Supporting information for: Mechanisms of Nanonewton Mechanostability in a Protein Complex Revealed by Molecular Dynamics Simulations and Single-Molecule Force Spectroscopy

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Protein Sequences

pET28a-ybbR-HRV3C-6xHIS-CBM(C63S)-CttA-XDoc

MGTDSLEFIASKLALEVLFQGPLQHHHHHHPWTSASMANTPVSGNLKVEFYNSNPS
DTTNSINPQFKVTNTGSSAIDLSKLTLRYYYTVDGQKDQTFWSDHAAIIGSNGSYNG
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DYSFKSASQFVEWDQVTAYLNGVLVWGKEPGGSVVPSTQPVTTPPATTKPPATTI
PPSDDPNAVVPNTVTSAVKTQYVEIESVDGFYFNTEDKFDTAQIKKAVLHTVYNEG
YTGDDGVAVVLREYESEPVDITAELTFGDATPANTYKAVENKFDYEIPVYYNNATL
KDAEGNDATVTVYIGLKGDTDLNNIVDGRDATATLTYYAATSTDGKDATTVALSP
STLVGGNPESVYDDFSAFLSDVKVDAGKELTRFAKKAERLIDGRDASSILTFYTKSS
VDQYKDMAANEPNKLWDIVTGDAEEE*

pET28a-ybbR-HRV3C-6xHIS-CBM(C63S)-ScaB-XDoc

MGTDSLEFIASKLALEVLFQGPLQHHHHHHPWTSASMANTPVSGNLKVEFYNSNPS
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PPSDDPNAVVPATNSGDNVSVYYTIETVAGYYFSHDTGVRGNGEAGGFDKNQVVK
ITKYTKDKNGNIIAINDLDLANVNYNGYTPNKAYIDRFGDPAQNPTDQTLANFADN
FAYDIPVYYGGDQLVDENGQPLTVKAYIGVKGDTNLDFIVDGRDATATLTYYARVS
TDNYTEADTPISPAPFITGADDPLDDLAAFLSDVDTNEWDKDNWKLAREDRILDGR
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pET28a-CohE-CBM(C63S)-HIS-ybbR

MGTALTDRGMTYDLDPKDGSSAATKPVLEVTKKVFDTAADAAGQTVTVEFKVSG
AEGKYATTGYHIYWDERLEVVATKTGAYAKKGAALEDSSLAKAENNGNGVFVAS
GADDDFGADGVMWTVELKVPADAKAGDVYPIDVAYQWDPSKGDLFTDNKDSAQ
GKLMQAYFFTQGIKSSSNPSTDEYLVKANATYADGYIAIKAGEPGSVVPSTQPVTTP
PATTKPPATTIPPSDDPNAMANTPVSGNLKVEFYNSNPSDTTNSINPQFKVTNTGSS

AIDLSKLTLRYYYTVDGQKDQTFWSDHAAIIGSNGSYNGITSNVKGTFVKMSSSTNN
ADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSNDYSFKSASQFVEWDQVTA
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Supplementary Figures

