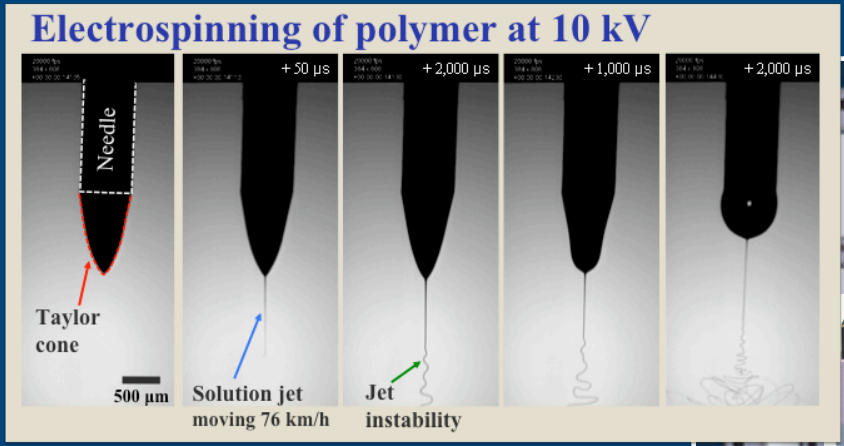
Alexander Bittner

Group leader "Self-Assembly", Ikerbasque Res. Prof. at CIC nanoGUNE in San Sebastian

Peptide fibers: Electrospinning from solutions, molecular vibrational analysis

Electrospinning (standard)

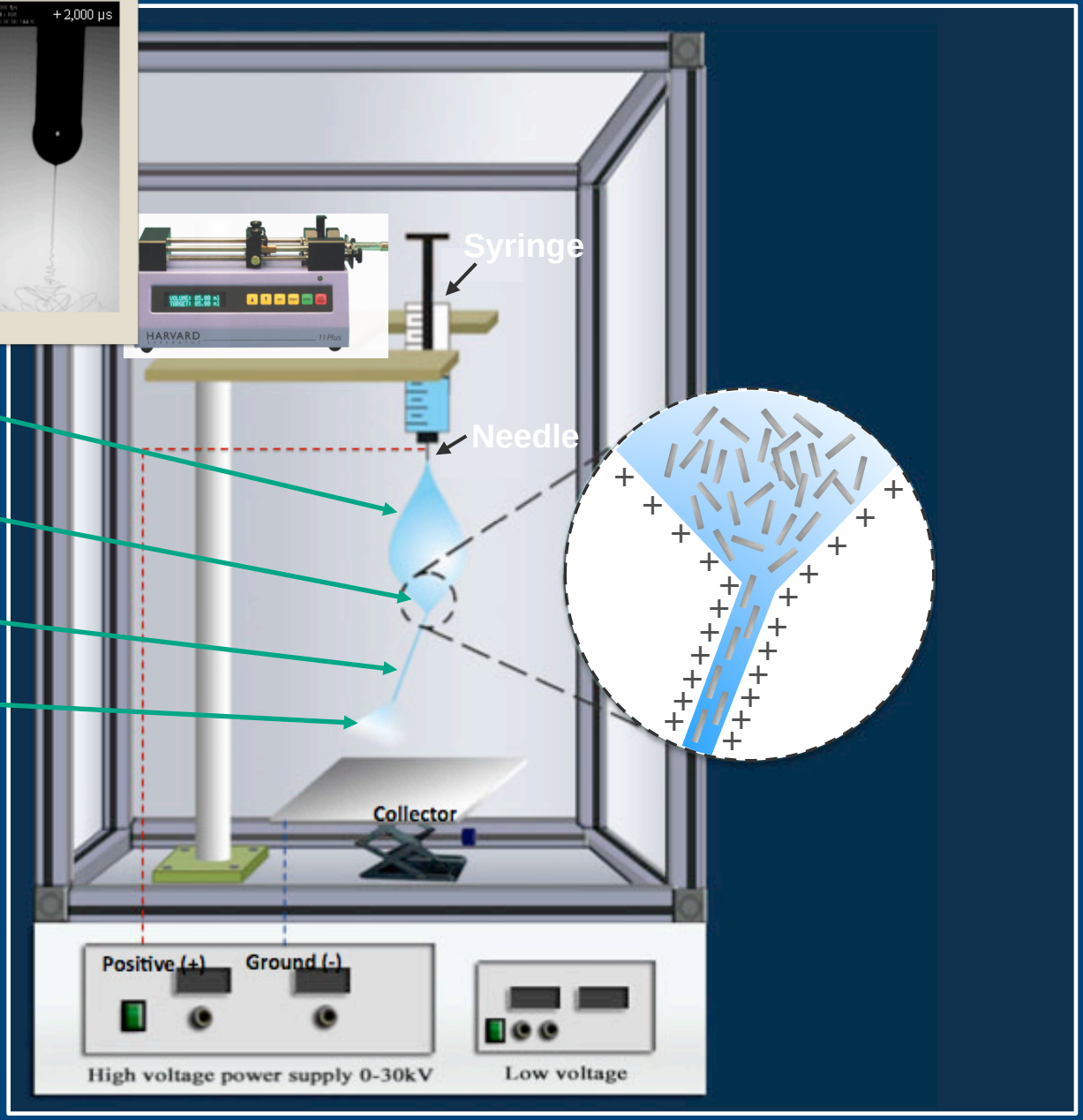


Droplet (1000 µm)

Taylor cone (100 µm)

Jet (50→1 µm)

Whipping jet (1→0.01 µm)



