Calmodulin Binds a Highly Extended HIV-1 MA Protein That Refolds Upon Its Release

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Supporting Material

Sample preparation for small-angle scattering

*Protein expression details for deuterated CaM***:** Deuterated CaM was produced by expression in *Escherichia coli*. The cDNA for CaM was subcloned from pET3a to pET28a, BL21 Star™(DE3) competent cells (Invitrogen) were transformed with saturating mini-prep plasmid, and allowed to recover for 2 hours in the rich media (Super Optimal Catabolic (SOC), Invitrogen). The transformed cells were added to a 50% minimal media culture, which was subsequently added to a second, larger start culture resulting in a media at 90% D_2O, such that the culture was maintained in exponential growth throughout. This second starter culture was used to inoculate a 1L 90% D_2O culture in a 2L bioreactor (Real time Engineering). At OD = 12, expression was induced with 1mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The culture was harvested 11 hours later, at OD = 22.5, yielding 42.7 g wet weight. The culture temperature was 37 °C.

Sample preparation details for SANS experiments: The conditions for preparing a 1:1 complex and the K_d value (170 nM) have been previously determined (27). At the concentration of the SAXS and SANS samples (0.2-0.24 mM), approximately >97% of the CaM and MA are complexed, and as they each are close to half the molecular mass of the complex their contribution to the scattering would be further reduced by a factor of 4 relative to the complex (i.e. 3% divided by 4) which is insignificant. In the Chow et al. study (27) we also showed, using SEC-MALLS, that in low salt conditions replicated in this study, Ca^{2+}-CaM-MA elutes as a single peak with relative mass as expected for the complex and the complex was characterised using SAXS. Confirmation that we successfully prepared the 1:1 complex with deuterated CaM for this study is in the replication of the SAXS data for the complex and derived structural parameters from the complex from the earlier study of Chow *et al*., including the R_g, D_{max}, overall $P(r)$ profile and estimate of the molecular mass for the complex from $I(0)$ (see paragraph 2 of **Results**.)

Matched solvent blanks for scattering experiments were obtained by using equilibrated dialysates from the CaM-MA SANS sample preparation. These were checked for D_2O content by densitometry (DMA 5000 Density Meter, Anton-Paar). Recorded values were 19.2%, 43.9%, 87.9% and 99.9% and are quoted throughout the text as 19%, 44%, 88% and 100%, respectively. A 0% D_2O sample was prepared but neutron scattering measurements on this sample were unsuccessful due to instrument difficulties.

Samples of the complex and respective dialysates were each spun for 20 minutes at 16,000 x *g* and a 300 µL aliquot was loaded into 1 mm banjo-shaped cells (product number 120-1 mm, HELLMA USA). The loaded solutions were degassed with gentle sonication. The cells then were placed in a twenty position sample changer with temperature control (set within a range of 10 - 20°C.)

Small-angle scattering data acquisition and reduction

SANS data were acquired using the SANS instrument Quokka at the Australian Nuclear Science and Technology Organisation (ANSTO.) The incident neutron beam for Quokka was monochromated to a triangular wavelength distribution by an Astrium high resolution helical slot neutron velocity selector (Astrium GmbH, Friedrichshafen, Germany) set for a wavelength, λ, of 4.94 Å with a time of flight measured $\Delta\lambda/\lambda$ of 6.65% (2.7% root mean square). The 44% and 100% D_2O samples were measured using sample-to-detector settings of 2 m and 7 m, whilst the 19% and 88% D_2O samples used 2m and 10 m settings. Data acquisition times were 30 min, 60 min, and 133 min for 2 m, 7 m, and 10 m, respectively. The different detector settings were a result of the data being collected in two different shifts and time constraints made it necessary to use the existing configuration or the second data collection period.

The Bruker Nanostar instrument at ANSTO used for the SAXS data acquisition has a copper rotating anode (0.3 mm filament) source operating at 45 kV and 110 mA to yield Cu Kα radiation (λ = 1.54 Å.) The Nanostar uses a 3 pinhole collimation and is fitted with a Vantec2000 2D detector. The sample chamber and optics were held under vacuum to avoid scattering from air. The sample-to-detector distance was 700 mm which provides for measurement of a q-range of 0.012-0.315 $Å^{-1}$.

