Backbone rigidity and static presentation of guanidinium groups increases cellular uptake of arginine-rich cell penetrating peptides

Supplementary Information

Supplementary Table S1:
Peptide sequences, linkers and molecular weight

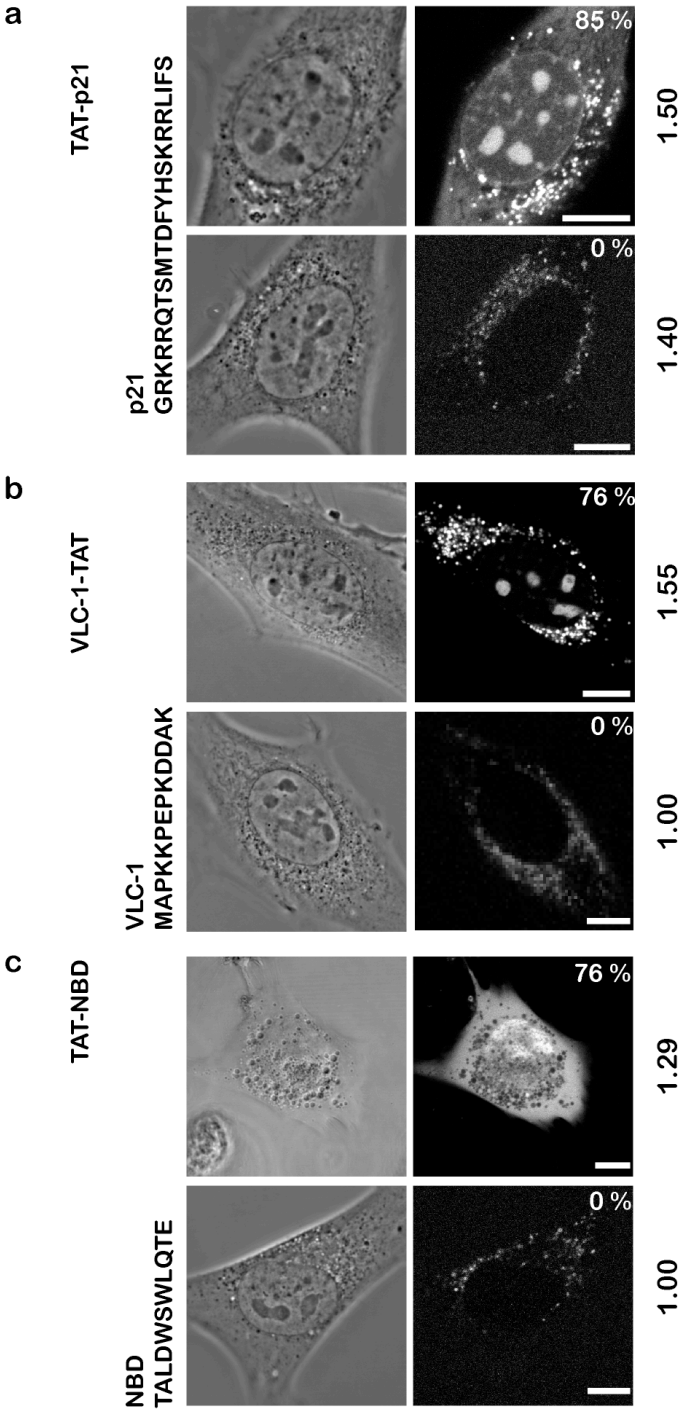
Peptide	Sequence	Label (linker)	MW [kDa]
TAT	rrrqrrkkrg	(Ahx)	1.396
TAT	rrrqrrkkrg	Fl (Ahx)	1.807
TAT	rrrqrrkkrg	FITC (Ahx)	1.897
TAT	rrrqrrkkrg	TAMRA (Ahx)	1.921
cyclic TAT	KrRrGrKkRrE	Fl (K1)	1.866
R10	rrrrrrrrrr		1.692
R10	rrrrrrrrrr	Fl	2.065
R10	rRrRrRrRrRq	Fl	
cyclic R10	KrRrRrRrRrRE	Fl	2.175
PTD4	YARAARQARA	Biotin (Ahx)	1.542
PTD4	araqraaray	TAMRA (Ahx)	1.615
TAT-p21	rrrqrrkkrgaaAGRKRRQTSMTDFYHSKRRLIFS	Fl	4.914
p21	GRKRRQTSMTDFYHSKRRLIFS	Fl	3.327
VLC-1-TAT	MAPKKPEPKDDAKAPAGRKKRRQRRRC		3.175
VLC-1	MAPKKPEPKDDAKAPAC		1.737
VLC-1-TAT	MAPKKPEPKDDAKAPAGRKKRRQRRRC	TAMRA (C)	3.856
VLC-1	MAPKKPEPKDDAKAPAC	TAMRA (C)	1.737
TAT-NBD	YGRKKRRQRRRTALDWSWLQTE		2.891
NBD	TALDWSWLQTE		1.348
TAT-NBD	YGRKKRRQRRRTALDWSWLQTE	Fl (Ahx)	3.559
NBD	TALDWSWLQTE	Fl (Ahx)	1.819

