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Supplemental Material to:

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The structure of dual-variable-domain immunoglobulin molecules alone and bound to antigen

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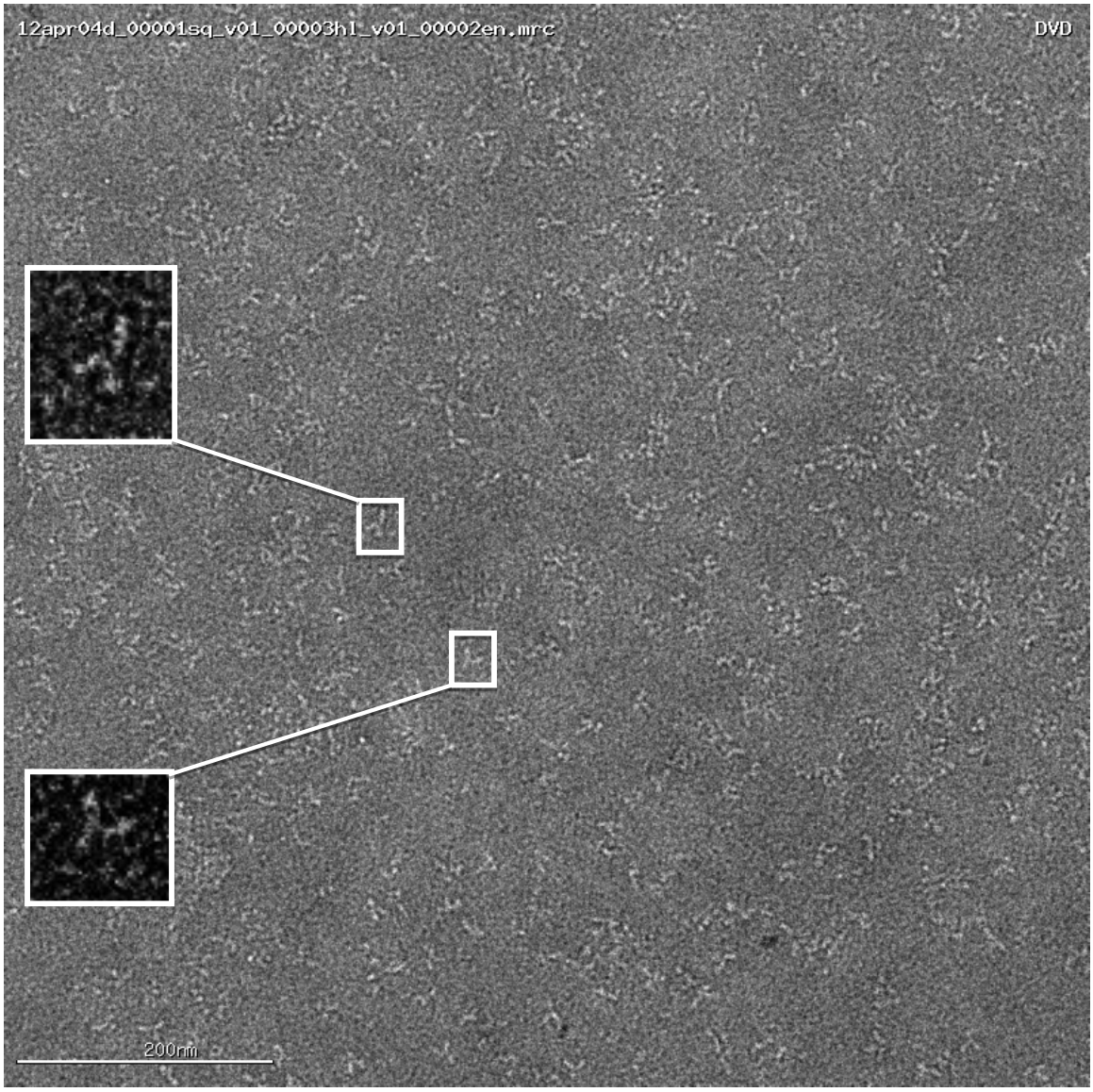
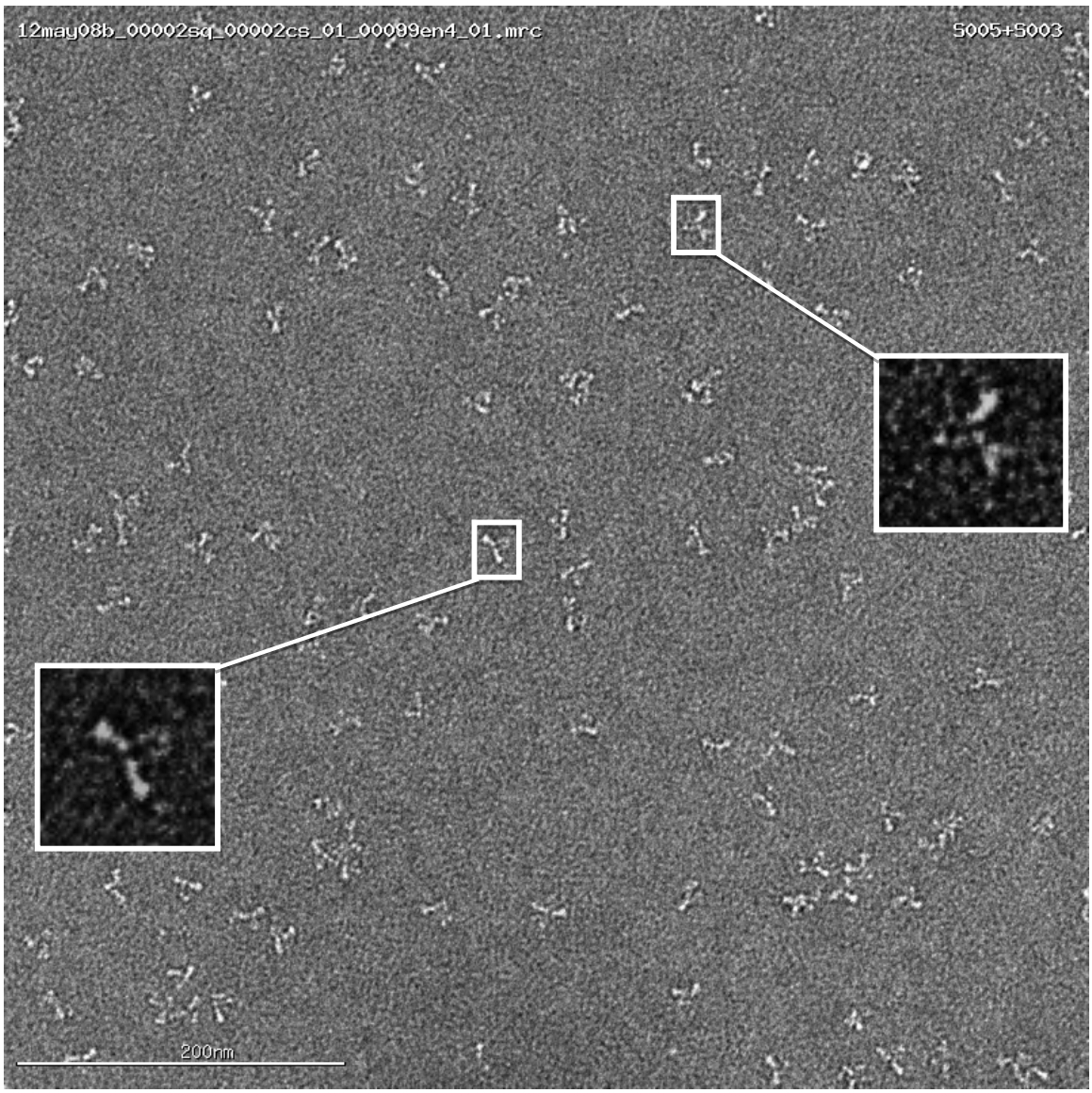
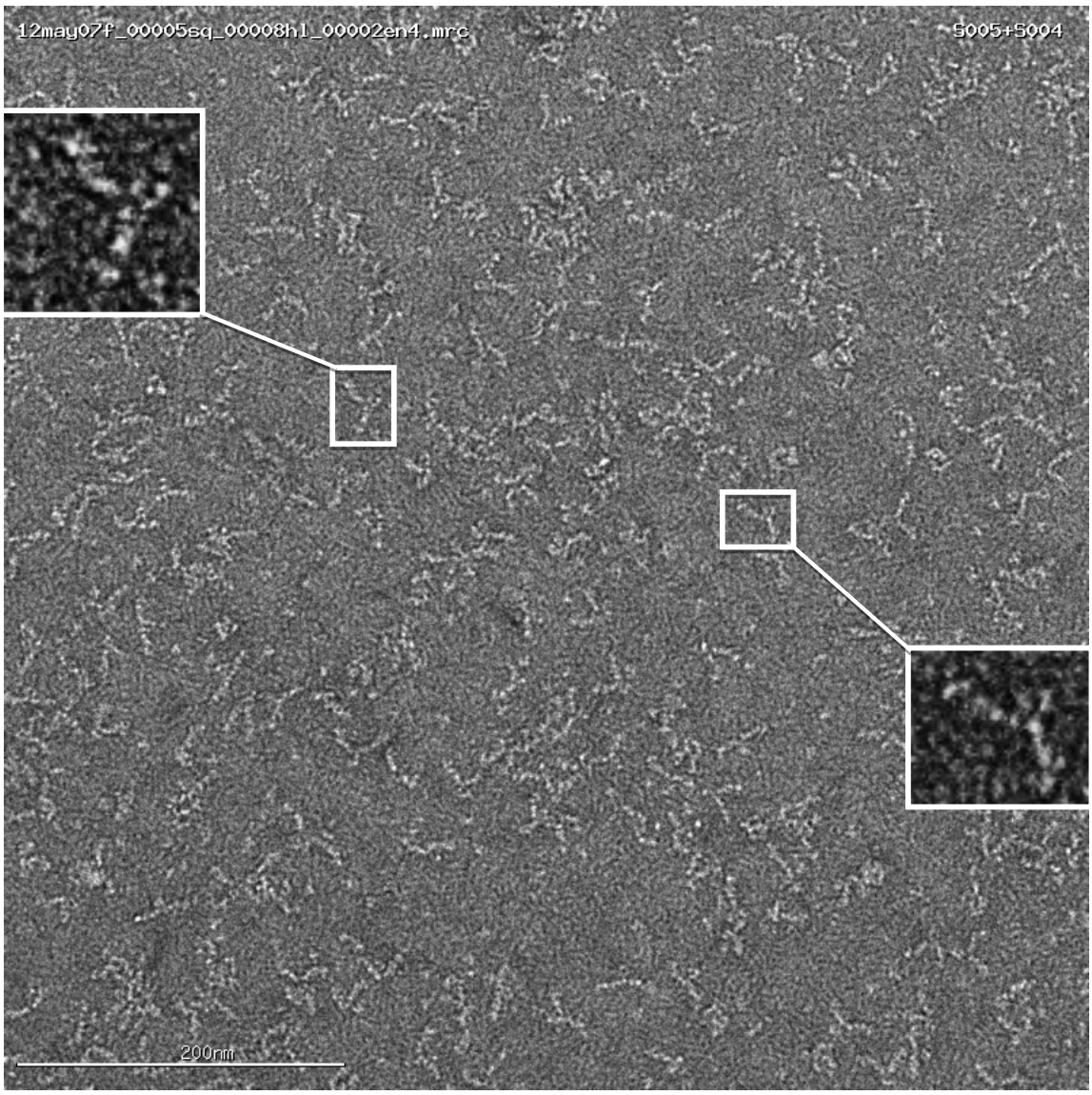
Supplemental Figure 1: Unprocessed images acquired at a nominal magnification of 52,000x of (a) a DVD-Ig™ molecule, (b) a DVD-Ig™ molecule bound to inner antigen, (c) a DVD-Ig™ molecule bound to outer antigen, (d) a DVD-Ig™ molecule bound to both inner and outer antigen. Individual particles are highlighted and shown at a larger scale.



