Rational design of polyfluorinated peptide-based materials: Self-assembly of an amphiphilic motif

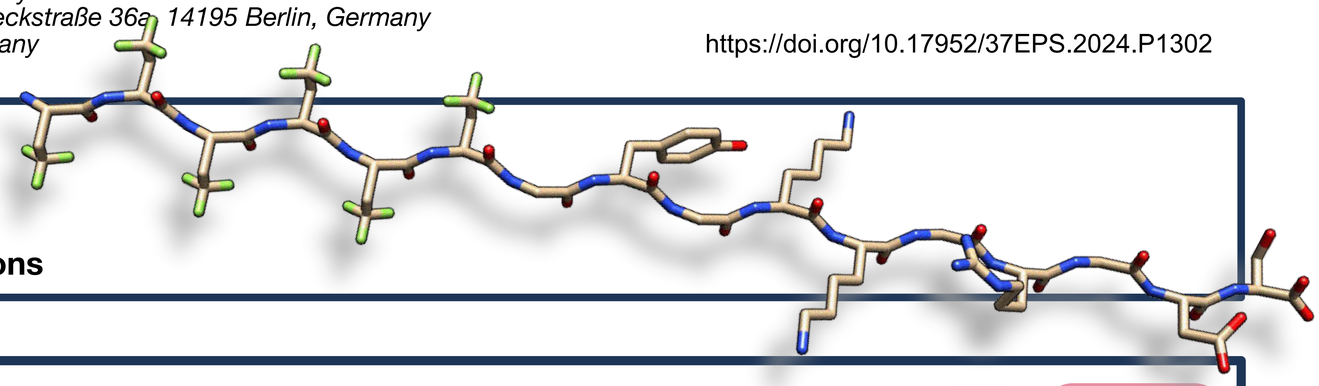
Maurizio Iannuzzi[1], Suvrat Chowdhary[1], Holly Fiedler[1], Kilian Haoues[1], Dr. Boris Schade[2], Dr. Andreas F. Thünemann[3], Elisa Quaas[1] and Prof. Dr. Beate Koksch[1]

[1]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Arnimallee 20, 14195, Berlin, Germany
[2]Institute of Chemistry and Biochemistry and Core Facility BioSupraMol, Freie Universität Berlin, Fabeckstraße 36a, 14195 Berlin, Germany
[3]Federal Institute for Materials Research and Testing (BAM), Unter der Eichen 87, 12205 Berlin, Germany

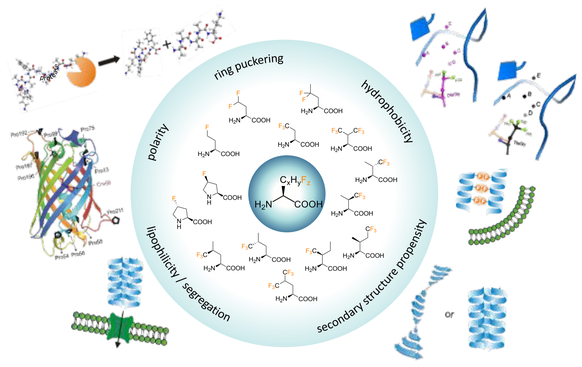
https://doi.org/10.17952/37EPS.2024.P1302

AIM

- Characterization of polyfluorinated **pH sensitive amphiphilic peptides for prospective receptor-specific drug delivery applications**
- Evaluation of self-assembly properties through **fluorine-specific interactions**

