Affibodies as valuable tool to counter β2-microglobulin aggregation.

Giulia Rizzi[1], *Cristina Visentin*[1], *Alexander Biedermann*[3], *Heiko Bruns*[3], *Stefano Ricagno*[1,2]

[1] Dipartimento di Bioscienze, Università degli Studi di Milano, via Giovanni Celoria, 26, 20133, Milan, Italy
[2] Institute of Molecular and Translational Cardiology, I.R.C.C.S. Policlinico San Donato, piazza Malan, 2, 20097, San Donato Milanese, Italy
[3] Department of Internal Medicine 5 Hematology and Oncology, University hospital of Erlangen, Hartmannstraße 14, 91052, Erlangen, Germany

Beta-2 microglobulin (β2m) is a small protein that physiologically constitute the invariant subunit of the Major Histocompatibility Complex I, with a crucial role in immune system regulation. Although monomeric β2m is stable in normal conditions, high local concentrations or the presence of mutations may prompt the misfolding of the protein, causing the formation of amyloid fibrils. The prolonged accumulation of such fibrils can trigger the development and worsening of several chronic and autoimmune diseases. Therefore, stabilizing the native state of β2m could be the first step towards preventing pathology progression and consequently favoring the recovery. To achieve this goal, small, customizable affinity proteins, called affibodies, will be used. Affibodies were designed from a scaffold, protein A of *S. aureus,* and are characterized by high solubility and thermal stability. Four affibodies were designed against β2m by phage display. After expression and purification, B2m and affibodies were incubated to favor complex formation. The binding was preliminary assessed by size-exclusion chromatography and subsequently confirmed by isothermal titration calorimetry and microscale thermophoresis techniques. The results revealed a dissociation constant in the nanomolar scale for two of the complexes. Parallelly, to assess whether the interaction between β2m and affibodies can reduce β2m aggregation propensity, ThT assays were performed. A significant reduction was observed upon incubation with one of the affibodies. Overall, the collected results suggest that affibodies can bind β2m *in vitro* with high affinity and significantly reduce its aggregation rate.