosis. *Annu Rev Med* 57:223–241
- Platt GW, Routledge KE, Homans SW, Radford SE (2008) Fibril growth kinetics reveal a region of beta2-microglobulin important for nucleation and elongation of aggregation. *J Mol Biol* 378:251–263
- Praetor A, Hunziker W (2002) beta(2)-Microglobulin is important for cell surface expression and pH-dependent IgG binding of human FcRn. *J Cell Sci* 115:2389–2397
- Regazzoni L, Colombo R, Bertolotti L et al (2011) Screening of fibrillogenesis inhibitors of beta2-microglobulin: integrated strategies by mass spectrometry capillary electrophoresis and in silico simulations. *Anal Chim Acta* 685:153–161
- Relini A, Canale C, De SS et al (2006) Collagen plays an active role in the aggregation of beta2-microglobulin under physiopathological conditions of dialysis-related amyloidosis. *J Biol Chem* 281:16521–16529
- Richardson JS, Richardson DC (2002) Natural beta-sheet proteins use negative design to avoid edge-to-edge aggregation. *Proc Natl Acad Sci U S A* 99:2754–2759
- Sacchettini JC, Kelly JW (2002) Therapeutic strategies for human amyloid diseases. *Nat Rev Drug Discov* 1:267–275
- Saito A, Pietromonaco S, Loo AK, Farquhar MG (1994) Complete cloning and sequencing of rat gp330/“megalin,” a distinctive member of the low density lipoprotein receptor gene family. *Proc Natl Acad Sci U S A* 91:9725–9729
- Sambashivan S, Liu Y, Sawaya MR, Gingery M, Eisenberg D (2005) Amyloid-like fibrils of ribonuclease A with three-dimensional domain-swapped and native-like structure. *Nature* 437:266–269
- Santambrogio C, Ricagno S, Colombo M, Barbiroli A, Bonomi F, Bellotti V, Bolognesi M, Grandori R (2010) DE-loop mutations affect beta2 microglobulin stability, oligomerization, and the low-pH unfolded form. *Protein Sci* 19:1386–1394
- Saper MA, Bjorkman PJ, Wiley DC (1991) Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J Mol Biol* 219:277–319
- Sasahara K, Yagi H, Naiki H, Goto Y (2007) Heat-induced conversion of beta(2)-microglobulin and hen egg-white lysozyme into amyloid fibrils. *J Mol Biol* 372:981–991

- Schneider A, Drechsler C, Krane V, Krieter DH, Scharnagl H, Schneider MP, Wanner C (2011) The effect of high-flux hemodialysis on hemoglobin concentrations in patients with CKD: results of the MINOXIS study. *Clin J Am Soc Nephrol* 7(1):52–59
- Schwalbe S, Holzhauser M, Schaeffer J, Galanski M, Koch KM, Floege J (1997) Beta 2-microglobulin associated amyloidosis: a vanishing complication of long-term hemodialysis? *Kidney Int* 52:1077–1083
- Shimizu S, Yasui C, Yasukawa K, Nakamura H, Shimizu H, Tsuchiya K (2003) Subcutaneous nodules on the buttocks as a manifestation of dialysis-related amyloidosis: a clinicopathological entity? *Br J Dermatol* 149:400–404
- Skinner M, Pepys MB, Cohen AS, Heller LM, Lian JB (1980) In: Freitas AF de, Glenner GG, Costa PP (eds) *Amyloid and amyloidosis: proceedings of the Third International Symposium on Amyloidosis*, Póvoa de Varzim, Portugal, 23–28 September 1979. Amsterdam: Excerpta Medica, pp 384–391
- Smith DP, Jones S, Serpell LC, Sunde M, Radford SE (2003) A systematic investigation into the effect of protein destabilisation on beta 2-microglobulin amyloid formation. *J Mol Biol* 330:943–954
- Snow AD, Kisilevsky R (1985) Temporal relationship between glycosaminoglycan accumulation and amyloid deposition during experimental amyloidosis. A histochemical study. *Lab Invest* 53:37–44