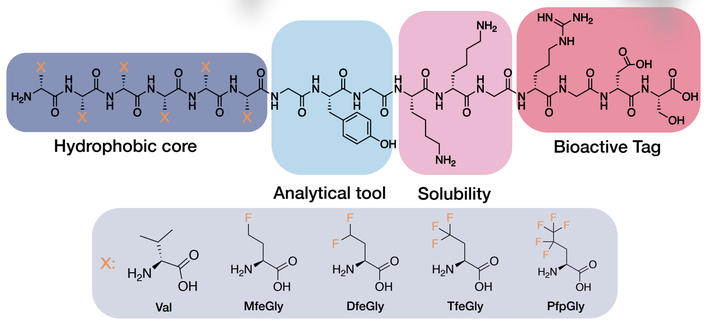


INTRODUCTION



Generation of a pH sensitive amphiphilic block oligopeptide containing the bioactive function RGD and a library of derivatives varying the length of the hydrophobic core and the degree of side chain fluorination. [4] Peptide rational design enables us to obtain desired features (pH sensitivity etc.). [1] The introduction of fluorine alters a wide range of peptide properties such as secondary structure propensity, folding, thermal and metabolic stability and proteolytic resistance. [2] The **RGD function** is highly effective at promoting the attachment of numerous cell types to a plethora of materials. This small sequence is the principal integrin-binding domain present within ECM proteins such as fiobronectin, vibronectin and fibrinogen. For this reason, RGD containing peptides offer several advantages for biomaterials applications. The use of RGD compared with native ECM proteins, minimized the risk of immune reactivity or pathogen transfer[3].

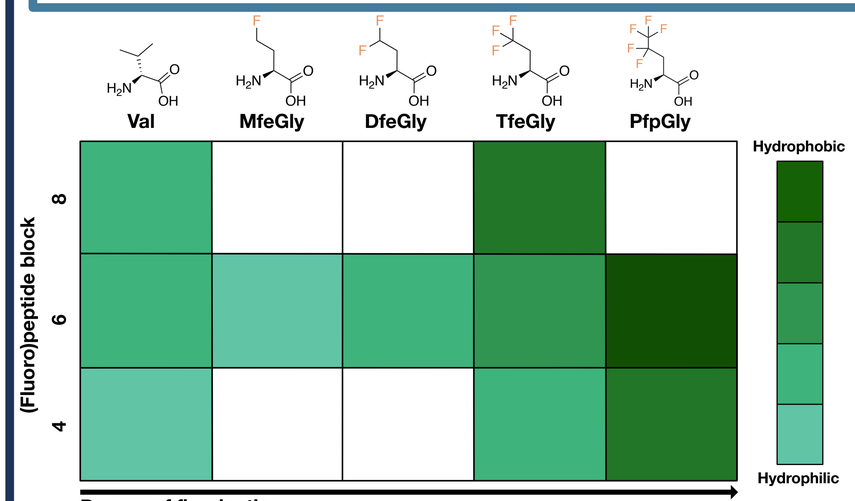
Herein in this work, we present the peptide motif X6RGD and its fluoro-derivates for prospective receptor-specific drug delivery in cancer theraphy. Overall, our results demonstrate that high degree of fluorination achieved triggers a selective modification of peptide self-assembly dramatically improving the structural properties, the carrier suitability,enzimatic degradation profiles and cytotoxic features of the fluoropeptide conjugate(s).



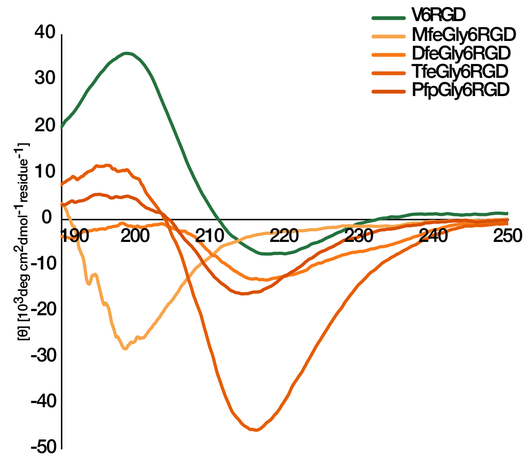
Sructure of the amphiphilic block co-polypeptide X6RGD (X6GYGKKGRGDS) and chemical structures of the (fluorinated) amino acids incorporated in the hydrophobic core.

RESULTS AND DISCUSSION

Hydrophobicity and secondary structure determination



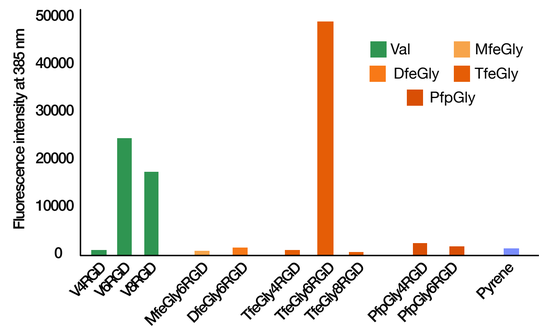
Heatmap giving an idea of the hydrophobicity of the peptides in the library. For (fluoro)peptide blocks: shorter than 6 residues no self-assembly was detected; longer than six residues the intrinsic aggregation propensity of the amphiphile made the process of sythesis and purification too complex.