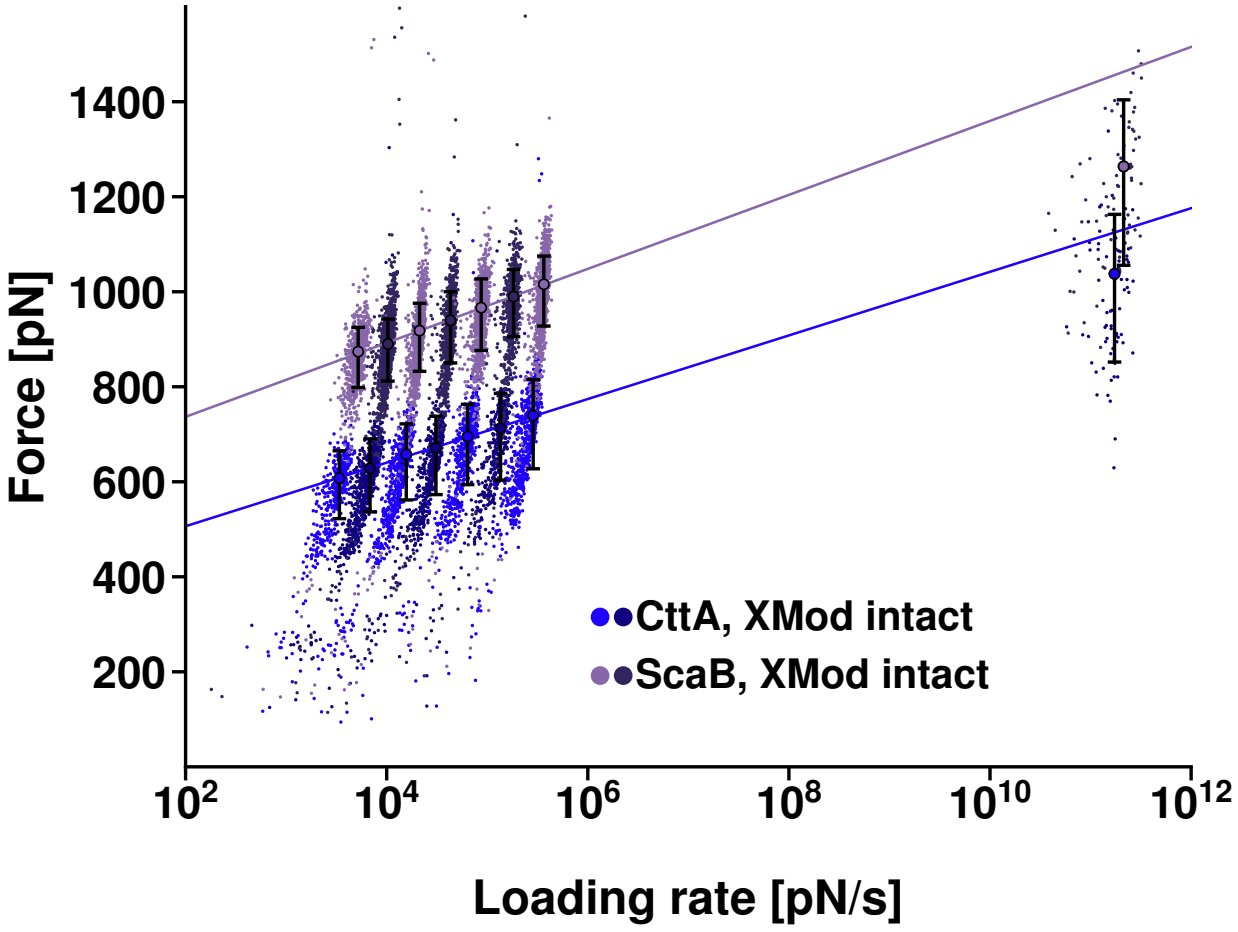

Figure S1: Dynamic force spectrum of curves with no apparent X-module unfolding fitted with a Bell Evans model. Extrapolating this fit to the simulated data reveals that the simulated rupture forces are approximately 8% lower than expected for the CttA-XDoc:CohE complex and 14% lower than expected for the ScaB-XDoc:CohE complex.

