

Supplementary information

Structural dynamics of mixed-subunit CaMKII α/β heterododecamers filmed by high- speed AFM

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Supplementary Video legends

Supplementary Video 1. HS-AFM videos of three representative CaMKII β homooligomers on a P[5]A+-modified mica surface. The trajectories of the kinase domains are shown on the right. Image size, 360 \times 98 pixels² (acquired in HS-AFM observations); scan area, 80 \times 68 or 69 nm²; frame rate, 3.3 fps.

Supplementary Video 2. HS-AFM videos of three representative CaMKII β homooligomers with 50 μ M bosutinib on a P[5]A+-modified mica surface. The trajectories of the kinase domains are shown on the right. Image size, 240 \times 96 pixels² (acquired in HS-AFM observations); scan area, 80 \times 64 nm²; frame rate, 3.3 fps.

Supplementary Video 3. HS-AFM videos of three representative CaMKII α/β 3:1 heterooligomers on a P[5]A+-modified mica surface. The trajectories of the kinase domains are shown on the right. Green arrowheads indicate the kinase domains of CaMKII β subunits. Image size, 240 \times 96 pixels² (acquired in HS-AFM observations); scan area, 80 \times 64 nm²; frame rate, 3.3 fps.

Supplementary Video 4. HS-AFM videos of three representative CaMKII α /GFP-CaMKII β 3:1 heterooligomers on a P[5]A+-modified mica surface. The trajectories of the kinase domains are shown on the right. Green arrowheads and arrows indicate the kinase domains of CaMKII β subunits and GFP, respectively. Image size, 120 \times 96 pixels² (acquired in HS-AFM observations); scan area, 80 \times 64 nm² or 100 \times 80 nm²; frame rate, 3.3 fps.

Supplementary Video 5. HS-AFM videos of three representative CaMKII β homooligomers treated with Ca²⁺/CaM (1 mM Ca²⁺ and 800 nM CaM) on a P[5]A+-modified mica surface. The trajectories of the kinase domains are shown on the right. White dotted circles indicate Ca²⁺/CaM bound to the kinase domains, and blue dotted squares indicate kinase domain complexes. Image size, 360 \times 96 pixels² (acquired in HS-AFM observations); scan area, 80 \times 82 or 80 nm²; frame rate, 3.3 fps.

Supplementary Video 6. HS-AFM videos of three representative CaMKII α/β 3:1 heterooligomers treated with Ca²⁺/CaM (1 mM Ca²⁺ and 800 nM CaM) on a P[5]A+-modified mica surface. The trajectories of the kinase domains are shown on the right. Green arrowheads indicate the kinase domains of CaMKII β subunits. White dotted circles indicate Ca²⁺/CaM bound to the kinase domains, and blue dotted squares indicate

kinase domain complexes. Image size, 240×96 pixels² (acquired in HS-AFM observations); scan area, 80×64 nm²; frame rate, 3.3 fps.

Supplementary Video 7. HS-AFM videos of three representative CaMKII β homooligomers treated with Ca²⁺/CaM and ATP (1 mM Ca²⁺, 800 nM CaM, and 1 mM ATP) on a P[5]A⁺-modified mica surface. The trajectories of the kinase domains are shown on the right. White dotted circles indicate Ca²⁺/CaM bound to the kinase domains, and blue dotted squares indicate kinase domain complexes. Image size, 360×96 pixels² (acquired in HS-AFM observations); scan area, $80 \times 77, 72$ or 76 nm²; frame rate, 3.3 fps.

Supplementary Video 8. HS-AFM videos of three representative CaMKII α/β 3:1 heterooligomers treated with Ca²⁺/CaM and ATP (1 mM Ca²⁺, 800 nM CaM, and 1 mM ATP) on a P[5]A⁺-modified mica surface. The trajectories of the kinase domains are shown on the right. Green arrowheads indicate the kinase domains of CaMKII β subunits. White dotted circles indicate Ca²⁺/CaM bound to the kinase domains, and blue dotted squares indicate kinase domain complexes. Image size, 240×96 pixels² (acquired in HS-AFM observations); scan area, 80×64 or 100×80 nm²; frame rate, 3.3 fps.

Supplementary Video 9. HS-AFM videos of three representative CaMKII β homooligomers treated with EGTA (2 mM) and ATP (1 mM) after Ca²⁺/CaM/ATP stimulation (1 mM Ca²⁺, 800 nM CaM, and 1 mM ATP) on a P[5]A⁺-modified mica surface. The trajectories of the kinase domains are shown on the right. Blue dotted squares indicate kinase domain complexes. Image size, 360×96 or 80×73 pixels² (acquired in HS-AFM observations); scan area, 80×74 or 73 nm²; frame rate, 3.3 fps.

Supplementary Video 10. HS-AFM videos of three representative CaMKII α/β 3:1 heterooligomers treated with EGTA (2 mM) and ATP (1 mM) after Ca²⁺/CaM/ATP stimulation (1 mM Ca²⁺, 800 nM CaM, and 1 mM ATP) on a P[5]A⁺-modified mica surface. The trajectories of the kinase domains are shown on the right. Green arrowheads indicate the kinase domains of CaMKII β subunits. Blue dotted squares indicate kinase domain complexes. Image size, 240×96 or 120×96 pixels² (acquired in HS-AFM observations); scan area, 80×64 nm²; frame rate, 3.3 fps.

84 **Supplementary Video 11. HS-AFM videos of CaMKII α _{T286A} and CaMKII β _{T287A}**
85 **homooligomers treated with Ca²⁺/CaM (1 mM Ca²⁺ and 800 nM CaM) on a P[5]A+-**
86 **modified mica surface.** An enlarged view of the white dotted box is presented on the
87 right. White dotted circles indicate Ca²⁺/CaM bound to the kinase domains, and white
88 dots indicate the center of the kinase domains. Frame rate, 3.3 fps.