

Supporting information

Modular site-specific conjugation of nanobodies using two co-associating tags

Eric Moeglin^{1,3}, Lina Barret², Bruno Chatton¹ and Mariel Donzeau^{*1}

mariel.donzeau@unistra.fr

Gene and protein sequences

Nano-HER2-E3

ATGGAAGTTCAACTGGTGAATCGGGCGGTGGTCTGGTTCAAGCGGGCGGCTCACTGCGTCT
GTCCTGTGCTACCTCGGGCATCACGTTTATGCGTTATGCACTGGGTGGTACCGTCAGAGCC
CGGGTAAACAACGTGAAATGGTTGCAAGTATTAAC TCCGGCGGTACCACGAATTATGCTGAT
TCAGTCAAAGGCCGTTTTACCATCTCGCGCGACAACGCAAAAAATACGGTGTACCTGCAGAT
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ACATCACCATCATCACCATTAA

MEVQLVESGGGLVQAGGSLRLSCATSGITFMRYALGWYRQSPGKQREMVASINSGGTTNYAD
SVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCNARWVKPQFIDNNYWGQGTQVTVSSAAA
TSEQKLI SEEDLNASTPLGDTTHTSGNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELN
EAL ELED AQAGKEPGSGGAPHHHHH

mScarlet-K3

ATGGCTAGTCACCATCACCATCACCATGCTGCCATGGTGAGCAAGGGCGAGGCAGTGATCAA
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MASHHHHHHAAMVSKGEAVIKEFMRFKVHMEGSMNGHEFEIEGEGEGRPYEGTQTAKLKVTK
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LEDGTLIYKVKLRGTNFPDGPVMQKKTMGWEASTERLYPEDGVLKGDIKMALRLKDGGRYL
ADFKTTYKAKKPVQMPGAYNVDRKLDITSHNEDYTVVEQYERSEGRHSTGGMDELYKASGNN
TSSSPQPKKKPLDGEYFTLQIRGRERFEMFRKLNKALELKDAQAGKEPGG

eGFP-K3

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GCCCCACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCG
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MASHHHHHHARMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTT
GKLPVPWPTLVTTLTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEV
KFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKNGIKVNFKIRHNIEDG
SVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLLEFVTAAGITLGMDEL
YEASGNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRKLNKALELKDAQAGKEPGG

K3 synthetic peptide

CALNNGEYFTLQIRGRERFEMFRKLNKALELKDAQA

Table S1 : Biochemical-properties of the different constructs

	Number of aa	Monomer MW (kDa)	pI	Oligomerization state	MW of the complex (kDa)	Calculated MW by SEC
Nano-HER2-E3	213	23.50	6.88	Homodimer	47	70
eGFP-K3	305	35	6.80	Homotetramer	138	160
mScarlet-K3	302	34	8.60	Homodimer	136	155
K3	35	4	10.11	Homotetramer	16	-
Nano-HER2-E3/eGFP-K3	n.a	n.a	n.d	Heterotetramer	115	175
Nano-HER2-E3/mScarlet-K3	n.a	n.a	n.d	Heterotetramer	115	-
Nano-HER2-E3/ K3	n.a	n.a	n.d	Heterotetramer	76	70