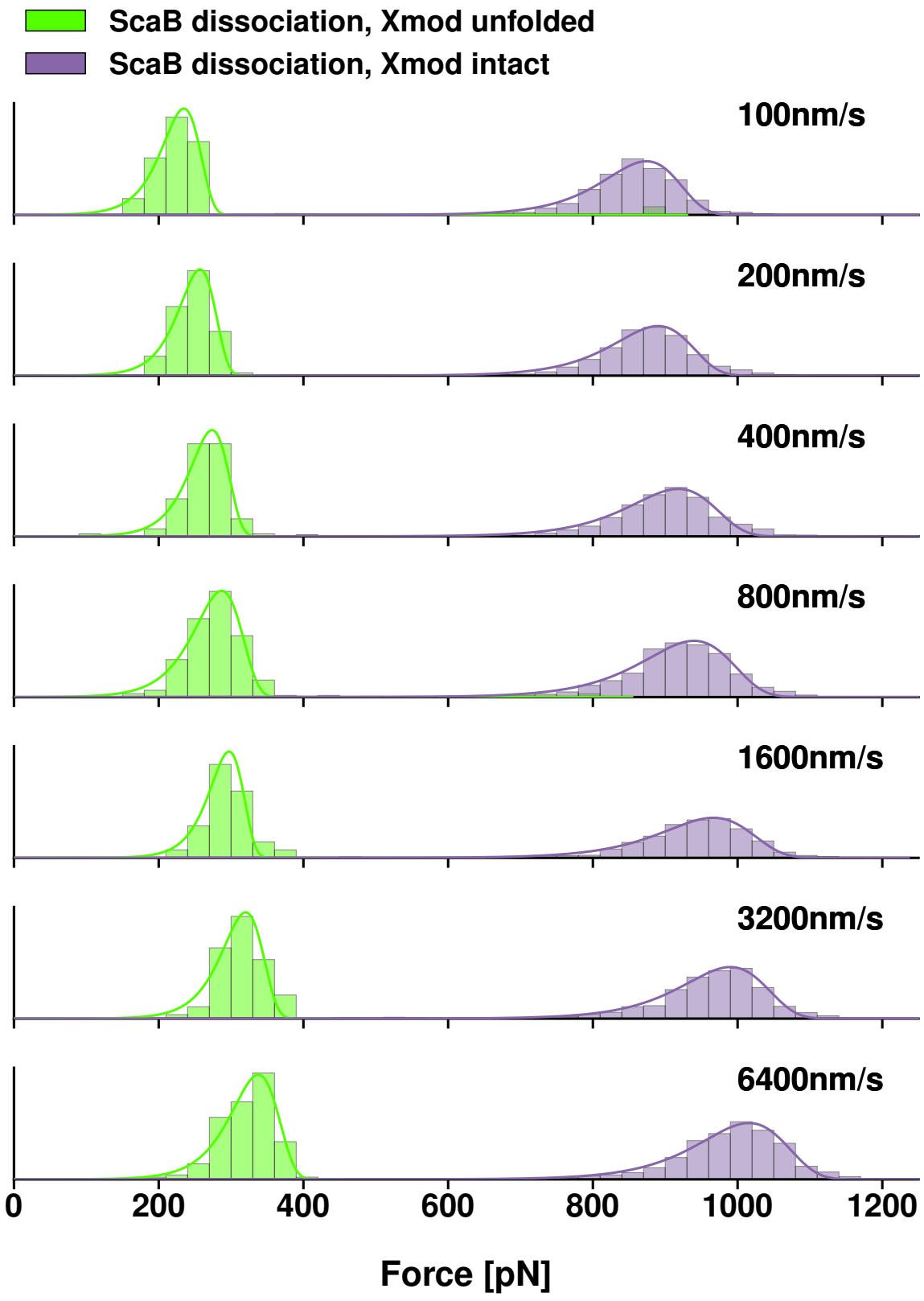

Figure S2: **Complex rupture forces of *Rf* ScaB-XDoc:CohE**. Green bars shows the rupture force of the complex following Xmod unfolding. Purple bars show the rupture force of the complex with Xmod intact.