CD spectras of amphiphilic (fluoro)peptides in phosphate buffer 10 mM pH 7.4 . Enhancing the content of fluorine in the hexapeptide hydrophobic core the secondary structure transitions from a canonical β-sheet conformation to a non-canonical one where the intensity of the bands is inverted in favor of the n -> π* UV transition

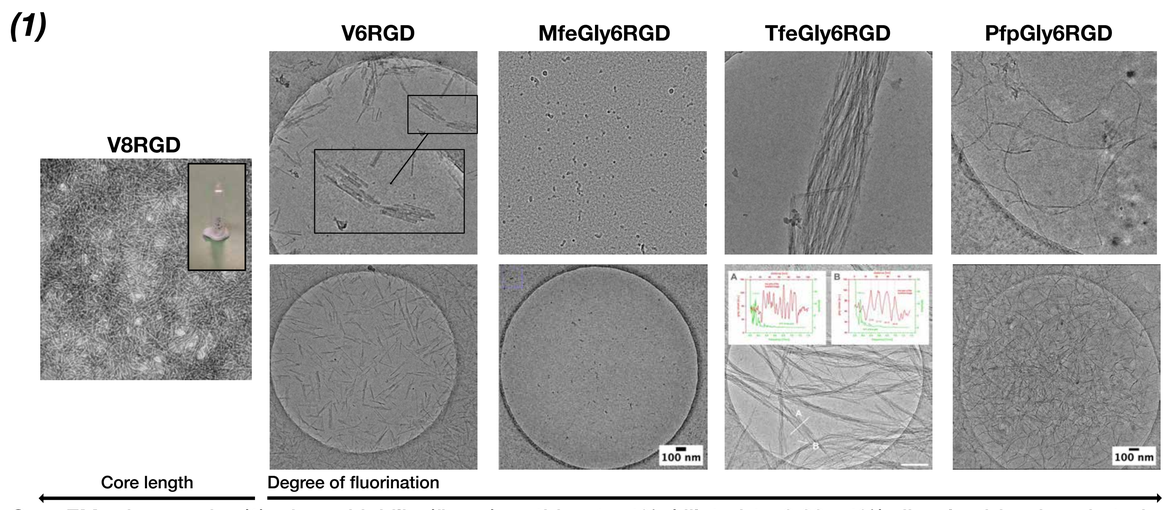
Carrier suitability

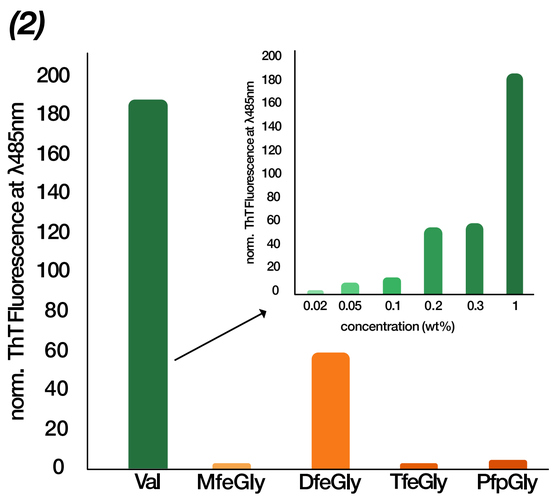
In oder to determine which peptide in the library was able to self-assemble incorporating a small molecule in the hydrophobic inner core, a fluorescence-based investigation through addition of water-insoluble probe pyrene was conducted. An enhancement in fluorescence intensity indicates the encapsulation and solubilization of pyrene in the core of the molecule.