Small-angle scattering data analysis and molecular modeling

Molecular weight determination from SAXS data: The mass of the CaM-MA complex in solution was determined using the equation:

$$M_r = \frac{I(0)N_A}{c\Delta\rho_M^2}$$

where c is the protein concentration (0.0072 $g.cm^{-3}$), N_A is Avogadro's number and $\Delta\rho_M = \Delta\rho\, v$ and $\Delta\rho$ is the contrast or difference in scattering density between the solvent and the scattering particle (3.084 x 10^{10} cm^{-2}), v is its partial specific volume (0.727 $cm^3\ g^{-1}$).

Rigid Body Modelling of CaM-MA: The program SASREF7 was used to create an atomistic model optimised to the SANS data. The NMR structure of MA (PDB accession number 2HMX) was divided into eight sections (residues 1-10, 11-25, 26-46, 47-72, 73-90, 91-95, 96-113, 114-133) based on the revised structure of MA (PDB accession code 1TAM). This division was chosen to allow a degree a movement between each alpha helix within MA and the tail region. A distance constraint of 5 Å was specified between the N- and C-termini of each MA fragment. The structure of CaM taken from the NMR structure of CaM complexed with the Munc13 peptide (PBD accession number 2DKU, first structure in the ensemble) was used as the CaM component as the neutron scattering data indicated this configuration of CaM is the best fit to the data for CaM in the complex (see results). A 10 Å distance constraint were specified between MA residues W16 and W36 and CaM residues 121-126 and 31-34, respectively, based on earlier evidence that these residues are involved in binding, either between CaM and MA or CaM and Munc13. The beam profiles for each instrument configuration were input so that corrections for the smearing effects of the beam geometry were accounted for. All models were visualized using the program PyMOL (Delano WL (2002) The PyMOL Molecular Graphics System. DeLano Scientific, San Carlos, CA, USA.)

Table S1

χ values for each MONSA reconstruction of CaM-MA					
	% D_2O in solvent				**SAXS**
	19	**44**	**88**	**100**	
1	1.0	0.6	1.0	0.9	1.0
2	0.9	0.6	0.9	0.8	1.0
3	0.9	0.6	1.0	0.9	1.0
4	0.9	0.6	1.0	0.9	1.0
5	0.9	0.6	0.9	0.9	1.0
6	0.9	0.6	0.9	0.9	1.0