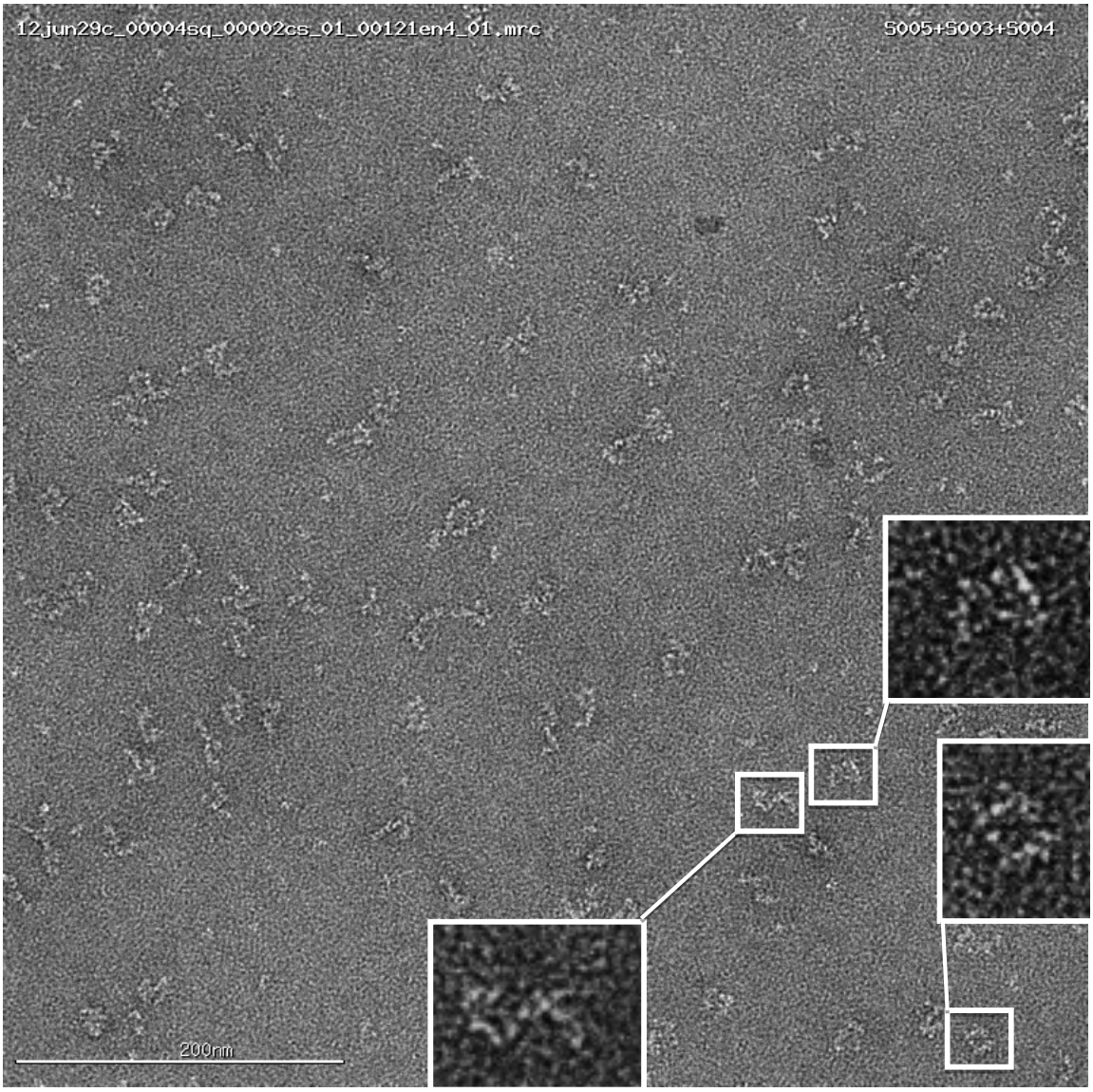
Supplementary figure 1a



Supplementary figure 1b

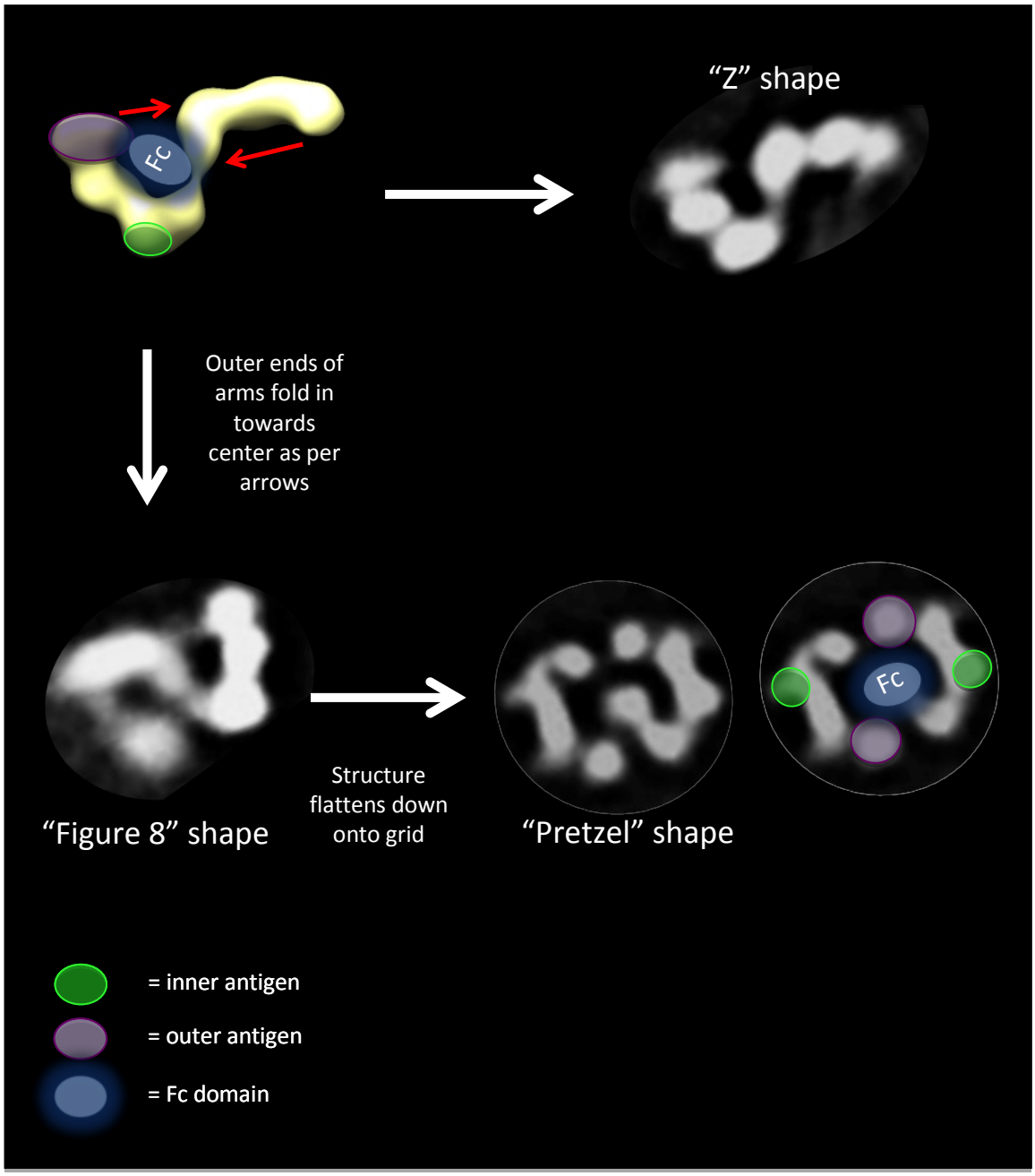


Supplementary figure 1c



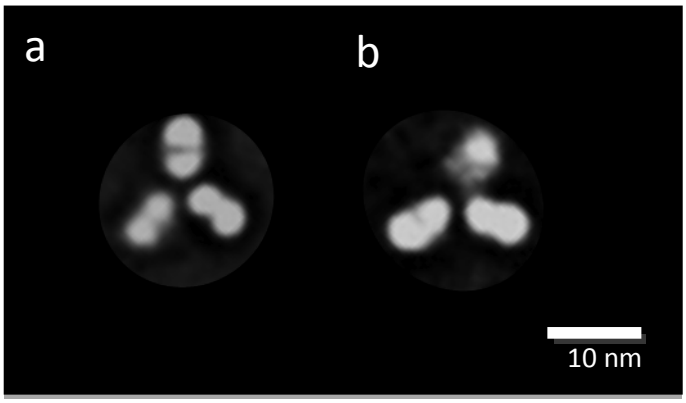
Supplementary figure 1d

Supplemental Figure 2: An interpretation of the 2D class averages observed for the DVD-Ig™ molecule bound to inner Antigen and outer Antigen as alternative conformations of the RCT map shown at top, left.