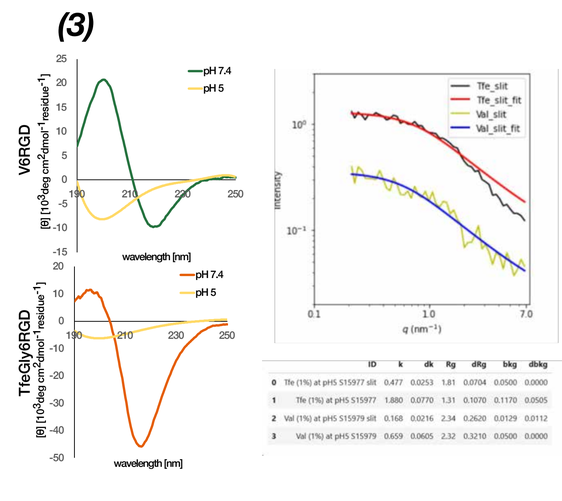


Among the whole library, V6RGD and TfeGly6RGD were the most suitable for carrier applications and therefore were subsequently tested for their drug release abilities. It's important to notice that PfpGly6RGD was also able to self-assemble but not to incorporate pyrene. A possible explanation is that due to the very strong fluorine-specific interaction that drives the assembly of this specific sequence it cannot accomodate molecules.

Morphology and self-assembly properties



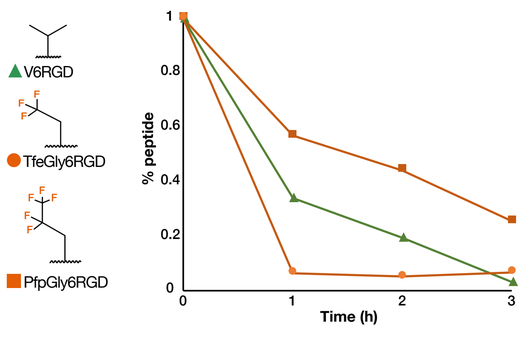




	ID	k	dk	Rg	dRg	bkg	dbkg
0	Tfe (1%) at pH5 S15977 slit	0.477	0.0253	1.81	0.0704	0.0500	0.0000
1	Tfe (1%) at pH5 S15977	1.880	0.0770	1.31	0.1070	0.1170	0.0505
2	Val (1%) at pH5 S15979 slit	0.168	0.0216	2.34	0.2620	0.0129	0.0112
3	Val (1%) at pH5 S15979	0.659	0.0605	2.32	0.3210	0.0500	0.0000

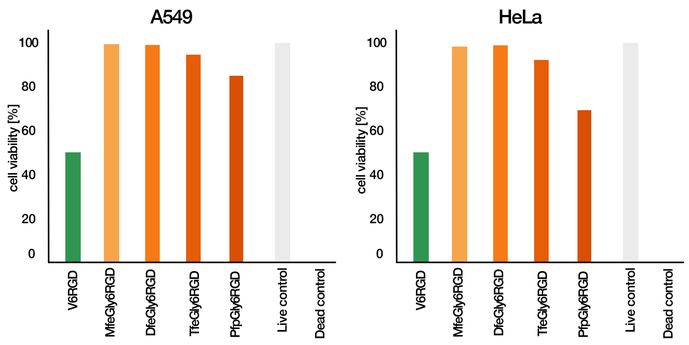
Cryo-EM micrographs (1) of amphiphilic (fluoro)peptides 1 wt% (diluted to 0.33 wt%) dissolved in phosphate buffer 10mM pH 7.4. V6RGD show platelet architectures compared to amyloid-like fibrils. Enhancing the fluorine content MfeGly6RGD forms a hint of fibrils probably at the air/water interface making difficult the capture of the image. TfeGly6RGD forms an homogeneous and highly organized elongated morphology, with a medium diameter centered around 6-7nm (zoom A,B). The single fibers tend to aggregate in bundles and twist. PfpGly6RGD forms instead a "snowflake"-like architecture consisting of shorter and more flexible fibers. On the other hand, elongating the chain of the hydrophobic core V8RGD forms a stiff hydrogel of bundled short and flexible fibrils. The rod-like architectures have been confirmed via SAXS measures revealing cilindrical structures with compact internal diameters according to the degree of fluorination. ThT fluorescence spectroscopy assay to confirm the amyloid nature of V6RGD (2). This dye display a strong fluorescence upon binding amyloid cross-β-sheet structures due to rotational immobilization. SAXS results confirmed the absence of assembled species of at least 1nm diameter at pH 5 (3) and CD spectra show a clear transition from β-sheet conformation in neutral medium (pH 7.4) to random coil in acidic (pH 5) conditions confirming the pH sensitivity of the motif.