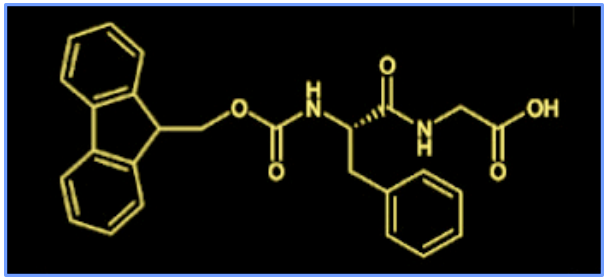
Substance Selection

Aromatic peptides

H-Phe-Phe-OH

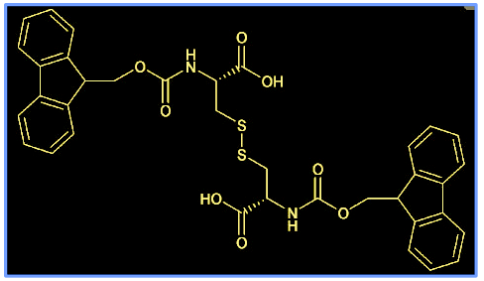
Phe-Phe

Fmoc-Phe-Gly-OH



Fmoc-FG

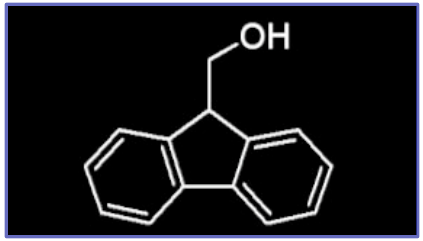
$(Fmoc\text{-}Cys\text{-}OH)_2$



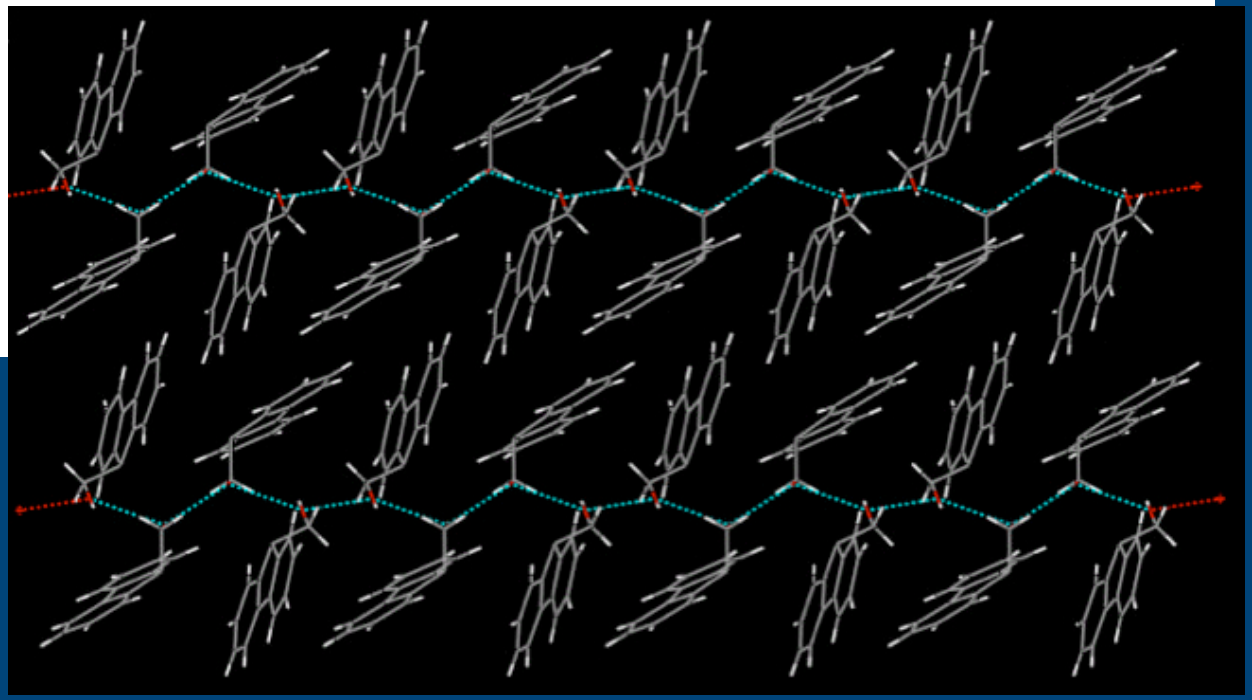
$(Fmoc\text{-}Cys)_2$

Fluorene derivates

9-Fluorenemethanol



9FM



Electrospinnability: Long fibres

Extremely long, coiled or straight fibers, 400-700 nm wide

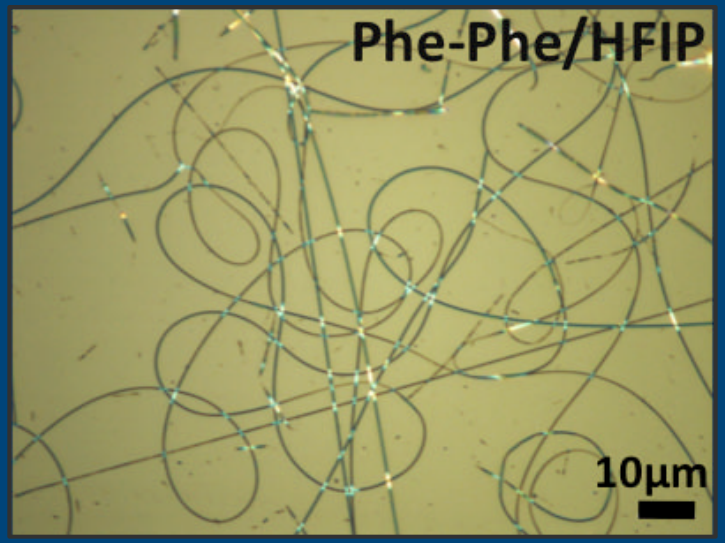
Phe-Phe/HFIP

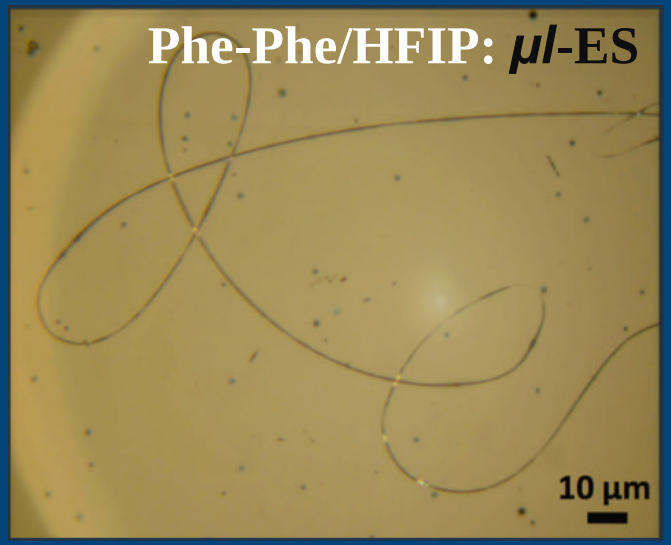
Con. = 16%wt

V = 15 kV

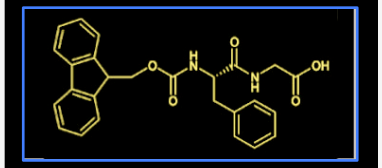
H = 15 cm

Q = 0.5 ml/h





Fmoc-FG/HFIP

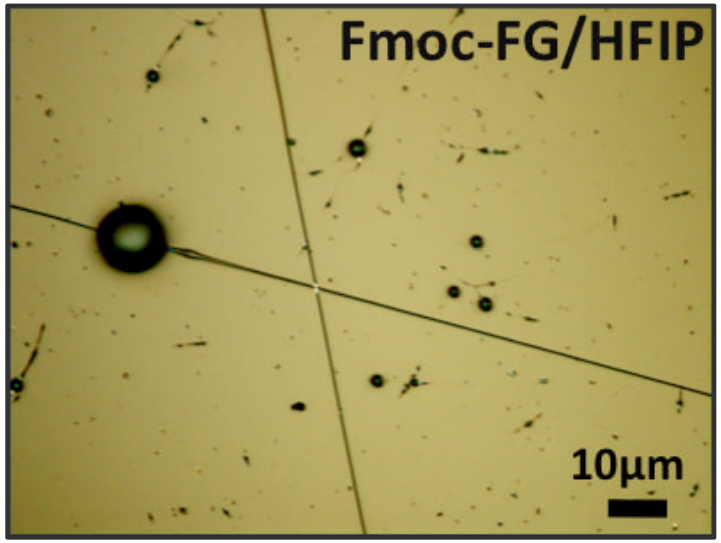