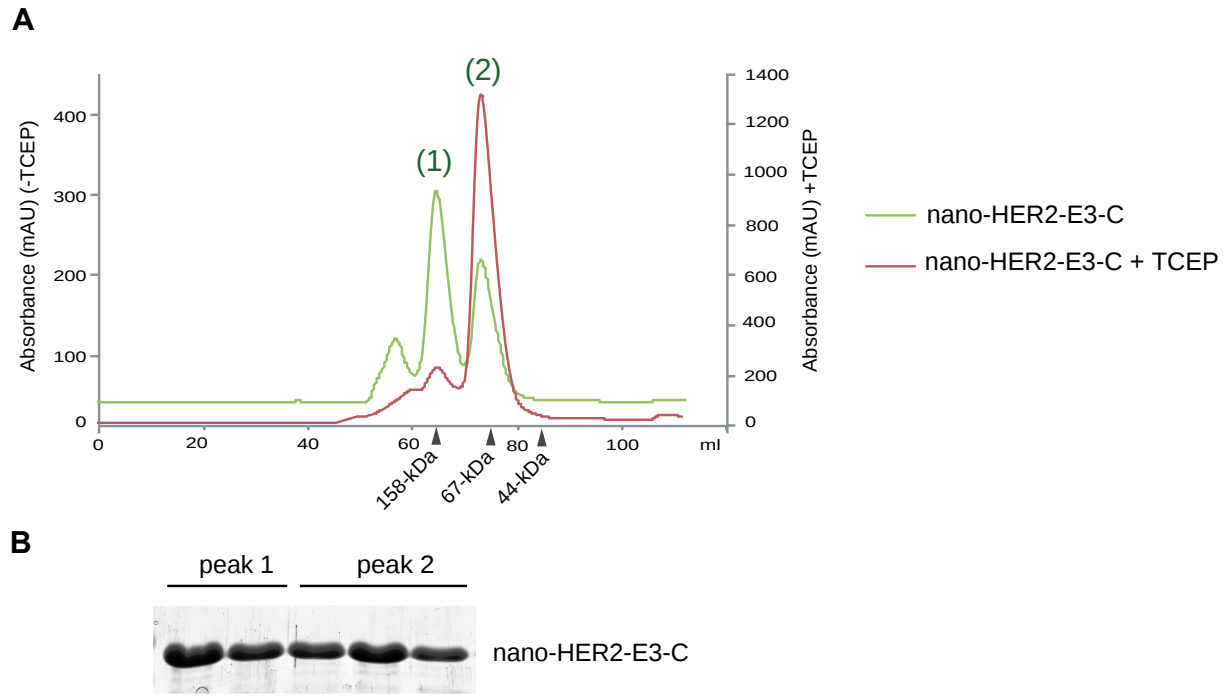


Figure S1: Purification of the nano-HER2-E3-C. (A) Purification of the nano-HER2-E3-C with (red) or without (green) TCEP on Hiload 16/160 Superdex 200pg after IMAC purification. (B) The two major peaks of nano-HER2-C without TCEP (1 and 2) were loaded on SDS-PAGE and stained by coomassie blue.

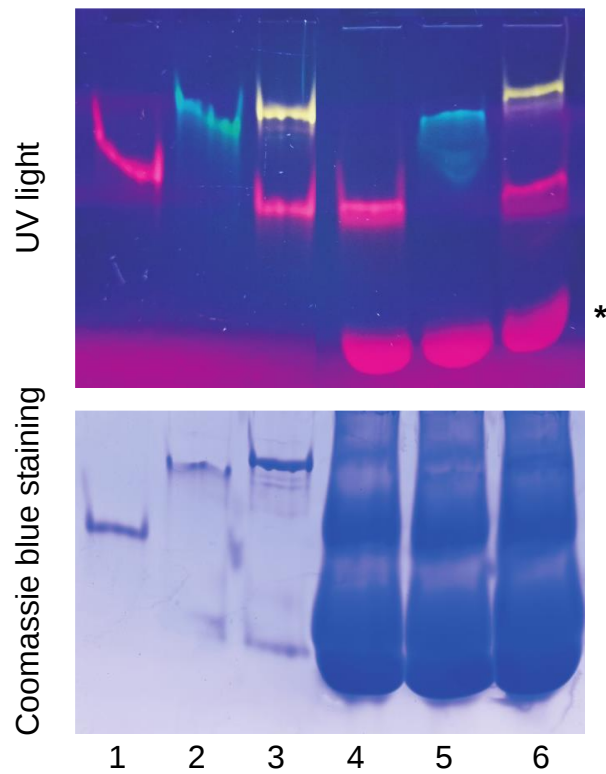


Figure S2: Complex formation in Serum. The eGFP-K3 purified protein was incubated with mCherry-E3 in PBS (lanes 1-3) or in the presence of 85 % foetal bovin serum (FBS) (lanes 4-6). The fluorescent moieties were revealed using UV light (upper panel) and FBS total proteins by Coomassie blue staining (lower panel): homotetramer mCherry-E3 (purple: lanes 1 and 4), eGFP-K3 (green: lanes 2 and 5), and heterotetramer mCherry-E3/eGFP-K3 (yellow: lanes 3 and 6). Intrinsic fluorescence of the Bovin Serum Albumin (BSA) is clearly visualized (upper panel, asterix).