Lower case letters indicate D- and upper case letters L-amino acids. The labeled form of PTD4 as well as several other peptides were synthesized in the retro-inverso (retro-all D) form. Linker sequences are underlined. The fluorophores are given together with the amino acid or chemical compound used for coupling at the N-terminus.

Supplementary Table S2:
Measured and deduced parameters obtained by analytical ultracentrifugation.

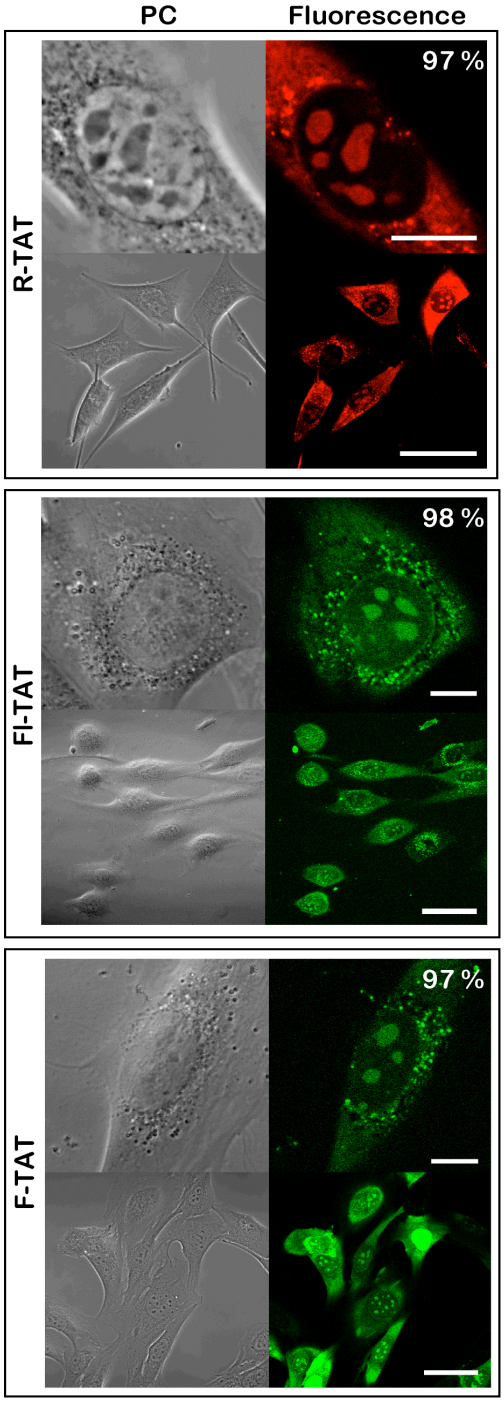
Construct	$s_{20,w}$	$D_{20,w}$ [10^7 cm^2 s^{-1}]	$M_{exp.}$ [kDa]	f/f_0	V_{dry} [nm^3]	d_{sphere} [nm]	d_a [nm]	d_b [nm]
TAT	0.291	18.6	1.445	1.39	1.71	1.48	5.21	0.79
R10	0.303	16.83	1.692	1.49	2.08	1.58	5.60	0.84
PTD_4	3.75	21.55	1.542	1.16	0.79	1.158	3.70	0.98
cyclicTAT (Fl)	0.368	19.99	1.87	1.14	2.31	1.64	2.50	0.70
TAT-p21 (Fl)	0.606	10.97	4.843	1.50	5.92	2.24	10.00	1.06
p21 (Fl)	0.521	13.30	3.081	1.40	3.76	1.55	7.39	0.98
VLC-1-TAT	0.410	12.43	3.135	1.55	4.07	1.00	9.20	0.46
VLC-1	0.420	24.26	1.626	1.00	2.00	1.56	1.56	1.56
TAT-NBD	0.539	14.80	3.274	1.29	3.97	1.96	6.24	1.11
NBD	0.397	25.96	1.349	1.00	1.61	1.45	1.45	1.45