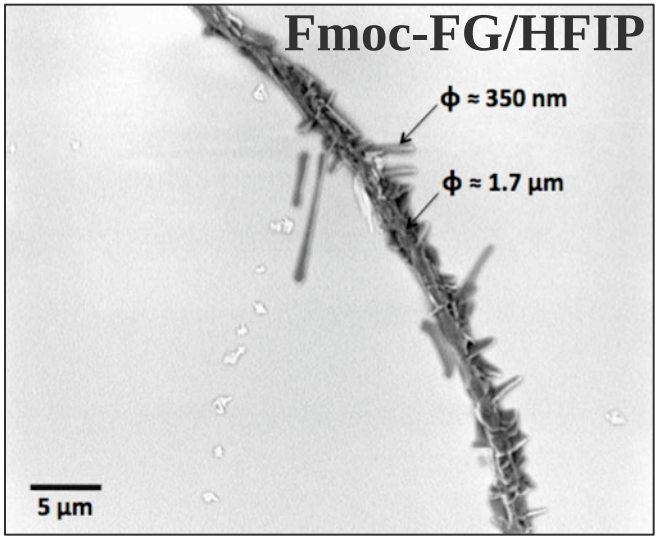
Con. = 18%wt

V = 15 kV

H = 15 cm

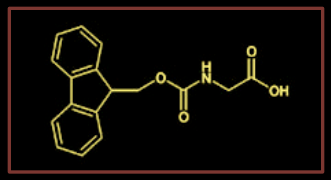
Q = 0.5 ml/h





Electrospinnability: Short Fibres

Fmoc-G/DMF

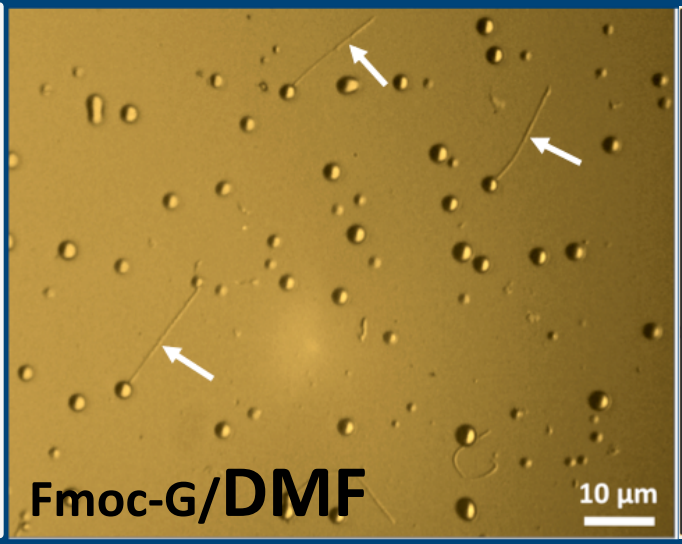


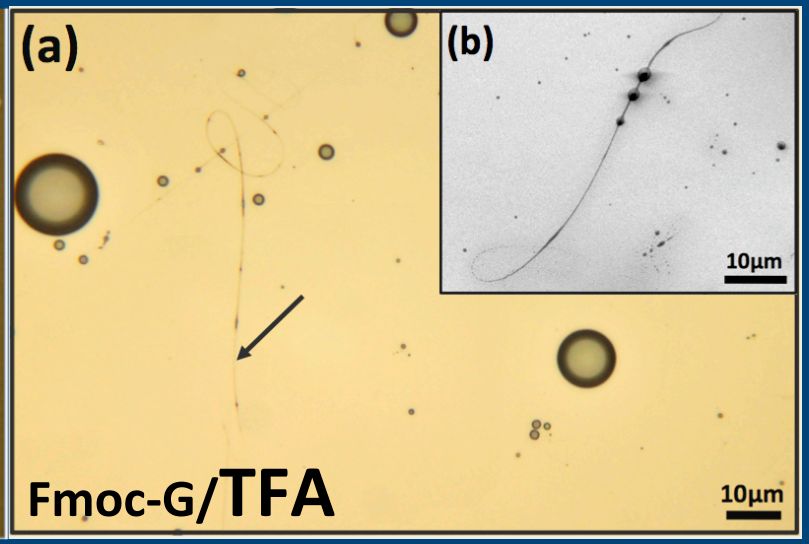
Con. = 20%wt

V = 10 kV

H = 25 cm

Q = 0.5 ml/h





Two-parameter approach (Gutmann, 1976; Malavolta et al., 2006)

Solvent	AN	DN	Electrospinnability
DMF	16.0	26.6	Droplets/short fibers
DMSO	19.3	29.8	Droplets
HFIP	**88.0**	0.0	**Long fibers**
TFA	**105.0**	0.0	**Long fibers**

AN : electron **A**cceptor **N**umber

DN : electron **D**onor **N**umber (nucleophilic)

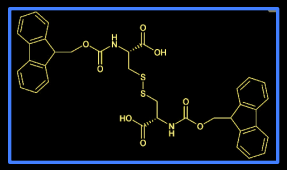
AN ≥ 88

Good acceptor!!