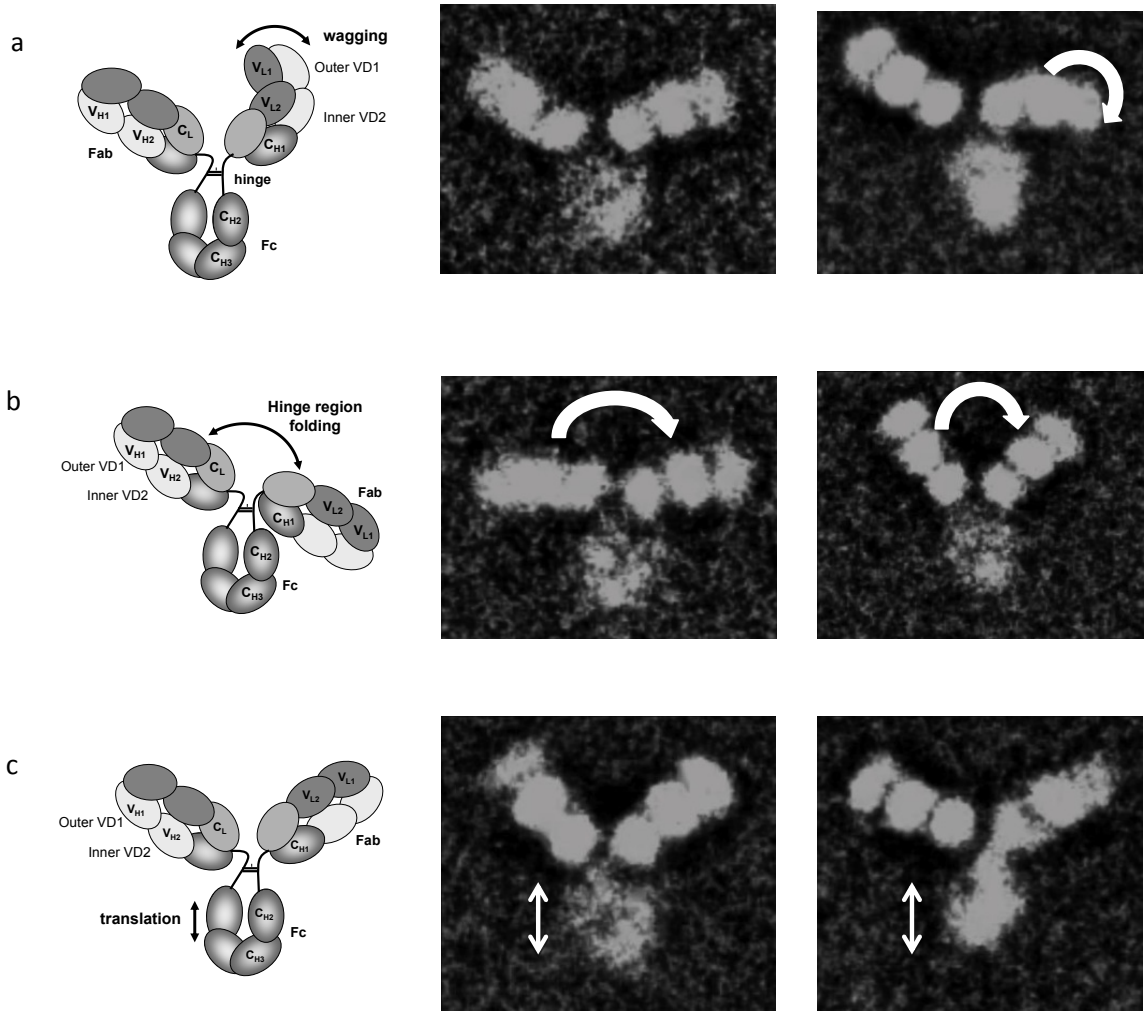
Supplementary figure 2

Supplement F**igure 3**: Class averages showing (a) Mab-1 (b) Mab-2.