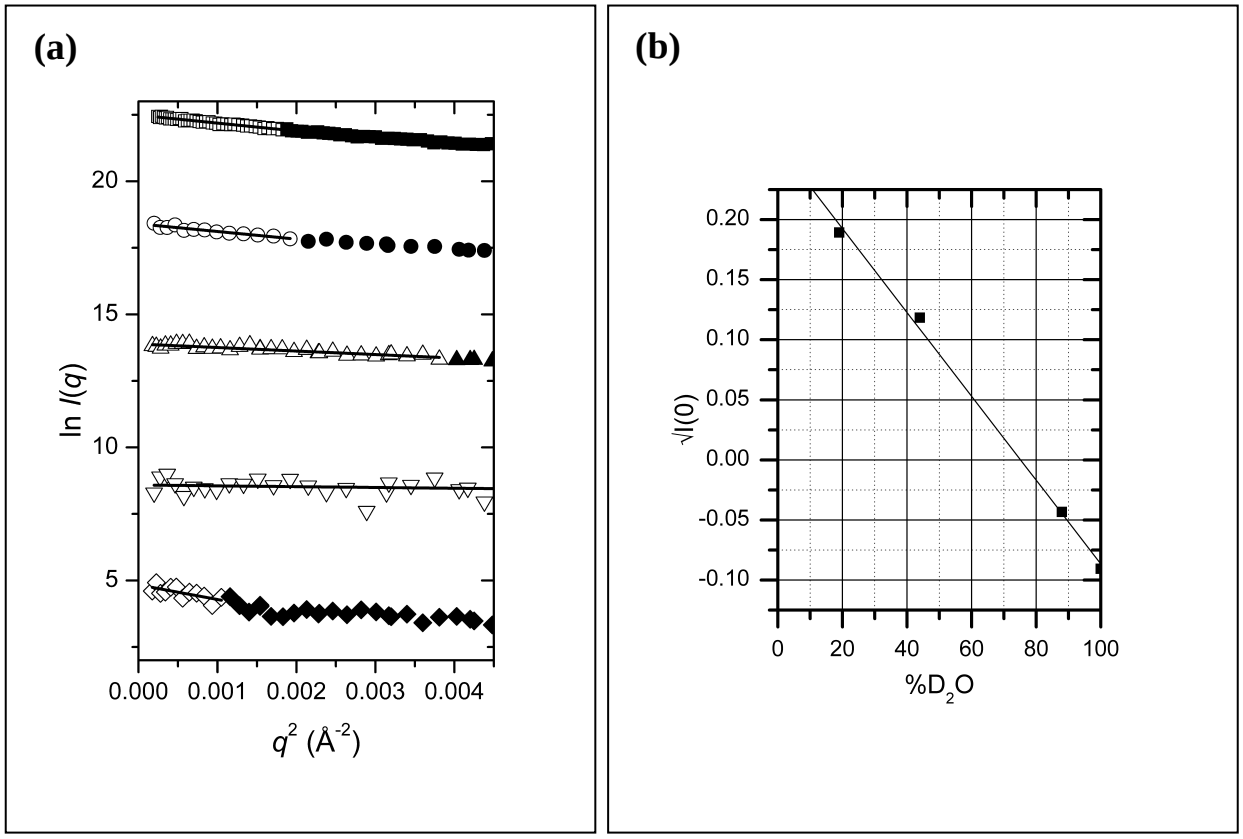

Figure S1. (a) Guinier plots for the scattering data. SAXS data CaM-MA (□) SANS data for $4Ca^{2+}$dCaM-MA in 19% D_2O, (○) 44% D_2O (Δ), 88% (∇), and 100% D_2O (◊). Open symbols indicate the Guinier region, filled symbols are beyond the Guinier region. (b) Contrast dependence of the forward scattering, $I(0)$, showing the total scattering particle solvent match point. Note, the negative values of √I(0) plotted are obtained by multiplication by -1 in order to obtain a single straight line for ease of fitting.