ES needs a **strongly electrophilic solvent**.

Electrospinnability: Solidified Droplets

(Fmoc-Cys)$_2$/DMF

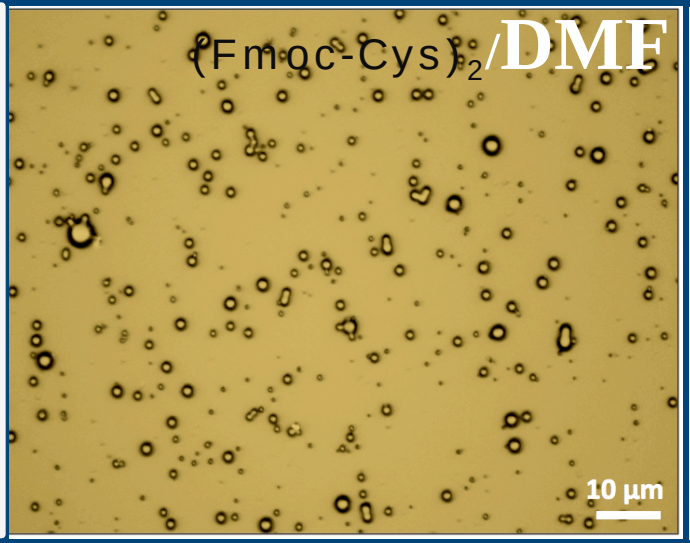


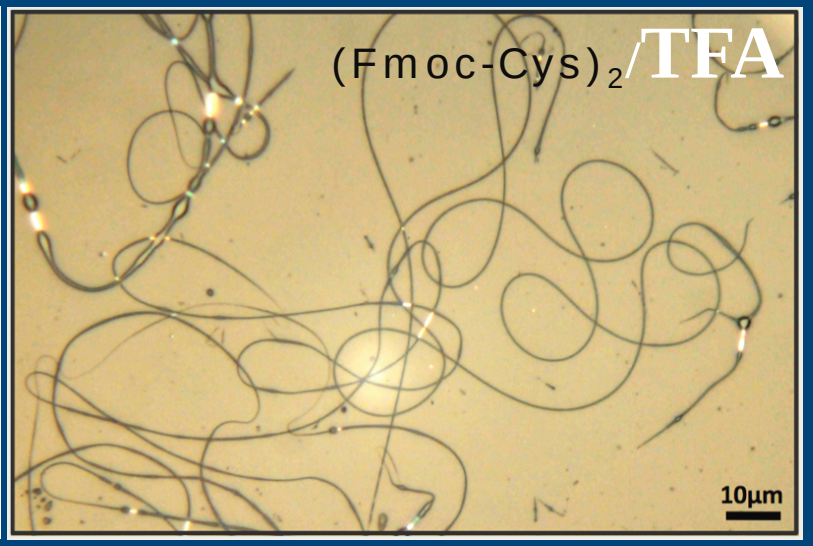
Con. = 20%wt

V = 10-30 kV

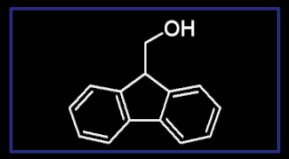
H = 10-15 cm

Q = 0.2-0.5 ml/h





Fluorene derivates substance

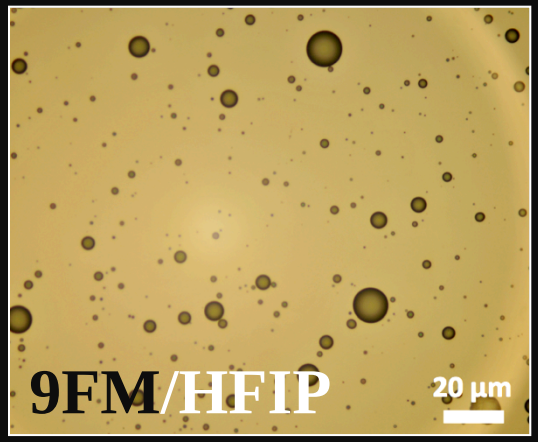


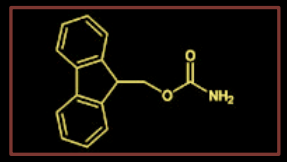
Con. = 10-18%wt

V = 10-30 kV

H = 10-15 cm

Q = 0.2-0.5 ml/h



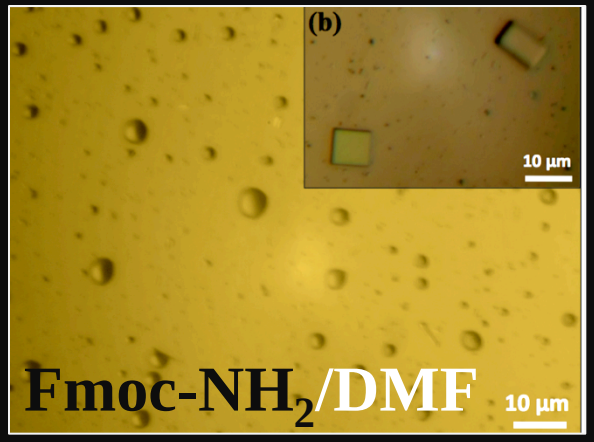


Con. = 10-19%wt

V = 10-30 kV

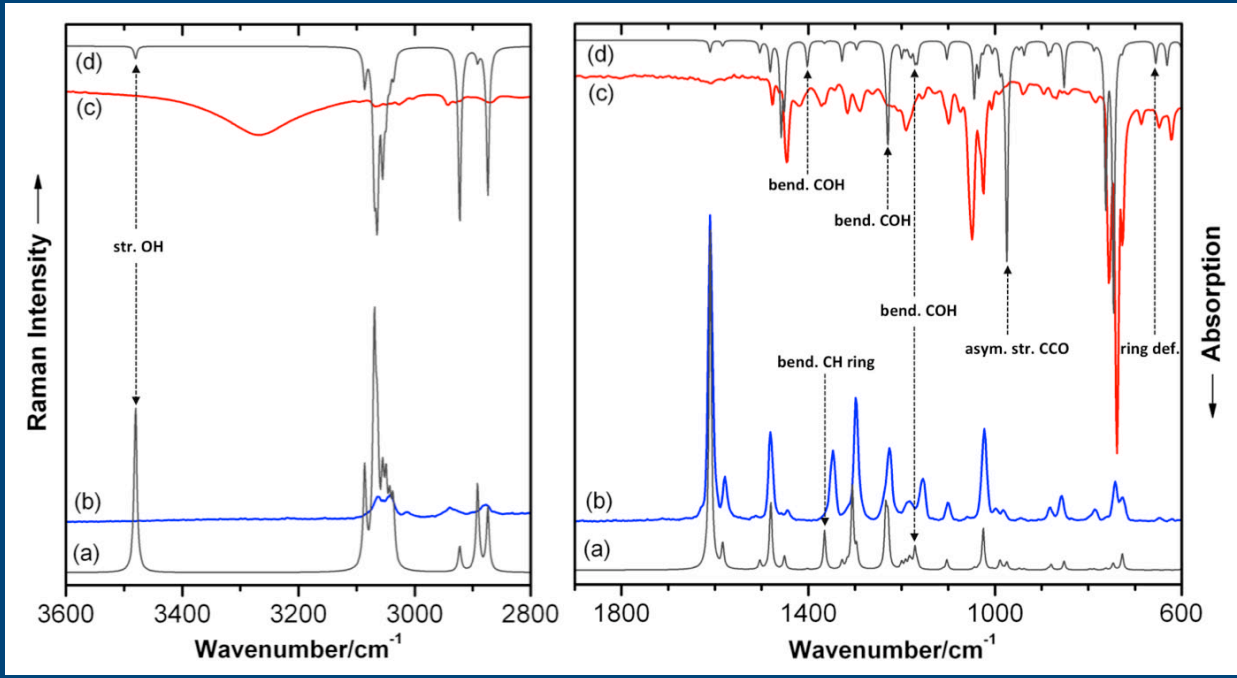
H = 10-15 cm

Q = 0.2-0.5 ml/h

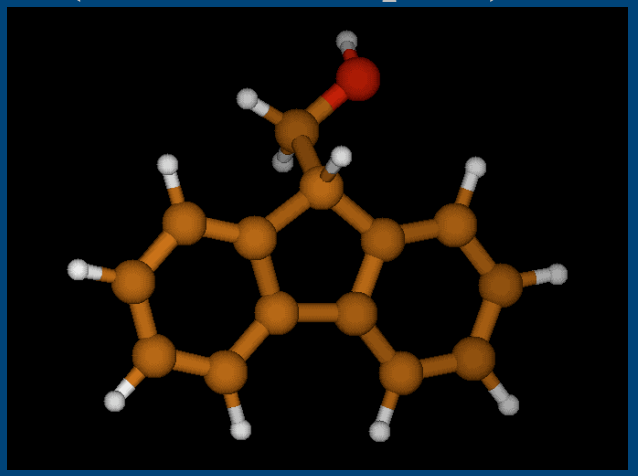


Only some substances (Phe-Phe, Fmoc-FG, (Fmoc-Cys)$_2$, Fmoc-G) can form fibres, when dissolved in an electrophilic solvent at high concentration.

Vibrational spectroscopy of 9FM

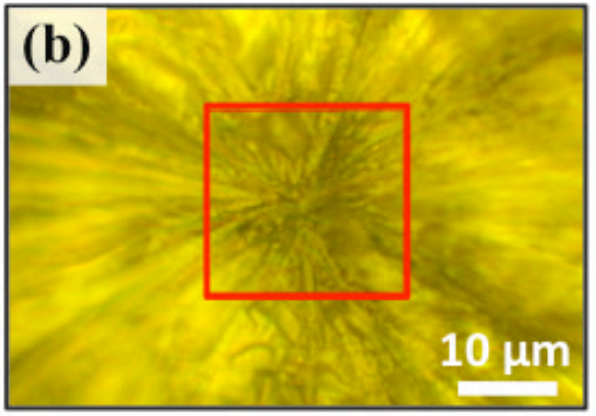


(solidified droplets)