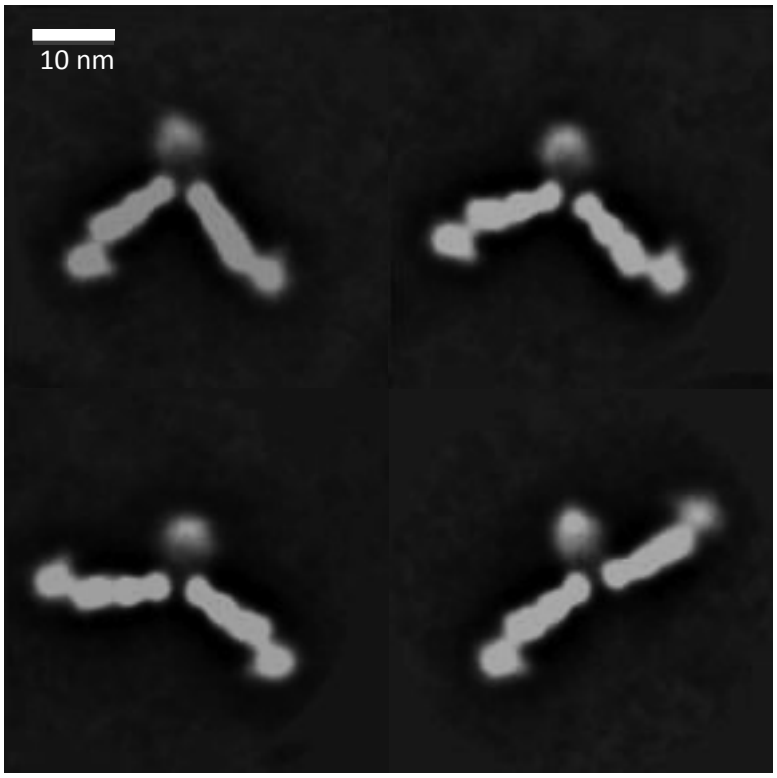
Supplementary figure 3

Supplemental F**igure 4**: Stacks of particles were classified, analyzed and sorted into sequential series and depicted as movie loops (see supplemental movie loops dvd1.mov and dvd2.mov). Individual frames from these movies have been extracted to highlight: (a) wagging of the outer domain, (b) hinge region folding, (c) translation of the Fc domain.



Supplementary figure 4

Supplemental F**igure 5:** Class averages showing a DVD-Ig^{TM} molecule bound to the outer antigen revealed varied conformations of the complex. Number of particles contributing to each class average is: n = 802 (top left), n = 943 (top right), n = 1222 (bottom left), n = 895 (bottom right).



Supplementary figure 5

Supplementary information:

Movies animations reveal flexibility of molecules

Stacks of CTF-corrected particles were evaluated using a coarse reference-free maximum-likelihood refinement [49] to identify and remove incorrectly boxed particles from the datasets. The filtered particle stacks were then iteratively aligned to a common reference, and the Maskiton package [50] was used to carry out a focused classification within a masked area to identify particle variations within specific regions. The resulting variations were then assessed to remove misalignment errors, before being converted to a movie format. Two movies are included. The first, dvd1.mov, was assembled from images within a single class average and reveals movement of the outer binding domain and translational movement of the Fc region. The second movie, dvd2.mov, was assembled from images across a number of class averages and shows flexibility of the Fab arms in the hinge region.

DVD1.mov

Supplement movie 1

Supplemental movie 1: *dvd1.mov* assembled from images within a single class average reveals movement of the outer binding domain as well as translational movement of the Fc region.

DVD2.mov

Supplemental movie 2

Supplemental movie 2: *dvd2.mov* assembled from images across a number of class averages reveals flexibility of the Fab arms in the hinge region of the DVD-Ig™ particle.

Supplemental fig