- Snow AD, Willmer J, Kisilevsky R (1987) A close ultrastructural relationship between sulfated proteoglycans and AA amyloid fibrils. *Lab Invest* 57:687–698
- Snow AD, Mar H, Nochlin D, Kimata K, Kato M, Suzuki S, Hassell J, Wight TN (1988) The presence of heparan sulfate proteoglycans in the neuritic plaques and congophilic angiopathy in Alzheimer's disease. *Am J Pathol* 133:456–463
- Snow AD, Bramson R, Mar H, Wight TN, Kisilevsky R (1991) A temporal and ultrastructural relationship between heparan sulfate proteoglycans and AA amyloid in experimental amyloidosis. *J Histochem Cytochem* 39:1321–1330
- Stoppini MS, Arcidiaco P, Mangione P, Giorgetti S, Brancaccio D, Bellotti V (2000) Detection of fragments of beta2-microglobulin in amyloid fibrils. *Kidney Int* 57:349–350
- Stoppini M, Mangione P, Monti M et al (2005) Proteomics of beta2-microglobulin amyloid fibrils. *Biochim Biophys Acta* 1753:23–33
- Takayama F, Miyazaki S, Morita T, Hirasawa Y, Niwa T (2001) Dialysis-related amyloidosis of the heart in long-term hemodialysis patients. *Kidney Int Suppl* 78:S172–S176
- Tang X, Sadeghi M, Olumee Z, Vertes A, Braatz JA, McIlwain LK, Dreifuss PA (1996) Detection and quantitation of beta-2-microglobulin glycosylated end products in human serum by matrix-assisted laser desorption/ionization mass spectrometry. *Anal Chem* 68:3740–3745
- Tennent GA, Lovat LB, Pepys MB (1995) Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis. *Proc Natl Acad Sci U S A* 92:4299–4303
- Terawaki H, Yoshimura K, Hasegawa T, Matsuyama Y, Negawa T, Yamada K, Matsushima M, Nakayama M, Hosoya T, Era S (2004) Oxidative stress is enhanced in correlation with renal dysfunction: examination with the redox state of albumin. *Kidney Int* 66:1988–1993
- Treuhart PS, MCCarty DJ (1971) Synovial fluid pH, lactate, oxygen and carbon dioxide partial pressure in various joint diseases. *Arthritis Rheum* 14:475–484
- Trinh CH, Smith DP, Kalverda AP, Phillips SE, Radford SE (2002) Crystal structure of monomeric human beta-2-microglobulin reveals clues to its amyloidogenic properties. *Proc Natl Acad Sci U S A* 99:9771–9776
- Uversky VN, Li J, Fink AL (2001) Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular NK between Parkinson's disease and heavy metal exposure. *J Biol Chem* 276:44284–44296
- van Ypersele dS, Jadoul M, Malghem J, Maldague B, Jamart J (1991) Effect of dialysis membrane and patient's age on signs of dialysis-related amyloidosis. The Working Party on Dialysis Amyloidosis. *Kidney Int* 39:1012–1019

- Verdone G, Corazza A, Viglino P, Pettirossi F, Giorgetti S, Mangione P, Andreola A, Stoppini M, Bellotti V, Esposito G (2002) The solution structure of human beta2-microglobulin reveals the prodromes of its amyloid transition. *Protein Sci* 11:487–499
- Villanueva J, Hoshino M, Katou H, Kardos J, Hasegawa K, Naiki H, Goto Y (2004) Increase in the conformational flexibility of beta 2-microglobulin upon copper binding: a possible role for copper in dialysis-related amyloidosis. *Protein Sci* 13:797–809
- Vincent C, Dendoroy L, Revillard J-P (1994) Molecular variants of b2-microglobulin in renal insufficiency. *Biochem J* 298:181–187