Vibrational	IR/Raman
o.o.p. CH ring bend.	728vs/m
i.p. CH ring bend.	vw/1610vs

IR downshifted	Raman downshifted
(1) str. OH	(3) i.p. CH ring bend.
(2) i.p. CH ring bend. + Roc. CH_2 + bend. COH	(4) i.p. CH ring bend. + bend. CH + bend. COH



Vibrational spectroscopy of 9FM

Vibrational Modes (9FM)	Experiments (cm^{-1})				Simulations (cm^{-1})				Shift	
	IR (Pow.)	IR (AIST)	Raman (Pow.)	Raman (Sigma)	Sim.	Scaled	IR inten.	Raman inten.	IR	Raman
str. OH		3323vs			3652	3480	3.78	235.90	↓	
wag. CH_2 + bend. COH	1418m				1451	1402	6.80	1.33	↑	
i.p. CH ring bend.		1367w	1347s	1348s	1413	1365	0.63	48.84		↓
bend. COH + bend. CH(H)	1208w		1227s	1226s	1277	1234	5.55	69.73	↓	
i.p. CH ring bend. + bend. CH(H) + bend. COH	1155w		1154m	1152m	1213	1172	4.58	26.63	↓	↓
asym. str. CCO	940w	940w	983w	983w	1009	975	59.17	9.57	↓	
i.p. ring def.	648m	622w	648vw		678	656	6.02	0.39	↓	
tor. COH			305w	304m	248	240	132.72	7.16		↑