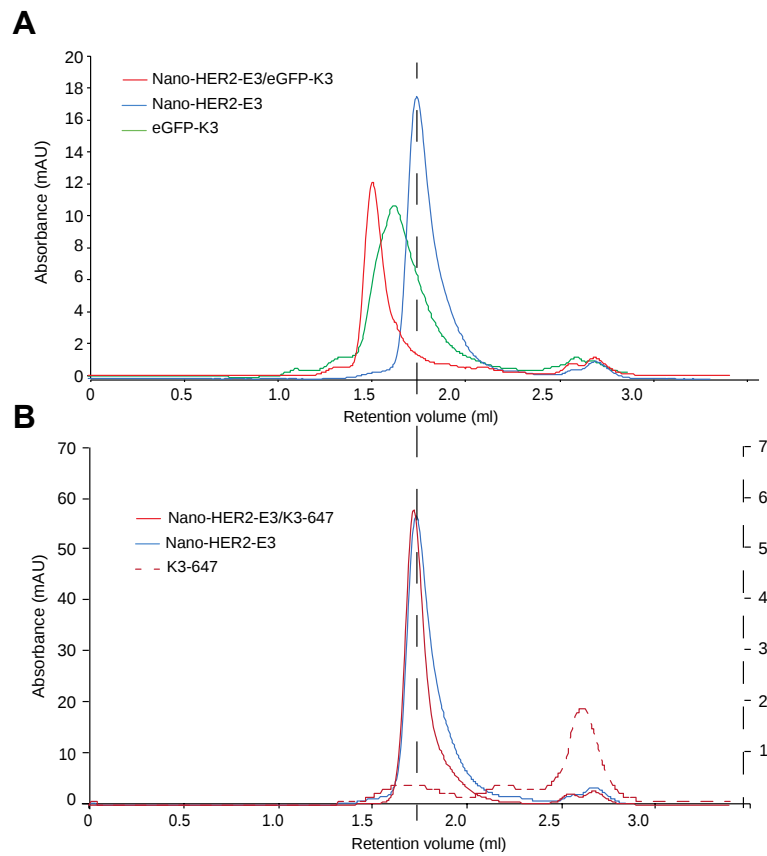


Figure S3: Analysis of complex formation. (A) Purified nano-HER2-E3 (blue), eGFP-K3 (green) and the heterotetrameric complex (red dashed line) or (B) nano-HER2-E3 (blue), mCherry-K3 (red) and the heterotetrameric complex (red dashed line) were mixed in equimolar amounts and analyzed by SEC.