- Vorbeck-Meister I, Sommer R, Vorbeck F, Horl WH (1999) Quality of water used for haemodialysis: bacteriological and chemical parameters. *Nephrol Dial Transplant* 14:666–675
- Wahlbom M, Wang X, Lindstrom V, Carlemalm E, Jaskolski M, Grubb A (2007) Fibrillogenic oligomers of human cystatin C are formed by propagated domain swapping. *J Biol Chem* 282:18318–18326
- Warren DJ, Otieno LS (1975) Carpal tunnel syndrome in patients on intermittent haemodialysis. *Postgrad Med J* 51:450–452
- White HE, Hodgkinson JL, Jahn TR, Cohen-Krausz S, Gosal WS, Muller S, Orlova EV, Radford SE, Saibil HR (2009) Globular tetramers of beta(2)-microglobulin assemble into elaborate amyloid fibrils. *J Mol Biol* 389:48–57
- Williams DB, Barber BH, Flavell RA, Allen H (1989) Role of beta 2-microglobulin in the intracellular transport and surface expression of murine class I histocompatibility molecules. *J Immunol* 142:2796–2806
- Winchester JF, Salsberg JA, Levin NW (2003) Beta-2 microglobulin in ESRD: an in-depth review. *Adv Ren Replace Ther* 10:279–309
- Wisniewski T, Castano EM, Golabek A, Vogel T, Frangione B (1994) Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am J Pathol* 145:1030–1035
- Woods LA, Platt GW, Hellewell AL, Hewitt EW, Homans SW, Ashcroft AE, Radford SE (2011) Ligand binding to distinct states diverts aggregation of an amyloid-forming protein. *Nat Chem Biol* 7:730–739
- Wu AHB (2006) *Tietz Clinical Guide to Laboratory Tests*, 4th edn. Saunders, St. Louis
- Yamaguchi I, Suda H, Tsuzuike N et al (2003) Glycosaminoglycan and proteoglycan inhibit the depolymerization of beta2-microglobulin amyloid fibrils in vitro. *Kidney Int* 64:1080–1088
- Yamamoto S, Hasegawa K, Yamaguchi I, Tsutsumi S, Kardos J, Goto Y, Gejyo F, Naiki H (2004a) Low concentrations of sodium dodecyl sulfate induce the extension of beta 2-microglobulin-related amyloid fibrils at a neutral pH. *Biochemistry* 43:11075–11082
- Yamamoto S, Yamaguchi I, Hasegawa K, Tsutsumi S, Goto Y, Gejyo F, Naiki H (2004b) Glycosaminoglycans enhance the trifluoroethanol-induced extension of beta 2-microglobulin-related amyloid fibrils at a neutral pH. *J Am Soc Nephrol* 15:126–133
- Yamamoto K, Yagi H, Ozawa D, Sasahara K, Naiki H, Goto Y (2008) Thiol compounds inhibit the formation of amyloid fibrils by beta 2-microglobulin at neutral pH. *J Mol Biol* 376:258–268
- Yamamoto S, Kazama JJ, Narita I, Naiki H, Gejyo F (2009) Recent progress in understanding dialysis-related amyloidosis. *Bone*. 45(Suppl 1):S39–S42. [Epub 2009, Mar 19]
- Young ID, Willmer JP, Kisilevsky R (1989) The ultrastructural localization of sulfated proteoglycans is identical in the amyloids of Alzheimer's disease and AA, AL, senile cardiac and medullary carcinoma-associated amyloidosis. *Acta Neuropathol* 78:202–209
- Young ID, Ailles L, Narindrasorasak S, Tan R, Kisilevsky R (1992) Localization of the basement membrane heparan sulfate proteoglycan in islet amyloid deposits in type II diabetes mellitus. *Arch Pathol Lab Med* 116:951–954
- Zhang P, Fu X, Sawashita J et al (2010) Mouse model to study human A beta 2M amyloidosis: generation of a transgenic mouse with excessive expression of human beta2-microglobulin. *Amyloid* 17:50–62
- Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R (1990) Beta 2-microglobulin deficient mice lack CD4-8<sup>+</sup> cytolytic T cells. *Nature* 443:742–746