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SUPPLEMENTARY INFORMATION

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Single-molecule displacement mapping unveils nanoscale heterogeneities in intracellular diffusivity

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Supplementary Table 1. List of plasmid constructs used in this work. Blue and red colors mark positively and negatively charged AAs that are varied between the different constructs.

Plasmid	Protein sequence	Size (AA)	Net charge[a]	Net charge[b]
mEos3.2-C1	mEos3.2-SGLRSRAQASNSAVDGTAGPGSTGSR (mEos3.2 = MSAIKPDMKIKLRMEGNVNGHHFVIDGDGTGKPFEGKQSMDLE VKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYFKQSFPKGY SWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDF RTTYKAKEKGVKLPGAHFVDHCIEILSHDKDYNKVKLYEHAVA HSGLPDNARR)	252	+2.2	+2.5
mEos3.2-NLS	mEos3.2-SGLRSRADPKKKRKVDPKKKRKVDPKKKRKVGSTGSR	262	+15.2	+15.5
mEos3.2 (−14)	mEos3.2-SGLRSRAQASNSDEDEEDDEEDEEDDEDNSAVDGTAGPGSTGSR	270	−13.8	−13.4
mEos3.2 (−7)	mEos3.2-SGLRSRAQASNSDEDEEDDEENSAVDGTAGPGSTGSR	263	−6.8	−6.5
mEos3.2 (0)	mEos3.2-SGLRSRAQASNSDESTQNSAVDGTAGPGSTGSR	259	+0.2	+0.5
mEos3.2 (+7A)	mEos3.2-SGLRSRAQASNSKKRKRNSAVDGTAGPGSTGSR	259	+7.2	+7.5
mEos3.2 (+14)	mEos3.2-SGLRSRAQASNSKKRKKRKKRKKRNSAVDGTAGPGSTGSR	266	+14.2	+14.5
mEos3.2 (+7B)	mEos3.2- SGRQKGHKCIRLPKVNQRMSR	247	+7.2	+7.5
mEosP5-C1 (+7C)	mEosP5-SGLRSRAQASNSAVDGTAGPGSTGSR (mEosP5 = MKSAIKPDMKIKLRMEGNVNGHHFVIDGDGTGKPFEGKQSMDLE VKKGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYFKQSFPKGY SWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDF RTTYKAKEKGVKLPGAHFVDHCIEILSHDKKYNKVKLYEHAVA HSGLPDNARR)	253	+7.2	+7.5
mEmerald-C1	MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKL TLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFFKS AMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDF KEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDG SVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDH MVLLEFVTAAGITLGMDELYKSGLRSRAQASNSAVDGTAGPGS TGSR	265	−3.7	−3.4

a: Calculated by summing the charges of each amino acid at pH 7.4 (ref 1): lysine = +0.999; arginine = +1.000; histidine = +0.048; glutamic acid = −0.999; aspartic acid = −1.000; cysteine = −0.085, and all other amino acids = 0.000.

b: Calculated by Protein Calculator v3.4 (http://protcalc.sourceforge.net/) for pH 7.4.

Supplementary Table 2. List of estimated net charges for the most abundant (>0.2% of total protein mass) cytoplasmic proteins, based on proteomics of the U2OS human cell line[2,3]. Protein sequences are from UniProt (https://www.uniprot.org/). Net charges are estimated for pH 7.4 using Protein Calculator (http://protcalc.sourceforge.net/). For each category, proteins are listed in the order of mass abundance (% of the total protein mass of the cell). This showed that most proteins in the categories of "cytoskeletal proteins", "chaperones and folding catalysts", and "others" are either strongly negatively charged (<−10) or neutral (within ±2). Half of the proteins in the "glycolysis" group are mildly (~+3) positive, possibly for their intended interactions with the negatively charged, phosphorylated glucose metabolites, which thereby neutralizing the total charge. Three proteins in the group of "ribosome" and one in "translation factors" are strongly positively charged, but these positive charges are more than compensated by their binding partner, the heavily negatively charged RNA[4,5].

Cytoskeletal proteins & regulators

Name	%mass	Net charge
Vim	2.7	-18.6
TubA1c	2.2	-22.7
ActB	2.2	-11.7
Cfl1	1.2	1.6
FlnA	0.84	-50.8
Plec	0.83	-76.9
myh9	0.71	-45.9
pfn1	0.68	1.8
FlnB	0.36	-60.9
FlnC	0.32	-54.6
TubB6	0.30	-24.7
LmnA	0.29	-2.1
Myl6	0.28	-14.0
SptAn1	0.27	-106.4

Chaperones and folding catalysts

Name	%mass	Net charge
Hsp90ab1	2.2	-39.3
HspA8	2.0	-12.8
cct2	1.1	-7.9
PPIA	0.97	0.9
HspD1	0.89	-5.2
HspB1	0.60	-2.7
cct6a	0.40	-4.5
cct5	0.20	-13.6

Glycolysis

Name	%mass	Net charge
Pkm2	3.1	2.4
Eno1	2.5	0.0
GAPDH	2.0	3.6
Tpi1	1.7	-5.0
AldOa	1.1	3.2
Pgk1	0.85	2.8
LdhA	0.53	3.2
Eno3	0.48	1.1
Eno2	0.22	-18.0

Ribosome

Name	%mass	Net charge
Rpl37a	0.54	18.7
Rps15a	0.26	10.1
Rpl7a	0.25	40.9
RplP0	0.20	-4.0

Translation factors

Name	%mass	Net charge
Eef1a1	2.7	11.4
Eef2	1.1	-4.8
Eif5a	0.50	-7.1
Eef1d	0.39	-14.8

Others

Name	%mass	Net charge
Mif	1.9	0.8
LgaLs1	0.79	-3.4
Tkt	0.47	1.7
Cltc	0.38	-39.8
GstP1	0.33	-3.3
Eif4a1	0.26	-9.0
FasN	0.23	-34.1

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