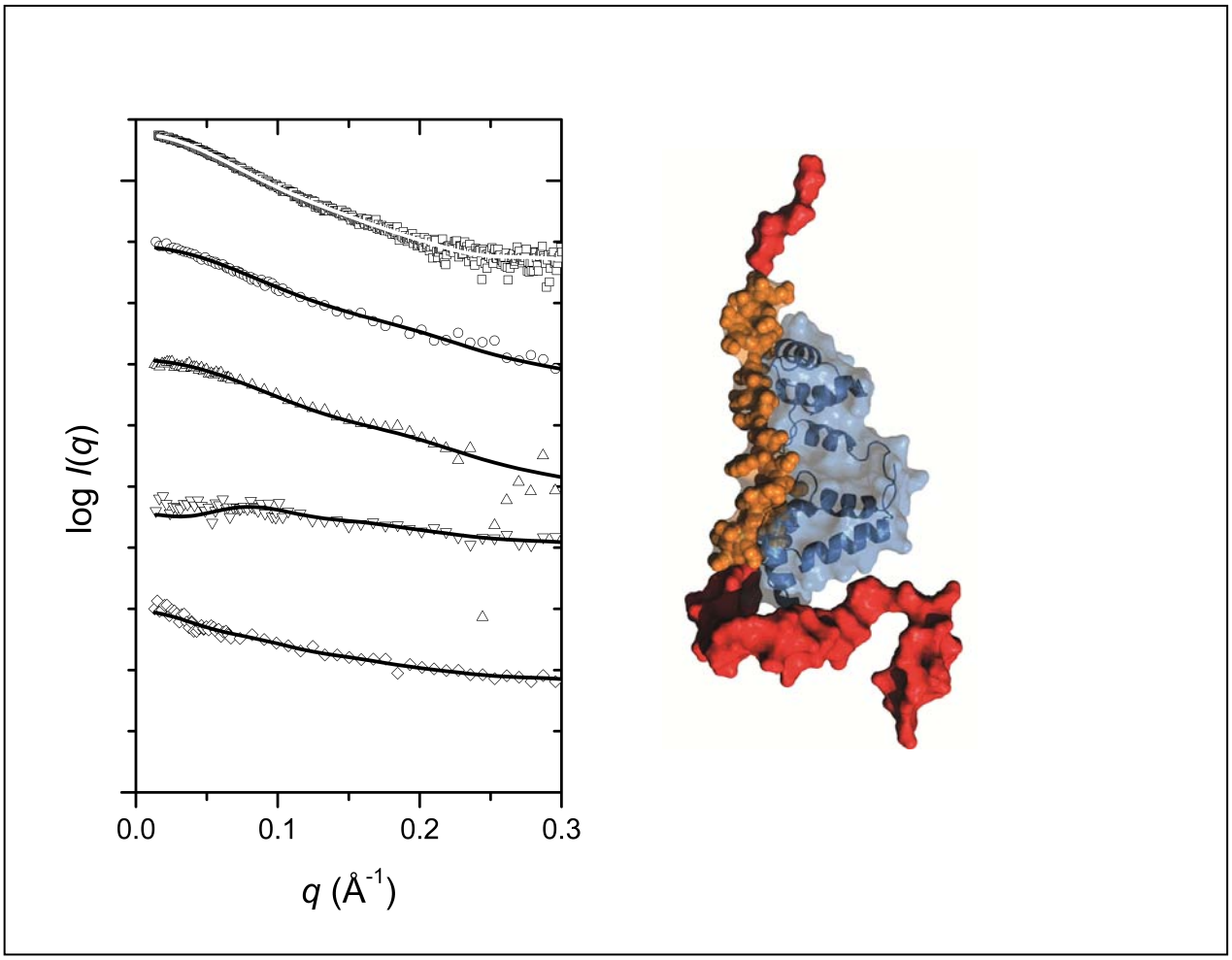

Figure S2. *Right*: Rigid body model of the CaM-(HIV-1) MA complex. In red, the N- and C-terminus of MA, as orange spheres; the binding sequence of MA that contacts CaM (Samal et al., 2011) and in blue, calmodulin. *Left*: The fits from the SASREF model to the experimental data have chi values of 0.9, 1.1, 0.7, 0.9, and 0.9 for the SAXS (□), 19% D_2O (○), 44% D_2O (Δ), 88% (∇), and 100% D_2O (◊) data, respectively. We note the relatively poor fit at the low-*q* for the 88% D_2O data. This lowest contrast data is the most sensitive internal density fluctuations and hence the details of the atomic structure. Comparing the fits here with those of the uniform density MONSA model it is evident that these same data are most impacted by assumptions about the internal density fluctuations.

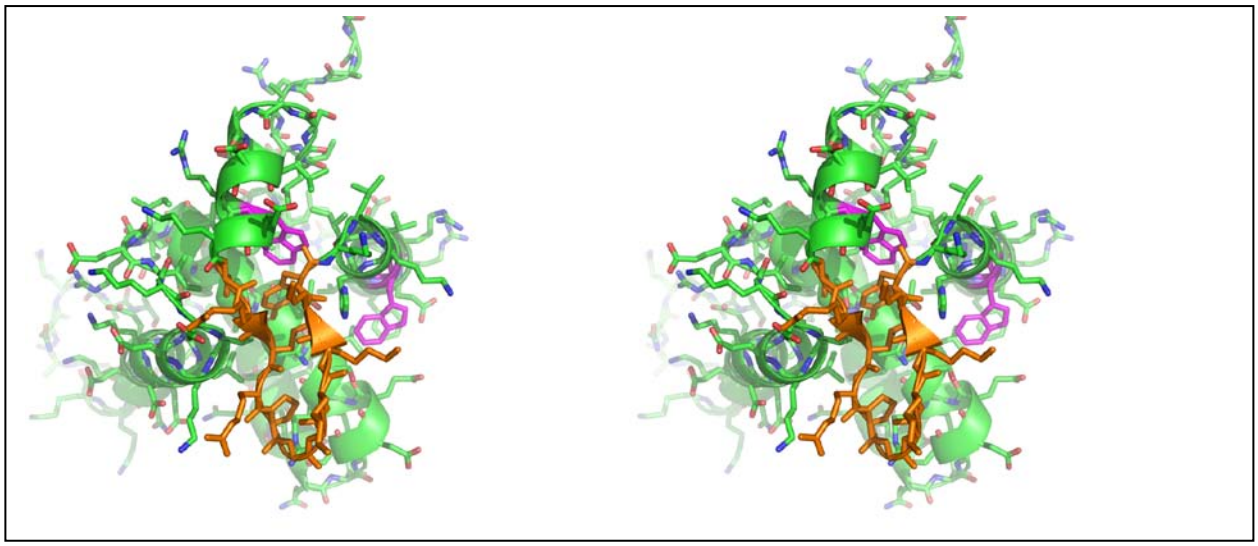

Figure S3. Arrangement of CaM-binding tryptophans in the folded native MA structure. Tryptophans W16 and W36 (magenta) are located in helices α1 and α2 (green ribbons), separated by a beta-turn-beta motif (orange) that results in their being spaced by only ~10-15 Å. The W16 side chain is completely buried within the hydrophobic core. In order for W16 and W36 to bind in the hydrophobic pockets of CaM in its semi-extended structure, a dramatic structural rearrangement of MA is required, exposing its hydrophobic core as it reorients the tryptophan side chains and spaces them by as much as 40 Å for interactions with each of the CaM lobes (coordinates from the averaged, refined NMR structure, PDB 1TAM).