s - sedimentation coefficient, D - diffusion coefficient, $M_{exp.}$- molecular weight (experimental), V - volume and d - diameter.



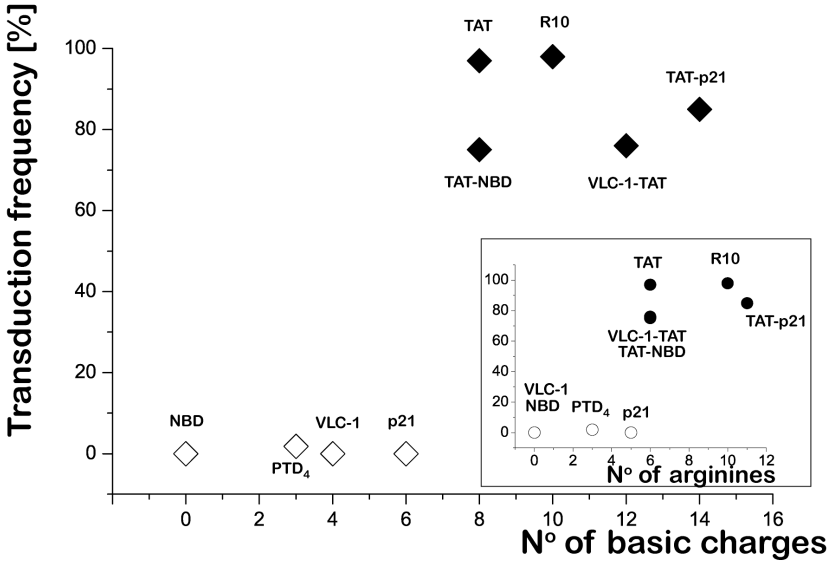
Supplementary Fig. S1: Live cell internalization of TAT cargo fusions and cargos alone

Ability of cargo peptides with and without TAT to transduce into living mouse myoblast cells after incubation for one hour at 15 μM in a volume of 200 μl PBS. Representative transmission images of the phase contrast (PC) and confocal optical sections of the fluorescent peptides are shown. Transduction efficiencies are given as percentages in the fluorescence images (n=150 cells) and the frictional ratio (f/f0) and the molecular dimension (d) calculated from the ultracentrifugation results are displayed on the right side of the images. PC: phase contrast, Scalebars = 10 μm.