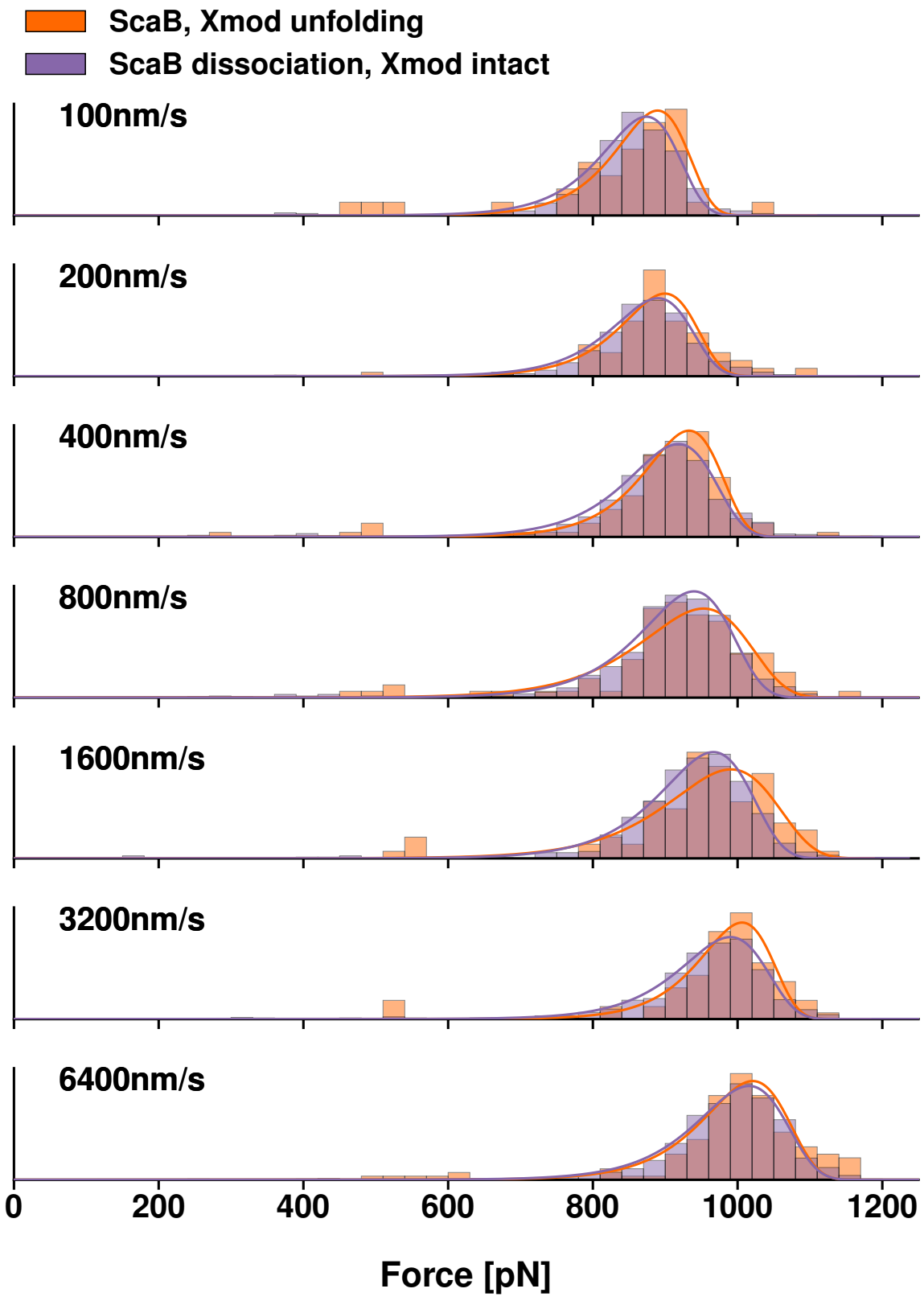

Figure S3: **Complex rupture forces with XMod intact vs. X-module unfolding forces for *Rf* ScaB-XDoc:CohE**. Orange bars show the unfolding force distribution of Xmod domains. Purple bars show the force distribution of complex rupture events with Xmod intact.

