Supporting Information

The role of protein thermodynamics and primary structure in fibrillogenesis of variable domains from immunoglobulin light-chains

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This document includes:

-Supporting Figures S1–S5

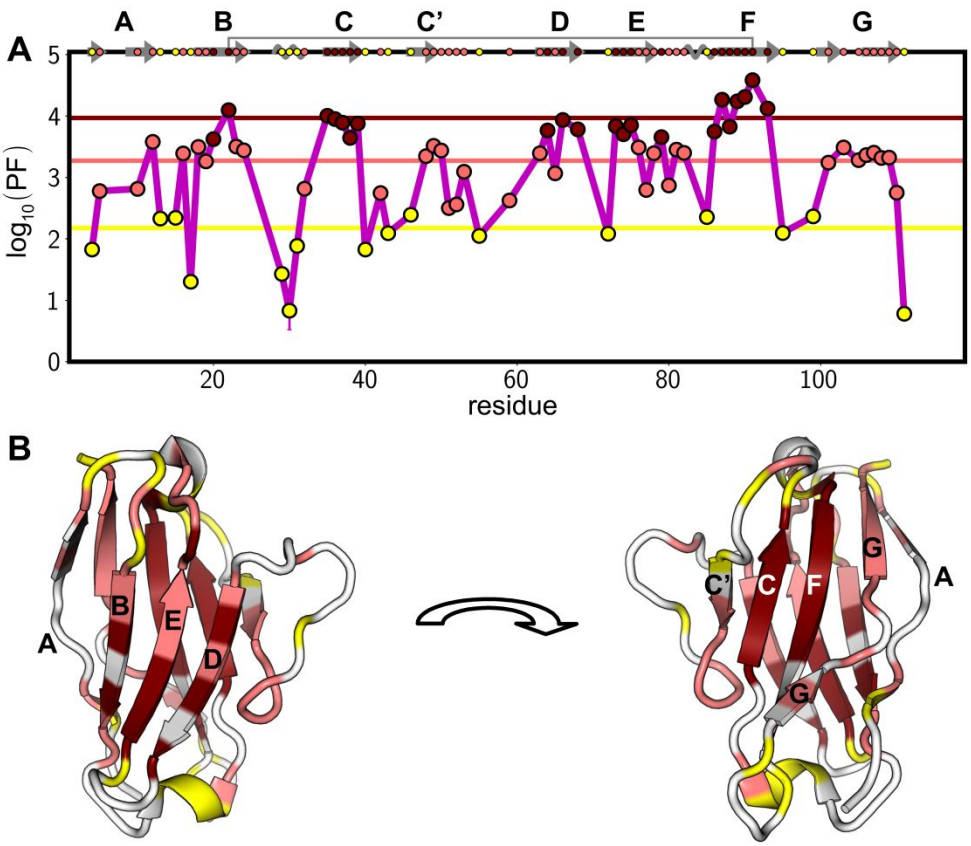

Figure S1. A) Protection factors (PF) from native state hydrogen exchange measurements performed on the WT 6aJL2-V_L domain. The color code indicates protection factors higher than 4000 (maroon), between 300 and 4000 (pink), and below 300 (yellow). B) PF values plotted on the 3D structure of the domain (PDB 2w0k[1]).

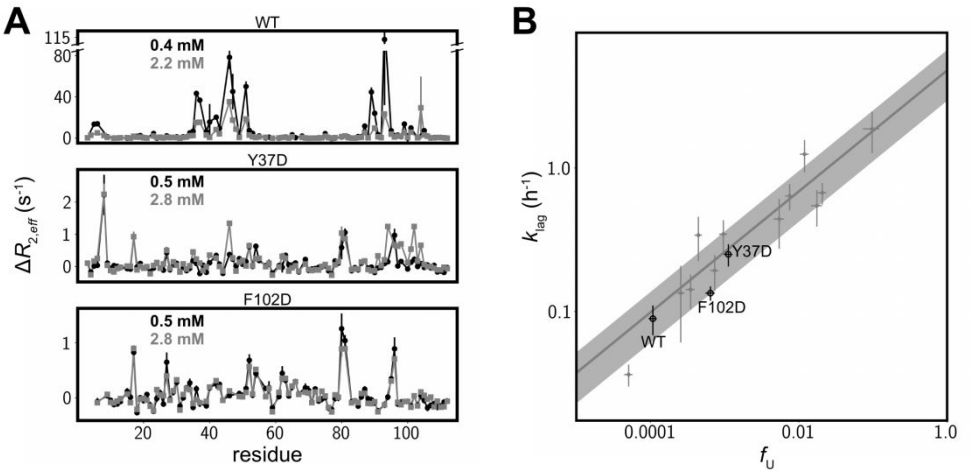

Figure S2. A) Size of the dispersion profiles, $\Delta R_{2,eff} = R_{2,eff}$(~30 Hz) – $R_{2,eff}$(1 kHz), for wild-type, Y37D and F102D 6aJL2-V_L at 2 different protein concentrations. The increase in $\Delta R_{2,eff}$ observed for residues in the WT domain as a function of decreasing protein concentration is consistent with an oligomerization process that is skewed towards the oligomer. Residues affected by the exchange are those at the V_L-V_L dimer interface, consistent with a monomer-dimer equilibrium, as characterized previously for JTO and WIL V_L domains[2]. Both Y37D and F102D mutations eliminate the exchange (note the different y-axis scales relative to the plot for the WT domain), as the V_L-V_L dimer interface is destabilized so that only folded monomeric domains are present[2]. B) Correlation, on a log-log scale, between f_U and k_{lag}, showing no significant deviation from the fitted curve of Figure 2D (grey line, shaded area indicates the standard error in k_{lag} values, see Materials and Methods) for Y37D and F102D mutants, indicating that the monomer-dimer equilibrium, as observed via the dispersion profiles recorded on the folded WT domain, is unrelated to the aggregation observed via the ThT assay. Unlabeled gray circles are the data points for the conservative mutations considered in Figure 2D.