Ezyme degradability



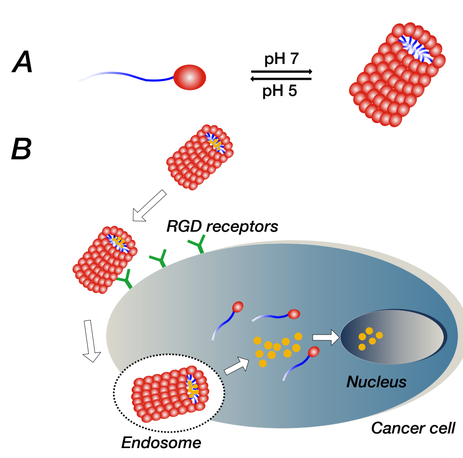
Digestion profiles of V6RGD, TfeGly6RGD, PfpGly6RGD 100uM in phosphate buffer 10 mM upon digestion with serine protease elastase 0.1 mg/mL during 3 hours of incubation time. Valine and TfeGly amino acids share similar hydrophobicity and van der vaals radius values. For this reason the substrate can accomodate the enzyme pocket resulting in an even faster digestion times in favor of the fluorinated peptide. Extending the sidechain length and fluorination content the enzyme is less selective towards the active site resulting in a much slower degradation kinetik.

Cytotoxicity

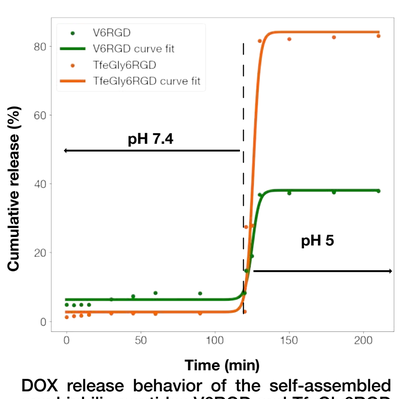


Cell viability (%) A549 and HeLa cell cultures treated with different amphiphilic oligopeptides V6RGD, MfeGly6RGD, DfeGly6RGD, TfeGly6RGD and PfpGly6RGD 1 mg/mL (circa 0.5mM) after 24h incubation. Values of viability are obtained via software automated quantification of fluorescence-imaged live/dead assays. The results are the media of two independent experiments. Countrary to any expectations, the non fluorinated V6RGD is the solely peptide to show a marked toxicity against both cell lines. The integration of even one fluorine atom is enough to bring the cell viability to safe values. For a very high fluorination degree (PfpGly6RGD - 26.6% in fluorine content of the total mw) a slighlty toxic effect is shown in the case of HeLa cells due to the intrinsic sensitivity ot the cell line.

Drug release: mechanism and in vitro experiment



Schematic illustration shows the pH responsive self-assembly of the amphiphilic peptide (A) and the targeted release of anti-tumor drug from the self-assembled architectures of the amphiphilic peptide (B).



DOX release behavior of the self-assembled amphiphilic peptides V6RGD and TfeGly6RGD in neutral (pH 7.4) or acidic medium (pH 5.0) at physiological temperature (37°C)

Outlook

MD simulations to determine the impact of fluorine-specific interactions on the self-assembly properties of the peptide motif.

References

[1] J. Liang, W.L. Wu, X.D. Xu, R.X. Zhuo, X.Z. Zhang Colloids and Surfaces B: Biointerfaces, 2014. 114: p. 398-403
[2] A. A. Berger, J.S. Völler, N. Budisa, B. Koksch, Acc. Chem. Res. 2017, 50, 2093–2103.
[3] S.L. Bellis, Biomaterials, 2011. 32(18): p. 4205-4210.
[4] U. I. M. Gerling, M. Salwiczek, C. D. Cadicamo, H. Erdbrink, C. Czekelius, S. L. Grage, P. Wadhwani, A. S. Ulrich, M. Behrends G. Haufe, B. Koksch, Chem. Sci. 2014, 5, 819–830.
[5] Y. Chen, Y. Hua, W. Zhang, C. Tang, Y. Wang, Y. Zhang and F. Qiu, Int. J. Nanomed,. 2018, 13, 2477–2489.
[6] A. Bertolani, L. Pirrie, L. Stefan, N. Houbenov, J. S. Haataja, L. Catalano, G. Terraneo, G. Giancane, L. Valli, R. Milani, O. Ikkala, G. Resnati and P. Metrangolo, Nat. Commun,.2015, 6, 7574.