Vibrational spectroscopy – complete assignment

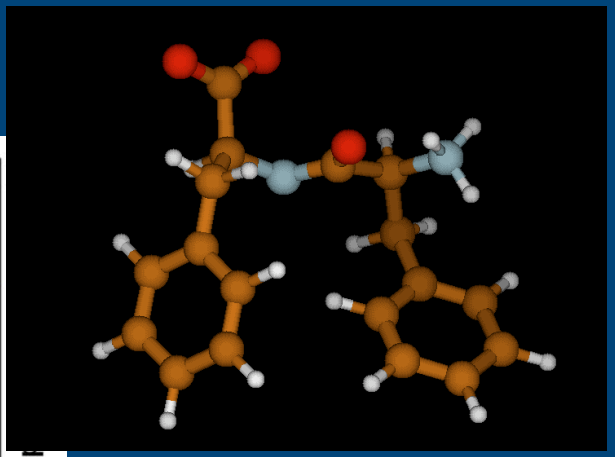
Vibrational Modes (9FM)	Experiments (cm^{-1})				Simulations (cm^{-1})				Shift	
	IR (Pow.)	IR (AIST)	Raman (Pow.)	Raman (Sigma)	Frq. Sim.	Frq. Scaled	IR inten.	Raman inten.	IR	Raman
str. OH		3323vs 3250vs			3652	3480	3.78	235.90	↓	
i.p. sym. str. CH ring	3095vw	3145w	3063m	3070m	3238	3086	10.77	140.49		
		3098w			3221	3070	35.54	325.58		
i.p. asym. str. CH ring	3065w	3063w			3216	3064	44.36	162.42		
	3049w	3039m	3040m	3048m	3206	3055	31.39	106.08		
	3028w		3014vw		3200	3049	14.74	101.84		
					3193	3043	7.06	71.07		
					3188	3038	1.50	60.09		
					3186	3036	6.22	33.68		
asym. str. CH_2 + str. C_9H	2943w		2939w	2943w	3067	2922	53.95	36.05		
	2930vw	2926w			3034	2892	3.70	125.22		
sym. str. CH_2	2870w	2877w	2877w	2883w	3016	2874	45.14	90.34		
i.p. bend. CH ring			1610s	1613vs	1667	1610	3.03	81.61		
			1578m	1578s	1667	1610	0.11	343.56		
					1640	1584	0.82	16.15		
					1638	1582	0.70	15.13		
bend. CH_2			1513vw		1557	1504	3.05	10.84		
i.p. bend. CH ring	1477m	1477w	1481s	1483s	1534	1485	7.27	27.36		
	1447s	1467m	1444w	1443w	1532	1482	1.02	63.53		
		1451s			1509	1480	23.37	0.86		
		1443s			1501	1451	15.22	16.62		
wag. CH_2 + bend. COH	1418m				1451	1402	6.80	1.33	↑	
i.p. CH ring bend.		1367w	1347s	1348s	1413	1365	0.63	48.84		↓
i.p. CH ring bend. + bend. C_9H	1316m	1329w			1374	1328	5.48	10.05		
		1316w			1363	1317	0.30	7.09		
i.p. CH ring bend.			1299s	1300s	1352	1306	0.26	104.71		
bend. C_9H	1289m	1284w			1342	1296	2.31	24.88		
bend. COH + bend. CH(H)	1208w		1227s	1226s	1277	1234	5.55	69.73	↓	
					1273	1229	26.42	57.21		
bend. CH(H)	1190m	1187w			1242	1200	4.12	8.49		
twi. CH_2 + bend. C_9H					1234	1192	1.60	9.46		
i.p. CH ring bend.		1178w	1183w	1187w	1225	1184	2.38	12.21		
					1221	1179	2.78	6.54		
i.p. CH ring bend. + bend. CH(H) + bend. COH	1155w		1154m	1152m	1213	1172	4.58	26.63	↓	↓
i.p. CH ring bend.	1100m	1098w		1104w	1208	1166	4.77	7.76		
					1151	1112	0.42	1.52		
					1142	1103	4.88	12.31		