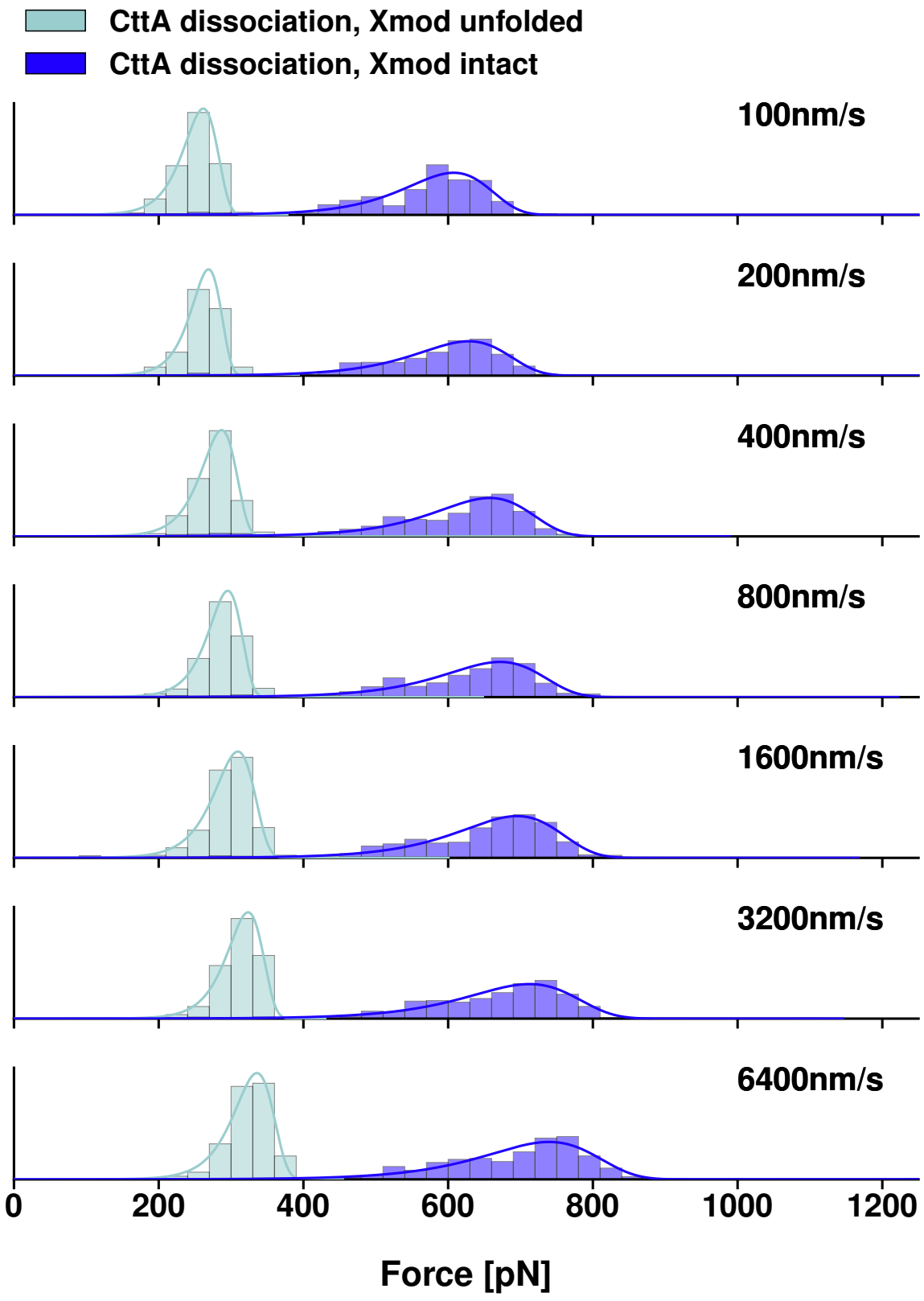

Figure S4: **Complex dissociation forces of *Rf* CttA-XDoc:CohE**. Teal bars shows the rupture forces of the complex following XMod unfolding. Blue bars show the rupture force distribution of the complex with XMod intact.