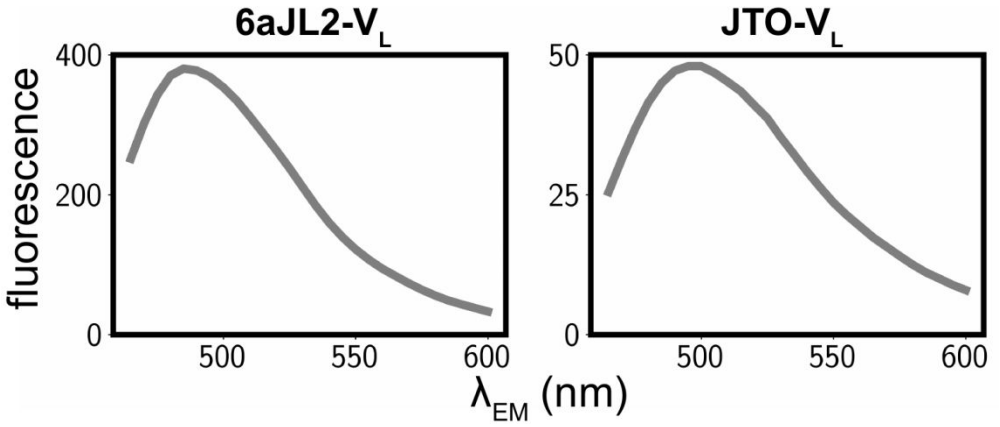

Figure S3. ThT emission spectra for 0.4 mM 6aJL2 and 0.6 mM JTO-V_L samples after the acquisition of ^{15}N CPMG NMR experiments.

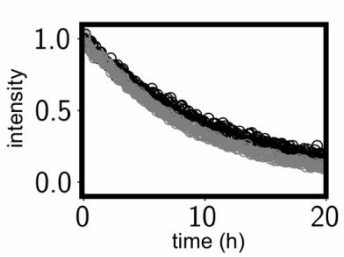

Figure S4. Solvent hydrogen/deuterium exchange time profiles for the slowly exchanging amide of I20 for 2 6aJL2-V_L mutants in the 16-24 region, S21D (black) and R24G (gray). The exchange of I20 reports on the global unfolding free-energy of each domain.

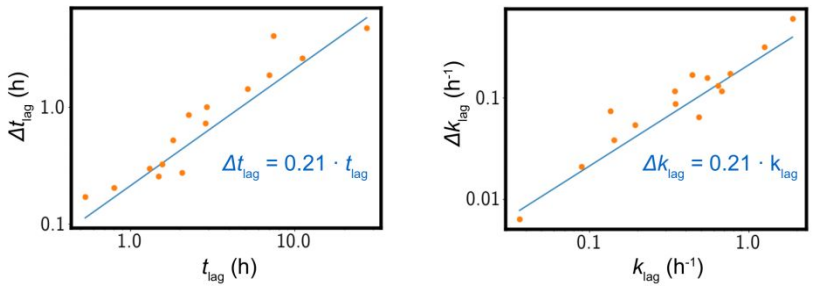

Figure S5. The experimental errors in t_{lag} and k_{lag}, Δt_{lag} and Δk_{lag}, are proportional to t_{lag} and k_{lag} values, respectively. (left) Experimental errors, Δt_{lag}, estimated from at least 6 duplicates for each of the protein domains shown in Figure 2D, plotted against t_{lag}. The observed correlation shows that the slower the

aggregation the larger the absolute variability in the lag time. (right) Experimental errors Δk_{lag}, derived from error propagation as $\Delta k_{lag} = \Delta t_{lag} \cdot k_{lag}^2$, plotted against k_{lag} values. Note that the proportionality constant (slope) is the same for both profiles since if $\Delta t_{lag} = C \cdot t_{lag}$, where C is the proportionality constant, then $\Delta k_{lag} = \Delta t_{lag} \cdot k_{lag}^2 = C \cdot t_{lag} \cdot k_{lag}^2 = C \cdot k_{lag}$.

References

1. Hernandez-Santoyo, A.; del Pozo Yauner, L.; Fuentes-Silva, D.; Ortiz, E.; Rudino-Pinera, E.; Sanchez-Lopez, R.; Horjales, E.; Becerril, B.; Rodriguez-Romero, A., A single mutation at the sheet switch region results in conformational changes favoring lambda6 light-chain fibrillogenesis. *J Mol Biol* **2010,** *396* (2), 280-92.
2. Rennella, E.; Morgan, G. J.; Kelly, J. W.; Kay, L. E., Role of domain interactions in the aggregation of full-length immunoglobulin light chains. *Proc Natl Acad Sci U S A* **2019,** *116* (3), 854-863.