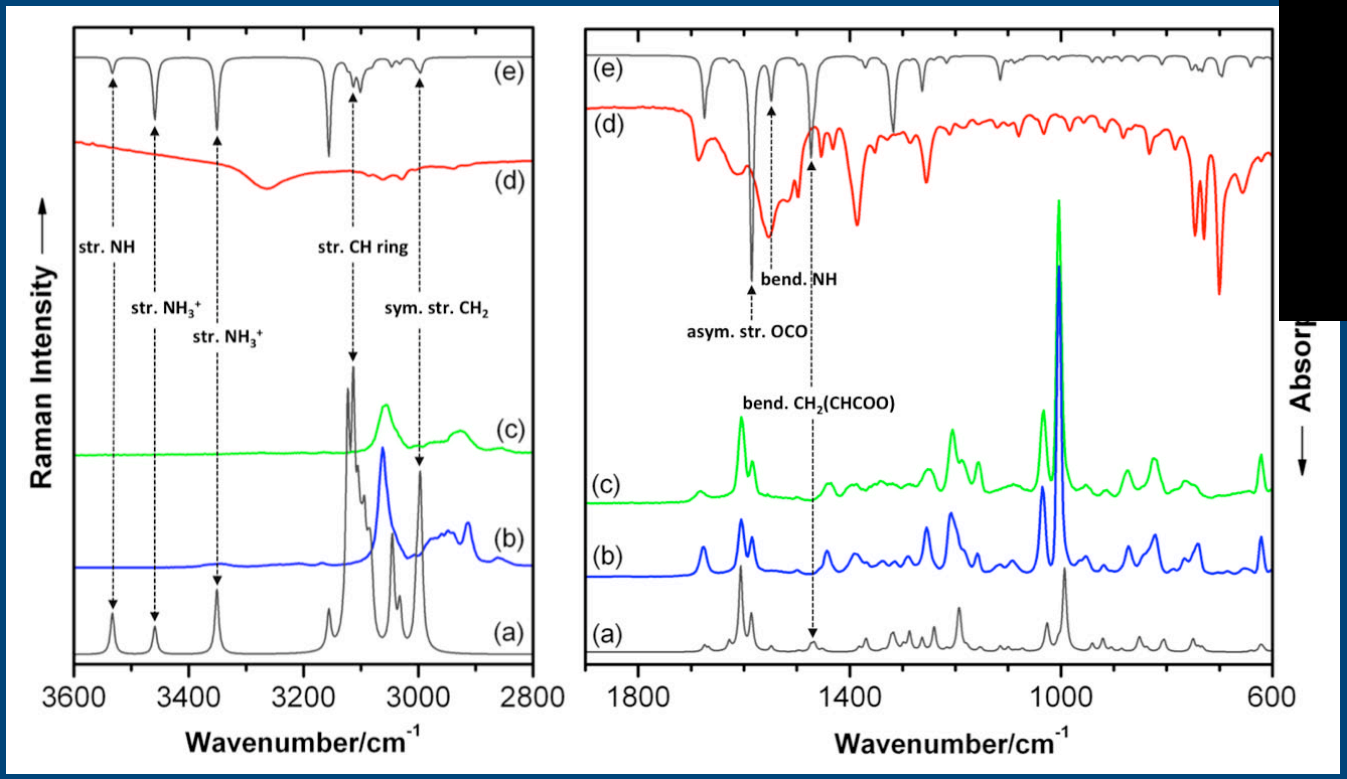
Table S1. *(Continued)*

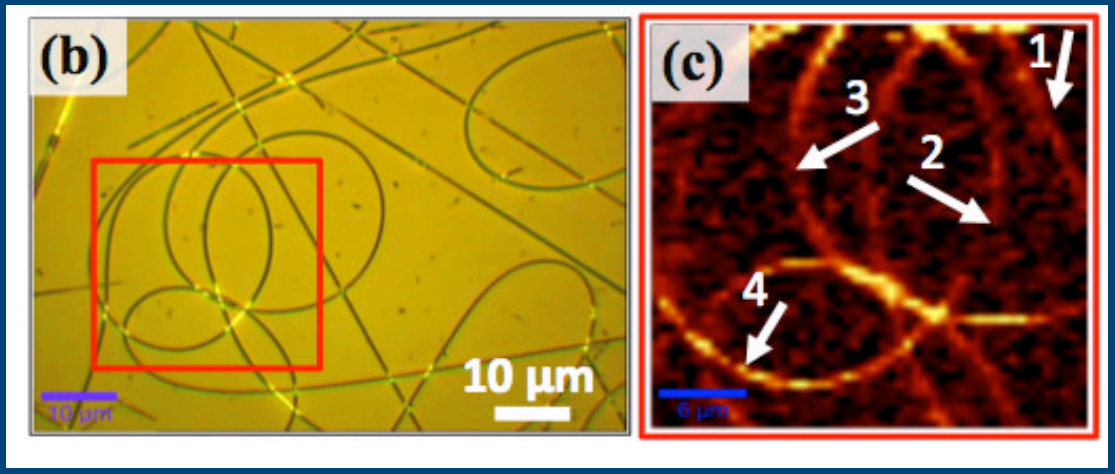
Vibrational Modes (9FM)	Experiments (cm^{-1})				Simulations (cm^{-1})				Shift	
	IR (Pow.)	IR (AIST)	Raman (Pow.)	Raman (Sigma)	Frq. Sim.	Frq. Scaled	IR inten.	Raman inten.	IR	Raman
i.p. CH ring bend. + roc. CH_2	1049s	1060s 1043s			1081	1045	14.90	2.46		
i.p. CH ring bend. + roc. CH_2 + bend. COH	1025s	1034m			1071	1035	8.40	2.04		
i.p. CH ring bend.		1022s 1017vs	1023s	1022s	1061	1025	2.23	51.62		
i.p. ring def.	1007w	1012s			1041	1006	2.53	1.07		
o.o.p. CH ring bend. + bend. CH + roc. CH_2	992w		999w	1000w	1024	989	5.99	10.79		
o.o.p. CH ring bend.					1021	986	1.11	1.66		
o.o.p. CH ring bend.					1016	982	3.68	1.51		
asym. str. CCO	940w	940w	983w	983w	1009	975	59.17	9.57	↓	
o.o.p. CH ring bend.	895w		881w	883w	982	948	1.43	2.05		
					971	938	3.45	1.34		
					917	886	3.47	1.78		
					911	880	2.21	6.67		
i.p. ring def.	868w	855w	857m	857m	882	852	12.31	10.82		
o.o.p. CH ring bend. + bend. C_9H					825	797	0.10	1.35		
i.p. ring def. + roc. CH_2	784w		786w	787w	816	788	1.79	1.42		
o.o.p. CH ring bend.	756s	758vs	743m	743m	790	763	43.15	2.16		
	739s	747m 741s	728m	726m	774	748	6.84	5.13		
	728s	730s 725m			772	727	65.91	3.74		
i.p. ring def.	648m	622w	648vw		753	698	1.69	20.48		↓
	622m				678	656	6.02	0.39		
					654	631	6.55	0.38		
i.p. ring def. + roc. CH_2		594m	593vw	591w	618	597	5.56	3.37		
o.o.p. ring def.		544w	543m	543m	589	569	0.37	0.59		
		537w		513m	556	537	11.68	4.85		
i.p. ring def.					528	510	0.38	3.98		
o.o.p. CH ring bend. + bend. C_9H					469	453	0.10	2.04		
o.o.p. ring def.					447	432	4.98	0.29		
i.p. ring def.			416m	417m	427	412	0.24	7.22		
o.o.p. ring def. + bend. CCO				391w	380	368	7.19	1.14		
bend. CCO				343w	341	329	2.46	3.35		
o.o.p. ring def. + bend. CCO					303	293	2.57	8.88		
tor. COH			305w	304m	248	240	132.72	7.16		↑
i.p. ring def.				213w	214	207	7.58	0.97		
tor. CCC				196w	186	179	9.32	3.66		
				157w	140	135	9.87	1.53		
tor. ring				130m	123	119	1.08	1.20		

Vibrational Modes (9FM)	Experiments (cm^{-1})				Simulations (cm^{-1})				Shift	
	IR (Pow.)	IR (AIST)	Raman (Pow.)	Raman (Sigma)	Frq. Sim.	Frq. Scaled	IR inten.	Raman inten.	IR	Raman
tor. ring					109	105	0.83	0.12		
					72	70	0.33	7.57		