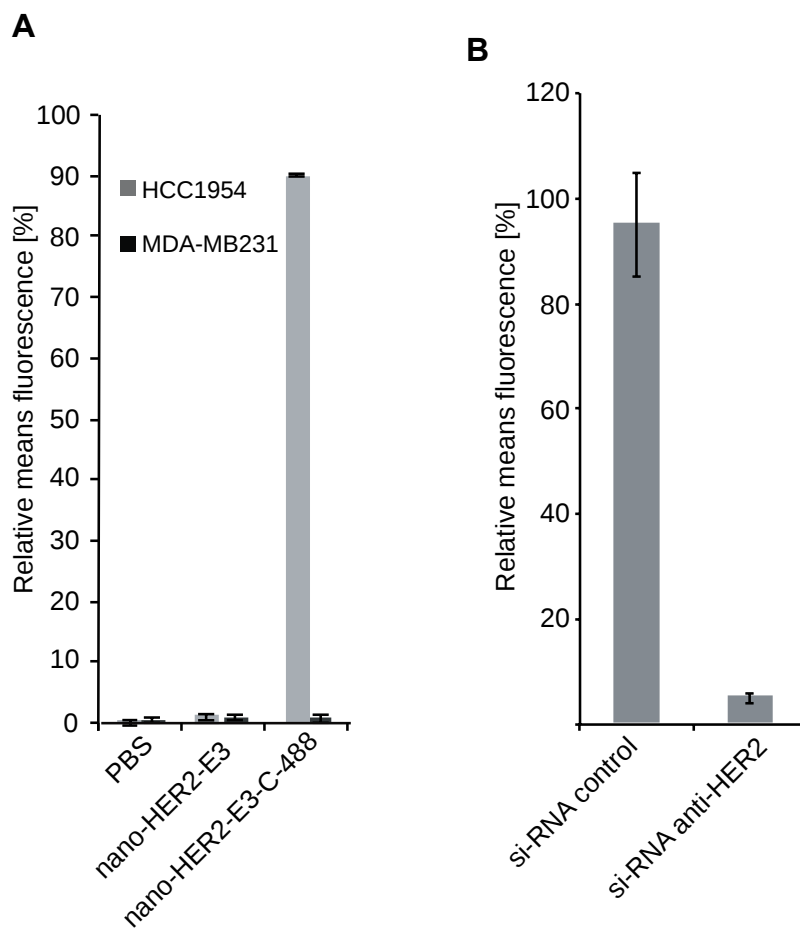


Figure S4: Analysis by flow cytometry of with the nano-HER2-E3-C-488 on HCC1954 and MDA-MB231 cell lines as indicated (A) or on HCC1954 treated with siRNA anti-HER2 or with a siRNA control (B). Relative means of fluorescence in % are represented.