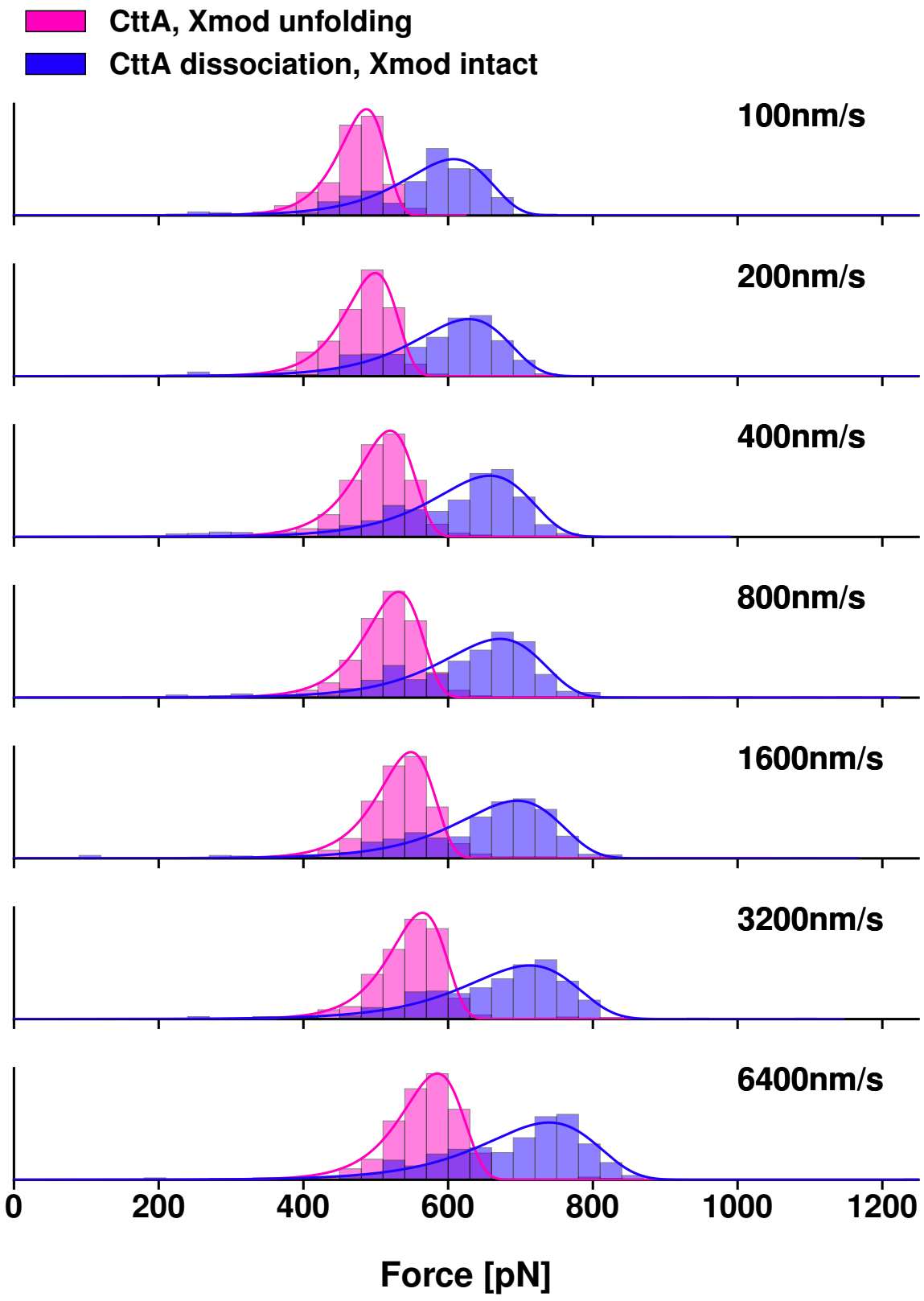

Figure S5: **Complex rupture forces with Xmod intact vs. X-module unfolding forces for *Rf* CttA-XDoc:CohE**. Pink bars show the unfolding force distribution of Xmod domains. Blue bars show the force distribution of complex rupture events with XMod intact.

