Supplementary Information

This section contains additional information on the force-independent binding rate of RAD51 to dsDNA in the applied force regime of 0.15 to 4 pN (Figures S1 and S2). Furthermore, we show that the obtained Hill coefficient for both nucleation and filament extension is independent of the binding size applied in the Monte Carlo simulations (Figure S3 and Table S1).
Introduction of 187 nM RAD51 in the presence of Mg\(^{2+}\) and ATP in the flow cell yields similar growth profiles on dsDNA at different stretching forces applied, i.e., 4, 2, and 0.6 pN. The blue line is a growth profile obtained from the Monte Carlo simulations (see also Figures 2C and 3C). The growth profile obtained at 0.6 pN is occasionally disturbed by aspecific interaction between the RAD51-DNA tether and the surface, causing pauses in the observed extension followed by rapid increments.
Independence of the extension rate on applied force. Experimental assembly curves of RAD51 on dsDNA in the presence of Mg$^{2+}$ and ATP obtained at different stretching forces (Figure S1) are fit with Monte Carlo simulated growth profiles by varying only the filament extension rate. The stretching force applied does not affect the binding rate of RAD51.
**Figure S3**

**RAD51-concentration dependence of nucleation and filament-extension rates.**

Experimental assembly curves of RAD51 in the presence of Ca$^{2+}$ (Figure 2A) are fit with Monte Carlo simulated growth profiles obtained for different binding sizes during nucleation and filament extension, i.e. (A) monomer and monomer, (B) pentamer and monomer, (C) pentamer and pentamer, and (D) monomer and pentamer, respectively. The top curves show an example of the data for [RAD51] = 187 nM. Because the fit in Figure D shows a growth profile that is clearly different from the experimental data, the rates for nucleation and extension per filament patch were not determined for this case. In the other three cases, the obtained concentration-dependent rates for nucleation and
extension per filament patch show a similar sigmoidal behavior independent of the binding size applied. The fit of the Hill equation (equation (2)) yielded Hill coefficients of 4.3 to 5.4 (Table S1), clearly indicating a multimeric binding size for both nucleation and extension. A pentameric binding size for both nucleation and extension per filament patch is thus internally consistent with the obtained fit parameters, whereas models with monomeric binding units fail to exhibit such a consistency.
Table S1

The concentration-dependent rates for nucleation and extension per filament patch obtained for different binding sizes applied in the Monte Carlo simulations (Figure S3), are fit with the Hill equation (equation (2)). The obtained Hill coefficients expressed in RAD51 monomers suggest a multimeric binding size for both nucleation as extension per filament patch.

<table>
<thead>
<tr>
<th></th>
<th>monomer</th>
<th>pentamer monomer</th>
<th>pentamer pentamer</th>
<th>monomer pentamer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleation</td>
<td>5.4 ± 0.8</td>
<td>5.4 ± 0.8</td>
<td>4.3 ± 0.6</td>
<td>N.D.</td>
</tr>
<tr>
<td>Filament extension</td>
<td>4.3 ± 0.6</td>
<td>4.3 ± 0.6</td>
<td>4.3 ± 0.5</td>
<td>N.D.</td>
</tr>
</tbody>
</table>