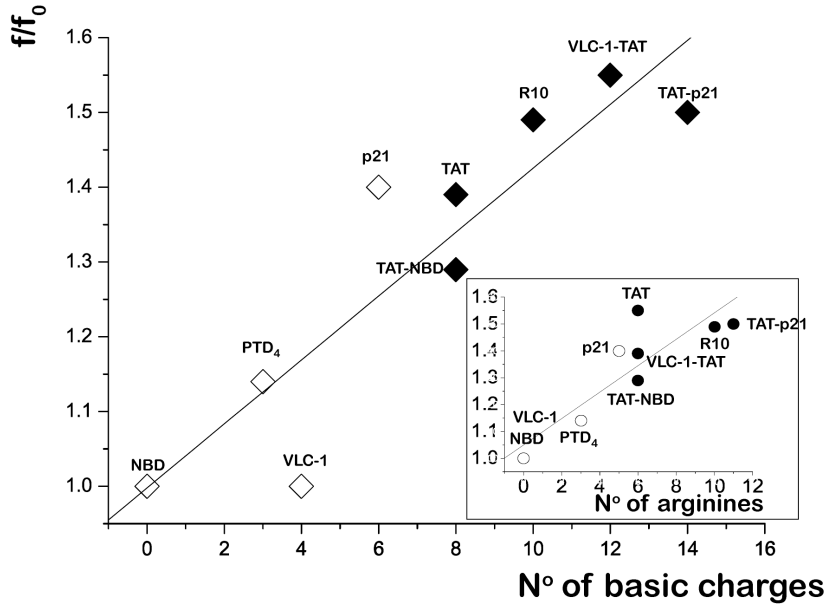


Supplementary Figure S2: Different labels do not change uptake behaviour of RRPs
To ensure that different labels did not influence the transduction assay, TAT (amino acids 48-57 from the transactivator of transcription of the human HIV-1 protein) coupled to fluorophores FITC (F), fluorescein (Fl) and TAMRA (T) utilized was tested for transduction ability and frequency as well as intracellular localization.

a

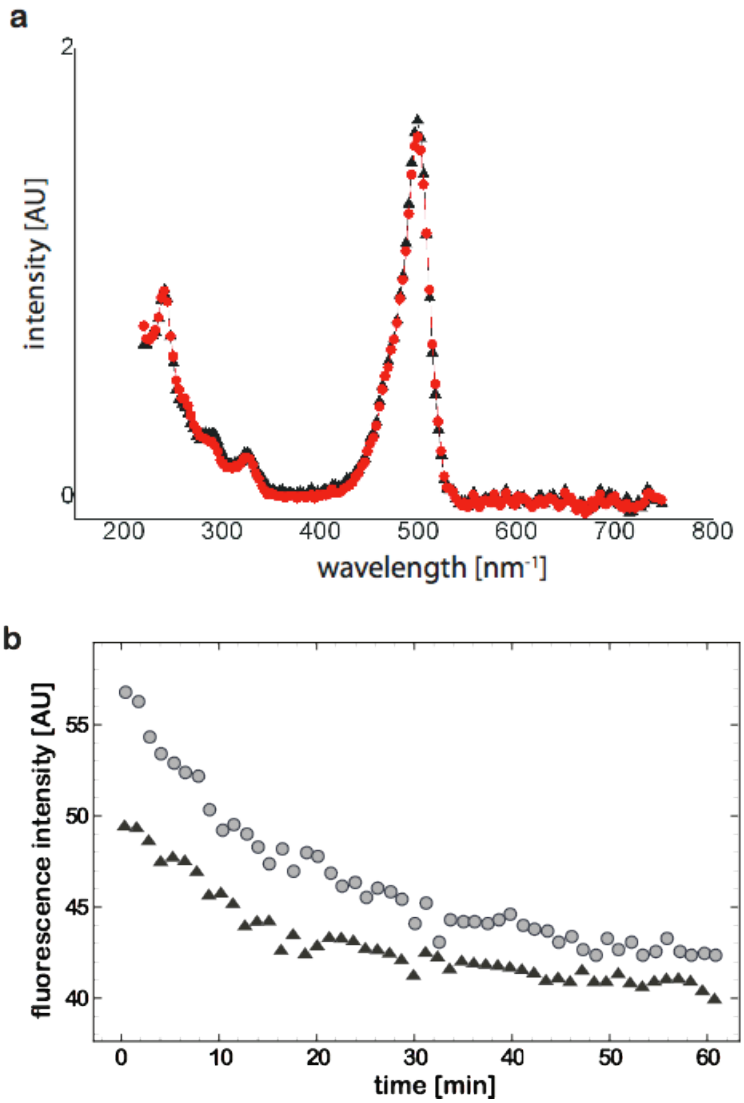


b



Supplementary Figure S3: Influence of basic charges on transduction frequency and structure

Transduction percentages (a) and frictional ratios f/f_0 of transducible (filled diamonds) and non-transducible (empty diamonds) peptides plotted against the number of basic residues and the number of arginines (inlay, transducible peptides filled circles and non-transducing peptides empty circles).



Supplementary Figure S4: **Comparison of the absorption spectra and extracellular fluorescence intensities during imaging of cyclic and linear TAT.**
a UV-VIS absorption spectra of cyclic and linear TAT peptide. Absorption of the peptides at 220 nm was used to assure that identical concentrations of both peptides were applied to living cells.
b Average intensities of extracellular fluorescence over time used to control for differences resulting from photobleaching and culture chamber inhomogeneities.