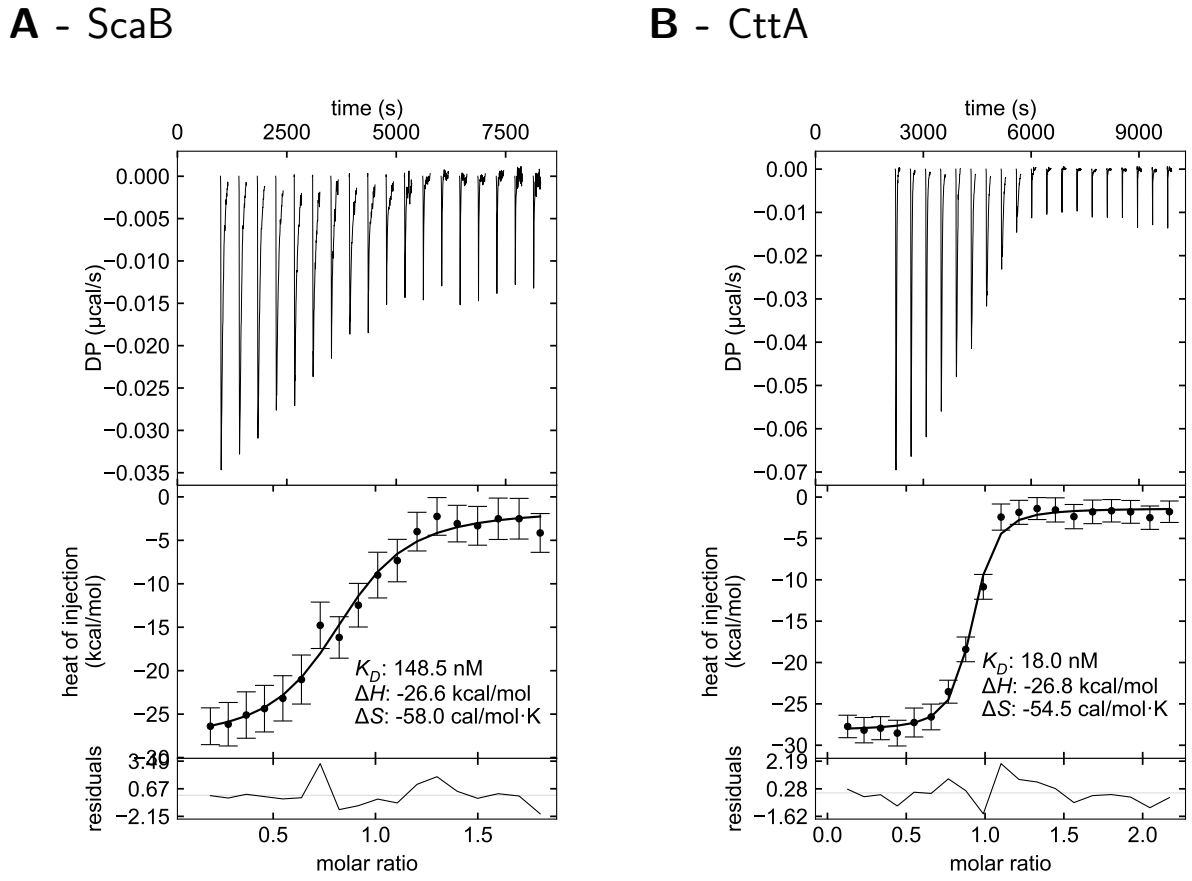

Figure S6: **Isothermal titration calorimetry. A:** The calorimeter's cell contained ybbR-CBM(C63S)-ScaB-XDoc at 4.04 µM, the injection syringe 35.0 µM CohE-NGL. **B:** The calorimeter's cell contained ybbR-CBM(C63S)-CttA-XDoc at 4.04 µM, the injection syringe contained 34.7 µM CohE-NGL.