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Supporting Information

Kinetic Analysis of the Interaction of *Mos1* Transposase with its Inverted Terminal Repeats Reveals New Insight into the Protein–DNA Complex Assembly

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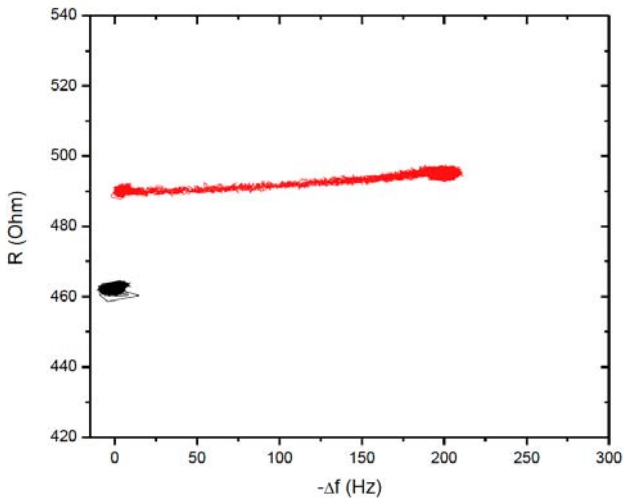
Supporting Information 1. Binding specificity

The QCM is a piezoelectric sensor, based on the measurement of the frequency variation (Δf in Hz) of the quartz crystal. The frequency variation depends on the propagation of the acoustic wave at the interface quartz crystal/solution. In the reported study, the DNA was first immobilized by its 3'OH single strand extremity onto the modified quartz crystal surfaces, and kept in solution. The protein is then injected into the solution. The resulting frequency variation corresponds to a modification of the acoustic wave propagation. Beyond the analysis of the frequency variation (by Sauerbrey's law) made in the main text, the resonant resistance (R in Ohm) of the quartz crystal can be measured during the assays. The variation of R in function of Δf (frequency variation) allows characterizing the origin of the frequency variation. R was plotted as a function of the frequency variation when MOS1 was added to a surface graphed with a double strand DNA that contained the *Mos1* 3'ITR (in red) and when MOS1 was added to a surface graphed with a double strand DNA that did not contained the *Mos1* 3'ITR (in black). In the first case, we reported a linear variation of R with Δf of 0.03 Ohm/Hz.



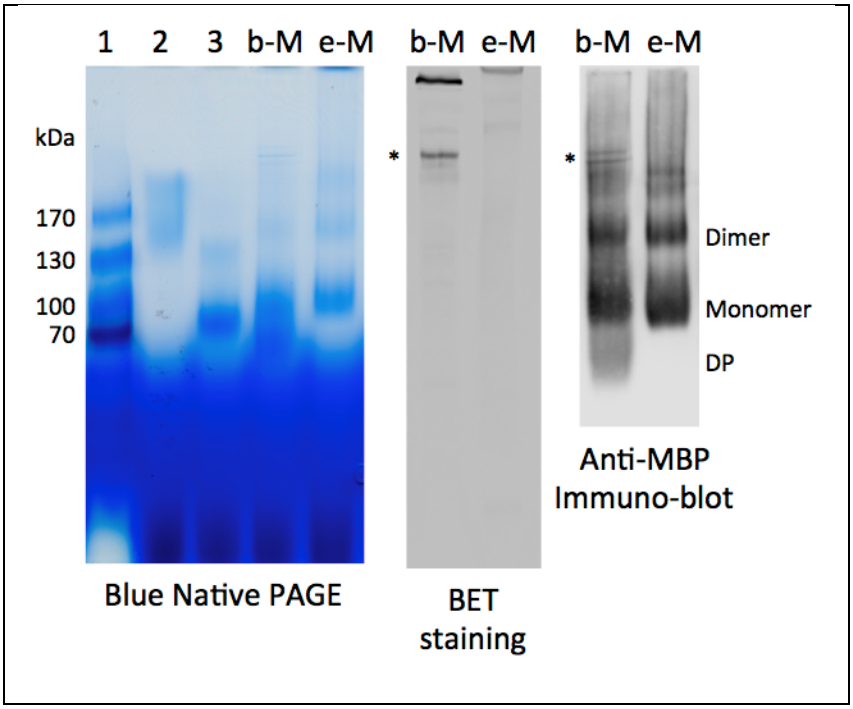
This quasi-non variation of R with the frequency variation is characteristic to a pure elastic event, indicating no variation of the solution viscosity at the crystal surface upon the protein injection. Consequently, the frequency variation only corresponds to a mass variation due to the interaction between the protein and the DNA grafted at the quartz crystal surface. The black "spot" obtained in the second case (no binding expected) confirms this.