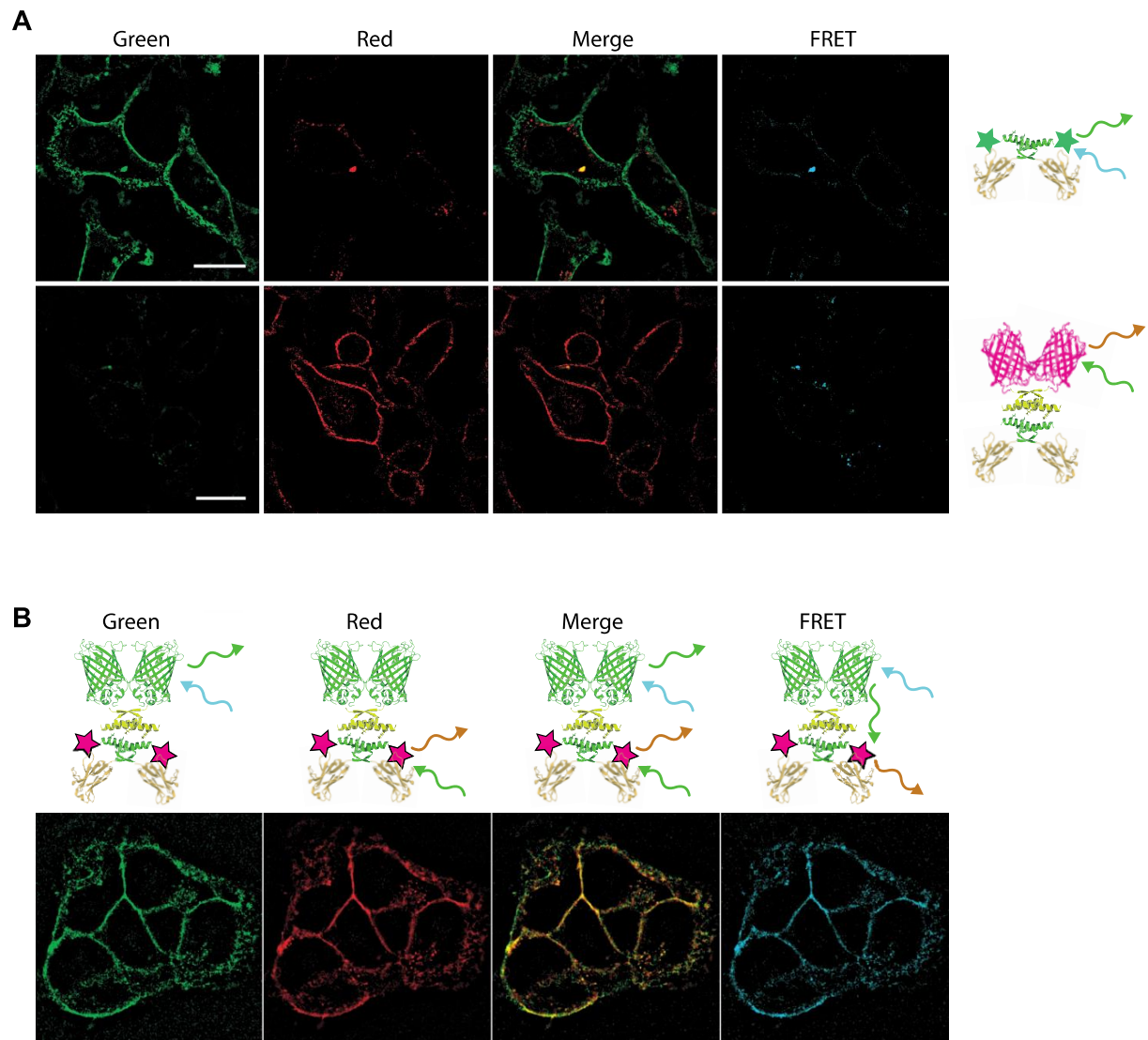


Figure S5: Complex formation at the cell surface receptor (A) control of the isolated fluorescent signals used in the FRET experiment. Upper panel: nano-HER2-E3-C-488, lower panel: nano-HER2-E3/mScarlet-K3. (B) FRET between and nano-HER2-E3-C-568 and eGFP-K3. HCC1954 cells were incubated with different complexes after fixation, as indicated. Images were taken by confocal microscopy and analyzed with Image J. Cartoons represent the complexes. Scale bar represent 10 μm .

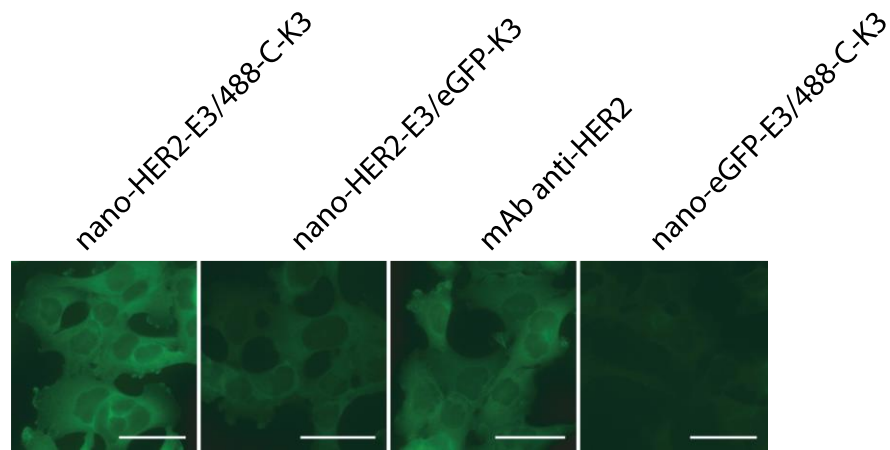


Figure S6: Indirect immunofluorescence. Fixed HCC1954 cells were incubated with either different Alexa-Fluor-488 labeled complexes or with an anti-HER2 mAb followed by a secondary Alexa-488 secondary mAb, as indicated. Images were taken by confocal microscopy and analyzed with Image J. Cartoons represent the complexes. Scale bar represent 50 μm .