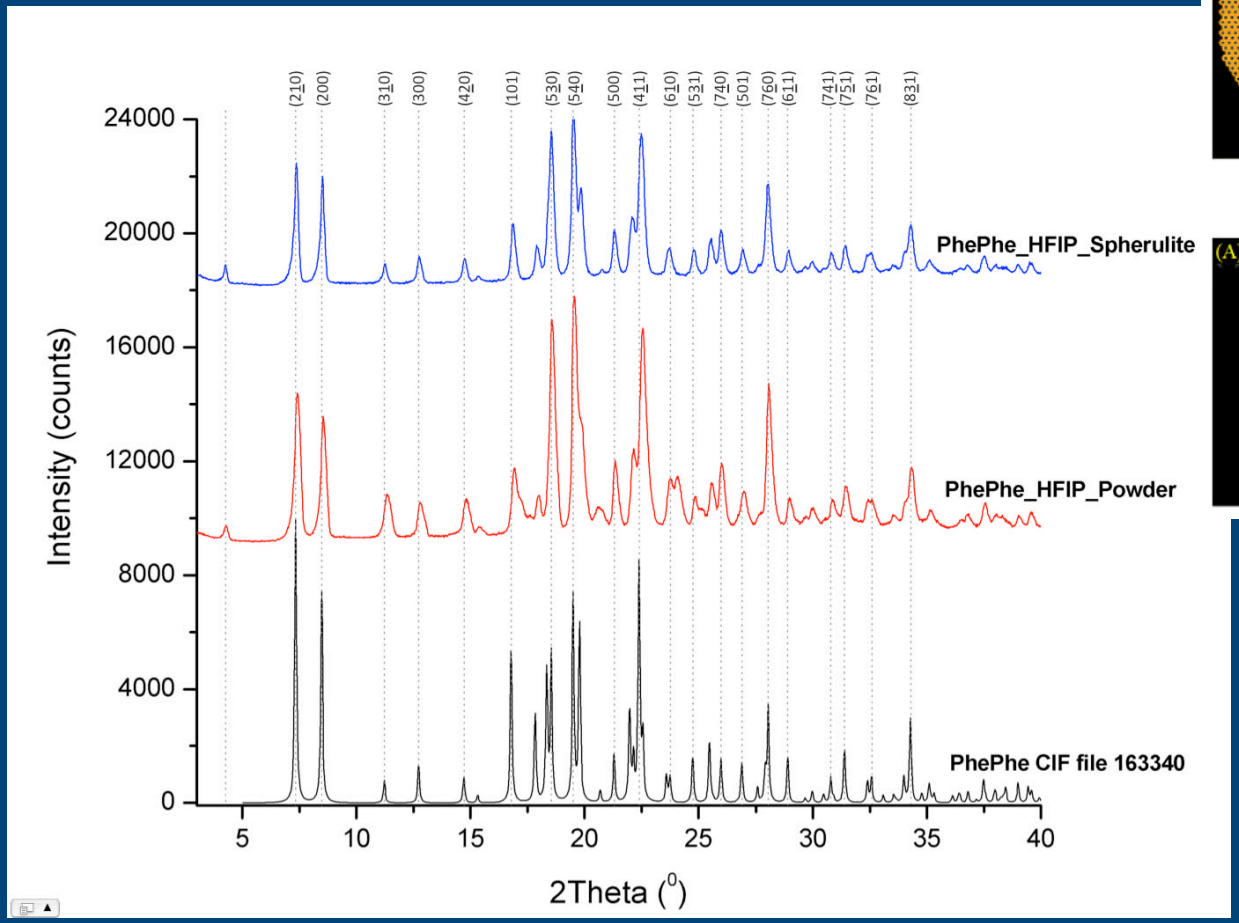
Vibrational spectroscopy of FF



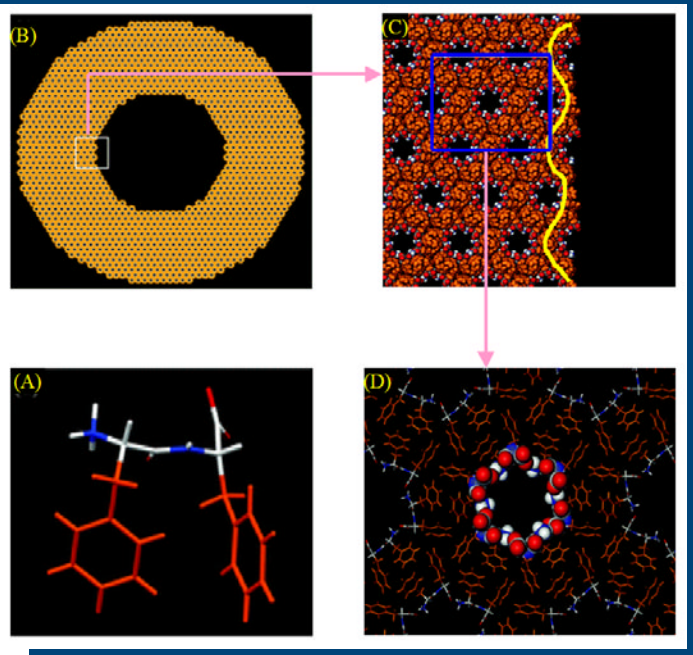




Structure of FF



Comparative powder X-ray diffraction



H. Görbitz

Role of π stacking vs. hydrogen bonding

→→ amyloid fibril aggregation?

Resume

Electrospinning of **monomers** is possible for aromatic peptide (derivates), which are able to **assemble (in 1D?)**

The **solvent** should be strongly electrophilic (AN ≥ 88), e.g. HFIP, TFA.

The upshifted/downshifted **vibrational frequencies** are related to H-bonding (and to π-stacking).

Protein spinning is straightforward, but the analysis is a major problem (solid state NMR?)

“µL ES” and “ExFFES” are simple and effective techniques

CIC nanoGUNE
nanoscience cooperative research center

Group “Self-Assembly”:

Wiwat Nuansing

Amaia Rebollo

Molecular vibrations:

Txema Mercero
(EHU/UPV, Donostia/ES)

XRD:

Javier Zuñiga
(EHU/UPV, Bilbo/ES)