Supporting Information 2. b-MOS1 and e-MOS1 dimerization

Different research teams (including ours) have previously shown that *mariner* transposases are produced and purified as dimers. This concerns transposases produced from *E. coli*, with or without MBP-tag (references in the main text). In contrast, the dimeric status of MBP-MOS1 produced and purified from insect cells (e-MOS1 in the main text) has not been investigated. We still know that MOS1 dimerization is needed to translocate it into the nucleus (Dematteï et al, 2011). We also know that PEC assembly and transposition rates are quite the same for e-MOS1 and b-MOS1 (references in the main text), suggesting that e-MOS1 is produced and purified as a dimer, as does b-MOS1. In an attempt to verify this point, we first performed gel filtration analyses as we previously and successfully done for b-MOS1 (6). After having sacrificed two gel filtration columns, it was necessary to admit that e-MOS1 behaviour did not allow such an analysis, whatever the pH, the buffer, the salt conditions and the protein concentration used. e-MOS1 never eluted from the column. Another strategy was then adapted, involving a new method known as Blue Native PAGE (Niepmann & Zheng, 2006) that allows the separation of proteins according to their size, oligomeric state, and shape. This gel system combined the addition of negative charges to the proteins by brillant Blue G with a discontinuous buffer system and gradient gels.

Using this system, b-MOS1 and e-MOS1 electrophoretic mobility were compared, assuming that obtaining identical or very similar profiles indicate a similar oligomeric organization. Briefly, 20µl of protein samples (5µg) were mixed to 10µl of loading buffer (100mM Tris-Cl pH 8, 40% glycerol, 0,5% Coomassie brillant blue G250 Merck) and incubated 10 min at RT. 5µg of conalbumin (75 kDa, GE-Healthcare) or 10µg of glyceraldehyde-3phosphate-dehydrogenase (GAPDH, 143 kDa, Sigma) were used as molecular mass standards. Pre-stained protein ladder (PageRuler, 10-170 kDa, Fermentas Life Sciences) without loading buffer were used as landmark. The samples were applied to a 4-15% polyacrylamide gradient gel (BioRad). The cathode buffer contained 100 mM Histidine (adjusted to pH 8 using Tris base without chloride) and 0,002% G-250. The anode buffer contained 100 mM Tris-Cl pH 8.8. The gel was run at 4°C and 100V for 3h. After the half of the time, the cathode buffer was changed for a buffer without G-250. Proteins are directly detected after electrophoresis since they are blue-coloured and the gels were scanned.

To detect the DNA (that could co-purified upon the purification of DNA-binding proteins), gels were incubated in a 10 µg/ml BET solution for a few minutes and imaged under UV-light. After electrophoresis, gels were transferred onto nitrocellulose membrane (Hybond ECL, GE-Healthcare) in liquid conditions at 4°C and 70V for 1h30 in Tris buffer (Tris base 25 mM, Glycine 192 mM, SDS 0,01%, Ethanol 20%). The membrane was then hybridized with an anti-MBP antibody (BioLabs) using standard procedures.



1: PageRuler
2: GAPDH (143 kDA)
3: Conalbumine (75 kDa)
b-M: b-MOS1
e-M: e-MOS1
DP: degradation products

In the blue native PAGE, GAPDH (lane 2) and Conalbumine (lane 3) give the approximated positions expected for MOS1 dimers (166 kDA due to the MBP-tag) and monomers (83 kDA) respectively. We note that the positions in the gel are likewise in agreement with the electrophoretic profile of the PageRuler (lane 1), which was not obligatory expected. The BET-stained gel confirms that DNA might be present in the protein preparation, giving bands of high molecular weight. For the bacterial sample, these bands (*) contain both DNA and MBP-MOS1, probably coming from non-specific interactions. The anti-MBP immuno-blot allows identifying the various conformations of MBP-MOS1 detected in the blue native PAGE. Dimers and monomers are detected for both samples, and the bacterial sample also contains degradation products (DP) as previously shown (ref 14 of the main text). Traces of higher order oligomers are also detected. The relative proportion of monomers and dimers are the same for both samples (bacterial *versus* eukaryotic), indicating that MOS1 dimerization is not affected

by the kind of producing cell. This is in agreement with what we know about MOS1 behaviour in eukaryotic cells, in which dimerization is needed to allow nuclear translocation and further activity (Dematteі *et al*. PlosOne, 2011).