

Table S3. Bulk dye anisotropy measurements.

Labeling position	No Cm	With Cm (100 μ M)
Cy5	0.275	0.273
N374C	0.217	0.221
V167C	0.230	0.235

Fluorescence anisotropy ($r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp})$) was measured for Cy5 free in solution or chemically attached to several different substituted Cys in MdfA using a bulk fluorimeter (HITACHI F-7000). Error in measured anisotropy values (*i.e.* standard deviation of 3 measurements) was $\leq 2\%$ in all cases.