Quantification of quaternary structure stability in aggregation-prone proteins under physiological conditions: the transthyretin case

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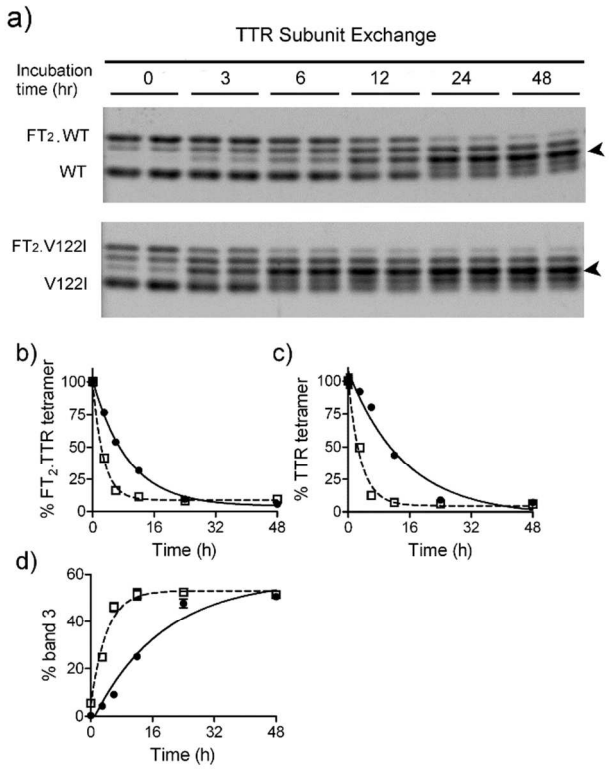
SUPPLEMENTAL FIGURES

Supplemental Figure S1: Subunit exchange reactions between WT TTR and FT_2.WT TTR and between V122I TTR and FT_2.V122I TTR over a 48h time course incubation at 37 °C. a) Semi-native PAGE stained with Sypro Ruby (arrowhead points to band 3). b) Quantitation of the FT_2-homotetramers over time; c) Quantitation of untagged homotetramers over time; d) Quantitation of the heterotetramers composed of 2 FT_2-subunits and 2 untagged subunits over time. Closed circles, WT/FT_2.WT reactions; Open squares, V122I/FT_2.V122I reactions.


a)
TTR Subunit Exchange
Incubation time (hr)
0
3
6
12
24
48
FT2.WT
WT
FT2.V122I
V122I
b)
% FT2.TTR tetramer
100
75
50
25
0
0
16
32
48
Time (h)
c)
% TTR tetramer
100
75
50
25
0
0
16
32
48
Time (h)
d)
% band 3
60
40
20
0
0
16
32
48
Time (h)

Supplemental Figure S2: Stabilization of V122I TTR and FT_2.V122I TTR by small molecules measured by subunit exchange. V122I TTR and FT_2.V122I TTR were pre-incubated with SOM0226, tafamidis or DMSO (vehicle control). Mixtures of V122I TTR and FT_2.V122I TTR with or without small molecules were prepared and incubated for up to 48 h at 37 °C. Subunit exchange rates were measured by quantifying the formation of mixed heterotetramers composed of 2 V122I and 2 FT_2. V122I subunits (arrowhead points to band 3). a) and c) Asymmetric mode where only V122I TTR was pre-incubated with small molecules; b) and d) Symmetric mode where both V122I and FT_2. V122I TTR were pre-incubated with small molecules. Symbols: DMSO, open diamonds; SOM0226, open circles; tafamidis, open triangles.